BE.342/442 Tuesday, November 15, 2005
Topics (guest lectures):
(1) Macromolecular Interactions and Protein and Adsorption
(2) Crystallography

(1) Larry Unsworth, Ph.D., Associate Research Office, National Institute for Nanotechnology

Objective: Presentation of general theory for macromolecular interactions. Specific examples of polymer and protein absorption.

Intermolecular and macromolecule-surface interactions are ubiquitous in nature and control most biological events. E.g., thrombosis, hemostasis, cell-biomaterial interactions, gene transcription, protein translation, etc.

Example of RNA translation: highly controlled selection to add amino acids to a peptide one at a time. Selective, *specific* interactions.

Nonspecific protein adsorption is not selective. Use of biomaterials in medical applications can cause protein adsorption, leading to inflammation, thrombogenesis, hydrolytic and oxidative processes, and bacterial adhesion and growth. In fact, venous prostheses cannot be made at all due to almost spontaneous protein adsorption, and other prostheses in the circulatory system can lead to deadly coagulation.

Interface: a region of a system in which there is an abrupt change of properties with distance. Interfacial phenomena direct macroscopic events, such as catalysis and bioactive species separation.

Thermodynamics of adsorption

Bulk solution

⇓⇑ native state

vicinal state
solute concentration and water structure differ from the bulk

⇓⇑

spreading
initial attachment : ⇔ multiple contact points
=====SURFACE==========

In a homogeneous multicomponent system, all extensive properties can be related by U =

$$\left(\frac{\partial U}{\partial S}\right)_{V, N_i, ..., N_r, Q} \equiv T$$

$$\left(\frac{\partial U}{\partial V}\right)_{S, N_i, ..., N_r, Q} \equiv -P$$

$$\left(\frac{\partial U}{\partial N_i}\right)_{V, S, Q} \equiv \mu$$

$$\left(\frac{\partial U}{\partial Q}\right)_{S, N_i, ..., N_r, V} \equiv F_j$$

$U(S,V,N_i,\ldots N_r,Q)$

Where U is the internal energy, V is the volume, N_i is the number of moles of species i, S is entropy, and Q is charge.

We can focus in on the internal energy of a surface by assuming zero volume and zero moles on the interface.

The intensive variables are defined as the partial derivatives of the internal energy with respect to their conjugate extensive variables, with all other variables held constant. This is how we define the interfacial surface tension γ^{LV}, interfacial chemical potential μ_i^{LV}, interfacial surface temperature T^{LV}, and interfacial electrical potential ϕ^{LV}.

The differential form of the interface internal energy can then be written for a liquid-vapor interface as the Gibbs dividing surface equation:

$$dU^{LV} = T^{LV}dS^{LV} + \gamma^{LV}dA^{LV} + \Sigma\mu_i^{LV}dN_i^{LV} + \phi^{LV}dQ^{LV}$$

Rearranging, we obtain the Gibbs Absorption Equation:

$$\gamma^{LV} = -SdT^{LV} - \Sigma\Gamma_i d\mu_i^{LV} - q^{LV}d\phi^{LV}$$

Here, Γ is the surface fraction due to non-adsorbed chains.

The total entropy of the system is the sum of the entropies of the liquid phase, the vapor phase, and the interface. Assuming equal pressure and temperature in all phases, this leads to the equilibrium condition that

$$\mu_i(P,T)^V = \mu_i(P,T)^L = \mu_i(P,T)^{LV}.$$

Because this argument relies on the approximations of zero volume and mole number, this only holds for monolayer coverage by small molecules, and for small, mobile homogeneous bulk

phases. This does *not* hold for solid-liquid interfaces, since they are chemically or physically homogeneous.

Macromolecular adsorption reaches “equilibrium” at solid-liquid interfaces, but it is not clear
- to what extent this equilibrium is controlled by the solid surface
- whether it is truly reversible
- the dependence on diffusion
- the dependence on pre-adsorbed molecules

Intramolecular surface forces in aqueous solution include:

1. van der Waals / London Dispersion, Keesom, and Debye forces

Van der Waals interactions are always present, arising from dipole-induced dipole interactions that result in adhesion, surface tension, and the condensed structure of macromolecules such as proteins and polymers in solution. These may be considered long-range, effective over distances from 0.2 nm to 10 nm depending on the system, and may be either repulsion or attraction. They may also lead to “weak” orientation effects.

The *interaction energy function* describes interaction energy as a function of the distance *r*:

$$w(r) = -\frac{3}{4}\frac{h\upsilon\alpha^2}{4(4\pi\varepsilon_0)^2 r^6}$$

2. electrostatic forces

Electrostatic interactions can be repulsive or attractive, arising from interactions between fixed surface charges and charges. The segregation of charged ions over the vicinal region is referred to as the ‘electrical double layer’: the Stern and the Gouy-Chapman layer.

-Stern layer is the closest layer, consists of ‘fixed’ counter-ions.

-Gouy-Chapman layer consists of a more diffuse ion conc. and extends to the point where the bulk ion distribution resumes.

Ion segregation induces a potential energy gradient, related to the inverse of the Debye length (k), the characteristic dimension of the electrical double layer, defined as:

$$\kappa = \left(2n_o z^2 e^2 / \varepsilon\, kT\right)^{0.5}$$

With bulk ion concentration (n_O), ion valence (z), electric charge (e), temperature (T), Boltzmann constant (k) and medium permittivity (ε).

Systems of low ionic strength, have greater Debye length and a more diffuse double layer, than systems of high ionic strength [van Oss, 1994].

3. hydrogen bonding

There is still no complete model for water interactions, but water is key to hydrogen bonding. The interaction energies of these bonds are ~10 - 40 kJ mol^{-1}, greater than either VDW or electrostatic interactions. The bonds are highly orientation dependent and responsible for subtle details of macromolecular structure, such as DNA conformation.

4. hydrophobic interactions:
In order to facilitate H-bonding when in the presence of non-polar molecules, water is forced into a highly oriented shell, sometimes referred to as a clathrate structure. Beyond a tight inner shell is a longer-range hydration shell of oriented water molecules.

The biological importance of adsorbed conformation is illustrated by the absorbed conformation of fibrinogen. Selectively allowing the alpha-C domain to interact with surfaces raises the elutability of fibrinogen 170-fold. We did not get to this in class -- see the powerpoint slides.

Adsorbed layer characterization

Ellipsometry: this cheap, non-destructive, method has a resolution of about 5 nm. The light from the monochromatic source passes through the Polarizer Prism (generating elliptical polarization) to the surface. Reflection from the surface changes the polarization. The reflected beam passes through the Analyzer Prism (which is used to determine the change in polarization), and finally through to the detector which senses the presence of the electromagnetic wave and its intensity.

Neutron reflectometry: neutrons are harvested from a reactor, selected for a certain wavelength by a monochrometer, and reflected off a surface. Spatial resolution of 0.1 to 1 nm. Plots the volume fraction of an absorbed substance as a function of distance from the surface.

The future of surfaces is likely to involve a biomimetic system design. For example, light-adsorbing antennae containing chomophores found in cyanobacteria and algae are currently being investigated for energy harvesting applications.

References:

- Callen, Thermodynamics and an Introduction to Thermostatistics, 2nd Ed., 1985.
- **Israelachvili, Intermolecular and Surface Forces, 1985**.
- Hunter, Foundations of Colloid Science, Vol. 1. 1987.
- Van Oss, Interfacial Forces in Aqueous Media, 1994.
- **Fleer, Polymers at Interfaces, 1993.**
- Jones, Polymers at Surfaces and Interfaces, 1999.
- de Gennes, Macromolecules, 9, 587, 1976.
- Carter, Adv. Protein Chem., 45, 153, 1994.

• Cosgrove, Coll. and Surf. 62, 199, 1992.
Phipps, Langmuir, 9, 3530, 1993

(2) Liselotte Kaiser, Center for Biomedical Engineering, MIT

Crystallization begins with cloning, expression, and purification of a protein. Next, crystallization trials attempt to crystallize the protein into a usable sample. This first part can take months or years, if successful at all. Afterward, the data collection and analysis can take only days or months. Let's focus on the first part.

What is protein crystallization?

It's a special case of phase separation in a thermodynamically non-ideal mixture controlled by kinetic parameters. To crystallize proteins, one must create the conditions that allow proteins to arrange themselves into a lattice to form a regular, repeating structure. The protein must be separate from aqueous solution, but will usually contain 30% to 60% water, so it will feel like Jell-O even in a crystalline form.

Crystallization solutions usually contain buffers to control the net charge of the protein, since certain charges are conducive to protein-protein interactions. Buffers include tris, phosphate, etc. – they depend on the pH you're trying to set.

In addition, dehydration agents, such a PEGs and salts (ammonium sulfate, ammonium chloride) help remove some of the water from the sample. These are rarely enough, so additives such as ethanol, ligands, and substrates to further remove water.

These days, you can purchase commercially available kits of 50 common crystallization solutions. A good start is to try these a see if you get a hit!

Some factors affecting crystal growth include:

- pH
- ionic strength
- temperature
- concentration of protein
- concentration of precipitant
- purity of protein
- additives and/or ligands
- organism that is the source of the protein
- substrates, coenzymes, inhibitors
- reducing or oxidizing environment
- metal ions
- surfactants or detergents
- vibrations
- contamination
- handling by the investigator!

- variations between batches or proteins

Crystallization Techniques

Vapor diffusion: a few microliters of protein are mixed with an equal amount of precipitant solution. A droplet of this mixture hands over a reservoir of water. Water from the drop slowly evaporates, causing the concentration in the drop to rise. Rather than rapidly precipitating the protein, the concentration must change so as to form crystals of the protein from the metastable zone of the system's phase diagram.

Classic setups for these methods are: hanging drop, sitting drop, and sandwich drop.

Tiny differences can be essential to finding the "sweet spot" between no reaction and protein precipitation. To find these exact conditions, 96 and 386 well formats are used to increase the number of samples and reduce the waste in materials. For smaller well sizes, robots are used to do the pipeting.

Less than a year ago, the Granada Box was discovered as a way to use diffusion methods to induce crystallization. Crystals are grown inside of capillaries gelled with a precipitating agent, so that a continuous concentration gradient can be sampled for favorable cystallization conditions. Microfluidics devices now on the market cans screen up to 8 samples in 96 conditions across concentration gradients. This Fluidigm system costs $300 per chip.

Back to low-tech methods: in a batch technique, the protein is simply mixed with the precipitant, immediately achieving supersaturation. This is useful for known crystallization conditions.

In microbatch techniques, droplets of the solution as smaller than 2 microliters are suspended in oil.

To speed up crystallization, seeding can be performed. Solids such as cat whiskers are touched onto an unusable crystal and streaked across an empty droplet, causing small portions of the crystal to streak through the droplet and nucleate what could become single crystals. This streak method is used to obtain bigger, better-diffracting crystals.

Solving the structure

When solving the structure of a protein, data is compared to a PDB (protein data bank) of known protein structures. The Swiss PDBViewer, PyMol, and other viewer programs read the PDB file, which includes data collection statistic, authors, and publications on each structure. It also includes remark lines, which contain detailed information about data collection and interpretation that may not be included in publications. The rest of the information identifies each atom in the crystal structure by its coordinates, occupancy, position in a chain, and other information.

Refinement statistics such as the R-factor and the RMS deviations for bond lengths depend not just on your data, but also on your model. B-factors (temperature factors) include both disorder and thermal motion.

The Ramachandran plot is usually included in the PDB or a publication, indicating the conformational freedom of the protein.