Supplementary Information:

For a bimolecular complex such as PLB-SERCA, the FRET pair separation distance may be obtained from the fit parameter $FRET_{max}$ using the Förster relationship $R = (R_0)[(1/FRET_{max})-1)^{1/6}$ (1). Pentameric PLB, however, presents an example of a FRET complex with multiple donors and acceptors. Li et al (2) developed a model for FRET within a ring-shaped oligomer, specifically for application to PLB but of general utility. This model assumes random mixing of donor:acceptor ratio and a symmetric ring-shaped assembly of PLB subunits. For a given donor, each additional subunit (indexed by *j*) will add energy transfer rate k_j with acceptor probability P_a to all previous terms, but will not affect the rate of any terms with donor probability $(1-P_a)$, such that the donor fluorescence intensity decay of n-mer is given by

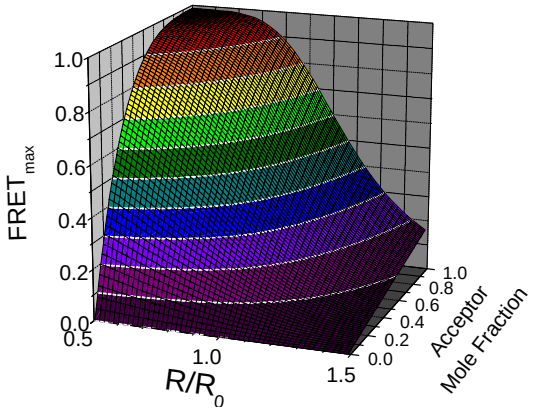
$$\frac{F(t)}{F(0)} = \exp(-k_D t)\prod_{j=2}^{n}\left[1 - p_a + p_a \exp(-k_j t)\right] \qquad \text{(Eqn. 1)}$$

where k_D is the decay rate of the donor alone. The term k_j is given by $k_j = k_D\left(r_j / R_0\right)^{-6} = k_D\left[\frac{R\sin(\frac{\pi(j-1)}{n})}{R_0\sin(\frac{\pi}{n})}\right]^{-6}$ where

R_0 is the Förster distance, r_j is the distance between the donor and the acceptor on subunit *j* in the *n*-mer ring and *R* is the distance between fluorophores on adjacent subunits. If oligomers are in equilibrium with a molar fraction *X* of monomers, and assuming that $E = 0$ for these monomeric donors, the observed steady-state energy transfer efficiency is given by

$$E_{OBS} = (1 - X)\{1 - \frac{F_{DA}(0)}{F_D(0)}\int_0^{\infty}\exp(-k_D t)\prod_{j=2}^{n}\left[1 - p_a + p_a \exp(-k_j t)\right]dk_D t\} \qquad \text{(Eqn. 2)}$$

A MatLab simulation that implements Eqn. 2 was used to analyze PLB-PLB FRET data, using a pentamer as the major oligomeric species. Since the measured $FRET_{max}$ value represents the intrinsic FRET of the complex, monomer fraction $X = 0$. The mole fraction acceptor is empirically determined from the starting fluorescence of YFP and the final fluorescence of CFP. This analysis yields the subunit distance R/R_0. Supplementary Fig. 1 is a 3D representation of the relationship plotted in Fig.1A.



Supplementary Fig. 1. Simulated dependence of FRET on probe separation and acceptor mole fraction.

1. Förster, T. (1948) *Ann. Phys. (Leipzig)* **2**, 55-75
2. Li, M., Reddy, L. G., Bennett, R., Silva, N. D., Jr., Jones, L. R., and Thomas, D. D. (1999) *Biophys J* **76**(5), 2587-2599