Supplementary information for:

Amyloid-β Nanotubes are Associated with Prion Protein-Dependent Synaptotoxicity

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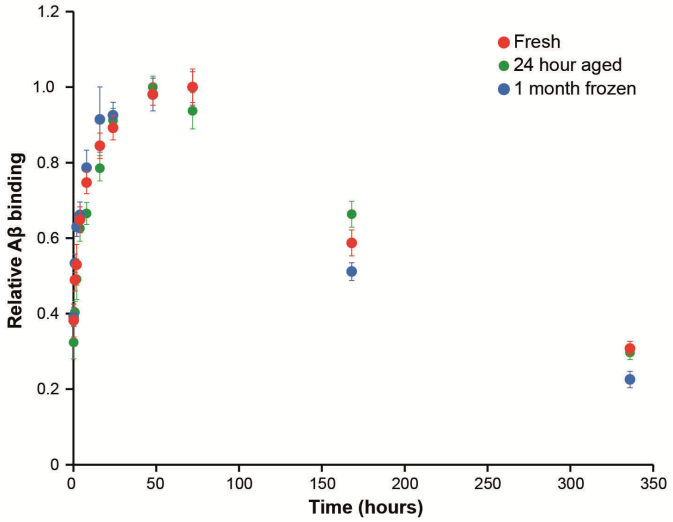
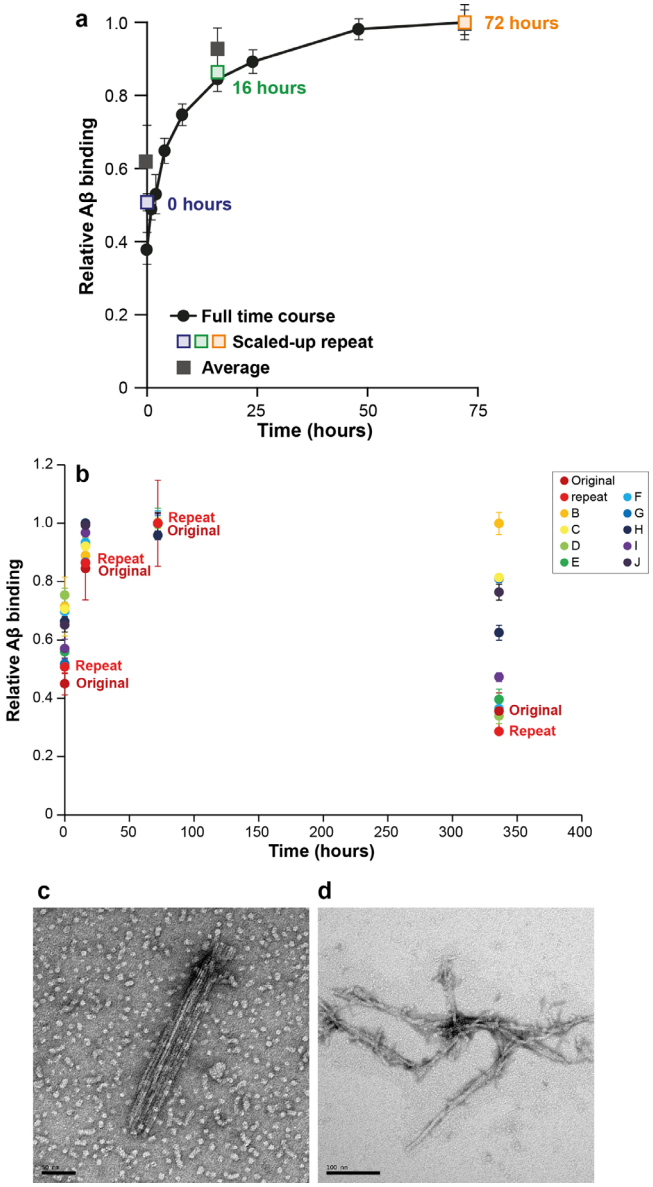
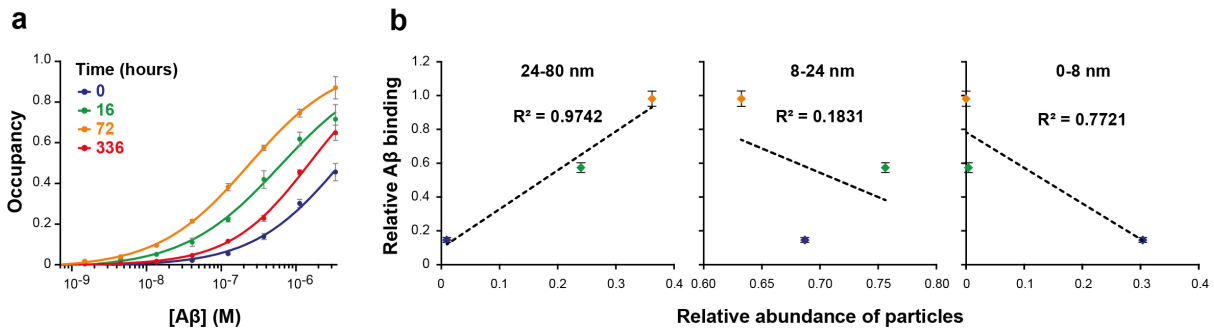


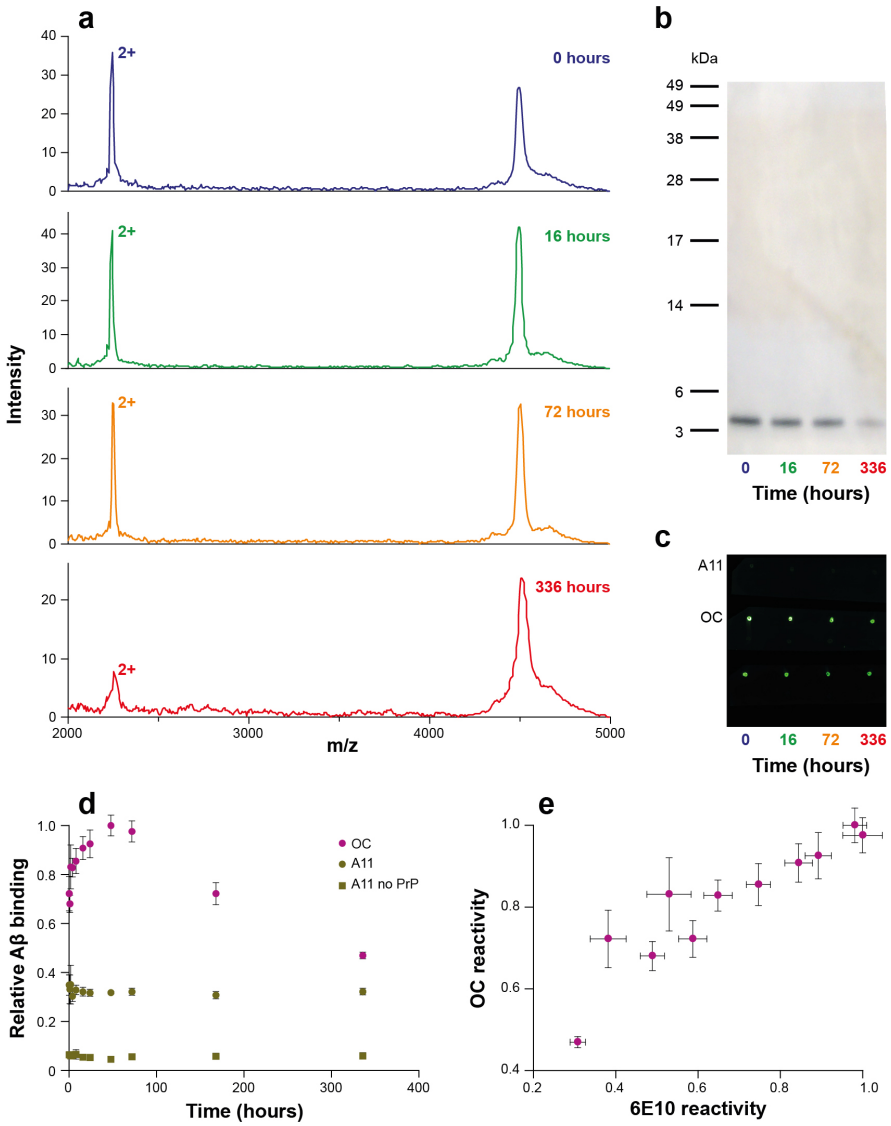
Supplementary Figure S1 **Long-term freezing or incubation of diluted samples for 24 hours at room temperature does not change the binding profile, suggesting the aggregation can be effectively blocked.** Original time course (red) with 11 time points was not systematically changed after dilution to 120 nM in PBS (0.05% Tween-20) and incubation at room temperature for 24 hours (green) or during storage of snap-frozen aliquoted samples at -80 °C for over 1 month (blue) (n = 3, error bars show standard deviation).



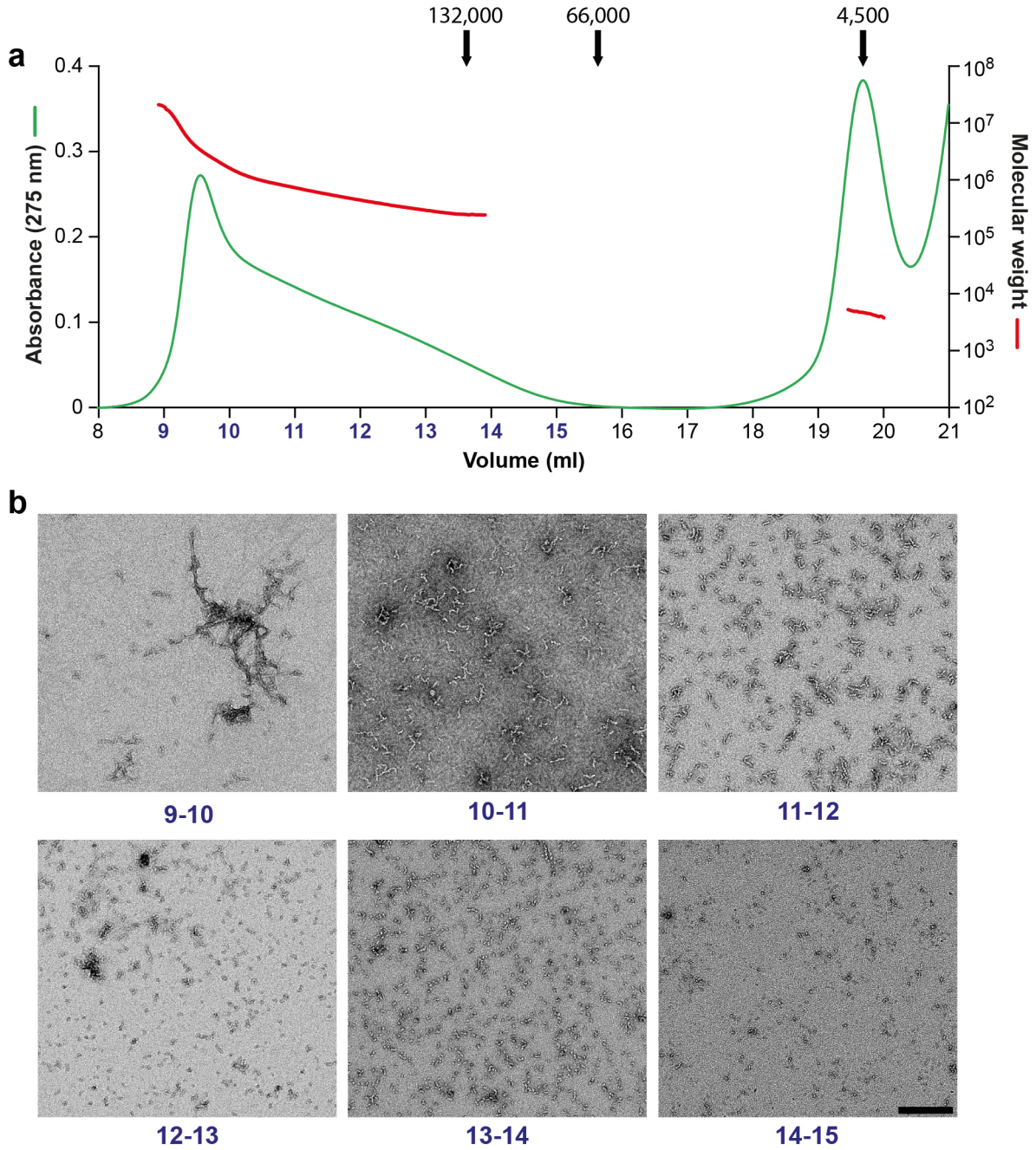
Supplementary Figure S2 **Repeat time-dependent aggregation of $A\beta_{1-42}$ and binding to PrP shows large variation, especially at the 336 hour time point.** Disaggregated films of $A\beta_{1-42}$ were dissolved in DMSO and Ham's F12 media, vortexed and centrifuged before incubating at 22 ^{o}C without agitation for different periods of time. **a**, A magnified version of Fig. 1d showing binding at 0, 1, 2, 4, 8, 16, 24, 48 and 72 hours (n = 3, error bars show standard deviation). **b**, Binding of Aβ to huPrP detected by DELFIA assay for the initial time course (dark red), a scaled-up repeat time course used for further characterisation including LTP (bright red) and 9 other separate time courses (orange to purple) (n = 3, error bars show standard deviation). **c**, Representative image of a 336 hour aggregated Aβ that retained PrP binding in the DELFIA which still contained short protofibrils (<50 nm) and globular structures (Batch B) seen by negative stain EM (Scale bar 50 nm) as well as a fibrillar clump. **d**, Representative image of a 336 hour aggregated Aβ that lost PrP binding in the DELFIA and no longer contained non-fibrillar Aβ assemblies (Batch D) seen by negative stain EM (Scale bar 100 nm).



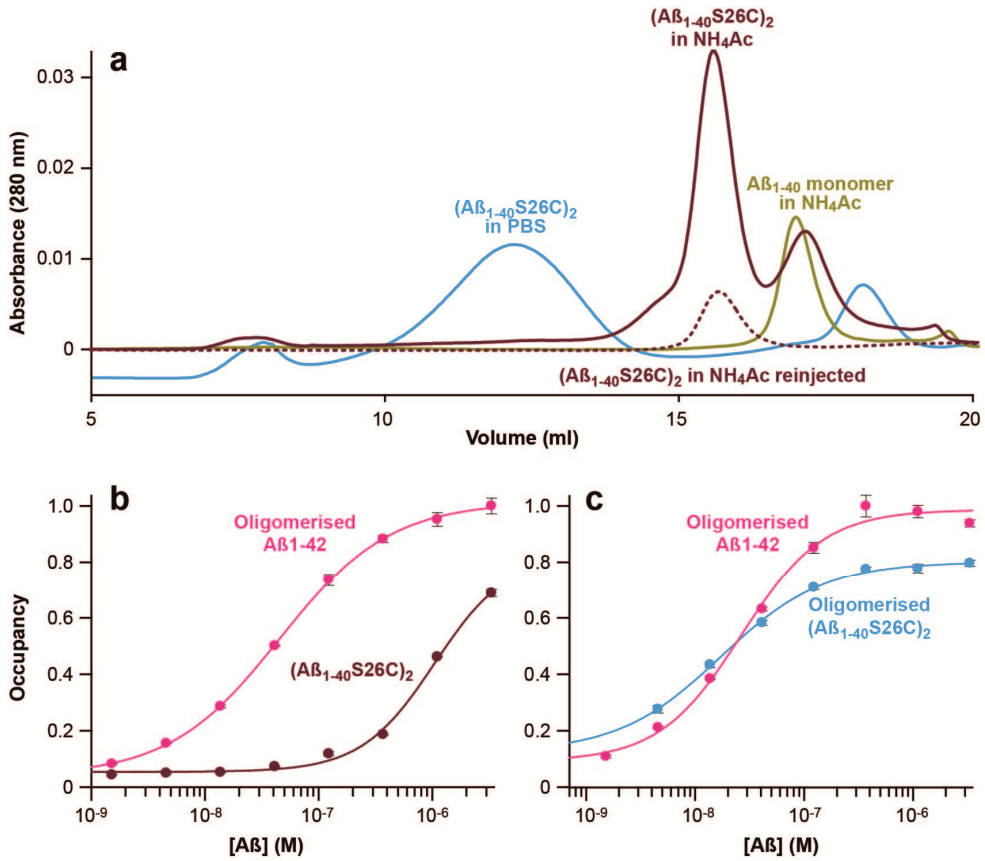
Supplementary Figure S3 **Binding to N1 fragment of PrP by differentially aggregated $A\beta_{1-42}$ shows greater selectivity for 72 hour time point containing most protofibrillar assemblies. a,** Dose response curve of the N1 fragment of PrP binding to Aβ aggregated for 0 (blue), 16 (green), 72 (orange) and 336 hours (red) (n = 3, error bars show standard deviation). **b,** Binding to N1 fragment of PrP at 120 nm Aβ concentration directly correlates with the presence of particles of lengths between 24-200 nm, as measured using EM (n = 3, error bars show standard deviation). To a lesser extent binding to particles with lengths 16-80 nm (R^2 = 0.9423) then 16-24 nm (R^2 = 0.8948) also showed a positive correlation. Particles with lengths 0-16 nm (R^2 = 0.9454) showed the strongest negative correlation suggesting the major PrP binding species are longer than 16 nm. The same batch was used for EM, DELFIA and most experiments in Fig. 1 and 2.



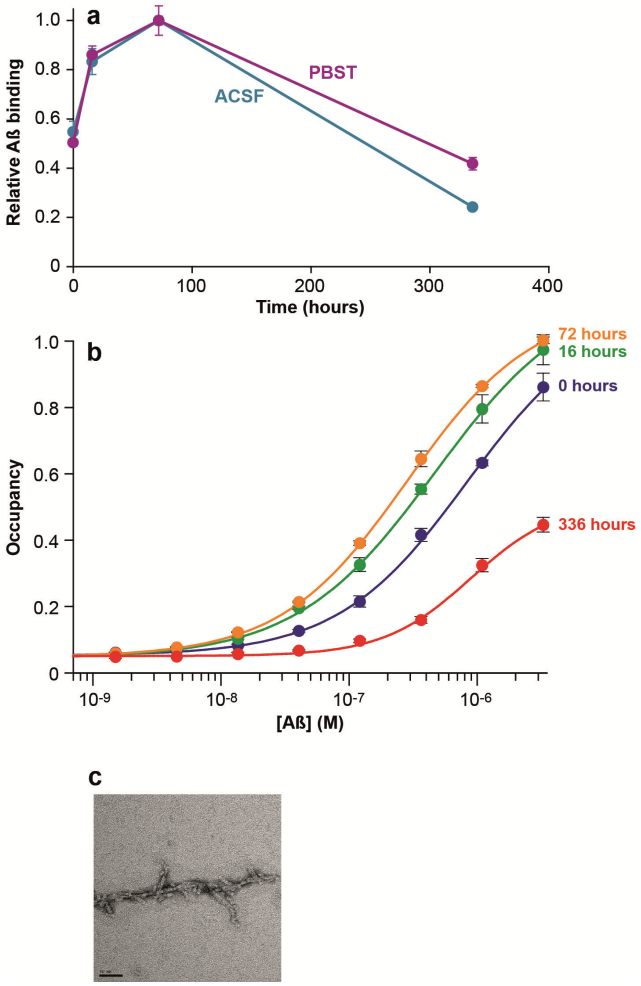
Supplementary Figure S4 **Biochemical characterisation of $A\beta_{1-42}$ present during time-dependent aggregation of $A\beta_{1-42}$. a**, SELDI-TOF mass spectra of Aβ aggregated for 0 (blue), 16 (green), 72 (orange) and 336 hours (red) show no significant peptide degradation over the course of the experiment. **b**, SDS-PAGE visualised by silver staining of Aβ aggregated for 0 (blue), 16 (green), 72 (orange) and 336 hours (red) shows most Aβ remains SDS-soluble up to the 72 hour time point. **c**, Dot blot analysis of Aβ aggregated for 0 (blue), 16 (green), 72 (orange) and 336 hours (red) shows that all time points contain "OC positive fibrillar oligomers" but very low levels of "A11 positive prefibrillar oligomers". The overall Aβ concentration remains constant, as seen by the anti-Aβ monoclonal antibody 6E10 (n = 3, error bars show standard deviation). **d**, DELFIA of initial time course experiment with detection by OC (purple) or A11 (olive) antibodies. A11 bound specifically to saturated, high density PrP found on the DELFIA plate, but showed no change on addition of Aβ. In contrast, OC detection showed a similar pattern of binding to that seen with 6E10 suggesting that all bound Aβ had similar degree of OC immunoreactivity. **e**, correlation of 6E10 and OC binding at different time points shows a direct correlation between their immunoreactivities (n = 3, error bars show standard deviation).



Supplementary Figure S5 **Characterisation of size-exclusion isolated Aβ oligomers. a**, SEC separation in PBS detected by UV and multi-angle light scattering of a batch of 16-hour incubated Aβ contains a mixture of monomer (4,500 ± 400) and different sized species with molecular masses ranging from 10^5 to 10^6. Arrows represent elution volumes for mass standards of NaOH-solubilised Aβ (4,500), BSA monomer (66,000) and BSA dimer (132,000). **b**, Representative negative stain EM images of different fractions of SEC-purified Aβ shows aggregated fibrillar material at 9-10 ml, with protofibrillar assemblies eluting between 10-15 ml with progressively decreasing lengths (Scale bar 200 nm).



Supplementary Figure S6 **Binding to PrP by purified $(A\beta_{1\text{-}40}S26C)_2$ and oligomers of $(A\beta_{1\text{-}40}S26C)_2$. a**, Initial purification by SEC of $(A\beta_{1\text{-}40}S26C)_2$ incubated overnight in 6 M guanidinium chloride and eluted in NH_4Ac (pH 8.5) (purple, solid line). Re-injection of purified $(A\beta_{1\text{-}40}S26C)_2$ after a 1 hour incubation at room temperature (purple, dashed line) showed that the sample contained pure $(A\beta_{1\text{-}40}S26C)_2$ and that no further aggregation was detected under these conditions. In contrast, purification by SEC of $(A\beta_{1\text{-}40}S26C)_2$ incubated overnight in 6 M guanidinium chloride and eluted in PBS (blue, solid line) lead to the formation of assemblies with masses between 70-200,000 as well as remaining reduced monomer (18 ml). **b**, Comparison of PrP binding by $(A\beta_{1\text{-}40}S26C)_2$ (purple) compared to Aβ aggregated using for 16 hours (magenta) show that ~100-fold greater Aβ concentration is required for a similar level of binding (n = 3, error bars show standard deviation). **c**, Comparison of PrP binding by $(A\beta_{1\text{-}40}S26C)_2$ assemblies (blue) and 16 hour oligomers [1, 2] (magenta) show a similar level of binding (n = 3, error bars show standard deviation).



Supplementary Figure S7 **Binding to PrP by differentially aggregated $A\beta_{1-42}$ is similar in PBST or artificial cerebrospinal fluid. a**, Comparison of the PrP binding of Aβ aggregated for 0, 16, 72 and 336 hours in ACSF (blue) compared to the standard PBST (0.1% BSA) (magenta) show buffer conditions do not markedly alter the PrP binding (n = 3, error bars show standard deviation). **b**, Dose response curve of PrP binding in ACSF of Aβ aggregated for 0 (blue), 16 (green), 72 (orange) and 336 hours (red) (n = 3, error bars show standard deviation). **c**, Supernatants of the same sample used in Fig. 3a and b after centrifugation at 16,100 x g for 20 min still contain fibrils (Scale bar 50 nm).