myosin VI has a rather small lever arm and they cannot be accounted for considering the structural transitions that occur within the myosin motor of other classes. Secondly, this motor produces force towards the minus end of actin filaments, which is the opposite direction of all other characterized myosins. In order to understand the molecular basis of these features, we would like to describe the structure of this myosin in different states of its ATPase cycle. During the ATPase cycle, myosin goes through states of strong and weak affinity for the actin filament. To this day, we solved the structure of three states of the cycle, one state that mimics the state of strong affinity for actin (at the end of the movement on the filament) and two states before the force production. One of them represents the starting point for movement on actin, the pre powerstroke state. The analysis of the structures from the beginning and the end of the powerstroke allows us to understand how myosin VI moves in the opposite direction (toward the minus-end of actin filaments) due to a unique insertion between the motor domain and the lever arm. These structures also allowed us to understand the origin of the large size of the myosin VI lever arm swing. Unexpectedly, we found that a conformational change occurs in the converter which allows an optimized movement of the lever arm during the stroke.

Keywords: myosin, molecular motors, mechanisms enzyme

MS.43.3

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How programmed cell death is regulated: Insights from structures of Bcl-2 family protein complexes

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Programmed cell death, or apoptosis, is controlled by the Bcl-2 family of proteins. The family consists of two main groups: the pro-survival group, which includes Mcl-1 and Bcl-xL, and the pro-death group, which includes the essential effector molecules Bax and Bak together with the more distantly related “BH3-only proteins” that are upregulated in response to various death stimuli. Interactions between members of these groups determine the fate of the cell and their deregulation underlies many disease conditions. Indeed, dysregulated apoptosis is a hallmark of many, if not all cancers. Thus, Bcl-2 family members are promising targets for anti-cancer therapeutics. Bax and Bak are thought to be able to adopt at least two distinct structural folds. One of these, the inactive fold, is well characterised and typical of the pro-survival Bcl-2 family proteins. Upon receipt of an apoptotic stimulus Bax and Bak are able to undergo a conformation change leading to an as yet undescribed activated form. One structural consequence is that a domain common to all Bcl-2 family members, the BH3 domain, becomes exposed. The pro-survival Bcl-2 family proteins are able to bind activated Bax and Bak via this BH3 domain and thus inhibit their activity. This provides an extra level of control over this important biological process. We have solved crystal structures for two such complexes, the Mcl-1:Bax BH3 (using a 34-mer BH3 peptide) complex and the Bcl-xL:Bak BH3 (34-mer) complex. In both cases significant structural changes must occur in the published structures of inactive Bax and Bak in order for these complexes to form.

Keywords: apoptosis, cancer, protein complexes

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Structural basis of spindle checkpoint activation and inactivation by Mad2 and p31comet

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In eukaryotes, the spindle checkpoint is a cell-cycle surveillance mechanism that ensures the fidelity of chromosome segregation in mitosis and thus prevents chromosome missegregation and aneuploidy. The status of spindle checkpoint signaling depends on the balance of two opposing dynamic processes that regulate the highly unusual two-state behavior of Mad2. In mitosis, a Mad1-Mad2 core complex recruits additional cytosolic Mad2 to kinetochores through Mad2 dimerization. Mad2 is then converted to a conformer amenable to Cdc20 binding, which facilitates checkpoint activation, halting mitosis. We report the crystal structure of an active Mad2 dimer. Combined with NMR and biochemical studies, we describe the features, kinetics and energetics of the dramatic conformational changes that initiate Mad2 structural activation. The opposing mechanism, spindle checkpoint inactivation, can be initiated by p31comet. This protein binds to Mad1- or Cdc20-bound Mad2 and thereby prevents Mad2 activation and promotes the dissociation of the Mad2-Cdc20 complex. We report the crystal structure of the Mad2/p31comet complex. Surprisingly, the sequentially unrelated p31comet adopts a fold strikingly similar to that of Mad2. It binds at the Mad2 dimerization interface and, by acting as an anti-Mad2 through structural mimicry, exploits the two-state behavior of Mad2 to block its activation. Through a combination of crystallographic and NMR studies, together with biochemical and cell biological studies, we provide a picture of the complex activation and inactivation processes during Mad2-dependent spindle checkpoint signaling.

Keywords: spindle assembly checkpoint, mitosis, structural mimicry

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Structural basis for the effects of PI3Kalpha oncogenic mutations

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PIK3CA, one of the two most frequently mutated oncogenes in human tumors, codes for p110alpha, the catalytic subunit of a heterodimeric PI3Kalpha kinase (p110alpha/p85) that plays a central role in signal transduction pathways. These somatic mutations increase PI3K kinase activity, leading to increased cell survival, cell motility, cell metabolism, and cell cycle progression. The 3.0 Å resolution structure of the complex between the catalytic subunit of PI3Kalpha, p110alpha, and two domains of its regulatory subunit, p85alpha reveals that the majority of the oncogenic mutations occur at the interfaces between p110 domains and between p110 and p85 domains. At these positions, the gain-of-function-mutations disrupt interactions resulting in changes in the kinase domain that may

increase enzymatic activity. The structure suggests that interaction with the membrane is mediated by one of the p85 domains (iSH2). The existence of p110alpha gain-of-function-mutants makes this protein an attractive therapeutic target. Since PI3Ks control a wide range of physiological functions, it would be desirable to inhibit only p110alpha, the isoform mutated in cancers. Structural differences among the isoforms can be exploited for that purpose. These findings may provide not only novel insight for the design of PI3K-isoform-selective drugs but also help on the design of mutation-specific drugs.

Keywords: PI3K, p110alpha, drug design

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Is there a steep learning curve in crystallography?

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The synthesis of aspirin is a part of many undergraduate organic classes. These courses teach synthesis skills and introduce students to purification using re-crystallization. Typically good quality crystals for single crystal x-ray investigation can be obtained within a few hours from the start of the experiment. As crystallography provides the most unambiguous structural information of all analytical tools it seems a natural progression to introduce students to this technique at an early stage and not reserve these tools for cutting edge research. Advances in chemical crystallography have traditionally focused on improving individual components of instrumentation and software algorithms. The next quantum leap is the development of fully integrated configurations, where several crystallographic methods are combined on a single accelerator platform. This allows for the full streamlining of the traditionally tedious and time consuming process of determining the three dimensional structures of molecular compounds. This talk tries to look critically at new, easy, and intuitive x-ray crystallographic methods for 3-D structural characterization. It questions whether user-friendly automated system operation generates reliable data without extensive crystallographic knowledge and can overcome the steep learning curve in crystallography.

Keywords: software automation, hardware automation, teaching

MS.44.2

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A tutorial for learning and teaching macromolecular crystallography

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Based on five diffraction data sets collected during the DGK(german crystallographic association)-workshop on X-Ray Diffraction Data Collection using Synchrotron Radiation held at the BESSY synchrotron (Berlin, Germany), we have assembled a tutorial for teaching and learning macromolecular crystallography with the emphasis on crystallization, diffraction data collection and processing, and automated structure solution. The tutorial consists of five experiments. It covers all the information needed to repeat the whole experiments or parts of it starting from the crystallization and ending with the structure determination. The material provided includes also the raw X-ray data. The five experiments are (1) structure determination by sulphur-SAD (single wavelength anomalous diffraction) on cubic insulin, (2) structure determination by MAD (multiple wavelength anomalous diffraction) on bromide-soaked thaumatin, (3) structure determination by molecular replacement on monoclinic hen egg-white lysozyme, (4) the identification of bound surface ions and (5) the identification of an active site ligand in tetragonal lysozyme crystals. For the later two projects, the diffraction data were collected using longer X-ray wavelengths. The tutorial and/or the provided X-ray data can be used in hands-on workshops in macromolecular crystallography or as material for lectures. The material can also help beginners in the field of macromolecular crystallography to gain first experience in crystallization, data collection and the use of crystallographic software to determine three-dimensional macromolecular molecule structures. Detailed description of the five experiments and all available material including the raw X-ray data will be made available for download from the authors'webpages.

Keywords: crystallographic teaching, X-ray diffraction of macromolecules, three-dimensional protein structure

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The web-based teaching in the Institute of Structural and Molecular Biology, University of London

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Birkbeck, University of London initiated the teaching of structural biology over the web in 1995, with the inaugural Principles of Protein Structure Course. This course can now be combined with a further web based modules in Protein Crystallography or Techniques in Structural Molecular Biology and project work to form the MSc in Structural Molecular Biology http://www.cryst.bbk.ac.uk/mscstructuralbiology.html. Students study blocks of course material released as password protected web pages. They interact with the module tutors via email/chat rooms and coursework is submitted electronically either via web forms or email. Written exams can be sat in universities or British Council offices around the world bypassing the need to travel to Birkbeck. The Commonwealth Scholarship Commission has awarded us scholarships for students based in developing Commonwealth nations, and internet bandwidth is still very much a consideration for these students. Nevertheless improved computer performance over the last 12 years has made delivery of the course easier. This is illustrated by the fact that in the early years, students would upload program scripts to servers at Birkbeck in order to perform crystallographic calculations, but can now run CCP4 software on their own machines. Similarly, Jmol images embedded in web pages have largely replaced downloading RasMol scripts.

Keywords: teaching aids, world wide web, teaching of crystallography