

Poster Sessions

Pelota and *S. cerevisiae* Dom34. Different overall conformations are due to conformational flexibility of the two linker regions between domains 1 and 2 and between domains 2 and 3. The observed inter-domain structural plasticity of Pelota proteins suggests that large conformational changes are essential for their functions [1].

[1] H.H. Lee, J.Y. Jang, H.J. Yoon, S.J. Kim, S.W. Suh, *Biochem Biophys Res Commun* **2010**, 399(4), 600-606.

Keywords: conformational flexibility, no-go decay, ribonuclease

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Structural studies on piRNA recognition by mammalian PIWI-like Argonaute proteins

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RNA silencing is a mechanism of gene regulation that affects virtually all biological processes. In combination with a family of proteins called the Argonautes (AGOs and PIWIs), small RNAs target larger nucleic acids (e.g. mRNAs) in a sequence-defined manner to turn down the expression of specific genes. Based on sequence analyses, different classes of small RNAs are enriched for specific nucleobases at their 5' end. The Argonaute MID domain anchors the 5'-phosphorylated end of the small RNA guide strand. This interaction is necessary for coupling small RNAs to one of the multiple Argonautes present in the cell, which can then perform its downstream effector activity. Our group recently discovered the molecular basis of human AGO2 selectivity for U and A at the 5'-end of micro RNAs (miRNAs). We showed that a short rigid loop with a specific conformation in the AGO2 MID domain confers this selectivity exclusively via protein backbone interactions [1]. Like miRNAs, PIWI-interacting RNAs (piRNAs) show a strong preference for U at the 5'-end of the guide RNA strand [2]. A question that arises from these observations is whether PIWIs can enforce the same type of selection on piRNAs via the identity of their 5'-nucleotides.

To answer this question, we determined the first crystal structures of MID domains from mammalian PIWI-like Argonautes. Although their structures show the same overall fold as human AGO2 there is a substantial difference in the positioning of the C-terminal alpha helix. This results in a loss of a critical lysine residue that is normally required to bind the phosphate group of the 5'-nucleotide in AGO-like Argonautes. Additionally, the nucleotide specificity loop in PIWI has a different conformation relative to that observed in AGO2 and suggests that side chains may also be involved in interacting with the base. The impact of these differences on interaction with piRNA will be studied using NMR titration experiments and functional assays on full-length PIWI-like Argonautes.

[1] F. Frank, N. Sonenberg & B. Nagar, *Nature* **2010**, 465, 818-822. [2] Thomson, T. & Lin, H. *Annu Rev Cell Dev Biol* **2009**, 25, 355-376.

Keywords: RNA silencing, Argonautes, MID domain

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Optimal fine ϕ slicing for single photon counting pixel detectors

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The data collection parameters used in a diffraction experiment have a strong impact on the quality of the acquired data. A careful choice of parameters leads to better data and can make the difference between success and failure in phasing attempts and better data will also result in a more accurate atomic model. The selection of data acquisition parameters has to account for the application of the data in various phasing methods or high-resolution refinement [1]. Furthermore, experimental factors like crystal characteristics, available experiment time, and the properties of X-ray source and detector have to be considered.

CCD detectors are for many years the prevalent type of detectors used in macromolecular crystallography. Most recommendations for data collection strategies as well as the experience of the experimenters are based on the characteristics of this detector type. Recently, hybrid pixel X-ray detectors that operate in single-photon-counting mode became available for macromolecular crystallography [2,3]. The commercially available PILATUS hybrid pixel detector is now in standard user operation at an increasing number of macromolecular crystallography synchrotron beamlines. Hybrid pixel detectors have fundamentally different characteristics and offer various advantages over CCD detectors [3,4]: (i) No readout noise and dark current. (ii) A sharp point spread function of one pixel. (iii) A short readout time in the millisecond range. (iv) A high dynamic range of 20 bits.

To fully exploit the advantages of hybrid pixel detectors different data collection strategies than those established for CCD detectors have to be applied because of the different characteristics of the two types of detectors. Fine ϕ slicing is a strategy particularly well suited for hybrid pixel detectors because of the fast readout time and the absence of readout noise. We systematically collected a large number of data sets from crystals of four different proteins to investigate the benefit of fine ϕ slicing on data quality with a noise-free detector in practice. Our results show that fine ϕ slicing can substantially improve scaling statistics and anomalous signal but potentially problems can arise when $\Delta\phi$ is only a small fraction of the crystal mosaicity.

[1] Z. Dauter, *Acta Crystallographica Section D* **2010**, 66, 389-392. [2] C. Broennimann, E.F. Eikenberry, B. Henrich, R. Horisberger, G. E. Huelsen, Pohl, B. Schmitt, C. Schulze-Briese, M. Suzuki, T. Tomizaki, et al., *Journal of Synchrotron Radiation* **2006**, 13, 120-130. [3] G. Hülsen, C. Broennimann, E.F. Eikenberry, A. Wagner, *Journal of Applied Crystallography* **2006**, 39, 550-557. [4] M.W. Tate, E.F. Eikenberry, S.M. Gruner, In *International Tables for Crystallography, Vol. F*. Edited by Rossmann MG, Arnold E. **2006**:148-153.

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Cases of Twinning at the Joint Center for Structural Genomics

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Twinning has been observed in a number of structures at the Joint Center for Structural Genomics (JCSG). Eighteen examples have been refined and deposited in the PDB of which fourteen were solved by MAD and the rest by molecular replacement. Several other targets that had twinned crystals also had non-twinned crystal forms that were ultimately solved and deposited. The twins can be classified as

merohedral (twin law matching a symmetry operation in the crystal system, but not crystal point group) and pseudo-merohedral (where the twin law belongs to a higher symmetry lattice than the structure obeys). The most common type of merohedral twinning is hemihedral involving two twin domains. We have also observed two cases with four twin domains (tetartohedral; PDBids 2PRX and 3NUZ). Improvements in software have simplified the detection and treatment of twinned data. When the project was started, twin refinement was limited to SHELXL and CNS or using detwinned data in cases of low twin fractions. The addition of twin refinement to phenix.refine and remlac has expanded the options for refining twinned data. A review of the twinning cases at the JCSG provides a guide for the characterization, solution and refinement of twinned structures.

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Keywords: twinning, structural genomics, macromolecular crystallography

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Advances in the CRANK software suite for automated crystal structure solution

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CRANK is a suite for automated macromolecular crystal structure solution that enables different crystallographic programs to seamlessly communicate. The current release can build structures automatically from single- and multiple-wavelength anomalous diffraction data and single isomorphous replacement with anomalous scattering data [1]. The latest release of CRANK includes several new algorithms which both increase robustness and speed up the automatic structure solution process.

Several improvements were made in substructure determination. While most programs use the absolute value of Bijvoet differences, $\Delta F = |F^+| - |F^-|$, as an estimate of $|F_A|$, a multivariate joint probability distribution, implemented in the AFRO program, is used in CRANK to obtain more accurate values for $|F_A|$. The substructure determination process was sped up substantially by allowing substructure detection to be terminated early without running all trials and by quickly evaluating whether a correct solution for the substructure was located.

In density modification it is often assumed that the initial and density-modified map are independent. We have developed a multivariate function for phase combination that rectifies this assumption by considering the observed Friedel pairs directly from a SAD experiment, accounting for the correlation between the initial and density-modified maps and refining the errors that can occur in a single-wavelength anomalous diffraction experiment. The maps produced by this multivariate phase combination program lead to many more structures being built automatically [2]. We also recently implemented a new cross-validated scheme for accurate error-parameter estimation in likelihood-based phase combination that results in improved phase probability and figure of merit estimates [3].

The use of experimental phase information in refinement is known to improve automated model building results. For SAD and SIRAS [4] experiments CRANK uses a multivariate likelihood function implemented in the program REFMAC [5], that takes as input the diffraction data, heavy atom coordinates and the calculated structure factors and accounts for the correlation between them. By using all

experimental information directly, the multivariate functions overcome limitations of the function that uses Hendrickson–Lattman coefficients to incorporate experimental phase information in refinement.

CRANK can be run either via a command line program GCX or through a *ccp4i* graphical user interface: both require only minimal input to run. Users however may also set-up a custom-made pipeline using any program at each step, customize variables for the individual steps and define the start and end step for a pipeline.

CRANK is licensed under GPL v2 and available from the CCP4 suite (www.ccp4.ac.uk) or www.bfsc.leidenuniv.nl/software/crank

[1] N.S. Pannu et al., *Acta Cryst.*, **2011**, *D67*, 331–337. [2] W.-J. Waterreus, P. Skubák et al., *Acta Cryst.*, **2010**, *D66*, 783–788. [3] P. Skubák, N.S. Pannu, *Acta Cryst.*, **2011**, *D67*, 345–354. [4] P. Skubák, G.N. Murshudov, N.S. Pannu, *Acta Cryst.*, **2009**, *D65*, 1051–1061. [5] Murshudov et al., *Acta Cryst.*, **2011**, *D67*, 355–367.

Keywords: automation, phasing, software

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Novel Approach to Automatic Scoring of Protein Drop Images Using UV Fluorescence

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Analyzing vast numbers of images is a time consuming bottleneck that affects most protein crystallization experiments. So far, software based image analysis tools for automatic scoring and ranking these images have generally failed because of various image artifacts caused by plates, lighting, and drop geometry. The recent commercial introduction of UV fluorescence imaging for protein crystallization has brought new opportunities for simpler and more reliable approaches to image analysis. Most existing analysis tools rank images using methods such as edge, shape, intensity, and frequency analysis.

With the new UV technologies it is now possible to rank based on protein fluorescence which also has the advantage of differentiating protein versus salt. We have developed a new analysis tool that when integrated with the CrystalTrak software provides the user with a fluorescence score which can be used to filter images from the image viewer those images with little to no fluorescence. This greatly reduces the number of images needing review. Due to the fact that the UV fluorescence images are free of many of the artifacts found in visible images the algorithm has been shown to be very reliable at eliminating clear drops with no false negatives. The methods used produce a score that is fundamentally a ratio of fluorescence signal versus background noise. The user then has the ability to set their own threshold based on this score determining how sensitive they want the algorithm to be and filter as many images as desired. Due to the fact that the Rigaku Minstrel HT UV uses the same optics for the visible and UV images the analysis tool also provides the ability to overlay the detected fluorescence signal over top of the visible images to highlight the items of interest in the visible image.

Keywords: biomacromolecule, crystallization, microscopy

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Fully Automated Cryogenic Crystal Screening System

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