Appendix 2 –

Calculation of R_0 from Spectral Overlap with MatLab®

This routine was written by Joshua Vaughan, Fall 2000.

```matlab
% This program will calculate the quantity Ro for FRET between given
% donor and acceptor molecules. The user will need to supply a file
% containing the fluorescence spectrum of the donor (two columns, the
% first with the wavelength range in nm, the second with the intensity
% of the fluorescence) and a file containing the absorption spectrum
% of the acceptor (also two columns, the first with the wavelength in
% nanometers, and the second with the absorption of the acceptor in
% molar decadic extinction coefficients).

% It is assumed that the user will have already cleaned up these spectra by
% performing any necessary baseline corrections, and by chopping the data,
% and including zeros in place of the chopped data. The user will also need
% to supply the refractive index of the solvent used, the quantum yield of
% the donor, and the parameter K (where K=k^2), which is usually 2/3.

% NOTE: since the absorbance spectrum is being interpolated at the set of
% fluorescence x-values, the absorbance spectrum must contain at least the
% same range of x-values that the fluorescence spectrum contains, even if
% it means adding some fake x values for which the corresponding y value
% is zero. One way to do this would be to use absorbance data that ranges
% from 500-800 nm, and fluorescence data that ranges from 532-790 nm.

q=.95;                       % Enter in the quantum yield of the donor
K=2/3;                       % Enter in the value K (usually 2/3)
n=1.361;

% Before Matlab can load your file, you must change the current directory to
% the directory containing your data (you can use unix commands to do this).

f=load('dcm_fl.txt');       % Load in the fluorescence spectrum
a=load('mg_abs.txt');       % Load in the absorption spectrum

% Interpolate the absorption curve at the set of fluorescence x values.
a1=[f(:,1) interp1(a(:,1),a(:,2),f(:,1))];

f1=[(1e7)*(f(:,1).^-1)   f(:,2)]; % Rewrite the x axis in wavenumbers
a2=[(1e7)*(a1(:,1).^-1) a1(:,2)]; % Rewrite the x axis in wavenumbers
A=sum(diff(f1(:,1)).*f1(2:end,2));% Calculate area of fluorescence curve
f2=[f1(:,1)  f1(:,2)/abs(A)];  % Unit normalize the fluorescence. Note that
                               % this means the fluorescence is now in units
                               % of reciprocal wavenumbers, or cm.

% Calculate the overlap function O.
O=[f2(:,1) (a2(:,2).*f2(:,2))./(f2(:,1).^4)];

% Calculate the overlap integral J.
J=sum(abs(diff(f2(:,1))).*O(2:end,2));

% Calculate Ro [cm].
  Ro=((8.8e-25)*(K*q/(n^4))*abs(J))^(1/6)
```