The Mechanism of the Sarco/Endoplasmic Reticulum ATP-Driven Calcium Pump

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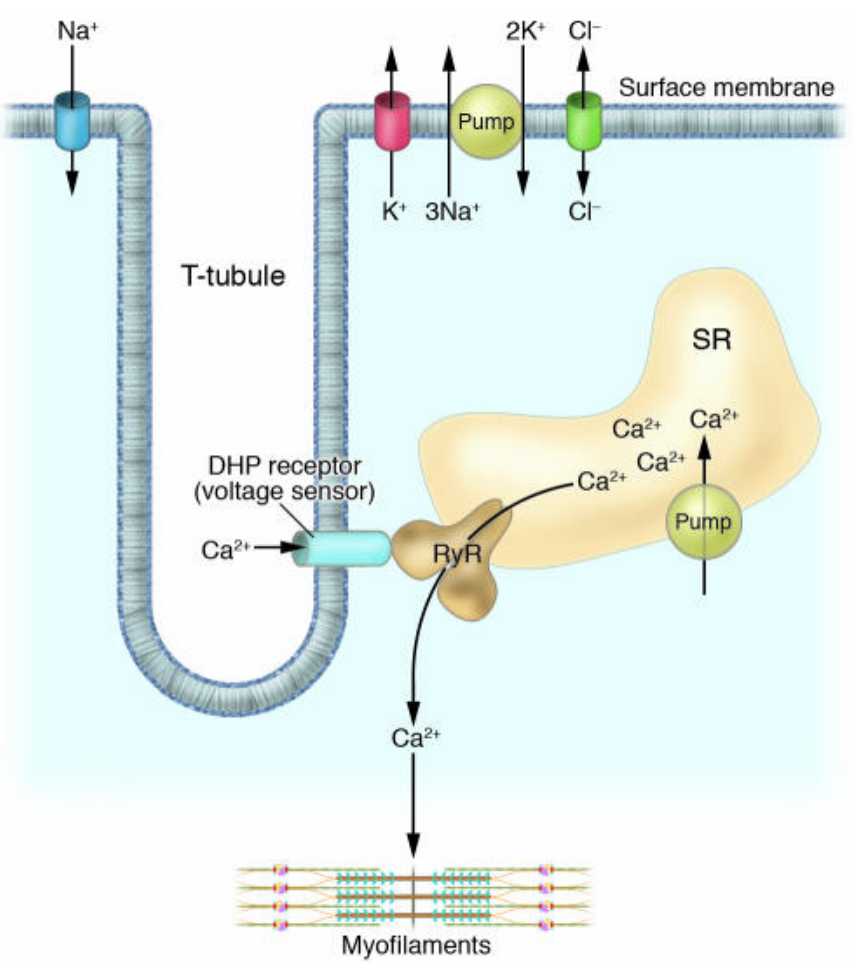
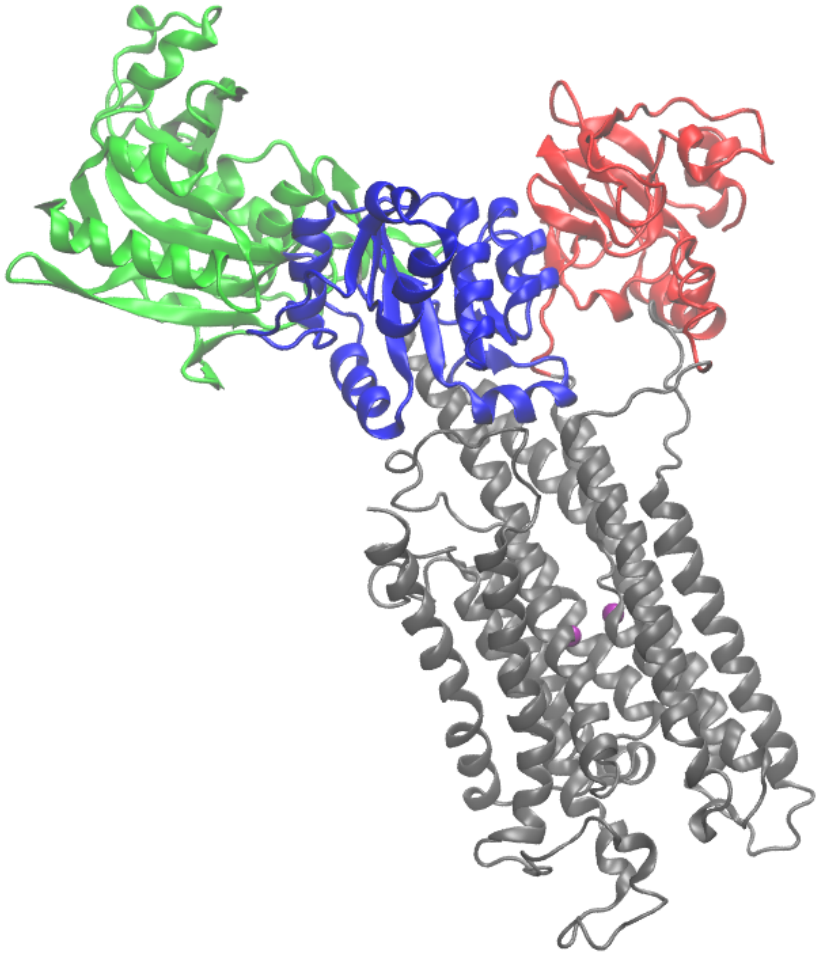
Calcium pump SERCA

- Sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) is an integral membrane protein
- Plays important role in the relaxation of skeletal muscle
- Transfers Ca^{2+} ions from the cytosol of the muscle cell to the lumen of the sarcoplasmic reticulum
- Maintains a 10000 times concentration gradient at the expense of ATP hydrolysis



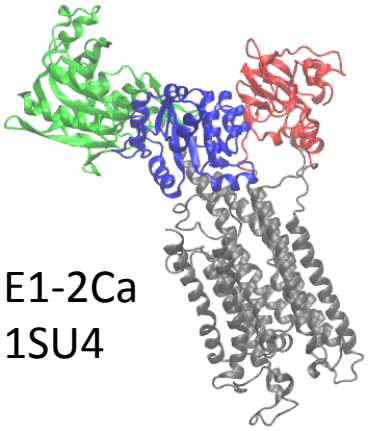
Architecture of the protein

- 994 amino acids
- Three cytoplasmic domains
  - Nucleotide binding domain, N (green)
  - Phosphorylation domain, P (blue)
  - Actuator domain A, (red)
- Ten transmembrane (TM) helices (M1-10)
- Two TM Ca^{2+} binding sites

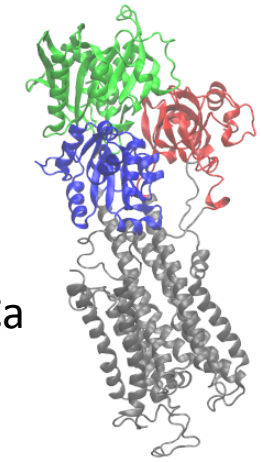


PDB ID: 1SU4 ; Toyoshima *et al.* Nature **405** 647 (2000)

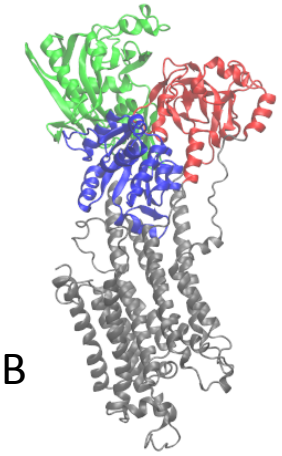
Pumping cycle and structures



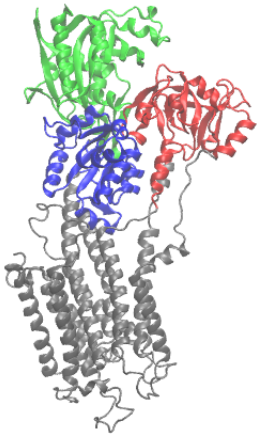
E1-2Ca
1SU4



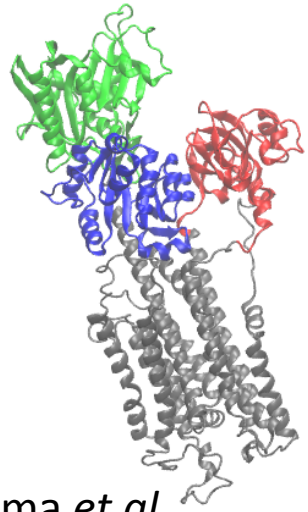
E1-ATP-2Ca
1VFP



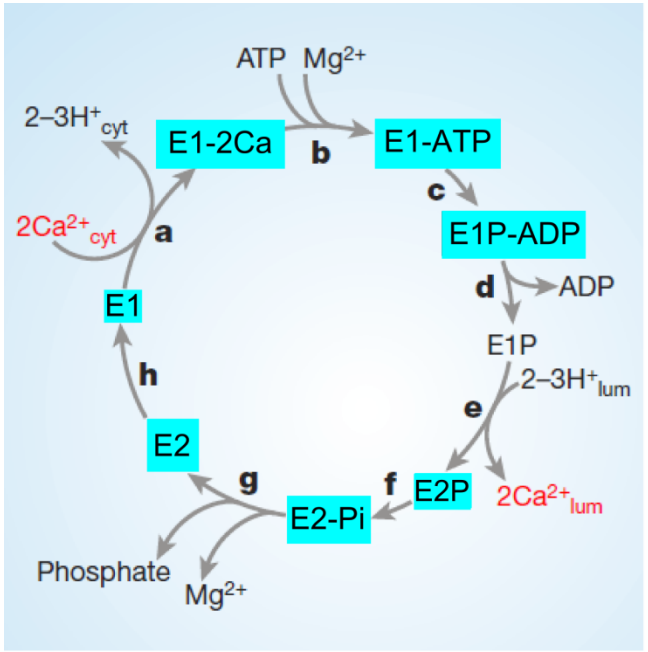
E2P
3B9B

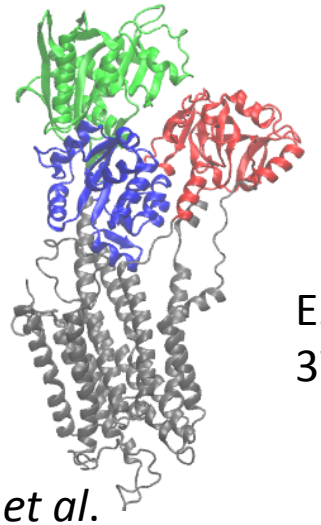


E2-Pi
1WPG



E1
3W5A





E2
3W5C

Toyoshima *et al.*
Nature **405** 647 (2000)
Nature **418** 605 (2002)
Nature **430** 529 (2004) , Nature **432** 361 (2004)
Nature **495** 260 (2013)

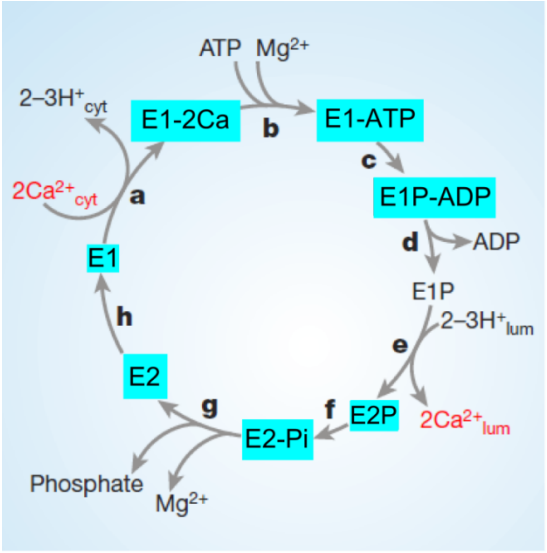
Nissen *et al.*
Science **304** 1672 (2004)
Science **306** 2251 (2004)
Science **450** 1036 (2007)
Nature **495** 265 (2013)

Objective and Importance of our project

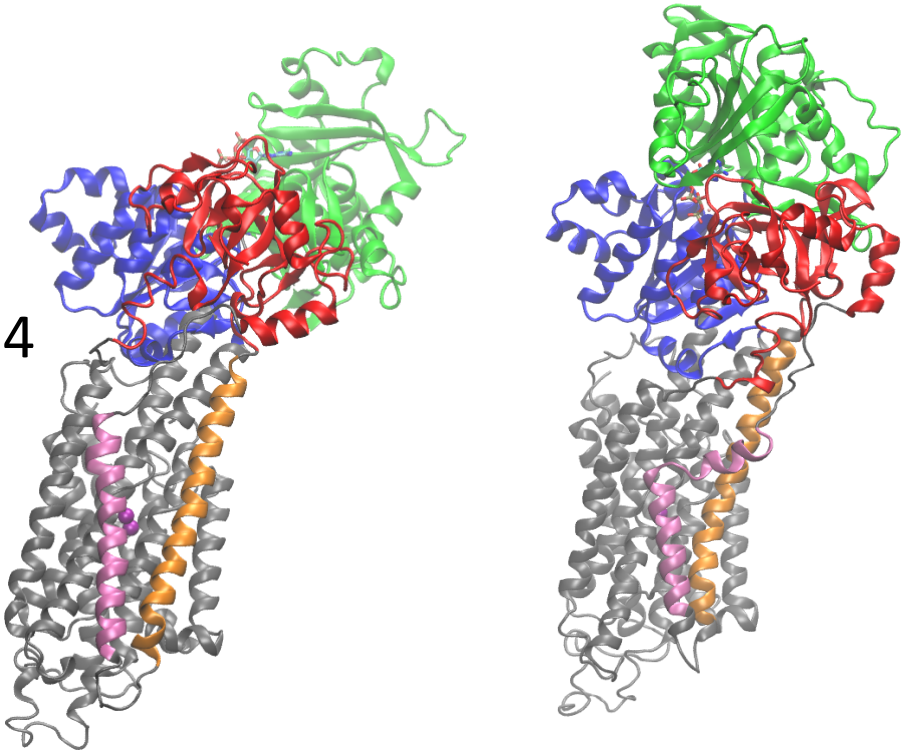
- Objective
  - How do the transitions take place?
  - How large and small scale motions are coupled ?
  - How information is transmitted over a long distance?
- Importance
  - Understanding the mechanism is of biomedical importance. SERCA is a drug target for several diseases e.g. heart failure
  - Good model system for understanding the mechanisms of a large class of ion pumps called P-type ATPases

Specific goal : understanding occlusion

- *Occlusion*: ions can not escape from the binding sites to the cytoplasmic medium
- This is important for transporting ions against the concentration gradient



E1-2Ca^{2+}
PDB ID: 1SU4



E1-2Ca^{2+}-ATP
(E1P-ADP)
PDB ID: 1VFP

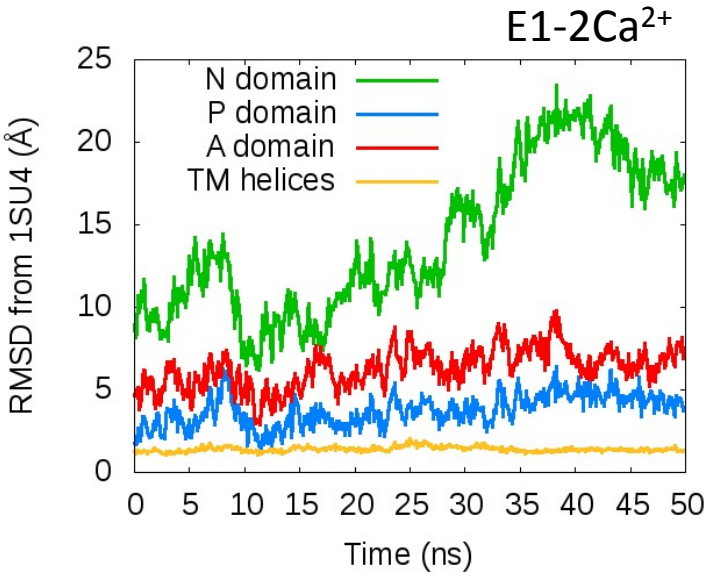
M1: Mauve
M2:Orange

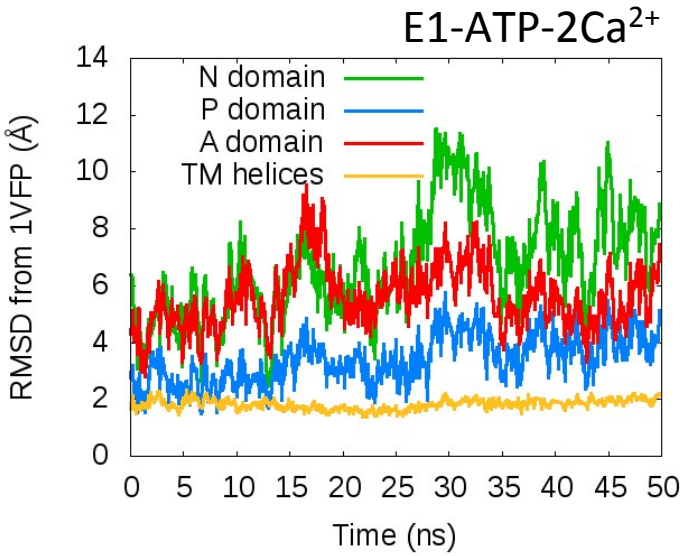
Computational strategy

- We like to simulate the transition at the all-atom level
- Brute-force molecular dynamics simulation is inadequate, e.g. after 500 ns the transition is not complete (Esponoza-Fonseca and Thomas PLoS One **6** e26936 (2011))
- We will employ a rare event method: string method with swarms of trajectory (Pan, Sezer and Roux J Phys Chem B **112** 3432 (2008))
- Several steps
  - MD simulations of the end points
  - Construction of a CG pathway, where protein is represented as a C^{α} trace
  - Reconstruct all-atom pathway from the CG pathway
  - Refine the pathway using string method

Simulation of end states

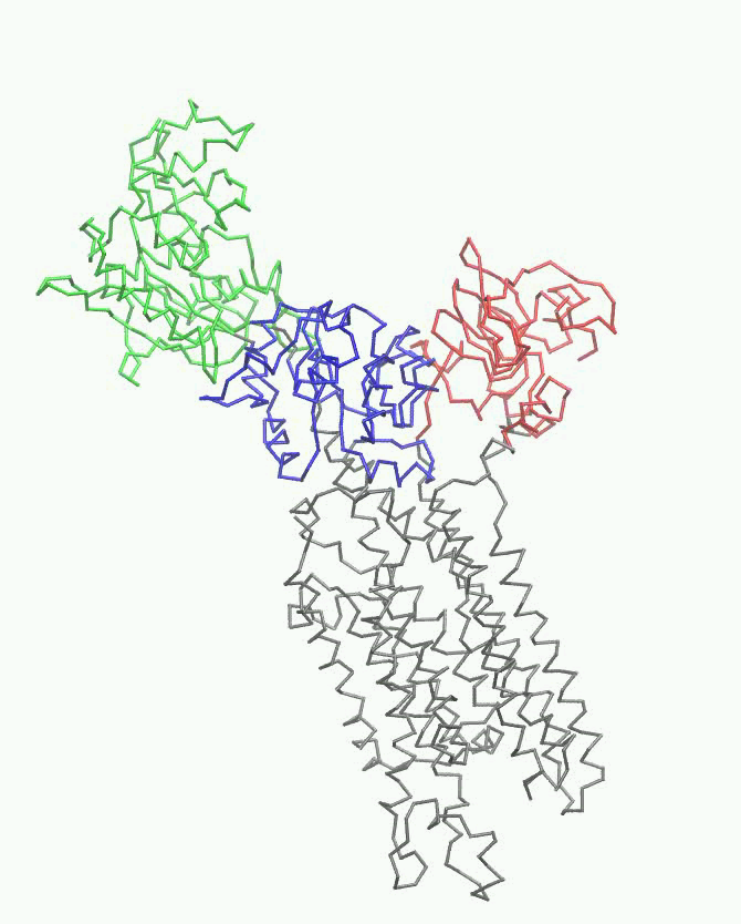
- Crystal structures were solvated with ATP and Mg^{2+} ion. E1-$2Ca^{2+}$ (PDB ID: 1SU4); E1-ATP-$2Ca^{2+}$ (PDB ID: 1VFP)
- 480 POPC lipids, ~70,000 TIP3P water, 0.15 M KCl. Total number of atoms ~291,000
- CHARMM 36 force field and NAMD 2.9
- 50 ns of simulation for each end state





CG pathway and all-atom reconstruction

- Protein is represented as a C^{α} trace
- Pathway was determined by the *ANMPathway* method



- 35 images in the CG pathway
- Steered MD is used for this purpose
- Image j+1 is prepared from image j with steering forces applied on the C^{α} atoms
- 500 ps of steered MD for a pair of consecutive images

http://anmpathway.lcrc.anl.gov/anmpathway.cgi

String method with swarms of trajectory

- Finds the "most probable transition path" between two minima on a free energy surface defined by a set of collective variables (CVs), $\mathbf{z} = \{z_1, z_2, \ldots, z_n\}$
- Pathway is a sequence of discrete images: $\{\mathbf{z}^1, \mathbf{z}^2, \ldots, \mathbf{z}^M\}$
- At each iteration every image is updated by adding a drift term

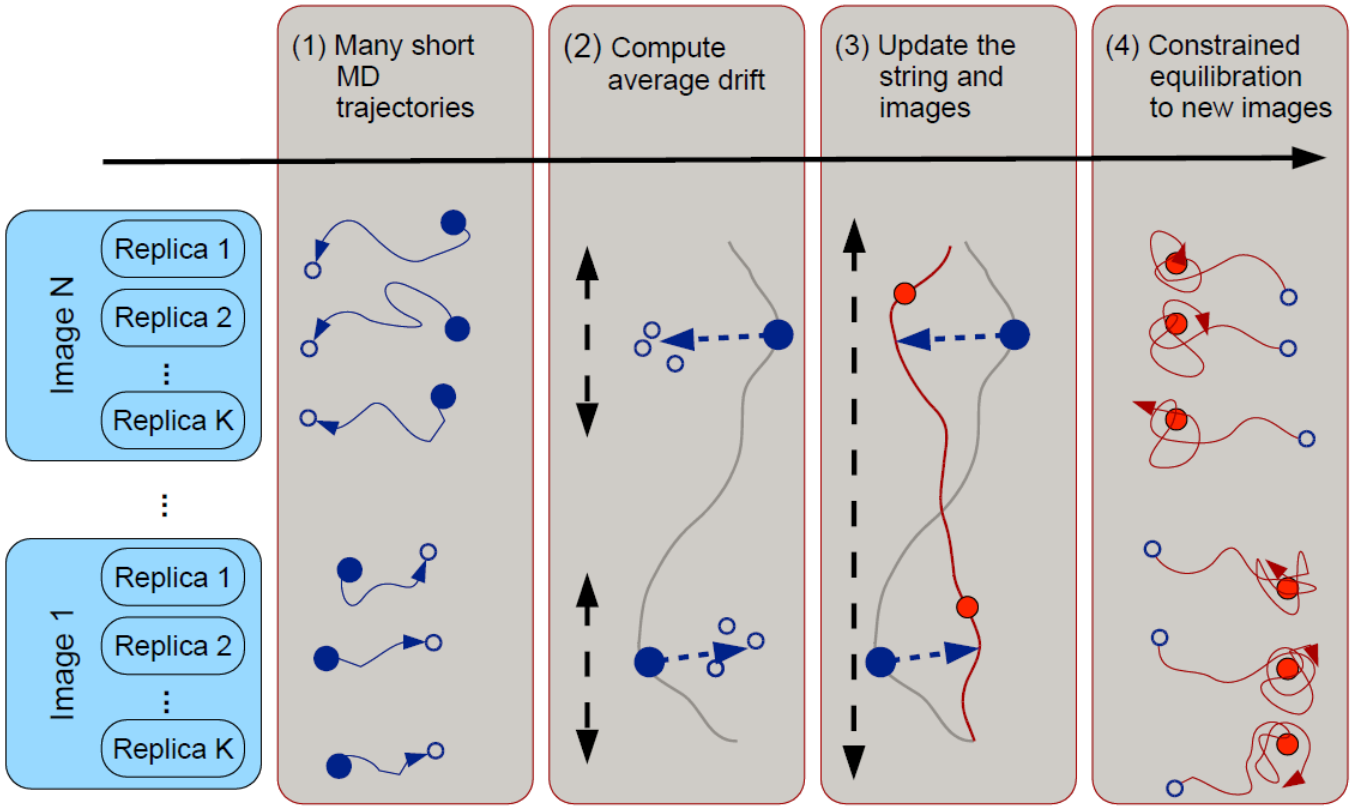
$$\mathbf{z}_{p+1}^k = \mathbf{z}_p^k + \Delta\mathbf{z}^k$$

- The drift is the average drift calculated from short unbiased MD trajectories (i.e. swarms) launched from each image

$$\Delta\mathbf{z} = \overline{\mathbf{z}(\tau) - \mathbf{z}(0)}$$

- After updating the CVs, all-atom representation of each image is generated by constrained equilibrations

Massively parallel *NAMD* implementation



N = 35

K = 32

Total number of replicas = N x K = 1120

Single production run can take up ~25% of *Blue Waters*

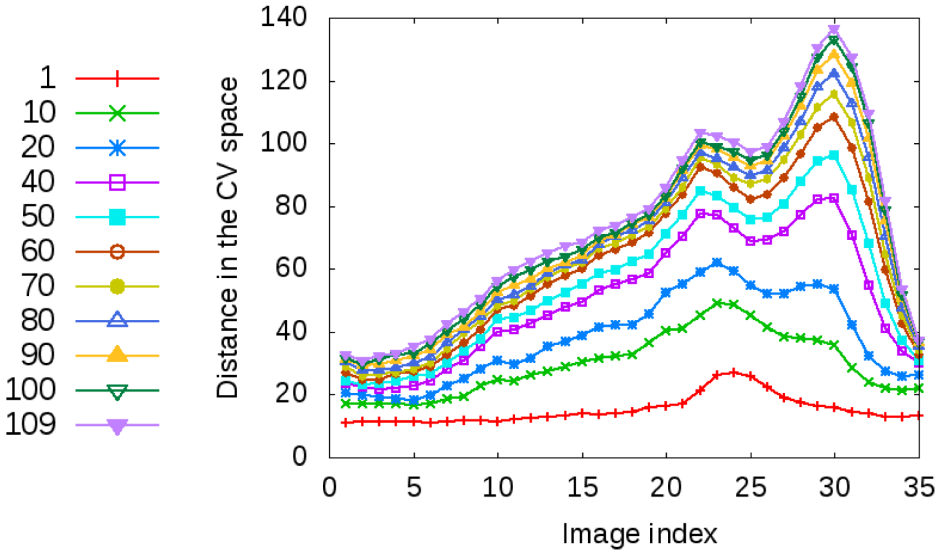
Dr. Mikolai Fajer

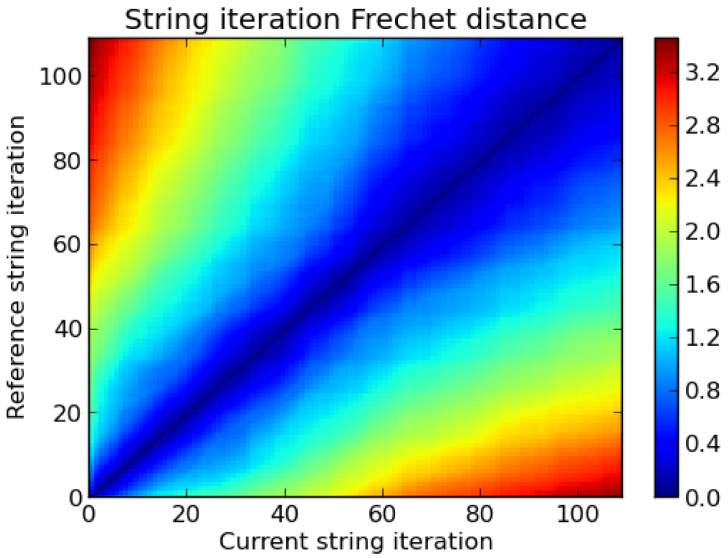
Convergence of string iterations

- Choice of CVs: Cartesian positions of all the C^{α} atoms in the cytoplasmic domains and M1-M6 helices. Several key side-chains in M1-M2 helices and in N, A and P domains. Total: 856 Cartesian positions

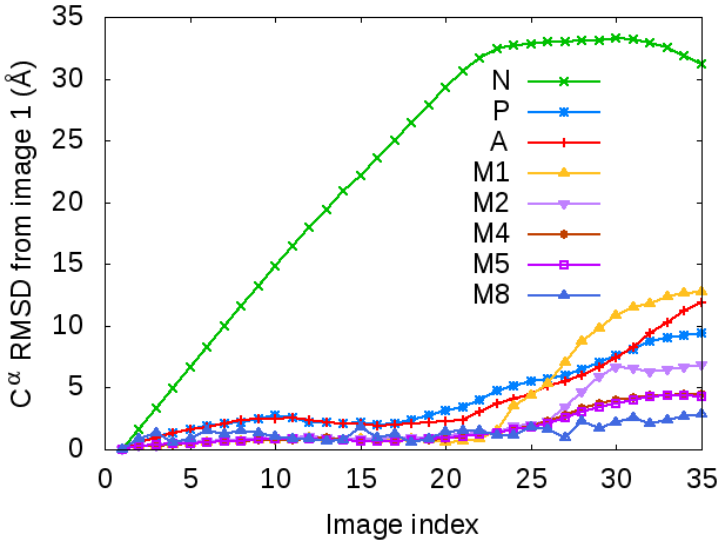
- 10 ps of swarm and 10 ps of constrained equilibration

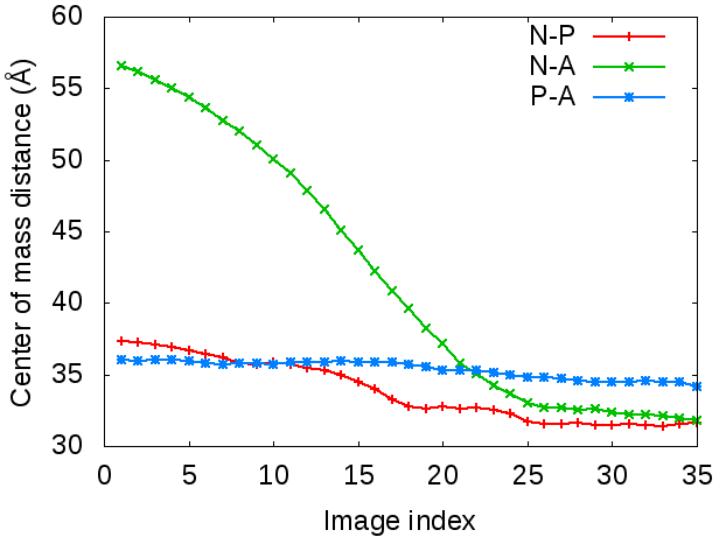
- 109 iterations

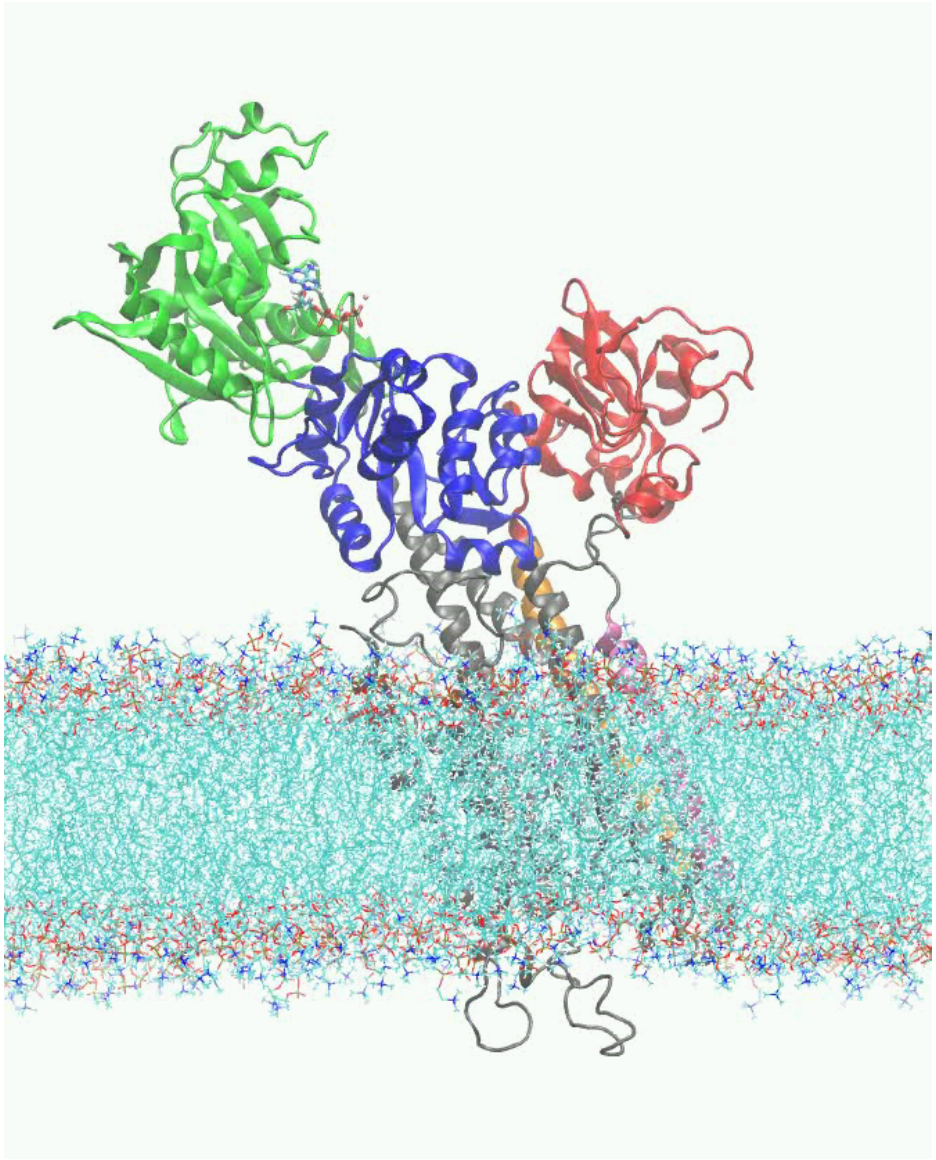




Results


35
30
25
20
15
10
5
0
0
5
10
15
20
25
30
35
Cα RMSD from image 1 (Å)
Image index
N
P
A
M1
M2
M4
M5
M8


60
55
50
45
40
35
30
0
5
10
15
20
25
30
35
Center of mass distance (Å)
Image index
N-P
N-A
P-A

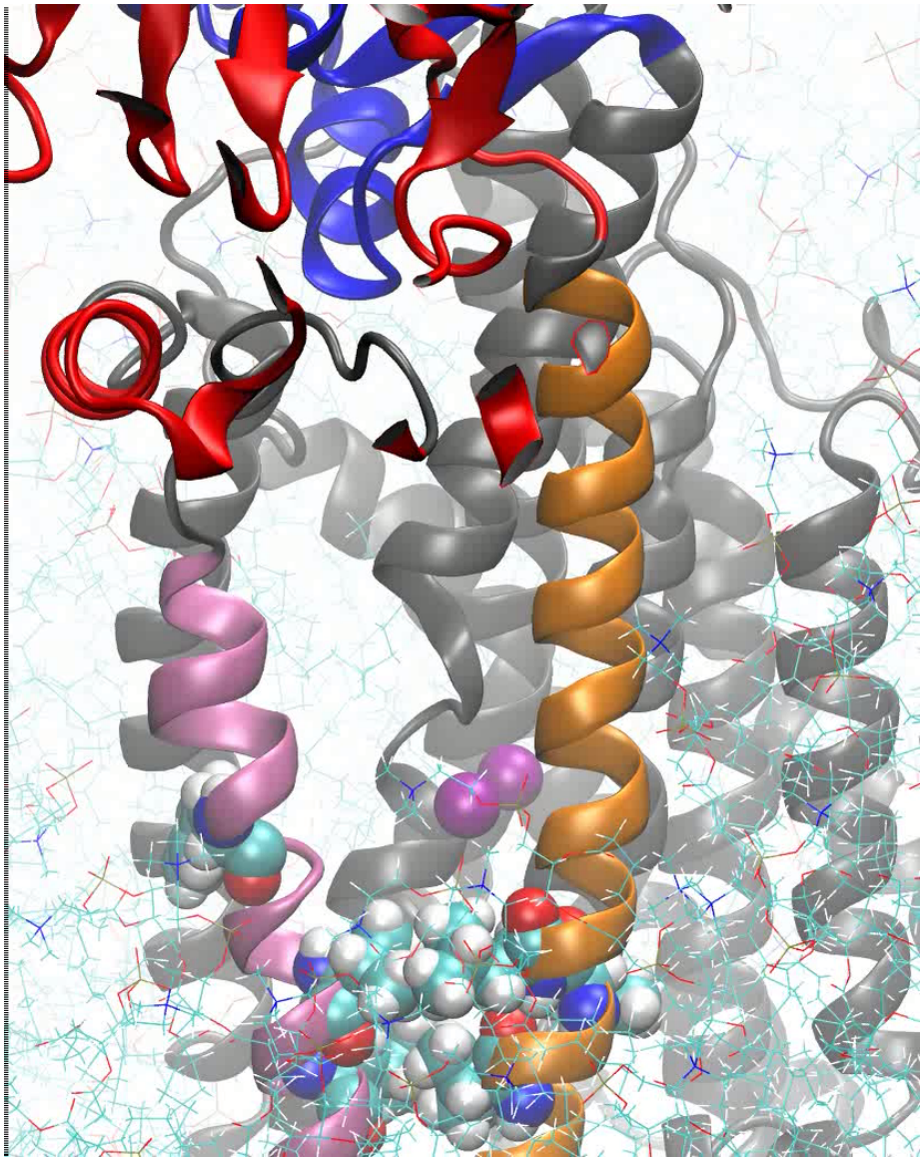


Result : role of key side chains

Due to upward movements of M1 and M2 and bending of M1 hydrophobic side chains block the ion pathway

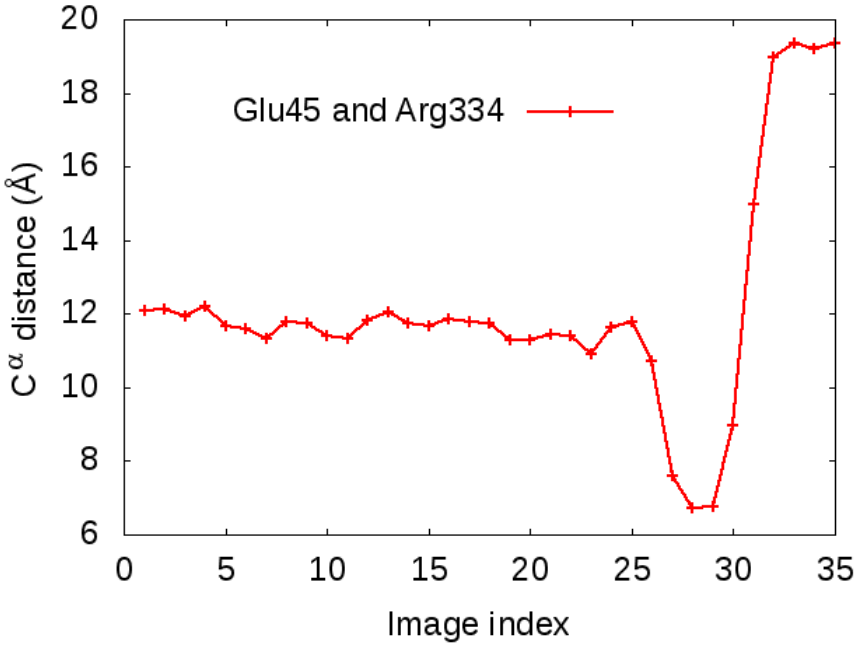
M1: Mauve
M2:Orange
Ca^{2+}: Purple spheres

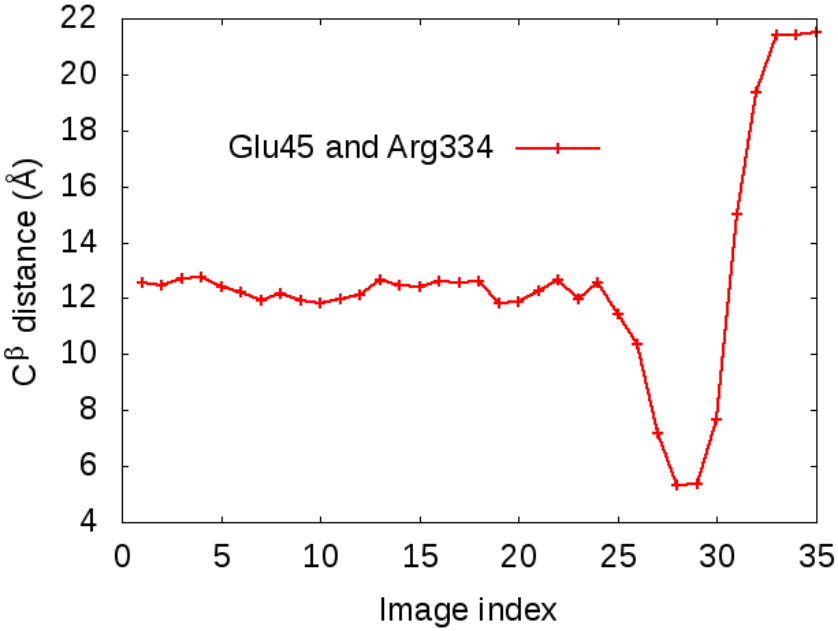
Space-filling representation : Phe57 Val62 Leu65 (M1); Ile94 Leu97 Leu98 (M2)



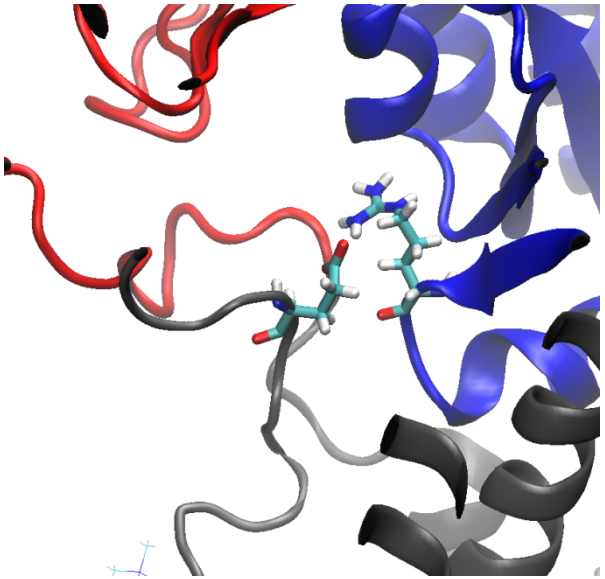
Prediction: formation of non-native contact

Non-native contact: Two residues that are far away in the end states but come close during the transition

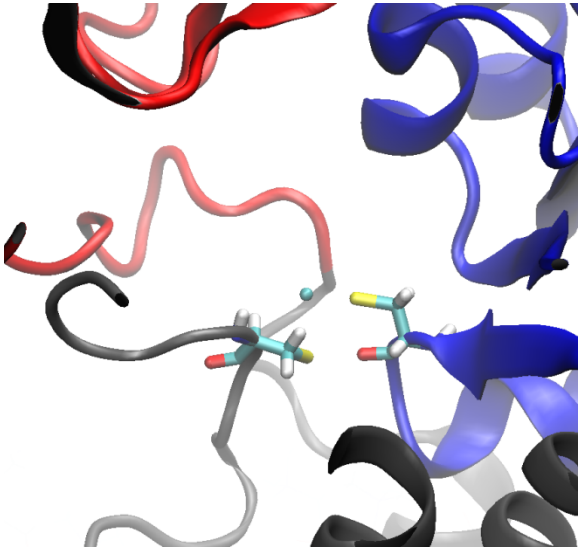




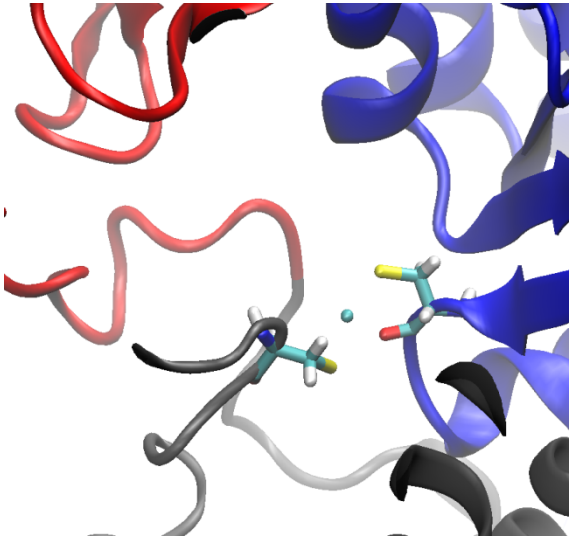
Probing non-native contact by metal bridge



Initial structure of closest contact



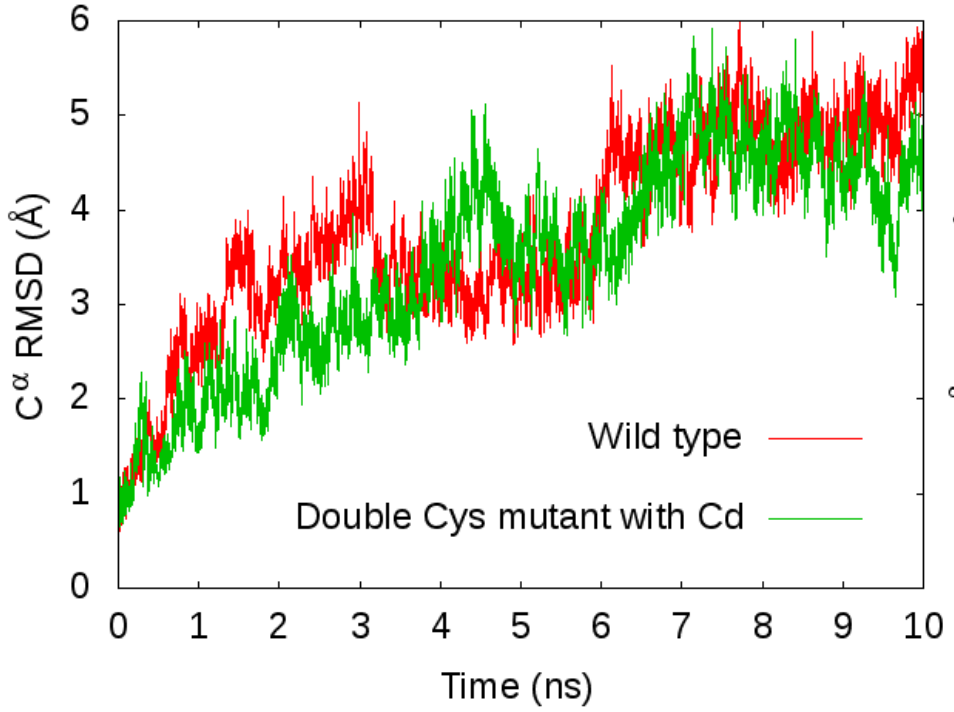
Mutation to negatively charged Cys and Cd^{2+} ion

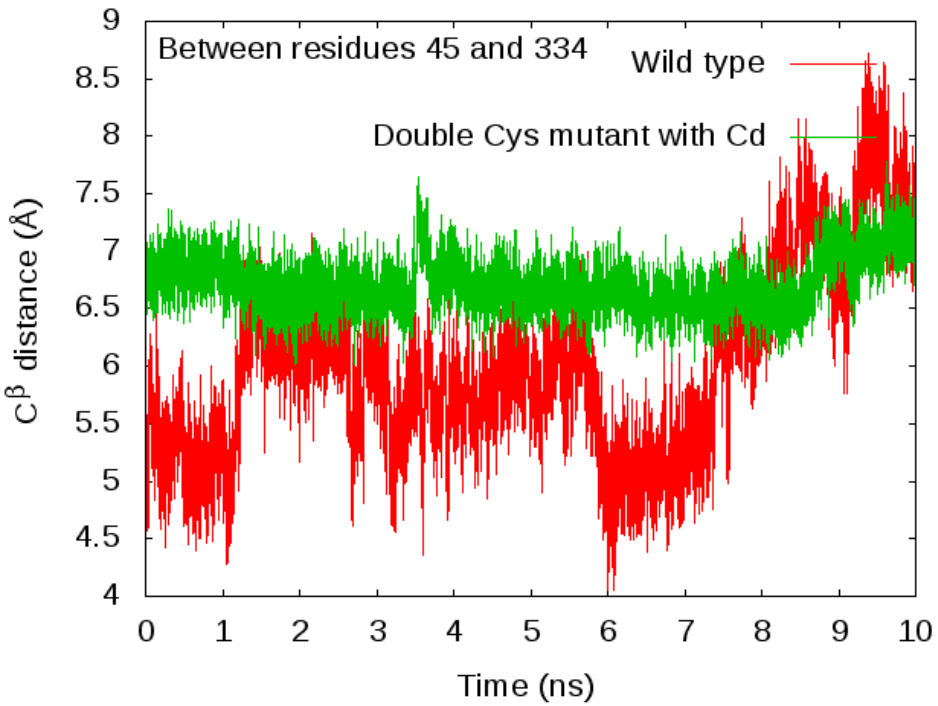


Metal bridge formation after restrained and free simulations

Probing non-native contact by metal bridge

Unbiased MD of wild type and double mutant with metal bridge





Conclusion

- We have simulated the conformational transition responsible for occlusion of Ca^{2+} ions
- The N domain moves freely at the initial stage of the transition which suggests that cytoplasmic domains can exist in more compact conformations in solution
- Mechanism: Large scale motions in the cytoplasmic domains cause the upward motions of M1 and M2 followed by bending of M1 which result in repositioning and flipping of key side chains
- Simulated pathway predicted formation of non-native contact which was probed by Cd bridge formation. Possible target for cross-linking experiment

Acknowledgement

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- Robert Brunner of NCSA/Blue Waters

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