Mutual chaperoning by amyloid forming proteins/ peptides

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Neurodegenerative diseases

Disease (amyloidoses)	**Protein**
Alzheimer's disease, Down's syndrome (Trisomy 21), Hereditary cerebral angiopathy (Dutch)	Amyloid precursor protein (Aβ 1-42)
Prion diseases : Kuru, Gerstmann-Straussler-Scheinker Syndrom (GSS), Creutzfeld-Jacob disease, Scrapie (sheep), Bovine spongiform encephalopathy ("mad cow")	**Prion protein**
Type II diabetes mellitus (adult onset)	Islet amyloid polypeptide (amylin)
Dialysis-associated amyloidosis	**β_2-microglobulin**
Senile cardiac amyloidosis	Atrial natriuretic factor
Familial amyloid polyneuropathy	**Transthyretin**
Reactive amyloidosis familial Mediterranean fever	Serum amyloid A
Familial amyloid polyneuropathy (Finnish)	**Gelsolin**
Macroglobulinemia	Gamma-1 heavy chain
Primary systemic amyloidosis	**Ig-lambda, Ig-kappa**
Familial polyneuropathy – Iowa (Irish)	Apolipoprotein A1
Hereditary cerebral myopathy – Iceland	**Cystatin C**
Nonneuropathic hereditary amyloid with renal disease	Fibrinogen alpha
Nonneuropathic hereditary amyloid with renal disease	**Lysozyme**
Familial British dementia	FBDP

Neurodegenerative diseases

Disease (non-amyloidoses)	**Protein**
Diffuse lewy body disease, Parkinson's disease	α-synuclein
Fronto-temporal dementia	Tau
Amyotropic lateral sclerosis	Superoxide dismutase-1
Triplet repeat diseases: (Huntinghton's, Spinocerebellar ataxias	Polyglutamine tracts (Huntingtin)
Spinal and bulbar muscural atropy	Androgen receptor
Spinocerebellar ataxias	Ataxins
Spinocerebellar ataxia 17	TATA box-binding protein

Table 1: Diseases of protein misfolding: amyloidoses and non-amyloidoses (reviewed in (Kagan and Thundimadathil, 2010))

Neurodegenerative diseases

SH3 domain p 85 phosphatidylinositol 3-kinase	Fibronectin type III phosphoglycenite linase acylphosphatase
HypF N-terminal domain (E. coli)	Amphoterin (human)
Apomyogloobin (equine)	Apocytochrome c
Endostatin (human)	Met aminopeptidase
Stefin B (human)	ADA2H
Fibroblast growth factor (N. viridescens)	Apolipoprotein CII
VI domain (murine)	B1 domain of IgG binding protein
Curlin CgsA subunit	Monellin
Serpins	

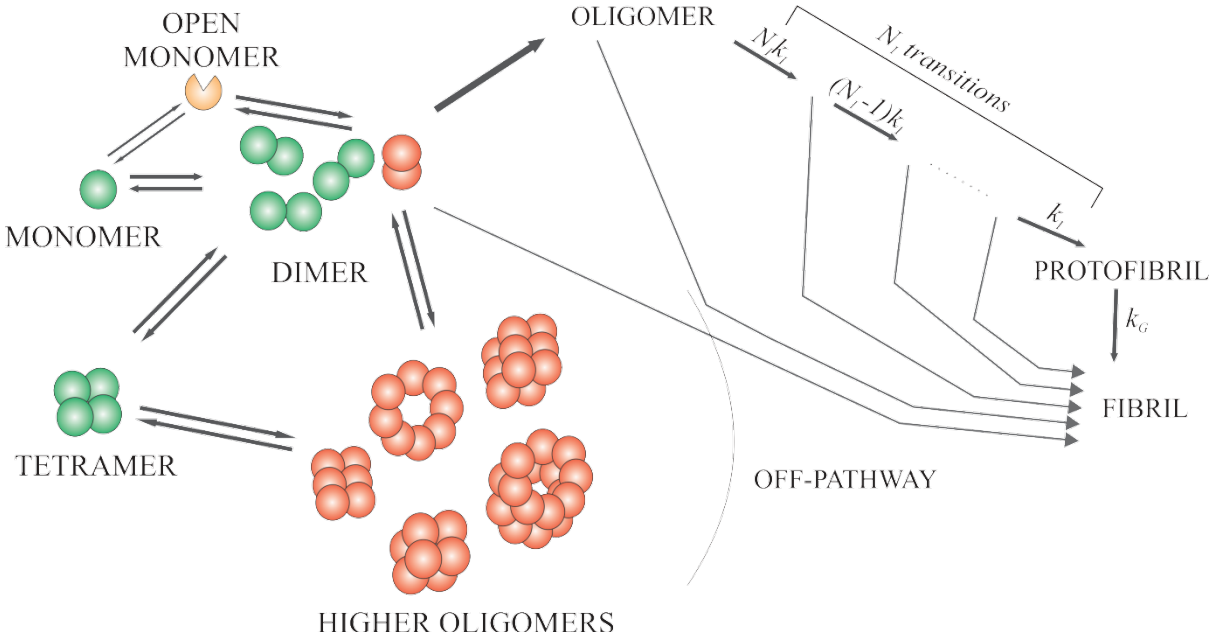
Table 2: Nondisease related amyloid forming proteins/peptides – model proteins (reviewed in (Kagan and Thundimadathil, 2010))

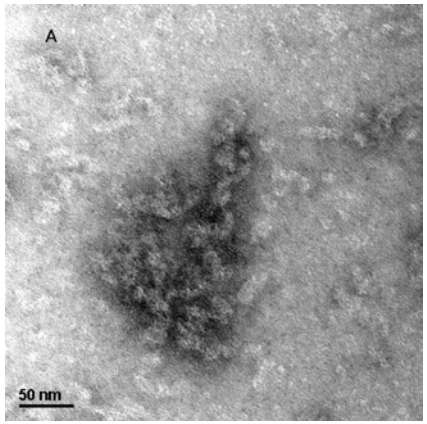
Cause of neurodegeneration?

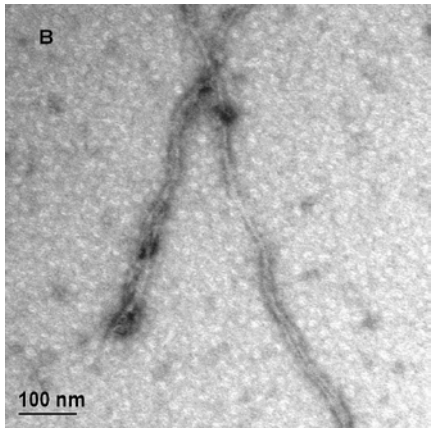
- Protein aggregation – hereditary cases - pathological mutants

- Examples of the mechanism
- a) stefin B
  (as a model protein and EPM1 gene)
- a) Amyloid-beta
- b) Beta2 - microglobulin

A possible in vitro mechanism



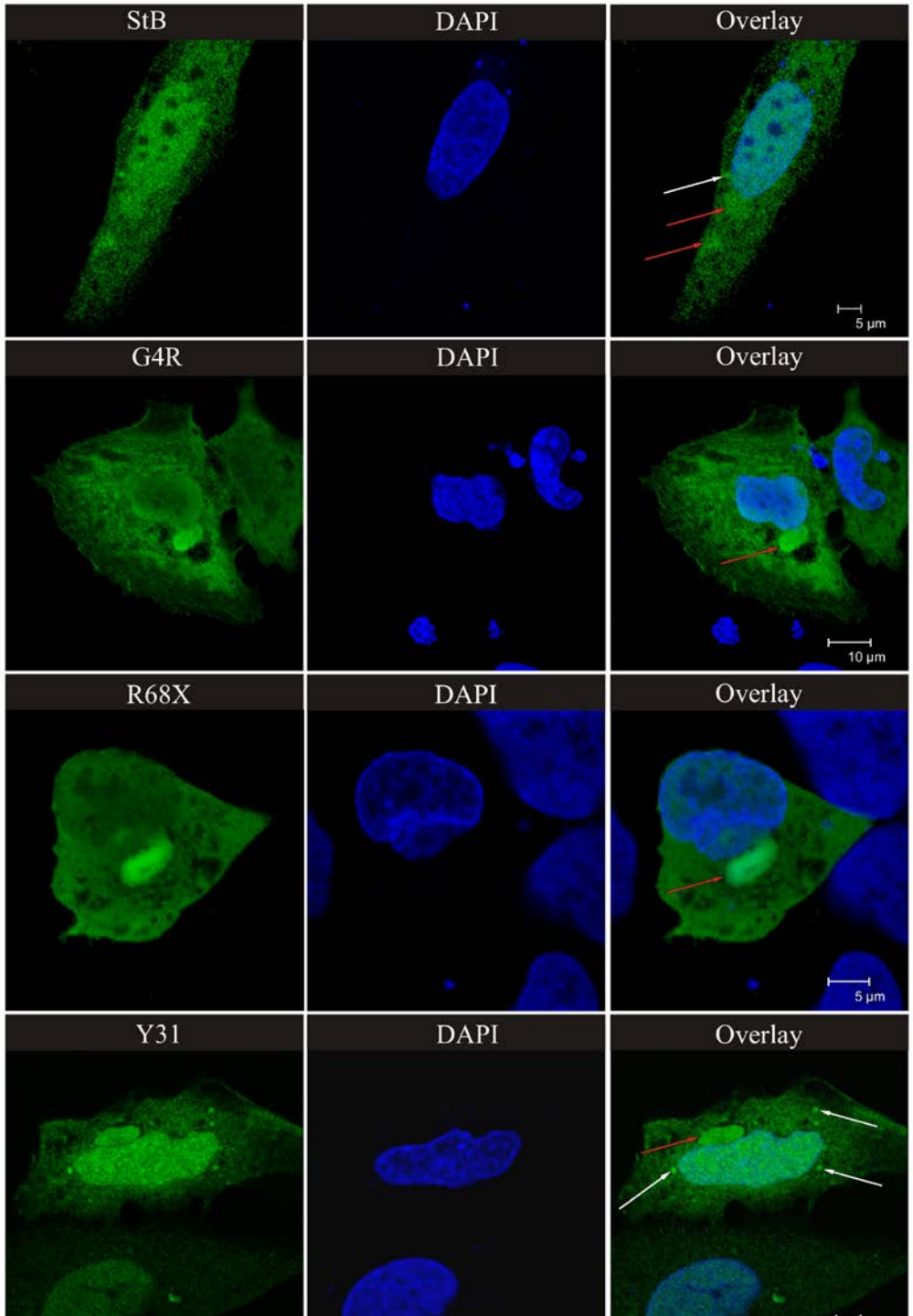




Protein aggregates in the cell

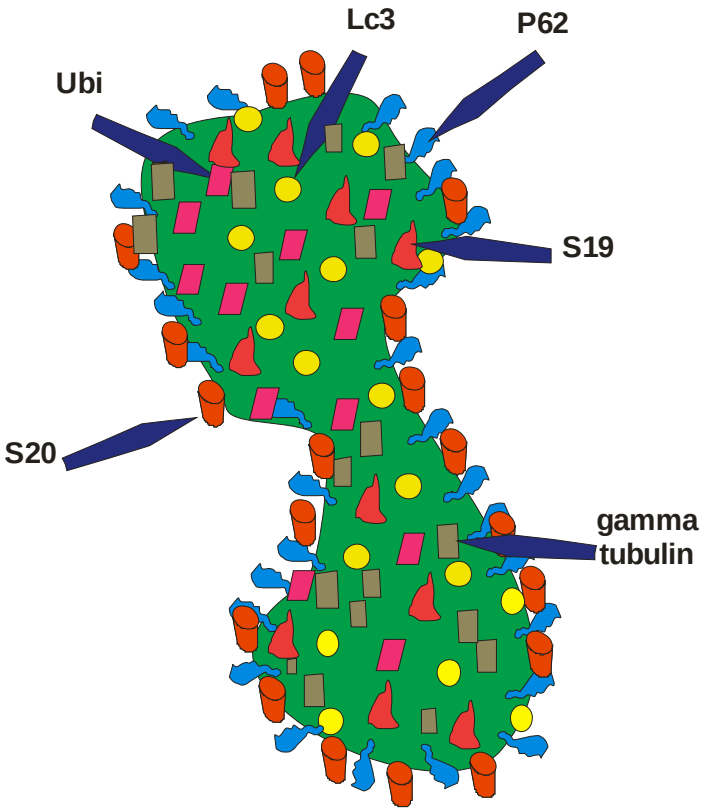
- We over expressed human stefin B (cystatin B) and some of its missense EPM1 mutants –
- maybe they also aggregate in patients?

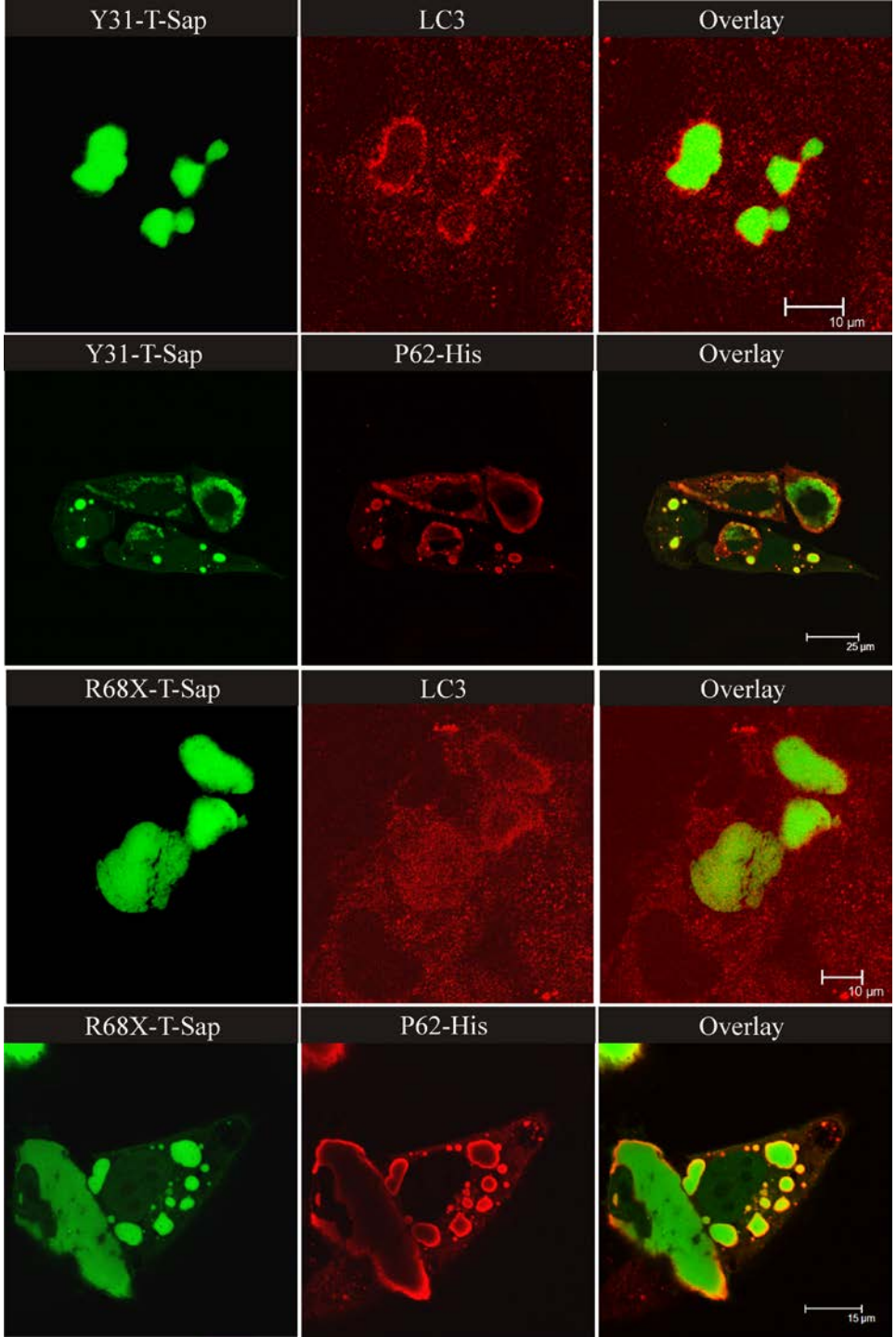
  (up to 40% expression level – as most of the mutations are dodecamer repeats and lead to lower protein expression)

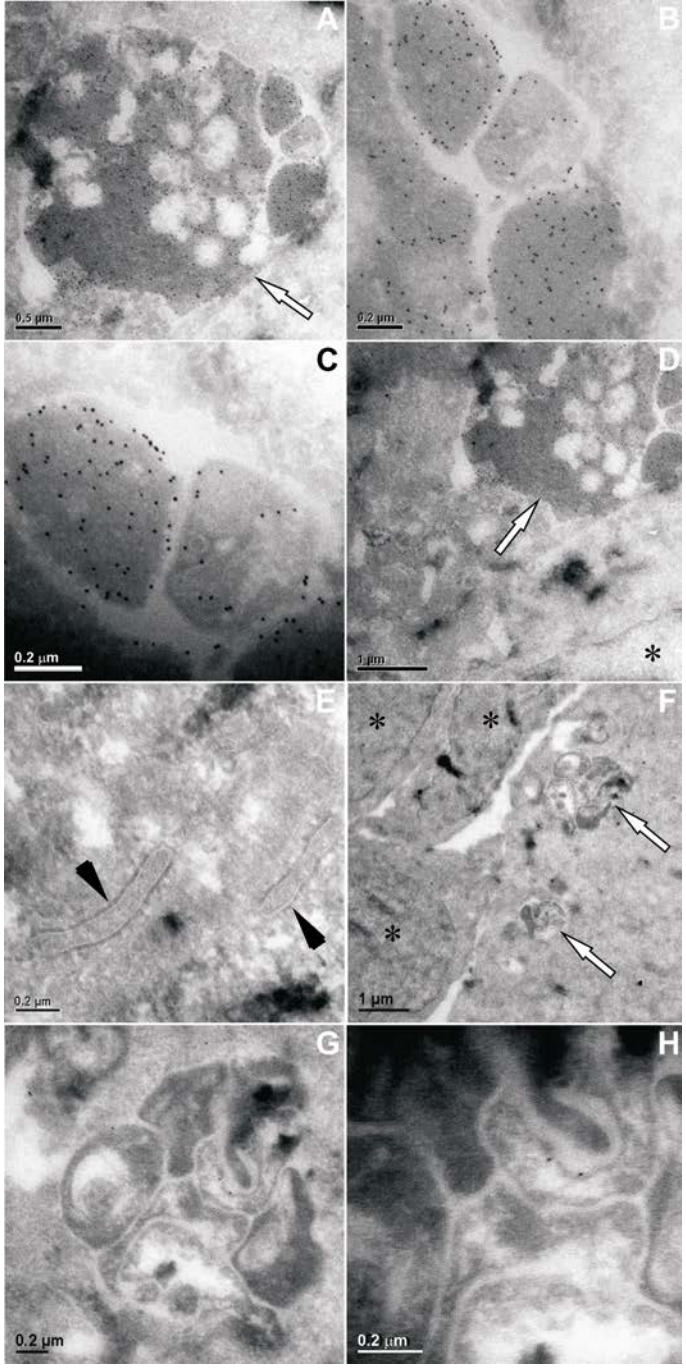


Stefin B and variants aggregation in cells – detected by stefin B polyclonal antibodies

Labeling of the aggregates by Ab’s




Y31-T-Sap
LC3
Overlay
10 µm
Y31-T-Sap
P62-His
Overlay
25 µm
R68X-T-Sap
LC3
Overlay
10 µm
R68X-T-Sap
P62-His
Overlay
15 µm



- Transmission Electron Microscopy

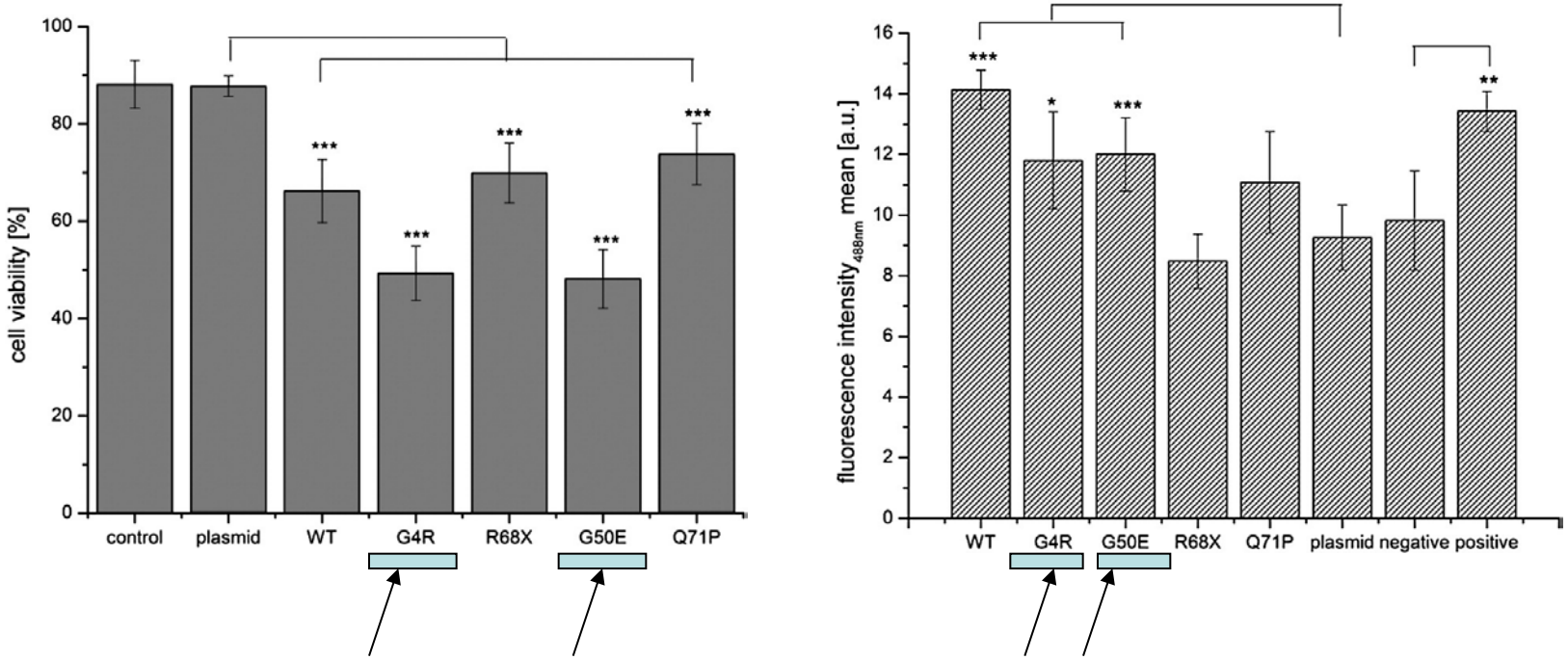
- Figs A-D is aggregate of G4R immuno-gold labeled

- Figs F-H are aggregates of R68X, which labels less well

- In Fig. E is rough ER
- * labels the nucleus

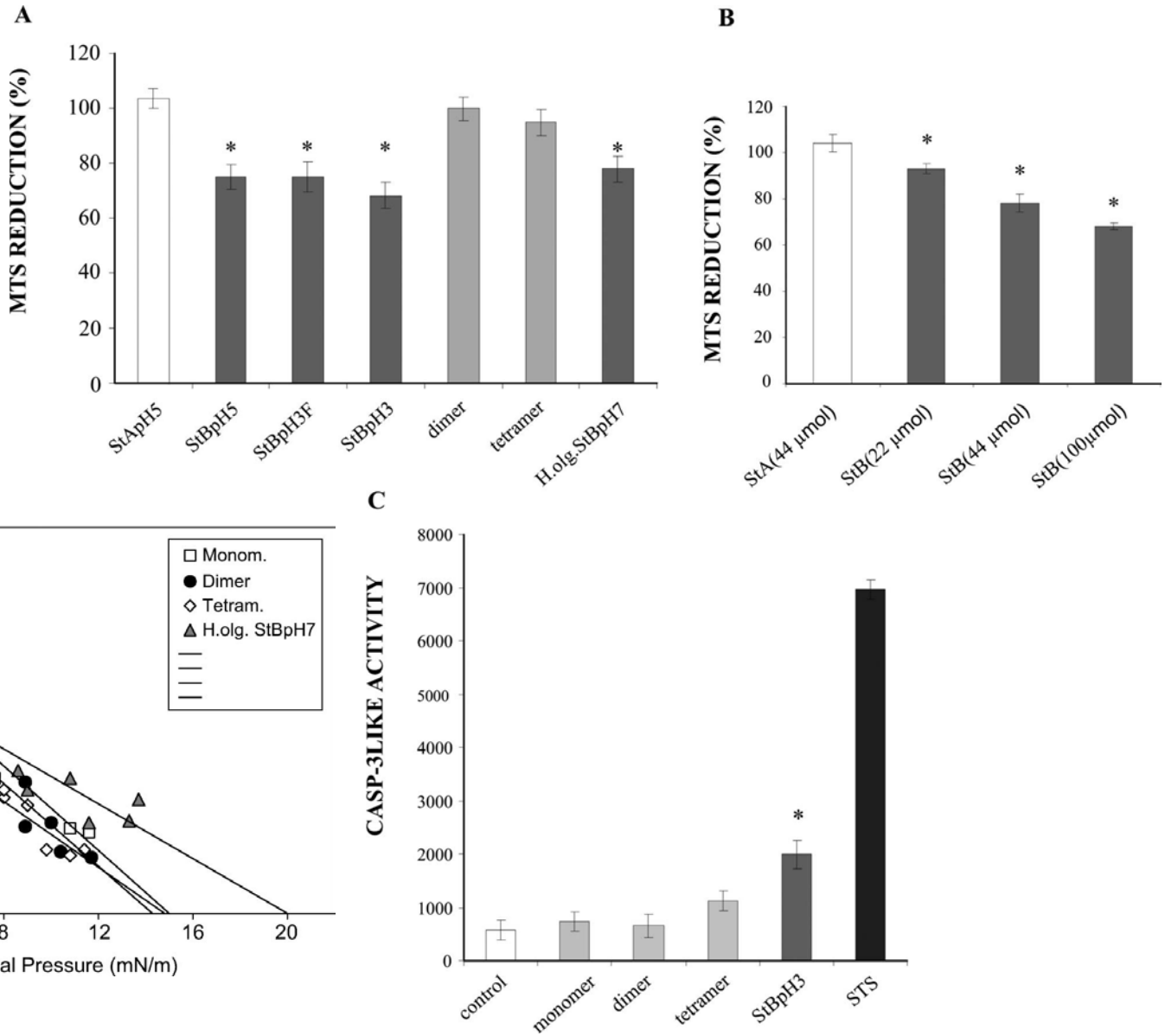
Toxicity of the aggregates

- *From Polajnar et al. 2013, BBA Molecular & Cellular Biology*

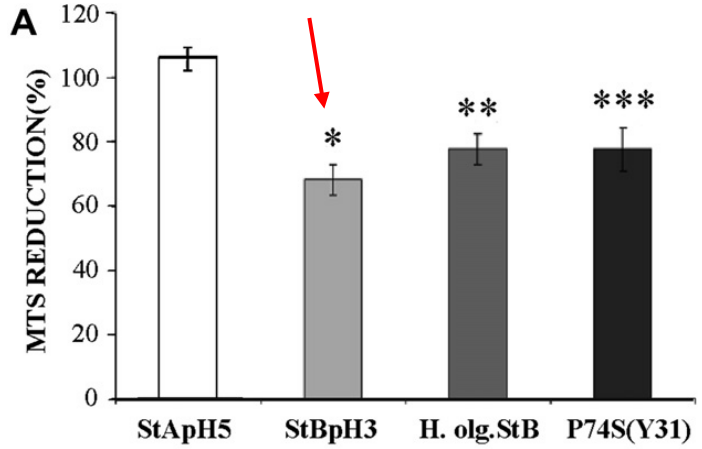


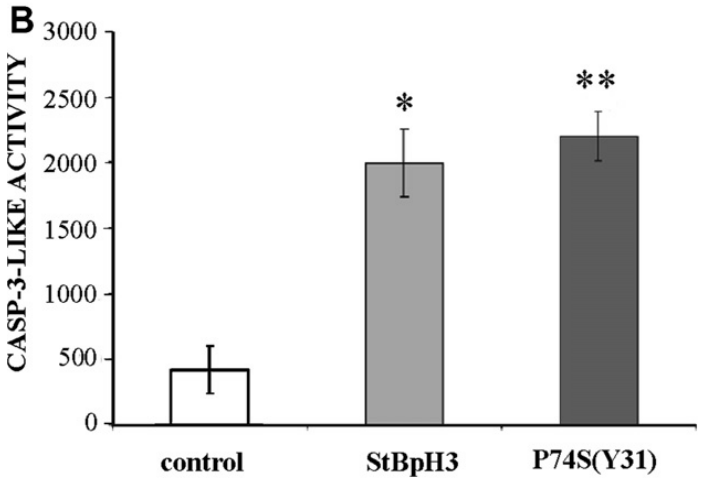
Cell death correlates with oxidative stress

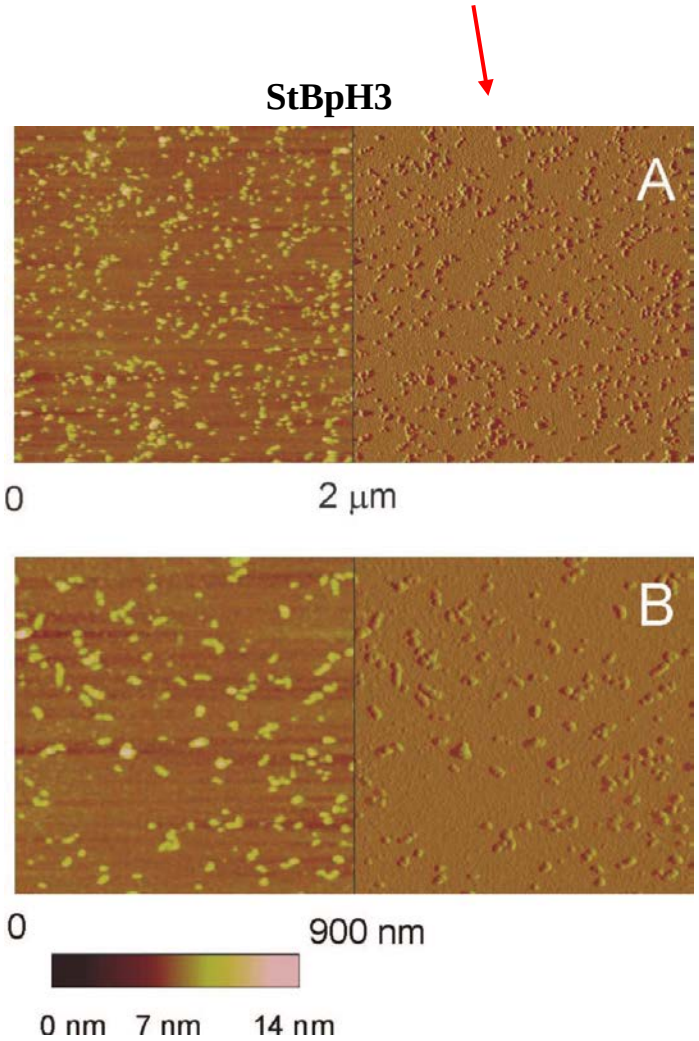
•*From Ceru et al., 2010, Biology of the Cell and*



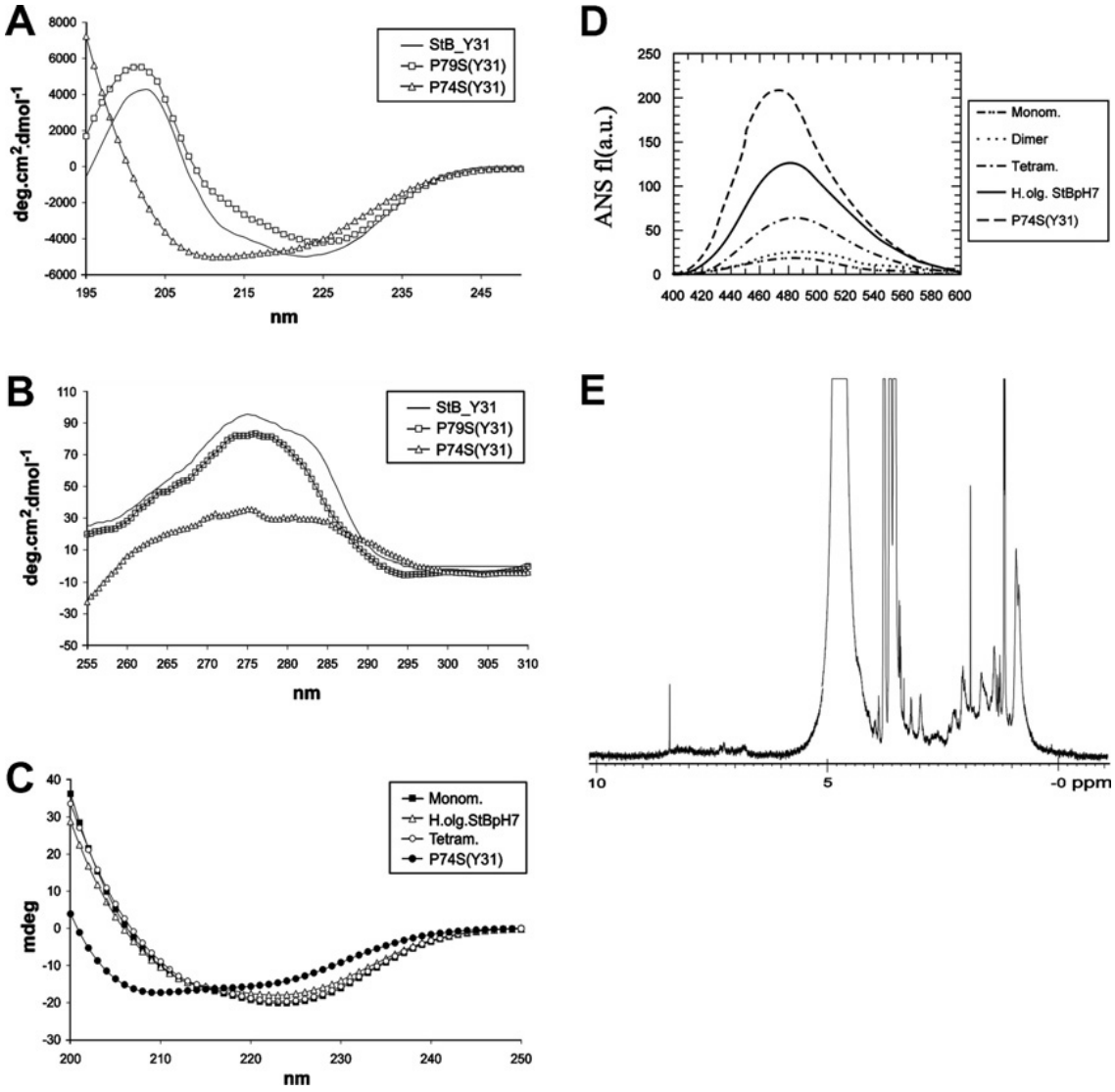
Molten globule conformation and oligomeric state are toxic







Characterizing the MG conformation



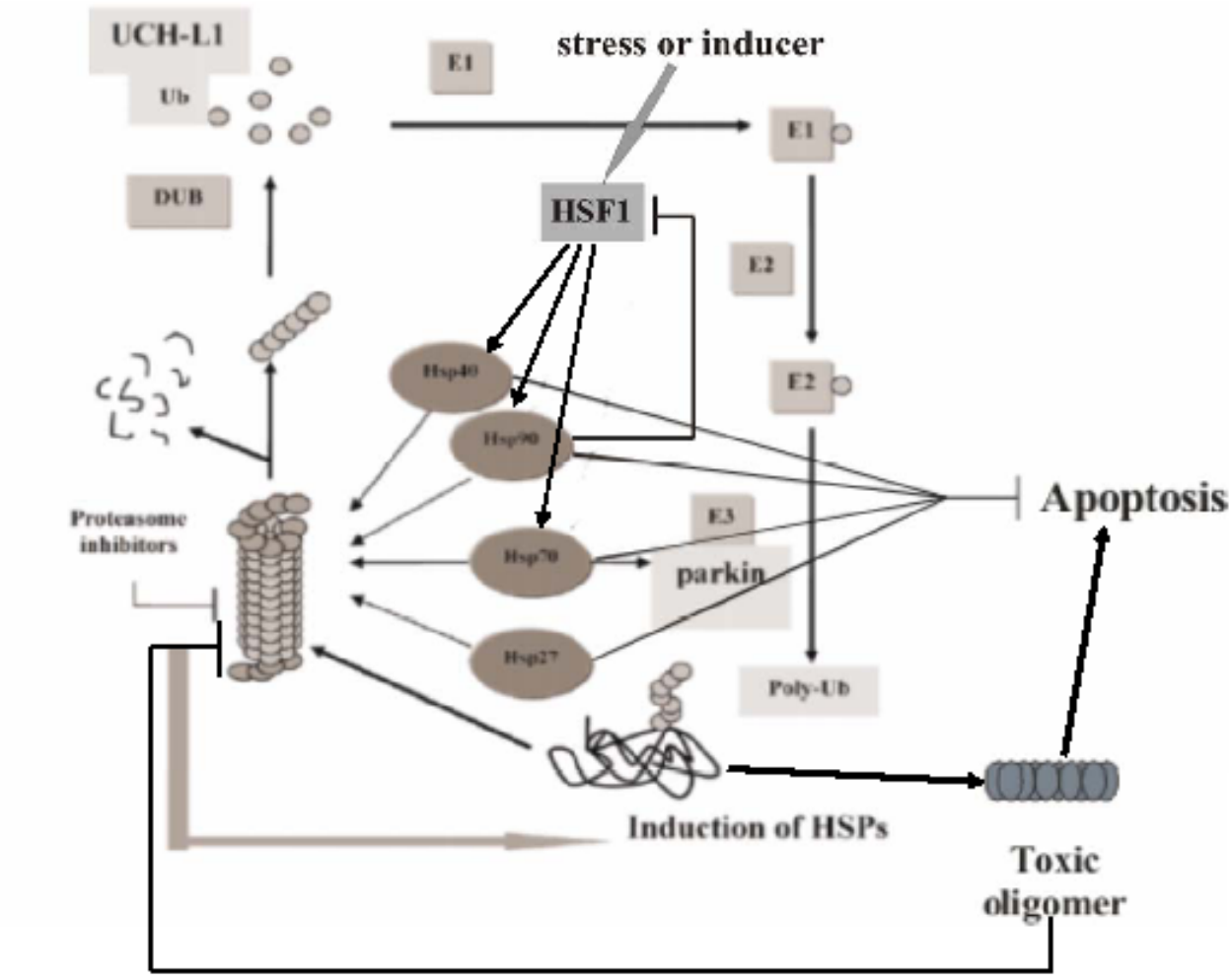
Managing protein aggregates by the cell

- Protein misfolding –

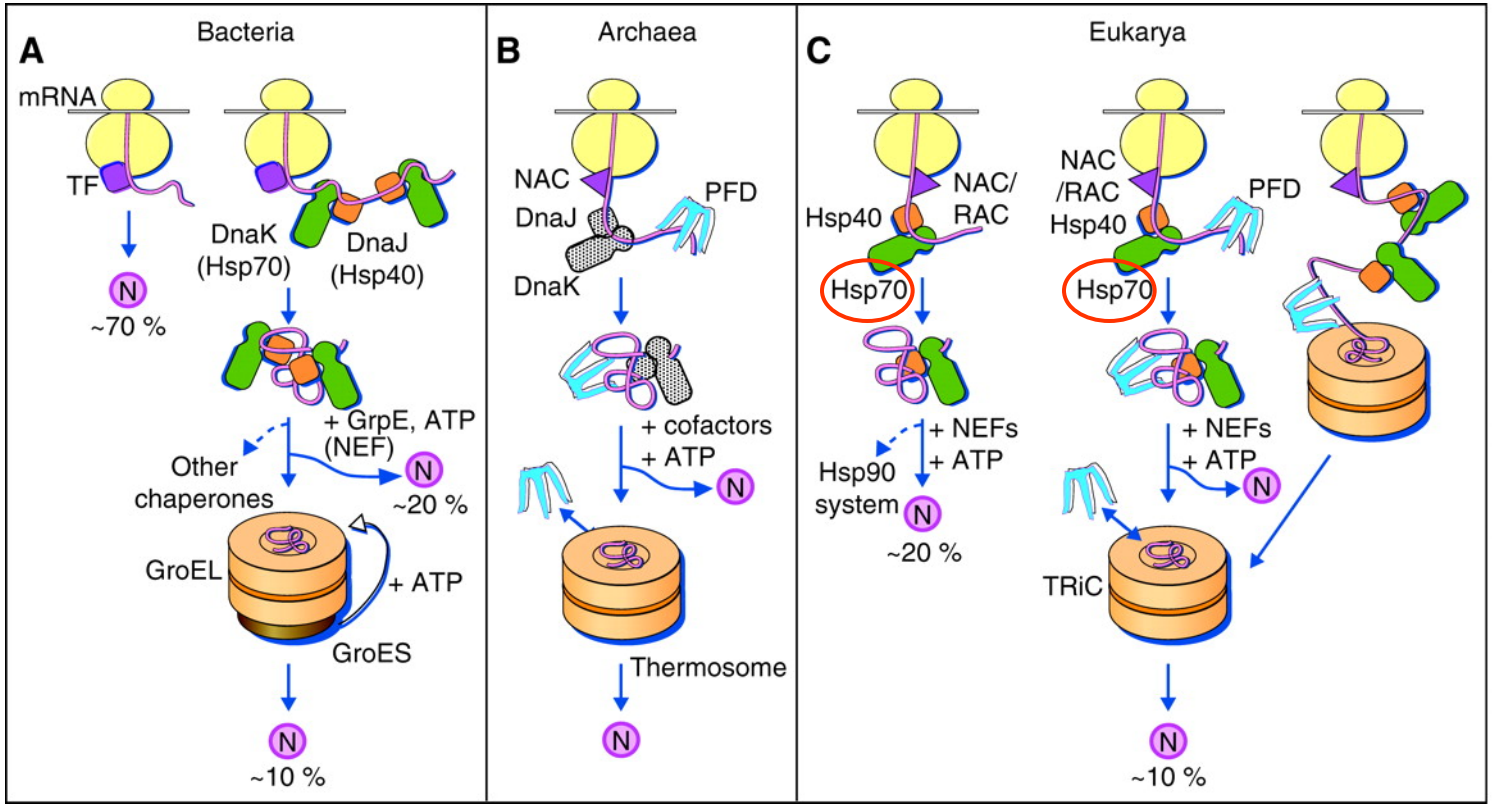
Protein homeostasis – quality system consists of :

- Regulation of the synthesis (signaling of cellular stress)
- Chaperones and chaperonins
- Degradation systems

Aggregation, chaperone system and UPS

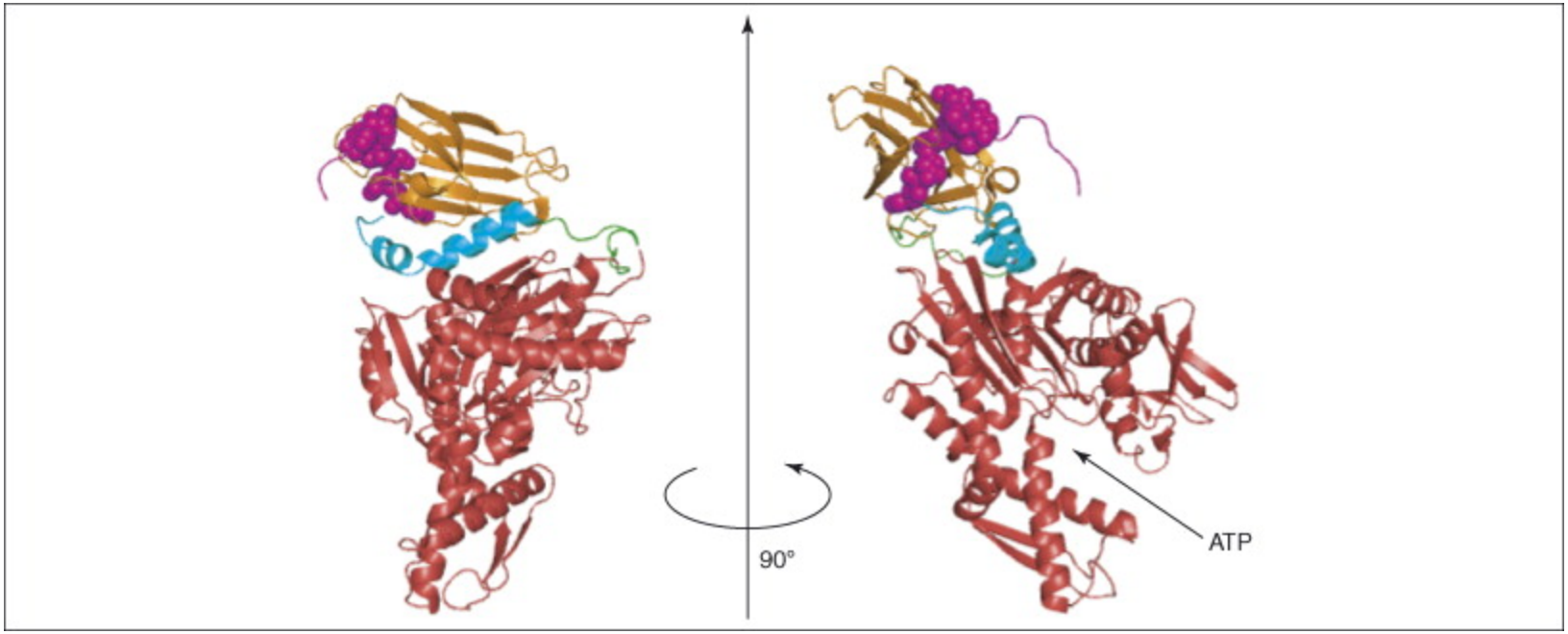


Protein folding in the cytosol.



R. Martin Vabulas et al. Cold Spring Harb Perspect Biol 2010;2:a004390

Heat shock protein 70 (Hsp 70) with a bound peptide



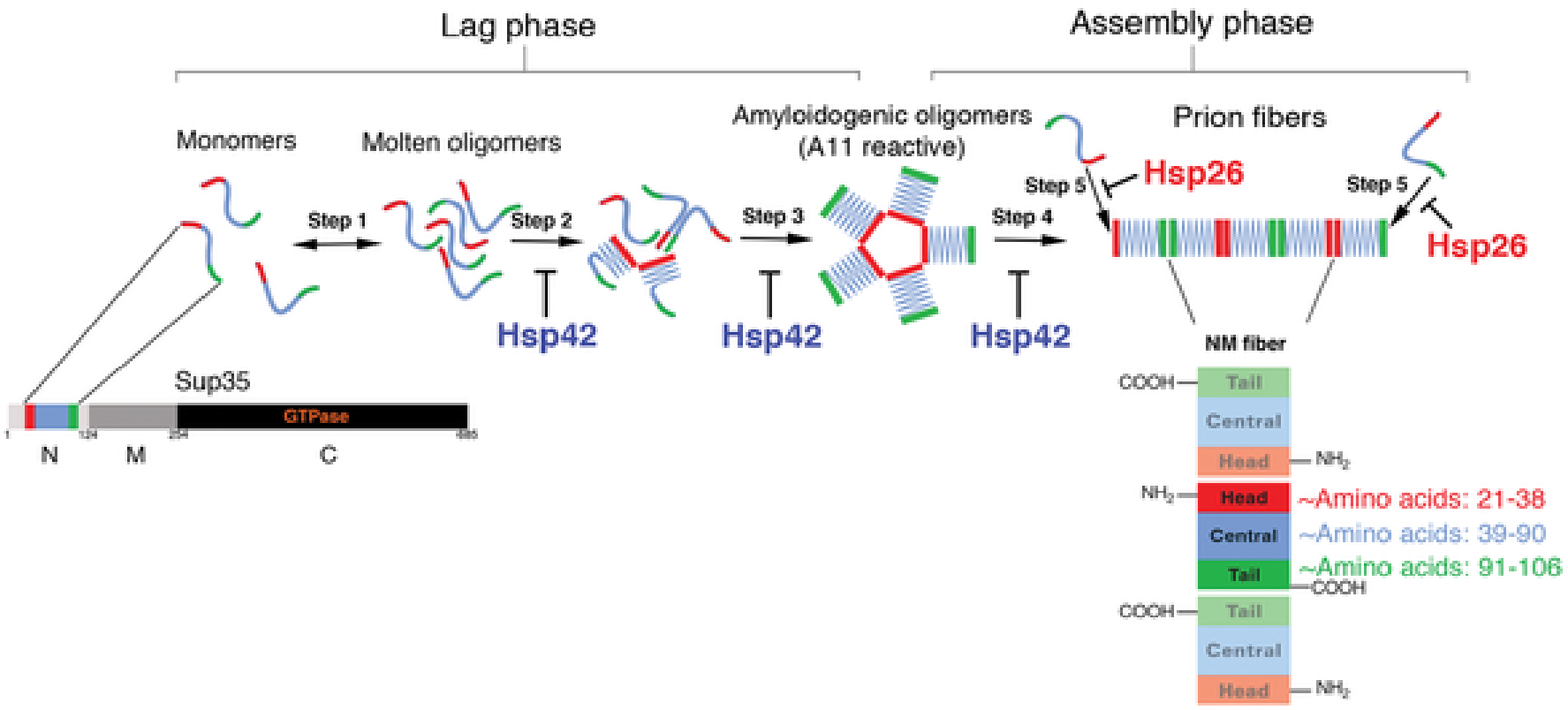
Role of Chaperone systems

- Heat shock Proteins: Hsp 70 and Hsp 104, are also dissagregases

- Small Heat Shock Proteins Potentiate Amyloid Dissolution by Protein Disaggregases –a PLOS One paper (2012), by Duennwald ML, Echeverria A, Shorter J

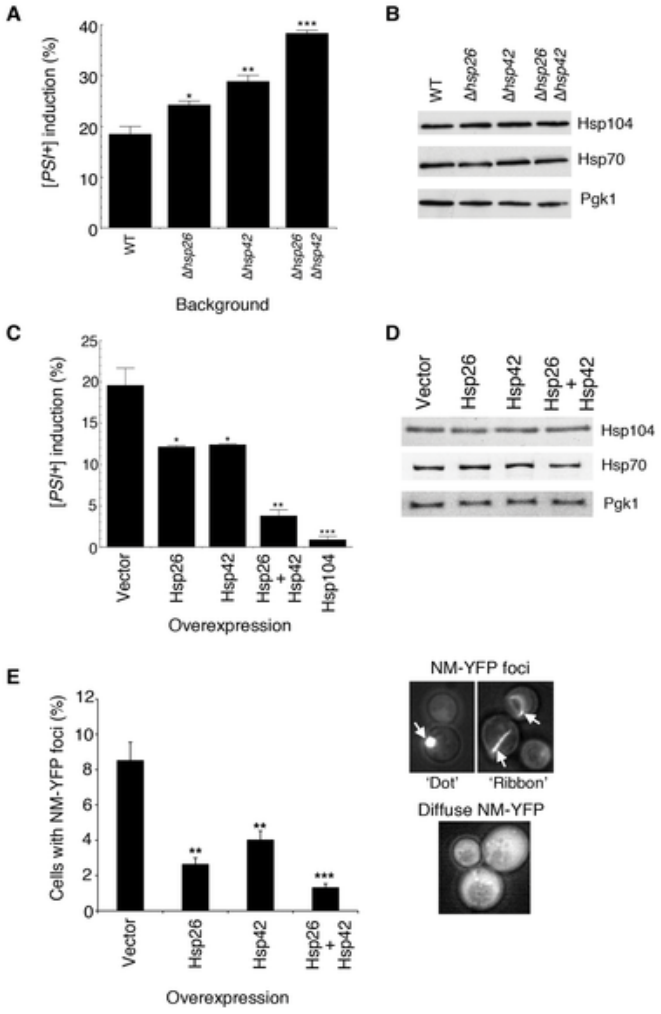
doi:10.1371

- **Figure 1. Mechanism of Sup35 prion assembly.**



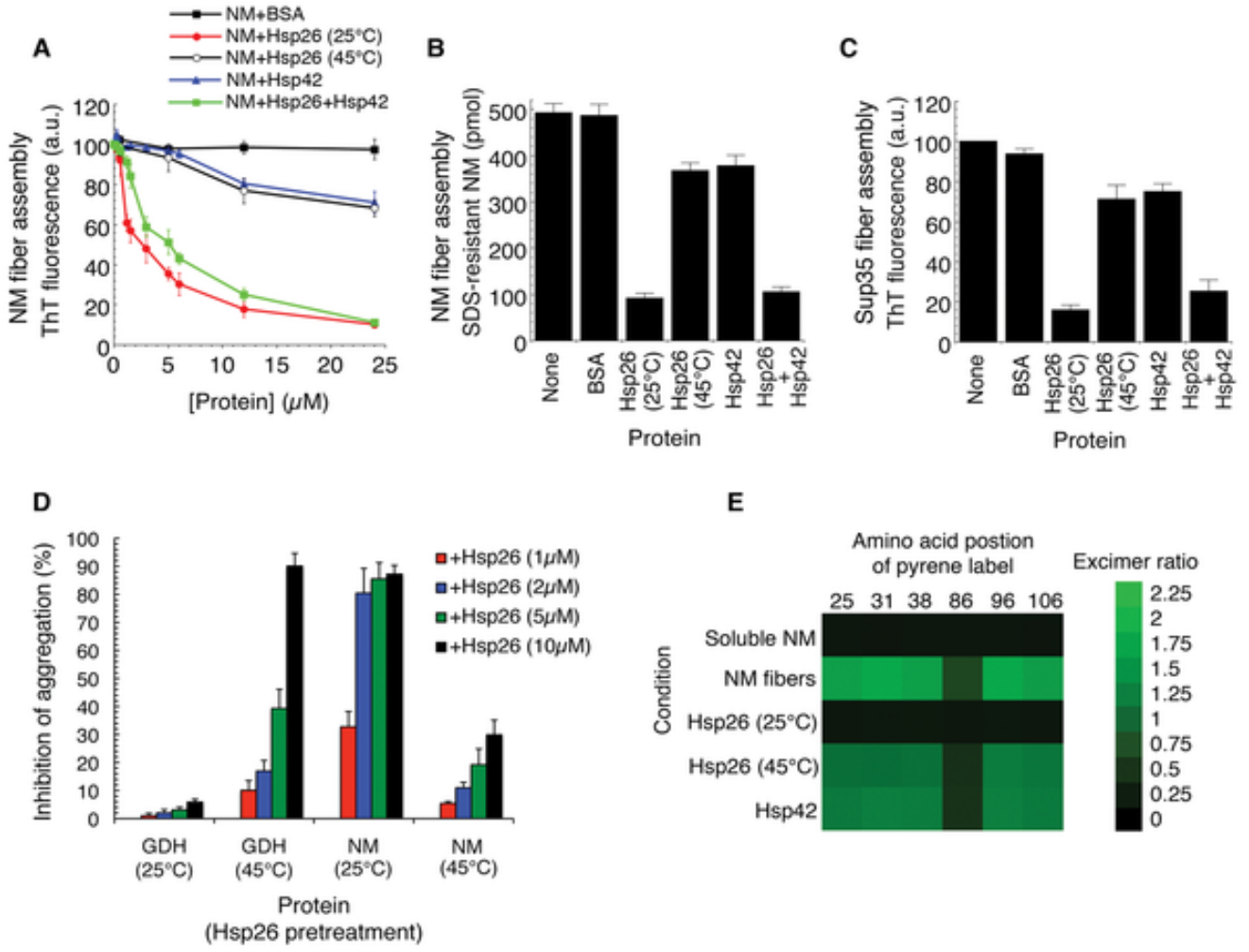
Duennwald ML, Echeverria A, Shorter J (2012) Small Heat Shock Proteins Potentiate Amyloid Dissolution by Protein Disaggregases from Yeast and Humans. PLoS Biol 10(6): e1001346. doi:10.1371/journal.pbio.1001346
http://127.0.0.1:8081/plosbiology/article?id=info:doi/10.1371/journal.pbio.1001346

- **Figure 4. Hsp26 and Hsp42 antagonize Sup35 prion formation in vivo.**



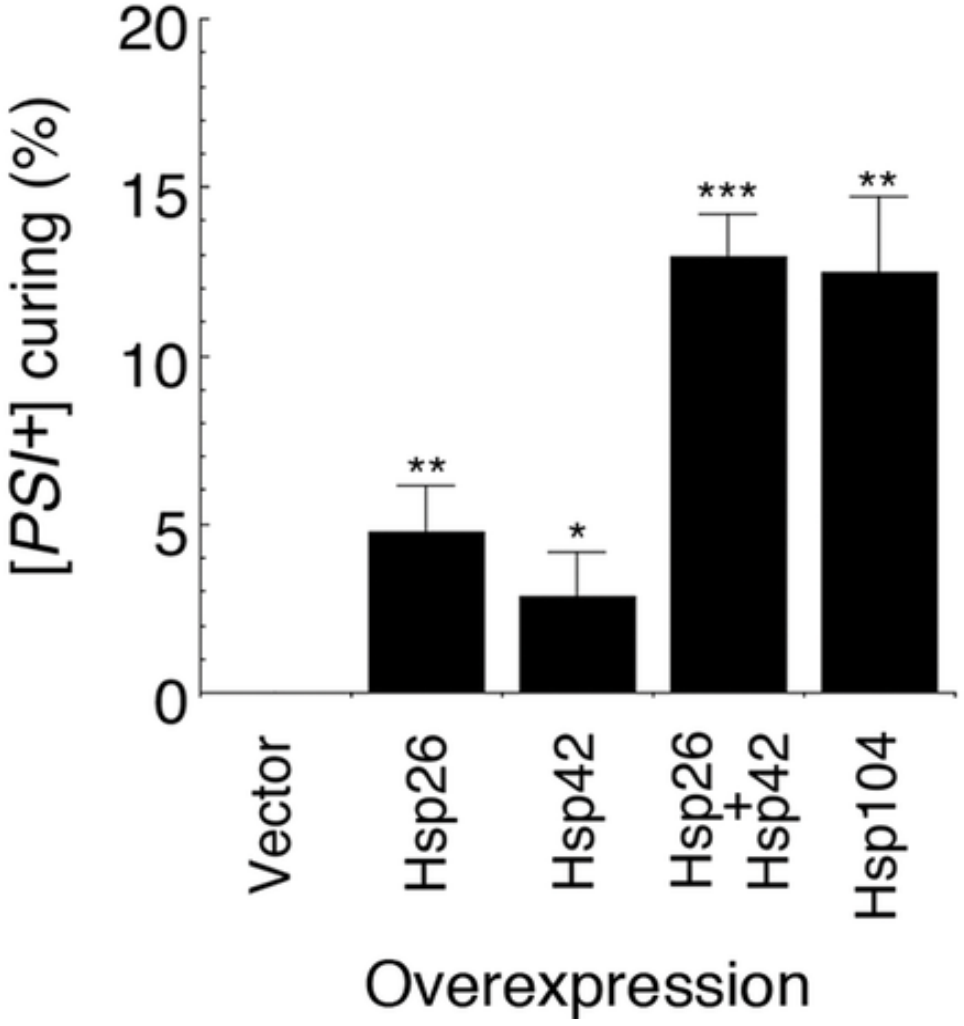
Duennwald ML, Echeverria A, Shorter J (2012) Small Heat Shock Proteins Potentiate Amyloid Dissolution by Protein Disaggregases from Yeast and Humans. PLoS Biol 10(6): e1001346. doi:10.1371/journal.pbio.1001346
http://www.plosbiology.org/article/info:doi/10.1371/journal.pbio.1001346

- **Figure 6. Hsp26 inhibits seeded assembly of Sup35 more potently than Hsp42 in a temperature-sensitive manner.**



Duennwald ML, Echeverria A, Shorter J (2012) Small Heat Shock Proteins Potentiate Amyloid Dissolution by Protein Disaggregases from Yeast and Humans. PLoS Biol 10(6): e1001346. doi:10.1371/journal.pbio.1001346
http://127.0.0.1:8081/plosbiology/article?id=info:doi/10.1371/journal.pbio.1001346

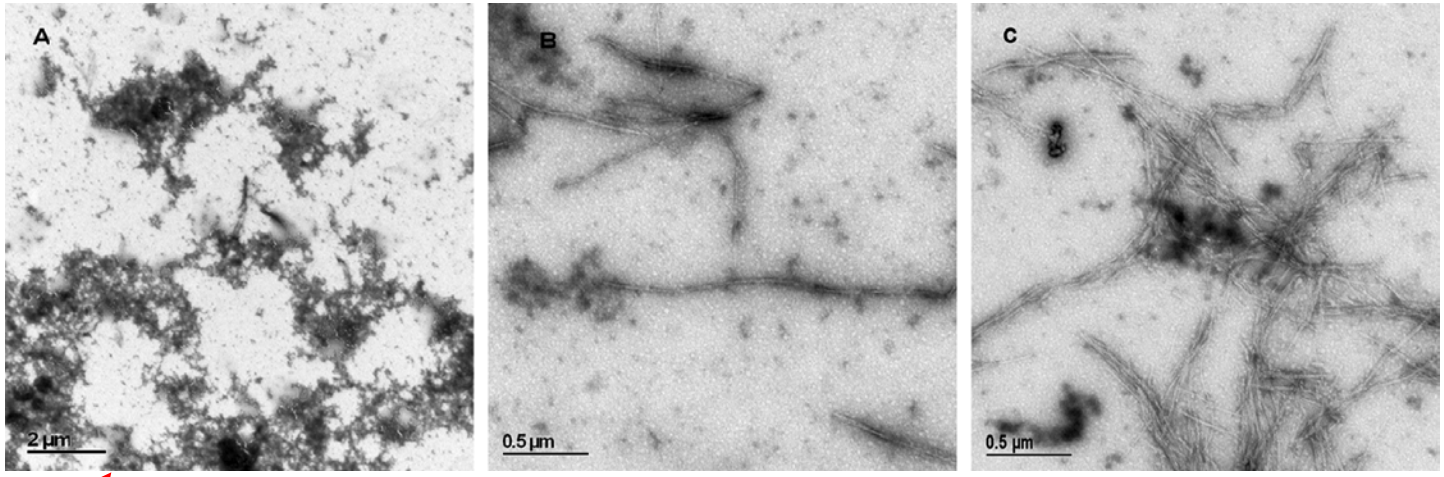
- **Figure 8. Elevated expression of Hsp26 and Hsp42 effectively cures [PSI+].**



Duennwald ML, Echeverria A, Shorter J (2012) Small Heat Shock Proteins Potentiate Amyloid Dissolution by Protein Disaggregases from Yeast and Humans. PLoS Biol 10(6): e1001346. doi:10.1371/journal.pbio.1001346
http://127.0.0.1:8081/plosbiology/article?id=info:doi/10.1371/journal.pbio.1001346

- Amateur chaperones
- Amyloid-beta binding proteins

A-beta binding proteins	description	Reference
MAP1B	Microtubule assoc. protein 1B This interaction may explain the loss of neuronal cytoskeletal integrity and impairment of microtubule-dependent transport	Gevorkian et al., Neurochem. Int. 52, p.1030 (2007)
ERAB	Endoplasmatic reticulum assoc. binding protein	Ray et al., Neuroch. Res. 23, p.1277 (1998)
αB-crystallin	αB - crystallin is a small heat shock protein, predominantly in human lenses /chataract Its binding inhibits fibril elongation	Shammas et al., Biophys. J. 101, p.1681 (2011)
Cystatin C	The binding site was shown on the monomer. Inhibition of A-beta fibrillation was shown.	Sastre et al., 2004
Stefin B (cystatin B)	The binding occurs with domain-swapped dimers, especially the tetramer and higher oligomers. Binding to oligomers of stefin B inhibits A-beta fibril elongation	Čeru et al., J. Biol. Chem. (2010)
Prion protein PrP^C	Oligomers of A-beta (1-42) bind prion PrP^C	Dohler et al., Brain 137, p. 873 (2014)

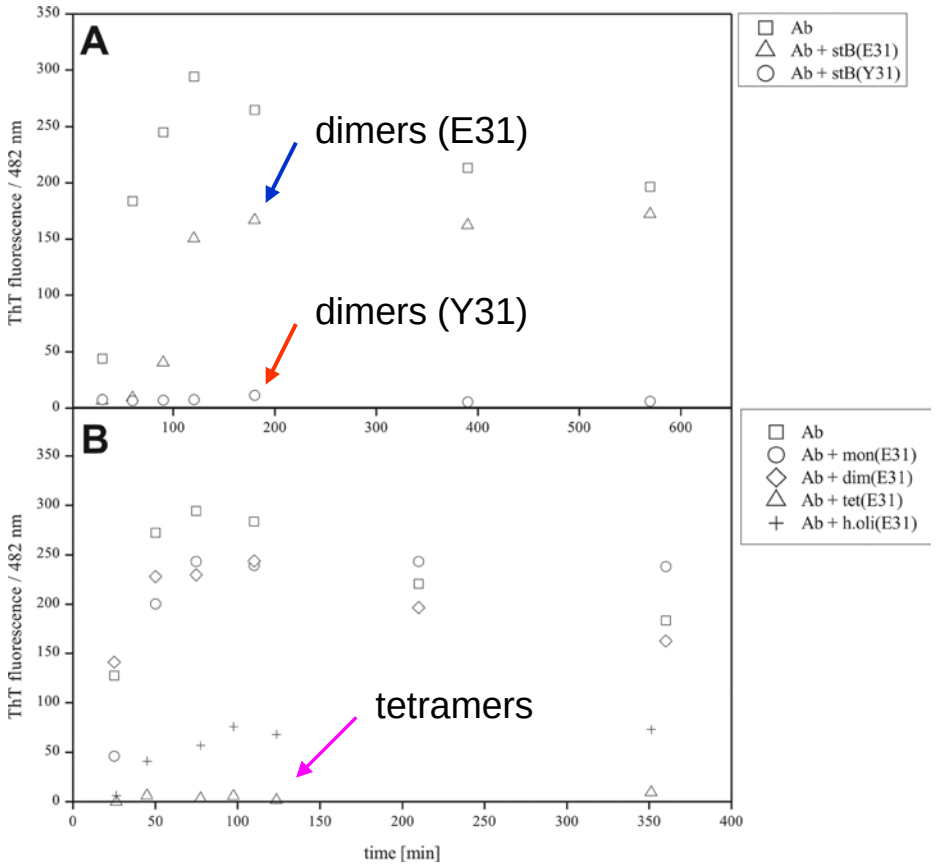


Inhibition of amyloid –fibril

formation **of A-beta** by

dimers of Y31-stefin B

tetramers of E31(wt)- stefin B

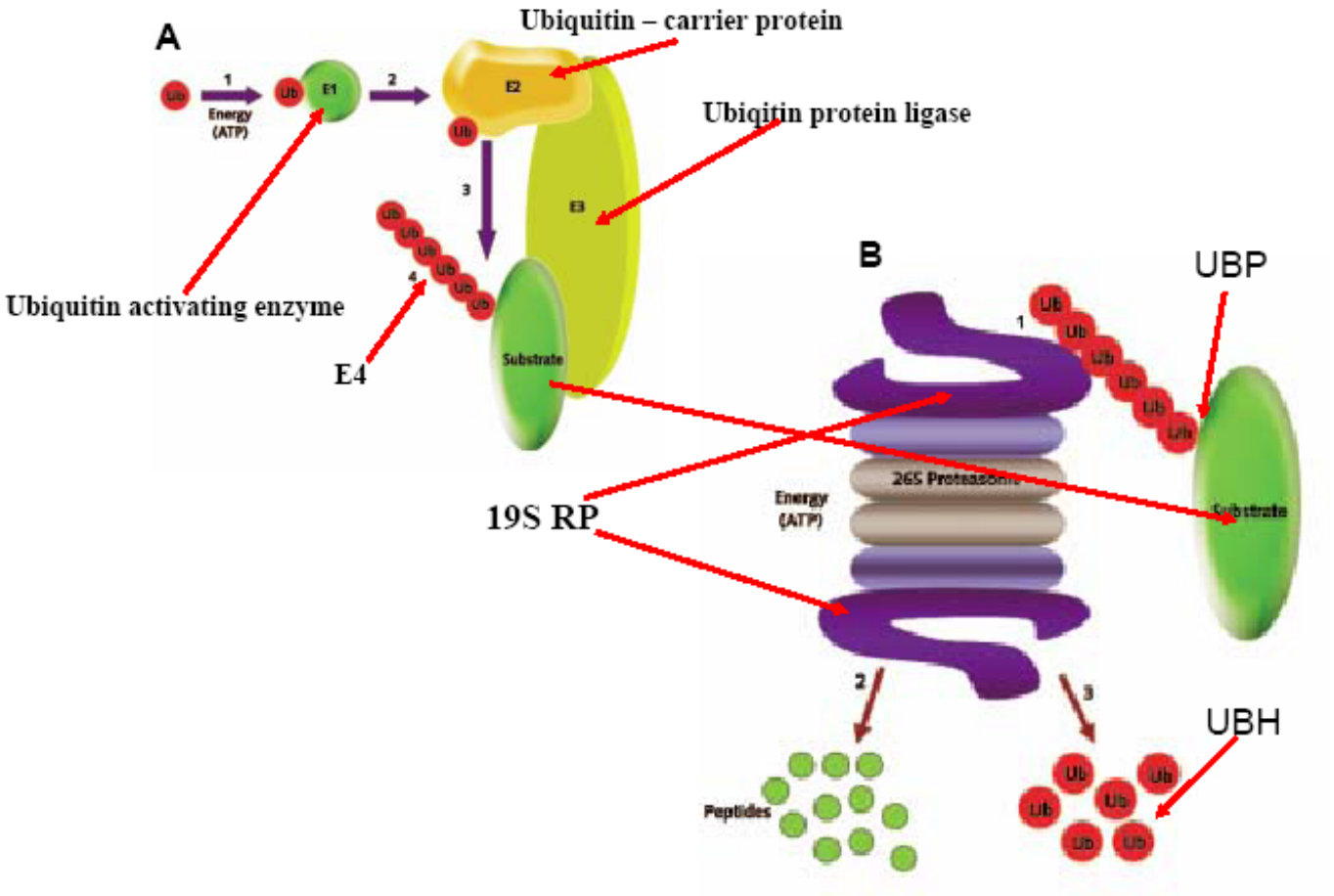


From Škerget et al., 2010, J.Biol.Chem.

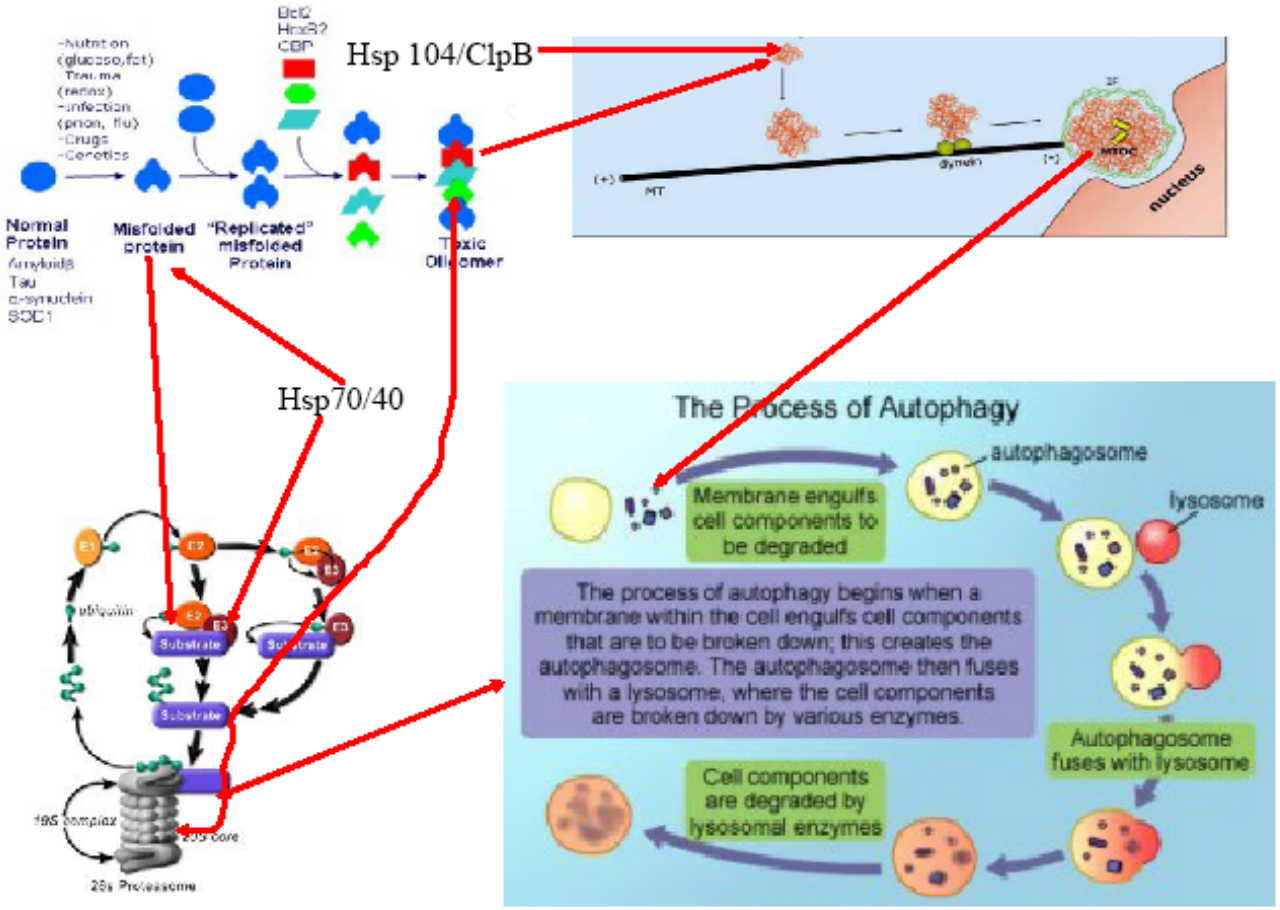
The 2 degradative systems

- UPS
- autophagy

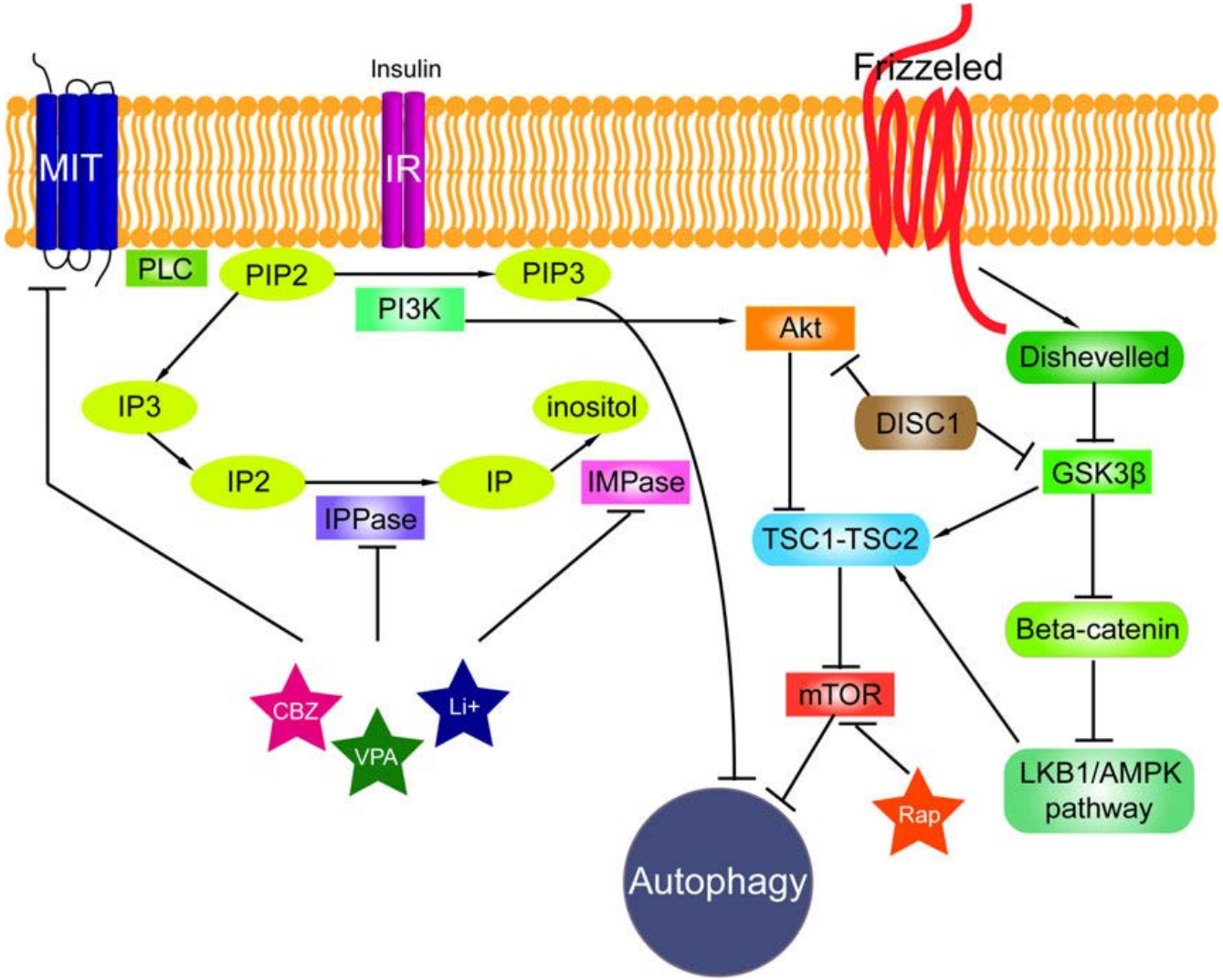
The complexity of proteasome system



Degradation of protein aggregates inside the mammalian cell



Signaling pathways connected to autophagy



From Polajnar et al., 2014

Journal of Cellular and Molecular Medicine, doi: 10.1111/jcmm.12349

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