Hsp70 and its co-chaperones in protein recovery from aggregates

Molecular crowding and rapidly changing environmental factors interfere with the process of newly synthesized polypeptide chain folding or promote loss of native conformation in mature polypeptides. Loss of native conformation not only leads to depletion of functional proteins, but leads to another problem in the cell, namely aggregation of polypeptides. A special class of proteins, called molecular chaperones, has evolved to counteract this process. In our grant application we will focus on the mechanisms by which chaperones from three different unrelated families, namely Hsp70 and its co-chaperones, Hsp100, and small Hsps act on protein aggregates to liberate and refold the polypeptides trapped inside these aggregates.

The main research methodology is based on reconstitution of disaggregation system from purified chaperones. The proteins whose native conformation is easy to monitor by fluorescence or enzymatic activity will be selected as disaggregation substrates. In our research we will use several variants of chaperone proteins. Presence of some of them confers characteristic phenotypes when analyzed *in vivo*. Analysis of their activities combined with structural insights will allow us to establish their mechanisms and cellular functions as well as details of their co-operation in refolding of proteins from aggregates. In our research we plan to apply several experimental techniques as bio-layer interferometry, enzyme assays, fluorescence measurements, electron microscopy, surface plasmon resonance and other methods.

The proposed comprehensive study of the mechanistic aspects of co-chaperones role in regulation of Hsp70 in disaggregation and refolding would improve our knowledge of the Hsp70 system that is a central regulator of protein homeostasis. The broad spectrum of Hsp70 functions has led to intense work on therapeutically useful modulators of this system. Early proof of concept studies have demonstrated that Hsp70 and its co-chaperones in cooperation with hyperactive version of Hsp104 disaggregase can reverse amyloid aggregation in animal models of neurodegeneration.