Investigating the dissociation process and binding energy of the p53-DBD/DNA complex by PaCS-MD and MSM

Mohamed Marzouk[1,2] and Akio Kitao[1]
[1]Tokyo Institute of Technology, Tokyo, Japan
[2]Ain Shams University, Cairo, Egypt

Abstract

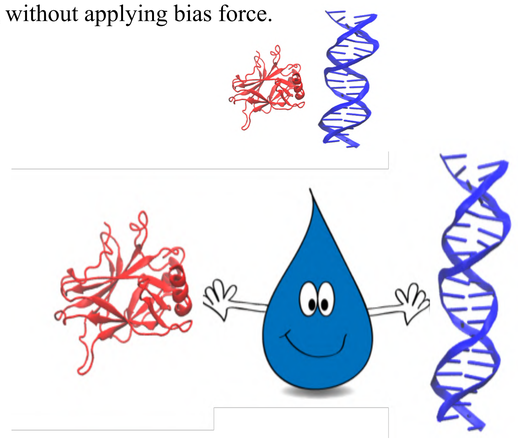
The binding of biological molecules to other molecules plays a crucial role in understanding their function, in mechanisms of diseases, and in the development of drug design. The binding of the p53 DNA binding domain (p53-DBD) to DNA is essential for the defensive role of p53 as a "guardian of the genome" against cancer. In the current study, we used PaCS-MD/MSM to study the dissociation pathways of p53-DBD. We used PaCS-MD to generate 75 dissociation pathways of the complex, which allowed us to investigate the key residues during the dissociation process. We built 3D MSM by vector coordinates, to generate free energy landscape of the dissociation of the complex. Our results showed free-energy-favorable directions during the dissociation.

1 Introduction

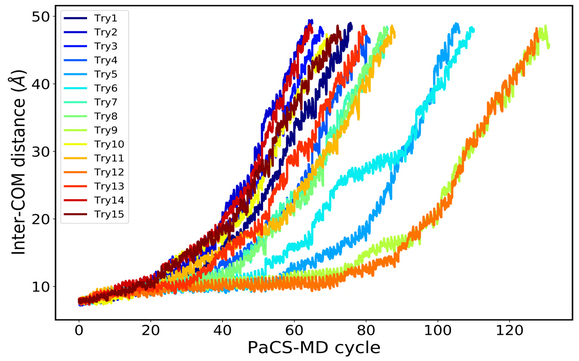
- p53 can induce expression of genes involved in many biological processes including cell cycle arrest, DNA repair, senescence, apoptosis, and metabolism in response of the cell to variety of stresses. Binding of p53 to specific DNA sequences is a mandatory step to activate gene expression.
- It is estimated that approximately 50% of human tumors contain mutations in p53. In fact, around 95% of p53 mutations occur in p53-DBD[1].
- It has been found that 30% of all p53-DBD mutations fall on six well known "hotspot" mutations. Two of these "hotspot" are contact mutations, i.e., R248 and R273, while four of these "hotspot" mutations are structural mutations R175, G245, R249, and R282.

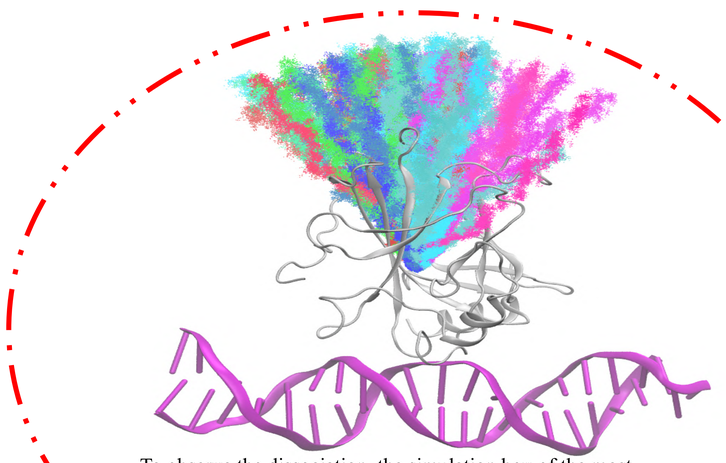
2 Dissociation by PaCS-MD

- A typical MD simulation can describe a variety of biological processes that take place on short timescales including confirmational change, ligand binding. However, crucial events such as protein-protein or protein-DNA binding/unbinding, most other protein folding events often take place on a much longer timescale and cannot generally be modeled through MD simulations. Therefore, in the last few years, a large variety of "enhanced sampling methods" have been developed which mainly speed up the description of the slow processes. Parallel cascade selection molecular dynamics (PaCS-MD)[2] is one of these approaches that enchance the samplinhg without applying bias force.

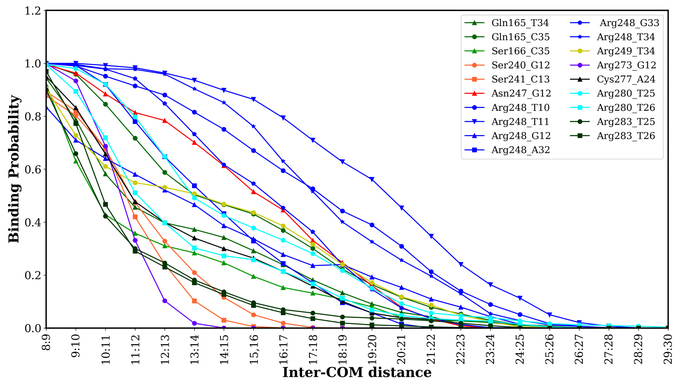


- PaCS-MD encompasses cycles of multiple independent parallel short MD simulations accompanied with criteria (here we use Inter center of mass distance, Inter-COM, as criteria for ranking the snapshots each cycle) for selecting initial structures of the next cycle. By repeating a series of cycles for the selected top ranked snapshots, PaCS-MD generates structures with a larger Inter-COM distance than those found in the previous cycle, which significantly enhances the probability of detecting the transition from bound to unbound state of the complex. The short simulation trajectories of one trial of a PaCS-MD simulation can be combined to generate a possible dissociation pathway which mutually overlaps in conformational space.





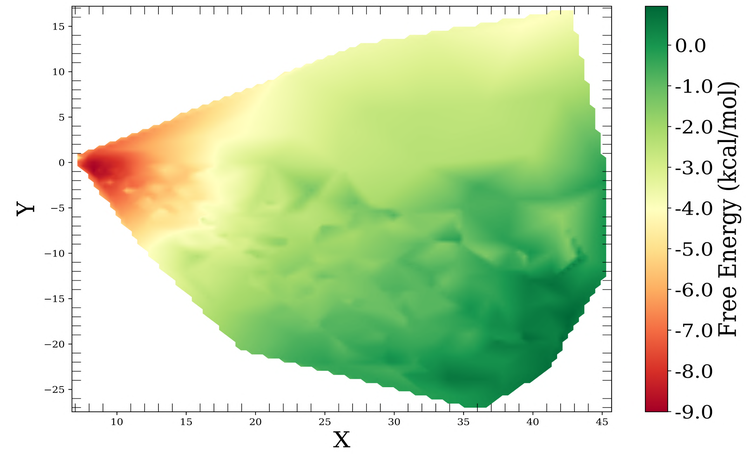
To observe the dissociation, the simulation box of the most populated cluster of the 1 μs conventional MD simulation extended, then PaCS-MD for five different conformations was ran, 15 trials for each one. Therefore, 75 dissociation pathways obtained.



To know the most crucial binding that keep the p53-DBD in bound state with p53, we calculated the probability of binding for 41 pairs detected as native contacts with threshold 3.5 Å. Only results of residue pairs that maintain binding more than 80% of time in the beginning of the dissociation are shown. The contacts of R248 with various DNA base pairs by hydrogen bonds and salt bridges have a curial role to keep the p53-DBD tightly bound to the DNA.

3 MSM

- The Markov state model (MSM) is widely used in computational biology as a technique that used to identifying stationary and kinetic information of protein dynamics for molecular dynamics simulation data. The MSM gives much awareness to transitions between metastable conformational states, which provide insights into pathways during dissociation in our case.
- We constructed the MSM from merged PaCS-MD trajectories of 75 trials using the three-dimensional COM coordinates between p53-DBD and DNA,3D-MSM.
- The obtained free energy landscape showed the most favorable dissociation pathway from the global minimum (here is the bound state) to the unbound state



4 Conclusion

- PaCS-MD is a powerful tool to enhance the sampling efficiency for rare events without applying any biasing force.
- The combination of PaCS-MD/MSM was shown to be useful in the investigation of dissociation pathways of a large protein/DNA complex and can assist to get the free energy landscape as well.

References

1- Braun, E. et al. Best Practices for Foundations in Molecular Simulations [Article v1.0]. Living J. Comput. Mol. Sci. 1, 1–28 (2019).

2- Harada, R. & Kitao, A. Parallel cascade selection molecular dynamics (PaCS-MD) to generate conformational transition pathway. J. Chem. Phys. 139, (2013).