Predicting the effect of Addition/Deletion mutations on protein stability by REST/FEP

Mohamed Marzouk and Shun Sakuraba

National Institutes for Quantum Science and Technology (QST), Institute of Quantum Life Science (iQLS), Molecular Modeling and Simulation (MMS) Team

1 Introduction

Can we express quantitively the protein stability by MD simulation?

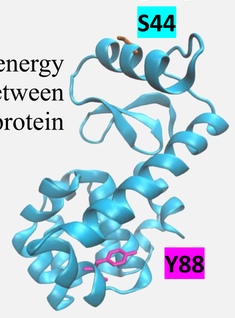
The free energy (ΔG) : The difference between the free energy of the folded and unfolded states of the protein (free energy of folding).
The free energy difference (ΔΔG) : (The difference in the difference of free energy)
- The difference in energy between free energy of folding for a mutated (mut) protein to its wild-type (wt) form.

$$\Delta\Delta G = \Delta G^{mut} - \Delta G^{wt}$$

Protein stability indicator

- Mutations (addition/deletion) impacts on the thermodynamic equilibrium between the folded and unfolded states of a protein which might propose different changes for enhancing protein stability. Computed ΔΔG, which is time-consuming and costly to measure experimentally, is a good measure to determine whether a mutation stabilizes or destabilizes the protein[1,2].
- In conventional molecular dynamics (MD) simulations, sampling the conformation space of proteins is challenging due to the energy barriers between the local minimum states in the potential energy surface. As a result, the simulation tends to become trapped in one of the local-minimum states for long periods of time which leads to poor estimation of ΔΔG.
- Therefore, we used Replica exchange with solute tempering (REST)[3,4], enhanced conformational sampling, which effectively resolves the problem of sampling with a reasonable computational cost.
- The REST approach has been integrated with the free-energy perturbation (FEP), hereafter REST/FEP, to compute ΔG between the folded and unfolded state of protein to examine the protein stability.

In this study :
We used the REST/FEP combination to study the effect of addition (A73-[A], Y88-[A]) and deletion (S44Δ, A73Δ) mutations on the stability of T4 lysozyme with PDB ID: 2LZM



PDB ID: 2LZM[6]

2 Materials & Method

A) Structure preparation
Generating → Starting structures & Topology file

Dual (pdb, top) & Ref (pdb, top)

Wildtype → PDB-ID 2LZM[5]
Mutant (add/delete) → AlphaFold2[6]

B) REST2/FEP
- Equilibration initial structure (~10 ns)
- All replicas (96) equilibration (~10 ns)
- MD production with replica exchange (4 ns every step)

→ **MD setting**

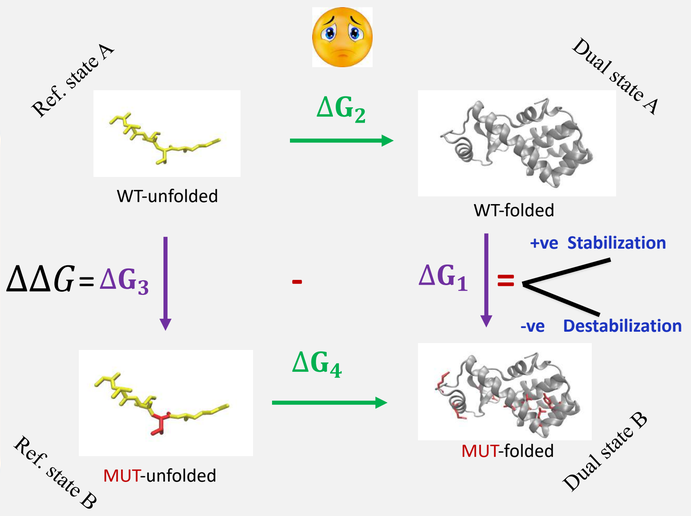
GROMACS v2021.5

FF : AMBER 14SB

# of replicas : 96

# of MD production steps : 6

Total simulation time : (6 * 4 + 0.1) * 96 = 2.3136 μs



C) Analysis
I) Replica Exchange validation II) Free energy prediction
III) Dihedral angles

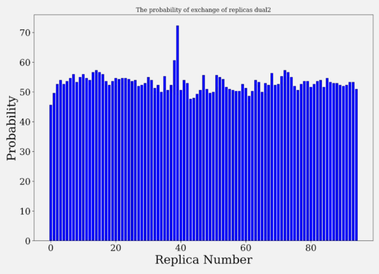
Why Free energy?

- Affinities are free energies (of binding)
  - Ligand/drug design
  - Protein/protein binding
  - Protein/DNA binding
- Stabilities are free energies (of folding).
- Rates of conformational transitions are determined by free energy barriers.
- PMFs are free energy profiles

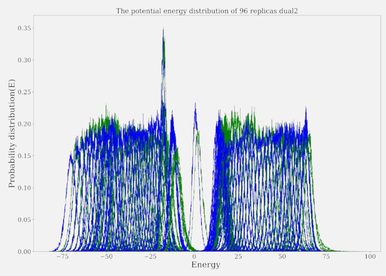
3 Results

I) Replica Exchange validation

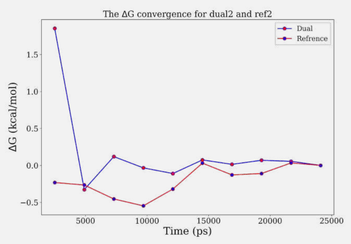
1) The probability of exchange between all the replicas is high enough

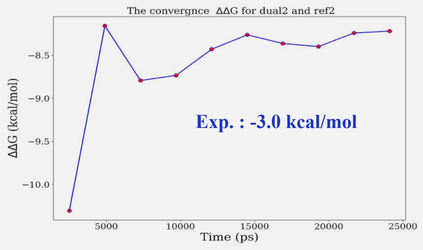


2) The overlap between potential energy distribution of neighboring replicas



II) Free energy prediction (ΔΔG)

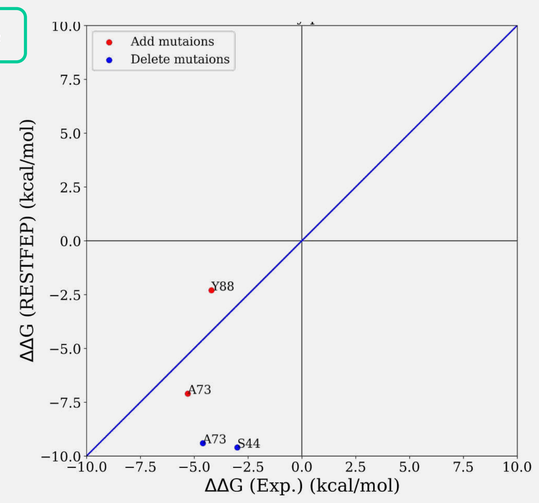




Different add/delete cases

Cases	Add/Del	ΔΔG Exp. (kcal/mol)	Predicted ΔΔG (kcal/mol) REST2/FEP
A73Δ	Delete	-4.6	-9.4
S44Δ	Delete	-3.0	-9.6
A73-[A]	Addition	-5.3	-7.1
Y88-[A]	Addition	-4.2	-2.3

The stability prediction curve

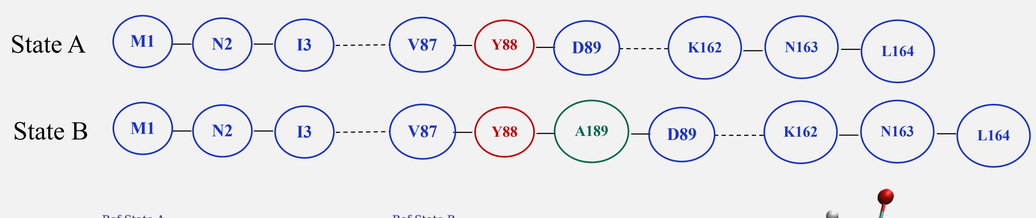


prediction of ΔΔG by REST/FEP still not so good in deletion cases.

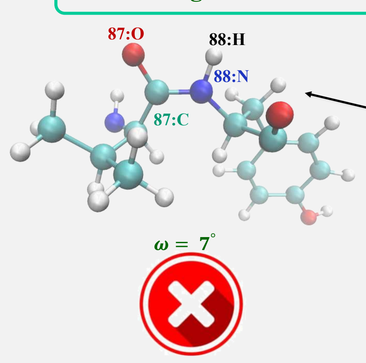
III) Dihedral angles for residues surrounding the mutation

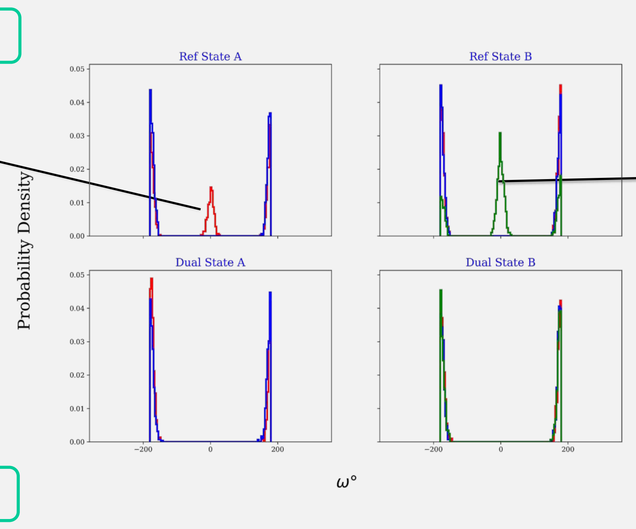
ω (Omega) : The peptide bond between the amide carbon C(i) and N(i+1) is the central bond.
φ (phi) : which involves the backbone atoms C-N-Cα-C.
ψ (psi) : which involves the backbone atoms N-Cα-C-N.

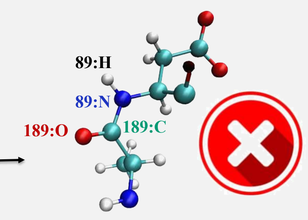
2) Addition Cases : Y88[A]

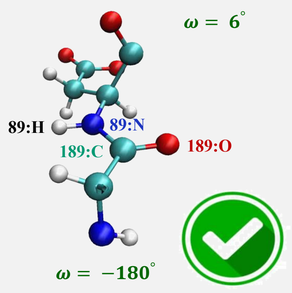


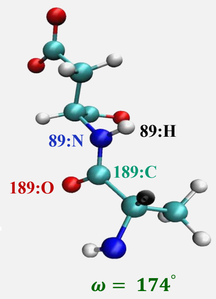
The ω angles for all states



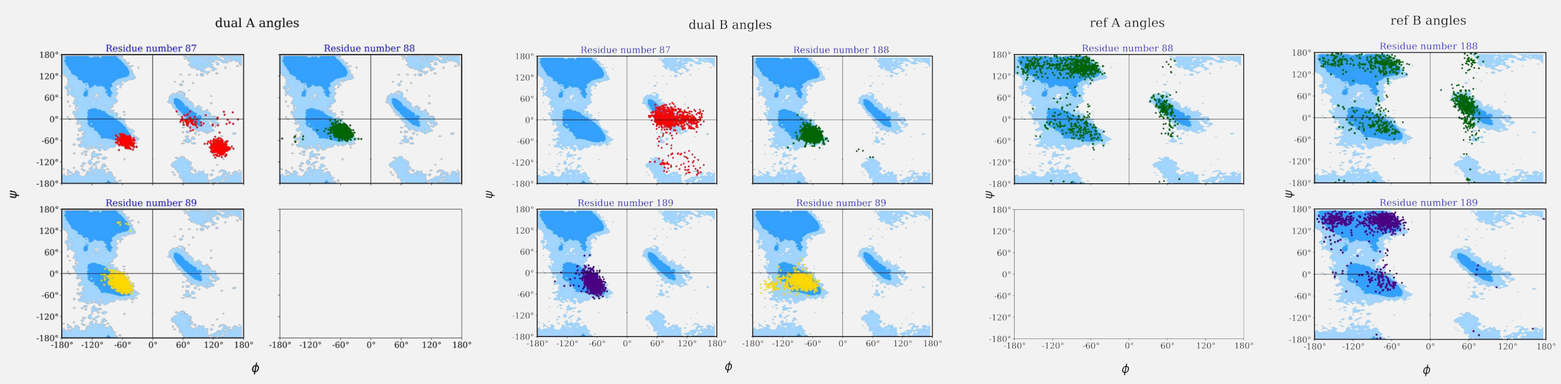








Ramachandran plots



4 Conclusion & Future Works

- The REST/FEP combination can be used to predict the free energy of proteins with add/delete mutations; however, some modifications are required to significantly improve the predicted value.
- Consequently, it can be used to investigate protein stability and develop future therapeutics.

Next steps :
- Investigate the effect of restrain force for dihedral angles for residues surrounding the added/deleted residue.
- Using crystal structures for add/del mutation instead of Alphafold predicted structure.
- Investigate the effect of different force fields on the prediction values for the ΔΔG

References

(1) Steinbrecher, T. et al. *J. Mol. Biol.* **2017**, *429*, 948–963.

(2) Dehghanpoor, R. et al. *Molecules* **2018**, *23*, 1–18.

(3) Liu, P. et al. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 13749–13754.

(4) Wang, L. et al. *J. Phys. Chem. B* **2011**, *115*, 9431–9438.

(5) Weaver, L. H. et al. *J. Mol. Biol.* **1987**, *193*, 189–199.

(6) Mirdita, M. et al. *Nat. Methods* **2022**, *19*, 679–682.