**The effect of JDP/Hsp70 chaperones on the conformation and stability of folded protein substrate**

In all organisms a class of proteins termed molecular chaperones assists other proteins throughout their life cycle from synthesis to degradation. They assist in protein folding, protein transport across cellular membranes, assembly of multi-protein complexes and biogenesis of cofactors required for protein activities. Among molecular chaperones, members of Hsp70 family are the most ubiquitous and the most versatile. In some cells, Hsp70s constitute up to 1-2% of total protein content. They perform chaperone functions during normal growth and development as well as during periods of stress and pathology. Thus, it is not surprising that Hsp70s are implicated in many pathologies including neurodegenerative diseases, metabolic dysfunctions, viral infections and cancer.

Quite remarkably all Hsp70's functions, including those related to pathologies, are based on a single biochemical mechanism: Hsp70s' ability to bind and release a variety of unfolded, partially folded and fully folded proteins termed 'substrates'. These interactions are controlled by other proteins termed co-chaperones. Among them, J-domain protein (JDP) co-chaperones play a pivotal role, as they bind substrate(s) independently and deliver it for interaction with partner Hsp70.

While the importance of the Hsp70-substrate binding cycle is well established, little is known about the impact of JDP/Hsp70 binding on structure and stability of protein substrate(s)- particularly when the substrate is a fully folded protein. In this proposal we will investigate how JDP/Hsp70 interaction affects the structure and stability of fully folded substrate. As a model we will use the JDP/Hsp70 system that specializes in biogenesis of iron-sulfur (FeS) clusters- cofactors critical for activity of many cellular proteins. This system has a dedicated JDP and a dedicated Hsp70 interacting with a single protein substrate- a scaffold protein on which FeS clusters are assembled before being transferred to target proteins. The JDP/Hsp70 system participates in the FeS cluster transfer.

In this proposal we will test the hypothesis, based on published research and our preliminary results, that JDP/Hsp70 binding affects the structure and stability of the scaffold protein in contrasting ways. We propose that, on one hand JDP binding stabilizes the structure of the scaffold, thus protecting it against proteolysis and stress factors; while, on the other hand, Hsp70 binding releases JDP from the scaffold and destabilizes its structure, thus facilitating the cluster transfer.

The contrasting effects of JDP/Hsp70 binding on the substrate structure and stability are new in the field of Hsp70 research- if confirmed they will challenge a view that the only role of JDP co-chaperones is substrate delivery for Hsp70. Therefore, our studies will also inform JDP/Hsp70 systems important for human pathologies- particularly neurodegenerative diseases and cancer, as JDPs roles beyond substrate delivery can constitute new targets for the future therapeutical interventions.