



UNIVERSITY OF LEEDS

This is a repository copy of *Assessing the causes and consequences of co-polymerization in amyloid formation*.

White Rose Research Online URL for this paper:
<http://eprints.whiterose.ac.uk/80193/>

Version: Published Version

Article:

Sarell, CJ, Stockley, PG and Radford, SE (2013) Assessing the causes and consequences of co-polymerization in amyloid formation. *Prion*, 7 (5). 359 - 368. ISSN 1933-6896

<https://doi.org/10.4161/pri.26415>

Reuse

Unless indicated otherwise, fulltext items are protected by copyright with all rights reserved. The copyright exception in section 29 of the Copyright, Designs and Patents Act 1988 allows the making of a single copy solely for the purpose of non-commercial research or private study within the limits of fair dealing. The publisher or other rights-holder may allow further reproduction and re-use of this version - refer to the White Rose Research Online record for this item. Where records identify the publisher as the copyright holder, users can verify any specific terms of use on the publisher's website.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



eprints@whiterose.ac.uk
<https://eprints.whiterose.ac.uk/>

Assessing the causes and consequences of co-polymerization in amyloid formation

Claire J Sarell, Peter G Stockley, and Sheena E Radford*

Astbury Centre for Structural Molecular Biology and School of Molecular and Cellular Biology; University of Leeds; Leeds, UK

How, and why, different proteins form amyloid fibrils is most often studied *in vitro* using a single purified protein sequence. However, many amyloid diseases involve co-aggregation of different protein species, including proteins with/without post-translational modifications (e.g., different strains of PrP), proteins of different length (e.g., β_2 -microglobulin and Δ N6, A β 40, and A β 42), sequence variants (e.g., A β and A β_{ARC}), and proteins from different organisms (e.g., bovine PrP and human PrP). The consequences of co-aggregation of different proteins upon the structure, stability, species transmission and toxicity of the resulting amyloid aggregates is discussed here, including the role of co-aggregation in expanding the repertoire of oligomeric and fibrillar structures and how this can affect their biological and biophysical properties.

Introduction

It has been well documented that amyloid fibrils can be highly polymorphic, even when formed from proteins with the same amino acid sequence under the same incubation conditions.¹ This polymorphism can be manifested through the formation of fibrils with different numbers and orientations of protofilaments, and also within the protofilament substructure, for example in the residues that contribute to the β -strand segments within the fibrils (reviewed in refs. 2–3). In a comprehensive study of an 11-residue transthyretin (TTR) peptide the structure and orientation of protofilaments

from three different fibril polymorphs was described in atomic detail using cryo-electron microscopy (EM), scanning transmission EM and magic angle spinning (MAS) NMR, revealing different numbers of protofilaments within each polymorph.⁴ The different packing arrangements available for an amyloid fibril core has also been explored using X-ray crystallography of 4–7-residue peptides from a variety of amyloidogenic proteins, the results revealing eight classes of polymorphs arranged as different steric zippers.⁵ The structures of the steric zippers differ in whether the β -strands are parallel or anti-parallel, whether the β -sheets pack face-to-face or back-to-back, and whether the sheets orientate themselves up or down relative to each other⁶ (see Fig. 1A).

Oligomeric intermediates of amyloid formation can also show polymorphism. HypF-N is the 91-residue N-terminal domain of HypF, a carbamoyl transferase in *Escherichia coli*.⁷ In a recent study, oligomers of HypF-N formed under different solution conditions were shown to be similar, morphologically and tinctorially, but differed in their ability to cause cell dysfunction.⁸ Further investigation using site-specific labeling with pyrene maleimide and binding of the dye 8-anilino-naphthalene-1-sulfonic acid (ANS) demonstrated that the oligomers that possess less structural order and expose more hydrophobic surface area are more toxic than oligomers with a more ordered core. The increased toxicity of one oligomer morphology over the other is thought to arise because the structural flexibility and hydrophobic exposure of the toxic oligomers allows them to cross the hydrophobic

Keywords: amyloid, fibril, seeding, heteropolymers, polymorph, strain

Submitted: 08/12/2013

Accepted: 09/07/2013

<http://dx.doi.org/10.4161/pri.26415>

*Correspondence to: Sheena E Radford;
Email: s.e.radford@leeds.ac.uk

Extra View to: Sarell CJ, Woods LA, Su Y, Debelouchina GT, Ashcroft AE, Griffin RG, Stockley PG, Radford SE. Expanding the repertoire of amyloid polymorphs by co-polymerization of related protein precursors. *J Biol Chem* 2013; 288:7327–37; PMID:23329840; <http://dx.doi.org/10.1074/jbc.M112.447524>

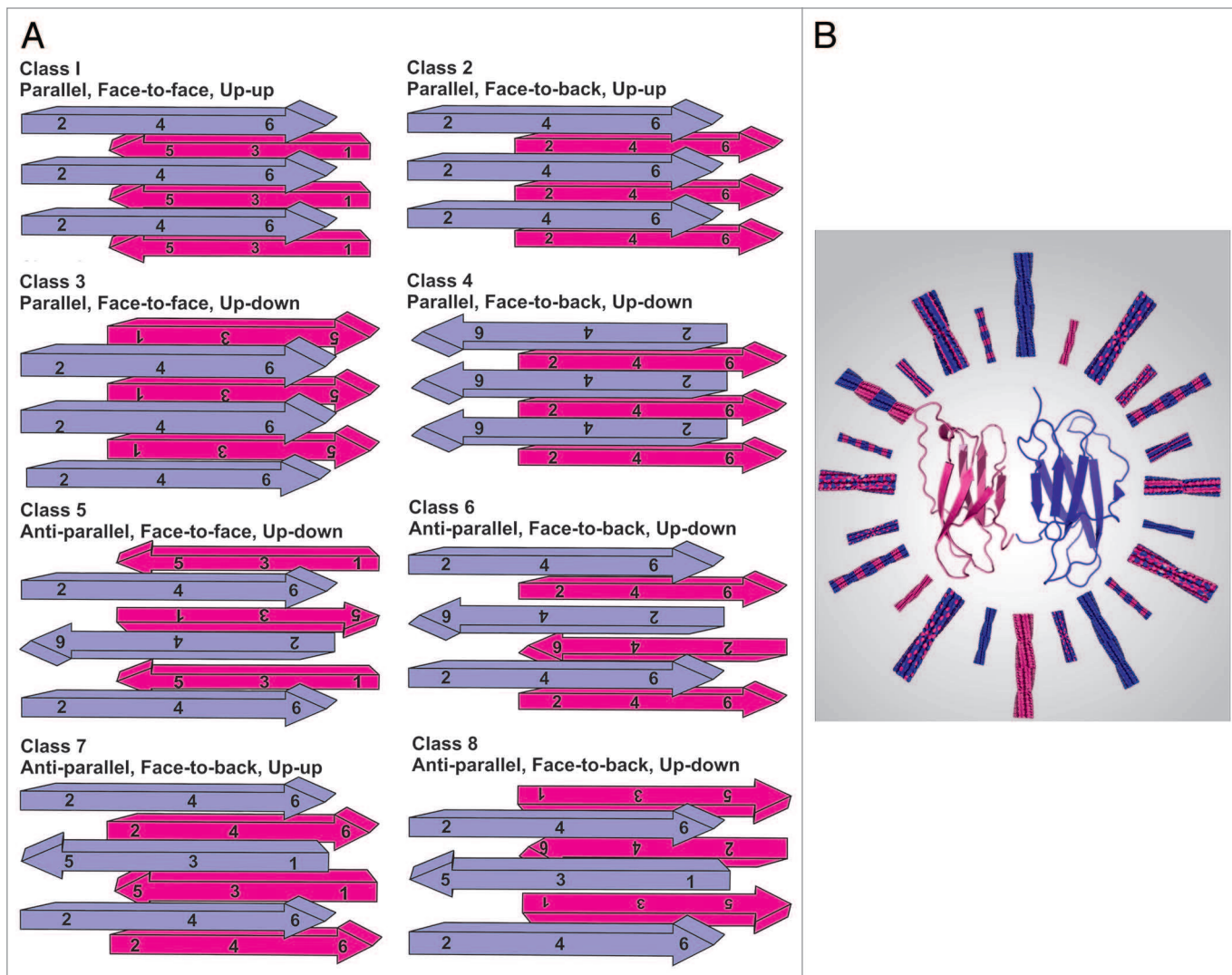


Figure 1. The potential repertoire of fibril polymorphs. **(A)** The eight classes of steric zipper available to a single polypeptide chain (re-drawn from Figure 4 of Sawaya et al.).⁵ **(B)** A schematic of different possible fibril polymorphs that can arise from co-aggregation of more than one protein (depicted are Δ N6 [PDB code 2XKU] in pink and β_2 m [PDB code 2XKS] in purple).²⁰

bilayer of the cell membrane, whereas the non-toxic oligomers can bind to, but not cross the membrane. In other examples the more structured oligomers were shown to be the most toxic.⁹ These examples, and many others,¹⁰⁻¹² show the ability of a single polypeptide sequence to adopt an array of oligomeric structures with differing physical, chemical, biophysical and biological functions, even for species of similar molecular weight. This poses significant challenges for the identification and structural characterization of each individual species in the amyloid cascade and in amyloid disease.

The above studies demonstrate that there is a large range of possible

polymorphic structures available to an amyloidogenic protein or peptide both as oligomeric intermediates in fibril formation and in the final fibrillar form. The potential assortment of polymorphs can be greatly expanded when mixtures of proteins can co-aggregate, either de novo or via cross-seeding. The resulting increased range of fibril structures expands the repertoire of aggregation propensities and amyloid stabilities. This can be useful, for example by providing enhanced opportunity for tailoring the physical properties of amyloid fibrils for use as nanomaterials. However, it may also be detrimental, for example, by increasing the possibility for toxicity and disease.

Here we discuss the repertoire of amyloid polymorphs formed from different protein and peptide sequences, (the interactions between amyloidogenic proteins and other important factors in amyloid fibril formation, such as chaperones, small molecules, metal ions, lipids, and glycolipids, are not included).¹³ We identify three different scenarios that can affect the likelihood of two proteins cross-seeding and/or co-polymerizing with each other. First, we discuss the requirements of sequence in determining co-aggregation propensity and its outcomes. Second, we describe scenarios in which one protein increases the amyloidogenic propensity of another. Finally, we review the extent to which

both proteins need to have the ability to adopt a similar fibril structure in order to co-assemble. A select number of studies are discussed in detail in each section, and a more comprehensive list of protein-protein interactions that expand the repertoire of fibril polymorphs is included in Table 1.

Condition 1: Co-Aggregation of Proteins/Peptides with Similar Primary Sequence

The intermolecular interactions that stabilize amyloid fibrils involve the peptide backbone and thus the ability to form fibrils is considered to be a generic property of all polypeptide chains.¹⁴ However side-chain interactions play a crucial role in the formation of amyloid fibrils and in determining the ability of one protein to seed polymerization of another. The importance of amino acid sequence similarity in cross-seeding (adding fibrillar seeds of one polypeptide sequence to monomers of a second polypeptide sequence) was shown in a study of hen lysozyme (Row 1, Table 1).¹⁵ Fibrillar seeds created from sequences that are 99.2% (I55T mutational variant of hen lysozyme) or 95% identical (turkey lysozyme) to that of the hen lysozyme monomer produced identical seeding behavior to homologous seeding. A seed with a sequence that is 60% identical to the hen lysozyme monomer (human lysozyme) showed faster fibril elongation than the rate of fibril formation of the unseeded monomer, but with a lag phase that is increased compared with self-seeding. Sequences with 36% identity or no identity to the hen lysozyme monomer had no effect on fibril formation.¹⁵ Thus the efficiency of seeding depends on the similarity between both the seed's and monomer's amino acid sequence.¹⁵

Similar primary sequences between amyloidogenic proteins may promote the likelihood of a cross-seeding event occurring, but this does not always result in a reciprocal ability for both partners to cross-seed each other's assembly. Islet amyloid polypeptide (IAPP), the amyloidogenic peptide involved in type II diabetes mellitus, is derived from the 89 amino acid precursor protein pro-islet amyloid polypeptide. A β , the neurotoxic

agent in Alzheimer disease (AD), is derived from the proteolytic processing of amyloid precursor protein which, depending on the splicing isoform, is composed of 365 to 770 amino acids. These precursor proteins are unrelated in sequence and have no obvious functional relationship. Despite this, residues 15–37 of A β (residues involved in the amyloid core of A β fibrils¹⁶) share 39% sequence identity and 65% sequence similarity with residues 10–33 of IAPP. The interaction between IAPP and A β 40 was studied to determine whether cross-seeding could occur.¹⁷ These *in vitro* studies showed that A β 40 fibrils, IAPP fibrils, and IAPP amorphous aggregates are all equally effective at seeding elongation with IAPP monomer. Conversely, IAPP aggregates will not cross-seed polymerization of A β 40, and IAPP fibrils are much less efficient at seeding A β 40 monomer compared with A β 40 fibrils, exhibiting only 2% of the seeding efficiency of A β fibrils on a weight basis.¹⁷ Thus, there is a surprising lack of equivalence in cross-seeding of A β 40 and IAPP (Row 2, Table 1).

The importance of primary sequence in determining the ability of proteins to co-aggregate is more complex than both partners simply having amyloid-prone primary sequences. In one example, the ability of a variety of different amyloid fibrils to act as a seed for elongation with A β 40 was compared with the efficiency of homologous seeding (A β 40 seeds).¹⁷ The majority of fibril types, including fibrils formed from the yeast protein Ure2p and the human protein β_2 -microglobulin (β_2 m), were as inefficient at cross-seeding A β 40 as non-amyloid protein aggregates such as collagen or denatured ovalbumin (Row 3 of Table 1). Thus simply being amyloidogenic does not necessarily result in an ability to seed elongation with other non-homologous amyloidogenic proteins; sequence similarity is required as well.

Sequence similarity is not the only important factor in determining the propensity for co-aggregation in amyloid formation as the precise positioning of compatible residues within the structure also plays a significant role. For example, co-incubation of murine and human A β 40 and A β 42 peptides revealed that interspecies fibrils will form for both

A β 40 and A β 42 alloforms (Row 4, Table 1)¹⁸ despite the three residue difference in the mouse and human amino acid sequences. This is because the residue differences between human and murine A β (Arg5Gly, Tyr10Phe, and His13Arg) are located toward the N-terminal region of the peptides, a region not thought to take part in the β -structure of A β fibrils.¹⁶ Interestingly the mixed fibrils containing murine and human A β 42 were more stable in solubilizing buffers than the homopolymeric human A β 42 fibrils.¹⁸ Murine A β peptide contains a larger amount of β -turn structure and is more strongly stabilized by hydrogen bonds than human A β .¹⁹ The increased number of hydrogen bonds may stabilize the mixed fibrils, resulting in a greater resistance to buffer solubilization compared with homopolymeric human fibrils. This study demonstrates how mixing proteins with different primary sequences can result in the emergence of new fibril polymorphs with different biophysical properties.

The ability of human A β and its murine homolog to co-polymerize is consistent with the other studies outlined above, as reflected by the percentage of overall sequence similarity between the two peptides (~93% identity for A β 40 and A β 42). Other studies show that the presence of a non-homologous amyloidogenic protein can inhibit fibril formation because both the number and position of differences in the primary sequences are incompatible with fibril elongation and/or nucleation, e.g., human and murine β_2 m (70% sequence similarity).²⁰ In an elegant study in which heterotetramers of transthyretin (TTR) were formed by mixing its human and murine subunits (Row 5, Table 1) Kelly and coworkers demonstrated that incorporation of murine TTR subunits protects the amyloidogenic human TTR from aggregating by stabilizing the native state.^{21,22} Similar results were observed for the mutant Thr119Met of human TTR which protects wild-type TTR tetramers from dissociation both *in vitro* and *in vivo* (Row 6, Table 1).²³ In both cases amyloid formation is inhibited via kinetic stabilization of the resulting heterotetramers relative to their homotetrameric human counterparts. Thus, using variants of amyloidogenic sequences

Table 1. Interactions between amyloidogenic proteins

	Protein 1	Protein 2	Type of cross-interaction	Effect on interaction	References
1	Hen lysozyme	I55T Hen lysozyme (99.2% sequence identity to hen lysozyme) Turkey lysozyme (95% identity) Human lysozyme (60% identity) Human α -lactalbumin (36% identity) Bovine insulin (no identity)	Cross-seeding	Sequences must be > 60% identical to cross-seed efficiently	15
2	A β 40	IAPP	Cross-seeding	A β fibrils will cross-seed IAPP monomer, but IAPP fibrils are inefficient at cross-seeding A β 40 monomer	17
3	A β 40	IAPP K ^{IV} light chain LEN-(1-30) Polyglutamine Gln ₃₀ Polyglutamine Gln ₅₀ Ure2p Λ^{IV} light chain JT05 β_2 -microglobulin Collagen Ovalbumin-RA	Cross-seeding	All the proteins listed showed less than 6% of the seeding efficiency of homologous seeding (A β 40 seeds with A β 40 monomer)	17
4	Human A β 40 and A β 42	Murine A β 40 and A β 42	Co-incubation	Interspecies fibril formation occurs and mixed fibrils are more stable than homopolymeric human A β fibrils	18
5	Human TTR	Murine TTR	Co-incubation	Murine TTR subunits stabilize the mixed tetramer and inhibition of fibril formation occurs	22
6	Human wild-type TTR	Human T119M TTR	Co-incubation	T119M TTR subunits stabilize the mixed tetramer and inhibition of fibril formation occurs	23
7	Human β_2 m	Human Δ N6	Co-incubation	Δ N6 monomer causes β_2 m fibril formation and formation of heteropolymeric fibrils with distinct morphology result	20,30
8	A β 40	A β 40 _{ARC}	Co-incubation	Stabilization of protofibrils occurs	32
9	Tau	α -synuclein	Co-incubation	Both proteins induce fibrillation of each other into homopolymers	33,57
10	A β 42	α -synuclein	Co-incubation	A β 42 and α -synuclein form hybrid nanopore oligomers	36
11	Human vCJD prions and human classical CJD prions	Mouse PrP	Cross-seeding	Human vCJD prions, but not classical CJD prions, induce formation of prions in mice with faithful strain replication	26,38
12	Hamster PrP R- and S-strain	Mouse PrP	Cross-seeding	The fibrillar R-strain of hamster PrP acts as a catalyst and a template for mouse PrP fibril formation, whereas the fibrillar hamster PrP S-strain could only act as a catalyst	39
13	Rnq1	Ure2p and Sup35	Cross-seeding	The ability to cross-seed Ure2p and Sup35 by the prion form of Rnq1 is strain dependent	41
14	Sup35	Ure2p	Cross-seeding	Sup35 prion inhibits fibril formation of Ure2p	41
15	A β 40	A β 42	Co-incubation	A β 42 monomer stimulates fibril formation of monomeric A β 40, but A β 40 monomer inhibits fibril formation of monomeric A β 42	42
16	A β 40	A β 42	Cross-seeding	A β 40 seeds both monomeric A β 40 and monomeric A β 42 equally well, but fibrillar A β 42 is inefficient at seeding A40 monomer compared with fibrillar A β 40	42
17	A β 40 and A β 42	PrP ^C	Co-incubation	PrP ^C inhibits A β fibril formation and traps it in an oligomeric state. Amyloid- β oligomers bind with nanomolar affinity to PrP ^C and the interaction is required for toxicity	43,44
18	Insulin	TTR	Co-incubation	The kinetics of fibril formation of both protein partners must be evenly matched for cross-seeding to occur, as with insulin and TTR	45
19	A β 40	Cystatin C	Co-incubation	Cystatin C inhibits A β 40 formation	49,50
20	A β 40	TTR	Cross-seeding	Preincubation of A β 40 with TTR reduced cytotoxicity	51
21	A β 40	Neuroserpin	Co-incubation	A β 40 acts as a catalyst of neuroserpin polymerization	58

Table 1. Interactions between amyloidogenic proteins (continued)

	Protein 1	Protein 2	Type of cross-interaction	Effect on interaction	References
22	A β 42	Neuroserpin	Co-incubation	Neuroserpin accelerates A β 42 aggregation to form non-toxic oligomers	59
23	Murine β_2m	$\Delta N6$	Co-incubation	Inhibition of fibril formation	20
24	Gliadin	Amylase/myoglobin	Co-incubation	Varied fiber morphologies form when the proteins are incubated together	60
25	A β 6–40 A β 26–42 A β 34–42 A β 26–43	A β 26–39 A β 26–40	Cross-seeding	All the proteins in the Protein 1 column accelerate fibril formation of monomeric A β 26–39 and A β 26–40	61
26	A β 42	Pyroglutamate A β 42	Cross-seeding	Cross-seeding accelerated fibril formation and resulted in increased toxicity	62
27	A β 40	α -synuclein	Cross-seeding	Fibrillar α -synuclein is more effective than fibrillar A β 40 at cross-seeding A β 40, oligomeric α -synuclein is less effective than oligomeric A β 40 at cross-seeding A β 40. A β 40 fibrils and oligomers are a poor seed of monomeric α -synuclein	63
28	A β 42	α -synuclein	Cross-seeding	Fibrillar and oligomeric α -synuclein are less effective than oligomeric and fibrillar A β 42 at seeding monomeric A β 42. A β 42 fibrils and oligomers are both less effective than α -synuclein fibrils and oligomers at seeding α -synuclein	63
29	A β 40	BRICHOS	Cross-seeding	Extension of the A β 40 lag phase in the presence of BRICHOS	64
30	A β 40	IAPP mimic ([N-Me]G24, [N-Me]I26)-IAPP	Cross-seeding	An IAPP mimic inhibits A β 40 fibril formation	65

Interactions between two amyloidogenic proteins of different primary sequences are shown. The types of interactions are either cross-seeding, where one protein is oligomeric or fibrillar and the other protein is monomeric, or co-incubation, where the two proteins are mixed as monomers. The effect of the interaction on the propensity for fibril formation is also described.

allows amyloid formation to be controlled and/or inhibited by introducing a non-homologous primary sequence into a fibril formation reaction.

Condition 2: Co-Aggregation Occurs by One Partner Protein Affecting the Rate of Fibril Formation of Another

Amyloidoses can occur when susceptible proteins are exposed to conditions that promote global unfolding of the native state²⁴ or enhance the population of amyloidogenic intermediates.²⁵ Statistically, mixed fibril formation is less likely than the generation of fibrils from a single sequence, as the production of heterofibrils requires a change in conformation of two proteins instead of one. However, this assumes that both partners must undergo a change to an amyloid conformer independently. By contrast with this observation, monomer-monomer interactions can promote protein unfolding, and/or formation of an amyloidogenic fold.^{20,26} Thus if one protein partner acts to enhance the amyloid potential of another, the probability of forming heteropolymeric assemblies is increased.

An excellent example of one protein enhancing the amyloid propensity of another has been shown for β_2m and its N-terminally truncated variant $\Delta N6$.²⁰ β_2m forms amyloid fibrils in vivo resulting in the disease dialysis-related amyloidosis (DRA).²⁷ However, in the absence of other co-factors or co-solvents human β_2m will not form fibrils in vitro at neutral pH within an experimentally tractable timescale,^{28,29} while $\Delta N6$ is highly aggregation prone.^{20,30} Surprisingly, when monomeric human β_2m is mixed with monomeric $\Delta N6$ fibril formation from both proteins occurs, resulting in heteropolymeric fibrils, even at neutral pH (Row 7, Table 1).^{20,30} Interestingly $\Delta N6$ was shown to stimulate fibril formation of human β_2m at sub-stoichiometric levels, with fibril formation occurring at a 1:99 $\Delta N6$: β_2m molar ratio.²⁰ An atomistic description of how $\Delta N6$ converts β_2m into an amyloidogenic form has been provided using NMR.²⁰ These studies showed that collision with $\Delta N6$ results in increased conformational dynamics of human β_2m , allowing β_2m to undergo further structural rearrangements, critically via cis-trans isomerization of Pro32,

that subsequently allows access to the amyloid state.

By enhancing the amyloid potential of β_2m , both β_2m and $\Delta N6$ are incorporated into heteropolymeric fibrils when incubated at a 1:1 ratio.³⁰ As discussed in the introduction, aggregation of a single polypeptide sequence can result in a range of fibril structures dependent on the experimental conditions employed, such as pH, temperature, ionic strength and agitation.³¹ In the case of mixed fibrils, for example those formed from β_2m and $\Delta N6$ where both proteins are incorporated into the fibril structure, the possibilities for polymorphism are vast. Perhaps unsurprisingly then, the heteropolymeric fibrils formed from β_2m and $\Delta N6$ do not resemble those formed from β_2m alone at acidic pH or from $\Delta N6$ alone at neutral pH. Instead biophysical experiments have shown that the heteropolymeric fibrils of β_2m / $\Delta N6$ form a unique polymorph that is thermodynamically less stable than both β_2m and $\Delta N6$ homopolymeric fibrils.³⁰ These findings show that the heteropolymorphic fibrils do not possess “average” structural or thermodynamic characteristics resulting from mixing of

the two precursors. Instead, through the enhancement of the amyloidogenic potential of one protein by another, a new area of the protein (mis)folding landscape is sampled, and a new polymorph is created.

The ability to endow an amyloid-prone form on a less amyloidogenic protein can also result in altered stabilities of on-pathway intermediates. The Arctic mutation (E22G) of A β 40 results in a highly amyloidogenic protein (A β 40_{ARC}) that forms both protofibrils and fibrils under conditions in which A β 40 remains monomeric.³² Interestingly in the absence of A β 40, A β 40_{ARC} protofibrils will convert to fibrils, but in the presence of A β 40 at a 1:1 molar ratio, the two proteins form mixed protofibrils that are incapable of further assembly. The mixed protofibrils are more stable than the protofibrils formed by A β 40_{ARC} alone and hence these species accumulate (Row 8, Table 1). Lashuel et al. postulate that these mixed protofibrils may be responsible for the enhancement of neurotoxicity and accelerated disease progression that is seen in patients carrying this mutation.³² Co-aggregation of two different peptides, therefore, can alter the kinetics of fibril formation, and thereby alter the morphology and stability of intermediates, resulting in a prolonged lifetime of toxic species.

A highly specific enhancement of one protein's amyloidogenic potential by another is demonstrated by the co-incubation of tau with a familial mutant of α -synuclein (Ala53Thr). This amino acid substitution results in increased α -synuclein and tau inclusions, suggesting that it not only increases the propensity for α -synuclein to form fibrils, but also promotes the formation of tau inclusions.³³ Further in vitro studies have shown that co-incubation of tau and α -synuclein induces polymerization of both proteins and this effect was observed for all six tau isoforms (Row 9, Table 1).³³ Immunogold labeling revealed that the individual fibrils are mostly homopolymers, but some fibrils were labeled for both α -synuclein and tau but in spatially separate domains, suggesting that end-to-end annealing of the homopolymers occurs.³³ Furthermore, at low concentrations of α -synuclein, the presence of tau

was required for α -synuclein fibrils to form. Similarly, tau requires α -synuclein pre-aggregation to induce the requisite conformational change to allow tau inclusion formation. Interestingly, this effect was specific to α -synuclein; other amyloidogenic proteins such as A β 40 are unable to initiate tau polymerization.³³

Enhancing a protein's amyloid potential not only results in increased formation of fibrils, but new species in the amyloidogenic pathway can also form by mixing protein monomers. A β has been shown to promote the aggregation and toxicity of α -synuclein in a dose- and time-dependent manner.³⁴ The two proteins may also interact in vivo as evidenced by an overlap in the pathology of AD and Parkinson Disease in Lewy Body Disease (LBD), where the initial signs are dementia followed by parkinsonism.³⁵ In samples of human brains from sufferers of LBD A β and α -synuclein were shown to co-immunoprecipitate, but no interaction between the two proteins was observed in non-demented controls. In vitro studies have shown that solubilized A β 42 induces formation of α -synuclein tetramers and higher-order oligomers (Row 10, Table 1).³⁶ Electron microscopy images of the mixture after 6 h show well-defined hybrid ring oligomeric structures 9–15 nm in diameter, which may form functional cation nanopores.³⁶ Mixing two amyloidogenic proteins, therefore, can change the stability and characteristics of species that form during amyloid assembly, in this case resulting in the formation of a hybrid nanopore that could be involved in disease progression.

Condition 3: Structure and Post-Translational Modifications Affect the Efficiency of Cross-Seeding

One of the most striking examples of post-translational modification altering protein co-aggregation is in prion diseases. In these disorders the efficiency of cross-species infectivity is partly determined by primary sequence, but also relies on compatible structures and post-translational modifications (typically glycosylation).³⁷ Furthermore, to efficiently cross-seed, both the seed and the monomer must

be able to adopt the same amyloidogenic conformation.

The importance of structure in prion propagation was demonstrated with the bovine spongiform encephalopathy (BSE) outbreak in the UK in the mid-1980s which resulted in the infection of more than 2 million UK cattle.³⁷ This was followed by an outbreak of a novel human prion disease, variant Creutzfeldt-Jakob disease (vCJD) in the mid-1990s. CJD is not a new disease: "classical" CJD prions, formed from spontaneous misfolding of the prion protein (PrP), occur at a rate of approximately one case per 1 million population per year.³⁷ However, despite sharing identical primary sequences, classical CJD prions and vCJD prions behave very differently in their ability to convert PrP^C (the non-infectious form of PrP) to PrP^{Sc} (the prion form) in a new species. This was shown when human classical CJD prions were used to infect wild-type mice, wherein no transmission was observed. However, when human vCJD prions were used instead, transmission was much more successful and the resultant mouse PrP showed faithful strain replication of the vCJD strain.³⁸ Fascinatingly, this shows that two proteins with identical amino acid sequences can experience different species barriers, confirming that prion propagation is conformation dependent (Row 11, Table 1).

Although the vCJD and classical CJD strains of PrP have identical primary sequences, they differ in their glycosylation patterns. Classical CJD strains are modified predominantly with low molecular mass glycosylation, whereas the vCJD strain has high molecular mass glycosylation.²⁶ Furthermore, the ratio of the three PrP glycoforms (unglycosylated, monoglycosylated, and diglycosylated) is faithfully maintained on passage in transgenic mice expressing human PrP.²⁶ Similarly, transmission of human prions and bovine prions to wild-type mice results in murine PrP with glycoform ratios that correspond to the initial inocula.²⁶ Interestingly, PrP glycosylation occurs before conversion to PrP, thus the different glycoforms may be determined by initial PrP conformation. Different cell types may also glycosylate proteins differently, thus particular PrP glycoform strains may replicate most

favorably in cell types with a similar PrP glycoform expressed on the cell surface.³⁷ Glycosylation similarities (or differences) between PrP and the infectious prion may help to explain the different incubation periods seen in individuals with prion disease.²⁶ Thus post-translational modifications can be crucial in determining the propensity of a protein to cross-seed, as well as affecting the location and timescale of disease developing.

In a recent study the importance of strain for cross-species infectivity was demonstrated using two morphologies of hamster prion protein, R and S.³⁹ The two strains were formed using the same stock of recombinant PrP under identical solvent conditions, but with different agitation modes; the S-strain was produced under shaking, the R-strain was produced with rotation. The R- and S-fibrils display substantial differences in their cross- β cores, epitope exposure and morphology.⁴⁰ Despite sharing the same amino acid sequence, the difference in morphology between the R- and S-fibrils has a profound effect on their ability to reproduce their strain faithfully in a different species (mouse). The hamster R-fibrils successfully recruit mouse-PrP into fibrils and maintain their morphology, thus acting both as a so-called “catalyst” and a template for fibril formation of mouse PrP. By contrast, the hamster S-fibril form also acts as a catalyst for structural conversion of mouse-PrP, but does not act as a template, i.e., the strain morphology was not propagated (Row 12, Table 1).³⁹ In the latter case, fibril elongation occurred but involved mouse-PrP switching to a new conformational state that resembled the R-fibril form. This demonstrates that primary sequence (condition 1) is not wholly responsible for the ability to act as an enhancer of amyloidogenicity in another protein (condition 2). Instead structure also affects the ability to transmit disease across a species barrier.

Yeast prion proteins have also provided a powerful model for developing understanding of both sequence and strain effects in prion propagation. When the yeast protein Rnq1 is aggregated into its prion form, it can promote prion formation of two other proteins, Sup35 and Ure2p.⁴¹ Interestingly the efficiency with

which the prion form of Rnq1 can cross-seed the latter two proteins is dependent on its strain. One Rnq1 prion strain will seed Ure2p inefficiently, but will seed Sup35 efficiently. However an alternative Rnq1 prion strain behaves in the opposite manner, inefficiently seeding Sup35, but efficiently seeding Ure2p. Both Rnq1 prion strains can propagate their morphology by homologous seeding, yet their ability to cross-seed other proteins is entirely dependent on their structure, rather than their (identical) amino acid sequence (Row 13, Table 1).⁴¹ Further revelations from yeast models show that not all prions can cross-seed, and some can even inhibit homologous seeding.⁴¹ For example, the prion form of Sup35 will inhibit Ure2p prion seeds from seeding non-prion Ure2p, through “poisoning” of the Ure2p prion seeds (Row 14, Table 1).⁴¹

The importance of structure in amyloid propagation is not limited to prion interactions. Despite A β 40 and A β 42 differing by only two residues, the two peptides have different effects on each other’s aggregation propensity. In co-polymerization experiments A β 42 monomers stimulate A β 40 monomers to aggregate, whereas A β 40 monomers shows a concentration-dependent inhibitory effect on A β 42 fibril formation (Row 15, Table 1).⁴² However, the same effect is not seen in cross-seeding experiments (Row 16, Table 1),⁴² where one partner is in fibrillar form and one partner is monomeric. In this case A β 42 fibrils are less efficient than fibrillar A β 40 at inducing fibril formation of monomeric A β 40. Why does A β 42 efficiently stimulate A β 40 amyloidogenesis only when it is monomeric, and not when in the fibrillar form? This phenomenon is explained by the importance of structure, as well as primary sequence for promoting amyloid formation. Accordingly, it is A β 42 oligomers, rather than fibrils, that are the optimal template for incorporating A β 40 monomers, and these are mainly populated during incubation of soluble A β 40 and A β 42 (co-polymerization), rather than when A β 42 is added as fibrils (cross-seeding). Thus, although primary sequence is important in allowing A β 42 and A β 40 co-aggregation to occur (condition 1), and A β 42 is an enhancer of A β 40 amyloidogenesis (condition 2), the inclusion of the

structure of A β 42 aggregates must also be considered in assessing the likelihood of co-aggregation occurring.

A final example of a structure-dependent interaction of two amyloidogenic proteins is between PrP and A β . Interactions between A β 42 oligomers and PrP leads to the inhibition of long-term potentiation in hippocampal slices from mice.⁴³ Interestingly, experiments using NMR have shown that PrP does not interact with disordered monomeric A β 40, but A β 40 must first change its conformation, possibly forming a misfolded monomer or dimer.⁴⁴ These studies not only highlight the importance of structure in determining co-aggregation, but also show that interactions are not limited to the monomer-monomer or monomer-fibril stages in fibril formation. Instead, recognition between two amyloidogenic proteins can be specific for a key intermediary structure in the amyloid formation pathway.

Conclusions

This review broadly categorizes interactions between two amyloidogenic proteins into “conditions” that prescribe the likelihood of a co-polymerization or cross-seeding event occurring. The first condition is that both partners must have a similar amino acid sequence. Although the importance of the protein backbone in fibril stability means most proteins may have the propensity to form fibrils under defined conditions,¹⁴ side-chain packing also plays a vital role.⁵ Interestingly the ability to cross-seed is not necessarily reciprocal, even between closely related amyloid proteins.¹⁷ The second condition is that at least one of the partners must enhance the amyloidogenic potential of the other, such as the effect of Δ N6 on β_2 m^{20,30} or α -synuclein on tau.³³ Finally, the third condition is that structure and/or post-translational modifications can affect the efficiency of cross-seeding, for example in prion propagation across different species. The three conditions are not mutually exclusive: primary sequence can influence fibril/oligomer structure, and a similarity in sequences between amyloidogenic proteins with their less amyloidogenic partners is important in defining the ability of

proteins to co-polymerize. The network of hydrogen bonds within a fibril is known to be vital for monomer addition, and this would be influenced by both side-chain and fold. The conditions outlined here are also not comprehensive: another property that can regulate the propensity for co-polymerization is similar aggregation kinetics of the co-incubated monomers (Row 18, Table 1).⁴⁵ Additionally the ratio of the two components may also be vital: under normal physiological conditions the A β 42:A β 40 ratio in the brain is ~1:9.⁴⁶ However, in familial AD patients a ratio of 3:7 A β 42:A β 40 is observed, suggesting that a change in the proportion of the two alloforms may be significant in the development of AD.⁴⁷

Examples of co-polymerization between unrelated sequences *in vivo* are relatively rare. The BSE crisis followed by the outbreak of vCJD has shown that cross-species prion transmission can occur.³⁷ An increased risk of AD has been shown for type II diabetes sufferers, suggesting a possible link between A β and IAPP aggregation.⁴⁸ In fact, A β seems to be promiscuous, with *in vivo* interactions reported between A β and cystatin c (Row 19, Table 1)^{49,50}, TTR (Row 20, Table 1)⁵¹, and neuroserpins (Rows 21 and 22, Table 1).⁵² The lack of more examples *in vivo* suggests that perhaps all three conditions described here need to be met, in a timely and spatially defined manner, for co-polymerization and/or cross-seeding to occur. Additionally the rapid increase in examples reported in the literature of co-polymerization and cross-seeding between different amyloidogenic proteins *in vitro* confirms the possibility for co-polymerization, giving weight to a possible *in vivo* relevance to at least some of these interactions.

Interactions between unrelated, or sequence distinct amyloidogenic proteins are fascinating and important because of the possibilities for polymorphism that arise from co-aggregation. In the introduction of this review the eight different classes of steric zippers that comprise the amyloid core were discussed⁵ and detailed studies of polymorphism in homopolymeric fibrils have been performed.^{1,4,53} Additionally polymorphism can occur at the earliest stages of aggregation, with

subsets of oligomers showing structural polymorphism that also affects the ability of the species to be toxic (reviewed in ref. 54). Polymorphism has been induced in proteins with the same sequence through alteration of solution conditions such as pH, agitation, temperature and/or ionic strength.^{31,55} Fibril length can also be controlled via fragmentation, allowing the physical dimensions and surface interactions of fibrils to be studied.⁵⁶ However, more parameters are also open to manipulation in systems that co-aggregate, such as the ratio of the protein species involved and the identity of the species that are mixed (monomers, oligomers or fibrils). By adjusting these different factors, in addition to altering experimental constraints, the potential repertoire of species that can form, both at the level of oligomers and fibrils is vast (see the schematic in Fig. 1B). Furthermore, as all proteins are potentially able to form amyloid,¹⁴ and some amyloidogenic proteins can cross-seed unrelated polypeptide sequences, the risk of co-polymerization could potentially extend to the entire proteome. Unravelling the different structural possibilities for oligomer and fibril architectures and defining their physical and biological characteristics will be challenging. However these multi-component assemblies will need to be defined for developing our understanding of the causative agents of amyloid disease, as well as to harness the future potentials of amyloid fibrils as designer nanomaterials.

Recent work has shown that a specific and transient protofibrillar species of Abeta42, with a novel triple helical structure, is the most potent aggregate involved in the interaction between PrP and Abeta.⁶⁶ This work supports the importance of structure for influencing amyloid interactions between different proteins.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by Medical Research Council Grant G0900958. We thank the Radford group for helpful discussions and critical reading of the manuscript.

References

1. Meinhardt J, Sachse C, Hortschansky P, Grigorieff N, Fändrich M. Abeta(1-40) fibril polymorphism implies diverse interaction patterns in amyloid fibrils. *J Mol Biol* 2009; 386:869-77; PMID:19038266; <http://dx.doi.org/10.1016/j.jmb.2008.11.005>
2. Eichner T, Radford SE. A diversity of assembly mechanisms of a generic amyloid fold. *Mol Cell* 2011; 43:8-18; PMID:21726806; <http://dx.doi.org/10.1016/j.molcel.2011.05.012>
3. Fändrich M, Meinhardt J, Grigorieff N. Structural polymorphism of Alzheimer Abeta and other amyloid fibrils. *Prion* 2009; 3:89-93; PMID:19597329; <http://dx.doi.org/10.4161/pri.3.2.8859>
4. Fitzpatrick AW, Debelouchina GT, Bayro MJ, Clare DK, Caporini MA, Bajaj VS, Jaronec CP, Wang L, Ladizhansky V, Müller SA, et al. Atomic structure and hierarchical assembly of a cross- β amyloid fibril. *Proc Natl Acad Sci U S A* 2013; 110:5468-73; PMID:23513222; <http://dx.doi.org/10.1073/pnas.1219476110>
5. Sawaya MR, Sambashivan S, Nelson R, Ivanova MI, Sievers SA, Apostol MI, Thompson MJ, Balbirnie M, Wiltzius JJ, McFarlane HT, et al. Atomic structures of amyloid cross-beta spines reveal varied steric zippers. *Nature* 2007; 447:453-7; PMID:17468747; <http://dx.doi.org/10.1038/nature05695>
6. Eisenberg D, Jucker M. The amyloid state of proteins in human diseases. *Cell* 2012; 148:1188-203; PMID:22424229; <http://dx.doi.org/10.1016/j.cell.2012.02.022>
7. Paschos A, Bauer A, Zimmermann A, Zehelein E, Böck A. HypF, a carbamoyl phosphate-converting enzyme involved in [NiFe] hydrogenase maturation. *J Biol Chem* 2002; 277:49945-51; PMID:12377778; <http://dx.doi.org/10.1074/jbc.M204601200>
8. Campioni S, Mannini B, Zampagni M, Pensalfini A, Parrini C, Evangelisti E, Relini A, Stefani M, Dobson CM, Cecchi C, et al. A causative link between the structure of aberrant protein oligomers and their toxicity. *Nat Chem Biol* 2010; 6:140-7; PMID:20081829; <http://dx.doi.org/10.1038/nchembio.283>
9. Cremades N, Cohen SI, Deas E, Abramov AY, Chen AY, Orte A, Sandal M, Clarke RW, Dunne P, Aprile FA, et al. Direct observation of the interconversion of normal and toxic forms of α -synuclein. *Cell* 2012; 149:1048-59; PMID:22632969; <http://dx.doi.org/10.1016/j.cell.2012.03.037>
10. Barghorn S, Nimmrich V, Striebinger A, Krantz C, Keller P, Janson B, Bahr M, Schmidt M, Bitner RS, Harlan J, et al. Globular amyloid beta-peptide oligomer - a homogenous and stable neuropathological protein in Alzheimer's disease. *J Neurochem* 2005; 95:834-47; PMID:16135089; <http://dx.doi.org/10.1111/j.1471-4159.2005.03407.x>
11. Bernstein SL, Dupuis NF, Lazo ND, Wyttenbach T, Condrón MM, Bitan G, Teplow DB, Shea JE, Ruotolo BT, Robinson CV, et al. Amyloid- β protein oligomerization and the importance of tetramers and dodecamers in the aetiology of Alzheimer's disease. *Nat Chem* 2009; 1:326-31; PMID:20703363; <http://dx.doi.org/10.1038/nchem.247>
12. Ono K, Condrón MM, Teplow DB. Structure-neurotoxicity relationships of amyloid beta-protein oligomers. *Proc Natl Acad Sci U S A* 2009; 106:14745-50; PMID:19706468; <http://dx.doi.org/10.1073/pnas.0905127106>
13. McLaurin J, Yang DS, Yip CM, Fraser PE. Review: modulating factors in amyloid-beta fibril formation. *J Struct Biol* 2000; 130:259-70; PMID:10940230; <http://dx.doi.org/10.1006/jsbi.2000.4289>
14. Dobson CM. Protein misfolding, evolution and disease. *Trends Biochem Sci* 1999; 24:329-32; PMID:10470028; [http://dx.doi.org/10.1016/S0968-0004\(99\)01445-0](http://dx.doi.org/10.1016/S0968-0004(99)01445-0)

15. Krebs MR, Morozova-Roche LA, Daniel K, Robinson CV, Dobson CM. Observation of sequence specificity in the seeding of protein amyloid fibrils. *Protein Sci* 2004; 13:1933-8; PMID:15215533; <http://dx.doi.org/10.1110/ps.04707004>
16. Petkova AT, Ishii Y, Balbach JJ, Antzutkin ON, Leapman RD, Delaglio F, Tycko R. A structural model for Alzheimer's beta-amyloid fibrils based on experimental constraints from solid state NMR. *Proc Natl Acad Sci U S A* 2002; 99:16742-7; PMID:12481027; <http://dx.doi.org/10.1073/pnas.262663499>
17. O'Nuallain B, Williams AD, Westermark P, Wetzel R. Seeding specificity in amyloid growth induced by heterologous fibrils. *J Biol Chem* 2004; 279:17490-9; PMID:14752113; <http://dx.doi.org/10.1074/jbc.M311300200>
18. Fung J, Frost D, Chakrabartty A, McLaurin J. Interaction of human and mouse Abeta peptides. *J Neurochem* 2004; 91:1398-403; PMID:15584916; <http://dx.doi.org/10.1111/j.1471-4159.2004.02828.x>
19. Hilbich C, Kisters-Woike B, Reed J, Masters CL, Beyreuther K. Human and rodent sequence analogs of Alzheimer's amyloid beta A4 share similar properties and can be solubilized in buffers of pH 7.4. *Eur J Biochem* 1991; 201:61-9; PMID:1915378; <http://dx.doi.org/10.1111/j.1432-1033.1991.tb16256.x>
20. Eichner T, Kalverda AP, Thompson GS, Homans SW, Radford SE. Conformational conversion during amyloid formation at atomic resolution. *Mol Cell* 2011; 41:161-72; PMID:21255727; <http://dx.doi.org/10.1016/j.molcel.2010.11.028>
21. Mirroy GJ, Lai Z, Lashuel HA, Peterson SA, Strang C, Kelly JW. Inhibiting transthyretin amyloid fibril formation via protein stabilization. *Proc Natl Acad Sci U S A* 1996; 93:15051-6; PMID:8986762; <http://dx.doi.org/10.1073/pnas.93.26.15051>
22. Reixach N, Foss TR, Santelli E, Pascual J, Kelly JW, Buxbaum JN. Human-murine transthyretin heterotetramers are kinetically stable and non-amyloidogenic. A lesson in the generation of transgenic models of diseases involving oligomeric proteins. *J Biol Chem* 2008; 283:2098-107; PMID:18006495; <http://dx.doi.org/10.1074/jbc.M708028200>
23. Hammarström P, Schneider F, Kelly JW. Trans-suppression of misfolding in an amyloid disease. *Science* 2001; 293:2459-62; PMID:11577236; <http://dx.doi.org/10.1126/science.1062245>
24. Johnson SM, Wiseman RL, Sekijima Y, Green NS, Adamski-Werner SL, Kelly JW. Native state kinetic stabilization as a strategy to ameliorate protein misfolding diseases: a focus on the transthyretin amyloidosis. *Acc Chem Res* 2005; 38:911-21; PMID:16359163; <http://dx.doi.org/10.1021/ar020073i>
25. Jahn TR, Parker MJ, Homans SW, Radford SE. Amyloid formation under physiological conditions proceeds via a native-like folding intermediate. *Nat Struct Mol Biol* 2006; 13:195-201; PMID:16491092; <http://dx.doi.org/10.1038/nsmb1058>
26. Collinge J, Sidle KC, Meads J, Ironside J, Hill AF. Molecular analysis of prion strain variation and the aetiology of 'new variant' CJD. *Nature* 1996; 383:685-90; PMID:8878476; <http://dx.doi.org/10.1038/383685a0>
27. Gejyo F, Yamada T, Odani S, Nakagawa Y, Arakawa M, Kunitomo T, Kataoka H, Suzuki M, Hirasawa Y, Shirahama T, et al. A new form of amyloid protein associated with chronic hemodialysis was identified as beta 2-microglobulin. *Biochem Biophys Res Commun* 1985; 129:701-6; PMID:3893430; [http://dx.doi.org/10.1016/0006-291X\(85\)91948-5](http://dx.doi.org/10.1016/0006-291X(85)91948-5)
28. Calabrese MF, Eakin CM, Wang JM, Miranker AD. A regulatable switch mediates self-association in an immunoglobulin fold. *Nat Struct Mol Biol* 2008; 15:965-71; PMID:19172750; <http://dx.doi.org/10.1038/nsmb.1483>
29. Platt GW, Radford SE. Glimpses of the molecular mechanisms of beta2-microglobulin fibril formation in vitro: aggregation on a complex energy landscape. *FEBS Lett* 2009; 583:2623-9; PMID:19433089; <http://dx.doi.org/10.1016/j.febslet.2009.05.005>
30. Sarell CJ, Woods LA, Su Y, Debelouchina GT, Ashcroft AE, Griffin RG, Stockley PG, Radford SE. Expanding the repertoire of amyloid polymorphs by co-polymerization of related protein precursors. *J Biol Chem* 2013; 288:7327-37; PMID:23329840; <http://dx.doi.org/10.1074/jbc.M112.447524>
31. Petkova AT, Leapman RD, Guo Z, Yau WM, Mattson MP, Tycko R. Self-propagating, molecular-level polymorphism in Alzheimer's beta-amyloid fibrils. *Science* 2005; 307:262-5; PMID:15653506; <http://dx.doi.org/10.1126/science.1105850>
32. Lashuel HA, Hartley DM, Petre BM, Wall JS, Simon MN, Walz T, Lansbury PT Jr. Mixtures of wild-type and a pathogenic (E22G) form of Abeta40 in vitro accumulate protofibrils, including amyloid pores. *J Mol Biol* 2003; 332:795-808; PMID:12972252; [http://dx.doi.org/10.1016/S0022-2836\(03\)00927-6](http://dx.doi.org/10.1016/S0022-2836(03)00927-6)
33. Giasson BI, Forman MS, Higuchi M, Golbe LI, Graves CL, Kottbauer PT, Trojanowski JQ, Lee VMY. Initiation and synergistic fibrillization of tau and alpha-synuclein. *Science* 2003; 300:636-40; PMID:12714745; <http://dx.doi.org/10.1126/science.1082324>
34. Masliah E, Rockenstein E, Veinbergs I, Sagara Y, Mallory M, Hashimoto M, Mucke L. beta-amyloid peptides enhance alpha-synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease. *Proc Natl Acad Sci U S A* 2001; 98:12245-50; PMID:11572944; <http://dx.doi.org/10.1073/pnas.211412398>
35. Galasko D, Hansen LA, Katzman R, Wiederholt W, Masliah E, Terry R, Hill LR, Lessin P, Thal LJ. Clinical-neuropathological correlations in Alzheimer's disease and related dementias. *Arch Neurol* 1994; 51:888-95; PMID:8080388; <http://dx.doi.org/10.1001/archneur.1994.00540210060013>
36. Tsigelny IF, Crews L, Desplats P, Shaked GM, Sharikov Y, Mizuno H, Spencer B, Rockenstein E, Trejo M, Platoshyn O, et al. Mechanisms of hybrid oligomer formation in the pathogenesis of combined Alzheimer's and Parkinson's diseases. *PLoS One* 2008; 3:e3135; PMID:18769546; <http://dx.doi.org/10.1371/journal.pone.0003135>
37. Collinge J. Prion diseases of humans and animals: their causes and molecular basis. *Annu Rev Neurosci* 2001; 24:519-50; PMID:11283320; <http://dx.doi.org/10.1146/annurev.neuro.24.1.519>
38. Hill AF, Desbruslais M, Joiner S, Sidle KC, Gowland I, Collinge J, Doey LJ, Lantos P. The same prion strain causes vCJD and BSE. *Nature* 1997; 389:448-50, 526; PMID:9333232; <http://dx.doi.org/10.1038/38925>
39. Makarava N, Ostapchenko VG, Savchenko R, Baskakov IV. Conformational switching within individual amyloid fibrils. *J Biol Chem* 2009; 284:14386-95; PMID:19329794; <http://dx.doi.org/10.1074/jbc.M900533200>
40. Makarava N, Baskakov IV. The same primary structure of the prion protein yields two distinct self-propagating states. *J Biol Chem* 2008; 283:15988-96; PMID:18400757; <http://dx.doi.org/10.1074/jbc.M800562200>
41. Bradley ME, Edsles HK, Hong JY, Wickner RB, Liebman SW. Interactions among prions and prion "strains" in yeast. *Proc Natl Acad Sci U S A* 2002; 99(Suppl 4):16392-9; PMID:12149514; <http://dx.doi.org/10.1073/pnas.152330699>
42. Pauwels K, Williams TL, Morris KL, Jonckheere W, Vandersteen A, Kelly G, Schymkowitz J, Rousseau F, Pastore A, Serpell LC, et al. Structural basis for increased toxicity of pathological aB42:aB40 ratios in Alzheimer disease. *J Biol Chem* 2012; 287:5650-60; PMID:22157754; <http://dx.doi.org/10.1074/jbc.M111.264473>
43. Laurén J, Gimbel DA, Nygaard HB, Gilbert JW, Strittmatter SM. Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. *Nature* 2009; 457:1128-32; PMID:19242475; <http://dx.doi.org/10.1038/nature07761>
44. Younan ND, Sarell CJ, Davies P, Brown DR, Viles JH. The cellular prion protein traps Alzheimer's Aβ in an oligomeric form and disassembles amyloid fibers. *FASEB J* 2013; 27:1847-58; PMID:2335053; <http://dx.doi.org/10.1096/fj.12-222588>
45. MacPhee CE, Dobson CM. Formation of mixed fibrils demonstrates the generic nature and potential utility of amyloid nanostructures. *J Am Chem Soc* 2000; 122:12707-13; <http://dx.doi.org/10.1021/ja0029580>
46. Suzuki N, Cheung TT, Cai XD, Odaka A, Otvos L Jr., Eckman C, Golde TE, Younkin SG. An increased percentage of long amyloid beta protein secreted by familial amyloid beta protein precursor (beta APP717) mutants. *Science* 1994; 264:1336-40; PMID:8191290; <http://dx.doi.org/10.1126/science.8191290>
47. Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton M, Kukull W, et al. Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat Med* 1996; 2:864-70; PMID:8705854; <http://dx.doi.org/10.1038/nm0896-864>
48. Janson J, Laedtke T, Parisi JE, O'Brien P, Petersen RC, Butler PC. Increased risk of type 2 diabetes in Alzheimer disease. *Diabetes* 2004; 53:474-81; PMID:14747300; <http://dx.doi.org/10.2337/diabetes.53.2.474>
49. Kaeser SA, Herzig MC, Coomaraswamy J, Kilger E, Selenica ML, Winkler DL, Staufenbiel M, Levy E, Grubb A, Jucker M. Cystatin C modulates cerebral beta-amyloidosis. *Nat Genet* 2007; 39:1437-9; PMID:18026102; <http://dx.doi.org/10.1038/ng.2007.23>
50. Mi W, Pawlik M, Sastre M, Jung SS, Radvinsky DS, Klein AM, Sommer J, Schmidt SD, Nixon RA, Mathews PM, et al. Cystatin C inhibits amyloid-beta deposition in Alzheimer's disease mouse models. *Nat Genet* 2007; 39:1440-2; PMID:18026100; <http://dx.doi.org/10.1038/ng.2007.29>
51. Li X, Masliah E, Reixach N, Buxbaum JN. Neuronal production of transthyretin in human and murine Alzheimer's disease: is it protective? *J Neurosci* 2011; 31:12483-90; PMID:21880910; <http://dx.doi.org/10.1523/JNEUROSCI.2417-11.2011>
52. Fabbro S, Schaller K, Seeds NW. Amyloid-beta levels are significantly reduced and spatial memory defects are rescued in a novel neuroserpin-deficient Alzheimer's disease transgenic mouse model. *J Neurochem* 2011; 118:928-38; PMID:21689108; <http://dx.doi.org/10.1111/j.1471-4159.2011.07359.x>
53. Jiménez JL, Nettleton EJ, Bouchard M, Robinson CV, Dobson CM, Saibil HR. The protofilament structure of insulin amyloid fibrils. *Proc Natl Acad Sci U S A* 2002; 99:9196-201; PMID:12093917; <http://dx.doi.org/10.1073/pnas.142459399>
54. Glabe CG. Structural classification of toxic amyloid oligomers. *J Biol Chem* 2008; 283:29639-43; PMID:18723507; <http://dx.doi.org/10.1074/jbc.R800016200>
55. Gosal WS, Morten IJ, Hewitt EW, Smith DA, Thomson NH, Radford SE. Competing pathways determine fibril morphology in the self-assembly of beta2-microglobulin into amyloid. *J Mol Biol* 2005; 351:850-64; PMID:16024039; <http://dx.doi.org/10.1016/j.jmb.2005.06.040>
56. Xue WF, Hellewell AL, Hewitt EW, Radford SE. Fibril fragmentation in amyloid assembly and cytotoxicity: when size matters. *Prion* 2010; 4:20-5; PMID:20305394; <http://dx.doi.org/10.4161/pri.4.1.11378>

57. Guo JL, Covell DJ, Daniels JP, Iba M, Stieber A, Zhang B, Riddle DM, Kwong LK, Xu Y, Trojanowski JQ, et al. Distinct α -synuclein strains differentially promote tau inclusions in neurons. *Cell* 2013; 154:103-17; PMID:23827677; <http://dx.doi.org/10.1016/j.cell.2013.05.057>
58. Chiou A, Hägglöf P, Orte A, Chen AY, Dunne PD, Belorgey D, Karlsson-Li S, Lomas DA, Klenerman D. Probing neuroserpin polymerization and interaction with amyloid-beta peptides using single molecule fluorescence. *Biophys J* 2009; 97:2306-15; PMID:19843463; <http://dx.doi.org/10.1016/j.bpj.2009.07.057>
59. Kinghorn KJ, Crowther DC, Sharp LK, Nerelius C, Davis RL, Chang HT, Green C, Gubb DC, Johansson J, Lomas DA. Neuroserpin binds Abeta and is a neuroprotective component of amyloid plaques in Alzheimer disease. *J Biol Chem* 2006; 281:29268-77; PMID:16849336; <http://dx.doi.org/10.1074/jbc.M600690200>
60. Ridgley DM, Ebanks KC, Barone JR. Peptide mixtures can self-assemble into large amyloid fibers of varying size and morphology. *Biomacromolecules* 2011; 12:3770-9; PMID:21879764; <http://dx.doi.org/10.1021/bm201005k>
61. Jarrett JT, Berger EP, Lansbury PT Jr. The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. *Biochemistry* 1993; 32:4693-7; PMID:8490014; <http://dx.doi.org/10.1021/bi00069a001>
62. Nussbaum JM, Schilling S, Cynis H, Silva A, Swanson E, Wangsanut T, Tayler K, Wiltgen B, Hatami A, Rönicke R, et al. Prion-like behaviour and tau-dependent cytotoxicity of pyroglutamylated amyloid- β . *Nature* 2012; 485:651-5; PMID:22660329; <http://dx.doi.org/10.1038/nature11060>
63. Ono K, Takahashi R, Ikeda T, Yamada M. Cross-seeding effects of amyloid β -protein and α -synuclein. *J Neurochem* 2012; 122:883-90; PMID:22734715; <http://dx.doi.org/10.1111/j.1471-4159.2012.07847.x>
64. Willander H, Presto J, Askarieh G, Biverstål H, Frohm B, Knight SD, Johansson J, Linse S. BRICHOS domains efficiently delay fibrillation of amyloid β -peptide. *J Biol Chem* 2012; 287:31608-17; PMID:22801430; <http://dx.doi.org/10.1074/jbc.M112.393157>
65. Yan LM, Velkova A, Tatarek-Nossol M, Andreetto E, Kapurniotu A. IAPP mimic blocks Abeta cytotoxic self-assembly: cross-suppression of amyloid toxicity of Abeta and IAPP suggests a molecular link between Alzheimer's disease and type II diabetes. *Angew Chem Int Ed Engl* 2007; 46:1246-52; PMID:17203498; <http://dx.doi.org/10.1002/anie.200604056>
66. Nicoll AJ, Panico S, Freir DB, Wright D, Terry C, Risse E, Herron CE, O'Malley T, Wadsworth JD, Farrow MA, et al. Amyloid- β nanotubes are associated with prion protein-dependent synaptotoxicity. *Nat Commun* 2013; 4:2416; PMID:24022506; <http://dx.doi.org/10.1038/ncomms3416>