

Mechanistic insight into the catalytic activity of $\beta\beta\alpha$ -metallonucleases from computer simulations: *Vibrio vulnificus* periplasmic nuclease as a test case

Bueren-Calabuig JA^{1,2}, Coderch C¹, Rico E³, Jiménez-Ruiz A³, Gago F¹.

¹ Department of Pharmacology, Universidad de Alcalá, E-28871 Alcalá de Henares, Madrid, Spain; E-mail: juan.bueren@edu.uah.es

² Present address: Department of Chemistry and Quantum Theory Project, University of Florida, Gainesville, Florida 32611-8435, United States.

³ Department of Biochemistry and Molecular Biology, Universidad de Alcalá, E-28871 Alcalá de Henares, Madrid, Spain.

Using information from wild-type and mutant *Vibrio vulnificus* nuclease (Vvn) [1-2] and I-PpoI homing endonuclease [3] co-crystallized with different oligodeoxynucleotides, we have built the complex of Vvn with a DNA octamer and carried out a series of simulations to dissect the catalytic mechanism of this metallonuclease in a stepwise fashion. The distinct roles played in the reaction by individual active site residues, the metal cation and water molecules have been clarified by using a combination of classical molecular dynamics simulations and quantum mechanical calculations. Our results strongly support the most parsimonious catalytic mechanism, namely one in which a single water molecule from bulk solvent is used to cleave the phosphodiester bond and protonate the 3'-hydroxylate leaving group [4].

[1] C.L. Li, L.I. Hor, Z.F. Chang, L.C. Tsai, W.Z. Yang, H.S. Yuan, EMBO J. 2003, 22, 4014–4025.

[2] Y. T. Wang, W. J. Yang, C. L. Li, L. G. Doudeva, H. S. Yuan, Nucleic Acids Res. 2007, 35, 584–594.

[3] E. A. Galburt, B. Chevalier, W. Tang, M. S. Jurica, K. E. Flick, R. J. Mon-nat, Jr., B. L. Stoddard, Nat. Struct. Biol. 1999, 6, 1096 – 1099.

[4] Bueren-Calabuig JA, Coderch C, Rico E, Jiménez-Ruiz A, Gago F. Chembiochem. 2011, 12, 2615-2622.

