MS08 Serial crystallography, obtaining structures from many crystals

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Xtrapol8: automatic elucidation of low-occupancy intermediate states in crystallographic studies

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Abstract

The ultimate goal of structural biology is to link structure to function but this connection remains difficult to make when only a single inanimate structural model is available. Accordingly, the structural characterization of intermediate states is of high interest and pursued by many structural biology groups. A big leap forward was taken with the advent of time-resolved serial crystallography at XFELs and synchrotrons, which enables researchers to follow the structural evolution of the crystalline structure after a specific trigger, opening avenues to produce movies of proteins at work. However, a major limitation remains that the occupancy of the intermediate state has to be large enough to become visible in the electron density map. This is generally not the case, with triggered crystals existing as mixtures of initial, intermediate and final state(s). Fortunately, powerful data processing strategies exist that can extract the intermediate state signal. Indeed, differences between the triggered and untriggered dataset can be visualized in Fourier difference (Fobs,triggered-Fobs,untriggered) electron density maps and extrapolated structure factor amplitudes (Fextr,triggered)[1] can be calculated that solely describe the intermediate state and can be used in crystallographic structure refinement. In the past, such data processing has been performed by some well-experienced crystallographers but remains up until today out of reach for most researchers.

Here we will present Xtrapol8, a program written in python, using the cctbx toolbox[2] (Phenix)[3] modules and CCP4 programs,[4] to make these approaches accessible to a wide audience of structural biologists, from well-experienced crystallographers to newcomers in the field. Briefly, Xtrapol8 allows the calculation of high-quality Fourier difference maps, estimation of the occupancy of the intermediate state(s) in the crystals, and generation of extrapolated structure factor amplitudes (Fig. 1). The program uses Bayesian statistics to weight structure factor amplitude differences[5,6] which are then used to generate extrapolated structure factor amplitudes for a range of possible intermediate state occupancies.[1,7] The correct occupancy of the intermediate state is determined based on the extrapolated electron densities and the automatically refined structures. With the possibility to launch Xtrapol8 via the command line or graphical user interface, and to control various parameters of which defaults are carefully chosen, the program is highly adapted to the user's expertise. We anticipate that it will ease and accelerate the handling of time-resolved structural data, and thereby the understanding of molecular processes underlying function in a variety of proteins.

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The main steps followed by Xtrapol8.