Interactive GUI for the calculation of Fobs-Fobs electron density difference maps and extrapolated structure factors based on the cctbx toolbox

De Zitter, E.¹, Coquelle, N.², Barends, T.³, Colletier, J.-P.¹

¹ Institut de Biologie Structurale, Université de Grenoble Alpes, CEA, CNRS, 38044 Grenoble, France, ² Structural biology Group, European Synchrotron Radiation Facility, 38044 Grenoble, France, ³ Department of Biomolecular Mechanisms, Max Planck Institute for Medical Research, 69120 Heidelberg, Germany

elke.de-zitter@ibs.fr

Since resolution of the first macromolecular structure, the goal of structural biology has been to link structure to function. It is now widely accepted that the latter emerges from the structural dynamics animating the macromolecule, making characterization of intermediate (and sometime excited) states of high interest to further understand molecular processes and possibly control them. With the advent of serial crystallography at X-ray free electron lasers and synchrotrons, time-resolved crystallography, performed following a specific perturbation of the crystalline system (laser excitation, substrate soak, etc), is on the verge of becoming feasible on virtually all systems opening avenues to characterize such excited and/or intermediates states. Because crystallography is an ensemble-averaged method, however, an inherent limitation is that the occupancy of intermediate states must be high enough for the "probed state" under investigation to become visible in the electron density. This is generally not the case, with "perturbed" crystals rather existing as mixtures of initial and/or final state(s) with the "probed" state. Differences in structure factor amplitudes between the reference and "perturbed" dataset can allow calculation of Fourier difference maps (Fobs, perturbed-Fobs, unperturbed), in which only the differences between the states are depicted. An even more powerful approach is to generate extrapolated structure factor amplitudes (F_{extr.perturbed}) solely describing the intermediate state and and to use these to refine its structure using conventional refinement tools. Such data processing has in the past been performed by a handful of well-experienced crystallographers with strong knowledge of existing software but remains out of reach for a wide audience.

Here, we will present a user-friendly program, Xtrapol8, written in python and exploiting the cctbx toolbox modules, that 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. Briefly, the program uses Bayesian statistics to weight structure factor amplitude differences [1] which are then used to generate extrapolated structure factor amplitudes for a range of possible intermediate state occupancies, with distinct weighting schemes [2, 3] (Figure 1). Based on the comparison between experimental and calculated differences, i.e. solely on experimental observations, the correct occupancy of the intermediate state is determined and its structure refined, shedding light on conformational changes not visible before. With various user-controllable parameters of which defaults are carefully chosen, the program is adapted to be used by a wide audience of structural biologists, ranging from well-experienced crystallographers to newcomers in the field. We anticipate that this program 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.

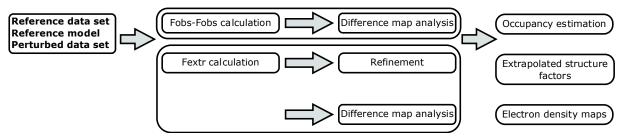


Figure 1. Xtrapol8 outline. The minimal input is shown in bold.

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