Quiz 8
Polymer Properties
March 10, 2016

This week we discussed the Hydrodynamic radius and the Ornstein-Zernike Function.

1) Wilkins et al. (*Biochemistry* **38** 16424 (1999)) determined the hydrodynamic radius for a variety of proteins using pulse field gradient NMR. In this measurement two timed magnetic pulses allow the determination of the number of molecules that move a certain distance in a magnetic field gradient during the time between the pulses. Dioxane is used as the probe molecule and the viscosity of a protein solution is determined by the translational motion of dioxane by diffusion. This viscosity is converted to R_h using the Stokes Einstein relationship. Suffice it to say that this is a rather indirect way to measure R_h, possibly subject to unexpected mitigating factors. Figure 3 shows the dependence of R_h, measured in this way, on N (molecular weight in terms of number of residues or monomers). The lower curve is for folded proteins (slope close to 1/3) and the upper curve is for unfolded proteins (slope close to 3/5).

a) Are the observed slopes of R_h expected for an expanded coil polyelectrolyte and a globular native state protein? (Temperature is not specified or controlled in this study.)
b) Would you expect R_g to equal R_h for globular (folded) proteins? Why? Which is larger?
c) Would you expect R_g to equal R_h for expanded coil chains? Why? Which is larger?
d) Sketch R_g and R_h versus T for a polymer chain. Under what condition does $R_g = R_h$?
e) Give Kirkwood's equation for R_h and the expression for R_g for a polymer chain.
f) Apply the condition of part "e" and explain how R_g could equal R_h. Under what meaningless condition could R_g equal R_h? (For a Gaussian chain the Kirkwood expression is evaluated to be $R_h \sim (3/11)\, R_{ete}$.)
g) Figure 5 shows R_h (dashed line) and R_g (solid line and points) for good solvent protein chains. Do the relative values of R_g and R_h make sense. Do you expect R_h to show the same scaling as R_g in this plot? (Temperature is not controlled in this plot.)

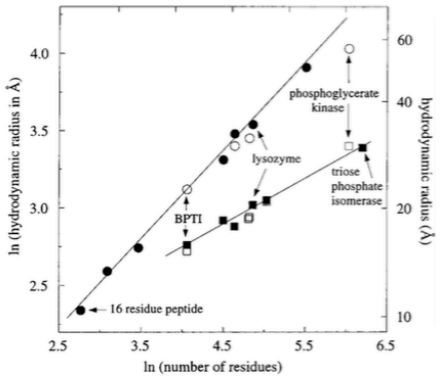

FIGURE 3: Plot of the $\log_e$ of the hydrodynamic radius versus the $\log_e$ of the number of residues in the polypeptide chain. The values determined in this work for native folded proteins (■) and highly denatured polypeptide chains (●) are shown with filled symbols. The line fitted to these data for the native proteins has a slope of 0.29 ± 0.02 and a *y*-axis intercept of 1.56 ± 0.1, while that fitted to the denatured protein data has a slope of 0.57 ± 0.02 and a *y*-axis intercept of 0.79 ± 0.07. Values of hydrodynamic radii reported in the literature from dynamic light scattering or PFG NMR studies are shown by open symbols (native folded proteins, □; highly denatured proteins, ○). The literature data used are for horse apocytochrome *c* (*62*), yeast phosphoglycerate kinase (*16, 63*), sperm whale apomyoglobin (*19*), bovine pancreatic ribonuclease A (*18*), bovine pancreatic trypsin inhibitor (*9*), and bovine α-lactalbumin (*23*). Selected data points are labeled with the protein name.

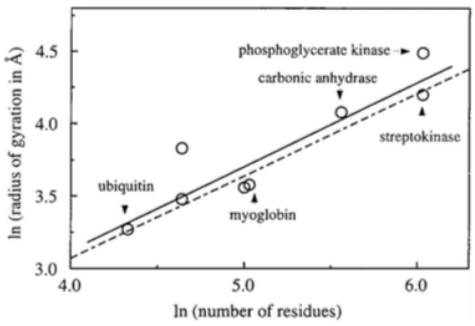

FIGURE 5: Plot of the $\log_e$ of the radius of gyration determined by SAXS or SANS versus the $\log_e$ of the number of residues in the polypeptide chain for proteins under strongly denaturing conditions. The solid line indicates the best fit to these data (slope 0.58 ± 0.11, *y*-axis intercept 0.80 ± 0.55) and the dashed line the fit for hydrodynamic radius data determined here by PFG NMR for highly denatured proteins (as shown in Figure 3). The data shown are for horse ferricytochrome *c* (*21, 62*), staphylococcal nuclease (*64*), horse myoglobin (*20*), bovine carbonic anhydrase B (*66*), *Streptococcus equisimilis* streptokinase (*16*), yeast phosphoglycerate kinase (*45*), and bovine ubiquitin (*68*). A number of the data points are labeled with the protein name.

2) The Ornstein-Zernike scattering function is widely used in simulations since there is a simple expression for the correlation function, $g(r) = 1/r \exp(-r/\xi)$ where ξ is the "correlation length". The Fourier transform of this correlation function yields $I(q) = G/(1+q^2\xi^2)$

a) By definition, $\gamma(r)$ at $r = 0$ is $\langle\rho^2\rangle V$, where $\langle\rho^2\rangle$ is the contrast and V is the particle volume. Similarly, $d\gamma(r)/dr$ at low r is related to the structural surface to volume ratio, $-S/(4V)$. Calculate these two values for the OZ function. Do these values make sense?

b) The OZ function is supposed to describe a completely random structural system. Should such a system produce a correlation function symmetric about 0? Explain your answer.

c) Compare the OZ scattering function with Guinier's Law using a low-q extrapolation and obtain an expression for R_g. Compare the OZ function with Debye scaling Bq^{-2} at high-q and obtain an expression for the scattering prefactor, B. How do these compare with those from the Debye scattering function for a polymer chain. ($B_{Debye} = 2G/R_g^2$).

ANSWERS: **Quiz 8**
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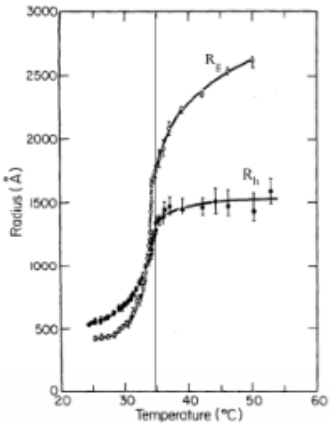
1) a) R_h is the radius of an equivalent sphere with the same drag coefficient as the structure. For the globular protein it is possible that R_h could reflect the globular size and therefore could scale with $N^{1/3}$. For the expanded coil the equivalent sphere size would not depend linearly on the molecular weight since a larger coil has a lower density, $\rho \sim N/V \sim N^{1-3/df} \sim N^{0.8}$ so larger coils have a lower density and would allow more penetration by a solvent compared to small coils. The scaling with N would be complex and not a single power-law relationship. It would change with N. The behavior is at odds with previous publications and with common sense. There is a strong temperature dependence of chain size. This doesn't seem to have any impact on scatter in this data set. Very odd.
b) R_h is larger than R_g for globular proteins. $R_g = \sqrt{(3/5)}\ R$ and $R_h = R$ for the radius of a sphere.
c) R_h is smaller than R_g for unfolded proteins. The degree of difference varies with temperature and molecular weight but should be large at high molecular weights and high temperatures.
d) They are equal just below the theta temperature, as the coil collapses.



e) $$\frac{1}{R_H} = \frac{1}{2N^2}\sum_{i=1}^{N}\sum_{j=1}^{N}\left\langle\frac{1}{|r_i - r_j|}\right\rangle \qquad R_g^2 = \frac{1}{2N^2}\sum_{n=1}^{N}\sum_{m=1}^{N}\left\langle(R_n - R_m)^2\right\rangle$$

f) For a Gaussian chain $R_h = 3/11\ R_{eted}$ and $R_g = \sqrt{6}\ R_{eted}$. So 0.27 and 2.4 or a ratio of 9. There is no stable state where $R_g = R_h$. It occurs as the coil is collapsing.
R_g could equal R_h if the number of monomers is 0, 1 or 2.
g) The plot shows R_h smaller than R_g, this makes sense. The ratio R_h/R_g should be a function of temperature, molecular weight, counter ion concentration, and other factors. It should not be constant for different proteins under randomly selected conditions. So the results are not expected. The R_g values are more believable than the R_h values. The scatter in R_g is expected.

2)
a) The function goes to $+\infty$ at r = 0 if approached from positive values and to $-\infty$ at r = 0 if approached from negative values of r. This means that it is the correlation function for an object that has infinite volume or infinitely negative volume.

We can find the surface area by expanding the exponential at small r, obtaining $d\gamma(r)/dr = 1/r - 1/\xi + r/(2\xi^2)$ - … at low r is the structural surface area, S/V is proportional to $-1/(2\xi^2)$. This doesn't have the correct sign or units.

b) The correlation function is calculated for random orientation of the vector "r". Therefore it is not possible to have an asymmetric correlation function.

c) At low q the denominator can be replaced by $\exp(q^2\xi^2)$. The inverse of this is $\exp(-q^2\xi^2)$. Comparison with Guinier's law yields $R_g = \sqrt{3}\,\xi$. At high q the function can be approximated by $1/(q^2\xi^2)$ so $B = G/\xi^2$. By comparing with the Debye scattering function expression for B it is found that $R_g = \sqrt{2}\,\xi$. The two limits for the OZ function do not result in a consistent value for ξ. The scattering function does not describe a polymer.