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Supplementary Information for

Amyloid β 42 fibril structure based on small angle scattering

Veronica Lattanzi[a,b], Ingemar André[a], Urs Gasser[c], Marija Dubackic[b], Ulf Olsson[b] and Sara Linse[a]

[a] Biochemistry and Structural Biology, Lund University, SE-22100 Lund, Sweden;

[b] Division of Physical Chemistry, Chemistry Center, Lund University, SE-22100 Lund, Sweden;

[c] Laboratory for Neutron Scattering and Imaging, Paul Scherrer Institut, 5232 Villigen PSI, Switzerland.

Paste corresponding author name here
Email: veronica.lattanzi@biochemistry.lu.se

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Supplementary Information Text

Aβ42 fibrils formed in the presence of ThT.

We have also investigated by SAXS/WAXS Aβ42 fibrils formed in the presence of the fluorescent amyloid dye Thioflavin T, ThT (molar ratio 1:1). ThT binds to many amyloid fibrils where it has a higher quantum yield, and is commonly used to quantitatively detect amyloids in for example time resolved experiments of fibril formation (1). The objective of present experiment was to investigate if the binding of ThT had any significant effect on the fibril cross-section dimension. The SAXS pattern is shown in Figure S1, where, for comparison, we also show the pattern from fibrils formed in the absence of ThT. As can be seen, there is essentially no effect on the fibril morphology or cross-section dimension when fibrils are formed in the presence of ThT. Also, the periodic β-sheet repeat distance, d_β=4.7 Å, remains unchanged.

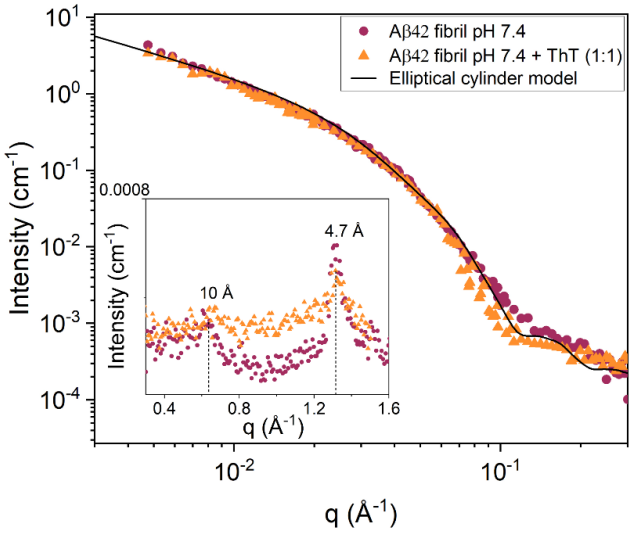

Fig. S1. Aβ42 monomer aggregation in presence of ThT; effect on fibril packing. Here we compare the absolute scale SAXS patterns of 350 μM Aβ42 fibrils formed in 20 mM phosphate buffer, 0.2 mM EDTA, 0.02% NaN_3 at pH 7.4 at 37 °C under quiescent conditions in absence (red dots) and in presence (orange triangles) of 350 μM ThT. The solid line is a calculated scattering pattern, where the fibrils are modeled as elliptical cylinders (see main text). The inset shows the wide-angle scattering pattern, where the dashed lines indicate the reflections at q=1.3 and 0.6 Å-1, respectively. No drastic changes in fibril morphology and internal packing were observed when fibrils were formed in presence of ThT.

Small angle neutron scattering and Aβ42 fibril cross-section.

A sample of Aβ42 was also investigated by small angle neutron scattering, SANS. Here, Aβ42 monomers were incubated in 20 mM sodium phosphate, 0.2 mM EDTA, 0.02% NaN_3 at pH 7.4, at 37°C under quiescent conditions for 5 days. Right before the SANS experiment, a washing procedure was used to change the buffer to fully deuterated D_2O buffer, for a maximum contrast between protein and solvent. In this work, the pD of the deuterated phosphate buffer corresponds

to the reading of the pH meter (2). The washing procedure was performed by centrifugation (using a Hettich MIKRO 220/220 R centrifuge, rotor Cat. No. 1195-A) at 22640 g-force for 20 min at r.t. and the supernatant was carefully retrieved without altering the fibril pellet at the bottom of the low-binding tube. The supernatant was replaced with fully deuterated buffer and between washing steps fibrils were re-dispersed by 30 s vortexing. In total 3 washing steps were executed, after which the buffer was added to obtain a final fibril concentration of 300 μM and a minimum volume of 450 μL. SANS experiments were performed at the SANS-II instrument at the Swiss spallation source, SINQ, located at the Paul Scherrer Institute in Villigen, Switzerland. Demountable stainless steel cells were used and placed on a rotating rack to prevent precipitation. The sample was held in the cell between two quartz windows, each window sealed by an o-ring. The o-ring were compressed by retaining rings that were held down by four screws for a final sample thicknesses of 1 mm and sample minimum volume of 450 μL.

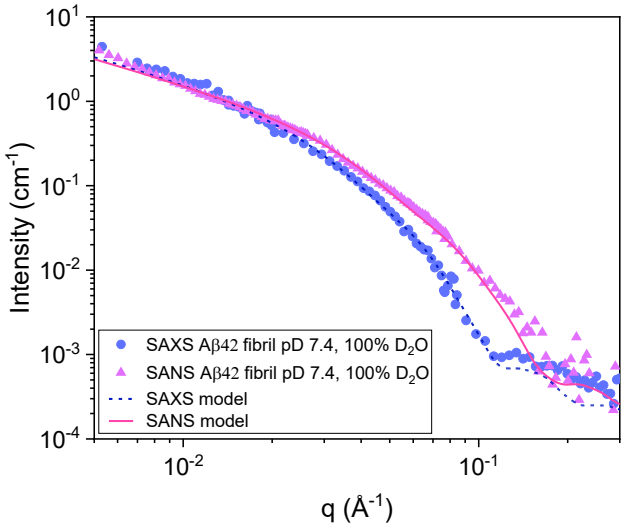

Fig. S2. Comparison between SAXS and SANS patterns. Here we compare the absolute scale SAXS (blue dots) and SANS (violet triangles) patterns of Aβ42 fibrils formed in 20 mM phosphate buffer, 0.2 mM EDTA, 0.02% NaN_3 at pD=7.4 at 37°C under quiescent conditions. The solid line and the short dash are calculated scattering patterns, where the fibrils are modeled as elliptical cylinders (see model fit parameters Table S1). SANS experiment shows a decrease in fibril cross-section. Again, no changes in the number of filaments per fibrils was observed (see Table S1).

The SANS scattering pattern is presented in Figure S2, where we also compare with reference SAXS data from a similar sample, also in a D_2O buffer. While the SAXS is essentially identical to the SAXS pattered obtained with H_2O buffer (Fig. 1 in the main paper), the SANS pattern is significantly different, consistent with smaller cross-section dimensions. Model scattering curves for the SAXS and SANS are shown as short dash and solid line, respectively. In the case of SANS modelling, the fact that Aβ42 contains 15% exchangeable protons was taken into account. For SAXS, all the model parameters were the same as for the cases when H_2O buffers were used (Table S1). In the SANS model curve the semi-axis dimensions were 2.0 and 8.0 nm respectively, which shall be compared with 3.0 and 9.0 nm, respectively for the SAXS sample (Table S1). A

similar difference between SANS and SAXS has been reported by Zhang et al. in a study of the model protein bovine serum albumin (3). They ascribed the difference to the contribution from a hydration shell in SAXS contrast. In any case, we note that the SANS data here are also consistent with N=2.

Chi-squared tests for the atomistic modeling.

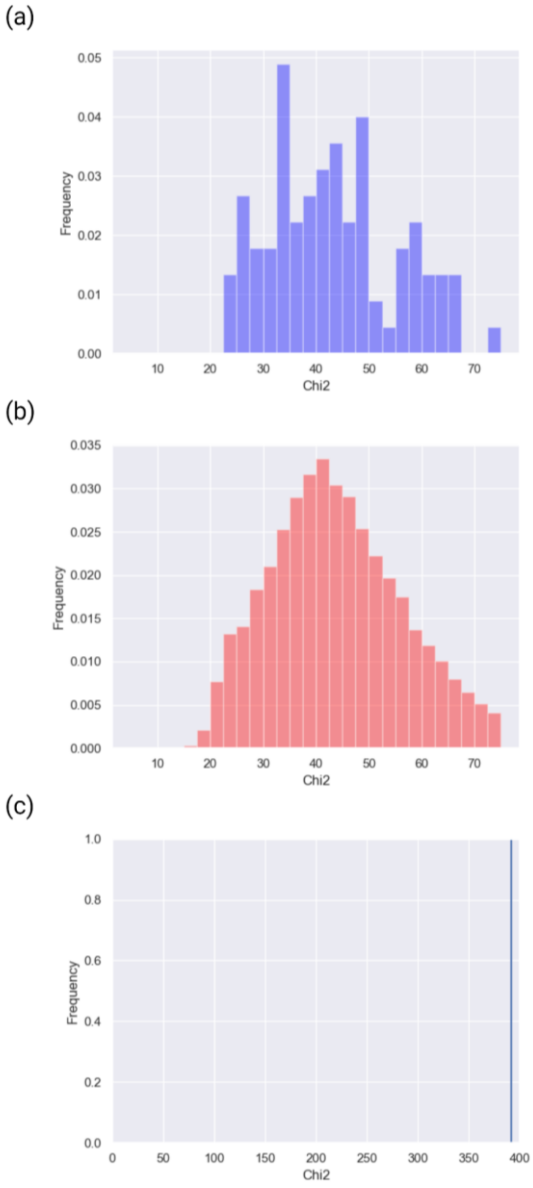

Fig. S3. Distribution of χ^2 values among models for the relative subunit rotation selected to be optimal. A) Single conformation of N-termini. B) Two different conformations of N-termini. C) No termini.

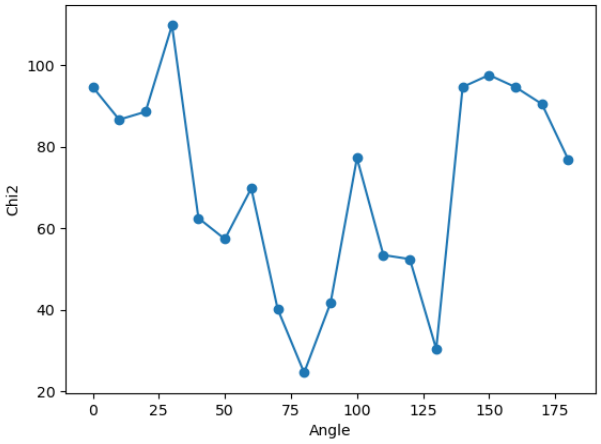

Fig. S4. Minimum χ^2 value for modeled ensemble as a function of rotation of the asymmetric unit. Models with single N-terminal conformation only.

Table S1. SAXS and SANS model fit parameters of Aβ42 fibrils formed in 20 mM phosphate buffer, 0.2 mM EDTA, 0.02% NaN_3 100% D_2O at pD 7.4.

Parameters	**SANS**	**SAXS**
Protein concentration [μM]	300	350
Molar weight [g/mol]	4645	4645
Protein mass density [g/mL]	1.43	1.43
Water (solvent) scattering length density [cm^{-2}]	$6.39*10^{10}$	$9.37*10^{10}$
rP: Protein scattering length density [cm^{-2}]	$2.83*10^{10}$	$12.7*10^{10}$
β-sheet repeat distance [Å]	4.7	4.7
Bg: background [cm^{-2}]	$2.0*10^{-4}$	$2.0*10^{-4}$
a: semi-axis 1 [nm]	2.0	3.0
b: semi-axis 2 [nm]	8.0	9.0
N: number of filaments	2	2

Comparison with circular cross-section.

In Fig. S5 we compare the form factors of the elliptical cylinder and a cylinder with circular cross-section, having the same cross-section area. In the figure we also show the experimental scattering data obtained at pH=7.4. No polydispersity is used, hence oscillations in the form factor is more pronounced in the case of the circular cross-section compared to the elliptical one. As can be seen, there is a clear difference between the two model form factors.

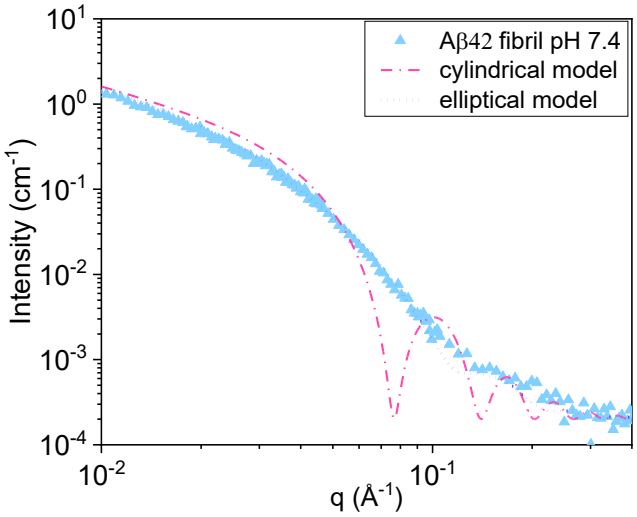

Fig. S5. Comparison between elliptical and circular cross-section. SAXS pattern of Ab42 fibrils, formed at pH=7.4 together with calculated form factors for cylinders having elliptical (a=3.0 nm and b=9.0 nm respectively) and circular (r=5.2 nm) cross-sections, respectively.

Here it is useful to recall that having an elliptical cross-section from a scattering point of view is essentially the same as having a circular cross-section together with a polydispersity in the cross-section radius covering the range from a to b. This is why the oscillations in the model form factor are not very pronounced in the case of the elliptical cross-section, even though no polydispersity in a or b is assumed. The parameter r in Eq. (4) in the main article can be seen as an effective radius, and φ in Eq. (4) is the 2D angular coordinate. The integral over φ in Eq.(3) thus essentially corresponds to an averaging over the effective radius r, with $a \leq r \leq b$, with a particular distribution function.

Thus, it would in principle be possible to describe the scattering patterns in Fig. 2 also with cylinders having circular cross-sections, if one includes the polydispersity in the cross-section radius covering the range from 3 to 9 nm. However, such a large polydispersity would then imply a corresponding broad polydispersity in the number of filaments, N. The fact that we reproduce the SAXS pattern in five individual experiments indicates that the cross-section degree of freedom has reached thermal

equilibrium in these experiments. While a broad equilibrium distribution of *N* cannot be completely excluded, a monodisperse cross-section with *N*=2, seems more likely, considering the reproducibility and the good agreement with the atomistic model form factor.

SI References

1. E. Hellstrand, B. Boland, D. M. Walsh, S. Linse, Amyloid β-protein aggregation produces highly reproducible kinetic data and occurs by a two-phase process. *ACS Chem Neurosci* **1**, 13-18 (2010).
2. K. A. Rubinson, Practical corrections for p(H,D) measurements in mixed H2O/D2O biological buffers. *Analytical Methods* **9**, 2744-2750 (2017).
3. F. Zhang *et al.*, Hydration and interactions in protein solutions containing concentrated electrolytes studied by small-angle scattering. *Physical Chemistry Chemical Physics* **14**, 2483 (2012).