Supporting Information:

Controlled Co-assembly Approach to Tune Temperature Responsiveness of Biomimetic Proteins

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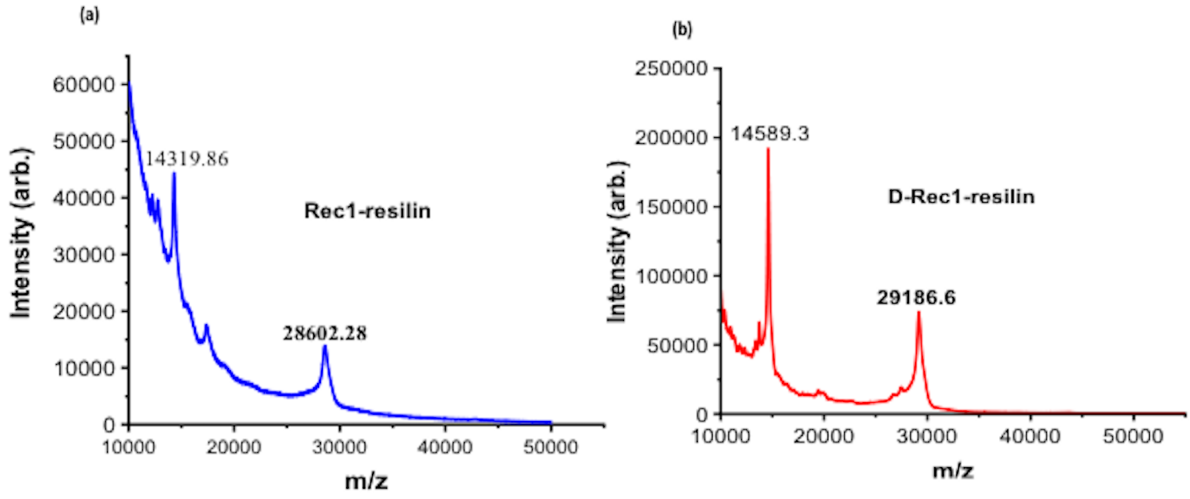

Figure S1: The mass spectrum of the synthesised (a) Rec1-resilin and (a) D-Rec1 protein polymers were obtained from a MALDI-TOF mass spectrometer utilising a linear positive mode. A total collection of 2000 shots were obtained for the calibration and sample measurement, where the spectrum range was set to 10,000 – 50,000 mz^{-1}. Protein calibration standard I and II (Bruker Daltonik GmbH, Leipzig) were used to establish an external calibration. Moreover, tuning the laser intensity and detector gain manually enabled optimal resolution to be achieved.[1] In turn, the molecular weight of the Rec1-resilin and D-Rec1 were determined to be 28.60 and 29.19 kDa.

Figure S2: Structural consensus and alignment of amino acid repeat sequence in rec1-resilin. Single-letter code is used.[2]

Table S.1: Amino acid composition of *Rec1-resilin*. The composition does not include the histidine tag segment after cleavage of the signal peptide.

Amino acid residue	Amino acid	Symbol	No of unit	Mol%
Non-polar side	Glycine	Gly/G	104	34.2
	Alanine	Ala/A	19	6.25
	Valine	Val/V	1	0.328
	Leucine	Leu/L	2	0.657
	Isoleucine	Ile/I	-	-
	Methionine	Met/M	-	-
	Proline	Pro/P	42	13.81
	Phenylalanine	Phe/F	1	0.328
	Tryptophan	Try/W	-	-
Uncharged polar side chain	Serine	Ser/S	44	14.47
	Threonine	Thr/T	6	1.97
	Asparagine	Asn/N	20	6.57
	Glutamine	Gln/Q	13	4.24
	Tyrosine	Tyr/Y	21	6.9
	Cysteine	Cys/C	-	-
Charge polar side chain	Lysine	Lys/K	2	0.657
	Arginine	Arg/R	16	5.26
	Histidine	His/H	-	-
	Aspartic acid	Asp/D	12	3.94
	Glutamic acid	Glu/E	1	0.328

MKIPYVLLFLVGVAVVNALPNPLFGGLVKSLSKKKQIFEDKFENLKENVGEKFENLKENVGEKVENLKENVGEKLENIKEKAGEKFENLKDN
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KQSNRVVEKSTDGDNEESYRSESSSSSSSSSSSSRSSSSSTYTGSHDDSSEE

Figure S3: The amino acid sequence of SS-2. Single-letter code for amono acide residues has been used.[3]

Table S.2: Amino acid composition of SS based on molar percentages. Reproduced from ref.[4] with permission from Elsevier © 2023.

Amino acid	Symbol	Aramwit et al. [5]	Keawkorn et al. [6]	Terada et al. [7]
Alanine	Ala/A	4.10	6.00	5.30
Arginine	Arg/R	2.87	3.10	1.80
Aspartic acid	Asp/D	15.64	16.70	18
Cysteine	Cys/C	0.44	0.20	<0.05
Glutamic acid	Glu/E	4.61	4.40	4.60
Glycine	Gly/G	15.03	13.50	15.70
Histidine	His/H	1.06	1.30	1.30
Isoleucine	Ile/I	0.56	0.70	0.70
Leucine	Leu/L	1.00	1.10	1.10
Lysine	Lys/K	2.35	3.30	2.50
Methionine	Met/M	3.39	0.04	<0.05
Phenylalanine	Phe/F	0.28	0.50	0.40
Proline	Pro/P	0.54	0.70	0.60
Serine	Ser/S	33.63	33.40	32.20
Threonine	Thr/T	8.16	9.70	8.40
Tryptophan	Try/W	0.00	0.20	0.00
Tyrosine	Tyr/Y	3.45	2.60	3.70
Valine	Val/V	2.88	2.80	3.70

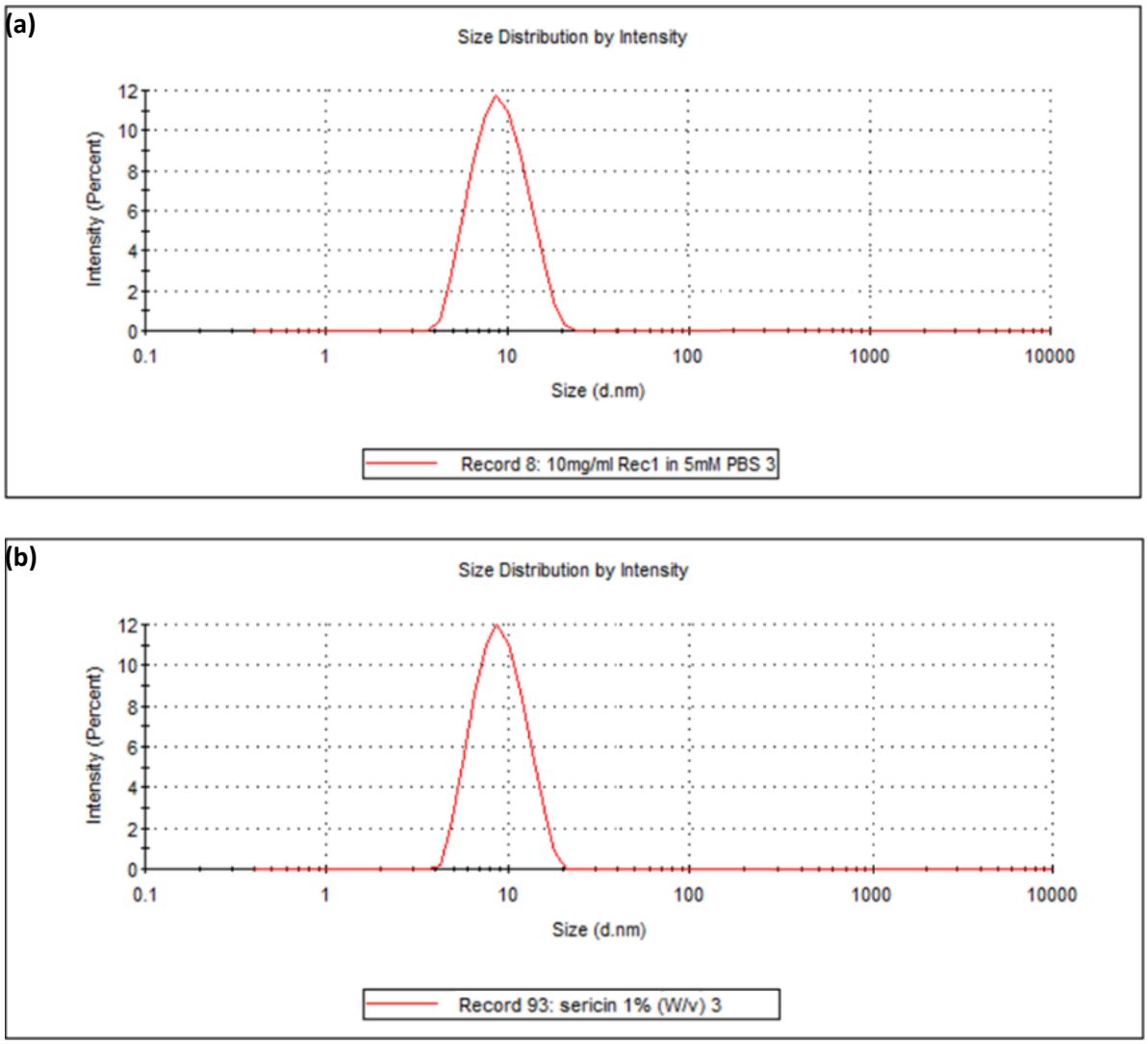

Figure S4: Intensity profiles of (a) Rec1-resilin and (b) SS via DLS size distribution curves

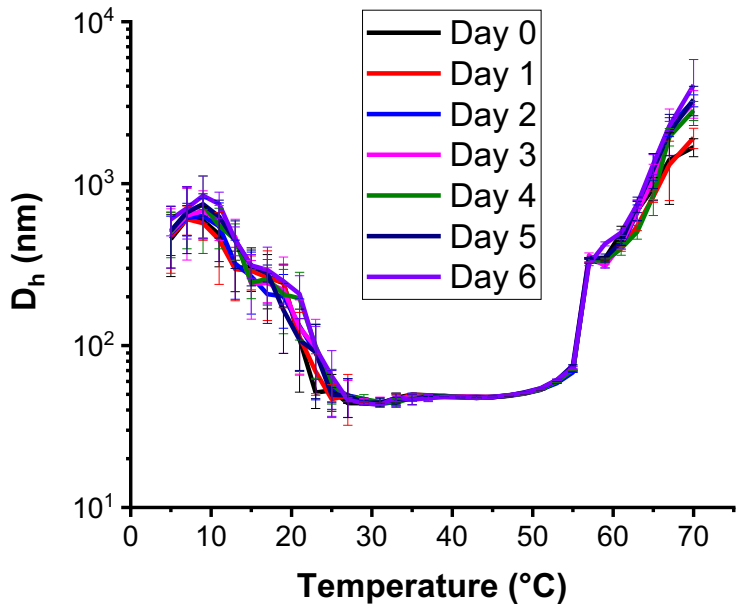

Figure S5: Rec1-resilin/Sericin co-assembled system D_h size measurements over an incubation period of 6 days heating from 4°C to 70°C.

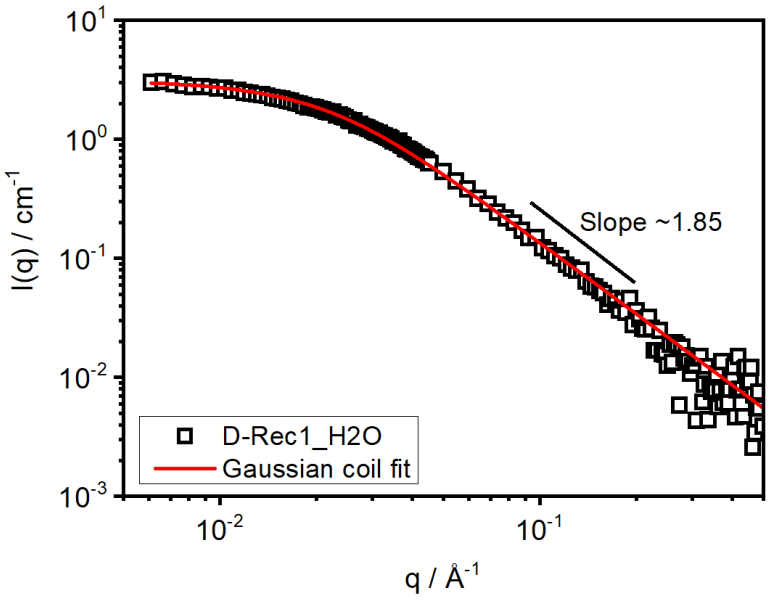

Figure S6a: SANS intensity profiles of D_Rec1 in water fit with model function to estimate structural parameters (adopted from ref 8).

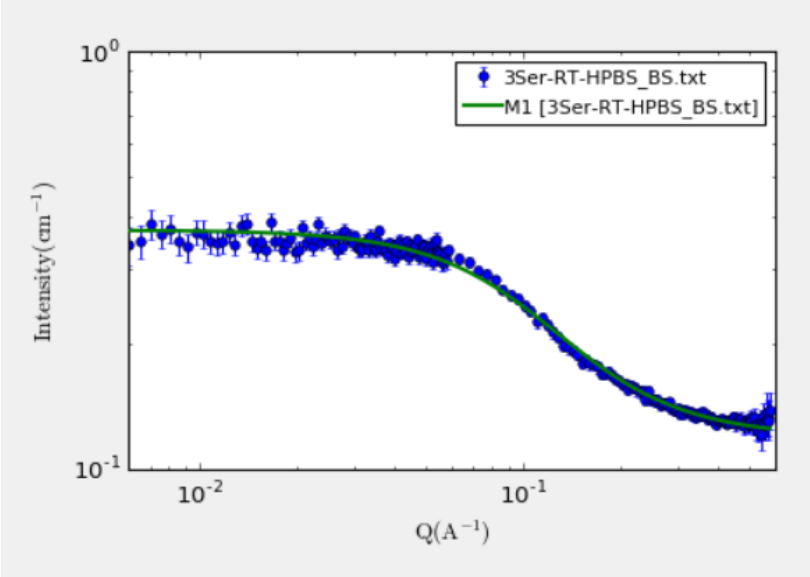

Figure S6b: SANS data of sericin in water fit with shape-independent model function to estimate structural parameters.

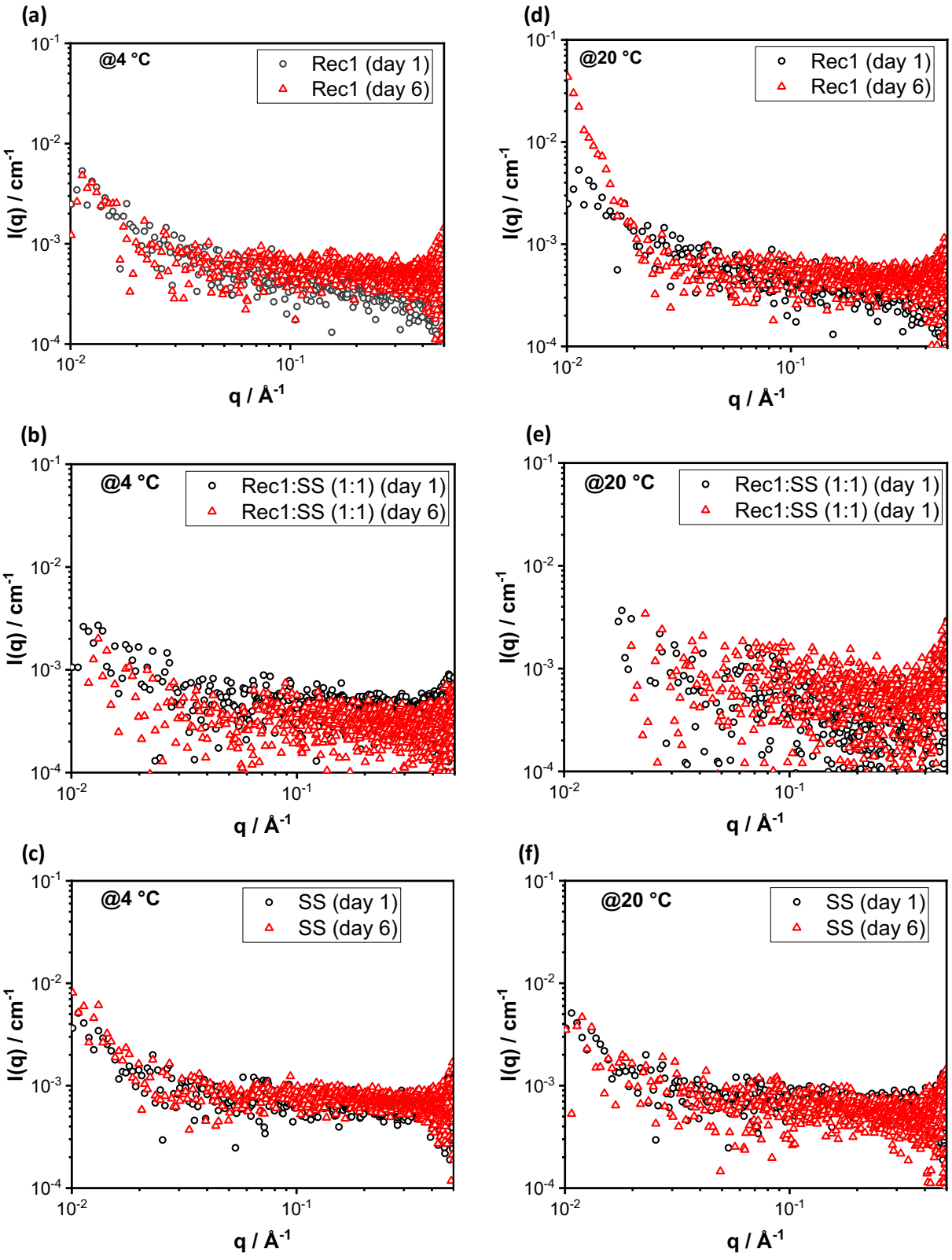

Figure S7: SAXS intensity profile of Rec1-resilin/SS co-assembled system (Rec1:Ser (1:1)) compared with pristine Rec1-resilin (Rec1) and SS (Ser) after 1 and 6 days of equilibration at UCST (at 4°C) and room temperature (at 20°C).

Table S3: Deconvoluted CD spectra fits interpreting the secondary structure of protein systems investigated on day 1 on incubation. Result 1 and 2 are based on the closest and average of all matching solutions with proteins, respectively.

System	Temp. (°C)	Result	Helix 1	Helix 2	Strand 1	Strand 2	Turns	Unordered	Total
Rec1- resilin	4	1	0.000	0.085	0.000	0.012	0.041	0.862	1.000
		2	0.003	0.031	0.000	0.015	0.033	0.918	1.000
	20	1	0.000	0.046	0.000	0.001	0.000	0.953	1.000
		2	0.002	0.017	0.001	0.012	0.059	0.908	0.999
	70	1	1.000	0.000	0.000	0.000	0.000	0.000	1.000
		2	0.958	0.039	0.000	0.003	0.000	0.000	1.000
Sericin	4	1	0.000	0.050	0.063	0.061	0.109	0.717	1.000
		2	0.000	0.019	0.054	0.050	0.056	0.821	1.000
	20	1	0.000	0.066	0.000	0.017	0.000	0.917	1.000
		2	0.002	0.016	0.041	0.034	0.051	0.856	1.000
	70	1	0.000	0.000	0.000	0.000	0.000	1.000	1.000
		2	0.000	0.000	0.346	0.042	0.288	0.324	1.000
Rec1- resilin/Sericin	4	1	0.000	0.048	0.062	0.036	0.067	0.786	0.999
		2	0.001	0.016	0.022	0.026	0.047	0.887	0.999
	20	1	0.000	0.049	0.000	0.006	0.000	0.945	1.000
		2	0.002	0.013	0.004	0.014	0.049	0.919	1.001
	70	1	0.000	0.000	0.000	0.000	0.000	1.000	1.000
		2	0.005	0.000	0.225	0.036	0.026	0.708	1.000

Table S4: Deconvoluted CD spectra fits interpreting the secondary structure of protein systems investigated on day 6 on incubation. Result 1 and 2 are based on the closest and average of all matching solutions with proteins, respectively.

System	Temp. (°C)	Result	Helix 1	Helix 2	Strand 1	Strand 2	Turns	Unordered	Total
Rec1- resilin	4	1	0.000	0.076	0.000	0.014	0.059	0.851	1.000
		2	0.003	0.031	0.001	0.010	0.027	0.928	1.000
	20	1	0.002	0.044	0.235	0.121	0.188	0.410	1.000
		2	0.002	0.022	0.263	0.133	0.198	0.283	1.001
	70	1	0.000	0.046	0.000	0.000	0.000	0.954	1.000
		2	0.006	0.013	0.016	0.020	0.111	0.834	1.000
Sericin	4	1	0.000	0.053	0.070	0.060	0.111	0.707	1.001
		2	0.003	0.022	0.035	0.038	0.055	0.847	1.000
	20	1	0.000	0.040	0.160	0.089	0.145	0.566	1.000
		2	0.001	0.016	0.155	0.086	0.120	0.620	0.998
	70	1	0.000	0.000	0.079	0.000	0.000	0.921	1.000
		2	0.002	0.004	0.126	0.031	0.062	0.774	0.999
Rec1- resilin/Sericin	4	1	0.000	0.055	0.039	0.040	0.097	0.770	1.001
		2	0.002	0.015	0.015	0.028	0.059	0.881	1.000
	20	1	0.000	0.047	0.184	0.093	0.140	0.535	0.999
		2	0.004	0.019	0.195	0.103	0.152	0.528	1.001
	70	1	0.027	0.137	0.000	0.064	0.185	0.587	1.000
		2	0.050	0.109	0.007	0.056	0.123	0.655	1.000

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