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Teaching Philosophy:

In my view teaching consists of two primary elements: one element is encouraging students to invest in their own education, and the other is to guide the student to an understanding of the course material. The first of these elements is, in my experience, more difficult for an educator to contend with, while the second is primarily a matter of helping the students help themselves.

A simple fact of life for a teacher is that students will not pay attention and subsequently learn unless they are motivated to do so. Therefore, while not listed in most teaching job descriptions, I believe a large part of a professor's job is to encourage students to invest time and effort in their own education. To some degree, the student is already motivated to obtain an education for one reason or another since a college education is voluntary. Additionally, students will be motivated by a range of different considerations, from students who attempt to pass a class with minimal effort simply because it's a requirement to others who will be fascinated by the particular subject at hand. An astute teacher will recognize that in any given class all of these motivations are present and be able to engage the different students on different levels. I believe a fundamental means of engaging the students at all levels is to ground the material in the student's own experience to help them recognize how the course material is directly relevant to their lives. For example, many general chemistry topics have clear consequences in everyday life such as mixing cleaning solvents or electrolytic reactions in batteries. The student may be vaguely aware of these topics but has not yet incorporated them into a formal conceptual framework. Helping students pin their vague knowledge to a formal conceptual framework will motivate them to learn the underlying foundations of the framework that allows them a fuller understanding of the material. This grounding provides a hook to get the student invested in the coursework, helps the student recognize the value of the coursework, and may provide the foundation for a longer lasting interest as well.

Even with motivated students, the teacher's job is to help the student learn by setting up the conditions for successful learning. As an undergrad, I tutored students in math and chemistry. Later as a graduate student teaching assistant, I taught hundreds of students general chemistry and senior level physical chemistry lab. During that time, I learned that given patient personally tailored instruction a motivated student could succeed in any particular course, but I also learned that a professor could either help or hinder their students' achievement by changing the overall learning environment. A successful learning environment is even more important in the context of modern classes sizes with students sometimes numbering in the hundreds. The critical aspects of a successful learning environment include setting clear expectations of individual achievement for the students, transparent means by which students can measure their own progress, and supportive mechanisms by which students get any help they need to succeed. Individual instruction should be provided by the course professor during office hours, but may also be provided by available campus tutoring programs and even class peers. All of these tools need to be fully utilized in creating a successful learning environment for the student.

At the end of a course, my goal is for students to both have a thorough understanding of the course material, and also an appreciation of how the course material is relevant in their lives.

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Past Research Accomplishments:

Overall, my research interests have been and will continue to be focused on developing solid-state NMR methods to determine structural constraints in biologically relevant systems that are difficult to fully characterize by other means.

Currently, I am working with Robert Tycko at the National Institutes of Health developing SSNMR methods of structure determination applicable to proteins and peptides. I have been applying these methods to the determination of the structure of the Alzheimer's β-amyloid peptide in its fibrillar form. At the NIH, I developed methods to generate high orders of multiple quantum with fast spinning magic angle spinning (MAS) suitable for rare spin nuclei with large chemical shift anisotropies (CSAs) [3] and a method to determine absolute orientation constraints on carbonyl CSA tensors in fibrillar aggregates [1]. These methods were used to obtain structural constraints for the Alzheimer's $A\beta_{1\text{-}40}$ peptide in its fibrillar form, which are consistent with a structural model recently proposed by Petkova et al. [PNAS, 2002. **99**(26): p.16742]. I have also been involved in an ongoing project to develop a heteronuclear double quantum (DQ) filtered REDOR (a heteronuclear dipolar recoupling technique) experiment that may be used to determine consecutive torsion angles ψ_i, ϕ_{i+1} in the peptide backbone.

As a graduate student with Gary Drobny at the University of Washington my research was focused on distance and peptide backbone torsion angle determination methods (making extensive use of the homonuclear dipolar recoupling sequence DRAWS developed in the lab), as well as computational aspects of NMR. I was intimately involved in developing the double quantum filtered DRAWS method to determine the distance between consecutive carbonyl carbons in peptides by following the buildup of DQ coherence. This method is particularly useful for determining the torsion angle ϕ_i in samples with a large natural abundance background signals [5]. I was also significantly involved in developing the 2D-DQDRAWS method to determine the torsion angles ϕ_i, and ψ_i simultaneously by following the time evolution of the DQ coherence between two consecutive carbonyls, which is strongly dependent on the relative orientation of the two carbonyl CSA tensors [7]. The 2D-DQDRAWS technique, also applicable to samples with high background signals, is a highly sensitive measurement of peptide secondary structure and can be used to easily distinguish between secondary structural motifs like α-helices, 3_{10}-helices and β-sheets.

Throughout my career, a significant part of my research efforts consisted of developing software for data analysis and interpretation. In particular, I developed an n-spin, n-dimensional solid-state NMR simulation program as well as a number of various data processing programs.

Additionally, I engaged in undergraduate research with Ludwik Adamowicz at the University of Arizona in theoretical *ab-initio* computational chemistry. I performed calculations with the Gaussian90 program to determine the electron affinity of various nucleic acid bases in the gas phase [9-11,13] as well as developed software used to determine multi-body effects in the potential energy surface for HF in the gas phase.

*Reference numbers refer to published papers listed in my *curriculum vitae*.

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Future Research Interests:

My research interests are focused on developing and applying solid-state NMR (SSNMR) methods to determine structural constraints in biologically relevant systems in order to contribute to our overall understanding of biological processes. In particular, systems that are difficult to fully characterize by other commonly used structure determination techniques. Here, I propose to study the internal structure of fibrils relevant to dialysis related amylodoisis as a means of both addressing a pressing medical problem and of probing the underlying biological processes that drive fibril formation.

Motivation:

Dialysis related amyloidosis [1] is a serious complication for patients receiving long term hemodialysis, resulting in carpal tunnel syndrome and/or arthritis-like pain in joints and tendons. Eventually, a majority of long-term hemodialysis patients experience the debilitating effects of the disease due to a buildup of fibrillar amyloid material in bone and joint tissues. β_2 microglobulin (Fig. 1) is a relatively small protein with 99 residues and is the major component of dialysis related amyloid. Because β_2 microglobulin is readily expressed in a variety of expression systems, it has been used extensively in amyloid formation studies, but little is known about its internal molecular structure in amyloid fibrils beyond the fact that it contains the 'cross-β' motif common to all amyloid fibrils. I propose to apply a set of SSNMR structure elucidation tools to answer questions about the structure of the β_2 microglobulin protein in its fibrillar form.

Filamentous fibrillar amyloid aggregates are interesting to study because of the many diseases with which they are associated, but also from the standpoint of understanding protein folding [2]. A large number of disparate proteins have been shown to form fibrils with morphologies similar to that found in Fig. 1B. Amyloid fibrils exhibit a characteristic 'cross-β' scattering pattern from X-ray fiber diffraction measurements indicating the presence of β-sheets oriented perpendicular to the long axis of the fibril. While the internal structures of at least a few of these different fibrils have been shown to be different in detail [3-6], it is unknown why the overall morphologies are so similar. Considerable effort is being expended in elucidating the intermolecular interactions that stabilize amyloid formation, the mechanisms of amyloid formation from peptide monomers or oligomers, and the internal molecular structure of peptides in amyloid fibrils.

Amyloid fibrils are ill suited to study by solution-state NMR since they are insoluble aggregates. Nor is the detailed internal structure of fibrils obtainable by crystallography because fibrils do not exhibit the long range crystalline order required. However, fibrils do exhibit local structural order as evidenced by the X-ray fiber diffraction results and NMR line narrowing between spectra of the unfibrilized peptide and the fibrillar peptide. Solid-state NMR is best suited to determine the internal structure of a peptide in an amyloid fibril since various SSNMR techniques can be used to measure dipolar couplings and electronic structure, site selectively, to give distance and orientation constraints at well defined locations in the peptide [7, 8].

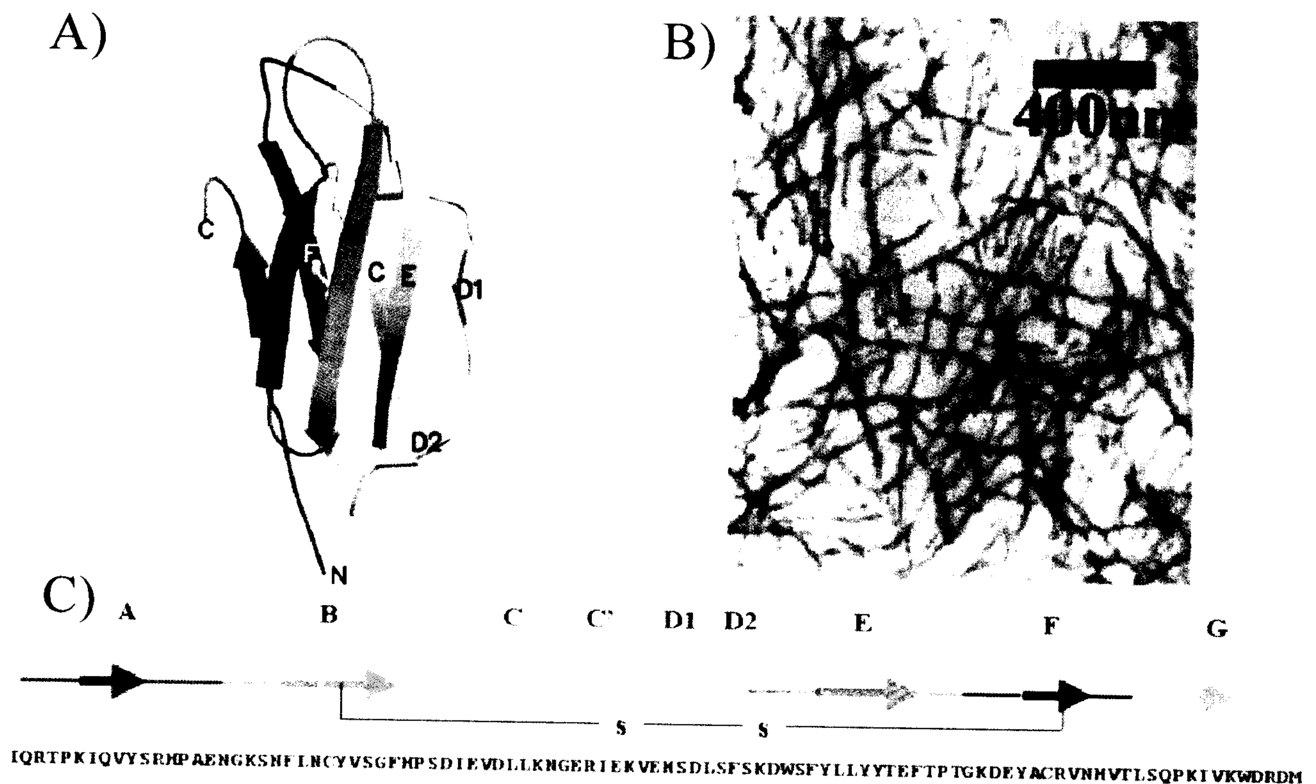

Figure 1: β_2 microglobulin. A) Native fold. B) Electron micrograph of β_2 microglobulin amyloid fibrils. C) Amino acid sequence. These images were taken from Jones et al., J. Mol. Biol. (2003) **325** pp249-257.

Primary Proposal:

The native protein structure of β_2 microglobulin (Fig. 1A) is a typical immunoglobulin fold consisting of seven β-strands organized into two β-sheets connected by a single disulfide bridge between Cys25 and Cys80. Researchers have identified fragments of the protein that will form amyloid fibrils *in vitro* [9-14], but are not agreed whether or not these fragments form the β-sheet core of the full-length protein amyloid. At least 3 different models have been proposed for the structure of β_2 microglobulin amyloid fibrils [14-16]: 1) Ivanova et al. [14] suggest that the C-terminal end (residues 72-99) of the peptide rearranges during formation of the fibril to give a new structural fold (β-strand – type I β-turn – β-strand) that then stacks to form the core of the fibril. The remainder of the peptide retains its native fold and is uninvolved in fibril formation. 2) Based on H/D exchange studies, Yamaguchi et al. [15] suggest that minimal amyloidogenic sequence(s) somewhere in the middle of the protein start to form an amyloid core. Then the induced β-sheet structure propagates through much of the rest of the protein. In their model the final amyloid core consists of the central part (approximately residues 20-80) of the peptide. 3) From conservation analysis and docking studies, Benyamini et al. [16] propose a different internal structure (not too dissimilar from the native fold) over a similar region of the peptide for the core of the amyloid.

Initially, I propose to study 3 fibril-forming fragments of β_2 microglobulin. I will validate the proposed (β-strand – type I β-turn – β-strand) structure for the C-terminal P72-M99 fragment. Additionally, I will determine the global fold of two candidates for the minimal amyloidogenic sequences in the middle of the protein, the so-called K3 fragment (obtained by digestion of the

amyloid) consisting of residues S20-K41 [15], and a central region consisting of residues D59-T71 that form the β-strand E in Fig. 1A [9], both of which have been shown to form fibrils *in vitro*. Since the fragments are all comparatively short, the selective isotopic labeling required for the SSNMR experiments may be achieved by producing these peptides synthetically and incorporating labeled amino acids as necessary. Later, I propose to study fibrils of the full-length β_2 microglobulin protein, which will be prepared depending on the isotopic labeling requirements, either by expression [17] or a 'total synthesis' method in which synthetic fragments of the protein are chemically ligated together to make the full length protein [18, 19].

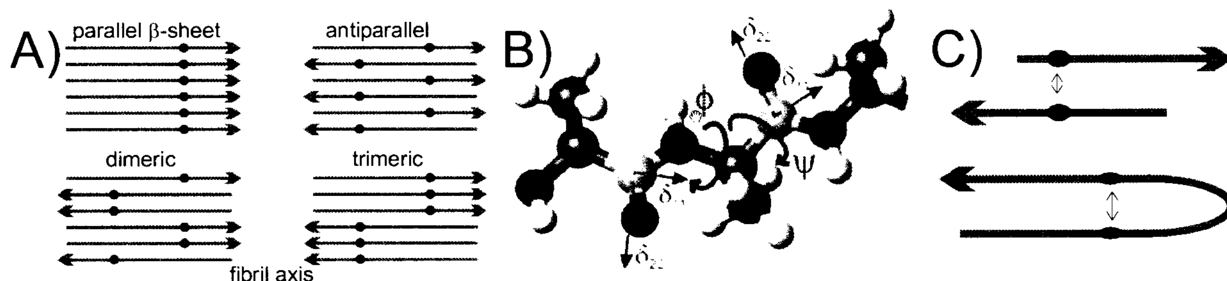

Figure 2: Experimental measurements to determine the global fold of the peptide in the amyloid fibril. A) Multiple quantum experiments to distinguish between different organizational structures. B) Backbone torsion angle measurements at particular locations. C) Proximity measurements between different parts of the peptide.

These structures will be determined using SSNMR methods similar to the ones used to determine structural constraints in amyloid fibrils of the Alzheimer's $A\beta_{1-40}$ peptide [3, 4, 7, 8], various fragments ($A\beta_{10-35}$, $A\beta_{16-22}$, and $A\beta_{34-42}$,) of the Aβ peptide [5], and the transthyretin fragment TTR(105-115) [6].

Dipolar couplings, chemical shift anisotropies (CSAs), and their cross correlations are measured at particular locations in the peptide to give structural constraints [3-8]. For instance, distances up to ~5-6 Å between two isotopic labels in a peptide (Fig. 2C) can be determined either by homonuclear or heteronuclear dipolar recoupling experiments. Such distance measurements can be used, for instance, to verify a hairpin structure, in which a peptide folds back on itself (as is postulated for the P72-M99 fragment). A variety of dipolar recoupling and cross-correlation experiments measure one or both of the peptide backbone torsion angles (Fig. 2B) at a single amino acid position for a direct determination of secondary structure at that position. Additionally, the correlation of secondary structure with the isotropic chemical shift of the C_α and CO carbons may be used to estimate whether a residue is in a helix or β-sheet conformation.

SSNMR may also be used to distinguish between fibril models with different organizations of peptides internal to the fibril. In particular, so-called 'spin counting' multiple quantum experiments can estimate the number of isotopic labels within a dipolar-coupling network. Fig. 2A shows a series of different internal organizations of peptides in a fibril that differ in the number of isotopic labels that are in close proximity to each other. Multiple quantum (MQ) spectra of a parallel β-sheet organization would show high orders of MQ coherence, while the anti-parallel case would show no higher orders since none of the isotopic labels are in proximity to each other. Dimeric and trimeric groupings would correspondingly show either 2 or 3 quantum coherences at most.

Secondary Proposal:

I also intend to continue developing new methods for structure determination in order to expand our ability to study biological systems. In particular, I will continue to explore methods to determine absolute orientations of dipolar vectors or CSA tensors internal to a fibril extending my previous work [4] with partially aligned fibrils samples on surfaces. Additionally, I will explore new methods to determine peptide backbone torsion angles (ϕ,ψ) in uniformly $^{13}C,^{15}N$ labeled residues of a peptide since many existing methods require more specific isotopic labeling schemes or are only sensitive in particular regions of the Ramachandran map. An extension of a heteronuclear DQ filtered dipolar dephasing experiment that I am currently working on looks promising. The experiment would be applicable to sequential pairs of uniformly ^{13}C, ^{15}N-labeled residues and should be sensitive over all values of ψ_i and ϕ_{i+1}.

Undergraduate Student Participation:

In my previous and current research efforts, I have performed and am intimately familiar with the majority of the required synthetic and SSNMR experimental methods and would feel comfortable training both graduate and undergraduate students in their use. Having experienced the benefits of undergraduate research myself, I would strongly encourage undergraduates to participate in my research program. Primarily, because I believe the experimental methods and techniques students would learn while contributing to one of these projects would be of practical benefit to their careers either directly in the workforce or as a foundation for future study.

Determining a global fold for an individual fibrillar peptide will require multiple sample preparations that vary in their labeling schemes. While the time commitment required to determine a global fold for each fibrillar peptide would be appropriate for graduate students, talented undergraduates should certainly be capable of contributing significantly to individual structural studies. Working on the various structural studies, undergraduates will be trained to perform peptide synthesis and purification, fibril formation studies, and SSNMR experiments and analysis. Long-term undergraduate researchers ($\geq$1 yr), or highly motivated ones, will be able to see a particular structural study through from beginning (synthesis) to end (NMR experiments and analysis). As a practical consideration, I anticipate that long-term undergraduate researchers will be able to contribute sufficiently to a project to deserve authorship on at least one or more journal articles.

Conclusion:

The proposed research projects allow exploration of both questions about a particular problem of pressing medical importance (dialysis related amyloidosis) and questions about fundamental protein folding interactions. Determining a global fold for the internal structure of β_2 microglobulin in dialysis related amyloid fibrils might be helpful for researchers designing a means of disrupting the amyloid formation process in patients on hemodialysis. Additionally, determining what particular peptide interactions are important in stabilizing the internal structure of different peptides in amyloid fibrils may give new insights into the fundamental interactions that drive disparate peptides to form fibrils of similar morphology.

References:

1. Kay, J., *beta(2)-microglobulin amyloidosis.* Amyloid, 1997. **4**(3): p. 187-211.
2. Dobson, C.M., *Principles of protein folding, misfolding and aggregation.* Sem. Cell Dev. Biol., 2004. **15**: p. 3-16.
3. Petkova, A.T., *et al.*, *A Structural model for Alzheimers beta-amyloid fibrils based on experimental constraints from solid state NMR.* PNAS, 2002. **99**(26): p. 16742-16747.
4. Oyler, N.A. and R. Tycko, *Absolute Structural Constraints on Amyloid Fibrils from Solid-State NMR Spectroscopy of Partially Oriented Samples.* J. Am. Chem. Soc., 2004. **126**(14): p. 4478-4479.
5. Gordon, D.J., *et al.*, *Increasing the amphiphilicity of an amyloidogenic peptide changes the beta-sheet structure in the fibrils from antiparallel to parallel.* Biophys. J., 2004. **86**(1): p. 428-434.
6. Jaroniec, C.P., *et al.*, *High-resolution molecular structure of a peptide in an amyloid fibril determined by magic angle spinning NMR spectroscopy.* PNAS, 2004. **101**(3): p. 711-716.
7. Tycko, R., *Applications of solid state NMR to the structural characterization of amyloid fibrils: methods and results.* Prog. Nuc. Mag. Res. Spec., 2003. **42**: p. 53-68.
8. Tycko, R., *Insights into the Amyloid Folding Problem from Solid-State NMR.* Biochemistry, 2003. **42**(11): p. 3151-3159.
9. Jones, S., *et al.*, *Amyloid-forming Peptides from beta2-Microglobulin -- Insights into the Mechanism of Fibril Formation in vitro.* J. Mol. Biol., 2003. **325**: p. 249-257.
10. Hasegawa, K., *et al.*, *Amyloidogenic synthetic peptides of beta2-microglobulin -- a role of the disulfide bond.* Biochem. Biophys. Res. Comm., 2003. **304**: p. 101-106.
11. Chiba, T., *et al.*, *Amyloid Fibril Formation in the Context of Full-length Protein: Effects of Proline Mutations on the Amyloid Fibril Formation of beta2-Microglobulin.* J. Biol. Chem., 2003. **278**(47): p. 47016-47024.
12. Ohhashi, Y., *et al.*, *Optimum Amyloid Fibril Formation of a Peptide Fragment Suggests the Amyloidogenic Preference of beta2-Microglobulin under Physiological Conditions.* J. Biol. Chem., 2004. **279**(11): p. 10814-10821.
13. Ivanova, M.I., *et al.*, *Role of C-Terminal 28 Residues of beta2-Microglobulin in Amyloid Fibril Formation.* Biochem., 2003. **42**: p. 13536-13540.
14. Ivanova, M.I., *et al.*, *An amyloid-forming segment of beta2-microglobulin suggests a molecular model for the fibril.* PNAS, 2004. **101**(29): p. 10584-10589.
15. Yamaguchi, K., *et al.*, *Core and Heterogeneity of beta2-Microglobulin Amyloid Fibrils as Revealed by H/D Exchange.* J. Mol. Biol., 2004. **338**: p. 559-571.
16. Benyamini, H., *et al.*, *beta2-Microglobulin Amyloidosis: Insights from Conservation Analysis and Fibril Modelling by Protein Docking Techniques.* J. Mol. Biol., 2003. **330**: p. 159-174.
17. Lian, L. and D.A. Middleton, *Labelling approaches for protein structural studies by solution-state and solid-state NMR.* Prog. Nuc. Mag. Res. Spec., 2001. **39**: p. 171-190.
18. Canne, L.E., *et al.*, *Chemical Protein Synthesis by Solid Phase Ligation of Unprotected Peptide Segments.* J. Am. Chem. Soc., 1999. **121**: p. 8720-8727.
19. Kochendoerfer, G.G. and S.B.H. Kent, *Chemical protein synthesis.* Curr. Opin. Chem. Biol., 1999. **3**: p. 665-671.