

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 (AGO_s and PIWI_s), 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.

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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