Poster Presentation

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In vivo protein crystallization in living insect cells

L. Redecke[1], M. Klinge[1], R. Schönherr[2], D. Rehders[1], D. Oberthür[3,4], B. Sommer[4,5], J. Martinez-Costas[6], M. Duszenko[5], R. Duden[2], C. Betzel[4]

[1]University of Hamburg and University of Lübeck, Joint Laboratory for Structural Biology of Infection and Inflammation, Hamburg, Germany, [2]University of Lübeck, Institute of Biology, Lübeck, Germany, [3]Center for Free-Electron Laser Science (CFEL), Hamburg, Germany, [4]University of Hamburg, Institute of Biochemistry, Hamburg, Germany, [5]University of Tübingen, Interfaculty Institute of Biochemistry, Tübingen, Germany, [6]University of Santiago de Compostela, Institute of Biochemistry, Santiago de Compostela, Spain

Spontaneous protein crystallization within living cells has been observed several times in nature, e.g. for storage proteins in seeds. In vivo crystal growth can also occur during gene over-expression, as particularly discovered in baculovirus-infected insect cells [1]. We have recently shown that these in vivo crystals represent valuable targets for structural biology after isolation from the cell. Applying serial crystallography techniques at an X-ray free-electron laser (XFEL) as well as using a highly brilliant synchrotron source, single crystal diffraction pattern were collected and combined to yield high-resolution structural information of the associated fully glycosylated protein [2,3]. So far, the cellular mechanisms involved in the in vivo crystallization process remain to be understood, preventing a more successful application of this novel approach. Thus, our study aims at identifying the parameters crucial for optimal crystal growth within baculovirus-infected Sf9 insect cells. Combining confocal microscopy with live-cell imaging techniques and compartment-specific staining methods, we systematically investigated the impact of the intracellular environment on in vivo crystallization by directing recombinant proteins into different cellular compartments using specific signal sequences. Moreover, the impact of cellular transport mechanisms and induced cellular stress on the quality and size of the in vivo crystals was investigated in detail. The presented results provide important insights into the process of protein crystallization within living cells and will therefore significantly contribute to increase the success rate for spontaneous crystal growth of other proteins. Considering that in vivo crystals represent highly suitable targets for structural biology, this approach offers exciting new possibilities for proteins that do not form crystals suitable for conventional X-ray diffraction in vitro.

[1] *R. Koopmann*, K. Kupelli*, L. Redecke* et al. Nat. Methods (2012) 9, 259-262,* ***[2]*** *L. Redecke*, K. Nass* et al. Science (2013) 339, 227-231,* ***[3]*** *C. Gati*, G. Bourenkov* et al. IUCrJ (2014) 1, doi:10.1107/S2052252513033939*

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