Supporting Information

The application of the hierarchical approach for the construction of foldameric peptide self-assembled nanostructures

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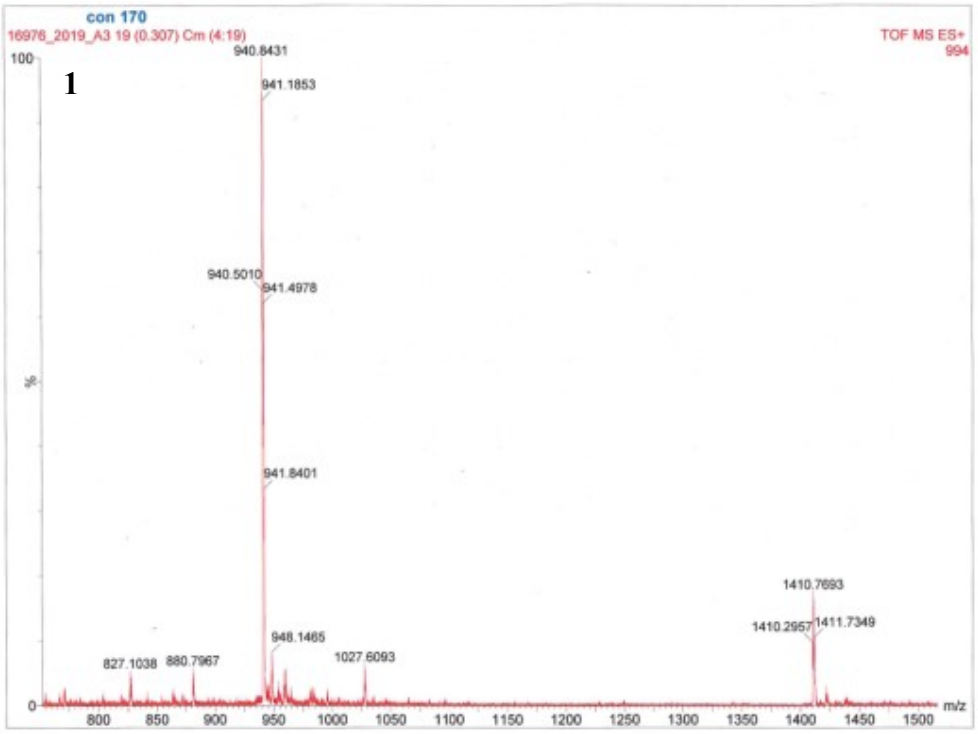
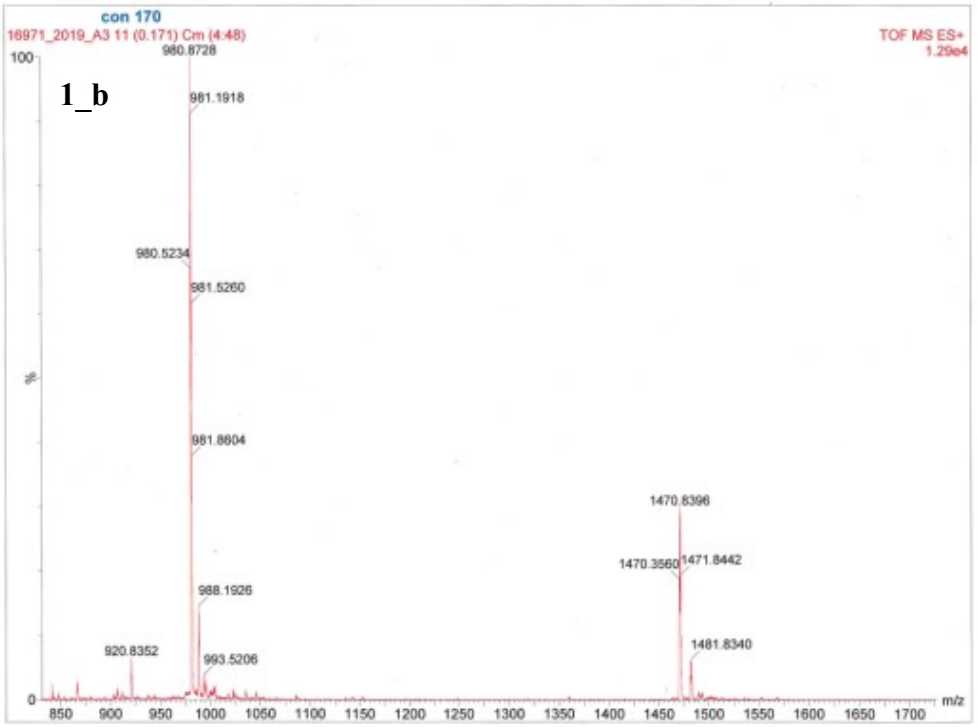
Table S1 Peptides analytical data.

Name	Formula	Calculated M/z	Experimental M/z	Analytical HPLC t_r [min]
1	$C_{130}H_{203}N_{33}O_{37}$	[(M+2H)/2] 1410.7693 [(M+3H)/3] 940.8431	[(M+2H)/2] 1410.7601 [(M+3H)/3] 940.8427	16.467
1_b	$C_{139}H_{215}N_{33}O_{37}$	[(M+2H)/2] 1470.8071 [(M+3H)/3] 980.8740	[(M+2H)/2] 1470.8396 [(M+3H)/3] 980.8728	16.733
1_c	$C_{139}H_{215}N_{33}O_{37}$	[(M+2H)/2] 1470.8071 [(M+3H)/3] 980.8740	[(M+2H)/2] 1470.7970 [(M+3H)/3] 980.8748	17.933
1_f	$C_{135}H_{209}N_{31}O_{35}$	[(M+2H)/2] 1413.7856 [(M+3H)/3] 942.8597	[(M+2H)/2] 1413.7716 [(M+3H)/3] 942.8586	17.900
2	$C_{119}H_{198}N_{32}O_{34}$	[(M+3H)/3] 874.5004 [(M+4H)/4] 656.3780	[(M+3H)/3] 874.4998 [(M+4H)/4] 656.3998	17.473
2_b	$C_{128}H_{210}N_{32}O_{34}$	[(M+2H)/2]1371.2937 [(M+3H)/3] 914.5317	[(M+2H)/2]1371.2964 [(M+3H)/3] 914.5308	17.053
2_c	$C_{128}H_{210}N_{32}O_{34}$	[(M+2H)/2]1371.2937 [(M+3H)/3] 914.5317	[(M+2H)/2]1371.2887 [(M+3H)/3] 914.5313	17.173
2_f	$C_{124}H_{204}N_{30}O_{32}$	[(M+2H)/2]1314.2722 [(M+3H)/3] 876.5174	[(M+2H)/2]1314.2855 [(M+3H)/3] 876.5172	17.440


con 170
16976_2019_A3 19 (0.307) Cm (4:19)
TOF MS ES+
994
1
940.8431
941.1853
940.5010
941.4978
941.8401
1410.7693
1410.2957
1411.7349
948.1465
827.1038
880.7967
1027.6093
%
m/z


con 170
16971_2019_A3 11 (0.171) Cm (4:48)
TOF MS ES+
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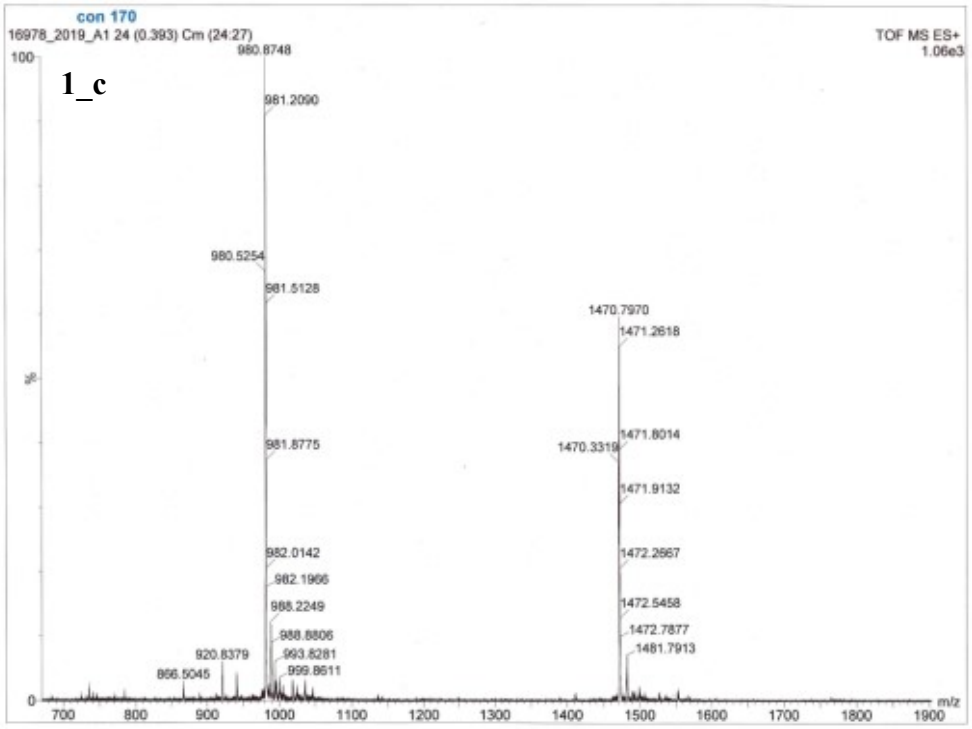
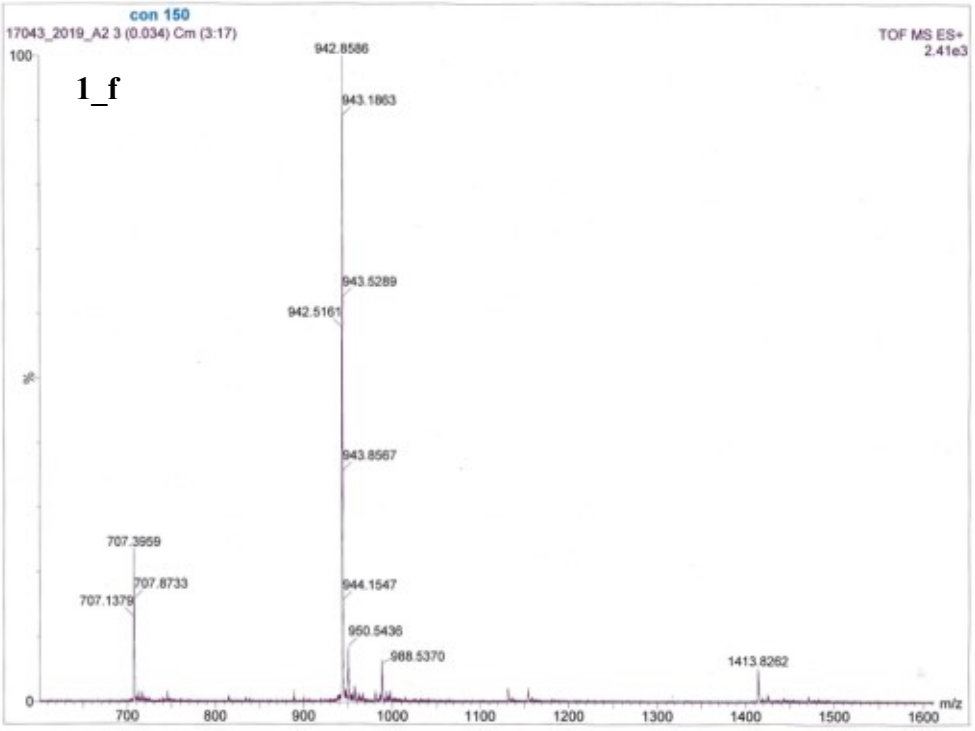
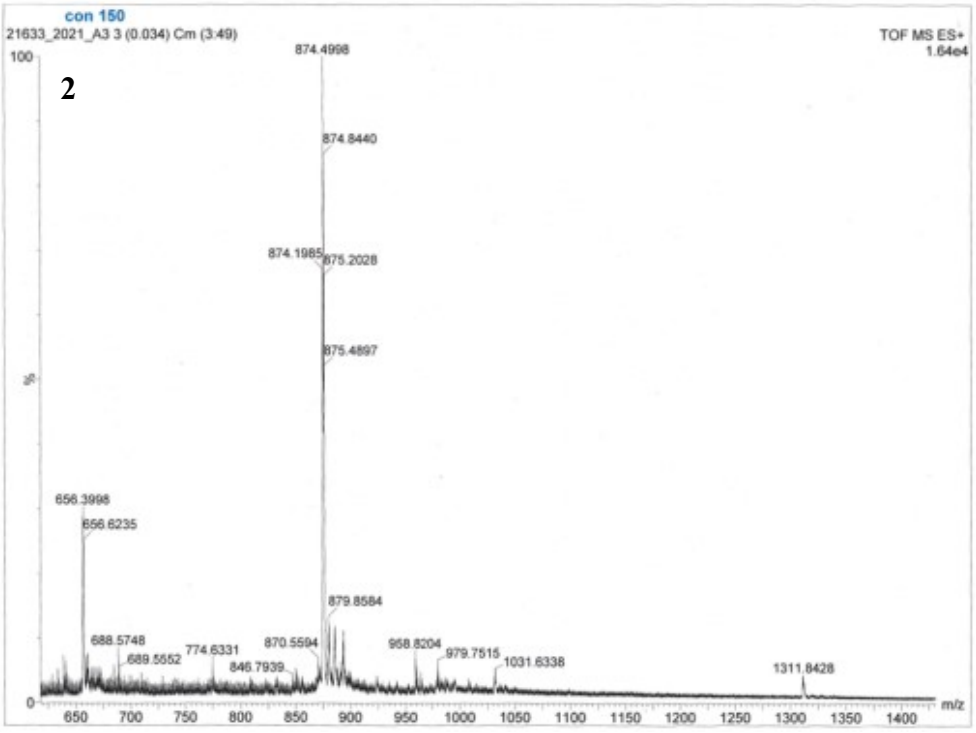
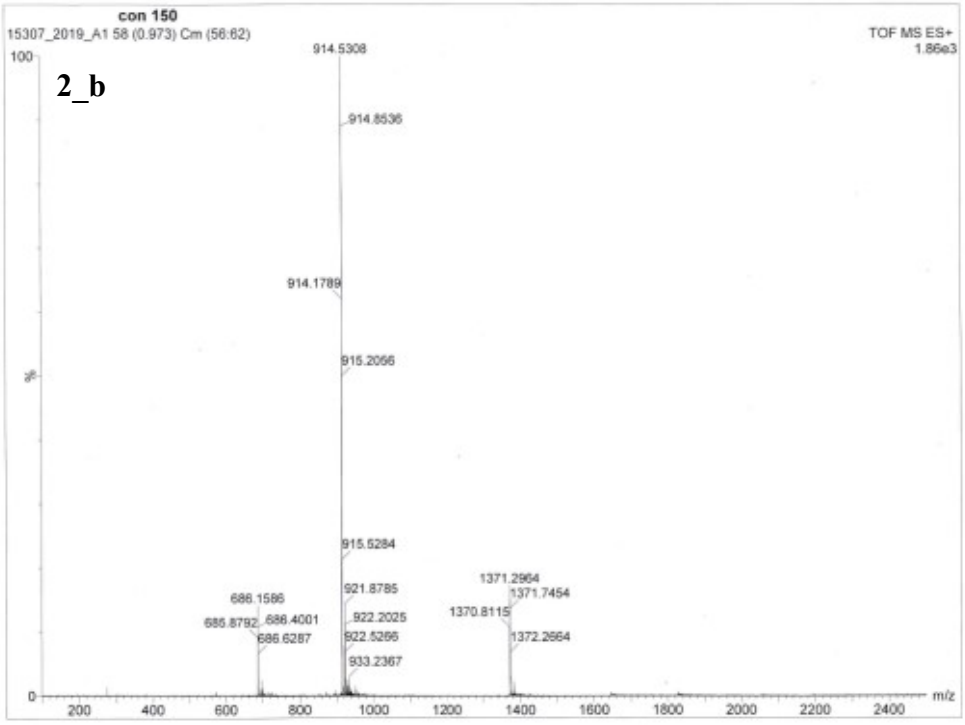
1_b
980.8728
981.1918
980.5234
981.5260
981.8604
1470.8396
1470.3560
1471.8442
988.1926
920.8352
993.5206
1481.8340
%
m/z


con 170
16978_2019_A1 24 (0.393) Cm (24:27)
TOF MS ES+
1.06e3
1_c
980.8748
981.2090
980.5254
981.5128
1470.7970
1471.2618
981.8775
1471.8014
1470.3319
1471.9132
982.0142
1472.2967
982.1966
988.2249
1472.5458
988.8806
1472.7877
920.8379
993.8281
1481.7913
866.5045
999.8611
%
m/z


con 150
17043_2019_A2 3 (0.034) Cm (3:17)
TOF MS ES+
2.41e3
1_f
942.8586
943.1863
943.5289
942.5161
943.8567
707.3959
707.8733
944.1547
707.1379
950.5436
988.5370
1413.8262
%
m/z


con 150
21633_2021_A3 3 (0.034) Cm (3:49)
TOF MS ES+
1.64e4
2
874.4998
874.8440
874.1985
875.2028
875.4897
656.3998
656.6235
879.8584
688.5748
689.5552
774.6331
846.7939
870.5594
958.8204
979.7515
1031.6338
1311.8428
m/z


con 150
15307_2019_A1 58 (0.973) Cm (56:62)
TOF MS ES+
1.86e3
2_b
914.5308
914.8536
914.1789
915.2056
915.5284
921.8785
1371.2964
1371.7454
686.1586
686.8792
686.4001
686.6287
922.2025
922.5266
1370.8115
1372.2964
933.2367
m/z

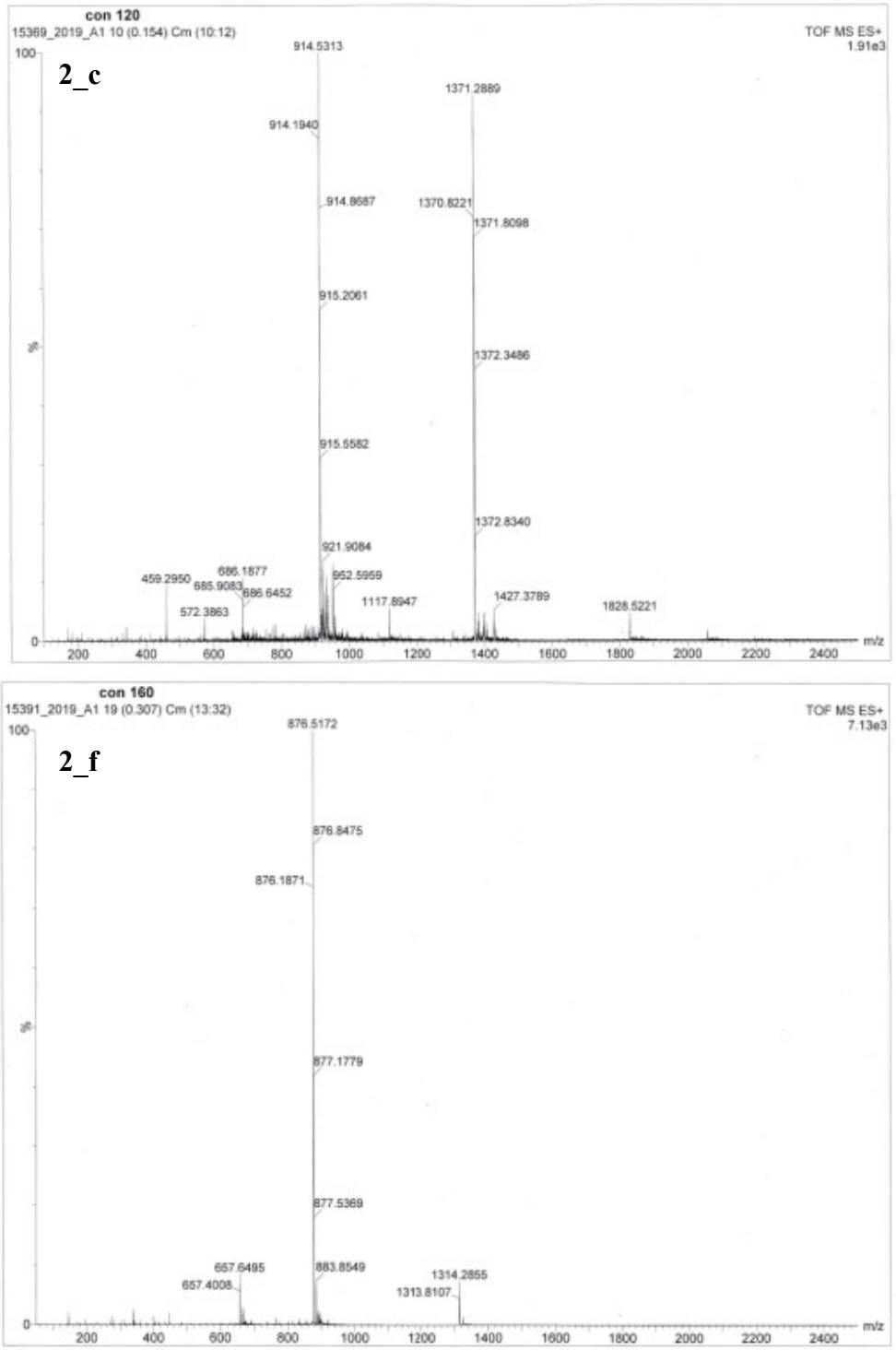
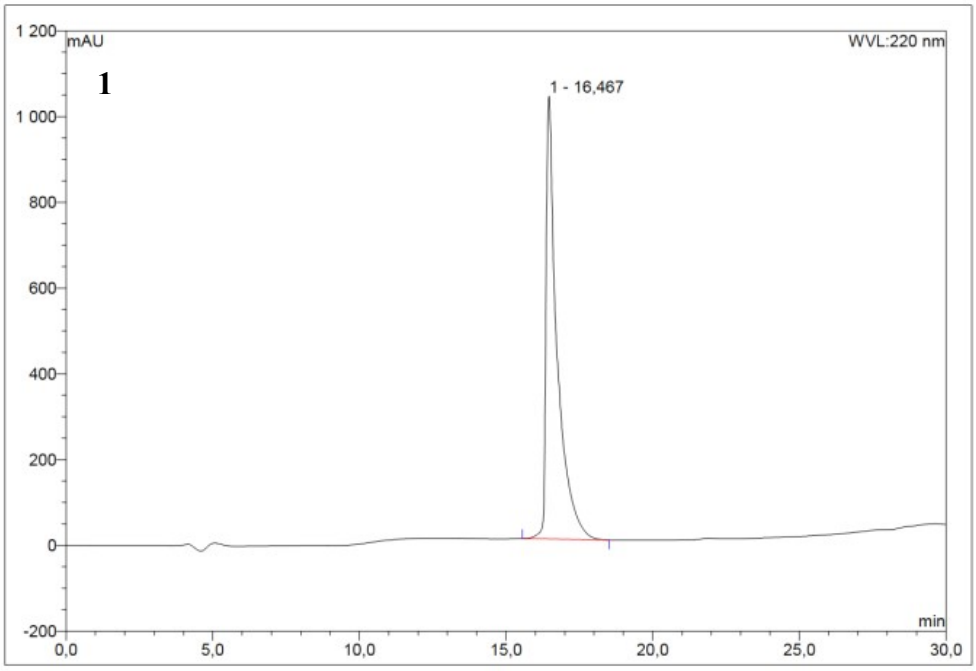
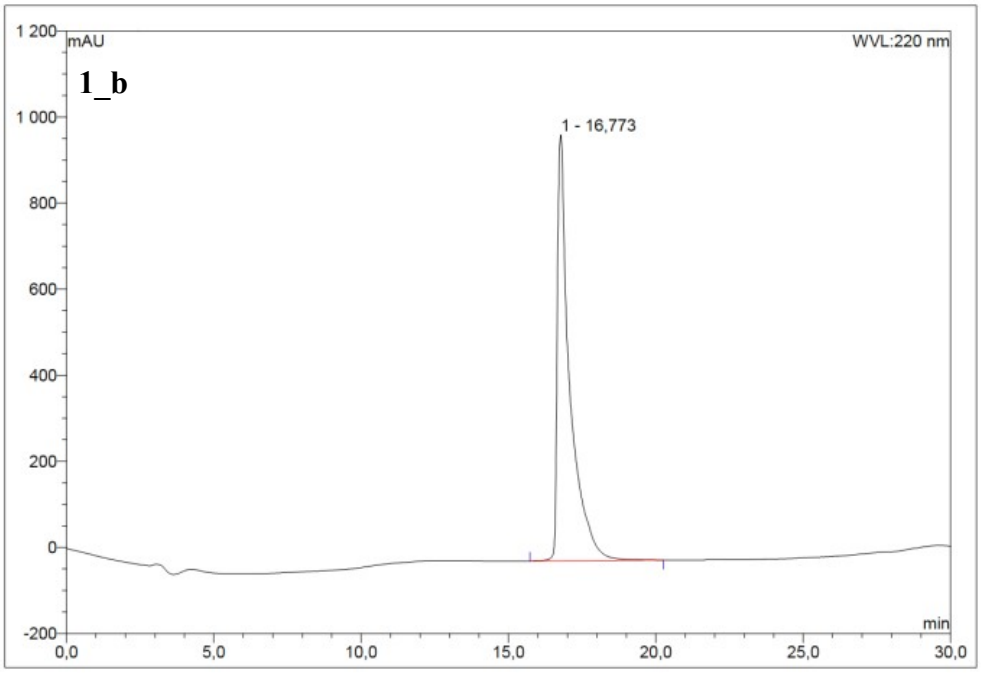
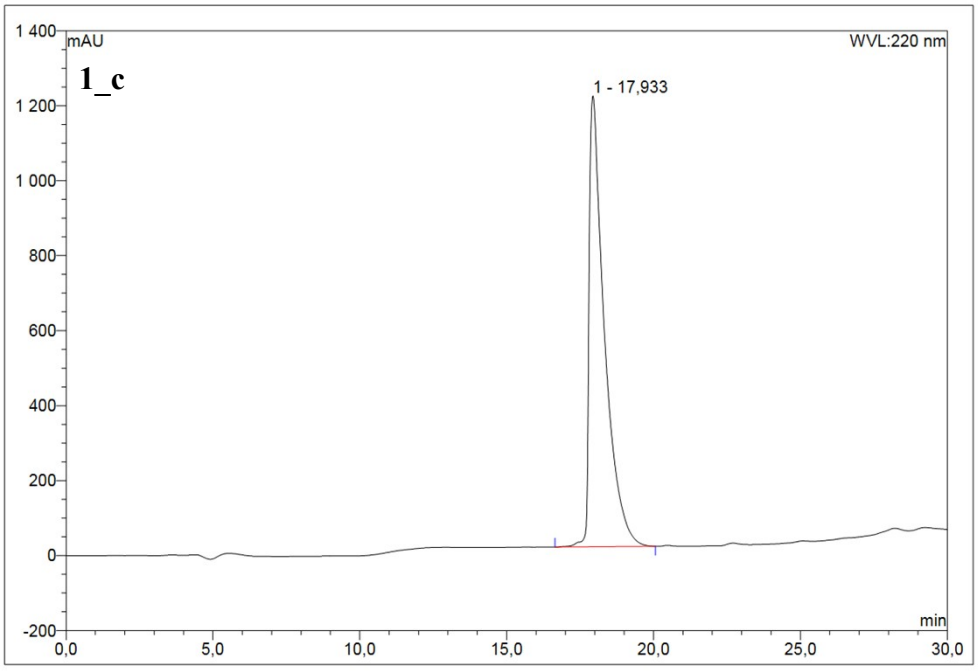
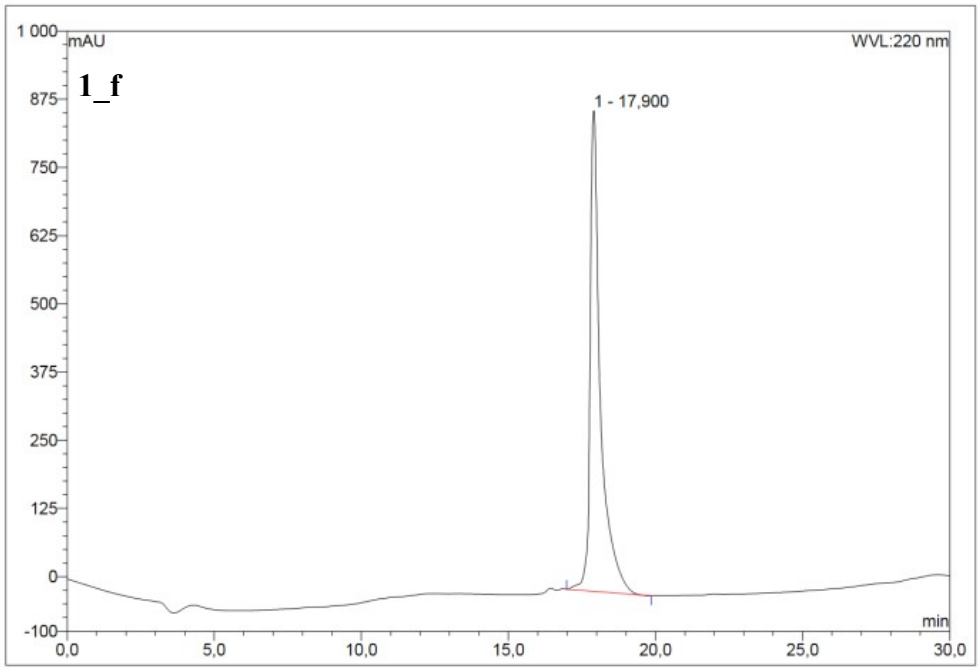


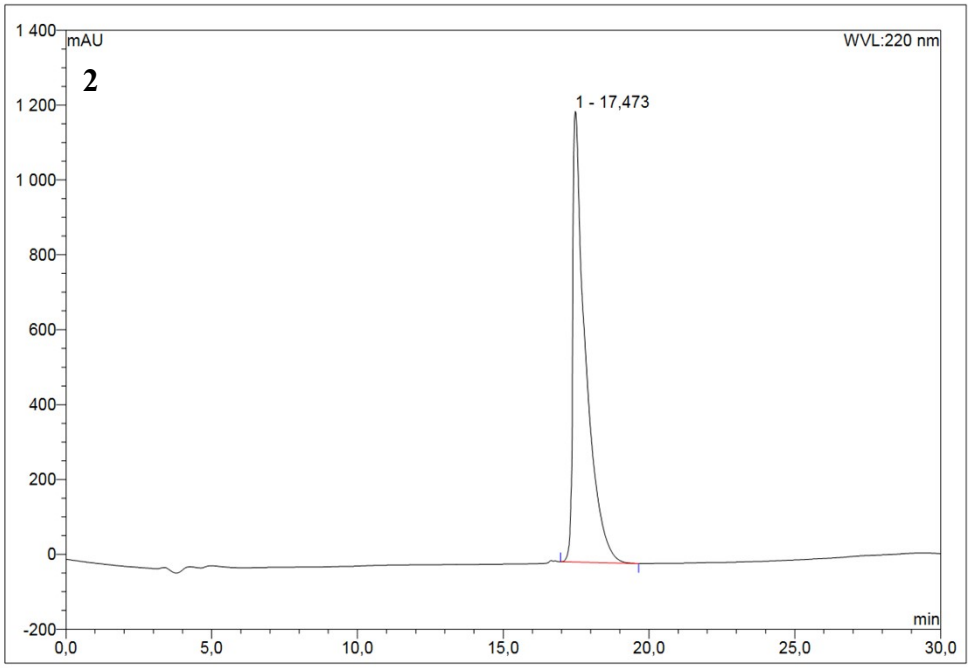
Fig. S2 Mass spectra of the studied peptides.

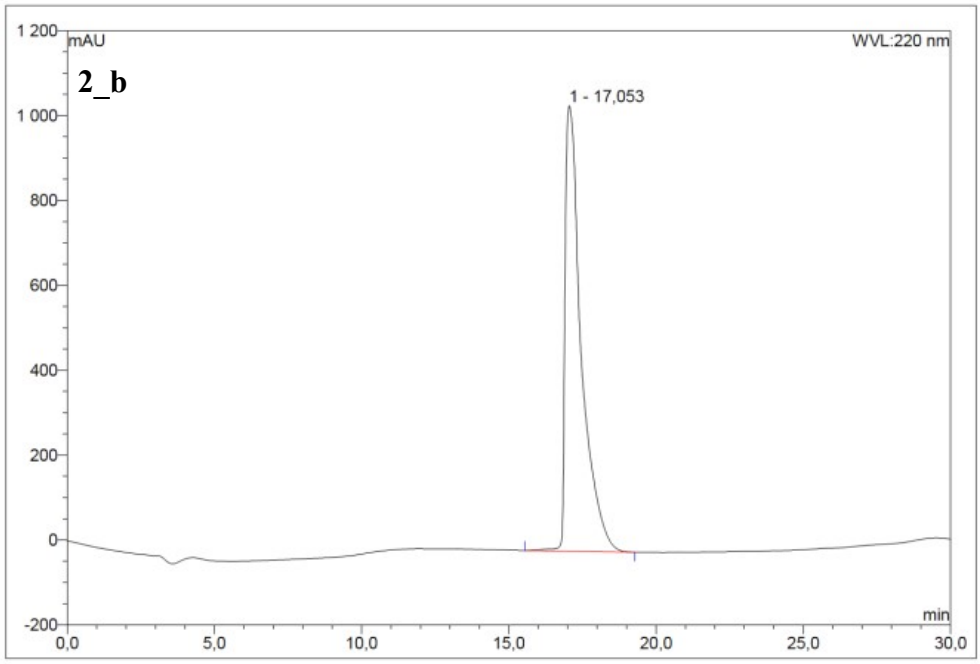

1
mAU
WVL:220 nm
1 - 16,467
1 200
1 000
800
600
400
200
0
-200
min
0,0
5,0
10,0
15,0
20,0
25,0
30,0


1_b
mAU
WVL:220 nm
1 - 16,773
1 200
1 000
800
600
400
200
0
-200
min
0,0
5,0
10,0
15,0
20,0
25,0
30,0


1_c
mAU
WVL:220 nm
1 - 17,933
1 400
1 200
1 000
800
600
400
200
0
-200
min
0,0
5,0
10,0
15,0
20,0
25,0
30,0


1_f
mAU
WVL:220 nm
1 - 17,900
1 000
875
750
625
500
375
250
125
0
-100
min
0,0
5,0
10,0
15,0
20,0
25,0
30,0


2
mAU
WVL:220 nm
1 - 17,473
1 400
1 200
1 000
800
600
400
200
0
-200
min
0,0
5,0
10,0
15,0
20,0
25,0
30,0


2_b
mAU
WVL:220 nm
1 - 17,053
1 200
1 000
800
600
400
200
0
-200
min
0,0
5,0
10,0
15,0
20,0
25,0
30,0

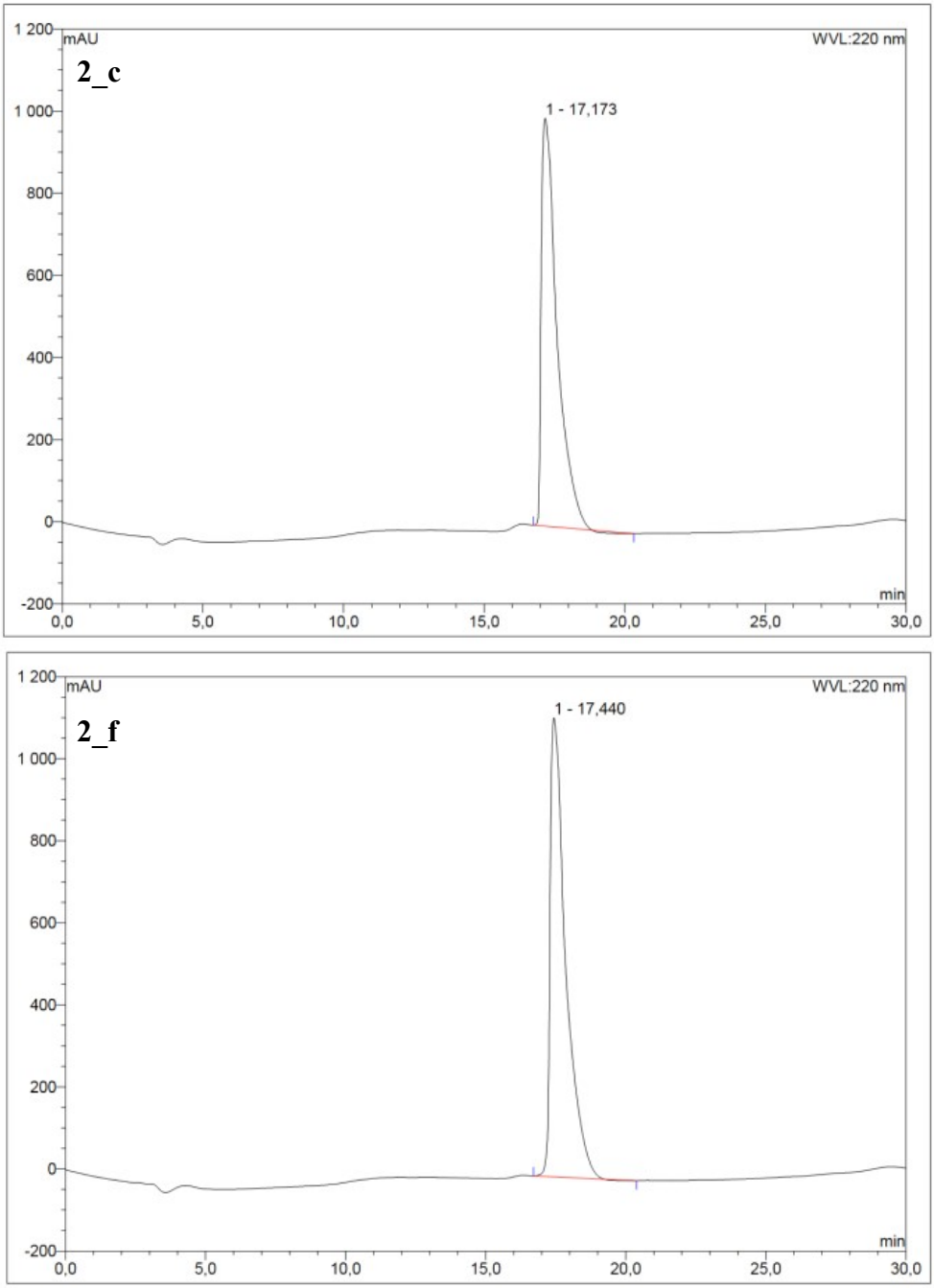


Fig. S3 Analytical HPLC chromatograms of the studied peptides.

Mean residue ellipticity was calculated using the equation (S4):

$$\theta = \frac{M\theta_{MRE}}{10cln} \qquad \text{(S4)}$$

θ = mean residue ellipticity

θ_{MRE} = ellipticity

c = concentration

l = path length

n = number of residues

$[\theta] = [\text{deg} \times \text{cm}^2 \times \text{dmol}^{-1}]$

Eqn. S4. Mean residue ellipticity calculation.

For each thermal denaturation experiment, the data were fit to a two-state folding model adapted and described by Kreitler *et al.* [I] using OriginPro 9.0.We used equation (S7):

$$\theta = \frac{1}{1 + e^{\frac{-\Delta H(1 - \frac{T}{T_m})}{RT}}} \left(b_f - b_u - m_u T + m_f T\right) + b_u + m_u T \qquad \text{(S7)}$$

where:

θ = measured ellipticity

b_f = y-intercept of folded baseline

b_u = y-intercept of unfolded baseline

m_f = slope of folded baseline

m_u = slope of unfolded baseline

T = temperature

T_m= melting temperature

ΔH = enthalpy of folding

R = ideal gas constant

Eqn. S5. The thermal denaturation experiment data fitting.

[I] D. F. Kreitler, *et al*. Effects of Single α-to-β Residue Replacements on Structure and Stability in a Small Protein: Insights from Quasiracemic Crystallization. *J. Am. Chem. Soc.* **2016**, *138*, 6498–6505.

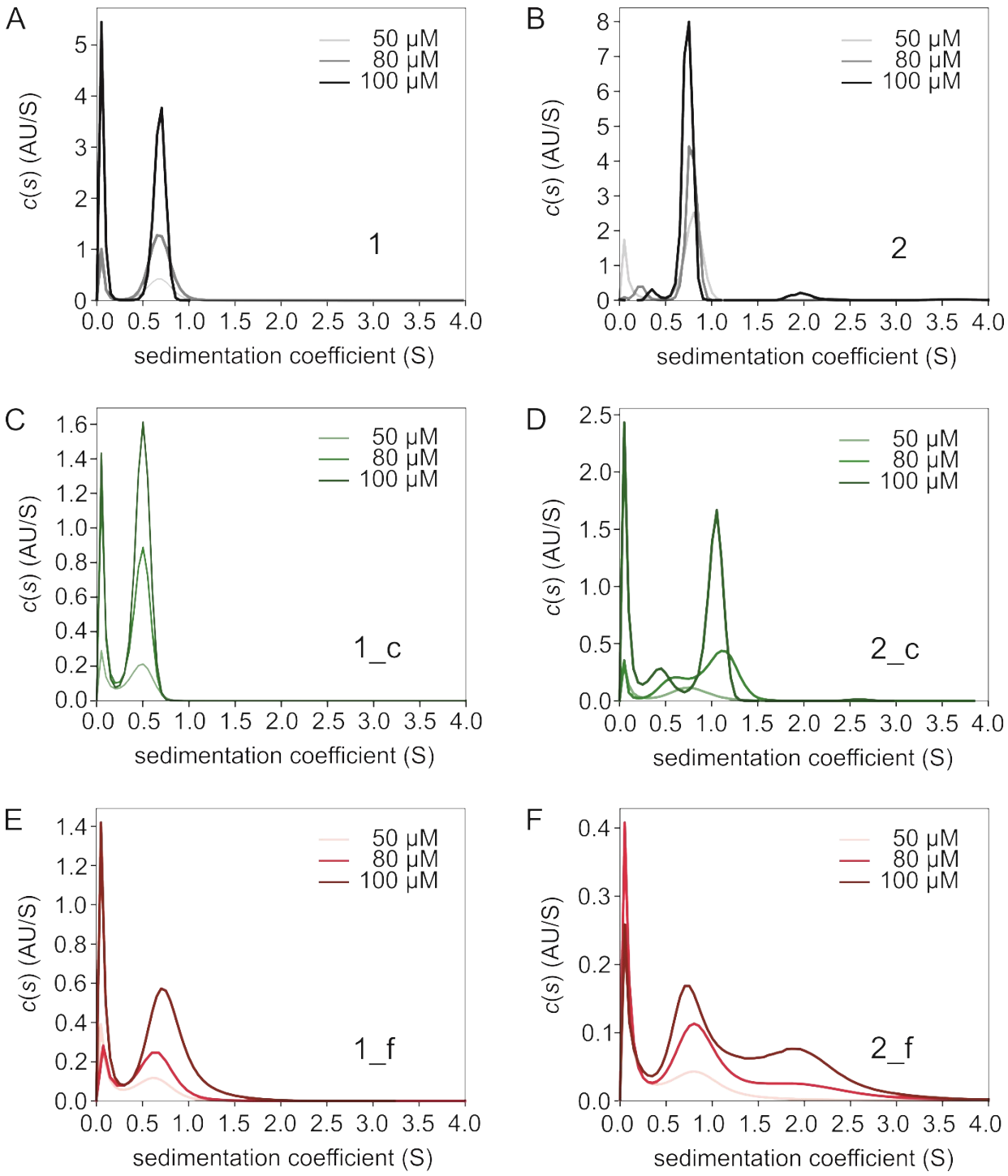


Fig. S6 Sedimentation coefficient distributions c(s) obtained for different concentrations of the studied peptides resuspended in water. Centrifugation was performed at 50 000 rpm and 20 °C.

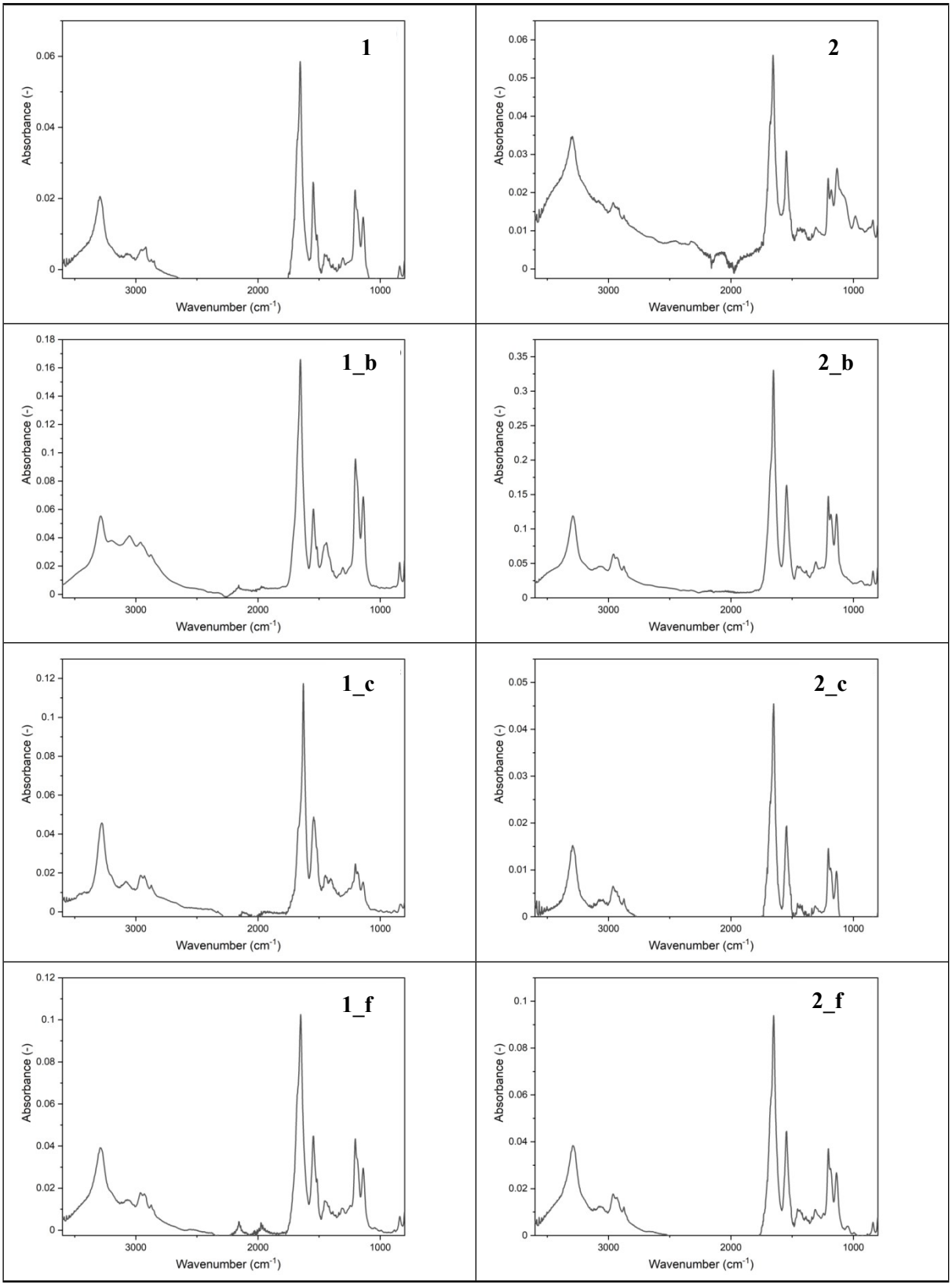
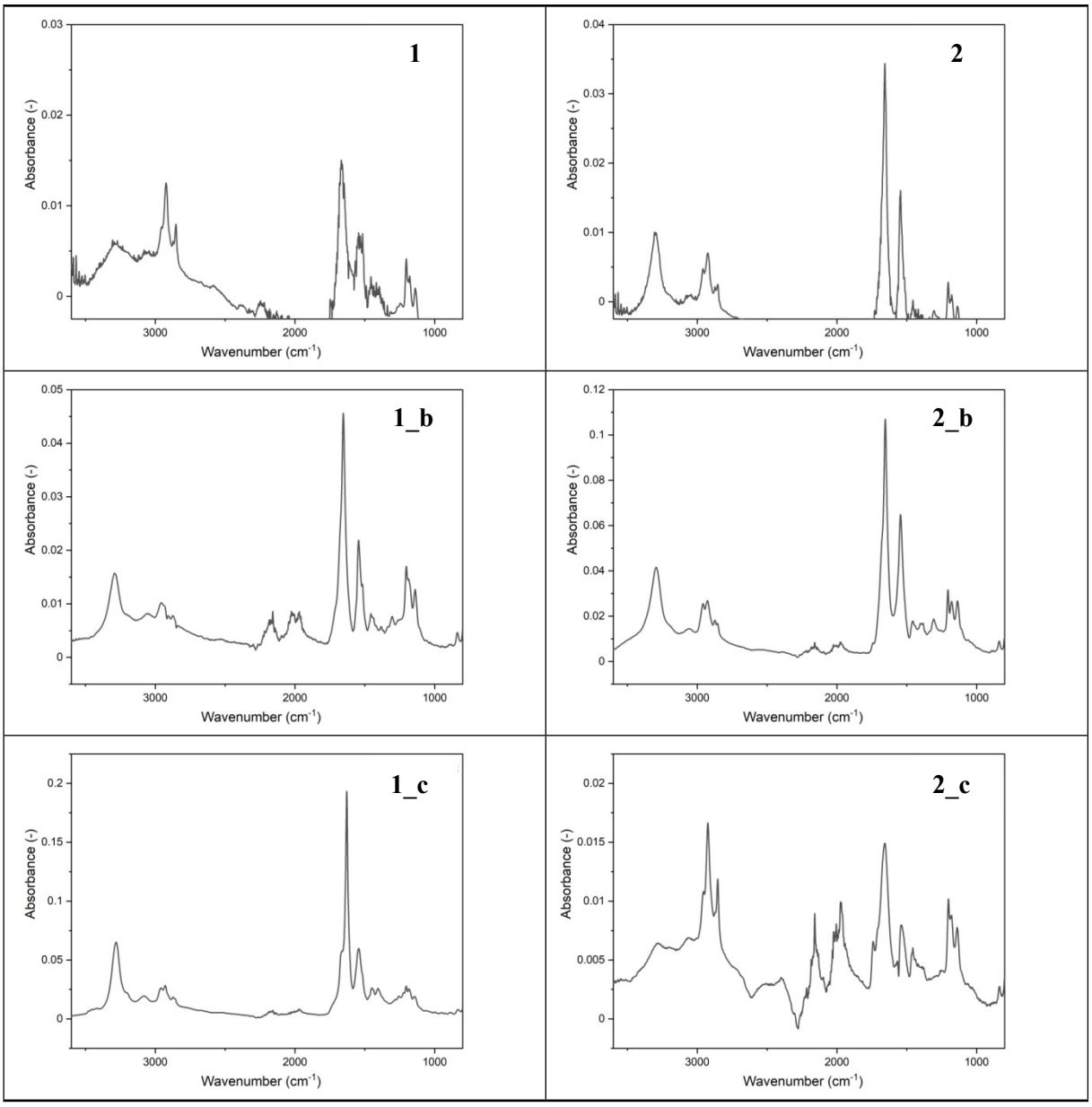

1
2
1_b
2_b
1_c
2_c
1_f
2_f
Absorbance (-)
Wavenumber (cm-1)

Fig. S7 Raw ATR-FTIR spectra of the studied peptides in the range of 3600-800 cm^{-1} on the day of dissolving. C_{pep} = 320 μM.



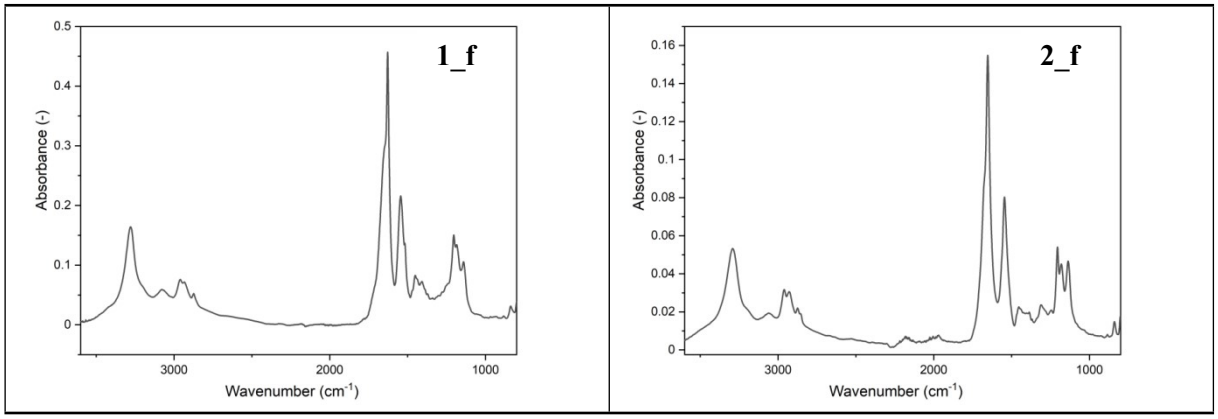
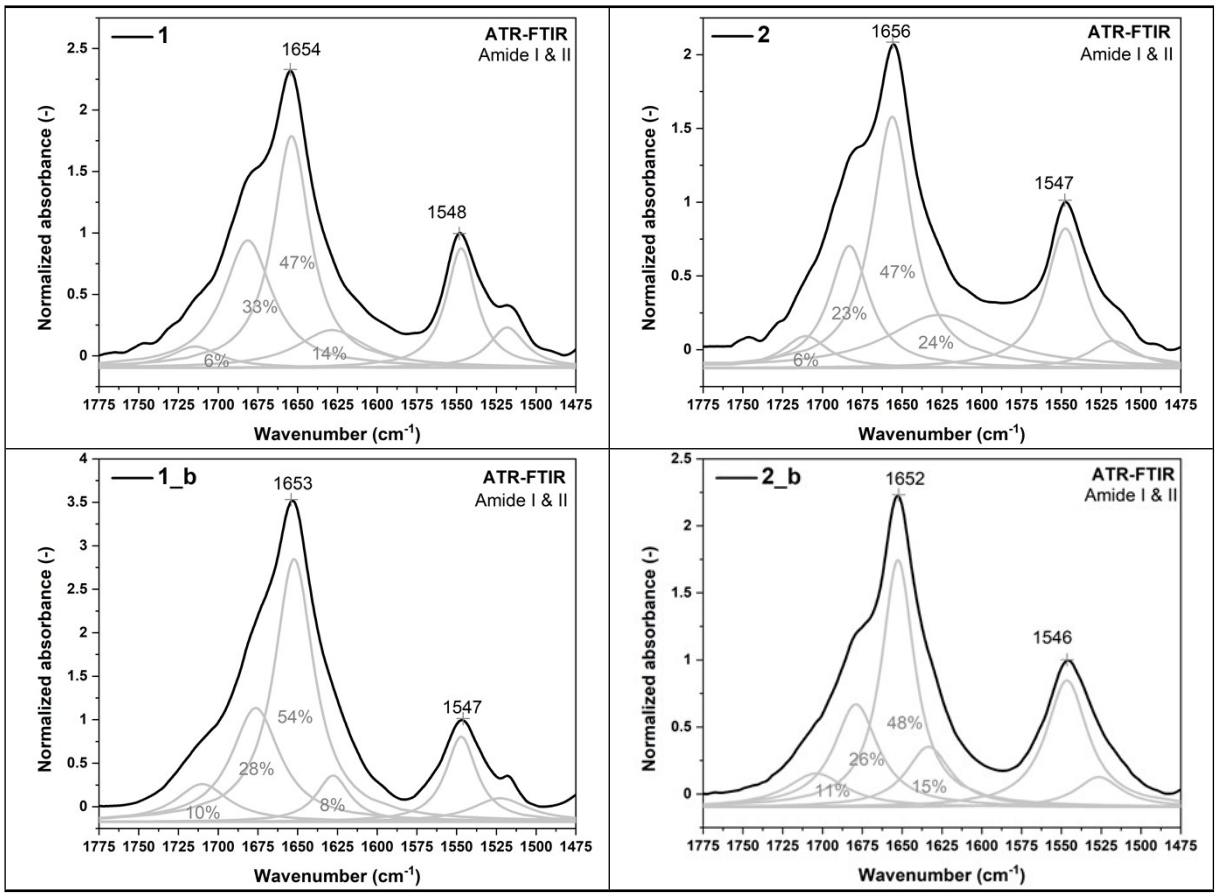


Fig. S8 Raw ATR-FTIR spectra of the studied peptides in the range of 3600-800 cm^{-1} after 30 days of incubation at 37 °C. C_{pep} = 320 μM.



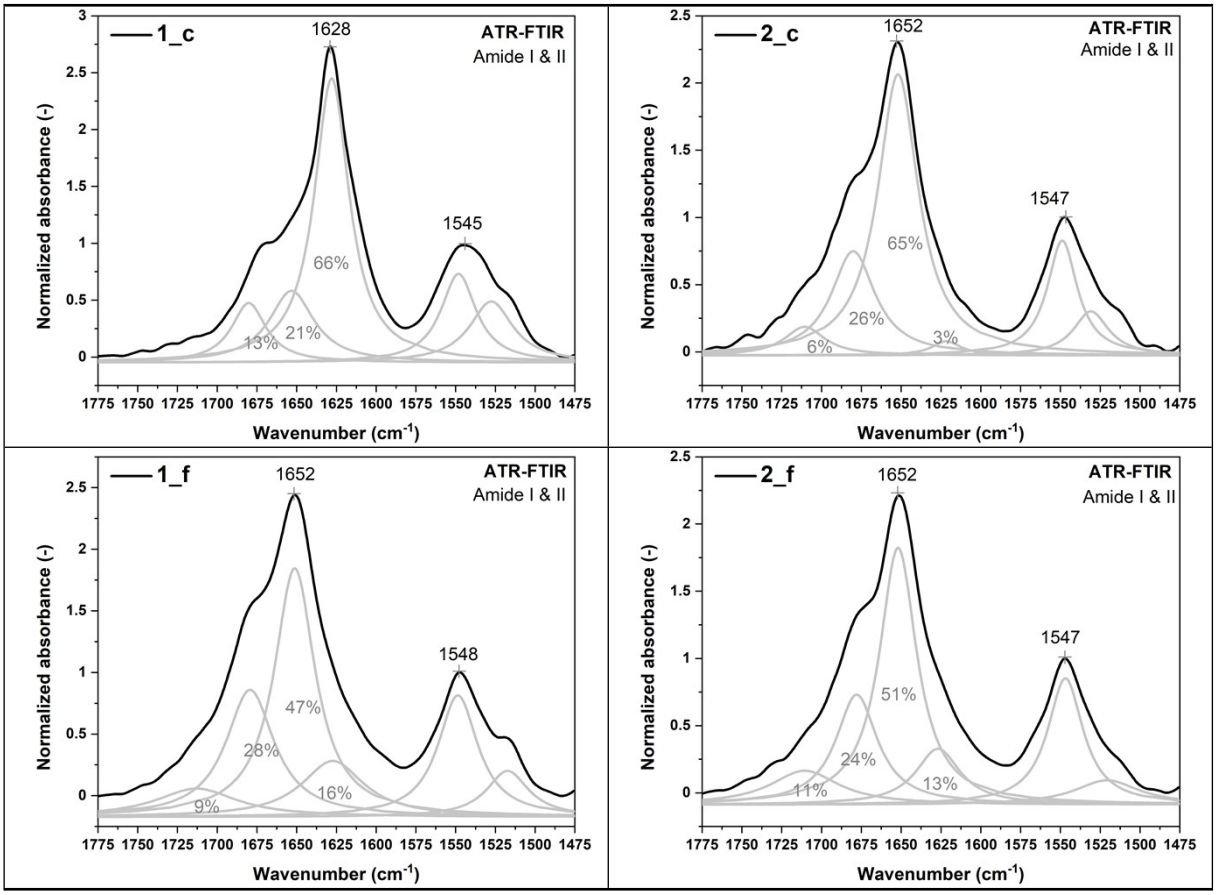
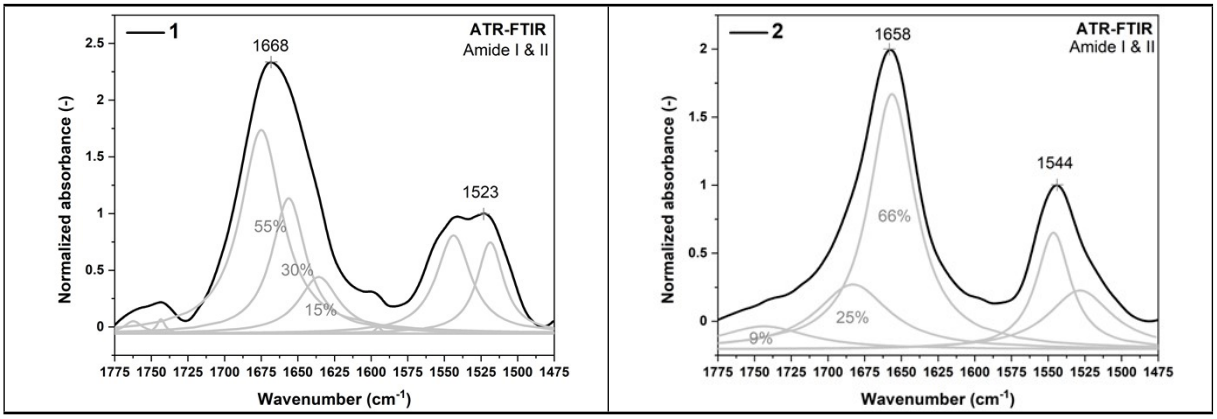


Fig. S9 Normalized ATR-FTIR spectra of air-dried films of studied peptides registered on the day of dissolving, with sub-bands obtained from the curve fitting procedure in the amide bands region (1775-1475 cm^{-1}). C_{pep} = 320 μM.



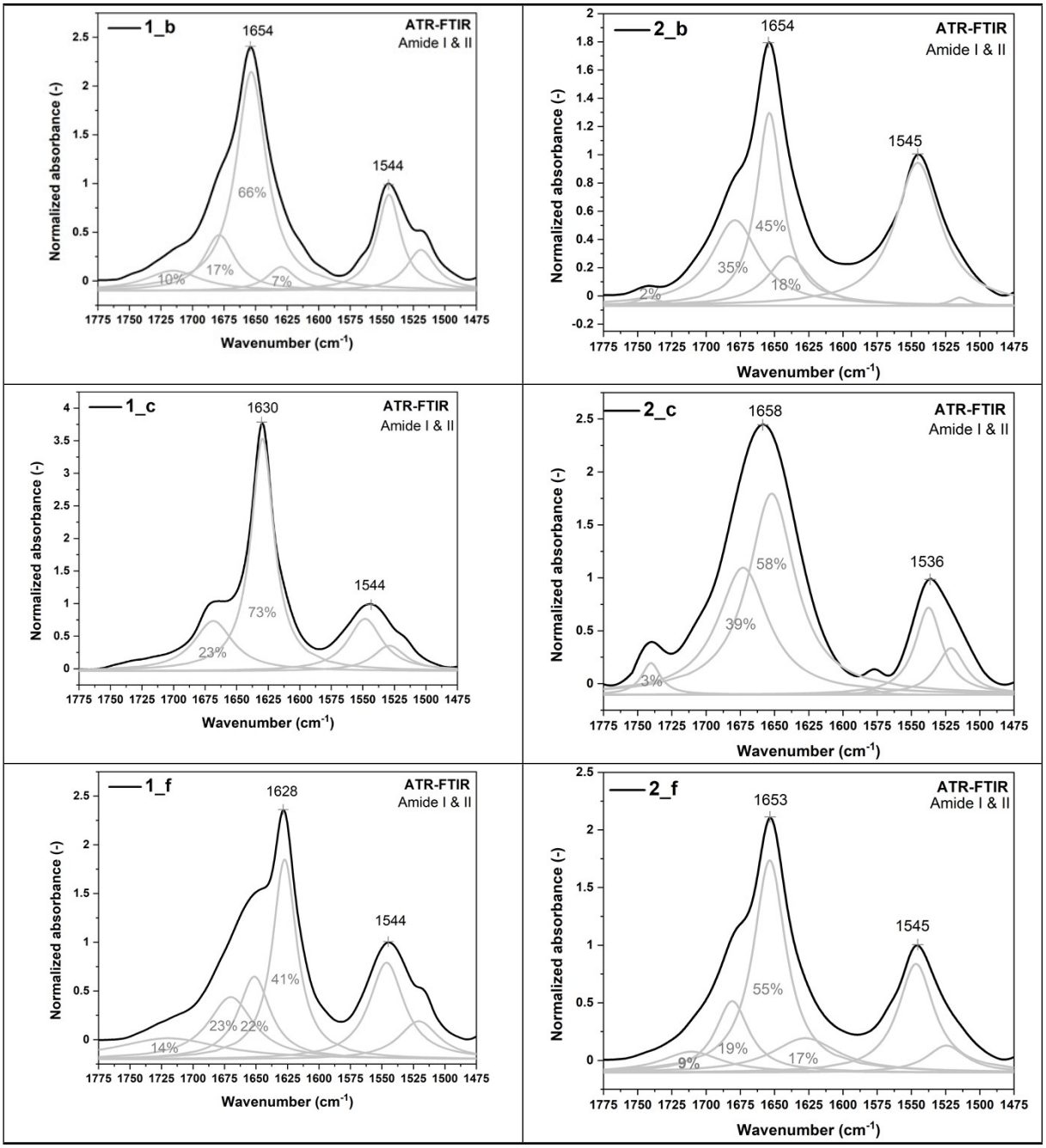


Fig. S10 Normalized ATR-FTIR spectra of air-dried films of studied peptides registered after 30 days of incubation at 37 °C, with sub-bands obtained from the curve fitting procedure in the amide bands region (1775-1475 cm^{-1}). C_{pep} = 320 μM.

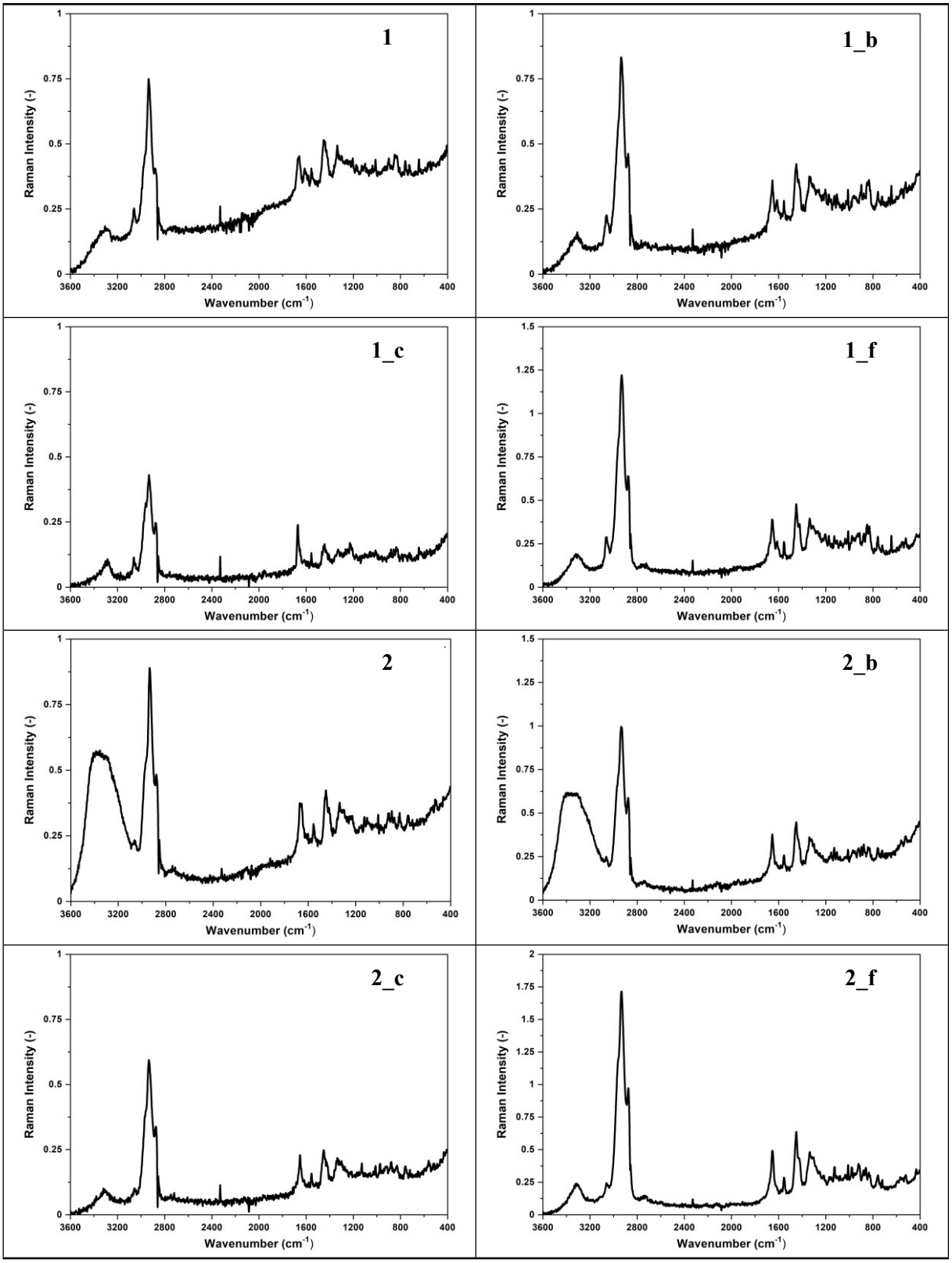


Fig. S11 Raw FT-Raman spectra of the studied peptides in the range of 3600-400 cm^{-1}. C_{pep} = 320 μM.

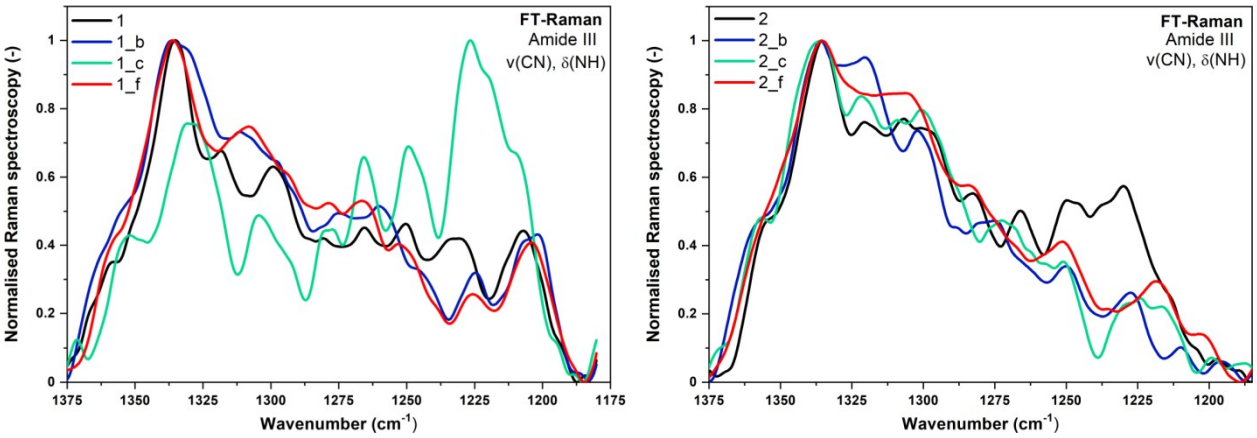


Fig. S12 Normalized FT-Raman spectra of the studied peptides smoothed with SG 35 (see Methods), in the wavenumber range of 1375–1175 cm^{-1} (Amide III). C_{pep} = 320 μM.

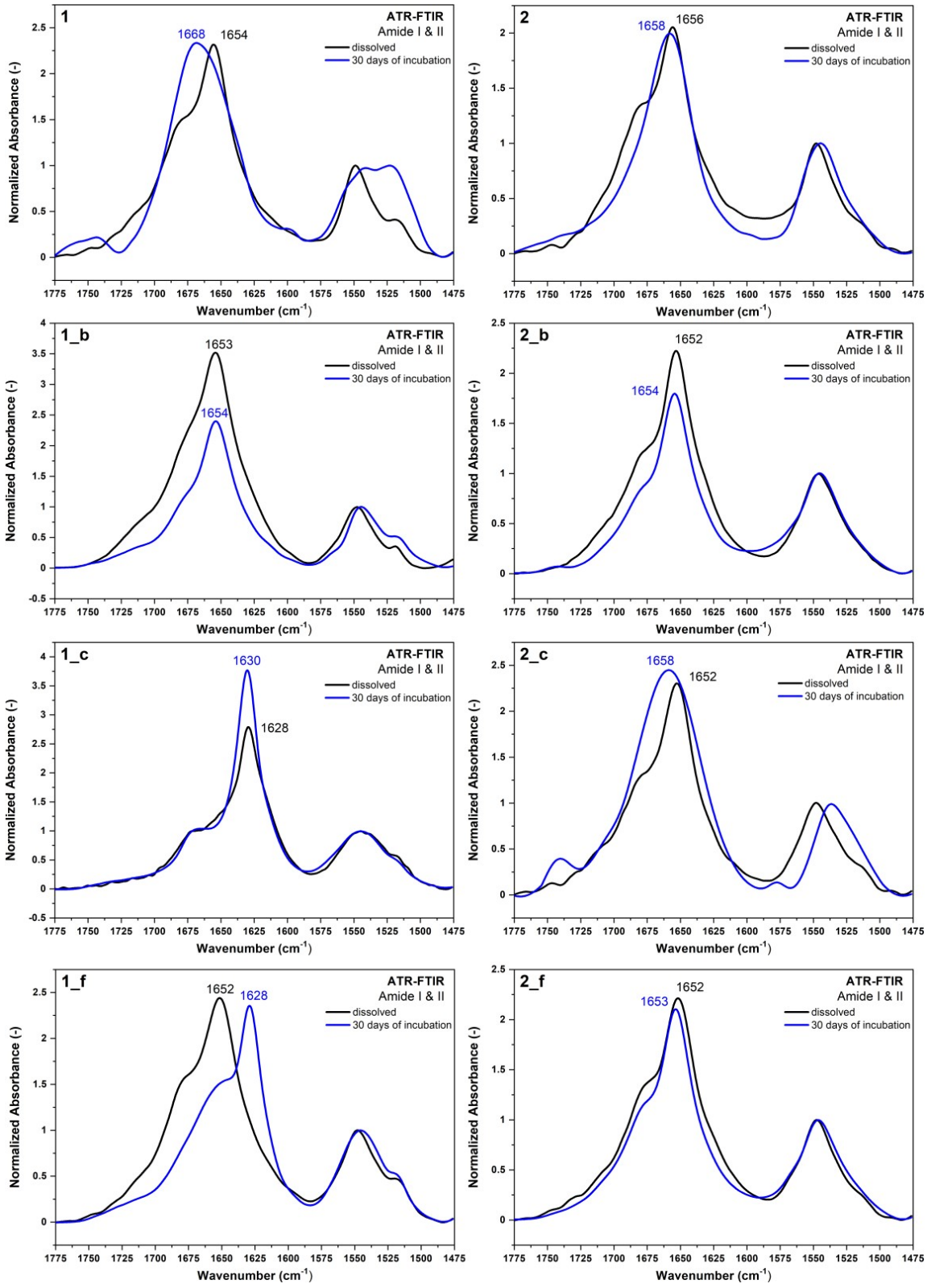


Fig. S13 The comparison of the normalized ATR-FTIR spectra after dissolving and after 30 days of incubation. C_{pep} = 320 μM.

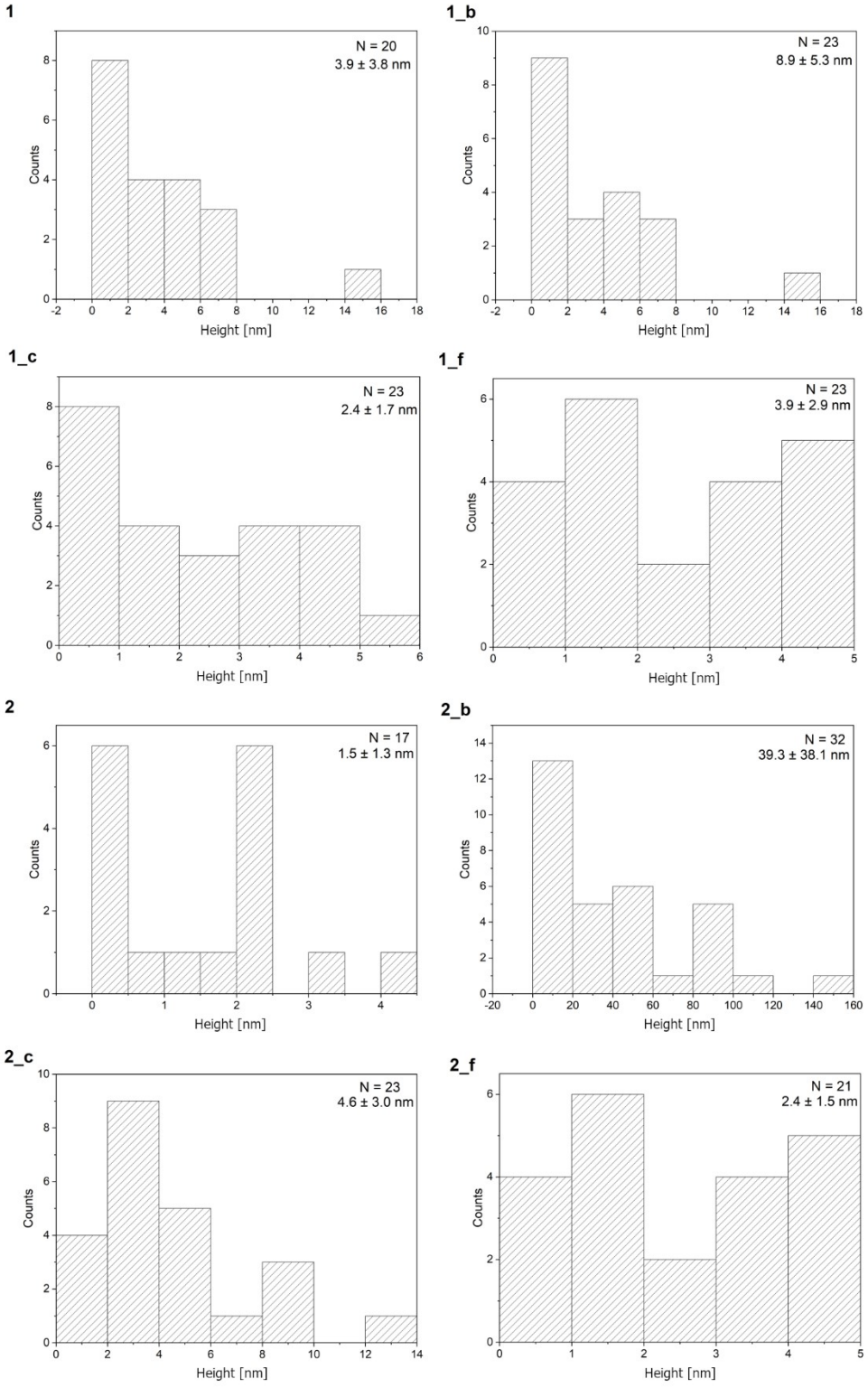


Fig. S14 Frequency distributions of the peptides height observed by AFM.

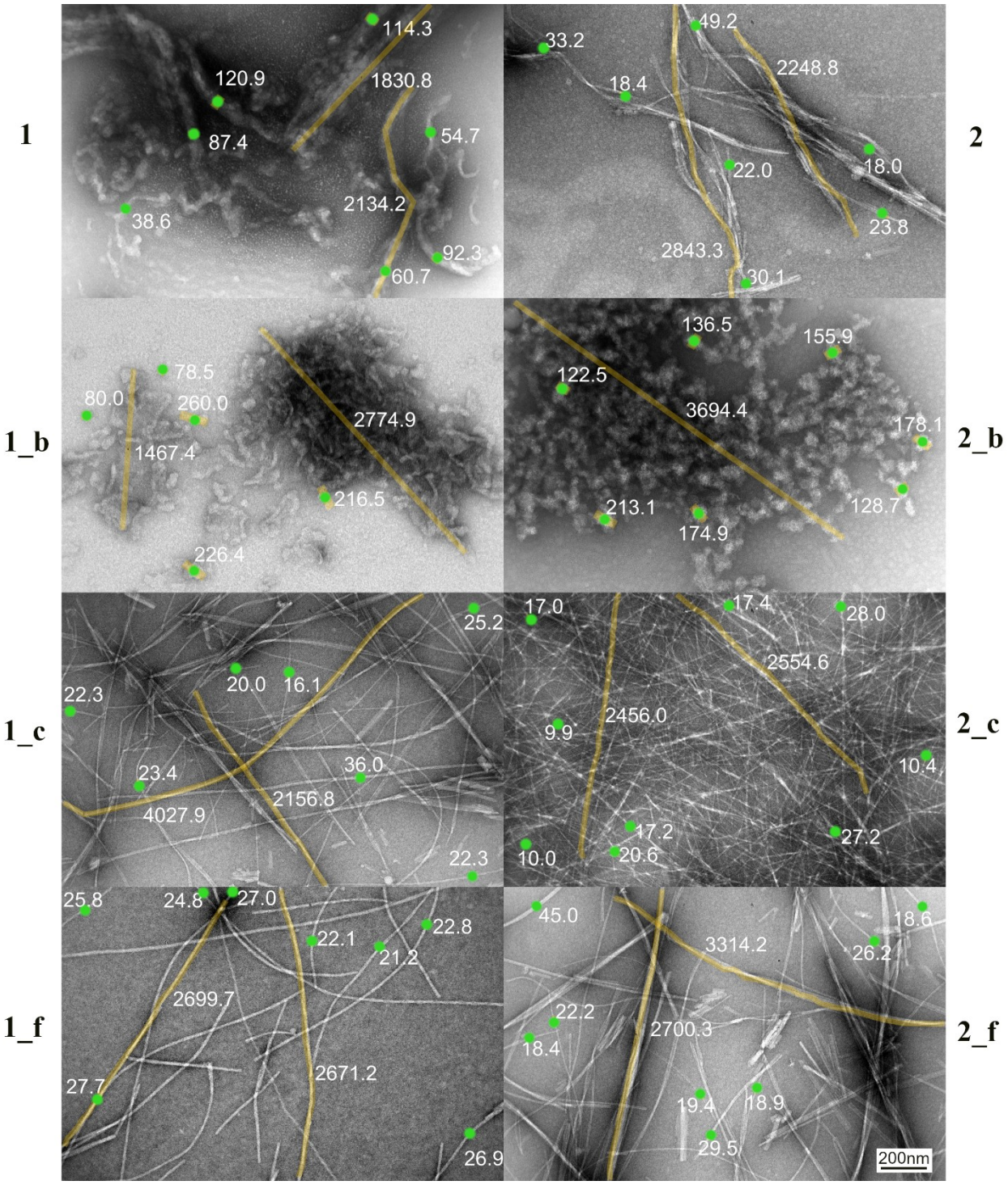
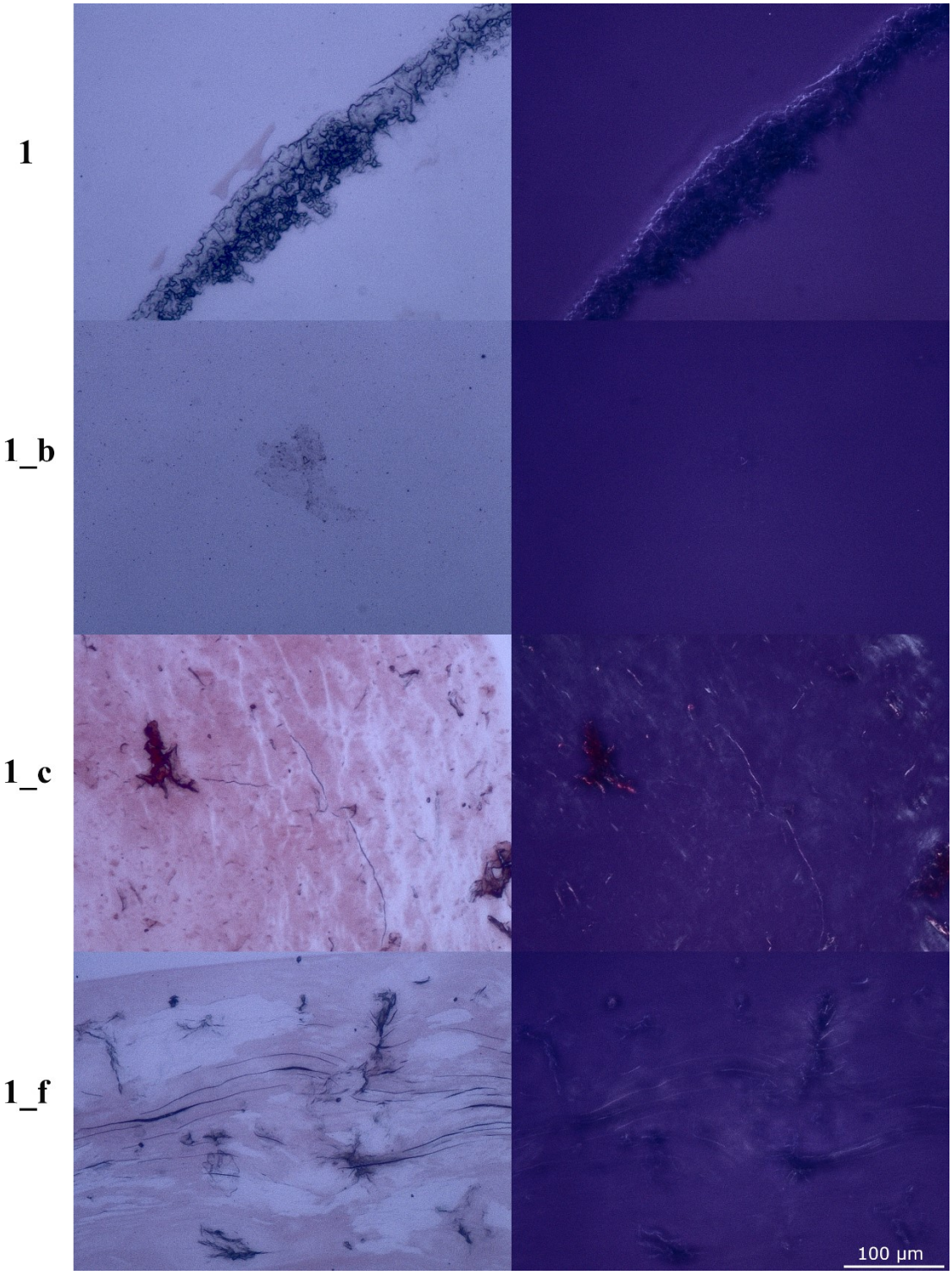


Fig. S15 Electron micrographs with size measurements (in nm) of the obtained nanostructures after 30 days of incubation at 37 °C (magnification of 20 000). The yellow marks correspond to the length dimension, whereas the green dots indicate specific points for measuring the diameter. $C_{pep.}$ = 160 μM.


1
1_b
1_c
1_f
100 µm

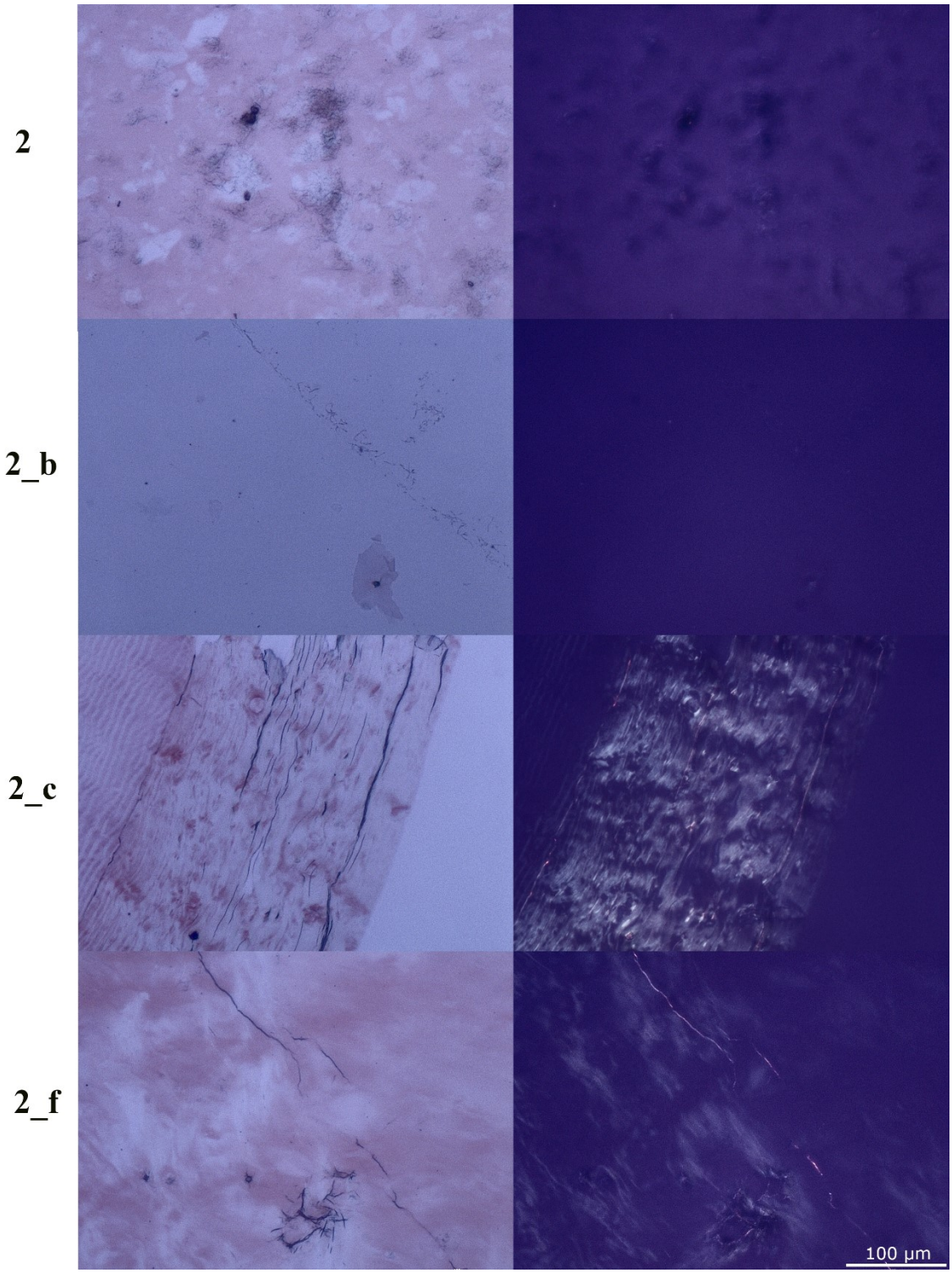


Fig. S16 The studied peptides stained with Congo red and examined under crossed polarized light (see right column). The original magnification x200.

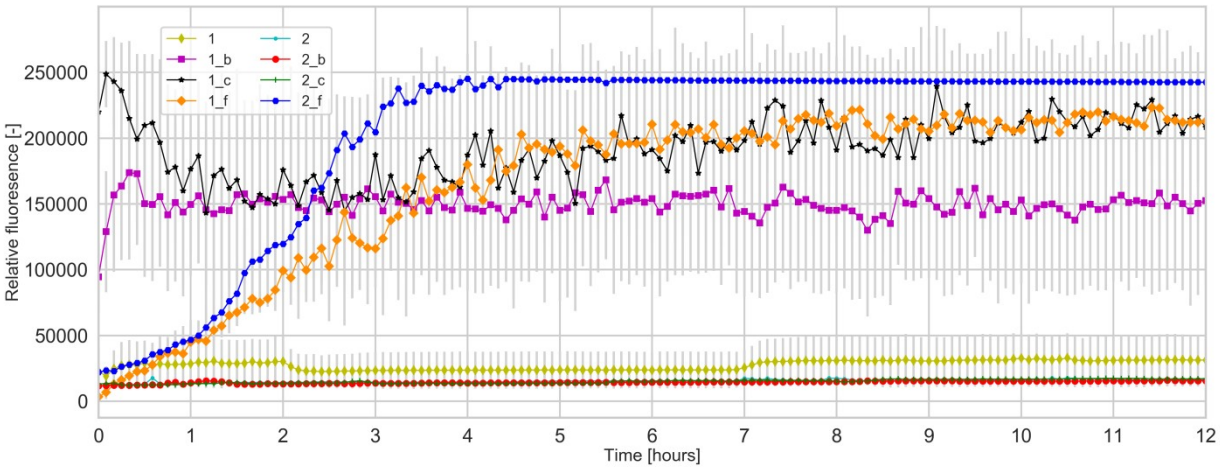


Fig. S17 Results of ThT fluorescence kinetic assay registered for the studied peptides.

Table S18 The mean values of relative fluorescence in the time range of 5 to 12 hours calculated for the studied peptides.

Sample	Relative fluorescence ± standard deviation [-]
1	29 018.83 ±17342.14
1_b	149 775.52 ± 62436.88
1_c	203 530.07 ± 62893.68
1_f	208 124.59 ± 51289.37
2	16 068.33 ± 1606.03
2_b	15 086.78 ± 844.59
2_c	16 152.38 ± 4755.09
2_f	243 411.35 ± 66.13