The Materials Science of Protein Aggregation

D.L. Cox, H. Lashuel, K.Y. C. Lee, and R.R.P. Singh

Abstract

Numerous human diseases are associated with conformational change and aggregation of proteins, including Alzheimer's, Parkinson's, prion diseases (such as mad cow disease), familial amyotrophic lateral sclerosis (ALS, or Lou Gehrig's disease), Huntington's, and type II (mature onset) diabetes. In many cases, it has been demonstrated that conformational change and aggregation can occur outside living cells and complex biochemical networks. Hence, approaches from materials and physical science have enhanced our understanding of the role of protein aggregation in these diseases at the molecular and nanoscale levels. In this article, we will review what is known about these protein structures from the perspective of materials science, focusing on the details of emergent oligomeric and nanotube-like structures, their interactions with model lipid bilayers, how the structures relate to observed biological phenomena, and how protein aggregation and amyloid formation can be employed for the good in biology and materials science.

Keywords: *amyloid diseases, complex adaptive matter, emergent behavior, prion diseases, protein aggregation, protofibrils, nanotubes, nanowires.*

Amyloid Diseases: A Truly Emergent Phenomenon

The primary goal of the study of complex adaptive matter is to identify the key organizing principles that govern materials phenomena at given length and time scales. In the case of the folding of individual globular proteins into their functional structures, we know a great deal. For example, the local rules governing α-helical secondary structure have been understood in detail for some 40 years;[1,2] indeed, the α-helix was predicted by Pauling and collaborators prior to discovery.[3] For the global tertiary structure of the protein, extensive experiments and simulation studies show that proteins engineered by evolution to experience minimal frustration in the interactions between closely contacted amino acids (or residues) develop funneled energy landscapes and relatively rapid (and multiple) pathways to folding after synthesis.[4] As discussed in the article by Ramirez in this issue, "frustration" refers to the effect of competing interactions that make it impossible to favorably lower the interaction energy.

However, we have comparatively little understanding of the organizing principles governing structure formation for proteins interacting with other proteins or membranes. This lack of knowledge is problematic, because proteins left alone tend to spontaneously aggregate, often by formation of β-sheet structures, which are not governed by local formation rules like α-helices (the distances between hydrogen bonding residues along the backbone can be great). β-sheets are formed from approximately linear stretches of peptide that hydrogen-bond from line to line. These structures are especially prone to protein aggregation due to favorable edge-to-edge hydrogen bonding between sheets.[5] This aggregation tendency is an obstacle in high-throughput proteomics, where one is interested in measuring the properties of individual proteins.[6]

Purposeful biological aggregation of monomeric proteins, as in the assembly of actin filaments or microtubules,[7] is usually highly regulated and energetically controlled (we will discuss tightly controlled and biologically useful β-sheet self-assembly later in the article). Living organisms have evolved an effective quality-control system to prevent protein misfolding and aggregation, where chaperone proteins provide "safe houses" for folding proteins,[8] and the ubiquitin/proteosome system ensures rapid degradation or disposal of misfolded proteins.

Table I conveys the tragic side of uncontrolled β-sheet self-assembly: it summarizes the key aspects of seven (out of dozens) prominent human amyloid diseases. Amyloid means "starch-like"—the aggregates stain like starch. These diseases typically arise in old- or middle-aged populations, and frequently arise spontaneously or sporadically rather than from genetic predisposition. Indeed, for spontaneous Alzheimer's, Parkinson's, prion, and immunoglobulin light chain diseases, incidence varies little between countries. Moreover, prion diseases, the lone infectious type of amyloid disease, show highly reproducible dose-versus-incubation-time distributions for intercerebrally inoculated animals.[9] Remarkably, infectious protein-only prion aggregates have been grown *in vitro*,[10] proving that the protein interaction properties alone, without additional biochemical guidance, account for the disease.

These observations suggest that these diseases can be studied from the perspective of materials growth, without extensive biological modulation. Indeed, it appears that the growth of amyloid structure has much in common with the oriented aggregation of inorganic, nearly monodispersed nanoparticles.[11] We seek here to portray the growing scientific movement toward the use of concepts and tools from materials science in the study of amyloidogenic proteins to elucidate the mechanisms of disease and design new materials.

Amyloid Structures: Plaques, Protein Nanotubes, and Oligomers

Plaques

The extracellular and/or intracellular accumulation of amyloid fibrils in the form of plaques or inclusions (Lewy bodies) in the brain is a defining hallmark for several neurodegenerative diseases (Figure 1). For example, Alzheimer's disease patients have large quantities of postmortem brain plaques, predominantly composed of 40–42-amino-acid-long Aβ peptides that are cleaved by protease proteins from the Alzheimer's precursor protein (BAPP).[13] These micrometer-scale plaques are composed of multi-polymeric strands of the peptide, called fibrils (Figure 1e), which

Table I: Aspects of Some Human Amyloid Diseases with Associated Protein Aggregation.

Disease	Protein/Peptide	Function	Heritable Component	Incidence	Onset Age* (Years)
Alzheimer's[51]	β-42 (from BAPP) and τ	?	~25%	~50% of post-85-yr-old population	>65
Parkinson's[52]	α-synuclein	?	5–10%	~1% of post-50-yr-old population	55–60 yrs
Huntington's[23]	huntingtin	?	100%	1 in 20,000 (Caucasian)	35–40
Familial ALS (Lou Gehrig's disease)[53]	Superoxide dismutase (20% of cases)	Lowers oxidative stress	100% (5–10% of all ALS)	2 in 10^6	46
Type II diabetes[54]	IAPP	?	High (obesity trigger)	14×10^6 per year (U.S.)	>40
Immunoglobulin light chain[55]	IG light chain	Immune response	Small/unknown	1 in 10^5	64
Prion diseases[35]	PrP^c	Lowers oxidative stress?	10–15%	~1 in 10^6	63

*Incidence/onset age is for non-heritable sporadic disease, unless otherwise noted.

have high quantities of β-sheet structure as revealed by crystallography, circular dichroism (which detects the different light polarization rotation tendencies of α-helices and β-sheets), and more recently, solid-state magnetic resonance studies. These β-strands are aligned perpendicular to the fibrillar axis, in a so-called "cross-β" structure, shown in Figure 1f. Postmortem plaques and inclusions from a variety of diseases are shown in Figure 1.[13] We note that the amyloid fibrils are protein nanotubes, hollow in the middle, with diameters of the order of 10–20 nm.

Despite a dominant research focus on both structure measurements and computer modeling on plaques, an emerging perspective is that the plaques may represent disease end points having little to do with toxicity.[14] This view is supported by observations such as (1) the abundant Aβ plaques observed in the brains of individuals displaying no symptoms of Alzheimer's disease and (2) the non-uniformity of plaque observation in prion diseases: for example, victims of kuru (a disease among the Fore people of New Guinea, arising from ritual cannibalism of deceased tribe members) exhibit them, while victims of spontaneous Creutzfeldt-Jakob disease usually do not.[15] In this view, toxicity is engendered by small β-sheet aggregates, possibly on the pathway to amyloid fibrils.

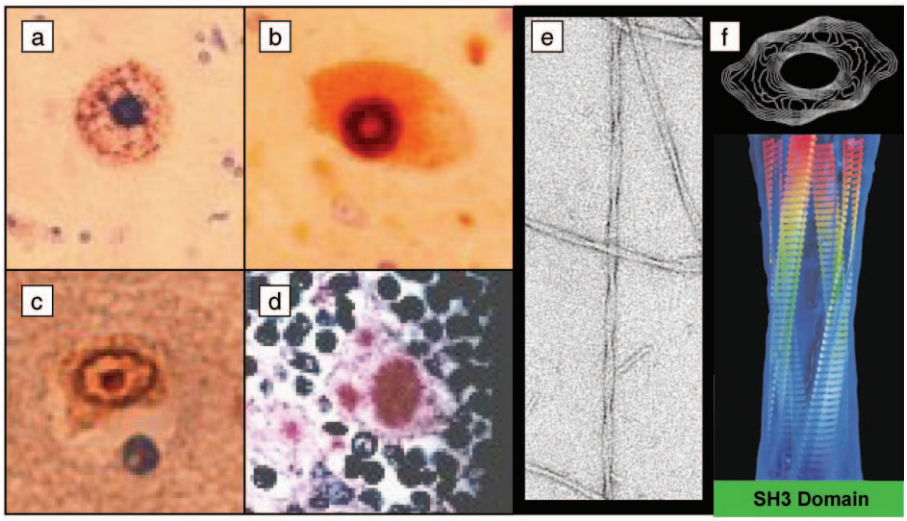

Figure 1. Plaques and fibrils. (a)–(d) Postmortem tissue plaques from human amyloid disease patients (from Reference 13): (a) Aβ42 (Alzheimer's) plaque, (b) α-synuclein (Parkinson's) plaque, (c) huntingtin plaque (Huntington's disease), and (d) PrP^{Sc} (kuru) plaque. (e) Transmission electron microscope images of amyloid fibrils (H. Lashuel, unpublished data). (f) Model of a hollow-core SH3 domain fibril: upper panel shows a density map cross section, lower panel shows the cross-β structure.[56]

β-Helices

Accordingly, attention has turned to small β-sheet motifs with multiple assembly outcomes, especially the left-handed β-helix (LHBH) structures shown in Figure 2,[16] recently proposed as the β-sheet unit for infectious mammalian prion trimers on the basis of cryogenic electron microscopic data.[17] The LHBH β-sheet structure has also been proposed for yeast prion-like proteins,[18] Alzheimer's disease,[19] and Huntington's disease.[16,20] This LHBH motif was first observed in several bacterial enzymes and the "antifreeze" protein of the spruce budworm; to date, there are 11 structures in the protein data bank (http://www.rcsb.org/pdb/) confirmed to have LHBHs. LHBHs are usually presumed (or, in two cases, observed)[21,22] to be in protein trimers. The LHBH motif has a fundamental repeat unit of triangular cross section, consisting of 18 amino acids with two per bend region and four per

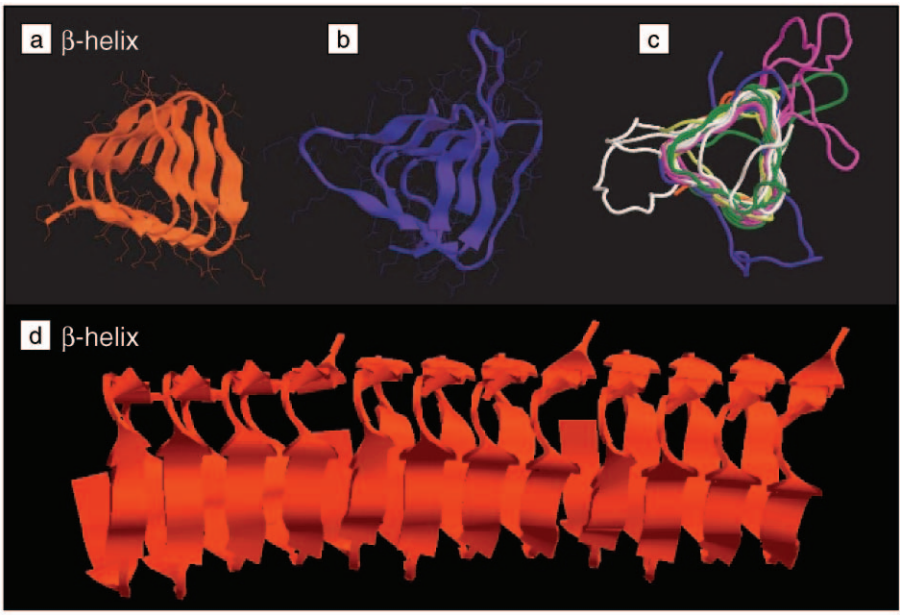

Figure 2. (a) Peptide backbone of measured left-handed β-helix (LHBH) structure of 1FWY protein. (b) Proposed LHBH structure of infectious prion protein (after Reference 16). Parallel, hydrogen-bonded β-sheets are shown as strips. (c) Superposed structure of six LHBH backbones, illustrating the uniform cross section of the helical structure (red, 1FWY protein; blue, proposed infectious prion protein; yellow, 1HMO protein; white, 1J2Z protein; green, 1L0S protein; fuchsia, 1T3D protein). (d) LHBH "nanotube" formed from a stack of three 1FWY helices. All images generated with the RASTOP molecular viewing program (see www.geneinfinity.org/rastop/); all files for the proteins 1FWY, 1HMO, 1J2Z, 1L0S, and 1T3D input to RASTOP are from the Protein Data Bank (http://www.rcsb.org), except for the PrP^{Sc} model, which is courtesy of C. Govaerts.

β-strand, alternating between hydrophobic and hydrophilic in the strand. The triangular cross section is very uniform across observed LHBHs (Figure 2c); each edge is ~1.9 nm in length. In Figure 2d, we show how multiple copies of the LHBH of an enzyme from *E. coli* (labeled 1FWY on the protein data base at www.rcsb.org/pdb) can be assembled into a kind of β-nanotube. This raises the natural and intriguing question: are amyloid fibrils composed of such "nanofilaments"?[19]

The LHBH has a tantalizing connection to Huntington's and other polyglutamine repeat diseases, where pathology derives from an inherited excess number of repeats of the amino acid glutamine on one end (the N-terminus) of the huntingtin protein.[23] A polyglutamine repeat number p of less than ~24 is normal; for $p > 36$, disease is certain. Note that 36 is the number of amino acids or residues in two turns of a LHBH that has fully saturated internal hydrogen bonds.[19]

The LHBH presents a challenge to theory and simulation. At present, this motif has not emerged from any molecular dynamics simulations or semi-analytic (Hamiltonian or cellular automata) approaches. The complicating factor is the long-range coupling along the backbone (bonded amino acids are separated by 18 residues). On the other hand, the remarkable conservation of the shape and helical cross section suggest that this is a motif ripe for study.

Nanoscale Oligomers

β-sheet oligomers obtained from *in vitro* growth of aggregates have been studied extensively.[13] One common form seems to be a spherical micelle-like aggregate of β-converted proteins, which have been implicated as precursors for the chain-like (protofibril) and annular oligomers that are also seen frequently during *in vitro* amyloid formation by most amyloidogenic proteins.[24] Figure 3 shows a compendium of oligomers and protofibrils from amyloid disease proteins.[25,26] The annular oligomers underlie the proposed toxicity mechanism discussed later in this article. We note that all atomic force microscope (AFM) measurements of spherical, chain-like, and annular oligomers share the same height and diameter, suggesting that the spherical aggregates are the precursors to the larger chain and annular pore-like structures.

The diversity of pre-fibrillar oligomer structures, the formation of which are highly dependent upon protein sequence and environmental conditions (e.g., pH, salt concentration, and levels of molecular crowding), may explain the mystery of prion disease strains. Strains, for a given mammal, have unique incubation-time versus dose distributions, tissue lesion profiles, and distributions of post-translationally attached sugars: the prion protein can have 0, 1, or 2 sugars attached.[27,28] Moreover, strains breed true upon multiple passage in animals. This means that upon passage from a diseased animal to a healthy animal, the same properties (incubation time, lesion profile, sugar binding) are preserved. There is considerable evidence that strain is encoded in prion conformation but no detailed understanding of the underlying mechanisms.[27,28] Prion oligomers[17] might have a spectrum of different shapes, and oriented aggregation of such protein "nanoparticles" might "breed" the conformation true (nonmatching shapes will be energetically unfavorable).

Domain Swapping

A separate way to generate fibrils and oligomers is by "domain swapping,"[29,30] in which, say, two identical copies (A,B) of a protein exchange a domain (a well-defined protein region attached to a flexible section of peptide). The domain of monomer A binds to the corresponding region of B and vice versa. The swapping is not limited to dimers; it can lead to filamentary structures in which the ith protein swaps with the $(i + 1)$th protein, for example, closed-chain oligomers (where filament ends are brought together to domain-swap) or two-dimensional structures.

Domain swapping has been proposed to play a role in prion strains.[31] Domain swapping in protein–protein interactions generically and amyloid diseases in particular seems certain to emerge as a critical theme in the coming years. At the conceptual level, the study of coarse-grained protein models with molecular dynamics has shown that the formation of domain-swapped dimers will proceed down a funneled landscape if the dimer enjoys minimal frustration.

Aggregation Pathways and Kinetics

AFM and kinetic modeling, well known to materials scientists, have been instrumental in advancing our understanding of the structural properties of the protein aggregates linked to disease and their growth kinetics. Figure 4 schematically shows accepted models on amyloid conversion/aggregation kinetics. We note

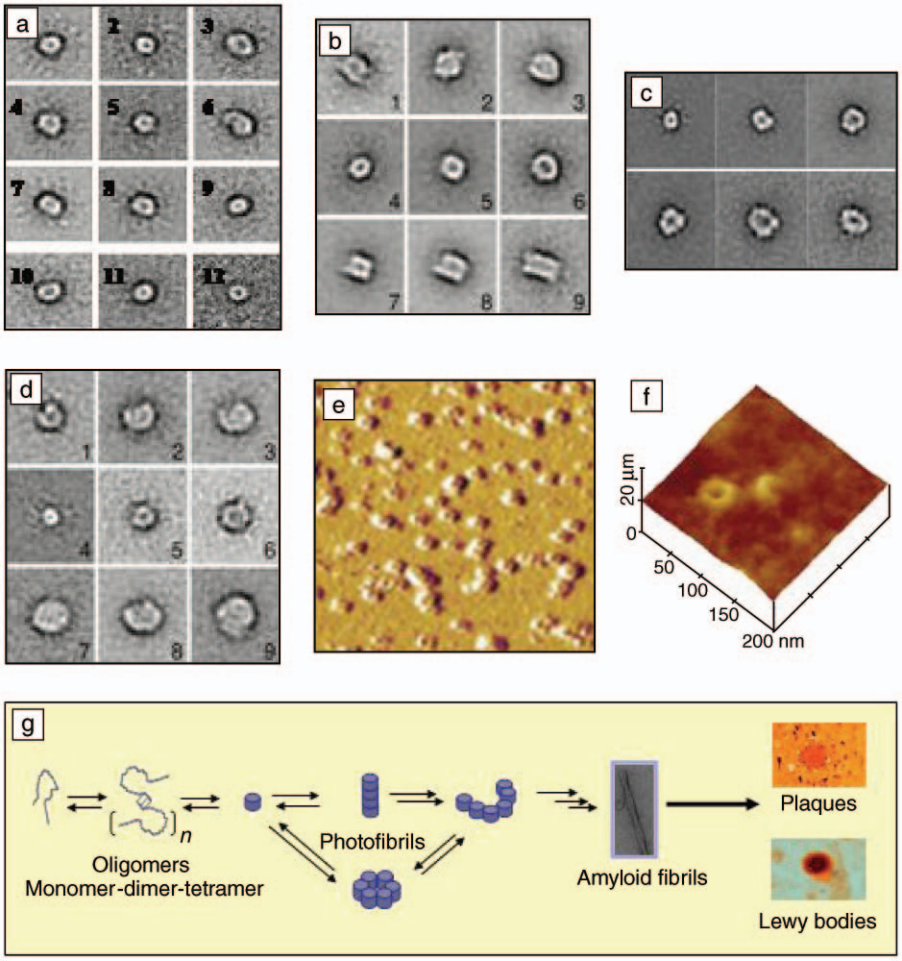

Figure 3. Amyloid oligomers. (a) Aβ peptide implicated in Alzheimer's disease, observed by electron microscopy (scale, 50 nm/box) (from Reference 24). (b) Nine different oligomers of mutant α-synuclein, implicated in Parkinson's disease, observed by electron microscopy (scale, 40.5 nm/box) (from Reference 25). (c) Six oligomers of mutant SOD1, implicated in familial ALS (from Reference 56).[57] (d) Nine mutant α-synuclein oligomers (scale, 40.5 nm/box) (from Reference 25). (e) Atomic force microscope (AFM) images of Aβ oligomers inserted in a supported lipid bilayer (from Reference 26). Clearly resolved annular oligomers have outer diameters of ~16 nm. (f) AFM image of α-synuclein oligomer on supported lipid bilayer (from Reference 25). (g) Schematic illustration of oligomerization pathways. Here, the subscript n *in the second stage represents the number of monomers in an oligomer.*

that β-sheet conversion is typically concomitant with aggregation (i.e., conformational changes and protein assembly are linked),[32,33] with the possible exception of polyglutamine proteins,[34] and requires templating either by spontaneously formed (and rare) nuclei or by external seeding of aggregates.[33] Also, fission of mammalian prions is necessary for disease propagation and exponentially growing aggregates in mammals; prion-like proteins in yeast have fission effected by another protein[35] (although the fission can occur spontaneously *in vitro*).[36] Why mammalian prions fission and other amyloidogenic proteins do not remains a mystery.

In vitro and theoretical studies of conversion/aggregation suggest that the late-age onset in amyloid diseases derives from slow underlying molecular processes. For example, *in vitro* kinetics experiments for polyglutamine peptides extrapolated to *in vivo* concentrations of huntingtin protein suggest that within the "sampling window" of a human lifespan, toxic aggregate concentrations should arise only for glutamine number $p > 36$, consistent with clinical observations.[34]

For prion diseases, theoretical modeling of two-dimensional aggregation and fission (prions live mostly on neuronal membranes) yielded a sporadic incubation time distribution that peaked at ~100 times that obtained from dilute seeding for physiological concentrations of the normally expressed protein called PrP^c that misfolds in the diseased form.[37] Given a mean incubation time for kuru of 12 years,[38] this suggests that endemic sporadic prion disease requires ~1000-year life spans! Meanwhile, the 1 in 10^6 sporadic disease background incidence may reflect the low-amplitude, pre-peak tail in the incubation time distribution.[38]

Amyloid–Membrane Interaction and Toxicity

Many amyloidogenic proteins associate with lipid membranes. AFM studies on supported bilayers and molecular modeling have helped support a potential unifying hypothesis for amyloid disease toxicity: that small oligomers pierce cell membranes, triggering cell death through superfluous ion pores. It was proposed in detail that a pair of β-coupled annular tetramers of Aβ peptides can insert into the neuronal membrane leaflets and create an ion pore.[39] *In vitro*-grown oligomers qualitatively consistent with this hypothesis have been found (Figure 3) and studied with AFM on supported bilayers (Figure 3f); these are evidently composed of 4 nm spherical oligomers, much larger than the peptide tetramers of Reference 39. These oligomers permit excess calcium flux, which is toxic to cultured neurons.[26] Intracerebral inoculation of rats and mice with a solution presumed rich in oligomeric Aβ particles leads to a reversible short-term memory deficit.[40] A recent theoretical study found strong correlations between the membrane insertion configuration and the pore model for 4 of 5 mutations leading to early-onset Alzheimer's disease.[41] While the pore model is not universally accepted, and may not apply to all the diseases (the prion trimer model, for example, will not allow ion passage), it remains a vibrant area of research.

Amyloids for Good in Biology and Materials Science

Biologically Useful Amyloids

Emerging evidence suggests that amyloid structures can provide useful biological functions. Some examples are

1. *Heritable amyloid structure in yeast.* As alluded to previously, prion-like proteins in yeast form aggregates that fission upon cell division, can actively confer phenotype, and may provide some stress protection.[42]
2. *Spider silk.* Spiders produce insoluble filaments of fibroin protein that possess 30× greater extensibility and toughness than steel. Recent circular dichroism studies show that significant amyloid-like cross-β structure develops in a region of reduced

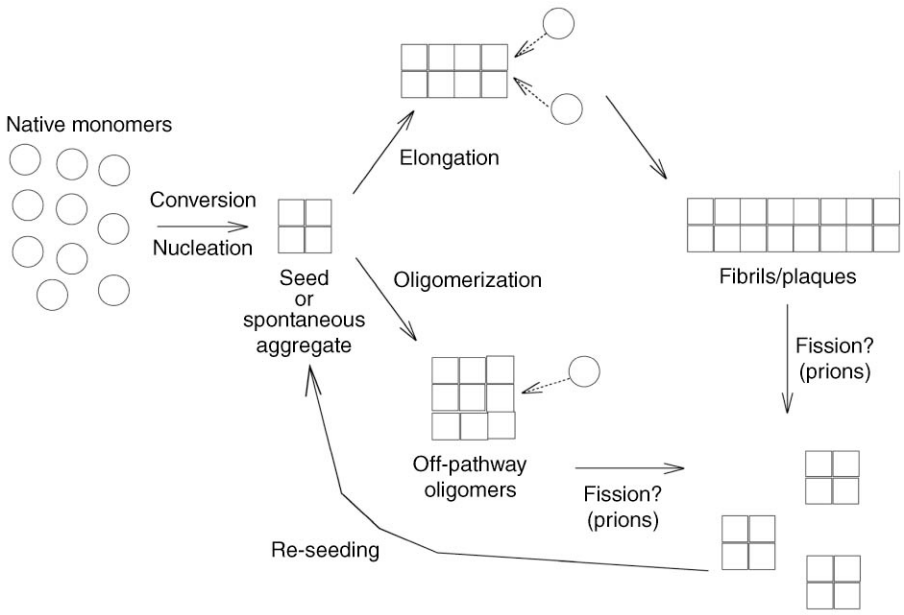

Figure 4. Schematic illustration of amyloid conversion and aggregation. Native monomers of the relevant peptides or proteins are shown as circles and high-β-content converted proteins as squares. Peptides can either spontaneously convert or be seeded. Subsequent aggregation and conversion can generate either proto-fibrils via the elongation step, which then form amyloid fibrils and plaques, or pass to "off-pathway" oligomers. In the case of prions, the oligomers and/or fibrils can fission, which then re-seeds the initial conversion/aggregation process. Prions are the only known amyloid proteins to spontaneously fission in the converted form either in vitro *or* in vivo*. It is assumed (but not shown explicitly here) that both oligomers and native monomers experience some level of removal by cellular systems* in vivo*.*

pH downstream from the initial extrusion site.[43]

3. *Chorion in fish and insect egg shells.* Truncated peptides from the central regions of two chorion proteins self-assembled into spherulites possessing β-structure, which then converted to fibrils upon maturation, suggesting amyloid character in actual egg shells.[44]

4. *Amyloid-like structure in synapses of Aplysia (slugs).* The N-terminus region of the *Aplysia* synapse protein CPEB is glutamine-rich, like huntingtin protein. Engineered expression of CPEB in yeast yields prion-like aggregates similar to the native ones discussed earlier; hence, prion-like states of CPEB might effect long-term strengthening of synaptic contacts.[45]

Amyloids in Materials Science

The regular diameter and periodicity of the amyloid fibrils make them good materials templates. One group employed yeast prion-like proteins to template ~100-nm-wide gold nanowire growth; the gold-coated protein filaments after initial decoration by small gold nanoparticles were found to bind to genetically engineered cysteine residues.[46] Silver nanowires of 20 nm width were grown *inside* filamentary cross-β tubes grown from a diphenylalanine peptide.[47] Engineering of controllably switched β-sheet materials could prove valuable for tissue growth scaffolding, as one example.[48] Hybrid molecules including eight amino acids—four per strand, but with non-amino bends—have been developed that controllably self-assemble into different β-sheet structures (as shown in Figure 5) depending upon the pH, analogous to spider silk.[49] Clearly, the future of engineered amyloid structures in materials science looks bright.[50]

Conclusion

In this article, we have developed themes that bring together the fields of amyloid diseases and materials science. Protein misfolding and aggregation phenomena are intimately linked to many serious public health issues. However, many aspects of the phenomena have close analogies in synthesized materials, and their full molecular understanding requires experimental and modeling tools more familiar in the physical and materials sciences. In addition to the possible medical breakthroughs that such multidisciplinary studies can lead to, there is a growing possibility that understanding the mechanisms of amyloid formation can have wide impact in fields ranging from basic neurobiology to materials science.

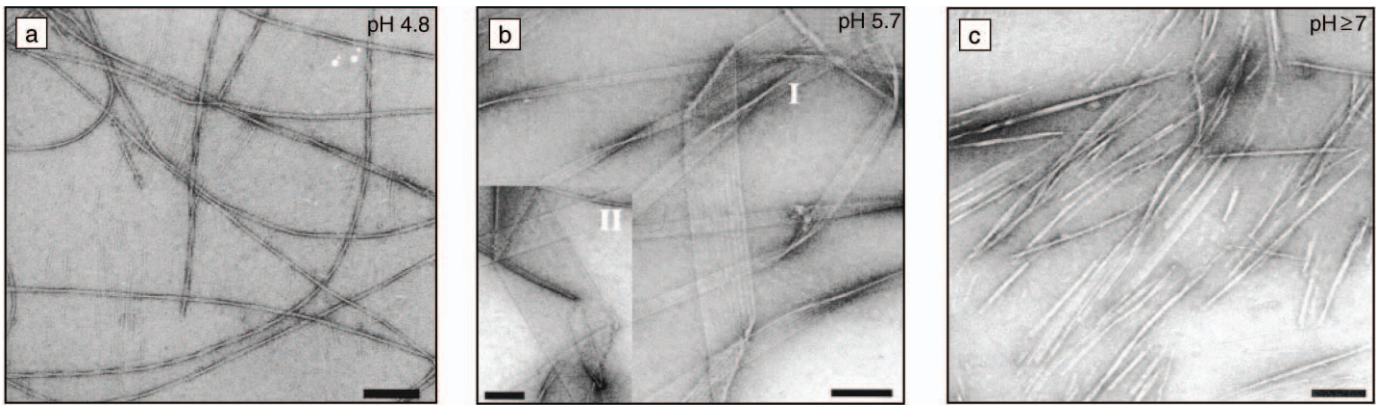

Figure 5. (a)–(c) Images from electron microscopy showing pH-dependent fibril growth of designed peptidomimetic molecules (from Reference 49). Scale bars are 100 nm.

Acknowledgments

D.L. Cox and R.R.P. Singh acknowledge the support of the U.S. Army Congressionally Directed Medical Research Fund, grant NP02013. D.L. Cox thanks the NSF Center for Theoretical Biological Physics, grant PHY 0120999, and S. Yang, H. Levine, and J. Onuchic for many useful discussions. We thank C. Govaerts for sharing the coordinates of the model prion trimer and R. Lal for sharing the second paper in Reference 26 prior to publication. This article is reflective of scientific discussions and presentations from two workshops on the physical properties of amyloids sponsored by the Institute for Complex Adaptive Matter (San Francisco, November/December 2001; Boston, February 2004).

References

1. B.H. Zimm and J.K. Bragg, *J. Chem. Phys.* **31 (**1958) p. 526.
2. S. Lifson and A.J. Roig, *J. Chem. Phys.* **34 (**1961) p. 1963.
3. L. Pauling, R.B. Corey, and H.R. Branson, *Proc. Natl. Acad. Sci. USA* **37** (1951) p. 205.
4. P.G. Wolynes, J.N. Onuchic, and D. Thirumalai, *Science* **267** (1995) p. 1619; J.N. Onuchic and P.G. Wolynes, *Curr. Opin. Struct. Biol.* **14** (2004) p. 70.
5. M. Stefani and C.M. Dobson, *J. Mol. Med.* **81** (2003) p. 678.
6. P.S. Lee and K.H. Lee, *Biotech. Bioeng.* **89** (2004) p. 195.
7. J.E. Bear, M. Krause, and F.B. Gertler, *Curr. Opin. Cell. Biol.* **13** (2001) p. 158; S. Inouye, *J. Struct. Biol.* **118** (1997) p. 87.
8. P.L. Clark, *Trends Biochem. Sci.* **29** (2004) p. 527.
9. S.B. Prusiner, J. Safar, and S.J. DeArmond, in *Prion Biology and Diseases* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2004) p. 143.
10. G. Legname, I.V. Baskakov, H.-O.B. Nguyen, D. Riesner, F.E. Cohen, S.J. DeArmond, S.B. Prusiner, *Science* **306** (2004) p. 673.
11. R.L. Penn, *J. Phys. Chem. B* **108** (2004) p. 12707.
12. P.H. St. George-Hyslop, *Sci. Am.* **283** (2000) p. 76.
13. All plaque/inclusion images are from the laboratory of M. Feany, http://feany-lab.bwh.harvard.edu/link2/ (accessed April 2005).
14. B. Caughey and P.T. Lansbury Jr., *Annu. Rev. Neurosci.* **26** (2003) p. 267.
15. S. J. deArmond, J.W. Ironside, E. Bouzamondo-Bernstein, D. Peretz, and J.R. Fraser, in *Prion Biology and Diseases* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2004) p. 777.
16. J. Jenkins and R. Pickersgill, *Prog. Biophys. Mol. Biol.* **77** (2001) p. 111.
17. C. Govaerts, H. Wille, S.B. Prusiner, and F.E. Cohen, *Proc. Nat. Acad. Sci. USA* **101** (2004) p. 8342.
18. A. Kishimoto, K. Hasegawa, H. Suzuki, H. Taguchi, K. Namba, and M. Yoshida, *Biochem. Biophys. Res. Commun.* **315** (2004) p. 739.
19. J.-T. Guo, R. Wetzel, and Y. Xu, *Proteins: Struct. Funct. Bioinformatics* **57** (2004) p. 357.
20. M.F. Perutz, J.T. Finch, J. Berriman, and A. Lesk, *Proc. Natl. Acad. Sci. USA* **99** (2002) p. 5591; R. Wetzel, *Structure* **10** (2002) p. 1031.
21. V.E. Pye, A.P. Tingey, R.L. Robson, and P.C.E. Moody, *J. Biol. Chem.* **279** (2004) p. 40729.
22. C. Kisker, H. Schindelin, B.E. Alber, J.G. Ferry, and D.C. Rees, *EMBO J.* **15** (1996) p. 2323.
23. H.Y. Zoghbi and H.T. Orr, *Annu. Rev. Neurosci.* **23** (2000) p. 217.
24. H.A. Lashuel, D.M. Hartley, B.M. Petre, J.S. Wall, M.N. Simon, T. Walz, and P.T. Lansbury, *J. Mol. Biol.* **332** (2003) p. 795.
25. H.A. Lashuel, B.M. Petre, J. Wall, M. Simon, R.J. Nowak, T. Walz, P.T. Lansbury, *J. Mol. Biol.* **322** (2002) p. 1089.
26. A. Parbhu, H. Lin, J. Thimm, and R. Lal, *Peptides* **23** (2002) p. 1265; A. Quist, I. Doudevski, H. Lin, R. Azimova, D. Ng, B. Frangione, B. Kagan, J. Ghiso, and R. Lal, et al., preprint submitted to *Nat. Struct. Biol.* (2005).
27. S.B. Prusiner, M.R. Scott, S.J. DeArmond, and G. Carlson, in *Prion Biology and Diseases* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2004) p. 187.
28. M. Scott, D. Peretz, R.M. Ridley, H.F. Baker, S.J. DeArmond, and S.B. Prusiner., in *Prion Biology and Diseases,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (2004) p. 435.
29. M.J. Bennett, M.P. Schlunegger, and D. Eisenberg, *Protein Sci.* **4** (1995) p. 2455; M.P. Schlunegger, M.J. Bennett, and D. Eisenberg, *Adv. Protein Chem.* **50** (1997) p. 61; Y. Liu, G. Gotte, M. Libonatti, and D. Eisenberg, *Nat. Struct. Biol.* **8** (2001) p. 211.
30. S.C. Yang, S.S. Cho, Y. Levy, M.S. Cheung, H. Levine, P.G. Wolynes, J.N. Onuchic, *Proc. Natl. Acad. Sci. USA* **101** (2004) p. 13786.
31. F.E. Cohen and S.B. Prusiner, *Annu. Rev. Biochem.* **67** (1998) p. 793.
32. T.R. Serio, A.G. Cashikar, A.S. Kowal, G.J. Sawicki, J.J. Moslehi, L. Serpell, M.F. Arnsdorf, and S.L. Lindquist, *Science* **289** (2000) p. 1317.
33. S. Chen, F.A. Ferrone, and R. Wetzel, *Proc. Natl. Acad. Sci. USA* **99** (2002) p. 11884.
34. J. Masel, V.A.A. Jansen, and M.A. Nowak, *Biophys. Chem.* **77** (1999) p. 139.
35. P. Chien, J.S. Weissman, and A.H. DePace, *Annu. Rev. Biochem.* **73** (2004) p. 617.
36. S.R. Collins, A. Douglass, R.D. Vale, and J.S. Weissman, *PLoS Biol.* **2** (2004) p. 1582.
37. A. Slepoy, R.R.P. Singh, F. Pazmandi, R.V. Kulkarni, and D.L. Cox, *Phys. Rev. Lett.* **87** 058101 (2001).
38. R.G. Will, M.P. Alpers, D. Dormont, and L.B. Schonberger, in *Prion Biology and Diseases* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2004) p. 629.
39. N. Arispe, H. B. Pollard, and E. Rojas, *Proc. Natl. Acad. Sci. USA* **90** (1993) p. 10573; N. Arispe, H.B. Pollard, and E. Rojas, *Mol. Cellu. Biochem.* **140** (1994) p. 119; S.R. Durell et al., *Biophys. J.* **67** (1994) p. 2137.
40. D.M. Walsh, I. Klyubin, J.V. Fadeeva, W.K. Cullen, R. Anwyl, M.S. Wolfe, M.J. Rowan, and D.J. Selkoe, *Nature* **416** (2002) p. 535.
41. D.L. Mobley, D.L. Cox, R.R.P. Singh, M.W. Maddox, and M.L. Longo, *Biophys. J.* **86** (2004) p. 3585.
42. H.L. True, I. Berlin, and S.L. Lindquist, *Nature* **431** (2004) p. 184.
43. C. Dicko, F. Vollrath, and J.M. Kenney, *Biomacromolecules* **5** (2004) p. 704.
44. S.J. Hamodrakas, A. Hoenger, and V.A. Iconomidou, *J. Struct. Biol.* **145** (2004) p. 226.
45. K. Si, S. Lindquist, and E.R. Kandel, *Cell* **115** (2004) p. 879.
46. T. Scheibel, R. Parthasarathy, G. Sawicki, X.M. Lin, H. Jaeger, and S.L. Lindquist, *Proc. Natl. Acad. Sci. USA* **100** (2003) p. 4527.
47. M. Reches and E. Gazit, *Science* **300** (2003) p. 625.
48. C.E. MacPhee and D.N. Woolfson, *Curr. Opin. Solid State Mater. Sci.* **8** (2004) p. 141.
49. H.A. Lashuel, S.R. LaBrenz, L. Woo, L.C. Serpell, J.W. Kelly, *J. Am. Chem. Soc.* **122** (2000) p. 5262.
50. S.H. Waterhouse and J.A. Gerrard, *Aust. J. Chem.* **57** (2004) p. 519.
51. S. Gao, H.C. Hendrie, K.S. Hall, and S. Hui, *Arch. Gen. Psychiatry* **55** (1998) p. 809.
52. S.C. Conley and J.T. Kirchner, *Postgrad. Med. Online* **106** (1) (1999), http://www.postgradmed.com/issues/1999/07_99/conley.htm (accessed April 2005).
53. M.J. Strong, A.J. Hudson, and W.G. Alvord, *Can. J. Neurol. Sci.* **18** (1991) p. 45.
54. S.R. Votey and A.L. Peters, "Diabetes Mellitus, Type II—A Review," http://www.emedicine.com/emerg/topic134.htm (accessed April 2005).
55. R.H. Falk, R.L. Comenzo, and M. Skinner, *New Eng. J. Med.* **337** (1997) p. 898.
56. J.L. Jimenez, J.I. Guijarro, E. Orlova, J. Zurdo, C.M. Dobson, M. Sunde, H.R. Saibil, *EMBO J.* **18** (1999) p. 815.
57. S.S. Ray, R.J. Nowak, K. Strokovich, R.H. Brown, T. Walz, and P.T. Lansbury, *Biochem. U.S.* **43** (2004) p. 4899. □