Supporting Information

for

Convergence of Free Energy Profile of Coumarin in Lipid Bilayer

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Recommended Simulation Protocol

Based on our results, we recommend the following simulation protocol:

1. Calculate RESP partial charges of the guest molecule
2. Run unbiased simulation to collect initial structures (this can be supplemented with very slow pulling simulation from a deepest position of drug in the bilayer, at most 1 $nm \cdot ns^{-1}$, with a pulling force constant of at most 500 $kJ \cdot mol^{-1} \cdot nm^{-2}$)
3. Run constraint simulation with at least 10 ns bins
    a. For a rough initial screening use bins spaced 4 Å apart
    b. For fine results use bins spaced 1 Å apart

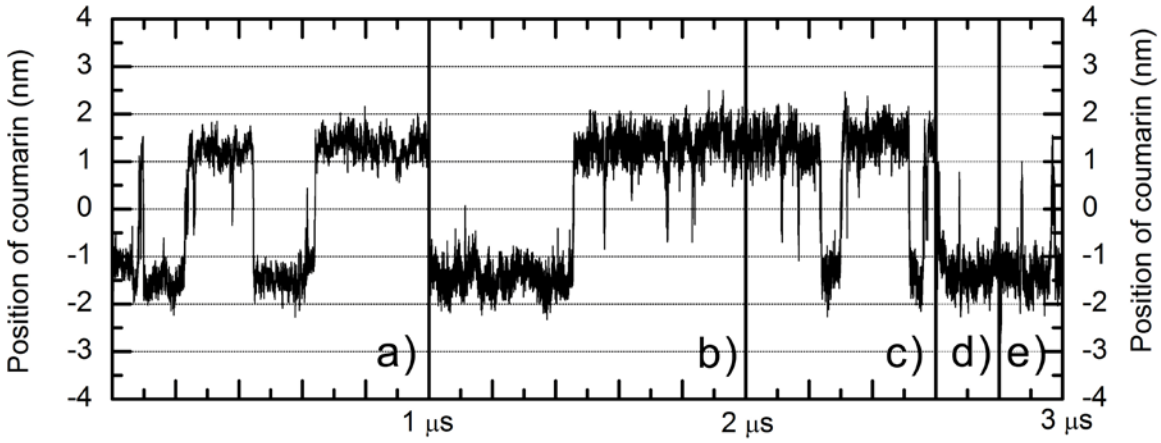

***Figure S1**: Position of coumarin in unbiased simulations, total time 3 μs. Coumarin preferentially stays at ~14Å, while it penetrates the bilayer freely with short stays in the middle of the bilayer. Coumarin enters the bilayer during a very short period of maximum 10 ns of each simulation.*

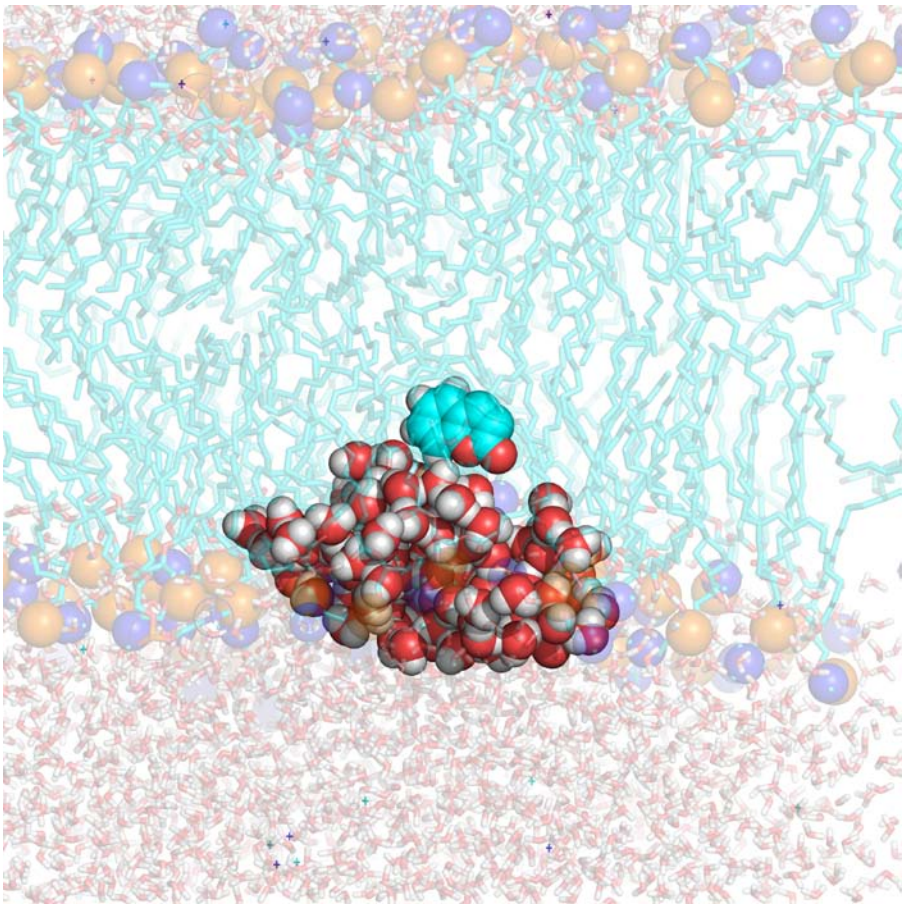

***Figure S2**: Funnel shape water defect caused by pulling coumarin into the membrane. The solvation shell follows coumarin and disturbs the bilayer surface, which leads to water artifacts in the free energy profile.*

Window spacing effects on the free energy profiles

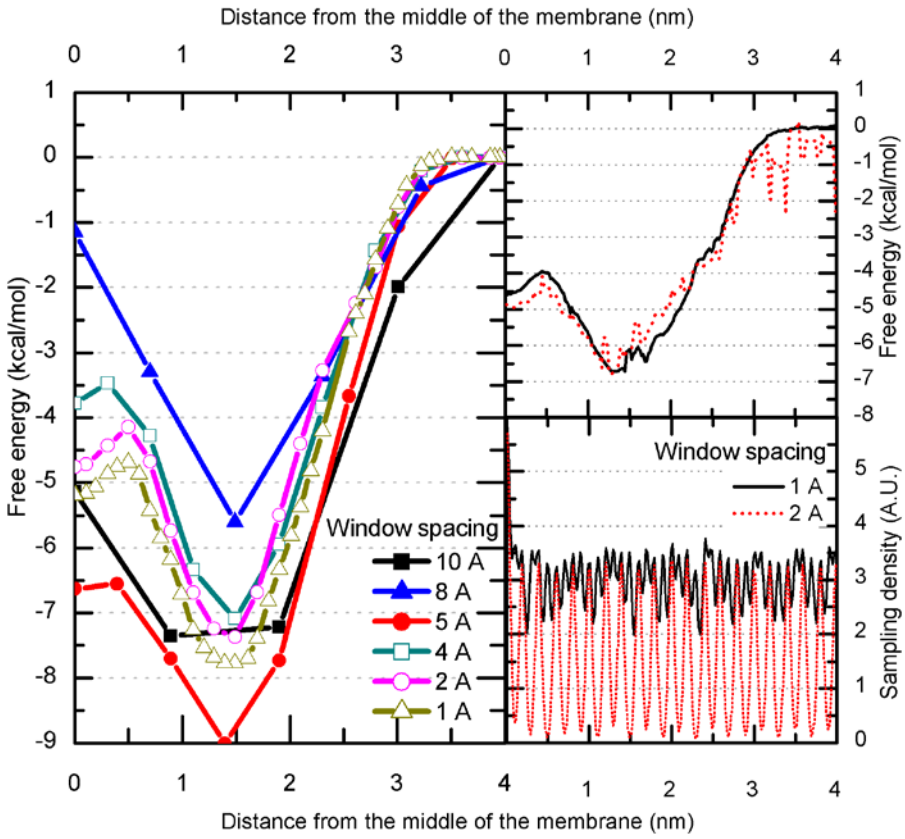

***Figure S3**: Effect of window spacing in constraint (CF, left) and umbrella (UF, right) simulations with initial structures obtained from free simulation. With 4Å between windows in constraint simulation the positions of minima can be still recognized, but when using umbrella simulation the area is not thoroughly sampled and the free energy profile cannot be plotted. Significant errors in the profile with 2 Å distance between simulation windows can be observed (upper right panel), as the sampling density is very low with a force constant of 2,000 kJ·mol^{-1}·nm^{-2} (lower right panel).*

The number of simulation windows along the z-axis significantly affects the quality and cost of the free energy profile calculation. The effect of window spacing in umbrella simulation has been previously studied, and too wide spacing has been found to lead to higher errors or even prohibit free energy profile plotting. Constraint simulation allows a wider windows spacing, especially in the areas of small changes in constraint force. The free energy profile can be plotted with any windows spacing, although its quality is dependent on this variable. It is possible (and recommended) to calculate a rough free energy profile at the beginning of simulation (with 4 Å between simulation windows) and then add more simulation windows in the areas of local minima and maxima. This approach leads to more efficient use of computer time, as attention is focused precisely on the areas of interest.

***Table S1**: Partial charges on coumarin atoms calculated by restraint electrostatic potential fit (RESP calculated at B3LYP/cc-pVDZ level), by PRODRG2Beta Server (PRODRG) and from Mulliken population analysis (MULLIKEN) calculated at HF/6-31G* level (in gas phase). Atom labels are shown on Figure S4.*

Atom label	RESP	PRODRG	MULLIKEN
OAA	-0.48	-0.59	-0.61
CAI	0.76	0.33	0.98
OAH	-0.42	-0.14	-0.52
CAK	0.50	0.18	0.56
CAF	-0.35	0.02	-0.40
HAF	0.18	0.06	0.22
CAC	-0.02	0.02	-0.04
HAC	0.12	0.06	0.15
CAB	-0.20	0.02	-0.24
HAB	0.14	0.06	0.17
CAE	-0.08	0.00	-0.13
HAE	0.12	0.00	0.16
CAJ	-0.16	0.00	-0.14
CAG	0.16	0.00	0.17
CAD	-0.26	0.00	-0.32

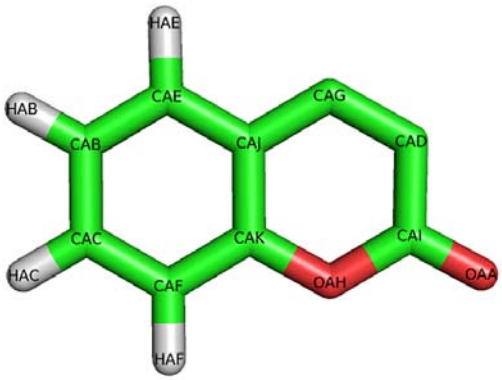

***Figure S4**: Atom labels of coumarin united atoms as used in Table S1.*

Probability of a spontaneous transition $p(\mathrm{A}\rightarrow\mathrm{B})$ from state A to B in time Δt via a barrier of $\Delta G^{\ddagger}$ can be estimated from the equation (Eq. S1)

$$p(\mathrm{A}\rightarrow\mathrm{B}) = 1 - \exp\left(-\frac{k_B T}{h}\exp\left(-\frac{\Delta G^{\ddagger}}{RT}\right)\Delta t\right), \qquad \text{Eq. S1}$$

where k_B refers to Boltzmann constant, h to Planck constant, R to the universal gas constant, and T is temperature.