Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Antibiotic gold: Tethering of antimicrobial peptides to gold nanoparticles maintains conformational flexibility of peptides and improves trypsin susceptibility

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Table S1: Antimicrobial activity of the peptides with and without CG_5 spacer.

peptide	*M. luteus* DSM 1790	*B. subtilis* DSM 346
	µg/ml	µg/ml
PGLa wt	32	4
xPGLa	64	32
MSI-103 wt	4	8
xMSI-103	8	4
MAP wt	8	2
xMAP	32	16
BP100 wt	8	4
xBP100	16	8
TP10 wt	8	4
xTP10	32	8

x = CG_5 (the slight loss of activity is regained upon binding of the peptides to the gold nanoparticles, see Figure 4)

Table S2: DLS data showing the size range for PFNPs

PFNP	**size** [nm]
AuNP-PGLa	5-7
AuNP-MAP	3-8
AuNP-MSI-103	5-7

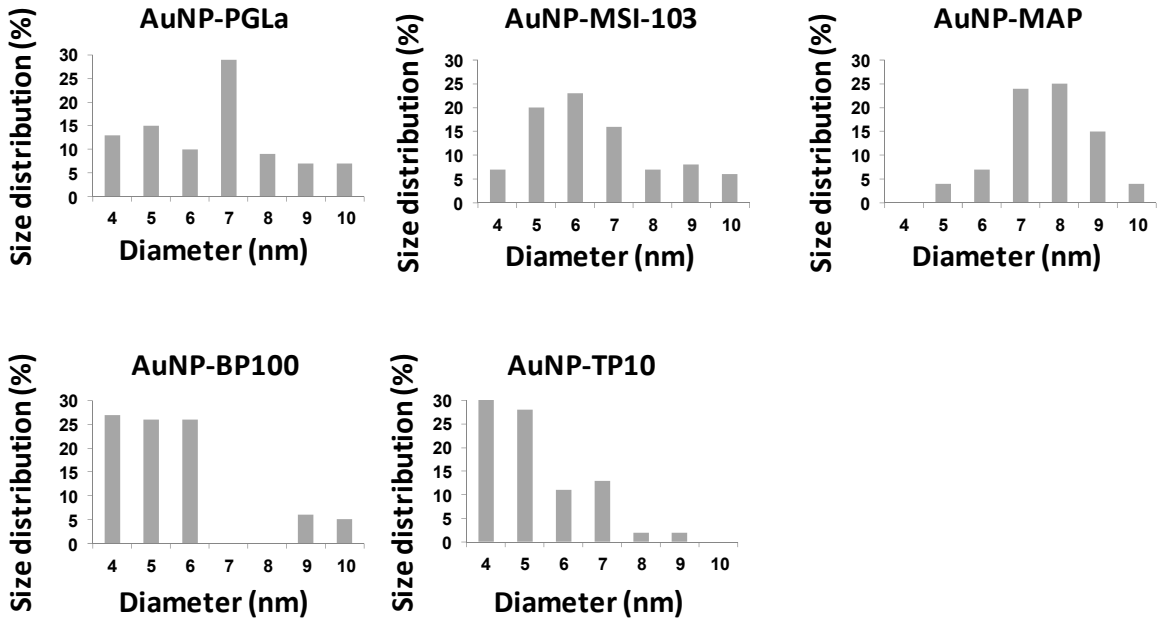

Figure S1: TEM image analysis show a narrow size distribution of the functionalized gold nanoparticles, with 80-99 % of population distributed between 4-9 nm.

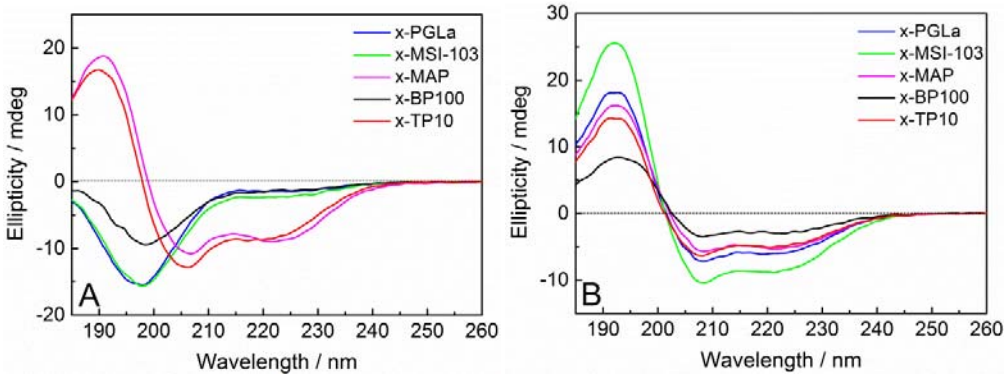

Figure S2: CD spectra of free x-peptides. In aqueous buffers free x-peptides are largely disordered with exception of TP10 and MAP (A) where as in a membrane-mimicking environment in the presence of lipid vesicles composed of DMPC and DMPG they show a distinct α-helical signature with minima at 208 and 222 nm (B).

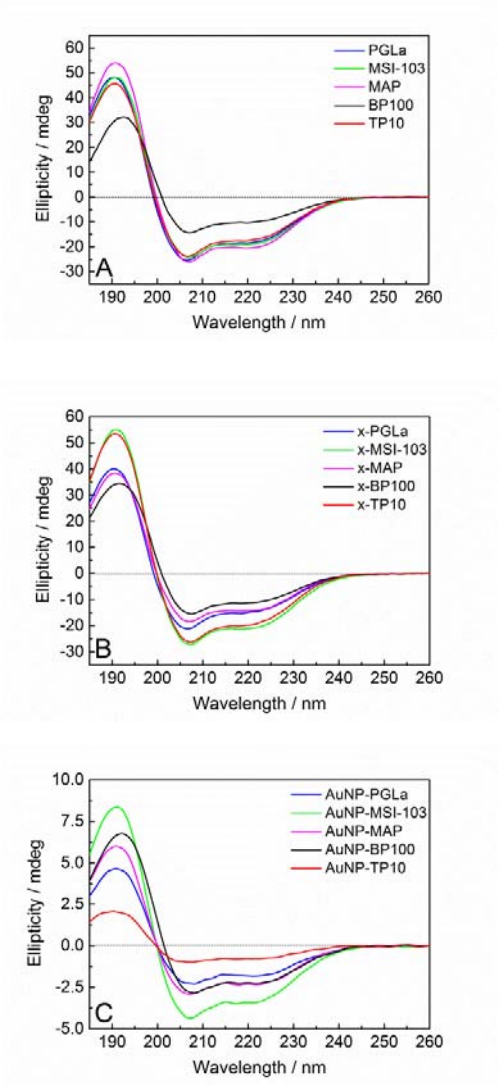

Figure S3: CD spectra of native peptides (A), free x-peptides (B) and the peptides bound to gold nanoparticles (C) in the presence of the helix-promoting solvent 2,2,2-trifluoroethanol (TFE, mixed 1:1 with phosphate buffer), all peptides show that they can fold into their characteristic α-helical form necessary for the antimicrobial action.

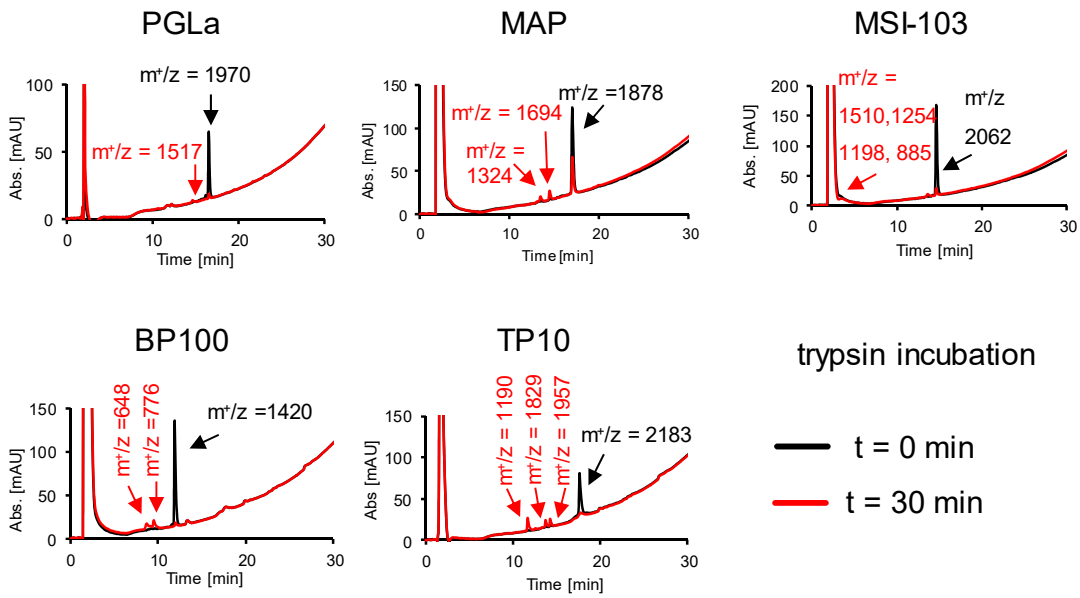

Figure S4: Overlapping LC-MS chromatograms showing the degradation of free peptides (black traces either without trypsin or t = 0 min). The red curves show almost completely degraded peptides after 30 minutes of incubation with trypsin.

PGLa
m^+/z=1970 g/mol (GMASKAGAIAGKIAKVALKAL)
m^+/z=1517 (1495+Na^+) g/mol (AGAIAGKIAKVALKAL)

MSI-103
m^+/z=2064 g/mol (KIAGKIAKIAGKIAKIAGKIA)
m^+/z=1510 g/mol (KIAGKIAKIAGKIAK)
m^+/z=1254 g/mol (IAGKIAKIAGKIA)
m^+/z=1198 g/mol (KIAGKIAKIAGK)
m^+/z=885 g/mol (IAKIAGKIA)

MAP
m^+/z=1878 g/mol (KLALKLALKALKAALKLA)
m^+/z=1694 g/mol (KLALKLALKALKAALK)
m^+/z=1324 g/mol (LALKALKAALKLA)

BP100
m^+/z=1420 g/mol (KKLFKKILKYL)
m^+/z=776 g/mol (KILKYL)
m^+/z=648 g/mol (ILKYL)

TP10
m^+/z=2183 g/mol (AGYLLGKINLKALAALAKKIL)
m^+/z=1957 g/mol (AGYLLGKINLKALAALAKK)
m^+/z=1829 g/mol (AGYLLGKINLKALAALAK)
m^+/z=1190 g/mol (AGYLLGKINLK)

Figure S5: Sequences of some of the identified peptide fragments (shown in red) that correspond to the molar masses observed in LC-MS (Figure S7), obtained after trypsin degradation of the parent peptides (shown in black).

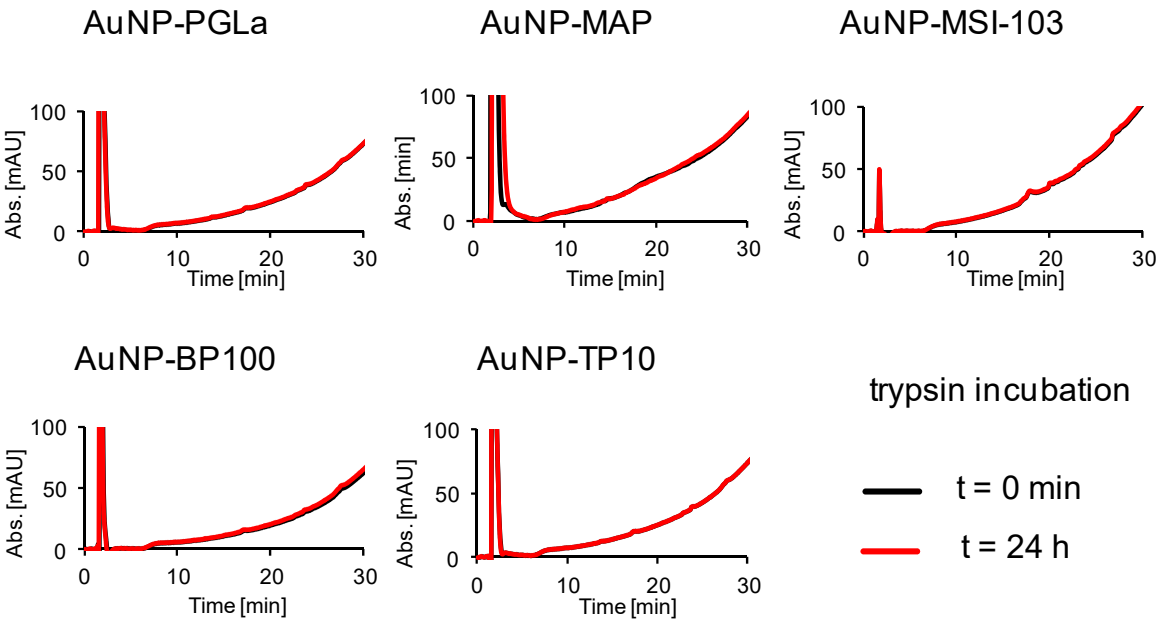

Figure S6: Overlapping LC-MS chromatograms showing that there is no degradation of the peptides when coupled to the PFNP: before (black lines) and after 24 h of incubation with trypsin (red lines).

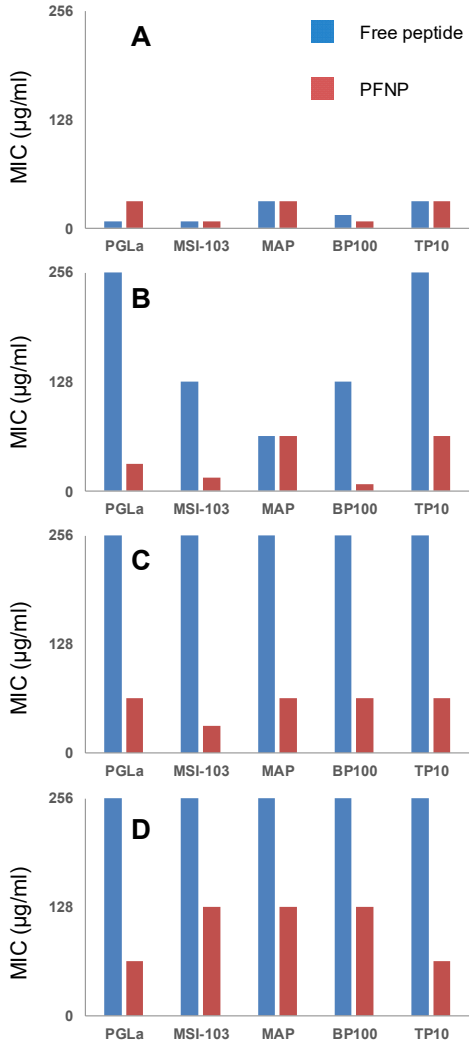

Figure S7. Decline in antimicrobial action (seen as increase in MIC value) as a function of trypsin incubation time (A= 0 min, B = 2 h, C=6 h and D=24 h) investigated on five free peptides (blue bars, left) and on PFNP (red bars on right). Free peptides are fully inactive within 2-6 h as seen by higher bars (blue) indicating loss of activity whereas PFNP retain their antimicrobial action even up to 24 h and show only slight decline in antimicrobial action. Each panel shows MIC values (µg/ml) for *E. coli* DSM 1103.

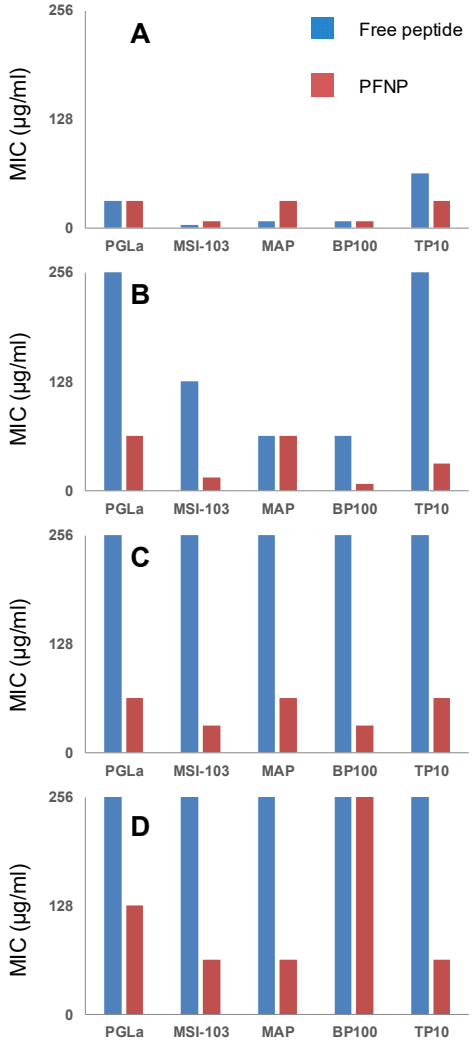

Figure S8. Decline in antimicrobial action (seen as increase in MIC value) as a function of trypsin incubation time (A= 0 min, B = 2 h, C=6 h and D=24 h) investigated on five free peptides (blue bars, left) and on PFNP (red bars on right). Free peptides are fully inactive within 2-6 h as seen by higher bars (blue) indicating loss of activity whereas PFNP (with an exception of BP100) retain their antimicrobial action even up to 24 h and show only slight decline in antimicrobial action. Each panel shows MIC values (µg/ml) for *M. luteus* DSM 1790.

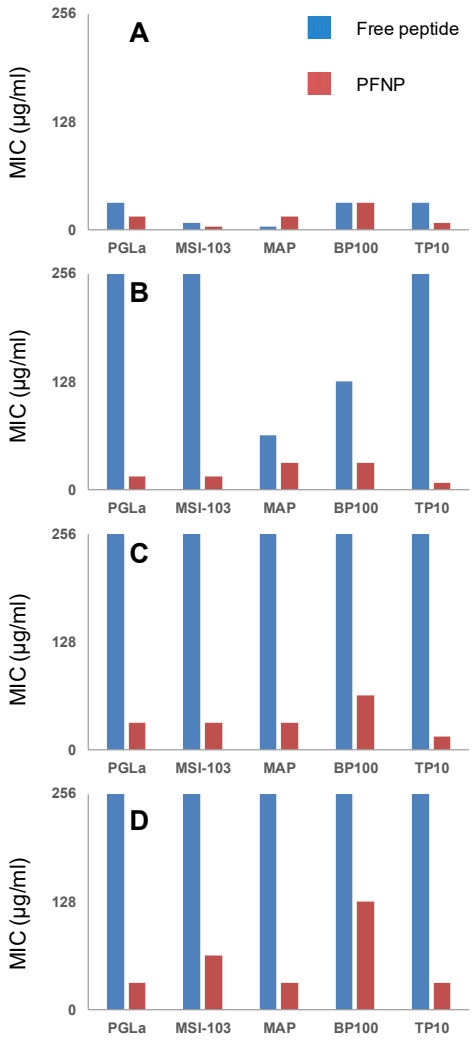

Figure S9. Decline in antimicrobial action (seen as increase in MIC value) as a function of trypsin incubation time (A= 0 min, B = 2 h, C=6 h and D=24 h) investigated on five free peptides (blue bars, left) and on PFNP (red bars on right). Free peptides are fully inactive within 2-6 h as seen by higher bars (blue) indicating loss of activity whereas PFNP retain their antimicrobial action even up to 24 h and show only slight decline in antimicrobial action. Each panel shows MIC values (µg/ml) for *S. aureus* DSM 1104.