Complex energy landscapes revealed: The phosphorylation reaction in protein kinase A

Phosphorylation reactions are ubiquitous biological processes. They are involved in fundamental processes such as signal transduction, cellular regulation, metabolic pathways, and gene transcription. Consequently, protein kinases, the enzymes catalysing phosphorylation reactions, are among the most widespread enzymes with about 2% of all eukaryotic genes encoding them. Due to their presence in almost any animal cell and the many roles they play in physiological processes, kinases are involved in numerous pathologies. Thus, there is pharmaceutical interest in designing highly specific inhibitors. Protein kinases have a highly conserved catalytic core in common, suggesting the existence of a common underlying reaction mechanism. Scientists from Daresbury Laboratory and Universitat Autònoma de Barcelona, Spain are collaborating to unravel this fundamental biochemical puzzle.

Protein kinases catalyse the transfer of phosphate from adenosine triphosphate (ATP) to specific residues of their substrate, serines, threonines, or tyrosines. Figure 1 shows a reaction scheme. We studied cyclic-Adenosine Monophosphate (cAMP) Dependent protein kinase A (PKA). It is the most rigorously investigated member of the serine/threonine kinase family.

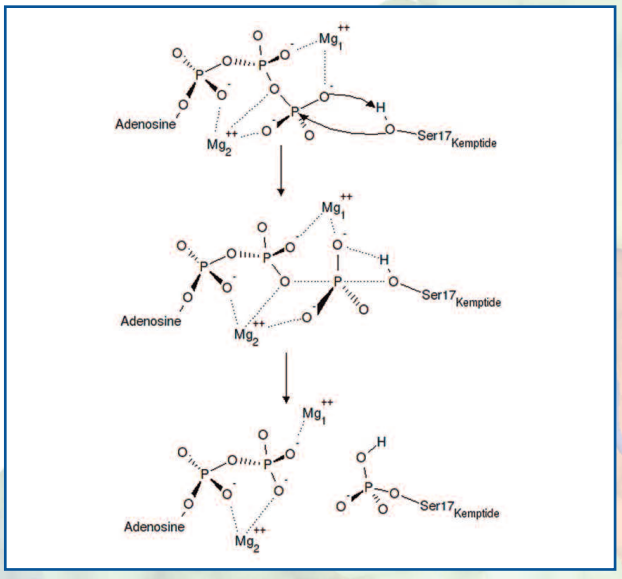

Figure 1: Schematic drawing of the phosphorylation reaction catalysed by PKA

The inactive enzyme is a tetramer consisting of two regulatory subunits and two catalytic subunits. Following activation by cAMP, the catalytic subunits (350 amino acids) become dissociated and work as independent monomers. The shape of the catalytic subunit and its substrate binding site are shown in Figure 2. The crystal structure [1] shows binding sites for cofactors and the substrate. The cofactors are one ATP molecule, which provides the phosphate when being transformed to adenosine diphosphate (ADP), two magnesium ions whose exact role in the process is still under investigation, and three catalytic water molecules. The active site of PKA recognises a specific pattern in the protein sequence of the substrate: Arg-Arg-X-P-Z, where Arg is arginine, X is a small residue, P is the phosphorylation site (serine or threonine), and Z is a large hydrophobic residue. We used the small synthetic substrate Kemptide as it has been extensively studied experimentally.

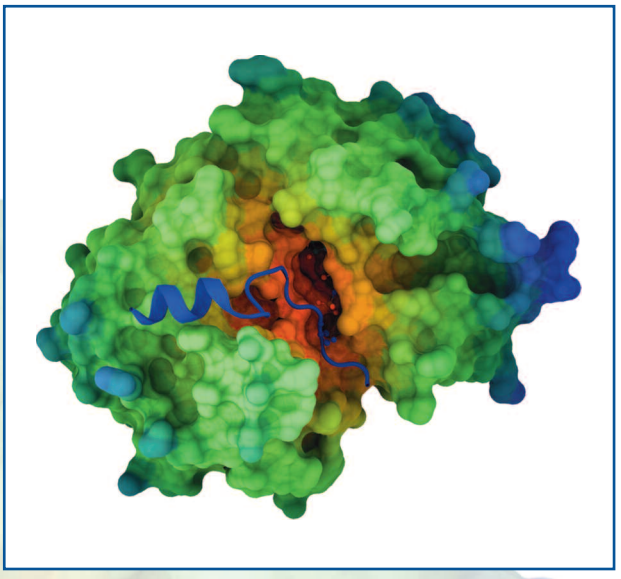

Figure 2: The active catalytic subunit of Protein Kinase A (surface representation), a substrate (blue ribbon) and ATP (ball-and-stick). Coordinates obtained from PDB entry 1CDK [1].

In order to shed light on the reaction mechanism, we described the protein with the classical CHARmm force field. After 6 nanoseconds of molecular dynamics (MD) to establish the structure at finite temperature [2], we used QM/MM to describe the chemical reaction. The MD simulation showed transitions between an open configuration and a closed configuration indicating the possibility of two distinct reaction mechanisms [2], see Figure 3. In the following we will only discuss the closed configuration, which leads to an associative mechanism. We used the Charmm program interfaced to MNDO97, the CHARmm-GAMESS(UK) interface, and the ChemShell code for the QM/MM simulations. While most of the calculations to date were carried out with the semiempirical AM1/d

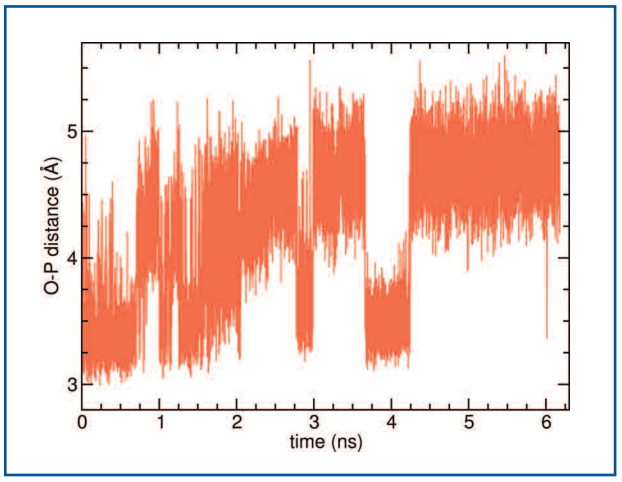

Figure 3: The O–P distance shows transitions during the molecular dynamics run between two possible reactant structures for a mechanism (short and long O–P distances).

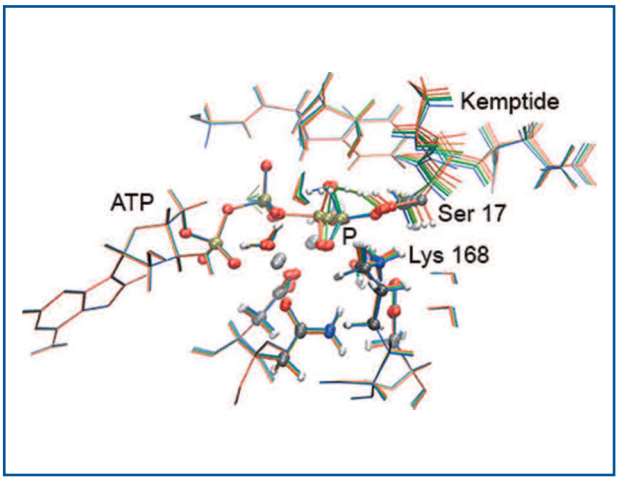

Figure 4: QM/MM reaction path obtained with the nudged-elastic band method as implemented in DL-FIND. Atoms as balls indicate the QM part. Bonds are coloured according to the reaction path from red (reactants) to blue (products). Besides the phosphate (P) and the substrate (Kemptide and Ser 17 which is part of it), Lys 168 and two water molecules play an important role in the reaction.

Hamiltonian as the QM method, one of us (Manuel Montenegro) spent three months in a collaborative visit at Daresbury Laboratory to get started on QM/MM with higher-level quantum chemistry methods. We are now investigating the potential energy surface based on Density Functional Theory (B3LYP).

The traditional way of estimating reaction barriers in large systems is to find a suitable reaction coordinate and to drive the system stepwise over the barrier by fixing the reaction coordinate while optimising all other degrees of freedom. While this method is likely to provide a reasonable estimate of the barrier when converged (i.e. when the resulting profile is smooth), the choice of the reaction coordinate is ambiguous. We tried a number of combinations of bond lengths (up to seven combined into a single reaction coordinate).

Using the ChemShell code, we applied the more rigorous NEB method as implemented in DL-FIND (see Frontiers 2007) to calculate the reaction path of an associative mechanism. While the results still depend on the starting conditions, they are independent of a manual choice of the reaction coordinate. The transition state resulting from the NEB path was refined with the dimer method. In both the NEB calculations as well as the dimer calculations, about 13 500 degrees of freedom were optimised. This would impede traditional mode-following methods. The resulting path is depicted in Fig. 4. One can clearly see the phosphate group being inverted in a classical S_N2 reaction. One proton is transferred from serine to the phosphate. The whole Kemptide substrate moves closer to the ATP cofactor. The amine group of Lys 168 provides a hydrogen bond to oxygen of serine mainly in the transition state, thus specifically stabilising the transition state. The large number of atoms involved in the transition mode led to a rather low imaginary frequency of the transition of 120 cm^{-1}.

Having found the most probable reaction mechanism we will now assess its relevance by simulating deactivated mutants known from experiment. If phosphotyrosine 197 is replaced by alanine, the system is known to be inactive. We will calculate the binding mode in this mutant and compare the reaction barrier to the wild-type enzyme.

Recent progress in optimisation algorithms allowed us to reliably calculate reaction barriers in systems as large as PKA. DL-FIND, the optimiser used to do the NEB and dimer method optimisations, is freely available under the L-GPL licence from http://ccpforge.cse.rl.ac.uk/projects/dl-find/.

M Montenegro, M Garcia-Viloca, À González-Lafont, JM Lluch.

Institut de Biotecnologia i de Biomedicina, Departament de Química, Universitat Autònoma de Barcelona, Spain

J Kästner, JMH Thomas, HJJ van Dam, and P Sherwood

CSED, Daresbury Laboratory

References:

[1] D Bossemeyer, RA Engh, V Kinzel, H Ponstingl, R Huber EMBO J. ***12****, 849 (1993)*

[2] M Montenegro, M Garcia-Viloca, À González-Lafont, JM Lluch J. Comput. Aided Mol. Des. ***21****, 603 (2007)*