Published in final edited form as:

Int J Biomed Nanosci Nanotechnol. 2011 April 1; 2(2): 167–176. doi:10.1504/IJBNN.2011.041000.

The impact of protein disulfide bonds on the amyloid fibril morphology

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Abstract

Amyloid fibrils are associated with many neurodegenerative diseases. Being formed from more than 20 different proteins that are functionally or structurally unrelated, amyloid fibrils share a common cross- β core structure. It is a well-accepted hypothesis that fibril biological activity and the associated toxicity vary with their morphology. Partial denaturation of a native protein usually precedes the initial stage of fibrillation, namely the nucleation process. Low pH and elevated temperature, typical conditions of amyloid fibril formation in vitro, resulted in partial denaturation of the proteins. Cleavage of disulfide bonds results typically in significant disruption of protein native structure and in the formation of the molten globular state. Herein we report on a comparative investigation of fibril formation by apo- α -lactalbumin and its analog that contains only one of the four original disulfide bonds using deep UV resonance and non-resonance Raman spectroscopy and atomic force microscopy. Significant differences in the aggregation mechanism and the resulting fibril morphology were found.

Keywords

α -lactalbumin; fibril; disulfide bonds; polymorphism; ultraviolet Raman spectroscopy; Raman spectroscopy; kinetics of aggregation; kinetics of fibrillation; protein structure; protein aggregation; fibrillation mechanism

1 Introduction

Protein unfolding and aggregation are associated with many neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and prion diseases, (Goldsbury et al., 2000; Sipe, 2005; Makarava and Baskakov, 2008). Moreover, amyloid fibril formation was found to be involved in many other maladies associated with protein aggregation including type II diabetes, prolactoma, primary amyloidosis, etc. (Brenner et al., 2004; Clark et al., 1996; Westermark et al., 1997). The postmortem analysis of organs and tissues of patients who have been affected by these diseases demonstrates the presence of β -sheet rich protein aggregates, commonly known as amyloid fibrils (Kisilevsky, 2000). The high structural complexity of amyloid fibrils and the various mechanisms of their growth result in one of the most intriguing phenomena in amyloid research – fibril polymorphism. The understanding of this phenomenon becomes

critical for a successful disease treatment due to the strong relationship between fibril toxicity and their morphology (Seilheimer et al., 1997; Makarava and Baskakov, 2008). It has been well documented that fibrils formed from the same protein can have various morphologies when prepared under different conditions (Petkova et al., 2005; Anderson et al., 2006). Two main hypotheses have been developed to explain the phenomenon of fibril polymorphism. According to the first, variations in the cross- β core structure, resulting from specific nucleation mechanisms, lead to the propagation of morphological differences in mature fibrils (Hoyer et al., 2002). Alternatively, fibril polymorphism could result from variations in the self assembly of protofibrils, small aggregated species that are found in the initial stages of fibrillation (Radovan et al., 2008). In the latter case, the cross- β core is established during protofibril formation and consequently, should have the same structure for various polymorphs.

The impact of a variety of physical factors such as temperature, pH, ionic strength and pressure on fibril formation is well documented in the literature (Goers et al., 2002; Bomhoff et al., 2006; Kim et al., 2006). Other factors such as protein or polypeptide amino acid composition and the presence of disulfide bonds also may cause significant changes in the protein aggregation mechanism (Zako et al., 2009). Point mutagenesis is a powerful technique for probing the role of a specific amino acid residue in the fibrillation process (Permyakov and Berliner, 2000; Kanski et al., 2002c; Ohhashi et al., 2002). Disulfide bonds and methionine amino acid residues are known to play a vital role in the stability of protein secondary and tertiary structure (Kay, 1997; Kanski et al., 2002a). It has been demonstrated that methionine amino acid residues of amyloid β ($A\beta$) may initiate free radical formation by a series of cascade reactions (Kanski et al., 2002b; Schoneich, 2002; Mozziconacci et al., 2008). At the same time, the disruption of disulfide bonds does not necessarily impact the physiological activity of some proteins, such as holo- α -lactalbumin and β -microglobulin (Ewbank and Creighton, 1993; Ohhashi et al., 2002).

We hypothesise that the roles of disulfide bonds may not be limited only to the conformational stability of protein molecules, but can also affect the protein aggregation at various stages of amyloid fibril formation. In order to investigate the role and the impact of disulfide bonds on fibril morphology, we utilised proteins that share the same amino acid sequence, namely apo- α -lactalbumin (LA) and 1-SS-carboxymethyl lactalbumin (1SS-LA), which possess different numbers of disulfide bonds. α -Lactalbumin is a small (~14 kDa) metal binding protein that is present in the whey milk of most mammal species as a regulatory subunit of lactose synthase (Permyakov, 2005). LA has four disulfide bridges (Cys 6–120; Cys 61–77; Cys 73–91 and Cys 28–111). 1-SS-LA is a protein mutant with only one disulfide bridge present, while the other S-S bonds are disrupted by carboxyl methylation. Both LA and 1-SS-LA form molten globules under acidic conditions (Kuwajima, 1996). The molten globule state of LA has a native-like secondary structure with a partial loss of specific tertiary interactions (Kataoka et al., 1997). 1-SS-LA adopts a molten globule-like state in aqueous solution. Protein molten globules play important physiological roles in living organisms, including transmembrane translocation and mislocation of proteins in cells, which that may provoke genetic diseases (Bychkova et al., 1988; Bychkova and Ptitsyn, 1995). Disease related mutations have also been shown to convert the native protein into molten globules (Morozova-Roche et al., 2000). The molten globule state of lysozyme has been shown to be a likely precursor for amyloid fibril formation (De Felice et al., 2004).

In this work, we investigated the role of disulfide bonds in fibrillation of bovine α -lactalbumin and its mutant, 1SS-LA, evaluated the differences in the aggregation mechanism for these two proteins and characterised the structure of the corresponding fibril polymorphs by Raman spectroscopy.

2 Materials and methods

2.1 Preparation of apo- α -lactalbumin and 1SS-LA fibrils

Bovine apo- α -lactalbumin, L6010 and 1SS-LA, L5888 (Sigma, St. Louis, MO, used as received) solutions (5 mg/ml, 150 mM NaCl, pH 2.0 adjusted by adding HCl) were incubated at 37°C with constant stirring for 72 h. The incubation was terminated by separating the gelatinous fraction by centrifugation at 14,000 g for 30 min at 37°C. The separated fibrils were redispersed in pH 2.0 HCl solutions with and without added NaCl at 25°C.

2.2 Atomic force microscopy

Atomic force microscopy (AFM) was used to visualise morphological variations of α -lactalbumin and 1SS-LA fibrils at different stages of aggregation. Redispersed fibrils were diluted with aqueous pH 2.0 HCl solution in a 1:400 ratio and deposited onto freshly-cleaved mica. AFM imaging was performed by using a MFP-3D™ Bio Asylum Research microscope (Asylum Research, CA, USA) in non-contact mode with Olympus AC160TS tips (tip radius was ~10 nm).

2.3 Non-resonance Raman spectroscopy

Fibril samples were lyophilised and deposited onto alumina foil. A Renishaw inVia confocal Raman spectrometer equipped with a research-grade Leica microscope, 20x long-range objective (numerical aperture of 0.35), and WiRE 2.0 software were employed for non-resonance Raman spectroscopy. A 785-nm-wavelength laser was used, and the laser power was reduced to approximately 11.5 mW to avoid sample photodegradation.

2.4 Deep UV resonance Raman spectroscopy

Deep UV resonance Raman spectroscopy (DUVRR) spectra (197-nm excitation) were measured using a home-built Raman spectrometer as described elsewhere (Lednev et al., 2005). A spinning quartz NMR tube with a magnetic stirrer inside was used for sampling. All reported Raman spectra were an average of at least three independent measurements. GRAMS/AI 7.0 software (thermo galactic, Salem, NH) was used for data processing.

2.5 H/D exchange

The application of hydrogen-deuterium exchange, combined with DUVRR spectroscopy for structural characterisation of the fibril core, is described in detail elsewhere (Xu et al., 2007a). Samples (1 mL) of fibrils dispersed in water were centrifuged at 14000g for 30 min. The precipitate was divided into two parts, each of which was washed two times either with D₂O or H₂O, by subsequent spinning-redispersion. The washed dispersions (in D₂O and H₂O) were used for Raman spectroscopic measurements.

3 Results and discussion

3.1 Morphology of amyloid aggregates

Both LA and 1-SS-LA proteins form molten globules under acidic pH, which are characterised by the partial loss of tertiary contacts and a small loss of secondary structure (Bomhoff et al., 2006). Formation of fibrillar aggregates was observed for both protein molten globules after the incubation at the denaturing conditions. However, fibrillation kinetics for LA and 1-SS-LA exhibit a significant difference in the lag phase. According to ThT fluorescence data, the lag phase of fibrillation at pH 2.0 (0.1 M NaCl, 2 mg/ml) is considerably shorter in the case of 1-SS-LA (1.6 ± 0.2 h) than that of LA (28.2 ± 0.3 h) (Goers et al., 2002). The main objective of our study was to compare the evolution of

structure and morphology for LA and 1-SS-LA aggregates during the fibrillation process. We utilised AFM for monitoring the fibrillation kinetics, and Raman spectroscopy for characterising the structure of mature fibrils. Aliquots of LA and 1-SS-LA solution were taken for analysis at various stages of fibrillation. Time periods between consecutive aliquots were significantly longer for the LA solution than those for 1-SS-LA solution in agreement with the lengths of the corresponding lag phases: after the initial ten hours and then every five hours for LA and every hour for 1-SS-LA. Aliquots were placed on freshly-cleaved mica and then immediately dried under a nitrogen flow.

No protofilaments or protofibrils with a typical elongated needle-type shape were found in the initial stages of 1-SS-LA fibrillation. Instead, spherical amorphous aggregates were observed by AFM [Figure 1(f)]. In contrast, thin, elongated protofilaments [Figure 1(b), red arrow] were observed among a large number of aggregated protein clumps in the initial stages of LA aggregation. After ten hours of 1-SS-LA aggregation, some of the initial, spherical aggregates stacked between each-other and formed fibril-like structures. These fibrils were relatively short and had 'bubble' like shapes [Figure 1(j)]. In the case of LA, we did not detect any spherical aggregates. Instead, the observed protofilaments tended to intertwine with each other and form protofibrils and mature fibrils [Figure 1(b) and Figure 1(c)] following the classical fibrillation mechanism.

3.2 Structural characterisation of LA and 1-SS-LA fibrils using Raman spectroscopy

Amyloid fibrils are non-crystalline and insoluble and consequently pose a serious problem in the application of conventional biophysical techniques such as x-ray crystallography and solution NMR spectroscopy for their structural characterisation (Chiti and Dobson, 2006; Sawaya et al., 2007). Recently, wide-angle x-ray scattering from flow-oriented fibrils has been utilised to estimate interstrand and intersheet spacing in cross- β structures (Squires et al., 2006). The application of fibre x-ray diffraction has been limited to short peptide microcrystals mimicking the core structure of amyloid fibrils (Nelson et al., 2005). Solid-state NMR spectroscopy probes the local secondary structure and side-chain conformations in amyloid fibrils (Tycko, 2006). This technique, however, requires site-specific ^{13}C and/or ^{15}N labels that limit its application for full-length protein fibrils. Application of FTIR is limited because of intense IR absorption by water. Deep ultraviolet resonance Raman (DUVRR) spectroscopy has been shown to be a powerful tool for characterising protein structure at all stages of fibrillation (Xu et al., 2005; Lednev, 2007; Shashilov et al., 2007).

DUVRR protein spectra of LA and 1-SS-LA (Figure 2, top row) were very similar, in agreement with the structural similarity of LA molten globules and the molten globule-like state of 1-SS-LA reported for their acidic solutions (Goers et al., 2002). The more intense $\text{C}_\alpha\text{-H}$ band in the Raman spectrum of 1-SS-LA indicated a larger contribution of unordered structure, while lower intensities of tyrosine and phenylalanine bands could be assigned to the variations of the local environment of the aromatic amino acid residues. A dramatic increase in the intensity of Amide I, Amide II and $\text{C}_\alpha\text{-H}$ bands in the gelatinous phase Raman spectra (Figure 2, central row) relative to that of the molten globule ones indicated the formation of β -sheet-rich fibrils. The Amide II band shifted to 1557 cm^{-1} in the spectrum of 1-SS-LA fibrils from 1550 cm^{-1} (LA fibrils). C-N stretching, N-H bending, and C-C stretching contribute to the Amide II Raman band. Asher and co-workers (Myshakina et al., 2008) have demonstrated that the Amide II frequency depends on the hydrogen bonding of the $-\text{N}(\text{H})-$ group of the polypeptide backbone. Consequently, the upshifted Amide II band in 1-SS-LA fibril spectrum indicated a higher hydration of these fibrils relative to that of LA fibrils. One can hypothesise that the upshift of the Amide II band should indicate that the cross- β -core in the 1-SS-LA fibrils was less protected from solvent (water) penetration than that in LA fibrils. The intensity of tyrosine and

phenylalanine bands in the spectrum of LA fibrils is lower than that in the spectrum of 1-SS-LA fibrils indicating a change in the tyrosine and phenylalanine local environment.

We applied a recently developed technique, hydrogen-deuterium exchange combined with DUVRR spectroscopy (Xu et al., 2007b), for structural characterisation of the fibril core. Mature fibrils prepared in water were lyophilised and redispersed in D₂O. Amide H-D exchange occurred only in the unordered parts of fibrils while the fibril core stays protonated providing that the core is protected from solvent penetration. Figure 2 (bottom row) shows the Raman spectra of LA and 1-SS-LA fibrils in D₂O. Amide H-D exchange causes a downshift of the Amide II band from 1,555 to 1,450 cm⁻¹ (Amide II') and the virtual disappearance of the Amide III band (Mikhonin and Asher, 2005). The large Amide II' band and the absence of the Amide II band in the D₂O Raman spectrum of 1-SS-LA fibrils indicated the complete H-D exchange of all amide protons. In other words, water penetrates easily to all parts of 1-SS-LA fibrils including the fibril core. In contrast, a significant Amide II band was evident in the D₂O Raman spectrum of LA fibrils (Figure 2, bottom row). Consequently, dissimilar resistance to the H-D exchange is the first significant difference between the properties of LA and 1-SS-LA fibrils.

Non-resonance Raman spectroscopy is a powerful technique for characterising protein disulfide bonds (Tu, 1982). Specifically, the conformation of the C-C-S-S-C-C segment, namely gauche-gauche-gauche (g-g-g), gauche-gauche-trans (g-g-t), or trans-gauche-trans (t-g-t) could be determined (Tu, 1982). We applied non-resonance Raman spectroscopy for the comparative characterisation of protein [Figure 3(a)] and fibril [Figure 3(b)] disulfide bonds.

LA has four disulfide bonds. Based on the obtained Raman spectra (Figure 3), three S-S bonds were in g-g-g conformation (Raman peak at 510 cm⁻¹) and one had (g-g-t) conformation, (a weak peak at 540 cm⁻¹). There was just one intense band (508 cm⁻¹) in the 450–550-nm spectrum range of LA fibrils [Figure 3(b)], indicating that all disulfide bonds in these fibrils had the same g-g-g conformation. The interpretation of non-resonance Raman spectra of 1-SS-LA protein and its fibrils is less obvious and requires additional studies. Very preliminarily, a single disulfide band of 1-SS-LA had t-g-t conformation (Raman peak at 540 cm⁻¹). Two small peaks (485 cm⁻¹ and 514 cm⁻¹) in the spectrum of 1-SS-LA could be tentatively assigned to the vibrational modes of sulphur-carboxymethyl groups (Tu, 1982). None of these peaks were evident in the non-resonance Raman spectrum of 1-SS-LA fibrils, which might indicate that the only disulfide bond in 1-SS-LA was broken. Alternatively, the conformation of the disulfide bond in 1-SS-LA fibrils might not be conserved and all conformations could be present. As a result, no distinct peak was evident in the Raman spectrum.

Overall, LA and its analogue 1-SS-LA, containing only one of four original disulfide bonds, showed different fibrillation kinetics, formed dissimilar aggregates at all stages of fibril formation and resulted in mature fibrils with different morphology. These observations allowed us to conclude that disulfide bonds play a very significant role in the fibrillation of apo- α -lactalbumin.

Acknowledgments

The authors are grateful to Professor P.S. Toscano for critical reading of the manuscript. This project is supported by Award Number R01AG033719 from National Institute of Aging.

References

- Anderson M, Bocharova OV, et al. 'Polymorphism and ultrastructural organization of prion protein amyloid fibrils: an insight from high resolution atomic force microscopy'. *Journal of Molecular Biology*. 2006; 358(2):580–596. [PubMed: 16519898]
- Bomhoff G, Sloan K, et al. 'The effects of the flavonoid baicalein and osmolytes on the Mg²⁺ accelerated aggregation/fibrillation of carboxymethylated bovine 1SS-alpha-lactalbumin'. *Archives of Biochemistry and Biophysics*. 2006; 453(1):75–86. [PubMed: 16530158]
- Brenner DA, Jain M, et al. 'Human amyloidogenic light chains directly impair cardiomyocyte function through an increase in cellular oxidant stress'. *Circulation Research*. 2004; 94:1008–1010. [PubMed: 15044325]
- Bychkova VE, Pain RH, et al. 'The 'molten globule' state is involved in the translocation of proteins across membranes'. *FEBS Letters*. 1988; 238(2):231–234. [PubMed: 3049159]
- Bychkova VE, Ptitsyn OB. 'Folding intermediates are involved in genetic diseases'. *FEBS Letters*. 1995; 359(1):6–8. [PubMed: 7531653]
- Chiti F, Dobson CM. 'Protein misfolding, functional amyloid, and human disease'. *The Annual Review of Biochemistry*. 2006; 75:333–366.
- Clark A, Chargé SB, et al. 'Islet amyloid in type 2 (non-insulin-dependent) diabetes'. *Acta Pathologica, Microbiologica et Immunologica Scandinavica*. 1996; 104(1–6):12–18.
- De Felice FG, Vieira MN, et al. 'Formation of amyloid aggregates from human lysozyme and its disease-associated variants using hydrostatic pressure'. *Faseb Journal*. 2004; 18(10):1099–1101. [PubMed: 15155566]
- Ewbank JJ, Creighton TE. 'Structural characterization of the disulfide folding intermediates of bovine alpha-lactalbumin'. *Biochemistry*. 1993; 32(14):3694–3707.
- Goers J, Permyakov SE, et al. 'Conformational prerequisites for alpha-lactalbumin fibrillation'. *Biochemistry*. 2002; 41(41):12546–12551. [PubMed: 12369846]
- Goldsbury CS, Wirtz S, et al. 'Studies on the in vitro assembly of A beta 1–40: implications for the search for A beta fibril formation inhibitors'. *Journal of Structural Biology*. 2000; 130(2–3):217–231. [PubMed: 10940227]
- Hoyer W, Antony T, et al. 'Dependence of alpha-synuclein aggregate morphology on solution conditions'. *Journal of Molecular Biology*. 2002; 322(2):383–393. [PubMed: 12217698]
- Kanski J, Aksenova M, et al. 'Substitution of isoleucine-31 by helical-breaking proline abolishes oxidative stress and neurotoxic properties of Alzheimer's amyloid beta-peptide'. *Free Radical Biology and Medicine*. 2002a; 32(11):1205–1211. [PubMed: 12031904]
- Kanski J, Aksenova M, et al. 'The hydrophobic environment of Met35 of Alzheimer's A beta (1–42) is important for the neurotoxic and oxidative properties of the peptide'. *Neurotoxicity Research*. 2002b; 4(3):219–223. [PubMed: 12829402]
- Kanski J, Varadarajan S, et al. 'Role of glycine-33 and methionine-35 in Alzheimer's amyloid beta-peptide 1–42-associated oxidative stress and neurotoxicity'. *Biochimica et Biophysica Acta*. 2002c; 1586(2):190–198. [PubMed: 11959460]
- Kataoka M, Kuwajima K, et al. 'Structural characterization of the molten globule of alpha-lactalbumin by solution x-ray scattering'. *Protein Science*. 1997; 6(2):422–430. [PubMed: 9041645]
- Kay CJ. 'Mechanochemical mechanism for peptidyl free radical generation by amyloid fibrils'. *FEBS Letters*. 1997; 403(3):230–235. [PubMed: 9091307]
- Kim YS, Randolph TW, et al. 'High-pressure studies on protein aggregates and amyloid fibrils'. *Methods of Enzymology*. 2006; 413:237–253.
- Kisilevsky R. 'Review: amyloidogenesis-unquestioned answers and unanswered questions'. *Journal of Structural Biology*. 2000; 130(2–3):99–108. [PubMed: 10940218]
- Kuwajima K. 'The molten globule state of alpha-lactalbumin'. *Faseb Journal*. 1996; 10(1):102–109. [PubMed: 8566530]
- Lednev, IK. 'Vibrational spectroscopy: biological applications of ultraviolet Raman spectroscopy, protein structures, methods in protein structures and stability analysis'. In: Uversky, VN.; Permyakov, EA., editors. *Biological Applications of Ultraviolet Raman Spectroscopy*. Nova Science Publishers, Inc; New York: 2007. p. 1-17.

- Lednev IK, Ermolenkov VV, He W, Xu M. 'Deep-UV Raman spectrometer tunable between 193 and 250 nm for structural characterization of proteins'. *Analytical and Bio-analytical Chemistry*. 2005; 381(2):431–437.
- Makarava N, Baskakov IV. 'The same primary structure of the prion protein yields two distinct self-propagating states'. *Journal of Biological Chemistry*. 2008; 283(23):15988–15996.
- Mikhonin AV, Asher SA. 'Uncoupled peptide bond vibrations in alpha-helical and polyproline II conformations of polyalanine peptides'. *Journal of Physical Chemistry B*. 2005; 109(7):3047–3052.
- Morozova-Roche LA, Zurdo J, et al. 'Amyloid fibril formation and seeding by wild-type human lysozyme and its disease-related mutational variants'. *Journal of Structural Biology*. 2000; 130(2–3):339–351. [PubMed: 10940237]
- Mozziconacci O, Williams TD, et al. 'Reversible intramolecular hydrogen transfer between protein cysteine thiol radicals and alpha C-H bonds in insulin: control of selectivity by secondary structure'. *Journal of Physical Chemistry B*. 2008; 112(49):15921–15932.
- Myshakina NS, Ahmed Z, Asher SA. 'Dependence of amide vibrations on hydrogen bonding'. *Journal of Physical Chemistry*. 2008; 112(38):11873–11877. [PubMed: 18754632]
- Nelson R, Sawaya MR, et al. 'Structure of the cross-beta spine of amyloid-like fibrils'. *Nature*. 2005; 435(7043):773–778. [PubMed: 15944695]
- Ohhashi Y, Hagihara Y, et al. 'The intrachain disulfide bond of beta (2)-microglobulin is not essential for the immunoglobulin fold at neutral pH, but is essential for amyloid fibril formation at acidic pH'. *Journal of Biochemistry*. 2002; 131(1):45–52. [PubMed: 11754734]
- Permyakov, E. a-Lactalbumin. Nova Science Publishers, Inc; New York: 2005.
- Permyakov EA, Berliner LJ. 'alpha-Lactalbumin: structure and function'. *FEBS Letters*. 2000; 473(3):269–274. [PubMed: 10818224]
- Petkova AT, Leapman RD, et al. 'Self-propagating, molecular-level polymorphism in Alzheimer's beta-amyloid fibrils'. *Science*. 2005; 307(5707):262–265. [PubMed: 15653506]
- Radovan D, Smirnovas V, et al. 'Effect of pressure on islet amyloid polypeptide aggregation: revealing the polymorphic nature of the fibrillation process'. *Biochemistry*. 2008; 47(24):6352–6360. [PubMed: 18498175]
- Sawaya MR, Sambashivan S, et al. 'Atomic structures of amyloid cross-beta spines reveal varied steric zippers'. *Nature*. 2007; 447(7143):453–457. [PubMed: 17468747]
- Schoneich C. 'Redox processes of methionine relevant to beta-amyloid oxidation and Alzheimer's disease'. *Archives of Biochemistry and Biophysics*. 2002; 397(2):370–376. [PubMed: 11795896]
- Seilheimer B, Bohrmann B, et al. 'The toxicity of the Alzheimer's beta-amyloid peptide correlates with a distinct fiber morphology'. *Journal of Structural Biology*. 1997; 119(1):59–71. [PubMed: 9216088]
- Shashilov V, Xu M, et al. 'Probing a fibrillation nucleus directly by deep ultraviolet Raman spectroscopy'. *Journal of American Chemical Society*. 2007; 129(22):6972–6973.
- Sipe, JD. Amyloid Proteins The Beta Sheet Conformation and Disease. WILEY-VCH; Berlin: 2005.
- Squires AM, Devlin GL, et al. 'X-ray scattering study of the effect of hydration on the cross-beta structure of amyloid fibrils'. *Journal of American Chemical Society*. 2006; 128(36):11738–11739.
- Tu, AT. Raman Spectroscopy in Biology: Principles and Applications. Wiley; New York: 1982.
- Tycko R. 'Molecular structure of amyloid fibrils: insights from solid-state NMR'. *Quarterly Reviews of Biophysics*. 2006; 39(1):1–55. [PubMed: 16772049]
- Westermarck P, Eriksson L, et al. 'Prolactin-derived amyloid in the aging pituitary gland'. *American Journal of Pharmacology*. 1997; 150(1):67–73.
- Xu M, Ermolenkov VV, et al. 'Lysozyme fibrillation: deep UV Raman spectroscopic characterization of protein structural transformation'. *Biopolymers*. 2005; 79(1):58–61. [PubMed: 15962278]
- Xu M, Shashilov V, et al. 'Probing the cross-beta core structure of amyloid fibrils by hydrogen-deuterium exchange deep ultraviolet resonance Raman spectroscopy'. *Journal of American Chemical Society*. 2007a; 129(36):11002–11003.
- Xu M, Shashilov V, et al. 'The first step of hen egg white lysozyme fibrillation, irreversible partial unfolding, is a two-state transition'. *Protein Science*. 2007b; 16(5):815–832. [PubMed: 17400924]

Zako T, Sakono M, Hashimoto N, Ihara M, Maeda M. 'Bovine insulin filaments induced by reducing disulfide bonds show a different morphology, secondary structure, and cell toxicity from intact insulin amyloid fibrils'. *Biophysical Journal*. 2009; 96(8):3331–3340. [PubMed: 19383476]

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Figure 1. Amplitude AFM images of LA aggregation after incubation (a) 10 h; (b) 20 h; (c) 40 h; (d) 72 h; and 1-SS-LA aggregation after incubation; (e) 1 h; (f) 2 h; (g) 10 h; (j) 48 h (see online version for colours)
Note: Scale bar is 500 nm

Figure 2.

DURR spectra of native proteins (top row), mature fibrils (central row), and mature fibrils in D_2O (bottom row) (see online version for colours)

Notes: DUVRR spectra in H_2O : LA (blue); 1-SS-LA (red);

DUVRR spectra in D_2O : LA fibrils (green); 1-SS-LA fibrils (black).

Figure 3.
Raman spectra of (a) LA (blue) and (b) 1-SS-LA (red) proteins and their lyophilised fibrils
(see online version for colours)