Supplementary Material (ESI) for Chemical Communications
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Electronic supporting information

PEGylation modulates the interfacial kinetics of proteases on peptide-capped gold nanoparticles

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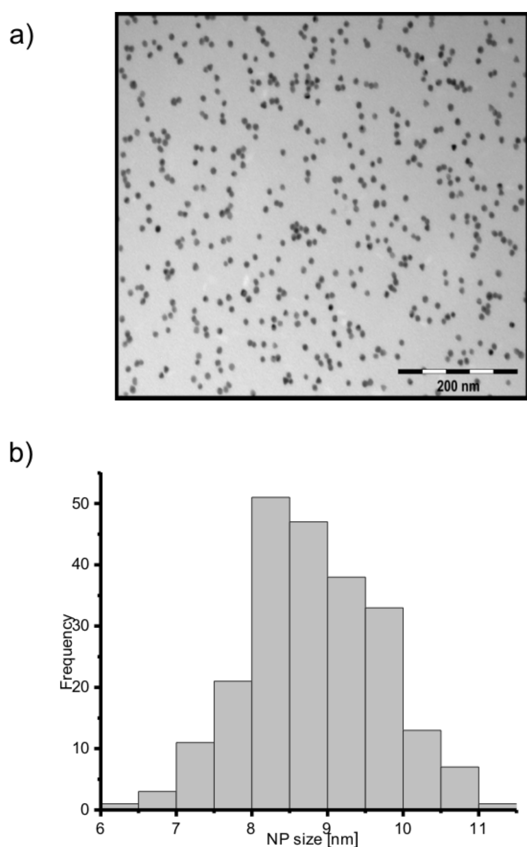

Fig. S1. Transmission Electron Microscopy (a) and size distribution (b) of the gold nanoparticles

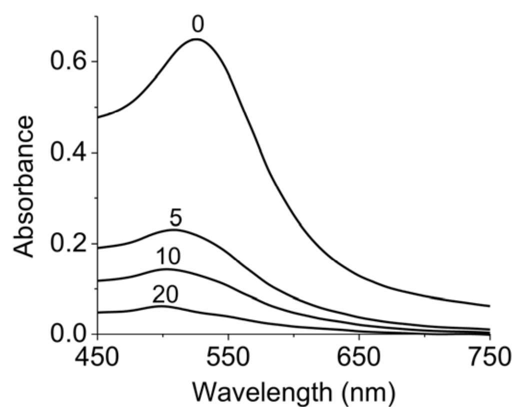

Fig. S2. KCN dissolution of peptide-capped nanoparticles: UV-visible absorbance spectra of CALNN-capped gold nanoparticles upon dissolution in 30 mM KCN (0, 5, 10 and 20 min after KCN addition)

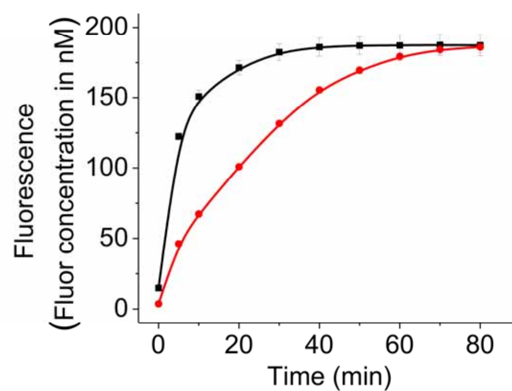

Fig. S3. KCN dissolution kinetics measured by the release of fluorescent molecules for two different self-assembled monolayer compositions (2% CCALNNThrFluor for both NP-SAMs), black boxes CALNN-capped nanoparticles, red circles CALNN/CCALNNPEG 7/3 capped nanoparticles

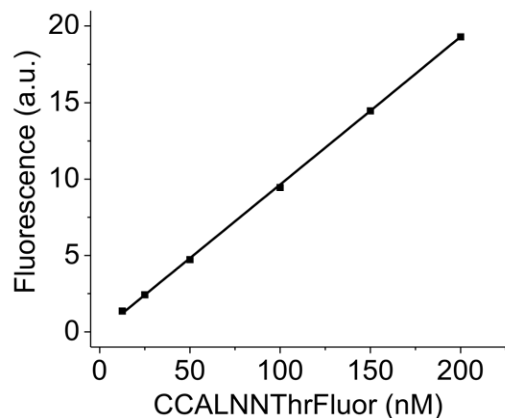

Fig. S4. Calibration curve for the fluorescence signal as a function of fluorescent peptide concentration

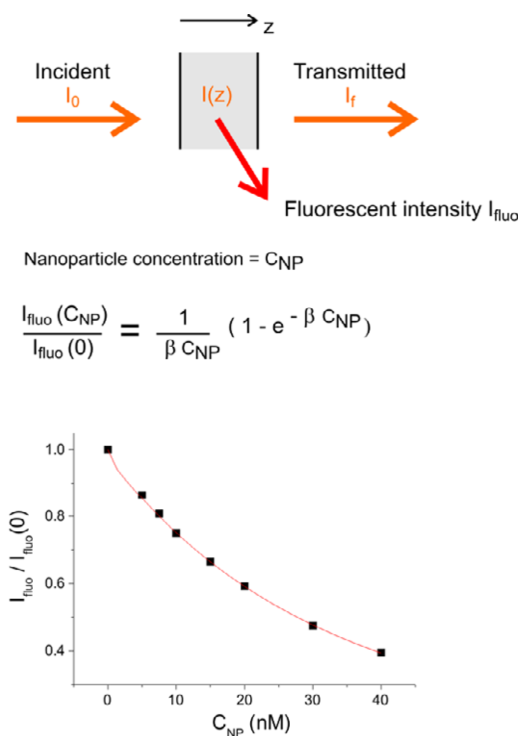

Fig. S5. Effect of the presence of an absorbing medium on the measured fluorescence intensity. Black squares: experimental points measured with free fluorescent peptide (0 – 60nM) in the presence of increasing nanoparticle concentration. Red line: single parameter fit with the expression above (deduced from Beer Lambert law, with an integration over z)

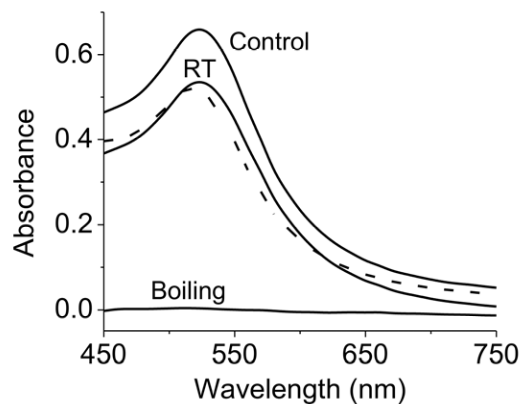

Fig. S6. Solid lines: UV-visible absorbance spectra of CCALNNPEG - capped gold nanoparticles: in the absence of KCN, 1h exposure to 30 mM KCN at either room temperature (RT), or, boiling; dashed line: UV-visible absorbance spectra of mix matrix[10] capped gold nanoparticles after 1h exposure to boiling with 30 mM KCN.

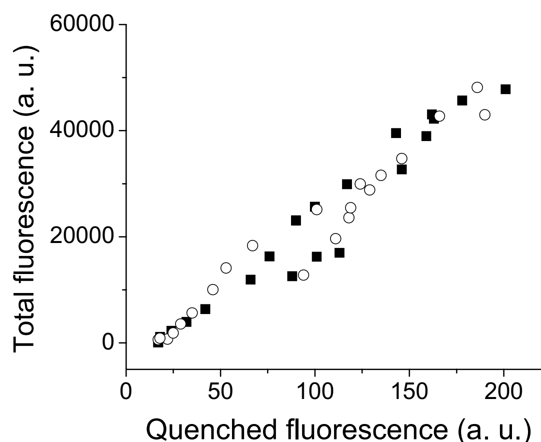

Fig. S7. Fluorescence intensity after dissolution of the gold core as a function of the original quenched fluorescence for a series of nanoparticles with different number of CCALNNThrFluor per nanoparticle. Black squares: CALNN-capped nanoparticles; Empty circles: CALNN/CCALNNPEG 7/3 ratio

Experimental procedures

Reagents and antibodies

Purified human cathepsin L, cathepsin L inhibitor Z-FF-fmk (Z-Phe-Phe-fluoromethyl ketone), purified bovine thrombin, purified bacillus thermoproteolyticus rokko thermolysin, purified human caspase-3, $HAuCl_4$, trisodium citrate, and tannic acid were from Sigma (St-Louis, MO, USA). Phosphate Buffered Saline (PBS), is composed of 137mM NaCl, 2.7mM KCl, 4.3mM Na_2HPO_4, 1.4mM KH_2PO_4, at pH 7.4. 10x PBS is a 10 times concentrated solution of PBS.

Nanoparticle synthesis

All glassware and apparatus are cleaned thoroughly with non-ionic detergent, ethanol, and water. To 395 ml of de-ionised water, 5 ml of 1% HAuCl4 was added and stirred for five minutes. Whilst this solution was heating to 60 ^oC, 8 ml 1% w/v trisodium citrate was added to 78.4 ml of water, and 1.6 ml of 1% w/v tannic acid. This 100 ml solution was quickly added to the 400 ml where the solution turned from a light yellow to a dark blue. The temperature was raised to 100 ^oC to complete the reaction, where the colour settled on a deep red. The resulting particle diameter was measured by Transmission Electron Microscopy TEM as 8.8 nm +/- 0.8 nm, averaged over 250 particles (Fig S1).

Peptides

The peptides were purchased from Anaspec (San Jose, CA, USA) and PPR Ltd (Fareham, UK) at > 70% purity.
Peptide sequences:
CCALNNPEG: CCALNN-(ethyleneglycol)$_6$-glycinol
CCALNNThrFluor: CCALNNGGGALVPRGSGTAK-(5-carboxyfluorescein)-NH_2
ccalnnthrfluor: ccalnnGGGalvprgsgtaK-(5-carboxyfluorescein)-NH_2
CCALNNCaspRhod: CCALNNGGGDEVDGK-(5-carboxy-tetrametylrhodamine)-NH_2
Mix matrix: 70% CVVVT-ol, 30% HS-$(CH_2)_{11}$-(ethyleneglycol)$_4$-ol
Stock solutions of CALNN peptide were made in 10x PBS at 2mM. Stock solutions of CCALNNPEG peptide were made in PBS at 2mM. Stock solutions of CCALNNThrFluor, ccalnnthrfluor, and CCALNNCaspRhod were made in dimethylsulfoxide at 2mM. CVVVT-ol stock solutions were made in 75% de-ionised water – 25% dimethylsulfoxide at 4mM. HS-$(CH_2)_{11}$-(ethyleneglycol)$_4$-ol stock solutions were made in MeOH at 10mM.

Peptide-capped gold nanoparticles

To 0.3 mL of the appropriate peptide mixture (1mM in 10x PBS), a solution of gold nanoparticles (2.7 ml, 13.9 nM) was added. They were briefly agitated before addition of 30 μl of 1% Tween-20. The solutions were left overnight at room temperature in the dark. The nanoparticle solutions were centrifuged (16,000*g*, 60 mins) and the pellet resuspended in 1 ml of PBS / 0.01% Tween-20 before a second centrifugation step (16,000*g*, 60 mins). This washing step was repeated once with PBS / 0.01% Tween-20, and then two times with PBS only, before resuspension in PBS at a final concentration of 100 nM. Concentration of nanoparticles was determined using the Beer-Lambert law, with extinction coefficients for CALNN or 7/3 CALNN/CCALNNPEG nanoparticles being determined prior to the washing of nanoparticles.

Fluorescence

Fluorescence was measured using a BMG labtech POLARstar fluorimeter (Offenburg, Germany) and Corning (Corning Inc., NY, USA) black – NBS 96-well plates.

Enzyme activity

Thrombin cleavage: each reaction (100 μl) was performed at 37 ^oC in PBS with 0.3M NaCl (pH 7.4), with 10nM of 95% CALNN – 5% CCALNNThrFluor nanoparticles and 50mU of purified thrombin.
Cathepsin L cleavage: each reaction (100 μl) was performed at 37 ^oC in 0.1M sodium-acetate buffer (pH 5.6) with 10nM of 98% CALNN – 2% CCALNNThrFluor nanoparticles and 1mU of purified cathepsin L. In some experiments, the addition of the cathepsin L inhibitor Z-FF-fmk (0.1 mM) was added to the nanoparticles before adding cathepsin L.
Thermolysin cleavage: each reaction (100 μl) was performed at 37 ^oC in 50mM potassium-phosphate buffer (dibasic, pH 7.5) with 10nM of 98% CALNN – 2% CCALNNThrFluor nanoparticles and 0.8mU of purified thermolysin at 37 ^oC.
Caspase-3 cleavage: each reaction (100 μl) was performed at 37 ^oC in caspase assay buffer with 10nM of nanoparticles and 4.3mU of purified caspase-3. Caspase assay buffer contains 20mM HEPES (pH 7.4), 2mM EDTA, 1mM DTT and 0.1% w/v CHAPS. Nanoparticles used were either 48% pep-PEG – 48% CALNN – 2% CCALNNCaspRhod, or 98% mixed-matrix peptides – 2% CCALNNCaspRhod.

KCN dissolution

CALNN-capped gold nanoparticles (10nM) were dissolved with potassium cyanide (30mM in PBS, pH 9.0) at room temperature (RT). 100% CCALNNPEG nanoparticles or mixed-matrix nanoparticles (10nM) were boiled for 1 hour at 100^oC in potassium cyanide (30mM in PBS, pH 9.0). Nanoparticles containing a percentage of CCALNNThrFluor in the SAM were boiled in potassium cyanide (30mM in PBS, pH 9.0) for 1 hour at 100^oC, placed on ice for 5 mins, mixed, and the fluorescence measured within 5 minutes. A calibration curve for the effect of KCN and boiling on the fluorescence of CCALNNThrFluor peptide was obtained, using the above procedure with a series of CCALNThrFluor concentrations; each experiment repeated in triplicate.