Crystals in living insect cells: Of detection, serial diffraction and genuine co-factor discovery

J.M. Lahey-Rudolph[1,2,3], J. Boger[2], R. Schönherr[2], L. Redecke[2,3]

[1] Technical University of Applied Sciences/ TH Lübeck, Mönkhofer Weg 231, 23562 Lübeck, [2] Institute of Biochemistry, University of Lübeck, Ratzeburger Allee 160, 23562 Lübeck, Germany, [3] DESY, Notkestraße 61, Hamburg, Germany
Mia.lahey-rudolph@th-luebeck.de

Proteins crystallize surprisingly often within living cells and across all domains of life. [1] Our research focuses on refining a systematic approach, InCellCryst, to leverage in cellulo crystallization in insect cells for structural biology. This crystallization approach can rapidly generate millions of micron-sized, high-quality protein crystals within the densely populated environment of the cell [2-3].

InCellCryst is a streamlined process, from cloning the gene of interest via crystallization in specific cellular compartments, over crystal detection and diffraction in living cells to structure solution of the target protein. [3] Detection of intracellular crystal formation via advanced microscopy methods is supplemented by an innovative screening approach combining SAXS and X-ray powder detection directly from infected cell cultures. [4] Upon successful intracellular crystallization, serial crystallography techniques are applied to the crystals inside the cells (Fig. 1). XFEL radiation or third-generation synchrotron sources can be utilized, based on the crystal diffraction volume and beam availability. [3,6-7] Notably, the insect cell supplies potentially binding cofactors which offers a highly interesting chance for discovering and identifying genuine high-affinity ligands in high-resolution electron density maps. We report GDP and ATP, binding in alternate conformations, as genuine co-factors of the IMPDH of *Trypanosoma brucei*, a pathogen causing the sleeping sickness [4,6].

The in cellulo crystallization approach presents a promising addition to traditional crystallization and soaking method. Limitations are mostly associated with limited manipulation possibilities of crystal nucleation events and crystal morphology for a given target, due to the protected environment of a membrane-surrounded cell compartment. The in cellulo technique is unsuitable for time-resolved serial crystallography experiments, and so far, 3D membrane proteins were not reported in living insect cells. Presented results pave the way to a more efficient use of crystal containing insect cells for serial diffraction data collection at synchrotrons and XFELs.

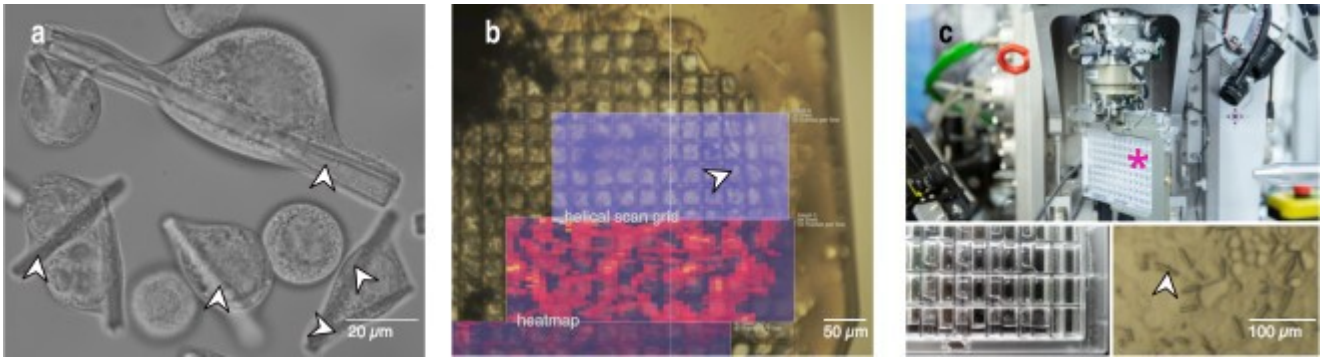

Figure 1. *a, Trypanosoma brucei* IMPDH protein crystalized in High Five insect cells; *b*, Serial diffraction data collection of intact crystal-containing insect cells with a MiTeGen MicroMesh™ [3]; *c*, In situ in cellulo diffraction data collection in mounted CrystalDirect™ plate, X-ray interaction region at *[3]. Arrows point at intracellular crystals.

[1] Schönherr, R. Lahey-Rudolph, JM. and Redecke, L., (2018). *Biol. Chem.,* **399** (7), 751–772.

[2] Schönherr*, R., Klinge*, M., Rudolph, J.M., Fita, K., Rehders, D., Lübber, F., Schneegans, S., Majoul, I.V., Duszenko, M., Betzel, C., Brandariz- Nunez, A., Martinez-Costas, J., Duden, R., Redecke, L. (2015), *Struct. Dyn*, **2**.

[3] Schönherr, R.*, Boger, J.*, Lahey-Rudolph, JM.*, Harms, M., Kaiser, J., Nachtschatt, S., Wobbe, M., Duden, R., König, P., Bourenkov, G., Schneider T.R. and Redecke, L. (2024), *Nat. Commun*., **15**:1709.

[4] Lahey-Rudolph, J.M., Schönherr, R., Jeffries, C.M., Blanchet, C.E., Boger, J., Ferreira Ramos, A.S., Riekehr, W.M., Triandafillidis, D.-P., Valmas, A., Margiolaki, I, Svergun, D., Redecke, L. (2020) *JAC*, **53**, 1168-1180.

[5] Nass*, K., Redecke*, L., (…), Lahey-Rudolph, J.M., (…) Chapman, H.N., Betzel, C. (2020), *Nat. Commun*., **11**, 620.

[6] Lahey-Rudolph, JM. et al. (2021) *IUCrJ*, 8, 665-677.

[7] Norton-Baker, B., Mehrhabi, P., Boger, J., Schönherr, R., von Stetten, D., Schikora, S., Kwok, A.O., Martin, R.W., Miller, R.J.D., Redecke, L., Schulz, E.C. (2021). *Acta Cryst.* **D77**, 820-834.