New research applies algorithms to help explain how homodimeric enzyme breaks one of the strongest chemical bonds found in nature

Recent research findings published by biochemistry groups from University of Toronto together with Adnan Sljoka of Kwansei Gakuin University, who has developed methods and algorithms founded in the area of mathematical rigidity theory with applications to protein structures have shed light on the role of protein dynamics and distant signal propagation (known as “allostery”) as key facets in functional control of an important bacterial enzyme fluoracetate dehalogenase. Their findings appeared in the January 20, 2017 issue of journal Science, in the research article “The role of dimer asymmetry and protomer dynamics in enzyme catalysis” (see full article here http://science.sciencemag.org/content/355/6322/eaag2355).

Background:

Almost all biological processes in the cell require enzymes which ensure that chemical reactions occur at specific rates crucial for biological life. Without enzymes, reactions (for instance in respiration and photosynthesis) would be too slow to maintain life. These fascinating biological machines (in most cases proteins) provide us with many difficult questions and depict a major scientific mystery, since the mechanism of their catalytic power still remains unresolved.

Over the last 50 years, a number of experimental approaches have been used in attempt to describe the mechanism of enzyme catalysis. Nuclear Magnetic Resonance (NMR) experiments have shown that enzymes are not static, but rather dynamic, enabling the them to sample many conformational states. Computational techniques such as Molecular Dynamics (MD) simulations, those founded in mathematical rigidity theory and others, have over the past 20 years also opened up many exciting opportunities for modelling protein dynamics.

A popular hypothesis is that dynamical effects play a central role in enzyme catalysis. Despite tremendous efforts, there has previously been no thorough experimental validation in how dynamics affect catalysis. One way dynamical changes are manifested in proteins is through allosteric effects, where a ligand or drug binding event at one part of the protein can cause changes in conformation and dynamics at other remote parts of the protein. Allostery, which has been coined as “a second secret of life” is essential to most biological functions, but the underlying mechanism of long-distance signal propagation is poorly understood.

Allostery and protein dynamics is of particular interest to Prof. Adnan Sljoka of Kwansei Gakuin University, also a Crest Member. Dr. Sljoka had previously developed algorithms founded in the area of mathematical rigidity theory applied to protein structures, particularly the Rigidity Transmission Allostery (RTA) method for analyzing and predicting the elusive allostery, in addition to a number of other computational biology techniques which aim to tackle difficult problems concerning protein dynamics and its function (see for example Sljoka et al article in Plos One, 2015 on the role of algorithms and computational biology in understanding key protein Tau involved in several neurodegenerative diseases). Some of Dr. Sljoka’s methods have recently attracted interest in Alzheimer’s disease research and drug development.

Main Achievements:

Research groups in biochemistry from University of Toronto, Dr. Sljoka and other researchers have studied how dynamics and allostery play an important role in bacterial enzyme fluoracetate dehalogenase; a bacterial homodimeric (composed of a pair of identical subunits) enzyme that breaks one of the strongest chemical bonds (carbon-fluorine). Biochemistry team performed X-Ray Crystallography experiments which provided a number of high resolution pictures of the enzyme's structure together with Nuclear Magnetic Resonance (NMR) which describes the dynamical changes that the enzyme undergoes during different stages of enzyme catalysis. Biochemical data had demonstrated cooperativity between the two subunits as crucial component of enzyme catalysis which occurs on longer millisecond time scale motions. The enzyme functions as a dimer but binding of a substrate only occurs in one subunit, while the second subunit remains empty which was shown to be critical for enzyme catalysis. The experiments have also demonstrated water molecules play an important role in the catalysis process.

Computationally, protein dynamics can be investigated with MD simulations, however due to the heavy computational cost, MD simulation is not widely applicable. It is nearly impossible to validate the functionally important longer time scale motions with MD simulations, especially those motions arising due to allosteric transmissions. To deal with this computational bottleneck, rigidity theoretical analysis using the RTA method were utilized to validate the experimental evidence that two subunits in enzyme are in allosteric communication. RTA analysis demonstrated the presence of physical allosteric pathways in the protein which are responsible in transmitting the allosteric signals between the two subunits. Moreover, the RTA method provided deep insights about energetic nature of allostery which highlighted key flexibility differences in allosteric communication in the enzyme during various stages of catalysis.

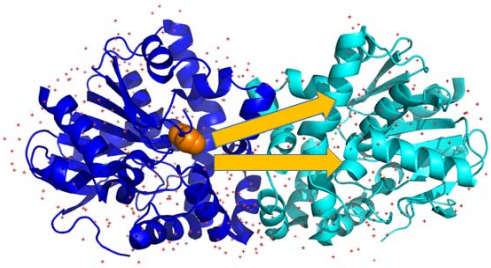

Figure: Fluoroacetate dehalogenase enzyme with substrate fluoracetate molecule (shown as orange spheres) and water in red dots exhibits allosteric communication between the two subunits (shown in distinct colours).

Significance of the result:

This paper has received positive response in the biochemistry community. In the same Science issue, Saleh and Kalodimos reviewed this work in the Perspective article "Enzymes at work are enzymes in motion" (http://science.sciencemag.org/content/355/6322/247), where they assert "Kim et al.'s study provides remarkable progress toward understanding the full range of functional mechanisms used by enzymes to achieve their catalytic power". This breakthrough research is expected to pave further research avenues that shed light on how enzymes work and

the role of allosteric communication in control of enzymes. It remains to be seen if other dimeric enzymes use similar mechanism for catalysis and the importance of water-protein interactions. Moreover, the techniques and methods inspired by rigidity theory, such as the RTA method, have exemplified how mathematical algorithms can be powerful tools to better understand the fundamental biological questions pertaining to protein function which are vital to understanding secrets of living organisms at the atomic level.