**Supporting Information**

Dissolution behaviour of Fluorescent Silica Nanoparticles in Biological Conditions

**Experimental**

**Silica Nanoparticle Syntheses**

*Materials*

Tetraethyl orthosilicate (TEOS) (cat. no. 86578), 3-Aminopropyltrimethoxysilane (APTMS, cat. no. 281778), Fluorescein Isothiocyanate (Isomer I) (FITC) (cat. no. F7250), L-Arginine (cat. no. A5006) and IGEPAL CO-520 (cat. no. 238643) were all purchased from Sigma-Aldrich and used as received. Commercially sourced silica NPs were purchased from Kisker.

*Stöber Type Fluorescent*

\(N\text{-}1\text{-}(3\text{-}\text{trimethoxysilylpropyl})\text{-}N\text{'}\text{-}\text{fluoresceyl thiourea (FITC-APTMS)}\) conjugate solution was prepared by dissolving 4mg of FITC in 2 ml of anhydrous ethanol. 20 µl of APTMS (11x molar excess) was then added immediately to this solution with the mixture then shaken at room temperature in darkness for 4hrs. This reaction was monitored by \(^{1}\text{H NMR (CD}_{3}\text{OD)}\) where a shift in the signal from the proton adjacent to the amine group (Si(OCH\(_3\))\(_3\)CH\(_2\)CH\(_2\)NH\(_2\)) upon coupling FITC reached an integration value constant and equal to the aromatic FITC signal. The reaction was deemed complete at 4 hours.

50nm fluorescent silica nanoparticles were then prepared as follows. To aqueous ammonia (28 %) (1.82 g) was added EtOH to a total volume of 49 ml and rapidly stirred. 1 ml of the conjugate solution was then added to this mixture followed 1 minute later by 1.9 ml of TEOS. The reaction was sealed and stirred at 25°C for a further 20 h. The particles were washed by centrifugation at 14000 rpm for 30 minutes with resuspension in EtOH (x3) and water (x3) using bath sonication (Branson 1510) and volume then adjusted following final resuspension in water to give a final particle concentration of 10 mg/ml (concentration measured by vacuum drying at 60°C for 12 hrs). Particle size can be modulated through aqueous ammonia concentration as described in the table below.

*Core-Stöber Shell*

Core shell particles detailed in figures S4 and S9 were prepared following a method similar to previously published. Briefly 50nm fluorescent cores were formed by mixing 0.5ml conjugate with 0.91 g aq. ammonia in 24.5ml EtOH in a polypropylene container stirring rapidly at 25°C. 0.95ml of TEOS was then added and the mixture was stirred at 600rpm for a further 20hrs. The shell was grown by addition of 1.45ml TEOS in aliquots at a rate of 50ul every 15mins which allowed the particle to be grown up to the desired size without secondary nucleation.

*Arginine --Silica Core Shell*

To a cleaned aqueous SiO\(_2\)--FITC NP dispersion (10mg/ml) was added L-Arginine to a concentration of 0.5mg/ml. The required amount of TEOS (estimated according to equation below) was then added.
and the two-phase reaction was left incubating at 70°C in darkness for 48hrs with slow stirring (100 rpm) to homogenise the lower aqueous phase. The resultant dispersion, now one homogenous phase, was then centrifuged at 14000 rpm for 20 mins followed by resuspension of the pellet in water. This cleaning cycle was then repeated three times.

\[
\rho_{\text{final}} = 3 \left( \frac{3V_{\text{SiO}_2}}{4\pi N} + \rho_{\text{core}}^3 \right)
\]

**Reverse Emulsion Method**

IGEPAL CO 520 1.5 ml was dissolved in 18 ml of cyclohexane with sonication for 1 minute. Aqueous ammonia (28 %) (480 µl) was then added giving a milky suspension which became clear with bath sonication for 5 minutes (Branson 1510) indicating formation of nano-emulsion. 100 µl of FITC-APTM conjugate (prepared as in previous) solution was then added followed by 240 µl TEOS. The suspension was mixed by swirling and then left stationary in the dark to react for 24 hrs. The resultant particles were precipitated from the emulsion by addition of Acetone (2 ml) and washed in series by i-propanol, ethanol (x3) and water (x3) to give a bright yellow particle suspension.

**SDS-PAGE**

Following set incubation periods 20µl of the resulting dispersions were mixed with 5 µl protein loading buffer [62.5 mM Tris-HCL pH 6.8, 2% (w/v) SDS, 10% glycerol, 0.04M DTT and 0.01% (w/v) bromophenol blue], 15 µl of this mixture was then loaded onto a 12% polyacrylamide gel. Gel electrophoresis was performed at 40mA for about 30 minutes or until the bromophenol blue mark could be seen about ¾ ways down the plate. The resultant gels were scanned using a Typhoon fluorescence scanner (Ex. Laser 488nm, Em. filter 526 band pass).

**Fluorescence Spectroscopy**

Supernatants were monitored for released molecular dye species using Fluorolog. After set incubation periods the particles were centrifuged for 30 mins at 14,000 rpm. Supernatant samples were taken from the top and monitored by fluorescence (Ex 485nm).

**Molybdenum blue colorimetric method for silicate determination**

Dissolved SiO$_2$ was measured by the colorimetric silicomolybdate method measuring dissolved silicate species up to trimeric (H$_4$SiO$_4$), dimeric (H$_6$Si$_2$O$_7$), and possibly trimeric (H$_8$Si$_3$O$_{10}$)$_2$ or tetrameric. Following desired incubation time the resulting particle dispersion was centrifuged at 14,000 rpm for 60 minutes after which 500 µL of the supernatant was transferred to a 2 ml eppendorf. To this 500 µl solution was added in sequence 250 µl each of 1M HCl, 0.27 M Na$_2$EDTA and 0.265 M Ammonium molybdate and the obtained yellow solution was left for ten minutes at room temperature, after the waiting period 250 µl of 0.67 M Tartaric acid was added and the solution was left for further five minutes. 500 µl of 1.35 M Sodium Sulfite was then added with a dark blue color indicating the presence of dissolved silicates. Solutions were left to fully develop for 30 minutes. The so prepared samples were measured in a Varian 6000i UV/Vis Spectrophotometer with absorption at 715 nm taken.
TEM

Imcubated particles were centrifuged at 14,000 rpm for 30 minutes in order to remove them from the saline incubatory solution. They were then resuspended in water to a concentration of ~200µg/ml followed by deposition and air evaporation of a 10 µl droplet on a carbon coated copper grid (S162 Agar Scientific). The samples were then analysed on the FEI Tecnai 120 instrument.

Size analysis

Dynamic light scattering (DLS) - Malvern instruments, Transmission electron microscopy (TEM) – FEI Tecnai 120, 300kV and CPS DC24000 disc centrifugation – CPS instruments Europe Inc. were used to determine the dispersion characteristics of the particles.

Cell Fluorescence Imaging

Tissue culture reagents were purchased from GIBCO Invitrogen Corporation/Life Technologies Life Sciences (Carlsbad, CA) unless otherwise specified. Lung cancer epithelial A549 cells (ATCC-CCL-185) were maintained as monolayer cultures in MEM supplemented with 10% foetal bovine serum (FBS), at 37°C and 5% CO₂ (cMEM).

For cell fluorescence imaging, A549 cells were grown on 15 mm glass coverslips inside a 12 well plate. 24 hours after seeding, cells were exposed to the nanoparticle by replacing the culture medium with the nanoparticle dispersion in complete MEM. Nanoparticle dispersions were freshly prepared just before addition to cells by diluting the nanoparticle stocks in cMEM to a final concentration of 50 µg/ml. After 22 hour exposure to nanoparticles, samples were washed with 3 x 1 ml PBS, fixed for 20 min with 4% formalin, permeabilized for 5 min in 1% saponin from Quillaja bark (Sigma), and incubated for 1 hour at room temperature with a primary antibody 1:100 mouse mAb to LAMP1 [H4A3] for lysosome staining. Then, cells were washed with 3 x 1 ml PBS, and incubated at room temperature for 1 h with 1:300 dilution of AlexaFluor 647 Goat Anti-mouse IgG (H + L) as a secondary antibody (Molecular Probes). Samples were washed with 3 x 1 ml PBS and incubated for 3 min with DAPI (Sigma) before mounting with MOWIOL (Calbiochem) on glass slides for imaging. The cells were observed using a Leica DMI6000B epifluorescence microscope.

Live Cell Imaging

hCMEC/D3 human brain endothelial cell line were grown on collagen coated 4 well live cell chamber (Lab-tek) and supplemented with growth factor depleted EBM-2 assay medium containing bFGF, 2% FCS, hydrocortisone and 10 mM HEPES. Cells were cultured in an incubator at 37 °C with 5% CO₂ and saturated humidity 2 days before the experiment. The cells were incubated with 100 µg/mL of nanoparticles in assay medium for 2 h at 37 °C then washed with PBS (3x). The plasma membrane was stained with CellMask™ Orange (10µg/mL) 10 min and washed with PBS (3x) before replaced with assay medium. The images were acquired using Nikon spinning-disk confocal microscope (40x, oil immersion objective). The cells were excited by switching between two lasers (Ex 488, 100%T and Ex 561, 10%T) to continuously acquire 600 images with 1 second interval. Images were processed using Imaris 7.2.3.
Supplementary Results

Figure S1. Dissolution Particle Size dependence for unlabelled Stober silica nanoparticles: Top: SiO$_2$ Nps incubated at conc. of 100µg/ml

Figure S2. 4hr incubations@ 37°C: Core-shell type in water (1), DMEM+10% FBS (2), 50nm SiO$_2$-FITC water(3,5,8) and DMEM+10% FBS (4,6,7), 10 nm SiO2-FITC in water(9) and DMEM+10% FBS(10)
Figure S3. 24 hr incubations @ 37°C: 50 nm SiO$_2$-FITC 24 hr incubation in water(1,2), PBS(3,4), DMEM (5,6) and DMEM+10% FBS (7,8).

Figure S4. 24 hr incubations @ 37°C: Commercial Silica(yellow-green) incubated 24 hr in water (batch I-1,2,batch II- 5,6), DMEM +10%FBS (batch I-3,4,batch II- 7,8) and synthesised 50nm SiO$_2$-FITC in water(9,10) and DMEM +10%FBS (11,12).

Figure S5. 7,24 and 48 hr incubation. Dye release comparison of Core-Shell synthesised and commercially available SiO$_2$ NPs for various media @ 37°C.(water, PBS, DMEM, DMEM +10% FBS)
Figure S6. 50 nm synthesised SiO$_2$-FITC incubated in saline PBS pH 7.4 @ 37°C. Scale bars 500 nm, 200 nm, 100 nm left to right. Top: 0 hours, second row: 6 hours, third row: 17 hr, bottom row: 26 hr.
Figure S7. Incubation of commercial yellow green fluorescently labelled silica in saline PBS @ 37°C top row 7 h, middle row 24 hr, bottom row 48 hr. Top: 6 hours, middle row: 17 hr, bottom row: 26 hr.
Figure S8. Incubation of commercial yellow green fluorescently labelled silica in DMEM 10%FBS @ 37°C. Scale bars: 500 nm, 200 nm, 100 nm left to right. Top: 6 hours, middle row: 17 hr, bottom row: 26 hr.
Figure S9 Higher magnification images showing top arginine shell fluorescent silica and bottom Stöber-FITC type nps incubated in HEPES pH 7.4, NaCl 148 mM, CaCl₂ 1 mM.
Figure S9. Top: Comparison of Arg-Sil Shell and Stöber Shell protection. 1-D SDS Page gel showing release of fluorescent species with time and molybdate assay showing dissolution. Bottom: Dissolution and dye release behaviour for reverse emulsion synthesised compared to arginine shell particles and those prepared by FITC encapsulation without APTMS conjugation.
Figure S10. T.E.M. images showing particle core, shell I, shell II and shell III particles following incubation in HEPES pH 7.4, NaCl 148 mM, CaCl₂ 1 mM. Scale bars represent 500 nm, 200 nm and 100 nm from left to right respectively.
Figure S11. Live Cell imaging Snapshots to monitor Photobleaching. hCMEC/D3 cells were grown in a live cell chamber for 2 days before exposure for 2 hours to nps (100 µg/ml) followed by washing with PBS (x3). They were then exposed to orange cell mask (10 µg/ml) for 10 minutes before PBS wash (x3) and replacement of cell medium (EBM-FBS 2%). The particles were continuously excited for 10 minutes with a 488nm laser at 100% transmission to maximise photobleaching effect. The mask was excited at 560nm with 10% transmission. Imaging was performed on a Nikon Eclipse Ti Spinning Disc Confocal Microscope.
Figure S12. Zeta Potential v pH for silica Nanoparticles (Measurements performed in 1mM KCl in alkaline to acidic direction).

Figure S13. DLS size/intensity profile showing particle colloidal stability of particle “Shell 1” in cell medium (DMEM) and serum containing cell medium (DMEM+FBS 10 %). (Measurements taken at 37°C, each profile is average of 33 measurements)

Particle Reaction Composition/Characterisation Table

<table>
<thead>
<tr>
<th>Particle</th>
<th>[TEOS]</th>
<th>[H2O]</th>
<th>[NH3]</th>
<th>FITC conjugate solution</th>
<th>Reaction temp</th>
<th>DLS (Z-Ave) (diam. nm)</th>
<th>DCS (wt av.)</th>
<th>TEM (diam. nm) (av)</th>
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<tbody>
<tr>
<td>Diameter (nm)</td>
<td>Concentration (µl/ml)</td>
<td>Temperature</td>
<td>RT (°C)</td>
<td>(diam. (nm))</td>
<td>30 measures</td>
<td></td>
<td></td>
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<td>-------------</td>
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<td>50</td>
<td>0.17</td>
<td>1.456</td>
<td>0.58</td>
<td>20 µl/ml</td>
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<td>0.91</td>
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<td>115.3</td>
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<tr>
<td>30</td>
<td>0.17</td>
<td>1.456</td>
<td>0.58</td>
<td>20 µl/ml</td>
<td>55°C</td>
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<td>Stober Shell Particles</td>
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<td>-</td>
<td>90 (0.15)</td>
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<tr>
<td>85nm Non-labelled</td>
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<td>-</td>
<td>RT</td>
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<tr>
<td>120nm non-labelled</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
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