Probing Molecular Conformation of Polymers and Proteins at a Surface Using Novel Time-Resolved Evanescent Wave-Induced Fluorescence Techniques

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Background:

We have developed novel variable-angle Time-Resolved Evanescent Wave-Induced Fluorescence Spectroscopic (VA-TREWIFS) techniques that allow us to probe spatially molecular conformation and chain segmental dynamics of a protein or polymer at an interface, *in situ*. These techniques have application in areas including protein biofouling, membrane proteins, annealing and wetting properties of thin polymeric films, etc

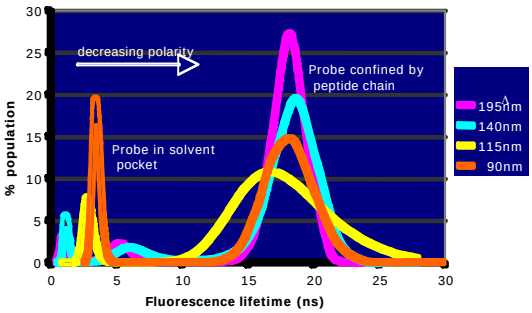
Outcomes:

- successful development of a method for monitoring molecular conformation and molecular mobility (ns time-scale) at an interface
- successful application of VA-TREWIFS to protein and polymer systems

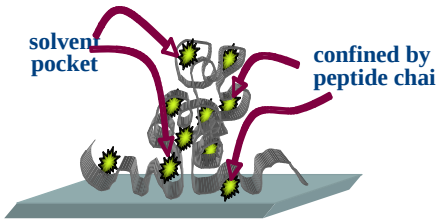
Progress to date:

VA-TREWIFS coupled with evanescent wave time-resolved anisotropy measurements, EW_TRAMS has given insights into:

- how interaction of a protein at an interface impacts on the protein's tertiary and secondary structure
- how the interplay between segment-segment, segment-surface and segment-solvent interaction affect the layer structure and surface mobility in cast polymer films



Maximum Entropy Method inversion of fluorescence decay curves as a function of the penetration depth, Λ, of the evanescent field for adsorbed protein. The fluorescent species is ANS associated with the polypeptide chain. Each peak corresponds to a population distribution of fluorescence lifetimes and reports on the polarity of the microdomain within the protein and hence the protein's secondary and tertiary structure.



Schematic representation of protein conformation at a surface as reported by Maximum entropy analysis of fluorescence decay kinetics: protein is partially denatured due to adhesive contacts with the surface thereby exposing fluorescent probe to more solvent. Away from the surface the protein maintains its secondary structure.

Funding is sought to:

- **broaden studies to more complex systems, both biological and polymeric, exploiting the proof of concept results obtained thus far**
- **extend the techniques to obtain full 3-D spatial information of macromolecular species at an interface**