Supplementary Material (ESI) for Chemical Communications

A facile method to clickable sensing polymeric nanoparticles

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General chemistry

Chemicals and solvents were purchased from standard suppliers and used without further purification. Subtilisin A (EC 3.4.21.62) was purchased from Calbiochem and chymotrypsin (from bovine pancreas) (EC 3.4.31.1) from Fluka. Deuterated solvents were purchased from Goss or Sigma Aldrich. Dichloromethane was dried over molecular sieves. All other solvents were used as supplied (Analytical or HPLC grade), without prior purification. Milli-Q water was used for chemical reactions and enzymatic cleavage reactions. All reactions using anhydrous conditions were performed using flame- or oven dried apparatus under an atmosphere of nitrogen. Reactions were monitored by analytical thin-layer chromatography on commercially available precoated aluminium packed plates (Merck Kieselgel 60 F_{254}). Visualization of the silica plates was achieved using a UV lamp (λ_{max} = 254 nm) and potassium permanganate staining. Organic solvents were evaporated under reduced pressure at ≤ 35 °C (water bath temperature).

Melting points were recorded on a Gallenkamp melting point apparatus and are uncorrected. Infrared spectra were measured on a Nicolet IR 200 FT-IR spectrophotometer in the range of 4000–500 cm^{-1} using KBr discs or NaCl discs. Absorption maxima (υ_{max}) are reported in wavenumbers (cm^{-1}) and classified as strong (s), medium (m) or broad (br). Only signals representing functional groups are reported. Absorptions from the fingerprint region are not listed.

Mass spectra (TOF-ES) were recorded on a Waters 2795 separation module/micromass LCT platform.

Proton nuclear magnetic resonance (δ^H) and carbon nuclear magnetic resonance (δ^C) spectra were recorded at 20 °C on a Bruker AV400 operating at 400.13 MHz and 101.62 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm), referenced to either $CDCl_3$ (^{1}H, 7.26 ppm; ^{13}C, 77.16 ppm) or [D_6]-DMSO (^{1}H, 2.50 ppm; ^{13}C, 39.51 ppm). Coupling constants (J) are recorded in Hz and significant multiplicities described by singlet (s), doublet (d), triplet (t), quadruplet (q), broad (br), multiplet (m) or doublet of doublets (dd). Spectra were assigned using appropriate COSY and DEPT sequences. Fluorescence spectra were recorded on a Cary Eclipse fluorimeter. UV-Vis spectra were recorded on a Cary 50 spectrophotometer.

Analytical high performance liquid chromatography (HPLC) was performed using either system 1 or system 2 to confirm purity.

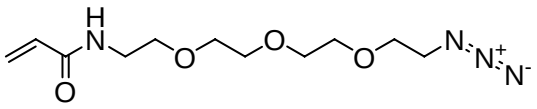
System 1: Phenomenex Onyx Monolithic C_{18} column (100 x 3.0 mm), flow rate of 1.2 mL min^{-1} and UV detection at 220 nm, 20–100% B in 15 min; solvent A: 0.06% TFA in water; solvent B: 0.06% TFA in MeCN/H_2O (90/10).
System 2: Hypersil™ Pep 100-C_{18} (4.6 x 150 mm), flow rate of 1.20 mL min^{-1} and UV detection at 220 nm. Linear gradient: 20–60% solvent B in 25 minutes. Solvent A: 0.06% TFA in water; solvent B: 0.06% TFA in CH_3CN/water (90/10).

Preparative HPLC was performed using either system 3 or system 4:

System 3: Kromasil (5 µ, 100 A) reversed phase C_{18} column (250 x 21.2 mm), a flow rate of 21.20 mL min^{-1} and UV detection at 220 nm, 28% B for 19 minutes, Solvent A: 0.06% TFA in water; solvent B: 0.06% TFA in MeCN.

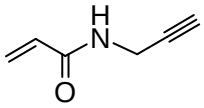
System 4: Phenomenex Onyx Monolithic reversed phase C_{18} column (100 x 10 mm), a flow rate of 14.10 mL min^{-1} and UV detection at 220 nm, Linear gradient: 10–40% B in 10 min , Solvent A: 0.06% TFA in water; solvent B: 0.06% TFA in MeCN.

_N_-(11-azido-3,6,9-trioxaundecanyl)acrylamide 4



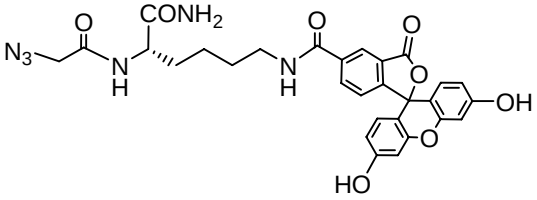
To a flame-dried round bottom flask was added 11-azido-3,6,9-trioxaundecan-1-amine (400 µL, 2.01 mmol) and dry DCM (5 mL). A solution of acryloyl chloride (197 µL; 2.44 mmol) in dry DCM (2.5 mL) was then dropped slowly to the reaction mixture at 0 °C. After adding triethylamine (337 µL; 2.42 mmol), the yellow reaction solution was left to stir at room temperature under a nitrogen atmosphere for 4 hours. The DCM was then removed on the rotavapor. THF was then added and the resulting white precipitate was removed by filtration. The THF was subsequently removed by rotary evaporation leaving a yellow oil which was dried *in vacuo* (517.3 mg, 1.90 mmol, 94%). The crude product was further purified by preparative HPLC (system 3) (450 mg of crude product yielded 106 mg of pure product in form of a yellow oil). ν_{max} (NaCl): 3321 (m, NH), 2868 (br, aliphatic), 2103 (s, N_3), 1661 (s, C=O), 1627 (m, C=C), 1537 (s, NH), 1119 (m, C-O-C) cm^{-1}; ^{1}H NMR (δ, $CDCl_3$, 400 MHz): 6.29 (dd, J = 16.8 and 1.6 Hz, 1H; CH_{trans}=CH), 6.29 (bs, 1H, N*H*) 6.12 (dd, J = 16.8 and 10.4 Hz, 1H; C*H*=CH_2), 5.63 (dd, J = 10.4 and 1.6 Hz, 1H, CH_{cis}=CH), 3.69-3.60 (m, 12 H, CH_2-O), 3.54 (m, 2H, CH_2-NH), 3.39 (t, J = 5.2 Hz, 2H, CH_2-N_3); ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 165.6 (C), 131.1 (CH), 126.4 (CH), 70.8 (CH_2), 70.7 (CH_2), 70.7 (CH_2), 70.4 (CH_2), 70.2 (CH_2), 69.9 (CH_2), 50.8 (CH_2), 39.4 (CH_2).

N-Propargyl acrylamide 5



N-Propargylacrylamide **5** was synthesized according to a modified procedure described by Malkoch and co-workers.[1] Propargylamine (0.60 mL, 9.37 mmol) and *N*,*N*-diisopropylethylamine (1.96 mL, 11.24 mmol) were added to a 2-neck round bottom flask and dissolved in dry DCM (3 mL). To the mixture was then added dropwise a solution of acryloyl chloride (0.91 mL, 11.20 mmol) in dry DCM (2 mL). The reaction was left to stir overnight under a nitrogen atmosphere, after which time the organic phase was removed by rotary evaporation. The crude product was subsequently dissolved in ethyl acetate (50 mL) and washed three times with an aq. bicarbonate solution, twice with a brine solution and twice with distilled water. After collecting and drying the organic phase over $MgSO_4$, ethyl acetate was evaporated *in vacuo* to dryness, to afford the product as a white crystalline solid (367 mg, 36%). The analytical data are in agreement with literature values.[1] mp: 39-40°C; ν_{max} (KBr): 3293 (s, alkyne), 3234 (m, NH), 1677 (s, C=O), 1649 (s, C=C), 1545 (s, NH) cm^{-1}; ^{1}H NMR (δ, 400 MHz,$CDCl_3$) : 6.32 (dd, *J* = 17.2 and 1.6 Hz, 1H, C$H_{2,trans}$=CH), 6.10 (dd, *J* = 17.2 and 10.4 Hz, 1H, C*H*=CH_2), 5.79 (bs, 1H, N*H*), 5.69 (dd, *J* = 10.4 and 1.2 Hz, 1H, C$H_{2,cis}$=CH), 4.14 (dd, *J* = 5.2 and 2.4 Hz, 1H, CH_2-NH), 2.25 (t, *J* = 2.4 Hz, 1H, C*H*-C); ^{13}C (δ,100 MHz, $CDCl_3$): 165.1 (C), 130.1 (CH), 127.3 (CH_2), 79.3 (C), 71.8 (CH), 29.3 (CH_2); *m*/*z*: 110.1333 (MH^+), calc. 110.1327.

N^{α}-2-Azidoacetyl-lysin(N^{ε}-5-carboxyfluoresceinyl)amide 7



Rink amide (MBHA) resin (200 mg, 0.14 mmol, 0.70 mmol g^{-1}) was placed in a reaction column and left to swell in DMF (4 mL) for 4 h. The resin was then washed with DMF (5 min, 2.4 mL min^{-1}), treated with 20% v/v piperidine in DMF and washed again with DMF (10 minutes, 2.4 mL min^{-1}) on the peptide synthesizer. Fmoc-Lys(ivDde)-OH (321.8 mg, 0.56 mmol), HATU (212.9 mg, 0.56 mmol) and DIPEA (195 μL, 1.12 mmol), which were previously dissolved in DMF (2 mL), were then added. The reaction mixture was gently stirred at room temperature overnight. The resin was then washed with DMF (5 min, 2.4 mL min^{-1}), treated with 10% v/v piperidine in DMF and DMF (10 min, 2.4 mL min^{-1}). After removing the excess of DMF, 2-azidoacetic acid[2] (56.5 mg, 0.56 mmol), HATU (212.9 mg, 0.56 mmol) and DIPEA (195 μL, 1.12 mmol) dissolved in DMF (2 mL) were added. The mixture was stirred intermittently for 6 h.

After washing the resin with DMF on the peptide synthesizer (10 min, 2.4 mL min-1), the resin was treated for 1 hour with 2% v/v hydrazine monohydrate in DMF (1 mL min^{-1}), followed by washing with DMF (15 min, 2.4 mL min^{-1}). To the reaction mixture was then added the mixture 5-carboxyfluorescein (102.8 mg, 0.27 mmol), HOAt (38.1 mg, 0.28 mmol) and DIC (48.2, 0.31 mmol), which were previously dissolved in DMF (2 mL). The reaction was left under intermittent

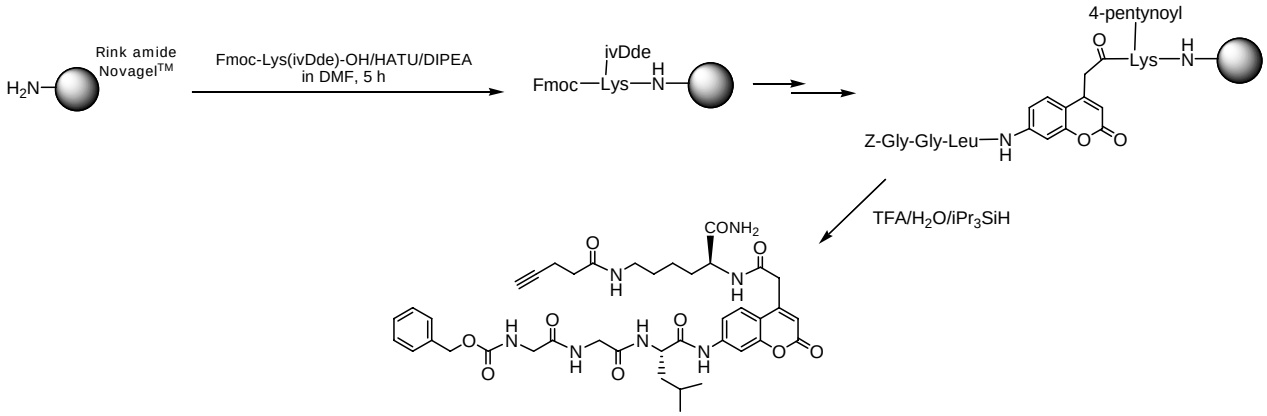
[1] M. Malkoch, R. J. Thibault, E. Drockenmuller, M. Messerschmidt, B. Voit, T. P. Russell and C. J. Hawker, *J. Am. Chem. Soc.*, 2005, **127**, 14942.

[2] S. Parkhouse, M. Garnett and W. C. Chan, *Bioorg. Med. Chem.*, 2008, **16**, 6641. ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 3.98 (s, CH_2), 8.98 (COO*H*); ^{13}C-NMR (δ, $CDCl_3$, 100 MHz): 174.4 (C), 50.0 (CH_2).

stirring in the dark overnight, after which time the resin product was washed with DMF, treated with 10 % v/v piperidine in DMF (30 min, 1.5 mL min^{-1}) and washed with DMF. The resin was subsequently collected by filtration, followed by successively washing with DMF, DCM and hexane. The resin was then dried *in vacuo* (247 mg).

The dry resin was placed in a 100 mL round bottom flask, to which triisopropylsilane (2 mL), water (2 mL) and TFA (38 mL) were added. The suspension was gently agitated in the dark for 4 h, after which time the suspension was filtered. The filtrate was evaporated to dryness *in vacuo* yielding an orange film. The orange film was gently washed with Et_2O (2 x 20 mL) and then dissolved in water (30 mL). After lyophilisation, the crude product was obtained as an orange solid (76.1 mg, 93%). Analytical HPLC (system 1): t_R = 4.49 min, *ca.* 95% purity. ν_{max} (KBr): 3393 (br, COOH), 2918 (br, aliphatic), 2110 (s, N_3), 1664 (s, C=O), 1638 (s, NH), 1541 (s, NH) cm^{-1}; ^{1}H-NMR (δ, [D_6]-DMSO, 400 MHz): 10.14 (bs, 2H, N*H*$_2$), 8.80 (t, *J* = 5.2 Hz, 1H, N*H*), 8.45 (d, *J* = 0.8 Hz, 1H, Ar-*H*), 8.23 (dd, *J* = 8.0/1.6 Hz, 1H, Ar-*H*), 8.17 (d, *J* = 8.0 Hz, 1H, N*H*), 7.45 (bs, 1H, O*H*), 7.36 (d, *J* = 8.0 Hz, 1H, Ar-*H*), 7.04 (bs, 1H, O*H*), 6.68 (d, *J* = 2.4 Hz, 2H, 2 x Ar-*H*), 6.59-6.52 (m, 6H, 6 x Ar-*H*), 4.22 (m, 1H, C*H*), 3.85 (d, *J* = 6.0 Hz, 2H, C*H*$_2$-N_3), 3.29 (m, 2H, CH_2-N*H*); *m*/*z*: 587.0926 (MH^+), calc. 587.1890.

Z-Gly-Gly-Leu-ACA-Lys(*N*$^{\varepsilon}$-pentynoyl)-NH_2 8



Rink amide Novagel™ resin (154 mg, 0.10 mmol, 0.67 mmol g^{-1}) was placed in a reaction column and left to swell in DMF (4 mL) for 4 h. The resin was then washed with DMF (5 min, 2.4 mL min^{-1}), treated with 20% v/v piperidine in DMF and washed again with DMF (10 minutes, 2.4 mL min^{-1}) on the peptide synthesizer. Fmoc-Lys(ivDde)-OH (237 mg, 0.41 mmol), HATU (157 mg, 0.41 mmol), HOAt (56 mg, 0.41 mmol) and DIPEA (144 µL, 0.83 mmol), which where were previously dissolved in DMF (1.5 mL), were then added. The reaction mixture was gently stirred at room temperature for five h.

The resin was then washed with DMF (5 mL, 2.4 mL min^{-1}), treated with 20% v/v piperidine in DMF (10 min, 2.4 mL min^{-1}) and washed with DMF (10 min, 2.4 mL min^{-1}). After removing the excess of DMF, 7-aminocoumarin-4-acetic acid[3] **9** (91 mg, 0.41 mmol), HOAt (56 mg, 0.41 mmol) and DIC (73 µL, 0.46 mmol) dissolved in DMF (1.5 mL) were added. The reaction mixture was left to stir intermittently for 24 h in the dark.

After washing the resin with DMF on the peptide synthesizer, the excess of DMF was removed and Fmoc-Leu-OH (148 mg, 0.69 mmol.), HATU (156 mg, 0.41 mmol), HOAt (56 mg, 0.41 mmol) and DIPEA (144 µL, 0.83 mmol) dissolved in DMF (2 mL) were added. The reaction mixture was then gently stirred in the dark overnight, after which time the resin was washed with DMF. Subsequently, a second coupling of Fmoc-Leu-OH was carried out using the same amount of amino acid and coupling reagents. Before the coupling of Fmoc-Gly-OH, the resin was washed with DMF,

[3] D. J. Maley, F. Leonetti, J. Backes, D. S. Dauber, J. L. Harris, C. S. Craik and J. A. Ellman, *J. Org. Chem.* 2002, **67**, 910; K. Welser, J. Grilj, E. Vauthey, J. W. Aylott and W. C. Chan, *Chem. Commun.* 2009, 671.

treated with 20% v/v piperidine in DMF and washed with DMF (3 x 10 min, 2.5 mL min^{-1}) on the peptide synthesizer. The excess of DMF was then removed and Fmoc-Gly-OH (127 mg, 0.43 mmol), HATU (163 mg, 0.43 mmol), HOAt (56 mg, 0.41 mmol) and DIPEA (144 μL, 0.83 mmol) dissolved in DMF (2 mL) were added. The reaction mixture was stirred on intermittent stir overnight, after which time the peptide column was washed with DMF, treated with 20% v/v piperidine in DMF, and washed with DMF (3 x 10 min, 2.5 mL min^{-1}) on the peptide synthesizer. Z-Gly-OH (86 mg, 0.41 mmol), HATU (157 mg, 0.41 mmol), HOAt (57 mg, 0.42 mmol) and DIPEA (144 μL, 0.83 mmol) dissolved in DMF (1 mL) were then added and the reaction mixture left to stir overnight in the dark for six hours.
The resin was then washed with DMF (10 min, 2.5 mL min^{-1}), treated with hydrazine monohydrate (2% in DMF) for 20 minutes and washed again with DMF (10 min, 2.5 mL min^{-1}). After removing the excess of DMF, 4-pentynoic acid (43 mg, 0.44 mmol), HATU (157 mg, 0.41 mmol), HOAt (57 mg, 0.42 mmol) and DIPEA (144 μL, 0.83 mmol) dissolved in DMF (2mL) were added to the peptide column. The reaction mixture was gently stirred in the dark overnight, after which time the resin was washed with DMF, collected by filtration followed by washing with DMF, DCM and hexane. The resin was then dried *in vacuo*.
The dry resin was placed in a 100 mL round bottom flask, to which triisopropylsilane (2 mL), water (2 mL) and TFA (38 mL) were added. The suspension was then left on intermittent stir in the dark for 4 hours, after which time the suspension was filtered. The filtrate was evaporated to dryness *in vacuo* yielding a greyish film. The greyish film was gently washed with Et_2O (2 x 20 mL). After lyophilisation, the crude product was obtained as a white solid (40 mg, 36%). Analytical HPLC (system 2): t_R = 18.62 min, *ca.* 50% purity. The crude product was further purified by preparative RP chromatography (system 4): 39.4 mg of crude product yielded 5.6 mg of pure product as a white solid. ^{1}H NMR (δ, 400 MHz, [D_6]-DMSO): 10.35 (bs, 1H, Ar-N*H*), 8.37 (d, *J* = 8.4 Hz, 1H, N*H*(Lys)), 8.17 (t, *J* = 5.6 Hz, 1H, N*H*(Leu)), 8.13 (d, *J* = 8.0 Hz, 1H, N*H*(Gly)), 7.83 (t, *J* = 5.2 Hz, 1H, N*H*(CH_2-Lys)), 7.80 (d, *J* = 2 Hz, 1H, C_8-*H* (coumarin)), 7.75 (d, *J* = 8.8 Hz, 1H, C_5-*H* (coumarin)), 7.54-7.50 (m, 2H, N*H*(Gly), C_6-*H* (coumarin)), 7.40-7.30 (m, 5H, Cbz-*H*), 6.99 (bs, NH_2), 6.32 (s, 1H, C_3-*H* (coumarin)), 5.03 (s, 2H, CH_2 (Cbz)), 4.47 (m, 1H, C*H*(Leu)), 4.17 (m, 1H, C*H*(Lys)), 3.78 (d, *J* = 5.6 Hz, CH_2(Gly)), 3.75 (s, 2H, CH_2(coumarin)), 3.66 (d, *J* = 6.0 Hz, CH_2(Gly)), 3.28 (s, 1H, C*H*-alkyne), 3.03-2.97 (m, 2H, CH_2-NH), 2.36-2.32 (m, 2H, CH_2(pentynoyl)), 2.25-2.22 (m, 2H, CH_2(pentynoyl)), 1.63-1.51 (m, 5H, C*H*(Leu). CH_2(Leu), CH_2(Lys)), 1.39-1.23 (m, *4*H, 2 x CH_2(Lys)), 0.92-0.87 (m, 6H, 2 x CH_3(Leu)); *m*/*z*: 788.3585 (MH^+), calc. 788.3619.

Synthesis of azide functionalized nanoparticles

A water-in-oil microemulsion was established by dissolving the surfactants Brij 30 (1.54 g) and AOT (0.80 g) in deoxygenated hexane (21 mL). To the mixture was then added the aqueous phase (1 mL), which consisted of the monomers acrylamide (265 mg, 3.73 mmol) and *N*-(11-azido-3,6,9-trioxaundecanyl)acrylamide **4** (12.5 mg, 0.046 mmol) and the crosslinker *N,N'*-methylene bisacrylamide (80 mg, 0.52 mmol). The polymerization was initiated by the addition of ammonium persulphate (15 μL, 10% w/v) and *N,N,N',N'*-tetramethylethylenediamine (7.5 μL). The reaction was allowed to proceed for 2 hours while being stirred under an argon atmosphere. After this time, the hexane was removed by rotary evaporation yielding an opaque, viscous residue. To remove surfactants and unreacted monomers, the particles were washed 10 times with absolute ethanol. The particles were then collected by vacuum filtration using a Millipore filtration system with a 0.02 μm Anodisc filter. After drying *in vacuo* the particles were obtained as a white powder. (257 mg, 72%).

Synthesis of alkyne functionalized nanoparticles

A water-in-oil microemulsion was established by dissolving the surfactants Brij 30 (1.54 g) and AOT (0.80 g) in deoxygenated hexane (21 mL). To the mixture was then added the aqueous phase (1 mL), which consisted of the monomers acrylamide (265 mg, 3.73 mmol) and *N*-propargylacrylamide **5** (12 mg, 0.11 mmol) and the crosslinker *N,N'*-methylene bisacrylamide (80 mg, 0.52 mmol). The polymerization was initiated by the addition of ammonium persulphate (15 µL, 10% w/v) and *N,N,N',N'*-tetramethylethylenediamine (7.5 µL). The reaction was allowed to proceed for 2 hours while being stirred under an argon atmosphere. After this time, the hexane was removed by rotary evaporation yielding an opaque, viscous residue. To remove surfactants and unreacted monomers, the particles were washed 10 times with absolute ethanol. The particles were then collected by vacuum filtration using a Millipore filtration system with a 0.02 µm Anodisc filter. After drying *in vacuo* the particles were obtained as a white powder (259 mg, 73%).

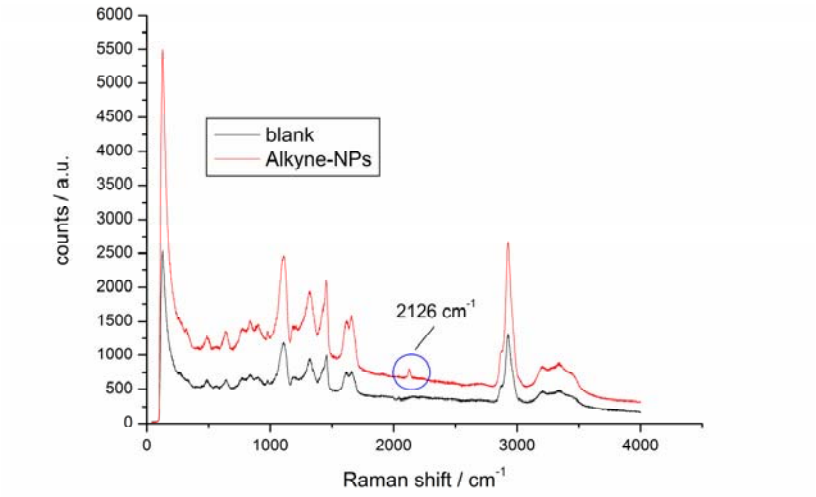
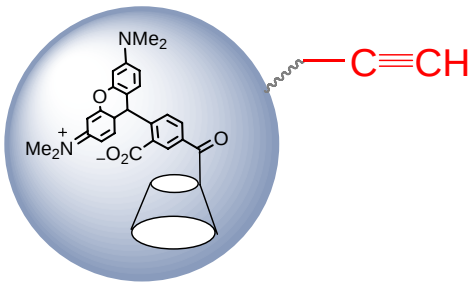


Figure S1 Raman spectra of the 'blank' (black solid line) and alkyne functionalized nanoparticles (red solid line).

Synthesis of alkyne functionalized nanoparticles with entrapped dextran-bound TAMRA fluorophore



The procedure is the same as for the synthesis of alkyne modified nanoparticles. In contrast to the procedure described above, the aqueous solution consisted of acrylamide (265 mg, 3.73 mmol), *N*-propargylacrylamide **5** (12 mg, 0.11 mmol), *N,N'*-methylene bisacrylamide (80 mg, 0.52 mmol) and TAMRA-dextran (100 µL, 5 mg mL^{-1} solution in water, MW 10,000) dissolved in water (800 µL) and DMSO (200 µL). After drying *in vacuo* the particles were obtained as a pink powder (207 mg, 58%).

Supplementary Material (ESI) for Chemical Communications
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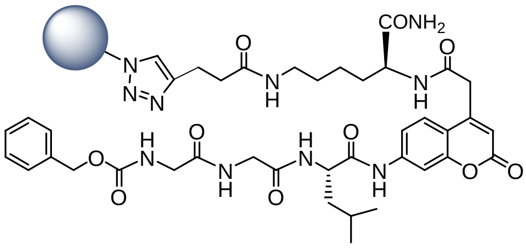
General procedure for clicking alkyne bearing compounds to azide functionalized nanoparticles

Click reaction with alkyne modified TAMRA fluorophore

Azide functionalized nanoparticles (10 mg) and the alkyne modified TAMRA fluorophore **6** (50 µL, 0.5 mg in 1 mL DMSO, 53.4 nmol) were added to a vial and were subsequently suspended in a solvent mixture of water:*t*-butanol:DMSO of 45:45:10. To the vial was then added the Cu(I) catalyst, tetrakis(acetonitrile) copper(I) hexafluorophosphate (10 mol%, 5.34 nmol) and the Cu(I) stabilizing ligand TBTA (10 mol%, 5.34 nmol), achieved by serial dilutions in DMSO. The reaction mixture was gently stirred for 48 hours in the dark, after which time the particles were washed with DMF (15 times, 1.5 mL) and EtOH (15 times, 1.5 mL) by centrifugation. The pinkish particles were subsequently dried *in vacuo* (8 mg). The negative control reaction was identical to above, except unmodified poly(acrylamide) nanoparticles were used.

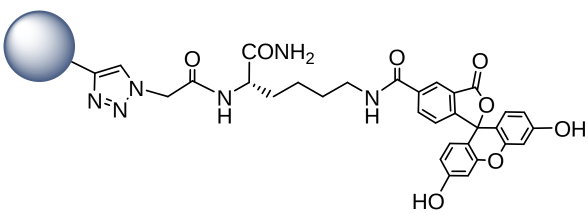
Click reaction with Z-Gly-Gly-Leu-ACA-Lys(N^{ε}-pentynoyl)-NH_2



Azide functionalized nanoparticles (17 mg) and Z-Gly-Gly-Leu-ACA-Lys(N^{ε}-pentynoyl)-NH_2 **8** (1.5 mg, 1.90 µmol) were added to a vial and were subsequently suspended in a solvent mixture of water:*t*-butanol:DMSO of 45:45:10. To the vial was then added tetrakis(acetonitrile) copper(I) hexafluorophosphate (10 mol%, 190 nmol) and TBTA (10 mol%, 190 nmol), achieved by serial dilutions in DMSO. The suspension was gently stirred for 48 hours in the dark, after which time the particles were washed with DMF (15 times, 1.5 mL) and EtOH (15 times, 1.5 mL) by centrifugation. After drying *in vacuo*, the particles were obtained as a white powder (18 mg). The negative control reaction was identical to above, except unmodified poly(acrylamide) nanoparticles were used

General procedure for clicking azide bearing compounds to alkyne functionalized nanoparticles

Click reaction with N^{α}-2-azidoacetyl-lysin(N^{ε}-5-carboxyfluoresceinyl)amide



Alkyne functionalized nanoparticles (20 mg) and N^{α}-2-azidoacetyl-lysin(N^{ε}-5-carboxy-fluoresceinyl)amide **7** (2.5 mg, 4.25 µmol) were added to a vial and were subsequently suspended

in a solvent mixture of water:*t*-butanol:DMSO of 70:20:10. To the vial was then added tetrakis(acetonitrile) copper(I) hexafluorophosphate (7 mol%, 295 nmol) and TBTA (7 mol%, 295 nmol), achieved by serial dilutions in DMSO. The reaction mixture was gently stirred for 48 hours in the dark, after which time the particles were washed with DMF (15 times, 1.5 mL) and EtOH (15 times, 1.5 mL) by centrifugation. After drying *in vacuo*, the particles were obtained as a white powder (19 mg). The negative control reaction was identical to above, except unmodified poly(acrylamide) nanoparticles were used.

Dynamic light scattering (DLS)

DLS measurements were carried out using a Viscotek Model 802 instrument equipped with an internal laser (825–832 nm) with a maximum radiation power of 60 mW. Samples of suspended nanoparticles in filtered deionised water (100 µl) were aliquoted into a black quartz cuvette with two small windows located at 90° as per the light path of this instrument. Measurements were taken at 20 °C and each analysis was made up of 10 correlation analyses lasting 10 seconds each. The intensity count was adjusted with sample dilution and laser intensity control (software manipulated), until a steady level between 500 and 1000 kilocounts was achieved. The software OmniSIZE3 was used to calculate the experimental correlation factors. The software uses a L-curve optimization algorithm to transpose the raw autocorrelation data into size distributions. Using the 'sum' and 'calc' options provided in the software, a mean hydrodynamic radius was ascertained for each sample. The resulting data was displayed showing the calculated population distribution of particle size and relevant peak analysis of the population.

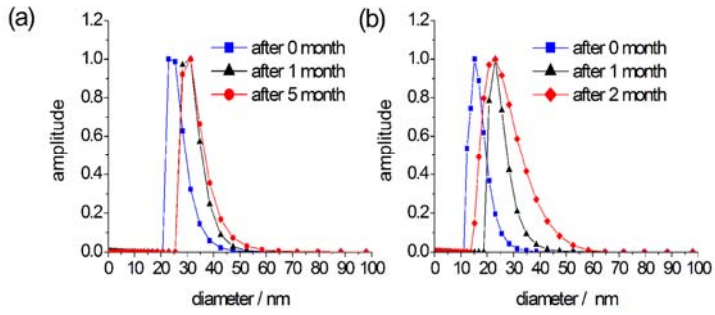


Figure S2 (a) Stability of azide functionalized nanoparticles (NPs) (b) Stability of alkyne functionalized NPs.

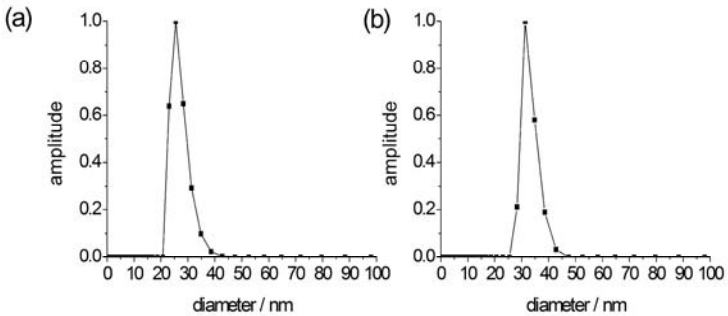


Figure S3 DLS of (a) alkyne functionalized NPs which were reacted *via* CuAAC with **7** and (b) azide functionalized NPs which were reacted *via* CuAAC with **6**.

Subtilisin mediated proteolytic reaction

Proteolytic cleavage reactions of peptide substrate bound nanoparticle (1.0 mg mL^{-1}) were performed at 37 °C in Tris–HCl buffer solutions (50 mM, pH = 8.20) using a Cary Eclipse fluorescence spectrophotometer. After pipetting the substrate (990 μL) into a quartz cuvette and placing the latter into the fluorimeter, the sample solution was incubated at 37 °C for 3 min. The cleavage reaction was initiated by the addition of a subtilisin solution (0.033 mM, 10 μL) in Tris–HCl buffer. The change in the fluorescence was monitored over a time period of 30 min (λ_{ex} = 380 nm).

pH responsive hybrid nanosensors

Alkyne functionalized nanoparticles, in which TAMRA-dextran (MW 10000) was incorporated and to which N^{α}-2-azidoacetyl-lysin(N^{ε}-5-carboxyfluoresceinyl)amide **7** was clicked to, were calibrated against a glass electrode Merohm 809 Titrando pH meter over a pH range of 5.3 to 7.6 in 0.1 M phosphate buffer saline (PBS). 1 mg mL^{-1} solutions were made up at each pH and 5-FAM and TAMRA-dextran excited at 490 and 555 nm and the emission recorded at 520 and 580 nm respectively on a Varian Cary Eclipse Fluorescence Spectrophotometer. Measurements were carried out in triplicate.