s1.m4.o5 **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. E-mail: marius@hexa.e17.physik.tu-muenchen.de*

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Enzyme kinetics describes reactions catalyzed by bio-molecules 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.

[1] Schmidt *et al*. (2003) *Biophys. J.* **84** 2112-2129
[2] Schmidt *et al*. (2004) *PNAS* **101** 4799-4804
[3] Rajagopal *et al*. (2004) *Acta Cryst.* (in press)

s1.m5.o1 **Adeno-associated Virus Replication Initiator Assembly**. Fred Dyda, Alison B. Hickman, Donald R. Ronning, *Robert M. Kotin Laboratory of Molecular Biology, NIDDK, NIH, Bethesda MD, 20892 U.S.A. E-mail: fred.dyda@nih.gov*

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The Rep protein of the adeno-associated virus (AAV) is essential both for viral replication and locus-specific integration of the viral genome into AAVS1, a silent region of human chromosome 19. Locus-specific integration, a property unique to AAV, is a desired property of vector systems used in gene therapy to establish safe and long term expression of the transgene. To understand the molecular mechanisms of AAV replication and integration, we have solved the crystal structure of the endonuclease domain of Rep, the first three-dimensionally characterized member of the Rolling Circle Replication superfamily We have also determined the structures of this domain of Rep bound to its DNA recognition element, or Rep Binding Site (RBS), in the viral inverted terminal repeat (ITR), and bound to a stem-loop of the ITR that is a key regulatory element in replication initiation. At the RBS, five Rep monomers bind to five direct GCTC tetranucleotide repeats, and each repeat is recognized by two Rep monomers from opposing sides of the DNA. At the same time, each Rep monomer recognizes two adjacent repeats, giving rise to a daisy-chain like assembly of Rep monomers on the DNA. The binding of two adjacent repeats by one molecule explains why Rep can tolerate substitutions (imperfect repeats) if a "perfect" GCTC is available next to it. The recognition of several direct repeats by multiple protomers spiraling around the axis of the DNA is an unusual mode of DNA binding. The same domain recognizes the ITR stem-loop structure using a completely different binding surface that interacts with the tip of the loop and recognizes a TxT motif. One of these bases is completely flipped out of the DNA helix and sits in pre-formed pocket of the protein where it is recognized using all possible hydrogen bond donors and acceptors. Taken together, the two complex structures suggest how the nuclease domain of Rep initiates hexameric helicase assembly on the ITR. To verify this, we have used analytical size-exclusion chromatography to show that on authentic ITR sequences the full length protein forms 6:1 complexes. Once assembled, the active helicase unwinds a region of the ITR so that the nuclease domain can introduce a strand and site specific nick, liberating a 3' OH group that can serve as the replication origin. Given its small size (21kD), the Rep endonuclease domain is remarkable in that it features three distinct DNA recognition sites, the two DNA binding sites and the nuclease active site. This is the structural manifestation of the economy that Rep has to observe given the very limited coding space available in the viral genome and the many functions that Rep must perform in viral replication.