Electronic Supplementary Information

Novel self-assembling cyclic peptides with reversible supramolecular nanostructures

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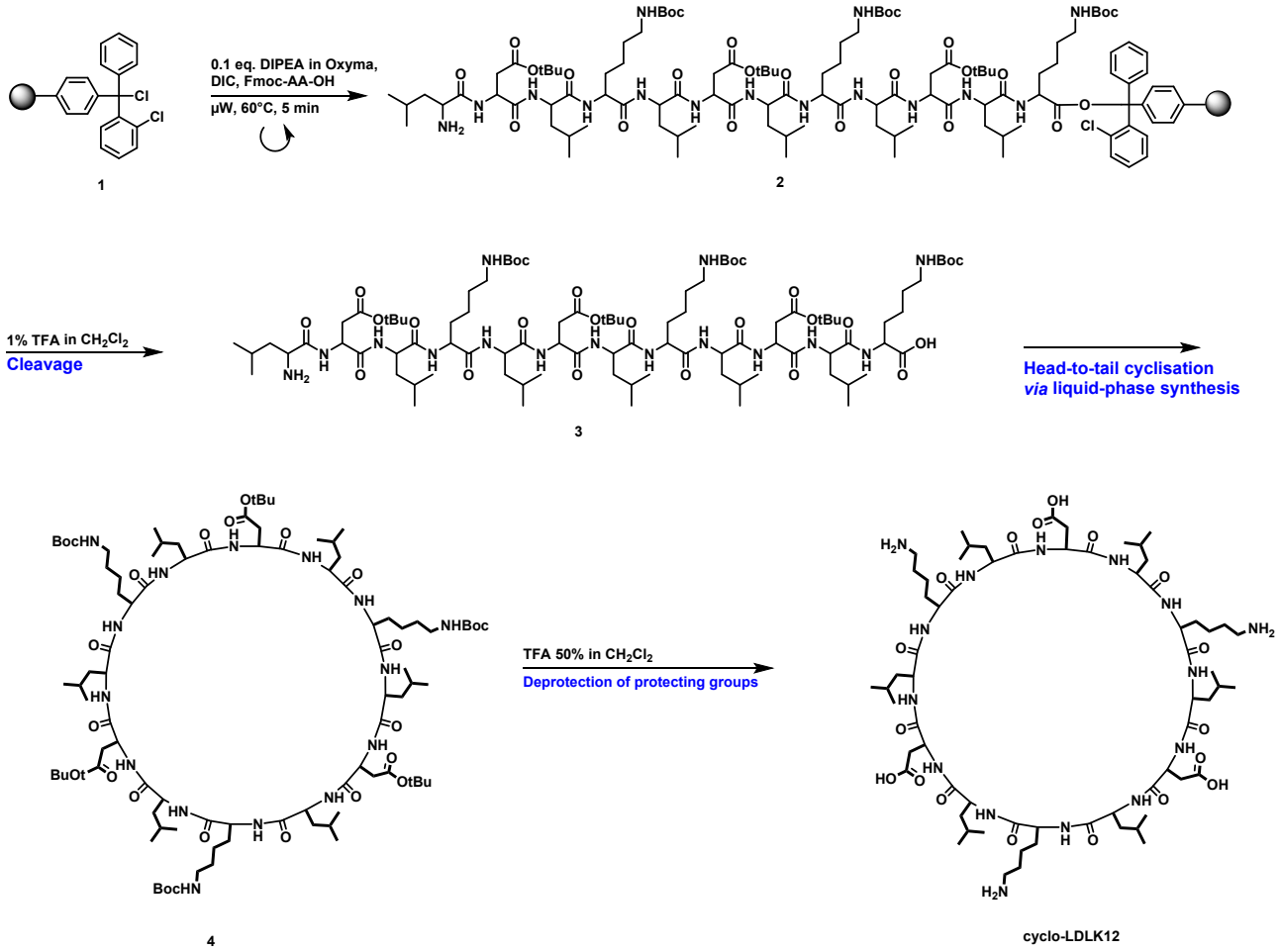


Scheme S1. Total synthesis of cyclo-LDLK12. The general procedure includes a Fmoc-based SPPS strategy, followed by a solution phase cyclization. Overall yield was 56%.

Entry	Peptide	Solvent	Activator	Conditions	Yield %
1	4	DMF, DCM	HBTU, DIPEA	RT, 1h	35
2	4	DMF, DCM	0.002 M HATU, DIPEA	RT, 4h	18
3	4	NMP	DIPEA, HOBt, PyBOP	RT, 2h	58
4	4	NMP	DIPEA, HOBt, PyBOP	RT, 6h	75

Table S1. Optimization of the solution phase cyclization. Yields are isolated yields for the purified final compounds.

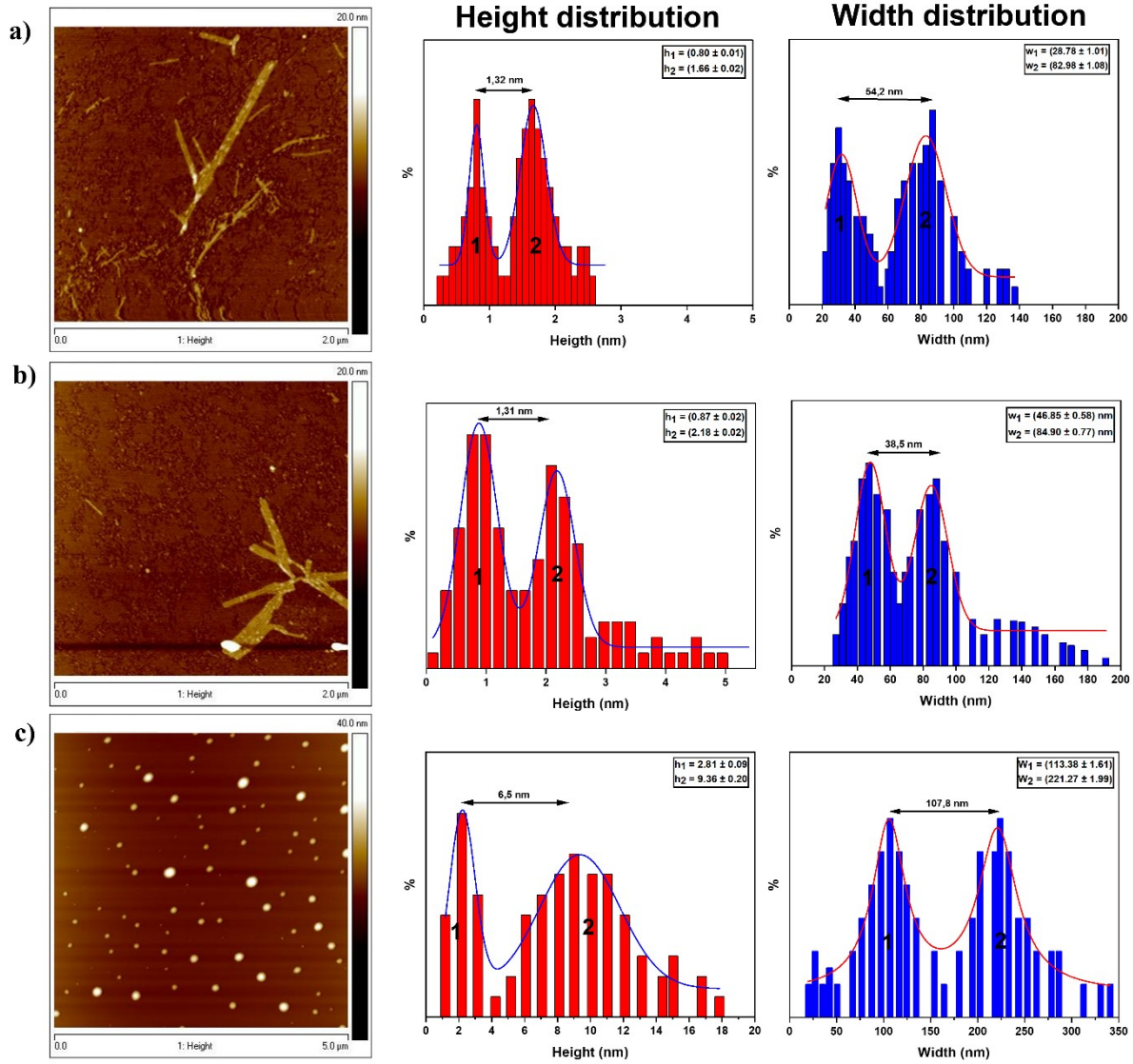


Figure S1. AFM profiles of cyclo-LDLK12 detected on mica by tapping mode. a) pH~2; b) pH~7; c) pH~10. All measurements were obtained with an image size 1x1 μm^2.

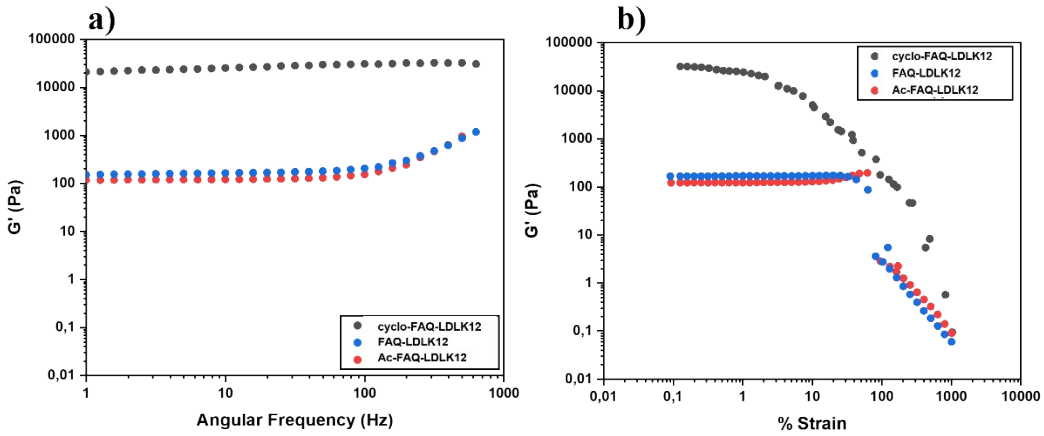


Figure S2. Characterization of cyclo-FAQ-LDLK12 by rheological experiments: a) frequency-sweep test performed to evaluate the stiffness of cyclo-FAQ-LDLK12, and b) strain-sweep experiment to evaluate the rheological stability of cyclo-FAQ-LDLK12.

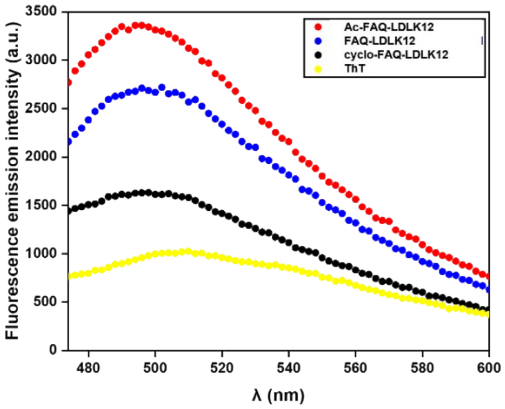


Figure S3. ThT-binding assay to study the *β*-sheet content of cyclo-FAQ-LDLK12 in comparison with the corresponding linear SAPs FAQ-LDLK12 and Ac-FAQ-LDLK12.

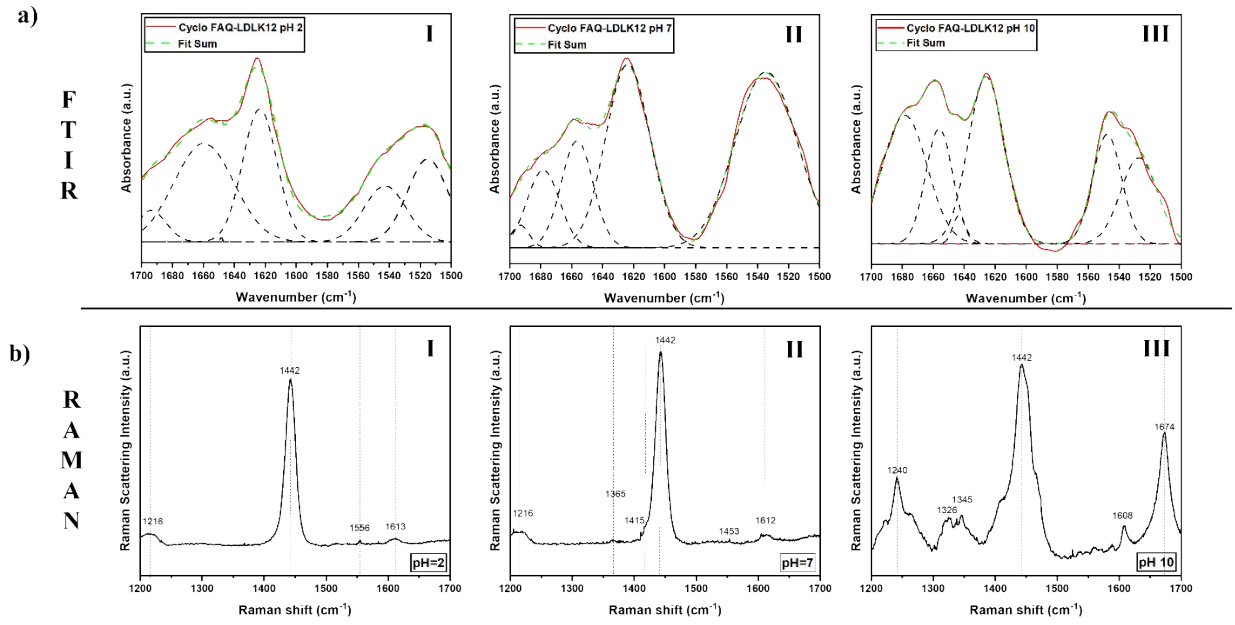


Figure S4. a) FT-IR absorption spectra and b) Raman spectra of cyclo-FAQ-LDLK12 at respectively I) pH 2, II) pH 7, III) pH 10; e) AFM images of cyclo-FAQ-LDLK12 at different pH conditions, showing liner nanostructures at I) pH 2 and II) pH 7, and round-shaped aggregates at III) pH 10.

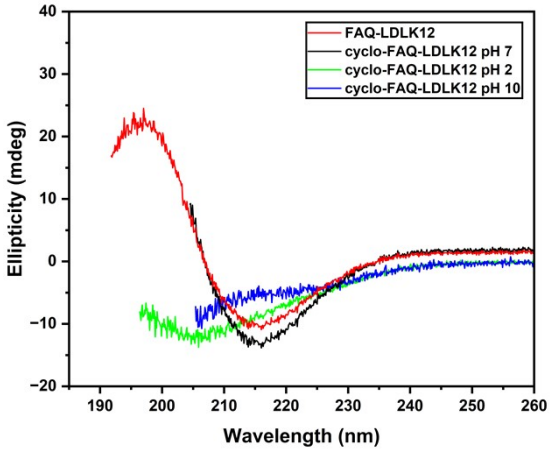


Figure S5. Far-UV spectra analysis by circular dichroism for cyclo-FAQ-LDLK12 at different pH.

pH	% β-pleated sheets		% turns	% helix	% random coil
	parallel	antiparallel			
2	3	37	11	16	33
7	2	52	23	11	12
10	6	24	17	25	28

Table S2. Deconvolution results of cyclo-FAQ-LDLK12 CD spectra performed by Spectra deconvolution version 2.1.

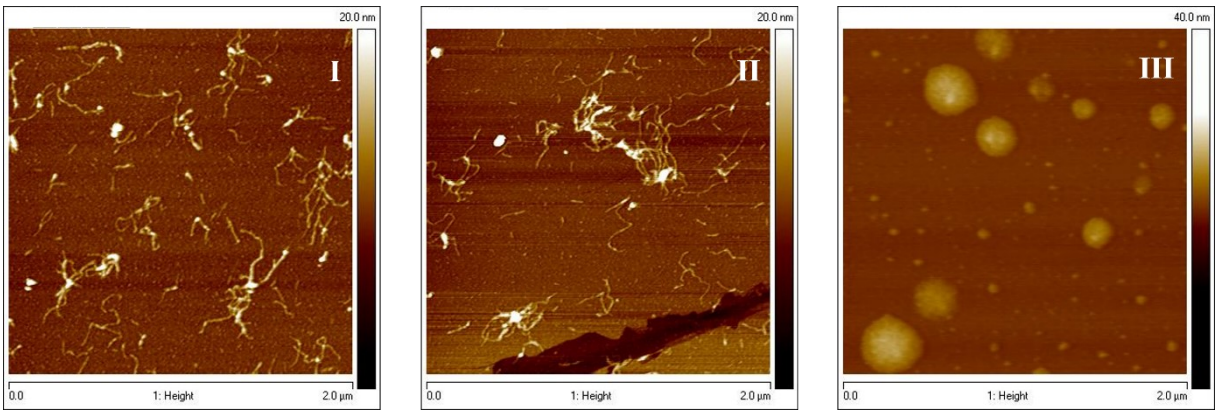


Figure S6. AFM images of cyclo-FAQ-LDLK12 at different pH conditions, showing liner nanostructures at I) pH 2 and II) pH 7, and round-shaped aggregates at III) pH 10.

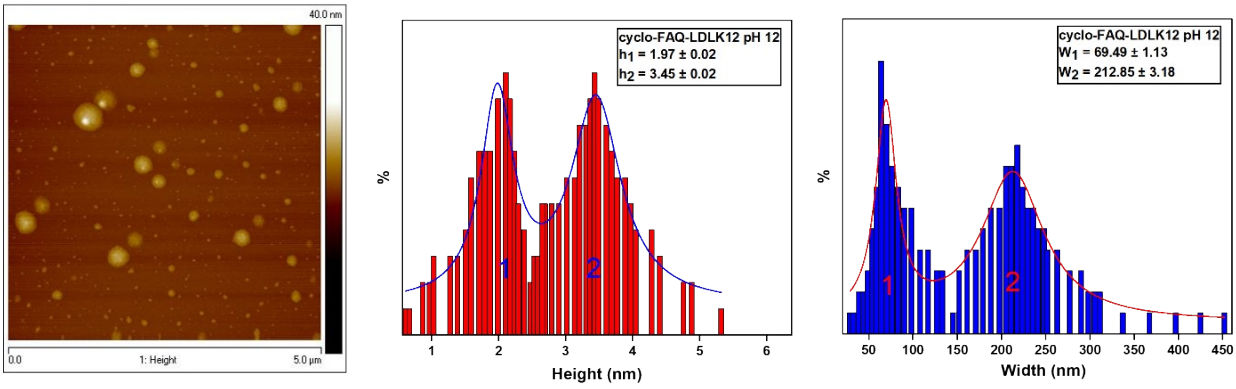


Figure S7. AFM image of cyclo-FAQ-LDLK12 at pH~10 with corresponding height and width profiles. All measurements were conducted with an image size of 1x1 μm^2.

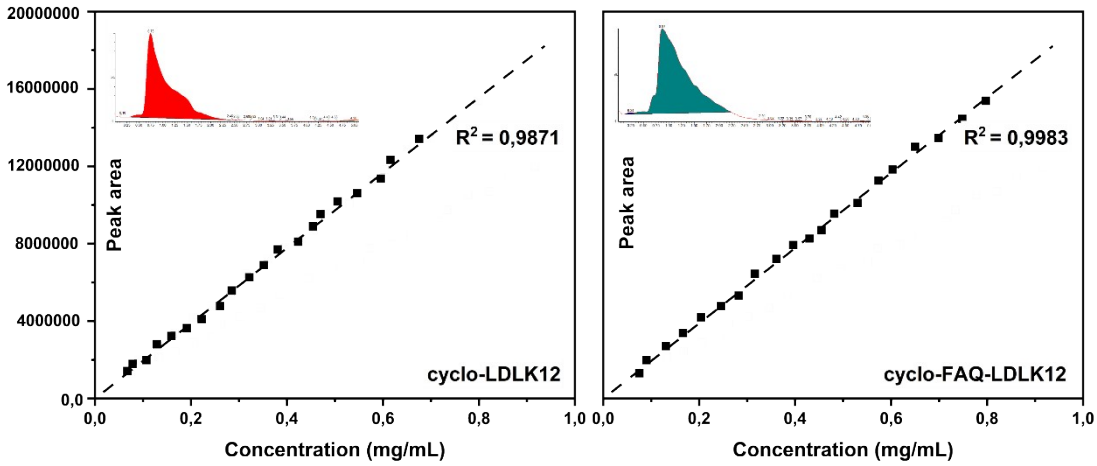


Figure S8. Twenty points calibration curves for LC-MS analysis were built (by using a peptide reference standard) in order to estimate the amount of nonhydrolyzed peptide after microwave-assisted acid hydrolysis. The correlation coefficient (R^2) was obtained for each linear regression curve.

Peptide	% nonhydrolized	Linear Counterpart	% nonhydrolized
Cyclo-LDLK12	43	LDLK12	12
Cyclo-FAQ-LDLK12	57	FAQ-LDLK12	18

Table S3. Comparison of experimental values between cyclic and linear peptides after partial hydrolysis *via* microwave-assisted acid hydrolysis.

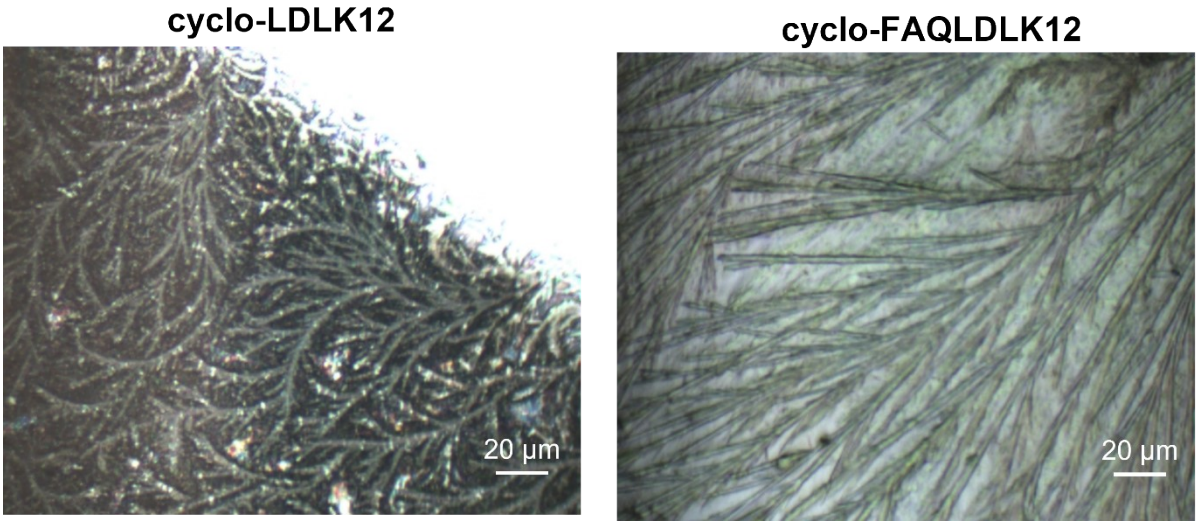


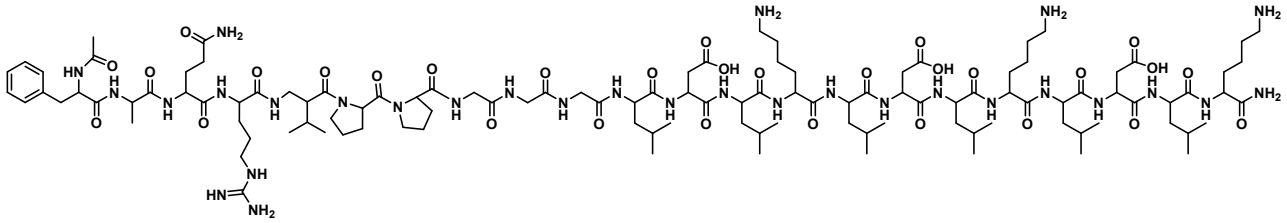
Figure S9. Micrographs of cyclo-LDLK12 and cyclo-FAQLDLK12 samples acquired with a 10x magnification right before Raman spectroscopy measurements at pH 7.

Mapping (force-field)	Multiplicity	Duration (ns)	Number of replicas
Gromos53a6	1	500	4
Gromos53a6	8	500	4
MARTINI	40	500	4
MARTINI	4 x 10-mer (nanotubes)	500	1
MARTINI	4 x 10-mer (aggregates)	500	1
MARTINI	100	2000	1

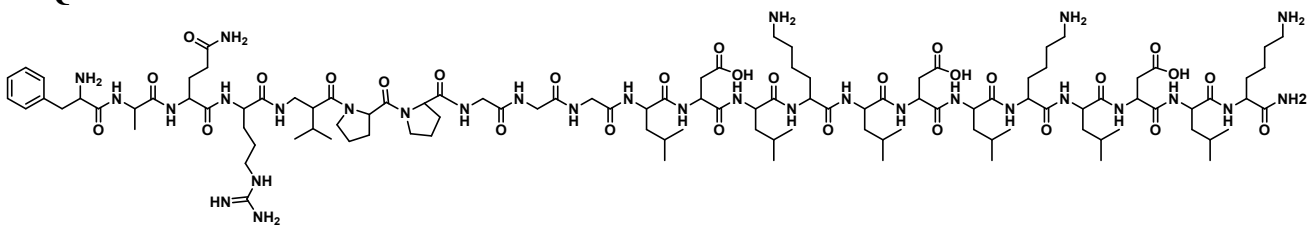
Table S4. Molecular Dynamics simulation details. Cyclo-LDLK12 self-assembling propensity was evaluated through united-atom molecular dynamics (UA-MD) simulations. Each system was represented according to Gromos53a6 force-field mapping. The peptide conformations, obtained from monomer simulations, have been used to prepare the starting configuration of 8-mer systems. Cyclo-LDLK12 SAPs aggregation propensity was evaluated through CG-MD simulations. Secondary structure (SS) parameters have been assigned according to experimental evidence. The 40-mers (fibril nuclei) and 100-mers (proto-fibril) were modeled adopting the standard MARTINI approach. Legend: mapping, force-field used for running MDs; Multiplicity, number of peptides in the simulation box; duration, length of the simulation; number of replicas, for statistical purposes.

Supplementary Chemical Structures

Ac-FAQ-LDLK12

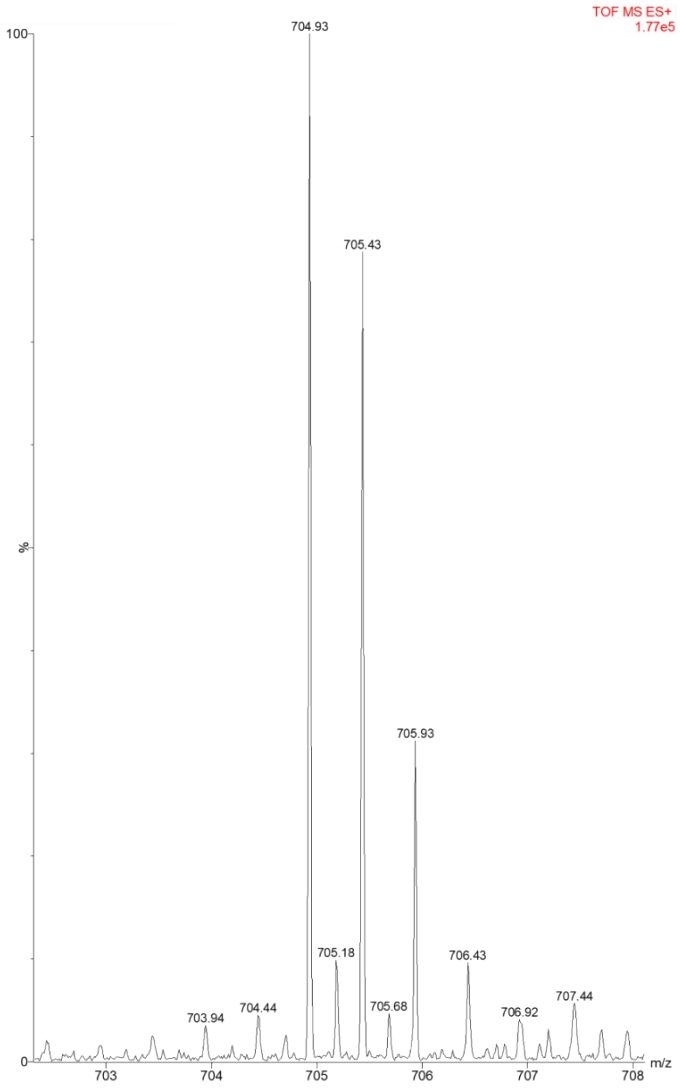


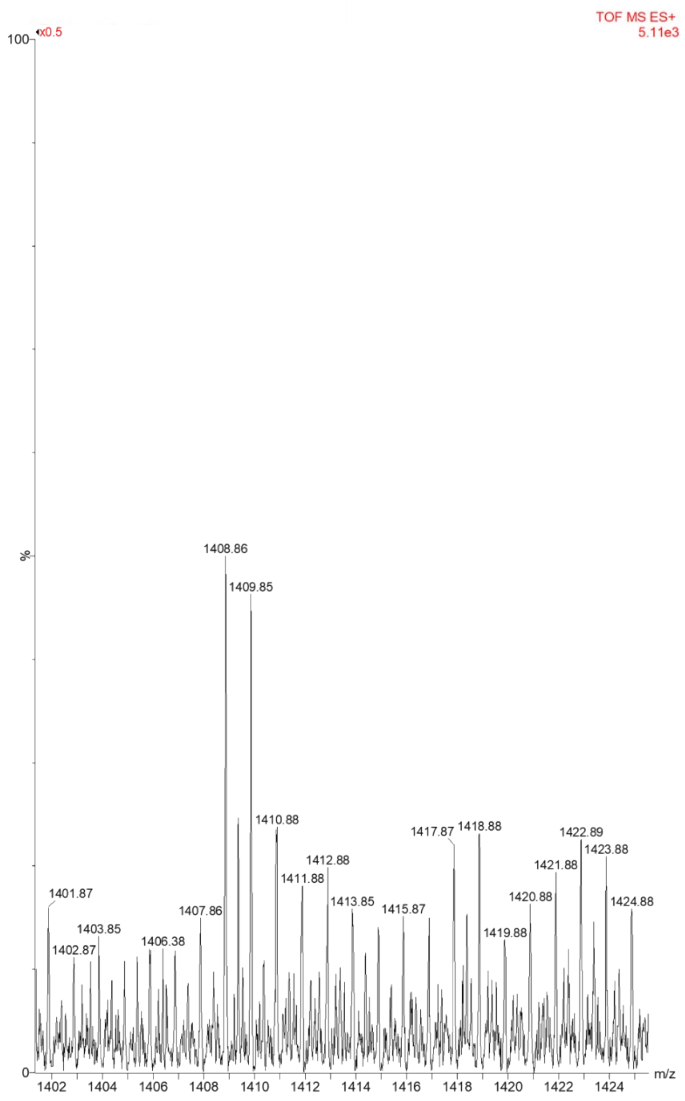
FAQ-LDLK12



Quadrupole time-of-flight (Qtof) Mass Spectra

cyclo-LDLK12
Calculated: m/z 1407,87; found: M +1 = 1408,86; M/2 +1 = 704,93




TOF MS ES+
5.11e3
x0.5
100
%
0
1408.86
1409.85
1401.87
1403.85
1402.87
1406.38
1407.86
1410.88
1411.88
1412.88
1413.85
1415.87
1417.87
1418.88
1419.88
1420.88
1421.88
1422.89
1423.88
1424.88
1402
1404
1406
1408
1410
1412
1414
1416
1418
1420
1422
1424
m/z

cyclo-FAQ-LDLK12
Calculated: m/z 2377,36; found: M/3 +1 = 793,18