Article:
Tipping, KW, Van Oosten-Hawle, P, Hewitt, EW et al. (1 more author) (2015) Amyloid fibres: inert end-stage aggregates or key players in disease? Trends in Biochemical Sciences, 40 (12). pp. 719-727. ISSN 0968-0004

https://doi.org/10.1016/j.tibs.2015.10.002

Trends Box

- Amyloid fibres are proteinaceous filaments that form as a consequence of protein misfolding. Their formation is linked to over 50 human diseases, including Parkinson's and Alzheimer's diseases, and type 2 diabetes mellitus.
- Amyloid fibres are structurally polymorphic even when formed from the same sequence. The structure can alter their length distribution, thermodynamic stability, mechanical properties, and biological activity.
- Amyloid fibres play a number of critical roles in disease, facilitating amyloid aggregate transmission, both between cells and, for prion-like species, between individuals.
- Amyloid fibres also sequester core components of the proteostasis network, disrupt membranes, and catalyse or cause the formation of cytotoxic oligomers.
- A comprehensive understanding of amyloid fibre biology will advance us towards our goal of therapeutic intervention.

Amyloid fibres: inert end-stage aggregates or key players in disease?

Kevin W. Tipping, Patricija Van Oosten-Hawle, Eric W. Hewitt and Sheena E. Radford

Astbury Centre for Structural Molecular Biology and School of Molecular and Cellular Biology, The University of Leeds, Leeds, LS2 9JT, United Kingdom

Corresponding author: Radford, S.E. s.e.radford@leeds.ac.uk

Key words: Amyloid, Fibres, Transmission, Disease

Abstract

The formation of amyloid fibres is a hallmark of amyloid disorders. Nevertheless, the lack of correlation between fibre load and disease as observed, for example, in Alzheimer's disease, means fibres are considered secondary contributors to the onset of cellular dysfunction. Instead, soluble intermediates of amyloid assembly are often described as the agents of toxicity. Here, we discuss recent experimental discoveries which suggest that amyloid fibres should be considered as disease-relevant species that can mediate a range of pathological processes. These include disruption of biological membranes, secondary nucleation, amyloid aggregate transmission, and the disruption of protein homeostasis (proteostasis). Thus, a greater understanding of amyloid fibre biology could enhance prospects of developing therapeutic interventions against this devastating class of protein misfolding disorders.

Historical perspective on the role of amyloid fibres in disease

Amyloid diseases are a group of protein misfolding disorders defined by the formation and deposition of insoluble protein fibres with a cross-β fold [1,2]. Although well known for their association with neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases, amyloid fibre formation is involved in a range of human conditions, in which misfolded protein aggregates deposit in a localised or systemic fashion [3]. Despite the increasing incidence of amyloidoses in our ageing population, these disorders are remarkably difficult to prevent or ameliorate, since the myriad of misfolded species that can form during amyloid assembly has precluded the precise identification of the originators of toxicity. In addition, many amyloid diseases are exacerbated by ageing due to the reduced efficiency of the proteostasis machinery. This leads to increased protein misfolding and aggregation events that accelerate the decline in protein homeostasis and enhance susceptibility to amyloid toxicity [4,5].

Since their discovery, the pathological role of amyloid fibres has undergone a shifting view; from the original findings implicating fibres as the causative agent of disease [6], to current opinions, which describe fibres as inert end-stage products of aggregation. Several reasons are responsible for this shift in opinion: i) amyloid fibres, including those composed of amyloid beta $(A\beta)_{1\text{-}40/42}$, α-synuclein, and islet amyloid polypeptide (IAPP), have been shown to display limited toxicity in comparison with oligomeric intermediates of their assembly [7], ii) the conserved cross-β fold of the amyloid fibre core has emerged as an important functional motif in a range of organisms, including prokaryotes, eukaryotes, and even humans [8,9], and iii) amyloid fibres are highly stable thermodynamically, and are amongst the

strongest and stiffest of any known proteinaceous material [10]. Prion fibres, which are structurally homologous to amyloid fibres, are the exception to this current view, and are firmly established as key facilitators of the spreading and infectivity observed in prionopathies [11]. Notably, recent discoveries have also shown that disease-associated amyloid fibres, including fibres formed from Aβ and α-synuclein, facilitate the spreading of amyloid formation *in vivo* [12–14].

Interest in amyloid fibres as disease-relevant agents has undergone a renaissance in recent years. Here, we examine evidence that places amyloid fibres as a key player in the persistence, progression and propagation of amyloid disease through an array of biological activities relevant to the long incubatory periods over which these disorders manifest. Thus, an understanding of amyloid fibre structure, dynamics, and biology may help to unravel the complexities of amyloid disease, and pave the way towards developing successful therapies against these disorders.

Biological membranes and fibre-induced toxicity

Amyloid formation proceeds, most often, when an unfolded or partially folded precursor partitions into an aggregation landscape in which intermolecular contacts drive the formation of multimeric protein complexes (Figure 1). Under 'normal' physiological conditions, the probability that a protein conformer aggregates can be enhanced by changes in the cellular environment. This can occur, for instance, during interactions with lipid bilayers, or changes in pH encountered upon entry into endosomes or lysosomes [15,16]. Mutation or truncation of the polypeptide sequence may also render a previously innocuous protein into an aggregation-prone species [17–19]. A myriad of structurally diverse oligomeric species can form on- or

off-pathway to the low energy minima occupied by amyloid fibres. These oligomers may initiate a cascade of diverse pathological responses [1].

Although oligomers have been shown to act as the primary deleterious aggregate in cell toxicity assays [7,20,21], several investigations have shown that amyloid fibres also possess cytotoxic properties [22]. Moreover, amyloid fibres composed of α-synuclein, β_2m and lysozyme have been shown to exhibit toxicity at low nM concentrations, when taking into consideration particle molarity within a fibrous sample [23,24]. In some instances, lipid membranes may be crucial mediators of fibre-induced cellular damage (Key Figure: Figure 2). For example, amyloid fibres composed of wild-type β_2-microglobulin (β_2m), the causative agent in dialysis-related amyloidosis (DRA), have been shown to interact with, and perturb, the bilayer structure of lipid vesicles (Key Figure: Figure 2A) [25,26]. The pH, the bilayer composition, ligand and co-factor binding, as well as the length of fibres can influence the extent to which these binding events cause membrane damage. For example, mild acidification of the solution conditions and a reduction in fibre length both enhance β_2m fibre-mediated bilayer disruption [24,27,28]. In addition, the presence of bis(monoacylglycero)phosphate (BMP) within the bilayer, a lipid enriched to ~15% of the total lipid composition within endosomal and lysosomal membranes, significantly increases the susceptibility of membranes to β_2m amyloid fibre-induced damage [27].

Other amyloid fibres can also compromise the integrity of synthetic and biological membranes: Fibres composed of polyQ-expanded Huntingtin exon 1 (HttEx1) or α-synuclein, have been shown to bind and cause damage to synthetic liposomes *in*

vitro [23]. Moreover, α-synuclein fibres disrupt intracellular calcium homeostasis by permeabilising cell membranes, leading to the onset of programmed cell death [23]. Other studies have reported that the binding of Aβ fibres to cell membranes inhibits long-term potentiation in mice hippocampal brain slices [29]. In addition, the interaction between Aβ fibres and lipids can lead to the resolubilisation of amyloid fibres into 'reverse' oligomers (Key Figure: Figure 2B) [30]. These oligomers were reported to be indistinguishable from those generated during the formation of amyloid fibres, were toxic to primary neurons, and caused memory impairment in mice models of disease. Thus, amyloid fibre-membrane interactions can expand the structural repertoire of cytotoxic species that can exert aggregate-mediated toxicity.

Direct binding to membranes is not the only mechanism by which fibres can induce membrane damage. Indeed, membrane disruption is caused by the elongation of IAPP fibres on the bilayer surface; hIAPP precursors embed within the membrane and structurally rearrange to form a seeding-competent nucleus [31]. The subsequent elongation of the β-sheet-rich seed leads to bilayer disruption [32]. A recent study also showed that membranes accelerate the rate of primary nucleation of α-synuclein fibres by >1,000 fold [33]. Thus, in addition to serving as platforms for fibre-mediated toxicity, membranes can also increase fibre load.

Secondary nucleation and fibre fragmentation

In addition to damaging membranes, recent studies have uncovered other mechanisms by which amyloid fibres can contribute to disease. For example, fibre surfaces have been shown to catalyse secondary nucleation mechanisms that proliferate the formation of oligomers from $A\beta_{1-42}$, α-synuclein or hIAPP monomers

(Key Figure: Figure 2C) [16,34–36]. Moreover, these secondary nucleation events can be the predominant pathway by which cytotoxic species are generated [35].

Additional secondary processes, such as fibre fragmentation [37], can also augment aggregation by increasing the number of templating fibre ends onto which soluble precursors are added (Key Figure: Figure 2D) [37,38]. Fibre fragmentation also enhances the uptake of amyloid fibres into endosomes and lysosomes, which can have devastating cellular consequences [24]. For example, the increased internalisation of fragmented β_2m fibres disrupts trafficking of membrane proteins to lysosomes and inhibits the capacity of lysosomes to degrade proteins (Key Figure: Figure 2E) [28]. Thus, secondary processes can drive amyloid disease by increasing the probability that fibres gain access to intracellular compartments (fragmentation), or by enhancing the local concentration of deleterious oligomers that form via surface-induced catalysis of nucleation (secondary nucleation). In support of the latter, a recent study showed that the molecular chaperone, BRICHOS, is able to inhibit secondary nucleation reactions catalysed by Aβ fibres and to reduce toxicity [34]. Thus, targeting secondary events may be a promising therapeutic strategy for ameliorating amyloid toxicity.

Transmission and spreading: From cellular toxicity to pathogenic progression in an organism

Perhaps the most significant activity attributed to amyloid fibres is the transmission of amyloid aggregation [11–14,39–41]. By contrast with their well-established toxicity, oligomers are relatively inefficient at propagating aggregation *in vivo*. This was recently demonstrated for α-synuclein aggregates in rat models of

synucleinopathies, whereby mature amyloid fibres, rather than oligomers, caused the most progressive motor impairment and cell death [41]. This phenomenon has been observed for other filamentous species within a multicellular organism. For example, in a *C. elegans* prion model cell-to-cell spreading of amyloid-like species occurs via endocytic uptake of fibrillar material from the extracellular space and is facilitated by autophagy-lysosomal mechanisms [42]. Rather than being degraded, prions accumulate in lysosomal, tubular structures that allow transmission between cells [42].

The immense thermodynamic stability of amyloid fibres may facilitate their enhanced infective capacity over their assembly intermediates by enabling fibres to persist in a variety of cellular environments [10]. Moreover, fibres are more resistant to proteolysis compared with their precursors, which further enhances their longevity in cellular compartments such as lysosomes [15,43]. Secondary processes can also enhance fibre transmissibility; fragmentation of α-synuclein fibres has been shown to lead to the more wide-spread deposition of fibres *in vivo* [44]. How aggregates traverse membrane barriers in order to seed aggregation within the cytosol of neighbouring cells is poorly understood. However, as described in this article, amyloid fibres bind to biological membranes, which may facilitate their penetration into the cytosol via membrane destabilisation and/or disruption.

The endolysosomal pathway is emerging as a common route hijacked by insoluble aggregates in order to transmit aggregation and cause cellular degeneration [28,45,46]. For example, both α-synuclein and polyglutamine expanded aggregates have been shown to be internalised by endocytosis and to seed aggregation within

the cytosol of previously uninfected cells [45,46]. Moreover, the presence of α-synuclein aggregates within endosomes compounds cellular toxicity by inhibiting lysosomal degradation [47]. Strikingly, the inhibition of lysosomal degradation increases the secretion of infective α-synuclein species via exosomes [47]. Thus, the observed targeting of endolysosomal pathways by amyloid fibres constitutes a key mechanism that can explain the basis for intercellular spreading. Phagocytic processes in the intact brain driven by phagocytic glia constitutes another key access route that allows spreading of pathogenic protein aggregates as was recently shown in a Drosophila Huntington disease model [48] . Glial phagocytosis is usually a neuro-protective clearance mechanism that allows removal of extracellular protein aggregates [49,50]. However, uptake of mutant huntingtin aggregates with a 91 Q expansion (HttQ91) from affected neuronal axons by glial cells in close proximity can induce prion-like conversion of otherwise soluble HttQ25 proteins in the glial cell cytoplasm, and thus add to the systemic toxicity associated with amyloid fibre formation observed in cellular and whole-animal studies (Figure 3).

Fibres as reservoirs of toxic oligomers

Most of the biological activities of amyloid fibres discussed thus far can be attributed to a fibre as a static entity. However, despite their formidable thermodynamic stability, fibres are dynamic and readily exchange subunits within the soluble milieu (Key Figure: Figure 2F) [51–53]. Although 'molecular shedding' is slow under conditions favouring fibre formation (~$1x10^{-4}.s^{-1}$, as determined for $A\beta_{40}$ and SH3 domain fibres [51,53]), such activity is likely to be biologically relevant, as suggested by the oligomeric halo surrounding Aβ fibrillar plaques in mice models of Alzheimer's disease [54]. The extent of molecular shedding can also be enhanced by changes in

the pH. As shown for β_2m fibres, a reduction in pH by a single unit (from pH 7.4 – 6.4) causes the shedding of oligomers ~30 nm in diameter that are not observed by incubating fibres at pH 7.4, nor during fibre formation at pH 2 [55,56]. In order to cause deleterious activity, β_2m fibres must first be internalised from the extracellular milieu [24,27,28]. The reduction in pH encountered by internalised β_2m fibres during endosome maturation, coupled with the increase in BMP content within the bilayer, may create an environment uniquely susceptible to β_2m fibre-mediated cellular dysfunction, by enhancing the release of membrane-active oligomers. For other fibres, the rate of molecular shedding can be influenced by protein sequence; the removal of the C-terminal two residues from Aβ_{1-42} enhances the rate of molecular shedding more than 60-fold [57].

The importance of molecular shedding in contributing to amyloid toxicity is supported by the amelioration of toxicity upon restricting fibre dynamics For example, the molecular chaperones clusterin, αB-crystallin, hsp70, or hsc70 retard the rate of molecular shedding and reduce toxicity upon binding to Aβ, β_2m or α-synuclein fibres, respectively [55,58,59]. Non-covalently bound small molecules may also be able to reduce molecular shedding of Aβ_{16-21} fibres [60]. Conversely, the human cytosolic hsp70 chaperone system involving hsc70, its co-chaperone DNAJB1 and nucleotide exchange factor Apg2 have been shown to fragment and disaggregate α-synuclein amyloid fibres into monomers in an ATP-dependent mechanism leading to reduced toxicity [61]. Thus, preventing shedding of toxic species from amyloid fibres, or resolubilizing amyloid fibres to non-toxic species including monomers, may be useful therapeutic strategies for treating a range of amyloid disorders.

The amyloid strain phenomenon

The concept of amyloid polymorphism and hence amyloid 'strains' adds to the complexity of understanding the development and progression of disease in different individuals. Fibres formed from familial variants associated with early-onset phenotypes of amyloid disease could, for example, favour the formation of a structurally distinct fibre that exhibits a distinct dynamic signature from its wild-type counterpart. Such structural differences between amyloid fibre 'strains', which may be compounded by the genetic diversity in humans and the conditions under which amyloid formation proceeds, (Figure 1) may contribute to the unpredictable disease progression observed in individuals suffering from the same amyloid disorder [41,62]. For example, different α-synuclein fibre 'strains' have been shown to exhibit specific toxicities and prion-like transmissible properties, suggesting that amyloid fibre conformation can influence cell-to-cell aggregate transmission and disease progression [41,63]. Such conformation-dependent fibre toxicities have also been reported for $A\beta_{1\text{-}40}$ [64]. A recent analysis using solid state NMR showed that single fibre polymorphs are faithfully propagated within the brain of Alzheimer's disease patients. Thus, distinct fibre 'strains' may facilitate differences in disease presentation, and, in some instances, may explain the lack of correlation between plaque load and disease progression [62].

Fibres as biological 'black holes'

While components of the proteostasis network (PN) have shown powerful inhibitory activity against amyloid toxicity, the unsolicited sequestration of proteins by amyloid fibres can also have catastrophic consequences. For example, fibres formed

intracellularly from *de novo* designed aggregation-prone polypeptides have been shown to sequester a wide range of cellular proteins [65]. These include molecular chaperones, as well as proteins involved in multiple cellular processes (Figure 3). Thus, fibres destabilise the PN by trapping vital components in dead-end interactions [66]. More specifically, insoluble intracellular deposits of β-sheet rich proteins sequester DnaJB1, an hsp40 co-chaperone, which is involved in transport of cytosolic misfolded proteins into the nucleus for proteasomal degradation by the ubiquitin-proteasome system (UPS) [65,67]. The enrichment of DnaJB1 within intracellular protein aggregates in turn inhibits the correct trafficking and degradation of natural misfolded substrates [67]. This leads to the proliferation of potentially toxic, non-amyloidogenic aggregates in the cell, thereby critically overloading the cellular capacity of the PN (Figure 3). A major contributing factor to this deleterious activity is the interaction of amyloid fibres with hydrophobic and flexible regions of pre-existent and newly synthesized proteins [65]. Therefore, in addition to sequestering key components of the PN, amyloid aggregates magnify their toxic and pathogenic potential by increasing the misfolding of already metastable components of the cellular proteome.

Whereas the discussed findings illustrate how amyloid species disrupt the PN within cells and at a systemic level, recent discoveries made in invertebrate model organisms suggest that the PN itself has evolved to communicate local disturbances between cells and tissues [68]. Thus, in the context of an organism, the PN is organized in a cell nonautonomous manner and has transcellular stress response mechanisms at place that can counteract systemic disease progression. Pre-emptive activation of such transcellular stress responses may hold the potential for more

effective therapeutic strategies against disease progression and systemic toxic potential caused by amyloid fibres (Figure 3).

Concluding remarks

Here, we have summarized compelling evidence illustrating that amyloid fibres should not be considered as inert products of aggregation, but rather as important facilitators of the cellular degeneration and propagation of aggregation observed in amyloid disease. Thus, the idea that oligomers are the primary progenitor of amyloid disease needs to be revisited, and indeed all potential misfolded species, including monomers, oligomers and fibres themselves, should be recognised as potential contributors to amyloid disease.

The study of amyloid fibre structure, dynamics, and biology has helped to illuminate the complex nature of amyloid diseases. Many key questions remain, including how do unique fibre 'strains' cause different biological effects by modulating fibre stability and dynamics, as well as the fibre 'interactome' (Outstanding Questions)? A better understanding of the degenerative effects that unique fibre morphologies can elicit will not only strengthen our understanding of the fundamental biology of amyloid disease, but further our goal of therapeutic intervention in this devastating group of protein-misfolding diseases.

Acknowledgements

The authors thank members of their groups for stimulating discussions. This work was supported by the Wellcome Trust (093794/Z/10/Z, K.W.T; 092896, E.W.H & S.E.R), and the European Research Council (ERC) ((FP7/2007-2013)/ERC Grant Agreement 32240, E.W.H & S.E.R).

Reference list

1 Knowles, T.P.J. *et al.* (2014) The amyloid state and its association with protein misfolding diseases. *Nat. Rev. Mol. Cell Biol.* 15, 384–396

2 Jahn, T.R. *et al.* (2010) The common architecture of cross-beta amyloid. *J. Mol. Biol.* 395, 717–727

3 Sipe, J. *et al.* (2012) Amyloid fibril protein nomenclature: 2012 recommendations from the Nomenclature Committee of the International Society of Amyloidosis. *Amyloid* 19, 167–170

4 Douglas, P.M. and Dillin, A. (2010) Protein homeostasis and aging in neurodegeneration. *J. Cell Biol.* 190, 719–729

5 Morimoto, R. (2008) Proteotoxic stress and inducible chaperone networks in neurodegenerative disease and aging. *Genes Dev.* 22, 1427–1438

6 Glenner, C. *et al.* (1971) Amyloid fibril proteins: Proof of homology with immunoglobulin light chains by sequence analyses. *Science* 172, 1150–1151

7 Kayed, R. *et al.* (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science* 300, 486–489

8 Blanco, L.P. *et al.* (2012) Diversity, biogenesis and function of microbial amyloids. *Trends Microbiol.* 20, 66–73

9 Maji, S.K. *et al.* (2009) Functional amyloids as natural storage of peptide hormones in pituitary secretory granules. *Science* 325, 328–332

10 Knowles, T.P. *et al.* (2007) Role of intermolecular forces in protein nanofibrils. *Science* 318, 1900–1903

11 Westermark, G.T. and Westermark, P. (2010) Prion-like aggregates: infectious agents in human disease. *Trends Mol. Med.* 16, 501–507

12 Goedert, M. (2015) Alzheimer's and Parkinson's diseases: The prion concept in relation to assembled amyloid-β, tau, and α-synuclein. *Science* 349, 12555

13 Jaunmuktane, Z. *et al.* (2015) Evidence for human transmission of amyloid-β pathology and cerebral amyloid angiopathy. *Nature* 525, 247–250

14 Prusiner, S.B. *et al.* (2015) Evidence for α-synuclein prions causing multiple system atrophy in humans with parkinsonism. *Proc. Natl. Acad. Sci. U.S.A.* 112, E5308–E5317

15 Teng, J. *et al.* (2014) An animal model of glomerular light-chain-associated amyloidogenesis depicts the crucial role of lysosomes. *Kidney Int.* 86, 738–746

16 Buell, A.K. *et al.* (2014) Solution conditions determine the relative importance of nucleation and growth processes in α-synuclein aggregation. *Proc. Natl. Acad. Sci. U.S.A.* 111, 7671–7676

17 Ramirez-Alvarado, M. *et al.* (2000) A systematic exploration of the influence of the protein stability on amyloid fibril formation in vitro. *Proc. Natl. Acad. Sci. U.S.A.* 97, 8979–8984

18 Buell, A.K. *et al.* (2011) Population of nonnative states of lysozyme variants drives amyloid fibril formation. *J. Am. Chem. Soc.* 133, 7737–7743

19 Eichner, T. and Radford, S.E. (2011) A diversity of assembly mechanisms of a generic amyloid fold. *Mol. Cell* 43, 8–18

20 Winner, B. *et al.* (2011) In vivo demonstration that α-synuclein oligomers are toxic. *Proc. Natl. Acad. Sci. U.S.A.* 108, 4194–4199

21 Bucciantini, M. *et al.* (2002) Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. *Nature* 416, 507–511

22 Pike, C.J. *et al.* (1991) In vitro aging of beta-amyloid protein causes peptide aggregation and neurotoxicity. 563, 311–314

23 Pieri, L. *et al.* (2012) Fibrillar α-synuclein and huntingtin exon 1 assemblies are toxic to the cells. *Biophys. J.* 102, 2894–2905

24 Xue, W.-F. *et al.* (2009) Fibril fragmentation enhances amyloid cytotoxicity. *J. Biol. Chem.* 284, 34272–34282

25 Milanesi, L. *et al.* (2012) Direct three-dimensional visualization of membrane disruption by amyloid fibrils. *Proc. Natl. Acad. Sci. U.S.A.* 109, 20455–20460

26 Sheynis, T. *et al.* (2013) Aggregation modulators interfere with membrane interactions of β_2-microglobulin fibrils. *Biophys. J.* 105, 745–55

27 Goodchild, S.C. *et al.* (2014) β_2-microglobulin amyloid fibril-induced membrane disruption is enhanced by endosomal lipids and acidic pH. *PLoS One* 9, e 104492

28 Jakhria, T. *et al.* (2014) β_2-microglobulin amyloid fibrils are nanoparticles that disrupt lysosomal membrane protein trafficking and inhibit protein degradation by lysosomes. *J. Biol. Chem.* 289, 35781–35794

29 Nicoll, A.J. *et al.* (2013) Amyloid-β nanotubes are associated with prion protein-dependent synaptotoxicity. *Nat. Commun.* 4, e2416

30 Martins, I.C. *et al.* (2008) Lipids revert inert Abeta amyloid fibrils to neurotoxic protofibrils that affect learning in mice. *EMBO J.* 27, 224–233

31 Lee, C.-C. *et al.* (2012) How type II diabetes-related islet amyloid polypeptide damages lipid bilayers. *Biophys. J.* 102, 1059–1068

32 Engel, M.F.M. *et al.* (2008) Membrane damage by human islet amyloid polypeptide through fibril growth at the membrane. *Proc. Natl. Acad. Sci. U.S.A.* 105, 6033–6038

33 Galvagnion, C. *et al.* (2015) Lipid vesicles trigger α-synuclein aggregation by stimulating primary nucleation. *Nat. Chem. Biol.* 11, 229–234

34 Cohen, S.I. a *et al.* (2015) A molecular chaperone breaks the catalytic cycle that generates toxic Aβ oligomers. *Nat. Struct. Mol. Biol.* 22, 208–213

35 Cohen, S.I. *et al.* (2013) Proliferation of amyloid-β42 aggregates occurs through a secondary nucleation mechanism. *Proc. Natl. Acad. Sci. U.S.A.* 110, 9758–9763

36 Ruschak, A.M. and Miranker, A.D. (2007) Fiber-dependent amyloid formation as catalysis of an existing reaction pathway. *Proc. Natl. Acad. Sci. U.S.A.* 104, 12341–12346

37 Xue, W.-F. *et al.* (2008) Systematic analysis of nucleation-dependent polymerization reveals new insights into the mechanism of amyloid self-assembly. *Proc. Natl. Acad. Sci. U.S.A.* 105, 8926–8931

38 Knowles, T.P.J. *et al.* (2009) An analytical solution to the kinetics of breakable filament assembly. *Science* 326, 1533–1537

39 Hamaguchi, T. *et al.* (2012) The presence of Abeta seeds, and not age per se, is critical to the initiation of Abeta deposition in the brain. *Acta Neuropathol.* 123, 31–37

40 Mougenot, A.-L.J. *et al.* (2011) Transmission of prion strains in a transgenic mouse model overexpressing human A53T mutated α-synuclein. *J. Neuropathol. Exp. Neurol.* 70, 377–385

41 Peelaerts, W. *et al.* (2015) α-Synuclein strains cause distinct synucleinopathies after local and systemic administration. *Nature* 522, 340–344

42 Nussbaum-Krammer, C.I. *et al.* (2013) Spreading of a prion domain from cell-to-cell by vesicular transport in Caenorhabditis elegans. *PLoS Genet.* 9, e1003351

43 Morten, I.J. *et al.* (2007) Investigation into the role of macrophages in the formation and degradation of β_2-microglobulin amyloid fibrils. *J. Biol. Chem.* 282, 29691–26700

44 Aulić, S. *et al.* (2014) Defined α-synuclein prion-like molecular assemblies spreading in cell culture. *BMC Neurosci.* 15, 69

45 Ren, P.-H. *et al.* (2009) Cytoplasmic penetration and persistent infection of mammalian cells by polyglutamine aggregates. *Nat. Cell Biol.* 11, 219–225

46 Desplats, P. *et al.* (2009) Inclusion formation and neuronal cell death through neuron-to-neuron transmission of α-synuclein. *Proc. Natl. Acad. Sci. U.S.A.* 106, 13010–13015

47 Danzer, K.M. *et al.* (2012) Exosomal cell-to-cell transmission of α-synuclein oligomers. *Mol. Neurodegener.* 7, 42

48 Pearce, M.M.P. *et al.* (2015) Prion-like transmission of neuronal huntingtin aggregates to phagocytic glia in the Drosophila brain. *Nat. Commun.* 6, 6768

49 Bae, E.-J. *et al.* (2012) Antibody-aided clearance of extracellular α-synuclein prevents cell-to-cell aggregate transmission. *J. Neurosci.* 32, 13454–69

50 DeMattos, R.B. *et al.* (2012) A plaque-specific antibody clears existing β-amyloid plaques in alzheimer's disease mice. *Neuron* 76, 908–920

51 Carulla, N. *et al.* (2005) Molecular recycling within amyloid fibrils. *Nature* 436, 554–558

52 Cremades, N. *et al.* (2012) Direct observation of the interconversion of normal and toxic forms of α-synuclein. *Cell* 149, 1048–1059

53 Narayan, P. *et al.* (2012) The extracellular chaperone clusterin sequesters oligomeric forms of the amyloid-β(1-40) peptide. *Nat. Struct. Mol. Biol.* 19, 79–83

54 Koffie, R.M. *et al.* (2009) Oligomeric amyloid beta associates with postsynaptic densities and correlates with excitatory synapse loss near senile plaques. *Proc. Natl. Acad. Sci. U.S.A.* 106, 4012–4017

55 Tipping, K.W. *et al.* (2015) pH-induced molecular shedding drives the formation of amyloid fibril-derived oligomers. *Proc. Natl. Acad. Sci. U.S.A.* 112, 5691–5696

56 Smith, D.P. *et al.* (2010) Elongated oligomers in β_2-microglobulin amyloid assembly revealed by ion mobility spectrometry-mass spectrometry. *Proc. Natl. Acad. Sci. U.S.A.* 107, 6794–6798

57 Sánchez, L. *et al.* (2011) Aβ40 and Aβ42 amyloid fibrils exhibit distinct molecular recycling properties. *J. Am. Chem. Soc.* 133, 6505–6508

58 Narayan, P. *et al.* (2012) Amyloid-β oligomers are sequestered by both intracellular and extracellular chaperones. *Biochemistry* 51, 9270–9276

59 Pemberton, S. *et al.* (2011) Hsc70 protein interaction with soluble and fibrillar alpha-synuclein. *J. Biol. Chem.* 286, 34690–34699

60 Jiang, L. *et al.* (2013) Structure-based discovery of fiber-binding compounds that reduce the cytotoxicity of amyloid beta. *Elife* 2, e00857

61 Huang, C. *et al.* (2006) Heat shock protein 70 inhibits alpha-synuclein fibril formation via interactions with diverse intermediates. *J. Mol. Biol.* 364, 323–336

62 Lu, J.-X. *et al.* (2013) Molecular structure of β-amyloid fibrils in Alzheimer's disease brain tissue. *Cell* 154, 1257–1268

63 Bousset, L. *et al.* (2013) Structural and functional characterization of two alpha-synuclein strains. *Nat. Commun.* 4, 2575

64 Petkova, A.T. *et al.* (2005) Self-propagating, molecular-level polymorphism in Alzheimer's β-amyloid fibrils. *Science* 307, 262–265

65 Olzscha, H. *et al.* (2011) Amyloid-like aggregates sequester numerous metastable proteins with essential cellular functions. *Cell* 144, 67–78

66 Cohen, E. *et al.* (2009) Reduced IGF-1 signaling delays age-associated proteotoxicity in mice. *Cell* 139, 1157–1169

67 Park, S.-H. *et al.* (2013) PolyQ proteins interfere with nuclear degradation of cytosolic proteins by sequestering the Sis1p chaperone. *Cell* 154, 134–145

68 Oosten-Hawle, P. van and Morimoto, R. (2014) Organismal proteostasis: role of cell-nonautonomous regulation and transcellular chaperone signaling. *Genes Dev.* 28, 1533–1543

Figure 1 The energy landscapes of protein folding and aggregation.

Folding of proteins into their functional, native states takes place via the formation of metastable folding intermediates *en route* to the low energy native state (blue). Under certain conditions, such as changes in pH, or upon alteration of polypeptide sequence, protein misfolding can occur more frequently (red polypeptides). This creates aggregation-competent monomers that partition into parallel 'misfolding' amyloid landscapes (red and yellow). The formation of amyloid is driven by intermolecular contacts and generates an array of multimeric protein complexes (oligomers) that precede the formation of highly-ordered, low-energy structures known as amyloid fibres. Depending upon the sequence or upon the conditions under which aggregation takes place (condition/sequence A or condition/sequence B), the ruggedness of the folding and amyloid landscapes can be altered. This can affect the probability of molecules misfolding, and entering the aggregation landscapes (not depicted here), and/or the stability and structure of fibres and oligomers formed (red and yellow landscapes). These alterations change the ability of an amyloid fibre, and of all species accessible within the particular landscape, to cause cellular dysfunction and degeneration.

Key Figure. Figure 2 Amyloid fibres can elicit different biological activities.

Fibres can (A) bind directly to lipid bilayers, causing deformations and membrane depolarisation (as indicated by red arrows). Similarly, (B) fibre:membrane interactions may promote the dissolution of fibres into cytotoxic oligomers that, among other activities, may also cause membrane disruption. (C) Fibre surfaces are

also catalytically active and can convert monomeric precursors into aggregation-competent structures, while (D) fibre ends can consume aggregation competent species via templated elongation. (E) Fragmentation enhances fibre activity by increasing the ability of fibres to be internalised into the endosomal pathway. (F) Amyloid fibres can also undergo molecular shedding, whereby oligomeric species dissociate from fibre ends to generate a localised pool of potentially toxic oligomers.

Figure 3 Proteostasis components affected by amyloid fibres and protective mechanisms.

Intracellular components of the proteostasis network are impaired by the presence of amyloid fibres in the cytosol (yellow). Trafficking components are hijacked by amyloid species to facilitate intercellular spreading between different cells. Multicellular organisms may have developed defence strategies that allow cell nonautonomous activation of protective stress responses by transcellular stress signalling and components of the organismal proteostasis network.

Outstanding Questions

- How does amyloid structure relate to disease pathology and progression? An array of structural polymorphisms is potentially accessible to aggregation-prone polypeptides. Understanding and identifying how distinct structural motifs drive ‘strain’-dependent toxicities may allow the progression of disease to become more predictable.
- Do different fibre morphologies have distinct interactomes? The trapping of vital cellular components by fibres can lead to the collapse of the proteostasis network. Identifying the interactions made with individual fibre morphologies could elucidate plausible points of intervention for targeted therapeutic strategies.
- What makes a fibril benign or toxic? The ‘strain’ phenomenon has the potential to explain why infectivity and fibre formation can be distinct processes. Some fibre polymorphs may possess a greater capacity to catalyze secondary nucleation events, or undergo fragmentation, for example, and thus increase toxicity over other fibre morphologies.
- How do amyloid fibres infect and spread between cells? Fibres are known to be able to access the cytosol when added extracellularly, but the mechanism by which this occurs remains poorly understood.

Figure 1

Click here to download Figure figure 1.jpg

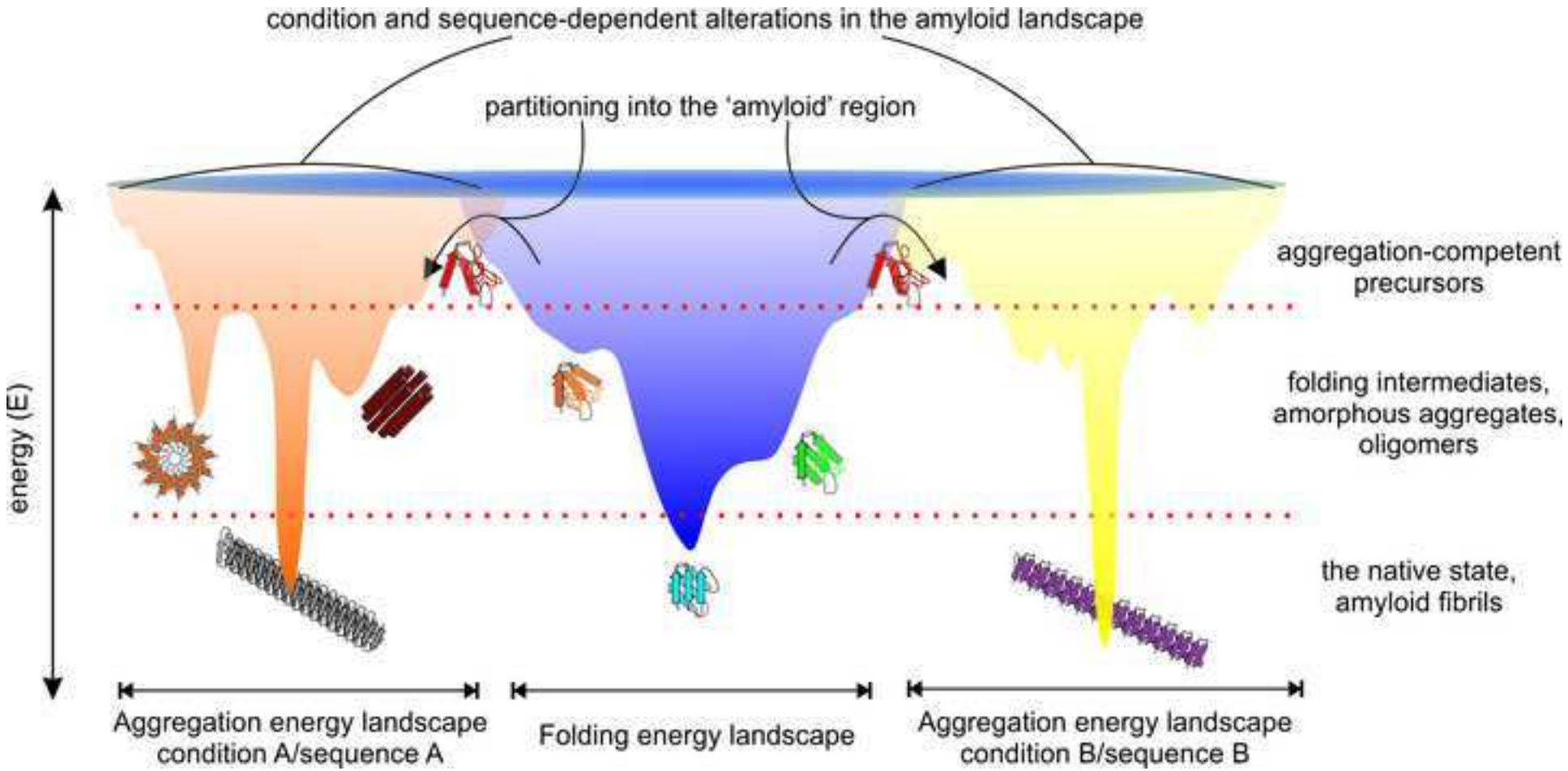
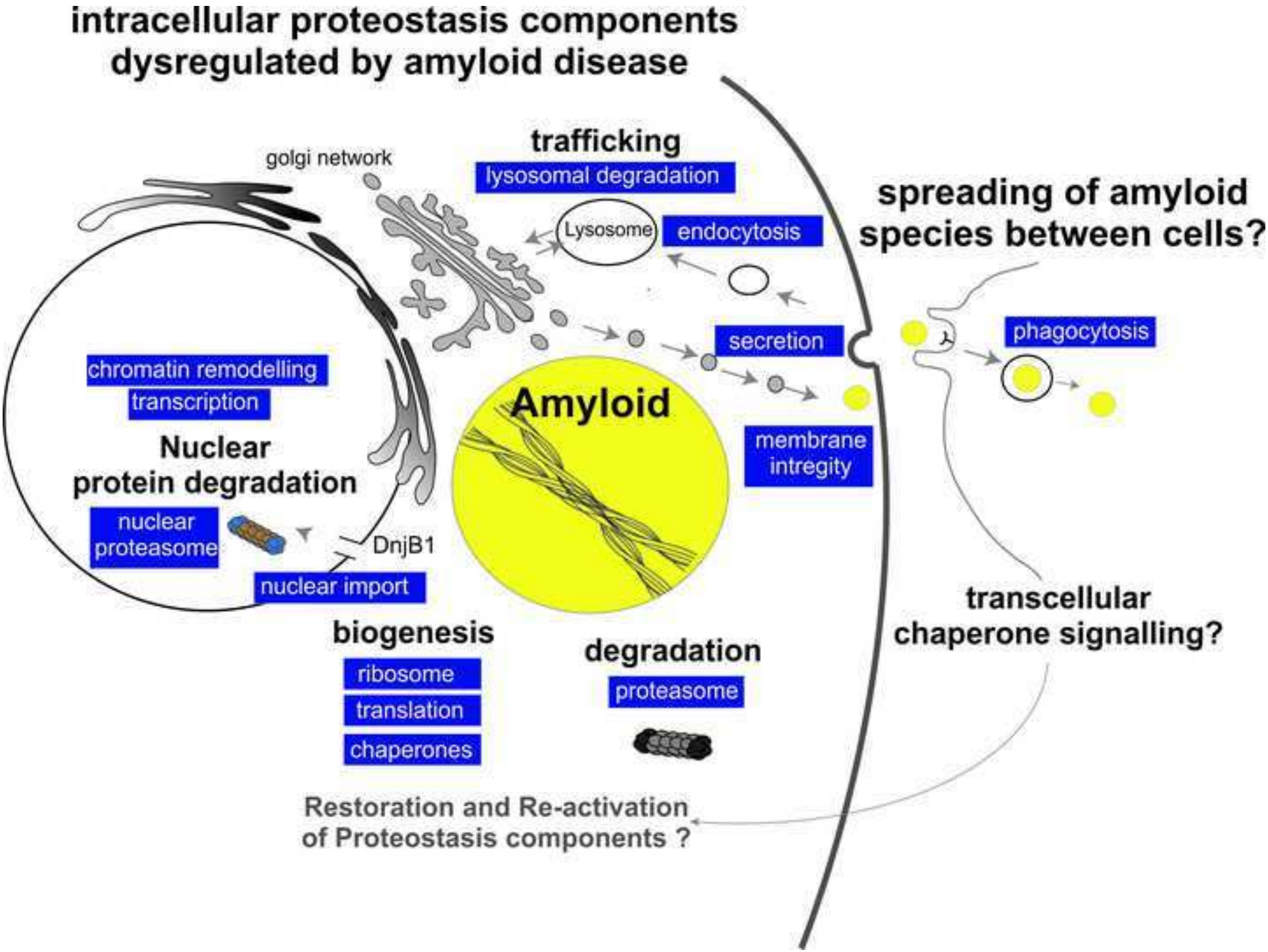


Figure 3

Click here to download Figure Figure 3 .jpg



Key Figure Figure 2 Click here to download Key Figure figure 2.jpg