Why study hydrodynamical methods?

The methods we study in this section are “low resolution” ones – one studies diffusional motion and friction to learn about:

1. molecular mass (and hence, aggregation)
2. molecular charge (for separation, or as a function of pH)
3. broad features of molecular shape (especially useful for very anisotropic systems, like DNA, collagen, filaments, etc.)
4. rigidity and flexibility (again, at low resolution)

There are two main classes of these methods: driven methods, that apply an external force (gravity, electric field) to the system, and usually come to a steady-state environment, and diffusion or Brownian motion methods, that measure the fluctuations that naturally occur (by thermal motion) in an unforced system at equilibrium.
Life at low Reynolds number

Even “macro” molecules are really quite small, and would ordinarily move very quickly at room temperature, if the were not constantly being buffeted about by the aqueous environment. Because of the viscosity of the environment, inertial forces are not very important in their motion: as Purcell says (p. 256 of your text):

“[at very low Reynolds number] what You are doing at the moment is entirely determined by the forces that are exerted on You at the moment, and by nothing in the past”

The relative effect of viscosity is represented in the Reynolds number $R = \frac{\rho \, ul}{\eta}$. For example, for a virus of diameter $5 \times 10^{-6}$ cm, in water ($\rho = 1$ gm cm$^{-3}$), with viscosity $\eta = 0.01$ poise $= 10^{-2}$ gm cm$^{-1}$s$^{-1}$, you get a Reynolds number of $5 \times 10^{-7}$. (See other examples on p. 256.)
Consider a particle moving with velocity $\mathbf{v}$ under the influence of some external force $\mathbf{F}$ (say a gravitational or electrostatic external force). In addition to the external force, there is a viscous drag inhibiting the motion, which is proportional to the velocity:

$$\mathbf{F} - f \mathbf{v} = m(\frac{d\mathbf{v}}{dt})$$

If the velocity at time 0, $\mathbf{v}_0$ is parallel to the applied force $\mathbf{F}$ (here assumed to be constant), then the linear differential equation is easy to solve:

$$\mathbf{v}(t) = \left(\frac{\mathbf{F}}{f}\right) + \left[\mathbf{v}_0 - \left(\frac{\mathbf{F}}{f}\right)\right]e^{-ft/m}$$

The velocity decays (quickly, if you plug in numbers) to a final value $\mathbf{F}/f$ which is linear in the applied force.
The frictional drag must depend on particle size, and on the viscosity of the medium (typically water, which has a viscosity $\eta$ of about 0.01 poise $= 0.01 \, \text{g cm}^{-1} \, \text{s}^{-1}$). Dimensional analysis can help here: suppose for a sphere of radius $r$:

$$f \propto \eta^x r^y$$

Now $f$ has units of g s$^{-1}$. The only possible values for $x$ and $y$ are 1, so that $f \propto \eta r$. For a sphere, where the molecules of the solvent “stick” to the surface, messy algebra can find Stokes law:

$$f_{\text{sph}} = 6\pi \eta r$$

For reference later on, the corresponding friction that retards rotation of a sphere of volume $V = 4\pi r^3 / 3$ is:

$$f_{\text{sph,rot}} = 6\eta V$$
It is common to compare the frictional coefficients of other simple shapes to that of a sphere of the same volume:

Table 1: Translational and rotational frictional coefficients of ellipsoids and cylindrical rods relative to spheres of the same volume [7–11]

<table>
<thead>
<tr>
<th></th>
<th>Prolate ellipsoid</th>
<th>Oblate ellipsoid</th>
<th>Cylinder</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R_e )</td>
<td>((ab^2)^{1/3})</td>
<td>((ab^2)^{1/3})</td>
<td>((3/2p^2)^{1/3}(L/2))</td>
</tr>
<tr>
<td>( F_t )</td>
<td>(\frac{\sqrt{1-q^2}}{q^{2/3}\ln(1+\sqrt{1-q^2})})</td>
<td>(\frac{\sqrt{q^2-1}}{q^{2/3}\arctan\sqrt{q^2-1}})</td>
<td>(\frac{(2p^2/3)^{1/3}}{\ln p+\gamma}, \gamma = 0.312 + \frac{0.565}{p} + \frac{0.100}{p^2})</td>
</tr>
<tr>
<td>( F_t(a) )</td>
<td>(\frac{4(1-q^2)}{3(2-2q^{4/3}/F_t)})</td>
<td>(\frac{4(1-q^2)}{3(2-2q^{4/3}/F_t)})</td>
<td>0.64 (1 + \frac{0.677}{p} - \frac{0.183}{p^2})</td>
</tr>
<tr>
<td>( F_t(b) )</td>
<td>(\frac{4(1-q^4)}{3q^2[2q^{-2/3}(2-q^2)/F_t-2]})</td>
<td>(\frac{4(1-q^4)}{3q^2[2q^{-2/3}(2-q^2)/F_t-2]})</td>
<td>(\frac{2p^2}{9(\ln p+\delta)}, \delta = -0.662 + \frac{0.917}{p} - \frac{0.050}{p^2})</td>
</tr>
</tbody>
</table>

Note: macromolecules always show a little more friction than they “should”, based on their size and shape. This is because (roughly speaking!) they drag along a certain amount of water with them (a “hydration layer”). See pp. 257-258 and 327-328 of your text.
Table 2: Translational and rotational friction coefficients for arrays of \( n \) identical spheres in the indicated geometries, relative to \( f_t \) and \( f_r \) for the spherical monomer. 33 corresponds to rotation around the axis of highest symmetry, and 11 is for either axis perpendicular to it. [13]

<table>
<thead>
<tr>
<th>( n )</th>
<th>Geometry</th>
<th>( F_t )</th>
<th>( F_r(11) )</th>
<th>( F_r(33) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sphere</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>2</td>
<td>Dimer</td>
<td>1.38</td>
<td>3.79</td>
<td>1.77</td>
</tr>
<tr>
<td>3</td>
<td>Triangle</td>
<td>1.61</td>
<td>4.24</td>
<td>5.71</td>
</tr>
<tr>
<td>3</td>
<td>Linear</td>
<td>1.71</td>
<td>9.26</td>
<td>2.52</td>
</tr>
<tr>
<td>4</td>
<td>Square</td>
<td>1.82</td>
<td>6.29</td>
<td>8.40</td>
</tr>
<tr>
<td>4</td>
<td>Tetrahedron</td>
<td>1.77</td>
<td>6.06</td>
<td>6.06</td>
</tr>
<tr>
<td>4</td>
<td>Linear</td>
<td>2.00</td>
<td>17.86</td>
<td>3.27</td>
</tr>
<tr>
<td>5</td>
<td>Pentagon</td>
<td>2.04</td>
<td>9.01</td>
<td>11.90</td>
</tr>
<tr>
<td>5</td>
<td>Bipyramid</td>
<td>1.92</td>
<td>8.47</td>
<td>6.41</td>
</tr>
<tr>
<td>6</td>
<td>Hexagon</td>
<td>2.25</td>
<td>12.35</td>
<td>16.39</td>
</tr>
<tr>
<td>6</td>
<td>Octahedron</td>
<td>2.02</td>
<td>8.70</td>
<td>8.70</td>
</tr>
<tr>
<td>6</td>
<td>Trigonal prism</td>
<td>2.07</td>
<td>10.00</td>
<td>9.17</td>
</tr>
<tr>
<td>8</td>
<td>Cube</td>
<td>2.31</td>
<td>13.33</td>
<td>13.33</td>
</tr>
</tbody>
</table>
The centrifugal force on the particles is $\omega^2 x$ per unit mass, where $\omega$ is the rotor speed and $x$ is the distance from the center of the rotor. The terminal velocity should equal this force divided by the friction coefficient. It is typical in the literature to report the Svedberg coefficient $s = v/(\omega^2 x)$, which has units of $s^{-1}$. Measurements are reported in Svedberg units, where 1S=10$^{-13}$ s.

In simple terms, you can just measure the velocity, and compare $s$ of the sample to that for standards of known molecular weight. However, realistically, the particles in the sample cell are experiencing both driven motion (because of the rotor speed) and diffusion (natural spreading because of concentration gradients. We need to examine diffusion first, then come back to the centrifuge problem later.
Fick’s first and second laws of diffusion

(Definition The flux $J$ is the mass transported across a boundary per second, divided by the area of the boundary. In the figure, the mass in the little box is $cA\,dx$ (concentration times volume). Flux $J$ is this mass divided by $A\,dt$; hence $J = c(dx/dt) = cv$, where $v$ is the velocity of the flow.)

Consider the tiny volume shown in the figure. The rate of mass transport from left to right through the zone must be proportional to the concentration at the left, $c(x)$, and inversely proportional to the thickness of the zone, $dx$. Transport in the opposite direction is proportional to $c(x+dx)/dx$. The net rate is given by Fick’s first law of diffusion:

$$J = \frac{[Dc(x) - Dc(x+dx)]}{dx} = -D(\partial c/\partial x)_t \quad (1)$$
Now, consider the change in mass inside the volume. This is just
\[ \frac{dm}{dt} = J(x) - J(x + dx). \]
In terms of concentrations, this is
\[ \frac{dc}{dt} = \frac{1}{V} \left( \frac{dm}{dt} \right) = \left( \frac{1}{dx} \right) \frac{dm}{dt}, \]
where we have taken \( A \) to be a unit area. Combining these:

\[ \left( \frac{dc}{dt} \right)_x = \left[ J(x) - J(x + dx) \right] / dx = - \left( \frac{\partial J}{\partial x} \right)_t \]  \hspace{1cm} (2)

Combining with Fick’s first law, we get Fick’s second law of diffusion:

\[ \left( \frac{dc}{dt} \right)_x = - \partial \left( -D \left( \frac{\partial c}{\partial x} \right) / dx \right) = D \left( \frac{\partial^2 c}{\partial x^2} \right)_t \]  \hspace{1cm} (3)
Solution to the diffusion equations

If you start with a spike (Dirac delta function) of material, it will spread by diffusion according to $c(x, t) = W_0 \exp\left(-\frac{x^2}{4Dt}\right)/\sqrt{4\pi Dt}$, where $W_0$ is the total mass.
Diffusion in the presence of an external force

Remember that a low Reynolds number, and accelerations caused by external forces are short-lived, and one quickly comes to a terminal velocity $v = F/f$, where $F$ is the external force and $f$ is the friction coefficient. In the presence of both an external force and diffusion (Fick’s first law):

$$J = -D \left( \frac{\partial c}{\partial x} \right) + \frac{cF}{f}$$  \hspace{1cm} (4)

By virtue of Eq. 2, we get the Smoluchowski equation:

$$\left( \frac{\partial c}{\partial t} \right) = D \left( \frac{\partial^2 c}{\partial x^2} \right) - \left( \frac{F}{f} \right) \left( \frac{\partial c}{\partial x} \right)$$  \hspace{1cm} (5)

Now, suppose you come to equilibrium ($J = 0$) where diffusion matches the external force (sedimentation equilibrium, say). Here gravity (or centrifugal force) is pulling particles down, creating a concentration gradient (higher concentration at the bottom). But diffusion works in the opposite direction, trying to equalize concentrations, and hence pulling particles up.
The Einstein-Smoluchowski relation

Rearranging Eq. 4 with $J = 0$ gives

$$D \frac{dc}{c} = \frac{F}{f} dx$$

Integrate both sides of this equation from $x = 0$ (top of the beaker) to position $x$, and set $w = -\int F dx$ where $w$ is the (reversible) work, minus sign because gravity pulls downward. Then

$$D \ln \frac{c(x)}{c(0)} = \frac{-w}{f}, \text{ or } c(x) = c(0) \exp\left(-\frac{w(x)}{fD}\right)$$

But at equilibrium we must also have the Boltzmann distribution law, so $fD = kT$, or $D = kT/f$, which is the Einstein-Smoluchowski equation. It is an example of a fluctuation-dissipation relation, connecting fluctuations ($D$) with friction, (dissipation, $f$).
Tumor necrosis factor alpha (TNF) was the first known member of a family of signaling molecules involved in inflammation, apoptosis, and many other important functions. A hallmark of this family is that these proteins normally occur as trimers in solution. A potential new member of this family was identified on the basis of sequence homology. However, when it was expressed in E. coli and refolded from inclusion bodies, it appeared to be a monomer based on its elution relative to standards on size-exclusion chromatography (SEC). Did this mean it was not truly a member of this family, or simply that it was not correctly refolded, or was the mass estimate from SEC wrong?

The graph to the right shows some sedimentation equilibrium data for this molecule, showing the concentration as a function of position within the cell as monitored by absorbance at 230 nm. Note that the total amount of protein for this experiment was <10 micrograms.
This next graph shows that data re-plotted as the natural log of absorbance vs. \( \text{radius}^2/2 \). In this type of plot a single species gives a straight line whose slope is proportional to mass. The theoretical slope calculated for the monomer mass (\(~17\ \text{kDa}\)). The dark blue line (mostly hidden behind the data points) has the theoretical slope for the trimer mass. This plot therefore makes it obvious that this protein is indeed a trimer, and therefore it is indeed a homolog of TNF (and presumably is correctly folded).
Measuring binding affinities

The function of many proteins is to bind to other proteins, and sedimentation equilibrium is a very powerful tool for studying such binding interactions. The graph at the right summarizes the data (points) and fitted curves for 8 experiments on mixtures of a monoclonal antibody and its ~25 kDa protein antigen. The data sets cover experiments at different mixing ratios of antibody to antigen, and cover a wide range of concentrations. (Note that this entire set of experiments used only ~80 micrograms of antibody.)

To analyze these data an appropriate binding model is needed. The model shown to the right is the simplest one possible for an antibody with two binding sites, and simply assumes that both sites have the same binding affinity and bind independently of one another (no cooperativity and no steric blocking of one site by antigen bound to the other). In fitting these data one is essentially asking: Is there a single value of the dissociation constant that can explain all 8 experiments? The solid lines in the graph above represent the best fit of this model, and the fact that the lines follow the data points quite well shows that this is a good fit.
Measuring sedimentation velocities

In the centrifuge we have both a flux driven by the centrifugal force $J = vc = \omega^2 x sc$, and a diffusive flux caused by concentration gradients that may be present:

$$J = \omega^2 x sc - D(\partial c / \partial x)$$

Now the change in mass in a small slice of the rotor is $dm/dt = JA(x) - JA(x + dx)$, where the cross section $A = ax\phi$. The concentration change is the mass change divided by the volume between the surfaces:

$$dc/dt = [1/(\phi x dx)] \{JA(x) - JA(x + dx)\}$$

$$= (-1/\phi xa) (dJA/dx) = -(1/x) (d(xJ)/dx)$$

Combining these two equations yields the Lamm equation for the ultracentrifuge:

$$dc/dt = D \left[ (d^2 c/dx^2) + (1/x)(dc/dx) \right] - s\omega^2 [x(dc/dx) + 2c]$$

(Note: we have ignored buoyancy, which opposes the centrifugal force; see pp. 354-355.)
Sample data one would fit to the Lamm equation

The graph shows scans across the centrifuge cell, recording the absorbance at 280 nm versus position within the cell. These scans were taken starting at 13 minutes after initiating a run at 45,000 rpm (the black data set in the graph), and then every ~12 minutes thereafter (blue, green, cyan, etc.). In the first data set the sedimentation of the antibody has already depleted its concentration at the left and formed a sedimentation boundary.

At later times in the run the depleted region expands and the boundary moves away from the center of the rotor, until by the time of the last data set the concentration of antibody has dropped to essentially zero throughout the upper half of the cell.
Some common data manipulations

What we often want to know is how much material is sedimenting at various sedimentation coefficients. By taking many scans close together in time, subtracting them in pairs, and doing some mathematical manipulation these data can be transformed into the sedimentation coefficient distribution, $g(s^*)$, which is shown at the right.

This distribution resembles a chromatogram, and the area under each peak gives the total amount of that species. For this antibody sample we see only one distinct peak, centered at a sedimentation coefficient of $\sim 6.5$ S, which corresponds to the native antibody 'monomer'. A sedimentation coefficient of 6.5 S is actually rather low for a 150 kDa species, which is consistent with high hydrodynamic friction from its highly asymmetric, non-globular 'Y' shape. The red curve is a fit of these data as a single species. This fit clearly fails to account for the data over the region from 8-12 S, indicating the presence of some dimer and possibly also some trimer.
Still more data analysis
A microscopic picture of friction and diffusion

We can write a microscopic description of a macromolecule encountering collisions with an (implicit) solvent as a Langevin equation:

\[
m \frac{d^2 x}{dt^2} = - \left( \frac{\partial V}{\partial x} \right) - f \left( \frac{dx}{dt} \right) + R(t)
\]  

The “random” force somehow has to be determined. It’s clear that \( \langle R(t) \rangle = 0 \), but beyond that, we will keep it undetermined right now. Let’s see how quickly velocity fluctuations decay, starting with a free particle, \( V = 0 \), where the Langevin equation is \( m(dv/dt) = -fv + R(t) \). Multiplying by \( v(0) \) and averaging over many collisions:

\[
m \left\langle v(0) \frac{dv(t)}{dt} \right\rangle = -f \left\langle v(0)v(t) \right\rangle + \left\langle v(0)R(t) \right\rangle
\]

The final term vanishes, so

\[
\frac{d}{dt} \left\langle v(0)v(t) \right\rangle = -\left( \frac{f}{m} \right) \left\langle v(0)v(t) \right\rangle \Rightarrow \left\langle v(0)v(t) \right\rangle = \left\langle v^2(0) \right\rangle e^{-ft/m}
\]
Kubo-Green relations

The mean KE, $1/2m\langle v^2 \rangle$ is given by $1/2kT$. Now integrate over all times $t$:

$$\int_0^\infty \langle v(0)v(t) \rangle \, dt = \frac{kT}{m} \int_0^\infty e^{-ft/m} \, dt = \frac{kT}{f} = D$$

This is a Green-Kubo relation, connecting an equilibrium time-correlation function to a transport coefficient. A similar relationship is

$$\int_0^\infty \langle R(0)R(t) \rangle \, dt = 2kTf \approx \langle R^2(0) \rangle$$