Supplementary Information

Steroids and steroid-like compounds alter the ion permeability of phospholipid bilayers via distinct interactions with lipids and interfacial water

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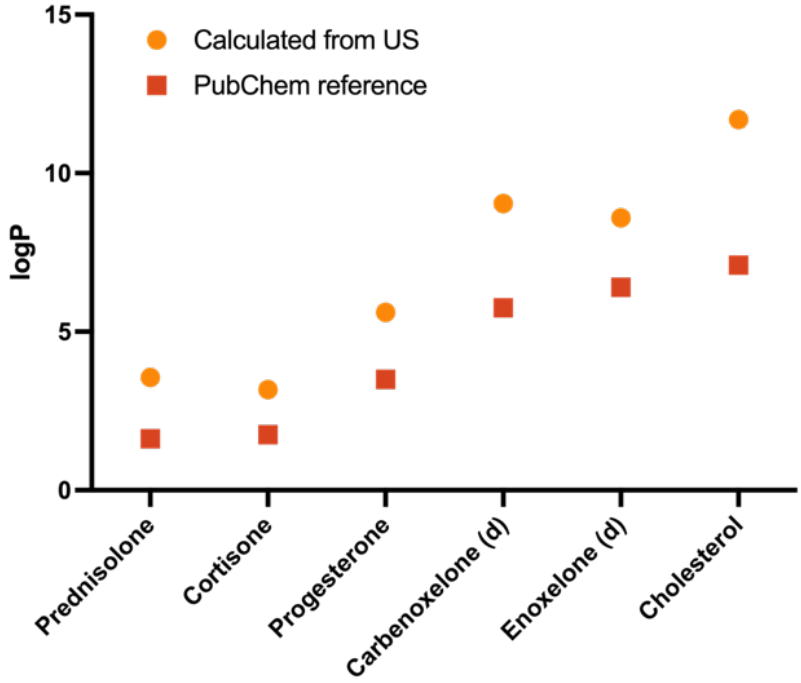

Figure S1. Comparisons between partition coefficient (LogP) values calculated from Umbrella Sampling simulations (US) in this work and values from PubChem calculated using XLOGP3. LogPs for carbenoxolone and enoxolone are from their protonated forms.

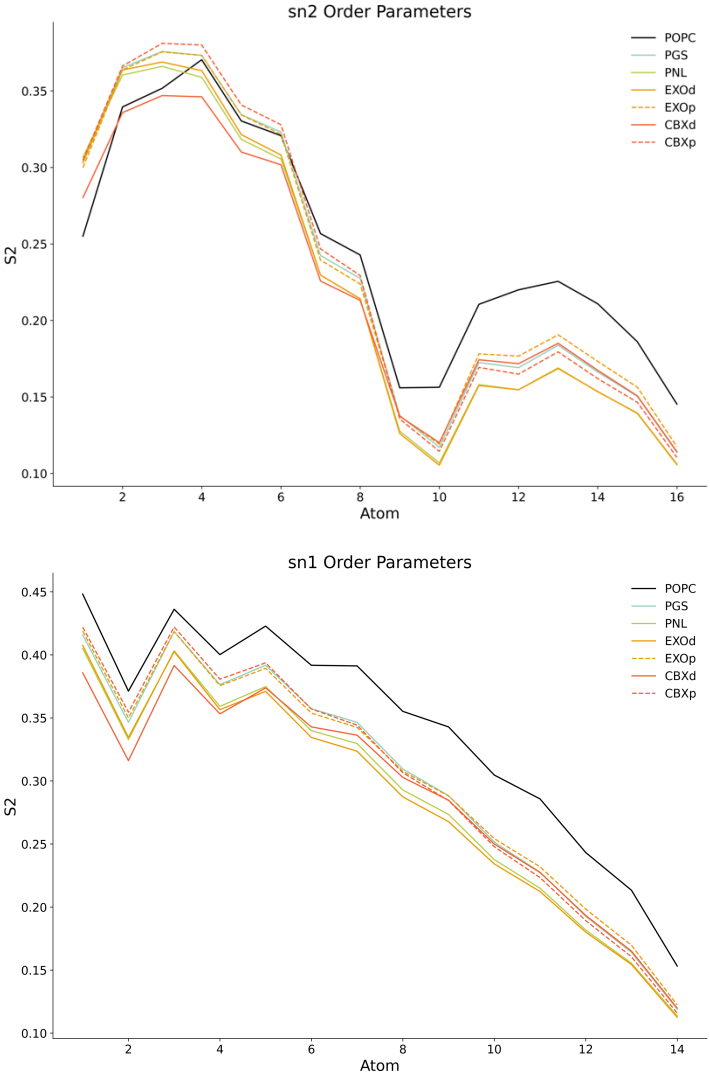

Figure S2. Order parameters for the *sn2* and *sn1* POPC lipid tails POPC bilayers in the absence and presence of steroid and steroid-like compounds. Order parameters were calculated using 1500 frames from the last 300 ns of a 1500-ns simulation.

Table S2. Hydrogen bond interactions of steroids with lipid headgroups, interfacial water and other steroids. The interactions were calculated using 1500 frames from the last 300 ns of a 1500-ns simulation using a 0.25 nm cutoff. The values given represent the average number of steroids involved in the interaction out of the 25 steroids in the system. Steroid atom numbers referenced in this table are shown in Figure S3. Interactions in bold represent interactions that occur in 5 or more steroids.

Cortisone (CTN)		
CTN-O5	**H2O**	**21.19**
CTN-O3	**H2O**	**13.66**
CTN-O2	**H2O**	**17.04**
H_2O	CTN-OH (H22)	3.55
H_2O	**CTN-OH (H28)**	**8.81**
POPC-C=O sn1	CTN-OH (H22)	0.61
POPC-C=O sn1	CTN-OH (H28)	1.35
POPC-C=O sn2	CTN-OH (H22)	0.46
POPC-C=O sn2	CTN-OH (H28)	2.62
POPC-PO4	**CTN-OH (H22)**	**20.29**
POPC-PO4	**CTN-OH (H28)**	**11.29**
CTN-O5	CTN-OH (H22)	0.16
CTN-O5	CTN-OH (H28)	0.05
CTN-O3	CTN-OH (H22)	0.05
CTN-O3	CTN-OH (H28)	3.14
CTN-O2	CTN-OH (H22)	0.00
CTN-O2	CTN-OH (H28)	0.00
Prednisolone (PNL)		
PNL-O5	**H_2O**	**21.45**
PNL-O3	**H_2O**	**13.92**
H_2O	PNL-OH (H22)	3.44
H_2O	**PNL-OH (H23)**	**8.66**
H_2O	**PNL-OH (H28)**	**8.98**
POPC-C=O sn1	PNL-OH (H22)	1.22
POPC-C=O sn1	PNL-OH (H28)	1.02

POPC-C=O sn2	PNL-OH (H22)	1.29
POPC-C=O sn2	PNL-OH (H28)	1.75
POPC-PO4	**PNL-OH (H22)**	**13.53**
POPC-PO4	**PNL-OH (H28)**	**12.08**
PNL-O5	PNL-OH (H22)	0.02
PNL-O5	PNL-OH (H23)	0.02
PNL-O5	PNL-OH (H28)	0.02
PNL-O3	PNL-OH (H22)	0.10
PNL-O3	PNL-OH (H28)	4.13

Progesterone (PGS)		
PGS-O1	**H_2O**	**18.91**
PGS-O2	**H_2O**	**9.51**

Carbenoxolone					
deprotonated (CBXd)			**protonated (CBXp)**		
CBXd-O3	**H_2O**	**21.66**	**CBXp-O4**	**H2O**	
CBXd-O4	**H_2O**	**22.51**	**16.85**		
CBXd-O2	**H_2O**	**17.15**	**CBXp-O2**	**H2O**	
CBXd-O5	**H_2O**	**13.48**	**17.27**		
CBXd-O6	**H_2O**	**20.82**	**CBXp-O5**	**H2O**	
CBXd-O7	**H_2O**	**21.36**	**16.24**		
			CBXp-O7	**H2O**	
			16.08		
			H_2O	**CBXp (H45)**	
			8.82		
			H_2O	**CBXp (H50)**	
			7.44		
			POPC-C=O sn1	CBXp (H45)	
			3.56		
			POPC-C=O sn1	CBXp (H50)	0.0
			POPC-C=O sn2	CBXp (H45)	
			3.56		

			POPC-C=O sn2	CBXp (H50)	
			1.78		
			POPC-PO4	**CBXp (H45)**	
			8.65		
			POPC-PO4	**CBXp (H50)**	
			14.74		
Enoxolone					
deprotonated (EXOd)			**protonated (EXOp)**		
EXOd-O3	**H_2O**		**EXOp-O4**	**H_2O**	
24.82			**17.11**		
EXOd-O4	**H_2O**		**EXOp-O2**	**H_2O**	
24.65			**18.49**		
EXOd-O2	**H_2O**		**H_2O**	**EXOp (H45)**	
23.03			**12.60**		
H_2O	**EXOd (H45)**		**H_2O**	**EXOp (H46)**	
10.41			**9.21**		
POPC-C=O sn1	EXOd (H45)	3.36	POPC-C=O sn1	EXOp (H45)	
POPC-C=O sn2	EXOd (H45)	4.73	2.12		
POPC-PO4	**EXOd (H45)**	**5.23**	POPC-C=O sn2	EXOp (H45)	
			4.11		
			POPC-C=O sn2	EXOp (H46)	
			4.00		
			POPC-PO4	**EXOp (H45)**	
			5.45		
			POPC-PO4	**EXOp(H46)**	
			7.88		

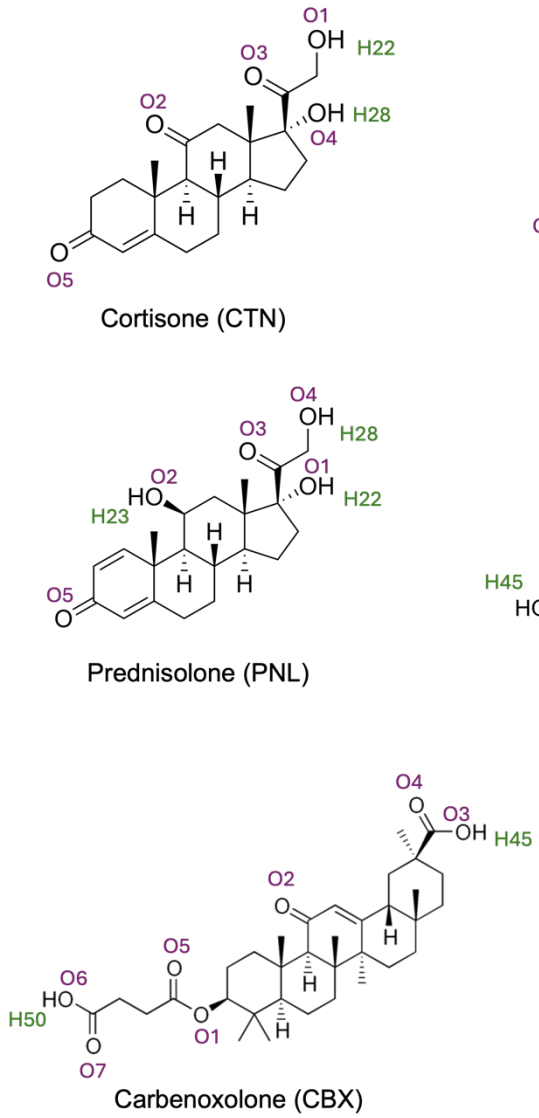

Figure S3. Atom numbers for steroids used in the analysis of steroid-lipid and steroid-water interactions reported in Table S2.

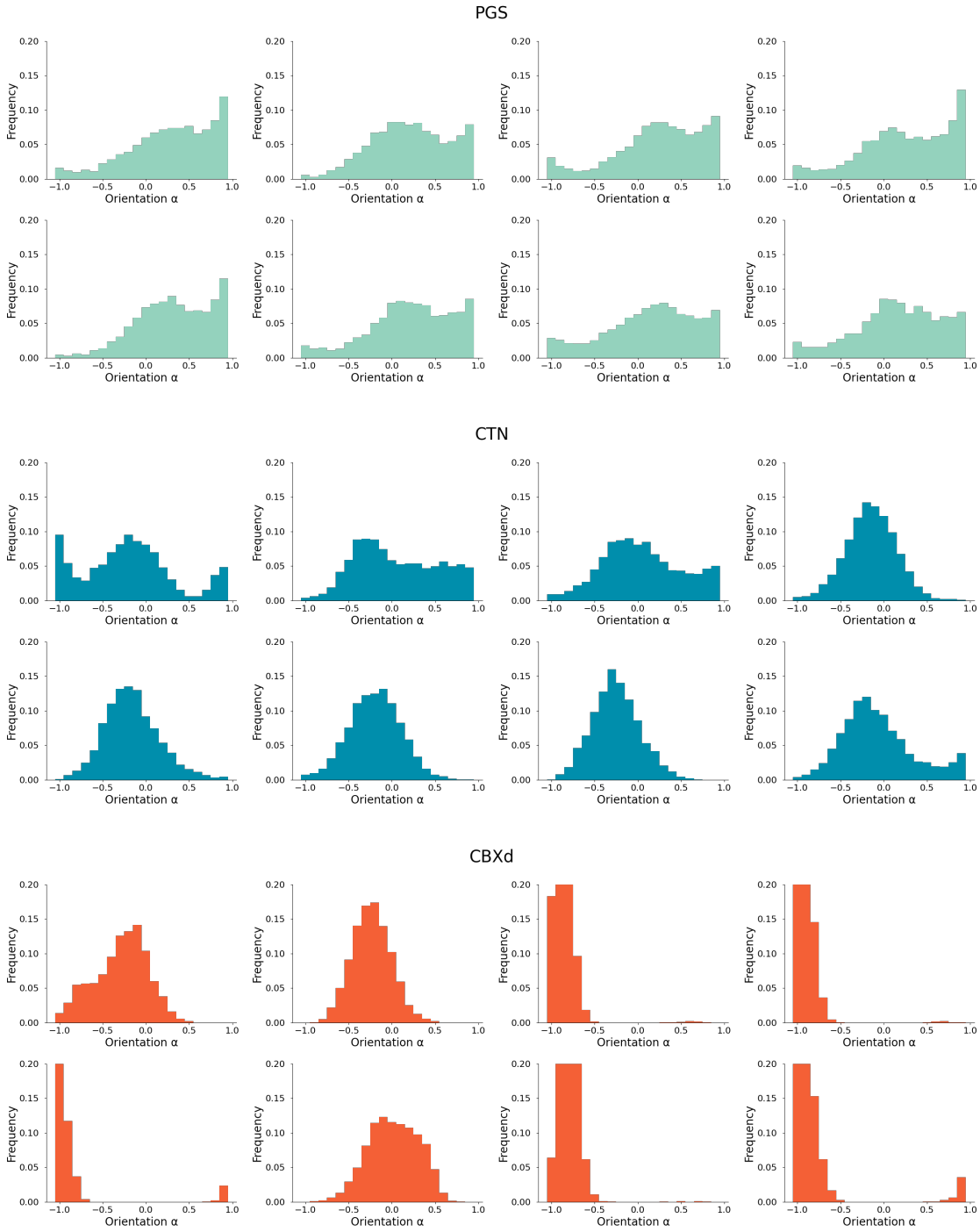

Figure S4. Orientation of PGS, CTN and CBX(d) in a POPC bilayer. Representative normalised distributions of orientation for eight of the 25 steroids in the system. Orientation was calculated using 6000 frames from the last 1200 ns of a 1500-ns simulation. Orientation was defined as the angle, α, formed by a vector connecting the C3 and C17 in the steroid and a vector running along the normal to the membrane.

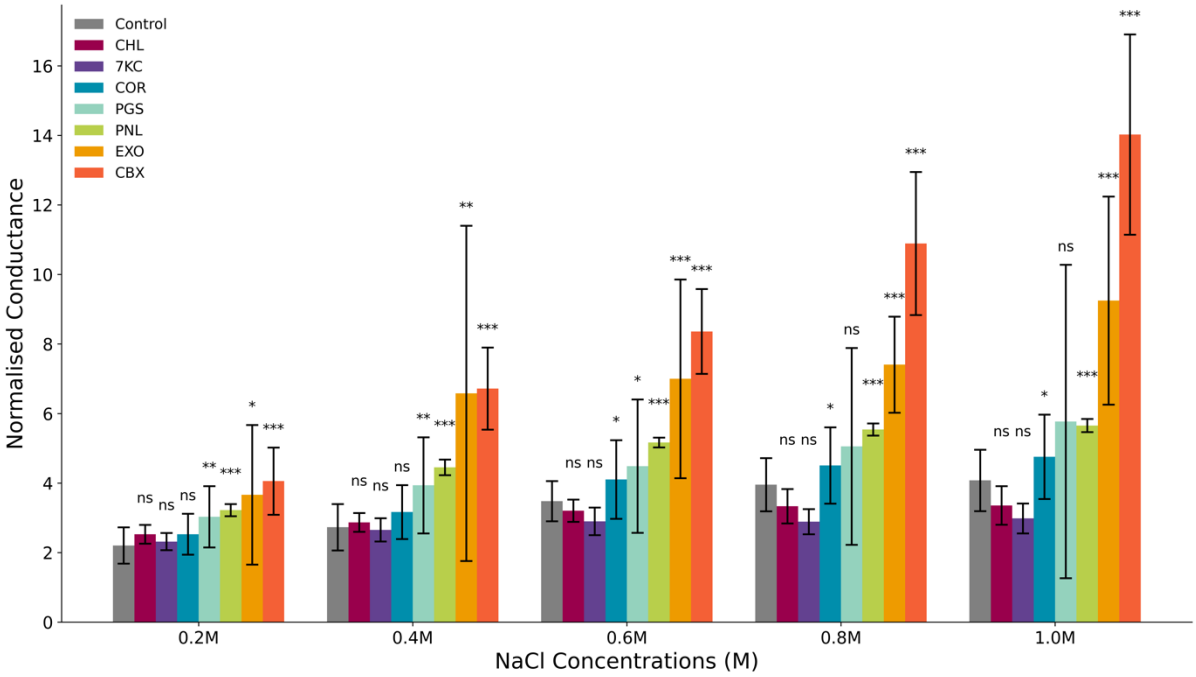

Figure S5. Normalised conductance of steroid-treated POPC bilayers for increasing NaCl concentrations from 0.2 M to 1.0 M. Membranes were treated with 100 μM drug in tris buffer (10 mM NaCl and 10 mM Tris) or tris buffer only for control experiments. Normalised conductance values are reported as average ± standard error of the means over at least 6 independent experiments (Control n = 17, Cholesterol (CHL) n = 10, 7-ketocholesterol (7-KC) n = 6, Cortisone (CTN) n = 18, Progesterone (PGS) n = 25, Prednisolone (PNL) n = 11, Enoxolone (EXO) n = 19, Carboxolone (CBX) n=12. Indicated p values from t-tests: ns = not significant, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

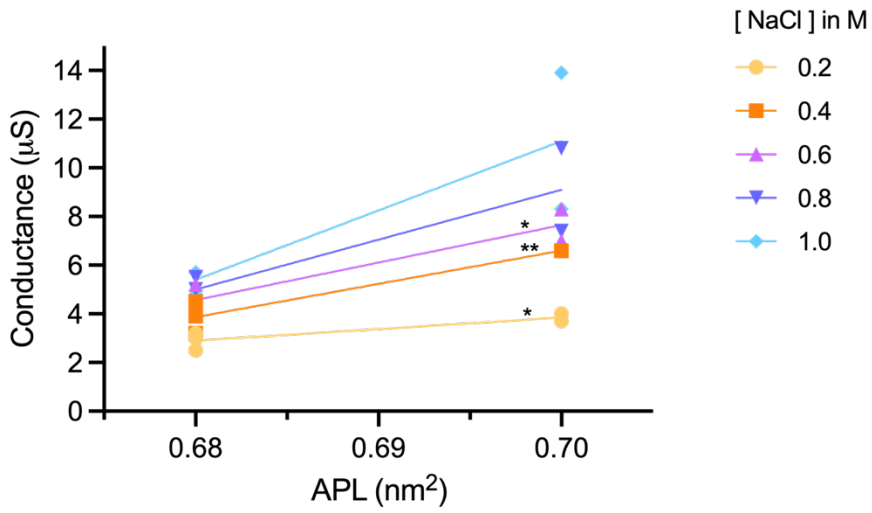

Figure S6. Normalised conductance vs Area per Lipid (APL). The normalised conductance for each of the five steroids or steroid-like compounds for increasing concentrations of NaCl from the EIS experiments is plotted against the APL calculated from the MD simulations. Indicated p values from t-tests comparing compounds with APL = 0.68 and APL = 0.70: * = $p \leq 0.05$, ** = $p \leq 0.01$.