Modulating of the free energy landscape of *E.coli* adenylate kinase

Raisa Kantaev[1], Inbal Riven[1], Adi Goldenzweig[2], Sarel Fleishman[2] and Gilad Haran[1]
Departments of Chemical Physics[1] and Biological Chemistry[2], Weizmann Institute of Science, Rehovot , 76100, Israel

Abstract

For most proteins, folding into their three-dimensional structure is crucial for their function. While fast folding of small globular proteins has been well characterized both experimentally and by simulation, much less is known about the slow folding of large, multi-domain and topologically complex proteins, for which a quite complex and rich energy landscape with multiple metastable intermediate states was suggested. Adenylate kinase (AK) is a monomeric enzyme whose fold is comprised of three domains: LID, NMP binding and CORE. We have previously studied the folding of this protein using single-molecule Förster resonance energy transfer (smFRET), and showed that its energy landscape involves a set of metastable intermediate states and multiple folding pathways. In this study we will check the hypothesis, inspired by recent theoretical work, that mutation-induced stabilization of the NMP domain may alter the folding of dominant pathways and/or the intrinsic stability of folding intermediates. Using the computer design program Rosetta we have already found a set of conservative point mutations that should stabilize the NMP domain, including a mutant in which a new salt bridge formed or mutants with improved side-chain packing within a subdomain. We prepared several of these mutants and are in the process of studying their folding on the single-molecule level. The work will pave the way to a deeper understanding of the folding mechanism of this large multi-domain protein**.**