

Molecular chaperones are proteins that are central players in maintaining protein homeostasis by limiting protein aggregation. Accumulation of protein aggregates can be associated with the onset of debilitating and/or often fatal diseases, and it is thought that chaperones can help mitigate these effects. Chaperones can limit the aggregation of protein by acting as disaggregases, assembling into complexes near the aggregate surface to disassemble protein aggregates, or holdases, binding to proteins or smaller aggregates and preventing their accumulation. In this dissertation we assess if molecular chaperones can limit the aggregation of human transthyretin (TTR) expressed within a yeast system and explore the relationship between chaperone concentration and activity. We find that the primary disaggregase in yeast, Hsp104, does not contribute disaggregase activity to TTR aggregates. Interestingly, an abundance of a Hsp104 variant associated with potentiated disaggregase activity, Hsp104<sup>A503S</sup>, increases the size of TTR aggregates. Concomitant overexpression of Hsp104<sup>A503S</sup> with yeast chaperones Sis1 or Ssa1 can at least partially compensate for the enlarged aggregate sizes. We offer evidence for the importance of Sis1 and Ssa1 in limiting the size of TTR aggregates and propose a model of Hsp104<sup>A503S</sup> titrating Sis1 or Ssa1 away from the aggregates surface, thereby impairing their effect on TTR. While Sis1 directly facilitates the limitation of TTR aggregate sizes, swapping the expression of Sis1 for its human homolog, DnajB1, results in a substantial increase in smaller aggregate populations. Our data supports that chaperones will limit the aggregation of TTR by a disaggregase model, as opposed to a holdase model. We propose chaperone complexes disassemble larger aggregates into smaller populations and offer evidence of chaperone variants being far more effective at this process. Understanding the relationship between these chaperones in limiting protein aggregation can allow future research into improving the disaggregase activity of chaperones maintaining protein homeostasis.