Objective

- Proteins
- Insulin protein
- Surfactant-protein interactions
- Photo-responsive surfactants (AzoTAB)
- UV-vis spectroscopy principle
- Concentration calculation of unknown sample by using UV-vis spectroscopy

Proteins

Proteins are organic macromolecules that take part in virtually all cell functions throughout living organisms. They may be enzymes responsible for catalyzing biochemical reactions, or have structural and mechanical functions, such as generating movement or providing a system of scaffolding that supports the cell structure. Many proteins also play an important role in cell signaling and adhesion, immune response and growth and differentiation. Proteins are polymers comprised of different combinations of 20 naturally occurring amino acids, known as protein residues. Each amino acid consists of a central alpha-carbon covalently bound to a carboxyl group, an amine group, a unique side chain, and a hydrogen atom (figure 1).

![Generalized structure of the amino acid](image)

**Figure 1.** Generalized structure of the amino acid. The nature of the R group determines classification.

The structure of the side chain attached to the alpha carbon can vary in size from a single hydrogen atom, as in glycine, to long chains or large heterocyclic groups, as in tryptophan, and determines many properties of the amino acid. The standard amino acid side chains may be generally classified as polar, non-polar, acidic or basic. Peptide linkages are formed between the individual amino acids through the translation process, in which messenger RNA produced by transcription is decoded by the ribosome, producing the main chain protein backbone. The function of a protein is heavily dependent on its structure, and the complex three dimensional interactions necessary for correct protein function require diverse and irregular protein structures. However, there are some aspects of protein structure common to all proteins, and specific, regular structural motifs have been, and continue to be identified.
Correct translation and subsequent structural organization and folding of the molecule leads to the formation of the so-called native state, or biologically active form of the protein. This native protein structure is not rigid, but a dynamic collection of equilibrium structures representing local minima in the potential energy of the system. Depending on the flexibility of the peptide structure, these motions may include hinge-bending, molecular breathing or conformational changes in addition to the atomic vibrations and backbone and side chain motions which take place on a sub-picosecond to nanosecond time scale. Though we only beginning to understand the relationship between these motions and the biochemical activity of the proteins, it is clear that they are functionally important. Such dynamics may be influenced by a number of factors such as pH, temperature, and ligand binding. While the modification of protein dynamics and domain motions may affect the function and activity of the protein, further structural alterations such as protein misfolding and protein malfunction arising from non-native conformations has been implicated in the progression of a number of debilitating and fatal diseases such as Parkinson’s disease, Alzheimer’s disease and Huntington’s disease. In these disorders, the misfolded proteins are observed to aggregate into large fibrillar species, as will be discussed in the following sections of this report. In order to better understand the protein folding/unfolding processes as well as the effects of structural fluctuations and the presence of metastable intermediates, a number of methods to induce protein unfolding have been examined, including chemical denaturants, pressure, temperature and pH. Surfactants, amphiphilic molecules with a hydrophobic tail and polar or charged hydrophilic head group, represent a class of small molecules that have proven quite useful in the study of these systems. When surfactants bind to proteins, the interaction of the surfactant tail with non-polar amino acids protects the hydrophobic core from interactions with the solvent, allowing the protein to unfold. In this report, we employ azobenzene based photoresponsive surfactants to control protein folding and conformation in the study of amyloidosis and the protein form-function relationship.

**Photoresponsive surfactants**

For photoresponsive surfactants, azobenzene is a common isomerizable unit in surfactant design. Azobenzene normally exists as the trans isomer, while the cis form cannot generally be made by chemical methods. Instead, the cis form is obtained through isomerization of the trans isomer. The trans-cis isomerization is controlled by the exposure wavelength, thus, different light illumination induces various yields of the cis isomer yield. For example, the azobenzene trans form can be easily isomerized to the cis conformation by illumination with light wavelengths from 330 to 380 nm, with a yield of the cis isomer ranging from 70-91%. Surfactants containing this azobenzene group (e.g., “azoTAB” or azobenzene-substituted phosphate amphiphiles) can be synthesized and applied to biological aspects. For example, azobenzene-substituted phosphate amphiphiles such as phosphoric acid mono-9-((4-phenylazo)-phenoxy)nonyl ester can be synthesized in two steps, first to synthesize the functionalized tails and then the tail is elongated via an ether linkage. This kind of surfactant is used to design biological membrane mimics, while the effects of the tran-cis isomerization on channel proteins has been studied. The photoresponsive cationic surfactant 4-ethyl-4’(trimethylaminobutoxy) azobenzene bromide (“azoTAB”) shown in Figure 2, an analog of the conventional surfactant dodecyltrimethylammonium bromide (DTAB), can be synthesized according to the published methods. Generally, azoTAB is prepared by azocoupling of alkylaniline with phenol, followed by alkylation and quarternization with dibromoalkane and trimethylamine. The final product purity can be determined by H-NMR in D2O.
The azoTAB surfactant exists as the *trans* isomer in solution in the dark (100% *trans*) or under visible light exposure (~75% *trans*), while conversion to the *cis* form can be achieved upon illumination with UV light (~95% *cis*). For a typical isomerization cycle of azoTAB, the absorption spectrum contains three transitions, 248 nm (*a π-π* transition), 350 nm (*a π-π* transition) and 440 nm (*a weak n-π* transition). Thus, using light at 365 nm from a mercury arc lamp, azoTAB is isomerized from *trans* isomer to the *cis* isomer leading to an increase in absorbance at 440 nm and a decrease at 350 nm. The photoisomeric state can be determined with UV-Vis spectroscopy as shown in Figure 1.5, with the *trans* isomer exhibiting a maximum absorbance at 350 nm and the *cis* isomer at 434 nm. The dipole moment across the nitrogen double bond is ~0.5 D for the *trans* isomer compared to ~3.1 D for the *cis* form. As a result, the *trans* isomer is significantly more hydrophobic than the *cis* form, allowing photo-induced changes in a variety of surfactant properties such as the critical micelle concentration (CMC), surface tension, and electrical conductivity. Furthermore, this property of azoTAB has also been used by our group to allow photo-reversible binding of the surfactant to various biomacromolecules, leading to photoreversible control BSA folding, the enzymatic activity of the lysozyme, solution structure of an amyloid-forming protein and DNA condensation.

**Insulin**

The Insulin monomer has a molecular weight of ~5800 kDa, and is comprised of two peptide chains linked by disulfide bridges. Both peptide chains contain large *α*-helical sections, and the longer B chain also contains a short *β*-structured section. In its native state, at neutral pH insulin exists primarily as a toroid-shaped hexamer. This is the form of the protein stored in the B-cells in the islets of Langerhans. It is thought that the hexameric assembly facilitates efficient storage of high concentrations of the hormone, as well as protecting the protein from degradation and aggregation. The active form of the molecule is the monomer, but destabilization of the monomer leads to self-association, and initiation of the fibrillation pathway.

While many proteins may take part in the fibrillation process, Insulin is an ideal model protein for studying the fibrillogenesis mechanism. While the large fibrillar structures formed in this system are morphologically very similar to those derived from disease proteins such as (Aβ) peptide...
fragments, insulin is readily available, relatively cheap, and well-studied. The primarily $\alpha$-helical secondary structure of the protein makes it a target for binding of the azoTAB surfactants. In the presence of the \textit{trans} isomer, the aggregation rate is increased with respect to the pure protein. Conversely, the \textit{cis} isomer extends the lag phase and apparently disrupts the formation of fibrils.