Small Heat Shock Proteins (sHSPs) are a ubiquitous family of ATP-independent molecular chaperones that are up-regulated in response to a variety of stresses that negatively impact protein homeostasis. In humans, mutations in sHSPs are linked to cataract formation, neurodegenerative disease and myopathies, all of which are associated with protein misfolding. sHSPs form homo-oligomeric complexes that dissociate during cellular stress, exposing hydrophobic patches that are capable of binding misfolding proteins, preventing their irreversible insolubilization. sHSP-substrate interaction results in the formation of large, soluble complexes that not only protect misfolded substrate from aggregation, but also allow for coordination with other components of the protein quality control network. sHSP-protected substrates can be refolded by ATP-dependent chaperones or targeted to proteases for degradation.

We are employing a combination of chemical and site-directed cross-linking, in conjunction with high resolution LC-MS/MS, in an effort to define sites of sHSP-substrate interactions. We have acquired a large data set using several different plant class-I cytosolic sHSPs complexed with model substrates (i.e. porcine mt-MDH and firefly luciferase). Our results validate previous observations, which implicated the N-terminal arm of the sHSPs as important for substrate recognition and binding. Additionally, examining several sHSP family members has provided insight into why different sHSPs have different protection efficiencies in vitro.

One important question that remains to be answered is, what is the mechanism in which ATP-dependent chaperones, such as HSP70, acquire substrate proteins from the sHSP-substrate complex for refolding. Previous studies have shown that the aggregation protection and substrate refolding action of sHSPs and HSP70 respectively can be recapitulated in vitro using recombinant proteins. However, determining the mode of substrate release from sHSP-substrate complexes followed by subsequent HSP70 binding and refolding has yet to be elucidated. Therefore, we propose to the crosslinking and LC-MS/MS approach to determine sites at which HSP70 binds substrates in sHSP-substrate complex. This is a first step to defining how partially misfolded, sHSP-captured substrate is targeted for refolding during heat stress.