Oral presentation

Fine slicing or instant crystal growth: two different approaches to prepare macromolecular crystalline samples for 3D electron diffraction experiments

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Electron diffraction of 3D nanometer sized crystals, most commonly named microED or 3D ED, has recently emerged as a new technique to solve the structure of both small organic molecules and proteins [1–3]. 3D ED is clearly a promising technique in structural biology, both quite easy to implement and very complementary to X-ray crystallography and single particle cryo-EM. Electrons are actually a very interesting probe for small samples as they strongly interact with matter, and more importantly, they deposit much less energy than X-rays per diffracted particle [4]. Sub-atomic resolution data can routinely be collected for small organic compounds and data up to 0.9 Å resolution have been obtained from protein crystals [5]. However, the major bottleneck of the technique when applied to protein samples is to produce nano-crystals that do not exceed 200 to 300 nm in at least one dimension and to deposit them on a grid while keeping the minimum amount of solvent around them. Here, we present two possible approaches. One uses cryo-sectioning after high pressure freezing of dextran embedded protein crystals. 150 to 200 nm thick cryo-sections of hen egg white lysozyme tetragonal crystals were used for electron diffraction experiments. Complete diffraction data up to 2.9 Å resolution were collected and the lysozyme structure was solved by molecular replacement and refined against these data [6]. The second approach used minute made lysozyme nano-crystals. By mixing lysozyme dissolved in water with a crystallant solution containing NaCl and Tb-Xo4 [7], a milky suspension of nanometer to micrometer size crystals were obtained that proved perfectly appropriate for 3D ED studies. Complete diffraction data up to 3.2 Å resolution were collected on 5 nano-crystals and the lysozyme structure was solved by molecular replacement with the Tb-Xo4 compound clearly visible in the residual $\{F_{obs} - F_{calc}\}$ Coulomb potential map [8]. Despite the speed of crystal growth, these crystals appear perfectly suitable for structural investigations.

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