

## Hydrodynamics Lecture 2

This lecture uses three levels of evaluation to analyze/predict the frictional coefficient (f) for compact globular macro-molecules based on MM size/shape:

- 1) zero-order: a non-hydrated sphere
- 2) first-order: a hydrated sphere and
- 3) second order: a hydrated non-spherical macromolecule.

Make note that calling these three evaluations zero, first and second order is only for the purposes of this class. At the end of the lecture we begin to discuss open, flexible polymers. And the boxed equations are ones we definitely have to know.

Remember from last class, the hydrated volume of a MM:

$$V_h \equiv (drymassMM) \cdot (\bar{v}_p) + \left( \frac{massH_2O}{\rho_w} \right) = \left( \frac{M}{N_o} \right) \cdot \left( \bar{v}_p + \left( \frac{\delta_w}{\rho_w} \right) \right)$$

Where  $\bar{v}_p$  = partial specific volume, M= molecular mass of MM, and  $N_o$ = Avagadro's #.

and  $\delta_w$  = (Mass of bound H<sub>2</sub>O)/(Mass of dry Molecule) → which seems to me like a fancy way of canceling out the mass of the MM (M) when you multiply everything in the right parentheses by  $\left( \frac{M}{N_o} \right)$ .

### Old Result for Hydrodynamics of Spheres:

$$f = 6\pi\eta_{solvent} \cdot r_{sphere}$$

The name of the game: to go from  $f$  (which you can get from things like sedimentation coefficient and diffusion coefficient) to properties of the MM such as size and shape (which can be described by  $r$ ).

### '0-level' spherical chicken analysis

→ If we're assuming an un-hydrated sphere:

$$\text{Then, } f_o = 6\pi\eta_{solvent} r_o$$

This also means that  $\delta_{H_2O} = 0$ . This simplifies the volume equation from above:

$$V_o = \text{volume of an un-hydrated sphere} = \left( \frac{M}{N_o} \cdot \bar{v}_p \right)$$

Using the general equation for the volume of a sphere:  $V_o = \left(\frac{4}{3}\pi \bullet r_o^3\right) = \left(\frac{M}{N_o} \bullet \bar{v}_p\right)$

Rearranging, we obtain:  $r_o = \left(\frac{3M\bar{v}_p}{4\pi N_o}\right)^{1/3}$  or the un-hydrated radius.

### **'1<sup>st</sup> order analysis'**

→ If we're allowing  $\delta_w > 0$ , but still assuming a sphere:

$$\text{Then } V_h = \left(\frac{M}{N_o}\right) \bullet \left(\bar{v}_p + \left(\frac{\delta_w}{\rho_w}\right)\right) = \left(\frac{4}{3}\pi \bullet r_h^3\right)$$

Rearranging:  $r_h = \left(\frac{3M \bullet \left(\bar{v}_p + \left(\frac{\delta_w}{\rho_w}\right)\right)}{4\pi N_o}\right)^{1/3}$  or the hydrated radius of a spherical MM.

### **'2<sup>nd</sup> order analysis' → Because real MM are hydrated, but they are not spherical:**

In this case:  $f = 6\pi\eta_{\text{solvent}} r_h F$

**F** is the Perrin factor or 'fudge-factor'. It is a function of shape only. Any well-defined shape has an associated F-value. For every given shape/size, there is a Perrin factor.

#### Qualitatively:

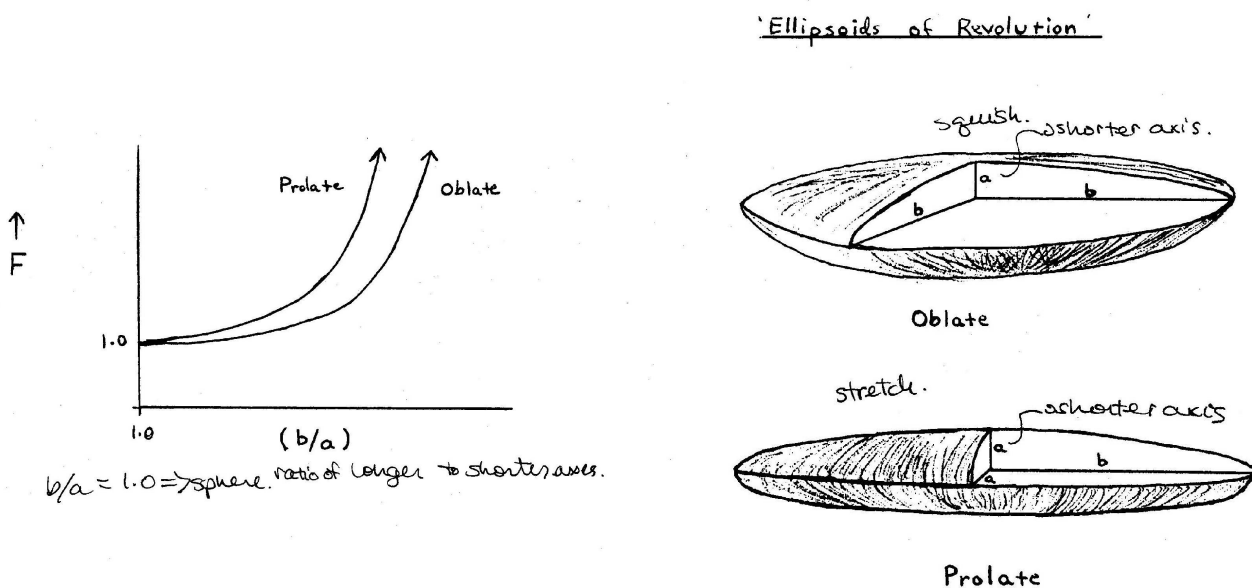
F=1.000 for a perfect compact sphere. F increases as the MM becomes more expanded (for example, maybe water could pass through the helices) and as the shape becomes more 'assymmetric'(less perfectly spherical). So, for example, F increases with partial denaturation of a protein.

F can never be less than 1. Not even for a particle optimally formed to move through a solvent.

For many MM the ellipsoid approximation is not perfect, but not too bad. Ellipsoids are described as either oblate or prolate and by their axial ratio ( $b/a$ ) = (long axis/ short axis). Note that when ( $b/a$ ) = 1.0, then the object is a sphere, and  $F = 1.0$ .

If we know  $F$ , we still do not know whether the object is prolate or oblate.

The graph below shows a plot of Perrin factors ( $F$ ) for a whole family of prolate and oblate ellipsoids (a table of these values is available in the course pack). It can be seen from the graph that if we know  $F$ , we still do not know whether the object is prolate or oblate.



#### An assumption:

2<sup>nd</sup> order analysis (i.e. the equation  $f = 6\pi\eta_{\text{solvent}} r_h F$ ) assumes that the MM is freely and randomly tumbling in solution.

**Why** do we need to assume this?

Well, consider a long rigid, rod-like molecule in a centrifuge:



The orientation of the rod in a) is more like a javelin, and there is less resistance than in b). With enough force, the entropic cost of ordering the molecules such that there is less resistance will be overcome, and the equation will be 'out of the window.' This is bad because this equation is critical for analyzing molecules in an electrophoretic field etc.

### Analysis of f:

If we know  $f$ ,  $M$  and  $\bar{v}_p$ , we can draw conclusions about the MM shape and hydration:

$$\left( \frac{f_{\text{exp}}}{f_o} \right) = \left( \frac{6\pi\eta_{\text{solvent}} r_h F}{6\pi\eta_{\text{solvent}} r_o} \right)$$

We can simplify to get the frictional ratio:

$$\left( \frac{f_{\text{exp}}}{f_o} \right) = \left( \frac{r_h}{r_o} \right) \bullet F$$

→  $f_{\text{exp}}$  is from experimental data.

→  $f$  is based on calculation.

→  $\left( \frac{r_h}{r_o} \right)$  is always  $\geq 1$ . it depends on  $\delta_{\text{H}_2\text{O}}$ , but not on MM shape.

→  $F$  is always  $\geq 1$ . it depends on shape only.

### Two ways to Analyze (f/f<sub>o</sub>):

- 1) Calculate the upper bounds for  $(r_h/r_o)$  and for  $F$  of our MM of interest. This restricts our values to a certain range.

If we know  $\left( \frac{f}{f_o} \right)$  (from experimental data), then the possible value (consistent with our data) of  $\left( \frac{r_h}{r_o} \right)$  will be maximum when  $F$  is at its minimum (i.e.  $F=1$ ).

So when  $\left( \frac{r_h}{r_o} \right)$  is maximum:  $\left( \frac{f}{f_o} \right) = \left( \frac{r_h}{r_o} \right)$

And likewise when  $F$  is maximum:  $\left( \frac{f}{f_o} \right) = F$ .

- 2) Assume an average value for  $\delta_{\text{H}_2\text{O}}$ .  
( $\delta_w$  is typically  $\approx 0.35$  g/gMM and  $\bar{v}_p = 0.73 \text{ cm}^3 \text{ g}^{-1}$ )

So: we can calculate an estimate of  $\left( \frac{r_h}{r_o} \right)$  and then use this value in the

$$\text{equation} \equiv \left( \frac{f_{\text{exp}}}{f} \right) = \left( \frac{r_h}{r_o} \right) \bullet F \text{ to estimate a 'best-guess' F-value.}$$

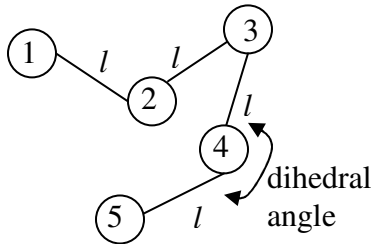
Remember when the Perrin factor (F) is found, we still don't know if the MM shape is oblate or prolate ellipsoid, so there are still two possibilities for shape (other than a value of 1.0). So you have to use information other than the hydrodynamics.

### **Model 2: 'Open, Flexible' Polymers:**

- Linear polymers of similar repeating units (monomers).
- No special interactions between different segments along the chain. (no secondary or tertiary structure).
  - for example denatured RNAs or proteins, and long double-stranded DNAs (these strands are floppy, not like the javelin mentioned earlier).

### **Model: Ideal Random Coil:**

- Infinitely flexible (floppy), and mass-less.



Shown above is a random walk in space. The distance between units is fixed, but there is no restraint upon the dihedral angle (it is completely random).

The prediction is that  $f$  proportional to  $M^{0.5}$ .