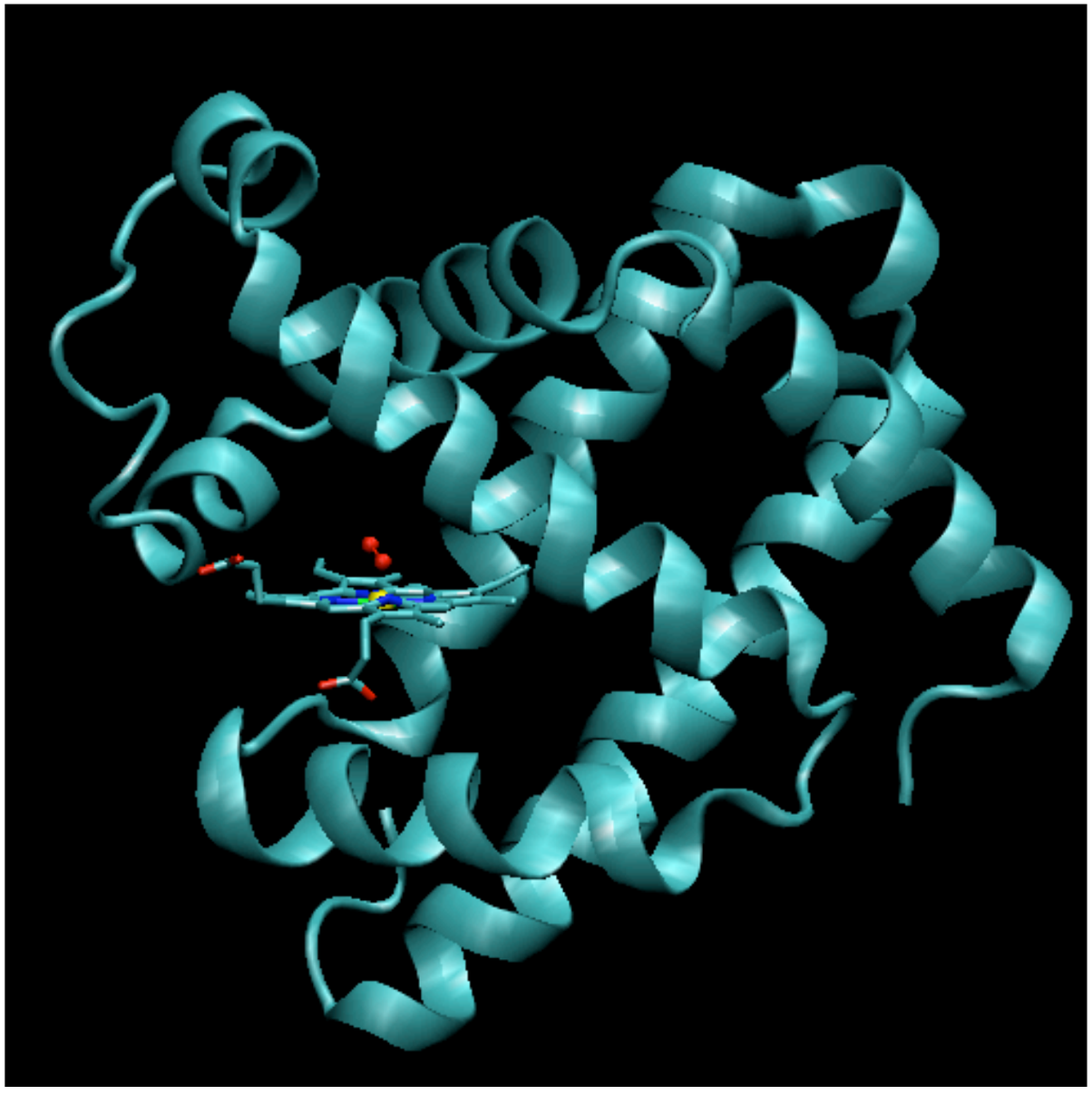
Ligand diffusion in myoglobin



Myoglobin is a small, monomeric protein which serves as oxygen storage and transport in mammals. It binds O2 and oxygen compounds (CO, NO) at the active site, the heme, with a bond to an Iron atom

L. M., G. Cottone, G. Ciccotti and E. Vanden-Eijnden *J. Am. Chem. Soc.*, 132, 1010 (2010)

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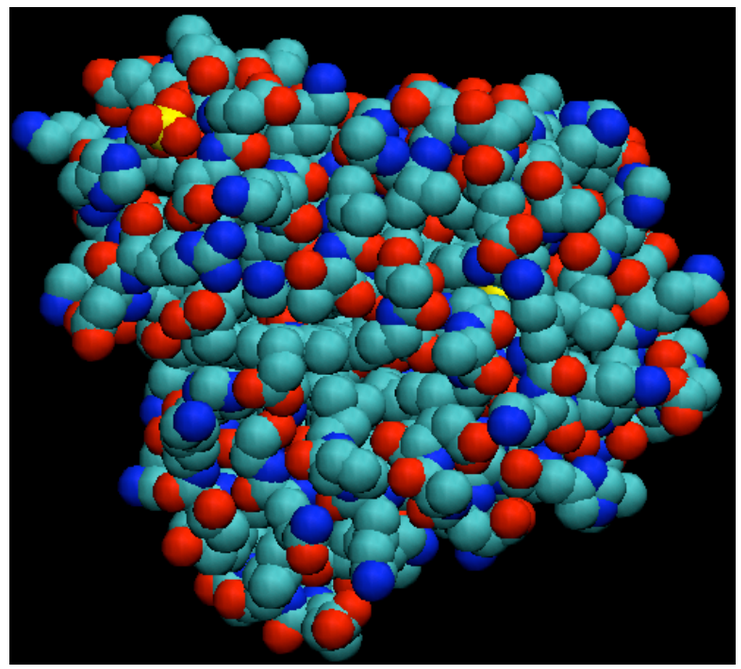
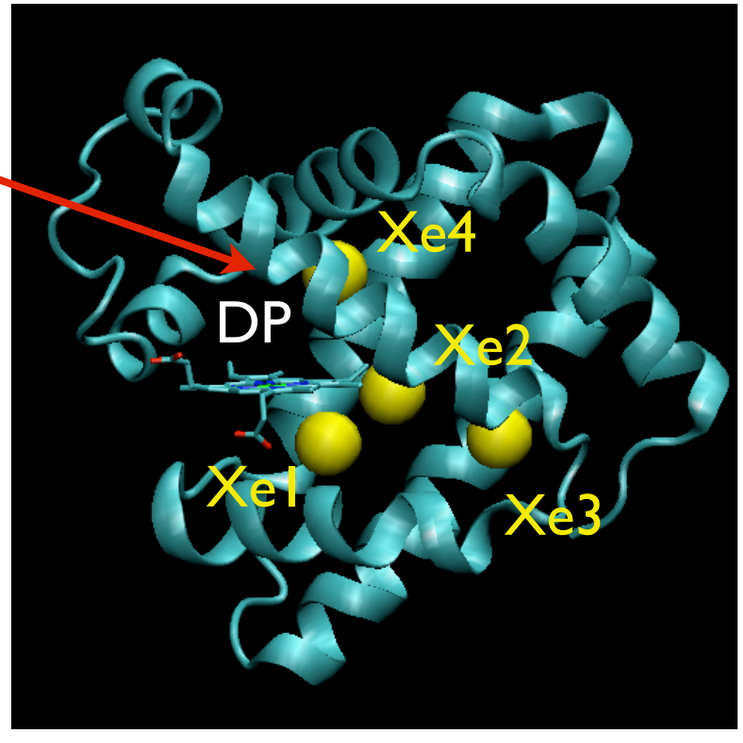


Quest for the complete network of possible paths for the ligand to reach (escape) the binding site.

Historically, the first guess was for a direct path from the Distal Pocket, DP (above the binding site) to the surface, passing close to a histidine side-chain (His gate).



Role of internal (Xenon) cavities emerged over the years.

Time-scale: >1000 ns

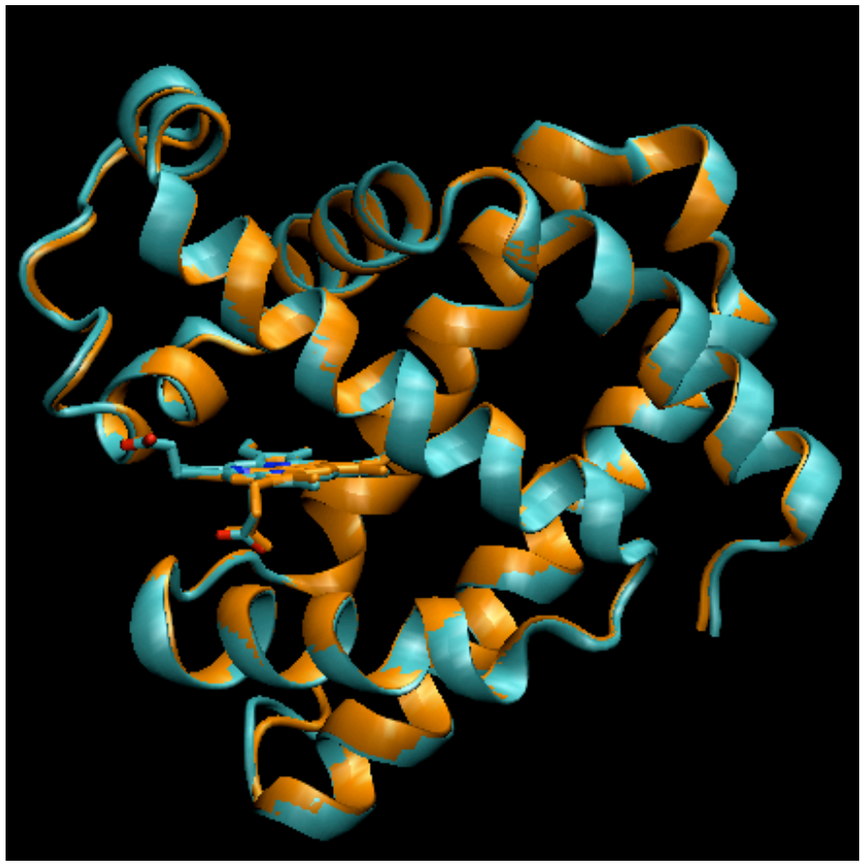
Ligand diffusion in myoglobin

Task: Compute the PMF landscape of CO location inside Mb

Collective variables: cartesian coordinates of CO center of mass (constrained rotations and translations of the protein)

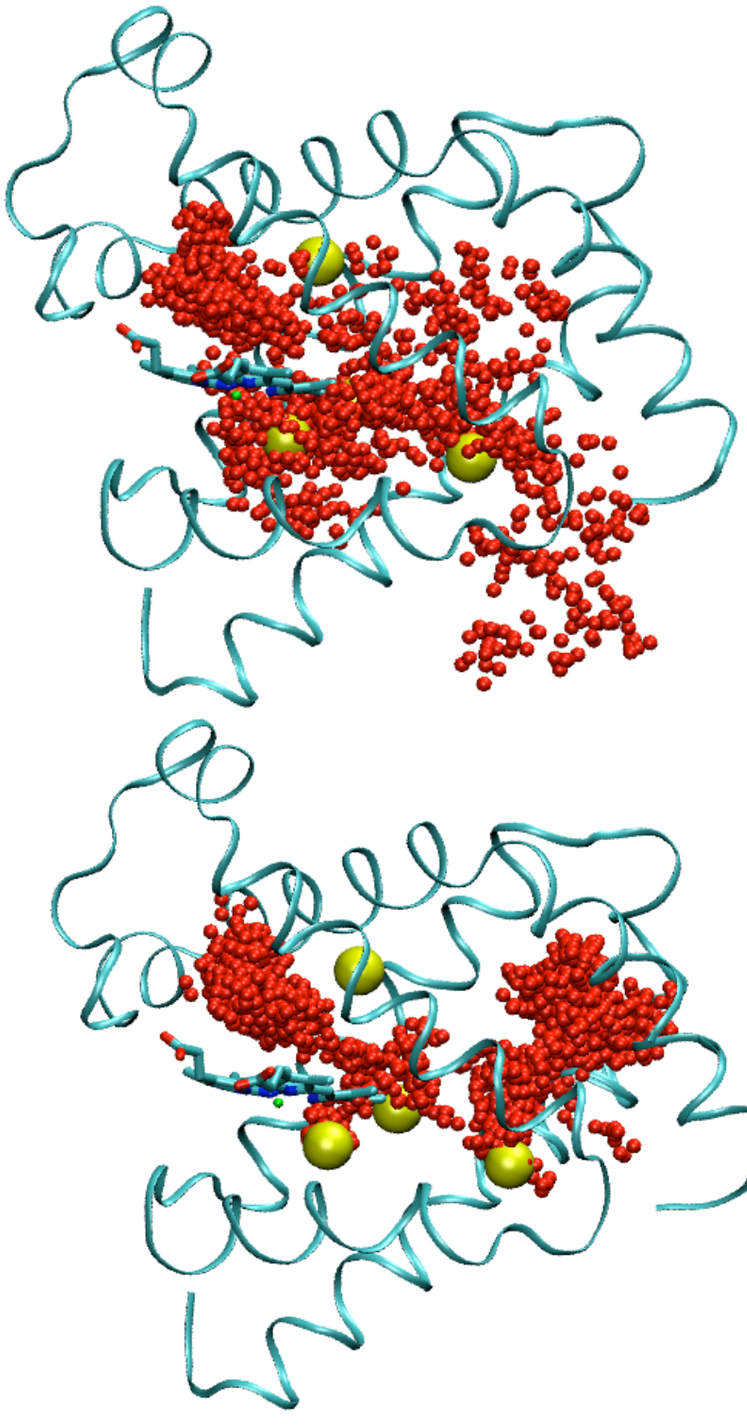
Assumption: no large scale conformational changes of the protein.

Cyan: MbCO, Orange: photolyzed Mb. Only localized change in Iron coordination and structure of the heme (time-scale < 500fs) represented by changing the parameters in the force field

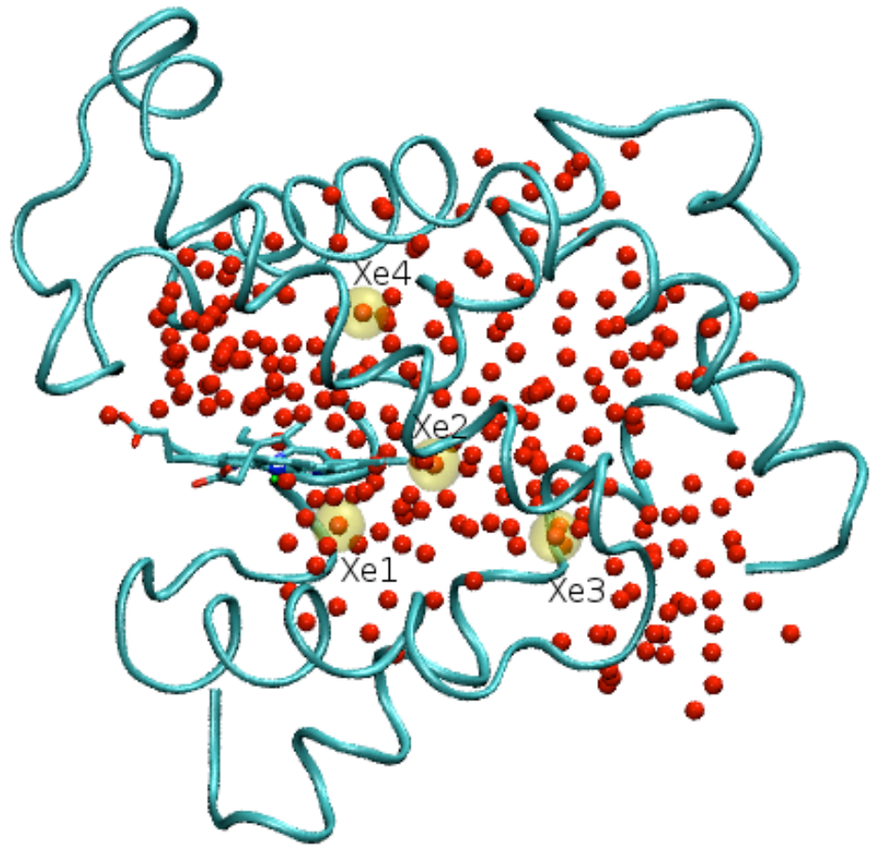


TAMD simulations

11 independent TAMD trajectories of the order of 100ps. Effective thermal energy 10 kcal/mol.



A collection of CO positions extracted from all TAMD trajectories:



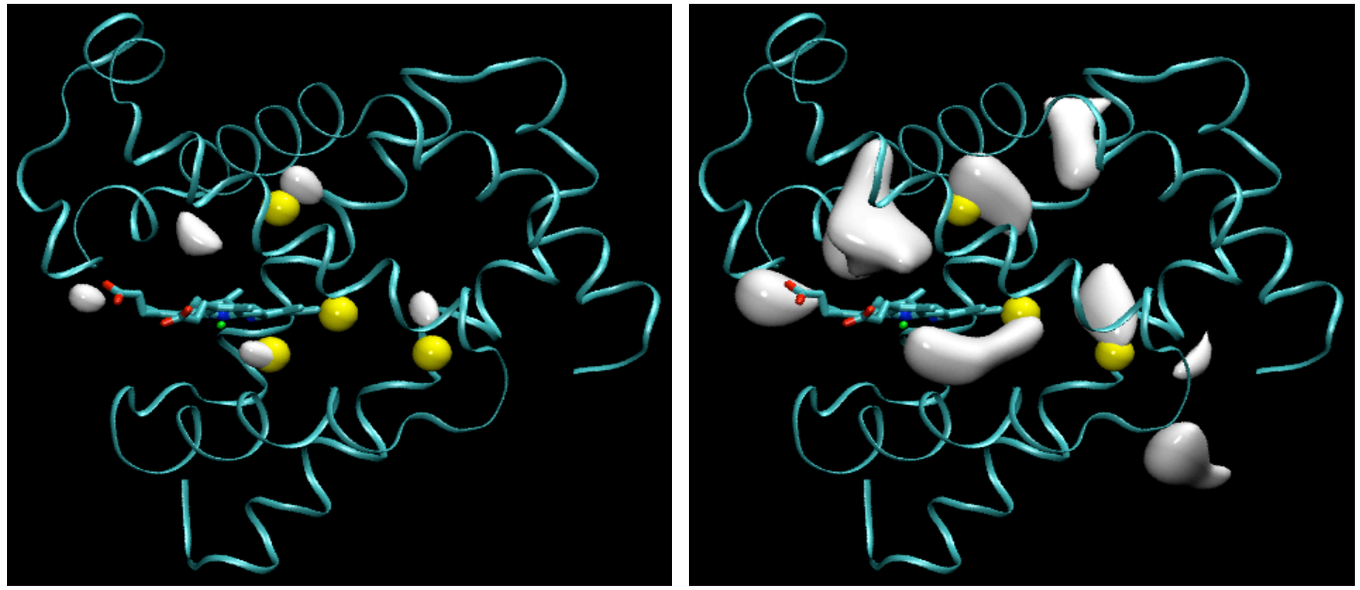
PMF map reconstruction

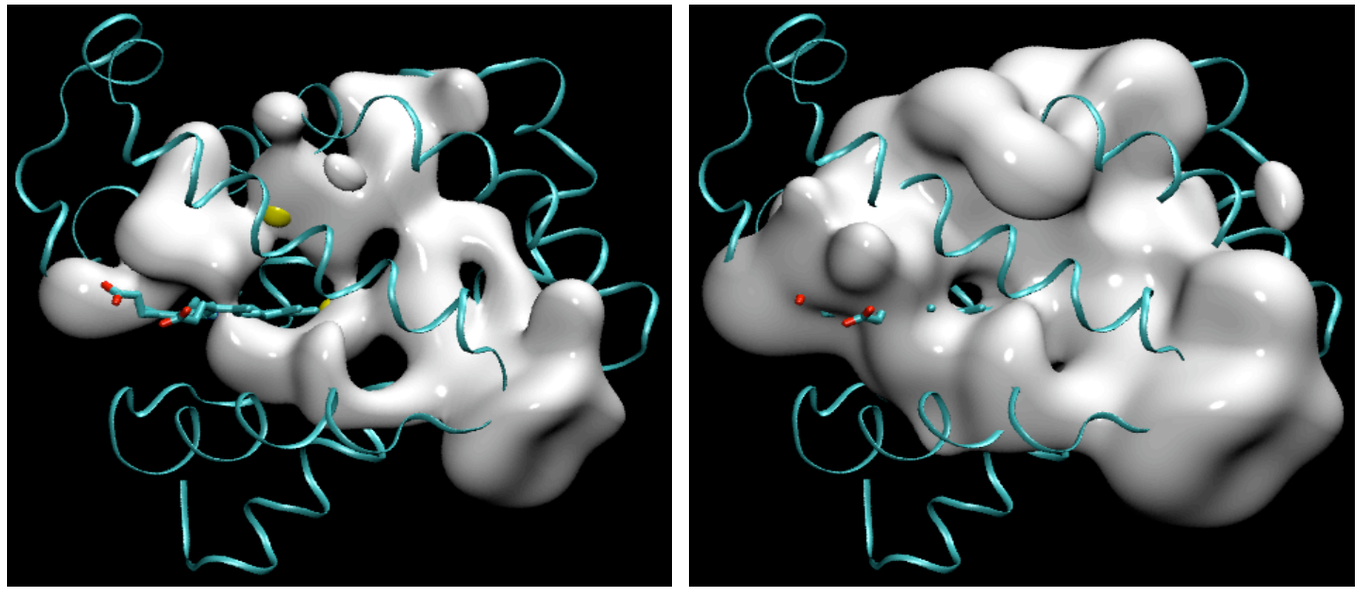
Mean forces computed in 239 centers, using 500ps long restrained MD trajs. Interpolation using gaussian RBF.

Isocontours at 1.5, 3.5, 5.5, 8.5 kcal/mol.

Global minimum in Xe4.

Other minima in DP, Xe cavities, and in other cavities, some observed in other studies (Elber, Brunori, Schulten, Ceccarelli, Onufriev).





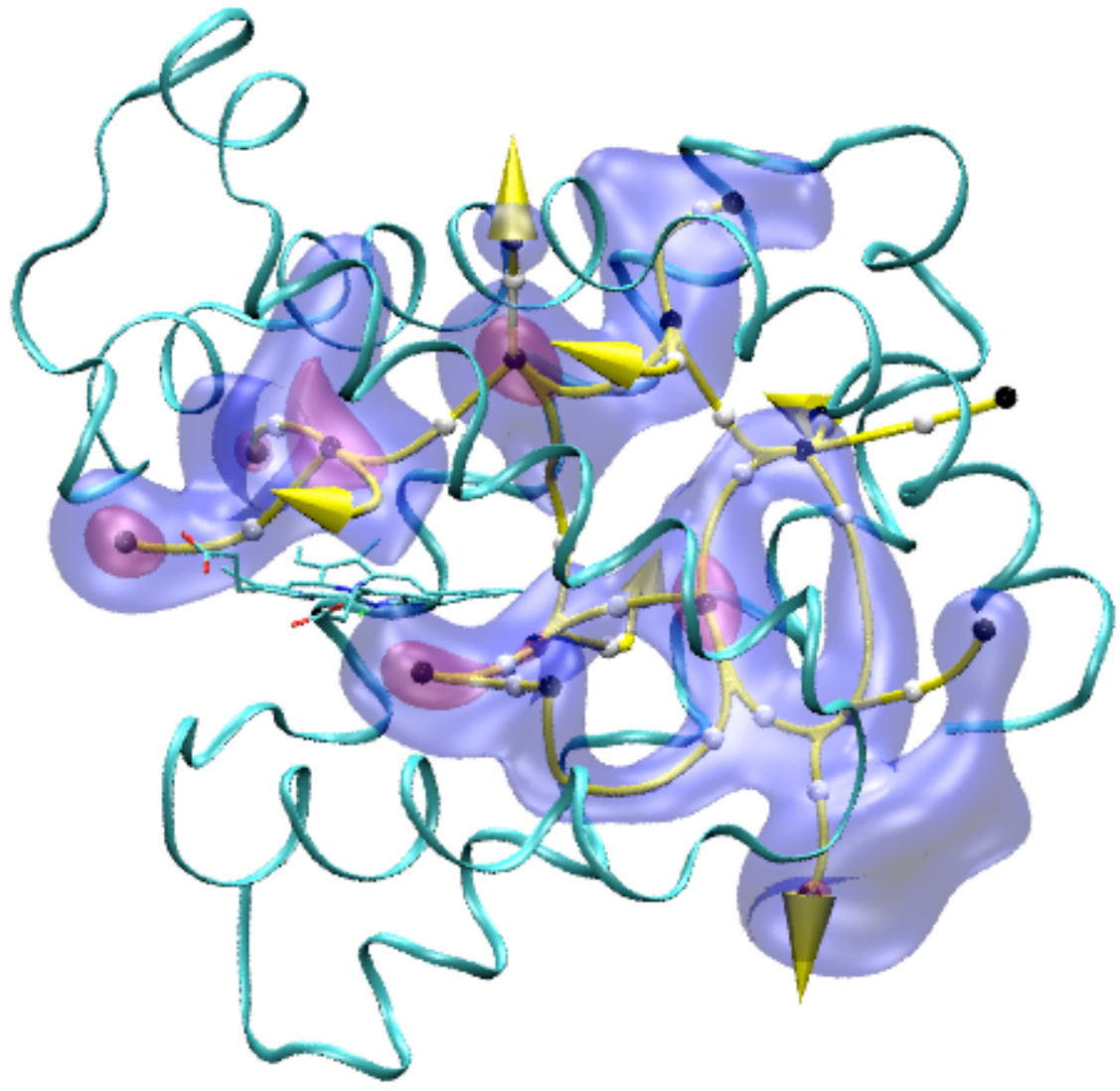
CO migration paths inside Mb as MFEPs from the string method on the reconstructed surface

No more MD required at this stage: we have an analytical representation of the PMF landscape from the interpolation

Yellow curves are MFEPs on the CO PMF map. Isosurfaces are 2.0 (red) and 5.0 kcal/mol (blue). White and black spheres indicate energy barriers and minima along the paths.

Arrows indicate where CO in TAMD trajectories exited to the solvent.

There is a network of paths connecting the DP to the cavities and to the protein surface.

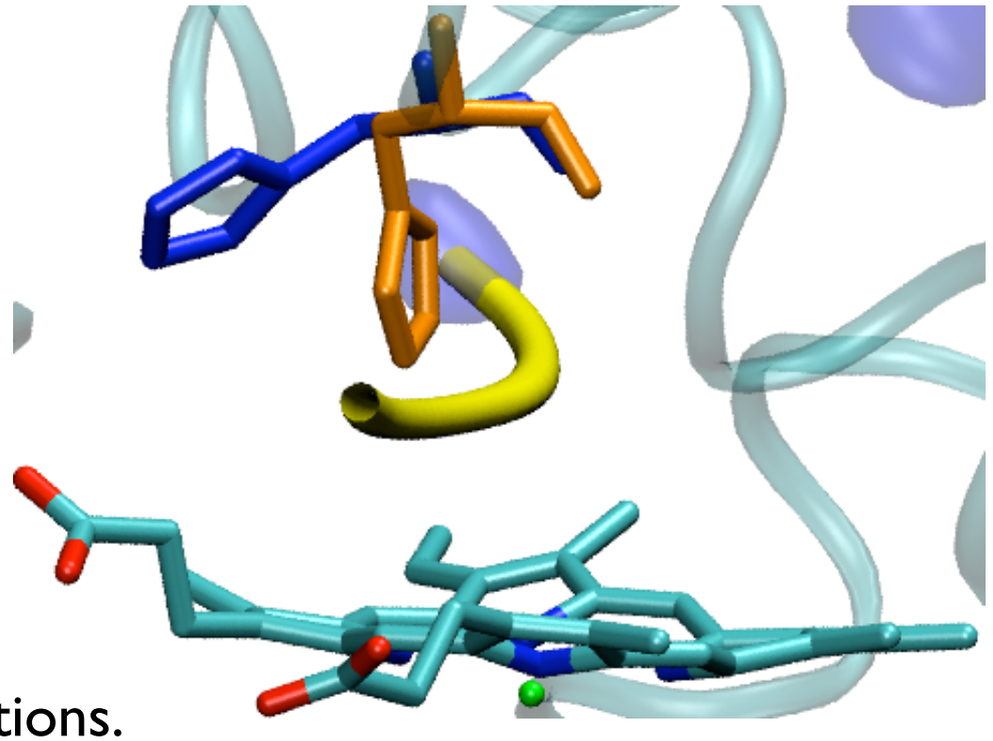


Histidine gate path

The yellow curve is the MFEP.

Orange sticks: proximal Histidine as in the crystal of Mb with CO bound.

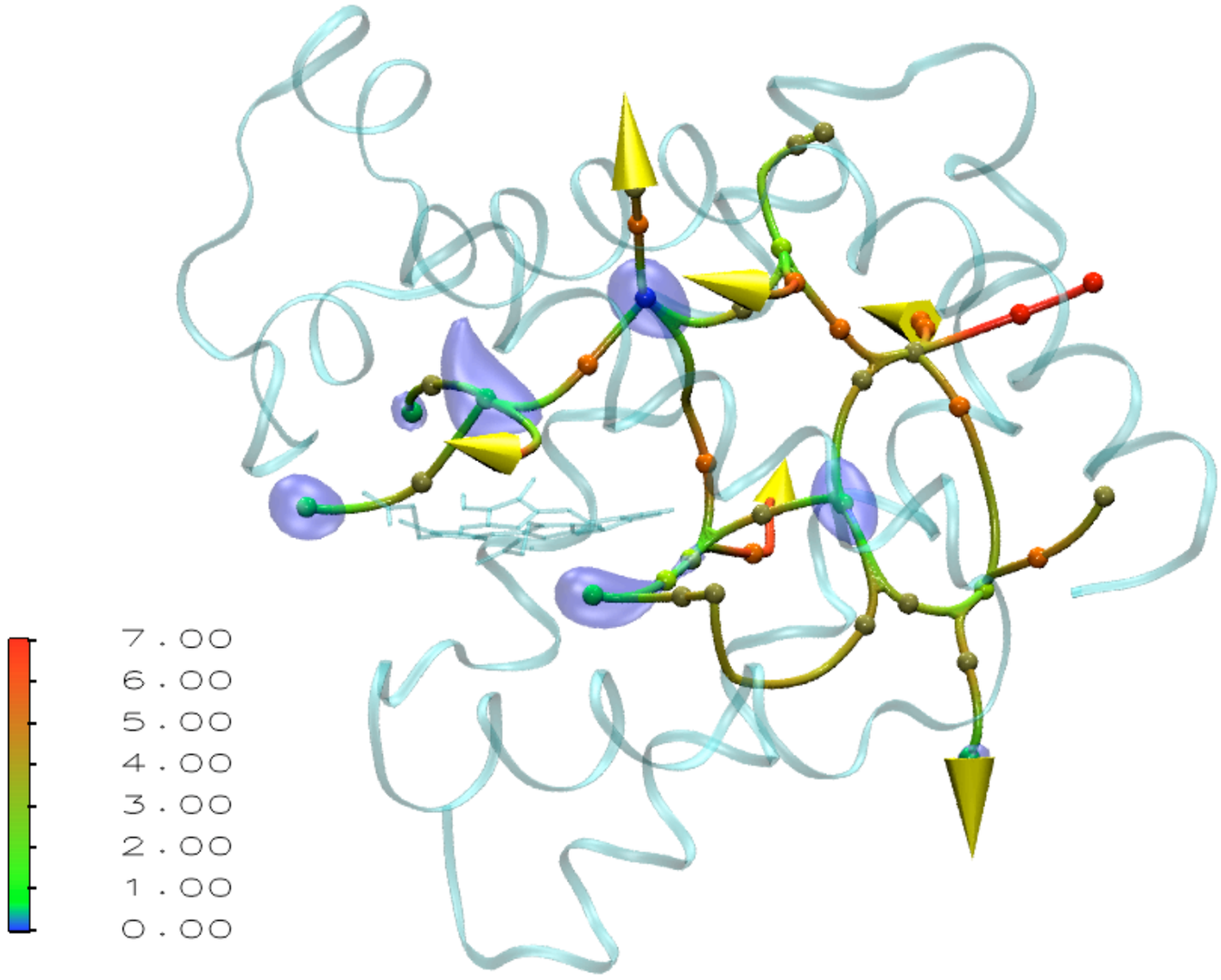
Blue sticks: deoxy Mb conformation from our simulations.



PMF barriers along the paths

Location of barriers correlates with random mutagenesis work of Huang & Boxer.

Values agree with literature where available (Schulten, Ceccarelli, Meuwly).



(X,Y)		(DP, HG)[a]	(DP, Xe4)	(Xe4, Xe2)	(Xe2, Xe1)	(Xe2, Xe3)
X to Y	ΔA_1[b]	6.7	4.5	5.3	0.2	2.7
Y to X	ΔA_2	0.1	5.1	3.5	1.3	3.9

Conclusions on Mb

CO can diffuse inside Mb across a network of paths passing through the Xenon cavities and several others not found by Xenon experiments.

The His gate is not the only possible path from the binding site to the protein surface. It is however the shortest from DP, and the only one without intermediate minima.

The energy barrier for the CO from DP to Xe4 (i.e. towards the interior of the protein) is smaller than the PMF barrier towards the His gate, which reveals the importance of internal paths.

Reconstruction of PMF surfaces and reaction pathways

Combine TAMD with other methods

Single Sweep for PMF surface reconstruction

⇨ String method for finding reaction pathways

The string method for calculating reaction paths

W. E, W. Ren, and E. Vanden-Eijnden *Phys. Rev. B.* 66, 052301 (2002)

L. M., A. Fischer, E. Vanden-Eijnden, and G. Ciccotti *J. Chem. Phys.*, 125, 024106 (2006)

The string method is an iterative algorithm to find minimum (free) energy paths (MFEPs) between given end points.

It evolves a discretized curve (replicas of the system) while preserving the parametrization (i.e., equal distance between the images).

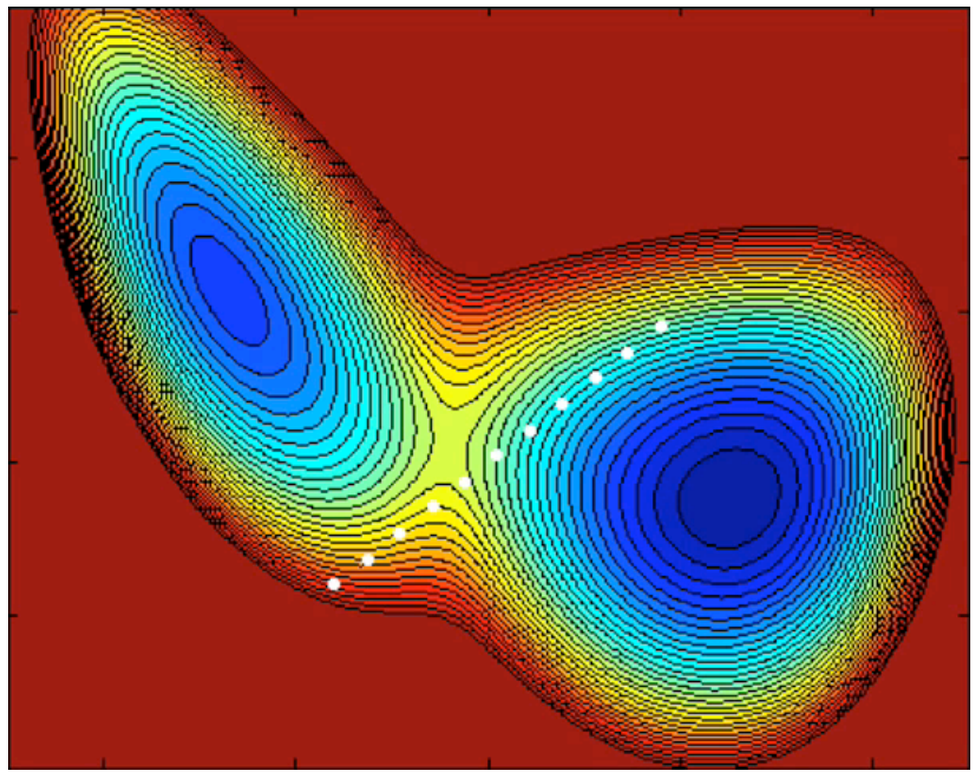
In collective variables space, mean-force based evolution of images:

$$\mathbf{z}(t+h) = \mathbf{z}(t) + h\ \mathbf{M}(z(t))\mathbf{F}(z(t))$$

with

$$\mathbf{M}_{ij}(z) = \left\langle \sum_k^N \frac{1}{m_k} \frac{\partial \theta_i}{\partial x_k} \frac{\partial \theta_j}{\partial x_k} \right\rangle_{\theta(x)=\mathbf{z}}$$

$$F_i = -\frac{\partial A}{\partial z_i}$$



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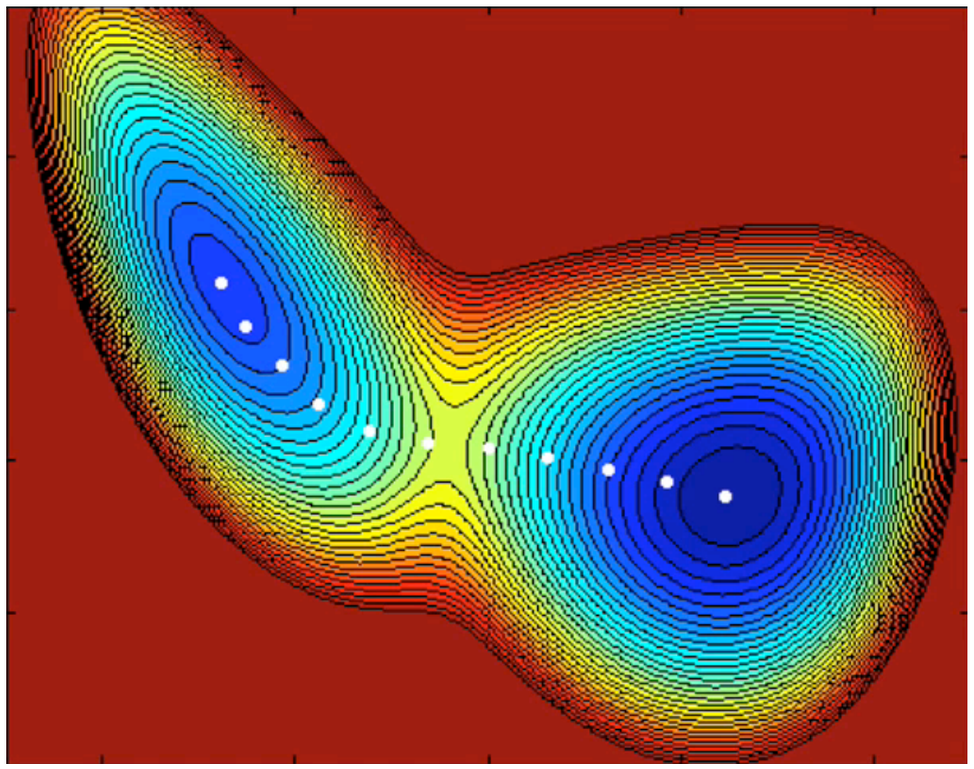
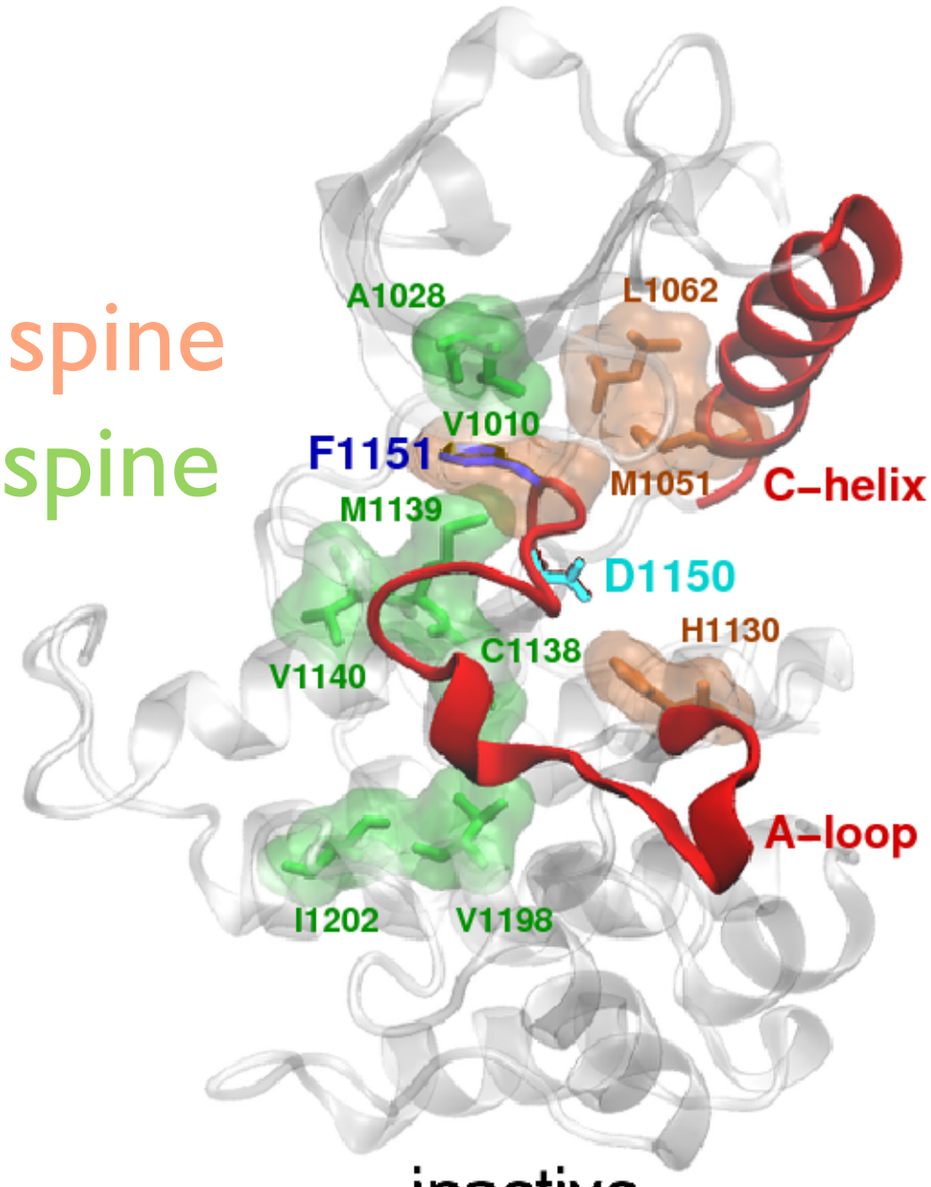
The string method requires an initial path:
it can be obtained from a TAMD trajectory



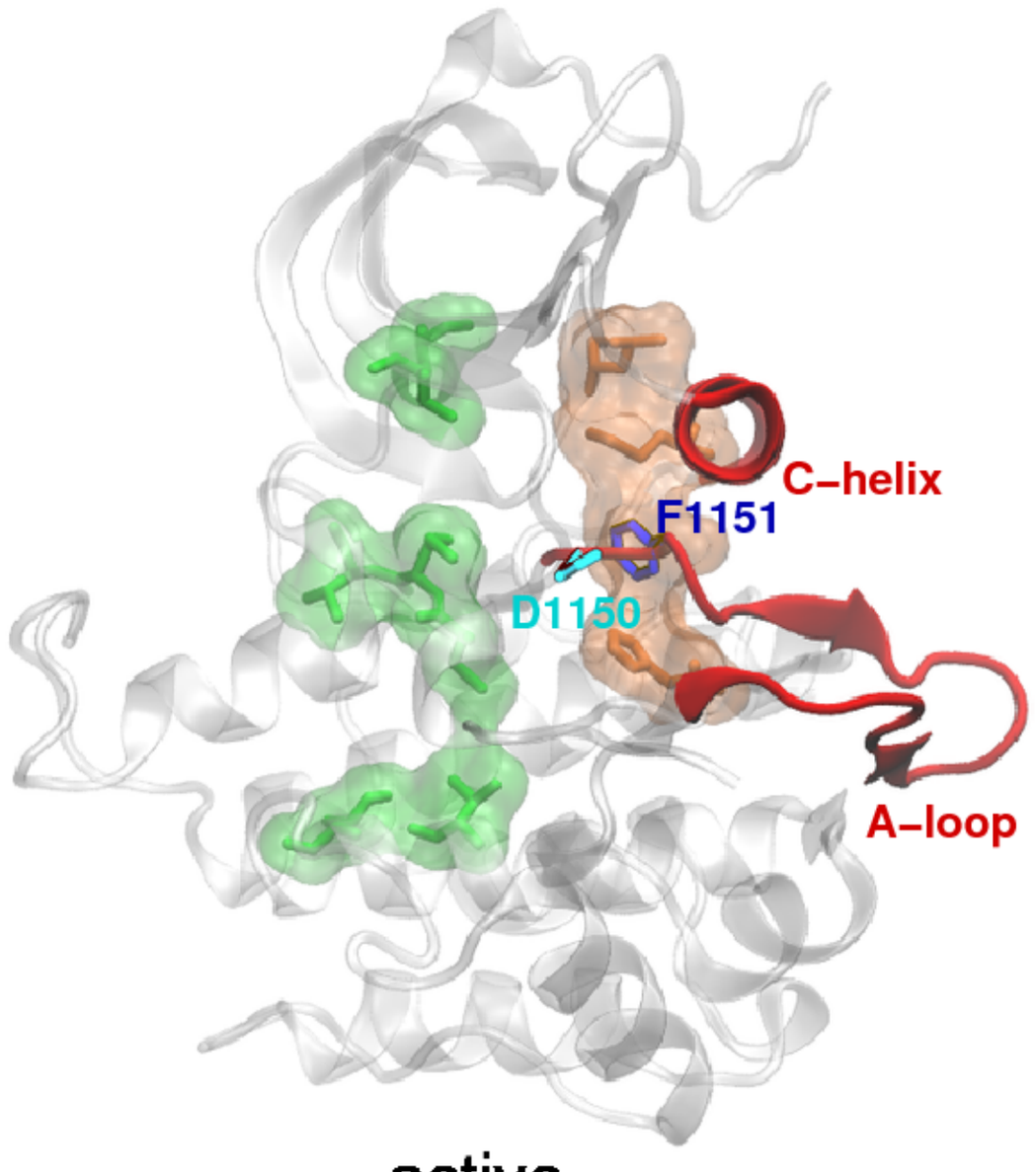
Conformational transition of kinase activation

C spine

R spine



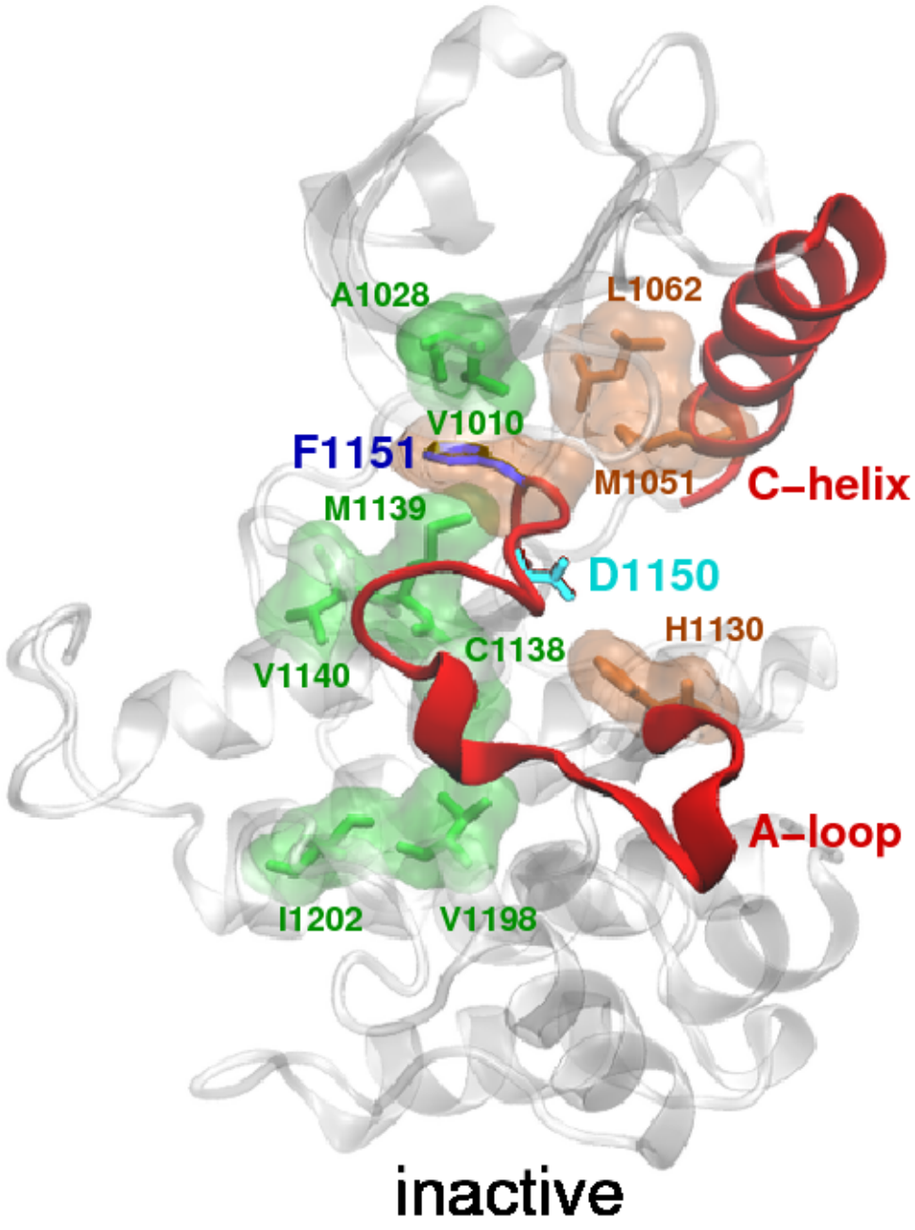
inactive



active

Harish Vashisth, **L. M.** and Cameron F. Abrams *Biophys. J.*, 102, 1979 (2012)

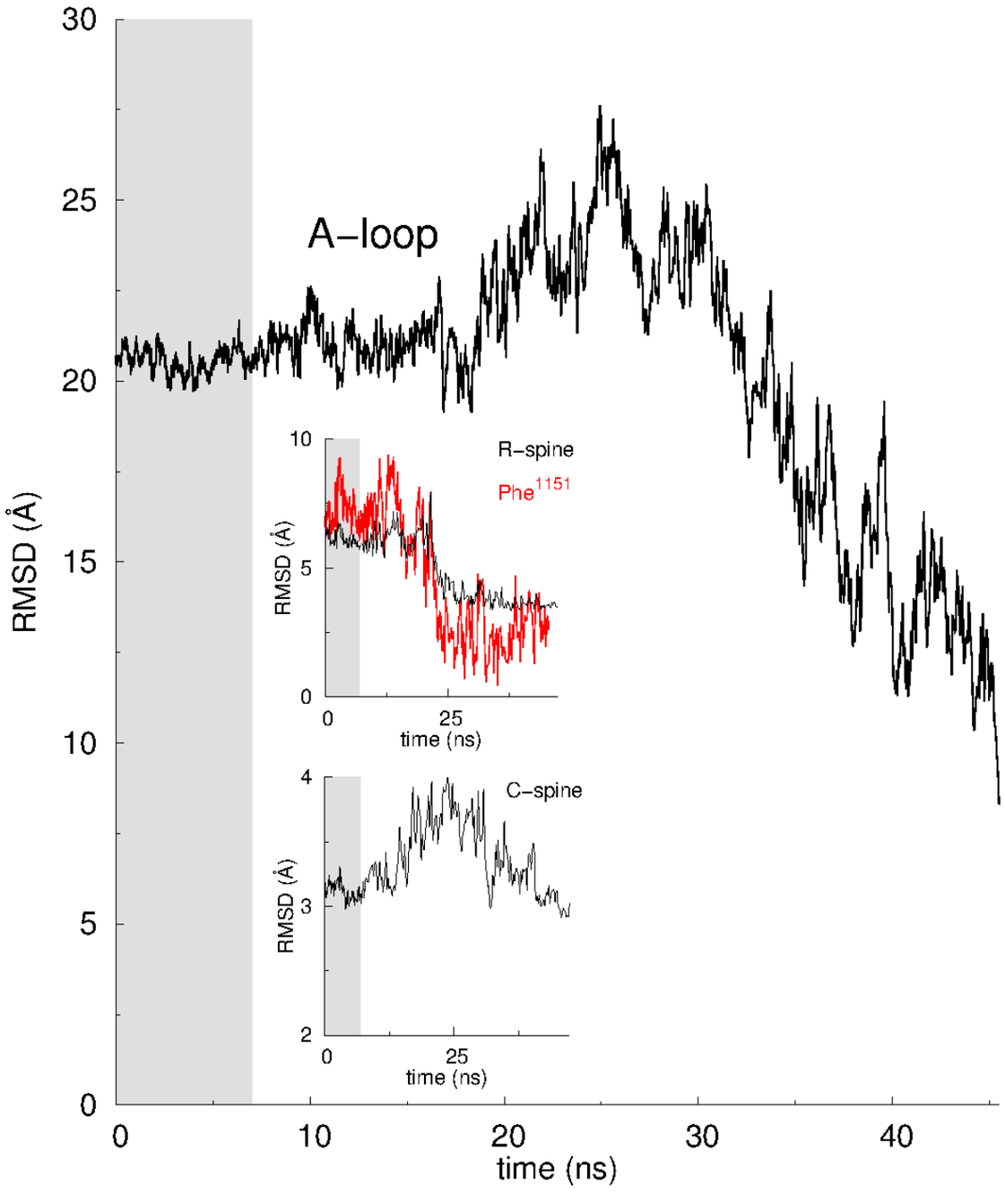
Conformational transition of kinase activation

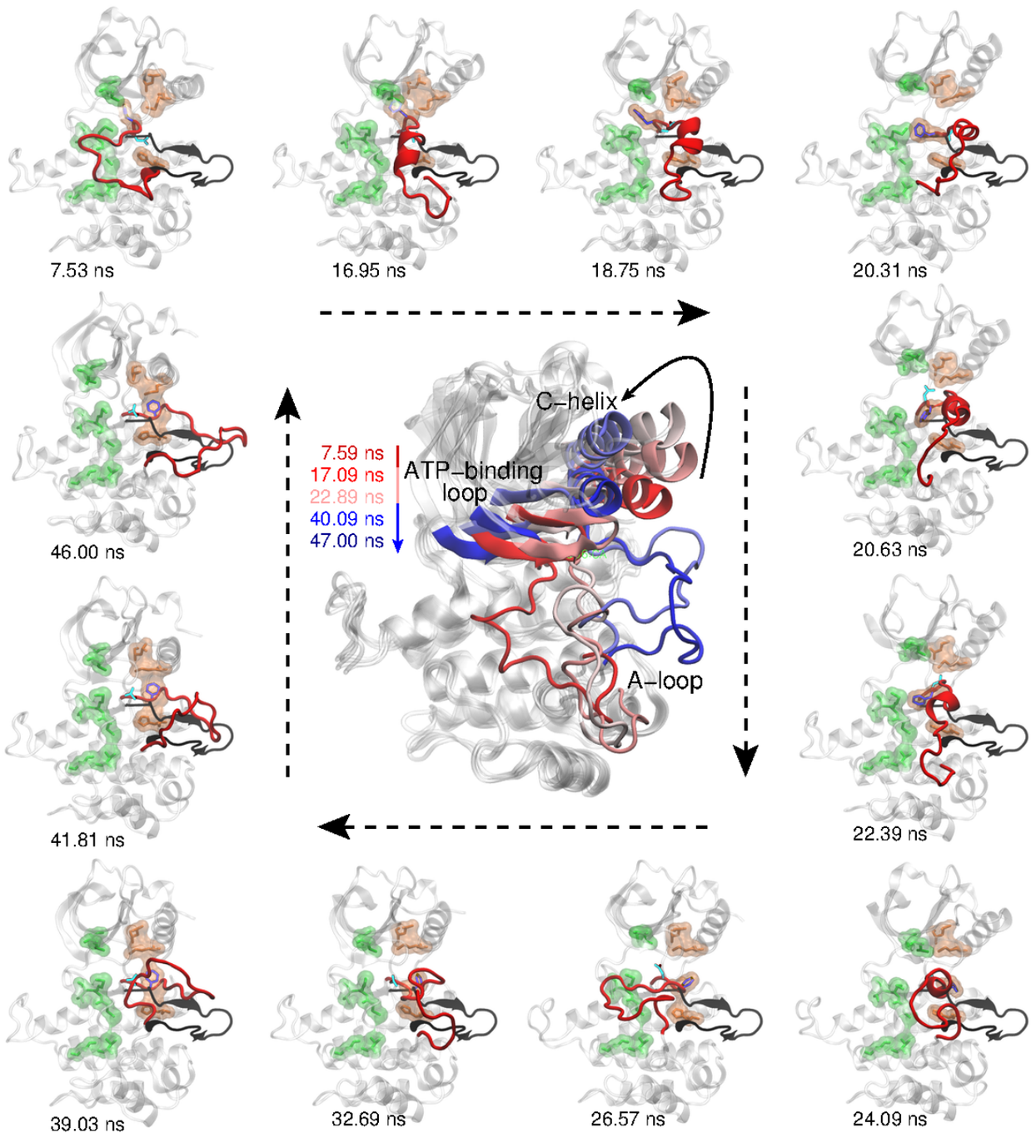


Collective variables: centers of mass coordinates of 4 residues groups belonging to the A-loop:
12 CVs

Effective thermal energy 5 kcal/mol

Conformational transition of kinase activation

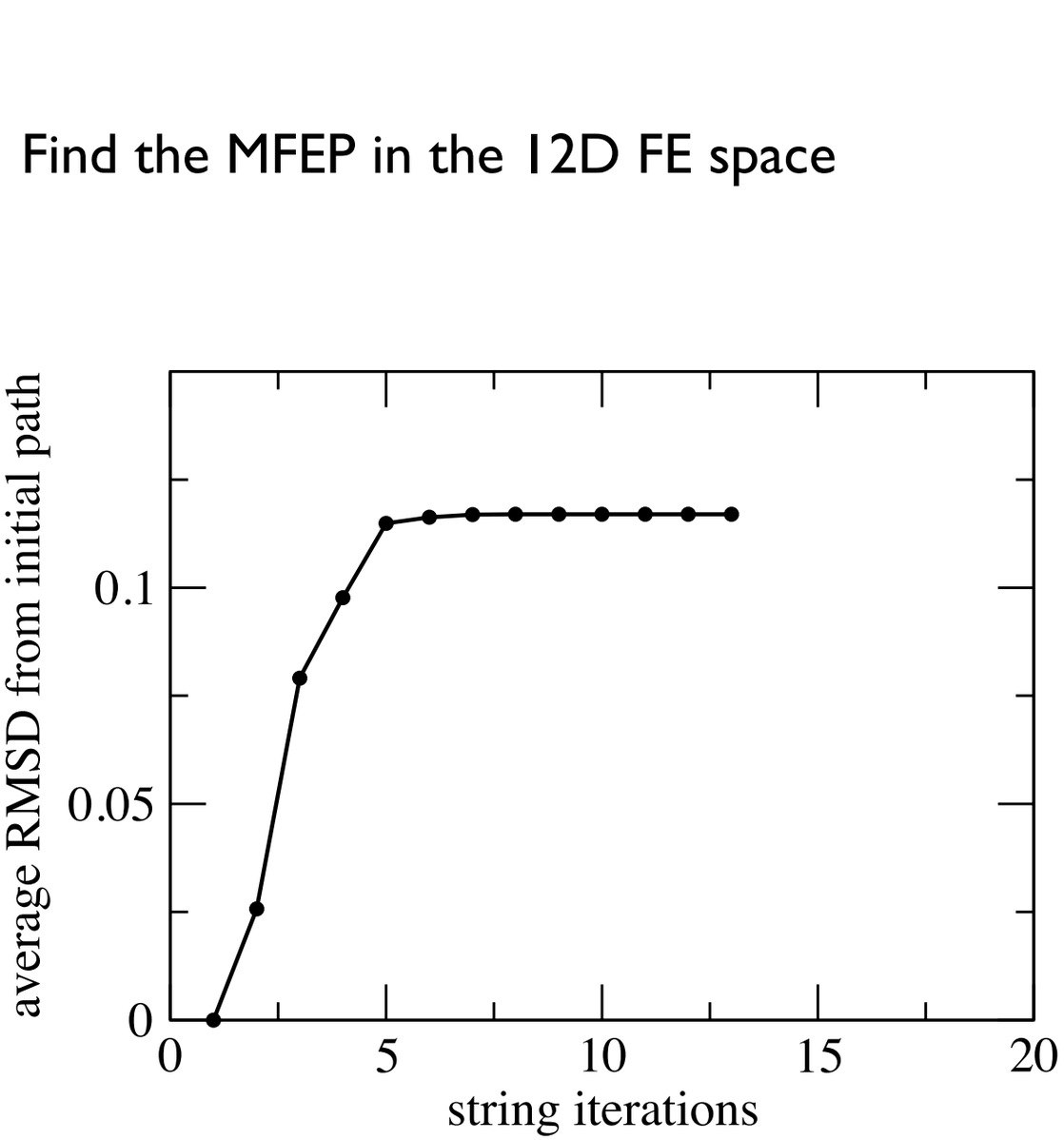


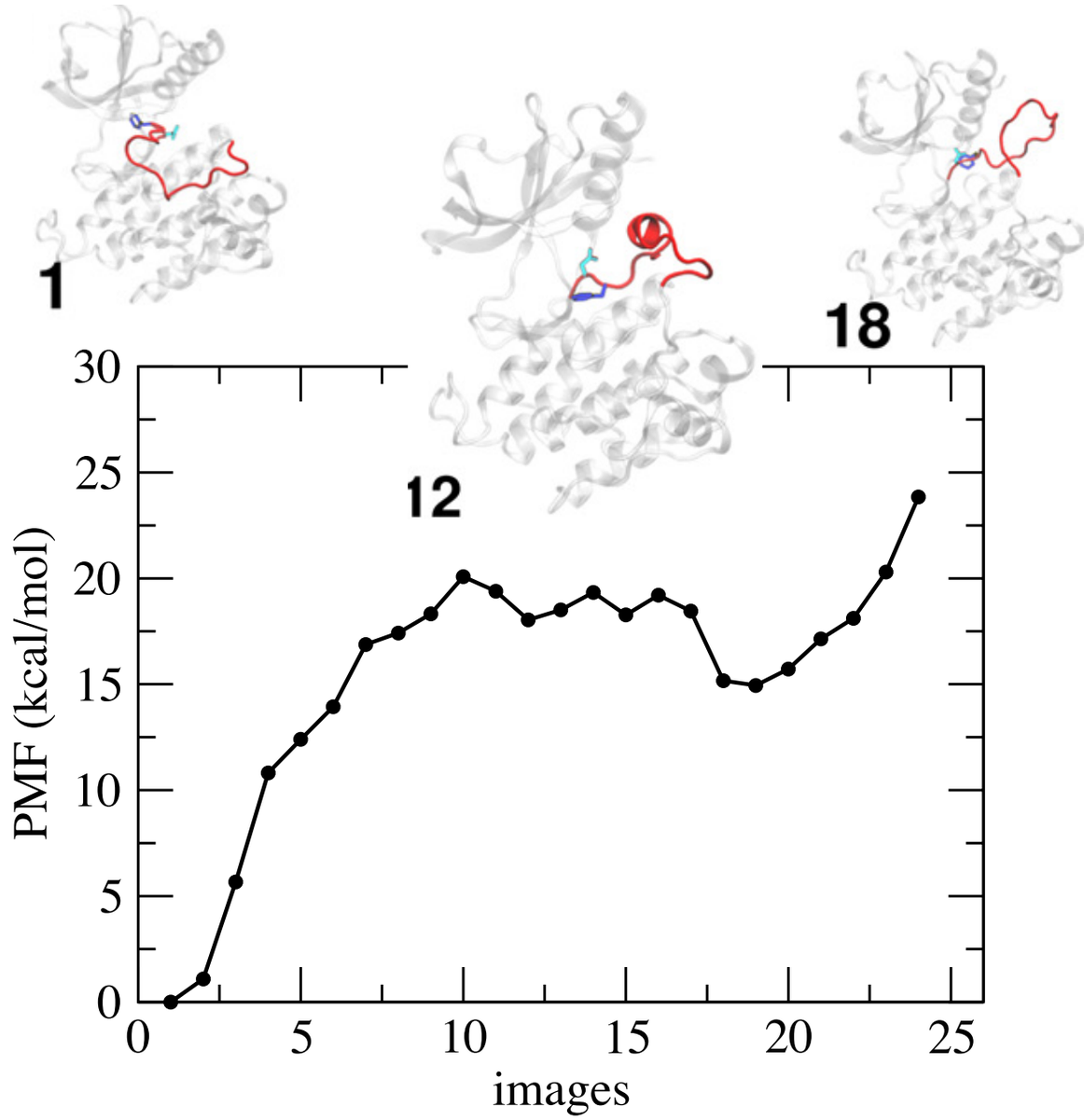


Presence of a helical conformation for the A-loop along the TAMD

Conformational transition of kinase activation
MFEP from the string method

Find the MFEP in the 12D FE space





The MFEP shows a metastable intermediate characterized by a helical configuration of the loop

Summary

TAMD can be used for exploration of unknown PMF landscapes, searching for new conformers (meta-stable states). It can give rough estimates of the PMF barriers by comparison with the effective energy on collective variables.

A large number of collective variables can be used (Abrams & Vanden-Eijnden PNAS 2010, 69 variables for the conformational transition of HIV-1 gp120)

More accurate PMF estimation and reconstruction obtained using TAMD in conjunction with the single-sweep method (multi-dimensional analogue of thermodynamic integration) and the string method (to specifically find MFEPs).