Supporting information for:

Prion soft amyloid core driven self-assembly of globular proteins into bioactive nanofibrils

*Weiqiang Wang[1], Susanna Navarro[1], Rafayel A. Azizyan[2], Manuel Baño-Polo[1], Sebastian A. Esperante[1] Andrey V. Kajava[2] and Salvador Ventura[1]**

[1]Institut de Biotecnologia i de Biomedicina and Departament de Bioquímica i Biologia Molecular; Universitat Autònoma de Barcelona; 08193 Bellaterra (Barcelona), Spain.

[2]Centre de Recherche en Biologie cellulaire de Montpellier, UMR 5237 CNRS, Université Montpellier, 1919 Route de Mende, 34293 Montpellier, Cedex 5, France

E-mail: salvador.ventura@uab.es

This PDF file includes Supporting Figures S1 to S6 and Tables S1 and S4.

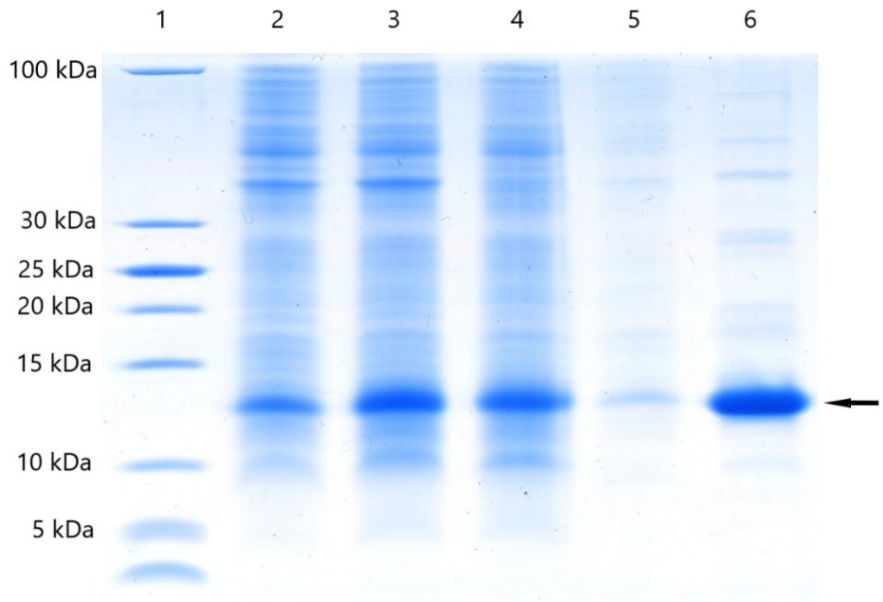

Figure S1. Analysis on SDS-PAGE of the expression and purification of Sup35-FF fusion protein. *Lane 1*, corresponds to molecular weight marker, *lane 2,* non-induced culture*, lane 3*, induced culture, *lane 4* and *5* are soluble (supernatant) and insoluble (pellet) fractions from total cell extract, and *lane 6* shows the purified fraction of Sup35-FF protein upon elution with 250 mM imidazole from a His-trap column. Black arrow indicates the band corresponding to Sup35-FF fusion protein.

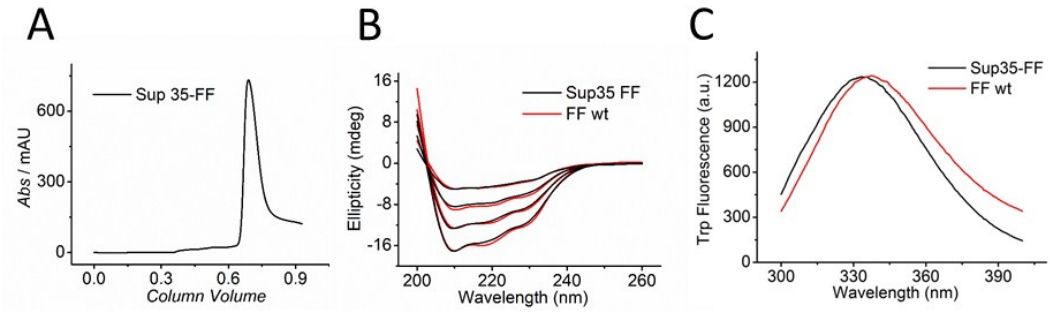

Figure S2. Conformational characterization of Sup35-FF protein. (A) Size-Exclusion Chromatography elution profile of Sup35-FF, (B) Far-UV CD spectra at different concentrations (5, 10, 15 and 20 µM) and, (C) Tryptophan intrinsic fluorescence spectra. Sup35-FF and FF-wt are shown in black and red, respectively.

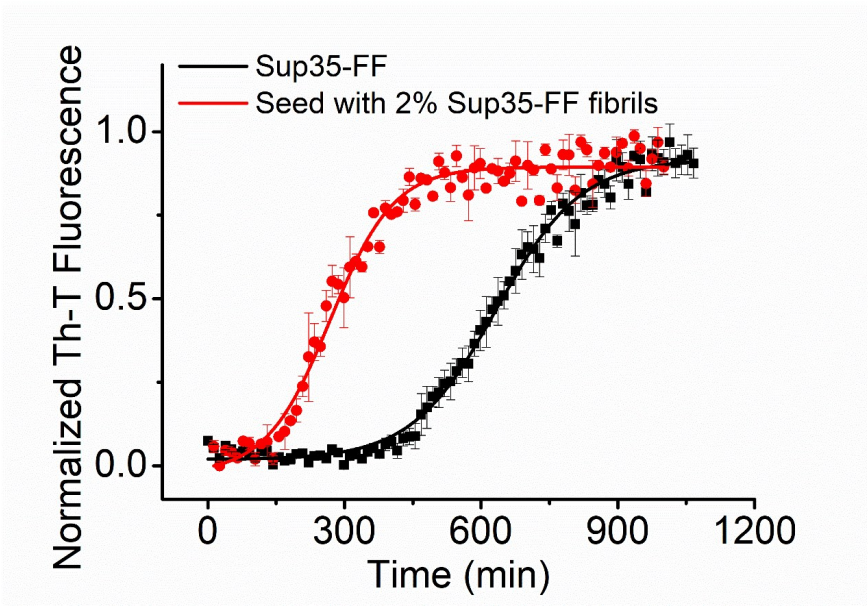

Figure S3. Aggregation kinetics and seeding reaction of Sup35-FF. Sup35-FF was dissolved at 50 µM in PBS containing 25 µM Th-T and the Th-T fluorescence emission was recorded along time in the absence (black squares) and, in the presence of 2% of pre-formed Sup35-FF fibrils as seeds (red dots).

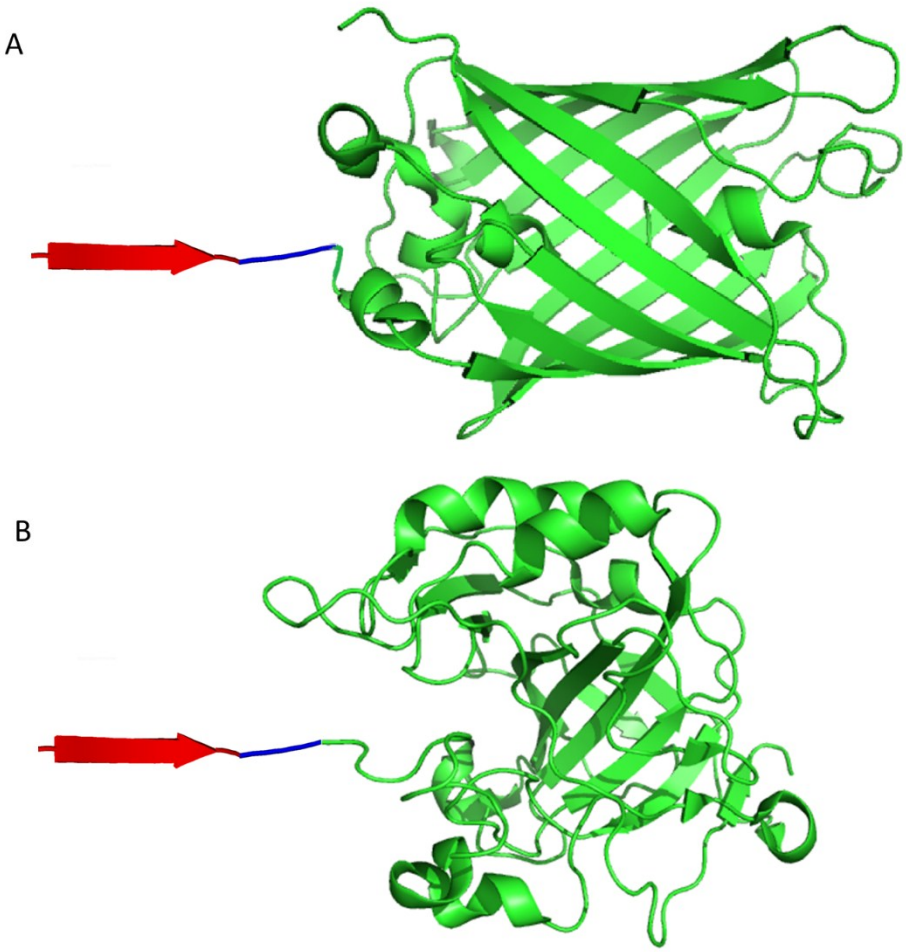

Figure S4. 3D representation of Green Fluorescent Protein and Carbonic anhydrase fused to the Sup35 soft amyloid core. Ribbon representation of (A) Sup35-GFP and (B) Sup35-CA using the PDB accession code 2B3Q and 1V9E for the GFP and CA structures, respectively. Sup35 soft amyloid core, spacer linker, GFP or CA are shown in red, blue and green, respectively.

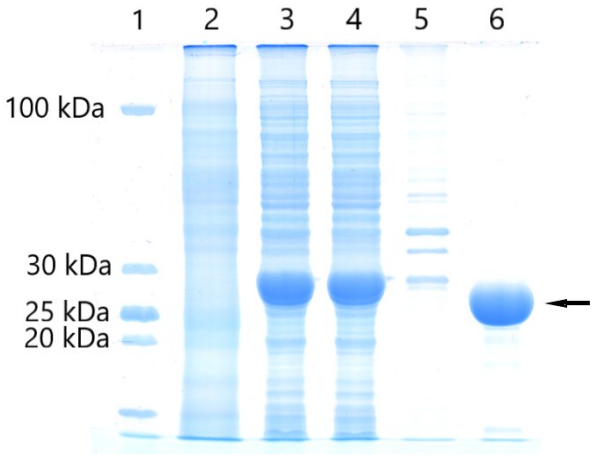

Figure S5. Analysis on SDS-PAGE of the expression and purification of Sup35-GFP fusion protein. *Lane 1*, corresponds to molecular weight marker, *lane 2,* non-induced culture, *lane 3*, total extract induced, *lane 4*, soluble fraction (supernatant), *lane 5*, insoluble fraction (pellet) and, *lane 6,* shows the purified fraction of Sup35-GFP by gel filtration. Black arrow indicates the band corresponding to Sup35-GFP fusion protein.

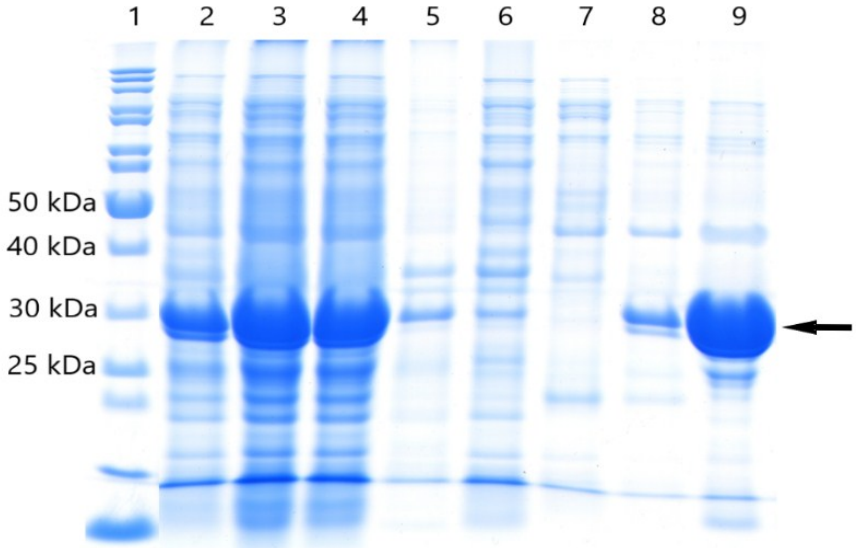

Figure S6. Analysis on SDS-PAGE of the expression and purification of Sup35-CA fusion protein. *Lane 1*, corresponds to molecular weight marker, *lane 2*, non-induced culture, *lane 3*, total extract induced*, lane 4*, soluble fraction (supernatant), *lane 5*, insoluble fraction (pellet) and *lanes 6-7*, flow through the His-trap column and*, lanes 8-9*, eluted protein at 250 mM imidazole. Black arrow indicates the band corresponding to Sup35-CA fusion protein.

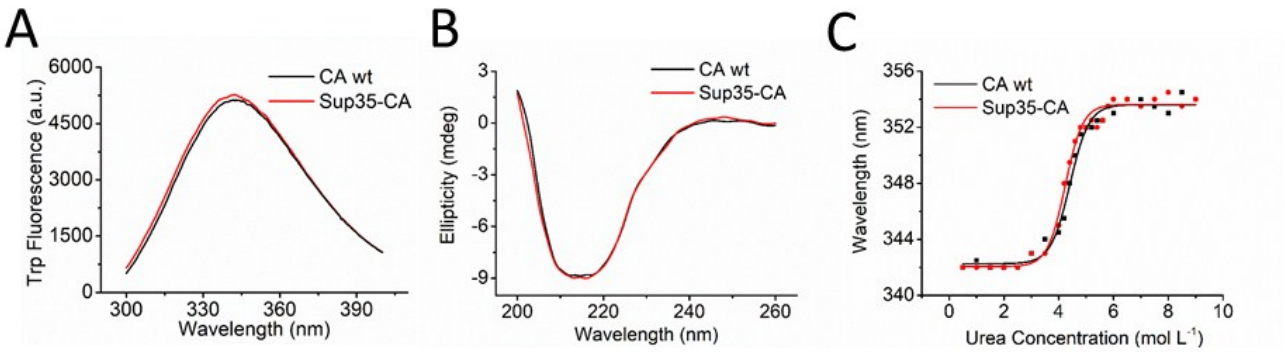

Figure S7. Conformational characterization and stability of Sup35-CA protein. (A) Tryptophan intrinsic fluorescence spectra, (B) Far-UV CD spectra, (C) Chemical equilibrium curves with urea were followed at 25 ºC by wavelength of maximum Trp fluorescence. CA-wt and Sup35-CA are shown in black and red, respectively.

A

Protein	Aggrescan	FoldAmyloid	PASTA 2.0
KLVFFA (Aβ)	**81.00**	**24.02**	**-4.53937**
Sup35-SAC	-32.60	20.84	-1.9920
Sup35-SAC-linker	-35.50	19.99	-1.9920
FF domain-His tag	-26.30	21.22	**-4.2112**
Sup35-FF	-27.60	20.81	**-4.2112**

B

Protein	PONDR (VSL2)	GlobPlot	PASTA 2.0	IUPRED
Sup35-SAC	Too short	100.00	100.00	56.52
Sup35-SAC-linker	100.00	100.00	100.00	54.84
FF domain-His tag	32.81	9.38	23.43	10.94
Sup35-FF	52.63	33.68	25.26	12.63

Table S1. Prediction of aggregation propensity and disorder. (A) Positive aggregation-prone predictions are shown in bold. Positive scores, values higher than 21.4 and values lower than -4.0, correspond to aggregation-prone proteins/regions according to Aggrescan, FoldAmyloid, and PASTA 2.0, respectively. The analysis of a classical amyloid core belonging to the Aβ-peptide is shown for comparison (B) For disorder prediction, the values indicate the percentage of polypeptide predicted to be disorder.

	[a] $\Delta G_{U-F}^{H_2O}$ (Kcal mol^{-1})		[b] m (Kcal mol^{-1} M^{-1})		[c] $[Urea]_{50\%}$ (mol·L^{-1})	
	Intrinsic Fluorescence	**CD**	**Intrinsic Fluorescence**	**CD**	**Intrinsic Fluorescence**	**CD**
Sup35-FF	4.04±0.18	4.02±0.28	0.72±0.03	0.76±0.05	5.62±0.04	5.32±0.08
FF-wt	4.11±0.11	4.21±0.20	0.72±0.02	0.77±0.04	5.68±0.04	5.44±0.04

[a] Gibbs energy of unfolding with urea determined from the equilibrium parameters.
[b] Dependence of the Gibbs energy of unfolding with urea.
[c] The urea concentration required to unfold 50% of the protein molecules.

Table S2. Thermodynamic characterization of soluble Sup35-FF and FF-wt proteins.

	T_m (℃)	
	Intrinsic Fluorescence	**CD**
Sup35-FF	62.72 ± 0.17	64.7 ± 0.4
FF-wt	64.61 ± 0.19	66.1 ± 0.5

Table S3. Melting temperatures of soluble Sup35-FF and FF-wt proteins.

Assignments	Sup35-FF fibrils	FF-wt
Inter β-sheet	27.25 % (1624 cm^{-1})	-
α-helix	59.50 % (1649 cm^{-1})	84.08 % (1650 cm^{-1})
Turns	13.25 % (1674 cm^{-1})	15.92 % (1673 cm^{-1})

Table S4. Assignment, % of area and wavenumber of the secondary structure components of Sup35-FF fibrils and soluble FF-wt in the amide I region of the FTIR spectra.