

Identification of a discrete allosteric pathway in a multidomain enzyme provides evidence for a specific inhibition mechanism

*Patrick A. Frantom*¹, *Huimin Zhang*^{2,3}, *Mark R. Emmett*^{2,4}, *Alan G. Marshall*^{2,4}, and *John S. Blanchard*¹

¹*Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY 10461 - USA*

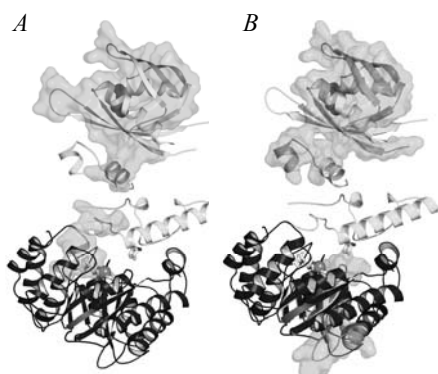
²*Ion Cyclotron Resonance Program, National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL, 32310 - USA*

³*Institute of Molecular Biophysics, Florida State University, Tallahassee, FL, 32306 - USA*

⁴*Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL, 32306 - USA*

Understanding the mechanism of allosteric regulation of proteins, where binding of an effector molecule influences the environment at a distal site, has been a central pursuit in the field of biochemistry for over 40 years. In cases where allosteric effectors induce large conformational changes such as the hinged motion of an entire domain, cause changes in quaternary structure, or proteins where T and R states can clearly be defined, physical mechanisms of allostery have been well defined. However, there are numerous cases where three-dimensional structures and biophysical characterization of a protein in the presence and absence of the effector molecule fail to identify any significant perturbations induced by binding the effector molecule. In these cases it is usually concluded that the binding of the effector molecule has altered the local dynamics of the protein, which are difficult to glean from static three-dimensional structures. Here we present one of the first reports mapping an interdomain allosteric pathway described by changes in local dynamics.

The enzyme α -isopropylmalate synthase from *Mycobacterium tuberculosis* (*MtIPMS*) catalyzes the first step in L-leucine biosynthesis and has been identified as a possible target for design of new anti-tubercular therapeutics. Recently it was shown that *MtIPMS* is subject to allosteric feedback inhibition by L-leucine. L-leucine binds to the regulatory domain of the enzyme, some 50 Å from the active site; however, structures in the presence and absence of L-leucine show no significant changes to the active site architecture. Backbone amide hydrogen/deuterium exchange experiments were performed to investigate structural changes which occur upon L-leucine binding. Based on changes in backbone amide dynamics, a mechanism of inhibition by L-leucine can be proposed where inhibitor binding causes a global conformational change in the regulatory domain creating a discrete signal which is sent through the linker domain to a single, specific peptide in the active site; this is the only site of change in the entire catalytic domain (Fig. 1A). Perturbation of this active site peptide, which includes conserved residues that bind an essential metal ion and properly orientate the substrate, appears to be the major inhibitory action caused by L-leucine binding. A mutant form of the enzyme (Y410F), which is insensitive to L-leucine inhibition, appears to bind L-



leucine, but miscommunicates the inhibitory signal to the opposite side of the active site (Fig. 1B). These results provide evidence that the interface between the catalytic and linker domains is critical for efficient inhibition by L-leucine and establish a physical description of the inhibitory mechanism.

Figure 1. Structural depiction of the allosteric mechanism. A monomer of IPMS made up of chain A (dark) and chain B (light) shown in ribbon form. Regions shown as surface are regions which show backbone amide protection for WT (A) and Y410F (B) IPMS upon L-leucine binding. L-leucine is shown as a space filling model in the regulatory domain (upper domain). The substrate α -KIV, the Mg^{2+} ion are also shown to denote the active site.