Protein Kinetics: Relaxations on Atomic Length Scales

M Schmidt[1], S Rajagopal[2], S Anderson[3], K Moffat[2,3,4]
[1] Physik-Department, TU-München, Germany, [2]Biochemistry and Molecular. Biology,[3]Consortium for Advanced Radiation Sources and [4]Institute for Biophyical Dynamics, University of Chicago, USA

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Enzyme kinetics describes reactions catalyzed by biomolecules in terms of chemical kinetics. Usually, relaxation times together with amplitudes which might be for example time-dependent absorption values are the only observables of such experiments. Intermediate states as well as possible chemical, kinetic mechanisms remain obscure and appreciable effort is necessary to extract them from the data.

On the atomic length scale transient kinetics can be observed by time-resolved macromolecular crystallography provided time is sampled in short intervals. At each time point the simultaneous population of multiple intermediate states generates structural admixtures. These mixtures must be separated to interpret the electron densities. This can be done by exploiting the time information present as the fourth dimension in the crystallographic experiment. The structures of the intermediates as well as the underlying chemical, kinetic mechanism can be extracted.

The key approach to this problem is to examine simultaneously the temporally varying difference electron densities of all grid points in the unit cell by employing a component analysis. Due to its numerical robustness the Singular Value Decomposition (SVD) is particularly well suited to analyze time-resolved crystallographic data [1]. After the SVD, the first few significant singular vectors contain the principal components of the decomposition whereas the remainder contains only noise; hence, the SVD is a convenient noise filter. As a by-product of the SVD time-smoothed difference maps are obtained. This process is called SVD-flattening. The improved maps can be used in further steps to extract pure and admixture free electron densities of the intermediates and to select compatible mechanism.

Photoactive Yellow protein is a role-molecule particularly well suited for a kinetic analysis after activation of its central chromophore by light. The singular value decomposition (SVD) was applied to time-resolved crystallographic data from the wild-type [2] as well as from the E49Q mutant [3] of PYP. The relaxation times of the transient kinetics were observable in the right singular vectors (rSV). From a fit of preliminary mechanisms, pure and time-independent difference electron densities of the intermediates were determined. These difference maps were used to derive the structures of the intermediates. In a following step, called posterior analysis, plausible kinetic mechanisms were derived which complete the picture of the PYP photocycle. With these new methods in hand time-resolved crystallography becomes an unrivaled tool for the kinetic analysis of processes of any kind in biological macro-molecules.

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