Early Stages of Apomyoglobin Folding

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Introduction

Apomyoglobin (apoMb) folding pathways contain several intermediate states, identified by time-resolved fluorescence spectroscopy, which form on the microsecond to millisecond time scale. (Cite Xu and Roder JPCB) While structural information about these intermediates is extremely useful in understanding protein folding mechanisms, such states are short-lived and sparsely populated, making experimental characterization is a challenging task. To gain further insight, we turn to molecular simulations to model folding intermediates in atomic detail. The folding of apoMb follows a U → M → N mechanism, where the M state is thought to be analogous to a highly-populated acid-denatured state at pH 4. Here, as a first step towards computational characterization of the M and N states, we perform large numbers of microsecond simulations at pH 4 and pH 7, restrained by experimentally determined H/D exchange protection factors. Our results are in good agreement with experimental observables, representing the first steps toward an atomically-detailed description of the conformational ensembles corresponding to the M and N states of apomyoglobin.

Objective

- Implement the H/D protection factors restraints in simulations.
- Characterize the N and M states at pH = 7 and pH = 4.
- Build a kinetic network model to describe apomyoglobin folding at low and neutral pH.

Methods and Simulations

Implementation of protection factor-restrained simulations in GROMACS

Maximum Entropy is the least biased way for enforcing restraints in simulations

$$U'(x) = U(x) + \alpha f(x)$$

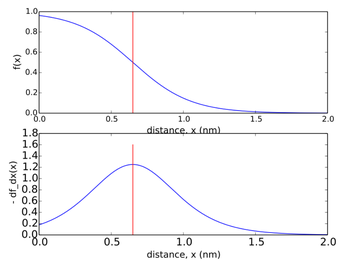
$$U = U_0 + \frac{e^{-b(x-x0)}}{1+e^{-b(x-x0)}} \times k$$

k = force constant ($\frac{kJ}{nm}$)

$$y = \frac{e^{-b(x-x0)}}{1+e^{-b(x-x0)}}$$

$x0 = 6.5$ Å

$b = 5.0$



Distances between amide hydrogen and all oxygen atoms within 6.5Å of amide groups were restrained.

Simulations

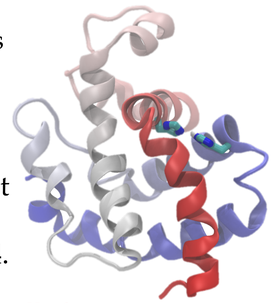
pH = 4	Temperatures (K)	300, 330, 350, 370, 400, 415
	Force Constants (kJ/nm)	0.0,0.1,0.2,0.3,0.4,0.5,0.6,0.7 ,0.8,0.9,1.0,1.1,1.2,1.5
pH = 7	Temperatures (K)	300, 330, 350, 370, 400, 415
	Force Constants (kJ/nm)	0.0,0.5,0.7,1.0,1.2,1.5,2.0

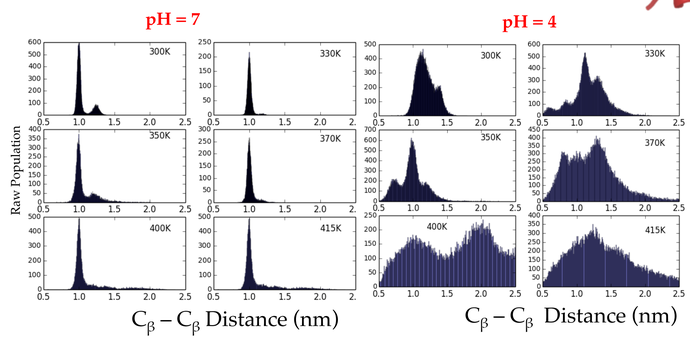
Total simulation times

- pH=7: ~ 100 μs
- pH=4: ~ 650 μs

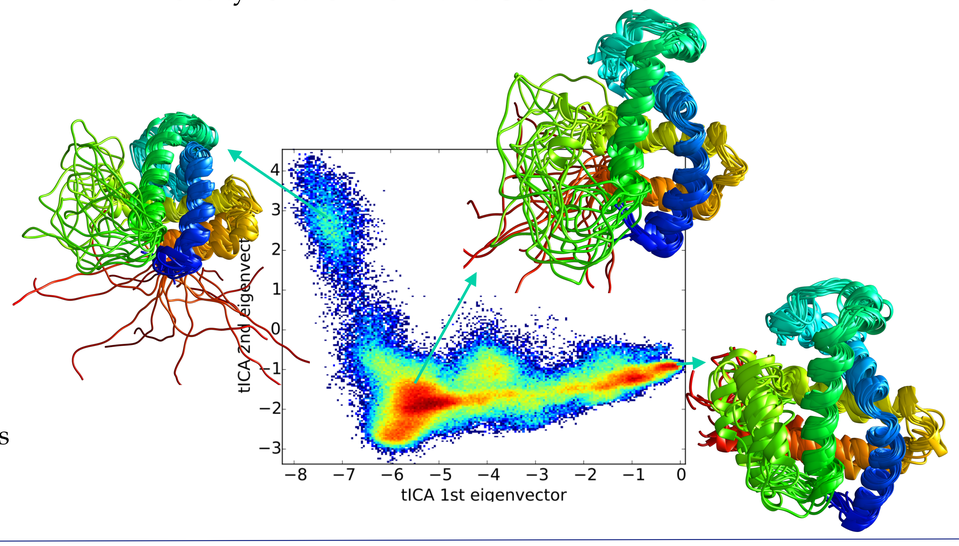
Results

His24 and His119 interaction is stable at pH = 7 even above melting temperature, but is disrupted at pH = 4.



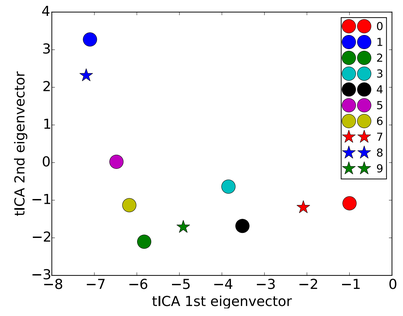


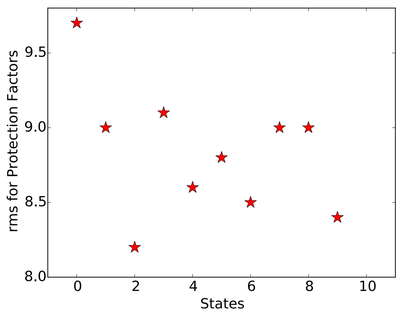
Free energy landscape of pH 7 restrained simulations contains several basins, which differ in degree of helicity for the F helix and C-terminal of the H helix.

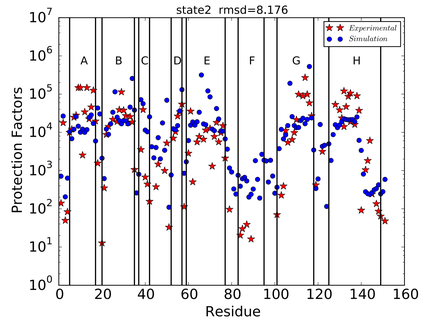


Results

Clustering the free energy landscape into 10 states and calculating the protection factors for each states, reveals state 2 as the most resembling of the apo state at neutral pH for myoglobin.







Conclusions

- At neutral pH, the His24-His119 interaction is highly stable, even as temperature increases to 415 K.
- At low pH (pH 4), the His24-His119 interaction is broken even at room temperature.
- We successfully implemented H/D restrains in our MD simulations and reproduced the experimental protection factors.

Future Direction

- Reweighting the restrained simulation data to obtain unbiased kinetic transitions for constructing Markov State Models of apoMB folding.

Acknowledgments

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