Reading for this week:


Polypeptides are expected to be unstable in membranes due to unsatisfied charges in the backbone.
The physical implications of a lipid bilayer membrane

\[ \Delta G_{\text{elec}}^{A \rightarrow B} = \frac{1}{2} \frac{g^2}{R_{\text{ion}}} \left( \frac{1}{E_B} - \frac{1}{E_A} \right) \]

\begin{align*}
\text{vacuum } & \varepsilon = 1 \\
\text{water } & \varepsilon = 80 \\
\text{lipid bilayer } & \varepsilon = 2-3
\end{align*}

Continuum electrostatics
Born equation
The importance of hydrogen bonding in membranes

Figure 3  Summary of the energies of peptide bond partitioning between water and nonpolar phases (kcal/mol). The cost of partitioning non-hydrogen-bonded peptide bonds into completely apolar phases is very high but is lower for partitioning into octanol or into the interface of palmitoyloleoylphosphocholine (POPC) bilayers. The cost of partitioning is dramatically reduced in all cases when peptide bonds participate in hydrogen bonds. This reduction is a major driving force for the formation of secondary structure in membranes and their interfaces. The free energy reduction associated with the partitioning of the most hydrophobic amino acid side chain, tryptophan, is too small to compensate for the cost of partitioning non-H-bonded peptide bonds. Therefore, a TM polypeptide segment composed of nonpolar amino acids can traverse the membrane only if there is complete backbone-backbone hydrogen bonding, as in α-helices or β-barrels (Figure 1). The free energies are from (a) Rosenman (130), (b) Ben-Tal et al (10), (c) Wimley et al (174), (d) Wimley & White (179), and (e) Wimley et al (176) and Ladokhin & White (78).
The lipid bilayer is not an isotropic solvent

**Figure 1.** Lipid bilayers contain large variations in density and polarity on a nanometer scale. (i) Snapshot of a DOPC bilayer. (ii) Partial density profile of a pure DOPC bilayer. The system is divided into four regions with different physicochemical properties [7]. Region I, the center of the bilayer, is hydrophobic and significantly disordered with properties similar to decane. In Region II, the lipid tails are more ordered and have a higher density, similar to a soft polymer. Region III contains a diverse mixture of functional groups including the carbonyl and glycerol groups of the lipid tails, most of the head group density and water. Region IV is defined by water that is perturbed by the lipid bilayer and can be quite deep.
H-bonded secondary structures in membranes

secondary structures

alpha helix

beta sheet

multi-TM Helices

Beta barrels
“Two-stage” model of membrane protein folding

https://doi.org/10.1016/j.jmb.2014.09.014
Alpha-helical membrane protein synthesis

https://doi.org/10.1016/j.bbamcr.2013.10.023
“Two-stage” folding of alpha-helical proteins

• Popot & Engelman - reassembly of proteolytic fragments of bacteriorhodopsin in membranes => two stage model of membrane protein folding
Beta-barrel folding

DOI: 10.1098/rstb.2015.0026
But what about the cost of side-chain partitioning?
Amino Acid side-chain transfer free energies
Amino Acid side-chain transfer free energies
Amino Acid side-chain transfer free energies
Amino Acid side-chain transfer free energies
Defects & Cooperativity

Figure 3: Arginine partitioning into lipid bilayers is non-additive. (a) Calculations show that arginine causes a water defect in the membrane, adding a second arginine to an existing defect causes almost no increase in free energy. Adapted with permission from 121. (b) Experimental observation of non-additivity of arginine partitioning. This panel summarizes five different experimental observations. Adapted with permission from 133.
Amino Acid side-chain transfer free energies

Figure 2. Summary of systems and environments in the different hydrophobicity scales addressed in this work. Each panel shows an overview of the system studied by the experiment and an indication of the environment encountered by an arginine residue.
Whole-Residue Hydrophobicity Scales

**Interface Scale**

POPC

### ΔG (water to bilayer, kcal mol⁻¹)

- **Charged**
- **Aromatic**

### ΔG (water to octanol, kcal mol⁻¹)

- **Charged**
- **Aromatic**

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B

**Hessa**

![Graph showing ΔG_{sa} values for Hessa amino acids](image)

**Amino acid**

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**Moon**

![Graph showing ΔG_{wi} values for Moon amino acids](image)

**Amino acid**
Amino Acid side-chain transfer free energies

Figure 4. The hydrophobicity scales are correlated but differ in overall magnitude. (i) Correlation between the normalized scales. In order to emphasize the correlation (r) among the scales, all scales are normalized so that the values range from -1.0 to 1.0. The scales differ in overall magnitude, as indicated by the slope (s). (ii) Hessa and Radzicka scales are correlated, but differ in overall magnitude scale factor. This panel plots the energies on an absolute scale to emphasize the difference in overall magnitude. Residues are colored by type. His, Pro, Gly were not present in all scales and are not shown. The values for Glu and Asp in Moon might represent a partially neutral state as the experiments were done at pH 3.8.
Hydropathy analysis

(A) GLYCOPHORIN

(B) BACTERIORHODOPSIN

Figure 10-20 Molecular Biology of the Cell (c) Garland Science 2015
“Two-stage” model of membrane protein folding

https://doi.org/10.1016/j.jmb.2014.09.014
A gold-mine of highly ordered membrane protein

- Stoeckeniuss & Rowen (1967) isolated purple membranes from H. Halobium archaea (> 4.3 M salt conc.)
- Osterhelt & Stoeckeniuss (1970) identified a single molecular species with MW 26 kDa formed the purple membrane. They showed that it was responsible for the color due to the binding of retinal, bound via a Schiff base linkage to lysine 216.
- First membrane protein structure in 1975 by Henderson & Unwin by EM diffraction

Patches of purple membrane, which contain bacteriorhodopsin in the archaeon Halobacterium salinarum.

Figure 5. Regeneration of BR chromophore from fragments refolded either separately or simultaneously in the absence of retinal. Purified fragments in SDS buffer were mixed with *Halobacterium* lipids (lipid to protein ratio 10:1, w/w) in the absence of retinal and taurocholate and reconstituted by PDS precipitation as described in Materials and Methods. Following dialysis, the vesicle suspensions were clarified by a brief sonication. (a) C-1 and C-2 in SDS buffer were mixed prior to PDS precipitation and simultaneously refolded in the same vesicles (top panel). Absorption spectra were recorded before (thin line) and after (thick line) addition of excess retinal (middle panel). Bottom: difference spectrum. (b) C-1 and C-2 were reconstituted into separate vesicles (top panel). The vesicles were mixed (equimolar ratio of the fragments) and absorption spectra recorded before (thin line) and after (thick line) addition of excess retinal (middle panel). Bottom: difference spectrum. (c) C-1 and C-2 were reconstituted into separate vesicles. The vesicles were mixed (equimolar ratio of the fragments) and freeze-thawed (top panel) in the absence (thin line) or presence (thick line) of excess retinal (middle panel). Absorption spectra were recorded after clarification by brief sonication. The identical result was obtained if retinal was added after freeze-thawing. Bottom: difference spectrum.
What is the role of lipids in protein reactions? Solvent or ligands?

- ESR reports lipid exchange between annular lipids as $\sim 10^7$ s$^{-1}$
- Lipid exchange in bulk $\sim 10^8$ s$^{-1}$
- Intrinsically different membrane environment around a protein
- Not lipid binding
Lipid trapping is detected by mass spectrometry
To understand this, we need to study reaction equilibria linked to lipid activity.

\[ K_{eq} = \frac{\chi_D}{(\chi_M^*)^2} \]
Gramicidin dimerization is sensitive to membrane properties

Non-conducting Monomers  Anesthetic  Non-conducting Monomers

Conducting Dimers

Time  Current

https://doi.org/10.1073/pnas.1611717114
Alpha-helical membrane protein folding

**Alpha helical folding from SDS**

partly denatured state in SDS

transition state

folded helical bundle

Steric-trapping studies folding in the native state

\[ \Delta G_B \]

\[ \Delta G_{U} + \Delta G_B \]
Oligomerization/aggregation as a model for the association reaction in membranes

Ernst & Robertson, JMB
2021
CLC dimers - binding via a greasy interface

CLC-ec1 from *E. coli*
Measuring membrane protein association equilibrium in membranes by single molecule subunit capture

Chadda & Robertson, MIE 2016
Quantifying the association reaction requires benchmarks to correct for artifactual association.

Chadda, Krishnamani, Mersch, Wong, Brimberry, Chadda, Kolmakova-Partensky, Friedman, Gelles & Robertson, eLIFE 2016 Chadda, Cliff, Brimberry & Robertson, JGP 2018
Quantifying the association reaction requires benchmarks to correct for artifactual association.

Chadda, Krishnamani, Mersch, Wong, Brimberry, Chadda, Kolmakova-Partensky, Friedman, Gelles & Robertson, eLIFE 2016 Chadda, Cliff, Brimberry & Robertson, JGP 2018
Quantifying the association reaction requires benchmarks to correct for artifactual association.

Chadda, Krishnamani, Mersch, Wong, Brimberry, Chadda, Kolmakova-Partensky, Friedman, Gelles & Robertson, eLIFE 2016 Chadda, Cliff, Brimberry & Robertson, JGP 2018
Equilibrium CLC-ec1 dimerization isotherm in membranes

\[ F_{\text{Dimer}} = \frac{1 + 4\chi^*K_{eq} - \sqrt{1 + 8\chi^*K_{eq}}}{4\chi^*K_{eq}} \]

\[ \Delta G^\circ = -RT \ln(K_{eq} \cdot \chi^*) \]

\[ \chi^* = \frac{\text{subunit}}{\text{lipid}} \]

\[ \Delta G_{\text{WT}}^\circ = -10.9 \pm 0.1 \text{ kcal/mole} \]

Chadda, Krishnamani, Mersch, Wong, Brimberry, Chadda, Kolmakova-Partensky, Friedman, Gelles & Robertson, eLIFE 2016 Chadda, Cliff, Brimberry & Robertson, JGP 2018
High-resolution atomic force microscopy of CLC-ec1 shows monomers, dimers & tetramers

Heath, Kots, Robertson, Lansky, Khelashvili, Weinstein & Scheuring, Nature 2021
Dynamic subunit exchange in membranes

\( \chi = 1 \) subunit per 100,000 lipids
Membrane: EPL
Buffer: 300 mM KCl, 20 mM citrate pH 4.5
Temperature: ~ 22 °C

Chadda, Lee, Sandal, Mahoney-Kruszka & Robertson (in preparation)
Modeling the membrane structure around CLC-ec1

Simulation details
Software: Gromacs
FF: MARTINI
Simulation time:
160 μs total
10 + 8 replicas

Lipid composition
headgroup:  67% PE, 33% PG
tails:      100% PO

Chadda, Bernhardt, Kelley, Teixeira, Griffith, Gil-Ley, Ozturk, Hughes, Forsythe, Krishnamani, Faraldo-Gomez & Robertson, eLIFE 2021
CLC-ec1 monomer introduces a thinned membrane defect

time-averaged lipid structures

Chadda, Bernhardt, Kelley, Teixeira, Griffith, Gil-Ley, Ozturk, Hughes, Forsythe, Krishnamani, Faraldo-Gomez & Robertson, eLIFE 2021
The effect of short-chain lipids on dimerization

\[ \chi_{\text{reconst.}} = 10^{-6} \text{ subunits/lipid} \]

\[ \rho = 1 \text{ subunit in (900 nm)}^2 \text{ box area} \]

\[ n > 3, \text{ mean } \pm \text{ sem} \]

Chadda, Bernhardt, Kelley, Teixeira, Griffith, Gil-Ley, Ozturk, Hughes, Forsythe, Krishnamani, Faraldo-Gomez & Robertson, eLIFE 2021
Dimerization shifts before changes in membrane thickness

- Bilayer thickness (SANS)
- function
- dimerization

\[ \Delta \Delta G \propto \log(DL) \rightarrow \text{preferential solvation} \]

Chadda, Bernhardt, Kelley, Teixeira, Griffith, Gil-Ley, Ozturk, Hughes, Forsythe, Krishnamani, Faraldo-Gomez & Robertson, eLIFE 2021
Short-chain lipids preferentially solvate the dimerization interface

Simulation details
Software: Gromacs
FF: MARTINI
Simulation time: 315 μs
8 replicas each

Lipid composition
headgroup:
67% PE, 33% PG
tails:
1-50% DL, 99-50% PO

Chadda, Bernhardt, Kelley, Teixeira, Griffith, Gil-Ley, Ozturk, Hughes, Forsythe, Krishnamani, Faraldo-Gomez & Robertson, eLIFE 2021
Short-chain lipids promote bilayer structure of the membrane defect

**Diagram: Time-averaged lipid structures**

Chadda, Bernhardt, Kelley, Teixeira, Griffith, Gil-Ley, Ozturk, Hughes, Forsythe, Krishnamani, Faraldo-Gomez & Robertson, eLIFE 2021
Short-chain lipids do not exhibit longer dwell times further supporting preferential solvation

**Lipid composition**
- **Headgroup:** 67% PE, 33% PG
- **Tails:** 1-50% DL, 99-50% PO

**Simulation details**
- **Software:** Anton2/Desmond
- **FF:** CHARMM36
- **Simulation time:** 10 + 40 μs