INTRODUCTION

In this course we will survey modern hydrodynamic techniques used for the characterization of biological macromolecules in the solution state. We will cover methods for probing biological structure and function, hydrodynamic theory of transport processes in solution, and discuss practical applications of these techniques to current problems in biophysics, biochemistry, and molecular biology, and provide a comprehensive introduction into analytical ultracentrifugation. You will gain insight in how these methods can be used for problem solving in modern research.

Hydrodynamic methods are part of solution biophysics.
Biophysics uses tools from physics and chemistry to study problems in biochemistry and molecular biology

INTRODUCTION

Life is characterized by exceptionally complex arrangements of atoms (molecular and supramolecular structures).

These arrangements form dynamically, and over time they undergo changes in conformation and composition. They also engage in complex interactions, carefully controlled by equilibria. Understanding these equilibria and interactions is at the core of many studies.

Dynamic interactions can best be studied in a physiological solution environment. Hydrodynamic methods are often used to identify and study these interactions.

Understanding life on a molecular level involves understanding the arrangements of these complex molecular structures, their conformational changes, and how their interactions are regulated and controlled.

The study of these processes gives insights into the fundamental structure and function of life's building blocks and allows us to study diseases that occur when these carefully calibrated equilibria fail (when regulation and control no longer function).

Hydrodynamic methods allow us to study the composition, conformation, size, stoichiometry, and thermodynamics of complex interactions.

Matter and Energy

In Hydrodynamics, we need to observe the molecules of interest in biophysical instruments. We use different frequencies of absorbed and scattered electromagnetic radiation to visualize their concentration in an experiment.

The concentration can be measured by different spectroscopic techniques:

- UV-visible Absorbance
- Refractive index
- Fluorescence emission

These observables are determined by the electronic and nuclear structure and the local environment around an atom or molecule. Spectroscopic methods therefore provide information on the composition, chemical environment & bound state of a molecule.

Matter and Energy

Instrumentation is used to generate fields of thermal, centrifugal, and electric fields that are used to interrogate biological material with electromagnetic radiation.

In some cases, the observed signal can be described by mathematical equations that model the process induced by the field and detected by the effect it has on the electromagnetic radiation. Experimental data can then be fitted to these models, and the parameters of the models provide molecular parameters.

In Hydrodynamics we need to:

- Understand the underlying chemistry
- Understand the capabilities of the instrumentation
- Ask the right questions
- Design the right experiments to test our hypotheses
- Build appropriate models
- Collect the appropriate data
- Properly analyze the data
- Interpret the results
- Combine information from multiple methods to arrive at an answer

Macromolecular Structure and Function

George Scatchard (1892-1973) – His Five Questions:

1. How many? (value of "n" for interacting components)
2. How tightly? (binding constants or free energies)
3. Why? (chemical nature of binding site)
4. Where? (physical location of binding site)
5. What of it? (significance of interaction)

Course Overview

Macromolecular Structure and Function

Selected parameters of interest to biophysical methods:

- Molar mass
- Hydrodynamic radius and hydration
- Anisotropy and shape vs. molecular structure
- Composition
- Partial concentration
- Sedimentation and diffusion coefficients
- Partial specific volume and density
- Charge
- Equilibrium constants and off-rates
- Molar extinction coefficients
- Excitation and fluorescence emission

Course Overview

Solution-based Methods and Techniques:

- Biospectroscopy
  - Absorption (UV and visible)
  - Circular Dichroism
  - Refractive index
  - Fluorescence spectroscopies (correlation, FRET)
- Calorimetry
- Analytical ultracentrifugation
  - Transport Properties
    - Diffusion
    - Sedimentation
    - Thermophoresis
  - Solvation/Hydration
- Light Scattering
- Small Angle Scattering
- Size exclusion chromatography
- Gel electrophoresis

Course Overview

Interrogation of Macromolecular Structure and Function

- Experimental Design
- Data Analysis and Modeling
  - Model Building
  - Data Fitting
    - Linear vs. nonlinear optimization, linearization
    - Grid searches and Monte Carlo
    - Effect of noise on results
    - Statistical analysis
  - Bead Modeling

Course Overview

Frequently considered questions:

- Is the sample homogeneous (*i.e.*, pure)?
- What is the molecular weight?
- If multiple components, what is the molecular weight distribution?
- Are interactions/associations thermodynamically reversible?
- What are the shapes and sizes of components and complexes?
- Do different macromolecules have (significantly) different densities (partial specific volumes)?
- Can conformational changes be measured?
- What are the oligomeric states? Equilibrium constants?
- Reversible mass action, aggregation, or non-interacting?
- How do molecules interact, and where does binding occur?
- Effect of solvent conditions on macromolecules