Lecture 5: 
Protein-Surface Interactions

Importance of Protein-Surface Interactions:

- Modulate cell adhesion
- Trigger the biological cascade resulting in foreign body response
- Central to diagnostic assay/sensor device design & performance
- Initiate other bioadhesion: e.g., marine fouling, bacterial adhesion

Fundamentals on Proteins:

- Largest organic component of cells (~18 wt% /H₂O =70%); extracellular matrix, and plasma (7wt% /H₂O=90%).
- Many thousands exist—each encoded from a gene in DNA.
- Involved in all work of cells: ex, adhesion, migration, secretion, differentiation, proliferation and apoptosis (death).
- May be soluble or insoluble in body fluids.

  *Insoluble proteins*—structural & motility functions; can also mediate cell function (ex., via adhesion peptides)

  *Soluble proteins*—strongly control cell function via binding, adsorption, etc.

- Occur in wide range of molecular weights.
“Peptides” (several amino acids): hormones, pharmacological reagents

e.g., oxytocin: stimulates uterine contractions (9 a.a.)
    aspartame: NutraSweet (2 a.a.)

“Polypeptides” (~10-100 amino acids): hormones, growth factors

e.g., insulin: 2 polypeptide chains (30 & 21 a.a.)
    epidermal growth factor (45 a.a.)

“Proteins” 100’s-1000’s of amino acids

e.g., serum albumin (550 a.a.)
    apolipoprotein B: cholesterol transport agent (4536 a.a.)

Protein Functions:

• *Structural/scaffold*: components of the *extracellular matrix* (ECM) that physically *supports cells*

  e.g., collagen—fibrillar, imparts strength;
  elastin—elasticity to ligaments;
  *adhesion proteins*: fibronectin, laminin, vitronectin—glycoproteins that mediate cell attachment (bonded to GAGs)

• *Enzymes*: catalyze *rxns* by lowering $E_a$ thru stabilized transition state, via release of binding energy

  e.g., urease—catalyzes hydrolysis of urea
• **Transport**: bind and deliver specific molecules to organs or across cell membrane

e.g., *hemoglobin* carries bound O\textsubscript{2} to tissues; *serum albumin* transports fatty acids

• **Motile**: provide mechanism for cell motion e.g., via (de)polymerization & contraction

e.g., *actin, myosin* in muscle

• **Defense**: proteins integral to the immune response and coagulation mechanism

e.g., *immunoglobulins (antibodies)*—Y-shaped proteins that bind to antigens (foreign proteins) inducing aggregate formation

  *fibrinogen* & *thrombin*—induce clots by platelet receptor binding

• **Regulatory**: cytokines—regulate cell activities

e.g., *hormones: insulin* (regulates sugar metabolism); *growth factors*
Protein Structure

Proteins have multiple structural levels…

1. **Primary Structure**

- comprised of amino acid residues: \(-\text{N-CHR-C}\) -
- peptide (amide) bond CONH is effectively rigid & planar (partial double-bond character)
- directional character to bonding: amino acids are L stereoisomers

Figure by MIT OCW.  

[Table showing L-alanine and D-alanine molecules]

Figure by MIT OCW.  
- side groups R have variable character

Figure by MIT OCW.  
2. Secondary Structure

Spatial configuration determined by the rotation angles $\varphi_i$ & $\psi_i$ about the single bonds of the $\alpha$-carbons


Figure by MIT OCW.

Ramachandran plots:
designate permitted ranges of $\varphi$ & $\psi$ for a.a. residues

3.051J/20.340J

β–sheets
- backbone has extended “zigzag” structure
- stabilized by intermolecular H-bonding between –NH and C=O of adjacent chains

α–helices
- stabilized by intramolecular H-bonding between C=O of residue $i$ and –NH of residue $i+3$ (requires all L or D stereoisomers)
natural abundance

- most common secondary structure in proteins
- in fibrous proteins: α-keratins (hair, skin,…)
- in globular proteins: avg. ~25% α-helix content

3. Tertiary & Quaternary Structure

- Tertiary: folded arrangements of secondary structure units
- Quaternary: arrangements of tertiary (polypeptide) units

Example: hemoglobin

## Synthetic Polymers vs. Proteins

<table>
<thead>
<tr>
<th>Property</th>
<th>Synthetic Polymers</th>
<th>Polypeptides</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Molecular Wt.</strong></td>
<td>1000-10^6 g/mol</td>
<td>1000-10^6 g/mol (typ. &lt;2000 a.a.)</td>
</tr>
<tr>
<td><strong>Molecular Wt. Distribution</strong></td>
<td>Always &gt; 1 (M_w/M_n)</td>
<td>Always ≡1</td>
</tr>
<tr>
<td><strong>Sequence</strong></td>
<td>i. 1-3 types of repeat units ii. many chemistries</td>
<td>i. many side groups ii. always amides</td>
</tr>
<tr>
<td><strong>Solution Structure</strong></td>
<td>Random coils or self-avoiding random coils</td>
<td>Globular – “condensed” chains (ρ~1.36 g/cm³) (hydrophobic R groups sheltered from H_2O)</td>
</tr>
<tr>
<td></td>
<td>R_g~N^{0.5} (θ solvent)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R_g~N^{0.6} (good solvent)</td>
<td>R_g~N_{aa}^{0.33}</td>
</tr>
<tr>
<td><strong>Available Conformations</strong></td>
<td>Ω_{ran} ~ z^{N} (z = # n.n.)</td>
<td>Ω~1 (can ↑ if bound or adsorbed!)</td>
</tr>
<tr>
<td></td>
<td>Ω_{SA} ~ z^{N} N^{1/6} &lt;&lt; Ω_{ran}</td>
<td></td>
</tr>
<tr>
<td><strong>Secondary Interactions</strong></td>
<td>van der Waals, H-bonds, electrostatic, “hydrophobic effect”</td>
<td>Same as synthetic, with “lock-and-key” topology</td>
</tr>
</tbody>
</table>

Polypeptides can *transform* to “random coil” conformations, through:

- changes in temperature
- changes in soln. pH or composition (e.g., added salts, urea)
- adsorption to surfaces

⇒ *changes physiological function!*
Protein Adsorption on Biomaterial Surfaces

**Background**

a) Protein activity varies in adsorbed vs. solvated state

*Why?*

1. **higher local concentration**—function may be conc. dependent

   e.g., cell adhesion increases with adhesion peptide concentration

2. **change in reactivity**—access to “active” a.a. sequence ↑ or ↓

   ⇒ enhanced or reduced binding capability

   e.g., fibrinogen: platelets adhere when adsorbed, not in soln.

3. **denaturation**—conformation varies from soln. conformation

   ⇒ different a.a. sequences exposed

   *enhance or deactivate normal function

   *elicit unintended function

   e.g., natural polymers used as biomaterials are more immunogenic than synthetic polymers
b) Driving forces for protein adsorption

1. secondary bond formation

- electrostatic > H-bonding > dispersive

2. entropic forces

- Configurations from “structured” H₂O adjacent to nonpolar surfaces (the “hydrophobic effect”)

- Less translational entropy loss ($\Delta S_{\text{mix}}$) for adsorbed proteins (macromolecules) vs. H₂O

\[
\frac{\Delta S_{\text{mix}}}{k} = n_p \ln \phi_p + n_{H_2O} \ln \phi_{H_2O}
\]

For a given $\phi_p$, $n_p$ decreases as protein MW increases $\Rightarrow \Delta S_{\text{mix}}$ decreases

- Configurations for denatured vs. solvated proteins
c) Adsorbed proteins initiate physiological responses to biomaterials

- coagulation mechanism
- alternative pathway of complement system (vs. antigen-antibody)
- *in vitro* protein adsorption experiments → 1\textsuperscript{st} test of “biocompatibility”

**Models for Protein Adsorption**

The simplest picture: *Langmuir model* for reversible adsorption

Makes analogy to chemical reaction kinetics:

\[
[P] = \text{protein concentration in solution (e.g.,#/vol)}
\]
\[
[S] = \text{density of unoccupied surface sites (e.g.,#/area)}
\]
\[
[PS] = \text{density of surface sites occupied by protein}
\]

\[
P + S \leftrightarrow PS
\]

Assumes: 1 protein binds 1 surface “site”—can involve multiple secondary bonds

Assuming the “reaction” follows 1\textsuperscript{st} order kinetics:

\[
\text{adsorption rate} = k_a[P][S]
\]
\[
\text{desorption rate} = k_d [PS]
\]

Assumes: dilute [P] (in plasma: 90% H\textsubscript{2}O)
At equilibrium: \( \text{adsorption rate} = \text{desorption rate} \)

\[ k_a[P][S] = k_d[PS] \]

Assumes: reversibility

Can define an “affinity” const, \( K \) (or \( K_a \)): \[
K = \frac{k_a}{k_d} = \frac{[PS]}{[P][S]}
\]

(a.k.a. “binding” or “association” const; typical units = L/mol)

\( K \) obtained experimentally by measure of fraction occupied sites:
\[
\nu = \frac{\# \text{ filled sites}}{\text{total } \# \text{ surface sites}}
\]

\[
\nu = \frac{[PS]}{[S]+[PS]} = \frac{K[P]}{1+K[P]}
\]

Binding plateaus at \( \nu = 1 \), monolayer coverage.

To obtain \( K \):

\[
\frac{1}{\nu} \text{ vs. } \frac{1}{[P]} \quad \text{slope} = \frac{1}{K}
\]
$K_a$ is an indicator of the favorableness of adsorption. Note that $K_a$ is the inverse of the dissociation constant, $K_d$, which has units of concentration, e.g., mol/L.

- For $[P] < K_d$, few occupied surface sites.
- For $[P] = K_d$, half of the surface sites will be occupied.

A second approach used to extract $K$ is known as a Scatchard plot.

Rearranging: \[ K[S] = \frac{[PS]}{[P]} \]

Defining the total number of surface sites: \[ [S]_0 = [S] + [PS], \]

And substituting for $[S]$: \[ K([S]_0 - [PS]) = \frac{[PS]}{[P]} \]

If the protein solution concentration is not significantly depleted upon adsorption, then $[P] \approx [P]_0$ (the initial protein concentration):

\[ \frac{[PS]}{[P]_0} = -K[PS] + K[S]_0 \]

Scatchard Plot

Provides a measure of $[S]_0$
In adsorption experiments, the value usually measured is a surface concentration, e.g., ng/cm$^2$ or µg/cm$^2$ – often denoted as $\Gamma$ or $\theta$

If we assume a monolayer coverage at $\Gamma_{\text{max}}$, we can calculate the effective area per protein molecule on the surface:

$$A_{\text{eff}} = \frac{M_{\text{protein}}}{N_A \Gamma_{\text{max}}}$$

Related to protein conformation on surface!

Note that $[S]_0$ (in #/area) is the inverse of the area per molecule:

$$A_{\text{eff}} = \frac{1}{[S]_0}$$