Biophysical Journal, Volume *115*

Supplemental Information

Fluorinated Alcohols' Effects on Lipid Bilayer Properties

Mike Zhang, Thasin Peyear, Ilias Patmanidis, Denise V. Greathouse, Siewert J. Marrink, Olaf S. Andersen, and Helgi I. Ingólfsson

TABLE S1:
Vapor pressure of the tested fluoroalcohols and their non-fluorinated counterparts

Name*	**Vapor pressure† (mm Hg)**
Ethanol	44‡
2-Propanol	33
tert-Butanol	44
TFE	70¶
HFIP	115
PFTB	270

***Name**, alcohol name. †**Vapor pressure** in mmHg at 20 °C except the ‡value at 19 °C and the ¶value at 25 °C. Values for the non-fluorinated alcohols were obtained from www.cameochemicals.noaa.gov; values for the fluoroalcohols are form SynQuest Laboratories, Inc. (Alachua, FL, www.synquestlabs.com).

TABLE S2:
Changes in bilayer partitioning with the addition of a methyl or trifluoromethyl group

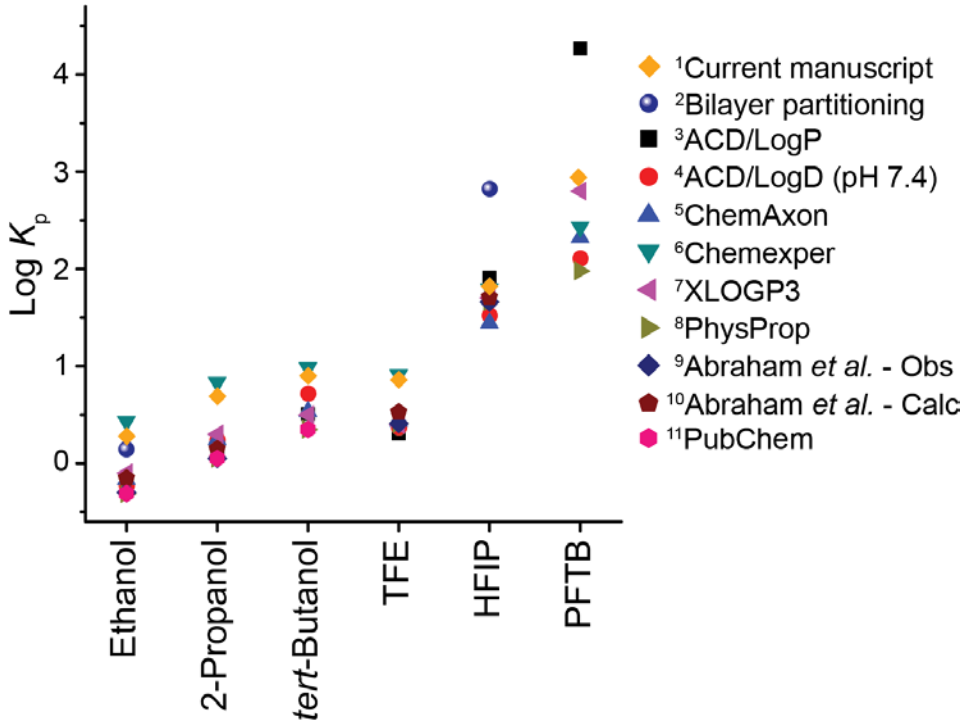
Change	From	To	Increment*	Geometric mean	Range/2 or sd	$\Delta\Delta G_p^{I\to II}$ (k_BT)
H→CH$_3$	Ethanol	2-Propanol	2.6			
	Ethanol	*tert*-Butanol	2.0	2.3	0.3	0.84
CH$_3$→CF$_3$	Ethanol	TFE	3.8			
	2-Propanol	HFIP	3.7			
	tert-Butanol	PFTB	4.8	4.1	0.6	1.41
H→CF$_3$	TFE	HFIP	9.3			
	TFE	PFTB	11.0	10.1	0.9	2.32

***Increment** is the relative increase in partitioning, which was estimated as $\left(K_p^{II}/K_p^{I}\right)^{1/n}$, where *n* is the number of substitutions (e.g., 2 when comparing ethanol and *tert*-Butanol).
$\Delta\Delta G_p^{I\to II} = k_BT\cdot\ln\{\text{Average Increment}\}$, where k_B is Boltzmann's constant and *T* the temperature in Kelvin.

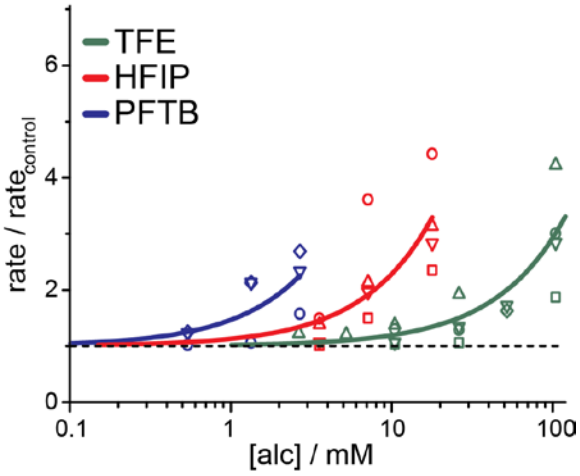
TABLE S3:
Changes in bilayer properties as measured by molecular dynamics simulations

Name*	Sim. length (ns)	Alcohol counts	Area per lipid† (nm^2)			Bilayer thickness‡ (nm)			Average tail order parameter¶		
			mean	sd	se	mean	sd	se	mean	sd	se
DOPC only	344	0	0.602	0.001	0.002	4.15	0.03	0.01	0.188	0.005	0.001
Ethanol	353	160	0.610	0.008	0.003	4.12	0.05	0.02	0.183	0.009	0.003
2-Propanol	341	90	0.603	0.004	0.001	4.16	0.02	0.00	0.190	0.004	0.001
tert-Butanol	433	62	0.608	0.005	0.001	4.15	0.03	0.01	0.189	0.006	0.001
TFE	344	60	0.604	0.004	0.001	4.16	0.02	0.01	0.191	0.001	0.001
HFIP	354	60	0.595	0.004	0.001	4.23	0.03	0.01	0.205	0.005	0.001
PFTB	379	58	0.601	0.005	0.002	4.20	0.04	0.01	0.201	0.006	0.002

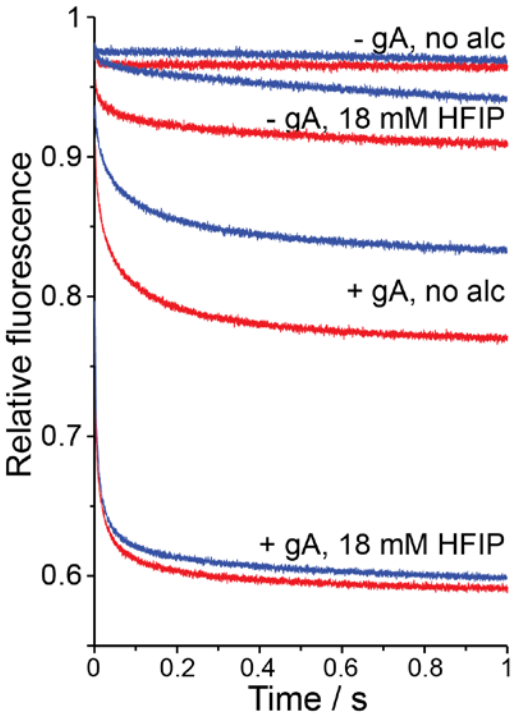
***Name**, alcohol name. †**Area per lipid** in nm^2 was measured as the average bilayer area divided by the number of lipids per leaflet. ‡**Bilayer thickness** in nm was measured as the average distance between the DOPC phosphates in the opposing bilayer leaflets. ¶**Average tail order parameter** was measured as the average second-rank order parameter (P_2) for the tail lipid backbone bonds. All values are calculated from the last 100 ns of the simulations and all reported standard errors of the mean (se) are obtained from block averaging. The number of blocks was increased from a single block to the point where each block had only five data points and the se estimated as the maximum of largest 20% of the block sizes studied.



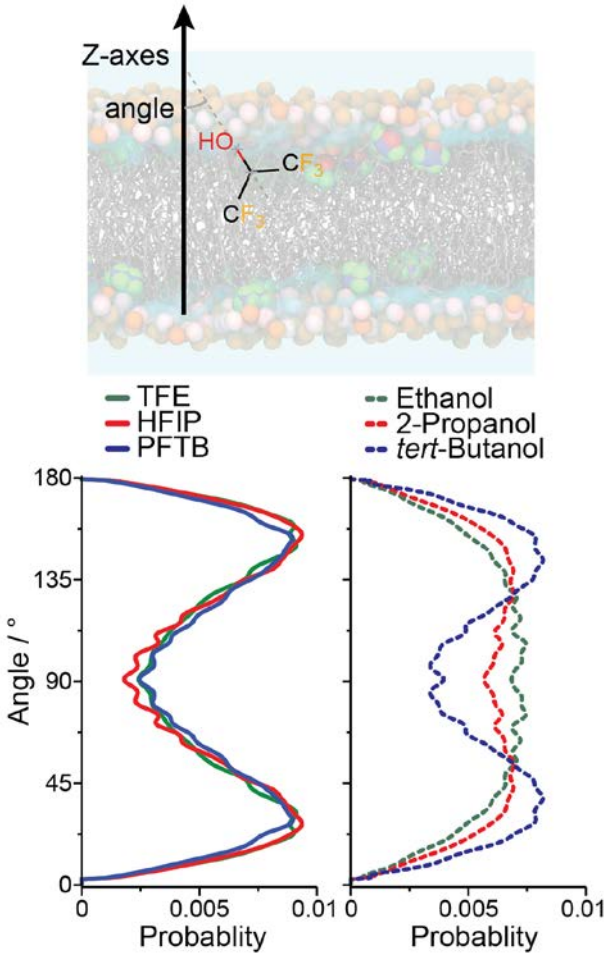
SUPPLEMENTARY FIGURE S1: Evaluating the partition coefficients (K_p) of the tested fluoroalcohols and their non-fluorinated counterparts. The values are either experimental or calculated octanol/water partition coefficients except for: ([1]) the lipid depletion experiments described in the main text; and ([2]) which is a water to 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) lipid bilayer partition coefficient from (1) for Ethanol and from (2) for HFIP. ([3, 4]) LogP and LogD (at pH 7.4), respectively, using the ACD/Labs algorithm (3), accessed Sept. 2015 from www.chemspider.com. ([5]) Predictions from www.chemicalize.org (ChemAxon Kft., Budapest, Hungary), accessed Sept. 2015. ([6]) Predictions from www.chemexper.com, accessed September 2015. ([7]) XLOGP3 algorithm (4), accessed Sept. 2015 from http://pubchem.ncbi.nlm.nih.gov/. ([8]) Experimental values and estimates from the PhysProp database (SRC, Syracuse, NY). ([9, 10]) Experimental observables and calculated estimated from (5). ([11]) Values from (6), accessed Sept. 2015 from http://pubchem.ncbi.nlm.nih.gov/.



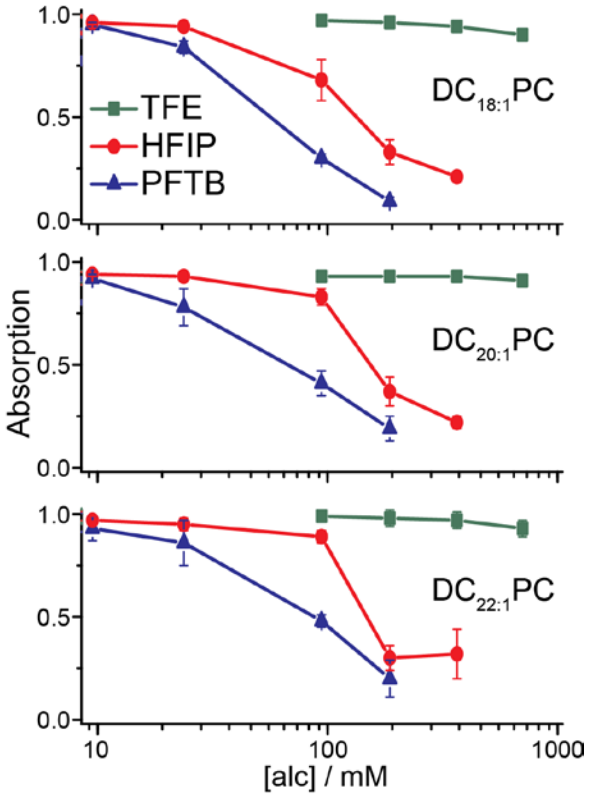
SUPPLEMENTARY FIGURE S2: Fluorinated alcohols bilayer-perturbing effect at pH 4.0. The dotted line represents no change, and the solid lines are f([alc]) = 1 + [alc]/D fits to the results; the differently shaped symbols for each alcohol are different days of experiment.



SUPPLEMENTARY FIGURE S3: Normalized fluorescence time courses observed with fluorophore-loaded LUVs incubated with and without HFIP, and with and without added gramicidin. Results from two experiments, one at pH 7 (red) and on at pH 4 (blue). Though the fluorescence quench at 1 s is slightly larger at pH 7 than at pH 4, the initial rates (the slopes of the fluorescence time course at 2 ms) vary little. (The different amplitudes reflect that the experiments were done with different LUV preparations.)



SUPPLEMENTARY FIGURE S4: The alcohols' orientation with respect to the bilayer normal. The alcohols angle with respect to the bilayer was approximated as the angle between the alcohols' oxygen-central carbon vector with respect to the *Z* axis of the simulation box (see schema). For all the alcohols, the angles were calculated based on the last 100 ns of each simulation and the binned, symmetrized angle distributions are shown. The increased density in the direction of 180 ° (aligned with the top leaflet) and 0 ° (aligned with the bottom leaflet) demonstrate the preferred orientation of the alcohols with the bilayer normal. The non-fluorinated, less hydrophobic alcohols have an overall wider distribution due to an increased likelihood of finding the alcohols in the aqueous phase. The fluorinated alcohols align more closely with the bilayer normal; the peak of the distributions for all three tested fluorinated alcohols is ~28 ° away from the box Z axes while *tert*-Butanol ~35 °.



SUPPLEMENTARY FIGURE S5: Fluorinated alcohols compromise bilayer integrity. Bilayer integrity was evaluated by monitoring the light absorption of multilamellar vesicle suspensions at increasing concentrations of TFE (green), HFIP (red), or PFTB (blue). Multilamellar vesicles were made from 1,2-dioleoyl-sn-glycero-3-phosphocholine ($DC_{18:1}PC$, top), 1,2-dieicosenoyl-*sn*-glycero-3-phosphocholine ($DC_{20:1}PC$, middle), and 1,2-dierucoyl-*sn*-glycero-3-phosphocholine ($DC_{22:1}PC$, bottom). The absorbances were normalized to the value in the absence of the fluoroalcohols. Average ± S.D. (n = 3) except for TFE with $DC_{22:1}PC$ vesicles, where n = 2 and the uncertainty is ± range/2. TFE had little effect at the concentrations tested, whereas HFIP and PFTB reduced the absorption (disrupted the vesicles) at the higher concentrations tested. For all three phospholipids tested, the liposomes were completely disrupted and formed a white precipitate at the highest HFIP and PFTB concentrations tested.

SUPPORTING REFERENCES

1. Terama, E., O.H. Ollila, E. Salonen, A.C. Rowat, C. Trandum, P. Westh, M. Patra, M. Karttunen, and I. Vattulainen. 2008. Influence of ethanol on lipid membranes: from lateral pressure profiles to dynamics and partitioning. J. Phys. Chem. B. 112: 4131–4139.

2. Ennaceur, S.M., and J.M. Sanderson. 2005. Micellar aggregates formed following the addition of hexafluoroisopropanol to phospholipid membranes. Langmuir. 21: 552–561.

3. Petrauskas, A., and E. Kolovanov. 2000. ACD/Log P method description. Perspectives in Drug Discovery and Design. 19: 99–116.

4. Cheng, T., Y. Zhao, X. Li, F. Lin, Y. Xu, X. Zhang, Y. Li, R. Wang, and L. Lai. 2007. Computation of octanol-water partition coefficients by guiding an additive model with knowledge. J. Chem. Inf. Model. 47: 2140–2148.

5. Abraham, M.H., H.S. Chadha, G.S. Whiting, and R.C. Mitchell. 1994. Hydrogen bonding. 32. An analysis of water-octanol and water-alkane partitioning and the delta log P parameter of Seiler. J. Pharm. Sci. 83: 1085–1100.

6. Hansch, C., A. Leo, and D.H. Hoekman. 1995. Exploring QSAR.: Hydrophobic, electronic, and steric constants. American Chemical Society.