Reversible Interactions - Oligomerization

Reversible reaction:
(Le Chatelier's principle, also called 'mass action')

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

Equilibrium Constant:

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

Kinetics:

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

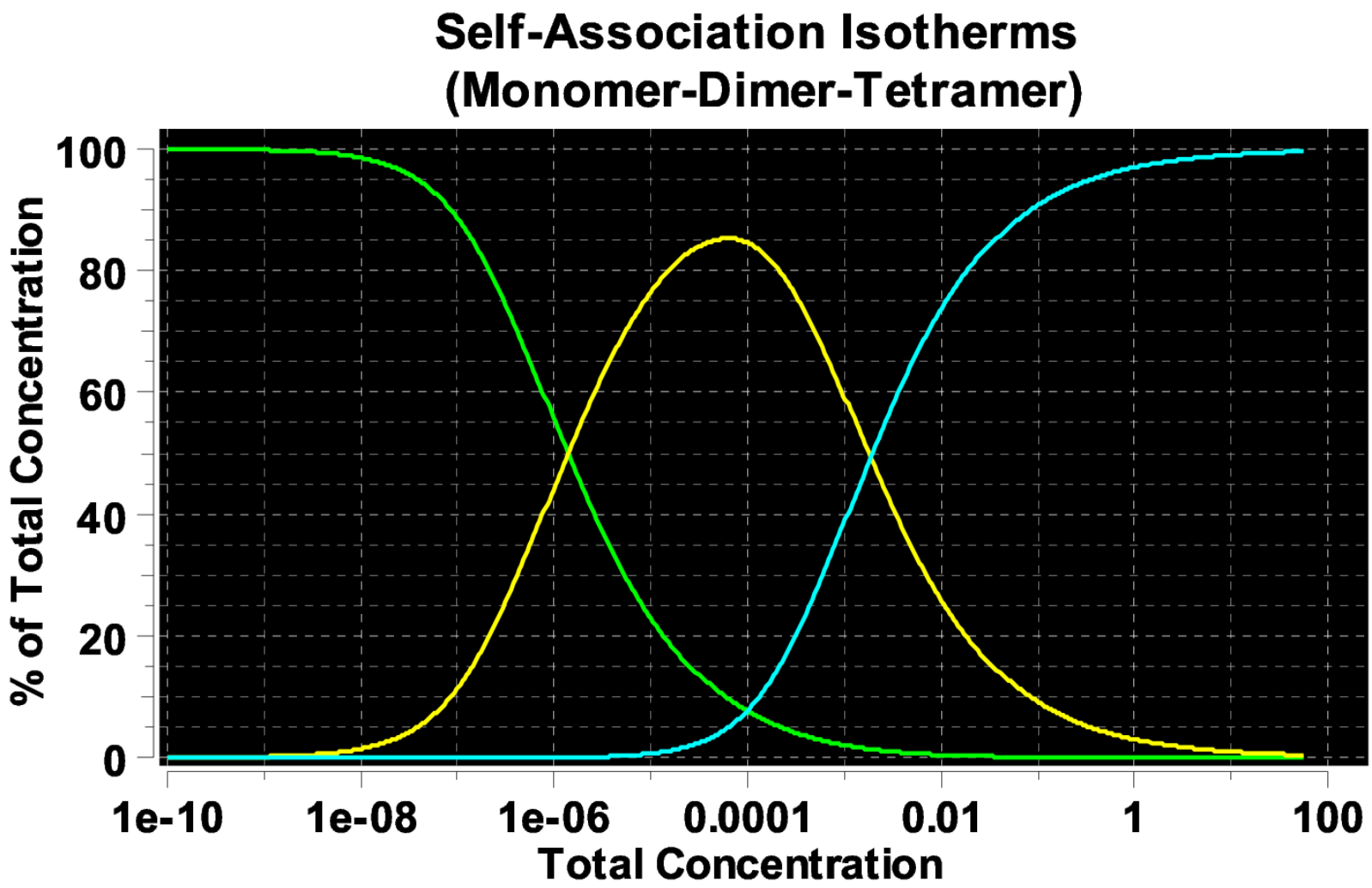
Solve Polynomial

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

Example, a monomer-dimer equilibrium:

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

Reversible Interactions - Oligomerization



SD

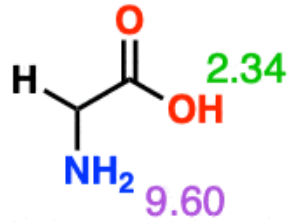
Monomer Dimer Tetramer

Macromolecular Interactions – Role of Solvent

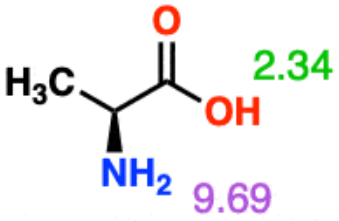
Interactions between molecules depend on the **surface properties** of the regions of the molecule that are interacting. Different **solvents** can amplify or eliminate these interaction effects, and change the Ka of interaction dramatically:

- 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**
- Conformational changes can induce **steric hindrance**, which can prevent proximity of interacting surfaces – for example, domains may change shape upon binding of small molecules.

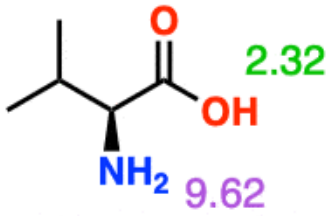
Structures (and pK_a values) of selected amino acids



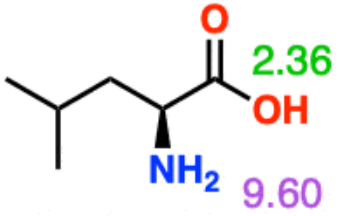
Glycine



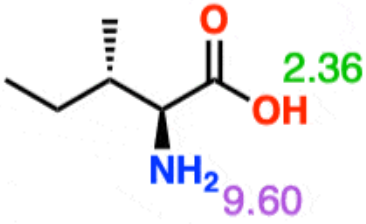
Alanine



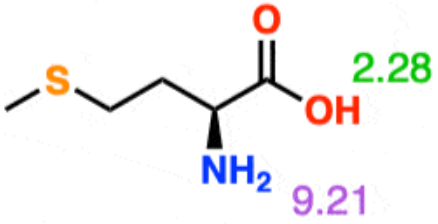
Valine



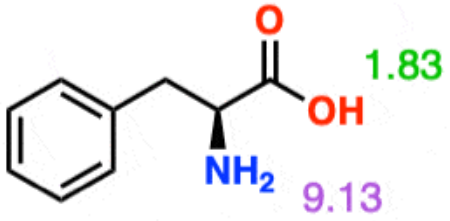
Leucine



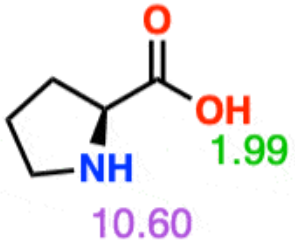
Isoleucine



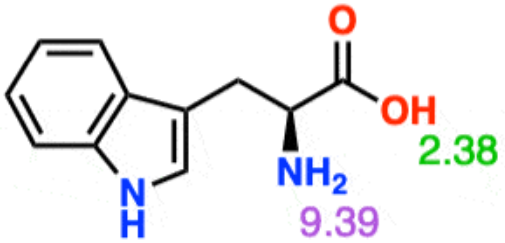
Methionine



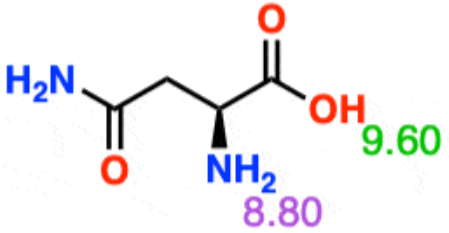
Phenylalanine



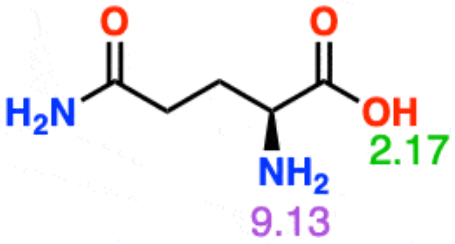
Proline



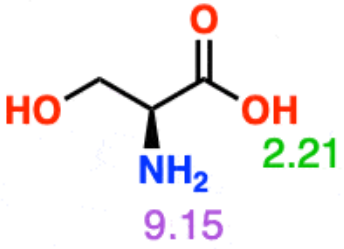
Tryptophan



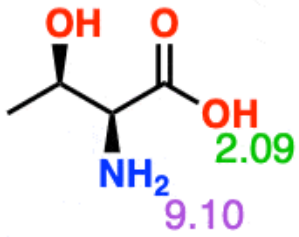
Asparagine



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

Homework (due before next 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-phenylalanine 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

3. (6 pts) You suspect a monomer-dimer interaction is caused by electrostatic interactions, how would you change the solution properties to test this (assume an aqueous solvent)? Would the k_D increase or decrease? What about the k_A?

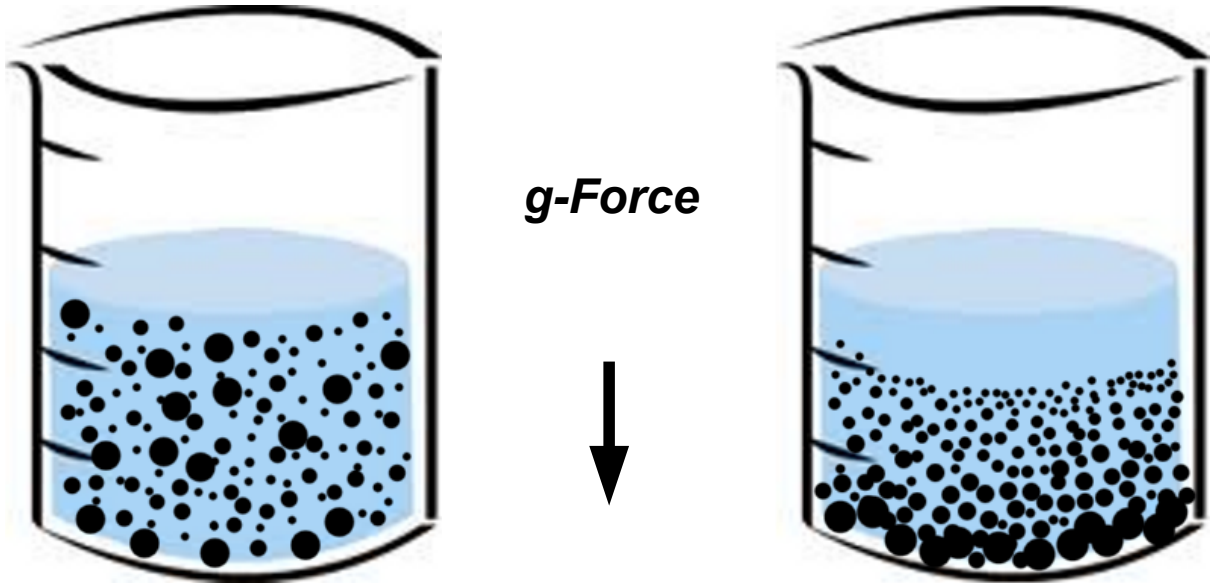
Please type your answer and e-mail me a PDF file.

Topic:

Sedimentation + Diffusion Transport

Macromolecular Transport - Sedimentation

Sedimentation of fine sand, coarse sand and pebbles by gravity, which particle sediments fastest?



Effect of size:

Bigger particles of the same material (i.e., same density) will sediment faster than smaller particles

Sedimentation is proportional to the MOLECULAR WEIGHT

Macromolecular Transport - Sedimentation

Effect of shape:

Measure speed of sedimentation of a piece of metal:

1. solid ball →*will sediment quickly*

2. flattened into a piece of foil →*will sediment slowly*

Shape matters!

Sedimentation is inversely proportional to ANISOTROPY

Macromolecular Transport - Sedimentation

Effect of partial specific volume:

Measure speed of sedimentation of a piece of metal:

1. flattened into a piece of foil →*will sediment slowly*

2. foil loosely folded into a ball →*will float*

All particles have the same weight – so what's different?

Sedimentation is inversely proportional to BUOYANCY and PARTIAL SPECIFIC VOLUME of sedimenting particle

Macromolecular Transport - Sedimentation

Effect of Solvent Density:

Measure speed of sedimentation of a protein:

1. in distilled water → *will sediment fast*

2. in salt water → *will sediment slowly*

It's the same particle – so what's different?

Sedimentation is affected by the DENSITY of the solvent

It’s impossible for anyone to get drown in Dead Sea!

Dead Sea is ten times more salty than the Mediterranean and three times more salty the Great Salt Lake in Utah

By TelanganaToday | Published: 22nd Jul 2020 7:01 pm

Macromolecular Transport - Sedimentation

Effect of Solvent Viscosity:

Measure speed of sedimentation of a protein:

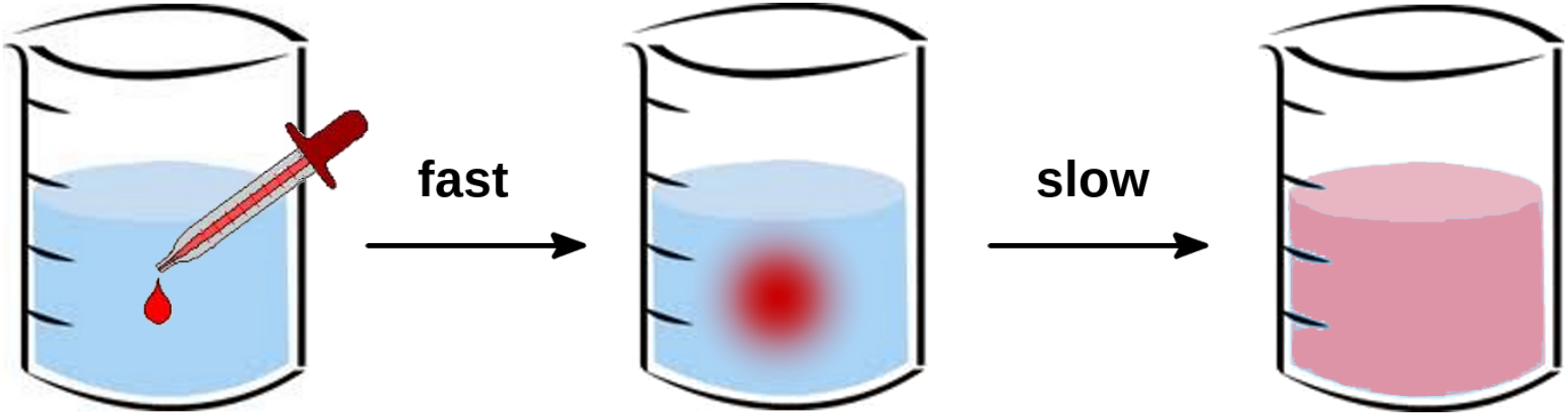
1. in distilled water → *will sediment fast*

2. in a sucrose solution → *will sediment slowly*

It's the same particle – so what's different?

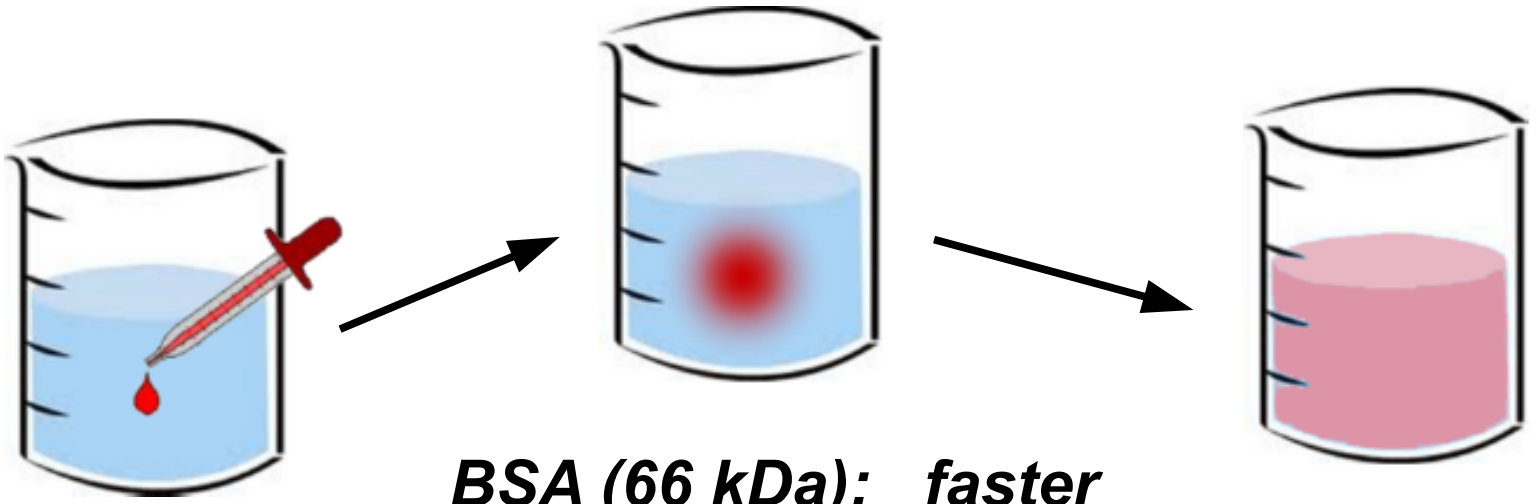
Sedimentation is affected by the VISCOSITY of the solvent

Macromolecular Transport - Diffusion

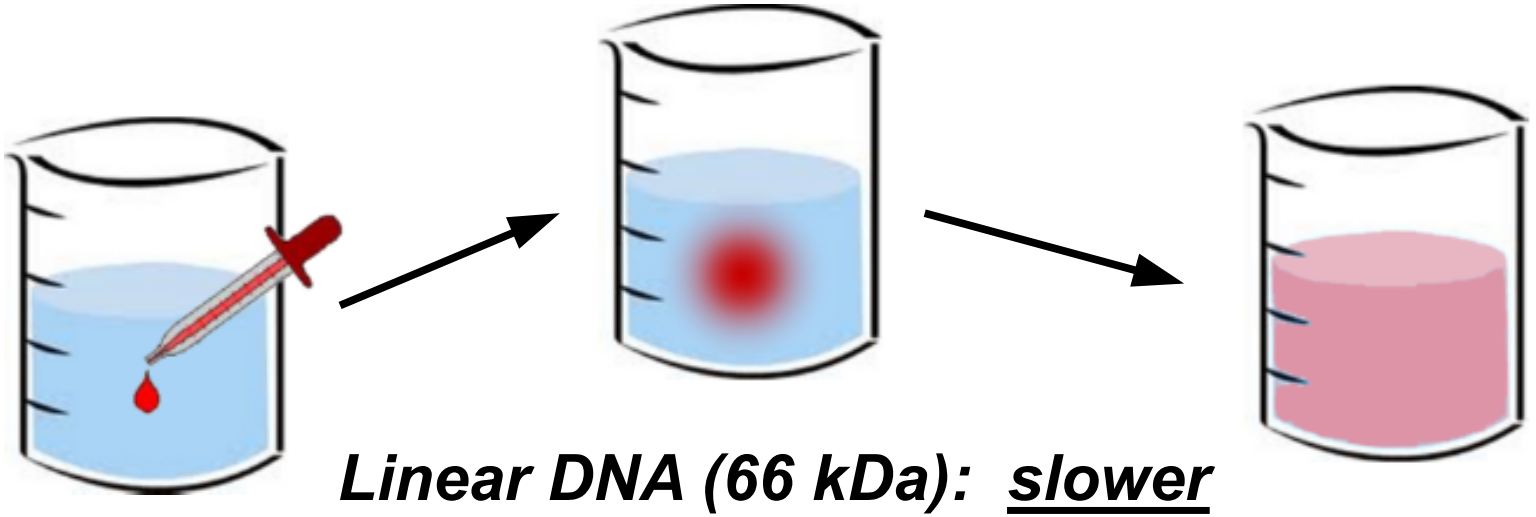


Diffusion is proportional to the CONCENTRATION GRADIENT

Macromolecular Transport - Diffusion



BSA (66 kDa): <u>faster</u>



Linear DNA (66 kDa): <u>slower</u>

Diffusion is inversely proportional to the friction of particle

Macromolecular Transport - Diffusion

Effect of Solvent Viscosity:

Measure speed of diffusion of a protein:

1. in a viscous solution containg sucrose → *slow*

2. in distilled water → *fast*

Diffusion is inversely proportional to

the VISCOSITY of the solvent

Macromolecular Transport - Diffusion

Effect of Solvent Density:

Measure speed of diffusion of a protein:

1. in solution with high density →

2. in solution with low density → *no change*

Diffusion is <u>NOT</u> affected by the

DENSITY of the solvent

Macromolecular Transport - Diffusion

Summary:

Sedimentation:

- Contribution from the solute:
  - Friction (includes effects from hydration)
  - Mass
  - Density (hydration)
- Contribution from the buffer:
  - Density
  - Viscosity

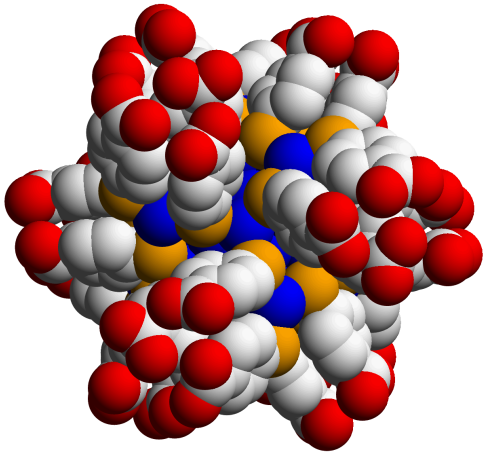
Diffusion:

- Contribution from the solute:
  - Friction
- Contribution from the buffer:
  - Viscosity

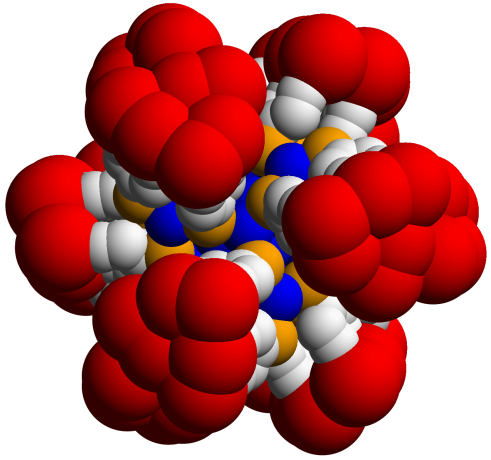
Partial Specific Volume ($\bar{v}$)

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

No hydration

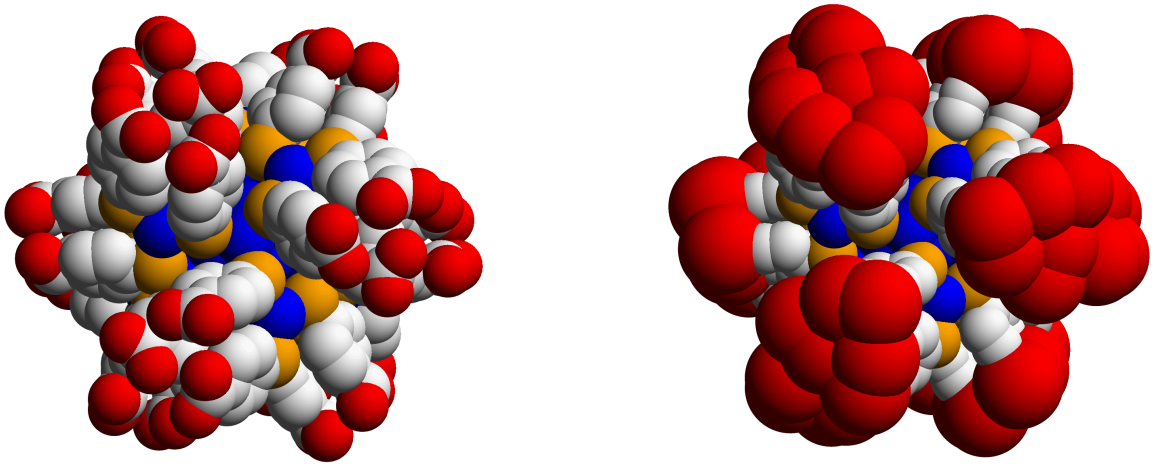


with hydration



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!

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:

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