Engineering therapeutic protein disaggregases

James Shorter*
Department of Biochemistry and Biophysics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104

ABSTRACT **Therapeutic agents are urgently required to cure several common and fatal neurodegenerative disorders caused by protein misfolding and aggregation, including amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and Alzheimer's disease (AD). Protein disaggregases that reverse protein misfolding and restore proteins to native structure, function, and localization could mitigate neurodegeneration by simultaneously reversing 1) any toxic gain of function of the misfolded form and 2) any loss of function due to misfolding. Potentiated variants of Hsp104, a hexameric AAA+ ATPase and protein disaggregase from yeast, have been engineered to robustly disaggregate misfolded proteins connected with ALS (e.g., TDP-43 and FUS) and PD (e.g., α-synuclein). However, Hsp104 has no metazoan homologue. Metazoa possess protein disaggregase systems distinct from Hsp104, including Hsp110, Hsp70, and Hsp40, as well as HtrA1, which might be harnessed to reverse deleterious protein misfolding. Nevertheless, vicissitudes of aging, environment, or genetics conspire to negate these disaggregase systems in neurodegenerative disease. Thus, engineering potentiated human protein disaggregases or isolating small-molecule enhancers of their activity could yield transformative therapeutics for ALS, PD, and AD.**

Monitoring Editor
David G. Drubin
University of California, Berkeley

Received: Feb 5, 2016
Revised: Mar 22, 2016
Accepted: Mar 23, 2016

We urgently need to pioneer game-changing solutions to remedy a number of increasingly prevalent and fatal neurodegenerative disorders, including amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and Alzheimer's disease (AD; Cushman *et al.*, 2010; Jackrel and Shorter, 2015). These disorders relentlessly erode our morale and economic resources. Aging is the major risk factor for all of these diseases, which threaten public health on a global scale and represent a severe impediment to living longer lives. A number of promising drugs have emerged to treat cancer and heart disease, but, distressingly, this is not the case for these and other neurodegenerative diseases, for which drug pipelines lie dormant and empty. This situation is unacceptable, and an impending healthcare crisis looms worldwide as population demographics inexorably shift toward older age groups.

ALS, PD, AD, and related neurodegenerative disorders are unified by a common underlying theme: the misfolding and aggregation of specific proteins (characteristic for each disease) in the CNS (Cushman *et al.*, 2010; Eisele *et al.*, 2015). Thus, in ALS, typically an RNA-binding protein with a prion-like domain, TDP-43, mislocalizes from the nucleus to cytoplasmic inclusions in degenerating motor neurons (Neumann *et al.*, 2006; Gitler and Shorter, 2011; King *et al.*, 2012; Robberecht and Philips, 2013; March *et al.*, 2016). In PD, α-synuclein forms toxic soluble oligomers and cytoplasmic aggregates, termed Lewy bodies, in degenerating dopaminergic neurons (Dehay *et al.*, 2015). By contrast, in AD, amyloid-β (Aβ) peptides form extracellular plaques and the microtubule-binding protein, tau, forms cytoplasmic neurofibrillary tangles in afflicted brain regions (Jucker and Walker, 2011). Typically, these disorders are categorized into ~80–90% sporadic cases and ~10–20% familial cases. Familial forms of disease often have clear genetic causes, which might one day be amenable to gene editing via clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 therapeutics if critical safety and ethical concerns can be successfully addressed and respected (Doudna and Charpentier, 2014; Baltimore *et al.*, 2015; Rahdar *et al.*, 2015; Callaway, 2016). However, the more common sporadic forms of disease often have no clear underlying genetics, and wild-type proteins misfold (Cushman *et al.*, 2010; Jucker and Walker, 2011; Robberecht and Philips, 2013; Dehay *et al.*, 2015). Consequently, therapeutic agents that directly target and safely reverse deleterious protein misfolding are likely to have broad utility (Eisele *et al.*, 2015).

DOI:10.1091/mbc.E15-10-0693

*Address correspondence to: James Shorter (jshorter@mail.med.upenn.edu).

Abbreviations used: Aβ, amyloid-β; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; CHIP, C-terminus of Hsp70-interacting protein; CRISPR, clustered regularly interspaced short palindromic repeats; PD, Parkinson's disease.

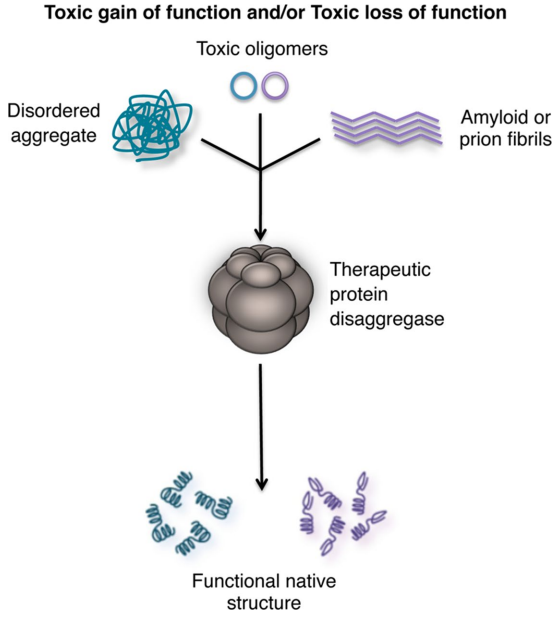

FIGURE 1: Therapeutic protein disaggregases. Two malicious problems are commonly associated with protein misfolding into disordered aggregates, toxic oligomers, and cross–β amyloid or prion fibrils: 1) a toxic gain of function of the protein in various misfolded states; and 2) a loss of function of the protein in the various misfolded states. These problems can contribute to the etiology of diverse neurodegenerative diseases in a combinatorial or mutually exclusive manner. A therapeutic protein disaggregase would reverse protein misfolding and recover natively folded functional proteins from disordered aggregates, toxic oligomers, and cross–β amyloid or prion fibrils. In this way, any toxic gain of function or toxic loss of function caused by protein misfolding would be simultaneously reversed. Ideally, all toxic misfolded conformers would be purged. Therapeutic protein disaggregases could thus have broad utility for various fatal neurodegenerative diseases.

There are no treatments that directly target the reversal of the protein-misfolding phenomena that underlie these disorders (Jackrel and Shorter, 2015). Strategies that directly reverse protein misfolding and restore proteins to native form and function could, in principle, eradicate any severely damaging loss-of-function or toxic gain-of-function phenotypes caused by misfolded conformers (Figure 1; Jackrel and Shorter, 2015). Moreover, therapeutic disaggregases would dismantle self-templating amyloid or prion structures, which spread pathology and nucleate formation of neurotoxic, soluble oligomers (Figure 1; Cushman *et al.*, 2010; Cohen *et al.*, 2013; Guo and Lee, 2014; Jackrel and Shorter, 2015). My group has endeavored to engineer and evolve Hsp104, a hexameric AAA+ ATPase and protein disaggregase from yeast (DeSantis and Shorter, 2012; Sweeny and Shorter, 2015), to more effectively disaggregate misfolded proteins connected with various neurodegenerative disorders, including ALS (e.g., TDP-43 and FUS) and PD (e.g., α-synuclein). Although wild-type Hsp104 can disaggregate diverse amyloid and prion conformers, as well as toxic soluble oligomers (Lo Bianco *et al.*, 2008; DeSantis *et al.*, 2012), its activity against human neurodegenerative disease proteins is suboptimal. Is it even possible to improve on existing Hsp104 disaggregase activity, which has been wrought over the course of millions of years of evolution?

Remarkably, the answer to this question is yes! We used nimble yeast models of neurodegenerative proteinopathies (Outeiro and Lindquist, 2003; Gitler, 2008; Johnson *et al.*, 2008; Sun *et al.*, 2011; Khurana *et al.*, 2015) as a platform to isolate enhanced disaggregases from large libraries of Hsp104 variants generated by error-prone PCR (Jackrel *et al.*, 2014b). In this way, we reprogrammed Hsp104 to yield the first disaggregases that reverse TDP-43, FUS (another RNA-binding protein with a prion-like domain connected to ALS), and α-synuclein (connected to PD) aggregation and proteotoxicity (Jackrel *et al.*, 2014a, 2015; Jackrel and Shorter, 2015; Torrente *et al.*, 2016). Remarkably, a therapeutic gain of Hsp104 function could be elicited by a single missense mutation (Jackrel *et al.*, 2014a, 2015; Jackrel and Shorter, 2015). Under conditions in which Hsp104 was ineffective, potentiated Hsp104 variants dissolved protein inclusions, restored protein localization (e.g., TDP-43 returned to the nucleus from cytoplasmic inclusions), suppressed proteotoxicity, and attenuated dopaminergic neurodegeneration in a *Caenorhabditis elegans* PD model (Jackrel *et al.*, 2014a; Jackrel and Shorter, 2015). Remarkably, these therapeutic modalities originated from degenerate loss of amino acid identity at select positions of Hsp104 rather than specific mutation (Jackrel *et al.*, 2014a; Jackrel and Shorter, 2015). Some of these changes were remarkably small (Jackrel *et al.*, 2014a, 2015; Jackrel and Shorter, 2015). Thus, potentiated Hsp104 variants could be generated by removal of a methyl group from a single side chain or addition or removal of a single methylene bridge from a single side chain (Jackrel *et al.*, 2014a, 2015; Jackrel and Shorter, 2015). Thus, small molecules that bind in accessible regions of Hsp104 rich in potentiating mutations might also be able to enhance activity. However, a small-scale screen for small-molecule modulators of Hsp104 revealed only inhibitors (Torrente *et al.*, 2014). Nonetheless, our work has established that disease-associated aggregates and amyloid are tractable targets and that enhanced artificial disaggregases can restore proteostasis and mitigate neurodegeneration (Jackrel and Shorter, 2015).

One surprising aspect of this work is just how many Hsp104 variants we could isolate with potentiated activity. We now have hundreds (Jackrel *et al.*, 2014a; Jackrel *et al.*, 2015). Typically, potentiated Hsp104 variants displayed enhanced activity against several neurodegenerative disease proteins. For example, $Hsp104^{A503S}$ rescued the aggregation and toxicity of TDP-43, FUS, TAF15, and α-synuclein (Jackrel *et al.*, 2014a; Jackrel and Shorter, 2014). By contrast, some potentiated Hsp104 variants rescued only the aggregation and toxicity of a subset of disease proteins. For example, $Hsp104^{D498V}$ rescued only the aggregation and toxicity of FUS and α-synuclein (Jackrel *et al.*, 2014a). A challenge that lies ahead is to engineer potentiated Hsp104 variants that are highly substrate specific to mitigate any potential off-target effects, should they arise (Jackrel and Shorter, 2015).

Very small changes in primary sequence yield potentiated Hsp104 variants. However, Hsp104 has no metazoan homologue (Erives and Fassler, 2015). Now comes the important point. Neuroprotection could be broadly achieved by making very subtle modifications to existing human chaperones with newly appreciated disaggregase activity—for example, Hsp110, Hsp70, and Hsp40 (Torrente and Shorter, 2013) and HtrA1 (Poepsel *et al.*, 2015).

Whether Metazoa even possess a powerful protein disaggregation and reactivation machinery had been a long-standing enigma (Torrente and Shorter, 2013). However, it has recently emerged that two metazoan chaperone systems—1) Hsp110, Hsp70, and Hsp40 (Torrente and Shorter, 2013) and 2) HtrA1 (Poepsel *et al.*, 2015)—possess disaggregase activity that could be therapeutically harnessed or stimulated to reverse deleterious protein misfolding in neurodegenerative disease. I suspect that Metazoa harbor additional disaggregase systems that remain to be identified (Guo *et al.*, 2014). Whether due to vicissitudes of

aging, environment, or genetic background, these disaggregase systems fail in the context of ALS, PD, and AD. Based on the surprising precedent of our potentiated versions of Hsp104 (Jackrel *et al.*, 2014a; Jackrel and Shorter, 2015), I hypothesize that it is possible to engineer and evolve potentiated variants of these human protein disaggregases to more effectively counter deleterious misfolding events in ALS, PD, and AD (Torrente and Shorter, 2013; Mack and Shorter, 2016).

Using classical biochemical reconstitution, it was discovered that one mammalian protein-disaggregase system comprises three molecular chaperones—Hsp110, Hsp70, and Hsp40—which synergize to dissolve and reactivate model proteins trapped in disordered aggregates and can even depolymerize amyloid fibrils formed by α-synuclein from their ends (Shorter, 2011; Duennwald *et al.*, 2012; Torrente and Shorter, 2013). Hsp110, Hsp70, and Hsp40 isoforms are found in the cytoplasm, nucleus, and endoplasmic reticulum, which suggest that protein disaggregation and reactivation can occur in several compartments (Finka *et al.*, 2015). Subsequent studies suggest that this system may be more powerful than initially anticipated (Rampelt *et al.*, 2012; Mattoo *et al.*, 2013; Gao *et al.*, 2015; Nillegoda *et al.*, 2015). Nonetheless, this system must become overwhelmed in neurodegenerative disorders. Perhaps selectively vulnerable neurons display particular deficits in this machinery. Hence, potentiating the activity of this system via engineering could be extremely valuable. It is promising that directed evolution studies yielded DnaK (Hsp70 in *Escherichia coli*) variants with improved ability to refold specific substrates (Aponte *et al.*, 2010; Schweizer *et al.*, 2011; Mack and Shorter, 2016), but whether this can be extended to human Hsp70 and the disaggregation of neurodegenerative disease proteins is uncertain.

It is exciting that recent studies have revealed that HtrA1, a homo-oligomeric PDZ serine protease, can dissolve and degrade AD-linked tau and Aβ42 fibrils in an ATP-independent manner (Tennstaedt *et al.*, 2012; Poepsel *et al.*, 2015). HtrA1 first dissolves tau and Aβ42 fibrils and then degrades them, as protease-defective HtrA1 variants dissolve fibrils without degrading them (Poepsel *et al.*, 2015). HtrA1 is found in the cytoplasm (~30%) but is also secreted (~70%; Poepsel *et al.*, 2015). Indeed, HtrA1 is known to degrade substrates in both the extracellular space and the cytoplasm (Chien *et al.*, 2009; Campioni *et al.*, 2010; Tiaden and Richards, 2013). Thus HtrA1 could dissolve Aβ42 fibrils in the extracellular space and tau fibrils in the cytoplasm and simultaneously destroy the two cardinal features of AD (Poepsel *et al.*, 2015). I suspect that this system becomes overwhelmed or is insufficient in AD, and thus potentiating and tailoring HtrA1 disaggregase activity could be a valuable therapeutic strategy. For example, it might be advantageous to simply degrade Aβ42 after disaggregation if the peptide has no beneficial function. Thus HtrA1 variants with enhanced disaggregation and degradation activity against Aβ42 could be extremely useful. However, Aβ42 (and related Aβ peptides) may have physiological functions that are presently underappreciated (Soscia *et al.*, 2010; Fedele *et al.*, 2015), in which case HtrA1 variants with enhanced disaggregase activity but reduced proteolytic activity could be vital. HtrA1 variants with enhanced disaggregase activity but reduced proteolytic activity may also be particularly important to recover functional tau from neurofibrillary tangles to reverse loss of tau function in AD and various tauopathies (Santacruz *et al.*, 2005; Trojanowski and Lee, 2005; Dixit *et al.*, 2008).

I suggest that relatively small changes in primary sequence will yield large increases in disaggregase activity for these systems as they do for Hsp104 (Jackrel *et al.*, 2014a; Jackrel and Shorter, 2015). If true, this would further suggest that small molecules that bind in the appropriate regions of Hsp110, Hsp70, Hsp40, or HtrA1 might also enhance disaggregase activity. Thus, isolating small-molecule enhancers of the Hsp110, Hsp70, and Hsp40 or HtrA1 disaggregase systems could yield important therapeutics. Indeed, I hypothesize that enhancing the activity of the Hsp110, Hsp70, and Hsp40 or HtrA1 disaggregase system with specific small molecules will enable dissolution of toxic oligomeric *and* amyloid forms of various disease proteins and confer therapeutic benefits in ALS, PD, AD, and potentially other neurodegenerative disorders.

It is intriguing that several small molecules are already known to enhance various aspects of Hsp70 chaperone activity (Pratt *et al.*, 2015; Shrestha *et al.*, 2016). These include MKT-077, JG-98, YM-1, YM-8, and 115-7c (Wisen *et al.*, 2010; Pratt *et al.*, 2015). It is not known whether any of these stimulates the disaggregase activity of the Hsp110, Hsp70, and Hsp40 system. MKT-077, JG-98, YM-1, and YM-8 are rhodocyanines that bind with low micromolar affinity to the nucleotide-binding domain of ADP- but not ATP-bound Hsp70, stabilizing the ADP-bound state (Pratt *et al.*, 2015). The ADP-bound state of Hsp70 engages clients with higher affinity, and consequently MKT-077, JG-98, and YM-1 activate binding of Hsp70 to misfolded proteins (Wang *et al.*, 2013; Pratt *et al.*, 2015). Thus, under some conditions, these small molecules can promote folding of certain Hsp70 clients (Morishima *et al.*, 2011; Pratt *et al.*, 2015). However, prolonged interaction of clients with Hsp70 promotes their CHIP-dependent ubiquitylation and degradation in vivo (Morishima *et al.*, 2011; Wang *et al.*, 2013; Pratt *et al.*, 2015). Intriguingly, YM-1 promotes clearance of polyglutamine oligomers and aggregates in cells (Wang *et al.*, 2013; Pratt *et al.*, 2015). MKT-0777, YM-1, JG-98, and YM-8 also promote clearance of tau and confer therapeutic benefit in tauopathy models (Abisambra *et al.*, 2013; Miyata *et al.*, 2013; Fontaine *et al.*, 2015). Of importance, YM-8 is long lived in vivo and crosses the blood–brain barrier (Miyata *et al.*, 2013). The dihydropyrimidine 115-7c activates Hsp70 ATPase turnover rate, promotes Hsp70 substrate refolding, and reduces α-synuclein aggregation in cell culture (Wisen *et al.*, 2010; Kilpatrick *et al.*, 2013). It binds to the IIA subdomain of Hsp70 and promotes the active Hsp70–Hsp40 complex (Wisen *et al.*, 2010). Small-molecule enhancers of HtrA1 protease activity have also emerged (Jo *et al.*, 2014). Thus it will be important to assess whether these small molecules enhance the activity of their respective disaggregases against various neurodegenerative substrates.

Although small molecules that enhance disaggregase activity of endogenous human proteins might be the most immediately translatable, gene-, mRNA-, or protein-based therapies can also be envisioned. For example, adeno-associated viruses expressing enhanced disaggregases might be used to target degenerating neurons (Dong *et al.*, 2005; Lo Bianco *et al.*, 2008; Deverman *et al.*, 2016). Alternatively, if viral vectors are undesirable, modified mRNAs might serve as an alternative to DNA-based gene therapy (Kormann *et al.*, 2011). Protein-based therapeutics could also be explored. For example, intraperitoneal injection of human Hsp70 increased lifespan, delayed symptom onset, preserved motor function, and prolonged motor neuron viability in a mouse model of ALS (Gifondorwa *et al.*, 2007; Gifondorwa *et al.*, 2012). Several other studies suggest that exogenous delivery of Hsp70 can have beneficial, neuroprotective effects in mice (Nagel *et al.*, 2008; Bobkova *et al.*, 2014; Bobkova *et al.*, 2015).

Ultimately, if safety and ethical concerns can be overcome in a circumspect, risk-averse manner, CRISPR-Cas9–based therapeutics might even be used to genetically alter the underlying disaggregase to a potentiated form in selectively vulnerable neuronal populations. This approach might be particularly valuable if enhanced disaggregase activity is not detrimental in the long term. Moreover, stem cell–based therapies for replacing lost neurons

could also be fortified to express enhanced disaggregase systems. Thus they would be endowed with resistance to potential infection by prion-like conformers that might have accumulated during disease progression (Cushman *et al.*, 2010).

Enhanced disaggregase activity is likely to be highly advantageous to neurons under circumstances in which protein misfolding has overwhelmed the system (Jackrel *et al.*, 2014a; Jackrel and Shorter, 2015). However, inappropriate hyperactivity of protein disaggregases might also have detrimental, off-target effects under regular conditions in which protein misfolding is not an overwhelming issue (Jackrel *et al.*, 2014a; Jackrel and Shorter, 2015). Thus it may be advantageous to engineer enhanced protein disaggregases to be highly substrate specific. In this way, off-target effects would be readily avoided. There is strong precedent for directed evolution or engineering of specialized chaperone or protein activity from a generalist antecedent (Wang *et al.*, 2002; Farrell *et al.*, 2007; Smith *et al.*, 2015). Thus, engineering specialist disaggregases for each disease substrate could be achieved. Alternatively, transient or intermittent doses of enhanced disaggregases at specific times or places where they are most needed would also minimize potentially toxic side effects. For example, enhanced disaggregase activity might be applied ephemerally to clear existing misfolded conformers and then be withdrawn once the endogenous proteostasis network regains control. Similarly, it is straightforward to envision administration of small-molecule enhancers of disaggregase activity in intermittent protocols that enable facile recovery from potential side effects (Fontaine *et al.*, 2015). In this way, any adverse effects of enhanced protein-disaggregase activity under normal physiological conditions would be avoided. Many barriers will need to be safely overcome to implement a successful therapeutic disaggregase, including how to deliver enhanced disaggregase activity to exactly where it is needed. However, these obstacles are not a reason to be pessimistic. On the contrary, the isolation of engineered disaggregases that efficaciously reverse deleterious misfolding of neurodegenerative disease proteins directs our attention to considerably expand the environs in which they should be sought. My closing sentences, therefore, are intended to be provocative.

I suspect that neuroprotection could be broadly actualized via precise but subtle alterations to existing protein-disaggregase modalities. The engineering and evolution of protein disaggregases could yield important solutions to avert an imminent plague of neurodegenerative disorders that promises to devastate our society. I strongly suspect that cures for various neurodegenerative disorders will be realized by pioneering as-yet-uncharted regions of disaggregase sequence space or chemical space to elucidate small-molecule enhancers of disaggregase activity.

ACKNOWLEDGMENTS

I thank Laura Castellano for the artwork. I am supported by National Institutes of Health Grant R01GM099836, a Muscular Dystrophy Association Research Award (MDA277268), an ALS Association Award, the Life Extension Foundation, a Linda Montague Pechinik Research Award, the Packard Center for ALS Research at Johns Hopkins University, and Target ALS.

REFERENCES

Abisambra J, Jinwal UK, Miyata Y, Rogers J, Blair L, Li X, Seguin SP, Wang L, Jin Y, Bacon J, *et al.* (2013). Allosteric heat shock protein 70 inhibitors rapidly rescue synaptic plasticity deficits by reducing aberrant tau. Biol Psychiatry 74, 367–374.

Aponte RA, Zimmermann S, Reinstein J (2010). Directed evolution of the DnaK chaperone: mutations in the lid domain result in enhanced chaperone activity. J Mol Biol 399, 154–167.

Baltimore D, Berg P, Botchan M, Carroll D, Charo RA, Church G, Corn JE, Daley GQ, Doudna JA, Fenner M, *et al.* (2015). Biotechnology. A prudent path forward for genomic engineering and germline gene modification. Science 348, 36–38.

Bobkova NV, Evgen'ev M, Garbuz DG, Kulikov AM, Morozov A, Samokhin A, Velmeshev D, Medvinskaya N, Nesterova I, Pollock A, *et al.* (2015). Exogenous Hsp70 delays senescence and improves cognitive function in aging mice. Proc Natl Acad Sci USA 112, 16006–16011.

Bobkova NV, Garbuz DG, Nesterova I, Medvinskaya N, Samokhin A, Alexandrova I, Yashin V, Karpov V, Kukharsky MS, Ninkina NN, *et al.* (2014). Therapeutic effect of exogenous hsp70 in mouse models of Alzheimer's disease. J Alzheimers Dis 38, 425–435.

Callaway E (2016). UK scientists gain licence to edit genes in human embryos. Nature 530, 18.

Campioni M, Severino A, Manente L, Tuduce IL, Toldo S, Caraglia M, Crispi S, Ehrmann M, He X, Maguire J, *et al.* (2010). The serine protease HtrA1 specifically interacts and degrades the tuberous sclerosis complex 2 protein. Mol Cancer Res 8, 1248–1260.

Chien J, He X, Shridhar V (2009). Identification of tubulins as substrates of serine protease HtrA1 by mixture-based oriented peptide library screening. J Cell Biochem 107, 253–263.

Cohen SI, Linse S, Luheshi LM, Hellstrand E, White DA, Rajah L, Otzen DE, Vendruscolo M, Dobson CM, Knowles TP (2013). Proliferation of amyloid-beta42 aggregates occurs through a secondary nucleation mechanism. Proc Natl Acad Sci USA 110, 9758–9763.

Cushman M, Johnson BS, King OD, Gitler AD, Shorter J (2010). Prion-like disorders: blurring the divide between transmissibility and infectivity. J Cell Sci 123, 1191–1201.

Dehay B, Bourdenx M, Gorry P, Przedborski S, Vila M, Hunot S, Singleton A, Olanow CW, Merchant KM, Bezard E, *et al.* (2015). Targeting alpha-synuclein for treatment of Parkinson's disease: mechanistic and therapeutic considerations. Lancet Neurol 14, 855–866.

DeSantis ME, Leung EH, Sweeny EA, Jackrel ME, Cushman-Nick M, Neuhaus-Follini A, Vashist S, Sochor MA, Knight MN, Shorter J (2012). Operational plasticity enables hsp104 to disaggregate diverse amyloid and nonamyloid clients. Cell 151, 778–793.

DeSantis ME, Shorter J (2012). The elusive middle domain of Hsp104 and ClpB: location and function. Biochim Biophys Acta 1823, 29–39.

Deverman BE, Pravdo PL, Simpson BP, Kumar SR, Chan KY, Banerjee A, Wu WL, Yang B, Huber N, Pasca SP, *et al.* (2016). Cre-dependent selection yields AAV variants for widespread gene transfer to the adult brain. Nat Biotechnol 34, 204–209.

Dixit R, Ross JL, Goldman YE, Holzbaur EL (2008). Differential regulation of dynein and kinesin motor proteins by tau. Science 319, 1086–1089.

Dong Z, Wolfer DP, Lipp HP, Bueler H (2005). Hsp70 gene transfer by adeno-associated virus inhibits MPTP-induced nigrostriatal degeneration in the mouse model of Parkinson disease. Mol Ther 11, 80–88.

Doudna JA, Charpentier E (2014). Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science 346, 1258096.

Duennwald ML, Echeverria A, Shorter J (2012). Small heat shock proteins potentiate amyloid dissolution by protein disaggregases from yeast and humans. PLoS Biol 10, e1001346.

Eisele YS, Monteiro C, Fearns C, Encalada SE, Wiseman RL, Powers ET, Kelly JW (2015). Targeting protein aggregation for the treatment of degenerative diseases. Nat Rev Drug Discov 14, 759–780.

Erives AJ, Fassler JS (2015). Metabolic and chaperone gene loss marks the origin of animals: evidence for Hsp104 and Hsp78 chaperones sharing mitochondrial enzymes as clients. PLoS One 10, e0117192.

Farrell CM, Baker TA, Sauer RT (2007). Altered specificity of a AAA+ protease. Mol Cell 25, 161–166.

Fedele E, Rivera D, Marengo B, Pronzato MA, Ricciarelli R (2015). Amyloid beta: walking on the dark side of the moon. Mech Ageing Dev 152, 1–4.

Finka A, Sharma SK, Goloubinoff P (2015). Multi-layered molecular mechanisms of polypeptide holding, unfolding and disaggregation by HSP70/HSP110 chaperones. Front Mol Biosci 2, 29.

Fontaine SN, Martin MD, Akoury E, Assimon VA, Borysov S, Nordhues BA, Sabbagh JJ, Cockman M, Gestwicki JE, Zweckstetter M, *et al.* (2015). The active Hsc70/tau complex can be exploited to enhance tau turnover without damaging microtubule dynamics. Hum Mol Genet 24, 3971–3981.

Gao X, Carroni M, Nussbaum-Krammer C, Mogk A, Nillegoda NB, Szlachcic A, Guilbride DL, Saibil HR, Mayer MP, Bukau B (2015). Human Hsp70 disaggregase reverses Parkinson's-linked alpha-synuclein amyloid fibrils. Mol Cell 59, 781–793.

Gifondorwa DJ, Jimenz-Moreno R, Hayes CD, Rouhani H, Robinson MB, Strupe JL, Caress J, Milligan C (2012). Administration of recombinant heat shock protein 70 delays peripheral muscle denervation in the

SOD1(G93A) mouse model of amyotrophic lateral sclerosis. Neurol Res Int 2012, 170426.
Gifondorwa DJ, Robinson MB, Hayes CD, Taylor AR, Prevette DM, Oppenheim RW, Caress J, Milligan CE (2007). Exogenous delivery of heat shock protein 70 increases lifespan in a mouse model of amyotrophic lateral sclerosis. J Neurosci 27, 13173–13180.
Gitler AD (2008). Beer and bread to brains and beyond: can yeast cells teach us about neurodegenerative disease? Neurosignals 16, 52–62.
Gitler AD, Shorter J (2011). RNA-binding proteins with prion-like domains in ALS and FTLD-U. Prion 5, 179–187.
Guo L, Giasson BI, Glavis-Bloom A, Brewer MD, Shorter J, Gitler AD, Yang X (2014). A cellular system that degrades misfolded proteins and protects against neurodegeneration. Mol Cell 55, 15–30.
Guo JL, Lee VM (2014). Cell-to-cell transmission of pathogenic proteins in neurodegenerative diseases. Nat Med 20, 130–138.
Jackrel ME, DeSantis ME, Martinez BA, Castellano LM, Stewart RM, Caldwell KA, Caldwell GA, Shorter J (2014a). Potentiated Hsp104 variants antagonize diverse proteotoxic misfolding events. Cell 156, 170–182.
Jackrel ME, Shorter J (2014). Potentiated Hsp104 variants suppress toxicity of diverse neurodegenerative disease-linked proteins. Dis Model Mech 7, 1175–1184.
Jackrel ME, Shorter J (2015). Engineering enhanced protein disaggregases for neurodegenerative disease. Prion 9, 90–109.
Jackrel ME, Tariq A, Yee K, Weitzman R, Shorter J (2014b). Isolating potentiated Hsp104 variants using yeast proteinopathy models. J Vis Exp 93, e52089.
Jackrel ME, Yee K, Tariq A, Chen AI, Shorter J (2015). Disparate mutations confer therapeutic gain of Hsp104 function. ACS Chem Biol 10, 2672–2679 .
Jo H, Patterson V, Stoessel S, Kuan CY, Hoh J (2014). Protoporphyrins enhance oligomerization and enzymatic activity of HtrA1 serine protease. PLoS One 9, e115362.
Johnson BS, McCaffery JM, Lindquist S, Gitler AD (2008). A yeast TDP-43 proteinopathy model: Exploring the molecular determinants of TDP-43 aggregation and cellular toxicity. Proc Natl Acad Sci USA 105, 6439–6444.
Jucker M, Walker LC (2011). Pathogenic protein seeding in Alzheimer disease and other neurodegenerative disorders. Ann Neurol 70, 532–540.
Khurana V, Tardiff DF, Chung CY, Lindquist S (2015). Toward stem cell-based phenotypic screens for neurodegenerative diseases. Nat Rev Neurol 11, 339–350.
Kilpatrick K, Novoa JA, Hancock T, Guerriero CJ, Wipf P, Brodsky JL, Segatori L (2013). Chemical induction of Hsp70 reduces alpha-synuclein aggregation in neuroglioma cells. ACS Chem Biol 8, 1460–1468.
King OD, Gitler AD, Shorter J (2012). The tip of the iceberg: RNA-binding proteins with prion-like domains in neurodegenerative disease. Brain Res 1462, 61–80.
Kormann MS, Hasenpusch G, Aneja MK, Nica G, Flemmer AW, Herber-Jonat S, Huppmann M, Mays LE, Illenyi M, Schams A, *et al.* (2011). Expression of therapeutic proteins after delivery of chemically modified mRNA in mice. Nat Biotechnol 29, 154–157.
Lo Bianco C, Shorter J, Regulier E, Lashuel H, Iwatsubo T, Lindquist S, Aebischer P (2008). Hsp104 antagonizes alpha-synuclein aggregation and reduces dopaminergic degeneration in a rat model of Parkinson disease. J Clin Invest 118, 3087–3097.
Mack KL, Shorter J (2016). Engineering and evolution of molecular chaperones and protein disaggregases with enhanced activity. Front Mol Biosci 3, 8.
March ZM, King OD, Shorter J (2016). Prion-like domains as epigenetic regulators, scaffolds for subcellular organization, and drivers of neurodegenerative disease. Brain Res 2016, S0006-8993(16)30096-8.
Mattoo RU, Sharma SK, Priya S, Finka A, Goloubinoff P (2013). Hsp110 is a bona fide chaperone using ATP to unfold stable misfolded polypeptides and reciprocally collaborate with Hsp70 to solubilize protein aggregates. J Biol Chem 288, 21399–21411.
Miyata Y, Li X, Lee HF, Jinwal UK, Srinivasan SR, Seguin SP, Young ZT, Brodsky JL, Dickey CA, Sun D, *et al.* (2013). Synthesis and initial evaluation of YM-08, a blood-brain barrier permeable derivative of the heat shock protein 70 (Hsp70) inhibitor MKT-077, which reduces tau levels. ACS Chem Neurosci 4, 930–939.
Morishima Y, Lau M, Peng HM, Miyata Y, Gestwicki JE, Pratt WB, Osawa Y (2011). Heme-dependent activation of neuronal nitric oxide synthase by cytosol is due to an Hsp70-dependent, thioredoxin-mediated thiol-disulfide interchange in the heme/substrate binding cleft. Biochemistry 50, 7146–7156.
Nagel F, Falkenburger BH, Tonges L, Kowsky S, Poppelmeyer C, Schulz JB, Bahr M, Dietz GP (2008). Tat-Hsp70 protects dopaminergic neurons in midbrain cultures and in the substantia nigra in models of Parkinson's disease. J Neurochem 105, 853–864.
Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, Bruce J, Schuck T, Grossman M, Clark CM, *et al.* (2006). Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 314, 130–133.
Nillegoda NB, Kirstein J, Szlachcic A, Berynskyy M, Stank A, Stengel F, Arnsburg K, Gao X, Scior A, Aebersold R, *et al.* (2015). Crucial HSP70 co-chaperone complex unlocks metazoan protein disaggregation. Nature 524, 247–251.
Outeiro TF, Lindquist S (2003). Yeast cells provide insight into alpha-synuclein biology and pathobiology. Science 302, 1772–1775.
Poepsel S, Sprengel A, Sacca B, Kaschani F, Kaiser M, Gatsogiannis C, Raunser S, Clausen T, Ehrmann M (2015). Determinants of amyloid fibril degradation by the PDZ protease HTRA1. Nat Chem Biol 11, 862–869.
Pratt WB, Gestwicki JE, Osawa Y, Lieberman AP (2015). Targeting Hsp90/Hsp70-based protein quality control for treatment of adult onset neurodegenerative diseases. Annu Rev Pharmacol Toxicol 55, 353–371.
Rahdar M, McMahon MA, Prakash TP, Swayze EE, Bennett CF, Cleveland DW (2015). Synthetic CRISPR RNA-Cas9-guided genome editing in human cells. Proc Natl Acad Sci USA 112, E7110–7117.
Rampelt H, Kirstein-Miles J, Nillegoda NB, Chi K, Scholz SR, Morimoto RI, Bukau B (2012). Metazoan Hsp70 machines use Hsp110 to power protein disaggregation. EMBO J 31, 4221–4235.
Robberecht W, Philips T (2013). The changing scene of amyotrophic lateral sclerosis. Nat Rev Neurosci 14, 248–264.
Santacruz K, Lewis J, Spires T, Paulson J, Kotilinek L, Ingelsson M, Guimaraes A, DeTure M, Ramsden M, McGowan E, *et al.* (2005). Tau suppression in a neurodegenerative mouse model improves memory function. Science 309, 476–481.
Schweizer RS, Aponte RA, Zimmermann S, Weber A, Reinstein J (2011). Fine tuning of a biological machine: DnaK gains improved chaperone activity by altered allosteric communication and substrate binding. Chembiochem 12, 1559–1573.
Shorter J (2011). The mammalian disaggregase machinery: Hsp110 synergizes with Hsp70 and Hsp40 to catalyze protein disaggregation and reactivation in a cell-free system. PLoS One 6, e26319.
Shrestha L, Patel HJ, Chiosis G (2016). Chemical tools to investigate mechanisms associated with HSP90 and HSP70 in disease. Cell Chem Biol 23, 158–172.
Smith BA, Mularz AE, Hecht MH (2015). Divergent evolution of a bifunctional de novo protein. Protein Sci 24, 246–252.
Soscia SJ, Kirby JE, Washicosky KJ, Tucker SM, Ingelsson M, Hyman B, Burton MA, Goldstein LE, Duong S, Tanzi RE, *et al.* (2010). The Alzheimer's disease-associated amyloid beta-protein is an antimicrobial peptide. PLoS One 5, e9505.
Sun Z, Diaz Z, Fang X, Hart MP, Chesi A, Shorter J, Gitler AD (2011). Molecular determinants and genetic modifiers of aggregation and toxicity for the ALS disease protein FUS/TLS. PLoS Biol 9, e1000614.
Sweeny EA, Shorter J (2015). Mechanistic and structural insights into the prion-disaggregase activity of Hsp104. J Mol Biol 2015, S0022-2836(15)00678-6 .
Tennstaedt A, Popsel S, Truebestein L, Hauske P, Brockmann A, Schmidt N, Irle I, Sacca B, Niemeyer CM, Brandt R, *et al.* (2012). Human high temperature requirement serine protease A1 (HTRA1) degrades tau protein aggregates. J Biol Chem 287, 20931–20941.
Tiaden AN, Richards PJ (2013). The emerging roles of HTRA1 in musculoskeletal disease. Am J Pathol 182, 1482–1488.
Torrente MP, Castellano LM, Shorter J (2014). Suramin inhibits Hsp104 ATPase and disaggregase activity. PLoS One 9, e110115.
Torrente MP, Chuang E, Noll MM, Jackrel ME, Go MS, Shorter J (2016). Mechanistic insights into Hsp104 potentiation. J Biol Chem 291, 5101–5115.
Torrente MP, Shorter J (2013). The metazoan protein disaggregase and amyloid depolymerase system: Hsp110, Hsp70, Hsp40, and small heat shock proteins. Prion 7, 457–463.
Trojanowski JQ, Lee VM (2005). Pathological tau: a loss of normal function or a gain in toxicity? Nat Neurosci 8, 1136–1137.
Wang AM, Miyata Y, Klinedinst S, Peng HM, Chua JP, Komiyama T, Li X, Morishima Y, Merry DE, Pratt WB, *et al.* (2013). Activation of Hsp70 reduces neurotoxicity by promoting polyglutamine protein degradation. Nat Chem Biol 9, 112–118.
Wang JD, Herman C, Tipton KA, Gross CA, Weissman JS (2002). Directed evolution of substrate-optimized GroEL/S chaperonins. Cell 111, 1027–1039.
Wisen S, Bertelsen EB, Thompson AD, Patury S, Ung P, Chang L, Evans CG, Walter GM, Wipf P, Carlson HA, *et al.* (2010). Binding of a small molecule at a protein-protein interface regulates the chaperone activity of hsp70-hsp40. ACS Chem Biol 5, 611–622.