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Femtosecond imaging of giant-hemeprotein with XFEL pulses

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Outrunning radiation damage, extremely intense femtosecond pulses of x-ray free-electron lasers (XFELs) open up the possibility of imaging the structure and dynamics of macromolecules frozen in time at room temperature. Single-Particle Imaging (SPI) of uncrystallized macromolecules at high- resolution is one of the most-desired foundational applications of XFELs and one of the ultimate goals of SPI is to image the light-induced ultrafast (ps/fs) dynamics in single-macromolecules. Biological proteins exhibiting photoinduced ultrafast dynamics are in the range of tens to few-hundreds of kDa, which are too small for SPI with the photon flux of current generation XFELs. Ideally, one needs MDa-sized, strongly scattering, photoactive proteins for such challenging endeavours to discern the signal above the background scattering and to obtain difference-maps with sufficient quality to elucidate photoinduced changes empirically. Photoactive proteins are sparse in nature and MDa-sized photoactive macromolecules are extremely rare. We have found one such rare MDa-sized photoactive, porphyrin-containing, large-metalloprotein-complex, the giant-hemeprotein—erythrocruorin (Ery) and show that Ery is likely suitable for validating the ultimate potential of SPI. We present the cryoEM characterization of stability of Ery in the unique experimental conditions of XFEL-SPI together with simulated single-molecule diffraction and 3D inten- sity reconstruction using expand-maximize-compress (EMC) algorithm and propose a potential SPI roadmap to demonstrate the imaging of ps/fs resolved bio-functional dynamics in Ery with XFEL pulses. Also, here we report the first in-solution ensemble fluctuation scattering results of Ery with the microfocus hard x-ray FEL pulses at the Linac Coherent Light Source (LCLS), USA—a step towards single-molecule imaging of Ery in-solution with nanofocus. We envisage that the robust giant-hemeprotein Ery could likely be an archetype biological macromolecular system enabling the imaging of light-induced ultra- fast (ps/fs) dynamics such as protein-quake following heme-doming in isolated single-proteins—“the Holy Grail” of XFEL-SPI.