# Protein Kinase A - inhibitor complex X-ray structures reveal major binding modes

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Protein kinases are key enzymes in signal transduction controlling the majority of all regulatory events in the eukaryotic cell. cAMP dependent protein kinase (or protein kinase A—PKA) is the prototype of the protein kinase family, a major target class for the design of cancer drugs. Protein kinase inhibitors are also highly useful in biological research exploring signal transduction pathways. Numerous studies of PKA/inhibitor complex structures have shown varying binding modes, but even for this single kinase, the full range of structural flexibility available to PKA is not known. In addition, successful therapeutic inhibitors will need to be selective for their target(s), but to a degree that is yet unknown. Selectivity is also crucial for inhibitors used for biochemical research associating cellular events with an individual protein kinase.

Studies of inhibitor complexes with mutants of PKA can provide a wealth of information regarding mechanisms to achieve protein kinase inhibitor selectivity, especially because PKA and mutants can readily be crystallized. The goal of our work is to determine multiple high resolution structures of inhibitor complexes with PKA and PKA mutants. Analysis of these structures combined with inhibition kinetic data will significantly enhance our understanding of protein kinase/inhibitor interactions essential for selectivity.

Protein kinase C was identified early as a potential cancer target. Various PKC inhibitors have been biochemically characterised, but not structurally. Due to the lack of a crystallisable PKC derivative, we used a PKA mutant to crystallize the PKC-inhibitor bisindolyl-maleimide 2 (Bim2). The resulting orthorhombic crystal contains a dimer in the asymmetric unit with two different conformations both of protein and inhibitor. The protein conformation of the molecule A is comparable to the staurosporine structure (1STC), whereas the molecule B is in the most open conformation of PKA crystallised up to now. The Bim2 inhibitor bound to the partially open conformation of molecule A has a highly complementary fit in the structure und shows significant

differences in binding in comparison to the planar staurosporine. Bim2 in molecule B binds in an opposite orientation in the catalytic cleft of the widely open kinase. Modelling of the clinical phase III inhibitor LY333531 into the electron density of Bim2 reveals interesting features of the probable binding mechanism. They can be explained by the differences of the catalytic site of PKC and PKA and by certain properties of the chemical structure of the inhibitor.

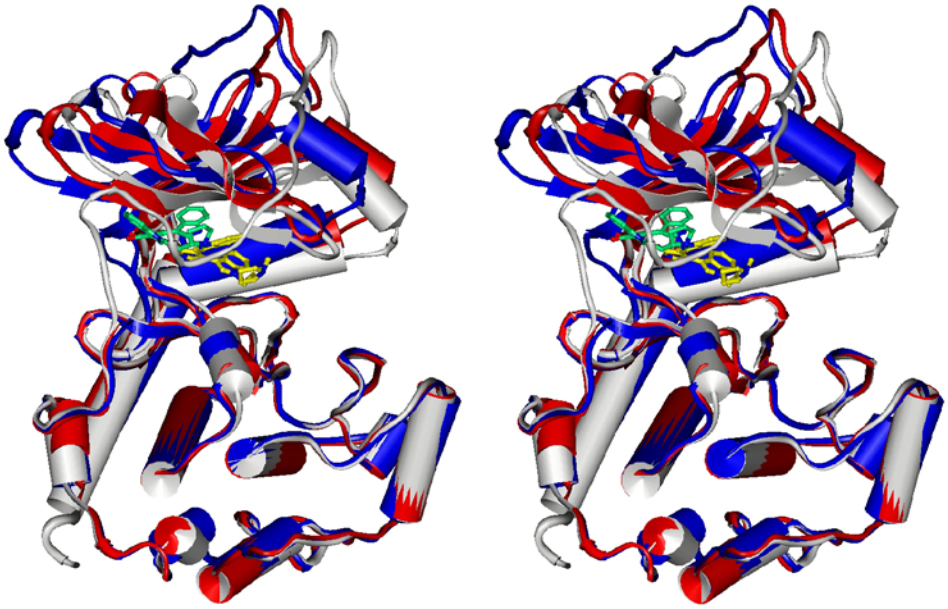

Figure 1: Crystal structure of a protein kinase A active site mutant in complex with the protein kinase C inhibitor bisindolyl-maleimide reveals two different conformations of the two molecules (including different inhibitor binding modes) in the asymmetric unit. The figure shows an overlay of the two molecules with 1CDK.

## References


[1] Gaßel, M., Breitenlechner C., Engh, R.A., and Bossemeyer, D. Crystal Structure of an Altered Catalytic Subunit of cAMP-dependent Protein Kinase in Complex with the PKC-Kinase Inhibitor Bisindolylmaleimide 2 in Two Different Conformations – Implications for Selectivity. Cellular & Molecular Biology Letters 8, 2A, p. 583 (2003)

[2] Michael Gaßel, Christine Breitenlechner, Norbert König, Robert Huber, Richard A. Engh and Dirk Bossemeyer, The Protein Kinase C Inhibitor Bisindolyl-maleimide II Binds with Reversed Orientations to Different Conformations of PKA, submitted.