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Internships in NanoSystems, Engineering and Technology

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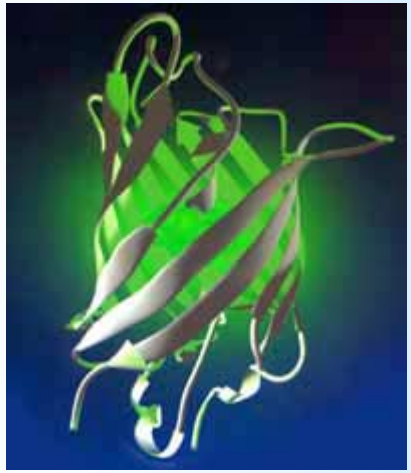
Abstract

In general, enzymes demonstrate high selectivity and reactivity under normal biological conditions, but are sensitive to denaturation and inactivation by temperature and pH extremes. Difficulty arises in maintaining stereochemical structure and activity under conditions of interest for practical applications. In particular, Green Fluorescing Protein (GFP), found in certain jellyfish, is optically active when in its native conformation. GFP is known to retain it fluorescence in a wide range of pH and temperature as compared to other proteins, but has a tendency to aggregate in solution and lose fluorescence. Immobilizing GFP, a small but stable protein, within a thin film provides information regarding protein conformation and the extent of aggregation. GFP behavior in the film provides a reference for immobilization of enzymes of comparable dimensions. Immobilization of enzymes or proteins on inorganic substrates also helps maintain structural integrity and function in less favorable environments. Mesoporous thin films are a suitable choice for enzyme supports due to their high surface areas (1000 m^2g^{-1}), large pore volumes (1.0mL g^{-1}) and tunable pore diameters (10-300Å). The synthesis of mesoporous silica materials as thin films makes them attractive for use as membranes, low dielectric interlayers and the immobilization of proteins for optical biosensing. Specifically, the optical transparency of mesoporous thin film could allow for convenient signal transduction in fluorescing systems such as GFP.

Green Fluorescent Protein

- *Aequorea victoria* is a jellyfish that contains a bioluminescent protein, GFP.
- Extremely stable protein able to retain fluorescence under a wide range of pH and temperature.



http://www.plantsci.cam.ac.uk

Why GFP ?

- GFP is a protein that will fluoresce when in it's native conformation.
- Immobilizing GFP provides information regarding protein conformation and the extent of aggregation with a mesopore.
- Enzymes are biological catalysts capable of degrading harmful chemicals ~ toxins and pesticides.

Protein Immobilization

Immobilization of enzymes on inorganic surfaces is of interest due to the potential application in biocatalysis and biosensoring.

Inorganic supports such as porous materials have many favorable qualities.

-Uniformed channels and tunable pore sizes

-Extremely high surface areas.

-Ability to functionalize

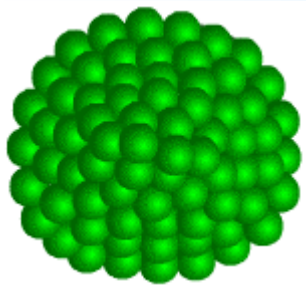
Thin Film Synthesis via Self-Assembly

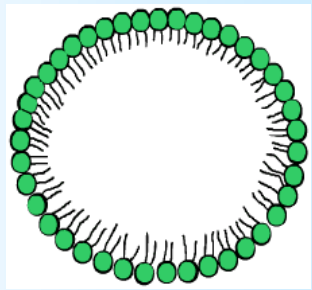
- Self-assembly is the spontaneous organization of materials through non-covalent interaction.
- In evaporative self assembly the progressively increasing surfactant concentration drives self-assembly of silica-surfactant micelles to organize into mesophases.
- The result is rapid formation of thin film mesophases that are highly oriented with respect to the substrate.

Micelle Role in Cooperative Self-assembly

- Micelle formation: Is a spherical assembly of surfactant in the solution where the interior of the assembly consists of the hydrophobic tails and the outside of the assembly being the hydrophilic head groups.

- Condensation of silica around the micelle forms a silica-surfactant complex. Burning off the surfactant results in a porous silica material





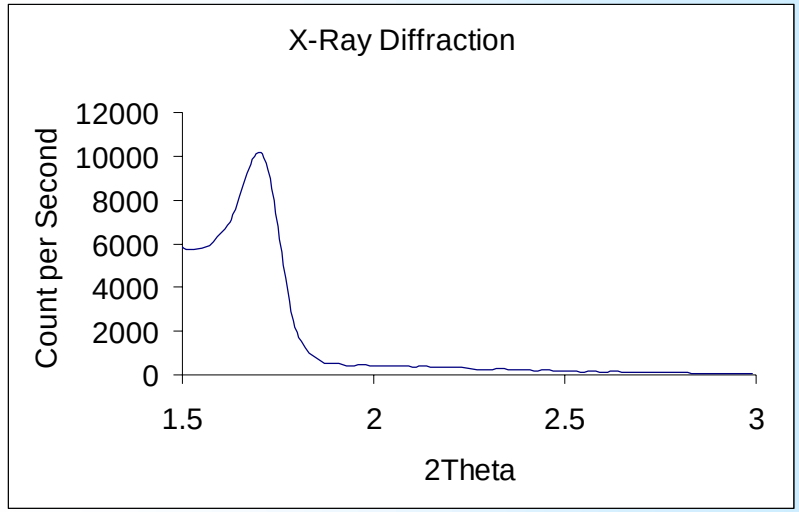
- Spherical & Cross sectional representation of a micelle

XRD Characterization of Silica Thin Film

- X-Ray Diffraction

  1D XRD provides evidence of meso-ordering (Left).

- Diffracted x-rays from different atoms can interfere with each other and because the atoms are arranged in a periodic fashion the diffracted waves will consist of sharp interference peaks with the same symmetry as in the distribution of atoms. Measuring the diffraction pattern therefore allows us to deduce the distribution of atoms in a material.



Incorporation of Aluminum

- Aluminum incorporation into porous material has many attractive applications.
  - Acid Catalysis
  - Ion Exchange
  - Hydrophilicity
    - Aluminum promotes hydrophilicity. GFP and Proteins in general desire a wet environment, this will lead to an increase in function and longevity.

ICP values are consistent with Al incorporation

Characterization of Aluminosilicate Thin Film

- Inductively Coupled Plasma (ICP). A tool for elemental analysis. The ICP uses a very hot argon plasma to excite atoms into high energy states. As these atoms relax they emit light at characteristic wavelenghts.

Current Goals

- Complete Characterization of thin film
  - 2D XRD
  - Transmission Electron Microscopy
- Immobilize Amino Acid
  - Characterize using Ultraviolet spectroscopy
- Immobilize GFP
  - Characterize and Publish

- Questions??
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