

## W0411

**X-ray Structural, Thermodynamic and Laser T-jump Kinetic Studies of Villin.** Thang K. Chiu, Jan Kubelka, James Hofrichter, William A. Eaton, David R. Davies. Lab of Molecular Biology, NIDDK, NIH, 9000 Rockville Pike, Bethesda, MD 20892 USA.

Understanding the fundamental processes which govern protein folding is medically important because protein misfolding are associated with numerous diseases. We have used x-ray crystallography, CD spectroscopy and laser T-jump kinetic measurements to study the folding of a 35 amino acid fragment of the villin headpiece domain. This is the smallest naturally occurring protein which folds without the need for ion or ligand, and has been the subject of numerous molecular dynamics simulations to study its folding behavior. Our atomic resolution x-ray structures reveal important details of packing of the hydrophobic core and some new features, such as inter-helical hydrogen bonds which bridge adjacent alpha helices. In addition, the structure and folding parameters of several mutants were determined to highlight the contribution of each residue to the overall folding of this small protein. We observed that removal of two buried charges reflected in the norleucine for lysine substitutions dramatically stabilizes the protein and increases its folding rate. Thus, relief of electrostatic repulsion among charged residues may play a key role in protein folding. This double mutant is the fastest-folding protein observed to date: ~700 ns. To better understand the contribution of the hydrophobic core to overall folding, we also determined the x-ray structure of a much smaller peptide fragment. This structure may shed some light on the folding pathway of its larger 35 amino acid counterpart.