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Combating neurodegenerative disease with chemical probes and model systems

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The disheartening results of recent clinical trials for neurodegenerative disease (ND) therapeutics underscore the need for a more comprehensive understanding of the underlying disease biology before effective therapies can be devised. One hallmark of many NDs is a disruption in protein homeostasis. Therefore, investigating the role of protein homeostasis in these diseases is central to delineating their underlying pathobiology. Here, we review the seminal role that chemical biology has played in furthering the research on and treatment of dysfunctional protein homeostasis in NDs. We also discuss the vital and predictive role of model systems in identifying conserved homeostasis pathways and genes therein that are altered in neurodegeneration. Integrating approaches from chemical biology with the use of model systems yields a powerful toolkit with which to unravel the complexities of ND biology.

Today's human population is facing the challenge of aging. Unfortunately, the short course of human evolution has not shaped our biology to cope with the consequences of greatly extended longevity. The greatest contributing risk factor for NDs is age. With an aging populace, the inevitable result is a steep rise in the incidence of NDs. The extended course of these diseases and the support required by those affected impose a vast economic and emotional burden on individuals, communities and governments worldwide.

Early investigations into ND biology revealed that protein aggregates were common pathological findings. Such aggregates have been viewed as hallmarks of these diseases for many years. Analyses of familial forms of NDs have implicated these aggregation-prone proteins as causative factors and have provided deeper insights into the processes and cellular players involved in disease progression. Many of the same biological processes disrupted in early-onset forms of various NDs, such as Alzheimer's (AD) and Parkinson's disease (PD), are thought to be similarly perturbed in the more common, sporadic disease forms. However, with the advent of whole-genome sequencing, it has become increasingly clear that sporadic forms of NDs have highly complex genetic and phenotypic architectures. Emerging at a time when (i) selective pressure on human evolution has been relaxed, (ii) somatic mutations have been acquired and (iii) lifespans have been increasing, ND states have tremendous biological heterogeneity[1].

Although intensive research efforts have been directed toward deciphering the mechanisms of NDs such as AD and PD, our understanding of neurodegeneration remains incomplete. The collective failure of recent clinical trials demonstrates a need for a better appreciation of the complex biology underlying these diseases to develop effective, targeted therapeutics[2]. Because one unifying characteristic of most NDs is a disruption of protein homeostasis (proteostasis), investigations into these mechanisms provide an opportunity for a deeper understanding of disease biology and the identification of efficacious treatment strategies[3].

In this review, we discuss the role of protein homeostasis in ND, highlighting relevant developments at the interface of chemistry and biology. We review several aspects of protein homeostasis that have been probed with small molecules and how these probes might serve as leads for therapeutic development. Additionally, we discuss how model systems can be used to further the current state of knowledge of the cellular biology associated with NDs.

Centrality of proteostasis to ND pathology

Proteins execute and mediate many essential functions in an organism and must therefore be exquisitely controlled and balanced on many levels. The processes that preserve this balance, collectively known as protein homeostasis, include protein (i) folding, (ii) synthesis and production as well as assembly (including post-translational modifications), (iii) degradation and disassembly and (iv) trafficking and localization (**Fig. 1**). It remains a daunting challenge to decipher how exactly the various arms of the proteostasis machinery are coordinated spatiotemporally in cells and tissues. For example, although a certain protein complex may be undergoing synthesis, folding and assembly, a subpopulation of the same complex could be on its way to a certain subcellular compartment while another subpopulation concomitantly undergoes degradation and disassembly. These processes are all influenced not only by the local function and availability of the protein in question but also by other interacting proteins and molecules. It is clear that these arms must be maintained in a state of delicate balance at all times. Moreover, disruptions in one or more of these processes have been implicated in a variety of disease states as well as in the aging process itself[4,5]. We will briefly introduce some key components of the proteostasis machinery and the relationship between the dysfunction of proteostasis and NDs.

The heat shock response (HSR) is a key regulator of cytoplasmic and nuclear protein homeostasis. In fact, many studies have shown that a balance in the various proteins involved in the HSR is crucial for maintaining normal physiology. An overabundance of heat shock proteins (HSPs) can result in unwanted stabilization of mutant proteins, as with mutant kinases associated with different cancers[6,7]. In contrast, underproduction of HSPs decreases protection against detrimental protein misfolding, mislocalization and aggregation processes. The unfolded protein response (UPR) is a pathway analogous to the HSR in the maintenance of protein homeostasis of the endoplasmic reticulum (ER). Like the HSR, the UPR is also involved in a broad range of physiological processes.

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Many model systems used to study NDs and their related protein pathologies are characterized by a high level of ER stress[8,9]. Such responses are not restricted to the ER, as UPR-like responses have also been observed in the mitochondrion[10], an organelle whose physiology is also perturbed in NDs and aging[11,12].

Autophagy is another critical class of processes in the protein homeostasis landscape. In different types of autophagy, cytosolic and various organelle components are targeted to the lysosome for degradation. These key pathways regulate both protein and organelle turnover, processes essential to cell survival. The efficient function of autophagy pathways is especially crucial to clear unwanted proteins and cellular components in post-mitotic cells such as neurons that do not experience dilution of intracellular components during mitosis[13]. The malfunction of autophagy processes has been implicated in various NDs. The loss of key autophagy-regulating genes has resulted in neurodegeneration in mouse models[14]. Additionally, many aggregating proteins associated with NDs are autophagy substrates, and impaired autophagy leads to the toxic accumulation of these proteins.

The aging process has been canonically associated with sporadic NDs, and, in fact, maintenance of proteostasis may be a crucial part of this relationship. Many studies have indicated that a key component of the aging process itself is the collapse of the protein homeostasis processes involved in the prevention of protein misfolding and aggregation, such as the HSR and UPR. This collapse of proteostasis and the onset of ND-associated proteotoxicity can be modulated by the primary metabolic signaling pathways that regulate cellular and organismal aging[4,15].

It is also important to mention that major changes in cellular proteostasis are not always detrimental to the cell. Many highly structured stable aggregates or aggregate bundles have been speculated to be protective, acting as sites for the sequestration of misfolded proteins[16]. Understanding how such assemblies vary in different neurodegenerative pathologies is just beginning.

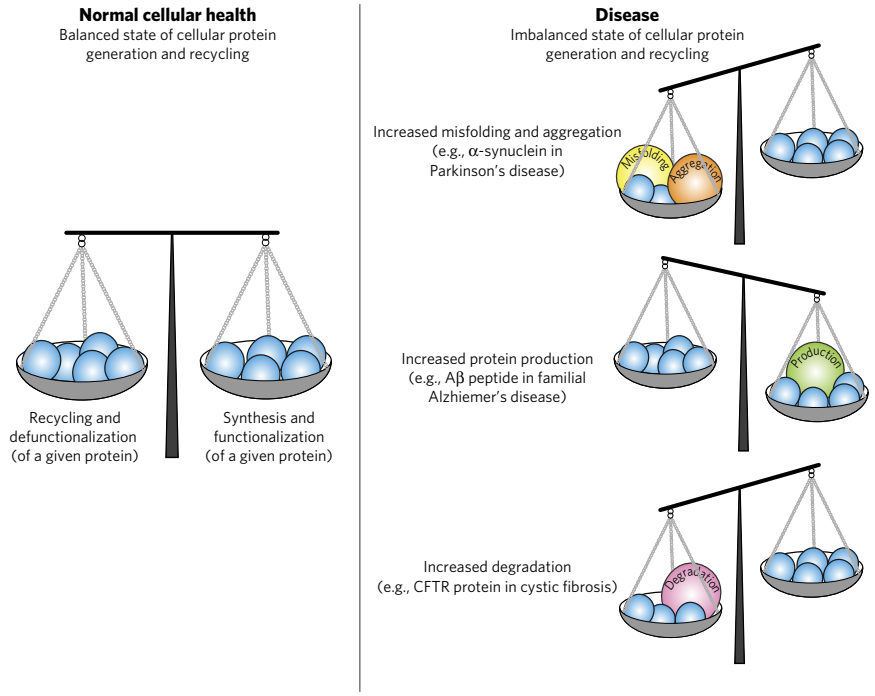


Figure 1 | Balancing act of proteostasis and potential perturbations driving disease. Under normal cellular homeostasis conditions, protein synthesis, assembly, proper folding and localization are balanced by timely degradation and disassembly (of protein complexes). In many human diseases, this balance is perturbed in multiple ways, in part due to changes in the misfolding and aggregation, production, and degradation of proteins. The scenarios presented do not capture the entirety of ND states and are meant to generalize common themes. Exceptions exist, for example, in the case of decreased protein degradation due to lysosomal defects identified in subsets of PD.

Disruption of neuronal proteostasis results in ND

Given that many non-NDs can also be characterized by dysfunction in protein homeostasis[17,18], what makes the brain[19] especially susceptible to changes in protein homeostasis? The key to this question may lie in the characteristic features that set neurons apart from other cell types. As noted earlier, neurons, along with certain muscle cells, are post-mitotic and cannot 'dilute out' long-lived proteins[20]. Their long cellular lifetime and high respiration rate can lead to the accumulation of large quantities of environmental damage, such as oxidative stress. Moreover, neurons are already tasked with the constant need to repair DNA breaks associated with neurodevelopment, learning and memory[21,22]. Therefore, additional DNA damage from environmental assaults or that accumulating with age could exceed the repair capacity of neurons, leading to dysfunction[23]. Nerve cells may also be aneuploid[24], which, coupled with few opportunities to correct chromosome replicating defects, subject neurons to increased proteotoxic stress[25]. Furthermore, neurons are physically extended cells whose function critically depends on the constant movement and transport of cargo across long biological distances and are therefore easily affected by problems in protein localization or trafficking. Additionally, the high surface area/volume ratio of neurons leads them to be especially susceptible to aberrant protein-membrane interactions[26]. Finally, neurons are also less efficient than other cell types at mounting a HSR[27], which is a cellular defense against proteotoxic stress. These various reasons render neurons particularly prone to dysfunctional proteostasis.

In many NDs, protein aggregates in and around human neurons are the signature cellular feature. For example, aggregates of α-synuclein are a pathological hallmark of a number of NDs, including most PD cases, as well as multiple system atrophy, dementia with Lewy bodies and neurodegeneration with brain iron accumulation[28,29]. Aggregates of the amyloid-β (Aβ) peptide are characteristic of AD. A number of pathologies are characterized by the misfolding and aggregation of proteins with expanded polyglutamine tracts (termed polyQ diseases), the most notorious of which is the protein huntingtin in Huntington's disease. Other examples of aberrant protein aggregation include that of TAR DNA-binding protein 43 (TDP-43) and ataxin-2 in amyotrophic lateral sclerosis (ALS)[30].

Although instances of protein misfolding and aggregation are the predominant examples of protein dyshomeostasis in neurodegeneration, they are certainly not the only ones. In familial AD, point mutations in the amyloid precursor protein (APP) and the enzyme complexes involved in its proteolysis to produce the Aβ peptide can lead to increased protein aggregation as well as abnormal levels of Aβ peptide production[31,32]. Moreover, mutations of the ubiquitin ligase Parkin (also known as PARK2) were shown to be strongly associated with some forms of early-onset PD starting in 1998, hinting at defects in the ubiquitin-proteasome degradation pathway in NDs[33]. Lastly, mislocalization of tau (AD), TDP-43 (ALS) and nucleoporin p62 (infantile bilateral striatal necrosis) highlight instances of inappropriate protein localization and trafficking[34]. Overall, it is evident that many NDs involve a complex set of disruptions to protein homeostasis.

In the following sections, we provide examples of how chemical biology can be used for the development of probes and, potentially, therapeutics directed at proteostasis dysfunction in NDs. We will also examine how the integration of various model systems can provide powerful insights into the fundamental conserved biology that underlies protein homeostasis for the design of more effective therapeutics.

Chemical biology for development of ND therapeutics

The development of therapeutics or useful probes for diseases related to protein homeostasis is a daunting challenge for a number of reasons. First, many pathways involved in protein homeostasis are highly conserved across many years of evolution, but their connectivity is not yet fully understood. Therefore, it is difficult to modulate a specific protein homeostasis pathway without affecting others. Second, many NDs involve the misfolding or aggregation of proteins that lack clearly defined structures or small molecule–binding pockets. For these reasons, the development of chemical compounds targeting the misfolded and aggregating species themselves has proven difficult for many proteins. Hence, most of the attention in this field has focused on modulating other aspects of protein homeostasis pathways. We briefly review some of the successes and challenges in the field of therapeutic and probe development.

Given that the chaperone machinery is a primary defense against misfolding and aggregation, targeting and modulating these proteins have been explored as a therapeutic option in a number of model systems. For example, recent work has reported that engineering a mutant yeast disaggregase, Hsp104, can bias its activity toward the dismantling of aggregates of the disease-associated proteins TDP-43, FUS and α-synuclein. This enhanced activity has the ability to protect dopaminergic neurons from protein aggregate-induced death in *Caenorhabditis elegans* as well[35]. Additionally, inhibitors of Hsp90 have been found to yield promising protective results in mouse models against polyglutamine toxicity[36]. Furthermore, small-molecule inhibition of Hsp90 in cases of spinal and bulbar muscular atrophy, a polyQ disease involving mutant androgen receptor, decreased toxicity in mice[37]. The authors noted, however, that the compound 17-AAG mildly induced two other chaperones (Hsp70 and Hsp40), pointing to the usual multifaceted effects of these small molecules on proteostasis. A later study showed that Hsp70 activation in *Drosophila* can promote polyQ degradation in a fruit fly model of androgen receptor toxicity[38]. Interestingly, polyQ proteins have been shown to sequester the yeast Hsp40 chaperone, an event that promotes the formation of cytoplasmic inclusions[39].

Modulators of the HSR have been discovered through various cellular screening methods. Most of these compounds act by modulating the transcription factor HSF1 and have been shown to reverse protein-aggregation phenotypes in cell culture–based and *C. elegans* model systems[40]. Nevertheless, modulating the HSR has to be approached with a considerable degree of caution as enhanced activation of HSF1, for example, is a potent enabler of malignancy[41]. Exacting finer control over the various arms of the HSR will allow for more effective therapeutics and probes.

The chemical modulation of autophagy has also proven to be an effective strategy for the amelioration of ND-associated phenotypes. For example, inducers of macro-autophagy pathways have shown promise in reducing huntingtin- and polyQ aggregate–induced toxicity while increasing lifespan in a number of model organisms and cellular systems, from fruit flies to mouse models[42]. Recently, the modulation of autophagy has been shown to rescue mouse and human neuronal models from the toxicity arising from TDP-43 aggregation[43]. Furthermore, a recent study has shown that the chemical induction of autophagy in human neurons from patients with the neurodegenerative condition Niemann-Pick disease type C can rescue the detrimental disease-associated phenotypes[44]. Other new strategies for therapeutic and probe discovery include phenotypic screening in mammalian cell lines. One such study identified compounds that rescued mutant huntingtin toxicity. These compounds led to the identification of protein disulfide isomerases, which are key ER-resident enzymes, as a new link between protein misfolding and apoptosis[45].

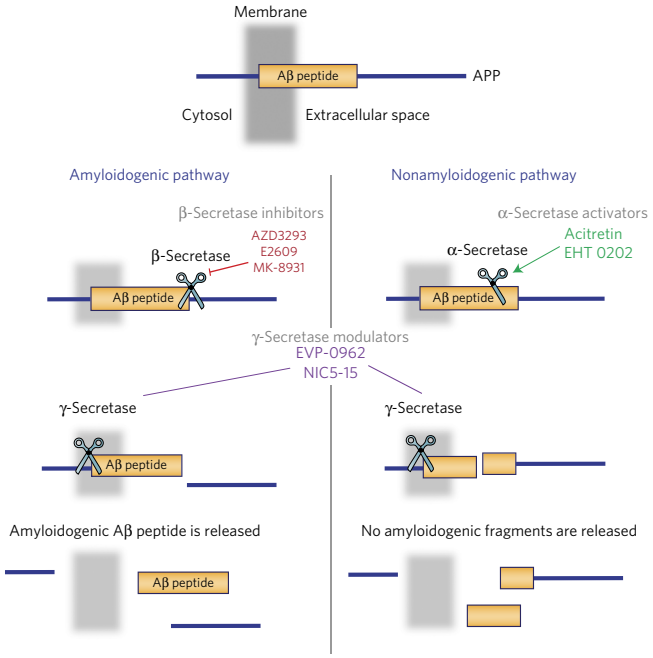


Figure 2 | Small molecules targeting the processing of APP. Three classes of secretase-modulating compounds are shown that are currently at various stages of clinical development. These candidate therapeutics inhibit APP processing via the amyloidogenic processing pathway (by β- and γ-secretase) and encourage a nonamyloidogenic processing pathway (mediated by α-secretase). This figure only includes compounds targeting these enzymes that are currently in clinical trials. Proteolytic processing concept adapted from ref. 111.

Another important example of the application of chemical biology to ND therapeutics is the modulation of the enzymatic processing of APP in AD. Overproduction of the Aβ peptide, which aggregates in AD, is due to the misregulation of the proteolytic enzymes that cleave APP (**Fig. 2**). APP is first cleaved by either α-secretase or β-secretase (BACE1). In AD, a predominant initial proteolytic cleavage by β-secretase generates an Aβ precursor peptide. Subsequent cleavage of the precursor by γ-secretase generates the Aβ peptide. The Aβ monomers (most commonly 40 or 42 amino acids in length) then aggregate into a heterogeneous ensemble of cytotoxic oligomers that are implicated in the neurotoxicity associated with AD. The proteolytic processing of APP into Aβ was identified as having a key role in AD when a number of familial AD cases were identified with mutations in APP around the cleavage sites or in the γ-secretase complex itself[46]. Such mutations were found to increase the total quantity of Aβ produced or to bias the lengths or primary sequence of the Aβ peptide toward those that are more aggregation prone. BACE1 inhibitors have been explored as potential therapeutic options for AD[47]. Other chemical approaches that modulate γ-secretase activity away from APP and toward its other substrates are also under development. A third avenue of therapeutic targeting aims to chemically neutralize the cytotoxic Aβ oligomers themselves by immunotherapy. Much of this research is in progress. Although results from clinical trials with similar compounds have been disheartening in the past, the compounds and therapeutics currently under development have promising initial data and will hopefully prove more fruitful than their predecessors (**Table 1**).

Table 1 | Therapeutic strategies for the treatment of AD.

Type	Mode of action	Target	Name	Phase	Company
Small molecule	Modulators of APP processing	α-Secretase	Acitretin	2	Stiefel/GSK
			EHT 0202	2	ExonHit Therapeutics
		β-Secretase	AZD3293	1	AstraZeneca
			E2609	1	Eisai/Biogen Idec
			LY2886721	Discontinued	Eli Lilly
			MK-8931	3	Merck
			RG7129	Discontinued	Roche
			Thalidomide	2/3	Celgene
		γ-Secretase	Avagacestat	Discontinued	Bristol-Myers Squibb
			Semagacestat	Discontinued	Eli Lilly
			EVP-0962	2	EnVivo Pharmaceuticals
			NIC5-15	2	Humanetics Pharmaceuticals
	Aggregation inhibitors or stabilizers	Aβ oligomers	ELND005	2	Élan/Perrigo
		Tau	Rember TM	Discontinued	TauRx Therapeutics
		Microtubules	Epothilone D	1	Bristol-Myers Squibb
	Enzymatic inhibition	Glycogen synthase kinase-3 (phosphorylates tau)	Tideglusib	Discontinued	Zeltia
	Metal homeostasis		PBT2	Discontinued	Prana Biotechnology
Immunotherapy	Aβ modulators	Aβ monomers	Affitope AD02	2	AFFiRiS/GSK
			Bapineuzumab	Discontinued	Janssen AI/J&J and Pfizer
			CAD106	2	Novartis
			Ponezumab	Discontinued	Pfizer
			Solanezumab	3	Eli Lilly
			Vanutide cridificar	Discontinued	Pfizer
			AN-1792	Discontinued	Janssen AI/J&J and Pfizer
		Aβ monomers and oligomers	Crenezumab	2	Genentech
		Aβ oligomers	BAN2401	2	Eisai
		Aβ oligomers and fibrils	BIIB037	1	Biogen Idec
			SAR228810	1	Sanofi
		Aβ fibrils	Gantenerumab	3	Chugai Pharmaceutical/Roche

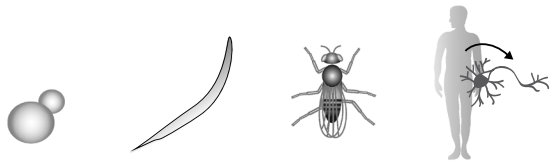
Strategies in **Table 1** are based on Alzforum's database of amyloid-related therapeutics.

Model systems

The complexity of NDs requires a deep understanding of the disease biology and the individual proteins involved. To that end, the research community has developed and refined the use of a number of model systems over the past few decades. Model systems provide powerful tools to understand the basic biology that underlies complex human diseases. As NDs result from a multifaceted interplay between many genetic and environmental factors, the ability to investigate these factors in model organisms offers a way to control variables and to examine the fundamental aspects of disease biology. Overall, the remarkable conservation of basic biological processes, including proteostasis pathways, among organisms separated by many years of evolution has enabled the fruitful use of these organisms as model systems to study the biology of human diseases. Although covering them all is beyond the scope of this review, the strengths of three popular nonmammalian model systems as well as an emerging human cell-based model are summarized in **Figure 3**. In the following section, we focus on a few examples of how these models have informed probe and therapeutic development. Although the contribution of these systems to the understanding of ND genetics has been profound, it is unfortunately beyond the scope of this review.

C. elegans. The nematode *C. elegans* is a popular model system that has been widely used to study NDs and disorders of protein homeostasis. Although worms and humans are separated by millions of years of evolutionary divergence, these tiny and transparent animals have differentiated tissues, including a nervous system. In fact, the tissue lineages of all cells have been tracked, and the connections between all 302 neurons have been mapped[48]. A toolbox of promoters even allows for the expression of aggregation-prone proteins in specific tissues of this anatomically transparent animal, such as muscles, intestines and neurons, which manifest in detectable, specific phenotypes such as constrained motion or neuronal loss.

Various models of protein aggregate–induced toxicity have been constructed in *C. elegans*, including the expression of Aβ, polyglutamine, tau, TDP-43 and α-synuclein, among others. When many of these toxic proteins are expressed in the nematode muscle tissue, paralysis or motor defects are observed[49,50]. These marked behavioral phenotypes provide a basis for medium-throughput genetic and chemical screening. For example, a 2012 study tested the efficacy of various antiaggregation compounds in a tau-expressing *C. elegans* model. By screening many chemicals, the authors were able to identify a compound that protected against the effects of toxic tau aggregation even in more complex mammalian systems[51]. Nematode models have also been exploited to evaluate the selective



	S. cerevisiae	*C. elegans*	*D. melanogaster*	Patient-derived iPS neurons
Ease of biochemical investigation	+++	++	++	+
Ease of genetic investigation	+++++	++	+++	+
Scale of chemical screens (number of compounds)	>500,000	~10,000	~2,000	~2,000
Neuronal subtype specificity	NA	++	++	+++
Approximate generation time	2 h	3–4 d	10 d	>20 d (to culture)
Number of homologous genes	~3,500	~7,000	~7,000	NA
Ease of imaging	+++	+++	+	+++
ND-associated protein toxicity models used for chemical screens	α-Synuclein	Aβ Tau LRRK2	Aβ CUG-repeat Fragile X	α-Synuclein
Select recent literature on chemical screens using model systems	Ref. 76	Refs. 51, 52	Refs. 67, 68	Ref. 82

Figure 3 | Comparison of select eukaryotic model systems for their potential for chemical biological approaches and drug discovery in NDs. A comparison of the salient features of the yeast, nematode, fruit fly and iPS neuron model systems, as discussed in this review, reveals the synergistic potential of using findings in more tractable model organisms to inform strategies and targets in more patient-relevant models.

inhibition of the hyperactive kinase activity of PD-associated mutant leucine-rich repeat kinase 2 (LRRK2) with small molecules, which led to the rescue of a dopaminergic deficit phenotype in *C. elegans*[52]. Likewise, the attenuation of LRRK2 neurodegeneration in both worm and fly models of PD has been reported[53]. The identification and efficacy of these kinase inhibitors in a whole-organism model shed light on the essential role of LRRK2 in PD pathogenesis while also suggesting promising avenues for future therapeutic development. *C. elegans* has been used not only to screen for compounds that alleviate toxic phenotypes but also to investigate a number of modulators of the HSR, which could prove useful leads in developing drugs to treat aggregation-based proteotoxicity[40].

One of the most valuable features of *C. elegans* is its translucency, allowing for direct imaging of fluorescent proteins in the tissues of live organisms using microscopy, paving the path for high-content screens[54]. An interesting way this feature has been adopted is its application to large-scale screens of live nematodes to identify chemical compounds that lead to neuronal regrowth after microsurgical alteration of axons[55]. As *C. elegans* has a short lifespan and shows distinct signs of aging, it is also an ideal organism with which to study the interaction between aging, metabolism, protein homeostasis and neurodegeneration.

As mentioned previously, the integration of data from *C. elegans* with findings from other complementary model systems is essential to the understanding of the complex biology of NDs. For example, a study in 2012 coupled a transgenic model of toxicity caused by the ALS-implicated proteins TDP-43 and FUS in *C. elegans* with an orthogonal model in zebrafish, creating a cross-species platform for testing compound efficacies[56]. These models linked dysfunction in the UPR machinery to TDP-43–induced toxicity[57]. This shows the power of using combinations of model systems to understand complex protein toxicity.

Drosophila melanogaster. As a multicellular eukaryote, *Drosophila* displays clear, tissue-specific pathologies and behavioral phenotypes. For example, the rough-eye phenotype, where the eye loses its exquisite structural regularity upon expression of a toxic protein, has been particularly useful in studying models of proteotoxicity[58]. Additionally, the expression of ND-related proteins often results in inclusions similar to those observed in post-mortem human brains. Flies have a short generation time (1–2 weeks) and the ability to express genes in a tissue-specific manner. For example, GAL4 nervous system–specific drivers have been used in many studies of ND-related proteotoxicity[59,60]. *Drosophila* also has the added advantage of defined multicell types and complex organs—notably the brain, which is composed of neurons and glia and ensheathed by a blood-brain barrier[61]—allowing for studies of functional brain regions and neural circuit interactions[62]. Importantly, *Drosophila* has well-understood genetics and a vast array of easily manipulatable approaches to exploit the genetics through crosses.

Seminal work in the study of ND *Drosophila* models has been performed on polyglutamine-, α-synuclein–, tau- and Aβ-expressing flies. Polyglutamine-[63], APP-[64] and tau-expressing[65] flies show the canonical signs of neurodegeneration, and an α-synuclein–expressing fruit fly model recapitulates many aspects of traditional PD in humans[66]. Furthermore, *Drosophila* models are well suited for the discovery of small-molecule compounds for biological probes or therapeutics. For example, a *Drosophila* model of CUG-repeat disease (that results in human myotonic dystrophy) displayed a rough-eye phenotype as well as semilethality. When screened for compounds that rescue this phenotype, ten candidates were identified that targeted G protein–coupled receptors and other receptors highly expressed in the brain[67]. Another chemical screen was performed on a *Drosophila* model of Fragile X syndrome. Here, using a lethal phenotype, a compound library of ~2,000 chemicals was screened to yield nine candidates, again targeting key G protein–coupled and cell surface receptors[68]. These examples illustrate the capacity of the *Drosophila* model system to be used for feasible *in vivo* medium-throughput screening. In the context of ND research, the most common uses of *Drosophila* models have been to validate compounds that have been uncovered in high-throughput screens in lower-order organisms and to define targets for efficacious compounds.

Similar to *C. elegans*, fruit fly models can be especially effective in unraveling new facets of ND pathomechanisms when used synergistically with orthogonal model systems. For example, a small-molecule inhibitor of translation initiation and stress granule formation (an inhibitor of the kinase PEK in *Drosophila* or PERK in humans that phosphorylates translation-initiation factor eIF2α) rescued TDP-43–initiated stress granule formation in both *Drosophila* and mammalian neurons, further corroborating the validity of these model systems in recapitulating select aspects of disease pathology[69]. Notably, the UPR-mediating PERK (PKR-like kinase) has recently been shown as a potentially efficacious

target for treating mouse models of prion disease[70] and AD[71]. As an additional example, a chemical compound screen against a polyglutamine-expressing yeast strain yielded compounds that were then validated in a *Drosophila* model expressing polyglutamine proteins. The compounds identified were able to modulate the aggregation and, consequently, toxicity of polyglutamine-expanded proteins[72]. Moreover, screening compounds that reduced polyglutamine toxicity in cell-free assays and tissue culture identified histone deacetylase (HDAC) inhibition as a key regulator of polyglutamine toxicity. These HDAC inhibitors were further validated in *Drosophila* model systems[73]. These studies reveal the utility of fruit fly models to uncover compounds that ameliorate different aspects of disease pathology in mammalian models and also shed light on the disease mechanism itself.

Yeast, a simple eukaryotic model. The budding yeast (*Saccharomyces cerevisiae*) is a simple eukaryotic organism that has been used to examine the cellular pathologies associated with NDs. Although this single-celled eukaryote does not have the complex connectivity of brain tissue, many of the fundamental biological features of eukaryotic cells are conserved[74]. These include organelles such as the nucleus, mitochondrion, ER and Golgi apparatus along with the actin and tubulin cytoskeletons. Moreover, yeast share with neurons the most central pathways of eukaryotic cell biology. These include protein chaperones, the proteasome and ubiquitin-mediated degradation pathways, autophagy, peroxisomes, apoptosis, vesicle trafficking, lipid metabolism and a host of highly conserved signal transduction pathways such as calcineurin-calmodulin, TOR, PKA, PKC and MAPK, all of which regulate adaptive responses to internal and external stressors. Yeast has also been an important model system in studying the HSR and continues to shed light on the nature of proteotoxic stress.

The primary advantages of yeast as a model organism lie in its genetic tractability and short doubling time. It is simple to integrate genes into the genome and delete or mutate genes in a highly controllable manner. In fact, many libraries exist that allow the overexpression, deletion and transposition of every open reading frame of the yeast genome. These can be exploited for high-throughput screens with overexpression, deletion and transposition of genes as well as for chemical screens performed in these various backgrounds[75,76]. The flexibility to work with both diploid and haploid strains allows the uncovering and masking of genetic traits through sporulation and mating. Moreover, recent developments in variomics libraries that encode collections of point mutants of every gene in the yeast genome will serve as key tools for target identification in chemical and genetic screens[77].

A recent chemical genetic study in yeast investigated the cellular pathologies related to the PD-associated protein α-synuclein[76]. This study identified a compound, NAB2, which rescued the growth deficits caused by expression of α-synuclein in the yeast model. Subsequently, three different types of genetic screens (genome-wide overexpression, polymorphism and transposition) were performed on a yeast strain overexpressing α-synuclein in the presence of NAB2 to identify the proximal protein target of the compound. A number of candidates emerged, all converging on the yeast E3 ubiquitin ligase Rsp5 (homolog of the human protein NEDD4). NAB2 was found to rescue α-synuclein toxicity by a new mechanism: activation of an E3 ubiquitin ligase. The compound was able to alleviate the effects of α-synuclein–induced dysfunction not only in the yeast model but also in induced pluripotent stem cell (iPSC)–derived human neurons developed from a patient with familial PD[78]. It is critical to note that Rsp5, because of its essential role in cell viability, was identified only through a combination of different types of genetic screens. These studies clearly demonstrate how a combination of high-throughput chemical genetics in yeast coupled with validation in higher-order model systems can result in the discovery of new probes and new pathways centrally involved in α-synuclein biology and its toxicity in disease. Overall, the ability to perform very high-throughput unbiased assays in yeast can markedly expand the already-successful application of phenotypic screens to the discovery of new molecular entities[79].

Simple model systems are primarily used to identify pathways to target with chemical approaches, and the translation of such discoveries between different model systems has been used effectively for understanding ND biology. However, simple model systems are not suitable for drug design optimization and pharmacokinetic studies. Although the number of currently marketed therapeutics discovered through studies in simple model organisms is small, this approach presents a promising outlook for future studies. In fact, the cholesterol-reducing blockbuster statin drugs were first identified for their antifungal action in inhibiting ergosterol biosynthesis in yeast, a pathway very similar to cholesterol biosynthesis in humans[80]. Such findings suggest that simple model systems can have a role in pathway, probe and therapeutic discovery for other human diseases as well. Furthermore, although some have raised questions about the interpretation and relevance of observations using overexpressed human proteins in model systems for which that species does not have evolutionarily conserved homologs, the proteins identified in lower-order organisms often have structural and phenotypic similarity to human proteins. Therefore, identification of affected proteins in model organisms that have no direct sequence relation to any human protein may still reveal pathways or processes that relate directly to the biology of human disease.

Extending findings from 'simple' model organisms

With the expanding use of large-scale sequencing technologies, the number of genetic associations with complex human diseases such as NDs has increased rapidly. Although the aforementioned model systems are highly suitable for unraveling the effects of these polymorphisms on basic cell physiology, they lack the specific cellular environments most relevant to the diseases. Two technical advances have recently provided new levels of relevance to the modeling of human disease: the development of iPSC technology and genome editing techniques. iPSCs allow for the creation of a disease-relevant cell type (e.g., neurons) from fibroblasts of a patient with a disease. This technology has opened new doors in studying cell type–specific effects of ND-related mutations[8,78,81,82]. Unlike tumor-derived cell lines, iPSCs provide a relevant human cell type with a genetic background related directly to a patient with the disease. The power and potential of these systems to illuminate human pathologies and test therapeutic strategies is immense. Although we will not be able to comprehensively review the specifics of such studies, they have been reviewed in greater detail elsewhere[83,84]. Here we emphasize that a number of key findings have been uncovered from and/or confirmed with studies of iPSCs. Many patient-derived iPSCs displayed various hallmarks of disrupted protein homeostasis including stress phenotypes, protein mislocalization, aggregation and sensitivity to various small-molecule compounds[85,86].

Initially, one of the challenges of using iPSC-derived cellular systems had been the inability to compare the patient-derived cells to an adequate control—the genetic backgrounds of fibroblasts from an AD patient and an age-matched, unaffected individual are considerably different[83]. Recent developments in genome editing techniques have helped investigators cross this hurdle. Systems such as zinc finger nucleases, transcription activator-like effector nucleases (TALENs) and the CRISPR-Cas nuclease systems derived from *Streptococcus pyogenes* enable the targeted modification of mammalian genomes[87–89]. These technologies have been adapted for screening studies in mammalian model systems[90] and have allowed for the generation of isogenic control lines for patient-derived cells with identical genetic backgrounds. Such isogenic cell-line pairs have already been used for investigations into the biology of AD, PD, Niemann-Pick disease type C and ALS[44,78,81,82].

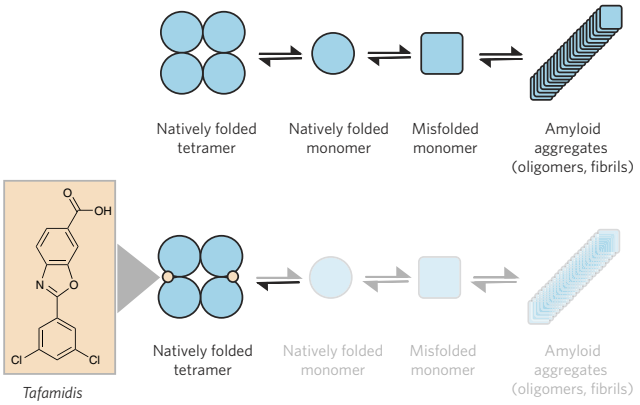


Figure 4 | Tafamidis targets TTR to stabilize folded, functional tetramers. TTR folds and assembles into a functional, thyroxine-binding homotetramer. However, certain point mutations within the coding sequence of TTR can favor the folded monomeric state compared to the tetramer. The folded monomer has a greater propensity to misfold and undergo amyloidogenic aggregation into a variety of species including oligomers and amyloid fibrils. In 2003, tafamidis was reported to be a potent inhibitor of TTR aggregation. The small molecule was designed to bind the thyroxine binding pockets at the intra-dimeric interface of the TTR tetramer. Tafamidis binding stabilizes TTR in the tetrameric structure and disfavors the monomeric state (and hence the amyloidogenic aggregation pathway).

As a result of these groundbreaking investigations, iPSCs are becoming an important addition to the arsenal of model systems to investigate the complexities of ND biology, and investigators are beginning to use them as screening platforms. The full adoption of such platforms is currently hindered by factors such as (i) the difficulty in scaling up iPSC-based assays or (ii) the differentiation times necessary to generate suitable cell lines, which at times can be extensive. These are therefore areas with great potential for innovative headways.

As with any new technology, it is important to consider possible caveats to this approach, primarily that the off-target effects of genome editing technologies have not yet been fully characterized. Moreover, a rising concern with using these systems as models of neurodegeneration is the conundrum of aging. Age is often considered to be the primary risk factor for the sporadic onset of many NDs. However, the reprogramming of iPSCs often resets the known molecular markers of cellular age. There have been efforts to artificially 'age' cells by genetic means[91]. However, the detailed molecular effects of these processes have yet to be understood. Nevertheless, iPSCs offer a promising new human cellular approach to help validate hypotheses generated from nonhuman model organisms. As demonstrated in the case of the NAB2 compound, for example, targets of a new small molecule can be discovered through the combinatorial power of multiple genome-wide genetic screens in yeast[92]. iPSC-derived neurons can subsequently be used to validate the targets and establish assays for compound optimization and target engagement.

Successes and failures of small-molecule mediators

The application of rigorous chemical screening approaches to model systems of ND proteotoxicity is a burgeoning field, and, simply put, not enough time has passed for validated compounds to go through clinical trials. There is an exception, however, in the case of tafamidis and the protein transthyretin (TTR). Other candidates, such as folding correctors in cystic fibrosis, are progressing through the pipeline as well[93].

Tafamidis and TTR, a rare success story. Tafamidis (trade name Vyndaqel) is one of the few successful therapies developed for a protein aggregation disease[94,95]. In the European Union and in a number of countries, tafamidis is approved for the treatment of familial amyloid polyneuropathy (FAP), a disease associated with the misfolding and consequent aggregation of the protein TTR. TTR functions as a homotetrameric hormone transporter. A number of point mutations have been identified in the coding sequence of the protein that result in changes in the monomer-tetramer equilibrium of TTR. The folded monomeric form has a greater potential to undergo misfolding and subsequent aggregation into toxic oligomers and β-sheet–rich amyloid fibrils, resulting in FAP. Tafamidis stabilizes the native tetramer and prevents the dissociation of mutant TTR into the monomeric state, thereby retaining the native quaternary structure of the protein and preventing amyloidogenic aggregation (**Fig. 4**). Tafamidis has been used successfully to treat a number of patients suffering from FAP.

The story of tafamidis is an example of a bench-to-bedside route of drug development, where a mechanistic understanding has been translated to an effective therapy. However, this is a rare occurrence among the attempts to find small-molecule drugs to treat diseases of protein homeostasis dysfunction. The primary reason for this lone success is that TTR is a well-folded protein that is inherently 'druggable' with deep structural pockets capable of specifically binding small molecules. Unfortunately, many other proteins involved in NDs do not have these desirable biophysical characteristics. For example, both Aβ and α-synuclein, which aggregate in AD and PD, respectively, are small and relatively disordered, which may render them 'undruggable'. It should be noted, however, that recent computational tools are allowing investigators to find less obvious pockets even in proteins of this type[96].

What lies ahead

Investigations to date have provided us with insights on the role of protein homeostasis networks in the pathogenesis of NDs. However, there are still many questions to answer. We suggest two avenues for future studies exploring the role and dysfunction of proteostasis in NDs.

Integration of intracellular systems. To modulate the proteostasis machinery for discovering ND therapeutics and probes, we need a greater understanding of how the various arms of the protein homeostasis hierarchy communicate and integrate inside the cell at a systems level. We first have to identify the players inside the cell and then decipher how they communicate with one another. Systematic approaches to look at protein homeostasis networks have been explored in organisms from bacteria to mammals[97]. Computational modeling has been a powerful tool to understand the basic protein homeostasis frameworks[98]. Now these approaches can be used to explore how protein homeostasis networks are altered in ND states as well. These investigations can extend beyond the roles of proteins alone. Chemical biology has helped uncover the role of other biomolecules in the pathology of these diseases. For example, a chemical screen in a Fragile X–associated tremor/ataxia syndrome *Drosophila* model revealed phospholipase A2 inhibitors as potential therapeutic measures[99], demonstrating the crucial role that phospholipids can have in altering the protein homeostasis state of the cell in certain NDs. Moreover, as technologies such as metabolite profiling develop, allowing further interrogation of model systems, we can begin to address challenging questions such as the complex role of metabolites[100], lipids[101] and RNAs[102] in protein and cellular homeostasis and how these are disrupted in ND states.

Integration of intercellular systems: looking beyond the cell membrane. Most of the work on ND biology has focused on intracellular pathologies and pathways. However, it is becoming increasingly apparent that the biology of these diseases is far more complex than can be constrained within the cell (see, for example,

a recent discussion in ref. 103 on the effects of circadian rhythms on neurodegeneration). In fact, there has been a growing body of experimental evidence supporting the concept of organismal proteostasis disruptions in these diseases. The source of disruption may be physically distant from the cells displaying the pathology. This has been demonstrated in recent discoveries of non-cell-autonomous mechanisms for proteostasis coordination[104,105]. Additionally, there has been increasing evidence postulating the spread of protein aggregates between cells, which can thereby propagate their associated toxic effects[106]. These observations have led to prion-like mechanisms to be proposed for the cell-to-cell spread of protein aggregates in NDs[107].

Moreover, the brain, the primary site of degeneration in many of these diseases, is a highly complex organ with a number of cell types that closely interact and communicate. Experimental evidence is now mounting to support the idea that glial cells (formerly considered mere support cells for neurons) may have a crucial role in ND biology[108]. Recent biochemical reports as well as genome-wide association studies have identified a strong immune component to many NDs, suggesting the involvement of glial cells such as astrocytes, oligodendrocytes and microglia in the pathogenesis of NDs[109]. iPSC cocultures may provide a key tool with which to examine these effects in a disease-relevant model system. As an example, astrocytes derived from ALS patient fibroblasts have been shown to be toxic to cocultured motor neurons, a phenotype that could be used for high-throughput compound screening[110].

Efforts integrating chemical biological approaches and model systems have resulted in seminal advances in understanding the role of protein homeostasis in NDs. The potential of these systems to provide additional insights is far from exhausted, and our knowledge remains far from complete. With ongoing development and refinement, we expect these techniques and systems to provide new platforms of discovery that will help us better understand, and eventually treat, the spectrum of NDs.

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Competing financial interests

The authors declare no competing financial interests.

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