ESRF	**Experiment title:** Room-temperature nanocrystallography on nanoslices of resin-embedded protein nanocrystals	**Experiment number**: LS-2253
Beamline: ID13	**Date of experiment**: from: April, 19th, 2014 to: April, 21st, 2014 July, 27th, 2014 July, 29th, 2014	**Date of report**: Sept. 10th, 2015
Shifts: 12	**Local contact(s)**: Manfred Burghammer	*Received at ESRF:*

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Report:

First of all, we wish to present our sincere apologies to the steering committee for not having submitted our report sooner. We understand the importance of such reports for the ESRF and shall not repeat this mistake.

The initial goal of our proposal was to develop a new sample preparation procedure, whereby crystals were flashcooled under high pressure (5kbar) in absence of cryoprotective agents, and the water replaced by a polymeric resin at cryogenic temperature. The obtained block of resin was then subjected to microtome slicing at RT, generating thin layers (200 to 2,000 nm thickness) of trapped nanocrystals. Our expectation was that this sample preparation procedure would ease crystals visualization, decrease background scattering and allow collecting data at room temperature using a serial, mesh-based nanocrystallography approach.

However, crystals did not survive when prepared in this fashion, and after two shifts of screening of nanosclices, we decided to resort to another sample presentation procedure. In this alternative approach, crystals are also presented to the X-ray micro-beam in a raster scanning fashion, but this time following the sandwiching of a crystal slurry between two silicon nitride wafers at RT. A compelling advantage of this technique is that it only requires 200-600 nl of settled crystals to yield a composite dataset. The sandwiching of the crystal slurry between two silicon nitride wafers indeed offers the advantage of drastically increasing the hit-rate and thereby the quality of the merged data.

We published part of these data in Acta Crystallographica section D this year (Coquelle et al., 2015) and the publication was highlighted on the IBS website (http://www.ibs.fr/science/faits-marquants/2015/), in the IBS Actualités magazine (http://www.ibs.fr/IMG/pdf/lettre_externe_2015_06_no34.pdf) and in the PSB newsletter (http://www.psb-grenoble.eu/IMG/pdf/july2015-web.pdf). The remaining data include that collected on an amyloid peptide from *Providencia stuartii* at sub-1Å resolution (Nasrallah et al., in preparation). Since then, the methodology has been used to study a number of other systems, including fluorescent proteins such as rsEGFP2 and IrisFP (LS-2463) and a functional amyloid oligomer secreted by

Staphylococcus aureus during biofilm formation (LS-2583; Moshe et al., in preparation). Furthermore, the data pre-processing software that we initially developed for 'easy' processing of serial diffraction data from ID13, NanoPealCell (Coquelle et al., 2015), can now also be used at XFEL sources such as SACLA and LCLS to both pre-process static and time-resolved crystallography data (Coquelle et al., in preparation). Briefly, NanoPealCell reads data from a variety of formats, hit-finds, background-subtracts and Bragg-peak searches images, and convert indexable files together with their metadata (experiment metrology and Bragg-peak locations) for seemingless integration with CrystFEL, cctbx.xfel (.pickle) and nXDS (.cbf).

We thank the steering committee for having allowed us to perform these experiments and progress in the field of serial crystallography. Below we past the abstract of the article that was published earlier this year (Coquelle et al. 2015) as well as the complete reference

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Raster-scanning serial protein crystallography using micro- and nano-focused synchrotron beams

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Supporting information: this article has supporting information at journals.iucr.org/d

High-resolution structural information was obtained from lysozyme microcrystals (20 µm in the largest dimension) using raster-scanning serial protein crystallography on micro- and nano-focused beamlines at the ESRF. Data were collected at room temperature (RT) from crystals sandwiched between two silicon nitride wafers, thereby preventing their drying, while limiting background scattering and sample consumption. In order to identify crystal hits, new multi-processing and GUI-driven Python-based pre-analysis software was developed, named *NanoPeakCell*, that was able to read data from a variety of crystallographic image formats. Further data processing was carried out using *CrystFEL*, and the resultant structures were refined to 1.7 Å resolution. The data demonstrate the feasibility of RT raster-scanning serial micro- and nano-protein crystallography at synchrotrons and validate it as an alternative approach for the collection of high-resolution structural data from micro-sized crystals. Advantages of the proposed approach are its thriftiness, its handling-free nature, the reduced amount of sample required, the adjustable hit rate, the high indexing rate and the minimization of background scattering.

Raster-scanning serial protein crystallography using micro- and nano- focused synchrotron beams. N. Coquelle, A. S. Brewster, U. Kapp, A. Shilova, *et al.* (2015). Acta Crystallogr D, 71, 1184-96.