Oral Contributions

[MS1] XFEL and time resolved crystallographic methods

Co-Chairs: John Helliwell (UK), Victor Lamzin (DE)

[MS1-01] Free electron laser radiation and *in vivo* grown nano-crystals open new routes in structural biology and options for time resolved Experiments

L. Redecke[a,b], Ch. Betzel[a], D. Rehders[a,b], K. Nass[c], D.P. DePonte[c], T. White[c], M. Duszenko[d], J. Spence[e], P. Fromme[f], I. Schlichting[g], H. Chapman[c],

[a]*University of Hamburg, Germany,*
[b]*University of Lübeck, Germany,*
[c]*Centre for Free Electron Laser Science (CFEL) DESY, Hamburg, Germany,*
[d]*University of Tübingen, Germany,*
[e]*Department of Physics, Arizona State University, Tempe, Arizona, USA,*
[f]*Department of Chemistry and Biochemistry, Arizona State University, Tempe, Arizona, USA,*
[g]*Max-Planck-Institut for Medical Research, Heidelberg, Germany.*

E-mail: Redecke@chemie.uni-hamburg.de

The recently established method of Free-Electron Laser (FEL) based Serial Femtosecond Crystallography (SFX) [1] was successfully utilized to collect high resolution diffraction of two key enzymes of the parasite *Trypanosoma brucei,* known to cause sleeping sickness, applying for the first time *in vivo* grown crystals for data collection. The refined structures, reliability factors and refinement statistics confirmed that the combination of *in vivo* grown microcrystals and the "diffraction before destruction" approach of XFELs provides a tremendous new option to analyse structure-function-relationships of proteins and protein complexes, which could not be analysed so far by conventional crystallography. Therefore, upon commissioning of future FELs, like the European XFEL in Hamburg, this method will certainly substantial speed up protein structure determination and will open also options to perform time resolved experiments, particular by eliminating the requirement for large and well-diffracting crystals.

Protein crystallization within cells has already been observed and reported several times before as a native process in cells. Nevertheless, no structural data of *in vivo* grown crystals have been obtained, because these crystals are rather small and were detected before mainly applying electron microscopy. During the last years we optimized methods and procedures to produce *in vivo* crystals of heterologously expressed proteins using the baculovirus/*Sf*9 system, as well as to harvest and to score *in vivo* grown crystals and micro crystal suspensions suitable for FEL applications [2]. Latest results and procedures will be presented accordingly.

Diffraction data of *in vivo* grown microcrystals were obtained at the Coherent X-ray Imaging (CXI) beamline [3] at LCLS by injecting TbIMPDH and TbCatB microcrystal suspensions across the FEL beam in vacuum. Hundreds of thousands of single-pulse diffraction pattern were recorded at room temperature. The protein structures were solved by molecular replacement and refined to high resolution. The room temperature TbCatB structure in complex with its pro-peptide provides most valuable information supporting future drug discovery investigations, particular considering the so far unknown native inhibition of TbCatB and essential structural differences caused by the extended pro-peptide [4].

In summary result to be presented revealed that high resolution structural information can be obtained from *in vivo* grown crystals applying FEL radiation. Therefore, the combination of *in vivo* crystallization and SFX open new routes in structural and systems biology.

[1] Chapman HN *et al*. Femtosecond X-ray protein nanocrystallography. *Nature* **470**, 73-77 (2011).

[2] Koopmann R, Kupelli K, Redecke L *et al*. *In vivo* protein crystallization opens new routes in structural biology. *Nat. Methods* **9**, 259-262 (2012).

[3] Boutet, S. & Williams, G.J. The Coherent X-ray Imaging (CXI) instrument at the Linac Coherent Light Source (LCLS). *New J. Phys.* **12**, 035024 (2010).

[4] Redecke L, Nass K *et al.* Native inhibition of *Trypanosoma brucei* cathepsin B revealed by an X-ray laser at 2.1 Å resolution. *Science* **339**, 227-230 (2012).

Keywords: in vivo crystallization, nano-crystals, free-electron laser radiation