Biology 5357: Chemistry & Physics of Biomolecules - Membranes & Membrane Proteins

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Lecture 5: Membrane proteins: folding and self-assembly

Required reading:

White, S., Wimley, W. (1999). **MEMBRANE PROTEIN FOLDING AND STABILITY: Physical Principles** Annual Review of Biophysics and Biomolecular Structure 28(1), 319-365. https://dx.doi.org/10.1146/annurev.biophys.28.1.319

Deisenhofer, J., Michel, H. (1989). **The photosynthetic reaction centre from the purple bacteriumRhodopseudomonas viridis** Bioscience Reports 9(4), 383-419. https://dx.doi.org/10.1007/bf01117044

Charges are energetically unstable in lipid bilayers. The lipid bilayer is made of oil which has a low dielectric constant (~2-3) compared to water (80). The dielectric constant describes how an electric field around a charge is reduced relative to a vacuum (1). Water molecules have electric dipoles, and each individual molecule is free to rearrange to stabilize the solvation of the charge. However, lipid molecules, and especially those constrained in a lipid bilayer, cannot rearrange, so the electrostatics are more similar to vacuum. The electrostatic component of the solvation free energy of a spherical point charge is described by the Born equation. This is the energy to take an ion from vacuum and solvate it in a continuum solvent like water. It is favorable to transfer into water, but unfavorable in the other direction (i.e. water to lipid). This means that charges, on ions or molecules, are all electrostatically prohibitive inside a stable low dielectric medium.

The cost of putting proteins in the membrane. Thus, with the partial charges on the peptide backbone, an unfolded polypeptide is inherently unstable in the membrane solvent. This cost can be experimentally measured by examining the partitioning of compounds from water into a non-polar solvent. N-methylacetamide is used as a model for the peptide backbone and is found to be unfavorable, ~ +6-7 kcal/mole into carbon tetrachloride and alkane.

It is important to note that the lipid bilayer is not an isotropic hydrophobic solvent like carbon tetrachloride or alkane. It has a particular structure and regions that are far more polar. It is slightly more stable in octanol or the interface of the POPC bilayer which both offer partially polar solvation, +1-2 kcal/mole.

However, if the hydrogen bonded form of the molecule is more favorable, stabilized by 3-6 kcal/mole. In addition, the side chain group offers additional stability, e.g. tryptophan of -3 kcal/mole, which provides favorable partitioning into the headgroup region of lipids, but is still insufficient for partitioning in the hydrophobic core.

The hydrogen bonded structures of membrane proteins. As a result, membrane proteins must fold into structural forms in which the secondary structure of the peptide backbone is hydrogen bonded. This comes in two types:

- beta-barrels - are found in prokaryotic outer membranes. Some toxins also form beta-barrel structures that can spontaneously partition into membranes
- alpha-helical structures - all eukaryotic membrane proteins

The two stage model of membrane protein folding. Therefore, membrane proteins differ from soluble proteins in this one essential way. In the first step of folding, they must fold and adopt specific secondary structures that reduce unpaired hydrogen bonds in order to transition from water to the membrane. To be stable, these segments must also be lined by non-polar residues to optimize stability of the unfolded structure. The second step is the equilibrium assembly of these non-polar segments while within the 2-dimensional hydrocarbon core of the lipid bilayer. Thus, we refer to this model as the two-stage model of membrane protein folding.

Biological membrane protein folding. The first step of partitioning is something that is rare to observe in a laboratory setting. While the free energy may favor the first step to occur, there appear to be high barriers to this step, and hydrophobic peptides will also aggregate in aqueous solution. Thus, biology has evolved chaperones and enzymes to assist in these steps.

- alpha helical proteins - membrane proteins are synthesized in the membrane, complexed with the SEC translocon complex that facilitates partitioning into the membrane. If the peptide is sufficiently hydrophobic, achieved via the formation of alpha-helical secondary structure, it partitions spontaneously into the lipid bilayer. Otherwise, it is translated into the periplasmic space, cytoplasm or ER.
- beta barrel proteins - in prokaryotes, are synthesized through the translocon complex but the peptide partitions into the periplasmic space. These hydrophobic peptides can spontaneously partition and fold into the membrane but there is a high kinetic barrier and many chaperones that are present to prevent aggregation. These complexes target the peptide to the outer membrane and the BAM chaperone complex which facilitates the folding into the membrane.

Amino acid partitioning. We know that the cost of partitioning N-methylacetamide into the hydrocarbon is reduced upon hydrogen bonding. It is further modulated depending on the side chains that are present. Tryptophan allows for favorable partitioning at the water-bilayer interface, but what about the other amino acids? How do we know that residues are hydrophobic, and which residues are the most hydrophobic?

This information is obtained by measuring the partitioning free energies of amino acids from water into different solvent phases that represent the lipid bilayer. Some examples include:

- Radzika - experimental measurements of amino acid partitioning from water into isotropic cyclohexane. The amino acid water/cyclohexane system is mixed and then left to equilibrate and the quantity of the amino acid is measured in each solvent phase.
- MacCallum - computational calculation of the potential of mean force of an amino acid as it crosses the membrane. This is calculated using molecular dynamics simulations and the umbrella sampling approach to target sampling across the entire reaction coordinate, here the position along the membrane. Since this is done with an atomistically detailed model of the system, it can model in rearrangements of the molecular structure of the membrane where continuum models cannot.
- Wimley - partitioning of a small pentapeptide with a different amino acids on the central residue. This has been done for interfacial models such as octanol, a partially polar solvent which contains aqueous pockets, and the POPC lipid bilayer interface. This does not capture the full energetic cost of partitioning within the hydrocarbon core, which is better represented by an isotropic hydrophobic solvent model.
- Moon - this scale is determined from the equilibrium folding free energies of the OMPLa in the membrane, with a guest amino acid at a position embedded within the membrane core. It is the cost to put that side chain into the membrane core, but it is not completely independent of the rest of the protein, and so it also decreases the free energy cost relative to isotropic solvent scales. This cooperativity with the backbone scaffold means that the cost to partitioning even charged amino acids is much lower than expected when calculated with continuum solvent models.
- Hessa - this scale is a biological partitioning model because it is measured in a cellular system, following the partitioning of non-polar alpha helices into the lipid bilayer via the translocon complex. To measure the proper insertion, they follow the glycosylation of two sites on the neighboring loops which will define whether the helix is transmembrane or it is in the aqueous solution. By varying the central amino acid on the helix, they can measure the change in the free energy of partitioning. Note, the same issues of cooperativity may apply here as in the OMPLa model, and the resultant protein may not be folded so other defects in the membrane may occur.

In general, from all of these scales, we obtain a quantification of the free energy cost to put amino acids into membrane environments. There is general agreement in the types of residues considered hydrophobic vs. polar, but the quantities of these energies depend on where these residues are partitioning in the experimental models. Comparing the different models highlights how these energies can differ.

Hydrophobicity plots. From these scales, one can predict the existence of transmembrane helices within protein sequences. This is known as hydropathy analysis and can use any one

of these scales as the quantitative input for calculating the score. Depending on the scale, different results may be obtained which can affect the output of the prediction algorithm. Keep in mind that membrane proteins can be quite complicated, with transmembrane helices that are broken or do not fully traverse the membrane, which can make these prediction methods inaccurate.

Membrane protein assembly in membranes. In the second stage of membrane protein folding, the helices come together and assemble into a folded structure, or multiple helices bind to one another to form complexes. Membrane proteins reside in a free energy minimum that is determined by the energy balance of interactions between the protein with water, other proteins segments, the lipid bilayer and cofactors.

Bacteriorhodopsin re-naturation. A key experiment was conducted in 1987 showing that membrane proteins are thermodynamically driven to specific folded forms in lipid bilayers. In this experiment, bacteriorhodopsin - the classic membrane protein model that showed the first structure of a membrane protein, was proteolytically cleaved into fragments. These fragments were reconstituted separately and then reintroduced into the same lipid bilayer by freeze-thawed fusion of the vesicles. A marker for proper folding is the absorbance of trans retinal form that is only obtained upon binding to the fully assembled protein. Thus, the components are driven to assembly through for some physical reason, encoded by the protein sequence.

Studying equilibrium protein folding/association in membranes. In the bacteriorhodopsin studies, they demonstrated that membrane proteins come together and are in principle thermodynamically driven, however it does not provide quantification of this reaction. This is a major challenge within the field because one cannot simply denature a membrane protein within the membrane. As we learned previously, detergents are required to solubilize membrane proteins. Sometimes they can lead to un-folded state, as is the case with SDS. However, these denaturants also dissolve the membrane structure and so the challenge is preserving the