Electronic Supplementary Material (ESI) for RSC Advances

Amyloidogenesis of proteolytic fragments of human elastin

Brigida Bochicchio [a]*, ***Antonietta Pepe***[a], ***Florian Delaunay*** [a,b] , ***Marina Lorusso***[a]

Stephanie Baud [c], ***Manuel Dauchez***[c]

[a]Department of Science, University of Basilicata, Potenza, Italy; [b]Institut Carnot CIRIMAT UMR CNRS 5085, Université Paul Sabatier, Toulouse, France,[c]University of Reims-Champagne Ardenne, Reims, France.

AUTHOR INFORMATION

Corresponding Author

* E-mail: brigida.bochicchio@unibas.it. Phone: 39-0971-205-481. Fax: 39-0971-205-678.

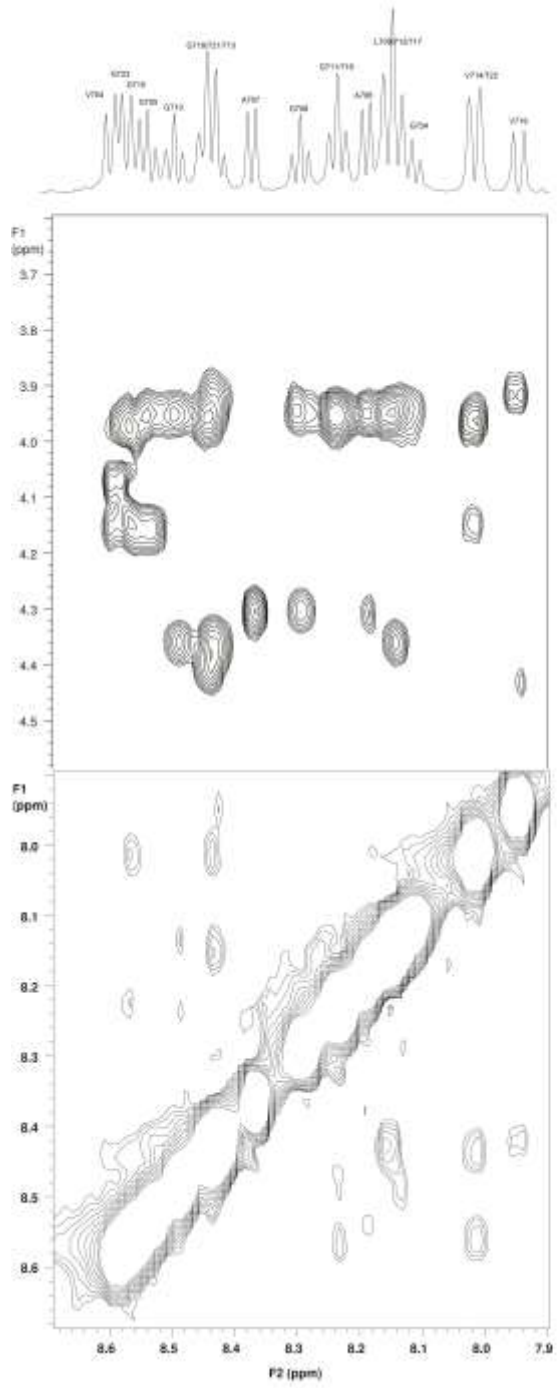

Figure S1: 1D spectrum of S4 peptide recorded in H_2O/D_2O (90:10, v/v) at 25°C (top). The fingerprint region (middle) and the amide region (bottom) of the ROESY spectrum recorded at 200 ms mixing time. The regions were displayed with the same threshold, in order to highlight the difference in intensity of the cross-peaks.

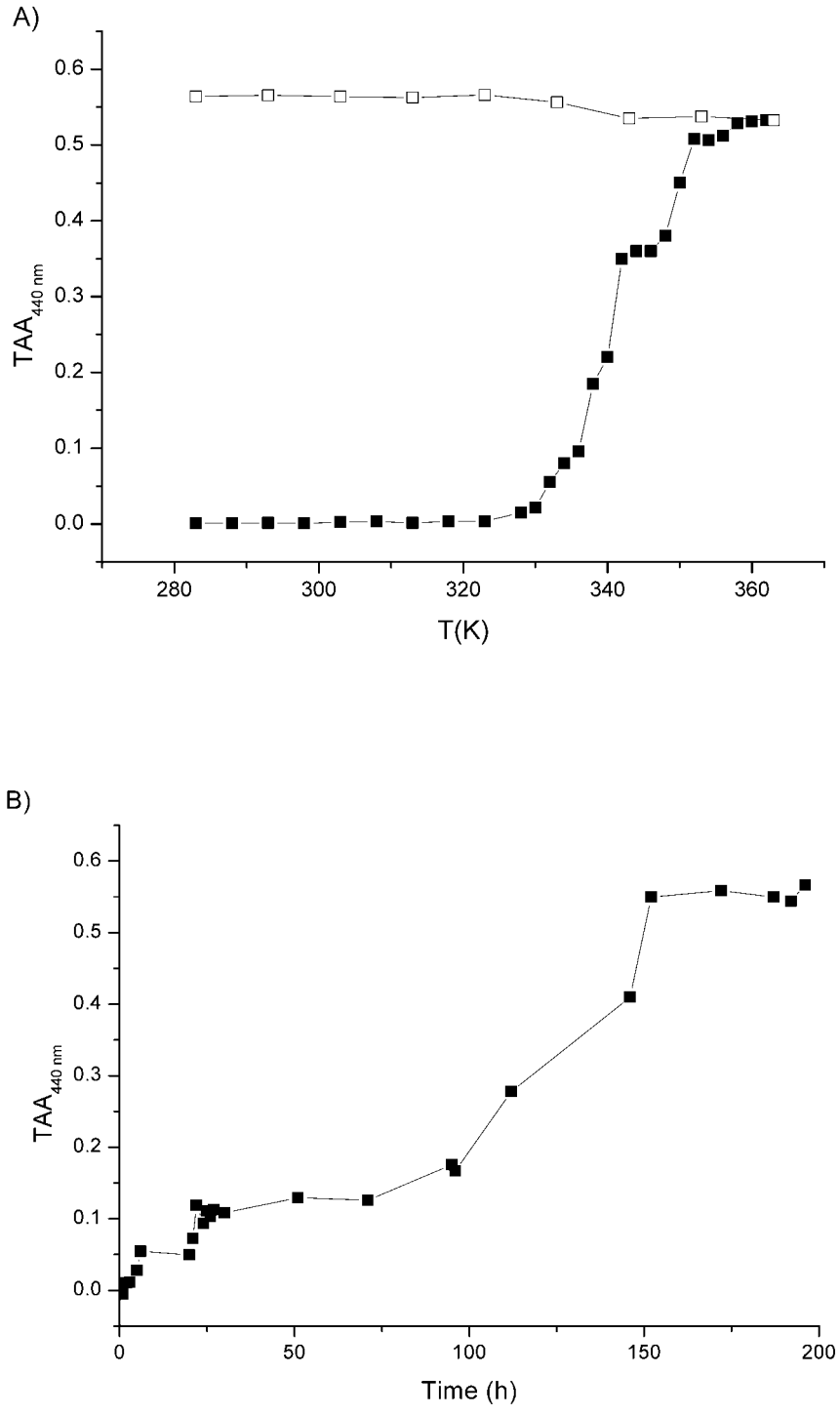

Fig. S2. Turbidimetry graphs of 1mM S4 in TBS buffer (pH 7.0): A) as a function of temperature (Filled squares: warming curve. Empty squares: cooling curve), B) at 37°C as a function of time.

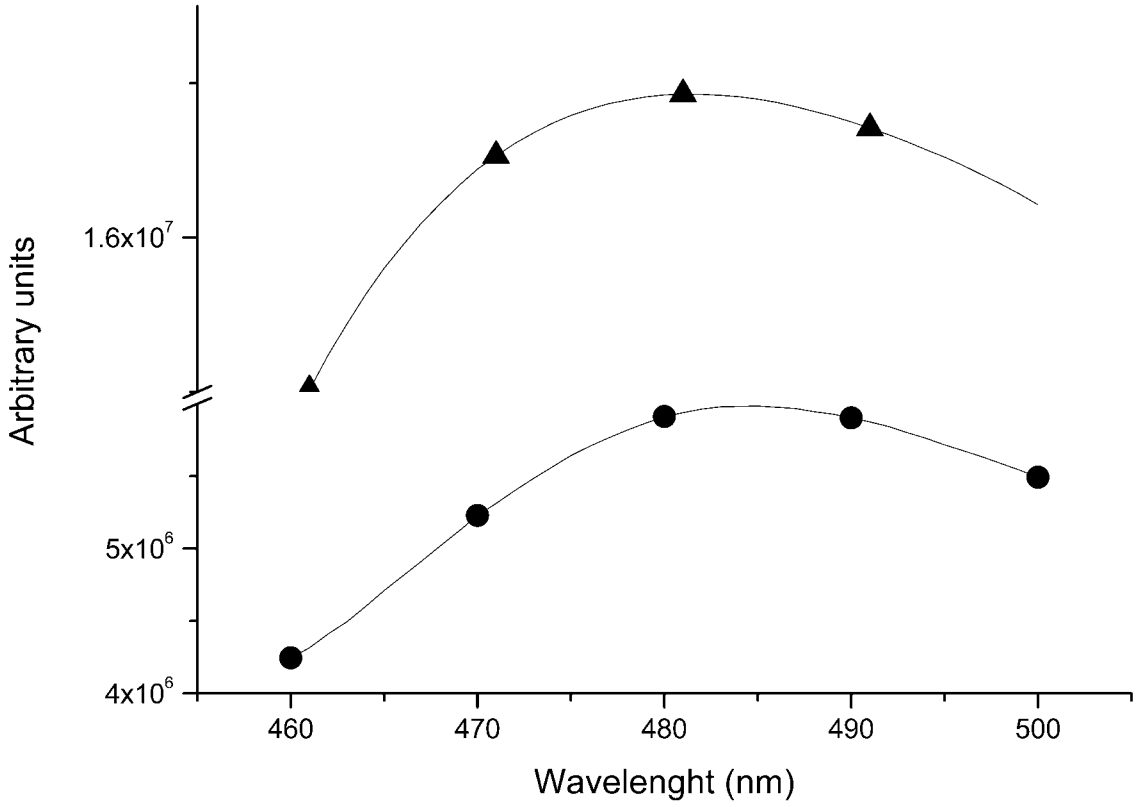

Figure S3: Thioflavin T fluorescence graph. Thioflavin T (ThT) 50 μM (circle); S4 fibers and ThT 50 μM (triangle).

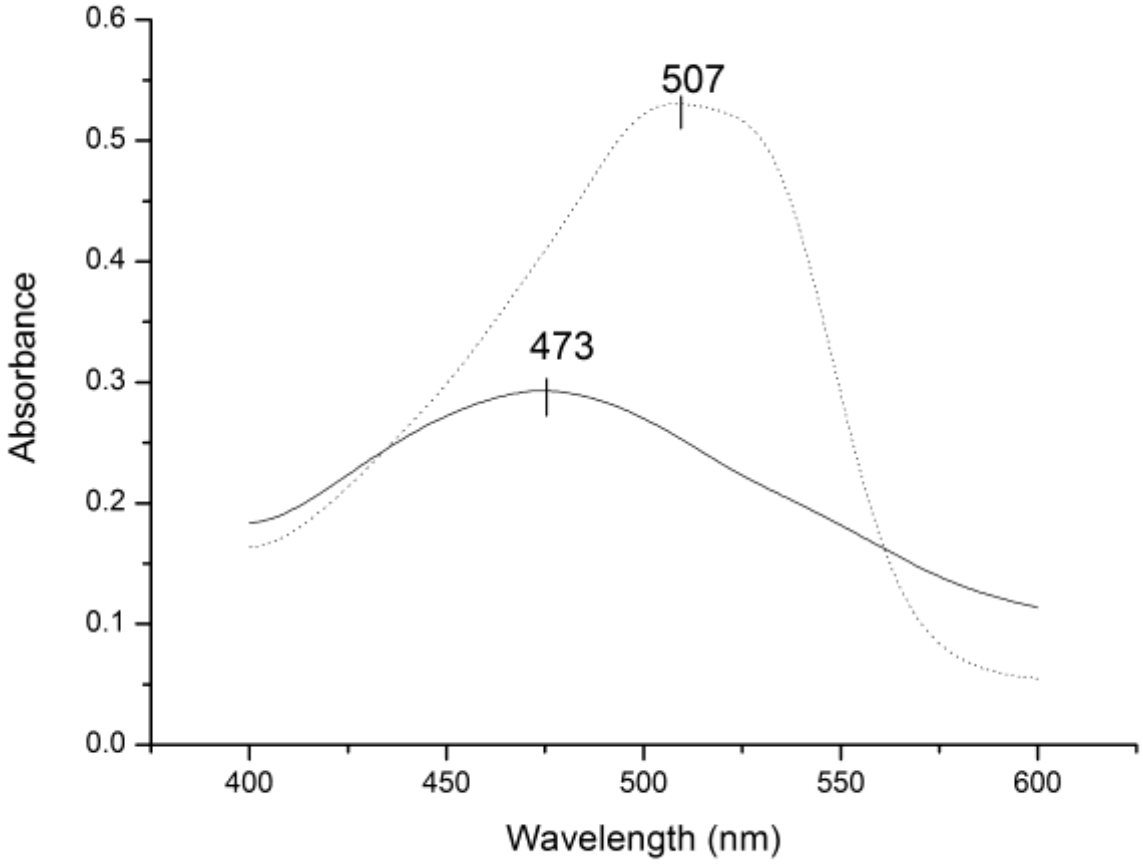

Figure S4: Affinity of Congo Red to S4 at room temperature.

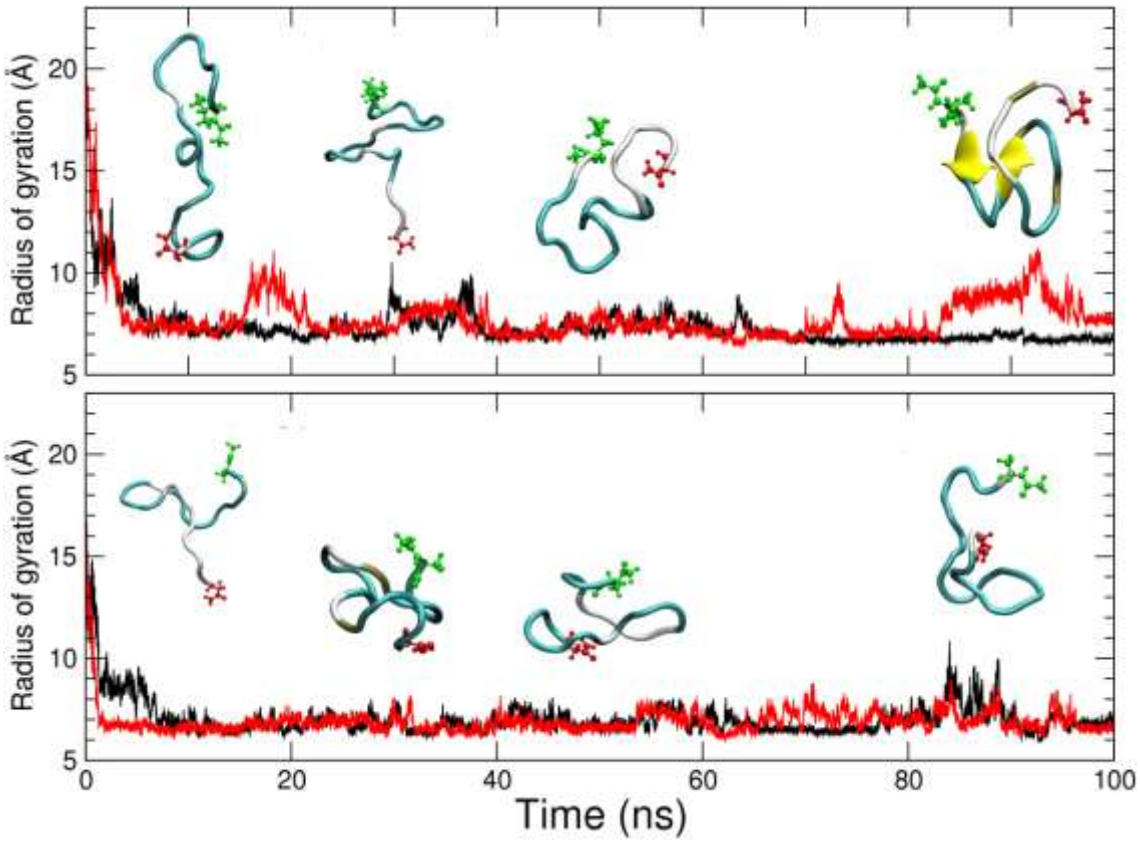

Fig.S5: Evolution of the radius of gyration of the S4 (top) and S3 (bottom) peptides along the trajectory. Fig.S5. Snapshots of S4 molecule at the following steps of the simulation: a) 5 ns; b) 10 ns; c) 50 ns; d) 90 ns. The peptide is colored according to the secondary structure. The N (green) and C (red) terminal residues are represented in ball and sticks.

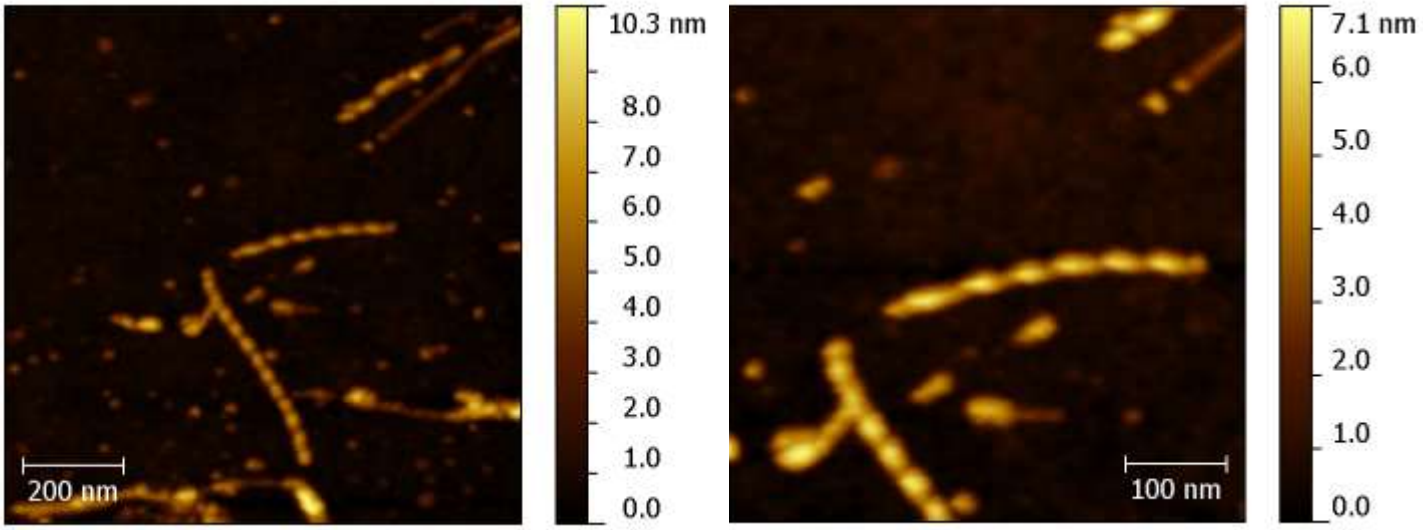

Fig. S6: AFM micrographies of S4 fibers deposited on Si (100).

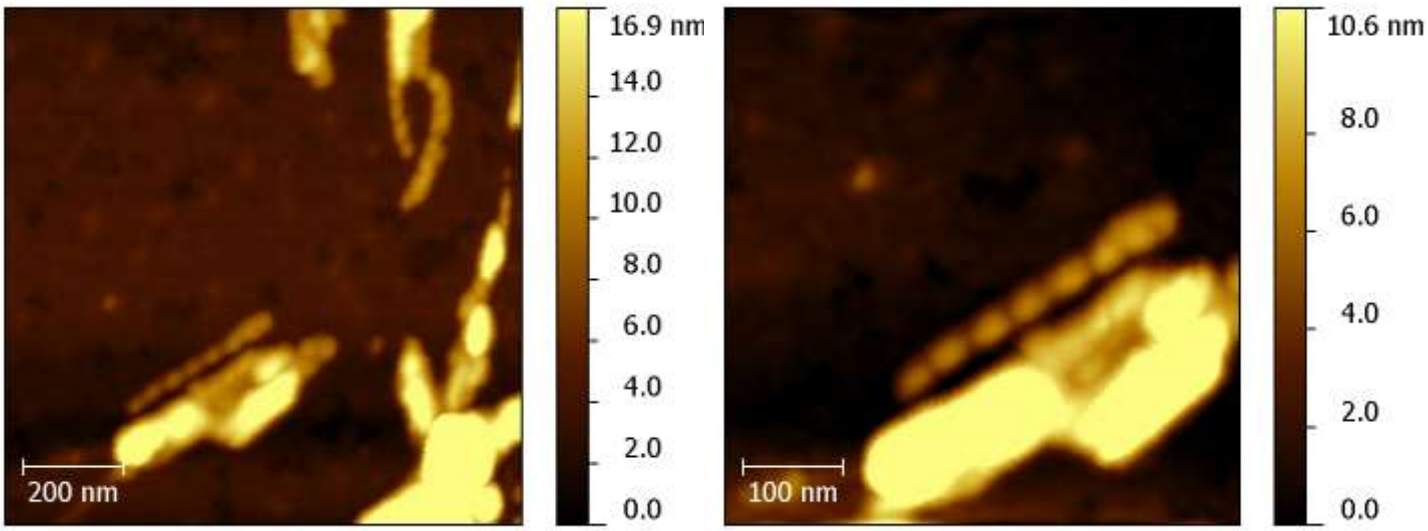

Fig. S7: AFM micrographies of S4 fibers deposited on mica.

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Supplemental Tables

Table S1. Assignments of proton resonances of S3 peptide in H_2O/D_2O (90/10, v/v) at 25°C.

Table S2. Assignments of proton resonances of S4 peptide in H_2O/D_2O (90/10, v/v) at 25°C.

Table S3. Analysis of FTIR decomposed spectra of lyophilized S1, S2, S3, S4 polypeptides and of S4 fibers.

Table S4. Elastin-derived peptide sequence compositions.

Supplemental Tables

Table S1: Assignments of proton resonances of S3 peptide in H_2O/D_2O (90/10, v/v) at 25°C

	Chemical shift of proton resonance (ppm)					
residue[a]	NH	Hα	Hβ	others	$^3J_{NH-H\alpha}$ (Hz)	-Δδ/ΔT (ppb/K)
Ala[706]		4.05	1.53		-	
Ala[707]	8.65	4.36	1.41		5.9	6.4
Gly[708]	8.43	3.92				6.4
Leu[709]	8.27	4.38	1.68	Hγ1.66 Hδ0.92/0.87	6.6	7.9
Gly[710]	8.54	3.97				7.5
Gly[711]	8.26	3.96				Nd
Leu[712]	8.13	4.36	1.63	Hγ1.67 Hδ0.92/0.87	6.2	6.4
Gly[713]	8.44	3.96				Nd
Val[714]	8.02	4.16	2.11	Hγ 0.92	7.3	7.8
Gly[715]	8.57	3.96				8.0
Gly[716]	8.23	3.98				Nd
Leu[717]	8.17	4.35	1.68	Hγ 1.68 Hδ0.92/0.87	6.2	6.6
Gly[718]	8.43	3.97				6.9
Val[719]	7.95	4.43	2.07	Hγ 0.97/0.91	8.1	8.0
Pro[720]	-	4.41	2.30/1.98	Hγ2.06 Hδ3.90/3.70		-
Gly[721]	8.43	3.97				6.0
Val[722]	8.02	4.15	2.12	Hγ 0.94	7.3	7.5
Gly[723]	8.56	4.00				7.7
Gly[724]	8.04	3.88				6.4

Table S2: Assignments of proton resonances of S4 peptide in H_2O/D_2O (90/10, v/v) at 25°C

	Chemical shift of proton resonance (ppm)					
residue[a]	NH	Hα	Hβ	others	$^3J_{NH-H\alpha}$ (Hz)	-Δδ/ΔT (ppb/K)
Leu[703]	-	4.08	1.67	1.66		-
Val[704]	8.59	4.16	2.05	0.97	7.3	-
Gly[705]	8.54	3.96				7.6
Ala[706]	8.18	4.31	1.39		6.2	7.2
Ala[707]	8.36	4.30	1.41		6.1	5.7
Gly[708]	8.29	3.94				7.6
Leu[709]	8.13	4.37	1.66	Hγ 1.66 Hδ 0.94/0.99	-	
Gly[710]	8.50	3.95				7.3
Gly[711]	8.23	3.95				
Leu[712]	8.13	4.37	1.66	Hγ 1.66 Hδ 0.94/0.99	-	
Gly[713]	8.43	3.96				
Val[714]	8.01	4.17	2.11	Hγ 0.95	8.5	7.5
Gly[715]	8.56	3.96				
Gly[716]	8.23	3.95				
Leu[717]	8.13	4.37	1.66	Hγ 1.66 Hδ 0.94/0.98	-	
Gly[718]	8.44	3.92				
Val[719]	7.95	4.44	2.08	Hγ 0.92/0.99	8.6	8.1
Pro[720]		4.41				
Gly[721]	8.44	3.97				
Val[722]	8.01	4.17	2.11	Hγ 0.95	8.5	7.8
Gly[723]	8.57	3.97				
Gly[724]	8.15	3.94				6.3

Table S3. Analysis of FTIR decomposed spectra of lyophilized S1, S2, S3, S4 polypeptides and of S4 aggregates (S4a). Wavenumber value, percentage area and secondary structure assignment for amide I and II components are given.

	S1 $\tilde{\upsilon}$ [cm^{-1}] (Area)	**S2** $\tilde{\upsilon}$ [cm^{-1}] (Area)	**S3** $\tilde{\upsilon}$ [cm^{-1}] (Area)	**S4** $\tilde{\upsilon}$ [cm^{-1}] (Area)	**S4a** $\tilde{\upsilon}$ [cm^{-1}] (Area)	**Assignment**
Amide I	1625/1696 (17%)	1631/1696 (17%)	-/-	1632/1697 (17%)	1630/1697 (32%)	Antiparallel β-sheet
	-	-	1643 (23%)	-	-	H_2O
	1652 (18%)	1652 (17%)	-	1648 (16%)	1655 (16%)	Random coil
	1664 (20%)	1667 (17%)	1661 (22%)	1662 (20%)	1675 (13%)	PPII
	1679 (16%)	1681 (12%)	1679 (13%)	1685 (18%)	–	β-turn
	-	-	1697 (6%)	-	-	β-turn
Amide II	1521 (8%)	1519 (11%)	1519 (12%)	1517 (10%)	1527 (12%)	β-sheet
	1538 (13%)	1539 (12%)	1537 (15%)	1537 (10%)	1547 (11%)	Random coil
	1554 (8%)	1557 (9%)	1555 (9%)	1552 (13%)	1558 (6%)	β-sheet

Table S4: Elastin-derived peptide sequence compositions of EX30, S1, S2, S3, S4 peptides

Elastin-derived peptide	% P	% G
EX30	0.040	0.520
S4	0.045	0.500
S3	0.053	0.530
S2	0.062	0.560
S1	0.125	0.500