Cell-free synthesis of amyloid fibrils with infectious properties and amenable to sub-milligram magic-angle spinning NMR analysis

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Supplementary information

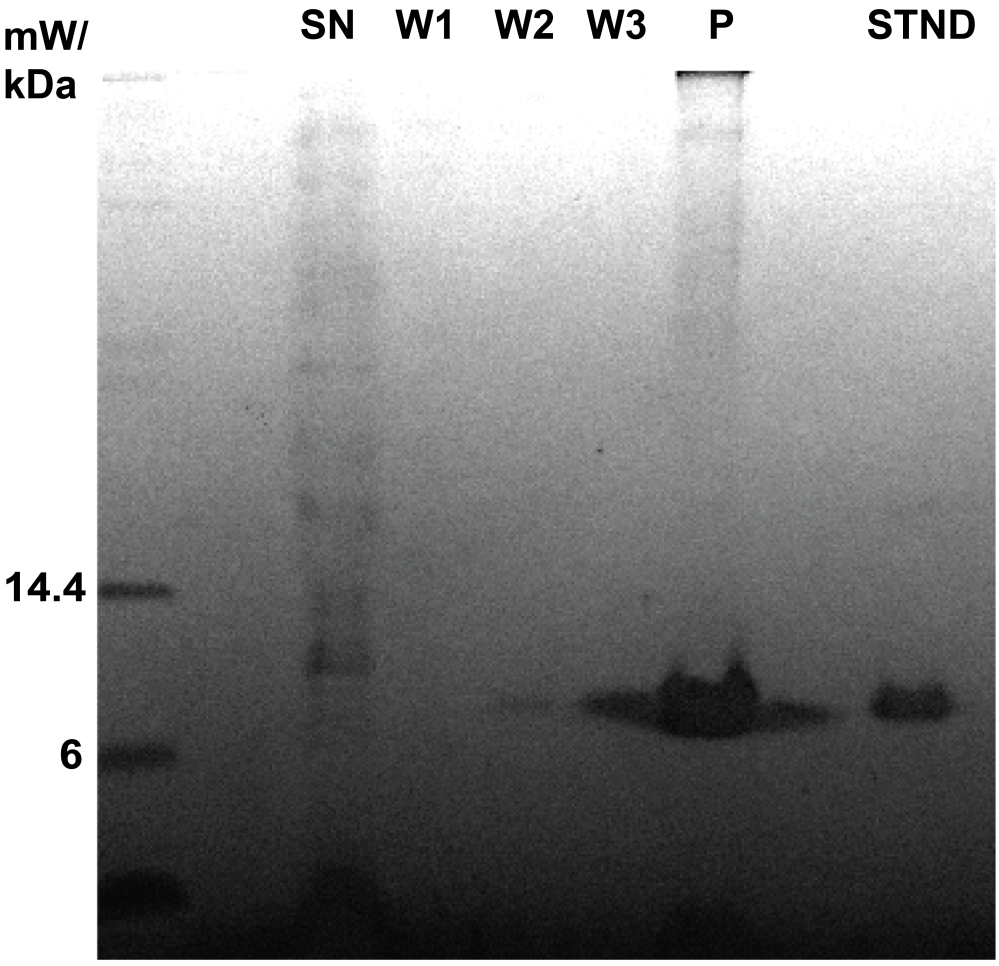

Figure S1: SDS-PAGE gel of cell-free synthesized HET-s (218-289). SN: supernatant. W1, W2, W3: washing fractions 1, 2 and 3, P – washed pellet. Standard (Stnd) is the recombinant HET-s (218-289) protein, with a molecular weight of 9.8 kDa. The pure HET-s (218-289) protein was found in the pelleted fraction.

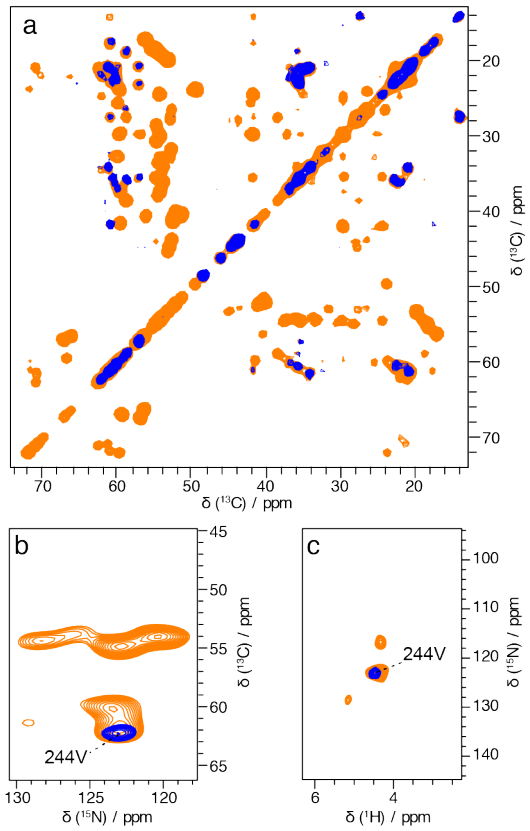

Figure S2: (a) Overlay of 2D ^{13}C-^{13}C projections from the 3D hCCH TOCSY of R-HET-s PFD (in orange) and CF-GVI-HET-s (in blue). (b) 2D ^{13}C-^{15}N and (c) ^{15}N-^{1}H of the 3D hNCaH of R-HET-s PFD (in orange) and CF-GVI-HET-s (in blue). Intra-residue correlations for V244 are highlighted.

Table S1. Reagents and concentrations for the master mixture (MM).

Reagent	Stock	Final	V, μL
NaN_3	10 % w/v	0,05%	90
PEG 8000	40 % w/v	2%	900
KOAc	4 M	150.8 mM	679
$Mg(OAc)_2$	1 M	7.1 mM	332
HEPES	2.5 M	0.1 M	660
Complete*	50 x	1 x	360
Folinic Acid	10 mg/ml	0.1 mg/ml	180
DTT	0.5 M	2 mM	72
NTP mix**	75 x	1 x	240
PEP	1 M	20 mM	360
Acetyl Phosphate	1 M	20 mM	360
RCWMDE	17 mM	1 mM	1078
AA-mix	5 mM	1 mM	2250
H_2O			1800
Total			9211

* Complete protease inhibitor mixture was prepared by dissolving 1 tablet in 1 mL H_2O.

**NTP mixture was prepared by mixing together 1.25 mL of 0.36 mM ATP, 1.25 mL of 0.24 mM CTP, 1.25 mL 0.24 mM GTP and 1.25 mL of 0.24 mM UTP.

Table S2. Reagents and concentrations for the feeding (FM).

Reagent	Stock	Final	V, μL
MM			8699
S30 Buffer	100%	30%	5950
AA-mix	5 mM	0.5 mM	2125
H_2O			226
Total			17000

Table S3. Reagents and concentrations for the reaction mixture (RM).

Reagent	Stock	Final	V, μL
MM			512
Pyruvate kinase	10 mg/ml	0.04 mg/ml	8
tRNA	40 mg/ml	1 mg/ml	12
T7 RNAP	420 U/µl	6 U/µl	14.3
RNA Guard	32 U/µl	0.3 U/µl	10.7
DNA	1000 µg/ µl	50 ng/µL	50
S30 extract	100%	30%	350
H_2O			47
Total			1000

Table S4. The extracted line widths (at FWHM) for ^{1}H amide resonances in both samples.

Residue	CFS	R-HET-s PFD
	^{1}HN Line widths/Hz	^{1}HN Line widths/Hz
244V	250	390
245V	252	390
264V	254	499
267V	252	366
268V	315	585
271I	267	315
275V	485	390
277I	218	476
278G	226	275
282G	301	377
283G	238	311
285G	163	355

Average	**268±78**	**394±87**

Table S5. The extracted line widths (at FWHM) for ^{1}Ha resonances in both samples.

Residue	**CFS**	**R-HET-s PFD**
	^{1}Ha Line widths/Hz	^{1}Ha Line widths/Hz
231I	155	195
239V	204	259
244V	269	269
245V	417	227
264V	141	228
267V	201	239
268V	251	259
277I	161	190
Average	225±90	223±29

Table S6. The experimental NMR parameters for 2D spectra.

	CFS GVI		R-HET-s PFD	
Experiment	**2D hNH**	**2D hCH**	**2D hNH**	**2D hCH**
MAS frequency/kHz	100	100	100	100
Field/T	18.8	18.8	18.8	18.8
Transfer I	HN-CP	HC-CP	HN-CP	HC-CP
^{1}H field/kHz	80	80	80	80
^{15}N field/kHz	20		20	
^{13}C field/kHz		20		20
Shape	ramp ^{1}H	ramp ^{1}H	ramp ^{1}H	ramp ^{1}H
Time/ms	1.3	0.3	0.9	0.3
Transfer II	NH-CP	CH-CP	NH-CP	CH-CP
^{1}H field/kHz	80	80	80	80
^{15}N field/kHz	20		20	
^{13}C field/kHz		20		20
Shape	ramp ^{1}H	ramp ^{1}H	ramp ^{1}H	ramp ^{1}H
Time/ms	0.7	0.3	0.8	0.3
^{13}C carrier/ppm		54		54
^{1}H decoupling	slTPPM12	slTPPM12	slTPPM12	slTPPM12
^{1}H decoupling field/ kHz	25	25	25	25
t1 increments	128	300	74	400
Windows function	QSine 3	QSine 3	QSine 3	QSine 3
Sweep width (t1)/kHz	32.4	18.1	42.4	18.1
Acquisition time (t1)/ms	19.7	11	11.4	11
^{15}N/^{13}C decoupling	WALTZ16	WALTZ16	WALTZ16	WALTZ16
^{15}N/^{13}C decoupling field/ kHz	10		10	10
t2 increments	1536	1536	1536	1536
Windows function	QSine 3	QSine 3	QSine 3	QSine 3
Sweep width (t2)/kHz	100	37	100	37
Acquisition time (t2)/ms	10.2	20.7	10.2	20.7
Interscan delay/s	1.3	1.6	1.3	1.6
Number of scans	64	32	32	32
Measurement time/h	1.5	5.7	0.75	5.7

Table S7. The experimental NMR parameters for 3D spectra

	CFS GVI		R-HET-s PFD	
Experiment	**3D hNCaH**	**3D hCCH**	**3D hNCaH**	**3D hCCH**
MAS frequency/kHz	100	100	100	100
Field/T	18.8	18.8	18.8	18.8
Transfer I	NH-CP	HC-CP	NH-CP	HC-CP
^{1}H field/kHz	80	80	80	80
^{13}C field/kHz		20		20
^{15}N field/kHz	20		20	
Shape	ramp ^{1}H	ramp ^{1}H	ramp ^{1}H	ramp ^{1}H
Time/ms	1.8	0.4	1.4	0.4
Transfer II	NC-CP	TOCSY	NC-CP	TOCSY
^{15}N field/kHz	20		20	
^{13}C field/kHz	80	25	80	25
Shape	ramp ^{15}N		ramp ^{15}N	
Time/ms	11	14	11	14
^{13}C carrier/ppm	54	40	54	40
^{1}H decoupling	slTPPM12			slTPPM12
^{1}H decoupling field/ kHz	25	25		25
Transfer III	CH-CP	CH-CP	CH-CP	CH-CP
^{1}H field/kHz	80	80	80	80
^{13}C field/kHz	20	20	20	20
Shape	ramp ^{1}H	ramp ^{1}H	ramp ^{1}H	ramp ^{1}H
Time/ms	0.4	0.3	0.3	0.3
t1 increments	32	102	40	124
Windows function	QSine2	QSine 3	QSine 3	QSine 2
Sweep width (t1)/kHz	10.4	12.4	8	14.1
Acquisition time (t1)/ms	3.3	4.1	2.5	4.4
^{1}H decoupling	slTPPM12	slTPPM12	slTPPM12	slTPPM12
^{1}H decoupling field/ kHz	25	25	25	25
t2 increments	48	104	40	124
Windows function	QSine 2	QSine 3	QSine 3	QSine 2
Sweep width (t2)/kHz	3.1	12.4	3.2	14.1
Acquisition time (t2)/ms	7.4	4.1	6.1	4.4
^{15}N/^{13}C decoupling	WALTZ16	WALTZ16	WALTZ16	WALTZ16
^{15}N/^{13}C decoupling field/ kHz	10	10	10	10
t3 increments	2048	2048	2048	2048
Windows function	QSine 3	QSine 3	QSine 3	QSine 3
Sweep width (t3)/kHz	81.9	48.5	82	48.5
Acquisition time (t3)/ms	12.5	21.1	12.5	21.1
Interscan delay/s	1.5	1.5	1.5	1.2
Number of scans	64	4	24	4
Measurement time/h	41	17.7	16	20.5