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# Luminescent Chemosensors Based on Silica Nanoparticles

Sara Bonacchi, Damiano Genovese, Riccardo Juris, Marco Montalti,  
Luca Prodi, Enrico Rampazzo, Massimo Sgarzi, and Nelsi Zaccheroni

**Abstract** The field of nanoparticles is amazingly many-sided and consequently their applications range between many different areas from industry to bio-analysis and catalysis. In particular, luminescent nanoparticles attract close attention in the areas of biology, medical diagnosis and therapy, where they already find many applications. In this so fascinating and wide framework we have focussed our attention on luminescent silica nanoparticles able to act as sensing materials. We highlight here the importance, especially with the aim of sensing, of gaining precise knowledge and control of their structures; the performance of a chemosensor is, in fact, totally dependent on its design. We then briefly present the state of the art and the progress both in the synthetic protocols and in the application of luminescent silica nanoparticles as chemosensors. We present many recent examples, organized into two main sections, the first dealing with systems presenting the signalling units on the surface (dye coated silica nanoparticles, DCSNs) and the second with systems entrapping the dyes inside the silica matrix (dye doped silica nanoparticles, DDSNs).

**Keywords** Chemical sensors, Fluorescence, Luminescence, Signal amplification, Silica nanoparticles

## Contents

1	Introduction .....	95
1.1	Nanoparticles in Bioimaging and Sensing .....	97
2	Synthesis of Fluorescent Silica Nanoparticles .....	102
2.1	Stöber Method .....	102
2.2	Reverse Microemulsion Method .....	105
2.3	Direct Micelles as Template .....	106
3	Luminescent Silica Nanoparticles as Chemosensors .....	109

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3.1 Introduction .....	109
4 Some General Remarks .....	130
References .....	131

## Abbreviations

AOT	Bis(2-ethylhexyl) sulfosuccinate sodium salt
APTES	3-Aminopropyltriethoxysilane
AuNPs	Gold colloids
BODIPY	Dye class containing boron-dipyrromethene (4,4-difluoro-4-bora-3a,4a-diaza- <i>s</i> -indacene) as core
CHEF	Chelation-enhanced fluorescence
CP	Conjugated polymers
DCSN	Dye coated silica nanoparticle
DDSN	Dye doped silica nanoparticle
DEDMS	Diethoxydimethylsilane
DETA	<i>N'</i> -[3-(Trimethoxysilyl)-propyl]diethylenetriamine
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPA	Dipicolinic acid
EDTA	Ethylenediaminetetraacetic acid
EDTAD	Ethylenediaminetetraacetic dianhydride
FITC	Fluoresceine isothiocyanate
FICFF	Flow field flow fractionation
FRET	Fluorescence resonance energy transfer
ICT	Internal charge transfer
MPS	Mercaptopropyltriethoxysilane
MRI	Magnetic resonance imaging
NBD	Nitrobenzoxadiazole
NIR	Near infrared spectral region
NP	Nanoparticle
ORMOSIL	Organic-modified silica
PBS	Phosphate buffered saline
PDT	Photodynamic therapy
PEG	Polyethylene glycol
PET	Photoinduced electron transfer
PPS	Diethynylbenzene and diiodo-dipropyloxy-sulphonate benzene units
PVP	Polyvinylpyrrolidone
QDs	Quantum dots
RES	Reticulo-endothelial system
RNA	Ribonucleic acid
SNP	Silica nanoparticle

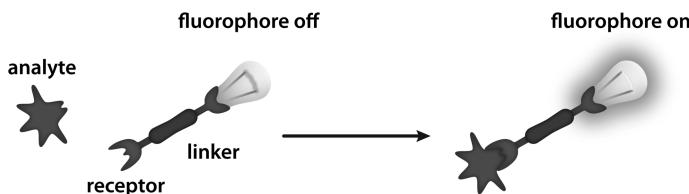
SPB	Surface plasmon band
TEM	Transmission electron microscopy
TEOS	Tetraethoxysilane tetraethyl orthosilicate
TMSCl	Trimethylsilylchloride
TNT	Trinitrotoluene
TSQ	6-Methoxy-8- <i>p</i> -toluensulfonamide
VTES	Triethoxyvinylsilane
$\varepsilon$	Molar extinction coefficient
$\Phi$	Fluorescence quantum yield

## 1 Introduction

Photochemistry, which deals with the fundamental interactions of light with matter, is attracting the interest of researchers from many different fields, both in fundamental and applied studies, spanning from investigations on processes involved in the origin of life on earth to design and engineering of new solutions useful for everyday life such as energy production, medical diagnosis and therapeutics, data storage, material and environmental sciences [1]. It is therefore not surprising that photochemistry is still undergoing tremendous development. In particular, interest is shifting from purely molecular systems to supramolecular architectures [1] and, more recently, nanostructures [2–5], where intermolecular interactions can result in novel photochemical and photophysical properties. Such architectures are in fact an ideal platform to couple elementary processes (light absorption and emission, energy and electron transfer) to give rise to more complex ones (directional excitation energy migration or multi electron photo injection) in order to design nanosized functional photochemical devices [1].

An extensive description of the theoretical bases of all the principles and processes typical of photochemistry is beyond the scope of this introduction, but can be found in the literature [6]. However, it is worth noting that all fluorescence parameters such as Stokes shift, fluorescence intensity and anisotropy, emission and excitation spectra, and fluorescence lifetime can be used to encode what is happening in the close neighbourhood of the monitored species. The versatility of fluorescence-based methods of analysis derives indeed from the wide number of variables that can be tuned and coupled to get the required information, allowing one to overcome even very complex analytical problems.

Fluorescent chemosensors in particular [7, 8] have already found wide applications in many fields, such as environmental monitoring, process control, food and beverage analysis, and also represent one of the most rapidly developing fields in biology and medicine [9]. The design of fluorescent chemosensors has been continuously evolving in the last decades and numerous are the reviews on this topic [10] and see, for example, [11, 12]. The classical design of a fluorescent probe includes two fundamental moieties, a receptor responsible for the molecular recognition of the analyte and a fluorophore responsible to signal the recognition event. There are three main strategies to approach the design of fluorescent molecular indicators for

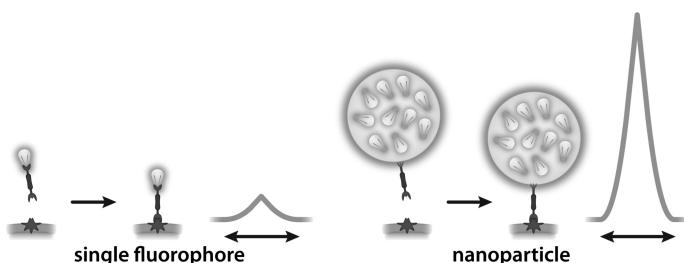


**Fig. 1** Schematic representation of a chemosensor

chemical sensing in solution. The first is based on intrinsic fluorescent probes [7], which are fluorescent molecules where the signal transduction mechanism involves the interaction of the analyte with a ligand site that is part of the  $\pi$ -system of the dye. The second involves extrinsic fluorescent probes, in which the receptor moiety and the fluorophore are covalently linked but are electronically independent [13–15] (Fig. 1). The third strategy is called chemosensing ensemble, based on a competitive assay in which a receptor-fluorophore ensemble is selectively dissociated by the addition of an appropriate competitive analyte for the ligand, resulting in a detectable response of the fluorophore [16–18].

Very recently, the use of more complex architectures has allowed one to overcome many of the typical limitations of conventional fluorophores (organic dyes) such as poor photostability, low quantum yield, unsuitability for physiological conditions and therefore for *in vitro* and *in vivo* applications. These systems include, for example, conjugated polymers (CP), QDs, gold nanoparticles and silica nanoparticles doped with luminescent species [19–21].

The performances of such photophysical multi-component devices are essentially dependent on the possibility of controlling the mutual interactions between the molecular components in the assembly, and hence their spatial organization. This condition is fundamental to optimize the efficiency by minimizing the occurrence of undesired processes leading to energy loss and photo-degradation. A rigorous traditional synthetic approach surely allows very good control of the geometry, yielding fascinating, outstandingly efficient devices. However, in many cases, the performance is also related to the number of molecular units and the covalent interconnection of hundreds or thousands of molecules would require a tremendous effort. Self-assembling and self-organization [10] provide a lower degree of control of the electronic interactions between active moieties compared to traditional synthesis. Nonetheless, a much longer-range spatial control and organization is achievable with these methods, allowing a much higher level of complexity to be reached. However, such methods provide non-covalent systems, which are not always appropriate for applications in complex matrices such as cells and tissues, since they do not guarantee the necessary robustness to ensure long-run lifetimes. In these cases a bottom-up approach, which allows the design of nanometric systems based on molecular units held together by covalent bonds, is one of the possible solutions.



**Fig. 2** Comparison between the signals arising from a molecular chemosensor and a nanoparticle-based one

Nanotechnology is an emerging science that exploits the characteristic and unique properties of these materials at the nanoscale level. An inventory of the nanotechnology-based consumer products currently on the market can be found in some specialized websites and, though not comprehensive, it includes more than 1,000 items, which are the fruit of more than 20 years of basic and applied research. In this context nanoparticles represent one of the main subjects of interest [22]. These systems have, in fact, rapidly found many industrial uses in a wide range of fields such as electronic, optoelectronic, biomedical, pharmaceutical, cosmetic, catalytic and materials areas. Among all these applications, one of the cut-edge research topics in the field of photoactive nanoparticles is the development of innovative nanosystems for biological imaging, medical diagnostics and therapeutics (Fig. 2).

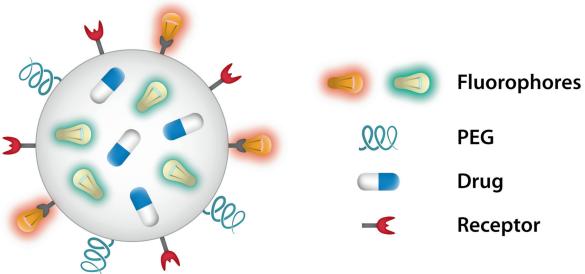
In this chapter we will focus our attention on properly modified silica nanoparticles that are able to conjugate unique photophysical and photochemical properties to simple and low-cost preparation. Moreover, the versatility of the chemistry of silica offers the possibility to obtain easily sophisticated, but robust, multifunctional systems [23, 24].

In the following sections different families of nanoparticles will be compared to emphasize the unique features of doped luminescent silica nanostructures, and to underline the challenges that these systems are expected to face, mainly in the field of sensing.

## 1.1 *Nanoparticles in Bioimaging and Sensing*

Luminescent organic or metallorganic species, polymers and proteins are the most commonly used moieties for imaging and sensing. It has to be noted, however, that in these applications dye molecules are exposed to a variety of harsh environments and often suffer from photobleaching and quenching due to interactions with solvent molecules and reactive species, such as oxygen or ions, dissolved in solution. Furthermore,  $\pi-\pi$  stacking can occur at high local concentrations, e.g. when the dyes are deposited on surfaces or interfaces, leading to energy transfer and

**Fig. 3** Multifunctional nanoparticle: receptor units recognize the proper target; polyethylene glycol (PEG) chains improve the particle stability; fluorophores provide a means for detection; drug can be hosted inside



self-quenching. All this has greatly limited the use of fluorophores for in vitro assays and in vivo cellular imaging and sensing, and much effort is devoted to improve their stability and sensitivity.

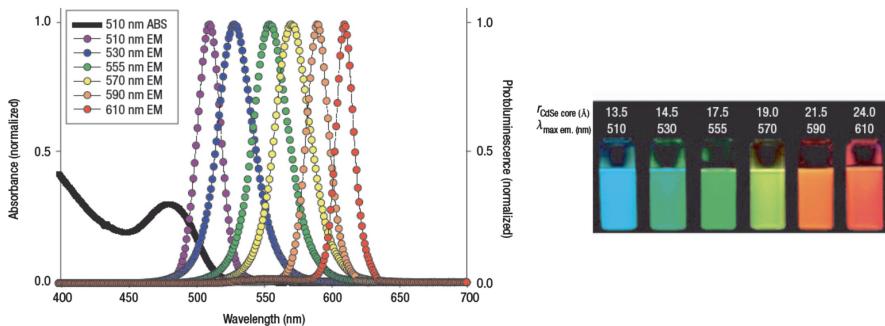
Nanoparticles often provide a protected environment where active species are not affected by external and unforeseeable triggers (such as the case of silica, titania, latex nanoparticles) [25], or are intrinsically less sensitive to the environment than molecular dyes (QDs, metal nanoparticles) [26, 27]. Moreover, unique features arise from their particular size-dependent opto-electronic properties, their size – similar to biomolecules such as proteins and polynucleic acids – and their high surface-to-volume ratio. With proper engineering and surface modification, nanoparticle probes can also be obtained featuring enhanced fluorescence signals, increased sensitivities, a prolonged detection time and a better reproducibility. Figure 3 schematizes the versatility of these systems, summarizing a few important desirable properties of multifunctional nanoparticles [28].

There are several classes of nanoparticles such as organic, inorganic and metallic, all of them currently employed as fluorescent emitters, present pros and cons, and are more or less suitable for each particular application. Hereafter we present a brief overview of each of these classes to introduce this fascinating field, while in Sect. 3 we will discuss in much more in detail silica nanoparticles that are the focus of this contribution.

### 1.1.1 Quantum Dots

Quantum dots (QDs) were developed in the early 1970s. These atomic clusters are luminescent nanometer-scale (1.5–12 nm) heterostructures, containing from a few hundred to a few thousand atoms of a semiconductor material (CdSe, CdS or InP and InAs). They can be coated with an additional semiconductor shell (e.g. zinc sulphide) to improve their optical properties such as their brightness, and the photostability of the material since, in the core–shell QDs, photobleaching effects are strongly reduced [29].

The optical properties of QDs are very characteristic [30]. The energy of their excited states depends not only on the constituent material but also upon the particle



**Fig. 4** Right: absorption and emission of six different QD dispersions. Left: photo demonstrating the size-tunable fluorescence properties and spectral range of the dispersions plotted on the right vs CdSe core size (excited with a 365 nm UV lamp). Adapted with permission from [29]

size (Fig. 4) and this *tunability* has been widely exploited for designing multicolour assays, thanks also to the narrow emission spectra of homogeneously sized QDs solutions. They also present a broad absorption spectrum, large molar extinction coefficients ( $\epsilon$ ), high fluorescence quantum yields ( $\Phi$ ), very long emission lifetimes (up to the range of  $\mu\text{s}$ ) and a much higher photostability than traditional fluorescent molecules.

QDs do not disperse well in water, but by modifying their coating it is possible to make them water soluble, facilitating their conjugation to biomolecules and making them useful for biological imaging [31]. Several different methods are used to make them biocompatible and to introduce binding specificity [32, 33]. Many reports have appeared regarding applications of QDs as *in vitro* or *in vivo* diagnostic tools. However, they present many drawbacks, the most significant being cytotoxicity: the toxicity of elements such as cadmium, which is present in many of these nanocrystals, is well known, and thus it is critical to know whether these cytotoxic substances can leak out of the QD particles over time, upon illumination or oxidation. It has to be said that this is still a controversial point since there are reports in the literature that provide evidence of cytotoxicity of QDs [34], and others in which no cytotoxicity is observed [35]. Recently much progress has been reported toward overcoming their limitations [36–39] and several appealing examples of QDs bio-sensors based on FRET [40] have appeared.

### 1.1.2 Gold Nanoparticles

In the twentieth century, various methods for the preparation of gold colloids (AuNPs) were reported and reviewed [41]; among them, the Brust–Schiffrin method for AuNPs synthesis [42], published in 1994, has had considerable impact on the field since it allowed, for the first time, the facile synthesis of thermally stable and air-stable AuNPs of reduced dispersity and controlled size.

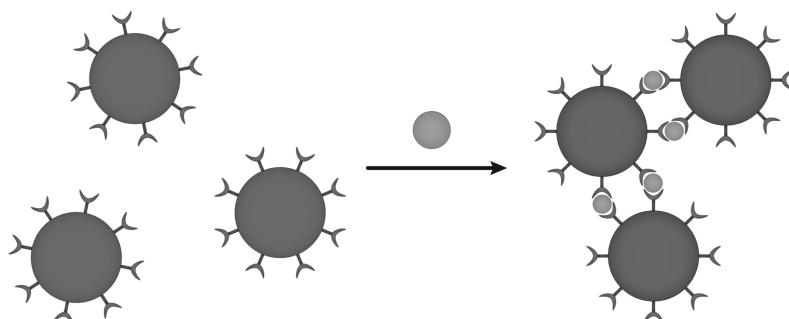
As far as physical properties are concerned, the deep red colour of AuNPs solutions or glasses is due to the so-called surface plasmon band (SPB), a broad absorption band in the visible region. The SPB arises from the collective oscillations of the mobile electrons at the surface of the nanoparticles induced by the electromagnetic field of the incoming light, and has been theoretically described by the Mie theory [43] and studied by many authors. This band is negligible in AuNPs with a diameter of less than 2 nm, as well as bulk gold, while for AuNPs of a diameter below 100 nm, the SPB maximum  $\lambda_{\max}$  was observed in aqueous media in the 510–580 nm range. The SPB maximum and bandwidth are indeed influenced by a number of factors, such as particle size, shape, solvent, dielectric properties and temperature, and also by aggregate morphology, surface functionalization and the refractive index of the surrounding medium (for a more detailed account on the photophysics of AuNPs see Daniel and Astruc [44]) [45, 46].

In the field of sensors and labels, interesting applications can be found based on SPB shift [47].

As schematized in Fig. 5, the binding of a target analyte can induce NPs aggregation. Since the plasmon-resonance spectrum of free single particles differs significantly from that of aggregated ones, it is possible to obtain quantitative measurements of the aggregating species present in solution by measuring the SPB shift caused by the recognition event [48, 49].

As far as luminescence is concerned, gold nanoparticles [50] generally quench the emission of fluorophores adsorbed or bound at their surface but it has to be said that both radiative and nonradiative rates critically depend on the size and shape of the AuNPs, the distance between the dye molecules on the surface, the orientation of the dipole with respect to the dye-nanoparticle axis and the overlap of the molecule's emission with the nanoparticles absorption spectrum [51]. It has also been observed that very small gold nanoparticles (with a diameter smaller than 1.5–2 nm) can present an intrinsic luminescence that is centred in the NIR spectral region [52, 53].

Finally, colloidal gold offers some unique advantages over other labelling agents, e.g. QDs or organic dyes. For instance, it does not undergo any photodecomposition,



**Fig. 5** Aggregation of gold nanoparticles induced by coordination leads to a red-shift of the surface plasmon resonance band

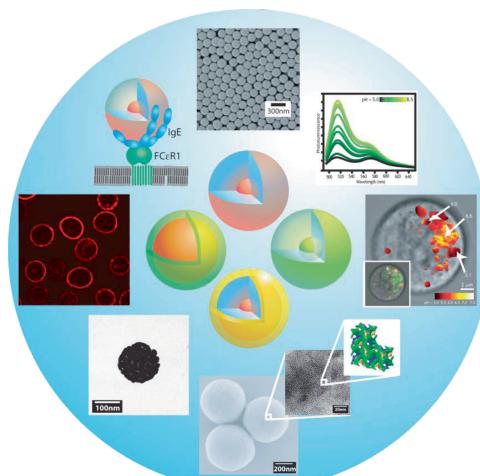
which is a common problem encountered while using fluorescent dyes. Second, it is not intrinsically toxic (gold, in fact, is one of the few metals that is not rejected by our body), it is reasonably stable and it can also be stored in a dry state.

### 1.1.3 Luminescent Silica Nanoparticles

In the past few years, many reports have described the distinct advantages of luminescent silica nanoparticles over traditional dye molecules [54, 55]. These advantages allow their convenient use as fluorescent probes for applications ranging from biosensors [56, 57] to interfacial interaction studies such as immunoassays [58], multiplexed bio-analysis [59–61], nucleic acid analysis [62] and drug delivery [63] to name but a few (Fig. 6).

There are many different synthetic methods to prepare silica nanoparticles and in particular dye doped silica nanoparticles (DDSNs), all characterized by simplicity, low costs, versatility and great control over the architecture of the resulting materials. We will discuss in detail the most common procedures in the next section, with some emphasis on a new synthetic strategy that we have developed and patented [64, 65] that affords monodisperse silica nanoparticles with a particularly versatile and readily obtained core–shell architecture.

The ability to control the spatial organization of the molecular components of complex architectures, in fact, is an essential condition to design systems able to perform specific functions with high efficiency without losing excitation energy into parasite processes. Speaking more generally about efficient photochemical devices (that also include luminescent sensors), they are required to be (photo) chemically stable, to be compatible with the milieu of use and to present photoophysical properties that are not dependent on the environment, or only from a specific analyte. Moreover, ease of synthesis and low cost starting materials are



**Fig. 6** An overview of the versatility of the fluorescent core–shell silica nanoparticle platform; illustrations of single and dual-emission particles as well as gold-nanoshell encapsulated core–shell particles are shown at the centre of the figure, while a variety of applications including bio-imaging, drug delivery, sensing and therapeutics are shown in the periphery. Reproduced with permission from [55]

also obviously desirable if the product is intended to be marketed. Finally, if the systems are prepared to be used for diagnosis or therapeutics (or both), they should be biocompatible and non-toxic, raising no concern about their disposal.

Luminescent silica nanoparticles are, in our opinion, the most promising and valuable of all the species presented till now in this brief introduction; they are potentially interesting for many applications like energy production and storage, catalysis and in particular sensing. After a description of the most common preparation methods in Sect. 2, we will present the state of the art for these particles as far as their application as chemosensors is concerned.

## 2 Synthesis of Fluorescent Silica Nanoparticles

The controlled preparation of monodispersed and stable colloidal silica was proposed for the first time by Stöber [66] and developed in the following years by many other scientists. They improved the method and increased the complexity of the resulting nanomaterial, with a consequential proliferation of possible applications, which proved once more the versatility of this kind of nanostructured scaffold. The concept of chemical functionalization was a crucial point in the design of new materials for practical applications. Van Blaaderen's idea of condensing fluorescent molecules with the monomeric tetraethoxysilane precursor (TEOS) during the growing step in the nanoparticles synthesis allowed the preparation of the first fluorescent silica nanoparticles containing an organic dye covalently linked to the silica matrix [67, 68]. Besides the methodology presented by Stöber and modified by van Blaaderen, other strategies have recently been developed, mainly based on the reverse microemulsion method [69, 70] or on the use of direct micelles as templates [71–72]. In addition to all these methods, our research group has recently developed a synthetic strategy that affords not only monodispersed but also ordered core–shell silica nanoparticles. We will discuss in detail in this section all these preparation approaches, in order to show the pros of each methodology as a function of the specific aim or application that the researcher has in mind.

### 2.1 Stöber Method

The chemical process at the basis of the silica formation is the controlled hydrolysis of tetraethoxysilane (TEOS) molecules and their ammonia catalysed condensation in ethanol/water/ammonia solution. This method allows the continuous and easy control of the nanoparticle dimensions by a suitable choice of the concentrations and ratios of the components of the reaction mixture (TEOS, water and ammonia). Several optimized synthetic protocols with well defined experimental conditions allow one to obtain nanoparticle samples in a dimensional range of about

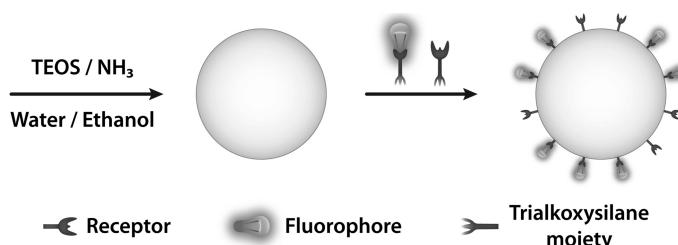
15–200 nm (up to ca. 800 nm with one pot procedure), that useful for bio-analytical applications.

Although the colloidal silica suspensions obtained with this method are already strongly stabilized in water solutions by electrostatic repulsion, controlling the properties of NPs surface is fundamental to tune their solubility and adhesion properties and to avoid irreversible aggregation. Moreover, exterior functionalization is often essential for many applications. Triethoxysilane groups are suitable for surface modifications and properly silanized molecules can be grafted onto the silica surface to modify its functionalities. Such reagents anyway do not guarantee complete passivation: the residual undesirable reactivity due to the external Si-OH sites may require further treatment with end-capping reactants such as  $(CH_3)_3SiCl$  or monoalkoxysilanes.

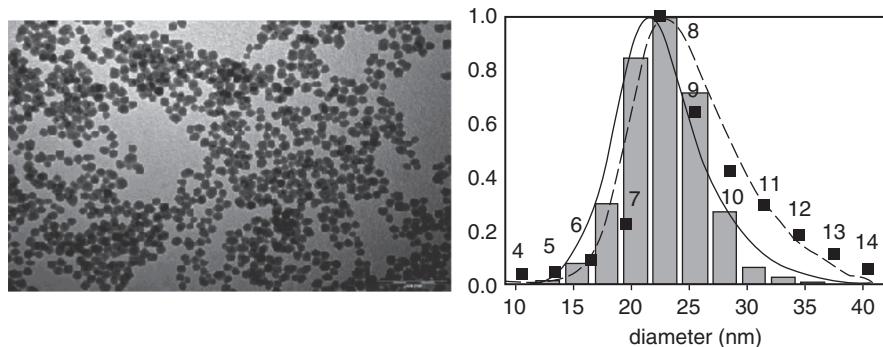
Such a covalent approach also allows one to obtain luminescent silica nanoparticles capping the surface with fluorophores. These emitting systems are usually indicated as dye coated silica nanoparticles (DCSNs) (Fig. 7).

The main synthetic procedures leading to alkoxy-functionalized fluorophores include the hydrosilylation reaction and especially the use of commercial alkoxysilanes bearing a useful reactive functional group. Among these reagents we often find triethoxy(3-isocyanatopropyl)silane as one of the most useful. Belonging to the *click chemistry* family, its reaction with fluorophore derivatives bearing an amine group is fast and quantitative. Furthermore, the formation of the ureidic group in the adduct often increases the solubility of lipophilic organic dyes in polar solvent (ethanol, water) to concentrations useful for the nanoparticle preparation.

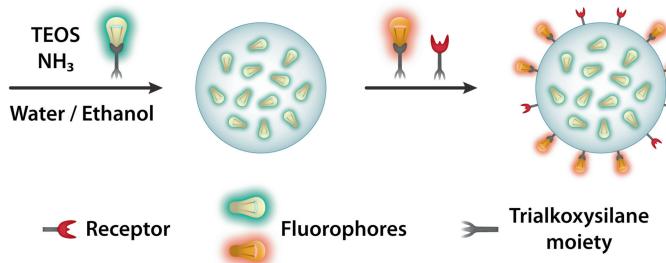
During the synthesis the condensation of trialkoxysilane derivative is almost quantitative, but sometimes the separation of the nanoparticles from the unreacted silane derivatives could be necessary and can be achieved by centrifugation, ultrafiltration or dialysis. Centrifugation/re-dispersion cycles are generally of use when nanoparticles are sufficiently heavy (in term of size, 100–200 nm of diameter and more) [73], and if their surfaces are tightly passivated/stabilized to prevent irreversible aggregation via condensation between the outer siloxanic groups. However, as nanoparticles re-dispersion is often obtained by sonication or vigorous stirring, this strategy is not generally applicable to delicate systems like nanoparticles/biomolecule conjugates. Ultrafiltration and dialysis are the best techniques to purify solutions of small nanoparticles; they in fact allow one to maintain the



**Fig. 7** Stöber synthesis of dye coated silica nanoparticles (DCSNs)



**Fig. 8** *Left:* TEM picture of the fluorescent silica nanoparticles. *Right:* size distribution of the nanoparticles obtained from TEM images (bars); absorbance at 500 nm during the FIFFF elution of the nanoparticles (dashed line); numerical size distribution curve calculated from FIFFF (continuous curve); fluorescence intensities of the fraction  $n$  with a diameter between  $3n - 1$  and  $3n$  (squares). Adapted with permission from [74]

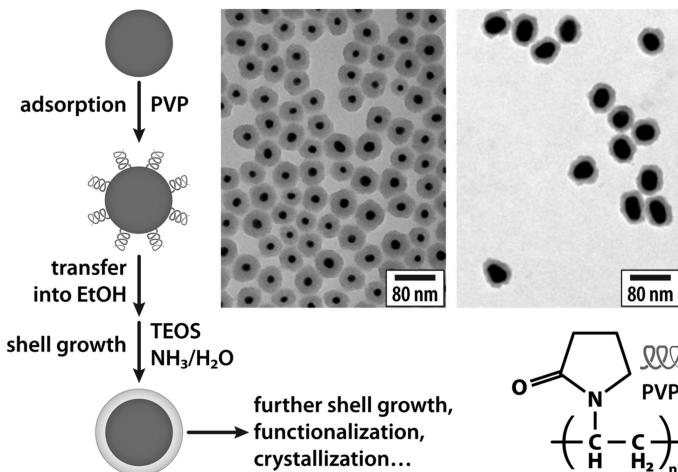


**Fig. 9** Van Blaaderen modification of the Stöber method for the synthesis of dye doped silica nanoparticles

monodispersity of the samples and, when required, to replace the alcoholic environment with aqueous solutions. We recently also showed that FIFFF (Flow Field Flow Fractionation) is a powerful technique to size sort and purify fluorescent colloids of nanometric dimension [74] (Fig. 8).

The Stöber method was brilliantly modified by van Blaaderen who had the idea of co-condensing fluorescent molecules with the monomeric tetraethoxysilane (TEOS) precursor during the growing step in the nanoparticles synthesis, yielding systems in which organic dyes are covalently linked to the silica matrix [67, 68]. These architectures are commonly addressed as DDSN and present a high versatility since different species can be inserted inside the nanoparticles and, moreover, the surface is still available for further functionalization (Fig. 9).

The photophysical properties of dye molecule in DDSNs can also be tuned by exploiting plasmonic effects, that is by growing the silica nanoparticle around a metal core. Experimentally, such sophisticated structures are achieved by carrying out the Stöber synthesis in the presence of preformed metal nanoparticles stabilized



**Fig. 10** Diagram of the general procedure for the coating of colloids with silica and TEM pictures of gold nanoparticles coated with silica (left: 7 nm Au NPs with 18 nm of silica shell; right: 20 nm Au NPs with 12 nm of silica shell). Adapted with permission from [75]

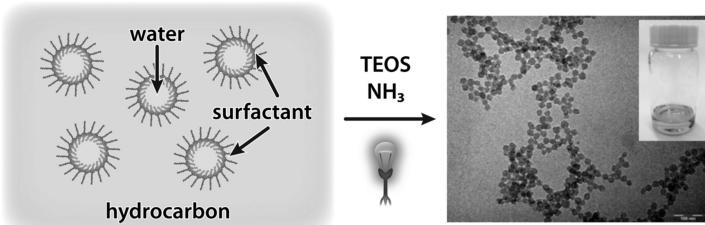
by polyvinylpyrrolidone (PVP) [75]. The reaction condition can be tuned to control the thickness of the resulting silica shell (Fig. 10).

## 2.2 Reverse Microemulsion Method

The reverse microemulsion method is based on the controlled hydrolysis of tetraethoxysilane (TEOS) molecules and their ammonia catalysed condensation like the Stöber method, but the reaction milieu is in this case a stable and macroscopically isotropic dispersion of a surfactant and water in a hydrocarbon. In this system the hydrolysis is confined inside the aqueous nuclei where precursors condense to form the nanoparticles. Optimized synthetic protocols and experimental conditions allow one to obtain nanoparticle samples in the dimensional range of about 15–200 nm [70, 76] (Fig. 11).

The main discriminating parameters to control the nanoparticle dimensions in the microemulsion method are the kind of surfactant and the surfactant to water molar ratio [77]. An advantage of this method is that it often does not require the functionalization of the fluorophores (when hydrophilic) that can be physically trapped inside the matrix or via non-covalent interactions. Derivatization of the dye molecules with a trialkoxysilane group is anyway preferable in order to avoid the leaching of the doping material, especially when small particles (with a diameter close to 20 nm) and a high level of doping (which usually varies between 0.1% and 1% but can be as high as 10% vs moles of TEOS) is required.

Also, in this case, the condensation of trialkoxysilane derivative is generally almost quantitative, but the separation of the nanoparticles from the unreacted



**Fig. 11** Schematic representation of the reverse microemulsion method for the synthesis of dye-doped silica nanoparticles

silane derivatives, and from the reaction media (surfactant, etc.) is necessary and can be obtained by precipitating the particles with an organic solvent. In this case the surface derivatization can be obtained through addition of silanized molecules before the purification step.

Microemulsion based methods can also allow one to prepare species that combine interesting optical and magnetic properties, thanks to the inclusion of an iron oxide magnetic nucleus (especially magnetite,  $\text{Fe}_3\text{O}_4$ ). The resulting hybrid materials, if properly synthesized, can merge a high biocompatibility and hydrophilicity with a magnetic behaviour suitable for several medical and technological applications. Precursor magnetic nanoparticles with a narrow size distribution and with tuneable diameter (4–20 nm) are prepared by reverse microemulsion based methods [78, 79] or thermal decomposition of iron precursors in organic media [80–83]. Silica shell formation may be based either on modified Stöber methods (as seen for metal cores) or reverse microemulsion methods. Interestingly, the presence of a magnetic core, which is advantageous for dual imaging and for a specific positioning in a controlled magnetic field, can also be exploited for the recovery and hence for the purification of these hybrid materials.

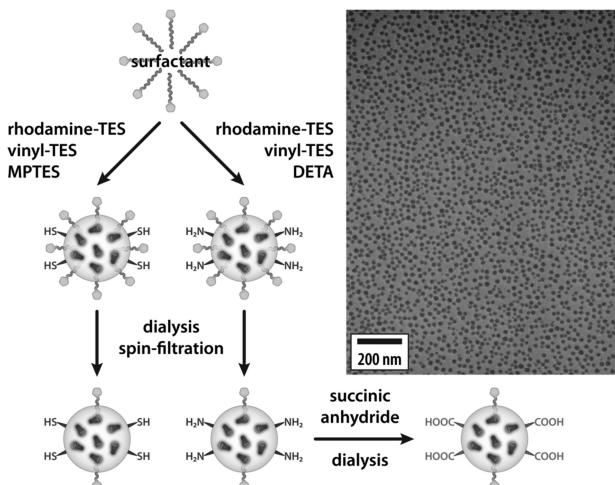
### 2.3 Direct Micelles as Template

This approach is probably the most recent within those described so far. It is based on the use of a surfactant in water solution (sometimes together with a co-surfactant) in which the micellar aggregates (or co-aggregates) behave as templates, where the formation of the nanoparticle structure takes place. The strong point of the strategy is the use of cheap reagents in aqueous solution, for a reaction which provides extremely mono-disperse water soluble nanoparticles, with diameters within quite a narrow range (10–50 nm), these being the most desirable dimensions in most *in vivo* and *in vitro* bio-analytical applications.

Within the examples appearing in the literature, the synthetic strategy used by Prasad and coworkers to obtain the so-called ORMSIL (ORganic-MOdified SILica) nanoparticles stands out for versatility and simplicity. It is based on an

oil in water method utilising a surfactant/1-butanol/DMSO and water mixture. The surfactants normally used are AOT [bis(2-ethylhexyl) sulfosuccinate sodium salt] or Tween 80, while the silica precursor is VTES (triethoxyvinylsilane). The organosilane condensation is promoted using APTES (3-aminopropyltriethoxysilane) or ammonia, and is followed by two purification steps, dialysis and ultrafiltration. The method provides highly monodisperse and stable aqueous suspensions of nanoparticles in the 20–30 nm range that exhibit some degree of mesoporosity. The multimodality of the nanoparticles, that is the ability to carry out multiple functions, is conferred mainly through surface functionalization and encapsulation. The introduction of functional groups on the nanoparticles surface ( $-\text{NH}_2$ ,  $-\text{COOH}$ ,  $-\text{SH}$ ) together with PEG chains [84] allow for targeting through the coupling with bioactive molecules such as transferrin, monoclonal antibodies [85] or DNA [86, 87], while the encapsulation of imaging or therapeutic agents such as single and two photon fluorophores [88], PDT agents [89, 90], QDs and magnetic nanoparticles ( $\text{Fe}_3\text{O}_4$ ) [91] address the ORMOSIL particles as imaging and therapeutic agents. Due to the porosity of the organo-silica matrix, in many cases the conjugation of the fluorophore and/or of the PDT agent with the silica matrix is required to avoid the possibilities of leakage [92] (Fig. 12).

Inspired by the work of Liu and co-workers who have described a new kind of core–shell (silica-PEG) nanoparticles as platform for drug-delivery [71], we have very recently proposed [93] a synthetic strategy that affords monodispersed and ordered core–shell silica nanoparticles. Such systems allow the irreversible inclusion of dye molecules in the silica core and present a stable biocompatible and water soluble polymeric protective shell. For these reasons these materials appear particularly promising in the development of luminescent probes for *in vitro* and, hopefully, *in vivo* medical and bio-analytical applications.



**Fig. 12** Synthetic scheme for the preparation of functionalized ORMOSIL nanoparticles. Adapted with permission from [85]

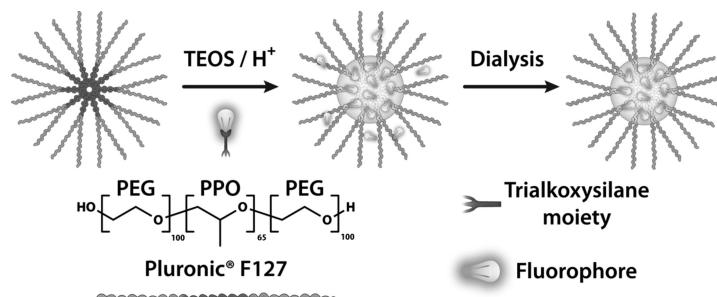
This synthetic strategy is based on the formation of direct micelles of Pluronic® F127 in water. Pluronic F127® is a non-ionic triblock copolymer surfactant terminating in primary hydroxyl groups, and presenting a poly(ethylene glycol)-poly(propylene oxide)-poly(ethylene glycol) structure (PEG-PPO-PEG, MW 12600), that is relatively non-toxic.

The subsequent addition of tetralkoxysilane (TEOS) in acidic (or even neutral) conditions leads to the formation of a silica core, due to the fact that, especially before hydrolysis, alkoxysilane are rather apolar species and tend to migrate and accumulate in the central part of the polymeric micelles, the more hydrophobic area where the silicate condensation is promoted. This induces the formation of the silica nanoparticles only inside the micelles, as the condensation proceeds, leading to the entrapment of the surfactants molecules and to the final silica core-PEG shell architecture (Fig. 13).

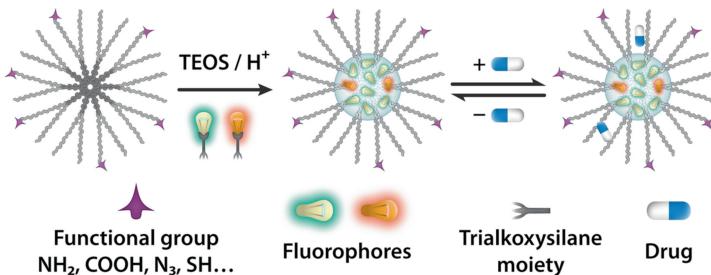
As already mentioned for other synthetic strategies, the silicate condensation needs to be controlled in order to avoid the inclusion in the matrix of the whole PEG segments, or the aggregation through inter-particles polymerization. This can be achieved by adding to the reaction mixture, in due time, DEDMS (diethoxydimethylsilane) or TMSCl (trimethylsilylchloride) that are capping agents able to stop the silica condensation.

The addition of dyes in the initial reaction mixture affords dye-doped silica cores. According to their solubility, in fact, they partition between water and hydrophobic micelles, the latter fraction remaining physically entrapped in the silica network. Derivatizing the dye with a trialkoxysilane group leads to its co-condensation with TEOS, resulting in robust luminescent systems. Thus, this method allows the physical or covalent entrapment of dozens of molecules to a small silica core, providing very bright nanosystems.

Targeting moieties exposed on the surface of silica nanoparticles would account for bio-recognition and bio-specificity, opening up a number of possibilities in biomedical and analytical applications. We are currently exploring the possibility of linking such targeting moieties to the surface with a versatile procedure: through either standard conjugation protocols or more recent click chemistry strategies, we have substituted the terminal -OH groups of the triblock copolymers by proper functional groups (-COOH, -NH<sub>2</sub>, -SH, -N<sub>3</sub>, alkynes...). The co-micelles of



**Fig. 13** Schematic representation of the Pluronic® assisted method for the synthesis of dye-doped silica nanoparticles



**Fig. 14** Diagram showing some features of the silica core-PEG shell nanoparticles

original and modified Pluronic® F127 afford nanoparticles that, as prepared, exhibit functional groups on the surface that can subsequently link the particle to a variety of targeting moieties (Fig. 14).

Transmission electron microscopy (TEM) images show very uniform spherical particles  $d = (10 \pm 1)$  nm. This is the image of the more dense silica cores, the polymeric shell being too soft to be observed with this technique. Light scattering measurements on the same samples provide a hydrodynamic diameter in the 20–30 nm range, very close to that measured for pure F127 surfactant solutions [94–96]. This larger hydrodynamic diameter also takes into account the contribution of the flexible PEG chains in solution.

These core–shell nanoparticles are extremely soluble and stable (up to several month) in water (or phosphate buffered saline solutions, PBS) in which they maintain an outstanding monodispersity. The strength of this strategy is mainly being a one-pot method, in which very cheap and basically non-toxic components are used even if the synthesis pertains to functionalized nanoparticles. Moreover, the PEG shell boosts the performances of the colloidal system looking at in vivo and in vitro bio-analytical applications. The PEG shell provides a stabilizing stealth layer [97] and as a matter of fact in simulated physiological or bio-analytical protocols work-up conditions (PBS 1x, bovine serum albumin up to 10 wt%) these colloidal systems retain their stability and mono-dispersion.

Another feature is the ability to host in the outer PEG shell water insoluble materials such as dyes [98] or chemosensors. In perspective these systems seem to be good candidates for the development in an easy and rapid fashion of chemosensors presenting valuable features like signal amplification due to light harvesting properties.

### 3 Luminescent Silica Nanoparticles as Chemosensors

#### 3.1 Introduction

Introducing this chapter, we have demonstrated the advantages in passing from conventional luminophores to complex architectures, and in particular why in our opinion luminescent silica nanoparticles are the most interesting and promising

nanosystems to be exploited in many fields like energy production and storage, catalysis, and, in particular, sensing for medical or environmental applications.

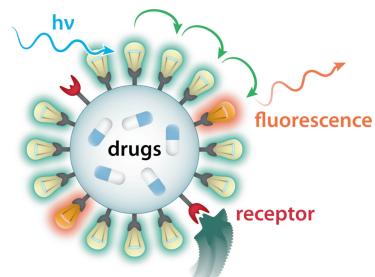
To obtain sensitive luminescent chemosensors, many requirements must be met. In particular the material should be (photo)chemically stable, compatible with the milieu of use, should present photophysical properties that do not depend on the environment or on a specific analyte; for marketing in general it should be obtained with an easy synthesis and low cost starting materials, and for their use in the medical field they should be biocompatible, non toxic and environmental friendly as far as their disposal is concerned.

Hereafter we discuss in more detail how luminescent silica nanoparticles can potentially fulfil all of these crucial features:

1. Silica is photophysically inert, i.e. it is transparent to visible light and is not involved in energy- and electron-transfer processes. For this reason, all the photochemical properties of the luminescent silica nanoparticles are mainly conferred by the doping material and, when present, by the capping agents. Photoactive matrices, in contrast, can be involved in photodecomposition processes (titania) [99] or simply cause quenching of the luminescence (gold) [100].
2. Silica does not present intrinsic toxicity, and for this reason silica NPs are environmentally friendly and can be suitable for *in vivo* applications because they do not undergo microbial attack. Although a deeper investigation is still necessary in this context, preliminary experiments are in favour of the benign nature of silica nanoparticles, also supporting their use for *in vivo* diagnosis and therapy [101]. From this point of view, QDs suggest much bigger concerns about their use in clinical applications [102–104] and their disposal, because of their constituting elements such as cadmium and selenium.
3. Each silica nanoparticle can contain a large number of photochemically active species; for example, a nanoparticle with a diameter of 60 nm used for labelling purposes can contain as much as  $10^4$ – $10^5$  fluorophores. Thanks to these large amounts of dyes incorporated in a small volume, the goal of obtaining a particle with brighter luminescence can easily be fulfilled [105] since its extinction coefficient is equal to the sum of those of the single chromophores.
4. The silica matrix has the capability to protect the active material segregated inside the nanoparticle from external chemicals. Large species cannot, in fact, permeate inside the nanoparticle, while small ones can but with a much reduced diffusion coefficient. This feature still allows, on one hand, the use of NPs as chemosensors for analytes of small dimensions (the dye interacts in its ground state), and on the other hand it decreases the possibility of undesired photoreactions (the excited state of the dye cannot undergo bimolecular reactions), thus increasing the photostability of the fluorophores inside the nanoparticle. The inclusion in this kind of matrix also helps to provide the active species with an almost constant environment in chemical terms.
5. There are many different methods to synthesize luminescent silica NPs, as reported in detail in Sect. 2, but they share valuable common features: they usually require inexpensive reagents and mild conditions, they are rather simple

and do not involve complicated separation procedures. Furthermore, the versatility of the synthesis allows one to design luminescent nanoparticles with chemical properties suitable for the desired applications, including *in vivo* ones. Surface modification with well known chemical procedures [76] allows one to optimize their already good compatibility with water, the solvent of choice for the largest part of purposes, and with the biological microenvironment (cellular membranes, biomolecules, etc.). A simple tuning of the diameter is also possible through the control of the condition of growth of the nanoparticles. A fine control of these two variables (size and functionalization) often has a synergic action to obtain long-life systems in which the nanoparticle head off by RES (Reticulo-Endothelial System) is delayed. RES is a mechanism by which foreign particles are removed from blood or lymph by macrophages in vertebrate organisms. In addition to this, the great versatility of the synthetic strategies opens also up the possibility to adapt these materials for very different applications without requiring each time the design of the synthesis from scratch. This is also facilitate by the ability to realize *onion-like* multilayer structures, i.e. formed by a core, as many layers as desired, and an external modifiable surface (in case one or more of these parts might be of a different material if the application should require it) (Fig. 15).

These five points make clear how luminescent silica nanoparticles are particularly suitable to be used to engineer efficient fluorescent chemosensors, due both to their intrinsic properties and to their versatility. It has to be underlined again, anyway, how all the photophysical properties of luminescent silica nanoparticles are conferred by the doping species, and therefore the photophysical properties of the dyes are the first determining point of the performance of these systems in sensing and imaging fields. This is not a trivial point since, besides their own characteristics, in the final objects their possible cross-interactions can also play a major role. Numerous works in literature highlight that FRET, steady-state and time-resolved fluorescence and fluorescence anisotropy measurements are powerful tools to provide information on the rotational mobility of the photoactive dyes, on the distance and communication between them and on signal amplification effects [106]. In DDSNs homo-energy transfer processes are very important, since they can, on the one hand, lead to undesired self-quenching phenomena, while, on the other hand, in more complex systems such as multilayered *onion-like* structures,



**Fig. 15** Versatility of silica nanoparticles: receptor units on the surface recognize their analytes; fluorophores may undergo energy transfer processes; drug can be hosted inside

they are essential to convey all the energy gathered in a single shell to an adjacent one, favouring directional energy transfer from the core to the periphery or vice versa.

As far as hetero-energy transfer processes are concerned, when DDSNs are loaded with different dyes, they can be intentionally avoided with a structure design able to prevent all electronic interactions, thus yielding ratiometric systems for quantitative measurements [107, 108]. On the other hand, it is also possible to take great advantage from interchromophoric interactions in DDSNs to optimize internal FRET to yield high fluorescence intensity, large Stokes shift and wide absorption with multiple emission colours for applications in multiplex FRET bioassays [109]. Nowadays, numerous papers show appealing results in this field [61, 73], and have shown that, by precisely controlling and varying the concentrations of the dyes within the NP, excitation with a single wavelength can lead to different emission signals, permitting the simultaneous and sensitive detection of multiple targets.

To summarize, energy transfer processes can induce in luminescent silica nanoparticles very valuable collective properties, yielding species that can be gathered in three main classes:

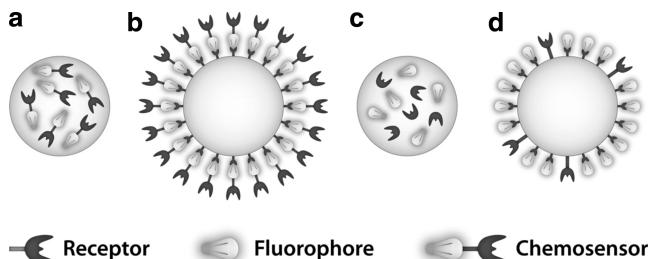
1. *Antenna systems*, which present an enhanced light-sensitivity obtained by an increase in the overall cross-section for light absorption.
2. *Systems that present spectral sensitization*, very important when the light absorption properties of a potentially photoactive (generally, luminescent) species does not permit efficient excitation in the desired wavelength range. This kind of phenomenon is crucial for many applications in different fields, such as, for example, the spectral sensitization of semiconductor electrodes in solar energy conversion.
3. Systems performing *light-energy up-conversion*, that is to say showing anti-Stokes luminescence, a very particular and precious function [110].

Within this very wide panorama we will discuss in this section DDSNs designed to work as sensors and that can be included in one or more of these classes. Efficient nanosensors, in fact, can exploit the antenna effect to obtain signal amplification [111] that leads to a large increase of the sensitivity, and, as a consequence, to lower detection limits.

It is clear at this stage that, to obtain any of these photophysical devices able to perform valuable functions, it is of great importance to know how the different dyes are located and distributed inside the nanoparticle. Such information is not easy to obtain, and we have spent much research effort in recent years in this direction [112].

They can also take advantage of spectral sensitization to obtain remarkable Stokes shifts that typically allow a dramatic increase of the signal to noise ratio for more sensitive and precise photoluminescence measurements.

There are two main approaches possible for the design of these innovative nanostructured sensors, in which the receptor and luminescent units can – or can not – be covalently linked together to form a chemosensor. These groups should be



**Fig. 16** Different approaches in the design of nanostructured sensors

successively derivatized with a triethoxysilane group, which permits their further condensation inside the nanoparticle (if this is porous or small enough to allow the interaction of the sensor with the target) [25] or on its surface (if the final solubility of the system results suitable for the analysis milieu) [113]. This will enable one to develop four different kinds of multichromophoric nanostructures, in which (1) the chemosensor is included in the core of the nanoparticle (Fig. 16a), (2) the chemosensor is linked to the surface of the nanoparticle (Fig. 16b), (3) the separate luminescent and receptor units are condensed in the core of the nanoparticle (Fig. 16c) and (4) the separate luminescent and receptor units are linked to the surface of the nanoparticle (Fig. 16d).

It is very important to note that all these structures do not show equivalent properties and performances. They present different pros and cons; for example when the active species are segregated inside, they are more stable and the water solubility of the matrix is maintained, but they can be much less accessible to the target. On the other hand, when the sensing species decorate the surface they are readily available for binding but this can also result in less stable and soluble systems. Moreover, from our recent studies, we have shown that the packing of the moieties on the surface of the nanoparticles is more efficient than that obtainable in the core. Therefore, on the surface the bound species are closer and their mobility is much higher, and this can cause an increase in electronic interactions that could, in some cases, yield the desired signal amplification effect [23, 114] and an increase of the complexation constants caused by synergic effects of more neighbouring receptors [115]. It is therefore very important to be able to optimize these structures in view of the application of interest, that is to say taking into account the environment, the mobility and steric hindrance of the target and the desired communication level between the various units. This does not sound like – and actually is not – an easy task.

We will discuss hereafter many recent examples of chemosensors based only on luminescent silica nanoparticles but, even if this can appear to be a narrow field, the scenario is instead very wide. Therefore, with the aim of clarity, we have divided them in two main sections, one dealing with systems presenting the signalling units on the surface (dye coated silica nanoparticles, DCSNs) and the other with systems presenting it segregated inside the silica matrix (DDSNs). Moreover, for both

architectures, as we have already mentioned, we have distinguished between species presenting the chemosensor directly bound to the matrix as such or self-assembled starting from separated receptor and luminescent signalling units.

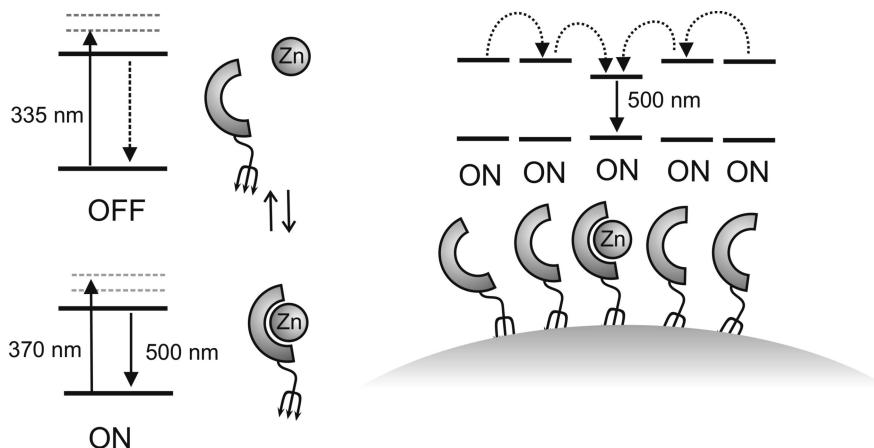
### 3.1.1 Silica Nanoparticles with Chromophores on Their Surface

#### Directly Bound Sensing Subunits

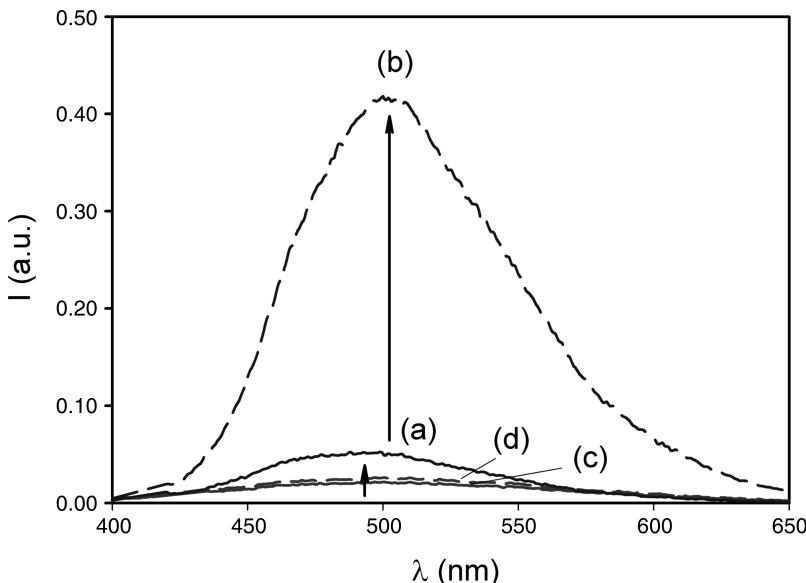
With the aim of sensing applications, multichromophoric systems obtained by the organization of active units on the surface of silica nanoparticles sound more promising than those resulting from silica doping, since they allow a higher local density of molecules and hence a stronger electronic communication.

Following the synthetic strategy of the DCSNs, we have demonstrated the possibility to take advantage of the spatial organization and electronic communication between chromophoric units on the surface of silica nanoparticles for the development of a self-organized Zn(II) fluorescent chemosensor [116]. We used a triethoxysilane derivative of TSQ (6-methoxy-(8-p-toluenesulfonamido)quinoline) to realize a multichromophoric network on the surface of preformed silica nanoparticles. TSQ is a widely used fluorescent chemosensor able to bind Zn(II) ions with good selectivity. It is characterized by an off-on response due to an internal charge transfer (ICT) in the Zn(II)TSQ and Zn(II)(TSQ)<sub>2</sub> complexes (Fig. 17).

In our system the off-on fluorescence signal was amplified by the energy transfer process from the uncomplexed non-fluorescent TSQ units to the neighbouring luminescent Zn(II) complexes. In a low Zn(II) concentration regime, these self-organized chemosensors showed a 50% increase of the response with respect to the reference system TSQ in the same conditions. Even if the total sensitivity gain is quite



**Fig. 17** *Left:* changes in the photophysical properties of TSQ upon complexation. *Right:* schematization of the processes occurring in silica nanoparticles coated with TSQ at low zinc concentration. Reproduced with permission from [24]

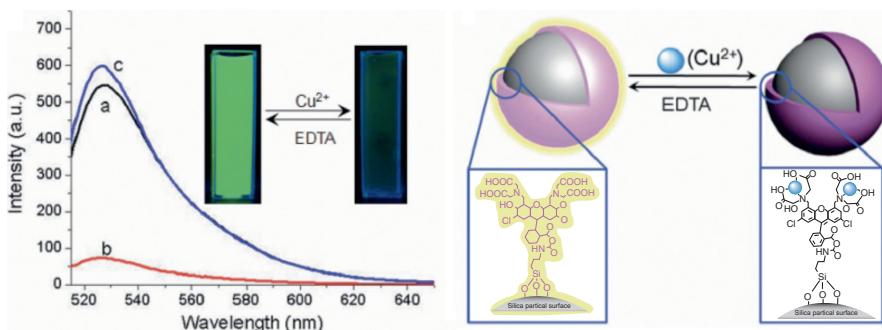


**Fig. 18** Fluorescence spectra of a solution of nanoparticles coated with TSQ before (a) and after (b) the addition of zinc ( $1 \times 10^{-6}$  M). The same addition to a solution of TSQ with the same concentration of dye cause very small changes (from c to d). Reproduced with permission from [24]

limited, this is, to our knowledge, the first example of an amplification effect in an off-on system (Fig. 18).

These phenomena, together with the enhanced affinity toward the substrate (the association constant increases of almost four orders of magnitude in the NPs), induced by the self-organized network on the surface of the nanoparticles, lead to a great increase in the sensitivity of the system, and provide interesting hints for the development of new fluorescent chemosensors. The same TSQ derivative was included in the silica matrix by Mancin and coworkers [25] that reported how its fluorescence was still sensitive to the presence of zinc ions but no amplification effect could be observed, as we will discuss more in detail in Sect. 3.1.2.

Another two nanosensors for copper ions proposed by Jong Hwa Jung and coworkers are based on the use of preformed silica nanoparticles 15 nm in diameter capped with silane derivatives of luminescent chemosensors. The first [117] presents a phenanthroline based sensing unit covalently bound to silica supporting structures of three different morphologies, including nanoparticles. They compared the fluorescent response and found that all the systems were very selective for copper, which was the only metal to induce a significant fluorescence quenching even in the presence of excess of other metal cations. The same group has recently published [118] a similarly selective but more efficient system to detect  $\text{Cu}^{2+}$  in living cells. They bound a fluorescein derivative bearing a trialkoxysilane moiety and two coordinating sites to the surface of commercial silica nanoparticles,



**Fig. 19** *Left:* fluorescence spectra of 10- $\mu\text{M}$  silica nanoparticles without (*a*) and with (*b*) 100 eq. of  $\text{Cu}^{2+}$  and after treatment with EDTA in water (*c*). *Right:* proposed structure of  $\text{Cu}^{2+}$ -bound nanoparticles before and after treatment with EDTA. Adapted with permission from [118]

yielding a selective and reversible (upon addition of EDTA) nanosensor for copper in water at pH 7.4, with a detection limit of 0.5  $\mu\text{M}$ . The incubation of human cancer cells (HeLa cells) with the sensitive nanoparticles showed that they are cell permeable, and still able to signal the presence of  $\text{Cu}^{2+}$  (Fig. 19).

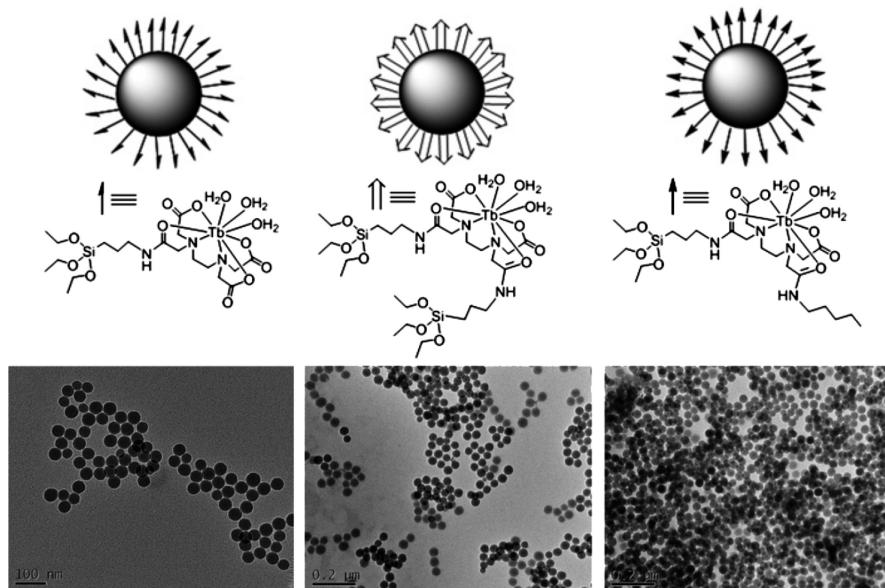
Besides selectivity, another very valuable characteristic of nanosensors is certainly the possibility of use to perform quantitative analysis, such as ratiometric measurements. Xi Chen and coworkers have reported a quite elaborate ratiometric fluorescent system for the detection of mercury ions in aqueous solution [119]. The nano-sensor architecture presents a silica core with a diameter of 100 nm surrounded by a shell of CdTe QDs embedded in silica, these nanospheres then being capped with a silane derivative of rhodamine 6G. This system presents a well-resolved dual fluorescence emission centred at 545 nm (rhodamine) and at 625 nm (QDs), but while the intensity of the second is unaffected by pH variations the first is pH dependent. In PBS buffered solution the rhodamine is present in the lactam form (cyclic amide) so that its fluorescence is quenched. Adding increasing amounts of  $\text{Hg}^{2+}$  a strong enhancement can be observed, since the interaction with mercury induces the same effect of protonation: ring opening of the lactam form of rhodamine. The high selectivity toward  $\text{Hg}^{2+}$ , together with the low detection limit (of the order of nanomolar), precision, reversibility and reproducibility, induced the author to envisage potential application to the monitoring and analysis of waters.

The same ratiometric approach is also the basis of another nanosensor proposed by Tristan Doussineau and coworkers [107]. In this case the rhodamine is segregated inside silica nanoparticles of diameter about 100 nm, prepared following the Stöber method, and then functionalized at the surface with a fluorescent naphthalimide derivative using bridges of different length. The two nanosensors do not show drastic differences in performance, but the one bearing the external fluorophore in close proximity of the surface was more dimensionally polydispersed, and slightly less stable towards aggregation. Both two-dye nanosystems revealed an interesting pH sensitivity in a physiologically relevant pH range. In particular, while the naphthalimide fluorescence intensity at 525–535 nm decreases with increasing pH, the

rhodamine emission at 585 nm remains unaffected, making these tools potentially non-invasive and selective systems for monitoring pH in biosamples, a field of general huge interest.

The great demand for suitable materials to be used in medical and biological analysis in the last few years has, unfortunately, been pared by the urge for sensors able to detect species relevant to security, such as spores and explosives.

Wenbin Lin and K. M. L. Taylor have proposed an interesting system for spore detection based again on a ratiometric approach. Dipicolinic acid (DPA) is a major component of endospores (for example *B. anthracis* spores) [120]. It is already known that there is an affinity of this species for Tb(III) ions, extensively used in fluorescence methods to detect DPA: when the complex is formed the ligand gives rise to an efficient energy transfer to the metal excited states that results in an enhanced luminescence. The authors present here a system that gathers many terbium EDTA complexes on the surface of silica nanoparticles prepared via a well-established water-in-oil reverse microemulsion procedure, and doped with a ruthenium trisbipyridyl complex, yielding quite monodispersed spheres about 37 nm in diameter. The terbium complex was covalently bound on the surface via one or even two silane bridging chains, and the metal complex luminescence intensity was found to be linearly enhanced by the addition of increasing amounts of DPA in solution, while it did not change in the presence of even large excesses of potentially competing ligands. As expected, the luminescence of the ruthenium complex was totally unaffected by the DPA presence acting as an internal standard, also allowing for ratiometric detection at nanomolar concentrations of the analyte (Fig. 20).



**Fig. 20** Schematic representation and TEM images of silica nanoparticles coated with three different modified EDTA-Tb complexes. Adapted with permission from [120]

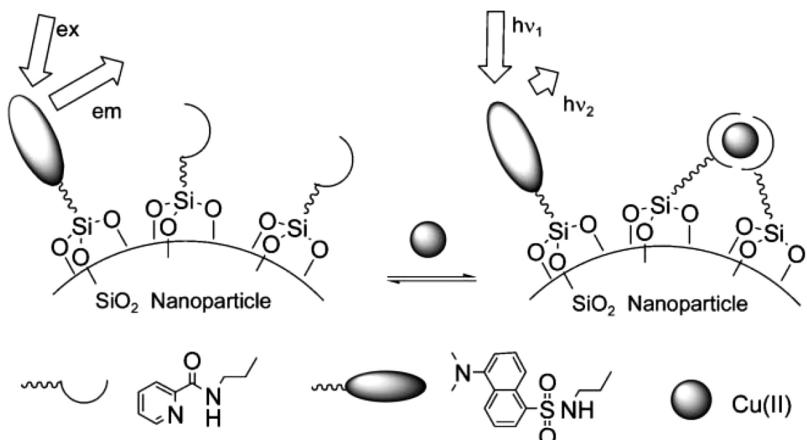
As mentioned above, the other important class that causes civil and military security concerns is that of explosives, and within them the nitroaromatics are particularly common. Conjugated polymers have been widely explored as chemosensors for the fluorescence detection of trinitrotoluene (TNT), as has their incorporation into organic-inorganic hybrid materials in order to tune their photophysical properties and to improve their stability. One very recent example in this direction is described by Yang Li and coworkers [121]. The binding of a conjugated polymer containing diethynylbenzene and diiodo-dipropoxy-sulphonate benzene units (PPS) on silica nanoparticles has been brought to a stable system showing an intense fluorescence that is efficiently quenched in the presence of TNT. The authors found that the fluorescence of the material and its detection performance are strongly dependent on both the solvent and the nanoparticle dimensions, but in all cases it demonstrated a high sensitivity toward the analyte in solution.

### Non-Directly Bound Sensing Subunits

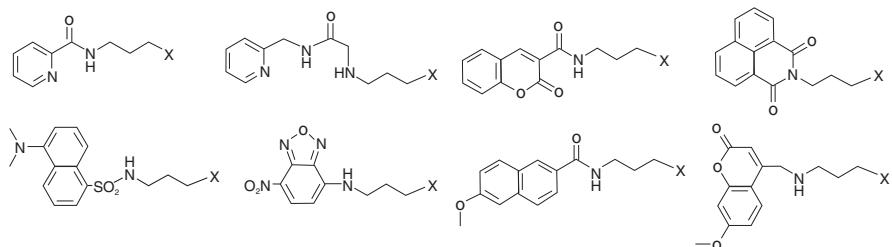
As already stressed in the introduction, silica nanoparticles can be used for an uncommon approach to the preparation of chemosensors. As suggested by Tonellato et al. [122], they can act as a template for the self-organization of the key subunits of a sensor, the fluorescent moiety and the receptor. Even if the components are not previously mutually connected, and therefore they do not directly interact, the self-assembly itself induces their spatial closeness that consequently ensures the electronic communication between the bound substrate and the dye. The appropriate transduction mechanism must be envisaged in the sensor design in order to have a sufficient electronic communication to make possible energy- and/or electron-transfer processes converting the recognition event in a drastic change of the photophysical properties of the signalling dye.

The same authors proposed this strategy to prepare a selective copper chemosensor self-assembling the receptor, a picolinamide, and the signalling unit, a dansylamide, on the surface of preformed silica nanoparticles (20 nm in diameter) [115]. The grafting via the silanization of the sensor components ensured their spatial proximity and yielded a nanomaterial able to detect selectively  $\text{Cu}^{2+}$  ions down to micromolar concentrations via dansyl fluorescence quenching. Moreover, the affinity of the ligand for the target ions increased, and this was ascribed to the organization of the picolinamide moieties on the surface that can induce cooperative effects among the neighbouring binding sites (Fig. 21).

These authors successively proved the versatility of the new approach, preparing different nanoparticles by varying the signalling units and/or the ligands grafted onto the silica nanoparticles [113]. With careful choice of the components and of their ratio, they were able to obtain an amplified quenching response in which each single copper binding event was able to quench up to ten surrounding dyes, a particularly important demonstration of collective effects in nanomaterials (Fig. 22).



**Fig. 21** Coated silica nanoparticles based self-organized fluorescence chemosensor. Adapted with permission from [113]



**Fig. 22** Ligands and fluorescent dyes used for the coating of silica nanoparticles (where  $X$  can be  $-\text{Si}(\text{OEt})_3$  or  $-\text{H}$ )

Ramón Martínez-Máñez et al. followed the identical approach to prepare anion sensors self-assembling anthracene and different thiourea silane derivatives on commercial silica nanoparticles (18 nm in diameter) [123]. Different methods of grafting were compared and particularly the consecutive and simultaneous grafting of the two sensing subunits. The authors prepared a batch of coated nanoparticles differing in the nature of the ligand and the ratio of the components and they carried out a systematic study on their fluorescence in solution in presence of organic (acetate, benzoate) and inorganic (phosphates, sulphates and halogenides) anions. All the materials were non-particularly selective and the fluorescence of the hybrid nanoparticles underwent only moderate changes, but the relatively low synthetic effort and the modular procedure were proved, opening up the possibility for great improvements of the performance.

They then focused their attention on polyanions and in particular on charged polysaccharides, proposing a new hybrid nanosensor for heparin [124]. In this case, the system presents only amine and thiol binding sites grafted onto the surface of

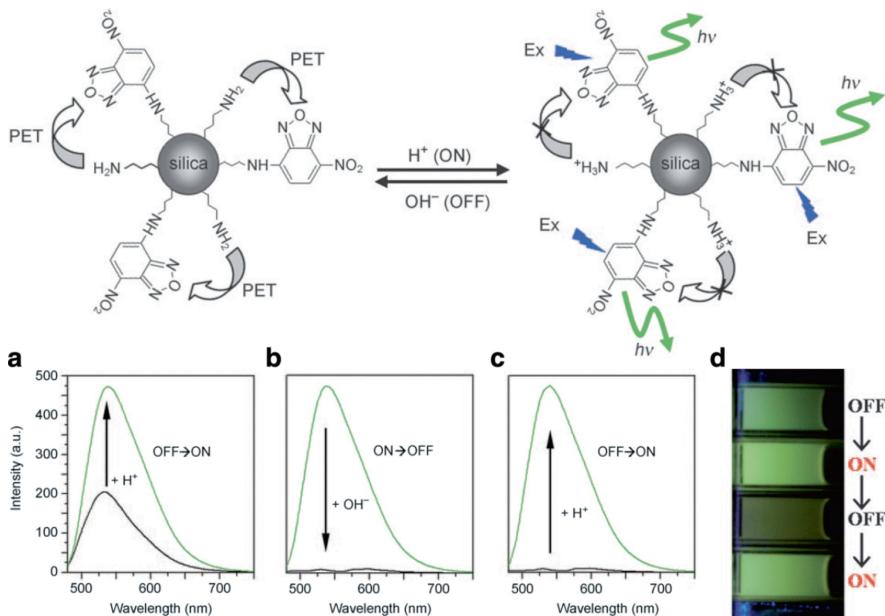
the commercial silica nanoparticles, and the mechanism of detection is based on the chemosensing ensemble strategy [17]. The signalling unit is now a squaraine free in solution that, in the absence of heparin, interacts with the thiols on the surface of the nanoparticles. This binding causes changes in the squaraine absorbance at 643 nm and quenching of its emission at 679 nm. The presence of heparin restores the typical values of the free dye in solution. The interactions of the polysaccharide with the amines on the surface of the nanoparticles in fact drives their wrapping by the heparin, making them no longer accessible to the signalling reporter that recovers its fluorescence. This system is therefore a sensitive (down to 2  $\mu$ M) and selective chromo-fluorogenic hybrid nanosensor for heparin, even in the presence of large amounts of inorganic anions, monosaccharides, charged disaccharides and other charged polysaccharides.

The research group led by Zhongping Zhang is also extensively exploring the same modular approach, that is to say the silica nanoparticle template self-assembly, but in this case for the preparation of chemosensors for explosives (TNT) and herbicides (2,4-dichlorophenoxyacetic acid) [125]. They used nitrobenzoxadiazole (NBD) and organic amines as dye and receptor to decorate the surface of silica nanoparticles (150 nm in diameter) where they experience special proximity. Their reciprocal distance is short enough to allow photoinduced electron transfer (PET) from the amines to the NBD that causes the quenching of the dye fluorescence. Protonation of the amino ligands leads to fluorescence enhancement due to inhibition of PET, making this system a good, reversible and stable pH nanosensor. The herbicide 2,4-dichlorophenoxyacetic acid, being able to exchange protons, can be effectively detected by taking advantage of the same mechanism (Fig. 23).

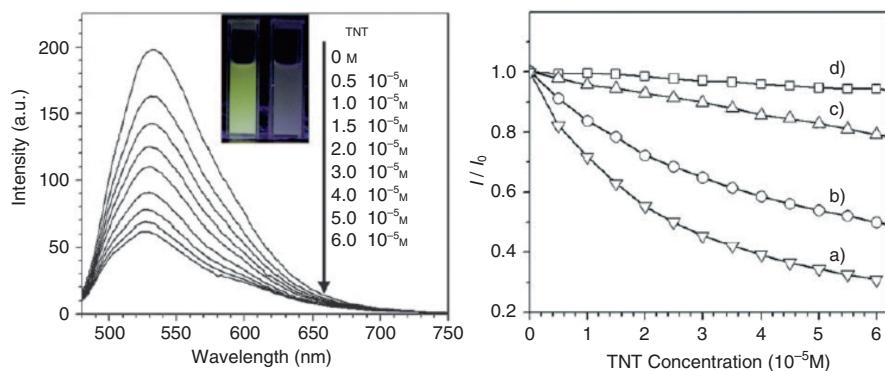
This hybrid nanomaterial also proved to be sensitive to TNT, but in this case the recognition event causes a quenching of the fluorescence of the system. This can be explained since in the presence of this analyte FRET from the dye to the complex formed between the primary amine and TNT becomes predominant on PET. The authors have also assembled these nanoparticles in etched microwells on a silicon chips to prepare inexpensive solid state sensing materials for protons, herbicide and TNT that could be detected down to micromolar concentrations (Fig. 24).

They have also used the same approach to detect TNT using capping silica nanoparticles (200 nm in diameter) with primary amine receptors and fluorescein or rhodamine dyes [126]. The analyte brings about induced quenching of the fluorescence for both species again via a FRET mechanism. Nanoparticle assembled chips were able to detect TNT in solution down to nanomolar concentrations while when deposited as a thin film could sense nitroaromatic vapours down to 4 ppb in air.

There is another interesting system that exploits the same FRET mechanism, to obtain in this case fluorescence amplification, but based on the addition of a non-bound second fluorophore. Bin Liu and Yusong Wang [127] have immobilized a DNA strand on monodispersed silica nanoparticles (100 nm in diameter) and then hybridized it with a fluorescein labelled complementary DNA engineered to induce in the final double strand three pairs of T-T mismatches that are specific coordination sites for mercury ions. After incubation with metal ions and thermal washing, a cationic conjugated polymer was added. This last component was selected in order



**Fig. 23** Above: schematic diagram of the reversible fluorescence switch by sequential titration of HCl and NaOH into the suspension of NBD-(NH<sub>2</sub>)-silica nanoparticles. Below: reversible fluorescence switch effect of NBD-(NH<sub>2</sub>)-silica nanoparticles in ethanol with sequential addition of 1 mM HCl (a), NaOH (b), and HCl (c), with corresponding visual fluorescence changes, excited with a 365 nm UV lamp (d). Adapted with permission from [125]



**Fig. 24** Left: evolution of the fluorescence spectra of NBD-(NH<sub>2</sub>)-silica nanoparticles with increasing TNT concentrations (the inset shows the fluorescence images before and after addition of TNT with excitation by a 365 nm UV lamp). Right: variation in fluorescence intensity with increasing TNT concentrations for NBD-(NH<sub>2</sub>)-silica nanoparticles with different ratios of NBD to amino groups: 1:40 (a), 1:10 (b), 3:1 (c), and free NBD-APTES conjugates (d). Adapted with permission from [125]

to present good spectral overlap with fluorescein so that energy transfer could occur, and the initial low fluorescence intensity of the labelled complex system was enhanced via sensitization. The system becomes very selective for  $\text{Hg}^{2+}$  ions since the formation of this complex induces a significant increase in the melting temperature of the material. Therefore, with careful thermal washing, it was possible to denature all the double strands containing metal ions different from mercury. The photoluminescence enhancement is linear in low concentration regimes both of metal ions and nanoparticles.

### 3.1.2 Silica Nanoparticles Containing Chromophores

As we have already mentioned, the trapping or co-condensation of luminophores (or luminescent chemosensors) within the silica matrix yields dye doped nanoparticles (DDSNs) that contain the photoactive units and present an unreacted surface. This opens up two different strategies to exploit these systems as luminescent chemosensors. The signalling units and the receptors (as a whole or as separate moieties) can be inserted inside the particle and the target analyte must diffuse through the silica matrix in order to interact with the ligand and be detected. This is obviously possible only for small species such as protons or metal ions. For bigger analytes another architecture can assure better performance, a setting with the signalling units located inside the particle and the receptor components bound on the surface.

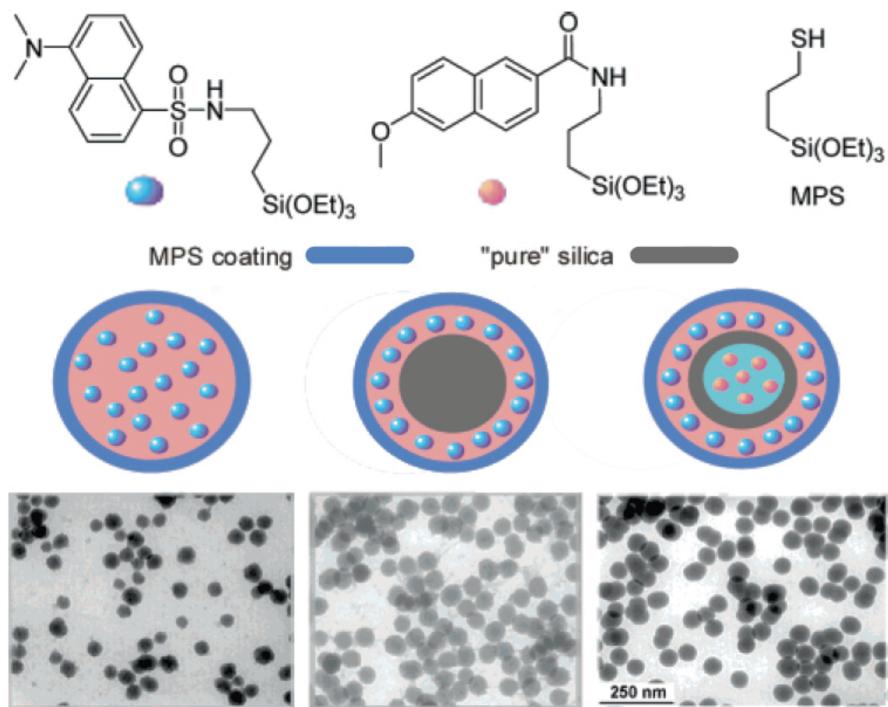
It is fundamental to highlight that, for both assemblies, the particle size is a very important parameter that influences the efficiency of the sensors. We have, in fact, shown experimentally that the particles are accessible to the solvent and the analyte only up to a certain depth [128]. When the particle is small, the solvent permeable layer is deep enough to make the whole body of the particle accessible to the analyte. On the other hand, the luminophores contained close enough to the surface are able to sense the state of the receptors so that all the fluorophores are effectively responding to the external signal. These two features are not guaranteed as the diameters of the colloid increases. It is therefore self-evident that many parameters have to be taken into account when designing a chemosensor and each architecture presents pros and cons. For example, we have already discussed the interesting results obtained binding TSQ [6-methoxy-(8-*p*-toluenesulfonamide)quinoline] to the surface of silica nanoparticles: the proximity of the chemosensors induced and enhancement of the binding constant and a 50% increase in the response with respect to the reference system TSQ in the same conditions. These very positive results are unfortunately accompanied by a substantial change in the solubility of the system that, after TSQ grafting, could be efficiently dissolved only in DMSO.

An elegant way to overcome this solubility problem is to include the same TSQ derivative in the silica matrix, preserving the hydrophilicity typical of this material. Mancin and co-workers [25] reported the preparation of  $\text{SiO}_2$  nanoparticles (15 nm in diameter) doped with covalently bound TSQ. This system is soluble in water/ethanol 1:1 and is also porous enough to allow the diffusion of zinc ions inside the

silica matrix. This was proved by the fact that the chemosensor fluorescence is still sensitive to the presence of zinc ions in solution. The drawback, in this case, is that the initial quantum yield of TSQ doped nanoparticles is higher than that of molecular TSQ in the same conditions, probably due to a partial protonation of the dyes by the acidic silanol groups. This has a negative consequence on the sensitivity of the material since the relative variation of the fluorescence caused by ion complexation is lower and the binding ability suffers a certain decrease. This could not be counterbalanced by the expected possible amplification effect due to chemosensors proximity, since no amplification could be observed in this case, probably because of the excessive distance between the receptor molecules. Mancin and coworkers have also prepared a totally similar system differing only in the addition of another dye in the particle. They used a coumarin derivative, indifferent to the analyte, that presents an emission band centred at 410 nm that remains constant in intensity and position during the whole titration with  $Zn^{2+}$  acting as an internal reference and validating the particles as ratiometric chemosensors.

The same authors had, shortly before, already proposed an even simpler and more versatile strategy to obtain silica nanosensors for metal cations [129]. They noticed that the silica itself provides a certain metal adsorption ability and they thought it could have been possible to exploit directly this feature to avoid the addition of a receptor moiety. They prepared silica particles doped with the dansylamide dye by co-polymerization of tetraethoxysilane (TEOS) and a dansyl triethoxysilane derivative by following the Stöber/van Blaaderen method. The resulting DDSNs showed a high sensitivity to the presence of  $Cu^{2+}$  ions that caused a strong fluorescence quenching, allowing the metal detection down to micromolar range. In this case, the silica itself or, better, the acidic silanol groups network on the particles surface are the receptor unit of the sensor. The small diameter of the spheres (about 15 nm) assures enough proximity of the  $Cu^{2+}$  ions bound on the particle and the fluorescent units segregated inside the core to quench their emission. Therefore the simple formation of the nanoparticles led to the straightforward conversion of fluorescent dyes into chemosensors. As mentioned above, the efficiency of sensors presenting architectures of this kind is greatly influenced by the particle size, and the authors have experimentally demonstrated this point, investigating the response to copper ions of analogous DDSNs with different diameters. Smaller particles show an almost complete quenching of the emission, while the larger ones present only a much smaller variation.

Even if the described system demonstrated an unexpected selectivity for  $Cu^{2+}$  ions, it is obvious that the modification of the outer shell of silica with other functional groups could increase the affinity of the system toward the desired metal ion, as the same authors have demonstrated for another self-organized fluorescent nanosensor able to detect  $Pb^{2+}$  ions [108]. Mancin et al. prepared in this case three different batches of DDSNs always following the Stöber/van Blaaderen procedure. The first ones (about 50 nm in diameter) presented dansyl units covalently bound inside and a surface coated with (mercaptopropyl)triethoxysilane (MPS). These particles are soluble in polar solvents (including water) and their photophysical properties are typical of the dansyl dye. The presence of thiols



**Fig. 25** Schematic representation of dye-doped silica nanoparticles with corresponding TEM images and structures of the dyes employed as sensors for  $\text{Pb}^{2+}$  ions. Adapted with permission from [108]

makes the system more selective for lead ions and their addition to a buffered (pH 7) solution of the nanoparticles causes a substantial quenching, even if in saturation conditions the system still presents a residual fluorescence of 60% with respect to the initial one (Fig. 25).

This can again be explained by the inaccessibility of the particle core: a second set of core–shell systems where a 50 nm diameter sphere of pure silica was coated with an approximately 5-nm dansyl doped silica shell capped with MPS showed in fact a remarkable sensitivity with only a 30% residual fluorescence. Mancin and coworkers have then designed a last batch of particles to obtain lead ratiometric detection. This was made by a multishell system with a silica core doped with a reference coumarin derivative surrounded by a 7-nm insulating pure silica layer and an outer 3-nm dansyl doped silica shell capped with MPS. The behaviour of this last system was analogous to the previous one but in this case ratiometric detection and calibration were possible thanks to the presence of the reference coumarin emission.

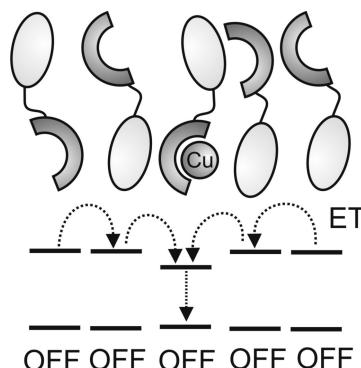
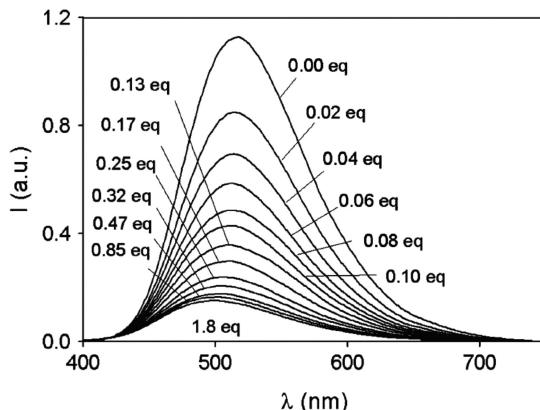
All the examples discussed till now make clear many advantages of using DDSNs to design sensors instead of dye capped silica nanoparticles or single dyes, but still no examples of signal amplification has been shown.

Our research group is indeed very interested in this phenomenon and, in order to demonstrate the possibility of amplifying the response of a fluorescence chemosensor by inclusion in SNPs, we synthesized a proof of principle system based on the dansylated 3-[2-(2-aminoethylamino)ethylamino]propyl-trimethoxysilane commercial receptor [23] (Fig. 26).

The nanoparticles were synthesized with the Stöber method, maximizing the density of the fluorophores with the aim of allowing the occurrence of multicomponent cooperative photophysical processes. Interestingly, the addition of copper, cobalt and nickel ions induced a strong quenching of the fluorescence intensity even at nanomolar concentrations (Fig. 27).

The ability of  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Co}^{2+}$  to quench the fluorescence of this dye had already been reported for polyaminic dansylated systems and had been explained on the basis of energy transfer processes involving the dansyl excited state and the

**Fig. 26** Fluorescence spectra of dansylated silica nanoparticles ( $\lambda_{\text{em}} = 335 \text{ nm}$ ) for different concentrations of added copper ions. Reproduced with permission from [23]



**Fig. 27** Schematization of the energy transfer processes occurring in silica nanoparticles doped with 3-[2-(2-aminoethylamino)ethylamino]propyltrimethoxysilane. Complexation of a single  $\text{Cu}^{2+}$  ion causes the average quenching of 13 fluorophores (only five are reported in the picture for simplicity). Reproduced with permission from [24]

metal ion. The results obtained with our system, however, suggest that each copper ion, the species having the highest affinity, could quench up to 13 dansyl units, leading to strong signal amplification. This fluorescence response to complexation showed by the nanoparticles is in our opinion extremely interesting since, not only does it prove that they are porous enough to let in small cations, as already reported by other authors, but also that the proximity of the chemosensor units allows the communication of each receptor moiety with all the neighbouring fluorophores.

If silica matrices are permeable to small cations, they will be even more permeable to protons, and many authors have thought to take advantage of this property to design silica nanoparticles for pH sensing [130–132]. Most interestingly, Kemin Wang and coworkers [130] have described a ratiometric system able to sense pH variations in a range between 4 and 7 in murine macrophages and in living human cancer cells (Hela cells) during apoptosis. The authors have synthesized DDSNs (average 42 nm in diameter) containing both a silane fluorescein derivative, the luminescent sensing species, and the tris(2,2'-bipyridyl)ruthenium (II) as an internal standard reference. After investigating the stability and photostability of the system they incubated the nanosensors with murine macrophages and then monitored the changes in lysosomal pH in real time after exposure to chloroquine, an antimalarial drug. The comparison of the variations in the ratio between the fluorescein fluorescence intensity and the ruthenium complex luminescence intensity in time for reference samples and for different amounts of added drug showed how chloroquine stimulates lysosomal pH changes that can be revealed with high sensitivity using this method. The results obtained with Hela cells also demonstrated that intracellular acidification in apoptotic cancer cells, after treatment with dexametasone, a glucocorticoid commonly used in medicine, could be monitored in real time with a response of less than 1 s. The system also presented good reversibility, high stability and excellent quantification performance also thanks to its ratiometric nature.

The same fluorescein isothiocyanate (FITC) silanized via its reaction with (3-aminopropyl)triethoxysilane (APTES) was used in dye doped silica nanoparticles with a diameter of about 100 nm by Feng Gao and coworkers to prepare stable water soluble species for hydrogen peroxide indirect sensing [133]. The reverse microemulsion method was used to synthesize the doped nanospheres that had then been aminated at the surface. They used the same strategy to obtain analogous species for glucose detection but with a europium(III) complex as dye doping unit [134]. The authors also performed the analysis of blood glucose in human serum samples with this fluorescence-based approach, and the results were in good agreement with the data obtained via other common clinical methods.

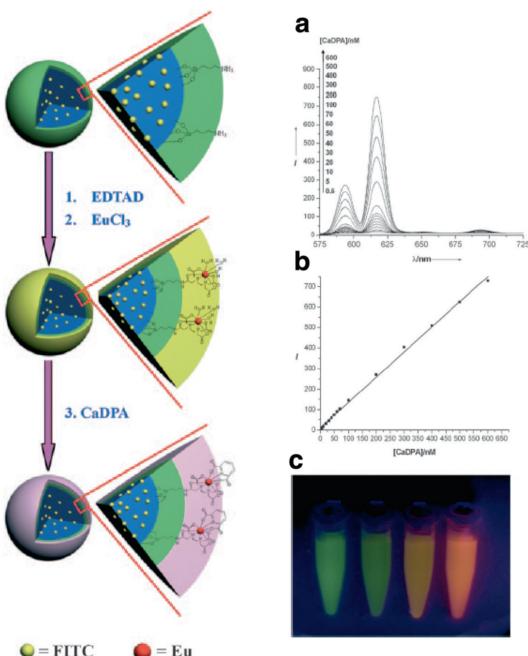
In general, lanthanide-based sensors have strongly attracted the attention of the scientific community worldwide for some decades due to their unique and outstanding photophysical characteristics such as long emission lifetimes, very narrow emission bands and large Stoke shifts. These spectroscopic features allow time resolved detection that is the most elegant way to clear measurements from background fluorescence with a consequent remarkable sensitivity enhancement. As already mentioned in Sect. 3.1.1.1 [120], DPA or better calcium dipicolinate

(CaDPA) has a high affinity for lanthanide ions and in particular terbium(III) complexes are considered the best probes for the detection of this species which is a unique biomarker for bacillus spores such as *B. anthracis*, known as a potential agent for biological terroristic attacks.

Lehui Lu and coworkers have recently published a very interesting study on a new nanosensor based on DDSNs for CaDPA that for the first time uses a europium complex as chemosensor [135]. The use of europium can offer some advantages over terbium such as a larger Stoke shift and a red emission. They have prepared fluorescein doped silica nanoparticles (65 nm in diameter) and then grafted onto their surface silanized ethylenediaminetetraacetic dianhydride (EDTAD) that was directly converted into its europium derivative via reaction with  $\text{EuCl}_3$ . In this complex, three of the coordinating positions of the lanthanide are occupied by water molecules that enhance non-radiative quenching of the metal excited state, the system in fact being non-emitting (Fig 28).

On the other hand, these water molecules are only weakly bound to the metal centre and they are readily substituted by DPA when present in solution, resulting in a significant enhancement of the complex emission intensity. The authors showed a linear correlation between the maximum emission intensity of the nanosensor and the concentration of CaDPA in solution in the range 0.6–600 nM with a detection limit down to 0.2 nM. Therefore it was very sensitive but also very selective over other aromatic ligands. The rapid and ultrasensitive detection of *B. anthracis* spores in water was also helped by the ratiometric nature of the material since the

**Fig. 28** Left: design strategy for Eu-based fluorescence nanoparticle sensor (EDTAD is ethylenediamine tetraacetic acid dianhydride). Right: (a) fluorescence response of the sensor ( $\text{Eu}^{II}$  content: 10  $\mu\text{M}$ ) upon addition of different concentrations of CaDPA at pH 6.5, (b) fluorescence intensity at 616 nm of the sensor as a function of CaDPA concentration. (c) visual fluorescence colour changes of the sensor ( $\text{Eu}^{II}$  content: 120  $\mu\text{M}$ ) upon addition of different concentrations of CaDPA (from left to right: 0, 25, 50, 100  $\mu\text{M}$ ). Adapted with permission from [135]



fluorescein emission of the nanoparticle core is totally unaffected by the presence of the CaDPA substrate.

### 3.1.3 Luminescent Silica Core–Shell Nanoparticles

In the previous section we have already described some multilayered systems [108] constituted by differently doped silica strata, but here we will report on a few examples of core–shell nanoparticles that combine the properties of different materials. Silica is a key component in this area since it offers unique characteristics of ease of synthesis, biocompatibility and a ductile chemistry able to merge many different substances including biomolecules, therefore opening up the possibility of using these new tools in the fields of biology and medicine [136, 137].

On the other hand, silica is often used as an insulating layer when direct *communication* between different components of a complex systems has to be avoided. For example, for a few years an unprecedented method has been proposed for increasing the efficiency of energy transfer exploiting the effect of plasmonic nanostructures [138–140]. In this systems *contact* of the fluorophores with the metal (usually silver) must be prevented since it would result in excitation energy dissipation and, most importantly, the increase in the efficiency of the energy transfer can be obtained only if the metal nanostructure is localized at a optimal distance.

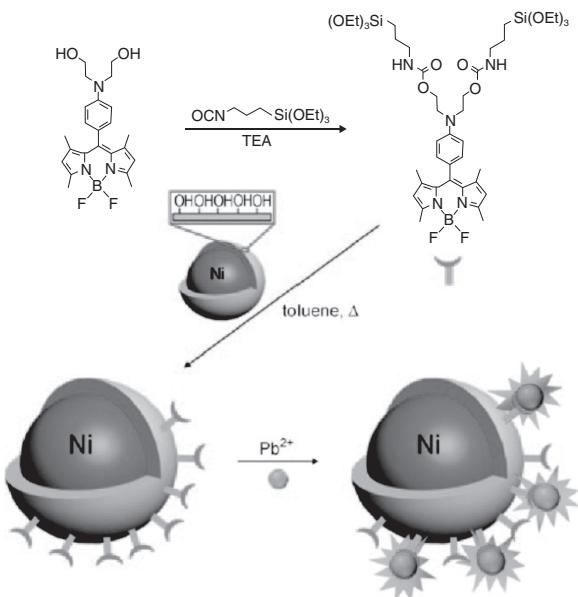
It is now clear how these hybrid core–shell materials present a very complex structure but also how, at the same time, they can assure the possibility of obtaining a great richness in functionalities, properties and performance [141].

In particular, magnetic silica nanoparticles are of great interest for research and applications in a variety of fields because they are stable and biocompatible. With the use of an external magnet they can be isolated, treated and repeatedly utilized. In biomedical and environmental applications they have been studied and used for bio-separation, enzyme immobilization, protein purification, as magnetic resonance imaging (MRI) contrast agents, and to remove toxins or pollutants different samples.

In this last area of sensing and separation, Weihong Tan and coworkers had proposed some years ago a very interesting *genomagnetic nanocapturer* for the collection of trace amounts of DNA/mRNA in cancer cells [142]. They prepared magnetic cores with a silica shell covalently functionalized at the surface with molecular beacons as a DNA probe for gene recognition and collection. These complex nano-objects presented a diameter of about 30 nm and some valuable features such as the possibility of monitoring the process in real-time, a very low limit of detection and collection (down to femtomolar) and an excellent specificity due to the use of molecular beacons.

The system was quite efficient and was probably an inspiration for Jong Hwa Jung et al. [143] who, more recently, have proposed a similarly engineered system for the sensing and separation of toxic species like lead and mercury in different matrices. They have synthesized nickel nanoparticles coated with a silica shell (30–40 nm in diameter), then grafted with a di-silanized 4,4-difluoro-4-bora-3a-,4a-diaza-*s*-indacene (BODIPY) derivative (Fig. 29). This dye, buffered at pH 7, is

**Fig. 29** Synthesis of BOD-IPY-functionalized magnetic silica nanoparticles. Adapted from [143]



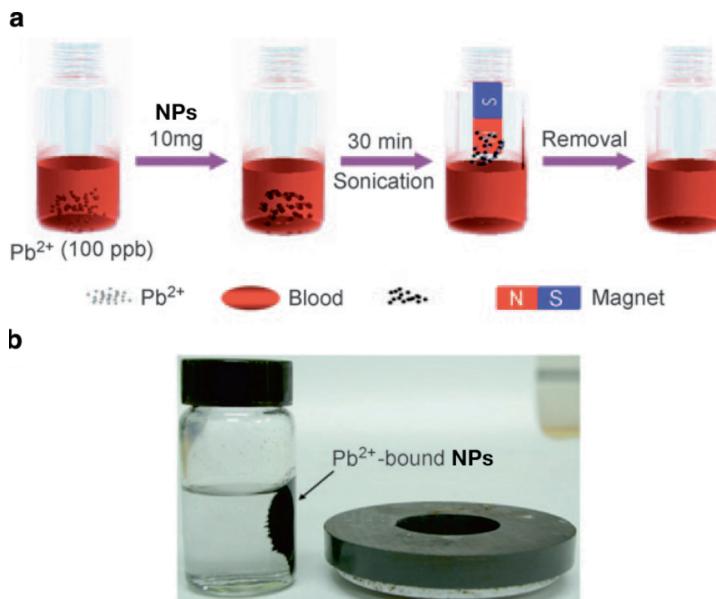
almost nonfluorescent due to an internal PET, but in the presence of lead ions it shows an about eightfold chelation-enhanced fluorescence CHEF at 510 nm, that is reversible with the addition of a strong base.

Titration experiments with  $\text{Pb}^{2+}$  revealed the formation of a 1:1 complex and a detection limit lower than 15 ppb that is the maximum limit allowed for lead in drinking water. Moreover, the preorganization on the silica surface of the chemosensors led to a high selectivity for  $\text{Pb}^{2+}$  over the other possible interfering cations, and this suggested to the authors that it could have been a promising candidate for the separation of this toxic ion. They performed pilot experiments to remove lead cations, using a small external magnet, from water and human blood, where they could separate 96% of the total contain (100 ppb) selected as the lower unsafe limit of  $\text{Pb}^{2+}$  content in children blood (Fig. 30).

The analogous experiment in water allowed them to remove the 97% of the initial 15 ppb of  $\text{Pb}^{2+}$ , validating the potentialities of this new type of magnetic biocompatible systems to detect and separate heavy metal toxins from different matrices.

The same authors, further developing this idea, prepared another core–shell nanosystem presenting an  $\text{Fe}_3\text{O}_4$  nucleus with a silica coating superficially functionalized with aminonaphthalimide units [144]. In this case the material proved to be sensitive and selective for mercury and methylmercury ions in the pH range 4–11, and the experiments to test its ability to remove these toxic agents from drinking water containing 100 ppb of both of them showed 100% efficiency.

The sensing of mercury ions in water was also the aim of Enrique García-España, Javier Alarcón and coworkers [145] who have reported on a core–shell material modified with a fluorescent chemosensor based on anthracene and simple secondary



**Fig. 30** (a) Illustration of the removal of  $\text{Pb}^{2+}$  from human blood. (b) Photograph of a magnet attracting  $\text{Pb}^{2+}$ -bound nanoparticles in water. Adapted from [143]

amines as receptors. The aluminosilicate nucleus is surrounded by a silica shell that allows the covalent bounding of the active units on the surface of the nanosystem (5–10 nm in diameter). In aqueous solutions with a pH in the range 3.5–5.5, the presence of  $\text{Hg}^{2+}$  caused a significant decrease of the luminescence of the nanosensor, allowing a detection limit in pure water of 0.2 ppb and very selective detection. A further advantage, in this case, is that the gelification at pH 11 of the material allows its recovery by simple centrifugation.

#### 4 Some General Remarks

Nanofunctional materials in general, and nanoparticles in particular, are the basic constituents of the nanosciences, a field characterized by an unprecedented interdisciplinarity that merges chemistry with engineering, physics with material sciences and medicine. The importance of this topic is definitely substantial, as testified by the astonishing and increasing number of related publications. This lively research indicates not only that a certain degree of maturity in the know-how has been reached, but also that the development of nanotechnological products, even if more and more extensive, is only in its *infancy*.

The world of nanoparticles is amazingly many-sided and manifold with huge versatility in exploitation from industrial areas to bio-analysis and catalysis.

In particular, luminescent and magnetic nanoparticles attract the utmost attention in biology, medical diagnosis and therapy, where they already find many applications.

In this so fascinating and wide framework we have focused our attention particularly on luminescent silica nanoparticles able to act as sensing materials. After highlighting the first important aim to gain very precise knowledge and control of their structures, we have briefly presented the state of the art and progress in the synthetic protocols that allow one to prepare differently organized and precise architectures. This is of fundamental importance for the aim of sensing; in fact, the performance of a chemosensor is totally dependent on its design.

Among the different possible signalling methods, luminescence offers great advantages and is therefore the preferred one in many kinds of applications. In particular, fluorescent chemosensors grafted or self-assembled and self-organized on/in nanoparticles find wide applications in two major areas: (1) the detection of analytes in bulk biological or environmental solutions, and (2) the intracellular measurement of pH, oxygen, and reactive oxygen species, cations, etc. In this last case, silica nanoparticles are particularly suitable not only for their intrinsic low cytotoxicity but also because the gathering of the signalling molecules inside them, or on their surface, brings down their toxicity in comparison with the use of the same species as free indicators.

With the examples reported we have tried to show how luminescent silica based nanosensors can be seen as very promising systems offering many advantages such as solubility and stability in aqueous solvents and in physiological conditions, the possibility to insert targeting moieties for bio-conjugation and an intrinsic multichromophoric nature. This last point also allows, with a suitable choice of dyes and close control of the structure, signal amplification effects via collective energy and electron transfer processes.

All these features make luminescent silica nanosensors unique platforms. Their versatility, in fact, opens up the possibility to implement them in so many ways and different approaches that it is impossible to predict now the potentialities of the resulting final materials and of their applications.

We are convinced that the field of nanoscience will quickly make substantial steps forward and that, in this framework, silica nanoparticles will be one of the main characters with a fundamental role in the new ambitious applications, probably now difficult to envisage, that will surely have a great impact on the quality of our lives.

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