Electronic Supplementary Material (ESI) for Faraday Discussions.

SUPPLEMENTARY INFORMATION

Ultrasmall silicon nanoparticles as a promising platform for multimodal imaging

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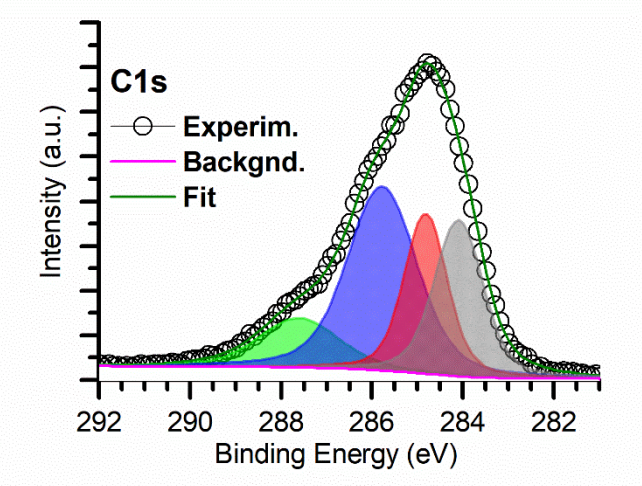


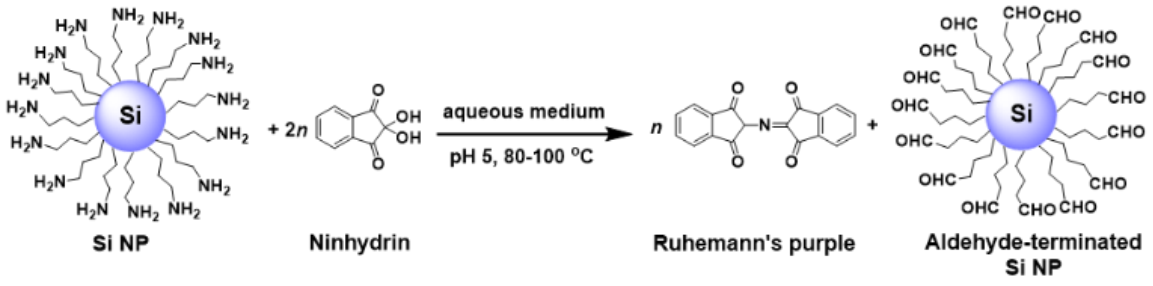
Figure S1. XPS high resolution carbon scan of Si NPs with deconvolution.

Table S1. XPS data for deconvolution of the high resolution carbon scan.

Component	Peak BE	FWHM eV	Area (P) C	Colour
C=O	287.6	2.08	5356.74	Green
C—O	285.8	1.80	17394.01	Blue
C—C	284.8	1.13	9420.01	Red
C=C	284.1	1.27	10413.35	Gray

Calculations for the quantification of amine groups on Si NPs' surface

Quantification of surface amine groups. In order to gain information on the concentration of amine groups per mass of Si NPs, the Kaiser test was employed, utilising a method similar to those employed in previous studies. Different known masses of sample are mixed and heated in separate solutions with ninhydrin reagent, which reacts with primary amine groups on the surface of the Si NPs to generate Ruhemann's purple that presents a characteristic peak in absorption spectra at 570 nm (**Scheme S1**). The quantity of this molecule is assessed by UV-Vis absorption spectroscopy to translate into the concentration of amine groups in each amount of sample.
The absorbance of each of the solutions was measured and the values at λ = 570 nm were recorded. As shown in **Figure S2**, a linear relationship exists between the masses of Si NPs and the absorption at 570 nm in four test solutions, meaning the data has a high degree of reliability. From the data collected, an average concentration of 4.16 µmol NH_2 per mg Si NPs was estimated, which was used to calculate a value of approximately 43 amine groups per particle (**Table S2/Equations S1, S2**).



Scheme S1. Reaction of amine terminated Si NPs with ninhydrin to form Ruhemann's purple and aldehyde terminated Si NPs in the Kaiser Test.

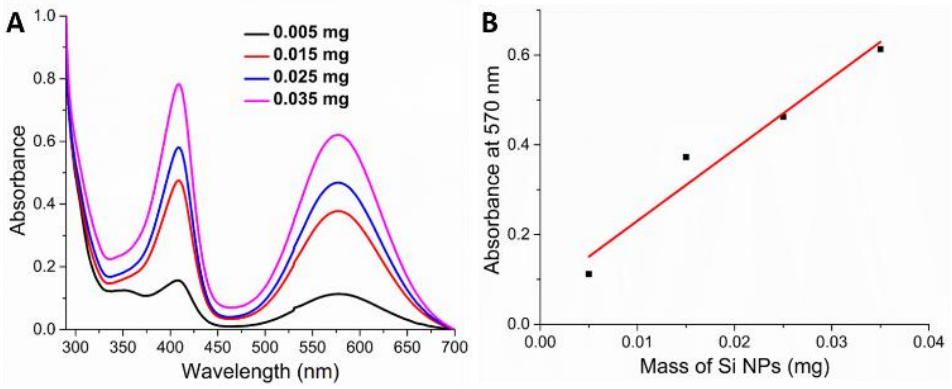


Figure S2. A) UV-Vis absorption spectra of Kaiser Test solutions containing different masses of Si NPs. B) Linear fitting of absorbance values against mass of Si NPs. Spectra measured in methanol.

Table S2. Data from the Kaiser Test to evaluate the concentration of amine groups on Si NPs (value of ε calculated from earlier calibration curve with ninhydrin).

Mass Si NPs (mg)	Absorbance (λ = 570 nm)	Volume of test solution (ml)	ε (dm^3 mol^{-1} cm^{-1})	Concentration of amine groups (µmol/mg)
0.005	0.11164	0.003	15000	4.465
0.015	0.37249	0.003	15000	4.966
0.025	0.46234	0.003	15000	3.698
0.035	0.61334	0.003	15000	3.504

Equation S1. Calculation for the concentration of amine groups (10^6 used to account for dilution of the test solution when measuring the absorbance).

$$conc.\,amine\ groups = \frac{(Abs\ at\ \lambda = 570\ nm)\ \times Vol \times 10^6}{\varepsilon\ \times mass\ Si\ NPs}$$

Equation S2. Calculation of the number of amine groups per Si NPs.

Mass of SiNPs:
One SiNP:
Diameter = 2.4 nm
Vol (V) = (4/3) π (1.2 x 10^{-9})^3 = 7.24 x 10^{-27} m^3 = 7.24 x 10^{-21} cm^3
Density (D) = 2.33 g/cm^3 (silicon)
Mass of one NP (M = V x D) = 1.7 x 10^{-20} g = ***1.7 x 10^{-17} mg = M_{SiNP}***
No. Si NPs per mg = 1/ M_{SiNP} ***= 5.88 x 10^{16}***

Mass of amine groups per mg (M = n x MW) = 4.16 x 10^{-6} mol x 16 g/mol
= 6.7 x 10^{-5}g ***= 6.7 x 10^{-2}mg***
No. amine groups per mg = n x N_A = 4.16 x 10^{-6} x 6.022 x 10^{23} ***= 2.5 x 10^{18}***

<u>Number of amine groups per particle:</u>
5.88 x 10^{16} particles per mg
2.5 x 10^{18} amine groups per mg
So amine groups per particle
= amine groups per mg/ particles per mg
= ***43 amine groups per particle***

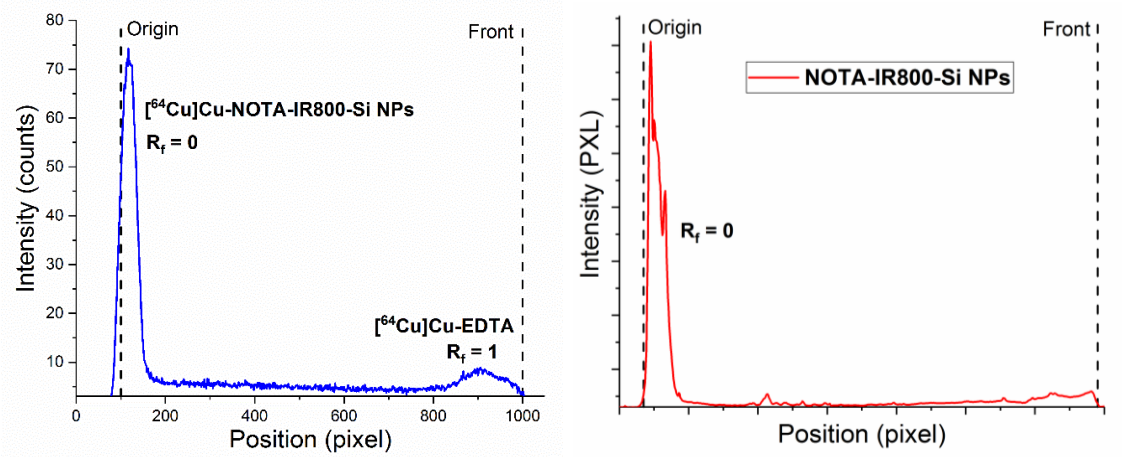


Figure S3. Complementary Radio-iTLC and fluorescence-TLC of NOTA-IR800-Si NPs. EDTA incubated ^{64}Cu(II)-labelled Si NP-IR800-NOTA. (Radio-iTLC SG; Mobile phase: NH_4OAc:MeOH 1:1 (v/v); [^{64}Cu]Cu-NOTA-IR800-Si NPs R_f = 0, [^{64}Cu]Cu-EDTA R_f = 1 (left); Fluorescence TLC of NOTA-IR800-Si NPs measured under IR short channel (710 – 730 nm), RP-TLC; Mobile phase: Methanol; Si NP-IR800-NOTA R_f = 0 (right).

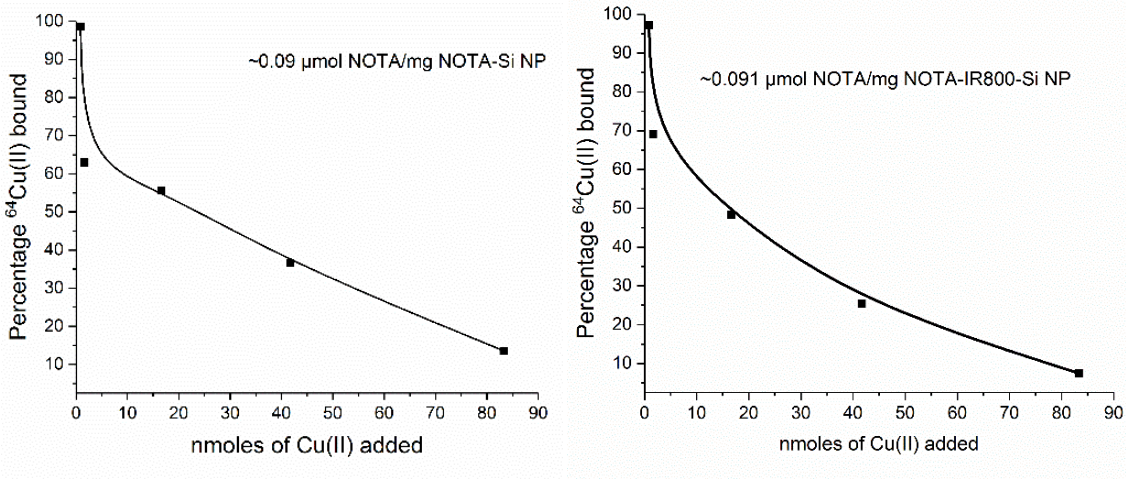


Figure S4. Radiometric titration curve for ^{64}Cu(II) and Cu(II) labeled [^{64}Cu]Cu-NOTA-Si NPs (left) and [^{64}Cu]Cu-NOTA-IR800-Si NPs (right) (Experimental value: ~0.09 µmol NOTA/mg NOTA-Si NP and ~0.091 µmol NOTA/mg NOTA-IR800-Si NP).

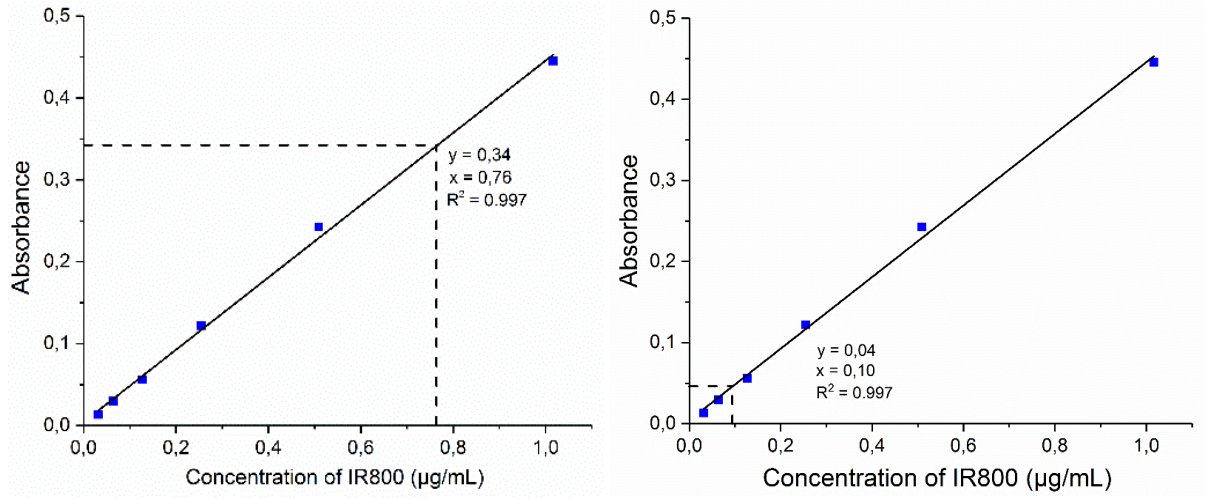


Figure S5. Quantification of IR800 dye present on IR800-Si NPs (left) and NOTA-IR800-Si NPs (right) by UV-Vis spectroscopy (Experimental value ~0.013 µmol IR800 dye/mg IR800-Si NP and ~0.010 µmol IR800/mg NOTA-IR800-Si NP).

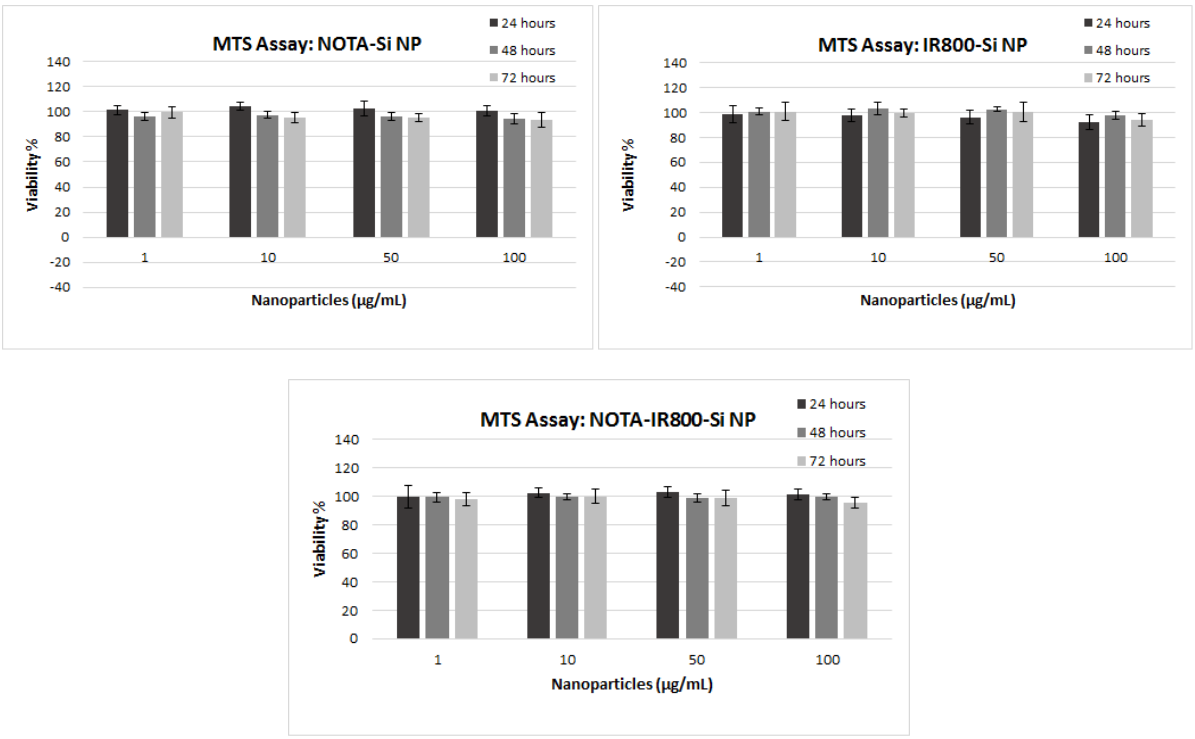


Figure S6. Effect of NOTA-Si NPs, IR800-Si NPs and NOTA-IR800-Si NPs on the viability of HEK293 cells. The cells were exposed to 1, 10, 50 and 100 µg/mL Si NPs dispersions for 24, 48 and 72 h respectively in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and viability was determined using MTS assay. Triton-X was used as positive control.

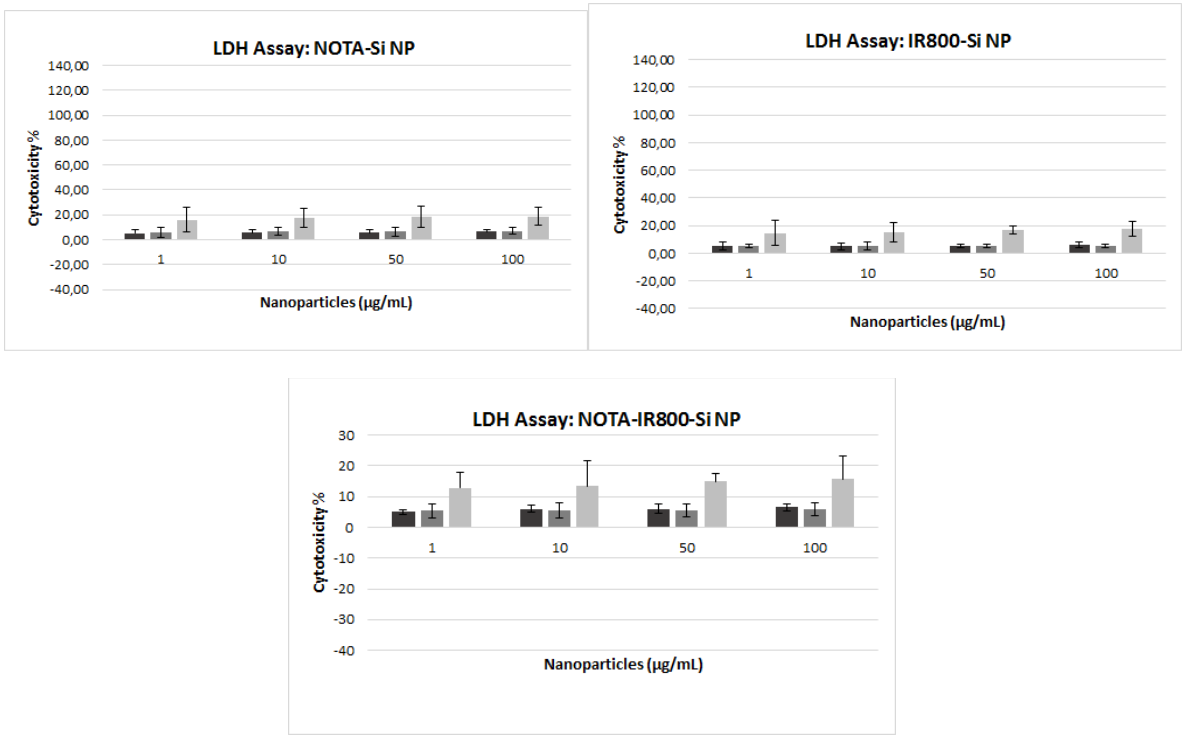


Figure S7. Effect of NOTA-Si NPs, IR800-Si NPs and NOTA-IR800-Si NPs on the viability of HEK293 cells. The cells were exposed to 1, 10, 50 and 100 µg/mL Si NPs dispersions for 24, 48 and 72 h respectively in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and viability was determined using LDH assay. Lysis buffer for maximum LDH release was used as a positive control.