Mechanically functional amyloid fibrils in the adhesive of a marine invertebrate as revealed by Raman spectroscopy and atomic force microscopy

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Summary. Amyloid fibrils are primarily known in a pathogenic context for their association with a wide range of debilitating human diseases. Here we show a marine invertebrate (*Entobdella soleae*) utilizes functional amyloid fibrils comparable to those of a unicellular prokaryote (*Escherichia coli*). Thioflavin-T binding and Raman spectroscopy provided evidence for the presence of amyloid in the adhesive of *Entobdella soleae*. We elucidated that for these two very different organisms, amyloid fibrils provide adhesive and cohesive strength to their natural adhesives. Comparing the nanoscale mechanical responses of these fibrils with those of pathogenic amyloid by atomic force microscopy revealed that the molecular level origin of the cohesive strength was associated with the generic intermolecular β -sheet structure of amyloid fibrils.

Functional adhesive residues were found only in the case of the functional amyloid. Atomic force microscopy provided a useful means to characterize the internal structural forces within individual amyloid fibrils and how these relate to the mechanical performance of both functional and pathogenic amyloid. The mechanistic link of amyloid-based cohesive and adhesive strength could be widespread amongst natural adhesives, irrespective of environment, providing a new strategy for biomimicry and a new source of materials for understanding the formation and stability of amyloid fibrils more generally.

Introduction

Nature has evolved many extraordinary adhesive strategies that are essential for the survival of a wide diversity of micro- and macro-organisms in marine, freshwater and terrestrial habitats. A large amount of work on natural adhesives has focused on marine organisms in order to prevent biofouling, or to develop aqueous-based biomimetic adhesives (Kamino, 2008). These adhesives vary in that a sessile marine organism depends upon permanent adhesion, but motile organisms rely on transitory adhesion during locomotion, or temporary adhesion to different surfaces (Walker, 1987).

While research in this area progresses, no common underlying strategy has emerged in natural adhesives. For example, attachment of the blue mussel is reliant upon an adhesive with a high content of 3,4-dihydroxy-

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L-phenylalanine (DOPA) (Waite and Tanzer, 1981; Waite, 1983), whereas the dry, temporary adhesion of the Tokay gecko is attributed to weak van der Waals forces between a surface and the fine setae on gecko toes (Autumn *et al.*, 2000., 2002). The merging of these two adhesion strategies has recently produced a superior biomimetic hybrid adhesive (Lee *et al.*, 2007). Here we study the temporary, marine-based adhesive of a fish skin parasite (*Entobdella soleae*) and a bacterial (*Escherichia coli*) biofilm adhesive, and find a surprising structural commonality between these two otherwise unconnected natural adhesives.

Entobdella soleae (Monogenea: Capsalidae) is a parasitic marine flatworm that firmly attaches to the skin of the common sole (*Solea solea*) (Kearn and Evans-Gowing, 1998). The parasite moves like a leech, using anterior pads on the head for temporary attachment, while the posterior attachment organ (the haptor) is detached and relocated (Kearn and Evans-Gowing, 1998). These pads attach not by suction, but by means of an adhesive secretion. This tissue adhesive is of intrinsic interest, not only because of its critical role in the parasite's establishment and subsequent survival, but also as it works rapidly to provide strong adhesion to wet surfaces (Kearn and Evans-Gowing, 1998). Previous chemical characterisation of this adhesive has shown that it does not contain lipid, polysaccharide or DOPA, but instead consists of a network of highly insoluble proteinaceous fibres (Hamwood *et al.*, 2002).

The fibres bare a similar morphology to curli, the proteinacious fibrils in the natural adhesive expressed by *Escherichia coli*. These extracellular adhesive fibres have been identified as amyloid fibrils using congo red and thioflavin T binding (Chapman *et al.*, 2002), and their primary function is to promote colonization of a surface (Olsen *et al.*, 1989). Surface-associated amyloid fibrils have since been described in the biofilms of bacteria within several phyla (Larsen *et al.*, 2007; Otzen and Nielsen, 2007). Amyloid fibrils are primarily known for their association with a wide range of degenerative human diseases, such as Alzheimer's and Parkinson's diseases (Lansbury, 1999). However, in recent years the identification of amyloid in a range of functional roles has emerged (Chiti and Dobson, 2006; Mostaert *et al.*, 2006; Fowler *et al.*, 2007; Cherny and Gazit, 2008).

The aim of this study was to investigate if the adhesive fibres of *Entobdella soleae* also have the generic amyloid core structure of an intermolecular β -sheet. Here we characterize the mechanical properties of the adhesive fibrils extruded from the parasite with atomic force microscopy (AFM), and compare these data to those obtained for the amyloid-based adhesive from *E. coli*.

Employing Raman spectroscopy and histochemical staining with AFM allowed both physical and chemical properties of the adhesive protein structures of *Entobdella soleae* to be made *in situ*. If the adhesive of this marine parasite is indeed amyloid-based, this would provide the first mechanistic link between two otherwise unrelated natural adhesives.

Materials and Methods

Pathogenic fibril preparation

Pre-formed fibrils of recombinant human α -synuclein were provided by Dr Shahin Zibae (Cambridge University, UK), and were prepared according to Zibae *et al.* (2007). For the Raman spectroscopy, A β_{25-35} fibrils were grown from a synthesized amyloid β -Protein₂₅₋₃₅ (H-1192, Bachem, UK). This peptide was selected due to its ability to form fibrils readily in H₂O (Yanker *et al.*, 1990), providing a means for deuterium exchange during the formation process. The peptide was dissolved in milliQ ultrapure water (Millipore, MA, USA) or 100% D₂O (Sigma, Switzerland) at a concentration of approximately 1 mM. Fibrils formed within one week at room temperature (verified by Congo red birefringence, thioflavin-T binding and AFM imaging).

Secreted functional fibrils

Curli fibrils were obtained from *E. coli* MC4100, the wild-type curli producing strain. The curli attachment method is based on that described previously (Pawar *et al.*, 2005). Diluted MC4100 culture (1:100) was placed into a polypropylene falcon together with a glass coverslip. The cells were allowed to attach to the glass surface at 28°C for seven days with slow orbital shaking (~ 50 rpm). The coverslip was then removed, and stored in a sealed Petri dish to keep fibrils hydrated until analysis. Specimens of *Entobdella soleae* were maintained in aquaria as parasites on the skin of *Solea solea*. The secreted adhesive was collected by manually manipulating live parasites to extrude adhesive 'prints' from their anterior end onto a clean glass slide. This procedure is described in full elsewhere (Hamwood *et al.*, 2002).

Thioflavin T binding

Binding of the benzothiazole dye thioflavin-T (Sigma-Aldrich, Ireland) to amyloid structures was detected by directly staining samples on glass slides with 10 μ m

aqueous thioflavin-T for five minutes, washing in milliQ water, and viewing immediately at 63X magnification with a Zeiss Confocal META system microscope (LSM 510, Carl Zeiss Ltd, UK). Emission of thioflavin-T dye binding to amyloid was previously determined (Mostaert *et al.*, 2006) as a peak around 550 nm after excitation at 488 nm and emission collected at 505 to 620 nm using a control sample of amyloid fibrils grown from A β_{25-35} (H-1192, Bachem, UK). An unstained A β_{25-35} amyloid fibril control emitted no fluorescence signal when excited the same way as the thioflavin-T stained control.

Raman spectroscopy

Raman spectra were collected with a Renishaw MicroRaman (Renishaw, UK) with a HeNe laser (633 nm) and an infrared (IR) laser (785 nm) and a magnification of 100x. The power measured at the sample in both cases was 2–5 μ W. Raman spectra were recorded on areas of adhesive deposited on glass slides in the cases of *Entobdella soleae* and *E. coli*, and directly on amyloid that was deposited onto glass slides from stock solution in the case of the pathogenic amyloid sample. Contamination or artefacts were identified by measuring spectra in different areas and comparing the intensities of the peaks. In this way, the peak at 1580 cm^{-1} in the spectrum measured with an IR laser and the peak at 1591 cm^{-1} in the spectrum measured with a HeNe laser could be identified as not belonging to the protein.

Atomic force microscopy (AFM)

For AFM experiments on α -synuclein fibrils, a 20 μ L drop was deposited onto freshly cleaved mica and allowed to incubate at room temperature for 1 h to allow sufficient adsorption of the fibrils onto the mica surface. The sample was rinsed gently with milliQ water to remove unbound fibrils and a further 150 μ L milliQ water added prior to AFM. The fibrils from *Entobdella soleae* and curli on glass substrates were probed directly in milliQ water. All force curves were obtained with an Asylum MFP-3D AFM (Asylum Research, Santa Barbara, CA), using Si $_3$ N $_4$ cantilevers (CSC38, MikroMasch, Estonia) with calibrated spring constants between 0.5 and 0.8 Nm^{-1} . Force measurements were taken with a tip velocity of 1 $\mu\text{m/s}$, and halted at a maximum loading force of 2 nN. Numerous force curves were obtained at different positions on different fibrils. AFM images were taken in intermittent-contact mode before and after force measurements to ensure data was being collected directly on fibrils and that there was no substantial damage or motion of the fibrils caused by the measurements. Image

processing and analysis of the force curves, including fitting each force peak with the worm-like chain model in order to calculate parameters such as mean contour length and persistence length, were performed using IGOR PRO (Wavemetrics, OR, USA) data analysis software.

Results and Discussion

Thioflavin-T binding indicates the presence of amyloid

In the case of *Entobdella soleae*, amyloid detection by congo red binding and birefringence is impractical because of the extremely small volume of adhesive produced by the parasite and the problems of harvesting the adhesive. However, direct binding of the adhesive fibres with the fluorochrome dye thioflavin-T was detected with confocal microscopy (Fig. 1). This dye exhibits specific fluorescence when bound to amyloid fibrils (Mostaert *et al.*, 2006), and is commonly used to detect amyloid fibrils, both *ex vivo* and *in vitro*. This is supportive, but not conclusive evidence that the adhesive fibrils of *Entobdella soleae* share structural characteristics with amyloid fibrils.

Fig. 1. Direct binding of thioflavin-T to β -sheet structures within the secreted adhesive from the marine parasite *Entobdella soleae*. Thioflavin T is a benzothiazole dye commonly used to detect amyloid fibrils, both *ex vivo* and *in vitro*. **A:** Confocal microscope image of the secreted adhesive stained with 10 μM aqueous thioflavin-T (scale bar, 50 μm). **B:** Confocal emission spectrum collected for the thioflavin-T stained adhesive within the region circled in Figure 2A. The spectrum was collected from 505 to 620 nm, after excitation at 488 nm. Emission of thioflavin-T binding to amyloid occurs as a peak around 550 nm which was determined using stained and unstained control samples of A β_{25-35} amyloid fibrils.

Fig. 2. A: Raman spectra observed for amyloid fibrils in the adhesive extruded from the marine parasite *Entobdella soleae*. The amide III band was typical for a β -sheet structure with maxima at 1228 and 1251 cm^{-1} . Attempts to unambiguously assign the amide III bands by exchanging the hydrogen in the peptide backbone with deuterium, were made by incubating for 7 days in D_2O at 37°C (red spectrum), but yielded no significant change in the Raman spectrum (compared to fibrils in H_2O , blue spectrum). **B:** Raman spectra of the amyloid fibrils produced from $\text{A}\beta_{25-35}$ in H_2O and D_2O . The amide III peaks at 1232 and 1263 cm^{-1} are not present in the spectrum of the amyloid fibrils grown in D_2O (red spectrum). As with the protein from *Entobdella soleae* (Fig. 3A), no significant exchange could be observed in the spectrum of the fibrils grown in H_2O after incubating in D_2O (blue spectrum). The Raman spectrum of the amyloid fibrils produced from $\text{A}\beta_{25-35}$ in H_2O (blue spectrum) contains a very sharp amide I peak at 1667 cm^{-1} that is only evident as a shoulder of the peak centred at 1650 cm^{-1} in the spectrum of the fibrils produced in D_2O (red spectrum).

Raman spectroscopy identifies intermolecular β -sheets

To evaluate if the adhesive fibres of *Entobdella soleae* possess the generic amyloid structure we used Raman spectroscopy to compare the structure of the proteins in the adhesive with amyloid fibrils formed from peptides of $\text{A}\beta_{25-35}$ (Fig. 2). This widely studied peptide was chosen due to its ability to form fibrils rapidly in H_2O (Yankner *et al.*, 1990), providing an avenue for deuterium exchange during the formation process.

In the Raman spectrum (HeNe, 633 nm) of $\text{A}\beta_{25-35}$ fibrils, a very sharp amide I peak was prominent at 1667 cm^{-1} (Fig. 2B, blue). For the adhesive fibrils of *Entobdella soleae*, a similar peak was observed at 1666 cm^{-1} (only measurable with an IR laser (785 nm), data not shown). In both cases, the peak was assigned to the $\nu(0,0)$ mode of the amide I band for an infinite antiparallel β -sheet. Peaks for the associated $\nu(\pi,0)$ mode were lower than expected in both spectra, lying in the region normally associated with phenylalanine, tryptophan or tyrosine. However, the $\text{A}\beta_{25-35}$ peptide does not contain any of these amino acids, and thus the peaks centred at 1619 and 1618 cm^{-1} (Fig. 2B, blue) were unambiguously attributed to the $\nu(\pi,0)$ mode. For *Entobdella soleae* fibrils, the peak at 1612 cm^{-1} (Fig. 2A, blue) was much too strong to be assigned to these amino acids, that are only present in low concentrations in the secreted adhesive (Hamwood *et al.*, 2002). Thus the peak at 1612 cm^{-1} was attributed to the $\nu(\pi,0)$ mode. The unusually low values for an amide I band are thought to be due to exceptionally strong hydrogen bonding (Barth and Zscherp, 2002). In amyloid fibrils there are extensive intermolecular networks of hydrogen bonding stabilizing the stacks of β -strands (Sunde and Blake, 1997). For both types of fibril the amide III band was typical for a β -sheet structure, with maxima at 1228 and 1251 cm^{-1} (*Entobdella soleae*, Fig. 2A, blue), and 1232 and 1263 cm^{-1} ($\text{A}\beta_{25-35}$, Fig. 2B, blue). Attempts were made to unambiguously assign the amide III bands by exchanging the hydrogen with deuterium. However, after 7 days incubation in D_2O at 37°C , no significant change was observed in the Raman spectrum of either type of fibril (Fig. 2). We postulate that in both cases the tight, insoluble amyloid fibrils did not allow the D_2O to penetrate into the structure. For comparison, new fibrils of $\text{A}\beta_{25-35}$ were grown in D_2O (Fig. 2B, red), and in this case the amide III peaks were not present, confirming the previous attribution of the amide III peaks.

The Raman spectroscopy thus enabled us to establish that the structure of infinite β -sheets, and exceptionally strong intermolecular hydrogen bonding is common to

Fig. 3. **A:** Light micrograph of the marine parasite *Entobdella soleae* (ventral view). Anterior pads (ap) bind to fish skin using a temporary adhesive secretion, and the haptor (h) attaches to host skin by suction (scale bar, 1 mm). **B:** Atomic Force Microscopy (AFM) image of fibres obtained directly from an adhesive pad print of *Entobdella soleae* without purification or additional isolation (scale bar, 3 μm). The dark to light height range = 0–15 nm. **C:** A typical force-extension curve taken from an adhesive fibre of *Entobdella soleae* (arrow indicates region of fibre where this particular curve was taken). Both the approach (red) and retraction (blue) force traces are shown. **D:** Transmission electron micrograph of negatively stained (0.2% uranyl acetate) bacteria (*E. coli*) surrounded by curli fibrils (scale bar, 0.5 μm). **E:** AFM image of curli fibrils (scale bar, 1 μm). The dark to light height range = 0–10 nm. **F:** Typical force-extension curve taken for curli fibrils (fibril probed is indicated by the arrow) from the adhesive biofilm of *E. coli*.

both the adhesive fibrils of *Entobdella soleae* and the amyloid fibrils of A β_{25-35} .

'Sacrificial bonds' and 'hidden length' provide cohesive strength

In order to understand how amyloid fibrils function as an adhesive we explored the inelastic response of the fibrils at the molecular level by making nanoscale mechanical measurements using AFM. Measurements were made directly on fibrils extruded from *Entobdella soleae* (Fig. 3A, B) without purification or additional isolation, and curli fibrils (Fig. 3C, D) from *E. coli* (bacteria surrounded by curli are shown in Fig. 3D). The fibrils were located

in AFM imaging mode, and the AFM tip was then brought into contact at a chosen point along the length of a fibril (e.g., as indicated in Fig. 3B, E) to establish non-specific adsorption to the tip (Fukuma *et al.*, 2006). The tip and substrate were then pulled apart under an increasing tensile load with the fibril attached between the two surfaces until the material finally failed and the connection between the tip and substrate, via the fibril, was broken.

Characteristic force versus displacement curves, showing the response of the fibrils on retraction of the AFM tip can be seen in Figure 3C, F. The fibrils display a series of sawtooth mechanical responses reflecting the repetitive breaking of 'sacrificial bonds'

Fig. 4. Comparison of the sawtooth response from amyloid structures using atomic force microscopy. **A:** Expansion of the sawtooth response in the force-extension curve from the amyloid fibril in the adhesive of *Entobdella soleae* shown in Figure 1C. **B:** Sawtooth expansion from the force-extension curve shown in Figure 1F for the curli fibril in the adhesive biofilm of *E. coli*. **C:** Expansion of the sawtooth response in a typical force-extension curve from amyloid fibrils grown from α -synuclein. Sawtooth responses are shown fit to the WLC model (dotted lines). Both the approach (red) and retraction (blue) force traces are shown.

within an intermolecular β -sheet as protein is pulled from the surface of the fibril, and the 'hidden length' of the amino-acid chain subsequently extended. This process leads to superior mechanical strength when compared with systems of similar overall length, but without such sacrificial bonds, due to the extra energy required in pulling the material apart (Smith *et al.*, 1999) (corresponding to the area under the curves in the expanded sections shown in Fig. 4A, B). This mechanism of 'hidden length' and 'sacrificial bonds' has been measured previously in modular proteins such as titin (Rief *et al.*, 1997), the natural adhesives of algae (Dugdale *et al.*, 2005; Mostaert *et al.*, 2006), and the 'glue' adding fracture resistance to bone (Fantner *et al.*, 2005). It does not have to be associated with adhesion *per se*, but instead influences the cohesive strength of the material. When the sawtooth peaks are equally spaced, as in Figure 4, this indicates an underlying repetitive structural unit within the material.

Worm-like chain fitting reveals differences related to constituent proteins

By fitting a number of curves using the worm-like chain (WLC) fit for polymers (Bustamante *et al.*, 1994; Rief *et al.*, 1997) we found quantitative variations between the fibrils. In the case of the *Entobdella soleae* fibrils, a consistent change in contour length of 15.5 nm (SD 4.2 nm, $n = 55$) was measured. The contour length corresponds to the hidden length which is exposed when sacrificial bonds are broken, and in the case of material composed of protein, the approximate number of amino

acids within this hidden length can be found by dividing the length by the approximate size of a single stretched amino-acid (0.36 nm), which in this case gives ~ 43 amino acids. The total length of the stretched polypeptide chain is given by the number of amino acids between the two attachment points (in this case the anchor within the intermolecular β -sheet and the anchor either directly to the tip or to an adjacent fully stretch polypeptide) multiplied by the length of a single stretch amino acid. The hidden length exposed when the protein is pulled from the fibril will always be less than the total length as there is no measure of the initial folded length of the protein within the β -sheet with this measurement technique (Rief *et al.* 1997). The number and type of sacrificial bonds cannot be deconvoluted, but the force involved in simultaneously breaking sacrificial bonds corresponds to the peak to trough force, which was found to be 63 pN (SD 40 pN).

As can be seen in Figure 4A and B, there is good qualitative agreement between the mechanical response of the adhesive fibrils of *Entobdella soleae* and curli. In the case of curli, WLC fitting ($n = 46$) revealed a consistent change in contour length of 27.3 nm (SD 15.2) corresponding to ~ 76 amino acids, and peak to trough force of 123 pN (SD 65 pN). It is interesting to note that approximately twice the number of amino acids are involved in the hidden length of curli, and that this corresponds to twice as much force associated with the breaking of sacrificial bonds. This could be an indication that the magnitude of the individual sacrificial bonds is the same for both systems and relates directly to the *number* of constituent amino acids involved in

the intermolecular β -sheet rather than the *nature* of the constituent amino acids. Non-specific bonding of this type would be wholly consistent with the generic nature of the amyloid structure and may correspond to the intermolecular hydrogen bonds indicated by Raman spectroscopy. For curli, the primary structural protein is CsgA, consisting of 151 amino acids, 21 of which make up an N-terminal Sec signal sequence and are cleaved off after translocation into the periplasmic space (Wang and Chapman., 2008). Of the remaining 130 amino acids, 109 are proposed to form the amyloid core region containing five imperfect repeating units forming a strand-loop-strand motif (Collinson *et al.*, 1999). If this model is correct then the folded width of the monomer within the amyloid core structure would be approximately 0.5 nm (width of a β -strand) $\times$ 5 (number of β -strands) giving a folded width of approximately 2.5 nm. The hidden length exposed as each monomer is removed from the amyloid core structure should then be the total length of the amino acid chain within the core structure ($109 \times 0.36 = 39.2$ nm), minus the folded length of approximately 2.5 nm, which would give a hidden length of approximately 36.7 nm. Although somewhat higher than our measured value of 27.3 nm (SD 15.2), this calculated value still lies within the standard deviation of our measurement. In the case of the *Entobdella soleae* adhesive, the constituent structural protein, or proteins, have yet to be identified, but the above results would indicate that from each constituent protein there are in the region of 60 amino acids associated with the amyloid core structure.

We have shown previously that fibrils formed from short peptide segments are susceptible to damage by the AFM tip during indentation due to the near-crystalline properties of the fibrils (Fukuma *et al.*, 2006). For this reason, the mechanical response of fibrils produced *in vitro* from wild type α -synuclein whole protein was explored for comparison (Fig. 4C). For this 140 amino-acid protein, force curves revealed a change in contour length of 17.0 nm (SD 6.5, $n = 20$), and the peak to trough force was 108.5 pN (SD 35), both values being intermediate between those for curli and the *Entobdella soleae* fibrils, and much larger than force peaks seen previously for amyloid formed from short peptides (Fukuma *et al.*, 2006). It should be noted that with these fibrils sawtooth responses were rare and, when observed the curves had very few peaks, hence the correspondingly low value of n for these measurements. This may indicate an underlying structural difference between fibrils formed *in vitro* and those measured *ex vivo*.

The adhesive component resides at the fibril surface

In contrast to the adhesive fibrils from the marine parasite and bacterial biofilm, the α -synuclein fibrils with no adhesive function show zero separation between the sawtooth response and the base line of zero force in their AFM force curves (Fig. 4C). For the adhesive amyloid fibrils, an initial tensile loading force is required before the structure of the fibrils themselves can be unravelled. This peak to baseline force is 76 pN (SD 46 pN) for curli, 46 pN (SD 23 pN) for fibrils of *Entobdella soleae*, and is likely to be associated with the specific adhesive function of these fibrils (Fig. 4A, B). The force probably serves to unravel an adhesive residue, which does not lie within the intermolecular β -sheet core structure of the fibril itself but, due to its function, will preferentially bind to the AFM tip. It is only after this residue has been extended that sacrificial bonds within the fibrillar core structure start to be broken and proteins removed. Thus the adhesive component of the material resides at the surface of the fibrils while the cohesive strength of the material exists within the intermolecular β -sheets making up the amyloid core structure. In the case of curli the glycine rich 21 amino acid residue thought to lie outside the core structure could be responsible for the observed adhesion. The calculated relative toughness for the three types of fibrils (as calculated from the area under the curves without normalizing for the cross-sectional area of the polypeptide chain) was 2.3 aJ (SD 1.8 aJ) for the adhesive fibrils of *Entobdella soleae*, 7.2 (SD 4.8) aJ for curli, and 1.2 aJ (SD 0.3) for α -synuclein.

Conclusions

Previously, no mechanistic link between different types of natural adhesive had been identified, and indeed strategies for adhesion were found to differ widely for organisms seeking adhesion in different environments with different mechanical demands placed upon them (Yule and Walker, 1987; Waite, 2002). Consequently, past biomimetic strategies have relied on mimicking the best match for a particular application or combining specific features of different adhesive strategies (Lee *et al.*, 2007). The beauty of the generic amyloid mechanism is that it does not appear to be specific to a particular environment, nor to a specific amino-acid sequence, thus giving it the potential to meet a broad variety of possible adhesive requirements.

While AFM is primarily used for imaging surface structures at the nanoscale, here we have shown that by using the AFM tip to carefully pull apart individual amyloid fibrils we can reveal the underlying core

structure and relate this to the biological utility of the fibrils in the case of functional amyloid. The molecular level insight provided here should add an additional controllable design parameter at the molecular level, as well as helping to establish any differences between the molecular level structure of functional and pathogenic amyloid fibrils.

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