Presenter: Borries Demeler

Topic:
Interactions, Mass Action, Transport

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Interactions, Mass Action, Transport

An important branch of biophysical research concerns itself with the study of molecular interactions.

In this lecture, we will review types of molecular interactions occurring in cellular systems, and how they can be studied by biophysical solution methods by observing their transport:

- Reversible interactions driven by mass action
- Irreversible interactions leading to aggregation
- Transport by sedimentation
- Transport by diffusion
- Solvent interactions

Macromolecular Interactions

Molecules in solution can display dynamic behavior in response to external perturbations and changes in their own concentration or their environments.

Of particular interest are **interactions** between molecules. Interactions can lead to **covalent bond formation** or they can be **non-covalent**.

Molecules can interact with other molecules to form higher order structures, or **assemblies**. When they interact with ***identical*** molecules (e.g., $A + A \rightarrow A_2$), we refer to it as **self-association**, interactions between ***different*** molecules (even if they are of the same type) are called **hetero-association** (e.g., $A + B \rightarrow AB$).

Macromolecular Interactions

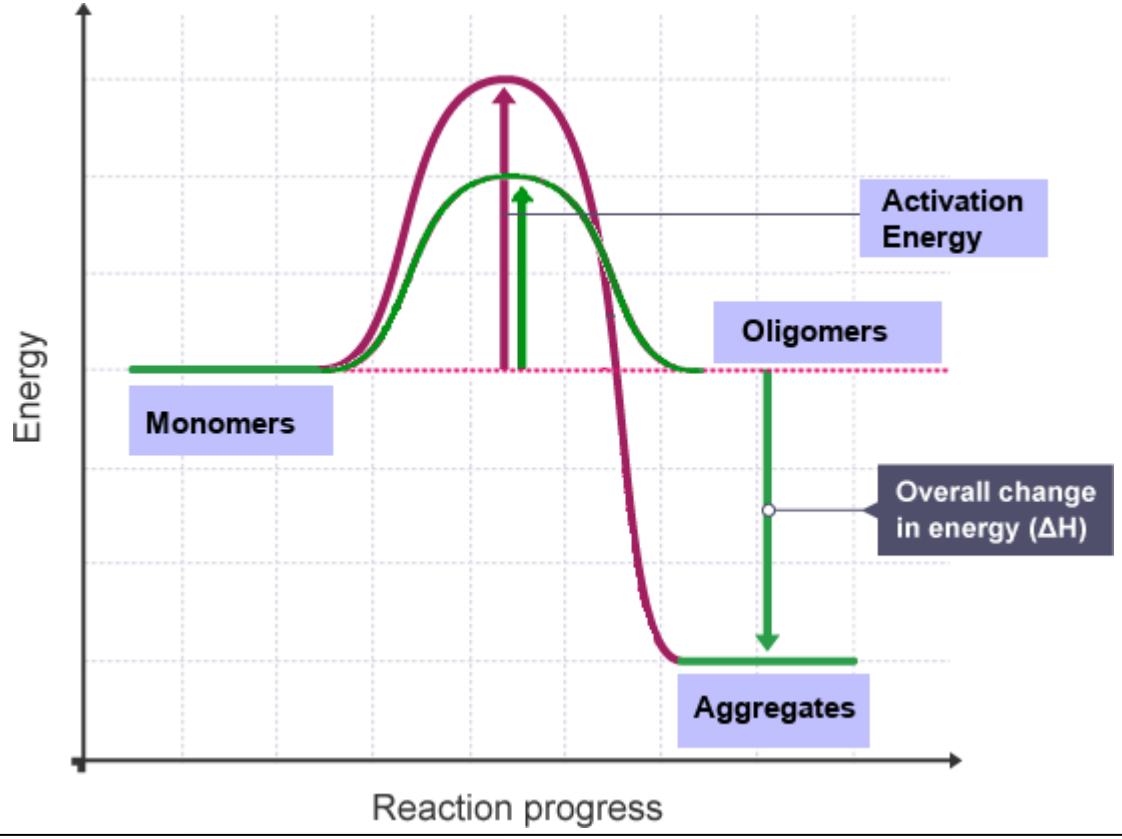
Biopolymers (most often proteins) can interact with biopolymers of the **same** type (protein-protein, RNA-RNA, DNA-DNA interactions) or **different** types (protein-DNA, protein-RNA, DNA-RNA, protein-lipid, protein-carbohydrates, protein-small molecules or drugs, nanoparticles, etc.). Biopolymers also interact with the **solvent.**

Interactions can be transient and **reversible**, or **irreversible**.

- Reversible interactions are caused by weak interactions (electrostatic, hydrophobic) and lead to **oligomerization**
- Irreversible interactions lead to **aggregation** which is typically pathologic or unphysiological.

Macromolecular Interactions

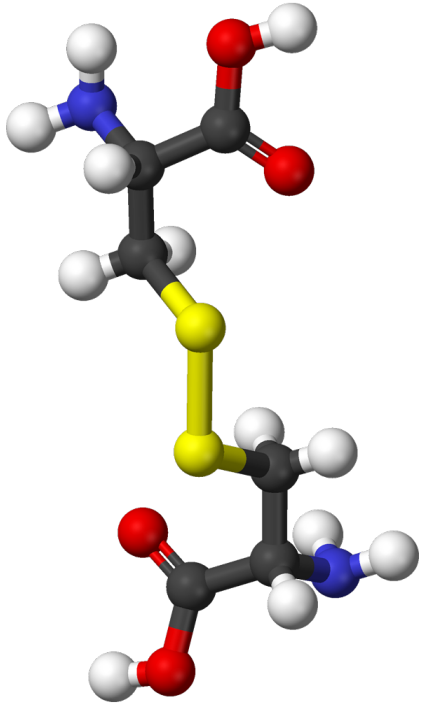
Which interaction occurs depends on the activation energy that needs to be overcome to interconvert between the forward and backward reaction. When the energy barrier becomes too high (under ambient conditions) to go backward, an interaction reaction is considered to be irreversible and this leads to aggregation.



Irreversible Interactions

Examples of events that cause irreversible solution interactions of macromolecules (aggregation events) are:

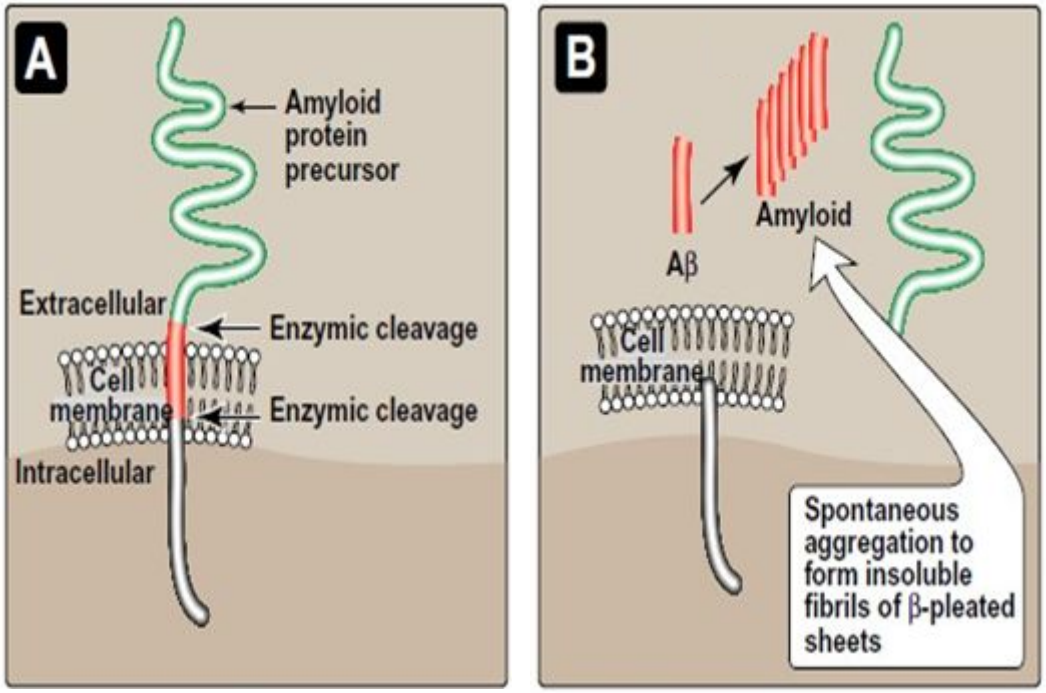
1. Oxidation: Covalent disulfide bond formations between cysteine side chains of proteins
2. Covalent cross-linking, either chemically or photo-activated
3. Hydrophobic, van der Waals interactions, coupled with entropic effects
4. Structural changes in the molecule (amyloid beta – beta-sheet, insulin fibrils)

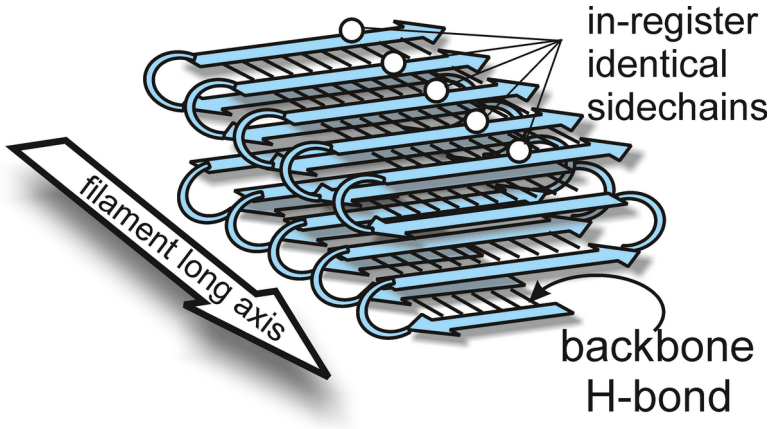


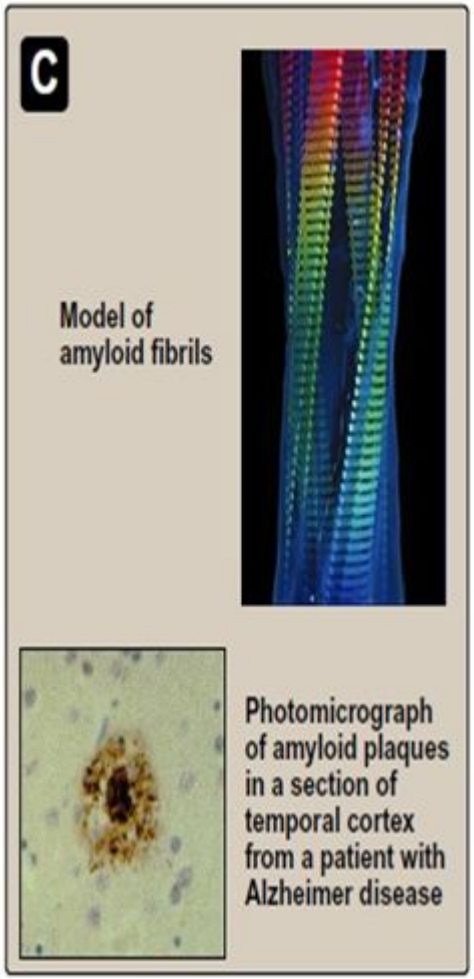
Cysteine disulfide link

Irreversible Interactions

Amyloid Beta aggregation



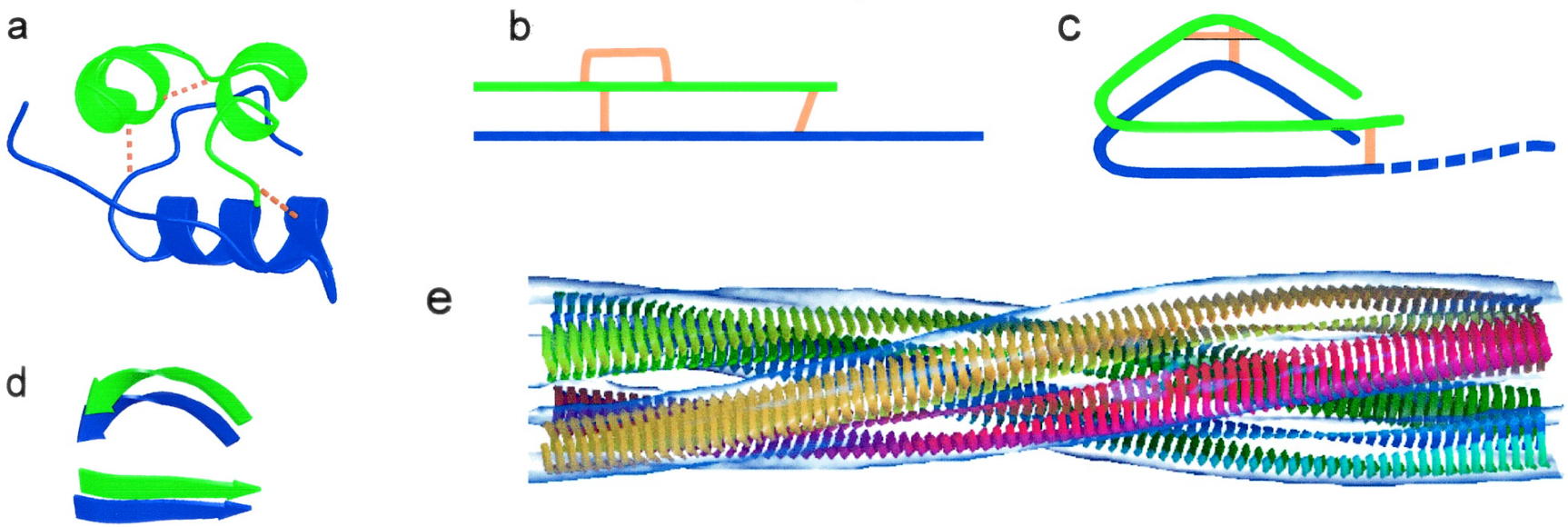




Credits: V. Joshi

Speransky, V. et al. (2001). J. Cell Biol., 153, 1327-1335

Irreversible Interactions

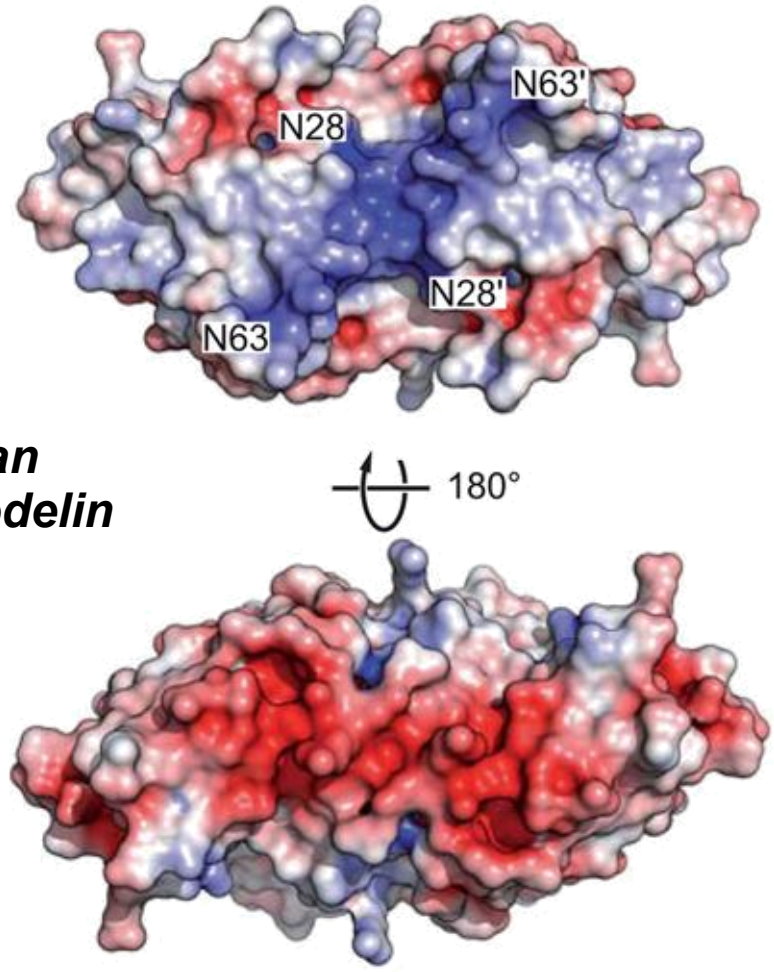


(a) Insulin structure showing the three native disulfide bonds. A chain, green; B chain, blue; disulfide bonds, gold. (b) Topology diagram of insulin color coded as in a. (c) Possible topology for the amyloid protofilament. Orientations of the termini and disulfide bonds within the curved structure are arbitrary. The C terminus of chain B (dashed) is not required for amyloid fibril formation (see ref. 43). (d) β-strand model of a protofilament. Each chain is shown in two segments, a straight and a curved β-strand (PDB accession no. 1umu, residues 93–100). Each insulin molecule would occupy two layers, connected by the interchain disulfide bonds. (e) A possible β-strand model docked into the EM density of the compact fibril (transparent gray surface). The four protofilaments are colored separately.

Credits: José L. Jiménez et al. PNAS July 9, 2002 99 (14) 9196-9201;

Reversible Interactions

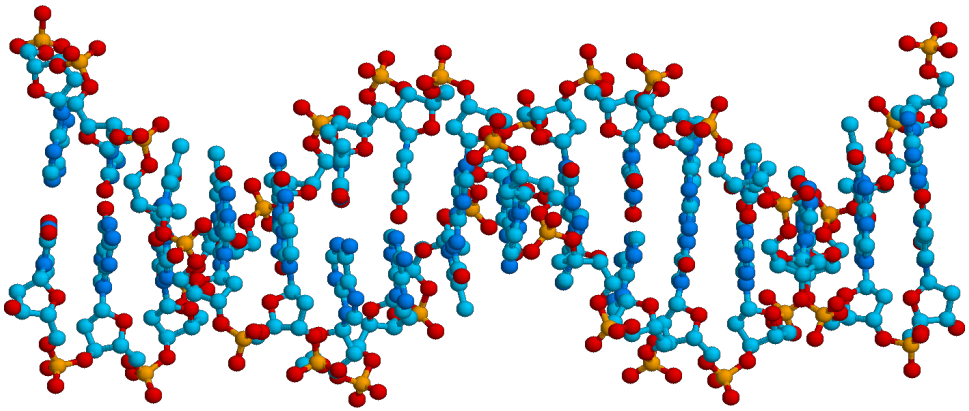
Reversible interactions typically require structural complementarity and are caused by weak forces and molecular attractions (electrostatic, hydrophobic, salt bridges, van der Waals forces, and hydrogen bonds).

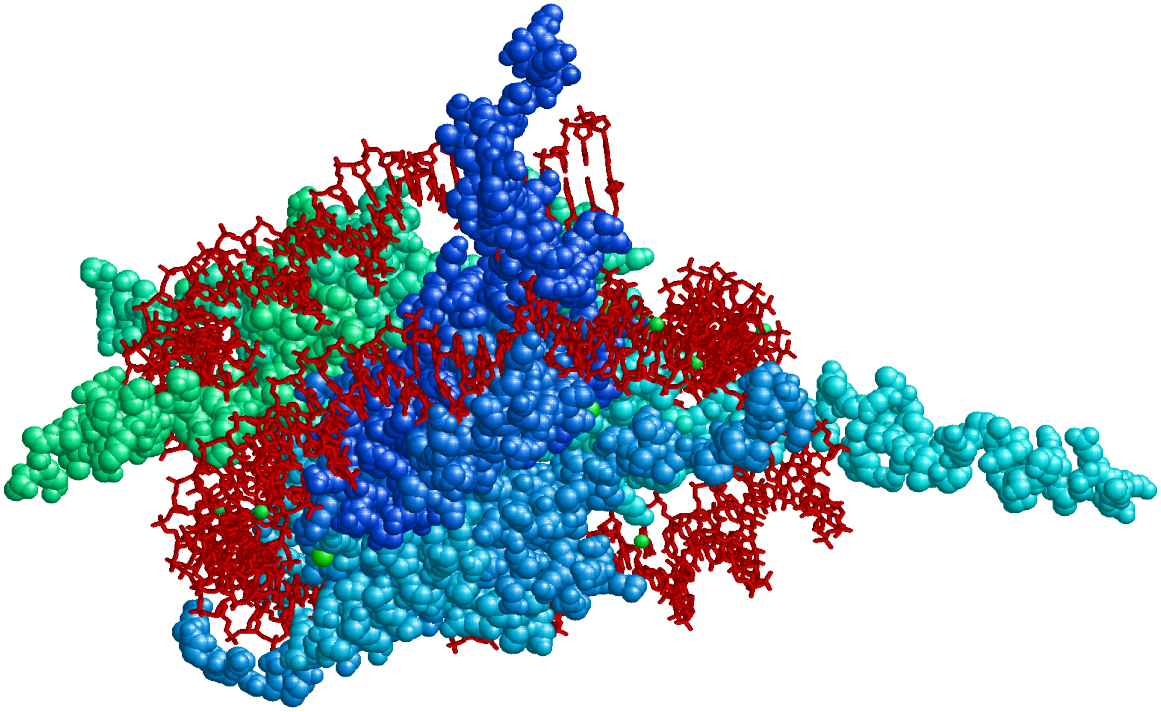


Human glycodelin

A. Schiefner et al., Biochem. J. 2015, 466 (1) 95-104

Reversible Interactions





Electrostatic interactions are very important for nucleic acid binding. DNA has a strongly negatively charged backbone (PO_4^-) which forms salt bridges with positively charged amines from lysine sidechains, allowing stiff, double-stranded DNA to curl around the nucleosome and become flexible.

Reversible Interactions

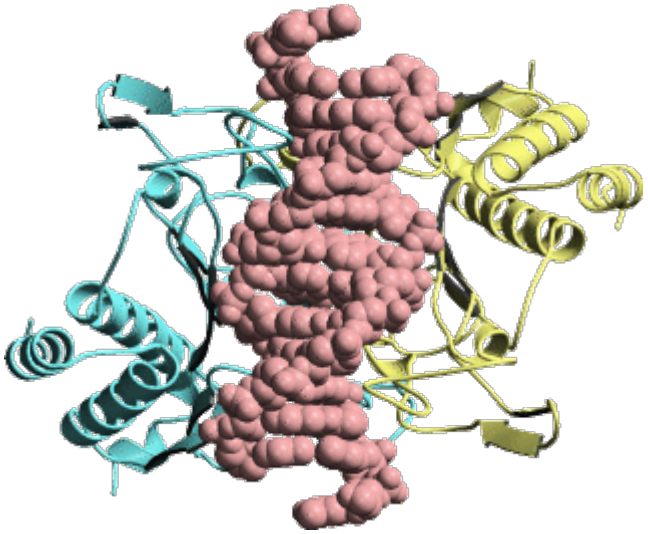
There can be multiple reactions:

$$A + A \leftrightarrows A_2$$
$$A_2 + B \leftrightarrows A_2 B$$

The concentration in one component affects the concentration of another

Reactions can be fast (diffusion controlled) or slow (kinetically limited)

...and they observe mass action laws



DNA restriction enzyme Eco RI

Reversible Interactions - Oligomerization

Reversible reaction (Le Chatelier's principle):

$$\sum_{i=1}^{n} M \rightleftarrows M_n \qquad \sum_{i=1}^{n} M \leftrightarrows M_n$$

Equilibrium Constant:

$$K_a = \frac{[M_n]}{[M]^n} \qquad K_d = \frac{[M]^n}{[M_n]}$$

Kinetics:

$$K_a = \frac{k_{on}}{k_{off}}$$

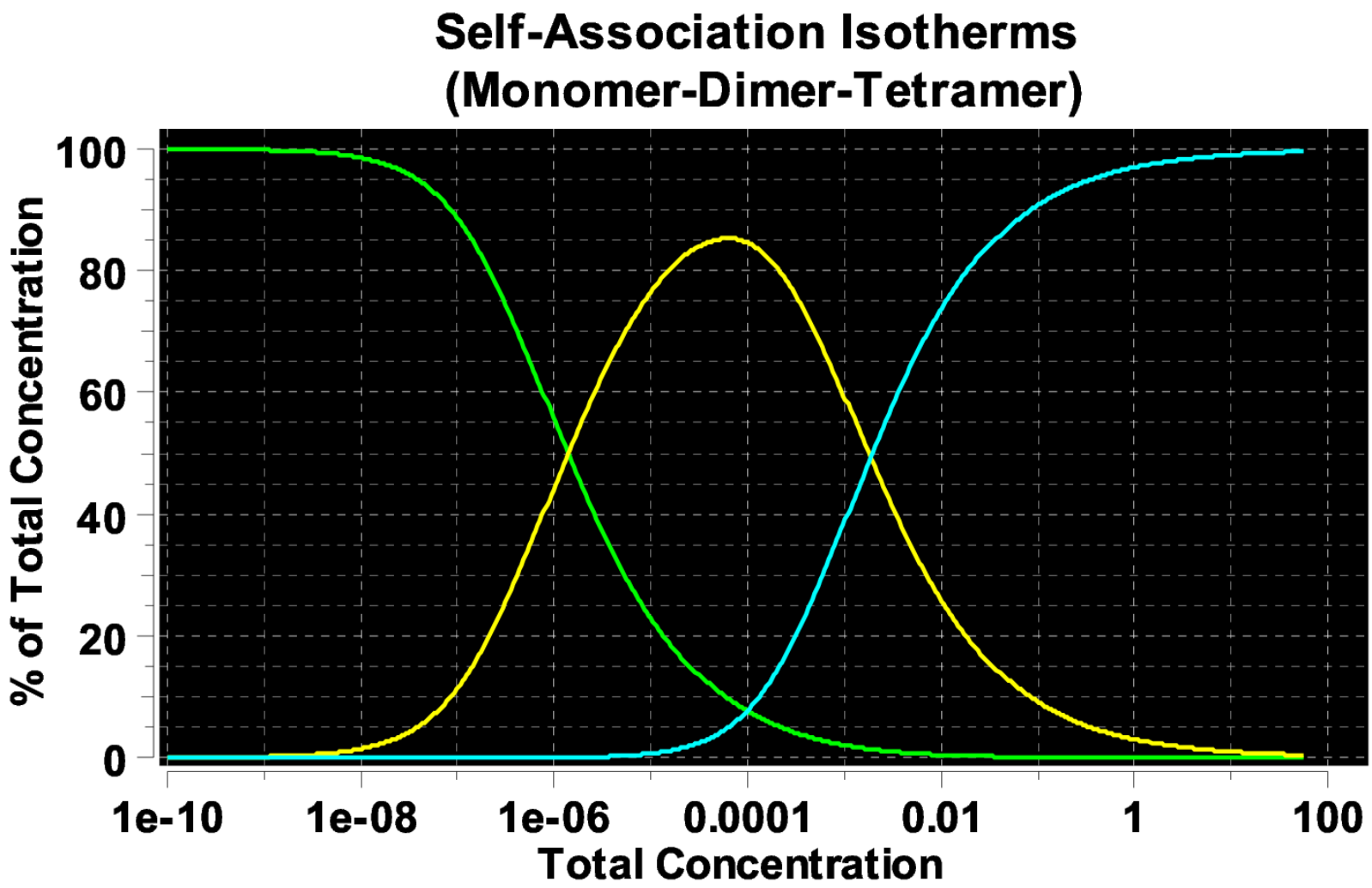
Solve Polynomial

$$[M] + n[M_n] = [C_{total}]$$
$$[M] + nK_A[M]^n - [C_{total}] = 0$$

Example, a monomer-dimer equilibrium:

$$A + A \leftrightarrows A_2$$
$$[A] + 2\,K_a\,[A]^2 - [C_{total}] = 0$$

Reversible Interactions - Oligomerization



SD

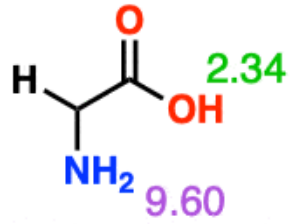
Monomer Dimer Tetramer

Macromolecular Interactions – Role of Solvent

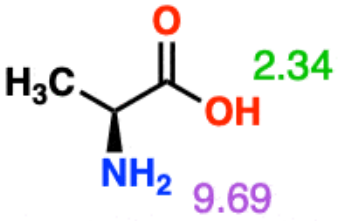
Interactions between molecules depend on the **surface properties** of the regions of the molecule that are interacting. Different **solvents** can amplify or eliminate these interaction effects, and change the Ka of interaction dramatically:

- Charge-charge interactions can be disrupted by increasing the **ionic strength**.
- **pH changes** may modify the charge on surface groups and alter the electrostatic interactions.
- Hydrophobic interactions can be disrupted by **amphiphilic detergents**
- **Steric hindrance** can prevent proximity of interacting surfaces – domains may change shape upon binding of small molecules.

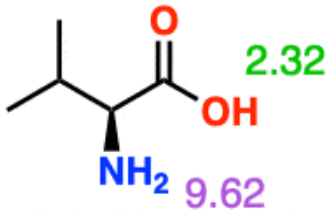
Structures (and pK_a values) of selected amino acids



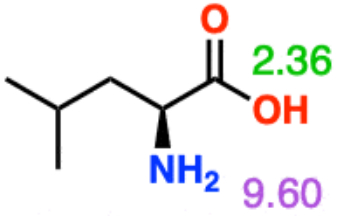
Glycine



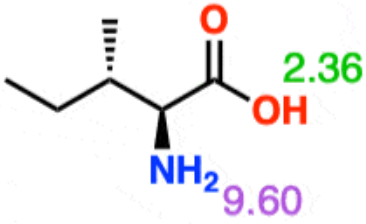
Alanine



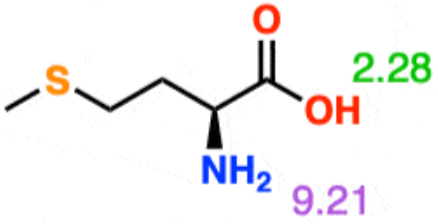
Valine



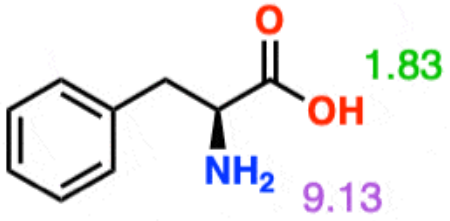
Leucine



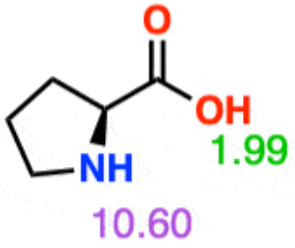
Isoleucine



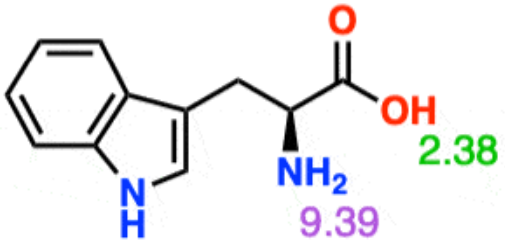
Methionine



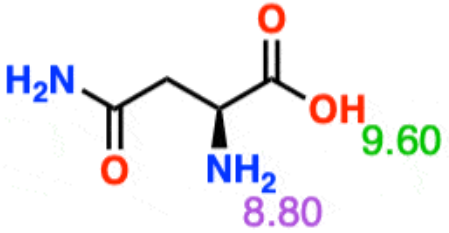
Phenylalanine



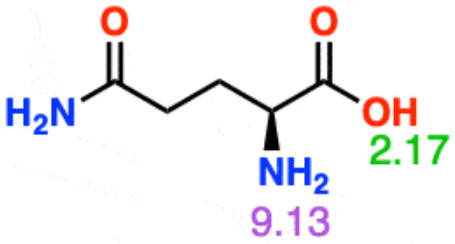
Proline



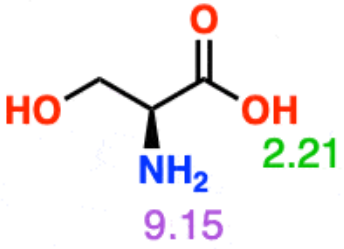
Tryptophan



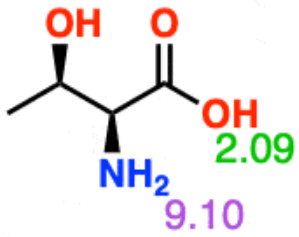
Asparagine



Glutamine



Serine



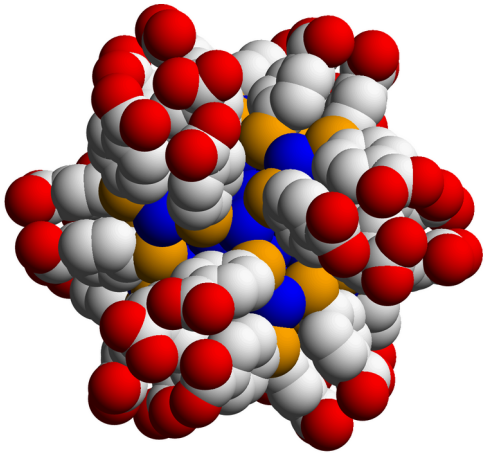
Threonine

	Amino Acid	pKa1 (α-COOH)	pKa2 (α-NH2)	pKa3 (Side Chain)	Isoelectric Point (pI)
Non-Polar Aliphatic Side Chains	Alanine (Ala)	2.35	9.69	-	6.02
	Glycine (Gly)	2.34	9.60	-	5.97
	Isoleucine (Ile)	2.36	9.68	-	6.02
	Leucine (Leu)	2.36	9.68	-	6.02
	Methionine (Met)	2.28	9.21	-	5.75
	Proline (Pro)	1.99	10.60	-	6.30
	Valine (Val)	2.32	9.62	-	5.97
Non-Polar Aromatic Side Chains	Phenylalanine (Phe)	1.83	9.13	-	5.48
	Tryptophan (Trp)	2.38	9.39	-	5.89
	Tyrosine (Tyr)	2.20	9.11	10.07	9.59
Polar Uncharged Side Chains	Asparagine (Asn)	2.02	8.84	-	5.43
	Cysteine (Cys)	1.71	10.78	8.33	9.56
	Glutamine (Gln)	2.17	9.13	-	5.65
	Serine (Ser)	2.21	9.15	-	5.68
	Threonine (Thr)	2.63	9.10	-	5.87
Polar Acidic Side Chains	Aspartic Acid (Asp)	2.09	9.82	3.86	2.98
	Glutamic Acid (Glu)	2.19	9.67	4.25	3.22
Polar Basic Side Chains	Arginine (Arg)	2.18	9.04	12.48	10.76
	Histidine (His)	1.82	9.17	6.04	7.61
	Lysine (Lys)	2.18	8.95	10.79	9.87

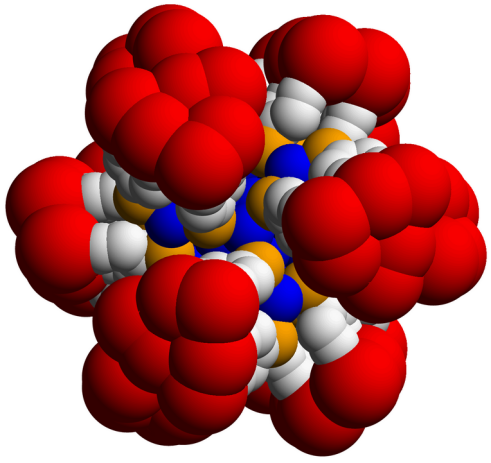
Partial Specific Volume ($\bar{\nu}$)

The partial specific volume of a molecule can be thought of as the inverse of the density (volume required for 1 gram of solute). In solution, the $\bar{\nu}$ value includes the bound solvent that migrates with the molecule in a sedimentation or diffusion experiment:

No hydration

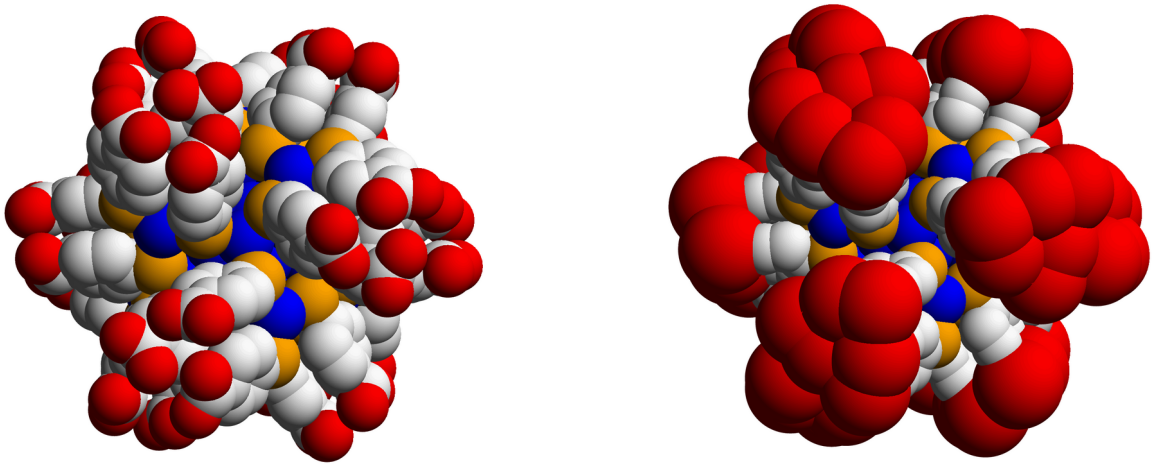


with hydration



Partial Specific Volume ($\bar{\nu}$)

No hydration **with hydration**



The sedimenting particle always carries along a solvation shell, which adds to the size of the particle. This solvation shell changes the volume and ALSO changes the density of the sedimenting particle. Because the size changes, also the friction changes. The volume and density changes are represented by the partial specific volume.

The partial specific volume is highly solvent dependent!

PSV calculation from MD simulations

- PSV is hard to compute experimentally; For example, for nucleic acids, the estimation of PSV is complicated by the fact that $\bar{\nu}$ depends on base composition, secondary structure, solvation and the concentrations and identities of ions in the surrounding buffer.
- PSV is intimately related to statistical-mechanical formulation of excess volume caused by insertion of the solute into the solvent:

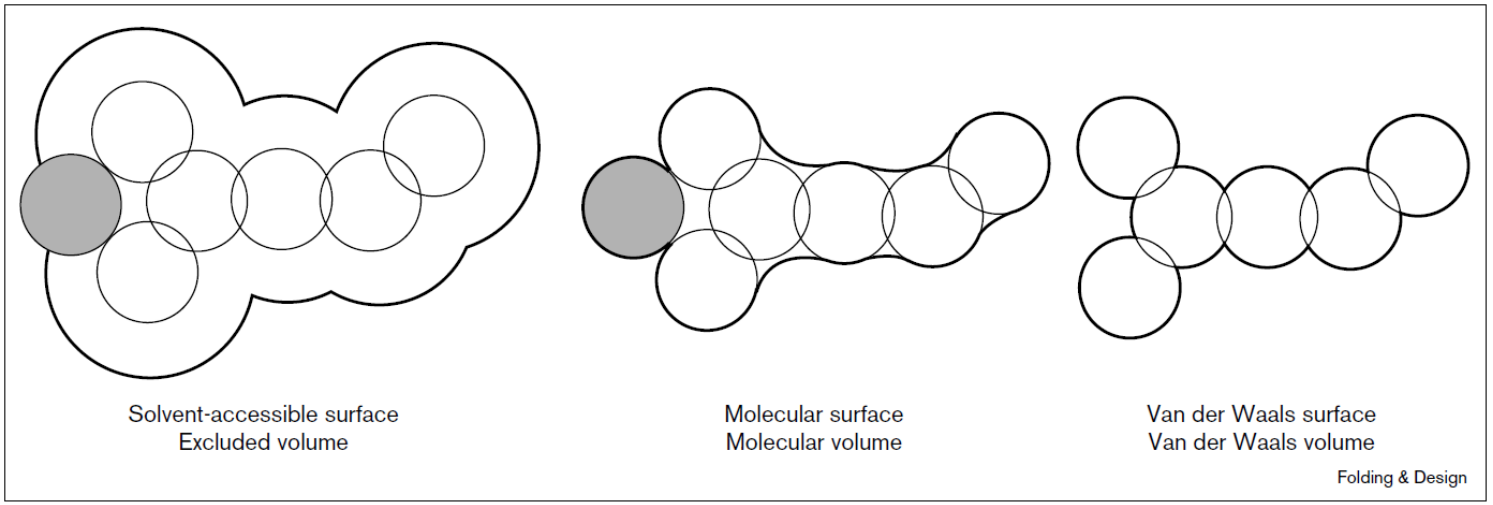
$$\Delta V = \int_{|\vec{r}|<\lambda} d\vec{r}\ \rho(\vec{r})\left(\frac{1}{\rho(\vec{r})} - \frac{1}{\rho_0}\right)$$

$$= -\int_{|\vec{r}|<\lambda} d\vec{r}\ (g_{uv}(\vec{r}) - 1) \overset{\text{def}}{=} \Delta V(\lambda)$$

$$\bar{v}_2 = v_2 + \delta_1(v_1 - v_1^0)$$

v_2 - "intrinsic" solute volume
δ_1 - # of waters in the hydration layer
v_1 - PSV of the water in hydration layer
v_0 - PSV of the water in the bulk

Surface area & intrinsic volume definitions



MD can address all these issues and provide closest correspondence with the theory!!

Intrinsic Viscosity

Macromolecules alter the viscosity of a solvent.
Linear polymers, such as unfolded polypeptide chains, nucleic acids and carbohydrates have the greatest effects.

Given a pure solvent viscosity of η_0 , and a macromolecule concentration of c, the measured viscosity can be formulated as:

$$\eta = \eta_0(1 + k_1 c + k_2 c^2 + \ldots)$$

The **relative viscosity** is the ratio of the solvent viscosity to the measured viscosity:

$$\eta_{\mathrm{rel}} = \eta / \eta_0 = (1 + k_1 c + k_2 c^2 + \ldots)$$

The **specific viscosity** is a measure of the effect of the macromolecule:

$$\eta_{\mathrm{sp}} = \eta_{\mathrm{rel}} - 1 = (k_1 c + k_2 c^2 + \ldots)$$

To a first approximation, the effect of a macromolecule on the viscosity of the solvent, is approximated by the **intrinsic viscosity** $[\eta]$:

$$[\eta] = \lim_{c \to 0} (\eta_{\mathrm{sp}} / c) = \lim_{c \to 0} (k_1 + k_2 c + \ldots) = k_1$$

with units typically of cm^3/g

Intrinsic Viscosity

Intrinsic viscosity $[\eta]$ is not sensitive to molecular weight, but is "exquisitely" sensitive to the shape of the macromolecule.

When macromolecules are hydrated spheres, $[\eta] = 2.5\ V_h\ N_A / M_r$ where V_h is the volume of the hydrated sphere, N_A is Avogadro's number, and M_r is its mass.

So globular macromolecules of any size will have approximately the same $[\eta]$.
On the other hand, rod like macromolecules can have enormous $[\eta]$.

Sample	**MW (kDA)**	**$[\eta]$ (cm^3/g)**
Globular:		
Ribonuclease A	14	3.3
Hemoglobin	68	3.6
Bushy stunt virus	10,700	3.4
Rod-like:		
Tropomyosin	93	52
Myosin	493	217
DNA	6,000	5000

Thus, measuring $[\eta]$ can be useful in monitoring the unfolding of approximately spherical globular proteins. The viscosity of a solution can be measured by Cannon-Ubbelohde type viscometers (determining the time a solution flows into a capillary) or with a rotating cylinder viscometer, which measures the force required to make the cylinder rotate. More recently, on-line differential viscosimeters following a SEC separation are producing quite accurate values with minimum amount of sample.

The intrinsic viscosity of a structure (e.g. PDB or bead model) can be computed.

Macromolecular Transport

Atoms and molecules have mass and charge: they will move in response to an external gravitational/centrifugal or electric field. Rates of their movement in response to such fields provide information on molecular mass, charge, size and shape.

Application of an external force field or perturbation from the equilibrium state will induce motion:

- Electric field → electrophoresis, motion of charged molecules
- Centrifugal force → sedimentation, motion due to mass
- Chemical potential → diffusion, osmosis
- Heat → Thermophoresis, Brownian motion → diffusion
- Pressure → volume changes

Macromolecular Transport

Transport processes are irreversible processes:

- System is in a non-equilibrium state and relaxes towards an equilibrium
- Transport occurs due to a potential applied to the system:

Process	Potential	Flow of	Equilibrium State	Experiment:
Electrical conduction	Electrostatic	Electrons	Uniform electrostatic potential	Electrophoresis
Heat Conduction	Temperature	Heat	Uniform temperature	Thermophoresis
Diffusion	Chemical Potential	Molecules	Uniform chemical potential	Light Scattering, Fluorescence Correlation, Analytical Ultracentrifugation
Sedimentation	Total potential (chemical potential + centrifugal potential energy)	Molecules	Uniform total potential	analytical ultracentrifugation

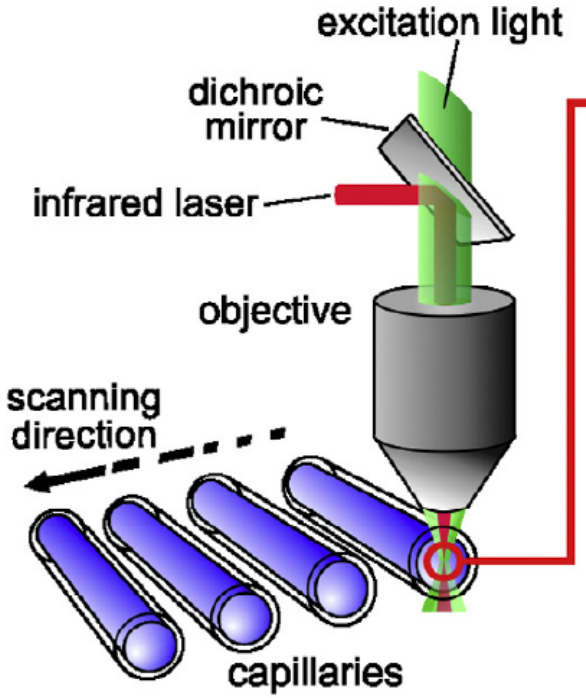
The flow is proportional to the gradient in the potential:

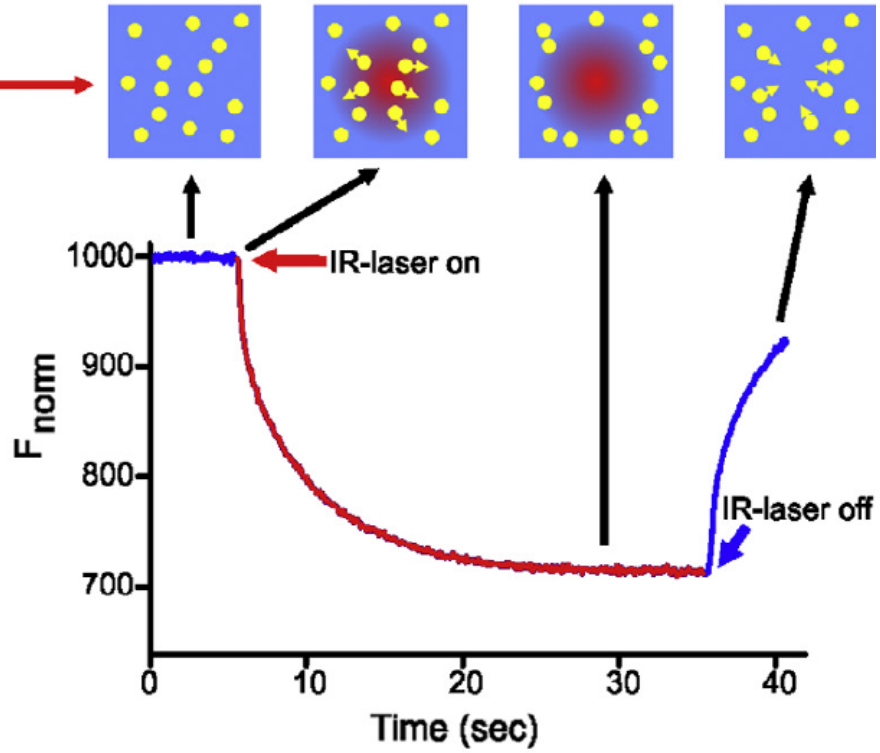
$$J_i = -L_i \frac{\partial U_i}{\partial x}$$

Transport Processes – Thermophoresis:

Two observables are measured in a microscale thermophoresis experiment: 1. decrease of fluorescence from the **T**emperature **R**elated **T**emperature **C**hange (TRIC), and 2. from the thermophoresis of the fluorescently tagged molecules. The thermophoresis can be described by:

$$\frac{C_{hot}}{C_{cold}} = e^{(-S_t \Delta T)}$$



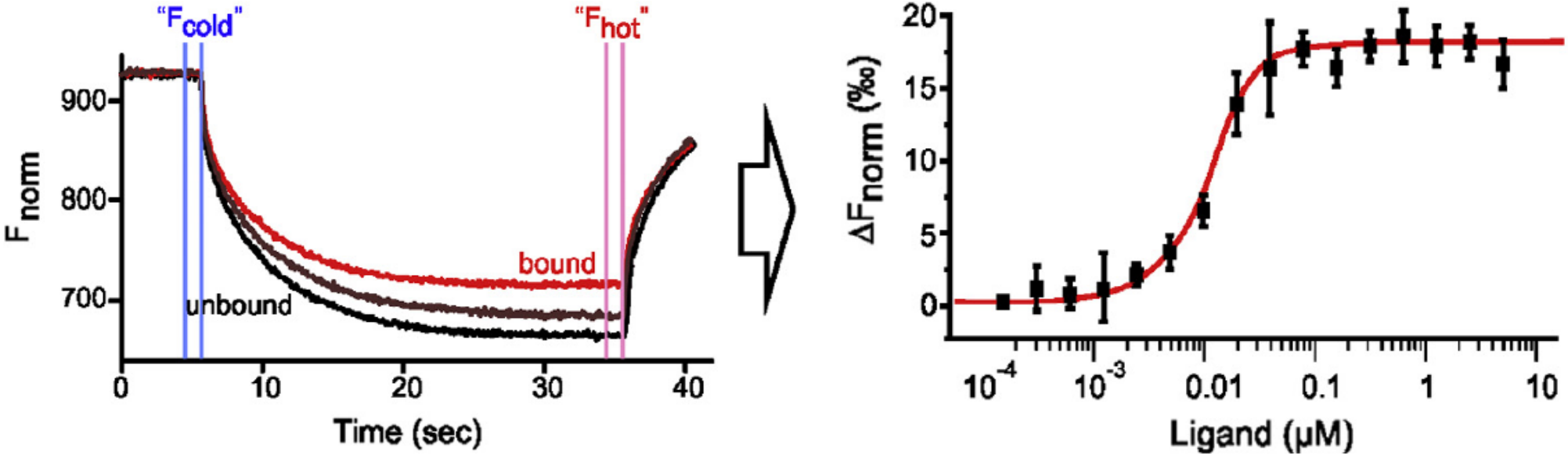


Credits: M. Jerabek-Willemsen et al., J. Mol. Struct., 2014 (1077), 101-113

Transport Processes – Thermophoresis:

It is important to realize that you can neither measure the local concentration nor the local temperature, so a true S_T is not really available.

Instead, plot the normalized ratio of F_{hot} over F_{cold} for different ligand concentrations to obtain a binding isotherm for the titration:



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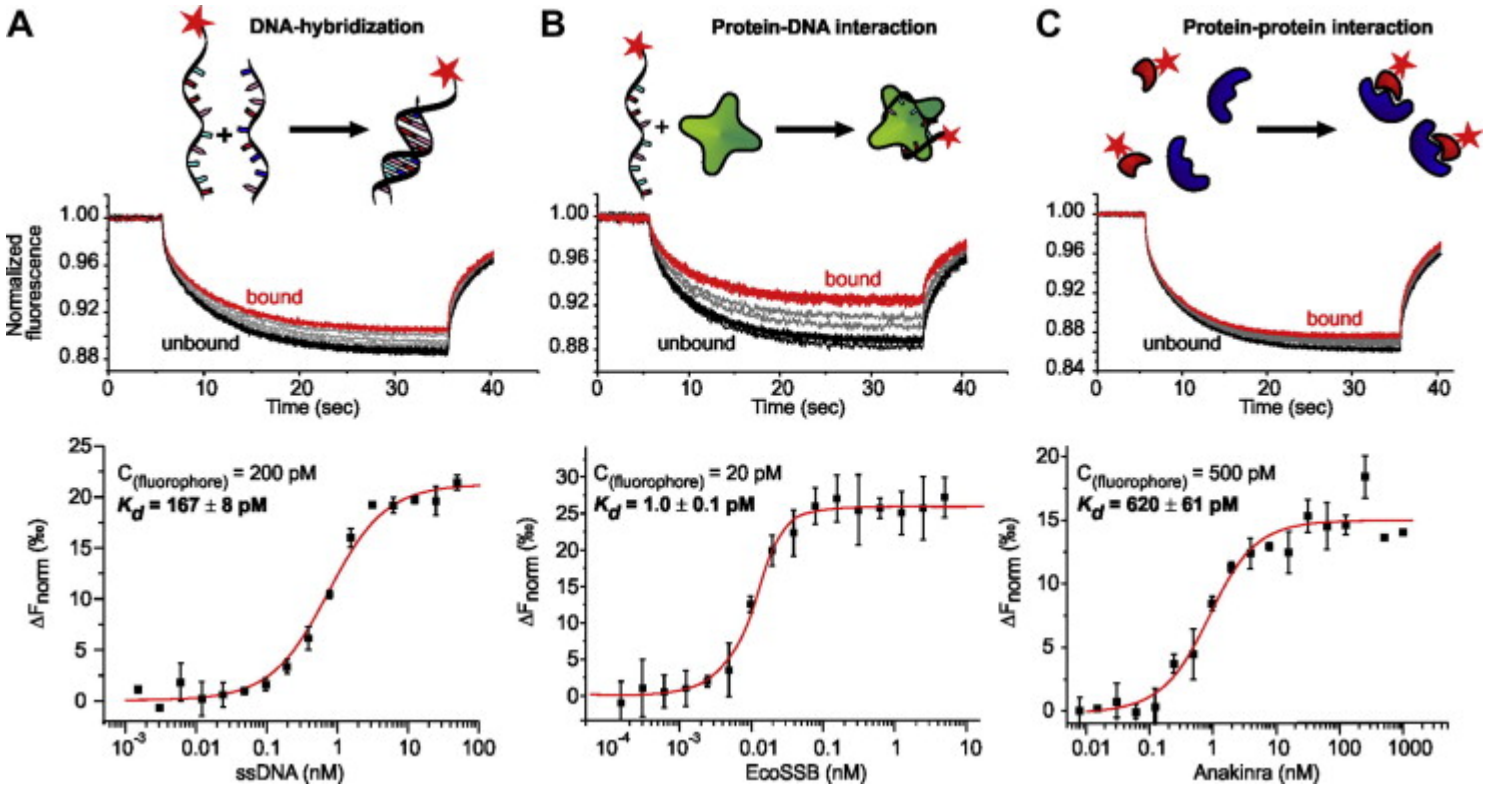
$$\frac{C_{hot}}{C_{cold}} = e^{\left(-S_t \Delta T\right)}$$



Homework (due Wednesday before the lecture)

1. (9 pts) A researcher measured the k_d for a protein's monomer-dimer equilibrium to be 6 μM. For each of these protein concentrations, what is the **molar ratio** of mols(monomer) to mols(dimer) in a solution that contains the following concentrations of this protein. Show your work:

- 60 nM
- 6 μM
- 60 μM

2. (12 pts) A poly-Phe peptide is placed into different buffers with varying pH. In which buffer do you expect the peptide to be positively, neutral, or negatively charged? If the sign of the charge is the same for two or more of the following conditions, indicate which one is more strongly charged. Justify your answer.

- pH 2.51
- pH 5.48
- pH 7.00
- PH 11.0

Please type your answer and e-mail me a PDF file.