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# Supporting Information



## Engineering Cytochrome-Modified Silica Nanoparticles To Induce Programmed Cell Death

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## Experimental Procedures

### Particle design and synthesis

*General*

All chemicals were purchased from Sigma-Aldrich and used as received, unless otherwise specified. 5/6-carboxyfluorescein succinimidyl ester (NHS-fluorescein) was purchased from Thermo Scientific.

*Native FITC-MSNs [type (1)] and primary amine modified MSNs [type (2)]*

Native FITC-MSNs were synthesised by a surfactant templated and base catalysed procedure. Initially, a mixture of triethanolamine (1.03g, 6.9 mmol, TEA), cetyltrimethylammonium bromide (0.65 g, 1.77 mmol, CTAB), $H_2O$ (16.02 ml, 0.89 mol), and ethanol (2.33 ml, 0.04 mol) was heated to 80˚C. Tetraethylorthosilicate (1.45 ml, 6.49 mmol, TEOS) was then added dropwise under vigorous stirring. 60 min later, equimolar amounts of a TEOS and (3-aminopropyl)triethoxysilane (APTES) mixture (15 µmol) were added. After a further 60 min of stirring, the reaction was stopped with centrifugation (13200 rpm, 20 min) and several washes with ethanol. The as-synthesised MSNs were sonicated in acidic ethanol for 30 min to remove the surfactant. The resulting aminated MSNs were subsequently reacted with NHS-fluorescein (7.1 mg, 15 µmol) in ethanol at room temperature for 48 h. The obtained FITC-MSNs, possessing internalised dye molecules, were collected by a series of washing and centrifugation. Type (2) aminated MSNs were prepared by post-grafting APTES (1.54 µl, 6.56 µmol, 7.68 µl, 32.80 µmol or 15.35 µl, 65.60 µmol for 1 mol%, 5 mol% or 10 mol% of amination, respectively) with 40 mg of the native FITC-MSNs in an ethanol/water mixture (2:1) at room temperature for 24 h and followed by a series of ethanol washing [mol% represents the molar percentage ratio of amine groups with respect to TEOS during the particle condensation reaction steps. Amine introduction and an ability to control its levels was confirmed by ζ-potential assessments and an ability to control, to high degrees of reproducibility, charging profiles (see tabulated summary in Figure 1)].
*Type (1):* IR: 963, 1000-1130, 3000-3460 $cm^{-1}$

*Type (2)*: IR: 963, 1000-1130, 1600, 3000-3460 $cm^{-1}$

*Imidazole modified MSNs [type (3), (4) and (5)]*

For imidazole-only [type (3)] and carboxyl/imidazole co-modified MSNs [type (5)], 30 mg of the above 1 mol% primary amine modified MSNs ($NH_2$-MSNs) were firstly converted to carboxylated MSNs (COOH-MSNs) by stirring with succinic anhydride (1 mg, 10 µmol) in anhydrous DMF overnight. The resulting carboxylated MSNs were then activated and conjugated with histamine or histidine (5 µmol) by N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (1.9 mg, 10 µmol, EDC) and N-hydroxysuccinimide (2.3 mg, 20 µmol, NHS) at pH 7 followed by a series of centrifugation and washing steps [note that the molar ratio of activated group:EDC:NHS remains at 1:2:4 throughout all modifications]. For primary amine and imidazole co-modified MSNs [type (4) particles]; 30 mg of 1 mol% of primary amine modified MSNs were reacted with EDC/NHS activated histidine (0.78 mg, 5 µmol) in pH 7 followed by a series of centrifugation and ethanol washing [mol% represents the molar percentage ratio of amine groups with respect to TEOS during the particle condensation reaction steps. Amine introduction at controlled doses was confirmed by reproducible associated charging profiles and the subsequent FTIR analysed introduction of coupled functional groups (see tabulated summary in Figure 1) and FTIR (Figure S2)].

*$NH_2$-MSNs:* IR: 963, 1000-1130, 1600, 3000-3460 $cm^{-1}$

*COOH-MSNs:* IR: 963, 1000-1130, 1552, 1647, 1719, 3000-3460 $cm^{-1}$

*Type (3)*: IR: 963, 1000-1130, 1552, 1647, 3000-3460 $cm^{-1}$

*Type (4)*: IR: 963, 1000-1130, 1678, 3000-3460 $cm^{-1}$

*Type (5)*: IR: 963, 1000-1130, 1552, 1647, 3000-3460 $cm^{-1}$

*Cytochrome c labelled MSNs [type (6), (7) and (8)) & BSA (or lysozyme) labelled MSNs (type (11)]*

40 mg of native FITC-MSNs were post-functionalised with APTES (15.4 µl, 65.60 µmol; yielding $NH_2$-MSNs) followed by succinic anhydride (13.1 mg, 131.2 µmol) carboxylation in anhydrous DMF to produce 10 mol% carboxylated MSNs (COOH-MSNs). 25 mg of the resulting carboxylated particles were then activated with EDC/NHS and labelled with cytochrome *c* (26 µL of 1 g/L, from saccharomyces cerevisiae) in pH 7 to obtain type (6) particles ([Cyt *c*] = 87.5 pmol/mg MSNs). 25 mg of 1 mol% primary amine modified MSNs were reacted with EDC/NHS activated cytochrome *c* or BSA or lysozyme (26 µL of 1 g/L) at pH 7 to produce type (7) cytochrome *c* or type (11) particles. For high cytochrome *c* dosage particles, type (8), the reaction was started with 0.5 mol% aminated MSNs (40 mg of native FITC-MSNs with 3.28 µmol of APTES) followed by reacting with succinic anhydride (0.16 mg, 1.64 µmol) in anhydrous DMF. 10 mg of the as-synthesised amine/carboxyl co-modified MSNs were then activated by EDC/NHS and conjugated with cytochrome *c* (410 nmol) at pH 7 generating type (8) particles. Particles were collected with a series of washing and centrifugation steps.

*FITC-MSNs:* IR: 963, 1000-1130, 3000-3460 $cm^{-1}$

*$NH_2$-MSNs:* IR: 963, 1000-1130, 1600, 3000-3460 $cm^{-1}$

*COOH-MSNs:* IR: 963, 1000-1130, 1552, 1647, 1719, 3000-3460 $cm^{-1}$

*Cleavable protein linker associated cytochrome c MSNs [type (9) and (10)]*

Acid-responsive hydrazone linked MSNs [type (9)] were prepared by modifying 50 mg of native FITC-MSNs with triethoxysilylbutyraldehyde (10.7 µl, 4.38 nmol) in 2:1 ethanol/water mixture (yielding aldehyde functionalised MSNs, CHO-MSNs) followed by nucleophilic addition of succinic dihydrazide (1.3 µg, 8.76 nmol) in pH 8.5 overnight. The resulting hydrazone modified MSNs ($CNNH_2$-MSNs) were then reacted with succinic anhydride (0.9 µg, 8.76 nmol) followed by post-functionalisation with APTES at the optimal 1 mol% level. The generated hydrazone and primary amine co-modified MSNs were subsequently activated with EDC/NHS and coupled with cytochrome *c* ([Cyt *c*] = 87.5 pmol/mg MSNs) at pH 7, thus resulting in the formation of the acid-responsive hydrazone linked MSNs (9). Redox-responsive disulfide linked MSNs [type (10)] were prepared by modifying 50 mg of FITC-MSNs with APTES (1.03 µl, 4.38 nmol) followed by succinic anhydride (0.9 µg, 8.76 nmol) carboxylation, EDC/NHS activation and cystamine (2.0 µg, 8.76 nmol) conjugation. The obtained cystamine MSNs were subsequently carboxylated by succinic anhydride (1.8 µg, 17.52 nmol) and post-grafted with 1 mol% of APTES. EDC/NHS activation was then carried out

with the generated disulfide and primary amine co-modified MSNs to conjugate cytochrome *c* ([Cyt *c*] = 87.5 pmol/mg MSNs) at pH 7, yielding the disulfide linked MSNs (10). The MSNs were collected after a series of $H_2O$ washing and centrifugation steps.

*FITC-MSNs:* IR: 963, 1000-1130, 3000-3460 $cm^{-1}$

*CHO-MSNs:* IR: 963, 1000-1130, 1721, 3000-3460 $cm^{-1}$

*$CNNH_2$-MSNs:* IR: 858, 963, 1000-1130, 1356, 3000-3460 $cm^{-1}$

*$NH_2$-MSNs:* IR: 963, 1000-1130, 1597, 3000-3460 $cm^{-1}$

*COOH-MSNs:* IR: 963, 1000-1130, 1557, 1632, 1719, 3000-3460 $cm^{-1}$

*$NH_2$-SS-MSNs: IR:* 963, 1000-1130, 1557, 1632, 3000-3460 $cm^{-1}$

*COOH-SS-MSNs: IR:* 963, 1000-1130, 1557, 1632, 1719, 3000-3460 $cm^{-1}$

**Cellular uptake and programmed cell death determination**

Human cervical carcinoma HeLa cells were grown in 2 mL Dulbecco's Modified Eagle Medium (DMEM) (Life Technologies) supplemented with 10% fetal bovine serum (Sigma-Aldrich) and 1% penicillin-streptomycin (Life Technologies) in 3.5 cm glass-bottom petri dishes for 24 h at 37°C in an atmosphere of 5% $CO_2$. After 24 h incubation, the culture medium was removed and the cells were washed with 2 mL phosphate buffer saline (PBS) twice. The MSNs suspended in a PBS/DMEM mixture (200 μg/mL) were then added to the cell dishes and incubated for 24 h. For confocal imaging, the cells were fixed with 4% paraformaldehyde and labelled with fluorescent tags following protocols provided by the manufacturers (LysoTracker Red and Hoechst33342, Life Technologies). The fixed cells were then imaged with a Nikon Eclipse Ti-E inverted microscope with attached A1 confocal laser system and Plan Apo oil immersion X100 objective. Confocal images of adherent cells are merged from three channels excited by lasers at 488 nm, 561 nm and 404 nm for FITC tagged MSNs (green), late endososome/lysosomes (red, stained with LysoTracker Red) and nuclei (blue, stained with Hoechst33342), respectively. For cell death determination, after total 48 h incubation, the cells were detached with Trypsin-EDTA (Life Technologies) and re-suspended in 100 μL of 1X annexin-binding buffer (Life Technologies). Propidium iodide (2 μL of 100 μg/mL, PI) and Pacific blue conjugated Annexin V (10 μL, AnnV) (Life Technologies) were added to the cell suspensions for 15 min followed by washing and resuspending in annexin-binding buffer. The cell suspensions were subsequently transferred to a haemocytometer (Immune Systems). Programmed cell death was then determined from the PI and AnnV staining ratio using confocal microscopy. For caspase 3/7 expression, the cells, after 24 h culture, were treated with particles and cultured for a further 9 h. After this time, the particle suspension was removed and the cells were washed with 2 mL DPBS twice and subsequently treated with a caspase 3/7 reporter (Image-iT LIVE Red Caspase-3 and -7 dection kit, using the protocols provided by the manufacturer, Life Technologies) for 1 h under culture conditions. After labelling, the cells were imaged using confocal fluorescent microscopy. The data shown were average observations across 6 repetitions and 2 separately prepared batches of particles. Images of cell suspensions shown in Figure 8 are merged from three channels

excited by lasers at 488 nm, 404 nm and 561 nm for MSNs (green), pacific blue conjugated Annexin V (blue) and propidium iodide (red), respectively.

## Particle Characterisation

*Transmission electron microscopy (TEM)*

TEM images were obtained on a Jeol JEM-2100, 200 kV, $LaB_6$ instrument, operated at 120 kV with a beam current of about 65 mA. Samples for TEM were prepared by deposition and drying of a drop of the nanoparticle suspension in water onto a formvar-coated 300-mesh copper grid. Diameters were measured using the ImageJ version 1.40 software; average values were calculated by counting a minimum of 100 particles.

*UV-visible Spectroscopy*

UV-visible spectroscopy (300 - 700 nm) was carried out using a Shimadzu UV PC-2401.

*Fourier transform infrared spectroscopy (FITR)*

IR spectra were recorded using FTS-6000 Fourier Transform IR spectrometer with a DuraSamplIR diamond ATR (Attenuated Total Reflection) accessory built by Bio-Rad.

*Dynamic light scattering (DLS) and zeta potential*

DLS measurements were carried out using a Malvern Zetasizer Nano operated at 25°C. A 532 nm laser was used as the light source and the measurements were recorded at a detection angle of 173° (backscatter). PDI indicated the polydispersity index of the suspensions. Zeta potential measurements were executed using a disposable capillary cell. Hydrodynamic diameters were determined in Ultrapure water (Elga water Flex3) as an average of 5 measurements. Zeta potentials were assessed in different pH buffers over 5 repetitions (pH 3 and pH 4.1 in formate acid buffer; pH 5.5 in 2-(N-morpholino)ethanesulfonic acid (MES) buffer; pH 7.1 in phosphate buffer; pH 8.5 in 2-amino-2-hydroxymethyl-propane-1.3-diol (tris base) buffer).

## Figures and Tables

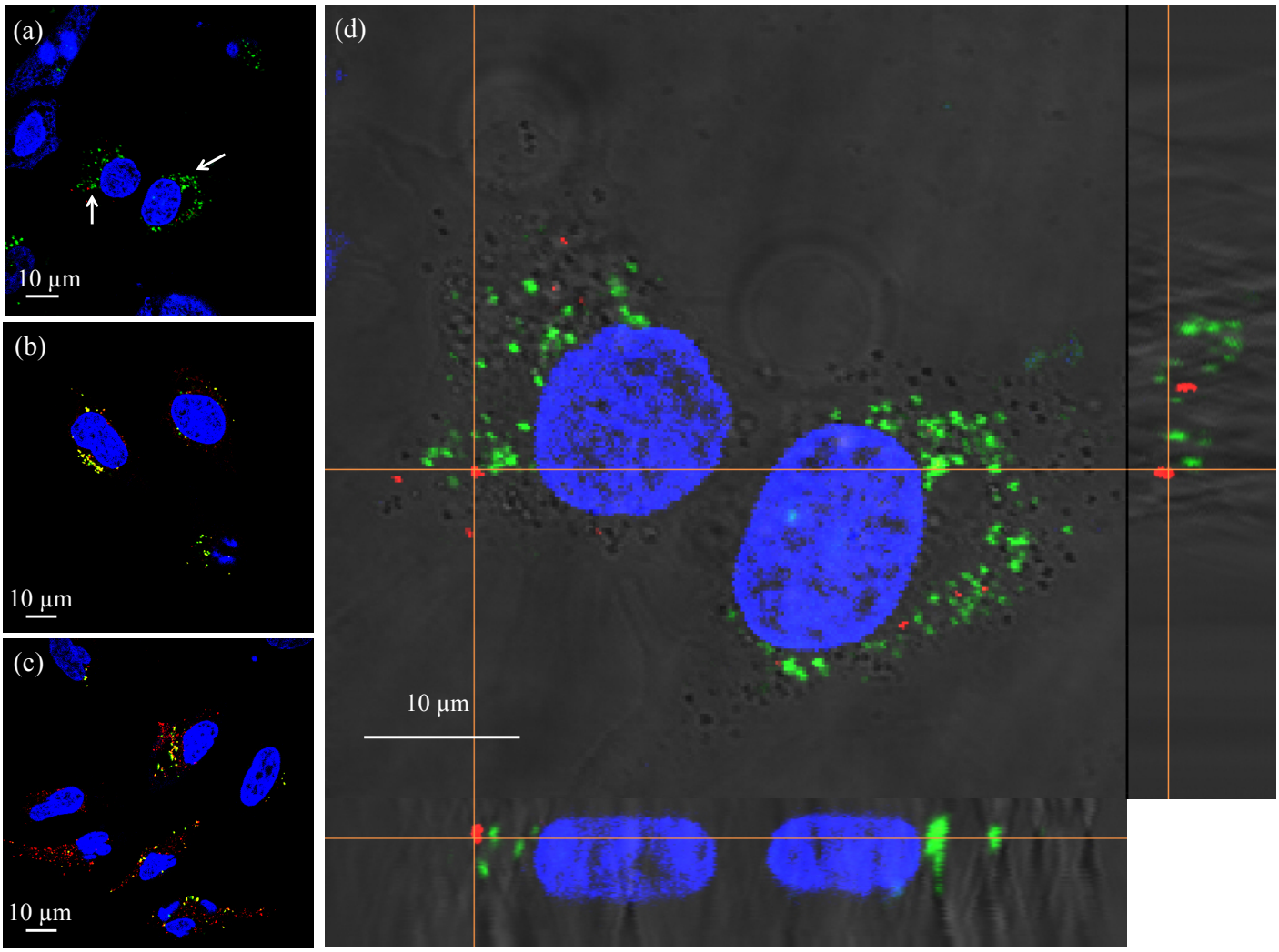


**Figure S1.** (a-c) Merged confocal fluorescent images of HeLa cells 24 h after incubation (~3x10$^3$ cells with 200 µg/ml of MSNs) summarising the effects of amine surface density on sub cellular localisation. Images from (a) to (c) are the corresponding results of type (2) particles with 1 mol%, 5 mol% and 10 mol% of primary amine modification designated as type (2)a, (2)b and (2)c, respectively. The green colour represents functionalised FITC-MSNs; red areas are indicative of endo/lysosomes; yellow colour shows co-localisation of MSNs and endo/lysosomes. Arrows in image (a) indicate MSNs having escaped endo/lysosomal entrapment (green). The data confirms only 1 mol% primary amine modified MSNs [type (2)a] are capable of effective endo/lysosomal escape. (d) Z-stack confocal fluorescent image [from image (a)] of HeLa cells 24 h after incubation (~3x10$^3$ cells with 200 µg/ml of MSNs) with type (2)a particles where the coplanar characteristics of green particles, red endo/lysosomes and blue nucleus and their perfect spatial separation in three dimensons indicate the successful internalisation and cytosol access of nanoparticles. Image is composed of a merge of green nanoparticles, red endo/lysosomes, blue nucleus and bright field with xz and yz cross sections shown on the bottom and right, respectively. Note that the bright field of cell bodies were resolved as the grey mottled structures which once more, confirmed the internalisation of nanoparticles. The images shown are entirely representative of the behaviour observed across the population of cells for each given particle type.

Figs. S2 (a) – (c) show characteristic FTIR absorptions of MSNs modified with amine containing moieties during the reaction steps. All MSN samples displayed strong absorption signals at 1065 cm$^{-1}$ and 963 cm$^{-1}$,

characteristic of asymmetric stretching of Si-O-Si bridges and skeletal vibration of the C-O bond stretch, respectively. Additional weak stretches at 1600 $cm^{-1}$ assigned to $–NH_2$ bending, were observed for $NH_2$-MSNs. Conjugation of $NH_2$ with histidine resulted in an additional peak at 1678 $cm^{-1}$, assigned to the amide I band of the secondary amide, indicative of successful histidine introduction [(b), type (4)]. Conversion of $NH_2$ to COOH led to the emergence of a distinctive absorption at 1719 $cm^{-1}$ representing C=O (COOH-MSNs). Peaks at 1647 $cm^{-1}$ and 1552 $cm^{-1}$ are assigned to the amide I band and amide II band of the secondary amide after carboxylation. After imidazole conjugation [(a), type (3)] or histidine conjugation [(c), type (5)], the carbonyl peak (at 1719 $cm^{-1}$) of carboxylic acid disappeared, indicating the successful introduction of the imidazole/histidine functional groups.

Figure S2 (d) and (e) show characteristic FTIR absorptions of MSNs modified with cleavable linkers during the reaction steps. All MSN samples display strong absorption signals at 1065 $cm^{-1}$ and 963 $cm^{-1}$, characteristic of asymmetric stretching of Si-O-Si bridges and skeletal vibration of the C-O bond stretching, respectively. (d) An emerging stretch at 1721 $cm^{-1}$ is observed for aldehyde modified particles, which can be assigned to C=O stretching (CHO-MSNs). Conjugation of succinic dihydrazide leads to the evolution of two additional stretches at 1356 $cm^{-1}$ ($CH_2$ deformation) and 858 $cm^{-1}$ (primary amine wagging; $CNNH_2$-MSNs). (e) Additional weak peaks at 1597 $cm^{-1}$ assigned to $–NH_2$ bending are observed for $NH_2$-MSNs. Conversion of $NH_2$ to COOH leads to the emergence of a distinctive absorption at 1719 $cm^{-1}$ representing C=O (COOH-MSNs). Peaks at 1632 $cm^{-1}$ and 1557 $cm^{-1}$ are assigned to the amide I band and amide II bands of the secondary amide after carboxylation. After cystamine conjugation, the carbonyl peak (at 1719 $cm^{-1}$) disappears indicating the successful introduction of the cystamine linker ($NH_2$-SS-MSNs). A further conversion of the $NH_2$ end group of cystamine to COOH results in an increase in the intensity of the C=O peak (1719 $cm^{-1}$) (COOH-SS-MSNs).

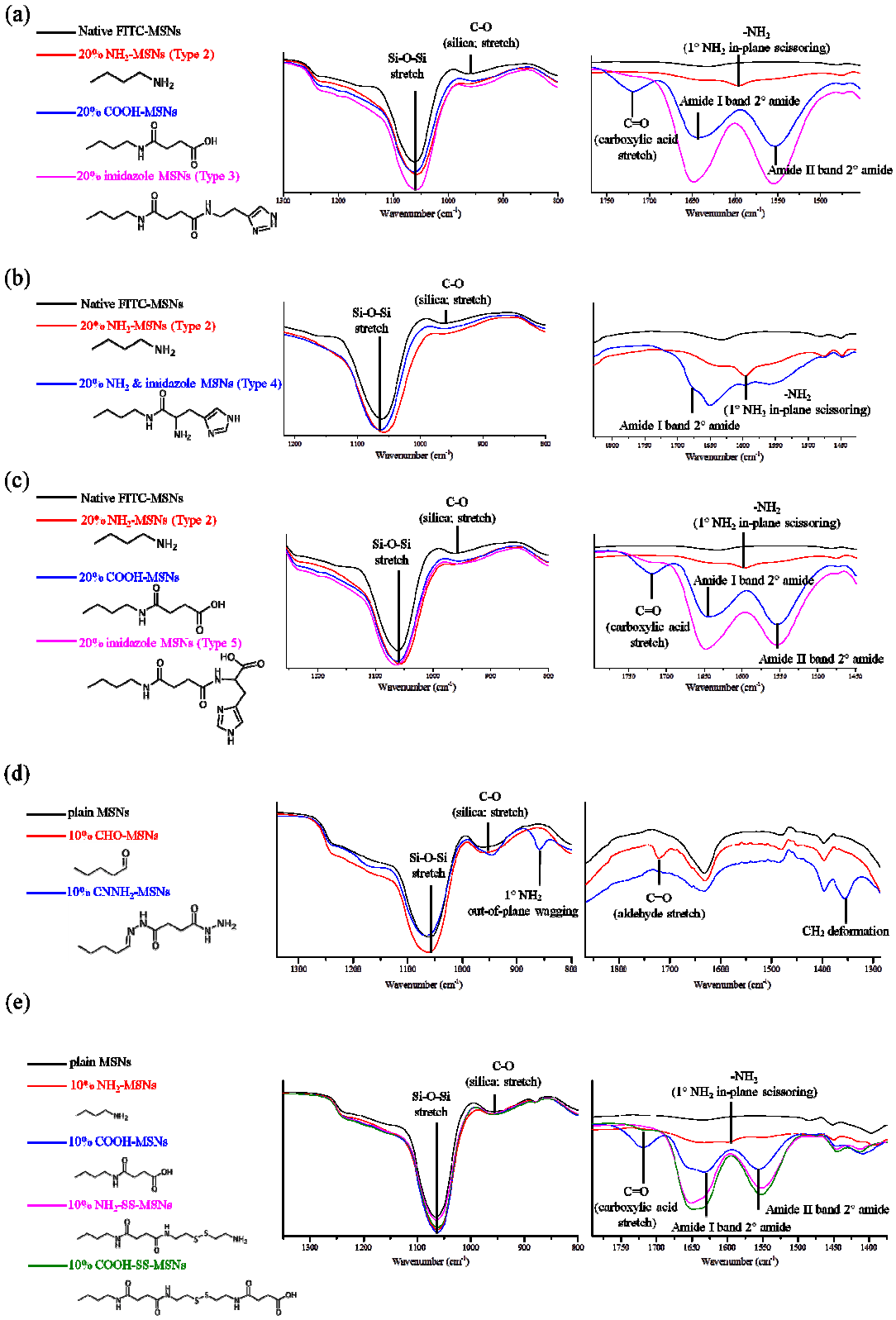


**Figure S2.** FTIR spectra corresponding to the surface chemistries of each preparative step of (a) native FITC-MSNs, 20 mol% aminated ($NH_2$-MSNs), carboxylated (COOH-MSNs) and imidazole containing particles, of types (2) and (3) particles; (b) native FITC-MSNs, 20 mol% aminated ($NH_2$-MSNs) and amine and imidazole containing particles, of types (2) and (4) particles; (c) native FITC-MSNs, 20 mol% aminated ($NH_2$-MSNs), carboxylated (COOH-MSNs) and carboxyl and imidazole containing particles, of type (2) and (5) particles. FTIR spectra corresponding to the surface chemistries of each preparative step of (d) acid responsive hydrazone-conjugated MSNs and (e) redox-responsive disulfide-conjugated MSNs [these analyses monitored each reaction step prior to cytochrome c conjugation; the concentration of cytochrome c of the subsequent protein conjugation falls below the sensitivity threshold of FTIR. Protein presence was confirmed (and semi-quantified) by the BCA assays shown in Figure S5]. Note that as 1 mol% modification was below the sensitivity threshold for detection by FTIR, the data presented are the results of an analogous 20 mol% (a-c) and 10 mol% (d and e) of surface modification.

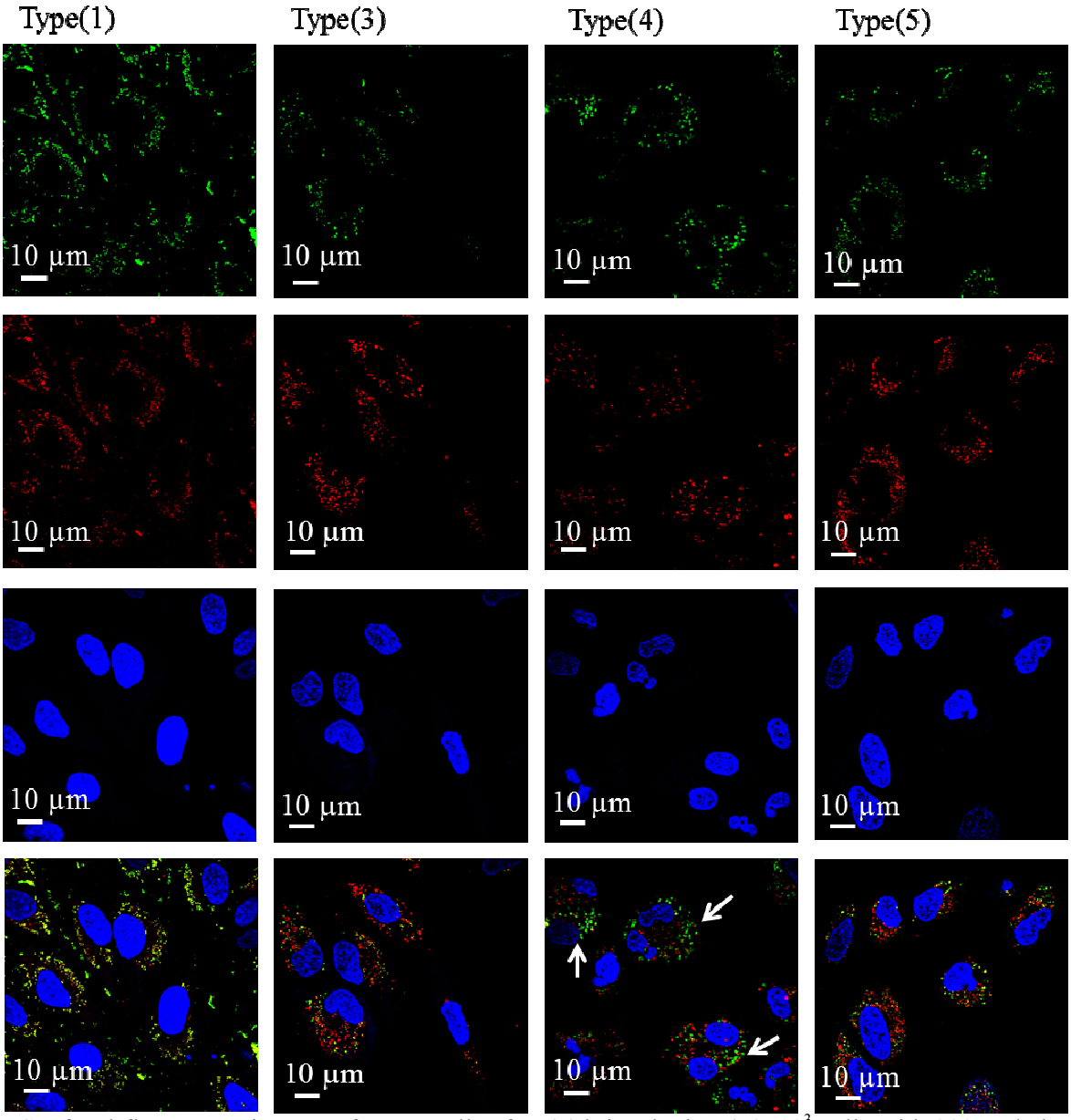


**Figure S3.** Confocal fluorescent images of HeLa cells after 24 h incubation (~3x10$^3$ cells with 200 µg/ml of MSNs). Images from left to right are the corresponding characteristic results associated with type (1), type (3), type (4) and type (5) particles, respectively. The spectrally resolved components shown from top to bottom are FITC-MSNs (green), late endosome/lysosomes (red, stained with LysoTracker Red) and nuclei (blue, stained with Hoechst33342). The merged composites of these images are shown at the very bottom. Yellow represents co-localised MSNs and endo/lysosomes. Arrows indicate MSNs having escaped endo/lysosomal entrapment (green). The data suggests only 1 mol% primary amine and imidazole co-modified MSNs [type (4)] are capable of effective endo/lysosomal escape. The images shown are entirely representative of the behaviour observed across the population of cells for each given particle type.

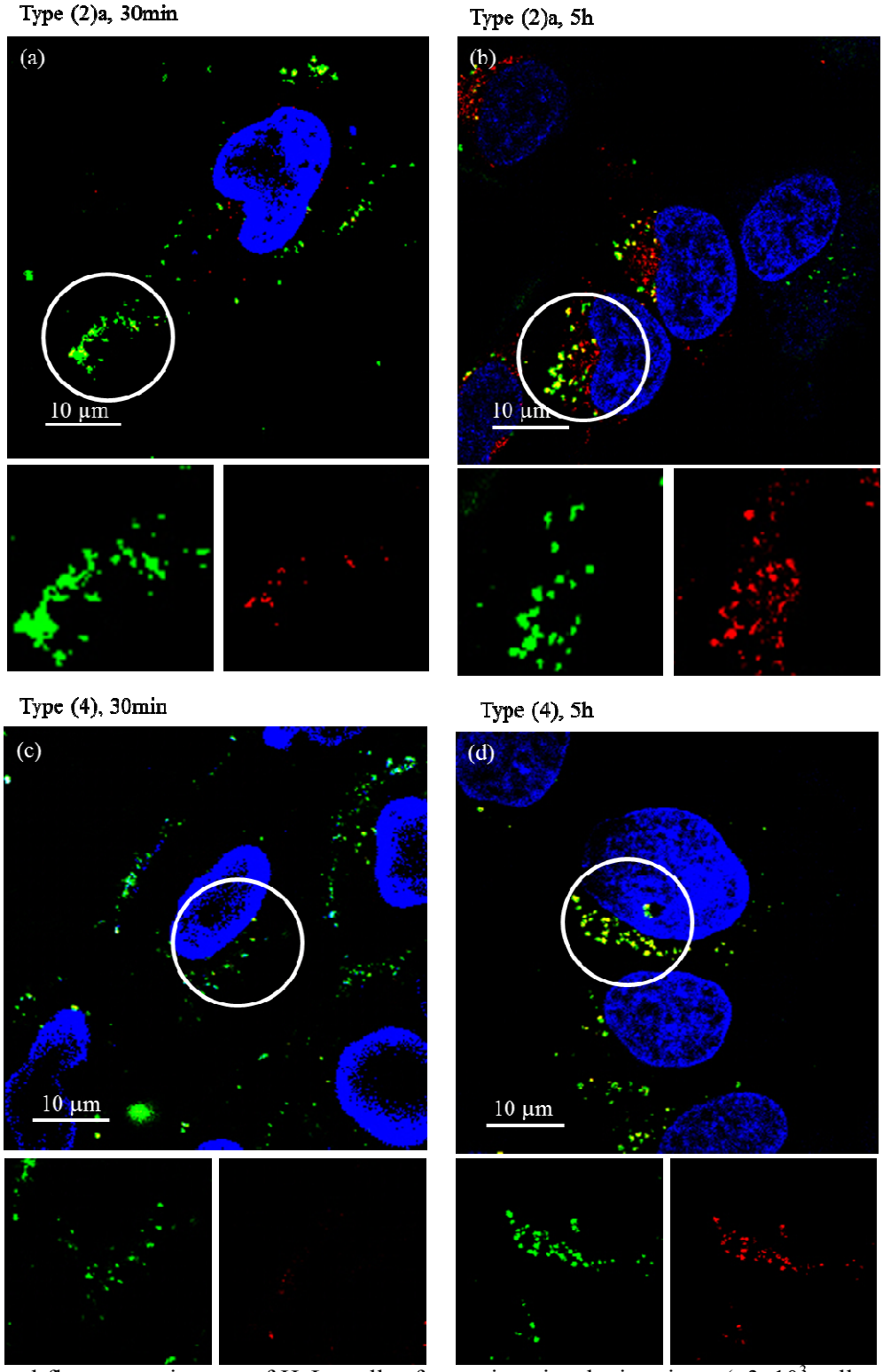


**Figure S4.** Confocal fluorescent images of HeLa cells after various incubation times (~$3x10^3$ cells with 200 µg/ml of MSNs). (a) Type (2)a particles after 30 min incubation; (b) Type (2)a particles after 5 h incubation; (c) Type (4) particles after 30 min incubation and (d) Type (4) particles after 5 h incubation. The spectrally resolved components of the circled regions are magnified and shown on the bottom-left and bottom-right for FITC-MSNs (green) and late endosome/lysosomes (red, stained with LysoTracker Red). After 30 min incubation, the lack of co-localisation of some type (2)a and (4) particles with late endosome/lysosomes (observed as green) indicates that the particles have not yet been transported or maturated into these late compartments of the endocytic pathway. After 5 h, the yellow colour demonstrating the perfect overlap of green particles and red endo/lysosomes indicates their fusion into these late compartments and particle entrapment therein. After 24 h, both particle types escaped from these organelles into the cytosol (as shown in Figure 2 and 5). The images shown are entirely representative of the behaviour observed across the population of cells for each given particle type.

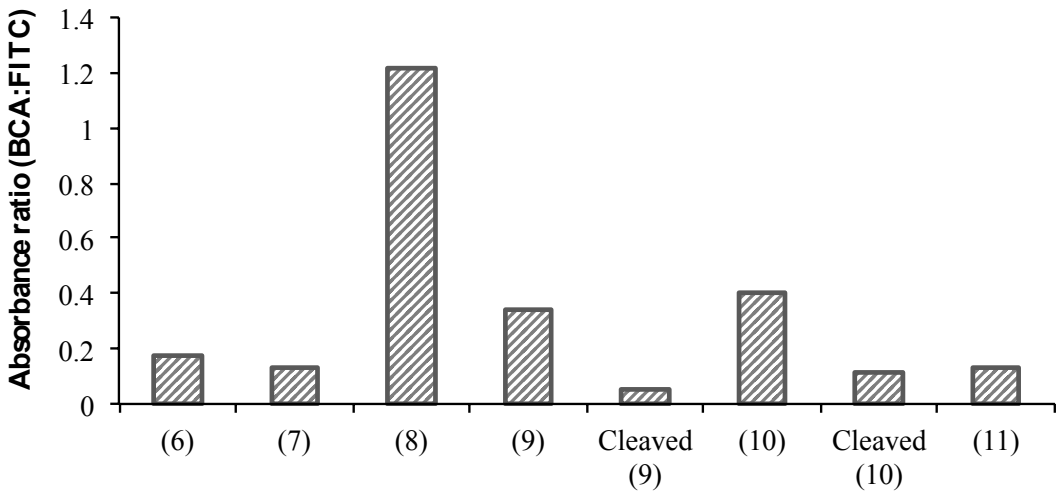


**Figure S5.** UV-vis absorbance ratiometric analysis of protein loading. Absorbance ratios are determined from the absorbance of protein induced bicinchoninic acid-cuprous complexes (BCA assay) with respect to that of FITC on particles, indicating particle types (6), (7) and (11) possess comparable protein levels (hence indicating that the subsequent different cellular physiological responses would result only from the different particle formulations rather than the variations in protein levels) whereas type (8) possesses a significantly higher degree of protein coating, as expected due to their significantly higher loading level. The protein levels on particle types (9) and (10) can not be directly compared with those on other particle types, as they contain strong BCA interfering species on their linkages (hydrazone and disulfide). The ratios of particles (9) and (10) after bond cleavage indicate that the protein levels on these particles are clearly reduced after cleavage using either an acidic pH 4.1 (a comparable pH to that in lysosomes) buffer solution or a reducing agent, tris(2-carboxyethyl)phosphine, respectively, showing the successful removal of the proteins. Note that, since the BCA method is not a true end-point analysis (that is, the final color continues to develop over time), the errors associated with measurements conducted across different timeframes are statistically meaningless and not included in this bar chart (BCA analyses are sufficient for qualitative comparison).

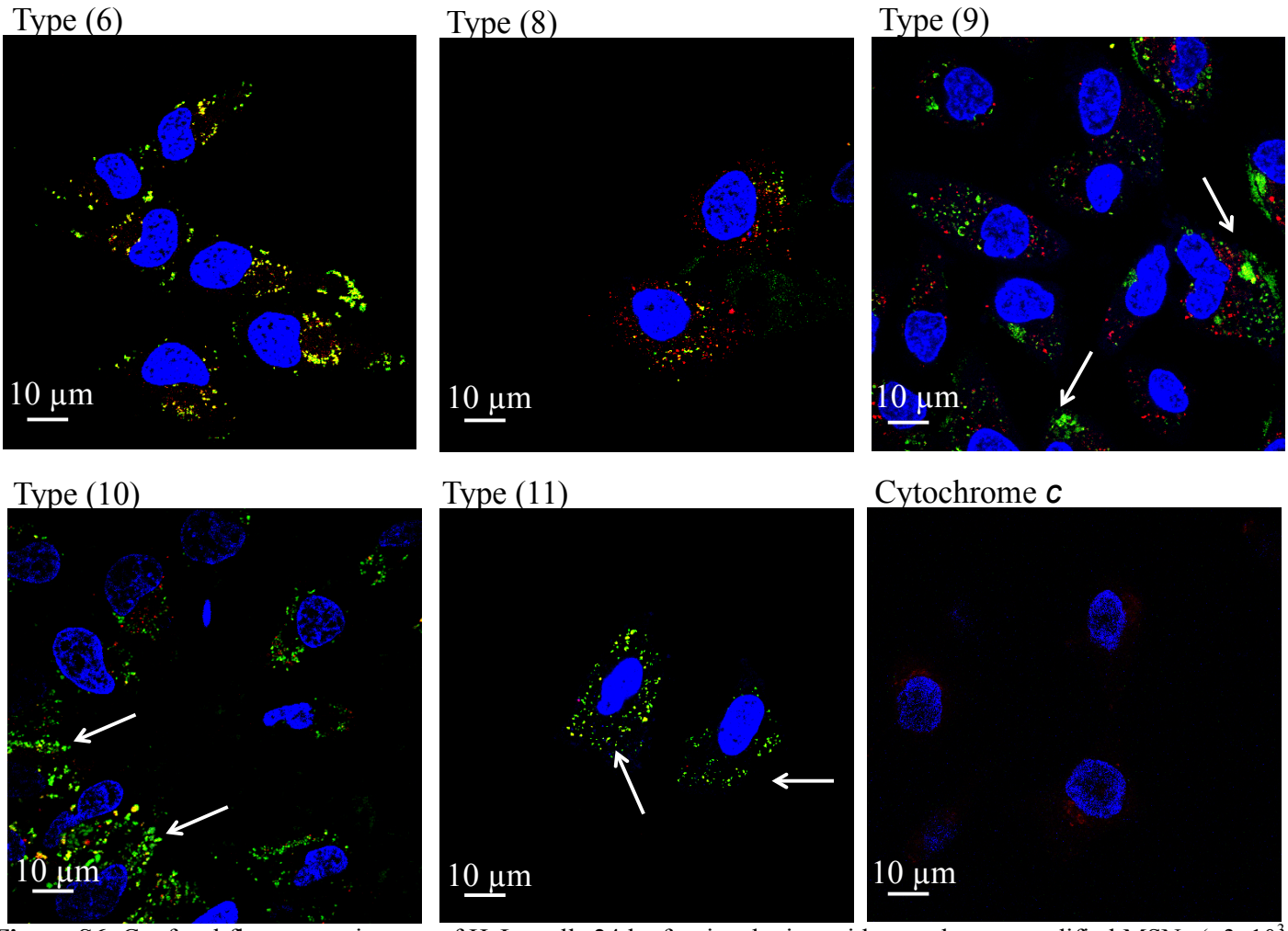


**Figure S6.** Confocal fluorescent images of HeLa cells 24 h after incubation with cytochrome modified MSNs (~$3x10^3$ cells with 200 µg/ml of MSNs). Images are the corresponding characteristic results associated with particle compositions (6), (8), (9), (10) and (11) and FITC-labelled cytochrome *c* (not particle bound) as labelled. Endosomes/lysosomes and nuclei are LysoTracker Red and Hoechst33342 blue stained, respectively. Yellow are the co-localised FITC-MSNs and endo/lysosomes. Arrows indicate MSNs having escaped endo/lysosomal entrapment (green). The data suggests type (9), type (10) and type (11) particles (acid-responsive, redox-responsive and BSA modified MSNs, respectively) are capable of effective endosomal escape whereas types (6) and (8) are not, as expected according to their charging profiles (Figure 3). Membrane impermeable FITC-labelled cytochrome (not particle bound) is not internalised and thus does not appear in the green channel. The images shown are entirely representative of the behaviour observed across the population of cells for each given particle type.

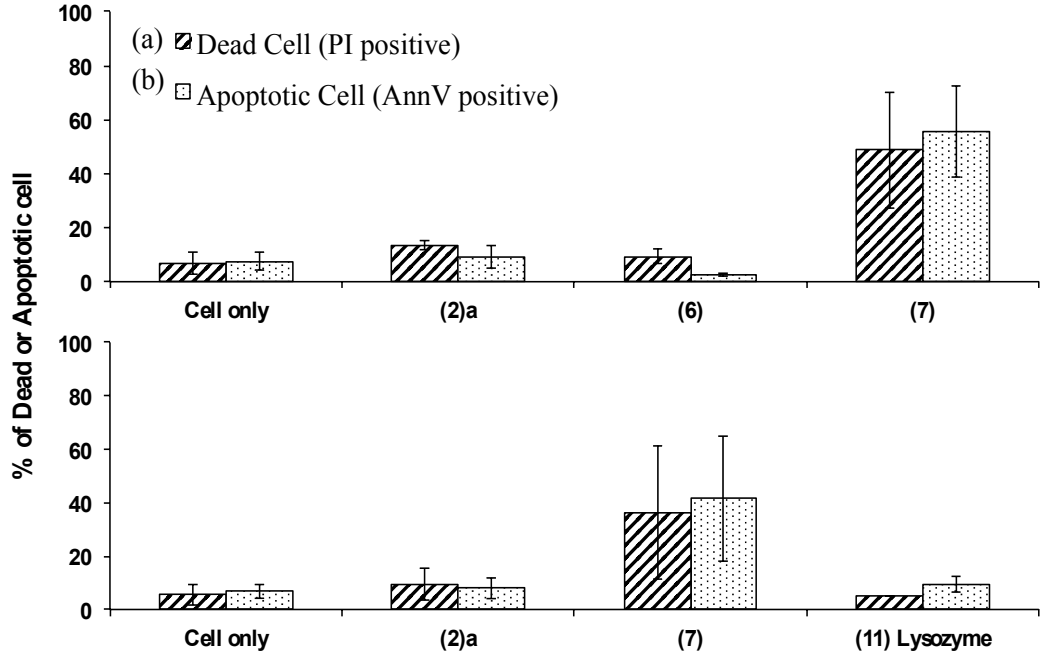


**Figure S7.** Haemocytometer data summarising the percentage of HeLa cells undergoing programmed cell death after treatment with nanoparticle types (2)a, (6), (7) and (11; lysozyme modified) determined from (a) the ratio of propidium iodide positive and negative cells, indicating cell death; (b) the ratio of Annexin V positive and negative cells, indicating an apoptotic mechanism of cell death (each dosage ~$3x10^3$ cells with 200 μg/ml MSNs, 24 h incubation). Significantly, the cytochrome labelled particles [type (7)] which induced the highest percentage of cell death have shown a correspondingly high percentage of AnnV-positive cells (compared to PI-positive cells), confirming that the cells died apoptotically (Top). The data indicates only particles appended through a non-cleavable linker to apoptosis inducing cytochrome *c* [type (7)], are able to cause a high level of apoptotic cell death. Particles without protein modification [type (2)a] and particles appended through a non-cleavable linker to lysozyme [*i.e.* not an apoptosis triggering protein; type (11)] demonstrate comparable cell death to a cell only control (Bottom). Data shown are the results of two repetitions.

**Table S1.** ζ-potential summary monitored stepwise through nanoparticle surface coupling reactions (ζ-potential values are an average of 5 measurements).

| Type (3) reaction steps | Mean ζ-potential (mV) pH 7.1 |
|---|---|
| Native FITC-MSNs | -30.3 |
| Amination | -17.5 |
| Carboxylation | -44.9 |
| Imidazole conjugation | -39.1 |

| Type (4) reaction steps | Mean ζ-potential (mV) pH 7.1 |
|---|---|
| Native FITC-MSNs | -30.3 |
| Amination | -17.5 |
| Histidine conjugation | -28.7 |

| Type (5) reaction steps | Mean ζ-potential (mV) pH 7.1 |
|---|---|
| Native FITC-MSNs | -30.3 |
| Amination | -17.5 |
| Carboxylation | -44.9 |
| Histidine conjugation | -38.5 |

| Type (6) reaction steps | Mean ζ-potential (mV) pH 7.1 |
|---|---|
| Native FITC-MSNs | -30.3 |
| Amination | 2.5 |
| Carboxylation | -60.2 |
| Cyt ***c*** conjugation | -49.0 |

| Type (7) reaction steps | Mean ζ-potential (mV) pH 7.1 |
|---|---|
| Native FITC-MSNs | -30.3 |
| Amination | -17.5 |
| Cyt ***c*** conjugation | -23.5 |

| Type (8) reaction steps | Mean ζ-potential (mV) pH 7.1 |
|---|---|
| Native FITC-MSNs | -30.3 |
| Amination | -23.8 |
| Carboxylation | -24.7 |
| Cyt ***c*** conjugation | -13.3 |

| Type (9) reaction steps | Mean ζ-potential (mV) pH 7.1 |
|---|---|
| Native FITC-MSNs | -30.3 |
| Aldehyde conjugation | -38.1 |
| Hydrazone conjugation | -35.5 |
| Amination | -29.6 |
| Cyt ***c*** conjugation | -13.0 |

| Type (10) reaction steps | Mean ζ-potential (mV) pH 7.1 |
|---|---|
| Native FITC-MSNs | -30.3 |
| Cystamine conjugation | -35.9 |
| Amination | -28.9 |
| Cyt ***c*** conjugation | -23.8 |

| Type (11) reaction steps | Mean ζ-potential (mV) pH 7.1 |
|---|---|
| Native FITC-MSNs | -30.3 |
| Amination | -17.5 |
| BSA conjugation | -21.6 |