Supporting Information

Boosting stability: a hierarchical approach for self-assembling peptide structures

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Table S1. Sequences and formulas of the synthesized peptides.

Peptide	Sequence	Formula
Di 1	*gabcdef* H-KIAALKQKIAALKKEIAWLEAENAALEQ-NH_2	$C_{141}H_{240}N_{38}O_{39}$
Di 2	H-KIAALKQKNAALKKEIAWLEAEIAALEQ-NH_2	$C_{141}H_{240}N_{38}O_{39}$
Di 1X	H-KIAALKXKIAALKXEIAWLEXENAALEX-NH_2	$C_{146}H_{243}N_{35}O_{37}$
Di 2X	H-KIAALKXKNAALKXEIAWLEXEIAALEX-NH_2	$C_{146}H_{243}N_{35}O_{37}$
CAM 1	H-KIAALKQKIASLKQEIDALEYENDALEQ-NH2	$C_{140}H_{235}N_{37}O_{46}$
CAM 2	H-KIRALKAKNAHLKQEIAALEQEIAALEQ-NH_2	$C_{138}H_{240}N_{42}O_{40}$
CAM 1X	H-KIAALKXKIASLKXEIDALEXENDALEX-NH_2	$C_{140}H_{238}N_{34}O_{42}$
CAM 2X	H-KIRALKXKNAHLKXEIAALEXEIAALEX-NH_2	$C_{144}H_{247}N_{39}O_{37}$

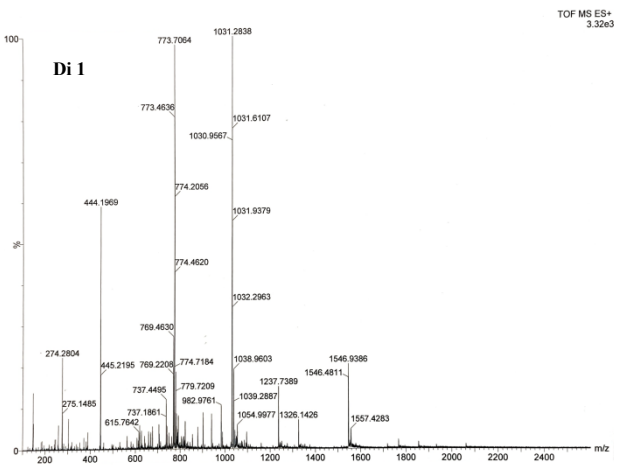
Table S2. Analytical data for the synthesized peptides.

Peptide	Calculated M/z	Experimental M/z	Analytical HPLC t_r [min]
Di 1	[(M+3H)/3] 1031.2743 [(M+4H)/4] 773.7077	[(M+3H)/3] 1031.2838 [(M+4H)/4] 773.7064	16.200[1]
Di 2	[(M+3H)/3] 1031.2743 [(M+4H)/4] 773.7077	[(M+3H)/3] 1031.2859 [(M+4H)/4] 773.7079	16.733[1]
Di 1X	[(M+2H)/2] 1540.9198 [(M+3H)/3] 1027.6158 [(M+4H)/4] 770.9638	[(M+2H)/2] 1540.9132 [(M+3H)/3] 1027.6144 [(M+4H)/4] 770.9599	18.233[1]
Di 2X	[(M+2H)/2] 1540.9198 [(M+3H)/3] 1027.6158 [(M+4H)/4] 770.9638	[(M+2H)/2] 1540.9789 [(M+3H)/3] 1027.6158 [(M+4H)/4] 770.9665	11.319[2]
CAM 1	[(M+3H)/3] 1058.2484 [(M+4H)/4] 793.9382	[(M+3H)/3] 1058.2490 [(M+4H)/4] 793.9403	9.453[2]
CAM 2	[(M+3H)/3] 1043.2767 [(M+4H)/4] 782.9601	[(M+3H)/3] 1043.2759 [(M+4H)/4] 782.9429	9.448[2]
CAM 1X	[(M+4H)/4] 768.1969 [(M+5H)/5] 614.7591	[(M+4H)/4] 768.0991 [(M+5H)/5] 614.6814	9.680[2]
CAM 2X	[(M+4H)/4] 779.9747 [(M+5H)/5] 624.1813	[(M+4H)/4] 779.8937 [(M+5H)/5] 624.0977	10.311[2]

[1]Analytical HPLC was performed using Waters C18 1.7 µm 50 × 2.1 mm column. Program (eluent A: 0.05% TFA in H_2O, eluent B: 0.05% TFA in acetonitrile, flow 0.5 mL/min): A: t = 0 min, 90% A; t = 30 min, 10% A.

[2]Analytical HPLC was performed using Kinetex 5 µm EVO C18 100 Å 150 × 4.6 mm column. Program (eluent A: 0.05% TFA in H_2O, eluent B: 0.05% TFA in acetonitrile, flow 0.5 mL/min): A: t = 0 min, 90% A; t = 25 min, 10% A.

A)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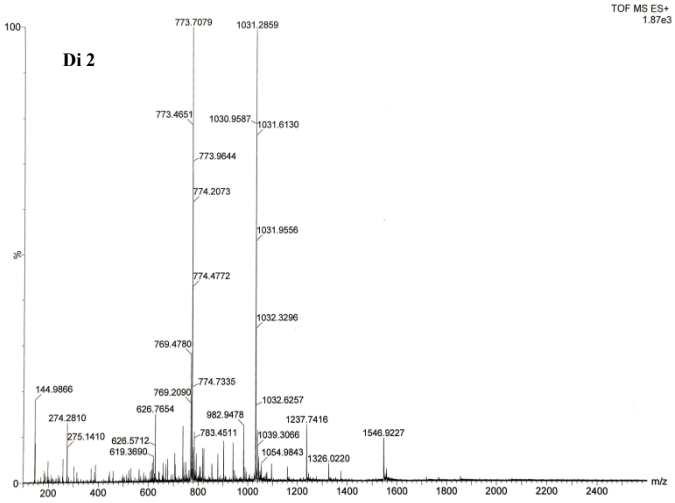
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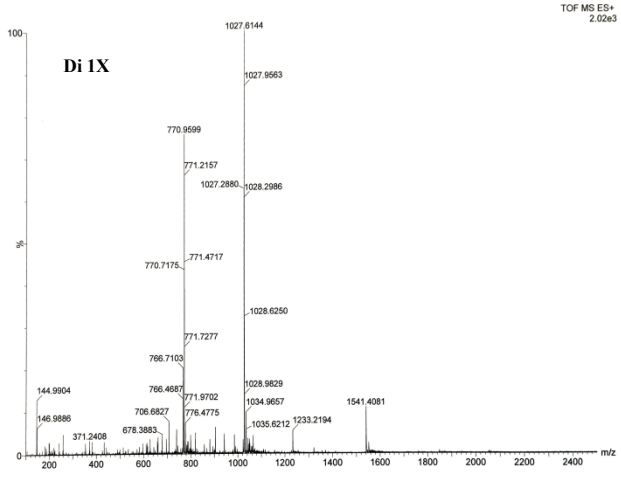
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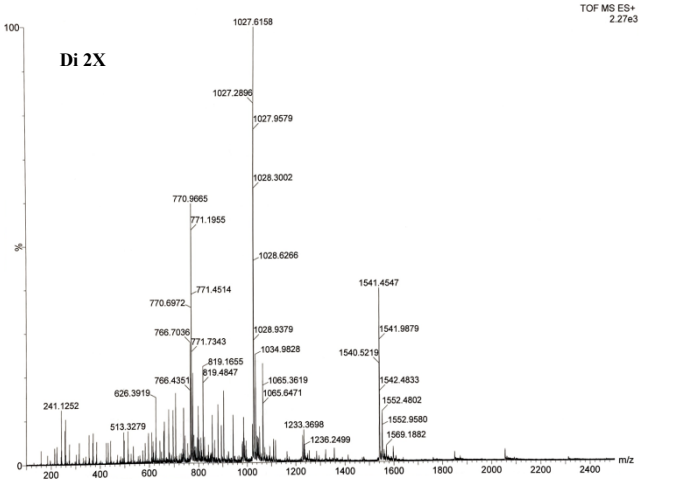
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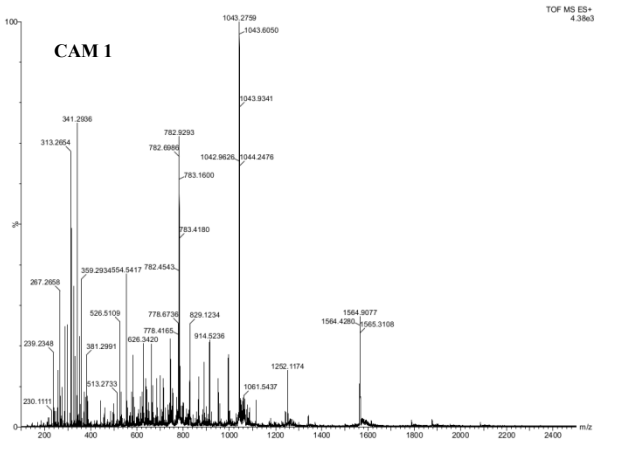
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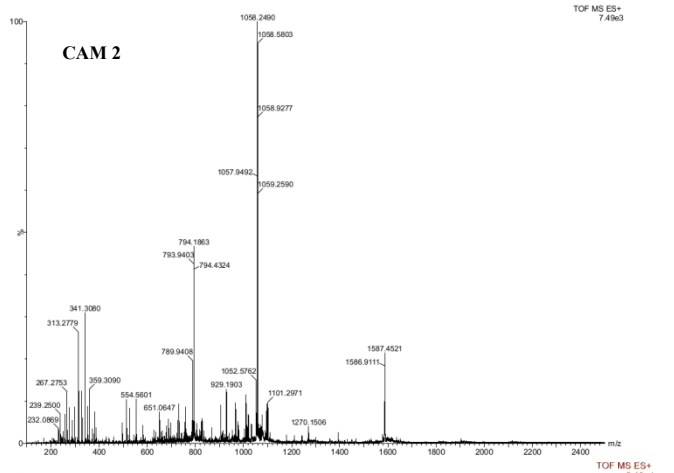
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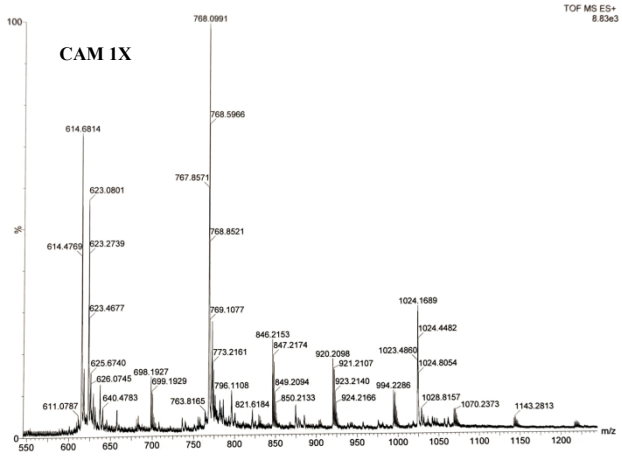
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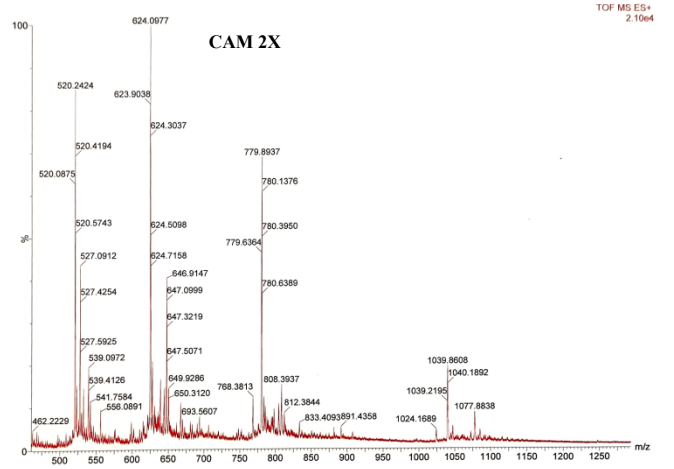
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B)

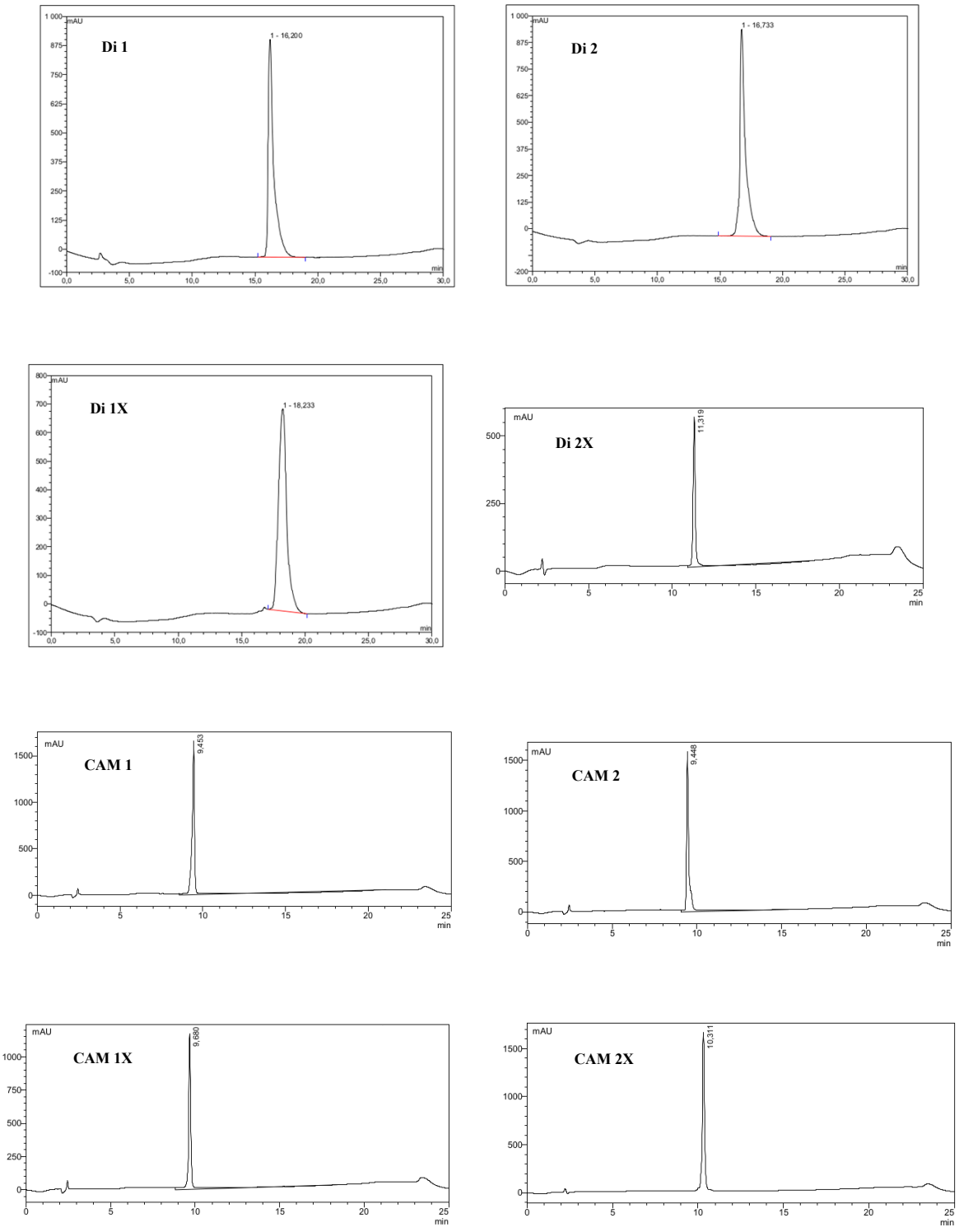

Figure S1. A) Mass spectra and B) analytical HPLC chromatograms of the studied peptides.

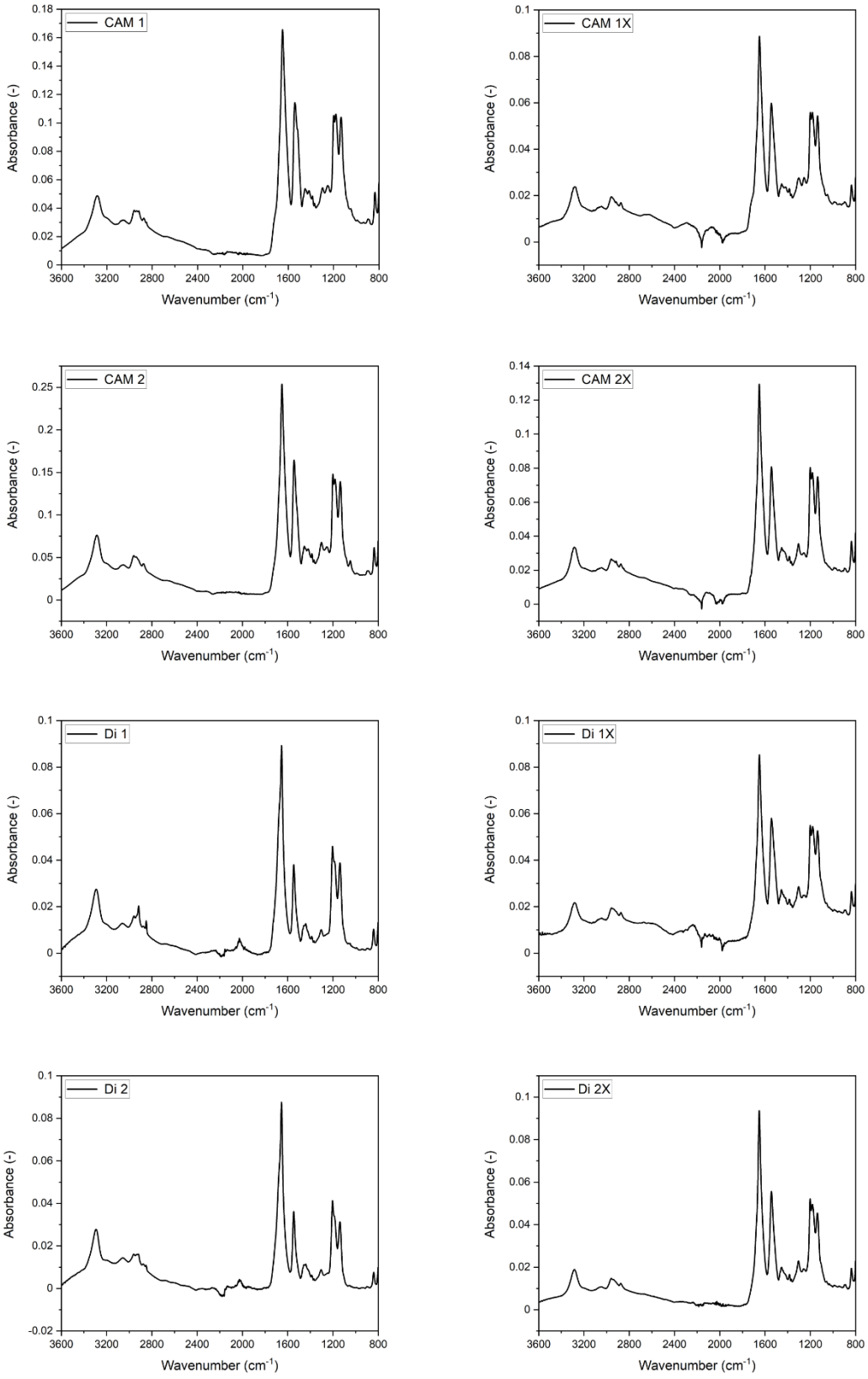

Figure S2. Powder ATR-FTIR spectra of the studied peptides in the range of 3600–800 cm^{-1}.

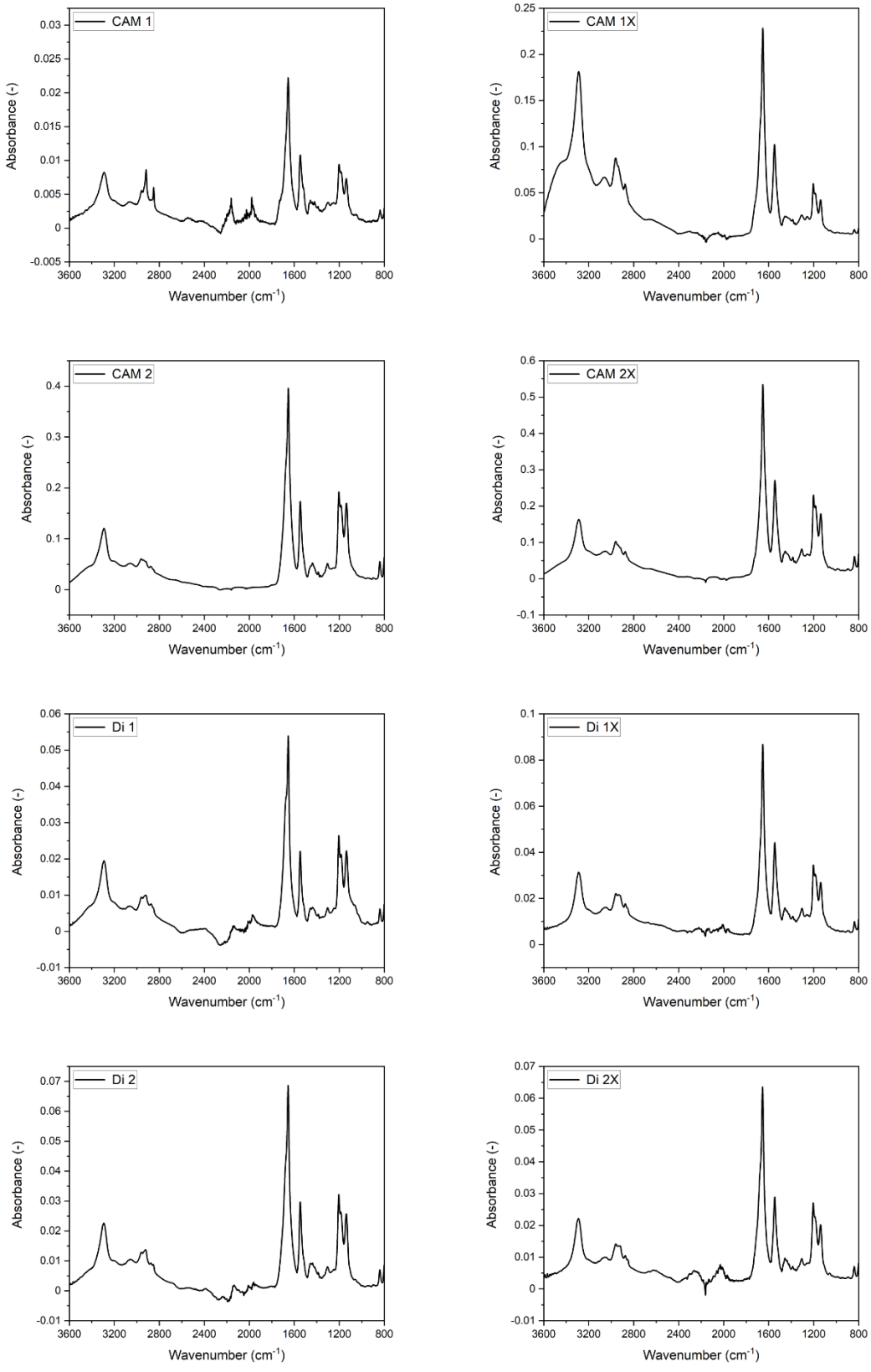

Figure S3. Raw ATR-FTIR spectra of the studied peptides in the range of 3600–800 cm^{-1} directly after dissolving. C_{pep}=1 mg/mL [0.317–0.327] mM.

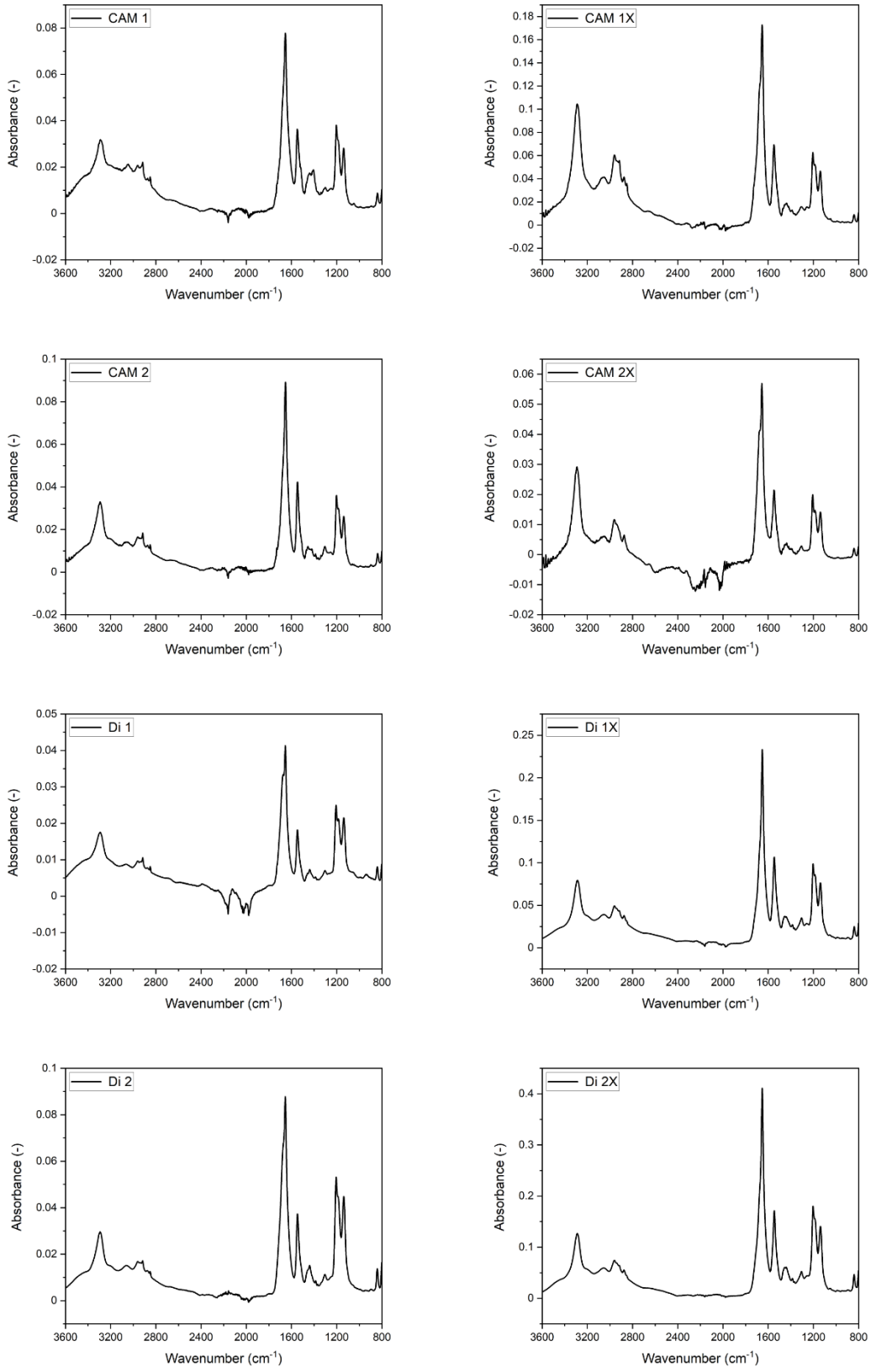

Figure S4. Raw ATR-FTIR spectra of the studied peptides in the range of 3600–800 cm^{-1} after 7 days of incubation at 37 ºC. C_{pep}=1 mg/mL [0.317–0.327] mM.

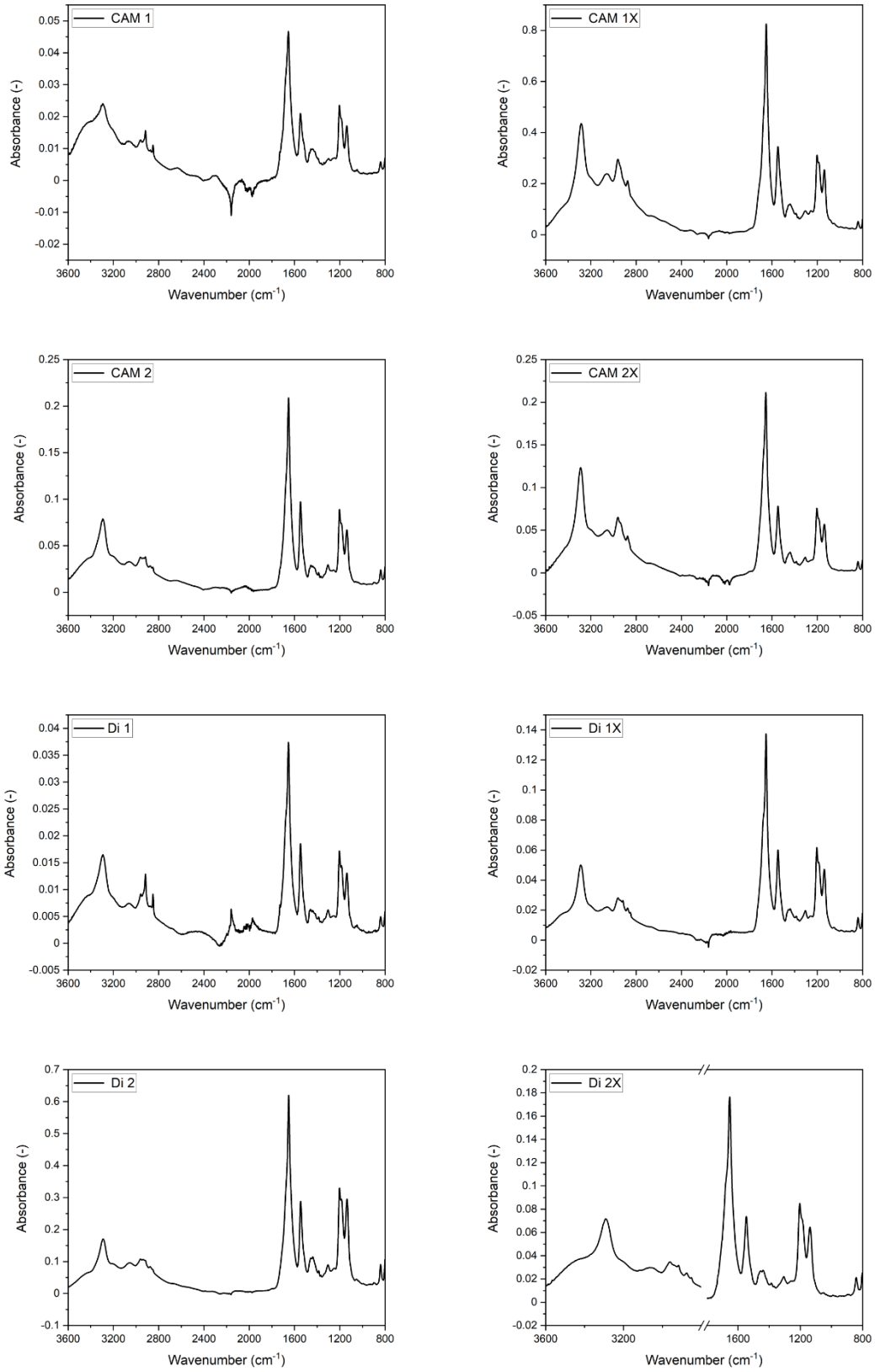

Figure S5. Raw ATR-FTIR spectra of the studied peptides in the range of 3600–800 cm^{-1} after 30 days of incubation at 37 ºC. C_{pep}=1 mg/mL [0.317–0.327] mM.

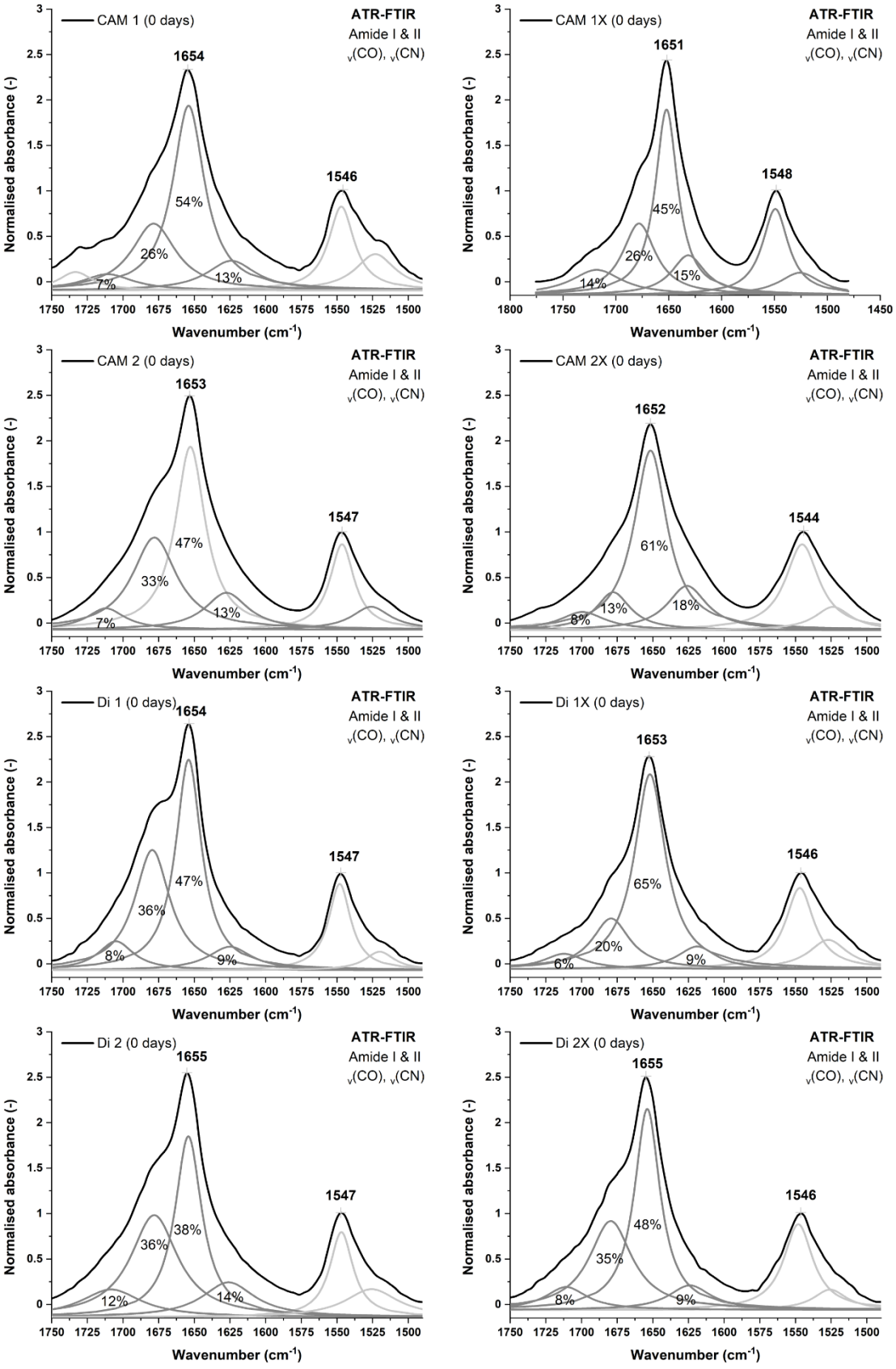

Figure S6. Normalized ATR-FTIR spectra of air-dried films of the studied peptides with sub-bands obtained from the curve fitting procedure in the amide bands region, directly after dissolving (1750–1490 cm^{-1}). C_{pep}=1 mg/mL [0.317–0.327] mM.

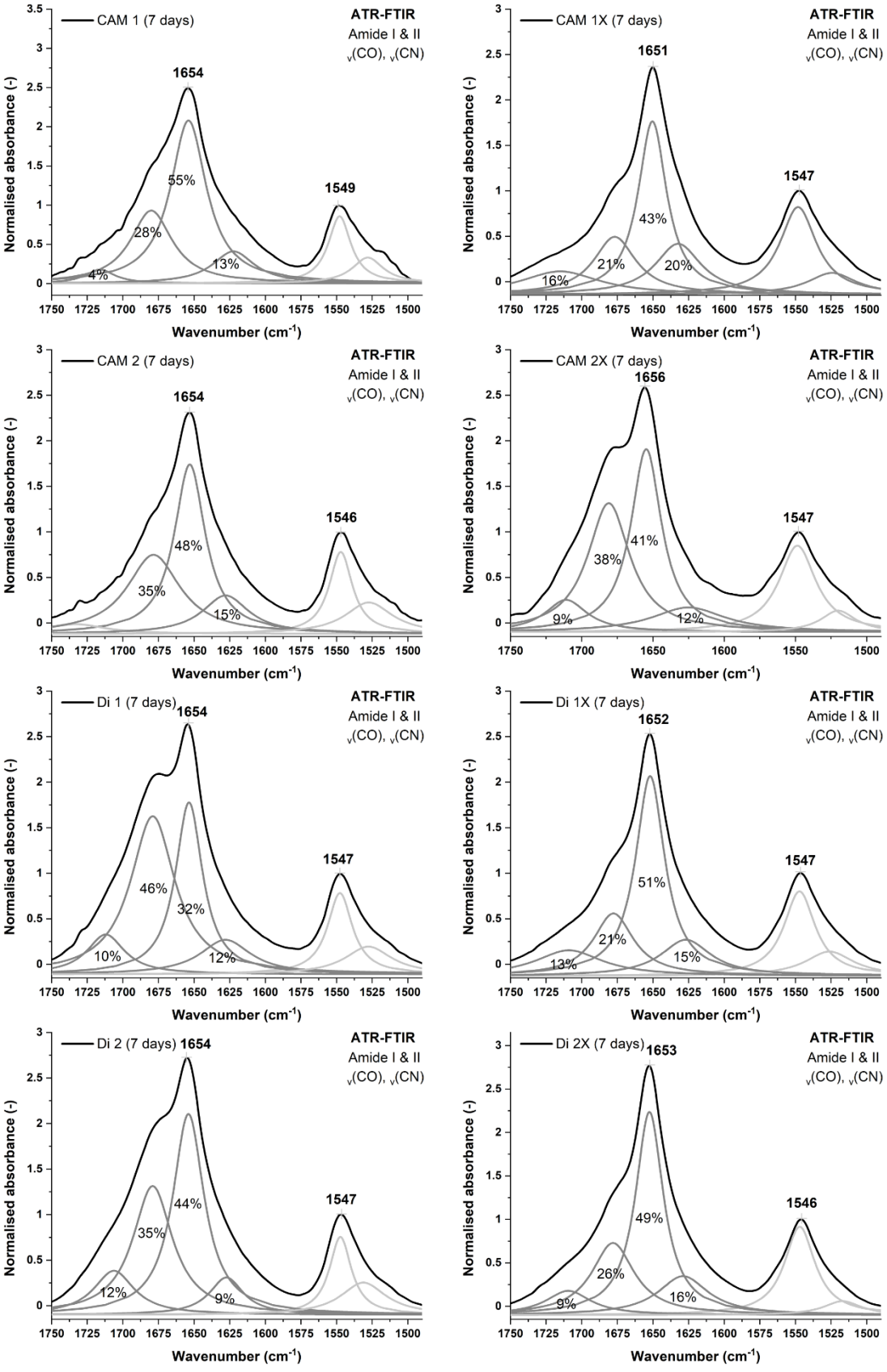

Figure S7. Normalized ATR-FTIR spectra of air-dried films of the studied peptides with sub-bands obtained from the curve fitting procedure in the amide bands region, after 7 days of incubation at 37 ºC (1750–1490 cm^{-1}). C_{pep}=1 mg/mL [0.317–0.327] mM.

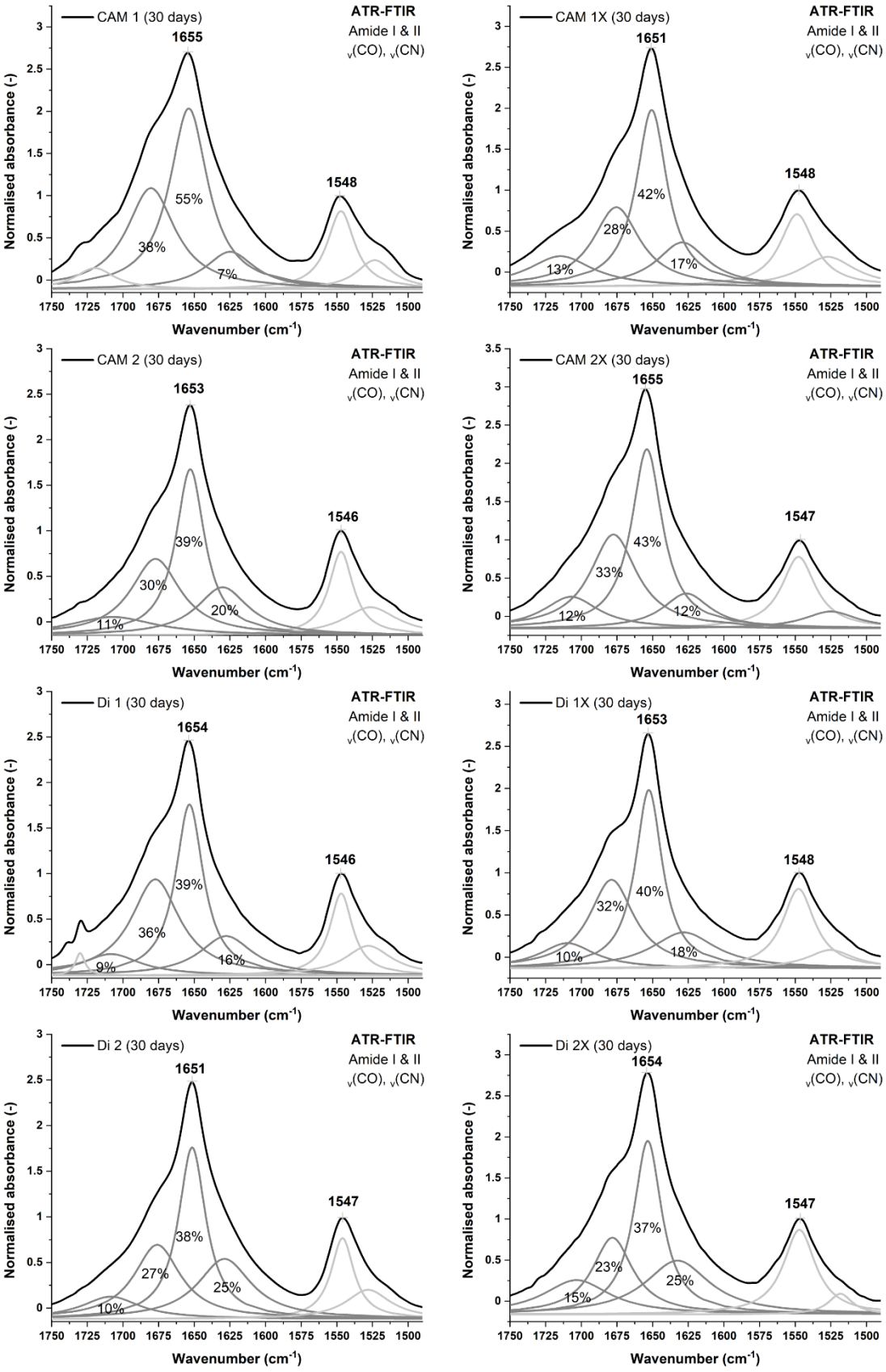

Figure S8. Normalized ATR-FTIR spectra of air-dried films of the studied peptides with sub-bands obtained from the curve fitting procedure in the amide bands region, after 30 days of incubation at 37 ºC (1750–1490 cm^{-1}). C_{pep}=1 mg/mL [0.317–0.327] mM.

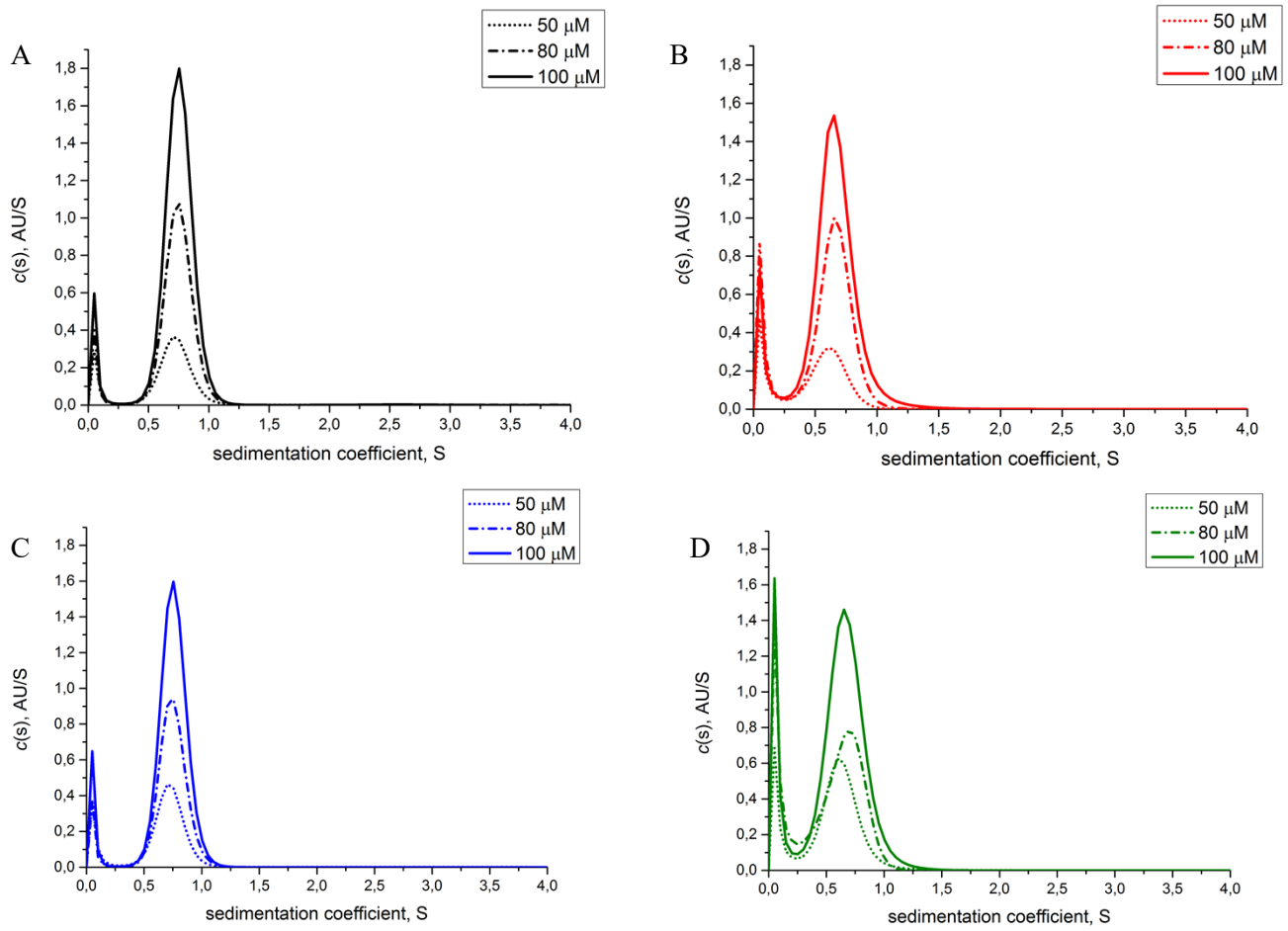

Figure S9. Sedimentation coefficient distributions *c*(s) obtained for different concentrations of the studied peptides resuspended in water. Centrifugation was performed at 50 000 rpm and 20 °C.

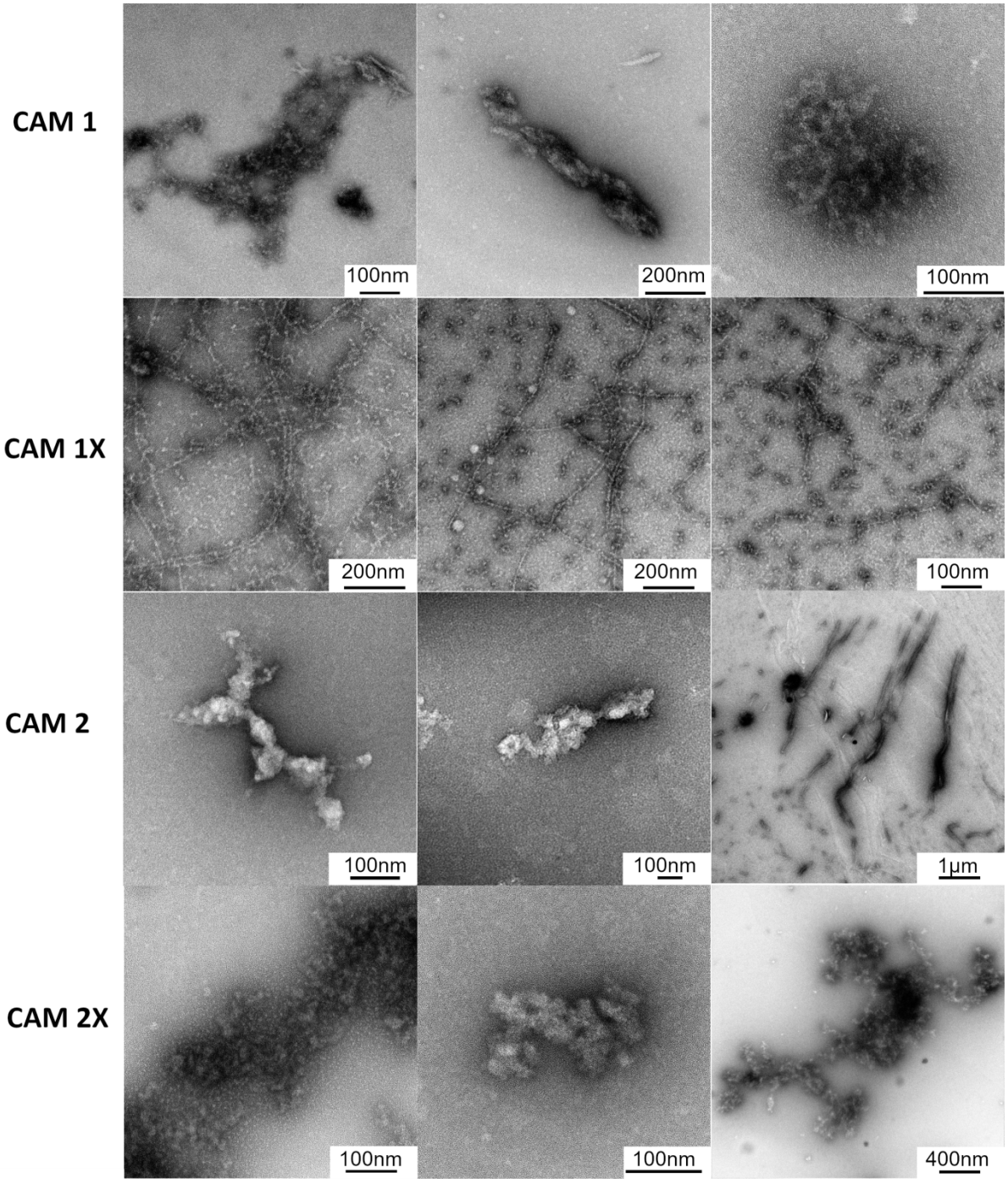

Figure S10. TEM micrographs of the CAM group peptides recorded 30 minutes after dissolving. C_{pep} = 0.5 mg/mL [0.159−0.164] mM.

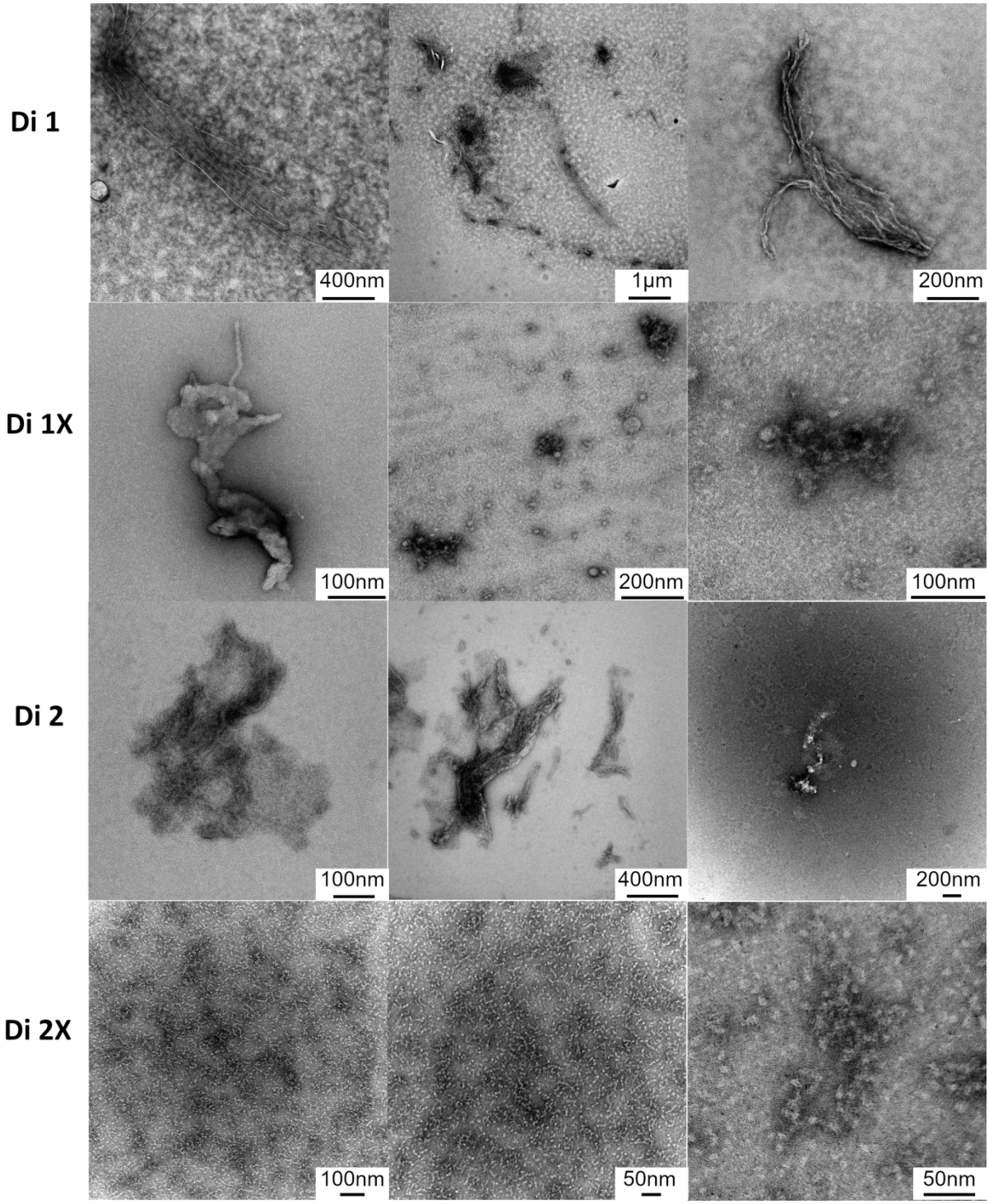

Figure S11. TEM micrographs of the Di group peptides recorded 30 minutes after dissolving. C_{pep}=0.5 mg/mL [0.159−0.164] mM.

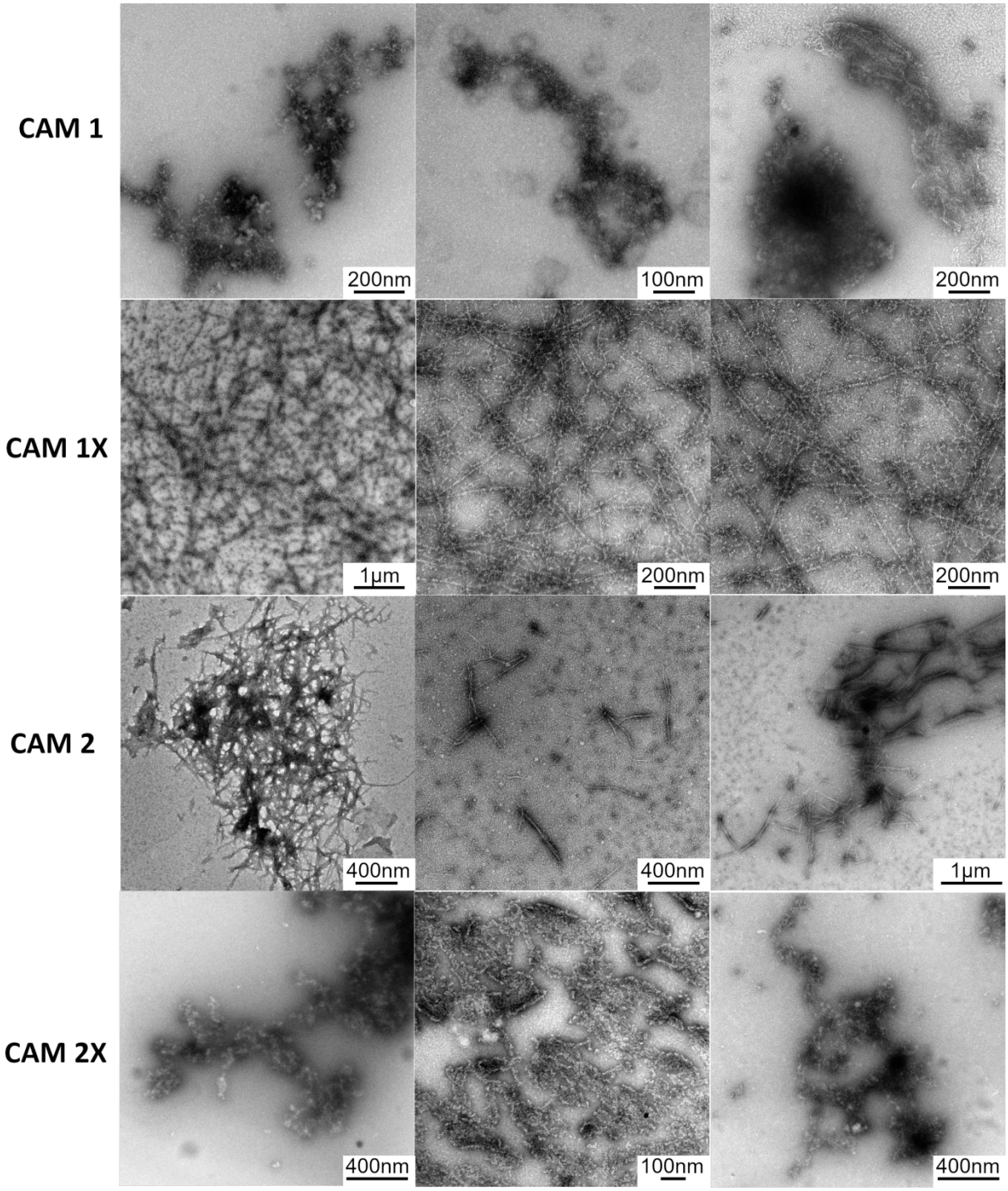

Figure S12. TEM micrographs of the CAM group peptides recorded after 48 hours of incubation at 37 °C. C_{pep} = 0.5 mg/mL [0.159−0.164] mM.

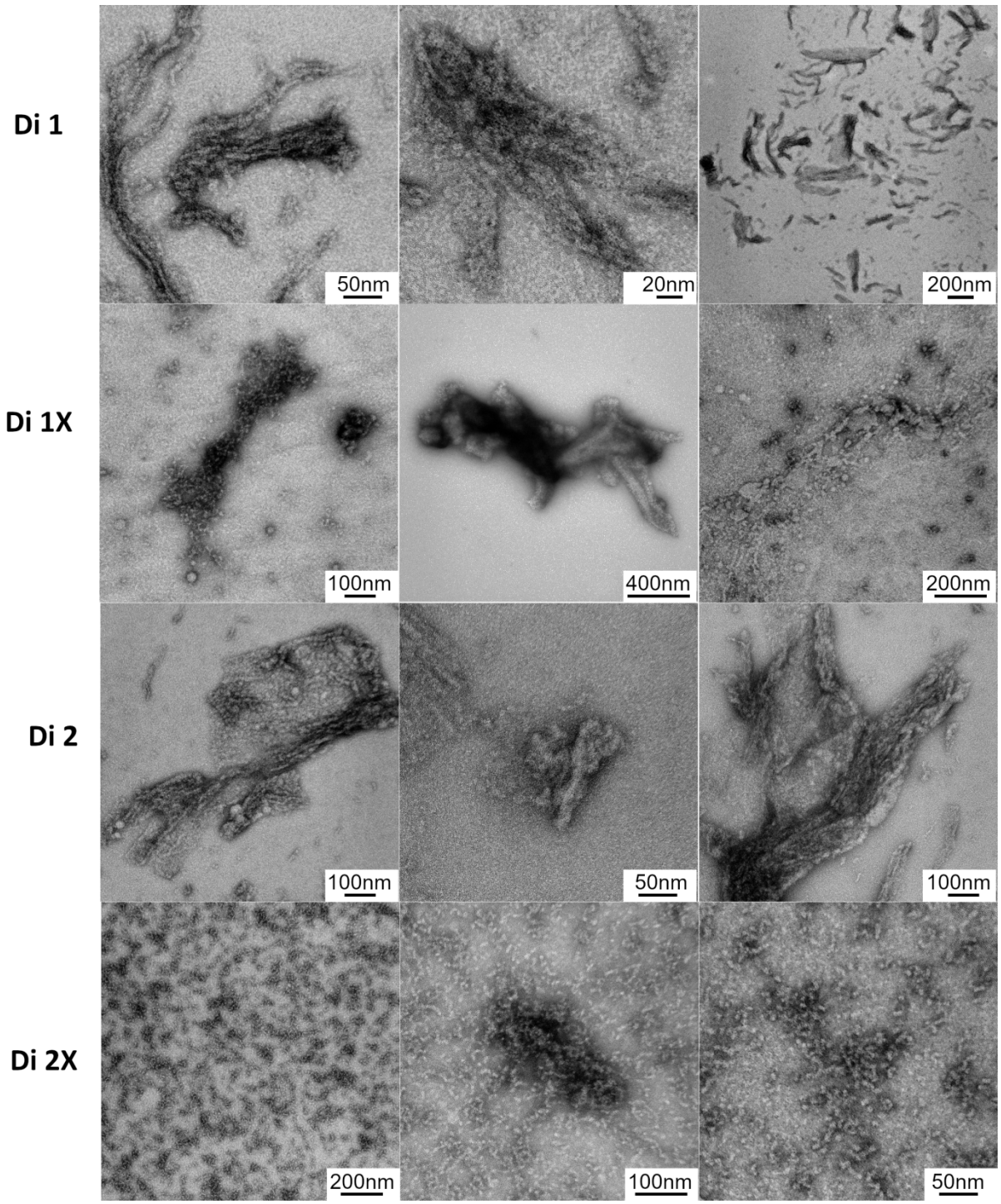

Figure S13. TEM micrographs of the Di group peptides recorded after 48 hours of incubation at 37 °C. C_{pep} = 0.5 mg/mL [0.159−0.164] mM.

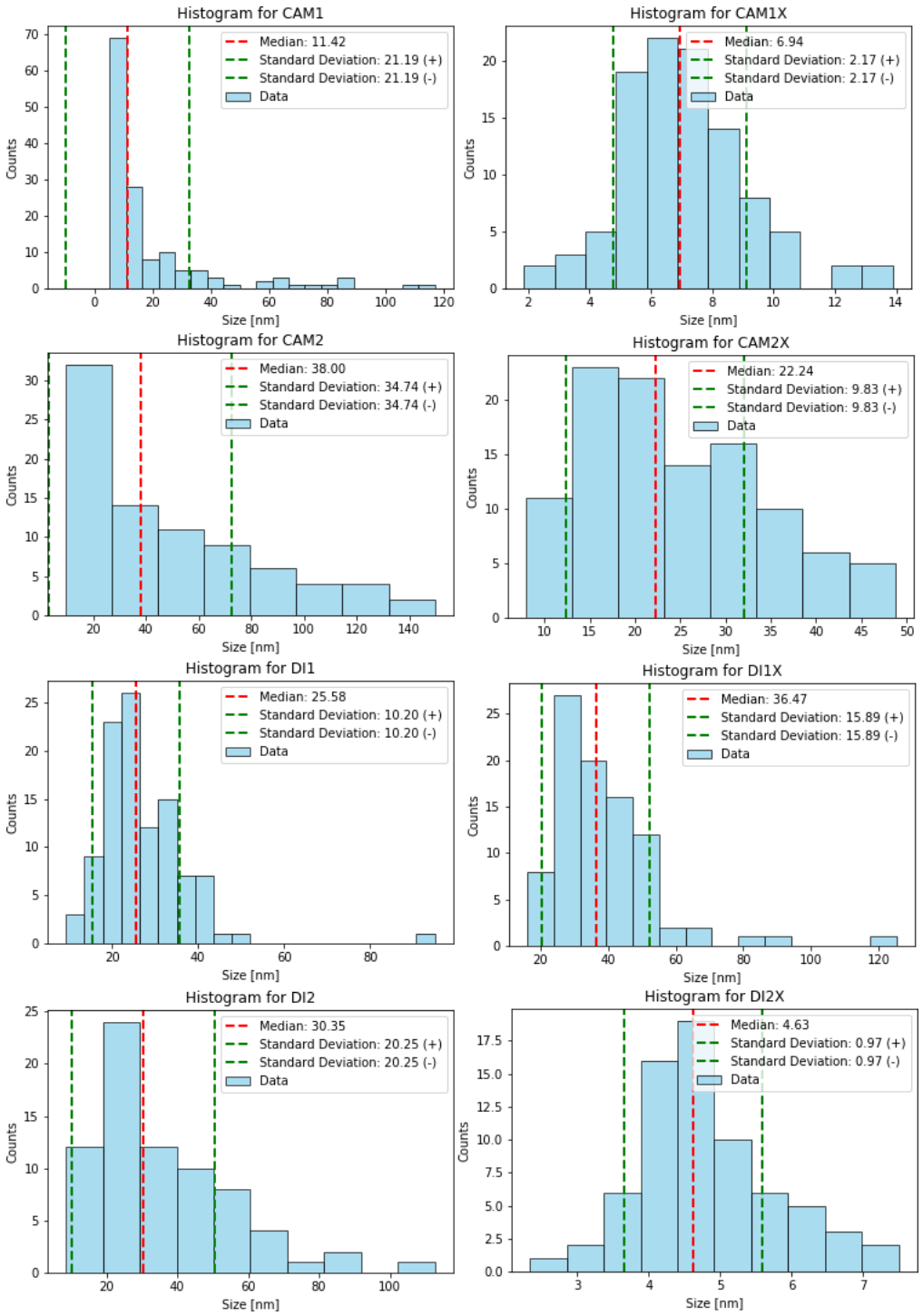

Figure S14. Diameter size distribution of the studied peptides recorded by TEM 30 minutes after dissolving. C_{pep} = 0.5 mg/mL [0.159−0.164] mM.

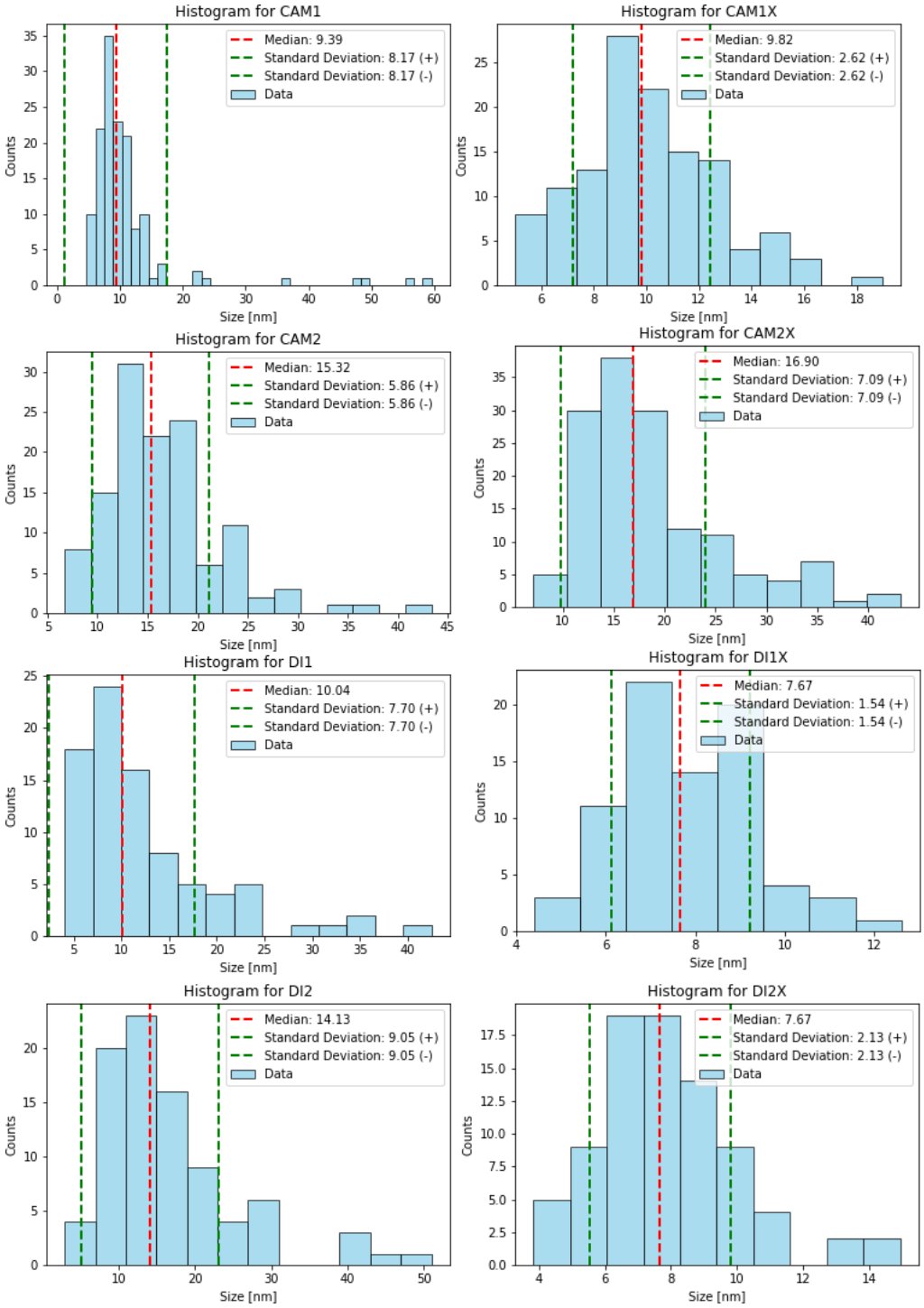

Figure S15. Diameter size distribution of the studied peptides recorded by TEM after 48 hours of incubation at 37 °C. C_{pep} = 0.5 mg/mL [0.159−0.164] mM.

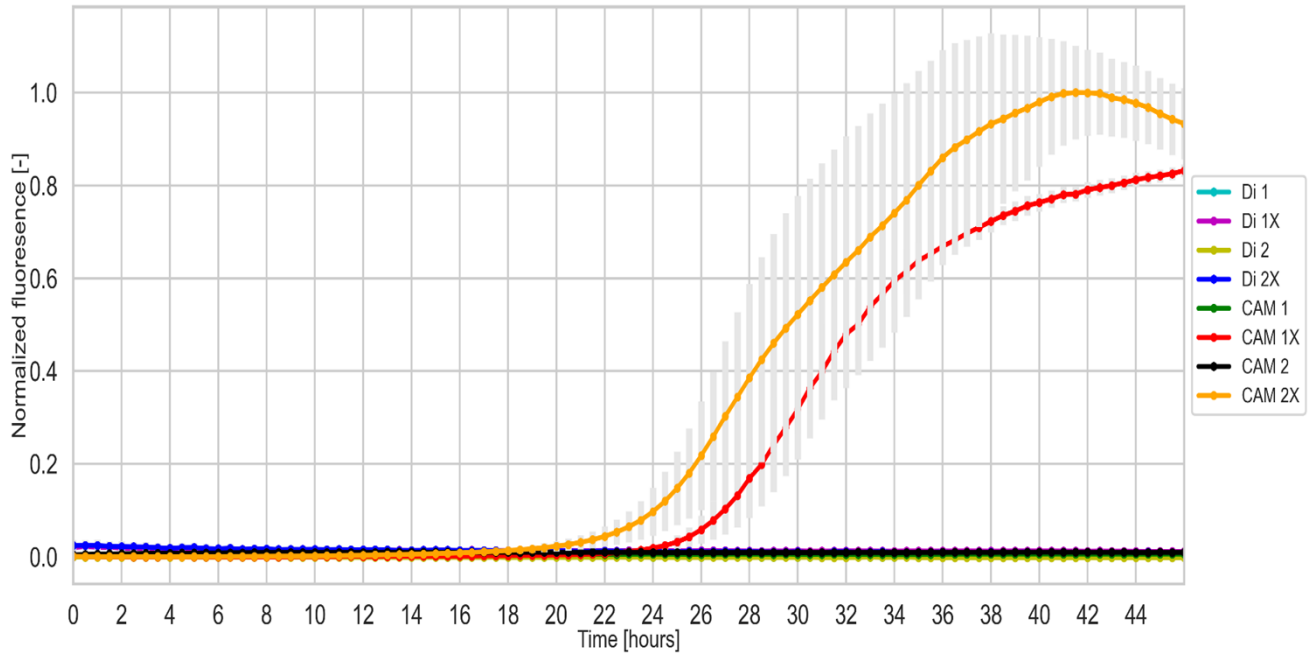

Figure S16. Normalized ThT fluorescence curves showing the aggregation kinetics of studied peptides. C_{pep} = 100 µM.

Table S3. Parameters obtained from modeling the aggregation kinetics of the peptides at a concentration of C_{pep} = 100 µM. In this model, y_i and y_f correspond to the initial and final intercepts on the y-axis, while m_i and m_f represent the slopes of these baselines. The elongation phase midpoint is given by t_{half}. The aggregation onset, or lag time, is determined as $t_{lag} = t_{half} - 2\tau$, where τ represents the time constant for elongation, following the method described by Malmos et al. 2017.

Sample	y_i [-]	y_f [-]	m_i [-]	t_{half} [hours]	τ [-]	t_{lag} [hours]
Di 1	0.00E+00	0.00	0.00	0.00	3.00	0.00
Di 1X	0.00E+00	0.00	0.00	0.00	2.50	0.00
Di 2	0.00E+00	0.00	0.00	0.00	2.99	0.00
Di 2X	0.00E+00	0.00	0.00	0.00	2.50	0.00
CAM 1	7.54E-04	0.00	0.00	1.20	2.50	0.00
CAM 1X	8.07E-23	0.75	0.02	28.80	3.00	22.80
CAM 2	5.61E-14	0.01	0.08	4.80	3.00	0.00
CAM 2X	2.48E-15	0.60	0.08	33.00	3.00	27.00

Table S4. Results of turbidity measurements based on absorbance at 340 nm.

Time [hours]	Di 1	Di 1X	Di 2	Di 2X	CAM1	CAM 1X	CAM2	CAM 2X	PBS
0	0.242	0.515	0.241	0.512	0.244	0.247	0.238	0.421	0.226
46	0.378	1.138	0.59	1.266	0.791	0.808	0.967	1.186	0.49