Outer membrane protein folding and topology from a computational transfer free energy scale

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Supporting information

Reduced discrete state model of conformational space

The reduce discrete state space Ω for the transmembrane (TM) region of an outer membrane β-barrel protein (OMP) is defined following reference.[1] Briefly, each TM strand has a length $L+1=16$. Each residue on a TM strand interacts with neighboring strands through strong H-bond, non H-bond, or weak H-bond.[2–4] One strand i can slide $d_i \in \{-l, \cdots, 0, \cdots, l\}$ residues away from its center position. A specific conformation of the TM region of an OMP with N TM strands is defined by a vector $\mathbf{d}$:

$$\mathbf{d} = (d_1, \cdots, d_N),$$

in which d_i denotes the offset position of strand i. The full configuration space Ω is:

$$\Omega = \{\mathbf{d} | \mathbf{d} = (d_1, \cdots, d_N) \in \mathbb{Z}^N\}.$$

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Because each offset position d_i can have a value from $-l$ to l, the size of the configuration space Ω is $(2l+1)^N$. We used $l = 3$ in this study.

TM strands, side chain orientations, and center positions

Sequences of TM strands are extracted from PDB structures of OMPs. The side-chain orientations of TM residues are determined by visualization using pymol.[5] The inter-strand interaction type between two residues on neighboring strands, *i.e.*, strong H-bond, non H-bond and weak H-bond, is determined using the DSSP software.[6] The midplanes of the lipid bilayers of OMPs are taken from the OPM server.[7] The center residue of a TM strand is defined as the residue with the shortest distance to the mid-plane and is assigned the position index $i = 0$. Position indice for other residues in the TM strands are assigned according to their relative positions to the center residue.

Energy calculation of an OMP in a specific configuration

The empirical potential function for calculating the energy of a TM strand is based on our previous empirical MSIP function,[4] which was derived from structures of OMPs using the combinatorial permutation model as the reference state to resolve the coupling effects in short TM strands. For a specific configuration d, the empirical energy function consists of four terms. First, each residue contributes to the single-residue burial energy $E_{\mathrm{B}}(\mathbf{d})$ based on the region in which the residue is located (*i.e.*, hydrophobic core, headgroup, and cap region) and its side-chain orientation (see reference[4] for numerical details). Detailed analysis on the distribution of amino acid residues in different regions of membrane bilayers was described in our previous study.[4] Second, each residue in the TM region interacts with residues in the neighboring strands through strong H-bond interaction (E_{SH}) and non H-bond interaction (E_{NH}) (see reference[4] for numerical details). The summation of E_{SH} and E_{NH} is the inter-strand interaction energy $E_{\mathrm{Inter}}(\mathbf{d})$. Third, each residue in the TM region of a strand interacts with two other residues on the neighboring strand through weak H-

bond ($E_{\mathrm{WH}}(\mathbf{d})$). Fourth, each residue interacts with the nearest residues on the same TM strand, which are of the same side-chain orientation. This term is the intra-strand interaction energy $E_{\mathrm{Intra}}(\mathbf{d})$ (see reference[8] for numerical details) . Following Wouters and Curmi,[2] the strand-strand interaction network is that of the ideal anti-parallel β-sheet (Fig S1). Residues that do not form β-pairing interactions in the native structures usually have less favorable interactions in the canonical model and contribute little in the Boltzmann factor.

The strand energy $E(i;\,\mathbf{d})$ for a TM strand i in configuration $\mathbf{d}$ is then calculated as:

$$
\begin{aligned}
E(i;\,\mathbf{d}) &= w_{\mathrm{B}} \cdot E_{\mathrm{B}}(i;\,\mathbf{d}) + w_{\mathrm{Intra}} \cdot E_{\mathrm{Intra}}(i;\,\mathbf{d}) + w_{\mathrm{Inter}} \cdot E_{\mathrm{Inter}}(i;\,\mathbf{d}) + w_{\mathrm{WH}} \cdot E_{\mathrm{WH}}(i;\,\mathbf{d}) \\
&= w_{\mathrm{B}} \cdot \sum_{k_i} E_{\mathrm{B}}(k_i;\,d_i) + w_{\mathrm{Intra}} \cdot \sum_{k_i} E_{\mathrm{Intra}}(k_i;\,d_i) \\
&\quad + w_{\mathrm{Inter}} \cdot [\sum_{k_i} \sum_{k_{i-1}} (E_{\mathrm{Inter}}(k_i,\,k_{i-1};\,d_i,\,d_{i-1})) + \sum_{k_i} \sum_{k_{i+1}} (E_{\mathrm{Inter}}(k_i,\,k_{i+1};\,d_i,\,d_{i+1}))] \\
&\quad + w_{\mathrm{WH}} \cdot [\sum_{k_i} \sum_{k_{i-1}} (E_{\mathrm{WH}}(k_i,\,k_{i-1};\,d_i,\,d_{i-1}) + \sum_{k_i} \sum_{k_{i+1}} (E_{\mathrm{WH}}(k_i,\,k_{i+1};\,d_i,\,d_{i+1})], \quad (1)
\end{aligned}
$$

where k_i is the position of a residue in strand i, w_{B}, w_{Inter}, w_{WH}, and w_{Intra} are weights for the energy terms. We assigned weight values of 1.0, 0.6, 0.4, and 0.8 for w_{B}, w_{Inter}, w_{WH}, and w_{Intra}, respectively. Our results suggest that position-dependent residue burial term plays the most important role. As long as the rank order of $w_{\mathrm{B}} \geq w_{\mathrm{Intra}}, w_{\mathrm{Inter}} > w_{\mathrm{WH}}$ is maintained, our model is not sensitive to specific values of the weights of these terms. Specifically, 38 sets of weight parameters were tested and the transfer free energy scales were derived at residue A210 using each parameter set. The correlation coefficient (R^2) of the derived scales with the Moon-Fleming scale is 0.81, with a small standard deviation of ± 0.04, indicating our model is robust with respect to the choice of weights. Previous studies have shown that the strength of a weak H-bond is slightly stronger than half of that of a conventional H-bond,[9–13] consistent with an assignment of $w_{\mathrm{Inter}} > w_{\mathrm{WH}}$.

The energy of the TM region of an OMP in a specific configuration $\mathbf{d}$ is then calculated

as the summation of strand energies of all TM strands:

$$E(\mathbf{d}) = \sum_i E(i; \mathbf{d}). \tag{2}$$

Calculating the folding free energy of the TM region of an OMP

Considering each configuration $\mathbf{d}$ as a microstate, the partition function Z_{lipid} of the TM region of an OMP in the lipid bilayer is then calculated by summing the Boltzmann factor of TM strands over all microstates:

$$Z_{\text{lipid}} = \sum_{\mathbf{d}\in\Omega} e^{-\frac{E(\mathbf{d})}{k_B T}}. \tag{3}$$

The free energy of the protein in the lipid bilayer is then computed as:

$$G_{\text{lipid}} = -k_B T \ln Z_{\text{lipid}}. \tag{4}$$

The folding free energy of the TM region of a protein is therefore:

$$\Delta G = G_{\text{water}} - G_{\text{lipid}}, \tag{5}$$

where G_{water} is assign a constant C. That is, the free energy of the ensemble of conformations of an OMP in the solution phase is not hugely affected by a point mutation. This assumption is reasonable, as OMPs in solution are unlikely to be in an extended linear form. For example, a significant amount of residual structures were reported for denatured OmpX by NMR and MD studies, even under the strong condition of 8 M urea.[14,15]

Calculating transfer free energy scale of a host residue in OmpLA

Following the work of reference,[16] we take differences in the ΔG of a mutant at the host residue of OmpLA and the ΔG of Ala at the same host residue as the transfer free energy

from water to bilayer $\Delta\Delta G$ of the mutated amino acid.

$$\Delta\Delta G^{\text{Host}}_{\text{mutant}} = \Delta G^{\text{Host}}(\text{Ala}) - \Delta G^{\text{Host}}(\text{mutant}). \tag{6}$$

We further simplify Equation 6 using Equation 5:

$$\begin{aligned}
\Delta\Delta G^{\text{Host}}_{\text{mutant}} &= \Delta G^{\text{Host}}(\text{Ala}) - \Delta G^{\text{Host}}(\text{mutant}) \\
&= [G^{\text{Host}}_{\text{water,Ala}} - G^{\text{Host}}_{\text{lipid,Ala}}] - [G^{\text{Host}}_{\text{water,mutant}} - G^{\text{Host}}_{\text{lipid,mutant}}] \\
&= G_{\text{lipid,mutant}} - G_{\text{lipid,Ala}},
\end{aligned} \tag{7}$$

assuming $G^{\text{Host}}_{\text{water,Ala}} = G^{\text{Host}}_{\text{water,mutant}} = C$. That is, the free energy of the ensemble of conformations of an OMP in the solution phase is not hugely affected by a point mutation. This assumption is reasonable as OMPs in solution are unlikely to be in an extended linear form. For example, a significant amount of residual structures were reported for denatured OmpX by NMR and MD studies, even under the strong condition of 8 M urea.[14,15]

Detecting transfer free energy scales with strong context dependency

For n_i number of host residues at the depth position i whose transfer free energy scales have been calculated, we further calculate the pairwise correlation coefficients between scales obtained for each of the the $\binom{n_i}{2}$ pair of residues, and then take the average. We then exclude each of the k-th residue in turn and recalculate the average correlation among the $\binom{n_i-1}{2}$ scales. If this new averaged correlation coefficient increases by $\geq 10\%$, we do not include this k-th residue in the calculation of the final averaged general scale. Therefore, both the average scale and the identification of deviating residues do not depend on the sequence in which residues were chosen for calculation.

Calculating depth-dependent transfer free energy profiles of amino acid residues

All depth-dependent transfer free energy profiles $\{\Delta\Delta G_{aa(i)}\}$ of amino acid residues, except those for Tyr and Trp, are approximated by an asymmetric stepwise Gaussian function:

$$\Delta\Delta G_{aa(i)} = \begin{cases} a_0 e^{(-i^2/2a_1^2)}, & \text{if } i \geq 0 \\ a_0 e^{(-i^2/2a_2^2)}, & \text{if } i < 0 \end{cases} \tag{8}$$

where $i \in \{-p, \cdots, 0, \cdots, p\}$ denotes the position index of the TM residue, with $i = 0$ corresponding to the center of the bilayer. We used $p = 4$ in this study. For Trp and Tyr, a double Gaussian function is used:

$$\Delta\Delta G_{aa(i)} = a_0 e^{(-(i-a_2)^2/2a_1^2)} + a_0 e^{(-(i+a_4)^2/2a_3^2)}. \tag{9}$$

The fitted $\Delta\Delta G_{aa(i)}$ values and the values of a_0, a_1, a_2, a_3, a_4 can be found in Table S3.

Comparing thermodynamic stability of OMPs in the asymmetric outer membrane and in the hypothetical symmetric outer membrane

The thermodynamic stability of the TM region of an OMP is approximated by the total transfer free energy of the lipid-facing TM residues. Denote the amino acid residue in the j-th position on the i-th strand as $A_{i,j}$, the stability of an OMP in the asymmetric outer membrane is calculated as:

$$\Delta\Delta G_{\text{asym}} = \sum_{i=1}^{N} \sum_{j=-p}^{p} \Delta\Delta G_{A_{i,j}(j)}, \tag{10}$$

where N is the number of strands in the OMP, and p is defined as above.

The stability of an OMP in the hypothetical symmetric outer membrane is calculated as:

$$\Delta\Delta G_{\text{sym}} = \sum_{i=1}^{N} \sum_{j=-p}^{p} \Delta\Delta G^{*}_{A_{i,\,j}(j)}, \tag{11}$$

with

$$\Delta\Delta G^{*}_{A_{i,\,j}(j)} = \begin{cases} \Delta\Delta G_{A_{i,\,j}(j)}, & \text{if } j \leq 0 \\ \Delta\Delta G_{A_{i,\,j}(-j)}. & \text{if } j > 0 \end{cases} \tag{12}$$

By subtracting Equation 10 from Equation 11, we obtain the difference in thermodynamic stability between the TM region of an OMP in the native asymmetric outer membrane and in the hypothetical symmetric outer membrane.

Comparing thermodynamic stabilities of OMPs in NC-IN and NC-OUT topologies

Additive model. When an additive model is used, we calculate the thermodynamic stability of the TM region as the total transfer free energy of lipid-facing TM residues of a given OMP. We denote $A_{i,\,j}$ as the amino acid in the j-th position of the i-th strand. The thermodynamic stability of an OMP in the NC-IN topology is calculated as:

$$\Delta\Delta G_{\text{NC-IN}} = \sum_{i=1}^{N} \sum_{j=-p}^{p} \Delta\Delta G_{A_{i,\,j}(j)} \cdot \delta_{ij}, \tag{13}$$

with

$$\delta_{ij} = \begin{cases} 1, & \text{if } A_{i,\,j} \text{ is lipid facing,} \\ 0, & \text{if } A_{i,\,j} \text{ is pore facing,} \end{cases} \tag{14}$$

Similarity, the thermodynamic stability of an OMP in NC-OUT topology is calculated as:

$$\Delta\Delta G_{\text{NC-OUT}} = \sum_{i=1}^{N} \sum_{j=-p}^{p} \Delta\Delta G_{A_{i,\,j}(-j)} \cdot \delta_{ij}, \tag{15}$$

with

$$\delta_{ij} = \begin{cases} 1, & \text{if } A_{i,j} \text{ is lipid facing,} \\ 0. & \text{if } A_{i,j} \text{ is pore facing.} \end{cases} \tag{16}$$

By subtracting Equation 13 from Equation 15, we obtain the difference in stability when adopting the two different topologies:

$$\begin{aligned} \Delta\Delta G_{\text{topo}} &= \Delta\Delta G_{\text{NC-OUT}} - \Delta\Delta G_{\text{NC-IN}} \\ &= \begin{cases} \geq 0, & \text{OMPs in NC-IN topology,} \\ < 0, & \text{OMPs in NC-OUT topology.} \end{cases} \end{aligned} \tag{17}$$

The predicted topology is chosen to be the one with lower $\Delta\Delta G$.

Nonadditive model. Alternatively, we directly calculate the folding free energies of the TM region of an OMP using Equation 5 by directly summing up the Boltzman factors of residues in the TM regions over all enumerated miscrostates. The difference in thermodynamic stability $\Delta\Delta G_{\text{topo}}$ between the two topologies is then:

$$\begin{aligned} \Delta\Delta G_{\text{topo}} &= \Delta G_{\text{NC-OUT}} - \Delta G_{\text{NC-IN}} \\ &= \begin{cases} \geq 0, & \text{OMPs in NC-IN topology,} \\ < 0, & \text{OMPs in NC-OUT topology.} \end{cases} \end{aligned} \tag{18}$$

Calculating cooperativity of residue pairs

The cooperativity of two residues at the positions (WT, WT) of a pair of host positions in OmpLA is calculated as:[16]

$$\Delta\Delta\Delta G^{\text{Host Pair}}_{\text{mutant}} = \Delta\Delta G^{\text{Host Pair}}_{(\text{WT, mutant})} + \Delta\Delta G^{\text{Host Pair}}_{(\text{mutant, WT})} - \Delta\Delta G^{\text{Host Pair}}_{(\text{mutant, mutant})}. \quad (19)$$

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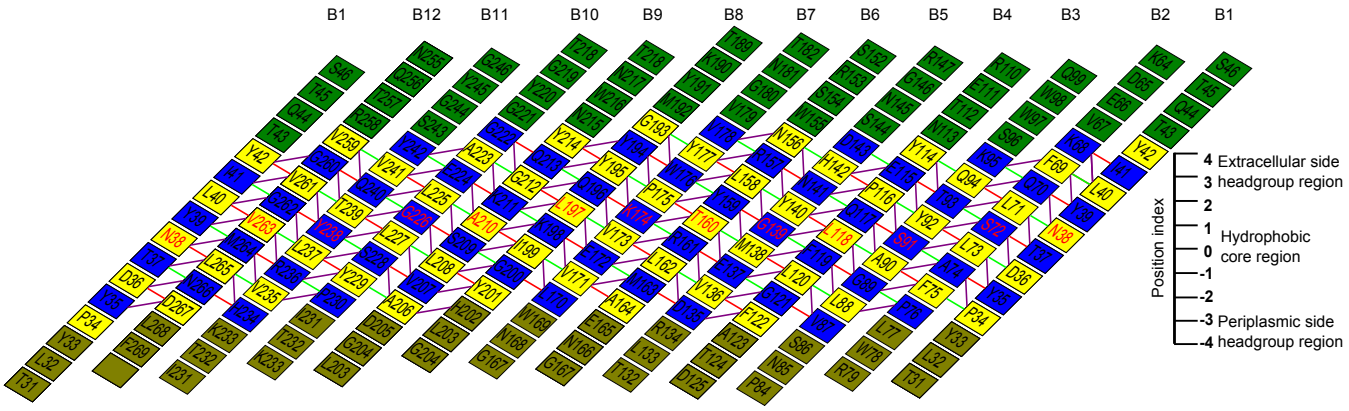

Fig. S1. The discrete model of the transmembrane region of outer membrane phospholipase A (OmpLA). In this model, a β-strand has 16 residues: 4 residues in the extracellular cap region (green), 9 residues in the TM region (pore-facing residues in blue, lipid-facing residues in yellow), and 3 residues in the periplasmic cap region (olive). Position index i of TM residues as integers are listed on the vertical line. Residues at position $i = 0$ are highlighted in red. Important inter-strand interactions between two neighboring TM residues are drawn in red (strong H-bond), green (non H-bond), and brown (weak H-bond) lines.

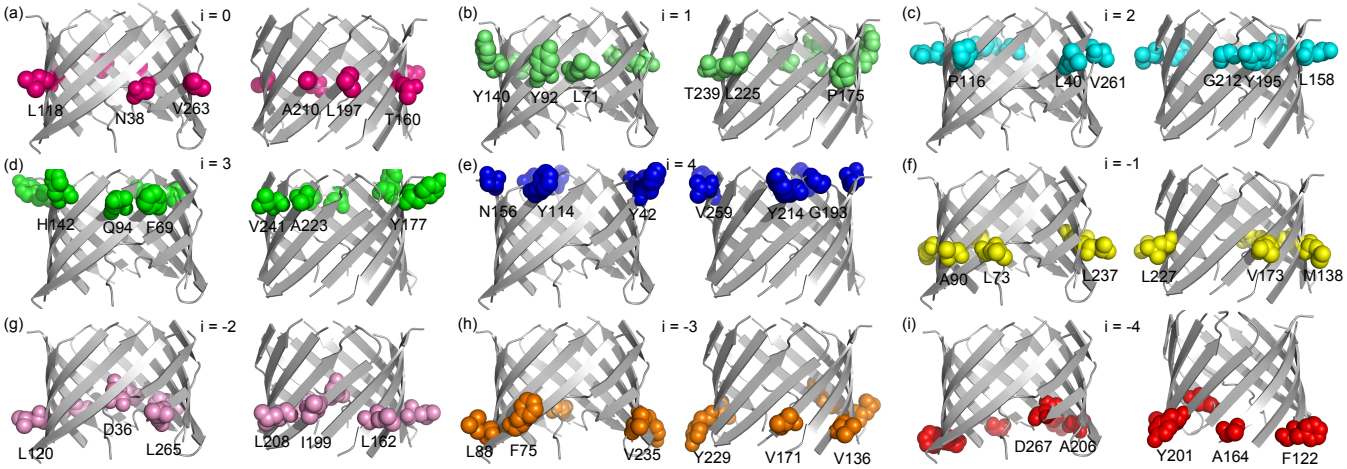

Fig. S2. 53 lipid-facing TM residues are used in deriving transfer free energy scales in OmpLA. For depth-position i, lipid-facing residues with the same position index i are used as host residues. Side-chains of the 53 host residues are shown in spheres.

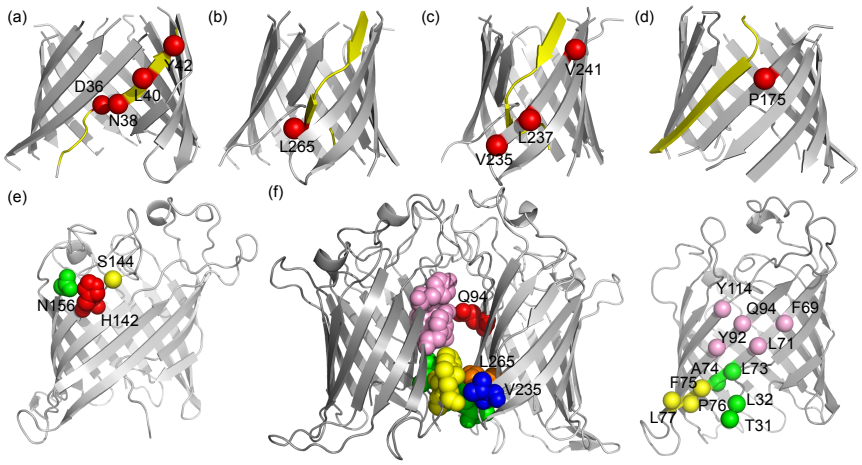

Fig. S3. Host residues demonstrating strong context dependency in transfer free energy scales are located in structurally deformed environment (a-d), active-site residues (e), or forming inter-molecular interactions for dimerization (f). (a) D36, N38, L40, and Y42 are located in β1, which contains a TM coil (residue 35-38). (b) L265 is located in β12, which also contains a TM coil (residue 260-263) (c) V235, L237, and V241 are next to a deformed strand β12 as their neighboring strand. (d) P175 in β7 is neighboring the deformed portion of strand β8. (e) H142 (red) and N156 (green) are part of the catalytic triad (H142-S144-N156) of OmpLA for its enzymatic function.[17] (f) Q94 (red), V235 (blue), and L265 (orange) provide inter-molecular interactions for the OmpLA dimerization: Q94 makes contacts with F69, L71, Y92, and Y114 (pink); V235 with F75, P76, and L77 (yellow); L265 with T31, L32, L73, A74, F75, P76, and L77 (yellow and green). Inter-molecule contacts are identified using a simplicial edge criteria[18]

(a)

	$\Delta\Delta G^{o}_{w,l}$	$\Delta\Delta G^{aa}_{app}$	$\Delta\Delta G_{Octanol}$
$Ez\beta$[a]	NA[c] / NA[d] / 0.65[e]	NA / NA / 0.84	NA / NA / 0.55
Slusky-Dunbrack[b]	NA / 0.31 / 0.30	NA / 0.44 / 0.43	NA / 0.52 / 0.51
E_b	0.47 / 0.64 / 0.64	0.45 / 0.78 / 0.76	0.50 / 0.68 / 0.68
$\Delta\Delta G^{A210}$	0.81 / 0.81 / 0.81	0.86 / 0.88 / 0.89	0.67 / 0.67 / 0.66

a Data of Cys, Met, and Thr not reported
b Data of Cys not reported
c R^2 is calculated for all 20 residues
d R^2 is calculated with Cys excluded
e R^2 is calculated with Cys, Met, and Thr excluded

(b)

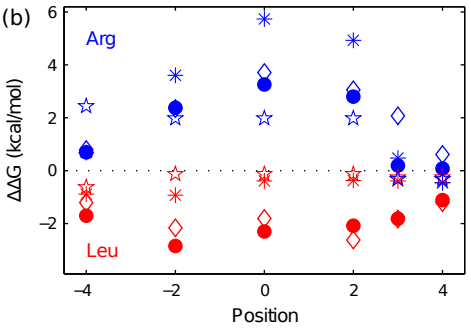

Fig. S4. (a) Three scales based on probabilities of finding residues in the TM strands (values see Table S6), $Ez\beta$,[19] Slusky-Dunbrack scale,[20] and E_b,[4] were compared with three experimental hydrophobicity scales. (b) Computed transfer free energies (closed circle) of Arg and Leu agree well with the experimentally measured values (diamond).[16] However, profiles of empirical energy values either from statistical distribution (empty star)[4] or from the earlier empirical energy function (asterisk)[1] were unable to reproduce the experimental values (diamond).

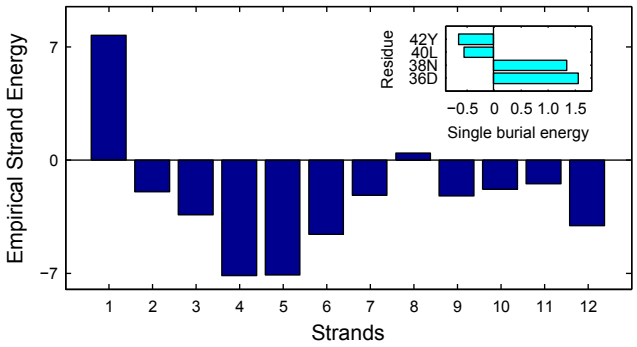

Fig. S5. Strand stability analysis of OmpLA detects unstable strand $\beta 1$ and its unstable residues. Strand energy of each TM strand of the native structure is calculated based on ref[1] . Strand $\beta 1$ is a relatively unstable TM strand, as it has significantly higher strand energy than other TM strands. Lipid-facing residues D36 and N38 are detected as the unstable residues of strand $\beta 1$ based on their high single burial energy (inset figure).

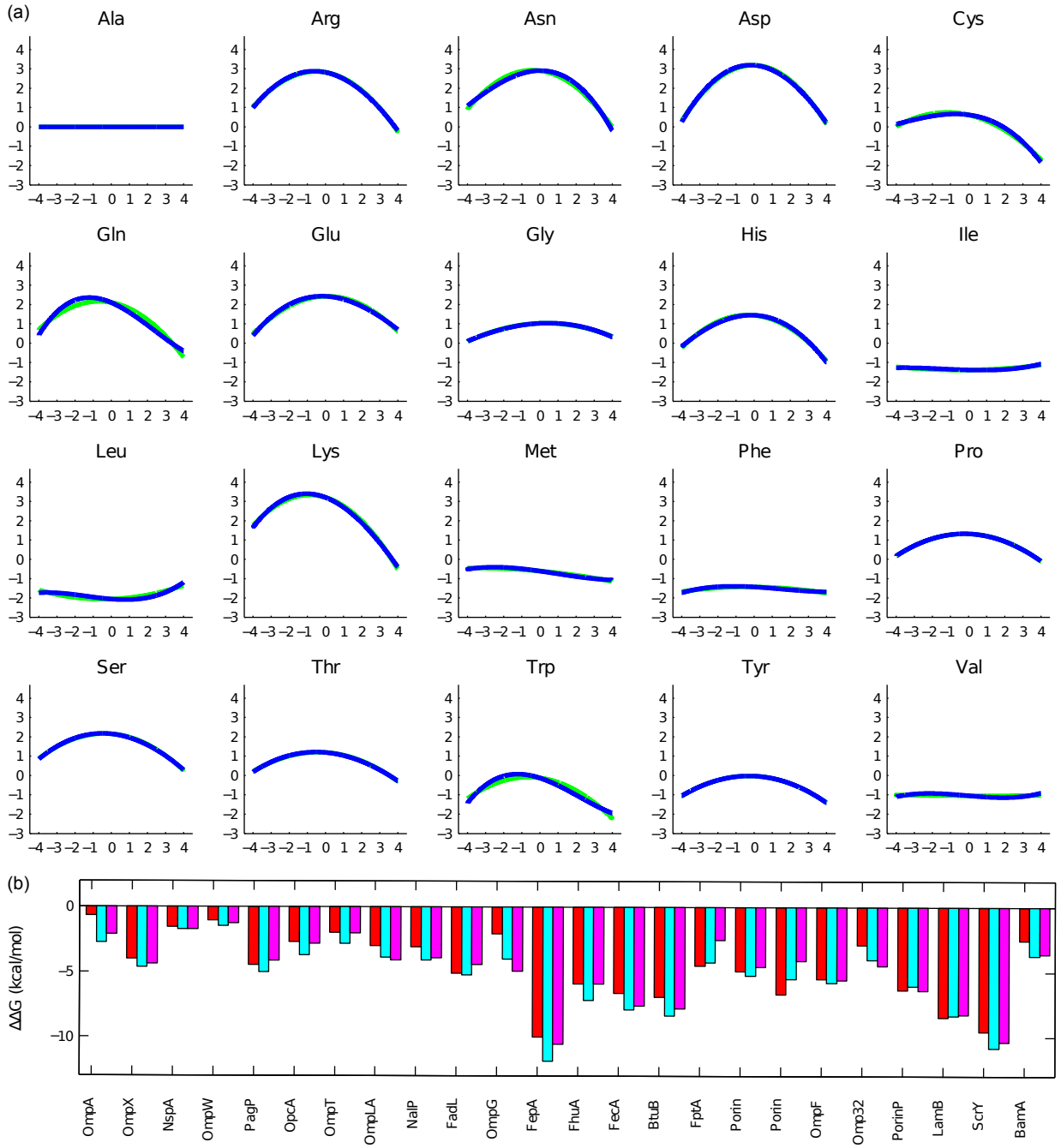

Fig S6. Approximation on thermodynamic stability is not sensitive to parameters of depth-dependent profiles. (a) Polynomial functions were used to derive depth-dependent transfer free energy profiles. Asymmetry in the profiles was observed in both second-degree (green) and third-degree (blue) polynomial function. (b) The stability differences of 24 OMPs in the asymmetric and a symmetric OM were determined using profiles derived from asymmetric Gaussian (red), second-degree polynomial (cyan), and third-degree polynomial (blue). All OMPs showed higher stability ($\Delta\Delta G < 0$) in the asymmetric OM.

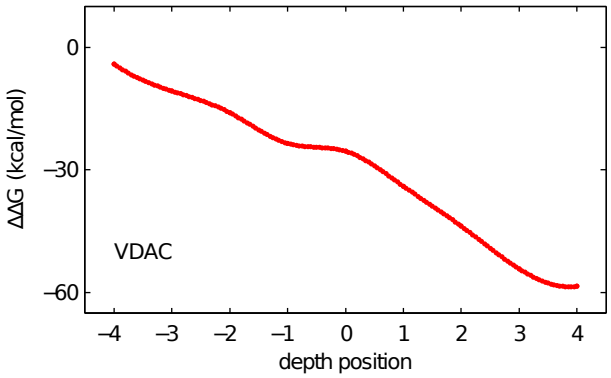

Fig. S7. Spontaneous insertion is predicted for mitochondria outer membrane protein VDAC. Folding energy of VDAC (PDB id: 3EMN) is approximated as the total transfer free energy of lipid-facing residues inserted into the bilayer. No energy barrier is observed during the folding process.

Table S1. Transfer free energy $\Delta\Delta G^{\text{host}}$ (kcal/mol) at different host residues of OmpLA and the general transfer free energy $\Delta\Delta G_{(i)}$ calculated from non-context dependent hosts at each depth-position i

A.A	$\Delta\Delta G(kcal/mol)$					
	$\Delta\Delta G^{\text{F122}}$	$\Delta\Delta G^{\text{A164}}$	$\Delta\Delta G^{\text{Y201}}$	$\Delta\Delta G^{\text{A206}}$	$\Delta\Delta G^{\text{D267}}$	$\Delta\Delta G_{(-4)}$
A	0	0	0	0	0	0±0
R	1.86	0.69	1.16	0.71	1.12	1.11±0.21
N	1.87	1.03	0.85	1.45	1.14	1.27±0.18
D	1.07	0.30	0.03	−0.4	−0.12	0.18±0.25
C	0.25	0.12	0.07	0.03	0.12	0.12±0.04
Q	0.82	0.57	0.30	0.73	0.36	0.56±0.10
E	1.14	0.14	0.24	0.71	0.42	0.53±0.18
G	0.62	0.12	0.25	−0.01	−0.03	0.19±0.12
H	0.20	−0.47	−0.32	0.62	0.22	0.05±0.20
I	−1.70	−1.14	−1.33	−0.65	−1.74	−1.31±0.20
L	−1.63	−1.70	−1.57	−1.78	−1.80	−1.70±0.04
K	2.13	1.89	2.08	2.18	2.24	2.10±0.06
M	−0.48	−0.38	−0.69	−0.48	−0.57	−0.52±0.05
F	−2.17	−2.01	−1.40	−1.63	−1.85	−1.81±0.14
P	−0.37	0.43	0.41	0.36	−0.49	0.07±0.20
S	1.75	0.90	0.76	0.24	1.36	1.00±0.26
T	0.29	0.28	0.23	−0.43	0.69	0.21±0.18
W	−1.86	−1.59	−1.53	−0.50	−1.37	−1.37±0.23
Y	−1.20	−1.10	−1.10	−1.00	−0.78	−1.04±0.07
V	−1.33	−0.84	−0.7	−1.15	−1.29	−1.06±0.12

	$\Delta\Delta G^{\text{V235}}$	$\Delta\Delta G^{\text{F75}}$	$\Delta\Delta G^{\text{L88}}$	$\Delta\Delta G^{\text{V136}}$	$\Delta\Delta G^{\text{V171}}$	$\Delta\Delta G^{\text{Y229}}$	$\Delta\Delta G_{(-3)}$
A	0	0	0	0	0	0	0±0

Continued on next page

Table S1 continued

A.A	$\Delta\Delta G\,(kcal/mol)$						
R	−0.66	0.67	1.4	3.27	1.41	1.70	1.69±0.43
N	0.34	0.33	1.06	2.27	1.59	1.49	1.35±0.32
D	1.10	0.21	0.72	3.17	3.17	1.31	1.72±0.62
C	0.28	0.15	0.22	0.81	0.83	0.26	0.45±0.15
Q	−0.13	0.79	0.41	0.58	2.22	2.15	1.23±0.39
E	−0.72	0.40	0.39	1.87	1.07	1.50	1.05±0.29
G	−0.32	0.26	−0.02	0.08	0.29	0.80	0.28±0.14
H	−0.90	0.20	0.79	−0.26	0.09	0.67	0.30±0.19
I	−0.31	−1.41	−1.15	−0.57	−0.65	−1.76	−1.11±0.23
L	−0.61	−2.16	−2.95	−1.06	−0.11	−2.25	−1.71±0.50
K	0.84	0.60	1.67	2.02	2.39	1.89	1.71±0.30
M	−0.39	−1.27	−1.58	0.64	0.61	−0.27	−0.37±0.46
F	−0.46	−1.00	−2.42	−0.77	−0.26	−2.11	−1.31±0.41
P	0.45	−0.02	0.94	0.33	1.76	0.75	0.75±0.30
S	−0.16	0.59	0.59	1.33	1.08	1.81	1.08±0.23
T	−0.05	0.15	0.86	0.57	0.32	1.17	0.61±0.18
W	−0.34	−0.23	−1.41	−0.59	−0.29	−0.63	−0.63±0.21
Y	−0.74	−0.48	−1.64	−0.16	−0.80	−0.17	−0.65±0.27
V	−0.45	−0.96	−0.92	−0.75	−0.70	−1.46	−0.96±0.13

	$\Delta\Delta G^{\mathrm{D36}}$	$\Delta\Delta G^{\mathrm{L265}}$	$\Delta\Delta G^{\mathrm{L120}}$	$\Delta\Delta G^{\mathrm{L162}}$	$\Delta\Delta G^{\mathrm{I199}}$	$\Delta\Delta G^{\mathrm{L208}}$	$\Delta\Delta G_{(-2)}$
A	0	0	0	0	0	0	0±0
R	−0.24	1.49	2.38	4.04	2.56	2.36	2.84±0.40
N	0.06	0.72	2.04	4.62	3.11	1.66	2.86±0.66
D	0.51	0.28	2.12	4.47	3.15	2.38	3.03±0.53

Continued on next page

Table S1 continued

A.A	$\Delta\Delta G\,(kcal/mol)$						
C	0.37	1.02	−0.34	0.82	0.60	0.41	0.37±0.25
Q	−0.37	1.30	1.87	4.83	3.40	1.34	2.86±0.79
E	−0.03	0.17	1.89	3.71	2.82	1.28	2.42±0.53
G	−0.96	0.42	0.33	1.11	0.92	0.58	0.74±0.17
H	−0.65	1.23	−0.05	1.27	0.47	1.22	0.73±0.32
I	0.28	−0.97	−2.02	−2.00	−1.73	−1.42	−1.79±0.14
L	−0.52	−1.36	−2.85	−1.89	−2.02	−2.27	−2.26±0.21
K	−0.09	1.83	4.36	4.82	3.67	2.46	3.83±0.51
M	−0.28	0.48	−1.29	0.06	0.24	−0.46	−0.36±0.34
F	−0.24	−1.53	−1.47	−1.96	−1.74	−1.59	−1.69±0.11
P	1.07	0.02	2.70	0.96	1.99	0.89	1.64±0.44
S	−0.49	1.43	2.06	4.01	2.70	1.20	2.49±0.59
T	−0.21	0.80	1.31	1.39	1.52	1.04	1.32±0.10
W	−0.51	0.14	0.25	0.16	0.19	−0.24	0.09±0.11
Y	−0.30	0.09	−0.41	−0.15	−0.25	0.49	−0.08±0.20
V	−0.29	−1.07	−1.43	−1.34	−1.40	−0.92	−1.27±0.12

	$\Delta\Delta G^{\mathrm{L237}}$	$\Delta\Delta G^{\mathrm{L73}}$	$\Delta\Delta G^{\mathrm{A90}}$	$\Delta\Delta G^{\mathrm{M138}}$	$\Delta\Delta G^{\mathrm{V173}}$	$\Delta\Delta G^{\mathrm{L227}}$	$\Delta\Delta G_{(-1)}$
A	0	0	0	0	0	0.00	0±0
R	1.98	2.28	3.43	1.76	4.47	1.53	2.69±0.55
N	2.34	1.96	3.56	1.06	3.14	1.71	2.29±0.46
D	1.82	1.86	3.51	1.53	3.72	1.19	2.36±0.52
C	1.25	0.27	1.31	0.81	1.52	0.01	0.78±0.29
Q	2.44	1.60	2.01	0.70	2.13	1.37	1.56±0.26
E	1.54	1.54	3.08	0.66	2.65	1.33	1.85±0.44

Continued on next page

Table S1 continued

A.A	$\Delta\Delta G\,(kcal/mol)$						
G	0.17	1.17	2.05	0.20	1.24	0.49	1.03±0.32
H	1.87	0.38	1.27	0.87	1.96	0.67	1.03±0.27
I	0.76	−1.60	−1.38	−0.08	−0.02	−1.06	−0.83±0.33
L	−0.10	−2.04	−2.20	−0.14	−0.35	−2.68	−1.48±0.52
K	2.42	2.78	2.98	2.05	4.15	2.46	2.88±0.35
M	1.22	−1.18	−1.85	−0.42	0.70	−1.46	−0.84±0.45
F	0.64	−1.63	−2.07	−0.02	0.22	−2.01	−1.10±0.50
P	0.73	−0.08	2.07	0.12	0.34	1.09	0.71±0.39
S	1.31	1.65	2.97	0.64	2.17	1.30	1.75±0.39
T	0.42	0.94	1.87	−0.35	1.33	0.50	0.86±0.38
W	1.87	−0.81	0.04	0.84	0.75	−1.16	−0.07±0.40
Y	0.50	−0.57	0.33	0.11	0.49	−0.71	−0.07±0.24
V	−0.09	−1.62	−1.22	0.39	−0.16	−0.43	−0.61±0.36

	$\Delta\Delta G^{\mathrm{N38}}$	$\Delta\Delta G^{\mathrm{L118}}$	$\Delta\Delta G^{\mathrm{T160}}$	$\Delta\Delta G^{\mathrm{L197}}$	$\Delta\Delta G^{\mathrm{A210}}$	$\Delta\Delta G^{\mathrm{V263}}$	$\Delta\Delta G_{(0)}$
A	0	0	0	0	0	0	0±0
R	1.04	3.29	5.08	1.76	3.26	2.08	3.09±0.58
N	0.87	3.96	3.54	3.74	3.10	2.88	3.44±0.20
D	1.14	4.30	3.78	1.83	4.04	3.89	3.57±0.44
C	0.85	0.57	0.47	0.64	0.38	0.91	0.59±0.09
Q	0.28	3.67	2.89	2.85	2.25	2.56	2.84±0.24
E	0.60	3.35	3.00	3.41	2.89	1.76	2.88±0.30
G	−0.06	1.05	0.88	1.20	1.15	1.32	1.12±0.07
H	0.83	2.82	3.04	2.00	3.23	2.43	2.70±0.22
I	0.50	−2.25	−1.30	−1.89	−1.53	−1.27	−1.65±0.19

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Table S1 continued

A.A	$\Delta\Delta G\,(kcal/mol)$						
L	0.15	−2.47	−2.46	−2.25	−2.30	−2.49	−2.39±0.05
K	0.16	4.60	6.88	4.57	3.97	2.15	4.43±0.76
M	0.18	−0.50	−0.18	0.48	−0.62	−0.45	−0.25±0.20
F	0.54	−1.79	−2.28	−2.24	−1.82	−1.65	−1.96±0.13
P	0.26	1.22	1.61	1.46	1.81	0.92	1.40±0.15
S	0.47	3.19	3.14	2.14	1.73	2.73	2.59±0.28
T	0.20	1.75	1.46	1.61	1.07	1.11	1.40±0.13
W	0.35	−0.69	1.37	0.22	−0.34	−0.23	0.07±0.36
Y	0.25	−0.09	−0.36	−0.41	−0.45	0.42	−0.18±0.16
V	0.52	−1.57	−0.11	−1.42	−1.39	−1.52	−1.20±0.27

	$\Delta\Delta G^{\mathrm{P175}}$	$\Delta\Delta G^{\mathrm{L71}}$	$\Delta\Delta G^{\mathrm{Y92}}$	$\Delta\Delta G^{\mathrm{Y140}}$	$\Delta\Delta G^{\mathrm{L225}}$	$\Delta\Delta G^{\mathrm{T239}}$	$\Delta\Delta G_{(1)}$
A	0	0	0	0	0	0	0±0
R	1.15	2.56	1.26	3.51	1.77	2.47	2.31±0.38
N	1.96	3.01	1.61	2.90	2.46	2.70	2.54±0.25
D	1.54	2.83	1.90	3.54	2.71	2.62	2.72±0.26
C	0.87	0.03	−0.61	1.27	−0.05	0.92	0.31±0.34
Q	0.98	1.53	1.19	2.06	0.23	2.84	1.57±0.44
E	0.72	2.80	2.30	2.57	1.84	2.10	2.32±0.17
G	0.78	0.78	1.14	1.24	1.05	0.43	0.93±0.15
H	1.82	0.37	0.11	1.88	−0.18	1.74	0.78±0.43
I	−0.09	−2.22	−0.93	−0.02	−2.39	−1.64	−1.44±0.44
L	−0.02	−2.84	−1.24	−0.36	−2.08	−1.77	−1.66±0.42
K	1.48	2.23	1.69	3.37	0.98	2.86	2.23±0.42
M	−0.56	−1.64	−1.00	0.68	−1.39	−0.25	−0.72±0.42

Continued on next page

Table S1 continued

A.A	$\Delta\Delta G\,(kcal/mol)$						
F	0.07	−1.39	−1.57	0.18	−1.38	−0.78	−0.99±0.32
P	−0.14	2.35	0.50	0.30	2.02	0.24	1.08±0.46
S	0.57	2.01	1.45	2.15	1.40	1.66	1.73±0.15
T	−0.06	0.91	1.08	1.35	0.62	1.05	1.00±0.12
W	0.54	−0.93	−1.60	0.70	−1.17	0.27	−0.55±0.44
Y	0.15	−0.27	−1.30	0.48	−0.22	0.57	−0.15±0.34
V	−0.07	−1.70	−0.79	−0.16	−1.05	−0.83	−0.91±0.25

	$\Delta\Delta G^{\mathrm{L40}}$	$\Delta\Delta G^{\mathrm{P116}}$	$\Delta\Delta G^{\mathrm{L158}}$	$\Delta\Delta G^{\mathrm{Y195}}$	$\Delta\Delta G^{\mathrm{G212}}$	$\Delta\Delta G^{\mathrm{V261}}$	$\Delta\Delta G_{(2)}$
A	0	0	0	0	0	0	0±0
R	−0.19	2.65	1.70	1.54	2.80	1.20	1.98±0.32
N	−0.06	2.51	2.50	2.49	2.64	2.60	2.55±0.03
D	0.39	3.16	2.06	2.09	3.17	3.22	2.74±0.27
C	0.48	0.17	0.25	−0.06	0.42	−0.12	0.13±0.10
Q	−0.77	0.73	0.52	1.25	1.20	0.43	0.83±0.17
E	−0.82	2.55	1.81	2.37	2.23	0.55	1.90±0.36
G	−0.44	0.97	0.64	1.26	1.15	0.61	0.93±0.13
H	0.18	0.97	0.63	2.60	−0.02	−0.16	0.80±0.49
I	−0.04	−1.13	−1.86	−1.31	−1.24	−1.66	−1.44±0.14
L	0.03	−2.59	−2.13	−2.68	−2.08	−2.74	−2.44±0.14
K	0.72	1.06	1.36	2.65	1.88	1.57	1.70±0.27
M	−0.91	−1.54	−0.51	−0.34	−0.78	−1.73	−0.98±0.28
F	0.70	−2.47	−1.63	−1.24	−2.18	−1.93	−1.89±0.21
P	1.20	−0.34	0.98	2.27	1.51	1.85	1.25±0.45
S	−0.74	1.80	1.43	1.83	2.36	1.51	1.79±0.16

Continued on next page

Table S1 continued

A.A	$\Delta\Delta G\,(kcal/mol)$						
T	−0.61	1.07	0.72	1.23	1.27	0.12	0.88±0.21
W	0.18	−1.41	−1.12	−0.23	−1.08	−1.25	−1.02±0.21
Y	0.27	0.41	0.00	0.22	−0.65	−0.76	−0.16±0.23
V	0.10	−1.90	−1.53	−1.67	−1.22	−1.08	−1.48±0.15

	$\Delta\Delta G^{\mathrm{Q94}}$	$\Delta\Delta G^{\mathrm{H142}}$	$\Delta\Delta G^{\mathrm{V241}}$	$\Delta\Delta G^{\mathrm{F69}}$	$\Delta\Delta G^{\mathrm{Y177}}$	$\Delta\Delta G^{\mathrm{A223}}$	$\Delta\Delta G_{(3)}$
A	0	0	0	0	0	0	0±0
R	0.71	−0.80	−0.16	0.55	1.29	0.19	0.68±0.32
N	−0.23	−0.32	0.92	0.14	0.46	0.50	0.37±0.11
D	−0.29	−0.46	0.26	0.70	0.92	1.26	0.96±0.16
C	−0.90	−0.80	0.21	−1.62	0.40	−1.80	−1.01±0.71
Q	−0.11	−0.53	1.15	−0.16	−0.70	0.45	−0.14±0.33
E	0.85	−0.52	0.26	0.86	0.44	1.29	0.86±0.25
G	0.02	−0.39	−0.05	0.63	0.67	0.44	0.58±0.07
H	0.02	−1.74	−0.54	−0.61	−0.30	−0.81	−0.57±0.15
I	−0.05	0.18	−0.39	−1.26	−0.66	−0.78	−0.90±0.18
L	−0.38	−0.44	−0.45	−1.71	−0.65	−1.82	−1.39±0.37
K	0.68	−0.14	0.11	0.28	1.28	−0.09	0.49±0.41
M	−0.26	−0.12	0.43	−1.39	−0.73	−1.28	−1.13±0.20
F	−0.34	−0.44	−0.71	−1.61	−0.81	−1.71	−1.38±0.28
P	−0.12	0.02	−0.36	0.29	−0.01	0.59	0.29±0.17
S	−0.15	−0.18	0.36	0.61	0.38	0.75	0.58±0.11
T	0.04	−0.33	−0.10	−0.09	−0.18	0.05	−0.07±0.07
W	0.07	0.09	−0.35	−1.27	−1.34	−2.33	−1.65±0.34
Y	0.15	−0.53	−0.65	−1.30	−0.84	−1.49	−1.21±0.19

Continued on next page

Table S1 continued

A.A	$\Delta\Delta G\,(kcal/mol)$						
V	0.08	0.27	−0.42	−1.29	−0.91	−0.19	−0.80±0.32

	$\Delta\Delta G^{\mathrm{Y42}}$	$\Delta\Delta G^{\mathrm{N156}}$	$\Delta\Delta G^{\mathrm{Y114}}$	$\Delta\Delta G^{\mathrm{G193}}$	$\Delta\Delta G^{\mathrm{Y214}}$	$\Delta\Delta G^{\mathrm{V259}}$	$\Delta\Delta G_{(4)}$
A	0	0	0	0	0	0	0±0
R	−0.13	1.16	0.53	−0.88	0.08	−0.08	−0.09±0.29
N	−0.41	−1.05	−0.13	−0.06	0.17	0.38	0.09±0.12
D	−0.35	0.28	0.62	−0.21	0.37	0.40	0.30±0.18
C	−0.84	−2.22	−1.82	−1.91	−1.63	−1.75	−1.78±0.06
Q	−0.27	−0.62	−0.48	−0.73	−0.24	0.59	−0.22±0.29
E	−0.18	−0.07	1.33	0.57	0.60	1.18	0.92±0.20
G	−0.02	0.48	0.42	0.52	0.17	0.39	0.38±0.07
H	0.27	0.41	−0.79	−1.63	0.61	−0.84	−0.66±0.47
I	−0.08	−1.89	−1.13	−1.46	−1.11	−1.06	−1.19±0.09
L	−0.21	−1.62	−1.04	−1.24	−1.11	−1.45	−1.21±0.09
K	0.17	−0.19	−0.53	−0.65	0.64	0.01	−0.13±0.29
M	−0.18	−0.31	−0.96	−1.07	−0.99	−0.97	−1.00±0.03
F	−0.04	−1.94	−2.03	−2.07	−1.32	−1.57	−1.75±0.18
P	0.20	1.37	−0.75	0.35	−0.14	0.10	−0.11±0.24
S	−0.25	−0.07	0.55	0.13	0.43	0.69	0.45±0.12
T	0.00	−0.99	−0.24	−0.23	−0.11	−0.10	−0.17±0.04
W	0.16	−1.97	−1.88	−2.53	−1.27	−1.86	−1.89±0.25
Y	0.40	−2.11	−1.47	−1.57	−0.78	−1.50	−1.33±0.18
V	0.54	−1.12	−0.97	−1.24	−0.82	−0.75	−0.95±0.11

Table S2. Correlation coefficient (R^2) of transfer free energy scales from different host residues ($\Delta\Delta G^{\text{host}}$ in kcal/mol) of OmpLA at the same depth-position i

position $i = 4$

	$\Delta\Delta G^{F122}$	$\Delta\Delta G^{A164}$	$\Delta\Delta G^{Y201}$	$\Delta\Delta G^{A206}$	$\Delta\Delta G^{D267}$
$\Delta\Delta G^{F122}$	1.00				
$\Delta\Delta G^{A164}$	0.88	1.00			
$\Delta\Delta G^{Y201}$	0.87	0.95	1.00		
$\Delta\Delta G^{A206}$	0.71	0.76	0.76	1.00	
$\Delta\Delta G^{D267}$	0.90	0.87	0.89	0.76	1.00

position $i = 3$

	$\Delta\Delta G^{F75}$	$\Delta\Delta G^{L88}$	$\Delta\Delta G^{V136}$	$\Delta\Delta G^{V171}$	$\Delta\Delta G^{Y229}$	$\Delta\Delta G^{V235}$
$\Delta\Delta G^{F75}$	1.00					
$\Delta\Delta G^{F75}$	1.00					
$\Delta\Delta G^{V136}$	0.43	0.53	1.00			
$\Delta\Delta G^{V171}$	0.39	0.48	0.65	1.00		
$\Delta\Delta G^{Y229}$	0.85	0.78	0.59	0.57	1.00	
$\Delta\Delta G^{V235}$	0.11	0.23	0.23	0.51	0.14	1.00

position $i = 2$

	$\Delta\Delta G^{D36}$	$\Delta\Delta G^{L120}$	$\Delta\Delta G^{L162}$	$\Delta\Delta G^{I199}$	$\Delta\Delta G^{L208}$	$\Delta\Delta G^{L265}$
$\Delta\Delta G^{D36}$	1.00					
$\Delta\Delta G^{L120}$	0.08	1.00				
$\Delta\Delta G^{L162}$	0.01	0.79	1.00			
$\Delta\Delta G^{I199}$	0.04	0.89	0.95	1.00		
$\Delta\Delta G^{L208}$	0.03	0.83	0.84	0.86	1.00	
$\Delta\Delta G^{L265}$	0.01	0.55	0.64	0.64	0.71	1.00

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Table S2 continued

position $i = 1$						
	$\Delta\Delta G^{L73}$	$\Delta\Delta G^{A90}$	$\Delta\Delta G^{M138}$	$\Delta\Delta G^{V173}$	$\Delta\Delta G^{L227}$	$\Delta\Delta G^{L237}$
$\Delta\Delta G^{L73}$	1.00					
$\Delta\Delta G^{A90}$	0.90	1.00				
$\Delta\Delta G^{M138}$	0.53	0.48	1.00			
$\Delta\Delta G^{V173}$	0.82	0.71	0.75	1.00		
$\Delta\Delta G^{L227}$	0.87	0.87	0.47	0.65	1.00	
$\Delta\Delta G^{L237}$	0.44	0.37	0.58	0.63	0.38	1.00

position $i = 0$						
	$\Delta\Delta G^{N38}$	$\Delta\Delta G^{L118}$	$\Delta\Delta G^{T160}$	$\Delta\Delta G^{L197}$	$\Delta\Delta G^{A210}$	$\Delta\Delta G^{V263}$
$\Delta\Delta G^{N38}$	1.00					
$\Delta\Delta G^{L118}$	0.14	1.00				
$\Delta\Delta G^{T160}$	0.13	0.86	1.00			
$\Delta\Delta G^{L197}$	0.04	0.89	0.82	1.00		
$\Delta\Delta G^{A210}$	0.18	0.95	0.88	0.84	1.00	
$\Delta\Delta G^{V263}$	0.19	0.92	0.72	0.76	0.88	1.00

position $i = -1$						
	$\Delta\Delta G^{L71}$	$\Delta\Delta G^{Y92}$	$\Delta\Delta G^{Y140}$	$\Delta\Delta G^{P175}$	$\Delta\Delta G^{L225}$	$\Delta\Delta G^{T239}$
$\Delta\Delta G^{L71}$	1.00					
$\Delta\Delta G^{Y92}$	0.81	1.00				
$\Delta\Delta G^{Y140}$	0.70	0.67	1.00			
$\Delta\Delta G^{P175}$	0.42	0.35	0.66	1.00		
$\Delta\Delta G^{L225}$	0.94	0.76	0.59	0.34	1.00	
$\Delta\Delta G^{T239}$	0.79	0.65	0.86	0.64	0.64	1.00

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Table S2 continued

position $i = -2$

	$\Delta\Delta G^{L40}$	$\Delta\Delta G^{P116}$	$\Delta\Delta G^{L158}$	$\Delta\Delta G^{Y195}$	$\Delta\Delta G^{G212}$	$\Delta\Delta G^{V261}$
$\Delta\Delta G^{L40}$	1.00					
$\Delta\Delta G^{P116}$	0.05	1.00				
$\Delta\Delta G^{L158}$	0.02	0.89	1.00			
$\Delta\Delta G^{Y195}$	0.00	0.72	0.86	1.00		
$\Delta\Delta G^{G212}$	0.03	0.87	0.92	0.75	1.00	
$\Delta\Delta G^{V261}$	0.01	0.74	0.85	0.74	0.88	1.00

position $i = -3$

	$\Delta\Delta G^{F69}$	$\Delta\Delta G^{Q94}$	$\Delta\Delta G^{H142}$	$\Delta\Delta G^{Y177}$	$\Delta\Delta G^{A223}$	$\Delta\Delta G^{V241}$
$\Delta\Delta G^{F69}$	1.00					
$\Delta\Delta G^{Q94}$	0.26	1.00				
$\Delta\Delta G^{H142}$	0.00	0.00	1.00			
$\Delta\Delta G^{Y177}$	0.57	0.10	0.05	1.00		
$\Delta\Delta G^{A223}$	0.83	0.15	0.00	0.39	1.00	
$\Delta\Delta G^{V241}$	0.19	0.01	0.00	0.11	0.25	1.00

position $i = -4$

	$\Delta\Delta G^{Y42}$	$\Delta\Delta G^{Y114}$	$\Delta\Delta G^{N156}$	$\Delta\Delta G^{G193}$	$\Delta\Delta G^{Y214}$	$\Delta\Delta G^{V259}$
$\Delta\Delta G^{Y42}$	1.00					
$\Delta\Delta G^{Y114}$	0.03	1.00				
$\Delta\Delta G^{N156}$	0.01	0.49	1.00			
$\Delta\Delta G^{G193}$	0.01	0.72	0.46	1.00		
$\Delta\Delta G^{Y214}$	0.01	0.68	0.57	0.54	1.00	
$\Delta\Delta G^{V259}$	0.02	0.82	0.48	0.82	0.71	1.00

Table S3. Values of depth-dependent profile $\Delta\Delta G_{aa(i)}$ (kcal/mol) and fitting parameters

$\Delta\Delta G_{aa(i)}$	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
-4	0.00	1.20	1.08	0.78	0.19	0.83	0.66	0.15	0.00	−1.08	−1.49	1.77	−0.31	−1.27	0.41	0.96	0.36	−1.41	−1.09	−0.85
-3	0.00	1.80	1.72	1.48	0.33	1.36	1.20	0.36	0.01	−1.24	−1.75	2.44	−0.47	−1.44	0.69	1.41	0.64	−0.53	−0.55	−0.97
-2	0.00	2.42	2.41	2.34	0.49	1.92	1.85	0.68	0.24	−1.37	−1.95	3.08	−0.63	−1.56	1.01	1.86	0.95	−0.10	−0.19	−1.06
-1	0.00	2.88	2.94	3.08	0.63	2.37	2.39	0.98	1.39	−1.45	−2.09	3.54	−0.76	−1.65	1.26	2.19	1.22	−0.03	−0.05	−1.12
0	0.00	3.06	3.15	3.38	0.68	2.54	2.61	1.11	2.50	−1.48	−2.13	3.71	−0.81	−1.68	1.36	2.31	1.32	−0.13	−0.01	−1.15
1	0.00	2.61	2.73	3.01	0.33	1.76	2.39	1.04	0.93	−1.44	−2.06	2.89	−0.80	−1.65	1.20	2.06	1.07	−0.43	−0.01	−1.12
2	0.00	1.62	1.77	2.12	0.04	0.58	1.83	0.85	0.05	−1.33	−1.86	1.37	−0.77	−1.57	0.81	1.46	0.57	−1.03	−0.19	−1.05
3	0.00	0.73	0.86	1.19	0.00	0.09	1.17	0.61	0.00	−1.17	−1.57	0.40	−0.73	−1.44	0.43	0.82	0.20	−1.67	−1.20	−0.95
4	0.00	0.24	0.32	0.53	0.00	0.01	0.63	0.38	0.00	−0.97	−1.23	0.07	−0.67	−1.28	0.17	0.36	0.05	−1.86	−1.32	−0.82

Para	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
a_0		3.06	3.15	3.38	0.68	2.55	2.61	1.11	2.50	−1.48	−2.13	3.71	−0.81	−1.68	1.36	2.31	1.32	−1.88	−1.57	−1.15
a_1		1.77	1.87	2.07	0.82	1.17	2.37	2.73	0.71	4.35	3.83	1.42	6.63	5.55	1.97	2.08	1.54	1.19	1.67	4.90
a_2		2.91	2.73	2.34	2.50	2.67	2.41	2.01	0.92	5.00	4.74	3.29	2.87	5.41	2.57	3.02	2.49	4.90	5.43	5.17
a_3																		1.625	0.754	
a_4																		0.758	3.558	

Table S4. Differences in the folding free energy of the TM regions of OMPs in the NC-IN and the NC-OUT topology calculated using the additive model by summing up the transfer free energy of all lipid facing TM residues ($\Delta\Delta G_{topo}^{Additive}$ in kcal/mol) and by using the partition function as in Eq 5 ($\Delta\Delta G_{topo}^{Ensemble}$ in kal/mol). The folding free energy ($\Delta G_{NC-IN}^{Ensemble}$ in kcal/mol) calculated using Eq. 5 is also listed.

Protein	PDB ID	$\Delta\Delta G_{topo}^{Additive}$	$\Delta\Delta G_{topo}^{Ensemble}$	$\Delta G_{NC-IN}^{Ensemble}$
OmpA	1BXW	−2.20	10.76	−85.80
OmpX	1QJ8	2.38	9.57	−80.80
NspA	1P4T	0.20	3.33	−85.03
OmpW	2F1T	−2.34	1.21	−79.58
PagP	1THQ	3.71	1.81	−78.90
OpcA	1K24	3.03	12.80	−101.65
OmpT	1I78	0.32	8.95	−97.57
OmpLA	1QD6	2.09	11.51	−115.17
NalP	1UYN	3.27	13.37	−119.08
FadL	1T16	−1.30	12.98	−140.39
OmpG	2F1C	−0.59	4.81	−144.21
FepA	1FEP	1.65	26.98	−201.88
FhuA	2FCP	−0.85	22.34	−205.09
FecA	1KMO	1.65	16.22	−206.17
BtuB	1NQE	−0.92	19.35	−189.49
FptA	1XKW	0.08	15.54	−203.87
Porin	1PRN	2.40	9.06	−138.77
Porin	2POR	3.95	7.03	−150.68
OmpF	2OMF	5.07	11.13	−141.27
Omp32	1E54	2.11	16.13	−146.27
PorinP	2O4V	2.73	18.44	−153.98
LamB	2MPR	4.66	7.60	−156.50
ScrY	1A0S	7.95	16.48	−157.27
BamA	4K3B	−2.17	8.32	−153.36

There is correlation between the folding free energy and the number of strands in the OMPs. However, OMPs of the same family can have different folding energy. Among the five OMPs belonging to the TonB-dependent transporter family, BtuB (PDB id: 1NQE) is less stable than the other four proteins (PDB id: 1FEP, 1KMO, 1NQE, and 1XKW). The folding free energy of BtuB is 7.2% lower than the average folding the other four members.

Table S5. Cooperativity of ionizable or polar residue pairs $\Delta\Delta\Delta G_{\mathrm{XX}}^{\mathrm{Hostpair}}$ (kcal/mol) of host pairs at different positions in OmpLA

XX	Host pair position $(4, 2)$				Host pair position $(2, 0)$			
	Y114P116	G193Y195	G212Y214	Average	P116L118	Y195L197	A210G212	Average
DD	0.44	−0.29	0.22	0.12	1.70	1.60	0.98	1.43
EE	0.82	0.15	0.05	0.34	1.13	1.21	0.25	0.86
RR	0.20	−1.11	0.02	−0.30	1.11	1.43	0.87	1.14
KK	−0.13	−0.81	−0.24	−0.39	0.29	1.25	0.81	0.78
HH	0.51	−0.90	−0.07	−0.15	1.18	1.03	0.11	0.77
NN	0.58	−0.32	0.14	0.13	1.73	1.49	0.32	1.18
QQ	0.06	−1.10	0.00	−0.35	0.65	1.37	0.10	0.71

XX	Host pair position $(0, -2)$				Host pair position $(-2, -4)$			
	L118L120	L197I199	L208A210	Average	L120F122	I199Y201	A206L208	Average
DD	2.37	1.84	3.22	2.48	0.84	1.78	0.04	0.89
EE	2.93	2.97	4.23	3.38	1.42	1.33	0.98	1.24
RR	2.61	1.51	2.66	2.26	1.36	1.50	0.53	1.13
KK	4.99	4.05	3.55	4.20	3.24	3.61	2.76	3.20
HH	0.78	1.00	2.34	1.37	0.51	0.34	0.68	0.51
NN	3.11	4.16	3.05	3.44	1.74	2.32	1.27	1.78
QQ	3.11	3.45	3.34	3.30	0.62	1.29	1.39	1.10

Table S6. Values of experimental and knowledge-based amino acid hydrophobicity scales in kcal/mol.

	experimental				knowledge-based	
A.A	$\Delta\Delta G^{o}_{w,l}$	$\Delta\Delta G^{aa}_{app}$	$\Delta\Delta G_{octanol}$	$E_z\beta$	Slusky-Dunbrack	E_b
A	0	0.11	0.50	−0.8	0.13	−0.69
R	3.71	2.58	1.81	1.4	−1.06	2.66
N	3.47	2.05	0.85	0.7	−0.69	2.30
D	2.95	3.49	3.64	1.3	−0.63	2.66
C	0.49	−0.13	−0.02	NA	NA	4.61
Q	3.01	2.36	0.77	0.7	−0.69	2.21
E	1.64	2.68	3.63	1.1	−2.48	4.61
G	1.72	0.74	1.15	0	0.04	0.27
H	4.76	2.06	0.11	1.2	−0.25	1.90
I	−1.56	−0.60	−1.12	−1.0	0.10	−0.83
L	−1.81	−0.55	−1.25	−2.0	0.35	−0.91
K	5.39	2.71	2.80	1.3	−0.98	3.51
M	−0.76	−0.10	−0.67	NA	0	−0.46
F	−2.20	−0.32	−1.71	−1.9	0.21	−0.25
P	−1.52	2.23	0.14	0.8	0.31	0.05
S	1.83	0.84	0.46	0.9	−0.11	1.31
T	1.78	0.52	0.25	NA	−0.30	0.40
W	−0.38	0.30	−2.09	−0.4	−0.44	0.26
Y	−1.09	0.68	−0.71	−0.0	−0.21	0.17
V	−0.78	−0.31	−0.46	−1.5	0.05	−0.96

$\Delta\Delta G^{o}_{w,l}$: Moon and Fleming's whole-protein scale[16]
$\Delta\Delta G^{aa}_{app}$: Hessa *et al*'s biological scale [21]
$\Delta\Delta G_{octanol}$: Wimley and White's octanol scale [22]
$E_z\beta$: Hsieh *et al*'s $E_z\beta$ scale[19]
Slusky-Dunbrack: Slusky and Dunbrack's scale[20]
E_b: Jackups and Liang's scale[4]

Table S7. Empirical strand interaction energy term values in kcal/mol.

E_{SH}	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	−0.17	−0.09	0.06	−0.08	−0.00	0.22	−0.09	0.01	0.18	0.02	0.15	0.12	0.56	0.17	0.36	−0.03	0.08	0.38	−0.07	−0.19
R	−0.22	0.07	0.01	−0.00	−0.16	0.04	0.21	−0.02	0.31	0.64	0.10	−0.23	0.75	−0.82	0.07	−0.19	−0.48	−0.01	−0.12	
N	−0.42	−0.54	−0.00	0.33	0.33	0.02	0.21	0.39	−0.06	−0.25	0.18	0.56	−0.47	−0.05	0.05	0.10	0.80	0.22		
D	1.78	−0.00	0.67	0.06	−0.11	−0.41	1.78	0.18	0.66	−0.45	1.78	−0.47	−0.08	0.03	−0.26	0.24	0.14			
C	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00				
Q	−0.03	0.19	−0.04	0.20	−0.38	−0.12	−0.19	−0.14	−0.25	−0.00	0.04	−0.06	−0.61	0.15	0.35					
E	−0.29	0.15	1.78	1.78	−0.09	0.17	−0.08	0.56	−0.00	−0.18	−0.27	−0.39	0.09	0.22						
G	0.10	−0.13	0.21	0.06	0.09	0.34	−0.33	−0.60	0.07	0.15	0.15	−0.23	−0.12							
H	1.78	1.78	−0.01	−0.74	−0.41	−0.08	−0.95	−0.26	0.59	−0.65	1.78	−0.08								
I	0.13	0.06	−0.39	−0.43	0.02	1.78	−0.17	−0.15	0.15	−0.27	0.10									
L	0.08	0.67	−0.03	−0.07	0.21	0.03	−0.01	−0.26	−0.21	−0.02										
K	−0.01	−0.41	0.12	1.78	−0.29	−0.26	0.22	0.95	0.45											
M	1.78	0.14	1.78	−0.37	−0.10	0.57	0.23	−0.13												
F	0.09	0.11	0.22	−0.01	−0.15	0.10	−0.17													
P	−0.00	−0.06	−0.06	1.78	−0.31	1.78														
S	−0.06	0.03	0.73	0.30	0.34															
T	−0.08	0.42	0.12	0.15																
W	−0.11	0.14	−0.24																	
Y	0.67	−0.11																		
V	0.26																			

E_{NH}	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	−0.19	0.39	0.34	0.59	−0.00	0.15	−0.04	0.05	0.62	−0.11	−0.14	0.47	−0.29	−0.09	−0.08	0.04	0.23	0.60	0.23	−0.15
R	−0.13	−0.32	−0.10	−0.00	−0.24	−0.32	0.34	1.78	0.22	0.87	−0.06	1.78	−0.22	0.13	0.35	−0.09	0.46	−0.36	0.07	
N	−0.29	−0.15	−0.00	0.09	0.10	−0.16	−0.25	0.62	0.49	−0.08	0.15	1.78	1.78	−0.27	0.01	0.45	0.66	0.69		
D	−0.26	−0.00	−0.02	0.43	0.07	−0.11	1.78	0.34	0.01	0.15	0.39	−0.54	−0.28	−0.00	−0.33	−0.09	0.26			

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Table S7 continued

C	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00
Q	0.47	0.59	−0.25	−0.06	1.78	0.01	−0.44	0.03	0.73	1.78	0.03	−0.18	−0.21	−0.03	1.78	
E	0.01	−0.12	−0.47	−0.06	0.23	−0.29	0.17	−0.56	1.78	0.66	0.09	−0.03	0.12	0.38		
G	0.16	0.28	−0.29	−0.17	0.73	−0.22	0.25	−0.11	−0.05	−0.01	0.12	0.33	−0.26			
H	1.78	0.19	0.13	0.08	1.78	−0.24	1.78	−0.40	−0.54	0.36	−0.17	0.08				
I	−0.01	−0.05	−0.06	−0.28	0.26	0.54	0.69	−0.10	0.15	0.08	−0.11					
L	−0.18	−0.09	0.11	0.18	−0.33	0.15	0.20	0.43	0.14	0.08						
K	−0.22	−0.02	0.32	0.18	−0.00	−0.21	−0.18	−0.14	0.31							
M	−0.26	−0.22	−0.30	0.11	0.06	0.32	0.27	0.53								
F	−0.02	0.31	−0.05	0.03	−0.18	−0.15	−0.05									
P	1.78	0.02	−0.10	0.26	0.19	−0.31										
S	−0.23	0.11	−0.08	0.02	0.47											
T	−0.24	0.31	0.15	0.08												
W	−0.08	−0.55	0.41													
Y	−0.09	−0.02														
V	−0.07															

E_{WH}	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	0.09	−0.24	−0.18	−0.00	−0.00	−0.06	−0.04	−0.09	−0.22	0.09	0.18	−0.24	0.25	−0.01	0.10	−0.01	0.21	0.12	0.01	−0.02
R	1.02	0.47	1.02	−0.00	0.22	0.66	0.27	0.29	−0.10	−0.30	0.67	−0.00	−0.35	0.12	0.47	−0.02	−0.18	0.03	−0.33	
N	0.25	0.92	−0.00	0.20	0.60	0.31	−0.03	−0.47	−0.05	0.35	−0.02	−0.27	−0.44	0.75	0.20	−0.30	−0.19	−0.24		
D	1.02	−0.00	0.02	0.87	0.31	1.02	−0.34	0.07	−0.07	0.15	−0.28	−0.84	0.75	−0.25	−0.38	−0.03	−0.41			
C	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00				
Q	0.07	0.34	0.40	−0.32	−0.14	−0.15	0.56	0.28	−0.18	−0.35	0.13	0.25	−0.29	−0.23	−0.12					
E	0.06	−0.03	0.14	−0.27	−0.32	1.01	−0.40	−0.29	−0.06	1.02	0.12	0.26	−0.09	−0.13						
G	0.04	0.10	−0.11	−0.16	0.18	0.16	−0.05	−0.54	0.12	0.10	−0.05	0.04	−0.16							
H	1.02	−0.35	0.20	0.05	0.08	−0.11	1.02	−0.33	−0.24	−0.16	0.42	0.05								
I	1.01	0.53	−0.24	−0.06	0.49	1.02	−0.35	−0.09	0.11	0.23	0.19									

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Table S7 continued

L	0.12	−0.21	0.45	0.12	0.41	−0.23	−0.11	0.16	0.25	0.34
K	1.02	0.53	−0.26	1.02	0.18	0.25	−0.11	−0.16	−0.11	
M	−0.34	0.11	−0.29	−0.33	−0.03	0.13	−0.34	−0.01		
F	0.24	0.19	−0.16	−0.10	0.35	0.26	0.95			
P	1.02	0.53	1.02	1.02	−0.12	0.80				
S	0.67	0.31	−0.29	−0.26	−0.22					
T	0.08	−0.18	−0.30	−0.14						
W	0.31	0.43	0.06							
Y	0.12	0.18								
V	0.82									

E_{Intra}	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	−1.61	−1.08	−1.09	−1.01	−2.11	−1.12	−0.89	−1.37	−1.43	−2.71	−2.91	−0.78	−2.33	−2.85	−1.20	−1.19	−1.37	−2.26	−1.99	−2.39
R	−0.92	−0.97	−1.36	−1.52	−1.07	−1.34	−1.02	−1.28	−2.15	−2.38	−0.35	−1.85	−2.36	−1.01	−0.96	−1.12	−2.02	−1.87	−1.82	
N	−0.99	−0.99	−1.53	−1.01	−0.89	−1.03	−1.23	−1.92	−2.21	−0.72	−1.75	−2.22	−0.91	−0.94	−1.11	−1.82	−1.63	−1.67		
D	−0.72	−1.43	−0.86	−0.60	−0.94	−1.37	−1.88	−2.01	−0.99	−1.52	−2.06	−0.79	−0.96	−1.07	−1.68	−1.63	−1.47			
C	−3.22	−1.69	−1.34	−1.87	−2.13	−3.25	−3.45	−1.15	−2.95	−3.43	−1.82	−1.69	−1.84	−2.93	−2.46	−2.94				
Q	−0.91	−0.84	−0.98	−1.17	−2.17	−2.39	−0.76	−1.95	−2.43	−1.02	−0.88	−1.12	−1.84	−1.76	−1.82					
E	−0.54	−0.72	−1.27	−1.94	−2.12	−1.07	−1.71	−2.11	−0.75	−0.88	−1.03	−1.77	−1.65	−1.58						
G	−1.33	−1.27	−2.24	−2.46	−0.68	−2.01	−2.44	−1.11	−1.08	−1.23	−2.02	−1.78	−2.00							
H	−1.80	−2.45	−2.69	−0.80	−2.36	−2.82	−1.33	−1.25	−1.43	−2.36	−2.08	−2.12								
I	−3.87	−4.17	−1.78	−3.56	−4.05	−2.23	−2.08	−2.38	−3.42	−3.11	−3.58									
L	−4.36	−1.99	−3.79	−4.31	−2.49	−2.32	−2.57	−3.63	−3.36	−3.83										
K	−0.07	−1.47	−1.99	−0.57	−0.62	−0.78	−1.59	−1.54	−1.47											
M	−3.23	−3.88	−2.04	−1.79	−2.08	−3.28	−2.91	−3.15												
F	−4.30	−2.52	−2.38	−2.53	−3.65	−3.35	−3.72													
P	−1.04	−0.93	−1.12	−2.21	−1.89	−1.96														
S	−0.99	−1.16	−1.77	−1.65	−1.80															

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Table S7 continued

T	−1.25	−1.91	−1.78	−2.05
W	−2.99	−2.76	−3.07	
Y	−2.47	−2.73		
V	−3.27			

Table S8. Single burial energy term values in kcal/mol.

AA	E-CAP	E-HG-IN	E-HG-OUT	C-IN	C-OUT	P-HG-IN	P-HG-OUT	P-CAP
A	0.20	0.19	0.28	0.01	−0.41	0.19	0.39	0.03
R	0.12	−0.24	0.83	−0.30	1.57	−0.15	0.06	−0.23
N	−0.23	−0.14	0.59	−0.09	1.36	−0.01	0.66	−0.24
D	−0.34	−0.15	1.90	0.25	1.57	0.02	0.23	−0.30
C	0.83	0.83	0.83	0.83	0.83	0.83	0.83	−0.88
Q	0.25	−0.10	0.21	−0.21	1.31	−0.47	−0.11	−0.10
E	−0.16	−0.26	0.83	−0.32	0.83	−0.26	0.41	−0.08
G	−0.06	0.20	0.25	−0.33	0.16	−0.06	0.64	0.02
H	−0.09	−0.18	−0.46	0.20	1.12	0.64	−0.54	0.06
I	0.13	0.50	−0.32	0.60	−0.49	0.09	0.01	0.25
L	0.08	0.46	−0.34	0.54	−0.54	0.25	0.12	0.40
K	0.03	−0.51	1.21	−0.03	2.08	0.22	−0.19	−0.26
M	0.15	0.49	0.35	0.02	−0.27	−0.29	−0.05	0.07
F	−0.17	0.98	−0.57	0.54	−0.15	0.73	−0.36	0.26
P	−0.57	0.15	0.77	1.43	0.03	0.83	0.20	−0.10
S	0.13	−0.38	0.57	−0.22	0.77	−0.19	0.43	−0.16
T	−0.01	−0.35	0.25	−0.06	0.24	−0.27	0.19	0.06
W	0.20	0.60	−0.67	0.40	0.15	0.46	−0.72	0.25
Y	0.82	0.33	−0.54	0.06	−0.10	0.16	−0.64	0.30
V	0.28	0.53	−0.32	0.64	−0.57	0.03	0.02	0.35