SUPPORTING INFORMATION

The flip-flop of steroids in phospholipid bilayers: effects of the chemical structure on transbilayer diffusion

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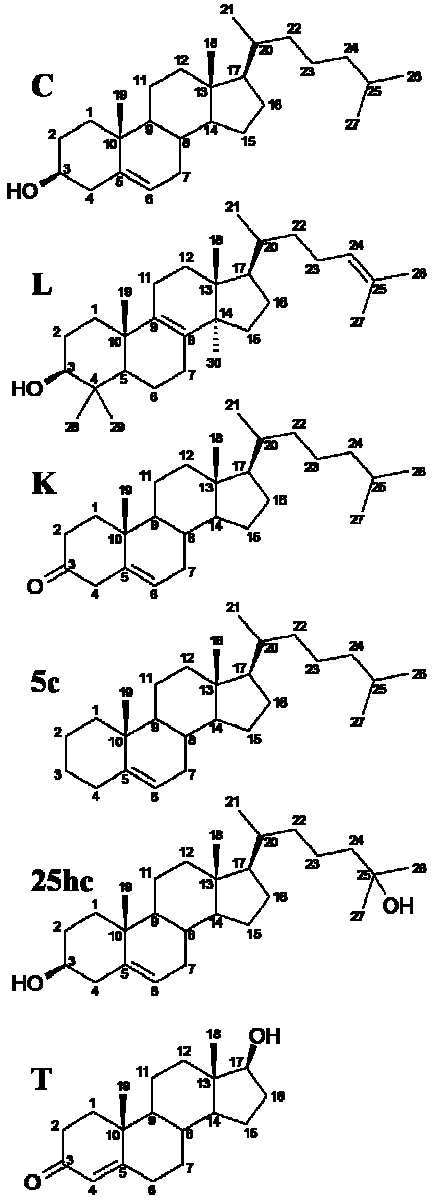
1. Parameters used in calculations for the steroids

Chart S1. Molecular structures of the steroids with atom numbering.[a]



[a] **C**=cholesterol, **L**=lanosterol, **K**=ketosterone, **5c**=5-cholestene, **25hc**=25-hydroxycholesterol, **T**=testosterone.

Table S1. Parameters used in calculations for the steroids [1].

van der Waals radii	H: 1.00Å; C: 1.85Å; O: 1.50Å [2]
Connolly surface	probe sphere radius = 3Å; vertex density = 13Å^{-2} [3]
atomic charges	OPLS force field [4] (see table S2)
atomic polarizabilities ($4\pi\varepsilon_0$ Å^3)	from bond polarizabilities [5] [a] H–C: 0.65; C–C: 0.57; C=C: 1.65; C–O: 0.64; O–H: 0.73; C=O: 1.43
internal dielectric constant	ε_{in} = 2 [6]
Born empirical parameters	F = 8; C_0 = 0.3028; C_1 = 1.009; D = 0.00 Å^{-1}; E = -0.16 Å^{-1} [b]

[a] Half of a bond polarizability is assigned to each of the two connected atoms, hence the polarizability of an atom is obtained as half the sum of the polarizabilities of all the bonds in which it is involved; no atomic polarizability is attributed to H atoms, so that the whole polarizability of any C–H and O–H bond is attributed to the C and O bonded atom, respectively.

[b] Obtained from optimization with respect to finite difference solution of the Poisson equation, for a test set of benzene derivatives (see SI of ref. [1]), according to the procedure reported in ref. [7].

Table S2. OPLS [4] atomic charges for the steroids (atom numbering as in chart S1).

	C	**L**	**K**	**5c**	**25hc**	**T**
1	–0.120	–0.120	–0.120	–0.120	–0.120	–0.120
2	–0.120	–0.120	–0.120	–0.120	–0.120	–0.120
3	+0.205	+0.205	+0.470	–0.120	+0.205	+0.470
4	–0.120	0.000	–0.120	–0.120	–0.120	–0.120
5	0.000	0.000	0.000	0.000	0.000	0.000
6	–0.115	–0.120	–0.115	–0.115	–0.115	–0.115
7	–0.120	–0.120	–0.120	–0.120	–0.120	–0.120
8	–0.060	0.000	–0.060	–0.060	–0.060	–0.060
9	–0.060	0.000	–0.060	–0.060	–0.060	–0.060
10	0.000	0.000	0.000	0.000	0.000	0.000
11	–0.120	–0.120	–0.120	–0.120	–0.120	–0.120
12	–0.120	–0.120	–0.120	–0.120	–0.120	–0.120
13	0.000	0.000	0.000	0.000	0.000	0.000
14	–0.060	0.000	–0.060	–0.060	–0.060	–0.060
15	–0.120	–0.120	–0.120	–0.120	–0.120	–0.120
16	–0.120	–0.120	–0.120	–0.120	–0.120	–0.120
17	–0.060	–0.060	–0.060	–0.060	–0.060	+0.205
18	–0.180	–0.180	–0.180	–0.180	–0.180	–0.180
19	–0.180	–0.180	–0.180	–0.180	–0.180	–0.180
20	–0.060	–0.060	–0.060	–0.060	–0.060	–
21	–0.180	–0.180	–0.180	–0.180	–0.180	–
22	–0.120	–0.120	–0.120	–0.120	–0.120	–
23	–0.120	–0.120	–0.120	–0.120	–0.120	–
24	–0.120	–0.115	–0.120	–0.120	–0.120	–
25	–0.060	0.000	–0.060	–0.060	+0.265	–
26	–0.180	–0.180	–0.180	–0.180	–0.180	–
27	–0.180	–0.180	–0.180	–0.180	–0.180	–
28	–	–0.180	–	–	–	–
29	–	–0.180	–	–	–	–
30	–	–0.180	–	–	–	–
$O(C_3)$	–0.700	–0.700	–0.470	–	–0.700	–0.470
$H(OC_3)$	+0.435	+0.435	–	–	+0.435	–
$O(C_{25})$	–	–	–	–	–0.700	–
$H(OC_{25})$	–	–	–	–	+0.435	–
$O(C_{17})$	–	–	–	–	–	–0.700
$H(OC_{17})$	–	–	–	–	–	+0.435
H(C=)	+0.115	+0.115	+0.115	+0.115	+0.115	+0.115
all other H	+0.060	+0.060	+0.060	+0.060	+0.060	+0.060

2. Free energy surfaces and diffusion tensors calculated for the steroids.

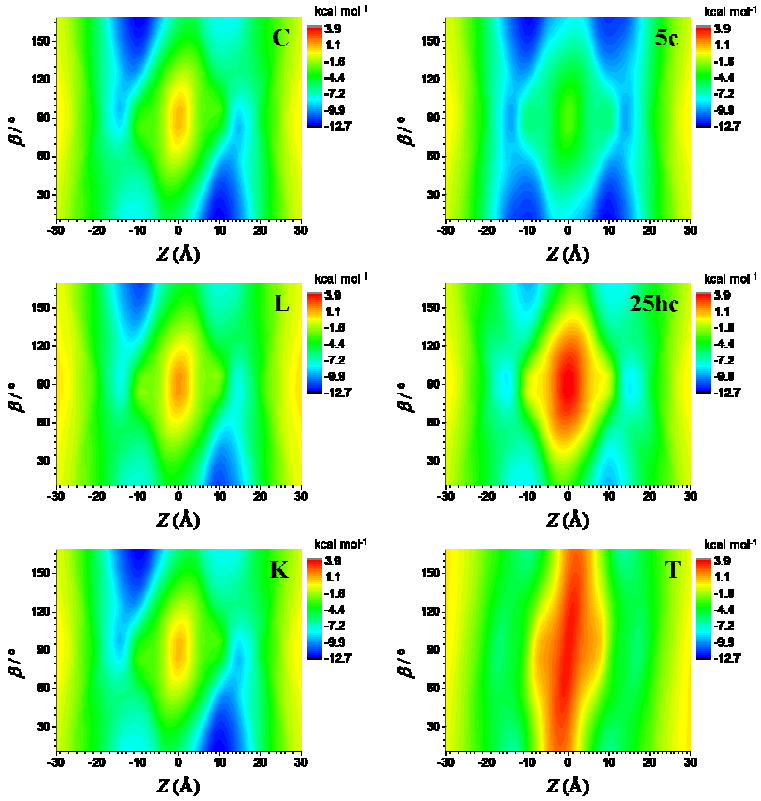


Figure S1. Free energy surface calculated for the steroids as a function of the position Z of the molecular centre of mobility (CM in figure 1 in the main text) along the bilayer normal (**N**), and of the angle β between **N** and the long molecular axis (z in figure 1 in the main text). Z=0 at the bilayer midplane.

Table S3. Diagonal elements of the roto-translational diffusion tensors calculated for the steroids.[a]

	D^T_{xx} (cm^2 s^{-1})	D^T_{yy} (cm^2 s^{-1})	D^T_{zz} (cm^2 s^{-1})	D^R_{xx} (cm^2 s^{-1})	D^R_{yy} (cm^2 s^{-1})	D^R_{zz} (cm^2 s^{-1})
C	$1.1\cdot10^{-6}$	$1.1\cdot10^{-6}$	$1.3\cdot10^{-6}$	$1.5\cdot10^{8}$	$1.5\cdot10^{8}$	$5.3\cdot10^{8}$
L	$1.1\cdot10^{-6}$	$1.0\cdot10^{-6}$	$1.3\cdot10^{-6}$	$1.7\cdot10^{8}$	$1.5\cdot10^{8}$	$5.0\cdot10^{8}$
K	$1.1\cdot10^{-6}$	$1.1\cdot10^{-6}$	$1.3\cdot10^{-6}$	$1.6\cdot10^{8}$	$1.6\cdot10^{8}$	$5.3\cdot10^{8}$
5c	$1.1\cdot10^{-6}$	$1.1\cdot10^{-6}$	$1.3\cdot10^{-6}$	$1.6\cdot10^{8}$	$1.6\cdot10^{8}$	$5.4\cdot10^{8}$
25hc	$1.1\cdot10^{-6}$	$1.0\cdot10^{-6}$	$1.3\cdot10^{-6}$	$1.5\cdot10^{8}$	$1.5\cdot10^{8}$	$5.2\cdot10^{8}$
T	$1.4\cdot10^{-6}$	$1.3\cdot10^{-6}$	$1.6\cdot10^{-6}$	$3.8\cdot10^{8}$	$3.8\cdot10^{8}$	$7.2\cdot10^{8}$

[a] D_{ii}^T and D_{ii}^R represent the diffusion coefficients for translation along and rotation around the i molecular axis, respectively.

3. Comparison between results obtained for cholesterol with OPLS [4] and RESP (B3LYP/6-31G) [8,9] atomic charges.**

Table S4. OPLS and RESP (B3LYP/6-31g**) atomic charges[a] for cholesterol (atom numbering as in chart S1).

	C-OPLS	C-RESP
1	–0.120 (+0.060)	–0.212 (+0.050)
2	–0.120 (+0.060)	–0.058 (+0.061)
3	+0.205 (+0.060)	+0.278 (+0.002)
4	–0.120 (+0.060)	–0.248 (+0.088)
5	0.000	–0.068
6	–0.115 (+0.115)	–0.228 (+0.113)
7	–0.120 (+0.060)	–0.054 (+0.045)
8	–0.060 (+0.060)	–0.016 (+0.035)
9	–0.060 (+0.060)	+0.011 (+0.002)
10	0.000	+0.285
11	–0.120 (+0.060)	–0.088 (+0.029)
12	–0.120 (+0.060)	–0.088 (+0.006)
13	0.000	+0.316
14	–0.060 (+0.060)	+0.022 (+0.006)
15	–0.120 (+0.060)	–0.136 (+0.036)
16	–0.120 (+0.060)	–0.080 (+0.026)
17	–0.060 (+0.060)	–0.008 (–0.012)
18	–0.180 (+0.060)	–0.377 (+0.079)
19	–0.180 (+0.060)	–0.277 (+0.067)
20	–0.060 (+0.060)	+0.108 (+0.004)
21	–0.180 (+0.060)	–0.349 (+0.086)
22	–0.120 (+0.060)	–0.082 (+0.024)
23	–0.120 (+0.060)	+0.064 (0.000)
24	–0.120 (+0.060)	–0.235 (+0.056)
25	–0.060 (+0.060)	+0.315 (–0.018)
26	–0.180 (+0.060)	–0.391 (+0.093)
27	–0.180 (+0.060)	–0.391 (+0.093)
$O(C_3)$	–0.700	–0.632
$H(OC_3)$	+0.435	+0.391

[a] The charges of hydrogen atoms are reported in brackets, in the line of the carbon atom to which they are linked.

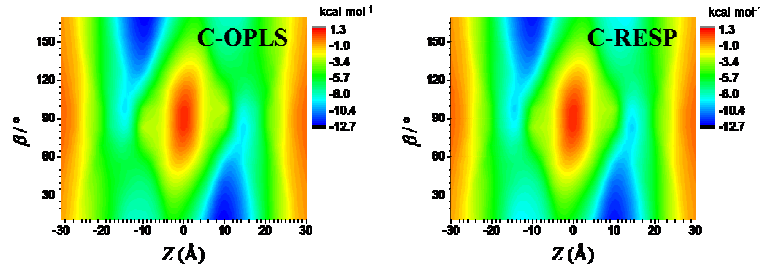


Figure S1. Free energy surface calculated for cholesterol with OPLS and RESP (B3LYP/6-31g**) atomic charges, as a function of the position Z of the molecular centre of mobility (CM in figure 1 in the main text) along the bilayer normal (**N**), and of the angle β between **N** and the long molecular axis (z in figure 1 in the main text). Z=0 at the bilayer midplane.

Table S5. Free energy barriers[a] and rates[b] for the transitions in figure 2 of the main text, and flip-flop rate constants, k_{ff}, calculated for cholesterol with OPLS and RESP (B3LYP/6-31g**) atomic charges.

	1→2 through **A**		**2→1** through **A**		**1→3** through **C**		**3→1** through **C**		k_{ff} (10^4 s^{-1})
	E_A-E_1	W_{21}	E_A-E_2	W_{12}	E_C-E_1	W_{31}	E_C-E_3	W_{13}	
C-OPLS	10.5	$1.6\cdot10^4$	4.6	$5.5\cdot10^6$	11.1	$7.3\cdot10^3$	5.3	$2.6\cdot10^6$	2.0
C-RESP	8.4	$1.2\cdot10^5$	3.8	$1.2\cdot10^7$	11.1	$1.3\cdot10^4$	6.5	$1.3\cdot10^6$	4.6

[a] E_s-E_j (eq. 5 in the main text) in k_BT units; [b] W_{ij} (eq. 7 in the main text) in s^{-1} units.

3. References.

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