Electronic Supplementary Information

Low-Power Microwaves: a Cell-Compatible Physical Treatment to Enhance Self-Assembling Peptides Mechanical Properties

Maria Gessica Ciulla,[1] Amanda Marchini,[1,2] Jacopo Gazzola,[3] Manuel Sambrotta,[4] and Fabrizio Gelain[1,2,*]

[1] Institute for Stem-Cell Biology, Regenerative Medicine and Innovative Therapies, IRCCS Casa Sollievo della Sofferenza, 71013 San Giovanni Rotondo, Italy.
[2] Center for Nanomedicine and Tissue Engineering (CNTE), ASST Grande Ospedale Metropolitano Niguarda, 20162 Milan, Italy.
[3] Department of Biotechnology and Bioscience, University of Milan – Bicocca, 20125 Milan, Italy.
[4] Department of Chemistry, Materials and Chemical Engineering “Giulio Natta”, Politecnico di Milano, 20133 Milan, Italy
* Corresponding author. E-mail: f.gelain@css-mendel.it; Tel.: +39-02-6444-7519

LDLK12

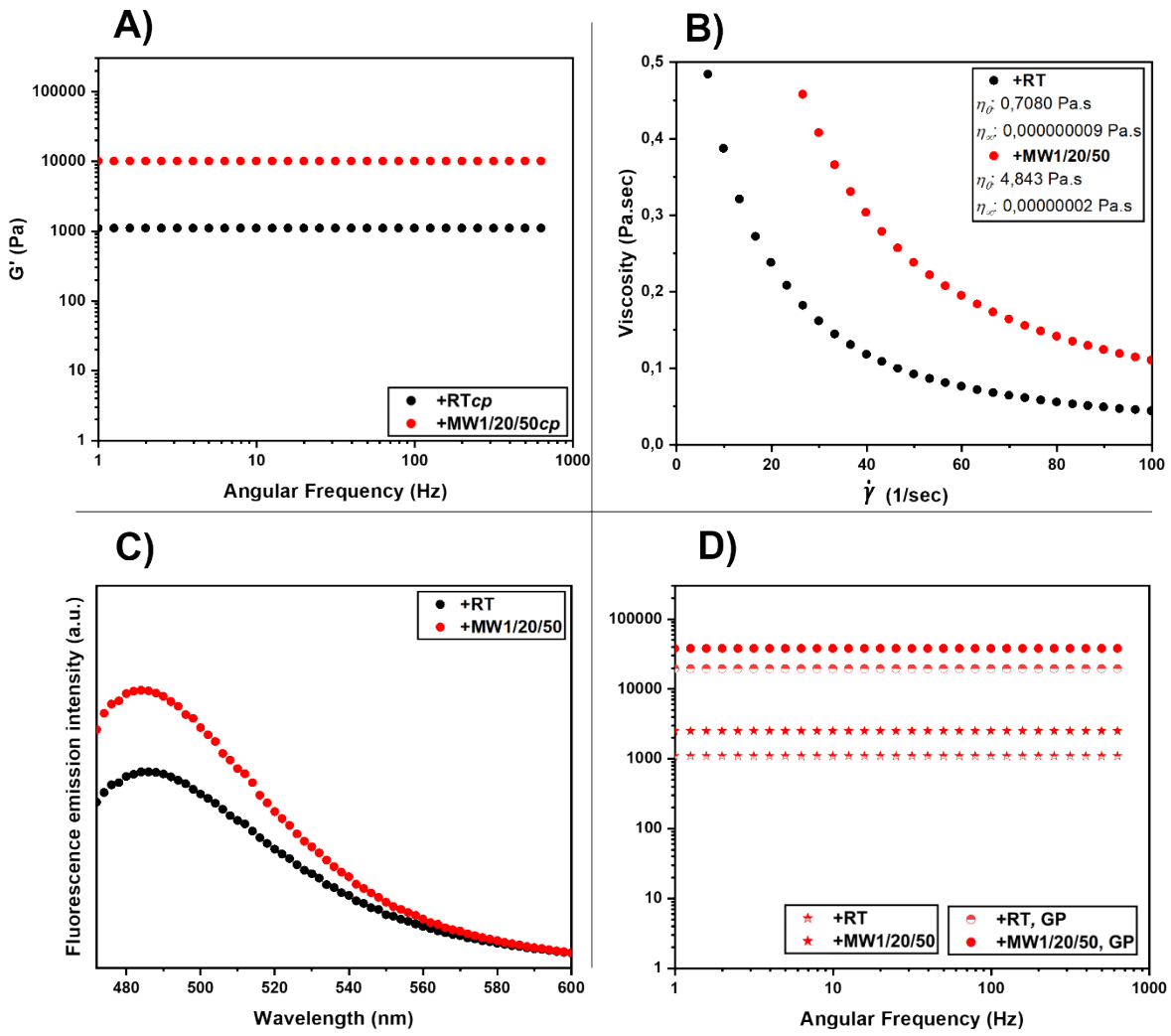


Fig. S1. +MW1/20/50 treatment on LDLK12. A), B) and D) Rheological experiments. A) Frequency sweep test using a cone-plate geometry. B) Viscosity test, performed in a cone-plate geometry *via* continuous shear ramp. C) ThT binding assay. D) Frequency sweep test in a parallel-plate geometry to investigate the effect of Genipin cross-linking after MW treatment.

FAQ-LDLK12

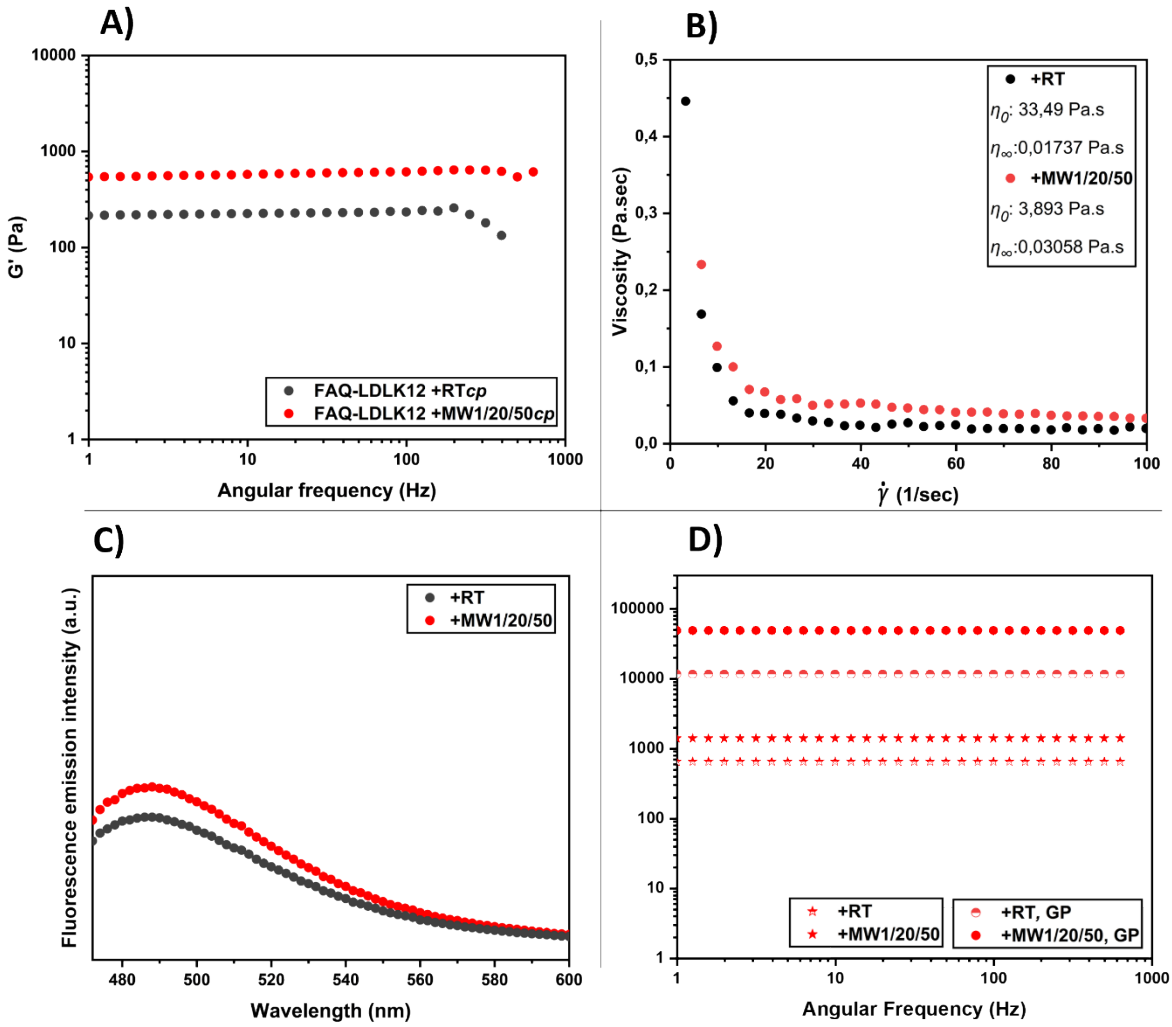


Fig. S2. +MW1/20/50 treatment on FAQ-LDLK12. A), B) and D) Rheological experiments. A) Frequency sweep test using a cone-plate geometry. B) Viscosity test, performed in a cone-plate geometry *via* continuous shear ramp. C) ThT binding assay. D) Frequency sweep test in a parallel-plate geometry to investigate the effect of Genipin cross-linking after MW treatment.

CK_1

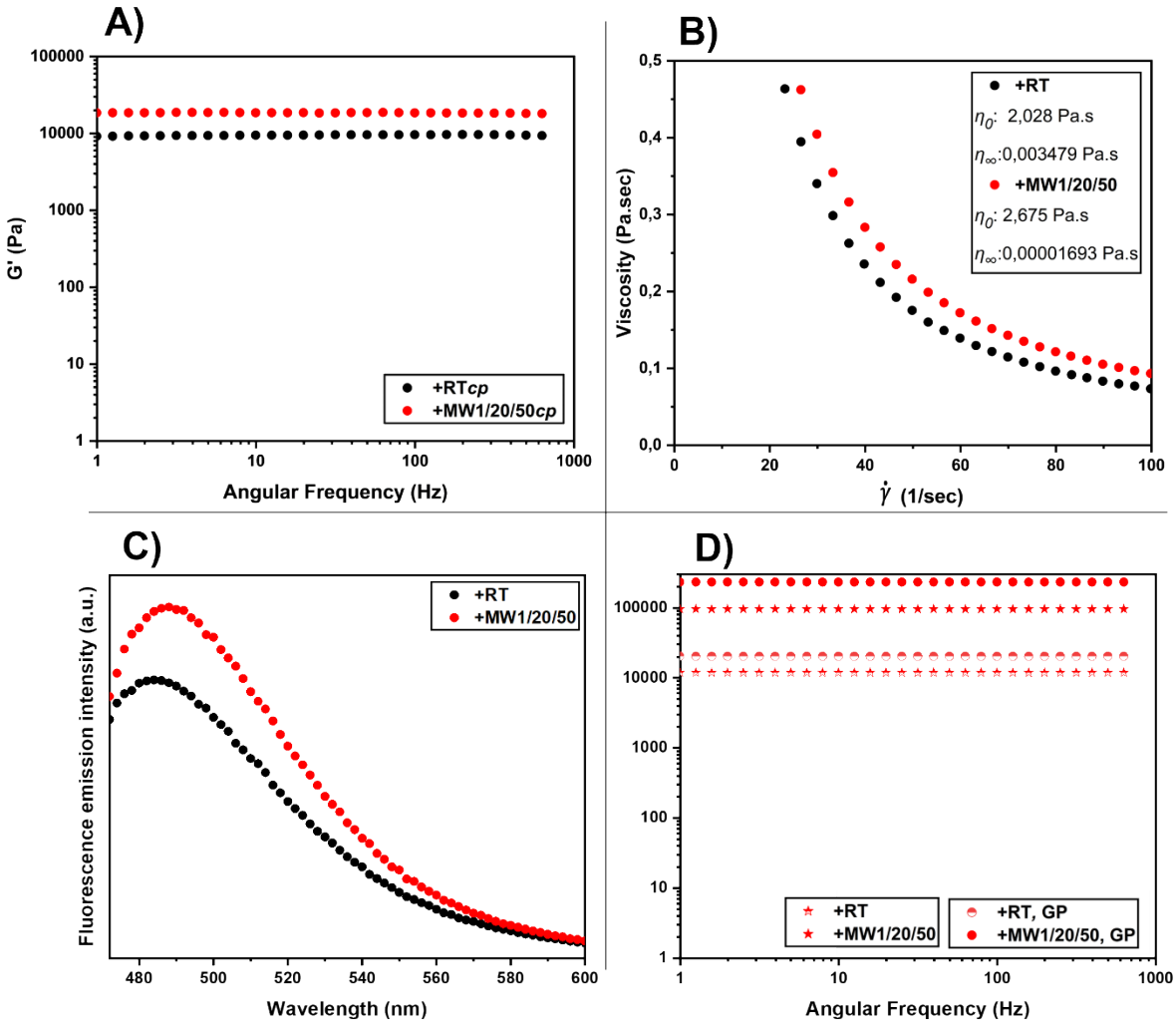


Fig. S3. +MW1/20/50 treatment on CK_1. A), B) and D) Rheological experiments. A) Frequency sweep test using a cone-plate geometry. B) Viscosity test, performed in a cone-plate geometry *via* continuous shear ramp. C) ThT binding assay. D) Frequency sweep test in a parallel-plate geometry to investigate the effect of Genipin cross-linking after MW treatment.

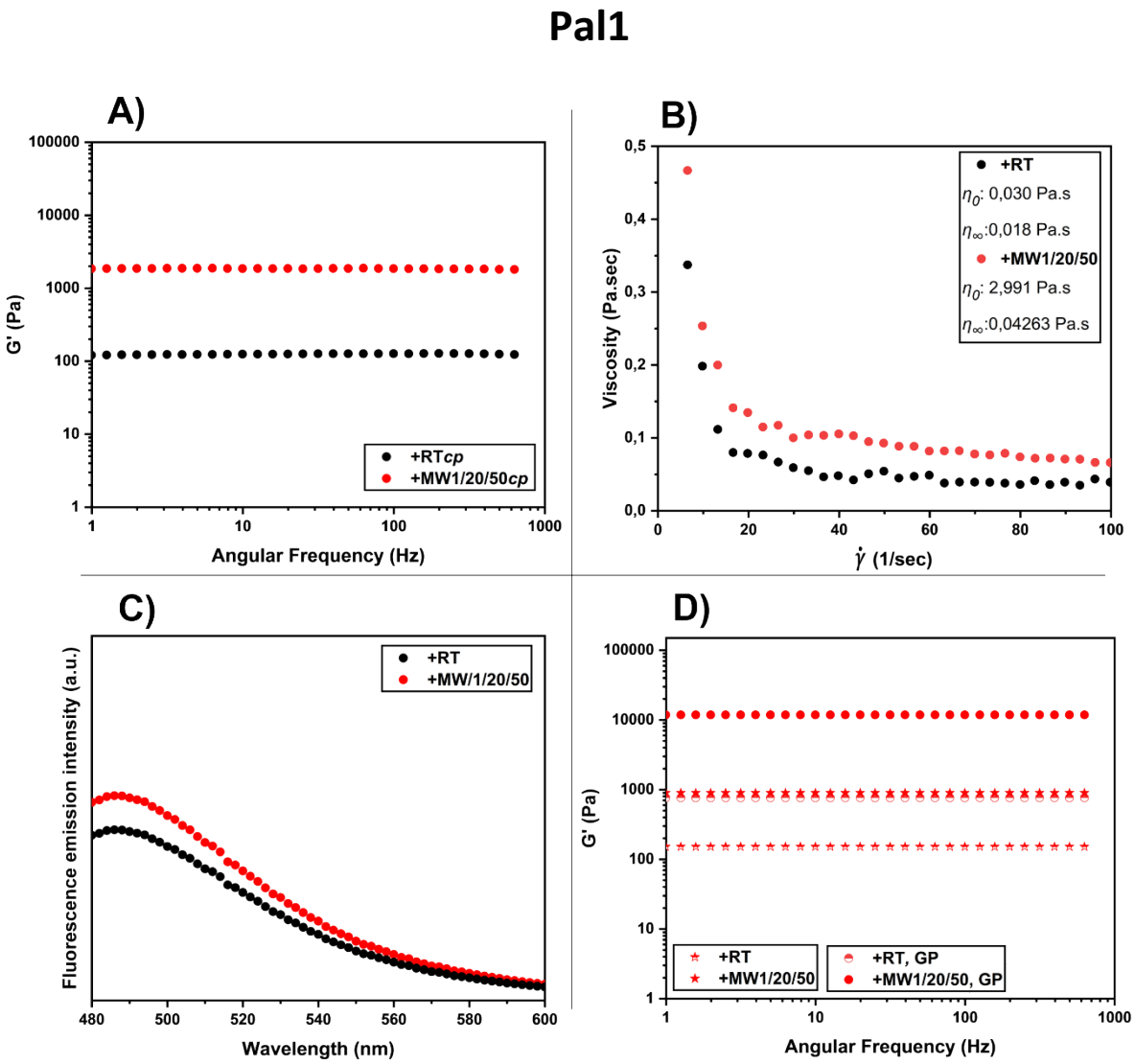


Fig. S4. **+**MW1/20/50 treatment on Pal1. A), B) and D) Rheological experiments. A) Frequency sweep test using a cone-plate geometry. B) Viscosity test, performed in a cone-plate geometry *via* continuous shear ramp. C) ThT binding assay. D) Frequency sweep test in a parallel-plate geometry to investigate the effect of Genipin cross-linking after MW treatment.

BM3

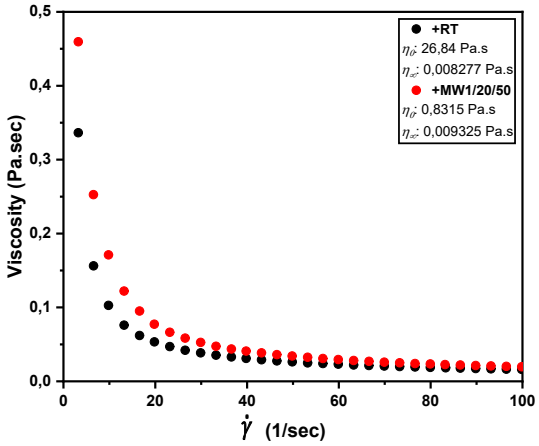


Fig. S5. Viscosity test of BM3 with and without MW treatment at 1 W, for 20 minutes and at a maximum temperature of 50 °C. The experiment was performed in a cone-plate geometry *via* continuous shear ramp.

HYDROSAP

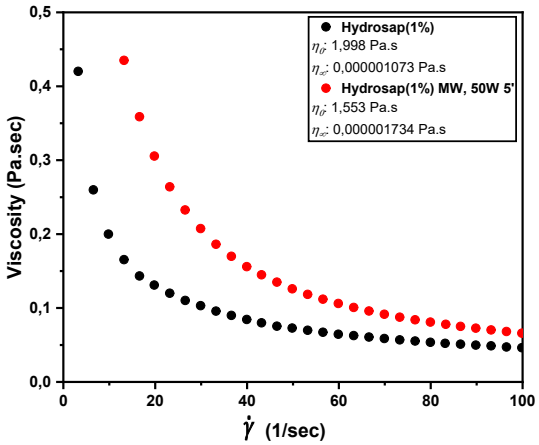


Fig. S6. Viscosity test of HYDROSAP with and without MW treatment at 1 W, for 20 minutes and at a maximum temperature of 50 °C. The experiment was performed in a cone-plate geometry *via* continuous shear ramp.

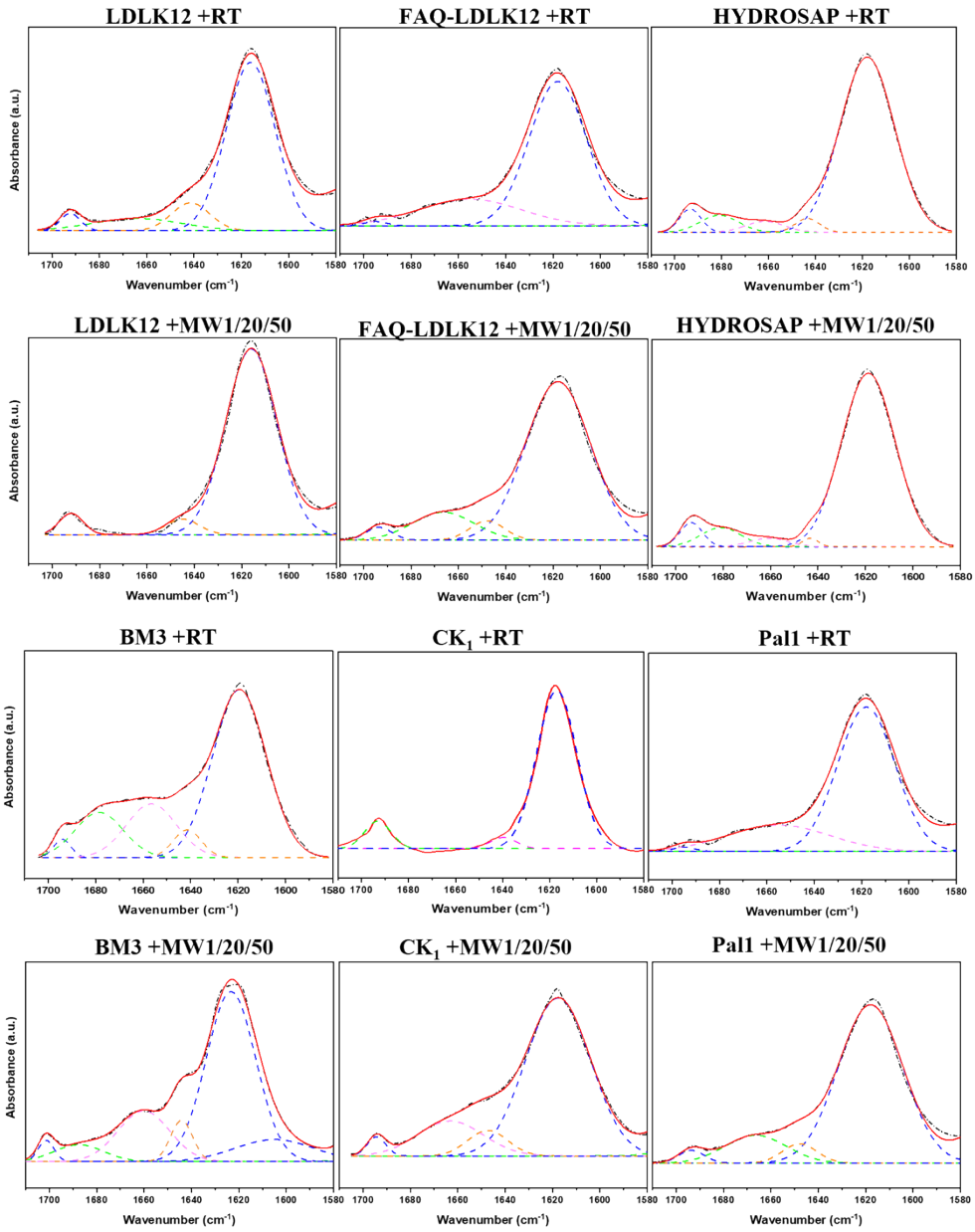
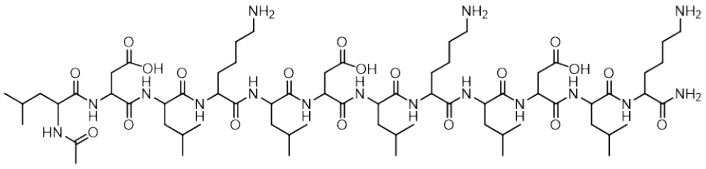


Fig. S7. Experimental deconvolution ATR-FTIR absorption spectra of LDLK12, FAQ-LDLK12, HYDROSAP, BM3, CK_1 and Pal1 for Amide I. To resolve the overlapping bands, after a baseline correction, the peaks were detected using the respective second derivatives, followed by smoothing with the 7–9 point Savitsky–Golay function with polynomial order of 2. Peak fitting/deconvolution was then performed with a Voigt function using OriginPro software.

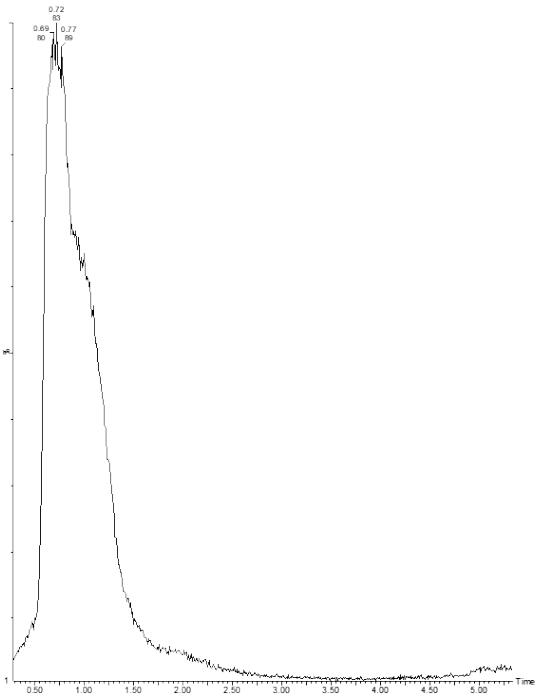
MASS SPECTRA

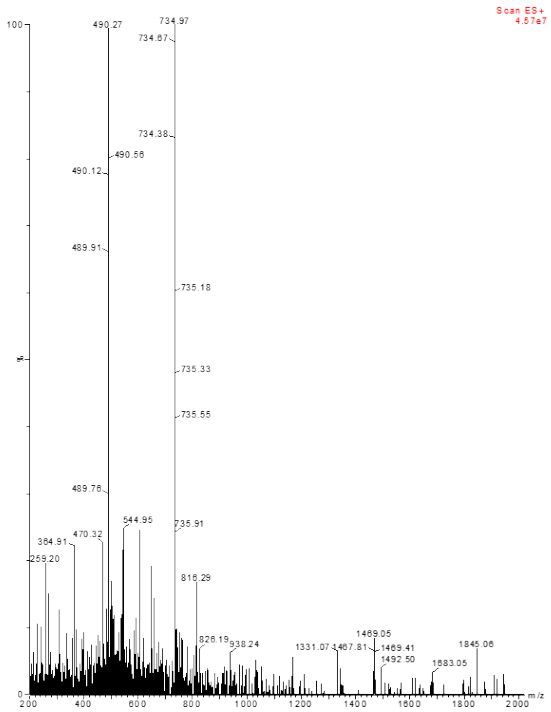
LDLK12

Calculated: m/z 1467,7; found: M +1 = 1468,10; M/2 +1 = 734,92; M/3 +1 = 490,27

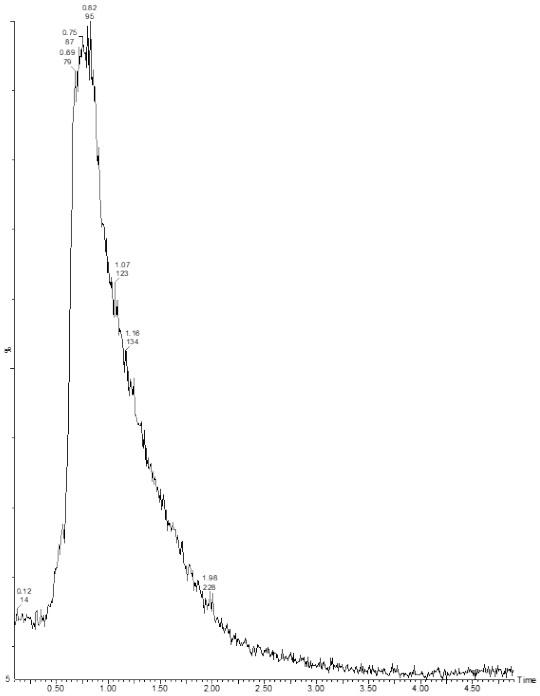


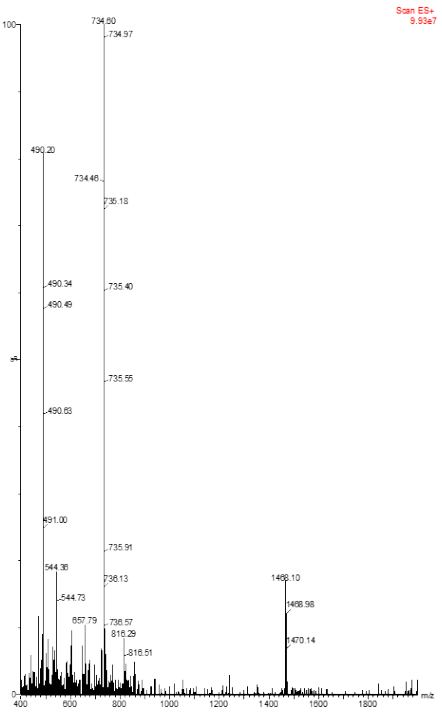
LDLK12





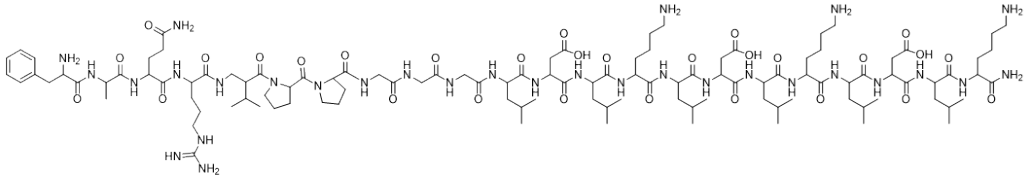
LDLK12 +MW1/20/50



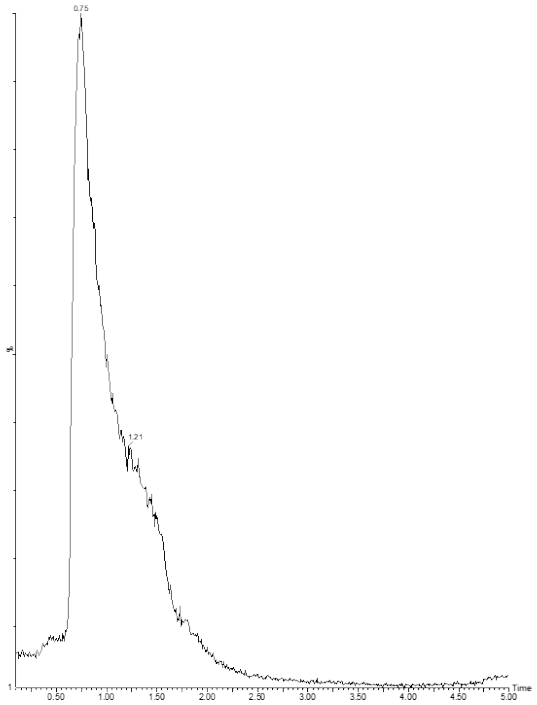


FAQ-LDLK12

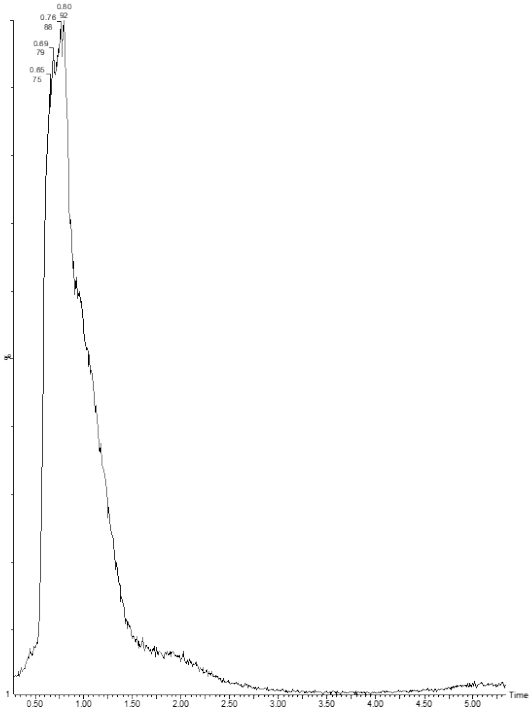
Calculated: m/z 2392,8; found: M/2 +1 = 1197,16; M/3 +1 = 798,67; M/4 +1 = 599,11

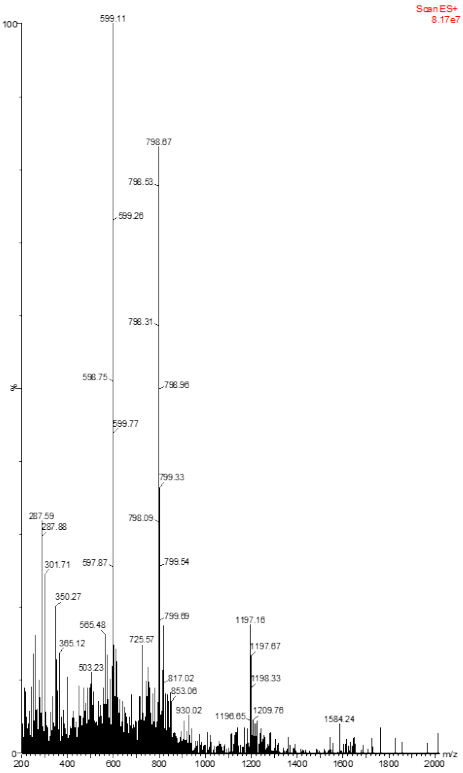


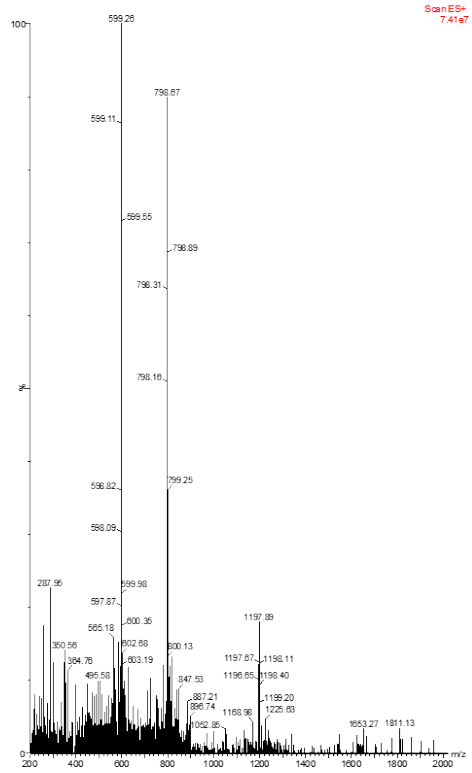
FAQ-LDLK12



FAQ-LDLK12 +MW1/20/50

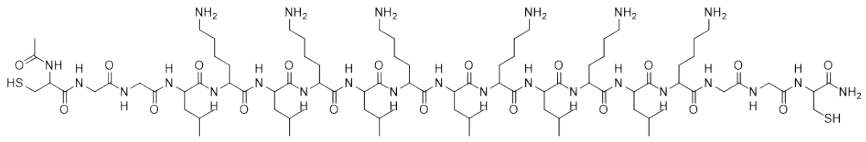




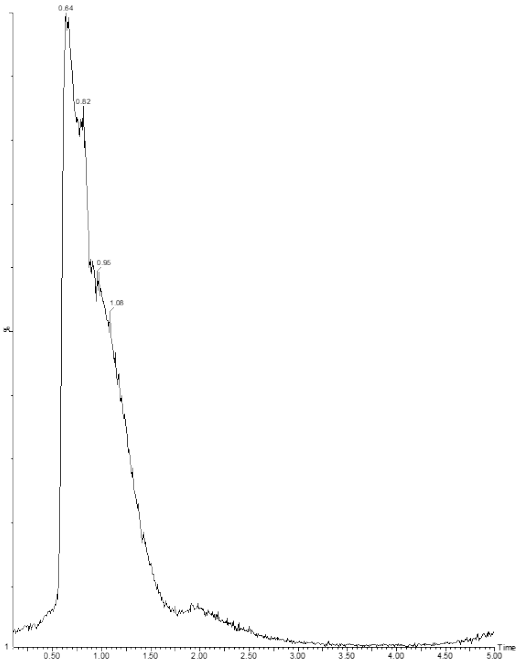


CK_1

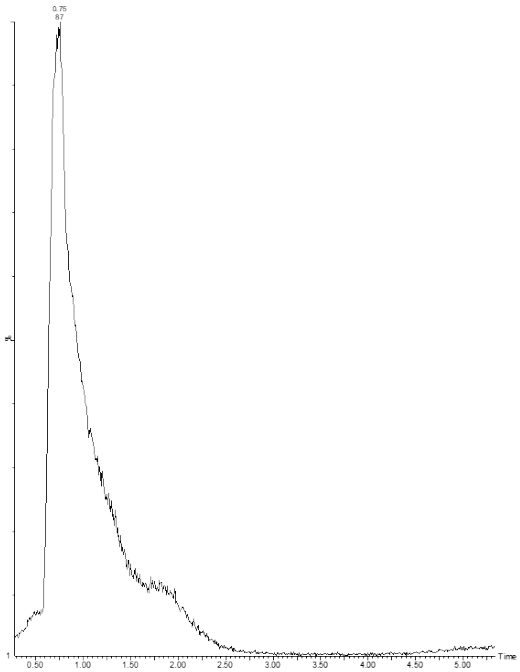
Calculated: m/z 1941,5; found: M/2 +1 = 972,61; M/3 +1 = 648,04

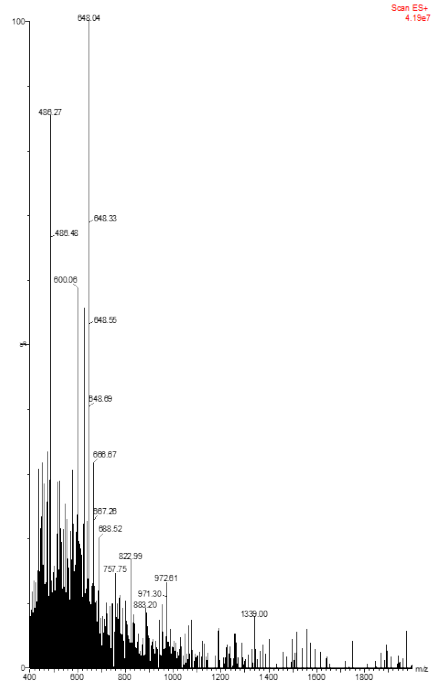


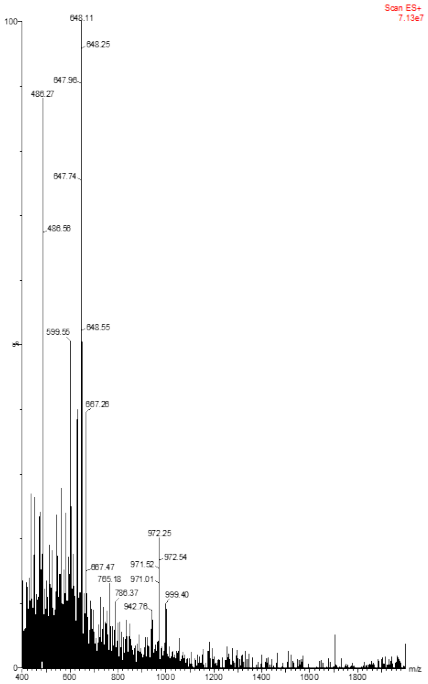
CK_1



CK_1 +MW1/20/50

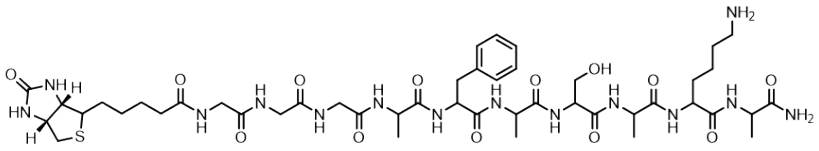


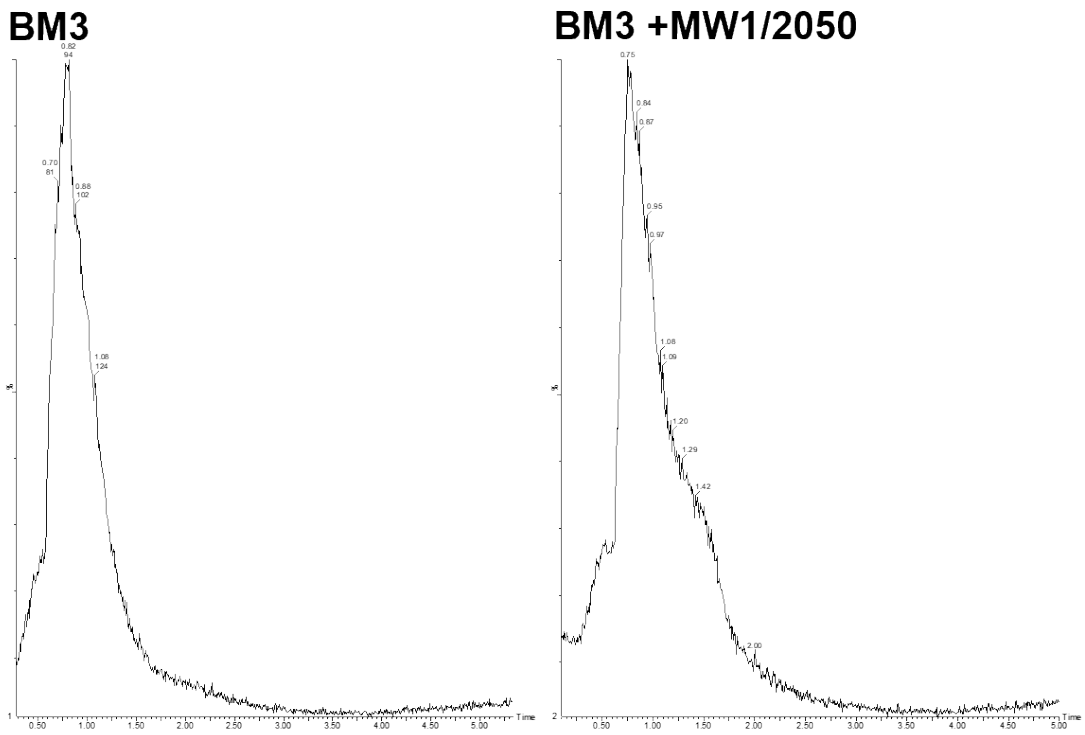


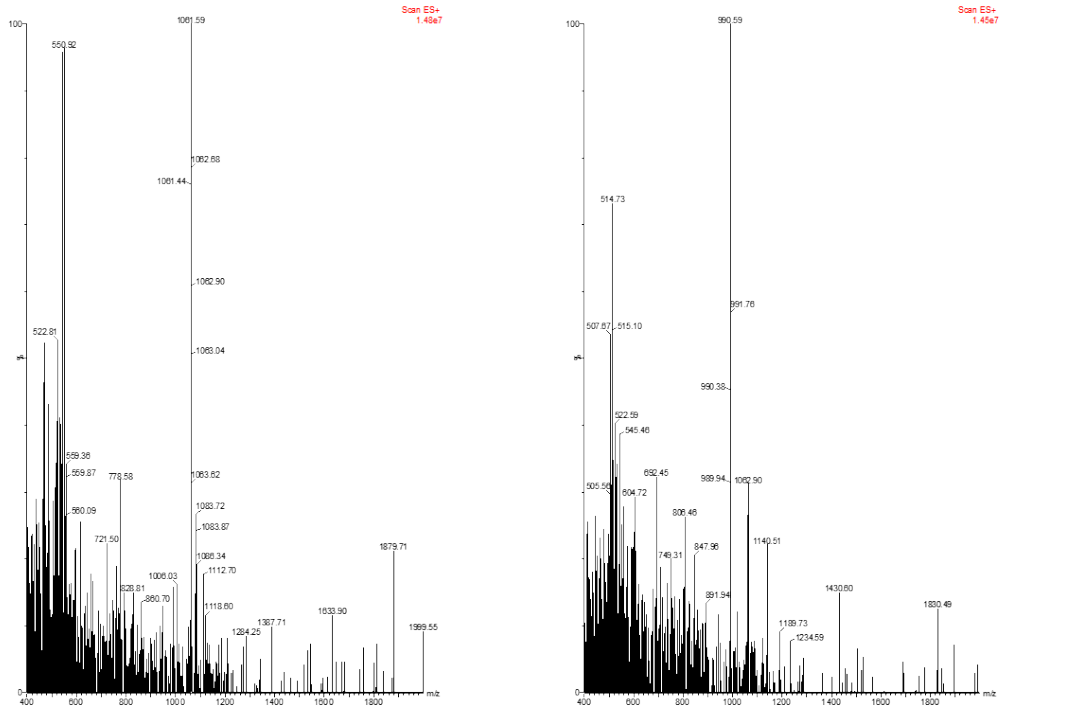


BM3

Calculated: m/z 1061,2; found M +1 = 1062,68

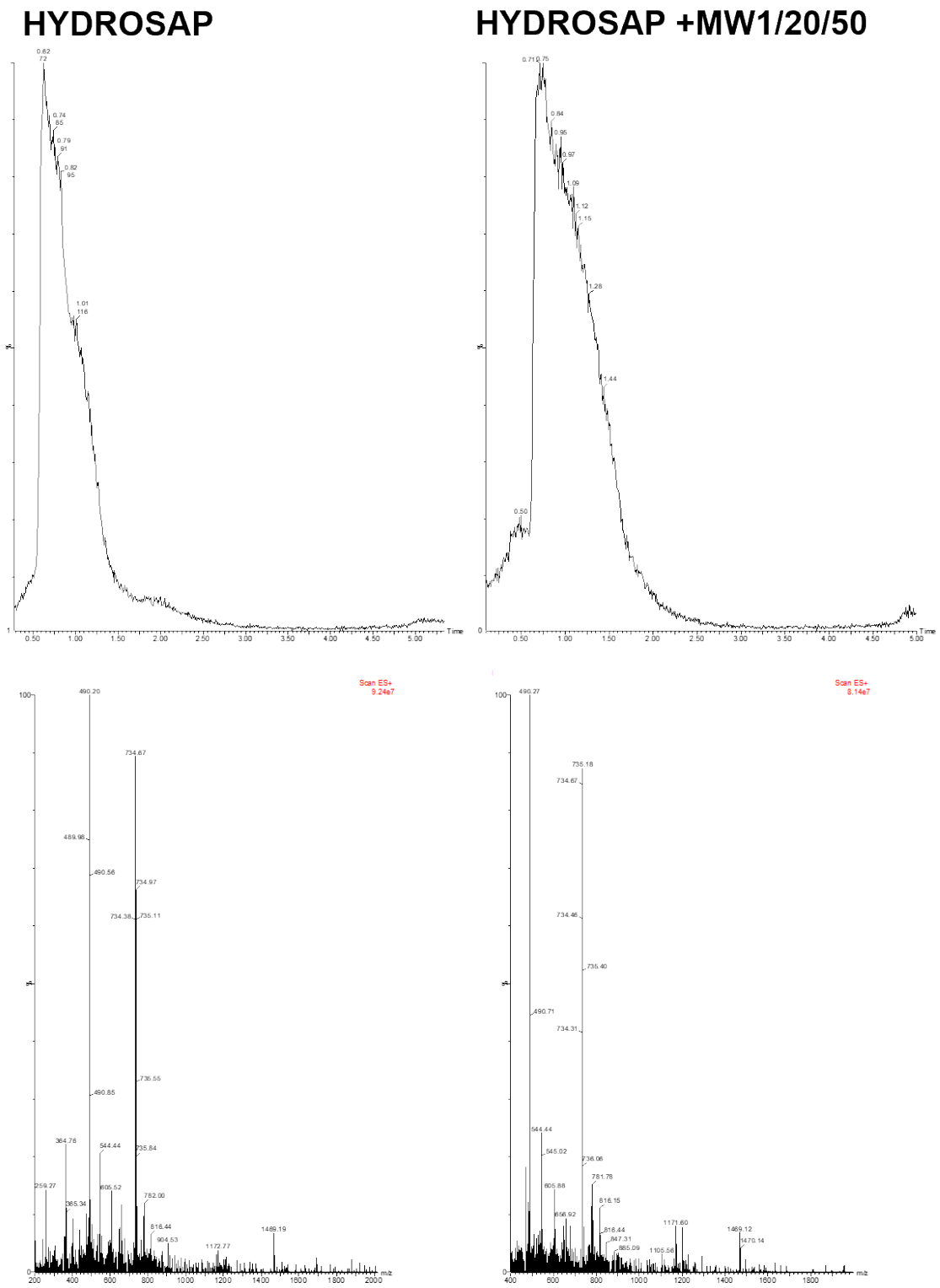






HYDROSAP

Calculated: m/z 1467,7; found: M/2 +1 = 734,67



Pal1

Calculated: m/z 1072,7; found: M +1 = 1073,74; M/2 +1 = 537,45

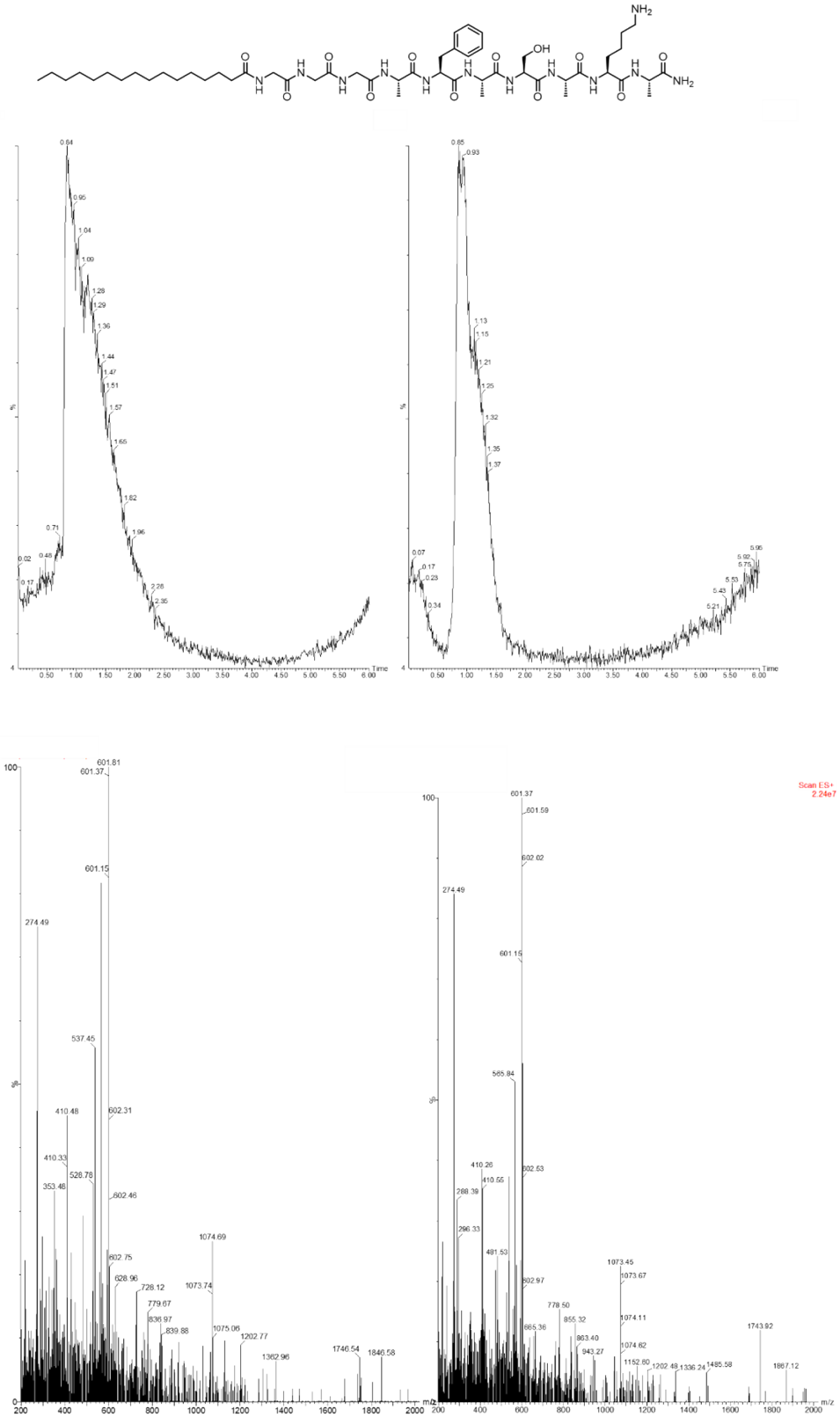


Fig. S8. Structural formulas and ESI-MS analyses of SAPs before and after +MW1/20/50 treatment. LC-MS/MS showed no secondary reaction involving chemical cross-linking after MW treatment in all SAPs.

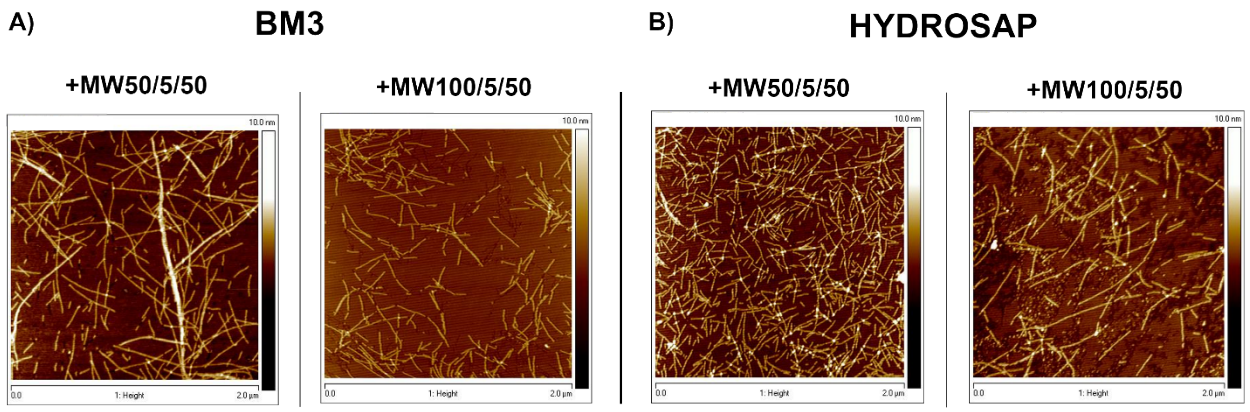


Fig. S9. AFM images of A) BM3 and B) HYDROSAP after or +MW100/5/50 treatments. Both peptides showed a stronger fiber fasciculation in the case of +MW50/5/50 treatment, while +MW100/5/50 caused partial defragmentation of nanofibers, likely because of high-power-induced SAP denaturation. These findings are in agreement with results reported in Fig. 1.

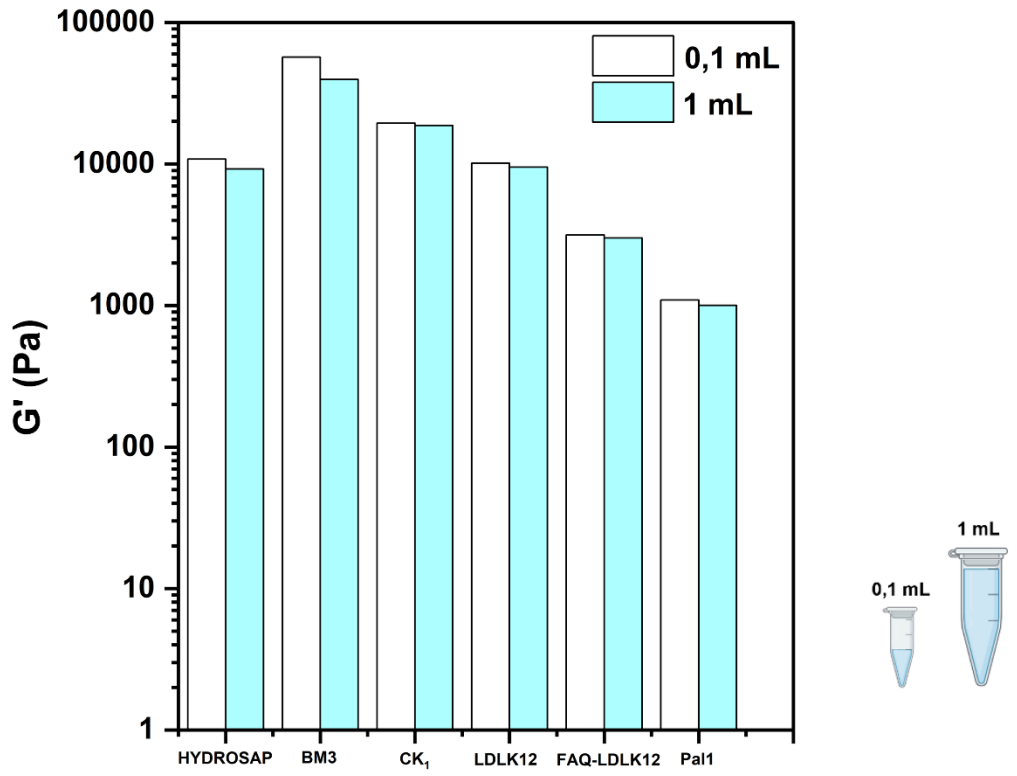


Fig. S10. Comparison between 100 µL and 1 mL scale vials to investigate the mechanical properties. All SAPs presented in this study revealed a similar behaviour when treated in a bigger scale.

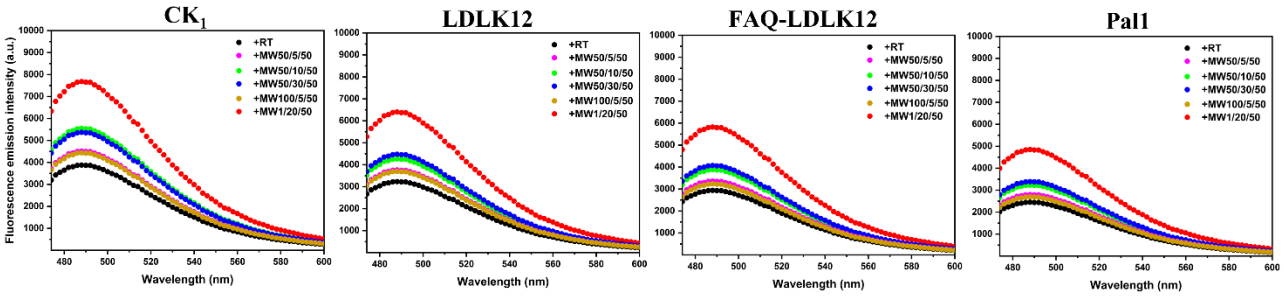


Fig. S11. ThT experiments to compare different MW protocols in the tested SAPs

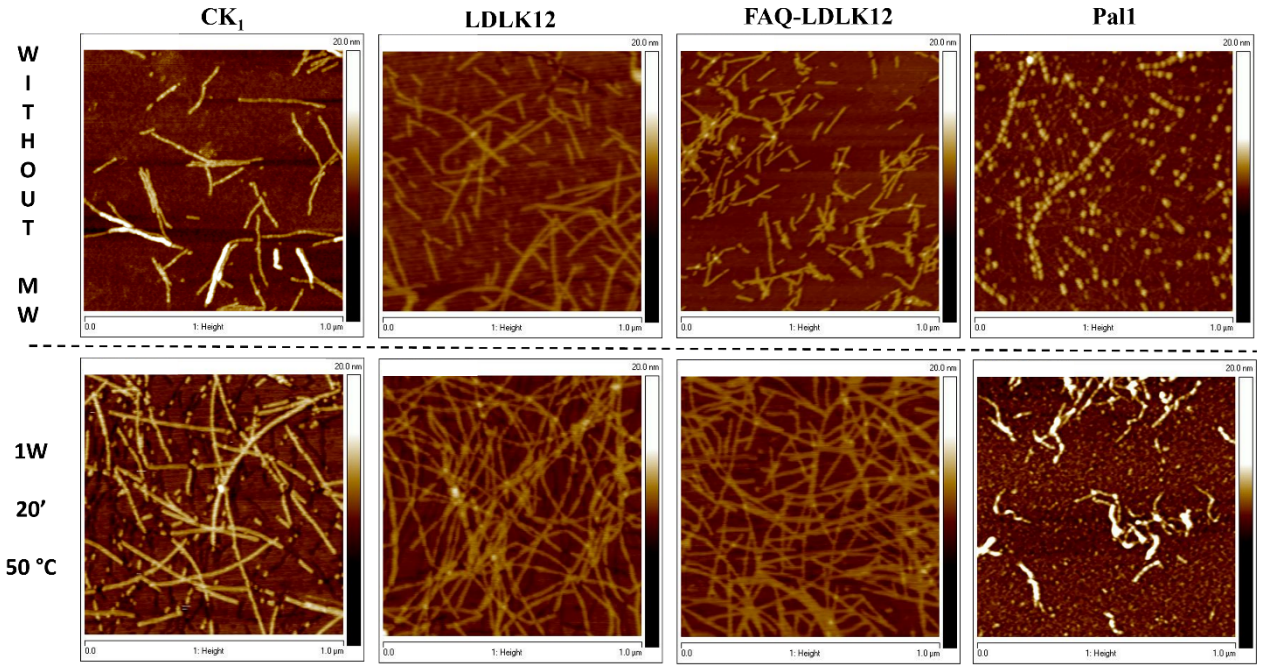


Fig. S12. AFM images of LDLK12, FAQ-LDLK12, CK_1, and Pal1, before and after MW treatment. All studied SAPs showed an increased population of fibers after treatment at 1 W, 20', 50 °C.

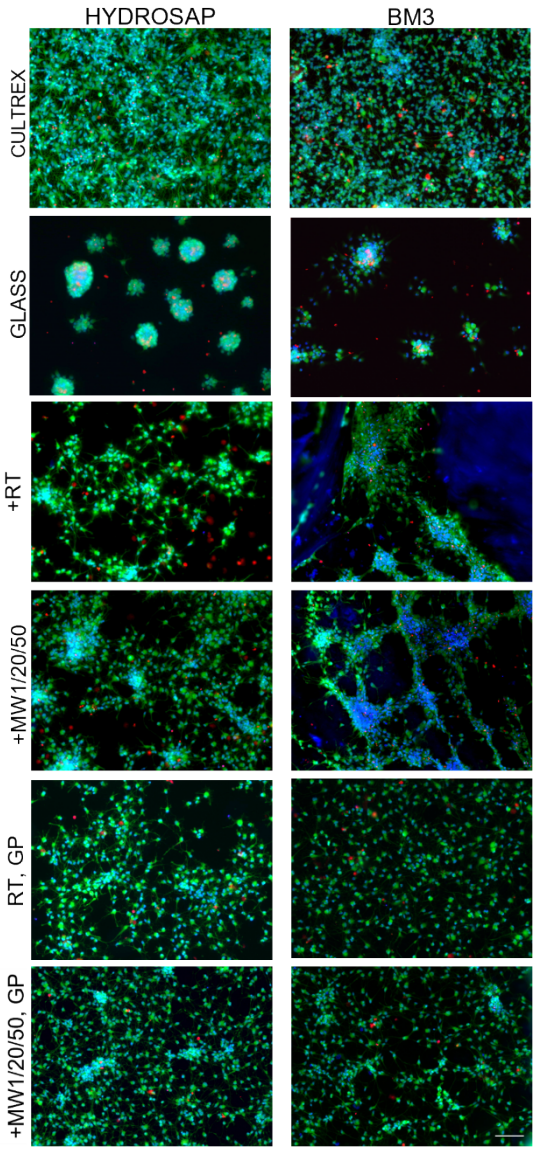


Fig. S13. Representative images (LIVE&DEAD viability assay) of hNSCs cultured for 7 days in vitro. hNSCs were seeded on HYDROSAP and BM3 at different conditions: +RT, +MW1/20/50, +RT, GP and +MW1/20/50, GP. hNSCs cultured on Cultrex and glass surface acted as positive and negative controls respectively. Live cells are stained in green and dead cells in red. Cell nuclei are visualized with HOECHST in blue. Scale bar 100µm.

Peptide	Conditions	Acronym	G′ (Pa)	G′′ (Pa)
BM3	MW, 50W, 5’, 50°C	+MW50/5/50*cp*	9351 ± 454	720 ± 137
BM3	MW, 50W, 10’, 50 °C	+MW50/10/50*cp*	26630 ± 980	2542 ± 52
BM3	MW, 50W, 30’, 50 °C	+MW50/30/50*cp*	37553 ± 146	3104 ± 73
BM3	MW, 50W, 60’, 50 °C	+MW50/60/50*cp*	35231 ± 243	3002 ± 53
BM3	MW, 100W, 5’, 50 °C	+MW100/5/50	6300 ± 115	674 ± 48
HYDROSAP	MW, 50W, 5’, 50 °C	+MW50/5/50*cp*	3386 ± 360	156 ± 15
HYDROSAP	MW, 50W, 10’, 50 °C	+MW50/10/50*cp*	4643 ± 870	176 ± 20
HYDROSAP	MW, 50W, 30’, 50 °C	+MW50/30/50*cp*	6384 ± 980	469 ± 36
HYDROSAP	MW, 50W, 60’, 50 °C	+MW50/60/50*cp*	6479 ± 837	532 ± 78
HYDROSAP	MW, 100W, 5’, 50 °C	+MW100/5/50	931 ± 70	61 ± 14
LDLK12	MW, 50W, 5’, 50 °C	+MW50/5/50*cp*	8334 ± 322	345 ± 69
FAQ-LDLK12	MW, 50W, 5’, 50 °C	+MW50/5/50*cp*	1136 ± 124	83 ± 22
CK_1	MW, 50W, 5’, 50 °C	+MW50/5/50*cp*	12210 ± 632	1157 ± 110
Pal1	MW, 50W, 5’, 50 °C	+MW50/5/50*cp*	699 ± 73	73 ± 11

Table S1. Different MW irradiation protocols vs SAP mechanical properties after overnight incubation followed by gelation with DPBS.

Peptide	GP	Conditions	Acronym	G′ (Pa)	G′′ (Pa)	Max Strain (%)
BM3		MW, 50W, 5', 50°C	+MW/50/5/50*pp*	9815 ± 475	882 ± 126	200-300
BM3		MW, 1W, 20', 50°C	+MW1/20/50*pp*	25910 ± 811	1948 ± 236	300-400
BM3		Without MW, RT	+RT*pp*	8890 ± 360	695 ± 83	200-300
BM3	✓	MW, 50W, 5', 50°C	+MW/50/5/50*pp*	25910 ± 811	1948 ± 236	500-600
HYDROSAP		MW, 50W, 5', 50°C	+MW/50/5/50*pp*	17690 ± 618	733 ± 79	200-300
HYDROSAP		MW, 1W, 20', 50°C	+MW1/20/50*pp*	18500 ± 1130	824 ±83	200-300
HYDROSAP		Without MW, RT	+RT*pp*	8553 ± 3825	457 ± 97	100-200
HYDROSAP	✓	MW, 50W, 5', 50°C	+MW/50/5/50*pp*	47300 ± 1999	4392 ± 310	400-500
LDLK12		MW, 1W, 20', 50°C	+MW1/20/50*pp*	2499 ± 173	136 ± 11	200-300
LDLK12		Without MW, RT	+RT*pp*	1100 ± 141	55 ± 6	100-200
FAQ-LDLK12		MW, 1W, 20', 50°C	+MW1/20/50*pp*	1405 ± 123	171 ± 14	100-200
FAQ-LDLK12		Without MW, RT	+RT*pp*	652 ± 62	49 ± 5	50-100
CK_1		MW, 1W, 20', 50°C	+MW1/20/50*pp*	20380 ± 796	1765 ± 75	600-700
CK_1		Without MW, RT	+RT*pp*	11870 ± 478	811 ± 68	400-500
Pal1		MW, 1W, 20', 50°C	+MW1/20/50*pp*	899 ± 41	56 ± 12	100-200
Pal1		Without MW, RT	+RT*pp*	152 ± 37	34 ± 9	50-100

Table S2. Assessment of the max strain (%) calculated after multiple cycles of strain-sweep tests with incremented maximum strains. Acronyms: overnight incubation (+); MW-treated SAPs or untreated ones (RT); power intensity; time of MW treatments; maximum temperature allowed for the sample; type of geometry used for rheological measurements (*pp*, parallel-plate). Working concentrations are respectively: BM3 2% w/v; HYDROSAP 1% w/v; LDLK12 1% w/v; FAQLDLK 2% w/v; CK_1 5% w/v; Pal1 2% w/v.

Peptide	% w/v	Conditions	Acronym	G′ (Pa)	G″ (Pa)
BM3	2%	without MW, RT	**-RT*cp***	285 ± 23	74 ± 9
HYDROSAP	1%	without MW, RT	**-RT*cp***	633 ± 14	82 ± 6
LDLK12	1%	without MW, RT	**-RT*cp***	446 ± 95	93 ± 12
FAQ-LDLK12	2%	without MW, RT	**-RT*cp***	225 ± 84	31 ± 10
CK_1	5%	without MW, RT	**-RT*cp***	129 ± 18	22 ± 12
Pal1	2%	without MW, RT	**-RT*cp***	85 ± 13	18 ± 7

Table S3. Rheological characterization of SAPs with no MW treatment and without overnight incubation was analysed after a 3 hours time-sweep experiment in presence of Dulbecco's phosphate buffer saline solution (DPBS 1X). As discussed in the main text, the use of these SAPs immediately after dissolution in water is disadvantageous in terms of overall scaffold mechanical properties.

Peptide	GP	Treatment	Wavenumber (cm^{-1})	% β-sheets secondary structure component
LDLK12		+RT	1616, 1692	80
LDLK12		+MW1/20/50	1616, 1692	95
FAQ-LDLK12		+RT	1617, 1692	59
FAQ-LDLK12		+MW1/20/50	1618, 1693	78
HYDROSAP		+RT	1618, 1693	67
HYDROSAP		+MW1/20/50	1618, 1693	82
BM3		+RT	1623, 1700	72
BM3		+MW1/20/50	1623, 1631, 1699	92
CK_1		+RT	1617, 1693	77
CK_1		+MW1/20/50	1618, 1694	91
Pal1		+RT	1617, 1692	49
Pal1		+MW1/20/50	1618, 1693	65
LDLK12	✓	+RT	1622, 1645, 1692	85
LDLK12	✓	+MW1/20/50	1622, 1645, 1692	97
FAQ-LDLK12	✓	+RT	1622, 1645, 1692	65
FAQ-LDLK12	✓	+MW1/20/50	1622, 1645, 1692	81
HYDROSAP	✓	+RT	1621, 1693	75
HYDROSAP	✓	+MW1/20/50	1621, 1693	89
BM3	✓	+RT	1623, 1645, 1694	80
BM3	✓	+MW1/20/50	1623, 1645, 1693	97
CK_1	✓	+RT	1622, 1693	82
CK_1	✓	+MW1/20/50	1622, 1694	94
Pal1	✓	+RT	1622, 1645, 1692	65
Pal1	✓	+MW1/20/50	1622, 1645, 1692	77

Table S4. Peaks analysis in the Amide I band and quantification of percentage of β-sheets secondary structures from ATR-FTIR-deconvolved spectra of LDLK12, FAQ-LDLK12, HYDROSAP, BM3, Pal1 and CK_1. Relative abundance of β-sheets was calculated after elimination of spectral noise and normalization of the deconvoluted individual peak area with the total amide I peak area, followed by multiplication by 100.

Peptide	GP	Treatment	% free primary amino groups
LDLK12	✓	+RT	38
LDLK12	✓	+MW1/20/50	31
FAQ-LDLK12	✓	+RT	35
FAQ-LDLK12	✓	+MW1/20/50	26
HYDROSAP	✓	+RT	36
HYDROSAP	✓	+MW1/20/50	24
BM3	✓	+RT	25
BM3	✓	+MW1/20/50	18
CK_1	✓	+RT	41
CK_1	✓	+MW1/20/50	33
Pal1	✓	+RT	39
Pal1	✓	+MW1/20/50	30

Table S5. Quantification of free amino groups of Genipin cross-linked scaffolds *via* TNBSA assay. All experiments showed a decreased percentage of free amines in samples pre-treated with microwaves.

Supplementary method

2,4,6-Trinitrobenzene Sulfonic Acid (TNBSA) Assay: TNBSA was used to indirectly quantify the degree of the cross-linking, calculated by estimating the free amino groups (lysine residues) present after Genipin cross-linking reaction. TNBSA solution in 0.1 M aqueous buffer at pH 8.5 was added to GP cross-linked samples and stirred for 24 h at 37 ◦C. The reaction mixture was quenched with a solution of 1 N HCl. The measurements were carried out in 1 cm path length micro-fluorescence cell, and the absorbance was quantitied at 335 nm using an Infinite M200 Pro plate reader (Tecan).