

SAXS and X-ray Crystallography to Probe the Catalytic and Regulatory Mechanisms of Aspartate Transcarbamoylase

Evan R. Kantrowitz, Boston College

Aspartate transcarbamoylase (ATCase) from *Escherichia coli* is the textbook example of an enzyme which exhibits molecular cooperativity and allosteric regulation. ATCase catalyzes the first reaction in the pyrimidine biosynthesis pathway, which produces the pyrimidine nucleotides, the building blocks for the nucleic acids. ATCase exhibits cooperativity for one of its substrates, L-aspartate, and is allosterically inhibited, via a feedback mechanism, by the end products of the pathway CTP and UTP. The enzyme is also activated by a product of the parallel purine biosynthesis pathway, ATP. ATCase exists in two conformational states, the low activity or T state and the high activity or R state. The alteration in the ratio of these two states is the basis of allosteric control. The T and R states can be easily resolved by SAXS, and time-resolved SAXS can be used to measure the time evolution of the conformational changes between the T and R states. Small-angle X-ray scattering in solution and X-ray crystallographic studies on the wild-type and mutant versions of the enzyme will be discussed. These experiments have been instrumental in obtaining a molecular level description for each of the steps in the catalytic mechanism as well as the time-evolution of the conformational changes critical for regulation of enzyme activity.