

Tunable surface modification of silica nanoparticles through ‘click’ chemistry

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Tuning the surface functionality of silica nanoparticles through a ‘click’ chemistry-based protocol is demonstrated. Pyrene, a fluorophore that displays excimer emission specifically at higher density/concentration is chosen for highlighting the fidelity of surface functionalization. The UV–visible absorbance of the pyrene chromophore capping the nanoparticle is used to determine the amount of pyrene units present on the surface of a silica nanoparticle.

Keywords: ‘Click’ chemistry, pyrene, silica nanoparticles, surface functionality.

SILICA nanoparticles find varied applications in chromatography¹, bio-separation², imaging³ and the synthesis of multifunctional nanocomposites^{3,4}. Functionalization of silica nanoparticle surfaces is commonly achieved using layer-by-layer assembly⁵, physical adsorption⁶ and silane coupling agents⁷. Silane functionalization protocols, that proceed by way of a covalent bond formation between the surface silanol groups and silane coupling agents, have been widely used for silica nanoparticle surface modification. While silanization can be used to impart a broad range of functionality, the method suffers from inherent drawbacks. Factors such as solvent polarity and reaction temperature play complicated and opposing roles in directing the course of the reaction². Self-condensation of the silane coupling agent poses problems in tuning the surface density of the functionalities and often results in irreproducible surface coverage². Conditions that promote large-scale self-condensation of silanes in the solvent usually affect the density and uniformity of the surface-capping agent. In a recent study by Bruce and coworkers², the effect of various reaction conditions was studied to assess the density of surface amine groups capping silica-coated magnetite nanoparticles. The density of the surface amine groups was varied from 5 to 24×10^{-9} mol/mg of the composite, by controlling various parameters such as reaction temperature, solvent composition and duration of the reaction. However, the authors clearly point out the difficulty in this procedure, wherein the surface density depends critically on various reaction conditions. Further utilization of this functionality is usually based on chemical reactions that either require anhydrous conditions (as in

Schiff’s base formations) or require special coupling agents (as in formation of amide bond) to be quantitative.

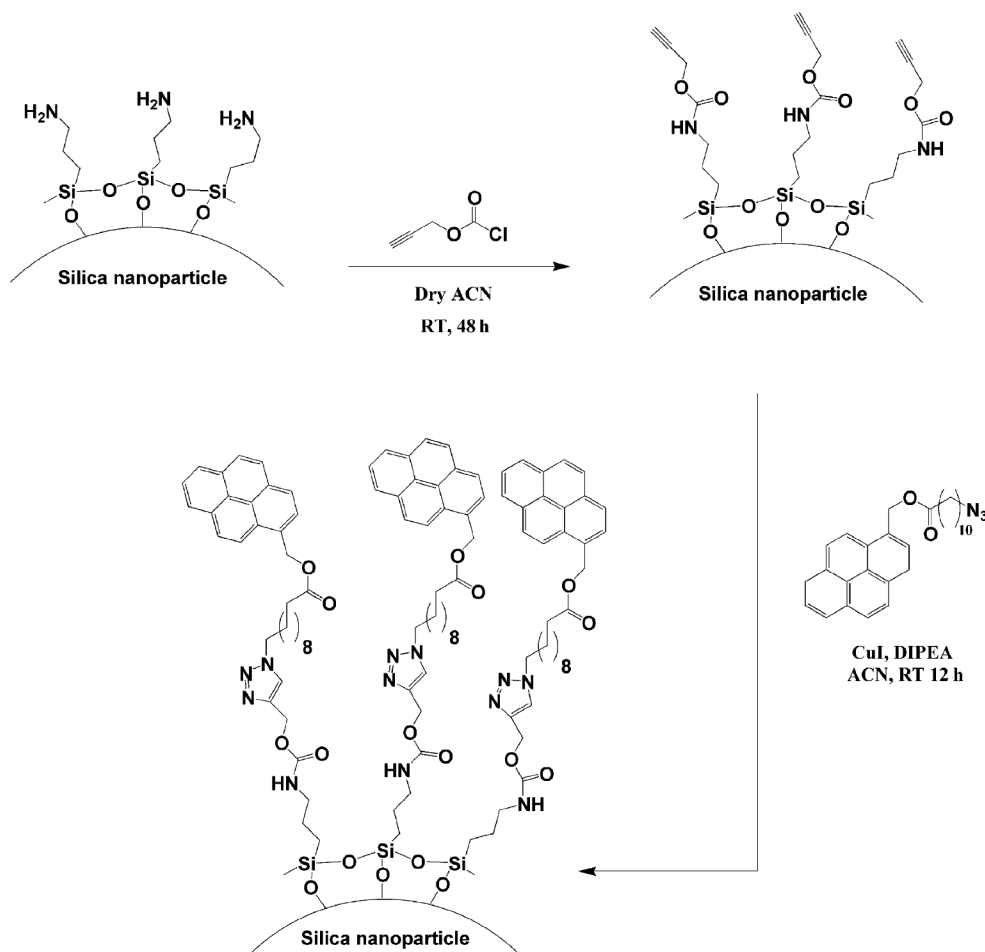
The Huisgen’s 1,3-dipolar cycloaddition reaction, one of the popular ‘click’ reactions, between an azide and a terminal alkyne resulting in 1,2,3-triazoles⁸, has been demonstrated to be a versatile tool in organic synthesis⁹, material and polymer synthesis¹⁰. The fact that these reactions proceed to give high yields even under ambient conditions make them a popular choice in bioconjugation procedures where the pH, medium of reaction and temperature conditions play a crucial role¹¹. Moreover, the modular nature of a ‘click’ reaction enables imparting of desirable functionalities, without the capping agent undergoing side reactions¹². Previous reports have demonstrated the utility of the ‘click’ reaction in the preparation of monolayers of surface functionality on silica substrates¹³ and suitable fabrication of silica gel for HPLC packings¹. However, a systematic study varying the surface density of functional groups on silica nanoparticles using ‘click’ chemistry-based protocols has not been reported.

Addressing this lacuna, we herein present a ‘click’ reaction-based procedure that builds on the functionality imparted by silane coupling agents to afford an easy protocol that can be used to tune the density of the silica nanoparticle surface capping (Scheme 1). Standard functionalization protocols are initially used to graft propargyl groups onto the silica nanoparticle surface. This is followed by a reaction with varying amounts of a pyrene chromophore bearing an azide (PA) group. The photoactive capping agent used enables estimation of the amount of fluorophore capping. The utility of the present method in studying the variance in surface functionalization can easily be validated through pyrene ‘excimer’ emission characteristic of the proximity of the pyrene molecules to one another. As proposed, we observe that by changing the initial amount of PA, the density of surface capping can be easily controlled. We add that the procedure is generic and could be adapted to functionalize silica with any other molecule bearing an azide group. Presented below are details of investigation.

Experimental

Tetraethyl orthosilicate, 99.9% (TEOS); (3-aminopropyl)-triethoxysilane, 99% (APTES); 1-pyrenemethanol, 98%;

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Scheme 1. Salient features of the 'click' chemistry-based protocol for functionalization of silica nanoparticles. See text for details.

11-bromoundecanoic acid, 95%; 4-N,N-(dimethylamino)pyridine, 95% (DMAP); N,N'-diisopropylcarbodiimide, 99% (DIC); N,N-diisopropylethylamine, 99% (DIPEA) and copper(I) iodide (CuI) were procured from Sigma Aldrich Chemicals. Absolute ethanol, ammonia (about 30% in water), sodium azide, petroleum ether and chloroform were procured from Merck. Dichloromethane, dimethylformamide and acetonitrile procured from Merck were dried following standard procedures and stored over molecular sieves. Propargylchloroformate was synthesized following a reported procedure¹⁴. Deionized Milli-Q water was used, wherever necessary.

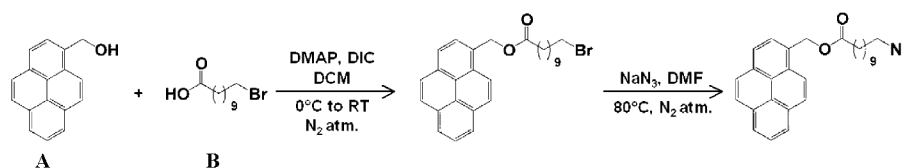
Synthesis and preliminary functionalization of silica nanoparticles

Silica nanoparticles were synthesized following the procedure reported by Stober and co-workers¹⁵. Purification of silica nanoparticles was carried out using three rounds of centrifugation (13,000 rpm for 15 min) interspersed with re-dispersion and washing with ethanol. The size of the

silica nanoparticles was determined to be about 65 nm using TEM studies.

APTES functionalization

APTES functionalization is the most preferred method for obtaining exposed amine groups on silica surface. For our study, we optimized the conditions available in several papers and the details are as follows². Bare silica nanoparticles were dispersed in absolute ethanol (150 mg in 15 ml) followed by the addition of 2% w/v of APTES. The above mixture was stirred for 6 h at reflux conditions. Purification of functionalized silica nanoparticles was carried out using three rounds of centrifugation (13,000 rpm for 15 min) interspersed with re-dispersion and washing with ethanol. Amine functionalization was qualitatively concluded by the ninhydrin test, taking the formation of blue colour as an indication for the amine groups. Further, the fact that these particles undergo a facile reaction with propargylchloroformate to yield alkyne functionalized silica particles, provide conclusive evidence for



Scheme 2. Protocol for the synthesis of methyl-(1-pyrene)-11-azido-undecanoate.

successful amine functionalization. Functionalized silica nanoparticles were subjected to TEM and FTIR characterization after purification.

Propargyl functionalization

In a typical reaction, 250 mg of APTES-capped silica nanoparticles was dispersed in 50 ml of dry acetonitrile followed by addition of 0.3 ml of propargylchloroformate. The above reaction mixture was stirred for 48 h at RT under nitrogen atmosphere. The propargyl-capped silica nanoparticles were purified using multiple rounds of centrifugation and washing with acetonitrile. These purified nanoparticles were characterized using FTIR and TEM.

Synthesis of organic molecules

Synthesis of the organic molecule bearing the azide functionality (PA) was accomplished following standard protocols¹⁶ (Scheme 2) and its structural authenticity was thoroughly established by various spectroscopic techniques, including ¹H and ¹³C NMR spectroscopy and MALDI-TOF mass spectrometry. All reactions were performed under nitrogen atmosphere. Removal of solvent *in vacuo* refers to distillation using a rotary evaporator attached to an efficient vacuum pump. Products obtained as solids or syrups were dried under high vacuum. Analytical TLC was performed on pre-coated silica plates (*F*₂₅₄, 0.25 mm thickness); compounds were visualized by UV light, iodine chamber or by staining with anisaldehyde or ninhydrin solution. ¹H, ¹³C NMR spectra were recorded on 200.13 MHz for ¹H and 50.32 MHz for ¹³C NMR spectrometers. Chemical shifts (δ_{H}) are quoted in ppm and are referenced to tetramethylsilane (internal). MALDI-TOF mass spectra were recorded on a VOYAGER-DE-STR instrument.

Synthesis of methyl-(1-pyrene)-11-bromo-undecanoate (PB)

To a solution of 1-pyrene methanol (**A**) (0.69 g, 0.297 mmol) and 11-bromoundecanoic acid (**B**) (0.7876 g, 0.297 mmol) in anhydrous CH₂Cl₂ (15 ml), was added DIC (0.693 ml, 0.445 mmol) and a catalytic amount of DMAP at 0°C under nitrogen atmosphere. The resulting solution was brought to room temperature (35°C) and stirred vigorously

for 2 h. At the end of the reaction, as adjudged by TLC analysis, the reaction mixture was diluted with 50 ml of freshly distilled CH₂Cl₂, washed thoroughly with water and extracted thrice with CH₂Cl₂ (3 × 50 ml). The combined organic layers were dried over anhydrous Na₂SO₄ and filtered. The filtrate was concentrated *in vacuo* to obtain a crude residue which was subsequently purified by silica gel column chromatography using a gradient solvent system starting with 10% ethyl acetate in petroleum ether and the product (PB; 1.31 g, 92%) was eluted with 30% ethyl acetate in petroleum ether.

Characterization data of compound PB

¹H NMR (200.13 MHz, CDCl₃): 1.13–1.45 (m, 12H, $-(\text{CH}_2)_6-$), 1.56–1.86 (m, 4 H, $-\text{COCH}_2\text{CH}_2-$ + $-\text{CH}_2\text{CH}_2\text{Br}$), 2.37 (t, 2 H, $J = 7.36$ Hz, COCH_2), 3.36 (t, 2 H, $J = 6.81$ Hz, CH_2Br), 5.83 (s, 2 H), 7.95–8.36 (m, 9 H); ¹³C NMR (50.32 MHz, CDCl₃): 25.0, 28.1, 28.6, 29.0, 29.1, 29.2, 29.3, 32.8, 33.8, 34.4, 64.5, 122.9, 124.6, 125.4, 125.5, 126.1, 127.3, 127.7, 127.8, 128.1, 129.1, 129.5, 130.7, 131.2, 131.7, 173.8; calculated molecular weight of C₂₈H₃₁BrO₂: 478.15, Found: 478.13, 480.13; FTIR: 3037 (m), 2916 (s), 2850 (s), 1739 (s).

Synthesis of methyl-(1-pyrene)-11-azido-undecanoate (PA)

Compound PB (0.84 g, 0.17 mmol) was dissolved in 20 ml of anhydrous DMF and NaN₃ (0.911 g, 1.4 mmol) was added to it under stirring condition. The mixture was heated at 80°C for 12 h in nitrogen atmosphere. The volume of the solution was reduced *in vacuo* and the light yellow solid was purified by silica gel column chromatography using a gradient solvent system starting with 10% ethyl acetate in petroleum ether and the pure product (PA) (0.734 g, 95%) was fully characterized by ¹H, ¹³C and DEPT NMR spectroscopy and MALDI-TOF spectrometry.

Characterization data of compound PA

¹H NMR (200.13 MHz, CDCl₃): 1.10–1.32 (m, 12 H, $-(\text{CH}_2)_6-$), 1.45–1.68 (m, 4 H, $-\text{COCH}_2\text{CH}_2-$ + $-\text{CH}_2\text{CH}_2\text{N}_3$), 2.36 (t, 2 H, $J = 7.33$ Hz, COCH_2), 3.19 (t, 2 H, $J = 6.89$ Hz, CH_2N_3), 5.81 (s, 2 H, OCH_2), 7.92–8.28 (m, 9 H); ¹³C NMR (50.32 MHz, CDCl₃): 24.9, 26.4, 28.8,

29.0, 29.1, 29.2, 29.3, 34.3, 51.4, 64.5, 122.9, 124.6, 124.8, 125.4, 125.4, 126.0, 127.3, 127.7, 127.8, 128.1, 129.0, 129.5, 130.6, 131.1, 131.6, 173.8; calculated molecular weight of $C_{28}H_{31}N_3O_2$: 441.24, Found: 441.27; FTIR: 3043 (m), 2928 (s), 2853 (s), 2094 (s) [azide stretching], 1733 (s) cm^{-1} .

Click reaction

In a typical reaction, 50 mg of purified propargyl-capped silica nanoparticles and 32 mg of CuI were dispersed in 5 ml of acetonitrile. The role of Cu(I) salt here is to catalyse the regioselective reaction of the azide and alkyne to yield 1,4-disubstituted 1,2,3-triazoles, rather than 1,5-disubstituted 1,2,3-triazoles⁸. This is followed by the addition of 4 mg of PA (concentration 1.8×10^{-3} M) and 0.36 ml of DIPEA. The above constituents were stirred for 12 h at RT. Purification of pyrene-capped silica nanoparticles was carried out as follows: three rounds of centrifugation interspersed with re-dispersion and washing with acetonitrile. The precipitate thus obtained was re-dispersed in 5 ml of acetonitrile and added to 15 ml of 0.01 N HCl. This led to the precipitation of silica nanoparticles and dissolution of excess copper(I) iodide. The precipitate was further washed with acetonitrile and chloroform to yield purified pyrene-capped silica nanoparticles (SiPy1).

The above process was repeated with varying amounts of PA (concentration 1.8×10^{-4} and 1.8×10^{-5} M) to yield silica nanoparticles with varying surface density of the pyrene moieties (SiPy2 and SiPy3 respectively). The nanoparticles thus obtained were characterized using transmission electron microscopy and spectroscopic techniques such as UV-Vis absorbance, fluorescence and FTIR.

Quantitative estimation of pyrene moieties on silica particles

In a typical procedure, 1 mg of pyrene-functionalized silica was dispersed in 1 ml of $CHCl_3$. The UV-Vis absorbance spectra of the dispersed solutions were then recorded. Background correction was performed using the JASCO spectral analysis software to remove contribution from the scattering of silica. The value of absorbance at 346 nm was then used to quantitatively estimate the amount of pyrene present in 1 mg of functionalized silica by comparison with a calibration plot of PA.

Instrument details

UV-Vis spectroscopic measurements were carried out on a JASCO model V-570 dual-beam spectrophotometer operating at a resolution of 2 nm. Fluorescence measurements

were carried out on a Perkin Elmer LS55 fluorescence spectrophotometer with a slit width of 5 nm. TEM samples of functionalized silica nanoparticles were prepared by placing drops of the dispersed samples over carbon-coated copper grids and allowing the solvent to evaporate. TEM measurements were performed on a JEOL model 1200EX instrument operating at an accelerating voltage of 80 kV. FTIR spectra were recorded on a Perkin-Elmer FTIR Spectrum One spectrophotometer in diffuse reflectance mode, operating at a resolution of $4 cm^{-1}$.

Results and discussion

Functionalization of silica nanoparticles was achieved following Scheme 1. Figure 1 shows the FTIR spectra of silica nanoparticles at different stages of functionalization. In comparison to the APTES functionalized silica (curve 1, Figure 1), propargyl chloroformate treatment leads to a peak at $1700 cm^{-1}$ (curve 2, Figure 1) due to the carbonyl stretching vibrations in urethanes¹⁷, clearly indicating successful functionalization. The spectrum from pure PA clearly reveals a strong peak at $2100 cm^{-1}$, which provides evidence for the presence of azide. Further, the 'click' product obtained on reaction of the propargyl functionalized silica with PA showed no feature at $2100 cm^{-1}$, indicating the absence of any azide moiety (compare curves 3 and 4, Figure 1) in the product. This also rules out the possibility of PA capping the silica nanoparticles by way of physical adsorption, in which case the azide group would be detected by FTIR. The spectral features due to the 1,2,3-triazole unit¹⁸, expected around $1650 cm^{-1}$, are not well observable as they overlap with the signal at $1650 cm^{-1}$, which is characteristic of water present in the silica¹⁹. Conclusive indication for the formation of the 'click' product was nevertheless obtained from optical characterization.

It is important to note that dispersibility of the nanoparticles can be tuned by choosing an appropriate capping agent. Silica nanoparticles obtained as products of the

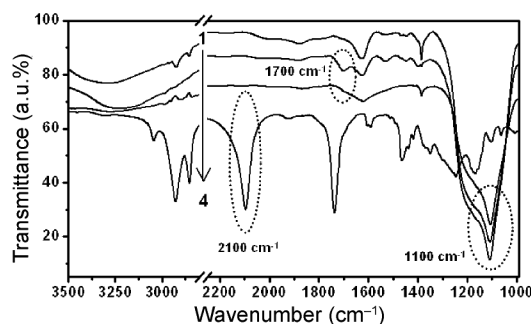
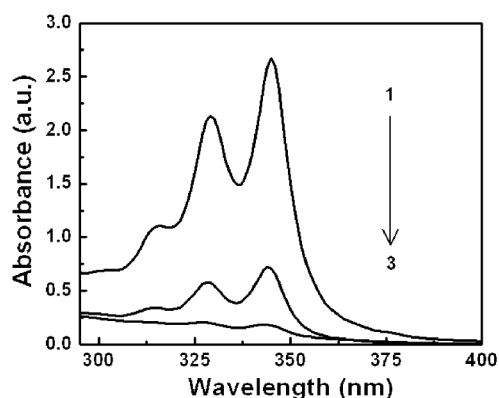
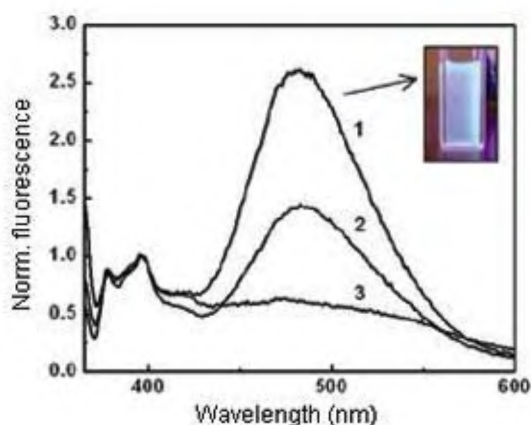


Figure 1. FTIR spectra of APTES, propargyl and pyrene-capped silica nanoparticles (curves 1–3 respectively). Note the intense band at $1100 cm^{-1}$, which arises due to the siloxane bonds present in silica (samples 1–3). Curve 4 corresponds to methyl-(1-pyrene)-11-azido-undecanoate (PA). See text for details.

Table 1. Surface density of pyrene units on silica nanoparticles under different conditions. See text for details

Sample	Stober silica content per mg of sample (g)	Number of particles per mg of sample	Amount of pyrene per mg of sample (mol)	Number of pyrene units per particle	Surface density of pyrene units (molecules/nm ²)
SiPy1	9.55×10^{-4}	3.35×10^{12}	4.5×10^{-8}	8.1×10^3	0.61
SiPy3	9.85×10^{-4}	3.46×10^{12}	3×10^{-9}	5.2×10^2	0.04

**Figure 2.** Background corrected UV-Vis absorbance profiles of SiPy1, SiPy2 and SiPy3 (curves 1–3 respectively). See text for details.**Figure 3.** Curves 1–3 show normalized emission profiles corresponding to SiPy1–SiPy3 respectively (excitation at 350 nm; normalization of the signals done wrt intensity of emission at 397 nm). (Inset) Photograph showing SiPy1 dispersed in CHCl₃ when irradiated with a 365 nm UV lamp.

‘click’ reaction are dispersible in chloroform (due to the non-polar nature of pyrene capping), that further corroborates the change in surface nature on ‘click reaction’. The dispersions in chloroform have been used to estimate concentration of the surface capping agent. Figure 2 shows UV-Vis absorbance profiles of pyrene-functionalized silica nanoparticles. Curves 1–3 corresponding to SiPy1–SiPy3 (see experimental section for details) respectively, indicate the presence of pyrene moieties on the silica nanoparticle surface. The spectral features at 315, 330 and 346 nm are typical of pyrene chromophores²⁰.

This observation along with the absence of any peak corresponding to azide functionality in FTIR spectra, clearly indicates the occurrence of ‘click’ reaction between the terminal alkyne functionality of propargyl-functionalized silica nanoparticles and the azide functionality of PA. Varying spectral intensities reflect a change in the surface density of pyrene chromophores on the nanoparticle surface. The amount of pyrene chromophore in 1 mg of the silica composite was estimated to be 4.5×10^{-8} mol for SiPy1. Similarly, for SiPy2 and SiPy3, the amount of pyrene in 1 mg of silica composite was determined to be 1.2×10^{-8} and 3×10^{-9} mol respectively. The number of pyrene chromophores on the surface of a single particle was calculated based on these estimates (Table 1 and Appendix 1). Typically a single silica nanoparticle in case of SiPy1 is functionalized to bear 8000 pyrene units (surface density 6×10^{-1} molecules/nm²) compared to 500 moieties on SiPy3 surface (surface density 4×10^{-2} molecules/nm²). Thus the ‘click’-based protocol can be used to vary surface concentration of the capping agent by orders of magnitude, with initial concentration of the azide playing a decisive role.

Additional evidence for the functionalization and the ease with which the surface functionalization density can be tuned was obtained from fluorescent measurements. Figure 3 shows the emission profiles of pyrene functionalized silica SiPy1–SiPy3 (curves 1–3 respectively) when excited at 350 nm. Examination of curve 1 (corresponding to emission from SiPy1) reveals peaks at 378 and 397 nm, and a hump at 389 nm. These emissions of the pyrene fluorophore^{20–22} provide proof for successful functionalization of the nanoparticle surface by way of the ‘click’ reaction. Moreover, a broad band centred at 480 nm characteristic of the pyrene excimer emission (curve 1, Figure 3) was observed²¹. Inset in Figure 3 shows the green emission of SiPy1 (corresponding to the excimer emission) dispersed in CHCl₃ when excited with a 365 nm UV lamp. Excimer emission is observed when the concentration of pyrene in a given analyte is either high enough or the pyrene moieties are in close proximity in constrained environments, allowing the excited-state interaction of pyrene units^{21,22}. Observation of pyrene excimer emission in the present case is attributed to the high density of the pyrene fluorophore on the nanoparticle surface. The fall in relative intensity of the excimer emission in comparison to monomer emission in case of SiPy2 and SiPy3 (curves 2 and 3 respectively, Figure 3) is attributed to the change in the surface density of pyrene fluorophores. Accord-

ingly, for SiPy3 having a surface density of 4×10^{-2} molecules/nm², an excimer emission of very low intensity was observed. Whereas for SiPy1, having a higher density of 6×10^{-1} molecules/nm², an intense excimer emission was observed. With decreasing surface density the pyrene units are distributed far apart on the surface of the silica nanoparticles, preventing them from interacting in the excited state. This leads to a low-intensity excimer emission. This particular observation has been previously made in the case of pyrene-functionalized gold nanoparticles by Thomas and co-workers²². Consequently, it is seen that in sample SiPy3, having a low concentration of pyrene capping, the intensity of the excimer is not significant as in SiPy2 and SiPy1. Formation of excimer in SiPy1 due to aggregation of nanoparticles can be ruled out as all samples have been diluted to the same extent and no aggregates could be observed either in solution or in the TEM images. The TEM images also indicate no change in the size and shape of the nanoparticles during the process of the functionalization (Figure 4 a–c).

Conclusion

We have demonstrated that ‘click’ chemistry can be used as a versatile tool for functionalization of silica nanoparticles. By this procedure the surface density of the pyrene capping agent can be tuned by varying the initial concentration of reactants. UV–Vis absorbance and fluorescence spectrophotometry were used to monitor the change in surface density of the capping agent and determine the number of pyrene units that are bound to the surface of a single nanoparticle. FTIR analysis indicates that the ‘click’ product does not contain any adsorbed surface capping agent. It is important to note that the particle

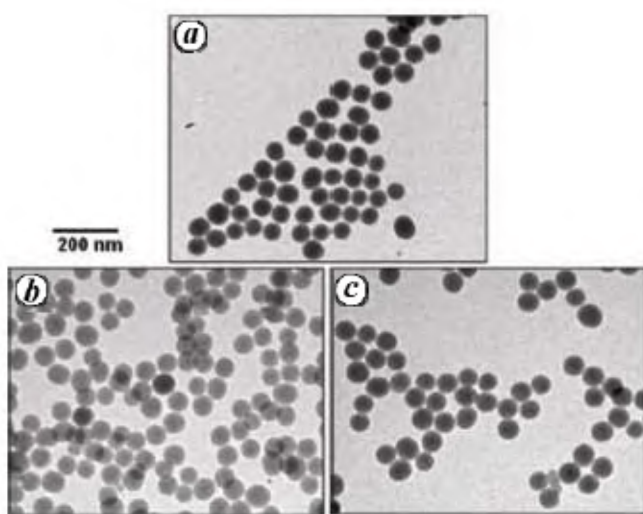


Figure 4. Transmission electron micrographs of functionalized silica nanoparticles: (a) Stober silica, (b) propargyl-capped silica and (c) pyrene-capped silica nanoparticles (SiPy1). Scale bar is common to all images.

size, shape and dispersibility are not affected during the course of this functionalization protocol. The procedure presented here is general and could be extended to impart desired functionality onto the nanoparticle surface by choosing appropriate coupling molecules having the azide functional group. Similar results can be obtained by starting with azide-bearing silica and alkyne functionalized capping molecule.

Appendix 1: Determination of pyrene density on functionalized silica nanoparticles

The pyrene chromophores were assumed to be uniformly distributed among all the silica nanoparticles and the particles themselves were assumed to be uniform spheres in the following calculations.

Density of Stober silica nanoparticles = 1.98×10^6 g/m³.

Volume of a Stober silica nanoparticle (diameter 65 nm) = 1.44×10^{-22} m³.

Weight of a Stober silica nanoparticle (diameter 65 nm) = 2.85×10^{-16} g.

Surface area of a Stober silica nanoparticle (diameter 65 nm) = 1.33×10^{-14} m².

Since the samples SiPy1 and SiPy3 contain organically functionalized silica, TGA analysis (not shown here) was used to determine the weight content of Stober silica in the respective samples. SiPy1 and SiPy3 respectively, recorded 4.5 and 1.5% greater weight loss at 800°C in comparison to Stober silica. These were incorporated in further calculations summarized in Table 1.

1. Guo, Z., Lei, A., Liang, X. and Xu, Q., Click chemistry: A new facile and efficient strategy for preparation of functionalized HPLC packings. *Chem. Commun.*, 2006, 4512–4514.
2. Campo, A. D., Sen, T., Lellouche, J. P. and Bruce, I. J., Multifunctional magnetite and silica–magnetite nanoparticles: Synthesis, surface activation and applications in life sciences. *J. Magn. & Mater.*, 2005, **293**, 33–40; Bruce, I. J. and Sen, T., Surface modification of magnetic nanoparticles with alkoxy silanes and their application in magnetic bioseparations. *Langmuir*, 2005, **21**, 7029–7035.
3. Burns, A., Ow, H. and Weisner, U., Fluorescent core–shell silica nanoparticles: Towards ‘Lab on a Particle’ architectures for nanobiotechnology. *Chem. Soc. Rev.*, 2006, **35**, 1028–1042.
4. Kim, J. *et al.*, Generalized fabrication of multifunctional nanoparticle assemblies on silica spheres. *Angew. Chem., Int. Ed. Engl.*, 2006, **45**, 4789–4793.
5. Johnston, A. P. R., Read, E. S. and Caruso, F., Ultrathin, responsive polymer click capsules. *Nano Lett.*, 2005, **5**, 953–956.
6. Roth, I., Simon, F., Bellmann, C., Seifert, A. and Spange, S., Fabrication of silica particles functionalized with chromophores and amino groups using synergism of poly(vinylamine) adsorption and nucleophilic aromatic substitution with fluoroaromatics. *Chem. Mater.*, 2006, **18**, 4730–4739.
7. Philipse, A. P. and Vrij, A., Preparation and properties of nonaqueous model dispersions of chemically modified, charged silica spheres. *J. Colloid Interface Sci.*, 1989, **128**, 121–136; Van Blaaderen, A. and Vrij, A., Synthesis and characterization of

- monodisperse colloidal organo-silica spheres. *J. Colloid Interface Sci.*, 1993, **156**, 1–18.
8. Rostovtsev, V. V., Green, L. G., Fokin, V. V. and Sharpless, K. B., A stepwise Huisgen cycloaddition process: Copper(I) catalyzed regioselective 'Ligation' of azides and terminal alkynes. *Angew. Chem., Int. Ed. Engl.*, 2002, **41**, 2596–2599.
 9. Zhang, L. *et al.*, Ruthenium catalyzed cycloaddition of alkynes and organic azides. *J. Am. Chem. Soc.*, 2005, **127**, 15998–15999.
 10. Binder, W. H. and Sachsenhofer, R., 'Click' chemistry in polymer and materials science. *Macromol. Rapid Commun.*, 2007, **28**, 15–54.
 11. Lewis, W. G. *et al.*, Click chemistry *in situ*: Acetylcholinesterase as a reaction vessel for the selective assembly of a femtomolar inhibitor from an array of building blocks. *Angew. Chem., Int. Ed. Engl.*, 2002, **41**, 1053–1057.
 12. Kolb, H. C., Finn, M. G. and Sharpless, K. B., Click chemistry: Diverse chemical function from a few good reactions. *Angew. Chem., Int. Ed. Engl.*, 2001, **40**, 2004–2021.
 13. Lummerstorfer, T. and Hoffmann, H., Click chemistry on surfaces: 1,3-dipolar cycloaddition reactions of azide-terminated monolayers on silica. *J. Phys. Chem. B*, 2004, **108**, 3963–3966.
 14. Ramesh, R., Bhat, R. G. and Chandrasekaran, S., Highly selective deblocking of propargyl carbonates in the presence of propargyl carbamates with tetrathiomolybdate. *J. Org. Chem.*, 2005, **70**, 837–840.
 15. Stober, W., Fink, A. and Bohn, E., Controlled growth of monodisperse silica spheres in the micron size range. *J. Colloid Interface Sci.*, 1968, **26**, 62–69.
 16. Hotha, S., Anigundi, R. I. and Natu, A., Expedient synthesis of 1,2,3-triazole-fused tetracyclic compounds by intramolecular Huisgen ('click') reactions on carbohydrate-derived azido-alkynes. *Tetrahedron Lett.*, 2005, **46**, 4585–4588.
 17. Yilgor, E., Burgaz, E., Yurtsever, E. and Yilgor, I., Comparison of hydrogen bonding in polydimethylsiloxane and polyether based urethane and urea copolymers. *Polymer*, 2000, **41**, 849–857.
 18. Hotha, S. and Kashyap, S., 'Click chemistry' inspired synthesis of pseudo-oligosaccharides and amino acid glycoconjugates. *J. Org. Chem.*, 2006, **71**, 364–367.
 19. Prado, A. G. S. and Airoldi, C., The pesticide 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) immobilized on silica gel surface. *J. Colloid Interface Sci.*, 2001, **236**, 161–165.
 20. Ipe, B. I., Thomas, K. G., Barazzouk, S., Hotchandani, S. and Kamat, P. V., Photoinduced charge separation in a fluorophore-gold nanoassembly. *J. Phys. Chem. B*, 2002, **106**, 18–21.
 21. Milosavljević, B. H. and Thomas, J. K., Solvent effects on the photophysical properties of pyrene-3-carboxylic acid. *J. Phys. Chem.*, 1988, **92**, 2997–3001; Thomas, J. K., Physical aspects of radiation-induced processes on SiO₂, γ -Al₂O₃, zeolites, and clays. *Chem. Rev.*, 2005, **105**, 1683–1734; Yamazaki, T., Tamai, N. and Yamazaki, I., Molecular association of pyrene in the Langmuir-Blodgett monolayer film: Analysis of picosecond time-resolved fluorescence spectra. *Chem. Phys. Lett.*, 1986, **124**, 326–330.
 22. Ipe, B. I. and Thomas, K. G., Investigations on nanoparticle-chromophore and interchromophore interactions in pyrene capped gold nanoparticles. *J. Phys. Chem. B*, 2004, **108**, 13265–13272.

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