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 circumvent 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: neutron diffraction, multiple reflections, bent perfect crystals

MS07.P17

Multiple Bragg Reflections in Cylindrically Bent Perfect Crystals

Pavel Mikula,¹ Miroslav Vrana,¹ Jan Saroun,¹ Vyacheslav Em,³ B.S. Seong,² *Nuclear Physics Institute ASCR, v.v.i., Rez (Czech Republic). Neutron Science Division, KAERI 1045 Duedok 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 azimuthal 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 thickness 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: sulfur phasing, XFEL, femtosecond, nanocrystal

MS08.P01

Modulation of microtubule protofilament interactions by modified taxanes

José Manuel Andreu,¹ Ruth Matesanz,¹ Javier Rodríguez-Salarichs,¹ Benet Pera,¹ Angeles Canales,¹ Jesús Jiménez-Barbero,¹ Wim Bras,³ Aurora Nogales,⁴ Wei-Shuo Fang,⁵ José Fernando Diaz,⁵ *Chemical and Physical Biology, Centro de Investigaciones Biológicas, CSIC. Ramiro de Maeztu 9, 28040 Madrid, (Spain). ¹Centro de Estudios Avanzados de Cuba. Carretera San Antonio km 1 1/2, Valle Grande, La Lisa, Ciudad Habana, CP. 17100, Cuba. ²Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO), DUBBLE CRG/ESRF BP 220 F38043 Grenoble Cedex, France. ³Instituto de Estructura de la Materia, CSIC, Serranìa 121, 28006 Madrid, (Spain). ⁴Institute 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 C13 result in changes of interprotomylament 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
MS08.P02

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

Simona Fermani, 1* Giuseppe Falini, 1 Anton Thumiger, 2 Lucia Marri, 2 Francesca Sparla, 2 Paolo Pupillo, 2 Xavier Trivelli, 2, 4 Paolo Trost, 2 Matteo Calvaresi, 2 Francesco Zerbetto, 2 Department of Chemistry “G. Ciamiciani” and Department of Experimental Evolutionary Biology, University of Bologna, Bologna, (Italy) 1 Univ Lille Nord de France, F-59000 Lille, France. 2 USTL, UGSF, F-59650 Villeneuve d’Ascq, France. 3 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.


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

MS08.P03

Rotor architecture in the yeast F1-c10-ring complex of F-ATP synthase

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

F_{10}F_{-}ATP synthase is a hybrid molecular nanomotor. F_{1} is a chemical motor driven by ATP hydrolysis while F_{10} is an electrical motor driven by the proton flow. The two stepping motors are mechanically coupled through a common rotary shaft. The F_{10}ATPase X-ray structures support binding change mechanism for catalysis. The empty and open catalytic site β_{0} is the OPEN site with low affinity for nucleotides, the β_{0}β_{o} site filled by ATP (or AMP-PNP) is the LOOSE conformation and the β_{0}β_{e} site filled by ADP is the TIGHT conformation where synthesis occurs. In F_{1} domain, the orientation of the γ-subunit relative to the (αβ), 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_{10}c_{10} subcomplex of the yeast F_{10}F_{-}ATP synthase were isomorphous [1] and [2] with a crystal form named yF_{10}c_{10} dII.

In the crystal structure of the Mg-ADP-inhibited state of the yeast F_{10}c_{10}ATP synthase, solved at 3.4 Å resolution, an ADP molecule was bound in both β_{0}β_{o} and β_{0}β_{e} catalytic sites [1]. The c_{0}β_{0}β_{o} pair is slightly open and resembles the novel conformation identified in the yeast F_{1} [3], whereas the α_{e}β_{e}β_{o} pair is very closed and resembles more a DP pair. In the F_{1} rotor ring, the essential Glu59 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_{10}c_{10}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_{-} rotor interface, resulting in an unbalanced machine.

Recently, we have solved a new crystal form of the yeast Saccharomymes cerevisiae F_{10}c_{10} complex, named yF_{10}c_{10}dII (II), inhibited by adenyllyl imidodiphosphate (AMP-PNP) and dicyclohexylcarbodiimide (DCCD), at 6.5 Å resolution in which the crystal packing has a weaker interaction between the F_{10}c_{10} and the rotor. The β_{o}σ-carboxyl group is replaced by a more efficient generator. The present yeast yF_{10}c_{10}dII (II) and the bovine bF_{10}c_{10} [4] models are comparable and together provide accurate models of the F_{10}c_{10} ring domain in the intact F_{10}F_{-}ATP synthase.


C262