Structural Biology of PrP Prions

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Prion diseases are characterized by the deposition of amyloids, misfolded conformers of the prion protein. The misfolded conformation is self-replicating, by a mechanism solely enciphered in the conformation of the protein. Because of low solubility and heterogeneous aggregate sizes, the detailed atomic structure of the infectious isoform is still unknown. Progress has, however, been made, and has allowed insights into the structural and disease-related mechanisms of prions. Many structural models have been proposed, and a number of them support a consensus trimeric β -helical model, significantly more complex than simple amyloid models. There is evidence that such complexity may be a necessary property of prion structure. Knowledge of the structure of prions will provide a greater understanding of the protein isoform conversion mechanism, and could eventually lead to rationally designed intervention strategies.

The central initiating event in prion diseases is the misfolding of a normal functional protein to acquire a β -sheet-rich, self-perpetuating structure. During this structural transition, the mammalian prion protein (PrP) is converted from its normal cellular isoform (PrP^C) to the infectious form (PrP^{Sc}), which serves as an autocatalytic template for the conformational change of PrP^C into PrP^{Sc}, promoting replication of its own isoform and spreading through the affected tissue (Prusiner 2007). The protein-only hypothesis states that this mechanism is solely enciphered in the structure of the PrP^{Sc} isoform (Prusiner 1982). The molecular structure of PrP^{Sc} is therefore particularly important to the understanding of self-replicating protein

conformations in neurodegenerative diseases, which could eventually lead to pharmaceutical intervention strategies.

The first structural information about PrP^{Sc} came from the search for the pathogen itself (Prusiner et al. 1981, 1982; Bolton et al. 1982). It was shown that PrP^{Sc} is insoluble and partially resistant to treatment with proteinase K (PK), in contrast to PrP^C (McKinley et al. 1983a; Pan et al. 1993). Furthermore, treatment of the highly infectious preparation with PK removed only ~65 amino acids from the N-terminal end of PrP^{Sc}, forming a molecule termed PrP 27–30, and did not reduce its infectious titer (McKinley et al. 1983a, 1991). This observation showed that the core of the prion conformation

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Figure 1. Electron micrograph of mouse PrP 27–30 (RML isolate). Fibrils exhibit a rod-like structure with diameters of ~5 nm. Scale bar, 100 nm. (From Wille et al. 2009a; reprinted, with permission, from the National Academy of Sciences © 2009.)

must reside in the PK-resistant C-terminal residues of PrP. This resistance to PK was used extensively in the development of purification methods for PrP^{Sc}; development of new experimental protocols allowed the preparation of high-titer, high-purity PrP^{Sc} and paved the way to obtaining more structural information. PrP^{Sc} was shown to form large aggregates; electron microscopy (EM) of high-titer fractions showed the presence of rod-shaped structures (Fig. 1), which exhibited the tinctorial properties of amyloid, including birefringence on binding Congo red (McKinley et al. 1983b; Prusiner et al. 1983). The similarity between PrP^{Sc} aggregates and the aggregates associated with Alzheimer's disease (AD) led to the speculation that PrP prion diseases and AD shared a common mechanism (Prusiner 1984), decades before the experimental proof that both diseases are based on self-propagating protein conformations (Holmes and Diamond 2012; Prusiner 2012).

The availability of highly purified prions encouraged more structural investigations of PrP^{Sc}. Fourier-transform infrared (FTIR) spectroscopy showed that PrP^{Sc} is rich in β -sheet structure (40%–50%), in contrast to PrP^C, which has very little (~3%) (Caughey et al. 1991; Pan et al. 1993). While it is recognized that amyloid properties and infectivity can be separated (Wille et al. 1996; Leffers et al. 2005), the realization that PrP^{Sc} has an amyloid structure has led to a multitude of structural studies

focusing on the amyloid state of natural and recombinant prion proteins.

AMYLOID STRUCTURE

Amyloids have commonly been described as sharing three properties: a long, unbranched fibrillar structure as seen by EM (Cohen and Calkins 1959), enhanced birefringence on binding Congo red (Divry and Florkin 1927), and cross- β structure (Astbury et al. 1935; Rudall 1946). Cross- β structure, which may be considered the defining feature of amyloid structure, consists of β -strands running approximately perpendicular to the fibril axis, forming long β -sheets running approximately in the direction of the axis. Despite this narrow definition, amyloids are derived from an enormous variety of denatured proteins, and in some cases form naturally functional, especially structural, proteins.

Astbury et al. (1935) first observed cross- β structure in an X-ray fiber diffraction study of denatured egg whites. The investigators recognized that the protein chains were arranged in what later came to be called β -sheets; early work on silk fibroin (Meyer and Mark 1928; Astbury 1933) had described the chain arrangement in a β -sheet, including the stabilizing main-chain hydrogen bonds, although these terms were not used. The denatured egg-white (albumin) structure, however, appears to be the first described in which the chains were running per-

pendicular rather than parallel to the fiber; this structure later came to be called cross- β . Fiber diffraction showed that amyloids had the cross- β structure (Eanes and Glenner 1968; Bonar et al. 1969), which was characterized in detail in a study of lacewing fly egg stalks (Geddes et al. 1968).

Fiber diffraction patterns from amyloids are characterized by strong diffracted intensity at about 4.75 Å in the meridional direction (parallel to the fibril axis), corresponding to the separation of strands in a β -sheet, and in many cases (including virtually all of the early work), broader but still distinct equatorial (perpendicular to the meridian) intensity at about 10 Å (Sunde et al. 1997; Jahn et al. 2010). The 10 Å intensity (whose position may vary considerably) derives from the distance between β -sheets stacked parallel to each other. This stacking is characteristic of the many amyloids formed by small peptides under suitable conditions, including many short peptide fragments of larger amyloidogenic proteins. Cross- β structures have been studied by fiber diffraction in molecules as simple as polyamino acids (Arnott et al. 1967; Fändrich and Dobson 2002), and in peptides ranging in size from as few as six amino-acid residues to full-length mammalian and yeast prions.

A wide variety of synthetic peptide amyloids have been studied by fiber diffraction. These include a number of fragments of the Alzheimer's-associated amyloid A β (Inouye et al. 1993), particularly A β (11–25) (Serpell et al. 2000; Sikorski et al. 2003), as well as full-length A β (Malinchik et al. 1998; Sikorski et al. 2003). Transthyretin (associated with familial amyloidotic polyneuropathy) and a ten-residue transthyretin fragment as well as amyloids isolated from patients have been studied (Blake and Serpell 1996; Sunde et al. 1997; Inouye et al. 1998). Diffraction patterns from full-length islet amyloid polypeptide (IAPP or amylin), associated with type II diabetes (Makin and Serpell 2004), and a fragment of IAPP (Sunde et al. 1997) have been reported. Kirschner's group has obtained fiber diffraction data from betabellins, designed proteins with amyloidogenic properties (Lim et al. 2000; Inouye et al. 2002).

In a series of important but difficult studies, fiber diffraction data were obtained from prion proteins and fragments (Nguyen et al. 1995; Inouye et al. 2000; Salmona et al. 2003). Although the quality of the data was limited, distinct cross- β patterns were obtained from a number of peptides, including several similar to PrP residues 106–126 (Tagliavini et al. 1993), as well as the 55-residue peptide MoPrP(89–143)P101L and the corresponding peptide from Syrian hamster.

While some investigators have required the 10 Å stacked- β -sheet intensity to characterize an amyloid, it is not strictly necessary, since several important examples have now been found of Congo-red-staining fibrils with cross- β structure, but without the stacked-sheet structure, and consequently without the dominant 10 Å intensity on the equator of the X-ray fiber diffraction pattern. These examples are architecturally more complex; they include the U-shaped two- β -strand structure found in A β (McDonald et al. 2012; Lu et al. 2013), which could be viewed as a two-sheet stacked-sheet structure, although the stacking is extremely irregular, and the β -solenoidal structure of the functional fungal prion HET-s (Van Melckebeke et al. 2010; Wan et al. 2012), which can only by a considerable stretch of the imagination be termed a stacked sheet. Both of these amyloids have been characterized structurally by solid-state nuclear magnetic resonance (ssNMR), which has proven to be a very powerful technique for the study of both fragments (Jaroniec et al. 2004) and complex amyloids (Lansbury et al. 1995; Lim et al. 2006; Shewmaker et al. 2006; Baxa et al. 2007; Luca et al. 2007; Wickner et al. 2008; Tuttle et al. 2016).

A number of small amyloidogenic peptides (four to seven residues) have been crystallized and their structures determined (Nelson et al. 2005; Sawaya et al. 2007; Apostol et al. 2011). While these crystal structures are not, strictly speaking, from amyloids, since they are not fibrillar structures, they do provide us with models of the molecular interactions in simple amyloids with unprecedented precision.

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PrP STRUCTURE

Prusiner et al. (1983) showed that PrP^{Sc} exhibits the fibrillar structure and Congo-red-binding properties of amyloids. Consistent with these observations, both PrP^{Sc} and PrP 27–30 were shown by FTIR and circular dichroism (Caughey et al. 1991; Gasset et al. 1993; Pan et al. 1993; Safar et al. 1993; Baron et al. 2011) to have very high β -sheet content. The high β -sheet content in PrP^{Sc} is in sharp contrast to that in PrP^C, which has been shown both spectroscopically (Pan et al. 1993) and by direct structure determination (Riek et al. 1996, 1997; Knaus et al. 2001) to be largely α -helical, with less than 10% β -sheet. Many early studies suggested that PrP 27–30 and PrP^{Sc} also contained substantial amounts of α -helix, although less than PrP^C. However, more recent studies (Baron et al. 2011; Smirnovas et al. 2011; Vázquez-Fernández et al. 2012) have found little or no α -helix in PrP 27–30 or PrP^{Sc}. Safar et al. (1993) also reported that PrP 27–30 contained no α -helical structure.

There has been no direct structure determination for PrP^{Sc}, but a remarkable variety of models has been proposed, all with a β -sheet core but having little else in common. The models can be broadly assigned to two groups, with either simple β -sheets or β -solenoids (also called β -helices) at the heart of the molecular fold. Simple sheet models have been developed by model building by Huang et al. (1996), Warwicker (2000), and Mornon et al. (2002), and by molecular dynamics simulation of the conversion of the PrP^C structure by DeMarco and Daggett (2004). All of these models were for single subunits. The Mornon et al. (2002) and DeMarco and Daggett (2004) models were both fitted into a trimeric density derived from EM by Wille et al. (2002). Warwicker (2000), Mornon et al. (2002), and DeMarco and Daggett (2004) constructed amyloid fibrils with cross- β -structure from their subunit models, although it is unclear how much distortion was required to satisfy the continuous hydrogen-bonding pattern in the fibrils. The Warwicker (2000) model has a repeating unit of two β -strands in the direction of the fibril axis, the

DeMarco and Daggett (2004) model four, and the Mornon et al. (2002) model five. These repeats should be considered in the light of the four-strand repeat found experimentally in fiber diffraction experiments by Wille et al. (2009a), discussed below. All of these models were developed before the most recent estimates of secondary structure content (Baron et al. 2011; Smirnovas et al. 2011; Vázquez-Fernández et al. 2012) and include substantial α -helical content, but the α -helices do not appear to interfere with the potential for fibrillization in any of them. Cobb et al. (2007) used site-directed spin labeling and electron paramagnetic resonance spectroscopy to study recombinant PrP (recPrP) amyloid, and developed an in-register parallel β -sheet model. This model did not include C-terminal α -helices. However, since the experimental data were derived from recPrP amyloid, whose structure is known to be very different from that of brain-derived PrP amyloid (Wille et al. 2009a), this model is not necessarily relevant to PrP^{Sc}, a possibility that the investigators did not discount.

A more complex stacked- β -sheet model has been proposed by Groveman et al. (2014). This model, in which the repeating unit has the thickness of a single β -strand, was derived from ssNMR studies of recPrP fibrils, but fibrillized by seeding from brain-derived PrP.

Downing and Lazo (1999) suggested that the β -structure in prions and other amyloids (Lazo and Downing 1997) could be β -helical rather than arranged in simple stacked β -sheets. Their model for PrP was constructed from intertwined chains forming a two-chain, antiparallel, eight-rung β -helix. Such a complicated β -helical fold has not been found in other protein structures and is not consistent with fiber diffraction data (Wille et al. 2009a). Nevertheless, the β -helix motif has been very useful in subsequent modeling studies.

Wille et al. (2002) used negative-stain EM to compare isomorphous two-dimensional crystals found in preparations of PrP 27–30 and a 106-residue fragment of PrP, PrP(Δ 23–88, Δ 141–176) (PrP^{Sc}106) (Muramoto et al. 1996). PrP^{Sc}106 had been shown to support prion propagation in transgenic mice (Supatta-

Figure 2. Trimeric model of PrP27–30 derived from two-dimensional crystals. (A) Two-dimensional crystals of MoPrP27–30 (RML isolate) prepared by PK digestion and precipitation using phosphotungstic acid. Scale bar, 100 nm. (B) Processed image of PrP 27–30 crystal lattice derived from A using averaging and symmetry. (Panels A and B are from Wille et al. 2009b; adapted, with permission, from the National Academy of Sciences © 2009.) (C) Trimeric model of mouse PrP 27–30 constructed using UCSF Chimera (Pettersen et al. 2004).

pone et al. 1999). Consideration of the packing of the PrP 27–30 molecules into the crystal lattice, the inferred locations of the *N*-linked oligosaccharides, and the β -sheet structure led these investigators to suggest that the core of the PrP 27–30 structure is a parallel β -helix. Govaerts et al. (2004) developed this model further, with improved EM data and a careful analysis of potential β -helical folds, taking into consideration a wide variety of known protein β -structures. They fitted projection maps at ~ 12 Å resolution with a trimeric model (Fig. 2) in which each PrP 27–30 subunit contained four turns of a left-handed β -helix, and suggested that PrP amyloid fibrils might consist of stacked trimers of PrP 27–30. The C-terminal portion of the molecule remained in its α -helical conformation.

Stork et al. (2005) presented two very similar four-layer β -helical models, both consistent with the density maps of Wille et al. (2002), but with different threading from the model of Govaerts et al. (2004). They refined these β -helical models in molecular dynamics simulations and found the β -helices to be stable. Yang et al. (2005) used the threading of Govaerts et al. (2004), but suggested that since there was no obvious stabilization of the trimer in the Govaerts model, a domain-swapping interaction between the trimer subunits could provide stability. In this model, the swapped domain of 11 amino acids corresponds to the first two

β -strands of the β -helix. Model building from the Govaerts model followed by molecular dynamics simulations found the domain-swapped model to be significantly more stable than the unmodified Govaerts model.

Langedijk et al. (2006) constructed left- and right-handed β -helices. However, neither Langedijk et al. (2006) nor Govaerts et al. (2004) were able to construct stable right-handed helical models. Molecular dynamics simulations by Langedijk et al. (2006) favored left-handed helices, and also found two-rung β -helices to be more stable than the four-rung β -helices of the Govaerts model. Again, the C-terminal α -helical helices were present, but did not appear to be important to the model structure.

Kunes et al. (2008) developed a number of models with separate left-handed β -helices in both the N- and C-terminal segments of the PrP protein. These models do not contain an α -helical structure, consistent with the data and interpretations of Baron et al. (2011), Smirnovas et al. (2011), and Vázquez-Fernández et al. (2012). A major problem with this group of models, however, is that they are specifically designed to fit recombinant mammalian PrP fibrils; the fibril model depends on the low-resolution negative-stain EM reconstruction of Tattum et al. (2006), which used recPrP amyloid.

The β -helical models of Govaerts et al. (2004), Stork et al. (2005), and Yang et al. (2005) are very similar, differing primarily in

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Figure 3. Fiber diffraction patterns from PrP amyloids. Black arrows indicate cross- β meridional diffraction at close to 4.8 Å resolution. (A) Syrian hamster PrP 27–30, strain Sc237. The inset in the center of the pattern uses a different color table to show an intense 63 Å reflection, indicative of the lateral packing of the fibrils. (B) Mouse PrP 27–30, RML isolate, purified by a different protocol. The inset uses a different color table to show the weak, broad 4.8 Å diffraction. White arrows indicate second and third orders of meridional 19.2 Å diffraction, indicative of a four- β -strand repeating unit. Because of the different purification methods, equatorial intensities are seen more clearly in A, meridional intensities in B. (C) Recombinant Syrian hamster PrP (residues 90–231) amyloid. White arrow, broad equatorial diffraction at about 10.5 Å resolution, not seen in A or B. (From Wille et al. 2009a; reprinted, with permission, from the National Academy of Sciences © 2009.)

details of threading. Details of threading remain uncertain, but the combination of EM, structural bioinformatics, and molecular dynamics stability studies appears to support a consensus on the trimeric β -helical model, consistent with the EM reconstructions of Wille et al. (2002) and Govaerts et al. (2004). While there are still many uncertainties about the structure of the C-terminal part of PrP, which is α -helical in all of these models, the models place this part of the protein molecule on the outside of the fibril, and none of them depends on the α -helical conformation for structural integrity or amyloidogenesis.

Fiber diffraction patterns from infectious brain-derived PrP 27–30 (Fig. 3) were obtained by Wille et al. (2009a) and compared with diffraction calculated from a large number of model structures. The patterns confirmed the cross- β structure of the fibrils, and were consistent with a fibril structure resembling a solid cylinder (for example, a β -helix) of diameter about 55 Å, rather than any type of stacked-sheet structure (Fig. 4). Low-angle diffraction from some samples corresponded to hexagonal paracrystalline packing of filaments with a unit cell edge of 72 Å, close to the spacing of the PrP 27–30 trimers measured from two-dimensional

crystal lattices (Wille et al. 2002, 2007; Govaerts et al. 2004). Many of the diffraction patterns included meridional diffraction (in the direction of the fibril axis) corresponding to the second, third, and fourth orders of a 19.2 Å axial repeat in the fibril structure, the size of a four-stranded β -sheet. The diffraction data thus supported both the general cylindrical shape of the β -solenoid and the four-rung structure of the Govaerts et al. (2004) model. Molecular volume calculations using the unit cell edge of 72 Å also supported the four-rung structure, and specifically excluded the possibility of a one-rung structure.

The 19.2 Å axial repeat corresponding to a four-rung β -helical structure has also been observed for PrP^{Sc}106 fibrils (Wan et al. 2015). This observation raises a question about the relationship between the PrP^{Sc}106 and PrP 27–30 structures, since the amino acid sequence of PrP^{Sc}106 is not consistent with the proposed threading of the PrP sequence into a β -solenoid (Govaerts et al. 2004). This question could be resolved by an alternative threading, or it may be that PrP^{Sc}106 adopts an alternative four-rung self-propagating structure.

Wille et al. (2009a) also obtained diffraction patterns (Fig. 3C) from infectious recombinant

Figure 4. Observed and calculated diffraction patterns for β -helical and stacked-sheet amyloid models. (A) Diffraction pattern from Syrian hamster PrP 27–30. (B) Calculated diffraction from a disordered noncrystalline trimeric β -helical model. (C) Model used to calculate data in B. (D) Diffraction pattern from recombinant Syrian hamster PrP 27–30 amyloid. (E) Calculated diffraction from a stacked-sheet model. (F) Model used to calculate data in E. In C and F, the filament axis is perpendicular to the figure plane. (From Wille et al. 2009a; reprinted, with permission, from the National Academy of Sciences © 2009.)

prions (Legname et al. 2004). Fibrils formed by recPrP have often been used for structural studies (Tattum et al. 2006; Cobb et al. 2007; Kunes et al. 2008; Groveman et al. 2014), although in most cases the recombinant material was not infectious at all. Groveman et al. (2014) seeded recPrP with brain-derived PrP^{Sc} in an attempt to make PrP^{Sc}-like recPrP amyloids. The resulting amyloid had PK-resistant fragments similar to those of PrP^{Sc}, although infectivity was not reported. Even the infectious recPrP diffraction patterns of Wille et al. (2009a) were distinctly different from those of brain-derived prions (Fig. 4). Although, like the brain-derived prion patterns, they showed the presence of cross- β structure, they were characteristic (Wille et al. 2009a; Wan et al. 2014b) of patterns from stacked sheets rather than β -helices, with broad, strong equatorial diffracted intensity at about 10 Å resolution, one of the hallmarks of stacked-sheet structure. Diffrac-

tion patterns from a synthetic prion isolate twice-passaged through mice, however, were almost indistinguishable from those of the naturally occurring RML prion isolate. These observations make it clear that the amyloid structure of infectious recombinant prions is very different from that of natural prions. They leave open the question of the structural basis for the infectivity of the recombinant prions (which is much less than that of brain-derived prions). Among other hypotheses, the infectivity of the recombinant amyloid could be because of a small fraction of molecules that do not make a detectable contribution to the fiber diffraction patterns, but are similar in structure to the brain-derived prions. Alternatively, the predominant structure of the recombinant amyloid may represent an incompletely matured form of PrP amyloid on a pathway leading to the fully replication-competent infectious prion structure. Such a pathway could involve het-

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erogeneous seeding processes, whereby an amyloid structure could nucleate the formation of a related but different architecture. Processes such as these could be important in the phenomena of prion strain adaptation and the crossing of species barriers (Makarava et al. 2011, 2012; Wan et al. 2013).

STRUCTURAL COMPLEXITY

The consensus β -helical models, as well as most of the competing models, are much more complex than the stacked-sheet amyloid models that have usually been presented to describe simpler amyloids such as those formed by short synthetic peptides (Sawaya et al. 2007; Jahn et al. 2010). A question that arises is whether this complexity is a necessary property of prion structure, beyond the structural elements found in simpler non-self-propagating amyloid structures (Wan and Stubbs 2014a; Wan et al. 2015).

The inherent difficulties in purification and high degree of structural disorder in PrP^{Sc} make this question difficult to answer. One approach to the difficulty is to take advantage of the greater tractability of other self-propagating amyloids, including non-mammalian prions and amyloids associated with other diseases. Non-mammalian prions, such as those found in yeasts and fungi, may be functional rather than pathological, better ordered than pathological prions, and more tractable to structural studies. The fungal prion HET-s has been particularly useful, and has been studied by ssNMR (Wasmer et al. 2008a,b; Van Melckebeke et al. 2010), cryo-EM (Mizuno et al. 2011), and fiber diffraction (Wan et al. 2012, 2013; Wan and Stubbs 2014a,b,c). It is now widely held (Holmes and Diamond 2012; Prusiner 2012) that many or all pathological amyloids, such as those associated with Parkinson's and Alzheimer's diseases, share the self-propagating properties of PrP^{Sc}, and thus may themselves be considered to be prions. The Alzheimer's-associated peptide A β in particular has been well-characterized structurally (see Tycko 2016), particularly by ssNMR (Petkova et al. 2006; Paravastu et al. 2008; Ahmed et al. 2010; Bertini et al. 2011; McDonald et al. 2012; Lu et al. 2013), fiber diffraction (Malin-

chik et al. 1998; Sikorski et al. 2003; Jahn et al. 2010; McDonald et al. 2012; Pauwels et al. 2012), and molecular dynamics simulations (Buchete et al. 2005; Fawzi et al. 2007; Miller et al. 2011). The Parkinson's-associated amyloid protein α -synuclein has also been characterized by ssNMR and fiber diffraction (Tuttle et al. 2016).

The complexity of self-propagating prion structures is illustrated by the β -solenoidal structure of HET-s, the Greek-key structure of α -synuclein, and even by the relatively simpler U-shaped two- β -strand structures of A β . Not only are these structures more complex than stacked sheets, but the structures, fibrillization properties, and, in the case of A β , clinical presentation, are strongly affected by mutations at the ends of the β -strands, and even in the loops connecting the strands (the loop between the two strands in A β and the loop connecting the two rungs of the solenoid in HET-s). The boundary between the first β -strand and the connecting loop in A β includes residues 21–23, which are often altered in families exhibiting early-onset AD (Levy et al. 1990; Hendriks et al. 1992; Miravalle et al. 2000; Grabowski et al. 2001; Nilsberth et al. 2001; Tomiyama et al. 2008). In HET-s, mutations in the connecting loop can have a large impact on fibrillization kinetics, stability, and structure of the fibril (Wan and Stubbs 2014a), as well as infectivity (Ritter et al. 2005), despite being in a region without β -structure.

Both HET-s and A β fibrils exhibit structural polymorphism. The structure of HET-s may be β -solenoidal or stacked-sheet (Mizuno et al. 2011; Wan et al. 2013), although the stacked-sheet form has little or no infectivity (Sabaté et al. 2007). This polymorphism is strongly reminiscent of the two structures observed for PrP, β -solenoidal for brain-derived PrP^{Sc} and stacked sheet for recPrP (Wille et al. 2009a). Under low-humidity drying conditions, the β -solenoid may collapse into a structure resembling stacked sheets (Wan and Stubbs 2014b), but the two-rung structure, quite distinct from the generic stacked-sheet structure (Wan et al. 2013), is maintained. A β forms a variety of fibril polymorphs in vitro, including two- and three-stranded fibrils (Petkova et al. 2005, 2006; Paravastu et al. 2008), and there are distinct struc-

tural differences at the molecular level between the three-stranded polymorph formed in vitro and a morphologically similar form formed by seeding with extract from human brain tissue. In addition, structures seeded from different patients were different from one another (Lu et al. 2013). These and other observations suggest that structural polymorphism is correlated with differences in strains of A β prions (Meyer-Luehmann et al. 2006; Stöhr et al. 2014), as it is in PrP (Legname et al. 2006) and yeast prions (Tanaka et al. 2006; Toyama and Weissman 2011). Structural differences have been observed between different amyloid forms of PrP (Ostapchenko et al. 2010), although these observations were made on recPrP fibrils, which have not been shown to adopt the structure of infectious PrP^{Sc} fibrils (Wille et al. 2009a).

Because the insolubility and inherent disorder of PrP prions have made their structures difficult to study, many investigators have preferred to work with peptide fragments, which might serve as structural and functional models for biologically active prions. These fragments have varied in size from six residues (Sawaya et al. 2007; Apostol et al. 2011) to 106 (Muramoto et al. 1996; Supattapone et al. 1999) or longer. PrP 27–30 is itself a fragment of PrP^{Sc}, although it includes most or all of the structurally ordered part of PrP^{Sc} and is fully infectious (McKinley et al. 1991). Wan et al. (2015) studied a number of these fragments by fiber diffraction. They found that the shortest fragments examined, including a 21-residue peptide human PrP(106–126) known to be neurotoxic although not shown to be infectious (Forloni et al. 1993; Walsh et al. 2009), formed generic stacked sheets, most probably with antiparallel β -strands. The 55-residue peptide MoPrP(89–143)P101L is infectious in transgenic mice expressing PrP(P101L) at a level similar to that of wild-type PrP (Kaneko et al. 2000; Tremblay et al. 2004). It appears to have the structure of a two-rung β -solenoid, and also appears to share with HET-s (Wan and Stubbs 2014b) the property of collapsing into a two-rung stacked sheet (a distorted solenoid) at low humidity. PrP^{Sc}106 shared the 19.2-Å repeat of PrP 27–30, and may be the best model among these

peptides for PrP^{Sc}. However, it exhibits disorder comparable to and often greater than that of PrP 27–30, and only yields acceptable diffraction data with difficulty. Comparisons of PrP^{Sc}106, PrP 27–30, and PrP^{Sc} may provide useful insights but, in general, longer fragments model the complexity of PrP 27–30 and PrP^{Sc} to a limited extent at best, and short fragments, although useful as sources of high-quality structural data relevant to amyloid structure in general, tell us little about the complexity required for self-propagation.

In some cases, the complexity of prion structures allows a variety of amyloid structures to form. These alternative structures are believed to be the basis of the phenomenon of prion strains. Strains are characterized by variation in phenotype (for example, behavioral variation among strains of PrP prions) (Pattison et al. 1961; Prusiner 2007), but the underlying basis of strain variation is believed to be structural variation. While self-propagation of prion structures generally refers to faithful replication of structure, prions of a given structure may on occasion seed the formation of different structures in the process of heterogeneous seeding. This phenomenon has been observed and characterized for HET-s (Wan et al. 2013) and PrP (Makarava et al. 2011, 2012). These observations showed that amyloids with distinctly different architectures can interact with one another, and that heterogeneous seeding does not require in vivo cofactors. The existence of heterogeneous seeding suggests that a preferred prion structure can be reached regardless of the structure of the nucleating agent. In the case of recPrP, this adaptation may require several passages through animals (Colby et al. 2009; Makarava et al. 2012; Ghaemmaghami et al. 2013). Heterogeneous seeding can thus provide a relatively simple mechanism for the transition from recPrP amyloid to infectious PrP^{Sc} prion, and more significantly in vivo, for strain adaptation and interspecies prion transmission.

CONCLUDING REMARKS

The difficulties inherent in structural studies of PrP^{Sc} remain substantial. Prusiner (2007) has

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provided an extensive list of structural studies of recombinant and synthetic PrP and PrP fragments. Wan et al. (2015) have compared the structural information available from a series of fragments of PrP varying in size from 21 to 106 amino acids. Wille et al. (2009a) compared the structures of brain-derived and infectious recombinant PrP prions. Two major problems emerge: the smaller fragments do not reflect the PrP^{Sc} structure, and recPrP amyloid does not have the same structure as brain-derived PrP^{Sc}. Low solubility and heterogeneous aggregate sizes are still a major challenge for high-resolution structural studies on PrP^{Sc}.

Nevertheless, great progress has been made over the past 20 years. The view that PrP^{Sc} has a β -helical structure (Govaerts et al. 2004; Stork et al. 2005; Yang et al. 2005; Wille et al. 2009a) has received considerable support, although it is not universally accepted and details of the structure at the molecular level remain uncertain. Some apparent difficulties are actually proving to be helpful in understanding the structural and pathological mechanisms involved in prion disease. The difference between the stacked-sheet structure of recPrP amyloid and the β -helical structure of brain-derived PrP^{Sc} has informed consideration of such structure-dependent phenomena as prion strains and species barriers (Wille et al. 2009a; Wan et al. 2013).

Finally, the recognition that many pathological amyloids share the self-propagating properties of PrP^{Sc}, and may thus be considered to be prions, greatly broadens the field of structural studies of prions. Studies of smaller and better-ordered prions such as A β , α -synuclein, and HET-s should considerably help our understanding of large, complex, disordered prions such as PrP.

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REFERENCES

*Reference is also in this collection.

- Ahmed M, Davis J, Aucoin D, Sato T, Ahuja S, Aimoto S, Elliot JI, Van Nostrand WE, Smith SO. 2010. Structural conversion of neurotoxic amyloid- β_{1-42} oligomers to fibrils. *Nat Struct Mol Biol* **17**: 561–567.
- Apostol MI, Wiltzius JJW, Sawaya MR, Cascio D, Eisenberg D. 2011. Atomic structures suggest determinants of transmission barriers in mammalian prion disease. *Biochemistry* **50**: 2456–2463.
- Arnott S, Dover SD, Elliott A. 1967. Structure of β -poly-L-alanine: Refined atomic co-ordinates for an anti-parallel β -pleated sheet. *J Mol Biol* **30**: 201–208.
- Astbury WT. 1933. Some problems in the X-ray analysis of the structure of animal hairs and other protein fibres. *Trans Faraday Soc* **29**: 193–205.
- Astbury WT, Dickinson S, Bailey K. 1935. The X-ray interpretation of denaturation and the structure of the seed globulins. *Biochem J* **29**: 2351–2360.
- Baron GS, Hughson AG, Raymond GJ, Offerdahl DK, Barton KA, Raymond LD, Dorward DW, Caughey B. 2011. Effect of glycans and the glycosphosphatidylinositol anchor on strain dependent conformations of scrapie prion protein: Improved purifications and infrared spectra. *Biochemistry* **50**: 4479–4490.
- Baxa U, Wickner RB, Steven AC, Anderson DE, Marekov LN, Yau W, Tycko R. 2007. Characterization of β -sheet structure in Ure2p1-89 yeast prion fibrils by solid-state nuclear magnetic resonance. *Biochemistry* **46**: 13149–13162.
- Bertini I, Gonnelli L, Luchinat C, Mao J, Nesi A. 2011. A new structural model of A β 40 fibrils. *J Am Chem Soc* **133**: 16013–16022.
- Blake C, Serpell LC. 1996. Synchrotron X-ray studies suggest that the core of the transthyretin amyloid fibril is a continuous β -sheet helix. *Structure* **4**: 989–998.
- Bolton DC, McKinley MP, Prusiner SB. 1982. Identification of a protein that purifies with the scrapie prion. *Science* **218**: 1309–1311.
- Bonar L, Cohen AS, Skinner MM. 1969. Characterization of the amyloid fibril as a cross- β protein. *Proc Soc Exp Biol Med* **131**: 1373–1375.
- Buchete N, Tycko R, Hummer G. 2005. Molecular dynamics simulations of Alzheimer's β -amyloid protofilaments. *J Mol Biol* **353**: 804–821.
- Caughey BW, Dong A, Bhat KS, Ernst D, Hayes SE, Caughey WS. 1991. Secondary structure analysis of the scrapie-associated protein PrP 27–30 in water by infrared spectroscopy. *Biochemistry* **30**: 7672–7680.
- Cobb NJ, Sönnichsen FD, Mchaourab H, Surewicz WK. 2007. Molecular architecture of human prion protein amyloid: A parallel, in-register β -structure. *Proc Natl Acad Sci* **104**: 18946–18951.
- Cohen AS, Calkins E. 1959. Electron microscopic observations on a fibrous component in amyloid of diverse origins. *Nature* **183**: 1202–1203.
- Colby DW, Giles K, Legname G, Wille H, Baskakov IV, DeArmond SJ, Prusiner SB. 2009. *Proc Natl Acad Sci* **106**: 20417–20422.

- DeMarco ML, Daggett V. 2004. From conversion to aggregation: Protofibril formation of the prion protein. *Proc Natl Acad Sci* **101**: 2293–2298.
- Divry P, Florkin M. 1927. Sur les propriétés optiques de l'amyloïde. *C R Soc Biol* **97**: 1808–1810.
- Downing DT, Lazo ND. 1999. Molecular modeling indicates that the pathological conformations of prion proteins might be β -helical. *Biochem J* **343**: 453–460.
- Eanes ED, Glenner GG. 1968. X-ray diffraction studies on amyloid filaments. *J Histochem Cytochem* **16**: 673–677.
- Fändrich M, Dobson CM. 2002. The behaviour of poly-amino acids reveals an inverse side chain effect in amyloid structure formation. *EMBO J* **21**: 5682–5690.
- Fawzi NL, Okabe Y, Yap E, Head-Gordon T. 2007. Determining the critical nucleus and mechanism of fibril elongation of the Alzheimer's $A\beta_{1-40}$ peptide. *J Mol Biol* **365**: 535–550.
- Forloni G, Angeretti N, Chiesa R, Monzani E, Salmona M, Bugiani O, Tagliavini F. 1993. Neurotoxicity of a prion protein fragment. *Nature* **362**: 543–546.
- Gasset M, Baldwin MA, Fletterick RJ, Prusiner SB. 1993. Perturbation of the secondary structure of the scrapie prion protein under conditions that alter infectivity. *Proc Natl Acad Sci* **90**: 1–5.
- Geddes AJ, Parker KD, Atkins EDT, Beighton E. 1968. "Cross- β " conformation in proteins. *J Mol Biol* **32**: 343–358.
- Ghaemmaghami S, Colby DW, Nguyen HB, Hayashi S, Oehler A, DeArmond SJ, Prusiner SB. 2013. Convergent replication of mouse synthetic prion strains. *Am J Pathol* **182**: 867–874.
- Govaerts C, Wille H, Prusiner SB, Cohen FE. 2004. Evidence for assembly of prions with left-handed β -helices into trimers. *Proc Natl Acad Sci* **101**: 8342–8347.
- Grabowski TJ, Cho HS, Vonsattel JP, Rebeck GW, Greenberg SM. 2001. Novel amyloid precursor protein mutation in an Iowa family with dementia and severe cerebral amyloid angiopathy. *Ann Neurol* **49**: 697–705.
- Groveman BR, Dolan MA, Taubner LM, Kraus A, Wickner RB, Caughey B. 2014. Parallel in-register intermolecular β -sheet architectures for prion-seeded prion protein (PrP) amyloids. *J Biol Chem* **289**: 24129–24142.
- Hendriks L, van Duijn CM, Cras P, Cruts M, van Hul W, van Harskamp F, Warren A, McInnis MG, Antonarakis SE, Martin J, et al. 1992. Presenile dementia and cerebral haemorrhage linked to a mutation at codon 692 of the β -amyloid precursor protein gene. *Nat Genet* **1**: 218–221.
- Holmes BB, Diamond MI. 2012. Cellular mechanisms of protein aggregate propagation. *Curr Opin Neurol* **25**: 721–726.
- Huang Z, Prusiner SB, Cohen FE. 1996. Scrapie prions: A three-dimensional model of an infectious fragment. *Fold Des* **1**: 13–19.
- Inouye H, Fraser PE, Kirschner D. 1993. Structure of β -crystallite assemblies formed by Alzheimer β -amyloid protein analogues: Analysis by X-ray diffraction. *Biophys J* **64**: 502–519.
- Inouye H, Domingues FS, Damas AM, Saraiva MJ, Lundgren E, Sandgren O, Kirschner DA. 1998. Analysis of X-ray diffraction patterns from amyloid of biopsied vitreous humor and kidney of transthyretin (TTR) Met30 familial amyloidotic polyneuropathy (FAP) patients: Axially arrayed TTR monomers constitute the protofilament. *Amyloid* **5**: 163–174.
- Inouye H, Bond J, Baldwin MA, Ball HL, Prusiner SB, Kirschner DA. 2000. Structural changes in a hydrophobic domain of the prion protein induced by hydration and by Ala $\rightarrow$ Val and Pro $\rightarrow$ Leu substitutions. *J Mol Biol* **300**: 1283–1296.
- Inouye H, Bond JE, Deverin SP, Lim A, Costello CE, Kirschner DA. 2002. Molecular organization of amyloid protofilament-like assembly of betabellin 15D: Helical array of β -sandwiches. *Biophys J* **83**: 1716–1727.
- Jahn TR, Makin OS, Morris KL, Marshall KE, Tian P, Sikorski P, Serpell LC. 2010. The common architecture of cross- β amyloid. *J Mol Biol* **395**: 717–727.
- Jaroniec CP, MacPhee CE, Bajaj VS, McMahon MT, Dobson CM, Griffin RG. 2004. High-resolution molecular structure of a peptide in an amyloid fibril determined by magic angle spinning NMR spectroscopy. *Proc Natl Acad Sci* **101**: 711–716.
- Kaneko K, Ball HL, Wille H, Zhang H, Groth D, Torchia M, Tremblay P, Safar J, Prusiner SB, DeArmond SJ, et al. 2000. A synthetic peptide initiates Gerstmann–Sträussler–Scheinker (GSS) disease in transgenic mice. *J Mol Biol* **295**: 997–1007.
- Knaus KJ, Morillas M, Swietnicki W, Malone M, Surewicz WK, Yee VC. 2001. Crystal structure of the human prion protein reveals a mechanism for oligomerization. *Nat Struct Biol* **8**: 770–774.
- Kunes KC, Clark SC, Cox DL, Singh RR. 2008. Left-handed β helix models for mammalian prion fibrils. *Prion* **2**: 81–90.
- Langedijk JPM, Fuentes G, Boshuizen R, Bonvin AMJJ. 2006. Two-rung model of a left-handed β -helix for prions explains species barrier and strain variation in transmissible spongiform encephalopathies. *J Mol Biol* **360**: 907–920.
- Lansbury PT, Costa PR, Griffiths JM, Simon EJ, Auger M, Halverson KJ, Kocisko DA, Hendsch ZS, Ashburn TT, Spencer RGS, et al. 1995. Structural model for the β -amyloid fibril based on interstrand alignment of an antiparallel-sheet comprising a C-terminal peptide. *Nat Struct Biol* **2**: 990–998.
- Lazo ND, Downing DT. 1997. β -helical fibrils from a model peptide. *Biochem Biophys Res Comm* **235**: 675–679.
- Leffers KW, Wille H, Stöhr J, Junger E, Prusiner SB, Riesner D. 2005. Assembly of natural and recombinant prion protein into fibrils. *Biol Chem* **386**: 569–580.
- Legname G, Baskakov IV, Nguyen HB, Riesner D, Cohen FE, DeArmond SJ, Prusiner SB. 2004. Synthetic mammalian prions. *Science* **305**: 673–676.
- Legname G, Nguyen HB, Peretz D, Cohen FE, DeArmond SJ, Prusiner SB. 2006. Continuum of prion protein structures enciphers a multitude of prion isolate-specified phenotypes. *Proc Natl Acad Sci* **103**: 19105–19110.
- Levy E, Carman MD, Fernandez-Madrid IJ, Power MD, Lieberburg I, van Duinen SG, Bots GTAM, Luyendijk W, Frangione B. 1990. Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type. *Science* **248**: 1124–1126.

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- Lim A, Makhov AM, Bond J, Inouye H, Connors LH, Grif-fith JD, Erickson BW, Kirschner DA, Costello CE. 2000. Betabellins 15D and 16D, de novo designed β -sandwich proteins that have amyloidogenic properties. *J Struct Biol* **130**: 363–370.
- Lim KH, Nguyen TN, Damo SM, Mazur T, Ball HL, Prusiner SB, Pines A, Wemmer DE. 2006. Solid-state NMR structural studies of the fibril form of a mutant mouse prion peptide PrP89–143(P101L). *Solid State Nucl Magn Reson* **29**: 183–190.
- Lu J, Qiang W, Yau W, Schwieters CD, Meredith SC, Tycko R. 2013. Molecular structure of β -amyloid fibrils in Alzheimer's disease brain tissue. *Cell* **154**: 1257–1268.
- Luca S, Yau W, Leapman R, Tycko R. 2007. Peptide conformation and supramolecular organization in amylin fibrils: Constraints from solid-state NMR. *Biochemistry* **46**: 13505–13522.
- Makarava N, Kovacs GG, Savtchenko R, Alexeeva I, Budka H, Rohwer RG, Baskakov IV. 2011. Genesis of mammalian prions: From non-infectious amyloid fibrils to a transmissible prion disease. *PLoS Pathog* **7**: e1002419.
- Makarava N, Kovacs GG, Savtchenko R, Alexeeva I, Ostapchenko VG, Budka H, Rohwer RG, Baskakov IV. 2012. A new mechanism for transmissible prion diseases. *J Neurosci* **32**: 7345–7355.
- Makin OS, Serpell LC. 2004. Structural characterisation of islet amyloid polypeptide fibrils. *J Mol Biol* **335**: 1279–1288.
- Malinchik SB, Inouye H, Szumowski KE, Kirschner DA. 1998. Structural analysis of Alzheimer's β (1–40) amyloid: Protofilament assembly of tubular fibrils. *Biophys J* **74**: 537–545.
- McDonald M, Box H, Bian W, Kendall A, Tycko R, Stubbs G. 2012. Fiber diffraction data indicate a hollow core for the Alzheimer's A β 3-fold symmetric fibril. *J Mol Biol* **423**: 454–461.
- McKinley MP, Bolton DC, Prusiner SB. 1983a. A protease-resistant protein is a structural component of the scrapie prion. *Cell* **35**: 57–62.
- McKinley MP, Bolton DC, Prusiner SB. 1983b. Fibril-like structures in preparations of scrapie prions purified from hamster brain. In *Proceedings of the Electron Microscopy Society of America*, pp. 802–803. San Francisco Press, San Francisco, CA.
- McKinley MP, Meyer RK, Kenaga L, Rahbar F, Cotter R, Serban A, Prusiner SB. 1991. Scrapie prion rod formation in vitro requires both detergent extraction and limited proteolysis. *J Virol* **65**: 1340–1351.
- Meyer KH, Mark H. 1928. Über den Aufbau des Seiden-Fibroins. *Ber Deutsch Chem Ges* **61**: 1932–1936.
- Meyer-Luehmann M, Coomaraswamy J, Bolmont T, Kaeser S, Schaefer C, Kilger E, Neuenschwander A, Abramowski D, Frey P, Jaton AL, et al. 2006. Exogenous induction of cerebral β -amyloidogenesis is governed by agent and host. *Science* **313**: 1781–1784.
- Miller Y, Ma B, Nussinov R. 2011. The unique Alzheimer's β -amyloid triangular fibril has a cavity along the fibril axis under physiological conditions. *J Am Chem Soc* **133**: 2742–2748.
- Miravalle L, Tokuda T, Chiarle R, Giaccone G, Bugiani O, Tagliavini F, Frangione B, Ghiso J. 2000. Substitutions at codon 22 of Alzheimer's A β peptide induce diverse conformational changes and apoptotic effects in human cerebral endothelial cells. *J Biol Chem* **275**: 27110–27116.
- Mizuno N, Baxa U, Steven AC. 2011. Structural dependence of HET-s amyloid fibril infectivity assessed by cryoelectron microscopy. *Proc Natl Acad Sci* **108**: 3252–3257.
- Mornon J, Prat K, Dupuis F, Boisset N, Callebaut I. 2002. Structural features of prions explored by sequence analysis. II. A PrP^{Sc} model. *Cell Mol Life Sci* **59**: 2144–2154.
- Muramoto T, Scott M, Cohen FE, Prusiner SB. 1996. Recombinant scrapie-like prion protein of 106 amino acids is soluble. *Proc Natl Acad Sci* **93**: 15457–15462.
- Nelson R, Sawaya MR, Balbirnie M, Madsen AØ, Riekel C, Grothe R, Eisenberg D. 2005. Structure of the cross- β spine of amyloid-like fibrils. *Nature* **435**: 773–778.
- Nguyen JT, Inouye H, Baldwin MA, Fletterick RJ, Cohen FE, Prusiner SB, Kirschner DA. 1995. X-ray diffraction of scrapie prion rods and PrP peptides. *J Mol Biol* **252**: 412–422.
- Nilsberth C, Westlind-Danielsson A, Eckman CB, Condron MM, Axelman K, Forsell C, Stenih C, Luthman J, Teplow DB, Younkin SG, et al. 2001. The “Arctic” APP mutation (E693G) causes Alzheimer's disease by enhanced A β protofibril formation. *Nat Neurosci* **4**: 887–893.
- Ostapchenko VG, Sawaya MR, Makarava N, Savtchenko R, Nilsson KPR, Eisenberg D, Baskakov IV. 2010. Two amyloid states of the prion protein display significantly different folding patterns. *J Mol Biol* **400**: 908–921.
- Pan K, Baldwin M, Nguyen J, Gasset M, Serban A, Groth D, Mehlhorn I, Huang Z, Fletterick RJ, Cohen FE, et al. 1993. Conversion of α -helices into β -sheets features in the formation of the scrapie prion proteins. *Proc Natl Acad Sci* **90**: 10962–10966.
- Paravastu AK, Leapman RD, Yau W, Tycko R. 2008. Molecular structural basis for polymorphism in Alzheimer's β -amyloid fibrils. *Proc Natl Acad Sci* **105**: 18349–18354.
- Pattison IH, Millson GC. 1961. Scrapie produced experimentally in goats with special reference to the clinical syndrome. *J Comp Pathol* **71**: 101–109.
- Pauwels K, Williams TL, Morris KL, Jonckheere W, Vandersteen A, Kelly G, Schymkowitz J, Rousseau F, Pastore A, Serpell LC, et al. 2012. Structural basis for increased toxicity of pathological A β 42:A β 40 ratios in Alzheimer disease. *J Biol Chem* **287**: 5650–5660.
- Petkova AT, Leapman RD, Guo Z, Yau W, Mattson MP, Tycko R. 2005. Self-propagating, molecular-level polymorphism in Alzheimer's β -amyloid fibrils. *Science* **307**: 262–265.
- Petkova AT, Yau W, Tycko R. 2006. Experimental constraints on quaternary structure in Alzheimer's β -amyloid fibrils. *Biochemistry* **45**: 498–512.
- Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. 2004. UCSF Chimera—A visualization system for exploratory research and analysis. *J Comput Chem* **15**: 1605–1612.
- Prusiner SB. 1982. Novel proteinaceous infectious particles cause scrapie. *Science* **216**: 136–144.
- Prusiner SB. 1984. Some speculations about prions, amyloid, and Alzheimer's disease. *N Engl J Med* **310**: 661–663.

- Prusiner SB. 2007. Prions. In *Fields virology* (ed. Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE), pp. 3059–3092. Lippincott Williams and Wilkins, Philadelphia.
- Prusiner SB. 2012. Cell biology. A unifying role for prions in neurodegenerative diseases. *Science* **336**: 1511–1513.
- Prusiner SB, McKinley MP, Groth DF, Bowman KA, Mock NI, Cochran SP, Masiarz FR. 1981. Scrapie agent contains a hydrophobic protein. *Proc Natl Acad Sci* **78**: 6675–6679.
- Prusiner SB, Bolton DC, Groth DF, Bowman KA, Cochran SP, McKinley MP. 1982. Further purification and characterization of scrapie prions. *Biochemistry* **21**: 6942–6950.
- Prusiner SB, McKinley MP, Bowman KA, Bolton DC, Bendheim PE, Groth DF, Glenner GG. 1983. Scrapie prions aggregate to form amyloid-like birefringent rods. *Cell* **35**: 349–358.
- Riek R, Hornemann S, Wider G, Billeter M, Glockshuber R, Wüthrich K. 1996. NMR structure of the mouse prion protein domain PrP(121–231). *Nature* **382**: 180–182.
- Riek R, Hornemann S, Wider G, Glockshuber R, Kurt Wüthrich K. 1997. NMR characterization of the full-length recombinant murine prion protein, mPrP(23–231). *FEBS Lett* **413**: 282–288.
- Ritter C, Maddelein M, Siemer AB, Lühns T, Ernst M, Meier BH, Saupé SJ, Riek R. 2005. Correlation of structural elements and infectivity of the HET-s prion. *Nature* **435**: 844–848.
- Rudall KM. 1946. The structure of epidermal protein. In *The society of dyers and colourists: Symposium on fibrous proteins*, pp. 15–23. Chorley and Pickersgill, Leeds, UK.
- Sabaté R, Baxa U, Benkemoun L, Sánchez de Groot N, Coullary-Salin B, Maddelein M, Malato L, Ventura S, Steven AC, Saupé SJ. 2007. Prion and non-prion amyloids of the HET-s prion forming domain. *J Mol Biol* **370**: 768–783.
- Safar J, Roller PP, Gajdusek DC, Gibbs CJ. 1993. Conformational transitions, dissociation, and unfolding of scrapie amyloid (prion) protein. *J Biol Chem* **268**: 20276–20284.
- Salmona M, Morbin M, Massignan T, Colombo L, Mazzoleni G, Capobianco R, Diomedea L, Thaler F, Mollica L, Musco G, et al. 2003. Structural properties of Gerstman-Sträussler-Scheinker disease amyloid protein. *J Biol Chem* **278**: 48146–48153.
- Sawaya MR, Sambashivan S, Nelson R, Ivanova MI, Sievers SA, Apostol MI, Thompson MJ, Balbirnie M, Wiltzius JJW, McFarlane HT, et al. 2007. Atomic structures of amyloid cross- β spines reveal varied steric zippers. *Nature* **447**: 453–457.
- Serpell LC, Blake CCF, Fraser PE. 2000. Molecular structure of a fibrillar Alzheimer's A β fragment. *Biochemistry* **39**: 13269–13275.
- Shewmaker F, Wickner RB, Tycko R. 2006. Amyloid of the prion domain of Sup35p has an in-register parallel β -sheet structure. *Proc Natl Acad Sci* **103**: 19754–19759.
- Sikorski P, Atkins EDT, Serpell LC. 2003. Structure and texture of fibrous crystals formed by Alzheimer's A β (11–25) peptide fragment. *Structure* **11**: 915–926.
- Smirnovas V, Baron GS, Offerdahl DK, Raymond GJ, Caughey B, Surewicz WK. 2011. Structural organization of brain-derived mammalian prions examined by hydrogen-deuterium exchange. *Nature Struct Mol Biol* **18**: 504–506.
- Stöhr J, Condello C, Watts JC, Bloch L, Oehler A, Nick M, DeArmond SJ, Giles K, DeGrado WF, Prusiner SB. 2014. Distinct synthetic A β prion strains producing different amyloid deposits in bigenic mice. *Proc Natl Acad Sci* **111**: 10329–10334.
- Stork M, Giese A, Kretschmar HA, Tavan P. 2005. Molecular dynamics simulations indicate a possible role of parallel β -helices in seeded aggregation of poly-Gln. *Biophys J* **88**: 2442–2451.
- Sunde M, Serpell LC, Bartlam M, Fraser PE, Pepys MB, Blake CCF. 1997. Common core structure of amyloid fibrils by synchrotron X-ray diffraction. *J Mol Biol* **273**: 729–739.
- Supattapone S, Bosque P, Muramoto T, Wille H, Aagaard C, Peretz D, Nguyen HB, Heinrich C, Torchia M, Safar J, et al. 1999. Prion protein of 106 residues creates an artificial transmission barrier for prion replication in transgenic mice. *Cell* **96**: 869–878.
- Tagliavini F, Prelli F, Verga L, Giaccone G, Sarma R, Gorevic P, Ghetti B, Passerini F, Ghibaudi E, Forloni G, et al. 1993. Synthetic peptides homologous to prion protein residues 106–147 form amyloid-like fibrils in vitro. *Proc Natl Acad Sci* **90**: 9678–9682.
- Tanaka M, Collins SR, Toyama BH, Weissman JS. 2006. The physical basis of how prion conformations determine strain phenotypes. *Nature* **442**: 585–589.
- Tattum MH, Cohen-Krausz S, Thumanu K, Wharton CW, Khalili-Shirazi A, Jackson GS, Orlova EV, Collinge J, Clarke AR, Saibil HR. 2006. Elongated oligomers assemble into mammalian PrP amyloid fibrils. *J Mol Biol* **357**: 975–985.
- Tomiyama T, Nagata T, Shimada H, Teraoka R, Fukushima A, Kanemitsu H, Takuma H, Kuwano R, Imagawa M, Ataka S, et al. 2008. A new amyloid β variant favoring oligomerization in Alzheimer's-type dementia. *Ann Neurol* **63**: 377–387.
- Toyama BH, Weissman JS. 2011. Amyloid structure: Conformational diversity and consequences. *Annu Rev Biochem* **80**: 557–585.
- Tremblay P, Ball HL, Kaneko K, Groth D, Hegde RS, Cohen FE, DeArmond SJ, Prusiner SB, Safar JG. 2004. Mutant PrP^{Sc} conformers induced by a synthetic peptide and several prion strains. *J Virol* **78**: 2088–2099.
- Tuttle MD, Comellas G, Nieuwkoop AJ, Covell DJ, Berthold DA, Kloepper KD, Courtney JM, Kim JK, Barclay AM, Kendall A, et al. 2016. Solid-state NMR structure of a pathogenic fibril of full-length human α -synuclein. *Nat Struct Mol Biol* **23**: 409–415.
- * Tycko R. 2016. Molecular structure of aggregated amyloid- β : Insights from solid-state nuclear magnetic resonance. *Cold Spring Harb Perspect Med* **6**: a024083.
- Van Melckebeke H, Wasmer C, Lange A, Ab E, Loquet A, Böckmann A, Meier BH. 2010. Atomic-resolution three-dimensional structure of HET-s(218–289) amyloid fibrils by solid-state NMR spectroscopy. *J Am Chem Soc* **132**: 13765–13775.
- Vázquez-Fernández E, Alonso J, Pastrana MA, Ramos A, Stitz L, Vidal E, Dynin I, Petsch B, Silva CJ, Requena JR. 2012. Structural organization of mammalian prions as probed by limited proteolysis. *PLoS ONE* **7**: e50111.

G. Stubbs and J. Stöhr

- Walsh P, Simonetti K, Sharpe S. 2009. Core structure of amyloid fibrils formed by residues 106–126 of the human prion protein. *Structure* **17**: 417–426.
- Wan W, Stubbs G. 2014a. The fungal prion HET-s as a model for structural complexity and self-propagation in prions. *Proc Natl Acad Sci* **111**: 5201–5206.
- Wan W, Stubbs G. 2014b. Fiber diffraction of the prion-forming domain HET-s(218–289) shows dehydration-induced deformation of a complex amyloid structure. *Biochemistry* **53**: 2366–2370.
- Wan W, Stubbs G. 2014c. Heterogeneous seeding of HET-s(218–289) and the mutability of prion structures. *Prion* **8**: 178–182.
- Wan W, Wille H, Stöhr J, Baxa U, Prusiner SB, Stubbs G. 2012. Degradation of fungal prion HET-s(218–289) induces formation of a generic amyloid fold. *Biophys J* **102**: 2339–2344.
- Wan W, Bian W, McDonald M, Kijac A, Wemmer DE, Stubbs G. 2013. Heterogeneous seeding of a prion structure by a generic amyloid form of the fungal prion-forming domain HET-s(218–289). *J Biol Chem* **288**: 29604–29612.
- Wan W, Wille H, Stöhr J, Kendall A, Bian W, McDonald M, Tiggelaar S, Watts JC, Prusiner SB, Stubbs G. 2015. Structural studies of truncated forms of the prion protein PrP. *Biophys J* **108**: 1548–1554.
- Warwick J. 2000. Modeling a prion protein dimer: Predictions for fibril formation. *Biochem Biophys Res Commun* **278**: 646–652.
- Wasmer C, Lange A, Van Melckebeke H, Siemer AB, Riek R, Meier BH. 2008a. Amyloid fibrils of the HET-s(218–289) prion form a β solenoid with a triangular hydrophobic core. *Science* **319**: 1523–1526.
- Wasmer C, Soragni A, Sabaté R, Lange A, Riek R, Meier BH. 2008b. Infectious and noninfectious amyloids of the HET-s(218–289) prion have different NMR spectra. *Angew Chem Int Ed* **47**: 5839–5841.
- Wickner RB, Dyda F, Tycko R. 2008. Amyloid of Rnq1p, the basis of the [PIN⁺] prion, has a parallel in-register β -sheet structure. *Proc Natl Acad Sci* **105**: 2403–2408.
- Wille H, Zhang G, Baldwin MA, Cohen FE, Prusiner SB. 1996. Separation of scrapie prion infectivity from PrP amyloid polymers. *J Mol Biol* **259**: 608–621.
- Wille H, Michelitsch MD, Guénebaud V, Supattapone S, Serban A, Cohen FE, Agard DA, Prusiner SB. 2002. Structural studies of the scrapie prion protein by electron crystallography. *Proc Natl Acad Sci* **99**: 3563–3568.
- Wille H, Govaerts C, Borovinskiy A, Latawiec D, Downing KH, Cohen FE, Prusiner SB. 2007. Electron crystallography of the scrapie prion protein complexed with heavy metals. *Arch Biochem Biophys* **467**: 239–248.
- Wille H, Bian W, McDonald M, Kendall A, Colby DW, Bloch L, Ollesch J, Borovinskiy AL, Cohen FE, Prusiner SB, et al. 2009a. Natural and synthetic prion structure from X-ray fiber diffraction. *Proc Natl Acad Sci* **106**: 16990–16995.
- Wille H, Shanmugam M, Murugesu M, Ollesch J, Stubbs G, Long JR, Safar JG, Prusiner SB. 2009b. Surface charges of polyoxometalates modulate polymerization of the scrapie prion protein. *Proc Natl Acad Sci* **106**: 3740–3745.
- Yang S, Levine H, Onuchic JN, Cox DL. 2005. Structure of infectious prions: Stabilization by domain swapping. *FASEB J* **19**: 1778–1782.

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