Summary:

Protein misfolding and aggregation are associated with various neurodegenerative disorders such as Alzheimer's Disease, Parkinson's disease, Huntington's disease, Amyotrophic lateral sclerosis and Prion's disease. Neurodegenerative disorders are characterized by loss of specific neurons in brain and affect mostly the old aged group people. The similarity between these disorders is that these are caused by the intracellular accumulation of amyloid protein particles. Yeast has extensively been studied as model of amyloid based disorders. In the study we have identified two cellular chaperones modulator Snl1 and Cpr7 which regulate [URE3] yeast prion propagation.

Snl1 is a novel ER anchored mammalian Bag homolog protein in yeast. Bag domain proteins have been known to modulate the activity of Hsp70. To examine the role of Snl1 in yeast prion propagation we had created knockout of Snl1 in [*PSI*$^+$] and [URE3] strains and found that deletion of Snl1 had no effect on yeast prion propagation. We also examined the Snl1 overexpression using galactose inducible promoter. It had no effect on yeast prion propagation. Snl1 has 2 domains: Snl1(1-39) ER transmembrane domain and cytoslic Snl1(40-159) (Snl1-M). Snl1-M consists of Bag homology domain Snl1(49-159) (Snl1-S) and 9 amino acid extension at its N-terminal. We overexpressed Snl1 variants from galactose inducible promoter in [URE3] and [*PSI*$^+$] and observed that overexpression of Snl1-S cures [URE3] prion specifically. Then we further examined whether Snl1-S mediated curing require Hsp70. The Snl1 residues critical for Hsp70 interaction were mutated and mutant was overexpressed in [URE3] strain. We found that Hsp70 interaction with Snl1 is critical for Snl1-S mediated curing of [URE3] prion. Cytosolic Hsp70s are functionally distinct for yeast

prion propagations. We also examined the effect of Snl1-S overexpression in strain expressing Ssa1 or Ssa2 as sole Ssa Hsp70 source. We observed that Snl1-S curing was independent of the presence of different Hsp70 isoforms. To check whether the Snl1-S mediated curing of [URE3] requires any factor other than Hsp70 we performed random mutagenesis of Snl1-S using hydroxylamine based mutagenesis. We screened for mutants which were unable to cure [URE3] upon overexpression of Snl1-S. Our *in vitro* data with purified Snl1-S mutant Snl1-S (L114V) and Snl1-S (S89N) suggest their ability to interact with Hsp70. However fractionation experiment with yeast lysate showed that mutants were largely fractionated in pellet fraction suggesting that mutants were inaccessible for interaction with cytosolic Hsp70. Overexpression of entire cytosolic Snl1-M protein, 9 amino acid extension outside the Bag domain has no effect on [URE3] propagation. Mutation of lysine to alanine at the 42, 44 and 47 residues was resulted in curing of [URE3] prion.

In another study we examined the role of Hsp90 & its co-chaperone in yeast prion propagation. It is well known that Hsp70 and its co-chaperones are crucial regulators for yeast prion strength and stability. The role of Hsp90 is not clear for yeast prion propagation. In our studies we have examined the role of Hsp90 and its co-chaperones in yeast prion propagation. We expressed individual isoform of Hsp90, either HSP82 or HSC82 and found that 1 isofom is sufficient to propagate [URE3] prion stably. We have examined the Hsp90 influence on [URE3] prion by expressing sole source of Hsp90 mutant, His$_6$-Hsp82Δ211-264 or His$_6$-Hsp82ΔMEEVD. We observed that wt type His$_6$-Hsp82 and His$_6$-Hsp82Δ211-264 has no effect on [URE3] prion stability, while deletion of highly conserved MEEVD motif, which is required for interaction with TPR containing co-chaperones, results in increase frequency of [ure-o] cells. This observation suggests that MEEVD motif is required for

[URE3] stability. We further created a single knockout of different Hsp90 co-chaperones in [URE3] strain. We observed that deletion of TPR containing co-chaperone Cpr7 results in profound effect of [URE3] stability as observed by red colony color phenotype and poor growth on Adenine deficient medium. Similar studies were carried out in [*PSI*$^+$] strains. We observed that deletion of any of Hsp90 co-chaperones have no effect on [*PSI*$^+$] prion propagation. We further knocked out Cpr7 in strong and weak [*PSI*$^+$] variants. We observed that Cpr7 deletion has no effect on [*PSI*$^+$] propagation, suggesting that Cpr7 is specifically required for [URE3] propagation. Cpr7 requirement for [URE3] propagation is further confirmed by sporulation and tetrad dissection of [URE3] diploid heterozygous for *cpr7Δ* (CPR7/*cpr7Δ*). We have examined the overall abundance of Hsp70 and its co-chaperones level in *cpr7Δ* strain. We observed a similar abundance of Ydj1, Sse1 and Hsp90 levels suggesting loss of [URE3] in *cpr7Δ* strain is not due to altered abundance of their chaperones. In contrast, we observed about 2 fold higher expression of Hsp70 in *cpr7Δ* strain compare to wt type strains. We overexpressed extra copy of cytosolic Hsp70 Ssa1, Ssa2, Ssa3 and Ssa4 under SSA2 promoter through plasmid borne gene. The expression from the plasmid borne gene has no effect on [URE3] stability while in *cpr7Δ* strain cells loss 99% of [URE3]. Similarly Cpr7 deletion also results in the loss of [URE3] prion in Ssa2 strains. Overexpression of Ssa2 has no effect on [URE3] stability in Ssa2 strain, suggesting that [URE3] loss in Ssa2 is unrelated to Hsp70 overexpression. Cpr6 and Cpr7 belongs to PPIase family of enzymes, sharing more than 50% of sequence similarity. We performed the genetic complementation assay to examine whether Cpr6 can complement the function of Cpr7 for [URE3] prion propagation and observed that Cpr6 is unable to do so. Further we performed Cpr6 and Cpr7 domain studies by exchanging PPIase domain and TPR domain between Cpr6

and Cpr7. We observed that TPR domain of Cpr7 alone can complement the function of Cpr7 for [URE3] prion propagation. Similarly we examined Cns1, a functional homolog of Cpr7, for its possible role in complementing the Cpr7 for [URE3] prion propagation. We observed that Cns1 overexpression can complement the function of Cpr7 for [URE3]. Cpr7 is required for folding and activity of heterologously expressed glucocorticoid receptor and v-Src kinasein yeast. Here we have examined the interaction of purified Cpr7 with Ure2 in yeast lysate using the pull down experiment and observed that Cpr7 interacts with Ure2 in Hsp82 wt type as well as Hsp82ΔMEEVD strains, suggesting that Cpr7 interacts with Ure2 independent of Hsp90. Cpr7 interacts with Ure2 and is required for [URE3] propagation. We hypothesized that Cpr7 might modulate Ure2 fibrillation. We performed *in vitro* fibrillation of Ure2 in the presence and absence of Cpr6, Cpr7 and Tpr domain of Cpr7 and we observed that Cpr7 or its TPR domain alone increases the Ure2 fibrillation while Cpr6 has no effect on Ure2 fibrillation. Thus it is possible that increase in Ure2 fibrillation by Cpr7 promotes [URE3] propagation.

We performed structural and biophysical characterization of Snl1 variants to understand their differential effects on [URE3] propagation. We examined the oligomeric structure of Snl1 variants. The size exclusion chromatography of Snl1 variants were performed using Superdex 75. The proteins were eluted at near its dimeric size, suggesting that they form dimer in solution or might posses non-globular structure. In order to understand the oligomeric status of Snl1 variants we performed AUC experiments. The Snl1 variants were predominantly sedimented to near its monomeric structures molecular weight. To know the secondary structural content of Snl1 variants, we performed far UV CD spectroscopy. We observed that Snl1-M was showing a higher helical content than Snl1-S. Mutations in Snl1-

M outside the Bag homology domain from lysine to alanine [Snl1-M(AAA)] resulted in decrease of secondary structure content compared to Snl1-M as well as Snl1-S, suggesting that increased flexibility of Snl1-S structure could be the basis of functional differences between Snl1-S and Snl1-M.

We examined interaction of Snl1 variants with Hsp70 using SPR. We observed that Snl1-M binds with Hsp70' Ssa2 with relatively higher affinity as compared to Snl1-S or Snl1-M(AAA). Snl1-S and Snl1-M(AAA) has similar affinity to Ssa2.

SAXS scattering was performed to gain the structural insights of Snl1 variants. We observed that Snl1 variants were monodisperse and free from aggregation as evident in the intensity plots. Snl1 variants, Snl1-S and Snl1-M were natively folded while Snl1-M(AAA) had some unfolded structures at 10mg/ml concentration, evident with kratky plots. We calculated R_g and D_{max} of Snl1 variants through (P)r vs R plots. We observed the average R_g and D_{max} of Snl1-S, Snl1-M(AAA) and Snl1-M to be 2.42±0.09 nm & 8.02 nm, 2.45±0.10 nm & 10.4 nm and 2.49 & 9.3 nm respectively. We performed *ab initio* modeling of Snl1 variants and observed that Snl1-M had elongated shape compared to Snl1-S or Snl1-M(AAA) which had slightly bend shapes.