Phasing macromolecular crystals (MX) with native elements such as sulfur and phosphorous has many advantages over heavy atom substitution methods, but it has always been hampered by systematic errors. At their K edges, sulfur (2.5 keV) and phosphorous (2.1 keV) exhibit comparable anomalous signal strength to that of selenium (f" ≈ 4 $e^-$), but conventional MX data collection is impractical at these wavelengths because the attenuation depth in protein crystals is only ~20 μm. Not only are the diffracted beams weak, but the uncertainty in the attenuation factor itself is a systematic error that is generally greater in magnitude than the anomalous difference to be measured. Using smaller crystals reduces the attenuation factor as well as the error it introduces, but at the expense of increasing another systematic error: radiation damage. In general, crystals small enough to have small absorption errors do not survive long enough for accurate anomalous differences to be measured. To date, successful sulfur phasing experiments have used relatively large crystals of sulfur-rich proteins and photon energies of about 7 keV. However, the new technique of femtosecond nanocrystallography has demonstrated significant reduction of radiation damage effects and good data quality at 2 keV from crystals much smaller than the attenuation depth. Unfortunately, each crystal may only be shot once, and it is geometrically impossible to simultaneously place a given h,k,l index and its Friedel mate (-h,-k,-l) onto the same Ewald sphere, so two opposing Ewald spheres must be generated. This is accomplished by illuminating the crystal with two X-ray beams, coming from opposite directions for a Colliding Beam Anomalous Measurement (CBAM). In this geometry, the diffracted rays of each Friedel pair emerge from the crystal in opposite directions with identical partialities and very similar attenuation factors. This makes it possible to directly measure the relative Bijvoet difference (ΔF/F) without any need to integrate the full spot intensity and circumvents the "partiality problem" of single-beam femtosecond nanocrystallography. At the sulfur K edge, 2.5 Å data may be collected, provided the detector surfaces are arranged to cover most exit angles, including backscattered rays. The simultaneous recording of patterns from two Ewald spheres does increase the likelihood of overlaps, but this is compensated by the "still" nature of the patterns. The design of a CBAM instrument for use at the Linac Coherent Light Source is currently underway.

**Keywords: sulfur phasing, XFEL, femtosecond, nanocrystal**

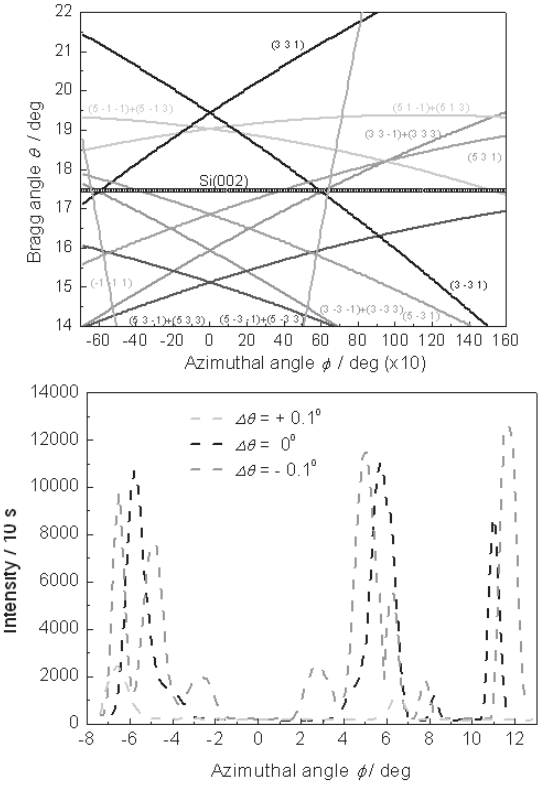
## MS07.P17

*Acta Cryst.* (2011) A**67,** C261

**Multiple Bragg Reflections in Cylindrically Bent Perfect Crystals**

Pavol Mikula,[a] Miroslav Vrana,[a] Jan Saroun,[a] Vyacheslav Em,[b] B.S. Seong,[b] [a]*Nuclear Physics Institute ASCR, v.v.i., Rez (Czech Republic).* [b]*Neutron Science Division, KAERI 1045 Daedok daero, Yuseong-gu, Daejeon, 305-353 (Republic of Korea).* E-mail: mikula@ujf.cas.cz

Multiple Bragg reflections (MBR) realized in one bent-perfect crystal (BPC) slab by sets of different lattice planes behave differently in comparison with the case of perfect nondeformed or mosaic crystal. Individual sets of lattice planes are mutually in dispersive diffraction geometry and the kinematical approach can be applied on this MBR process. Then, MBR process can be considered as one or several parallel double Bragg reflection events. By using neutron diffraction and the method of azimutal rotation of the Si crystal around the scattering vector related to the forbidden primary reflection (002) at the wavelength 0.1625 nm, several strong multiple reflections were investigated with a possible exploitation in high resolution diffractometry. The intensities of the monochromatic beam obtained on the basis of MBR effect depend on the thicknes of the crystal and its curvature as well as on the orientation of the individual participating planes with respect to the crystal deformation vector.




**Keywords: neutron diffraction, multiple reflections, bent perfect rystals**

## MS08.P01

*Acta Cryst.* (2011) A**67,** C261-C262

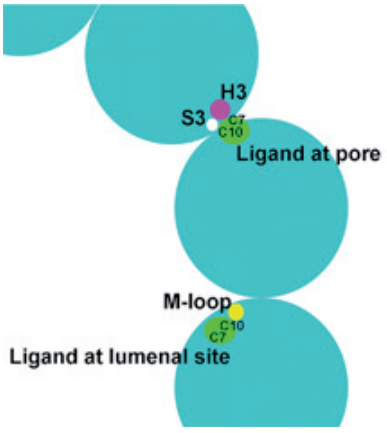
**Modulation of microtubule protofilament interactions by modified taxanes**

José Manuel Andreu,[a] Ruth Matesanz,[a] Javier Rodríguez-Salarichs,[a,b] Benet Pera,[a] Angeles Canales,[a] Jesús Jiménez-Barbero,[a] Wim Bras,[c] Aurora Nogales,[d] Wei-Shuo Fang,[e] José Fernando Díaz,[a] [a]*Chemical and Physical Biology, Centro de Investigaciones Biológicas, CSIC. Ramiro de Maeztu 9, 28040 Madrid, (Spain).* [b]*Centro de Estudios Avanzados de Cuba. Carretera San Antonio km 1 1/2 , Valle Grande, La Lisa, Ciudad Habana, CP. 17100, Cuba.* [c]*Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO), DUBBLE CRG/ESRF BP 220 F38043 Grenoble Cedex, France.* [d]*Instituto de Estructura de la Materia, CSIC, Serrano 121, 28006 Madrid, (Spain).* [e]*Institute of Materia Medica, Chinese Academy of Medical Sciences, 1 Xian Nong Tan Street, Beijing 100050, China.* E-mail: j.m.andreu@cib.csic.es

The antitumor compounds paclitaxel (taxol) and docetaxel modify the association between αβ-tubulin molecules promoting their assembly into microtubules. These drug-induced microtubules have different numbers of protofilaments [1]. The modification of the microtubule structure, through a non-yet characterized mechanism, is probably related to the changes in the tubulin-tubulin interactions responsible of the stabilizing activity. The effects of taxanes modified in positions C2, C7, C10 and C13 [2] on microtubule structure have been characterized using Small Angle X-ray Scattering. Modifications in positions C7, C10 and C2 result in changes of interprotofilament angles and thus in alterations of the microtubule structure, while modifications in position C13 do not induce any changes.

The observed effects have been explained using NMR-based

docking techniques and molecular dynamics simulations. Modeling results indicate that positions C7 and C10 affect the conformation of three key elements (the M-loop, S3 and H3) in the lateral interactions that modulate the contacts between adjacent protofilaments. Alternatively, the change in C2 slightly rearranges the ligand in the binding site, thus modifying the interaction of the ligand C7 position with the M-loop.




[1] J.M. Andreu, J. Bordas, J.F. Diaz, J. Garcia de Ancos, R. Gil, F.J. Medrano, E. Nogales, E. Pantos and E. Towns-Andrews. J. Mol. Biol. **1992**, *226*, 169-184. [2] R. Matesanz, I. Barasoain I, C.G. Yang , L. Wang, X Li, C. de Ines, C. Cordech, F. Gago, J. Jimenez-Barbero, J.M. Andreu, W.S. Fang and J.F. Díaz. Chemistry & Biology **2008,** *15*, 573-585

Scheme of the interactions of taxane ligands at the pore and luminal binding sites of microtubules, with the structural elements responsible for the interprotofilament interaction. The microtubule protofilaments are seen from the plus end

**Key words: SAXS, protein, assembly, NMR, molecular dynamics**

## MS08.P02

*Acta Cryst.* (2011) A**67,** C262

**A redox-controlled synergistic mechanism regulates the binding of the intrinsically disordered protein CP12 to photosynthetic glyceraldehyde-3-phosphate dehydrogenase**

Simona Fermani,[a] Giuseppe Falini,[a] Anton Thumiger,[b] Lucia Marri,[b] Francesca Sparla,[b] Paolo Pupillo,[b] Xavier Trivelli,[c,d,e] Paolo Trost,[b] Matteo Calvaresi,[a] Francesco Zerbetto,[a] [a]*Department of Chemistry "G. Ciamician" and* [b]*Department of Experimental Evolutionary Biology, University of Bologna, Bologna, (Italy).* [c]*Univ Lille Nord de France, F-59000 Lille, France.* [d]*USTL, UGSF, F-59650 Villeneuve d'Ascq, France.* [e]*CNRS, UMR 8576, F-59650 Villeneuve d'Ascq, France.* E-mail: simona.fermani@unibo.it

Carbon assimilation in plants is regulated by the reduction of specific protein disulfide bridges by light and their re-oxidation in the dark [1]. The redox switch, CP12, is a small, intrinsically-disordered protein that carries two disulfides groups. In the dark, it forms an inactive supramolecular complex with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [2],[3]. Here we show that binding of CP12 to GAPDH follows a synergistic mechanism that includes both conformational selection and induced folding steps. Initially, a conformation characterized by a circular structural motif of the C-terminal disulfide of CP12 is selected by GAPDH. Subsequently, the induced folding of the flexible C-terminal tail of CP12 in the active site of GAPDH site stabilizes the binary complex. Formation of several hydrogen bonds compensates the entropic cost of CP12 fixation and terminates the synergistic mechanism that controls carbon assimilation.

[1] B.B. Buchanan, Y. Balmer *Annu. Rev. Plant Biol*. **2005**, *56*, 187–220 [2] E. Graciet, P. Gans, N. Wedel, S. Lebreton, J. M. Camadro, B. Gontero *Biochemistry* **2003**, *42*, 8163–8170 [3] L. Marri, P. Trost, P. Pupillo, Sparla, F. *Plant Physiol*. **2005**, *139*, 1433-144.

**Keywords: photosynthesis, enzyme, complex**

## MS08.P03

*Acta Cryst.* (2011) A**67,** C262-C263

**Rotor architecture in the yeast $F_1$-$c_{10}$-ring complex of F-ATP synthase**

Alain Dautant, Jean Velours, Jean-Claude Talbot, Claire Stines-Chaumeil, Daniel Brèthes, Marie-France Giraud, *IBGC, UMR 5095 CNRS Université de Bordeaux, Bordeaux (France).* E-mail: a.dautant@ibgc.cnrs.fr

$F_1F_o$-ATP synthase is a hybrid molecular nanomotor. $F_1$ is a chemical motor driven by ATP hydrolysis while $F_o$ is an electrical motor driven by the proton flow. The two stepping motors are mechanically coupled through a common rotary shaft. The $F_1$-ATPase X-ray structures support binding change mechanism for catalysis. The empty and open catalytic site $\beta_E$ is the OPEN site with low affinity for nucleotides, the $\beta_{TP}$ site filled by ATP (or AMP-PNP) is the LOOSE conformation and the $\beta_{DP}$ site filled by ADP is the TIGHT conformation where synthesis occurs. In $F_1$ domain, the orientation of the γ-subunit relative to the $(\alpha\beta)_3$ component determines the catalytic state of the enzyme and, according to the binding change mechanism, a 120° rotation of the γ-subunit during the ATP hydrolysis cycle results in E→TP, TP→DP and DP→E interconversions.

Up to now, the three available crystal structures of the $F_1c_{10}$ sub-complex of the yeast $F_1F_o$-ATP synthase were isomorphous [1] and [2] with a crystal form named $yF_1c_{10}$(I).

In the crystal structure of the Mg.ADP-inhibited state of the yeast $F_1c_{10}$-ATP synthase, solved at 3.4 Å resolution, an ADP molecule was bound in both $\beta_{DP}$ and $\beta_{TP}$ catalytic sites [1]. The $\alpha_{DP}$-$\beta_{DP}$ pair is slightly open and resembles the novel conformation identified in the yeast $F_1$ [3], whereas the $\alpha_{TP}$-$\beta_{TP}$ pair is very closed and resembles more a DP pair. In the $F_o$ rotor ring, the essential cGlu59 carboxylate group is only surrounded by apolar residues. Its closest hydrogen bond acceptor, the cLeu57 carbonyl oxygen of the adjacent c-subunit, is too far away to make a direct hydrogen bond. The proton binding has specific features compared to the bacterial $Na^+$-transporting or the cyanobacterial and chloroplastic $H^+$-transporting F-type ATP synthase rotor structures. In the crystal, significant interactions of the $c_{10}$-ring with the $F_1$-head of neighboring molecules affect the overall conformation of the $F_1$-*c*-ring complex. The symmetry axis of the $F_1$-stator and the inertia axis of the *c*-ring are tilted near the $F_1$-$F_o$ rotor interface, resulting in an unbalanced machine.

Recently, we have solved a new crystal form of the yeast *Saccharomyces cerevisiae* $F_1c_{10}$ complex, named $yF_1c_{10}$(II), inhibited by adenylyl imidodiphosphate (AMP-PNP) and dicyclohexylcarbodiimide (DCCD), at 6.5 Å resolution in which the crystal packing has a weaker influence over the conformation of the $F_1$-*c*-ring complex.

Despite a low resolution, the overall fold is clearly visible with a more straight C-terminal helix of subunit γ. Though the $F_1$-stator is 8° tilted relative to the rotor axis, its center of mass is located approximately on this axis. Therefore, $yF_1c_{10}$(II) provides a model of a more efficient generator. The present yeast $yF_1c_{10}$(II) and the bovine $bF_1c_8$ [4] models are comparable and together provide accurate models of the $F_1$-*c*-ring domain in the intact $F_1F_o$-ATP synthase.

[1] A. Dautant, J. Velours, M.-F. Giraud, *The Journal of. Biological Chemistry* **2010**, *285*, 29502-29510. [2] D. Stock, A.G. Leslie, J.E. Walker, *Science* **1999,** *286*, 1700-1705. [3] V. Kabaleeswaran, N. Puri, J.E. Walker, A.G.W. Leslie,