Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Cell-Targeted Platinum Nanoparticles and Nanoparticle Clusters

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Supporting Information

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1. Experimental Procedures

1.1 Reagents and General Methods

All reagents were purchased as reagent grade from commercial sources and used as supplied. Solvents for reactions were purchased as synthesis grade and used as supplied. RP-HPLC solvents were purchased as HPLC grade and used without further purification. All amino acids were purchased from either CEM corp. or GL Biochem as L-enantiomers with the following side chain protection: Fmoc-His(Trt)-OH, Fmoc-Ala-OH, Fmoc-Leu-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asp-OtBu and Fmoc-Glu(OtBu)-OH. ESI-MS were recorded on a HP 1100 Series LC/MSD spectrometer. Semi-preparative RP-HPLC was performed on a Dionex Ultimate 3000 system using the following column: Phenomenex Gemini $C_{18,}$ 110 Å, 10 mm · 250 mm, 5 μm at a flow rate of 5 mL/min. Analytical RP-HPLC was performed on a Dionex Ultimate 3000 system using the following column: Phenomenex Gemini $C_{18,}$ 110 Å, 4.6 mm · 150 mm, 3 μm at a flow rate of 1 mL/min. A linear gradient of 0.1% trifluoroacetic acid-water (A) and 0.1% trifluoroacetic acid-acetonitrile (B) was used with detection at 210 nm. Dynamic light scattering (DLS) measurements were conducted with a Malvern Zetasizer Nano Series Nano-ZS at 25°C using a Malvern ZEN 1002 dip cell kit. Light microscopy images have been acquired with a Leica DMRE microscope fitted with a Leica DC500 digital camera. Transmission electron microscope (TEM) images have been acquired using a JEOL 2100F microscope operated at an acceleration voltage of 200 keV and equipped with a Gatan SC1000A1 digital camera or a FEI Tecnai T12 electron microscope operated at an acceleration voltage of 120 keV and equipped with a Gatan Ultrascan 1000 digital camera. Electron diffraction patterns and STEM-EDS analyses have been generated with a JEOL 2010 transmission electron microscope operated at an acceleration voltage of 200 keV and equipped with an Oxford Inca EDS detector. Image analysis of the light microscopy and TEM images was performed with ImageJ software for the average size of cell clumps and core size of PtNPs, respectively. SEM images were acquired with a FEI (Philips) XL30 S-FEG SEM fitted with a SiLi (lithium drifted) EDS detector with a super ultra thin window and a Gatan Alto 2500 cryo unit and with a FEI Quanta 200 field emission Environmental SEM.

1.2 Preparation of HALNN

Peptide HALNN was assembled manually on a 0.2 mmol scale using standard Fmoc SPPS in a glass reaction vessel. The synthesis was performed on aminomethylated polystyrene resin

(0.2 mmol, 200 mg, loading 1.0 mmol/g) derivatised with Rink amide linker. For linker attachment, the resin was swollen in DMF for 15 mins, then the DMF was drained. Rink amide linker (0.6 mmol, 324 mg) and Oxyma Pure (0.76 mmol, 108 mg) were dissolved in 3 mL DMF, DIC (0.6 mmol, 92 µL) was added and the reaction mix added to the resin. After shaking for 2 h, the reaction mix was drained and the resin washed with DMF (3x) and DCM (3x).

For the attachment of C-terminal asparagine, the Rink amide linker was deprotected with 20% piperidine/ DMF (4 mL, 2 x 5min), the resin washed with DMF (3x), Fmoc-Asp-OtBu (1 mmol, 412 mg, attachment via side chain) dissolved in 4 mL 0.24 M HBTU/ DMF and 1 mL 2 M *N*-methylmorpholine/ DMF and the mixture added to the resin. After shaking for 45 min, the reaction mix was drained and the resin washed with DMF (3x) and DCM (3x).

For peptide chain assembly, the preceding amino acid was deprotected with 20% piperidine/ DMF (2 x 4 mL, 2 x 5min), then the resin washed with DMF (3x). 1 mmol of the respective amino acid (His: 620 mg, Ala: 312 mg, Leu: 353 mg, Asn: 412 mg) was dissolved in 4 mL 0.24 M HBTU/ DMF and 1 mL 2 M *N*-methylmorpholine/ DMF and added to the resin. After shaking for 1 h, the reaction mix was drained and the resin washed with DMF (3x) and DCM (3x). After final Fmoc deprotection, the peptide was released from resin with concomitant removal of the side-chain protecting groups by shaking for 3 h in 15.2 mL TFA, 400 µL H_2O and 400 µL TIS. The resin was filtered, the peptide precipitated from the cleavage mix with cooled Et_2O (5°C) and the supernatant decanted. After washing with fresh Et_2O, the peptide was freeze-dried from H_2O/ACN solution, yielding 143.7 mg of crude peptide.

The whole amount of crude peptide was purified in a single run by semi-preparative HPLC, initially running for 10 min at 0% B followed by a linear gradient of 0% to 50% B over 13 min, yielding 105 mg of purified peptide (93 %) as a white solid in *ca.* 99% purity according to analytical RP-HPLC. R_t 13.5 min (1-60% B over 20 min, 1 mL/min); *m/z* (ESI-MS): observed mass = 568.0, calculated mass = 567.6 [$M+H^+$].

1.3 Preparation of HALNNE$_6$

Peptide HALNNE$_6$ was assembled using a Tribute peptide synthesiser on a 0.1 mmol scale using standard Fmoc SPPS. The synthesis was performed on aminomethylated polystyrene resin (0.1 mmol, 100 mg, loading 1.0 mmol/g). The resin was swollen in DCM (5 mL) for 15 min and then the solvent was drained. Fmoc-L-Glu(OtBu)-$OCH_2PhOCH_2CH_2CO_2H$ (0.2 mmol, 120.7 mg) was dissolved in 2 mL of DCM, DIC (0.2 mmol, 31 µL) was added and the reaction mixture was added to the resin and shaken for 1h. The mixture was drained and the

resin washed with DMF (3 ×) and DCM (3 ×). Couplings of the subsequent amino acids (0.5 mmol) were carried out for 45 min at room temperature in the presence of HBTU (0.46 mmol) and NMM (1 mmol) in DMF. The N^{α}-protecting groups were removed with 20% piperidine solution in DMF (2 × 3 mL, 2 × 5 min). After final Fmoc deprotection, the assembled peptide was released from resin with concomitant removal of the side-chain protecting groups by shaking for 3 h in 9.5 mL TFA, 250 μL H_2O and 250 μL TIS. The resin was filtered, the peptide precipitated from the cleavage mix with cooled Et_2O (5°C) and the supernatant decanted. After washing with fresh Et_2O, the peptide was freeze-dried from H_2O/ ACN solution, yielding 122.8 mg of crude peptide. 23.37 mg of crude peptide were purified by semi-preparative HPLC with a linear gradient of 0% to 50% acetonitrile over 20 min, yielding 15.6 mg of purified peptide as a white solid in *ca.* 95% purity according to analytical RP-HPLC. R_t 18.7 min (1-60% acetonitrile over 20 min, 1 mL/min); *m/z* (ESI-MS): observed mass = 671.8, calculated mass = 672.2 $[M+2H]^{2+}$.

1.4 Preparation of H_2N-mini-PEG-CO_2H

Boc-8-amino-3,6-dioxaoctanoic acid (Boc-mini-PEG™, 0.51 mg, 1.92 μmol) was dissolved in 100 μL of neat TFA and shaken for 30 mins. The TFA was evaporated under a stream of nitrogen gas and then the sample was dissolved in 100 μL of water and freeze-dried yielding a white solid.

1.5 Preparation of H_2N-mini-PEG-GRGD-CO_2H

Peptide mini-PEG™-GRGD was assembled manually using a sintered glass reaction vessel on a 0.1 mmol scale using standard Fmoc SPPS. The synthesis was performed on aminomethylated polystyrene resin (0.1 mmol, 102 mg, loading 0.98 mmol/g). The resin was swollen in DCM (5 mL) for 15 min and then the solvent was drained. Fmoc-L-Asp(OtBu)-$OCH_2PhOCH_2CH_2CO_2H$ (0.2 mmol, 118 mg) was dissolved in 2 mL of DCM, DIC (0.2 mmo 31 μL) was added and the reaction mixture was added to the resin and shaken for 1h. The mixture was drained and the resin washed with DMF (3 ×) and DCM (3 ×). Couplings of the subsequent amino acids (0.5 mmol) were carried out in 45 min at room temperature in the presence of HCTU (0.46 mmol) and NMM (1 mmol) in DMF. The N^{α}-protecting groups were removed with 20% piperidine solution in DMF (2 × 3 mL, 2 × 5 min). After Fmoc deprotection of Gly-4, Fmoc-8-amino-3,6-dioxaoctanoic acid (Fmoc-mini-PEG™, 193 mg, 0.5 mmol) was dissolved in 2 mL of 0.23M HCTU in DMF, NMM (110 μL, 1 mmol) was

added and the mixture added to the resin and shaken for 1 h. The mixture was drained and the resin washed with DMF and DCM. After final Fmoc deprotection, the assembled peptide was released from resin with concomitant removal of the side-chain protecting groups by shaking for 3 h in 9.4 mL TFA, 250 µL H_2O, 250 µL DODT and 100 µL TIS. The resin was filtered, the peptide precipitated from the cleavage mix with cooled Et_2O (5°C) and the supernatant decanted. After washing with fresh Et_2O twice, the peptide was freeze-dried from H_2O/ acetonitrile solution, yielding 65.7 mg of crude peptide in *ca.* 81% purity according to analytical RP-HPLC. R_t = 7.51 min (0-41% acetonitrile over 20 min, 1 mL/min); *m/z* (ESI-MS): observed mass = 275.2, calculated mass = 275.8 $[M+2H]^{2+}$.

1.6 Preparation of PtNPs-HALNN (Precursor for ConA conjugates 1-3)

K_2PtCl_6 (4.9 mg, 10 µmol) and HALNN (0.09 mg, 0.16 µmol) were dissolved in 10 mL distilled H_2O and stirred for 30 min at room temperature. Then $NaBH_4$ (40 µL, 80 µmol) was added and the reaction mix stirred for 30 min during which it changed colour from yellow to dark brown due to PtNP formation. The PtNPs were centrifuged (14500 rpm, 20 min) and the supernatant decanted. The PtNPs were resuspended in PBS buffer (1x, pH 7.4) using sonication, centrifuged and the supernatant decanted.

For TEM sample preparation 1 mL of the crude PtNP suspension was centrifuged (14500 rpm, 20 min) and the light brown supernatant was decanted. The black pellet was resuspended in 500 µL EtOH using sonication, centrifuged (14500 rpm, 10 min) and the supernatant decanted (2x). Then the pellet was resuspended in 100 µL EtOH using sonication, several drops of the suspension were casted onto a TEM grid and the grid was air dried in a dust-free environment.

1.7 Preparation of PtNPs-HALNN (Precursor for mini-PEG and RGD conjugates 6-7)

K_2PtCl_6 (9.72 mg, 20 µmol) and HALNN (0.18 mg, 0.32 µmol) were dissolved in 20 mL distilled H_2O and stirred for 30 min at room temperature. Then $NaBH_4$ (80 µL, 160 µmol) was added and the reaction mix stirred for 30 min during which it changed colour from yellow to dark brown due to PtNP formation. The PtNPs were centrifuged (26000 rpm, 2 h) and the supernatant decanted. The PtNPs were resuspended in 10 mL PBS buffer (1x, pH 7.4) using sonication, freeze-dried and stored at -20°C.

1.8 Preparation of PtNPs-HALNNE$_6$ (Precursor for mini-PEG and RGD conjugates 8-9)

K_2PtCl_6 (9.72 mg, 20 µmol) and HALNNE$_6$ (0.43 mg, 0.32 µmol) were dissolved in 20 mL distilled H_2O and stirring for 30 min at room temperature. $NaBH_4$ (80 µL, 160 µmol) was added and the reaction mix stirred for 30 min during which it changed colour from yellow to dark brown due to PtNP formation. The PtNPs-HALNNE$_6$ were centrifuged (27000 rpm, 2 h) and the supernatant decanted. The PtNPs-HALNNE$_6$ were resuspended in 10 mL PBS buffer (1x, pH 7.4) using sonication, freeze-dried and stored at -20°C.

PtNPs-HALNNE$_6$ for the **fibroblast binding assay** were prepared following the procedure above, but in the final step they were resuspended in 2 mL PBS buffer (instead of 10 mL), divided into 10 aliquots à 200 µL, freeze-dried and stored -20°C.

For **TEM sample preparation** 1 mL of the crude PtNP suspension was centrifuged (14500 rpm, 20 min) and the brown supernatant was decanted. The black pellet was resuspended in 500 µL EtOH using sonication, centrifuged (14500 rpm, 5 min) and the supernatant decanted (2x). Then the pellet was resuspended in 500 µL EtOH using sonication, several drops of the suspension were casted onto a TEM grid and the grid was air dried in a dust-free environment.

1.9 Preparation of PtNPs-HALNN-ConA 1

EDC·HCl (0.184 mg, 0.96 µmol, 6 eq. relative to HALNN) and sulfo-NHS (0.05 mg, 0.24 µmol, 1.5 eq. relative to HALNN) were dissolved in 500 µL PBS and added to the PtNPs-HALNN conjugates prepared according to section 1.6. After short sonication to resuspend the NPs, the reaction mix was shaken for 30 min, centrifuged (14500 rpm, 15 min) and decanted. The activated PtNPs-HALNN conjugates were washed with 250 µL PBS, centrifuged and the supernatant decanted. Concanavalin A (270 µg) and the activated PtNPs-HALNN conjugates were dissolved/ resuspended in 1 mL PBS each. The two solutions were combined and shaken at 900 rpm for 3 h at room temperature. The PtNP-HALNN-ConA **1** clusters were centrifuged (5000 rpm, 5 min) and the supernatant decanted. 1 mL of PBS was added followed by sonication, centrifugation and decantation to remove non-bound protein (3x). Finally, 1 mL of PBS was added and the solution separated into two aliquots à 500 µL. The samples were freeze-dried and stored at -20°C (after reconstitution 500 µL of the solution were used for DLS analysis and the other 500 µL for the rbc aggregation assay, see section 1.13).

1.10 Preparation of PtNPs-HALNN-mini-PEG 6

K_2PtCl_6 (9.72 mg, 20 μmol) and HALNN (0.18 mg, 0.32 μmol) were dissolved in 20 mL distilled H_2O and stirred for 30 min at room temperature. Then $NaBH_4$ (80 μL, 160 μmol) was added and the reaction mix stirred for 3 h during which it changed colour from yellow to dark brown due to PtNP formation. The PtNPs-HALNN conjugates were centrifuged (26000 rpm, 2 h) and the supernatant decanted. The PtNPs-HALNN conjugates were then resuspended in 10 mL of PBS buffer (1x, pH 7.4) using sonication and freeze-dried yielding a grey powder.

The freeze-dried mixture was resuspended in 10 mL distilled H_2O using sonication, EDC (1.92 μmol, 0.37 mg) and sulfo-NHS (0.48 μmol, 0.10 mg) were dissolved in 500 μL each and added to the suspension of PtNPs-HALNN conjugates. The mixture was shaken at 750 rpm at rt for 30 mins to activate the C-termini of the HALNN peptides. Then the mixture was centrifuged (26000 rpm, 1 h) and the supernatant decanted. The activated PtNPs-HALNN c9onjugates were resuspended in 10 mL PBS using sonication and H_2N-mini-PEG-CO_2H (1.92 μmol, 0.31 mg) dissolved in 1 mL of PBS was added. The mixture was shaken at 750 rpm at rt overnight, then centrifuged (20000 rpm, 30 min) and the supernatant decanted. The PtNPs-HALNN-mini-PEG conjugates were resuspended in 2 mL of PBS, divided into 10 aliquots à 200 μL, freeze-dried and stored -20°C.

1.11 Preparation of PtNPs-HALNN-mini-PEG-GRGD 7

K_2PtCl_6 (9.72 mg, 20 μmol) and HALNN (0.18 mg, 0.32 μmol) were dissolved in 20 mL distilled H_2O and stirred for 30 min at room temperature. Then $NaBH_4$ (80 μL, 160 μmol) was added and the reaction mix stirred for 3 h during which it changed colour from yellow to dark brown due to PtNP formation. The PtNPs-HALNN conjugates were centrifuged (26000 rpm, 2 h) and the supernatant decanted. The PtNPs-HALNN conjugates were then resuspended in 10 mL of PBS buffer (1x, pH 7.4) using sonication and freeze-dried yielding a grey powder.

The freeze-dried mixture was resuspended in 10 mL distilled H_2O using sonication, EDC (1.92 μmol, 0.37 mg) and sulfo-NHS (0.48 μmol, 0.10 mg) were dissolved in 500 μL each and added to the suspension of PtNPs-HALNN conjugates. The mixture was shaken at 750 rpm at rt for 30 mins to activate the C-termini of the HALNN peptides. Then the mixture was centrifuged (26000 rpm, 1 h) and the supernatant decanted. The activated PtNPs-HALNN conjugates were resuspended in 10 mL PBS using sonication and H_2N-mini-PEG-GRGD-CO_2H (0.64 μmol, 0.35 mg) dissolved in 1 mL of PBS was added. The mixture was shaken at

750 rpm at rt overnight, then centrifuged (20000 rpm, 30 min) and the supernatant decanted. The PtNPs-HALNN-mini-PEG-GRGD conjugates were resuspended in 2 mL of PBS, divided into 10 aliquots à 200 µL, freeze-dried and stored -20°C.

1.12 Preparation of PtNPs-HALNNE$_6$-mini-PEG 8

K_2PtCl_6 (9.72 mg, 20 µmol) and HALNNE$_6$ (0.43 mg, 0.32 µmol) were dissolved in 20 mL distilled H_2O and stirred for 30 min at room temperature. Then $NaBH_4$ (80 µL, 160 µmol) was added and the reaction mix stirred for 3 h during which it changed colour from yellow to dark brown due to PtNP formation. The PtNPs-HALNNE$_6$ conjugates were centrifuged (27000 rpm, 2 h) and the supernatant decanted. The PtNPs-HALNNE$_6$ conjugates were then resuspended in 10 mL of PBS buffer (1x, pH 7.4) using sonication and freeze-dried yielding a grey powder.

The freeze-dried mixture was resuspended in 10 mL distilled H_2O using sonication, EDC (1.92 µmol, 0.37 mg) and sulfo-NHS (0.48 µmol, 0.10 mg) were dissolved in 500 µL each and added to the suspension of PtNPs-HALNNE$_6$ conjugates. The mixture was shaken at 750 rpm at rt for 30 mins to activate the carboxylates of the HALNNE$_6$ peptides. Then the mixture was centrifuged (27000 rpm, 2 h) and the supernatant decanted. The activated PtNPs-HALNNE$_6$ conjugates were resuspended in 10 mL PBS using sonication and H_2N-mini-PEG-CO_2H (1.92 µmol, 0.31 mg) dissolved in 1 mL of PBS was added. The mixture was shaken at 750 rpm at rt overnight, then centrifuged (27000 rpm, 1 h) and the supernatant decanted. The PtNPs-HALNNE$_6$-mini-PEG conjugates were resuspended in 2 mL of PBS, divided into 10 aliquots à 200 µL, freeze-dried and stored -20°C.

1.13 Preparation of PtNPs-HALNNE$_6$-mini-PEG-GRGD 9

K_2PtCl_6 (9.72 mg, 20 µmol) and HALNNE$_6$ (0.43 mg, 0.32 µmol) were dissolved in 20 mL distilled H_2O and stirred for 30 min at room temperature. Then $NaBH_4$ (80 µL, 160 µmol) was added and the reaction mix stirred for 3 h during which it changed colour from yellow to dark brown due to PtNP formation. The PtNPs-HALNNE$_6$ conjugates were centrifuged (27000 rpm, 2 h) and the supernatant decanted. The PtNPs-HALNNE$_6$ conjugates were then resuspended in 10 mL of PBS buffer (1x, pH 7.4) using sonication and freeze-dried yielding a grey powder.

The freeze-dried mixture was resuspended in 10 mL distilled H_2O using sonication, EDC (1.92 µmol, 0.37 mg) and sulfo-NHS (0.48 µmol, 0.10 mg) were dissolved in 500 µL each and added to the suspension of PtNPs-HALNNE$_6$ conjugates. The mixture was shaken at 750

rpm at rt for 30 mins to activate the carboxylates of the $HALNNE_6$ peptides. Then the mixture was centrifuged (27000 rpm, 2 h) and the supernatant decanted. The activated PtNPs-$HALNNE_6$ conjugates were resuspended in 10 mL PBS using sonication and H_2N-mini-PEG-GRGD (0.64 µmol, 0.35 mg) dissolved in 1 mL of PBS was added. The mixture was shaken at 750 rpm at rt overnight, then centrifuged (27000 rpm, 1 h) and the supernatant decanted. The PtNPs-$HALNNE_6$-mini-PEG-GRGD conjugates were resuspended in 2 mL of PBS, divided into 10 aliquots à 200 µL, freeze-dried and stored -20°C.

1.14 Red Blood Cell Aggregation Assay

600 µL of fresh rat blood were drawn into BD microtainer tubes containing lithium heparin. The sample was inverted 8 x to ensure mixing with the anticoagulant and then refilled into a 15 mL centrifuge tube. The blood was spun at 600 g for 10 min, then the white blood cells and plasma were decanted. 3 mL of L-15 medium (Leibovitz) with 0.2 mg/ mL BSA were added, the red blood cells (rbcs) resuspended by passing them through a pipette tip several times, spun at 1200 g for 10 min and the supernatant decanted. 5 mL of L-15 medium with 0.2 mg/ mL BSA were added, the rbcs resuspended, spun at 1200 g for 10 min, and the supernatant decanted. 5 mL of L-15 medium with 0.2 mg/ mL BSA were added and the sample stored at 5°C.

The freeze-dried PtNP-HALNN-ConA adducts were dissolved in 500 µL dist. H_2O containing 0.133 mg/ mL $CaCl_2 \cdot 2\ H_2O$, 0.1 mg/ mL $MgCl_2 \cdot 6\ H_2O$ (to produce a PBS solution with Ca^{2+} and Mg^{2+}) and 0.2 mg/ mL BSA using sonication and 500 µL of L-15 medium with 0.2 mg/ mL BSA were added. 20 µL of the rbc suspension were added to the NP suspension and the sample shaken at 100 rpm at 37°C for 3 h. The negative control consisted of 20 µL of the rbc suspension in 1 mL L-15 medium with 0.2 mg/ mL BSA.

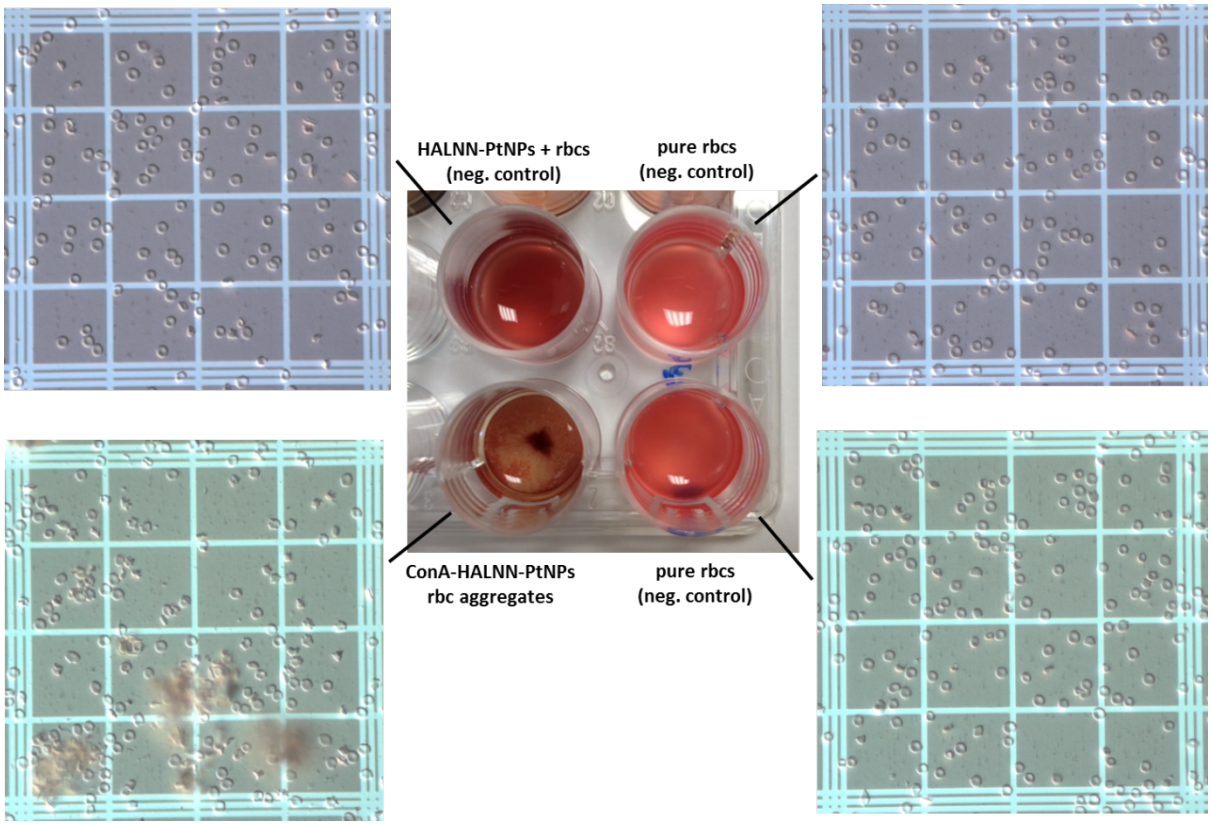

Figure S1: Rbc aggregation assay after 1.5 h shaking at 100 rpm at 37 °C: Only the sample containing PtNPs-HALNN-ConA **1** agglutinated, the two sets of negative controls (pure rbcs and rbcs exposed to HALNN-PtNPs) did not aggregate. Samples were deposited on a haemocytometer and images were taken with a digital camera fitted to a light microscope: The bottom left image confirms that the agglutinated rbcs released haemoglobin, the rbcs of the negative controls stayed intact.

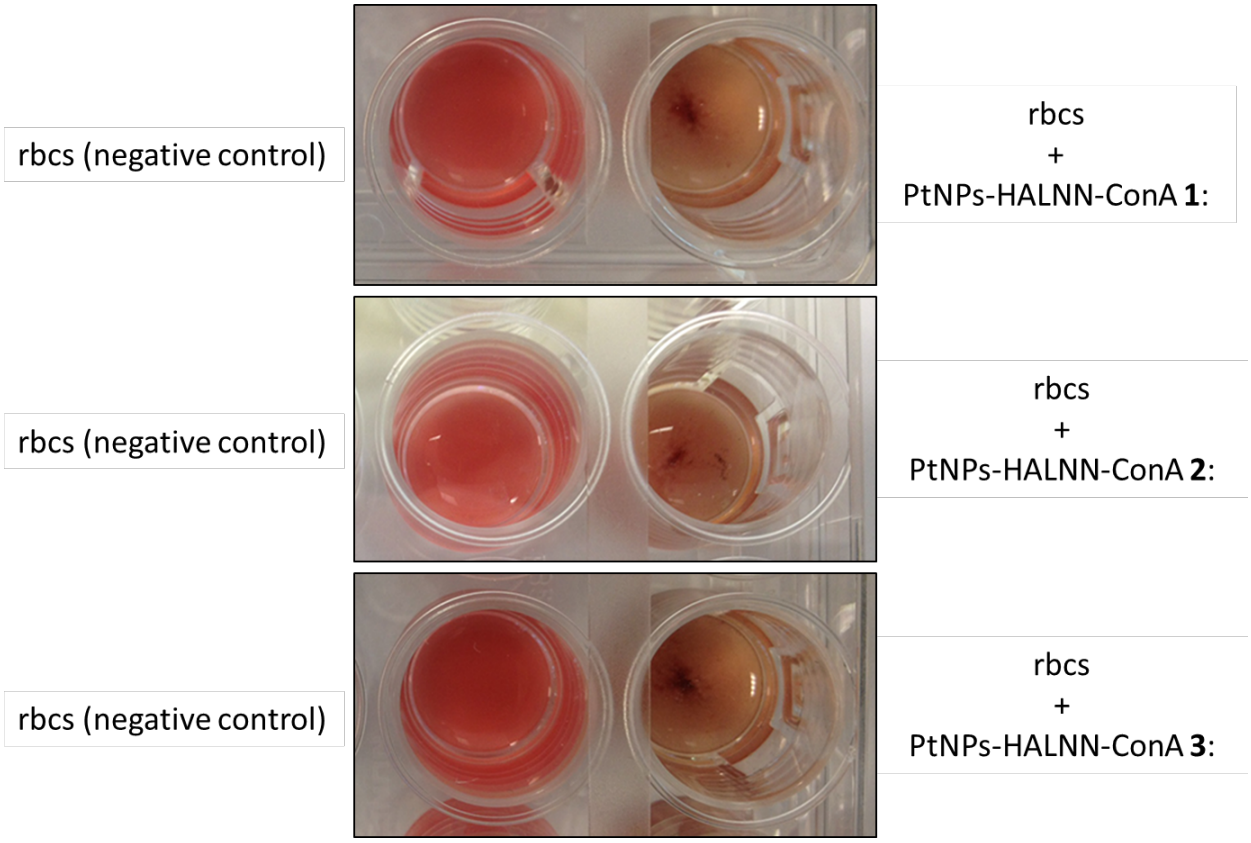

Figure S2: Rbc aggregation assay after 3 h shaking at 100 rpm at 37 °C: ConA concentration dependent agglutination of rbcs incubated with PtNPs-HALNN-ConA adducts **1-3**.

1.15 Cover Slip Preparation for SEM Analysis

The cover slips were soaked in glacial acetic acid for 1 h, then washed with dist. H_2O and dried. A solution of 0.1 mg/ mL of poly-L-lysine in PBS buffer (1x) was prepared and the clean cover slips were soaked in this solution for 2 h. The solution was removed using a pipette and the cover slips left to dry in a dust-free environment.

1.16 Sample Preparation for SEM Analysis

The rbcs aggregated with PtNPs-HALNN-ConA **1** were transferred into a 2 mL Eppendorf tube and centrifuged at 800 rpm for 2 min, then the supernatant was decanted. The rbcs-PtNPs-HALNN-ConA aggregates were resuspended in 1.8 mL Dulbecco's PBS with calcium chloride and magnesium chloride (1x) and 200 µL 25% glutaraldehyde were added. The sample was fixed at 5 °C overnight. The fixative was decanted, the aggregates washed with 1 mL Dulbecco's PBS with calcium chloride and magnesium chloride (1x), centrifuged (800 rpm, 2 min), and the supernatant decanted. The aggregates were resuspended in 100 µL

Dulbecco's PBS with calcium chloride and magnesium chloride (1x) and one drop of the suspension was deposited on a cover slip coated with poly-L-lysine. The aggregates were left to react with the poly-L-lysine surface for 1 h, then the cover slip was placed in a petri dish and the sample soaked in 30, 50, 70, 90 and 100% EtOH for 10 min each to remove H_2O from the specimen. Finally, the alcohol was removed from the specimen using a liquid CO_2 critical point dryer and the sample was stored in a desiccator.

1.17 SEM Analysis of rbc-PtNPs-HALNN-ConA 1 Aggregates

The SEM specimen prepared in section 1.15 was sputter coated with a half platinum coating using a Quorum Q150R sputter coater. Images were acquired with a Philips XL30S FEG SEM using the through-the-lens (TLD) detector and the backscattered electron (BSE) detector.

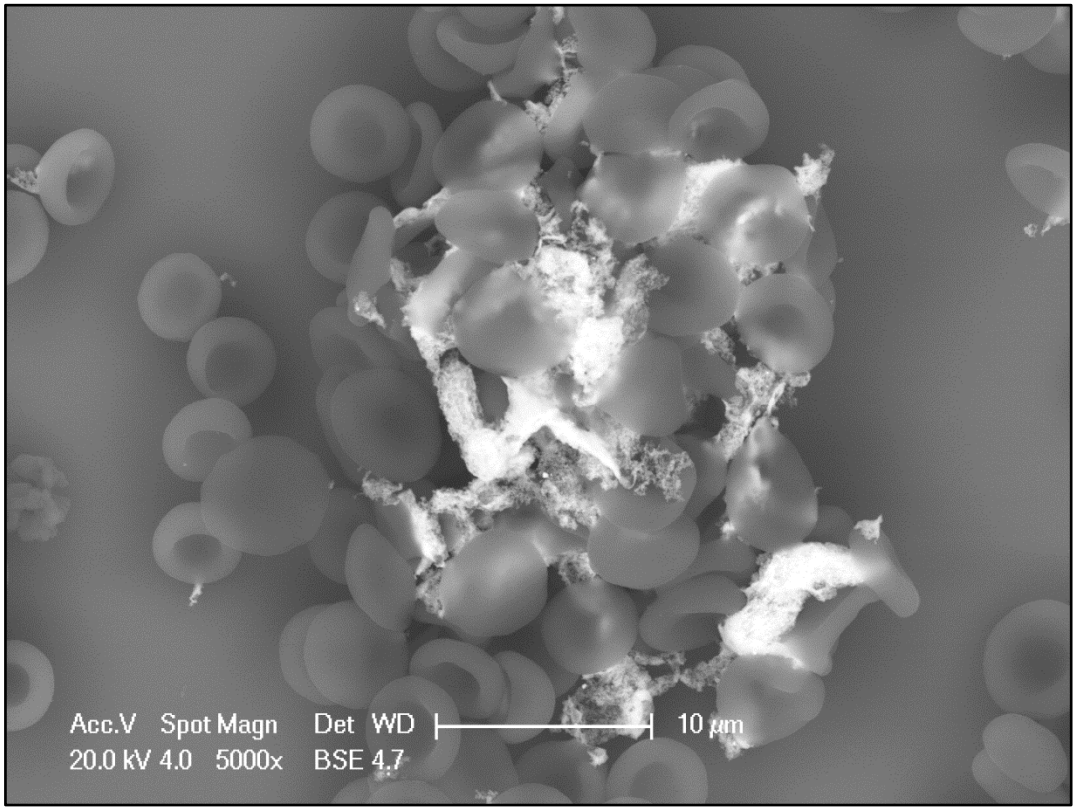

Figure S3: SEM image of rbc-PtNPs-HALNN-ConA aggregates **1** acquired with the backscattered electron detector.

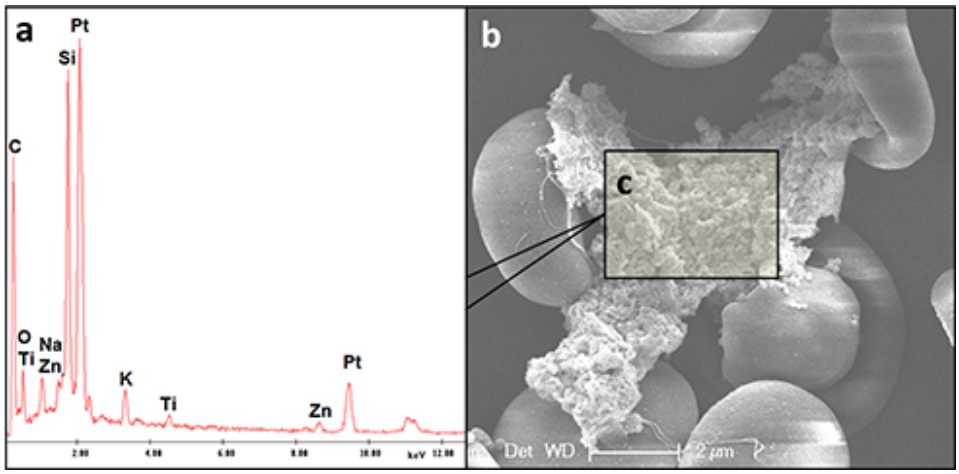

Figure S4: SEM analysis of the rbcs-PtNP-HALNN-ConA **1** aggregates: a) EDS analysis of area c) confirming high platinum content of the sample; b) SEM image acquired using the through-the-lens detector.

1.18 Cell culture and flow cytometry

3T3-L1 cells (ATCC® CL-173™) and primary human fibroblasts (S61) were cultured at sub-confluence at 37°C/5% CO_2 in DMEM supplemented with 10% fetal bovine serum (FBS), 1% Penicillin/Streptomycin and 2 mM GlutaMAX-1 ('DF10'; all Gibco®, Life Technologies™). To assess surface expression of integrin subunits S61 fibroblast cells were labelled on ice with fluorescent antibodies against: integrin-β1/CD29 (-APC, clone MAR4); integrin-α4/CD49d (-APC, clone 9F10); integrin-α5/CD49e (-PE, clone IIA1) and isotype control antibodies (-APC and -PE clone MOPC-21, all from BD Biosciences). Unbound antibodies were removed by serial washes with PBS/1% FBS. Live cells were selected using DAPI exclusion. Data were acquired using a FACSAria II (BD Biosciences) and analysed using FlowJo software (Treestar, vX.0.6.). S61 Fibroblasts were found to express both $\alpha_4\beta_1$ and $\alpha_5\beta_1$ integrins (Figure S5, below)

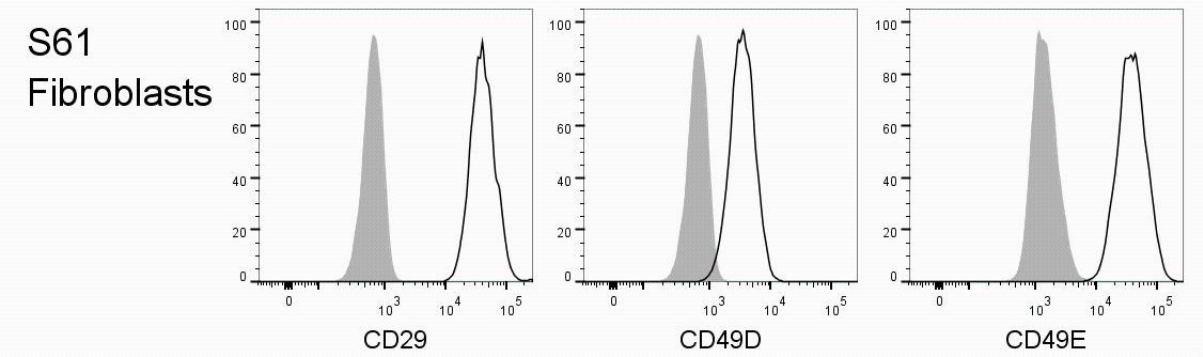

Figure S5: Surface expression of integrin subunits by S61 primary human fibroblasts. Filled grey peaks represent isotype control staining, and solid black lines represent CD29/49D/49E staining.

1.19 S61 Fibroblast and 3T3-L1 Cell Binding Assay

3T3-L1 and S61 fibroblasts were seeded into wells of a 24-well plate onto sterile 13 mm glass cover slips at $5x10^4$ cells/cm^2 in DF10, and allowed to grow overnight. Cover slips were washed to remove non-adherent cells, and 800 µL fresh DF10 supplemented with 200 µL of PtNPs-HALNNE$_6$ **5**, PtNPs-HALNNE$_6$-miniPEG **8** and PtNPs-HALNNE$_6$-miniPEG-GRGD **9** added to individual wells. Cells were incubated overnight and cover slips were then washed 5 times with PBS to remove remaining free PtNPs, and images acquired at 200x magnification prior to sample fixation (Leica DMI3000B; LASv4 software). PtNPs-HALNNE$_6$ **5** were easily removed by washing, whereas PtNPs-HALNNE$_6$-miniPEG **8** and PtNPs-HALNNE$_6$-miniPEG-GRGD **9** stayed firmly attached to the cells as large clumps visible under the light microscope (Figure S6, below).

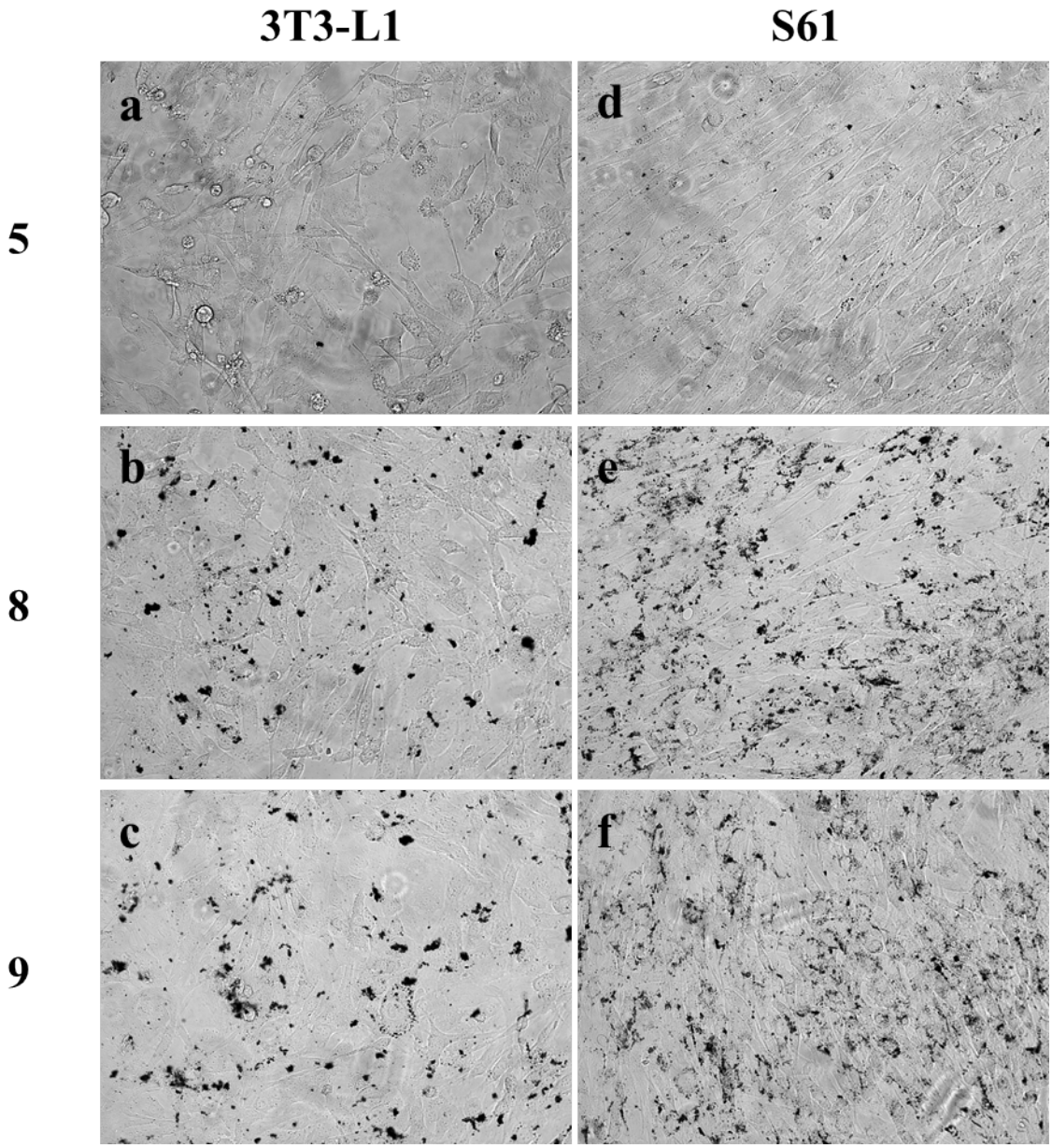

Figure S6: Light microscopy images of a) 3T3-L1 cells incubated with **5**, b) 3T3-L1 cells incubated with **8**, c) 3T3-L1 cells incubated with **9**, d) S61 cells incubated with **5**, e) S61 cells incubated with **8**, and f) S61 cells incubated with **9** acquired at 200x magnification. PtNPs appear dark under bright-field.

1.20 Sample Preparation for SEM Analysis

The S61 and 3T3-L1 cells incubated with PtNPs-HALNNE$_6$ **5**, PtNPs-HALNNE$_6$-mini-PEG **8** and PtNPs-HALNNE$_6$-mini-PEG-GRGD **9** were fixed with 500 uL each of 2.5% glutaraldehyde in PBS for 2 h at 4°C. The aqueous solution was then removed and replaced with 30, 50, 70, 90, 100% EtOH (10 mins each). Finally, the alcohol was removed from the specimens using a liquid CO_2 critical point dryer and the samples were stored in a desiccator.

1.21 SEM Analysis of 3T3-L1 and S61 Cell Binding with Compounds 5, 8 and 9

The SEM specimens prepared in section 1.20 were examined without sputter coating in a FEI Quanta 200 field emission Environmental SEM. SEM images were acquired using a large field detector (LFD) or solid state backscattered electron detector (SSD) at 100-8000x magnification.

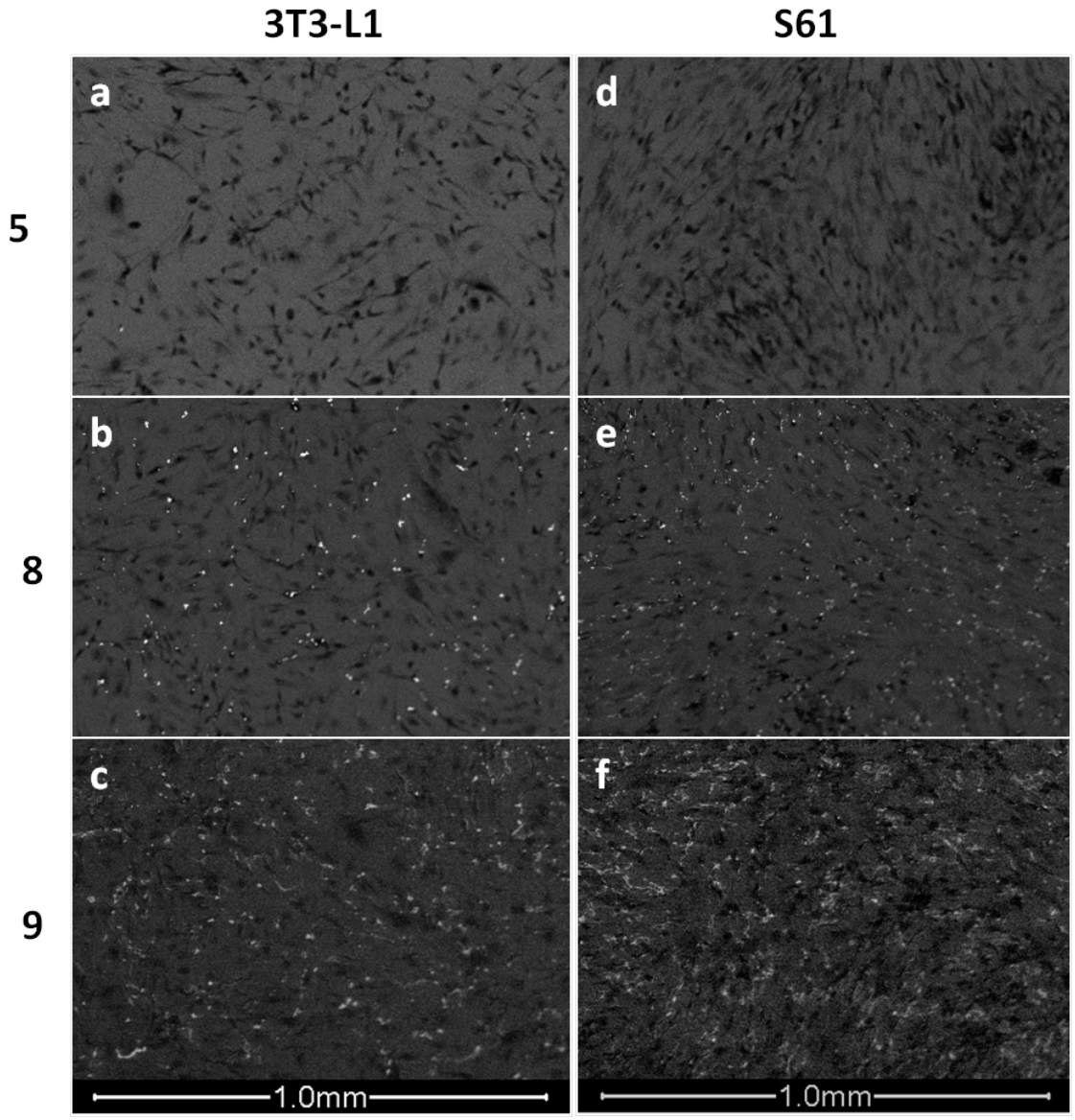

Figure S7: SEM images of a) 3T3-L1 cells incubated with **5**, b) 3T3-L1 cells incubated with **8**, c) 3T3-L1 cells incubated with **9**, d) S61 cells incubated with **5**, e) S61 cells incubated with **8**, and f) S61 cells incubated with **9** acquired at 100x magnification. PtNPs appear white due to the high atomic number of platinum.

2. TEM Analysis

2.1 PtNPs-HALNN

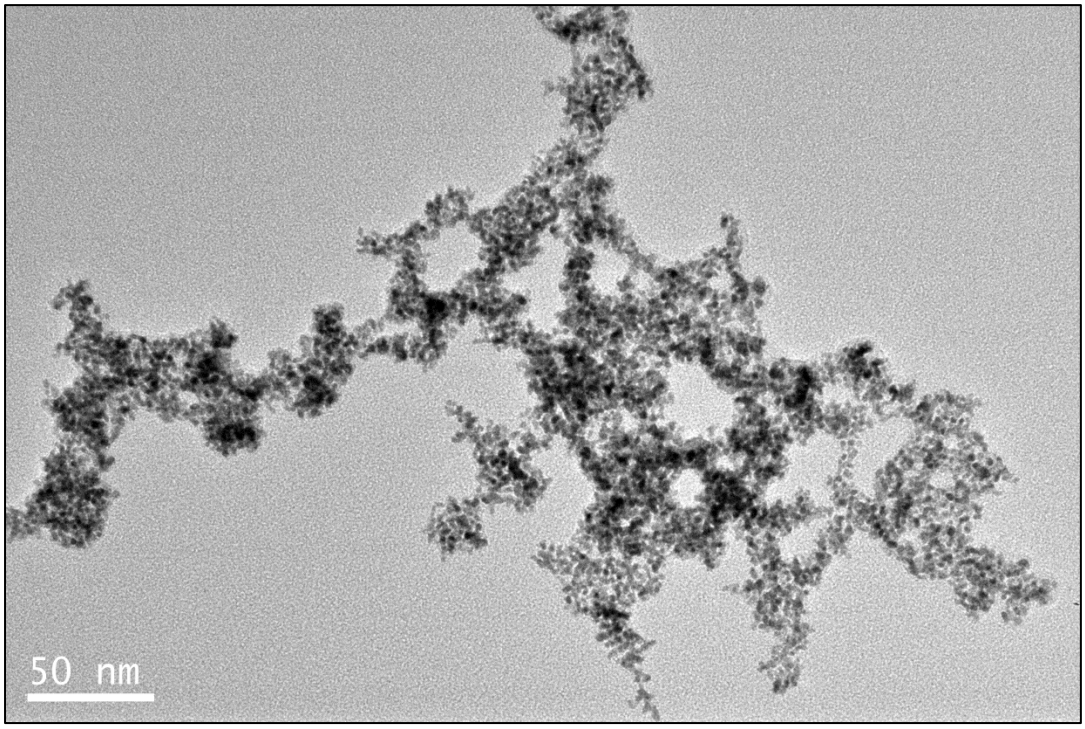

Figure S8: Low magnification TEM image of PtNPs-HALNN.

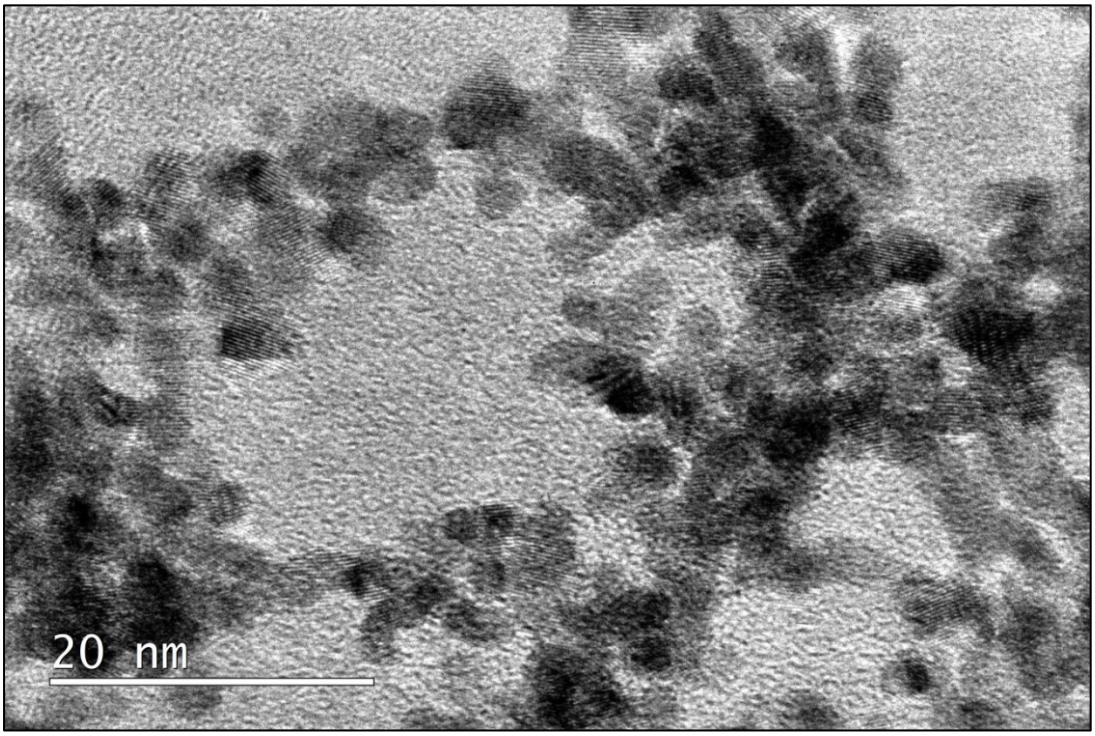

Figure S9: HRTEM image of PtNPs-HALNN.

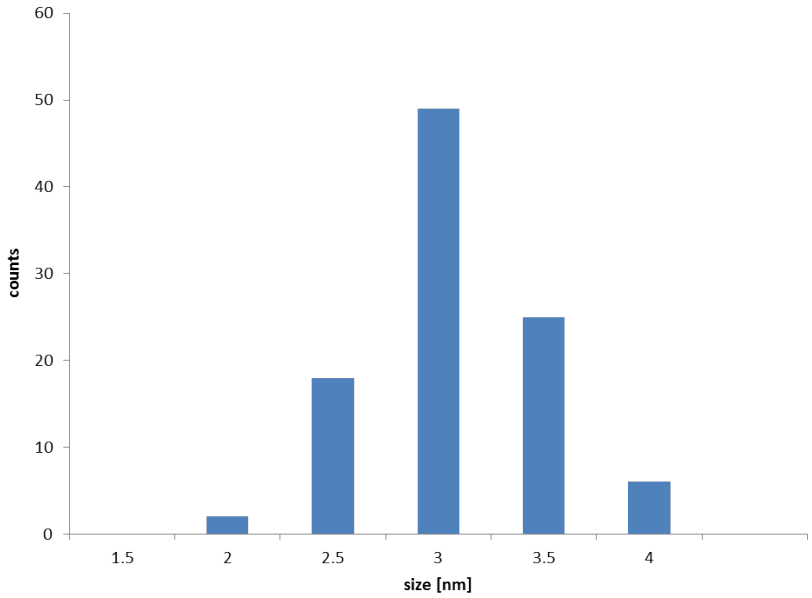

Figure S10: Size distribution histogram of ~ 100 PtNPs-HALNN: mean cross diameter = 2.8 ± 0.4 nm.

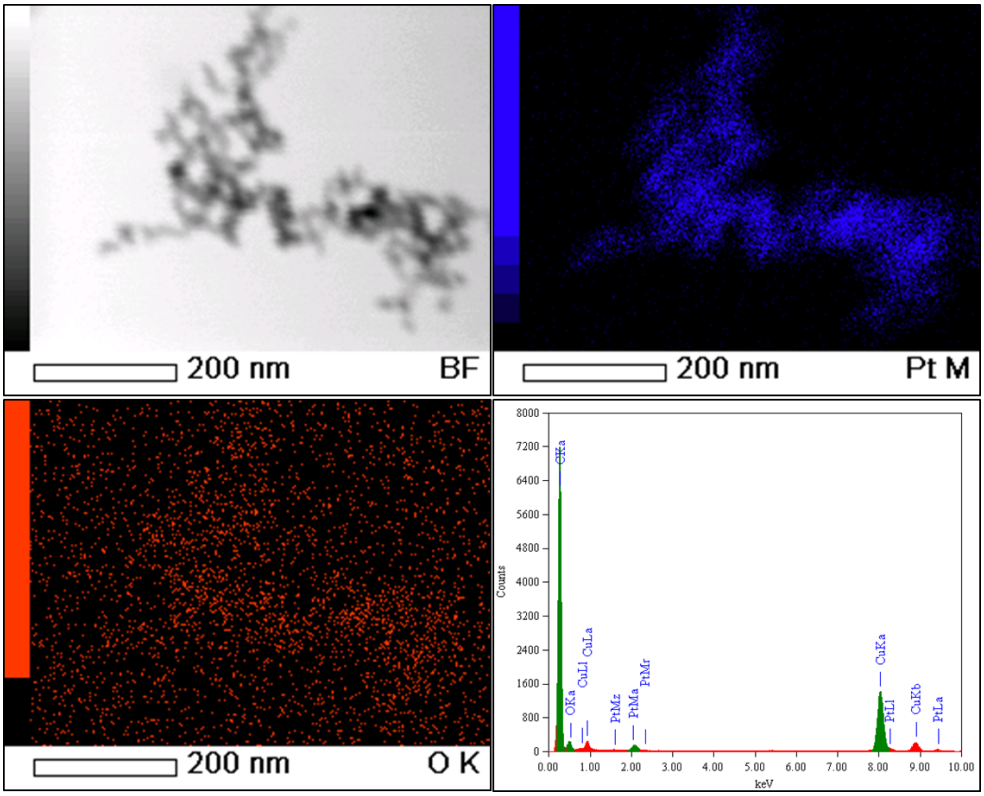

Figure S11: STEM-EDS analysis of PtNPs-HALNN confirming the presence of platinum (top right image) and oxygen (bottom left image) in the specimen.

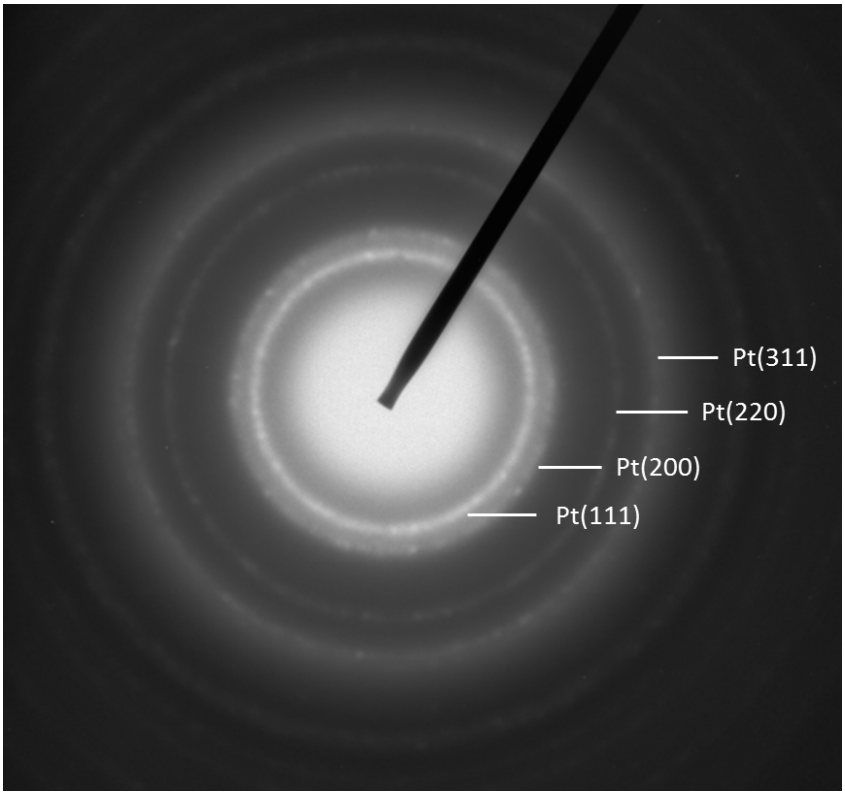

Figure S12: Indexed SAED pattern of PtNPs-HALNN.

2.2 PtNPs-HALNNE$_6$

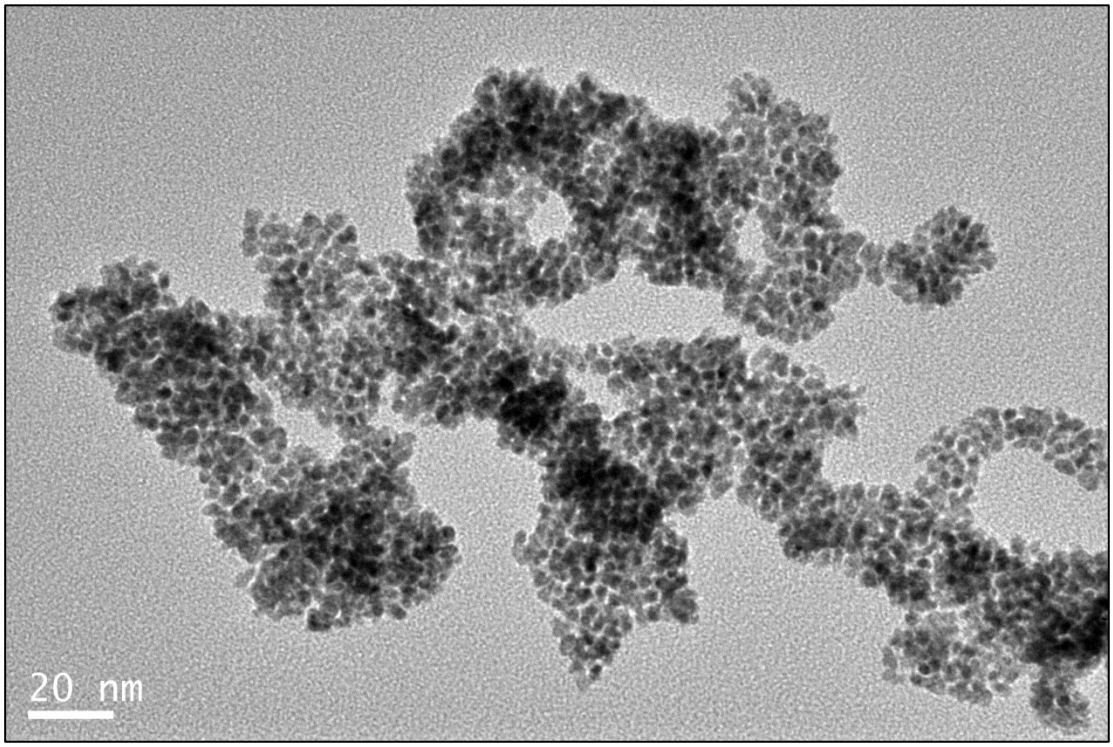

Figure S13: Low magnification TEM image of PtNPs-HALNNE$_6$.

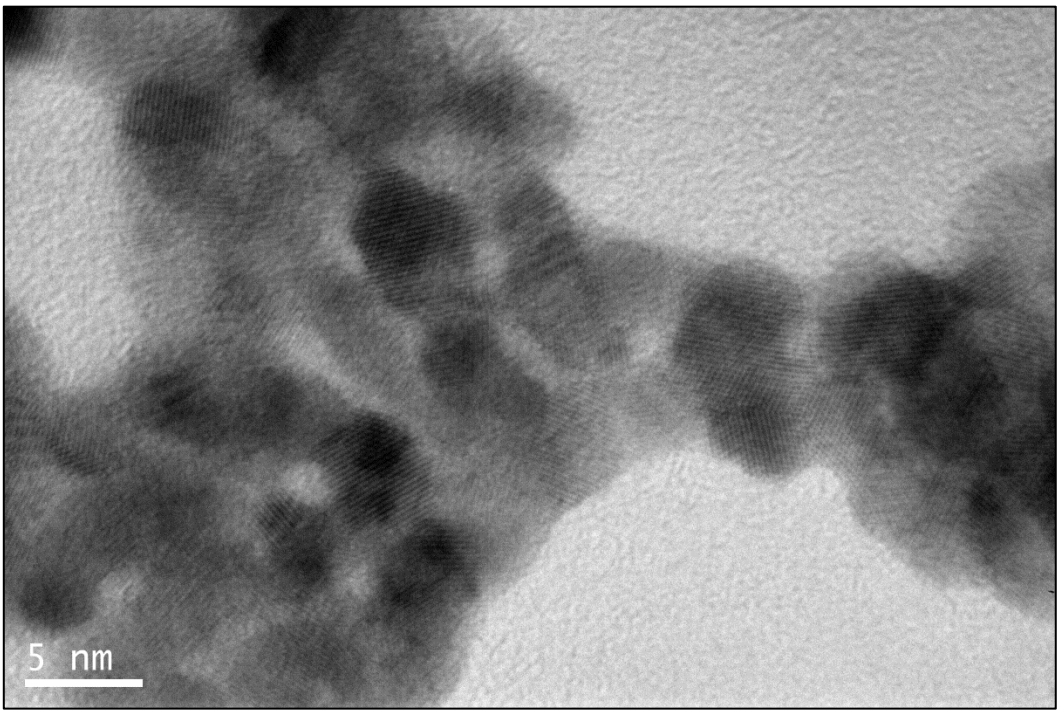

Figure S14: HRTEM image of PtNPs-HALNNE$_6$.

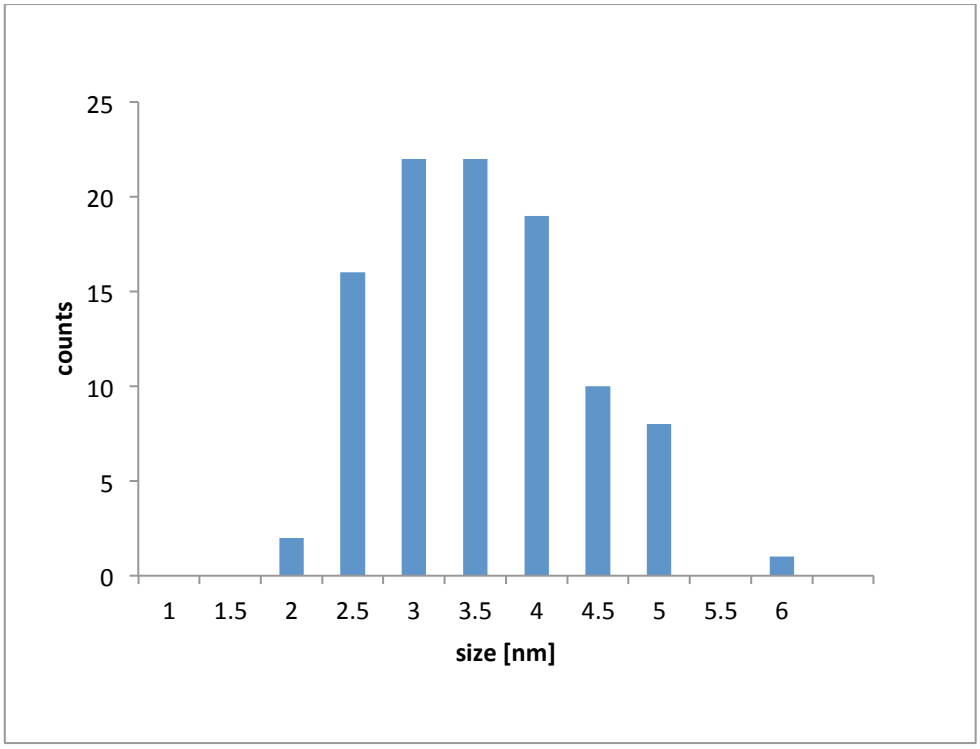

Figure S15: Size distribution histogram of ~ 100 PtNPs-HALNNE$_6$:

mean cross diameter = 3.3 ± 0.8 nm.

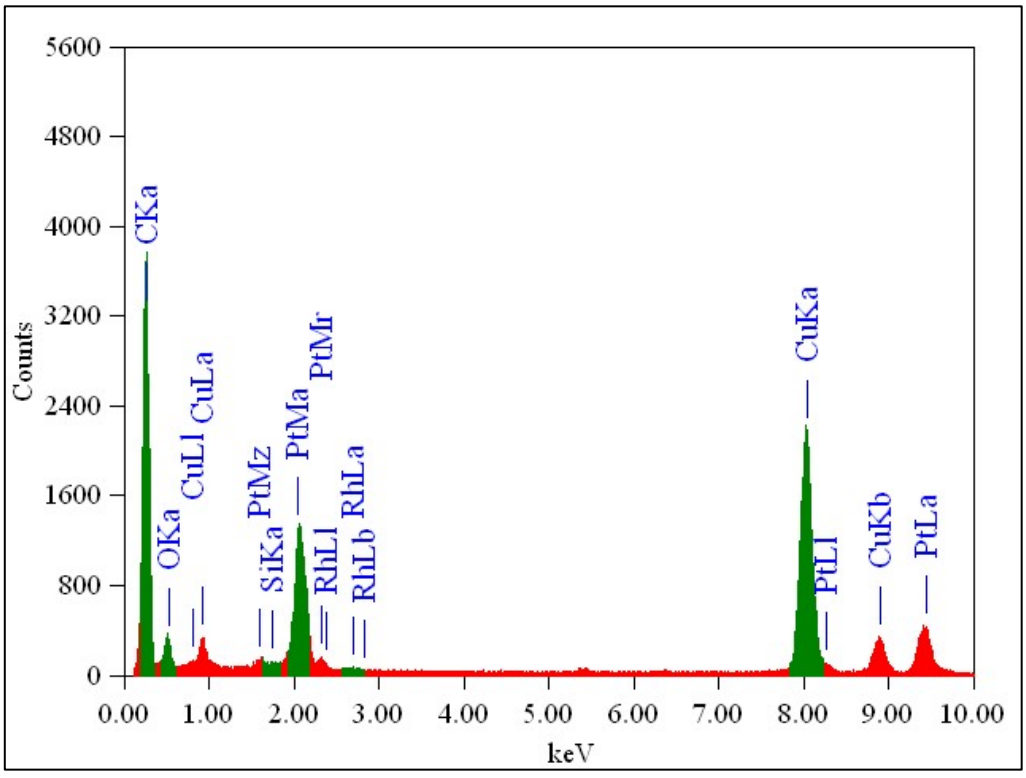

Figure S16: EDS analysis of PtNPs-HALNNE$_6$ confirming the presence of platinum.

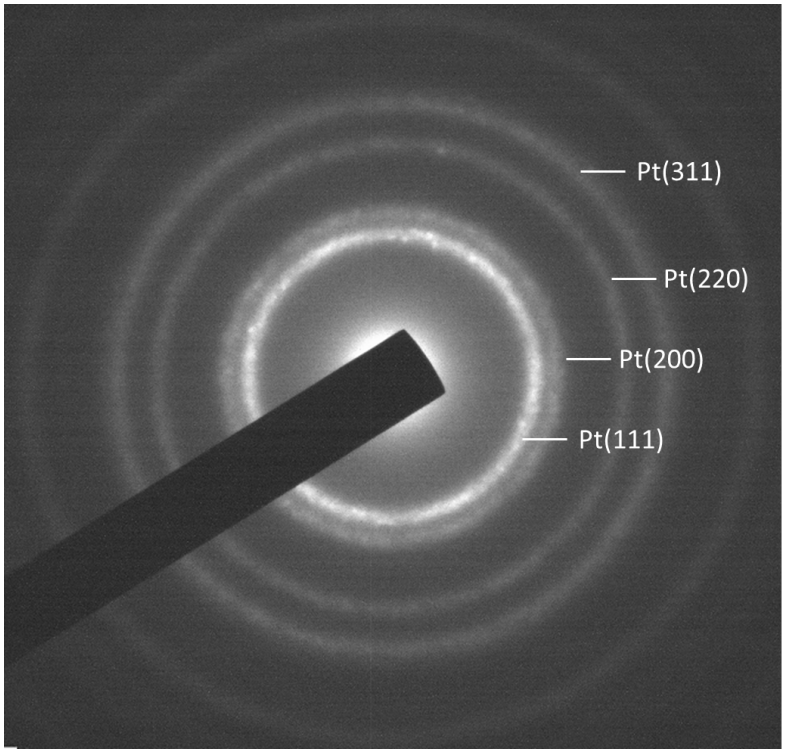

Figure S17: Indexed SAED pattern of PtNPs-HALNNE$_6$.

2.3 PtNPs-HALNN-ConA 1

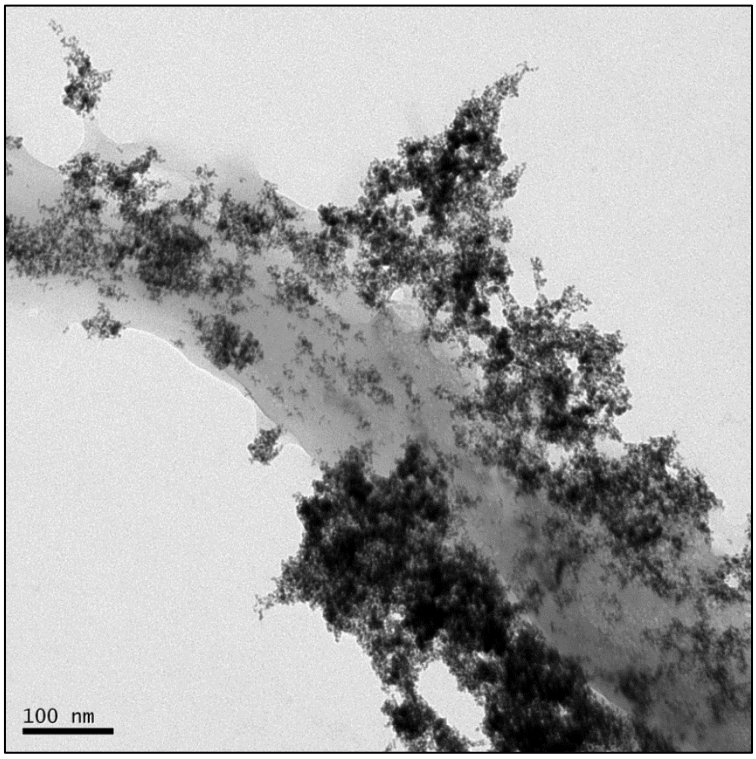

Figure S18: Low magnification TEM image of PtNPs-HALNN-ConA **1** acquired at an acceleration voltage of 120 keV.

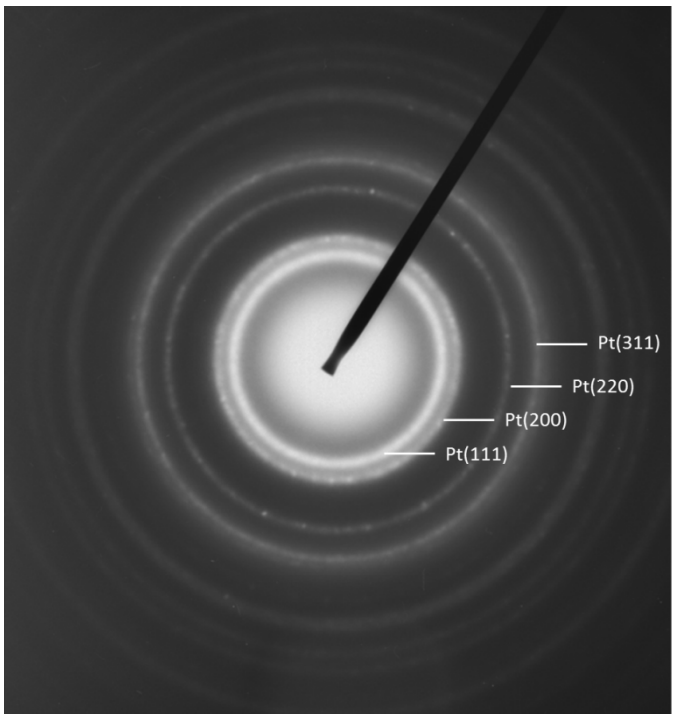

Figure S19: Indexed SAED pattern of PtNPs-HALNN-ConA **1**.

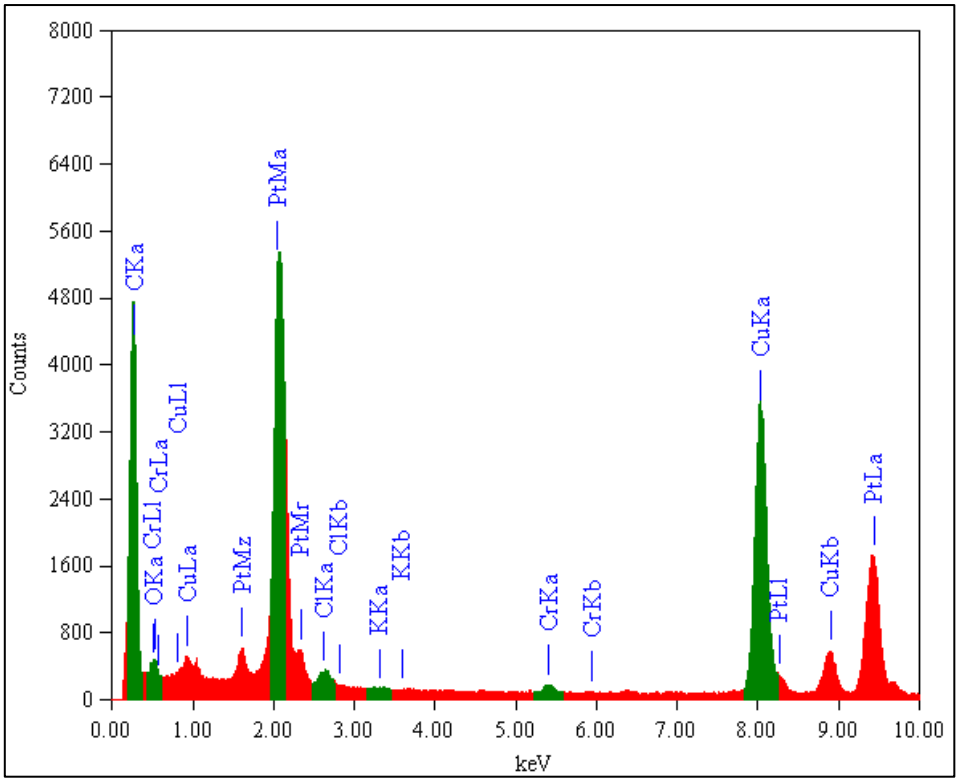

Figure S20: EDS analysis of PtNPs-HALNN-ConA **1**.

3. DLS Analysis

Table 2: Dynamic light scattering analysis showing the Z-average size of the PtNP conjugates before and after freeze-drying/ resuspension in aqueous solution and the Zeta potential of the PtNP conjugates after freeze-drying/ resuspension in aqueous solution.

	Size				Zeta Potential	
Sample	Z-average* (nm)	PDI	Z-average** (nm)	PDI	Mean (mV)	Zeta deviation (mV)
PtNPs-HALNN	37.8	0.12	153.9	0.36	-35.1	5.51
PtNPs-HALNNE$_6$	187.4	0.29	174.5	0.25	-37.3	7.36
PtNPs-HALNN-ConA **1**	-	-	426.7	0.3	-27.6	6.17
PtNPs-HALNN-ConA **2**	-	-	373.5	0.25	-20.3	9.56
PtNPs-HALNN-ConA **3**	-	-	829.7	0.31	-24.1	5.91
PtNPs-HALNN-miniPEG	105.6	0.29	687.1	0.58	-22.6	7.96
PtNPs-HALNN-miniPEG-GRGD	82.1	0.23	648.7	0.9	-23.4	7.56
PtNPs-HALNNE$_6$-miniPEG	349.1	0.25	392.9	0.42	-29.5	10.8
PtNPs-HALNNE$_6$-miniPEG-GRGD	129.7	0.19	338.5	0.4	-27.5	11.3

* Sample taken before freeze-drying, **Sample taken after freeze-drying