Methods in Molecular Biophysics: Structure, Dynamics, Function

BME, Tuesdays, 5PM

Instructors: David Case & Babis Kalodimos
<table>
<thead>
<tr>
<th>Date</th>
<th>Subject</th>
<th>Chapter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan 20</td>
<td>Introduction to Biophysics and macromolecular structure</td>
<td>A</td>
</tr>
<tr>
<td>Jan 27</td>
<td>Thermodynamics, calorimetry and surface plasmon resonance</td>
<td>C</td>
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<tr>
<td>Feb 3</td>
<td>Hydrodynamics: diffusion, electrophoresis, centrifugation, fluorescence anisotropy and dynamic light scattering</td>
<td>D</td>
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<tr>
<td>Feb 17</td>
<td>Midterm exam (1/3 of final grade)</td>
<td></td>
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<tr>
<td>Feb 24</td>
<td>Introduction to NMR: spin Hamiltonians, chemical shielding, spin-spin coupling, dipolar interactions</td>
<td>J1</td>
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<tr>
<td>Mar 3</td>
<td>Experimental NMR: multi-dimensional spectroscopy &amp; pulse sequences</td>
<td>J2</td>
</tr>
<tr>
<td>Mar 10</td>
<td>Protein NMR: assignment strategies, protein structure determination</td>
<td>J2</td>
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<td>Mar 17</td>
<td>Spring break</td>
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<tr>
<td>Mar 24</td>
<td>NMR studies of dynamics: spin relaxation, chemical exchange and H/D</td>
<td>J3</td>
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<tr>
<td>Mar 31</td>
<td>IR and Raman spectroscopy</td>
<td>E</td>
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<tr>
<td>Apr 7</td>
<td>Molecular dynamics simulations. Theory and practice of force-field based studies of macromolecules</td>
<td>I</td>
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<tr>
<td>Apr 14</td>
<td>Optimal microscopy: light, fluorescence and atomic force microscopy, single molecule studies</td>
<td>F</td>
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<tr>
<td>May 12?</td>
<td>Final exam (1/3 of final grade)</td>
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Biophysics: An integrated approach

Why?
The ideal biophysical method

would have the capability of observing atomic level **structures** and **dynamics** of biological molecules in their physiological environment, i.e. *in vivo*

would also permit visualization of the structures that form throughout the course of conformational changes or chemical reactions, regardless of the time scale involved
From *in vivo* to *in vitro*

A realistic drawing of the *E. coli* bacterium by David Goodsell
What Biophysics can do for Biochemistry?

Biochemistry describes in molecular terms the structures, mechanisms, and chemical processes shared by all organisms and provides organizing principles that underlie life in all its diverse forms, principles we refer to collectively as the molecular logic of life.

Development of high-throughput techniques:

- Bioinformatics (analysis of genomic information)
- Functional proteomics (identification of all the proteins present in a cell)
- Dynamic proteomics (determining how this population responds to external conditions)
- Structural genomics (protein structure determination)
What Biophysics can do for Biochemistry?

Dramatic changes are transforming the field of biochemistry, which is rapidly progressing from a science performed almost entirely at the laboratory bench to one that may be explored through computers.
Evolution path leading to modern living systems

How the remarkable properties of living organisms arise from the thousands of lifeless biomolecules?

The study of Biochemistry shows how the collections of inanimate molecules that constitute living organisms interact to maintain and perpetuate life animated solely by the physical and chemical laws that govern the nonliving universe!

- amino acids
- nucleotides
- lipids etc
All organisms are remarkably uniform at the molecular level. This uniformity reveals that all organisms on Earth have arisen from a common ancestor!
Structural hierarchy in the molecular organization of cells

Level 4: The cell and its organelles

Level 3: Supramolecular complexes
- Chromosome
- Plasma membrane

Level 2: Macromolecules
- DNA
- Protein
- Cellulose

Level 1: Monomeric units
- Nucleotides
- Amino acids
- Sugars
The size of organisms and its components

- Nanometers
  - Small molecules: Atoms, Glucose
  - Macromolecules: Hemoglobin, Ribosome

- Micrometers
  - Cells: Mitochondrion, Bacterium
  - Assemblies: Red blood cell

- Millimeters
  - Multicellular organisms: C. elegans

- Meters
  - Newborn human
The size of organisms and its components

Relative sizes and detection devices

<table>
<thead>
<tr>
<th>λ (m)</th>
<th>10^{-15}</th>
<th>10^{-12}</th>
<th>10^{-9}</th>
<th>10^{-6}</th>
<th>10^{-3}</th>
<th>1</th>
<th>10^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ν (s^{-1})</td>
<td>10^{+24}</td>
<td>10^{+21}</td>
<td>10^{+18}</td>
<td>10^{+15}</td>
<td>10^{+12}</td>
<td>10^{+9}</td>
<td>10^{+6}</td>
</tr>
</tbody>
</table>

- Cosmic rays
- Gamma rays
- X-ray rays
- Ultraviolet
- Visible
- Infrared
- Radio
Time scales in biology

Electronic rearrangements in vision: $10^{-15}$ sec
Bond vibrations: $10^{-12}$ sec
Macromolecular thermal motions: $10^{-9}$ sec
DNA unfolding: $10^{-6}$ sec
Enzyme catalysis: $10^{-3}$ sec
Protein synthesis: 1 sec
Molecular evolution: $10^{16}$ sec
Time scales in biology
Associated energies and temperatures of biophysical methods

<table>
<thead>
<tr>
<th>Energy (electron volts)</th>
<th>Time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^{-15}</td>
<td>10^{-12}</td>
</tr>
<tr>
<td>10^{-9}</td>
<td>10^{-6}</td>
</tr>
<tr>
<td>10^{-3}</td>
<td>1</td>
</tr>
</tbody>
</table>

- DLS
- NMR
- FB
- EB
- FD
- NS, FTIR
- LS, 2-D IR
Biological Molecules

Genes contain instructions for making proteins.

Proteins act alone or in complexes to perform many cellular functions.
The central dogma of biology

Gene 1 → Transcription of DNA sequence into RNA sequence → RNA 1

Gene 2 → Transcription of DNA sequence into RNA sequence → RNA 2

Gene 3 → Transcription of DNA sequence into RNA sequence → RNA 3

RNA 1 → Translation (on the ribosome) of RNA sequence into protein sequence and folding of protein into native conformation → Protein 1

RNA 2 → Translation (on the ribosome) of RNA sequence into protein sequence and folding of protein into native conformation → Protein 2

RNA 3 → Translation (on the ribosome) of RNA sequence into protein sequence and folding of protein into native conformation → Protein 3

Protein 1 → Formation of supramolecular complex

Protein 2 → Formation of supramolecular complex

Protein 3 → Formation of supramolecular complex
The genetic code

DNA Genetic Code Dictates Amino Acid Identity and Order

DNA Sequence Is the Genetic Code.

Growing Protein Chain

GCA  AGA  GAT  AAT  TGT...

Ala  Arg  Asp  Asn  Cys...
Protein structure

Proteins are built from a repertoire of 20 amino acids

[Chemical structure diagram]
Protein structure

Proteins are built from a repertoire of 20 amino acids
Protein structure

Primary structure
- Lys
- Lys
- Gly
- Gly
- Leu
- Val
- Ala
- His

Secondary structure
- $\alpha$ Helix

Tertiary structure
- Polypeptide chain

Quaternary structure
- Assembled subunits
Protein structure

hemoglobin
Protein structure

MOLECULAR STRUCTURE
- Primary (sequence)
- Secondary (local folding)
- Tertiary (long-range folding)
- Quaternary (multimeric organization)
- Supramolecular (large-scale assemblies)

FUNCTION
- Regulation
- Signaling
- Structure
- Movement
- Transport
- Catalysis
Macromolecules are the major constituents of cells

<table>
<thead>
<tr>
<th>E. coli Cell</th>
<th>Percentage of total weight of cell</th>
<th>Approximate number of different molecular species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>70</td>
<td>1</td>
</tr>
<tr>
<td>Proteins</td>
<td>15</td>
<td>3,000</td>
</tr>
<tr>
<td>Nucleic acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>RNA</td>
<td>6</td>
<td>&gt;3,000</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Lipids</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Monomeric subunits</td>
<td>2</td>
<td>500</td>
</tr>
<tr>
<td>and intermediates</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Inorganic ions</td>
<td>1</td>
<td>20</td>
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</table>
Central to its function is the arrangement of the molecule's constituent atoms in three-dimensional space—its **stereochemistry**

Three-dimensional structure is described by **configuration** and **conformation**

**Stereoisomers**: molecules with the same chemical bonds but different stereochemistry—that is different configuration

Interactions between biomolecules are invariably **stereospecific**, requiring specific stereochemistry in the interacting molecules
Structure foundations

**Configuration** is conferred by the presence of either:

**double bonds**, around which there is no freedom of rotation

![Chemical structures of 11-cis-Retinal and All-trans-Retinal](image)
Structure foundations

**Configuration** is conferred by the presence of either:

- **chiral centers**, around which substituent groups are arranged in a specific sequence

Similar or identical chemical properties but different physical and biological properties
Distinct from configuration is molecular conformation.

Conformers are free to assume different positions in space because of the freedom of rotation about single bonds.
Structure foundations

Interactions between biomolecules are stereospecific

Complementary fit between a macromolecule and a small molecule
Chemical bonds - Foundations

The noncovalent molecular interactions are responsible for the strength and specificity of recognition among biomolecules.

- Electrostatic interactions
- Hydrogen bonds
- van der Waals interactions
Electrostatic interactions

An electrostatic interaction depends on the **electric charges on atoms**

\[ E = k \frac{q_1 q_2}{D r^2} \]

E.g., the electrostatic interaction between two atoms bearing two single opposite charges separated by 3 Å in water (D=80) has an energy of **1.4 Kcal mol⁻¹**.
Hydrogen bonds

They are highly directional and have energies of $1 - 3 \text{ Kcal mol}^{-1}$. 
Chemical bonds - Foundations

van der Waals interactions

Energies are quite small (0.5–1 Kcal mol\(^{-1}\)).
Chemical bonds - Foundations

Energy scale

Noncovalent interactions
- Electrostatic
- van der Waals
- Thermal energy

Covalent bonds
- Hydrogen bonds
- Hydrolysis of ATP phospoanhydride bond
- C–C
- C=C

Energy scale:
- $0.24 \times 10^0$
- $0.24 \times 10^1$
- $0.24 \times 10^2$
- $0.24 \times 10^3$ kcal/mol
Molecular recognition

Noncovalent interactions govern molecular recognition
Molecular recognition

Noncovalent interactions govern molecular recognition

Noncovalent interactions govern molecular recognition.
The role of water

The properties of water affect biomolecular interactions

Water is the most abundant substance in living systems, making up 70% or more of the weight of most organisms.

Water is polar

Water is cohesive

Hydrogen bond: 0.177 nm
Covalent bond: 0.0965 nm
The role of water

The properties of water affect biomolecular interactions

Water is the most abundant substance in living systems, making up 70% or more of the weight of most organisms.
The role of water

The properties of water affect biomolecular interactions

Hydrophobic- nonpolar molecules do not dissolve in water
The role of water

The properties of water affect biomolecular interactions

Hydrophobic- nonpolar molecules do not dissolve in water

Dispersion of lipids in H₂O
Each lipid molecule forces surrounding H₂O molecules to become highly ordered.

Clusters of lipid molecules
Only lipid portions at the edge of the cluster force the ordering of water. Fewer H₂O molecules are ordered, and entropy is increased.
The role of water

**water release** - driving binding force

- Ordered water interacting with substrate and enzyme
- Disordered water displaced by enzyme-substrate interaction
- Enzyme-substrate interaction stabilized by hydrogen-bonding, ionic, and hydrophobic interactions
The role of water

water binding in hemoglobin
The role of water

water mediated interactions stabilize protein structures