Two-component sHsp bacterial system cooperation in protection from heat shock-induced protein aggregation.

Small heat shock proteins (sHsps) are evolutionary conserved class of ATP-independent chaperones that protect cells against proteotoxic stress. sHsps form assemblies with aggregation-prone misfolded proteins, which facilitates subsequent substrate solubilization and refolding by ATP-dependent Hsp70 and Hsp100 chaperones. Upon heat shock, sHsps rapidly dissociate from oligomeric complexes and bind unfolding substrate proteins - preventing their aggregation by forming sHsps-substrate assemblies. Although assemblies architecture is unknown, they were shown to keep substrate proteins in refolding-competent state until Hsp70 system breaks sHsps-substrate interaction and starts disaggregation by recruiting Hsp100.

Bacteria usually harbour one or two sHsp-coding genes, whose products interact with each other, forming multimeric complexes. It was shown that in contrast to single-sHsps systems, sHsps from two-component systems differ in function, yet the mechanism of their action remains unknown. In my study I functionally compare two-component sHsp system of IbpA/IbpB from *E. coli* with single-sHsp systems from *V. harveyi* and *E. amylovora* to unravel *E. coli* IbpA/IbpB cooperation principles.

In order to have an insight is sHsps evolution a phylogenetic tree of Gammaproteobacteria-derived sHsps-coding genes was calculated. We typed two sHsps for comparison with *E. coli* IbpA/B that represent sHsp system that have always been a single protein system (*V. harveyi*) and another that have lost the second protein across the evolution (*E. amylovora*). First we analyzed morphological properties of investigated sHsps. To test their ability to form characteristic assemblies with substrate, we employed sedimentation in glycerol gradient and dynamic light scattering analysis. We also plan to asses these *via* AFM imaging. Secondly, we looked into functional sHsps analysis. In order to inspect sHsps binding to unfolded/aggregated substrate we developed a BLI-based method that allowed us to have an insight in aggregate-sHsps and aggregate-Hsp70/100 complex formation. We also performed a series of *in vitro* reactivation of Luciferase aggregated in presence or absence of sHsps checking their effectiveness in providing refolding-competent substrate to *E. coli* Hsp70/100 systems. Finally, we are planning *in vivo* studies of protein disaggregation after heat shock in *E. coli* variants.

Our already obtained results suggest that, as in single sHsp system one protein has to effectively bind (upon heat shock) and release (at permisive conditions) misfolded substrate, two-component sHsps system splits these functions. Namely, one sHsp becomes responsible for tight binding of unfolding substrate and another one somehow weakens this interaction allowing for efficient substrate protein reactivation. This work should provide an insight in sHsps regulation *via* subfunctionalization.