s1.m5.p13 **Modelling crystal contacts by site-directed mutagenesis.** Katja Schirwitz, Andrea Schmidt and Victor Lamzin, *EMBL, Notkestrasse 85, 22603 Hamburg, Germany. E-mail: katja.schirwitz@embl-hamburg.de*

Keywords: Crystal contacts; Surface modelling; Mutagenesis

Improvements in recombinant protein purification technologies as well as robot-assisted protein crystallisation, X-ray data collection and structure determination have greatly advanced the crystallographic investigation of proteins. Prediction of protein crystal contact formation could become an interesting tool for improvement of macromolecular crystallisation. It is hypothesised that surface residues with high conformational entropy, specifically lysines and glutamates, impede protein crystallization. Mutation often results in improved crystal growth and enhanced X-ray diffraction. We initiated a pilot investigation on the rational mutagenesis of surface residues with the aim to improve the crystal diffraction. We have chosen formate dehydrogenases as our first model system as they show variable sequences and crystallisation tendencies. We have isolated the genomic DNA from *Candida boidinii* and cloned the formate dehydrogenase gene into an *E.coli* expression vector. Based on the purification procedure described for native fdh isolated from *Candida boidinii* [1] and *Candida methylica* [2] a two-step routine purification protocol was established, yielding up to 10 mg pure and homogenous recombinant Cbfdh protein with a mean specific activity of 5 U/mg at 22°C from one litre *E.coli* - suspension. By aligning regions of predicted disorder with the model of the Cbfdh protein (Labrou *et al.*, 2001) we identified amino acids potentially problematic for the crystallisation process. We defined a first set of target mutants, cloned them, expressed the recombinant mutant protein, characterised the biochemical properties and set up crystallisation trials to monitor the influence of the mutations. The results of this study will be presented. In a long-term prospective we expect to proceed towards developing a mechanism for the prediction of amino acids that interfere with the formation of intermolecular contacts needed for nucleation and proper crystal growth.

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s1.m5.p14 **Crystallization Of Photosystem II Core Complex From Pisum Sativum.** Kuta Smatanova I.[a], Vacha F.[b], Gavira J.A.[c], Garcia-Ruiz J.M.[c], *[a]Institute of Physical Biology USB CB & Institute of Landscape Ecology AS CR, Zamek 136, 373 33 Nove Hrady, Czech Republic, [b]Institute of Physical Biology USB CB, Zamek 136, 373 33 Nove Hrady, & Institute of Plant Molecular Biology AS CR, Branisovska 31, 370 05 Ceske Budejovice, Czech Republic, [c]Laboratorio de Estudios Cristalograficos, IACT CSIC-UGRA, Facultad de Ciencias, Avd. Fuentenuva s/n, 18002 Granada, Spain. E-mail: ivas@bf.jcu.cz*

Keywords: Membrane protein; Photosystem II; Crystallization

Membrane proteins are responsible for enzymatic reactions, which play an important role in all fundamental processes of life. The fact that protein crystals are quite unstable, highly temperature- and light-sensitive along with complicated composition of membrane proteins are responsible for difficult crystal growing and solving their structure.

Monomeric photosystem II (PSII) core complex consisting of reaction center proteins D1 and D2, the chlorophyll-containing inner-antenna subunits CP43 and CP47, α and β subunits of cytochrome b_{559}, several low molecular weight subunits, pigments and cofactors and three extrinsic proteins of oxygen-evolving complex [1] was isolated from *Pisum sativum*, purified and concentrated to 4-6 mg/ml chlorophyll *a*. Freshly isolated and frozen samples of monomeric PSII core complex were crystallized using the counter-diffusion technique implemented in single capillaries [2] and traditional vapor diffusion method in sitting drops. In both cases the protein solution was gelled with tetramethyl orthosilicate (TMOS) or agarose at different concentration. Gel free experiments were prepared in parallel. Different types of precipitants, inorganic salts, different pH values and variant protein:precipitant concentration ratios were tested experimentally. As a general observation, only fresh purified and non-frozen protein was suitable for crystallization trials. Both, the use of gels and crystallization in capillaries, were proved as useful crystallization method.

Plate crystals of monomeric PSII core complex were grown in sitting drops from precipitant solution containing PEG4000, NaCl, Bis-Tris pH 7.00 at 283K. Needle-shaped crystals of protein were grown in sitting drops and in capillaries from the same precipitant solution in the presence of TMOS or agarose at RT. Green crystals were tested at synchrotron DESY (Hamburg) at 100K. Crystallization experiments on PSII membrane protein complexes are still in the progress.

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