Including H-bonding And Lipid Exposure In Near-atomic Level Folding Simulations Of Helical Membrane Proteins

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Abstract

We are interested in simulating the folding of transmembrane helical proteins (TMHs), leveraging our ***Upside*** molecular dynamics program that can reversibly fold some soluble proteins up to 97 AA in CPU-hours without the use of fragments or homology.

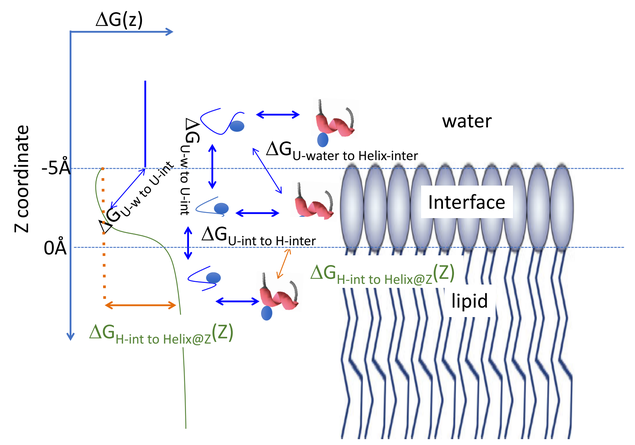
Upside utilizes a number of unique features including a rapid side chain packing and free energy calculation at each time step. This procedure enables the dynamics to be conducted on only three backbone atoms and avoids having the side chain rattling and friction that often slows all-atom methods.

To apply *Upside* to TMHs, we include lipid-protein interactions and energies of backbone exposure within the membrane, and do so in a dynamic manner to **avoid "double-counting" of protein-lipid and protein-protein interactions** as helices come together.

Energies are obtained from the statistics of large and curated protein training set, **accounting for both depth in the membrane and exposure levels**, E(Z, exposure)∝ln(frequency).

We also incorporate membrane depth dependent energies for unsatisfied backbone H-bond donors and acceptors.

Background & Purpose of Study



Accurate membrane potential

→ transfer energy from aqueous to lipid-water interface and to lipid center (hydrocarbon core)

→ folding/insertion pathways for membrane-associated proteins

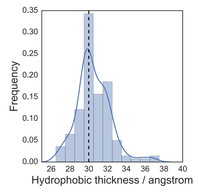
Conclusions

We calculate the distribution of conformations for a variety of transmembrane helical proteins (TMHs), demonstrating that they are located in the correct position within the membrane (as defined by OPM [1]). For multimeric complexes, we find that many monomers retain their native orientation when separated suggesting that docking is a viable assembly strategy. However, monomers from some ion channels experience larger movement due to the exposure of charged and polar residues normally solvated within the channel cavity. The assembly of these TMHs may involve a partial induced-fit mechanism. Comparisons involving tests of existing knowledge-based membrane potential assess their ability to correctly reproduce the native orientations as successfully predicted by our membrane potentials for TMHs [2, 3].

References:
[1] Lomize, Mosberg *et al.*, Bioinform. 22 (2006) 623
[2] Senes, DeGrado *et al.*, J. Mol. Biol. 366 (2007) 436
[3] Schramm, DeGrado, Samish *et al.*, Structure 20 (2012) 924

Reference to the Bilayer Interface Allows for Adjustable Membrane Thickness & Only Use Lipid-exposed Residues

Hydrophobic Thickness Distri. Of Training Set: 140 TMHs



Burial level: count the # side chains beads within a fixed radius of the CB atom in a hemisphere above the atom

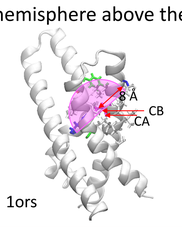
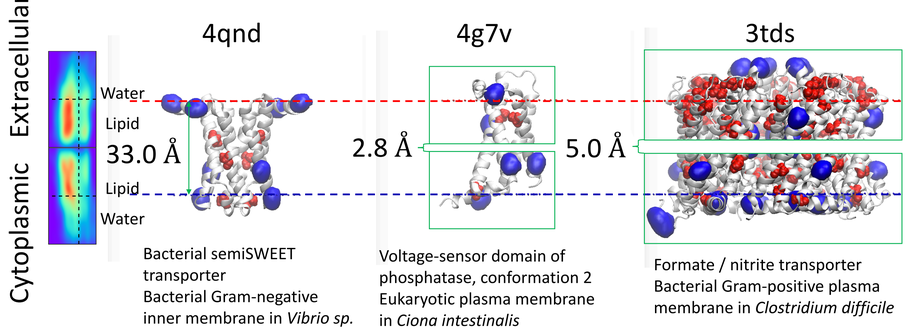


Illustration of ALA distribution on the extracellular and cytoplasmic leaflets

Exposed ALAs: blue QuickSurf; Buried ALAs: red VDW

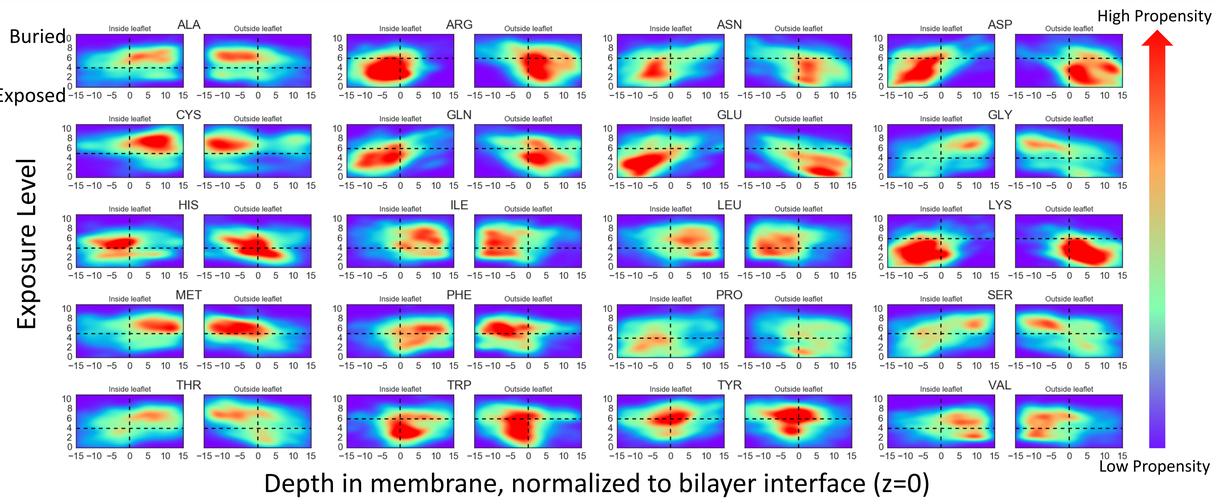


Bacterial semiSWEET transporter
Bacterial Gram-negative inner membrane in *Vibrio sp.*

Voltage-sensor domain of phosphatase, conformation 2
Eukaryotic plasma membrane in *Ciona intestinalis*

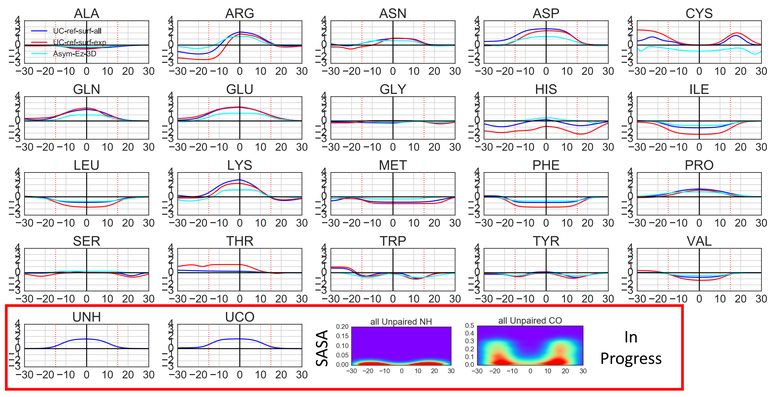
Formate / nitrite transporter
Bacterial Gram-positive plasma membrane in *Clostridium difficile*

Propensity(depth, side chain exposure to lipid)



Depth in membrane, normalized to bilayer interface (z=0)

Membrane Potential Profile at Thickness = 30 Å（unit: RT）



In Progress

Potential profiles in comparison:

UC-ref-surf-all: UChicago potential derived from all residues regardless of the burial level

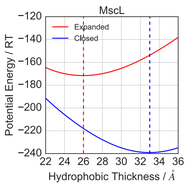
UC-ref-surf-exp: UChicago potential derived from residues exposed to lipid

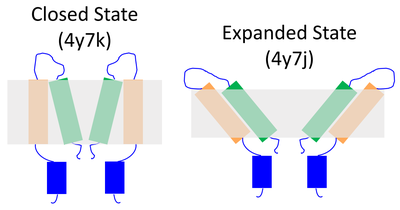
Asym-Ez-3D: Schramm, DeGrado, Samish *et al.*, Structure 20 (2012) 924

Novel Features:

1. Adjustable hydrophobic thickness
2. Incorporation of unsatisfied H-bond donors and acceptors
3. Derived from and applied to only lipid-exposed residues

Example: Predict Bilayer Thickness for MscL Expanded & Closed State



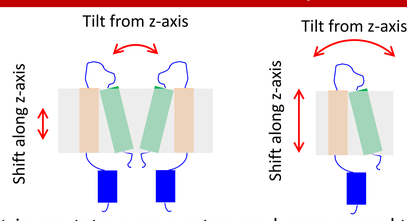


Experiment confirmed that thicker membrane bilayer constituted of lipid molecules with longer chain tends to stabilize the closed state of MscL, while thinner bilayer tends to stabilize the expanded state, as expected by hydrophobic mismatch. [1]

Our prediction: 26.0 Å for expanded state, 33.0 Å for closed state
OPM prediction: 26.8 Å for expanded state, 31.8 Å for closed state [2]

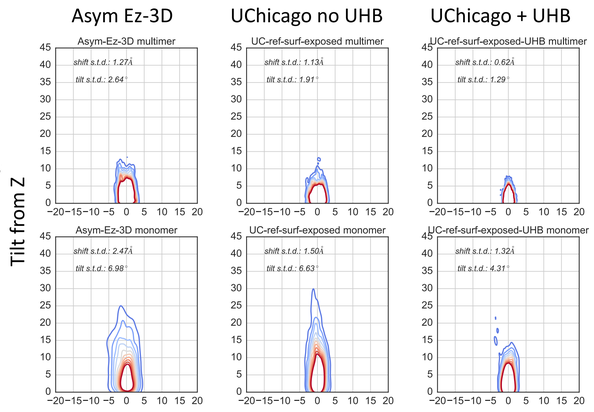
[1] Perozo *et al.*, Nat. Struct. Biol. 9 (2002) 696
[2] Lomize, Mosberg *et al.*, Bioinform. 22 (2006) 623

Upside Dynamic Orientation Tests

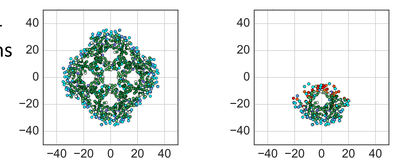


- 23 dimers, trimers, tetramers, pentamers, hexamers and their monomeric units are simulated.
- TMHs are restrained as rigid bodies in the simulation, and the deviations of tilt angle and shift distance to the native state are calculated.
- Membrane potentials (both UChicago potential and Asym Ez-3D) are only applied to the lipid-exposed residues. (Note: Asym Ez-3D was derived from all residues.)
- Unsatisfied H-bond (UHB) energies are applied to all residues with unpaired backbone H-bond partner regardless of the exposure status.

Distribution of (shift, tilt) of all trajectories from 23 systems

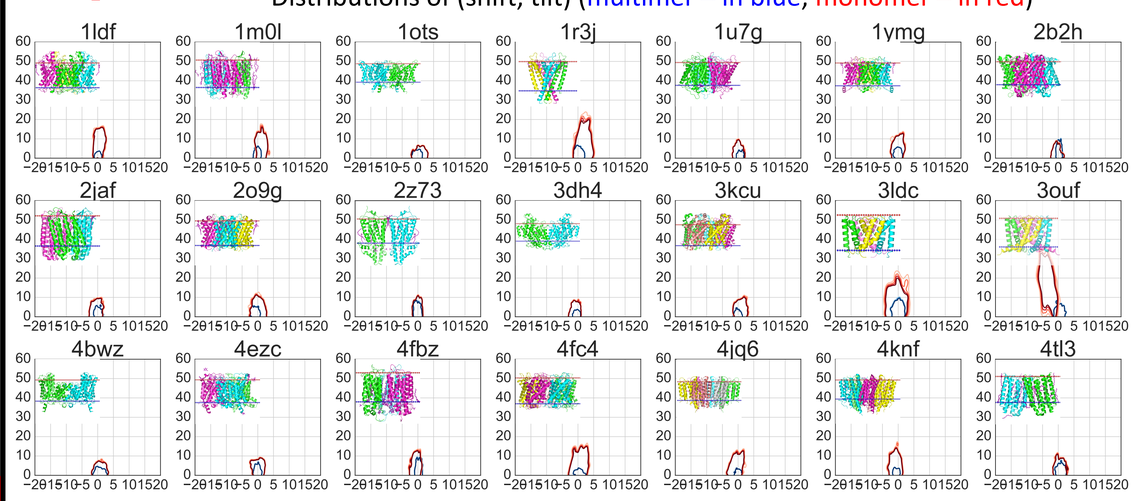


Exposure level projection on XY-plane of CA atoms

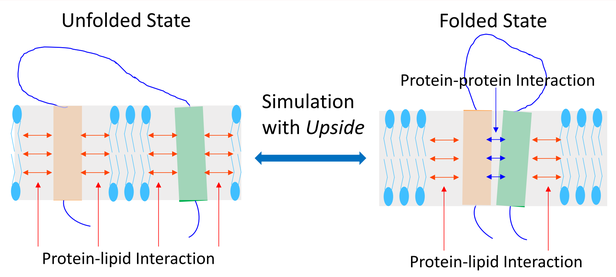


Exposed residues – Blues
Buried residues – Greens
Exposed in monomer but buried in multimer -- Reds

Distributions of (shift, tilt) (multimer – in blue; monomer – in red)



Upcoming: Folding Simulations Eliminating The Double-counting Issue



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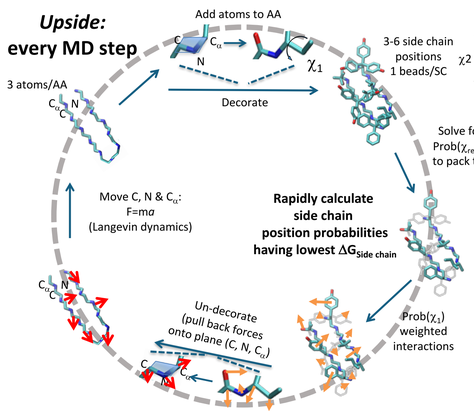
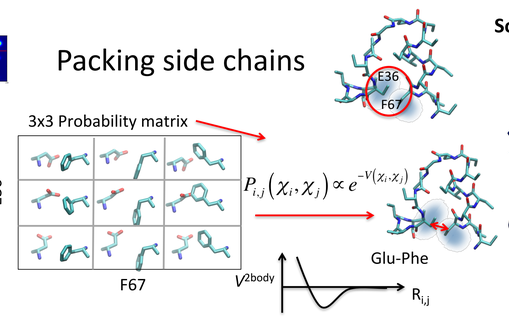
Upside: Fast MD Simulations Using 3 Atoms per AA But With Molecular Details

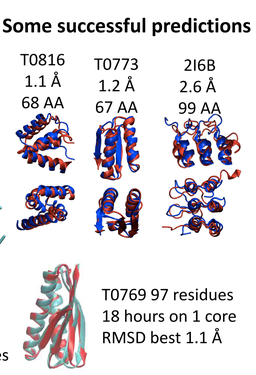


Packing side chains



For 3 interacting residues, find self-consistent set of pairwise $P(\chi_i, \chi_j)$, $P(\chi_i, \chi_k)$ & $P(\chi_j, \chi_k)$... (3x3) probability matrices that minimizes $G_{protein} = \langle V \rangle - TS$

Some successful predictions



More details: Jumper et al. arXiv:1610.07277

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