Influence of S100A9 on Prion Protein Amyloid Aggregation

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Supplementary information

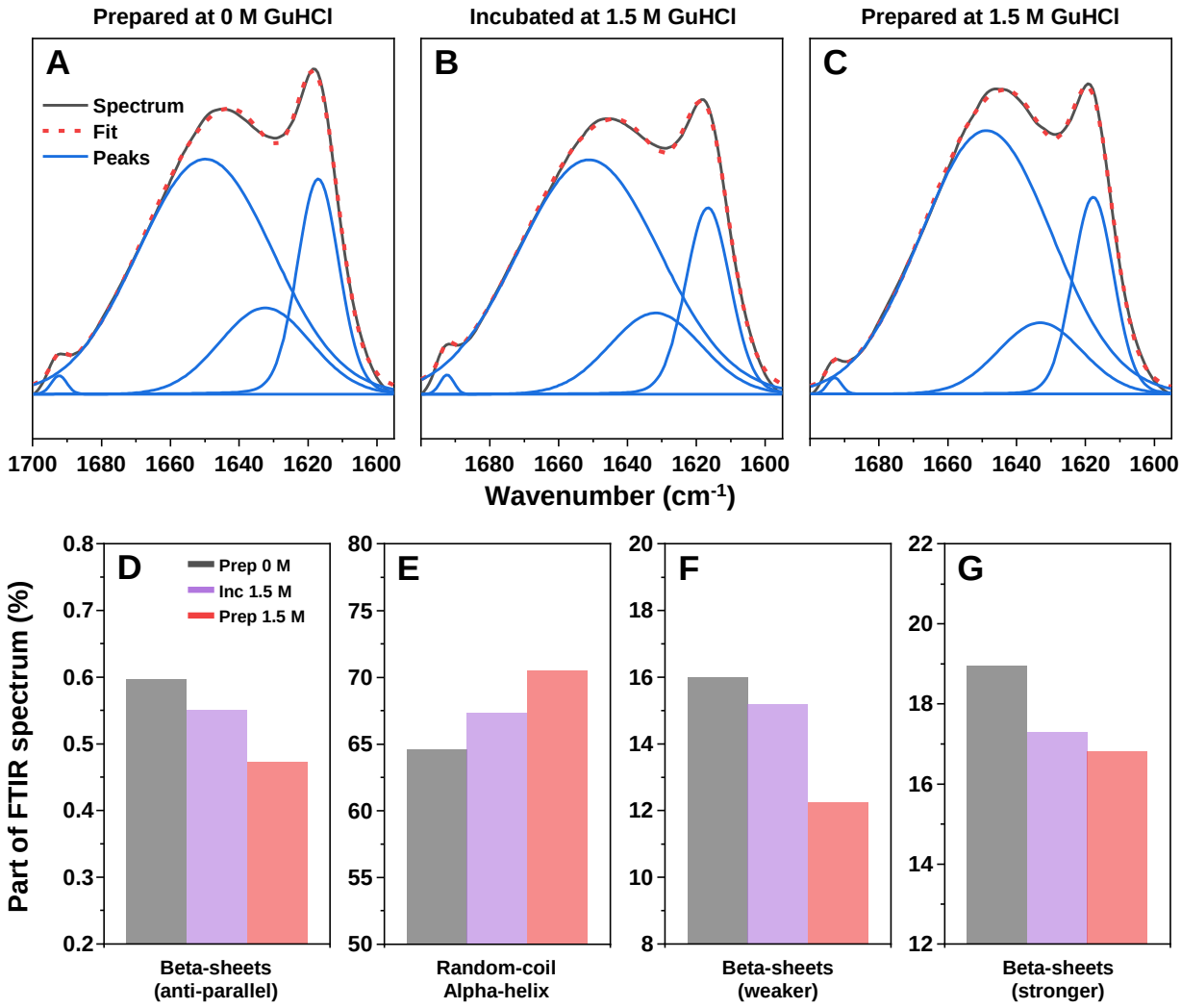

Figure S1. Deconvolution of S100A9 fibril FTIR spectra when they were formed under 0 M GuHCl conditions (A), resuspended and incubated under 1.5 M GuHCl (B) or formed under 1.5 M GuHCl conditions (C). The parts of FTIR spectra associated with anti-parallel beta-sheets (D), random-coil or alpha-helical motifs (E), weaker (F) and stronger (G) hydrogen bond strength parallel beta-sheets.

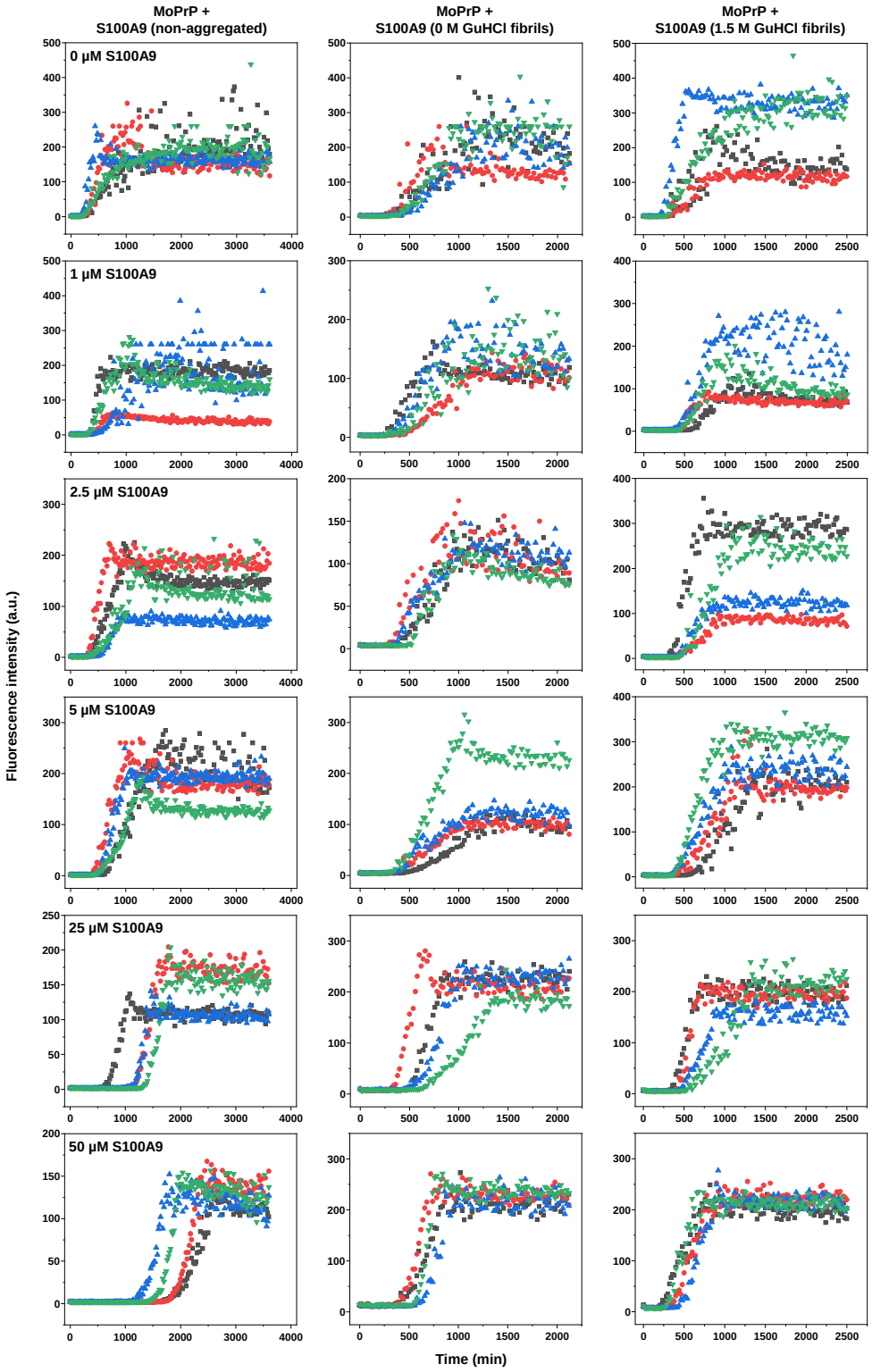

Figure S2. Representative aggregation curves (n=4) of PrP under different concentrations of non-aggregated S100A9 (first column) and S100A9 aggregated under 0 M GuHCl (second column) or 1.5 M GuHCl (third column) conditions.

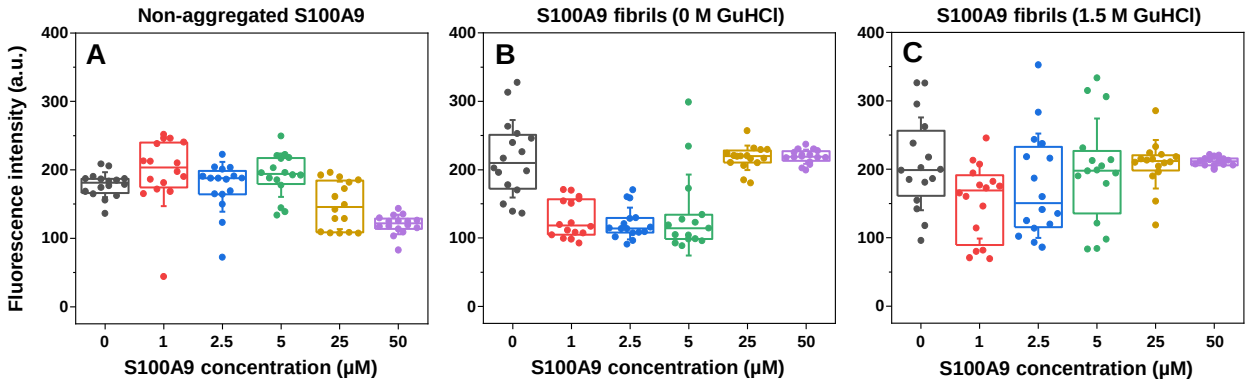

Figure S3. Fluorescence intensity distributions of PrP samples prepared under different conditions of non-aggregated S100A9 (A) and S100A9 fibrils prepared under 0 M (B) and 1.5 M GuHCl (C) conditions.

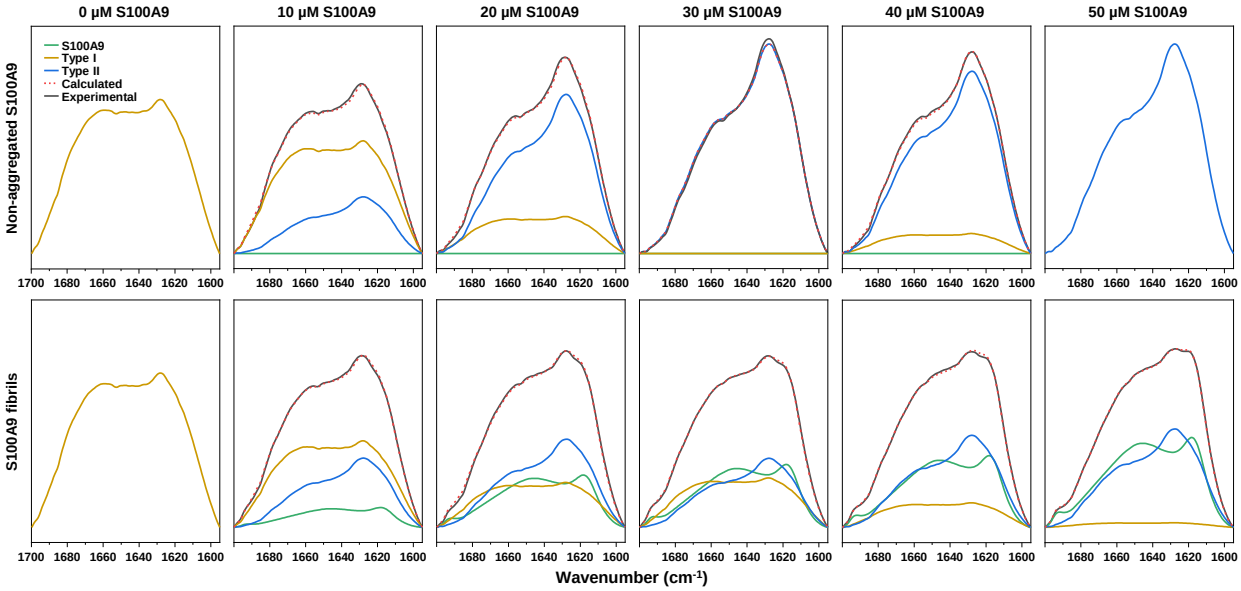

Figure S4. Deconvolution of PrP-S100A9 sample aggregate FTIR spectra. Part of FTIR spectra related to S100A9 fibrils (green), type I (yellow) and type II (blue) PrP fibrils. Combined S100A9, type I and II PrP fibril spectra (red dashed line) comparison with experimentally acquired S100A9-PrP spectra (black).

S100A9 cloning, expression and purification

Plasmid encoding His6-SUMO-S100A9 gene was derived from constructs of S100A9 (a gift of Ludmilla Morozova-Roche (Umeå)) and pET28_SUMO_PDZ1PDZ2 (a gift of Björn M. Burmann (Gothenburg)]. Briefly, the S100A9 gene and the His6-SUMO tag were amplified and fused by standard PCR methods, yielding the His6-SUMO-S100A9 insert which was cloned into the pET28a(-) vector (5'-NcoI; 3'-BamHI). All primers used for cloning are listed in Table 1.

Table 1. Plasmid, primers and primer sequences used for S100A9 cloning.

Plasmid	Primer	Sequence
pDS99 (6xHis-SUMO-S100A9	pet15_5206	5' ATCGAGATCTCGATCCCGCG 3'
	DS99_S100A9_BamHI	5' CGGGATCCTTAGGGGGTGCCCTCCCC 3'
	DS99_S100A9_frw	5' GATTGGCGGTATGACTTGCAAAATGTCGCAG 3'
	DS99_S100A9_rev	5' GCAAGTCATACCGCCAATCTGTTCCAGATG 3'

The plasmid containing His6-SUMO-S100A9 was chemically transformed into One ShotTM E. coli BL21 StarTM (DE3) (Fisher Scientific) cells, that afterwards were grown at 37°C in LB medium containing kanamycin (50 mg/ml). Once optical density was reached at 600 nm ≈ 0.7, protein production was induced with 0.4 mM isopropyl-β-d-thiogalactopyranoside (Fisher Scientific), and the cells were left to grow overnight at 25°C. Cells were harvested by centrifugation at 6000 x g for 20 min at 4°C and subsequently resuspended in 50 ml of lysis buffer (25 mM Hepes/NaOH, 0.5 M NaCl, and 10 mM imidazole (pH 7.5)). The suspension was disrupted with a Sonopuls (Bandelin) homogeniser (10 s on, 30 s off, 30% power, total time 30 min). Cell debris was removed by centrifugation at 18,000 x g for 45 min at 4°C, and the supernatant was applied to a Ni2+ Sepharose 6 Fast Flow (Cytiva) loaded gravity column, followed by stepwise elution with 20 ml of lysis buffer supplemented with 75 and 300 mM imidazole, respectively. Fractions containing the His6-SUMO-S100A9 protein were dialyzed two times against 10 mM Tris buffer (pH 7.4) and the His6-SUMO tag was cleaved with human Sentrin-specific protease 1 (SENP1) catalytic domain (derived from pET28a-HsSENP1, that was a gift from Jorge Eduardo Azevedo (Addgene plasmid #71465) at 4°C overnight [1,2]. The cleaved proteins were applied again to a Ni^{2+} column, and the flow-through was collected. The proteins were concentrated using Amicon centrifugal filters [10k Molecular weight cut-off (MWCO), Merck Millipore] and purified further by size exclusion chromatography (HiLoad® 26/600 Superdex® 75 pg, Cytiva) in PBS.

References

1. Panavas, T., Sanders, C. & Butt, T. R. SUMO Protocols: Chapter 20. *Methods Mol. Biol.* **497**, 303–317 (2009).

2. Mendes, A. V., Grou, C. P., Azevedo, J. E. & Pinto, M. P. Evaluation of the activity and substrate specificity of the human SENP family of SUMO proteases. *Biochim. Biophys. Acta - Mol. Cell Res.* **1863**, 139–147 (2016).