Supplementary Information

Influence of peptides chirality on their protein-triggered supramolecular hydrogelation

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Materials

Materials, synthesis and characterization of peptide ***L***-1, ***D***-1, ***L***-2 and ***D***-2

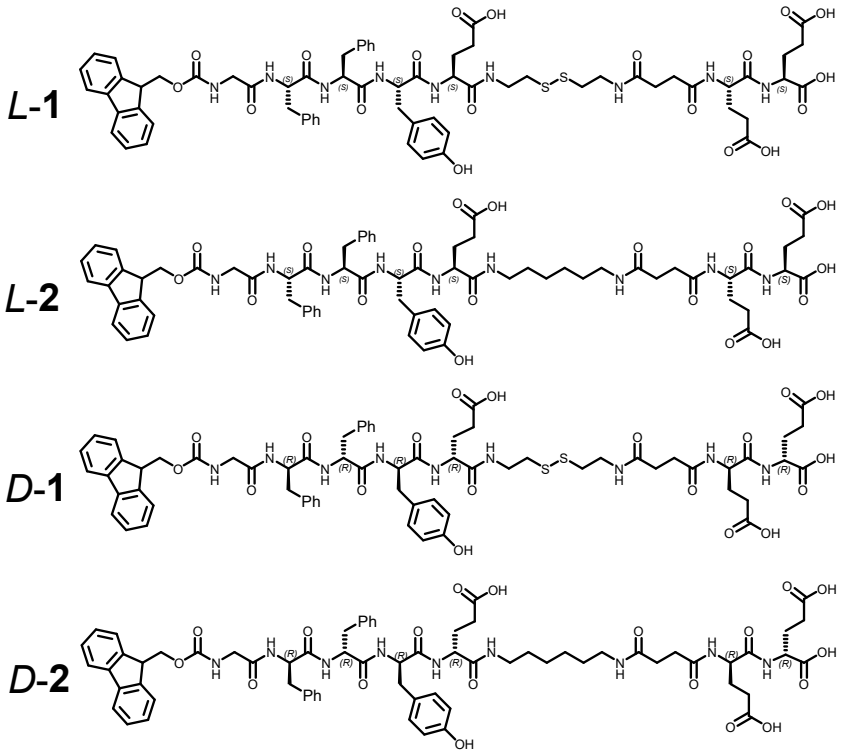
List of chemicals and abbreviations: All chemicals used are listed in the table given. They were all used as received, without purification.

Name, acronym (abbreviation)	MW (g.mol^{-1})	Provider	CAS number
Bovine Serum Albumin ≥ 99%, essentially Globulin free (**BSA**)	66 500	Sigma-Aldrich	9048-46-8
Alkaline Phosphatase from bovine intestinal mucosa (**AP**)	170 000	Sigma-Aldrich	9001-78-9
Urease (**U**) from Canavalia ensiformis (Jack bean)	480 000	Sigma-Aldrich	9002-13-5
β-Galactosidase (**β-GAL**) from Escherichia coli	450 000	Sigma-Aldrich	9031-11-2
Carbonic anhydrase from bovine erythrocytes (**CA**)	30 000	Sigma-Aldrich	9001-03-0
Dimethylformamide (**DMF**)	73.09	Acros Organics	68-12-2
Dichloromethane (**DCM**)	84.93	Acros Organics	75-09-2
Trifluoroacetic acid (**TFA**)	114.02	Thermo Scientific	76-05-1
N,N-Diisopropylethylamine (**DIPEA**)	129.24	Sigma-Aldrich	7087-68-5
Fmoc-L-phenylalanine (**Fmoc-F-OH**)	387.43	Sigma-Aldrich	35661-40-6
Fmoc-L-Tyrosine-OH (**Fmoc-Tyr(tBu)-OH**)	459.53	Sigma-Aldrich	71989-38-3
Triisopropylsilane (**TIPS**)	158.36	Sigma-Aldrich	6485-79-6
Resin 2-chlorotrityl chloride (**2-CTC**)	-	Iris Biotech	42074-68-0
1-Hydroxybenzotriazole hydrate (**HOBt**)	135.12	Sigma Aldrich	123333-53-9
N,N,N',N'-Tetramethyl-o-(1Hbenzotriazol-1-yl)uranium hexafluorophosphate (**HBTU**)	379.24	Sigma-Aldrich	94790-37-1
Diethyl ether	74.12	Acros Organics	60-29-7
Fmoc-Glu(OtBu)-OH	425.47	Sigma-Aldrich	71989-18-9
Fmoc-Gly-OH	297.31	Sigma-Aldrich	29022-11-5
Succinic Acid	118.09	Sigma-Aldrich	110-15-6
mono-Fmoc1,6-diaminohexane-hydrochloride	374.9	Sigma-Aldrich	945923-91-1
Fmoc-Cystamine-Suc	474.59	Iris Biotech	946849-80-5
Thioflavine T (**ThT**)	318.86	TCI	2390-54-7
Phosphate buffered saline (Tablet P4417)	-	Sigma-Aldrich	

Synthesis, purification and characterization of peptides: Synthesis of peptide *L*-**1**, *D*-**1**, *L*-**2** and *D*-**2** listed in the scheme S1 were prepared using solid support chemistry by employing "Fmoc strategy" on 2-CTC resin following slightly changing the previously reported procedures (Rodon Fores et *al*. *Chem Sci.* 2019, **10**, 4761–6). In this previous report, succinic acid and cystamine spacer was assembled by

coupling one after the other on the growing peptide chain. Whereas herein, Fmoc-Cystamine-Succinic acid is added as commercially available in one step. Synthetic pathway and general experimental procedures for peptide *L*-**1** is given below and similar protocol was used to synthesize *D*-**1**, *L*-**2** and *D*-**2** peptides by incorporating L or D amino acid monomers in the respective coupling step.

Scheme S1. List of peptides



General Experimental Procedures:

Step a) Loading of resin: 0.5 g of 2-CTC resin (1.6 mmol/g; 0.8 mmol) was soaked in a solid phase extraction (SPE) tube in dry DCM (9 mL) for 2 hrs. DCM was removed and a solution of Fmoc-L-Glu(OtBu)-OH (1.02 g, 2.4 mmol, 3 eq.) and DIPEA (0.9 mL) in dry DCM (8 mL) was added and the tube was stirred at RT for 4 hrs. Solution was filtered off and beads were washed twice with methanol, and stirred for 1 hour at room temperature in 10 mL solution of DCM: MeOH: DIPEA (80:15:05) and then filtered off.

Step b) A solution of 20% piperidine in DMF (8 mL) was added to the beads and the tube was stirred for 20 minutes. The solution was filtered off and the beads were washed thoroughly with DCM, DMF, DCM (5 Times with each solvent). A Kaiser test* was performed and it was positive (free amine present).

Step c) A solution of Fmoc-L-Glu(O*t*Bu)-OH (1.02 g, 2.4 mmol, 3 eq.), HBTU (0.91 g, 2.4 mmol, 3 eq.) and HOBt (0.325 g, 2.4 mmol, 3 eq.) and DIPEA (0.9 mL) in dry DMF (7 mL) was added. The tube was

stirred for 4-5 hrs. The solution was filtered off and the beads were washed thoroughly with DCM, DMF, DCM (5 Times with each solvent). A Kaiser test* was performed and test was negative (free amine absent).

Step d) A solution of Fmoc-Cystamine-Suc-OH (1.14 g, 2.4 mmol, 3 eq.), HBTU (0.91 g, 2.4 mmol, 3 eq.) (0.325 g, 2.4 mmol, 3 eq.) and DIPEA (0.9 mL) in dry DMF (7 mL) was added. The tube was stirred overnight at RT. The solution was filtered off and the beads were washed thoroughly with DCM, DMF, DCM (5 Times with each solvent). A Kaiser test* was performed and test was negative (free amine absent). In CH_2-CH_2 spacer containing peptides (*L*-2 & *D*-2), this step was conducted first coupling with succinic acid followed by coupling with mono-Fmoc1,6-diaminohexane-hydrochloride. Each of this step was monitored by performing Kaiser test.

Step e) A solution of Fmoc-L-Tyr(O*t*Bu)-OH (1.1 g, 2.4 mmol, 3 eq.), HBTU (0.91 g, 2.4 mmol, 3 eq.) and HOBt (0.325 g, 2.4 mmol, 3 eq.) and DIPEA (0.9 mL) in dry DMF (7 mL) was added. The tube was stirred for 4-5 hrs. The solution was filtered off and the beads were washed thoroughly with DCM, DMF, DCM (5 Times with each solvent). A Kaiser test* was performed and test was negative (free amine absent).

Step f) A solution of Fmoc-L-Phe-OH (0.92 g, 2.4 mmol, 3 eq.), HBTU (0.91 g, 2.4 mmol, 3 eq.) and HOBt (0.325 g, 2.4 mmol, 3 eq.) and DIPEA (0.9 mL) in dry DMF (7 mL) was added. The tube was stirred for 4 hrs. The solution was filtered off and the beads were washed thoroughly with DCM, DMF, DCM (5 Times with each solvent). A Kaiser test* was performed and test was negative (free amine absent).

Step g) A solution of Fmoc-Gly-OH (0.71 g, 2.4 mmol, 3 eq.), HBTU (0.91 g, 2.4 mmol, 3 eq.) and HOBt (0.325 g, 2.4 mmol, 3 eq.) and DIPEA (0.9 mL) in dry DMF (7 mL) was added. The tube was stirred for 4 hrs. The solution was filtered off and the beads were washed thoroughly with DCM, DMF, DCM (5 Times with each solvent). A Kaiser test* was performed and test was negative (free amine absent).

Between each coupling, step b i.e. deprotection step is carried out after which rinsing of resin was executed by using 5 times 3 mL of DMF and DCM and then a Kaiser test is made to confirm the free amine of growing peptide chain.

Step h) Cleavage of the resin and side chains deprotection: 5 mL of a solution containing 95% TFA + 2.5 % H_2O + 2.5 % triisopropylsilane is added in the extraction tube and stirred at RT for 2 h. The filtrate was recovered and evaporated on rotatory evaporator until a thick oil was obtained. Diethyl ether (20 mL) was added and peptide was precipitated by keeping solution at 4°C for overnight. The resulting precipitate was recovered as a white powder.

***** Kaiser test: (Ninhydrin Test): three drops of solution A, B and C is added in a test tube containing more than 10 beads of the resin. The test tube is heated at 100 °C.

When the Kaiser test is positive, the beads and the solution turn into blue color. This indicates the presence of free amine that conclude Fmoc deprotection step succeeded or a failed coupling step.

When the Kaiser test is negative, the beads and solution remain uncolored. This indicates that the coupling step is completed or a failed the Fmoc deprotection step. In case of a Fmoc deprotection or a coupling step failed, the step is repeated after a washing the beads until the Kaiser test leads to the required color.

Protocol for preparation of Kaiser test solution:

Solution A:

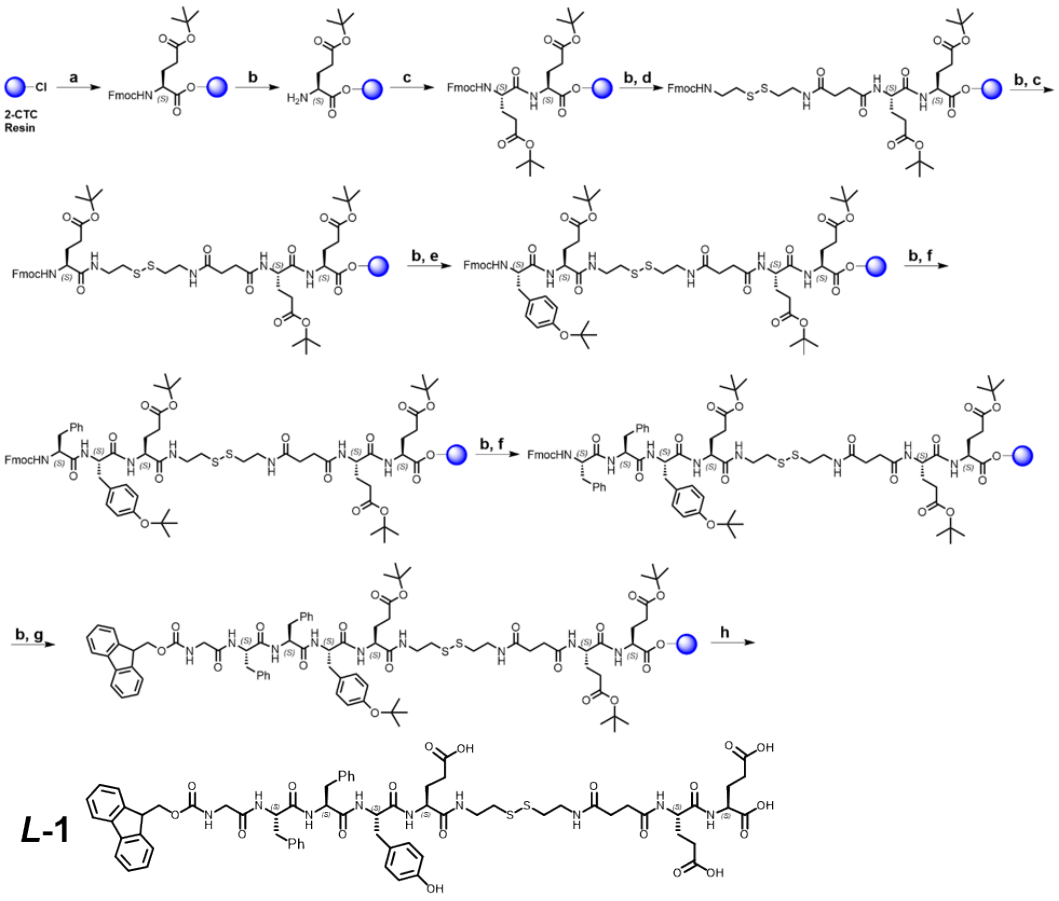
- Dissolve 16.5 mg of KCN in 25 mL of distilled water.
- Dilute 1.0 mL of above solution with 49 mL of pyridine.
- Pour it into a small reagent bottle and label it “A”

Solution B:

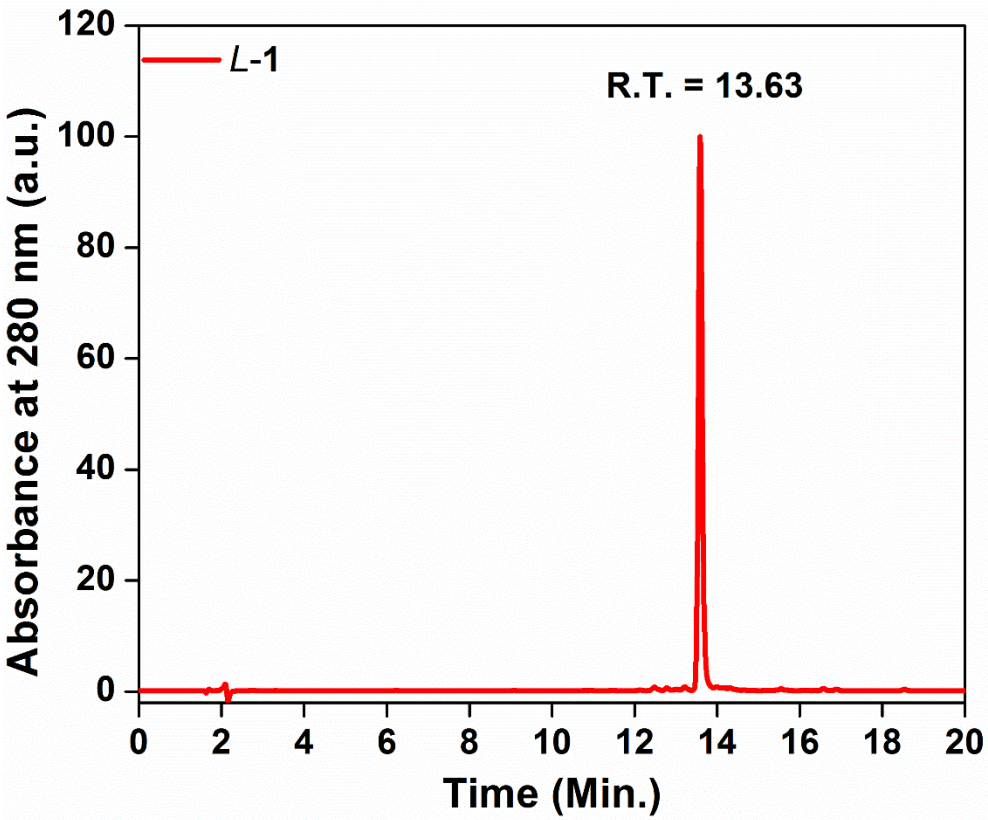
- Dissolve 1.0 g of ninhydrin in 20 mL of BuOH.
- Pour into a small reagent bottle and label it as “B”.

Solution C:

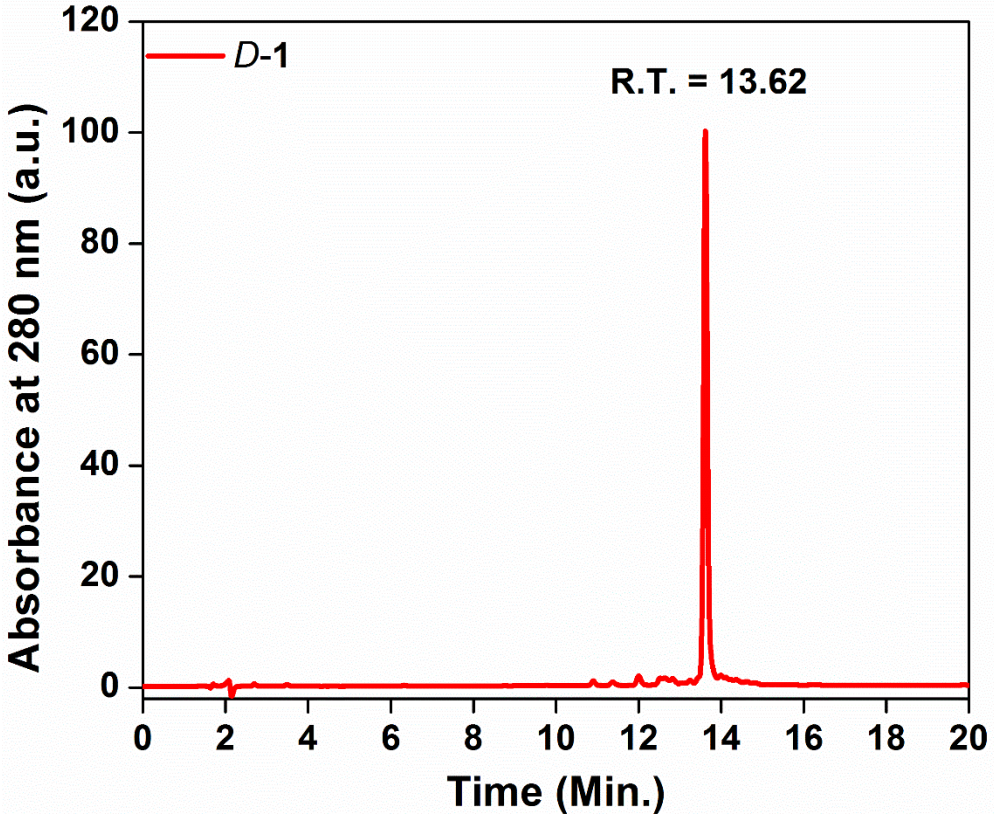
- Dissolve 40 g of PhOH in 20 mL of BuOH.
- Pour it into a small reagent bottle and label it “C”.



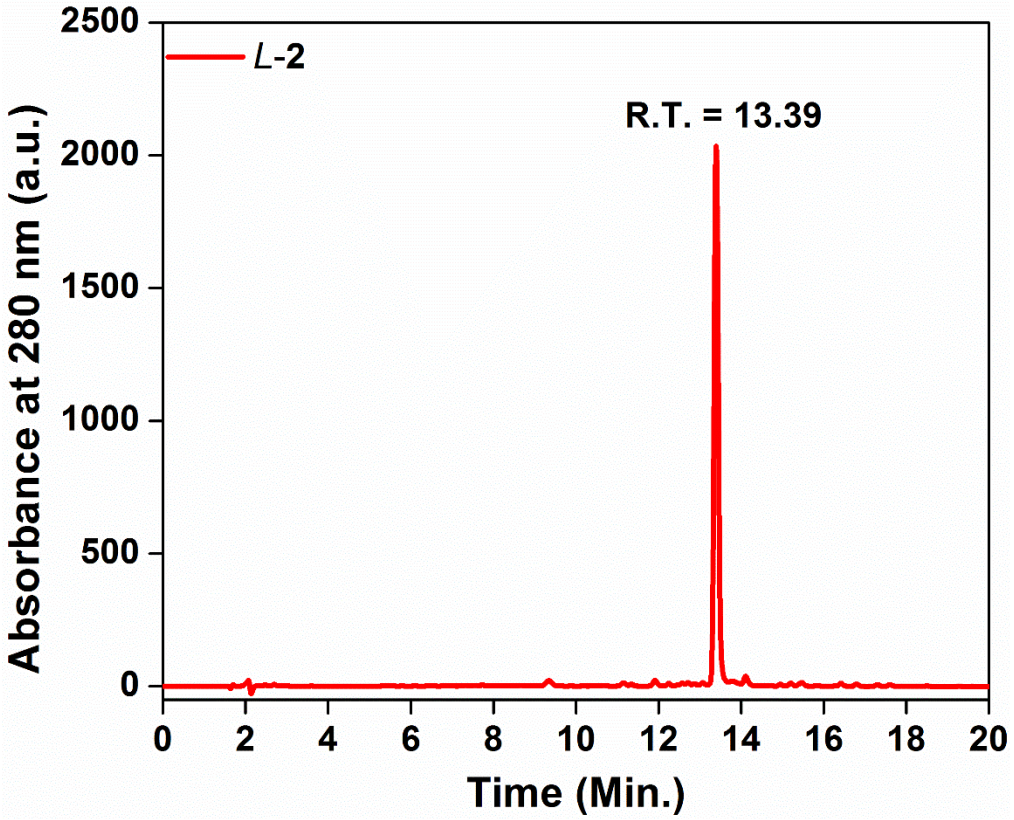
HPLC-Profile of ***L***-1



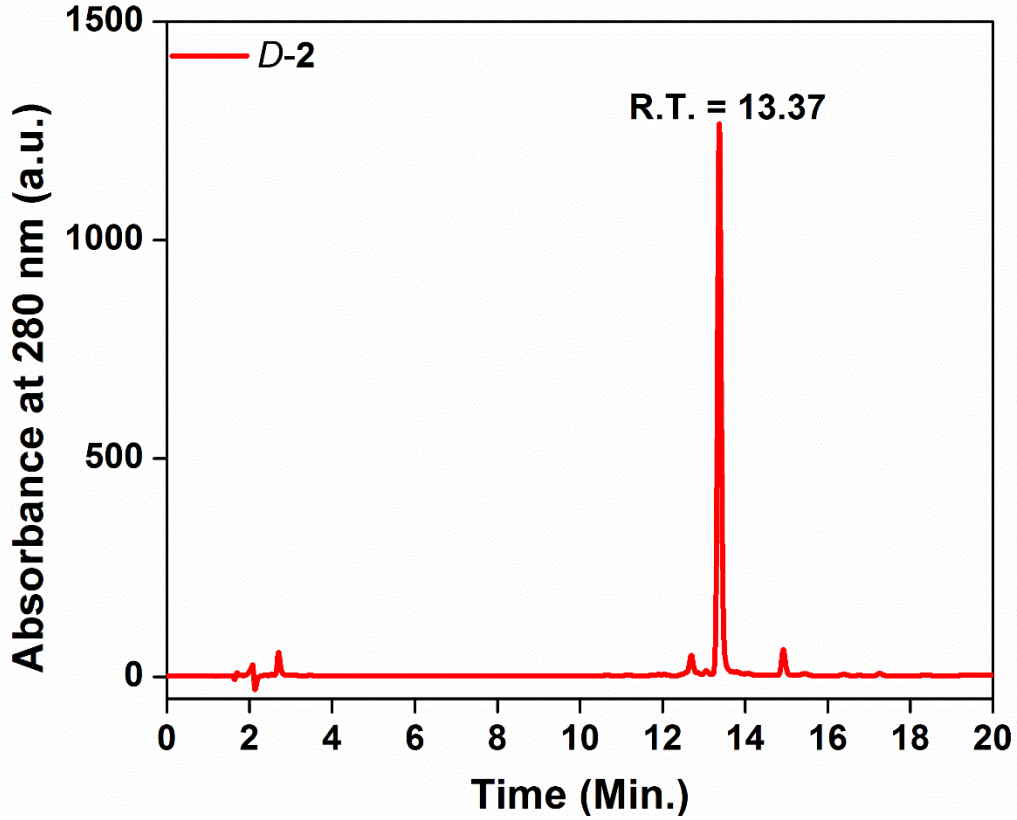
HPLC-Profile of ***D***-1



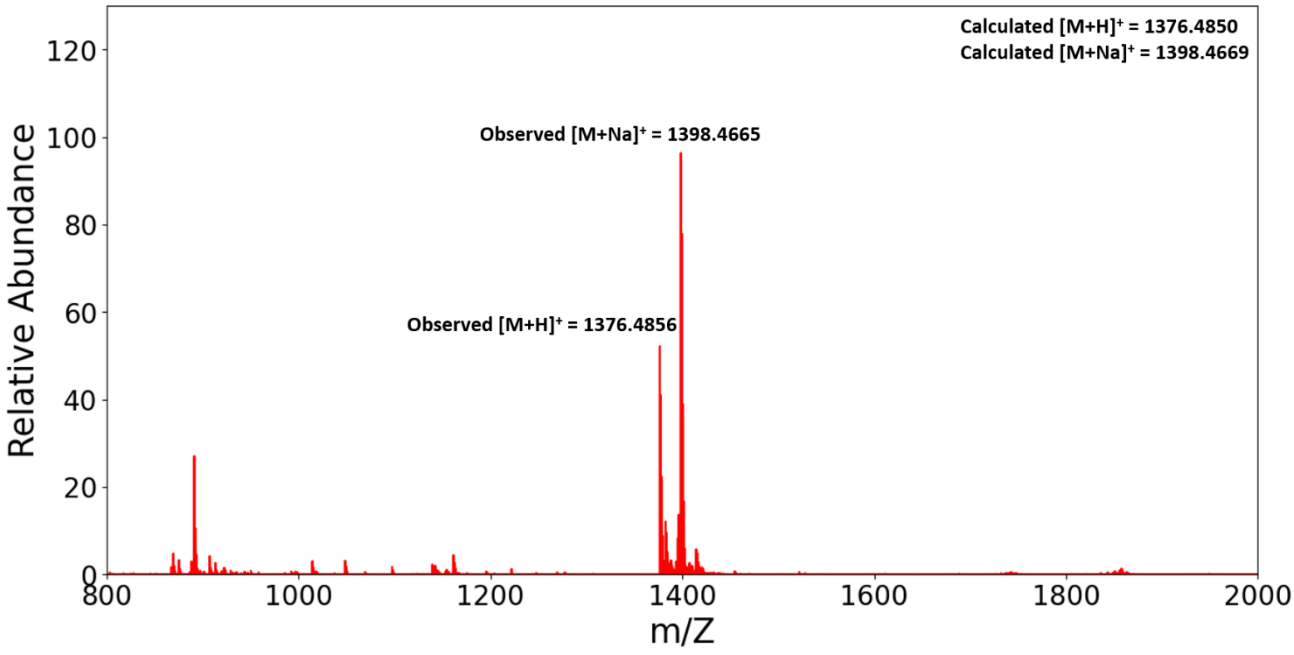
HPLC-Profile of ***L***-2



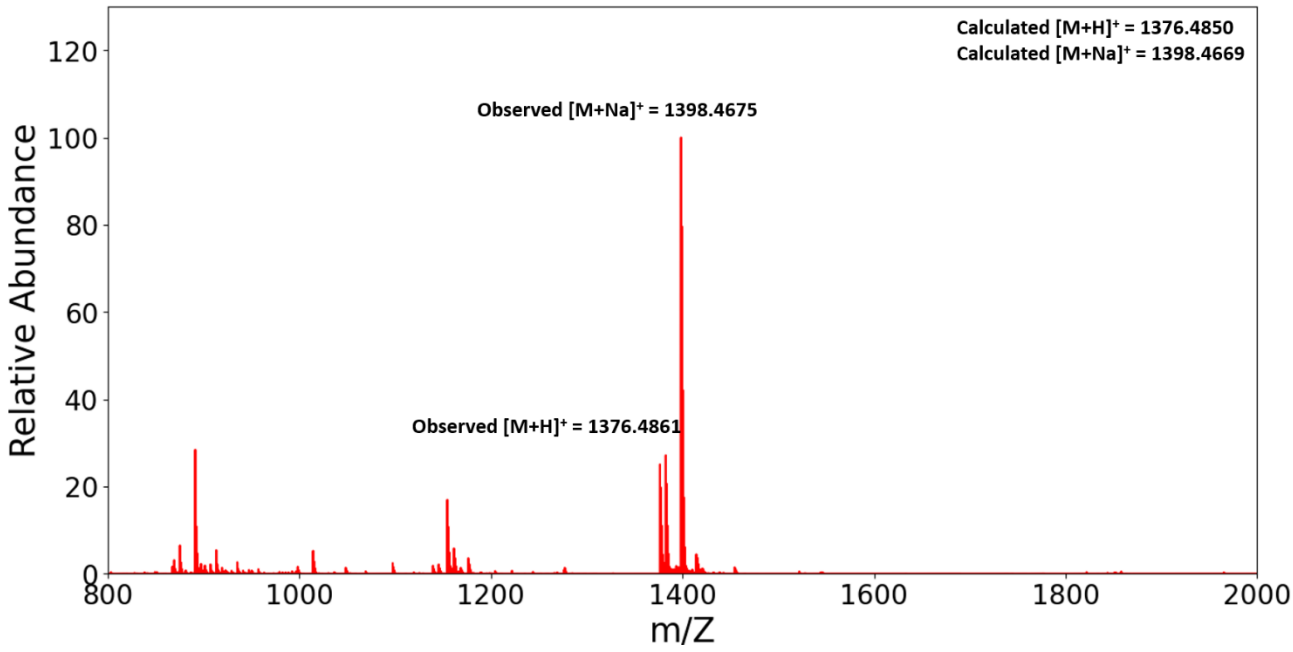
HPLC-Profile of ***D***-2



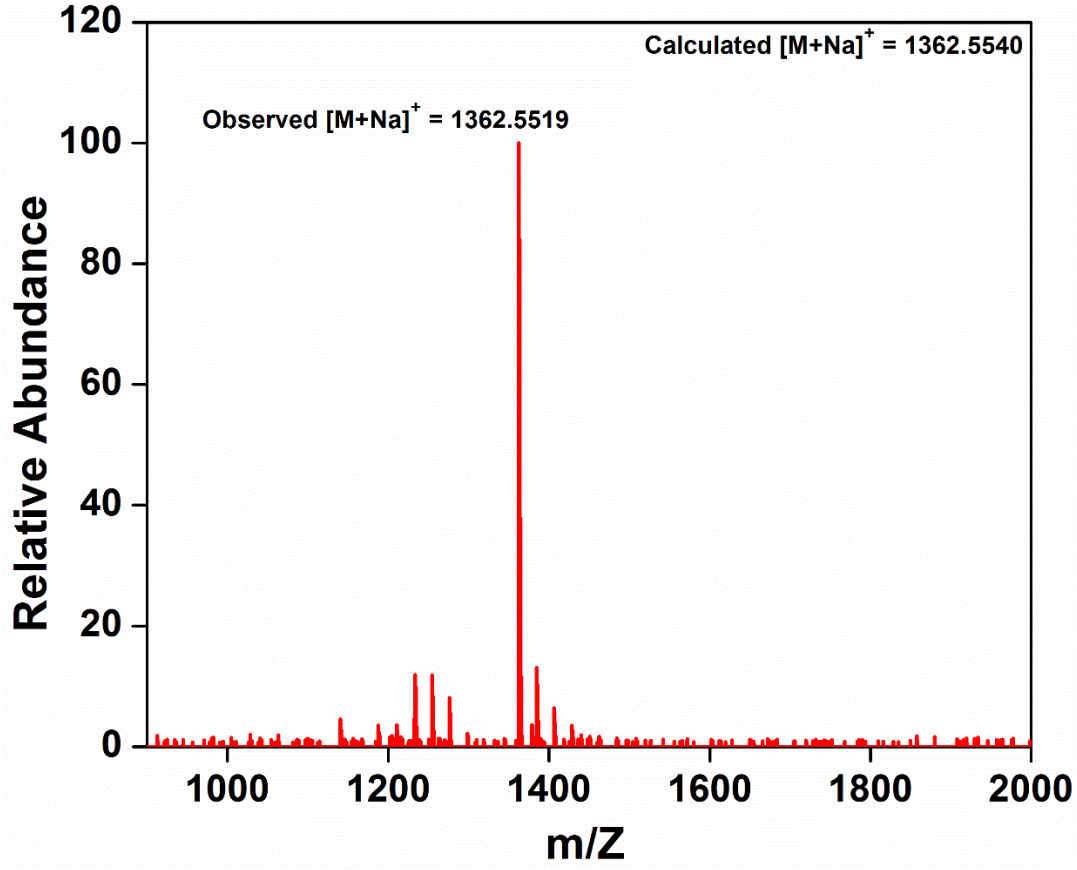
HRMS of ***L***-1



HRMS of ***D***-1


Calculated [M+H]+ = 1376.4850
Calculated [M+Na]+ = 1398.4669
Observed [M+Na]+ = 1398.4675
Observed [M+H]+ = 1376.4861
Relative Abundance
m/Z
0
20
40
60
80
100
120
800
1000
1200
1400
1600
1800
2000

HRMS of ***L***-2


Calculated [M+Na]+ = 1362.5540
Observed [M+Na]+ = 1362.5519
Relative Abundance
m/Z
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100
80
60
40
20
0
1000
1200
1400
1600
1800
2000

HRMS of ***D***-2

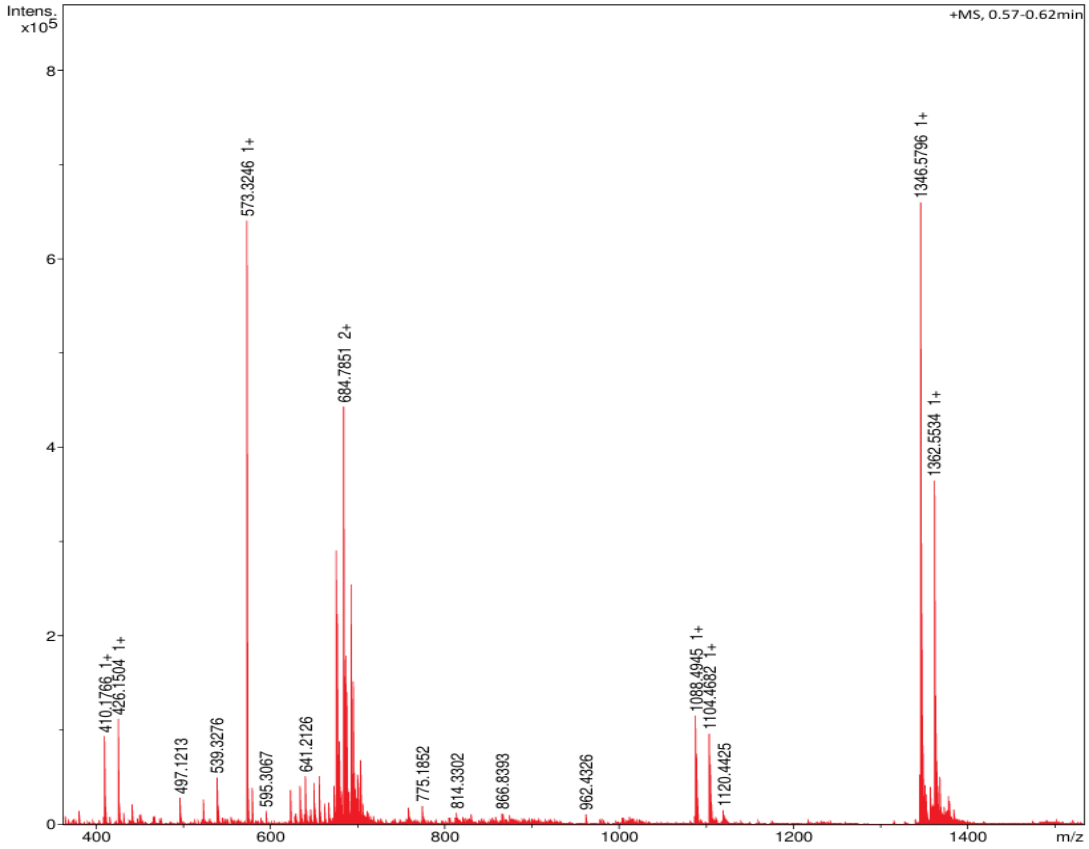
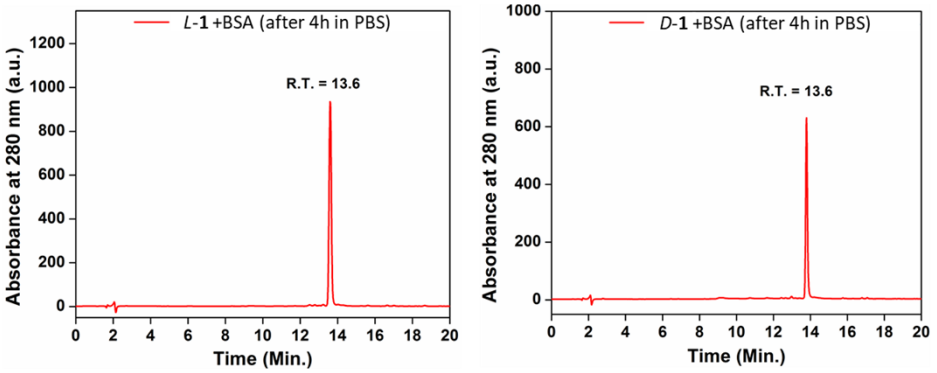

Intens.
x10^5
+MS, 0.57-0.62min
8
6
4
2
0
410.1766 1+
426.1504 1+
497.1213
539.3276
573.3246 1+
595.3067
641.2126
684.7851 2+
775.1852
814.3302
866.8393
962.4326
1088.4945 1+
1104.4682 1+
1120.4425
1346.5796 1+
1362.5534 1+
400
600
800
1000
1200
1400
m/z

Figure S1. HPLC chromatogramm analyses of (**a**) *L*-**1** +BSA and *D*-**1** +BSA gelation/solution respectively, and (**b**) after 3 months-old solution of *L*-**1** and *D*-**1**.

a.



b.

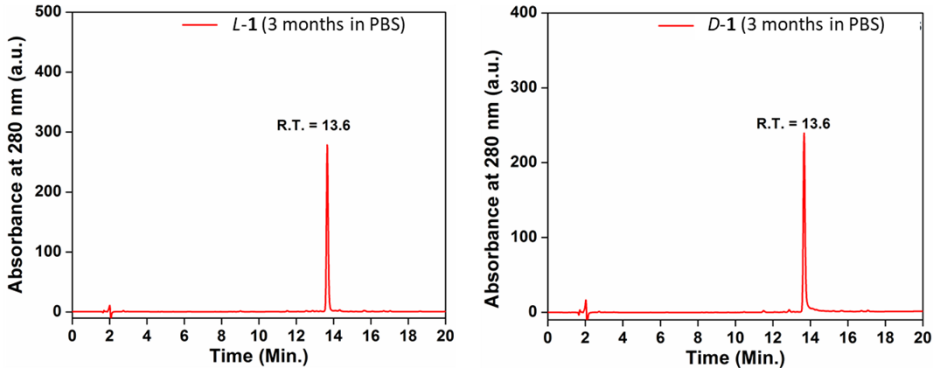


Figure S2. High Tension (HT) values corresponding to the CD spectra of *L*-**1**, *D*-**1**, *L*-**1**+BSA and *D*-**1**+BSA.

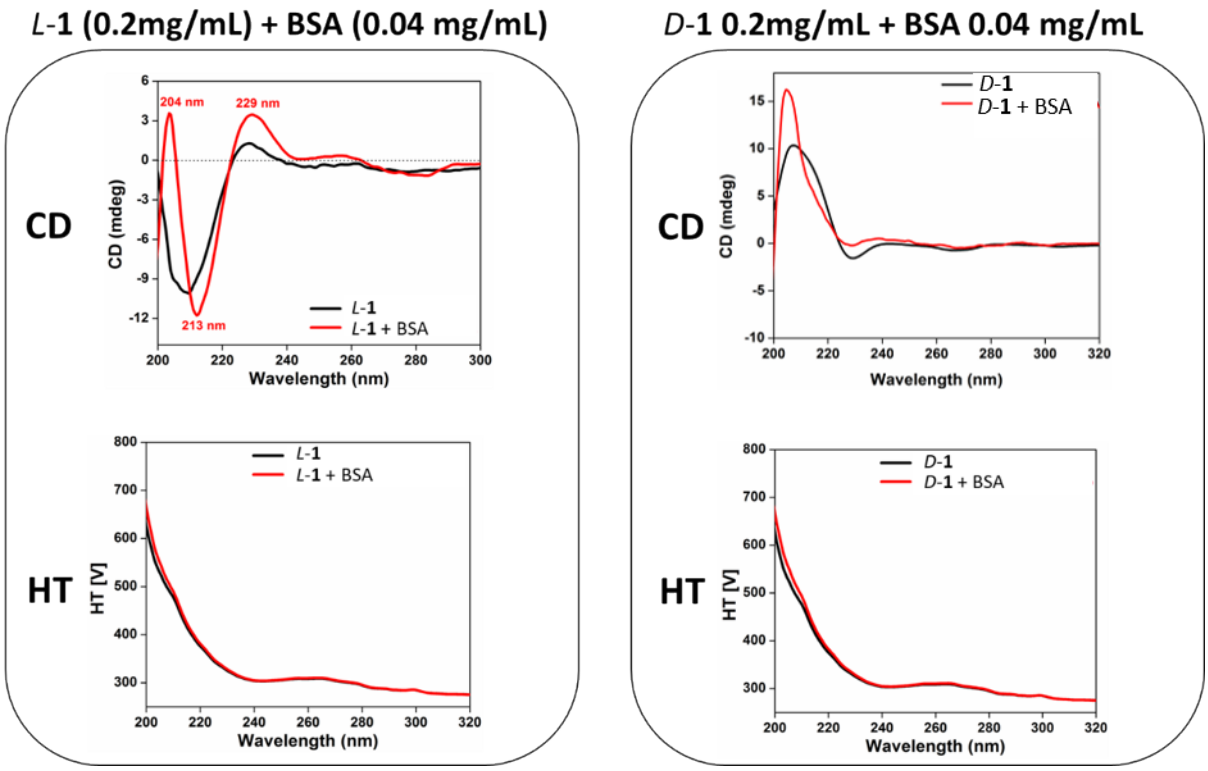
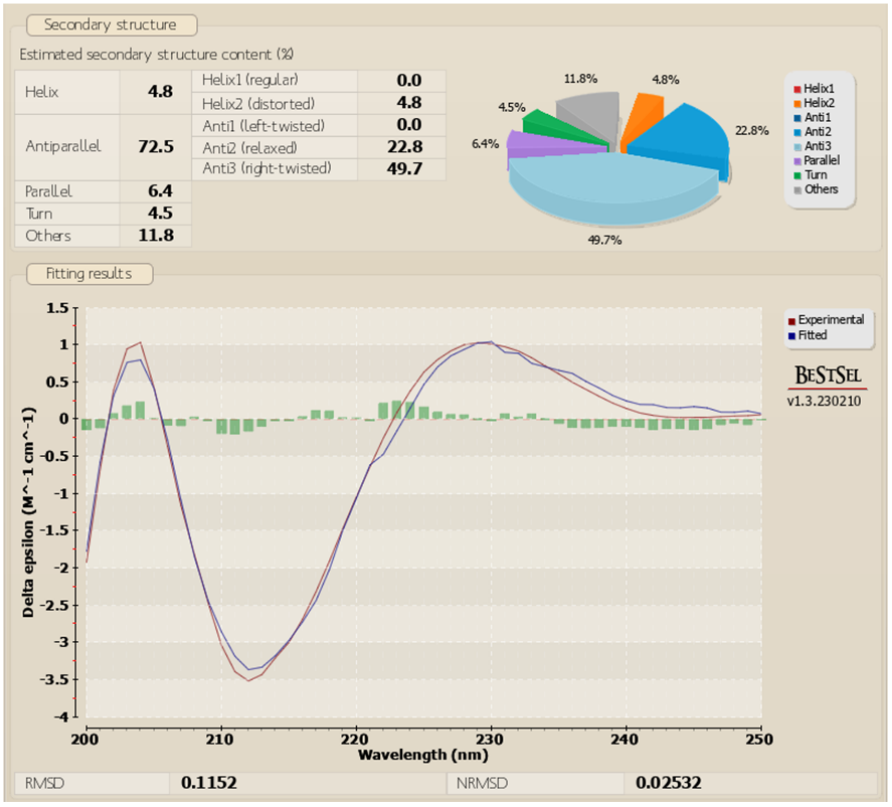


Figure 3. BESTSEL online software evaluation of the CD spectra of (**a**) *L*-**1** +BSA and (**b**) *L*-**1**.

a.



b.

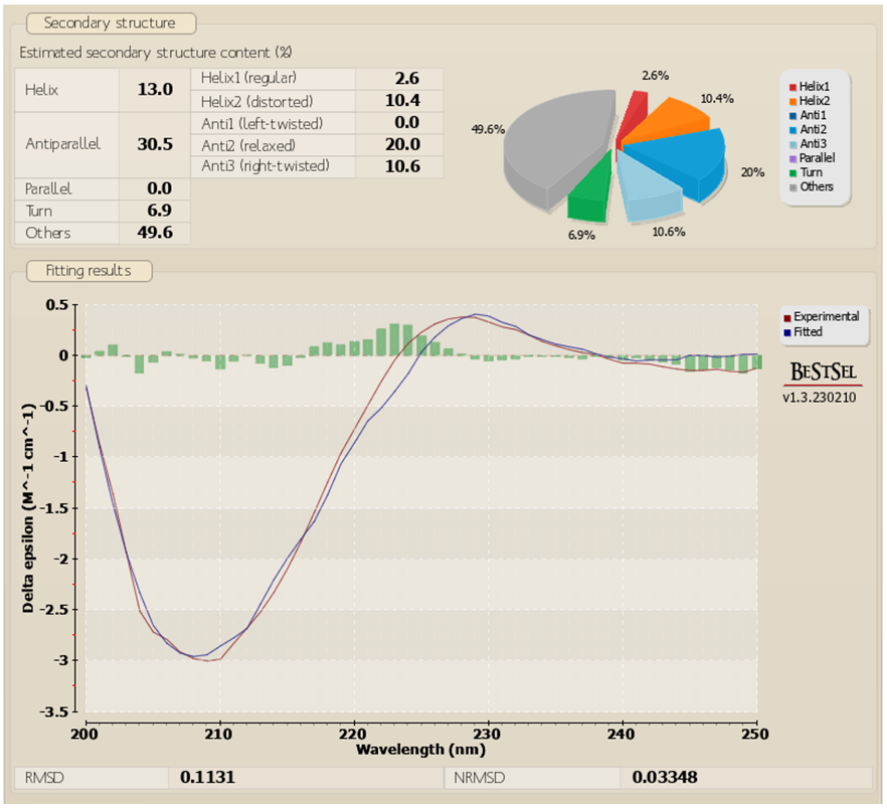
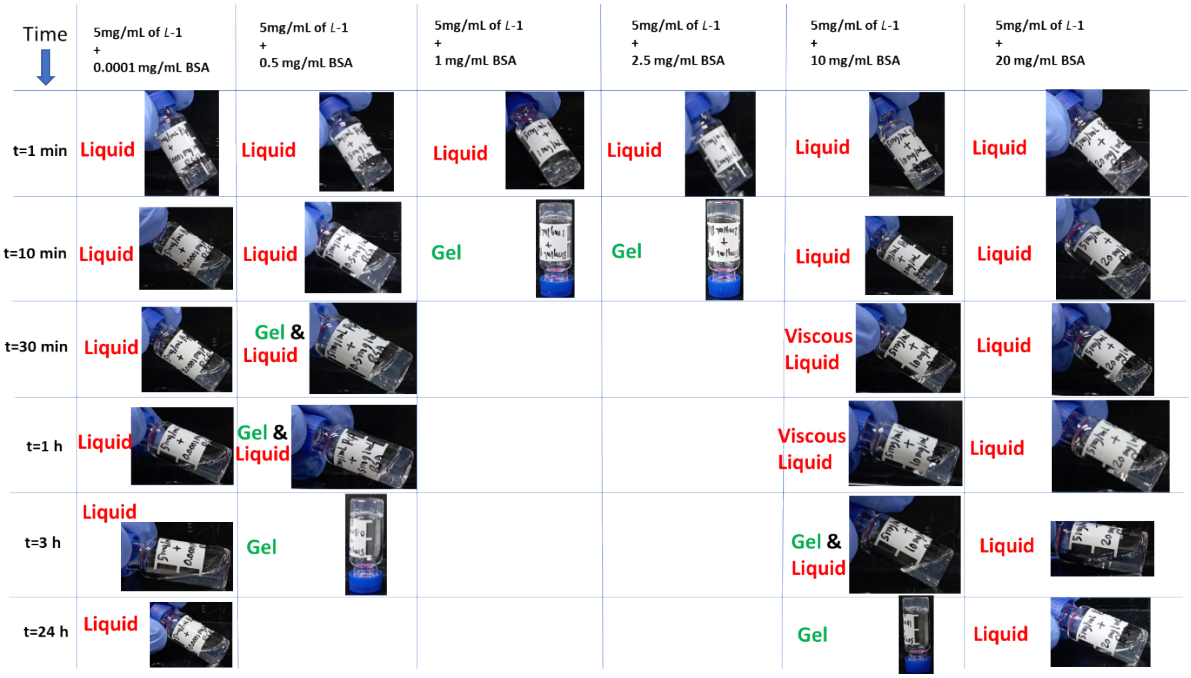


Figure S4. (*top*) Pictures of self-supported hydrogel formation through inverted vial tests over time for constant concentration of L-1 peptide (5 mg/mL, PBS 10 mM pH 7.4) and various concentration of BSA. (*bottom*) Summary table of observations. The state « Gel & Liquid » or « G&L » means we observed the presence of pieces of hydrogel in a liquid.



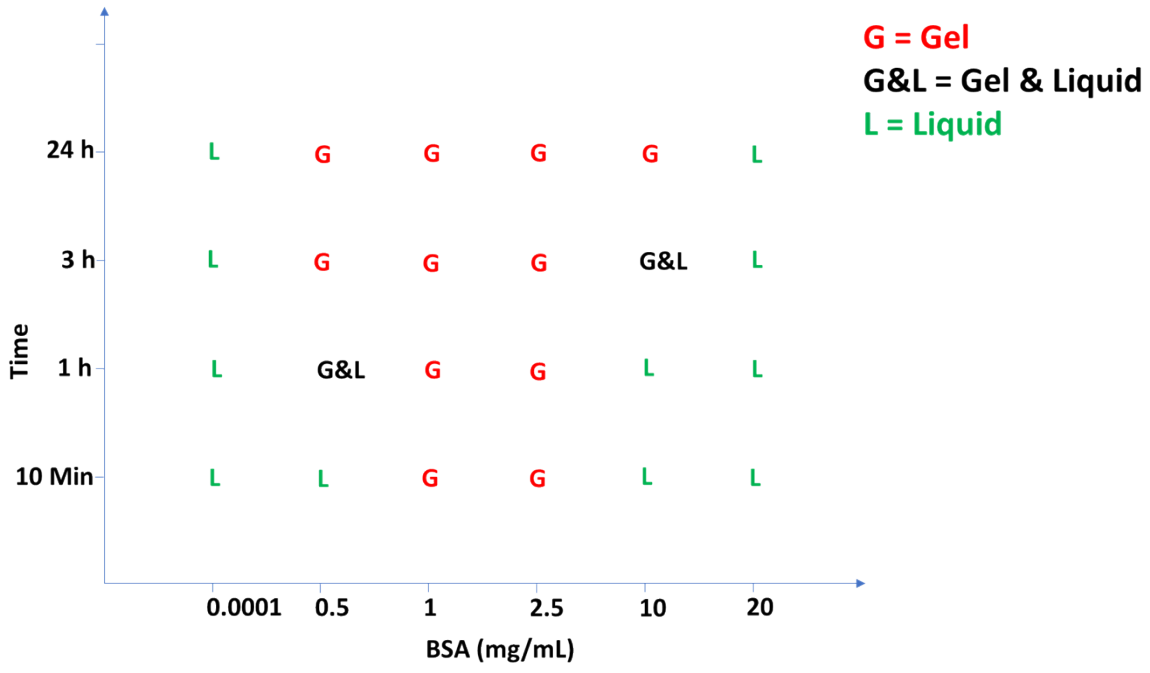


Figure S5. (*left*) Self-supported hydrogel obtained 3 months later the mixture of 5 mg/mL of *L*-**1** peptide and 20 mg/mL of BSA in PBS 10 mM, pH 7.4. (*right*) Typical TEM image

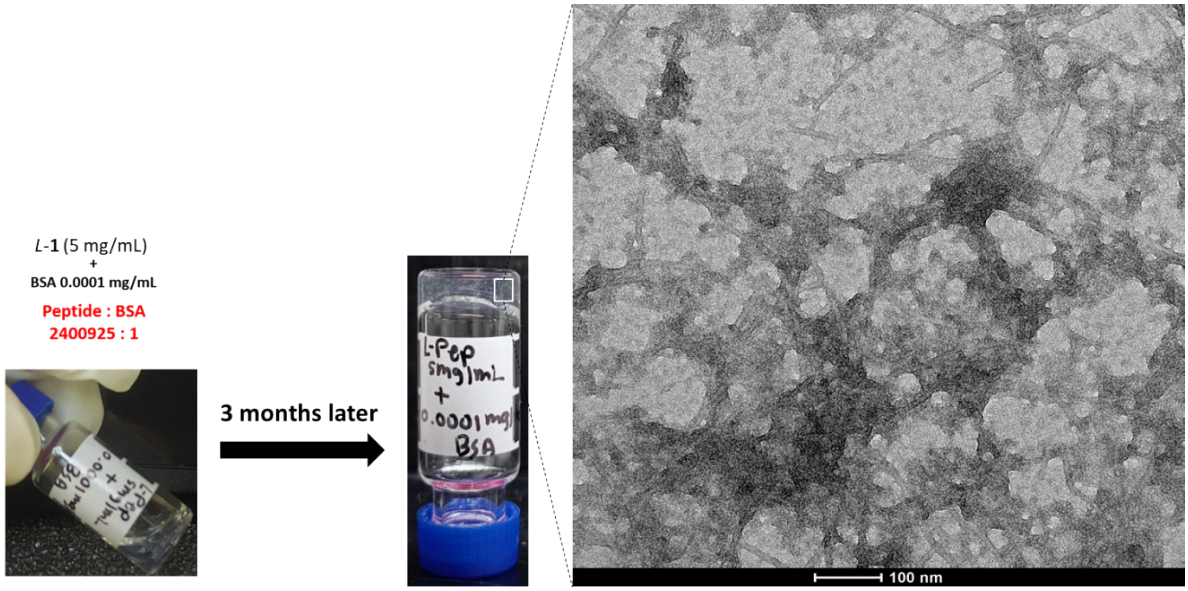


Figure S6. Storage modulus (G' –red color) and loss modulus (G'' – blue color) as a function of the time (hydrogel point determination, *left column*), the frequency (once hydrogel formed, *middle column*) and the strain (once hydrogel formed, right column) of *L*-**1** based hydrogel prepared in presence of BSA at 20, 2, 1, 0.5 or 0.0001 mg/mL

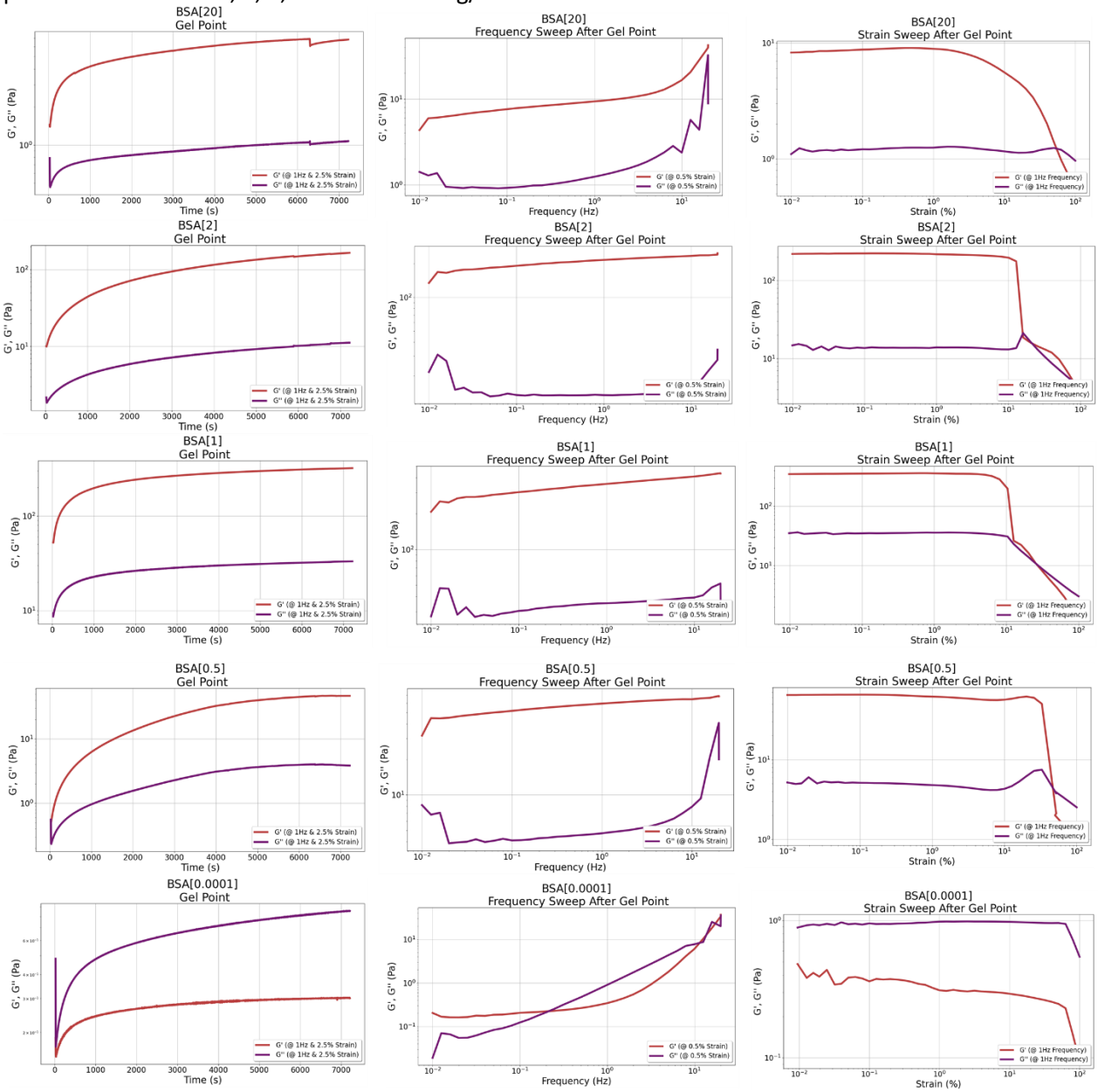
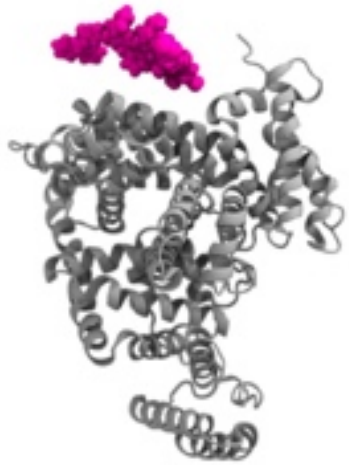
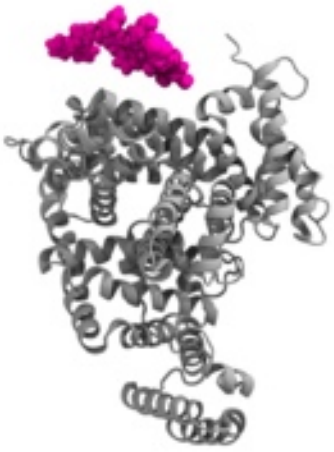
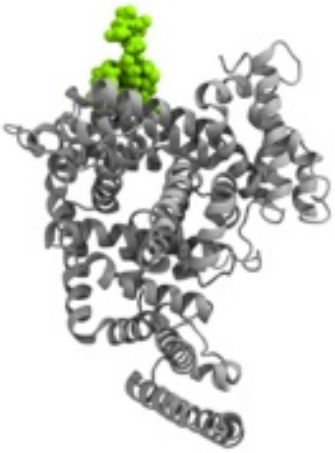
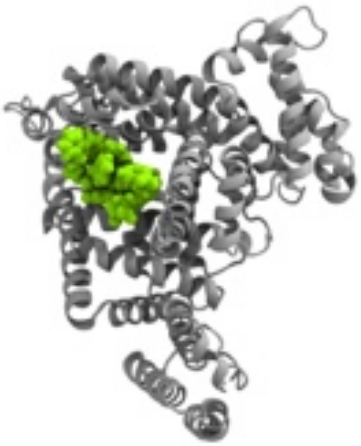
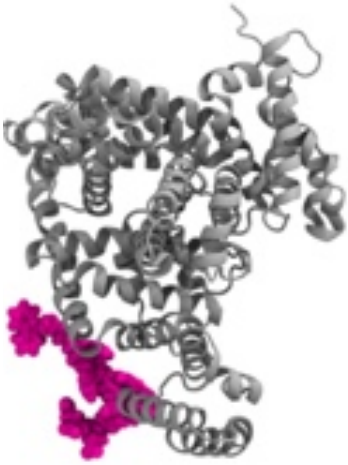
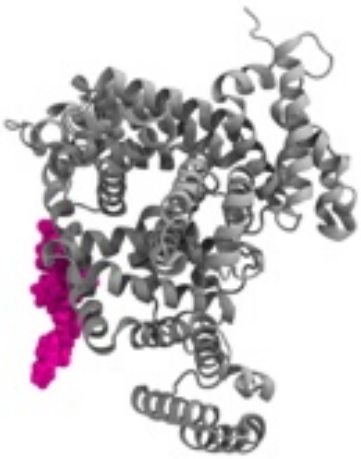
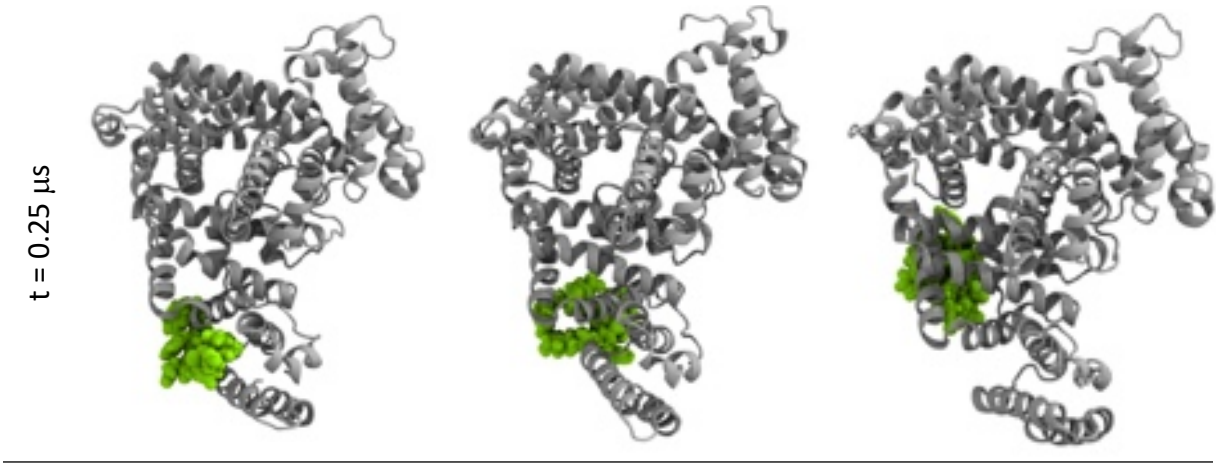


Figure S7. Peptide (**a**) *D*-**1** or (**b**) *L*-**1** at the surface of BSA. Initial (t=0 ms) and final (t=0.25 ms) snapshots of the different simulated system starting from different regions. Purple in initial position and green peptide in final position.

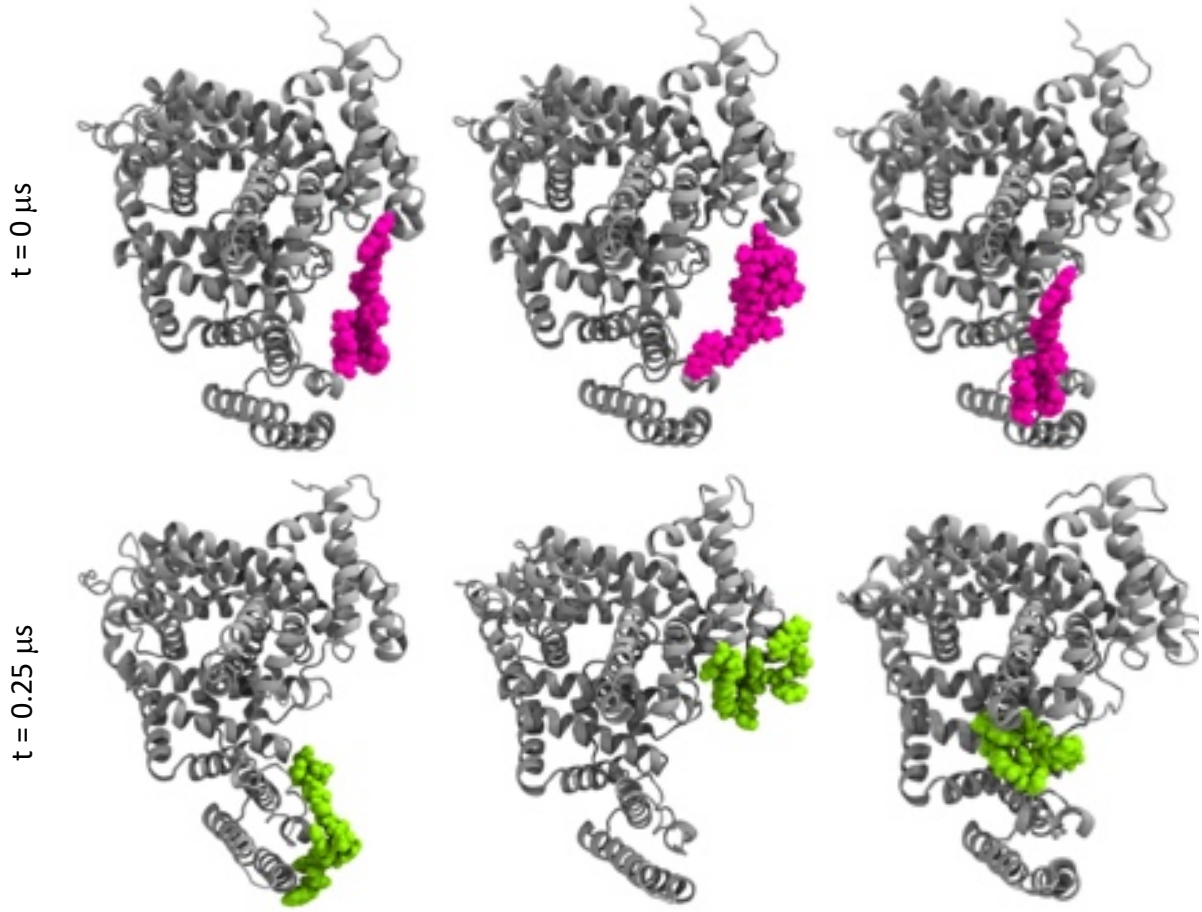
a.

1 x Peptide *D*-1

	RUN – 1	RUN – 2	RUN – 3
Site I			
t = 0 µs			
t = 0.25 µs			
Site II			
t = 0 µs			



Site III

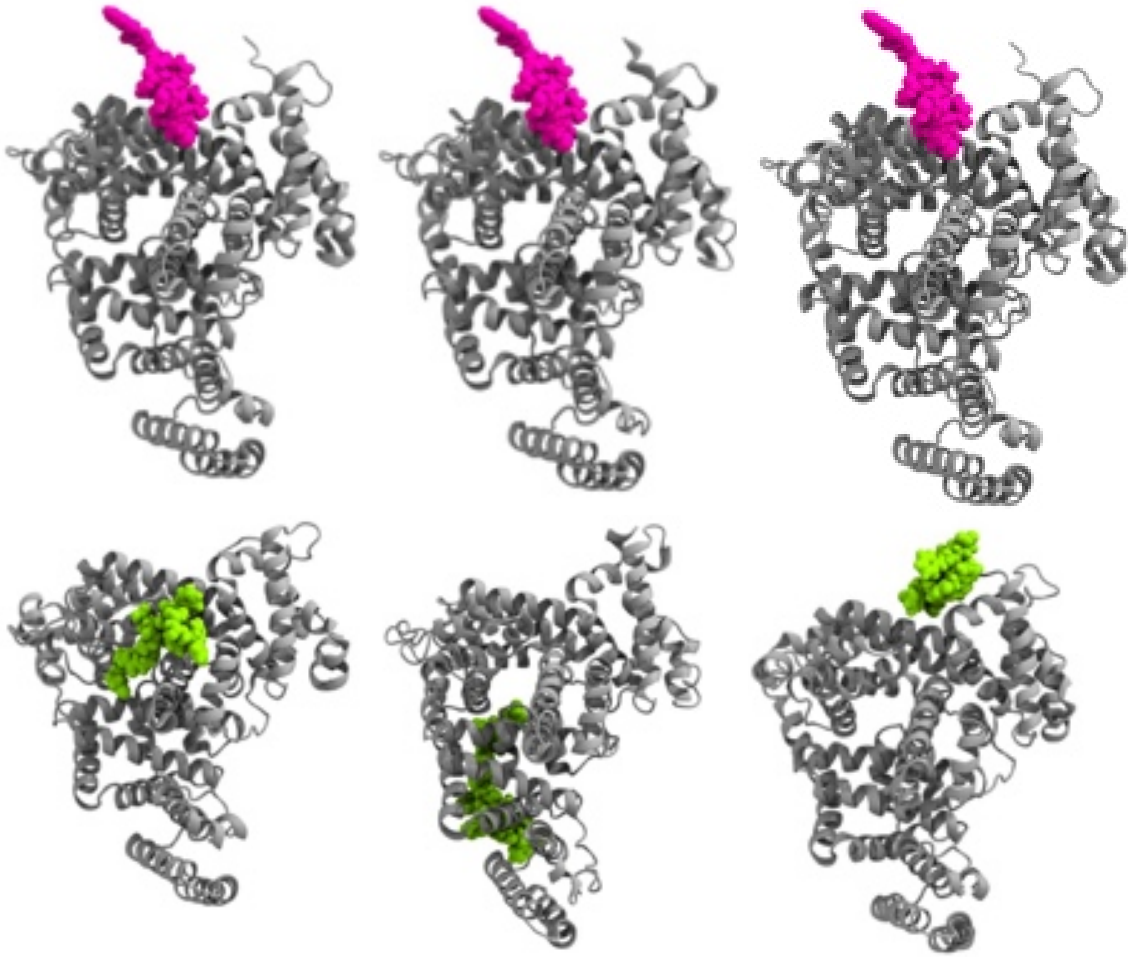


b.

1 x Peptide *L*-1		
RUN – 1	**RUN – 2**	**RUN – 3**
Site I		

t = 0 µs

t = 0.25 µs



Site II

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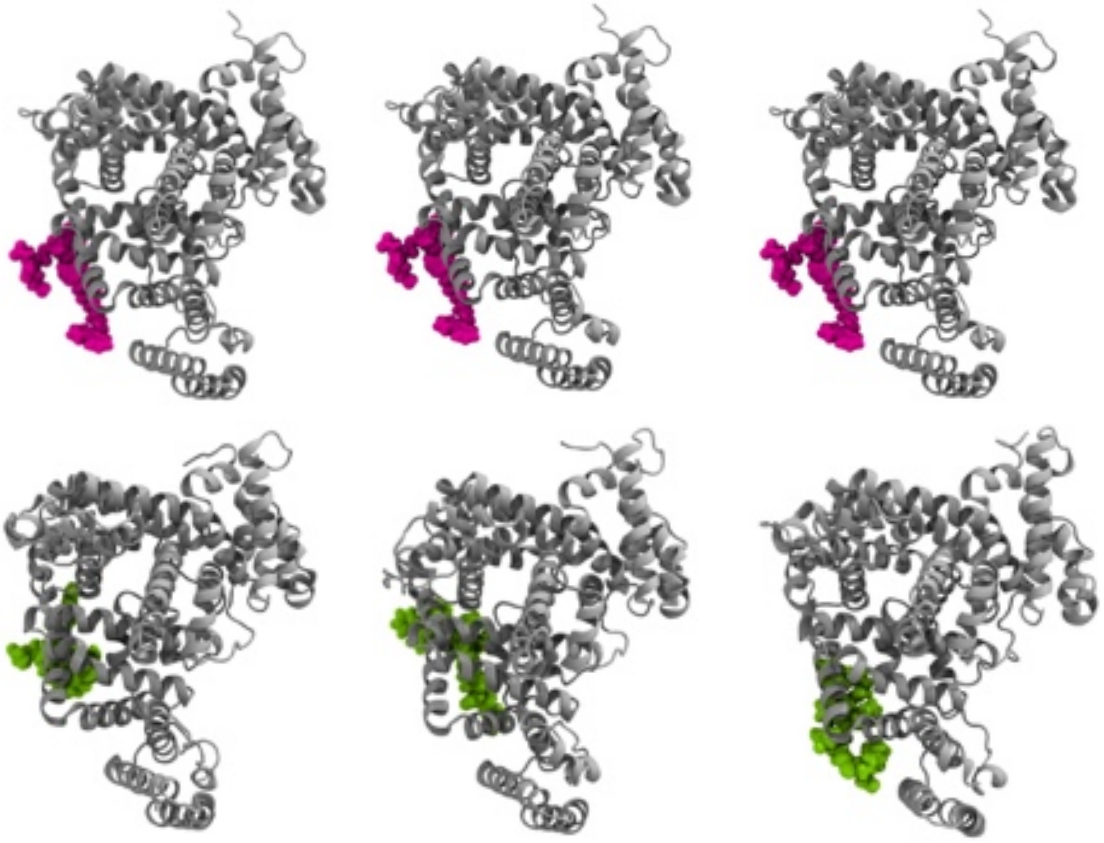
t = 0.25 µs



Site III

t = 0 μs

t = 0.25 μs

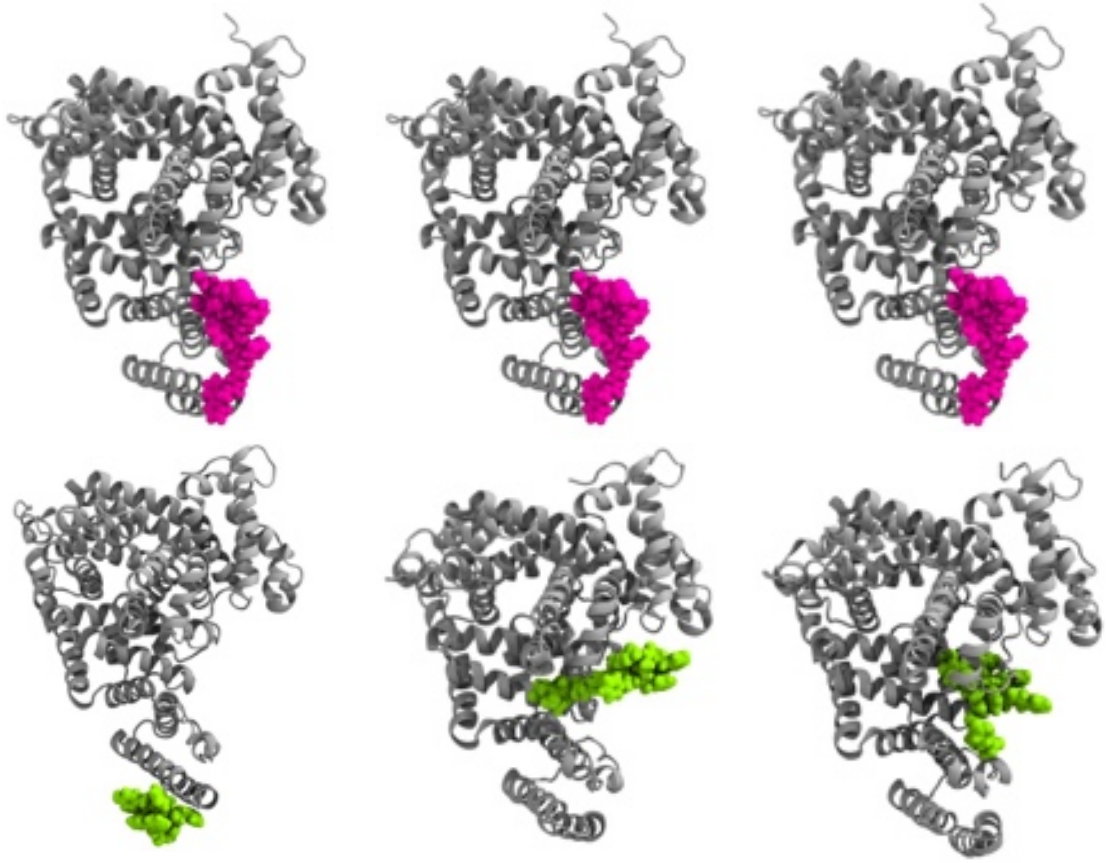


Figure S8. Snapshots of the starting configurations of the four *L*-**1** peptide (*top*) or four *D*-**1** peptide (*bottom*) in the vicinity of the BSA protein (water molecules are not shown for sake of clarity). The red colored peptide is the one initially adsorbed on BSA surface.

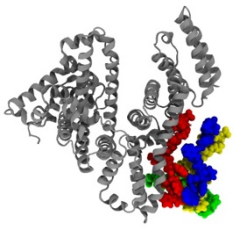
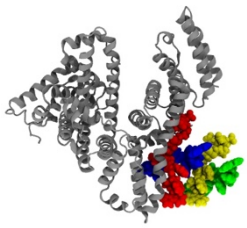
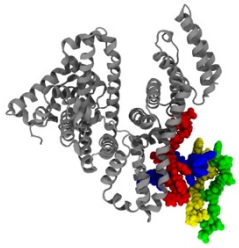
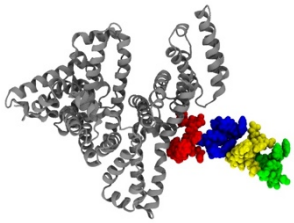
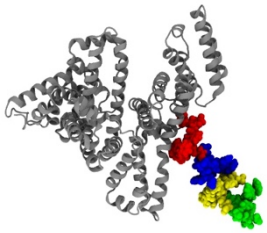
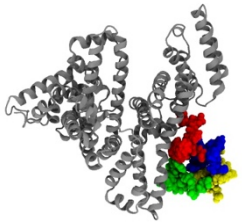
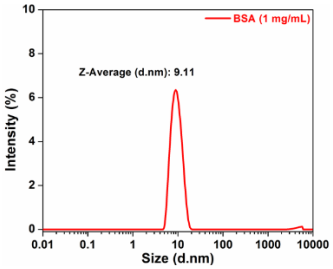
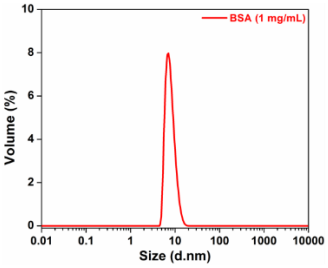
4 x *L*-**1** peptide		
RUN – 1	**RUN – 2**	**RUN – 3**
		
4 x *D*-**1** peptide		
RUN – 1	**RUN – 2**	**RUN – 3**
		

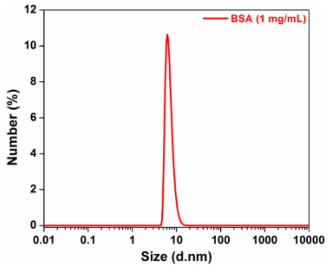
Figure S9. Dynamic Light scattering of BSA (1 mg/mL) in PBS (10 mM, pH 7.4)



	Size (d.nm)	% Intensity	St Dev (d.nm)
Peak 1	9.268	98.2	2.211
Peak 2	4630	1.8	807.4
Peak 3	0	0	0



	Size (d.nm)	% Intensity	St Dev (d.nm)
Peak 1	7.944	100	1.784
Peak 2	0	0	0
Peak 3	0	0	0



	Size (d.nm)	% Intensity	St Dev (d.nm)
Peak 1	7.081	100	1.327
Peak 2	0	0	0
Peak 3	0	0	0

Z-Average (d.nm): 9.11
PDI: 0.146
Intercept: 0.884
Result quality: Good