Crowded, Cell-like Environment Accelerates the Nucleation Step of Amyloidogenic Protein Misfolding

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Running Title: Crowding accelerates human Tau and prion protein misfolding

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Supplemental Data

FIGURE S1. **Verification of phosphorylated status of $Tau_{244-441}$ fragment by GSK-3β.** SDS-PAGE profiles of non-phosphorylated and GSK-3β hyperphosphorylated $Tau_{244-441}$ fragments (*A*). *Lane* 1, protein molecular weight marker: β-galactosidase (116.0 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), lactate dehydrogenase (35.0 kDa), restriction endonuclease *Bsp*98 I (25.0 kDa), β-lactoglobulin (18.4 kDa), and lysozyme (14.4 kDa). *Lane* 2 represents non-phosphorylated $Tau_{244-441}$ and *lane* 3 represents GSK-3β hyperphosphorylated $Tau_{244-441}$. Proteins in the gel were visualized by Coomassie Blue staining. Sequencing by tandem MS/MS in IDA mode corresponding to phosphorylated residue Ser400 in $Tau_{244-441}$ (*B*). A bivalent precursor ion was sequenced by tandem MS/MS. Phosphopeptide SPVVpSGDTSPR (Mr 1180.51; residues 396-406, precursor ion $[M+2H]^{2+}$ with *m*/*z* 590.98^{2+}). The peptide mass and *m*/*z* of the fragment ions identify a phosphopeptide with phosphate on Ser400 of $Tau_{244-441}$ fragment. The y ion series (y7-y10) demonstrate that Ser400 is dehydroalanine because of the loss of phosphoric acid (98 Da) from phosphoserine in the fragmentation reaction.

FIGURE S2. **Effects of macromolecular crowding on amyloid formation of human prion protein monitored by ThT fluorescence (*A*) and assay of the absorbance at 400 nm (*B*).** The concentration of PrP was 0.25/0.125 and 0.50 mg/ml for (*A*) and (*B*) respectively. For 0.25 or 0.50 mg/ml PrP, the crowding agent

concentrations were 0 (*open square*), 50 g/l (*solid circle*), 100 g/l (*solid triangle*), and 150 g/l (*inverted solid triangle*), respectively. For 0.125 mg/ml PrP, the crowding agent concentrations were 0 (*open circle*), 100 g/l (*open triangle*), and 150 g/l (*inverted open triangle*), respectively.

FIGURE S3. **Proteinase K-digestion assays of human PrP fibrils formed in the absence of a crowding agent (*A*) and in the presence of 150 g/l Ficoll 70 (*B*).** Human PrP fibril samples were prepared in 100 mM Tris-HCl (pH 7.5) with 0.5% Triton X-100 (*lanes* 2-4 and 9-11) or without Triton X-100 (*lanes* 5-7 and 12-14) and heated in water bath for 15 min at 80 ^{0}C, cooled down, and then incubated with PK for 1 h at 37 ^{0}C at PK/PrP molar ratios: 1:1000 (*lanes* 2, 5, 9, and 12), 1:500 (*lanes* 3, 6, 10 and 13), and 1:100 (*lanes* 4, 7, 11, and 14). The controls with zero protease in the absence of a crowding agent and in the presence of 150 g/l Ficoll 70 were loaded on *lanes* 1 and 8 respectively. Amyloid fibrils were produced from human PrP incubated in the absence and presence of crowding agents for 12 h. Protein fragments were separated by SDS-PAGE and detected by silver staining.

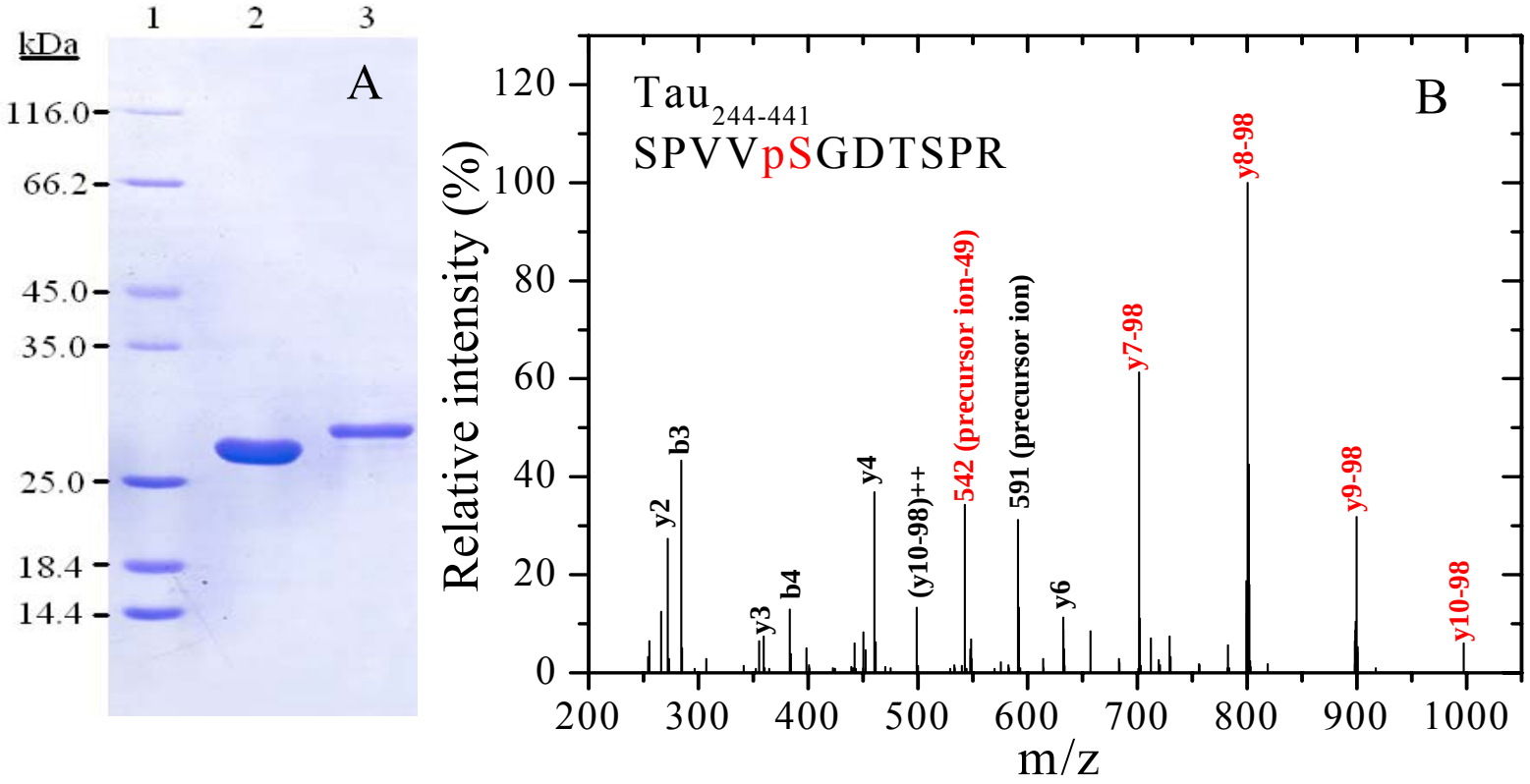
1
2
3
kDa
A
116.0
66.2
45.0
35.0
25.0
18.4
14.4
B
Tau244-441
SPVVpSGDTSPR
Relative intensity (%)
m/z
y2
b3
y3
b4
y4
(y10-98)++
542 (precursor ion-49)
591 (precursor ion)
y6
y7-98
y8-98
y9-98
y10-98

Figure S1

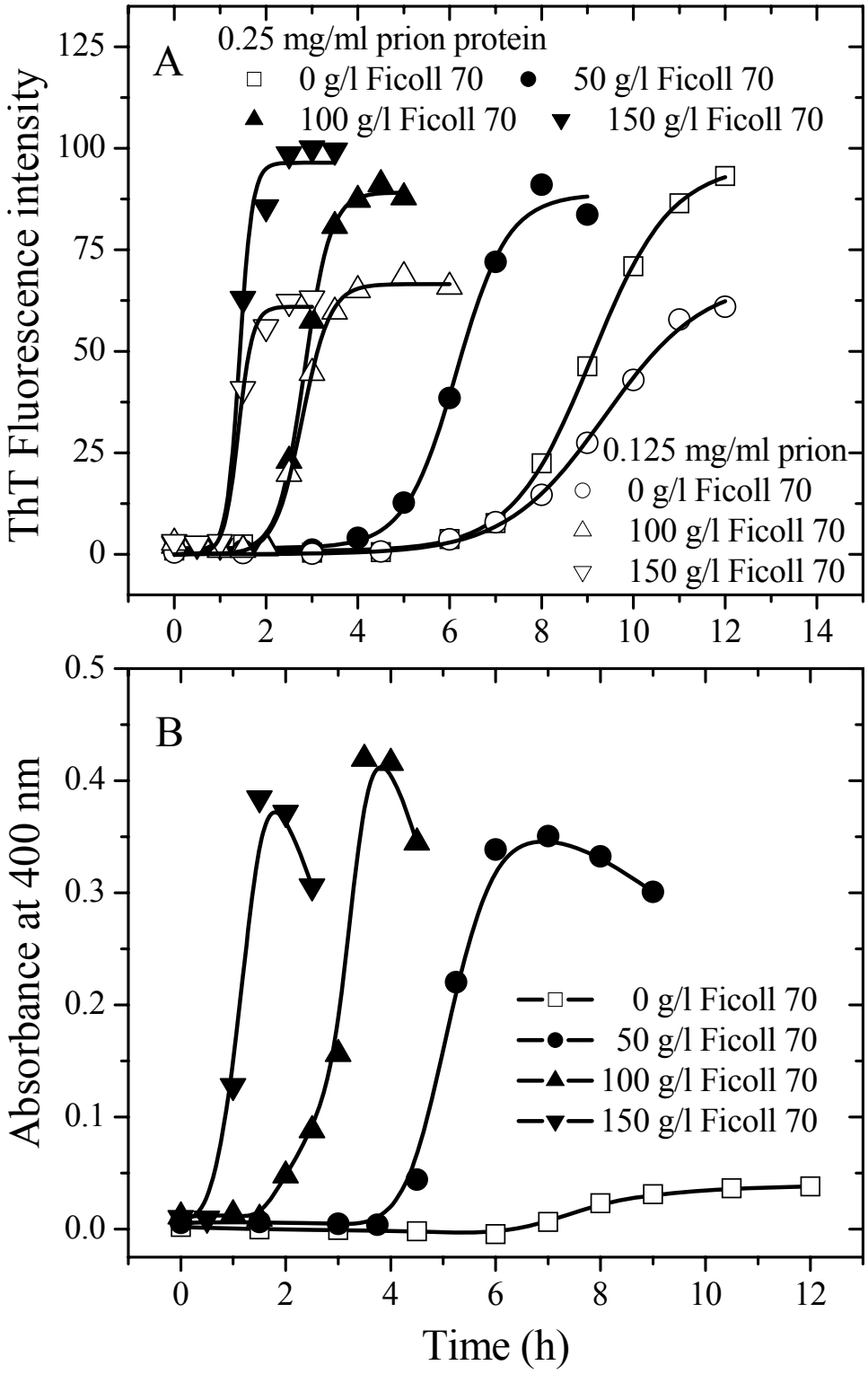
A
0.25 mg/ml prion protein
0 g/l Ficoll 70
50 g/l Ficoll 70
100 g/l Ficoll 70
150 g/l Ficoll 70
0.125 mg/ml prion
0 g/l Ficoll 70
100 g/l Ficoll 70
150 g/l Ficoll 70
ThT Fluorescence intensity
B
0 g/l Ficoll 70
50 g/l Ficoll 70
100 g/l Ficoll 70
150 g/l Ficoll 70
Absorbance at 400 nm
Time (h)

Figure S2

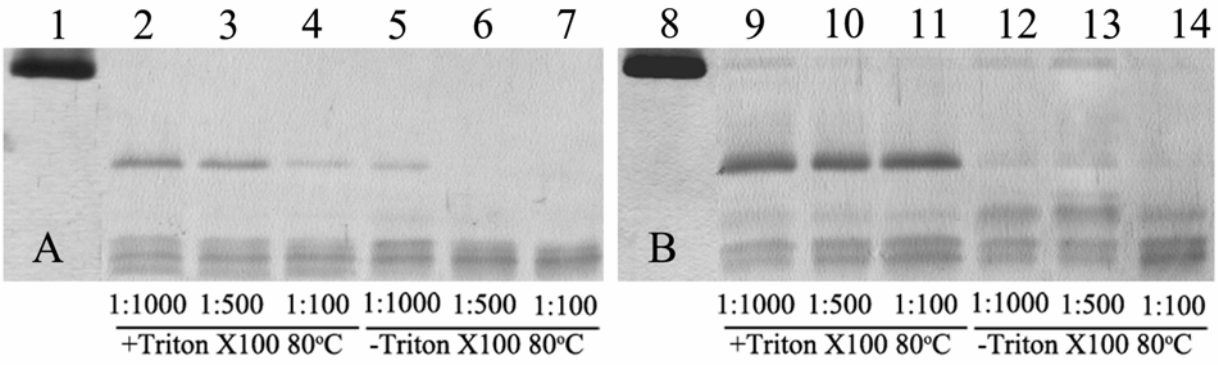
1 2 3 4 5 6 7
8 9 10 11 12 13 14
A
B
1:1000 1:500 1:100 1:1000 1:500 1:100
+Triton X100 80°C
-Triton X100 80°C
1:1000 1:500 1:100 1:1000 1:500 1:100
+Triton X100 80°C
-Triton X100 80°C

Figure S3

TABLE S1

Kinetic parameters for fibril formation of human Tau fragments in the absence and in the presence of crowding agents as determined by ThT binding assays at 37 ^{0}C. Best-fit values of these kinetic parameters were derived from non-linear least squares modeling of a sigmoidal equation as described in the "Materials and Methods" and the Hill function to the data plotted in Fig. 1. Errors shown are standard errors of the mean.

[Crowding agent] (g/l)		k (h^{-1})	t_m (h)	Lag time (h)	t_{50} (h)	$F(\infty)$
Hyperphosphorylated $Tau_{244-441}$						
Absence		0.23 ± 0.01	14.8 ± 0.3	6.10 ± 0.68	20.0 ± 1.3	59 ± 5
	100	2.27 ± 0.43	1.52 ± 0.15	0.64 ± 0.32	2.71 ± 1.1	45 ± 27
Ficoll 70	200	2.13 ± 0.06	1.07 ± 0.01	0.13 ± 0.04	1.67 ± 0.07	179 ± 7
	300	2.65 ± 0.04	0.77 ± 0.01	0.02 ± 0.02	0.98 ± 0.02	175 ± 4
	100	2.34 ± 0.21	1.60 ± 0.08	0.74 ± 0.16	2.56 ± 0.18	48 ± 6
Dextran 70	200	2.01 ± 0.09	1.08 ± 0.02	0.08 ± 0.06	1.75 ± 0.05	273 ± 8
	300	2.99 ± 0.07	0.69 ± 0.01	0.02 ± 0.03	1.02 ± 0.02	272 ± 5
Non-phosphorylated $Tau_{244-441}$						
Crowding agent (g/l)		k (h^{-1})	t_m (h)	Lag time (h)	t_{50} (h)	$F(\infty)$
Absence		3.07 ± 0.17	0.89 ± 0.02	0.24 ± 0.06	1.36 ± 0.03	293 ± 6
	50	4.80 ± 0.21	0.68 ± 0.01	0.26 ± 0.03	0.745 ± 0.010	219 ± 4
Ficoll 70	100	4.76 ± 0.25	0.52 ± 0.01	0.10 ± 0.03	0.560 ± 0.013	211 ± 8
	200	5.29 ± 0.25	0.45 ± 0.01	0.07 ± 0.03	0.492 ± 0.008	256 ± 8
	50	4.28 ± 0.20	0.73 ± 0.01	0.26 ± 0.03	0.788 ± 0.007	295 ± 4
Dextran 70	100	4.62 ± 0.25	0.52 ± 0.01	0.09 ± 0.03	0.573 ± 0.003	346 ± 3
	200	5.53 ± 0.27	0.39 ± 0.01	0.03 ± 0.03	0.440 ± 0.003	420 ± 6

TABLE S2

Kinetic parameters for amyloid fibril formation of human prion protein in the absence and in the presence of crowding agents as determined by ThT binding assays at 37 ^{0}C. Best-fit values of these kinetic parameters were derived from non-linear least squares modeling of a sigmoidal equation and the Hill function to the data plotted in Fig. 2*A*. Errors shown are standard errors of the mean.

[Crowding agent] (g/l)		k (h^{-1})	t_m (h)	Lag time (h)	t_{50} (h)	$F(\infty)$
Ficoll 70	0	1.51 ± 0.07	7.38 ± 0.04	6.06 ± 0.10	7.39 ± 0.03	127 ± 1
	50	1.46 ± 0.20	4.89 ± 0.11	3.52 ± 0.30	4.85 ± 0.09	124 ± 3
	100	2.25 ± 0.19	2.89 ± 0.04	2.00 ± 0.12	2.90 ± 0.02	150 ± 1
	150	6.29 ± 1.07	1.17 ± 0.03	0.86 ± 0.08	1.16 ± 0.03	209 ± 7
	200	6.64 ± 1.15	1.11 ± 0.03	0.81 ± 0.08	1.09 ± 0.03	262 ± 8

TABLE S3

Kinetic parameters for amyloid fibril formation of human α-synuclein in the absence and in the presence of crowding agents as determined by ThT binding assays at 37 ^{0}C. Best-fit values of these kinetic parameters were derived from non-linear least squares modeling of a sigmoidal equation and the Hill function to the data from Uversky and co-worker's study of amyloid formation of human α-synuclein (32). Errors shown are standard errors of the mean.

[Crowding agent] (g/l)		k (h^{-1})	t_m (h)	Lag time (h)	t_{50} (h)
Absence		0.20 ± 0.01	34.7 ± 0.2	24.7 ± 0.7	35.0 ± 0.2
150 g/l	Dextran 138	0.28 ± 0.01	16.5 ± 0.2	9.36 ± 0.46	16.4 ± 0.2
	Ficoll 70	0.43 ± 0.02	8.80 ± 0.12	4.15 ± 0.34	8.6 ± 0.1
	Ficoll 400	0.40 ± 0.02	7.35 ± 0.12	2.35 ± 0.37	7.0 ± 0.1