MS5-O2 Crystal structures of the human doublecortin C-terminal and N-terminal domains in complex with specific antibodies

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Doublecortin is a microtubule-associated protein essential for human brain development and for proper transport of presynaptic vesicles. Missense mutations in the doublecortin gene cause defective cortical neuronal migration leading to the brain formation disorders X-linked lissencephaly (XLIS) and subcortical band heterotopia (SBH). Anti-doublecortin-antibodies are widely used research tools to detect neurogenesis. In order to enable better characterization of the doublecortin interaction with tubulin we raised novel domain specific antibodies against doublecortin. Only the antibody specific for the C-terminal domain prevented doublecortin binding to microtubules. The antibodies epitopes and binding geometries were observed in their crystal structures in complex with their doublecortin domains. With help of the specific antibody, the first crystal structure of the C-terminal domain of human doublecortin was determined. Several new structures of the doublecortin N-terminal domain illustrate the conformational flexibility of the linker region connecting the two domains, that is necessary for doublecortin function. Taken together, our results show that doublecortin interacts with microtubules by its C-terminal domain.

Keywords: protein crystallization, antibodies, drug discovery, drug design

MS5-O3 Combining “dry” co-crystallization with in situ diffraction to facilitate ligand screening by X-ray crystallography

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Structure-Based Drug Design is well established as a powerful technique to develop new binders for a given therapeutic target. Indeed, nanomolar ligands can be built from fragments showing millimolar affinity to the target. To optimally guide the drug-design process, one have to unravel protein-ligand interaction at atomic details.

Recently, structure determination has been dramatically accelerated. New beamlines have been recently set up to allow automatic data collections of a few hundred of macromolecular crystals per day. However, ligand deliveries is still a tedious and often limiting step.

We have recently developed a new method for pre-coating crystallization plates with the desired ligands. This lead to a solvent-free ligand delivery to protein crystals compliant with most (if not all) media used for protein crystal growth.

Ligand pre-coating prior to co-crystallization can be combined with in situ diffraction thanks to dedicated robotic suites compatible with 96-well plates[1] in order to make ligand screening by X-ray crystallography fully automatic. The use of this integrated approach to discover new ligands for important drug targets will be discussed [2].

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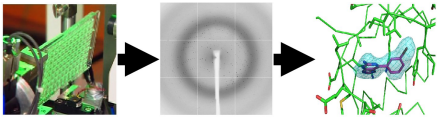

Figure 1. Toward automatic ligand screening using ligand pre-coating and *in situ* X-ray crystallography

Keywords: solvent-free co-crystallization; fragment approach ; therapeutic targets, drug design ; automation; solvent evaporation