UNRAVELING FUNCTIONAL HOLE HOPPING PATHWAYS IN THE [FE_4S_4]-CONTAINING DNA PRIMASE

Darius Teo, Duke University
2018–2019 Graduate Fellow

EXECUTIVE SUMMARY

This work has resulted in a Python module (EHPath.py) for characterizing charge hopping pathways in proteins and nucleic acids, which is the first computational tool that maps and ranks hopping pathways according to their mean residence time. The functionality of the module has been evaluated in several proteins including the oxygen-utilizing model enzyme cytochrome p450. Force field parameters that describe the high-potential iron–sulfur cluster have also been developed for molecular dynamics (MD) simulations. These two advances will enable the investigation of the role of an amino acid mutation found in gastric tumors in attenuating primase–DNA charge transfer and, in turn, primer handoff to polymerase α in DNA replication.

RESEARCH CHALLENGE

Recent work [1] suggests that primer handoff from the human DNA primase to polymerase α (Polα), as part of the lagging strand synthesis in DNA replication, is driven by charge (electron or hole) transfer and the modulation of the redox states of the high-potential iron–sulfur (*i.e.*, $[Fe_4S_4]^{2+/3+}$) clusters housed in the p58c and p180c domains of primase and Polα, respectively. Experiments have investigated the efficiency of charge transfer between $[Fe_4S_4]^{2+/3+}$ in wild-type/mutant p58c and the protein-bound RNA/DNA duplex. Both Y345C and Y345F mutations in primase were found to reduce $[Fe_4S_4]^{2+/3+}$-RNA/DNA charge transfer by approximately 60 to 95% [1]. The Y345C somatic mutation is of particular interest owing to its presence in gastric tumors [2].

In order to investigate the impact of mutation on charge transfer computationally, two challenges have to be met. First, a computational tool that can map and rank charge hopping pathways in proteins/nucleic acids has to be developed; this module can then be utilized to examine the hopping pathways between the RNA/DNA duplex and $[Fe_4S_4]^{2+/3+}$ in both the wild-type and mutant Y345C primase. Second, as there are no current force field parameters that can treat the high-potential $[Fe_4S_4]^{2+/3+}$ cluster, new parameters have to be developed. Once these two challenges are overcome, molecular dynamics (MD) simulations of the mutant and wild-type proteins can be performed; the trajectories obtained from these simulations would then help evaluate the possible attenuation of mutant primase binding to nucleic acid through Generalized Born/Poisson–Boltzmann methods, as well as the modulation of charge hopping pathways between the RNA/DNA duplex and $[Fe_4S_4]^{2+/3+}$.

METHODS & CODES

This work has resulted in a Python module (EHPath.py) that can map and rank charge hopping pathways in proteins/nucleic acids according to the mean residence time detailed in [3]. This code has also been used to evaluate hopping pathways in proteins of interest (manuscript submitted). The Python module is available at https://github.com/etransfer/EHPath. With regard to developing force field parameters to treat $[Fe_4S_4]^{2+/3+}$ for MD simulations, this work utilized broken-symmetry [4] density functional theory (BS–DFT) to optimize the geometries of the cluster in the two redox states (with relevant redox layer spin assignments), followed by force constant calculations using Seminario's method [5]. Existing Lennard–Jones 6–12 parameters were used for the MD simulations. Partial atomic charges for the cluster were also derived. The force field parameters were derived and tested for robustness in MD simulations.

RESULTS & IMPACT

High-potential Fe_4S_4 clusters (in the 2+/3+ oxidation states) are important because they are commonly found in enzymes related to DNA replication and repair, including DNA primase and Polα. Investigating the impact of the Y345C mutation in primase will advance the understanding of the primer handoff process driven by charge transfer as well as other cellular redox processes that are paramount in the regulation of major metabolic pathways. In addition, this will help inform the design of inhibitors that target such mutations. Furthermore, the newly developed force field parameters can be widely used by the research community for MD simulations of high-potential iron–sulfur cluster-containing proteins. Similarly, the Python module is available to the community for evaluating charge hopping pathways in proteins/nucleic acids that are relevant to other research areas.

WHY BLUE WATERS

The Blue Waters fellowship has greatly advanced the progress of this research—both in terms of time and computation. Access to the top-notch computational power of the Blue Waters supercomputer has been very useful for running and completing BS–DFT and Hessian DFT calculations efficiently. The Blue Waters Point of Contact has also provided advice and engaged in helpful discussions through email exchanges and in-person meetings.

PUBLICATIONS & DATA SETS

R. D. Teo, E. R. Smithwick, A. Migliore, and D. N. Beratan, "A single AT–GC exchange can modulate charge transfer-induced p53–DNA dissociation," *Chem. Commun.*, vol. 55, no. 2, pp. 206–209, Nov. 2018, doi: 10.1039/C8CC09048C.

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R. D. Teo, E. R. Smithwick, and A. Migliore, "2'-Deoxy-2'-fluoro-arabinonucleic acid: a valid alternative to DNA for biotechnological applications using charge transport," *Phys. Chem. Chem. Phys.*, vol. 21, no. 41, pp. 22869–22878, Nov. 2019, doi: 10.1039/C9CP04805G.

Darius Teo is in the third year of a Ph.D. program in chemistry, working under the direction of David N. Beratan at Duke University. He expects to graduate in May 2020.