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# Pipeline for in cellulo structure determination

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The production of diffraction-quality crystals remains the major bottleneck in X-ray crystallography, as shown by data from the main structural biology consortia. By contrast, in certain systems, crystals grow readily in the complex environment of the cell used to express the protein, be it in the natural context or in a recombinant system for overexpression. Recent interest in these in vivo crystals have
emerged in the context of a growing impact of microcrystallography brought by serial microcrystallography at synchrotron and X-ray free electron laser facilities.

Our aim is to set up a pipeline to streamline in cellulo diffraction, direct exposure of crystals to X-rays directly through the cells. Using in vivo crystals of the recombinant cypovirus polyhedrin, we show that crystal-containing cells could be selectively sorted by flow cytometry based on their higher side scattering. Crystal-containing cells were dyed with Trypan blue to achieve better visualisation, mounted on micromeshes and flash-frozen. Analysis of these cells on the MX2 beamline of the Australian Synchrotron show that high-quality diffraction data can be collected from in cellulo crystals. The structure of the polyhedrin protein determined by molecular replacement closely matches the model previously determined from purified micro-crystals. Advantages of this approach over conventional crystallography and future developments will be discussed in the presentation.

## Keywords or phrases (comma separated)

In vivo crystals, in cellulo diffraction

## Summary

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