Can You Get Shape Information From Dynamic Light Scattering?

Yes, you can get shape information from dynamic light scattering (DLS). However, extraction of this information from the DLS measurement, requires some apriori information. By definition, the DLS measured hydrodynamic radius is the radius of a hypothetical hard sphere that diffuses at the same rate as the particle under examination. This definition is somewhat problematic with regard to visualization however, since hypothetical hard spheres are non-existent. In practice, macromolecules in solution are non-spherical, dynamic (tumbling), and solvated. As such, the radius calculated from the diffusional properties of the particle is indicative of the *apparent* size of the dynamic hydrated/solvated particle. Hence the terminology, 'hydrodynamic' radius.

If the mass (or molecular weight) and the partial specific volume (inverse density) for the particle being measured are known, a mass equivalent spherical size can be calculated. The closer the particle is to being spherical, the closer the mass equivalent spherical radius will be to the DLS measured hydrodynamic radius. In fact, it is the difference in these two values that allows us to extract particle shape information.

Let us consider lysozyme for example. From the crystallographic structure shown in Figure 1, lysozyme can be described as a 26 x 45 Å ellipsoid with an axial ratio of 1.73. The molecular weight of the protein is 14.7 kDa, with a partial specific volume or inverse density of 0.73 mL/g. The mass equivalent spherical radius (R_M) can be calculated using the expression shown below, where M_w is the molecular weight, V bar is the partial specific volume, and N_A is Avogadros number. For lysozyme, R_M = 1.62 nm, which is roughly 15% smaller than the measured hydrodynamic radius of 1.9 nm.

$$R_M = \left(\frac{3M_w \overline{V}}{4\pi N_A} \right)^{\frac{1}{3}} = 1.62 \text{ nm}$$

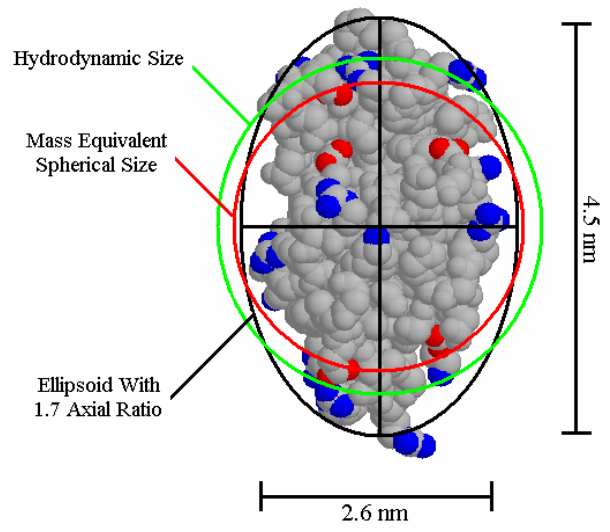


Figure 1: Space filling model of lysozyme showing representative hydrodynamic and mass equivalent spherical sizes, along with an ellipsoid having an axial ratio consistent with the structural dimensions.

The frictional coefficient for the mass equivalent hard sphere (f_M) can be calculated as shown below, where η is the medium viscosity. The frictional coefficient for the hydrodynamic sphere (f_H), can be calculated in a similar fashion, where the subtraction of the solvent layer thickness is integrated to account for hydration effects.

$$f_M = 6\pi\eta R_M = 6\pi\eta\left(\frac{3M_w\overline{V}}{4\pi N_A}\right)^{\frac{1}{3}}$$

$$f_H = 6\pi\eta\left(R_H - \text{Solvent Layer}\right)$$

The Perrin or shape factor (F) is defined as the ratio of the measured frictional coefficient to that of the mass equivalent sphere. After subtracting the thickness of a single layer of solvent (SL = 0.24 nm) from the measured hydrodynamic radius, the Perrin factor for lysozyme is calculated as 1.02.

$$F = \frac{f_H}{f_M} = \left(\frac{4\pi N_A}{3M_w\overline{V}}\right)^{\frac{1}{3}}\left(R_H - SL\right) = \frac{\left(R_H - SL\right)}{R_M} = \frac{1.90 - 0.25}{1.62} = 1.02$$

The Perrin factor can then be used to calculate the axial ratio for ellipsoidal shaped particles. Graphical representations of the Perrin equations for prolate and oblate ellipsoids are shown in Figure 2. For the lysozyme example, a Perrin factor of 1.02 is consistent with an oblate ellipsoid axial ratio of 1.62, which is virtually identical to the axial ratio calculated using structural dimensions (see Figure 1).

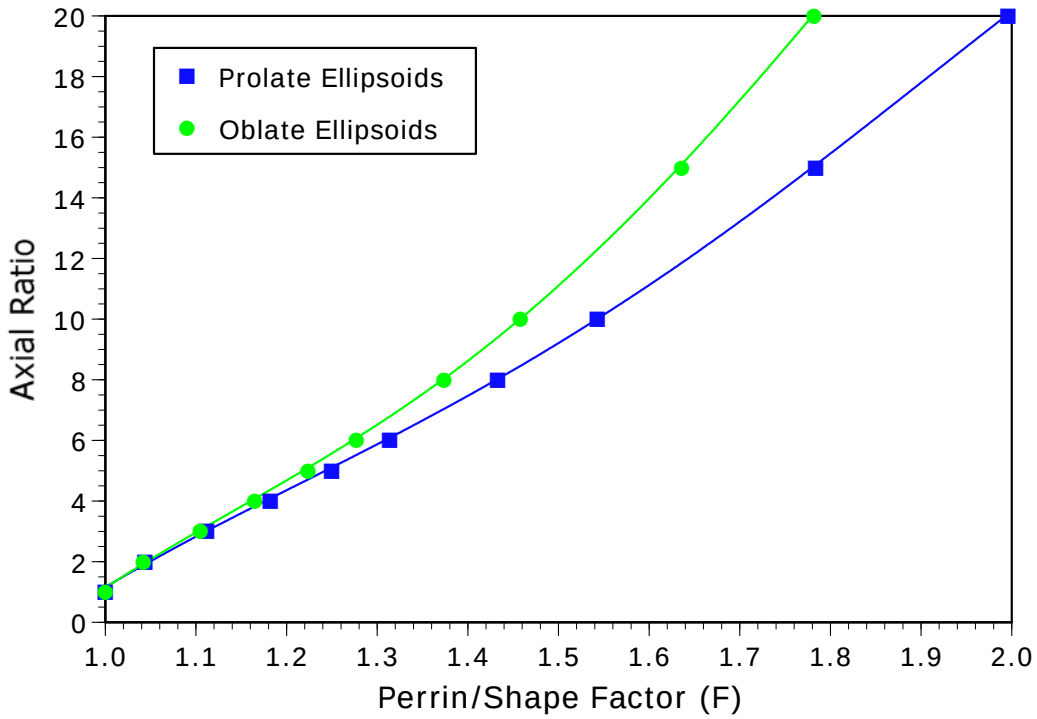
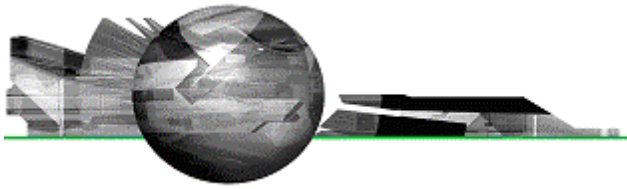


Figure 2: Graphical representations of the Perrin equations for prolate and oblate ellipsoids.



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