Electronic Energy Migration/Transfer as a Tool to Explore Biomacromolecular Structures

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av filosofie doktorsexamen framläggs till offentligt förvar i sal KB3B3, KBC fredagen den 4 april, kl. 09:00. Avhandlingen kommer att förvaras på engelska.

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Fluorescence-based techniques are widely used in bioscience, offering a high sensitivity and versatility. In this work, fluorescence electronic energy migration/transfer is applied to measure intramolecular distances in two types of systems and under various conditions.

The main part of the thesis utilizes the process of donor-acceptor energy transfer to probe distances within the ribosomal protein S16. Proteins are essential to all organisms. Therefore, it is of great interest to study protein structure and function in order to understand and prevent protein malfunction. Moreover, it is also important to try to study the proteins in an environment which resembles its natural habitat. Here two protein homologs were investigated; S16\textsubscript{thermo} and S16\textsubscript{meso}, isolated from a hyperthermophilic bacterium and a mesophilic bacterium, respectively. It was concluded that the chemically induced unfolded state ensemble of S16\textsubscript{thermo} is more compact than the corresponding ensemble of S16\textsubscript{meso}. This unfolded state compaction may be one reason for the increased thermal stability of S16\textsubscript{thermo} as compared to S16\textsubscript{meso}.

The unfolded state of S16 was also studied under highly crowded conditions, mimicking the environment found in cells. It appears that a high degree of crowding, induced by 200 mg/mL dextran 20, forces the unfolded state ensemble of S16\textsubscript{thermo} to become even more compact. Further, intramolecular distances in the folded state of five S16 mutants were investigated upon increasing amounts of dextran 20. We found that the probed distances in S16\textsubscript{thermo} are unaffected by increasing degree of crowding. However, S16\textsubscript{meso} shows decreasing intramolecular distances for all three studied variants, up to 100 mg/mL dextran. At higher concentrations, the change in distance becomes anisotropic. This suggests that marginally stable proteins like S16\textsubscript{meso} may respond to macromolecular crowding by fine-tuning its structure. More stable proteins like S16\textsubscript{thermo}, however, show no structural change upon increasing degree of crowding.

We also investigated the possibility of local specific interactions between the protein and crowding agent, by means of fluorescence quenching experiments. Upon increasing amounts of a tyrosine labelled dextran, a diverse pattern of fluorescence quantum yield and lifetime suggests that specific, local protein-crowder interactions may occur.

In a second studied system, electronic energy migration between two donor-groups, separated by a rigid steroid, was studied by two-photon excitation depolarization experiments. Data were analysed by using recent advances, based on the extended Förster theory, which yield a reasonable value of the distance between the two interacting donor-groups. To the best of our knowledge, this is the first quantitative analysis of energy migration data, obtained from two-photon excited fluorescence.

**Keywords**
Fluorescence, electronic energy transfer, two-photon excitation, small ribosomal protein S16, macromolecular crowding, dextran 20