ESRF

ESRF	Experiment title: **Structures of Michaelis and Product Complexes of Plant Cytokinin Dehydrogenase: Implications for Flavoenzyme Catalysis**	**Experiment number**: MX129
Beamline: ID14-EH2 ID14-EH1 ID14-EH4	**Date of experiment**: ID14 2 :: 10 July 2003 / 11 July 2003 ID14 1 :: 04 October 2003 / 06 October 2003 ID14 4 :: 10 November 2003 / 11 November 2003	**Date of report**: 11 May 2004
Shifts: 4	**Local contact(s)**: **Stéphanie MONACO, Dominique BOURGEOIS, Pierre LEGRAND**	*Received at ESRF:*

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Report:

Abstract of the relevant manuscript submitted to Journal of Molecular Biology in April 2004

Cytokinins are a diverse class of compounds that are essential for plant growth. Cytokinin dehydrogenase (CKX) has a major role in the control of the levels of these plant hormones by catalysing their irreversible oxidation. The crystal structure of *Zea mays* CKX displays the same two-domain topology of the flavoenzymes of the vanillyl-alcohol oxidase family but its active site cannot be related to that of any other family member. The X-ray analysis reveals a bipartite architecture of the catalytic centre, which consists of an internal cavity lined by the flavin ring and a funnel shaped region on the protein surface. A pore with a diameter of about 4 Å connects the two sub-sites. Snapshots of two critical steps along the reaction cycle

were obtained through the elucidation of the complexes with a slowly reacting substrate and the reaction product, which correspond to the states immediately before (Michaelis complex) and after (product complex) oxidation has taken place. The substrate displays a "plug-into-socket" binding mode that seals the catalytic site and precisely positions the carbon atom undergoing oxidation in close contact with the reactive locus of the flavin. A polarising H-bond between the substrate amine group and an Asp-Glu pair may facilitate oxidation. Substrate to product conversion results in small atomic movements that lead to a planar conformation of the reaction product allowing double bond conjugation. The mechanism of amine oxidation in CKX differs from that observed in other flavin-dependent amine oxidases.

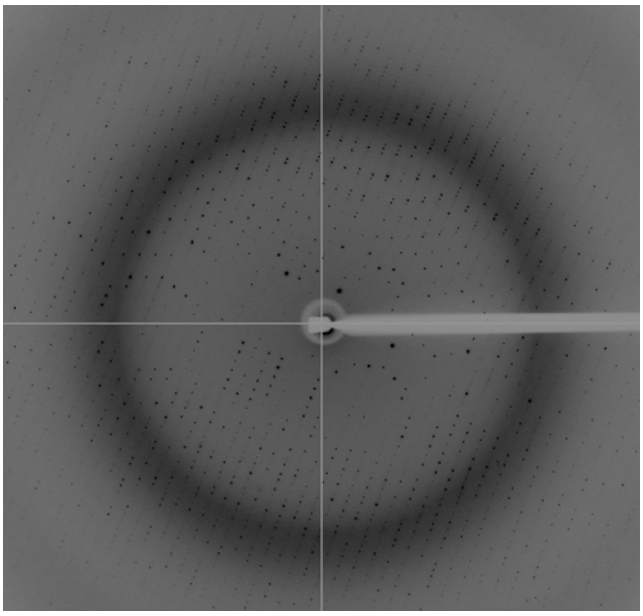

Figure 1. Diffraction pattern for CKX measured on the ID14-EH2 beam line. Resolution at the edge is 1.5 Å.

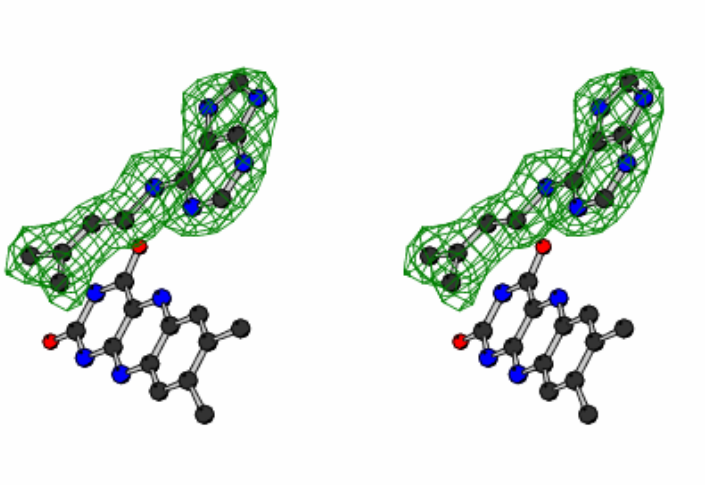

Figure 2. Unbiased 2Fo-Fc electron density maps observed in the active sites of the complex obtained by soaking in N^6-isopentenyladenine. The map was calculated before the ligand atoms were included in the refinement.

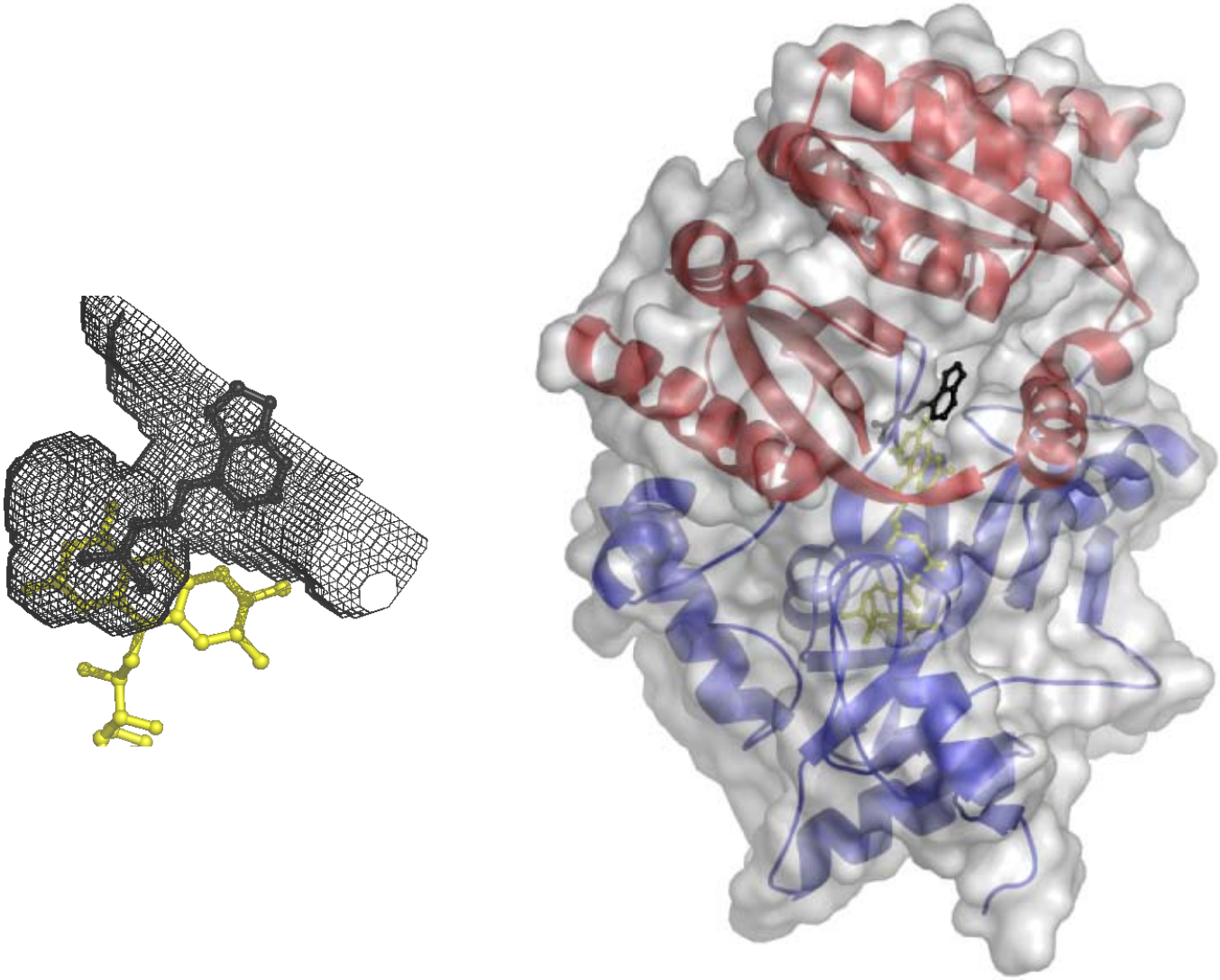

Figure 3. Shape and architecture of the active site of CKX. (left) Close-up view of the protein surface in the region surrounding the active site. The picture outlines the internal cavity located in front of the flavin ring (yellow) connected through a pore to the outside surface. The oxidised N^6-isopentenyladenine ligand is shown in black. (right) View of the CKX monomer surface. The isopentenyl substituent is buried inside the internal cavity whereas the adenine ring sticks out on the surface with its edge accessible to the solvent.