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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

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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<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, (NH<sub>4</sub>)<sub>2</sub>Cl and CaCl<sub>2</sub>) 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*

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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  $\beta$ -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 of 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.

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

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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  $\beta$ -fold structure (Ernst et al, 2004, EMBO Rep, 5:297-303). However, the mode of binding to cognate DNA has remained elusive.