

Determination of Membrane-Insertion Free Energies by Molecular Dynamics Simulations

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Supplemental Information

Table S1: Self-energy correction for non-neutral systems.

System	L (Å)	Δu (kcal/mol)
OmpLA in membrane	85.0	-5.53
OmpLA in water	70.0	-6.72
Arg on seven-residue strand	46.0	-10.23
Isolated Arg	33.0	-14.26

Table S2: Overview of the free-energy changes associated with specific FEP simulations.

transformation	ΔG (kcal/mol)		
	forward	backward	BAR ^a
$\Delta G_{\text{DLPC}}^{\text{OmpLA}}(\text{Ala210} \rightarrow \text{Arg})$	-41.87	+47.42	-44.19±0.16
$\Delta G_{\text{aq.}}^{\text{OmpLA}}(\text{Ala210} \rightarrow \text{Arg})$	-46.64	+47.67	-46.76±0.21
$\Delta G_{\text{aq.}}^{7\text{-res.}}(\text{Ala} \rightarrow \text{Arg})$	-40.43	+40.48	-40.55±0.14
$\Delta G_{\text{aq.}}^{\text{iso.}}(\text{Ala} \rightarrow \text{Arg})$	-37.83	+38.29	-38.12±0.12
$\Delta G_{\text{aq.} \rightarrow \text{DLPC}}^{\text{polyL}}(\text{Arg}) - \Delta G_{\text{aq.} \rightarrow \text{DLPC}}^{\text{polyL}}(\text{Ala})$	+10.70	-2.55	+6.09±0.19
$\Delta G_{\text{DLPC}}^{\text{OmpLA}}(\text{Ala210} \rightarrow \text{Leu})$	-1.24	+2.14	-1.58±0.05
$\Delta G_{\text{aq.}}^{\text{OmpLA}}(\text{Ala210} \rightarrow \text{Leu})$	-0.56	+0.79	-0.67±0.04
$\Delta G_{\text{aq.}}^{7\text{-res.}}(\text{Ala} \rightarrow \text{Leu})$	+1.23	-0.99	+1.06±0.07
$\Delta G_{\text{aq.}}^{\text{iso.}}(\text{Ala} \rightarrow \text{Leu})$	+0.44	-0.55	+0.48±0.06
$\Delta G_{\text{DLPC}}^{\text{OmpLA}}(\text{Ala210} \rightarrow \text{Ser})$	-3.90	+3.54	-3.74±0.03
$\Delta G_{\text{DLPC}}^{\text{OmpLA}}(\text{Ala210} \rightarrow \text{Ser})^*$		+4.75	
$\Delta G_{\text{aq.}}^{\text{OmpLA}}(\text{Ala210} \rightarrow \text{Ser})$	-7.92	+7.75	-7.82±0.03
$\Delta G_{\text{aq.}}^{7\text{-res.}}(\text{Ala} \rightarrow \text{Ser})$	-7.01	+7.43	-7.26±0.05
$\Delta G_{\text{aq.}}^{\text{iso.}}(\text{Ala} \rightarrow \text{Ser})$	-9.16	+8.76	-8.98±0.05

^a The Bennett acceptance ratio (BAR) estimates were obtained by combining the forward and backward transformations (1). The error bars associated to the free-energy differences correspond to the statistical precision, but not the overall reliability of the BAR estimator (2, 3). All free energies involving Arg are pre-corrected values (see Methods and Table 1). The asterisk on the second Ala210→Ser mutation indicates that the perturbation, only carried out in the reverse direction, utilized a different starting state in which a water molecule was embedded in the membrane with the Ser210 residue.

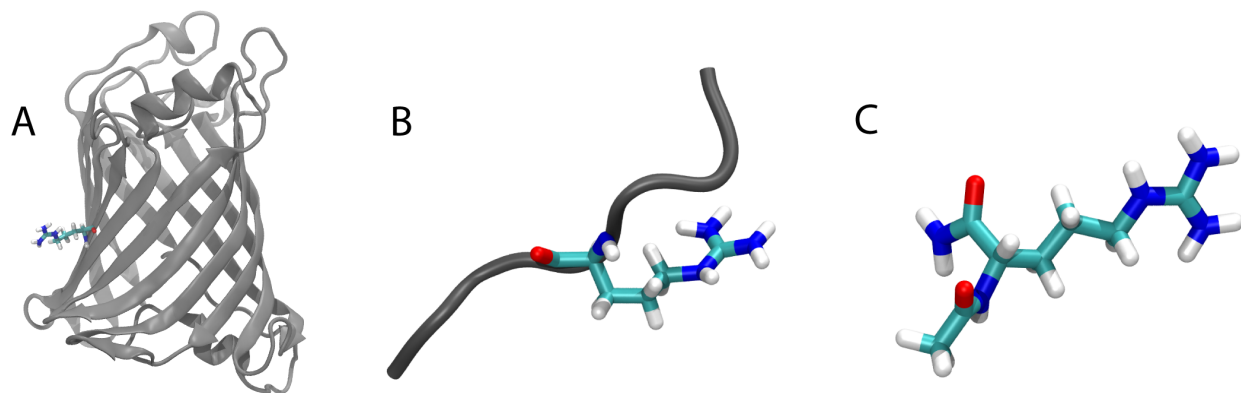


Figure S1: Three contexts considered for the aqueous state of residue 210. (A) Arg210 on fully-folded OmpLA. (B) Arg on a seven-residue oligopeptide taken from OmpLA. (C) Isolated Arg amino acid.

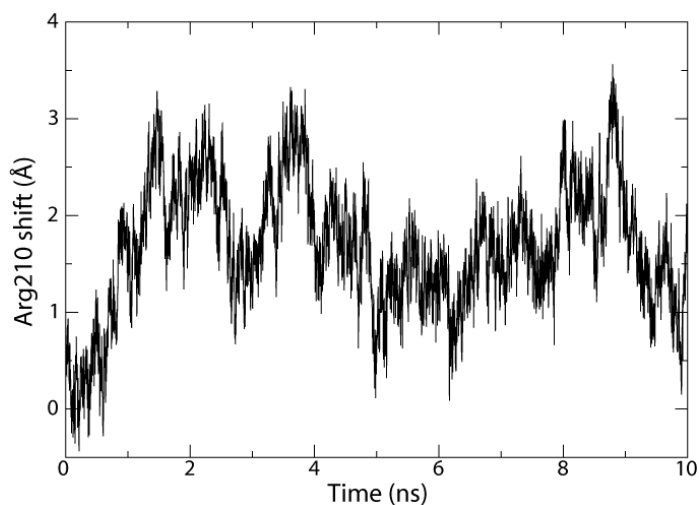


Figure S2: Shifting of OmpLA in the DLPC bilayer. Shown is the deviation between the position in z of the C_{α} atom of Arg210 and the center of the membrane. Data was taken over the course of a 10-ns equilibration of the OmpLA:Arg210 mutant.

Corrections to the insertion cost for Ser

Arg, due to its size and positive charge, can quickly induce the formation of a water defect in the membrane; in the forward FEP simulation, the partially coupled Arg began interacting with water molecules already near $\lambda = 0.2$, i.e., with only a small fraction of its full charge. Conversely, Ser, although still polar, is smaller and uncharged, and, therefore, cannot disturb the membrane as greatly when embedded inside it. While in the FEP simulations the serine residue primarily interacted with the OmpLA β barrel, during a 10-ns equilibration of a fully coupled Ser210 in the

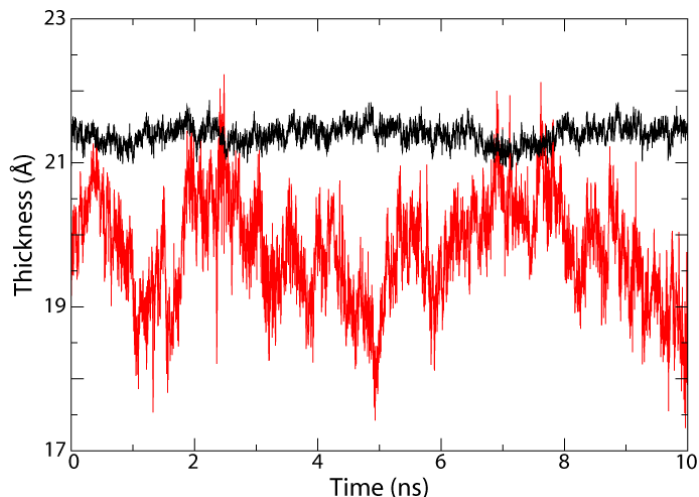


Figure S3: Local thinning of the membrane. The black curve is the hydrophobic thickness of the DLPC membrane during equilibration of the OmpLA:Arg210 mutant. The red curve is the thickness of the membrane within 15 Å of Arg210.

membrane, a water molecule was observed to penetrate the membrane core and hydrogen bond with it. To account for the possibility of water-Ser interactions in the free energy of insertion for Ser, an additional FEP simulation was carried out in the reverse (Ser \rightarrow Ala) direction in which the initial state included a water molecule bonded to Ser210. In this simulation, the free-energy gain for transforming Ser to Ala was 1.21 kcal/mol higher (see Table S2), making the insertion cost for Ser on OmpLA 1.21 kcal/mol lower, i.e., as low as 2.31 kcal/mol, in much better agreement with the experimental value of 1.8 kcal/mol.

An additional effect on the Ser insertion free energy may result from the fact that the optimal hydrogen bonding geometry in MD simulations differs from that observed in crystal structures (4, 5). For Ser210 the hydrogen bond formed with the backbone of the OmpLA β barrel matches the ideal physical geometry closely, but this geometry is underrepresented in simulations that use the CHARMM force field (5). Therefore, it is likely that formation of this hydrogen bond results in a smaller free energy gain in simulations than in experiments, leading to a greater cost of insertion for Ser than otherwise expected. To quantify this effect, multiple snapshots (10 in total) were taken from the simulations in which Ser was hydrogen bonded to the barrel and the corresponding residues were converted to an ethanol and an N-methyl acetamide, respectively. The interaction energy between these two residues was calculated using ab initio quantum chemical simulations (QM) and also using the CHARMM forcefield (MM). QM calculations were carried out using Gaussian at the MP2 level of theory (6). On average, the QM energy was 0.46 kcal/mol lower than

the MM energy, indicating that the forcefield does not favor formation of this hydrogen bond as greatly as it should and, thus, unduly penalizes the insertion free energy of Ser. On the other hand, Arg, which is accommodated by perturbing the membrane rather than OmpLA, does not suffer from this force-field deficiency.

References

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