optimized. It was shown that oxygen-evolving complex is possible to wash out from thylakoid membrane by contain of different salts. According preliminary crystallization experiments it was shown that success of crystallization trials is dependent on purification steps of this complex. It was shown that degradation of subunits is caused by presence of Na^+, K^+, Ca^{2+} and Mg^{2+} ions, but Ca^{2+} are sufficient additive for crystallization. Our results showed that development of reproducible purification protocol is a crucial step toward reproducible crystallization experiments.

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Keywords: plant photosystem II, oxygen-evolving complex

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ProSMART - procrustes structural matching alignment and restraints tool

Robert Nicholls, Garib Murshudov, *Structural Biology Laboratory, Department of Chemistry, University of York, Heslington, York, YO10 5YW, (England).* E-mail: nicholls@ysbl.york.ac.uk

ProSMART (Procrustes Structural Matching Alignment and Restraints Tool) is a tool to aid in the comparative analysis and refinement of protein structures, intended to be complementary to existing resources. Primarily, it is used for conformational invariant/independent pairwise structural alignment, allowing identification of local similarities. The tool provides residue-based dissimilarity scores for assessment of local similarity, identifies rigid substructures, and outputs sets of superposed coordinates. Utilising information from external structures, atomic distance restraints may be generated for subsequent use during crystallographic refinement.

Pairwise structural alignment is achieved by performing an all-on-all comparison of n-residue structural fragments between two chains. Individual structural fragments are compared using Procrustes analysis, quickly achieving local backbone root mean square deviation. A fragment alignment is achieved using a dynamic programming algorithm, which is then further refined. In order to maintain conformation-invariance, the alignment is filtered to enforce global rigidity of neither chains nor domains. This feature makes the tool suited to the analysis of domain movement and other conformational changes, as well as for the identification of structural units that are conserved between seemingly different structures.

Following identification of the alignment, the structures are searched for conserved rigid substructures. For each identified substructure, superposed coordinates are output. ProSMART has a variety of features, useful in different applications. One such feature is the ability to colour (superposed) structures according to various residue-based dissimilarity scores, to be viewed in PyMOL. ProSMART also allows dissimilarity scoring of side chain conformation, relative to local coordinate frame. This feature is of particular use when comparing the side chains of two near-identical structures in different conformations and/or bound states, due to the ability to detect subtle changes.

Given an alignment, ProSMART can be used to generate external restraints on the distances between relatively close, non-bonded, atoms. Using one or more similar structures, the software generates restraints that are intended to help the target protein adopt a conformation that is more reasonable for structures in the class in which it belongs, whilst allowing global flexibility. The assertion is that the target structure's local atomic distances should be reasonably similar to those from similar structures. External restraints from ProSMART can be applied during crystallographic refinement by REFMAC5. These restraints have been found to stabilise refinement in some cases, especially at low resolution (3-6Å) where experimental data alone may not be sufficient. Tests are promising, suggesting that external restraints might be used to improve reliability in future.

ProSMART may be used with data from various methods, including structures from crystallography and electron microscopy, and ensembles from nuclear magnetic resonance and molecular dynamics simulations. A pre-release version of ProSMART is available by contacting the author.

Keywords: alignment, comparison, restraints

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Towards the complete structure of the S-layer protein SbsC

Anđela Đordić,[a] Tea Pavkov-Keller,[a] Eva Maria Egelseer,[b] Uwe B. Sleytr,[b] Walter Keller,[a] *[a]Institute of Molecular Biosciences, K.F. University Graz, (Austria).* [b]*Center for Nanobiotechnology, University of Natural Resources and Applied Life Sciences, Vienna, (Austria).* E-mail: andela.dordic@uni-graz.at

Monomolecular paracrystalline surface layers (S-layers) are composed of a single (glyco)protein and are the most commonly observed cell surface structures of bacteria and archaea. Because of their diverse properties S-layers have various potential applications in nanobiotechnology [1]. However, detailed structural information on S-layer proteins is very scarce. In order to determine the structure-function relationship of SbsC, the S-layer protein from *Geobacillus stearothermophilus*, deletion mutants were produced. It was shown that the N-terminal part is responsible for binding to the secondary cell wall polymer (SCWP) and that the C-terminal part is essential for self-assembly [2]. Recently, the crystal structure of the C-terminally truncated form $rSbsC_{(31-443)}$ was solved to 2.4 Å [3].

We continued the work with different N-terminal truncations and crystals of 3 different protein constructs were obtained. The structure of one construct was solved by producing different heavy atom derivatives. The structure consists of 3 Ig-like domains connected with the short linker. The refinement of the crystals from two other constructs is in progress.

Small angle X-ray scattering measurement of all constructs was performed. All constructs consist of domains similar in size and shape. We can conclude that the full length SbsC protein consists of 9 domains. The first coiled-coil domain followed by 8 Ig-like domains.

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Keywords: S-layer, crystallization, SAXS

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A study of how different ligands and ph may influence insulin crystallisation by using powder diffraction

Anastasia Giannopoulou, F. Karavasili, Yves Watier, Jonathan

Wright, Andrew Fitch, Irene Margiolaki, Mathias Norrman, Gerd Schluckebier, *University of Patras, Greece, Novo Nordisk A/S, Copenhagen*, (Denmark). E-mail: natevdokia@gmail.com

Modern developments of the powder diffraction technique have allowed the investigation of systems with large unit cells as proteins. Protein powder specimens consist of a large number of randomly oriented diffracting micro-crystals, which can be formed rapidly by batch crystallisation. Insulin is a hormone central to regulating carbohydrate and fat metabolism in the body. In this study, we investigate the effects that different ligands (such as Resorcinol, Phenol and more), as well as the pH, have on the structural characteristics of insulin. Powder diffraction data were collected at room temperature at the ESRF (Grenoble, France). Preliminary data interpretation correlating the crystallisation conditions with the structural and micro structural characteristics of insulin will be presented.

Keywords: proteins, powder diffraction, synchrotron radiation

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Structural studies of urate oxidase using powder diffraction

Foteini Karavasili,[a] Anastasia Giannopoulou,[a] Sotonye Dagogo,[b] Jon Wright,[b] Andy Fitch,[b] Marion Giffard,[c] Francoise Bonneté,[c] Irene Margiolaki,[a] [a] *University of Patras, (Greece).* [b]*European Synchroton Radiation Facility (ESRF), Grenoble, (France).* [c]*Centre de Recherche en Matiere Condensée et Nanosciences (CRMC-N), (Marseille).* E-mail: fkar@upatras.gr

Polycrystalline protein precipitants are frequently obtained under a variety of crystallisation conditions and thus powder methods can be employed for structural characterisation of small proteins when single crystals are unavailable. Urate Oxidase from *Aspergillus flavus* (Uox) is a protein used to reduce toxic uric acid accumulation and also for the treatment of hyperuricaemia which occurs during chemotherapy. In this study, we investigate the effects of different concentrations of salts (such as NaCl, KCl, $(NH_4)_2SO_4$, $(NH_4)_2Cl$ and $CaCl_2$) as well as polyethylene glycol (PEG6000 and PEG8000) concentration on the structural characteristics of Uox, uncomplexed and complexed with 8-azaxanthin (AZA). Powder diffraction data were collected at room temperature at the ESRF (Grenoble, France).

Keywords: proteins, powder diffraction, synchrotron radiation

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Engineering cysteine residues to facilitate protein crystallization: a case study with the beta galactosidase from *Lactobacillus plantarum*

Yanaisis Álvarez,[a] Iván Angulo,[a] José Antonio Curiel,[b] Blanca de las Rivas,[b] Rosario Muño,[b] José Miguel Mancheño[a] [a]*Department of Crystallography and Structural Biology, Instituto de Química Física "Rocasolano", CSIC, Madrid, (Spain).* [b]*Group of Bacterial Biotechnology, Instituto de Ciencia y Tecnología de la Alimentación y Nutrición, CSIC, Madrid, (Spain).* E-mail: xyanaisis@iqfr.csic.es

Currently, carbohydrate-processing enzymes are being exploited for many biotechnological applications due to their exquisite stereoselectivity and high efficiency. In particular, much attention has been focused on the use of β-glucosidases for the enzymatic hydrolysis of flavorless glycoconjugates present in juices and wine beverages for the release aroma volatiles. With the aim to analyze a novel glycosidase with potential applications food industry we have produced a novel glycosidase from the food lactic acid bacterium *Lactobacillus plantarum*. Thus, we have cloned and heterologously expressed the bgl gene (lp_3629) in *Escherichia coli*. Despite the initial experimental approach we follow for producing and purifying recombinant BGL protein was successful in that BGL was crystallized [1] and its structure solved by molecular replacement, subsequent trials for producing BGL systematically failed, rendering massive protein precipitation. Careful inspection of the BGL structure revealed unexpected electron density around the solvent exposed cysteine residues Cys-211 and Cys-292 that was explained in terms of the chemical modification of the above cysteine sulfurs by the cacodylate buffer in presence of DTT. In particular, the electron density observed is consistent with the presence an arsenic atom covalently bound, similarly to other reported studies [2-3]. To test the hypothesis that these cysteine residues are somehow involved in the precipitation of BGL we have produced the double-mutant Cys-211-Ser and Cys-292-Ser (BGL-2M). This recombinant protein was easily produced and purified, showing excellent solubility properties in contrast to native BGL. Furthermore, BGL-2M was subsequently crystallized in several conditions containing PEG3350. Diffraction quality crystals have been measured at the ID23-2 beamline (ESRF; Grenoble, France) and provided us with an almost complete dataset at 2.4 Å resolution. These data permitted to solve the structure of BGL-2M at the above resolution, confirming the designed mutations and revealing no conformational changes with respect to the native structure. Moreover, since this protein exhibits similar enzymatic properties as BGL, it opens the possibility to analyse in detail the structural basis of catalysis by means of protein complexes with substrate analogues.

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Keywords: biocrystallography, galactosidase, protein precipitation

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Structural insights in the DNA binding mechanism of a NAC transcription factor

Leila Lo Leggio,[a] Ditte H. Welner,[a] Søren Lindemose,[b] Addie N. Olsen,[b] Karen Skriver,[b] [a]*Biophysical Chemistry Group, Department of Chemistry, University of Copenhagen, (Denmark).* [b]*Section for Protein Science, Department of Biology, University of Copenhagen, (Denmark).* E-mail: leila@chem.ku.dk

The NAC (NAM/ATAF/CUC) proteins constitute a large group of transcription factors in plants, playing important roles in stress responses as well as plant development (reviewed in Olsen et al, 2005, Trends in Plant Science, 10:79-87). Controlling their action has potential applications in agriculture *e.g.* for improving the nutrition value in crop plants (Uauy C. et al, (2006) Science, 314:1298) or biofuel production (Hu et al., 2010, BMC Plant Biol, 10:145 ; Shen et al., 2009, Bioenerg Res, 2(4):217-232).

NAC proteins consist of two regions: a conserved N-terminal region (NAC domain) with DNA binding and oligomerization abilities, and a diverse C-terminal region which functions as a transcriptional activator. We have previously determined the structure of the DNA-binding NAC domain of *Arabidopsis thaliana* ANAC019 to a maximum resolution of 1.9 Å, and revealed a dimeric and predominantly β-fold structure (Ernst et al, 2004, EMBO Rep, 5:297–303). However, the mode of binding to cognate DNA has remained elusive.