Molecular insights into the membrane affinities of model hydrophobes

Jianguo Li[a,b], Roger W. Beuerman[a,e,f]*, Chandra S. Verma[a,b,c,d]*

[a] *Singapore Eye Research Institute, 11 Third Hospital Avenue, #06-00, Singapore 168751*
[b] *Bioinformatics Institute (A*-STAR), 30 Biopolis Street, #07-01 Matrix, Singapore 138671*
[c] *School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551*
[d] *Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543*
[e] *Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, 5 Lower Kent Ridge Road, Singapore 119074*
[f] *Duke-NUS, SRP Neuroscience-Behavioural Disorders, Singapore*

*Corresponding authors: Roger W. Beuerman (rwbeuerman@gmail.com)
Chandra S. Verma (chandra@bii.a-star.edu.sg)

Simulation details of each system

Table S1. Details of each conventional MD simulation system.

Simulation	Box dimension	No. lipids	No. water molecules	simulation time	Initial conformation
16 mangostin	7.5x7.5x7.5 nm	0	13450	150 ns	in water (60 mM)
16 ciprofloxacin, (zwitterionic)	7.5x7.5x7.5 nm	0	13527	150 ns	in water (60 mM)
16 xanthone	7.5x7.5x7.5 nm	0	13621	150 ns	in water (60 mM)
16 tetracycline	7.5x7.5x7.5 nm	0	13476	150 ns	in water (60 mM)
16 alpha-mangostin	6.05x5.96x10.8 nm	128	7256	1000 ns	on membrane
16 ciprofloxacin, (neutral)	6.02x5.95x9.73 nm	128	5841	1000 ns	on membrane
16 ciprofloxacin, (neutral)	6.4x6.3x8.5 nm	128	5841	1000 ns	in membrane

Table S2. Details of each umbrella sampling simulation system.

simulation	compound	temperature	No. windows	Size of each window	Simulation time of each window	No. water molecules	No.lipid molecules	Box length (nm)
1	2 mangostin molecules	310	24	0.15	50 ns	11367	0	7x7x7
2	2 ciprofloxacin molecules (zwitterionic)	310	24	0.15	50 ns	9097	0	6.5x6.5x6.5
3	2 xanthone molecules	310	24	0.15	50 ns	7136	0	6x6x6
4	2 tetracycline molecules	310	24	0.15	50 ns	9086	0	6.5x6.5x6.5
5	1 mangostin molecule	310	36	0.1 nm	400 ns	3841	72	4.5x4.5x10.2
6	1 mangostin molecule	323	28	0.15 nm	400 ns	3841	72	4.5x4.5x10.2
7	1 Ciprofloxacin molecule (neutral)	310	28	0.15 nm	400 ns	3267	72	4.51x4.51x9.18
8	1 Ciprofloxacin molecule (neutral)	323	28	0.15 nm	400 ns	3267	72	4.51x4.51x9.18
9	1 xanthone molecule	310	28	0.15 nm	200 ns	3866	72	4.5x4.5x10.2
10	1 tetracycline molecule	310	28	0.15 nm	200 ns	3935	72	4.47x4.47x10.5

Convergence of PMF calculations: Convergence depends on sufficient sampling of the phase space. We believe our PMF simulations are converged because of the following. Firstly, the four compounds used in this study are small uncharged molecules. These compounds have relatively rigid conformations, resulting in significant sampling of the phase space during the simulations. It has been reported that the main error in PMF calculations of the interactions of small molecules with lipid bilayers arises from hidden free energy barriers which largely arise from the formation of lipid defects induced by charged molecules[1] and in such cases, complex reaction coordinates are required. For example, analogues of the arginine side chain are known to induce lipid defects filled with water molecules during the translocation of the molecules across the membrane, which, if not accounted for carefully, result in insufficient sampling. In contrast, the four compounds in our study are all uncharged and translocate across the membrane without significant membrane deformations. Additionally, the PMFs in our study were computed using umbrella sampling (US) simulations. In each umbrella window, the simulation was run for 200-400 ns, which, to our knowledge, is the longest time scale used in US simulations for calculating the free energies of such processes of solutes traversing membranes. In another work by Chris Neale and Pomès [2], 200 ns windows were used to calculate the PMF of the translocation of a charged molecule, n-propylguanidinium, across the membrane. Other studies have generally used much shorter windows in US simulations of solute-bilayer systems. For example, Cramariuc et al. used 15 ns windows to calculate the PMF of the translocation of ciprofloxacin across the membrane[3]. Indeed, shorter time scale windows (50 ns) were also used to study even flexible molecules such as peptides translocating across the membrane [4]. Finally, we also calculated error bars in our study using the boot strap method, and they suggest convergence of the PMFs.

Results

Figure S1. Number of hydrogen bonds during the aggregation of each compound.

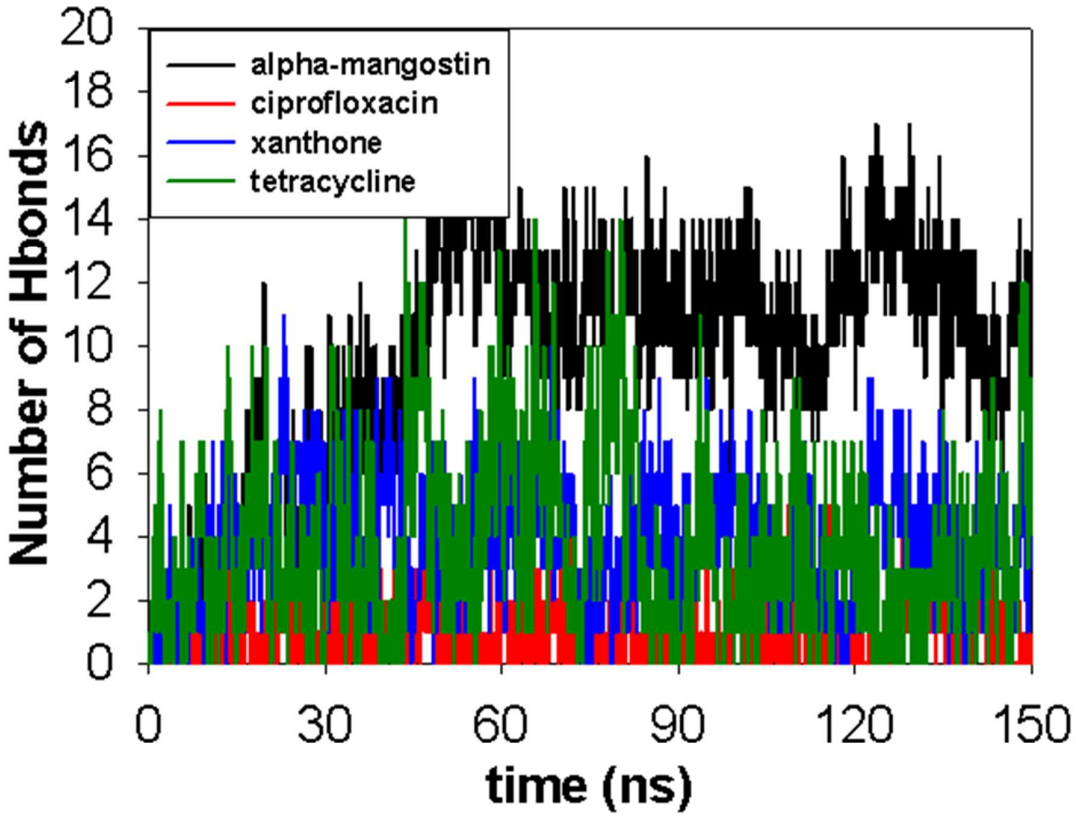


Figure S2. Temperature dependence of transfer free energies of alpha-mangostin and ciprofloxacin across a membrane

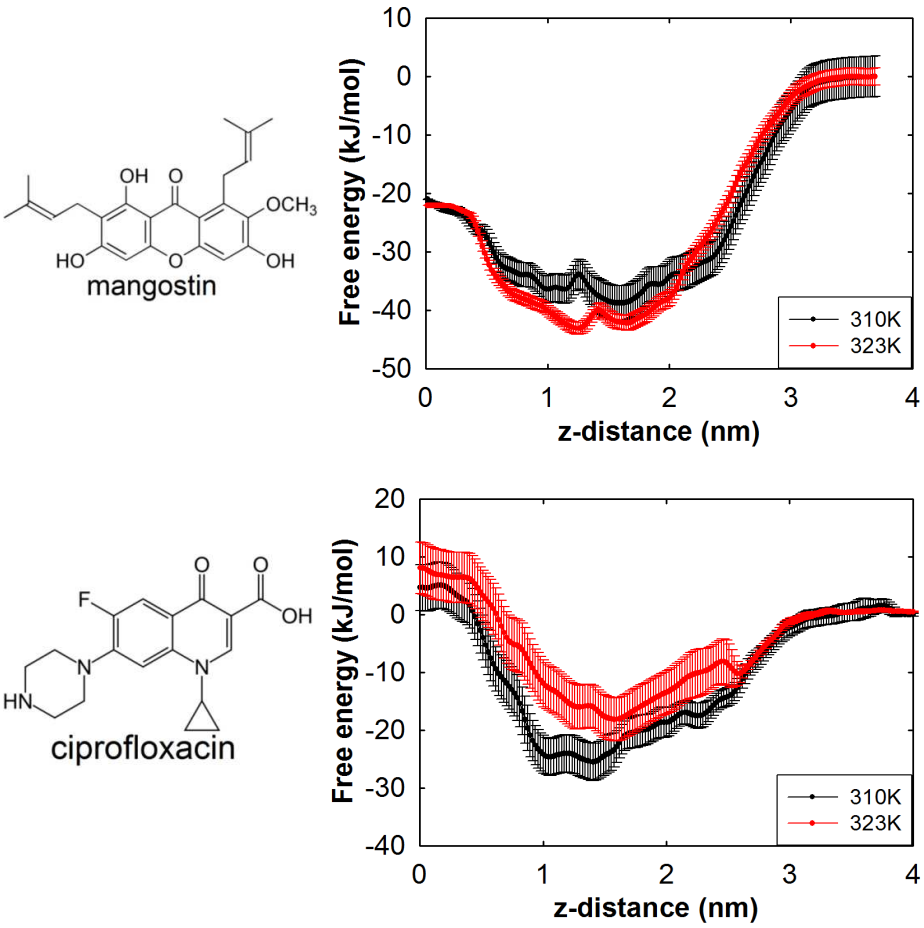


Figure S3. The orientation distributions of mangostin and ciprofloxacin at different distances from the membrane bilayer center. Two angles theta and phi were defined to characterize the orientation of each compound, with theta being the angle between the long axis of the molecule and the bilayer normal, and phi being the vector normal to the aromatic plane and the bilayer.

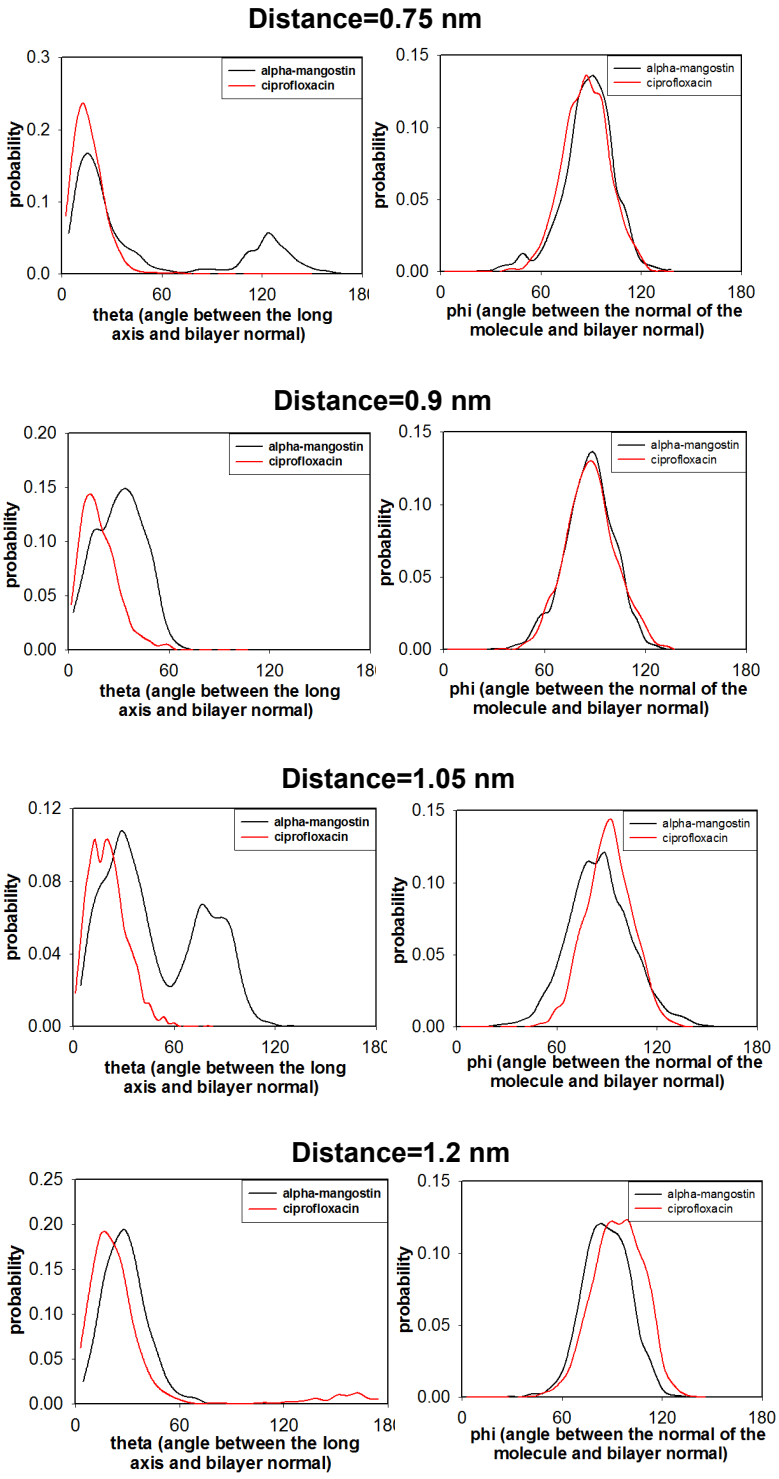
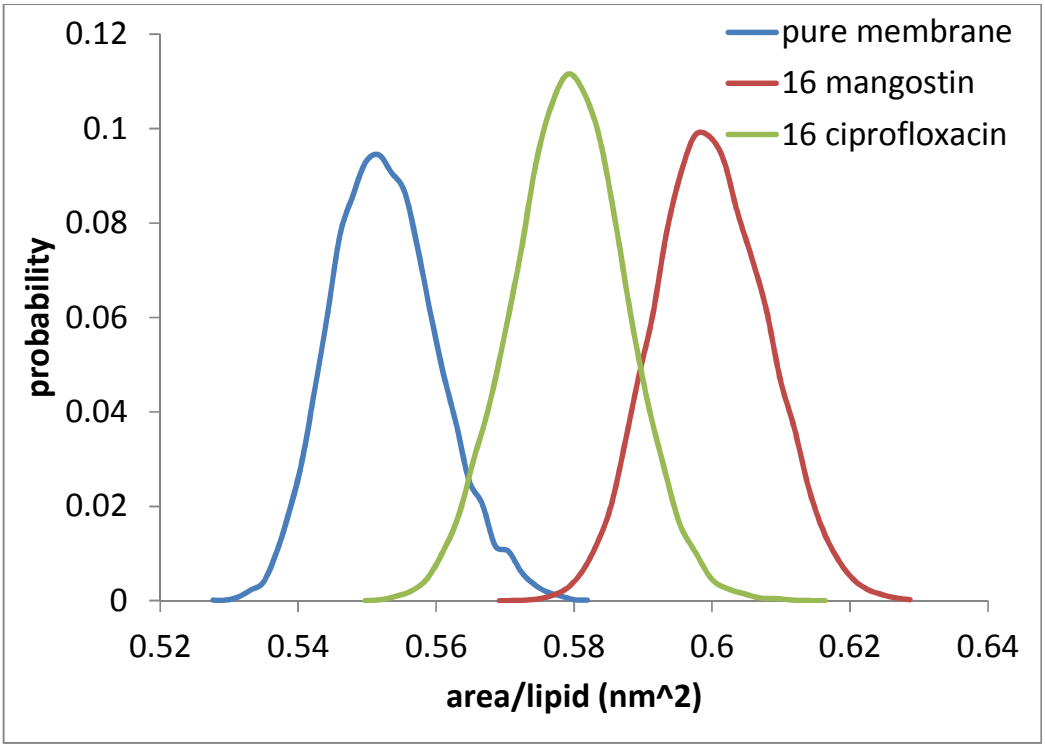


Figure S4. Area per lipid of the bacterial membrane in the presence of 16 mangostin and 16 ciprofloxacin molecules.



References:

(1) Neale, C.; Pomès, R. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **2016**, *1858*, 2539.

(2) Neale, C.; Bennett, W. F. D.; Tieleman, D. P.; Pomès, R. *Journal of Chemical Theory and Computation* **2011**, *7*, 4175.

(3) Cramariuc, O.; Rog, T.; Javanainen, M.; Monticelli, L.; Polishchuk, A. V.; Vattulainen, I. *Biochimica et Biophysica Acta (BBA) - Biomembranes*.

(4) Bennett, W. F. D.; Hong, C. K.; Wang, Y.; Tieleman, D. P. *Journal of Chemical Theory and Computation* **2016**, *12*, 4524.