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Novel fluorescent silica nanoparticle probe for Förster Resonance Energy Transfer

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ABSTRACT

The preparation and utilization of a novel particulate label based on fluorescent hybrid silica nanoparticles are reported in this article. These fluorescent nanoparticles have shown several unique advantages over existing dye molecules, quantum dots, and latex-based fluorescent particles in easy preparation, good photostability and high sensitivity. Alexa Fluor® dyes, the leading and most trusted fluorescent dyes available today were used to make the grafting onto silica nanoparticles. Alexa Fluor® was modified with 3-aminopropyl-triethoxysilane (APS) to provide reactive groups Alexa-APS. Alexa-APS can be grafted onto silica nanoparticles *via* silylation process. The silica nanoparticles were prepared by Stöber methods and treated by Etching to functionalized their surface. A mixture of Alexafluoro donor A488 and Alexafluoro acceptor A568 coated silica nanoparticles leads to the Förster Resonance Energy Transfer (FRET) by excitation of the acceptor when the particles are distant by less than 10nm.

Keywords:*Synthesis of silica nanoparticles; Fluorescent hybrid silica nanoparticles; functionalization; Fluorescence*

1. INTRODUCTION

Colloidal particles and their modification have a long history of importance in a broad range of applications in technology and materials processing. They can be made from many different materials and suspended in a wide variety of solvents. The rheological properties of colloidal suspensions have traditionally been of primary concern in their technological applications, and our understanding of these properties continues to evolve. However, new uses of colloidal particles are also emerging. Because they can be produced to a precise size, colloidal particles are now also being used in novel ways as building blocks for engineering completely new materials, including high-precision filters, controlled-porosity substrates, and photonic devices. In addition, new methods are evolving to alter the shape of the particles and create controlled structures with non-spherical particles. New experimental techniques are allowing improved measurement and increased understanding of the structure, properties, and behavior of colloidal suspensions. The possibility to control the size of spheres, linked with the modification of the surface, makes the silica particles like a product of departure for the preparation of colloidal model systems that have various applications, for instance in studies of the division of light, or sedimentation [1,2,3,4,5]. Silica is a model system, very practical and very controlled, the structure and properties of silicon materials depend on the

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method of preparation of these materials. In this work, the spheres of monodisperse colloidal silica are prepared by controlled hydrolysis of tétraéthoxysilane (TEOS) in a water / ammonia / ethanol according to the Stöber method [6]. The functionalization of silica nanoparticles has been the object of great interest and intensive investigation because the surface properties of silica materials are largely influenced by the nature of the surface functional groups. Functionalized silica is a versatile material, and ways to effectively introduce organic functional groups onto the surfaces [7] and insides [6] of the particles are now under control for many applications.

Many literature studies have been done on grafting and chemical modification of silica nanoparticles, they can be classified into two categories, grafting onto the surface and grafting into the volume. Most studies in the chemical modification has been used with grafting into the silica nanoparticles to modified the optical properties of dye-doped silica particles, the encapsulation of organic dye molecules in sieves leads to composite materials with novel optical properties, which enable potential applications as pigments or as materials for optical data storage, frequency doubling, micro-lasing, gas sensing, or photocatalaysis [8,9,10,11], the incorporation of fluorescent dyes into mesoporous molecular sieves, have been restricted to rhodamine or fluorescein derivatives [12,13,14,15], the incorporation of magnetic compounds [16,17,18,19] or organic [20,21,22,23] or inorganic dyes [24,25,26], into silica nanoparticles is well reported by condensation of the reactive species with tetraethoxysilane in a Stöber-type reaction or a reverse microemulsion system [27] for instance, the synthesis of monodisperse air-stable super -paramagnetic α-Fe nanocrystals encapsulated in nanospherical silica particles of 50nm in diameter [17], the optimization of Dye-doped silica nanoparticles prepared using a reverse microemulsion method to facilitates unique applications in bioanalysis and bioseparation [21], the use of the Stöber synthesis method to prepare silica nanoparticles in which ruthenium diimine complexes were physically encapsulated and their application in streptavidin-biotin affinity assays [25], a novel amperometric glucose biosensor based on ferrocene-doped silica (FcDs) nanoparticles as an electron transfer mediator conjugated with a biopolymer chitosan (CHIT) membrane was developed for the determination of glucose in rat brain coupled to in vivo microdialysis, [28] the grafting into the silica has done too with a luminescent probes as fluorescein isothiocyanate (FITC)-incorporated silica-coated core-shell SPIO nanoparticles,SPIO@SiO2 (FITC) has been developed with diameter of 50nm, as a bifunctionally magnetic vector that can efficiently label human mesenchymal stem cells [29].

The grafting onto the surface on the silica nanoparticles has been done in the literature for many applications, recently, the chemical modification of nanosized silica particles being used in chemical sensor synthesis, for instance, a chemical modification of surfaces by nitrogen-containing organic reagents (γ-aminopropyltriethoxysilane, imidazole, or N-vinyl-2-pyrrolidone) and also with hexamethyldisilazane and hydrocarbon oligomers was proposed to enhance the adsorptive capacity of silica with respect to monocarboxylic and bile acids [30], a silica nanoparticles surfaces have been modified with enzyme molecules (glutamate dehydrogenase (GDH) and lactate dehydrogenase (LHD)) and a biocompatible reagent for cell membrane staining, the nanoparticles surface biochemical functionalization demonstrates the feasibility of using nanoparticles for biosensing and biomarking applications [31], some modification use thiol/disulfide-coupling chemistry for the immobilization of oligonucleotides onto silica nanoparticles and subsequently demonstrated the properties of the resulting DNA nanoparticles [32] and compare several synthetic methods of grafting silica with strong and weak anion-exchanging groups starting with commercially available chloroalkyl- and γ–aminopropylsilanes [33].

In the present work, we made the grafting of Alexa fluorescein onto the surface of silica particles to study the Förster Resonance Energy Transfer (FRET) phenomenon. The silica nanoparticles were prepared by Stöber methods [6] and treated with Etching [34]. According to this method we can control the particle size distribution, especially in a range of 30-200nm [35]. AlexaFluor was modified with 3-aminopropyl-triethoxysilane (APS) to provide reactive groups Alexa-APS. APS is known for its stability in basic solutions. The amine functionality of APS allows the coupling with carboxyl group COOH of Alexa Fluor, and then the etched silica nanoparticles were added to the mixture Alexa-APS and leads finally to the formation of fluorescent silica nanoparticles. A mixture of Alexa 488 and Alexa 568 coated silica nanoparticles leads the FRET phenomenon by laser excitation of acceptor if the particles are distant by less than 10 nm. Particles were characterized by FTIR Spectrum, UV-visible Spectrometer, FX-Spectroscopy, elemental analysis and images have been made on home built confocal fluorescent microscope.

2. EXPERIMENTAL SECTION

2.1. Materials. The materials used in this study were tetraoxydiethylsiloxane TOES (Aldrich, 99%), Ammonia solution (CARLO EBRA, 30%), 3 Aminopropyltrietoxysilane APS (ACROS, 99%), methylsulfonic acid CH_3SO_3H (Fluka, 98%), AlexaFluoro 488 carboxylic acid succinimidyl ester (INVITROGENE, Molecular Probes), AlexaFluoro 568 carboxylic acid succinimidyl ester (INVITROGENE, Molecular Probes) Ethanol absolute GR for analysis (MERCK, 99%).

2.2. Syntheses of silica nanoparticles by Stöber method. Silica nanoparticles with diameter 200nm were synthesized by Stöber method. This synthesis is based on hydrolysis and condensation of tetraoxydiethylsiloxane TOES ($Si(OC_2H_5)_4$) in a basic middle. A mixture was made by 7.4ml EtOH, 8mL NH_3 and 19mL H_2O is placed with stirring a few minutes at room temperature, then a quantity of TEOS 12mL were added to the mixture, the reaction was allowed to continue under stirring for 12 hours at room temperature by using mechanical agitation with a speed of rotation 700 rpm. After the reaction was completed, the nanoparticles were isolated by centrifuging and washing with ethanol and water several times to remove any surfactant molecules or any physically adsorbed from the surface of the particles. Finally the silica nanoparticles were obtained.

2.3. Etching of the silica nanoparticles by CH_3SO_3H. 5mL of silica nanoparticles obtained with 200nm of diameter were added to 100mL of 10% CH_3SO_3H aqueous solution, the mixture was refluxed at 110°C for 48h with stirring. The etched particles were recovered by filtration, washed several times with water, and then dried at 120°C under vacuum for 24h.

2.4. Grafting of APS with Alexa 568 Fluorocarboxilic acid. 300µg of Alexa 568 were added to 100µl of APS and 5mL of ethanol EtOH, the mixture is placed with stirring for 48h for completing reaction under nitrogen atmosphere at room temperature to get finally the solution Alexa-APS.

2.5. Grafting of Alexa-APS solution onto silica nanoparticles. The etched silica nanoparticles were dispersed in 30ml EtOH, then 5mL of Alexa-APS were added, the resulting mixture was heated at 75°C and stirred for 48h. The supernatant was poured off and the modified particles were extracted by a several centrifugation in ethanol. Finally, the particles were dried at 120°C for 24h under vacuum.

2.6. Characterization. The APS and organic dyes grafting on the surface of silica nanoparticles were studing by, UV-visible Perkin Elmer Precisely Lambda 950 UV/Vis Spectrometer, FX-Spectroscopy, MD-5020-PTI, LPS-220B-PTI, 814-Photomultipler Detection System-PTI, The images have been made on home build Confocal fluorescent microscope, FTIR Spectrum, VARIAN FTS 3000, FTIR spectrum were obtained by using Perkin-Elmer series, 1600, FTIR Spectrometer with a resolution 4 cm^{-1} and 32 scans, and elemental analysis (performed by the service analysis, physical measurements and optical spectroscopy, CNRS- Strasbourg University).

3. RESULTSAND DISCUSSIONS

A several kinds of AlexaFluorophoreA488, A568andA647 were used to make their modifications with APS. They arecovalently boundtothecoupling agentbyanaddition reaction betweenthe amine function of APS and the acid group(-COOH) of Alexafluorophore forming a peptide bond. The Table 1 present the characteristics of each Alexa obtained from Molecular Probes, Inc (Leiden).

Table1: Absorption andemission ofdifferent Alexafluorophores, from MolecularProbes

Fluorophore	Absorption (nm)	Emission (nm)	Color
Alexa Fluor 488	495	519	Green
Alexa Fluor 568	578	603	Orange-Red
Alexa Fluor 647	650	668	Red-Far
R0 Förster	A488/A568 = 6,2 nm	A488/A647 = 5,6nm	A568/A647 = 8,2nm

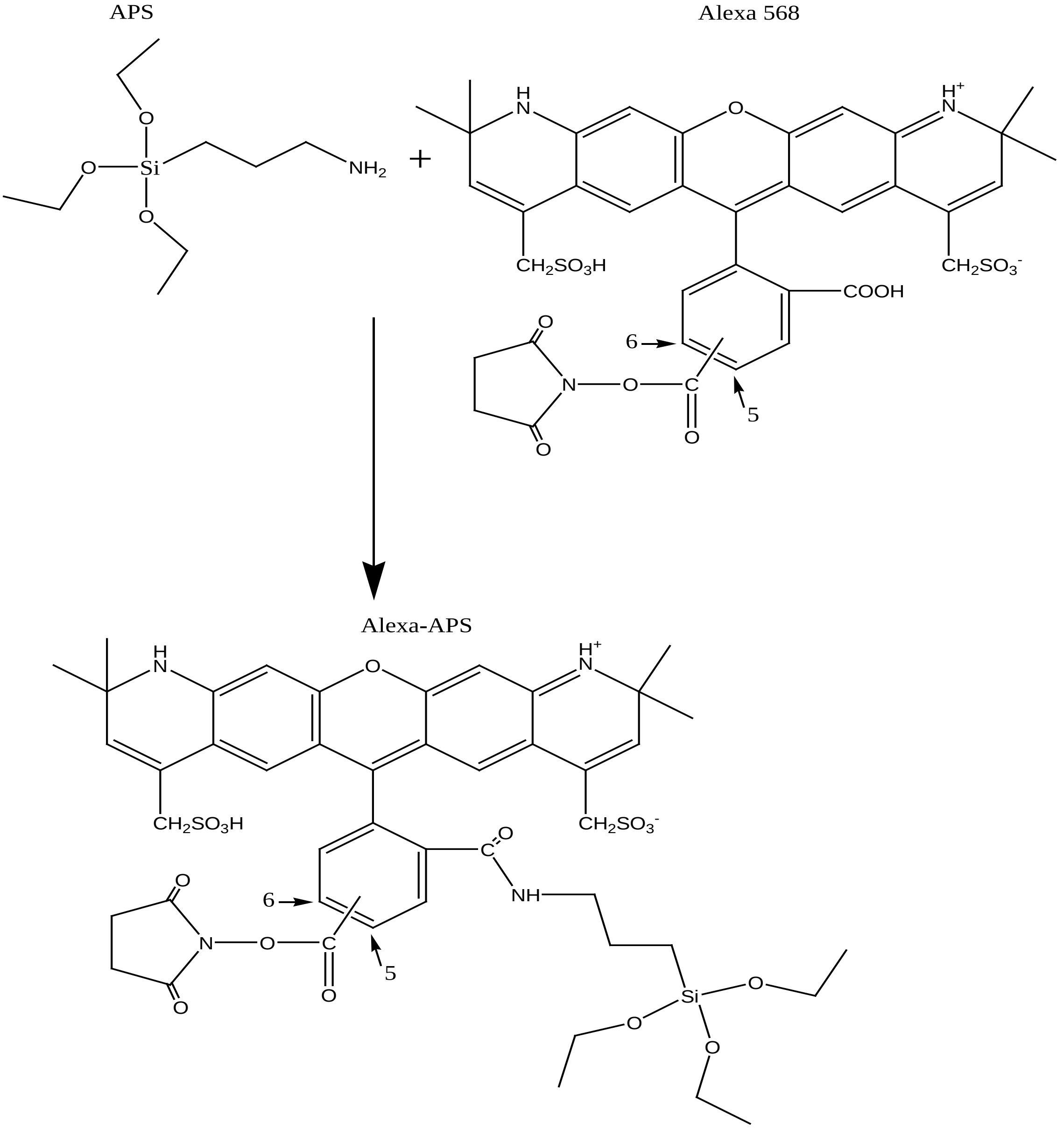

Figure 1: Schematic of chemical modification of AlexaFluoro A568 with APS

We present in this article the modification of Alexa 568 fluorescent dye with APS. The amino groups of 3-Aminopropyltrietoxysilane APS, play an important role in the formation of the amide link with Alexa fluorescent dye as shown in the Figure 1. The silanol SiOH groups on the silica nanoparticles surface which are the reactive sites for silylation, play an important role in the surface modification process. Under anhydrous conditions, three possible reactions can occur, as shown in Figure 2.

Based on elemental analysis there was 0.3035 wt % carbon, 1.214 wt % hydrogen and 0.045 wt % nitrogen associated with the starting silica nanoparticles. After grafting with Alexa-APS, the silica nanoparticles contained 1.1935 wt % carbon, 1.304 wt % hydrogen and 0.218 wt % nitrogen, thus we grafted onto the surface of silica nanoparticles 0.09wt % hydrogen (0.09mole), 0.89wt % carbon (0.074mole) and 0.173wt % nitrogen (0.012mole).Theoretically if Alexa-APS is totally grafted on silica nanoparticles, we will have 8.08 wt% of hydrogen, 7.07 wt% of nitrogen and 84.8% of carbon, the experimental results obtained after grafting were 7.8wt%of hydrogen, 77,2wt% carbon and 15wt% nitrogen. The mole ratio of carbon (C) to hydrogen (H) after grafting is 0.82, which is similar to the C/H mole ratio in equation (1).

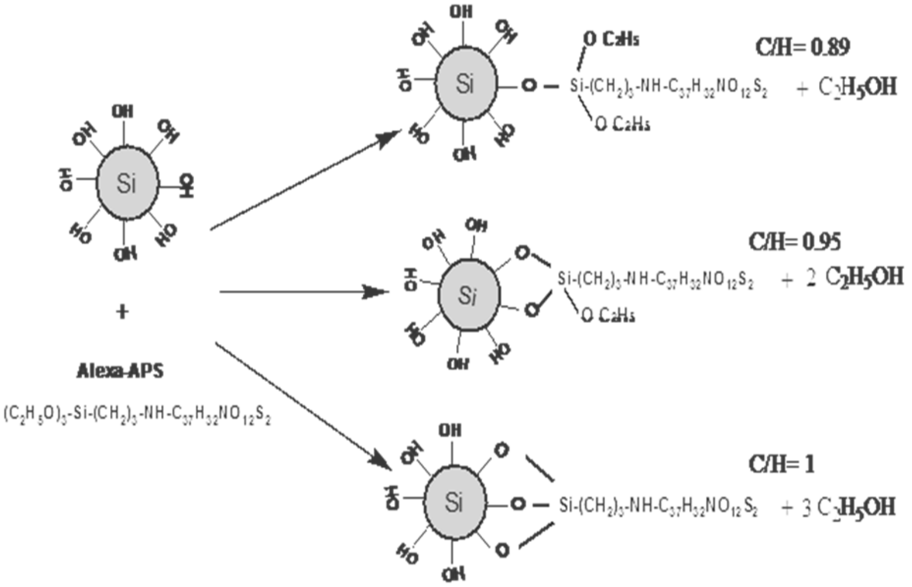

Figure2: Schematic of grafting of Alexa-APS onto the surface of silica functionalized. The molar ratio of carbon to hydrogen(C/H) is given by each reaction

The grafting of Alexa-APS onto silica nanoparticles was investigated by FT-IR spectra of pure SiO_2 nanoparticles (Figure3a), APS-Alexa modified silica nanoparticles (Figure 3b) and APS (Figure 3c). The assignments for the main FT-IR absorption bands are listed in Table2.

Table2: Assignments of IR absorption bands

Frequency (cm^{-1})	Assignment
~ 3450	O-H stretching of Si-OH group and water
~ 2850-2980	C-H stretching vibration
~ 1720	Amide group -CONH-
~ 1650	CO (amide) stretching vibration
~ 1545	N-H/C-N (amide)
~ 1464, ~ 1380	CH_3 bending vibration
~ 1097, ~ 940, ~ 805	Si-O-Si stretching vibration
~ 720	NH^+ rocking

In the FT-IR spectrum of the Alexa-APS modified silica nanoparticles, apart from the characteristic vibration bands for Si-O, the CH_2 absorption band at 2950 cm^{-1} (asymmetric stretching) and 2860 cm^{-1} (symmetric stretching) can be clearly observed. These bands are also present in FT-IR spectrum of APS. The formation of amide group bands is also present in the spectrum as shown in the Figure 3.

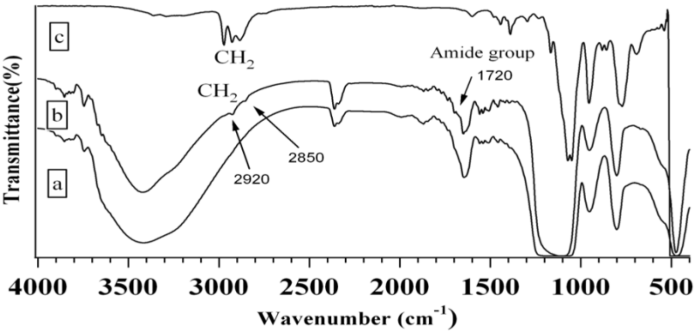

Figure 3: FT-IR spectra of (a) silica nanoparticles, (b) APS-Alexa grafted onto silica nanoparticles and (c) APS

The grafting of Alexa-APS onto silica nanoparticles was investigated too by absorption and fluorescence emission spectra as shown in the Figure 4. Figure 4A shows the absorption and fluorescence emission spectra of Alexa Fluor 568 dispersed in ethanol, Figure 4B the absorption spectra of Alexa 568 grafted silica nanoparticles by UV-visible spectrometer in ethanol and the emission spectra of Alexa 568 grafted silica nanoparticles dispersed in ethanol by FX spectroscopy.
As indicated by the FT-IR, UV absorption, FX emission spectra and elemental analysis, the grafting of modified silica with APS-Alexa onto the surface of silica nanoparticles was successfully realised.
After the grafting, we focused on the observation of Förster Resonance Energy Transfer (FRET) between fluorescent silica particles in order to study their contacts. The achievement of this non-radiative energy transfer between fluorescent particles, makes the subject of another publication who focuses on the study of contacts between fluorescent colloidal particles. This paper presents the technique we used to show this contact. The image has been made on home build confocal fluorescent microscopy. Our goal is focused here on the observation of non-radiative energy transfer in a concentrated colloidal suspension consisting of fluorescent silica particles from 1 to 10µm. We chose here to image large ball with diameter 3µm to have enough power to resolve two neighboring contact points in our confocal fluorescent microscopy.

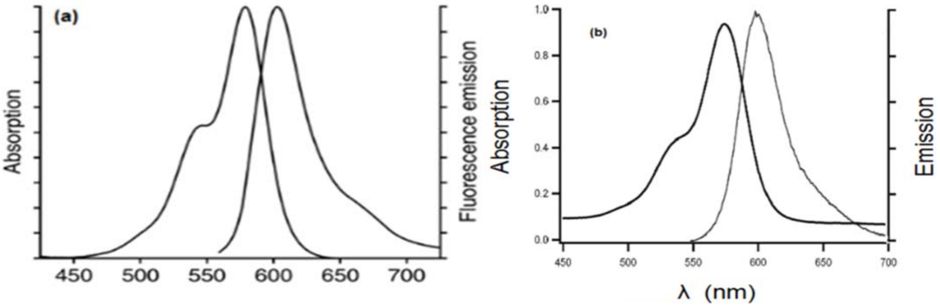

Figure 4: (a) Absorption and Emission of AlexaFluoro A568 in ethanol from *Molecular probes* and **(b)** Absorption and Emission of AlexaFluoro A568 modified with APS and coated silica particles

The pinholes of excitation and collection are formed by the openings of two optical fibbers of 50 microns of heart. The sample is placed on a piezoelectric plate, and is illuminated with a laser wavelength λ=400 nm. A target illumination and collection is used, the fate of our optical magnification is 100 times. Fluoresced light then passes through a notch filter that illuminates the intensity at the emission wavelength. It is then separated by a spectrometer. Two pictures are taken, the first to the wavelength of emission of the donor fluorophore, and the second to the Wavelength of the acceptor fluorophore. For a typical acquisition image, the size of a pixel is 250x250x250 nm and that of a picture is 40x40 µm in a plane perpendicular to the optical axis and 20µm along the optical axis. Thus, the number of pixels per plane perpendicular to the optical axis is 25600. The time required for the measurement of the intensity emitted by the acceptor fluorophore is 0.015s per pixel. The sample is prepared by allowing the particles settle. We observe a layer of 20µm thick on the underside of the sample.

The Figure 5 shows a mixture of Alexa 488 and Alexa 568 coated silica particles with diameter 3µm and leads to the FRET phenomena by excitation of acceptor when the particles are distant by less than 10nm.

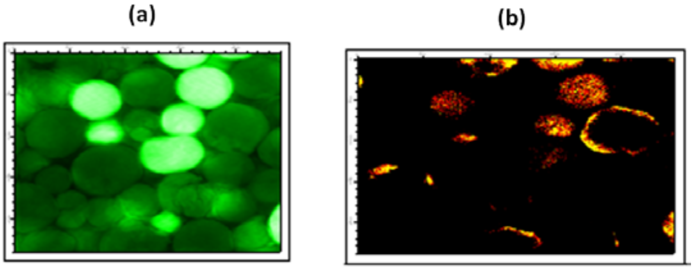

Figure 5: Image of silica nanoparticles grafted by Alexa 488 and Alexa 568, observed by Confocal microscopy: (a) the mixture of Alexa coating silica particles before excitation and (b) after excitation

The particles which are more clear and illuminated corresponding to donor particles, and those that are more darker corresponding to acceptor particles Figure5 (a). An AlexaFluoro donor A488 grafted onto silica particles, initially in its electronic excited state, transfer energy to AlexaFluoro acceptor A568 (in proximity, typically less than 10 nm) through nonradiative dipole–dipole couplingFigure5 (b). This mechanism is termed "Förster resonance energy transfer". FRET is analogous to near field communication, in that the radius of interaction is much smaller than the wavelength of light emitted. In the near field region, the excited fluorophore Alexa 488 emits a virtual photon that is instantly absorbed by a receiving fluorophore Alexa 568. These virtual photons are undetectable, since their existence violates the conservation of energy and momentum, and hence FRET is known as a *radiationless* mechanism.

4. CONCLUSIONS

The following conclusions can be drawn from the present study. Alexa fluorophore modified with APS can be grafted on silica particles via silylation process. The presence of Et_3N group and the functionalization of the surface of silica particles with etching phenomena enhances the grafting efficiency. A mixture of Alexa 488 and Alexa 568 coated silica particles leads to the Förster Resonance Energy Transfer (FRET) when the particles are distant by less than 10 nm. The choice of fluorophores was very delicate, and specially made for the non-radiative energy transfer. Several conditions are necessary for this kind of energy transfer. The calculations that we did to achieve this FRET and the experimental conditions required will be detailed in another publication that focuses on the study of contacts between fluorescent colloidal particles.

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