Proteasome, Neurodegeneration, and Prions

Journal Club

20.08.2019

Interactive Journal Club - Outline

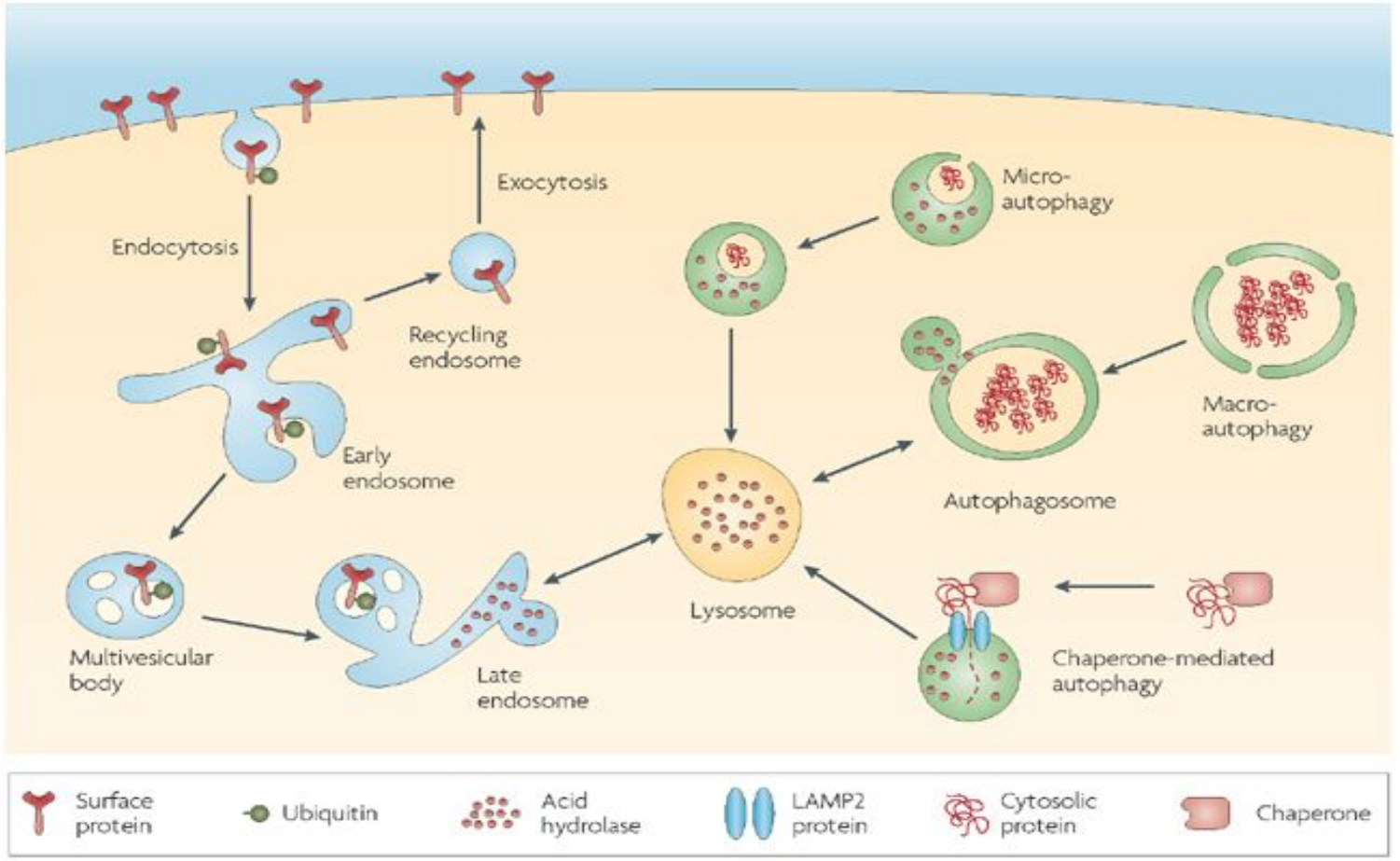
- Proteasome basics
- Proteasome in neurodegenerative diseases
- Proteasome in prion biology
  - PrP^{C} and the Proteasome
  - PrP^{Sc} and the Proteasome

Proteasome basics

Two major ways to degrade proteins

Lysosomal pathways

- Degrades most membrane and endocytosed proteins
- Can also degrade cytosolic proteins through autophagy



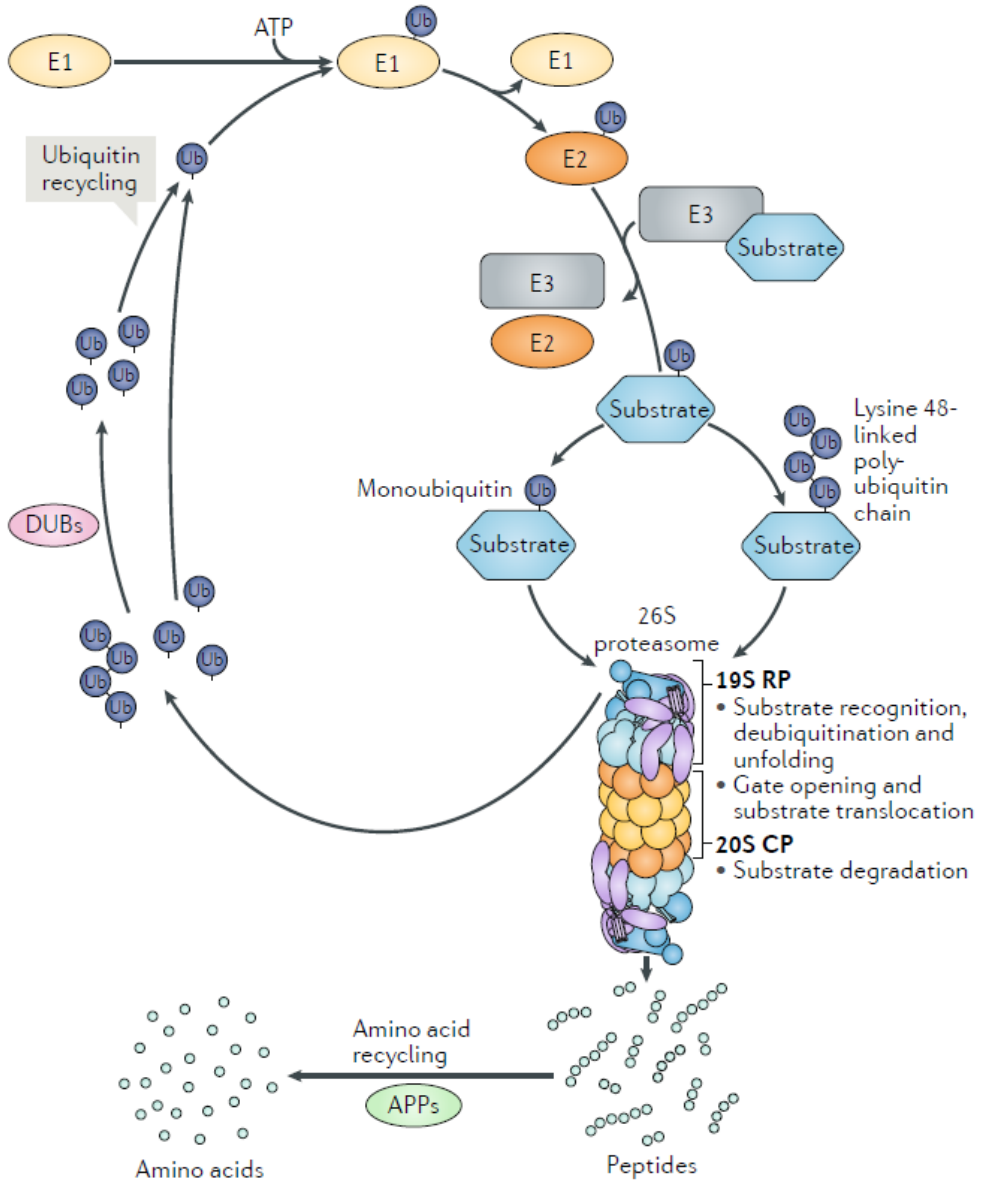
Nature Reviews | Neuroscience

Proteasome basics

Two major ways to degrade proteins

Ubiquitin–proteasome system

- Responsible for degrading most intracellular, soluble proteins
- can also degrade transmembrane proteins if they are extracted from the membrane into the cytosol



Proteasome basics

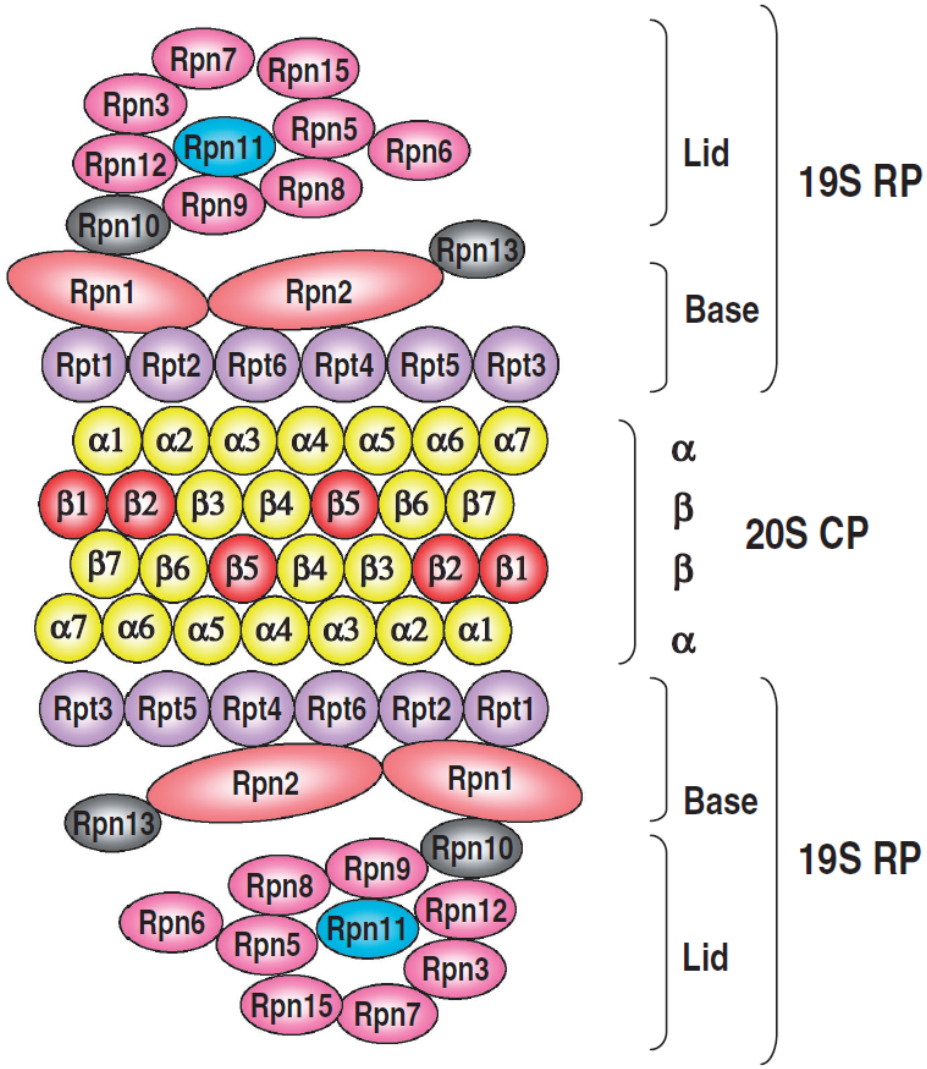
General proteasome composition

19S regulatory particle (lid)

- Reponsible for identification of proteins that are targeted to the proteasome
- Responsible for deubiquitination of tagged proteins so that they can enter the catalytically active core
- Prevents unregulated access to the proteolytic core

20S core particle (base)

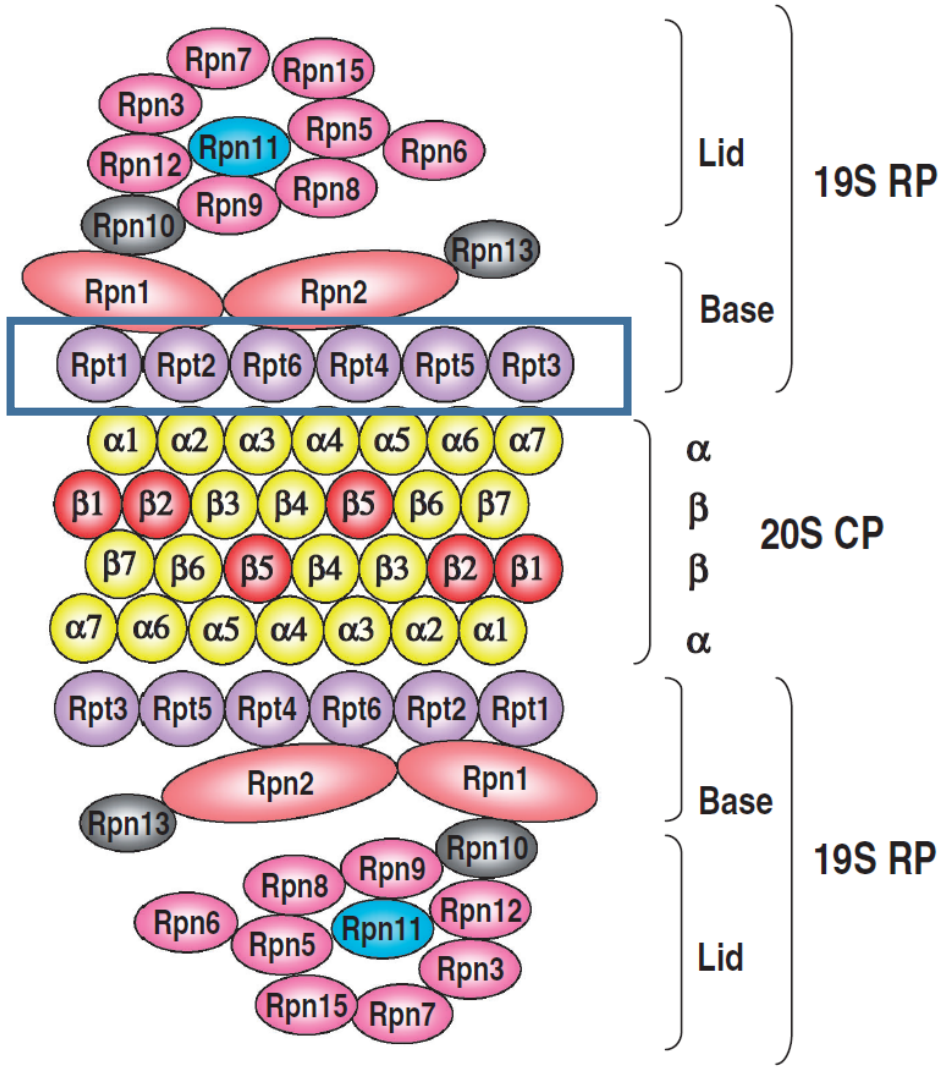
- Catalytically active subunits stacked in four heteroheptameric rings
- Certain subunits have proteolytic functions; break down proteins to amino acid chains, which will be degraded further by aminopeptidases



Proteasome basics

General proteasome composition

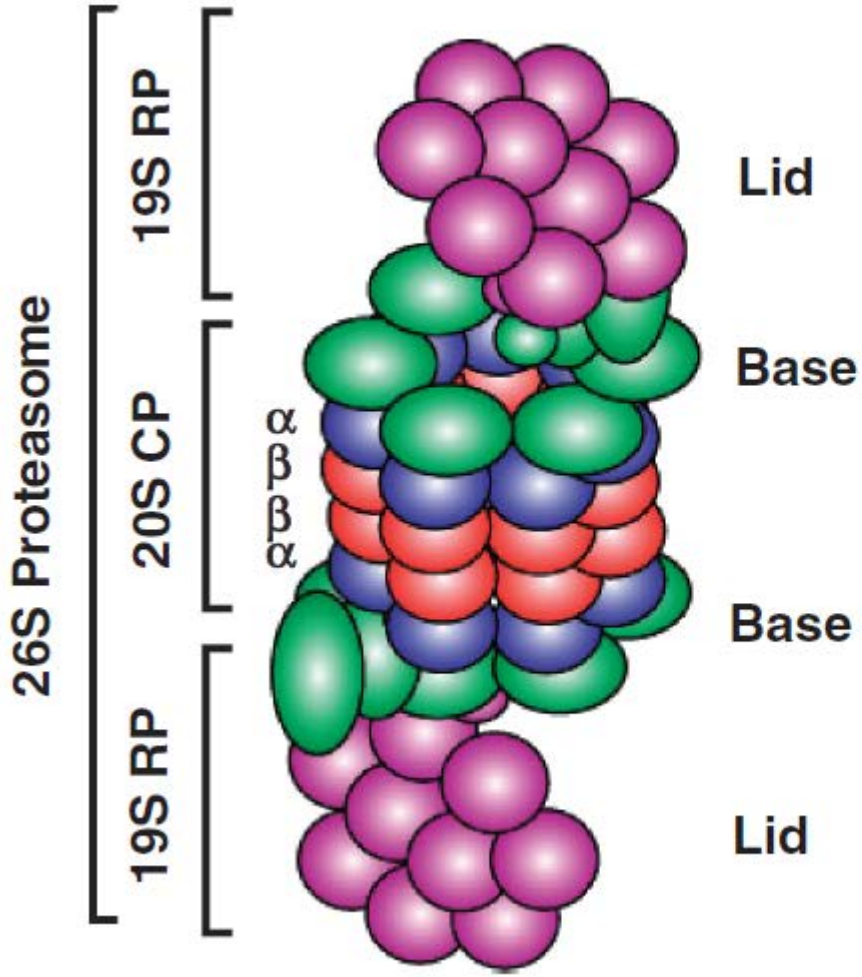
Six ATPases arranged in a hexameric ring within the base of the RP, which couples ATP hydrolysis to substrate unfolding and translocation through its channel pore



Proteasome basics

Proteasome mode of action

https://www.youtube.com/watch?v=TgOe7aPVpoM



Capture polyubiquitylated proteins (Rpn10, Rpn13)

↓

Deubiquitylate (Rpn11)

↓

Unfold & Open α-gate (Rpt1 - Rpt6)

↓

Proteolysis (β1, β2 & β5)

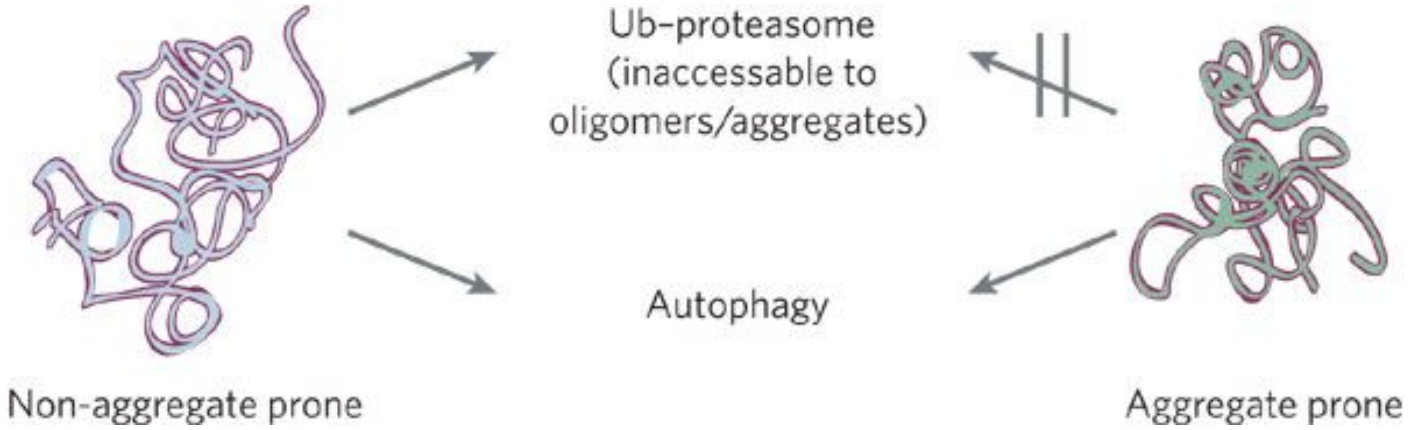
⇐

Keep in mind for later

If a proteins is captured and deubiquitinated, a conformational change leads to the opening of the lid to allow access to the proteolytic core

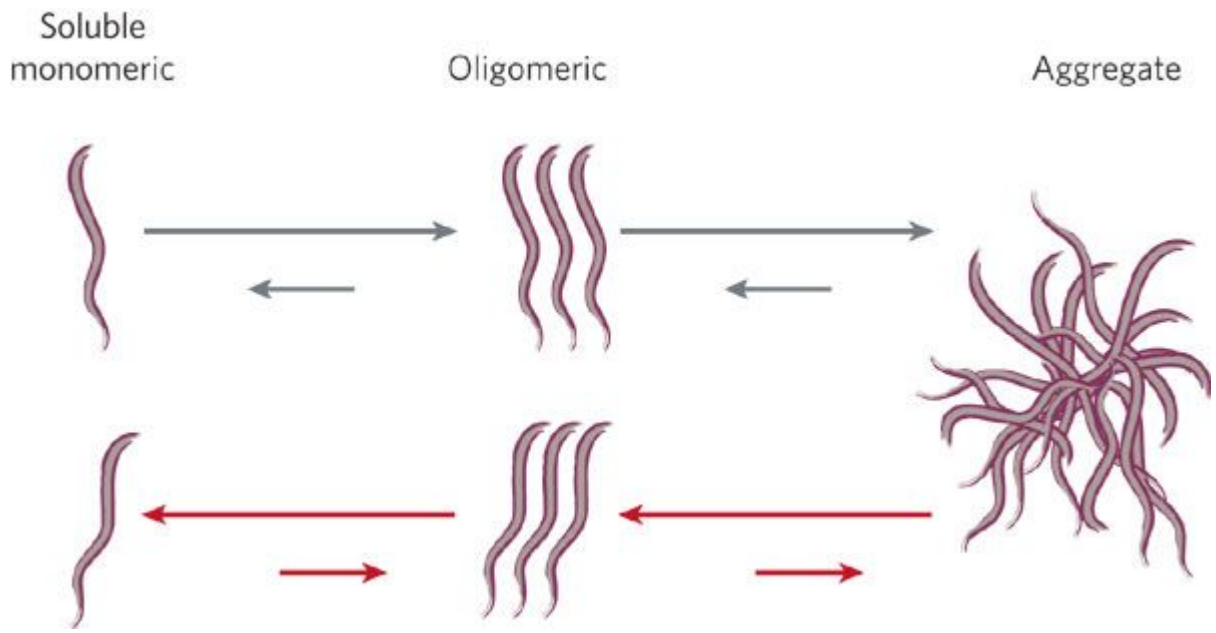
Mediated by a HbYX motif on specific subunits of the lid

Proteasome in neurodegeneration



- UPS as the main mechanism for protein degradation of cytosolic proteins
- Aggregates are thought to be too big to be degraded by it
- Macroautophagy as the main aggregate degradation pathway

Proteasome in neurodegeneration



“Under normal conditions of substrate synthesis and basal autophagy, the direction of the equilibrium of an aggregate-prone protein is towards aggregate formation. If substrate synthesis is stopped or clearance of the soluble/oligomeric forms is enhanced (for instance, by autophagy) then this equilibrium can be reversed and aggregates are indirectly cleared”

Proteasome in neurodegeneration

Other relations to neurodegeneration?

- Strong correlation between aging and decrease in proteasomal activity
- Certain proteins related to neurodegenerative diseases are proteasomal substrates (e.g. Tau, a-syn, Htt)
- Mutations of related proteins, or inhibition of the proteasome can lead to neurodegeneration (e.g. PARKIN1 as an E3 ligase)

- **Aggregates linked to neurodegeneration are thought to inhibit proteasomal function**

Proteasome in other neurodegenerative diseases

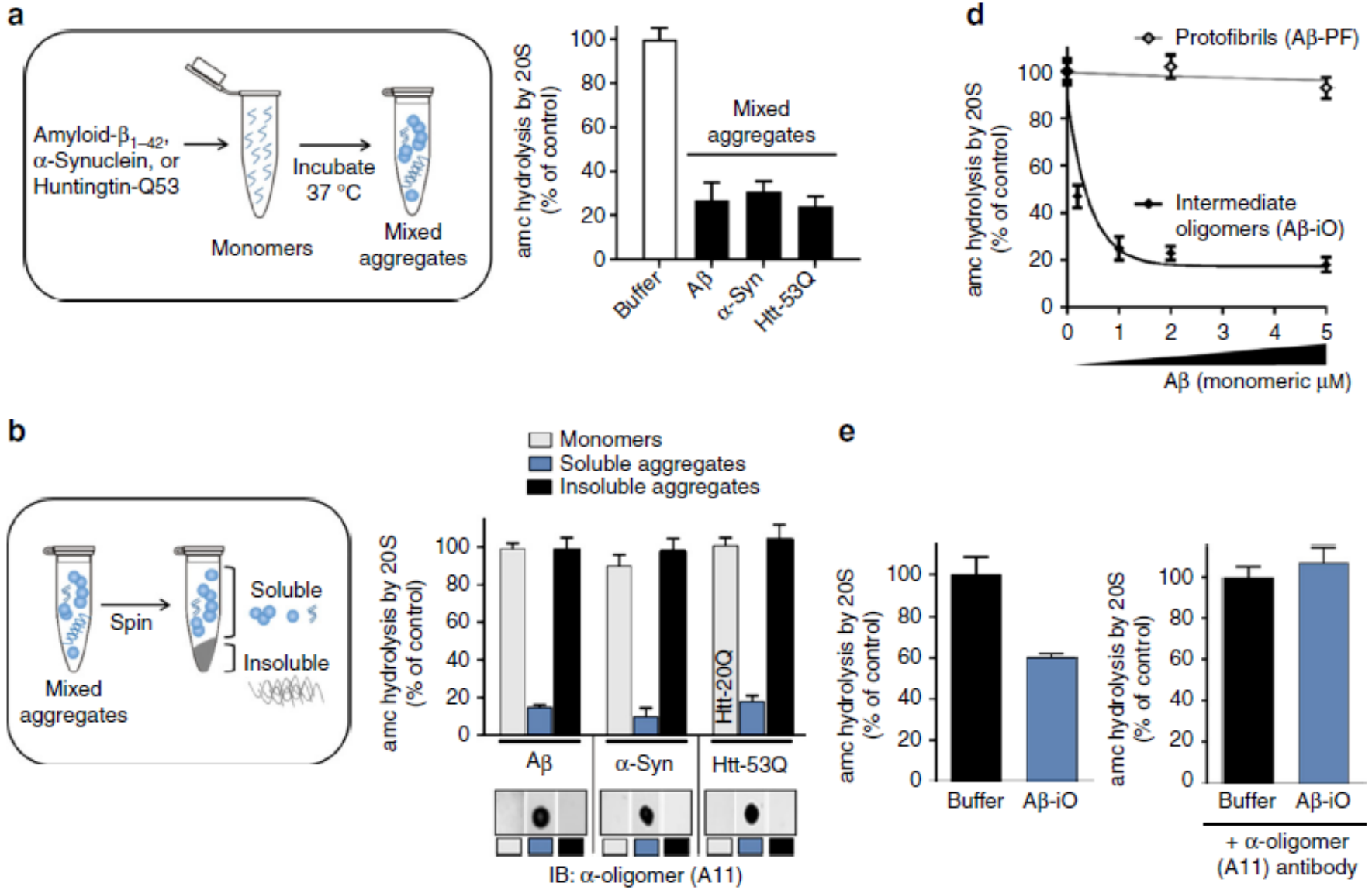
ARTICLE

DOI: 10.1038/s41467-018-03509-0 OPEN

A common mechanism of proteasome impairment by neurodegenerative disease-associated oligomers

Tiffany A. Thibaudeau[1], Raymond T. Anderson[1] & David M. Smith[1]

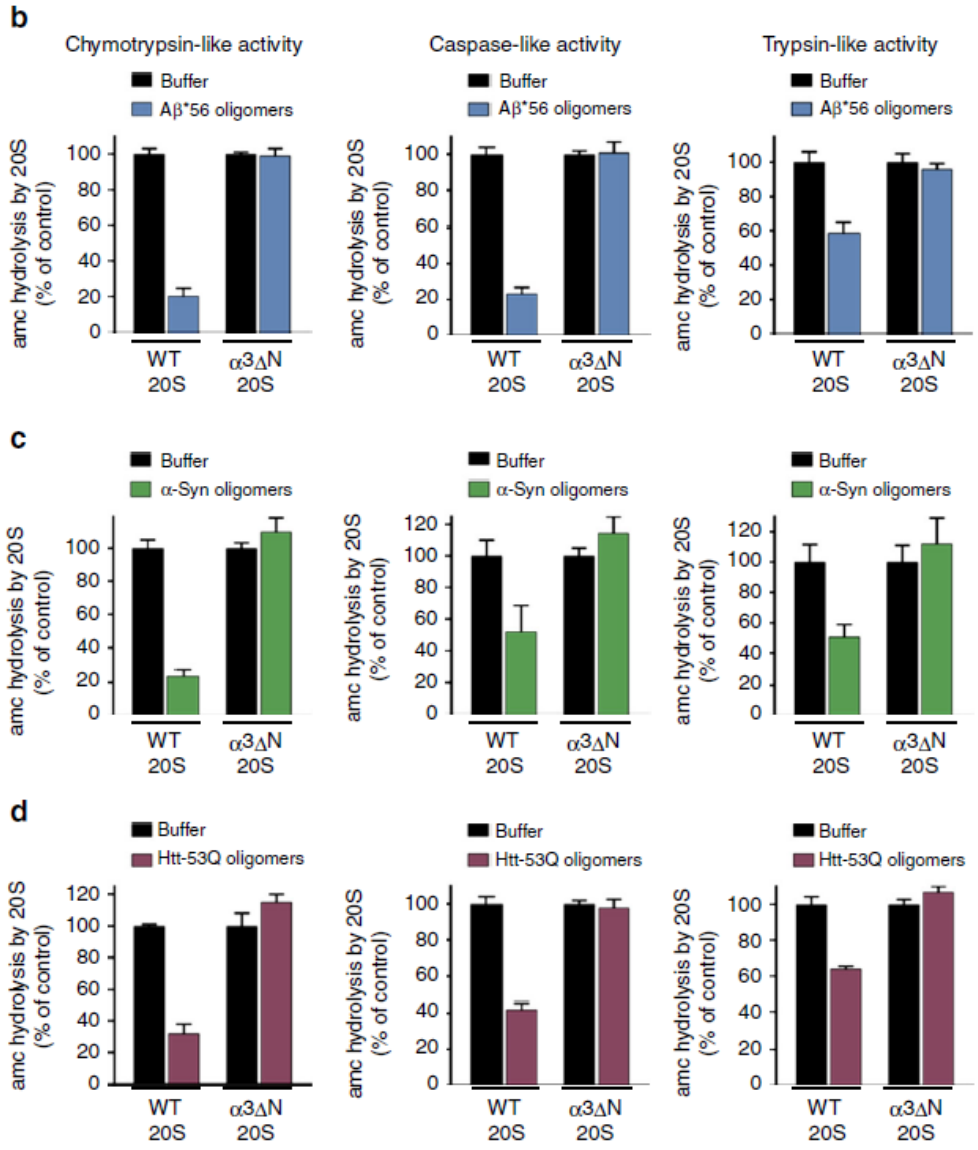
Proteasome in other neurodegenerative diseases



Proteasome in other neurodegenerative diseases

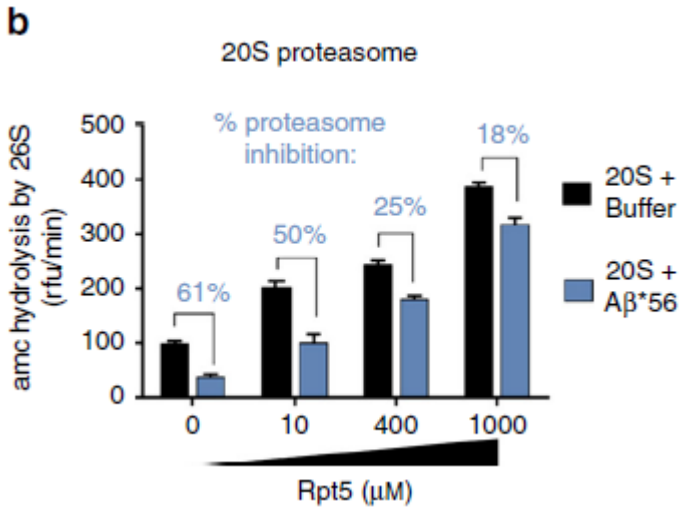
Different oligomers related to neurodegeneration can inhibit the 20S core part of the proteasome via impairment of substrate entry

If the α3-20S core subunit was mutated to have a constitutively open proteasome, inhibitory effect was abolished

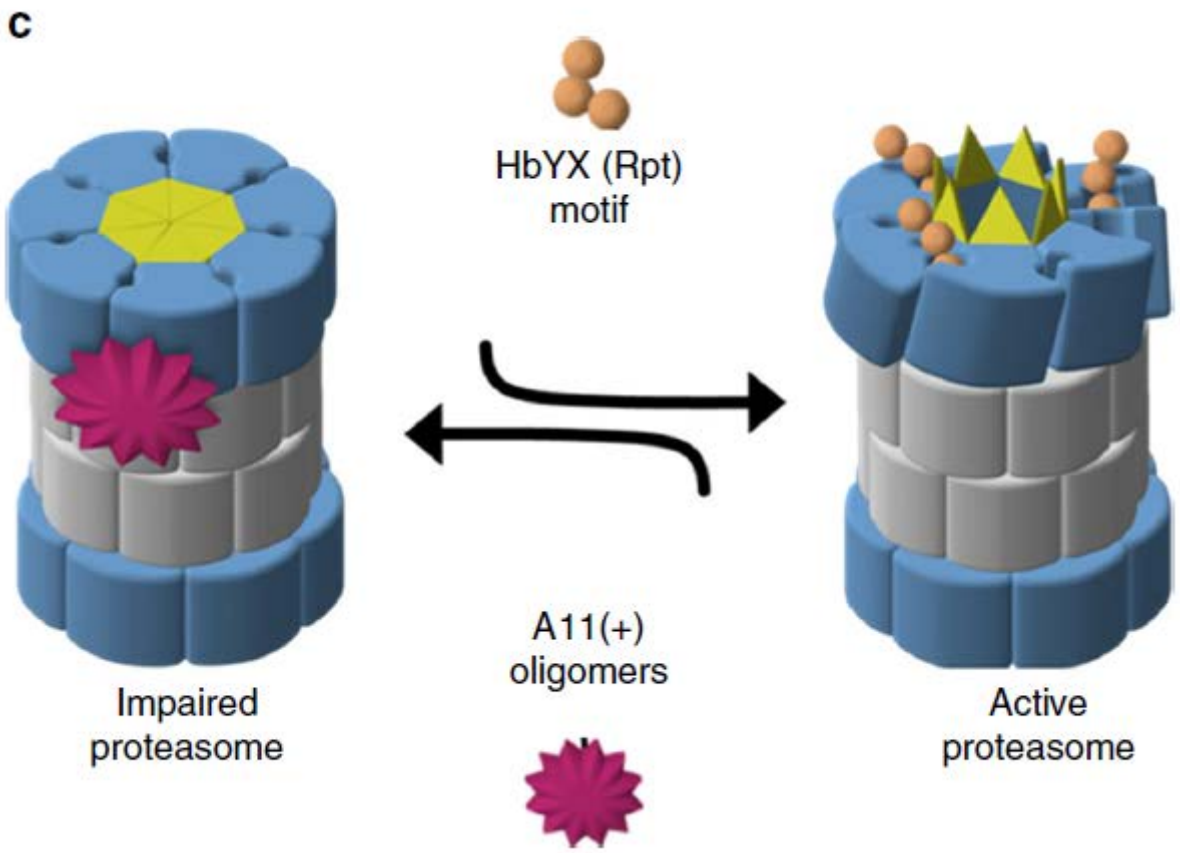


Proteasome in other neurodegenerative diseases

A small peptide mimicing the HbYX motif could decrease the effect of proteasomal inhibtion



Proteasome in other neurodegenerative diseases



Intermediate oligomers of a-syn, Aβ and Htt can impaire the proteasomal function by inducing closed gate structure

Latest research

Cell Reports

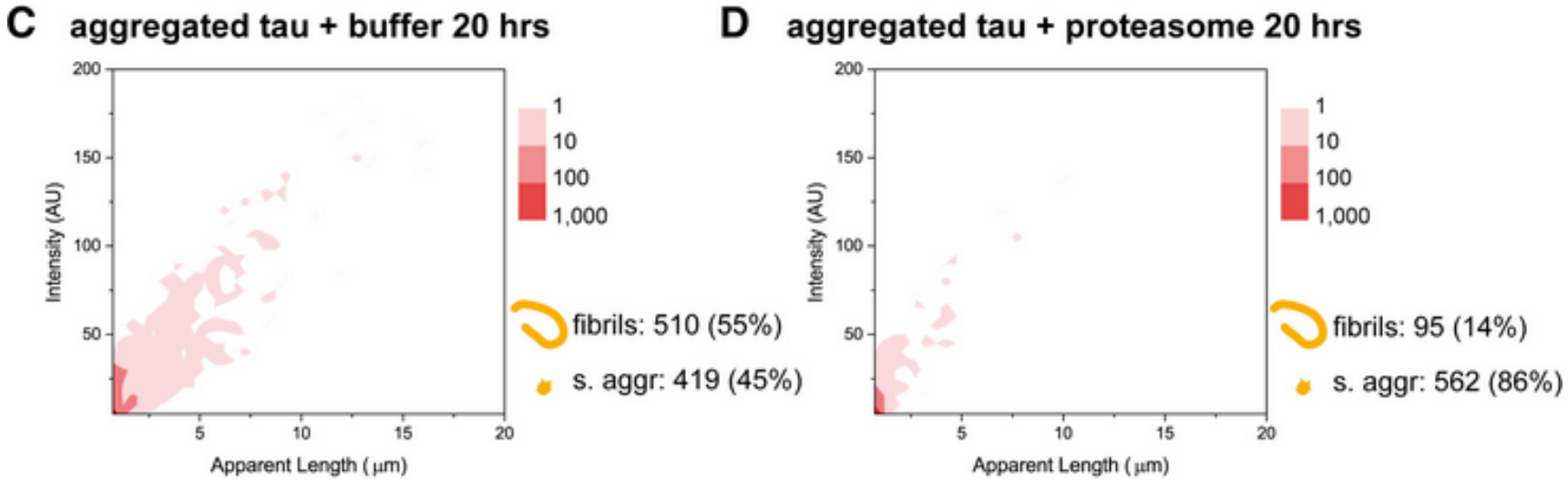
CellPress

Volume 26, Issue 8, 19 February 2019, Pages 2140-2149.e3

Article

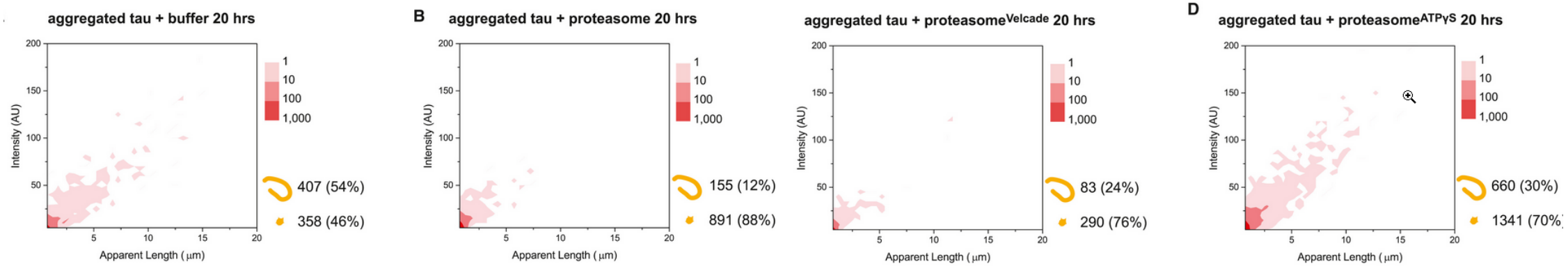
Filamentous Aggregates Are Fragmented by the Proteasome Holoenzyme

Rachel Cliffe [1, 4], Jason C. Sang [1], Franziska Kundel [1], Daniel Finley [2] ✉, David Klenerman [1, 3] ✉, Yu Ye [1, 2, 4, 5] ✉

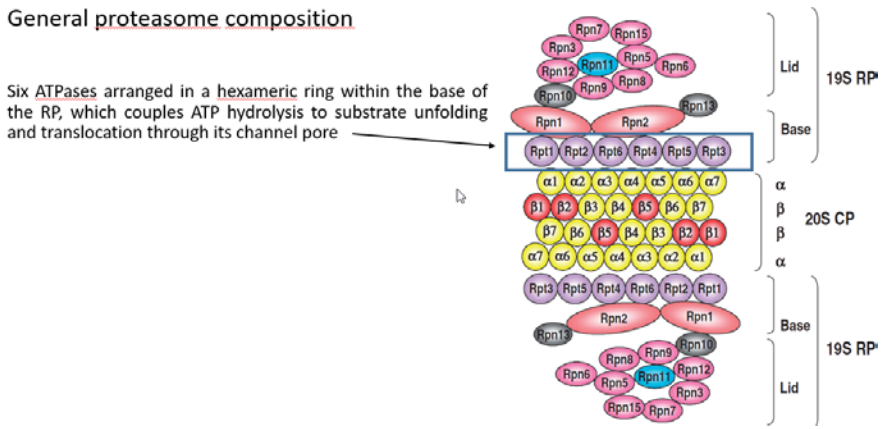


Aggregate length measured by total internal reflection fluorescence microscopy: TIRFM

Latest research



Study claims that ATPase activity of the proteasome (which is totally unrelated to the proteolytic part), is able to fragment filamentous fibrils



Proteasome in other neurodegenerative diseases

To summarize:

- Oligomeric (A11+) species of different disease relevant proteins can inhibit the proteasome via interacting with the 20S core part, preventing substrate entry
  - Proteasomal inhibition might lead to a vicious cycle in proteinopopathies, especially with regards to proteins that are normally a substrates of the proteasome
- Inhibtion of the proteaseome potentially elicits toxic effects
- Effect can be alleviated by appliying and anti-A11 antibody or a small peptide mimicing the HbYX motif enhancing open-gate confirmation

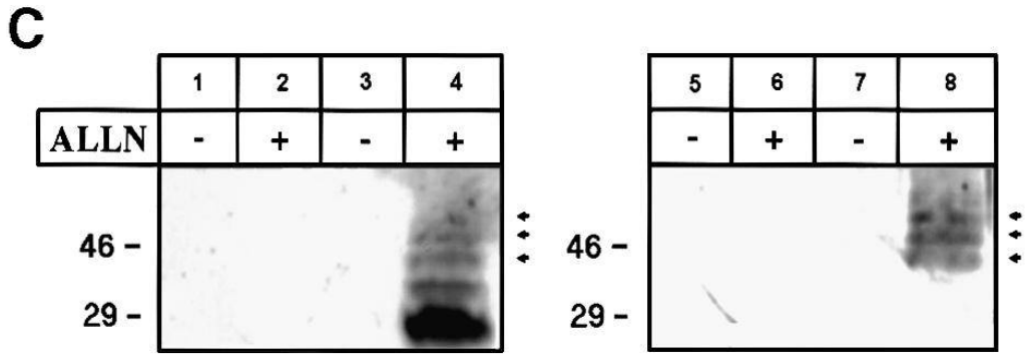
- ATPase activity of the proteasome enhances the fragmentation of fibrils *in vitro*
  - *Potentially unanticipated function of the proteaseome as a disaggregase*

Proteasome in prion biology - PrP^C

- PrP^C as a cell membrane protein is mainly degraded by the lysosomal pathway

Controversial findings:

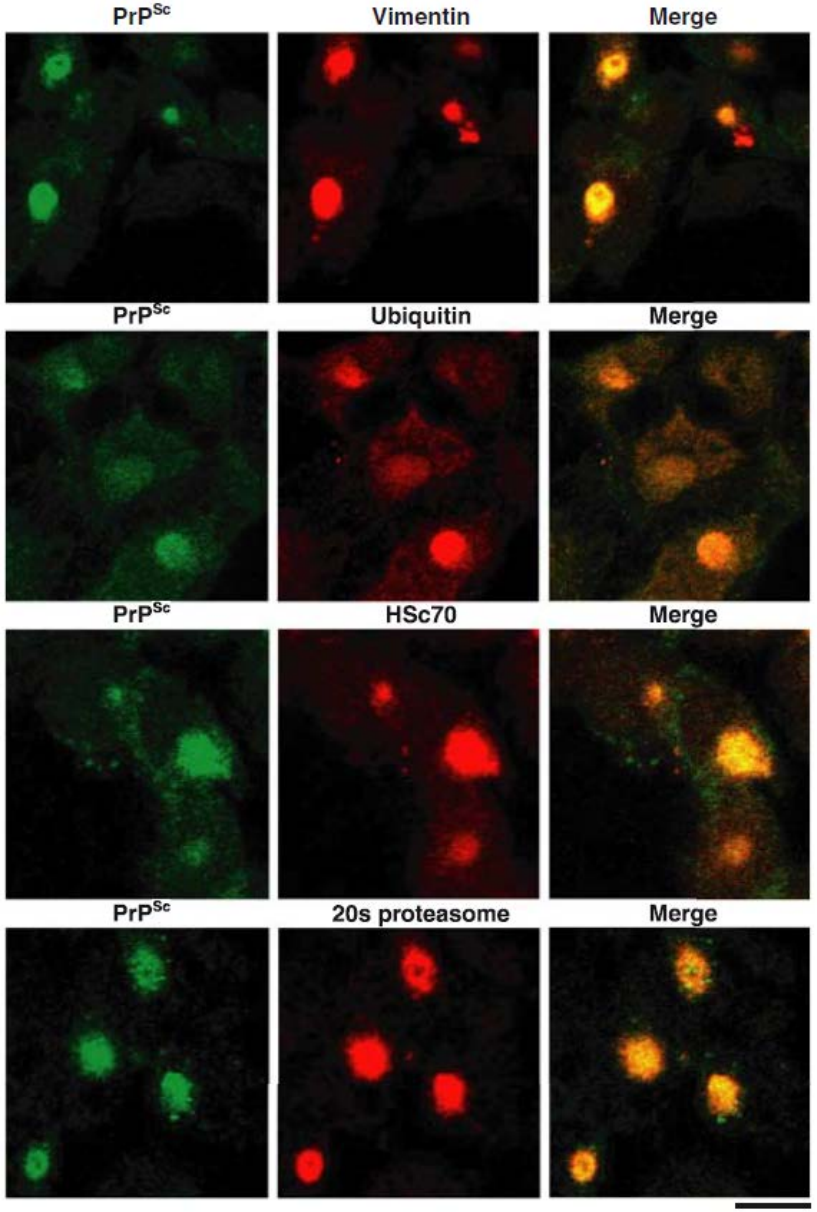
- Ma & Lindquist (2001) and Yedida (2001) showed that treatment of cells with different proteasome inhibitors was leading to PrP^C accumulation in the cytoplasm. (Drisaldi (2003), showed an increase of PRNP mRNA upon proteasomal inhibition)
- Yedida also showed the existence of ubiquitinated PrP^C (however in CHO cells expressing the human PrP…)



- PrP^C might partially be a proteasomal substrate and reach the cytoplasm via different speculative mechanisms such as retrotranslocation. However, the question remains controversial

Proteasome in prion biology - PrP^{Sc}

After
1uM Lactacystein

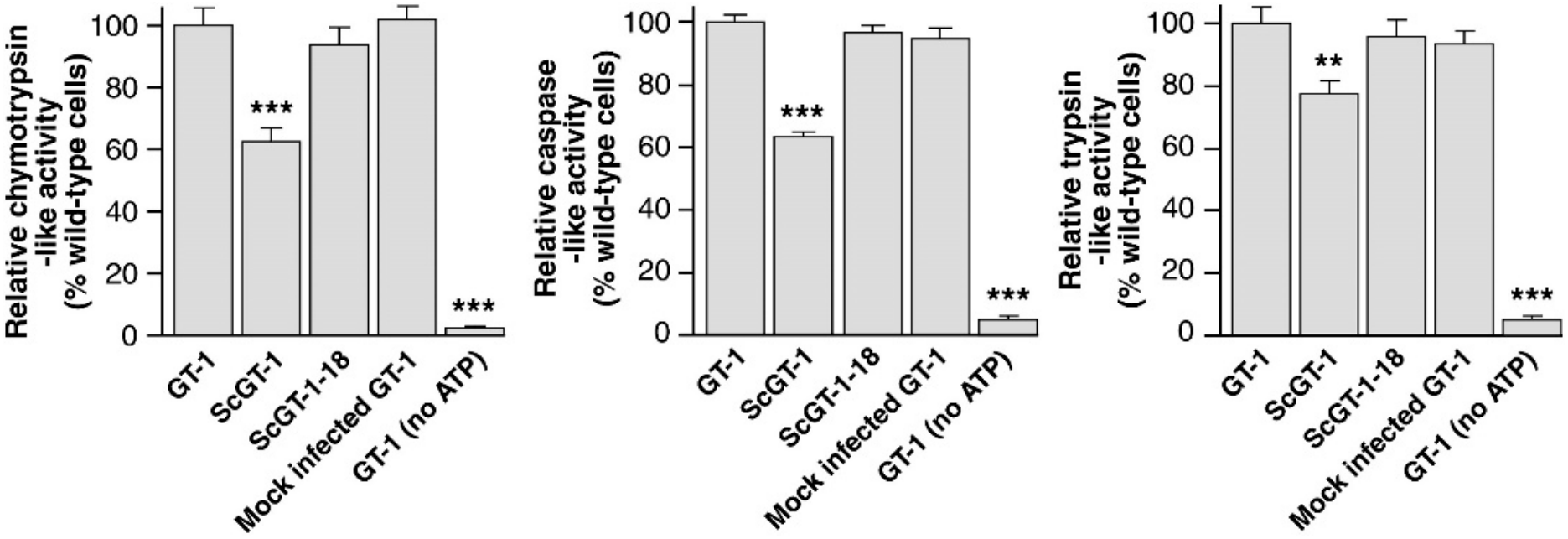


- PrP^{Sc} has been shown to be localized at least partially in the cytoplasm and therefore might interact with the proteasome
- Kristiansen (2005) showed cytosolic aggresome formation containing PrP^{Sc} after mild proteasome inhibition of prion-infected cells

=> Proteasomal inhibition does not seem to be beneficial for prion clearance, either related to enhanced PrP^{C} production or via direct or indirect mechanisms

Proteasome in prion biology - PrP^{Sc}

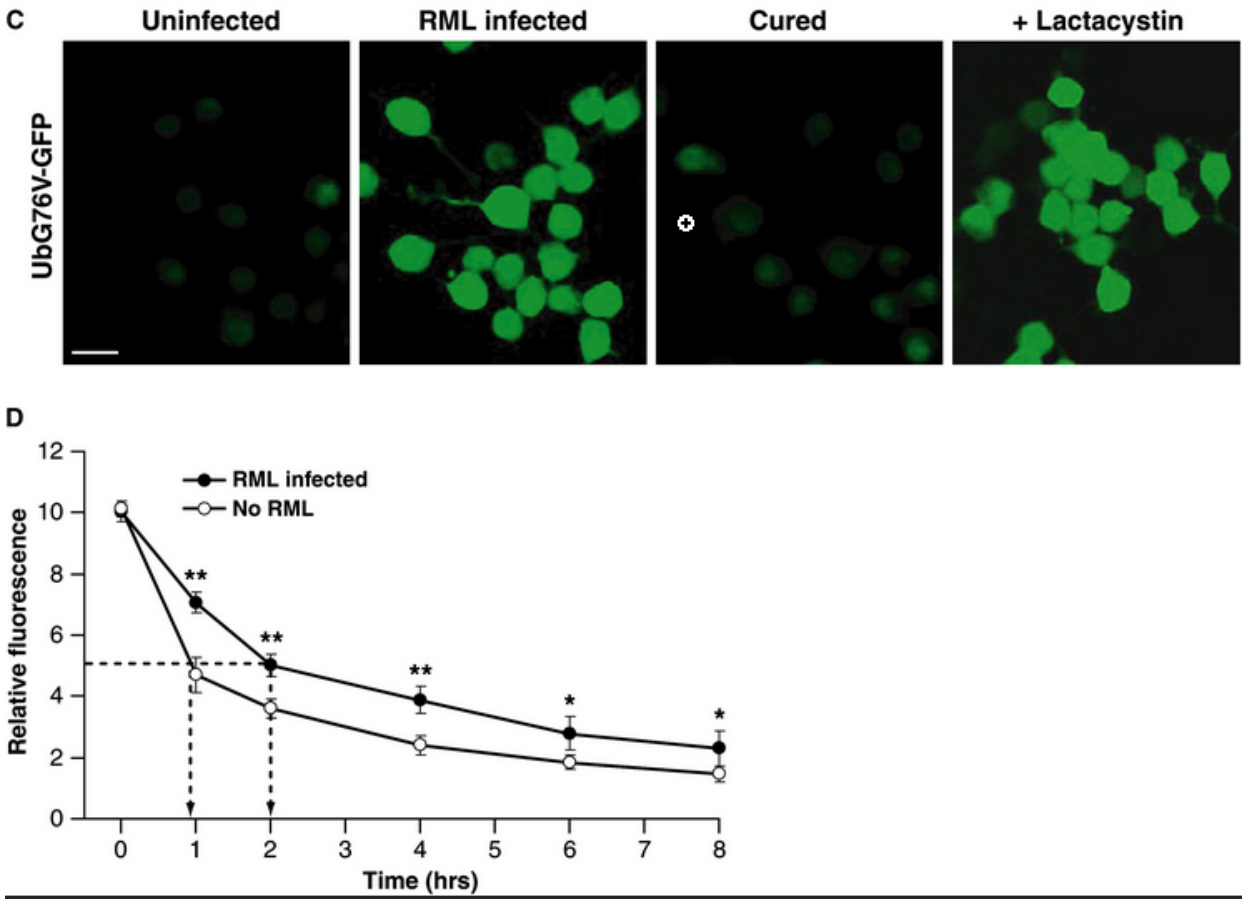
However, additional effect of prions on the Proteasome (Kristiansen et al. (2007))



Prion infection decreased proteasomal activity in two different prion infected cell lines (data only shown for one), which could be abrrogated by applying an anti-PrP antibody treatment

Proteasome in prion biology - PrP^{Sc}

However, additional effect of prions on the Proteasome (Kristiansen et al. (2007))

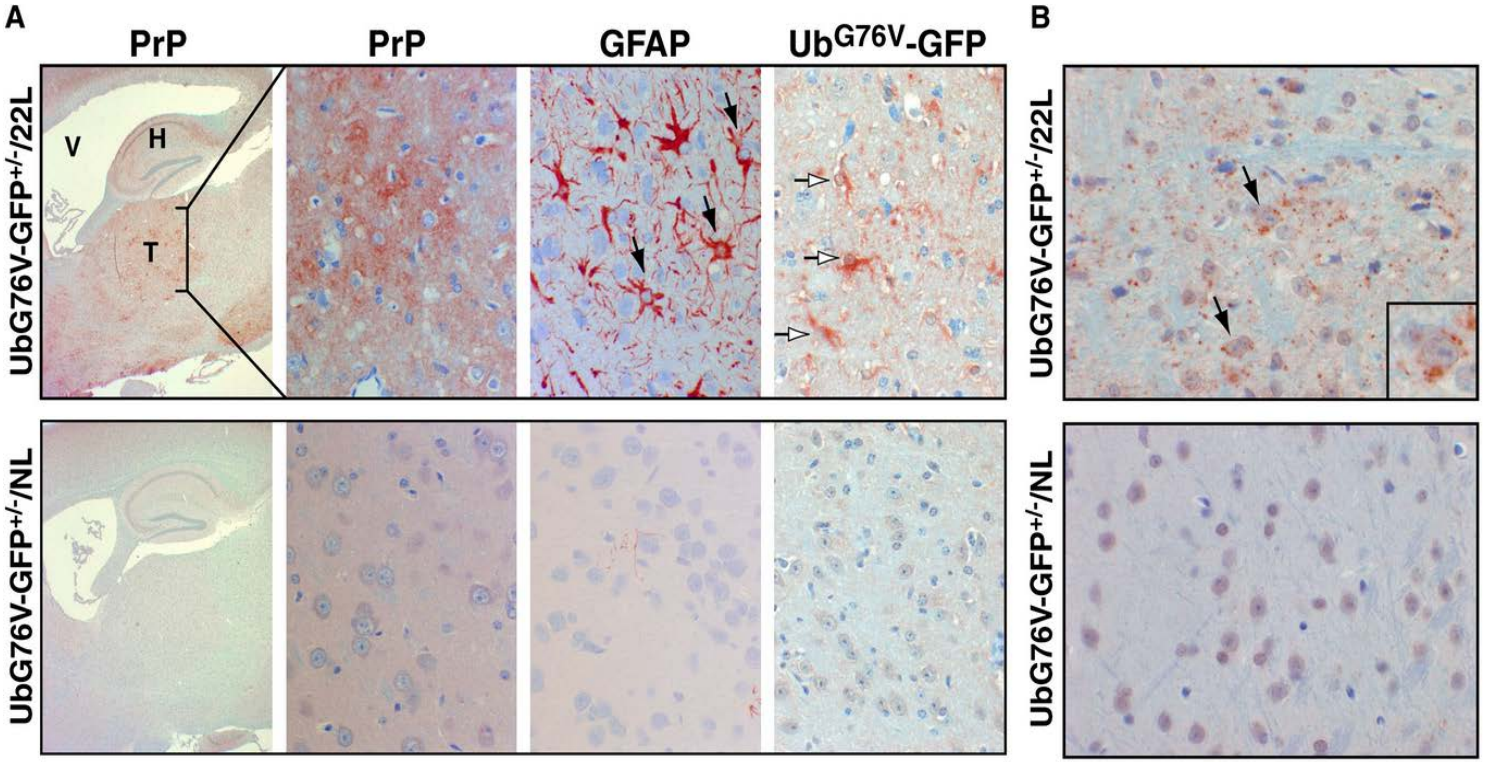


Accumulation of the fluorescent GFP reporter (for proteasomal activity) occurs in prion-infected Ub^{G76V}-GFP expressing N2aPK-1 cells or lactacystin-treated (50 µM) N2aPK-1 cells

ScN2aPK-1 cells took twice as long as N2aPK-1 cells to clear 50% of the accumulated Ub^{G76V}-GFP

Proteasome in prion biology - PrP^{Sc}

However, additional effect of prions on the Proteasome (Kristiansen et al. (2007))

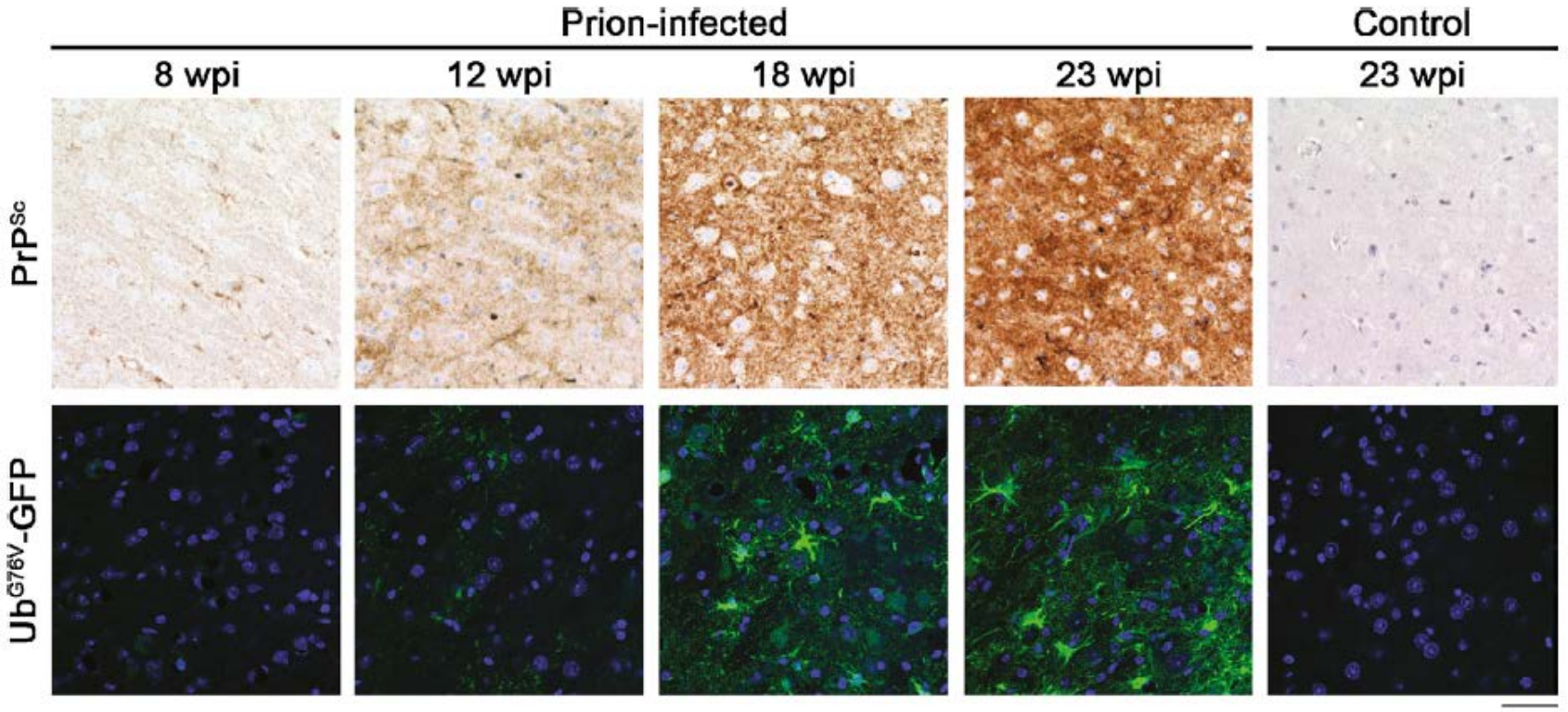


Prion infection inhibited degradation of the Ub^{G76V}-GFP reporter

Proteasomal inhibition by prions *in vivo*

Proteasome in prion biology - PrP^{Sc}

Time course experiment of prion inoculated mice for proteasomal activity (McKinnon et al (2016))

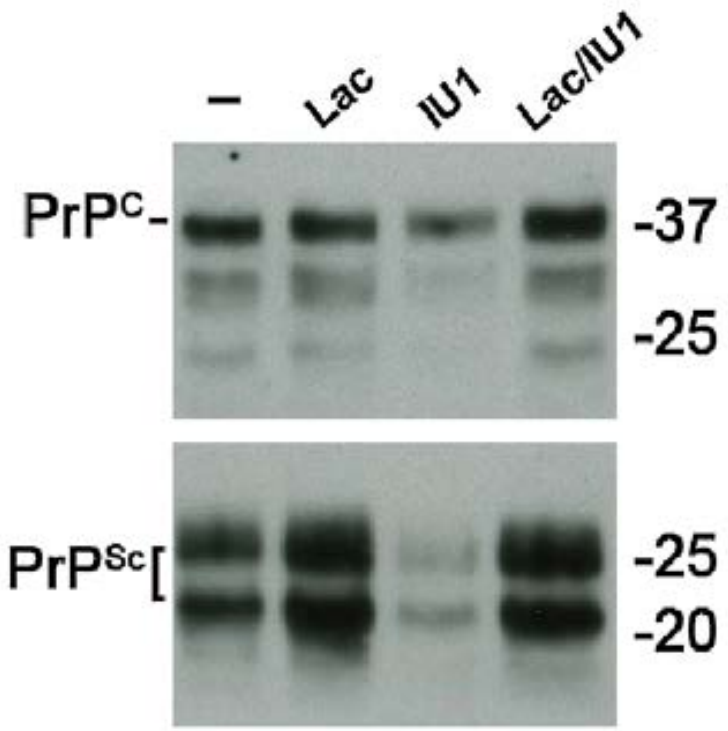


Prion infection inhibited degradation of the Ub^{G76V}-GFP reporter already early on

Proteasomal inhibition by prions *in vivo*

Proteasome in prion biology - PrP^{Sc}

Proteasomal inhibition in scCAD5 (McKinnon et al (2016))



Also, prion infected scCAD5 show higher amount of PrP^{Sc}, which can be abbrogated by the treatment with a «proteasomal activator»

Proteasome in prion biology - PrP^{Sc}

Further studies showed, that PrP^{Sc} directly interacts with the 20S core part of the proteasome, thereby preventing substrate entry.

(Deriziotis, P., et al. (2011). "Misfolded PrP impairs the UPS by interaction with the 20S proteasome and inhibition of substrate entry." EMBO J 30(15): 3065-3077.)

The same effect could be elicited by using β-PrP, a predominantly β-sheet species with similar physico-chemical properties to PrP^{Sc}.

Novel findings from the neurodegenerative field have already been known for prions since years…

Proteasome in prion biology - PrP^{Sc}

To summarize:

- The involvement of the proteasomal in the degradation of PrP^{C} remains controversial
  - Parts of PrP^{C} might be degraded by the proteasome upon retrotranslocation
  - Proteasomal inhibitors lead to accumulation of PrP^{C} in the cytoplasm
  - There are ubiquitinated species of PrP^{C} (in CHO cells expressing human PrP)

- Prions inhibit the proteasomal activity *in vitro* and *in vivo* via direct interaction with the 20S core part, preventing substrate entry
- Inhibition of the proteasome could potentially elicit neurotoxic effects

$\Rightarrow$If PrP^{C} is partially a proteasomal substrate, again, inhibition of the proteasome could lead to a vicious cycle

$\Rightarrow$If prions inhibit the proteasome, regulation of subunits could lead to dishinhibition and restore normal proteasomal function => cells can work normally and clear prions via direct or indirect mechanisms

$\Rightarrow$If Proteasome has a disaggregase function, regulation of subunits could decrease/increase this function

References:

- http://www.cureffi.org/2013/07/25/the-role-of-the-proteasome-in-prp-degradation-and-prion-disease/
- Yedidia Y, Horonchik L, Tzaban S, Yanai A, Taraboulos A. Proteasomes and ubiquitin are involved in the turnover of the wild-type prion protein. EMBO J. 2001;20(19):5383–5391. doi:10.1093/emboj/20.19.5383
- Tai, H.-C. and E. M. Schuman (2008). "Ubiquitin, the proteasome and protein degradation in neuronal function and dysfunction." Nature Reviews Neuroscience 9: 826.
- Ciechanover, A. and Y. T. Kwon (2015). "Degradation of misfolded proteins in neurodegenerative diseases: therapeutic targets and strategies." Experimental &Amp; Molecular Medicine 47: e147.
- M. Kristiansen, M.J. Messenger, P. Klohn, S. Brandner, J. Wadsworth, J. Collinge, S.J. Tabrizi. Disease-related prion protein forms aggresomes in neuronal cells leading to caspase-activation and apoptosis. J. Biol. Chem., 280 (2005), pp. 38851-38861
- Kristiansen, M., et al. (2007). "Disease-associated prion protein oligomers inhibit the 26S proteasome." Mol Cell 26(2): 175-188.
- McKinnon, C., et al. (2016). "Prion-mediated neurodegeneration is associated with early impairment of the ubiquitin-proteasome system." Acta Neuropathol 131(3): 411-425.
- Deriziotis, P., et al. (2011). "Misfolded PrP impairs the UPS by interaction with the 20S proteasome and inhibition of substrate entry." EMBO J 30(15): 3065-3077.
- Rubinsztein, D. C. (2006). "The roles of intracellular protein-degradation pathways in neurodegeneration." Nature 443(7113): 780-786