Molecular aspects of the interaction with Gram- and Gram+ bacteria of hydrothermal carbon nanoparticles associated to Bac8c2,5Leu antimicrobial peptide

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SUPPORTING INFORMATION

Table S1. Desorption Indexes (DI) following incubation of SL-Bac8c^{2,5Leu}@CNP-S with BSA

	DI values at pH 7.4	**DI values at pH 4.0**
In the absence of BSA	0.052	0.61
+ Equimolar BSA	0.30	0.22
+ Excess BSA	0.42	0.18

Table S2. Analysis of the logarithmic differences of bacterial viability between all the samples tested and the CTRL- or the unloaded CNP-S at the three concentrations tested for *S. aureus* SH1000. The positive values are referred to bacterial growth while the negative ones to bacterial decrease. *T tests* were performed to determine the statistical significance of the differences between the means of the couple of samples considered. Stars: * *p value* < 0.05; ** *p value* < 0.01.

CNP 53.3 µg/ml	AMP 16 µg/ml		
Time (h)	**Samples**		
	AMP – CTRL-	**CNP-S – CTRL-**	**Bac8c^{2,5Leu}@CNP-S – CTRL-**	**Bac8c^{2,5Leu}@CNP-S - CNP-S**
0	0,00	0,00	0,00	0,00
2	-0,09	0,17	-0,06	-0,22
5	-0,15	** 0,57	** 0,44	-0,13
24	** -2,57	** 1,82	** -1,19	** -3,01

CNP 26.7 µg/ml	AMP 8 µg/ml		
Time (h)	**Samples**		
	AMP – CTRL-	**CNP-S – CTRL-**	**Bac8c^{2,5Leu}@CNP-S – CTRL-**	**Bac8c^{2,5Leu}@CNP-S - CNP-S**
0	0,00	0,00	0,00	0,00
2	-0,30	0,18	-0,06	-0,24
5	-0,16	** 0,60	** 0,52	-0,09
24	** -2,04	** 1,76	-0,03	** -1,80

CNP 13.3 µg/ml	AMP 4 µg/ml		
Time (h)	**Samples**		
	AMP – CTRL-	**CNP-S – CTRL-**	**Bac8c^{2,5Leu}@CNP-S – CTRL-**	**Bac8c^{2,5Leu}@CNP-S – CNP-S**
0	0,00	0,00	0,00	0,00
2	0,18	0,11	** -0,24	-0,35
5	0,33	** 0,64	* 0,27	-0,37
24	** -0,69	** 1,41	0,00	** -1,41

Table S3. Analysis of the logarithmic differences of bacterial viability between all the samples tested and the CTRL- or the unloaded CNP-S at the three concentrations tested for *E. coli* MG1655. The positive values are referred to bacterial growth while the negative ones to bacterial decrease. *T tests* were performed to determine the statistical significance of the differences between the means of the couple of samples considered. Stars: * *p value* < 0.05; ** *p value* < 0.01.

CNP 53.3 µg/ml | AMP 16 µg/ml

Time (h)	Samples			
	AMP – CTRL-	CNP-S – CTRL-	Bac8c^{2,5Leu}@CNP-S – CTRL-	Bac8c^{2,5Leu}@CNP-S - CNP-S
0.25	0,00	0,00	0,00	0,00
2	** -5,36	** -0,45	** -0,38	0,07
5	** -5,98	-0,07	** -0,87	** -0,80
24	** -6,49	0,15	0,04	-0,11

CNP 26.7 µg/ml | AMP 8 µg/ml

Time (h)	Samples			
	AMP – CTRL-	CNP-S – CTRL-	Bac8c^{2,5Leu}@CNP-S – CTRL-	Bac8c^{2,5Leu}@CNP-S - CNP-S
0.25	0,00	0,00	0,00	0,00
2	** -3,03	** -0,33	** -0,32	0,01
5	** -5,98	-0,11	** -0,82	** -0,71
24	** -6,49	0,06	-0,05	-0,11

CNP 13.3 µg/ml | AMP 4 µg/ml

Time (h)	Samples			
	AMP – CTRL-	CNP-S – CTRL-	Bac8c^{2,5Leu}@CNP-S – CTRL-	Bac8c^{2,5Leu}@CNP-S - CNP-S
0.25	0,00	0,00	0,00	0,00
2	* -0,91	-0,02	-0,13	-0,11
5	** -1,99	-0,07	** -0,51	** -0,45
24	** -2,72	0,13	-0,08	-0,21

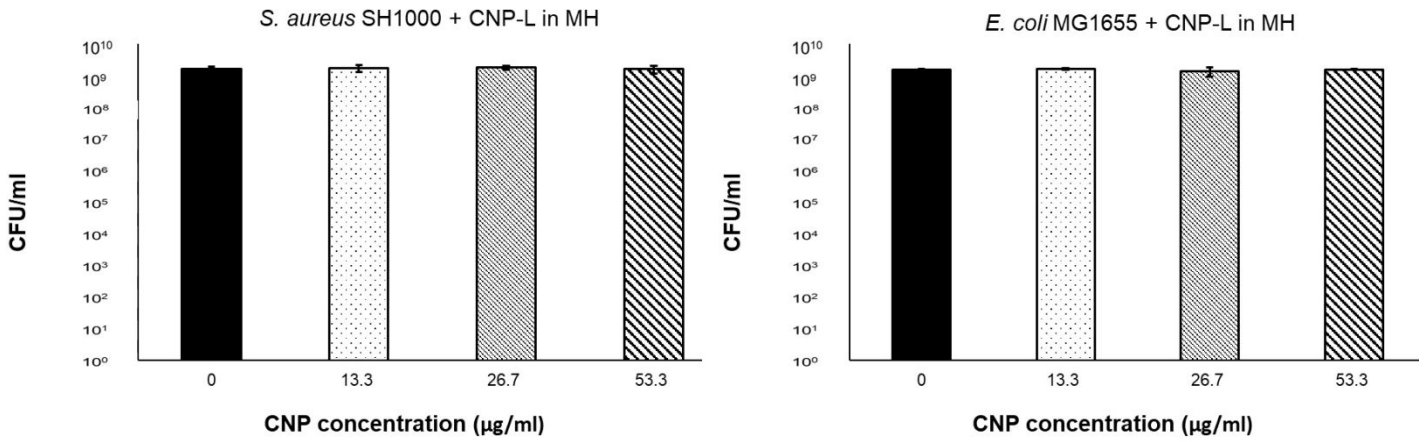


Figure S1. Viability assay of *S. aureus* SH1000 and *E. coli* MG1655 treated with CNPs at three concentrations. Counted CFU/ml of bacteria grown in MH only as negative control (CTRL -), or in presence of CNPs at concentrations of: 13.3 µg/ml, 26.7 µg/ml and 53.3 µg/ml for 24 h. Each experiment was replicated three independent times.

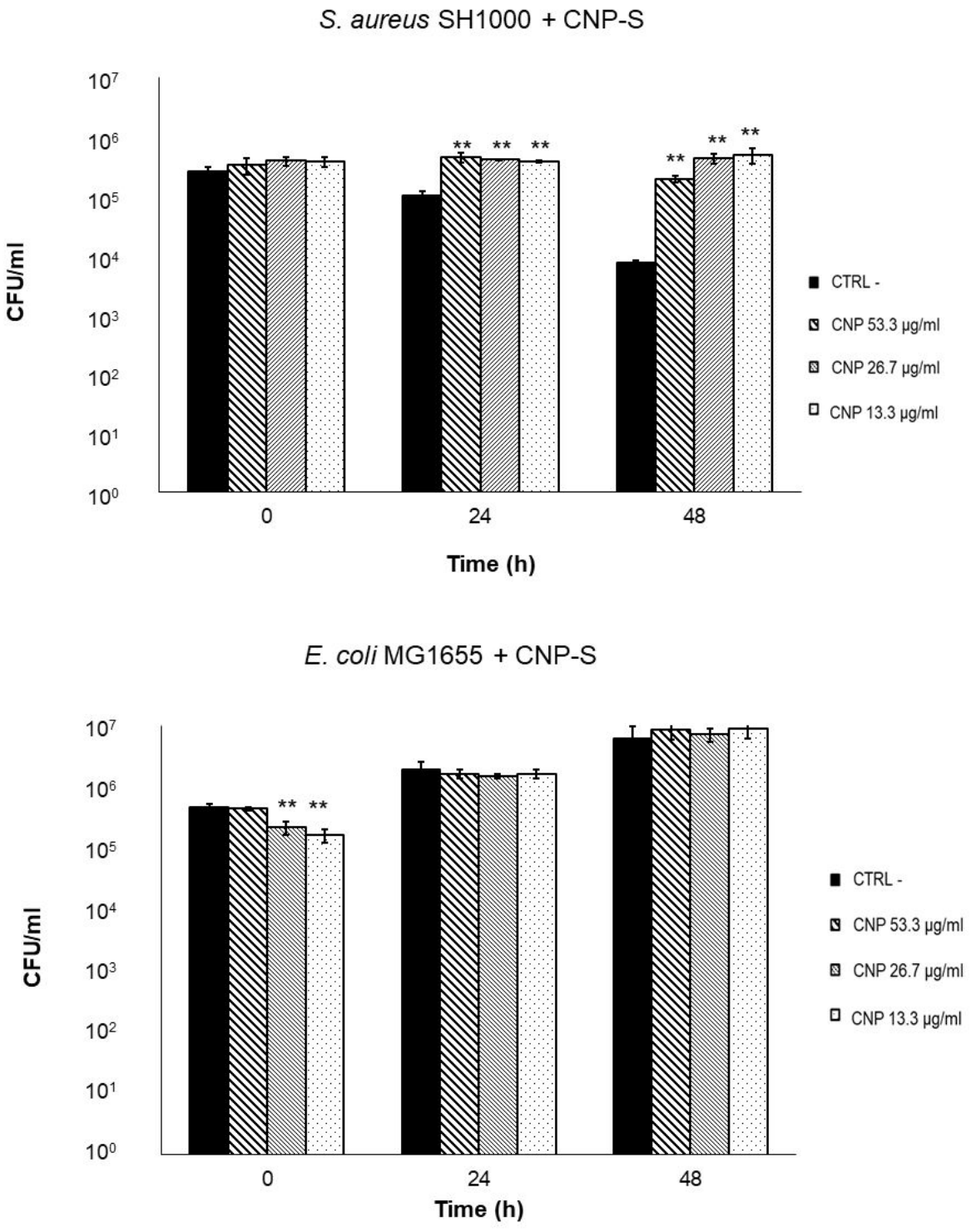


Figure S2. Effect of CNP-S on the vitality of S. aureus SH1000 and E. coli MG1655. Bacteria were grown in PBS (black, CTRL -), or in presence of CNP-S at concentrations of: 53.3 µg/ml, 26.7 µg/ml and 13.3 µg/ml. Each experiment was replicated three independent times. The double stars represent a *p* value < 0.01.

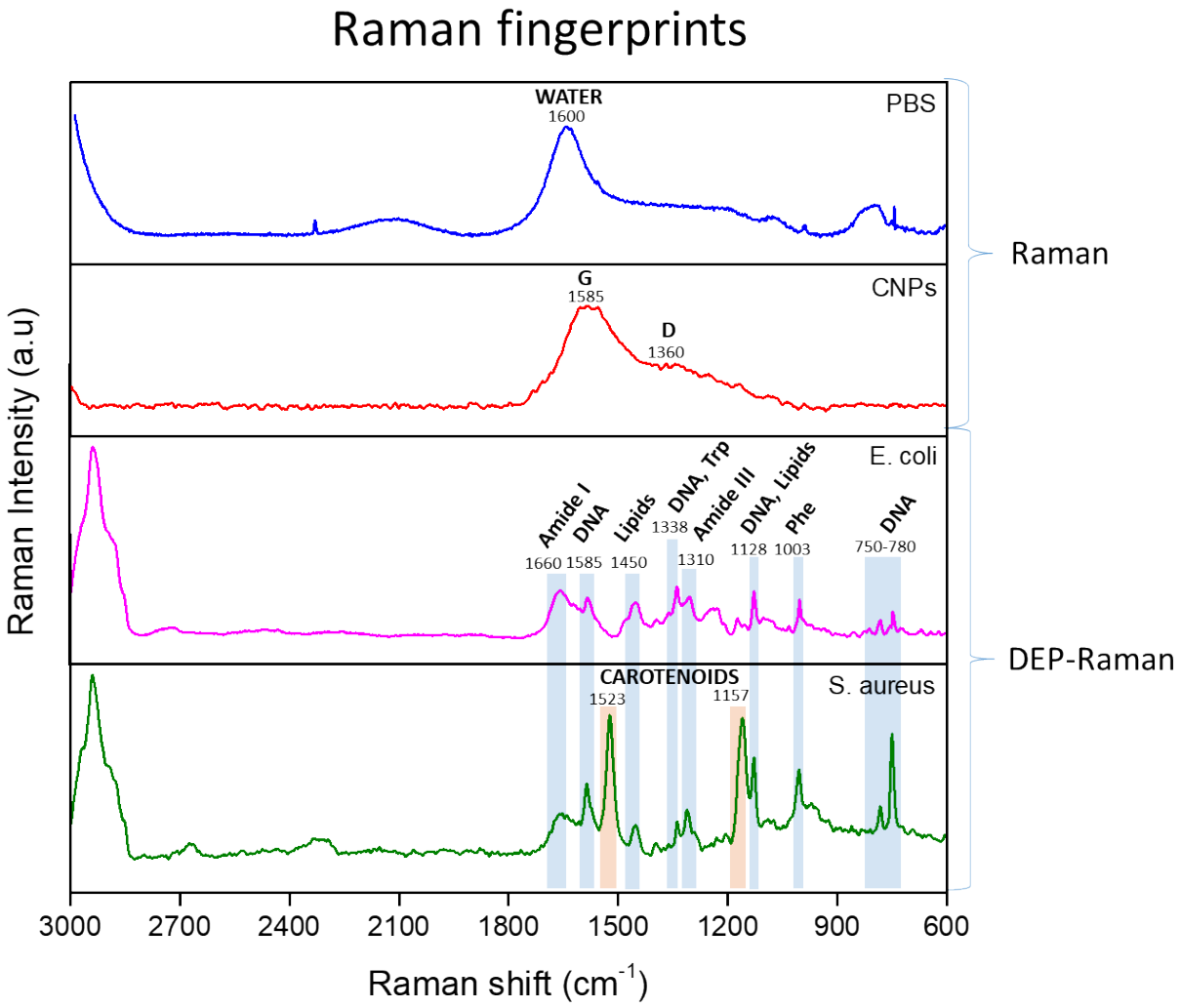


Figure S3. Raman spectra of phosphate buffered saline solution (PBS); CNPs; *E. coli* MG1655 and *S. aureus* SH1000.

In the spectra of PBS the vibrational band of water 1600 cm^{-1} is the dominant signal. CNP-L exhibited the typical G and D bands of the turbostatic graphite (1585cm^{-1} and 1360 cm^{-1}) [13] The band were broad, and with a low G/D intensity ratio, indicating a preponderant amorphous carbon atomic structure.

The Raman spectra of bacteria showed the typical signals referred to bacterial cells structure but the two strains were clearly different also for the very visible carotenoid signals present in the *S. aureus* spectrum.

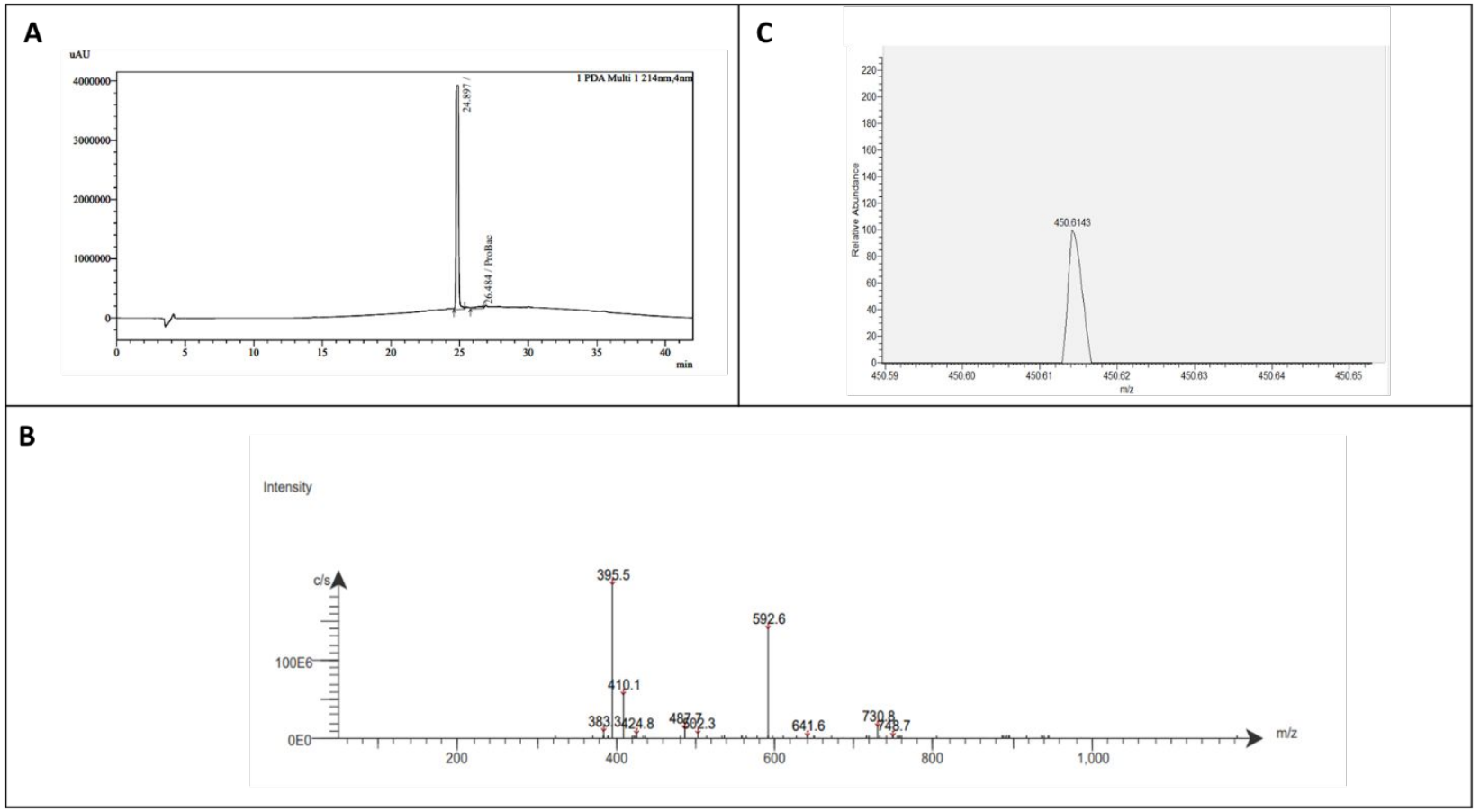
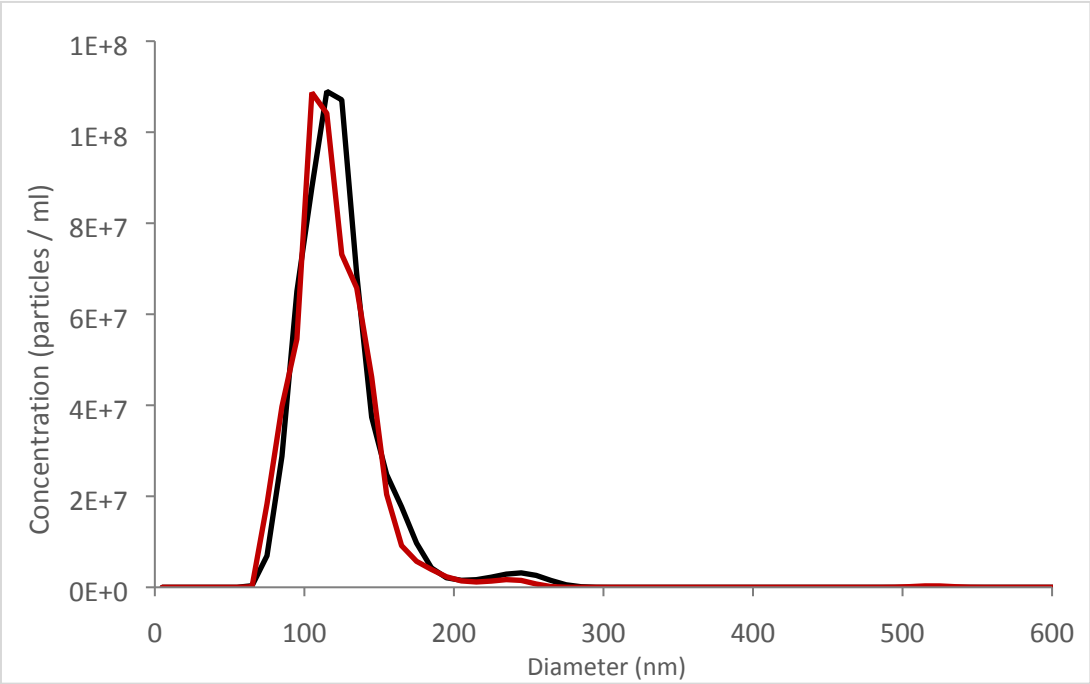


Figure S4. (A) Chromatogram of purified Bac8c^{2,5Leu}, obtained by RP-HPLC, PDA detector with a wavelength of 214 nm. (B) Mass spectra of Bac8c^{2,5Leu} Formula: $C_{57}H_{90}N_{20}O_8$, [Bac8c^{2,5Leu} +2H]$^{2+}$ Calculated MW: 592.6, Measured MW: 592.6; [Bac8c^{2,5Leu} +3H]$^{3+}$ Calculated MW: 395.5, Measured MW:395.5. (C) MS analysis of peptide SL- Bac8c^{2,5Leu}. Formula $C_{66}H_{105}N_{21}O_{10}$, [Bac8c-SL+3H]$^{3+}$ Calculated MW: 450.6, Measured MW: 450.6



	CNP	Bac8c^{2,5Leu}@CNP
Mean	123,8	119,8
Mode	119,2	109,2
SD	29,4	30,8
D10	83,5	79,5
D50	109,6	105,7
D90	146,5	138,8

Figure S5. Size distribution of Bac8c2,5Leu@CNP-S and CNP-S evaluated by Nanoparticle Track Analysis.

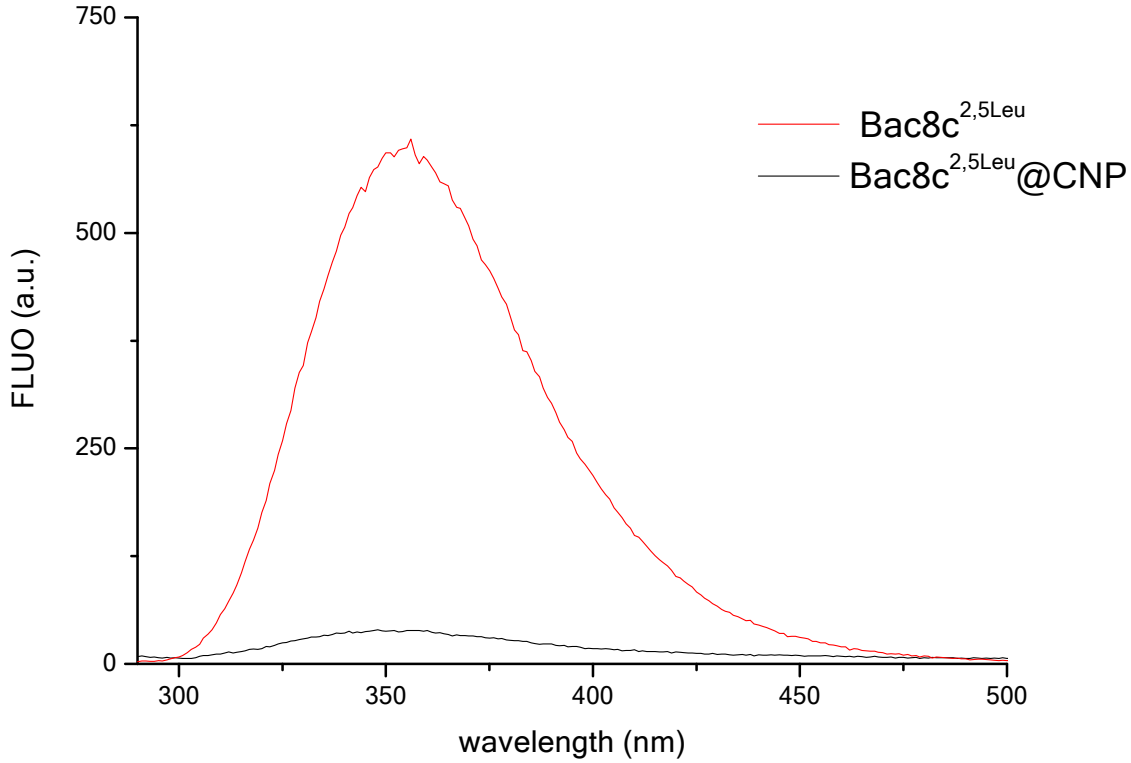


Figure S6. Loading of CNP-S with Bac8c^{2,5Leu} peptide. Fluorescence spectra of the supernatant obtained after incubation of the peptide with CNP and removal of the nanoparticles Bac8c$^{2,5\ Leu}$@CNP by centrifugation (black line) in comparison with the spectra of the loading solution (red line).

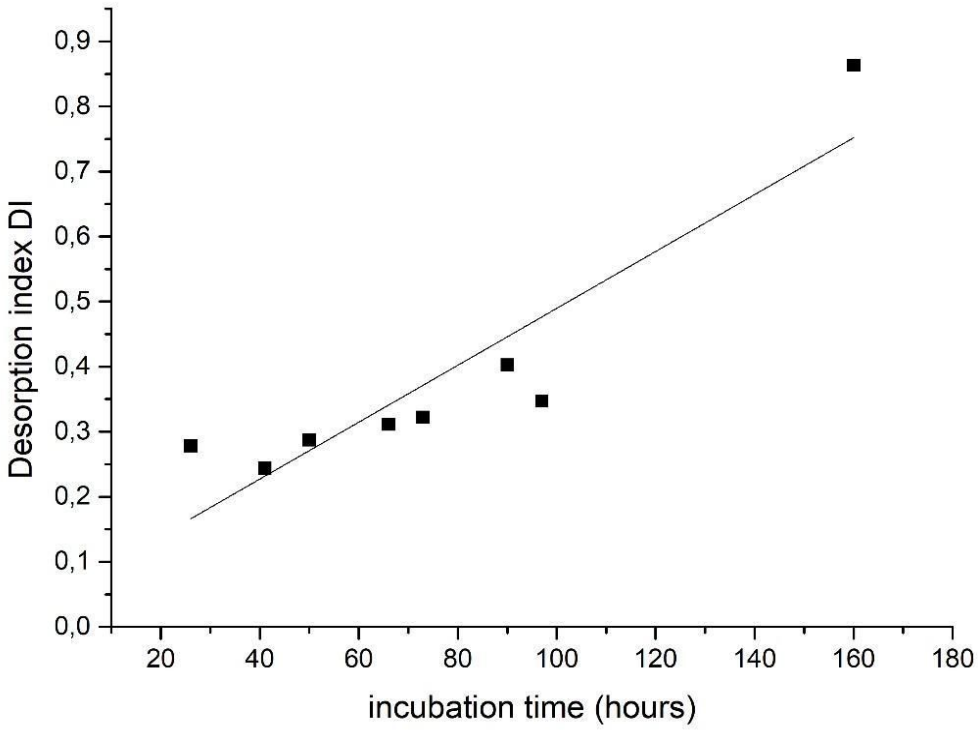


Figure S7. Desorption kinetic of SL-Bac8c^{2,5Leu} @CNP monitored by EPR spectroscopy at pH 7.4.

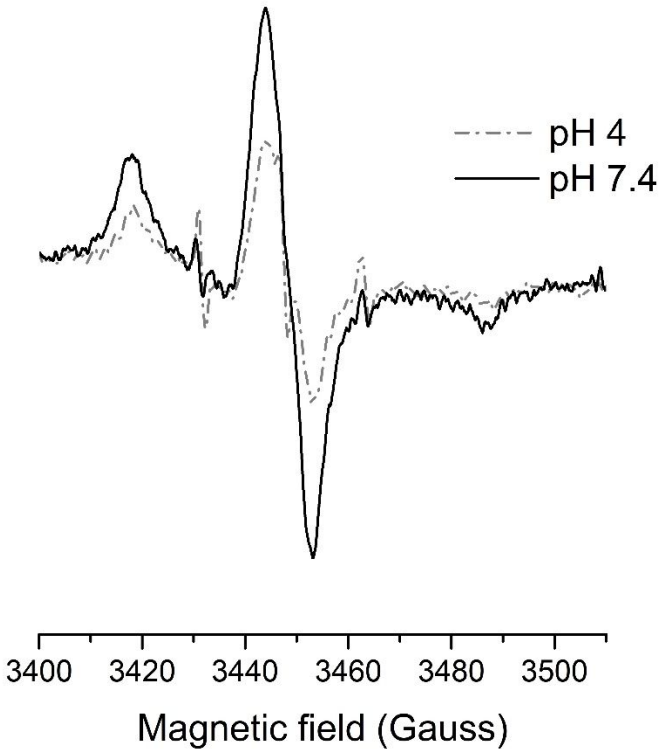


Figure S8. **Effect of pH on peptide desorption from SL-Bac8c^{2,5Leu}@CNP-S.** EPR spectra of SL-Bac8c^{2,5Leu}@CNP suspension in 10 mM phosphate buffer at neutral and acidic pH (Instrumental settings: microwave power 5 mW; modulation amplitude 1 G; flat cell at RT)

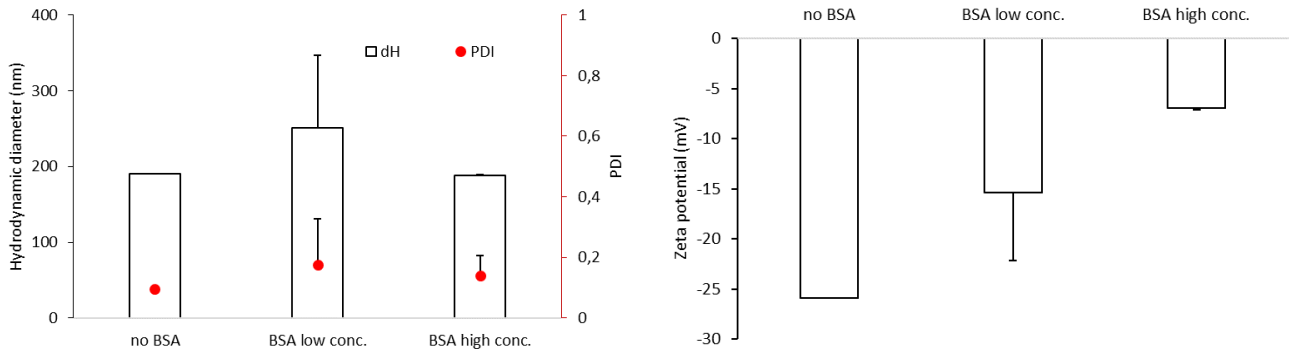


Figure S9. Effect of BSA on the hydrodynamic diameter (A) and ζ-potential (B) of Bac8c^{2,5Leu} @CNP