Supplementary Information

The derivations of the transfer efficiency equations

From the Förster equation:

$$k_{RET} = \frac{9\ln(10)\kappa^2\Phi_D}{128\pi^5 N_A \tau_D^0 n^4 R^6}\int_0^\infty I_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \qquad \text{(A)}$$

The efficiency of FRET is,

$$E = \frac{k_{RET}}{\tau_D^{0\,-1} + k_{RET}} \qquad \text{(B)}$$

If the separation of donor and acceptor are at the Förster critical distance, $R = R_0$, the transfer efficiency is 1/2. By combining equation A and B,

$$\frac{1}{2} = \frac{k_{RET}}{\tau_D^{0\,-1} + k_{RET}} = \frac{1}{\left(\tau_D^0 k_{RET}\right)^{-1} + 1} = \frac{1}{\left(\frac{9\ln(10)\kappa_{R=R0}^2\Phi_D}{128\pi^5 N_A n^4 R_0^6}\int_0^\infty I_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda\right)^{-1} + 1}$$

Therefore,

$$\frac{9\ln(10)\kappa_{R=R0}^2\Phi_D}{128\pi^5 N_A n^4 R_0^6}\int_0^\infty I_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda = 1$$

$$R_0^6 = \frac{9\ln(10)\kappa_{R=R0}^2\Phi_D}{128\pi^5 N_A n^4}\int_0^\infty I_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda$$

$$\frac{R_0^6}{\kappa_{R=R0}^2} = \frac{9\ln(10)\Phi_D}{128\pi^5 N_A n^4}\int_0^\infty I_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \qquad \text{(C)}$$

In equation C, $\kappa^2_{R=R0}$ is the orientation factors at the critical distance. By substituting equation C into equation A, we can get,

$$k_{RET} = \frac{\kappa^2 R_0^6}{\kappa_{R=R0}^2 \tau_D^0 R^6} \qquad \text{(D)}$$

Substituting equation D into equation B,

$$E = \frac{1}{\frac{\kappa^2_{R=R0} R^6}{\kappa^2 R_0^6} + 1}$$

If $\kappa^2_{R=R0}$ is taken as 2/3, the equation becomes,

$$E = \left(1 + \frac{2R^6}{3\kappa^2 \overline{R}_0^6}\right)^{-1} \qquad \text{(E)}$$

$\overline{R}_0$ means the Förster critical distance at which the transfer efficiency is 1/2 and the orientation factor, $\kappa^2_{R=R0}$, is 2/3.

This is how equation 6 was derivated. The equation C thus becomes equation 5

$$\overline{R}_0^6 = \frac{9\ln(10)(2/3)\Phi_D}{128\pi^5 N_A n^4} \int_0^\infty I_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \qquad \text{(F)}$$

If the orientation factor of donor and acceptor, κ^2, is isotropic and as 2/3, the transfer efficiency is,

$$E = \left(1 + \frac{R^6}{\overline{R}_0^6}\right)^{-1}$$

This is the most popular equation of transfer efficiency, but it is only valid when the motions of donor and acceptor are almost random.