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Auto-Rickshaw: An online tool for validation of an X-ray diffraction experiment

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We present automated crystal structure determination platform, Auto-Rickshaw[1], that combines invoking of a number of macromolecular crystallographic software packages with several decision-making steps in between. A large number of structure solution paths are encoded in the system and the optimal path is selected by the decision-makers as the structure solution evolves. The Auto-Rickshaw phasing protocols include SAD, SIRAS, MAD, MR and MRSAD.The primary aim of the pipeline is to validate the crystallographic experiment at the synchrotron site while the crystal is still at or near the beamline. Thus, the system has been optimized for speed, so that typically within a few minutes the answer is provided whether the collected data will be of sufficient quality to allow successful structure determination. The EMBL-Hamburg beamline user have been successfully using this system for a little over three years. It is now available as a web serivce (http://www.embl-hamburg.de/Auto-Rickshaw) in the structural biology community. An overview of the Auto-Rickshaw pipeline with its design, functionality, decision making and some examples and the way this platform is used as a feedback system for X-ray data collection or validation of X-ray experiment, will be discussed.

Keywords: auto-Rickshaw, phasing, data validation

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AutoPROC - A framework for automated data processing

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autoPROC is a set of tools and programs to automate the steps performed during data processing: (a) determination of correct direct beam coordinates, (b) indexing of diffraction images, (c) determination of accurate cell parameters, (d) integration of a series of images, (e) scaling and merging of each sweep separately as well as of a collection of related sweeps, (f) analysis of anomalous signal, (g) automatic determination of most-likely space group symmetry. It is designed to be independent of the actual program(s) used for the various steps. The current version offers complete processing streams based on either MOSFLM [1] or XDS [2], with crossing points between them at various levels. The scaling and merging step - which is also available as a stand-alone module - has been implemented using SCALA [3]. autoPROC has been developed towards the simultaneous treatment of multi-component data sets such as MAD, inverse-beam SAD or multi-sweep data sets related by an action of a kappa goniometer. This involves pooling, converting and re-using common information about indexing matrices at various wavelengths and for different kappa settings, and ensuring totally coherent indexing between all images sets.

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Keywords: data processing, automation, crystallographic software

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Structure of AcrB: A novel mechanism for multidrug resistance

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The AcrA-AcrB-TolC complex is the major multidrug efflux pump in Escherichia coli. The asymmetric structure of the trimeric inner-membrane component AcrB (1,2) implies functional rotation of the monomers and a peristaltic mode of drug efflux. This mechanism suggests the occurrence of conformational changes in the periplasmic pore domain through the movements of subdomains during cycling of the monomers through the different states loose (L), tight (T) and open (O). Recently, we tested this hypothesis by introducing cysteines at the interfaces of potentially moving subdomains, and obtained disulfide bond formation as quantified by alkylation of free cysteines and MALDI-TOF analysis (3). Cross-linking indeed increased susceptibility to noxious compounds , and caused inhibition of pump function which could be regained upon exposure to the reducing agent DTT.

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Keywords: antibiotic resistance, membrane proteins, membrane transport

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Structural description of the ATPase cycle of a myosin that moves backward

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Myosins are molecular motors that use the energy obtained from the hydrolysis of ATP to move along actin filaments. Among myosin family, class VI myosins are very intriguing because of their atypical motility properties. First, dimeric myosin VI is capable of taking multiple steps (processive movement) of 30-36 nm along actin filament. These steps are surprisingly very large considering that

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

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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