Origin Assignment 2 –Estimates of Hydrodynamic Radii from Dynamic Light Scattering Data.

Objective: Analyze experimental correlation functions of scattered light to determine the mean hydrodynamic radius and distribution width of a monomodal population of large unilamellar vesicles (LUVs).

Introduction: The time scales of fluctuations in scattered light from a sample can be used to estimate the diffusion coefficients, and from that the hydrodynamic radius (radii) of particles (or a collection of particles) in a liquid sample. As we discussed in class, particles that have refractive indices different from the solvent, scatter light. The light scattered intensity depends on the number of particles in the scattering volume (the volume of sample from which the scattered light is detected) and the (intrinsic) scattering strength of the particle. In a DLS experiment we are interested in scattering from particles that are typically much smaller than the wavelength of light, which exhibits Rayleigh scattering (see the Wikipedia page for an explanation). The scattering cross section of a particle depends, among other things, on the size and refractive index of the particle.

The correlation function is simplest to analyze for a simple sample, *e.g.* one that consists of a non-aggregating protein. The correlation function for light scattered from such a *monodisperse* solution of a macromolecule decays by a single exponential:

$g^2(\tau) = y_0 + y_1 * \exp[-2\Gamma\tau]$

where τ is the delay time and the decay rate, Γ, is related to the diffusion coefficient, D, by

$D = \Gamma/q^2$

The scattering vector, q, is given by

$$q = \frac{4\pi n_0}{\lambda_0} \sin\left(\frac{\theta}{2}\right)$$

From D, the hydrodynamic radius is estimated for the equivalent sphere by the Stokes equation.

Lipid vesicles (and most synthetic polymers) do not have a single molecular weight. Instead the samples are comprised of distribution of weights, and therefore a distribution of hydrodynamic radii. The analysis of these samples then requires an analysis of this underlying distribution. If it is well described by a single Gaussian distribution, then the method of cumulants can be used (Koppel, 1972). Such samples are *polydisperse* and *monomodal*, which is to say the distribution is characterized by a mean and a distribution width (variance).

$g^2(\tau) = y_0 + y_1 * \exp[-2\Gamma\tau] * (1 + \mu_2\tau^2/2)^2$

where μ_2 is the variance of the distribution, and all the other variables have the previous definitions.

Assignment Tasks

1. Login to the course website (http://people.umass.edu/rmweis/chem728/). Navigate to the assignments page.
2. Download the data in the Excel file: "Origin_Assign2_DLS_data.xls". The file has three data sets, one experimental correlation function for sonicated unilamellar vesicles (SUVs) and two sets obtained with large unilamellar vesicles (LUVs).
3. Open Origin. Copy and paste the SUV data set from the Excel file into an Origin worksheet. (Eventually, you will analyze all three sets.)
4. Plot the data as a *Scatter* plot.
5. Fit the Data to a single exponential decay with the nonlinear least squares 'fitting engine'. **cps**(x) = y_0 + y_1*exp[-G*x]
6. Make a note of the fit parameters (y_0, y_1, G).
7. Create a new column in the worksheet and use the fit parameters generate a column of values that is given by **cps**(x) = y_0 + y_1*exp[-G*x].
8. Create another new column in the worksheet, fill the column with residuals: residuals = **cps**(data) - **cps**(fit) and plot the residuals.
9. Repeat steps 5-8. Plot the data in a new graph window and fit the data to **cps**(x) = y_0 + y_1*exp[-G*x]*(1+(mu/2*x^2))^2 (where mu is an additional variable related to the width of the particle size distribution)
10. Plot the residuals generated with the two different functions in the same window. How do they compare? Is either fit satisfactory?
11. Compute the diffusion coefficient from the two different fits and the corresponding hydrodynamic radii. From the second fit, compute the distribution width. In these calculations, you will need to use the following parameters from the scattering experiment:

Scattering angle, $\theta = 90^o$
Wavelength of incident radiation, $\lambda_0 = 685$ nm
refractive index, $n_0 = 1.333$
viscosity, $\eta = 1.002$ cP (centiPoise)
Temperature = 293 K

12. Repeat these manipulations for the other two data sets (LUV50 and LUV100). What do learn from an analysis of all these data?