Energetics of the Transmembrane Peptide Sorting by Hydrophobic Mismatch

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Abstract: Hydrophobic mismatch between a lipid membrane and embedded transmembrane peptides or proteins plays a role in their lateral localization and function. Earlier studies have resolved numerous mechanisms through which the peptides and membrane proteins adapt to mismatch, yet the energetics of lateral sorting due to hydrophobic mismatch has remained elusive due to the lack of suitable computational or experimental protocols. Here, we pioneer a molecular dynamics simulation approach to study the sorting of peptides along a membrane thickness gradient. Peptides of different lengths tilt and diffuse along the membrane to eliminate mismatch with a rate directly proportional to the magnitude of mismatch. We extract the 2-dimensional free energy profiles as a function of local thickness and peptide orientation, revealing the relative contributions of sorting and tilting, and suggesting their thermally accessible regimes. Our approach can readily be applied to study other membrane systems of biological interest where hydrophobic mismatch, or membrane thickness in general, plays a role.

Cellular membranes display varied lipid compositions and hence physico-chemical properties supporting the processes taking place in and on them.[1] The membranes of the endoplasmic reticulum, the Golgi apparatus, and the plasma membrane differ in their thicknesses,[2] suggesting that hydrophobic mismatch (MM) controls the sorting and targeting of transmembrane (TM) peptides and proteins along the secretory pathway. MM is the difference in the hydrophobic extents of the transmembrane domain (TMD) and the host membrane[3,4] which—if unaccounted for—leads to an energetic penalty. Apart from organelle-level sorting, MM also affects protein function, conformation, stability, orientation, oligomerization, and dynamics.[5–8] Moreover, the plasma membrane[9] and organelle membranes[10] are heterogeneous with different local lipid pools manifested in different membrane properties—including thickness—leading to lateral sorting of proteins.[11] Sometimes, MM cannot be eliminated by lateral sorting, but instead either the membrane, the protein, or both adapt to eliminate the mistmatch. For multi-pass TMDs, this is not straightforward without conformational changes. However, single-pass TMDs can cope with mismatch by multiple mechanisms.

In the case of positive MM (TMD longer than membrane thickness), the TMD can either tilt or bend,[5,12–15] and the membrane can also respond by locally adjusting its thickness.[16,17] This mechanism is intriguing, since it can drive TMD aggregation by lowering the total energetic penalty associated with membrane deformation.[14] In the case of negative MM, the membrane can bulge, possibly coupled with TMD aggregation.[13,16,17] Alternatively, water can penetrate the headgroup region to hydrate charged protein residues.[13] In extreme MM scenarios, the TMDs might alternate their anchoring between membrane leaflets, or even abandon their TM orientation.[15,16] Of exceptional interest is the related energetics, as it maps the TMD and membrane properties to the preferred response. For example, a single-pass TMD with MM>0 might partition to a thicker domain or stay put and increase its tilt. Both actions would at least partially eliminate MM, but which one is favoured? Or are both active within the limits of the thermal energy? Surprisingly, these questions have escaped earlier computational and experimental examinations, likely due to methodological challenges.

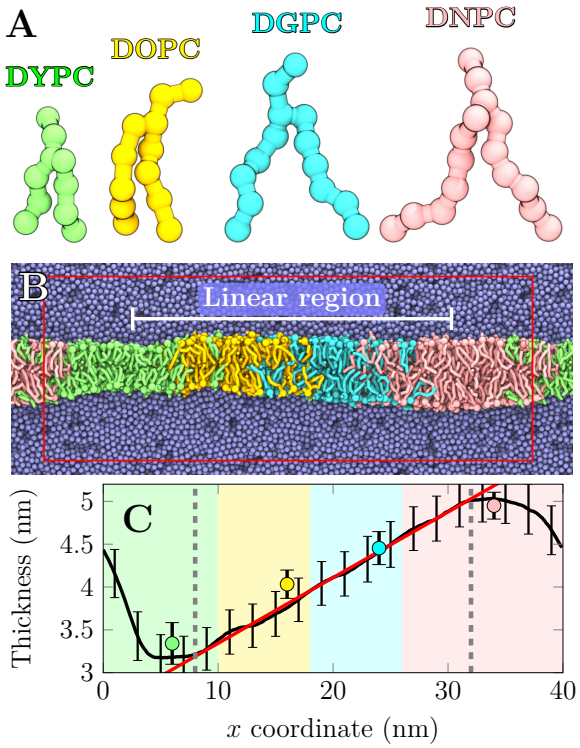

Figure 1. Thickness gradient generation. A) The four lipid types used in this study (see SI). Naming follows the Martini convention (`cgmartini.nl`). **B)** Membrane after 5 µs of simulation. Colouring as in A). Water in iceblue. The red rectangle shows the unit cell and white line the linear region (see panel C). **C)** Local membrane thickness (black). A linear thickness gradient with a slope of 0.077 nm/nm (red) is present in between gray dashed lines. Markers show single-component membrane thicknesses at the x coordinate in the middle of the corresponding patch in the assembled membrane. Shaded areas show positioning of membrane patches (overlap omitted).

Here, we tackle these questions using coarse-grained (CG) molecular dynamics (MD) simulations. We set up a lipid membrane containing a thickness gradient by merging four single-component membranes made of different phosphatidylcholine (PC) lipids with increasing acyl chain lengths: DYPC (3 coarse-grained beads per chain), DOPC (4 beads), DGPC

Table 1. Peptides used in this study. "l_{TM}" is the length of the hydrophobic region (0.15 nm per leucine). Equilibrium values are provided for local thickness (d_{eq}), peptide tilt angle (θ_{eq}), and projected peptide length (l_{proj}), and l_{mis} is the calculated MM.

Peptide properties			Unbiased simulations				US simulations	
Name	Sequence	l_{TM}	d_{eq} (nm)	θ_{eq} (°)	l_{proj} (nm)	l_{mis} (nm)	d_{kT} (nm)	θ_{kT} (°)
Leu17	$K_2L_{17}K_2$	2.55	3.3±0.5	23±10	2.3±0.4	−1.0±0.9	3.2–3.3	4–45
Leu19	$K_2L_{19}K_2$	2.85	3.5±0.6	25±11	2.6±0.5	−0.9±1.1	3.2–3.3 / 3.6–3.9	14–49 / 7–32
Leu21	$K_2L_{21}K_2$	3.15	3.8±0.6	26±12	2.8±0.6	−1.0±1.2	3.7–4.0 / 4.3–4.4	8–39 / 11-25
Leu23	$K_2L_{23}K_2$	3.45	4.3±0.4	17±8	3.3±0.3	−1.0±0.7	4.27–4.54	4–27
Leu25	$K_2L_{25}K_2$	3.75	4.5±0.4	23±9	3.4±0.5	−1.1±0.9	4.32–4.56	10–36
Leu27	$K_2L_{27}K_2$	4.05	4.8±0.6	16±8	3.9±0.3	−0.9±0.9	4.4–4.6 / 4.9–5.0	8–33 / 4–23
Leu29	$K_2L_{29}K_2$	4.35	5.1±0.2	23±8	4.0±0.5	−1.1±0.7	4.32–4.55	27–48

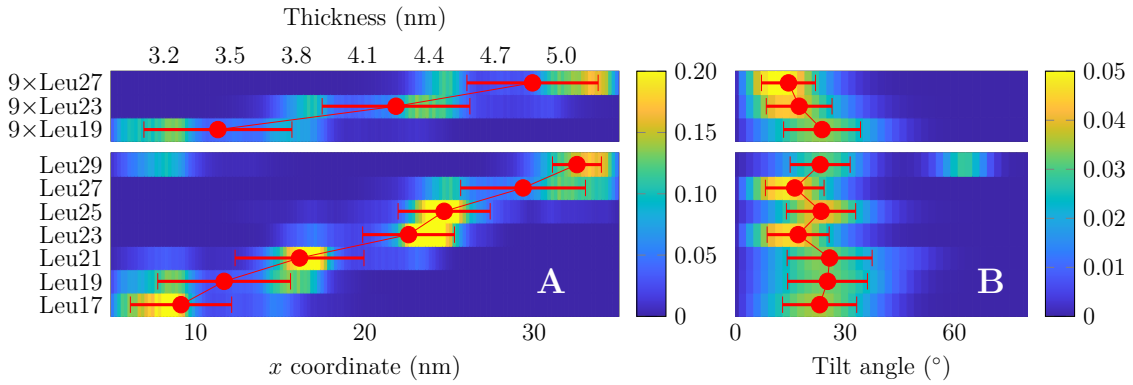

Figure 2. Spontaneous lateral sorting and tilting of the peptides. A) Density profiles of the peptides along the thickness gradient extracted from the last 50 µs of the unbiased simulations. Data shown for multi- (top) and single-peptide (bottom) systems. For single-peptide systems, data are gathered from 3 replicas with different initial peptide location. The mean value and standard deviation of the peptide center(s) of mass is shown in red markers. When initiated from a thin membrane, Leu29 did not find its equilibrium location and was omitted from the calculation of these values. **B)** Histograms of the peptide tilt during the last 50 µs of the unbiased simulations. Red markers again show mean value and standard deviation with one replica for Leu29 omitted. All profiles integrate to 1, and the numeric values for red markers are listed in Table 1.

(5 beads) and DNPC (6 beads), corresponding to 12–24 carbon atoms per chain in the Martini model[18,19] (Fig. 1A). The thicknesses of corresponding single-lipid membranes—ranging from 3.34 to 4.95 nm—are indicated by markers in Fig. 1C. To generate a permanent thickness profile along the x axis, the different lipid types are maintained at certain x-coordinate intervals using flat-bottom potentials (Fig. 1B). A smooth profile in the x coordinate range of 8–32 nm is generated with an optimized amount of lipid type overlap (see methods and Fig. S2 in the Supporting Information (SI)), resulting in maximum thickness difference of ≈1.85 nm. Due to periodic boundary conditions, there is an abrupt thickness jump at the edge of the simulation box, but analyses here focus on the linear region. Fig. S1 demonstrates that lipid density was uniform along the x coordinate and that the local area per lipid values were in reasonable agreement with values from single-component membrane simulations. Details of system setup and simulations are available in the SI.

We next embedded polyleucines (Leu17–Leu29) whose hydrophobic thicknesses l_{TM} ranged from 2.55 to 4.35 nm to this membrane (Table 1). Polyleucines have demonstrated tolerance for large MM and maintain their TM orientation.[20] The peptides were capped by two lysines to anchor them to the membrane–water interfaces.[21] See SI for further details.

We first studied whether the thickness gradient sorts the peptides, or whether alternative mechanisms—such as peptide tilt or membrane deformation— dominate. The secondary structure of the peptides is fixed in the Martini 2.2 model,[18,19] disallowing stretching, bending, or helix breaking, yet these are not expected to be important.[15,16] We performed 3 sets of 100 µs-long unbiased simulations with varying initial positions of the peptides with a flat-bottom potential maintaining them within the linear regime of the thickness gradient (x=5–35 nm). As the peptides diffuse at rates of D≈0.015–0.027 nm^2/ns, they cover $\delta x=\sqrt{4D\Delta}$≈100 nm in the simulation time of Δ=100 µs, allowing them to spontaneously find their preferred membrane environment, while (large) MM likely renders this search faster. Rapid sorting indeed occurs as evidenced by the time traces of the peptide positions (Fig. S3 and movie at DOI: 10.6084/m9.figshare.24105606). We also repeated this calculation with 9 copies of Leu19, Leu23, or Leu27 present in the membrane and initially placed along the thickness gradient at constant intervals (Fig. S4 & DOI: 10.6084/m9.figshare.24105606). The TM peptide density profiles and tilt angle distributions are shown as colormaps in Fig. 2. Red markers show mean ± standard deviation of the position of the peptide center of mass (and hence preferred thickness d_{eq}, defined as inter-leaflet phosphate distance) or tilt angle (θ_{eq}), which are also listed in Table 1. In one replica, Leu29 never left its initial position in the thin membrane and was omitted from the calculation of d_{eq} and θ_{eq}.

The d_{eq} values are 0.6–0.9 nm larger than the hydrophobic peptide lengths (l_{TM}). Despite lateral sorting, the peptides still demonstrate a significant tilt θ_{eq} of 22±4° on average without a systematic dependence on peptide length (Fig. 2B). Curiously, Leu23 and Leu27 tilt somewhat less (≈17°) than other peptides (≈24°), and this is reproduced

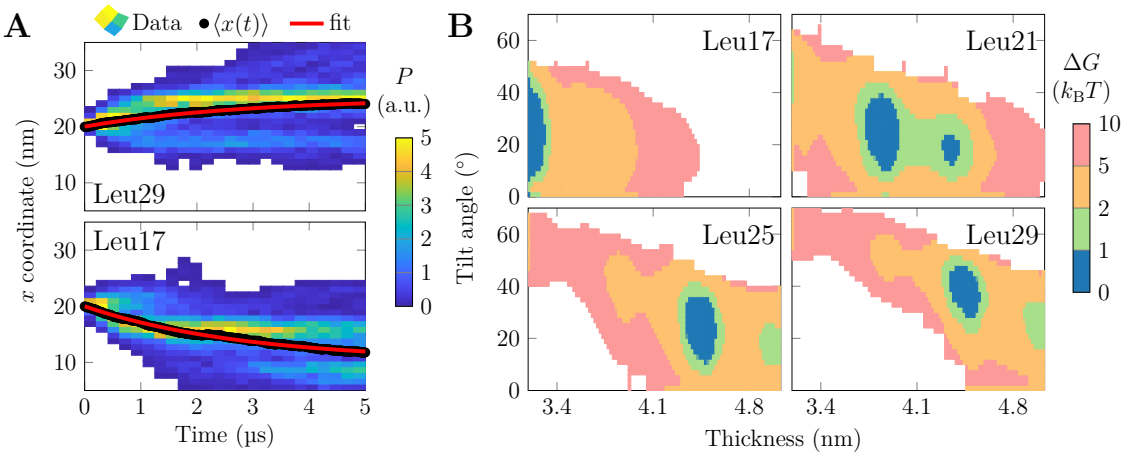

Figure 3. Energetics of lateral peptide sorting. A: Density of TM peptide center of mass as a function of time and x coordinate for the 100 replicas. Mean x shown in black and a fit of $x = x_0 - A\,(1 - \exp(-t/\tau))$ in red. **B:** 2D free energy surfaces as a function of membrane thickness and peptide tilt for selected systems. The rest are shown in Fig S5. Regions accessible from the global minimum within 1×, 2×, 5×, or 10 × $k_\mathrm{B}T$ are highlighted, and $\Delta G > 10 \times k_\mathrm{B}T$ discarded.

in the multi-peptide system. Tilting leads to the projected peptide length l_proj along the z axis (normal to the membrane) being ≈0.3 nm shorter than l_TM. Thus, the realized MM value (l_mis=d_eq–l_proj) is consistently 1.0±0.1 nm for all studied peptides, whereas an extrapolation to zero lentgh provides a mismatch value of 0.8 nm (see TOC graphic). Concluding, spontaneous sorting takes place on the simulation time scale, yet tilting also contributes to MM elimination.

To obtain physical insight into the sorting, we placed a single Leu17 or Leu29 at the center of the thickness gradient, and performed 100 independent unbiased simulations (Fig. 3A). We hypothesized that the velocity along the gradient (x) is linearly proportional to the distance of the peptide from its equilibrium location, $v = \frac{\mathrm{d}x}{\mathrm{d}t} = k(x_\mathrm{eq} - x)$, in other words proportional to MM. We solve for $x = x_0 - A(1 - \exp(-k/\tau))$, which fits the data in Fig. 3A remarkably well ($R^2 > 0.99$). This validates that v∼MM, which in viscous overdamped media where F∼v also indicates that F∼MM, *i.e.* a harmonic force. Curiously, both Leu17 (here MM<0) and Leu29 (MM>0) were found to relax towards x_eq with a time constant of 2.8 µs, suggesting that the force due to MM is symmetric around 0. Our peptides mainly respond to MM by tilting, yet membrane thickness deformation due to larger proteins was found to have a similar dependence on mismatch.[22]

The peptides occasionally sample different thicknesses and tilt angles during the unbiased simulations (Fig. S3), indicating that the underlying free energy minima are shallow. To verify this, we performed umbrella sampling (US) simulations to extract potentials of mean force (PMFs) for the peptide position along the thickness gradient. We combined this PMF with the free energy profile of peptide tilt angle obtained from the US windows as $\Delta G(\theta)_\mathrm{tilt} = -RT \ln(\theta/\theta_0)$ with RT the thermal energy and θ_0 the most likely tilt ($\Delta G(\theta_0) = 0$). We combined these two profiles to construct a 2D free energy surface that provide both the minima as well as the thermally accessible ranges of thickness and tilt angle for each peptide (Selected ones in Fig. 3B, rest in Fig. S5). All profiles demonstrate minima which cover 0.1–0.3 nm in thickness (d_{kT}) and 14–41° in tilt angle (θ_{kT}) within $\Delta G = k_\mathrm{B}T$ from the global minimum (ranges listed in Table 1; for peptides with two minima, values for both are reported). The profiles also reveal that doubling the threshold to 2 × $k_\mathrm{B}T$ barely increases the accessible ranges, whereas within 5 × $k_\mathrm{B}T$, the peptides can already sample a thickness range > 1 nm. The accessible tilt angle range is less sensitive. Finally, within the 10 × $k_\mathrm{B}T$ threshold, the peptides sample the entire available thickness range. The profiles demonstrate the expected trend; the thinner the membrane, the more tilted the peptides.

While the energetics of sorting by MM has not been previously studied, Kim and Im extracted the thermally accessible tilt angle ranges for WALP23/WALP27 peptides in POPC and DMPC membranes.[12] WALP23/WALP27 have l_TM values similar to our Leu17/Leu21 peptides. We performed simulations of single-component POPC and DMPC membranes and identified the US windows with similar thicknesses. In the window corresponding to POPC, Leu17/Leu21 sample tilt angles of 5–29°/10–36°, whereas the values for WALP23/WALP27 in POPC were similar at 7–26°/14–46.[12] For DMPC-like thickness, Leu17/Leu21 showed tilts of 6–34°/18–47, in reasonable agreement with WALP23/WALP27 in DMPC with 14–39°/32–51°.[12] The small differences likely arise from different peptide sequences, especially the residues anchoring them to the membrane–water interfaces.

The US windows provide a systematic set of different (fixed) MM conditions. We first analyzed how the peptides respond to MM *via* tilting. Fig. 4A demonstrates tilt angles larger than 60° in the case of significant positive MM. With thicker membranes, the tilt angles decrease monotonously until saturation at ≈10°. Our membrane does not contain a thick enough region to observe this saturation for the three longest peptides. We used these tilt values to calculate the projected peptide lengths (l_proj) along the z axis (membrane normal), as for the unbiased simulations (Table 1). We then estimated the real MM defined as l_mis=d_eq–l_proj (Fig. 4B). Ideal MM (assuming peptide orientation along membrane normal) is also shown. In the case of positive ideal MM, peptide tilting leads to a fairly constant projected MM of –1 nm, in line with our unbiased simulations (Table 1). There is no obvious peptide length dependence. In the thicker membrane with ideal MM<–1 nm, the peptides can no longer tilt but rather maintain a constant tilt (Fig. 4A), and the projected MM follows the ideal behavior. Curiously, the magnitude of l_proj for each peptide is smallest right before it starts to follow the ideal MM scenario, *i.e.* at the least tilted conformation. This suggests that in too thin membranes, the peptides overcompensate for the MM by tilting.

Concluding, we have presented a novel simulation approach to study phenomena affected by membrane thickness. Using a combination of different lipid species and flat-bottom restraints, a thickness gradient is maintained along one axis of the simulation box. As the first example, we have focused on single-span peptides, which serve as model systems for the TMDs of physiologically important receptor tyrosine kinases. Our results demonstrate that peptides of different lengths are spontaneously sorted over distances of dozens of nanometers in the microsecond time scale. This indicates that our setup can be efficiently used to study the sorting of lipids, peptides, proteins and their complexes, and other membrane-embedded objects. Moreover, with the x coordinate one-to-one mapped to thickness, conformational changes of proteins induced by the latter are readily studied. Free energy profiles of thickness-dependent properties—such as sorting or conformation—can be extracted using a simple reaction coordinate. We facilitate these and other yet unconsidered applications by providing all the simulation inputs and outputs in the Zenodo repository at DOI: to be added. Here, we used the CG Martini[18,19] model, yet the future extension to atomistic resolution is straightforward. Morever, the CG approach has limited resolution, and although the smoothness of our thickness gradient is optimized (Fig. S2), there is still a detectable bias of the peptides towards the single-component membrane regions (see Fig. 3), rendering our approach only semi-quantitative. This is potentially an entropic effect, as a peptide in the overlap region decreases the total amount of lipid mixing permitted within the flat-bottom restraints. This is likely overcome with the additional resolution and hence smoother profiles of atomistic models.

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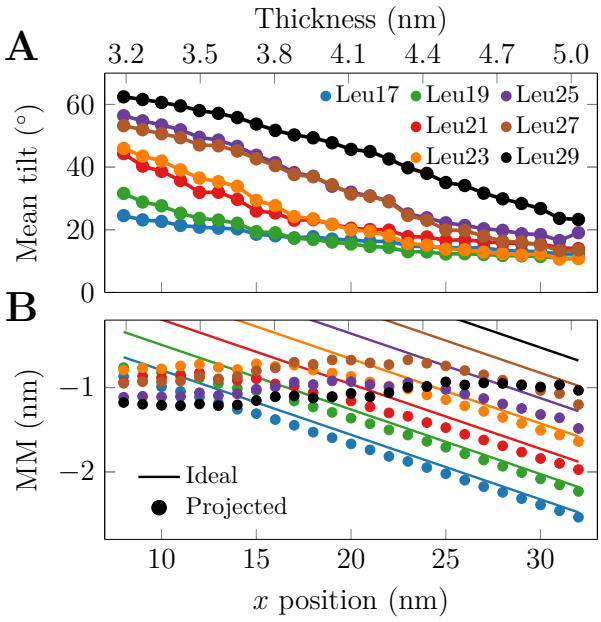

Figure 4. Peptide response to MM in US windows. A) Peptide tilt as a function of thickness. Even at significant MM, the peptides maintain a tilt angle of 10–20° due to entropy.[23] **B)** Ideal and projected MMs. The peptides tilt to maintain a MM of –1 nm, yet at larger thicknesses the peptides adopt a small tilt angle and the projected MM follows the ideal MM.

Supporting Inf. Available

Details on simulation setup and analysis. Additional results.

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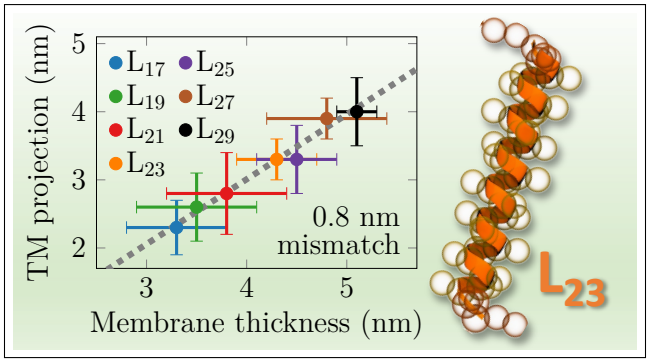
TOC Graphic


TM projection (nm)
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4
5
Membrane thickness (nm)
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