Chaperone Machinery in Protein Refolding from Amorphous and Fibrillar Aggregates

In the bustling environment of a cell, molecular crowding and swiftly changing conditions can interfere with the proper folding of newly synthesized polypeptide chains. These disruptions can also cause mature polypeptides to lose their native conformation. When this happens, not only do functional proteins become depleted, but there's also a risk of polypeptide aggregation, which pose a challenge for the cell. Fortunately, a special class of proteins, known as molecular chaperones, has evolved to counteract these issues.

Our research will delve into how Hsp70, along with its co-chaperones and the Hsp100 disaggregase, manages to act on protein aggregates, liberating and refolding the trapped polypeptides. We will reconstitute the disaggregation system using purified chaperones. For our study, we will select proteins whose native conformation can be easily monitored through fluorescence or enzymatic activity, making them suitable substrates for disaggregation.

We will use several variants of chaperone proteins, some of which produce distinctive phenotypes when analyzed in vivo. By combining activity analysis with structural insights, we aim to uncover the mechanisms and cellular functions of these chaperones, as well as how they cooperate to refold proteins from aggregates. Our research will employ a variety of experimental techniques, including bio-layer interferometry, enzyme assays, fluorescence measurements, electron microscopy, and surface plasmon resonance, among others.

This comprehensive study of the mechanistic roles of co-chaperones in regulating Hsp70 during disaggregation and refolding will enhance our understanding of the Hsp70 system, a crucial regulator of protein homeostasis. Given the broad spectrum of Hsp70 functions, there is significant interest in developing therapeutic modulators of this system. Early proof-of-concept studies have shown that Hsp70, in cooperation with its co-chaperones and a hyperactive version of Hsp100 disaggregase, can reverse amyloid aggregation in animal models of neurodegenerative diseases.