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Electronic Supplementary Information (ESI)

Multifunctional Core-Shell Magnetic Cisplatin Nanocarriers

Benjamin Thierry*, Fares Al-Ejeh, Aparajita Khatri, Zhe Yuan, Pamela J. Russell, Sue Ping, Michael P. Brown, Peter Majewski

Ian Wark Research Institute, University of South Australia, Mawson Lakes, SA 5095, Australia, Experimental Therapeutics Laboratory, Hanson Institute, Adelaide, SA 5000, Australia, Oncology Research Centre, Prince of Wales Hospital, University of New South Wales, Sydney, NSW 2031, Australia, Department of Medical Oncology, Royal Adelaide Hospital, and School of Medicine, The University of Adelaide, Adelaide, SA 5000, Australia

Nanoparticles synthesis

Monocrystalline iron oxide nanoparticles were synthesized following a one-pot procedure recently reported by Niederberger et al[1]. Briefly, $Fe(acac)_3$ powder was mixed with benzyl alcohol in a glove-box under nitrogen atmosphere. The reaction mixture was sealed in a Teflon cup, which was then carefully placed and tightly sealed in a steel container. The container was transferred from the glove-box to a furnace preheated to 175ºC and kept at 175ºC for 48 h.

A ligand exchange procedure with the ene-diol ligand 2-(3,4-dihydroxyphenyl)ethylamine (dopamine) was used to cap the nanoparticles and transfer them to an aqueous phase. The black particle suspension

in benzyl alcohol was mixed with a dopamine solution in MilliQ water. A 10-minute sonication, which was followed by half-day settling, was applied to the mixture. The nanoparticles were thus transferred from the organic layer to the aqueous layer. The aqueous phase was collected and washed three times using high speed centrifugation to remove excess dopamine. TEM analyses indicated a primary size distribution of approximately 15 nm as shown in figure IS1

Gold Nanoparticles were synthesized using a standard method based on the citrate reduction of gold chloride. Briefly, a 1% trisodium citrate solution in water was added to a 0.01% solution of $NaAuCl_4$ under reflux and kept under strong agitation.

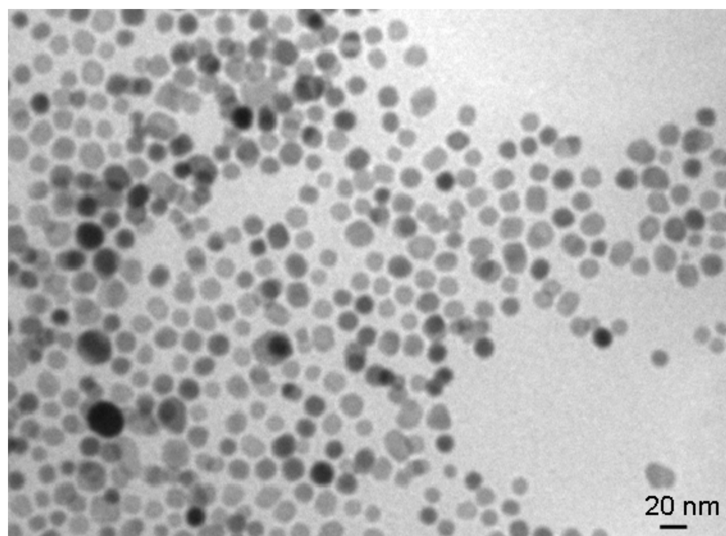

Figure ESI1. TEM image of dopamine-stabilized nanoparticles in water

S2. Self-assembly of the PAH/PAA shell

The dopamine coated nanoparticles were purified using 3 round of centrifugation at 19000 rpm and resuspended in 18 MΩ Milipore water. Poly(allylamine hydrochloride) (PAH, MW 15 000) and poly(acrylic acid) (PAA) (35 % aqueous solution, MW 15 000) were obtained from Sigma-Aldrich and used without further purification. 8 mg/mL solutions of the polyelectrolytes were prepared in 18 MΩ Milipore water and filtered using a 0.4 um filter. The pH of the PAA and PAH solutions were adjusted before use to respectively 9 and 5 using either sodium hydroxide or hydrochloric acid.

The layer-by-layer assembly process was initiated by adding drop-by-drop an equivolume of the dopamine-coated nanoparticles to the PAA solution under simultaneous mechanical agitation and sonication. The mixture was left to react for 1 h and the excess polyelectrolyte was then removed by 2 round of centrifugation/resuspension in water. The PAA-coated nanoparticles were next added under simultaneous mechanical agitation and sonication to an equivolume of the PAH solution. This process was repeated until reaching the desired number of polyelectrolyte layer. The layer-by-layer coating process was followed using dynamic light scattering and zetasizer measurements. In addition, a small aliquot of the core-shell nanostructures was collected at each step and used for XPS elemental analyses. The same layer-by-layer coating procedure was used to coat the prepared 20 nm nanoparticles. The procedure was initiated by coating a PAH layer onto the citrate-gold nanoparticles.

Prior to the loading with cisplatin and complexation with adenovirus, PAA terminated core-shell nanoparticles were coated either with a polyethyleneimine - polyethylene glycol (PEI-PEG_{5000}) copolymer or with PEI (MW ~25 000, Sigma). The electrostatic interaction between PAA and PEI or PEI-PEG was used here. PEI-PEG with a grafting ratio of PEG (MW 5000) to primary amines of 1:4.5 was prepared as previously reported[2].

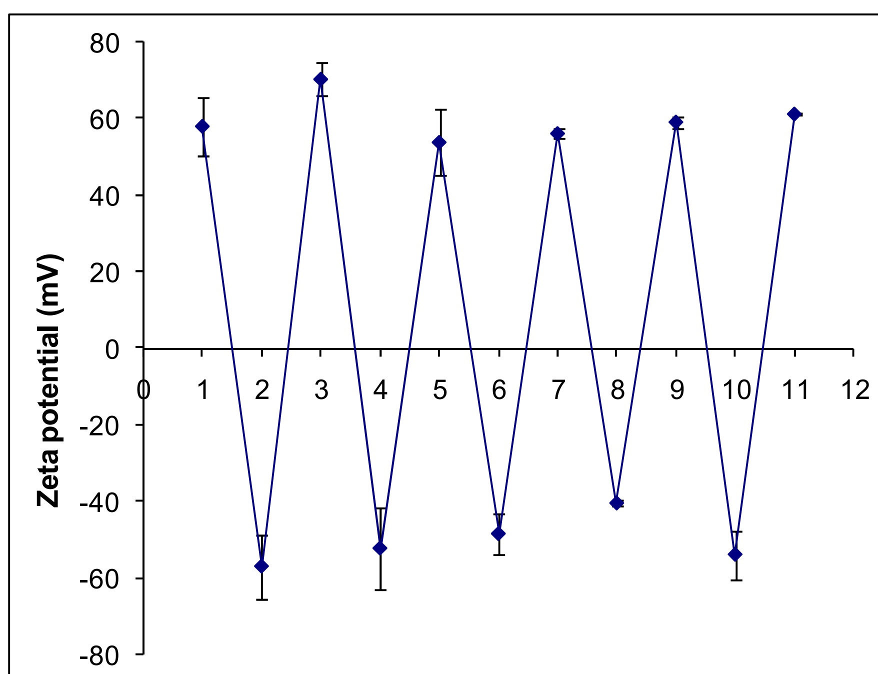

Figure ESI2. Zeta-potential measurements as a function of the number of polyelectrolyte layers.

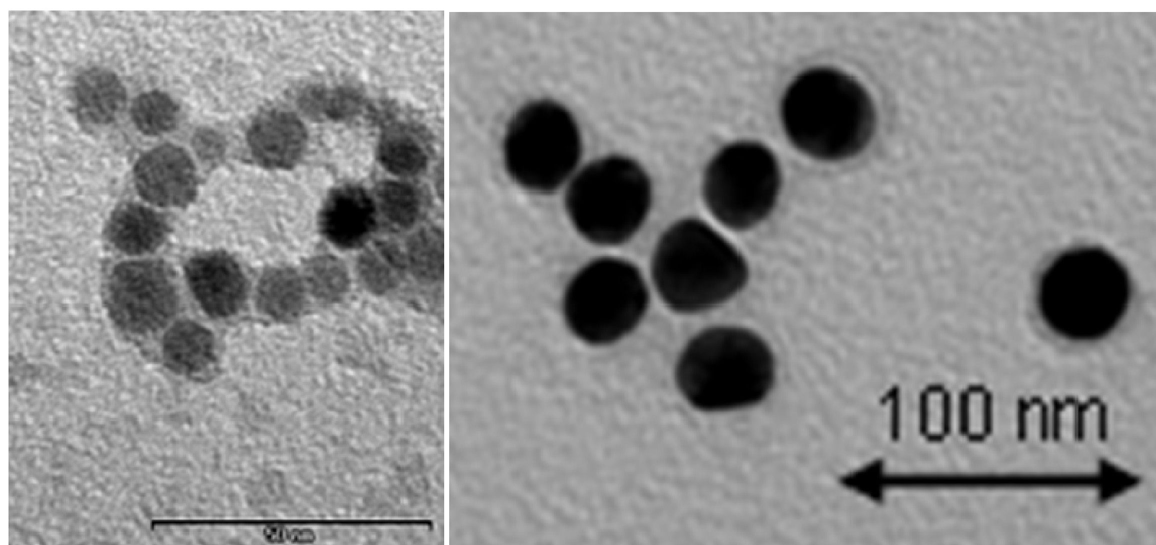

Figure ESI3. TEM of cisplatin-loaded 10 layers shell iron oxide and gold nanoparticles. The presence of the organic shells is visible around the nanoparticles.

S3. Cisplatin loading and release study

Cisplatin was obtained from the Royal Adelaide Hosptital Cytotoxics Pharmacy (Adelaide, South Australia). The 1 mg/mL cisplatin solution contained Cisplatin USP, sodium chloride and mannitol (Pfizer Australia). The PEGylated core-shell nanocarriers (0.05 M Fe) were mixed with an equivolume of the cisplatin solution and the mixture was incubated at 37 C in the dark for 24 h. The excess cisplatin was then removed by 3 rounds of centrifugation and the cisplatin loaded core-shell nanostructures resuspended in PBS.

To determine the release profile of the drug, a suspension of the cisplatin loaded nanostructures was incubated in PBS at 37 C in the dark. At various time intervals, the suspension was aliquotted and the sample was immediately centrifugated and washed twice to remove unbound drugs. The nanostructures were then resuspended in a concentrated ethanolic solution and prepared for elemental XPS analyses. The amount of cisplatin within the polymeric shell was calculated using an over-layer model. Advanced models for such XPS elemental analyses of core-shell nanostructures (see for instance Ref[3]) were not used in this study as the standard over-layer model was found accurate enough when taking into consideration the experimental error associated with the techniques used to determine the size distribution of the nanoparticles.

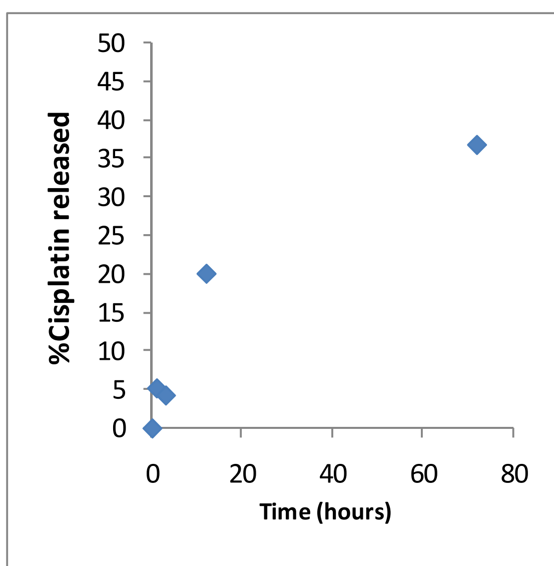

Figure ESI4. *In vitro* release of cisplatin from the core-shell nanostructure in PBS at 37 °C.

S4. Cytotoxicity of cisplatin loaded nanocarriers

Suspension cultures of Jurkat cells (American Type Culture Collection, ATCC) were maintained in RPMI-1640 (JRH Biosciences Inc., Lenexa, KS) containing 5% fetal calf serum FCS (JRH Biosciences Inc.) and passaged by splitting at 1:10 every 72h. Jurkat cells were incubated in triplicates (96-wells plates) in the absence or presence of increasing concentrations of cisplatin-loaded nanoparticles prepared in 200µL RPMI-1640/5% FCS. Plates were incubated without or with the placement of magnet under the plates for72 hours at 37°C, 5 % CO_2 humidified incubator before analysis of cell viability. Cell viability was assessed by the exclusion of 7-AAD (2µg/mL for 15min at RT) and flow cytomerty using a Becton-Dickinson FACScan flow cytometry system (BD Biosciences, San Jose, CA) to detect the far-red fluorescence of 7-AAD on channel FL-3 of FACScan flow cytometer. Acquisition was standardized to 10,000 events. Flow cytometry data were analyzed using WinMDI v2.8 (Scripps Research Institute, La Jolla, CA). The percentage of dead cells, which were positive for 7-AAD (7-ADD^+), to the total cell population was calculated from flow cytometry data analysis and plotted as a function of the concentration of cisplatin-loaded nanoparticles.

S5. Synthesis of the NIR dye and conjugation

A derivative of the NIR dye IR820 was synthesized using a modification of the procedure reported by Masotti et al. [4] Briefly, a solution of the IR820 dye (Sigma) was reacted in anhydrous DMF with triethylamine and 6-aminohexanoic acid under nitrogen atmosphere. The reaction was left to proceed for 3 hours at 90 C and the carboxyl-derivatized IR820 was recovered by flash chromatography (AcOEt/MeOH 70/30 to 0/100). The carboxylic groups were activated using an 10 time excess of EDC (1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide Hydrochloride) and reacted with PEI in anhydrous DMF at a dye to primary amines in the PEI backbone ratio of 1:100. The IR820-PEI conjugate was dialyzed against a 70:30 % water/methanol mixture then water for 3 days before being recovered by

freeze-drying.

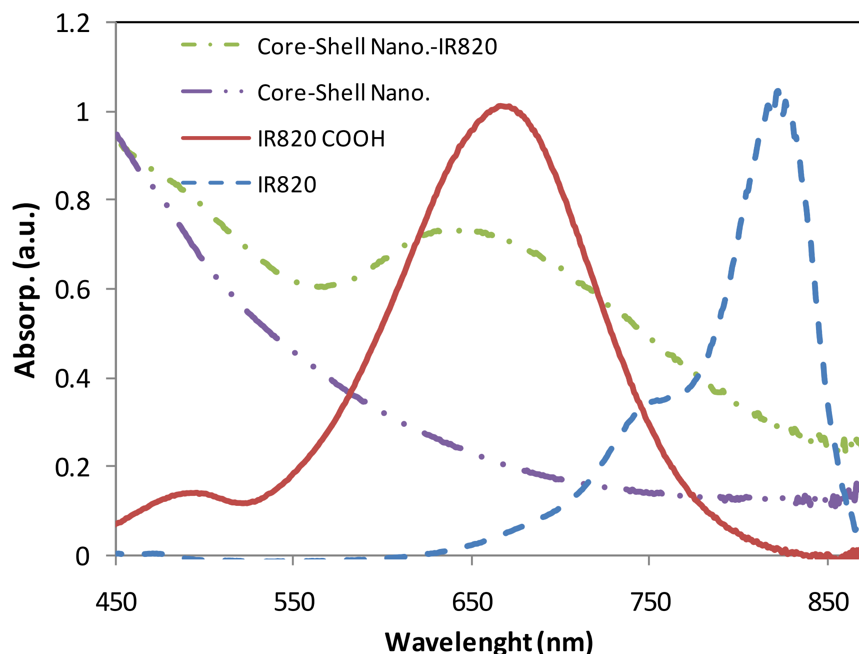

Figure ESI5. UV-Vis absorption of the IR-820 dye.

S6. Adenovirus – nanostructure complex and transfection study

Replication incompetent E1/E3 deleted Adenoviral vector expressing green fluorescent protein (Ad.CMV.GFP) was coupled with IR820-PEI-MNP by incubating over 20 minutes at room temperature (RT) in serum free media. To form these complexes, Ad.CMV.GFP at a multiplicity of infection (MOI) of 100 transduction units/cell was incubated with IR-820-PEI-MNP (6.7ug Fe/mL, 40 pg Fe per cell) in total volumes of 100µl serum-free DMEM (Gibco-BRL, Carlsbad, CA, USA) media. This corresponded to an uptake of ~15 pg Fe/cell as measured by Inductively-coupled Plasma optical emission spectrometry (ICP-OES)., For these experiments, $5x10^4$ PC3 cells were grown on round glass coverslips (12mm) placed in wells of a 24 well plate for 24 h. For magneto-transduction, cells were incubated with IR820-PEI-MNP-Ad complexes at 37^oC and 5% CO_2 with magnets (neodymium-iron-

boron (Nd-Fe-B), diameter 15mm, height 5mm) (Chemicell, Berlin) applied for 1 hour. Alternatively, for standard transduction, cells were incubated with complexes for 4 h without the magnets. After this, fresh complete DMEM (1 mL) was added to each well and cells were incubated for another 12h (magnetotransduction) or 52 h (transduction) prior to fixation in 4% para-formaldehyde for 15 minutes at RT. The fixed cells were imaged using confocal microscopy, red particles were visualized at the wavelength of 650nm and above. The GFP expression was visualized by UV microscopy at ~500 nm. Images were acquired using FluoView software. Similarly, Luciferase (luc) expressing Adenovirus (Ad.CMV.Luc; moi=100 i.e.100 plaque forming units /cell) was complexed with IR820-PEI-MNP as described above and PC3 cells seeded in 96 well plate ($6x10^3$) were magnetotransduced (1h) with Ad.CMV.Luc+IR820.PEI.MNP complexes . Gene expression was assessed 48 h later in cultured live cells by measurement of bioluminescence using Luciferase imaging (Xenogen, IVIS lumina, USA).

S7. Characterization of the nanoparticles

XPS analyses were conducted using a Kratos AXIS Ultra DLD X-ray photoelectron spectrometer with a monochromatic Al Kα X-ray source and a hemispherical analyzer. The pass energy was 20 eV with a resolution of 0.3 eV for high-resolution spectra. Spectra were collected at a photoelectron takeoff angle of 90°. Binding energies were referenced to the C1s hydrocarbon carbon peak at 285.0 eV to compensate for surface charging effects. Component fitting of the high resolution spectra was performed using CasaXPS version 2.3.12 software. Shirley-type backgrounds were used and constrained to a full width at half-maximum between 0.9 and 1.5 eV. The peak fits used 70% Gaussian/30% Lorentzian peak shapes. Concentrated nanoparticle solutions were drop-cast onto silicon wafers and dried at room temperature. Nanoparticles were examined using a JEOL 2010F scanning transmission electron microscope (TEM) equipped with a field emission gun electron source operated at 200 kV (JEOL, Japan). Staining was achieved using 2% phosphotungstic acid (PTA). UVvisible absorption

measurements were carried out with a Varian Cary 5 UV-vis-NIR spectrophotometer at room temperature. Nanoparticle size distribution and zeta potential measurements were carried out using a Zetasizer Nano ZS (Malvern Instruments) equipped with a 633nm He-Ne laser. Measurements were performed on dilute nanoparticle suspensions in MilliQ water.

The iron content of nanoparticle suspensions was determined using Prussian blue titration and atomic absorption spectroscopy. Standard curve was prepared using $FeCl_3$ $6H_2O$. Absorbance was read at 690nm. Nanoparticle suspensions were treated for 1 h with 6 N hydrochloric acid. The concentration of iron oxide magnetic nanoparticles could be estimated from the iron concentration of particle suspension knowing the size-distribution from TEM and dynamic light scattering.

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