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# Part I: Syntheses, Characterizations, and Investigations of Molecular Micelles and Imidazolium-Based Ionic Liquids for Protein Separations in Electrophoresis PART II: Investigating the Mentoring Canon Through Exploration of Thirty Years of Mentoring by an Exemplar

Monica R. Sylvain
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**PART I:**
**SYNTHESES, CHARACTERIZATIONS, AND INVESTIGATIONS OF MOLECULAR MICELLES AND IMIDAZOLIUM-BASED IONIC LIQUIDS FOR PROTEIN SEPARATIONS IN ELECTROPHORESIS**

**PART II:**
**INVESTIGATING THE MENTORING CANON THROUGH EXPLORATION OF THIRTY YEARS OF MENTORING BY AN EXEMPLAR**

**A Dissertation**

**Submitted to the Graduate Faculty of the**
**Louisiana State University and**
**Agricultural and Mechanical College**
**in partial fulfillment of the**
**requirements for the degree of**
**Doctor of Philosophy**

**in**

**The Department of Chemistry**

**By**
**Monica R. Sylvain**
**B.S., Howard University, 1990**
**December, 2010**

## DEDICATION

I dedicate this work to my wonderful husband and confidant, Martin A. Sylvain. I am eternally grateful to you for your loving, patient and caring support, which helped make this goal a realization. When I was unsure, you encouraged me with your sound wisdom; and when I was weary, you strengthened me with your words and kind acts. We are one, and our walk together is supernatural. I have been blessed with a King sent from heaven. Thank you, Martin. I Love You.

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## LIST OF ABBREVIATIONS

| Abbreviation | Name |
|---|---|
| AGP | alpha-1-acid glycoprotein |
| α-CHY | alpha-chymotrypsinogen A |
| α-LAC | alpha-lactalbumin |
| ANS | 1-anilinonaphthalene-6-sulfonic acid |
| β-ME | β-mercaptoethanol |
| BSA | bovine serum albumin |
| BMOEOEIMBr | 1-butyl-3-methoxyethoxyethylimidazolium bromide |
| BMOEIMBr | 1-butyl-3-methoxyethylimidazolium bromide |
| BMOMIMBr | 1-butyl-3-methoxymethylimidazolium bromide |
| CD | circular dichroism |
| CE | capillary electrophoresis |
| CMC | critical micelle concentration |
| CYTC | cytochrome C |
| $D_2O$ | deuterium oxide |
| DMOEOEIMBr | 1-dodecyl-3-methoxyethoxyethylimidazolium bromide |
| DMOEIMBr | 1-dodecyl-3-methoxyethylimidazolium bromide |
| DMOMIMBr | 1-dodecyl-3-methoxymethylimidazolium bromide |
| DLS | dynamic light scattering |
| $d_6$-DMSO | dimethyl sulfoxide |
| DTT | dithiothreitol |
| EOF | electroosmotic flow |

| | |
|---|---|
| GLY | Glycine |
| GnHCl | guanidine hydrochloride |
| IL | ionic liquid |
| LYS | lysozyme |
| MMOEOEIMBr | 1-methyl-3-methoxyethoxyethyimidazolium bromide |
| MMOEIMBr | 1-methyl-3-methoxyethylimidazolium bromide |
| MMOMIMBr | 1-methyl-3-methoxymethylimidazolium bromide |
| MEKC | micellar electrokinetic chromatography |
| MALDI-MS | matrix assisted laser desorption ionization mass spectrometry |
| MW | molecular weight |
| NMR | nuclear magnetic resonance |
| OT-CEC | open tubular electrochromatography |
| OVA | ovalbumin |
| Phe | phenylalanine |
| PHOS *b* | phosphorylase b |
| pI | isoelectric point |
| poly-SUS | poly (sodium *N*-undecanoyl sulfate) |
| RB | running buffer |
| RNase *A* | ribonuclease A |
| RNase *B* | ribonuclease B |
| $R_f$ | relative mobility |
| RSD | relative standard deviation |
| RTIL | room temperature ionic liquid |

| | |
|---|---|
| SB | sample buffer |
| SDS | sodium dodecyl sulfate |
| SDS-PAGE | sodium dodecyl sulfate polyacrylamide gel electrophoresis |
| TSIL | task-specific ionic liquid |
| $T_f$ | Transferrin |
| TCEP | tris(2-carboxyethyl) phosphine |
| Tris | tris(hydroxymethyl)aminomethane |
| Trp | tryptophan |
| Tyr | tyrosine |

## ABSTRACT

The research reported in this dissertation is presented in two parts. The first part is the Syntheses, Characterizations, and Investigations of Molecular Micelles and Imidazolium-Based Ionic Liquids for Protein Separations in Electrophoresis, and the second part is a case study entitled Investigating the Mentoring Canon through Exploration of Thirty Years of Mentoring by an Exemplar.

Protein separations in the gel electrophoretic format are ubiquitous analyses used on a daily basis throughout biochemistry, analytical chemistry, and biotechnology. The goal of the research reported in the first part of this dissertation is to synthesize and characterize molecular micelles and ether-functionalized imidazolium-based ionic liquids and investigate their relevance and practical utility for protein separations in polyacrylamide gel electrophoresis (PAGE). Four experimental parameters were optimized for poly (sodium *N*-undecanoyl sulfate) (poly-SUS) protein separations. The analytical techniques, fluorescence spectroscopy and circular dichroism, proved useful for investigating the extent and mechanism of interaction that the molecular micelles and ionic liquids had with a combination of globular and glycosylated proteins. Based on the spectroscopic results, postulations were made and experiments were designed using validation samples in one-dimensional PAGE. Poly-SUS binding to p53, a major target for anticancer therapy, was highly efficient. The addition of 20% v/v 1-methoxyethoxyethyl-3-methyl imidazolium bromide and 1- methoxyethoxyethyl-3-butyl imidazolium bromide to the sample buffer containing poly-SUS resulted in significant gel band shifting of transferrin, a well known biomarker for chronic alcoholism and congenital disorders of glycosylation (CDG).

The goal of the research reported in part two of this dissertation was to investigate the mentoring of an exemplar over a thirty year period to determine why his mentoring ‘works’ as evidenced by the many invited talks, publications as well as local and national mentoring awards received. Study participants’ conceptions of what made the exemplar an effective mentor and why his mentoring philosophy worked were determined by item analysis of 40 mentor interview questions, 3080 protégé in-depth survey responses, and 2 mentoring award nomination packages. A theory of why the exemplar’s mentoring ‘worked’ was determined using constructivist grounded theory analysis. The theory that was constructed consisted of three stages: intra-psychological positioning, inter-psychological exchange, and intra-psychological transformation.

# PART I.: SYNTHESES, CHARACTERIZATIONS, AND INVESTIGATIONS OF MOLECULAR MICELLES AND IMIDAZOLIUM-BASED IONIC LIQUIDS FOR PROTEIN SEPARATIONS IN ELECTROPHORESIS

# CHAPTER 1

# INTRODUCTION

## Part I. Introduction to Protein Structure and Stability

### 1.1 Protein Structure

Proteins are a class of organic macromolecules that are typically constructed from the twenty different common L-amino acids. Structurally, each amino acid contains common features: an α-carbon, an amino group, a carboxyl group, and a side chain or R group. The amino group, carboxyl group, and variable side chain are covalently bound to the α-carbon, which is the central atom for each amino acid. Nineteen of the twenty most common amino acids follow this pattern. The remaining amino acid, proline, deviates from this basic structure because the α-carbon exists as a cyclic imine. In addition, most amino acids are asymmetric since the α-carbon is bound by four different constituents (i.e. a chiral center) (Figure 1.1). The resulting two stereoisomers are optically active due to differences in how each isomer rotates plane polarized light; thus, leading to L- and D- amino acids. Amino acids can be linked together to form higher ordered structures, such as proteins. Proteins may be constructed from as few as a hundred amino acids or thousands of them. However, proteins containing less than one hundred amino acids (or < 10kDa) are referred to as polypeptides. Polypeptides and proteins are formed by linking multiple amino acids via peptide bonds that are formed during a condensation reaction between the α-carboxyl group and the α-amino group of adjacent amino acids.

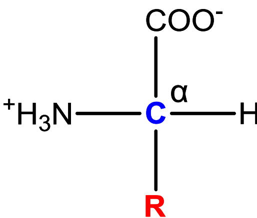



**Figure 1.1** General structure of an amino acid

When dissolved in an aqueous solution, a protein exhibits amphoteric properties existing as a zwitterion. Under these conditions, the dipolar ion can act as an acid (a proton donor) or a base (a proton acceptor). The sum of charges on a protein is called its net charge. At the isoelectric point of a protein, the equimolar concentrations of the proton donor ($^{+}H_3N$-CHR-COOH) and proton acceptor ($^{+}H_3N$-CHR-$COO^{-}$) species are equal, and its net charge is zero. The net charge of a protein is determined by the medium in which it is suspended. If the protein solution is at a pH below the protein's isoelectric point, the protein bears a net positive charge. For solutions whose pH is above its isoelectric point, the protein's overall charge is negative.

### 1.2 Classification of Proteins

The protein side chains or R groups (Figure 1.1) are essential components having various sizes, structures, and polarities which influence the structure, stability, solubility, and function of the resulting protein. Structurally, proteins contain four distinct classifications (Figure 1.2): primary (1º), secondary (2º), tertiary (3º), and quaternary (4º). The primary structure is defined by the linear order and number of amino acids in the chain sequence. Conventionally, the order of amino acids is interpreted from the N-terminus to the C-terminus, which is the amine-terminated group and carboxyl-terminated group of the protein, respectively. When amino acid residues along the peptide backbone form hydrogen bonds, an ordered secondary structure is formed. The secondary structure contains several motifs including alpha helix, beta sheet, random coil, turns, and loops. Studies by Chakrabarty et al. [1] and Blaber et al. [2] among others have demonstrated that amino acids in aqueous solution prefer specific conformations particularly α-helix and β-sheet that influence protein structure and folding. Therefore, the alpha helix and beta sheet (Figure 1.3) are the two most common and stable secondary structural motifs that exist. As the protein chain folds driven primarily by the

hydrophobic effect, it forms a three-dimensional shape or tertiary structure where the non-polar amino acid side chains pack in the core of the protein and the more polar

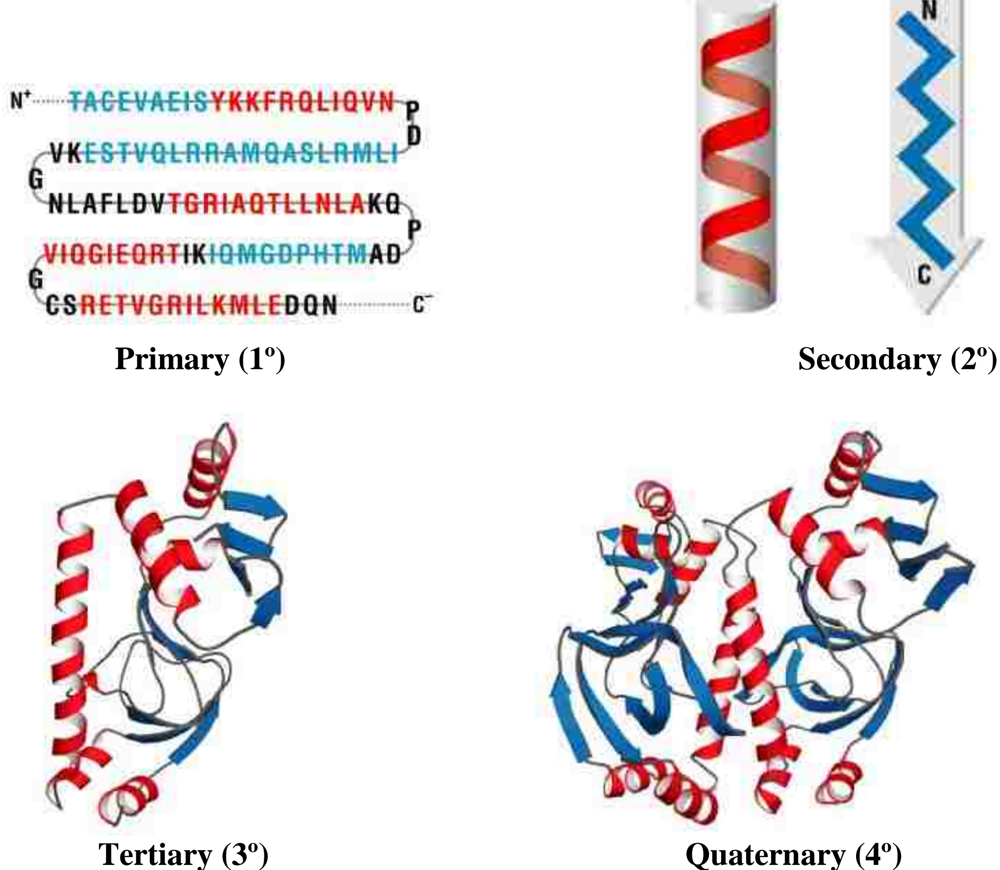



**Figure 1.2** The four protein classifications: primary (1º), secondary (2º), tertiary (3º), and quaternary (4º). (http://www.new-science-press.com/info/illustration_files/nsp-protein-1-0-1_2.jpg, accessed January 23, 2010)

side chains reside near the water-rich surface [3]. Other covalent and non-covalent interactions such as disulfide bonds, salt bridges, and hydrogen bonds also contribute to the stability of tertiary structure. In addition, the quaternary structure is an assembly of monomer chains or subunits into a larger complex typically joined by disulfide bonds and often stabilized by many of the non-covalent interactions that stabilize tertiary structure. Quaternary structures, where

greater than two subunits form a complex, are called multimers (i.e., dimer, trimer, tetramer, etc.). To understand the classification of protein structure, it is important to note that Christian Anfinsen in 1954 was the first to show that the amino acid sequence determines the folding pattern and subsequent higher-order structure [4]. Changes to protein structure or conformation will be discussed in the next section.

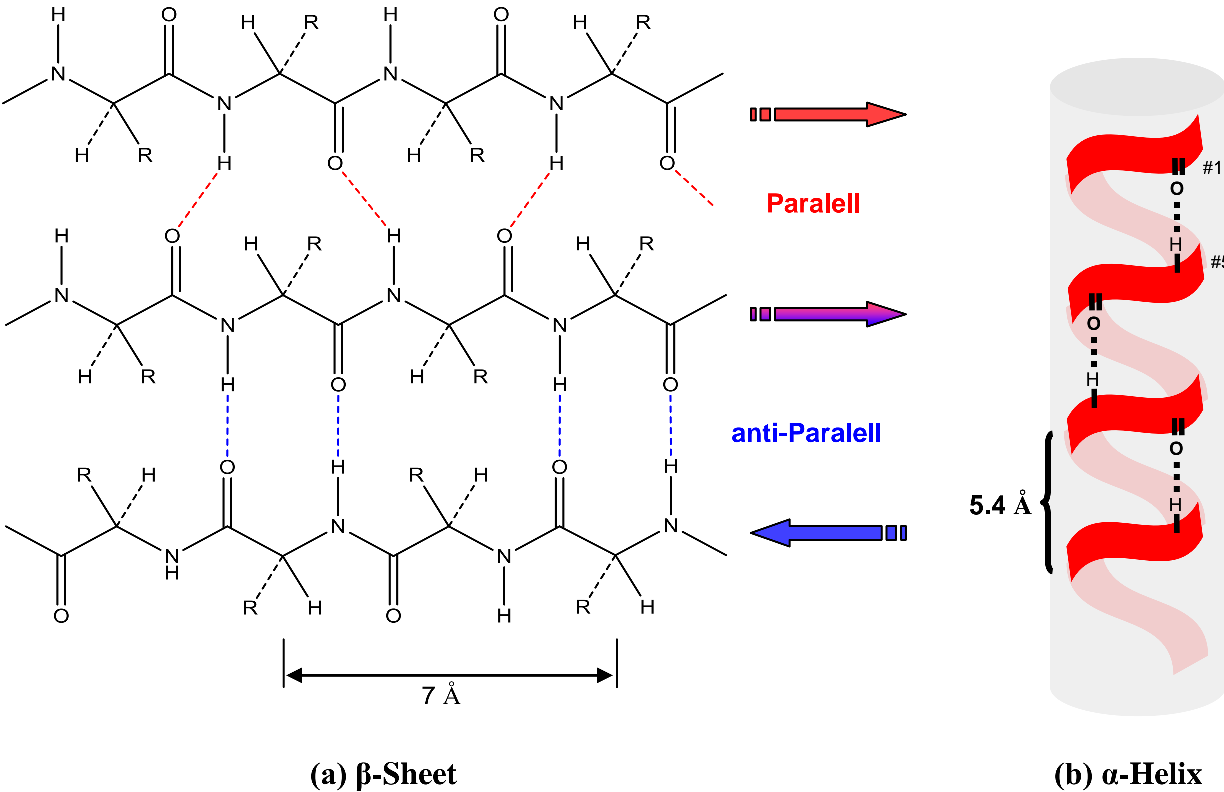



**Figure 1.3** Schematic representation of (a) β-sheet and (b) α-helix in protein secondary structure.

## 1.3 Protein Denaturation

In the two-state model, it is generally believed that proteins exist in the native (folded) or denatured (unfolded) conformation [5]. Under physiological conditions the stability of the native state of most proteins is only marginally greater (~ 10 cal/mole) than the denatured state

[6]. Transitioning from the lower to higher state of configurational entropy requires the introduction of a perturbation. Such molecular disturbances are often caused by denaturants which are known to alter the equilibrium between these two conformational states as shown below:

$$F \leftrightarrow U \tag{1.1}$$

Here, $F$ is the concentration of protein in the folded or native conformation, and $U$ is the concentration of protein in the unfolded or denatured state at a particular denaturant concentration. When a protein transitions to the denatured or unfolded state the previously buried hydrophobic patches become exposed to the polar environment of the solvent, usually water. Contributing to the conformation of the protein are several intermolecular forces (Figure 1.4) namely disulfide linkages, salt bridges, hydrogen bonding, and dispersion forces (or hydrophobic interactions). The existence of the hydrophobic force in protein folding was first introduced in 1938 by Irving Langmuir although it received little attention [7]. Today, it is generally accepted that the hydrophobic force is the dominant energetic force that contributes to folding of proteins into compact globules.

Protein denaturation or unfolding is achieved by several means including chemical denaturants, temperature, pH, alcohols, and organic solvents [8]. The latter two methods are used to a lesser extent than the former three methods, which are discussed in detail. High concentrations of the two chaotropic agents, urea and guanidine hydrochloride, are believed to disrupt hydrophobic interactions within the protein secondary structure causing denaturation. The first accounts of denaturation by urea were reported by Spiro [9] and Ramsden [10] around 1900. Comparable effectiveness in protein denaturation by the use of guanidine hydrochloride (GnHCl) for determining sulfhydryl content in egg albumin appeared in the work of Greenstein

in 1938 [11]. Although high concentrations (5-6 M) are required [12-14] the widespread use of urea and guanidine hydrochloride as chemical denaturants has prevailed because protein unfolding is generally greater than can be achieved by other means [15]. Other methods of denaturing proteins include exposing the analyte to acidic conditions (pH ~2) [16-17] or relatively high temperatures (~60 ºC – 100 ºC) [18]. However, prediction of complete transitions from the folded to the unfolded state is nearly impossible under these conditions because of the complexity of the stabilizing and destabilizing forces experienced by individual proteins [19]. Lastly, detergents or surfactants have also been found to denature proteins [20-22]. In 1939, Anson first demonstrated that surfactants were efficient denaturants of hemoglobin [23]. Today, the most commonly used surfactant for denaturing proteins is sodium dodecyl sulfate (SDS), an anionic amphiphilic compound [24-25]. A more comprehensive treatment of the binding of SDS to proteins will be provided in a later chapter. Next, a detailed look at the structure, characterization, and classification of surfactants follows.

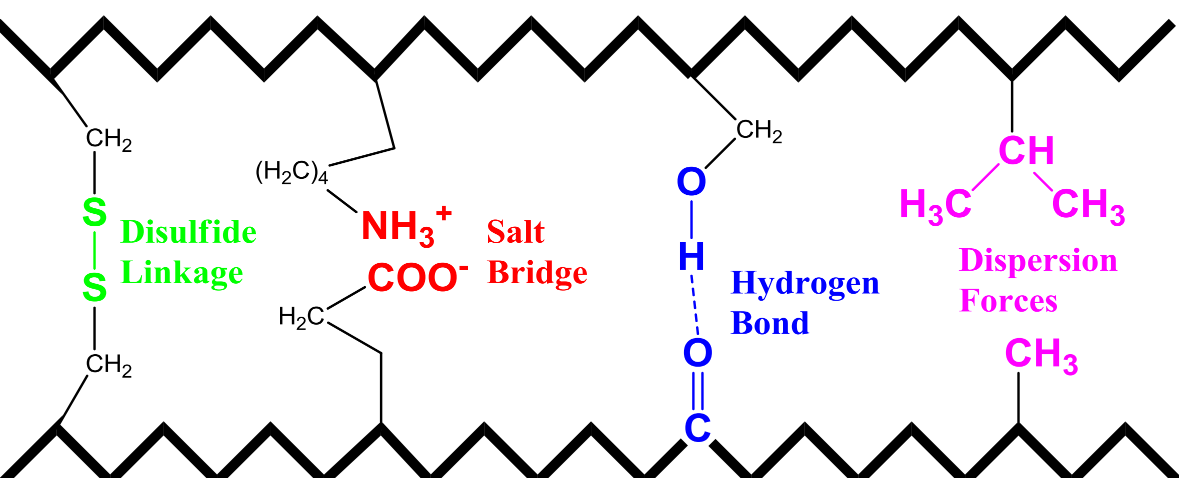



**Figure 1.4** Schematic of intermolecular forces that contribute to protein conformation.

## Part II. Introduction to Surfactants, Molecular Micelles, and Ionic Liquids

### 1.4 Surfactants and Conventional Micelles

Surfactants are amphiphilic molecules known as "surface active agents" that structurally contain a hydrophilic head group and an aliphatic chain designated as the tail (Figure 1.5). The head group is polar and thus is compatible with the aqueous environment while the hydrocarbon tail is nonpolar and resists interaction with the aqueous environment. The hydrophobic moiety may consist of one chain or up to four chains. In general, surfactants are broadly classified by the charge on the head group into four categories: anionic, cationic, nonionic, and zwitterionic [26]. Each surfactant type is presented in Table 1.4.1 indicating the typical groups used and at least one representative example.

**Table 1.1** Typical surfactant structures

| Surfactant type | Structure | Example |
|---|---|---|
| Anionic | $R\text{-}X^-M^+$ | $C_{12}H_{25}SO_4^-Na^+$ |
| Nonionic | $[R\text{-}(OCH_2)CH_2))_nOH))]$ | $C_{13}H_{21}(OCH_2)CH_2))_nOH$ |
| Cationic | $R\text{-}N^+(CH_3)_3X^-$ | $(C_{16}H_{33})N^+(CH_3)_3Br^-$ |
| Zwitterionic | $R\text{-}N^+(CH_3)_2CH_2X^-$ | $CH_3(CH_2)_{12}N^+(CH_3)_2CH_2SO_3^-$ |

R: alkyl group, $M^+$: metal ion, $X^-$: halogen, carboxylate, sulfonate or sulfate, n: integer

Surfactants exhibit unique properties in water and are capable of spontaneous self assembly into a structure known as a micelle. The aggregation of the surfactant monomer into a micelle is typically spherical and concentration dependent. At low concentrations of surfactant in an aqueous solution, surfactant monomers are dispersed and form a layer at the solution/air

interface. The hydrophilic head points inward and interacts with the polar solvent and the hydrophobic tail points upward and remains above the surface. As the concentration of monomeric species increases and reaches the critical micelle concentration (CMC), where no more monomer is able to assemble at the solution/air interface, spontaneous aggregation of the surfactant monomer results in the formation of a micelle. In the spherical micellar structure, consistent with the Hartley model [27], the hydrophobic tails are oriented toward the core or middle forming a hydrophobic domain and the hydrophilic head groups are exposed to the solvent. A schematic representing this process is shown in Figure 1.5.

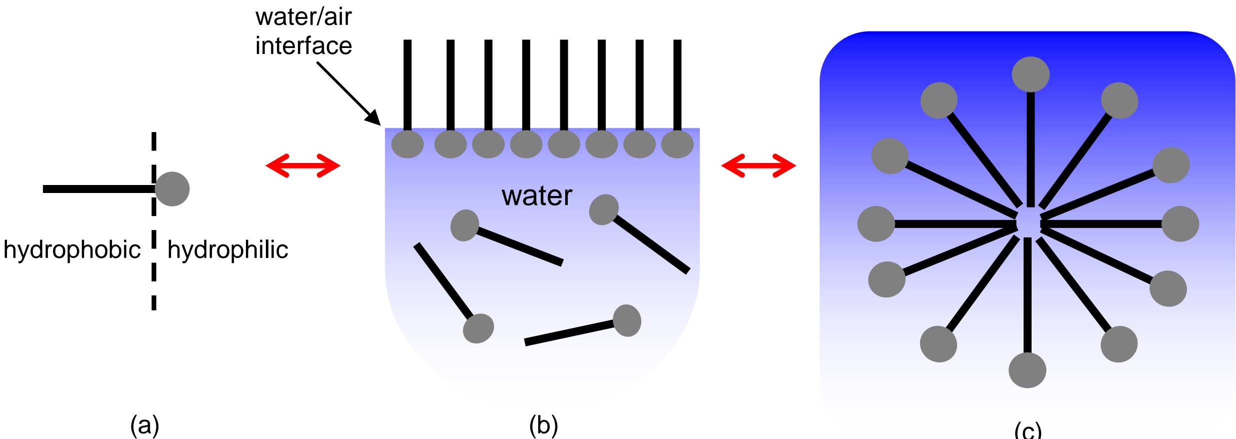



**Figure 1.5** Schematic of the formation of a conventional micelle: (a) surfactant monomer, (b) surfactant monomer layer at water/air interface, (c) surfactant micelle in aqueous solution

The size of micelles is a combined effect [28]. It is controlled by a balance of attraction of the hydrophobic moieties that favor micelle growth and the repulsion between hydrophilic head groups that limit micelle size [29]. The repulsive forces arise from electrostatic and/or steric interactions between the polar head groups. Thus, the radius of a conventional micelle may be

estimated from the length of the hydrocarbon chain. The average number of alkyl chains found in a micelle is the aggregation number. It typically ranges from approximately 50 - 120 monomer units. For example, SDS at concentrations greater than its CMC has an aggregation number of approximately 62 and a radius (assuming a spherical shape) of approximately 1.72 nm [30], which is consistent with the length of the dodecyl moiety [31]. The aggregation number may be found by using the equation

$$N = \frac{[\text{Surfactant}] - CMC}{MW} \tag{1.2}$$

where MW is the molecular weight of the surfactant monomer. It can be determined by analytical techniques such as light scattering [32], fluorescence [33], and nuclear magnetic resonance (NMR) [34].

The CMC is an important parameter, which provides significant information about the surfactant molecule and the micelle that are in equilibrium. At the critical micelle concentration, the dynamic assembly and disassembly processes coexist. Therefore, conventional micelles are considered poly-dispersed species. The analytical techniques that are typically used to determine the CMC are tensiometry, conductivity, absorbance, fluorescence, and light scattering. When the specific physical property is plotted versus the surfactant monomer concentration, a change in the slope of the curve is observed at the CMC as shown in Figure 1.6. For example, in a surface tension measurement above the CMC, the surface tension remains constant (i.e. the slope is zero) because only the numbers of micelles increase with increasing concentration. The surface tension is no longer dependent on the concentration of the monomeric species. A dynamic equilibrium exists between the surface monolayer, the monomeric solute, and the micellar phase. Other solution parameters such as surfactant concentration, type of solvent, solvent additives, and temperature affect the CMC [35]. Additionally, the CMC can also be affected by simple

changes in the molecular structure. For example, the CMC decreases (N increases) as the hydrocarbon chain length increases. Furthermore, the addition of a terminal double bond lowers the hydrophobicity of the alkyl chain and increases the CMC by a factor of two [36]. The CMC increases when branching of the hydrocarbon chain is introduced into the molecule. The addition of salt decreases repulsion between the ionic head groups and thus lowers the CMC [37]. Lastly, the increased hydrophobicity of nonionic surfactants results in lower CMCs than ionic surfactants. The CMC values of the surfactants used in this dissertation were determined using tensiometry. Tensiometry or other techniques used to determine CMC are not needed when molecular micelles are used. The following section describes how molecular micelles are different from conventional micelles and the advantages that they offer over conventional surfactants.

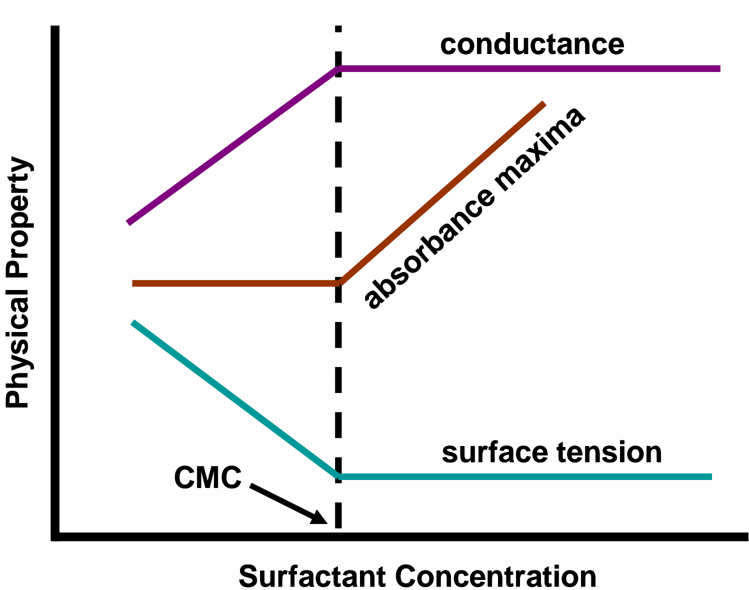



**Figure 1.6** Determination of critical micelle concentration (CMC) using different physical properties.

## 1.5 Molecular Micelles

Molecular micelles are amphipathic molecules that have unique physicochemical properties. Each molecule contains a hydrophilic head group and a hydrophobic tail as with conventional surfactants. However, molecular micelles are distinct from conventional micelles in that the hydrophobic region is covalently bound. Briefly, a double bond (terminal or along the aliphatic chain) is polymerized using γ-irradiation at concentrations well above the CMC (Figure 1.7). This concentration ensures that a spontaneous self-assembled phase exists. When the irradiation/polymerization is complete, the dynamic assembly and disassembly process has been largely eliminated. The molecular micelle has no CMC. In addition, the irradiation process imparts a unique morphology to the molecular micelle through formation of a covalently bound highly hydrophobic micro-domain. Such molecular micellar hydrophobicity is in stark contrast to conventional and second generation surfactants, which contain either double hydrocarbon chains or double polar head groups. The covalent binding structurally increases the stability and rigidity of molecular micelles in solution and upon interaction with other molecules. These properties among others have been successfully utilized in capillary electrophoresis (CE) and in gel electrophoresis, the subject of this dissertation.

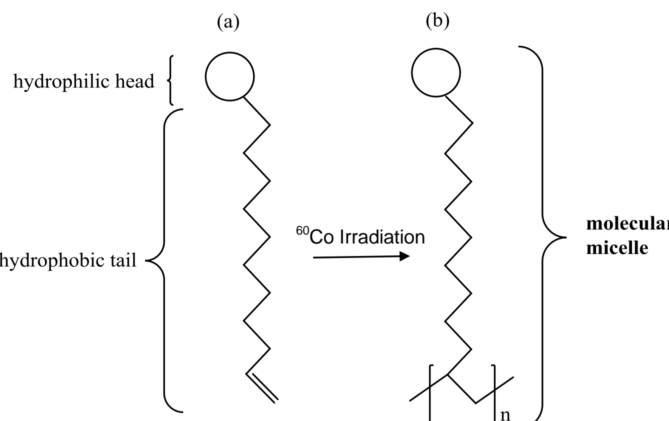



**Figure 1.7** Schematic of a typical molecular micelle synthesized in our laboratory: (a) monomer, (b) polymer.

### 1.5.1 Molecular Micelles in Capillary Electrophoresis

Along with other research groups [38-39], previous studies reported by our laboratory have demonstrated that interactions with molecular micelles provide superior separation schemes relative to conventional micelles due to their improved interactions [40-42]. Mwongela and coworkers use of ionic liquids in the background electrolyte (BGE) allowed for the resolution of enantiomeric peak overlap when the molecular micelles, poly sodium N-undecanoyl sulfate (poly-SUS) and poly sodium oleyl-L-leucylvalinate (poly-*L*-SOLV), were used as the pseudostationary phases in micellar electrochromatography (MEKC) [43]. The use of molecular micelles provided the selectivity needed for the separation whereas the ionic liquids greatly influenced the migration times and peak efficiencies. Additional data from our laboratory suggests that molecular micelles have several distinct advantages over conventional micelles in open tubular capillary electrochromatography (OT-CEC) using polyelectrolyte multilayer (PEM) coatings [44]. In this work, alternating layers of an anionic molecular micelle and a cationic polymer formed a PEM coating which was used to separate four globular proteins. The use of the chiral dipeptide molecular micelle, poly-*L*-sodium undecanoyl leucylvalinate (poly-*L*-SULV), in the adsorbed wall coating resulted in reversal of elution order for lysozyme and cytochrome C demonstrating partitioning and selectivity. Each of these reports demonstrated reduction in Joule heating by the use of lower molecular micelle concentrations compared to conventional micelles that generally require concentrations in great excess (2 - 10 times higher) of the CMC.

### 1.5.2 Achiral Molecular Micelles in Capillary Electrophoresis

Achiral molecular micelles have been shown to be suitable for MEKC separations [45-49]. Moreover, molecular micelles have been successfully used in place of conventional micelles

as pseudostationary phases in MEKC achieving distinct molecular recognition advantages as evidenced in chiral separations. In one MEKC study, poly-SUS provided better baseline resolution of sixteen priority pollutants than SDS during separation of polycyclic aromatic hydrocarbons [50]. One advantage of using molecular micelles in MEKC is that higher amounts of organic modifiers can be used without affecting its structural integrity. For example, up to twice as much methanol or acetonitrile can be added to the background electrolyte when molecular micelles are used in MEKC compared to SDS micelles [50, 51]. In addition, our laboratory was able to alleviate early problems with achiral molecular micelles by polymerizing them under γ-irradiation yielding high purity (97-99%) and high yield. [50] The achiral molecular micelle, poly-SUS, was found to be more suitable than other polymeric surfactants in OT-CEC separations of benzodiazapines by Kamande *et al*. [52] because of its stability over a wider pH range.

The success of molecular micelles for protein separations in CE inspired the hypothesis that they may be useful reagents in gel electrophoresis. Therefore, one aspect of this dissertation is the investigation of molecular micelles and ionic liquids for protein separations in 1D gel electrophoresis (see Chapters 2 and 4). Next, an introduction to ionic liquids, their biological applications, and use in CE is presented.

## 1.6 Ionic Liquids

Ionic liquids (ILs) are often defined as a class of ionic compounds that melt below 100 °C. If an ionic liquid melts below ambient temperature, it is called a room temperature ionic liquid (RTIL). These low-temperature molten salts contain an anion that is coupled to a cation. Common anions and cations used in the synthesis of ionic liquids are shown in Figure 1.8. The cations are typically organic and the anions are organic or inorganic. The most actively used

cations are the 1-alkyl-3-methyl imidazoliums followed by pyridinium, pyrrolidinium, tetraalkylammonium, and tetraalkylphosphonium ions. Depending on the end use, some anions that have found use are tetrafluoroborate, hexafluorophosphate, dycyanamide, halides, and bis(trifluoromethanesulfonyl)amide [53]. Although ILs contain anions and cations like the high-melting (≈ 800 °C) inorganic salt, NaCl (i.e., table salt), a comparison of the two types of molecules reveals obvious differences. Structurally, an inorganic salt has a symmetrical anion-cation pair. In contrast, ionic liquids typically have asymmetrical cations [54], which prevent them from having ordered packing structure. Thus, crystallization of ionic liquids is uncommon. In general, ionic liquids are characterized by a complex interplay of molecular interactions that are still under investigation.

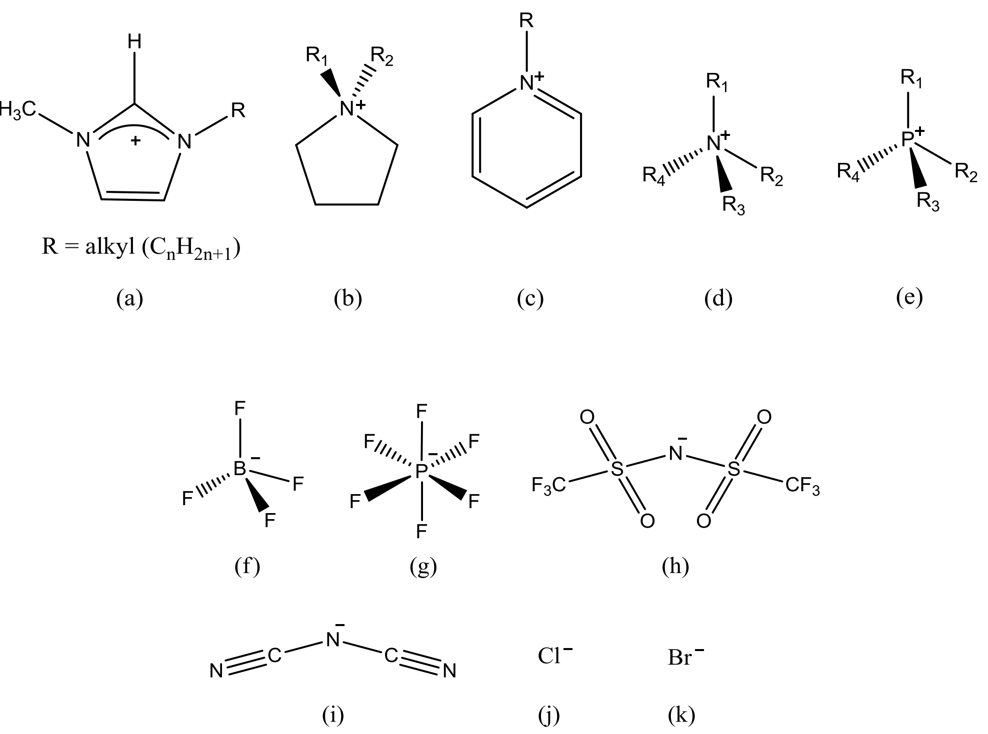



**Figure 1.8** Common cations and anions used in synthesis of ionic liquids. Cations: (a) 1-alkyl-3-methylimidazolium, (b) pyridinium, (c) pyrrolidinium, (d) tetralkylammonium, (e) tetralkylphosphonium. Anions: (f) tetrafluoroborate, (g) hexafluorphosphate, (h) bis(trifluoromethanesulfonyl)amide, (i) dicyanamide, (j) chloride, (k) bromide.

Certainly, the most desirable property of ionic liquids is the ability of the scientist to synthesize tunable molecules by simply modifying the cation or the anion moieties [55]. Other properties of ionic liquids that have been exploited in analytical applications such as liquid-liquid extraction [56], spectroscopy [57], electrochemistry [58], and sensors [59] include their dissolution properties, varying miscibility with solvents, ionic conductivity, selectivity, durability, resistance to thermal degradation, and negligible vapor pressure. A few analytical examples have been cited, but the diverse applications of ionic liquids are numerous and will undoubtedly continue to grow.

### 1.6.1 Biological Applications of Ionic Liquids

One emerging area in which ionic liquids are being explored is biological applications. They have been used as solvents to solubilize cellulose [60] and deoxyribonucleic acid (DNA) [61], as reaction media for enzymatic reactions [62], as well as precipitants and additives during protein crystallization [63]. Judge and coworkers screened sixteen ionic liquids as precipitants and additives during crystallization of six model proteins including lysozyme and trypsin. Crystallization was achieved in the presence of 1-butyl-3-methylimidazolium trifluoroacetate for trypsin and at least ten imidazolium-based ionic liquids with varying anions for lysozyme.

Of particular interest in bio-applications is the use of ionic liquids in the dissolution, stabilization, unfolding, and renaturation of proteins. It has been shown that some proteins are stable, remain active, and refold in some ILs [64-66]. For example, Fujita *et al.* reported that cytochrome C retained its native state and its electron transfer activity after eighteen months of storage in choline dihydrogen phosphate (with 20 wt% water) at room temperature [67]. As a comparison, cytochrome C experienced inactivity after only one week when stored in conventional aqueous media such as Tris and phosphate buffers. Lange and coworkers [68]

studied the use of imidazolium-based ionic liquids as renaturation reagents for proteins extending the initial work in this area by Summers and Flowers [69]. Protein activity and % refolding yield were monitored during the examination of two model proteins, lysozyme and the single-chain antibody fragment ScFvOx, in the presence of N'-alkyl and N'-(ω-hydroxyalkyl)-N-methylimidazolium chlorides. Refolding was enhanced by the ionic liquids tested and was found to be comparable to, and in some instances even better than, L-arginine hydrochloride (L-ArgHCl), one of the most effective renaturants to date. These studies are of practical relevance since some proteins exhibit instability, particularly insoluble aggregates from inclusion bodies of recombinant expressed protein, when handled *in vitro* [68]. Stabilizing agents and refolding buffers are required to induce renaturation and ensure their long-term stability. Lack of stability and solubility are two limitations that have hindered the widespread use of some protein therapeutics for pharmaceutical applications [70].

Three methods exist for dissolution of biological materials in ionic liquids: (1) direct dispersion into neat ionic liquid, (2) protein modification by polyethylene glycol (PEG) followed by dissolution in an ionic liquid, and (3) addition of small quantities of water (i.e. hydration) to the ionic liquid solvent. First, in 2001 Kimizuka and Nakashima developed sugar-philic ether-derivatized ionic liquids that were capable of directly dissolving glycolipids that formed stable bilayer membranes [71]. Second, incubation in and covalent attachment of PEG to a protein have been used to increase protein solubility in ionic liquids [72-73]. Generally, conjugation of the protein with PEG (or pegylation) increases the solubility of the target molecule among other benefits. Recently, Chen *et al.* [74] successfully performed the first protein conjugation using ionic liquids as solvents to increase protein solubility. A one pot high-yielding synthesis of poly(methacryloyloxyethylphosphorylcholine) (polyMPC) and lysozyme-polyMPC conjugation

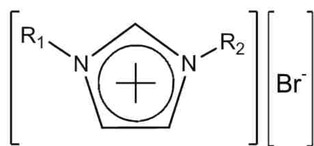
was successfully performed in the ionic liquids, 1-butyl-3-methylimidazolium tetrafluoroborate ($BMIMBF_4$), 1-ethyl-3-methylimidazolium trifluoromethane sulfonate ($EMIMCF_3SO_3$), and 1-butyl-3-methylimidazolium trifluoromethane sulfonate ($BMIMCF_3SO_3$). Third, hydrated ionic liquids where small quantities of water are present [66] may be providing the requisite hydrogen-bonding environment for protein solubilization [75] while retaining the properties of the ionic liquid. Recently, Vijayaraghavan and coworkers [76] reported the dissolution of DNA and its long-term stability (> 1 year) in choline-based hydrated ionic liquids. Such studies provide for the potential that new ionic liquids (i.e., "designer solvents") may be effective solvents for other biomacromolecules and biological materials. These experimental successes have been paralleled by the use of ionic liquids in CE albeit not at a similar rate. In the next section, CE separations of proteins using ionic liquids will be discussed.

### 1.6.2 Ionic Liquids in Capillary Electrophoresis

Ionic liquids have been used in CE for the separation of many analytes in various applications including covalent wall coatings [77], background electrolyte modifiers [78], and pseudo-stationary phases [79]. However, it was Stalcup and coworkers who first described a mechanistic theory for the separation of polyphenols using alkylimidazolium-based ionic liquids in CE [80]. The imidazolium cation offered two advantages in that it dynamically coated the capillary wall causing a reversal of the electroosmotic flow (EOF), which allowed separation of neutral species, and provided analyte interaction sites in the bulk solution. They also noted that the separation was fairly insensitive to changes in the anion ($BF_4^-$ vs $PF_6^-$), but was highly sensitive to changes in the cation (ethyl vs butyl at $N_3$). Moreover, wall adsorption was alleviated when Wu and coworkers used 1-butyl-3-methylimidazolium tetrafluoroborate as a dynamic coating to separate cytochrome *C,* lysozyme, ribonuclease A, albumin, and α-

lactalbumin [81]. They were able to baseline separate both acidic and basic proteins in fourteen minutes with peak efficiencies as high as 670,000 plates/m. In the work by Mwongela et al., 1-alkyl-3-methylimidazolium tetrafluoroborate ionic liquids were compared to organic solvents as additives to aqueous buffer solutions to study their effect on chiral separations in MEKC [82]. The authors noted that the presence of the IL BGE modifiers resulted in faster migration times requiring smaller volumes compared to the organic solvents.

Although reports of using ionic liquids in capillary electrophoresis have emerged, their use in gel electrophoresis has not been investigated. Gel electrophoresis makes use of electrolytes that are salts in the aqueous buffer system. Since ILs are typically viscous and conductive, it would be difficult to use the neat ionic liquid as the solvent. Factors that would restrict their use in gel electrophoresis would include a very high current and/or applied voltage, extremely high joule heating, and a low electric field. However, due to their tunability and success in CE, imidazolium-based ionic liquids (Figure 1.9) have been explored in this research as buffer additives or modifiers to investigate the feasibility of their use for protein separations in gel electrophoresis. An introduction to the theory and uses of gel electrophoresis, particularly sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) follows.




| | | | |
|---|---|---|---|
| (1) | [DMOMIM][Br] | $R_1 = CH_3OCH_2$ | $R_2 = CH_3(CH_2)_{10}CH_2$ |
| (2) | [DMOEIM][Br] | $R_1 = CH_3OCH_2CH_2$ | $R_2 = CH_3(CH_2)_{10}CH_2$ |
| (3) | [DMOEOEIM][Br] | $R_1 = CH_3OCH_2CH_2OCH_2CH_2$ | $R_2 = CH_3(CH_2)_{10}CH_2$ |
| (4) | [MMOMIM][Br] | $R_1 = CH_3OCH_2$ | $R_2 = CH_3$ |
| (5) | [MMOEIM][Br] | $R_1 = CH_3OCH_2CH_2$ | $R_2 = CH_3$ |
| (6) | [MMOEOEIM][Br] | $R_1 = CH_3OCH_2CH_2OCH_2CH_2$ | $R_2 = CH_3$ |
| (7) | [BMOMIM][Br] | $R_1 = CH_3OCH_2$ | $R_2 = CH_3CH_2CH_2CH_2$ |
| (8) | [BMOEIM][Br] | $R_1 = CH_3OCH_2CH_2$ | $R_2 = CH_3CH_2CH_2CH_2$ |
| (9) | [BMOEOEIM][Br] | $R_1 = CH_3OCH_2CH_2OCH_2CH_2$ | $R_2 = CH_3CH_2CH_2CH_2$ |

**Figure 1.9** Basic structure of ether-functionalized imidazolium-based ionic liquids

## Part III. Introduction to Electrophoretic and Spectroscopic Techniques

### 1.7 Gel Electrophoresis

Gel electrophoresis is an electrophoretic separation technique in which the separation is achieved by analyte migration through a porous gel medium as a voltage is applied. The gel serves as a matrix through which proteins traverse effectively being separated based on size, net charge, and hydrophobicity [83]. Theoretically, the fundamental gel electrophoresis process occurs as an electric field with a potential gradient E forces a particle (or analyte) with an electric charge q through a viscous medium. Opposing the forward motion of the analyte is a frictional force that must be overcome for the separation to be completed. In solution the frictional resistance, $f$, obeys Stoke's Law given by $f = 6\pi r v \eta$ (1.3) where r equals the particle radius, v is the particle velocity, and $\eta$ is the viscosity of the medium. This phenomenon is expressed mathematically by the equations below:

$$qE - f = 0 \tag{1.4}$$

$$qE = 6\pi r v \eta \tag{1.5}$$

The electrophoretic mobility, $\mu_{ep}$, of an analyte during gel electrophoresis is given by

$$\mu_{ep} = \frac{d}{tE} = \frac{v}{E} = \frac{q}{6\pi r v \eta} = \text{constant } x \frac{q}{r} \tag{1.6}$$

where d represents the migration distance of the particle in time, t. Other important parameters in the gel electrophoretic format are joule heating and ionic strength. Typically, gel electrophoresis is performed on gels as small as 8 x 8 cm and as large as 20 x 20 cm. Joule heating may result in loss of resolution, increased analyte mobility, and increased free diffusion. Therefore, the gel electrophoresis module is generally cooled by an ice bath. The joule heating phenomenon is governed by the following equation:

$$Q = \frac{V^2}{RA} = \frac{VI}{A} \tag{1.7}$$

where Q = heat, V = voltage, R = resistance, A = area, and I = current. In general, the larger gels experience less joule heating at the same applied voltage. However, for smaller gels this can be circumvented by using a lower applied voltage for the separation. The need to use lower voltages is disadvantageous in gel electrophoresis because it may take up to four hours to generate an electropherogram under this condition. However, others view the need for lower voltages as an advantage because the researcher has control over maintaining the integrity of their analyte.

### 1.7.1 Polyacrylamide Gel Electrophoresis (PAGE)

Separation of proteins into zones began on filter paper [84] then moved to columns of sucrose [85]. However, the first use of gel medium in this electrophoresis separation technique originated with the work of Consden in 1946 [86]. A slab gel made of either silica jelly or agar was set between two glass plates and cooled by a water bath during electrophoresis. The silica gel medium proved suitable for the separation of amino acids with different ion mobilities since post-separation of the agar from the amino acids proved troublesome. Increased resolution was realized in the work of Smithies in 1955 when he discovered molecular sieving gel electrophoresis by using a starch-based gel in a plastic tray to separate β-lactoglobulin, cabbage enzyme, and proteins from human sera [87]. Appearing in the work of Raymond and Wang in 1960 [88], the use of synthetic polyacrylamide for the gel matrix was introduced. The polyacrylamide used in these gels and still used today is synthesized using the reactions in Figure 1.10. Briefly, the acrylamide monomer is reacted with N,N methylene bisacrylamide, the co-monomer, in the presence of tetramethylethylenediamine (TEMED) and ammonium persulfate to

yield polyacrylamide. The ammonium persulfate catalyzes the free-radical based reaction. Several advantages are offered by the use of polyacrylamide gels: 1) gel stability, 2) neutral polymer charge yields no electro-endosmotic flow effects on the analytes, 3) the clear gel is suitable for densitometry measurements, and 4) flexible pore size distribution that can be manipulated by changing the monomer and the co-monomer concentrations. Synthesis of polyacrylamide gels is a function of two parameters, %T and %C. Percent T (%T) is the percentage of acrylamide plus bisacrylamide (or total monomer concentration), and %C is the percentage of bisacrylamide crosslinker. The effective pore size is inversely proportional to the total monomer concentration. Thus, increasing %T decreases the pore size. Such flexibility in pore-size adjustment is often used in separations of proteins with widely varying hydrodynamic sizes. In these situations, a gradient gel is used which has larger pores at the top of the gel and subsequently smaller pores going vertically down the gel.

**Figure 1.10** Polymerization reaction involved in polyacrylamide gel synthesis.

Thereafter, many advances in gel electrophoresis were achieved. Other such advances followed including the work by Ornstein and Davis, who in separate experiments, used disc gels to reduce the size of the protein separation bands into narrow zones [89,90]. Maizel demonstrated that a mechanical fractionator, which would extrude the polyacrylamide gel continuously through an orifice, would produce comparable band patterns and was less laborious than manual gel sectioning [91]. The emergence of these alternative polyacrylamide gel electrophoresis (PAGE) procedures for protein separations had significant impact for years to come.

### 1.7.2 Sodium Dodecyl Sulfate in Polyacrylamide Gel Electrophoresis (PAGE)

SDS-PAGE is the simplest and most widely used analytical technique for the analysis of proteins in the life sciences. Most life scientists do protein analysis using the discontinuous method of Laemmli [92], in which a two buffer system is employed for contiguous stacking and resolving regions of the gel. SDS-PAGE is suitable for purification, identification, quantification, and oligomeric analysis of proteins, as well as a preparative technique for two-dimensional gel electrophoresis (2DGE)/mass spectrometry. A strength of SDS-PAGE is the ability of the technique to be coupled with 2DGE/MS [93] and western blots [94].

SDS is an anionic detergent that has a twelve-carbon hydrophobic chain and a sulfate head group. The first description of using SDS in PAGE appeared in the work of Maizel and coworkers in 1965 [95]. The use of SDS as an effective solubilizing agent for poliovirus particles, which was the subject of their investigation, had been reported earlier [96]. In this early work, Maizel and coworkers suggested that the basis of the separation was not the size and charge of the native protein, but the size and charge of the "protein-SDS complex." Today, it is well-known that the electrophoresis of proteins through the porous gel matrix is a function of the

size and charge [97]. However, when SDS is present, it binds to most proteins in a relatively uniform stoichiometric ratio having the same charge to mass ratio. Thus, charge is no longer a factor in the migration mechanism, and separation through the porous polyacrylamide gel is based on molecular weight (or size) alone. For most globular proteins at saturation, the binding ratio is approximately 1.4 g SDS/g protein [98]. However, subsequent investigations have found that the binding ratio more accurately reflects a range of 1.5-2 g SDS/g protein for globular proteins [99]. Rath and coworkers established that this ratio diverges further from this narrow range when separating membrane proteins in gel electrophoresis [100]. The binding ratio was estimated to be as high as 10 g SDS/g protein in many cases.

There are two modes of SDS-PAGE that are typically employed in molecular biology and other areas of research that use the technique: denaturing PAGE and native PAGE. Each separation mode is used for different purposes. In denaturing PAGE the proteins are subjected to denaturation by interaction with SDS, disulfide reduction, and heating beyond the protein's melt (or denaturation) temperature. In normal denaturing PAGE protocol, the concentrations of SDS used in the sample buffer and running buffer are 2% w/v (≈ 69 mM) and 0.1% w/v (≈ 3.5 mM), respectively. The use of reducing agents such as β-mercaptoethanol (β-ME), dithiothreitol (DTT), and tris(2-carboxyethyl) phosphine (TCEP) breaks disulfide bonds (S-S) converting them to sulfhydryl groups (-SH). This ensures that only the monomeric form of the protein is electrophoresed. It is generally accepted that SDS covers the surface of the protein producing a constant charge to mass ratio effectively forming an SDS-protein complex whose overall charge is highly negative. A schematic of this phenomenon is shown in Figure 1.11. In contrast, native PAGE is performed in the absence of SDS and heating, and the separation is based on net charge, hydrodynamic size, and shape of the protein. In effect, the electric charge on the protein,

which is a function of the pH of the buffer, drives the electrophoresis. The native conformation of the protein also has an effect on this PAGE separation. The more compact species will migrate faster, while the less compact species will migrate slower, based on retardation by the frictional force. Native PAGE is sensitive to any external factor that may change the charge or the conformation of the protein. Thus, it is suitable for investigating quaternary structure, aggregation, chemical degradation, and protein binding events among others. However, native PAGE finds most use when the biological activity of the protein has to be retained for subsequent testing. Between these two modes of PAGE, denaturing PAGE is used most often.

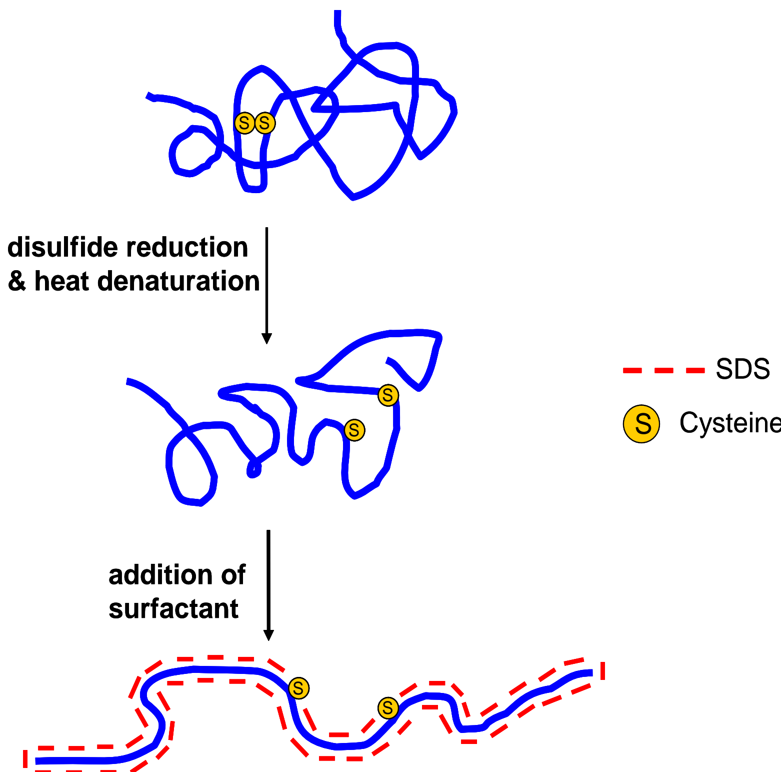



**Figure 1.11** Protein denaturation induced by (a) disulfide reduction and heating, and (b) addition of SDS.

In both PAGE operating modes, the SDS-protein complex sieves through the gel toward the anode. Experimentally, a gel separation based on the Laemmli method offers the advantage of optimal resolution. This is achieved by the analyte traveling through a stacking gel where it is concentrated into a thin zone having chloride as the leading ion and Glycine as the trailing ion. Second, upon entering the resolving gel, the pH of the medium increases approaching the pKa of Glycine, and it becomes more negatively charged. Thus, as electrophoresis proceeds Glycine overtakes the concentrated analyte zone as shown in Figure 1.12. The SDS-protein complex migrates until the size of the complex matches the pore size of the gel, and its migration stops. Then, a relatively thin band is formed filling the width of the lane. Since SDS-PAGE is a semi-quantitative analytical technique, certain band characteristics are used to describe the effectiveness of the separation. These are resolution, band sharpness, and peak intensity.

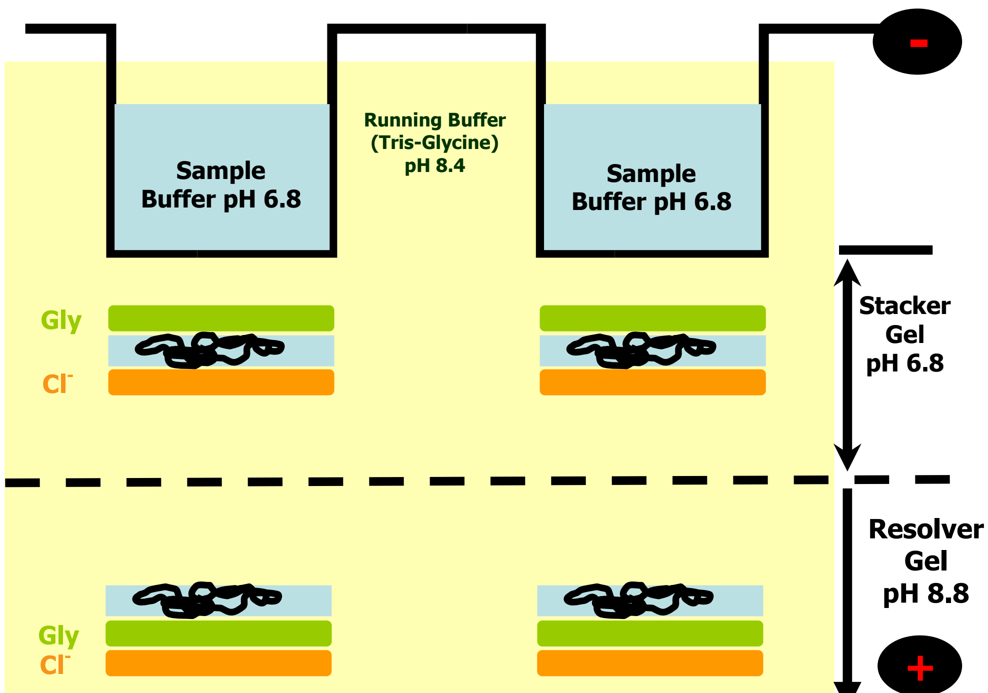



**Figure 1.12** Schematic representation of the electrophoretic process in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Typically in an adjacent lane, the molecular weight standard protein mixture is separated that has known relative mobilities ($R_f$). The relative mobility is a ratio of the distance the analyte traveled divided by the total length of the gel. Shapiro et al. showed that the log MW is inversely proportional to the relative mobility of each protein band [101]. Thus, a plot of log MW vs. relative mobility of the standard proteins may be used to determine the molecular weight of an unknown species. A representation of this method is given in Figure 1.13.

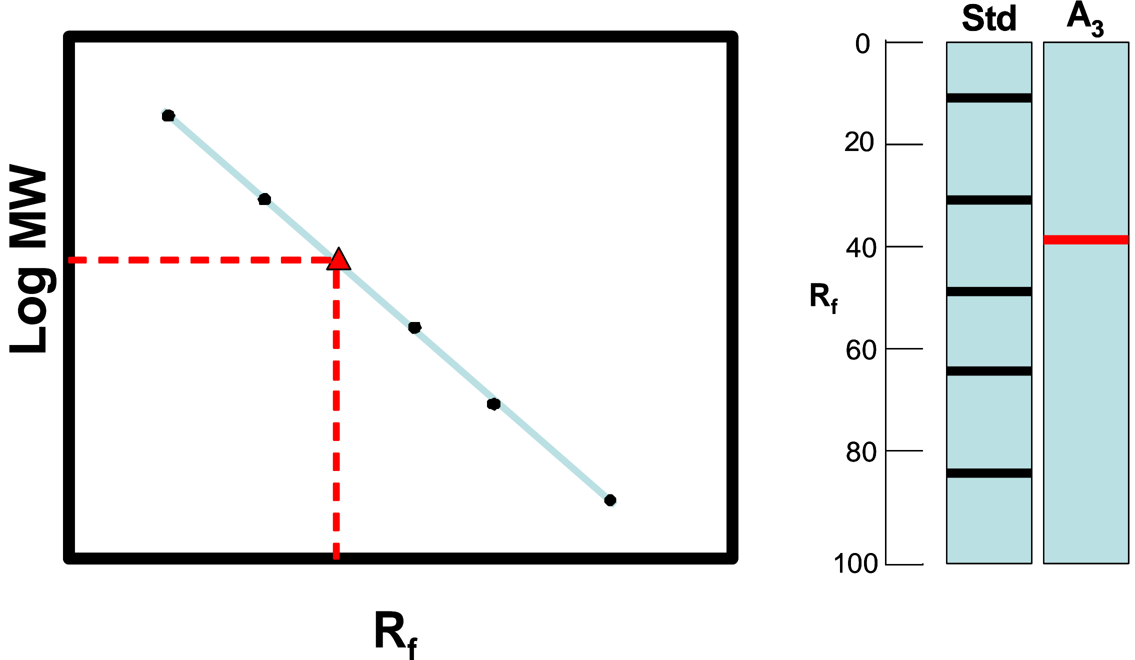



**Figure 1.13** Method for determining the molecular weight of an unknown in SDS-PAGE (Std = Standard Proteins; $A_3$ = unknown, $R_f$ = relative mobility, MW = molecular weight).

### 1.7.3 Visualization and Detection in SDS-PAGE

Visualization of proteins in PAGE is based on various staining or detection methods. Many of these methods rely on non-covalent interactions, which are generally reversible. Coomassie brilliant blue (G-250) and coomassie colloidal blue (R-250) [102] dyes are the most

widely used reagents for staining in PAGE. These hydrophobic organic dyes bind to proteins through ionic interactions between dye sulfonic acid groups and positive protein amine groups, as well as non-covalently binding to basic and hydrophobic protein regions. In principle, the binding efficiency is a function of protein density in each band and the relative amounts of hydrophobic and basic amino acid residues present in each protein analyte. Thus, these dyes are limited when protein abundance is low as in samples of complex mixtures derived from cells or tissues. The detection limit for Coomassie colloidal blue, the more sensitive of the two dyes, is approximately 10 nanograms of protein per band. For additional sensitivity, some researchers use silver staining [103]. This method was first introduced by Oakley et al. in 1980 [104] as an ultrasensitive staining technique. Silver staining is able to detect as little as 0.5 nanograms of protein per band, which is 50-100 times more sensitive than Coomassie colloidal blue. However, one disadvantage to silver staining is the interference in downstream protein characterization by the presence of crosslinking agents such as glutaraldehyde that are inherent in the process. In addition, silver has a low linear range and suffers from run-to-run variability. Within the last fifteen years, more sensitive visualization stains have emerged such as fluorescent dyes like the family of Sypro dyes [105,106]. These dyes offer several advantages: (1) high compatibility with mass spectrometry, (2) comparable sensitivity to silver stain, and (3) good linear dynamic range.

## 1.8 Circular Dichroism

In this dissertation research, circular dichroism (CD) was used to determine the extent of interaction of the molecular micelles and the ionic liquids with globular and glycosylated proteins. Circular dichroism is an analytical technique that is frequently used to study the conformational changes of proteins in solution under different conditions [107]. Far UV (180 -

240 nm) CD spectroscopy is the characteristic region of the electromagnetic spectrum where secondary structure transitions in proteins are gauged. In this region, the peptide backbone is a highly sensitive chromophore. As stated in section 1.1, the α-carbon for most amino acids (except glycine) is chiral and is optically active. Therefore, proteins rotate plane polarized light, which is the basis of CD or polarized light spectroscopy. When plane polarized light interacts with a protein, electronic excitation energy transitions give rise to characteristic bands in specific wavelength regions of the CD spectrum [108]. The secondary structural elements, α–helix, β-sheet, β-turn and random coil, induce distinctive shapes and magnitudes in the far UV [106]. The characteristic minima and maxima in the CD spectrum are indicative of that conformational state. Typical CD peak characteristics in the far UV region are shown in Table 1.2. A protein that is primarily α-helical in structure exhibits two strong negative bands at 208 nm and 222 nm. Thus, as the 208 nm ($-\theta_{208nm}$) or the 222 nm ($-\theta_{222nm}$) minima increase with increasing concentration, the protein begins to unfold and approaches a random coil conformation. Proteins that are primarily β-sheet have a characteristic minimum at 216 nm and a maximum at 195 nm. Lastly, a protein that is in the random coil (or unfolded/disordered extended chain) conformation exhibits a maximum at 218 nm and a strong minimum at 195 nm.

**Table 1.2** Characteristic CD peaks for protein secondary structure.

| Secondary Structure | Minima (nm) | Maxima (nm) |
|---|---|---|
| α–helix | 208, 222 | none |
| β-sheet | 216 | 195 |
| β-turn | 180 | 205 |
| random coil | 195 | 218 |

Like other optical techniques, CD obeys Beer's law ($\Delta A = \Delta \varepsilon cl$ where A = difference in absorbance from the right ($A_r$) and left ($A_l$) circularly polarized light, $\varepsilon$ = difference in molar absorptivity ($\varepsilon_r$ - $\varepsilon_l$), c = concentration, and l = pathlength) [109]. The CD spectrum of each component is directly proportional to its concentration, and the total spectrum is the sum of the percentages of all possible components (or secondary structural motifs). Therefore, it is possible to determine the relative contributions of each secondary structure type by using software to deconvolute the far UV spectrum into a sum of the fractional multiples of the reference spectra [110]. Much software is now available for this purpose [111]. Any spectral data in this dissertation decomposed into relative proportions of secondary structure elements were obtained from CDNN ver. 2.0 [112].

## 1.9 Fluorescence Spectroscopy

Fluorescence spectroscopy is a highly sensitive analytical technique that allows studies to be performed at micromolar concentrations, which minimizes analyte consumption. In fluorescence, the fluorophore absorbs a photon, which is promoted to a higher energy excited state in the same spin state. Before emission, the photon experiences non-radiative deactivation processes that lower it to the singlet excited state ($S_1$). These deactivation processes, often referred to as internal conversion, may be either vibrational or rotational losses due to collisions with the solvent or with other species in the sample. The lifetime of the fluorescence or emission ranges between $1x10^{-7}$ to $1x10^{-9}$ s. Although fluorescence is statistically more probable, a transition of the fluorophore to the triplet state (T) through intersystem crossing will result in phosphorescence. In the former case, the fluorophore emits a photon at a longer wavelength (or lower energy state). This movement of the emission to a longer wavelength relative to the excitation wavelength is called the Stokes Shift. Factors that may cause the Stokes shift include

solvent effects, excited state reactions, energy transfer, and complex formation. A representative schematic of the Jablonski diagram, which is a visual image of the interaction between radiation and matter that shows possible transitions between electronic states of a molecule, is given in Figure 1.13 [113].

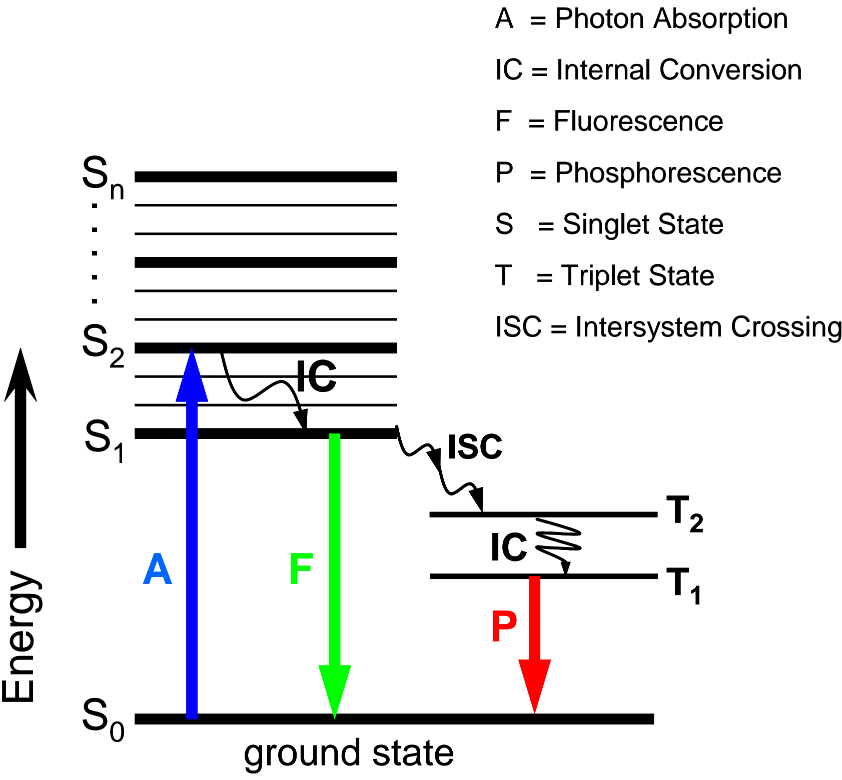



**Figure 1.14** The Jablonski Diagram.

The basic steady-state instrumentation consists of a light source, excitation and emission monochromators, a sample chamber, and a detector or photomultiplier tube (Figure 1.14). Of the many light sources used in fluorescence spectroscopy including lasers, photodiodes, and lamps, a 450W xenon arc lamp was used as the light source for all fluorescence

experiments in this dissertation. Xenon arc lamps provide continuous light output from 250 to 700 nm [114]. Transmitted light from the excitation monochromator is filtered through adjustable slits before passing through the sample. The emission monochromator is at a 90° angle from the excitation light path to minimize the detection of excitation radiation. The light that exits the emission monochromators, whose wavelength is specified by the user, is filtered through adjustable slits before entering the detector. The photomultiplier amplifies the signal and the output is displayed as a fluorescence spectrum.

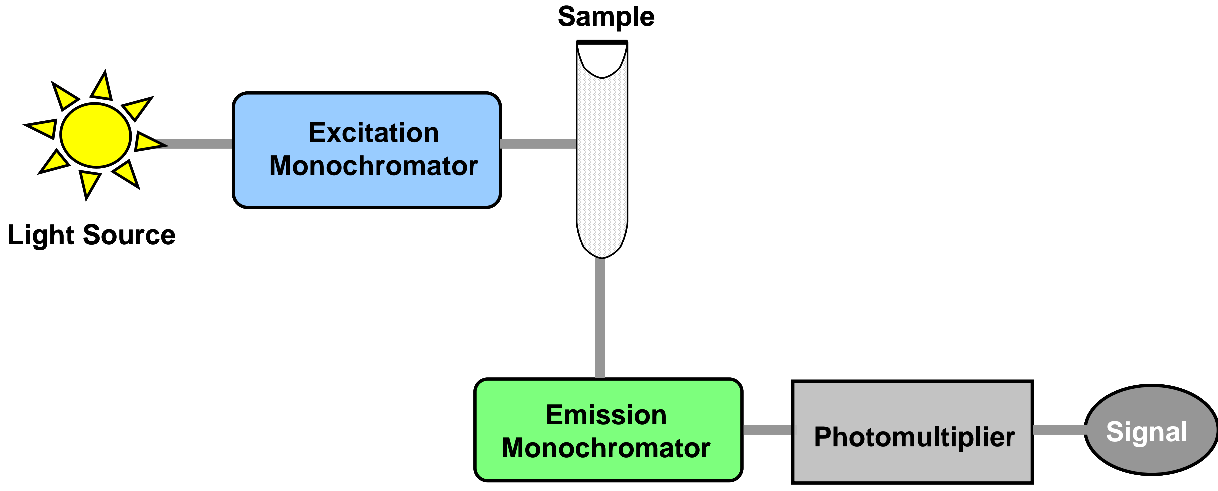



**Figure 1.15** Schematic diagram of a spectrofluorometer.

The two types of fluorescence used in this dissertation to quantify protein interactions with poly-SUS and imidazolium-based ionic liquids are steady-state and anisotropy fluorescence. Intrinsic and extrinsic protein fluorescence, FRET, and anisotropy have been exploited for detailed examinations of these interactions. A brief overview of these steady state fluorescence techniques are below.

### 1.9.1 Intrinsic Protein Fluorescence

Intrinsic protein fluorescence serves as a useful probe in biochemical applications of fluorescence spectroscopy. Though rarely found in large quantities in proteins, three aromatic amino acid residues, tryptophan (W), tyrosine (Y), and phenylalanine (F) display intrinsic ultraviolet fluorescence (Figure 1.16). Much of the present-day research using intrinsic fluorescence was pioneered by Gregorio Weber, who first hypothesized and then spectrally resolved the fluorescence emission from the aromatic amino acids found in proteins [115,116]. Of the three aromatic amino acids, the most abundant is tryptophan, which is why it is often used as a reporter molecule [117]. Tryptophan absorbs light at the longest wavelength (295 – 305 nm), and its emission maximum in an aqueous environment is near 350 nm. Tyrosine possesses a quantum yield similar to tryptophan (0.14 vs. 0.13) [118] while the quantum yield of phenylalanine is typically small (~ 0.02) [113]. Therefore, fluorescence emission from phenylalanine is rare for proteins.

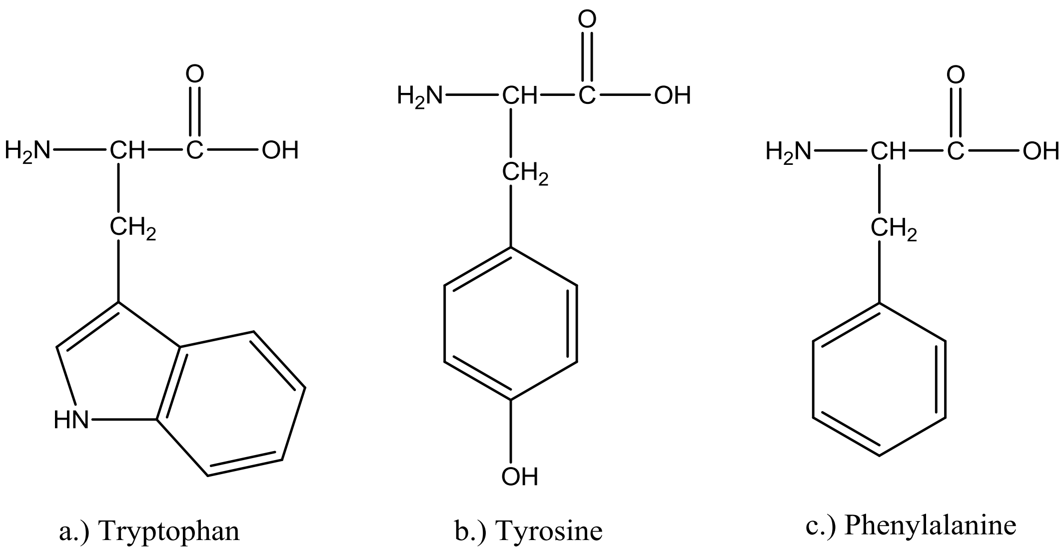



**Figure 1.16** Aromatic amino acids in proteins: (a) tryptophan, (b) tyrosine, (c) phenylalanine.

Protein intrinsic fluorescence, particularly spectral shifts in the tryptophan emission, is useful in understanding protein structural changes experienced in different associations such as ligand binding and protein-protein interactions. Specifically, changes in protein conformational transitions, denaturation, and substrate binding may be elucidated from the high sensitivity of tryptophan's indole ring to its local environment. When monitoring tryptophan emission, it is widely reported that the fluorophore should be excited at wavelengths near 295 nm to minimize spectral emission overlap from tyrosine [119-121]. Emission wavelengths range from ≈ 308 nm (azurin) to ≈ 355 nm (glucagon) [122]. In general, the tryptophan emission reflects its average

**Table 1.3** Factors affecting the spectral properties of tryptophan [113]

- quenching by electron deficient amino or carboxyl groups
- homotransfer among the tryptophan residues
- electron transfer quenching by peptide bonds in the protein backbone
- electron transfer quenching by disulfides and amides

environment whether the protein contains one or more tryptophanyl residues. Wide variations in tryptophan emission have been observed, partially due to the unequal contribution of individual tryptophan residues to the total emission [123]. The emission from tryptophan is affected by several factors, which are listed in Table 1.3. When tryptophan is in a completely apolar environment, a hypsochromic shift (≈ 310 nm) is observed. As the tryptophan residue moves to a more polar environment, the fluorescence emission shifts to longer wavelengths. Ultimately, tryptophan becomes solvent accessible and forms hydrogen bonds with water resulting in an

even greater bathochromic shift (≈ 350 nm) as demonstrated in Figure 1.17. For example, denatured or unfolded proteins transition through many different conformational states and exhibit variable tryptophan emissions reflecting the different environments experienced during the structural transitions.

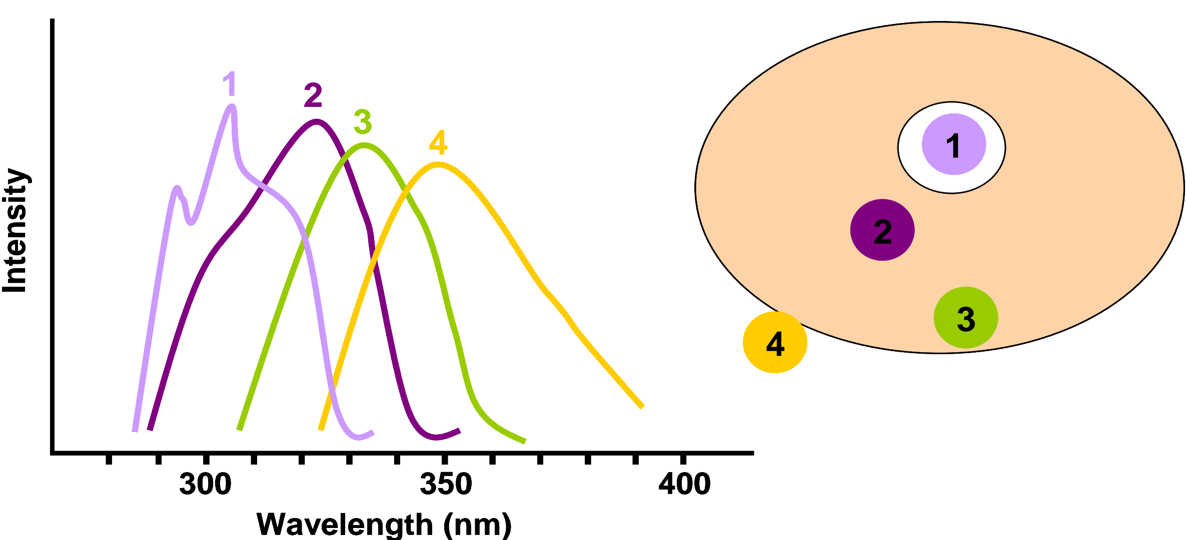



**Figure 1.17** Effect of local environment on emission spectra of tryptophan [111].

Another useful way to monitor protein association using intrinsic protein fluorescence is through fluorescence resonance energy transfer (FRET). This requires measurement of the rate of non-radiative energy transfer from the excited state of the intrinsic protein fluorophore (donor) to another fluorophore or binding partner (acceptor). The efficiency of the energy transfer is governed by equation 1.6 and varies inversely as the sixth power of the distance between the donor and acceptor [113].

$$E = \frac{1}{1 + \left(\frac{r}{R_o}\right)^6} \tag{1.8}$$

where E = efficiency of energy transfer, r = distance between donor and acceptor, $R_o$ = Förster distance, i.e., the distance between donor and acceptor where the energy transfer is 50% efficient. The distance between the two dipoles (< 10 nm) and favorable spatial orientation are key determinants of the rate of energy transfer [124]. Other factors that affect the rate of energy transfer are listed in Table 1.4. Moreover, FRET has been used as a spectroscopic ruler to measure distances between the two species [125] and has opened up new opportunities at the nanometer scale. In this dissertation research, intrinsic and extrinsic fluorescence resonance energy transfer was used as a measure of the extent of interaction or association of poly-SUS and ether-functionalized imidazolium-based ionic liquids with proteins. A brief review of extrinsic fluorescence measurements is available in section 1.9.5.

Table 1.4 Factors affecting the rate of energy transfer

1. extent of spectral overlap of the emission spectrum of the donor with the absorption spectrum of the acceptor
2. distance between the donor and acceptor
3. quantum yield of the donor
4. relative orientation of the donor and acceptor transition dipoles

### 1.9.2 Binding Isotherm Models

Steady state intrinsic fluorescence data can be used to determine the nature of cooperative binding between proteins and ligands [126,127]. Cooperative binding can be described as the binding of a ligand to a macromolecule that affects additional binding of the ligand to the macromolecule. Subsequent binding may be to the same site or to different sites on the substrate. Three types of cooperativity have been described: positive cooperative, negative cooperative, and non-cooperative. Positive cooperative binding is characterized by an increase in subsequent ligand binding. In negative cooperativity, subsequent ligand binding is impaired, and in non-cooperative binding the ligand binds at different sites independently. For both positive and negative cooperativity, the mechanism of interaction is referred to as allosterism. Allosteric interactions are thought to be mediated by a transition in protein conformation [128].

The classic example of cooperativity is the binding of oxygen to hemoglobin in which oxygen binding affinity is increased by initial oxygen binding [129]. At the molecular level, the binding of a ligand or surfactant to a protein is typically illustrated by a binding isotherm. A plot of fraction of ligand bound versus ligand concentration or denaturant concentration, for example, produces a sigmoidal curve. In this curve, one binding state (mainly bound or mainly free) predominates, which produces the sigmoidal shape (Figure 1.18). A sigmoidal binding isotherm also results when SDS interacts with most proteins [127]. As stated before, SDS is an anionic surfactant that forms micelles at its CMC. Interaction of anionic surfactants with proteins is believed to involve both electrostatic and hydrophobic interactions. The anionic head group interacts with the cationic amino acid side chains, namely lysyl, arginyl, and hystidyl. If denaturation is induced exposing protein hydrophobic regions, then hydrophobic interactions between the alkyl chain of the anionic surfactant and the hydrophobic amino acid residues ensue.

Saturation of all potential protein binding sites occurs when the CMC of the anionic surfactant is approached [130].

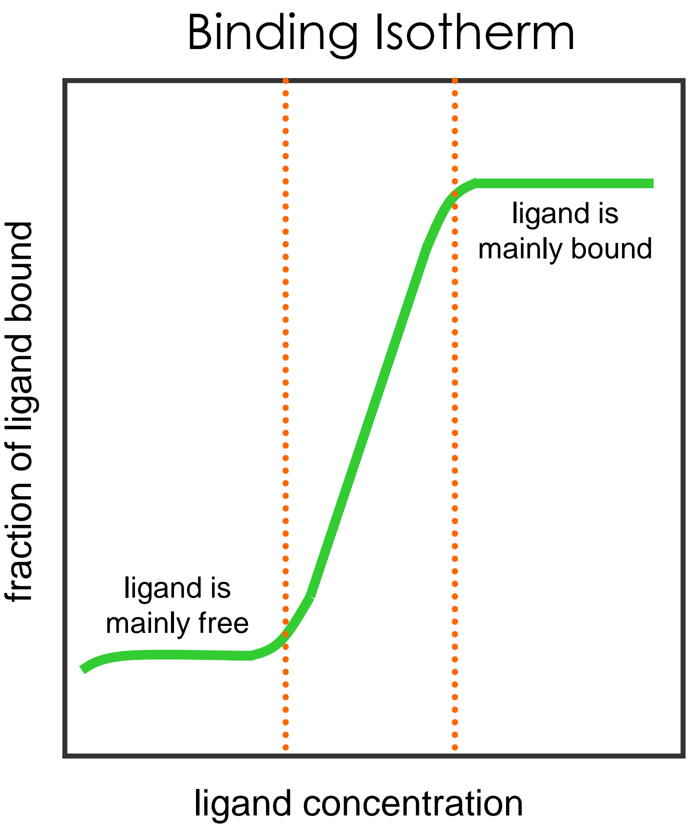



**Figure 1.18** Characteristic sigmoidal shape of a binding isotherm

Several models exist that have been applied in the analysis of steady state fluorescence data for binding isotherm determination. Common models for the analysis of surfactant binding to a protein are the Scatchard equation, closely related to the Hill equation, and the Satake-Yang model [131]. Of those models available, the research reported here utilized the Scatchard model. It was selected for its relevance to the protein-ligand associations that were investigated, particularly because multi-site binding was expected [132] which is readily seen in the scatchard

plot. In addition, the Satake-Yang model does not include micelle size or packing effects [133], which are important characteristics of the ligands investigated.

Scatchard analysis has been used extensively in biochemistry [134]. However, it is a special form of the Hill equation shown below. The Hill equation was developed in 1910 and used to interpret the binding of oxygen to hemoglobin.

$$\nu = \frac{n_H K[S]^{n_H}}{1 + K[S]^{n_H}} \tag{1.9}$$

where ν is the average number of surfactant molecules bound per protein molecule, $n_H$ is the cooperativity coefficient, *K* is an intrinsic binding constant, and [S] is the surfactant or ligand concentration. Briefly, the Hill equation assumes that the formation of protein-surfactant complexes (i.e., $PS_i$) proceeds as a series of equilibria (i.e, $P + S_{i-1} \leftrightarrow PS_i$). It accounts for the varying degrees of cooperativity between binding sites by use of the cooperativity coefficient, $n_H$. The Scatchard equation, shown below, is a special form of the Hill equation where $n_H = 1$ [18].

$$\frac{\nu}{[S]} = K(n - \nu) \tag{1.10}$$

Quantitative treatment using the Scatchard equation provides diagnostic information about the protein-surfactant system such as type of cooperativity, number of binding sites, and binding regions.

### 1.9.3 Extrinsic Fluorescence Spectroscopy

Extrinsic protein fluorescence was first introduced in the work of Gregorio Weber in 1954 [135]. He postulated that critical information about proteins and their interactions could be indirectly retrieved from labeling the proteins with external fluorophores. Sometimes the molecule of interest, namely a protein, DNA, etc., is non-fluorescent or possesses minimal

intrinsic fluorescence. In these situations, an external fluorophore (often called a fluorescent probe [136], reporter group [137], or dye) provides a label that acts as a fluorometric detection reagent. In widespread use today because of their versatility and sensitivity [138], extrinsic fluorophores are classified into two categories: covalent and non-covalent. The primary covalent and non-covalent probes, respectively, that Dr. Gregorio Weber developed were 5-(dimethylamino)naphthalene-1-sulfonyl chloride (dansyl chloride) and 1-anilinonaphthalene-6-sulfonic acid (ANS) (Figure 1.12.1). Dansyl chloride (DNS-Cl) is a non-fluorescent molecule until it reacts with the N-terminus of a protein forming a bioconjugate that is environmentally sensitive and has a large Stokes shift [139]. Anari and coworkers developed a sensitive and specific method for quantitating ethinylestradiol by derivatizing it with dansyl chloride [140]. Another probe important to biophysical studies is ANS. ANS is an aromatic naphthalene derivative containing a secondary amine and a sulfonate group. It is virtually non-fluorescent in an aqueous environment and becomes highly fluorescent in apolar environments through non-covalent contact. [135] ANS has been one of the most widely used probes for protein characterization. Several types of interactions that contribute to the binding mechanisms of ANS to proteins have been proposed, namely hydrophobic, [141] electrostatic, [142] and lower contributing forces such as Van der Waals [143]. Recently, Togashi and Ryder revisited the binding of ANS to BSA and reported a more detailed estimation of binding parameters [144]. Among the applications of fluorescent dyes in protein biochemistry are (1) protein-surfactant interactions, (2) molten globule intermediate states [145], (3) protein aggregation [146,147], and (4) unfolding and refolding processes [148].

5-(dimethylamino)napthalene-1-sulfonyl chloride (DNS-Cl) 1-anilinonapthalene-6-sulfonic acid (ANS)

**Figure 1.19** Examples of covalent (DNS-Cl) and non-covalent (ANS) extrinsic probes

Steady state fluorescence spectroscopy is the most common mode of fluorescence used for protein characterization using an extrinsic probe. The fluorescent dye is used as a reporter of the binding that occurs between a protein and another protein or a ligand such as a surfactant. The information that is extracted from the fluorometric experimental data is typically the binding parameters which include the number of binding sites, the binding association constants, and the type of binding. These parameters reveal the affinity and specificity of the ligand of interest toward a protein. In general, the fluorometric titration technique is used due to its simplicity and sensitivity. Briefly, a non-fluorescing (or very low fluorescing) dye is added in a sufficiently high molar ratio to a marginally fluorescent protein solution, which produces a highly fluorescent dye-protein complex (Figure 1.20). The steady state emission of the complex is measured followed by the addition of increasing concentrations of surfactant or new ligand. Following each subsequent addition of surfactant, the fluorescence intensity is measured. As the

dye molecule is displaced by the surfactant molecule, distinct changes in the emission intensity and wavelength location are observed. When the protein is completely saturated with the surfactant, no change in fluorescence will be observed upon addition of more surfactant. If an affinity exists for the dye, the surfactant or ligand will form a dye-surfactant complex. An increase in dye emission is observed at a new wavelength location. In summary, extrinsic fluorescence is desirable since it is complementary to intrinsic fluorescence measurements in that the external reporter molecule is sensitive to changes in surface hydrophobicity [149] and the emission is independent of the aromatic amino acid residues [150].

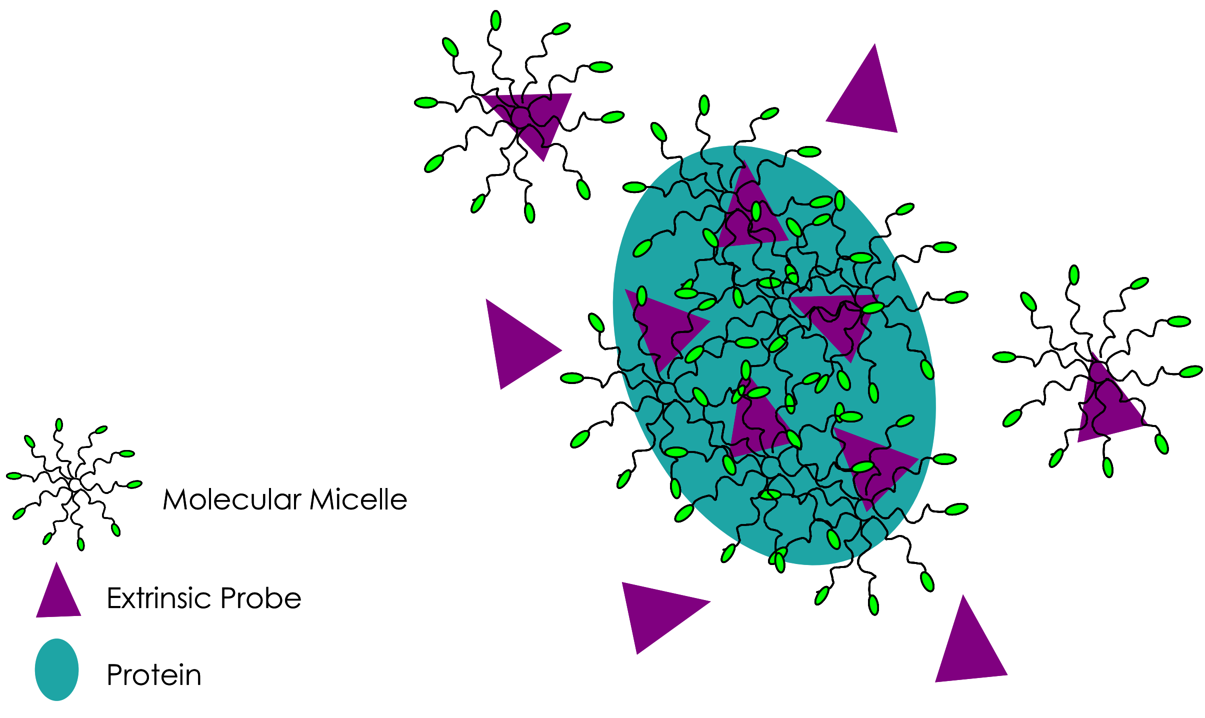



**Figure 1.20** Schematic of an extrinsic probe fluorometric titration

### 1.9.4 Ionic Liquids and Fluorescence

For ionic liquids to have sustainability as new solvent systems in biological applications, an understanding of their structure, intermolecular interactions, and dynamics at the molecular level is required. Fluorescence spectroscopy is one analytical technique that has been used to study many of these properties of ionic liquids including solvent polarity [151], micelle formation of surface-active ionic liquids [152], aggregation of surfactants in ILs [153], and protein stability in ionic liquids [154]. Reichardt [155] summarized reports of imidazolium IL polarity ($E_T(30)$ determined by long-wavelength solvent-dependent solvatochromic dyes. He noted, in agreement with the well-known weak acid proton at $C_2$ [156], that the polarity of the imidazolium ILs that were alkylated at $C_2$ were less polar than the 1,3-dialkylimidazolium counterparts. Carmichael and Seddon used the probe, nile red, and found the polarity of imidazolium ILs is comparable to short-chain primary and secondary alcohols such as methanol, ethanol, and butan-1-ol [157]. These characteristics may make them advantageous in separations requiring aqueous solutions. In another study, Baker and coworkers [154] were the first to employ fluorescence spectroscopy to study the thermostability and conformation of the protein, monellin, in 1-butyl-1-methylpyrrolidinium bis(trifluoromethane sulfonyl)imide. The ionic liquid, equilibrated with 2% v/v water, shifted the melt temperature of monellin from 40 °C in aqueous media to over 100 °C in the ionic liquid. Moreover, as the temperature was increased the emission maximum of monellin in the ionic liquid was highly blue-shifted approaching wavelengths that are typical of complete shielding of Trp by nearby aromatic residues suggesting significant alteration of the protein structure. From these studies, it is evident that ionic liquids are promising solvents for bio-applications. In this dissertation, spectroscopic investigations of protein interactions with imidazolium-based ionic liquids and subsequent use as additives in gel

electrophoresis buffer systems are reported. Steady-state resonance energy transfer and fluorescence anisotropy are used to study these interactions. A brief introduction to fluorescence anisotropy is found in the next section.

### 1.9.5 Fluorescence Anisotropy

Fluorescence anisotropy is also a steady-state measurement that is able to monitor the binding of small molecules to proteins, to study conformational changes in proteins, and to study protein-protein and protein-surfactant interactions [158,159]. In this method, which typically uses an L-polarization format (Figure 1.21), linearly plane polarized light is used to excite the fluorophore. The transition moments in the fluorophore that are parallel to the incident polarized light vector become preferentially excited. When the fluorescence emission is detected, the two components, the one parallel and the one vertical, to the excitation beam are recorded. On the molecular level, the fluorescence anisotropy provides a measure of the angular displacement between the fluorophore excitation and photon emission [113]. This is related to the rotational diffusion, which occurs within the lifetime of the excited state. The anisotropy is dependent on the size and shape of the rotating molecule and the viscosity of the solution. Anisotropy is sensitive to differences in the molecular rotational rates of a bound and a free form of protein in the presence of a ligand, and the magnitude of this difference is a measure of the selectivity. Anisotropies quantified for protein-ionic liquid interactions were used in this research for optimizing gel electrophoresis separation conditions. To quantify the anisotropy, calculations involving the equation below are performed.

$$r = \frac{I_{vv} - GI_{vh}}{I_{vv} + 2GI_{vh}} \qquad (1.11)$$

where r = anisotropy, G = G factor = $I_{HV}/I_{HH}$, a constant which is the ratio of the sensitivity of the detection system for the horizontally and vertically polarized light, $I_{VV}$ = the vertically polarized excitation and the vertically polarized emission, and $I_{VH}$ = the vertically polarized excitation and the horizontally polarized emission [113].

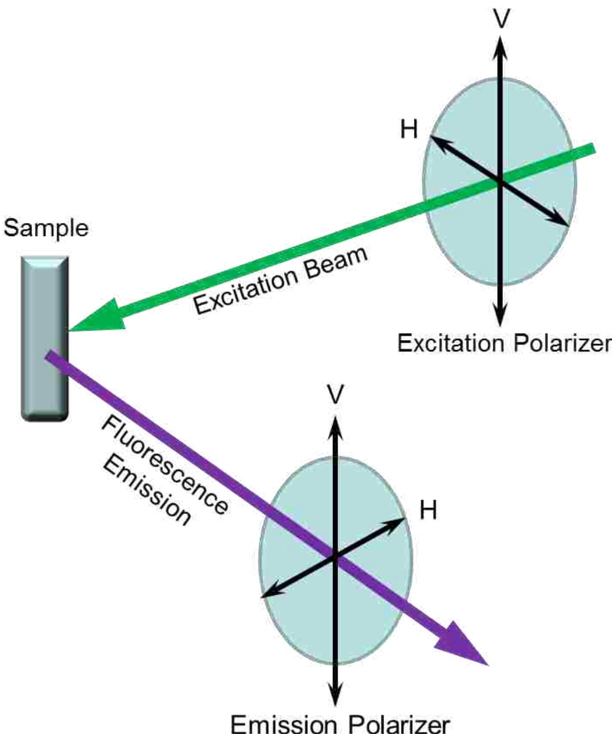



**Figure 1.21** Diagram of the L-Polarization format for fluorescence anisotropy [113]. Vertical (V) and horizontal (H) orientations of each polarizer are shown.

## 1.10 Scope of Dissertation

The research reported in this dissertation is presented in two parts. The first part is the Syntheses, Characterizations, and Investigations of Molecular Micelles and Imidazolium-Based Ionic Liquids for Protein Separations in Electrophoresis, and the second part is a case study

entitled Investigating the Mentoring Canon Through Exploration of Thirty Years of Mentoring by an Exemplar.

The goal of the research reported in the first part of this dissertation is to investigate the use of the achiral molecular micelle, poly-SUS, and imidazolium-based ionic liquids that have ether functionality for protein separations in one dimensional gel electrophoresis. All ionic liquids and molecular micelles were synthesized and characterized by analytical techniques such as elemental analysis, $^{1}H$ NMR, $^{13}C$ NMR, light scattering, and tensiometry. Chapter 2 highlights the simultaneous 1D gel electrophoresis separation of acidic and basic proteins under optimized conditions for poly-SUS, which is compared to the standard protocol using the conventional surfactant, SDS. Optimal conditions were established for poly-SUS by varying the sample buffer concentration, running buffer concentration, sample buffer temperature, and running buffer pH. In Chapter 3 the mechanism of interaction between poly-SUS and model proteins is investigated using intrinsic and extrinsic fluorescence spectroscopy and circular dichroism. The results were used to determine the gel electrophoresis separation conditions for the p53 protein in the presence of poly-SUS, SUS, and SDS. Superior binding efficiency and separation of p53 was demonstrated at low concentrations (i.e., 0.08 %w/v) of poly-SUS compared to SUS and SDS. It has been predicted that in the next decade ionic liquids will probably replace conventional solvents in many applications. One such application is presented in Chapter 4 where for the first time ionic liquids are used as additives in the aqueous sample buffer of gel electrophoresis. The feasibility of their use gel electrophoresis was first investigated using fluorescence spectroscopy. Several ether-functionalized imidazolium-based ionic liquids are used over a wide concentration range for protein separations in one dimensional gel electrophoresis. The band for the protein, transferrin, found in human serum was found to

shift in the gel upon treatment with the ionic liquids, 1-methyl-3-methoxyethoxyethyl imidazolium bromide and 1-butyl-3-methoxyethoxyethyl imidazolium bromide. Lastly, Chapter 6 concludes the studies in this dissertation and highlights the future direction involving the use of molecular micelles and ionic liquids as reagents in two-dimensional gel electrophoresis.

In part two of this dissertation, a case study was performed, in which thirty years of mentoring in the sciences by an exemplar was investigated. The goal of this research was to move beyond descriptions of mentoring attributes to explore why the mentoring employed by the exemplar was deemed successful as evidenced by the many local and national mentoring awards that have been bestowed upon him. Chapter 1 describes the rationale of the research, explains the research questions, and provides a graphical representation of the metacognitive processes traversed during this work. In Chapter 2, a review of the literature on mentoring and the theories that served as the theoretical framework of the study are surveyed. A description of the research methods is provided in Chapter 3. Constructivist grounded theory was used as the research methodology. The results of the research and a detailed discussion of the generalized theory, which is grounded in the data inquiry, are provided in Chapter 4. Finally, concluding remarks and implications of the study follow.

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# CHAPTER 2

# USE OF POLY (SODIUM *N*-UNDECANOYL SULFATE) FOR THE GEL ELECTROPHORESIS SEPARATION OF ACIDIC AND BASIC PROTEINS

## 2.1 Introduction

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) has become and remains the standard approach for identification, quantification, purification and structural analysis of simple, oligomeric and conjugated proteins [1-5]. The establishment and propagation of its continuous selection as the method of choice for biological separations began with several seminal papers including those published separately by Ornstein [6] and Davis [7] in 1964, Maizel in 1965 [8], and Laemmli in 1970 [9]. In this electrophoretic technique, polyacrylamide gels made with contiguous stacking and resolving gels and detergents having both hydrophobic and hydrophilic moieties are used. In the Laemmli system [9], gel electrophoresis measurements are routinely carried out with SDS as the surfactant. The amphipathic nature of SDS is essential for the separation. At concentrations greater than the critical micelle concentration (CMC), surfactant monomers spontaneously self assemble into micellar structures. Typically spherical, the surfactant micelles are reported to promote denaturation and solubilization then bind to proteins in a relatively uniform stoichiometric ratio of 1.4 g SDS/g protein at saturation [10]. However, subsequent studies have found that this ratio more accurately reflects a range of 1.5-2 g SDS/g protein for globular proteins [11]. Negatively charged SDS-protein complexes are formed having the same charge density. Thus, charge is eliminated from the migration mechanism, and separation through the porous polyacrylamide gel is only a function of size. Despite the obvious limitations of PAGE such as joule heating and long measurement times, a simple citation search of all scientific research that utilizes this technique confirms its

importance. Widespread usage of PAGE, particularly SDS-PAGE, remains at an all time high due to its simplicity, availability, and relatively low cost.

Although SDS has been used in PAGE for decades, anomalous migrations[11-13] exist and deleterious effects subsist which have yet to be resolved. The presence of longer chain alkyl sulfates in unpurified SDS may lead to multiple bands as a single protein complexes with one or more SDS monomer [14]. Even with the use of pure SDS, carbohydrate-bearing, highly basic, and highly acidic proteins migrate anomalously in gel electrophoresis [15-18]. Certain proteins such as ribonuclease and collagen seem not to migrate according to molecular weight (i.e. "gel shifting") [19-20] suggesting a need for greater understanding of the binding and transport mechanism involved. Rath and coworkers investigated "gel shifting" in SDS-PAGE of helical membrane proteins noting that stoichiometric binding ranged from 3.4–10 g SDS/g protein and that the anomalous migrations of these types of proteins originated from the wide variations in SDS binding [21]. In addition, SDS may crystallize at low temperatures [3, 22] producing artifacts in the gel which sometime compromise resolution [23]. Moreover, when SDS is used in mass spectrometry, it forms sodium adducts with proteins and thus reduces the accuracy of protein identification [24].

To overcome these limitations in SDS-PAGE, the developments of protocols that modify native and denaturing PAGE in various ways have been proposed. For example, in 1984 two cationic detergents, cetyltrimethylammonium bromide (CTAB) and cetylpyridinium chloride, were used to replace SDS in a search for an alternative surfactant for PAGE [25]. Unfortunately, these cationic detergents required a ten-fold increase in concentration as compared to SDS. In another study, mixing of anionic surfactants with alcohols of equivalent aliphatic chain length resulted in resolution of three fractions of the NS1 influenza virus, which

was not previously achieved with conventional SDS-PAGE [26]. Modifications of gels were previously reported by Bustamante *et al.* [27]. and Casas-Terradellas *et al.* [28] where double-inverted gradient polyacrylamide slab gels for the electrophoretic separation of proteins under nonreducing conditions and large porosity gels coupled with conventional gradient gels for the simultaneous separation of giant and small proteins were used, respectively. More recently, Zilberstein and coworkers [29] essentially altered the separation mechanism from molecular sieving to isoelectric focusing by introducing a linear gradient of positively charged immobiline as an additive to the polyacrylamide gel. Other attempts at changing SDS in the first dimension of 2-D Gel Electrophoresis have been described. An acid-labile surfactant (ALS) reportedly exhibited similar denaturing and electrophoresis properties as SDS [3]. However, the authors revealed that ALS decomposes at low pH, and the apparent negative charge on the ALS-protein complex may not be as high as with SDS.

Over the years, other modifications in normal SDS-PAGE protocol finding necessity have included changes to the running buffer [30-32], gel composition [33, 34] as well as finding more sensitive stains such as copper [35], silver [36], sypro ruby [37], and fluorescent chameleon labels [38]. Furthermore, to reduce the negative effects associated with protein modification during separation, the Bis-Tris-Mes gel, which operates at near neutral conditions, has been validated and is now commercially available [39].

These primary limitations in SDS-PAGE suggest a need for surfactants that can discriminate a wide range of protein types and molecular weights with little or no modification in the separation protocol. Therefore, the use of molecular micelles as alternatives to SDS in gel electrophoresis is an attractive approach. In this study, we describe the novel use of the molecular micelle, poly (sodium *N*-undecanoyl sulfate) (poly-SUS) (Figure 2.1), which

consistently produced baseline resolution of eleven globular proteins, as a potential replacement for SDS in PAGE. For comparison, the present investigation has included data from similar protein separations completed with SDS-PAGE.

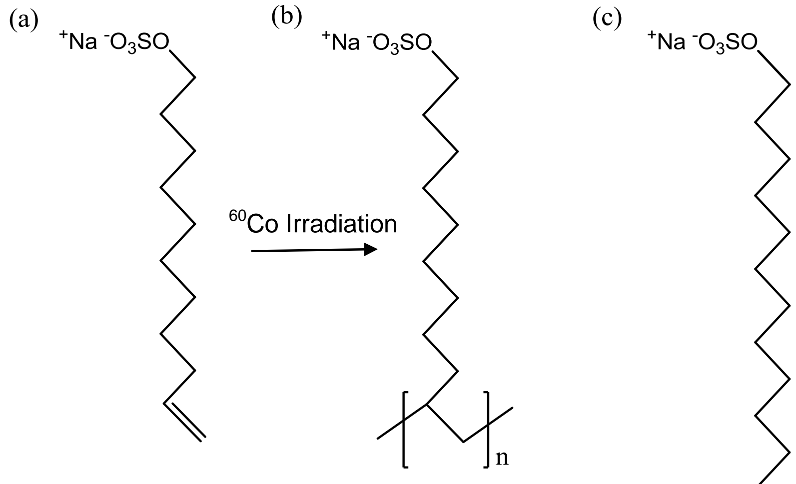


**Figure 2.1**. Structures of the (a) monomer, sodium undecylenic acid (SUS), (b) molecular micelle, poly (sodium *N*-undecanoyl sulfate) (poly-SUS), and (c) the conventional surfactant, SDS, investigated.

## 2.2 Materials and Methods

### 2.2.1 Materials

Lysozyme (chicken egg white, 14.7 kDa), ribonuclease A (bovine pancreas, 13.7 kDa), carbonic anhydrase (bovine erythrocytes, 29 kDa), phosphorylase B (rabbit muscle, 97 kDa), ovalbumin (egg, 45 kDa), BSA (bovine, 66 kDa), cytochrome C (bovine heart, 12.3 kDa), β-galactosidase (*Escherichia coli*, 116 kDa), α-chymotrypsinogen A (bovine pancreas, 26 kDa), and α-lactalbumin (bovine milk, 14.2 kDa) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Myosin (rabbit muscle, 205 kDa) was purchased from Fisher Scientific (Pittsburgh, PA, USA). Each protein was ≥ 95% pure, and the molecular weight was verified by MALDI MS or ESI MS for 9 of the 11 proteins. All proteins were reconstituted into 1 mg/ml aliquots with 10 mM phosphate buffer at pH 7.4 and stored at -20°C. Standard precast 4-20% Tris HCl gradient

polyacrylamide mini gels were obtained from Bio-Rad Laboratories (Hercules, CA, USA). Reagents used to prepare the running buffer, the sample buffer, and the destaining solution were purchased from Invitrogen Corporation (Carlsbad, CA, USA). Ultrapure water (18.2 MΩ) was obtained using an Elga PURELAB Ultra water purifier (Lowell, MA, USA). All reagents were used as received without further purification.

### 2.2.2 Synthesis of the Molecular Micelle, poly-SUS

Poly-SUS was synthesized according to a procedure previously reported by Warner *et al*. [22] and Bergstrom [31]. The critical micelle concentrations (CMC) of monomeric SUS [~25 mM (aq)] and SDS [~8 mM (aq)] were estimated from surface tension measurements at room temperature by use of a KSV Sigma 703 digital tensiometer. Polymerization of SUS was achieved at a concentration of five times the CMC under γ-irradiation from a $^{60}Co$ source. Monomeric SUS and polymeric SUS (poly-SUS) were characterized by use of $^{1}H$ NMR in deuterium oxide ($D_2O$) on a Bruker–250 MHz instrument. Complete polymerization was confirmed by disappearance of the NMR chemical shift signals (5.0 – 6.0 ppm) associated with the terminal vinyl group. All poly-SUS solutions are reported using the equivalent monomer concentration, namely calculations were based on the molecular weight of the individual surfactant unit (i.e., SUS, 272 g/mol).

### 2.2.3 Gel Electrophoresis Instrumentation

A Bio-Rad Laboratories Mini-PROTEAN 3 Electrophoresis Module was used for PAGE separations (Hercules, CA, USA). A constant voltage of 200 V was applied for each separation by a 1000 V Bio-Rad power supply. During staining and destaining, gels were placed in plastic containers and set on a rocker (Midwest Scientific, St. Louis, MO, USA). Typical

staining and destaining times for SDS-PAGE were used. The protein bands were analyzed for each gel using a Kodak Gel Logic 200 Image Analyzer (Rochester, NY, USA).

### 2.2.4 Preparation of Sample and Running Buffers

Standard 10x RB stock solution contained 25 mM Tris and 192 mM Glycine. The running buffer solution was prepared by measuring an appropriate amount of poly-SUS (or SDS) into a volumetric flask, dissolving it with 50 mL of running buffer stock solution, and diluting it to a final volume of 500 mL with ultrapure water (18.2 MΩ). Desired pH values of the running buffer were achieved by the addition of either 1 M NaOH or 1 M HCl. All poly-SUS solutions were prepared by using the equivalent monomer concentration, namely calculations were based on the molecular weight of the individual surfactant unit (i.e. SUS, 272 g/mol). Concentrations of 0.0125%, 0.025%, 0.0375%, and 0.053% w/v poly-SUS were used in the running buffer for the optimization and validation separations. The sample buffer was prepared in 1.7 mL eppendorf tubes by combining appropriate amounts of ultrapure water, 50 mM Tris HCl, pH 6.8, glycerol, bromophenol blue, and 0.078%, 0.156%, 0.313%, 0.625%, 1.25%, 2.0%, or 2.51% w/v poly-SUS (or SDS). Eleven model proteins with MW ranging from 12.3 to 205 kDa of equal concentration were added in the SB at a protein:SB ratio of 2:1. The reducing agent, β-mercaptoethanol, was added at 5% v/v of the SB.

### 2.2.5 Electrophoretic Separation

Running buffer totaling 325 mL was loaded into the upper and lower chambers of the Mini-PROTEAN 3 module. Each sample was heated at 95 °C for 5 minutes on a dry bath incubator from Fisher Scientific (Pittsburgh, PA, USA) unless noted otherwise. Dry bath incubator temperatures were adjusted during optimization of the poly-SUS separation protocol. Twenty microliters (20 μL) of sample was loaded into each well of the 4-20% Tris HCl gradient

mini gels. When SDS was in the sample, a wide range SDS marker (6.5 – 205 kDa) from Sigma-Aldrich (St. Louis, MO, USA) was used. The migration time was less than thirty-five minutes for all separations. After each separation, gels were rinsed with ultrapure water (18.2 MΩ), stained with approximately 25 mL of Colloidal Blue Stain, and placed on a rocker. Gels were destained with ultrapure water (18.2 MΩ) until a clear background was visible.

### 2.2.6 Circular Dichroism Measurements

Circular dichroism spectra were recorded in the range from 200 to 320 nm with an AVIV Model 62DS (AVIV Associates, Lakewood, N.J.) spectrophotometer at 25 °C. CD spectra of native protein samples in 25 mM Tris/192 mM Glycine, pH 8.4 were acquired at concentrations that produced optimal CD signal. The Tris/Glycine buffer was filtered with a 0.45 μm nylon filter prior to sample preparation. All protein CD spectra were taken with samples in a 1 mm pathlength quartz cell. The CD scans were done in triplicate, average spectra obtained, and corrected for the background intensity of the buffer. SDS and poly-SUS are transparent in the far and near UV which required no spectral correction. Protein secondary structure analysis was carried out using CDNN ver. 2.0. CD response is reported as ellipticity and displayed in units of millidegree (θ).

## 2.3 Results and Discussion

Molecular micelles are amphipathic molecules with hydrophilic head groups and covalently bound hydrophobic tails. Previous studies reported by our laboratory indicated that molecular micelles were superior to conventional micelles for many separation techniques [40-43]. For example, molecular micelles have been successfully used in place of conventional micelles as pseudo-stationary phases in micellar electrokinetic chromatography (MEKC) achieving distinct molecular recognition advantages as evidenced in chiral separations. In one

MEKC study, poly-SUS provided better resolution of sixteen priority pollutants than SDS during separation of polycyclic aromatic hydrocarbons [40]. Additional data from our laboratory suggests that molecular micelles have several distinct advantages over conventional micelles in open tubular capillary electrochromatography (OT-CEC) using polyelectrolyte multilayer (PEM) coatings [44]. Furthermore, molecular micelles have physicochemical properties that would make them attractive alternatives to the conventional micelle, SDS, in PAGE. For instance, molecular micelles are formed from polymerizing the terminal double bond of amphipathic molecules at concentrations higher than the CMC (Figure 2.2). Subsequently, the dynamic equilibrium between the surfactant monomer and the micelle is eliminated because of the covalently-bound hydrophobic core. Therefore, molecular micelles have no CMC, and we hypothesized that PAGE separations of proteins would be achieved at much lower concentrations of poly-SUS than SDS. Since molecular micelles have no CMC, in principle there is no free monomer that will carry current and contribute to joule heating. Thus, we proposed slower

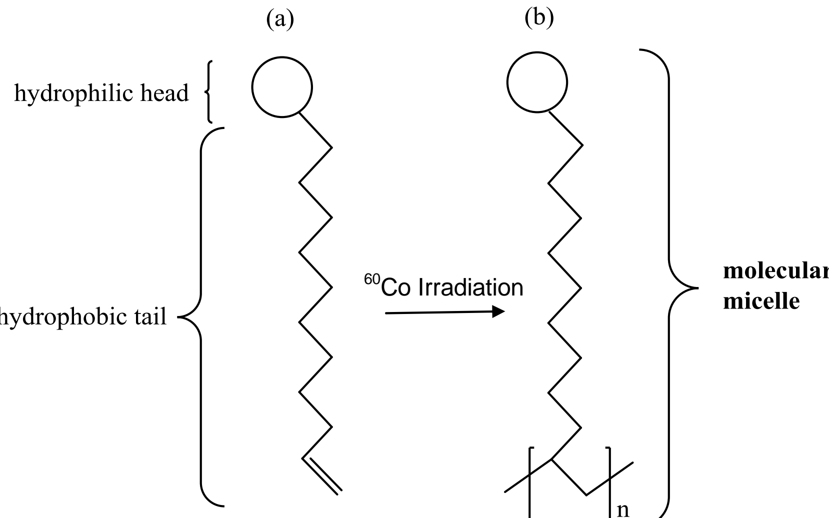


**Figure 2.2** Schematic of a typical molecular micelle synthesized in our laboratory: (a) monomer, (b) polymer.

mobility of the protein/molecular micelle complex allowing for more efficient separations and minimal loss of resolution. In addition, the stability [40] of the molecular micelle structure is not compromised and analyte resolution should be improved. Thus, in this study we expected sharper bands and resolution of a greater number of proteins in a complex mixture of acidic and basic proteins of varying molecular weight (12 – 200 kDa). Moreover, molecular micelles exhibit improvements in rigidity and controllable size [40] that may be beneficial during complexation with certain proteins, e.g. hydrophobic proteins, and thus may allow for separations of proteins with varying hydrophobicity and charge.

### 2.3.1 Optimization

#### 2.3.1.1 Running Buffer pH

Polyacrylamide gel electrophoresis of eleven proteins with molecular weights ranging from 12 - 205 kDa was performed at four running buffer pH levels (7.5, 8.0, 8.5, and 9.0). As shown in Figure 2.3, the SB concentrations included 2.51%, 2%, 1.25%, 0.625%, 0.313%, 0.156%, and 0.078% w/v poly-SUS. The poly-SUS running buffer concentration used was 0.05% w/v. We propose that just as with conventional surfactants, molecular micelles bind to proteins through coulombic interactions with charged amino acid residues and hydrophobic interactions with the nonpolar polypeptide backbone. However, little or no separation was observed at pH 7.5 and pH 8.0 of the running buffer. When the pH was increased to 8.5, the protein bands became visible with some resolution (i.e. a qualitative determination), but the band intensity decreased with decreasing sample buffer concentration. At pH 6.0 (~pI) glycine would be slowly migrating toward the anode as a zwitterion. As the pH is raised and approaches the second acid dissociation constant, the $-NH_3^+$ group loses its proton and the molecule has an overall negative charge. Thus, we suppose that the glycine ion migrates faster as the running

buffer pH is increased causing greater stacking which concentrates the poly-SUS/protein complexes into a thinner starting zone in the stacking region of the gel. This concentration of the poly-SUS/protein complexes resulted in visible and sharp bands at the optimum running buffer pH of 9.0. At this pH, the protein bands were sharp, the band peak intensities exceeded three times the background intensity (i.e. noise), and baseline resolution (determined qualitatively) was achieved for bands identified within the sample buffer concentration range. At pH 9.0 the number of bands identified ranged over the entire concentration range (0.078% - 2.5% w/v poly-SUS) were from 6 to 11 with a median of 9.

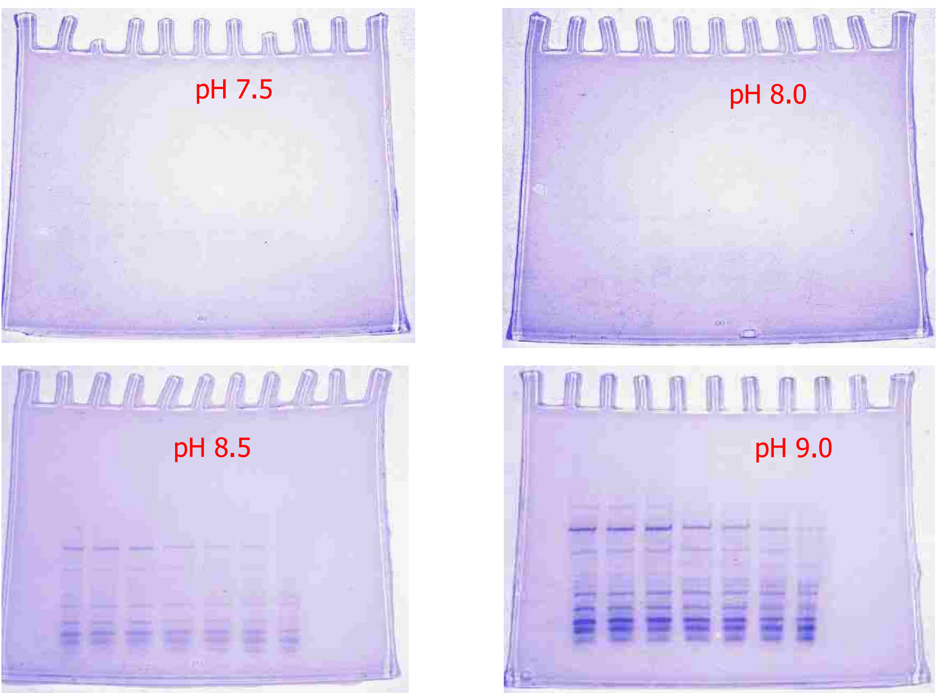


**Figure 2.3** Effect of running buffer pH on protein separations using poly-SUS. RB pH is as indicated on each gel (7.5, 8.0, 8.5, and 9.0). Lanes 2 to 8: 2.5%, 2%, 1.25%, 0.63%, 0.31%, 0.16% and 0.078% w/v poly-SUS in the SB. Poly-SUS RB concentration was 0.05% w/v.

### 2.3.1.2 Sample Buffer Temperature

Figure 2.4 illustrates a representative gel from the sample buffer temperature ($T_{sb}$) study. In standard SDS-PAGE all samples are heated to 95°C. In the poly-SUS $T_{sb}$ study, separations were performed at the optimum running buffer pH of 9.0 and at five different temperatures: 25, 65, 75, 85, and 95°C. All samples were heated for 5 minutes and the poly-SUS running buffer concentration was 0.05% w/v. Temperature did make a substantial difference in resolution as evidenced by the optimum temperature for the poly-SUS protein separations (75°C) was twenty-one percent lower than the standard temperature (95°C) used in SDS-PAGE. At 75°C,

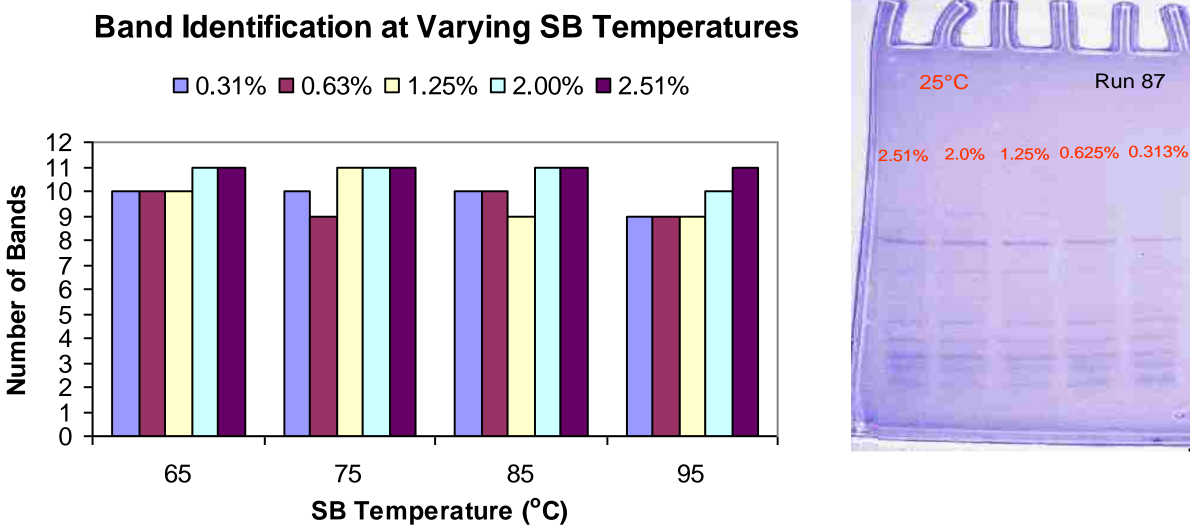


**Figure 2.4** Determination of optimum sample buffer temperature. Histogram of the number of bands identified (out of 11) at various sample buffer temperatures and poly-SUS concentrations (on the left). Gel of room temperature (25°C) poly-SUS separation. Poly-SUS SB concentrations are as indicated for each lane. Poly-SUS RB concentration was 0.053% w/v. (on the right).

for the sample buffer concentrations greater than 1.25% w/v poly-SUS, eleven bands were identified and by qualitative determination they were baseline resolved. A lower sample buffer temperature may indicate that the poly-SUS efficiently solubilizes the proteins in its hydrophobic

environment subsequently disrupting its secondary structure and denaturing the protein. Thus, complete denaturation from near boiling at 95°C may not be necessary. However, it should be noted that additional comparisons with separations at temperatures below 65°C may be warranted. This is evidenced by the resolution (qualitatively determined) of greater than 50% of the proteins for the separation at room temperature, 25°C, (Figure 2.4) though the band intensity was low. The low band intensity may only point to the need to determine the optimum staining procedure when using molecular micelles, in this case poly-SUS. Lower required sample buffer temperatures will have a direct impact on reduction in energy usage when multiple samples are being prepared simultaneously.

A plot of log molecular weight versus relative mobility for a molecular weight marker yields a straight line in conventional SDS-PAGE. Similar plots for poly-SUS separations were completed to compare the linearity of the separations at 75°C and 95°C, the standard $T_{sb}$ used in SDS-PAGE. As shown in Figure 2.5, consistent and superior correlation coefficients ($R^2$) ranging from 0.94 – 0.97 over the entire sample buffer concentration range (0.078% - 2.51% w/v) were achieved at 75°C as compared to 95°C. Thus, this data confirmed that 75°C was the optimum temperature for the poly-SUS separations. In addition, it is interesting to note that $R^2$ increased as the SB concentration decreased. This data is consistent with our original hypothesis that poly-SUS has no CMC and therefore lower concentrations of the molecular micelle would be required for complexation with the proteins.

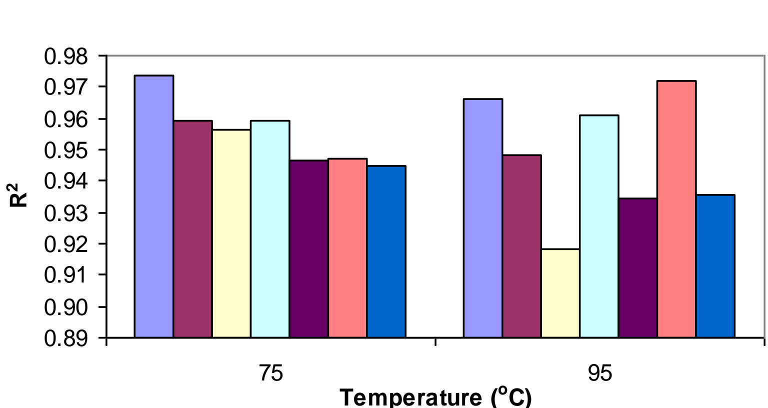


**Figure 2.5** Determination of optimum sample buffer temperature. Histogram showing the linearity of the poly-SUS separations at two sample buffer temperatures, 75°C and 95°C, and varying sample buffer concentrations.

### 2.3.1.3 Sample Buffer Concentration

In order to verify the effectiveness of the molecular micelle over a wide range of concentrations, the poly-SUS concentration in the sample buffer was varied for the protein separations. Figure 2.6 shows two representative separations at concentrations in lanes 2 to 8 of 2.51%, 2%, 1.25%, 0.625%, 0.313%, 0.156% and 0.078% w/v of poly-SUS in the SB. The poly-SUS running buffer concentration was 0.0125% w/v and 0.053% w/v for run the two different separations, respectively. As hypothesized, the lack of dynamic equilibrium between SUS monomer and micelle in the molecular micelle permitted sample buffer concentrations as low as 0.078% w/v or a 26-fold reduction as compared to the standard SDS concentration (2% w/v) in PAGE. The average number of bands identified at the lowest poly-SUS sample buffer concentration (0.078% w/v) was 10 (see Tables 1 and 2 in the *supporting information*). At poly-SUS sample buffer concentrations of 0.625% w/v and lower, the first protein band was no longer visible. We believe this indicated a need to understand the binding mechanism of the association

between poly-SUS and different proteins. To begin probing this question, PAGE data were collected removing either the disulfide reductant or the thermal heating (or both) in the presence of poly-SUS (2.5% - 0.0098% w/v in the SB). The results suggested that binding of poly-SUS to the proteins is more efficient when free thiols are present. At concentrations lower than 0.078% w/v, poly-SUS promoted denaturation, but aggregation and precipitation occurred when the hydrophobic effect was disrupted by the molecular micelle. Taken in aggregate, examination of these data suggests that with further investigation sample buffer concentrations lower than 0.0098% w/v, which would represent a >99% reduction relative to SDS in the SB, may be attainable.

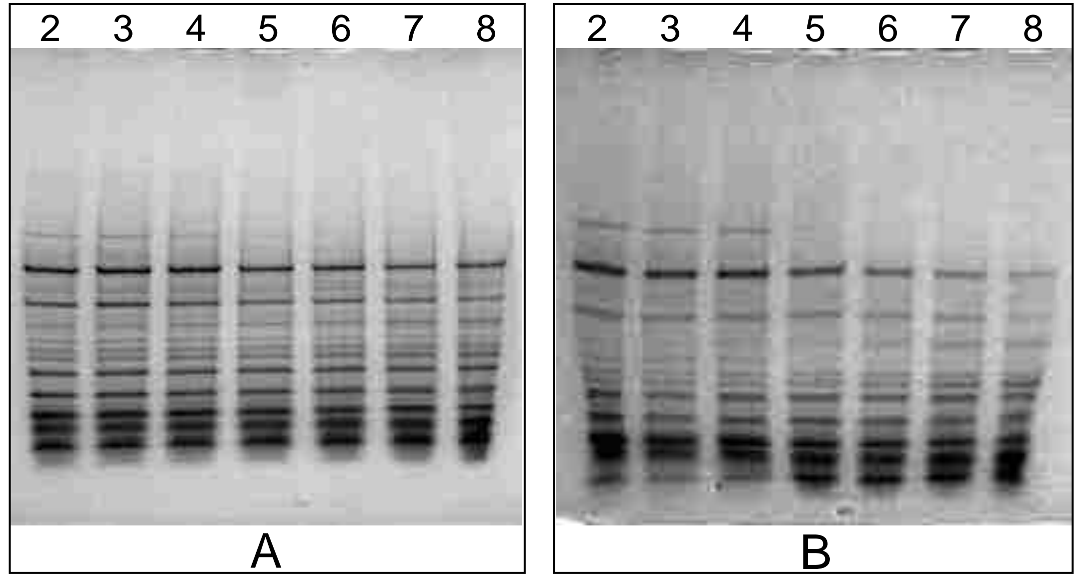


**Figure 2.6** Effect of sample buffer concentration on protein separations using poly-SUS. SB concentrations shown on each gel in lanes 2 to 8 are 2.5%, 2%, 1.25%, 0.625%, 0.313%, 0.156% and 0.078% w/v poly-SUS, respectively.

#### 2.3.1.4 Running Buffer Concentration

The effect of the poly-SUS concentration in the running buffer on the separation and migration pattern of the proteins is shown in Figure 2.7. The poly-SUS running buffer concentrations investigated were 0.0125%, 0.025%, 0.0375%, and 0.053% w/v. Few differences

were noted in the separations at the four RB concentrations. The migration patterns and the number of bands identified remained consistent for all sample buffer concentrations. Band identification and qualitatively determined resolution were maintained even at running buffer concentrations that were 8 times lower (0.0125% w/v) than the standard SDS concentration of 0.1% w/v. These data suggest that further reductions in poly-SUS running buffer concentration may result in preservation of band identification, band resolution, and peak intensity.

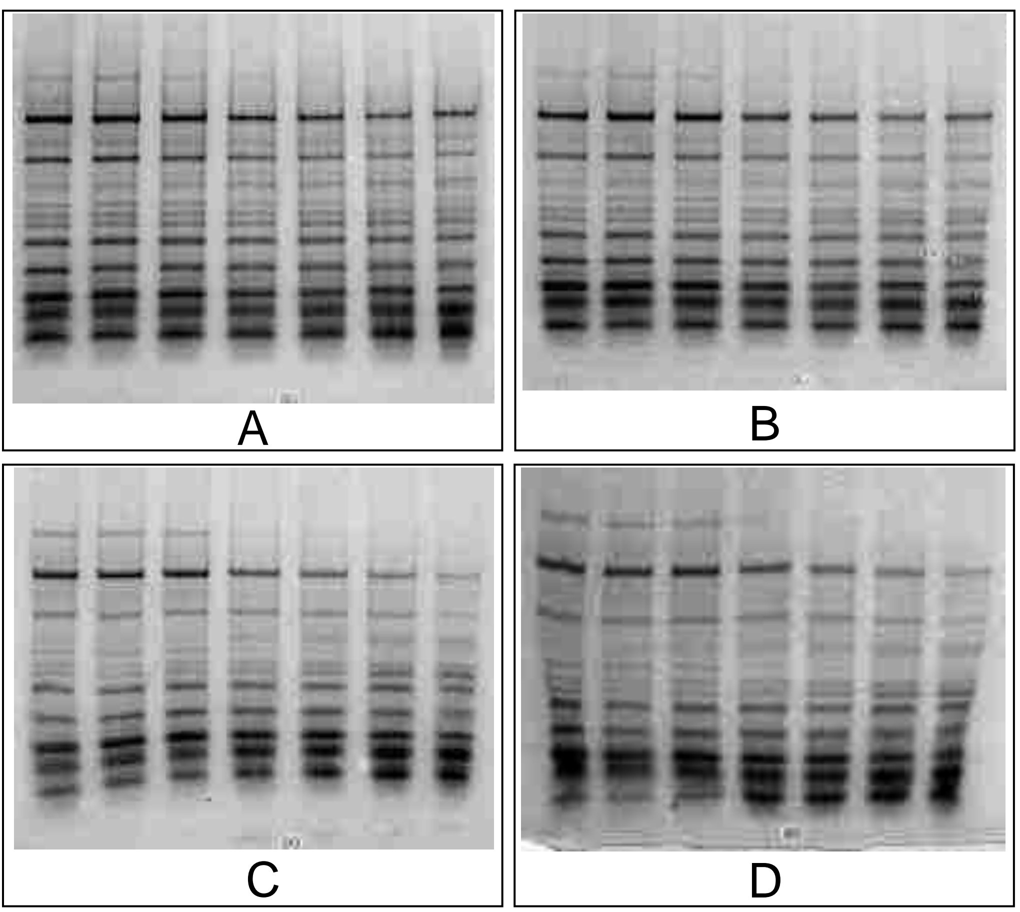


**Figure 2.7** Effect of running buffer concentration on protein separations using poly-SUS. Poly-SUS RB concentrations are as indicated on each gel (A – 0.0125%, B – 0.025%, C – 0.0375%, and D - 0.053% w/v).

### 2.3.2 Method Validation

The reproducibility of the poly-SUS protein separations was determined by measuring the number of bands using $S/N \geq 3$ as the minimum acceptable intensity for the inter- and intra-day runs. At least three inter-day separations were completed over a one week period, while four intra-day separations were completed within the same day. The percent relative standard deviations are shown in Tables 2.1 and 2.2. An average of 9.8 and 10.3 proteins (out of 11) were identified for the inter- and intra-day separations for all running buffer and sample buffer concentrations, respectively. However, it should be noted that at the lowest running buffer concentration (0.0125% w/v) for the intra-day separations the average number of bands identified exceeded the number of proteins placed in the SB although the measurements were relatively precise (%RSD < 7.8%). This may be due to possible systematic errors introduced during the freeze/thaw cycle between separations which may have caused protein instability and degradation. Overall, the majority of the poly-SUS separations were reproducible for the sample buffer concentrations (0.0781% - 2.51%) and running buffer concentrations (0.0125% - 0.053%) used as indicated by %RSD lower than 10%. Based on these data, the poly-SUS successfully reproduced the separations of the eleven proteins.

**Table 2.1** Inter-Day Reproducibility data of poly-SUS protein separations with $T_{sb}$ = 75 °C and $EB_{pH}$ = 9.0. Avg = average number of identifiable bands ($S/N \geq 3$); St.Dev. = standard deviation; RSD% = percent relative standard deviation.

| ***Inter-Day Reproducibility (n=3)*** | | **Sample Buffer Concentration, w/v% poly-SUS** | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Electrode Buffer Concentration, w/v% poly-SUS** | | **2.51%** | **2.00%** | **1.25%** | **0.625%** | **0.313%** | **0.156%** | **0.0781%** |
| **0.0125%** | Avg # bands | 10.7 | 11 | 11.3 | 10.7 | 10.7 | 10.3 | 10 |
| | St.Dev. | 0.58 | 0 | 0.58 | 1.2 | 1.2 | 0.58 | 0 |
| | RSD% | 5.4 | 0 | 5.1 | 10.8 | 10.8 | 5.6 | 0 |

**Table 2.1 cont'd.**

| *Inter-Day Reproducibility (n=3)* | | Sample Buffer Concentration, w/v% poly-SUS | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Electrode Buffer Concentration, w/v% poly-SUS** | | **2.51%** | **2.00%** | **1.25%** | **0.625%** | **0.313%** | **0.156%** | **0.0781%** |
| **0.0250%** | Avg # bands | 10 | 9.3 | 9.7 | 9.3 | 9.7 | 10 | 10 |
| | St.Dev. | 0 | 1.15 | 0.58 | 0.58 | 0.58 | 0 | 0 |
| | RSD% | 0 | 12.4 | 6.0 | 6.2 | 6.0 | 0 | 0 |
| **0.0375%** | Avg # bands | 10.7 | 10.7 | 10.3 | 10 | 10 | 10.3 | 10 |
| | St.Dev. | 0.58 | 0.58 | 0.58 | 1.7 | 1 | 1.2 | 1 |
| | RSD% | 5.4 | 5.4 | 5.6 | 17.3 | 10 | 11.2 | 10 |
| **0.0530%** | Avg # bands | 10.3 | 10.3 | 11 | 10.3 | 9.7 | 9.3 | 10.3 |
| | St.Dev. | 1.2 | 1.2 | 0 | 0.6 | 0.6 | 1.2 | 1.2 |
| | RSD% | 11.2 | 11.2 | 0 | 5.6 | 6.0 | 12.4 | 11.2 |

**Table 2.2** Intra-Day Reproducibility data of poly-SUS protein separations. All other parameters are the same as in Table 1.

| *Intra-Day Reproducibility (n=4)* | | Sample Buffer Concentration, w/v% poly-SUS | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Electrode Buffer Concentration, w/v% poly-SUS** | | **2.51%** | **2.00%** | **1.25%** | **0.625%** | **0.313%** | **0.156%** | **0.0781%** |
| **0.0125%** | Avg # bands | 13 | 13 | 13 | 11.8 | 12.3 | 11.5 | 10.8 |
| | St.Dev. | 0 | 0 | 0 | 0.5 | 0.96 | 0.58 | 0.5 |
| | RSD% | 0 | 0 | 0 | 4.3 | 7.8 | 5.0 | 4.7 |
| **0.0250%** | Avg # bands | 10.5 | 11 | 10.5 | 9.8 | 10.3 | 10.5 | 9.3 |
| | St.Dev. | 1.7 | 0 | 1 | 1.3 | 0.5 | 0.58 | 0.96 |
| | RSD% | 16.5 | 0 | 9.5 | 12.9 | 4.9 | 5.5 | 10.4 |
| **0.0375%** | Avg # bands | 10.3 | 10.3 | 10.3 | 9 | 9.8 | 10 | 8.75 |
| | St.Dev. | 1.5 | 1.5 | 1.5 | 1.2 | 0.5 | 1.2 | 1.7 |
| | RSD% | 14.6 | 14.6 | 14.6 | 12.8 | 5.1 | 11.5 | 19.5 |
| **0.0530%** | Avg # bands | 10.5 | 10.8 | 11.0 | 10.3 | 10.5 | 10.3 | 10.8 |
| | St.Dev. | 0.58 | 0.5 | 0 | 0.96 | 0.58 | 0.5 | 0.5 |
| | RSD% | 5.5 | 4.7 | 0 | 9.3 | 5.5 | 4.9 | 4.7 |

### 2.3.3 Method Comparison

Separations were completed with the surfactants, SDS and poly-SUS, in order to make a comparison and to validate the separation efficiency of poly-SUS. A visual comparison of the separations for a range of SB concentrations (0.625% - 2.51% w/v) for both SDS and poly-SUS are shown in Figure 2.8. Near complete baseline resolution of the eleven globular proteins were observed in the poly-SUS system. Three separations were run at the standard SDS conditions,

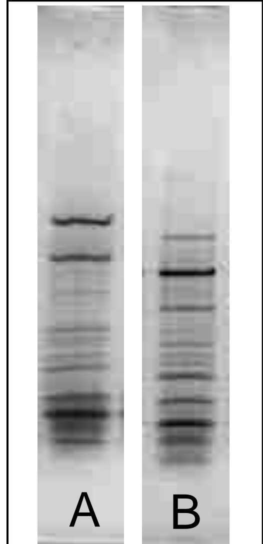


**Figure 2.8** Visual comparison of separations using standard SDS protocol and optimized poly-SUS protocol for a sample buffer concentration of 2% w/v. A - SDS (2%,w/v), and B – poly-SUS (2% w/v).

and only ten protein bands were identifiable (i.e. S/N ≥3) in each case. The sharpness of the bands for the poly-SUS separation is apparent when compared to the SDS separation. As shown in Figure 2.9, the electrophoretic mobility of the poly-SUS separated proteins was inversely proportional to the log of the MW with a high correlation coefficient ($R^2$ = 0.95). At the optimized conditions for the PAGE separation using poly-SUS, the migration of the proteins through the gel followed a similar sieving pattern as seen in an SDS gel. A strong correlation ($R^2$ = 0.997) between the relative mobility of poly-SUS with respect to SDS is shown in Figure

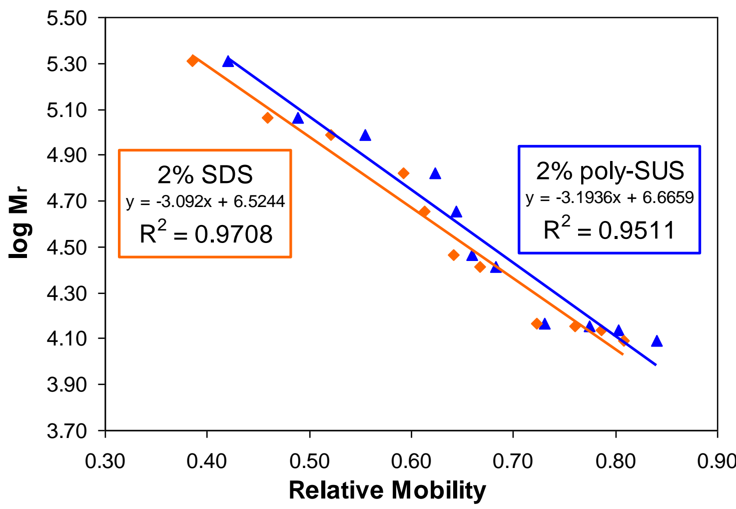


**Figure 2.9.** Comparison of the electrophoretic separation for SDS and poly-SUS at equal sample buffer concentrations (2% w/v). (SDS shown in orange; poly-SUS shown in blue).

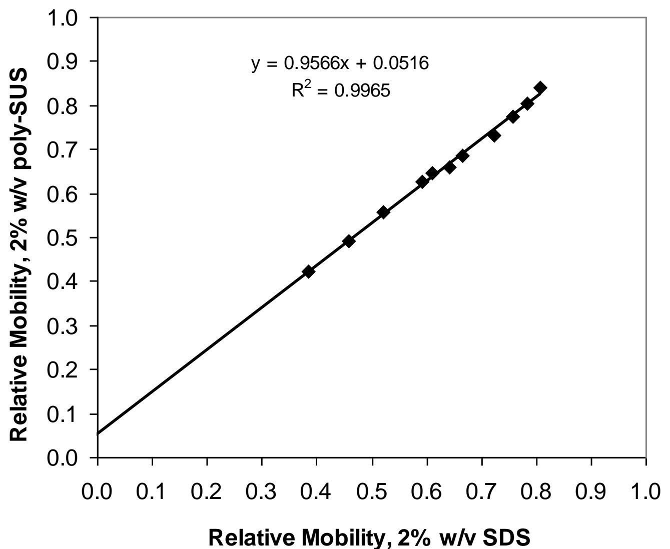


**Figure 2.10** Correlation of relative mobility data obtained from protein separations using SDS and poly-SUS (SB concentration = 2% w/v). The trend line was extended for emphasis.

2.10. At zero relative mobility for SDS, the poly-SUS relative mobility had reached 0.05 demonstrating that the poly-SUS/protein complex migrates faster.

### 2.3.4 Effect of Poly-SUS on Globular Protein Conformation

In general, it is accepted that the native protein structure is disrupted when binding occurs between proteins and surfactants. The nature of poly-SUS binding to proteins is of interest because of the ability of the molecular micelle to separate all eleven proteins in this study, and the general trend seen in the gels that the poly-SUS/protein complex migrated faster suggesting smaller size and greater overall negative charge compared to the SDS/protein complex. The latter point garners attention because of the longstanding ambiguity surrounding the common occurrence of anomalous protein migrations (particularly dimeric species and slow SDS/protein complex migration) in denaturing SDS-PAGE. To investigate this phenomenon, changes in the secondary structure of phosphorylase b, BSA, lysozyme, and α-lactalbumin in the absence and presence of poly-SUS and SDS were studied with circular dichroism. Phosphorylase b (an acidic protein, 97 kDa) was selected because it was the only protein in the complex mixture that SDS could not resolve. Three other representative proteins, BSA (an acidic protein, 66 kDa), lysozyme (a basic protein, 14.7 kDa) and α-lactalbumin (an acidic protein with structural homology with lysozyme, 14.2 kDa), also used in the complex mixture for the PAGE separations were selected.

Structurally, the alpha helix in the far UV (180-240 nm) is shown by a minimum at 222 nm and a minimum at 208 nm, which represent the $n \rightarrow \pi^*$ and the $\pi$ to $\pi^*$ electronic transition of the carbonyl on the peptide backbone, respectively. A greater predominant shift in the minimum at 208 nm is observed for poly-SUS relative to SDS in the presence of phosphorylase b for the

concentrations from 0 – 20 mM (Figure 2.11). In principle the CD spectrum of a protein is the sum of percentages of all possible secondary structural motifs (i.e. α–helix, β-sheet, β-turn, and random coil). Thus, as the 208 nm minimum increases with increasing concentrations of poly-SUS, the secondary structure of phosphorylase b approaches the random coil conformation.

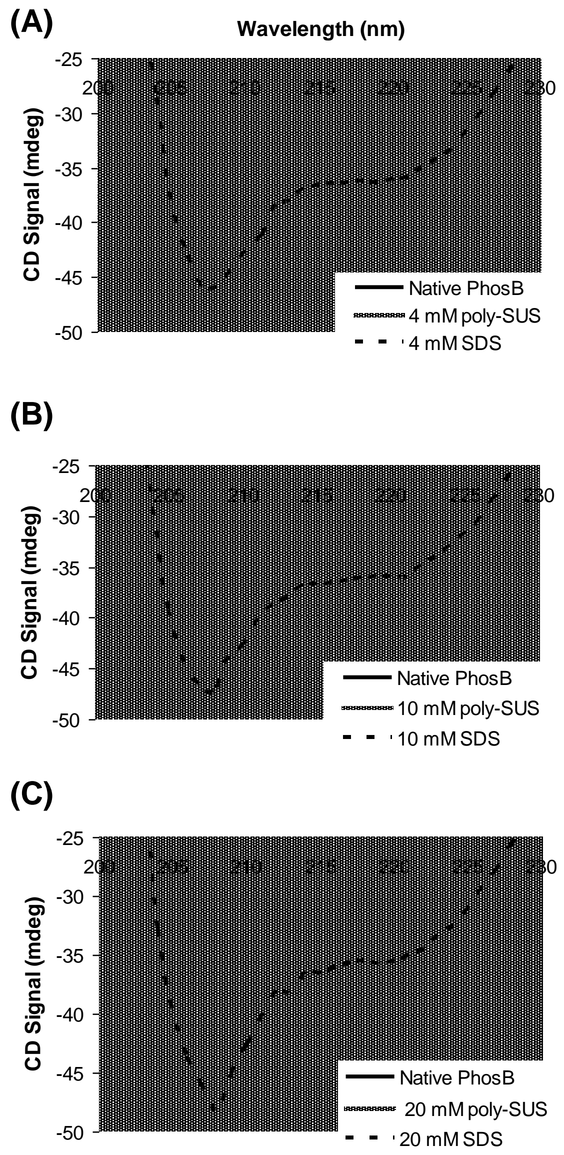


**Figure 2.11** Circular dichroism spectra of 0.7 mg/ml **p**hosphorylase b (solid line) in the presence of 4 mM (A) 10 mM (B),and 20 mM (C) poly-SUS (shaded line) and SDS (dashed line). The buffer used was 25 mM Tris/192 mM Glycine, pH 8.4 at 25 °C.

Further evidence for the poly-SUS-induced secondary structural change in phosphorylase b is presented in Figure 2.12. In the concentration range from 0-1 mM, the reduction in % α-helix is similar for poly-SUS and SDS. At concentrations greater than 4 mM, the critical micelle concentration of SDS in the Tris/Glycine buffer, a much greater reduction in % α-helix is observed for poly-SUS than SDS.

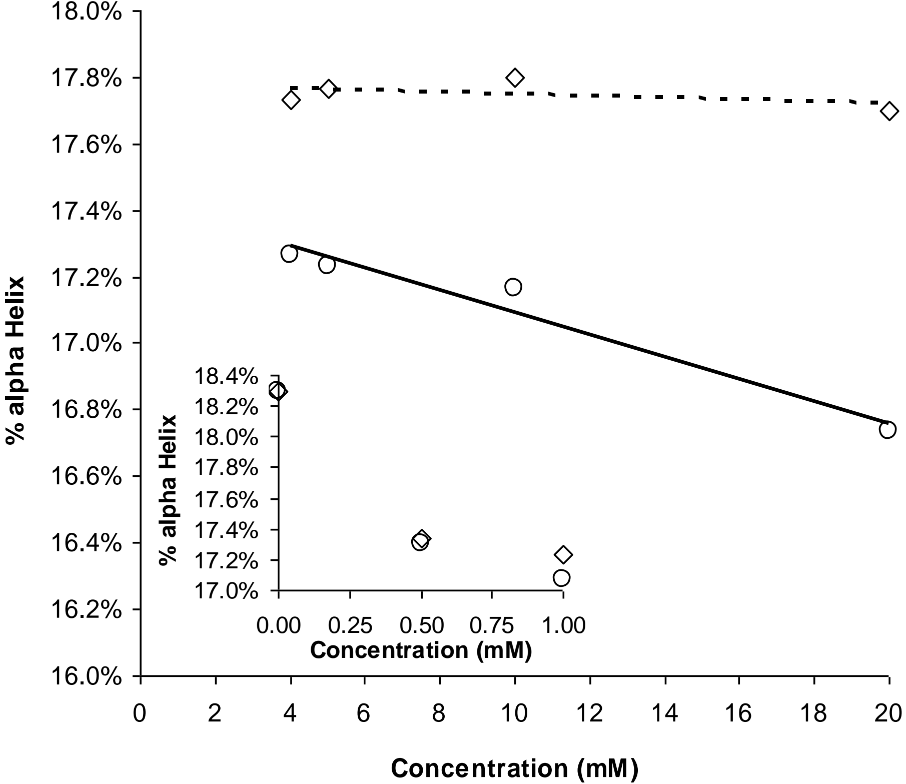


**Figure 2.12** Changes in secondary structure of phosphorylase b in the presence of 0 – 20 mM poly-SUS (open circles, solid line) and SDS (open diamonds, dotted line) in 25 mM Tris/192 mM Glycine, pH 8.4 at 25 °C.

Comparisons of the CD data for phosphorylase b, BSA, and α-lactalbumin, for differences between the poly-SUS and SDS signal at 208 nm, indicate a molecular weight dependence (Figure 2.13). These results demonstrate that as the molecular weight decreases, the molecular micelle, poly-SUS, binds more readily to the acidic proteins and induces more of a

change in the conformation compared to SDS. These results can be explained by the fact that at the buffer pH of 8.4 the acidic proteins have an overall negative charge where the poly-SUS aliphatic chain would interact hydrophobically with the hydrophobic amino acid residues. We believe that as the molecular weight of the acidic protein decreases the hydrophobic interaction dominates requiring low concentrations for unfolding due to the highly hydrophobic covalently bound core of the molecular micelle.

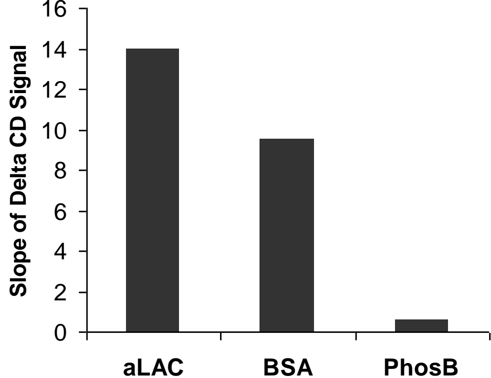


**Figure 2.13** Influence of protein molecular weight on the slope of the plot of CD difference between poly-SUS and SDS monitored at 208 nm versus concentration (0 – 20 mM).

For the CD studies, lysozyme was the only protein with an overall positive charge at the pH of the buffer. The circular dichroism data for the interactions with lysozyme showed that neither poly-SUS nor SDS affected the secondary structure over the 0 – 20 mM concentrations studied. We suppose that the time-scale of the electrostatic interaction of poly-SUS or SDS with lysozyme is much faster than the hydrophobic interaction and thus, prohibits the unfolding of the protein to any extent. This inference is consistent with the work by Arunachalam and Gautham where the results of dynamic simulations of 781 protein structures revealed two types of

structures, which included a large cluster forming the hydrophobic core believed to dictate the protein fold [46].

## 2.4 Conclusion

In this study, the novel use of the molecular micelle, poly (sodium *N*-undecanoyl sulfate) (poly-SUS), as the surfactant in polyacrylamide gel electrophoresis was investigated. Systematic investigations of the separation conditions were explored, in which the sample buffer (SB) concentration, running buffer (RB) concentration, RB pH, and SB temperature were varied. Eleven proteins with varying molecular weights (12 - 200 kDa), hydrophobicity, and isoelectric point were used. When poly-SUS was used as the surfactant in MoMi-PAGE, the optimum pH and SB temperature were determined to be 9.0 and 75°C, respectively. The results suggested that lower SB and RB concentrations may be possible as evidenced by comparable band identification over the entire range of RB (0.0125% - 0.053%) and SB (0.078% - 2.51%) concentrations used. The logarithm of the protein molecular weight was found to be inversely proportional to the relative mobility for MoMi-PAGE as with conventional SDS-PAGE. As compared to the standard Laemmli protocol for SDS-PAGE, a 26-fold reduction in SB concentration, an 8-fold reduction in RB concentration, and a 20-degree reduction in SB temperature were achieved. Examination of the circular dichroism data suggested that poly-SUS may be well suited for the separation of low molecular weight acidic proteins or peptides.

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## CHAPTER 3

## PROTEIN INTERACTIONS WITH A MOLECULAR MICELLE AND A CONVENTIONAL SURFACTANT STUDIED BY FLUORESCENCE SPECTROSCOPY AND CIRCULAR DICHROISM

### 3.1 Introduction

Intrinsically disordered proteins (IDPs) are a class of proteins that lack fixed order (i.e., natively disordered) in the full structural motif or in specific amino acid sequences under physiological conditions *in vitro*. Such protein disorder is atypical and was first predicted in 1997 by Romero, et al. [1] and subsequently confirmed experimentally by this group and others [2-5]. This discovery revolutionized long-held views about the structure of proteins, particularly the accepted wisdom that proteins only existed in three states (ordered, molten globule, and random coil). Furthermore, it challenged the idea that the three dimensional structured state is a prerequisite for protein function [6] consistent with the 'lock and key' [7] and the 'induced fit' [8] theories. More importantly, it has recently been suggested that 40% of the human proteome is partially or fully instrinsically disordered [9]. This rationalization is significant since many of these IDPs are associated with diseases [10,11]. In addition, the binding of IDPs to their targets rely heavily on hydrophobic interactions, as compared to proteins that are more globular [12]. Given the structural diversity of IDPs, a wide range of conformational propensities are expected, and as noted by Wright and Dyson [13], the characterization of these conformational states represent a major challenge. Therefore, investigations into IDP structure and binding events will certainly benefit from and more probably require the development of new analytical tools and experimental approaches.

Even as characterization of IDP interactions with their target partners remains a challenge, protein interactions with sodium dodecyl sulfate (SDS) continue to be a long-standing area of ambiguity. For example, in standard denaturing SDS-PAGE (polyacrylamide gel electrophoresis) protocol, a protein and SDS (2% w/v ≈ 69 mM) interact in the sample buffer. In addition, a disulfide reducing agent and melt temperature heating are introduced, producing changes in the secondary and tertiary protein structure. This conformational change is believed to allow SDS access to previously buried hydrophobic amino acid residues (i.e., disruption of the hydrophobic effect). In most cases SDS, binds to each protein in a relatively uniform stoichiometric ratio of 1.4 g SDS/g protein [14,15] at saturation. Subsequent studies have found that this ratio more accurately reflects a range of 1.5-2.0 g SDS/g protein for globular proteins [16] and up to 10 g SDS/g protein for membrane proteins [17]. During PAGE separation, the SDS-protein complex migrates toward the anode with a constant charge/mass ratio and sieves through the porous gel, with retention times roughly proportional to molecular weight. It is this generally accepted stoichiometry, where one SDS molecule associates with every two amino acid residues [18], seemingly independent of the protein sequence and structure, which stifles a complete understanding of SDS-protein complexation. Globular protein-SDS complexes and IDP-target interactions may seem to have little in common. However, it may be this kind of complexity in ordered and disordered protein interaction systems that will force us out of old paradigms into new avenues and ways of perceiving this ubiquitous type of phenomenon.

Recent investigations of protein-surfactant interactions have primarily focused on single- and double-chained surfactants, including cetyltrimethylammonium bromide (CTAB), SDS, bis(cetyldimethylammonium)butane dibromide, and 1,2-ethane bis(dimethyldodecylammonium bromide) among others [19-21]. These studies have almost always been restricted to single or

double chain monomeric species. However, to the best of our knowledge interactions of proteins with polymerized surfactants (in this work termed molecular micelles) have not been reported. We believe this issue is of practical interest and relevance to using molecular micelles as separation reagents in 2D gel electrophoresis for membrane proteins and as probes to further our understanding of their solubilization [22]. In this work, the interaction of five proteins in solution with a molecular micelle, poly (sodium *N*-undecanoyl sulfate) (poly-SUS), its monomeric species, SUS, and the conventional surfactant, SDS, have been surveyed.

Molecular micelles garner attention because of their unique physicochemical properties in comparison to those of single and double chain surfactants. The molecular micelle, poly-SUS, used in this study is an amphipathic molecule with an achiral hydrophilic head group and a covalently bound hydrophobic tail (Figure 1). In general, poly-SUS is formed by polymerizing the double bond at concentrations higher than the critical micelle concentration (CMC) using γ-irradiation. This concentration ensures that a spontaneous self-assembled phase exists. Subsequently, the dynamic equilibrium between the surfactant monomer and the micelle is largely eliminated after irradiation/polymerization. Therefore, molecular micelles do not have a CMC, and their overall stability is not compromised when interacting with proteins. The irradiation process imparts a unique morphology to the molecular micelle through formation of a covalently bound highly hydrophobic micro-domain. Such molecular micellar hydrophobicity is in stark contrast to conventional and second generation surfactants. Thus, it is expected that poly-SUS should have access to greater numbers of sites on proteins that are improbable as a result of the dynamic assembly and disassembly of a conventional micelle. As a result, we hypothesize that protein binding would be achieved at much lower concentrations using a molecular micelle than is achieved with a conventional surfactant such as SDS. Moreover, our

laboratory has demonstrated that interactions with molecular micelles provide superior separation schemes relative to conventional micelles due to their improved interactions 23-25]. Thus, the present work was undertaken to study the mechanism of interaction of five proteins with the achiral molecular micelle, poly-SUS, with the aim of providing a basis for the development of a new analytical tool for use in biochemistry and biotechnology.

## 3.2 Materials and Methods

### 3.2.1 Reagents

Serum albumin (bovine, 66 kDa, BSA), ovalbumin (egg, 45 kDa, OVA), α-chymotrypsinogen A (bovine pancreas, 26 kDa, aCHY), α-lactalbumin (bovine milk, 14.2 kDa, aLAC), cytochrome *c* (bovine heart, 12.3 kDa, CYTC), and 8-anilino-1-napthalenesulfonic acid (ANS) were obtained at the highest purity available from Sigma-Aldrich (St. Louis, MO, USA) and used as received. The protein molecular mass was verified by use of MALDI MS, and each protein was reconstituted into 1 mg/ml aliquots with 25 mM Tris/192 mM Glycine, pH 8.4 and stored at the required temperature. Selection of Tris/Glycine as the buffer was critical to understanding the potential application of poly-SUS in 1D gel electrophoresis. Ultrapure water (18.2 MΩ) was obtained using an Elga PURELAB Ultra water purifier (Lowell, MA, USA). SDS (>98%), Tris, and Glycine were obtained from Invitrogen Corporation (Carlsbad, CA, USA) and the Precision Plus Protein All Blue Standard marker was obtained from Bio-Rad Laboratories (Hercules, CA). Each was used as received without further purification.

### 3.2.2 Synthesis of the Molecular Micelle, Poly-SUS

The synthesis, characterization, and polymerization of poly-SUS and the monomer, SUS, were described in Chapter 2.

### 3.2.3 Instrumentation

Fluorescence spectra were recorded at 25 °C using a SPEX Fluorolog-3 spectrofluorimeter (Jobin Yvon, Edison, NJ) equipped with a 450-W xenon lamp and R928P photomultiplier tube (PMT) emission detector. A quartz cuvette with an optical pathlength of 1 cm was used, and bandwidths for both the excitation and emission monochromators were 3 nm unless otherwise stated. Excitation was performed at 295 nm (Trp) and 364 nm (ANS), while emission spectra were respectively measured in the ranges of 335 - 360 nm and 460 – 525 nm. Reported fluorescence spectra were obtained from proteins at concentrations of 1 mg/ml in 25 mM Tris/192 mM Glycine, pH 8.4 unless otherwise indicated. Circular dichroism data were obtained using an AVIV Model 62DS (AVIV Associates, Lakewood, N.J.) spectrophotometer at 25 °C fitted with a 1 mm pathlength quartz cell. CD spectra of native protein samples in 25 mM Tris/192 mM Glycine, pH 8.4 were acquired at concentrations that produced optimal CD signal. The Tris/Glycine buffer was filtered with a 0.45 µm nylon filter prior to sample preparation. All CD scans were done in triplicate in the far UV (200-240 nm) and near UV (240-320 nm) regions of the spectrum, respectively, and average spectra recorded. All CD spectra were corrected for background intensity of the buffer. CD response is reported as ellipticity and displayed in units of millidegree (mdeg).

### 3.2.4 Binding of Poly-SUS to Proteins

A spectrophotometric titration procedure was used to determine the characteristic binding parameters of poly-SUS, SUS, and SDS interacting with the five proteins employed in this study. The proteins (1 mg/ml) were allowed to equilibrate with a range of concentrations of poly-SUS, SUS, and SDS (0 – 20 mM) in 25 mM Tris/192 mM Glycine, pH 8.4 at 25 °C. The binding isotherms, stoichiometries, and dissociation constants were determined using Scatchard

Analysis using the relationship $\nu/S_f = Kn - K\nu$, where $K$ corresponds to the equilibrium binding constant, $n$ is the number of binding sites, $S_b$ is the bound surfactant concentration, $S_f$ is the free surfactant concentration, and $\nu$, which was determined from $S_b$[surfactant]/[protein]. The concentration of the bound poly-SUS was determined using the relationship, [poly-SUS]$(I - I_0)/(I_m - I_0)$, where $I_0$ is the fluorescence intensity of the protein in the absence of poly-SUS (or SUS, SDS), I is the fluorescence intensity when the protein and poly-SUS (or SUS, SDS) are in equilibrium, and $I_m$ is the fluorescence intensity when the protein is completely saturated with poly-SUS (or SUS, SDS). The concentration of free poly-SUS, [free poly-SUS], was determined by 1-[bound poly-SUS].

## 3.3 Results and Discussion

### 3.3.1 Intrinsic Fluorescence Spectroscopy

Intrinsic Trp fluorescence of the protein was used to determine the relative binding properties of the three ligands, poly-SUS, SUS, and SDS, to the five proteins. In biological systems where a ligand L binds to a receptor (macromolecule), scatchard analysis [26] of the data is typically used to determine the regions of binding in the isotherm, the binding constant for each region, and the number of ligand binding sites. Analyses of Scatchard plots reveal the nature of binding, particularly when multi-site ligand binding is suspected [27]. Generally, the binding isotherm displays four characteristic regions with increasing surfactant concentration: (1) specific binding to high energy sites on the protein, which are believed to be electrostatic, (2) noncooperative association, (3) cooperative binding as evidenced by a marked increase in binding and where protein unfolding is believed to occur, and (4) saturation in which no further binding takes place and micelles co-exist with the saturated protein [18, 28]. Moreover, distinct differences in binding, namely positive or negative cooperative binding of the ligand L (or

surfactant) to the target are obtainable [29]. For positive cooperative binding, the protein's affinity for additional surfactant molecules increases upon binding, which is facilitated by prior surfactant loading while in the latter case the protein's affinity for bound surfactant molecules decreases after initial interaction with the surfactant.

The intrinsic fluorescence of a protein, contributed by the aromatic amino acids of Trp, Tyr, and Phe, is often used to study peptides and proteins. Fluorescence is used most frequently because of its high selectivity and sensitivity, as well as low analyte consumption. In this work, shifts in the Trp emission intensity and emission wavelength maximum ($\lambda_{max}$) were monitored. These shifts are sensitive to exposure of Trp to different environments due to changes in protein conformation upon interaction with surfactants. A hypsochromic shift in $\lambda_{max}$ and an increase in emission intensity are indicative of the Trp residue moving into a more hydrophobic environment, while observation of a bathochromic shift in $\lambda_{max}$ and decrease in emission intensity suggests a more hydrophilic environment. The five proteins studied have intrinsic Trp residues in the native state, which are either buried in hydrophobic pockets or located toward the outer surfaces, as indicated in Table 1. Trp excitation at 295 nm was used to minimize excitation of tyrosine residues and subsequent heterotransfer to Trp. In the absence of poly-SUS, SUS or SDS, the intrinsic Trp fluorescence of BSA, OVA, aCHY, aLAC, and CYTC displayed typical emission maxima (i.e., $\lambda_{max}$) of 352 nm, 346 nm, 338 nm, 346 nm, and 371 nm, respectively (Figure 3.1). Increasing the concentrations of poly-SUS, SUS, or SDS in the presence of the five proteins resulted in various fluorescence emission responses and emission maxima shifts (Appendix A). The variability in emission responses was expected due to heterogeneity in the number of Trp residues and their locations in each native protein. For example, BSA and aCHY have two (1 solvent accessible, 1 buried) and eight (6 solvent accessible, 2 buried) Trp residues,

**Table 3.1** Physical properties of proteins studied.

| Protein | Accession # | MW (kDa) | Trp[a] | % α-helix[b] | % beta sheet[b] | Theoretical pI[c] | Theoretical Charge at pH 8.4[c] |
|---|---|---|---|---|---|---|---|
| cytochrome C (CYTC) | P62894 | 12.3 | 1 (1/0) | 40 | 1 | 9.5 | +6 |
| α-lactalbumin (aLAC) | P00711 | 14.7 | 4 (2/2) | 43 | 11 | 5 | -12 |
| α-chymotrypsinogen A (aCHY) | P00766 | 26 | 8 (6/2) | 14 | 32 | 8.2 | -2 |
| ovalbumin (OVA) | P01012 | 45 | 3 (1/2) | 32 | 32 | 5.3 | -16 |
| albumin, bovine serum (BSA) | P02768 | 66 | 2 (1/1) | 70 | - | 5.9 | -37 |

[a] Tryptophan (Trp) residues that are solvent accessible (sa) and buried (b) are indicated as (sa, b).
[b] The secondary structure data was obtained from http://www.pdb.org.
[c] The theoretical values were obtained from http://www.scripps.edu/~cdputnam/protcalc.html.

respectively. With increasing concentration of poly-SUS, the Trp emission of aCHY was gradually quenched and bathochromically shifted until a point of saturation was reached, where further increase in [poly-SUS] did not result in additional quenching or shifting of emission maxima. When aCHY was saturated with the ligand e.g., poly-SUS (≈ 1 mM), the emission wavelength maxima no longer red shifted and remained essentially constant. For BSA, we

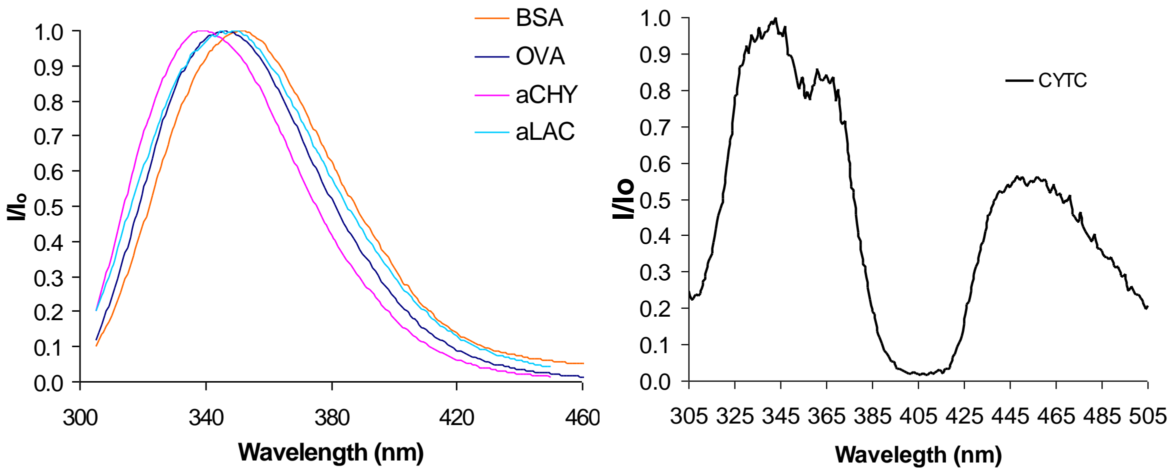


Figure 3.1 Intrinsic Trp fluorescence for BSA, aCHY, OVA, aLAC, and CYTC. The buffer used was 25 mM Tris/192 mM Glycine, pH 8.4 at 25 °C.

observed quenching of the Trp emission and an initial hypsochromic shift followed by a continual bathochromic shift in emission wavelength maxima with increasing poly-SUS (SUS, and SDS) concentration. Similarly, saturation of BSA with poly-SUS (SUS, and SDS) coincided with no further Trp quenching or red shifting in the emission wavelength maxima (Figure 3.2). The bathochromic shift and quenching (data not shown) in the Trp emission maxima of aCHY and BSA with increasing concentration of the ligand are attributed to changes in the native

conformation of aCHY [30] and BSA [20]. Observation of such a conformational change induced by the binding of ligand to the protein suggests a hydrophilic microdomain in which the Trp residues could reside.

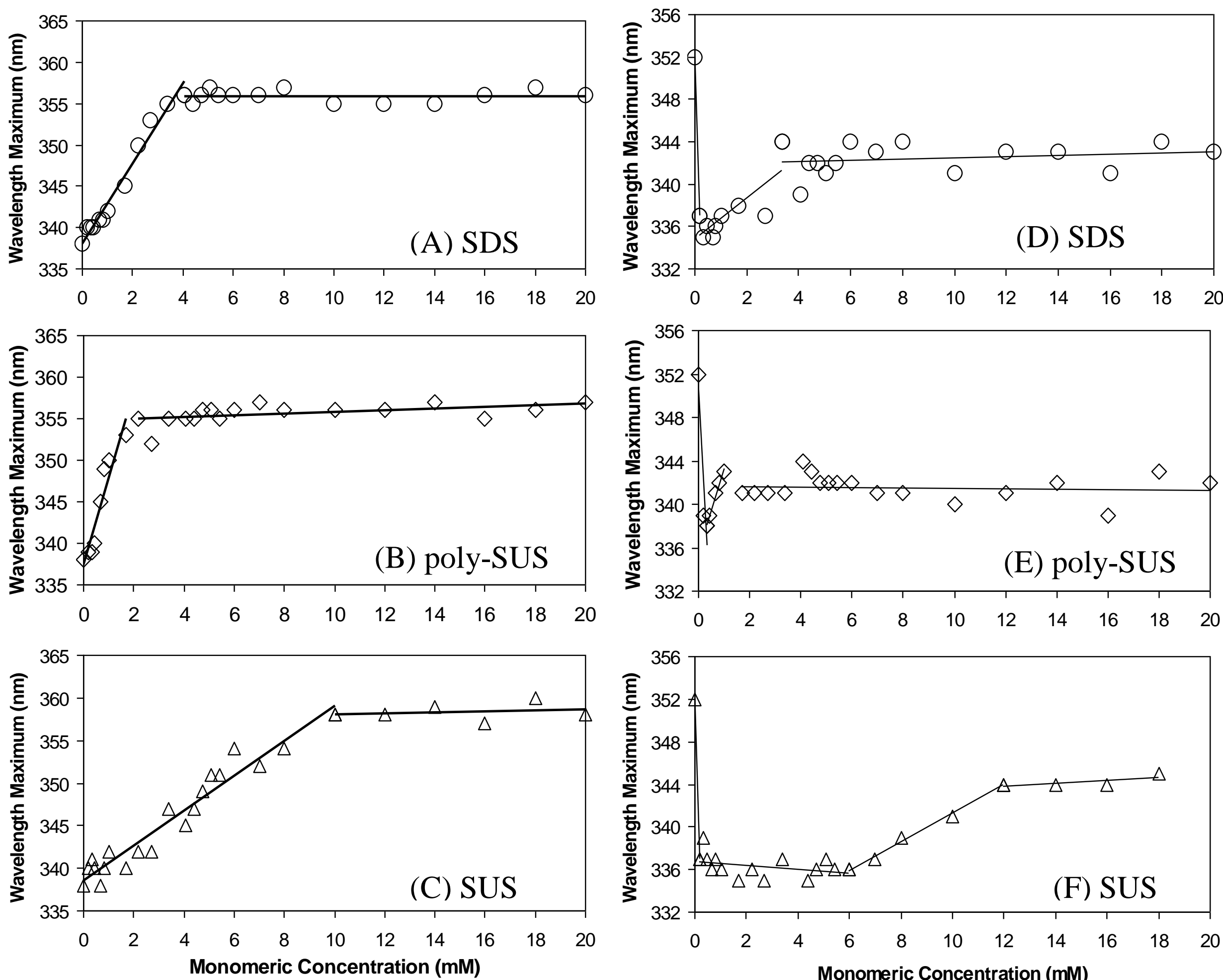


**Figure 3.2** Fluorescence wavelength maxima shifts of $Trp_{aCHY}$ and $Trp_{BSA}$ in the presence of increasing monomeric concentration (0 – 20 mM) of SDS (open circles), poly-SUS (open diamonds), and SUS (open triangles) in association with aCHY (38 μM) and BSA (15 μM), respectively, determined by steady state fluorescence ($\lambda_{ex}$ = 295 nm, 25°C). The lines have been included to guide the eye.

In the lower concentration regime (i.e., 0 < [ligand] < 1.0 mM), it was observed that binding of poly-SUS to all of the proteins occurred much faster than with SDS or SUS. Interestingly, for BSA at concentrations between 0 and 0.8 mM, the slope of the fraction bound curve (*see inset of* Figure 3) for poly-SUS is observed to be 8.4 ($\approx 2^3$) and 16.6 ($\approx 2^4$) times that for SDS and SUS, respectively, suggesting that poly-SUS binding to BSA is immediate and

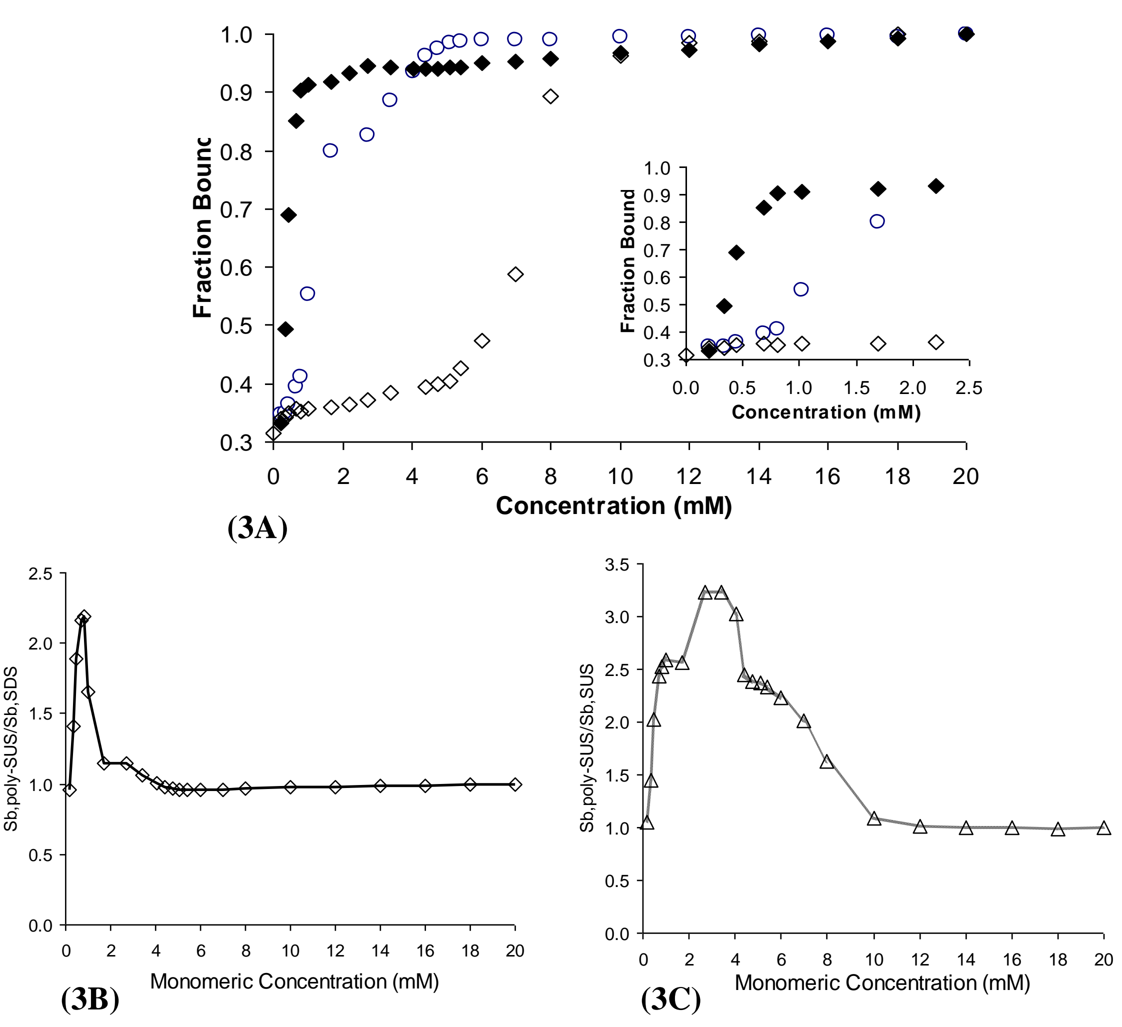


**Figure 3.3** (A) Fraction bound of poly-SUS (solid diamonds), SUS (open diamonds), and SDS (open circles) to BSA (15 μM) with increasing monomeric surfactant concentration (0 – 20 mM).(B) Ratio of poly-SUS bound to SDS bound for BSA. (C) Ratio of poly-SUS bound to SUS bound for BSA

rapid. Thus, a surfactant with one more methylene group (SDS) binds BSA twice as fast as a surfactant with one less carbon (SUS), while overall binding is exponentially greater when the terminal double bond of the monomer is polymerized (poly-SUS). At concentrations as low as 0.8 mM, the fraction of poly-SUS bound to BSA reached 90% while it required five times more SDS and twelve times more SUS to reach the same bound fraction (Figure 3). Moreover, a similar trend was observed for the other four proteins in that the slope of the fraction bound curve at low concentrations ([poly-SUS] < 1.0 mM) was greater for poly-SUS than SDS or SUS (Table 3.2). To understand this significantly better binding of poly-SUS to BSA relative to SDS and SUS, a new plot was constructed (Figure 3). This plot revealed that between 0 and 0.8 mM the amount of poly-SUS bound to BSA exceeded two and three times that of SDS and SUS, respectively. While the same amount of SDS binding to BSA could not be attained until the concentration of SDS approached the CMC (~4.7 mM, determined experimentally by tensiometry) [31], a similar phenomenon was observed for the other conventional surfactant, SUS. Furthermore, we believe that the better performance of poly-SUS as compared to the other two surfactants is due to the absence of any transition from a monomeric species to a low-

**Table 3.2** Comparison of Slopes of Fraction Bound vs Concentration (< 1.0 mM)

| Protein | $m_{poly\text{-}SUS}/m_{SUS}$ | $m_{poly\text{-}SUS}/m_{SDS}$ | $m_{SDS}/m_{SUS}$ |
|---|---|---|---|
| cytochrome C (CYTC) | 4.6 | 1.4 | 3.2 |
| α-lactalbumin (aLAC) | 51 | 1.2 | 41 |
| α-chymotrypsinogen A (aCHY) | 2.1 | 1.4 | 1.5 |
| albumin, bovine serum (BSA) | 16.6 ($\approx 2^4$) | 8.4 ($\approx 2^3$) | 1.97 ($\approx 2^1$) |

aggregated state followed by a full micellar state to saturate the proteins [20]. Poly-SUS followed an "all or nothing" binding mechanism, wherein it was either unbound or fully bound over small changes in concentration spending little time in partially bound intermediate states [32], presumably due to polymerization of the terminal double bond. We believe these differences in observed protein binding represent an important step towards understanding the hydrophobic effect.

For the number of binding sites, values of 24 and 5 were found on BSA for poly-SUS and SDS, respectively. Although the number of SDS binding sites is in agreement with previous reports [33,34], it is significantly lower than the number of binding sites found for poly-SUS. The large number of poly-SUS binding sites on BSA offers an explanation for the efficiency with which poly-SUS was observed to interact with BSA (reaching 90% fraction bound at concentrations as low as 0.8 mM). In addition, the amount of poly-SUS bound to BSA as compared to SDS at concentrations $< 1.0$ mM may be explained by the large number of sites made available for binding by poly-SUS-induced conformational changes to BSA (see section entitled Effect of Poly-SUS on Protein Denaturation: Circular Dichroism Studies). Further, Scatchard analysis of the data revealed an intriguing mechanism of binding for poly-SUS to BSA that is in direct opposition to what has been offered in the literature for conventional surfactants. First, the binding constant for poly-SUS in the low concentration regime ($0.2 <$ [poly-SUS] $< 0.8$ mM) was $1.3 \times 10^4$ $M^{-1}$, which is two orders of magnitude greater than the observed value for SDS in the same range. Such a large binding constant is generally observed in the third region of the binding isotherm, cooperative binding, where massive increases in ligand binding [i.e., high K ($M^{-1}$)] to the protein (or target) occur. Second, the Scatchard plot in this concentration range was curvilinear with concavity pointed downward (concave), which is indicative of positive

cooperative binding. In this situation, the binding of poly-SUS to one site on BSA increases the poly-SUS binding affinity to subsequent sites on BSA. In contrast, with SDS, the Scatchard plot was concave upward (convex) in this concentration range indicating negative cooperative binding. Typically observed in this low concentration region, negative cooperative binding occurs when the surfactant monomer binds to a site on the protein with no allosteric effects present to facilitate additional ligand binding. Thus, SDS binding is highly specific in the low concentration regime (0.2 < [poly-SUS] < 0.8 mM). In addition, positive cooperative binding of poly-SUS was observed up to 2.7 mM, followed by negative cooperative binding suggesting that below this concentration BSA became saturated with poly-SUS and no additional binding occurred. The binding of SDS agreed with the isotherm observed by Takeda et al. (1981) who used conductometric and chromatographic methods to study the binding events for SDS associating with BSA [35]. The highest binding constant for SDS was calculated to be 4.5 X $10^4$ $M^{-1}$ in region III (4.1 – 5.4 mM) where the onset of SDS micellization occurs. By comparison of the cooperativity profiles for all five proteins (Table 3.3), it is apparent that positive cooperativity dominated for poly-SUS in the low concentration region (0 < [poly-SUS] < 1.0 mM). This observation suggests maximum molecular micelle complexation with each biopolymer. The positive cooperative binding to all five proteins in this concentration range suggests that poly-SUS binding to the biomacromolecules studied is independent of protein size and charge. We attribute this phenomenon to the rigidity (absence of micelle assembly and disassembly due to the covalently bound micellar structure), flexibility (ability to adopt different conformations), and hydrophobicity (large hydrophobic microdomain) that exists in poly-SUS, giving it access to more binding sites and thus, promotion of positive allosteric modulation. Also, an increase in positive cooperativity at such low concentrations may be exploited as a

result of the greater number of anionic head groups on the polymerized molecular micelle compared to its monomeric species. Taken together, results from this intrinsic fluorescence data demonstrate that poly-SUS binds to the biomacromolecules studied more rapidly than SDS and SUS, at much

**Table 3.3** Cooperative binding profiles for poly-SUS, SUS and SDS (0-20 mM) and BSA, aCHY, CYTC, aLAC, and OVA

| | | BSA | OVA | aCHY | aLAC | CYTC |
|---|---|---|---|---|---|---|
| Region 1 | poly-SUS | + | + | + | + | + |
| | SUS | + | - | + | - | + |
| | SDS | - | - | + | - | + |
| Region 2 | poly-SUS | + | - | - | - | - |
| | SUS | o | - | - | - | - |
| | SDS | o | - | - | - | - |
| Region 3 | poly-SUS | - | + | - | o | - |
| | SUS | - | - | - | - | - |
| | SDS | + | - | + | - | - |
| Region 4 | poly-SUS | - | - | - | + | - |
| | SUS | - | - | - | - | - |
| | SDS | - | - | - | - | - |

\+ positive cooperative binding, - negative cooperative binding, O noncooperative binding

lower concentrations (<1.0 mM) than SDS (> 4.1 mM) and SUS (> 10 mM), (3) in larger quantities than SDS and SUS, (4) in a positive cooperative manner, and (5) through a primarily hydrophobic interaction under the experimental conditions explored.

### 3.3.2 Extrinsic Fluorescence Spectroscopy

In addition to intrinsic fluorescence spectroscopy, the extrinsic probe, 8-anilino-1-naphthalene-sulfonate (ANS), has been used to monitor surfactant saturation concentrations and surfactant-induced unfolding of the five proteins. Fluorescence data were collected for excitation wavelengths of 295 nm and 364 nm representing Trp and ANS, respectively. ANS is both hydrophobic, i.e. containing a naphthalene moiety and hydrophilic, i.e. possessing an anionic sulfonate head group. At pH 8.4, four of the proteins had a net negative charge, while one protein had a net positive charge (Table 1); therefore, the interaction of ANS with the four acidic proteins was expected to be primarily hydrophobic although electrostatic contacts were possible, but to a lesser extent. In addition, we concluded that the interaction between the acidic proteins and poly-SUS, SDS, and SUS would be primarily hydrophobic due to the hydrophobicity of the core of poly-SUS (or hydrophobicity of the aliphatic chain in the conventional micelle). As poly-SUS (or SDS or SUS) was titrated into the cuvette and allowed to equilibrate, we envisaged ANS amphipathic contacts would be replaced by poly-SUS amphipathic contacts on the proteins. When the protein-surfactant interaction was complete, we supposed that ANS would be found either in the hydrophobic core of the molecular micelle, poly-SUS, in the bulk aqueous environment due to its hydrophilicity, or sterically situated between the two environments. It is well known that the fluorescence emission of ANS increases in the presence of an apolar environment such as the hydrophobic patches within proteins. As expected, an increase in ANS emission in the presence of each protein due to hydrophobic interaction and subsequent energy transfer from Trp was observed although the greatest intensity increase resulted from the interaction with BSA (Figure 3.4). Efficient resonance energy transfer from intrinsic Trp residues in BSA to bound ANS suggests that these

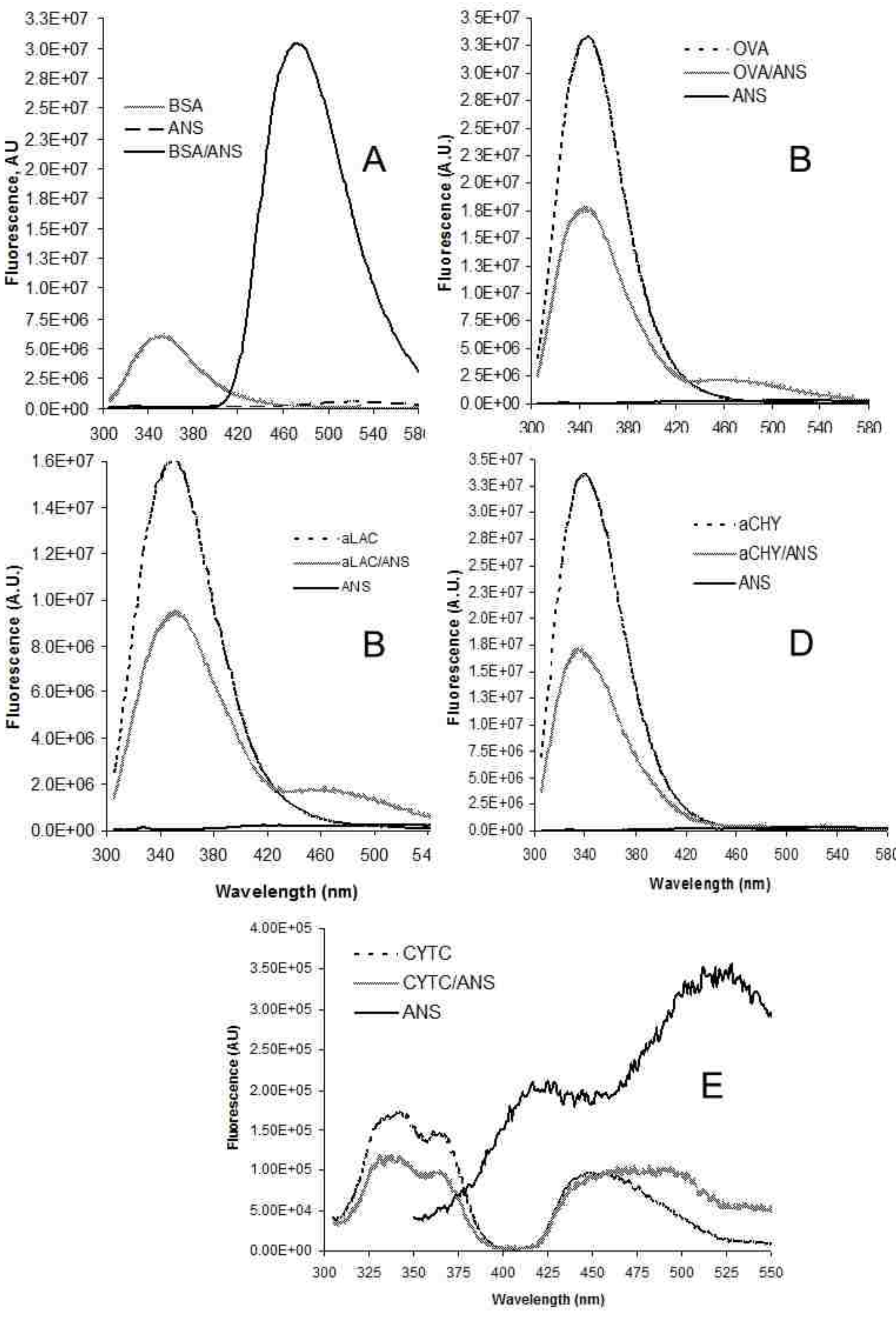


**Figure 3.4** Energy transfer from (A) BSA, (B) OVA, (C) aLAC, (D) aCHY, (E) CYTC to ANS (dark line).

two molecules were in close proximity (< 10 nm). Little energy transfer from $Trp_{aCHY}$ to ANS was observed. For CYTC, the ANS emission decreased presumably due to the more favorable heterotransfer from Trp to the intrinsic heme group [36] in lieu of energy transfer to ANS. Also, a greater increase in ANS emission in the presence of poly-SUS over SDS was observed suggesting that poly-SUS is significantly more hydrophobic than SDS (Figure 3.5).

Titration of the sample containing 1.0 mg/ml protein and 20 µM ANS with increasing concentrations of poly-SUS (0 – 6 mM) resulted in decreased ANS emission, as it was displaced from its non-covalent contact with the protein by the molecular micelle due to greater hydrophobicity of poly-SUS. In addition, the ANS emission maximum red shifted indicating

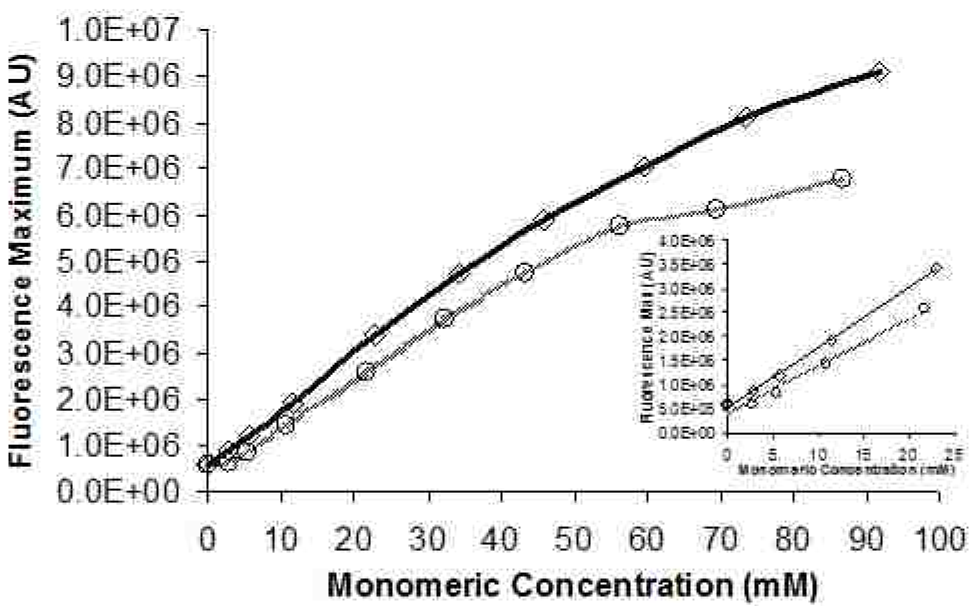


**Figure 3.5** Relative hydrophobicity of poly-SUS compared with SDS determined by titrating increasing concentrations of poly-SUS (open diamonds) and SDS (open circles) into buffered ANS. [ANS] = 20 µM. [poly-SUS or SDS] = 0 – 100 mM on a monomeric basis. Inset (0 – 25 mM): $R^2_{poly\text{-}SUS} = 0.9984$. $R^2_{SDS} = 0.9835$.

ANS was moving to a more hydrophilic environment. When ANS was completely displaced from the protein and found a new thermodynamically stable state solubilized in what we suspect is the hydrophobic molecular micelle, poly-SUS, the ANS emission increased. If ANS was released into the aqueous phase and did not interact with poly-SUS, its emission would have been essentially quenched which did not occur. The concentration of poly-SUS coinciding with complete ANS displacement was believed to be the saturation concentration of poly-SUS, interacting with the protein since excess poly-SUS was then available for hydrophobic interaction with ANS. Similar trends in emission maxima were observed for increasing concentrations of SUS and SDS in the presence of all of the proteins (BSA, aLAC, OVA, and aCHY) and ANS. The saturation concentrations determined by extrinsic fluorescence spectroscopy (Figure 4) were consistent with the values observed when monitoring the intrinsic Trp fluorescence of the proteins studied. For example, the saturation concentration of poly-SUS, SUS, and SDS for interaction with aLAC was observed to be 1.1 mM, 2.9 mM, and 4.1 mM, respectively using extrinsic fluorescence which is close to the values obtained using intrinsic fluorescence (1.0 mM, 2.9 mM, and 4.4 mM, for poly-SUS, SUS, and SDS respectively). Thus, it was confirmed that poly-SUS binds to aLAC at concentrations at least four times less than SDS. When taken in aggregate, analyses of the results using the extrinsic reporter molecule indicate binding of poly-SUS to the proteins studied is primarily hydrophobic.

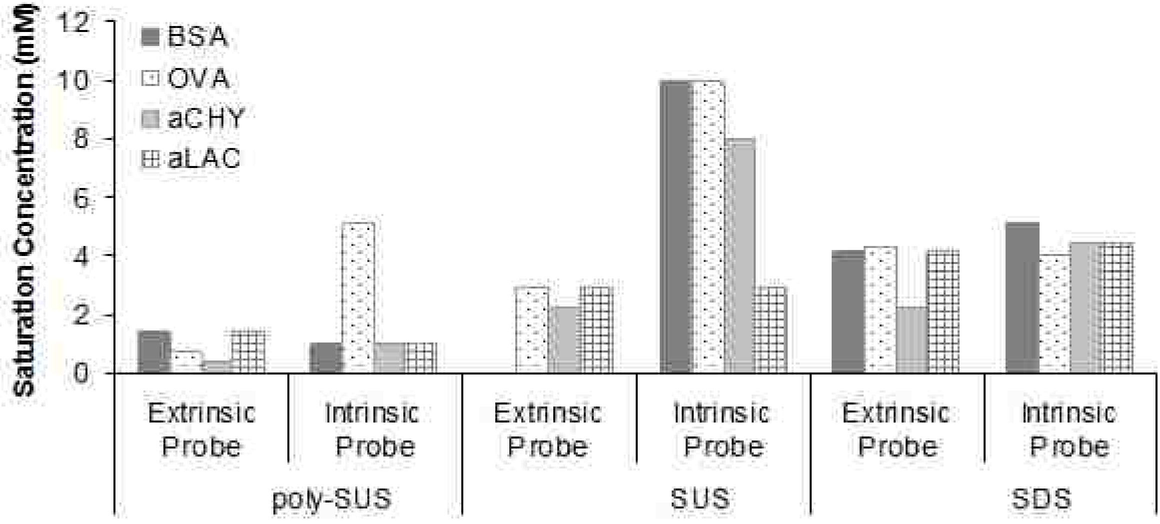


**Figure 3.6** Comparison of saturation concentrations (mM) for poly-SUS, SUS, and SDS associating with the proteins, BSA, OVA, aCHY, and aLAC (1 mg/ml), determined from intrinsic and extrinsic fluorescence spectroscopy. Saturation was not attained for SUS/BSA using the extrinsic probe; therefore, no concentration is indicated.

### 3.3.3 Protein Denaturation Monitored by Circular Dichroism

To evaluate the effect of poly-SUS on protein conformation, circular dichroism measurements were used to monitor changes in the secondary structure. Far UV CD spectroscopy is the characteristic region of the electromagnetic spectrum where secondary structure transitions in proteins are gauged. In this region, the peptide backbone is the chromophore; a protein that is primarily α-helical in structure exhibits two negative bands at 208 nm and 222 nm. Alterations in the far UV spectra for aLAC in the absence and presence of poly-SUS and SDS are shown in Fig. S4. A greater predominant shift in the minimum at 208 nm is observed for poly-SUS relative to SDS in the presence of aLAC at a concentration of ≈ 4 mM (or 0.12% w/v). No

appreciable differences in CD spectra were observed for poly-SUS and SDS at ≈ 2.9 mM (or 0.08 %w/v). In principle the CD spectrum of a protein is the sum of percentages of all possible secondary structural motifs (i.e., α–helix, β-sheet, β-turn, and random coil). Thus, as the 208 nm minimum ($-\theta_{208nm}$) increases with increasing concentration of poly-SUS, aLAC begins to unfold. The shift in the CD signal for the 208 nm minimum at ≈ 4 mM (or 0.12 %w/v) suggests a transition in aLAC conformation toward random coil in the presence of poly-SUS. Further evidence for the poly-SUS-induced secondary structural change in aLAC was observed upon addition of greater amounts of poly-SUS resulting in dramatic shifts in the minimum at 208 nm relative to SDS (data not shown). In addition, when binding to BSA in either the native or reduced states, poly-SUS perturbs the secondary structure causing significant unfolding (Figure 5, *A-D*) as compared to SDS. To further probe the concentration effect of poly-SUS on the secondary structure change in BSA, we have monitored the change in ellipticity at 208 nm. In the concentration range from 0 - 25 mM the ellipticity at 208 nm ($-\theta_{208nm}$) for poly-SUS increased dramatically compared to SDS (Figure 3.9) suggesting decreased α-helical content and significant unfolding of BSA. Though an increase in ellipticity was observed at 222 nm, the trend was not as pronounced as at 208nm. Over the concentration range studied, SDS did not appreciably change the conformation of BSA. From these data one can deduce that poly-SUS overcomes the low dielectric constant of the hydrophobic interior [37] to induce major conformational changes, which exponentially increases the capacity of poly-SUS to bind to the interior sites complementing its ability to bind the solvent accessible hydrophobic regions.

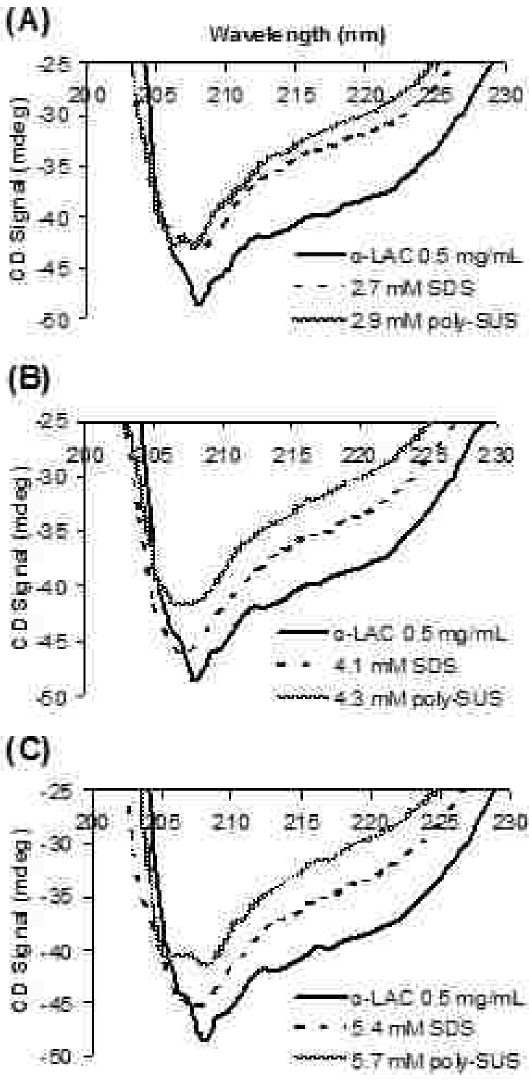


**Figure 3.7** Far-UV CD spectra of 0.5 mg/ml native aLAC (dark solid line) in the presence of (A) 0.078 %w/v poly-SUS (solid line) and SDS (dotted line), (B) 0.117 %w/v (solid line) and SDS (dotted line), and (C) 0.156% w/v poly-SUS (solid line) and SDS (dotted line) as indicated. The buffer was 25 mM Tris/192 mM Glycine at pH 8.4 and 25 °C. A 1 mm pathlength quartz cuvette was used.

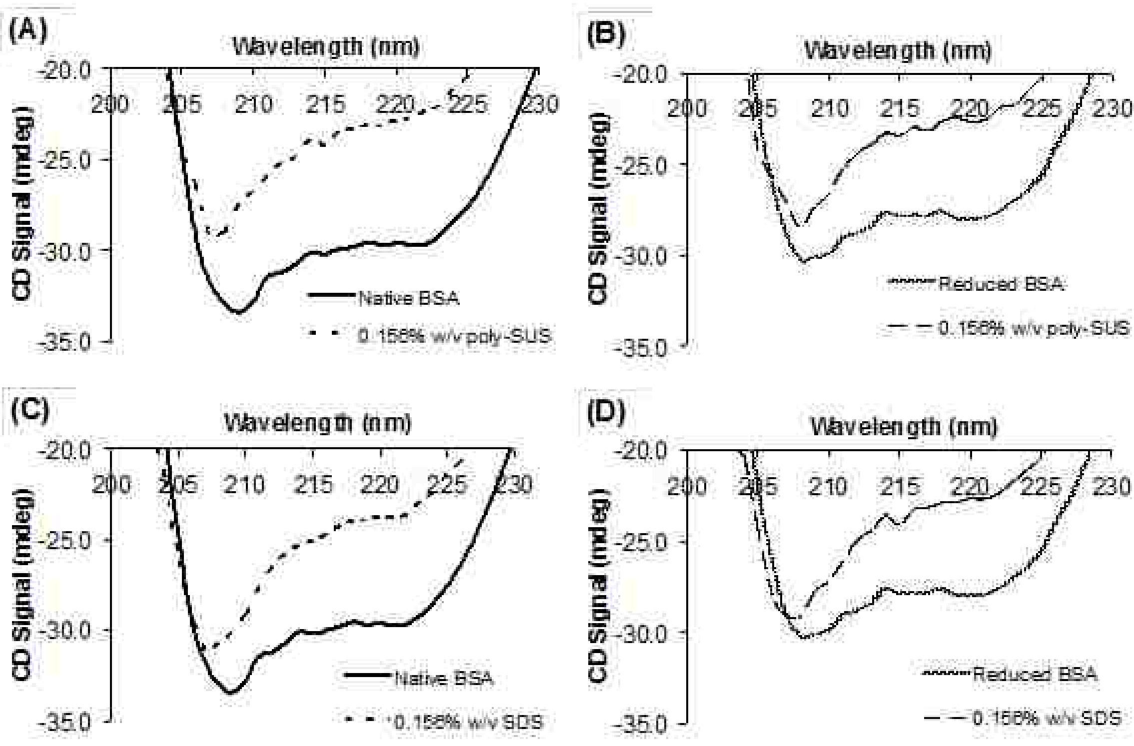


**Figure 3.8** Changes in secondary structure of BSA in the native (A) & (C) and reduced (B) & (D) states in the presence of 0.156 % w/v poly-SUS and 0.156 % w/v SDS as indicated. The buffer was 25 mM Tris/192 mM Glycine at pH 8.4 and 25° C. A 1 mm pathlength quartz cuvette was used.

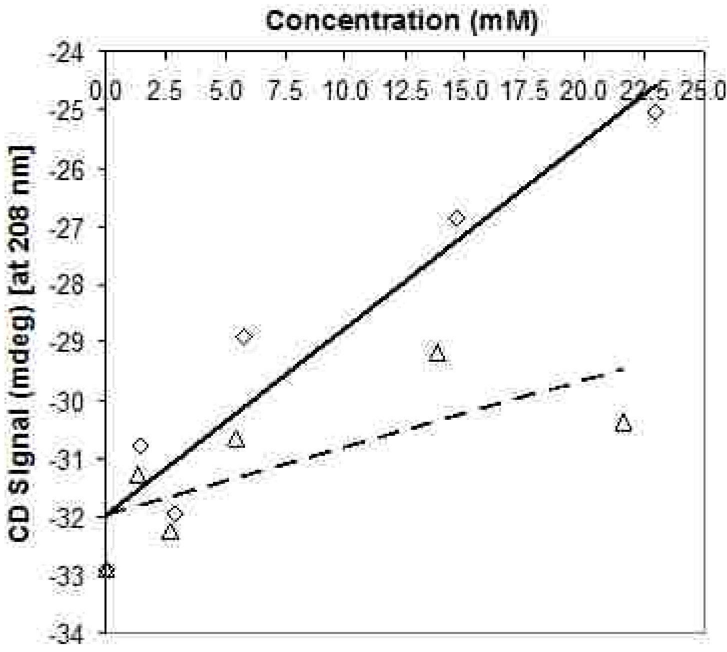


**Figure 3.9** CD Signal monitored at 208 nm for poly-SUS (diamonds; solid line) and for SDS (triangles; dotted line) bound to BSA (0.2 mg/ml) with increasing monomeric concentration (0 – 25 mM). The solid and dotted lines were included to guide the eye.

Summarizing thus far, the present work emphasizes the critical interactions that contribute to the binding mechanism involving a molecular micelle and five proteins with the aim of contributing to the growing need for new and enhanced analytical tools for biochemistry and biotechnology. By intrinsic and extrinsic fluorescence spectroscopy, we have determined that the molecular micelle, poly-SUS, binds the proteins studied at lower concentrations than SUS and SDS exhibiting a positive cooperative binding mechanism. Further, our circular dichroism results show that poly-SUS disrupts the secondary structure of BSA and aLAC at low concentrations, which is in stark contrast to SDS.

### 3.3.4 Comparison of Poly-SUS and SDS in PAGE

We postulated that poly-SUS would perform superior to SDS at low concentrations in polyacrylamide gel electrophoresis due to greater hydrophobic interactions and greater binding constants observed from our spectroscopic data. We chose the His-tagged protein, p53, which contains long regions of unordered structure [39] as the analyte of interest. This protein was selected because it is a major target for anticancer therapy due to mutations that alter its ability to contribute to tumor suppression [40,41]. Facile detection of poly-SUS, SUS, and SDS binding efficiency to p53 was evaluated in the porous gel medium. Representative PAGE-SDS and PAGE-poly-SUS separations are shown in Figure 6. Migration of p53 through the gel was influenced by the ligand binding efficiency as judged by the location of the band in the gel. In a gradient gel, which was used here, the stacking region of the gel contains large pores that are capable of accommodating large aggregates whereas the resolving region of the gel contains progressively smaller pores as the gel is traversed. It was observed (Figure 6, *A*) that at a loading of 0.08% w/v (2.9 mM) SDS in the sample buffer a trail of p53 was essentially smeared down the lane presumably due to a lack of binding with SDS. We suppose that the water molecules that solvate the hydrophilic residues on the protein surface began interacting with the hydrophilic head groups of the SDS monomers and micelles causing a reduction in protein-protein repulsive forces and subsequent aggregation or precipitation of p53. The SUS monomer separation exhibited bands that were diffuse (Figure 3.11). Examination of this gel suggests lower protein binding with the addition of one degree of unsaturation in the molecule, which is consistent with the intrinsic fluorescence results. From the work by Sprague et al. [41], we know that the presence of unsaturation originating from the terminal double bond in SUS increases the CMC by a factor of two over its saturated counterpart. Thus, micellar species with widely differing

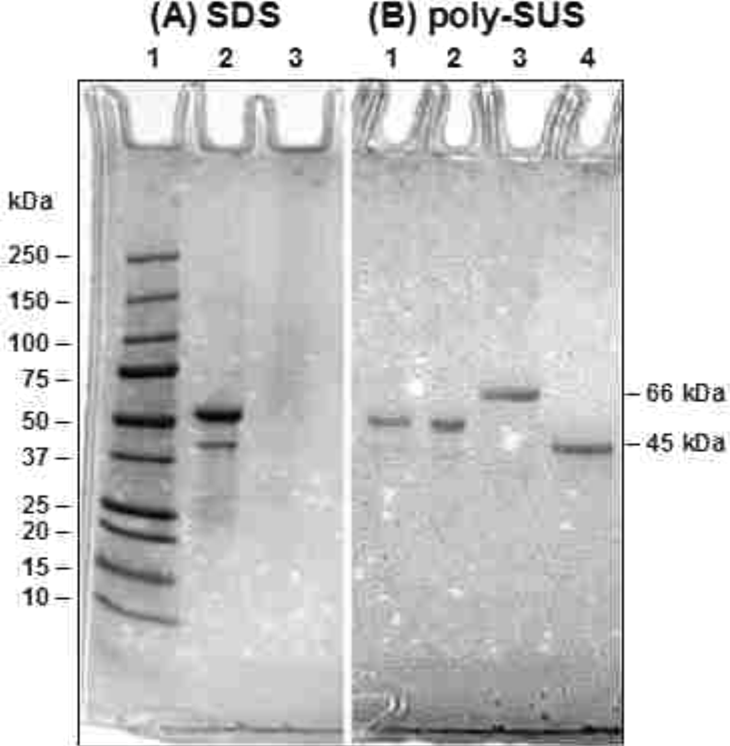


**Figure 3.10** PAGE of p53 on a 4-20% gradient gel using (A) the conventional surfactant, SDS, and (B) the molecular micelle, poly-SUS. (A) SDS: lane 1, precision plus molecular weight marker (MWM, 10-250 kDa); lane 2, 2% w/v SDS; lane 3, 0.08% w/v SDS. (B) poly-SUS: lane 1, 2% w/v poly-SUS; lane 2, 0.08% w/v poly-SUS; lane 3, BSA standard and 2% w/v poly-SUS; lane 4, OVA standard and 2% w/v poly-SUS. Lanes 3 and 4 were used as markers to identify the approximate molecular weight of p53 in the poly-SUS separation.

aggregation numbers interacting with the protein may contribute to the diffuse bands and streaking. Conversely, poly-SUS binding to p53 is highly efficient down to concentrations in the sample buffer as low as 0.08% w/v (2.9 mM) as observed in Figure 6, *B*. Thus, it was concluded from these data, as predicted by our fluorescence spectroscopy results, that poly-SUS has robust association with water soluble proteins like p53 that is attainable at concentrations as high as 2% w/v and as low as 0.08% w/v. This robustness is attributed to poly-SUS being polymerized,

having conformational flexibility, and having highly hydrophobic microdomains providing it with access to sites on the protein that are not attainable with a conventional micelle. Collectively, these PAGE separations are consistent with our hypothesis that poly-SUS at low concentrations would exhibit greater binding efficiency and separation effectiveness in PAGE compared with SDS.

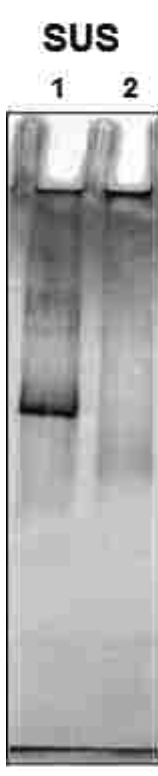


**Figure 3.11** SUS monomer gel electrophoresis separation of p53 at 2% w/v and 0.08% w/v of SUS in lanes 1 and 2, respectively. At the higher concentration (2% w/v) multiple diffuse bands are evident. Note p53 left in well at both SUS concentrations suggesting low to no binding of SUS with p53.

### 3.4 Model Proposal

On the basis of our measurements, a model is proposed that may explain the stronger interactions of poly-SUS with the biomacromolecules at much lower concentrations than with

SDS. Moreover, we offer this model as a basis for theorizing how molecular micelles may contribute to our understanding of protein binding to SDS.

In general, a protein maintains a stable conformational state because of its intrinsic hydrophobic interactions (e.g., the hydrophobic effect), and becomes unstable resulting in aggregation when the hydrophobic patches are exposed to a polar solvent such as water. As the molecular micelle equilibrates into the lowest entropic state we expect no alkyl portion of the structure of poly-SUS to interfere with electrostatic interactions. Further, that the cationic amino acid residues are bound more tightly to the anionic hydrophilic moiety resulting in a much stronger electrostatic interaction than with SDS. Though it is generally accepted that surfactants bind only the hydrophobic regions of proteins, the positively charged amino acids may facilitate the positive cooperative binding indicated by the Scatchard plots for poly-SUS at concentrations below 1.0 mM. Likewise, energetically favorable hydrophobic interactions may result when hydrophobic regions of a protein are initially exposed to a polar environment such that the flexible conformations of and multiple hydrophobic regions of poly-SUS specifically and immediately bind to the protein. Furthermore, we propose that the onset of these interactions (both hydrophobic and electrostatic) is dependent on the thermodynamic favorability of the interaction between poly-SUS and the biomacromolecules. Because of its flexibility, poly-SUS is expected to adopt conformations that essentially allow it to incorporate into the structural motif of the protein. However, in doing so not all interactions are thermodynamically favorable resulting in secondary structure rearrangements in the protein until a stabilized state is reached. This new stable state is then thermodynamically favorable for quantitatively increased efficiency and greater binding with poly-SUS. We suppose that this secondary rearrangement and ligand-to-protein binding occurs at a rate wherein there is an intimate time-scale link between

electrostatic and hydrophobic binding. Moreover, the hydrophobic specificity and affinity exhibited by poly-SUS toward the proteins provide a basis for furthering our understanding of SDS binding to proteins. It is well known that for SDS larger numbers of micellar species (i.e., higher concentrations) must exist before the protein binding capacity reaches saturation [20, 42]. This suggests that although there are electrostatic interactions that probably occur the efficiency of binding increases dramatically only when the hydrophobicity of the micellar species increases (i.e., increased aggregation numbers). In contrast, the saturation binding capacity of poly-SUS to proteins is exponentially increased by simply polymerizing the terminal double bond, thereby eliminating the dynamic assembly and disassembly process and creating a highly hydrophobic microdomain. Thus, the need for increased hydrophobic micellar species to facilitate efficient binding is eliminated and strong poly-SUS hydrophobic interactions at concentrations in the low millimolar regime dominate.

some residues like tryptophan and tyrosine are highly conserved in disordered proteins. This is due to their important role in forming protein–protein interfaces

### 3.5 Conclusion

Detailed examination of these data show that poly-SUS is a robust surfactant that is facile, flexible, and hydrophobic with high affinity for globular proteins at concentrations in the low millimolar concentration range. The salient positive cooperative binding mechanisms of the five proteins and their poly-SUS affinities at low concentration may be general features of many water-soluble proteins when they associate with molecular micelles. These properties were exploited by the coupling of intrinsic and extrinsic fluorescence spectroscopy, circular dichroism, and polyacrylamide gel electrophoresis thereby exposing the potential applications of molecular micelles. Extrapolating from our data, we propose molecular micelles as solubilizing

agents for membrane proteins and as probes for studying the hydrophobic interactions of IDPs to their targets. The extraordinary hydrophobicity and flexibility of molecular micelles may offer a new way of studying these phenomenon. Moreover, results from the data presented here suggest that water soluble, hydrophobic, amino acid-based molecular micelles may exhibit greater recognition and higher binding affinity of proteins, which may facilitate enhanced solubilization, separation, and identification in proteomics studies.

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# CHAPTER 4

# SYNTHESIS, CHARACTERIZATION, AND INVESTIGATION OF ETHER-LINKED IMIDAZOLIUM-BASED IONIC LIQUIDS AS GEL SEPARATION REAGENTS USING STEADY STATE FLUORESCENCE SPECTROSCOPY

## 4.1 Introduction

Considerable interest in the use of ionic liquids in analytical chemistry continues to grow as the myriad of potential cation/anion combinations [1] are explored for many different applications. Within chemical analysis, ionic liquids have been used as non-polar stationary phases in gas chromatography [2], as biphasic constituents in liquid-liquid extraction [3], and as matrices in matrix-assisted laser desorption ionization mass spectrometry [4]. One area of interest that continues to emerge is the application of ionic liquids in electrically-driven separations, particularly HPLC [5,6] and CE [7,8]. The primary physicochemical properties of ionic liquids that make them desirable for these analytical techniques are: (1) negligible vapor pressure [9], (2) solubility in polar and nonpolar solvents [10], (3) good electrical conductivity, (4) high ionic mobility, and (5) excellent chemical stability [11].

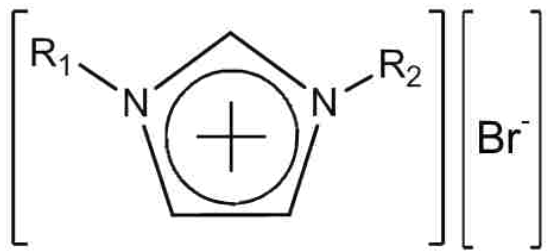
In both HPLC and CE ionic liquids have shown excellent potential by demonstrating enhanced resolution, peak efficiency, and peak symmetry. However, use of ionic liquids in CE has displayed promise for a broad range of compound types [10]. In CE, they have been used to covalently modify the capillary wall and separate analytes such as DNA and as polar [12] or nonpolar [13] stationary phases to separate polar and nonpolar compounds, respectively. In an easier approach, baseline separation of phenolic compounds [14], metal ions [15], basic proteins [16], and amino acids [17] have been achieved by simply using ionic liquids as coatings, BGEs or buffer additives. Wu and coworkers recently baseline separated a mixture of five acidic and

basic proteins by use of 1-butyl-3-methylimidazolium tetrafluoroborate as a wall coating and buffer modifier [18]. In addition, ionic liquids have been used as additives in nonaqueous CE [19].

Applications of ionic liquids in CE have extended into chiral separations where they are used as the chiral selector [20,21] or as an achiral solvent for a chiral selector [22]. Our laboratory investigated the use of imidazolium-based ionic liquids for the separation of a mixture of two binaphthyl derivatives whose enantiomer peaks overlapped [22]. In another report, we compared ionic liquid additives to molecular organic solvent additives in the MEKC separation of binaphthyl derivatives including enantiomers with a stereogenic carbon [23]. It was demonstrated that the 1,3-dialkylimidazolium tetrafluoroborate ILs as BGE modifiers resulted in faster migration times requiring smaller volumes compared to the organic solvents. In general, the application of ionic liquids in CE remains an emerging research area with many new and interesting possibilities.

In this work, the feasibility of examining gel electrophoresis separation systems by means of intrinsic protein fluorescence and fluorescence anisotropy is investigated using a selection of ether-functionalized imidazolium-based ionic liquids, i.e., dual-functionalized ionic liquids [24]. Moreover, the fluorescence characterization of protein-ionic liquid interaction was used to determine which ILs might be useful reagents for buffer modification in PAGE. Subsequent PAGE separations using the molecular micelle, poly-SUS, and ether-functionalized imidazolium-based ionic liquids as additives in the buffer system were explored based on the fluorescence results. Protein band resolution and band gel shifting compared to equivalent separations using SDS were used as gauges of interaction and binding efficiency. The nine ILs investigated are shown in Figure 4.1. Significant physicochemical properties of these ILs, i.e.,

solubility in aqueous solutions, good conductivity, hydrophobicity, and anion similarity to the anion of the salt in the PAGE buffer system, were considered favorable for this investigation. The high solubility of the ILs in aqueous solutions is attributed to the bromide anion. It should be noted that these ILs were varied in cation ether functionality (i.e., 1 – 2) and cation alkyl chain length (i.e., methyl, butyl, dodecyl). The IL with the dodecyl moiety had an experimentally determined CMC of approximately 4.5, which was in good agreement with the report by Baltazar and coworkers [25] for 1-dodecyl-3-methylimidazolium bromide having a CMC of 4.3.




[DMOMIM][Br] $R_1 = CH_3OCH_2$ $R_2 = CH_3(CH_2)_{10}CH_2$
[DMOEIM][Br] $R_1 = CH_3OCH_2CH_2$ $R_2 = CH_3(CH_2)_{10}CH_2$
[DMOEOEIM][Br] $R_1 = CH_3OCH_2CH_2OCH_2CH_2$ $R_2 = CH_3(CH_2)_{10}CH_2$
[MMOMIM][Br] $R_1 = CH_3OCH_2$ $R_2 = CH_3$
[MMOEIM][Br] $R_1 = CH_3OCH_2CH_2$ $R_2 = CH_3$
[MMOEOEIM][Br] $R_1 = CH_3OCH_2CH_2OCH_2CH_2$ $R_2 = CH_3$
[BMOMIM][Br] $R_1 = CH_3OCH_2$ $R_2 = CH_3CH_2CH_2CH_2$
[BMOEIM][Br] $R_1 = CH_3OCH_2CH_2$ $R_2 = CH_3CH_2CH_2CH_2$
[BMOEOEIM][Br] $R_1 = CH_3OCH_2CH_2OCH_2CH_2$ $R_2 = CH_3CH_2CH_2CH_2$

**Figure 4.1** Structure of ether-derivatized imidazolium-based ionic liquids investigated

## 4.2 Materials and Methods

### 4.2.1 Reagents

Ribonuclease A (bovine pancreas, 13.7 kDa, RNase A), Ribonuclease B (bovine pancreas, 15 kDa, RNase B), transferrin (human, 80 kDa, $T_f$), and $\alpha_1$-acid glycoprotein (human, 40 kDa, AGP) were obtained at the highest purity available from Sigma-Aldrich (St. Louis, MO, USA) and used as received. Each protein was reconstituted into 2-4 mg/ml stock solutions in 20 mM phosphate buffer dibasic, pH 7.5 and stored at the required temperature. Three of the proteins (RNase B, Tr, and AGP) studied were glycosylated with N-linked glycans attached at asparagine (Asn) (Figure 4.2). Poly-SUS was synthesized and characterized as reported in chapter 2. Standard precast 4-20% Tris HCl gradient polyacrylamide mini gels and the Precision Plus Protein All Blue Standard marker were obtained from Bio-Rad Laboratories (Hercules, CA, USA). SDS and the other reagents used to prepare the running buffer, the sample buffer, and the destaining solution were purchased from Invitrogen Corporation (Carlsbad, CA, USA). Ultrapure water (18.2 MΩ) was obtained using an Elga PURELAB Ultra water purifier (Lowell, MA, USA). All reagents were used as received without further purification.

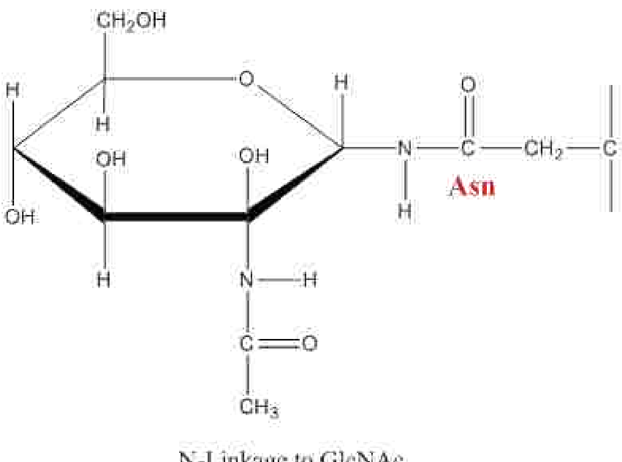



**Figure 4.2** Glycoprotein N-linkage of N-acetylglucosamine to asparagine (Asn)

### 4.2.2 Synthesis and Characterization of Imidazolium-Based Ionic Liquids

The ILs were synthesized according to a previously reported procedure by Dyson et al. in 2007 [26]. This is the first report of six of the nine ILs synthesized. All ILs were characterized by use of $^{1}$H NMR, and $^{13}$C-NMR in $d_6$-DMSO on a Bruker–250 MHz instrument (Appendix B). Additional characterization such as elemental analysis was performed by Atlantic Microlab (Norcross, GA). The critical micelle concentration (CMC) of 1-dodecyl-3-methoxymethylimidazolium bromide was estimated from surface tension measurements at room temperature by use of a KSV Sigma 703 digital tensiometer.

### 4.2.3 Instrumentation and Conditions

Steady-state fluorescence and anisotropy spectra were recorded at 25 °C using a SPEX Fluorolog-3 spectrofluorometer (Jobin Yvon, Edison, NJ) equipped with a 450-W xenon lamp and R928P photomultiplier tube (PMT) emission detector. A quartz cuvette with an optical pathlength of 1 cm was used, and bandwidths for both the excitation and emission monochromators were 3 nm unless otherwise stated. Excitation was performed and emission spectra were detected at the wavelengths indicated in Table 4.1 Reported fluorescence spectra were obtained from IL concentrations in the range of 0.05 – 0.5% w/v and protein concentrations in the range of 0.2 – 1.0 mg/ml in 20 mM phosphate buffer dibasic, pH 7.5 unless otherwise indicated. All samples were allowed to equilibrate for thirty minutes to ensure equilibrium had been reached.

**Table 4.1** Excitation and emission wavelengths of the proteins and ionic liquids investigated

| Protein/Ionic Liquid | Excitation Wavelength (nm) | Emission Wavelength (nm) |
|---|---|---|
| Ribonuclease A | 280 | 307 |
| Ribonuclease B | 280 | 310 |
| Transferrin, human | 295 | 337 |
| $\alpha_1$-acid glycoprotein | 295 | 348 |
| MMOEOEIMBr | 360 | 426 |
| BMOEOEIMBr | 360 | 425 |
| DMOEOEIMBr | 360 | 424 |
| MMOEIMBr | 350 | 421 |
| BMOEIMBr | 350 | 421 |
| DMOEIMBr | 350 | 420 |
| MMOMIMBr | 350 | 422 |
| BMOMIMBr | 373 | 443 |
| DMOMIMBr | 350 | 408 |

## 4.3 Results and Discussion

### 4.3.1 Intrinsic Fluorescence Spectroscopy

Proteins of various sizes and carbohydrate content were studied by intrinsic fluorescence spectroscopy in the absence and presence of ether-derivatized imidazolium-based ionic liquids. Intrinsic tyrosine (Tyr) fluorescence from RNase A and RNase B and intrinsic tryptophan (Trp) fluorescence from $T_f$ and AGP were monitored over an IL concentration range of 0.2% - 0.5% w/v. The selection of RNase A and RNase B was due to the fact that RNase B is glycosylated at Asn-34 with a high mannose structure, whereas RNase A is unglycosylated. It

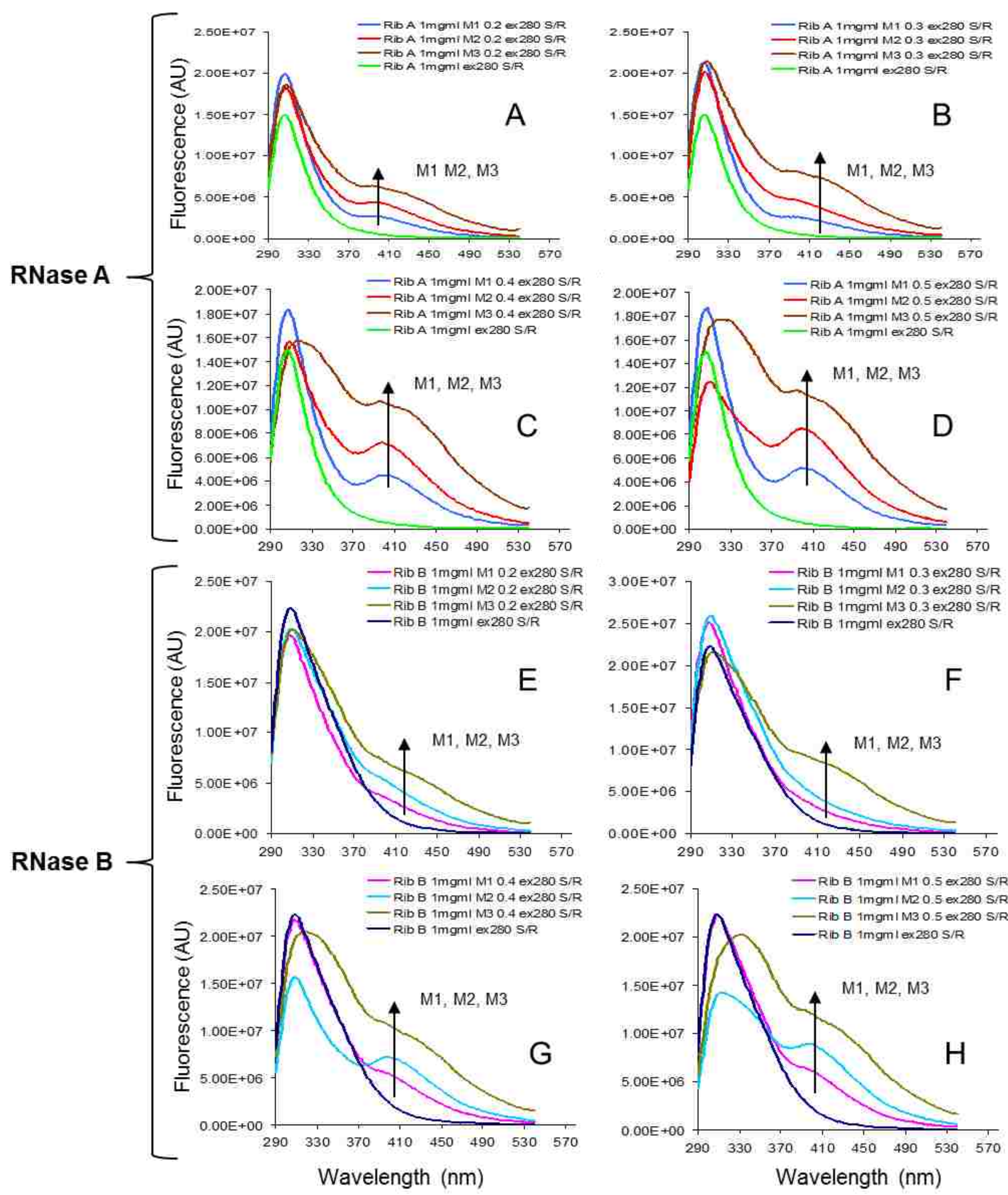



**Figure 4.3** Steady-state fluorescence spectra of RNase A (A - D) and RNase B (E - H) in the presence of increasing concentration (0.2–0.5% w/v) of 1-methyl-3-methoxyethoxyethylimidazolium bromide (M1), 1-butyl-3-methoxyethoxyethylimidazolium bromide (M2), and 1-dodecyl-3-methoxyethoxyethylimidazolium bromide (M3) in 20 mM phosphate buffer dibasic, pH 7.5.

has been shown that the polypeptide backbone of the two proteins is statistically identical with high homology between their primary structures [27]. We hypothesized that the ILs would interact differently with the glycosylated protein compared to its non-glycosylated counterpart.

### 4.3.2 Interaction of Rib A and Rib B with Ionic Liquids

Electrophoresis As expected, the IL fluorescence intensity increased with increasing concentration of IL in the presence of RNase A and RNase B. The steady-state fluorescence spectra of RNase A and RNase B in the presence of 1-methyl-3-methoxyethoxyethylimidazoliumbromide [MMOEOEIM][Br], 1-butyl-3-methoxyethoxyethylimidazolium bromide [BMOEOEIM][Br], and 1-dodecyl-3-methoxyethoxyethylimidazolium bromide [DMOEOEIM][Br] are illustrated in Figure 4.3. As the concentration of IL increased, the tyrosine fluorescence was quenched and red-shifted indicating Tyr was becoming exposed to a more hydrophilic environment. In addition, the energy transfer to the IL increased for RNase A and RNase B as the length of the alkyl chain increased (i.e., dodecyl > butyl > methyl) at $N_1$ in the imidazolium ring. The two-ether containing ILs with the dodecyl moiety at $N_1$ had the greatest effect on IL/protein interaction and subsequent energy transfer to the ILs. In contrast, little to no interaction or energy transfer was observed for the ILs containing only one ether group and a methyl group adjacent to $N_3$ in the imidazolium ring, but containing the same alkyl chain length functionality (Figure 4.4). This observation may possibly suggest that maximum interaction is facilitated by a longer "spacer" between the imidazolium ring and the ether moiety. A steady state fluorescence experiment, where the singular ether group was maintained and the "spacer" was increased to an ethyl group, seems to corroborate this supposition (Figure 4.5). It should be noted here that the interaction of the two-ether containing ILs was greater for RNase A than RNase B. Arnold and coworkers [28]

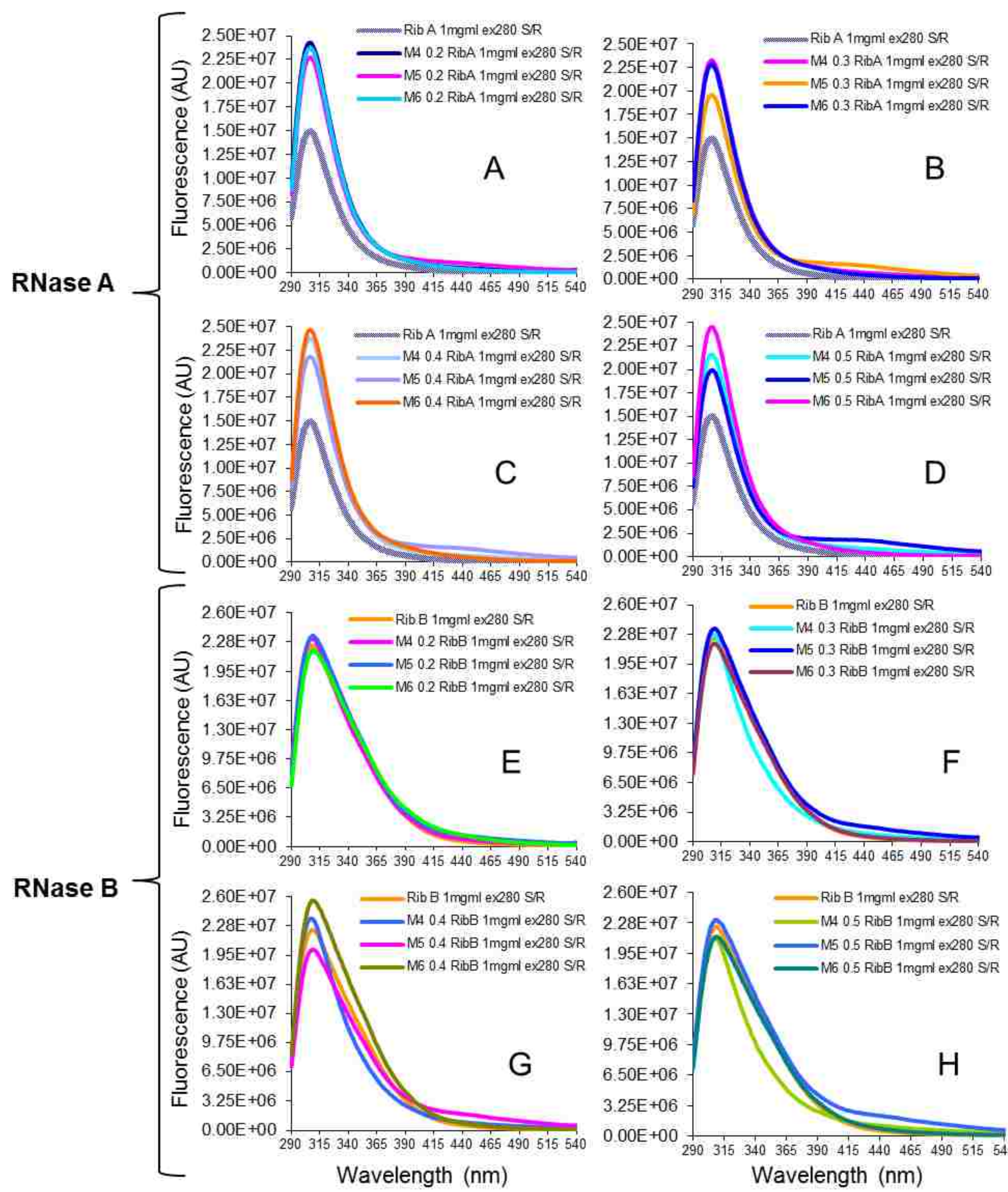



**Figure 4.4** Steady-state fluorescence spectra of RNase A (A - D) and RNase B (E - H) in the presence of increasing concentration (0.2 – 0.5 %w/v) of 1-methyl-3-methoxymethylimidazoliumbromide (M6), 1-butyl-3-methoxymethylimidazolium bromide (M5), and 1-dodecyl-3-methoxymethylimidazolium bromide (M4) in 20 mM phosphate buffer dibasic, pH 7.5.

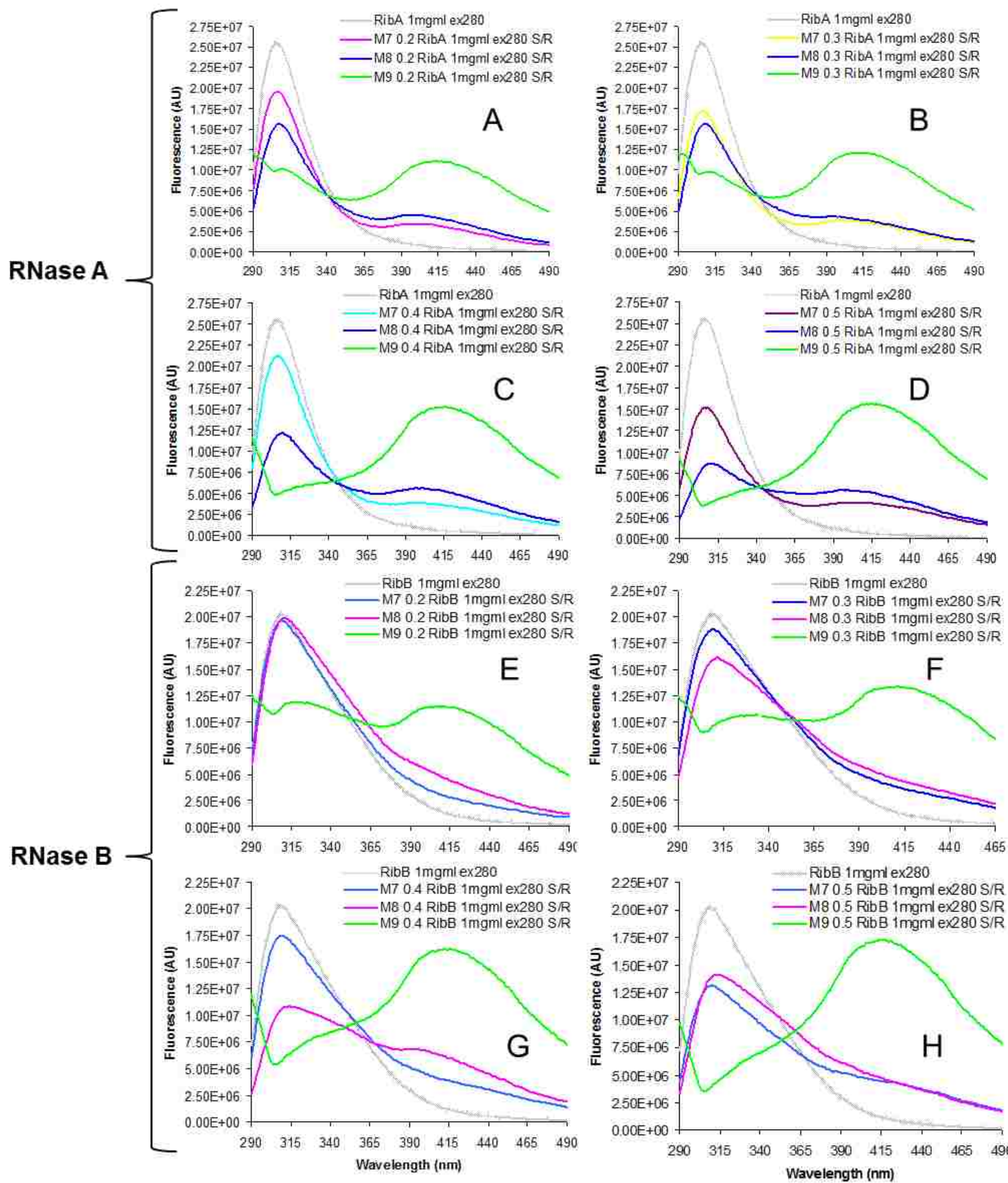



**Figure 4.5** Steady-state fluorescence spectra of RNase A (A - D) and RNase B (E - H) in the presence of increasing concentration (0.2 – 0.5 %w/v) of 1-methyl-3-methoxyethylimidazolium bromide (M7), 1-butyl-3-methoxyethylimidazolium bromide (M8), and 1-dodecyl-3-methoxyethylimidazolium bromide (M9) in 20 mM phosphate buffer dibasic, pH 7.5.

showed that RNase B stabilization by the single carbohydrate moiety at Asn-34 is ≈ 3 kJ/mol greater than RNase A, which contains no sugar. In a kinetics study, this group also concluded that the carbohydrate chain in RNase B sterically hindered trypsin proteolysis at the primary cleavage site (Asn34) [29]. Therefore, it is plausible to infer that the high mannose structure in RNase B prevents any greater interaction with the ILs.

The effect of varying the RNase A and RNase B concentrations while maintaining a static IL concentration was also investigated. The results of the steady-state fluorescence are shown in Figure 4.6. When the RNase A and RNase B concentrations were varied from 0.2 – 1.0 mg/ml, the greatest interaction was observed for the two ILs, [MMOEOEIM][Br] and [BMOEOEIM][Br], in the presence of RNase A. The energy transfer from Tyr in RNase A to both ILs and quenching of Tyr were a maximum at the lowest protein concentration (0.2 mg/ml). As the RNase A concentration increased the energy transfer to [MMOEOEIM][Br] and [BMOEOEIM][Br] decreased. In addition, the interaction of RNase A with [DMOEOEIM][Br] was also a maximum at the lowest protein concentration. Similarly, the distance of the IL with the dodecyl moiety from Tyr in RNase A increased with increasing protein concentration. Examination of these observations suggests that protein concentrations near the 0.2 mg/ml range for these ILs might be optimal in a gel separation. Moreover, the interaction of RNase B with the two-ether functionalized ILs increased with increasing hydrocarbon chain length as indicated by the extent of energy transfer from Tyr to the IL, [DMOEOEIM][Br]. Little to no energy transfer was observed for the interaction of RNase B with [MMOEOEIM][Br] and [BMOEOEIM][Br]. However, it was observed that the energy transfer from Tyr in RNsse A and RNase B to [DMOEOEIM][Br] were similar over the entire protein concentration range. The

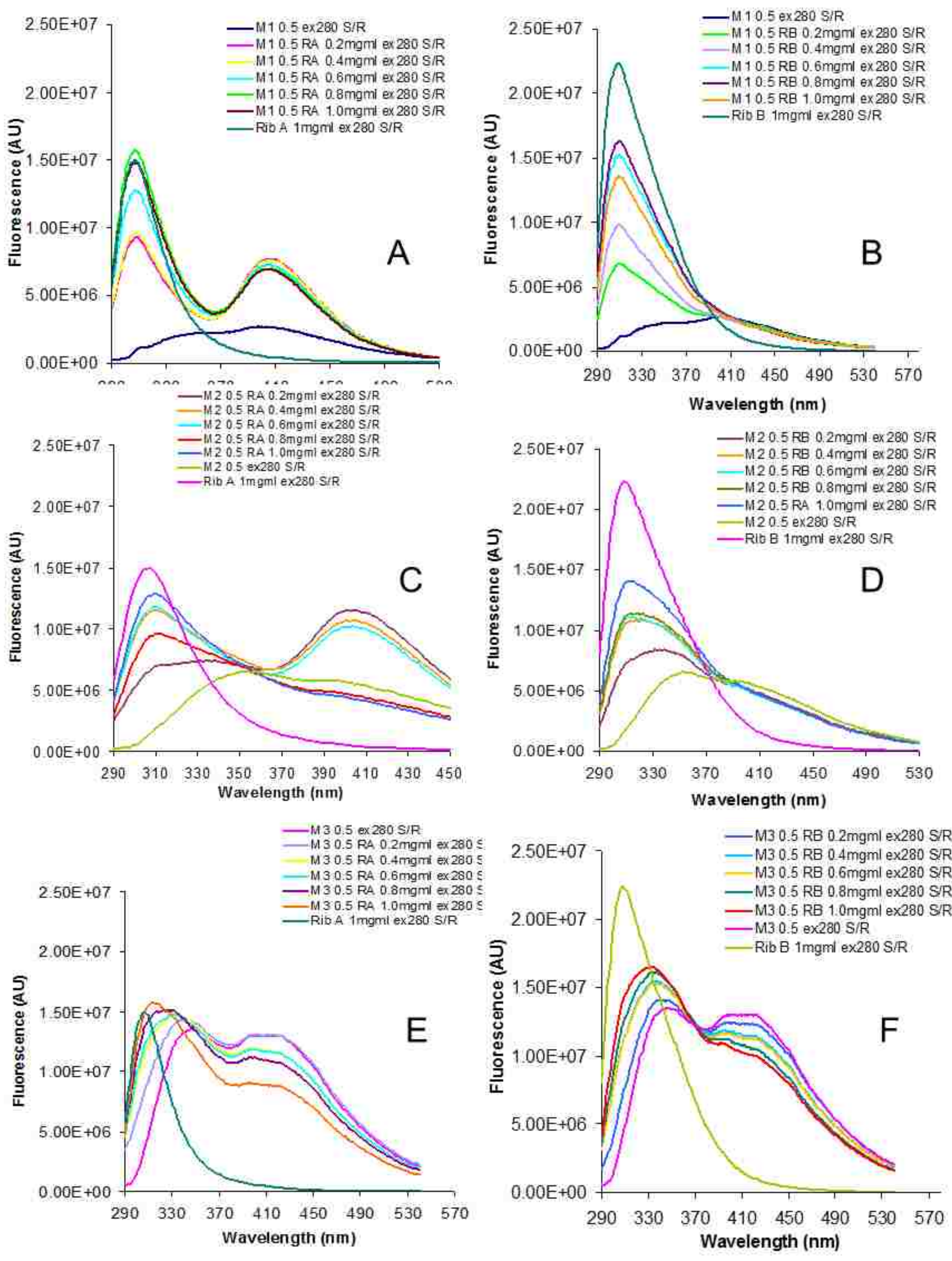



**Figure 4.6** Steady-state fluorescence spectra of increasing concentrations (0.2 – 1.0 mg/ml) of RNase A (A,C,E) and RNase B (B,D,F) in the presence of 0.5% w/v of 1-methyl-3-methoxyethoxyethylimidazolium bromide (M1), 1-butyl-3-methoxyethoxyethylimidazolium bromide (M2), and 1-dodecyl-3-methoxyethoxyethylimidazolium bromide (M3) in 20 mM phosphate buffer dibasic, pH 7.5.

structure and the intensities of the resulting fluorescence spectra for both proteins were quite similar. From this data, one can conclude that [DMOEOEIM][Br] interacts with RNase A and RNase B in analogous ways.

### 4.3.3 Effect of Imidazolium-Based Ionic Liquids as Reagents in Gel Electrophoresis: Rib A and Rib B

The steady-state fluorescence results for RNase A and RNase B were applied in denaturing PAGE using the sample buffer reagents, SDS and poly-SUS, along with three of the nine ILs investigated. An IL concentration of 20% v/v was used for the IL buffer additives, namely, [MMOEOEIM][Br], [BMOEOEIM][Br], and [DMOEOEIM][Br]. These ILs were selected due to the extent of interaction with RNase A and RNase B indicated by the fluorescence data. Gel separations for this system are shown in Figure 4.7. In both gels, the RNase A and RNase B separations are in lanes 2-5 and lanes 6-9, respectively. The first ionic liquid, [MMOEOEIM][Br], was added in lanes 3 and 7, while the ionic liquids with the butyl and dodecyl functionality were added in lanes 4 and 8 and lanes 5 and 9, respectively. The x-axis in each of the profile plots is a 0 to 100% scale measuring the protein migration from the top to the bottom of the gel. For the RNase B separations, profiles C and F in Figure 4.7 clearly show that there is no effect of the three ILs as buffer modifiers for the native state of the analyte. It should be noted, however, that the combination of poly-SUS and the ILs efficiently prevent the formation of higher order RNase A species. In addition, a distinct protein band shift is observed for all three ILs for the RNase A separation in the presence of both SDS and poly-SUS. This may suggest that the ILs have a similar effect on the complex formation involving SDS and poly-SUS with RNase A

### 4.3.4 Interaction of $T_f$ and AGP with Ionic Liquids

The effect of increased numbers of glycosylation sites was explored for the two proteins, human transferrin and $\alpha_1$-acid glycoprotein. Transferrin contains two N- linked glycans at Asn-413 and Asn-611, and AGP contains five oligosaccharide moieties. A spectroscopic investigation using intrinsic tryptophan (Trp) fluorescence from $T_f$ and AGP was employed over IL concentration range of 0.2% - 0.5% w/v. Significant interaction between $T_f$ and the ILs,

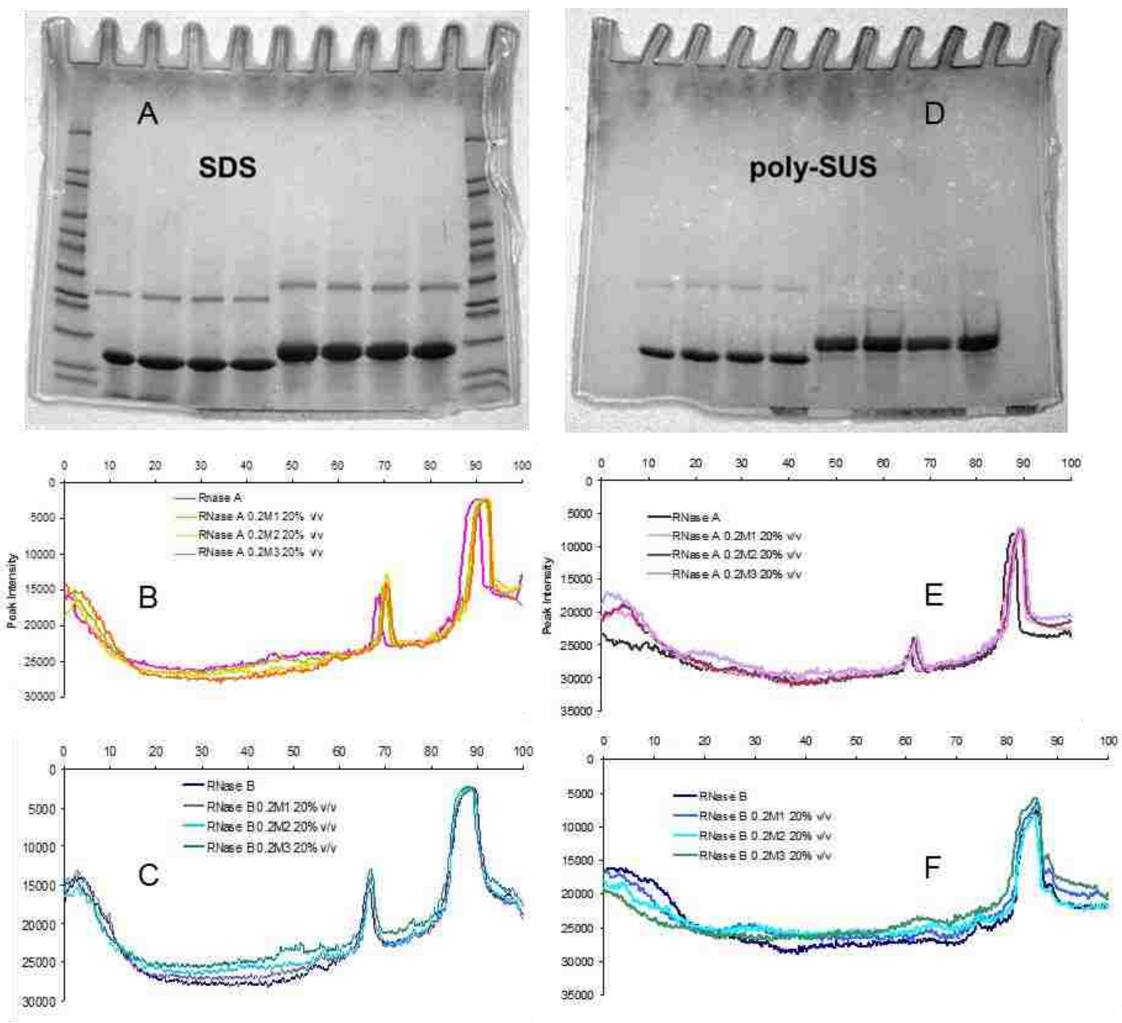



**Figure 4.7** PAGE of RNase A (lanes 2-5) and RNase B (lanes 6-9) on a 4-20% gradient gel using (A) SDS at 2% w/v and (D) poly-SUS at 0.08% w/v with 20% v/v ILs: [MMOEOEIM][Br] (lanes 3,7), [BMOEOEIM][Br] (lanes 4, 8), and [DMOEOEIM][Br] (lanes 5, 9). (A) SDS: lanes 1,10 precision plus molecular weight marker (MWM, 10-250 kDa.

[MMOEOEIM][Br] and [BMOEOEIM][Br], was observed at 0.2% w/v IL. At this IL concentration, the energy transfer from Trp in $T_f$ to the ILs was greatest. For the IL with the dodecyl moiety, the solution was visibly turbid, which typically indicates protein aggregation. In addition, there was no interaction observed for this IL with $T_f$ in the fluorescence measurements. We suppose that the long alkyl chain of the IL perturbed the protein exposing hydrophobic regions that formed large aggregates and precipitated out of solution. As the IL concentration was increased, a marked decrease in Trp energy transfer is observed. At an even lower IL concentration (i.e., 0.05% w/v), the energy transfer and hence interaction with the protein was less than at the IL concentration of 0.2% w/v. For AGP, interaction with all nine ionic liquids was surveyed using intrinsic tryptophan (Trp) fluorescence as with $T_f$. Significant shifts in the fluorescence intensity and wavelength maxima were evident for the methoxyethyl ionic liquid having the dodecyl functionality. As the ionic liquid concentration was increased, the efficiency of the energy transfer from Trp to [DMOEIM][Br] increased. For the remaining ionic liquids, little to no interaction was evident from the fluorescence spectra. In each case the Trp wavelength maximum remained at 348 nm, but the fluorescence intensity was quenched. This observation is consistent with Trp being quenched by neighboring amino acid residues, peptide bonds, or disulfide groups. Since the wavelength maximum stayed constant, these quenching effects are not accompanied by conformational changes in the protein structure. Thus, neither the Förster distance nor the steric orientation requirement was met for energy transfer to occur. In addition, the interactions of the ionic liquids with $T_f$ and AGP revealed no relationship between number of glycosylation sites and ionic liquid energy transfer. However, the significant energy transfer observed from two of the ionic liquids to Tf and one of the ionic liquids to AGP suggest that they may be feasible buffer modifiers in gel electrophoresis.

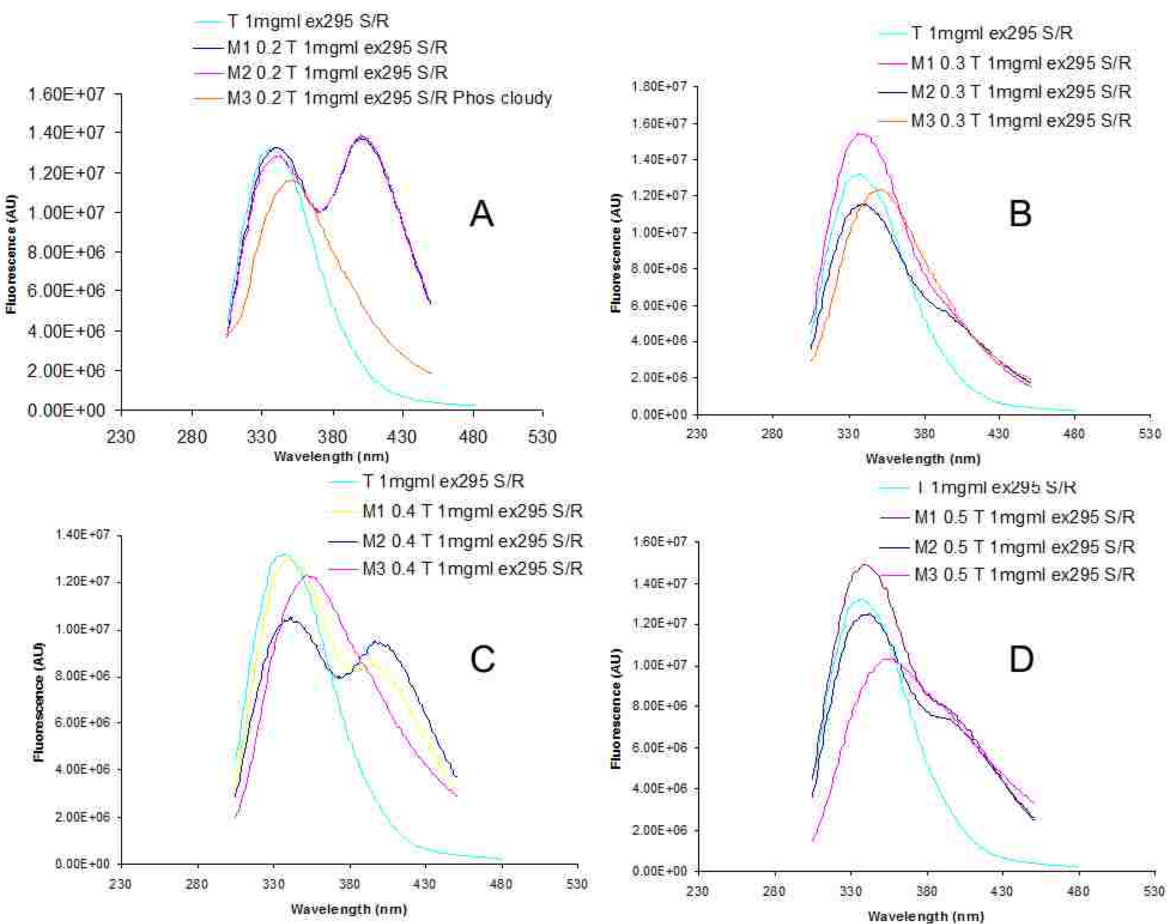



**Figure 4.8** Steady-state fluorescence spectra of transferrin (A - D) in the presence of increasing concentration (0.2–0.5% w/v) of [MMOEOEIM][Br] (M1), [BMOEOEIM][Br] (M2), and [DMOEOEIM][Br] (M3) in 20 mM phosphate buffer dibasic, pH 7.5.

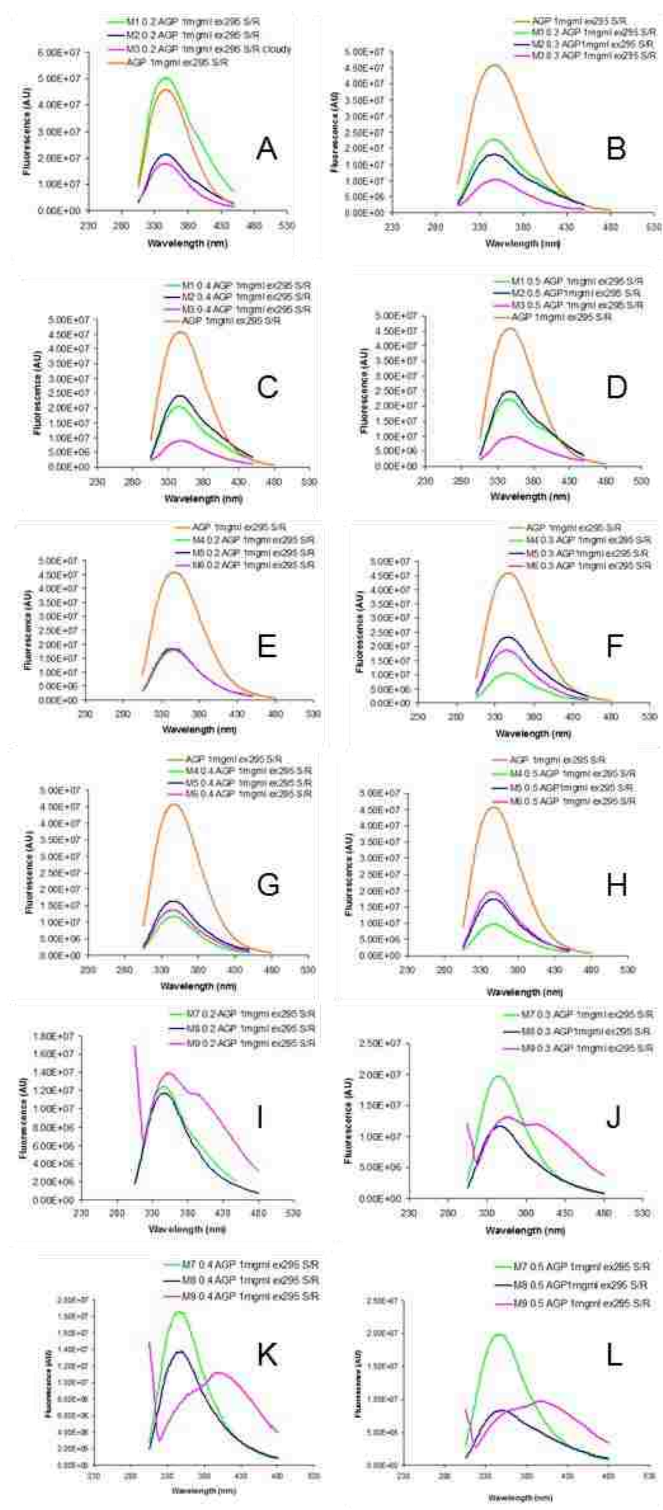



**Figure 4.9** Steady-state fluorescence spectra of AGP (A - L) in the presence of increasing concentration (0.2–0.5%w/v) of [MMOEOEIM][Br] (M1), [BMOEOEIM][Br] (M2), [DMOEOEIM][Br] (M3), [DMOMIM][Br] (M4), [BMOMIM][Br] (M5), [BMOMIM][Br] (M6), [MMOEIM][Br] (M7), [BMOEIM][Br] (M8), and [DMOEIM][Br] (M9) in 20 mM phosphate buffer dibasic, pH 7.5.

### 4.3.5 Effect of Imidazolium-Based Ionic Liquids as Reagents in Gel Electrophoresis: $T_f$ and AGP

From these steady state fluorescence spectroscopy results, PAGE separations of $T_f$ and AGP were performed in the presence of the ionic liquids that demonstrated significant interaction with the proteins. The buffer modifiers for the $T_f$ separations were [MMOEOEIM][Br], [BMOEOEIM][Br], and [DMOEOEIM][Br]. In the AGP separations, the ionic liquid sample buffer additives were [MMOEIM][Br], [BMOEIM][Br], and [DMOEIM][Br]. The initial ionic liquid concentration in the sample buffer was 20% v/v. Two sets of separations were performed in which the surfactant in the sample buffer and running buffer was either SDS or poly-SUS. Figure 4.10 is the representative gel and profile plots of the $T_f$ and AGP separations for both surfactant systems. In lanes 2, 3 and 4 for the SDS separation, the relative migration of the major protein band for $T_f$ was the same in the absence and in the presence of ionic liquid. This observation is more clearly seen in the densitometry plot where the curves for each experiment are overlaid. In contrast, when poly-SUS was the surfactant in the sample buffer, a protein band shift is observed compared to the separation in the absence of ionic liquid. This shift is consistent with a protein/poly-SUS/ionic liquid complex (lanes 3 and 4) that migrates faster and is presumably smaller than the protein/poly-SUS complex in lane 2. Again, this effect is more pronounced in the band profile plot. It should also be noted here that when SDS is present in the sample buffer not band shift is observed and there are a significant number of smaller species toward the bottom of the gel. The gel where poly-SUS is in the sample buffer indicates species that are larger with no significant smaller species present as in the case with SDS. We can only speculate about the origin of these dissimilarities. These observations could be due to dimerization or complete $T_f$ unfolding in the presence of poly-SUS and the ionic liquids where a larger complex would be possible or it could be due to disulfide

reduction into smaller polypeptides by the reductant in the presence of SDS and the ionic liquids. Moreover, the AGP separations reveal no differences of the major protein band relative migration in the absence and presence of ionic liquid for both the SDS and poly-SUS separations. Interestingly, the SDS separation consistently produced one major protein band peak and a minor protein band peak indicative of a larger species. However, the poly-SUS separation in the presence of the ionic liquid showed a merging of the major AGP band with the

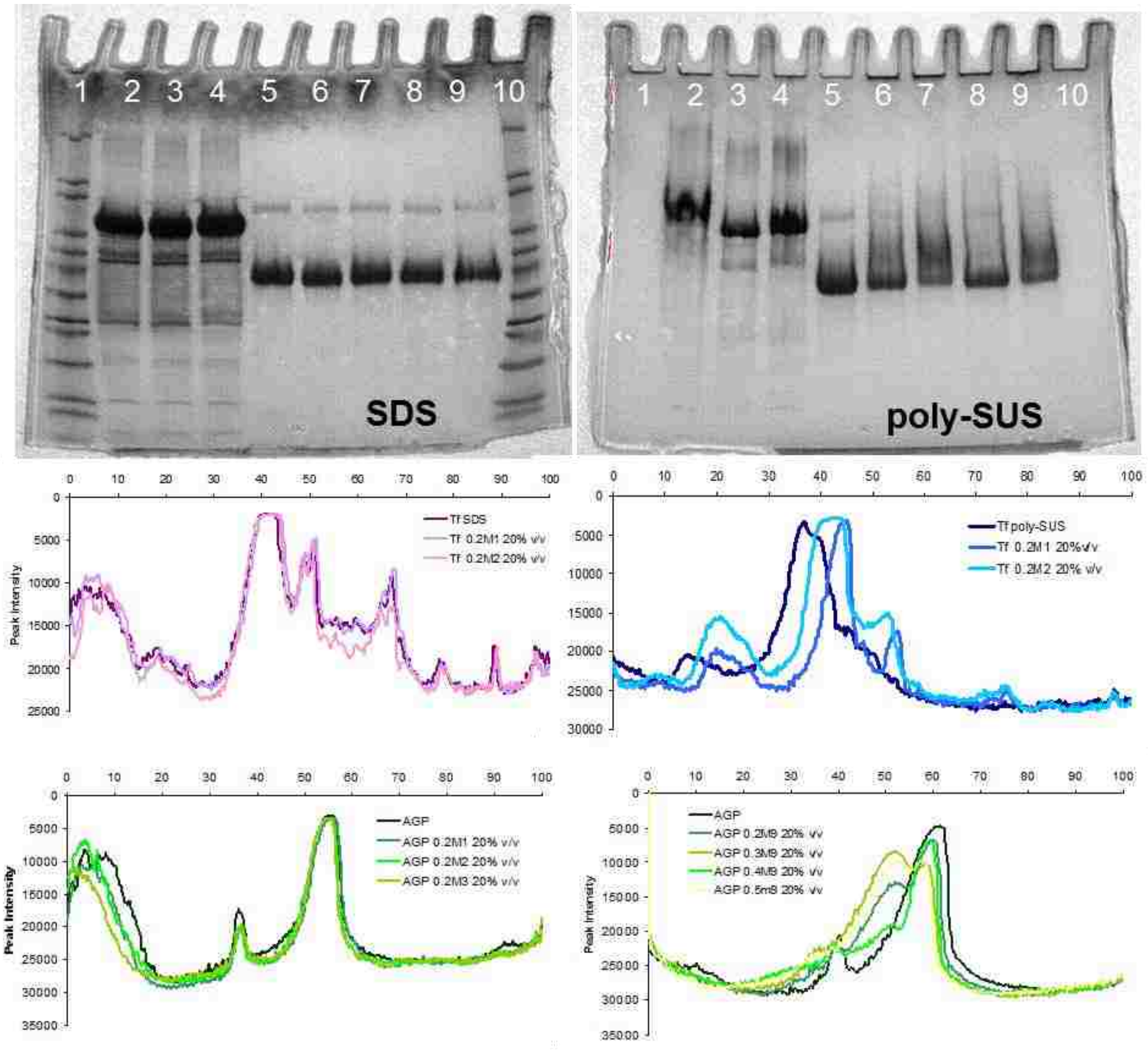



**Figure 4.10** PAGE of Transferrin (lanes 2-4) and AGP (lanes 5-9) on a 4-20% gradient gel using (A) SDS at 2% w/v and (D) poly-SUS at 0.08% w/v with 20% v/v ILs: [MMOEOEIM][Br] (lane 3), [BMOEOEIM][Br] (lanes 4), [MMOEIM][Br] (lane 7), [BMOEIM][Br] (lane 8), [DMOEIM][Br] (lanes 9). (A) SDS: lanes 1,10 precision plus molecular weight marker (MWM, 10-250 kDa.

larger complex species for all concentrations of [DMOEIM][Br]. The relative migration of the larger complex species was between the major band and the minor band of the separation in the absence of ionic liquid.

## 4.4 Conclusion

In this work, we have demonstrated the feasibility of using ether-derivatized imidazolium-based ionic liquids as buffer modifiers in gel electrophoresis by studying the propensity of the ionic liquids to interact with many types of proteins using steady state fluorescence spectroscopy. Significant interaction of several ionic liquids with Rib A, Rib B, $T_f$, and AGP was observed in the fluorescence studies. No effect of was observed when comparing the unglycosylated Rib A with the one glycan-containing homologous protein, Rib B. In addition, the relative numbers of glycosylation sites (i.e., 1, 2, or 5) were insignificant predictors of ionic liquid interaction. However, several of the ionic liquids demonstrated interaction with the proteins as evidenced by the energy transfer that was observed in the fluorescence investigations. When moving to the slab gel format, no effect of the three ILs as buffer modifiers was observed as seen in the electropherograms for Rib A and Rib B. The presence of poly-SUS along with the ILs did, however, prevent the formation of higher order species. When AGP was the analyte of interest, the relative mobility of all gel bands in the absence and the presence of the ILs were the same. Interestingly, in the presence of two ILs and poly-SUS, a distinct shift in the $T_f$ band was observed while the shift was not observed in the absence of the ILs. In the presence of the same ILs with SDS, no band shifting was observed for $T_f$. These results are significant because this is the first time that ILs have been employed in separations in gel electrophoresis. Moreover, they offer insight into the use of ILs in the slab gel format and

show promise that ILs may be useful in resolving proteins that are highly homologous and within a few hundred Daltons in molecular weight.

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# CHAPTER 5

# CONCLUSIONS AND FUTURE DIRECTION

In this body of work, part I of this research focused on applications of a molecular micelle and ionic liquids in one dimensional gel electrophoresis. One analytical technique was employed, but two different applications were investigated. The application of the molecular micelle and the ionic liquids in PAGE were complemented by spectroscopic studies.

In Chapter 2, the use of the achiral molecular micelle, poly-SUS, as the surfactant in the sample buffer and running buffer in polyacrylamide gel electrophoresis protein separations was investigated. The performance of poly-SUS in PAGE was demonstrated by the simultaneous separation of acidic and basic proteins in one run. Poly-SUS proved to be a useful surfactant in polyacrylamide gel electrophoresis because of its ability to discriminate proteins with widely varying molecular weights, isoelectric points, and hydrophobicity. As compared to the conventional surfactant, SDS, the bands produced during PAGE separations with poly-SUS were more intense and sharper. In addition, the reproducibility of the poly-SUS protein separations was evaluated by calculating the relative standard deviation (%RSD). The day-to-day and intra-day RSD values were found to be less than 17.3% and 19.5%, respectively.

Chapter 3 reports the investigation of the mechanism of interaction of poly-SUS with a representative set of acidic and basic proteins that were used in the PAGE separations. The coupling of intrinsic and extrinsic fluorescence spectroscopy, circular dichroism, and polyacrylamide gel electrophoresis demonstrated that poly-SUS is flexible and highly hydrophobic with high affinity for globular proteins at concentrations in the low millimolar concentration range. Poly-SUS was able to resolve the tumor suppressor protein, p53, at concentrations 24 times lower than the standard PAGE surfactant, SDS.

In chapter 4, the feasibility of using imidazolium-based ILs as buffer modifiers in PAGE was demonstrated using fluorescence spectroscopy as a gauge of the interactions between the ILs and several proteins. No trend was observed when comparing the interactions of the ILs with non-glycosylated and glycosylated proteins or for increasing numbers of glycosylation sites. However, a consistent and significant gel band shift for $T_f$ was observed in the presence of the ILs and poly-SUS, which did not occur in the absence of the ILs or when SDS was present.

The use of poly-SUS in PAGE proved useful for many different protein types containing various molecular weights, sugar moieties, isoelectric points, and hydrophobicity. The ability of poly-SUS to solubilize membrane proteins using its extraordinary hydrophobicity should be investigated. In addition, molecular micelles with tunable hydrophilic head groups may offer some selectivity in solubilization when investigating certain types of highly hydrophobic proteins. These studies should include 2D gel electrophoresis, where the solubilization of these types of proteins proves to be troublesome. In these types of studies, the typical surfactants used, particularly SDS, suppress the mass spectrometry signal and adversely affect protein identification. These studies could be extended to investigate the effect of the molecular micelles used for solubilization on the mass spectrometry signal and subsequent identification of the proteins.

Studies to further characterize the interaction of the ILs, [MMOEOEIM][Br] and [BMOEOEIM][Br], with $T_f$ should be investigated for the future. Techniques such as circular dichroism to study changes in the secondary structure upon interaction with the ILs and dynamic light scattering to study the size of the IL/protein complex compared to the native protein could be used. In addition, solution studies should be performed to investigate the hypothesis that the ILs may be catalyzing the loss of carbohydrate from $T_f$, which leaves a smaller protein that

migrates further down the gel. The gel electrophoresis separations in the presence of the ILs should also be optimized. Several parameters that could have significant impact upon the band shifting would be investigated, namely IL concentration in the buffer, gel percentage (or pore size), and separation voltage. Lastly, a series of ionic liquids (ILs) based on the methoxy- and methoxyethoxy-imidazolium moiety with various anions ([$PF_6$], [$NTf_2$], [$ClO_4$], [DCA], [$NO_3$], [AC], and [Cl]) and their corresponding non-methoxy ILs should be investigated as buffer modifiers in the separation of proteins in gel electrophoresis.

# PART II: INVESTIGATING THE MENTORING CANON THROUGH EXPLORATION OF THIRTY YEARS OF MENTORING BY AN EXEMPLAR

# CHAPTER 6

# INTRODUCTION

## 6.1 Rationale

For the last several decades, American leadership has been evident around the world in the science and technology (S&T) arenas. However, an increasingly competitive global environment, where countries are focusing on innovation and growing their economies, has begun to challenge our S&T lead. According to Rising Above the Gathering Storm (viewed 05/25/10), recapturing its S&T leadership role will require the United States to take bold, creative steps to ensure a continuum of recruiting, training, and graduating the brightest students. This recommendation was echoed in 2009 by President Obama in a speech at the National Academy of Sciences (NAS). He challenged NAS members to "think about new and creative ways to engage young people in science and engineering, like science festivals, robotics competitions, and fairs that encourage young people to create, build, and invent -- to be makers of things, not just consumers of things" (retrieved 09/07/10).

Central to increasing the number of science, technology, engineering, and mathematics (STEM) scientists will be piquing the interest of high school students (Hulleman, C. S., Harackiewicz, J. M., 2009) and lowering the attrition of undergraduate STEM students who were highly qualified upon entry (Seymour, E., Hewitt, N. M., 1997; Tobias, S., 1990). Others like Dr. John P. Holdren, assistant to President Obama for Science and Technology and Director of the White House Office of Science and Technology Policy, notes that “formal” mentors “can be very important in the preparation and training of our Nation’s scientists and engineers.” He also reminded us in his blog that “strong mentoring relationships are also key for recruiting and retaining diverse students in science and engineering” (viewed 05/27/10).

In general, it is agreed among scientists in industry, academia and government that mentoring has the potential to reposition America as a science and technology (S&T) leader around the world once again. However, further research in STEM mentoring is needed that investigates what Paglis, Green, and Bauer (2006) call "construct definition and measurement development for mentoring in the academic context." Although most professors agree that mentoring one's science protégés is not new and has long been an honored mainstay in graduate education (Bird, S. J., 1994), little is known beyond a list of 'good' mentoring attributes. Oftentimes, effective mentoring is evident only after the protégé has moved on and is many years removed. It is clear that there is a need to bridge the 'what is' of mentoring to the 'effectiveness' of mentoring by the 'how does it work' of mentoring.

Given the importance of mentoring to the entry and progression of all students in the sciences (Bargar, R. R., Mayo-Chamberlain, J., 1983; Nettles, M., Millett, M., 2006), it is surprising that little research has focused on the mentoring practices of individual mentor exemplars, why they have been honored for their work, and what can be learned from them. During a search to locate research related to mentoring in the sciences, particularly STEM, few references were found (Bird, S. J., 1994; Feldman, G. C., 2001; Packard, B., 2005; Peter, G., 2004; Shellito et. al., 2001; Watkins, Gayle Harris, 2005). A large number of websites, articles, and books were found that raise the issue of the importance of mentoring in STEM and provide summaries and training steps that are common to successful mentoring relationships (Collins, S. N., et al., 2001; Handelsman, J., et . al., 2005; National Academy of Sciences, viewed 09/08/10). One dissertation by Gayle Harris Watkins (2005) enumerated how exemplar mentors practice mentoring. Having an intrinsic motivation toward mentoring, the mentor exemplars focus on retention in STEM, have a definite method of mentoring, and teach the intangible aspects of the

discipline. To our knowledge no research reports exist in the literature that closely examine a science mentor exemplar, one who has garnered prestigious awards for his/her mentoring efforts. More specifically, this study focused on going beyond simply *describing* what was done in the mentoring relationship to *explaining* why it has worked in order to further our understanding of the mentoring canon.

## 6.2 Research Questions

The main research question of this study was: Why does the exemplary mentoring of Dr. Isiah M. Warner work?

Additional sub-questions were:

1. What factors were critical in the development and sustaining of the mentor/protégé relationship with Dr. Isiah Warner?
2. What are the specific characteristics, attributes, and actions that Dr. Isiah M. Warner exhibits during the mentoring relationship that make him an effective mentor?
3. What were the benefits received by the protégé as a result of being mentored by Dr. Isiah M. Warner?

## 6.3 Research Vee Diagram

A graphical representation of the study called a research Vee diagram (Gowin, 1981) is presented in Figure 1. The Vee diagram is designed to aid the researcher's metacognitive thought processes in connecting the theory to the final outcomes (i.e., knowledge and value claims). The structure of the diagram is separated into ten parts and further subdivided into four general categories: (1) research and sub-questions, (2) theoretical and conceptual basis of the research, (3) major research events, and (4) the methods which lead to the knowledge and value claims.

Research Question

Why does the exemplary mentoring of Dr. Isiah M. Warner work?

Theory

Social Development Theory
Social Learning Theory
Invitational Learning Theory

Principles

For learning to occur, a more knowledgeable other (MKO) must be present who employs scaffolding during the dynamic social interaction with the protégé.

Cognitive acquisition involves concept and propositional learning, which are key aspects of meaning making.

Concepts

Mentoring, social interaction, respect, mutual exchange, reciprocity, cognitive acquisition, trust, conceptual change, intentionality, optimism, , modeling, scaffolding, more knowledgeable other

Events

Mentor Interview

Protégé Survey

Self-Selection Survey

Mentoring Award Nomination Packages

Sub-Questions

What factors were critical in developing and sustaining the mentor/protégé relationship with Dr. Isiah Warner?

What are the specific characteristics, attributes, and actions that Dr. Isiah M. Warner exhibits during the mentoring relationship that make him an effective mentor?

What were the benefits received by the protégé as a result of being mentored by Dr. Isiah M. Warner?

Value Claims

The PET (Positioning, Exchange, Transformation) Science Mentoring Theory is a powerful tool for assessing whether mentoring efforts in the sciences are working.

Knowledge Claims

Mentoring in the sciences that works requires the existence of three stages: (1) intra-psychological positioning, (2) inter-psychological exchange, and (3) intra-psychological transformation. Omission of one stage results in mentoring that does not work.

Transformations

Transcription and analysis of digitally recorded mentor interview

Statistical quantification of survey responses

Analysis of nomination package materials

Constant comparative analysis of interview, survey, and nomination package content/open responses

Records

Digital recording of mentor interview

Self-selection essay

Protégé survey open responses

Nomination package letters

**Figure 6.1** Research Vee Diagram.

## 6.4 Flow Chart of Research

A flow chart of research, which outlines the study from conception to completion, is shown in Figure 2. The diagram outlines the chronology, key research activities, and data that were collected over the course of this research.

## 6.5 Definition of Terms

- case study - a type of research methodology which is based on an in-depth investigation involving a single person, event or group
- efficacy - the ability to produce an effect
- grounded theory - a qualitative mode of inquiry in which the theory emerges from the data that has been collected, analyzed, conceptualized, and generalized
- constructivism - a view of meaning making where humans actively construct their knowledge of the external and knowable world
- invitational learning theory - a paradigm for personal and professional functioning that uses four key functions (respect, intentionality, optimism, and trust) in understanding the nature of human potential
- social development theory - a theory of learning through social interaction which results in cognitive development
- social learning theory - a theory of learning which posits that people learn from each other in a social context through observation and emulation
- STEM - science, technology, engineering, and mathematics
- triangulation – a research methodology in which the data collected originates from multiple sources to increase the credibility and validity of the study

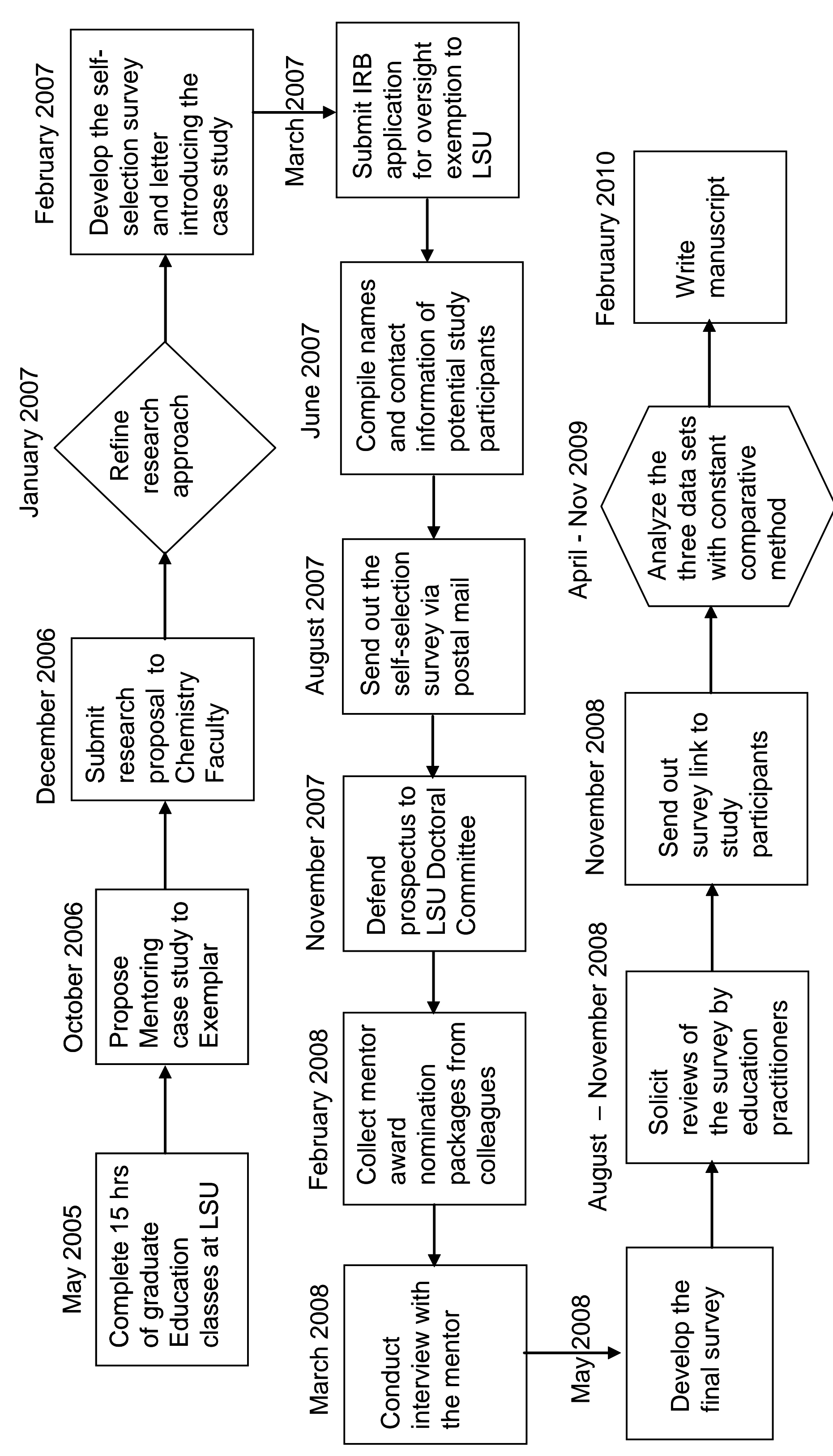


**Figure 6.2** Flow Chart of Research.

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# CHAPTER 7

# LITERATURE REVIEW

The purpose of this study is to determine 'why' the mentoring of an exemplar works. Exploration of his mentoring practices over a thirty-year period was investigated. The participants in the study were current or former protégés who confirmed being in a mentoring relationship with the exemplar.

This chapter begins by exploring different aspects of mentoring in STEM including the types of mentoring, roles and responsibilities of a mentor, and mentoring outcomes. Next, the researcher examines the theoretical frameworks for this study. A detailed review of invitational learning theory, social development theory, and social learning theory is outlined. Each theory contributes to the basic tenets of understanding mentoring as a construct and how its effectiveness should be gauged. Lastly, the constructivist grounded theory research methodology will be reviewed and discussed in the context of this study.

## 7.1 Mentoring in the Sciences

It is generally agreed among scientists and practitioners alike that mentoring should be incorporated either formally or informally into the developmental process of young people into scientific professionals (viewed 05/27/10) and is a critical factor in the entry and progression of women and minorities in the sciences where they are under-represented (Grant, Linda et. al., 1993). In an effort to diversify the sciences, national programs designed to serve as change-agents at all levels of education have been funded throughout the U.S. In most cases, mentoring is cited as an integral part of the programmatic framework (Handelsman, J., et al., 2005; U. of Iowa, viewed 05/27/10). This concept of providing mentoring to one's science protégés is not new and has long been an honored mainstay in graduate education. Yet, we are still unclear

about the mentoring canon and even less is known about how or why it translates into effectiveness. This may be, in part, due to the fact that mentoring graduate students remains only an implicit part of the new faculty member's duties. For the assistant professor on tenure-track, the pursuit of developing a sustainable research agenda, securing competitive funding, and accumulating peer-reviewed manuscripts, takes precedence. Unless a faculty member agrees that mentoring is essential to the development of his/her scholarship (Coppola, B. P., 2001), it remains in the distant background. Yet, there are programs at the undergraduate level which have demonstrated that mentoring may be part of the answer (Brainard, S., Ailes-Sengers, L., 1994; Matsui, J. et al., 2003; Summers, M. F., Hrabowski III, F. A., 2006), and they may be catalyzing a mentoring renaissance. For example, the highly regarded Meyerhoff Scholars Program (Maton, K., Hrabowski III, F., 1999) at the University of Maryland, Baltimore County centers its efforts on mentoring and has consistently produced large numbers of students who are intent on driving scientific and technological progress and thus pursue post-graduate work (graduate school, MD/PhD programs, and medical school). Moreover, for many years the Biology Scholars Program (BSP) at UC Berkeley (Matsui, J., et al., 2003), in which mentoring is a central part of the program components, has produced Biology graduates at significantly higher rates than non-BSP students.

Thus, mentoring in the sciences (i.e., STEM) continues to evolve as individuals and programs attempt to harness its power and prove its worth. However, academic mentoring falls short of the "construct development and measurement work" (Paglis, L. L. et. al., 2006) that exists in the organizational behavior literature. Moreover, mentoring in STEM has essentially been a 'hit or miss' enterprise. Yet, there are exceptions. In some cases, like the one in the department of Mathematics at the University of Iowa, faculty have committed to systemic

departmental change and embraced the role of mentoring in achieving their recruitment, retention, and graduation goals. A "communal effort by the department" resulted in a faculty that is now described as a "community of mentors" (viewed 05/27/10). Although the cultural change began as a minority recruitment initiative, today the graduate student population is majority American which is one example of many unexpected results. Their ten-year effort culminated in the department of mathematics receiving the Presidential Award for Excellence is Science, Mathematics, and Engineering Mentoring (PAESMEM) in 2005. A key aspect of the change at the University of Iowa was the intentional restructuring of the graduate program, which included them instituting mentoring. Institutionalized mentoring can exist in many forms, and the next section will provide an overview of the different structures of mentoring.

#### 7.1.1 Mentoring Structure

There are essentially two types of mentoring relationships: formal and informal. Formal mentoring refers to a highly structured system in which the mentor is assigned a protégé. Often found in organizations or businesses, these mentoring programs typically use goals, training, schedules and evaluations as the benchmarks for success. The assigned mentor could be someone from a closely related discipline, a different discipline, or even a different department. However, there are situations like the one at the University of Iowa where mentors may be assigned or graduate students are allowed to select a mentor. This, too, is typical of formal mentoring, particularly in academia. On the other hand, informal mentoring relationships develop on their own. This type of mentoring is best described as informal and mutually agreeable. Two potential mentor/protégé participants unofficially volunteer in a process of self-selection to engage in mentoring with the other person. Over time, the mentoring relationship is constructed and experienced by both parties. This construction of mentoring, whether formal or

informal, is further explored in the next section where a review of the basic elements or functions of the mentoring relationship is discussed.

### 7.1.2 Mentor Functions and Attributes

Early research efforts by Astin (1977), Pascarella et al. (1978) and Wilson et al. (1975) emphasized the impact of faculty-student relations on educational experience and outcomes. More recently, a study conducted by the University of Wisconsin, Madison for the Pew Charitable Trusts found that faculty-student relations at the graduate level needed improvement (Schrope, M., 2001). More than half of the 4000 graduate students in the third year or above had no clear understanding of what or how long it would take them to graduate. Therefore, the authors called for annual reviews between advisors and students to discuss needs, expectations, and progress. Although mentoring in the sciences is the focus of this research, many of the elements of mentoring identified in science-focused research is similar to the elements espoused in other discipline-specific research on mentoring. In 1988 Anderson and Shannon developed a model of mentoring in which three specific mentoring aspects were highlighted. These included relationship, functions, and dispositions. More specifically, these three categories were further expressed as role modeling, teaching, sponsoring, befriending, encouraging, caring, and concern. Moreover, Johnson found in 2002 that the personality characteristics associated with being a good mentor were intelligent, empathetic, patient, flexible, ethical, and well-known as a scholar. Rose (2003) investigated the qualities of an ideal faculty mentor from the perspective of doctoral students. In this study, it was determined that the top two attributes desired were communication effectiveness and honest feedback. A more detailed study by Punyanunt-Carter and Wrench (2008) explored the advisor-protégé communication relationship. In this work, participants' perceptions of the advisor's credibility and trustworthiness were positively correlated with

solution-oriented conflict management strategies and non-confrontational conflict management strategies, respectively. Thus, the researchers suggested that advisors could increase protégé degree completion by being more cooperative, supportive and non-confrontational when resolving conflict.

Much of the research on scientific mentoring is still in the infancy stage, but a substantial body of work on mentoring has been provided by doctoral dissertations (Filippelli, L. A., 1997; Johnson, B. A., 2001; Moore, E., 1999; Watkins, G. H., 2005). In general, these studies have concluded that a mentor's role is categorized broadly as being psychosocial and career-related. Oftentimes, the functions of and the attributes of a 'good' mentor have been identified simultaneously. In 2001 Johnson, explored the mentoring relationship of faculty and graduate students across different academic departments at the same university. Interview and web surveys were conducted. In general, students' mentoring experiences were positive. Those who had a mentor did better than those who did not, and students wanted the benefits of more than one mentor. However, more closely related to this dissertation research was the study conducted by Moore in 1999. An in-depth study comparing the 'ideal' and 'received' mentoring of male and female graduate students in mathematics, the physical sciences and engineering was pursued. The participants expressed two functions and two attributes of an ideal mentor. The functions were as teacher and role model, which can be broadly classified in the psychosocial and career categories, respectively. The participants also expressed the desire for encouragement and support from an ideal mentor. However, the female and male participants agreed that they had seldom received encouragement or support from their advisors. Moreover, Filippelli (1997) extended our understanding of the social relationships of accomplished scientists by exploring the differences in mentoring received from the doctoral advisor and student colleagues during

graduate studies. The study found that men and women disagreed on the psychosocial and career-related mentoring from advisors and colleagues. Females believed that the career-enhancement mentoring by the doctoral chairperson was more important compared to men. In addition, women identified spouses and friends as positive influencers of their development into scientists. In contrast, the male scientists indicated that the major advisor was the positive influencer. This review of mentoring in the sciences is not exhaustive, but rather focuses on research that is noteworthy because of its relevance to the research in this dissertation, namely 'how and why does mentoring in the sciences work?'

### 7.1.3 Mentoring Outcomes

The benefits of mentoring have been known to span undergraduate, graduate and post-graduate education. For example, Campbell and Campbell (1997) found that mentored undergraduate students had higher GPAs, completed more credit hours per semester, and had greater retention rates than their non-mentored peers. At the graduate level, students reported benefits such as enhanced self-confidence, career advice, guidance, support, listening, and role modeling (Luna, G. and Cullen, D., 1998). In particular, a supportive advisor positively affected the time-to-degree. A similar finding was reported by Nettles and Millett (2006). In addition, they indicated that mentored graduate students compared to non-mentored colleagues were more likely to publish manuscripts while in school and present papers at national conferences. Yet, of the 9000 graduate students surveyed over a ten year period, only 30% felt they had a faculty mentor. Even with these inconsistencies in the numbers of students having a mentor, evidence suggests that the benefits of having a mentor are numerous. For example. Kirchmeyer (2005) reported how mentors develop their protégés with the skills necessary to progress and use their influence to open doors for them. Moreover, Reskin (1979) concluded from his study on

academic chemists that a significant effect of mentoring is evident during the early years of a scientist's career. These mentoring outcomes are significant and provide concrete reasons for why mentoring is viewed as a powerful tool. In the following sections, the underlying theory used as the framework during exploration of the mentoring of the exemplar is reviewed.

### 7.2 Invitational Learning Theory

In STEM doctoral programs, an adviser invites a student to conduct research with him/her on a project. This invitation is believed to contribute to the protégé's success (Bargar, R. R., and Mayo-Chamberlain, J., 1983; Cameron, S. W. and Blackburn, R. T., 1981). However, a simple invitation by a faculty member, according to William Purkey, is of the utmost value and has the potential to generate great end results. It was Purkey who, in 1978, first offered the theory of Invitational Learning as a new paradigm of personal and professional functioning. At the core of invitational learning is the idea that a person's existence can be enriched and their development facilitated in a helping relationship. According to the theory, this is made possible by the four value-tenets: respect, trust, optimism, and intentionality. Respect is a vital element made manifest by understanding that each individual is able, valuable, and responsible. Thus, they are consistently and dependably treated in accordance with this idea. Trust is the second quality of Invitation Learning. "It recognizes the interdependence of human beings and emphasizes that professional helping should be a collaborative, cooperative activity where process is as important as product" (Purkey, W. and Schmidt, J. J., 1990). In addition, invitational learning deviates from other approaches in that it begins with a desire to help or a genuine response to concern for someone else's well-being. It does not require that trust has already been established. In contrast, Invitational Learning begins by deliberately preparing all conditions required to establish trust. The third notion of Invitational Learning is optimism.

This element is critical because its basis is the thought that human beings have not yet discovered any limits in our development. Thus, it is a natural corollary that having a dream of what may be possible for you becomes a necessary experience when one is part of a helping relationship. A belief in the untapped potential of human beings is also required. Intentionality is the last tenet of Invitational Learning. “An invitation can be defined as an intentional act designed to offer something beneficial for consideration” (Purkey, W. and Schmidt, J. J., 1990). Therefore, intentionality underlies the actions of a helper and is a major responsibility in this theory. Overall, invitational learning provides a way of engaging in the process of creating educative relationships with a focus on encouraging the realization of human potential.

### 7.3 Social Development Theory

Social development theory was introduced by Lev Vygotsky (1978). This theory purports that cognitive development occurs in a social context where people act and interact in shared experiences. The idea is that learning or development is a consequence of social interaction. Social learning is a life-long process in which cognition or knowledge is acquired. In this theory, a person knows and is able to perform certain tasks on her/his own. However, a difference in current and potential development exists that is called the zone of proximal development. Vygotsky described it as "the distance between the actual development level as determined by independent problem solving and the level of potential development as determined through problem solving under adult guidance or in collaboration with more capable peers" (Vygotsky, L. S., 1978). That is, Vygotsky believed that learning occurred in this gap between what is known and what can be known. Moreover, this theory suggests that learning occurs between people in an inter-psychological way followed by within the person in an intra-psychological way (Hua Liu, C. and Matthews, R., 2005). Vygotsky’s theory of social

development is relevant to this dissertation research because social interaction is a major part of a mentoring relationship and the distinct differences between the potential development of the protégé and what the protégé is currently capable of achieving singularly.

### 7.4 Social Learning Theory

A closely related theory to social development theory is the social learning theory of Albert Bandura (1977). Although originally applied to the understanding of aggression and psychological disorders (Bandura, A., 1973), it has recently been applied in educational contexts (Jonassen, D., 1998). In this theory, cognitive development is believed to occur in social contexts, but it emphasizes the role of observation and modeling. Bandura suggests that observation leads to encoding of behaviors, attitudes, and emotions that guides a person's action in later situations (Bandura, A., 1977). Thus, the person who is attentive and motivated rehearses the behavior profile and begins modeling it in her/his own life. In addition, Kearsley (retrieved 09/13/10) reminds the researcher that social learning theory is most successful when the modeled behavior originated with someone who is admired, and it results in outcomes that the person values. These principles are key aspects of the mentor-protégé relationship and clearly link social learning theory to this work.

### 7.5 Research Methodology

Grounded theory is used in education-based research and other disciplines because of the power that it offers in creating a substantive theory about certain phenomena or occurrences which has yet to be revealed. The exploratory and explanatory nature of this research methodology is desirable in these situations. Over the years, grounded theory has gone through many different interpretations since its introduction by Glaser and Strauss in 1967. In this dissertation, the researcher uses constructivist grounded theory as the theoretical frame for

analysis and interpretation of the data. In constructivist grounded theory, the researcher is no longer the 'distant expert' (Charmaz, K., 2000), but is acknowledged as the author of the co-constructed theory that is grounded in the data from the study participants. "Research participants' implicit meanings, experiential views—and researchers' finished grounded theories—are constructions of reality" (Charmaz, K., 2006). Similar to traditional grounded theory (Glaser, B. G. and Strauss, A., L., 1967), coding, category development, relationships, and saturation are key components of the data analysis process. In general, there are three main types of coding: word by word, incident to incident, and line by line. When beginning to generate codes, the researcher is instructed to concentrate on remaining open, staying close to the data, coding quickly, generating short codes, comparing data with data, and focusing on actions and process instead of topics. Saturation is achieved when the addition of data to the analysis process does not generate any new theoretical insight. Then, a story line emerges from iteratively conceptualizing, interpreting, categorizing, and inter-relating the participant data. This story line is the theory generated from the data.

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# CHAPTER 8

# METHODS

## 8.1 Research Design

This research study is an exploratory investigation aimed at deepening our understanding of the mentoring canon as revealed by protégés who were mentored by a Chemistry professor considered an exemplar in this field. Mentoring relationships of the chemistry faculty member spanning a thirty-year career were investigated. Particularly, this mentor was selected because of the number of awards he had received which all recognized him not only as a mentor but also as one of the most exemplary mentors in the United States. After taking one glance at the mentor exemplar's list of awards, publications, and invited talks about mentoring, it was clear that his mentoring relationships had been long lasting, widespread, and productive. To the best of the researcher's knowledge, this is the first time that anyone has used the case study of a mentor exemplar to expand our understanding of the theory and practice of mentoring in the sciences.

The research question was best answered using a case study, which employed qualitative methodology during data collection and analyses. As Marshall and Rossman (1995) note, a case study is most often used when the research question is exploratory. It provides an in-depth study of each particular phenomenon under investigation using multiple forms of data. In this study, the exploratory nature of the research question afforded the researcher the opportunity to use triangulation. This method of data collection, in which data is generated from multiple sources, allows the researcher to strengthen the credibility and validity (Patton, M. Q., 1990; Tashakkori,A. and Teddlie, C., 1998) of the study through corroboration and convergence. Thus, the researcher could ensure and sustain co-construction of meaning during data analysis.

The units of analyses were a self-selection survey answered by prospective participants, an in-depth survey answered by the protégés, a transcribed interview with the mentor, and mentoring award nomination packages.

Constructivist grounded theory was used as the theoretical inquiry method for this work. It is appropriate for fields of study about which little is known (Glaser, B. G. and Strauss, A. L., 1967). As noted before, this is true of mentoring in the STEM disciplines. It aims to be exploratory and descriptive resulting in a fundamental theory about an area of importance. The principles of this approach are used to develop a theory that is grounded in the data generated by the inquiry. In this work the researcher used a constructivist grounded theory research design, which relied on a discursive approach involving the common methods of concurrent data generation and analysis, coding and developing relationships, and construction of a story line (Strauss, A. and Corbin, J., 1998). Overall, the researcher sought to demonstrate relevance, in that the grounded theory connected to the experiences of the participants in the mentoring relationship while remaining open to reinterpretation reflective of the multiple truths and realities experienced by them while being engaged in the mentoring relationship. The results were a wholistic, integrated view of why the mentoring of the exemplar worked.

This study provides a number of advantages for the investigation of mentoring in the sciences: (1) It is a longitudinal study which spans thirty years; (2) The sample consisted solely of students in the ‘hard’ sciences, such as chemistry, physics, and engineering; and (3) The exemplar is well known for his mentoring efforts as evidenced by the many mentoring awards received. In particular, others like Hunt and Michael (1983) have expressed the importance of longitudinal research in examining mentoring because of the duration and the interpersonal dynamics of these kinds of relationships.

## 8.2 Sampling

The mentor exemplar (i.e. "Mentor") was a full professor in the Chemistry Department at a large flagship university classified by the Carnegie Foundation as a "Research University (very high research activity)". Sixty-three prospective study participants, who formerly conducted research with the Mentor, were solicited through contacts offered by the same. Eligibility criteria for participants were two-fold: (1) each participant self-defined being a protégé of the Mentor, and (2) each participant affirmed having experienced a mentoring relationship with the Mentor. Thirty participants (48%) voluntarily continued on in the study through the self-selection process after providing specifics about how they were mentored. Each participant was informed that to fully participate in the study he/she would be asked to complete a detailed online survey which would take approximately 45 minutes. Of the thirty initial participants, twenty (67%) became final participants by completing the online survey. Study participants confirmed initiation of the mentor/protégé relationship with the Mentor during one of four different stages of scientific development (high school student, undergraduate student, graduate student, post doctoral researcher) spanning three different universities over twenty-eight years. Fifty-six percent and forty-four percent of the sample were female and male, respectively, with ages ranging from 21 to $46^{+}$. At least fifty percent of the sample indicated that they were protégés of the Mentor for more than four years while only one participant stated that the Mentor was their first and only. Overall, the sample population was quite diverse, which allowed for greater understanding of the Mentor's best practices over the last twenty-eight years. Tables 1 and 2 summarize the survey response rates and the demographic characteristics of the sample, respectively.

**Table 8.1.** Survey Response Rates

| | Self-Selection Survey | Detailed Mentoring Survey |
|---|---|---|
| Number of Potential Participants | 63 | 30 |
| Response Rate | 48% | 67% |

**Table 8.2.** Demographic characteristics of the sample

| Variable | Total |
|---|---|
| Stage (n=18) | |
| High school student | 10.0% |
| Undergraduate Student | 55.0% |
| Graduate Student | 30.0% |
| Post doctoral researcher | 5.0% |
| Other | 0.0% |
| Age Range (n=20) | 21-46$^{+}$ |
| Ethnicity (n=20) | |
| Hispanic | 5.0% |
| African American | 45.0% |
| Caucasian | 35.0% |
| Asian/Pacific Islander | 10.0% |
| African | 5.0% |
| Years Knowing the Mentor | 1- 28 |

## 8.3 Protection of Human Subjects

The risk posed to the study participants was minimal; therefore, an application for exemption from the researcher's university Institutional Review Board (IRB) was submitted for approval. Assurances included anonymity for all participants and the use of non-identifying pseudonyms in all subsequent data analysis, public presentation, and peer-reviewed publication. The application was approved receiving IRB #E3582. A copy of the application, approval form,

participant solicitation letter, and participant consent form are in Appendices C and E, respectively. The researcher's Human Participant Protection Education for Research Teams Completion Certificate from the National Institute of Health is included in Appendix D.

## 8.4 Data Collection

The generation of data for this study required the collection of (a) protégé self-selection survey responses; (b) protégé mentoring survey responses; (c) transcribed interview responses from the Mentor; and (d) mentoring award nomination packages submitted on behalf of the Mentor. Initially, potential study participants were contacted by letter using postal mail. Due to a less than favorable response, a subsequent invitation was sent to them electronically via email with phone call follow-ups and automated emails to encourage completion of surveys (a) and (b). In the self-selection survey not only did the potential study participants affirm being mentored by the faculty member (i.e. Mentor), but also wrote an essay describing in detail how they were mentored. The protégé detailed mentoring survey (Appendix I) probed the mentoring relationship further specifically addressing issues such as the Mentor's disposition and behavior, attributes of the mentoring that were effective, influence on the protégé, and long-term impact of the mentoring association. The protégé mentoring survey was constructed by consulting several references (Anderson, E. M., and Shannon, A. L., 1988; Bell-Ellison, B. A. et. al., 2008; Berk, R. A. et al., 2005; Bova, B. and Phillips, R., 1984; Brown II, M. C. et al., 1999; Burlew, L., 1991; Erkut, S. and Mokros, J. R., 1984; Handelsman, J. et al., 2005; Jacobi, M., 1991; Koro-Ljungberg, M. and Hayes, S., 2006; Paglis, L. L. et al., 2006). The survey was administered online through www.surveymonkey.com. A semi-structured interview with the Mentor was designed to explore and delineate, from the Mentor's perspective, what exactly he does when mentoring and why it is so effective. The interview with the Mentor was digitally recorded and

transcribed with all identifying references deleted. Lastly, two nomination packages for prestigious mentoring awards were collected; each one provided data from academic colleagues who believed the Mentor should be honored for the efficacy of his craft.

## 8.5 Data Analysis

Four sets of data from different sources were analyzed in this study. First, the protégé self-selection survey had two specific questions and open-response essay. Second, the protégé mentoring survey comprised 16 specific questions, 14 open response questions, and 4 questions, which contained a total of 126 responses, developed using a 5 point likert scale. Third, the researcher developed fifteen interview questions for the Mentor, but used a reflexive mode of interviewing in which concurrent data generation and analysis could be achieved. Thus, *ad hoc* questions were asked during the interview process to clarify responses and explore specific emergent topics more deeply. This type of questioning resulted in over forty questions being asked of the exemplar. Data collection ceased when saturation was reached (i.e. no new patterns in the Mentor's responses were emerging in the data). Fourth, two prestigious mentoring award nomination packages were analyzed. Each nomination package contained different sections because they were for two different awards. Not all of the sections from each nomination package were provided to the researcher. For each set of data, the researcher analyzed them for recurrent themes through a process of individual analysis followed by the development of a coding structure. Constant comparison of coding and ideas between the four data sources were considered. Inter-relationships began to emerge across data collection instruments. These themes were further analyzed by determining what theoretical categories the data suggested. Then, the categories were integrated until saturation occurred where new incidences revealed no

new aspects in the categories. Thus, a "unified theoretical proposition" or "generalized theory" (Glaser, B. G., 1965) of why the mentoring by the exemplar worked was produced.

## 8.6 Limitations

This study employed a qualitative case study design. Case study relies on in-depth inquiry of a specific phenomenon in its natural context. The focus during analysis is on the viewpoint of each individual participant (Gall, M. D. et al., 1996). Case studies are viewed as 'limited' by Gall, Borg, and Gall (1996) for two reasons: (1) issues with transferability of the research findings, and (2) difficulty with disguising the identity of the persons participating in the study. In this study, the generalizability of the results to mentors and students in other academic disciplines may be problematic. The experiences of a mentor and/or a protégé in the hard sciences (i.e., chemistry, physics, and engineering) may be quite different from the mentoring relationship experienced in other disciplines (i.e., history, education, etc.).

Although the research approach was primarily qualitative, some of the qualitative survey responses were converted to quantitative data to assist the researcher in rankings to determine "why" the mentoring of the exemplar was successful. One challenge with conversion of qualitative data to quantitative data is the loss of process orientation only viewable in qualitative data. However, in this case the converted data was not from open-ended response items. Moreover, another limitation is that the researcher functioned as the data collection instrument during the interview with the exemplar. Functioning in this role is seen by some (Patton, M. Q., 1990) as one of the greatest weaknesses of qualitative methods. Human bias and lack of interviewing skills may threaten the reliability and validity of the data. To address this limitation, the researcher used a reflexive mode of data collection and analysis in which *ad hoc*

clarifying questions and additional probing questions were asked, particularly during the interview of the exemplar.

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# CHAPTER 9

# RESULTS AND DISCUSSION

In this chapter, a discussion of the results obtained from analyzing each data source is provided. Each set of data contributed significantly to the generalized theory of why the mentoring of the exemplar worked. Thus, it is important to describe specific aspects of the results in the context of the exploration.

## 9.1 Mentor's Conceptions of His Mentoring Strategies

Three major issues emerged from the exemplar interview data. These were the exemplar's motivation, the exemplar's ways of doing, and protégé transformation. The primary motivation of the exemplar stemmed from a desire to make a difference and the pride in helping someone. During the interview, the exemplar made reference to the 'mentors' who helped him know which path to proceed on along the way. In particular, the exemplar was interested in mentoring the disenfranchised and/or the less privileged, which as he stated was not necessarily linked to race. The exemplar worked in ways that emphasized that the protégé was the central focus while mentoring. For example, the exemplar would take a special and active interest in the person even before they would become a protégé. Through observation, the exemplar would 'see something' in the potential protégé. According to this study in at least 85% of the cases, an informal mentoring relationship would begin by mutual agreement. From the exemplar's perspective, he would always look out for the individual, and never let the science (i.e., his career) override the individual's needs and concerns. Much of the exemplar's ways of doing centered on the protégé being the agent of her/his own destiny. The exemplar would help the protégé to figure out his/her passion (i.e., what would they do in life for free if they were wealthy?). One approach, in particular, would be the exemplar figuring out or determining the

strategies or 'keys' that would 'unlock' or motivate them to pursue her/his passion. The focus would be upon getting the maximum out of everything she/he was doing. Therefore, the exemplar would set extremely high expectations and standards, but never give up on a protégé. Moreover, the exemplar would keenly listen and provide guidance as the protégé would engage the pursuit of this passion. When decisions were required, the exemplar would list possible solutions, but allow the protégé to make the final decision. In this way among others, the exemplar would teach the protégé 'survival skills.' All protégés would be able to figure things out for themselves. In some cases, the exemplar used 'tough love' as a means of emotional support. The exemplar described a protégé who needed this kind of support as an undergraduate in his laboratory. Therefore, he recommended him to a graduate advisor who had high standards. Interestingly, the advisor has commented that this was the best Ph.D. he has ever produced and several times a year asks the exemplar to send him more just like him. Through this example, the exemplar opened the idea of his mentoring being transformative for the protégé. The exemplar described another transformation that occurred in one of his protégés because of his ways of doing. In that case, the protégé wanted to remain in his research group after conducting undergraduate research with him. The exemplar refused to allow her to stay and is certain that "pushing people out of the door, exposing them to new environments helps them to grow up."

### 9.2 Colleagues' Conceptions of the Mentor Exemplar

The colleagues who submitted nomination packages for mentoring awards on behalf of the mentor exemplar were consistent in their assessment. His is a genuine mentor. In agreement with the exemplar's desire to make a difference, the colleagues identified his motivation for mentoring as the desire to turn on one young, unengaged mind. His approach to mentoring was captured in two distinct statements. One, the exemplar's mentoring was enabled

by his personal standards and drive for excellence. Two, the exemplar always held a long-term view for the protégés' best interests. In addition, the colleagues enumerated characteristics of the mentor that they believed were critical to the success of his mentoring. These included deep personal concern, gifted, gentle, professional, humorous, good heart, devoted, dedicated, and nurturing. Moreover, specific actions were identified that described the exemplar's mentoring style. Among them, emphasizing the primacy of the student, guiding students, constant encouragement, and uncompromising demand for the highest academic and professional standards were listed. The entire list is shown in Table 4.1.

**Table 9.1** Actions Associated with the Mentoring Style of the Exemplar

---

- Leads by example
- Supportive
- Emphasizes the primacy of the student
- Spends valuable time assisting others
- Guides students
- Constant encouragement
- Accommodates students who want to work in his lab and finds funding or them
- Counsels and directs students many years after they have left the university
- Helps where he can
- Advises
- Listens
- Door always open to students
- Uncompromising demand for the highest academic and professional standards
- Influences people who are not his students/colleagues

---

One colleague highlighted the impressiveness of the number of protégé interactions, but emphasized the "broadness and depth" of the protégé interactions. He described the effect of the exemplar's intense mentoring efforts in Kenya, which resulted in the creation of a pipeline of excellent African students pursuing Ph.D. degrees at LSU. In addition to the exemplar's

mentoring moving beyond LSU and the United States, both colleagues spoke about it being devoid of racial and cultural boundaries (foreigners, majority, minority) as well as age boundaries (students, mid-career faculty, peers). Moreover, the other colleague remarked about the reproducibility of the exemplars' mentoring success personally witnessed by him in over a dozen other young scientists. Lastly, both colleagues agreed that many of the exemplar's protégés and even peers emulate his mentoring philosophy reproducing his legacy in their own careers.

### 9.3 Protégés' Conceptions of the Mentor Exemplar

The protégés' conceptions of the mentor exemplar began with an understanding of their definition of a mentor. In over 50% of the responses, the protégés identified five key functions and attributes of a mentor. They are guide, advise, more experienced, focus is protégé success and trustworthy. A schematic representing the most important (largest circle) to the least important (smallest circle) mentor attributes and functions in the protégés' definition of a mentor are shown in Figure 4.1. Interestingly, two of the five major aspects of the protégés' mentoring definition were among the first impressions of the mentor exemplar when he was first met. These are successful researcher (or more experienced) and focus on full potential of students (or focus is protégé success). A third aspect, caring, that was a part of their definition of mentoring was also recognized in the exemplar. In addition, the protégés indicated that the exemplar's interest in them and that he encouraged them be the best they could be were the two principal reasons that convinced them that he would be a good mentor. It should be noted here that the protégés' first impressions of the exemplar influenced their decision to be mentored by him. The two primary reasons given were also consistent with their definition of a mentor, namely focus is

protégé success.  In contrast, the protégés initially hoped to primarily receive research experience from the mentoring relationship with the exemplar.  Many of them had no expectations and

**Figure 9.1** Schematic of the protégés' definition of mentoring.  The most important aspect is indicated by the largest circle leading to the least important aspect, which is represented by the smallest circle. The highlighted circles were also indicated as part of the protégés' first impressions of the exemplar.

hoped to receive nothing in particular.  However, in terms of whether or not their expectations were fulfilled the majority of the protégés expressed that the mentoring relationship exceeded anything that they had hoped for.  Ninety-five percent (95%) of the protégés indicated that the exemplar was exceptionally effective and very effective as a mentor.  In addition, they indicated

that the exemplar was 60% more effective and much more effective in comparison to other mentors that they had. These responses corroborate the sentiments of the exemplar's colleagues that his mentoring is effective and sometimes described as legendary.

When asked in open response to identify the personality traits that contribute to the exemplar's effectiveness as a mentor, the protégés identified caring, leader, sense of what is right, energy & passion for what he does, tenacity, encourager, seriousness, and good listener. This question was followed up by asking the protégés to identify the characteristics of the exemplar's mentoring that contribute to his effectiveness. Many of the responses were echoed in the protégés' definition of a mentor, their first impressions of the exemplar, and the reasons why they wanted to be mentored by him. A schematic of the responses is given in Figure 4.2. To delve deeper into the issue of effectiveness with the hopes of eliciting responses that would provide insight into why mentoring by the exemplar worked, the protégés were asked to specifically recount what the exemplar did (or does) that was (is) effective as a mentor. The

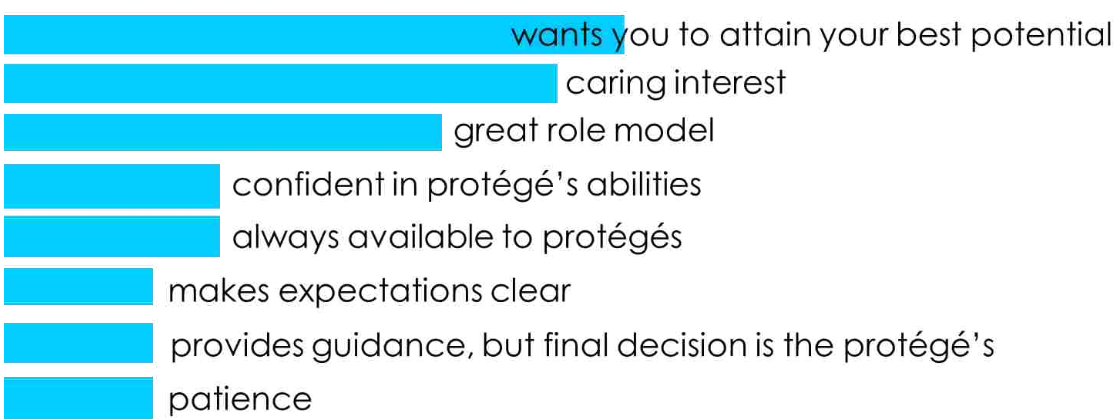


**Figure 9.2** Schematic of the key characteristics identified by the protégés that contribute to the effectiveness of the mentoring by the exemplar.

same themes continued to emerge from the responses: (1) encouragement through diverse means, (2) supportive of protégés interests and goals, (3) maintains open line of communication, (4) made me feel important, and (5) instilled a dialogue about science that is without boundaries. More specifically, the protégés were asked what top three things the exemplar does as a mentor that all mentors should do? The top three items were that he cares, encourages, and challenges his protégés. The consensus by the protégés was that the exemplar does one thing that most contributes to his effectiveness in mentoring, which is continuously helping others to reach their goals and succeed. This consensus was confirmed in the data from the exemplar and the colleague nomination packages. Thus, it was recognized as what Glaser (1965) calls an underlying uniformity, which ultimately becomes a part of a generalized theory. Every aspect of the exemplar's mentoring style stemmed from wanting each protégé to succeed in their passion, which generated an intentional effort on the part of the exemplar to help them in all ways that were available to him. The overall result was that the protégés experienced a positive transformation in their life, and the transformation was independent of age, race or gender. From the mentoring relationship with the exemplar, the protégé learned (i.e., 60%) that he/she was capable of accomplishing anything.

## 9.4 Generalized Theory: Why the Mentoring of the Exemplar Worked

Exploration and analysis of the data generated in this study suggests that the mentoring of the exemplar worked because there were three stages of the mentoring process: (1) intra-psychological positioning by the mentor, (2) inter-psychological exchange between the mentor and the protégé, and (3) intra-psychological transformation within the protégé (Figure 4.3). In stage one the mentor possessed an internal locus of control in which he believed that he was capable of positively impacting a protégé. With the intra-psychological positioning, the

mentor possessed specific motivation, intention, and commitment toward potential protégés. The mentor exemplar in this study was motivated by taking pride in helping someone. He created an invitational learning (respect, trust, intention, and optimism) environment that focused on the protégé identifying and pursuing his/her passion. The intention of the exemplar was to informally invite potential protégés into a mentoring relationship with the expectation that he would make a difference in his/her life. The commitment of the exemplar was evident in that the protégés' needs and concerns took precedence over the science (i.e. the career). Thus, every thought and action produced by the exemplar for the protégé was motivated by the internal commitment to help she/he reach their goals and succeed. The motivation, intent, and commitment to the protégés were confirmed in the nomination packages and the in-depth protégé survey. In stage two, mutual exchanges occur between the mentor and the protégé. Initially, a close match existed between the protégé's definition of a mentor and his/her first impressions of the exemplar in this study. In turn, the mentor's authenticity was verified by the protégé. This required that the exemplar's actions remained consistent with the protégés' initial first impressions. In

**Figure 9.3** Schematic of the generalized theory of why the mentoring by the exemplar worked.

addition, the protégé's willingness to change must be gauged by the mentor. This factor was expressed as being very critical to the development and sustainment of the mentoring relationship. Through this process of exchange, trust, which was identified in this study as one of the two most critical factors in the development and sustainment of the mentoring relationship, is established. The other most critical factor was effective communication, a key to any kind of intra-psychological exchange. During the on-going dynamic social interaction of the exemplar with the protégés, cognitive acquisition occurred. The researcher presumes that the protégé processed new information, conceptualized and internalized its use, and selected new preferences. In particular, the exemplar provided intentional ways of assisting the protégé in moving to new levels in the developmental process. He looked for 'keys' to 'unlock' the protégé and motivate them. Without consciously doing so, the exemplar employed scaffolding (Brown, A. and Ferrara, R., 1985) in which he adjusted the level of his help in response to the protégé's level of performance. For example, the exemplar expressed using 'tough love' for some of the protégés and not for others. In another case, a protégé explained in an open response question that the exemplar believed in him until he could believe in himself. The exemplar seemed to mediate the development of the protégé in the inter-psychological stage although the exchange was reciprocal (Brown, A. and Ferrara, R., 1985). In stage three, the intra-psychological transformation within the protégé is experienced. As the protégé continued to trust the exemplar and actively seek out advancements in the zone of proximal development, internal belief systems began to change. The protégés in this study received more value than expected from the mentoring relationship with the exemplar. As described by Bandura, the protégé observes the belief systems, attitudes, emotions, and behaviors of the exemplar. Over time, she/he models them often enough that they become permanent fixtures in their own belief systems and personal

attributes. Such transformations were described in the triangulation data. The nomination package data revealed how protégés of the exemplar emulate his mentoring philosophy producing his legacy in their own careers. One protégé described his life-changing transformation in detail. “My success is due to the emotional change that ‘the exemplar’ was able to make in me at that time in my life. It is really amazing how much one person can change someone’s life like ‘the exemplar’ did for me.” Most of the protégés (60%) believed that they could accomplish anything as a result of their mentoring relationship with the exemplar. Moreover, other significant transformations were experienced by the remaining protégés. Each one benefitted in ways that exceeded what was expected.

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# CHAPTER 10

## CONCLUSIONS AND IMPLICATIONS

This study was an exploration of the mentoring of an exemplar over a thirty year period with the aim of furthering our knowledge of the mentoring canon. The research was centered on one main question and three sub-questions. To explore the research questions, the researcher developed a participant self-selection survey, an in-depth protégé survey, and semi-structured interview questions. Examples of these instruments are found in Appendices F, H, and I.

The main research question was: Why does the exemplary mentoring of Dr. Isiah M. Warner work?

Examination of the data in this study suggests that the exemplary mentoring of Dr. Isiah Warner worked because of a dynamic three-stage process that occurs throughout the life of the mentoring relationship. Stage one is a pre-conceptual positioning by the mentor where the exemplar is motivated, intentional, and committed to having a positive impact on the protégé's life. In stage two, a series of exchanges occur between the exemplar and the protégé and cognitive acquisition happens. In this stage, the basic tenets of trust, authenticity, developmental engagement, and effective communication are experienced. Stage three is the intra-psychological phase for the protégé. During this stage, the protégé observes and internalizes aspects of the exemplar's attitude, behavior, emotions, and belief systems that eventually become permanent parts of his/her own personal attributes. Throughout stages two and three, maladaptive protégé behaviors are replaced by positive developmental outcomes.

Additional sub-questions were:

1. What factors were critical in the development and sustaining of the mentor/protégé relationship with Dr. Isiah Warner?

Nine factors were determined to be critical in the development and sustainment of the mentoring relationship. The two most critical factors were trust and effective communication. The very critical factors were honesty, openness in the exemplar, willingness by the protégé to change, and the exemplar's knowledge of the protégé's strengths. Other factors deemed critical were relationship flexibility, openness in the protégé, and protégé's knowledge of her/his strengths.

2. What are the specific characteristics, attributes, and actions that Dr. Isiah M. Warner exhibits during the mentoring relationship that make him an effective mentor?

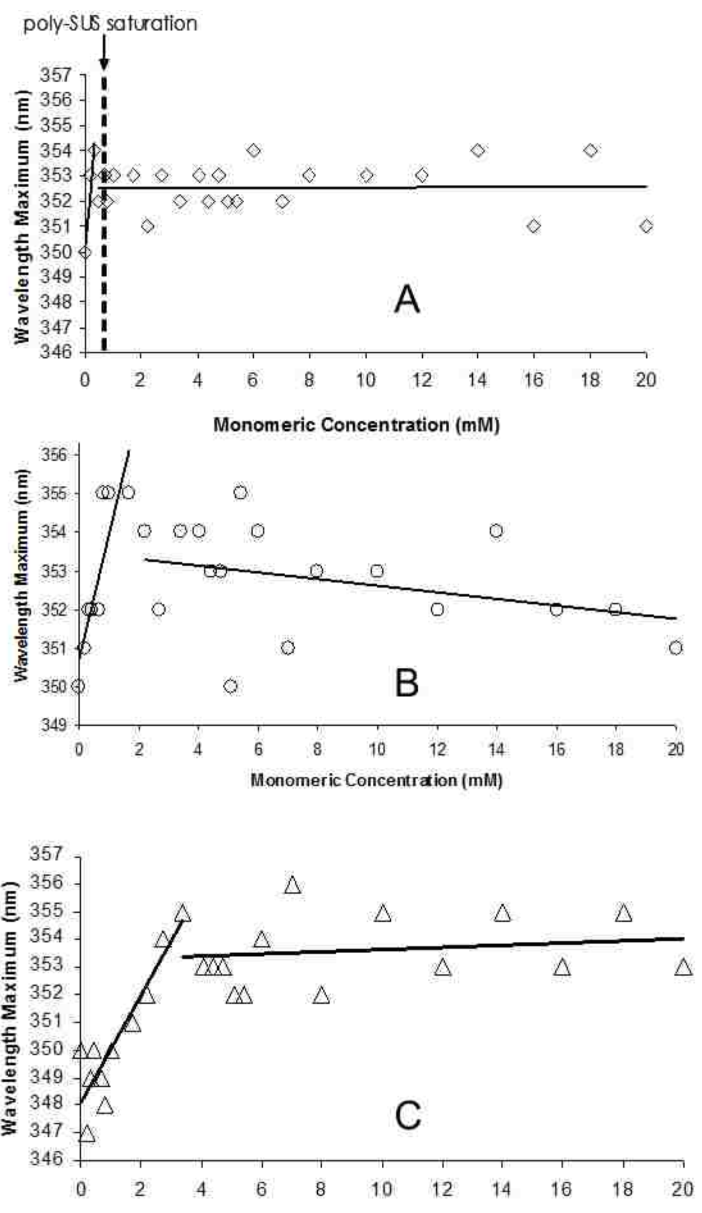
Many characteristics, attributes, and actions used by the exemplar were identified throughout the triangulation data, but three main gauges of his effectiveness were that he cared, he used constant encouragement, and he challenged his protégés.

3. What were the benefits received by the protégé as a result of being mentored by Dr. Isiah M. Warner?

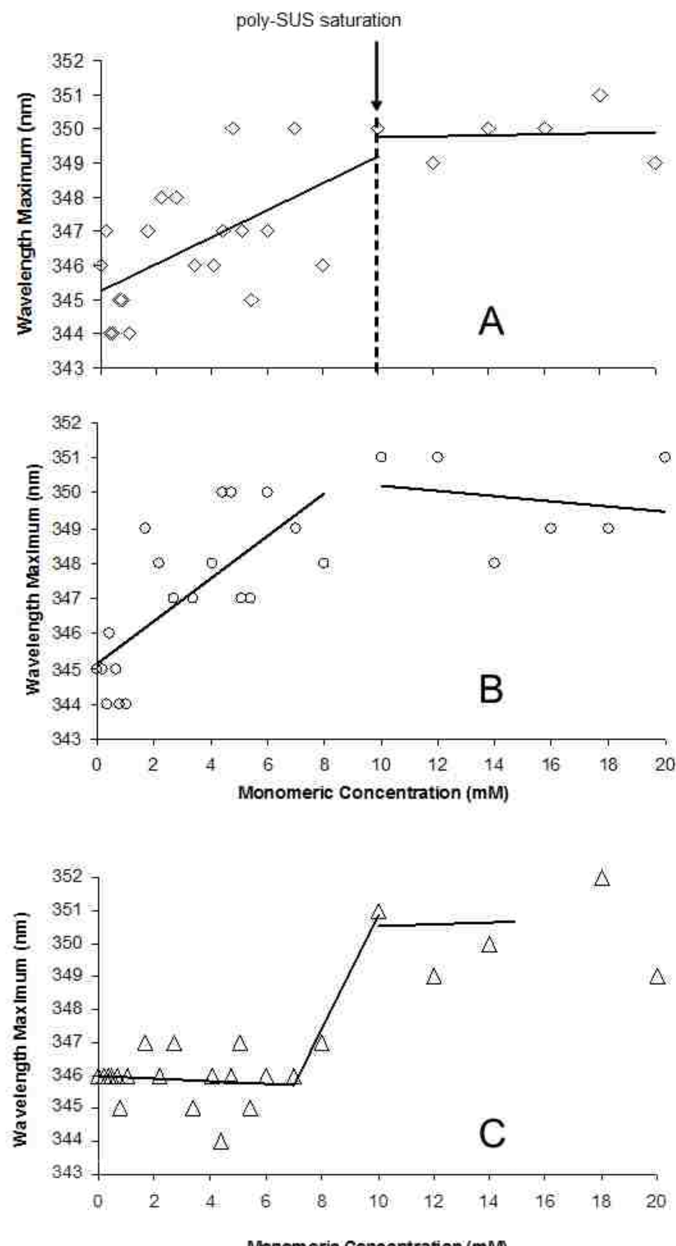
The protégés responded to this question in at least two ways. One, the benefits were psycho-social transformations. More than half of the protégés expressed that they believed in their ability to be a scientist or anything they desired. Others explained how they learned how to believe in their abilities and have a balance between their professional and personal life. Two, the benefits were career-related. Forty-five percent of the protégés said that a publication, poster presentation, or an oral presentation was a direct result of them being mentored by the exemplar. Thirty-three percent said that a job promotion, a prestigious award, an organizational leadership position, or a job change was directly attributed to the mentoring relationship by the exemplar.

This research is beneficial because it is the first time that a case study has been done on a mentor exemplar to determine why his mentoring efforts have been so successful. It offers a general theory for why the mentoring of an exemplar works. It also lays the groundwork for subsequent case studies and explorations of why individual or group mentoring efforts, like in the Mathematics department at the University of Iowa, work. In addition, the research could be expanded to determine why certain percentages of the mentoring relationships by mentor exemplars do not work. Because of the exploratory nature of this research on mentoring, many other opportunities for further research are also available. First, grounded theory is a qualitative inquiry method. The generalized theory obtained from this type of research is typically followed up by testing the theory using a quantitative approach. Second, a case study methodology was used. Additional research is needed to ascertain whether or not these findings are transferable even within the context of the same areas of science.

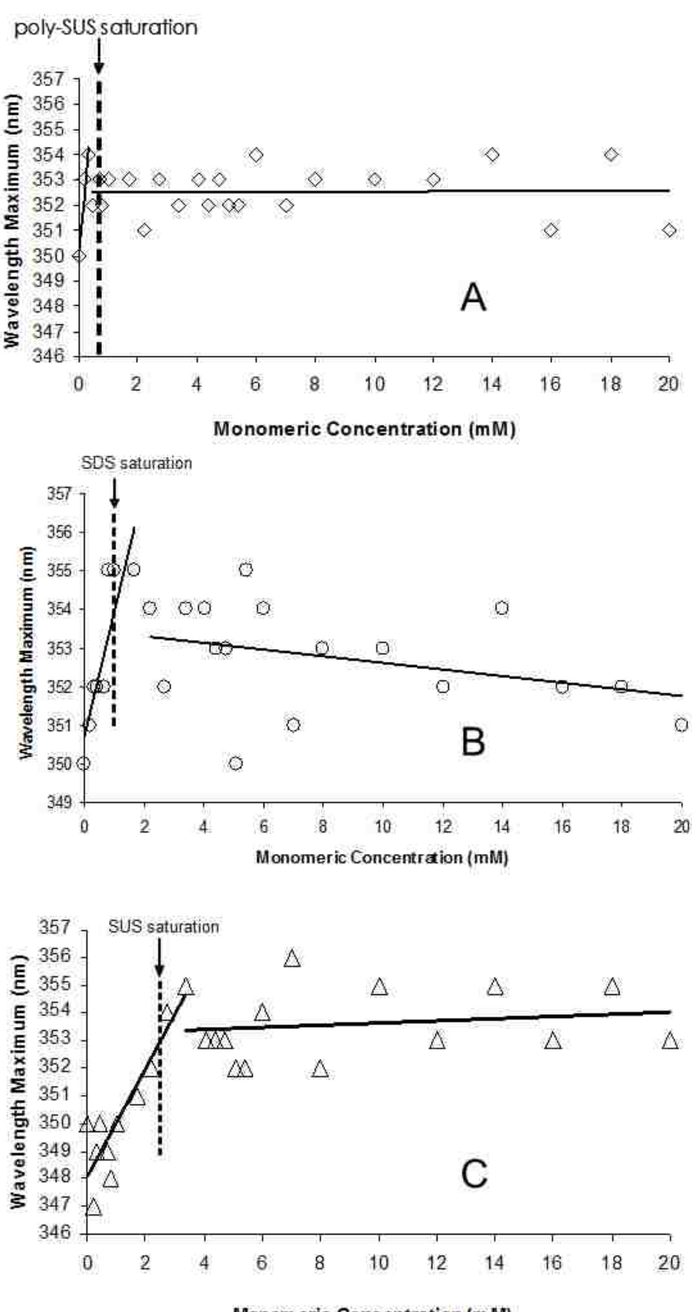
## APPENDIX A: FLUORESCENCE WAVELENGTH MAXIMA SHIFTS OF CYTC, OVA, AND α-LAC




**APPENDIX A.1** Fluorescence wavelength maxima shifts of $Trp_{aLAC}$ in the presence of increasing monomeric concentration (0 – 20 mM) of (A) poly-SUS (open diamonds), (B) SDS (open circles), and (C) SUS (open triangles) in association with aLAC (68 µM) determined by steady state fluorescence ($\lambda_{ex}$ = 295 nm, 25°C). The lines have been included to guide the eye.

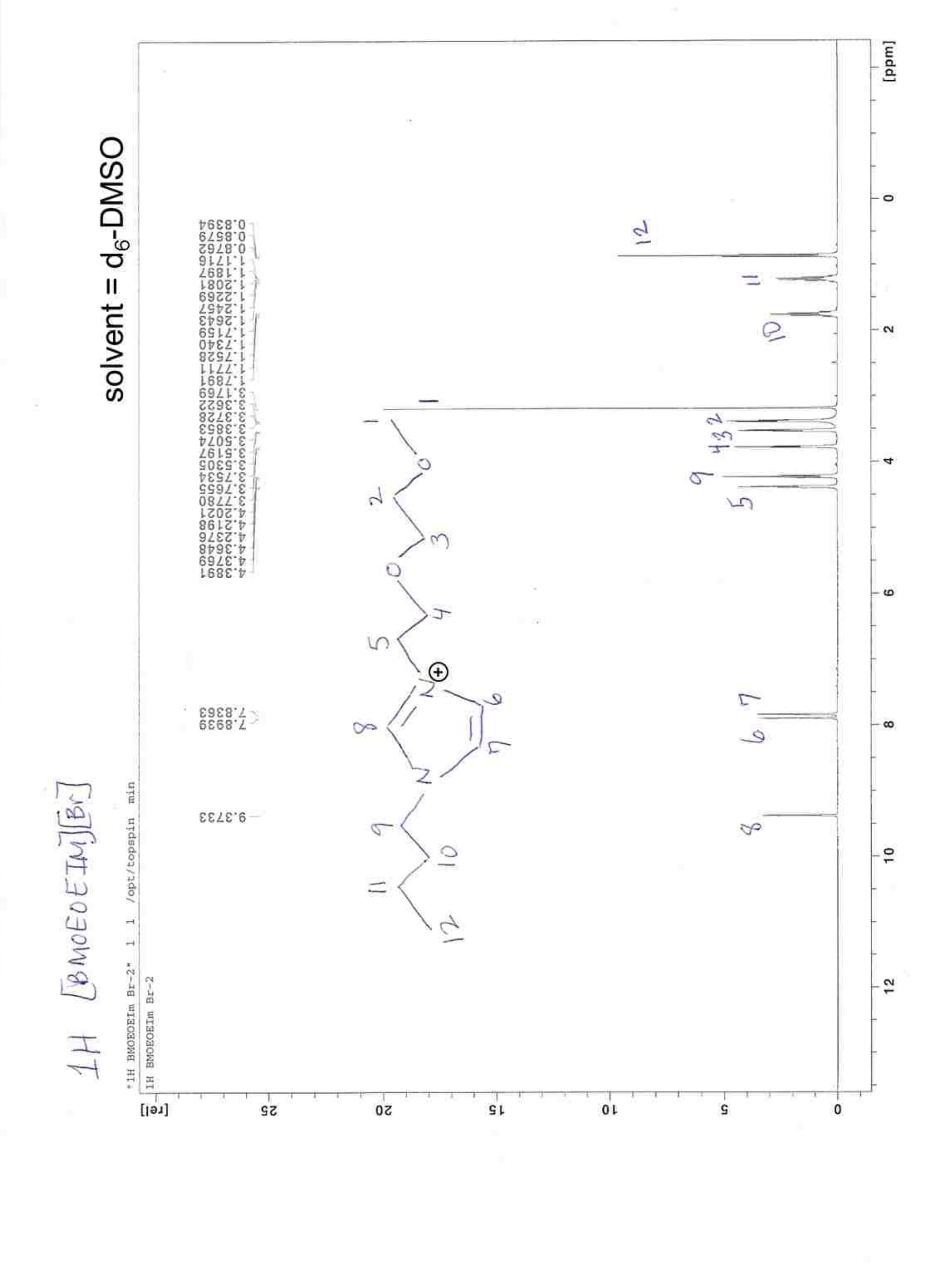


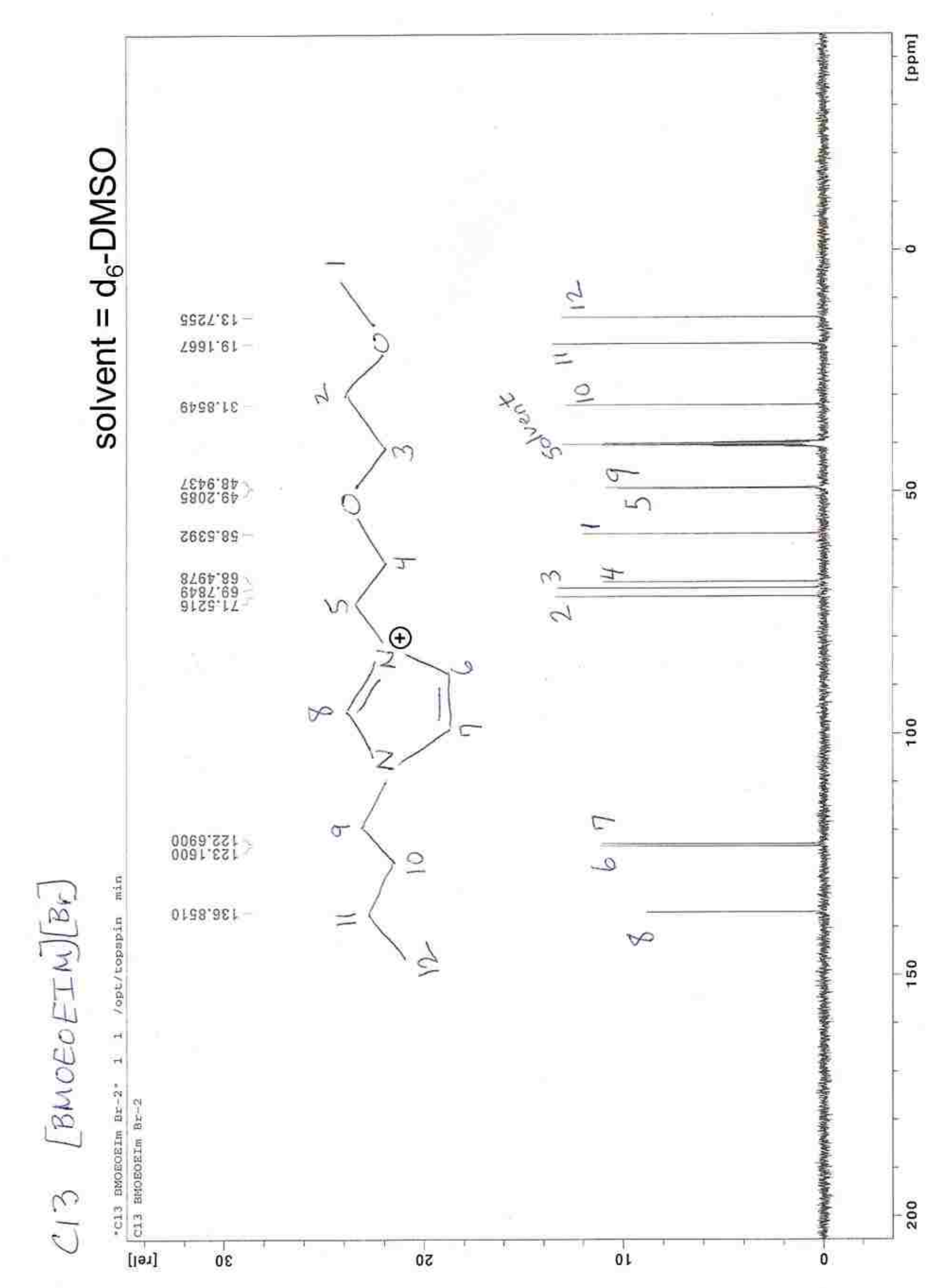

**APPENDIX A.2** Fluorescence wavelength maxima shifts of $Trp_{OVA}$ in the presence of increasing monomeric concentration (0 – 20 mM) of (A) poly-SUS (open diamonds), (B) SDS (open circles), and (C) SUS (open triangles) in association with aLAC (68 μM) determined by steady state fluorescence ($\lambda_{ex}$ = 295 nm, 25°C). The lines have been included to guide the eye.




**APPENDIX A.3** Fluorescence wavelength maxima shifts of $Trp_{OVA}$ in the presence of increasing monomeric concentration (0 – 20 mM) of (A) poly-SUS (open diamonds), (B) SDS (open circles), and (C) SUS (open triangles) in association with aLAC (68 μM) determined by steady state fluorescence ($\lambda_{ex}$ = 295 nm, 25°C). The lines have been included to guide the eye.

## APPENDIX B: ^{1}H-NMR AND ^{13}C-NMR SPECTRA OF IONIC LIQUIDS

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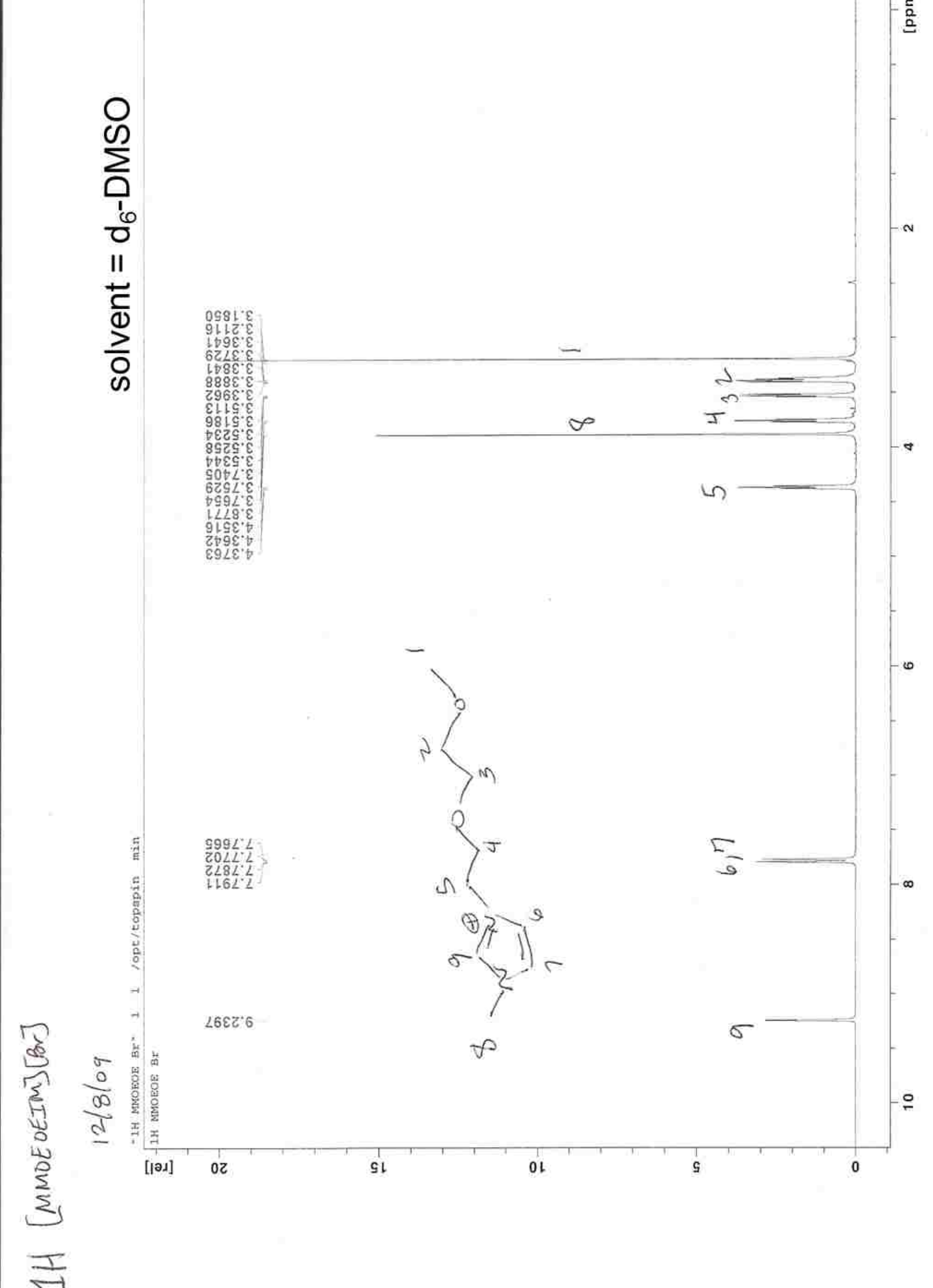
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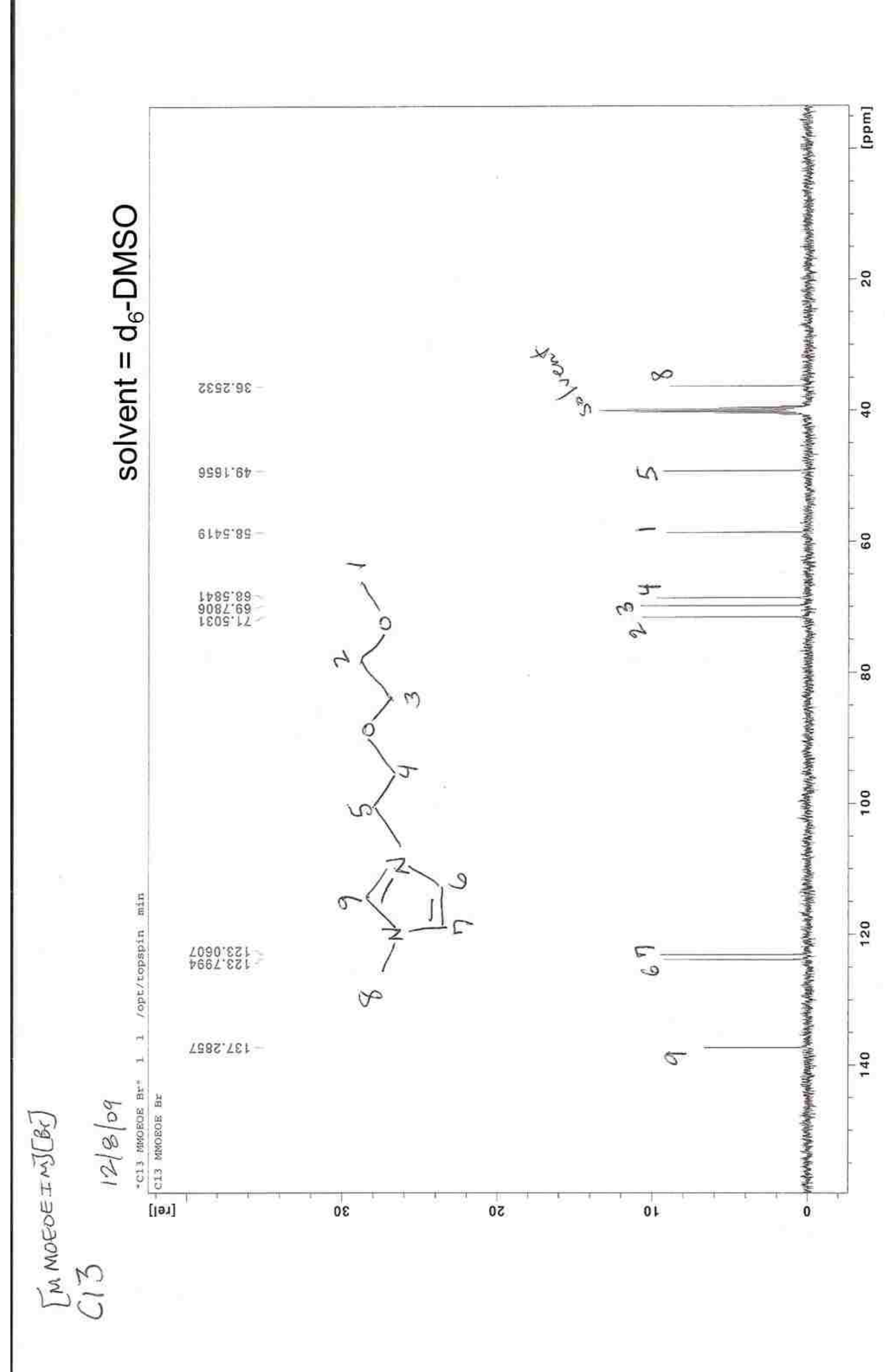
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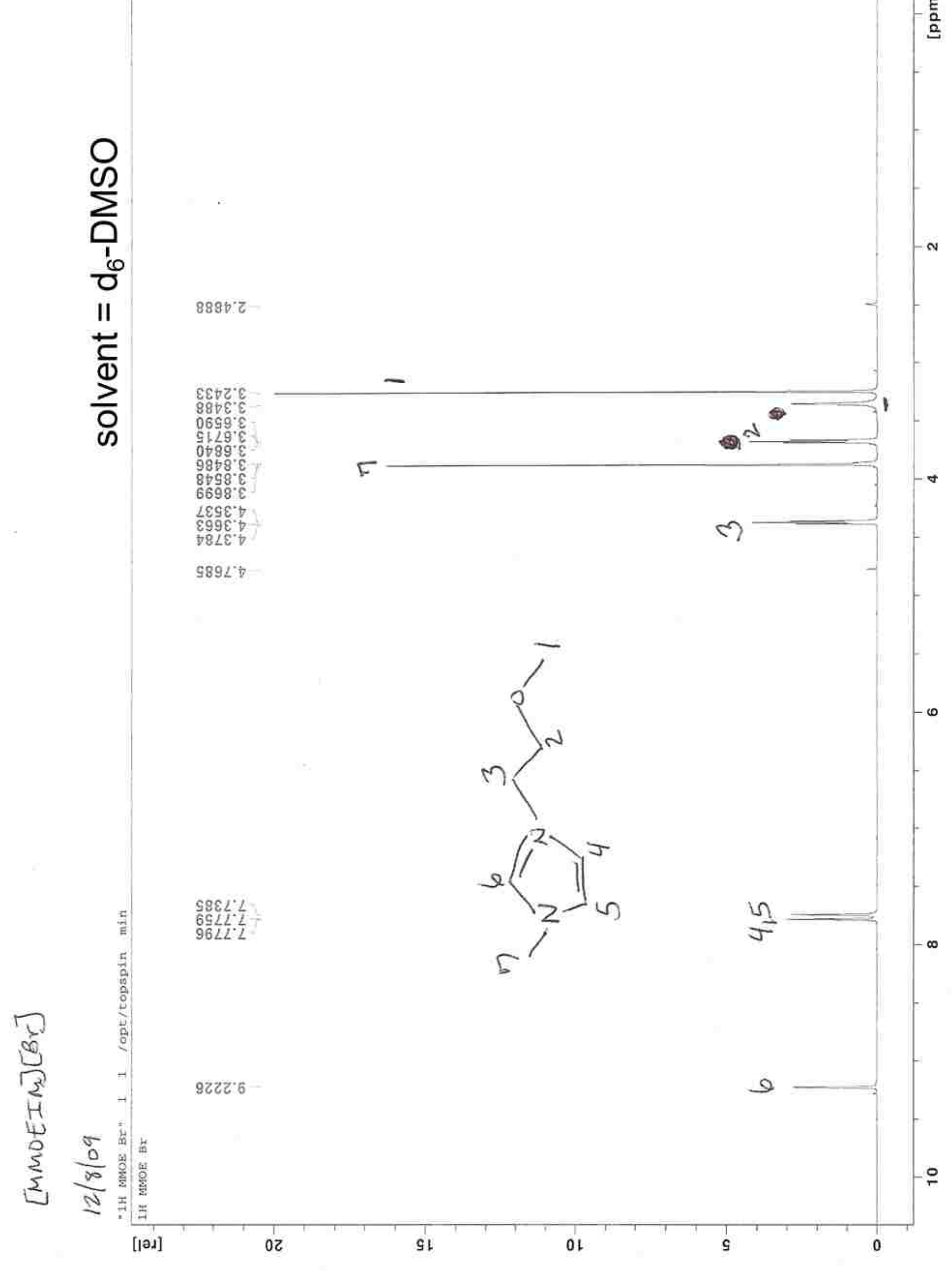

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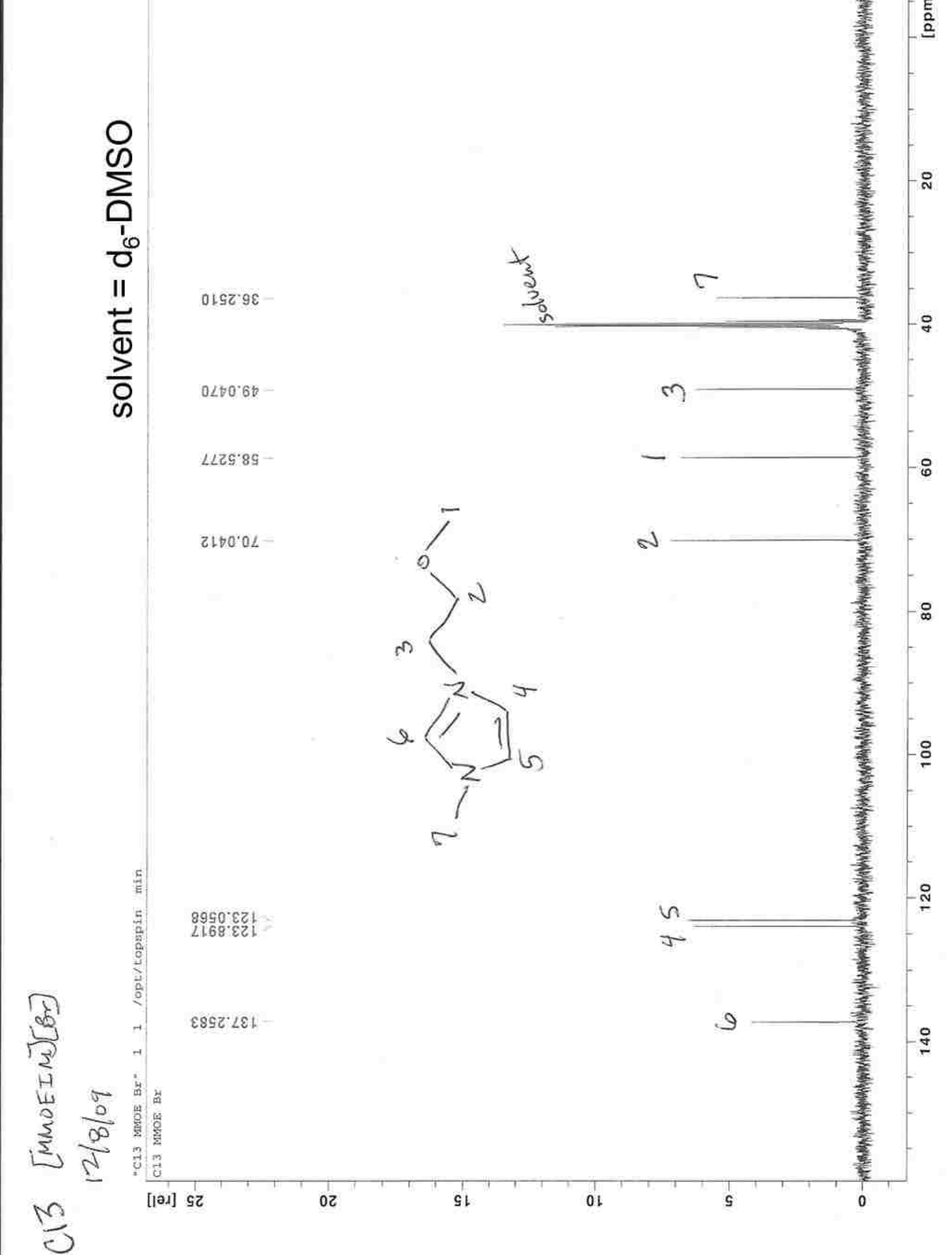
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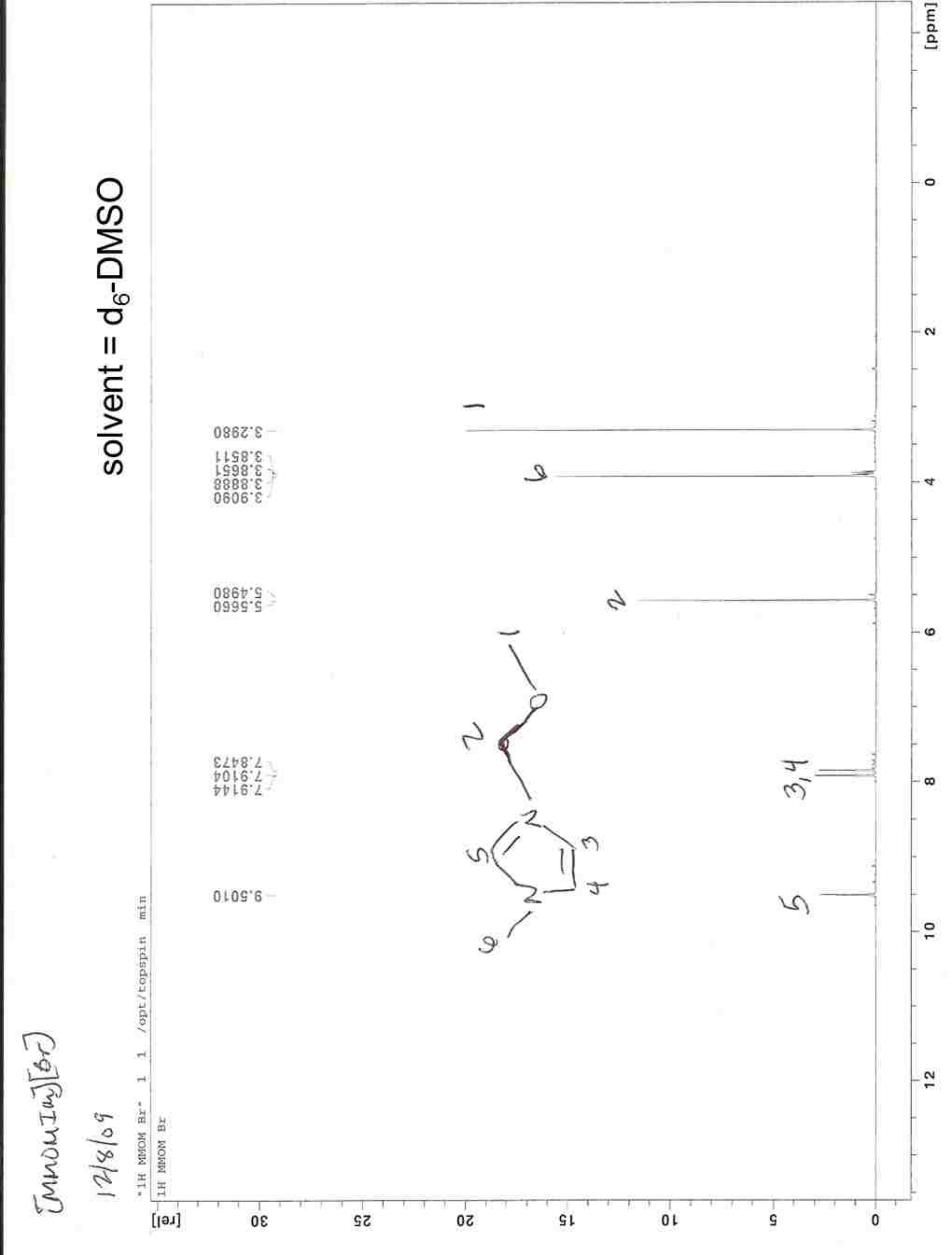
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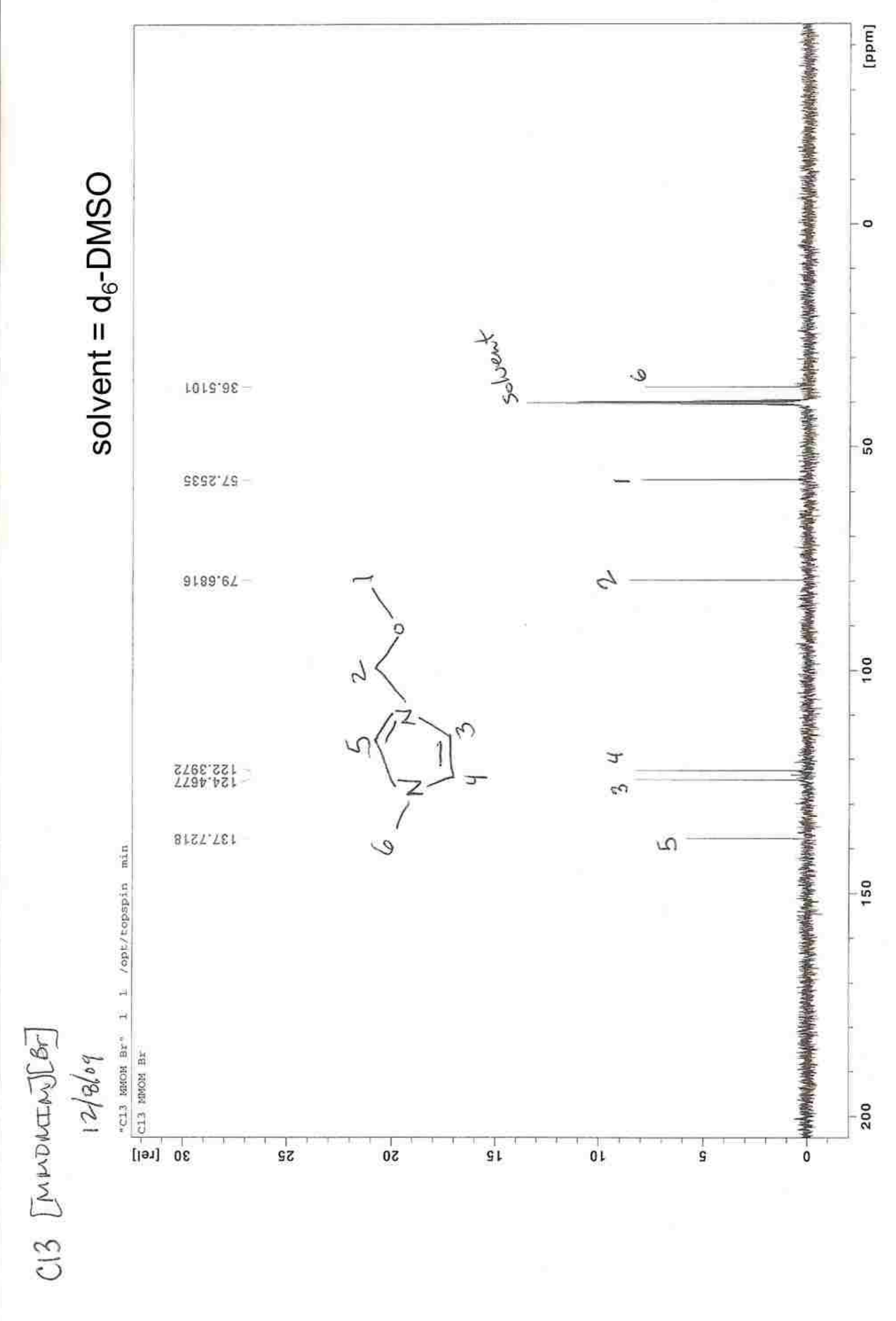
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solvent
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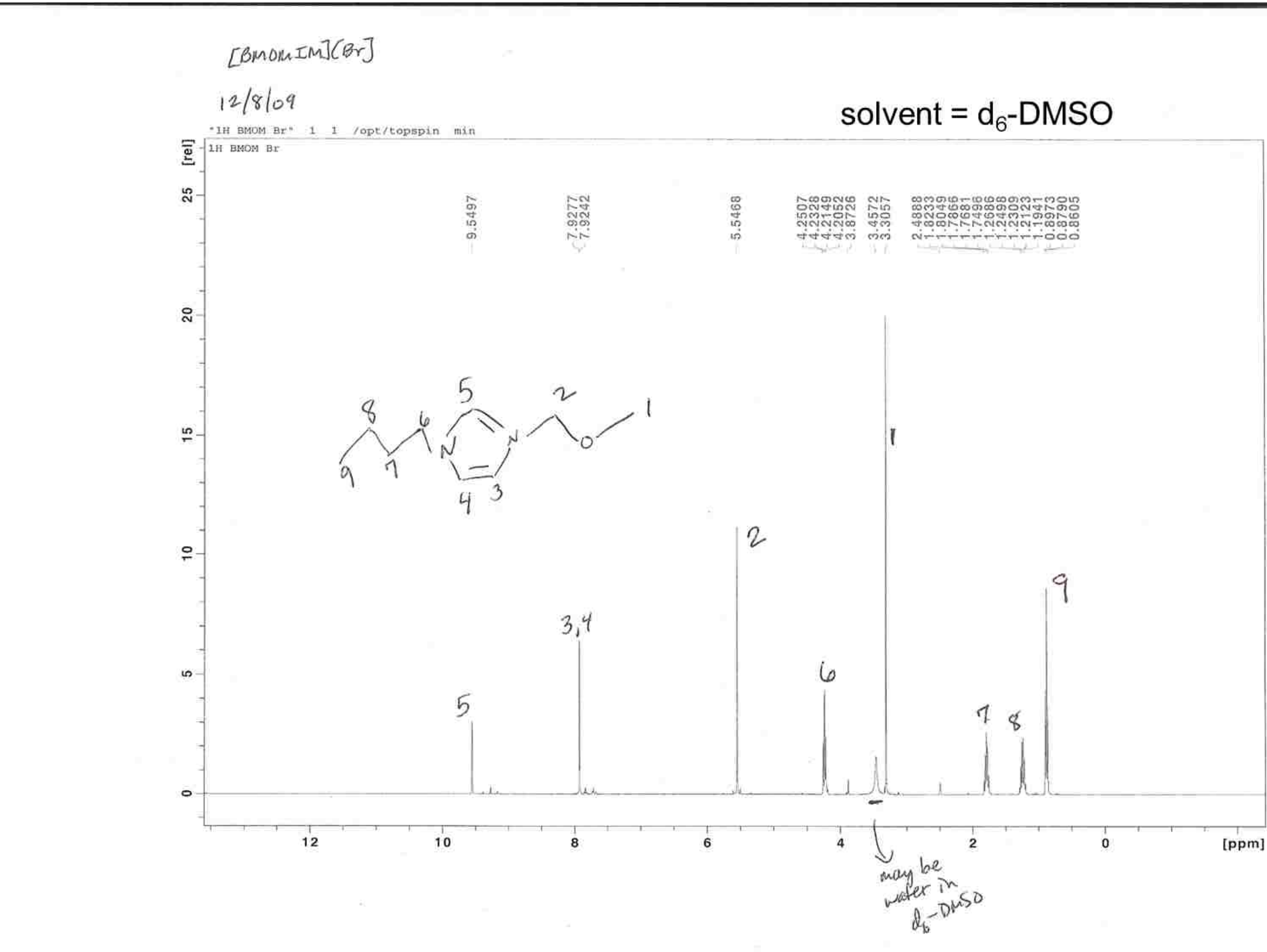
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12/8/09
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4.2149
4.2052
3.8726
3.4572
3.3057
2.4888
1.8233
1.8049
1.7866
1.7681
1.7496
1.2686
1.2498
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0.8605
may be water in $d_6$-DMSO
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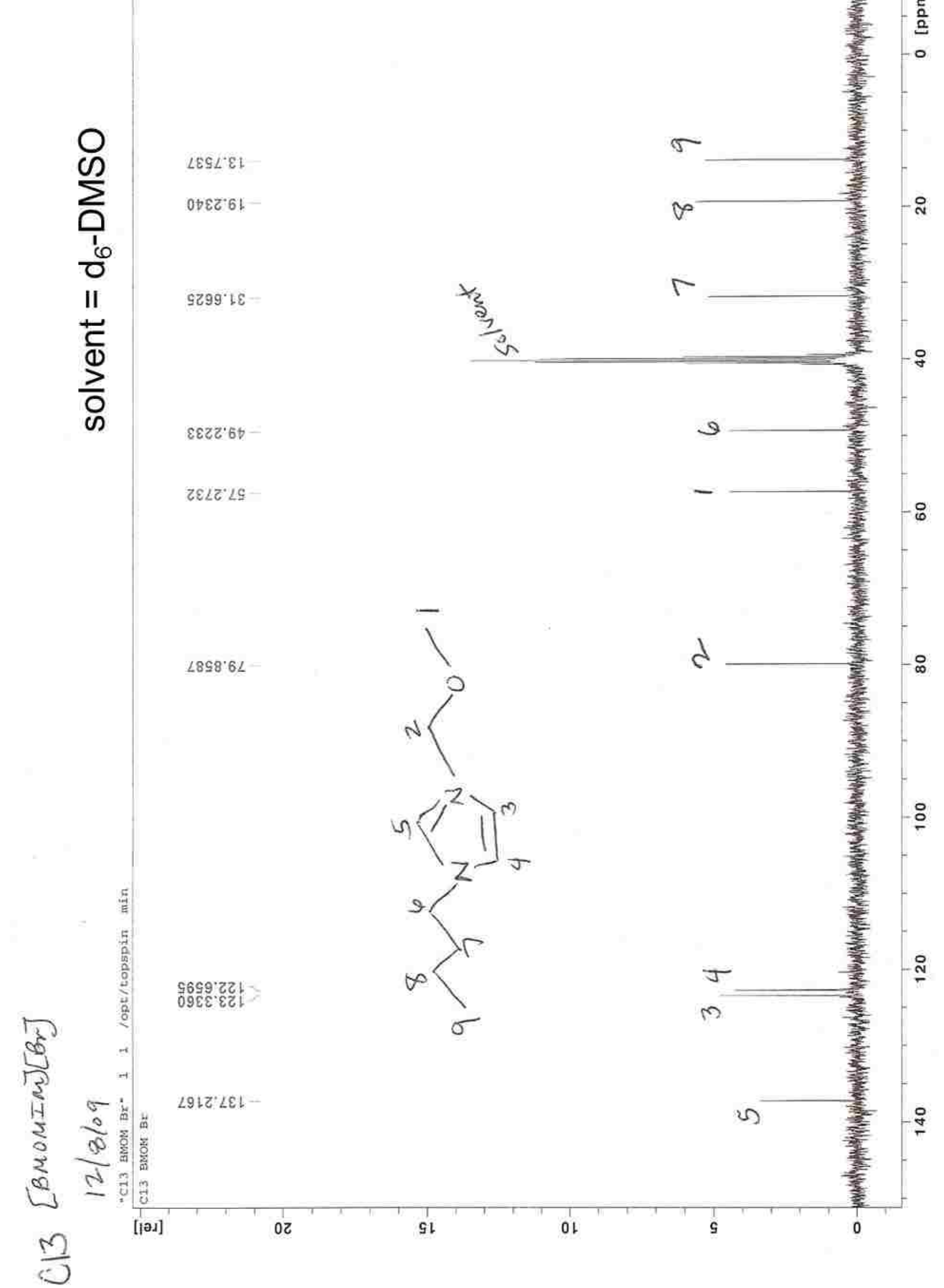
[rel]



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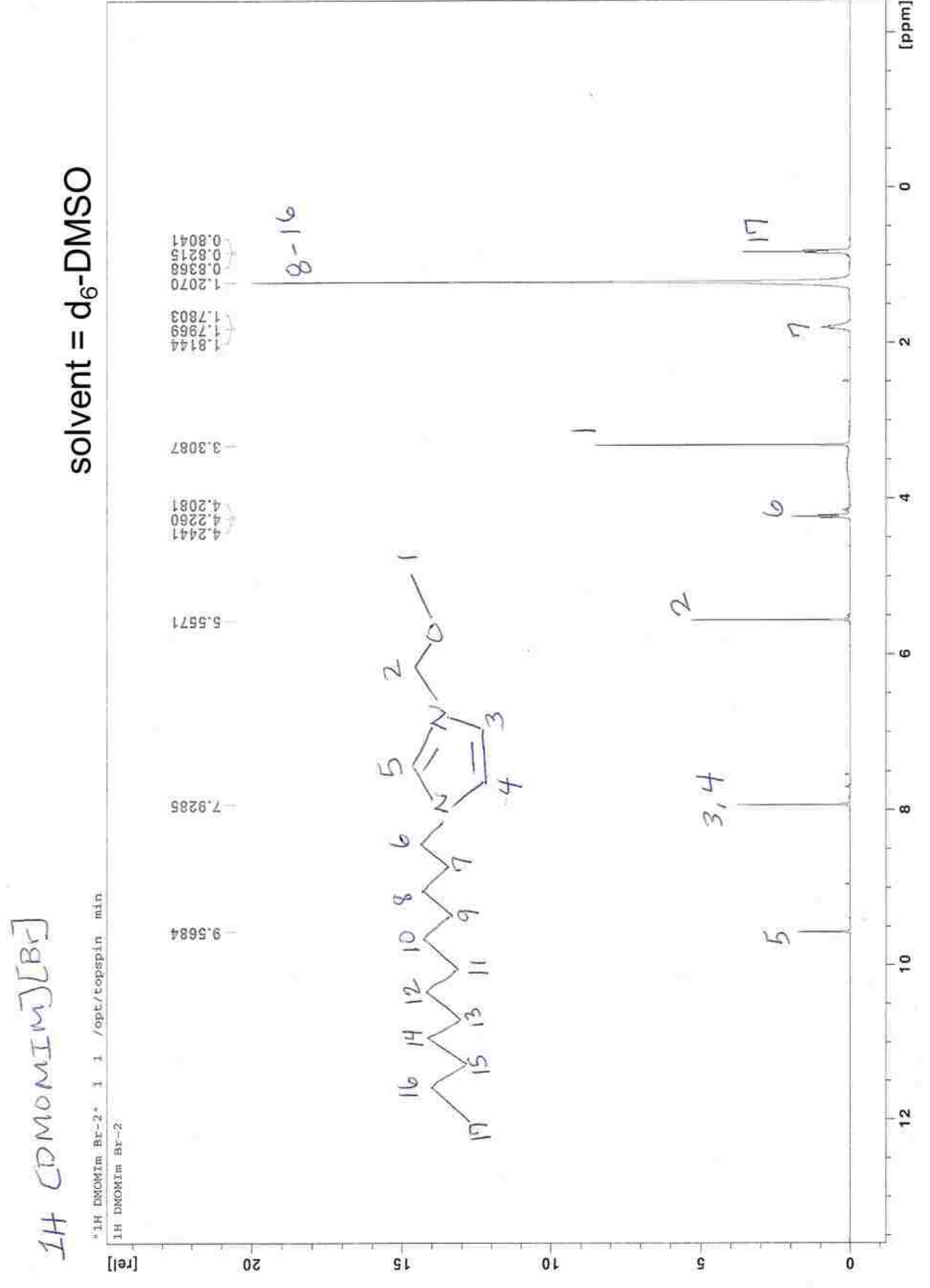
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17
7
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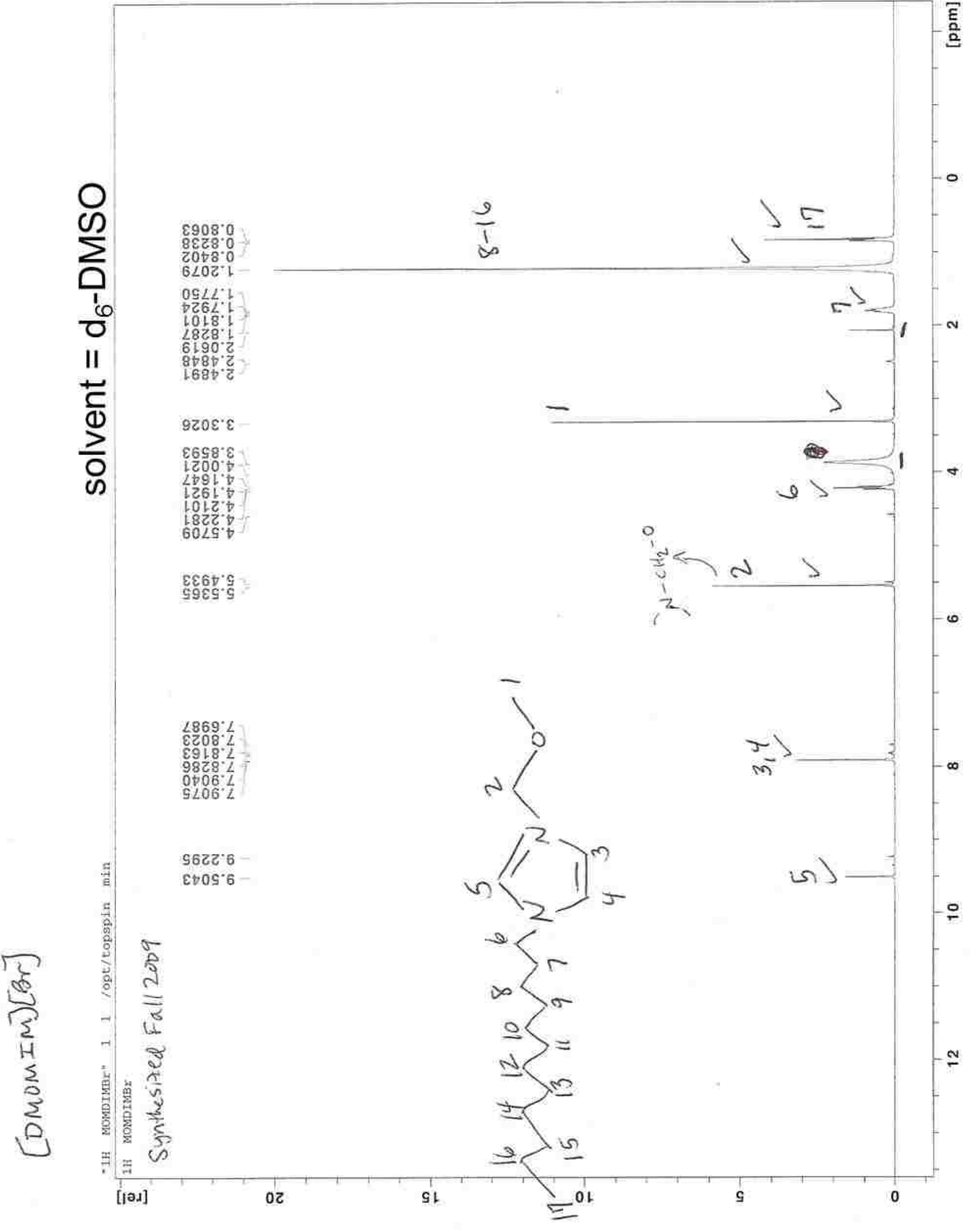
6
2
3,4
5



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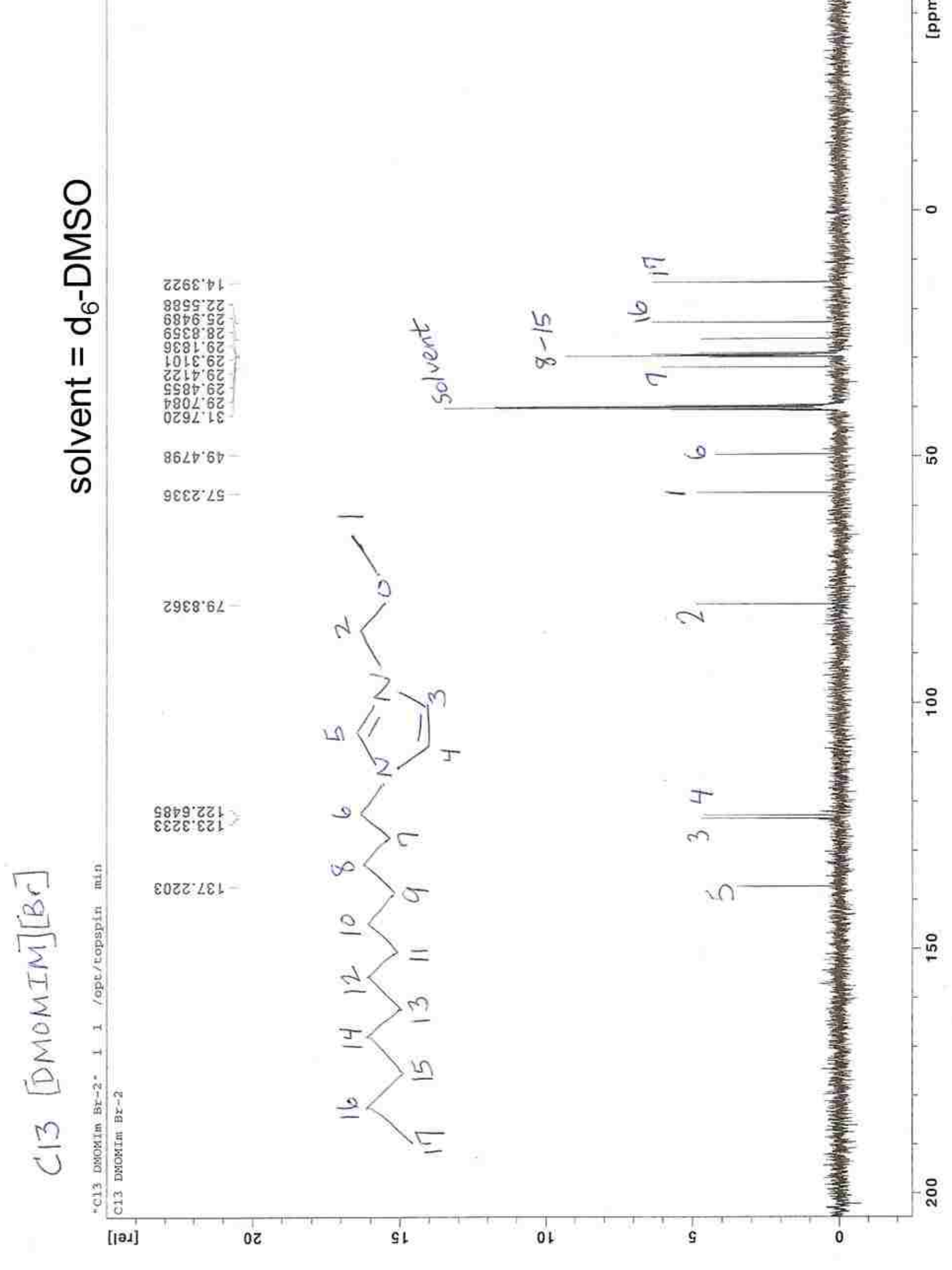

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14.3922
solvent
8-15
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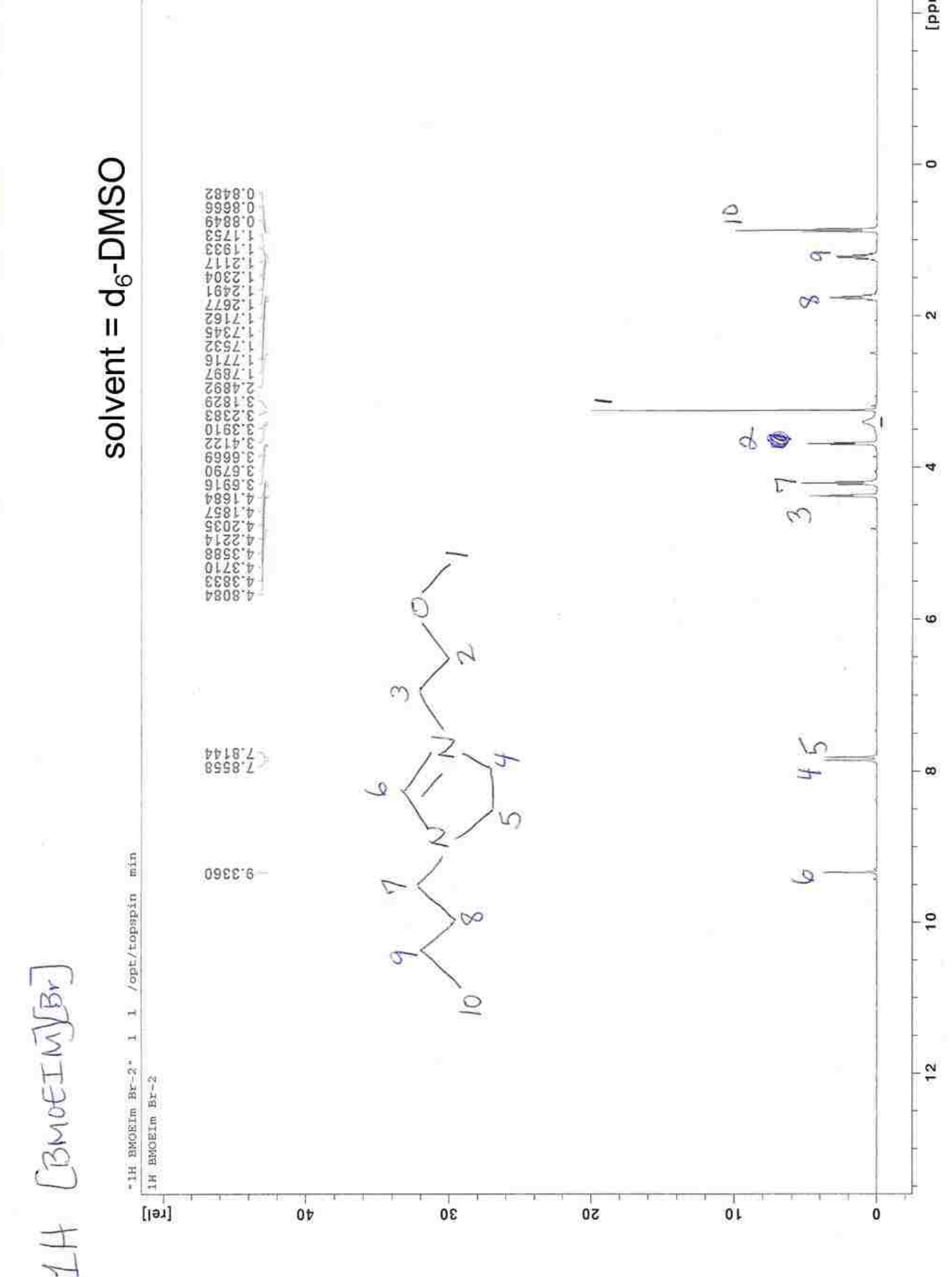
[ppm]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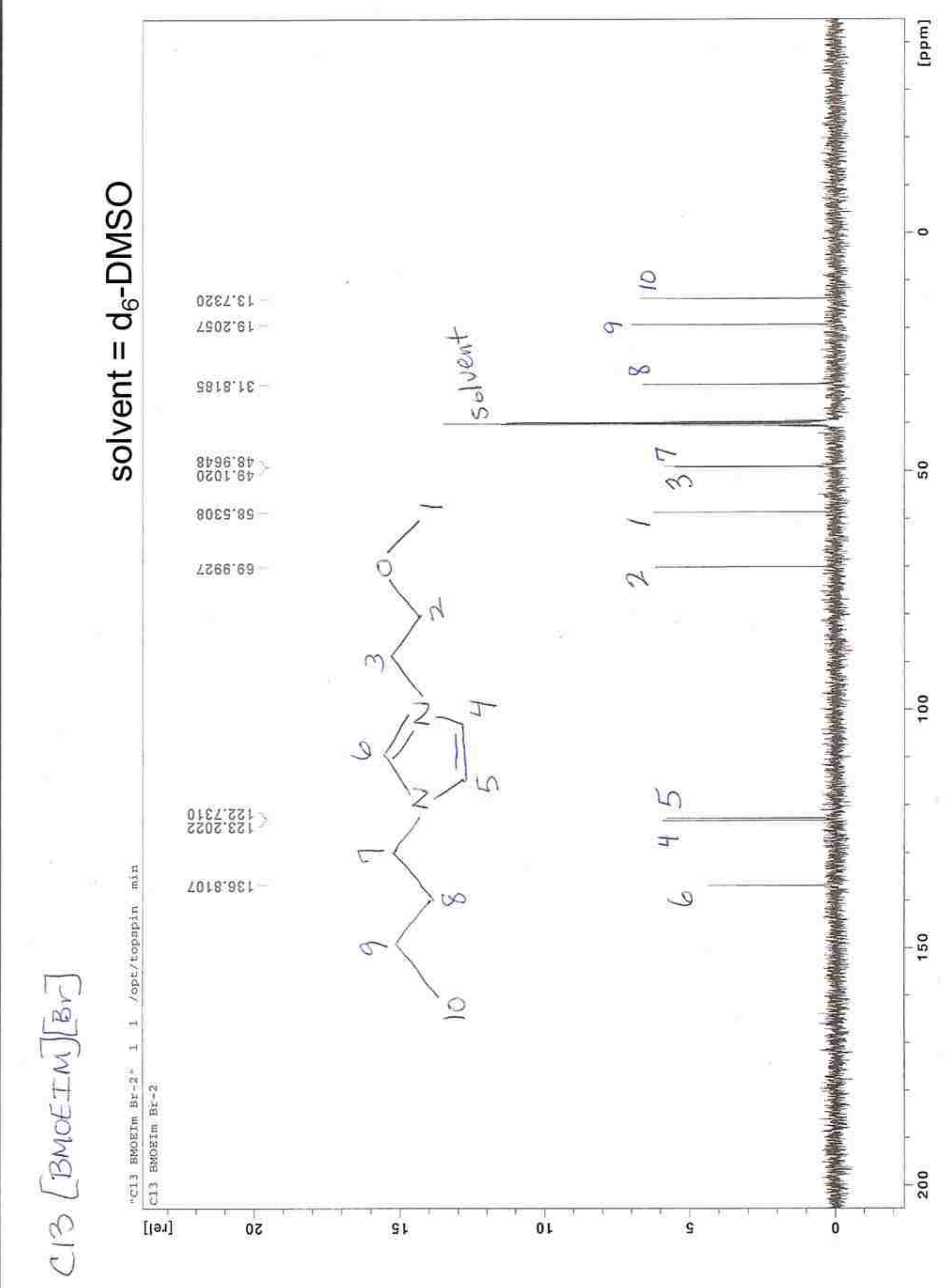
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49.1020
48.9648
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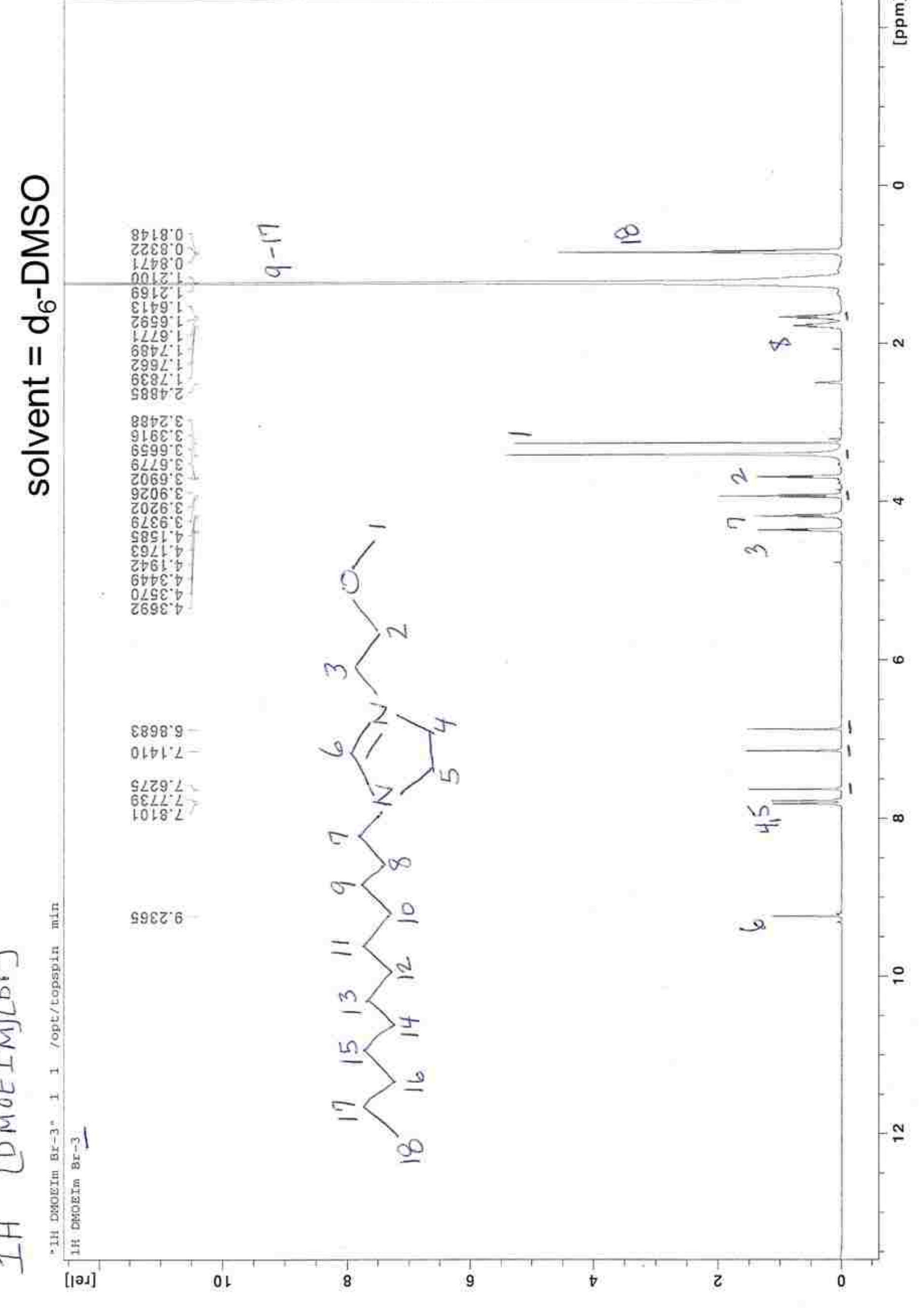
13.7320
solvent
[ppm]
[rel]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3.9379
3.9202
3.9026
3.6902
3.6779
3.6659
3.3916
3.2488
2.4885
1.7839
1.7662
1.7489
1.6771
1.6592
1.6413
1.2169
1.2100
0.8471
0.8322
0.8148
9-17
18
8
1
2
7
3
45
6
[ppm]
[rel]

## APPENDIX C: LSU IRB APPLICATION WITH APPROVAL FORM

*Institutional Review Board*
*203 B-1 David Boyd Hall*
*Louisiana State University and A&M College*
*Baton Rouge LA 70803*

(225) 578-8692

FAX: 578-6792
IRB@LSU.EDU

TO: Monica Sylvain
Chemistry

FROM: Robert C. Mathews, Chairman

DATE: March 26, 2007

Re: IRB# E3582

We have received your application for exemption titled "The Pedagogical Impact of Dr. Isiah M. Warner's Mentoring practices on Student Success"and have given it file number E3582.

You have received the exemption and I am sending you a copy of the stamped approved application. IRB contact information should be added to the consent letter. The correct IRB information is Robert C. Mathews, Chairman, at 203 B-1 David Boyd Hall, Phone - 225-578-8692.

Please forward the requested information/revisions to the IRB office so that we may stamp approved on the latter and return a copy to you for your files.

IRB #: E3582 LSU Proposal #: ____________ Revised: 10/04/2006

LSU INSTITUTIONAL REVIEW BOARD (IRB) for HUMAN RESEARCH SUBJECT PROTECTION

578-8692 FAX 6792
Office:203 B-1 David Boyd Hall

## APPLICATION FOR EXEMPTION FROM INSTITUTIONAL OVERSIGHT

Unless they are qualified as meeting the specific criteria for exemption from Institutional Review Board (IRB) oversight, ALL LSU research/projects using living humans as subjects, or samples or data obtained from humans, directly or indirectly, with or without their consent, must be approved or exempted in advance by the LSU IRB. This Form helps the PI determine if a project may be exempted, and is used to request an exemption.

**Instructions:** Complete this form.

Exemption Applicant: **If it appears that your study qualifies for exemption send:**

**(A) Two copies of this completed form,**
**(B) a brief project description (adequate to evaluate risks to subjects and to explain your responses to Parts A & B),**
**(C) copies of all instruments to be used. If this proposal is part of a grant proposal include a copy of the proposal and all recruitment material.**
**(D) the consent form that you will use in the study. A Waiver of Written Informed Consent is attached and must be completed only if you do not intend to have a signed consent form.**
**(E) Certificate of Completion of Human Subjects Protection Training for all personnel involved in the project ( including students who are involved with testing or handling data) at http://cme.cancer.gov/clinicaltrials/learning/humanparticipant-protections.asp. (Unless already on file with the IRB.)**

**to: ONE screening committee member (listed at the end of this form)in the most closely related department/discipline or to IRB office.**

If exemption seems likely, submit it. If not, submit regular IRB application. Help is available from Dr. Robert Mathews, 578-8692, irb@lsu.edu or any screening committee member.

Principal Investigator Monica R. Sylvain Student? yes Y/N

Ph: 578-3919 E-mail msylva1@lsu.edu Dept/Unit Chemistry

If Student, name supervising professor Dr. Isiah M. Warner Ph: 578-2829
Mailing Address 434 Choppin Hall Ph.
Project Title The Pedagogical Impact of Dr. Isiah M. Warner's Mentoring Practices on Student Success
Agency expected to fund project N/A
Subject pool (e.g. Psychology Students) Dr. Warner's former graduate, undergraduate, high school, and post-doctoral mentees
Circle any "vulnerable populations" to be used: (children <18; the mentally impaired, pregnant women, the aged, other). Projects with incarcerated persons cannot be exempted.
I certify my responses are accurate and complete. If the project scope or design is later changed I will resubmit for review. I will obtain written approval from the Authorized Representative of all non-LSU institutions in which the study is conducted.

PI Signature Monica R Sylvain Date 3/21/07 (no per signatures)

==================================================================

Screening Committee Action: Exempted ✓ Not Exempted ___ Category/Paragraph 1

Reviewer Mathews Signature Robert C Mathews Date 3/26/07

Study Exempted By:
Louisiana State University
Institutional Review Board
203 B-1 David Boyd Hall
225-578-8692
Robert C. Mathews, Chair

Part-A: DETERMINATION OF "RESEARCH" and POTENTIAL FOR RISK

This section determines whether the project meets the Department of Health and Human Services (HSS) definition of research involving human subjects, and if not, whether it nevertheless presents more than "minimal risk" to human subjects that makes IRB review prudent and necessary.

1. **Is the project involving human subjects a systematic investigation, including research, development, testing, or evaluation, designed to develop or contribute to generalizable knowledge?**

(Note some instructional development and service programs will include a "research" component that may fall within HSS' definition of human subject research).

[X] YES

[ ] NO

2. **Does the project present physical, psychological, social or legal risks to the participants reasonably expected to exceed those risks normally experienced in daily life or in routine diagnostic physical or psychological examination or testing?** You must consider the consequences if individual data inadvertently become public.

[ ] YES **Stop.** This research cannot be exempted–submit application for IRB review.

[X] NO **Continue** to see if research can be exempted from IRB oversight.

3. Are any of your participants incarcerated?

[ ] YES **Stop.** This research cannot be exempted–submit application for IRB review.

[X] NO **Continue** to see if research can be exempted from IRB oversight.

4. **Are you obtaining any health information from a health care provider that contains any of the identifiers listed below?**

A. Names

B. Address: street address, city, county, precinct, ZIP code, and their equivalent geocodes. Exception for ZIP codes: The initial three digits of the ZIP Code may be used, if according to current publicly available data from the Bureau of the Census: (1) The geographic unit formed by combining all ZIP codes with the same three initial digits contains more than 20,000 people; and (2) the initial three digits of a ZIP code for all such geographic units containing 20,000 or fewer people is changed to '000'. (Note: The 17 currently restricted 3-digit ZIP codes to be replaced with '000' include: 036, 059, 063, 102, 203, 556, 692, 790, 821, 823, 830, 831, 878, 879, 884, 890, and 893.)

C. Dates related to individuals

i. Birth date

ii. Admission date

iii. Discharge date

iv. Date of death

v. And all ages over 89 and all elements of dates (including year) indicative of such age. Such ages and elements may be aggregated into a single category of age 90 or older.

D. Telephone numbers;

E. Fax numbers;
F. Electronic mail addresses;
G. Social security numbers;
H. Medical record numbers (including prescription numbers and clinical trial numbers);
I. Health plan beneficiary numbers;
J. Account numbers;
K. Certificate/license numbers;
L. Vehicle identifiers and serial numbers including license plate numbers;
M. Device identifiers and serial numbers;
N. Web Universal Resource Locators (URLs);
O. Internet Protocol (IP) address numbers;
P. Biometric identifiers, including finger and voice prints;
Q. Full face photographic images and any comparable images; and
R. Any other unique identifying number, characteristic, or code; except a code used for re-identification purposes; and
S. The facility does not have actual knowledge that the information could be used alone or in combination with other information to identify an individual who is the subject of the information.

[ ] **YES Stop.** This research cannot be exempted--**submit application for IRB review.**

[X] **NO Continue** to see if research can be exempted from IRB oversight.

---

**Part B: EXEMPTION CRITERIA FOR RESEARCH PROJECTS**

**Research is exemptable when <u>all</u> research methods are <u>one or more of the following five categories</u>. Check statements that apply to your study:**

---

[X] **1. In education setting, research to evaluate <u>normal educational practices</u>.**

---

[X] **2. For research not involving vulnerable people [prisoner, fetus, pregnancy, children, or mentally impaired]: <u>observe</u> public behavior (including participatory observation), or do <u>interviews</u> or <u>surveys</u> or <u>educational tests</u>:**

**The research must also comply with <u>one</u> of the following:**
**<u>either that</u>**

[ ] a) the **participants cannot be identified**, directly or statistically;

***<u>or that</u>***

[X] b) the **responses/observations could not harm participants if made public**;

***<u>or that</u>***

[ ] c) federal statute(s) completely protect all participants' confidentiality;

*or that*

---

[ ] 3. For research not involving vulnerable people [prisoner, fetus, pregnancy, children, or mentally impaired]: observe public behavior (including participatory observation), or do interviews or surveys or educational tests:

- all respondents are elected, appointed, or candidates for public officials.

---

[ ] 4. Uses only existing data, documents, records, or specimens properly obtained.

*The research must also comply with one of the following:*

*either that:*

[X] a) subjects cannot be identified in the research data directly or statistically, and no-one can trace back from research data to identify a participant;

*or that*

[ ] b) the sources are publicly available

---

[ ] 5. Research or demonstration service/care programs, e.g. health care delivery.

*The research must also comply with all of the following:*

[ ] a) It is directly conducted or approved by the head of a US Govt. department or agency.

*and that*

[ ] b) it concerns only issues under usual administrative control (48 Fed Reg 9268-9), e.g., regulations, eligibility, services, or delivery systems;

*and that*

[ ] c) its research/evaluation methods are also exempt from IRB review.

---

[ ] 6. For research not involving vulnerable volunteers [see "2 & 3" above], do food research to evaluate quality,taste, or consumer acceptance.

*The research must also comply with one of the following:*

*either that*

[ ] a) the food has no additives;

*or that*

[ ] b) the food is certified safe by the USDA, FDA, or EPA.

---

NOTE: Copies of your IRB stamped consent form must be used in obtaining consent. Even when exempted, the researcher is required to exercise prudence in protecting the interests of research subjects, obtain informed consent if appropriate, and must conform to the Ethical Principles and Guidelines for the Protection of Human Subjects (Belmont Report), 45 CFR 46, and LSU Guide to Informed Consent. (Available from OSP or http://www.lsu.edu/irb)

*Institutional Review Board*
*203 B-1 David Boyd Hall*
*Louisiana State University and A&M College*
*Baton Rouge LA 70803*

---

**LSU IRB**
**REQUEST FOR WAIVER OF SIGNED INFORMED CONSENT**

***** A copy of the script you will use for oral consent should be included with this form. This script should contain the necessary elements for written informed consent (see http://appl003.lsu.edu/osp/osp.nsf/$content/LSU%20IRB%20Documents/$File/chklst.txt)**

***** This form may not be used for exemptions involving children.**

**FROM:** Name: Monica R. Sylvain
Department: Chemistry

**TO:** Robert C. Mathews, Chairman
Institutional Review Board for Research with Human Subjects

**DATE:** 3/21/07

**RE:** **IRB#** ___________

**TITLE:** The Pedagogical Impact of Dr. Isiah M. Warner's Mentoring Practices on Student Success

**I am requesting waiver of signed Informed Consent because:**

☐ (a) The consent document would create the ***principal risk*** of participating in the study.

Or

☒ (b) The research presents ***no more than minimal risk*** of harm to subjects and involves no procedures for which written consent is normally required.

## APPENDIX D: CERTIFICATE OF COMPLETION - HUMAN PARTICIPANTS PROTECTION EDUCATION FOR RESEARCH TEAMS

# Completion Certificate

---

This is to certify that

### Monica Sylvain

has completed the **Human Participants Protection Education for Research Teams** online course, sponsored by the National Institutes of Health (NIH), on 03/21/2007.

This course included the following:

- key historical events and current issues that impact guidelines and legislation on human participant protection in research.
- ethical principles and guidelines that should assist in resolving the ethical issues inherent in the conduct of research with human participants.
- the use of key ethical principles and federal regulations to protect human participants at various stages in the research process.
- a description of guidelines for the protection of special populations in research.
- a definition of informed consent and components necessary for a valid consent.
- a description of the role of the IRB in the research process.
- the roles, responsibilities, and interactions of federal agencies, institutions, and researchers in conducting research with human participants.

---

National Institutes of Health
http://www.nih.gov

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A Service of the National Cancer Institute

## APPENDIX E: STUDY OVERVIEW LETTER AND CONSENT FORM

September 4, 2007

Xxx
Xxx
Xxx
Xxxx

Dear xxx:

I am Monica Sylvain, a graduate student with Professor Isiah M. Warner at Louisiana State University. I am currently conducting research about Dr. Isiah M. Warner's mentoring practices over the last 35+ years, with the primary goal of elucidating why his efforts have been successful for his present and former students/mentees. Other *goals* of the project are: (1) to use the case study as a mentoring model for primary and secondary schools as well as undergraduate and post-graduate institutions and (2) to disseminate a DVD that chronicles the study's findings to primary and secondary schools throughout the United States.

You have been selected to participate because you have interacted in some capacity with Professor Isiah M. Warner, scientist, educator, and mentor. In an effort to document the impact of Dr. Isiah Warner's mentoring efforts, you are being asked to voluntarily participate in a pre-screening process from which final case study participants will be selected. Participation is simple and will not take much of your time.

Please answer the series of questions below and complete the essay if it is applicable to you. There is also a place for you to provide any additional information. If you wish instead to participate in a telephone pre-screening, please email back to this address, msylva1@lsu.edu, or call 504-559-6000 and we can set up a time to talk. A self-addressed stamped envelope has been enclosed for you to return the paper survey.

I should also note that with your permission, select contributions may be published as a text on mentoring. This may require the gathering of additional information. Any questions related to your participation as a human subject in this study may be forwarded to Robert C. Mathews, Chairman, Institutional Review Board, 203 B-1 David Boyd Hall, Louisiana State University, Baton Rouge, LA 70803, (225) 578-8692. Thank you for your participation.

Sincerely,

Monica R. Sylvain

**Louisiana State University** **IRB # E3582**

| | |
|---|---|
| **Study Title:** | Mentoring Efficacy in the Sciences: Effects of Dr. Isiah M. Warner's Mentoring Practices on Mentee Success |
| **Investigators:** | The following investigators are available for questions about this study M-F, 8:00 am – 4:30 pm CST: Monica R. Sylvain 504/559-6000 |
| **Purpose:** | The purpose of this research project is to identify the key mentoring practices of Dr. Isiah M. Warner and elucidate why and how his mentoring efforts have contributed to the success of his mentees. |
| **Subject Inclusion:** | Individuals who are or have been present or former mentees of Dr. Isiah M. Warner (i.e. high school, undergraduate, and graduate students as well as post doctoral associates) are eligible to participate. |
| **# of Subjects:** | No limit. This number will depend on the number of volunteers. |
| **Procedures:** | The study will be conducted in two phases. In the first phase, subjects will spend approximately 45 minutes completing a questionnaire. In the second phase, selected case study subjects will spend approximately one hour completing a detailed mentoring survey. |
| **Consent:** | By answering the questions and returning the questionnaire, the subject is providing and documenting consent to participate in this study. |
| **Benefits:** | The study may yield valuable information about mentoring that may affect issues such as retention, under representation in the sciences, etc. |
| **Risks:** | There is little or no personal risk in this study. Every effort will be made to maintain the confidentiality of your study records. Files will be kept in secure cabinets to which only the investigator will have access. |
| **Privacy:** | Results of the study may be published, but no names or identifying information will be included in the publication. Subject identity will remain confidential unless disclosure is required by law. |
| **Right to Refuse:** | Subjects may choose not to participate or to withdraw from the study at any time without penalty. |

# APPENDIX F: INTERVIEW FACESHEET AND QUESTIONS

## FACESHEET

| | |
|---|---|
| Interviewer: | Monica R. Sylvain |
| Date: | March 27, 2008 |
| Time: | 9:30 – 11:00 am |

| | |
|---|---|
| Interviewee: | Isiah M. Warner |
| Title: | LSU Vice Chancellor for Strategic Initiatives, Boyd Professor, Howard Hughes Medical Institute Professor and Philip W. West Chair in Air Quality/Environmental Analytical Chemistry |
| Hometown: | Bunkie, LA |
| Education: | B.S. Chemistry, Southern University, 1968<br>Ph.D. Chemistry, U. of Washington, 1977 |

## QUESTIONS

1. Why do you choose to mentor others?

2. Do you get anything out of being a mentor? If so, what?

3. Would you say that mentoring is an inherent part of being a scientist? Why? Why Not?

4. Are there any particular ages, ethnic groups, etc. that you particularly like to mentor?

Why? Why not?

5. You have a demonstrated drive for excellence, as evidenced by graduating at the top of your high school class, completing a Ph.D. in three years, being the first analytical chemist to earn tenure at Texas A&M in over a decade, serving as Department Head at LSU, serving as a National Science Foundation Program Officer, publishing in excess of 250 research articles, and training more than 40 Ph.D. students. Do your own personal standards for excellence facilitate your ability to mentor others? If so, how?

6. On average, you have mentored more African Americans and women than other faculty do. Did you set out to mentor students from underrepresented groups?

If not, why do you think that happened?

7. How would you define the role of a mentor?

In your opinion, would this definition be specific to chemistry only?

8. In your own words, what is your approach to mentoring a student?

Do you employ a different approach for different types of students?

Do you think that you could effectively mentor any student or only students with certain characteristics?

9. Are there specific actions that you consistently find yourself employing when mentoring someone?

If yes, what are they? Why are they important?

Is there feedback from your mentees that allows you to identify specific actions that you employ as you mentor them?

10. Do you consider yourself a role model for the students you mentor?

If yes, how do you integrate this belief into your mentoring practices?

11. When do you know that you have been effective at mentoring someone?

Is it the positive feedback that motivates you to continue to mentor or would you mentor without the positive feedback?

12. What, in your opinion, are the characteristics of an effective mentor?

Have you encountered other colleagues who are effective mentors?

What makes them effective mentors?

13. Are there specific skills that you hope to develop in the protégés that you mentor?

14. Although you have received feedback from people at all stages of professional development, do you consider each one of them to be your mentee?

15. Why would you nominate someone for the NSF PAESMEM or the AAAS Lifetime Mentor Award?

## APPENDIX G: INTERVIEW TRANSCRIPTION

**Monica**: Why do you choose to mentor others?

**Dr. Warner**: You'll find out these answers might vary somewhat. Why, because I say the first thing that comes to my mind. But, what really comes to my mind is the fact that I was mentored by other people. Coming from my background, a very country background, (Bunkie, Louisiana) if I didn't have mentors in place I wouldn't have known which path to proceed along, and so I try to make sure that I'm there for students who need similar guidance.

**Monica**: So do you get anything out of being a mentor and if so what do you receive?

Dr. Warner: I would say the primary thing I receive is the pride in knowing that I helped someone. The pride when the students come back and give me feedback that I've made a difference. There was a young woman, I don't know if you've ever met Chanel Fortier, and Chanel has given me that sort of feedback. When I was at PITTCON, I guess it was, she told me how much she appreciated me being there for her.

**Monica**: So would you say that mentoring is an inherent part of being a scientist?

**Dr. Warner**: No, I would not say it's inherent, in fact I feel that those two things are contradictory. I feel that being a scientist means that you want to get the results out, new results, novel results, out as fast as possible. Being a mentor means that you are looking out for the individual, and sometimes those two things conflict. And, typically one, depending on the individual, will take priority over the other. A good mentor will never let the science, in my opinion, override the individual's needs and concerns.

**Monica**: So how would you recommend someone to balance that, since you think that sometimes they contradict one another or conflict one another?

**Dr. Warner**: I wouldn't be a good person to make that recommendation, because I think sometimes I don't do a good job of balancing them, the two. Sometimes I let the mentoring override the scientific needs. I was just thinking about that, as a matter of fact today, that there are a number of students who never produce papers out of their dissertation because I let them out without at least a draft. And they promise and swear 'oh we'll get it, we'll get it out', and it just doesn't happen. I think this is one area in which I'm going to let the scientist outweigh the mentor. Students, because in the end it's good for them to have manuscripts from their studies, so I'm going to make sure that no one gets out unless they have a draft, no exceptions. Even those students who say 'I will be exceptional.' How did I get on that topic, what was the question?

**Monica**: The question was you said that you think that being a scientist and being a mentor can conflict sometimes, and I asked how would you suggest that one balance that?

**Dr. Warner**: Now, it's not easy to balance the two. I think that it should be played on a case-by-case basis.

**Monica**: So, are there particular ages, ethnic groups, or other characteristics of persons that you particularly like to mentor?

**Dr. Warner**: Because of my background, I think I'm always interested in those that are disenfranchised, those that have less privileges than others, and that may or may not be because of race. That could be, I mentioned earlier about a young man who was in my class, came from a rural background, had never heard of MIT or didn't even know that MIT existed, or that it was a good school. And I identified with him; he was a white male, so I think I have a particular affinity for underprivileged, regardless as to what that underprivileged is about.

**Monica**: So, since you've demonstrated a drive for excellence, as evidenced by a number of things, 1. you completed your Ph.D. in 3 ½ years, 2. you were the first analytical chemist to earn tenure at Texas A&M in over a decade; you've published in excess of 250 research articles, and you've trained more than 40 Ph.D. students. Does your own personal standards for excellence facilitate your ability to mentor others?

**Dr. Warner**: I do know that my own standards for excellence help me to encourage other students to pursue excellence. I'll give you an example of a young man who's coming; you should really meet him, Troy Alexander. Have you met him? (Monica: I have not met him.) Okay, did he fill out a form, did you give him a form. (Monica: I may have given him a form, I don't remember everyone's name.) Okay. (Monica: Is this the gentleman who…) He's at army research office. (Monica: I have met him before. Yes, in OSI.) He's coming a week from Friday, to give a seminar. So you'll want to catch him, and talk to him. But in his particular case, he was referred to me by Dr. Robinson because the young man was bright, but didn't have the grades for various reasons, and he and I clashed all the time. Because I'd ask him to write a paragraph and he would just throw something together, and you know improper English and I'd say this is not acceptable. You don't turn anything in to me like this. So I got him a school to go to for a Ph.D. and I immediately received a call from the chair of that chemistry department. He wanted to know if I knew what I was doing based on this young man's record he was going to have trouble and plus the advisor I wanted him to have was going to kick him out within a week. And, I assured them that would not be the case. Well, needless to say, that young man is a very successful Ph.D. right now because I did stick with my standards, because I sent him to someone who I knew would have high standards. And right now when I see that young man, he runs a whole laboratory for Army Research Office. And when I see that young man, it's difficult for me to associate that young man with the same one that was in my laboratory.

**Monica:** What made you know that he was going to be able to rise to the standards of the advisor that you suggested he be under?

**Dr. Warner**: Because he had all of the elements. He was very bright, he had all of the potential, except exactly what I mentioned earlier, that he was from a rural background and had some family problems (I won't go in to that), but I knew that if he could get around all of those kinds of problems that he would be very successful. The magnitude of his success, I don't think I would have predicted. I mean, he's gone beyond what I would have even expected. And, as I

indicated it's just hard for me to say is that the same person who was in my lab'. It's like I'm talking to a different person.

**Monica**: So Dr. Warner, on average you have mentored more African Americans and women than other faculty do, on average. (Dr. Warner: I imagine. I've never counted them though. I imagine that's true.) Did you set out to mentor students from underrepresented groups?

**Dr. Warner**: No. As a matter of fact, it wasn't until I came to LSU that my group turned majority African American. Before that at Texas A&M and at Emory, I had a majority group of Whites. But I guess because it's a large population of African Americans here a lot of them migrate toward my group.

**Monica**: Did you have a larger percentage, what percentages of women did you have at Texas A&M and Emory and now at LSU.

**Dr. Warner**: I've never counted that. There may be in some of my NSF reports. But we can go through and see all the students that have graduated from my group.

**Monica**: How would you in your own words define the role of a mentor?

**Dr. Warner**: A mentor takes a special interest in the individual, much as a father to the son or a mother to the son or daughter. That same sort of interest, because I take the same interest and pride with my mentees as I do with my own kids. If a mentee of mine is successful I'm just as proud as if it were one of my sons that's successful.

**Monica:** So would you in your opinion say that this definition is specific to only chemistry?

**Dr. Warner**. No. I think that's a general definition. In my opinion. Because I mentor people outside of chemistry also.

**Monica**: And are you drawn to those people because they come from disadvantaged backgrounds or are there other reasons?

**Dr. Warner**: What do you mean, the people outside of chemistry? (Monica: Yes Sir.) Typically, I interact and meet people inside of chemistry. When I meet people outside of chemistry, typically they are coming to see me because someone sent them to me. And so, invariably they have some problem that they want me to help them solve. So I guess, yes that would be the case.

**Monica**: We've heard stories, stories go around about you, about how you meet these different mentees. Sometimes you meet them at conferences, sometimes you meet them in the elevator. How does all that happen? Or do you know?

**Dr. Warner**: No, I don't know. I don't know how it happens. I just pick up conversations and start talking with people. Quite often it happens as a joke. I mentioned Chanel Fortier, for example. I met her in the elevator, and she was a chemical engineering major. And, I started

joking with her and I said oh you need to be in chemistry.  And she just laughed until one day she showed up at my door and said "I think I'm going to take your advice and switch to chemistry."  And now, she's getting her Ph.D. this summer in chemistry.

**Monica**:  So again in your own words.  What is your personal approach to mentoring a student, outside of having an interest in them and wanting to take pride in their success?

**Dr. Warner**:  I think that each individual tends to have different levels of passion for different areas.  And my approach is to try to get them to figure out what it is, what that passion is about, and what it is that they want to do, what is it that they would do in life, if they were wealthy and they were going to work for free, what is it they would do.

**Monica**: So you do employ a different approach for different types of students?

**Dr. Warner**: Different students have different personalities, so yes; I try to figure out what is the key that unlocks them, what helps to motivate that particular person.  So, I try to find different strategies to motivate them to purse their passion, to maximize what they get out of whatever they are doing.

**Monica**: So would you say then that your approach to mentoring stems from figuring out how to motivate these students?

**Dr. Warner**:  Yes.  That's part of it.  It depends on the particular needs of the student.

**Monica**: Is there anything else?

**Dr. Warner**:  I listen.  That's one thing I do, I listen to the person.  I observe.  I'll give you another example.  I was on the selection committee for our LA-STEM program just recently. There was a young woman from Admissions who happened to be on my committee.  We were introducing ourselves to the various students, and she says "my name is Misty Johnson and I am not in science.  I'm in Admissions but I had a passion for science in high school."  My ears perked up and so after the Selection Weekend was all over with, I emailed her and said I detected something in your voice.  I'm still interested in Arts and Science, come by sometimes and let's talk.  She wrote me an email back and said, "Dr. Warner, this is divine intervention."  She said, "I don't know how you picked up on that."  She said, "but it's something that's been nagging at me for the longest."  And so, we had lunch one day and we were talking about some strategies for her to get back into science.  But those are the kinds of things that I just pick up on. Particularly if there's a science tie there.

**Monica**:  So would you say then that over the years, because of your desire to want to help disadvantaged students or more importantly to help people who are interested in the sciences to find what their passion is, that you have purposely, almost, you've purposely made sure that you key in on those kinds of things.

**Dr. Warner**:  I think over the years I've developed an ear for those kinds of things.  I don't think I did initially.  Because initially I didn't think I was doing anything special.  People were just

making up things.  I had mentioned to you earlier I attended a NOBCChE Conference.  Maybe I had been in the field maybe 10 or 15 years, and I walked in the door and this guy asks," (don't know who the guy was until this day), but he said, "Isiah Warner, the greatest mentor of all times."  And, I said why (in hell) did he say something like that, I mean just to be talking to himself.  But afterwards, I started paying attention to how many more people would say the same thing, but in different words.  And I began to say well maybe I'm doing something different that I don't realize that I'm doing.  And that's where I started paying attention to that.  Particularly when I started getting feedback from students saying that "you did this for me, you did that for me."  I had no idea what they were talking about until I thought about it.

**Monica**: So in what ways have you received feedback from students that you've been an effective mentor?

**Dr. Warner**: Just recently when I won this AnaChem Award I received an email from a young man at Texas A&M.  I have no idea whether he's white or black.  My inclinations are that he's probably white from the way he wrote this.  I can usually tell whether it's a minority student by the way they thank me for being a minority.  But this young man doesn't even associate with being a minority.  He told me how I had made a difference for him, and he noticed that I had received this award and it prompted him to write me an email thanking me for that.

**Monica**:  So was this a former student of yours?

**Dr. Warner**: I don't think he worked in my lab.  He took a class under me.  And would come by and talk to me occasionally.  If he walked through this door right now I would have no idea who this young man is.

**Monica**: Do you find that you are only able to mentor students with certain characteristics?  Or you can mentor anyone?

**Dr. Warner**: I haven't seen any limitations in terms of gender, race, whatever.  I haven't seen any difference.

**Monica**: When you mentor, your protégés, whether they are from high school, undergrad, grad or post docs, are there specific actions that you consistently find yourself employing when you mentor them?

**Dr. Warner**: One thing that always happens, I always let them make their own decisions.  If they want to figure out something, I might give them multiple paths to achieve something, but ultimately the decision is theirs.  That's one thing; I try to get them to reach the decision.  I mentioned Ebony Spikes, who would come in with a problem, and I would give her five or six solutions.  "Well, which one should I take," and I would say Ebony it's your life.  You pick the one that you are most comfortable with.

**Monica**: Why would you say that that's important, that they make their own decisions even though you're serving as a mentor to them?

**Dr. Warner**: Because it is their lives and in the end, if something doesn't work out they can honestly say well "I made the decision." I suppose it would be an easy out if I made the decision for them to blame Dr. Warner. Dr. Warner made the decision, so it's all his fault.

**Monica**: So if one of your mentees comes to you with a problem what's your first inclination towards helping them?

**Dr. Warner**: The first inclination is to have them talk out the problem. Have them indicate, have you thought about possible solutions. If you have, what are the possible solutions. If I think of some that they haven't thought about, I'll list some other possible solutions. And I say you have to pick out the one that's most comfortable for you. Sometimes it's not easy. A young man just came by the other day, and he says he's working hard on his grades, he's having emotional problems, but he thinks he's prepared for an exam, but he just can't seem to get things right. The only I can do is refer them to psychologists or to Dr. McGuire who can sometimes pick up certain things better than I can on the academic side.

**Monica**: Would you say that the issues that you've seen that your mentees have had over the years are issues and/or problems that all students have?

**Dr. Warner**: I haven't found a very unique problem to any student yet. I don't think all students have the same problems, but many students have variations of some of those same problems. And over the years I can't think of any I have encountered any one particular that was unique.

**Monica**: So then do you consider yourself a role mode for the students you mentor?

**Dr. Warner**: I imagine some students identify with me as a role model. I don't think all of them think of me as a role model, but I think some of them do.

**Monica**: Do you feel that being a role model to the mentees, is that important in mentoring?

**Dr. Warner**: No. I often say that students sometimes need more than one mentor. Sometimes they need a mentor as a role model, sometimes they don't. If you are a minority student and you have a white male or a white female who's your mentor, they could or could not be a role model. It depends on what you need the role model for. If you need a role model as a chemist and they're a chemist, then they can be a role model. But if you need a role model of a minority chemist they can't be that role model. And so you might have to engage with a minority chemist to satisfy that other need that you have.

**Monica**: So, after you've mentored one of your students, let's say it's a post doc or a graduate student, when do you know and/or are satisfied that you've been effective at mentoring that person?

**Dr. Warner**: I don't ever know until they give me the feedback that I've been effective, or I see evidence of changes in behavior that promotes a more effective career choice.

**Monica**: Can you expound on that. When you said evidence?

**Dr. Warner**: Well, like the young man I mentioned that I sent to another university. I think the average person would have given up on him and said this guy will never make a descent Ph.D. But I saw something in him that made me recognize that this guy can be outstanding if he takes the right path. I don't think there are many people who started out with the right path and ended up with a Ph.D. who are more successful than he is. He started out on the wrong path and ended up on the right path, and in the end I think he as good a scientist as any of the guys who started on the right path completely.

**Monica**: Specifically in that situation did you continue to stay in contact with that young man?

**Dr. Warner**: Periodically we would interact with one another, particularly from the professor. I told you about the chair who said that the professor had such high standards that this guy was going to kick him out of his group in a week. Well, that professor calls me up every year, "do you have any more students like him. Please send me some more students like him." The effect that the chair predicted was just the opposite. The professor considers this guy the best student he's ever produced, and he wants me to send him more. I talk to him on the phone probably once a month. He must ask me six times a year to send me another student like him.

**Monica**: So what in your opinion, Dr. Warner, are the characteristics of an effective mentor?

**Dr. Warner**: They have to take an active interest in the individual. They have to allow the individuals to make their own choices. They have to be able to provide a variety of pathways for that person to be successful. Sometimes, I can think of a couple of instances where there was only one pathway that I could see, and that person ended up being successful.

**Monica**: Are there ways in which you provide emotional support to your mentees, that in the end you felt like, were effective?

**Dr. Warner**: Yes. Sometimes emotional support might be tough love. Like the young lady I told you about, Chanel Fortier. She wanted to stay in my group and get her Ph.D. in my group. And she'd probably be done with her Ph.D. now had she stayed in my group. But when I saw her at PITTCON I saw a level of strength in her. The fact that I pushed her out of the door, I think I saw a level of strength in her that she would never have gotten to if she had stayed in my group and used my group as sort of a crutch. I think pushing people out of the door, exposing them to new environments helps them to grow up. She's really grown and matured quite a bit.

**Monica**: When you start mentoring a mentee, again, whether they are from high school, undergrad, grad or post doc, are there specific skills that you aim to teach them during this process of mentoring them?

**Dr. Warner**: The ultimate skills are survival skills. Being able to figure these things out for themselves, that you don't need the guidance. There's a young woman who graduated from Emory probably around '86 or '87 (I believe) in chemistry, 3.6 or 3.7 average. The highest African American average I have ever heard of at Emory because Emory brings in these students and then destroys them, particularly minority students. I mean bright students and they just

destroy them. This young lady graduated with a 3.6 or 3.7 GPA, went on to medical school, had some problems, was diagnosed as bipolar. So maybe once a year she'll call me. I assume it's because that's the positive side in her life. She remembers the good times when she was doing well at Emory and I was there. So she'll call me and ask me for advice on something. The last call I received from her was probably a couple of years ago, and she was saying "Dr. Warner, my mom thinks that I,. I'm off my bipolar medication, my mom thinks that I need to be back on it. What do you think?" I said I think you ought to listen to your mom because she was clearly distraught and maybe that's why she hasn't called me back. But, I tried to reach her mom to let her mom know that she was on the right path and she needs to encourage her to get back on her medication because this young lady was clearly having problems.

**Monica**: You mentioned that you received a call from someone, but do you continue to receive calls from current or former mentees?

**Dr. Warner**: All the time. Yes.

**Monica**: What kinds of things are they asking you about?

**Dr. Warner**: More often than not, they are just calling to check on me to see how I'm doing. Letting me know some recent success. Very rarely are there negative things in their lives. Most often it's the positive things that they want me to be aware of.

**Monica**: So even in terms of the positive things, do they ask you for advice, let's say what grad school to go to, or what job you think they should take, or anything like that?

**Dr. Warner**: Well sometimes they might call me and say I need a letter of reference. I'm going to be moving into a new job or even, I have a very personal relationship with a lot of my students, "we've just had our first kid, we just had a new kid." I can remember a young man. He got his Ph.D. from the University of Colorado. When he got engaged, he called and let me know. That's an interesting young man by the way. He's a white male with a very privilege background, and he was an Emory undergraduate. And, if I went to him and said that I really need to talk to you sometime, he would pull out his book and say "well Dr. Warner I'm available on Thursday at 1 o'clock, how does that fit with your schedule?" This is the honest God's truth, he wasn't trying to be pretentious. He was just that busy. He ran cross country, he was a chemistry major, graduated with a 3.8 GPA, and he volunteered with the homeless. One day all of my graduate students were gone out of the lab and I said 'where is everyone.' Oh, Tony's got them volunteering for the homeless. At this time, I mean they were all gone and they would all go at the same time. This is an undergraduate who's got my graduate students out doing these things. And then he had a Rhodes Scholarship, he was a shoe-in for a Rhodes Scholarship. Instead he went and spent two years in Africa, in Libya working. And then the next thing I hear from him (2 years later) he's brought some young African man with him and he's trying to get him into college. He's calling all his friends up to give money so I donated (I don't remember how much I donated) but I donated something.

**Monica**:  You've already received very prestigious mentoring awards like the NSF PAESMEM Award and the AAAS Lifetime Mentor Award.  Why would you nominate someone for these kinds of awards?

**Dr. Warner**: Because I think they need good role models, but I think there needs to be many more role models out there.  And, when I recognize somebody doing something special, I think, and quite often, just like me, they don't think 'well, I don't expect to be rewarded for these things,' but I think there should be some recognition for them, particularly if there are awards for those kinds of things. And usually when I nominate someone, they win, I'll get some feedback from the committee, "thank you for continuing to nominate great people for these awards."  I've gotten that kind of feedback from AAAS and from PAESMEM also.

**Monica**: So what do you see in the people that you've nominated for these kinds of awards that they're doing that is special in terms of their mentoring?

**Dr. Warner**: Well, they all have their careers. They all have their jobs, but they're doing these extra things that they're not getting paid for.  I'll give you an example, I nominated Dr. McGuire for an award.  And so, I had to reach her for something, so I got in early one morning, about 6 o'clock in the office.  So I called her office so I could leave a message because I knew I would forget about it, and she picked up the phone. I said, "what are you doing in this early?"  She said, "what are you doing in this early?  I recognized that she puts in this extra time because she wants to have the time for the students.  As a supervisor she doesn't have to do that.  But, she can't leave that alone, she can't help but to interact with students.  That's the most important thing to her, is to interact with the students.  And so, somebody that dedicated needs to be recognized for what they're doing.

**Monica**:  Is there anything else, Dr. Warner, you'd like to say about the way that you mentor students or do you have any other stories about students that you've mentored over the years that really stand out to you?

**Dr. Warner**: Well, just that I'm still learning.  There's one story I always tell about a young woman who came in (I don't remember her name or else I would have listed her as one of the persons) and asked me to be her advisor.  I asked her what was her major and she said pre-med. I said I am not a pre-med advisor.  I said the pre-med advisor is next door.  She said, "Well I know that."  And I said, well isn't he advising you?  "Yeah he's advising me to give up medicine.  She said that with my grades, he keeps saying that I'll never get into a reputable medical school.  I said, what's your grades.  She said a 2.6.  I said well he's just trying to be realistic.  He's not trying…, "well Dr. Warner he doesn't have to be so negative about it."  So, I finally was her advisor for a couple of years and she didn't get into medical school.  But, I saw that she had a passion.  She had been working full-time and all that, so she had a passion and was bright enough so I suggested she go across town and take a full curriculum of science courses at Georgia State.  Because as I indicated Emory just tore down any kind of confidence minority students had (she was an African American female).  Emory would just tear down any kind of confidence these students would have, and 3 years later she walks into my office and said "do you remember me?" And I said, well, vaguely, I remember the face.  And so, she recanted the story to me.  I thought about how if I hadn't been there to advise her.  I said what are you doing?

She said, "I'm in medical school." She said I did exactly what you said, went across to Georgia State and took all of these classes in science and did very well and then applied to medical school. She got in.

**Monica**: Wow, that's fantastic.

**Dr. Warner**: So, that story sticks out, and that story tells me that you should never give up on anyone. And, had she not come to me as an advisor, that other advisor, the other pre-med advisor would have had her convinced that she never would have gotten into medical school.

**Monica**: So earlier, you said, you have really high standards for your mentees, do you ever find that's a problem for some of them. That your expectations, that they have trouble reaching the expectations that you have for them?

**Dr. Warner**: I've learned to adjust my expectations for certain students. There are certain students that never reach these expectations, but I don't have a problem in believing that they have those capabilities. I don't think I'm ever wrong on those things. Maybe I am wrong, but I don't ever believe that I'm wrong. Because I see all the qualities that are necessary for success. Like the young man that I told you about. What I do recognize is that sometimes students don't have the same aspirations that I have for them. But, I think that if you have God-given talents, it's a sin and a shame not to use all of those talents. That is my belief.

**Monica**: Okay, that actually concludes the interview, unless you have anything else you'd like to add.

**Dr. Warner**: No, I don't think so.

**Monica**: Okay. Thanks.

## APPENDIX H: PARTICIPANT SELF-SELECTION SURVEY

In what capacity do you know Dr. Isiah M. Warner?

Do you consider Dr. Warner a mentor? ____ yes _____ no

If you answered yes to question (2), please write a one page essay describing in detail how Dr. Warner has mentored you. You may use the back of this sheet if you need additional space.

Additional comments ______________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

_________________________________________________________________

## APPENDIX I: IN-DEPTH MENTORING SURVEY

### A Survey of Dr. Isiah M. Warner's Efficacy as a Mentor in the Sciences

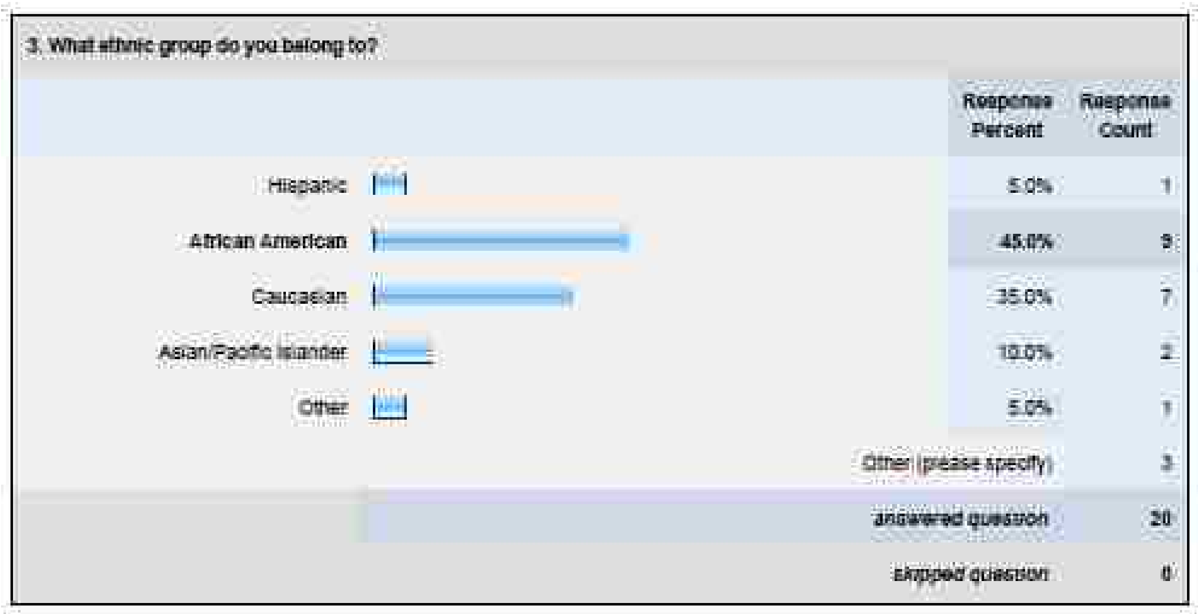
**1. What is your current age?**

| | Response Percent | Response Count |
|---|---|---|
| < 20 years old | 5.0% | 1 |
| 21 – 25 years old | 20.0% | 4 |
| 26 – 30 years old | 10.0% | 2 |
| 31 – 35 years old | 0.0% | 0 |
| 36 – 40 years old | 25.0% | 5 |
| 41 – 45 years old | 25.0% | 5 |
| 46+ years old | 15.0% | 3 |
| | *answered question* | 20 |
| | *skipped question* | 0 |

**2. What country are you originally from?**

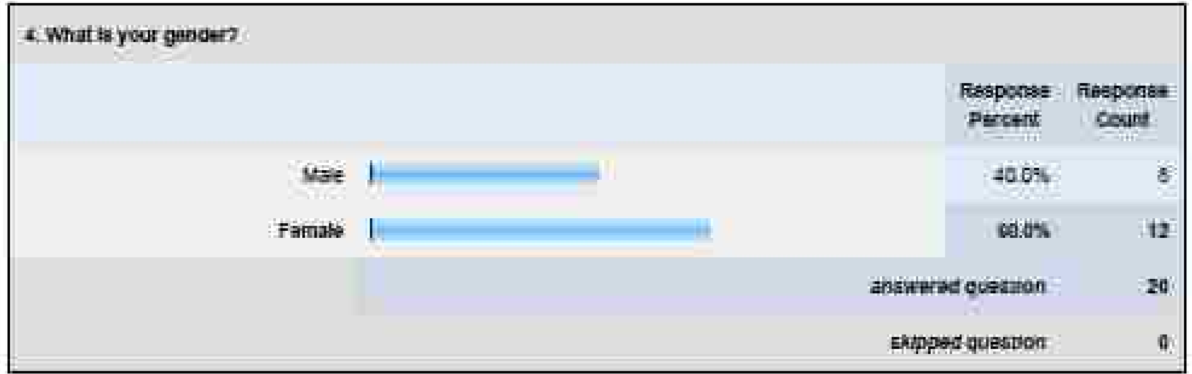
| | Response Count |
|---|---|
| | 20 |
| *answered question* | 20 |
| *skipped question* | 0 |

Page 1



**3. What ethnic group do you belong to?**

| | Response Percent | Response Count |
|---|---|---|
| Hispanic | 5.0% | 1 |
| African American | 45.0% | 9 |
| Caucasian | 35.0% | 7 |
| Asian/Pacific Islander | 10.0% | 2 |
| Other | 5.0% | 1 |
| | Other (please specify) | 3 |
| | answered question | 20 |
| | skipped question | 0 |



**4. What is your gender?**

| | Response Percent | Response Count |
|---|---|---|
| Male | 40.0% | 8 |
| Female | 60.0% | 12 |
| | answered question | 20 |
| | skipped question | 0 |

**5. What is your current job title?**

| | Response Count |
|---|---|
| | 20 |
| answered question | 20 |
| skipped question | 0 |

**6. What is your definition of a mentor?**

| | Response Count |
|---|---|
| | 20 |
| *answered question* | 20 |
| *skipped question* | 0 |

**7. When did you meet Dr. Warner?**

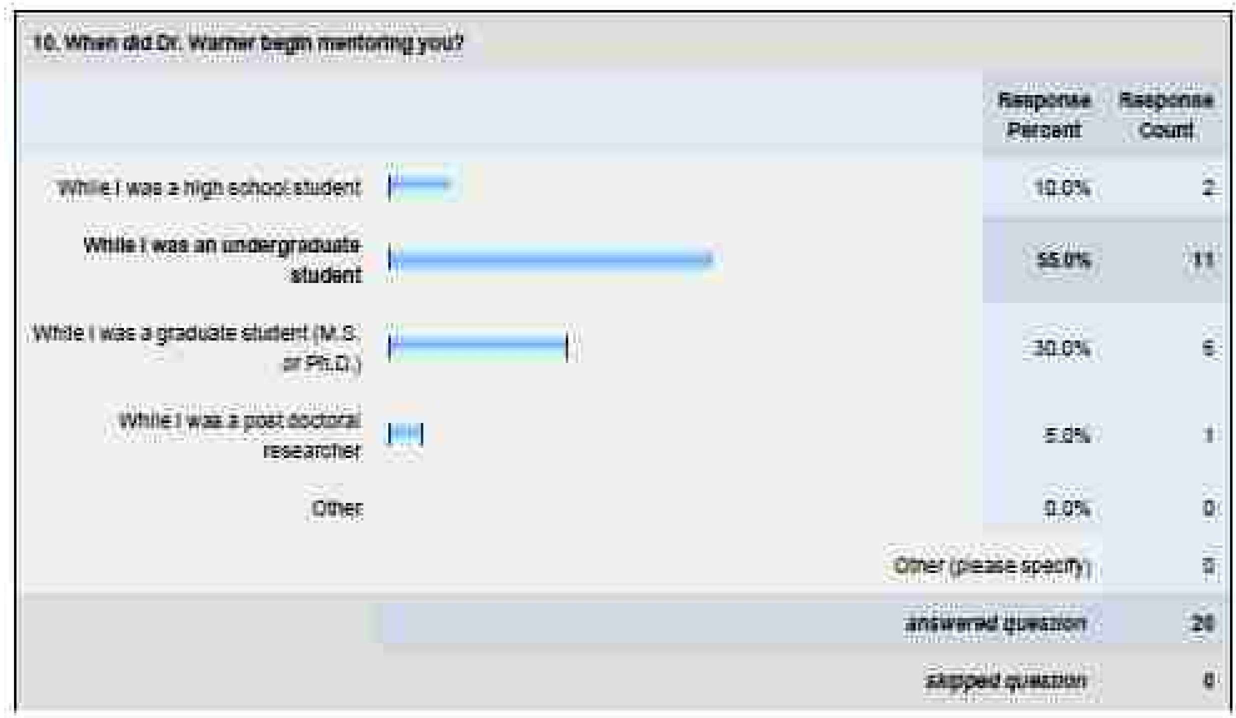
| | Response Count |
|---|---|
| | 20 |
| *answered question* | 20 |
| *skipped question* | 0 |

**8. What were your first impressions of Dr. Warner?**

| | Response Count |
|---|---|
| | 20 |
| *answered question* | 20 |
| *skipped question* | 0 |

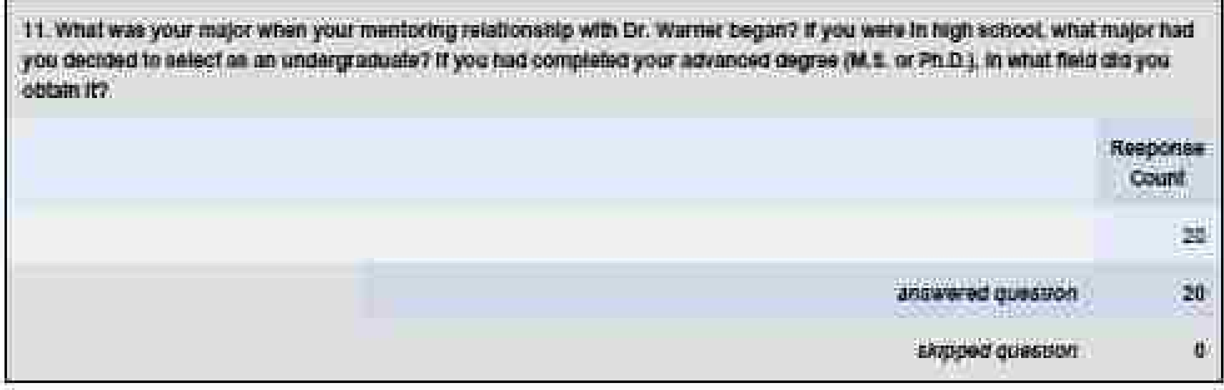
**9. When did you decide that you wanted to be mentored by Dr. Warner? (i.e. What happened to convince you that he would be a good mentor?)**

| | Response Count |
|---|---|
| | 20 |
| *answered question* | 20 |
| *skipped question* | 0 |



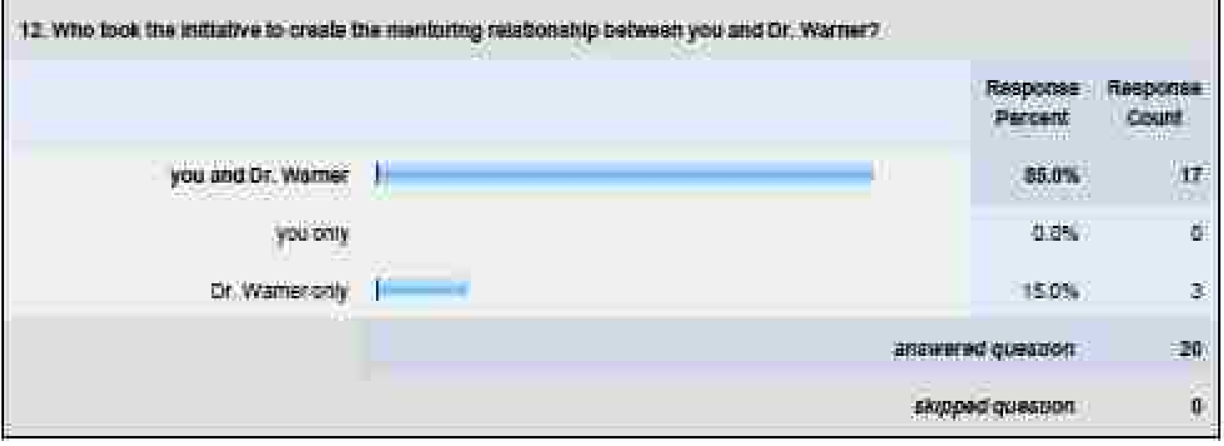
10. When did Dr. Warner begin mentoring you?

| | Response Percent | Response Count |
|---|---|---|
| While I was a high school student | 10.0% | 2 |
| While I was an undergraduate student | 55.0% | 11 |
| While I was a graduate student (M.S. or Ph.D.) | 30.0% | 6 |
| While I was a post doctoral researcher | 5.0% | 1 |
| Other | 0.0% | 0 |
| | Other (please specify) | 0 |
| | answered question | 20 |
| | skipped question | 0 |



11. What was your major when your mentoring relationship with Dr. Warner began? If you were in high school, what major had you decided to select as an undergraduate? If you had completed your advanced degree (M.S. or Ph.D.), in what field did you obtain it?

| | Response Count |
|---|---|
| | 20 |
| answered question | 20 |
| skipped question | 0 |

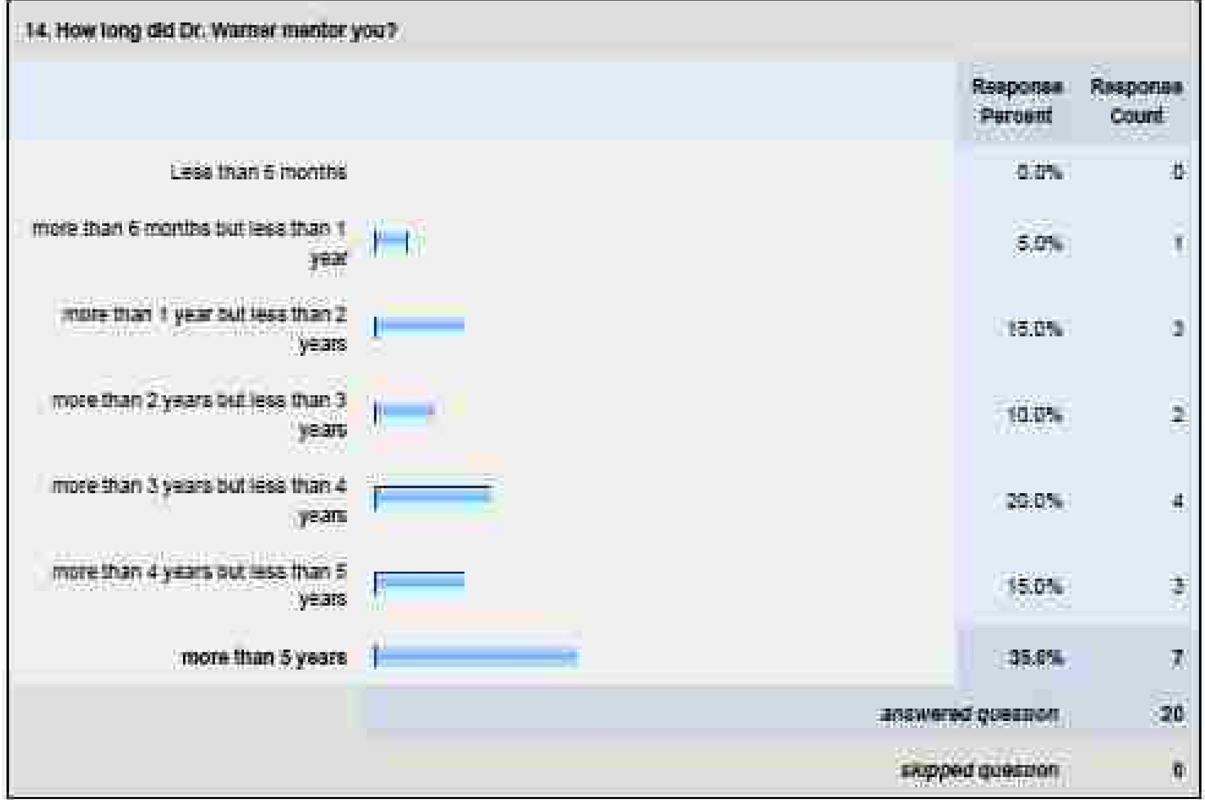


12. Who took the initiative to create the mentoring relationship between you and Dr. Warner?

| | Response Percent | Response Count |
|---|---|---|
| you and Dr. Warner | 85.0% | 17 |
| you only | 0.0% | 0 |
| Dr. Warner only | 15.0% | 3 |
| | answered question | 20 |
| | skipped question | 0 |

**13. What did you hope to get out of your mentoring relationship with Dr. Warner? Were your expectations fulfilled?**

| | Response Count |
|---|---|
| | 20 |
| answered question | 20 |
| skipped question | 0 |



**14. How long did Dr. Warner mentor you?**

| | Response Percent | Response Count |
|---|---|---|
| Less than 6 months | 0.0% | 0 |
| more than 6 months but less than 1 year | 5.0% | 1 |
| more than 1 year but less than 2 years | 15.0% | 3 |
| more than 2 years but less than 3 years | 10.0% | 2 |
| more than 3 years but less than 4 years | 20.0% | 4 |
| more than 4 years but less than 5 years | 15.0% | 3 |
| **more than 5 years** | **35.0%** | **7** |
| answered question | | 20 |
| skipped question | | 0 |

Page 1

15. How effective do you think Dr. Warner is as a mentor?

| | Response Percent | Response Count |
|---|---|---|
| Exceptionally effective | 65.0% | 13 |
| Very effective | 30.0% | 6 |
| Effective | 5.0% | 1 |
| Not very effective | 0.0% | 0 |
| Not effective | 0.0% | 0 |
| | answered question | 20 |
| | skipped question | 0 |

16. What characteristics of Dr. Warner's mentoring contributed to his effectiveness as a mentor?

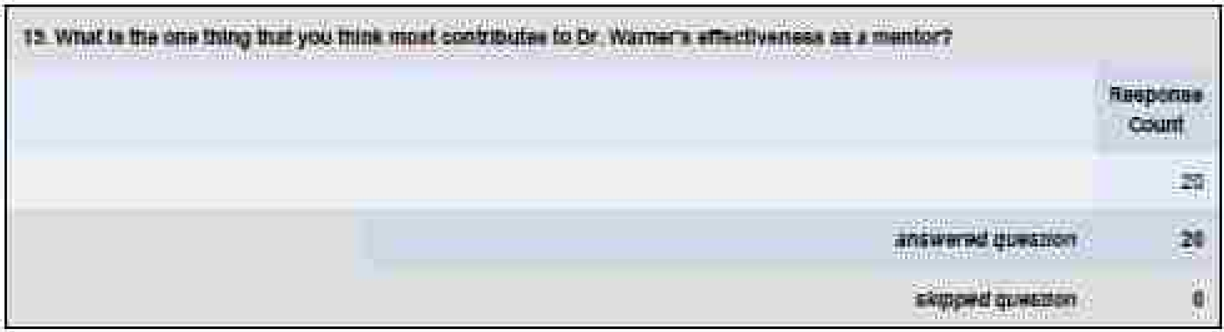
| | Response Count |
|---|---|
| | 20 |
| answered question | 20 |
| skipped question | 0 |

17. What traits in Dr. Warner's personality contribute to his effectiveness as a mentor?

| | Response Count |
|---|---|
| | 20 |
| answered question | 20 |
| skipped question | 0 |

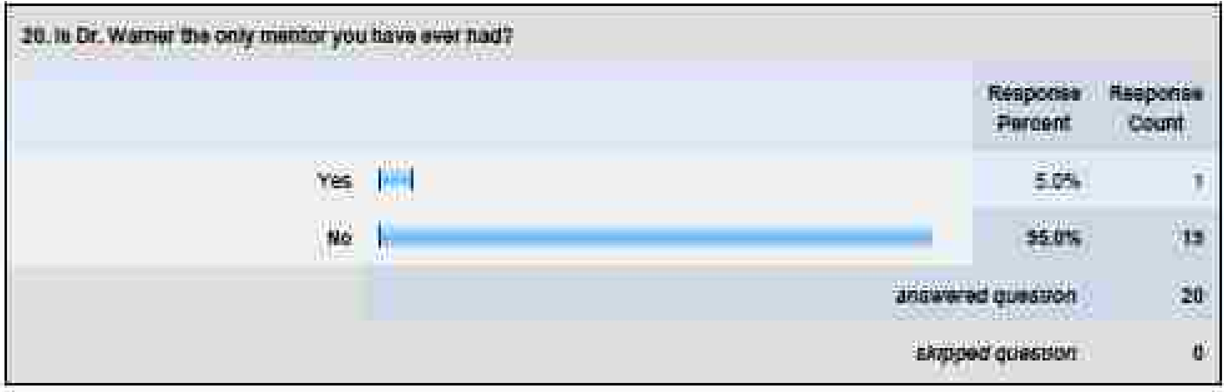
**18. What specifically did he do (continue to do) that was (is) effective? Please list as many as you are able to think of.**

| | Response Count |
|---|---|
| | 20 |
| answered question | 20 |
| skipped question | 0 |



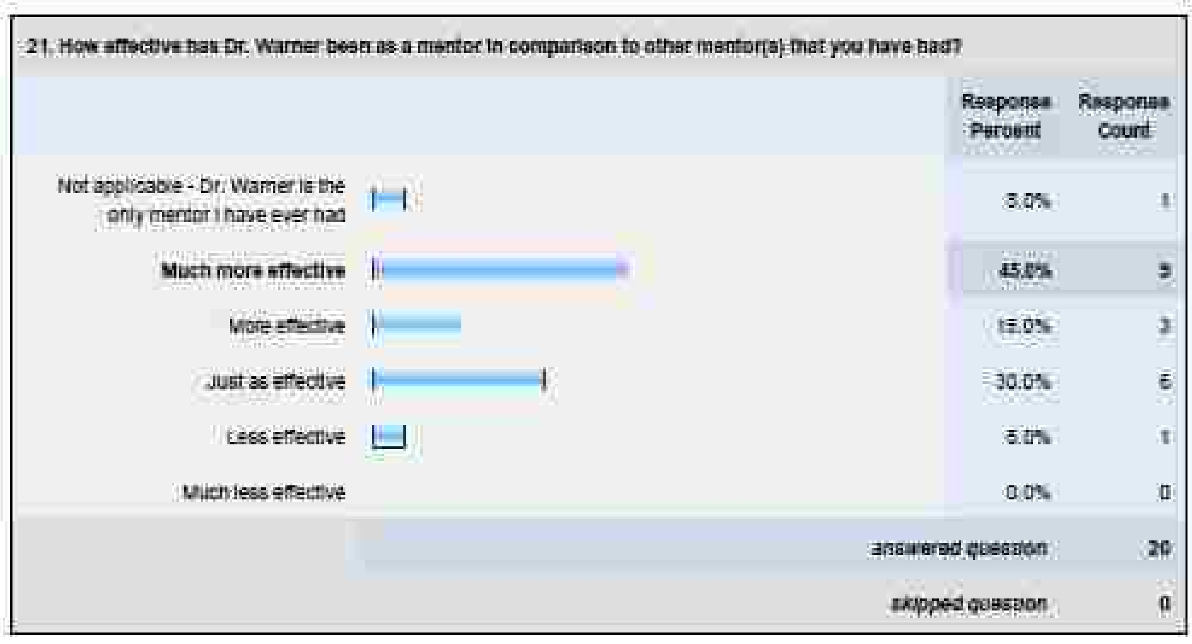
**19. What is the one thing that you think most contributes to Dr. Warner's effectiveness as a mentor?**

| | Response Count |
|---|---|
| | 20 |
| answered question | 20 |
| skipped question | 0 |



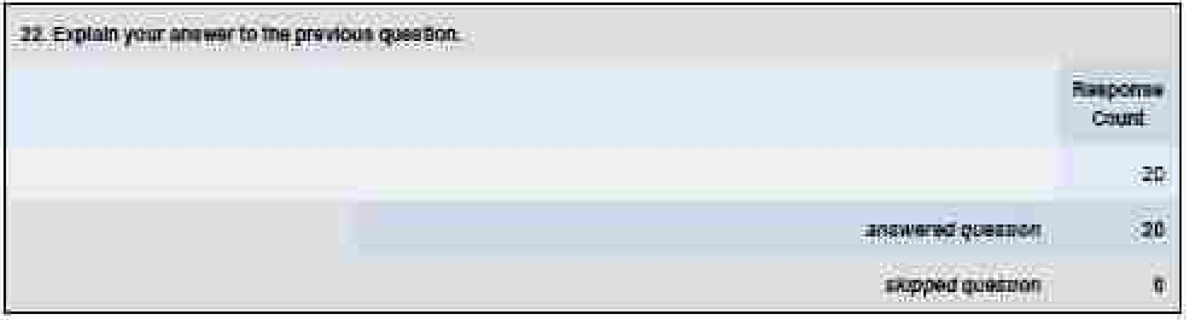
**20. Is Dr. Warner the only mentor you have ever had?**

| | Response Percent | Response Count |
|---|---|---|
| Yes | 5.0% | 1 |
| No | 95.0% | 19 |
| answered question | | 20 |
| skipped question | | 0 |



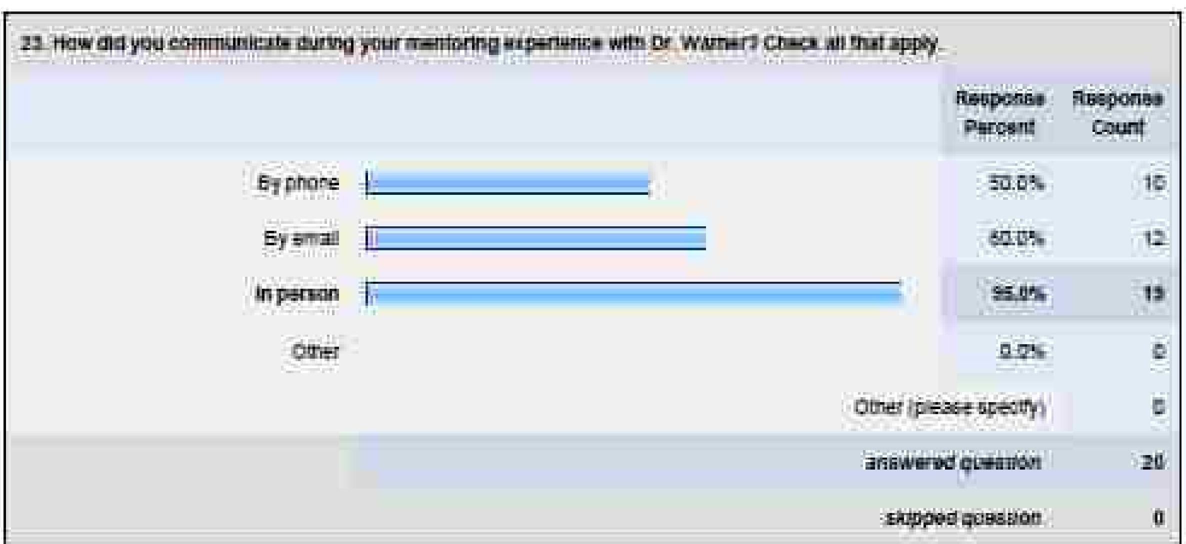
**21. How effective has Dr. Warner been as a mentor in comparison to other mentor(s) that you have had?**

| | Response Percent | Response Count |
|---|---|---|
| Not applicable - Dr. Warner is the only mentor I have ever had | 5.0% | 1 |
| **Much more effective** | **45.0%** | **9** |
| More effective | 15.0% | 3 |
| Just as effective | 30.0% | 6 |
| Less effective | 5.0% | 1 |
| Much less effective | 0.0% | 0 |
| | *answered question* | **20** |
| | *skipped question* | **0** |



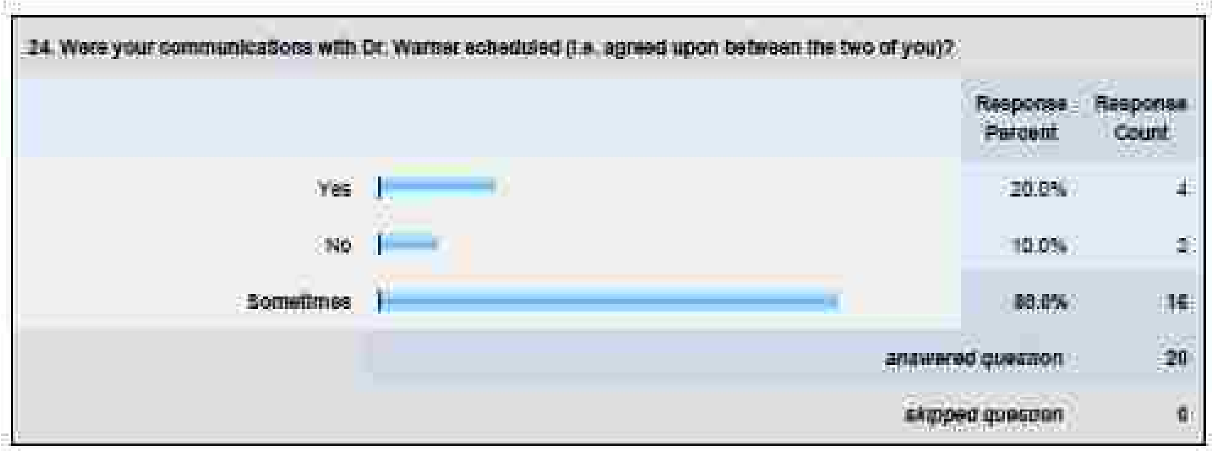
**22. Explain your answer to the previous question.**

| | Response Count |
|---|---|
| | 20 |
| *answered question* | **20** |
| *skipped question* | **0** |



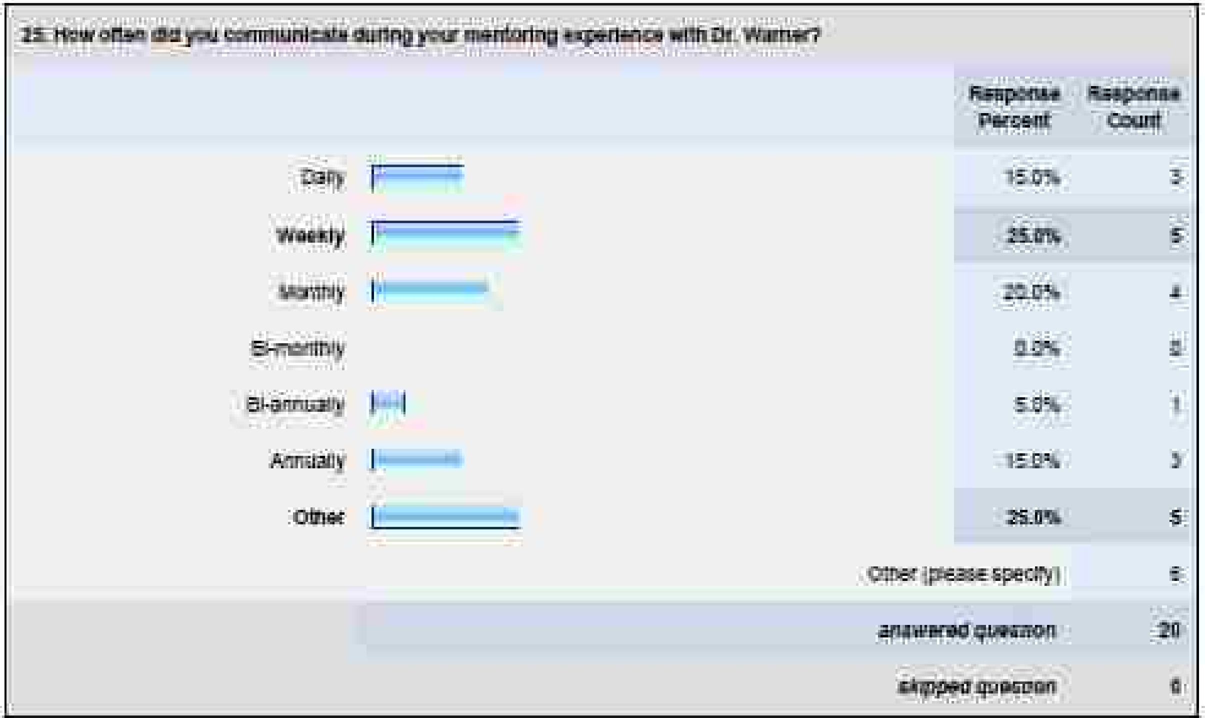
**23. How did you communicate during your mentoring experience with Dr. Warner? Check all that apply.**

| | Response Percent | Response Count |
|---|---|---|
| By phone | 50.0% | 10 |
| By email | 60.0% | 12 |
| **In person** | **95.0%** | **19** |
| Other | 0.0% | 0 |
| | Other (please specify) | 0 |
| | *answered question* | **20** |
| | *skipped question* | **0** |



24. Were your communications with Dr. Warner scheduled (i.e. agreed upon between the two of you)?

| | Response Percent | Response Count |
|---|---|---|
| Yes | 20.0% | 4 |
| No | 10.0% | 2 |
| Sometimes | 80.0% | 16 |
| answered question | | 20 |
| skipped question | | 6 |



25. How often did you communicate during your mentoring experience with Dr. Warner?

| | Response Percent | Response Count |
|---|---|---|
| Daily | 15.0% | 3 |
| Weekly | 25.0% | 5 |
| Monthly | 20.0% | 4 |
| Bi-monthly | 0.0% | 0 |
| Bi-annually | 5.0% | 1 |
| Annually | 15.0% | 3 |
| Other | 25.0% | 5 |
| Other (please specify) | | 6 |
| answered question | | 20 |
| skipped question | | 6 |

26. How critical to the development and sustainment of your mentor/protégé relationship with Dr. Isiah Warner was each item in the list below ?

| | extremely critical | very critical | critical | not very critical | not critical | N/A | Rating Average | Response Count |
|---|---|---|---|---|---|---|---|---|
| Willingness by you to participate? | 47.4% (9) | 31.6% (6) | 15.8% (3) | 0.0% (0) | 5.3% (1) | 0.0% (0) | 4.16 | 19 |
| Willingness by Dr. Warner to participate? | 68.4% (13) | 15.8% (3) | 10.5% (2) | 0.0% (0) | 0.0% (0) | 5.3% (1) | 4.61 | 19 |
| Openness in you? | 35.0% (7) | 30.0% (6) | 30.0% (6) | 0.0% (0) | 5.0% (1) | 0.0% (0) | 3.90 | 20 |
| Openness in Dr. Warner? | 52.6% (10) | 31.6% (6) | 15.8% (3) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 4.37 | 19 |
| Trust by you in Dr. Warner? | 65.0% (13) | 25.0% (5) | 10.0% (2) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 4.55 | 20 |
| Trust by Dr. Warner in you? | 60.0% (12) | 30.0% (6) | 10.0% (2) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 4.50 | 20 |
| Honesty in you? | 60.0% (12) | 25.0% (5) | 15.0% (3) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 4.45 | 20 |
| Honesty in Dr. Warner? | 50.0% (10) | 35.0% (7) | 15.0% (3) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 4.35 | 20 |
| Flexibility in you? | 25.0% (5) | 30.0% (6) | 30.0% (6) | 5.0% (1) | 5.0% (1) | 5.0% (1) | 3.68 | 20 |
| Flexibility in Dr. Warner? | 25.0% (5) | 25.0% (5) | 30.0% (6) | 20.0% (4) | 0.0% (0) | 0.0% (0) | 3.55 | 20 |
| Your awareness of your weaknesses? | 22.2% (4) | 27.8% (5) | 22.2% (4) | 16.7% (3) | 5.6% (1) | 5.6% (1) | 3.47 | 18 |
| Dr. Warner's awareness of your weaknesses? | 40.0% (8) | 35.0% (7) | 20.0% (4) | 0.0% (0) | 5.0% (1) | 0.0% (0) | 4.05 | 20 |
| Your awareness of your strengths? | 35.0% (7) | 20.0% (4) | 30.0% (6) | 15.0% (3) | 0.0% (0) | 0.0% (0) | 3.75 | 20 |
| Dr. Warner's awareness of your strengths? | 55.0% (11) | 25.0% (5) | 20.0% (4) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 4.35 | 20 |
| Willingness by you to change? | 35.0% (7) | 35.0% (7) | 15.0% (3) | 0.0% (0) | 5.0% (1) | 10.0% (2) | 4.06 | 20 |
| Willingness by Dr. Warner to change? | 5.0% (1) | 15.0% (3) | 25.0% (5) | 25.0% (5) | 20.0% (4) | 10.0% (2) | 2.56 | 20 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| The effectiveness of the communication between the two of you? | 70.0% (14) | 25.0% (5) | 5.0% (1) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 4.65 | 20 |
| | | | | | | | answered question | 20 |
| | | | | | | | skipped question | 0 |

**27. During your mentoring experience with Dr. Isiah Warner, how well did he ....**

| | extremely well | very well | well | not very well | not well at all | N/A | Response Count |
|---|---|---|---|---|---|---|---|
| understand your personal goals | 42.1% (8) | 42.1% (8) | 15.8% (3) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 19 |
| respect your personal goals | 47.4% (9) | 47.4% (9) | 5.3% (1) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 19 |
| evaluate your talents | 50.0% (10) | 45.0% (9) | 5.0% (1) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| build your talents | 45.0% (9) | 55.0% (11) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| understand what motivated you | 20.0% (4) | 50.0% (10) | 30.0% (6) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| hold you accountable | 40.0% (8) | 45.0% (9) | 5.0% (1) | 0.0% (0) | 0.0% (0) | 10.0% (2) | 20 |
| express care about your needs and concerns | 55.0% (11) | 30.0% (6) | 15.0% (3) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| suggest appropriate resources (primary sources, people, etc.) | 65.0% (13) | 20.0% (4) | 15.0% (3) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| provide you with feedback | 55.0% (11) | 40.0% (8) | 5.0% (1) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| encourage you to pursue excellence | 80.0% (16) | 15.0% (3) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| inspire you to pursue your passion | 85.0% (17) | 15.0% (3) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| take a special interest in you as an individual | 60.0% (12) | 20.0% (4) | 20.0% (4) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| actively listen | 55.0% (11) | 40.0% (8) | 5.0% (1) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| challenge you to extend your abilities (i.e. risk taking, write a section of an article, etc.) | 80.0% (16) | 5.0% (1) | 15.0% (3) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| allow you to make your own decisions | **60.0% (12)** | 25.0% (5) | 10.0% (2) | 0.0% (0) | 0.0% (0) | 5.0% (1) | 20 |
| nurture new thoughts or ideas (scientific or otherwise) generated by you | **55.0% (11)** | 20.0% (4) | 20.0% (4) | 0.0% (0) | 0.0% (0) | 5.0% (1) | 20 |
| provide you with suggestions of how to achieve something | **60.0% (12)** | 15.0% (3) | 25.0% (5) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| help you to deal with setbacks | **45.0% (9)** | 25.0% (5) | 20.0% (4) | 0.0% (0) | 0.0% (0) | 10.0% (2) | 20 |
| assist you to identify possible solutions to problems | **42.1% (8)** | 21.1% (4) | 31.6% (6) | 0.0% (0) | 0.0% (0) | 5.3% (1) | 19 |
| serve as a role model for you | **85.0% (17)** | 10.0% (2) | 5.0% (1) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| provide you with emotional support | 30.0% (6) | 15.0% (3) | **35.0% (7)** | 10.0% (2) | 0.0% (0) | 15.0% (3) | 20 |
| encourage the pursuit of your personal interests | **40.0% (8)** | 30.0% (6) | 15.0% (3) | 0.0% (0) | 0.0% (0) | 15.0% (3) | 20 |
| help you to mature | 25.0% (5) | **40.0% (8)** | 25.0% (5) | 0.0% (0) | 0.0% (0) | 10.0% (2) | 20 |
| demonstrate his commitment to your intellectual and professional development | **70.0% (14)** | 15.0% (3) | 15.0% (3) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| make himself available to you when you were in need | **50.0% (10)** | 25.0% (5) | 25.0% (5) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| understand how you learned best | 25.0% (5) | **35.0% (7)** | 15.0% (3) | 0.0% (0) | 0.0% (0) | 25.0% (5) | 20 |
| foster your independence | **45.0% (9)** | 30.0% (6) | 10.0% (2) | 0.0% (0) | 0.0% (0) | 15.0% (3) | 20 |
| acknowledge your contributions | **45.0% (9)** | 25.0% (5) | 25.0% (5) | 0.0% (0) | 0.0% (0) | 5.0% (1) | 20 |
| communicate his expectations of you | **45.0% (9)** | 30.0% (6) | 15.0% (3) | 0.0% (0) | 0.0% (0) | 10.0% (2) | 20 |
| create an environment where it was safe to make mistakes | 35.0% (7) | **40.0% (8)** | 15.0% (3) | 0.0% (0) | 0.0% (0) | 10.0% (2) | 20 |
| give you challenging assignments | **50.0% (10)** | 30.0% (6) | 5.0% (1) | 0.0% (0) | 0.0% (0) | 15.0% (3) | 20 |
| introduce and expose you to his professional colleagues | **60.0% (12)** | 15.0% (3) | 20.0% (4) | 5.0% (1) | 0.0% (0) | 0.0% (0) | 20 |
| protect you from risks | 15.0% (3) | **30.0% (6)** | **30.0% (6)** | 0.0% (0) | 0.0% (0) | 25.0% (5) | 20 |
| assess the skills and knowledge that you were gaining in the lab | 25.0% (5) | **45.0% (9)** | 20.0% (4) | 0.0% (0) | 0.0% (0) | 10.0% (2) | 20 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| foster open communication | 60.0% (12) | 30.0% (6) | 10.0% (2) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| stimulate your creativity | 45.0% (9) | 20.0% (4) | 25.0% (5) | 0.0% (0) | 0.0% (0) | 10.0% (2) | 20 |
| encourage you to be confident in your ability | 70.0% (14) | 15.0% (3) | 15.0% (3) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| remain patient | 70.0% (14) | 20.0% (4) | 10.0% (2) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| help you to establish career connections | 55.0% (11) | 25.0% (5) | 10.0% (2) | 5.0% (1) | 0.0% (0) | 5.0% (1) | 20 |
| give you access to publishing opportunities | 50.0% (10) | 25.0% (5) | 10.0% (2) | 0.0% (0) | 0.0% (0) | 15.0% (3) | 20 |
| coach you through a poster presentation, the next job application, etc. | 40.0% (8) | 20.0% (4) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 40.0% (8) | 20 |
| help you with academic work | 35.0% (7) | 15.0% (3) | 5.0% (1) | 0.0% (0) | 0.0% (0) | 45.0% (9) | 20 |
| provide feedback on the quality of your lab work | 49.0% (9) | 10.0% (2) | 25.0% (5) | 0.0% (0) | 0.0% (0) | 20.0% (4) | 20 |
| encourage you to pursue additional scientific work | 57.9% (11) | 15.8% (3) | 21.1% (4) | 0.0% (0) | 0.0% (0) | 5.3% (1) | 19 |
| explore career options with you | 35.0% (7) | 15.0% (3) | 25.0% (5) | 5.0% (1) | 0.0% (0) | 20.0% (4) | 20 |
| provide you with opportunities for recognition | 65.0% (13) | 10.0% (2) | 20.0% (4) | 0.0% (0) | 0.0% (0) | 5.0% (1) | 20 |
| advise you on balancing responsibilities | 49.0% (9) | 30.0% (6) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 25.0% (5) | 20 |
| establish a collegial interaction with you | 55.0% (11) | 25.0% (5) | 15.0% (3) | 0.0% (0) | 0.0% (0) | 10.0% (2) | 20 |
| create a safe space for expression of your voice, thoughts and interests | 57.9% (11) | 21.1% (4) | 10.5% (2) | 0.0% (0) | 0.0% (0) | 10.5% (2) | 19 |
| aid you in discovering your own career path | 42.1% (8) | 31.6% (6) | 26.3% (5) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 19 |
| bring to life the potential within you | 50.0% (10) | 25.0% (5) | 25.0% (5) | 0.0% (0) | 0.0% (0) | 5.0% (1) | 20 |
| remain proactive in his mentoring role | 50.0% (10) | 35.0% (7) | 15.0% (3) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| teach you to set high standards and not compromise them | 55.0% (11) | 25.0% (5) | 10.0% (2) | 0.0% (0) | 0.0% (0) | 10.0% (2) | 20 |

| | | |
|---|---|---|
| | answered question | 20 |
| | skipped question | 0 |

26. During your mentoring experience with Dr. Isiah Warner, how often did he...

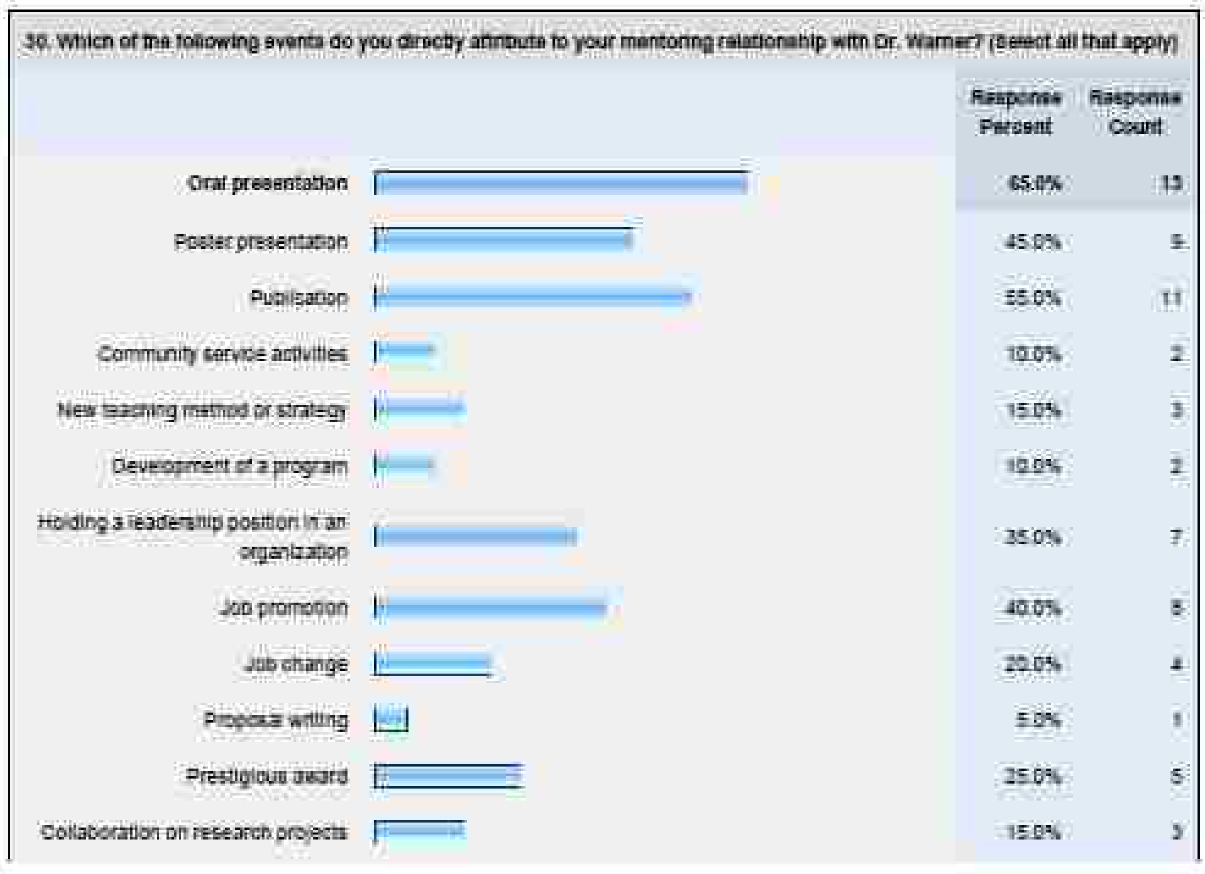
| | always | quite often | sometimes | not often | never | N/A | Response Count |
|---|---|---|---|---|---|---|---|
| hold you accountable | 63.2% (12) | 26.3% (5) | 5.3% (1) | 0.0% (0) | 0.0% (0) | 5.3% (1) | 19 |
| provide you with feedback | 45.0% (9) | 50.0% (10) | 5.0% (1) | 0.0% (0) | 0.0% (0) | 5.0% (1) | 20 |
| encourage you to pursue excellence | 80.0% (16) | 15.0% (3) | 5.0% (1) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| inspire you to pursue your passion | 65.0% (13) | 25.0% (5) | 5.0% (1) | 0.0% (0) | 0.0% (0) | 5.0% (1) | 20 |
| take a special interest in you as an individual | 65.0% (13) | 25.0% (5) | 10.0% (2) | 0.0% (0) | 0.0% (0) | 5.0% (1) | 20 |
| allow you to make your own decisions | 70.0% (14) | 25.0% (5) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 5.0% (1) | 20 |
| provide you with suggestions of how to achieve something | 50.0% (10) | 30.0% (6) | 20.0% (4) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| challenge you | 55.0% (11) | 40.0% (8) | 5.0% (1) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| help you to deal with setbacks | 45.0% (9) | 40.0% (8) | 0.0% (0) | 5.0% (1) | 0.0% (0) | 15.0% (3) | 20 |
| assist you to identify possible solutions to problems | 35.0% (7) | 35.0% (7) | 25.0% (5) | 0.0% (0) | 0.0% (0) | 5.0% (1) | 20 |
| provide you with emotional support | 15.0% (3) | 35.0% (7) | 10.0% (2) | 5.0% (1) | 5.0% (1) | 30.0% (6) | 20 |
| demonstrate his commitment to you as a protege | 55.0% (11) | 35.0% (7) | 5.0% (1) | 0.0% (0) | 0.0% (0) | 5.0% (1) | 20 |
| make himself available to you when you were in need | 47.4% (9) | 47.4% (9) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 5.3% (1) | 19 |
| foster your independence | 50.0% (10) | 35.0% (7) | 5.0% (1) | 0.0% (0) | 0.0% (0) | 10.0% (2) | 20 |
| communicate his appreciation of your contributions | 52.6% (10) | 26.3% (5) | 5.3% (1) | 5.3% (1) | 0.0% (0) | 10.5% (2) | 19 |
| communicate his expectations of you | 50.0% (10) | 35.0% (7) | 10.0% (2) | 0.0% (0) | 0.0% (0) | 5.0% (1) | 20 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| create an environment where it was safe to make mistakes | 45.0% (9) | 35.0% (7) | 5.0% (1) | 0.0% (0) | 0.0% (0) | 15.0% (3) | 20 |
| give you challenging assignments | 45.0% (9) | 35.0% (7) | 5.0% (1) | 0.0% (0) | 0.0% (0) | 15.0% (3) | 20 |
| introduce and expose you to his professional colleagues | 45.0% (9) | 35.0% (7) | 20.0% (4) | 5.0% (1) | 0.0% (0) | 0.0% (0) | 20 |
| protect you from risks | 30.0% (6) | 30.0% (6) | 15.0% (3) | 0.0% (0) | 0.0% (0) | 25.0% (5) | 20 |
| assess the skills and knowledge that you were gaining in the lab | 30.0% (6) | 40.0% (8) | 20.0% (4) | 0.0% (0) | 0.0% (0) | 10.0% (2) | 20 |
| foster open communication | 57.9% (11) | 36.8% (7) | 5.3% (1) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 19 |
| stimulate your creativity | 40.0% (8) | 35.0% (7) | 20.0% (4) | 0.0% (0) | 0.0% (0) | 5.0% (1) | 20 |
| encourage you to be confident in your ability | 65.0% (13) | 30.0% (6) | 5.0% (1) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| help you to establish connections | 40.0% (8) | 40.0% (8) | 20.0% (4) | 0.0% (0) | 0.0% (0) | 5.0% (1) | 20 |
| give you access to publishing opportunities | 40.0% (8) | 30.0% (6) | 20.0% (4) | 0.0% (0) | 0.0% (0) | 15.0% (3) | 20 |
| coach you through a poster presentation, the next job application, etc. | 35.0% (7) | 25.0% (5) | 10.0% (2) | 0.0% (0) | 0.0% (0) | 35.0% (7) | 20 |
| help you with academic work | 30.0% (6) | 10.0% (2) | 20.0% (4) | 0.0% (0) | 0.0% (0) | 40.0% (8) | 20 |
| provide feedback on the quality of your lab work | 30.0% (6) | 20.0% (4) | 30.0% (6) | 0.0% (0) | 0.0% (0) | 20.0% (4) | 20 |
| encourage you to pursue additional scientific work | 50.0% (10) | 45.0% (9) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 5.0% (1) | 20 |
| explore career options with you | 35.0% (7) | 25.0% (5) | 15.0% (3) | 5.0% (1) | 0.0% (0) | 20.0% (4) | 20 |
| provide you with opportunities for recognition | 45.0% (9) | 40.0% (8) | 15.0% (3) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 20 |
| advise you on balancing responsibilities | 25.0% (5) | 35.0% (7) | 10.0% (2) | 0.0% (0) | 5.0% (1) | 25.0% (5) | 20 |
| | | | | | | answered question | 20 |
| | | | | | | skipped question | 8 |

25. To what extent did your mentoring relationship with Dr. Isiah Warner positively influence your...

| | greatly influenced | very influenced | influenced | not very influenced | not influenced | N/A | Rating Average | Res[illegible] C[illegible] |
|---|---|---|---|---|---|---|---|---|
| academic performance? | 20.0% (4) | 50.0% (10) | 20.0% (4) | 0.0% (0) | 0.0% (0) | 10.0% (2) | 4.00 | |
| pursuit of the kind of student you wanted to become? | 55.0% (11) | 25.0% (5) | 5.0% (1) | 0.0% (0) | 0.0% (0) | 15.0% (3) | 4.59 | |
| ability to read scientific papers? | 55.0% (11) | 15.0% (3) | 5.0% (1) | 10.0% (2) | 5.0% (1) | 10.0% (2) | 4.17 | |
| skill in using research equipment? | 70.0% (14) | 5.0% (1) | 5.0% (1) | 0.0% (0) | 10.0% (2) | 10.0% (2) | 4.39 | |
| ability to develop a research hypothesis? | 50.0% (10) | 25.0% (5) | 5.0% (1) | 0.0% (0) | 10.0% (2) | 10.0% (2) | 4.17 | |
| ability to analyze data? | 50.0% (10) | 20.0% (4) | 10.0% (2) | 0.0% (0) | 10.0% (2) | 10.0% (2) | 4.11 | |
| ability to independently work on your research project? | 70.0% (14) | 15.0% (3) | 5.0% (1) | 0.0% (0) | 0.0% (0) | 10.0% (2) | 4.72 | |
| ability to collaborate with others? | 60.0% (12) | 15.0% (3) | 20.0% (4) | 0.0% (0) | 0.0% (0) | 5.0% (1) | 4.42 | |
| ability to organize your time better? | 40.0% (8) | 30.0% (6) | 10.0% (2) | 5.0% (1) | 5.0% (1) | 10.0% (2) | 4.06 | |
| self confidence? | 50.0% (10) | 25.0% (5) | 20.0% (4) | 0.0% (0) | 0.0% (0) | 5.0% (1) | 4.32 | |
| scientific confidence? | 70.0% (14) | 25.0% (5) | 5.0% (1) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 4.65 | |
| ability to write scientifically? | 45.0% (9) | 15.0% (3) | 10.0% (2) | 10.0% (2) | 5.0% (1) | 15.0% (3) | 4.00 | |
| ability to do oral presentations? | 50.0% (10) | 25.0% (5) | 15.0% (3) | 0.0% (0) | 0.0% (0) | 10.0% (2) | 4.39 | |
| scholarly interests? | 55.0% (11) | 20.0% (4) | 15.0% (3) | 5.0% (1) | 0.0% (0) | 5.0% (1) | 4.32 | |
| ability to navigate through the process of becoming a professional scientist? | 60.0% (12) | 25.0% (5) | 10.0% (2) | 0.0% (0) | 0.0% (0) | 5.0% (1) | 4.53 | |
| overall scientific competency? | 65.0% (13) | 20.0% (4) | 10.0% (2) | 0.0% (0) | 0.0% (0) | 5.0% (1) | 4.58 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| decisions about college, graduate school, a job, or a career? | 50.0% (10) | 30.0% (6) | 15.0% (3) | 0.0% (0) | 0.0% (0) | 5.0% (1) | 4.37 |
| overall development into a scientific professional? | 57.9% (11) | 31.6% (6) | 5.3% (1) | 0.0% (0) | 0.0% (0) | 5.3% (1) | 4.56 |
| commitment to continue working toward becoming a scientist? | 55.0% (11) | 20.0% (4) | 15.0% (3) | 0.0% (0) | 5.0% (1) | 5.0% (1) | 4.26 |
| commitment to becoming an exceptional scientist? | 65.0% (13) | 20.0% (4) | 10.0% (2) | 0.0% (0) | 0.0% (0) | 5.0% (1) | 4.58 |
| personal values? | 20.0% (4) | 30.0% (6) | 25.0% (5) | 0.0% (0) | 10.0% (2) | 15.0% (3) | 3.59 |
| lifestyle? | 25.0% (5) | 20.0% (4) | 25.0% (5) | 0.0% (0) | 10.0% (2) | 20.0% (4) | 3.63 |
| effectiveness as a scientist? | 50.0% (10) | 40.0% (8) | 10.0% (2) | 0.0% (0) | 0.0% (0) | 0.0% (0) | 4.40 |
| | | | | | | answered question | |
| | | | | | | skipped question | |



| | | | |
|---|---|---|---|
| Grant funded | | 20.0% | 4 |
| None | | 10.0% | 2 |
| | | answered question | 20 |
| | | skipped question | 0 |

**31. Is there anything unique about your mentoring relationship with Dr. Warner? Have you learned or gained things from him that you don't think you would have learned or gained any place else?**

| | | Response Count |
|---|---|---|
| | | 20 |
| | answered question | 20 |
| | skipped question | 0 |

**32. In your opinion, what are the top three things that Dr. Warner does as a mentor that you think ALL mentors should do?**

| | | Response Count |
|---|---|---|
| | | 20 |
| | answered question | 20 |
| | skipped question | 0 |

**33. What did you learn about yourself as a result of your participation in the mentoring experience with Dr. Isiah Warner?**

| | | Response Count |
|---|---|---|
| | | 20 |
| | answered question | 20 |
| | skipped question | 0 |

**34. Is there anything else you would like to share about your mentoring experience with Dr. Isiah Warner?**

| | Response Count |
|---|---|
| | 20 |
| *answered question* | 20 |
| *skipped question* | 0 |

**35. Please provide us with your contact information below just in case we need to follow up with you for clarification of a response. Only data extracted from this survey will be used. All other information will remain confidential. All of the answers will be pooled and no one will see the original data before it is aggregated except the evaluator. Thank you for your participation in this survey.**

| | Response Percent | Response Count |
|---|---|---|
| Name: | 100.0% | 20 |
| Company: | 70.0% | 14 |
| Address: | 100.0% | 20 |
| Address 2: | 15.0% | 3 |
| City/Town: | 100.0% | 20 |
| State: | 100.0% | 20 |
| ZIP/Postal Code: | 100.0% | 20 |
| Country: | 100.0% | 20 |
| Email Address: | 100.0% | 20 |
| Phone Number: | 100.0% | 20 |
| *answered question* | | 20 |
| *skipped question* | | 0 |

## VITA

Monica R. Sylvain was born in Omaha, Nebraska, in 1968. She is the third born child in a family of four children having one elder brother, one elder sister, and one younger brother. She attended Wakonda Elementary School and Joslyn Elementary School during her primary years and thereafter moved to Lewis and Clark Junior High School for middle school. Her high school education was completed at Central High School in 1986 where she graduated number three out of a class of four hundred sixty - nine. After receiving her high school diploma, Monica entered college with a full academic scholarship. Over the course of her undergraduate studies, she interned at Rohm and Haas, Incorporated, and performed independent research at the Omaha Public Power District, Hercules, Incorporated, and Sandia National Laboratory. She received numerous honors and awards during her undergraduate career. However, her most cherished is the Dow Chemical USA Outstanding Undergraduate Chemical Engineer Award for four consecutive years. Graduating at the top of her class, Monica completed her Bachelor of Science in Chemical Engineering (*Magna Cum Laude*) from Howard University in 1990. She began her technical career as a process engineer by accepting a position with American Cyanamid in Westwego, Louisiana, in 1991. After eight years, she changed careers to explore her passion for education and became a certified high school Honors Chemistry and Honors Physics teacher at West St. John High School in Edgard, Louisiana. In 2002, she brought her high school science students to the chemistry department at Louisiana State University for a class field trip where she met Dr. Isiah M. Warner, who later became her employer and her graduate school advisor. Before beginning graduate school, Monica managed the multi-million dollar National Science Foundation Math and Physical Sciences (NSF-MPS) grant named the Louisiana Science, Technology, Engineering and Mathematics (LA-STEM) Research Scholars

Program for Vice Chancellor Isiah M. Warner in the Office for Strategic Initiatives at Louisiana State University. In fall 2005, she began graduate studies in analytical chemistry at Louisiana State University. The following year Monica gained approval from the chemistry faculty to pursue research on a science education topic alongside her analytical chemistry research, which was a first for the department. During her graduate tenure, she received several awards including the *National Science Foundation Graduate Research Fellowship*, the *National Science Foundation Bridge to the Doctorate Fellowship*, the *United Negro College Fund/Merck and Company Graduate Science Research Dissertation Fellowship*, and the *Analytical Chemistry Outstanding Oral Speaker Award.* Monica graduated with the degree of Doctor of Philosophy from Louisiana State University in December 2010.

A compilation of publications and conference presentations from her dissertation research work in analytical chemistry and science education follow:

Sylvain, Monica R.; Das, Susmita.; Losso, Jack N.; El-Zahab, Bilal; Warner, Isiah M. "Positive Cooperative Mechanistic Binding of Proteins at Low Concentrations: A Comparison of poly (sodium N-undecanoyl sulfate) and Sodium Dodecyl Sulfate," *submitted to Langmuir*

Sylvain, Monica R.; Wandersee, James "The Exemplar Mentoring of Isiah M. Warner: Why Does it Work?," *in preparation for Science (Education Forum)*

2010 – March 29th Oral Presentation "Spectroscopic Characterization of Protein-Ligand Interactions: A Comparison of poly-SUS and SDS," Sylvain, Monica R.; Das, Susmita; Losso, Jack N.; El- Zahab, Bilal ; Warner, I. M. – National Organization for the Professional Advancement of Black Chemists and Chemical Engineers (NOBCChE) National Conference, Atlanta, GA

Gates, Arther T.; Moore, Jr., Leonard; Sylvain, Monica R.; Jones, Christina; El-Zahab, Bilal; Lowry, Mark; Robinson, James W.; Strongin, Robert M.; Warner, Isiah M. "Mechanistic Investigation of N-Homocysteinylation-Directed Gold Nanoparticle Assembly," *Langmuir*, 2009

2009 – April 28th Oral Presentation "University Faculty as Mentors: Lessons from a PAESMEM Awardee," Sylvain, Monica R. **-** Louisiana State University Office for Strategic Initiatives Faculty Mentoring Research Workshop, Baton Rouge, LA

2009 – March 19th Oral Presentation “Science Teachers as Mentors: Lessons from a PAESMEM Awardee,” Sylvain, Monica R. National Science Teachers Association (NSTA) National Conference, New Orleans, LA

2008 – August 4th Poster Presentation “Molecular Micelle-based Discontinuous Gel Electrophoresis Method for the Analysis of Globular Protein Mixtures,” Sylvain, Monica R.; Villar, Emily; Fakayode, Sayo O.; Lee, Jessica; El-Zahab, Bilal.; Lowry, Mark; Losso, Jack N.; Warner, Isiah M. - NSF Fostering US-Australian Collaborations in Materials, Sydney, Australia

2008 – March 20th Oral Presentation “A Novel Method for Separation of Globular Proteins by Use of Polyacrylamide Gel Electrophoresis,” Sylvain, Monica R.; Villar, Emily; Fakayode, Sayo O.; Lee, Jessica; El-Zahab, Bilal; Lowry, Mark; Losso, Jack N.; Warner, Isiah M. – National Organization for the Professional Advancement of Black Chemists and Chemical Engineers (NOBCChE) National Conference, Philadelphia, PA

2008 – March 3rd Poster Presentation “Identification and Quantification of Protein-Surfactant Complexes using Polyelectrolyte Multilayer Coated Capillaries,” Sylvain, Monica R.; Luces, Candace; Fakayode, Sayo O.; Lowry, Mark; Losso, Jack N.; Warner, Isiah M. - Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (PITTCON), New Orleans, LA