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Serial femtosecond X-ray diffraction of in vivo crystals in intact yeast cells

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Peroxisomes are membrane-enclosed organelles in eukaryotic cells with important roles in lipid metabolism and the scavenging of reactive oxygen species. Peroxisomes are capable of carrying an unusually high load of proteins, which under appropriate nutrient conditions results in the in situ crystallization of peroxisomal proteins in several yeast species and vertebrate hepatocytes [1,2]. In the methylotrophic yeast H.polymorpha, the predominant peroxisomal protein alcohol/methanol oxidase (AO) oligomerizes into octameric assemblies with a molecular mass of 600 kDa that spontaneously form 200-500 nm crystallites within peroxisomes [1]. We exposed H.polymorpha cell suspensions containing peroxisome-confined AO crystallites to femtosecond X-ray pulses at the Coherent X-ray Imaging (CXI) experimental endstation at the Linac Coherent Light Source. Peak detection routines mining the resulting scattering profiles identified >5000 Bragg-sampled diffraction patterns, providing the proof of concept that background scattering from the cells does not deteriorate the signal-to-noise ratio to an extent precluding observation of diffraction from individual AO crystallites. Summation patterns assembled from the individual frames match low-resolution powder diffraction patterns from concentrated suspensions of purified peroxisomes collected at the P14 beamline at the PETRAIII synchrotron, confirming that the observed diffraction mainly results from Bragg scattering of peroxisomal crystallites. To the best of our knowledge our data are the first to report room temperature X-ray diffraction from functional protein crystals in their native cellular environment. Currently the maximum resolution achieved in the diffraction patterns is limited to 20-15 Å. Future work will need to address improved sample preparation protocols in order to assess whether diffraction to a resolution sufficient to permit structure solution can be obtained. Protein crystal formation in vivo has been observed under physiological or pathological conditions in a number of other systems [3]. We hope that our results will help to establish serial femtosecond X-ray diffraction (SFX) as a method for structural characterization of cellular structures with crystalline content and provide a proof of concept for using in situ crystallization of proteins as a means to generate nanocrystalline samples for SFX.

[1] *M Veenhuis et al., Substructure of crystalline peroxisomes in methanol-grown Hansenula polymorpha: evidence for an in vivo crystal of alcohol oxidase. Mol Cell Biol 10: 949-957 (1981),* ***[2]*** *F Angermuller and HD Fahimi, Ultrastructural cytochemical localization of uricase in peroxisomes of rat liver. Histochem Cytochem 34: 159-165 (1986),* ***[3]*** *JPK Doye and WCK Poon, Protein crystallization in vivo. Curr Opin Colloid Interface Sci 11: 40-46 (2006)*

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