University of Massachusetts Medical School
UMASS

# GRADUATE SCHOOL OF BIOMEDICAL SCIENCES
# BIOCHEMISTRY AND MOLECULAR PHARMACOLOGY

## Ph.D. THESIS DEFENSE


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"Early folding biases in the folding free-energy surface of β/α -repeat proteins"


Early events in folding can determine if a protein is going to fold, misfold, or aggregate. Understanding these deterministic events is paramount for *de novo* protein engineering, the enhancement of biopharmaceutical stabilities, and understanding neurodegenerative diseases including amyotrophic lateral sclerosis and Alzheimer's disease. However, the physicochemical and structural biases within high energy states of protein biopolymers are poorly understood.

A combined experimental and computational study was conducted on the small β/α-repeat protein CheY to determine the structural basis of its sub-millisecond misfolding reaction to an off-pathway intermediate. Using permutations, we were able to discriminate between the roles of two proposed mechanisms of folding; a nucleation condensation model, and a hydrophobic collapse model driven by the formation of clusters of isoleucine, leucine, and valine (ILV) residues. We found that by altering the ILV cluster connectivity we could bias the early folding events to either favor on or off-pathway intermediates.

Structural biases were also experimentally observed in the unfolded state of a *de novo* designed synthetic β/α-repeat protein, Di_III-14. Although thermodynamically and kinetically 2-state, Di_III-14 has a compact unfolded state that is only observable under native-favoring conditions. This unfolded state appears to retain some native-like structure, consisting of a hydrophobic core (69% ILV) stabilized by solvent exposed polar groups and long range electrostatic interactions.

Together, these results suggest that early folding events are largely deterministic in these two systems. Generally, low contact order ILV clusters favor local compaction and, in specific cases, long range electrostatic interactions may have stabilizing effects in higher energy states.


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