

**E0050**

**Unveiling Functional Protein Motions with Picosecond X-ray Crystallography and Molecular Dynamics Simulations.** P.A. Anfinrud, F. Schotte, G. Hummer, Laboratory of Chemical Physics, National Institutes of Health, Bethesda, MD 20892, USA.

The recent development of picosecond time-resolved X-ray crystallography has allowed us to visualize in real time and with atomic detail the conformational evolution of a protein. However, these measurements depict the average structure of an ensemble of intermediates, not a single molecule. To gain single-molecule insights into mechanisms of protein function, a joint analysis of all-atom molecular dynamics (MD) calculations and picosecond time-resolved X-ray structures was performed. Ensemble-averaged MD simulations of the L29F mutant of myoglobin following ligand dissociation reproduce the direction, amplitude, and timescales of crystallographically-determined structural changes. This close agreement with experiments at comparable resolution in space and time validates the individual MD trajectories. From single-molecule trajectories, we have identified and structurally characterized a conformational switch that directs dissociated ligands to one of two nearby protein cavities. Subsequent ligand migration proceeds through a network of transiently interconnected internal cavities. This unique combination of simulation and experiment unveils at an atomic level relationships among protein structure, dynamics, and function.