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

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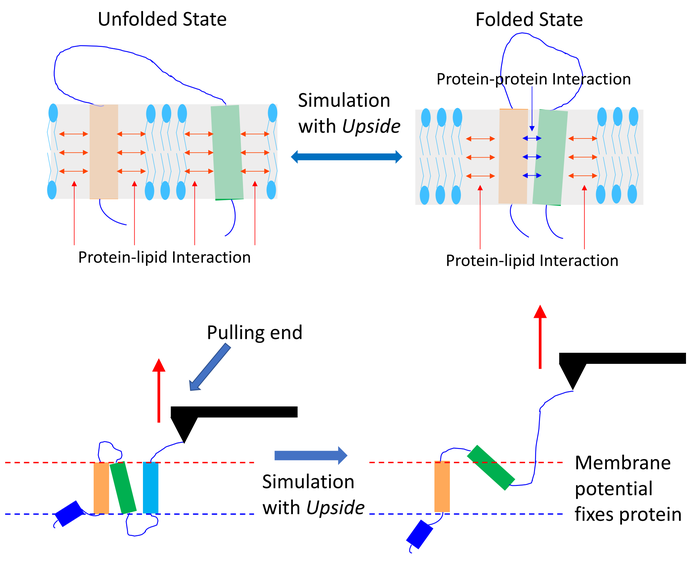
Abstract

Single-molecule force spectroscopy (SMFS) is a powerful technique to study the unfolding of soluble and membrane proteins. Coarse-grained (CG) models expedite the MD simulations and allow for slower and more realistic extraction velocities and lower pulling forces for simulating SMFS experiments. These factors increase the likelihood of observing transient intermediates.

We simulate the forced unfolding of membrane proteins using our CG model, *Upside*, with 6 atoms/AA and incorporating new membrane potentials. *Upside* can reversibly fold some soluble proteins up to 100 AA in CPU-hours without the use of fragments or homology. Our membrane potentials are derived from statistics of known structures, accounting for burial depth in the membrane and side chain exposure levels.

Background & Purpose of Study



Conclusions

In the simulations of the forced unfolding of bacteriorhodopsin (bR), we are able to rapidly reproduce the characteristic features displayed in experiments, including the unfolding of individual and pairs of helices, worm-like chain behavior of the elastic unfolded segments and the back-and-forth transitions between states with a comparable resolution as the experiments. The difference in the unfolding pathway are compared for the isolated monomer and in trimeric form. We can observe more intermediate states in the unfolding of a monomeric bR from the trimer.

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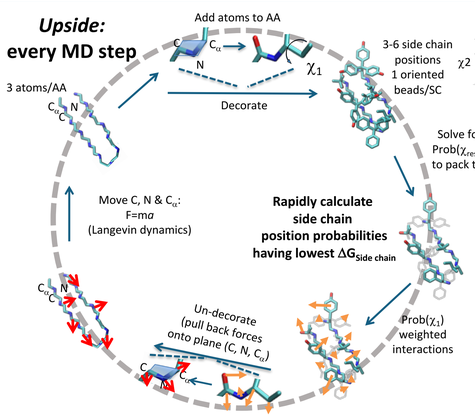
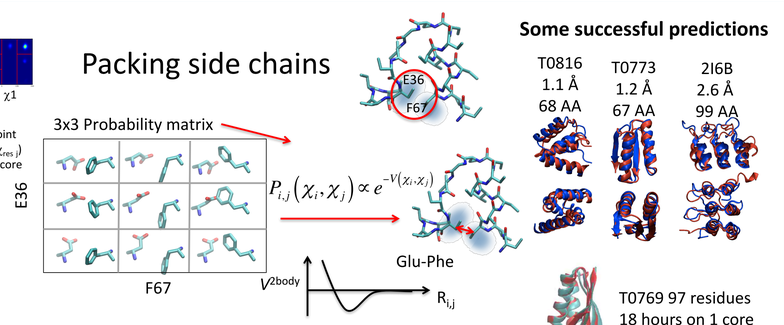
Upside: Fast MD simulations on 3 atoms/AA but with molecular details (6 atoms/AA)





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

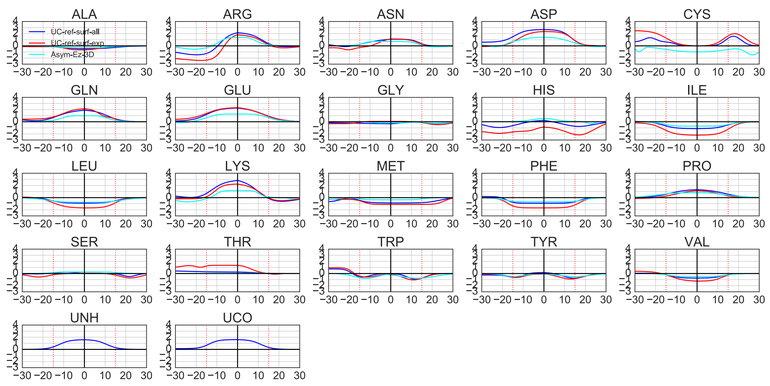
$$G_{protein} = \langle V \rangle - TS$$

Some successful predictions

More details: Jumper et al. arXiv:1610.07277

Try *Upside*: https://github.com/Freed-and-SosnickLab/upside-md

Membrane potential with unsatisfied H-bonding groups and variable thickness (unit: RT)



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

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

Manuscript in preparation.

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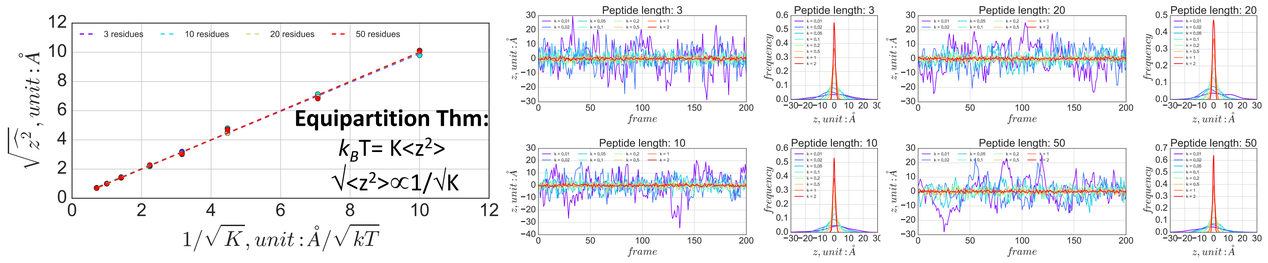
Simulating SMFS – Obtain correct physics

Thermal fluctuation of cantilever matches spring constant



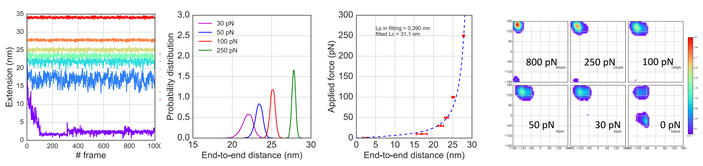
We took the first 3, 10, 20, 50, residues of bR, restrained the peptide as rigid body, and ran simulations with the first residue fixed on the cantilever.

→ measure the thermal fluctuation of the tip of the cantilever via the fluctuation of the fixed residue

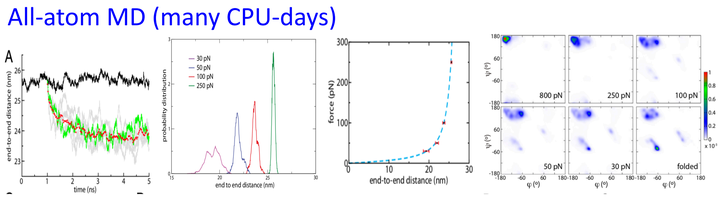
Reproduce all-atom MD of pulling ubiquitin

(N-term fixed, pull C-term)

Upside (1 CPU-day)



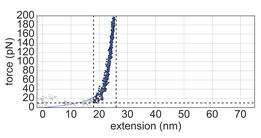
All-atom MD (many CPU-days)

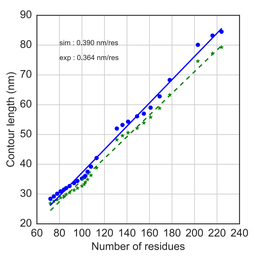


Stirnemann et al., PNAS 110 (2013) 3847

Calibrate Lp & Lc of bR

A160, force-extension



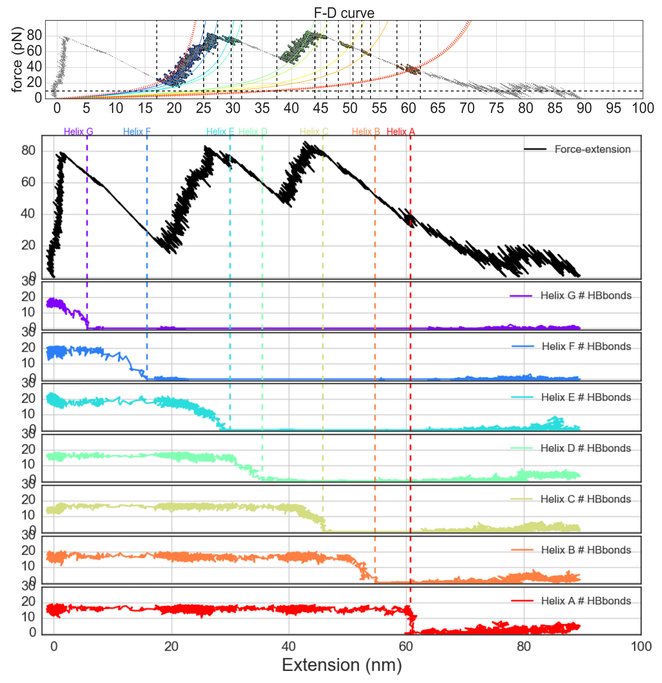


Yu et al., Science 355 (2017) 945

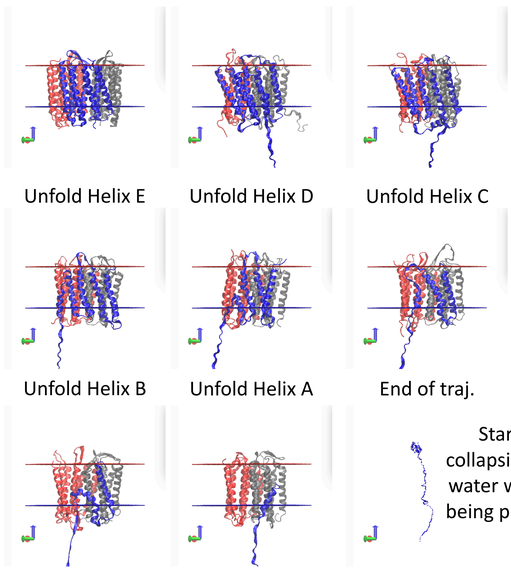
For each of the intermediates detected in the exp., we truncated the protein, applied forces to both termini of the polypeptide in opposite direction, and fitted to the WLC model to obtain the Lc.

Structural position	Lc, simulation (nm) (Fitted with Lp = 0.4 nm)	Lc, Sci. paper (Yu. et al.) (nm)
A160 72 res before	28.4	26.9
L127 105 res before	37.5	34.9
V101 131 res before	52	48.2
Y83 149 res before	56.1	52.1
G63 169 res before	62.8	58.8
V29 203 res before	80.1	74.6
P8 224 res before	84.5	79.3

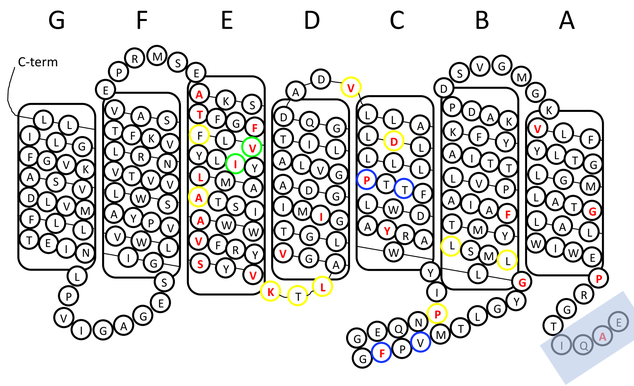
Unfold bR – One typical force-extension curve of unfolding a monomeric bR from the trimer



Snapshots of unfolding trajectory



Unfold bR – Comparison between pulling an individual monomer & pulling from a trimer



Sim. monomer: 1 traj. < 3 CPU-days

Sim. trimer : 1 traj. ~ 8 CPU-days

Spring const. = 0.01 kT/Å² = 4.1 pN/Å² at 298 K

Pulling vel. = 0.001 Å/step.

A Intermediate detected in exp. [Yu et al., Science 355 (2017) 945]

A Intermediate detected in Upside sim. of pulling from trimer

A Intermediate detected in Upside sim. of pulling from monomer

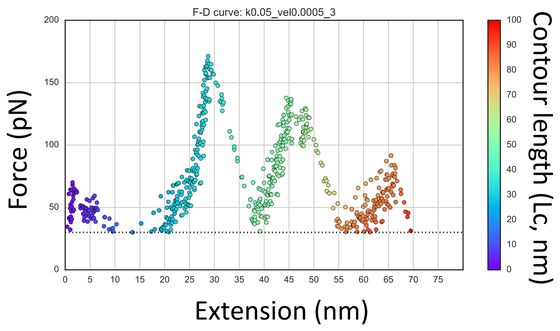
A Intermediate detected in both Upside sim. of pulling from monomer & trimer

→ Not in the model in sim.

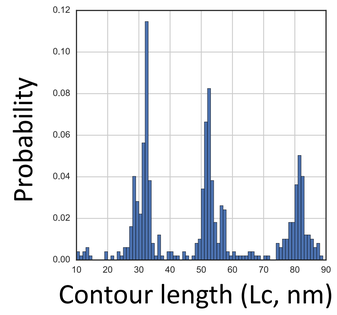
Pulling from a monomer: 18/20 traj. useful in analysis; 4/ 6 intermediate states (detected in ≥ 4 traj.) in the exp.

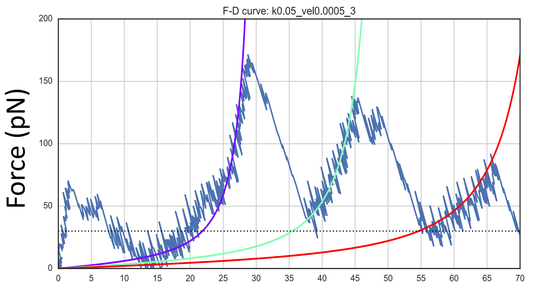
Pulling from a trimer. : 20/20 traj. useful in analysis; 8/12 intermediate states (detected in ≥ 4 traj.) in the exp.

Unfold bR – Trajectory analysis



1. Compute the contour length (Lc) for each point in traj.
2. Convert to the Lc space
3. Obtain the major states for each segment from the histogram of Lc's





I. Assign all the points in the Force-Extension Curve (FEC) to the nearest state by minimizing the objective function below:

$$dist\big(p(x,f), FEC(Lc)\big), \forall\ p(x,f), \forall\ Lc$$

II. Update/refine Lc's by minimizing the objective function below:

$$\sum_{p\ \in\ state\ Lc} dist\big(p(x,f), FEC(Lc)\big), \forall\ Lc$$

III. Iterate I and II till $\Delta Lc\ (= 0.01nm)$ until convergence (no further change in Lc needed).

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