Supplementary Material for "The Effect of Membrane Lipid Composition Upon the Formation of Lipid Ultra-Nanodomains" by Pathak, P. and London, E.

Estimating the Effect of Membrane Domain Formation Upon Donor Quenching

In the presence of an acceptor, the concentration dependence of donor quenching in a homogeneous bilayer can be approximated by (Chattopadhyay and London (1987) Biochemistry 26, 39-45):

$$(1)\quad F/Fo = \exp(-1.21\pi R_o^2 C_a)$$

Where C_a is the acceptor concentration in acceptors/area. This expression is strictly valid for a process with very close to an all-or-none distance dependence, and is a good approximation to FRET quenching of donor fluorescence by acceptor within a few percent (Chattopadhyay and London (1987) Biochemistry 26, 39-45). The donor fluorescence in a domain- containing membrane sample is the sum of the donor fluorescence in each domain. For a probe with fluorescence that is not dependent upon lipid phase, fluorescence will be given by:

$$(2)\quad F/Fo = fr.\ D\ Ld\ [\exp(-1.21\pi R_o^2 C_{a\ Ld})] + fr.\ D\ Lo[\exp(-1.21\pi R_o^2 C_{a\ Lo})]$$

Where fr. D Ld is the fraction of the donor in the Ld state, and fr. D Lo is the fraction of the donor in the Lo state, C_{Ld} is the concentration of the acceptor in the Ld domains, and C_{Lo} is the concentration of the acceptor in the Lo domains. The relationship between C_a, C_{Lo} and C_{Ld} is:

$$(3)\quad C_a = (fr.\ Ld)(C_{a\ Ld}) + (fr.\ Lo)(C_{a\ Lo})$$

Where fr. Ld and fr. Lo are the fractions of the bilayer in the Ld and Lo states, respectively. An analogous expression can be written for donor concentrations:

$$(4)\quad C_d = (fr.\ Ld)(C_{d\ Ld}) + (fr.\ Lo)(C_{d\ Lo})$$

The partition coefficients between Lo and Ld domains for acceptor and donor, respectively, are defined as:

$$(5)\quad Kp_a = C_{a\ Lo} / C_{a\ Ld}$$

$$(6)\quad Kp_d = C_{d\ Lo} / C_{d\ Ld}$$

Combining equations (3) and (5), and using the relationship that fr. Ld + fr. Lo = 1 yields the equations:

$$(7)\quad C_{a\ Ld} = C_a / [fr.Ld + (1\text{-}fr.\ Ld)(Kp_a)]$$

$$(8)\quad C_{a\ Lo} = [(Kp_a)(C_a)] / [fr.Ld + (1\text{-}fr.\ Ld)(Kp_a)]$$

Similarly:

(9) $C_{d\ Ld} = C_d /[fr.Ld+(1-fr.\ Ld)(Kp_d)]$

(10) $C_{d\ Lo} = [(Kp_{\ d})(C_d)]/[fr.Ld+(1-fr.\ Ld)(Kp_d)]$

The fraction of the donor in the Ld domains is given by:

(11) $fr.\ D_{Ld} = [(C_{d\ Ld})(fr.\ Ld)]/ [(C_{d\ Ld})(fr.\ Ld)+ (C_{d\ Lo})(fr.\ Lo)]$

Substitution of (9) and (10) into (11) gives:

(12) $fr.\ D_{Ld} = fr.\ Ld/ [(fr.\ Ld)+ (Kp_{\ d})(1-fr.\ Ld)]$

And thus:

(13) $fr.\ D_{Lo} = 1- fr.\ D_{Ld} = 1-[fr.\ Ld/ [(fr.\ Ld)+ (Kp_{\ d})(1-fr.\ Ld)]]$

Substituting (7),(8),(12), and (13) into equation (2) gives the final expression:

(14) $F/Fo = (fr.\ Ld/ [(fr.\ Ld)+ (Kp_{\ d})(1-fr.\ Ld)])\ (exp((-1.21\pi R_o^2 C_a)/[fr.Ld+(1-fr.\ Ld)(Kp_a))+ (1-[fr.\ Ld/ [(fr.\ Ld)+ (Kp_{\ d})(1-fr.\ Ld)])(exp((-1.21\pi R_o^2)([(Kp_{\ a})(C_a)]/[fr.Ld+(1-fr.\ Ld)(Kp_a)]))$

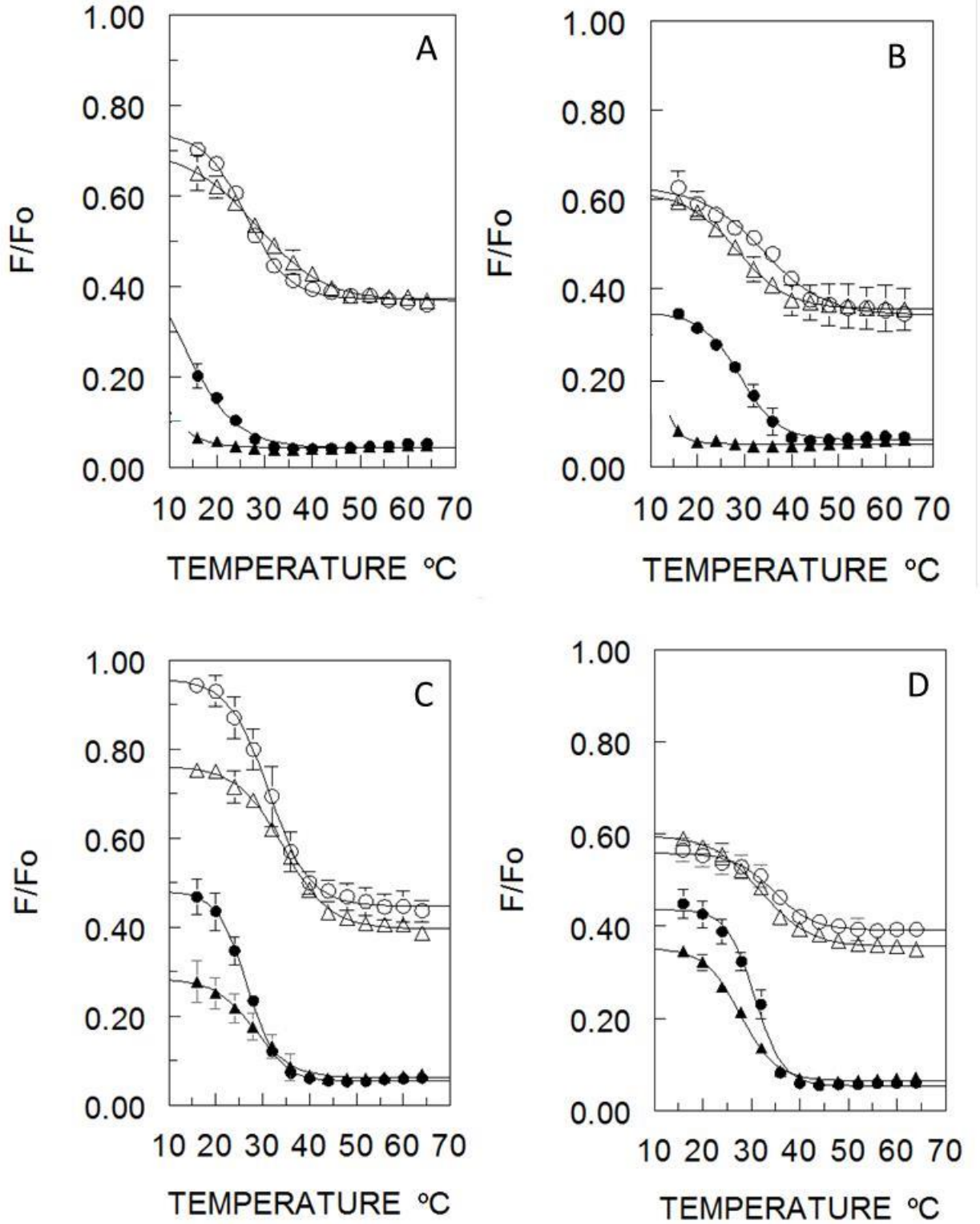
This gives the value of F/Fo as a function of the donor and acceptor partition coefficients, acceptor concentration, and the fraction of the bilayer in the Ld and Lo states.

Supplementary Figure 1: F/Fo data showing sigmoidal curves fit to data for calculation of T mid. A. bSM/POPC/chol, B. bSM/DOPC/chol, C. DPPC/POPC/chol, D. DPPC/DOPC/chol. Samples contained (triangle) 38 mol% or (circle) 28mol% cholesterol. Samples also contained (open symbols) 0.05 mol% pyrene-DPPE or (filled symbols) 0.1 mol% NBD-DPPE, and when acceptor was present 2 mol% rhodamine-DOPE. The sigmoidal fits were calculating using the SlideWrite program (Advanced Graphics Software Inc., Rancho Santa Fe, CA). In samples with NBD-DPPE, bSM, and either POPC or 38% cholesterol curves are very incomplete, and fits were calculated assuming that F/Fo reaches a limiting value of 0.5 at low temperature. In the case of bSM/DOPC/28mol% cholesterol this may have led to a slight underestimate of Tmid.

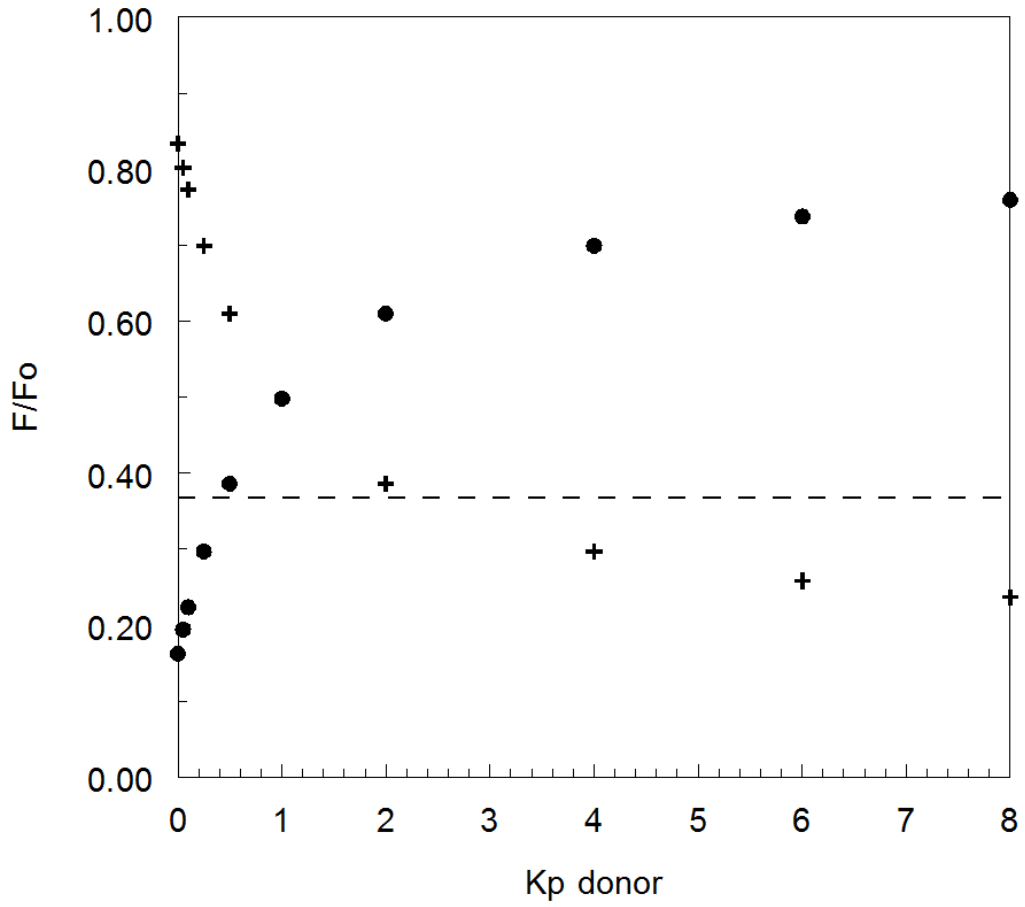
Supplementary Figure 2: Calculated FRET vs. donor partition coefficients in domain-containing membranes in which acceptor partitions strongly into Ld domains shown at intermediate acceptor concentration and/or intermediate Ro conditions. F/Fo is shown for acceptor Kp (Lo/Ld) values of 0.1 (circles) and 10 (plus symbols). Values chosen to approximate the behavior of the pyrene-DPPE, rhodamine-DOPE FRET pair in the experiments in this report (acceptor conc. $0.79/Ro^2$). F/Fo values calculated for a bilayer that is 50% liquid ordered domains. F/Fo values for homongeneous membranes lacking domains is shown by the dashed line.

Supplementary Figure 3: Calculated FRET vs. donor partition coefficients in domain-containing membranes in which acceptor partitions strongly into Ld domains shown at high acceptor concentration and/or large Ro conditions at different fractions of the bilayer in ordered domains. F/Fo is shown for acceptor Kp (Lo/Ld) values of 0.1 and (plus symbols) 25%, (squares) 50%, or (diamonds) 75% of the bilayer in Lo doamins. Values chosen to approximate the behavior of the NBD-DPPE, rhodamine-DOPE FRET pair in the experiments in this report (acceptor conc. $0.79/Ro^2$). F/Fo values for homongeneous membranes lacking domains is shown by the dashed line.

Supplementary Figure 1:



Supplementary Figure 2:



Supplementary Figure 3: