SUPPORTING INFORMATION

All-on-solid-phase (AOSP) glycopeptide synthesis.

The following example refers to the coupling of the 5-amino-pentanyl-α-D-mannopyranoside for simplicity, but the same strategy was adopted for the coupling of 5-amino-pentanyl β-D-galactopyranosyl- (1→2)-α-D-mannopyranoside.

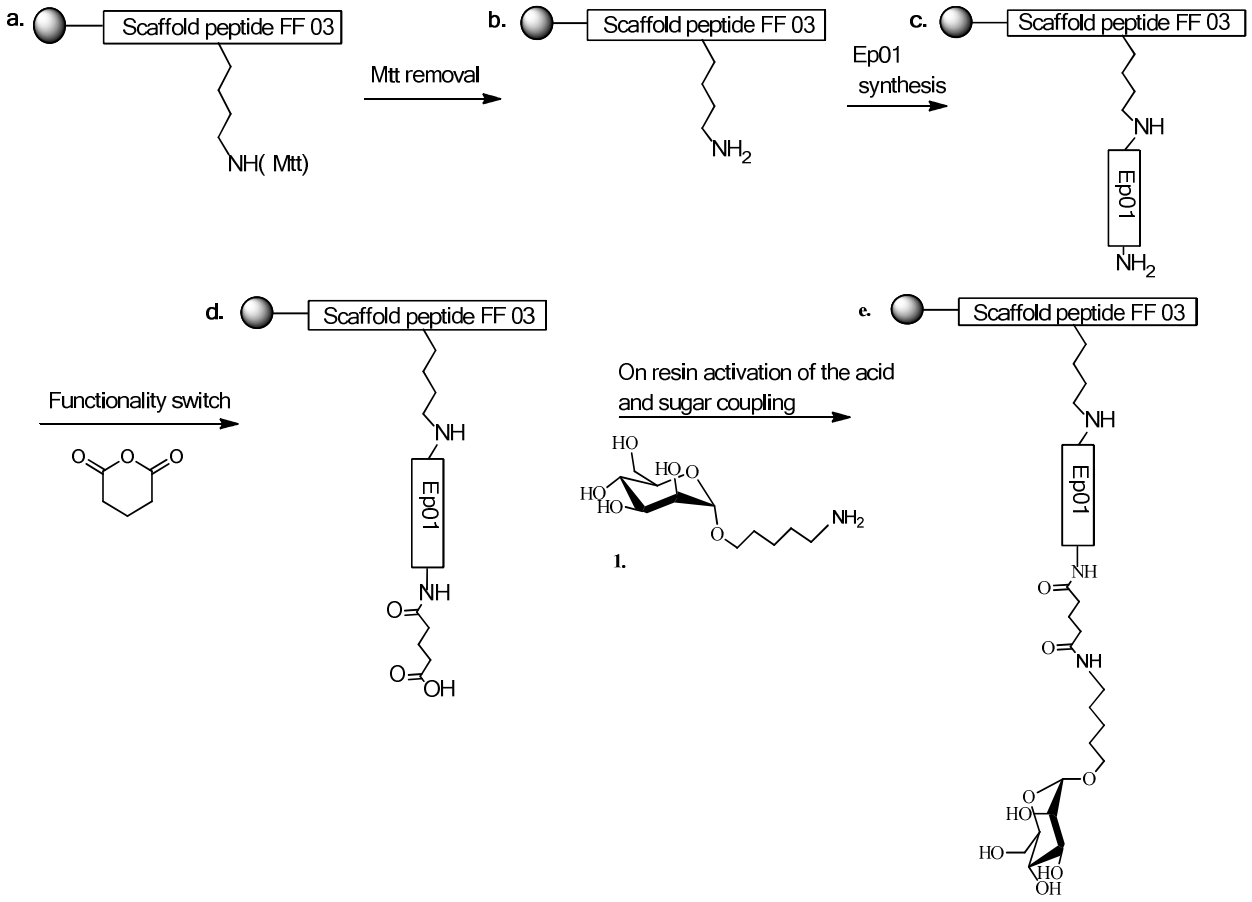
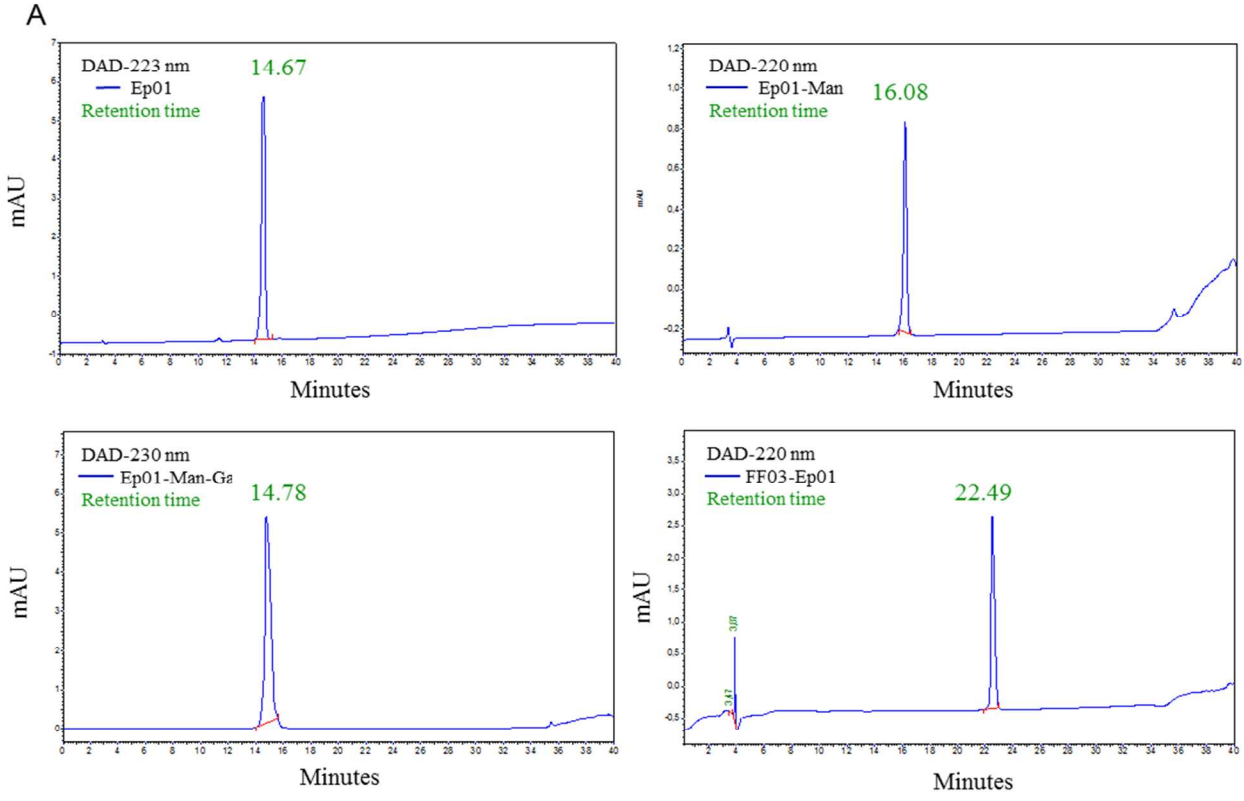


Figure S1. Schematic representation of the synthetic strategy of the glycopeptides FF03-Ep01-Man.

Peptide purification.

Peptide name	Column	Gradient (ACN in H_2O)	Elution time (min.)
Ep01	CAPCELL PAK C18, *Shiseido*	5%-70% in 30 minutes	14.6
Ep01-Man	CAPCELL PAK C18, *Shiseido*	10%-30% in 30 minutes	16.1
Ep01-Man-Gal	CAPCELL PAK C18, *Shiseido*	10%-30% in 30 minutes	14.8
FF03-Ep01	KINETEX 5u C18, Phenomenex	5%-70% in 30 minutes	22.5
FF03-Ep01-Man	KINETEX 5u C18, Phenomenex	30%-60% in 30 minutes	14.1
FF03-Ep01-Man-Gal	KINETEX 5u C18, Phenomenex	30%-60% in 30 minutes	14.6

Table S1: Details relative to peptides purification via analytical HPLC.



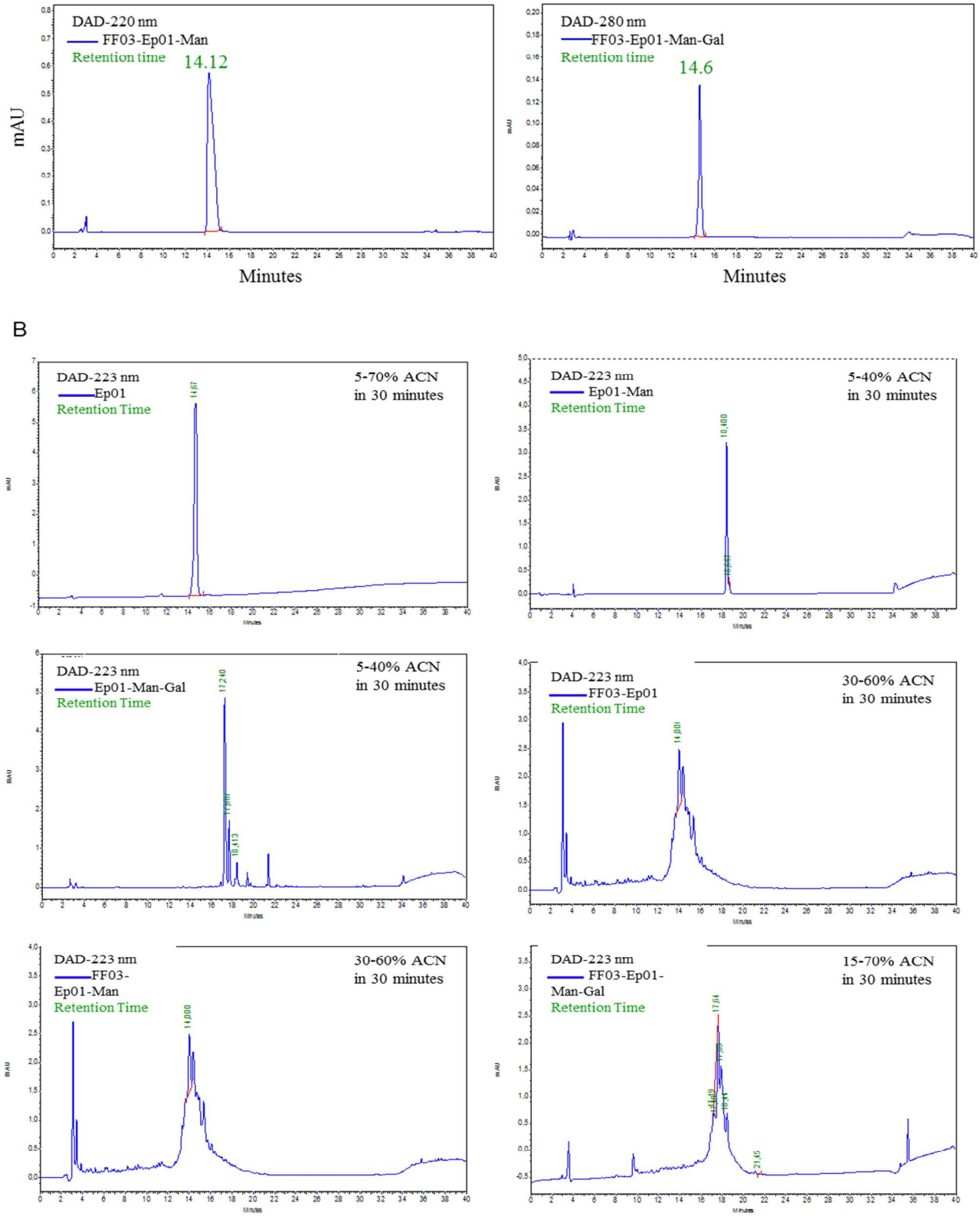
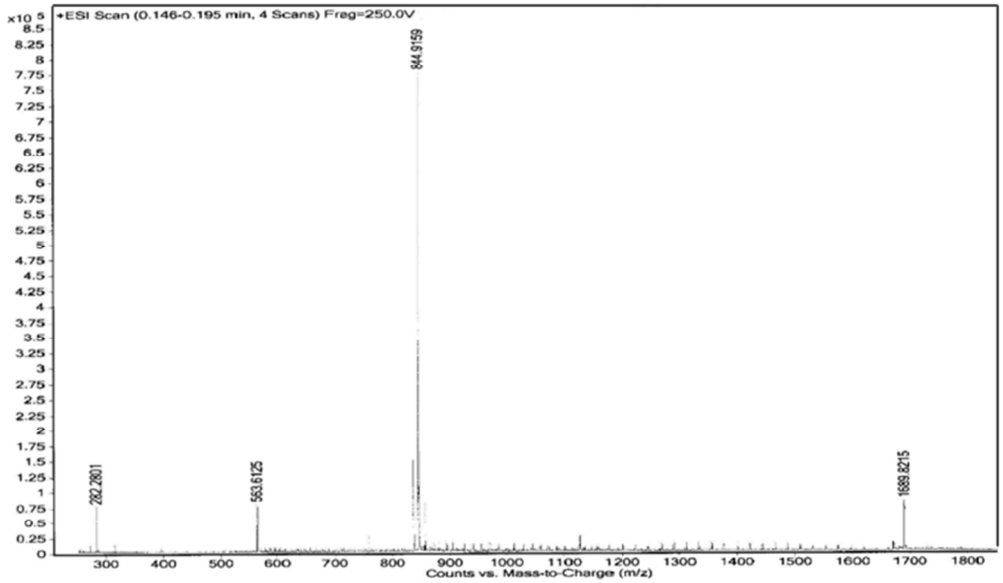


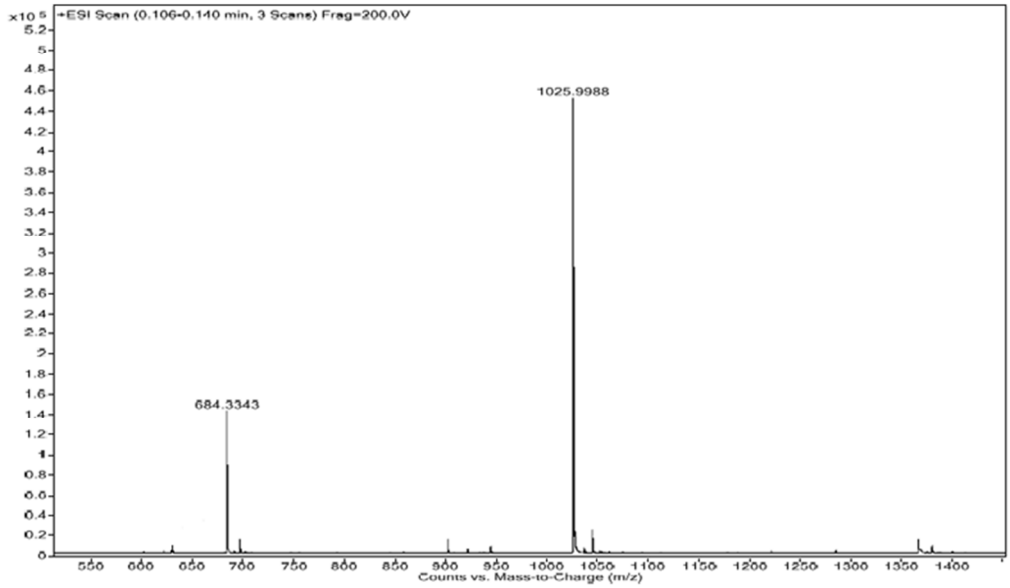
Figure S2. A: HPLC chromatograms of pure peptides. **B**: HPLC chromatograms of crude peptides, with the gradient of acetonitrile (ACN) in water reported on the top right of every picture.

Mass spectroscopy.

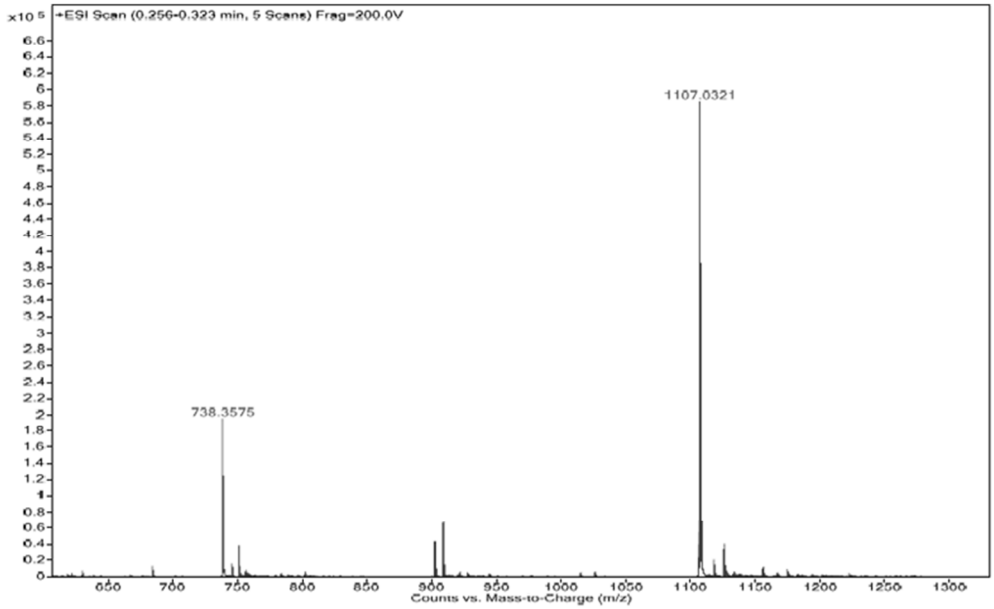
Peptide name	Calculated molecular mass*	Experimental molecular mass
Ep01	1687.806, (+1) 1688.814, (+2) 844.911, (+3) 563.610, (+4) 422.959, (+5) 338.56	(+1) 1689.82, (+2) 844.91, (+3) 563.61



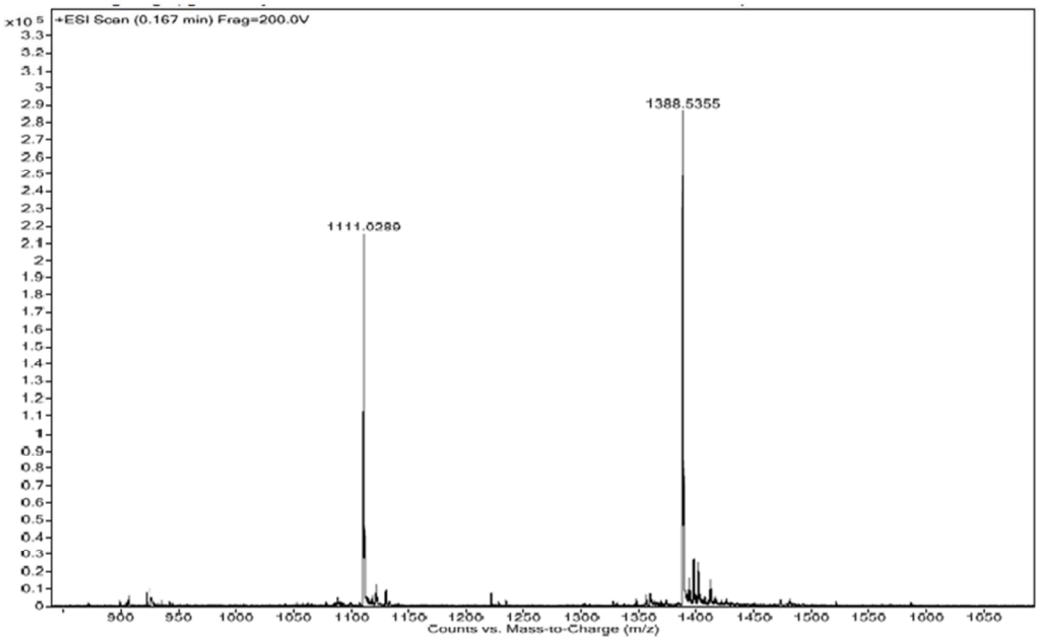
Ep01-Man	2048.956, (+1) 2049.964, (+2) 1025.486, (+3) 683.993, (+4) 513.247, (+5) 410.799	(+2) 1025.99, (+3) 684.33



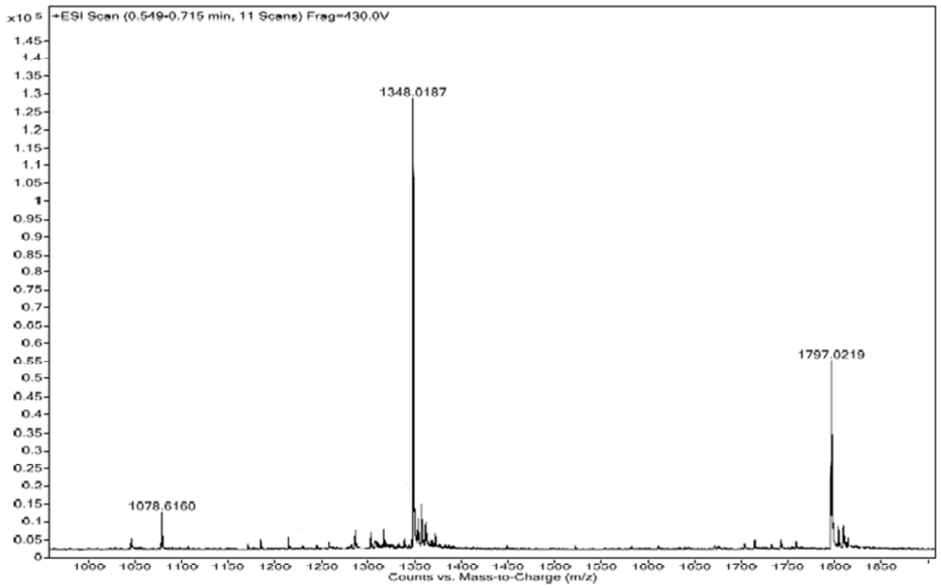
Ep01-Man-Gal	2212.006, (+1) 2212.014,(+2) 1106.511, (+3)738.010, (+4) 553.759, (+5) 443.2091	(+2) 1107.028, (+3) 738.35



FF03-Ep01	5023.961, (+1) 5024.969, (+2) 2512.988, (+3) 1675.661, (+4) 1256.998, (+5) 1005.8, (+6) 838.334, (+7) 718.716	(+3) 1676.63, (+4) 1257.72



FF03-Ep01-Man	5385.112, (+1) 5386.119, (+2) 2693.563, (+3) 1796.045, (+4) 1347.285, (+5) 1078.03	(+3) 1797.022, (+4) 1348.018



FF03-Ep01-Man-Gal	5548.161, (+1)5548.169, (+2) 2774.588, (+3) 1850.061, (+4) 1387.798, (+5) 1110.440	(+3) 1851.03, (+4) 1388.52, (+5) 1111.02

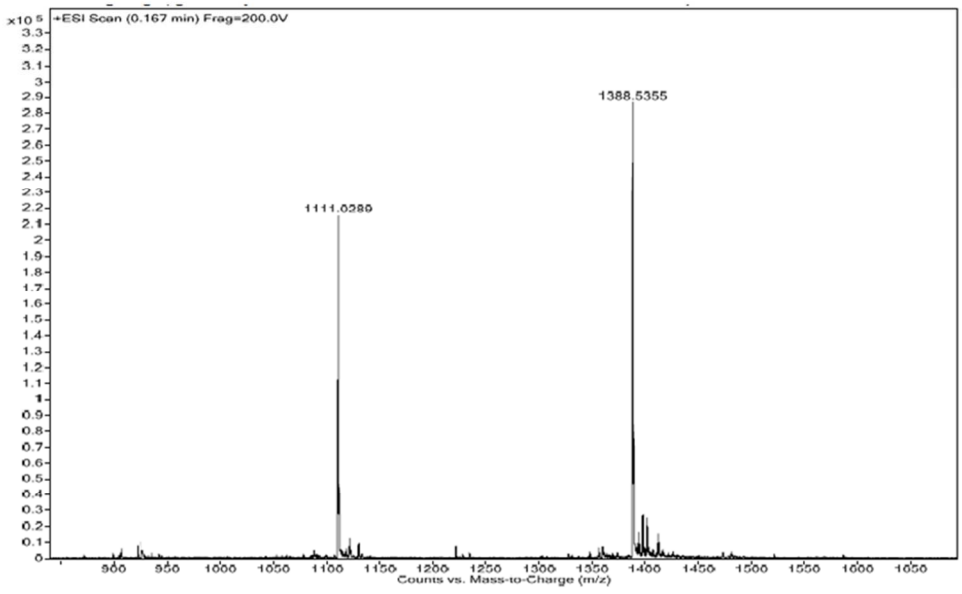


Table S2: Monoisotopic molecular masses of the peptides, *calculated on positive mode with Peptide Mass Calculator v3.2 by Jef Rozenski and experimentally obtained.

CD.

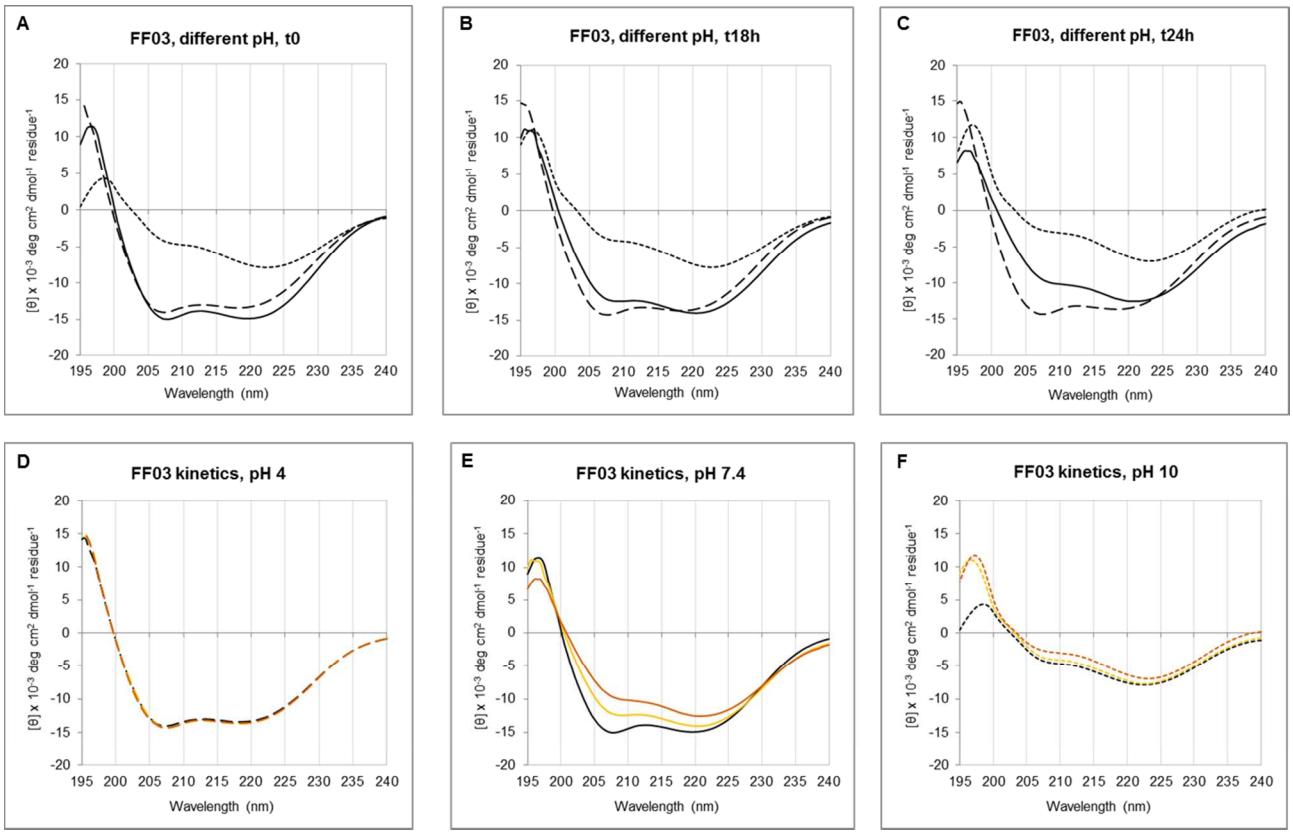


Figure S3. FF03 CD spectra collected at concentrations of 200 μM, at different pH and time from sample preparation. Values normalized on the basis of the extinction coefficient, the path length, the peptide concentration and the number of residues. Solid line: FF03 at pH 7.4; short dashes: FF03 at pH 10; long dashes: FF03 at pH 4. **A-C**: FF03 secondary structure comparison at three different pH values immediately (A), after 18 hours (B) and after 24 hours (C) from sample preparation. **D-F**: FF03 secondary structure comparison at different time points at the pH values 4 (D), 7.4 (E) and 10 (F); black lines indicate t0, yellow lines indicate t18h and orange lines indicate t24h.

CD spectra acquired at different pH are in agreement with the peptide design. At physiological pH and at a concentration of 200 μM, the intensity of the minimum at 208 nm gradually decreases, indicating stronger tendency to form higher supramolecular structures. CD spectra recorded at pH 4

and 10 show opposite behaviour: at pH 4 no change on the spectrum was detected within the 24 hours-analysis, indicating that, probably, under these conditions, only smaller oligomers are formed; at pH 10, FF03 is mostly insoluble (white precipitate can be observed during sample preparation), but the aggregates that are formed still conserve a certain degree of ellipticity. This could indicate rapid formation of larger bundles, which would become insoluble due to their size.

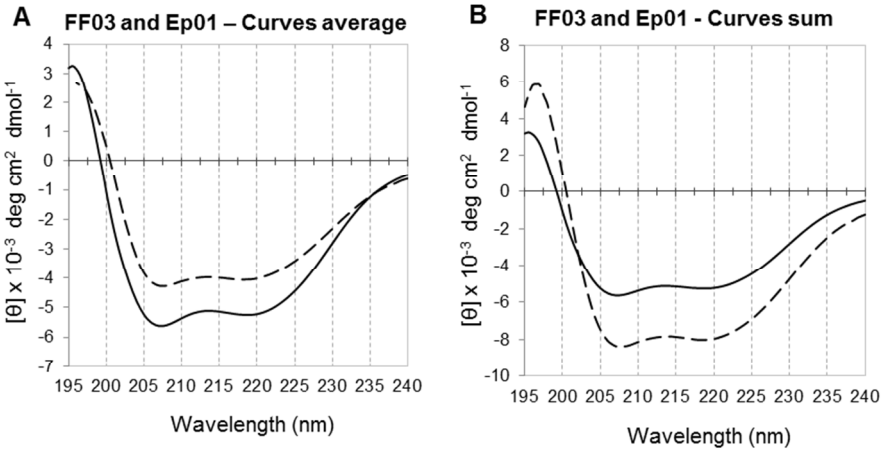
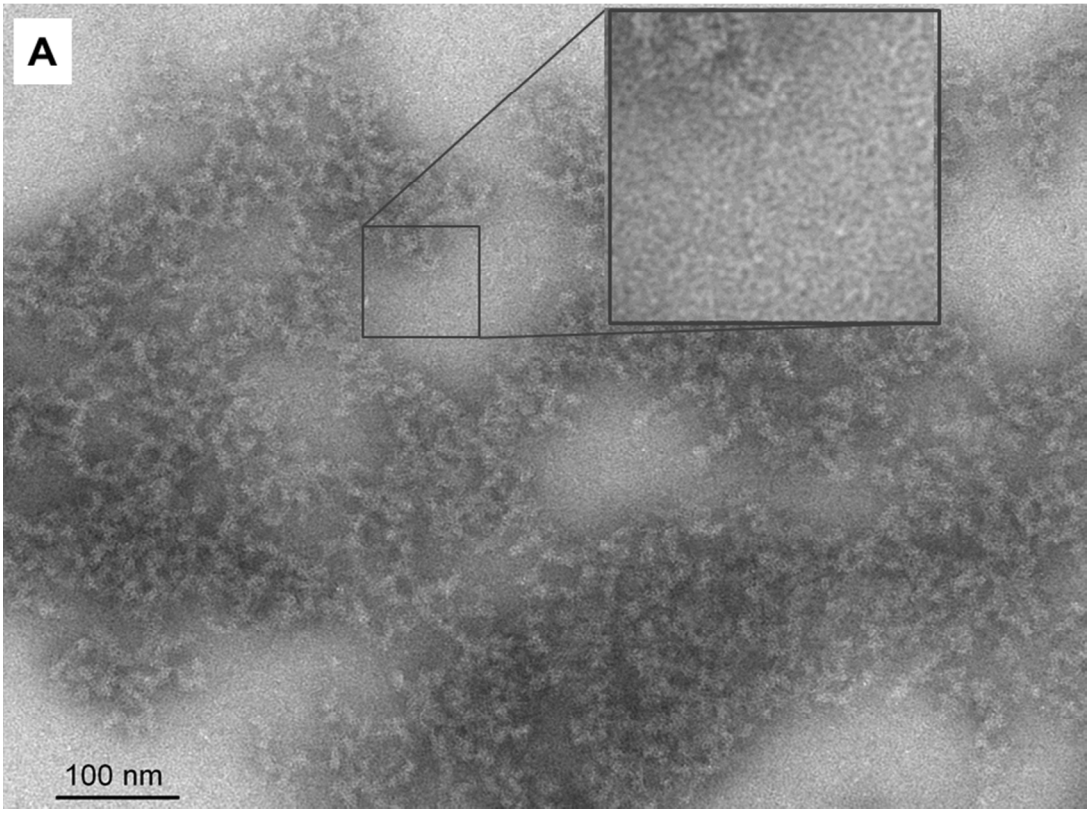
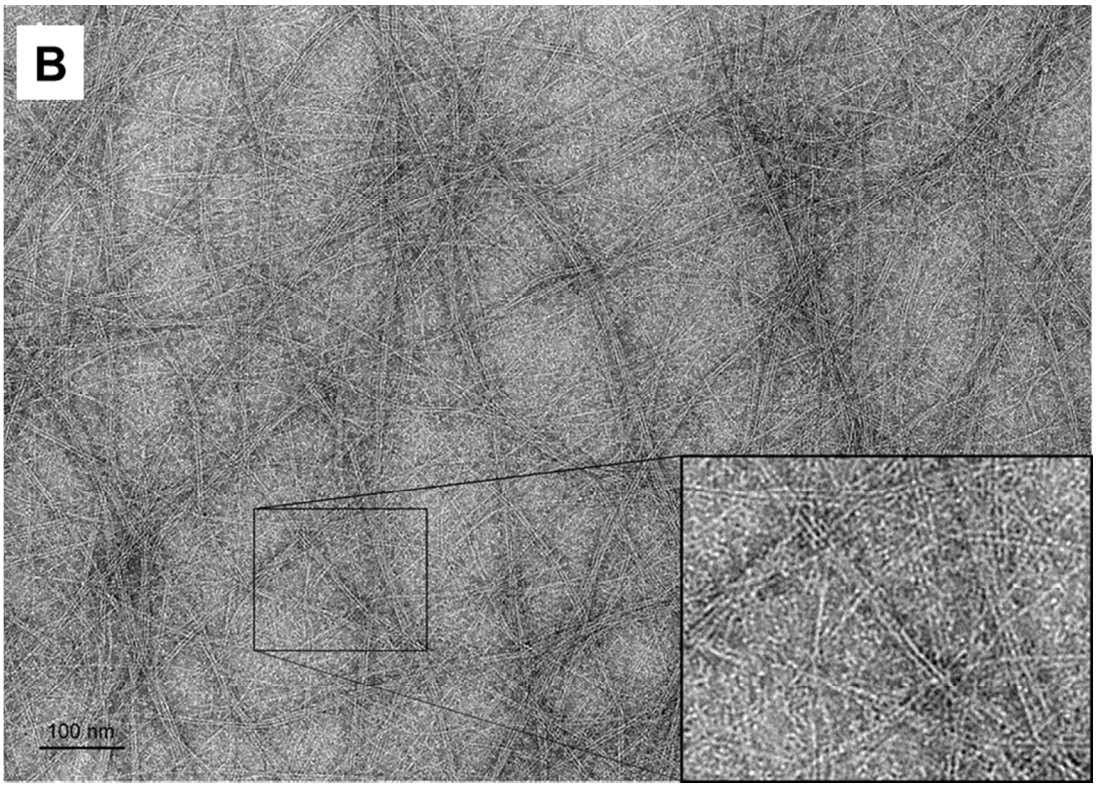


Figure S4. FF03-Ep01 CD spectrum simulations from combined FF03 and Ep01 single curves, resolved with the software OriginPro 9G. Solid line: experimentally obtained FF03-Ep01 peptide CD spectrum; short dashes: CD spectrum simulation obtained by averaging (**A**) or summing (**B**) FF03 and Ep01 separate CD spectra.

By mathematically averaging or adding the two separate curves obtained from the recorded CD spectra of FF03 and Ep01, the restoration of a more intense minimum at 208 nm for FF03-Ep01 is confirmed. This supports out thesis according to which the difference between the CD spectra of FF03 and FF03-Ep01 is not due to a loss of ability to form higher supramolecular structures but to the overall effect of the separate CD profiles of FF03 and Ep01.

TEM and cryo-TEM.





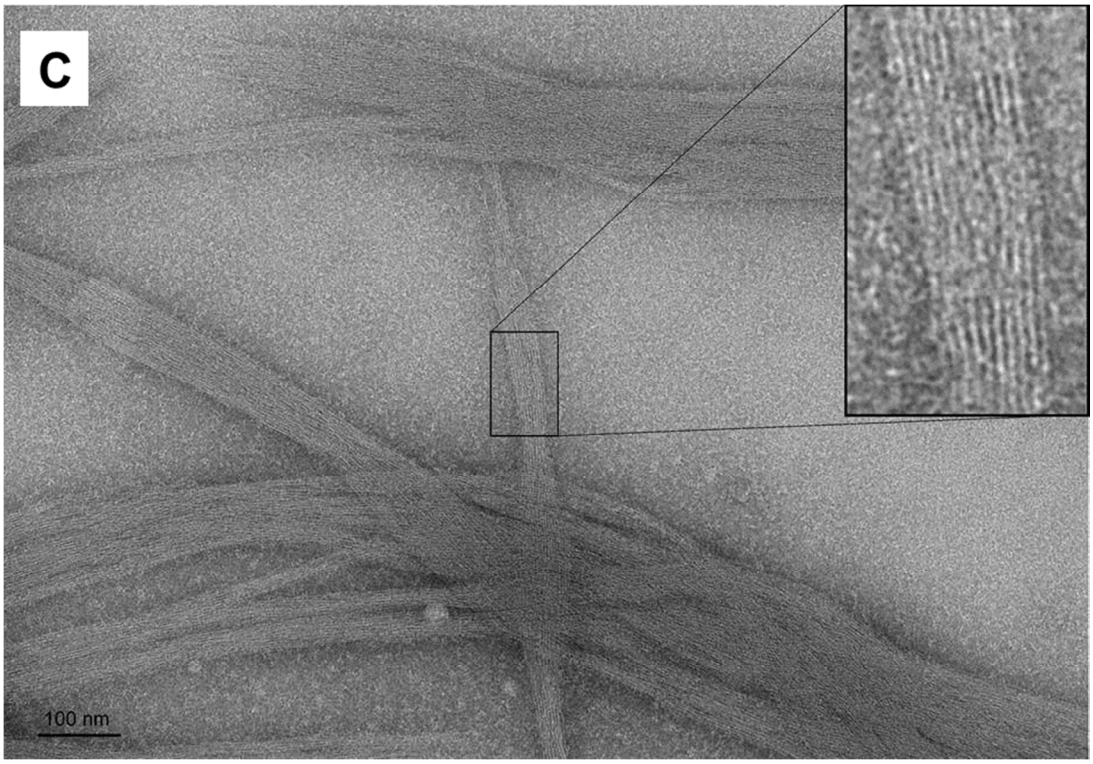
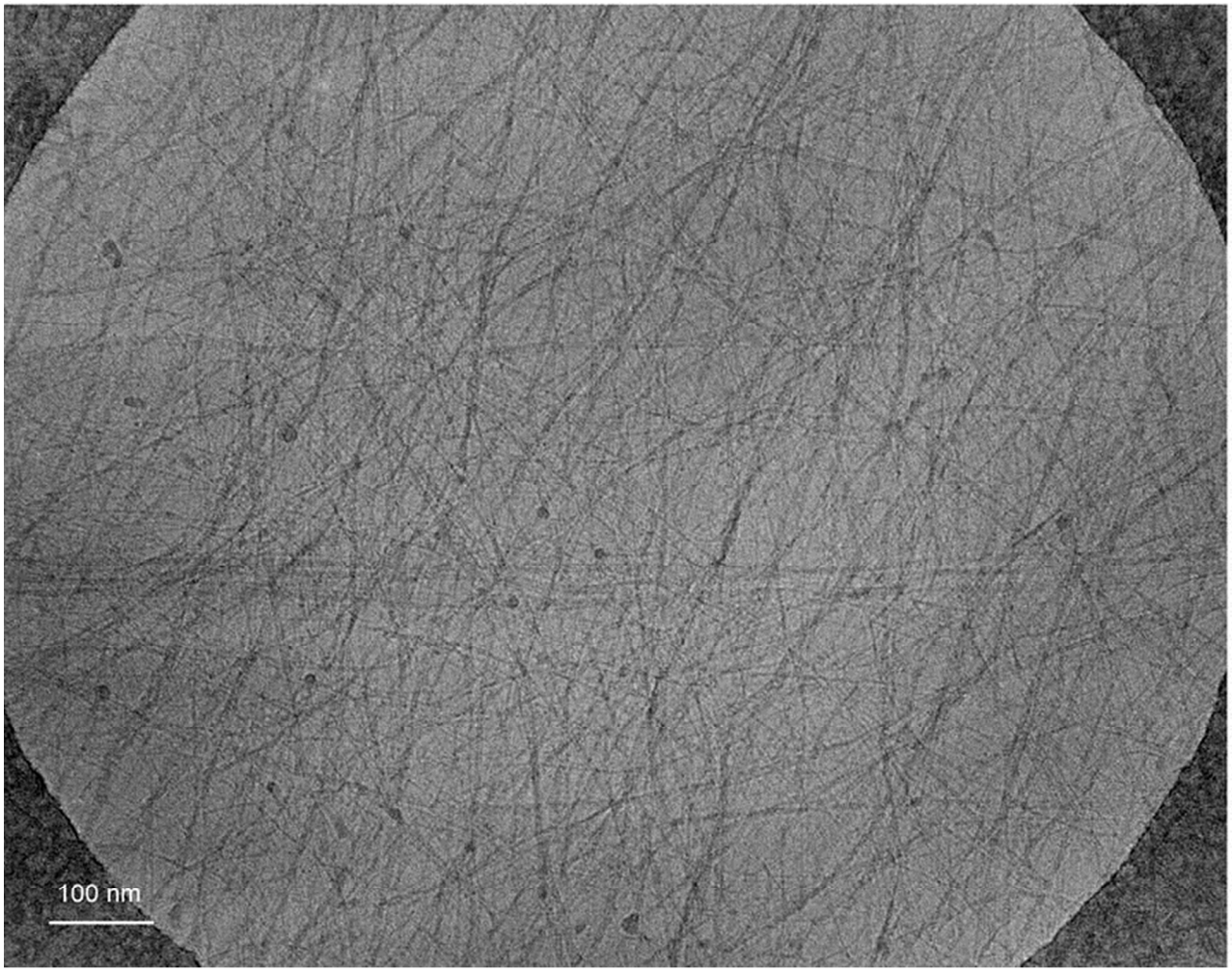
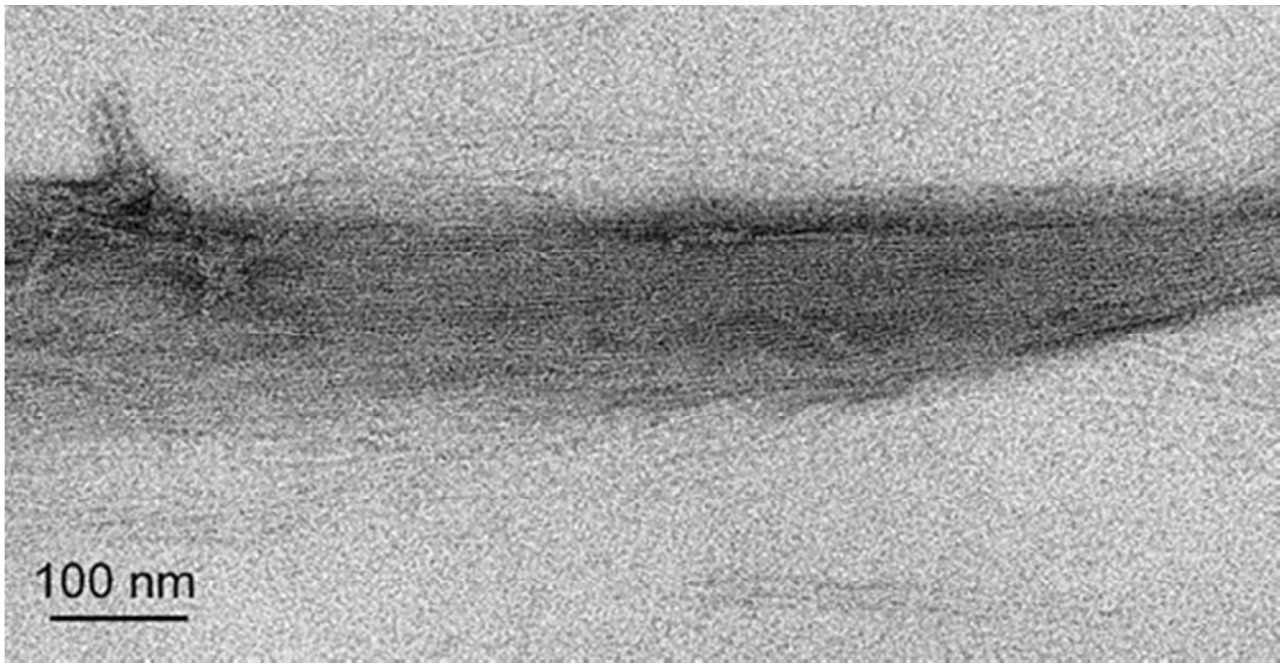


Figure S5. TEM micrographs of FF03 acquired at a concentration of 200 μM, two hours from sample preparation. **A**: pH 4; **B**: pH 7.4; **C**: pH 10. Squares: zoom on a section of the micrograph.

TEM micrographs of FF03 at pH 4 show no long fibers, but only flocculent, most likely a preparation effect. At neutral pH, FF03 form long fibers and bundles, and its appearance is the one of a hydrogel. Basic pH leads FF03 to assemble into very thick bundles and does not allow for a complete dissolution of the peptide.


100 nm


100 nm

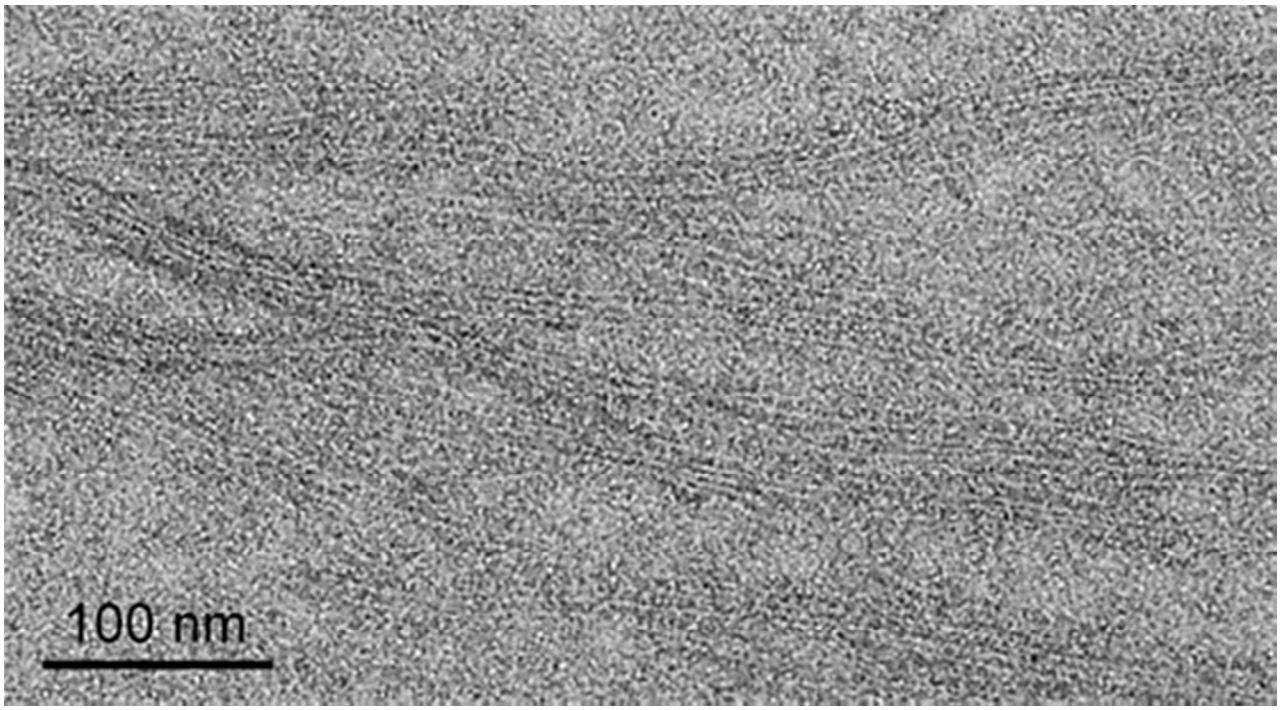
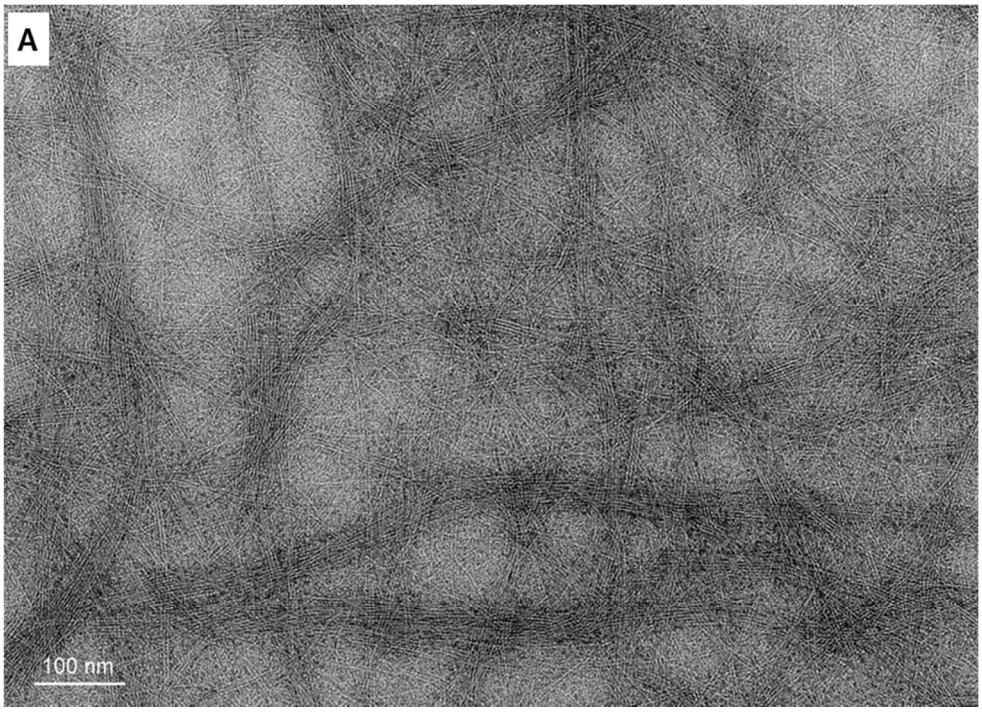


Figure S6. Top: Cryo-TEM of FF03 acquired at a concentration of 200 μM, 24 hours from sample preparation at pH 7.4. **Center and bottom:** TEM micrographs of peptide FF03-Ep01-Man acquired at a concentration of 200 μM, 24 hours from sample preparation.



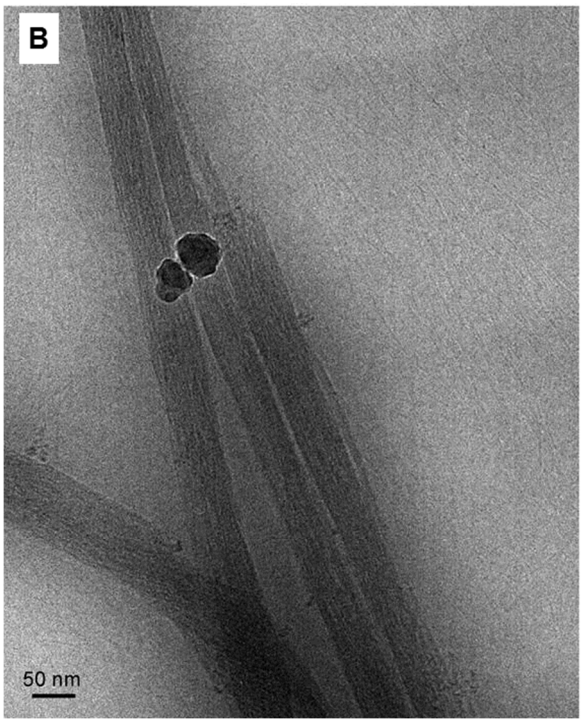
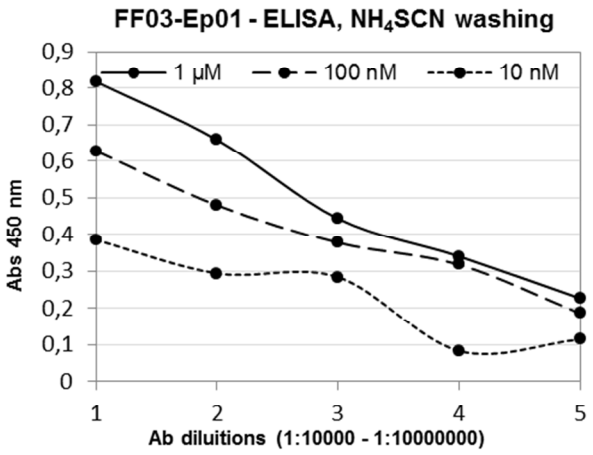


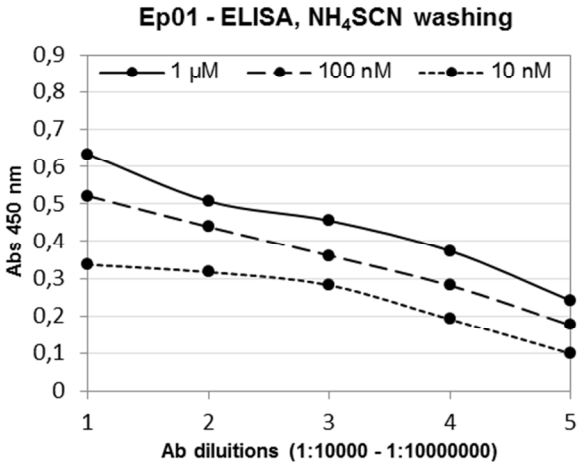
Figure S7. TEM (**A**) and cryo-TEM (**B**) micrographs of FF03 acquired at a concentration of 200 μM, seven days after sample preparation

ELISA.

FF03-Ep01 Abs 450 nm - triplicates (average)			
Serum	Peptide concentration		
dilution	1 μM	100 nM	10 nM
1:10000	0,8165333	0,6272667	0,3833
1:100000	0,6585334	0,4789667	0,29345
1:1000000	0,4425	0,3761	0,28335
1:10000000	0,3392667	0,3167333	0,08275
1:100000000	0,2263333	0,1856667	0,11405



Ep01 Abs 450 nm - triplicates (average)			
Serum	Peptide concentration		
dilution	1 μM	100 nM	10 nM
1:10000	0,634734	0,5219	0,336834
1:100000	0,507834	0,4393	0,316817
1:1000000	0,455634	0,361034	0,281917
1:10000000	0,3736	0,281367	0,190767
1:100000000	0,241	0,1748	0,098217



CRM197 Abs 450 nm - triplicates (average)			
Serum	Protein concentration		
dilution	1 μM	100 nM	10 nM
1:10000	0,748533	0,775267	0,7642
1:100000	0,702633	0,739867	0,74685
1:1000000	0,699233	0,7201	0,67485
1:10000000	0,669767	0,637133	0,60965
1:100000000	0,615733	0,608933	0,5519

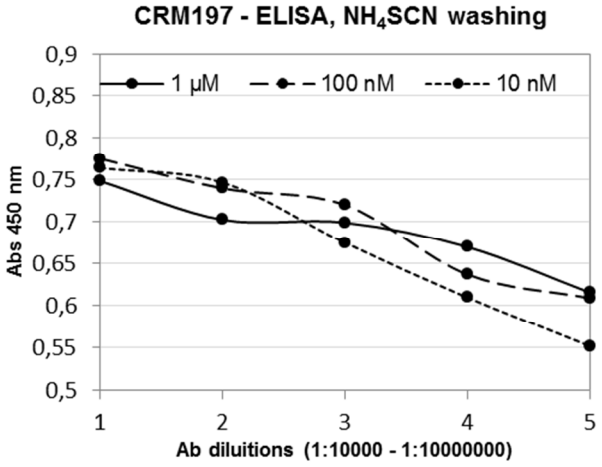


Figure S8. Left: average of triplicates for absorbance values recorded at 450 nm during ELISA for FF03-Ep01, Ep01 and CRM197 (positive control). **Right**: data plot of the results.

Optical microscope images.

The peptide macro-aggregates were observed using the optical microscope Axiovert 40 C by Zeiss, with the objective LD A-Plan 40x/0.5 Ph2 Var2.These over-1-µm- wide structures are found in highly concentrated samples (from 1 mg/ml) but they are not easy to detect at lower concentrations.

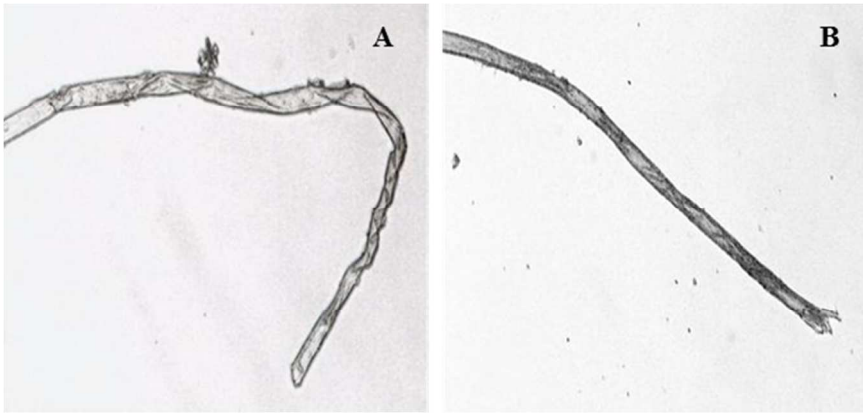
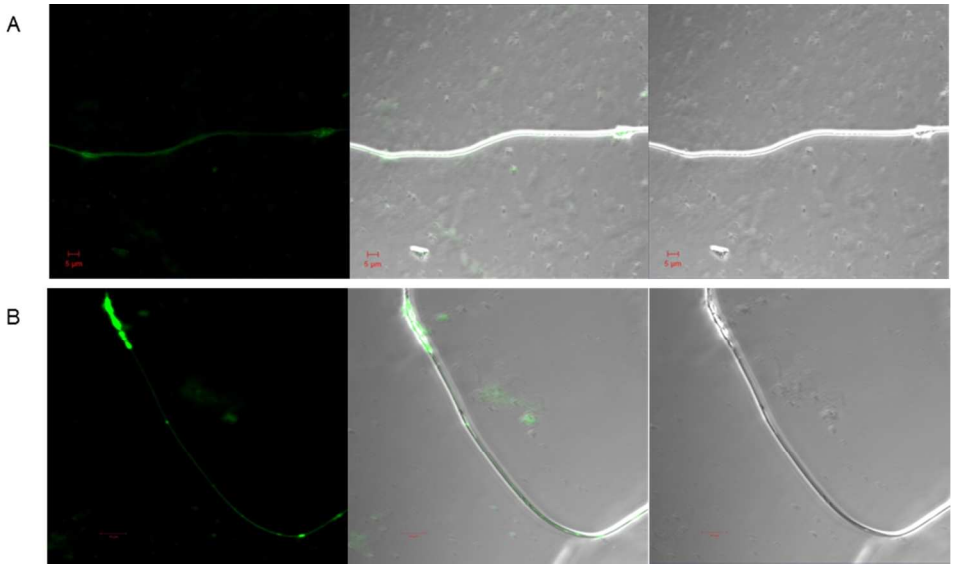


Figure S9. Confocal microscope images of **A:** FF03-Ep01; and **B:** FF03-Ep01-Man on poli-lysine plates, 1.5 mg/ml in PBS, pH 7.4.

ConA-FITC binding assay.

Additional CLSM pictures of ConA-FITC with FF03-Ep01 and FF03-Ep01-Man are provided.



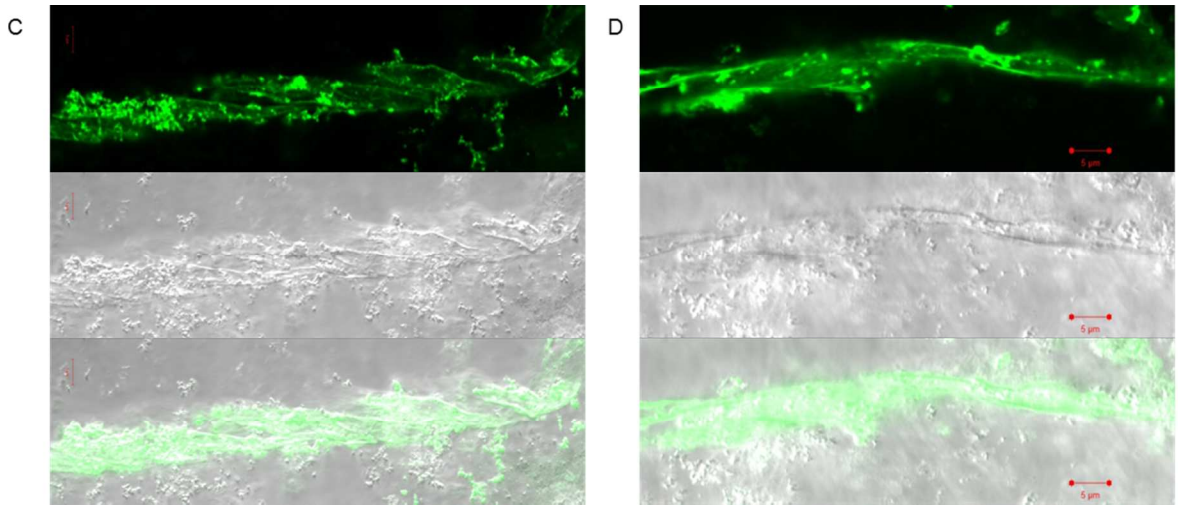


Figure S10. CLSM images of ConA-FITC incubated with FF03-Ep01 (**A** and **B**) and FF03-Ep01-Man (**C** and **D**).

Mannose-binding bacteria assay.

Images relative to the 2-hours-incubation of DAPI-stained *E. coli* ORN 178 with FF03-Ep01 and FF03-Ep01-Man.

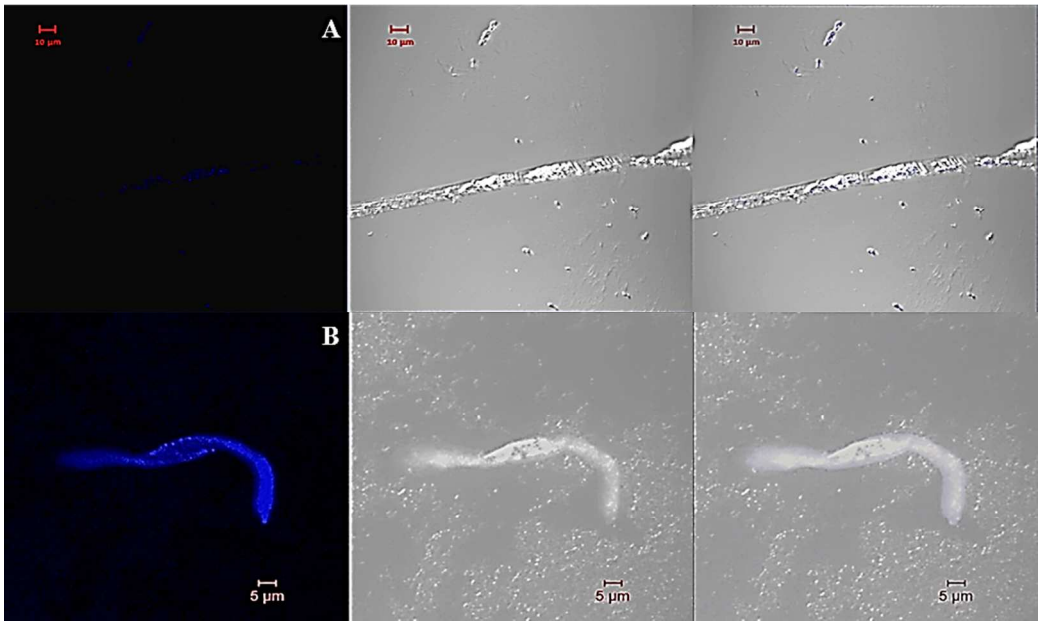


Figure S11. Optical sections of confocal laser-scanning images of FF03-Ep01 (**A**) and FF03-Ep01-Man (**B**).

Microdot assay.

	A	B	C	D	E	F
1	CRM 10 µM	CRM 100 µM	Ep01 10 µM	Ep01 100 µM	FF03-Ep01 10 µM	FF03-Ep01 100 µM
2		CRM 100 µM	Ep01 10 µM	Ep01 100 µM	FF03-Ep01 10 µM	FF03-Ep01 100 µM
3	Man 10 µM	Man 100 µM	Ep01-Man 10 µM	Ep01-Man 100 µM	FF03-Ep01-Man 10 µM	FF03-Ep01-Man 100 µM
4	Man 10 µM	Man 100 µM	Ep01-Man 10 µM	Ep01-Man 100 µM	FF03-Ep01-Man 10 µM	FF03-Ep01-Man 100 µM
5	Man-Gal 10 µM	Man-Gal 100 µM	Ep01-Man-Gal 10 µM	Ep01-Man-Gal 100 µM	FF03-Ep01-Man-Gal 10 µM	FF03-Ep01-Man-Gal 100 µM
6	Man-Gal 10 µM	Man-Gal 100 µM	Ep01-Man-Gal 10 µM	Ep01-Man-Gal 100 µM	FF03-Ep01-Man-Gal 10 µM	FF03-Ep01-Man-Gal 100 µM

Figure S12. Representation of a microdot assay slide, indicating position and concentration of peptide/protein.

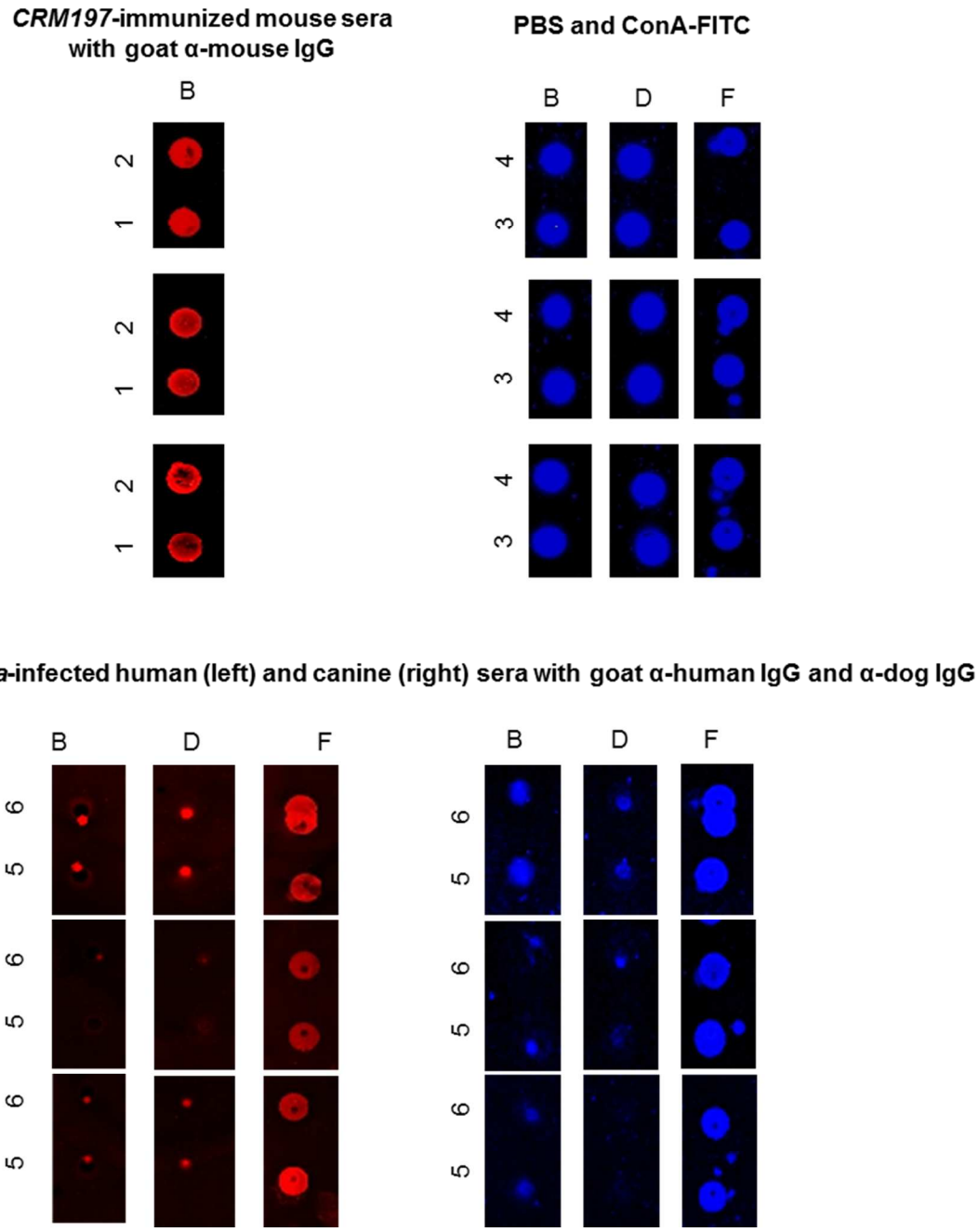


Figure S13. Examples of three of the 64 grids used in the microdot assay. The fluorescent are relative to the peptide/protein/carbohydrate concentration reported in Figure 9 of this manuscript (100 μM).