Computing Ligand Release Pathway Efficiency: A Comparison between Robotics Approach and Molecular Dynamics Simulations

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Enzyme activity and flexibility are intimately connected. Notably, enzymes dynamics contribute not only to catalysis, but also to ligand specificity and affinity (Falke, 2002). An *in silico* tool that can predict quickly dynamic properties into enzymes responsible for efficient substrate binding or product release would be very useful for driving further experimental or modeling studies. We have developed a new approach based on robotics motion planning, which is dedicated to determine if a specific motion is feasible with respect of geometrical constraints into the system enzyme-ligand. Our approach can compute simultaneously small to large protein motion (like catalytic groups, loop or domain movement) and ligand trajectories through deep cavities in enzymes (Cortes *et al.*, 2005).

We present an application of our robotics approach on the release of xylosetriose from GH-11 xylanase, a highly substrate specific enzyme. GH-11 xylanase possesses a thumb shaped loop, characterized by an opening and closing position that can modify the shape of the catalytic site. Robotic calculations coupled with geometric analysis were carried out to simplify large loop movements and to model the product release pathway out of the active site (Cortes *et al.*, 2003). Nevertheless, energetic terms are missing, and might induce an approximation less or more important on predicted pathways or protein deformations.

To gain insight on the accuracy of our robotics approach, we have compared trajectories issued from robotic and molecular dynamics (MD) simulations. We have performed 10 ns MD runs on GH-11 xylanase / xylosetriose with Gromacs software (Van Der Spoel *et al.*, 2005) using an explicit representation of solvent. We have observed a complete product release and a large opening loop motion. Pathways obtained with these two methods are globally similar, which enhances the ability of our robotics approach to provide satisfying estimations of dynamics trajectories. Finally, we show that our new approach can help in understanding how mutations in enzymes can modify the efficiency of product release. Experimental results reinforce our predictions (Paës *et al.*, 2007).

Cortes J, Simeon T, Remaud-Simeon M and Tran V. Geometric algorithms for the conformational analysis of long protein loops. J. Comput. Chem. 25(7):956-967 (2004).

Cortés J, Siméon T, Ruiz de Angulo V, Guieysse D, Remaud-Siméon M and Tran V. A path planning approach for computing large-amplitude motions of flexible molecules. Bioinformatics. 21(Suppl1):i116-25 (2005).

Paës G, Tran V, Takahashi M, Boukari I and O'Donohue MJ. New insights into the role of the thumb-like loop in GH-11 xylanases. Protein Eng Des Sel. 20(1):15-23 (2007).

Van Der Spoel D, Lindahl E, Hess B, Groenhof G, Mark AE and Berendsen HJ. GROMACS: fast, flexible, and free. J Comput Chem. 26(16):1701-18. (2005)

Falke JJ. Enzymology. A moving story. Science. 22;295(5559):1480-1 (2002).