Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Assessment of a Host-Guest Interaction in a Bilayer Membrane Model

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1 NMR

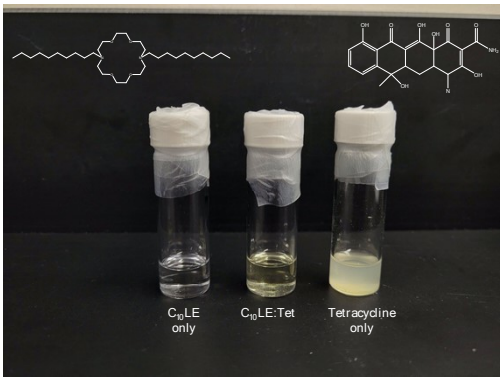

Figure S1. Vials containing C_{10}LE, tetracycline and C_{10}LE complexed with tetracycline.

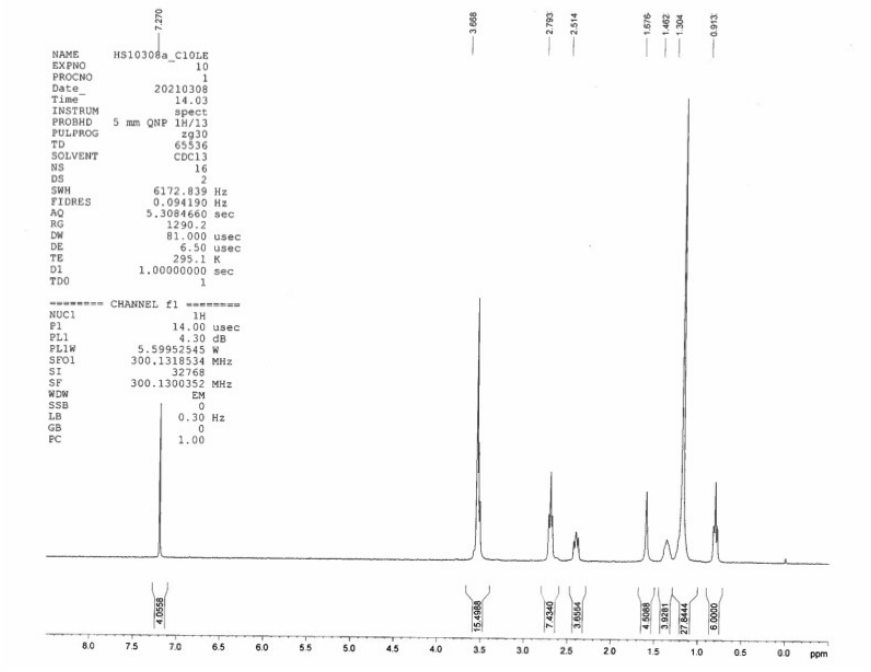

Figure S2. ^{1}H NMR spectrum of C_{10}LE

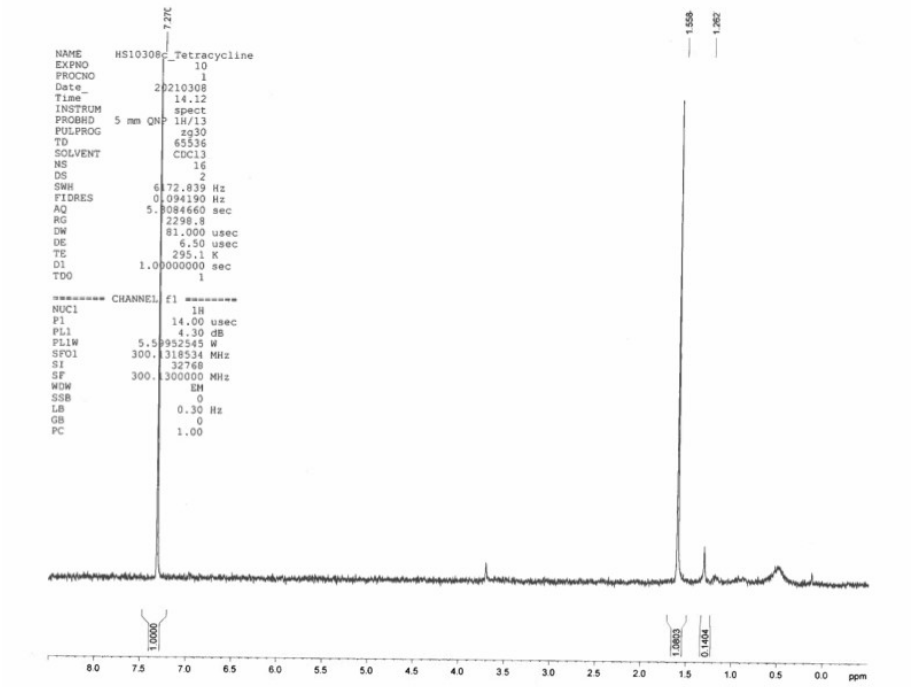

Figure S3. ^{1}H NMR spectrum of tetracycline hydrochloride

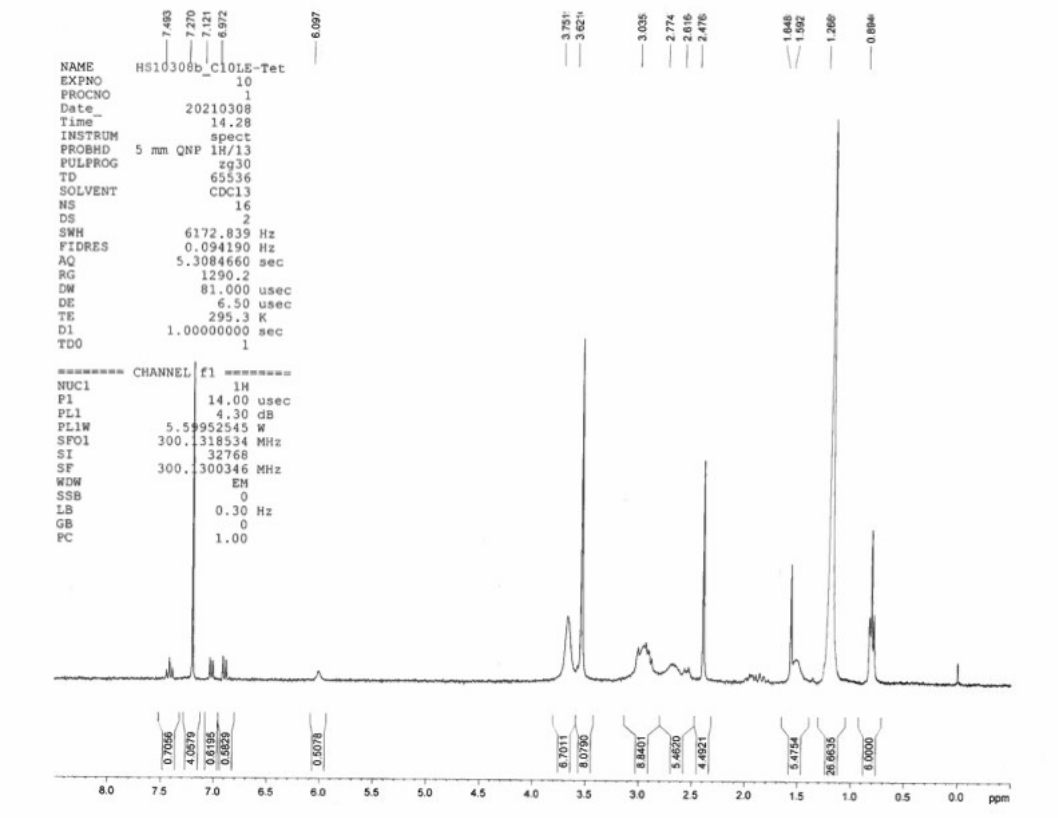

Figure S4. ^{1}H NMR spectrum of C_{10}LE complexed with tetracycline hydrochloride

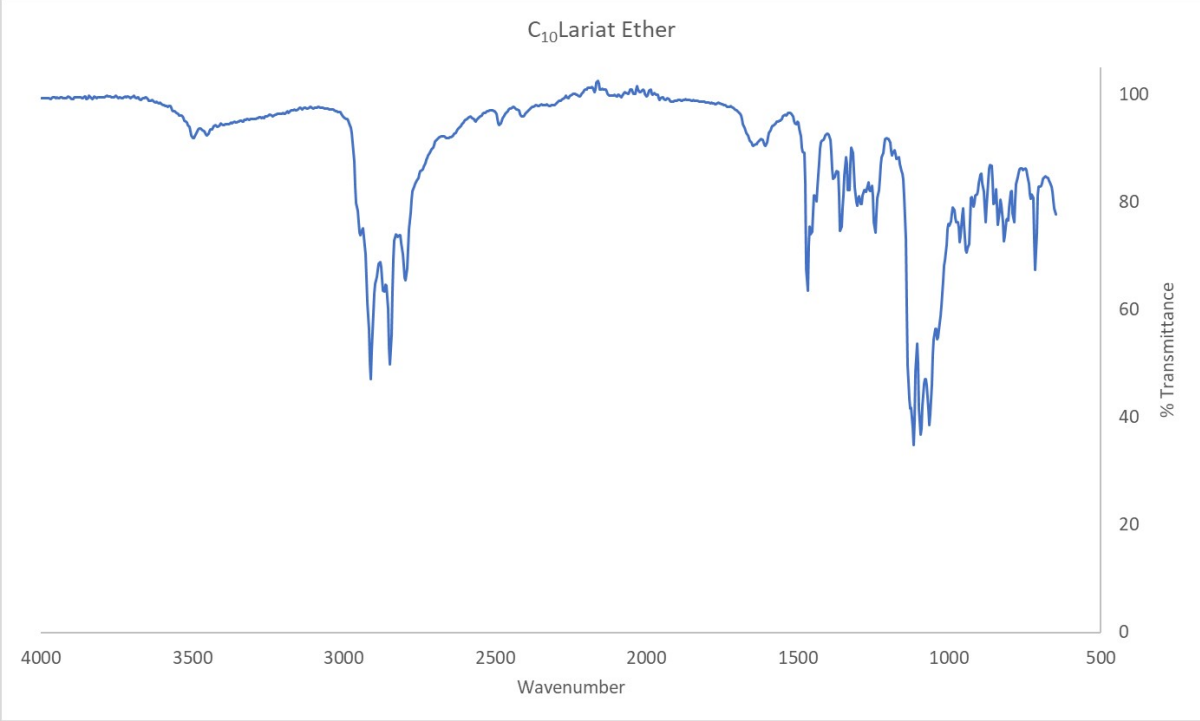

Figure S5. FT-IR spectrum of C_{10}LE

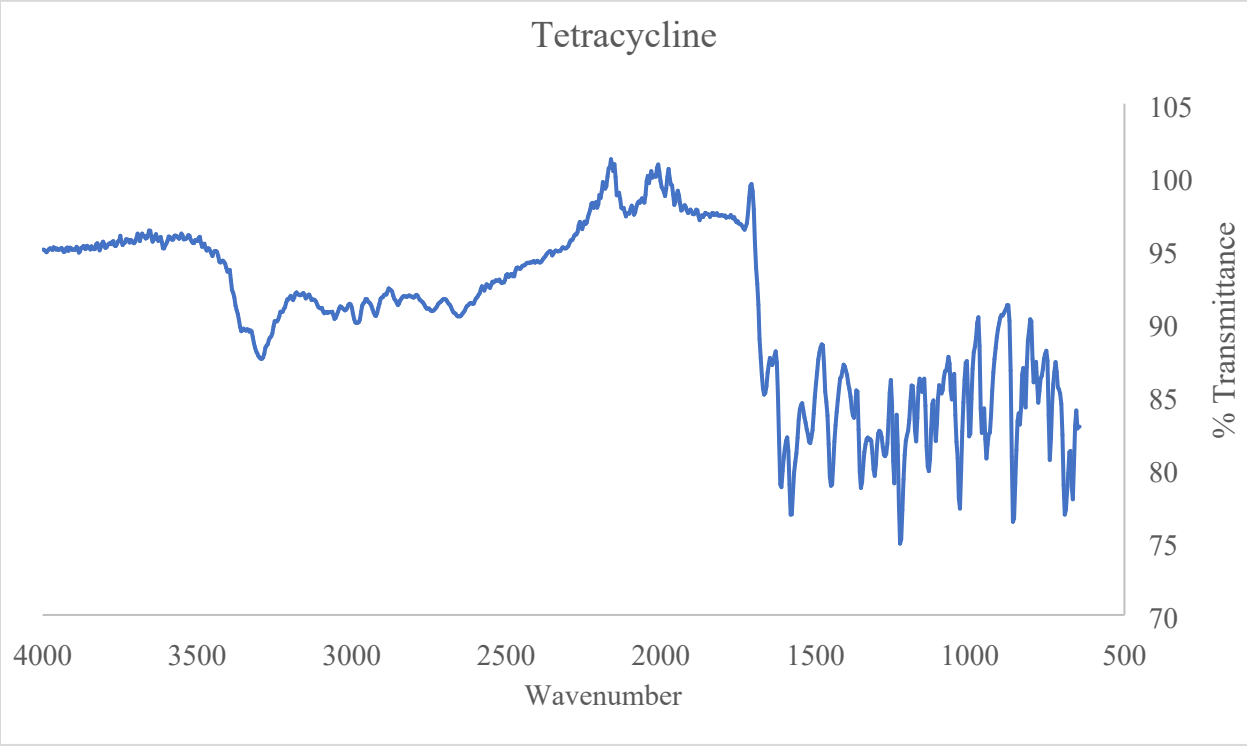

Figure S6. FT-IR spectrum of tetracycline hydrochloride.

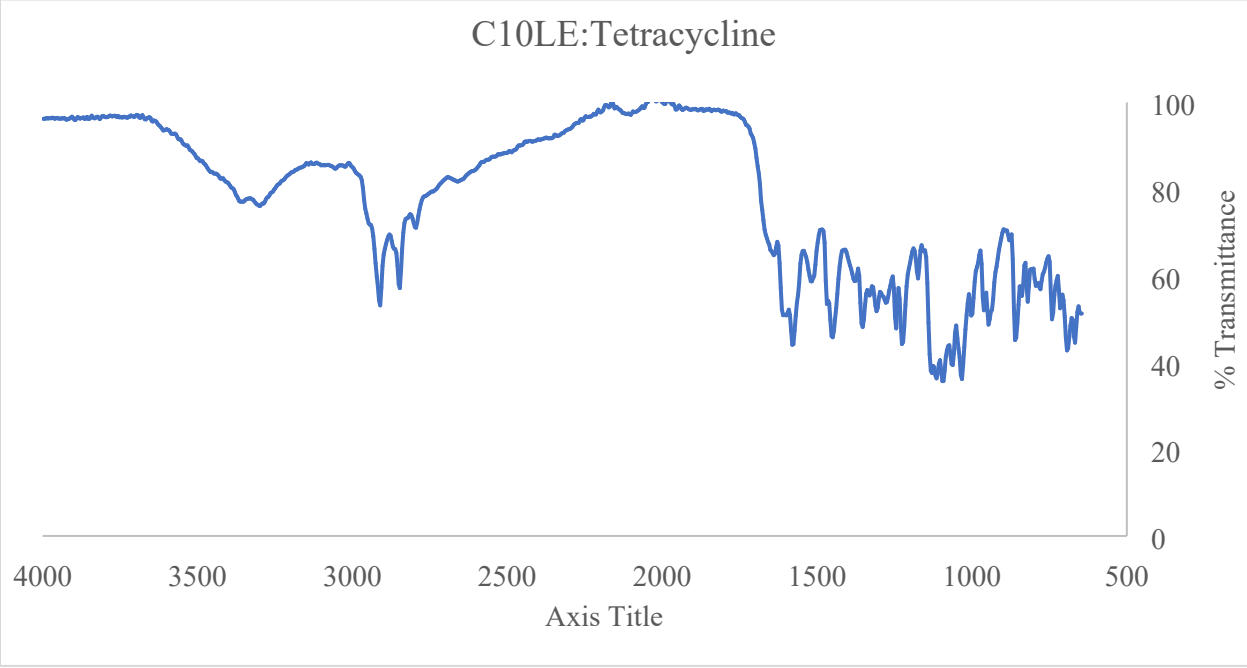

Figure S7. FT-IR spectrum of C_{10}LE complexed with tetracycline hydrochloride.

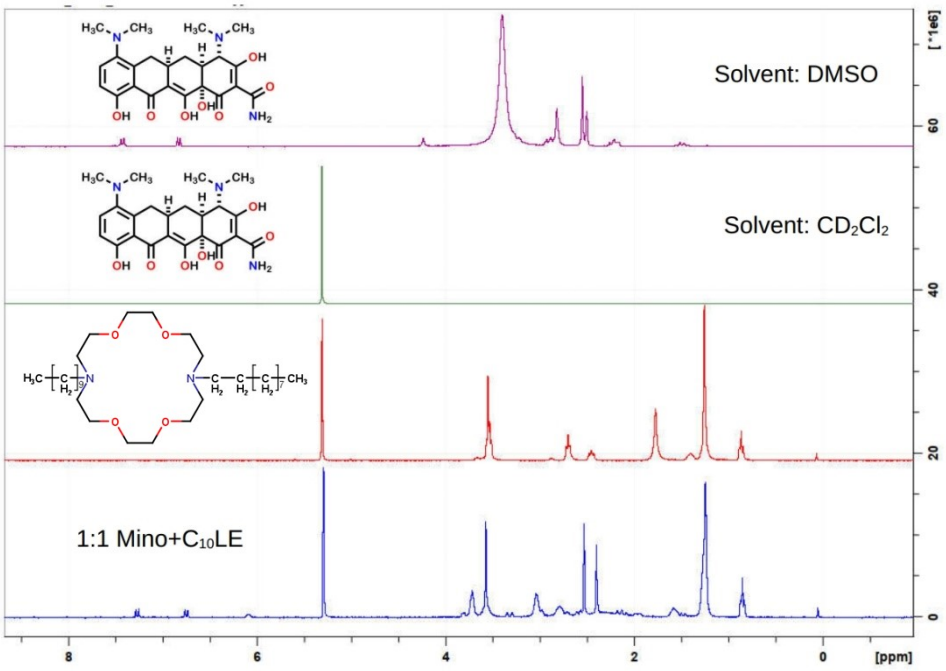

Figure S8. Stackplot ^{1}H NMR spectrum of C_{10}LE complexed with minocycline.

Table S-1. ^{1}H-NMR Spectra of *N,N*-Didecyl-4,13-diaza-18-crown-6 and Tetracycline Hydrochloride Recorded in $CDCl_3$.

Compound	Chemical Shift and Multiplicity[a]											
C_{10}LE	—	—	—	—	—	3.7 q	—	2.8 t	2.5 t	1.5 s	1.3 s	0.9 t
TET	7.6 t	7.1 d	6.9 d	5.1 s	—	—	—	2.7	—	—	—	—
1:1 Mixture	7.5 t	7.1 d	6.9 d	6.1 s	3.8 s	3.6 s	3.0 m	2.8 m	2.5 s	1.6 s	1.3 s	0.9 t

Notes: d = doublet, m = multiplet, q = quartet, s = singlet, t = triplet.

The NMR spectrum of the solution containing both tetracycline and lariat ether shows significant distortions in the peaks corresponding to the crown protons, notably at 3.03 and 2.77 ppm. For this reason, all calculations relating to a complexation ratio were made using the aliphatic signal at 1.28, which corresponds to the internal aliphatic protons of the two sidearms. This signal was then compared with peaks which corresponded to the aromatic and dimethylamine protons found in tetracycline. The three aromatic protons appear between 6.9 and 7.5 ppm, while the dimethylamine protons appear at about 2.4 ppm. Each trial then averaged the complexation values obtained from the comparison of these signals with the aliphatic LE signal using the equation below. In summary, the NMR complexation experiment was replicated 5 times, although the average of 1.00 ± 0.03 is ultimately the result of 20 individual calculations.

$$\frac{Integral_{LE}}{Integral_{Tet}} * \frac{Proton_{Tet}}{Proton_{LE}} = Complexation\ Ratio$$

Included is a sample calculation, which compares the dimethylamine signal of tetracycline with the aliphatic signal of LE.

$$\frac{27.3298}{5.8771} * \frac{6}{28} = 0.99675$$

2 NR DATA: C_{10}LE / Tetracycline hydrochloride

2.1 Experimental

All experiments were carried out in pure water or heavy water. Solution of small molecules was aided by DMSO, where necessary.

Table S1. A table with the scattering length densities used in the fits

Compound	Formula	Volume (Å^3)	Neutron scattering length (Å)	Neutron scattering length density (Å^{-2})
tetracycline	$C_{22}H_{24}N_2O_8$	470	1.2E-03	2.6E-06
tetracycline, labile protons exchanged to deuterons	$C_{22}H_{18}D_6N_2O_8$	470	1.8E-03	3.9E-06
C_{10}LE	$C_{32}H_{66}N_2O_4$	700	7.8E-05	0.1E-06
C_{10}	$C_{20}H_{42}$	460	-2.5E-04	-0.5E-06
LE	$C_{12}H_{24}N_2O_4$	240	3.2E-04	1.3E-06
d_{31}-POPC chains	$C_{32}H_{33}D_{31}$	925	3.0E-03	3.2E-06
h-POPC chains	$C_{32}H_{64}$	925	-2.72E-04	-0.3E-06
POPC headgroup	$C_{10}H_{18}O_8NP$	331	6.0E-04	1.8E-06

2.2 C_{10}LE / tetracycline complex

Table S2: Median fit parameter values and 68% confidence limits for the simultaneous analysis of the C_{10}LE / tetracycline hydrochloride experiments using a solid supported lipid bilayer composed of either d_{31}-POPC or h_{31}-POPC.

	12 µM C_{10}LE / 5 µM tetracycline incubation	87 µM C_{10}LE / 36 µM tetracycline incubation	Rinse
Substrate			
Thickness silicon oxide h_{31}/d_{31}-POPC / Å	8.8±0.4 / 8.0±0.9	8.7±0.6 / 8.2±0.7	9.0±0.6 / 7.9±0.6
Substrate roughness, standard deviation σ / Å	4.6±0.1 /2.6±0.6	4.5±0.4 / 2.6±0.6	4.7±0.5 / 2.7±1.0
Bilayer			
Thickness sub-membrane space / Å	1.0±0.4 / 1.8±0.8	1.3±0.5 / 1.6±0.7	1.5±0.5 / 1.2±0.7
Hydrocarbon thickness inner lipid leaflet neat bilayer	16.3±0.6 / 15.7±1.2	15.5±0.5 / 14.9±0.7	15.5±0.5 / 14.9±0.7
Hydrocarbon thickness outer lipid leaflet neat bilayer	12.9±0.5 / 13.6±1.0	13.3±0.5 / 13.8±0.7	12.9±0.5 / 13.4±0.6
Thickness change per leaflet after adding avobenzone	-0.3±0.2 / -0.3±0.4	-0.8±0.2 / -0.2±0.3	-1.3±0.4 / -0.7±0.7
Bilayer completeness neat bilayer	1.00±0.01 / 0.99±0.01	1.00±0.01 / 0.98±0.01	1.00±0.01 / 0.98±0.01
Bilayer completeness after incubation	1.00±0.01 / 0.99±0.01	1.00±0.01 / 1.00±0.01	1.00±0.01 / 1.00±0.01

C_{10}LE / tetracycline			
Peak position of C10LE distribution with respect to headgroup / bulk solvent interface / Å	n/a	-33.7 ± 2.6	-32.8 ± 1.7
C_{10}LE / tetracycline amount associated with bilayer (volume surface density) / $Å^3/Å^2$	total: 1.3 ± 0.6 C10LE: 0.7 ± 0.4 tetracycline: 0.4 ± 0.2	total: 6.2 ± 0.8 C10LE: 5.0 ± 0.4 tetracycline: 1.3 ± 0.6	total: 6.7 ± 1.3 C10LE: 5.4 ± 1.0 tetracycline: 1.2 ± 0.5

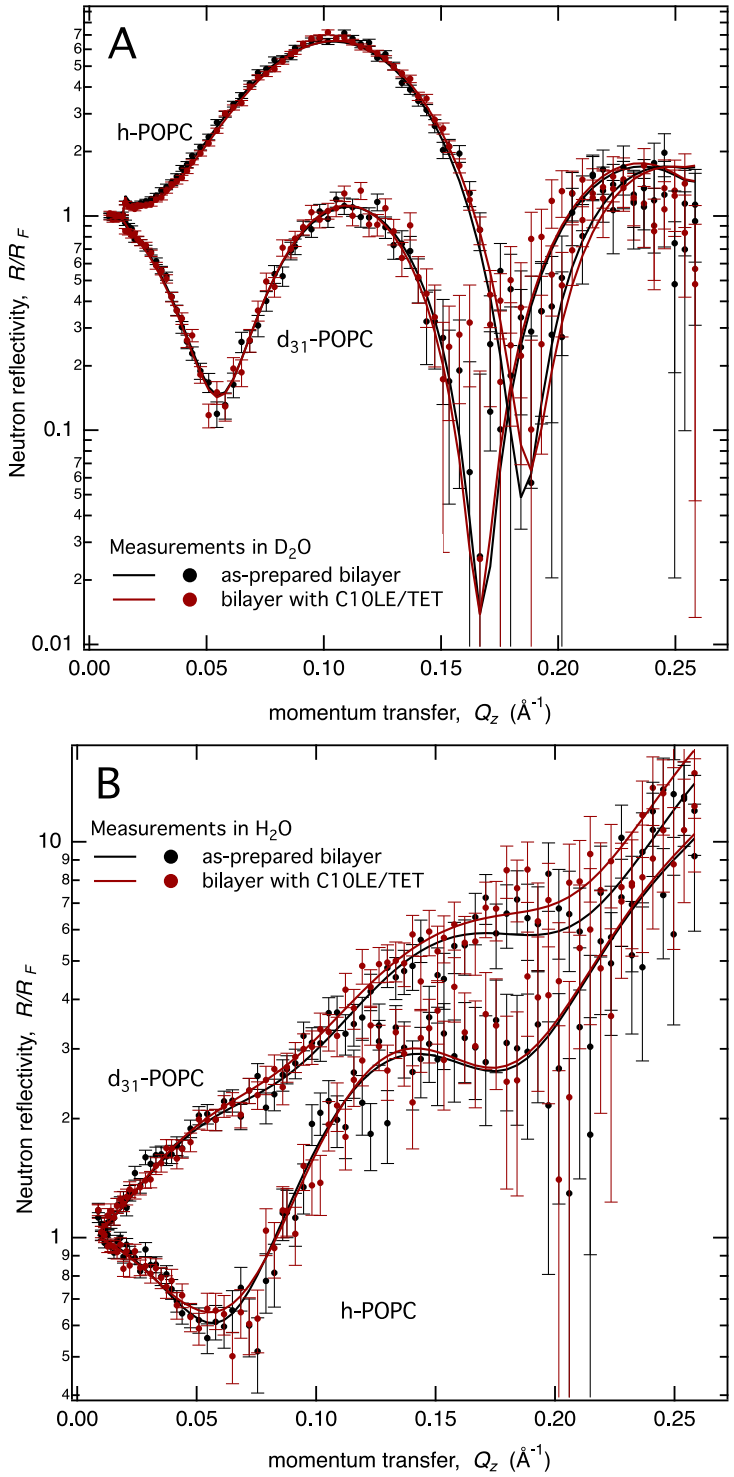

Figure S9: Fresnel normalized neutron reflectivity for the measurements of the as-prepared lipid bilayer and after adding of 12 µM C_{10}LE / 5 µM tetracycline hydrochloride. Reflectivities for the h_{31}-POPC and d_{31}-POPC lipid bilayers are shown.

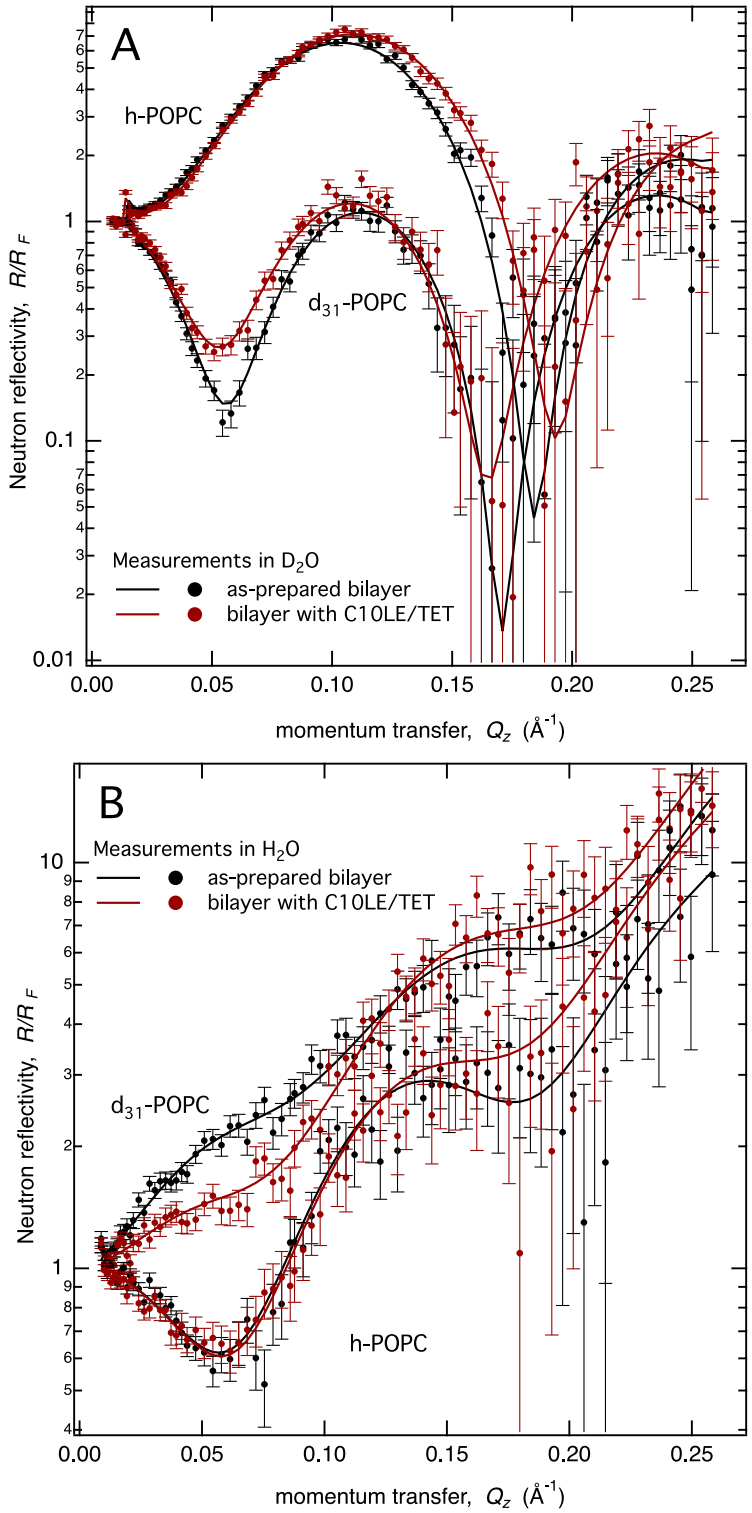

Figure S10: Fresnel normalized neutron reflectivity for the measurements of the as-prepared lipid bilayer and after adding of 87 µM C_{10}LE / 36 µM tetracycline hydrochloride. Reflectivities for the h_{31}-POPC and d_{31}-POPC lipid bilayers are shown.

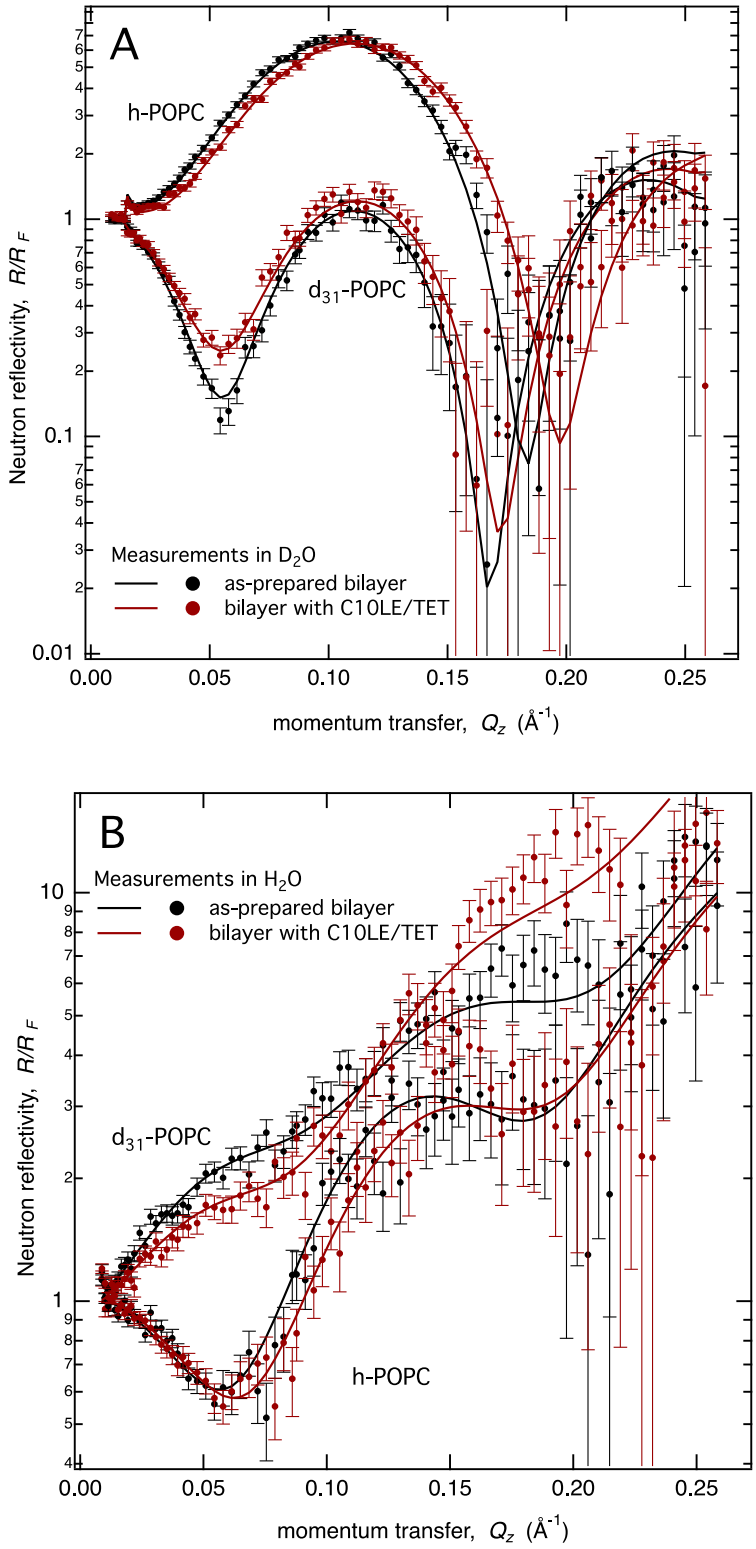

Figure S11: Fresnel normalized neutron reflectivity for the measurements of the as-prepared lipid bilayer and after a final rinse with pure water. Reflectivities for the h_{31}-POPC and d_{31}-POPC lipid bilayers are shown.

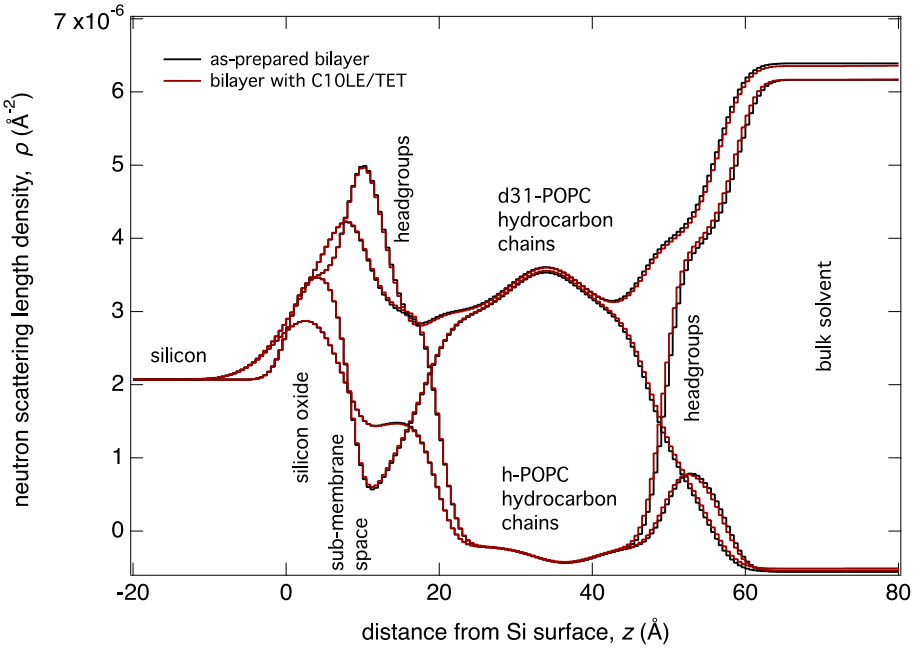

Figure S12: Best-fit nSLD profiles for the measurements of the as-prepared d_{31}-POPC and h_{31}-POPC lipid bilayers and after adding 12 µM C_{10}LE / 5 µM TET.

C_{10}LE/tetracycline hydrochloride 87µM/36 µM

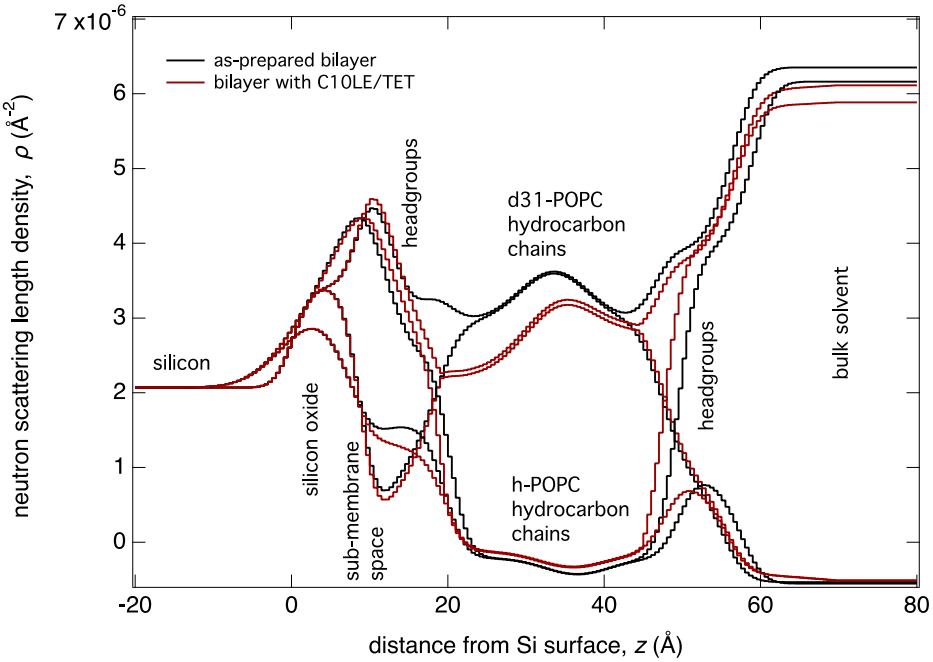

Figure S13: Best-fit nSLD profiles for the measurements of the as-prepared d_{31}-POPC and h_{31}-POPC lipid bilayers and after adding 87 µM C_{10}LE / 36 µM TET.

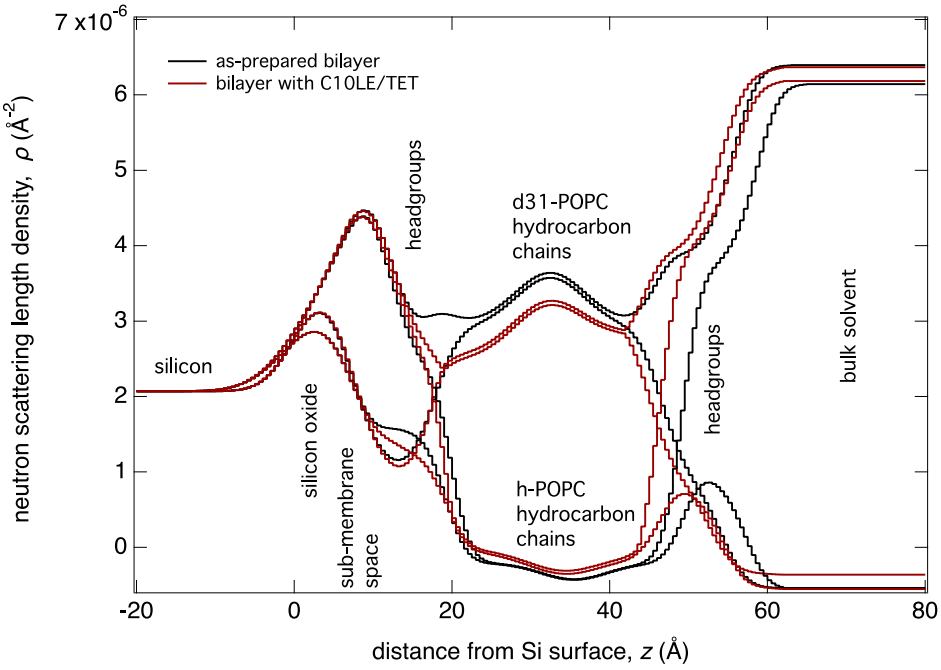

Figure S14: Best-fit nSLD profiles for the measurements of the as-prepared d_{31}-POPC and h_{31}-POPC lipid bilayers and after a final rinse with pure water.

2.3 C10LE

Table S3: Median fit parameter values and 68% confidence limits for the analysis of the C10LE experiments.

	12 µM C10LE	58 µM C10LE	Rinse
Substrate			
Thickness silicon oxide h31/d31-POPC / Å	8.0 ± 0.8 / 8.2 ± 0.6	7.2 ± 0.6 / 9.7 ± 0.5	7.6 ± 0.7 / 8.2 ± 0.5
Substrate roughness, standard deviation σ / Å	4.3 ± 0.4 / 3.6 ± 0.7	0.8 ± 0.4 / 2.0 ± 0.4	4.4 ± 0.4 / 4.6 ± 0.4
Bilayer			
Thickness sub-membrane space / Å	1.9 ± 0.9 / 3.9 ± 0.6	1.9 ± 0.6 / 3.0 ± 0.6	1.7 ± 0.6 / 3.0 ± 0.7
Hydrocarbon thickness inner lipid leaflet	16 ± 1 / 19 ± 1	14 ± 1 / 12 ± 1	14 ± 1 / 10 ± 1
Hydrocarbon thickness outer lipid leaflet	13 ± 1 / 10 ± 1	12 ± 1 / 13± 1	11 ± 1 / 14 ± 1
Thickness change per leaflet after incubation	-0.1 ± 0.2 / +0.2 ± 0.2	-1.5 ± 0.2 / -1.9 ± 0.3	-1.9 ± 0.2 / -2.3 ± 0.3
Bilayer completeness neat bilayer	0.98 ± 0.01 / 0.98 ± 0.01	0.96 ± 0.01 / 0.94 ± 0.01	0.96 ± 0.01 / 0.97 ± 0.02
Bilayer completeness after adding C10LE	0.99 ± 0.01 / 0.96 ± 0.01	1.00 ± 0.01 / 0.99 ± 0.01	0.99 ± 0.01 / 0.98 ± 0.01
Area per lipid outer leaflet with C10LE / Å^2	90 ± 7 / 70 ± 5	75 ± 7 / 70 ± 5	80 ± 5 / 65 ± 5
C10LE			
C10LE amount associated with bilayer (volume surface density) / Å^3/Å^2	3.0 ± 0.4	7.4 ± 0.4	4.5 ± 0.7

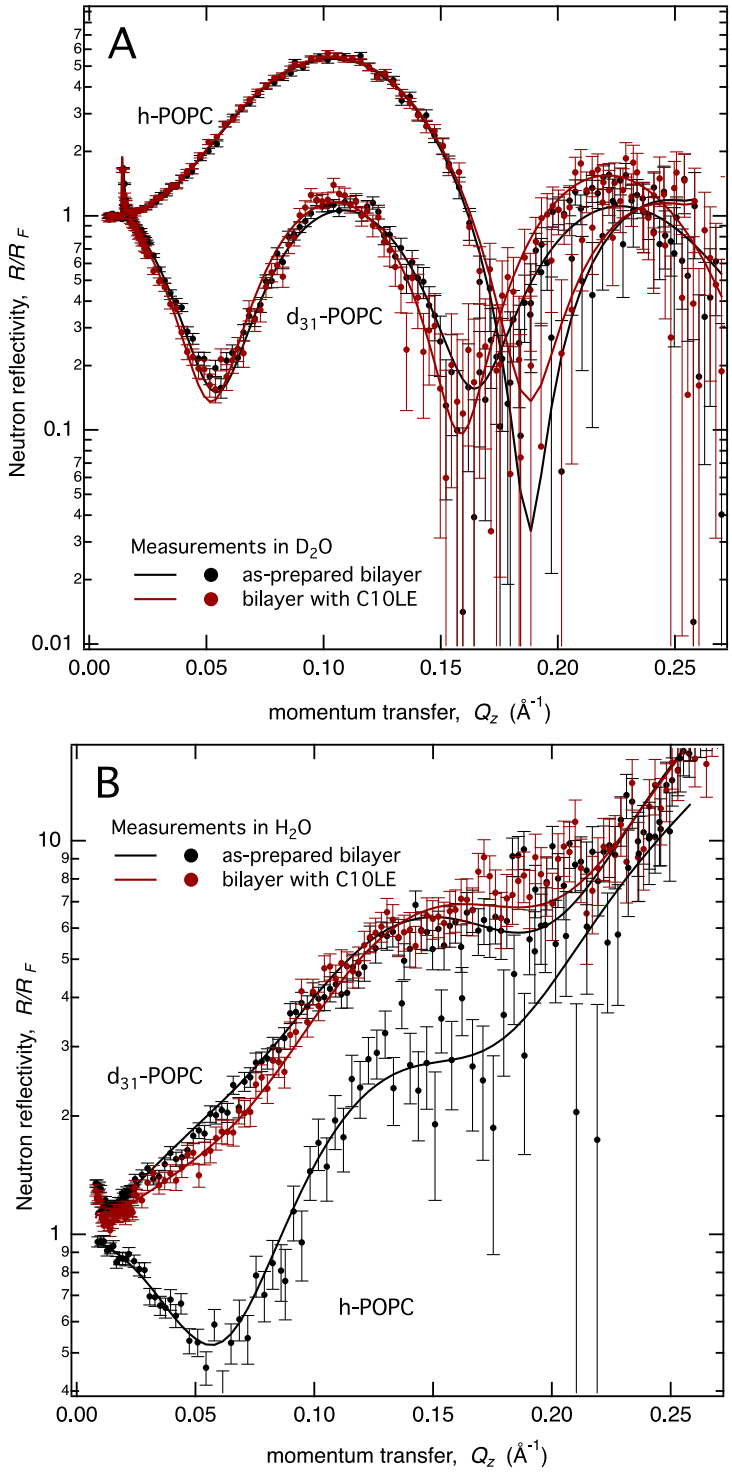

Figure S15: Fresnel normalized neutron reflectivity for the measurements of the as-prepared lipid bilayer and after adding of 12 μM C_{10}LE. Reflectivities for the h_{31}-POPC and d_{31}-POPC lipid bilayers are shown.

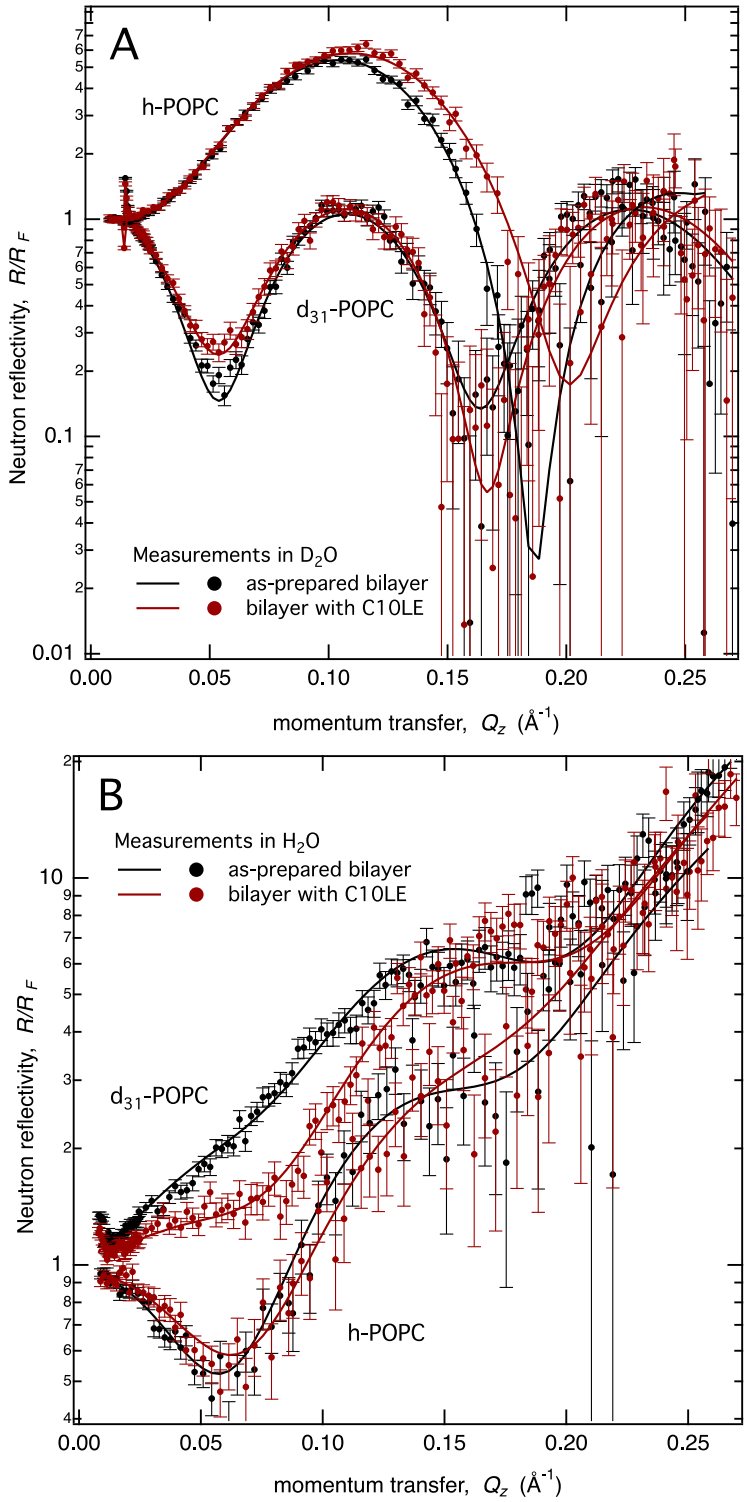

Figure S16: Fresnel normalized neutron reflectivity for the measurements of the as-prepared lipid bilayer and after adding of 58µM C10LE. Reflectivities for the h31-POPC and d31-POPC lipid bilayers are shown.

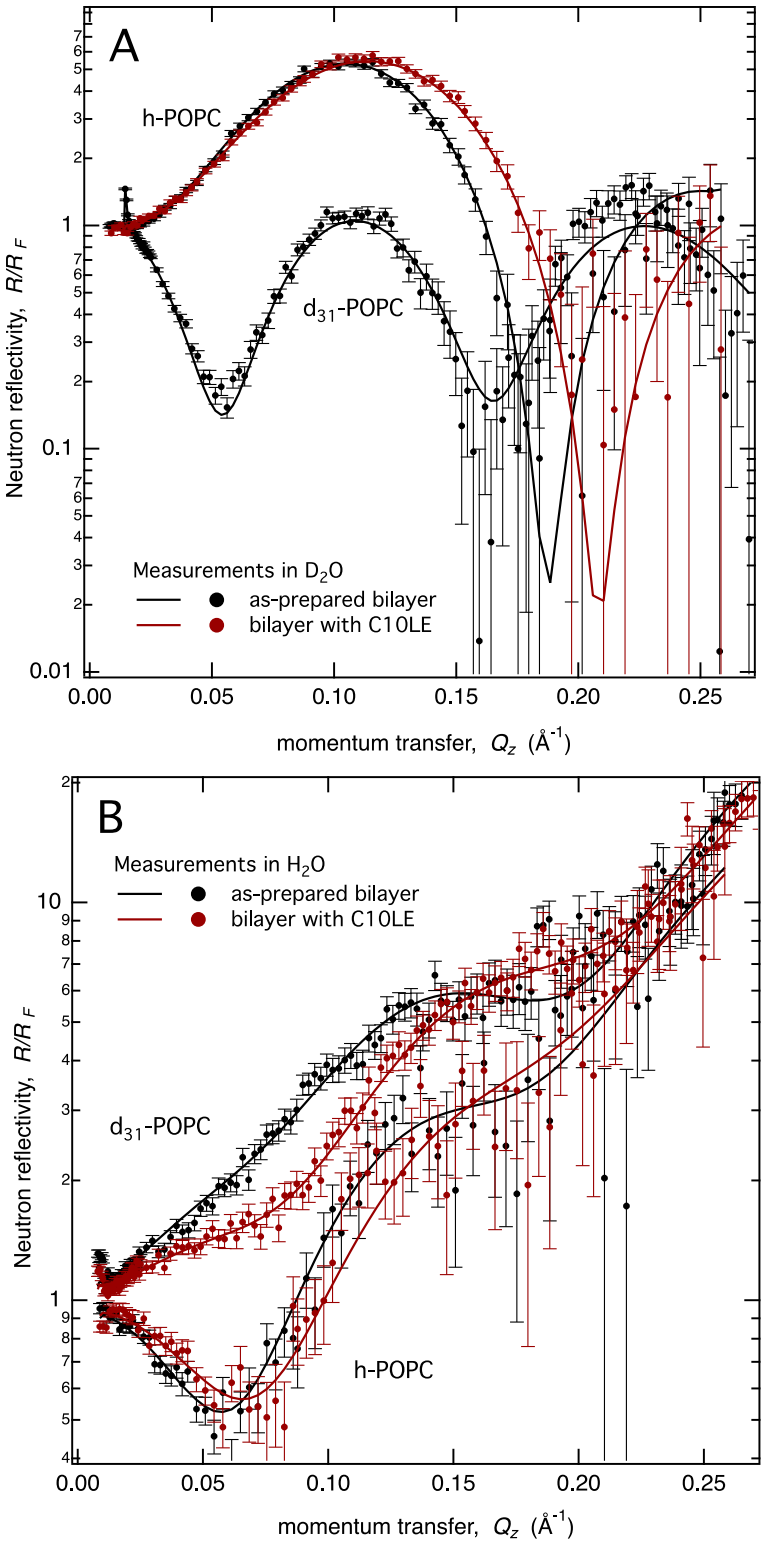

Figure S17: Fresnel normalized neutron reflectivity for the measurements of the as-prepared lipid bilayer and after a final rinse with pure water. Reflectivities for the h31-POPC and d31-POPC lipid bilayers are shown.

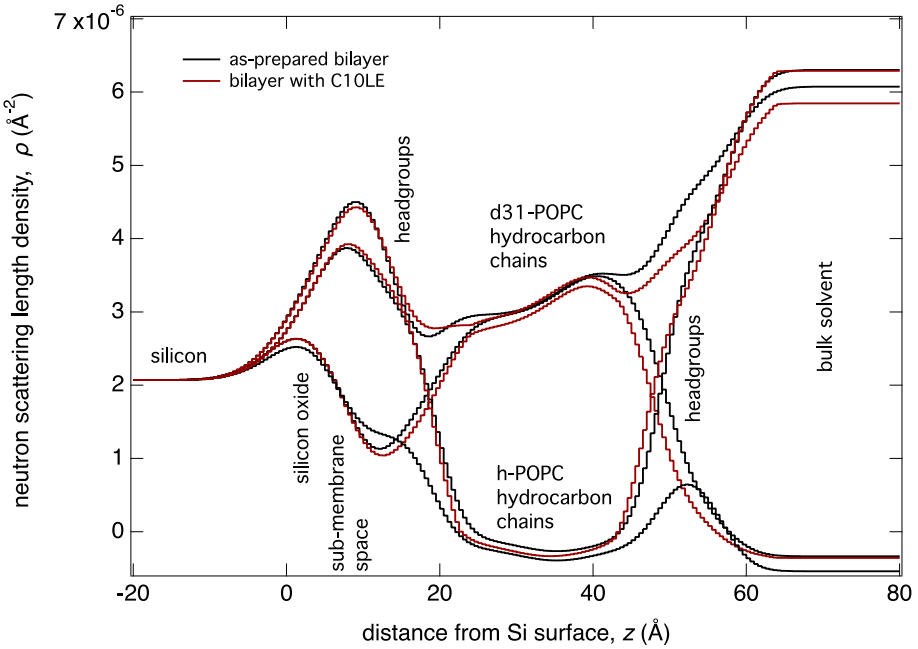

Figure S18: Best-fit nSLD profiles for the measurements of the as-prepared d_{31}-POPC and h_{31}-POPC lipid bilayers and after adding 12 µM C10LE.

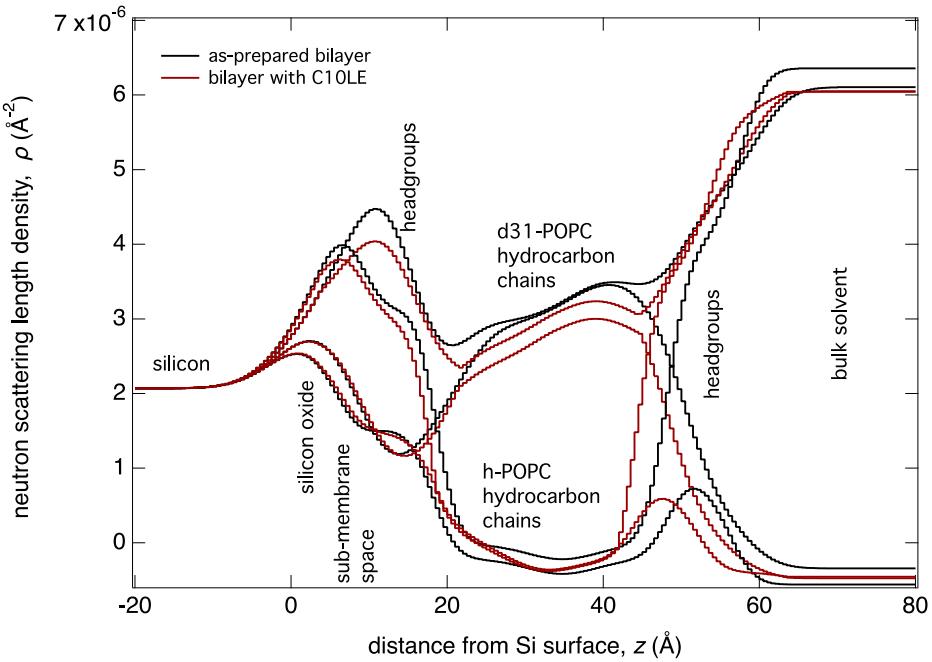

Figure S19: Best-fit nSLD profiles for the measurements of the as-prepared d_{31}-POPC and h_{31}-POPC lipid bilayers and after adding 58 µM C10LE.

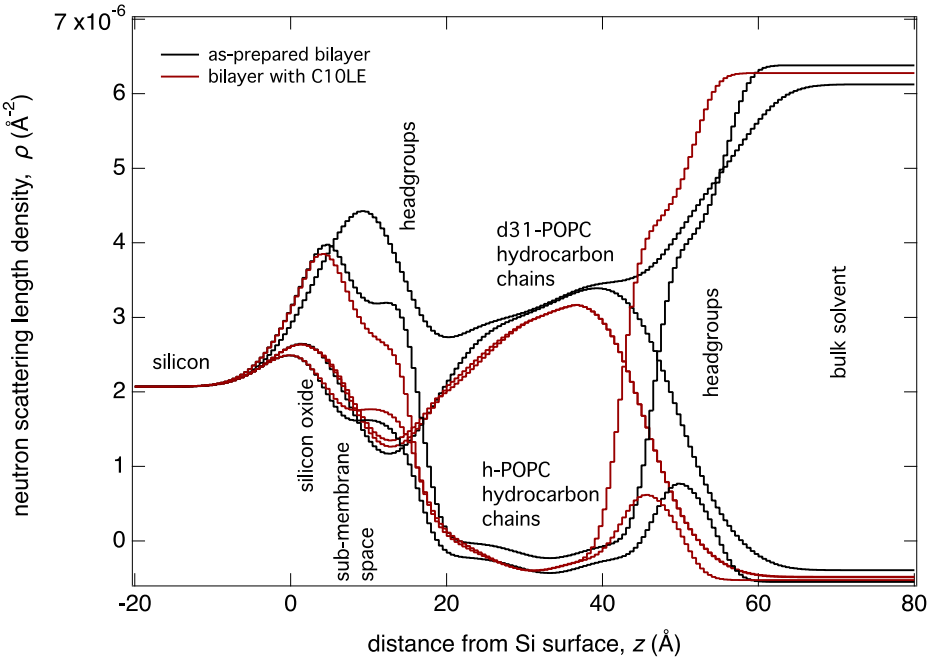

Figure S20: Best-fit nSLD profiles for the measurements of the as-prepared d_{31}-POPC and h_{31}-POPC lipid bilayers and after a final rinse with pure water.

2.4 Tetracycline

Table S4: Median fit parameter values and 68% confidence limits for the analysis of the tetracycline experiments.

	5 µM tetracycline	36 µM tetracycline	Rinse
Substrate			
Thickness silicon oxide / Å	6.9±0.7	6.7±0.6	6.7±0.7
Substrate roughness, standard deviation σ / Å	5.1±0.4	5.2±0.4	4.9±0.5
Bilayer			
Thickness sub-membrane space / Å	1.6±0.6	2.1±0.6	1.9±0.6
Hydrocarbon thickness inner lipid leaflet neat bilayer	14.6±0.6	14.6±0.6	15.0±0.6
Hydrocarbon thickness outer lipid leaflet neat bilayer	15.6±0.5	15.7±0.4	15.7±0.4
Thickness change per leaflet after incubation	-0.1±0.1	0.0±0.1	0.0±0.1
Bilayer completeness neat bilayer	1.00±0.01	1.00±0.01	1.00±0.01
Bilayer completeness after adding avobenzone	1.00±0.01	1.00±0.01	1.00±0.01
Tetracycline			
tetracycline amount associated with bilayer (volume surface density) / ($Å^3/Å^2$)	1.0 ± 0.5	0.6 ± 0.4	1.3±0.5

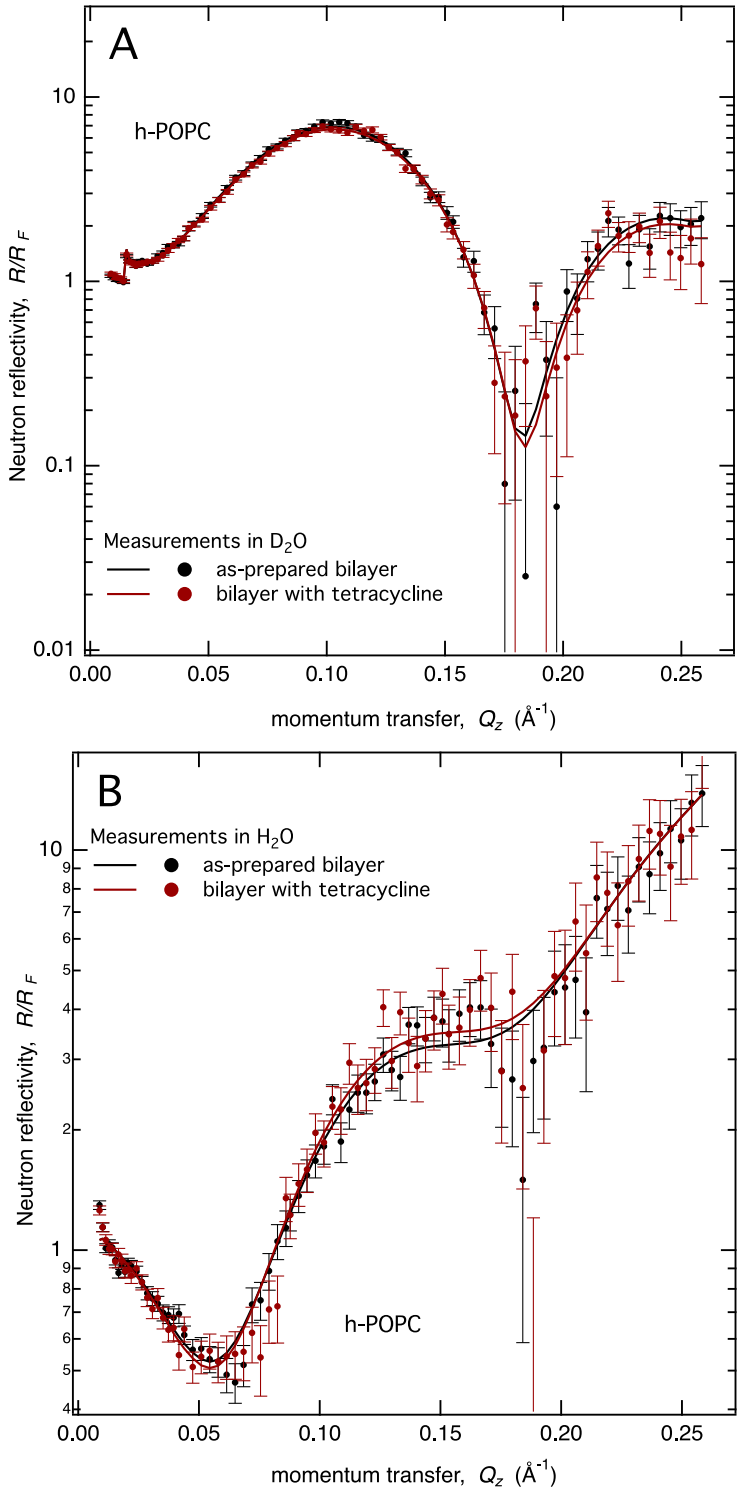

Figure S21: Fresnel normalized neutron reflectivity for the measurements of the as-prepared lipid bilayer and after adding of 5 µM tetracycline.

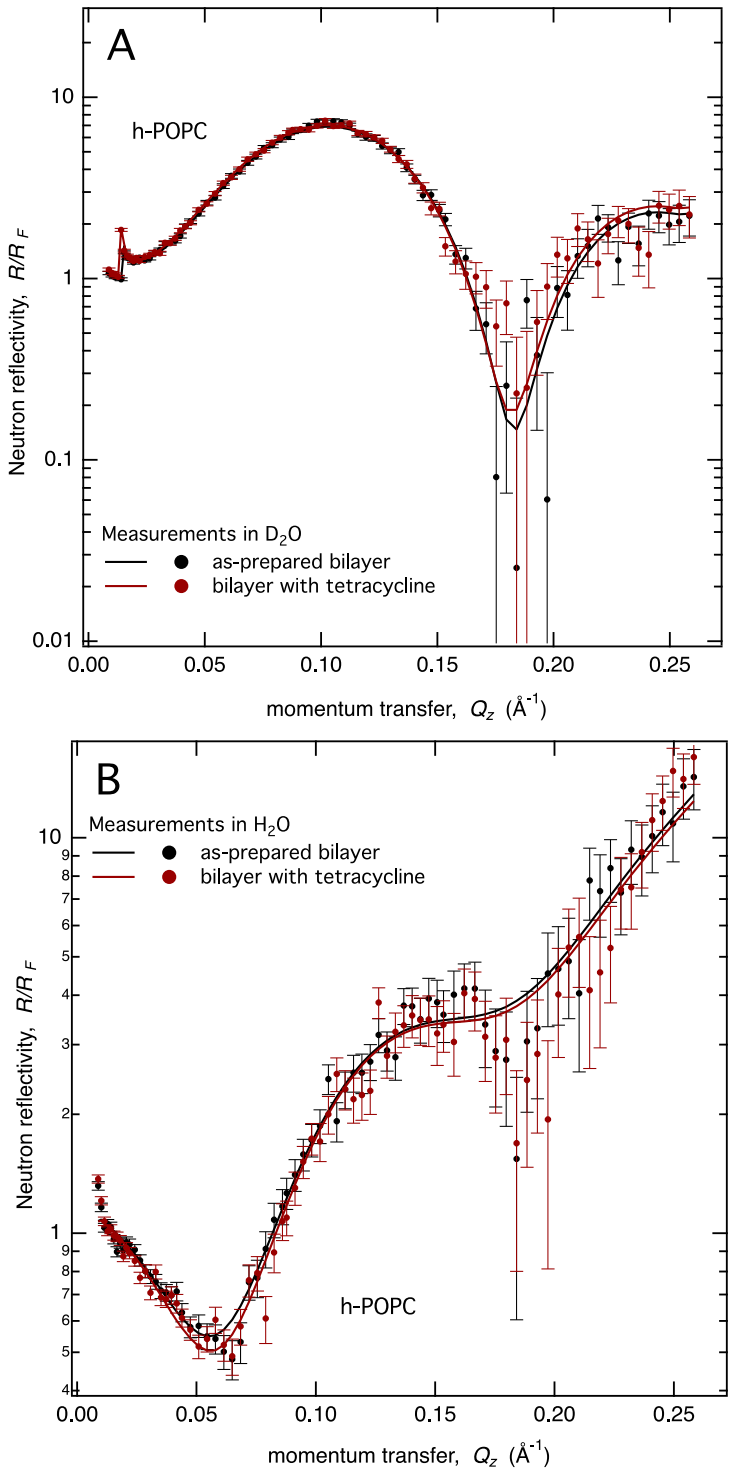

Figure S22: Fresnel normalized neutron reflectivity for the measurements of the as-prepared lipid bilayer and after adding of 36 µM tetracycline.

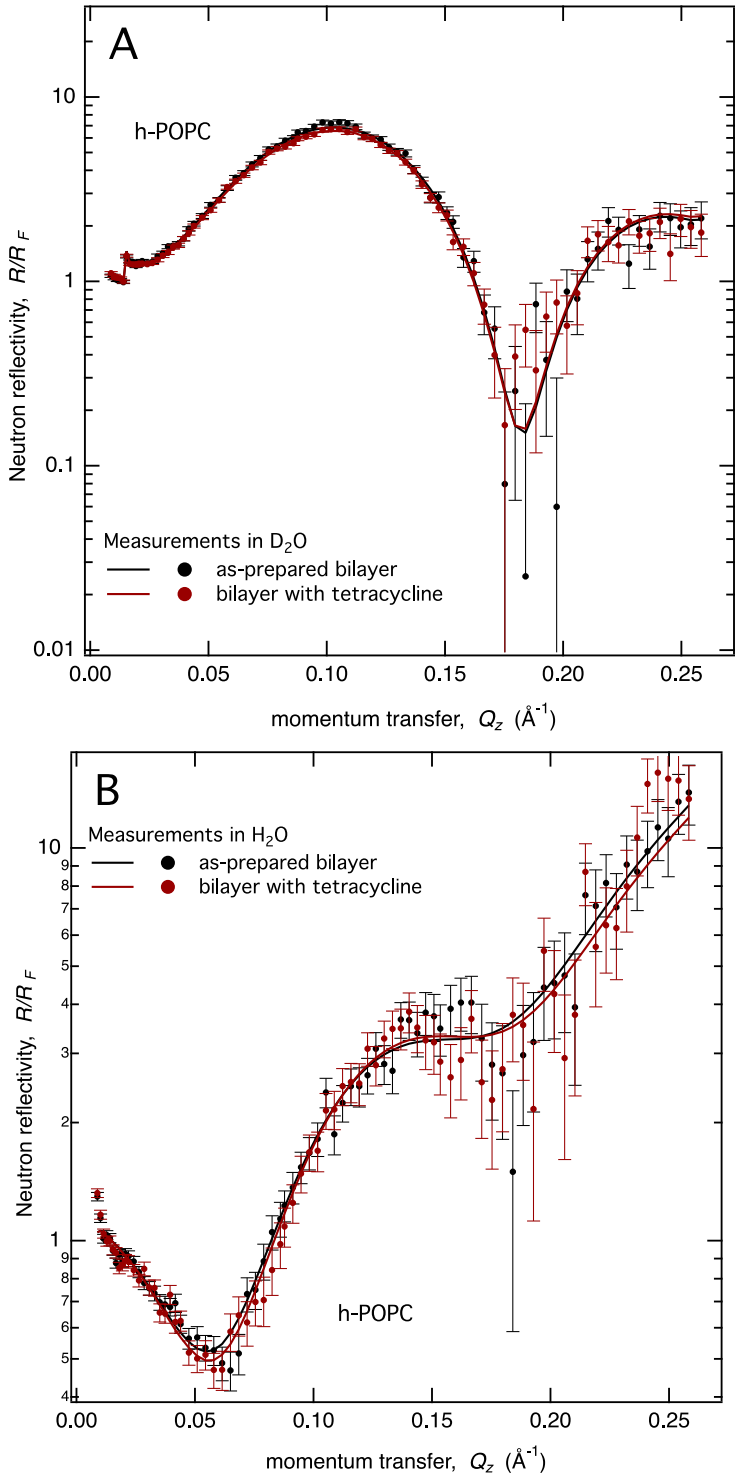

Figure S23: Fresnel normalized neutron reflectivity for the measurements of the as-prepared lipid bilayer and after a final rinse with pure water.

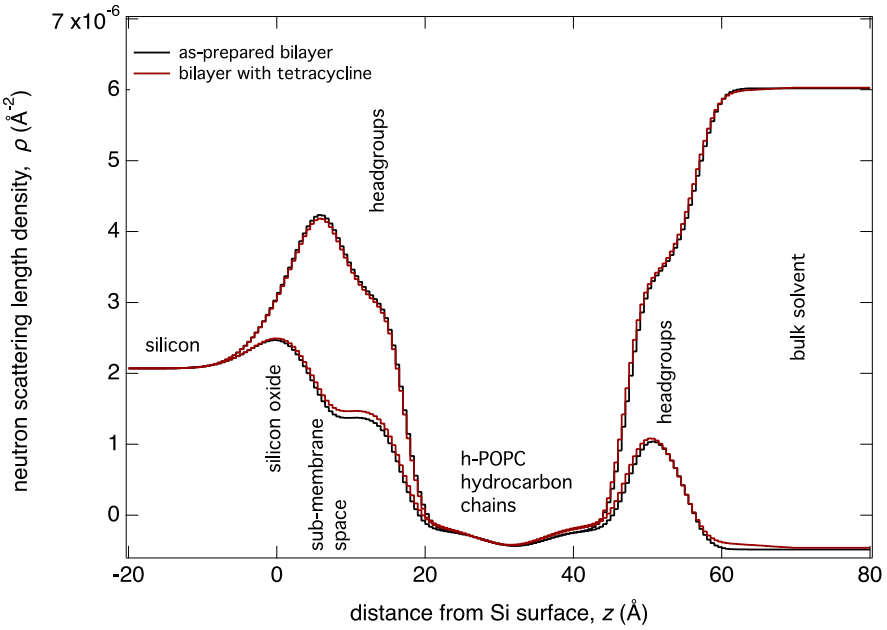

Figure S24: Best-fit nSLD profiles for the measurements of the as-prepared lipid bilayer and after adding 5 µM tetracycline.

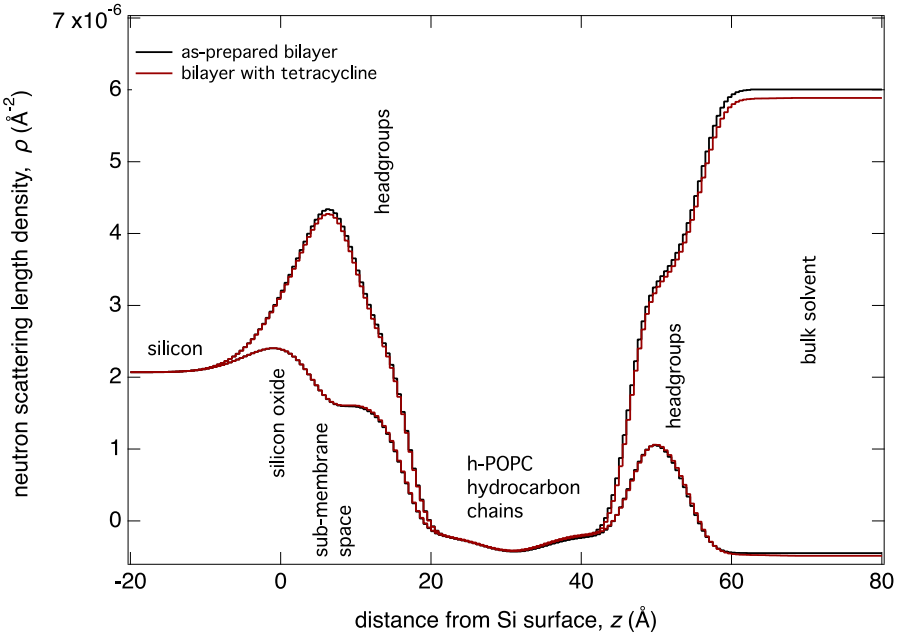

Figure S25: Best-fit nSLD profiles for the measurements of the as-prepared lipid bilayer and after adding 36 µM tetracycline.

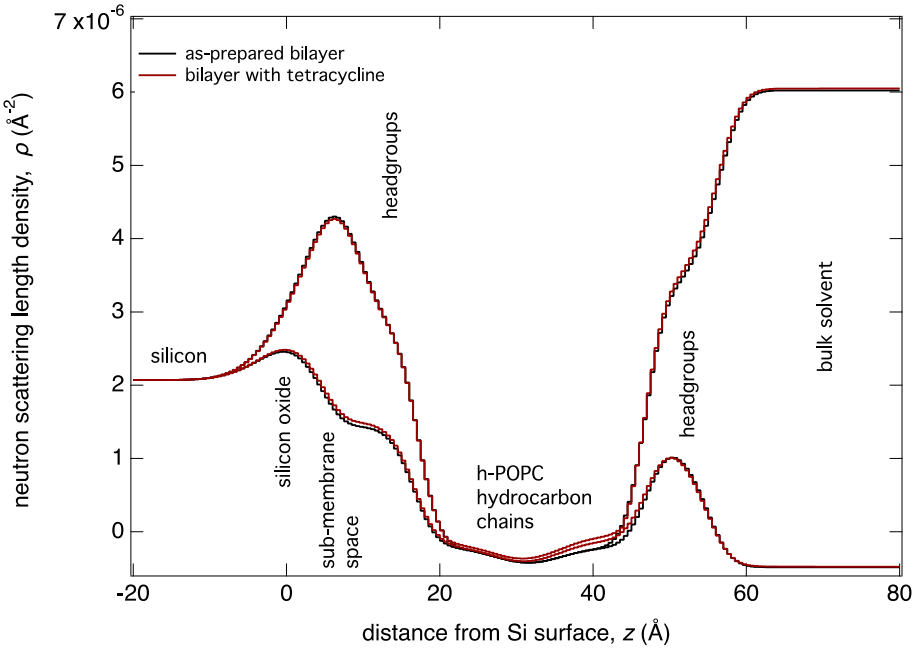

Figure S26: Best-fit nSLD profiles for the measurements of the as-prepared lipid bilayer and after adding 36 µM tetracycline.