Revealing key processes in enzyme efficiency through high performance computing

Davide Mercadante, School of Chemical Sciences, University of Auckland

Processivity is a word used to define the ability that some enzymes have to catalyze several reactions in a single binding cycle to their substrate. In other words, an enzyme binds its substrate and does not dissociate from it until more than one modification to it has been performed. Usually, processivity is exerted on substrates that have a linear conformation such as polymeric chains of DNA or polysaccharides, which need to be chemically modified in order to prompt a function.

In the plant world, there are many examples of substrates as long polymeric chains: for example the polysaccharides that are embedded in the plant cell wall, which need processive enzymatic activity in order to be dynamically re-arranged. Interestingly, plant pathogen bacteria also express enzymes that potentially have a processive activity on plant cell wall polysaccharides. One of the most interesting class of these enzymes are the bacterial Pectin methylesterases (PME), which catalyze the conversion of pectin linear regions from highly methylesterified to lowly methylesterified chains. By doing this, the chemical and physical properties of the plant cell wall are modified in a way that favors the breach of the plant cell by the pathogen.

Through means of Molecular Dynamics simulations carried out using the NeSI Pan High Performance Computing (HPC) cluster, I investigated the ability of a bacterial PME, expressed by the plant pathogen *Erwinia chrysanthemi*, to slide along pectin chains after each reaction cycle. Molecular Dynamics simulations represent a gold standard in the study of molecular motions through the application of principles that describe molecular mechanics.

The study revealed for the first time the mechanism, at an atomic level, by which the protein accomplishes a processive run along the polysaccharide. It was possible to solve the puzzling problem posed by the conformation of each monosaccharide along the chain. Indeed, the functional groups that are processed by the enzyme are alternately-facing conformations suggesting that a simple sliding of the enzyme along the chain would not be sufficient to assure any processive catalysis (Figure 1).

Simulations revealed that after the first catalytic event the conformational freedom of the substrate is locally enhanced by the electrostatic repulsion between the enzyme and the freshly generated product of reaction. This repulsion promotes a rotation of the functional groups along the polysaccharides so that a new group, susceptible to catalysis, can be approached by the enzyme (Figure 2). The above described study was useful to sample both the phenomenon of monosaccharide rotations around the glycosidic bond and the subsequent sliding of the enzyme along the substrate.

These findings are remarkable with respect to the mechanism adopted by these enzymes and give insights into a strategy for processive catalysis that does not need the hydrolysis of any externally available high-energy co-factor. Instead it uses the potential already embedded in the product of reaction to yield the energy useful to catalyze more reactions along the polymer. Moreover, on a point of view more closely related to the investigated system, the discovery of this mechanism provides new evidence of how the plant cell wall is dynamically modified to favor physiological process in plants and how the infection of plant hosts by bacteria is efficiently carried out.

The sampling of substrate conformational variations crucial to bacterial infection of plant hosts will be of great help to develop new compounds able to modify pathogen defense responses in plant cells.
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Molecular Dynamics simulations compute the motions of particles (which can be part of a biological or a non-biological system) by integrating, at each time-step, Newton’s equation of motion. In this equation the force acting between two particles is described as proportional to the mass of the particles and their acceleration at each time-step.

\[ F = m \ddot{a} \]

It is interesting to see that this is a very simple equation that, given all the parameters, can be easily solved by any simple calculator. Nevertheless, proteins and whatever surrounds them (water, ions, membranes or some other biological structures) are constituted by hundreds of thousands of particles (sometimes millions) that exert forces over both short (these are Lennard-Jones, hydrogen bonding and hydrophobic forces) and long-range (electrostatic forces). Computing all the forces at each time-step for hundreds of thousands or sometimes millions of particles is not an easy task although the equations involved are fairly simple. Scaling the calculations for some defined subsets of particles across different CPUs of multiple-core computers can considerably speed up the Molecular Dynamics simulations.

The simulations were carried out on the Pan cluster using GROMACS 4.5.5 software as the Molecular Dynamics engine. The high scaling performances of the hardware, in association to an appropriate compilation of the software relative to the cluster architecture, were fundamental in achieving the results as the conformational variations described above do not only belong to large molecules but occur on timescales for which Newton’s equation needs to be integrated thousands of times. Thanks to the high computing power provided by the New Zealand e-Science infrastructure (NeSI) simulations were highly parallelized. In addition, with the available general parallelized file-system (GPFS), the collection of data after computation on the required CPU cores was remarkably sped up. These advances have lowered the barrier between the integration of the equations to be solved and the writing the calculated results on the file-system - previously a bottleneck for large systems.

The results of the study have been published and featured on the cover of Biophysical Journal - a high-impact factor journal in the field of biophysics and computational or experimental structural biology. Such results are also part of my successfully completed PhD in Chemistry at the University of Auckland. Moreover, part of the results obtained from the collected trajectories calculated using the Pan cluster has been submitted and is under revision in the journal PLoS Computational Biology.

What's next?
The identification of the conformational variations characterizing the substrate during the processive activity of the investigated plant pathogen enzyme are of enormous importance for the design of a compound that is able to block the pathogenic activity of the organism. Indeed, starting from the conformations of intermediates between reaction cycles, it will be possible to create analogues to the substrate that are able to impede the conversion of substrate to product or, on the other hand, the ability of the protein to efficiently act on the substrate by blocking the sliding along the polymer. Moreover, the identification of this mechanism for a plant pathogenic enzyme opens questions about whether plants are able to process plant cell wall polysaccharides in this way or if there are different mechanisms underlying the fine regulation of plant cell wall composition during different plant life stages. Future simulations will be carried out partially on the Pan cluster for a higher scalability of the software and partially on the high performance supercomputing center located at JUPLICH in Germany. At the Heidelberg Institute of Theoretical Studies (HITS) in Heidelberg, I am continuing the research that I started during my PhD at the University of Auckland.

Contact Details:
Dr. Davide Mercadante
Postdoctoral Research Fellow
Heidelberger Institute of Theoretical Studies (HITS), Heidelberg – Germany.
e-Mail: davide.mercadante@h-its.org
Phone: +49 (0)6221 – 533 – 276
Fax: +49 (0)6221 – 533 – 298
Webpage on HITS: http://www.h-its.org/english/research/davide_mercadante

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