SUPPLEMENTARY MATERIALS

Fig. S1 A disulfide bond in 1N3R$^{S\text{-}S}$ and 1N4R$^{S\text{-}S}$ remains intact during fibrillation. After monitoring the kinetics of ThT fluorescence increase (Figs. 1B and C), insoluble fibrils of 1N3R$^{S\text{-}S}$ and 1N4R$^{S\text{-}S}$ were collected by ultracentrifugation at 110,000 x *g* for 30 min and re-dissolved in PBS containing 2 % SDS. Two μg of those re-dissolved tau fibrils was modified with 1 mM (methyl-$PEO_{12})_3$-PEO_4 maleimide (PEG) and examined by SDS-PAGE (lanes 2 and 4). As controls, soluble forms of 1N3R^{SH} and 1N4R^{SH} that are modified with PEG were also loaded (lanes 1 and 3). Samples of 1N3R$^{S\text{-}S}$ and 1N4R$^{S\text{-}S}$ aggregates did not contain the corresponding disulfide-reduced tau proteins, showing that the disulfide bond remains intact during fibrillation.

Fig. S2 Extent of tau aggregation was examined by SDS-PAGE analysis. After monitoring the kinetics of ThT fluorescence changes, protein samples were ultracentrifuged at 110,000 x *g* for 30 min and fractionated into soluble supernatant and insoluble pellets. Pellets were re-dissolved in the same volume of a PBS containing 2 % SDS with that of the corresponding supernatant. Then, equal volumes of supernatant (s) and pellet (p) fractions were loaded on a 12.5 % SDS-PAGE gel and stained with Coomassie Brilliant Blue. **(A)** Spontaneous aggregation of tau after 24 hours with plate shaking for 5 sec in every 2 min (Fig. 1B and C, also see Experimental Procedures). **(B)** Spontaneous aggregation of tau after 24 hours without plate shaking (black curves in Fig. 4). **(C)** Self-seeded aggregation of tau in the presence of heparin after 24 hours without plate shaking (self-seeding in each panel of Fig. 4). **(D)** Self-seeded aggregation of tau in the absence of heparin after 24 hours without plate shaking. A percentage indicated on the gel represents the fraction of tau in supernatant or pellets that is estimated from band intensities.

Fig. S3 Representative MALDI-TOF mass spectra of tau fibrils treated with trypsin. **(A)** 1N3R^{CA}, **(B)** 1N3R$^{S\text{-}S}$, **(C)** 1N4R^{CA}, and **(D)** 1N4R$^{S\text{-}S}$ fibrils. As mentioned in Experimental Procedures, tau fibrils were first digested with trypsin, ultracentrifuged to purify the trypsin-resistant insoluble peptides and then analyzed by a MALDI-TOF mass spectrometry. Mass spectra of the soluble tau proteins after trypsin digestion and ultracentrifugation are also shown. Identification of mass peaks was performed and summarized in Table S1. Mass peaks that could not be assigned to any tryptic fragments of tau were shown gray.

Figure S1

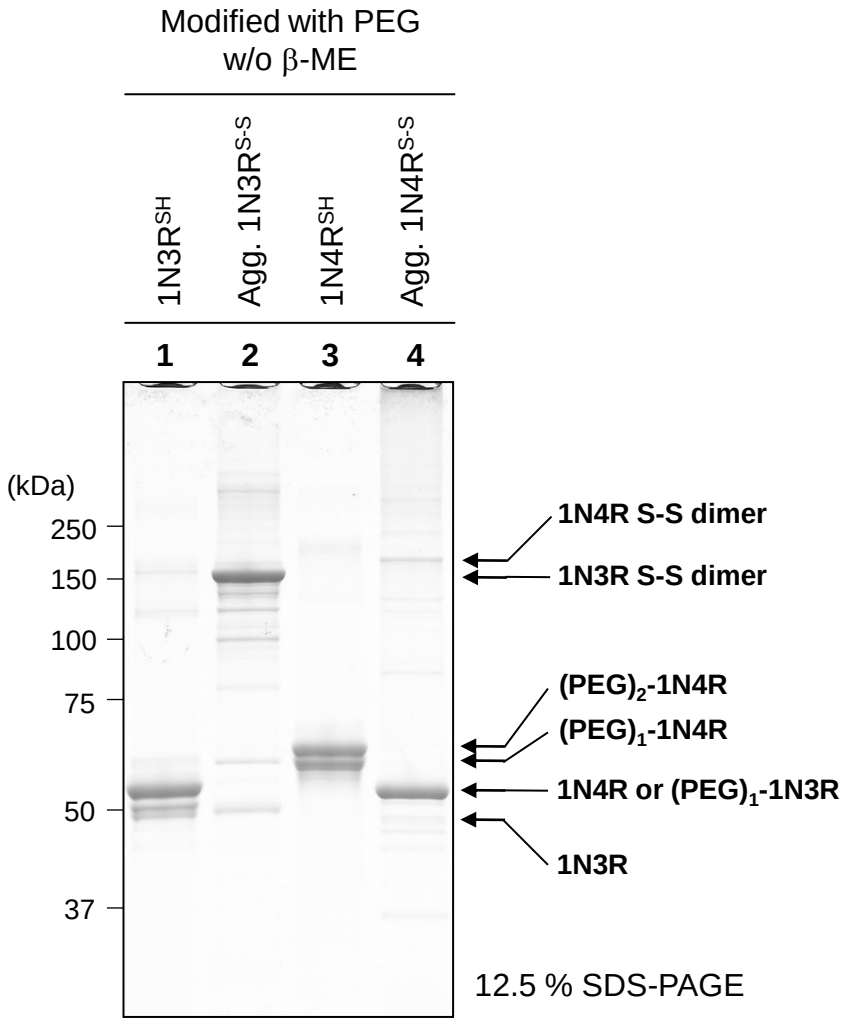
Modified with PEG
w/o β-ME
1N3R^SH
Agg. 1N3R^S-S
1N4R^SH
Agg. 1N4R^S-S
1
2
3
4
(kDa)
250
150
100
75
50
37
1N4R S-S dimer
1N3R S-S dimer
(PEG)2-1N4R
(PEG)1-1N4R
1N4R or (PEG)1-1N3R
1N3R
12.5 % SDS-PAGE

Figure S2

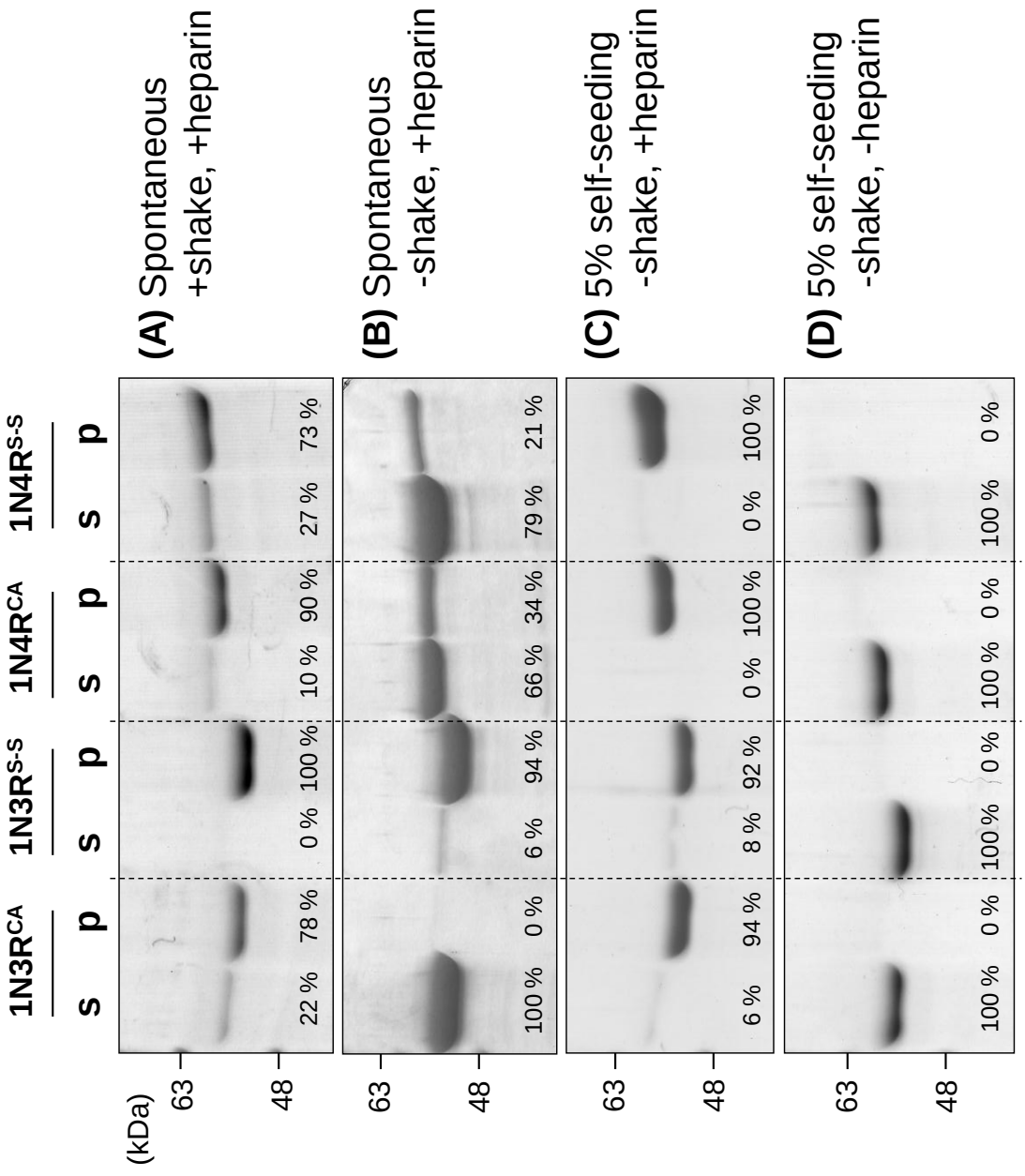
1N3R^CA
1N3R^S-S
1N4R^CA
1N4R^S-S
s p s p s p s p
(A) Spontaneous
+shake, +heparin
22 % 78 % 0 % 100 % 10 % 90 % 27 % 73 %
(B) Spontaneous
-shake, +heparin
100 % 0 % 6 % 94 % 66 % 34 % 79 % 21 %
(C) 5% self-seeding
-shake, +heparin
6 % 94 % 8 % 92 % 0 % 100 % 0 % 100 %
(D) 5% self-seeding
-shake, -heparin
100 % 0 % 100 % 0 % 100 % 0 % 100 % 0 %
(kDa)
63
48
63
48
63
48
63
48

Figure S3

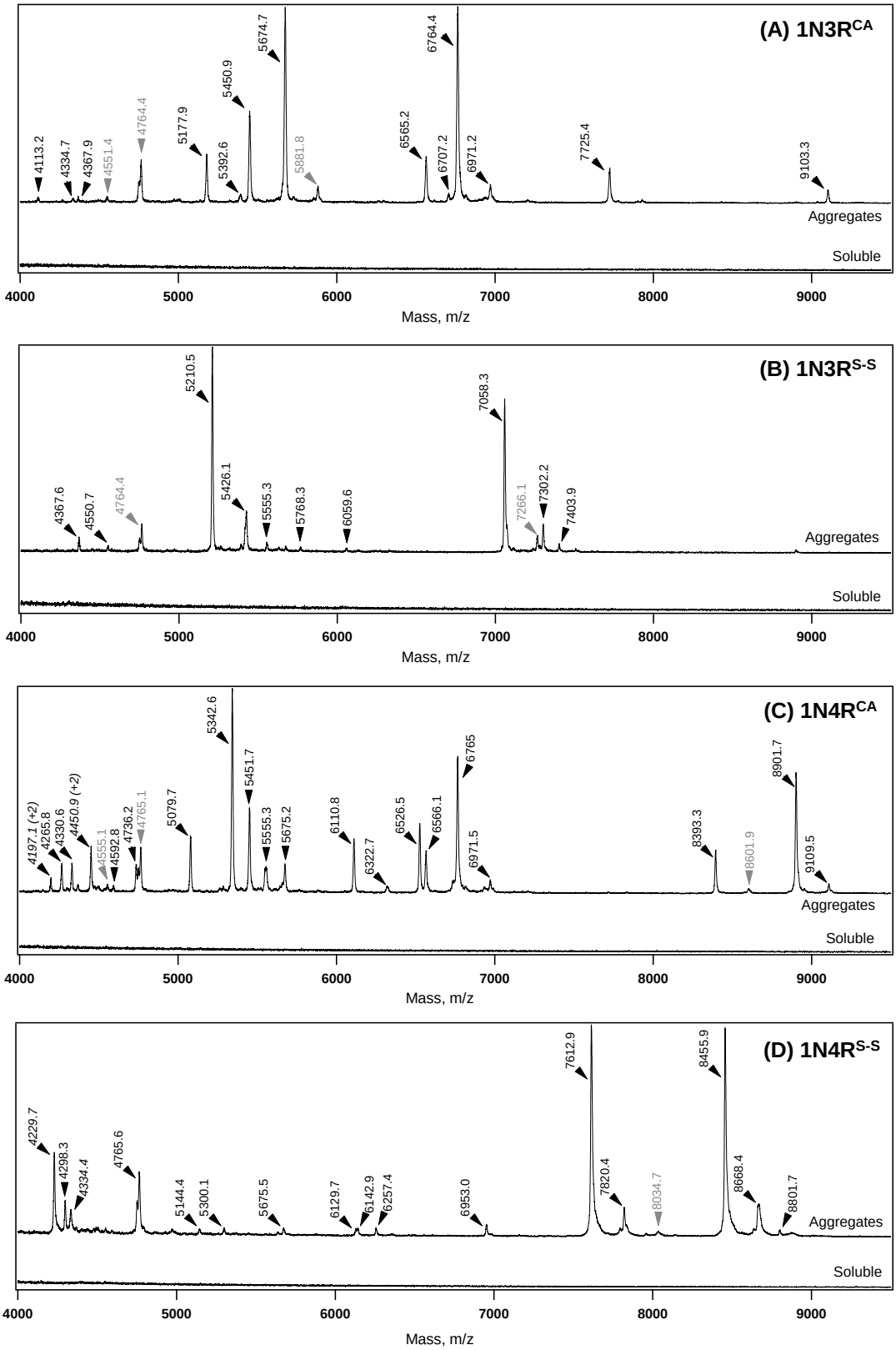
(A) 1N3R^CA
4113.2
4334.7
4367.9
4551.4
4764.4
5177.9
5392.6
5450.9
5674.7
5881.8
6565.2
6707.2
6764.4
6971.2
7725.4
9103.3
Aggregates
Soluble
4000
5000
6000
7000
8000
9000
Mass, m/z
(B) 1N3R^S-S
4367.6
4550.7
4764.4
5210.5
5426.1
5555.3
5768.3
6059.6
7058.3
7266.1
7302.2
7403.9
Aggregates
Soluble
4000
5000
6000
7000
8000
9000
Mass, m/z
(C) 1N4R^CA
4197.1 (+2)
4265.8
4330.6
4450.9 (+2)
4555.1
4592.8
4736.2
4765.1
5079.7
5342.6
5451.7
5555.3
5675.2
6110.8
6322.7
6526.5
6566.1
6765
6971.5
8393.3
8601.9
8901.7
9109.5
Aggregates
Soluble
4000
5000
6000
7000
8000
9000
Mass, m/z
(D) 1N4R^S-S
4229.7
4298.3
4334.4
4765.6
5144.4
5300.1
5675.5
6129.7
6142.9
6257.4
6953.0
7612.9
7820.4
8034.7
8455.9
8668.4
8801.7
Aggregates
Soluble
4000
5000
6000
7000
8000
9000
Mass, m/z

Table S1 Identification of the mass peaks observed in MALDI-TOF mass spectra (Fig. S3). Amino acid regions that possess observed m/z were identified using MS-Bridge and listed together with the calculated m/z values and the difference.

	Region	Observed m/z	Calculated m/z	Difference
1N3R^{CA}	370 – 406	4113.2	4112.66	0.54
	268 – 340	4334.7	4334.02	0.68
	407 – 447	4367.9	4366.81	1.1
	N.D.[a)]	4551.4	---	---
	N.D.[a)]	4764.4	---	---
	260 – 340	5177.9	5176.97	0.93
	258 – 340	5392.6	5392.23	0.38
	268 – 349	5450.9	5453.27	-2.4
	354 – 406	5674.7	5673.41	1.3
	N.D.[a)]	5881.8	---	---
	386 – 447	6565.2	6564.21	0.99
	322 – 383	6707.2	6705.63	1.6
	384 – 447	6764.4	6763.46	0.94
	222 – 317	6971.2	6974.26	-3.1
	376 – 447	7725.4	7723.57	1.8
	322 – 406	9103.3	9102.29	1.0
1N3R^{S-S}	268 – 340	4367.6	4366.08	1.5
	306 – 347	4550.7	4552.33	-1.6
	N.D.[a)]	4764.4	---	---
	260 – 340	5210.5	5209.03	1.5
	258 – 340	5426.1	5424.29	1.8
	260 – 343	5555.3	5553.40	1.9
	258 – 343	5768.7	5768.66	0.040
	260 – 347	6059.6	6057.01	2.6
	243 – 340	7058.3	7057.29	1.0
	N.D.[a)]	7266.1	---	---
	241 – 340	7302.2	7300.55	1.6
	243 – 343	7403.9	7401.66	2.2
1N4R^{CA}	(260 – 340)	4197.1 (+2)[b)]	---	---
	299 – 340	4265.8	4263.92	1.9
	396 – 438	4330.6	4328.78	1.8
	(354 – 438)	4450.9 (+2)[b)]	---	---
	N.D.[a)]	4555.1	---	---
	354 – 395	4592.8	4590.23	2.6
	295 – 340	4736.2	4734.45	1.8
	N.D.[a)]	4765.1	---	---
	295 – 343	5079.7	5078.82	0.88
	141 – 194	5342.6	5343.11	-0.51
	396 – 447	5451.7	5449.98	1.7
	318 – 370	5555.3	5552.31	3.0
	354 – 406	5675.2	5673.41	1.8
	260 – 317	6110.8	6109.06	1.7
	258 – 317	6322.7	6324.31	-1.6
	260 – 321	6526.5	6524.55	1.9
	386 – 447	6566.1	6564.21	1.9
	384 – 447	6765.0	6763.46	1.5
	156 – 225	6971.5	6968.79	2.7
	260 – 340	8393.3	8391.66	1.6
	N.D.[a)]	8601.9	---	---
	354 – 438	8901.7	8899.99	1.7
	268 - 353	9109.5	9110.48	-0.98
1N4R^{S-S}	(260 – 340)	4229.7 (+2)[b)]	---	---
	299 – 340	4298.3	4295.99	2.3

(258 – 340)	4334.4 (+2)[b)]	---	---
295 – 340	4765.6	4766.51	-0.91
299 – 347	5144.4	5143.96	0.44
268 – 317	5300.1	5298.17	1.9
354 – 406	5675.5	5673.41	2.1
282 – 340	6129.7	6127.08	2.6
260 – 317	6142.9	6141.12	1.8
281 – 340	6257.4	6255.25	2.1
275 – 340	6953.0	6951.12	1.9
268 – 340	7612.9	7612.84	0.060
281 – 353	7820.4	7817.02	3.4
N.D.[a)]	8034.7	---	---
260 – 340	8455.9	8455.79	0.11
258 – 340	8668.4	8671.05	-2.6
260 – 343	8801.7	8800.16	1.5

[a)] We could not identify the amino acid regions from the indicated m/z value. [b)] Mass peaks corresponding to +2 charged ions.