SUPPORTING INFORMATION

Membrane Fluidity Modulates Thermal Stability and Ligand Binding of Cytochrome P4503A4 in Lipid Nanodiscs

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Table S1. Optimal lipid:MSP1D1 ratios for mixed lipid nanodiscs.

System	Total Lipid:MSP1D1
0% DMPC	65.0
20% DMPC	59.0
50% DMPC	56.5
80% DMPC	70.0
100% DMPC	80.0

Table S2. Laurdan generalized polarization (G.P.) values for empty mixed lipid nanodiscs.

System	G.P.
0% DMPC	-0.055
25% DMPC	-0.004
50% DMPC	0.087
75% DMPC	0.165
100% DMPC	0.343

Table S3. Recovered calorimetric and van't Hoff enthalpies for deconvoluted thermograms.

System	T_m (°C)	ΔH_{cal} (cal/mol) [a]	ΔH_{vH} (cal/mol) [b]	$\Delta H_{vH}/\Delta H_{cal}$
0% DMPC	49.9	6.6×10^4	8.9×10^4	1.35
	53.9	5.8×10^4	1.4×10^5	2.41
	56.9	2.7×10^4	2.2×10^5	8.15
0% DMPC + KTZ[c]	51.6	6.6×10^4	7.9×10^4	1.20
	56.9	6.3×10^4	1.2×10^5	1.90
	60.5	6.5×10^4	2.0×10^5	3.08
	62.8	3.0×10^4	3.1×10^5	10.33
25% DMPC	50.2	8.2×10^4	8.7×10^4	1.06
	53.4	6.9×10^4	1.3×10^5	1.88
	56.4	4.9×10^4	1.7×10^5	3.47
25% DMPC + KTZ[c]	51.6	6.0×10^4	7.7×10^4	1.28
	56.7	6.9×10^4	1.3×10^5	1.88
	59.9	6.1×10^4	2.0×10^5	3.28
	62.0	2.8×10^4	2.9×10^5	10.36
50% DMPC	52.9	8.6×10^4	8.1×10^4	0.94
	57.4	9.4×10^4	1.4×10^5	1.49
	60.6	5.9×10^4	2.2×10^5	3.73
50% DMPC + KTZ[c]	51.0	7.5×10^4	8.2×10^4	1.09
	56.9	8.4×10^4	1.2×10^5	1.43
	60.2	7.0×10^4	1.9×10^5	2.71
	62.4	3.5×10^4	2.8×10^5	8.00
75% DMPC	50.4	6.6×10^4	1.1×10^5	1.67
	53.9	5.8×10^4	1.6×10^5	2.76
	56.7	2.8×10^4	2.2×10^5	7.86
75% DMPC + KTZ[c]	52.1	3.3×10^4	9.2×10^4	2.79
	56.6	5.0×10^4	1.4×10^5	2.80
	59.8	4.7×10^4	2.0×10^5	4.26
	62.0	2.9×10^4	2.7×10^5	9.31

[a] calorimetric enthalpy
[b] van't Hoff enthalpy
[c] 50 µM ketoconazole

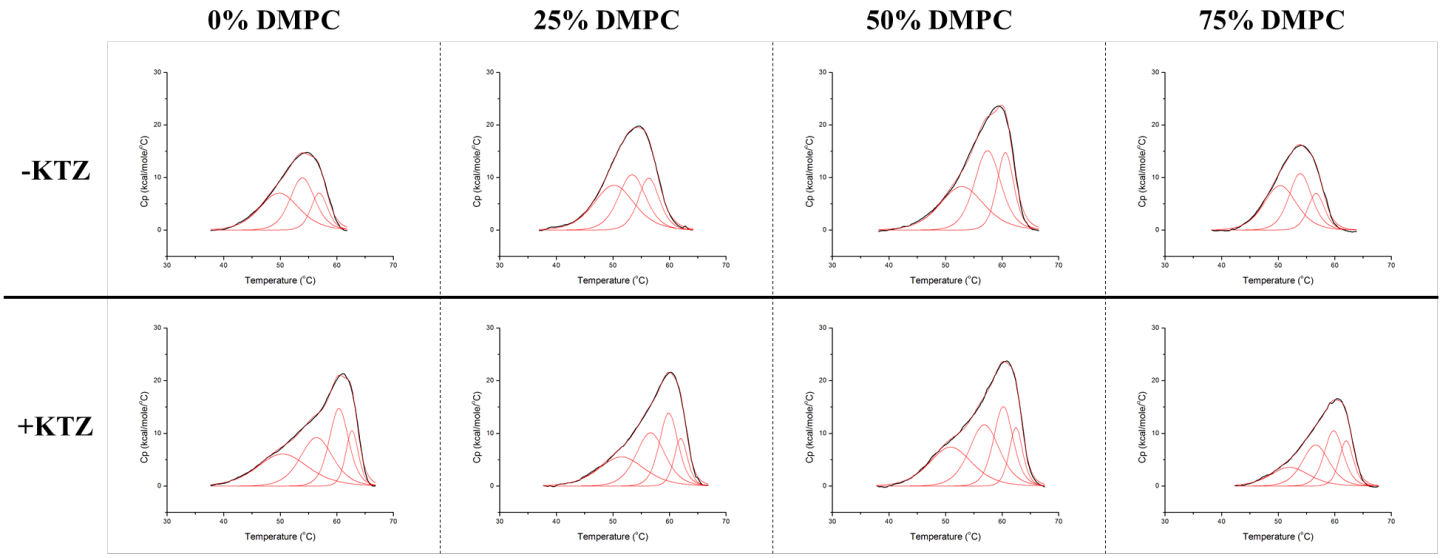

Figure S1. Deconvolution of CYP3A4 nanodisc thermograms containing varying lipid ratios, in the presence and absence of 5 μM KTZ. The recovered values for the deconvolutions are summarized in Table S3.

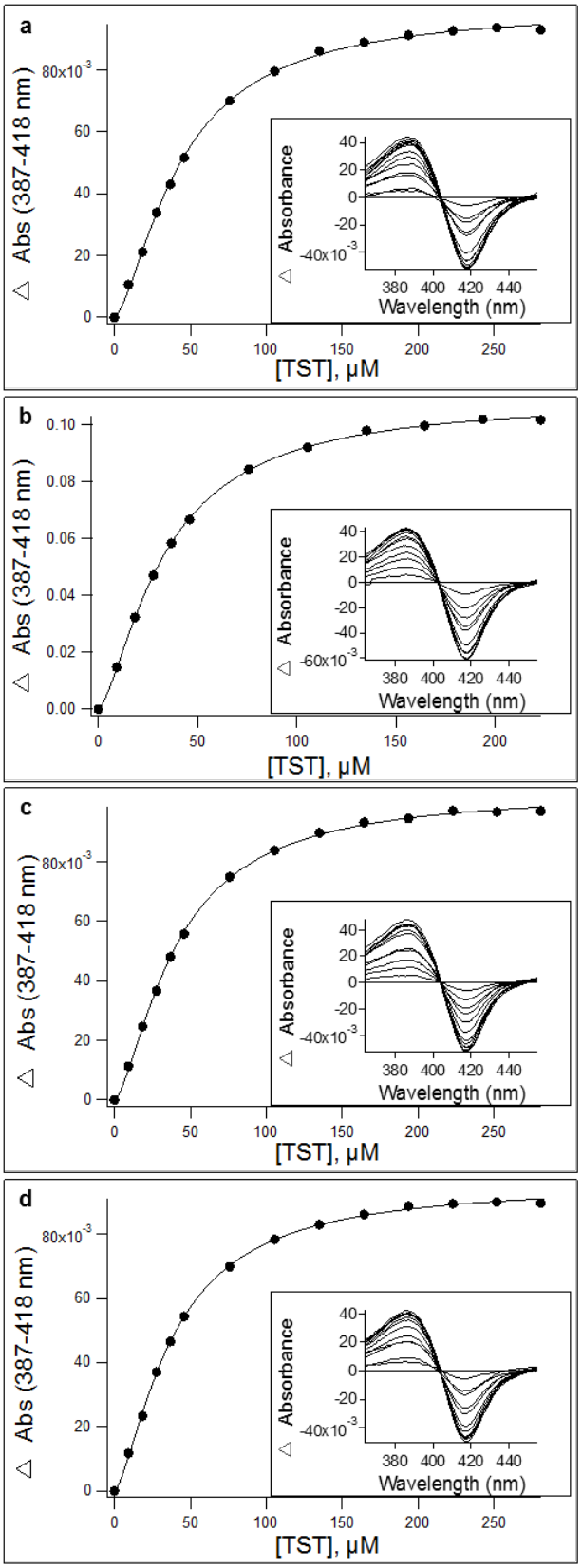

Figure S2. Binding isotherms for TST binding to CYP3A4 in nanodiscs composed of (a) pure POPC, (b) 3:1 POPC:DMPC (c) 1:1 POPC:DMPC, and (d) 1:3 POPC:DMPC CYP3A4 nanodiscs. *Inset:* corresponding difference absorbance spectra.

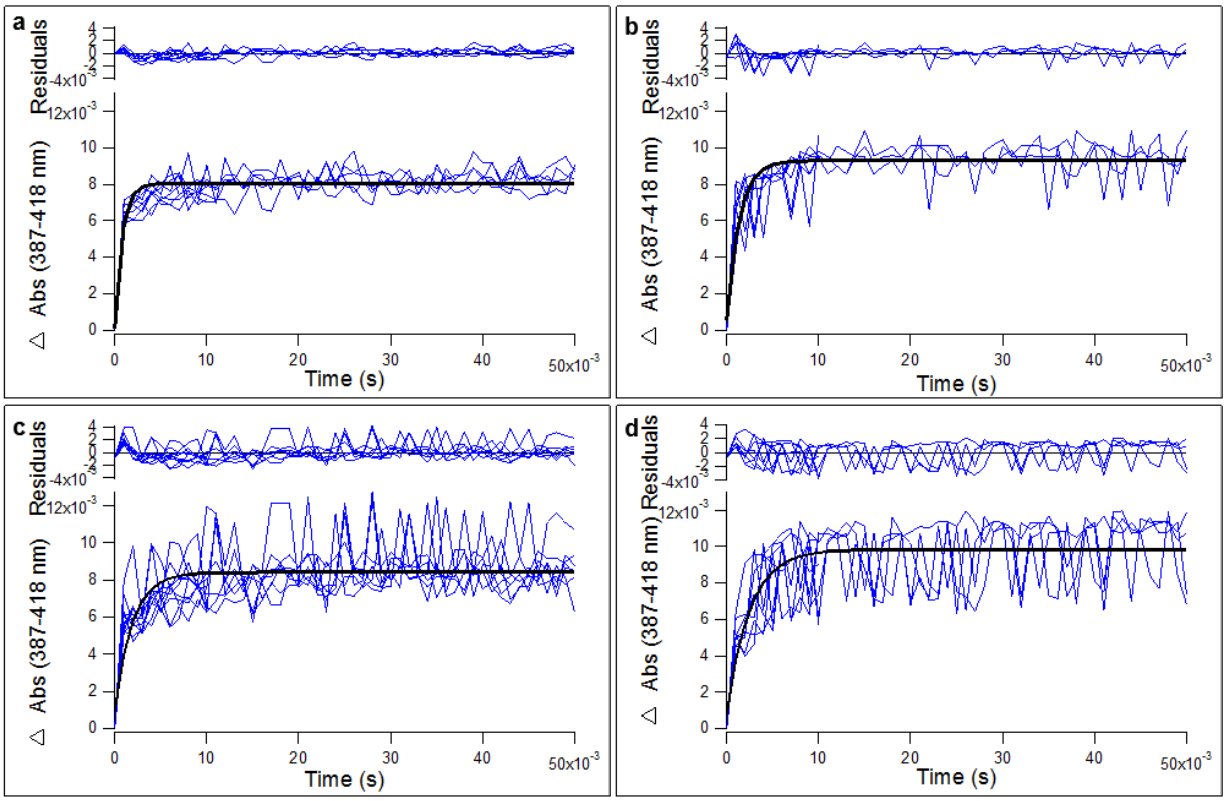

Figure S3. Stopped-flow kinetic analysis of TST binding to CYP3A4-nanodiscs composed of (a) pure POPC, (b) 3:1 POPC:DMPC, (c) 1:1 POPC:DMPC, and (d) 1:3 POPC:DMPC. Solid black lines is the result of fitting the kinetic data to equation 5. The saturating TST concentration was 200 μM.