Measuring the size and shape of macromolecules

Hydrodynamics: study of the objects in water

How do the move?

Translation

Rotation

1) Movement with no external force-

free diffusion

2) Movement under the influence of an external force-

e.g. centrifuge

electrophoresis

Hydrodynamics techniques measure the frictional resistance of the moving macromolecule in solution:

1. **Intrinsic viscosity**
   the influence of the object on the solution properties

2. **Free translational diffusion**

3. **Centrifugation/sedimentation**

4. **Electrophoresis**

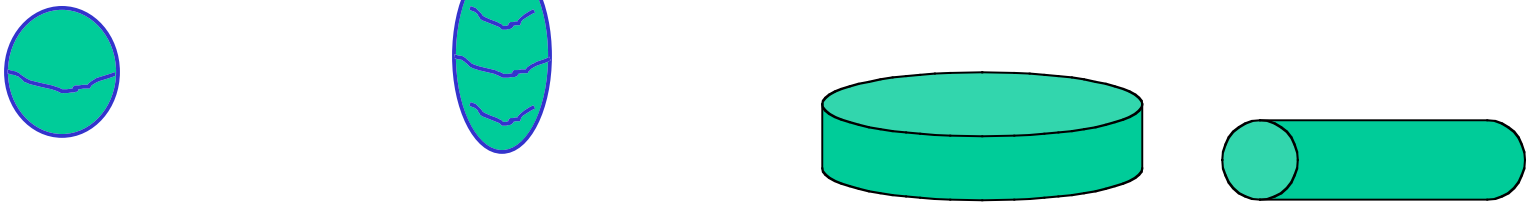
5. **Gel filtration chromatography**

6. **Free rotational diffusion/fluorescence anisotropy**

Hydrodynamics

Measurements of the motion of macromolecules in solution form the basis of most methods used to determine molecular size, density, shape, molecular weight

Equations based on the behavior of macroscopic objects in water can be used successfully to analyze molecular behavior in solution

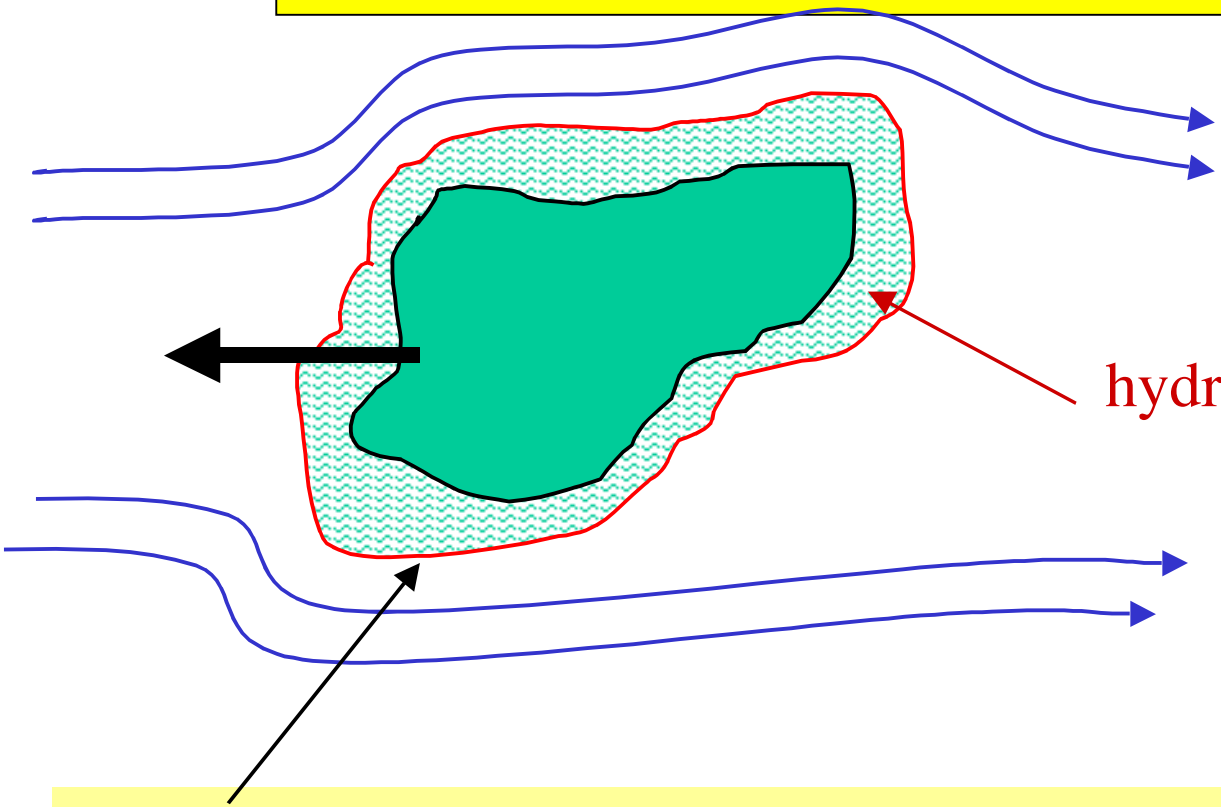


sphere ellipsoid (prolate, oblate) disk rod etc.

Techniques

	Free Motion	**Mass Transport**
Viscosity	translational diffusion rotational diffusion	sedimentation electrophoresis gel filtration

The hydrodynamic particle



hydrodynamically bound water

shear plane: **frictional resistance to motion depends on**

- size
- shape

Hydrodynamic Parameters

1. **Specific volume**: (Inverse of density)

$$\frac{volume}{gram} = V_i$$

1 = solvent
2 = solute

Volume = $V_1 g_1 + V_2 g_2$

$\left\{ \frac{\text{vol}}{\text{gram}} \right\}$ x grams

Proteins: V_2 ~ 0.7 - 0.75 mL/g **Na^+ DNA: V_2 ~ 0.5 - 0.53 mL/g**

Hydrodynamic Parameters

2. **Partial specific volume**: change in volume per gram under specific conditions

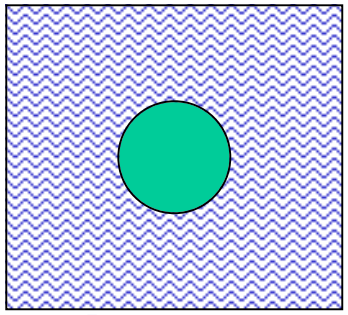
$$\bar{V}_i = \left(\frac{\partial V}{\partial g_i}\right)_{T,P,g_j}$$

Volume = $\bar{V}_1 g_1 + \bar{V}_2 g_2$ Gibbs - Duham relationship

We will assume that specific volume is approximately equal to the partial specific volume: $V_i \approx \bar{V}_i$

Proteins: V_P ~ 0.7 - 0.75 mL/g **Na^+ DNA: V_{DNA} ~ 0.5 - 0.53 mL/g**

3. Effective mass



Take into account buoyancy
by subtracting the mass of the displaced water

[mass - (mass of displaced water)]

$$[m - m(\overline{V}_2\rho) = m(1 - \overline{V}_2\rho)]$$

$$\frac{M(1 - \overline{V}_2\rho)}{N}$$

$m\overline{V}_2$ = volume of particle

$m\overline{V}_2\rho$ = mass of displaced solvent

ρ = solvent density

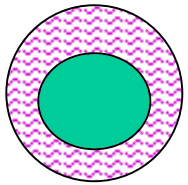
$$\overline{V}_2 = \frac{\text{Vol}}{g} \text{ of the molecule}$$

m = mass of molecule

m = (M/N)

4. **Hydration**

$$\delta_1 = \frac{g_{H2O}}{g_{protein}}$$



$$m_h = m + m\,\delta_1$$

$$m_h = \frac{M\,(1 + \delta_1)}{N}$$

Mass of hydrated protein

$$V_h = \frac{M\,(\bar{V}_2 + \delta_1\,\bar{V}_1)}{N}$$

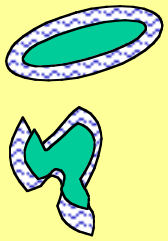
Volume of hydrated protein

$$V_h = (\bar{V}_2 + \bar{\delta}_1\,V_1)$$

volume of hydrated protein per gram of protein

Problems of hydrodynamic measurements in general--

Properties depend on : 1) size
too many parameters! 2) shape
3) solvation



Some solutions to obtain useful information:

1 Use more than one property and eliminate one unknown.
Example: sedimentation (S^o) and diffusion (D) can eliminate shape factor

2 Work under denaturing conditions to eliminate empirically the shape factor and solvation factors. These are constants for both standards and the unknown sample - then find **Molecular weight**.

Examples: 1) gel filtration of proteins in GuHCl; 2) SDS gels.

3 If **Molecular weight** is known: compare results with predictions based on an unhydrated sphere of mass M. Then use judgement to explain deviations on the basis of shape or hydration.

Behavior of limiting forms - eg; rods, spheres- are calculated for comparisons

Next we consider:

1 **What effect does the interaction of the hydrodynamic particle and the solvent have on solution properties?**

viscosity

2 **What effect does the particle-solvent interaction have on the motion of the particle?**

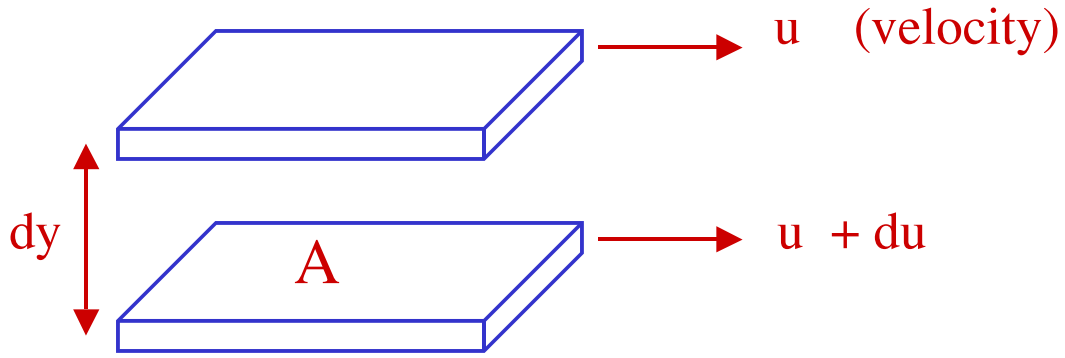
translational motion

- free
- in a force field

rotational motion

Viscosity

Frictional interactions between "layers" of solution results in energy dissipation



$$F_f \propto \frac{du \bullet A}{dy} = \eta \bullet \left(\frac{du}{dy}\right) \bullet A$$

(shearing force)

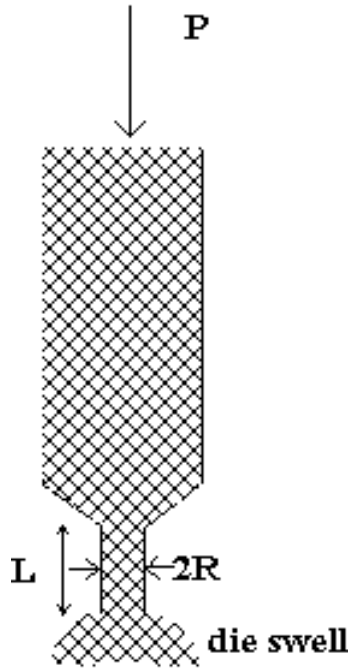
η ≡ coefficient of viscosity

cgs units ≡ Poise

η is related to the amount of energy dissipated per unit volume per unit time

Many ways to measure solution viscosity

capillary viscometer: measures the rate of flow of solution through a capillary with a pressure drop P



Effect of macromolecules on viscosity: only 2 parameters

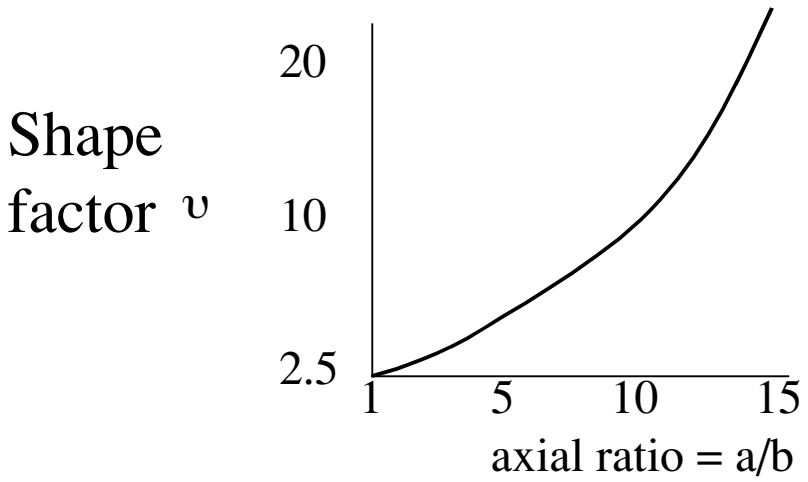
plus solute

$$\left(\frac{\eta}{\eta_o}\right) = 1 + \upsilon \bullet \phi$$

solvent alone

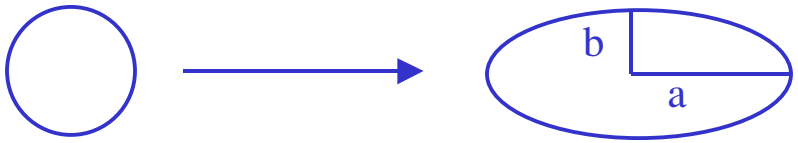
fraction of volume occupied by "particles"

"shape" factor



For a sphere: υ = 2.5

Shape factor is very large for highly asymmetric molecules



viscosity effects are very large for very elongated polymers **(large υ)** (e.g. actin) or molecules that "occupy" large volume **(large ϕ)** (e.g. DNA)

Relative viscosity and Specific viscosity

1 $\left(\frac{\eta}{\eta_o}\right) = \eta_r$ relative viscosity $= 1 + \upsilon\phi$

2 $\left(\frac{\eta - \eta_o}{\eta_o}\right) = \eta_{sp}$ specific viscosity

$$\eta_{sp} = \eta_r - 1 = \upsilon\phi$$

But $\phi = \overline{V}_h \bullet c$

$$\frac{\eta_{sp}}{c} = \upsilon \bullet \overline{V}_h$$

so

ν = shape factor

ϕ = fraction of solution volume occupied by solvent particles

$\underline{c}$ = g / mL solvent

$\overline{V}_h$ = volume per gram of protein occupied by solvated particle

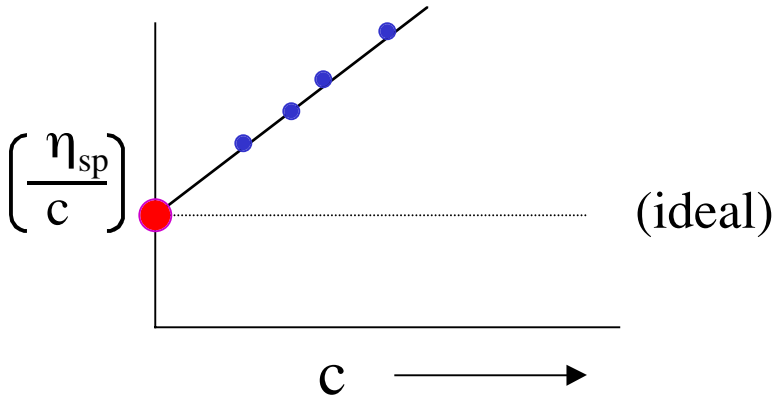
ideally, the increase in specific viscosity per gram of solute should be a constant ($\upsilon \bullet \overline{V}_h$). Often it is not due to "non-ideal" behavior

Shape factor

vol/g of protein of hydrated particle

Non-ideal case (i.e.,reality):

$$\left(\frac{\eta_{sp}}{c}\right) = \upsilon \bullet \overline{V_h} + (\text{const.}) \bullet c + \ldots.$$



Extrapolate measurement to infinite dilution: Intrinsic viscosity

$\lim_{C \to 0} (\eta_{sp}/c) = [\eta]$ limit at low concentration (intercept in graph above)

Intrinsic viscosity: $[\eta]$, units = mL / g

$$[\eta] = \upsilon \bullet \overline{V_h}$$

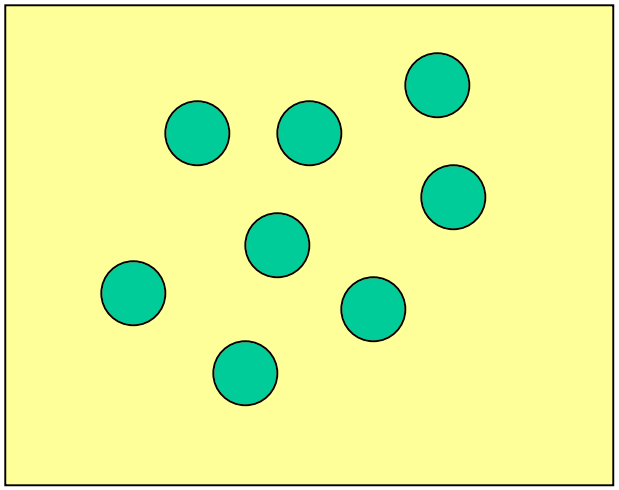
Intrinsic viscosity is dependent only on size and properties of the isolated macromolecule.

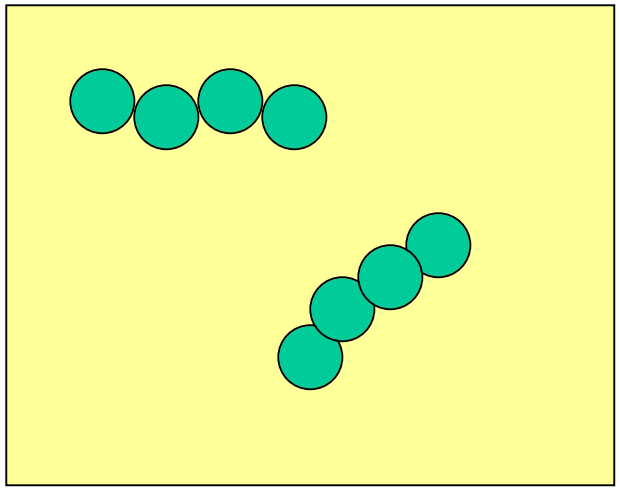
Intrinsic viscosity

$$[\eta] = \upsilon \cdot \bar{V}_h$$

ν increases

monomer → polymer





$\nu = 2.5$

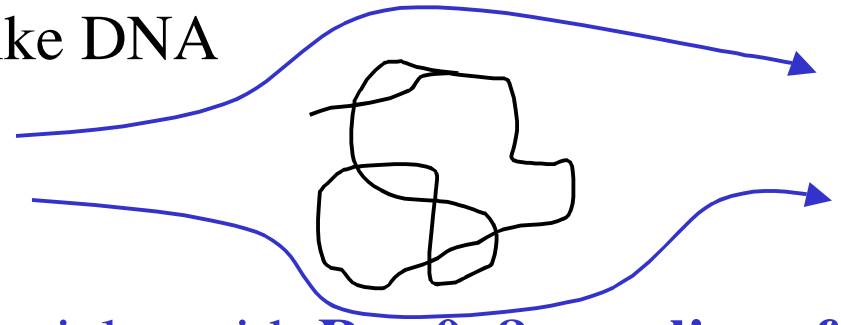
$$[\eta] = \upsilon \cdot \bar{V}_h$$

if $\nu = 25$ then $[\eta]$ is 10x larger

$$[\eta] = \upsilon \cdot \bar{V}_h$$

if we assume a spherical shape, $\nu = 2.5$, we will calculate an incorrect and very large value for $\bar{V}_h$

For a flexible polymer like DNA
or denatured protein



Behaves like compact particles with **R ≈ 0. 8 x radius of gyration**
(radius of gyration measures mass distribution and can be obtained from light scattering)

example: $[\eta] \approx 4$ mL / g for a native, globular protein

But for a random coil, if there is ~ 100 g "bound" solvent per gram solute

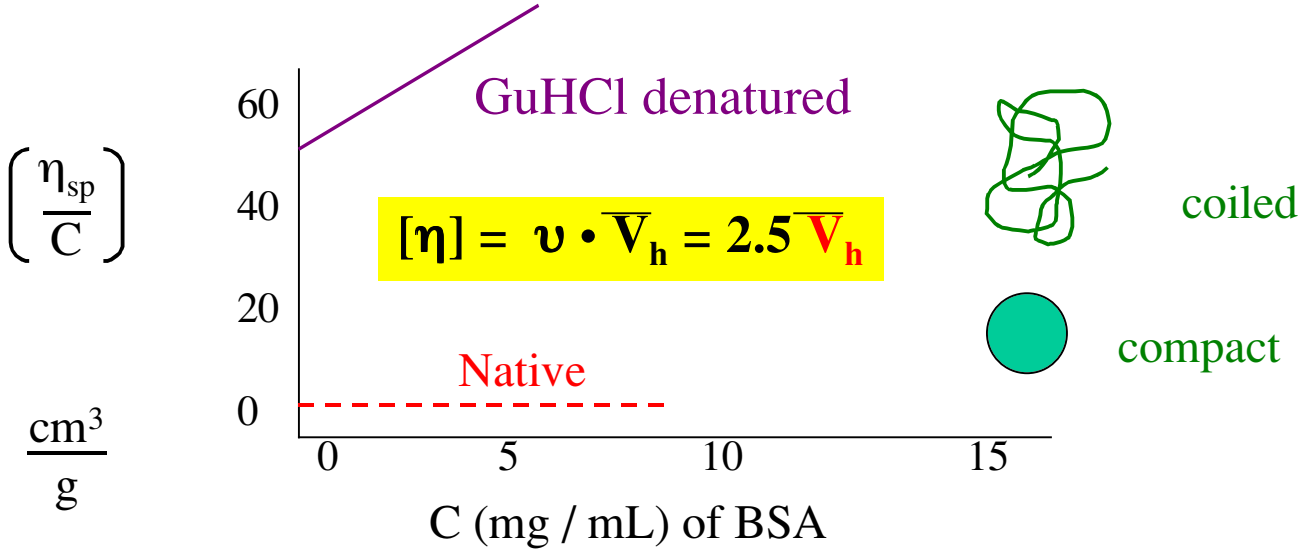
$$\overline{V}_h \approx \overline{V}_2 + 100 \approx 100 \text{ mL/ g}$$

$$[\eta] = 2.5 \bullet \overline{V}_h$$

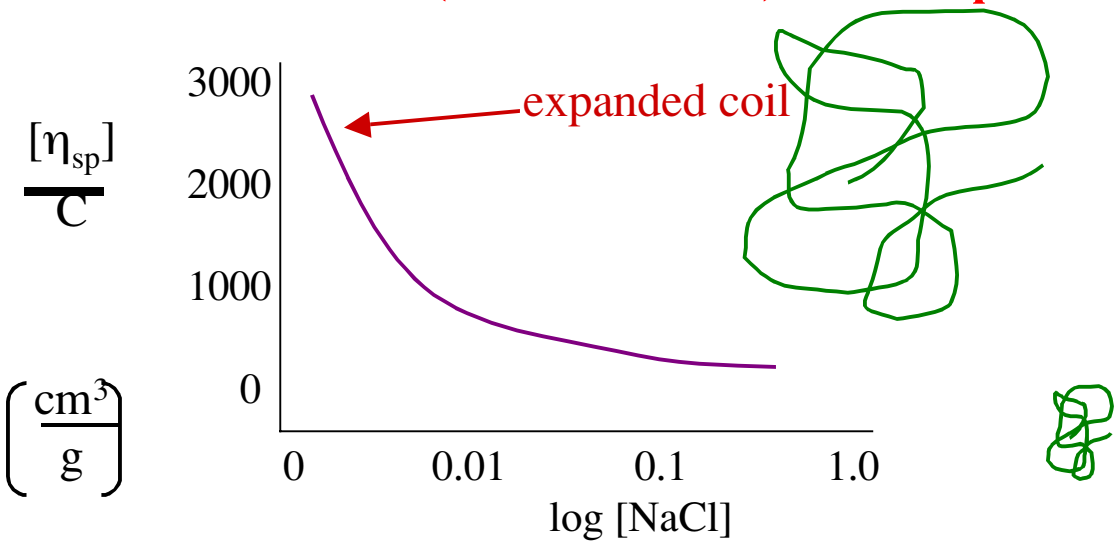
very large "coil"

Viscosity : examples

I Native Hemocyanin (M = 10^6) native vs GuHCl denatured

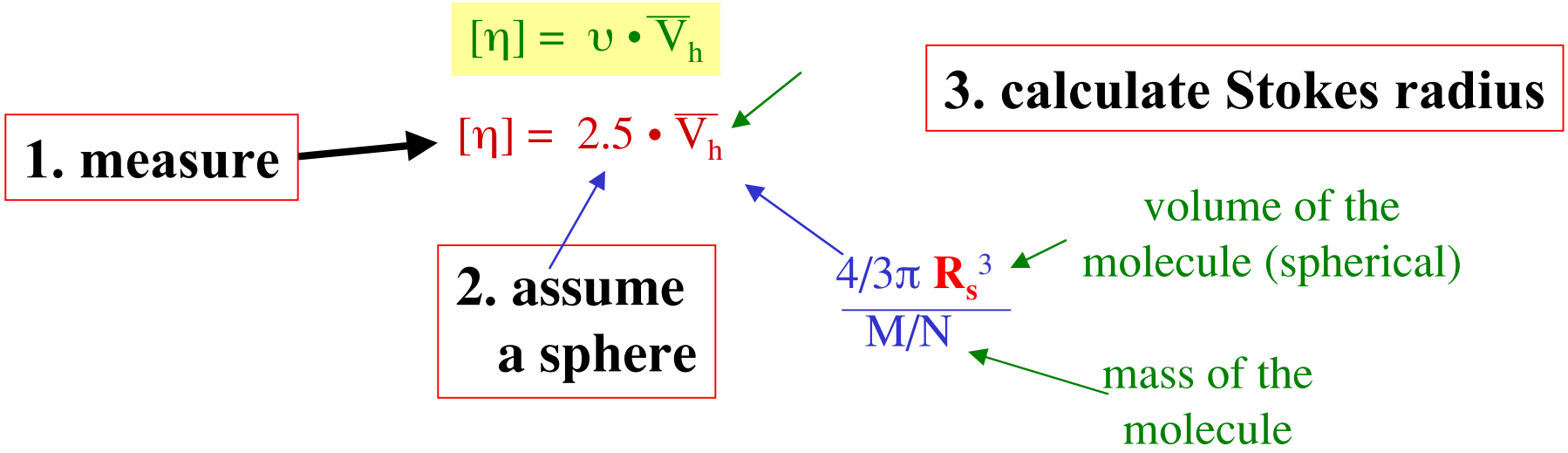


II T7 DNA (double strand) : salt dependence



Stokes Radius

Radius of the sphere which has the hydrodynamic properties consistent With the hydrodynamic measurement



Solve for the Stokes radius:

$$R_s = [\frac{M}{N} \bullet \frac{3}{4\pi} \overline{V}_h]^{1/3} \quad \text{where } \overline{V}_h = [\eta]/2.5$$

MINIMUM RADIUS

The radius expected if the molecule is an anhydrous sphere

$$\overline{V}_2 = \frac{[4/3\ \pi\ R_{min}^{\ 3}]}{(M/N)}$$

anhydrous sphere - point of reference

$$R_{min} = \left[\ \frac{M}{N}\left(\frac{3}{4\pi}\right)\overline{V}_2\right]^{1/3}$$

CALCULATED

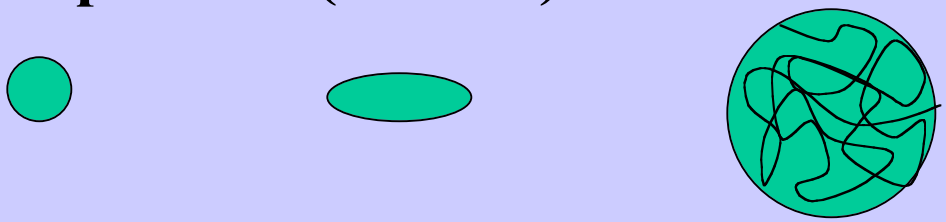
Compare the Stokes radius (measured) to the "minimum radius" expected assuming the molecule is an anhydrous sphere

Question: How close does the assumption of an anhydrous sphere come to explaining the value of $R_{s?}$

If R_s/R_{min} is not much larger than 1.0, then the assumption of the molecule being spherical is likely reasonable.

R_s should be slightly larger than R_{min} due to hydration

If R_s/R_{min} is much larger than 1.0, then either the molecule is not spherical ($\nu >> 2.5$) or it is not compact ($\bar{V}_h >> \bar{V}_2$)



For an anhydrous particle:

$$[\eta] = \upsilon \bullet \bar{V}_2$$

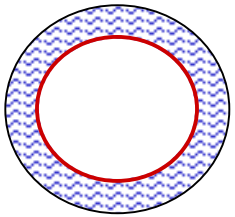
anhydrous vol/gram

for an anhydrous sphere: υ = 2.5

$$[\eta] = 2.5 \bullet \bar{V}_2$$

For real macromolecules, the intrinsic viscosity will vary from the above due to

1. correction due to **hydration**

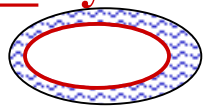


$\upsilon = 2.5$ $\quad \bar{V}_h$ **(hydrated vol/g = $\bar{V}_2 + \delta_{H20}$)**

2. correction due to **asymmetry**

$\upsilon > 2.5$ $\quad \bar{V}_h = \bar{V}_2$ (anhydrous vol/g)

or both **hydration and asymmetry**



$\upsilon > 2.5$ and **$\bar{V}_h = \bar{V}_2 + \delta_{H2O}$**

Comparison of intrinsic viscosity values for two proteins:

1. Ribonuclease:

   mol wt: 13, 683

   $\bar{V}_2 = 0.728$ ml/g

   $R_{min} = 17$ Å

2. Collagen:

   mol wt: 345,000

   $\bar{V}_2 = 0.695$ ml/g

   $R_{min} = 59$ Å

Interpreting Viscosity Data:

Does a reasonable amount of hydration explain the measured value of [η]?

protein	[η] mL/g	R_s (Å)	maximum solvation δ_{H2O} (g/g)	maximum asymmetry υ	(a/b)
Ribonuclease	3.3	19.3	**0.59**	4.5	3.9
Collagen	1150	400	460	**1660**	**175**

$$[\eta] = \upsilon \, \bar{V}_h = \frac{\upsilon \, [4/3 \, \pi \, R_s^3] \, N}{M}$$

Stokes Radius $\quad R_s = \left(\frac{3 \, [\eta] \, M}{4 \, \pi \, N \, \upsilon}\right)^{1/3}$

2.5

Appropriate for collagen

R_s/R_{min} = 6.8 (400/59)

$\bar{V}_h = \bar{V}_2$ (anhydrous value)

shape correction: **$\upsilon > 2.5$**

hydration correction: **$\bar{V}_h = \bar{V}_2 + \delta_{H2O}$**

Appropriate for ribonuclease

R_s/R_{min} = 1.14 (19.3/17)

$\upsilon = 2.5$