Where does Physical Biochemistry/Biophysics fit into ‘molecular biology’?

Central Dogma of Molecular Biology:

![Central Dogma Diagram]

Gene: Unit of heritable information
Codes for protein(s) or RNA(s)

<table>
<thead>
<tr>
<th>Year</th>
<th>Genome</th>
<th>Size (base pairs, bp)</th>
<th>Number of genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1982</td>
<td>Bacteriophage λ</td>
<td>48,500</td>
<td>71</td>
</tr>
<tr>
<td>1986</td>
<td>HIV virus</td>
<td>~10,000</td>
<td>9</td>
</tr>
<tr>
<td>1997</td>
<td>Escherichia coli K12</td>
<td>4.6 Mbp</td>
<td>4,288</td>
</tr>
<tr>
<td>1997</td>
<td>Saccharomyces cerevisiae</td>
<td>12 Mbp</td>
<td>6,000</td>
</tr>
<tr>
<td>2002</td>
<td>Schizosaccharomyces pombe</td>
<td>13.8 Mbp</td>
<td>4,824</td>
</tr>
<tr>
<td>1998</td>
<td>Caenorhabditis elegans</td>
<td>97 Mbp</td>
<td>19,000</td>
</tr>
<tr>
<td>2000</td>
<td>Arabidopsis thaliana</td>
<td>125 Mbp</td>
<td>25,498</td>
</tr>
<tr>
<td>2000</td>
<td>Drosophila melanogaster</td>
<td>180 Mbp</td>
<td>13,600</td>
</tr>
<tr>
<td>2001</td>
<td>Homo sapiens</td>
<td>3,000 Mbp</td>
<td>30 – 40,000</td>
</tr>
</tbody>
</table>
What does a simple gene look like?

ATG

enhancer promoter

“open reading frame”

TAA

polyA signal term.

transcription

mRNA

AUG

AAAAAA

translation (genetic code)

UAA

Protein

MAKSDYPILL..........STVAGPQKQ*

Further levels of diversity of protein product from a single ‘gene’:

genomic

mRNA splicing

Protein isoforms

Post-translational modifications:

e.g. phosphorylation, acetylation, ubiquitination, glycosylation...

Proteolysis

Greatly expands the total number of proteins possible from a single genome
PROTEOME (PROTEOMICS)

Proteome: Complement of expressed proteins produced by an organism or tissue/cell/organelle...

Within an organism:

Different cell type, different proteome.

Some proteins common to more than one cell type

Others specific to one cell type

Proteome can change during development/differentiation and in response to stimuli

2D-GEL ELECTROPHORESIS OF HUMAN HEART PROTEINS

SDS-PAGE

PROTEINS

VENTRICLE

IEF

ATRIUM

Proteins found in atrium not ventricle

Need to identify and characterize molecules (mass spectrometry)
How do molecules work?

Anfinsen expt. (1955): Ribonuclease

Native fold
Active

Denature

Unfolded
(denatured)
Inactive

Renature

Native fold
Active

1) Molecular sequence sufficient to determine 3D-fold (structure)
2) Activity (interactions) depends on correctly folded structure

Every cellular process depends on molecular interactions:
- Enzyme-substrate interactions
- Receptor-ligand interactions
- Protein-nucleic acid interactions: transcription, translation...
- Protein-protein: e.g. actin-myosin...

Permanent interactions
(obligate)
- stable interaction (strong binding energy)

Temporary/transient interactions
(non-obligate)
- weaker, reversible association (weaker binding energy)
- molecules exist as complex or as free components

Molecular structure can change upon association/dissociation and play a central role in functional output of interaction
Second Dogma of Molecular Biology:

MOLECULAR SEQUENCE

MOLECULAR STRUCTURE

MOLECULAR INTERACTIONS

MOLECULAR FUNCTION (BIOLOGY)

MDELTAGEEANDKEQKDFREALLYMATTERS

\[ \Delta G = \Delta H - T \Delta S = -RT \ln K_{eq} \]

\[ K_{eq} = \frac{[\text{folded}]}{[\text{unfolded}]} \]

\[ K_d = \frac{[X][Y]}{[XY]} \]

\[ \Delta G = \Delta H - T \Delta S = RT \ln K_d \]
What are the underlying driving forces for molecular structure and interactions?

Weak, interatomic interactions:

i. Electrostatic interactions
ii. Van der Waal's interactions (dipole moments)
iii. Hydrogen bonds

Directional component to magnitude - so shape is important!

Stability of folded structure depends partly on interplay of 100s - 1000s of weak interatomic forces (contribute to favorable enthalpy of folding)

Stability of complex depends on interplay of 10-100s of weak interatomic forces (contribute to favorable enthalpy of interaction) and shape of interacting surfaces (stereochemical complementarity):
Can we predict protein structure and function?

Similar sequence \[\rightarrow\] Similar structure? \[\rightarrow\] Similar interactions?? \[\rightarrow\] Similar function???

Generally, for two protein sequences:
If greater than \(~30\%\) sequence identity will have similar structure

Families/superfamilies of proteins/structural folds that share similar sequence/structure - often have similar biochemical properties/function

Query sequence \[\rightarrow\] BLAST \[\rightarrow\] sequence database matches

Databases:
Pfam (pfam.wustl.edu) - protein sequence families
Protein Data Bank (www.rcsb.org) - molecular structures
SCOP (http://scop.berkeley.edu/) - structural folds classification
GH5 vs. CAP
13.4 % identity

GH5 vs. HNF3
12.5 % identity

TATA-Box Binding Protein (TBP)

Human

40% sequence identity
75% sequence similarity

Extreme thermophile
(Pyrococcus furiosus)
What does physical biochemistry/biophysics seek to do?

What do biophysics techniques allow us to do?

1. Identify and characterize macromolecules

2. Identify, characterize and quantify molecular interactions

$$K_d = \frac{[P]_f [A]^n_f}{[PA_n]}$$

$$\Delta G = \Delta H - T \Delta S = RT \ln K_d$$

3. Determine atomic resolution structures of macromolecules and macromolecular complexes
CRITICAL PARAMETERS DESCRIBING MOLECULAR INTERACTIONS

MOLECULAR INTERACTION PARAMETERS

1. STOICHIOMETRY composition of complex composition of interacting species

2. AFFINITY strength of interaction(s)

3. CONCENTRATION OF INTERACTING SPECIES

4. ENERGY $\Delta H, \Delta S, \Delta G, \Delta C$
   “thermodynamic signature”

5. INFLUENCE OF SOLUTION CONDITIONS temperature, pH, ionic strength ...

6. INFLUENCE OF COMPETING FACTORS specificity, regulation

7. KINETICS rate(s) of association/dissociation

8. CELLULAR LOCALIZATION

*In vitro* and *in vivo*
What factors can influence degree of complex vs. free components at any instant in time?

Why does P interact with A but not B, C, D, ....?
Fraction complex (PA) = \frac{[P] [A]}{[PA]}

Units: M

\[ K_d = \frac{[P] [A]}{[PA]} \]

Concentration of components

\[ \text{Fraction complex} = \frac{(K_d + [P]_T + [A]_T) - \sqrt{(K_d + [P]_T + [A]_T)^2 - 4[P]_T [A]_T}}{2[P]_T} \]

\[ [P]_T = [P]_F + [PA] \]

\[ [P]_T = [A]_F + [PA] \]

\[ [P]_T \leq 0.1 \text{ } K_d \]

Never interact \quad Interaction can be regulated \quad Permanent complex
a) Hormone circulating in blood

If signal $\propto$ % complex then if $K_d = 10^{-6}$ M, will need [hormone] $\geq 10^{-6}$ M
In reality $K_d$ lower than this (< nM)

b) Hemoglobin ($\alpha_2\beta_2$ heterotetramer) binds and releases $O_2$ efficiently

But $[\text{Hemoglobin}]_{\text{blood cell}} > 1 \times 10^{-3}$ M (mM) so mostly $\alpha_2\beta_2$
Biophysical techniques exploit physical and/or chemical attributes of macromolecules.

<table>
<thead>
<tr>
<th>PHYSICAL ATTRIBUTES</th>
<th>TECHNIQUE</th>
<th>CHEMICAL ATTRIBUTES</th>
<th>TECHNIQUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MASS</td>
<td>Mass spectrometry</td>
<td>COMPOSITION</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td></td>
<td>Electrophoresis</td>
<td></td>
<td>Spectroscopy</td>
</tr>
<tr>
<td></td>
<td>Chromatography</td>
<td></td>
<td>Electrophoresis</td>
</tr>
<tr>
<td>SHAPE or VOLUME or DENSITY</td>
<td>Centrifugation</td>
<td>MOLECULAR STRUCTURE</td>
<td>Spectroscopy</td>
</tr>
<tr>
<td></td>
<td>Chromatography</td>
<td></td>
<td>X-ray crystallography</td>
</tr>
<tr>
<td></td>
<td>Electrophoresis</td>
<td></td>
<td>NMR spectroscopy</td>
</tr>
<tr>
<td></td>
<td>X-ray crystallography</td>
<td></td>
<td>Electron microscopy</td>
</tr>
<tr>
<td></td>
<td>NMR spectroscopy</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Electron microscopy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHARGE</td>
<td>Electrophoresis</td>
<td>SOLUBILITY</td>
<td>Chromatography</td>
</tr>
<tr>
<td></td>
<td>Chromatography</td>
<td></td>
<td>Crystallography</td>
</tr>
<tr>
<td>ENERGY</td>
<td>Spectroscopy</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calorimetry</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Physical and chemical attributes of a macromolecule depend on:
- chemical composition (e.g. protein amino acid sequence)
- three dimensional structure (arrangement of chemical groups in space).

Influenced by environment (solution conditions)
- pH, ionic strength etc.

From known sequence MKDKTIL...GHPFVKDE

- predict attributes
  - mass net charge (pI)
- predict behavior
  - chromatography
  - electrophoresis

Differences in attribute(s)
- separate and/or identify molecules
  - e.g. chromatography, mass spectrometry

Changes in attribute(s)
- interactions
- change in structural conformation
Important contributions of selected biophysical methods for identifying and characterizing molecules and molecular interactions
Mass and Isotopic Mass

Molecular mass (m) is expressed in Daltons (Da) or Atomic Mass Units (amu)
One Da = 1/12th mass of $^{12}\text{C}$

Molecular weight or Relative Molecular Mass ($M_r$) is the ratio of the mass of the molecule to 1/12th mass of $^{12}\text{C}$ and is dimensionless

It is not correct to express $M_r$ in Da (http://www.jbc.org/misc/itoa.TI.shtml)

<table>
<thead>
<tr>
<th>Element</th>
<th>Stable Isotopes</th>
<th>Isotopic Mass (a.m.u.)</th>
<th>Abundance (%)</th>
<th>Average Isotopic Mass (a.m.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>$^1\text{H}$</td>
<td>1.00784</td>
<td>99.985</td>
<td>1.007991</td>
</tr>
<tr>
<td></td>
<td>$^2\text{H}$</td>
<td>2.01410</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>$^{12}\text{C}$</td>
<td>12.00000</td>
<td>98.9</td>
<td>12.01104</td>
</tr>
<tr>
<td></td>
<td>$^{13}\text{C}$</td>
<td>13.00336</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>$^{14}\text{N}$</td>
<td>14.00307</td>
<td>99.634</td>
<td>14.00672</td>
</tr>
<tr>
<td></td>
<td>$^{15}\text{N}$</td>
<td>15.00011</td>
<td>0.366</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>$^{16}\text{O}$</td>
<td>15.99491</td>
<td>99.762</td>
<td>15.9993</td>
</tr>
<tr>
<td></td>
<td>$^{17}\text{O}$</td>
<td>16.99913</td>
<td>0.038</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$^{18}\text{O}$</td>
<td>17.99916</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>$^{31}\text{P}$</td>
<td>30.97376</td>
<td>100.0</td>
<td>30.97376</td>
</tr>
<tr>
<td>S</td>
<td>$^{32}\text{S}$</td>
<td>31.97207</td>
<td>95.02</td>
<td>31.97207</td>
</tr>
<tr>
<td></td>
<td>$^{33}\text{S}$</td>
<td>32.97146</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$^{34}\text{S}$</td>
<td>33.96787</td>
<td>4.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$^{35}\text{S}$</td>
<td>35.96708</td>
<td>0.08</td>
<td></td>
</tr>
</tbody>
</table>

Average Isotopic Mass = $\sum (\text{Isotopic mass} \times \text{abundance}) / 100$

Example: Hypothetical 60 amino acid residue protein

Composition: $C_{150}\ H_{310}\ O_{200}\ N_{160}\ S_6$

Using simple mass values: $M_r = 7,742$

($H = 1, \ C = 12, \ N = 14, \ O = 16, \ S = 32$)

Using average isotopic mass values, $M_r = 7,747.584$

- a difference of 5.584!
Basic mass spectrometer components:

IONIZATION CHAMBER

create molecular ions

sample molecules

accelerate ions

+ 

ANALYZER

SEPARATE BASED ON

\[ \frac{\text{mass}}{\text{charge}} = \frac{m}{z} \]

DETECTOR

photomultiplier

IONIZATION METHODS (for small molecules)

1. ELECTRON IMPACT IONIZATION (EI)

\[ \text{e}^- \rightarrow \text{M} \rightarrow \text{M}^+ \text{ radical} \rightarrow \text{F}^+ \text{ fragment} \rightarrow \text{ANALYZER} \]

Molecular ion radical

uncharged radical fragment

cation fragment

2. ELECTRON CAPTURE IONIZATION

\[ \text{M} + \text{e}^- \rightarrow \text{M}^- \rightarrow \text{M}^- \rightarrow \text{ANALYZER} \]

molecular anion

3. CHEMICAL IONIZATION (~ EI in presence of methane or ammonia)

Methane:

\[ \text{CH}_4 \rightarrow \text{CH}_4^+, \text{CH}_3^+, \text{CH}_2^+ \]

\[ \text{CH}_4^+ + \text{CH}_4 \rightarrow \text{CH}_5^+, \text{CH}_3^- \]

\[ \text{M} + \text{CH}_5^+ \rightarrow \text{MH}^+ + \text{CH}_4 \]

Ammonia:

\[ \text{NH}_3 \rightarrow \text{NH}_3^+, \text{NH}_2^+, \text{NH}^+ \]

\[ \text{NH}_3^+ + \text{NH}_3 \rightarrow \text{NH}_4^+, \text{NH}_2^- \]

\[ \text{M} + \text{NH}_4^+ \rightarrow \text{MH}^+ + \text{NH}_3 \]
IONIZATION OF BIOLOGICAL MACROMOLECULES

Commonly embed molecule in a matrix of some kind

1) FAST ATOM BOMBARDMENT (FAB)

![Diagram of FAB process]

Produces \((M+H)^+\) and \((M-H)^-\) ions

2) MATRIX-ASSISTED LASER DESORPTION IONIZATION (MALDI)

![Diagram of MALDI process]

Produces mostly \((M+H)^+\), but also some \((M+2H)^2+\), \((M+3H)^3+\),... ions
3) ELECTROSPRAY IONIZATION (ESI)

Sample in \rightarrow 4 \text{kV} \rightarrow \text{Sample syringe} \rightarrow \text{Spray formation} \rightarrow \text{Matrix evaporation} \rightarrow \text{N}_2 \rightarrow \text{To analyser and detector}

Coulombic explosion

**Advantage:** requires very little energy so very little molecular fragmentation

Ions formed by protonation, deprotonation, or addition of another ion (e.g. Na\(^+\), K\(^+\)) - (reflects acid-base equilibria of analyte)

Generates multiply charged ions: average 1 charge / 1000 amu.

e.g. Protein \(M_r \) 20,000 \(\text{M}^+, \text{M}^{2+}, \text{M}^{3+}, \text{M}^{4+}, \ldots, \text{M}^{19+}, \text{M}^{20+} \rightarrow \text{M}^-, \text{M}^{2-}, \text{M}^{3-}, \text{M}^{4-}, \ldots, \text{M}^{19-}, \text{M}^{20-}\)
TIME OF FLIGHT (TOF) ANALYZER

Electrical field, $E$

No voltage, no force exerted: ions drift at constant velocity

Detector

Source region

Drift region

Kinetic energy = $zES$

Kinetic energy = $\frac{1}{2}mv^2$

$zES = \frac{1}{2}mv^2$

so, $v = \sqrt{\frac{2zES}{m}}$

Time to traverse drift region, distance $D$, to detector, $t = \frac{D}{v}$

$$\frac{m}{z} = 2ES\left(\frac{t}{D}\right)^2$$

$$\frac{m}{z} \propto \left(\frac{t}{D}\right)^2$$

A MALDI-TOF mass spectrum:

$M_r \approx 18,000$

$\frac{m}{1} \approx 18,000$

$\frac{m}{2} \approx 9,000$

Figure 5.33 A mass spectrum of myoglobin. Both singly and doubly charged ions are present. [Adapted from Senko and McLafferty (1994). Annu. Rev. Biophys. Biomol. Str. 23, 763–785, with permission of Annual Reviews, Inc.]
A mass spectrum produced by ESI (for a positive ion)

$[M + nH]^n^+$

$[M + (n+1)H]^{(n+1)+}$

An ESI spectrum contains a set of m/z peaks = molecule at a range of charge states: (M+H)$^+$, (M+2H)$^{2+}$, (M+3H)$^{3+}$ ... or [M+nH]$^n^+$, [M+(n+1)H]$^{(n+1)+}$, [M+(n+2)H]$^{(n+2)+}$...

m/z peaks usually range from 800 - 2,000 regardless of molecular weight of molecule.

Deconvolution is required to calculate M from the set of peaks:

\[
998.25 = \frac{[M + nH]}{n} \\
942.75 = \frac{[M + (n+1)H]}{(n+1)}
\]

2 equations
2 unknowns

rearrange for M

\[
M = n(998.25) - nH^+ \\
M = (n+1)(942.75) - (n+1)H^+
\]

so:

\[
n(998.25) = n(942.75) + 942.75 - H^+
\]

therefore:

\[
n(998.25 - 942.75) = 942.75 - H^+
\]

and:

\[
n = \frac{(942.75 - H^+)}{(998.25 - 942.75)}
\]

but M $H^+$ = 1.007991

\[
n = \frac{942.75 - 1.007991}{(998.25 - 942.75)} = \frac{941.74}{55.5} = 16.97 \rightarrow 17
\]

\[
M = 17(998.25) - 17(1.007991) = 16970.25 - 17.14 = 16953.11
\]

Repeat for each sequential pair of peaks each gives estimate of M

Calculate mean and standard deviation $16952.08 +/- 1.74$ amu

M (theoretical) = 16950.7 Difference = 1.38 (<0.01 %)
What if the solution contains >1 type of molecule
e.g. two different proteins, A and B

Deconvolution becomes more difficult as number of molecular species with different mass increases

<table>
<thead>
<tr>
<th>MALDI</th>
<th>vs. ESI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td></td>
</tr>
<tr>
<td>femtomole ($10^{-15}$ mole)</td>
<td></td>
</tr>
<tr>
<td>attomole ($10^{-18}$ mole)</td>
<td></td>
</tr>
<tr>
<td>zeptomole ($10^{-21}$ mole)</td>
<td></td>
</tr>
<tr>
<td>(~100-1000 molecules)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spectral simplicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Each molecule gives mostly single charged ion so complex mixtures can be analyzed</td>
</tr>
<tr>
<td>Each molecule gives series of charged ions so complex mixtures difficult to analyze</td>
</tr>
</tbody>
</table>
Excitation energy $\Delta E_1 >$ Fluorescence or Emission Energy $\Delta E_2$

therefore emission wavelength $\lambda_2 >$ excitation wavelength $\lambda_1$
Fluorescence parameters:

1) Fluorescence lifetime (excited state lifetime) - $\tau$
   
   length of time from absorption to emission of photon (1 - 10 ns)

2) Quantum yield, $Q = \frac{\text{number of photons emitted}}{\text{number of photons absorbed}} \leq 1.0$

Factors affecting fluorescence:

1) Molecular structure (electronic and non-electronic transitions)
2) Quenching:  
   - Internal (due to structure)
   - External (interactions with other molecules)

STATIC QUENCHING  COLLISIONAL/DYNAMIC QUENCHING

Both involve NON-RADIATIVE loss of energy from excited fluor
a) Reduces Quantum yield
b) Changes Absorption wavelength
c) Changes Emission wavelength
**FLUOROPHORES**

<table>
<thead>
<tr>
<th>Tyrosine</th>
<th>Tryptophan</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Tyrosine structure" /></td>
<td><img src="image2.png" alt="Tryptophan structure" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fluorophore</th>
<th>$\lambda_{\text{exc}}$ (nm)</th>
<th>$\lambda_{\text{em}}$ (nm)</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosine</td>
<td>274</td>
<td>303</td>
<td>0.14</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>280</td>
<td>348</td>
<td>0.2</td>
</tr>
<tr>
<td>Fluorescein</td>
<td>494</td>
<td>518</td>
<td>~0.9</td>
</tr>
</tbody>
</table>

**GREEN FLUORESCENT PROTEIN**

<table>
<thead>
<tr>
<th>Protein</th>
<th>$\lambda_{\text{exc}}$ (nm)</th>
<th>$\lambda_{\text{em}}$ (nm)</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFP</td>
<td>395</td>
<td>510</td>
<td>0.79</td>
</tr>
<tr>
<td>Engineered GFP mutants</td>
<td>Cyan (CFP)</td>
<td>434</td>
<td>476</td>
</tr>
<tr>
<td></td>
<td>Yellow (YFP)</td>
<td>513</td>
<td>528</td>
</tr>
<tr>
<td></td>
<td>Blue (BFP)</td>
<td>384</td>
<td>448</td>
</tr>
</tbody>
</table>
**FLUORESCENCE RESONANCE ENERGY TRANSFER (FRET)**

A “SPECTROSCOPIC RULER”

Two different fluorophores separated by distance $R$

- **Fluorophore A** ('Donor')
- **Fluorophore B** ('Acceptor')

Absorbance or Emission

If $R < \sim 10 \text{ nm}$ ($\sim 100 \text{ Å}$) ‘resonance energy transfer’ can occur between A and B

```
\text{Non-radiative transfer of energy}
```

Efficiency of transfer $\propto \frac{1}{R^6}$

- **Excite at** $\lambda_1$
- **R > 10 nm**
- **Emission at** $\lambda_2$
  - No FRET

- **Excite at** $\lambda_1$
- **R < 10 nm**
- **FRET**
- **Emission at** $\lambda_3$
Some applications of FRET

1. Measuring interactions

Donor fluorophore (D) covalently attached to protein X, acceptor fluorophore (A) to protein Y

$$\begin{align*}
\text{X} & \quad + \quad \text{Y} \\
\lambda^D_{\text{exc}} & \quad \lambda^D_{\text{em}} \quad \lambda^A_{\text{em}}
\end{align*}$$

Fusion proteins \textit{in vivo}

2. Structural information  e.g. conformational change

Example: Oct. 4th Discussion paper by Violin et al.
A fluorescence polarimeter (similar to a spectrofluorimeter except for polarizers)

Polarization: Measure of orientation(s) of plane of oscillation of light wave

Normal unpolarized light

Selects one plane of polarization of incident light

Polarizer A

Polarizer B

or

Measure intensity of emitted light:

parallel to plane of incident light

perpendicular to plane of incident light

Intensity of emitted light:

\[ I_\parallel \]

\[ I_\perp \]

Polarization, \( P \):

\[ P = \frac{I_\parallel - I_\perp}{I_\parallel + I_\perp} \]

\( I_\parallel \) = intensity of parallel component

\( I_\perp \) = intensity of perpendicular component

Unpolarized or depolarized

P = 0

Polarized

P = 1
Molecular rotation and rotational relaxation time ($\rho$)

Molecules tumble!

For a spherical molecule:

$$\rho_0 = \rho_x = \rho_y = \rho_z$$

Rotational relaxation time, $\rho$ (seconds) is a measure of rate of tumbling (= average time take to rotate 90°)

$$\rho_0 \propto \text{Molecular Volume, } V \propto M_r$$

$M_r A << M_r B$

Tumbles quickly

Tumbles slower than free B and much slower than free A

Tumbles slowly
**Fluorophore** emits photon after absorbing a photon. It emits light that is more polarized than the incident light. Larger complexes rotate more slowly, leading to more polarized emitted light. Covalently attaching a fluorophore to a molecule changes the orientation of the emitted light's plane of polarization. The fluorescence lifetime ($\tau$) of fluorophores is typically 1 - 10 ns. This method has been used in Jacobs et al. & Fischle et al. (Discussion papers for Oct 11).
Useful literature for lecture 1 (Sept 29, 2004)

General:


Fluorescence methods:


Mass Spectrometry:


