Supplementary Information

Peptide Functionalized Gold Nanoparticles for Colorimetric Detection of Matrilysin (MMP-7) Activity

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Experimental Section

Materials:
Ethylenediaminetetraacetic acid (EDTA), Gold (III) Chloride Trihydrate (>=99.9%), sodium citrate tribasic dihydrate (ACS reagent, >=99.0%), α-Cyano-4-hydroxycinnamic acid (CHCA) and calcium chloride were purchased from Sigma-Aldrich. Phosphate Buffered Saline (PBS) Ultra Pure Grade and Bis-tris buffer were purchased from 1st base. MMP-7 human recombinant, E. Coli and MMP-2 human recombinant, E. Coli and MMP inhibitor II (N-Hydroxy-1,3-di-(4-methoxybenzenesulphonyl)-5,5-dimethyl-[1,3]-piperazine-2-carboxamide) were purchased from Merck Chemicals. The SH-$(CH_2)_{16}$-OH and the HO-EG_6-C_{11}-S-S-C11-EG_6-NHCO-Maleimide used as the PDMS inking and backfilling anchoring molecules, respectively, in the ellipsometric microarray experiments, were purchased from Prochimia, Poland. O-(7-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate was purchased from TBTU, Alexis Biochemicals.

Peptide synthesis:
The peptide JR2EC (NAADLEKAIE**AL**EKHLEAKGPCDA**AQL**EKQLEQAFEAFERAG) was synthesized on a Pioneer automated peptide synthesizer (Applied Biosystems) using standard fluorenylmethoxycarbonyl (Fmoc) chemistry with O-(7-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate as the activating reagent. The peptide was dissolved in 10 mM sodium citrate buffer with pH 6.

Gold Nanoparticle synthesis and functionalization:
Gold nanoparticles with a diameter of ~17 nm were obtained by the citrate reduction of $HAuCl_4$[1, 2] and stored at 4 °C until further use. UV-Vis extinction spectrum of the as-synthesized and citrate capped AuNPs showed a plasmonic peak around 520 nm. Functionalization of the AuNPs was performed by incubation of the particle in the 10 μM peptide solution at pH 6 (10 mM sodium citrate). The unbound peptide was removed by 5 repeated centrifugations followed by washing with PBS buffer at pH 7.4, and stored until further use. Before use, the buffer of the JR2EC functionalized AuNPs was changed to PBS buffer with 1 mM $CaCl_2$ at pH 7.4. After functionalization, the extinction peak of the AuNPs appeared at 521 nm.

Peptide patterning:
Patterning of the peptide on gold surface was prepared by micro contact printing of a hydroxyl-terminated alkane thiol to form a frame followed by backfilling with a maleimide disulphide in the non-printed domains (diameter 30 μm).[3] In a typical printing process, the PDMS stamp was soaked in a 5 mM SH-$(CH_2)_{16}$-OH solution for 1 min followed by drying in nitrogen gas before it was brought into conformal contact with the gold surface for 5 min. The printed gold surface was then incubated in a 50 μM maleimide (HO-EG_6-C_{11}-S-S-C_{11}-EG_6-NHCO-Maleimide) for 1 hr followed by incubation in 40 μM peptide solution in PBS buffer for 12 hrs. Imaging ellipsometry was conducted using a modified EP3 spectroscopic ellipsometer from Accurion, Germany. During the ellipsometric profile measurements the OH thiol areas were used as reference positions for calculating the incremental thickness changes upon

exposure to the maleimide disulphide, immobilization of the JR2EC peptide and after digestion. For example, the thickness difference between the OH-thiol and the maleimide disulphide was approximately 0.5 nm. This particular thickness must be subtracted when evaluating the thickness of peptide before and after digestion.

Digestion of peptide by MMP-7 and characterization:
The MMP-7 mediated hydrolysis of the immobilized peptides was performed at 25ºC and real time extinction spectra were recorded with the Lambda 35 spectrometer during 30 min. Control experiments were performed using 2 µg/mL MMP-2, 2 µg/mL MMP-7 with 10 µM MMP inhibitor II and 2 µg/mL MMP-7 with 10 mM ethylenediaminetetraacetic acid (EDTA), respectively. Digestion of the peptide in PBS buffer was characterized by MALDI-TOF-MS using a Shimadzu mass spectrometer with α-Cyano-4-hydroxycinnamic acid (CHCA) as matrix. A solution containing 10 µM of the peptide in PBS pH 7.4 was digested with 1 µg/mL MMP-7 for 2 hr at 25 ºC. The digested sample was desalted before MALDI-TOF experiment. Digestion of the peptides on planar gold by MMP-7 was evaluated by measuring the height profile of peptide-patterned gold surfaces using imaging ellipsometry (Accurion, Germany).

MALDI-TOF analysis of MMP-7 activity

Matrix metalloproteinase-7 (MMP-7), Fig.S1a) is an enzyme that in humans is encoded by the MMP-7 gene. It is one of the 24 zinc dependent proteases in the matrix metalloproteinases (MMPs) family which play essential roles in breaking down components of the extracellular matrix (ECM).

Fig.S1a) shows the structure of MMP-7 rendered with PyMOL.The black spheres are zinc ions and blue spheres are calcium ions. Fig.S1b) shows the MALDI-TOF spectrum of MMP-7. Two peaks were observed around 19170 m/z and 9587 m/z.

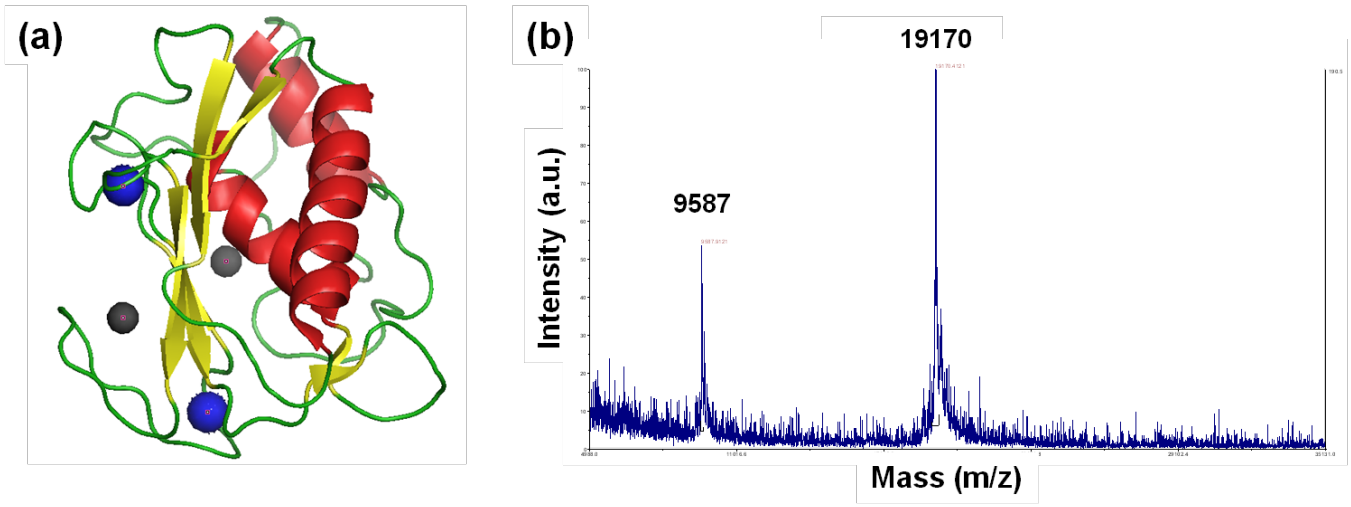

Fig.S1. (a) Structure of MMP-7 rendered with pyMOL, (b) MALDI-TOF spectrum of 2µg/mL MMP-7 in Milli-Q water.

Peptide Digestion Scheme

The positive amino acids and negative amino acids in neutral pH are shown in blue and red colors. It can be calculated the net charges decrease from -5 to -1 after digestion. Electrostatic potential of the intact and digested peptide is also plotted, where is red color stand for negative potential and blue color stand for positive potential. It's clearly seen that the negative potential decreases significantly after digestion with MMP-7.

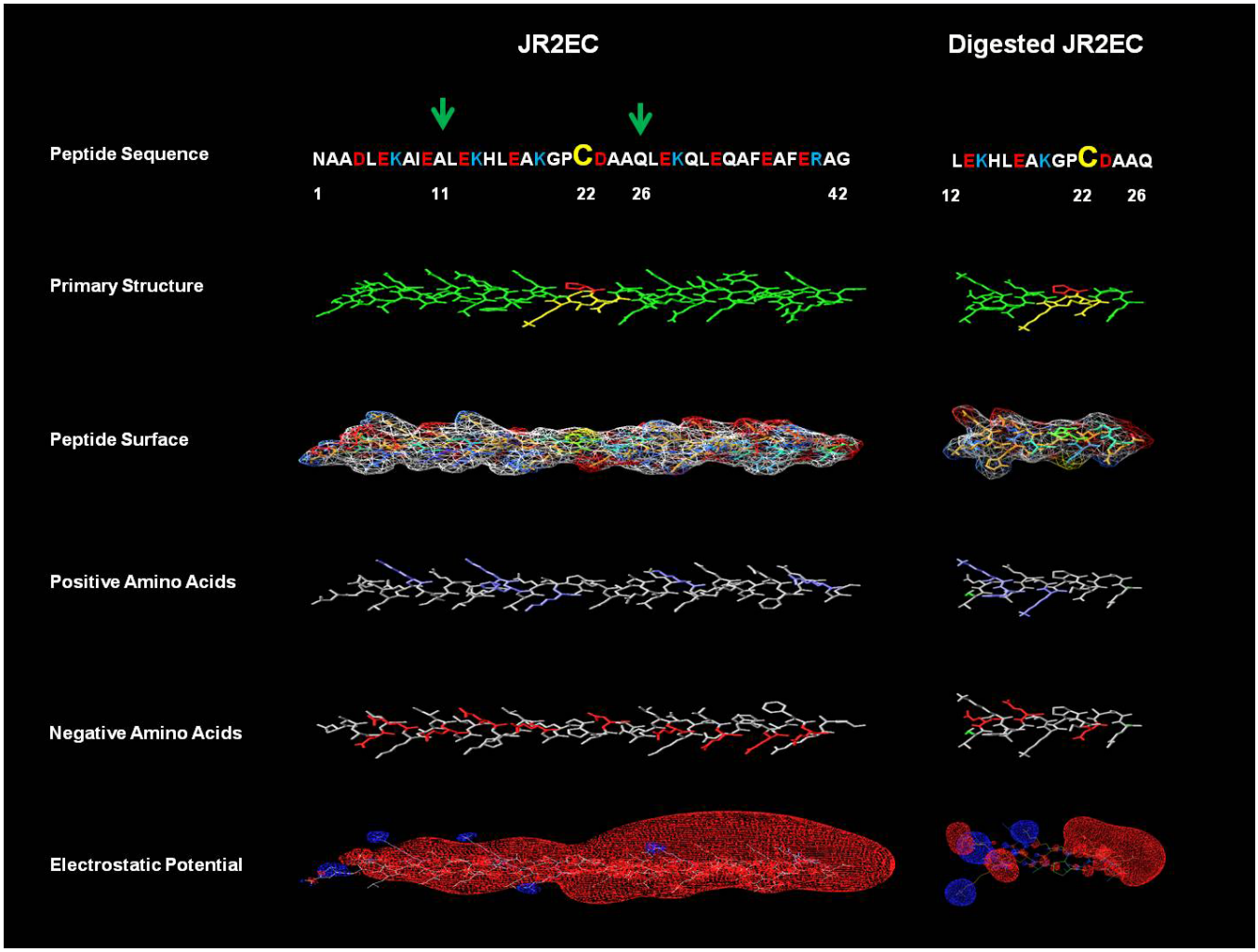

Fig.S2. Structure, charge distribution and calculated electrostatic potential of intact and digested JR2EC peptide at neutral pH.

TEM analysis of dispered and aggregated AuNPs

Transmission Electron Microscopy (TEM) image of JR2EC functionalized AuNPs in Fig. S3a) clearly shown that the AuNPs are dispersed, with an average interparticle distance calculated around 70 nm. This distance is far more than the interparticle coupling distance, which is roughly the diameter of the particle. With incubation of 2 μg/mL of MMP-7 for 30 min, aggregates of AuNPs can be seen from Fig.S3b). The average distance of AuNPs in the aggregates is 3.7 ±1.2 nm.

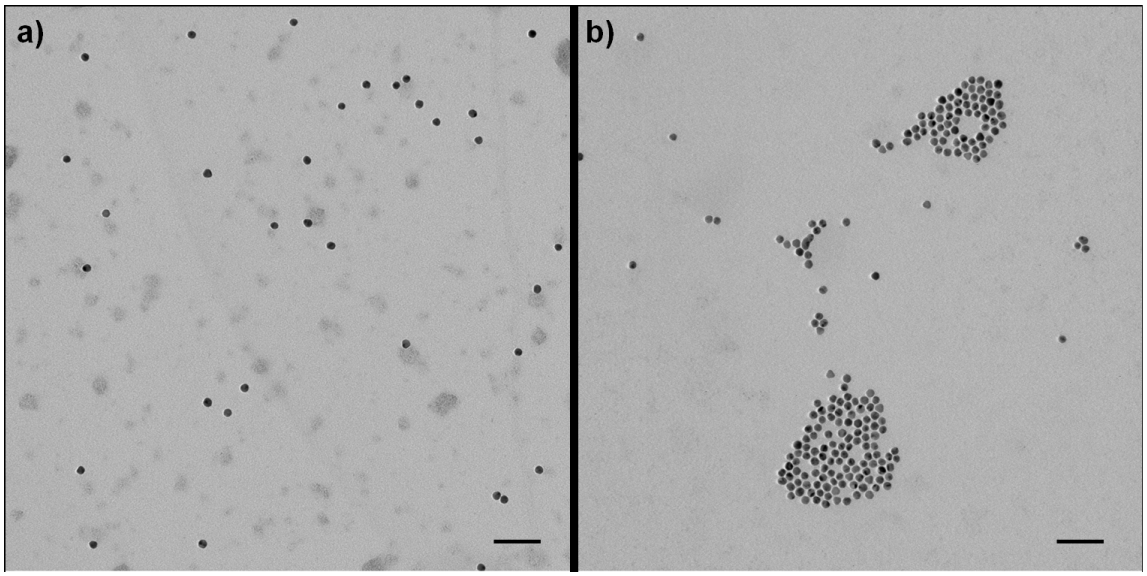

Fig.S3 Transmission Electron Microscopy (TEM) image of JR2EC functionalized AuNPs before a) and after b) digestion with MMP-7. Scale bar: 100 nm.

Naked eye detection of MMP-7-induced NP aggregation

Fig.S4 shows a photo of the JR2EC modified AuNPs incubated with 2 μg/mL MMP-7 for 30 min compared with that without MMP-7. From the photo we can see a clear colorimetric transition from red toward blue. The red-blue color change is corresponding to a peak shift from 521 nm to 561 nm.

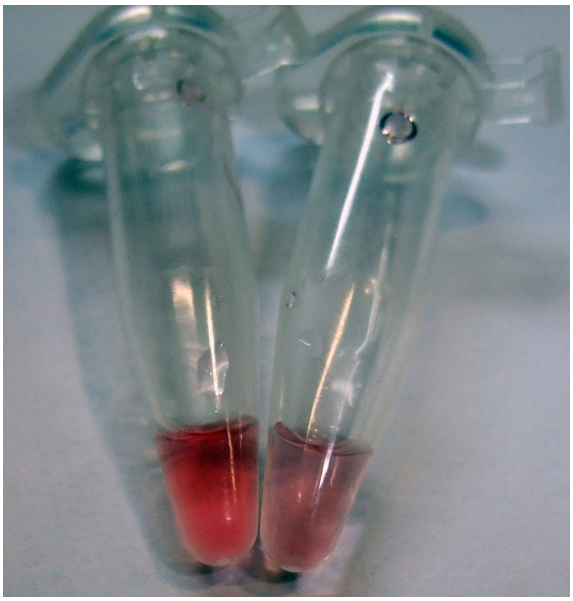

Fig.S4. Photo of AuNPs without presence of MMP-7 (left) and 2 μg/mL MMP-7 (right) by CCD camera.

Reference

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