Fluorescent Nanoparticles

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Luminescent Silica Nanoparticles: Extending the Frontiers of Brightness

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Silica nanoparticles are versatile platforms with many intrinsic features, such as low toxicity. Proper design and derivatization yields particularly stable colloids, even in physiological conditions, and provides them with multiple functions. A suitable choice of dyes and synthetic strategy may, in particular, yield a very bright nanosystem. Silica nanoparticles thus offer unique potential in the nanotechnology arena, and further improvement and optimization could substantially increase their application in fields of high social and economic impact, such as medical diagnostics and therapy, environmental and food analysis, and security. This paper describes silica-based, multicomponent nanosystems with intrinsic directional energy- and electron-transfer processes, on which highly valued functions like light harvesting and signal amplification are based.

1. Introduction

1.1. Photochemistry and Nanotechnology

Photochemistry is a constantly developing field, and over the last decades the interest of researchers has shifted from the study of purely molecular aspects to supramolecular architectures[1] and nanostructures.[2–6] Multicomponent systems are, in fact, an ideal platform for making nanosized functional photochemical systems that mix elementary processes (light absorption and emission, energy transfer and electron transfer) to obtain more complex processes (directional excitation energy migration, or multielectron photoinjection).[2] These architectures can also produce an increased signal-to-noise ratio compared to traditional dyes. All these features are very appealing for analytical applications in diverse fields of great social and economical impact, such as medical diagnosis, imaging, food and beverage analysis, environmental monitoring, and security.[7] In this context, luminescent nanoparticles are particularly versatile components that have already been used in many fields thanks in part to their extreme brightness. Their intrinsic adaptability derives both from the wide range of materials that can be used to engineer them (carbon, metals,[8] metal oxides, semiconductors, silica, polymers[9]) and from their modular design. Of all the various possibilities, we believe that silica-based luminescent nanoparticles offer the most appealing solutions to important analytical problems, and particularly those related to medical diagnostics and imaging[10] and to the development of nanotheranostic devices.[10–17] Silica is not in fact intrinsically toxic, although more in-depth investigations are under way to completely rule out possible hazards related to the tiny dimensions of nanoparticles. Preliminary experiments point in favor of their benign nature, even supporting their use for in vivo imaging and therapy.[12,13,18,19] Moreover, their quite simple and affordable synthesis and the ability to tune them helps to make dye-doped silica nanoparticles (DDSNs) one of the best candidates for biomedical applications.[13,19]

There are other nanocolloids that may in many cases offer a valuable alternative to DDSNs, such as dye-doped latex nanoparticles or intrinsically luminescent nanomaterials. The first are already used and commercialized[20–22] and offer some of the advantages presented by silica, while among the latter family, the most important class is that of semiconductor

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nanocrystals, generally called quantum dots (QDs). These require harsh conditions of synthesis but also have very interesting photophysical properties (resistance to photobleaching, broad and intense absorption, narrow and generally intense fluorescence, large Stokes shift) which can easily be tuned by changing the size and material.[12,18,23] However, their most common formulations contain toxic constituent elements like cadmium, and this could raise concerns about disposal as well as induce cytotoxicity as a result of accumulation caused by oxidative degradation in cells.[13] To eliminate such possible drawbacks, recent research trends are moving towards the development of poison-free QDs.

In this article we will focus on DDSNs, starting from a description of their synthesis methods, to then discuss several systems, largely taken from our experience, that are designed, trying to maximize their brightness, for applications as luminescent or electroluminescent labels and chemosensors.

1.2. Dye-Doped Silica Nanoparticles

Silica is a hydrophilic material that is photophysically inert; that is, it is transparent to visible light and is not involved in energy- and electron-transfer processes. Consequently, the photochemical properties of DDSNs are mainly conferred by the doping species and, when present, by capping agents. Photoactive matrixes, on the contrary, may be involved in photodecomposition processes (titania) or simply cause quenching of the luminescence of chromophores via electron- or energy-transfer processes (silver and gold).[24] Furthermore, each DDSN may contain many fluorophores and reach a molar absorption coefficient ε that easily overcomes $10^6\,L\,mol^{-1}\,cm^{-1}$ with only a dozen dyes. The silica matrix can also protect the dyes segregated inside the nanoparticle from external chemicals, thus increasing their (photo)stability and, in many cases, their luminescence quantum yield Φ. For these reasons, DDSNs generally show impressively high brightness, which is defined as the product $\varepsilon\Phi$.[7]

Silica nanoparticles are also particularly interesting nanomaterials with regard to their synthesis, which is based on modular approaches, is flexible, and requires very mild conditions, as will be discussed in more detail in Section 2.

2. Synthesis of Functionalized Silica Nanoparticles

The controlled preparation of colloidal silica was proposed for the first time by Kolbe in 1956 and then developed by Stöber in the late 1960s.[25] Preparation of the first DDSNs was made possible thanks to an idea by van Blaaderen of condensing trialkoxysilane derivatives of fluorescent molecules with the monomeric tetraethoxysilane (TEOS) precursor during nanoparticle growth, yielding systems in which organic dyes are covalently linked to the silica matrix.[26,27] Apart from the methodology presented by Stöber and modified by van Blaaderen, other strategies have recently been developed, based mainly on reverse microemulsions[28,29] or on direct micelles as templates (see Figure 1).[12,30] The chemical process underlying the formation of silica nanoparticles is in all cases the controlled hydrolysis of TEOS molecules and their catalyzed condensation; the methods basically differ in their reaction medium.

The Stöber method uses an ethanol/water/ammonia solution, while the reverse microemulsion method uses a stable and macroscopically isotropic dispersion of a surfactant

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Riccardo Juris received his MSc in Chemistry at the University of Bologna in 2006 with a thesis on the design and synthesis of new ligands for integrins $\alpha v\beta 3$. He is currently a PhD student in the Photochemical Nanosciences Laboratory at the same university. His research interests include the synthesis of new fluorescent molecules along with the fabrication of dye-doped silica nanoparticles. Moreover, he collaborates with Aczon S.r.l. for the exploitation of nanoparticles in biotechnology applications.

Damiano Genovese received his MSc in Photochemistry and Chemistry of Materials at University of Bologna in 2007. He spent six months working on a molecular device for electrochemical release of calcium ions at the Ecole Normale Superieure with C. Amatore in Paris (France). He also recently worked on the flow of fluorescent colloidal pastes in constricted microfluidic channels in the group of D. Weitz at Harvard University (USA). He is currently a PhD student working on the photophysics of interacting dyes in nanosystems.

Marco Montalti was born in Cesena (Italy) in 1971. He obtained a PhD in Chemical Sciences in 2001 at the University of Bologna. In 1999 he was a research assistant at Tulane University (LA, USA); since 2002 he has been an assistant professor at the Faculty of Mathematical Physical and Natural Sciences of the University of Bologna. His research interests are in luminescent supramolecular systems and nanoparticles for sensing and energy processing.

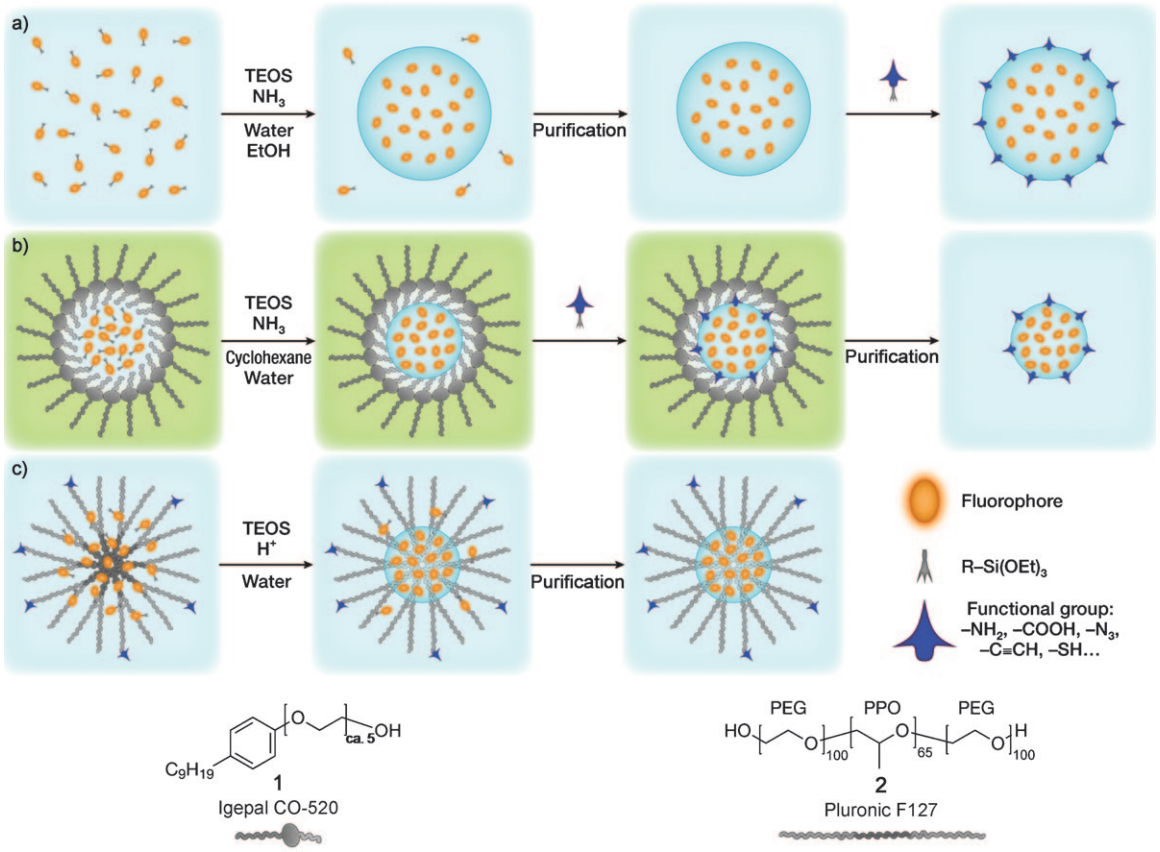

Figure 1. Strategies for the synthesis of silica nanoparticles described in the text. a) Stöber–van Blaaderen method; b) reverse microemulsion or water-in-oil method; c) synthesis of silica-core/PEG-shell nanoparticles according to Ref. [30].

and water in a hydrocarbon. In the latter case, hydrolysis and condensation are confined inside the aqueous nuclei of the inverted microemulsion, where TEOS and the organic precursors diffuse during the reaction. In general, with both preparations it is possible to obtain highly monodisperse nanoparticles with a diameter ranging from 15 to 800 nm, by maintaining careful control of the reaction conditions.

One very interesting alternative proposed by Maitra et al. in 2002[31] and then investigated in depth by Prasad and co-workers is the use of lipophylic organosilane derivatives, such as octyltriethoxysilane (OTES) or vinyltriethoxysilane

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Enrico Rampazzo studied chemistry and completed his PhD (2005) at the University of Padua (Italy) under U. Tonellato. After a postdoctoral stay in Padua with Fabrizio Mancin, he was a FIRB research fellow at the University of Bologna until 2008. He is now a postdoctoral fellow with Luca Prodi at the Photochemical Nanosciences Laboratory (Bologna). His research interests focus on the synthesis of luminescent dyes, sensors, and (electro)luminescent self-organized systems based on dye-doped silica nanoparticles for the development of sensors and labels.

Nelsi Zaccheroni was born in 1968 and has had a permanent position as Assistant Professor in Chemistry at the University of Bologna since January 1999. She obtained a degree in Chemistry and then a PhD in Chemical Sciences in 1997 at the same University in the Laboratory of Photochemistry and Supramolecular Chemistry. She spent one year as a postdoctoral fellow within a TMR-CEE project at the University College of Dublin (Ireland). Her research interests are mainly focused on luminescent systems for imaging and sensing, including those based on metal and silica nanoparticles.

(VTES), in direct micelle solution. The resulting nanoparticles, called ORMOSIL (organic modified silica), have a diameter in the 20–30 nm range, some degree of mesoporosity, and are particularly suitable for developing diagnosis (bioimaging)[32] and therapy (PDT) systems.[12] Using a similar strategy, Mancin and co-workers recently developed different PEGylated DDSNs ($d = 20$, 50, 100 nm) containing an alkoxysilane derivative of the cyanine dye IR775.[33]

The common feature of all these methods of synthesis is the need to add a certain amount (usually ≤ 1 mol% versus TEOS) of the fluorophore required to obtain a fluorescent material. The choice of method consequently depends mostly on the solubility of the fluorophore itself; the microemulsion, for example, is preferred when the emitting species is very soluble in water. The luminophore can be added directly during growth of the nanoparticles without any derivatization to be simply physically entrapped in the structure, or previously derivatized with an alkoxysilane moiety to enable its covalent binding to the silica matrix. The latter method is generally preferred to overcome dye leaching, even though proper functionalization of the fluorophores is not always an easy step. During the preparation of these nanomaterials, the condensation of trialkoxysilane derivatives is almost quantitative, but a purification step such as centrifugation,[28] ultrafiltration, or dialysis may be needed. One very interesting alternative is flow field–flow fractionation, a powerful technique to size sort and purify fluorescent colloids of nanometric dimensions.[34]

It should be noted that for the derivatization of the nanoparticle surface with dyes, chemosensors, PEG moieties, or bioactive molecules, such as oligopeptides, proteins, antibodies, or DNA, proper functional groups need to be introduced, such as those used in the most common coupling methods ($-NH_2$, $-COOH$, $-SH$),[12,18,19,32] or in click-chemistry-based reactions (azide/alkyne).[35] In this context, the development of one-pot synthesis methods could considerably stimulate the field, and with this goal in mind we recently proposed a one-pot approach (Figure 1c) for the synthesis of functionalized core–shell DDSNs.[30] The method is based on the preparation of micelles of Pluronic F127 in water. This surfactant is a nonionic triblock copolymer terminating in primary hydroxy groups with a poly(ethylene glycol)–poly(propylene oxide)–poly(ethylene glycol) structure (PEG-PPO-PEG; MW 12600). TEOS and many kinds of dyes can be added to the Pluronic water solution, thereby leading to their inclusion inside the micelles. The condensation of TEOS in acidic conditions then encourages the formation of a silica core containing dozens of covalently linked or physically entrapped dyes, surrounded by an outer shell formed by the PEG part of the surfactant. The final material is a versatile multicompartment system characterized by high water solubility, excellent monodispersity, stability, and brightness. The structural properties of these nanoparticles are independent of the dye included, which may be either an organic fluorophore or a metal complex. In particular, as can be seen from TEM images, the silica core has a diameter $d_C = (10 \pm 1)$ nm, while the hydrodynamic diameter d_H, measured by dynamic light scattering (DLS), is (25 ± 3) nm (Figure 2). These silica-core/PEG-shell nanoparticles also maintain their

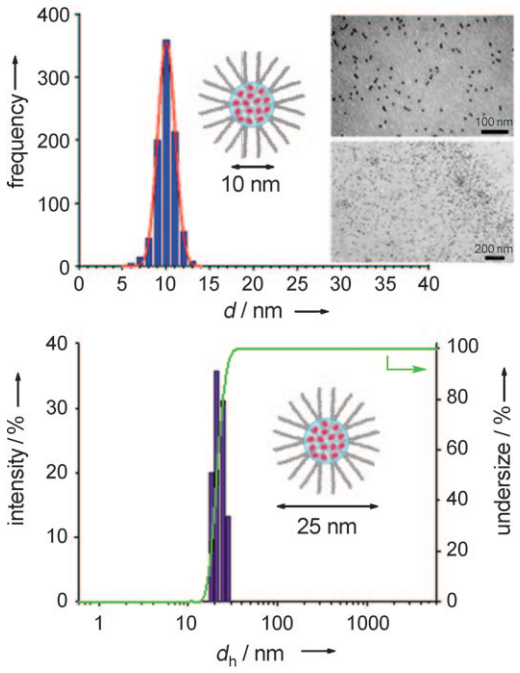

Figure 2. Size distribution of silica-core/PEG-shell nanoparticles according to a) TEM images and b) DLS distribution (in water). (Adapted from Ref. [30].)

characteristics under simulated physiological conditions (phosphate-buffered saline and bovine serum albumin; PBS-BSA), and in environments typical of most immunochemical protocols. In particular, the presence of the outer PEG shell is of great importance, especially for biological applications. PEG is, in fact, an FDA approved substance and it is the gold standard for stealth polymers in the emerging field of polymer-based drug delivery.[36] As it is not readily recognized by the immune system, this PEG shell increases the chances of this kind of DDSN to reach target sites in patients before being cleared from the body.

3. Applications of Luminescent Silica Nanoparticles

3.1. Applications in Photoluminescence

3.1.1. Labels

As already mentioned in Section 1, DDSNs are particularly suitable materials for producing bright labels, which is of great interest for imaging and in particular medical diagnostics for methodologies including histology and flow citometry.[18,19,37] To optimize their performance, it is however very important to further comprehend their structure. The mutual disposition of the fluorophores inside the silica matrix may indeed have a strong influence on the formation of aggregates and on the efficiency of homo- and hetero-energy transfer processes inside the system, thus determining its quantum yield and its ability to perform complex functions. Significant efforts have in fact been made to investigate the organization of active molecules within nanostructures. Wiesner and co-workers, for example, studied the changes

in brightness of SNs ($d = 30$ nm) covalently doped with the same amount of tetramethylrhodamine (TRITC) but presenting different core–shell structures by means of fluorescence correlation spectroscopy (FCS) and fluorescence lifetime analysis. Depending on this latter feature, a brightness factor of 7.5 to 27 times the TRITC molecules was measured for these systems.[38] In this research, our approach was to synthesize different families of pyrene-doped nanoparticles by adopting the Stöber strategy and monitor their formation and growth (Figure 3).[39]

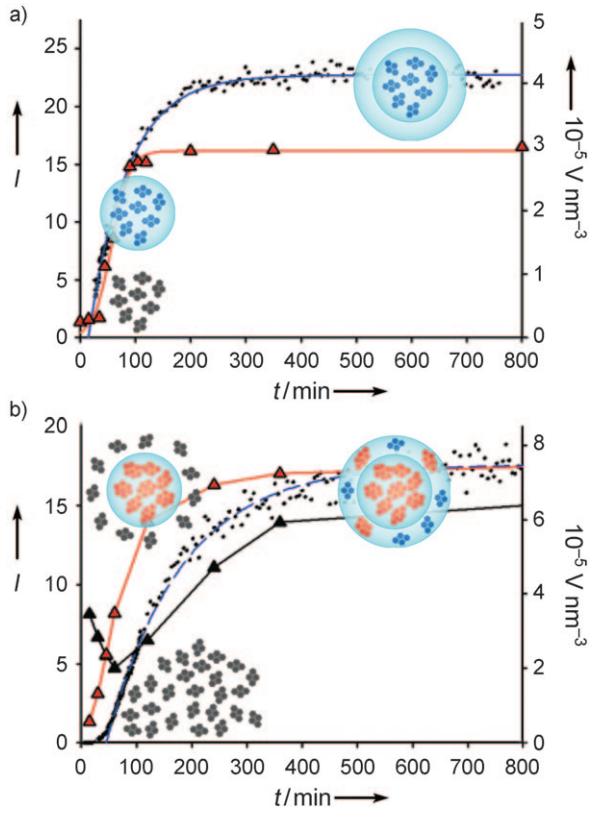

Figure 3. a) Fluorescence intensity ($\lambda_{exc} = 345$ nm) at 375 nm (red triangles) during the formation of nanoparticles with 0.1% doping. b) Fluorescence intensity ($\lambda_{exc} = 345$ nm) at 375 (black triangles) and 480 nm (red triangles) during the formation of nanoparticles with 1% doping. The black dots represent the hydrodynamic volume measured by DLS. (Adapted from Ref. [39].)

We exploited the dependence of pyrene photophysical properties on the local environment, and on the concentration used to explore the inner structure of the nanoparticles during their growth. In particular, by using its fluorescence quantum yield and the excimer and monomer emission ratio and correlating these with the hydrodynamic volume of the system as measured by DLS, we were able to trace the inclusion of the dye inside the nanoparticle. The structure of the material turned out to differ substantially in relation to the initial concentration of the dye. In a low-concentration regime of pyrene molecules, they are incorporated during nucleation and growth into an inner nucleus around which an undoped protective silica shell forms spontaneously. In contrast, at higher fluorophore concentration regimes, the formation of a small, densely doped nucleus is followed by the growth of a less densely doped shell. The Stöber strategy thus induces the spontaneous formation of core–shell structures, and the conventional picture of dyes homogenously dispersed in the nanospheres is unrealistic. This effect has to be taken into account when designing new materials to optimize their efficiency. For example, when using dyes such as fluorescein and rhodamine, which may give rise to self-quenching processes, their induced proximity in highly doped architectures could lead to a decrease of their quantum yield.[34] An elegant way of reducing this parasite process is to include a metal core (Au or Ag) in the structure to induce the so-called metal-enhanced fluorescence (MEF) effect. It has been hypothesized and observed that fluorophores that are held in close proximity to nanoscale noble metal surfaces present an increase in the quantum yield and a reduction of the fluorescence lifetime.[40–42] The MEF effect in fact leads to an increase of the radiative rate constant, thus making the fluorescent process more efficient at the expense of all of the nonradiative processes. An additional contribution to non-homogeneous behavior in DDSNs is made by environmentally sensitive fluorophores located near the surface presenting different properties to those located internally. The inner dyes are in fact shielded from the solvent while those on the surface can interact with it.[43] The relative number of fluorophores located in the two compartments can be controlled by changing the size of the nanoparticles, as the thickness of the solvent-permeable layer is not affected by their diameter. One possible strategy to overcome this problem is the creation of core–shell structures, as indicated, for example, in reference [18] or as recently elucidated by McDonagh and co-workers, who studied the internal architecture of SNs doped with a triethoxysilane derivative of an indodicarbocyanine (NIR664) by means of FCS and fluorescence lifetimes analysis.[44]

It must be stressed however, that different methods of synthesis may induce different dye distributions; for example, small nanoparticles prepared by micelle formation generally experience a more homogeneous fluorophore allocation, as happens to the structures synthesized using the method presented in Figure 1c. This method enables the preparation of systems with luminescence maxima spanning from the violet to near-infrared region (Figure 4). Recently,[45] we elucidated the structure and the photophysical properties of these core–shell SNs doped with a trialkoxy silane derivative of rhodamine B. Using an innovative approach, it was possible to determine the average number of fluorophores per nanoparticle (10), which is a fundamental parameter for quantifying the brightness of the single particle. A comparison between this system and QD585[46] is summarized in Figure 5 and in Table 1. The brightness of such DDSNs upon excitation at 532 nm (a common laser wavelength) reaches that of these highly luminescent probes, and even exceeds it at a high dye doping ratio (25 dyes per particle). We also explored the possibility of inducing energy transfer from the core to the periphery by hosting a cyanine dye (**4**; Figure 6) in the PEG shell. This process proved efficient and almost quantitative (FRET efficiency > 95%), thus shifting particle emission to the 700–750 nm region. This wavelength range is

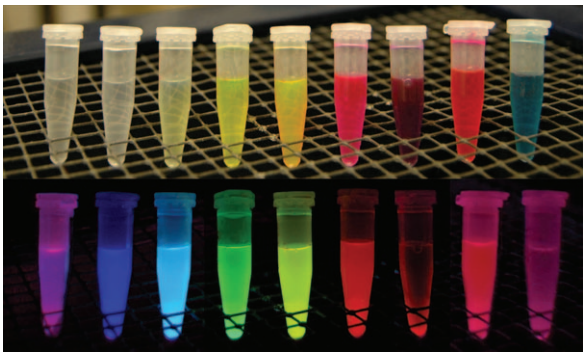

Figure 4. Images of different solutions of silica-core/PEG-shell nanoparticles prepared as described in Ref. [30] under ambient light conditions (above) and UV light (below).

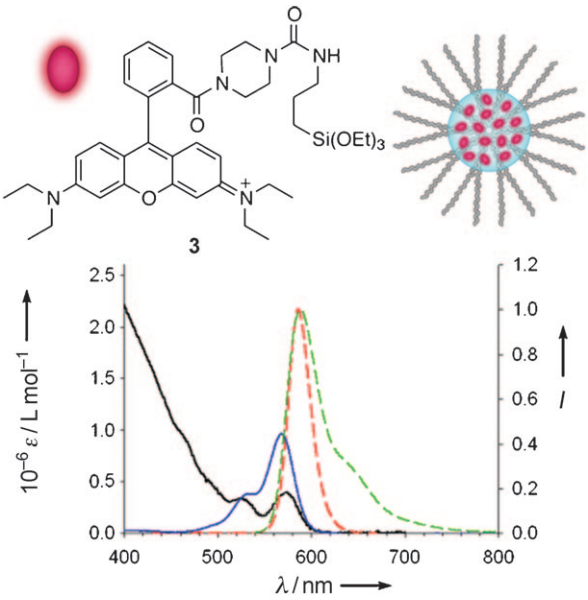

Figure 5. Absorption (full line) and fluorescence spectra (dotted line) of QD585 (black-red) and $\mathbf{3}_{10}$@NP (cyan-green) in aqueous solutions.

Table 1: Absorption, quantum yields, and brightness of free **3**, $\mathbf{3}_{10}$@NP, $\mathbf{3}_{25}$@NP, and QD585.[45,46]

	3 (EtOH)	$\mathbf{3}_{10}$@NP	$\mathbf{3}_{25}$@NP	QD585
ε [L mol^{-1} cm^{-1}] (532 nm)	3.7×10^4	3.8×10^5	9.5×10^5	3.0×10^5
Φ	0.70	0.40	0.40	0.40
brightness	2.6×10^4	1.5×10^5	3.8×10^5	1.2×10^5

of particular interest for in vivo studies as it is located in the tissue-transparency window,[9,47] and DDSNs offer some advantages compared to other nanoparticle-based systems. In fact, this kind of application requires both excitation and emission wavelengths to be above 650 nm, and a proper choice of the doping emitting dye in DDSNs can easily maximize their excitation efficiency in this window. In contrast, for other materials such as NIR-QDs, which have intrinsically quite poor and decreasing extinction coefficients

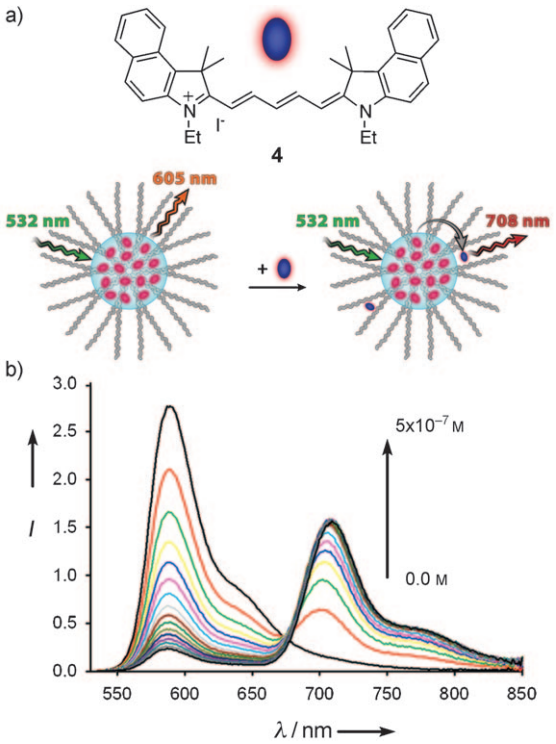

Figure 6. a) Representation of the energy-transfer mechanism between the hosted **4** and $\mathbf{3}_{10}$@NP (see Figure 5). b) Fluorescence spectra of a 1×10^{-7} M aqueous solution of $\mathbf{3}_{10}$@NP during the titration with **4** upon excitation at 532 nm. Each variation corresponds to the addition of 3.3×10^{-8} mol L^{-1} of **4**. (Adapted from Ref. [45].)

above 600 nm,[48] this may be a rather complex matter. Silica nanoparticles showing an interesting emission in the NIR region are already available,[49,50] but in our opinion there is a plenty of room for improvement.

Besides brightness, DDSNs have other very valuable features. For example they can be engineered to present a large Stokes shift, which by a substantial suppression of scattering and Raman effects facilitates the development of suitable species for bar-coding and multiplexing analysis. The simplest strategy is the one used by Wiesner and co-workers,[51] who synthesized DDSNs containing commercial fluorophores characterized by an intrinsic large Stokes shift and derivatized with an alkoxy silane group. This approach is straightforward but may be limited by the relatively small number of organic dyes with this property. Another approach is to exploit efficient energy transfer processes between two or more species, such as metal complexes or organic dyes, as mentioned above. Zhao and co-workers developed a silica-based system doped with ruthenium(II) and terbium(III) complexes,[52] while Konovalov and co-workers proposed an analogous system, but even more red-shifted, based on species containing ruthenium(II) and ytterbium(III).[53] This strategy may also make it possible to obtain a set of nanoparticles presenting emissions of different colors but which can be efficiently excited at the same wavelength,[54,55] a feature which can only otherwise be achievable using QDs. A proper design of efficient, intraparticle FRET could yield

DDSNs able to perform other highly valuable functions, such as light harvesting, signal processing, and energy conversion.

When the silica core of a nanoparticle is surrounded by a porous shell, FRET can also be diagnostic of the reversible inclusion of a molecule in this external part of the system, as in the case presented above of the cyanine dye **4**. In such an architecture, the hosted molecules can be extracted (released) by apolar matrixes. In general, this result shows that hydrophobic moieties can be hosted in the outer shell and can subsequently be released in a more lipophilic environment. These nanoparticles may therefore be particularly suitable for the design of nanotheranostic systems, which is one of the main subjects of our current research activity. With a proper choice of the components, they can in fact simultaneously behave as fluorescent labels (for imaging applications) and as carriers for the delivery of hydrophobic compounds (drugs).

3.1.2. Chemosensors

The need to develop sensors for different target analytes is well recognized and confirmed by the increasing research efforts spent on the preparation of increasingly efficient sensory devices.[56] Many strategies can be applied in this field. The first is based on the well-established receptor–spacer–luminophore method and on the optimization of all the steps that lead to the chemosensor response. More innovative solutions are however also being proposed that often involve a multidisciplinary approach. The advent of nanotechnology has opened up a wealth of new possibilities, as described in part by a very recent review.[57] The design of analyte-sensitive fluorescent probes based on silica nanoparticles may, for example, offer important advantages compared to their molecular counterparts. The solubility and stability of the system in solution is conferred mainly by the silica structure; therefore, many interesting compounds that cannot be used in an aqueous environment can instead be conveniently inserted, or covalently bound, to the silica matrix and also work in water.

Moreover, the ability to easily obtain ratiometric systems permits quantitative determinations. This can be achieved by inserting a fluorescent probe together with an analyte-independent fluorophore in the silica-based nanosensor. The most common methods use a design that can be structured to prevent all of the electronic interactions between the two fluorescent units, thus eliminating the possibility of energy-transfer processes. Typically, the reference dye is included in the core of the nanoparticles, while the probe is inserted in an outer shell or bound to the surface.[6,57,58] In other cases, interchromophoric interactions are on the contrary not only desirable but essential to obtain valuable functions such as signal amplification effects. Regarding chemosensors, signal amplification makes for much higher sensitivity, as the binding of each single target analyte causes changes in the photophysical properties of many fluorophores at the same time.

Tonellato et al.[60] have proposed a very elegant approach to bring fluorescent dyes and receptors close enough to communicate with each other and to possibly undergo collective processes. They used silica nanoparticles (NPs) as a template for self-organization in close proximity of the two separated subunits. This method offers interesting advantages, as it minimizes the synthetic effort and maximizes versatility, thus making it possible to tune the properties of the structure by simply adjusting the nature and ratio of the components. They showed interesting collective effects in these systems, demonstrating an amplified quenching luminescence response of the signaling units to the presence of the target analyte (copper ions). The same effect was shown by silica nanoparticles prepared following a more traditional approach and simply doped with chemosensor **5**. In this system, the binding of a single Cu^{2+} ion was in fact able to quench 13 dansyl moieties through energy- and electron-transfer processes (Figure 7).[59]

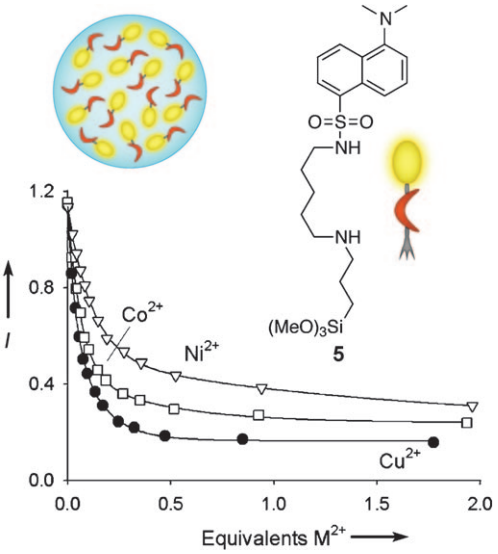

Figure 7. Fluorescence intensity changes at 510 nm (λ_{exc} = 335 nm) caused by the addition of different quenching metal ions to a solution of silica nanoparticles functionalized with **5**. (Adapted from Ref. [59]).

Amplified enhancement of the luminescence, that is, an amplified off/on chemosensor response, would be an even more valuable process that would yield materials with an extremely high signal-to-noise ratio output. Unfortunately, this effect is also much more difficult to achieve, as the energy, wherever absorbed, must be efficiently conveyed only towards the probe complexing the analyte (Figure 8), which in turn should emit a photon. To achieve this goal, a very fast homo-energy transfer between the noncomplexed units must take place accompanied by a very efficient hetero-energy transfer between the uncomplexed units (acting as donors) and the complexed units (acting as acceptors). This process requires high overlap integrals for both homo- and hetero-energy transfer processes and short distances between all the different units. Bearing this in mind and taking advantage of previous results,[61] we grafted **6** (a very well-known probe for Zn^{2+} ions) onto the surface of the nanoparticles rather than inside the matrix, as has already been reported for the same chemosensor.[58] As a result, the system showed a 50% off/on amplified response, which although modest was the first

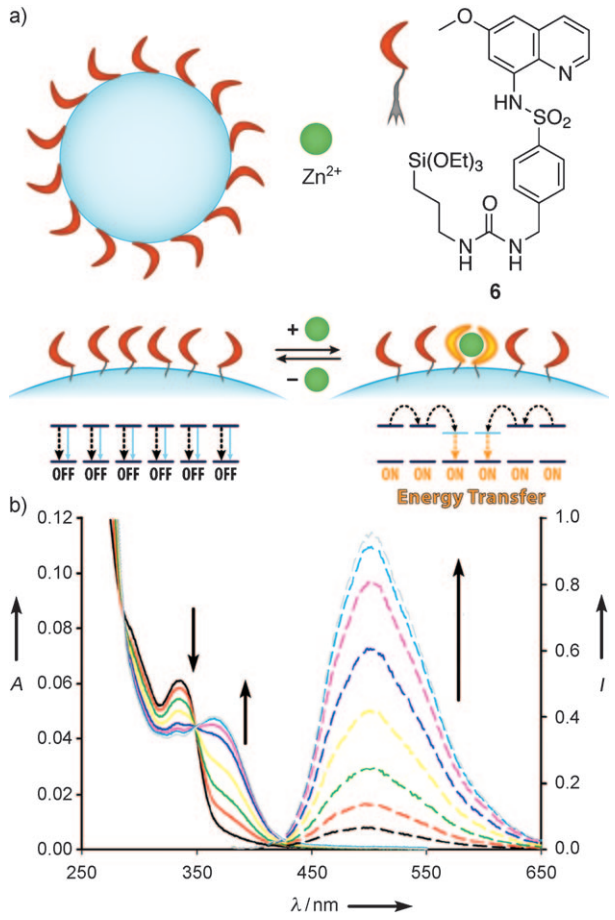

Figure 8. a) Representation of off/on amplification processes in multichromophoric systems. b) Absorption and emission spectra of NPs functionalized with **6** upon addition of increasing amounts of Zn^{2+} ions. (Adapted from Ref. [62].)

example ever reported of amplified enhancement of the luminescence signal in nanoparticle-based chemosensors.[62] Furthermore, the proximity between the different receptors also led to an increase of the association constant, and therefore of the affinity of the system to the target analyte.

3.2. Applications in Electrochemiluminescence

Electrochemiluminescence (ECL) offers better performance than photoluminescence (PL) in many applications.[63] The possibility of avoiding excitation sources in fact leads to remarkably low noise, signal specificity, and easier design of the device. At the moment, the search for increasing sensitivity and wider applications are the main targets of researchers working in this field. ECL has also been used recently, for example, in microscopy imaging,[64] thus opening up new horizons for advanced immunoassays and fluorescent in situ hybridization (FISH) analyses. Moreover, the increasing importance of multiplex analysis leads towards the design of species presenting ECL signals in spectral windows different from that (around 610 nm) typical of $[Ru(bpy)_3]^{2+}$ and related complexes, which are by far the most widely used ECL dyes.

As for PL, the use of multichromophoric units such as DDSNs increases the sensitivity of ECL thanks to a number of synergic contributions, such as the higher number of active units, the higher luminescence quantum yield of the excited state generated by the electrochemical reaction, and the possibility of using very bright dyes that are otherwise insoluble in water. Furthermore, the versatility of NPs means a magnetic core[65] can be used for their pre-concentration, or to derivatize their surface to induce NPs cluster formation to maximize the number of active species.[66]

As ECL is a diffusion-sensitive technique, the use of DDSNs with rather low diffusion coefficients does however present some possible drawbacks. For this reason, the best results reported to date have been obtained in applications that bypass the diffusion step, immobilizing the NPs on the electrode surface. We would like to mention here just two interesting examples, the monitoring of mouse IgC through an immunosensor based on immobilized DDSNs on a glassy carbon electrode,[67] and the detection of biomarkers through the creation of an immunosensor on a gold electrode.[68]

Another possible problem is related to the fact that the speed of the (electro)chemical processes leading to the formation of the excited state may be significantly slower in the silica matrix, even when the small molecules of co-reactant and of intermediates can diffuse through the pores of the silica structure. To minimize this effect, it is best to prepare smaller NPs and to choose synthetic strategies that do not lead to core–shell structures in which the dyes are concentrated mainly in the core, as typically happens using the Stöber method. It is in any case essential to estimate how many of the dyes segregated in the NP can actively participate in generating the ECL signal. In this context, we developed a model that we applied to DDSNs having a diameter of 18 nm obtained using the silane derivative **7**, demonstrating that about 35 % of the $[Ru(bpy)_3]^{2+}$ units were not electrochemically active. Despite this drawback, it was possible to obtain from a self-assembled monolayer (SAM) of these DDSNs on a gold electrode (Figure 9) an ECL signal that was a thousand-fold higher than that obtained from a compact SAM of an analogous $[Ru(bpy)_3]^{2+}$ complex.[29] Such result opens up new development perspectives for ultra-sensitive bioanalysis to prevent or limit the use of amplification methods such as the polymerase chain reaction (PCR). Chen et al.[69] recently obtained interesting results in this direction, coupling the amplified ECL signal of multiple $[Ru(bpy)_3]^{2+}$ units assembled on gold nanoparticles with the barcode method for protein or DNA detection.[70]

As already mentioned, there is another very important possible contribution of DDSNs to the improvement of the ECL technique that must not be ignored: the possibility of using them as vehicles to carry very bright dyes that are otherwise insoluble in water into aqueous media. Ruthenium(II) complexes are in fact by far the most dominant ECL-active species used in aqueous solutions, but there is a large library of other very efficient ECL active compounds that cannot be exploited in most applications owing to their poor solubility.[71] In this context, we recently trapped the completely insoluble neutral iridium(III) complex **8** inside silica-core/PEG-shell nanoparticles that showed stable ECL emis-

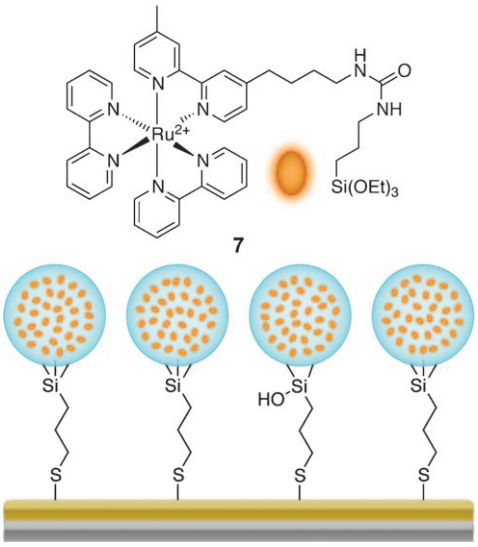

Figure 9. Representation of a NP SAM on a gold electrode. The NPs are doped with **7**.

sion in water (Figure 10).[30] We are now following the same approach to investigate other iridium(III) complexes and hydrophobic organic emitters to enhance ECL emission intensity and obtain a large set of different colors. This approach would increase the already high potential of the ECL technique, introducing multicolor systems for an easier and more efficient multiplex analysis.

4. Conclusions and Perspectives

Recent developments in the design and preparation of nanosized materials have provided powerful and versatile

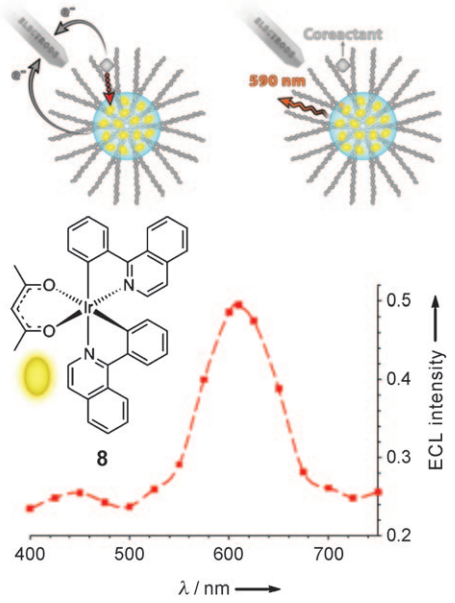

Figure 10. Above: representation of a mechanism observed for the generation of the ECL signal; below: ECL spectrum of the NPs doped with **8**. (Adapted from Ref. [30].)

platforms for addressing crucial issues in sensing, imaging, and molecular testing. The most important steps forward are expected in the field of nanomedicine, which is expected to lead to important breakthroughs in the near future in the treatment and prevention of many diseases, including tumors, with significant benefits to our quality of life.

In this context, dye-doped silica nanoparticles may be seen as a very promising platform for obtaining bright systems that perform complex and valuable functions for all the applications in which luminescence-based techniques are needed. In particular, with the proper choice of synthetic procedures, DDSNs can present very high monodispersity and remarkable stability and solubility in aqueous solutions even in physiological conditions combined with low intrinsic toxicity. They can contain several active species per nanoparticle, such as (electrochemi-)luminescent dyes, and as a consequence, they can present very high absorption coefficients (much more than $10^6\,L\,mol^{-1}\,cm^{-1}$) and luminescence quantum yields, and therefore impressively high brightness. Furthermore, these multichromophoric systems can show collective energy- and electron-transfer processes on which highly valuable functions, such as signal amplification and light harvesting, are based. This last function, besides applications in solar energy conversion,[72] makes it possible to obtain materials presenting emissions of different colors but with the same excitation wavelength.

We have also discussed above how silica-core/PEG-shell nanoparticles can provide additional advantages owing to the presence of a stealth layer and the possibility of hosting water-insoluble materials in the outer PEG shell. Interestingly, a proper choice of the hosted molecules can tune the fluorescence color and open up the possibility for drug-delivery experiments and the preparation of nanotheranostic systems.

All of these features make silica nanoparticles unique platforms in the nanotechnology arena. DDSNs in particular are already competitive in many applications with the most commonly used commercial dyes, including fluorescent proteins and QDs. Nevertheless, we strongly believe that new powerful possibilities in the field of silica nanoparticles have still to be explored. Improvements of the methods of synthesis, greater control of collective processes, and an enlargement of the library of the doping dyes will in fact enable ambitious applications that could have a potentially very high impact on our everyday life.

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[1] V. Balzani, F. Scandola, *Supramolecular Photochemistry*, Ellis Horwood Ltd, Avon (U.K.), **1991**.
[2] V. Balzani, A. Credi, M. Venturi, *Molecular Devices and Machines*, Wiley-VCH, Weinheim, **2008**.
[3] D. M. Bassani, *Chimia* **2006**, *60*, 175–178.

[4] A. P. de Silva, Y. Leydet, C. Lincheneau, N. D. McClenaghan, *J. Phys. Condens. Matter* **2006**, *18*, S1847 – S1872.
[5] S. Fukuzumi, T. Kojima, *J. Mater. Chem.* **2008**, *18*, 1427 – 1439.
[6] S. Bonacchi, D. Genovese, R. Juris, F. Mancin, M. Montalti, L. Prodi, E. Rampazzo, N. Zaccheroni in *Handbook of Porphyrin Science*, *Vol. 12* (Eds.: K. M. Kadish, K. M. Smith, R. Guilard), World Scientific, New York, **2010**, chap. 5.
[7] L. Prodi, *New J. Chem.* **2005**, *29*, 20 – 31, and references therein.
[8] E. Boisselier, D. Astruc, *Chem. Soc. Rev.* **2009**, *38*, 1759 – 1782.
[9] X. He, K. Wang, Z. Cheng, *Nanomed. Nanobiotech.* **2010**, *2*, 349 – 366.
[10] D. Shi, *Adv. Funct. Mater.* **2009**, *19*, 3356 – 3373.
[11] K. Riehemann, S. W. Schneider, T. A. Luger, B. Godin, M. Ferrari, H. Fuchs, *Angew. Chem.* **2009**, *121*, 886 – 913; *Angew. Chem. Int. Ed.* **2009**, *48*, 872 – 897.
[12] K.-T. Yong, I. Roy, M. T. Swihart, P. N. Prasad, *J. Mater. Chem.* **2009**, *19*, 4655 – 4672, and references therein.
[13] U. A. Gunasekera, Q. A. Pankhurst, M. Douek, *Targ. Oncol.* **2009**, *4*, 169 – 181.
[14] N. Doshi, S. Mitragotri, *Adv. Funct. Mater.* **2009**, *19*, 3843 – 3854.
[15] L. Wang, M. B. O'Donoghue, W. H. Tan, *Nanomedicine* **2006**, *1*, 413 – 426.
[16] J. Choi, A. A. Burns, R. M. Williams, Z. Zhou, A. Fleskен-Nikitin, W. R. Zipfel, U. Wiesner, A. Y. Nikitin, *J. Biomed. Opt.* **2007**, *12*, 064007.
[17] J. Kim, Y. Piao, T. Hyeon, *Chem. Soc. Rev.* **2009**, *38*, 372 – 390.
[18] A. Burns, H. Ow, U. Wiesner, *Chem. Soc. Rev.* **2006**, *35*, 1028 – 1042, and references therein.
[19] L. Wang, K. Wang, S. Santra, X. Zhao, L. R. Hilliard, J. E. Smith, Y. Wu, W. Tan, *Anal. Chem.* **2006**, *78*, 646 – 654.
[20] Y.-K. Gong, K. Nakashima, *Chem. Commun.* **2001**, 1772 – 1773.
[21] L. C. Katz, A. Burkhalter, W. J. Dreyer, *Nature* **1984**, *310*, 498 – 500.
[22] S. Prilloff, J. Fan, P. Henrich-Noack, B. A. Sabel, *Eur. J. Neurosci.* **2010**, *31*, 521 – 528.
[23] I. L. Medintz, H. T. Uyeda, E. R. Goldman, H. Mattoussi, *Nat. Mater.* **2005**, *4*, 435 – 446.
[24] S. Kühn, G. Mori, M. Agio, V. Sandoghdar, *Mol. Phys.* **2008**, *106*, 893 – 908.
[25] W. Stöber, A. Fink, E. Bohn, *J. Colloid Interface Sci.* **1968**, *26*, 62 – 69.
[26] A. Van Blaaderen, A. Imhof, W. Hage, A. Vrij, *Langmuir* **1992**, *8*, 1514 – 1517.
[27] N. A. M. Verhaegh, A. Van Blaaderen, *Langmuir* **1994**, *10*, 1427 – 1438.
[28] R. P. Bagwe, C. Yang, L. R. Hilliard, W. Tan, *Langmuir* **2004**, *20*, 8336 – 8342.
[29] S. Zanarini, E. Rampazzo, L. Della Ciana, M. Marcaccio, E. Marzocchi, M. Montalti, F. Paolucci, L. Prodi, *J. Am. Chem. Soc.* **2009**, *131*, 2260 – 2267.
[30] S. Zanarini, E. Rampazzo, S. Bonacchi, R. Juris, M. Marcaccio, M. Montalti, F. Paolucci, L. Prodi, *J. Am. Chem. Soc.* **2009**, *131*, 14208 – 14209.
[31] S. Das, T. K. Jain, A. Maitra, *J. Colloid Interface Sci.* **2002**, *252*, 82 – 88.
[32] R. Kumar, I. Roy, T. Y. Ohulchanskky, L. A. Vathy, E. J. Bergey, M. Sajjad, P. N. Prasad, *ACS Nano* **2010**, *4*, 699 – 708.
[33] I. M. Rio-Echevarria, F. Selvestrel, D. Segat, G. Guarino, R. Tavano, V. Causin, E. Reddi, E. Papini, F. Mancin, *J. Mater. Chem.* **2010**, *20*, 2780 – 2787.
[34] M. Montalti, L. Prodi, N. Zaccheroni, A. Zattoni, P. Reschiglian, G. Falini, *Langmuir* **2004**, *20*, 2989 – 2991.
[35] H. Mader, X. Li, S. Saleh, M. Link, P. Kele, O. S. Wolfbeis, *Ann. N. Y. Acad. Sci.* **2008**, *1130*, 218 – 223.
[36] K. Knop, R. Hoogenboom, D. Fisher, U. S. Schubert, *Angew. Chem.* **2010**, *122*, 6430 – 6452; *Angew. Chem. Int. Ed.* **2010**, *49*, 6288 – 6308.
[37] M.-C. Estévez, M. B. O'Donoghue, X. Chen, W. Tan, *Nano Res.* **2009**, *2*, 448 – 461.
[38] D. R. Larson, H. Ow, H. D. Vishwasrao, A. A. Heikal, U. Wiesner, W. W. Webb, *Chem. Mater.* **2008**, *20*, 2677 – 2684.
[39] E. Rampazzo, S. Bonacchi, M. Montalti, L. Prodi, N. Zaccheroni, *J. Am. Chem. Soc.* **2007**, *129*, 14251 – 14256.
[40] K. Aslan, M. Wu, J. R. Lakowicz, C. D. Geddes, *J. Am. Chem. Soc.* **2007**, *129*, 1524 – 1525.
[41] M. Martini, P. Perriat, M. Montagna, R. Pansu, C. Julien, O. Tillement, S. Roux, *J. Phys. Chem. C* **2009**, *113*, 17669 – 17677.
[42] M. Lessard-Viger, M. Rioux, L. Rainville, D. Boudreau, *Nano Lett.* **2009**, *9*, 3066 – 3071.
[43] M. Montalti, L. Prodi, N. Zaccheroni, G. Battistini, S. Marcuz, F. Mancin, E. Rampazzo, U. Tonellato, *Langmuir* **2006**, *22*, 5877 – 5881.
[44] S. Roy, R. Woolley, B. D. MacCraith, C. McDonagh, *Langmuir* **2010**, *26*, 13741 – 13746.
[45] E. Rampazzo, S. Bonacchi, R. Juris, M. Montalti, D. Genovese, N. Zaccheroni, L. Prodi, D. C. Rambaldi, A. Zattoni, P. Reschiglian, *J. Phys. Chem. B* **2010**, 114, 14605 – 14613.
[46] Y. Wu, G. P. Lopez, L. A. Sklar, T. Buranda, *Anal. Biochem.* **2007**, *364*, 193 – 203.
[47] E. İ. Altınoğlu, J. H. Adair, *Nanomed. Nanobiotech.* **2010**, *2*, 461 – 477.
[48] M.-K. So, C. Xu, A. M. Loening, S. S. Gambhir, J. Rao, *Nat. Biotechnol.* **2006**, *24*, 339 – 343.
[49] J. F. Bringley, T. L. Penner, R. Wang, J. F. Harder, W. J. Harrison, L. Buonemani, *J. Colloid Interface Sci.* **2008**, *320*, 132 – 139.
[50] E. Herz, H. Ow, D. Bonner, A. Burns, U. Wiesner, *J. Mater. Chem.* **2009**, *19*, 6341 – 6347.
[51] E. Herz, A. Burns, D. Bonner, U. Wiesner, *Macromol. Rapid Commun.* **2009**, *30*, 1907 – 1910.
[52] C. Wu, J. Hong, X. Guo, C. Huang, J. Lai, J. Zheng, J. Chen, X. Mu, Y. Zhao, *Chem. Commun.* **2008**, 750 – 752.
[53] S. V. Fedorenko, O. D. Bochkova, A. R. Mustafina, V. A. Burilov, M. K. Kadirov, C. V. Holin, I. R. Nizameev, V. V. Skripacheva, A. Yu. Menshikova, I. S. Antipin, A. I. Konovalov, *J. Phys. Chem. C* **2010**, *114*, 6350 – 6355.
[54] L. Wang, W. Tan, *Nano Lett.* **2006**, *6*, 84 – 88.
[55] X. Chen, M.-C. Estévez, Z. Zhu, Y.-F. Huang, Y. Chen, L. Wang, W. Tan, *Anal. Chem.* **2009**, *81*, 7009 – 7014.
[56] L. Basabe-Desmonts, D. N. Reinhoudt, M. Crego-Calama, *Chem. Soc. Rev.* **2007**, *36*, 993 – 1017.
[57] T. Doussineau, A. Schulz, A. Lapresta-Fernandez, A. Moro, S. Körsten, S. Trupp, G. J. Mohr, *Chem. Eur. J.* **2010**, *16*, 10290 – 10299.
[58] P. Teolato, E. Rampazzo, M. Arduini, F. Mancin, P. Tecilla, U. Tonellato, *Chem. Eur. J.* **2007**, *13*, 2238 – 2245.
[59] M. Montalti, L. Prodi, N. Zaccheroni, *J. Mater. Chem.* **2005**, *15*, 2810 – 2814.
[60] M. Arduini, E. Rampazzo, F. Manin, P. Tecilla, U. Tonellato, *Inorg. Chim. Acta* **2007**, *360*, 721 – 727.
[61] M. Montalti, L. Prodi, N. Zaccheroni, G. Falini, *J. Am. Chem. Soc.* **2002**, *124*, 13540 – 13546.
[62] S. Bonacchi, E. Rampazzo, M. Montalti, L. Prodi, N. Zaccheroni, F. Mancin, P. Teolato, *Langmuir* **2008**, *24*, 8387 – 8392.
[63] L. Hu, G. Xu, *Chem. Soc. Rev.* **2010**, *39*, 3275 – 3304.
[64] L. S. Dolci, S. Zanarini, L. Della Ciana, F. Paolucci, A. Roda, *Anal. Chem.* **2009**, *81*, 6234 – 6241.
[65] D.-J. Kim, Y.-K. Lyu, H. N. Choi, I.-H. Min, W.-Y. Lee, *Chem. Commun.* **2005**, 2966 – 2968.
[66] S. W. Bae, J.-W. Oh, I.-S. Shin, M. S. Cho, Y.-R. Kim, H. Kim, J.-I. Hong, *Analyst* **2010**, *135*, 603 – 607.
[67] X. Yang, R. Yuan, Y. Chai, Y. Zhuo, L. Mao, S. Yuan, *Biosens. Bioelectron.* **2010**, *25*, 1851 – 1855.
[68] J. Qian, Z. Zhou, X. Cao, S. Liu, *Anal. Chim. Acta* **2010**, *665*, 32 – 38.

[69] D. Zhu, Y. Tang, D. Xing, W. R. Chen, *Anal. Chem.* **2008**, *80*, 3566 – 3571.
[70] J.-M. Nam, C. S. Thaxton, C. A. Mirkin, *Science* **2003**, *301*, 1884 – 1886.
[71] M. M. Richter, *Chem. Rev.* **2004**, *104*, 3003 – 3036.
[72] S. Bonacchi, D. Genovese, R. Juris, E. Marzocchi, M. Montalti, L. Prodi, E. Rampazzo, N. Zaccheroni in *Reviews in Fluorescence 2008* (Ed.: C. D. Geddes), Springer, New York, **2010**, pp. 119 – 137.

