University of Massachusetts UMASS Medical School

GRADUATE SCHOOL OF BIOMEDICAL SCIENCES

BIOCHEMISTRY AND MOLECULAR PHARMACOLOGY

Ph.D. THESIS DEFENSE

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MENTOR: C. Robert Matthews, PhD

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"EXPLORING THE ROLE OF LARGE CLUSTERS OF BRANCHED ALIPHATIC RESIDUES ON THE FOLDING FREE ENERGY LANDSCAPE OF $(\beta\alpha)_8$ TIM BARREL PROTEINS"

$(\beta\alpha)_8$ TIM barrel proteins are one of the most common structural motifs found in biology. They have a complex folding free energy landscape that includes an initial off-pathway intermediate as well as two on-pathway intermediates. The formation of these intermediates is hypothesized to be driven by large clusters of the branched chain amino acids, isoleucine, leucine, and valine (ILV).

All-atom MD simulations and circular dichroism experiments on polar mutants of the hydrophobic clusters of α-Trp synthase, a TIM barrel protein, revealed the importance of dehydrating the clusters on intermediate states. Custom, single-piece microfluidic chips were interfaced with small angle x-ray scattering and time resolved FRET experiments to monitor the role of a large ILV cluster on the microsecond timescale in a second TIM barrel protein, sIGPS. Dimensional analysis of the initial misfolded intermediate showed an ILV cluster was responsible for the initiation of structure in the intermediate. Early structure formation in the ILV cluster was confirmed by coarse grained simulations. Native state hydrogen exchange experiments were used to probe the higher energy species that are in equilibrium with the native state. Results from the NMR experiment complement the kinetic studies as the core of stability found by NMR mapped back to the same region of the ILV cluster that was found to initiate folding.

When taken together, the results show the importance of hydrophobic clusters on the entire free energy surface of TIM barrel proteins.

Mentor(s)

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