Origin Assignment 9 (Optional) – Due Tuesday, May 8, 2012, 5 PM

Fluorescence Resonance Energy Transfer In PolyProline

Stryer & Haugland (1976) published a widely cited article (974 citations) that established the use of FRET as a means to measure distances in biological systems. Using a series of proline oligopeptides with donor and acceptor chromophores attached to the ends of the peptide, the authors measured the percent transfer as a function of separation distance.

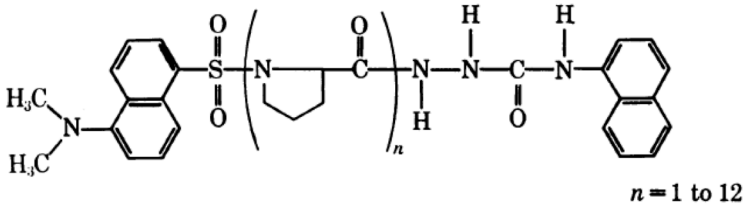

Figure 1. The polyproline oligopeptide series, *n* = 1 to 12, with the naphthalene donor at right and the dansyl acceptor at left.

Questions/Procedures - Show all your work for full credit.

(1) Stryer and Haugland use of FRET as a spectroscopic ruler required the synthesis of relatively well-defined rulers. Why was it important to use optical rotary dispersion (circular dichroism) to establish that the proline oligopeptides were type II helices? (Brief word answer, two to four sentences.)

(2) Stryer and Haugland measured the FRET efficiency for each of the oligoprolines (of which chromophore to chromophore distances were known by molecular modeling) to establish

(i) the r^{-6} relationship between transfer efficiency and separation distance

(ii) to test the equation for the Förster radius

$$r_0 = (9.79 \times 10^3)(\kappa^2 Q J n^{-4})^{1/6}$$

where

κ accounts for the orientation between chromophores. Rapid averaging gives a value of 2/3

Q is the quantum yield of donor fluorescence in the absence of acceptor (0.6 for naphthalene)

J is the overlap integral between the normalized donor fluorescence emission and the acceptor absorbance spectrum

n is the refractive index of the solvent (n = 1.4 for ethanol)

2.1 Use the transfer efficiency versus distance data in Origin to generate estimates of r_0 and the r^{-6} dependence as Stryer and Haugland did. Show all your work, it should lead you to the estimates Haugland & Stryer obtained. Note that Stryer and Haugland generated these estimates in fits where r_0 or r^{-n} were fit separately. In addition, conduct the analysis of the transfer efficiency versus distance where both r_0 and r^{-n} are adjustable parameters in the fit. Does this change the result significantly? (Three plots, three sets of fit results with written comments, ~ one paragraph.)

2.2 Estimate the value of r_0 from Förster theory, following to the method that Stryer and Haugland used, or according to some other procedure; just be careful of the units! (There are many treatments of the Förster theory that can be found on the web and in textbooks.)

$$r_0 = (9.79 \times 10^3)(\kappa^2 Q J n^{-4})^{1/6}$$

where the variables were defined above. Calculate *J* in Origin using the acceptor absorbance and donor emission spectra provided in 'Origin_Assign_9_FRET_Data.xlsx' at the course website. The overlap integral is given by

$$J = \int f_D(\lambda) \cdot \varepsilon_A(\lambda) \cdot \lambda^4 \, d\lambda$$

where
$f_D(\lambda)$ is the ***normalized*** fluorescence donor spectrum*
$\varepsilon_A(\lambda)$ is the absorbance spectrum, in units of molar extinction coefficient, of the acceptor
λ is the wavelength

Your calculation should yield the same result as Stryer and Haugland (1976) reported. Show all your work and provide a description of the procedure. (One plot, at least, step-by-step calculations, and a description of procedure.)

(3) Forty years later, Eaton and coworkers has revisited the analysis of oligoprolines using FRET as a 'spectroscopic ruler'. There are new developments. Compare Stryer and Haugland (1967) to Schuler et al (2005) and Best et al (2007). What are the significant new developments? Choose two to three specific points of comparison. Write one paragraph of discussion for each point of comparison. All the papers are posted at the website.

P20. Lubert Stryer & Richard P. Haugland. 1967. Energy transfer: A Spectroscopic Ruler. *Proc. Natl. Acad. Sci. USA* **58:**719-726.

P21. Benjamin Schuler, Everett A. Lipman, Peter J. Steinbach, Michael Kumke & William A. Eaton. 2005. Polyproline and the ''spectroscopic ruler'' revisited with single-molecule fluorescence. *Proc. Natl. Acad. Sci. USA* **102:**2754–2759.

P22. Robert B. Best, Kusai A. Merchant, Irina V. Gopich, Benjamin Schuler, Ad Bax & William A. Eaton. 2007. Effect of flexibility and cis residues in single-molecule FRET studies of polyproline. *Proc. Natl. Acad. Sci. USA* **104:**18964–18969.

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*Normalization of the integral is accomplished by integrating the fluorescence to obtain the area under the curve; then divide each fluorescence data point through by this area. Integration of the normalized fluorescence intensity data will yield the normalized integral (which equals one). The normalized intensities can be used in the equation for *J* given in the text. Alternately, the *J* can be calculated according to

$$J(\lambda) = \frac{\int_0^\infty f_D(\lambda) \cdot \varepsilon_A(\lambda) \cdot \lambda^4 \, d\lambda}{\int_0^\infty f_D(\lambda) \, d\lambda}$$

Note that the limits of the integral are chosen simply to account for the relevant nonzero values of $f_D(\lambda)$ and $\varepsilon_A(\lambda)$. In practice the limits are greater than 0 and less than ∞.