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Aim for the Core: Suitability of the ubiquitin-independent 20S proteasome as a drug target in neurodegeneration

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Abstract

Neurodegenerative diseases are a class of age-associated proteopathies characterized by the accumulation of misfolded and/or aggregation-prone proteins. This imbalance has been attributed, in part, to an age-dependent decay in the capacity of protein turnover. Most proteins are degraded by the ubiquitin-proteasome system (UPS), which is composed of ubiquitin ligases and regulatory particles, such as the 19S, that deliver cargo to the proteolytically active 20S proteasome core. However, a subset of clients, especially intrinsically disordered proteins (IDPs), are also removed by the action of the ubiquitin-independent proteasome system (UIPS). What are the specific contributions of the UPS and UIPS in the context of neurodegeneration? Here, we explore how age-associated changes in the relative contribution of the UPS and UIPS, combined with the IDP-like structure of many neurodegenerative disease-associated proteins, might contribute. Strikingly, the core 20S proteasome (20S) has been shown to predominate in older neurons and to preferentially act on relevant substrates, such as synuclein and tau. Moreover, pharmacological activation of the 20S has been shown to accelerate removal of aggregation-prone proteins in some models. Together, these recent studies are turning attention to the 20S proteasome and the UIPS as potential therapeutic targets in neurodegeneration.

INTRODUCTION TO THE PROTEASOME

The proteasome is a central protein degradation machine in eukaryotes[1]. Through hydrolysis activities, it removes damaged proteins and ensures the delivery of amino acids to support ongoing biosynthesis. In addition, the proteasome has been co-opted for more specialized tasks in regulating the cell cycle, differentiation, the inflammatory response, antigen presentation and apoptosis[2,3]. To enable these functions, the proteasome makes up a staggering 1 to 2% of the entire proteome in healthy cells. However, a decline in proteasome activity has been broadly implicated in ageing and age-associated diseases, including neurodegeneration. Presumably, this decline contributes to a catastrophic imbalance in proteostasis and accumulation of damaged and/or misfolded proteins. In this review, we

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explore the structure-function of the proteasome and its implications in the onset and progression of neurodegenerative disease. In addition, we focus on emerging therapeutic opportunities through pharmacological activation of this degradation machine.

The 20S proteasome (20S) is a barrel-shaped complex comprised of four heptameric rings: two stacked β-rings that are sandwiched by two α-rings (Fig 1A). Three of the seven subunits (β1, β2, & β5) that make up the β-ring are proteases that hydrolyze peptide bonds of substrates. These active sites are sequestered in the interior of the 20S chamber, such that substrates must first traverse through the exterior α-rings. In its closed state, the α-rings have a narrow pore that occludes the entry of most proteins[4]. Thus, one key to understanding proteasome regulation is to learn how substrates are granted access to the proteolytic chamber. Substrates are targeted to the proteasome through two major pathways, the ubiquitin-proteasome system (UPS) and the ubiquitin-independent proteasome system (UIPS). Proteasome activators (PA), which are predominantly multi-protein complexes, help facilitate degradation by the 20S. There are many types of PAs and the specific one that is bound determines whether that 20S proteasome is coupled to the UPS or UIPS (Fig 1A). However, most of the PAs share a conserved tripeptide sequence, the HbYX (Hydrophobic-TYRosine-unspecified residue 'X'), at their C-termini that interacts with pockets in the α-rings of the 20S to allosterically open the pore[5].

Ubiquitin-proteasome system (UPS)

Proteasomal degradation by the UPS first requires the conjugation of multiple ubiquitin (Ub) proteins onto the substrate, generating the polyUb signal that designates it as a substrate of the proteasome. Recent work has shown that conjugation of two or more polyUb chains is needed on the tagged substrate to efficiently interact with the UPS machine[6]. Thus, regulation of this pathway by the activity of the E1, E2 and E3 Ub ligases is a critical component of its function[7], but will not be described in detail here. The canonical regulatory particle of the UPS is PA700 (or 19S), which is a 700 kDa proteasome activator complex that associates with the 20S to create the 26S proteasome (26S)[8]. PA700 is comprised of a 'base' and a 'lid'. The lid contains subunits that bind to polyUb chains, as well as deubiquitinating enzymes (DUBs) that regulate association with the particle. The base contains the HbYX motifs that interact with the α-rings, and ATPases that unfold the substrate so that it can access the proteolytic chamber[9]. Recent reviews provide additional information about the structure of the 26S and its biological fuction[6].

Ub-independent-proteasome system (UIPS)

Ub-independent degradation is coordinated by the 20S and may be amplified with UIPS-specific PAs, including PA200 and the heptameric PA28[10]. PA200 is a monomeric protein that uses a C-terminal HbYX motif to bind to and activate the 20S. PA28 is composed of multiple, different subunits (alpha, beta, and gamma) and it relies on an alternative (e.g. non-HbYX) motif for association with the 20S[11,12]. The UIPS-specific PAs typically lack the unfolding activity of PA700; rather, they open the α-ring gate through a binding-induced conformational change and increase the flux of suitable substrates into the proteolytic chamber[13]. As discussed below, this mechanism restricts UIPS substrates to unfolded proteins that can fit into the channel without an active unfoldase. However, PA700-bound

26S has an open α-ring gate too and is thus capable of facilitating ubiquitin-independent substrate turnover[14]. The relative contributions of the 26S in the UPS and UIPS pathways remain unclear (Fig 1B); and, for simplicity, we will only mention the contributions of the 26S to the UIPS in passing in this review. Finally, the free 20S (no PA) is likely to be a contributor to the UIPS. Although the 20S has relatively low enzymatic activity in the absence of PAs (see below), some small or unfolded substrates may be able to traverse the closed gates and be degraded by the minimal machine.

Substrate-targeting by the UPS and UIPS

Over 90% of the human proteome is regulated by the UPS[15]. These substrates include a vast array of structured (or folded) proteins, intrinsically disordered proteins (IDPs) and proteins containing intrinsically disordered regions (IDRs). Structured proteins must be unfolded prior to their degradation and can therefore only be cleared by the UPS[16,17]. Essentially, folded proteins cannot fit through the narrow axial pore of the 20S, making them inaccessible to degradation by the UIPS particles[18]. However, IDPs and IDR-containing proteins, which lack this three-dimensional structure, are thought to readily traverse the α-ring gate[19]. Twenty percent of cellular proteins are classified as IDPs and as many as 41% of the eukaryotic proteome is predicted to contain IDRs[20,21], suggesting that the substrate pool of the UIPS may be considerably large. These substrates are particularly relevant for this discussion because they include the proteins that accumulate in neurodegenerative disorders, such as amyloid beta, tau, TDP-43 and α-synuclein (Table I)[22,23].

In cells, IDPs typically have shorter half-lives relative to structured proteins[24]. The UPS and UIPS have both been shown to facilitate the rapid proteasomal degradation of IDPs, such as p53 and p73[25]. Recognition of the IDPs by these pathways is mediated, in part, by disordered regions that act as signals (or degrons)[24]. Evidence for the UIPS in this process comes from experiments in which removal of the ubiquitinated lysine has been found to have little effect on turnover[26,27]. Thus, it seems that both the UPS and UIPS can contribute to the turnover of IDPs.

ROLE OF THE PROTEASOME IN AGEING AND NEURODEGENERATIVE DISEASES

Neurodegenerative disorders, such as Alzheimer’s disease (AD), Parkinson’s disease (PD) and amyotrophic lateral sclerosis (ALS), are characterized by the progressive structural and functional impairment of neurons, resulting in neuronal death[28]. Although these diseases have different clinical symptoms, they are all associated with the accumulation of aggregated proteins[29,30]. This observation implicates the age-dependent decline in proteostasis and the contributing role of proteasomal dysfunction[31]. Consistent with this idea, species with increased longevity and long-lived individuals within a given species exhibit higher proteasome activity and are less susceptible to diseases, including neurodegeneration[32,33].

Many of the proteins implicated in neurodegeneration are IDPs[34]. Due to their conformational flexibility, IDPs are prone to aggregation[35] and present a particular risk in

aged neurons, where the protein quality control network has deteriorated[36]. Against this backdrop, the proteasome likely plays a critical role because it rids neurons of damaged and misfolded proteins that may cause neuronal dysfunction. The role of the UPS in this process has received substantial attention due, in part, to the early observation that ubiquitinated proteins are found within disease-associated aggregates in postmortem brains[31]. Indeed, many recent reviews have focused on the connection between the UPS and neurodegeneration[37]. Similarly, the immunoproteasome, composed of alternative subunits[38], likely plays a role, especially in the immune regulation of the diseases. The structure-function of the immunoproteasome has also been reviewed[39] and will not be discussed here. Instead, we focus on the relatively under-studied position of the UIPS.

Cells employ the UIPS to cope with proteotoxicity

During neurodegeneration, neurons are challenged with a higher substrate load, potentially causing an imbalance in proteostasis and creating a feed-forward loop that compromises proteasome function[41]. Specifically, increased levels of misfolded proteins are though to "clog" the proteasome and impair its function[42]. There are several proposed mechanisms for this inhibition, including stabilization of the proteasome in its closed-gate conformation[43,44]. The feed-forward aspect of the relationship then comes about when the resulting proteotoxicity triggers mitochondrial dysfunction and increases reactive oxygen species (ROS) and DNA damage[45]. These processes contribute to further protein misfolding and inactivation of the proteasome through direct oxidation of its subunits[46]. Cells have adopted approaches to counteract this problem; for example, a rise in ROS induces expression of PA28α, which promotes UIPS activity[47]. Additionally, oxidative stress induces expression of 20S subunits through the oxidative stress sensor, Nrf2 (SKN-1)[48]. Interestingly, the levels of the UPS-associated PA700 remain constant under those conditions[49], suggesting that oxidatively damaged proteins may be preferentially cleared by the UIPS. Consistent with this idea, oxidative stress also mediates the Ecm29-dependent disassembly of 26S (Fig 2A)[50]. Collectively, these findings suggest that challenged cells rely on the UIPS to degrade oxidatively damaged and unfolded proteins.

Aged cells contain a latent pool of free (*e.g.* unbound) 20S proteasome

What are the relative contributions of the free 20S, the UPS and the UIPS to proteotoxicity and neurodegeneration? The answers are not yet clear, but some clues come from reports that the relative levels of 20S and PA-bound 20S change with ageing and disease. Using label-free proteomics in nine different human cell lines, Fabre *et al* estimates that 21 – 35% of the total proteasome pool is PA700-bound (26S) and less than 10% is bound to UIPS-specific PAs, while the remaining ~66% is unbound 20S[51] (Fig 2B). This is an interesting result because it suggests that cells may contain a latent pool of 20S proteasome that is not bound to any PA. Because PAs enhance the rate of proteolysis by as much as 20-fold[52], these findings suggest that some cells have a pool of 20S that is poised to be activated. How does this ratio change during ageing and disease? It is known that this downregulation is mediated, in part, by diminished expression of proteasome genes themselves[54]. To date, the distribution of PA-bound and unbound 20S has not been surveyed in a neurodegeneration model, but the ratio of unbound (*e.g.* free 20S) proteasome to UPS-specific 26S has been reported to increase with age. For example, in cultured human fibroblasts from old

individuals, the amount of 20S proteasome is decreased by 2.5-fold whereas the level of PA700 is lowered by 6.5-fold, compared to fibroblasts from young individuals[55]. Conversely, the levels of PA28 remained unchanged,[55] suggesting a relative switch from UPS to UIPS pathways during ageing (Fig 2B). Although it is difficult to conclude a direct cause-and-effect, this increased availability of 20S roughly coincides with a decrease in total proteasome activity in many cells, tissues, and organisms during ageing[53].

Proteasomal upregulation accelerates the clearance of pathogenic proteins

Together, these findings suggest that decreased proteasome activity, driven by a combination of reduced expression of 20S and an increase in free (*i.e.* non-PA bound) proteasome, may contribute to neurodegeneration. Accordingly, this model suggests that boosting proteasome activity may counteract the process. This goal could theoretically be achieved in a number of ways. For example, delivery of purified 20S to cells through direct injection has been shown to accelerate clearance of tau[56]. Similarly, cells expressing a 20S mutant, in which the gating N-termini of the α-subunits are deleted, are also partially protected from tau aggregation[57]. Pharmacologically, Finley *et al* identified small molecules that inactivate the deubiquitinating activity of USP14, the PA700-associated DUB, allosterically activate proteasomal degradation of polyUb-conjugated proteins and, consistent with the model, accelerate turnover of tau *in vitro*[58,59]. These findings have motivated others to search for molecules that directly bind the 20S to promote its activity (see below). Such a strategy might take advantage of the fact that only <10% of total proteasomes in a neurodegenerative disease model are intact 26S and a large pool is free 20S (Fig 2B). Consistent with this idea, over-expression of PA28 enhances protein clearance in ageing models[60]. The pharmacological equivalent of this approach would be to activate the 20S, in the absence of a PA. In the next sections, we discuss the opportunities and challenges of this possibility.

PHARMACOLOGICAL ACTIVATORS OF THE 20S PROTEASOME

The proteasome has long been the subject of studies to identify inhibitors. Early work identified natural products, such as lactacystin, that inactivate it by mimicking peptide substrates and covalently modifying the β-subunits[61]. These compounds became widely used chemical probes and, subsequently, a few were approved for the treatment of multiple myeloma patients[53], where they seem to exploit the need of cancer cells for high protein turnover. More recently, other strategies have been developed to create inhibitors with different mechanisms[62]. Activators of the proteasome, in contrast, have received less attention; as mentioned above, such molecules would ideally bind to the unbound 20S and open its pore, acting as “artificial activators” to compensate for the loss of natural PAs and proteasome activity during ageing (Fig 3). However, the size and complexity of the 20S coupled with an incomplete understanding of the mechanisms of activation, has made the discovery of activators a difficult task. Furthermore, the field of 20S activators lacks the serendipitous discovery of natural product leads (such as lactacystin) as starting points. The next sections introduce the early chemical efforts to create proteasome activators and point out the substantial challenges that remain in the discovery, optimization and deployment of these molecules. For clarity, the sections are divided based on the chemical composition of the compounds.

Denaturants

The first reported proteasome activator was the detergent, sodium dodecyl sulfate (SDS), which was identified *in vitro* by measuring the chymotryptic activity of the 20S against the fluorogenic peptide substrate, succinyl-Leu-Leu-Val-Tyr-7-amino-4-methylcoumarin (LLVY-amc). Addition of SDS increases the turnover of LLVY-amc by 20-fold, but at levels above its critical micellar concentration. This result suggests that SDS acts as a detergent, likely activating by partially denaturing the α-ring gate and allowing access of substrate into the pore. Another sign of this mechanism is that stimulation occurs within a narrow concentration range: lower concentrations activate turnover, while higher levels inhibit activity, likely by more extensive denaturation of the 20S[63,64]. Similar trends are seen for polycations (*e.g.* polylysine), polyanionic lipids (*e.g.* cardolipin & heparin), fatty acids (*e.g.* oleic, linoleic, & linolenic acids) and the natural product oleuropein[65–69] (Table IIA and B). Molecules with this type of concentration-activity signature are likely to be rather non-specific activators, with mechanisms that make them difficult to optimize.

Small molecules

Betulinic acid is a triterpene natural product that stimulates the 20S at low micromolar concentrations[70]. Unlike the detergents, betulinic acid preferentially stimulates the chymotryptic activity of the 20S (and not the other two activities). However, attempts to improve the potency of betulinic acid through medicinal chemistry have only yielded analogs that inhibit (rather than stimulate) proteasome activity, suggesting complex structure-activity relationships (SAR)[71]. More recent reports have turned to high-throughput chemical screens to identify alternative molecules. These assays often rely on measurement of proteasome activity through the hydrolysis of LLVY-amc, while secondary assays are used to measure degradation of biologically relevant substrates[72,73]. For example, Trader *et al* screened a subset of the NIH Clinical Collection (NCC) library to discover two compounds, AM-404 and MK-866, that stimulate the 20S *in vitro* and enhance turnover of α-synuclein by 3 to 4-fold in cells[72]. In a parallel screen of the Natural Product Library (NPL) of the NCC, Coleman & Trader identified three additional molecules (denoted NPL-1, 2, & 3) that activate the 20S at low micromolar concentrations[74] (Table IIC). Beyond the specific molecules identified, one of the highlights of that work is the introduction of a rigorous suite of assays to triage non-specific mechanisms. However, additional medicinal chemistry and structural biology efforts will be needed to advance chemical probes with the desired selectivity and potency.

In a parallel effort, Jones *et al* screened the NCC and Prestwick libraries, revealing chlorpromazine as a putative agonist of the 20S. This compound stimulated the activity of the 20S *by* 20-fold *in vitro*. Unbiased docking of chlorpromazine to the 20S predicted possible interactions with the intersubunit pocket of the α-ring, reminiscent of the natural HbYX motif. Thus, this chemical series may take advantage of intrinsic allosteric mechanisms to promote turnover. Chlorpromazine is a well-known dopamine D2 agonist, but chemical modifications suggested that the SAR for proteasome activation was distinct. However, the potency of these compounds remains limited (Table IIC)[73].

Peptides

Another strategy for stimulating the proteasome is to directly create mimics of the HbYX motif. Indeed, peptides inspired by the HbYX-containing C-termini of PAs can stimulate the 20S *in vitro* at mid-micromolar concentrations[5]. Moreover, the potency of these HbYX peptides seems to depend on which subunits of PA700 they were taken from [75,76]. Some of these HbYX peptides can enhance proteasomal degradation of a model substrate and rescue the 20S from inhibition by toxic amyloid-β oligomers[43]. While promising, peptide-based activators of the 20S have intrinsic challenges that will need to be overcome. For example, they have poor membrane permeability, uncertain selectivity and typically low metabolic stability. Despite these hurdles, the recently reported crystal structure of the HbYX peptide-bound eukaryotic 20S[77] might provide a structure-guided way to advance this approach. It is important to note that any compounds that bind this site would expectedly displace the UPS and UIPS-specific PAs. Thus, even though they might stimulate the pool of free 20S, their pharmacology is expected to be complex.

CONCLUSION and PROSPECTUS

There is mounting evidence that decreased activity of the proteasome contributes to neurodegeneration. Although decreased 26S is assuredly a key component of this decline, we have focused here on the contributions of the UIPS and the free 20S. This focus is based on evidence that both the PA28-bound and free 20S seem to become more prominent during ageing. Moreover, many of the neurodegenerative disease-associated proteins, such as tau, are IDPs and thus particularly good substrates for these complexes.

The increased level of free 20S during ageing has interesting implications for drug discovery. Specifically, molecules that boost the function of this “low activity” 20S pool might partially compensate for diminished proteasomal function. While a compelling idea, there are major challenges to pursuing this concept and the current molecules are not yet up to this task. One challenge is that many compounds can have detergent-like activity that is potent, but ultimately untenable for the creation of activators. Thus, any screening effort is highly likely to produce denaturation-like artifacts that must be removed through subsequent secondary assays and careful analysis of their dose dependence. In addition, one cannot use the typical approach of adding Tween or Triton-X in screening buffers to minimize the discovery of aggregators or pan-assay interference (PAINS) molecules[78]. Thus, additional types of artifacts are likely to populate the list of apparent “hits”. These two challenges are expected to result in higher-than-normal failure rates during compound triage and special care will be needed to select high quality scaffolds.

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Abbreviations

20S	core 20S proteasome

AD	Alzheimer's disease
ALS	amyotrophic lateral sclerosis
ATP	adenosine triphosphate
BBB	blood-brain barrier
DUB	deubiquitinating enzyme
HbYX	Hydrophobic-Tyrosine-unspecified residue 'X'
IDP	intrinsically disordered protein
IDR	intrinsically-disordered region
LLVY-amc	succinyl-Leu-Leu-Val-Tyr-7-amino-4-methylcoumarin
NCC	NIH (National Institute of Health) Clinical Collection
NPL	Natural Product Library
PA	proteasome activators
PAINS	pan-assay interference compounds
PD	Parkinson's disease
ROS	reactive oxygen species
Rpt	regulatory particle of triple-ATPase
SAR	structure-activity relationship
tau	microtubule-associated protein tau (MAPT)
TDP-43	trans-activation response element (TAR) DNA-binding protein 43
Ub	ubiquitin
UIPS	Ubiquitin-independent proteasome system
UPS	ubiquitin-proteasome system
USP-14	Ubiquitin-specific-processing protease

References

1. Smith DM, Benaroudj N, Goldberg A. Proteasomes and their associated ATPases: A destructive combination. J Struct Biol. 2006; 156(1):72–83. DOI: 10.1016/j.jsb.2006.04.012 [PubMed: 16919475]
2. Goldberg AL. Functions of the proteasome: from protein degradation and immune surveillance to cancer therapy. Biochem Soc Trans. 2007; 35(1):12–17. http://www.biochemsoctrans.org/content/35/1/12.abstract. [PubMed: 17212580]
3. Chen LH, CH. Proteasome Regulators: Activators and Inhibitors. Curr Med Chem. 2009; 16(8):931–939. DOI: 10.2174/092986709787581860 [PubMed: 19275603]

4. Kish-Trier E, Hill CP. Structural Biology of the Proteasome. Annu Rev Biophys. 2013; 42(1):29–49. DOI: 10.1146/annurev-biophys-083012-130417 [PubMed: 23414347]
5. Smith DM, Chang SC, Park S, Finley D, Cheng Y, Goldberg AL. Docking of the proteasomal ATPases' carboxyl termini in the 20S proteasome's α Ring Opens the Gate for Substrate Entry. Mol Cell. 2007; 27(5):731–744. DOI: 10.1016/j.molcel.2007.06.033 [PubMed: 17803938]
6. Finley D. Recognition and Processing of Ubiquitin-Protein Conjugates by the Proteasome. Annu Rev Biochem. 2009; 78(1):477–513. DOI: 10.1146/annurev.biochem.78.081507.101607 [PubMed: 19489727]
7. Finley D, Ulrich HD, Sommer T, Kaiser P. The ubiquitin-proteasome system of Saccharomyces cerevisiae. Genetics. 2012; 192(2):319–360. DOI: 10.1534/genetics.112.140467 [PubMed: 23028185]
8. DeMartino GN, Moomaw CR, Zagnitko OP, et al. PA700, an ATP-dependent activator of the 20 S proteasome, is an ATPase containing multiple members of a nucleotide-binding protein family. J Biol Chem. 1994; 269(33):20878–20884. [PubMed: 8063704]
9. Glickman MH, Rubin DM, Fried Va, Finley D. The regulatory particle of the Saccharomyces cerevisiae proteasome. Mol Cell Biol. 1998; 18(6):3149–3162. [PubMed: 9584156]
10. Schmidt M, Hanna J, Elsasser S, Finley D. Proteasome-associated proteins: Regulation of a proteolytic machine. Biol Chem. 2005; 386(8):725–737. DOI: 10.1515/BC.2005.085 [PubMed: 16201867]
11. Sadre-Bazzaz K, Whitby FG, Robinson H, Formosa T, Hill CP. Structure of a Blm10 Complex Reveals Common Mechanisms for Proteasome Binding and Gate Opening. Mol Cell. 2010; 37(5): 728–735. DOI: 10.1016/j.molcel.2010.02.002 [PubMed: 20227375]
12. Li J, Rechsteiner M. Molecular dissection of the 11S REG (PA28) proteasome activators. Biochimie. 2001; 83(3–4):373–383. DOI: 10.1016/S0300-9084(01)01236-6 [PubMed: 11295500]
13. Förster A, Masters EI, Whitby FG, Robinson H, Hill CP. The 1.9 Å structure of a proteasome-11S activator complex and implications for proteasome-PAN/PA700 interactions. Mol Cell. 2005; 18(5):589–599. DOI: 10.1016/j.molcel.2005.04.016 [PubMed: 15916965]
14. Strickland E, Hakala K, Thomas PJ, DeMartino GN. Recognition of misfolded proteins by PA700, the regulatory subcomplex of the 26S proteasome. J Biol Chem. 2000; 275(8):5565–5572. DOI: 10.1074/jbc.275.8.5565 [PubMed: 10681537]
15. Kwon YT, Ciechanover A. The Ubiquitin Code in the Ubiquitin-Proteasome System and Autophagy. Trends Biochem Sci. 2017; 42(11):873–886. DOI: 10.1016/j.tibs.2017.09.002 [PubMed: 28947091]
16. Rock KL, Gramm C, Rothstein L, et al. Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class I molecules. Cell. 1994; 78(5):761–771. DOI: 10.1016/S0092-8674(94)90462-6 [PubMed: 8087844]
17. Craiu aGaczynska M, Akopian T. , et al. Lactacystin and clasto-lactacystin beta-lactone modify multiple proteasome beta-subunits and inhibit intracellular protein degradation and major histocompatibility complex class I antigen presentation. J Biol Chem. 1997; 272(20):13437–13445. DOI: 10.1074/jbc.272.20.13437 [PubMed: 9148969]
18. Hagai T, Levy Y. Ubiquitin not only serves as a tag but also assists degradation by inducing protein unfolding. Proc Natl Acad Sci. 2010; 107(5):2001–2006. DOI: 10.1073/pnas.0912335107 [PubMed: 20080694]
19. Orlowski M, Wilk S. Ubiquitin-independent proteolytic functions of the proteasome. Arch Biochem Biophys. 2003; 415(1):1–5. DOI: 10.1016/S0003-9861(03)00197-8 [PubMed: 12801506]
20. Baugh JM, Viktorova EG, Pilipenko EV. Proteasomes can degrade a significant proportion of cellular proteins independent of ubiquitination. J Mol Biol. 2009; 386(3):814–827. DOI: 10.1016/j.jmb.2008.12.081 [PubMed: 19162040]
21. Dunker AK, Obradovic Z, Romero P, Garner EC, Brown CJ. Intrinsic protein disorder in complete genomes. Genome informatics. 2000; 11:161–171. DOI: 10.11234/gi1990.11.161 [PubMed: 11700597]

22. Weinreb PH, Zhen W, Poon AW, Conway KA, Lansbury PT. NACP, a protein implicated in Alzheimer's disease and learning, is natively unfolded. Biochemistry. 1996; 35(43):13709–13715. DOI: 10.1021/bi961799n [PubMed: 8901511]
23. Uversky VN, Oldfield CJ, Dunker AK. Intrinsically disordered proteins in human diseases: introducing the D2 concept. Annu Rev Biophys. 2008; 37(1):215–246. DOI: 10.1146/annurev.biophys.37.032807.125924 [PubMed: 18573080]
24. van der Lee R, Lang B, Kruse K, et al. Intrinsically disordered segments affect protein half-life in the cell and during evolution. Cell Rep. 2014; 8(6):1832–1844. DOI: 10.1016/j.celrep.2014.07.055 [PubMed: 25220455]
25. Asher G, Tsvetkov P, Kahana C. A mechanism of ubiquitin-independent proteasomal degradation of the tumor suppressors p53 and p73 service A mechanism of proteasomal degradation of the tumor suppressors p53 and p73. Genes Dev. 2005; 19:316–321. DOI: 10.1101/gad.319905 [PubMed: 15687255]
26. Peña MMO, Xing YY, Koli S, Berger FG. Role of N-terminal residues in the ubiquitin-independent degradation of human thymidylate synthase. Biochem J. 2006; 394(1):355–363. DOI: 10.1042/BJ20051479 [PubMed: 16259621]
27. Wiggins CM, Tsvetkov P, Johnson M, et al. BIMEL, an intrinsically disordered protein, is degraded by 20S proteasomes in the absence of poly-ubiquitylation. J Cell Sci. 2011; 124(6):969–977. DOI: 10.1242/jcs.058438 [PubMed: 21378313]
28. Popovic D, Vucic D, Dikic I. Ubiquitination in disease pathogenesis and treatment. Nat Med. 2014; 20(11):1242–1253. DOI: 10.1038/nm.3739 [PubMed: 25375928]
29. Walker LC, LeVine H. The cerebral proteopathies: neurodegenerative disorders of protein conformation and assembly. Mol Neurobiol. 2000; 21(1–2):83–95. DOI: 10.1385/MN:21:1-2:083 [PubMed: 11327151]
30. Sherman MY, Goldberg AL. Cellular defenses against unfolded proteins: A cell biologist thinks about neurodegenerative diseases. Neuron. 2001; 29(1):15–32. DOI: 10.1016/S0896-6273(01)00177-5 [PubMed: 11182078]
31. Dantuma NP, Bott LC. The ubiquitin-proteasome system in neurodegenerative diseases: precipitating factor, yet part of the solution. Front Mol Neurosci. 2014 Jul.7:1–18. DOI: 10.3389/fnmol.2014.00070 [PubMed: 24574959]
32. Ruby JG, Smith M, Buffenstein R. Naked mole-rat mortality rates defy gompertzian laws by not increasing with age. Elife. 2018; 7:1–18. DOI: 10.7554/eLife.31157
33. Chondrogianni N, Petropoulos I, Franceschi C, Friguet B, Gonos ES. Fibroblast cultures from healthy centenarians have an active proteasome. Exp Gerontol. 2000; 35(6–7):721–728. DOI: 10.1016/S0531-5565(00)00137-6 [PubMed: 11053662]
34. Deger JM, Gerson JE, Kayed R. The interrelationship of proteasome impairment and oligomeric intermediates in neurodegeneration. Aging Cell. 2015; 14(5):715–724. DOI: 10.1111/acel.12359 [PubMed: 26053162]
35. Levine ZA, Larini L, LaPointe NE, Feinstein SC, Shea J-E. Regulation and aggregation of intrinsically disordered peptides. Proc Natl Acad Sci. 2015; 112(9):2758–2763. DOI: 10.1073/pnas.1418155112 [PubMed: 25691742]
36. Ciechanover A, Brundin P. The ubiquitin proteasome system in neurodegenerative diseases: sometimes the chicken, sometimes the egg. Neuron. 2003; 40(2):427–446. The ubiquitin proteasome system in neurodeg. doi:S0896627303006068 [pii]. [PubMed: 14556719]
37. Dennissen FJA, Kholod N, van Leeuwen FW. The ubiquitin proteasome system in neurodegenerative diseases: Culprit, accomplice or victim? Prog Neurobiol. 2012; 96(2):190–207. DOI: 10.1016/j.pneurobio.2012.01.003 [PubMed: 22270043]
38. Kniepert A, Groettrup M. The unique functions of tissue-specific proteasomes. Trends Biochem Sci. 2014; 39(1):17–24. DOI: 10.1016/j.tibs.2013.10.004 [PubMed: 24286712]
39. Ferrington DA, Gregerson DS. Immunoproteasomes: Structure, Function, and Antigen Presentation. 2012; doi: 10.1016/B978-0-12-397863-9.00003-1
40. Uversky VN. Alpha-synuclein misfolding and neurodegenerative diseases. Curr Protein Pept Sci. 2008; 9(5):507–540. DOI: 10.1038/35081564 [PubMed: 18855701]

41. Huang Y, Mucke L. Alzheimer mechanisms and therapeutic strategies. Cell. 2012; 148(6):1204–1222. DOI: 10.1016/j.cell.2012.02.040 [PubMed: 22424230]
42. San Martín Á, Rodriguez-Aliaga P, Molina JA, Martin A, Bustamante C, Baez M. Knots can impair protein degradation by ATP-dependent proteases. Proc Natl Acad Sci. 2017; 114(37): 201705916.doi: 10.1073/pnas.1705916114
43. Thibaudeau TA, Anderson RT, Smith DM. A common mechanism of proteasome impairment by neurodegenerative disease-associated oligomers. Nat Commun. 2018; doi: 10.1038/s41467-018-03509-0
44. Bennett EJ, Bence NF, Jayakumar R, Kopito RR. Global impairment of the ubiquitin-proteasome system by nuclear or cytoplasmic protein aggregates precedes inclusion body formation. Mol Cell. 2005; 17(3):351–365. DOI: 10.1016/j.molcel.2004.12.021 [PubMed: 15694337]
45. Lin MT, Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature. 2006; 443(7113):787–795. DOI: 10.1038/nature05292 [PubMed: 17051205]
46. Reichmann D, Voth W, Jakob U. Maintaining a Healthy Proteome during Oxidative Stress. Mol Cell. 2018; 69(2):203–213. DOI: 10.1016/j.molcel.2017.12.021 [PubMed: 29351842]
47. Li J, Powell SR, Wang X. Enhancement of proteasome function by PA28 overexpression protects against oxidative stress. FASEB J. 2011; 25(3):883–893. DOI: 10.1096/fj.10-160895 [PubMed: 21098724]
48. Li X, Matilainen O, Jin C, Glover-Cutter KM, Holmberg CI, Blackwell TK. Specific SKN-1/NrF stress responses to perturbations in translation elongation and proteasome activity. PLoS Genet. 2011; 7(6):9–11. DOI: 10.1371/journal.pgen.1002119
49. Pickering AM, Staab TA, Tower J, Sieburth D, Davies KJA. A conserved role for the 20S proteasome and Nrf2 transcription factor in oxidative stress adaptation in mammals, Caenorhabditis elegans and Drosophila melanogaster. J Exp Biol. 2013; 216(4):543–553. DOI: 10.1242/jeb.074757 [PubMed: 23038734]
50. Wang X, Chemmama IE, Yu C, et al. The proteasome-interacting Ecm29 protein disassembles the 26S proteasome in response to oxidative stress. J Biol Chem. 2017; 292(39):16310–16320. DOI: 10.1074/jbc.M117.803619 [PubMed: 28821611]
51. Fabre B, Lambour T, Garrigues L, et al. Label-free quantitative proteomics reveals the dynamics of proteasome complexes composition and stoichiometry in a wide range of human cell lines. J Proteome Res. 2014; 13(6):3027–3037. DOI: 10.1021/pr500193k [PubMed: 24804812]
52. Masson P, Lundin D, Söderbom F, Young P. Characterization of a REG/PA28 proteasome activator homolog in dictyostelium discoideum indicates that the ubiquitin- And ATP-independent REGγ proteasome Is an ancient nuclear protease. Eukaryot Cell. 2009; 8(6):844–851. DOI: 10.1128/EC.00165-08 [PubMed: 19411624]
53. Schmidt M, Finley D. Regulation of proteasome activity in health and disease. Biochim Biophys Acta. 2014; 1843(1):13–25. DOI: 10.1016/j.bbamcr.2013.08.012 [PubMed: 23994620]
54. Lee C, Klopp RG, Weindruch R, Prolla TA. Gene Expression Pro le of Aging and Its Retardation by Caloric Restriction. Science (80-). 1999 Aug.285:1390–1393. DOI: 10.1126/science.285.5432.1390
55. Hwang JS, Hwang JS, Chang I, Kim S. Age-associated decrease in proteasome content and activities in human dermal fibroblasts: restoration of normal level of proteasome subunits reduces aging markers in fibroblasts from elderly persons. J Gerontol. 2007; 62(5):490–499. 62/5/490 [pii].
56. Han DH, Na H-K, Choi WH, et al. Direct cellular delivery of human proteasomes to delay tau aggregation. Nat Commun. 2014; 5:5633.doi: 10.1038/ncomms6633 [PubMed: 25476420]
57. Choi WH, de Poot SAH, Lee JH, et al. Open-gate mutants of the mammalian proteasome show enhanced ubiquitin-conjugate degradation. Nat Commun. 2016; 7:10963.doi: 10.1038/ncomms10963 [PubMed: 26957043]
58. Lee B-H, Lee MJ, Park S, et al. Enhancement of proteasome activity by a small-molecule inhibitor of USP14. Nature. 2010; 467(7312):179–184. DOI: 10.1038/nature09299 [PubMed: 20829789]
59. Boselli M, Lee BH, Robert J, et al. An inhibitor of the proteasomal deubiquitinating enzyme USP14 induces tau elimination in cultured neurons. J Biol Chem. 2017; 292(47):19209–19225. DOI: 10.1074/jbc.M117.815126 [PubMed: 28972160]

60. Vilchez D, Saez I, Dillin A. Organismal Ageing and Age-Related Diseases. Nat Commun. 2014; 5:1–13. DOI: 10.1038/ncomms6659
61. Kim KB, Myung J, Sin N, Crews CM. Proteasome inhibition by the natural products epoxomicin and dihydroeponemycin: Insights into specificity and potency. Bioorg Med Chem Lett. 1999; 9(23):3335–3340. DOI: 10.1016/S0960-894X(99)00612-5 [PubMed: 10612595]
62. McDaniel TJ, Lansdell TA, Dissanayake AA, et al. Substituted quinolines as noncovalent proteasome inhibitors. Bioorganic Med Chem. 2016; 24(11):2441–2450. DOI: 10.1016/j.bmc.2016.04.005
63. Orlowski N, Wilk S. A multicatalytical protease complex from pituitary that forms enkephalin and enkephalin containing peptides. Biochem Biophys Res Commun. 1981; 101(3):814–822. DOI: 10.1016/0006-291X(81)91823-4 [PubMed: 7030330]
64. Wilk S, Orlowski M. Evidence that Pituitary Cation-Sensitive Neutral Endopeptidase Is a Multicatalytic Protease Complex. J Neurochem. 1983; 40(3):842–849. DOI: 10.1111/j.1471-4159.1983.tb08056.x [PubMed: 6338156]
65. Ohkubo I, Gasa S, Namikawa C, Makita A, Sasaki M. Human Erythrocyte multicatalytic proteinase: Activation and binding to sulfated galacto- and lactosylceramides+ Biochem Biophys Res Commun. 1991; 174(14):1133–1140. [PubMed: 1825464]
66. Ruiz de Mena I, Mahillo E, Arribas J, Castaño JG. Kinetic mechanism of activation by cardiolipin (diphosphatidylglycerol) of the rat liver multicatalytic proteinase. Biochem J. 1993; 296(Pt 1):93–97. DOI: 10.1042/bj2960093 [PubMed: 8250860]
67. Watanabe N, Yamada S. Activation of 20S proteasomes from spinach leaves by fatty acids. Plant Cell Physiol. 1996; 37(2):147–151. DOI: 10.1093/oxfordjournals.pcp.a028925 [PubMed: 8665092]
68. Tanakas K, Yoshimura T, Ichihara A, et al. A High Molecular Weight Protease in the Cytosol of Rat Liver. J Biol Chem. 1986; 261(32):15204–15207. [PubMed: 3533938]
69. Katsiki M, Chondrogianni N, Chinou I, Rivett AJ, Gonos ES. The olive constituent oleuropein exhibits proteasome stimulatory properties *in vitro* and confers life span extension of human embryonic fibroblasts. Rejuvenation Res. 2007; 10(2):157–172. DOI: 10.1089/rej.2006.0513 [PubMed: 17518699]
70. Kashiwada Y, Hashimoto F, Cosentino LM, Chen CH, Garrett PE, Lee KH. Betulinic acid and dihydrobetulinic acid derivatives as potent anti-HIV agents. J Med Chem. 1996; 39(5):1016–1017. DOI: 10.1021/jm950922q [PubMed: 8676334]
71. Huang L, Ho P, Chen CH. Activation and inhibition of the proteasome by betulinic acid and its derivatives. FEBS Lett. 2007; 581(25):4955–4959. DOI: 10.1016/j.febslet.2007.09.031 [PubMed: 17904555]
72. Trader DJ, Simanski S, Dickson P, Kodadek T. Establishment of a suite of assays that support the discovery of proteasome stimulators. Biochim Biophys Acta. 2017; 1861(4):892–899. DOI: 10.1016/j.bbagen.2017.01.003
73. Jones CL, Njomen E, Sjögren B, Dexheimer TS, Tepe JJ. Small Molecule Enhancement of 20S Proteasome Activity Targets Intrinsically Disordered Proteins. ACS Chem Biol. 2017; 12(9):2240–2247. DOI: 10.1021/acschembio.7b00489 [PubMed: 28719185]
74. Coleman RA, Trader DJ. Development and Application of a Sensitive Peptide Reporter to Discover 20S Proteasome Stimulators. ACS Comb Sci. 2018; acscombsci.7b00193. doi: 10.1021/acscombsci.7b00193
75. Gillette TG, Kumar B, Thompson D, Slaughter Ca, DeMartino GN. Differential roles of the COOH termini of AAA subunits of PA700 (19 S regulator) in asymmetric assembly and Activation of the 26 S proteasome. J Biol Chem. 2008; 283(46):31813–31822. DOI: 10.1074/jbc.M805935200 [PubMed: 18796432]
76. Kim Y-C, DeMartino GN. C Termini of Proteasomal ATPases Play Nonequivalent Roles in Cellular Assembly of Mammalian 26 S Proteasome. J Biol Chem. 2011; 286(30):26652–26666. DOI: 10.1074/jbc.M111.246793 [PubMed: 21628461]
77. Witkowska J, Gizyńska M, Grudnik P, et al. Crystal structure of a low molecular weight activator Blm-pep with yeast 20S proteasome - Insights into the enzyme activation mechanism. Sci Rep. 2017; 7(1):1–11. DOI: 10.1038/s41598-017-05997-4 [PubMed: 28127051]

78. Baell JB, Nissink JWM. Seven Year Itch: Pan-Assay Interference Compounds (PAINS) in 2017 - Utility and Limitations. ACS Chem Biol. 2018; 13(1):36–44. DOI: 10.1021/acschembio.7b00903 [PubMed: 29202222]

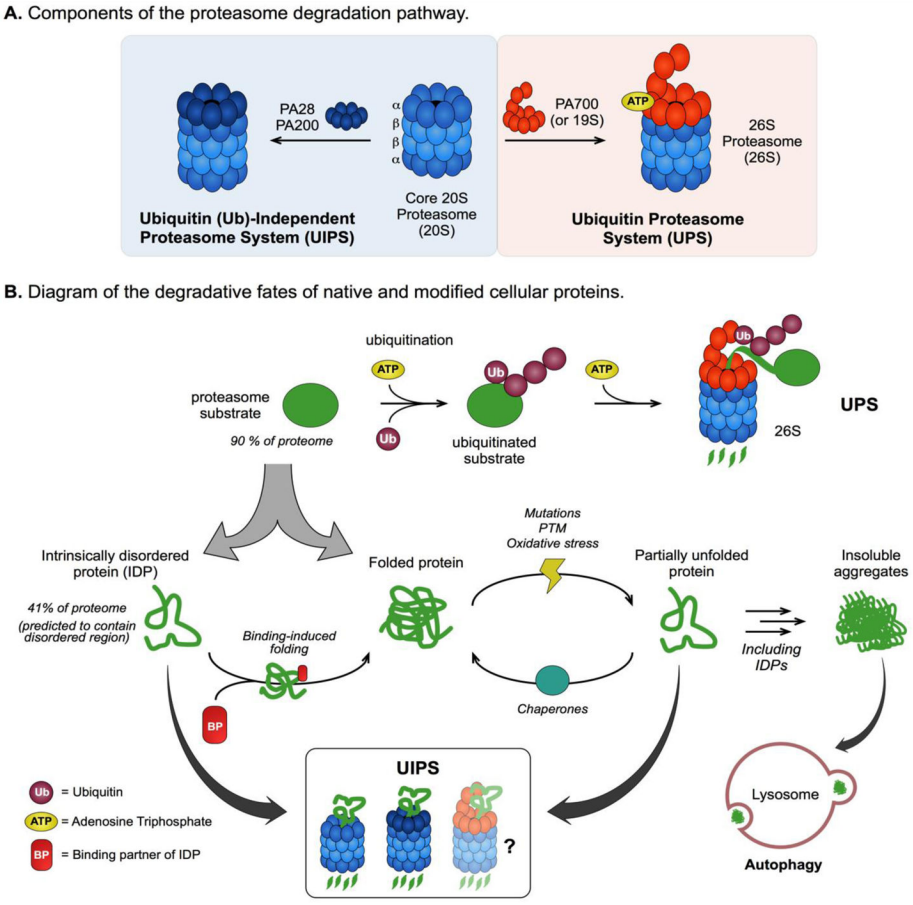

Figure 1. Structure, Function, and Substrate Profile of the Proteasome Degradation Pathway

(A) Components of the ubiquitin proteasome system (UPS) and the ubiquitin (Ub)-independent proteasome system (UIPS). (B) Diagram of substrate turnover by the different proteasome pathways. The 26S proteasome degrades over 90% of the proteome, including intrinsically disordered proteins (IDPs) and folded proteins, by the Ub- and ATP-dependent UPS. The UIPS targets substrates independent of Ub-conjugation and can effectively degrade IDPs, which constitute up to 41% of the proteome, but not folded proteins due to their three-dimensional structure. Cellular stresses, including mutations, post-translational modifications (PTMs) and oxidative damage, can partially unfold structured proteins making them susceptibility to turnover by the UIPS. Partially unfolded proteins (and IDPs) can self-associate to form insoluble aggregates, which cannot be processed by the proteasome, and are predominantly cleared from the cell through the lysosome-autophagy pathway.

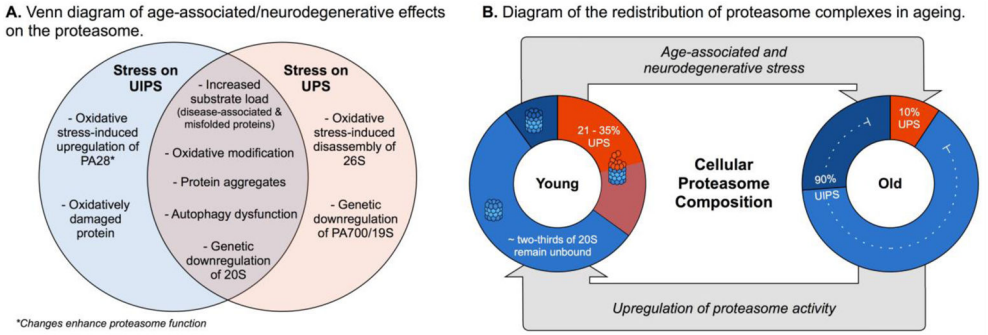

Figure 2. Proteasomal Regulation in Ageing and Neurodegenerative Disease Models

(A) Partial list of stresses that effect UPS (orange) and UIPS (blues) function. (B) Summary of the cellular composition of proteasome complexes (20S, 26S, and UIPS-specific) in young vs. aged fibroblasts. See the text for citations. Briefly, proteasome complexes from young or old fibroblasts were placed into categories based on whether they were free 20S or whether they contain PA700 (26S/UPS) or PA28 (UIPS)[51,55]. Note that this simplification does not account for the contribution of the UPS to ubiquitin-independent turnover. (C) Schematic representation of the general decline in proteasome activity during ageing. In theory, small molecules might be used to partially restore this activity to boost function. [51,55]

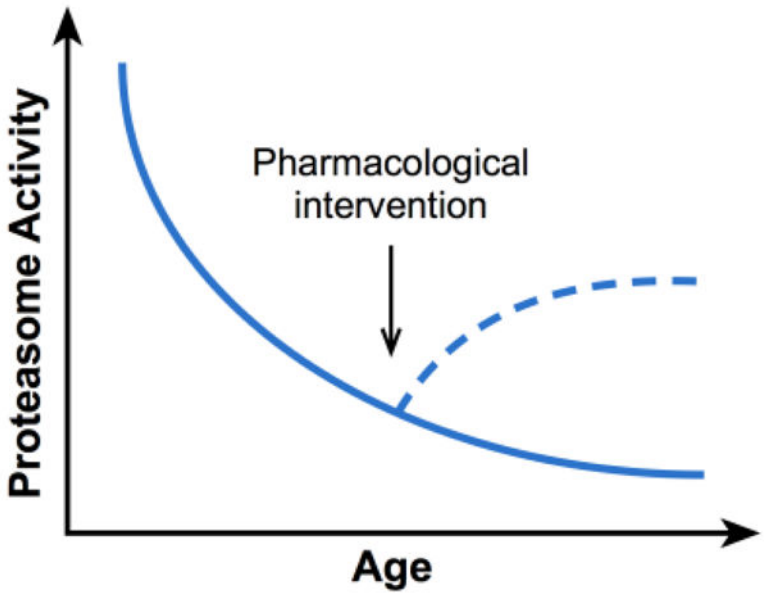

Figure 3. Schematic representation of the general decline in proteasome activity during ageing
In theory, small molecules might be used to partially restore this activity to boost function.

Table I

IDPs and associated neurodegenerative diseases. Adapted from *Uversky*[40].

Protein	Disorder by prediction (%)[a]	Function	Disease(s)
Amyloid-β	16.9	Peptidic fragment of APP, which regulates synapse formation, and neuronal plasticity	Alzheimer's disease Amyloidosis
Tau	77.6	Promote the assembly of and stabilizes neuronal microtubules	Tauopathies Alzheimer's disease Corticobasal degeneration Pick's disease Progressive supranuclear palsy
TDP-43	57.3	Transcriptional repression, pre-mRNA splicing, and translational regulation	Amyotrophic lateral sclerosis and frontotemporal lobar degeneration
α-Synuclein	90.7	Regulate synaptic vesicles	Alzheimer's disease (α) Multiple system atrophy(α) Parkinson's disease (α, β, γ) Diffuse Lewy body disease (α, β γ)
β-Synuclein	87.3		
γ-Synculein	100		
FUS	90.7	Transcriptional regulation (initiation & repression), and RNA-binding	Amyotrophic lateral sclerosis

[a]Disorder was predicted by *PONDR® VSL2*.

Table II

Activators of the 20S proteasome.