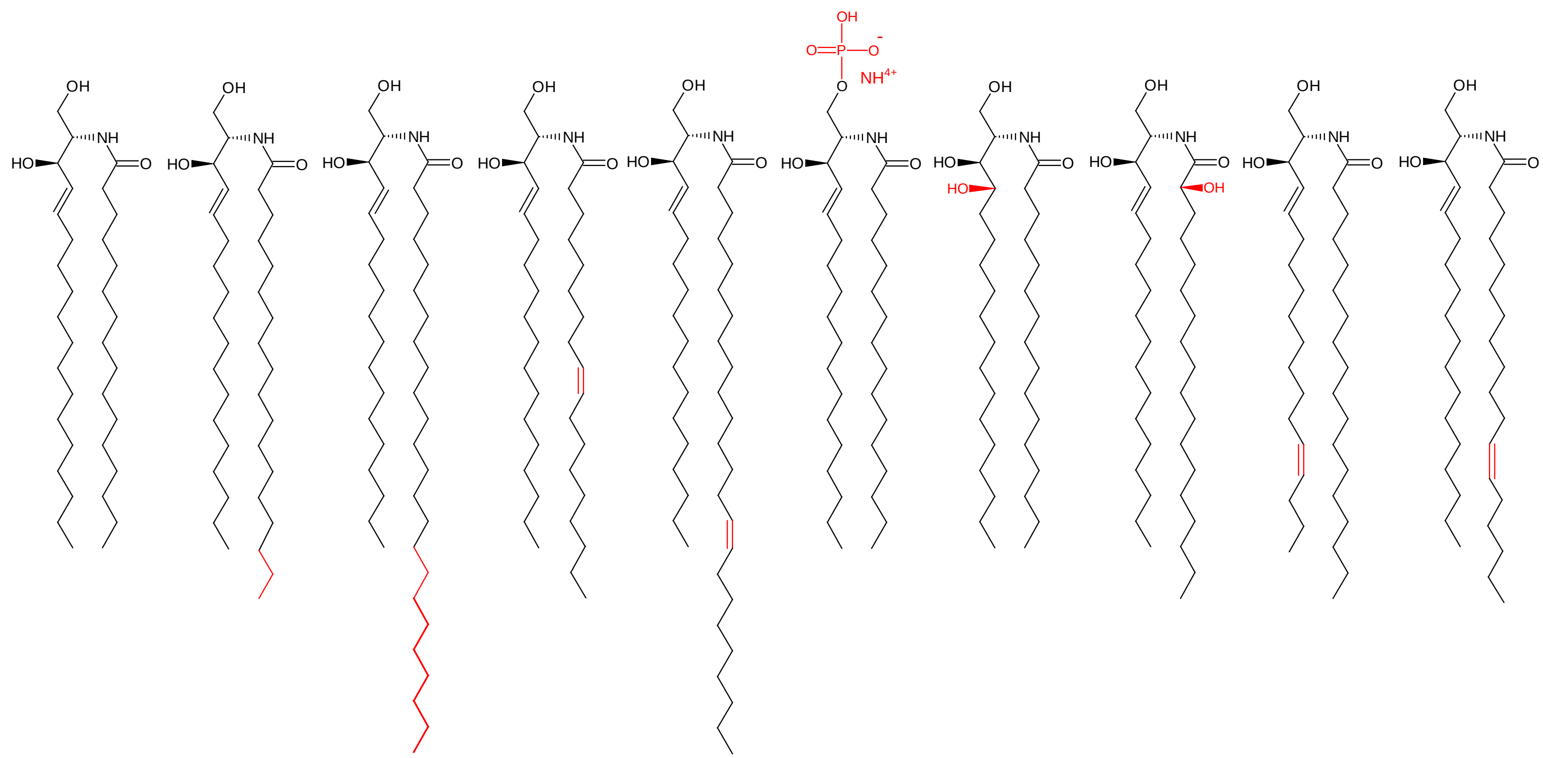
Supplemental information

Influence of Hydroxylation, Chain Length, and Chain Unsaturation on Bilayer Properties of Ceramides

Terhi Maula*, Md. Abdullah Al Sazzad, J. Peter Slotte

Biochemistry, Faculty of Science and Engineering, Åbo Akademi University, 20520 Turku, Finland

*Corresponding author



Scheme S1. Molecular structures of (from left to right) C16-cer, C18-cer, C24-cer, C18:1$^{\Delta 9c}$-cer, C24:1$^{\Delta 15c}$-cer, Cer-1-P, C16-phyto-cer, C18^{2OH}-cer, C18-sphingadiene-cer and C18:1$^{\Delta 12c}$-cer.

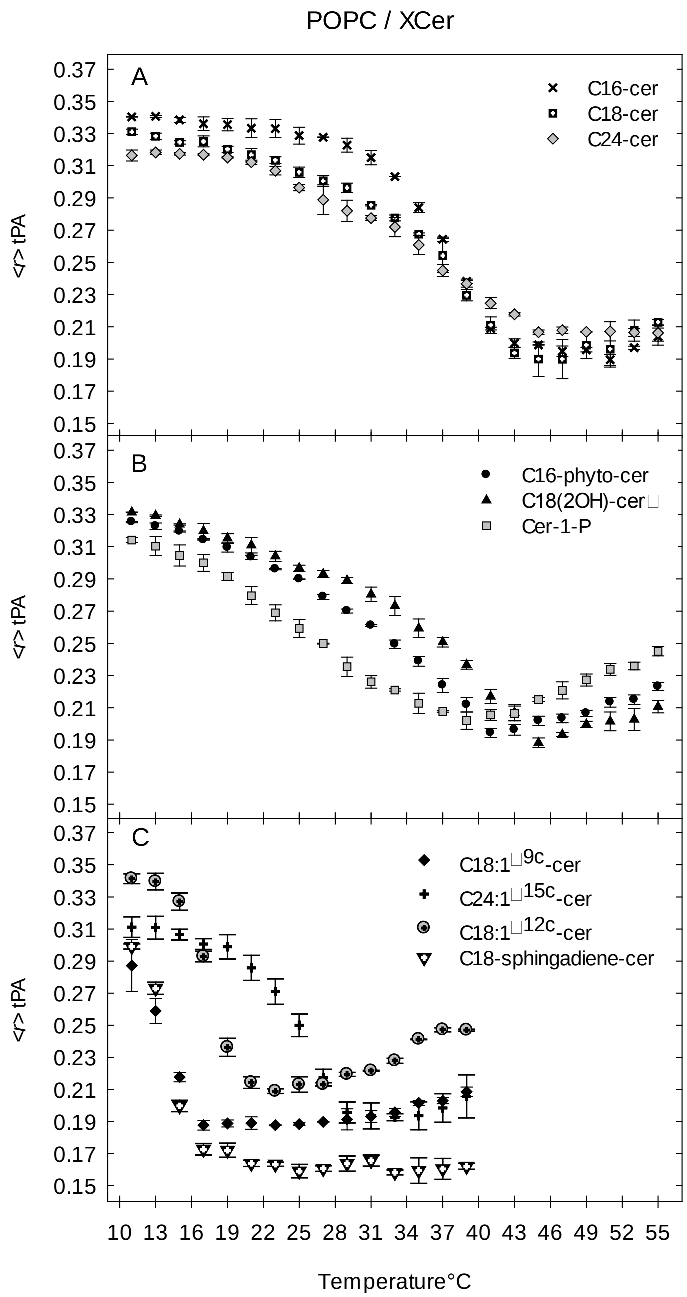


Figure S1. Steady-state fluorescence anisotropy of tPA (1 mol%) as a function of temperature in POPC/XCer bilayers (60:15 by mol). N=2-3 ± SD.

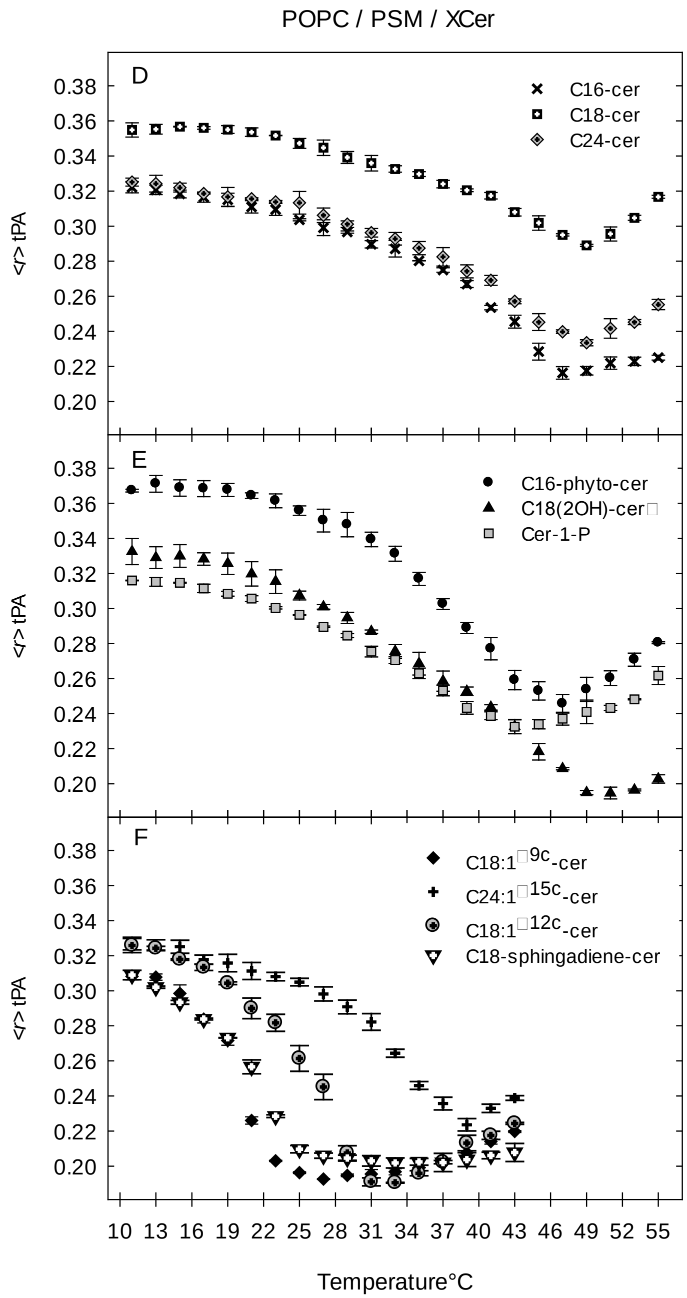


Figure S2. Steady-state fluorescence anisotropy of tPA (1 mol%) as a function of temperature in POPC/PSM/XCer bilayers (60:15:15 by mol). N=2-3 ± SD.

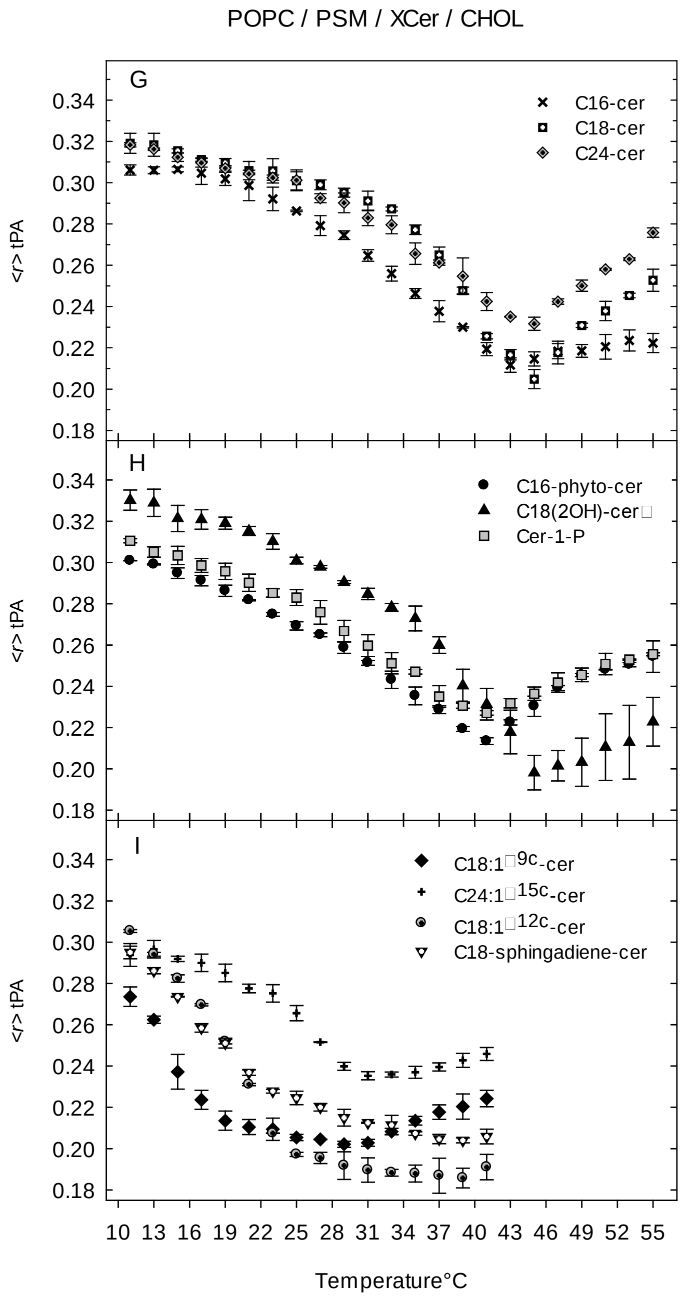


Figure S3. Steady-state fluorescence anisotropy of tPA (1 mol%) as a function of temperature in POPC/PSM/XCer/Chol bilayers (60:15:15:10 by mol). N=2-3 ± SD.

<u>Table S1.</u> <u>Time-resolved fluorescence decays of tPA (1 mol%) in binary and ternary mixed bilayers at 23°C.</u>

Sample	τ_1	f_1	α_1	τ_2	f_2	α_2	τ_3	f_3	α_3	τ_{AV}
POPC/C16-cer	49.1 ± 2.6	38.7 ± 3.1	14.8 ± 1.1	27.4 ± 0.7	45.0 ± 3.1	30.8 ± 2.1	5.6 ± 0.1	16.2 ± 1.4	54.2 ± 1.7	32.3 ± 1.9
POPC/C18-cer	49.0 ± 2.2	38.6 ± 3.0	14.6 ± 1.4	28.0 ± 1.1	45.0 ± 3.2	29.8 ± 2.1	5.4 ± 0.1	16.3 ± 0.8	55.4 ± 1.5	32.4 ± 2.4
POPC/C24-cer	48.2 ± 0.1	38.6 ± 1.3	15.7 ± 0.8	27.4 ± 0.1	46.8 ± 1.2	33.4 ± 0.3	5.6 ± 0.1	14.6 ± 0.1	50.9 ± 0.4	32.2 ± 0.3
POPC/C16-phyto-cer	42.0 ± 1.7	33.0 ± 1.5	7.5 ± 0.9	19.1 ± 1.7	18.2 ± 1.0	9.1 ± 0.4	5.5 ± 0.04	48.7 ± 2.5	83.3 ± 1.2	20.0 ± 0.4
POPC/C18(2OH)-cer	52.1 ± 3.7	43.5 ± 6.7	15.7 ± 2.3	29.7 ± 1.0	39.0 ± 6.3	24.6 ± 3.1	5.4 ± 0.04	17.3 ± 0.4	59.6 ± 1.7	35.4 ± 2.9
POPC/Cer-1- P	44.1 ± 2.5	39.3 ± 2.7	9.6 ± 1.5	19.6 ± 2.7	20.0 ± 2.4	11.0 ± 0.7	5.5 ± 0.05	40.6 ± 3.9	79.2 ± 2.3	23.5 ± 1.7
POPC/C18:1$^{\Delta 9c}$-cer	8.5 ± 0.2	32.7 ± 2.3	23.5 ± 2.0	5.3 ± 0.1	67.2 ± 2.3	76.5 ± 2.0				6.3 ± 0.09
POPC/C24:1$^{\Delta 15c}$-cer	37.8 ± 1.2	26.7 ± 3.2	8.1 ± 1.2	20.7 ± 1.0	40.0 ± 5.0	23.1 ± 3.4				20.1 ± 1.6
POPC/C18:1$^{\Delta 12c}$-cer	10.7 ± 0.5	14.8 ± 2.6	8.7 ± 1.3	5.9 ± 0.1	85.2 ± 2.6	91.2 ± 1.4				6.6 ± 0.1
POPC/C18-sphingadiene-cer	9.3 ± 0.4	25.1 ± 0.6	16.7 ± 0.8	5.5 ± 0.1	74.8 ± 0.6	83.2 ± 0.8				6.5 ± 0.1
POPC/PSM/C16-cer	52.5 ± 3.7	49.2 ± 2.6	16.7 ± 1.4	26.5 ± 1.5	31.1 ± 2.5	21.0 ± 2.6	5.6 ± 0.1	19.6 ± 2.9	62.2 ± 3.8	35.2 ± 3.4
POPC/PSM/C18-cer	49.3 ± 5.0	44.6 ± 3.8	18.0 ± 1.8	25.5 ± 1.9	40.9 ± 2.8	32.0 ± 2.3	5.6 ± 0.2	14.3 ± 1.7	49.9 ± 2.8	33.4 ± 4.3
POPC/PSM/C24-cer	48.0 ± 5.6	38.8 ± 8.9	17.4 ± 5.0	24.7 ± 1.7	49.0 ± 6.9	42.2 ± 5.6	6.2 ± 0.1	12.1 ± 3.5	40.3 ± 7.0	31.8 ± 5.8
POPC/PSM/C16-phyto-cer	47.9 ± 1.1	41.0 ± 0.6	9.6 ± 0.7	20.8 ± 2.0	20.0 ± 3.2	10.8 ± 1.6	5.4 ± 0.1	38.9 ± 3.7	79.5 ± 2.4	26.0 ± 1.7
POPC/PSM/ C18(2OH)-cer	52.4 ± 6.5	47.9 ± 2.7	18.9 ± 1.7	27.6 ± 2.9	37.4 ± 1.9	28.1 ± 2.7	5.7 ± 0.2	14.6 ± 2.3	52.8 ± 4.1	36.3 ± 5.3
POPC/PSM/Cer-1-P	49.0 ± 2.5	43.5 ± 1.7	12.2 ± 1.4	22.8 ± 2.3	26.0 ± 3.0	15.6 ± 2.2	5.7 ± 0.1	30.3 ± 4.5	72.2 ± 3.6	29.1 ± 3.0
POPC/PSM/C18:1$^{\Delta 9c}$-cer	34.9 ± 0.1	21.3 ± 0.5	5.5 ± 0.1	15.9 ± 0.4	25.6 ± 0.5	14.7 ± 0.1	6.0 ± 0.1	52.9 ± 1.0	79.7 ± 0.07	14.8 ± 0.2
POPC/PSM/C24:1$^{\Delta 15c}$-cer	39.3 ± 2.3	30.5 ± 5.6	10.7 ± 1.6	19.7 ± 2.4	47.7 ± 4.2	33.5 ± 1.8	5.4 ± 0.9	21.7 ± 1.7	55.7 ± 0.2	22.5 ± 0.8
POPC/PSM/C18:1$^{\Delta 12c}$-cer	38.1 ± 2.8	28.4 ± 8.1	8.6 ± 3.2	18.5 ± 2.4	38.0 ± 1.8	23.7 ± 4.9	5.5 ± 0.7	33.4 ± 9.6	67.6 ± 8.1	19.6 ± 1.0
POPC/PSM/C18-sphingadiene-cer	37.1 ± 1.8	24.8 ± 7.1	6.7 ± 2.4	16.6 ± 2.3	31.0 ± 3.7	18.9 ± 5.7	5.7 ± 0.7	44.0 ± 10.8	74.3 ± 8.1	16.9 ± 1.1

The bilayer composition was 60/15 (by mol) and 60/15/15 (by mol) for the binary and ternary mixtures, respectively. Each value is the average of at least three independently repeated experiments (±SD). τ, lifetime (ns); f, fractional intensity (%); α, fractional amplitude (%); τ_{AV}, intensity-weighted average lifetime (ns).

Table S2. Time-resolved fluorescence decays of tPA (1 mol%) in binary and ternary mixed bilayers at 10°C.

Sample	τ_1	f_1	α_1	τ_2	f_2	α_2	τ_3	f_3	α_3	τ_{AV}
POPC/C18:1$^{\Delta 9c}$-cer	50.3 ± 2.0	42.7 ± 3.5	21.5 ± 1.2	27.9 ± 1.8	44.0 ± 5.8	40.0 ± 5.8	8.7 ± 0.4	13.2 ± 2.5	38.4 ± 5.0	35.0 ± 1.7
POPC/C24:1$^{\Delta 15c}$-cer	51.7 ± 1.4	48.4 ± 5.7	25.5 ± 4.0	28.9 ± 1.6	40.0 ± 4.6	37.5 ± 2.1	8.4 ± 0.5	11.5 ± 1.2	36.9 ± 2.6	37.6 ± 1.9
POPC/C18:1$^{\Delta 12c}$-cer	53.1 ± 2.0	45.6 ± 4.2	24.8 ± 1.3	29.8 ± 2.1	44.9 ± 5.6	43.6 ± 5.6	8.7 ± 0.5	9.5 ± 1.4	31.4 ± 4.3	38.4 ± 1.2
POPC/C18-sphingadiene-cer	50.6 ± 1.7	47.6 ± 1.3	21.9 ± 1.9	25.0 ± 2.6	35.7 ± 3.4	33.4 ± 3.6	8.5 ± 0.4	16.5 ± 2.9	44.6 ± 5.1	34.5 ± 2.3
POPC/PSM/C18:1$^{\Delta 9c}$-cer	52.4 ± 1.2	43.0 ± 3.1	23.2 ± 1.5	29.0 ± 0.6	47.2 ± 3.3	46.0 ± 2.6	8.8 ± 0.3	9.6 ± 0.2	30.7 ± 1.2	37.2 ± 1.2
POPC/PSM/C24:1$^{\Delta 15c}$-cer	53.1 ± 0.8	45.2 ± 3.0	23.5 ± 1.9	28.4 ± 0.8	44.1 ± 2.5	42.6 ± 1.3	8.6 ± 0.2	10.6 ± 0.4	33.8 ± 0.8	37.4 ± 0.1
POPC/PSM/C18:1$^{\Delta 12c}$-cer	51.6 ± 2.4	50.0 ± 4.9	26.7 ± 1.1	27.2 ± 2.6	42.4 ± 4.2	43.1 ± 4.3	7.1 ± 2.8	7.5 ± 1.4	30.0 ± 3.1	37.9 ± 1.2
POPC/PSM/C18-sphingadiene-cer	52.4 ± 3.6	48.3 ± 7.7	25.8 ± 3.7	27.3 ± 4.0	43.9 ± 7.0	45.4 ± 8.3	7.8 ± 2.5	7.7 ± 3.1	28.6 ± 9.2	37.9 ± 2.2

The bilayer composition was 60/15 (by mol) and 60/15/15 (by mol) for the binary and ternary mixtures, respectively. Each value is the average of at least three independently repeated experiments (±SD). τ, lifetime (ns); f, fractional intensity (%); α, fractional amplitude (%); τ_{AV}, intensity-weighted average lifetime (ns).

Table S3. Time-resolved fluorescence decays of tPA (1 mol%, 23 °C) in complex mixed bilayers containing cholesterol.

Sample	τ_1	f_1	α_1	τ_2	f_2	α_2	τ_3	f_3	α_3	τ_{AV}
POPC/PSM/C16-cer/CHOL	50.3 ± 1.0	36.3 ± 9.5	10.7 ± 1.9	21.9 ± 3.7	31.1 ± 7.3	21.5 ± 4.4	7.3 ± 1.3	32.5 ± 2.4	67.6 ± 2.4	27.7 ± 2.4
POPC/PSM/C18-cer/CHOL	47.5 ± 2.8	35.8 ± 2.1	12.30 ± 0.7	22.3 ± 1.2	37.1 ± 2.8	27.29 ± 4.6	7.3 ± 0.7	27.0 ± 0.9	60.4 ± 5.2	27.3 ± 1.5
POPC/PSM/C24-cer/CHOL	44.1 ± 3.6	35.1 ± 4.26	13.3 ± 2.2	21.1 ± 2.8	42.5 ± 1.6	33.6 ± 2.3	7.0 ± 0.8	22.3 ± 3.3	53.0 ± 4.5	26.0 ± 1.4
POPC/PSM/C16-phyto-cer/CHOL	43.8 ± 0.3	18.0 ± 6.3	4.2 ± 1.4	15.5 ± 0.7	36.5 ± 8.8	24.4 ± 6.6	6.5 ± 0.2	45.3 ± 2.5	71.3 ± 5.1	16.5 ± 1.8
POPC/PSM/C18(2OH)-cer/CHOL	47.6 ± 2.5	40.6 ± 1.1	14.6 ± 1.6	22.4 ± 1.3	33.8 ± 4.9	26.0 ± 5.0	7.3 ± 0.4	25.5 ± 3.9	59.3 ± 6.6	28.8 ± 0.7
POPC/PSM/Cer-1- P/CHOL	50.0 ± 0.4	26.8 ± 4.8	6.9 ± 1.2	17.2 ± 0.3	35.8 ± 5.1	27.0 ± 3.9	7.4 ± 0.4	37.4 ± 0.6	65.9 ± 2.6	22.3 ± 1.2
POPC/PSM/C18:1$^{\Delta 9c}$-cer/CHOL	12.7 ± 0.4	52.7 ± 2.0	37.6 ± 1.9	6.9 ± 0.2	47.3 ± 2.0	62.3 ± 1.9				9.9 ± 0.4
POPC/PSM/C24:1$^{\Delta 15c}$-cer/CHOL	39.1 ± 3.1	16.4 ± 4.2	5.4 ± 1.9	18.7 ± 0.8	47.4 ± 4.5	32.2 ± 0.6	7.4 ± 0.5	36.1 ± 0.3	62.2 ± 1.4	17.9 ± 0.1
POPC/PSM/C18:1$^{\Delta 12c}$-cer/CHOL	13.8 ± 0.8	43.7 ± 3.2	28.0 ± 2.9	6.9 ± 0.3	56.3 ± 3.3	72.0 ± 2.9				9.9 ± 0.5
POPC/PSM/C18-sphingadiene-cer/CHOL	12.9 ± 0.6	48.4 ± 3.7	32.5 ± 3.6	6.6 ± 0.5	51.6 ± 3.7	67.5 ± 3.6				9.7 ± 0.7

The bilayer composition was 60/15/15/10 (by mol) for the quaternary mixtures. Each value is the average of at least three independently repeated experiments (±SD). τ, lifetime (ns); f, fractional intensity (%); α, fractional amplitude (%); τ_{AV}, intensity-weighted average lifetime (ns).

Table S4. Time-resolved fluorescence decays of tPA (1 mol%, 10 °C) in complex mixed bilayers containing cholesterol.

Sample	τ_1	f_1	α_1	τ_2	f_2	α_2	τ_3	f_3	α_3	τ_{AV}
POPC/PSM/C18:1$^{\Delta 9c}$-cer/CHOL	48.5 ± 2.2	28.0 ± 0.5	13.7 ± 0.9	24.4 ± 1.7	60.1 ± 2.5	58.3 ± 4.3	6.5 ± 4.8	11.7 ± 2.4	27.9 ± 4.6	29.5 ± 1.5
POPC/PSM/C24:1$^{\Delta 15c}$-cer/CHOL	45.7 ± 3.5	45.0 ± 7.2	22.2 ± 3.0	21.5 ± 3.3	46.5 ± 5.0	48.7 ± 2.7	6.6 ± 2.3	8.5 ± 2.2	29.0 ± 0.3	31.1 ± 1.2
POPC/PSM/C18:1$^{\Delta 12c}$-cer/CHOL	50.7 ± 0.9	31.8 ± 7.8	14.9 ± 5.8	23.7 ± 3.3	55.2 ± 3.6	53.2 ± 2.8	9.0 ± 0.9	12.9 ± 4.7	31.9 ± 7.2	30.4 ± 4.9
POPC/PSM/C18-sphingadiene-cer/CHOL	47.6 ± 3.3	29.2 ± 3.8	12.9 ± 3.4	22.1 ± 1.5	57.9 ± 2.2	54.2 ± 4.2	7.9 ± 0.5	12.9 ± 4.5	32.7 ± 47.2	27.7 ± 2.0

The bilayer composition was 60/15/15/10 (by mol) for the quaternary mixtures. Each value is the average of at least three independently repeated experiments (±SD). τ, lifetime (ns); f, fractional intensity (%); α, fractional amplitude (%); τ_{AV}, intensity-weighted average lifetime (ns).