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.

[1] I. Acebrón, *et al. Prot. Express. Purif. Biol Chem.* **2009**, *68*, 177-182. [2] Y. Goldgur, *et al. Proc. Acad. Sci. USA* **1998**, *95*, 9150-9154. [3] J.P. Noel, et al. *Nature* **1993**, *366*, 102-106.

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).

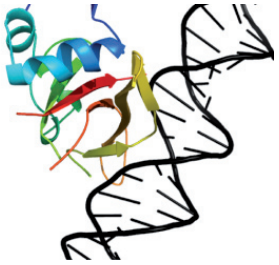
NAC proteins consist of two regions: a conserved N-terminal region (NAC domain) with DNA binding and oligomerization abilities, and a diverse C-terminal region which functions as a transcriptional activator. We have previously determined the structure of the DNA-binding NAC domain of *Arabidopsis thaliana* 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.

In this study, we present the X-ray structure of the NAC:DNA complex to 4.4 Å. The structure was solved with molecular replacement (MR) using as search models the apo-structure of NAC (1UT4) and a B-DNA model of the oligonucleotide (X.-J. Lu and W. K. Olsen (2003) Nucleic Acids Res 31(17):5108-5121). Refinement proved complicated due to the low resolution. The best strategy turned out to include NCS restraints on all atoms, reference-model restraints on protein atoms, and TLS. Phase restraints were available but did not improve refinement. Neither did simulated annealing.

The exact position of the individual DNA bases along the main DNA axis could not be determined by MR alone. Instead, the DNA sequence was assigned based on a uranyl photoprobing assay. The resulting model could be refined to R_{work}=24.1% and R_{free}=34.4%.

The structure provides for the first time experimental evidence for the speculated evolutionary relationship between the plant-specific NAC proteins and other transcription factors with a surprising phylogenetic breath. The structure shows how the NAC domain inserts the edge of its core beta-sheet in the major groove (figure), while leaving the DNA largely un-distorted. Recognition of DNA with the edge of a beta-sheet is also believed to be a feature of the WRKY family of transcription factors (Rushton et al. (2010) Trends Plant Sci 15(5):247-258), which are found in early eukaryotes and plants. Further, the animal GCM transcription factors use the same binding motif (Cohen et al. (2003) EMBO J 22(8):1835.1845). In addition, these three transcription factors share a core beta-sheet with a very similar topology.

The presented NAC:DNA complex structure provides a framework for studying the effects of single amino acids as well as structural features on DNA binding affinity and specificity. Further, we have evidence of limited flexibility of the NAC dimer arrangement, which could explain the limited tolerance in NAC binding site spacing that has been reported (Olsen et al. (2005) Plant Sci 169:785-797, Xue (2005) Plant J 41:638-649)



Keywords: transcription factor, NAC, low resolution

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Optimising low resolution structural biology techniques

Andrew A. McCarthy,[a] Sandor Brockhauser,[a] Sean McSweeney,[b] and the EMBL-Grenoble/ESRF Joint Structural Biology Group**,** *[a]European Molecular Biology Laboratory, Grenoble Outstation, 6 rue Jules Horowitz, Grenoble 38042, (France).[b]European Synchrotron Radiation Facility, 6 rue Jules Horowitz, BP 220 38043 Grenoble, (France).* E-mail: andrewmc@embl.fr

The investment in modern equipment and the development of highly automated beamline control software on the public MX-beamlines at the ESRF now allows standard X-ray diffraction experiments, even multiple anomalous diffraction (MAD), to be easily and robustly performed. However the success of X-ray diffraction experiments is still dependent on the quality of the crystals obtained. Most modern structural biology projects have become ever more complex and their success is now often dependent on a combination of low resolution techniques such as EM, X-ray small angle scattering and macromolecular crystallography. Many large and complex macromolecular assemblies often fail to crystallise or at best form few poorly diffracting crystals that are radiation sensitive. Such projects require newly developed equipment and a much more careful approach for data collection [1, 2]. In Grenoble we are developing improved instruments and methods for optimised low resolution data collection possibilities. Here I will present our current abilities and some future developments for new and challenging structural biology experiments.

[1] A.A. McCarthy, S. Brockhauser, D. Nurizzo, P. Theveneau, T. Mairs, D. Spruce, M. Guijarro, M. Lesourd, R.B.G. Ravelli, S. McSweeney, *J. Synchrotron Rad.* **2009**, *16,* 803-812. [2] S. Brockhauser, K.I. White, A.A. McCarthy, R.B.G. Ravelli *Acta Cryst. A* **2011**, *67*, 219-228.

Keywords: macromolecular crystallography, small angle X-ray scattering

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Preferential enrichment of cocrystals of amino acids and achiral dicarboxylic acid classified as racemic compound

Sekai Iwama, Rajesh Gonnade, Yuko Mori, Kazunori Kuyama, Hiroki Takahashi, Hirohito Tsue, Rui Tamura, *Graduate School of Human and Environmental Studies, Kyoto University, Kyoto (Japan). 606-8501.* E-mail: sekai.i@ax2.ecs.kyoto-u.ac.jp

Preferential enrichment is a symmetry-breaking chiral separation phenomenon that is initiated by the solvent-assisted solid-to-solid transformation of the first-formed metastable polymorphic form into a thermodynamically stable one during crystallization from the supersaturated solution of certain kinds of racemic mixed crystals (i.e., solid solutions or pseudoracemates) composed of two enantiomers in organic solvents without the aid of any external chiral element [1].

We anticipated that the preferential enrichment phenomenon might be observed even for a so-called 'racemic compound', if the following three requirements are satisfied: i) The solubility of the enantiopure sample is higher than that of the corresponding racemic sample. ii) Upon recrystallization of racemic or nearly racemic sample from organic solvents under kinetic conditions, a solid-to-solid polymorphic transition of the initially-formed metastable mixed crystals composed of the heterochiral 1D *R* and *S* chains into the stable mixed crystals mainly comprising heterochiral (*R,S*) chains occurs. iii) In the resulting deposited crystals, the fairly random arrangement of two enantiomers can be retained, without undergoing further solvent-mediated polymorphic transition to give exact racemic compound crystals.

Recently, we reported that other racemic crystals having a regular packing of a pair of *R* and *S* enantiomers in their crystal could also display the 'preferential enrichment' phenomenon. We have found that neutral amino acids, alanine and leucine, exhibited a very similar phenomenon to 'preferential enrichment' [2]. Here we report the successful enantiomeric resolution of other amino acids, such as phenylalanine, histidine, and cysteine with a racemic compound structure, which could be spontaneously resolved into its enantiomeric components by co-crystallization with achiral dicarboxylic acid. For example, repeated recrystallization of the cocrystals of DL-phenylalanine and fumaric acid from the 6-fold supersaturated aqueous solution led to a remarkable alternating enrichment of the two enantiomers up to 85% *ee* in the mother liquor, together with slight enrichment (< 6% *ee*) of the opposite enantiomer in the deposited crystals [3]. The mechanism of preferential enrichment is proposed on the basis of i) the observation of polymorphic transition during crystallization by in situ ATR-FTIR and Raman spectroscopy, ii) the characterization of deposited crystals by X-ray crystallographic analysis and powder XRD measurement, and iii) the optical microscopic and AFM observations of the crystal shape and surface, respectively.

[1] (*a*) R. Tamura, S. Iwama, H. Takahashi, *Symmetry* **2010**, *2*, 112-135; (*b*) R. Tamura, H. Takahashi, D. Fujimoto, T. Ushio, *Top. Curr. Chem* **2007**, *269*, 53-82; (*c*) R. Tamura, S. Iwama, R.G. Gonnade, *CrystEngComm,* **ASAP**. [2] S.