# Functionalized phosphorescent nanoparticles in (bio)chemical sensing and imaging – A review

Pablo Llano Suárez[a], Marta García-Cortés[a], María Teresa Fernández-Argüelles[a,b,*],

Jorge Ruiz Encinar[a], Marta Valledor[c], Francisco Javier Ferrero[c], Juan Carlos Campo[c],

José Manuel Costa-Fernández[a,*]

[a] Department of Physical and Analytical Chemistry. University of Oviedo, Avda. Julián Clavería 8, Oviedo 33006, Spain.

[b] Life Sciences Department, International Iberian Nanotechnology Laboratory (INL), Av. Mestre José Veiga, 4715-330 Braga, Portugal.

[c] Department of Electric, Electronic, Computer and Systems Engineering, University of Oviedo, Spain

[*] Corresponding authors:

María Teresa Fernández-Argüelles:　　　fernandezteresa@uniovi.es

José Manuel Costa Fernández:　　　jcostafe@uniovi.es

## ABSTRACT

Inorganic nanoparticles are a fascinating class of materials which promise great potential in numerous fields, including optical (bio)sensing. Many different kinds of such nanoparticles have been widely used for fluorescent sensing and imaging due to the different merits of fluorescent nanoparticles compared to molecular fluorophores. Progress made in the rational design of nanomaterials also allowed the synthesis of hybrid phosphorescent nanoparticles, that finds growing applications in sensing due to the combination of the interesting size- and shape-dependent properties of nanomaterials with a phosphorescence-type emission.

In this review, we intend to highlight some of progress made in this active research area and update the database of various phosphorescent nanoparticles-based sensors on the basis of different sensing targets of interest in environmental, industrial and biomedical areas.

Following an introduction and a discussion of merits of the synergy between nanomaterials and phosphorescence detection as compared to molecular luminophores the article assesses the kinds and specific features of nanomaterials often used in phosphorescence sensing. Specific examples on the use of phosphorescence nanoparticles in chemical sensing and bioimaging are given next. A final section intends to provide an overview of the prospects of such type of nanomaterials in the design of future devices for analytical chemistry.

## 1. Introduction

Luminescence spectroscopy is an essential tool nowadays in the bioanalytical area, as the analytical signal allows highly sensitive and selective measurements along with the possibility to perform remote monitoring for direct and in-situ chemical sensing [1]. Particularly, key advantage of luminescent sensors with respect to other systems rely on their excellent sensitivity (just a single photon can be enough for quantifying emission), combined with the appropriate selectivity that electronic photo-excitation imparts to these devices (the wavelength-dependence of the emission and of the excitation is a source of selectivity). In addition, the high spatial resolution of photoluminescence microscopy has enabled the development of powerful imaging applications for medical and biological purposes.

Due to the simplicity of photoluminescence methods and their inherent advantages in terms of sensitivity, selectivity, insensitivity to electrical interferences and considering that the luminescence signal is localized, many different non-destructive fluorescent methodologies that can provide real time, in situ, and dynamic information have been developed so far. For such purpose, a huge number of luminescent molecules have been used over the years. However, one the main drawbacks of such methodologies derive from the short fluorescent lifetimes and the low absorption coefficients and weak signals of the typical dyes used as labels, resulting in a low sensitivity. Additionally, many of the photoluminescence species suffer of photobleaching problems, limiting their use for long-term tracking [2].

Last decades a strong research effort has been done on the development of new nanomaterials for luminescent sensing, trying to overcome some of the major problems observed with the use of molecular luminescent dyes. The recent advances in nanotechnology resulted in the synthesis of novel hybrid nanoparticles with advantageous optical features. Some nanoparticles, such as Quantum Dot nanoparticles, are intrinsically fluorescent and, in contrast to molecular probes, provide excellent optical properties including improved brightness (high quantum yields and long emission lifetimes) and high resistance to photobleaching. Moreover, metal and semiconductor nanoparticles exhibit interesting size- and shape-dependent properties [3]. Therefore, nanoparticle-based detection platforms can provide capability for

multiplexing [4]. Additionally, the small size of the nanoparticles allows signal amplification by improving signal to noise ratio, therefore improving the analytical sensitivity, and improving the response time. Not surprisingly, as soon as fluorescent nanoparticles became available, they have been widely exploited as substitutes of traditional organic dyes in the development novel fluorescent sensing systems and imaging methodologies [5,6].

Today a huge number of nanoparticle-based fluorescent chemosensors and biosensors are available, with sensing schemes based on quenching or enhancement of the fluorescence emission, energy or electron transfer processes, etc. The advantages of the use of nanoparticles in the field of optical sensing and imaging were also recently extended to the development of methodologies based on a phosphorescence detection. Several strategies, including synthesis of hybrid nanomaterials, entrapment of phosphorescent dyes in nanoparticles or controlled doping quantum dots with rare earth impurities result in nanoparticles with a phosphorescence-type emission.

Phosphorescence or persistent luminescence has been attracting high attention for molecular imaging and sensing because such emission can last for a certain amount of time after removal of illumination source, permitting in vivo imaging without real-time external excitation. Compared to the fluorescence methods, room temperature phosphorescence offers several important advantages for optical sensing including an improved selectivity and sensitivity, longer emission lifetimes and wider gap between excitation and emission spectra [7]. The longer lifetime of the triplet-excited state facilitates the design of relatively inexpensive sensing systems based on measurement of decay-time measurements. Additionally the measurement of the emission delayed from the excitation makes it possible to remove spectral interferences coming from system and light scattering. Therefore, the combination of the use of nanoparticles as improved luminescent tags, due to their exceptional optical features, with the benefits of the phosphorescence detection justifies the growing interest in the synthesis and application of novel phosphorescent nanoparticles for the development of luminescence optical sensors and imaging systems.

Considering the aforementioned properties of phosphorescent nanomaterials, this review aims to provide a broad snapshot of the different types and specific features of nanomaterials exhibiting phosphorescence-type emission, aiming to perform a critical evaluation of the characteristics and performance of these nanomaterials. We are aware

that numerous excellent reviews focus on the use of phosphorescent nanoprobes have been produced during the last decade. However, most of them are devoted either to developments and fundamentals of specific kinds of photoluminescent nanoparticles (e.g. upconversion [8] or metal-doped QDs [9]) or to specific applications (e.g. bioimaging and biomedicine [10,11]). In this review we intend to give an overall view of the topic, from the design and synthesis of the different types of nanoprobes, with fundamentals of their corresponding phosphorescent properties, to their specific fields of applications that cover not only imaging and biomedicine but also determination of analytes, which in turn range from small inorganic ions to target proteins (i.e. biomarkers). Representative examples of key applications of such phosphorescent nanoparticles are next presented.

## 2. The transduction element: phosphorescent-type nanoparticles

A purpose of this review is to give the reader an overview of the wealth of phosphorescent nanoparticles that exist for use in phosphorescence sensing and imaging. The different types and specific features of nanomaterials showing phosphorescence are schematically presented in Figure 1 and explained in detail along this section. These include metal-doped colloidal inorganic nanoparticles, nanoparticle-loaded phosphorescent dyes, metal-enhanced phosphorescent nanoparticles, and upconversion nanoparticles.
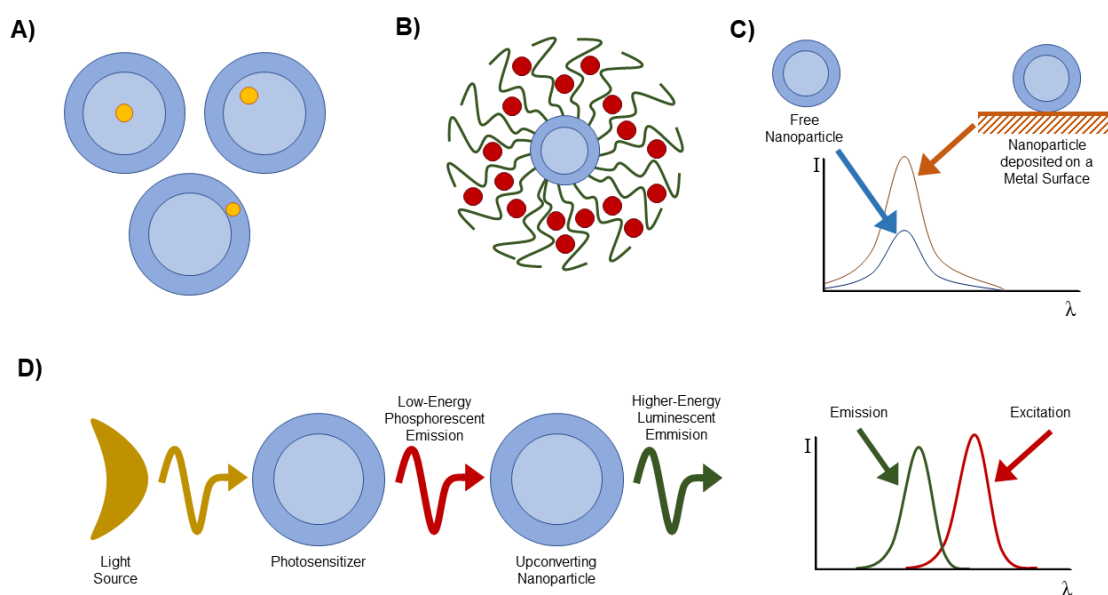
**Figure 1.** Different types of phosphorescent nanoprobes. A) Metal Doped Colloidal Inorganic Nanoparticles - dopant is part of the core-shell structure. B) Nanoparticle-Loaded Phosphorescent Dyes – Trapped dye in the ligand structure. C) Metal-Enhanced Phosphorescent Nanoparticles - phosphorescence emission increases by depositing the nanoparticle on a metal surface. D) Upconversion Nanoparticles – final luminescence observed is of a higher energy than excitation source.

## 2.1. Metal-doped colloidal inorganic nanoparticles.

Doping inorganic nanoparticles with different metals yield hybrid nanomaterials with novel properties and functions. Using this strategy, it is possible to modulate the optical properties of the nanomaterials. In this context, the incorporation of appropriate atoms or ions into host lattices is performed to give rise to a new class of nanomaterials to be exploited in bioanalytical applications including (bio)sensing and imaging due to the longer emission lifetimes obtained [12,13].

In this sense, ZnS, ZnSe, ZnO, CdS and CdSe quantum dots (QDs) have been evaluated as host lattices to obtain novel luminescent properties using a variety of transition-metal and lanthanide ions, including $Mn^{2+}$, $Cu^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Ag^+$, $Pb^{2+}$, $Cr^{3+}$, $Eu^{3+}$, $Tb^{3+}$, $Sm^{3+}$ and $Er^{3+}$ as dopant agents [9]. Among them, ZnS and ZnO are the most studied host lattices because they offer promising applications in bioanalysis and bioimaging. Since they do not contain toxic metals, they present potentially lower toxicity, and they also have wider energy bandgaps that allow the incorporation of more dopant agents, opening the possibility of developing dual doped or co-doped QDs [14,·15]. The presence of the transition metal or lanthanide dopant usually increases the lifetime of the emission, giving rise to a phosphorescence-like emission that overcomes the limitations of fluorescent nanoparticles or dyes due to the elimination of the biological background fluorescence for biosensing and bioimaging.

In order to incorporate the doping agent into the nanocrystal host, most of the reports found in the literature are based on wet chemistry procedures that take place in both aqueous or organic media, and the most common approaches described include chemical precipitation, microemulsion and organic high temperature chemical synthesis methods. In this way, a homogeneous distribution of the dopant in the host matrix can

6

be achieved, while a good control over the size, shape and production yield can be also obtained [13].

In aqueous-based routes, the metal-doped nanoparticle synthesis is typically performed via co-precipitation of the cationic precursors and doping agent with the anionic reagent (typically $Na_2S$) in presence of the desired surface ligands, that will provide appropriate functional groups for further (bio)conjugation, such as thiol [16], phosphates [17], polymers [18], proteins [19], etc. Experimental synthetic conditions such as temperature and pH have a strong influence on the optical and physical properties of the obtained doped nanocrystals. Reverse micellar synthesis has been also used to obtain Mn-doped CdS QDs, although overcoating with a ZnS shell must be performed in order to improve the luminescence efficiency due to the presence of surface dangling bounds [20]. One of the limitations of the aqueous-based routes is the maximum temperature that can be achieved, that gives rise to the generation of surface defects, and therefore, to nanoparticles with poor optical properties. Thus, in order to overcome this limitation, hydrothermal and microwave-based approaches, where the precursors are loaded in closed vessels, allow to increase the temperature of the media above the boiling point of water, giving rise to doped nanocrystals with excellent luminescence performance [21].

On the other hand, it is well-known that high quality doped QDs are typically obtained working at high temperatures in organic solvents that allow an effective annealing of the surface [22]. In this regard, there are three main approaches based on the timing of the dopant addition (see Figure 2) [23]. When the dopant is added along with the host precursors, the nanoparticles obtained present poor homogeneity and a mixture of doped and non-doped nanocrystals are obtained [24]. A second approach is based on the addition of the doping agent during the nucleation of the host [25]. Then, after nucleation, the reaction conditions are tuned to minimize the activity of the dopant, being the growth of the host the only process that takes place and giving rise to a nanoparticle with the dopant in the centre of the QDs. Finally, in a third approach the doping agent is added during the nanoparticle growth [26]. In this type of synthesis, once the nuclei of the NP is formed, the reaction temperature is lowered, and the doping agent is added. Afterwards, a new layer of host is used to coat the surface-doped nuclei.

Recently, a new generation of nanoparticles, whose long-lasting luminescence emission can last several hours, are being explored. They are known as persistent-luminescence nanoparticles (PLNPs), and the signal-to-noise ratio can be significantly improved due

to the removal of the background noise (especially high in biological media) originated from in situ excitation of the NPs in biological media [27]. Because the emission after excitation arises from a trapping and detrapping, the lifetimes of persistent luminescence materials are 10 orders of magnitude longer than the spin-forbidden transitions in phosphorescent molecules and metal–chelate compounds [28]. Alkaline earth silicates are the most well-known PLNPs, and depending on the dopant, they will emit at different wavelengths, e.g.: $Ca_2MgSi_2O_7:Eu^{2+}$ and $Sr_2MgSi_2O_7:Eu^{2+}$, emit in the blue (470 nm) and green (535 nm) regions respectively [29], or $Eu^{2+}$- and $Dy^{3+}$-doped $Ca_{1.86}Mg_{0.14}ZnSi_2O_7$ nanoparticles emit at 520 nm for 6 hours after excitation [27].
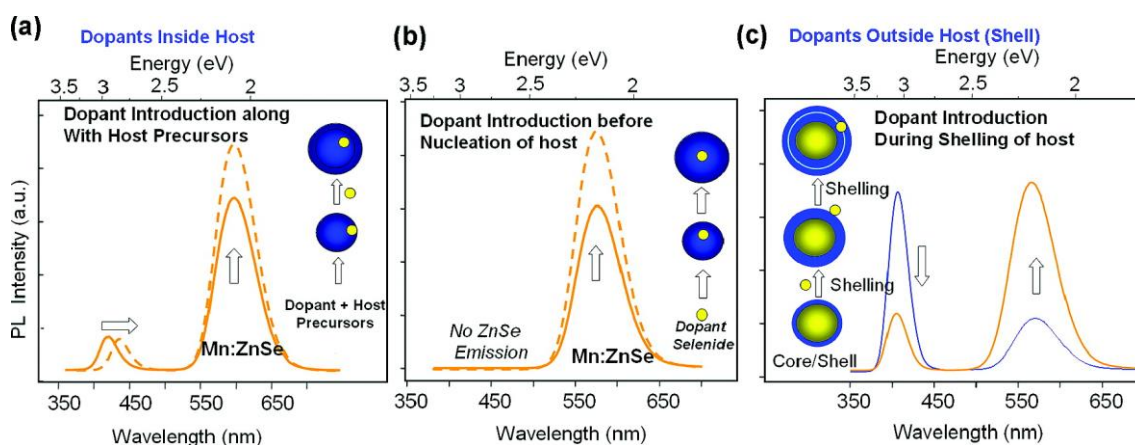


**Figure 2**. PL spectra obtained during different doping processes depending on the time of the introduction of the dopant. (a) along with host precursors, (b) before nucleation of the host, and (c) during formation of the shell of a core-shell structured nanocrystal. Every PL spectra were recorded with an excitation wavelength of 350 nm. *Reprinted with permission from N. Pradhan, D. D. Sarma, 'Advances in Light-Emitting Doped Semiconductor Nanocrystals', J. Phys. Chem. Lett. 2011, 2, p. 2818–2826. Copyright 2011 American Chemical Society.*

Latest developments achieved in the synthesis of doped nanocrystals have significantly widened the range of novel photophysical properties that display the doped quantum dots, that can be exploited in bioanalytical applications (e.g. as luminescent labels or in chemical sensing). Among the different doped-nanoparticles, those based on Mn as

dopant have experienced a higher degree of development as luminescent labels for bioimaging and chemical sensing applications, since interesting photophysical properties can be obtained when the host lattice of the nanoparticle is modified with Mn. In this context, several reports showed that the amount of the metallic element used as dopant is critical, since it has a direct impact in both, structural and optical properties of the NPs. In this sense, *Chen et al.* [30] showed that an increase of the levels of Mn acting as dopant gives rise to more defects in the nanostructure which leads to lower luminescent Quantum Yield (QY). Luminescence lifetimes , a parameter closely related to the electron-hole recombination processes and that therefore helps to understand the luminescence mechanism, were also affected by the level of Mn. In fact, Mn-doped core-shell AIZS/ZnS nanoparticles (AIZS = Ag-In-Zn-S) present lifetimes much longer than the same NPs with no doping agent, suggesting that different luminescence emission mechanisms may take place depending on the presence or absence of the dopant.

Several studies have been focused on how the doping agent modifies the optical properties of the NPs. In this sense, R. Beaulac et al. [31] proposed three different photophysical scenarios for different types of colloidal $Mn^{2+}$-doped II–VI semiconductor quantum dots with different energy band gaps in order to explain the emission of phosphorescence, summarized on figure 3. Energy levels corresponding to the excited states of both the $Mn^{2+}$ ion and the host semiconductor are displayed in Figure 2. In scenario I, an efficient energy transfer from the excitonic states to the excited levels of the dopant produces a quenching of the luminescent emission of the semiconductor to enhance the emission of the $Mn^{2+}$ $^4T_1$-$^6A_1$ transition. Excitons are quenched by $Mn^{2+}$ photoionization states (dopant-bound excitons) that relax nonradiatively to the ground state, so no luminescence is observed in the case of scenario II. The $Mn^{2+}$ excited states are outside the semiconductor band gap in scenario III. Since no quenching takes place, nanocrystals show their typical excitonic emission independently of the presence and amount of $Mn^{2+}$
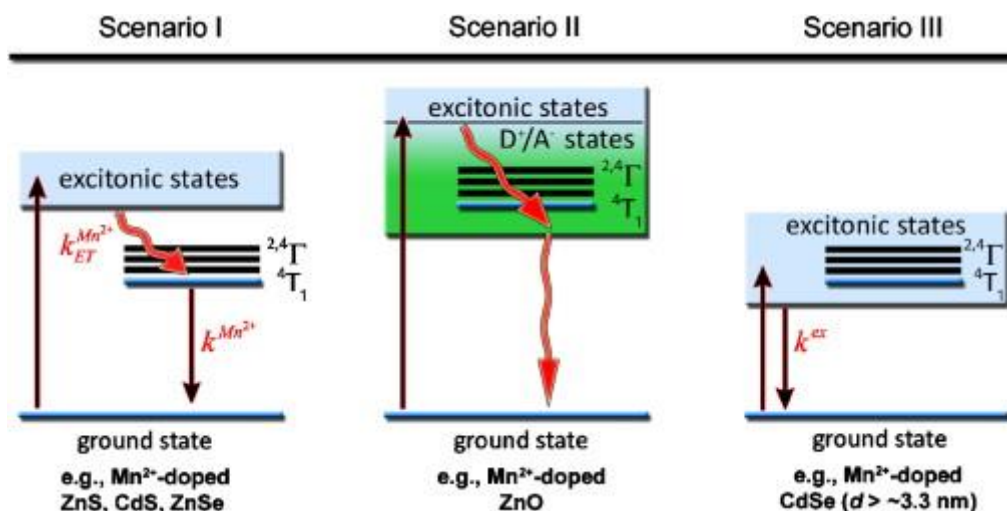
**Figure 3:** Different photophysical scenarios of colloidal $Mn^{2+}$-doped II–VI semiconductor nanocrystals. Radiative and nonradiative processes are indicated by straight and curved arrows, respectively. *Reprinted from Journal of Solid State Chemistry 181 (7), R. Beaulac, P.I. Archer, D.R. Gamelin, 'Luminescence in colloidal Mn2+-doped semiconductor nanocrystals', p. 1582-1589. Copyright 2008, with permission from Elsevier.*

Different photophysical effects are also likely to occur for doped core/shell nanocrystals, anisotropic nanostructures, and heteroarchitectures or superlattices integrating doped nanocrystals with other functional inorganic or organic materials. It is clear that additional research is still needed in order to better understand the role of dopants and their effects on the resulting nanoparticle luminescence. The higher control over the doping process will surely allow finer tuning of the resulting luminescent properties to overcome some of the present limitations of functionalized phosphorescent nanoparticles. In fact, it is not stretch to think in fit-for-purpose doped inorganic nanoparticles that could be designed for specific applications in chemical sensing and imaging.

## 2.2. Nanoparticle-loaded phosphorescent dyes

Another strategy described for preparation of NPs with phosphorescence-type emission is the loading of NPs with phosphorescent dyes. Such type of phosphorescent dye-encapsulated nanoparticles has shown to be very effective in the bioanalytical field. An important enhancement in the phosphorescence analytical signal is expected by this

approach due to the possibility to entrap or covalently bond thousands of indicator dyes on the NP surface.

Besides the inherent advantages of the phosphorescence emission, nanomaterials doped with phosphorescent molecules have also gained much attention due to their outstanding features as nanocarriers for drug delivery and bioimaging applications. One of the advantages of these systems is that both, core and shell, can be tailored to meet the requirements in order to encapsulate and release a specific molecule (e.g. a phosphorescent dye or a drug) that are typically hydrophobic, while the shell can be designed to ensure that the system is soluble in aqueous media [32]. Moreover, the surface of the nanoparticles can be easily modified with inert and biocompatible coatings, such as poly(ethylene glycol) (PEG), which endow them with the ability to evade capture and degradation by the reticuloendothelial system [33]. Furthermore, the surface of the nanoparticles can be also modified with biorecognition molecules in order to perform "active targeting" [34]. Last but not least, another important advantage of the use of these type of nanoparticle formulations is the possibility of performing co-immobilization of other types of imaging agents, such as Magnetic Resonance Imaging (MRI) [35] and Positron Emission Tomography (PET) probes [36]. This allows to perform multimodal imaging that will provide complementary anatomical and physiological information

As an example, cyclometalized iridium(III) complexes have attracted increasing interest as molecular probes in sensing, biological labelling, and bioimaging applications due to their long-lived excited states and high phosphorescent quantum yields. Quite recently, polymeric nanoparticles loaded with such iridium(III) complex have been successfully developed for luminescence bioimaging. Here the iridium(III) complex $[Ir(pq)_2]^+$ acts as both, drug and as phosphorescent emitter in a nanopolymeric 2-phenylquinoline (pq) core, and a folic acid end-capped polyethylene glycol (PEG) as the shell to provide biocompatibility to enter the cell nucleus [37].

The use of polymers for encapsulation of phosphorescent dyes is particularly advantageous for bioimaging, since the size of the nanospheres can be tuned by controlling the polymer chain length, while inclusion of appropriate functional groups can be used to tailor the nanosphere surface to provide biocompatibility or to attach bioactive molecules [38].

One of the most common methods to obtain polymer nanospheres is emulsion polymerization, where small droplets of monomer are dispersed in a continuous phase of water prior to polymerization. However, the droplets have to be stabilized by surfactants, which can affect negatively for certain bioanalytical applications [39]. Thus, surfactant-free methods as water-induced precipitation are also being used. These methods involve codissolution of a hydrophobic phosphorescent molecule and an amphiphilic polymer in a small amount of an organic solvent miscible with water. The addition of water under vigorous stirring generates a suspension of nanospheres with hydrophilic polymer groups at the surface and the phosphorescent molecules in the hydrophobic inner part of the spheres [40]. The precipitation method has been developed for encapsulation of phosphorescence-emitters such as ruthenium diphenyl phenanthroline [Ru(dpp)$_3$Cl$_2$] [39], Pt(II) and Pd(II) porphyrins [41] using polymer materials synthesized at the nanoscale, including acrylonitrile, acrylic acid, poly(vinyl chloride- co-ethyl acetate-co-maleic acid), poly(vinyl chloride-covinyl acetate-co-epoxy monomer) or poly(styrene-comaleic acid). Subsequent crosslinking of the polymer chains provides high quantum efficiencies and better photostability to equivalent non-crosslinked nanospheres due to a better protection of the phosphors against oxygen and other emission quenchers [41]. Recently, the use of small-molecule crosslinking agents, such as bisphenol A diglycidyl ether (BPA) in conjunction with a single amphiphilic polymer, has been proposed as an alternative to the use of multiple polymers with crossreactive functional groups, achieving a better control over the size, morphology and surface chemistry of the nanospheres [38] as shown on Figure 4.

Another common approach to encapsulate phosphorescent molecules is the use of phospholipid nanomicelles. In this sense, near-infrared phosphorescent Pt(II)-tetraphenyltetranaphthoporphyrin (Pt(TPNP)) molecules immobilized in 100 nm phospholipid nanomicelles were found to be highly stable in aqueous media, keeping its luminescent properties after immobilization. Such nanomaterial demonstrated to be highly useful for *in vivo* high-contrast optical imaging, targeting, and detection of tumors in small animals [42].
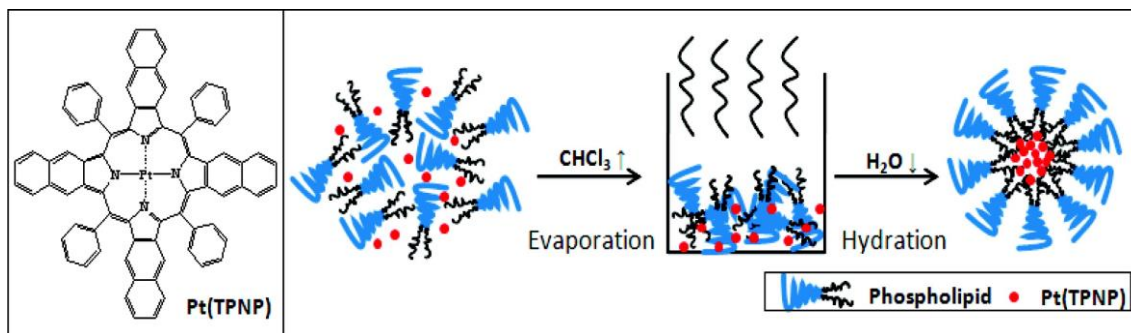
**Figure 4:** Preparation of polymeric nanomicelles by encapsulation of Pt(TPNP) within a PEG-modified phospholipid micelle. *Reprinted with permission from R. Kumar et al., 'Near-Infrared Phosphorescent Polymeric Nanomicelles: Efficient Optical Probes for Tumor Imaging and Detection', ACS Appl. Mater. Interfaces, 1 (7), p. 1474–1481. Copyright 2009 American Chemical Society.*

Silica nanoparticles were among the first ones to be used in bioimaging. Synthesis of such NPs is today well known, and materials produced have narrow size distribution and controlled pure sizes [43]. The low cytotoxicity of such NPs, and the capabilities to easily dope the NPs with different types of organic luminescent dyes explain the expansive literature existing on their use for sensing development. Phosphorescent silica nanoparticles have been also prepared through the immobilization of an Ir-tpy complex [Ir(tpy)(2)]X-3 (tpy = 2,2':6',2 "-terpyridine; X = $PF_6$ or $NO_3^-$), following three different strategies including the Stober, water-in-oil and direct micelle synthetic approaches. It is worth to mention that the first two approaches gave rise to a silica matrix that prevents the diffusion of oxygen and blocks the mobility of the complex, providing enhanced photochemical stability and higher phosphorescence efficiency compared to the free Ir-tpy complex. However, when following the direct micelle synthesis route the final structure presents a certain degree of mesoporosity, and the phosphorescent emission is not improved when compared to the free Ir-tpy complex [44].

Silica NPs and other NPs are often doped with phosphorescent dyes (e.g. lanthanides showing long-lived luminescent emission). Their use in biosensing and imaging has been recently reviewed by Chen *et al.* [45]. Lanthanide-doped SiNPs can be easily be obtained bu adding lanthanide ions to the precursor mixture during the polymerization of the SiNPs, or by grafting the SiNPs with lanthanide-complexes. Resulting NPs with

long luminescence decay times (a few ms) do not suffer form photobleaching and can be used for gated spectroscopy, facilitating efficient background suppression.


## 2.3. Metal-enhanced phosphorescent nanoparticles

These kinds of phosphorescent nanomaterials come in addition to more conventional (phosphorescent/dyed loaded) metal NPs. Such nanostructures are made of nanoparticles comprising a metallic core with a surface coating consisting on a polymeric or oxide material having phosphorescence emitters contacting the surface coating.

Interactions of luminophores with metallic nanoparticles (that is the base of the so-called metal-enhanced luminescence spectroscopy) has been used to obtain photoluminescence enhancements and increased photostability, reduced blinking in single molecule fluorescence spectroscopy, and increased transfer distances for fluorescence resonance energy transfer [46].

The majority of the metal enhanced phosphorescence applications to date have been performed on 2-dimensional surfaces, where glass microscope slides or plastics are used as the primary substrates that feature the metal nanostructures deposited using either wet-chemistry, electrochemically or by lithography. An example of such process is that produced by silver island films (SiFs) coupled with Rose Bengal [47], a dye that has been found to produce excited triplet states, in which the phosphorescence was enhanced on a 5-fold. In such nanostructure, a metal-enhanced phosphorescence process occur. Such process is a surface plasmon coupled phenomena in which non-radiative energy transfer occurs from excited luminophores to the surface plasmon electrons in metal surfaces, which in turn produces a phosphorescent-like emission. The surface plasmon phenomenon persists when the X-axis component of the emissive light from the chromophore is equal to the wavevector of the surface plasmon [48].

Based on this mechanism, a 2.5-fold metal-enhanced room temperature phosphorescence was registered when the phosphor diiodofluorescein was adsorbed on Ag nanoparticles-deposited on paper [49]. Similarly, platinum octaethylporphyrin (PtOEP) in conjunction with a nanogold metal substrate was employed to produce a significant surface plasmon coupled resonance [48]. In this study, there was no

noticeable spectral shifts for the phosphorescence emission, hinting that the emission is not due to changes in photophysical properties. Metallic nanoparticles that can be employed for plasmon-enhanced phosphorescence must be fabricated from any metal that enhances luminescence (e.g. noble metals such as silver, gold, platinum, aluminum, copper, zinc, palladium and composites thereof).

An alternative mechanism producing enhanced phosphorescence is related to the generation of localized plasmon resonance using metallic nanoparticles that resonate with the $O_2(a^1\Delta_g) \rightarrow O_2(X^3\Sigma_g^-)$ transition, and an oxygen sensitizer such as tetraphenylporphyrin (TTP) [50]. The metal nanostructure was extremely relevant for the efficiency of this phenomenon: nanosandwiches, gold nanorods or gold nanoshells do not generate an increase on the phosphorescence, whereas gold nanodiscs produce more than 3.5-fold increase in phosphorescence, and no increase of the fluorescence is observed, indicating that there is no metal-enhanced effect.


## 2.4. Phosphorescent Upconversion Nanoparticles

Upconversion Nanoparticles (UCNPs, also referred to as upconversion nanocrystals) typically consist of hexagonal NaYF4 nanocrystals, with nanoparticle sizes ranging from 10 to 100 nm, doped with trivalent lanthanide ions such as Er(III), Yb(III) or Tm(III). Here, the dopant is the emitter and additional doping with fluorophores is not needed. UCNPs are capable of converting low-energy radiation (Usually Near-Infrared) into higher energy wavelengths (usually UV-Vis) by combining two or more excitation photons into each emitted photon. Like quantum dots, UCNPs have several important advantages over traditional luminophores to be used as indicator dyes, including narrow bandwidths, excellent photostability and low photobleaching. Although NP size typically ranges from 10 to 100nm (and this affects the emission quantum yields), the control of the size and excitation/emission spectra of such NCs is still a challenge [51]. UCNPs are especially interesting in bioimaging due to the high penetration, low damage to biological tissue and low autofluorescence on NIR region.

Most UCNPs synthesis protocols yield water insoluble NPs, that must be later surface-modified to enable phase transfer to aqueous solutions. Such surface modification must be fine controlled to avoid a serious interference on the NPs optical properties. Remarkably, and unlike other luminescent NPs, quantum yield of UCNPs strongly

depends on the power density of the excitation source, and on the particle size (as small particle size, smaller quantum yield obtained) [52].

There are several different mechanisms that might produce upconversion, including atomic-like transitions in rare-earth ions, and photochemical upconversion. Perhaps the most common mechanism that can produce photochemical upconversion in which phosphorescent nanoparticles take part is based on the storage of energy in triplet states on a first chemical species ("sensitizer"), coupled to the "emitter" which collects the energy by Dexter energy transfer, combining the energy of two photons by triplet-triplet annihilation (TTA), as summarized in Figure 5. All these processes require close proximity between sensitizer and emitter. The sensitizer is usually a phosphorescent nanoparticle, and to ensure that sensitizer and emitter stay close, this type of system is usually immobilized on a solid support like silica or organogels.
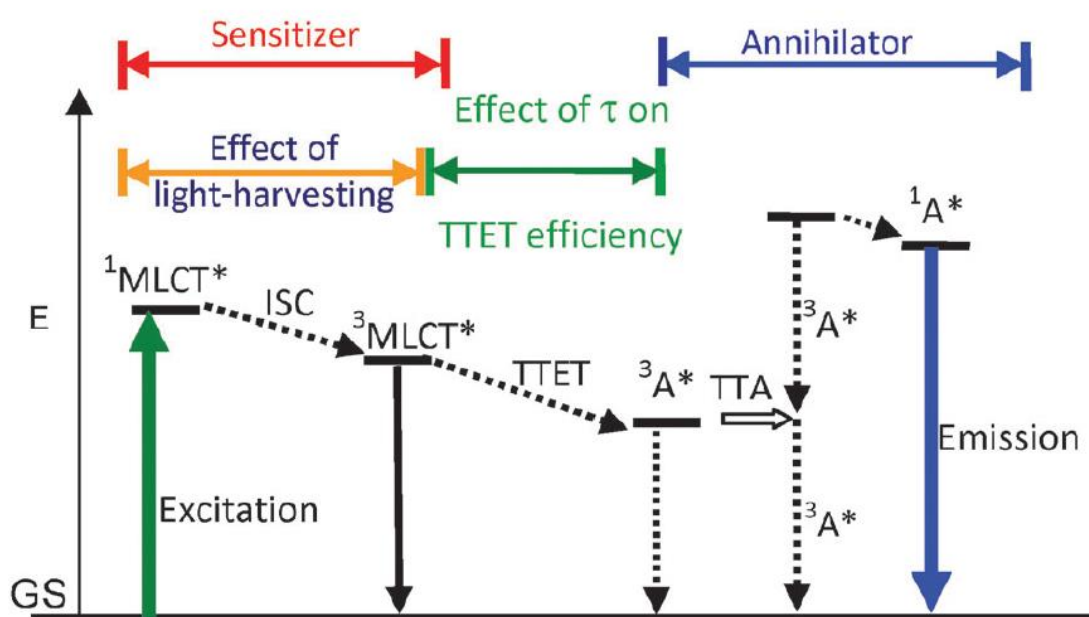


**Figure 5**: Jablonskii Diagram illustrating the mechanism of TTA upconversion. Reproduced from Ref. [53] with permission from The Royal Society of Chemistry. "E is energy. GS is the ground state ($S_0$). $^3$MLCT* is the metal-to-ligand-charge-transfer triplet excited state. TTET is triplet–triplet EnT. $^3$A* is the triplet excited state of the triplet acceptor. $^1$A* is the singlet excited state of the annihilator. The emission bands observed for the upconversion are the delayed fluorescence from the acceptor"

In general terms, upconverting nanoparticles typically produce fluorescence, and the study of nanoparticles that generate upconverted phosphorescence is scarce, usually restricted to their use as sensitizers [54,55], and sometimes the phosphorescent nanoparticle presents dual functionality, as it can also produce upconverted fluorescence [56]. It must be mentioned that, at present, one drawback of such UCNPs is the poor quantum yields measured, that often limits their applicability in real samples.

## 2.5. Other phosphorescence nanomaterials

Some other nanomaterials have been described for use as phosphorescent NPs, most with the potential of acting as probes for chemical sensing and imaging. These include, among others, carbon-based nanomaterials, organic semiconducting polymers or noble metal nanoparticles functionalized with complexing agents. These phosphorescent nanomaterials will not be discussed here in depth, as reflect only examples of recent developments from nanotechnology and almost no analytical applications of these nanomaterials have been proposed yet. However, considering their great potential for future imaging or sensing applications, a selection is given.

Carbon-based nanomaterials offer the possibility to be used as phosphorescent emitters in photoluminescence applications. Particularly, fluorescent carbon-based nanoparticles, so-called carbon dots (CDs) have various attractive properties and have been used in many different analytical applications. However, much less attention was paid to their phosphorescent phenomenon. A kind of highly efficient CD-based phosphorescent material was synthesized by a subtle design method that incorporated N-doped CDs into composite matrices (the melting recrystallization urea and biuret from the heating urea) by a one-pot heating treatment for the mixture of urea and NCDs [57]. Authors proposed that C$=$N bonds generated on the surface of the NCDs can create new energy level structures explaining the origin of phosphorescence emission registered. Here, composite matrices play a critical role to suppress the vibrational dissipation of long-lived triplets allowing the measurement of ultralong phosphorescent lifetime of seconds. Very recently, carbon dots exhibiting a ultralong RTP of few seconds were synthesized via microwave-assisted heating of ethanolamine and phosphoric acid aqueous solution [58]. The doping of the CDs with N and P elements was found to be critical to facilitate

intersystem crossing (n→π* transition) for effectively populating triplet excitons. Such carbon-based long-lifetime room-temperature phosphorescence nanomaterials, with a lower expected citotocixicty (they are metal free) as compared to other phosphorescent metal nanoparticle, have a bright potential for future bioanalytical applications.

Very recently, Zhen *et al.* [59] reported the synthesis of water-soluble organic semiconducting nanoparticles (OSNs) with ultralong phosphorescence for in vivo phosphorescence imaging. The approach developed forces the creation of nanoparticle aggregates through a top-down design to significantly stabilize the triplet excited states of a phosphorescent molecule. This extends the particle phosphorescence to the timescale that can be easily detected in a commercial whole-animal imaging system after removal of external light source. The phosphorescence emission from OSNs was found to be insensitive to the presence of oxygen. This study present a universal design principle to extend the lifetime of phosphorescent molecules to the level that can be effective for molecular imaging.

Functionalized gold nanoparticles were also proposed as phosphorescent nanomaterials [60]. Here, a hybrid nanoparticle was synthesized by capping monothiol derivatives of 2,2'-dipyridyl onto the surface of Au nanoparticles. The chelating agent 2,2'-bipyridine was complexated with $Eu^{III}$/$Tb^{III}$ ions yielded red-emitting and green-emitting phosphorescent nanomaterials. An attractive feature of such system is the capability to hold a large number of phosphorescent lanthanide complexes around Au nanoparticles thus amplifying the analytical signal while maintaining the long-lived emission properties. Considering that gold nanoparticles can be easily functionalized with appropriate functional groups able to selectively bind analytes or target molecules, such phosphorescent nanohybrid systems might have a great potential to be used in development of novel bioanalytical methodologies (e.g. chemical sensors or imaging tools).


## 3. SENSING MECHANISMS AND APPLICATIONS

A wide variety of assay formats for sensing and imaging (summarised in Figure 6) have been proposed employing the previously described phosphorescent nanoprobes. A critical overview of recent published literature is provided in this section
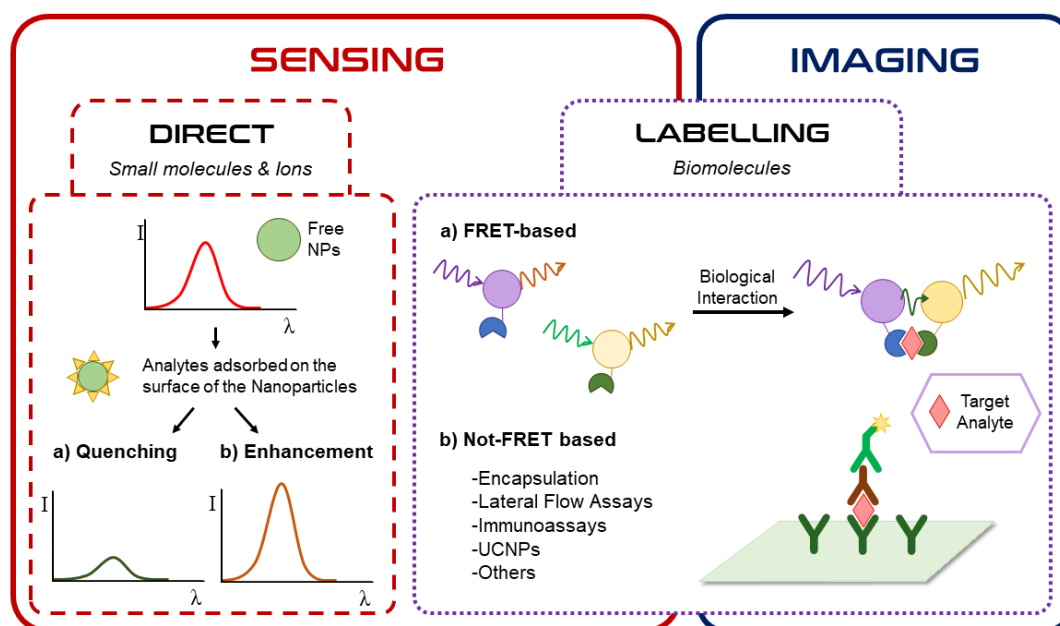
**Figure 6:** Assay formats of the different phosphorescent nanoprobes reviewed.

## 3.1. Direct Sensing of small molecules and ions

Luminescence-based sensors occupy a superior place over other types of chemical sensors because, among several other features, they present a great sensitivity, low cost, and capability for remote and in-situ measurements, making them excellent choices for environmental and industrial monitoring. This section aims to summarize some examples of phosphorescence sensors based on nanoparticles for environmental applications, including the detection of metals, antibiotics, pesticides and other contaminants, as well as some industrial applications which are mainly focused to oxygen and temperature sensing. In all cases, analytical signal comes from the effect that the presence of the target analyte in the solution makes over the phosphorescent emission of the NP. Some selected applications with their corresponding analytical figures of merit are given in Table 1.

Table 1. Compilation of direct sensing applications using phosphorescence nanoprobes

| Analyte/matrix | Phosphorescent sensor | Precision | Working Range | Detection limit | Ref. |
|---|---|---|---|---|---|
| Cations (Alkali/Alkanine/Transition metals) in water | Eu3+-AuNPs | a | a | Screening Purposes | 60 |
| Thiram in fruit peels | ZnS:Mn2+ QDs | a | 50nM–2.5µM | 25 nM | 61 |

| | | | | | |
|---|---|---|---|---|---|
| 2-mercaptibenzothiazole in water | MPA-capped Mn-doped ZnS QDs | 2.7% RSD | 0.6-72 mM | 45 nM | 62 |
| oxygen dissolved in microdroplets | dye embedded in poly(styrene-block-vinylpyrrolidone) nanobeads | a | Low O2 to atmospheric pressure | 0.07 µM (Low O2) 0.12 µM (1 atm, 20ºC) | 63 |
| oxygen in cellular media | hydrogel nanoparticles | a | 0-20% O2 | Imaging Purposes | 64 |
| sulfide anion in discharged water | TGA-coated Mn-doped ZnS QDs | <6% RSD | 2.5 - 38 µM | 0.15 µM | 65 |
| 2,4,6-Trinitrotoluene in water | L-cysteine Mn-doped ZnS | 2.3% RSD | 0.0025-0.45 µM | 0.8 nM | 66 |
| 2,4,6-Trinitrotoluene in aqueous solutions | L-cysteine Co-doped ZnS QDs | a | 0.025-0.5 M | 25 nM | 67 |
| 2,4,6-Trinitrotoluene in aqueous solution | APTES-functionalized Mn-doped ZnS QDs | 3.5% RSD | 0.05–1.8 µM | 50 nM | 68 |
| Pentachlorophenol in water | Mn-doped QDs with a molecanly imprinted polymer (MIP) | 2.8% RSD | 0.2-3.9 µM | 86nM | 69 |

a: Data not available

Quenching of the phosphorescent emission of ligand-capped gold nanoparticles containing $Eu^{3+}$ by addition of alkaline earth metal ions ($Ca^{2+}$ and $Mg^{2+}$) and transition metal ions ($Cu^{2+}$, $Zn^{2+}$, $Ni^{2+}$) makes such nanoparticles useful as probes for screening of such ions in aqueous media. Such quenching effect was the consequence of an isomorphous substitution of $Eu^{III}$ ions by the metals. Other ions such as $Na^+$ or $K^+$ did not influence the RTP emission [60].

Thiram has been detected in fruits by using $ZnS:Mn^{2+}$ QDs, whose phosphorescence was quenched by $Ag^+$ ions. The formation of a complex between thiram and silver ions gives rise to an increase of the phosphorescence signal which can be related to the concentration of thiram [61].
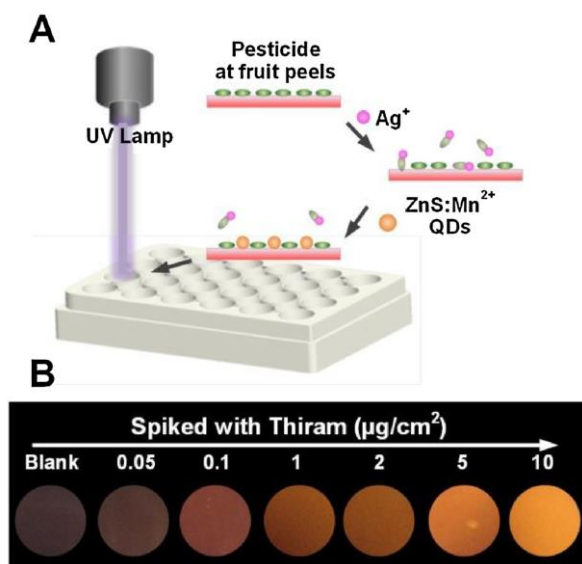
**Figure 7.** (A) On-site detection of thiram at fruit peels. (B) Digital photos apple peels spiked with different amounts of thiram under 312 nm UV light illumination. *Reprinted from Sensors and Actuators B: Chemical 252, C. Zhang et al., 'Selective phosphorescence sensing of pesticide based on the inhibition of silver(I) quenched ZnS:Mn$^{2+}$ quantum dots', p. 1083–1088. Copyright 2017, with permission from Elsevier.*

Oxygen quenching of phosphorescence has been widely studied, and a wide assortment of sensors for different analytes like pH, $CO_2$, orthophosphate, chloride, 1-butylamine, antibacterial oxolinic acid and antibiotic flumequine have been developed based on measurement of changes in $O_2$ levels detected by resorting to phosphorimetric oxygen sensing [7]. In this context, phosphorimetric nanoparticles have been evaluated for monitoring the level of oxygen dissolved in microdroplets [63]. The oxygen sensor nanoparticles consist of a phosphorescent indicator dye embedded in poly(styrene-block-vinylpyrrolidone) nanobeads. The phosphorescent nanoparticles, excitable by red light, present an emission in the near-infrared spectra region which minimizes background fluorescence from biological matter. Such nanoparticles probes were read out by a miniaturized phosphorimeter, able to measure phosphorescence lifetimes, overcoming the drawbacks of intensity-based measurements. Monitoring cell and tissue oxygenation is of key relevance in clinical and biological studies. Recently, a phosphorescent nanosensor material able to penetrate cellular media has been developed by entrapment of a highly photostable phosphorescent reporter dye along with a fluorophore acting as Förster resonance energy transfer (FRET) donor and two photon

antennae in hydrogel nanoparticles [64]. Such nanoprobe allowed a high resolution monitoring of dissolved oxygen in cellular media based on ratiometric intensity and phosphorescence lifetime based sensing. The developed nanoprobe combines along with a high versatility and excellent analytical performance for oxygen detection a high photo and chemical stability, low toxicity, and ease of fabrication and use.

2,4,6-Trinitrotoluene (TNT), a compound of major concern due to its biological persistence, toxicity and mutagenicity, has also been detected using different RTP methods. One of those methods is based on the formation of complexes through the L-cysteine ligands anchored at the surface of Mn-doped ZnS, that provides excellent selectivity in environmental and biological samples even in the presence of the main relevant metal ions ($Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$, among others) and other organic compounds [66]. Another study based on the same mechanism uses L-cysteine capped Co-doped ZnS QDs, and reports excellent sensitivity for TNT in solution without interference from a matrix of real water sample and other nitroaromatic compounds [67]. Finally, other approach developed for the detection of TNT in water samples makes use of APTES-functionalized Mn-doped ZnS QDs. In this case, the analyte binds to the APTES molecules through acid–base pairing interaction between electron-rich amino ligands and electron-deficient aromatic rings [68].

Sensing of reactive species by measuring changes in the phosphorescence emission (intensity or lifetime) after interaction of the analyte with the nanoparticle has attracted a great research interest. Simplicity of this sensing-scheme and the typically high sensitivity of the systems developed are two analytical features common to the different developed methodologies. Probably the main drawback observed is the reduced selectivity, because any concomitant species present in the sample able to interact with the nanoparticle surface could affect the phosphorescent signal as well. Surface modification of the phosphorescent NPs with ligands or biomolecules able to interact specifically with the analyte would improve the selectivity of such systems. In this sense, a strategy was proposed based on modifying the surface of Mn-doped phosphorescent QDs with a molecularly imprinted polymer (MIP) able to recognize with high selectivity and sensitivity traces of pentachlorophenol dissolved in water [69]..The analytical signal consisted of the measurement of the RTP quenching occurring after analyte recognition by the MIP layer.

In brief, a variety of simple methods have been reported so far for determination of small reactive species (e.g. ions or small molecules) based on the measurement of changes on the photoluminescence emission from the nanoparticles resulting after a direct interaction of such species with the surface of the nanoparticle or with appropriate ligands modifying the surface of the nanoprobe. Unfortunately, such approaches are restricted to a few number of chemical species able to directly interact with the nanostructure typically resulting in poor selectivity as some other different chemical compounds, different to the target analyte, can produce a change of the optical behavior of the nanoparticles as well. This explains that recent progress on the topic has been mostly focused on the use of the phosphorescent nanoparticles as labels of antibodies or other recognition elements in order to provide the desired selectivity. This approach allows analysts to extend the use of phosphorescent nanoparticles for the indirect detection/quantification of a wide variety of different targets. This will be revised in the following sections

## 3.2. Biomaging applications

Imaging methodologies for clinical and biological applications are based on many diverse techniques including near infrared and Raman spectroscopy, nuclear magnetic resonance, radioimaging, positron emission tomography, electrochemical imaging, elemental mass spectrometry coupled to laser ablation, luminescence, etc.

Luminescent imaging techniques are becoming increasingly important in clinical diagnostics. Particularly, the high sensitivity and suitability for image-guided surgery can complement conventional imaging methods. These advantages are based on the use of new sensitive and optically and biologically stable probes. In addition, an ideal photoluminescent probe for bioimaging should fulfill more requirements including: (i) high quantum yield, (ii) an appropriate excitation wavelength to avoid phototoxicity, and (iii) emission in the "tissue/biological transparency window". In this sense, near-infrared (NIR) excitation/emission wavelengths allow deeper tissue penetration and minimized photodamage [70-72]. Although imaging in the NIR range reduces autofluorescence and light scattering effects, *in vivo* fluorescence imaging still presents several limitations due to tissue autofluorescence that produces a poor signal-to-noise

ratio. In this sense, time-gated imaging using nanophosphors is an emerging technique to avoid or minimize the undesirable biological and tissue autofluorescence.

The use of phosphorescent nanoparticles as contrast agent for in vivo bioimaging is therefore a strategy with an exciting furuer in the topic. An important issue concerning *in vivo* and *in vitro* applications is the toxicity and biodistribution of the nanoparticles. In fact, size, surface coating and composition of NPs are closely related with their transport and effects in living organisms [73,74] .

This section aims to summarize those nanoparticle-based phosphorescent bioimaging applications reported so far according to the type of nanomaterial employed, such as encapsulated phosphorescent complexes, upconverting nanoparticles, and persistent luminescent nanoparticles.

*3.2.1. Polymer and silicate encapsulated phosphorescent complex.*

As aforementioned in the *Types of phosphorescent nanostructures* section, an efficient strategy to obtain nanophosphors is by encapsulating or trapping a phosphorescent complex (e.g. Ir, Eu, Ru) in a polymer or silica matrix.

An example of this strategy are phosphorescent Pt(II) porphyrins introduced in polymer dots that have been used as $O_2$ sensing photoluminescent probes. The nanoplatform for $O_2$ sensing is based on conjugated polymers acting as antenna for energy transfer to the platinum porphyrin that is sensitive to $O_2$ concentration, and emits around 650 nm [75,76]. This strategy has allowed to carry out ratiometric measurements as well as phosphorescence lifetime imaging (PLIM) and time-gated luminescence imaging for intracellular monitorization of $O_2$ levels. In these studies, it was demonstrated that polymer nanophosphors did not produce noticeable cell toxicity effects. Nevertheless, direct *in vivo* application might be limited for the excitation wavelength at 405 nm. Hence, in order to overcome this limitation, the two-photon cross section can be increased due to the polyfluorene polymer and the antenna effect, and therefore, two photon excitation using a near-infrared wavelength was possible []. A similar strategy based on the antenna effect from the polymer to a red emmiting long life-time complex was followed to produce Eu-poly(9-vinylcarbazole) polymer dots [77]. In this case, the polymer dots were functionalized with an antibody to target the cell surface receptor EpCAM, allowing to perform time-gated imaging of cells.

Luminescent Ru(II) and Eu(III) complexes have been also convalently encapsulated in silica NPs for time-gated luminiscence imaging [78,79]. The visible light-excited NPs were modifiyed with a secondary antimouse-IgG antibody to detect specific antibodyes for environmental pathogen detection using time-gated imaging. Another example of lanthanide encapsulated silica NPs has been reported to perform ratiometric hypochlorous acid (HClO) detection [80]. For this purpose, a dual-emission nanoprobe has been obtained by surface modification of silica NPs with an hypochlorous sensitive Eu-complex and an inert Tb-complex encapsulated in the NPs core. Combination of simultaneos ratiometric and time-gated detection has allowed to perform imaging of exogenous and endogenous HClO in cancerous cells. Moreover, NPs uptake by zebrafish as in vivo model can be monitorized using this detection mode with hight specificity and contrast.

### 3.2.2. Phosphorescent Upconverting nanoparticles (UCNPs).

The above mentioned photoluminescent probes provided high sensitivity and specificity for *in vitro* and small animal imaging models and present most of the spectroscopic requirements. However, UV or Vis light-excitation is not suitable for deeper penetration and could damage cells or tissue, limiting *in vivo* studies. In this sense, UCNPs are very attractive due to the possible NIR excitation and also Vis or NIR emission, which already has been applied for *in vivo* imaging [81]. Unfortunately, excitation of UCNPs usually requires high-power lasers due to their low quantum yield obtained when they are used in small animals, leading to a significant scattering background that limits the sensitivity as well as detrimental thermal effects. UCNPs present long and tunable luminescence lifetime, however their application has not been so exploited for time-gating applications, and only very few reports are available in the literature.

In this sense, Jin *et al.* have recently developed a time-gated imaging system (shown on Figure 8) using a pulsed 980 nm fibre coupled diode laser to be used in mice injected with water-soluble $Yb^{3+}/Tm^{3+}$ co-doped $NaLuF_4$ UCNPs emitting at 800 nm [82]. They achieved an enhancement of 31 times in signal-to-noise ratio comparing the time-gating mode versus the steady state mode. Moreover, the exposure duration to the excitation light was reduced to 35%, which minimizes thermal effects.
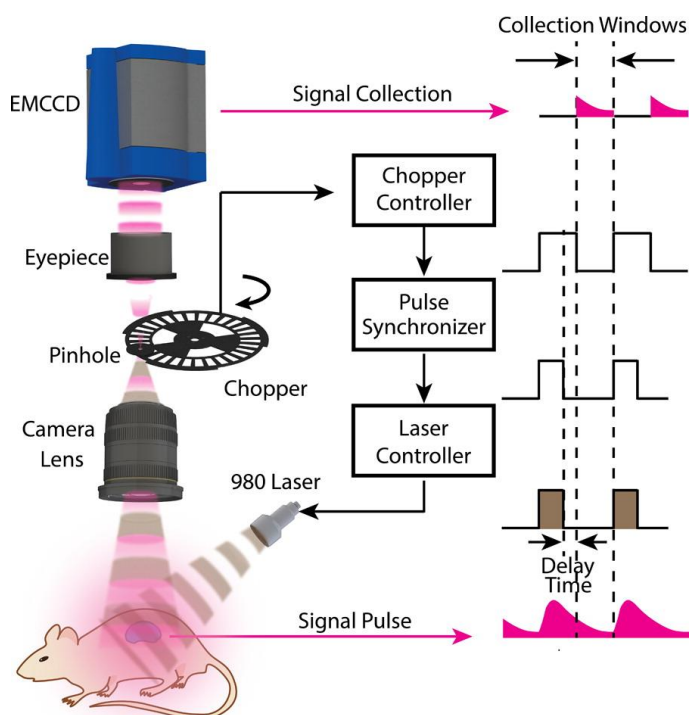
**Figure 8.** Schematics of the in vivo NIR imaging system developed by Jin *et al.* *Reprinted with permission from D. Jin et al., 'High-Contrast Visualization of Upconversion Luminescence in Mice Using Time-Gating Approach', Anal. Chem. 88, p. 3449–3454. Copyright 2016 American Chemical Society.*

### 3.2.3. Persistent luminescent nanoparticles.

Persistent luminescent nanoparticles (PLNPs) are especially attractive for *in vivo* imaging applications because can be optically excited before administration, avoiding any potential photodamage. The long lasting-afterglow allows nanoparticle detection during several hours without *in situ* excitation, and consequently, background from the excitation source or tissues can be completely avoided improving the contrast and the signal-to-noise ratio. Moreover, it is possible to synthetize NIR emitting PLNPs that are especially suitable for *in vivo* deep-tissue imaging [10,83]. These spectroscopic features allow to perform studies of real-time biodistribution of nanoparticles even hours after their administration [84], which is very important, besides their use as photoluminescent probes, in order to study their toxicity effect on living organisms.

Chen and Zhang were pioneers using PLNPs as *in vivo* contrast agents for photodynamic therapy [85]. Later Scherman and co-workers reported PLNPs application for *in vivo* imaging, using NIR emitting $Ca_{0.2}Zn_{0.9}Mg_{0.9}Si_2O_6$:

$Eu^{2+}, Dy^{3+}, Mn^{2+}$ PLNPs as passive tumour targeting agent in a mouse model []. Despite all the impressive spectroscopic features of PLNPs for *in vivo* imaging, they observed important limitations since the PLNPs suffered high liver and spleen reticuloendothelial system (RES) uptake. Hence, in order to overcome this limitation, the NPs surface coating was optimized in terms of PEG length and surface charge, trying to extend the NPs blood circulation necessary to perform passive tumour targeting, as well as to minimize RES uptake [86]. Results showed that a better biodistribution was achieved with non-charged NPs (metoxi-PEG particles) and smaller particle size. However, PLNPs emission intensity is size dependent, and such smaller NPs that are more convenient for tumour targeting, are not detectable in deep tissues and *ex vivo* imaging is required to study biodistribution.

New PLNPs using different host and luminescent dopants has been proposed to extend the afterglow emission since many bioanalytical applications, especially passive tumour targeting, require longer monitoring time than that reached with the first generation of PLNPs (1-3 h *in vivo*). In these sense, zinc gallate (ZGO) and gallogermanate (ZGGO) hosts have demonstrated to overcome the limitations previously mentioned due to the possibility to re-excite PLNPs *in vivo* using orange/red light-emitting diodes (LEDs), allowing NPs detection for several days in small animals [89,90]. This new generation of PLNPs has been further functionalized with different biomolecules to be successfully used in active targeting [87,88,89]. Moreover, multimodal contrast agents have been developed combining PLNPs with a metal complex or other kind of NPs to obtain multimodal probes that present persistent luminescence and simultaneously, magnetic resonance [90,91] or X-ray computed tomography features [92].

The current challenge in the development of PLNPs for imaging applications is to control the synthesis process to obtain monodispersed PLNPs with smaller sizes to minimize RES uptake and to improve biocompatibility, to increase the quantum yield and to enlarge the afterglow [93,94]. In this sense, triple-doped ZGGO NIR emitting NPs with high quantum yield (9.86%), superlong afterglow time (>20 days) and 44 nm size have been synthesized and successfully applied to tumour targeting bioimaging after oral administration [95].

**3.3. Biosensing applications using phosphorescent nanoprobes as labels**

It is well known that time-gated phosphorescence is a powerful method to perform highly sensitive detection of trace analytes. By using a pulsed excitation source and measuring the phosphorescence emission after a "delay time", it is possible to eliminate any contribution that comes from the excitation source or from the fluorescent background. Therefore, phosphorescence-based methodologies are an excellent option to develop analytical strategies in biological media where scattering macromolecules and luminescent species abound [38].

### 3.3.1. Mn-doped QDs quenching or enhancement-based biosensing

Due to the long-lived phosphorescence of Mn-doped QDs on the order of the ms attributed to the $^4T_1$ to $^6A_1$ transition, they have been explored as phosphorescent probes in biological matrices since their scattering and fluorescence background can be eliminated. Most of these applications are based on a quenching or enhancement of the phosphorescence. In this sense, the first biosensing application was performed for the detection of Enoxacin in urine, without the need of oxygen scavengers or other RTP inducers. Quenching of the emission of L-cysteine Mn-doped ZnS QDs allowed the detection of the aforementioned antibiotic with a limit of detection of 58.6 nM, and good selectivity was achieved in presence of the main relevant metal ions in biological fluids, biomolecules including aminoacids, glucose, and other types of antibiotics [96]. Detection of glucose was also performed based on quenching of the phosphorescence. In this case, the enzyme glucose oxidase was used as capping agent of Mn-doped ZnS QDs, and the quenching of the phosphorescence was performed by the generation of $H_2O_2$ produced upon oxidation of glucose catalysed by the enzyme. A detection limit for glucose detection in human serum of 3 μM was achieved, without the need for any complicated sample pretreatment [97]. Quenching of the phosphorescence of Mn doped ZnS QDs with different surface coatings has been also exploited for the detection of different types of analytes including acetone in water and urine [98,99], racenisodamine hydrochloride and atropine sulphate in human serum and urine [100] (all of them containing a L-cysteine capping), arginine in urine (using ATP as capping agent) [101], or DNA in urine (using octa(3-aminopropyl)octasilsequioxane octahydrochloride as capping ligand) [102].

Although most of the biosensing applications of Mn-doped QDs are based on quenching of the luminescence, there are also reported some methodologies based on phosphorescence enhancement. In this sense, sodium tripolyphosphate-capped Mn-doped ZnS QDs have been used for the turn-on RTP detection of ascorbic acid in human urine and plasma avoiding sample pretreatment and in presence of the main metal and molecules present in biological fluids (LOD 9 nM). Ascorbic acid is capable to chelate the Mn and Zn from the surface of the QDs, generating more holes which are subsequently trapped by $Mn^{2+}$ while ascorbic acid reduces $Mn^{3+}$ to $Mn^{2+}$ in the excited state, giving rise to an enhancement of the excitation and emission of the QDs.

QDs exhibit exceptional optoelectronic properties, making them ideal species to be used as donor moieties in Förster resonance energy transfer systems (FRET). Phosphorescent Mn-doped ZnS QDs surface labelled with Thrombin-binding aptamers were used as donors in the development of a FRET sensor for Thrombin quantification in biological fluids [103]. In this system carbon nanodots were used as acceptor energy species. After the recognition of the Thrombin by the aptamer-QD-CD system, the phosphorescence originally quenched due to the π-π stacking interaction between the aptamer and the CDs, is "turned on" due to a formation of quadruplex-thrombin complexes releasing the energy acceptor CDs from the energy donors. The aptamer-based "turn-on" phosphorescent nanosensor displays a detection limit as low as 0.013 nM in pure buffers with high selectivity.


### 3.3.2. Lateral Flow Tests

Lateral Flow Tests (LFTs) have gained an increasing interest for point-of-care (POC) applications due to its simplicity, low cost and user friendliness. They have been developed for the detection of an enormous amount of analytes including small molecules, macromolecules or microorganisms, and have already reached the market for detection of pregnancy, infections, cardiovascular disorders, or certain drugs-of-abuse. Most of these products are based on changes on the colour and suffer problems of sensitivity. Therefore, a great effort is being pursued in order to use new types of detection techniques. Among them, the use of phosphorescent nanoparticles as labels is starting to be explored, since fluorescence LFTs present also some disadvantages as the high background of the membrane or the sample matrix (e.g., blood or serum), and the small Stokes shifts of traditional fluorophores, which typically range from 10 to 30 nm.

Although little success has been achieved mainly due to oxygen quenching, Pt-based and Pd-based halogen-containing polymeric nanoparticles, with emission wavelengths at 650-750 nm and lifetimes of 100 µs and 500 µs respectively [104], have been evaluated as phosphorescent labels for the detection of C-reactive protein (CRP), an inflammatory biomarker, in serum. The use of these phosphorescent nanoparticles with low photobleaching effect, conjugated to an appropriate antibody, have allowed the development of a disposable test strip that uses a cheap LED as excitation source, and the fluorescent background of biological matrices and nitrocellulose membranes is dramatically reduced, achieving a detection limit for CRP of 0.2 ng mL$^{-1}$ [105].

Persistent luminescence nanoparticles (PLNPs) that emit intense visible light for several minutes after excitation have been also evaluated as labels in lateral flow assays (LFA). For this purpose, strontium aluminate PLNPs, encapsulated in silica nanoparticles to provide stability in water and with a final diameter of 250 nm, were functionalized with NeutrAvidin and used as reporters in LFAs with biotinylated hen egg lysozyme (bHEL) as a model analyte, achieving a sensitivity in the low low-picomolar level (7 pM) [106].

### 3.3.3. Phosphorescent immunoassays

Highly sensitive immunoassays can be developed by using phosphorescent labels that present decay times much longer than those of the background. Traditionally, phosphorescent immunoassays were based on the use of lanthanide ion chelates which show long lifetimes. However, this strategy has certain limitations due to problems of contamination and unspecific adsorptions of lanthanide ions in the different steps of the immunoassay [107,108]. In order to overcome these limitations, phosphorescent nanocrystals are an appealing alternative, and they have been already evaluated for different applications. In this sense, a phosphorescent sandwich immunoassay was developed using phosphorescent nanoparticles as labels, and prepared through the encapsulation of phosphorescent dyes inside beads synthesized by glutaraldehyde polymerization. The phosphorescent beads were then covalently linked to an antibody, and the sandwich immunoassay was performed with high sensitivity and specificity for human IgG as model analyte [109].

Despite PLNPs are mainly exploited in bioimaging applications as consequence of their long-lasting afterglow nature, PLNPs can be also employed in biosensing applications.

Hence, a FRET-based strategy was employed for the detection of PSA in human serum. For this purpose, a PSA antibody (PS6) was bioconjugated to PLNPs (PS6-PLNPs), and another PSA antibody (8A6) was labelled with Rhodamine B (8A6-RhB). PS6-PLNPs. After excitation at 360 nm of PS6-PLNPs, acting as energy donor, its long-lasting emission was transferred to 8A6-RhB, acting as acceptor. Therefore, an increase of the concentration of PSA in the sample gives rise to an increase of the ratio of the luminescence intensity of RhB (at 585 nm) to the luminescence intensity of PLNP (at 524 nm), allowing to perform an excitation-free type analysis (see Figure 9) [110].
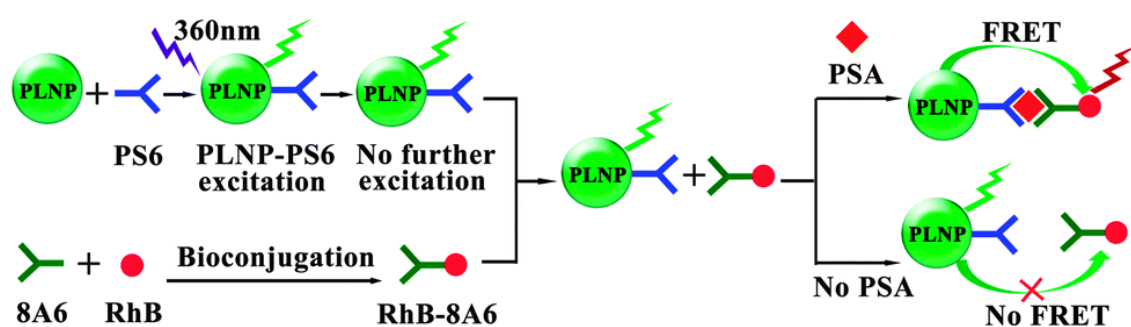


**Figure 9** Schematic illustration of the PLNP based FRET immunoassay for PSA detection. Reproduced from Ref. [110] with permission from The Royal Society of Chemistry.

Water stabilized PLNPs (Eu2+- and Dy3+-doped Ca1.86Mg0.14ZnSi2O7 nanoparticles) were also used as energy donors in another type of inhibition immunoassay. In this work, the presence of analyte produced a shortage of the distance between the donor (PLNPs), and an antibody labelled with gold nanoparticles (AuNPs) acting as acceptor. Therefore, due to the spectral overlap between the emission of the donor and the absorption of the acceptor, an inhibition of the luminescence takes place. This strategy was evaluated for the detection of α-fetoprotein (AFP), a serum biomarker for early diagnosis of hepatocellular carcinoma, and has allowed to perform the detection without external light source (after a pre-excitation stage), thus removing the autofluorescence and scattering light from the biological matrix found when in situ excitation is performed [27].

Other approaches also employed to entirely remove the interferent signal from cell or tissue autofluorescence and scattered light are based on the long-lived emission of lanthanide ions. In this sense, water-stabilized NaYF4:Ce/Tb NPs and ZrO2–Ln3+ NPs were evaluated as probes to perform the detection of trace levels of avidin in a typical avidin–biotin model system with detection limit of 5 nM and 3 nM respectively [111112]; and CaF2:Ln3+ NPs were used to detect a tumor biomarker (suPAR) at clinically relevant levels with a low detection limit of 328 pM [113].

It is clear from the written so far that FRET-based strategies are behind most common phosphorescent immunoassays that make use of nanoparticles. However, a sandwich-type phosphorescent immunoassay for PSA was recently developed. In this case, the secondary antibody was labelled with phosphorescent DHLA-coated Mn-doped QDs (see Figure 8). Bidentated DHLA ligands provided QDs with excellent stability giving rise to an intense phosphorescent emission. Extremely low detection limit (17 pg PSA mL$^{-1}$) could be then achieved. Notably, since the developed approach avoids bioconjugation of the QDs to the specific corresponding primary antibodies, it becomes almost generic as it could be applied for the detection of any other target protein just by changing the corresponding non-labelled specific capture and recognition antibodies [114]. This is in fact a great practical advantage because the most critical and cumbersome step in the development of NP-based immunoassays is the control and optimization of the NP-antibody bioconjugation reaction
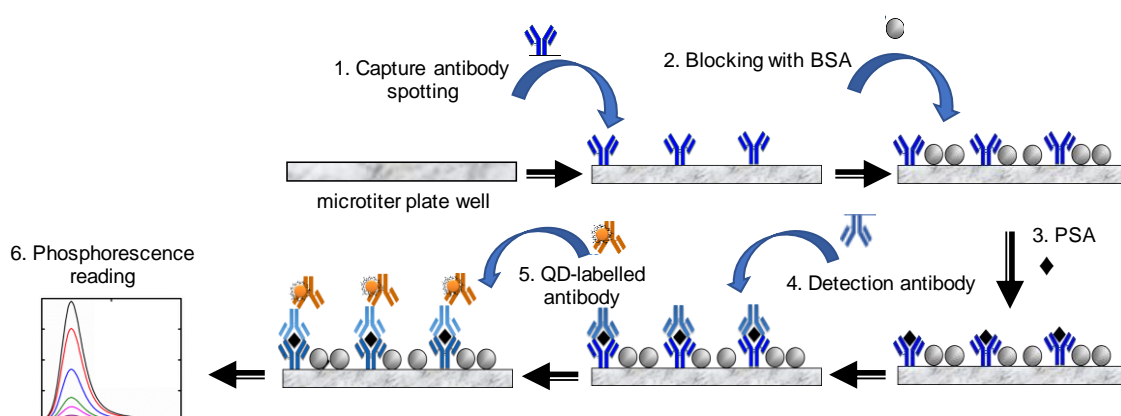


**Figure 10**. Schematic diagram of the antibody array strategy for the phosphorescence detection of PSA [114]. *Reprinted from Analytica Chimica Acta 987, M. García-Cortés*

## 4. Concluding Remarks and Perspectives

A wide number of phosphorescence-emitter nanoparticles have been recently reported. Such recent marriage between nanotechnology and phosphorescence detection is an exciting achievement both in sensing and bioimaging applications, even considering that it is still in its infancy and further research is needed to fully realize its potential in bioanalytical chemistry. Besides the documented exceptional properties of nanomaterials, the phosphorescent analytical signal allows to overcome many of the drawbacks reported for luminescence-based techniques. In this context, a complex challenge in biosensing of species of clinical interest is to achieve the required limits of sensitivity while maintaining selectivity. Photoluminescence time-gated detection played a key role in determining the sensitivity and selectivity of the sensing platforms because photoluminescence emission background coming from the biological media is greatly reduced. Additionally, hybridation of multiple small NPs emitting phosphorescence (e.g. metal doped-QDs) with a single biological recognition element brings with an amplified analytical signal, thus reducing the detection limits of the methodologies.However, to fully exploit the capabilities of phosphorescent nanomaterials for sensing and imaging purposes, it is necessary to develop novel robust approaches allowing to rapidly and accurately measuring the luminescence lifetimes from the relatively slow-decaying signals. Additionally, novel and improved phosphorescence lifetime imaging microscopy systems are required in order to monitor the phosphorescent signals from the nanoparticles allowing the acquisition of biologically relevant chemical information through lifetime measurements, Förster resonance energy transfer and phosphorescence quenching detection. Current developments of such new time-gating sensing and imaging systems for spectral and lifetime multiplexing will undoubtly contribute to extend the application field of the novel phosphorescent nanoparticles [115,116,117].

Another exciting research area in chemical sensing is the development of novel phosphorescent nanomaterials allowing optical multiplexing a property highly desirable in the development of extremely sensitive biosensing systems with multiplexing capabilities. Research on this area include the design of novel nanocomposites with wide-range, well-separated, and tuneable lifetime ultrabright phosphorescence or nanoassemblies obtained using the combination of long-lifetime dyes and semiconductor quantum dots for development of sensor systems based on time-gated Förster resonance energy transfer detection [118,119].

A big challenge in optical sensors development is related to the absolute intensity-dependent signal readout, resulting in erroneous sensing and imaging results due to different analyte-independent factors producing undesirable changes in the absolute optical signal intensity [120]. To minimize such effects, ratiometric measurements provide built-in self-calibration for signal correction. Advances in nanomaterial science make feasible the synthesis of optical nanoprobes with multiple functionalities (e.g. analyte-dependent phosphorescence emission along with analyte insensitive fluorescence or absorbance) [121] allowing the development of ratiometric strategies that can overcome many of the limitations met by traditional optical nanoprobes .

Last, but not least, a huge amount of research work is being done to produce nanostructures for multimodal imaging, combining the advantages of phosphorescence emission with an improved imaging resolution thanks to the simultaneous detection by complementary imaging techniques. These multimodal imaging agents can be later detected using phosphorescence along with a selection from radioisotope, magnetic resonance and optical imaging, among others. Nanoparticles offer great scope in this area as they lend themselves, via facile modification procedures, to act as multifunctional constructs.

To conclude, we believe that analytical applications of phosphorescent nanoparticles will continue to rise and novel and improved sensing and imaging schemes using such nanoparticles will appear in the next future in parallel with developments from nanoscience and nanotechnology.

**Acknowledgements**

**References**

1 G. Orellana, 'Luminescent optical sensors' Anal. Bioanal, Chem. 2004, 379, p. 344–346

2 R.C. Somers, M.G. Bawendi, D.G. Nocera, 'CdSe nanocrystal based chem-/bio-sensors', Chem. Soc. Rev. 2007, 36 (4), p. 579-591

3 C. Burda, X. Chen, R. Narayanan, M. A. El-Sayed, 'Chemistry and Properties of Nanocrystals of Different Shapes', Chem. Rev. 2008, 105 (4), p. 1025-1102

4 H. Goesmann, C. Feldmann, 'Nanoparticulate Functional Materials', Angew. Chem. Int. Ed. 2010, 49, p. 1362–1395

5 M.F. Frasco, N. Chaniotakis, 'Semiconductor Quantum Dots in Chemical Sensors and Biosensors', Sensors 2009, 9 (9), p. 7266-7286

6 J.M. Costa-Fernandez, 'Optical sensors based on luminescent quantum dots', Anal Bioanal Chem 2006, 384, p. 37–40

7 I. Sánchez-Barragán, J.M. Costa-Fernández, A. Sanz-Medel, M. Valledor, J.C. Campo, 'Room Temperature Phosphorescence for Optical Sensing' Trends in Analytical Chemistry 2006, 25 (10), p. 958-967

8 W. Zheng, P. Huang, D. Tu, E. Ma, H. Zhuab, X, 'Lanthanide-doped upconversion nano-bioprobes: electronic structures, optical properties, and biodetection', ChenChem. Soc. Rev., 2015, 44, p. 1379-1415

9 P. Wu, X.-P. Yan, 'Doped quantum dots for chemo/biosensing and bioimaging', Chem. Soc. Rev. 2013, 42, p. 5489-5521

10 T. Lécuyer, E. Teston, G. Ramirez-Garcia, T. Maldiney, B. Viana, J. Seguin, N. Mignet, D. Scherman, C. Richard, 'Chemically engineered persistent luminescence nanoprobes for bioimaging', Theranostics 2016, 6, p. 2488–2523

11 J. Wang, Q. Ma, Y. Wang, H. Shena, Q. Yuan, 'Recent progress in biomedical applications of persistent luminescence nanoparticles', Nanoscale, 2017, 9, 6204-6218

12 J.D. Bryan, D.R. Gamelin, 'Doped Semiconductor Nanocrystals: Synthesis, Characterization, Physical Properties, and Applications', Prog. Inorg. Chem. 2005, 54, p. 47–126

13 A.A. Bol, A. Meijerink, 'Doped semiconductor nanoparticles – a new class of luminescent materials?', J. Lumin. 2000, 87–89, p. 315–318

14 S. K. Panda, S. G. Hickey, H. V. Demir, A. Eychmüller, 'Bright White-Light Emitting Manganese and Copper Co-Doped ZnSe Quantum Dots', Angew. Chem., Int. Ed. 2011, 50, p. 4432–4436

15 O. Ehlert, A. Osvet, M. Batentschuk, A. Winnacker, T. Nann, 'Synthesis and spectroscopic investigations of Cu- and Pb-doped colloidal ZnS nanocrystals', J. Phys. Chem. B 2006, 110, p. 23175–23178

16 J. Q. Zhuang, X. D. Zhang, G. Wang, D. M. Li, W. S. Yang, T. J. Li, 'Synthesis of water-soluble $ZnS:Mn^{2+}$ nanocrystals by using mercaptopropionic acid as stabilizer', J. Mater. Chem., 2003, 13, p. 1853–1857

17 J. F. Suyver, S. F. Wuister, J. J. Kelly, A. Meijerink, 'Synthesis and Photoluminescence of Nanocrystalline ZnS:Mn$^{2+}$', Nano Lett., 2001, 1, p. 429–433.

18 A. A. Bol, A. Meijerink, 'Luminescence Quantum Efficiency of Nanocrystalline ZnS:Mn$^{2+}$. 1. Surface Passivation and Mn$^{2+}$ Concentration', J. Phys. Chem. B 2001, 105, p. 10197–10202

19 W. B. Zhou, F. Baneyx, 'Aqueous, Protein-Driven Synthesis of Transition Metal-Doped ZnS Immuno-Quantum Dots', ACS Nano 2011, 5, p. 8013–8018

20 H. Yang, P. H. Holloway, 'Efficient and Photostable ZnS-Passivated CdS:Mn Luminescent Nanocrystals', Adv. Funct. Mater. 2004, 14, p. 152–156.

21 L. M. Gan, B. Liu, C. H. Chew, S. J. Xu, S. J. Chua, G. L. Loy, G. Q. Xu, 'Enhanced Photoluminescence and Characterization of Mn-Doped ZnS Nanocrystallites Synthesized in Microemulsion', Langmuir 1997, 13, p. 6427–6431

22 P. I. Archer, S. A. Santangelo, D. R. Gamelin, 'Inorganic Cluster Syntheses of TM2+-Doped Quantum Dots (CdSe, CdS, CdSe/CdS): Physical Property Dependence on Dopant Locale', J. Am. Chem. Soc. 2007, 129, p. 9808–9818

23 N. Pradhan, D. D. Sarma, 'Advances in Light-Emitting Doped Semiconductor Nanocrystals', J. Phys. Chem. Lett. 2011, 2, p. 2818–2826

24 F. V. Mikulec, M. Kuno, M. Bennati, D. A. Hall, R. G. Griffin, M. G. Bawendi, 'Organometallic Synthesis and Spectroscopic Characterization of Manganese-Doped CdSe Nanocrystals', J. Am. Chem. Soc., 2000, 122, 2532–2540, DOI: DOI: 10.1021/ja991249n.

25 N. Pradhan, X. G. Peng, 'Efficient and Color-Tunable Mn-Doped ZnSe Nanocrystal Emitters: Control of Optical Performance via Greener Synthetic Chemistry', J. Am. Chem. Soc. 2007, 129, p. 3339–3347

26 P. V. Radovanovic, D. R. Gamelin, 'Electronic Absorption Spectroscopy of Cobalt Ions in Diluted Magnetic Semiconductor Quantum Dots: Demonstration of an Isocrystalline Core/Shell Synthetic Method', J. Am. Chem. Soc. 2001, 123, p. 12207–12214

27 B.-Y. Wu, H.-F. Wang, J.-T. Chen, and X.-P. Yan, 'Fluorescence Resonance Energy Transfer Inhibition Assay for α-Fetoprotein Excreted during Cancer Cell Growth Using Functionalized Persistent Luminescence Nanoparticles', J. Am. Chem. Soc. 2011, 133 (4), p. 686–688

28 E. Finley, A. Cobb, A. Duke, A. Paterson, J. Brgoch, 'Optimizing Blue Persistent Luminescence in $(Sr_{1-\delta}Ba_{\delta})_2MgSi_2O_7{:}Eu^{2+},Dy^{3+}$ via Solid Solution for Use in Point-of-Care Diagnostics', ACS Appl. Mater. Interfaces, 2016, 8 (40), p. 26956–26963.

29 S. H. M. Poort, H. M. Reijnhoudt, H. O. T. van der Kuip, G. Blasse, 'Luminescence of Eu$^{2+}$ in Silicate Host Lattices with Alkaline Earth Ions in a Row', J. Alloys Compd. 1996, 241, p. 75– 81

30 S. Chen, M.S. Zaeimian, J.H.S.K. Monteiro, J. Zhao, A.G. Mamalis, A. de Bettencourt-Dias, X. Zhu, 'Mn doped AIZS/ZnS nanocrystals: Synthesis and optical properties', Journal of Alloys and Compounds, 725, 2017, p.1077-1083

31 R. Beaulac, P.I. Archer, D.R. Gamelin, 'Luminescence in colloidal Mn2+-doped semiconductor nanocrystals', Journal of Solid State Chemistry 2008, 181 (7), p. 1582-1589

32 J.F. Wang, W.M. Liu, Q. Tu, J.C. Wang, N. Song, Y.R. Zhang, N. Nie, J.Y. Wang, 'Folate-decorated hybrid polymeric nanoparticles for chemically and physically combined paclitaxel loading and targeted delivery', Biomacromolecules 2011, 12, p. 228–234

33 T. K. Jain, I. Roy, T. K. De, A. Maitra, 'Nanometer Silica Particles Encapsulating Active Compounds: A Novel Ceramic Drug Carrier', J. Am. Chem. Soc. 1998, 120, p. 11092-11095

34 J. D. Byrne, T. Betancourt, L. Brannon-Peppas, 'Active targeting schemes for nanoparticle systems in cancer therapeutics', Adv. Drug Delivery Rev. 2008, 60, p. 1615-1626

35 J. H. Park, G. von Maltzahn, E. Ruoslahti, S. N. Bhatia, M. J. Sailor, 'Micellar Hybrid Nanoparticles for Simultaneous Magnetofluorescent Imaging and Drug Delivery', Angew. Chem., Int. Ed. 2008, 47, p.7284-7288

36 M. Shokeen, N.M. Fettig, R.Q. Rossin, 'Synthesis, in vitro and in vivo evaluation of radiolabeled nanoparticles', J. Nucl. Med. Mol. Imaging 2008, 52, p.267-277

37 Y. Wang, Y. Wu, F. Li, D. Chen, 'Folic acid-modified iridium(III) coordination polymeric nanoparticles facilitating intracellular release of a phosphorescent residue capable of nuclear entry', Inorg. Chem. Commun. 2014, 40, p.143–147.

38 A.N. Edwards, M.Yamazaki, S.H. Krishnadasan, T.W. Phillips, L.Rowlands, R. Jourdain, A.M. Nightingale, J.C. de Mello, 'Photostable phosphorescent polymer nanospheres for high sensitivity detection', J. Mater. Chem. C 2015, 3, p.6565-6572

39 J.M. Kürner, I. Klimant, C. Krause, H. Preu, W. Kunz, O.S. Wolfbeis, 'Inert Phosphorescent Nanospheres as Markers for Optical Assays', Bioconjugate Chem., 2001, 12, p.883–889

40 H. Fessi, F. Puisieux, J.P. Devissaguet, Google Pat., publication number CA1293170 C, application number CA 555153, also published as DE3777793D1, EP0274961A1, EP0274961B1, US5049322, 1988.

41 X. Song, L. Huang, B. Wu, 'Bright and Monodispersed Phosphorescent Particles and Their Applications for Biological Assays', Anal. Chem. 2008, 80, p.5501–5507

42 R. Kumar, T. Y. Ohulchanskyy, I. Roy, S. K. Gupta, C. Borek, M. E. Thompson, P. N. Prasad, 'Near-Infrared Phosphorescent Polymeric Nanomicelles: Efficient Optical Probes for Tumor Imaging and Detection', ACS Appl. Mater. Interfaces 2009, 1 (7), p. 1474–1481.

43 K. Ma, H. Sai, U. Weisner, 'Ultrasmall Sub-10 nm Near-Infrared Fluorescent Mesoporous Silica Nanoparticles', J. Am. Chem. Soc. 2012, 4, p. 13180-13183

44 S. Titos-Padilla, E. Colacio, S.J.A. Pope, J.J. Delgado, M. Melgosa and J.M. Herrera, 'Photophysical properties of $[Ir(tpy)_2]^{3+}$-doped silica nanoparticles and synthesis of a colour-tunable material based on an Ir(core)–Eu(shell) derivative', J. Mater. Chem. C 2013,1, p.3808-3815

45 X. Chen, Y. Liu, D. Tu, 'Lanthanide-Doped Luminescent nanomaterials', Nanomedicine and Nanotoxicology, Springer Verlag, Berlin, 2014. Ch. 7 p. 145-164. DOI: 10.1007/978-3-642-40364-4

46 Radiative decay engineering:metal enhanced fluorescence, In Principles of fluorescence spectroscopy. J.R. Lakowicz (ed), Springer, New York, USA, p 841.

47 Y. Zhang, K. Aslan, S. N. Malyn, C. D. Geddes, 'Metal-enhanced phosphorescence (MEP'), Chem. Phys. Lett. 2006, 427, p. 432–437

48 M.J.R. Previte, K. Aslan, Y. Zhang, C. D. Geddes, 'Surface Plasmon coupled phosphorescence (SPCP)', Chemical Physics Letters 2006, 432, p. 610-615.

49 M. C. He, Y. Q. Li, Y. X. Zhu, Y. Zhang, 'Metal nanoparticle-enhanced room temperature phosphorescence of diiodofluorescein on the filter paper substrate', Chin. Chem. Lett. 2010, 21, p. 109–112

50 R. Toftegaard, J. Arnbjerg, H. Cong, H. Agheli, D.S. Sutherland, P.R. Ogilb, 'Metal nanoparticle-enhanced radiative transitions: Giving singlet oxygen emission a boost', Pure Appl. Chem. 2011, 83 (4), p. 885–898

51 L.-D. Sun, Y.-F. Wang, C.-H. Yan, 'Paradigms and Challenges for Bioapplication of Rare Earth Upconversion Luminescent Nanoparticles: Small Size and Tunable Emission/Excitation Spectra', Acc. Chem. Res., 2014, 47 (4), p. 1001–1009

52 M. Pollnau, D. R. Gamelin, S. R. Lüthi, H. U. Güdel, M. P. Hehlen, 'Power dependence of upconversion luminescence in lanthanide and transition-metal-ion systems', Phys. Rev. B 2000, 61, p.3337-3346

53 J. Zhao, W. Wu, J. Sun, S. Guo, 'Triplet photosensitizers: from molecular design to applications', Chem. Soc. Rev. 2013, 42, p.5323-5351

54 J. Zhao, K. Xu, W. Yang, Z. Wang, F. Zhong, 'The tripled excited state of Bodipy: formation, modulation and application', Chem. Soc. Rev. 2015, 44, p. 8904-8939

55 K. Poorkazem, A. V. Hesketh, T. L. Kelly, 'Plasmon-Enhanced Triplet−Triplet Annihilation Using Silver Nanoplates', J. Phys. Chem. C 2014, 118, p. 6398−6404

56 D. Chen, Y. Chen, H. Lu, and Z. Ji, 'A Bifunctional Cr/Yb/Tm:Ca3Ga2Ge3O12 Phosphor with Near-Infrared Long-Lasting Phosphorescence and Upconversion Luminescence', Inorg. Chem. 2014, 53, p.8638−8645

57 Q. Li, M. Zhou, Q. Yang, Q. Wu, J. Shi, A. Gong, M. Yang, 'Efficient Room-Temperature Phosphorescence from Nitrogen-Doped Carbon Dots in Composite Matrices', Chem. Mater. 2016, 28 (22), p. 8221–8227

58 K. Jiang, Y. Wang, X. Gao, C. Cai, H. Lin, 'Facile, Quick, and Gram-Scale Synthesis of Ultralong-Lifetime Room-Temperature-Phosphorescent Carbon Dots by Microwave Irradiation', Angew. Chem., Int. Ed. 2018, 57, https://doi.org/10.1002/anie.201802441

59 X. Zhen, Y. Tao, Z. An, P. Chen, C. Xu, R. Chen, W. Huang, K. Pu, 'Ultralong Phosphorescence of Water-Soluble Organic Nanoparticles for In Vivo Afterglow Imaging' Advanced Materials 2017, 29, 1606665

60 B. I. Ipe, K. Yoosaf, K.G. Thomas, 'Functionalized Gold Nanoparticles as Phosphorescent Nanomaterials and Sensors', J. Am. Chem. Soc., 2006, 128 (6), p. 1907–1913

61 C. Zhang, K. Zhang, T. Zhao, B. Liu, Z. Wang, and Z. Zhang, 'Selective phosphorescence sensing of pesticide based on the inhibition of silver(I) quenched ZnS:Mn$^{2+}$quantum dots', Sens. Actuator B-Chem 2017, 252, p. 1083–1088

62 D. Li, Z. Fan, 'Phosphorescence detection of 2-mercaptobenzothiazole in environmental water samples by Mn-doped ZnS quantum dots', New J. Chem. 2017, 41, p. 4763-4766

63 M. Horka, S. Sun, A. Ruszcak, P. Garstecki, T. Mayr, 'Lifetime of Phosphorescence from Nanoparticles Yields Accurate Measurement of Concentration of Oxygen in Microdroplets, Allowing One To Monitor the Metabolism of Bacteria',Anal Chem. 2016, 88 (24), p. 12006-12012

64 A.V. Kondrashina, R.I. Dmitriev, S.M. Borisov, I. Klimant, I. O'Brien, Y.M. Nolan, A.V. Zhdanov, D.B. Papkovsky, 'A Phosphorescent Nanoparticle-Based Probe for Sensing and Imaging of (Intra)Cellular Oxygen in Multiple Detection Modalities', Advanced Functional Materials, 2012, 22 (23), p. 4931-4939

65 B-H. Zhang, F-Y. Wu, Y-M. Wu, X-S. Zhan, 'Fluorescent Method for the Determination of Sulfide Anion with ZnS:Mn Quantum Dots', J Fluoresc. 2010, 20, p. 243–250

66 W-S. Zou, D. Sheng, X. Ge, J-Q. Qiao, H-Z Lian, 'Room-Temperature Phosphorescence Chemosensor and Rayleigh Scattering Chemodosimeter Dual-Recognition Probe for 2,4,6-Trinitrotoluene Based on Manganese-Doped ZnS Quantum Dots', Anal. Chem. 2011, 83, p. 30–37

67 W-S. Zou, J-Q. Qiao, X. Hu, X. Ge, H-Z. Lian, 'Synthesis in aqueous solution and characterisation of a new cobalt-doped ZnS quantum dot as a hybrid ratiometric chemosensor', Ana. Chim. Acta 2011, 708, p. 134– 140

68 Y-Q. Wang, and W-S. Zou, '3-Aminopropyltriethoxysilane-functionalized manganese doped ZnS quantum dots for room-temperature phosphorescence sensing ultratrace 2,4,6-trinitrotoluene in aqueous solution', Talanta 2011, 85, p. 469–475

69 H.F. Wang, Y. He, T.R. Ji, X.P. Yan, 'Surface molecular imprinting on Mn-doped ZnS quantum dots for room-temperature phosphorescence optosensing of pentachlorophenol in water', Anal Chem. 2009, 81 (4), p. 1615-1621

70 R. Weissleder, 'A clearer vision for in vivo imaging', Nat. Biotechnol. 2011, 19, p.316–317

71 J. V. Frangioni, 'In vivo near-infrared fluorescence imaging', Curr. Opin. Chem. Biol. 2003, 7, p. 626–634

72 V.J. Pansare, S. Hejazi, W.J. Faenza,  R.K. Prud'Homme, 'Review of long-wavelength optical and NIR imaging materials: Contrast agents, fluorophores, and multifunctional nano carriers', Chem. Mater. 2012, 24, p. 812–827

73 J. Lazarovits, Y.Y. Chen, E.A. Sykes, W.C.W. Chan, 'Nanoparticle-blood interactions: the implications on solid tumour targeting.', Chem. Commun. (Camb). 2014, 51, p. 2756–2767

74 L.A. Lane, X. Qian, A.M. Smith, S. Nie, 'Physical Chemistry of Nanomedicine: Understanding the Complex Behaviors of Nanoparticles in Vivo', Annu. Rev. Phys. Chem. 2015, 66, p. 521–547

75 R.I. Dmitriev, S.M. Borisov, H. Düssmann, S. Sun, B.J. Müller, J. Prehn, V.P. Baklaushev, I. Klimant, D.B. Papkovsky, 'Versatile Conjugated Polymer Nanoparticles for High-Resolution O2 Imaging in Cells and 3D Tissue Models', ACS Nano. 2015, 9, p. 5275–5288

76 Q. Zhao, X. Zhou, T. Cao, K.Y. Zhang, L. Yang, S. Liu, H. Liang, H. Yang, F. Li, W. Huang, 'Fluorescent/phosphorescent dual-emissive conjugated polymer dots for hypoxia bioimaging', Chem. Sci. 2015, 6, p. 1825–1831

77 W. Sun, J. Yu, R. Deng, Y. Rong, B. Fujimoto, C. Wu, H. Zhang, D.T. Chiu, 'Semiconducting polymer dots doped with europium complexes showing ultranarrow emission and long luminescence lifetime for time-gated cellular imaging', Angew. Chemie - Int. Ed. 2013, 52, p. 11294–11297

78 C. Song, Z. Ye, G. Wang, D. Jin, J. Yuan, Y. Guan, and J. Piper, 'Preparation and time-gated luminescence bioimaging application of ruthenium complex covalently bound silica nanoparticles', Talanta 2009, 79, p. 103–108

79 L. Tian, Z. Dai, L. Zhang, R. Zhang, Z. Ye, J. Wu, D. Jin, J. Yuan, 'Preparation and time-gated luminescence bioimaging applications of long wavelength-excited silica-encapsulated europium nanoparticles', Nanoscale 2012, 4, p. 3551-3357

80 H. Ma, B. Song, Y. Wang, D. Cong, Y. Jiang, J. Yuan, 'Dual-emissive nanoarchitecture of lanthanide-complex-modified silica particles for in vivo ratiometric time-gated luminescence imaging of hypochlorous acid', Chem. Sci., 2017, 8, p. 150–159

81 J. Zhou, Z. Liu, F. Li, 'Upconversion nanophosphors for small-animal imaging.', Chem. Soc. Rev. 2012, 41, p. 1323–1349

82 X. Zheng, X. Zhu, Y. Lu, J. Zhao, W. Feng, G. Jia, F. Wang, F. Li, D. Jin, 'High-Contrast Visualization of Upconversion Luminescence in Mice Using Time-Gating Approach', Anal. Chem. 2016, 88, p. 3449–3454

83 Y. Li, M. Gecevicius, J. Qiu, 'Long persistent phosphors—from fundamentals to applications', Chem. Soc. Rev. 2016, 45, p. 2090–2136

84 Q. le Masne de Chermont, C. Chanéac, J. Seguin, F. Pellé, S. Maîtrejean, J.-P. Jolivet, D. Gourier, M. Bessodes, D. Scherman, 'Nanoprobes with near-infrared persistent luminescence for in vivo imaging', Proc. Natl. Acad. Sci. U. S. A. 2017, 104, p. 9266–9271

85 W. Chen, J. Zhang, 'Using Nanoparticles to Enable Simultaneous Radiation and Photodynamic Therapies for Cancer Treatment', J. Nanosci. Nanotechnol. 2006, 6, p. 1159–1166

86 T. Maldiney, C. Richard, J. Seguin, N. Wattier, M. Bessodes, D. Scherman, 'Effect of Core Diameter, Surface Coating, and PEG Chain Length on the Biodistribution of Persistent Luminescence Nanoparticles in Mice', ACS Nano 2011, 5, p. 854–862

87 A. Abdukayum, J.T. Chen, Q. Zhao, X.P. Yan, 'Functional near infrared-emitting $Cr^{3+}/Pr^{3+}$ Co-doped zinc gallogermanate persistent luminescent nanoparticles with superlong afterglow for in vivo targeted bioimaging', J. Am. Chem. Soc. 2013, 135, p. 14125–14133

88 S.Q. Wu, C.W. Chi, C.X. Yang, X.P. Yan, 'Penetrating Peptide-Bioconjugated Persistent Nanophosphors for Long-Term Tracking of Adipose-Derived Stem Cells with Superior Signal-to-Noise Ratio', Anal. Chem. 2016, 88, p. 4114–4121

89 H.-X. Zhao, C.-X. Yang, X.-P. Yan, 'Fabrication and bioconjugation of B III and Cr III co-doped ZnGa 2 O 4 persistent luminescent nanoparticles for dual-targeted cancer bioimaging', Nanoscale 2016, 8, p. 18987–18994

90 A. Abdukayum, C.X. Yang, Q. Zhao, J.T. Chen, L.X. Dong, and X.P. Yan, 'Gadolinium complexes functionalized persistent luminescent nanoparticles as a multimodal probe for near-infrared luminescence and magnetic resonance imaging in vivo', Anal. Chem. 2014, 86, p. 4096–4101

91 E. Teston, Y. Lalatonne, D. Elgrabli, G. Autret, L. Motte, F. Gazeau, D. Scherman, O. Clément, C. Richard, T. Maldiney, 'Design, properties, and in vivo behavior of superparamagnetic persistent luminescence nanohybrids', Small 2015, 11, p. 2696–2704

92 Y.-C. Lu, C.-X. Yang, X.-P. Yan, 'Radiopaque tantalum oxide coated persistent luminescent nanoparticles as multimodal probes for in vivo near-infrared luminescence and computed tomography bioimaging', Nanoscale 2015, 7, p. 17929–17937

93 Z. Li, Y. Zhang, X. Wu, L. Huang, D. Li, W. Fan, G. Han, 'Direct Aqueous-Phase Synthesis of Sub-10 nm "Luminous Pearls" with Enhanced in Vivo Renewable Near-Infrared Persistent Luminescence', J. Am. Chem. Soc. 2015, 137, p. 5304–5307

94 J.-P. Shi, X. Sun, J. Zhu, J.-L. Li, H.-W. Zhang, 'One-step synthesis of amino-functionalized ultrasmall near infrared-emitting persistent luminescent nanoparticles for in vitro and in vivo bioimaging', Nanoscale 2016, 8, p. 9798–9804

95 Y.-J. Li, X.-P. Yan, 'Synthesis of functionalized triple-doped zinc gallogermanate nanoparticles with superlong near-infrared persistent luminescence for long-term orally administrated bioimaging', Nanoscale 2016, 8, p. 14965–14970

96 Y. He, H.-F. Wang, X.-P. Yan, 'Exploring Mn-Doped ZnS Quantum Dots for the Room-Temperature Phosphorescence Detection of Enoxacin in Biological Fluids', Anal. Chem. 2008, 80 (10), p. 3832–3837

97 P. Wu, Y. He, H.-F. Wang, X.-P. Yan, 'Conjugation of Glucose Oxidase onto Mn-Doped ZnS Quantum Dots for Phosphorescent Sensing of Glucose in Biological Fluids', Anal. Chem. 2010, 82 (4), p. 1427–1433

98 E. Sotelo-Gonzalez, M. T. Fernandez-Argüelles, J. M. Costa-Fernandez, A. Sanz-Medel, 'Mn-doped ZnS quantum dots for the determination of acetone by phosphorescence attenuation', Anal. Chim. Acta 2012, 712, p. 120–126.

99 E. Sotelo-Gonzalez, A. M. Coto-Garcia, M. T. Fernandez-Argüelles, J. M. Costa-Fernandez, A. Sanz-Medel, 'Immobilization of phosphorescent quantum dots in a sol–gel matrix for acetone sensing', Sens. Actuators B: Chem. 2012, 174, p. 102–108

100 H. Wu, Z. Fan, 'Mn-doped ZnS quantum dots for the room-temperature phosphorescence detection of raceanisodamine hydrochloride and atropine sulfate in biological fluids', Spectrochim. Acta, Part A 2012, 90, p. 131–134.

101 H.-B. Ren, X.-P. Yan, 'Ultrasonic assisted synthesis of adenosine triphosphate capped manganese-doped ZnS quantum dots for selective room temperature phosphorescence detection of arginine and methylated arginine in urine based on

supramolecular Mg2+–adenosine triphosphate–arginine ternary system', Talanta 2012, 97, p. 16–22

102 Y. He, H.-F. Wang, X.-P. Yan, 'Self-Assembly of Mn-Doped ZnS Quantum Dots/Octa(3-aminopropyl)octasilsequioxane Octahydrochloride Nanohybrids for Optosensing DNA', Chem. Eur. J. 2009, 15, p. 5436–5440.

103 L. Zhang, P. Cui, B. Zhang, F. Gao, 'Aptamer-Based Turn-On Detection of Thrombin in Biological Fluids Based on Efficient Phosphorescence Energy Transfer from Mn-Doped ZnS Quantum Dots to Carbon Nanodots', Chem. Eur. J. 2013, 19 (28), p. 9242-9250

104 X. Song, L. Huang, B. Wu, 'Bright and Monodispersed Phosphorescent Particles and Their Applications for Biological Assays', Anal. Chem. 2008, 80 (14), p. 5501–5507

105 X. Songa, M. Knotts, 'Time-resolved luminescent lateral flow assay technology', Anal. Chim. Acta 2008, 626, 2, p. 186–192

106 A. S. Paterson, B. Raja, G. Garvey, A. Kolhatkar, A. E. V. Hagström, K. Kourentzi, T. R. Lee, R. C. Willson, 'Persistent Luminescence Strontium Aluminate Nanoparticles as Reporters in Lateral Flow Assays', Anal. Chem. 2014, 86 (19), p. 9481–9488

107 R. P. Ekins, S. Dakubu, 'The Development of High-Sensitivity Pulsed-Light, Time-Resolved Fluoroimmunoassays', Pure Appl. Chem. 1985, 57 (3), p. 473–482

108 A. Scorilas, E. P. Diamandis, 'Polyvinylamine-Streptavidin Complexes Labeled with a Europium Chelator: a Universal Detection Reagent For Solid-Phase Time Resolved Fluorometric Applications', Clin. Biochem. 2000, 33 (5), p. 345–350

109 B. Q. Sun, G. S. Yi, S. Y. Zhao, D. P. Chen, X. Y. Zhou, J. Cheng, 'Solid substrate phosphorescent immunoassay based on bioconjugated nanoparticles', Anal. Lett. 2007, 34 (10), p. 1627-1637

110 Bo-Yue Wu, Xiu-Ping Yan, 'Bioconjugated persistent luminescence nanoparticles for Föster resonance energy transfer immunoassay of prostate specific antigen in serum and cell extracts without in situ excitation', Chem. Comm. 2015, 51, p. 3903-3906.

111 D. Tu, L. Liu, Q. Ju, Y. Liu, H. Zhu, R. Li, X. Chen, 'Time-resolved FRET biosensor based on amine-functionalized lanthanide-doped NaYF4 nanocrystals', Angew. Chemie - Int. Ed. 2011, 50, p. 6306–6310

112 Y. Liu, S. Zhou, D. Tu, Z. Chen, M. Huang, H. Zhu, E. Ma, X. Chen, 'Amine-functionalized lanthanide-doped zirconia nanoparticles: Optical spectroscopy, time-resolved fluorescence resonance energy transfer biodetection, and targeted imaging', J. Am. Chem. Soc. 2012, 134, p. 15083–15090

113 W. Zheng, S. Zhou, Z. Chen, P. Hu, Y. Liu, D. Tu, H. Zhu, R. Li, M. Huang, X. Chen, 'Sub-10 nm lanthanide-doped $CaF_2$ nanoprobes for time-resolved luminescent biodetection', Angew. Chemie Int. Ed. 2013, 52, p. 6671–6676

114 M. García-Cortés, M. T. Fernández-Argüelles, J. M. Costa-Fernández, A. Sanz-Medel, 'Sensitive prostate specific antigen quantification using dihydrolipoic acid surface-functionalized phosphorescent quantum dots', Anal. Chim. Acta, 2017, 987, p. 118-126.

115 Y. Lu, J. Lu, J. Zhao, J. Cusido, F.M. Raymo, J. Yuan, S. Yang, R.C. Leif, Y. Huo, J.A. Piper, J. Paul Robinson, E.M. Goldys, D. Jin, 'On-the-fly decoding luminescence lifetimes in the microsecond region for lanthanide-encoded suspension arrays', Nat. Commun. 2014, 5, Article ID-3741

116 S.S. Howard, A. Straub, N.G. Horton, D. Kobat, C. Xu, 'Frequency-multiplexed in vivo multiphoton phosphorescence lifetime microscopy', Nat. Photonics., 2012, 7 (1), p. 33–37

117 L. Zhang, X. Zheng, W. Deng, Y. Lu, S. Lechevallier, Z. Ye, E. M. Goldys, J. M. Dawes, J. A. Piper, J. Yuan, M. Verelst, D. Jin, 'Practical Implementation, Characterization and Applications of a Multi-Colour Time-Gated Luminescence Microscope', Sci. Rep., 2014, 4, Article ID-659

118 J. Zhang, F. Song, Z. He, Y. Liu, Z. Chen, S. Lin, L. Huang, W. Huang, 'Wide-Range Tunable Fluorescence Lifetime and Ultrabright Luminescence of Eu-Grafted Plasmonic Core-Shell Nanoparticles for Multiplexing', Small 2016, 12, p. 397–404

119 H.S. Afsari, M. Cardoso Dos Santos, S. Linden, T. Chen, X. Qiu, P.M.P. van Bergen en Henegouwen, T.L. Jennings, K. Susumu, I.L. Medintz, N. Hildebrant, and L.W. Miller, 'Time-gated FRET nanoassemblies for rapid and sensitive intra- and extracellular fluorescence imaging', Sci. Adv. 2016, 2 (6), Article ID-e1600265

120 X. Huang, J. Song, B. C. Yung, X. Huang, Y. Xiong, X. Chen, 'Ratiometric optical nanoprobes enable accurate molecular detection and imaging', Chem. Soc. Rev., 2018, 47, p.2873-2920

121 K.D. Duncan, Ingela Lanekoff, 'Oversampling To Improve Spatial Resolution for Liquid Extraction Mass Spectrometry Imaging', Anal. Chem., 2018, 90 (4), p. 2939–2945