Supporting Information for

Local partition coefficients govern solute permeability of cholesterol-containing membranes

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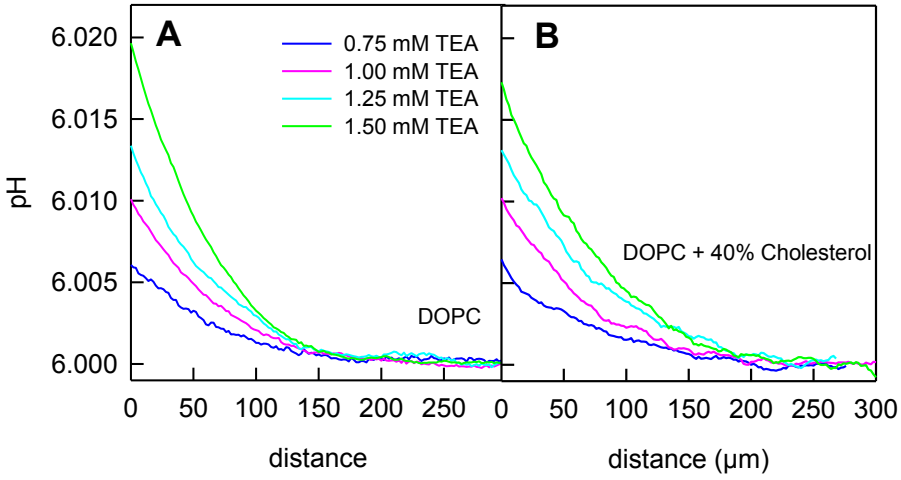

Figure S1: Representative pH profiles in the *trans* USL of a DOPC bilayer, which were induced by TEA added at the *cis* side of the membrane. The aqueous solutions contained 100 mM NaCl and 5 mM Mes (pH 7).

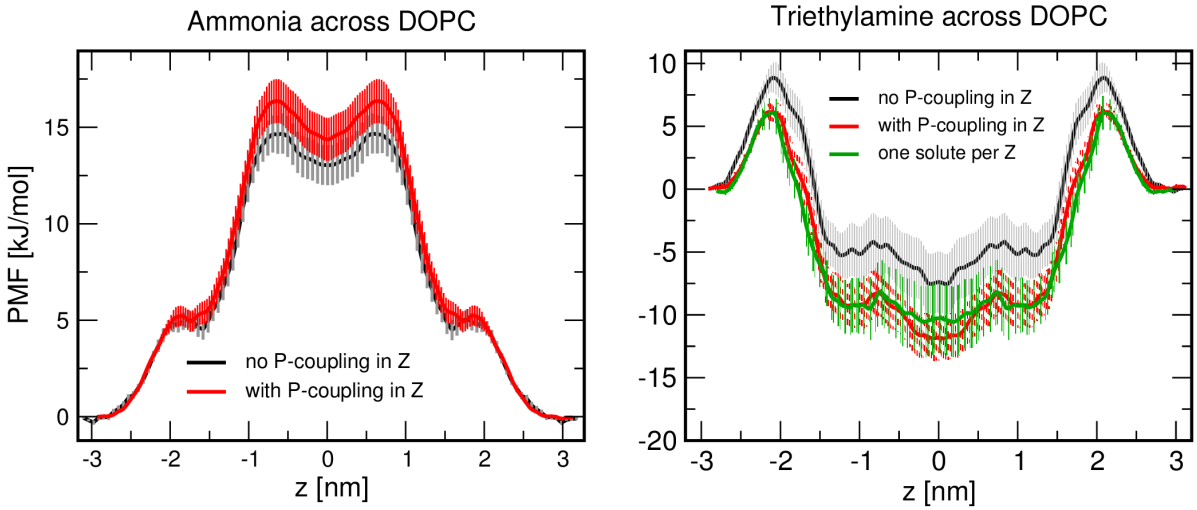

Figure S2: (A) Ammonia across DOPC: on the effect of pressure coupling in Z direction (membrane normal) during umbrella sampling simulations. The PMFs with pressure coupling in Z turned on (red) and off (black) agree within statistical errors. Error bars indicate one standard deviation. (B) TEA across DOPC: same analysis as in A. In addition, the umbrella simulation systems were set up with one TEA molecule per Z coordinate (instead of four, see Methods). Only marginal effects on the PMFs are found.

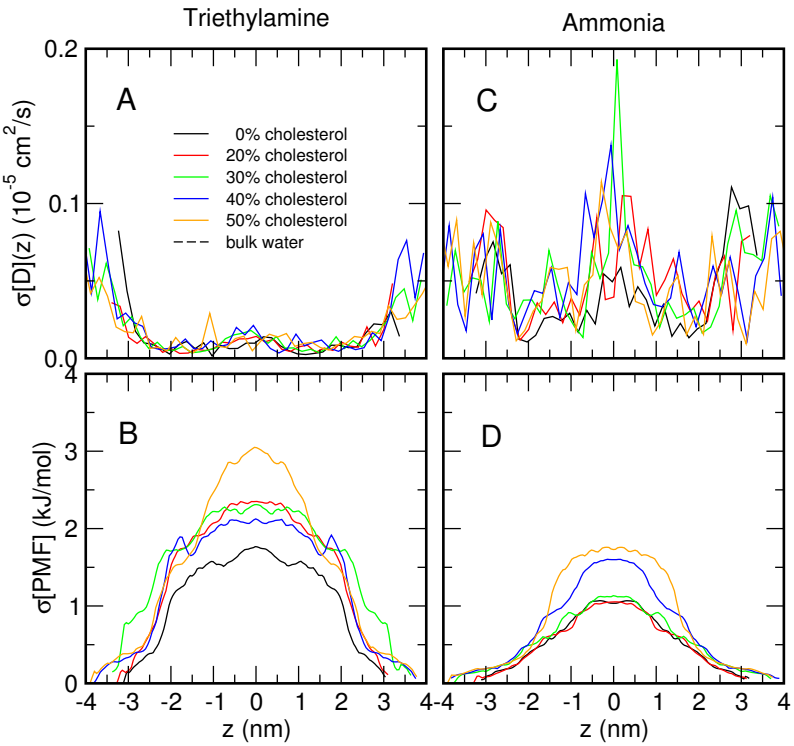

Figure S3: Statistical uncertainty shown as one standard deviation of the computed diffusion constants of (A) TEA and (C) ammonia shown in Figure 4 of the main text. Those uncertainties were computed from the standard deviation of diffusion constants of four independent solutes at each z position divided by $(N-1)^{1/2}$, where N=4. Thus, the estimate for the uncertainty is approximate but unbiased. (B/D) Uncertainty of the PMF computed using the Bayesian bootstrap of complete histograms [Hub et al., J. Chem. Theory Comput. 6, 3713-3720 (2010)]. The uncertainties of P_m (Fig. 3C/F) were computed from the uncertainties in A-D via Gaussian error propagation.

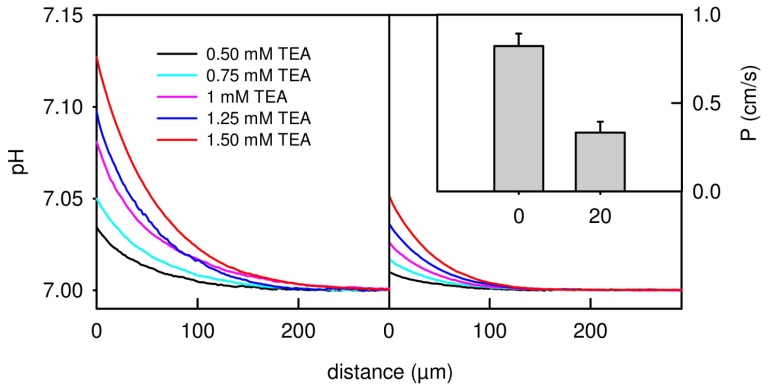

Figure S4: Representative pH profiles in the *trans* USL of a brain extract bilayer, which were induced by TEA added at the *cis* side of the membrane. Left: pure brain extract. Right: plus 20% cholesterol. Inset: TEA permeabilities at 0 and 20% cholesterol.

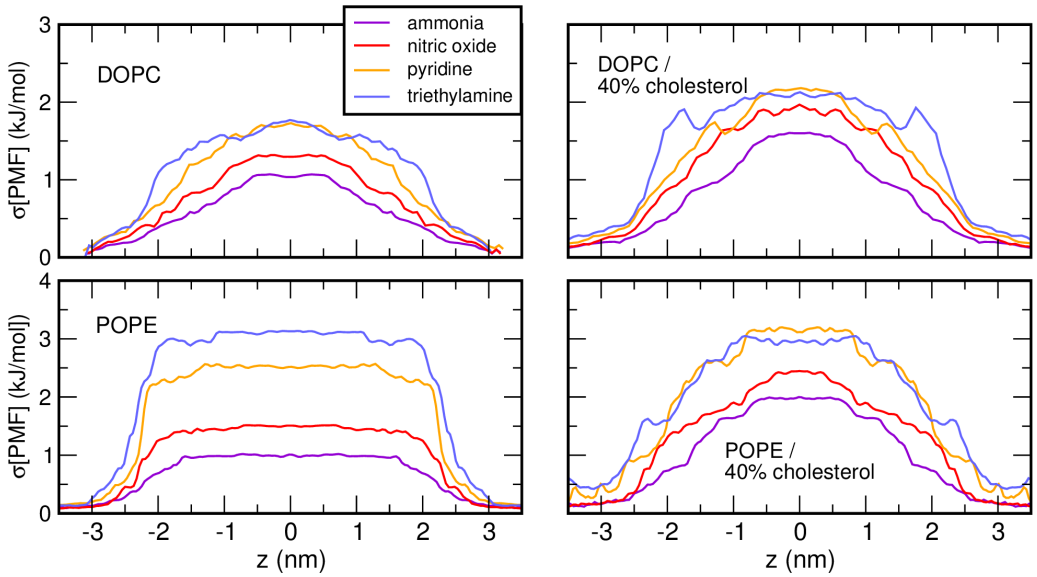

Figure S5: Statistical uncertainties (one standard deviation) of the PMFs shown as position dependent partition coefficients *K*(*z*) in Figure 6. $\sigma_{PMF}(z)$ translates into the uncertainties of K(z) as a factor of $\exp(-\sigma_{PMF}(z)/kT)$. Uncertainties were computed using the Bayesian bootstrap of complete histograms [Hub et al., J. Chem. Theory Comput. 6, 3713-3720 (2010)].