Hsp70 plays a major role in maintaining protein homeostasis, which is constantly endangered by stress-induced protein misfolding and aggregation. In response, one of cellular pathways developed by a cell is protein recovery from aggregates by molecular chaperones. To cope with aggregation, Hsp70 collaborates with a disaggregase from the Hsp100 family. The activity of Hsp70 is regulated by nucleotide exchange factors (NEFs) and J-domain proteins (JDPs). Different JDP classes, namely class A or class B, determine the mechanism of the Hsp70 interaction with misfolded protein substrates and the total disaggregation efficacy. To gain more insight into the interplay between Hsp70, its co-chaperones and protein substrates, I addressed how Hsp110, the most abundant cytosolic NEF, impacts Hsp70 activity in the context of different JDP classes.

By using a reconstituted yeast chaperone system, I investigated the impact of Sse1, a NEF belonging to Hsp110 family, at individual stages of protein disaggregation by Hsp70 (Ssa1), when paired with either class A (Ydj1) or class B (Sis1) JDPs. It appears that Sse1 acts at early stages of protein disaggregation rather than during the final folding of protein substrates. Sse1 improves both the disaggregation capacity and binding to aggregates by Hsp70, however the stimulation occurs particularly with class B JDPs. The significantly enhanced protein disaggregation is achieved through the Sse1-mediated more abundant recruitment of Hsp70 to the aggregate, leading to its modification observed as an emergence of aggregate species smaller in size. My results imply that class B-specific interaction between the C-terminal domain of Sis1 and the C-terminal motif EEVD of Ssa1 is vital for these processes.

In accordance with the reported concentration-dependent impact of Hsp110 on Hsp70, I elucidated the basis of Hsp70 inhibition by the NEF. Based on my results, I propose a novel mechanism of inhibition by Sse1 of Hsp70 with class B JDPs, involving competition between these co-chaperones for binding to Hsp70.

Since Metazoa lack an Hsp100 disaggregase and rely solely on Hsp70, I wanted to dissect how the disaggregation activity of the human Hsp70 system is affected by Hsp110. Similarly as in yeasts, the human Hsp110 potentiates the disaggregation activity and recruitment of the Hsp70 system to the aggregate. These effects are more pronounced for class B JDPs. Together, my results shed light on the mechanisms, by which Hsp110 regulates activity of Hsp70 chaperone machinery and provide a basis for further research on the role of NEF in the functioning of the Hsp70 system in humans and other eukaryotes.