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Upconversion Nanoprobes: Recent Advances in Sensing Applications

Zhiming Zhang,1,# Swati Shikha,2,# Jinliang Liu,1 Jing Zhang,1,* Qingsong Mei,2,* and Yong Zhang2,*

1School of Environmental and Chemical Engineering, Shanghai University, 99 Shangda Road, 200444, Shanghai, China
2Department of Biomedical Engineering, Faculty of Engineering, National University of Singapore, Singapore 117583, Singapore

# These authors contributed equally to this article

Corresponding author: lilyjzhang@shu.edu.cn (Jing Zhang), bmeqsmei@gmail.com (Qingsong Mei), biezy@nus.edu.sg (Yong Zhang)
1. Introduction

Lanthanide ions doped upconversion nanoparticles (UCNPs) are capable of stepwisely converting lower energy near-infrared (NIR) excitation light into higher energy visible or ultraviolet emission with a large anti-Stokes shift.\textsuperscript{1-4} Because lanthanide ions have plenty of energy levels, UCNPs exhibit unique optical properties including optical tunability over emission wavelengths and lifetimes, better photo-stability and improved monochromatic color-purity and spatial resolution. However, the low luminescent efficiency, mainly attributing to the weak and narrow absorptions, has greatly hampered the translation of UCNPs-based technologies from experimental research to real world applications.\textsuperscript{5} Recently, increasing efforts have been paid on boosting the upconversion luminescence brightness and efficiency, including inert shells coating, high concentration of activators doping, organic dyes modification, and so on.\textsuperscript{6-7} Many strategies even aimed to explore high quality UCNPs with some more unique optical properties, such as orthogonal emissions or encoding luminescent signals, and broaden their applications.\textsuperscript{1}

The marriage of UCNPs with sensing is one of the intriguing applications, which can greatly overcome some disadvantages faced by traditional optical labels, such as strong background interference of complex samples, photo damages to sensing targets, spectral overlap of multi-detection signals, and so on.\textsuperscript{8-11} To date, a number of UCNPs-based detection systems have been developed benefiting from the marriage.\textsuperscript{12-13} Most of these systems are based on the Förster resonance energy transfer (FRET) mechanism wherein the detection relies on the luminescence signals switch off/on or ratiometric luminescence variations.\textsuperscript{14} In the FRET process, UCNPs act as excellent energy donors. By taking advantages of versatile
surface coating strategies, a collection of energy acceptors, such as organic dyes, metal nanoparticles, two-dimensional nanomaterials, and so on, have been decorated onto the surface for detection of various targets. These upconversion nanosensors have been further integrated with several detection platforms including test strips and microscopy imaging to contribute towards their practical applications.

Figure 1. Overview of UCNPs-based nanosensors from luminescence mechanism to sensing applications.

In this review, we will focus on the recent two years literatures in the field of UCNPs-based sensing applications (Figure 1). First, recent progresses on the synthesis of specifically designed UCNPs and their surface modification strategies will be surveyed. Next, we will give a brief overview of general detection principles of the upconversion nanosensors, discuss the detection formats, and sum up various sensing applications including environmental hazard detection, food assays, biological analysis and medical diagnostics. Finally, we will outline future trends and challenges in the upconversion analysis field. We hope this review will offer a full-scale insight of upconversion nanosensors for readers to help
the further exploration of exceptional luminescent UCNPs and expand their application boundaries.

2. Properties of upconversion nanoparticles

Despite the umpteen benefits, UCNPs are encountered with the poor luminescence efficiencies. Notably, the upconversion process in UCNPs is based on the absorption and emission-related radiative transitions with long radiative lifetimes (about hundreds of microseconds). Consequently, a large amount of excitation energy is dissipated via faster and nonradiative pathways that primarily includes surface quenching and phonon coupling. Recently, various strategies, including coating of inert shells, modifications of organic dyes to enhance the absorption of excitation lights, further annealing of the as-prepared UCNPs, and so on, have been developed to improve the upconverting efficiencies, which will be reviewed in detail hereinafter. The as-prepared UCNPs capped with hydrophobic molecules should be transferred to hydrophilic surface for the further conjugation to realize chemo/biosensing applications. Recent advances in the surface modifications of UCNPs are also summarized in this section.

2.1 Synthesis of UCNPs with specific luminescent properties

2.1.1 Upconversion luminescence efficiency
The luminescence efficiency of UCNPs is attenuated by a few deleterious energy migration processes, including non-radiative decay, cross-relaxation, energy back-transfer, and energy transfer to the high-energy vibrations of surface ligands. Many vibrational transitions of organic molecules are dipole-allowed, thus the coupling of a luminescent dopant in UCNPs to the surrounding solvent molecule can induce significant luminescence quenching even when the distance is more than the Förster critical distance (10 nm). It was reported that solvent quenching affected all energy levels of Er\(^{3+}\) ions involved in upconversion luminescence.\(^{16}\) CH-stretch vibrations around 3000 cm\(^{-1}\) were found to matched with the transition energies of Er\(^{3+}\) ions in the process of \(^{4}S_{3/2} \rightarrow {4}F_{9/2}\) and \(^{4}F_{9/2} \rightarrow {4}I_{9/2}\), which induced the non-radiative decay of red and green emissions. To address these challenges, several efforts have been paid to improve the luminescence efficiency of UCNPs by changing the design of nanosturcture, dopant positions of lanthanide ions, and so on.

In order to restore the surface defects (such as disorder, vacancy, and interstitial defects), Xu et al. prepared KLu\(_2\)F\(_7\):Yb/Er bare core UCNPs and subjected them to a wet chemical annealing process. They used aberration-corrected high-angle annular dark field scanning transmission electron microscopy to compare surface state of the UCNPs with and without annealing.\(^{17}\) It was observed that the edge of the as-prepared UCNPs had an amorphous phase. Contrary to this, the further annealed UCNPs exhibited a well-defined edge. Additionally, the corresponding upconversion luminescence intensity of the annealed UCNPs was enhanced by an order of magnitude, thereby indicating the restoration of surface defects. As mentioned previously, energy transfer to surface via adjacent dopant ions dramatically decreased luminescence intensities. With regards to this, coating an inert shell of NaYF\(_4\) over the core...
UCNPs has been developed as an alternative strategy to enhance the UCNPs quantum yields. Homann et al. systematically studied the upconversion quantum yields of NaYF$_4$:Yb/Er@NaYF$_4$ nanoparticles with different shell thickness. They found that the maximum upconversion quantum yield was increased from 3.4% to approximately 9% when the size of the core-shell nanoparticles increased from 15 nm to 45 nm. Resch-Genger et al. also investigated the surface passivation effects and the possible intermixing influence between core and shell by coating NaYF$_4$ shells with varying thicknesses onto the surface of ultrasmall hexagonal-phase NaGdF$_4$:20%Yb/2%Er UCNPs. It was found that the optimum shell thickness was 5 nm, and varying the shell thickness resulted in tuning the lifetimes and quantum yields.

To effectively minimize the deleterious interactions between the sensitizer lanthanide ion and the luminescent activators, Zhou et al. reported an interfacial energy transfer (IET)-mediated approach to facilitate upconversion from more lanthanide ions and with more excitation strategies. They systematically interrogated the photon upconversion performance from the traditionally studied lanthanide ions including Er$^{3+}$, Tm$^{3+}$, Ho$^{3+}$, Tb$^{3+}$ and Eu$^{3+}$ through the IET strategy. The studies revealed that the separation of sensitizer-activator pairs (Yb-Er/Tm/Ho, Gd-Eu/Tb, and Nd-Yb) for efficient energy transfer was confined in a range less than 1.6-2.1 nm. Contrary to the common believe that the upconversion emission dynamics is dependent on the activator ions and their interactions with neighboring sensitizers, Zuo et al. demonstrated that excitation energy migration significantly affected the dynamics of upconversion luminescence. They designed NaYF$_4$:20%Yb/2%Er @ NaYF$_4$:20%Yb @ NaYF$_4$:10%Nd/20%Yb nanostructures with ‘spatially separated dopant ions’ to distinguish...
the basic steps of upconversion process (absorption of excitation energy, energy migration, and emission) that enabled them to investigate the temporal effect of energy migration.\textsuperscript{21} By using a binary pulsed (980 nm and 800 nm) excitation setup, it was observed that the strongest emission occurred when the 980 nm pulse was approximately 200 µs later the 800 nm pulse, while not illuminated at the same time. Moreover, the required time of the energy migration from Yb\textsuperscript{3+} to Er\textsuperscript{3+} can be well manipulated by the layer thickness, that changing from 0 to 4.5 nm would result in prolongation of 540 nm emission from 195 to 390 µs.

Thermal quenching, which is commonly caused by the increased activity of phonons that leverages the non-radiative relaxation pathways, broadly limits luminescent efficiency of optical materials at higher temperature. Jin \textit{et al.} recently reported that ‘heat-favorable phonons’ existing at the UCNPs’ surface (Figure 2A) were effective in circumventing the thermal quenching.\textsuperscript{22} It was found that the oxygen moiety of the surface molecules chelating with the exposed Yb\textsuperscript{3+} ions, [Yb···O], generated surface phonons. These phonons were observed to get further activated with an increased temperature. The study also illustrated that the [Yb···O] complexes upconverted the trapped phonon energy in a stepwise manner that subsequently led to a brighter emission from the UCNPs. Owing to this process, a 2,000-fold enhancement (approximately) in blue emission of 9.7 nm sized Yb\textsuperscript{3+}-Tm\textsuperscript{3+} co-doped nanoparticles was observed when the temperature was increased from room temperature to 453 K. Along with solving the issue of temperature-based luminescence quenching, this strategy also opened up the possibility of exploring new pathways to develop brighter UCNPs.

In a completely different approach, quenching process was rather employed as a tool to
prepare UCNPs with single emission. Kim et al. reported a liquid-quenched amorphous matrix of NaYF₄ created by completely melting the thin silica-coated NaYF₄ nanocrystals under excitation with 980 nm continuous wave laser. After the liquid quenching process, it exhibited highly efficient single-band green upconverting emissions. This was attributed to the elevated transition probability of the host sensitive transition that facilitated ultrafast decay of emissions as short as 0.2 µs. This ultrafast transition effectively prevented the other states from populating by dominating the photon consumption process, thereby resulting in the single-band green emissions.

2.1.2 Dye-functionalized UCNPs

Attaching organic dye antennas to UCNPs surfaces has been reported to dramatically increase their optical cross sections and absorption bandwidths, thereby resulting in a significantly enhanced upconversion luminescence. Schuck discovered that the NIR dye IR806 could play as an efficient antenna to harvest 808 nm excitations for leveraging the dye-UCNPs with a 33000-fold increase in brightness and a 100-fold increase in efficiency compared with bare UCNPs (Figure 2B). Upon excitation at 791 nm, the dye IR806 exhibited an emission centered at 981 nm that was close to the maximum absorption of Yb³⁺ transition ⁴F₇/₂ → ⁴F₅/₂. The spin-triplet states in the dye IR806 played as important intermediates in the energy migration, and the increase of effective nuclear charge of lanthanide ions on the surface of UCNP enhanced intersystem crossing (ISC) within the dye from singlet to triplet excited states. Thus, by increasing the amount of Gd³⁺ ions, the spin-orbit coupling and ISC kinetics within the dye were increased, leading to an additional enhancement in upconversion luminescence. ATTO 542 is a commercially available dye
having significant spectral overlap with the green emission of Er\textsuperscript{3+}, facilitating efficient energy transfer from Er\textsuperscript{3+} ions to ATTO 542. Wisser et al. decorated the surfaces of Na(Y/Gd/Lu)\textsubscript{4}:Yb/Er(18/2\%) UCNPs with ATTO 542, and demonstrated that the brightness of dyes decorated UCNPs increased two times and ten times than that of unmodified UCNPs in organic solution and water solution, respectively.\textsuperscript{25} It was pointed out that the brightness enhancement was attributed to the increased radiative rate after the dyes modification.

2.1.3 Heavy doping UCNPs

Concentration quenching is a well-known phenomenon in UCNPs, which was commonly explained by the deleterious cross-relaxation between activators and/or sensitizers in close proximity, or enhanced energy transformation to the surface defects. Therefore, relatively low concentrations of sensitizers (around 20 mol \%) and activators (lower than 2 mol \%) are used in conventional UCNPs. Recent research demonstrated that a high-power density of excitation (ca. \(10^6\) Wcm\(^{-2}\)) can effectively attenuate luminescence quenching in heavily doped UCNPs.\textsuperscript{26} Almutairi and co-workers found out that the main quenching at high dopant concentrations was predominantly attributed to energy migration to surface defects. In their work, it was found that the emission intensity decreased with increasing Er\textsuperscript{3+} concentration in NaYF\textsubscript{4} UCNPs, and both the upconversion and downshifted luminescence intensities increased monotonically after coating a NaLuF\textsubscript{4} shell with the thickness of 10 nm (Figure 2C).\textsuperscript{27} Furthermore, they developed an Er\textsuperscript{3+} heavily doped triple-layer core-shell-shell UCNPs, NaYb\textsubscript{0.2}/Er\textsubscript{0.8}F\textsubscript{4}@NaLuF\textsubscript{4}@NaGdF\textsubscript{4}, as triple-modal imaging contrast agents. For the NaYb/ErF\textsubscript{4} core nanoparticles, heavily doping of Er\textsuperscript{3+} (80\%) accelerated the excitation energy transformation to surface defects and quenched the luminescence. A NaLuF\textsubscript{4} epitaxial shell
growth onto its surface also sequestered the excitation energy in the core, thereby preventing surface quenching, and the emissions recovered immediately.28

Through combining the Tm$^{3+}$-mediated transient energy trapping effects and inert-shell coating, Liu’s group also demonstrated that an appropriate doping of Tm$^{3+}$ would further enhance the luminescence intensity in Er$^{3+}$-enriched core-shell NaErF$_4$;Tm (0.5 mol%)@NaYF$_4$.29 After calculation based on density function theory, they found that Tm$^{3+}$-doping led to a local realignment of 4f/5d orbitals of Er$^{3+}$, which facilitated the transportation of excitation energies to Er$^{3+}$ activator, thus leading to the enhanced upconversion luminescence. Almost at the same time, Zuo et al. reported that this heavily doped core-shell nanostructure, i.e. NaErF$_4$@NaYF$_4$ or NaErF$_4$:0.5% Tm@NaYF$_4$, exhibited high efficient monochromatic red emissions under multi-band excitations (~800 nm, ~980 nm, ~1530 nm).30 By investigations of the steady-state and time-resolved spectroscopic experiments, it was certified that the suppression of the concentration quenching effect was the result of “quenching site-free” environment of the activator.

For Tm-doped system, high Tm$^{3+}$ concentration (more than 1%) led to an intense cross-relaxation and luminescence quenching, as recently demonstrated by Jin and co-workers. The group found that the emission of UCNPs highly doped with 8% Tm$^{3+}$ ions under continuous-wave (CW) 980 nm excitation was clearly inhibited once a CW 808 nm probe beam was applied at the same time, while low doping UCNPs showed negligible optical switching effects (Figure 2D).31 They believed that the reduced distance between Tm$^{3+}$ ions generated a photon avalanche-like effect that quickly populated the $^3\!H_4$ level of Tm$^{3+}$ ions. In addition, the excitation energy of 808 nm matched well with the transition energy of $^3\!H_4 \rightarrow ^3\!H_6$. 11
which would induce the discharge of $^3\text{H}_4$ intermediate level and inhibit the upconversion pathway for blue emissions. They utilized these novel properties to achieve low-power super-resolution stimulated emission depletion (STED) microscopy and improve optical resolution to 28 nm. He’s group also reported an efficient optical depletion in Tm$^{3+}$ ions heavily doped NaYF$_4$ UCNPs. They found out that when co-excitation with an 808 nm CW laser, the blue emission at 455 nm was significantly depleted with a efficiency up to 96%, and the emissions at 475 nm and 650 nm were quenched less significantly, while the emission at 700 nm was distinctly enhanced. However, in low Tm$^{3+}$ doping (0.5%) NaYF$_4$ UCNPs, the emission at 455 nm was strongly enhanced after co-irradiation with 808 nm laser.

For the Nd$^{3+}$ highly doped UCNPs, after anchoring an indocyanine green (ICG) dye on the surface of nanoparticles, the optimal doping amount of Nd$^{3+}$ was shifted from 2 to 20 mol%, along with ~10 folds increment in upconversion brightness. The absorption cross-section of ICG dye was about 30 000 folds higher than that of Nd$^{3+}$ ions at 800 nm. The emission peak of ICG strongly matched with the absorption bands of Nd$^{3+}$ ions, facilitating the energy transfer between ICG and Nd$^{3+}$ ions. The enhanced harvesting of excitation light and efficient energy transformation among Nd$^{3+}$ ions led to the brightness increase in the Nd$^{3+}$-heavily doped UCNPs.

2.1.4 Orthogonal emission UCNPs

Orthogonal emission is an excitation-dependent luminescence, in which the emission can be modulated between different lanthanide activators by changing the external excitation light. With the multi-compartment core/shell structure, UCNPs can activate different energy transfer pathways and generate versatile emission colors from the UV to visible range under varied
excitations. Recently, many works have focused on the modulations of the different doping patterns in various layers of UCNPs to achieve the orthogonal emissions.

Zhang’s group reported a new core/shell UCNPs, NaGdF$_4$:Yb/Er@NaYF$_4$:Yb@NaGdF$_4$:Yb/Nd@NaYF$_4$@NaGdF$_4$:Yb/Tm@NaYF$_4$. By introducing a filtration layer (NaYF$_4$) and tuning its thickness in the core-shell structured UCNPs, two unique independent emissions could be achieved, including UV/blue emissions from Tm$^{3+}$ ions under illumination with 980 nm laser and green/red emissions from Er$^{3+}$ at 796 nm excitation. Yan’s group also synthesized a new orthogonal emission UCNPs (NaGdF$_4$:Yb/Er@NaYF$_4$:Yb/Tm@NaYbF$_4$:Nd@NaYF$_4$) with Er$^{3+}$ and Tm$^{3+}$ doped in different regions, in which, the green emission from Er$^{3+}$ ions and blue emission from Tm$^{3+}$ ions were separately activated with 808 and 980 nm lasers. They found that by depositing a NaYF$_4$ interlayer with the thickness of 1 nm, emissions of Er$^{3+}$ could be significantly inhibited under 808 nm excitation, and the inhabitation efficiency was enhanced when increasing the thickness of NaYF$_4$ interlayer. Moreover, with coating additional NaGdF$_4$:Tb layer next to the NaGdF$_4$:Yb,Tm layer, the green emission from Tb$^{3+}$ was introduced and lifetime was prolonged from 0.13 ms (Er$^{3+}$) to 3.6 ms (Tb$^{3+}$), which enabled the multiplexed fingerprint and time-gated luminescence imaging in the manners of wavelength or lifetime.

Romanowski et al. demonstrated that the UV emission from NaYF$_4$: Yb/Tm UCNPs could be turned on and off by changing the excitation pulse width. It was found that a short pulse width of 10 $\mu$s produced NIR emission and nearly undetectable UV luminescence. On the other hand, a longer 2 ms pulse width produced comparable bright UV and NIR emission. Zuo et al. developed Nd$^{3+}$-free UCNPs, NaErF$_4$@NaYF$_4$@NaYbF$_4$:0.5%Tm@NaYF$_4$, in which
UV-blue emissions from Tm$^{3+}$ ions were switched on after excitation with 980 nm, which can be entirely switched off by 800 nm light, leaving the emission at only 660 nm.$^{37}$ Very recently, Liu’s group demonstrated a power-independent orthogonal luminescence with ultrahigh spectral purity in multilayer UCNPs. For this, NaYF$_4$:Er@NaYF$_4$:NaYF$_4$:Yb/Tm@NaYF$_4$ multilayer nanoparticles were prepared and the thickness of NaYF$_4$:Yb/Tm layer was tuned to achieve UV emission of Tm$^{3+}$ under 980 nm excitation and green emission of Er$^{3+}$ at 1532 nm excitation (Figure 2E).$^{38}$ In addition, it was important to restrict the energy transfer from Yb$^{3+}$ to Er$^{3+}$ and excitation energy cross-relaxation between Er$^{3+}$ and Tm$^{3+}$ for the efficient orthogonal luminescence. This was done by incorporating an extra inert layer of NaYF$_4$ shell between the core and NaYF$_4$:Yb/Tm layer. As hypothesized by the group, the heavy Yb$^{3+}$ doping in NaYF$_4$:Yb/Tm shell led to a strong absorption of 980 nm photons by Yb$^{3+}$, thereby restricting them from being absorbed by Er$^{3+}$ present in the core region of the multilayer UCNPs.

2.2 Surface functionalization of UCNPs
The as-synthesized UCNPs are hydrophobic in nature attributed to the surface capping molecules such as oleic acid (OA), which is a pivotal challenge for their chemo/biosensing applications that require water-soluble nanoprobes. Therefore, it is important to explore efficient strategies to modify the UCNPs surfaces for hydrophilicity and subsequent conjugation to chemical or biological moieties in order to meet cater the needs of different applications. The most direct approach is getting rid of the hydrophobic OA molecules on the surface by treating them with a hydrochloric acid (HCl) solution, followed by the attachment of new ligands in a separate step. Wang’s group recently reported that solvothermal treatment of the HCl-treated UCNPs made the new ligands more firmly combined with UCNPs surface under high temperature and pressure (Figure 3A). Without the solvothermal treatment, most ligands cannot establish a strong bond with the nanoparticles that could be due to the electrostatic repulsion and steric hindrance.

Many other strategies, including reaction with strong oxidants, coating with amphiphilic molecules or silica shell, have also been widely explored to improve the hydrophility of UCNPs. For example, by using host-guest interactions, Jin et al. used cucurbit[7]uril (CB[7]) to efficiently substitute the surfactant OA molecules to form CB[7]-coated UCNPs (CB-UCNPs). The seven ketone groups with high electronegativity in CB[7] made it easy to replace OA and convert UCNPs to be hydrophilic. Furthermore, the molecular architecture of CB[7] comprised of a hydrophobic host-environment, that enabled the modifications of biomolecules via host-guest inclusion. Amphiphilic interactions were another effective strategy to convert hydrophobic surface into hydrophilic and create new reactive sites for conjugations of various target molecules. Jo et al. explored polyethylene glycol polymer to
modify UCNPs and further conjugate aptamers by using EDC/NHS coupling (Figure 3B). To generate long-term stability of functionalized UCNPs, Duong et al. systematically compared the adsorption capabilities of phosphate, carboxylic acid and sulphonic acid onto the UCNPs surfaces. It was found that the adsorption energies of carboxylic acid, sulphonic acid and phosphate groups were -77.9 kcal/mol, -80.0 kcal/mol and -90.4 kcal/mol, respectively. After ligand exchange with phosphate group, the UCNPs remained stable in water, PBS buffer and MES buffer for one week without any aggregations, whereas the UCNPs capped with carboxylic or sulphonic groups were only stable for the first few hours. Based on these features, Zhang’s group also reported that the specific phosphorylation of peptides dramatically enhanced the bonding strength without affecting the original target recognition performance of peptides (Figure 3C).

Surface modification of UCNPs with cellular membranes offered them many natural cellular properties. Liu’s group demonstrated a general method to coat the cellular membrane onto UCNPs surfaces (Figure 3D). The encapsulation of Cancer cell (CC)-vesicles onto the surface of UCNPs was achieved by a physical extrusion of the mixture of CC-vesicles and UCNPs through a membrane. The obtained CC-UCNPs kept stable in many medium, enabling the feasibility of the subsequent experiments. The same group also reported that the red blood cell (RBC) membranes coated UCNPs (RBC-UCNPs) efficiently prevented the protein corona formation upon exposure to human plasma. After further modification of cancer-targeting molecules onto the cell membrane, the targeting efficiency of RBC-UCNPs were significantly improved. By use of the unique nature of RBC as an oxygen carrier in the blood, RBC-UCNPs could be used for photodynamic therapy of cancers and endowed with
stealth capability to escape from the reticuloendothelial system.  

As we know, UCNPs are hexagonal cylinders having two (001) planes at the ends and six (100)/(010) planes at lateral surfaces. Different charge distributions on these planes endow them with different ligands-binding abilities. With regards to this, Jin et al. investigated the differences in binding affinity of phosphate groups and phosphodiester bonds on DNA to the different facets of UCNPs. It was found that the phosphodiester bonds on DNA backbone had binding affinity stronger than OA on (001) planes, and weaker than oleate anions (OA') on (100)/(010) facets. On the other hand, phosphate groups on DNA terminus were found to completely replace surfactant molecules on all the facets of UCNPs (Figure 3E), rendering the surface hydrophilic. Therefore, the anisotropic surface properties of UCNPs could be achieved by using phosphate or phosphodiester groups for functionalization.

3 Design of upconversion nanosensors

UCNPs have been widely applied as the energy donors for designing the sensing applications due to their advantageous optical properties such as long fluorescence lifetimes, low photobleaching, and narrow emission peaks. Besides, the UCNPs are less disturbed by the background interference because of the low-energy NIR light excitation. The most used detection principles in UCNP-based nanosensors is resonance energy transfer, usually known as FRET. In addition, some other detection principles, such as inner filter effect (IFE) and electron transfer, have also been applied in UCNPs-based nanosensors. Furthermore, to achieve rapid, easy to operate, portable, and cost-effective assays, many platforms have been developed, including lateral flow strips, test paper, microarrays, microfluidic devices, and so on. In this section, we will focus on the recent advances in the detection principles and...
forms.

### 3.1 Detection principles


Other than the luminescence donors (UCNPs), there remain three other prerequisites for
FRET: energy acceptors (luminescence quencher), recognition units for analytes, distance between the donors and acceptors. Generally, the energy acceptors should satisfy the spectral overlap between their absorption spectra and emission spectra of UCNPs, and the distance must be in close proximity (preferably <10 nm). The detection is based on quenching or recovering of UCNPs fluorescence after additions of analytes, through modulating the absorption of energy acceptors or the distance between donors and acceptors (Figure 4A). Energy acceptors could be modified onto the surface of UCNPs through physical adsorption, covalent coupling, coordination reaction, and so on (Figure 4B). Physical adsorption involves noncovalent interactions such as van der Waals force, electrostatic force, hydrophobic attraction, etc. For example, hydrophobic acceptors could be encapsulated into the pores of the mesoporous silica coated UCNPs.\textsuperscript{48} The hydrophilic mesoporous silica permits the dispersion of UCNPs in aqueous solution and subsequent functionalization. Covalent coupling reaction involves modifications of the UCNPs surface with the reactive functional groups followed by covalent conjugation of the acceptors. For example, citric acid bearing a pendant carboxylic acid is usually utilized as the hydrophilic ligand for the surface modification of UCNPs, which can be used for binding to a wide range of amino-functioned acceptors.\textsuperscript{49} In coordination reaction, the original hydrophobic ligand such as OA or oleylamine, are replaced by a chromophoric complex or organic fluorophores that displays higher coordination ability towards lanthanide ions. For example, chromophoric iridium (III) complex with carboxylic group is suitable for coordination reaction to the surface of the UCNPs though a one-step ligand exchange method.\textsuperscript{50}

Another detection principle in upconversion nanosensors is based on IFE, which is a
non-radiative, direct and sensitive technology wherein the excitation and/or emission light is absorbed by the absorbers.\textsuperscript{51} When the probe interacts with analytes, an increase/decrease or a shift in the acceptor’s absorption band appears, and this in turn, tunes the emission of UCNPs. In contrast to FRET, the IFE process is more flexible as it does not rely on the donor-acceptor distance. Significant progresses have been made using this approach to achieve the upconversion emission-based detection.\textsuperscript{52}

3.1.1 Energy transfer efficiency

In FRET-based upconversion nanosensors, the energy transfer efficiency ($E$) is described as the following equation:

\[
E = \frac{1}{1 + \left(\frac{R}{R_0}\right)^6}
\]

where, $R_0 =$ Förster distance at which the energy transfer efficiency is 50%, and $R =$ distance between the donor and the acceptor. From this equation, it is expected that the distance between the donor and acceptor is the primary factor for fabricating an efficient upconversion energy transfer process. However, different from classical molecular donor-acceptor pairs, the emitting lanthanide ions are distributed throughout the nanoparticles, leading to the distances between every emitting ion and the acceptor molecules on the surface of UCNPs are different from each other. Therefore, the particle sizes play a very important role in the energy transfer efficiencies. Muhr \textit{et al.} explored a series of different sized UCNPs modified with rose Bengal and sulforhodamine B to investigate the FRET efficiency (Figure 4C).\textsuperscript{53} It was found that the FRET efficiency was the highest of up to 60% when the diameter of UCNPs was 21 nm. For large-sized UCNPs hybrid systems, the FRET efficiency could
reduce drastically, which is attributed to the greater dimensions of the particle in comparison to the Förster critical distance, at which the donor’s internal decay rate becomes equivalent to the energy transfer rate. To overcome this flaw, Li et al. proposed a sandwich structure of core-inner shell-outer shell UCNPs that harbored the emitting lanthanide ions in the inner shell, bringing them nearer to particle surface and hence, closer to the external energy acceptors.\textsuperscript{54} Such a sandwich structured UCNPs exhibited a more than 7-fold enhancement of FRET efficiency than the conventional UCNPs. Deng et al. recently found out that Gd\textsuperscript{3+} sub-lattice could extract upconverted photon energy from the core of Yb\textsuperscript{3+}/Tm\textsuperscript{3+} co-doped UCNP system and transport it to the shell.\textsuperscript{55} This process allowed the migration of the excitation energy to the surface of the particle, subsequently promoting the energy transfer from the particle to surface-bound acceptors (Figure 4D).

3.1.2 Energy acceptors

In upconversion nanosensors, energy acceptors with the recognition units for detection of analytes are very important. In the past years, several energy acceptors such as organic dyes, metal nanoparticles, carbon nanomaterials, and two-dimensional nanosheets were used to construct UCNP-based nanosensors for assays of various ions, small molecules, proteins, nucleic acids, and so on.

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**Organic dyes.** Organic dyes have been extensively explored in upconversion nanosensors because of their broad and adjustable absorption band, ease of chemical functionalization for specific recognition of targets, low cost, and good water solubility.\(^{56-57}\) A myriad of UCNPs-organic dye based FRET systems have been developed for diverse detection applications, in which the cyanine family, rhodamine derivatives, FITC, and Texas Red are used as energy acceptors.\(^{58}\) Herein, we have presented three commonly used organic dyes for overlapping the luminescence of UCNPs at blue, green and red emissive regions, respectively (Figure 5A). The azo dye, structure shown in Figure 5A(a), shows an intensive absorption peak at 490 nm that precisely matches with the blue emission of UCNPs.\(^{59}\) The dyes Rhodamine B isothiocyanate and Nickel (II) phthalocyanine-tetrasulfonic acid tetrasodium salt (Figure 5A b and c) exhibit absorption peaks around 555 nm and 657 nm which greatly overlaps with green and red emissions, respectively.\(^{60}\)

**Metal nanoparticles.** Metal nanoparticles, especially gold nanoparticles (AuNPs), have received great attentions in developing upconversion nanosensors due to their excellent luminescence quenching capability in a broad spectral range.\(^{61-64}\) Unlike organic dyes, the energy transfer from UCNPs to AuNPs is a result of a dipole-surface effects-induced collective resonant oscillation. This implies that the distance between UCNPs and AuNPs could be in the range of 70-100 nm, which is effectively longer than typical FRET systems. Gold nanoparticles (AuNPs) and other noble metal nanoparticles, such as silver nanoparticles, could also be explored as energy acceptors in upconversion nanosensors because of their high sensitivity towards surrounding environment offering the opportunity for applications in biological and chemical probes.\(^{65-66}\) For instance, Gao *et al.* have developed gold nanorods...
(AuNRs) coated UCNPs satellite assemblies for intracellular messenger RNA (mRNA) analysis, in which the luminescence of UCNPs was significantly reduced by AuNRs (Figure 5B). Moreover, AuNPs also have been widely used as the fluorescence acceptors for the IFE systems due to their larger absorption superficial area coupled with larger extinction coefficient in comparison to the conventional dye quenchers.

**Carbon nanomaterials.** As a star carbon nanomaterials, graphene oxide (GO) nanosheets have been recognized as a super nano-quencher for universal fluorophores, including UCNPs (as Figure 5C showed). GO possesses a large rigid π-conjugated planar structures, coupling with carboxyl groups on the edge and hydroxyl/epoxy groups on basal plane, offering several advantages for constructing nanosensors. First, GO enables FRET without limitation of spectral overlap because of its broad range of absorption (200 nm to 800 nm). Second, GO offers longer working distance and better FRET efficiency (30 nm) than the conventional FRET acceptors (10 nm). Third, GO-based sensing leads to lower background signals due to their high fluorescence quenching efficiency. Fourth, the π-π stacking interactions between aromatic ring of GO and DNA/peptides backbones offers several immobilization prospects. At last, the cost-effectiveness of GO further encourages their usage for FRET-based sensing applications.

**Two-dimensional nanosheets.** Inspired from GO nanosheets, many other two-dimensional nanosheets, such as manganese dioxide (MnO$_2$) and molybdenum disulfide (MoS$_2$) nanosheets, have been used as the energy acceptors in UCNPs-based nanosensors. MnO$_2$ nanosheets have been found to quench the emission of UCNPs, which was recovered in the presence of glutathione (GSH) or H$_2$O$_2$ (as Figure 5D showed). Therefore, the
MnO2-modified UCNPs nanosystem can be used as nanosensor for the sensitive detection of GSH and H\textsubscript{2}O\textsubscript{2}. In another work, MoS\textsubscript{2}-UCNPs-based nanosensors were deployed for the detection of microcystin-LR (MC-LR) by utilizing the high fluorescence quenching ability of MoS\textsubscript{2} and their specificity towards ssDNA.\textsuperscript{78}

### 3.2 Detection formats

Detection strategies usually rely on suspension formats that offer benefits such as better analyte binding to the nanoprobes, convenient handling, flexibility in introducing nanoprobes to the detection system, and cost-effective operations. However, suspension assays could be time taking. Therefore, to achieve rapid, easy to operate, portable, and cost-effective assays, UCNP-based probes have been incorporated with various substrate-based sensing platforms including lateral flow strips, test paper, microarrays, and microfluidic devices. Moreover, smartphones have been integrated with these platforms to further contribute towards on-site detection and reach out to resource poor communities. This section provides an overview of different detection platforms developed for the UCNPs probes-based sensing, including their set up, advantages, and limitations.

3.2.1 Lateral flow strip

Lateral flow strip platform relies on the capillary action and immune reaction for the detection of target analyte. The lateral flow assay (LFA) or immunochromatographic assay performed on these strips are highly favoured due to their rapid result delivery, cost-effectiveness, ease of use and scalability. Typically, the strip harbours four parts for
sample, conjugation, nitrocellulose (NC) membrane, and absorbent (Figure 6A). The sample pad is where the analytical sample is dropped and buffer for the assay is stored. Additionally, the pores of the sample pad assist in filtering out some of the sample impurities. The migrated sample reaches conjugation pad where the target-specific nanoprobe is present. After conjugation with analytes, the nanoprobe further migrates to the porous NC membrane that is coated with specific antigen or antibody in the form of lines. The flow of sample is assessed by the control line, whereas, the detection of target analyte is observed through the signal response at the test line. The end of the strip is attached with adsorbent pad that helps in maintaining the flow directionality of the sample solution. In recent years, UCNPs-based probes have been incorporated with this platform to achieve detection of chemical and biological analytes. Lee’s group reported a portable LFA platform integrated with smartphone for fast and sensitive on-site sensing of avian influenza H5N2 and H5N6 viruses. In addition, LFA platform has also been designed for the detection of multiple analytes. Zhou’s group synthesized NaYF₄:Yb/Er UCNPs co-doped with Li⁺ (7 mol%) and of K⁺ (3 mol%) and fabricated the lateral flow nanoplatform for simultaneous detection of large bacteria Yersinia pestis and Burkholderia pseudomallei. Similarly, Xu’s group reported a household LFA prognosis platform for multiplexed prognosis of Heart Failure (HF) by simultaneously detecting the associated biomarkers namely, Brain natriuretic peptide (BNP) and suppression of tumorigenicity 2 (ST2). In conclusion, lateral flow strip serves as a promising platform to develop sensitive, rapid and portable assays.

3.2.2 Test paper

Test papers have drawn tremendous attention as platforms to establish rapid assays owing
to their cost-effectiveness, portability and simplicity. Amongst the different types, cellulose paper is the most used platform due to their inherent benefits such as high stability, biodegradability, chemical compatibility, and visibility. Test papers can also be patterned using hydrophobic inks to make test zones for the assay. However, these platforms are prone to interferences by background signals arising from complex samples. With regards to this, UCNPs with their superior optical properties have been incorporated to the test paper platform for sensing applications. Liu’s group synthesized poly(ethylenimine) (PEI)-functionalized UCNPs and covalently immobilized them on cellulose paper for the detection of cocaine with the help of aptamers. The developed UCNPs-based test paper device was showed to successfully track the cocaine in saliva and blood samples, demonstrating their potential for road-side testing of drug abuse. Mei et al. prepared UCNPs-based paper sensors by immersing the filter paper into NaYF₄:Yb/Tm UCNPs solution for the sensitive, fast qualitative and quantitative detection of pesticide thiram in spiked apple juice (Figure 6B). Very recently, UCNPs-based cellulose paper microzone device was fabricated to perform the detection of telomerase biomarker. The cellulose paper was functionalized using telomerase substrate (TS) oligonucleotide for capturing the target telomerase and corresponding telomeric repeat complementary oligonucleotide conjugated UCNPs were used as the reporter molecule. The detection was realized based on the UCNPs signal enhancement induced by the hybridization between target telomeric repeats and the complementary oligonucleotides.

3.2.3 Microarray

This platform encompasses multiple microwells enabling high throughput detection using
small sample volume in the single run of the assay. The platform has gained popularity for detection of multiple analytes in clinical diagnostics. It comprises of an array of multiple wells whose surfaces are functionalized with recognition molecules for capturing the target analytes. Using UCNPs-conjugated detection antibody, the captured analytes are labeled and measured qualitatively and quantitatively based on the UCNPs optical signal. Soukka’s group prepared NaYF₄:Yb/Er UCNPs for the simultaneous quantitative detection of prostate specific antigen (PSA), thyroid stimulating hormone, and luteinizing hormone using this platform. Multiple capture antibodies were printed onto different areas of a 96-well microtiter plates and secondary antibody was labeled with UCNPs. The presence of the analytes in the sample was reported by the fluorescence of UCNPs in the microwells. Recently, Soukka’s group reported a spectral and spatial two-color upconversion fluorescence-based microarray immunoassay for the simultaneous detection and classification of IgG and IgM antibodies for influenza A and human adenoviruses (Figure 6C). The virus antigens and antibodies were immobilized in the microtiter wells. The antibody responses against influenza and adenovirus infection were identified based on the spatial response, whereas, the antibody class was distinguished by the spectral response from the UCNPs-conjugated detection antibodies against the respective targets.

3.2.4 Microfluidics

Microfluidics has emerged as a powerful sensing platform by facilitating control over the fluid flow and assay miniaturization requiring small volumes of samples as well as assay reagents. The microfluidic devices can be fabricated using polydimethylsiloxane or paper. Taking the inherent advantages of UCNPs into account, they have been incorporated with
microfluidic assays for varied sensing applications. Wang et al. prepared an array of microfluidic silicon nanowire and integrated it with antibody-coated multifunctional magnetic upconversion nanoparticles (MUNPs) for the detection of circulating tumor cells (CTCs). Using this platform, the MUNPs were demonstrated to perform the specific target detection in the clinical blood sample as well. Furthermore, the use of magnetic field also allowed the convenient release of the captured CTCs for further analysis and culturing. Liu’s group reported a simple upconverting paper-based microfluidic device (UC-µPAD) for detection of matrix metalloproteinase-2. UCNPs were modified with a specific peptide substrate and immobilized on the test zone of UC-µPAD. A tetramethylrhodamine dye was conjugated to the end of the peptide, that led to the quenching of UCNPs fluorescence thereby allowing the FRET-based target detection.

4. Chemo/bio sensing applications

UCNPs have been extensively employed for wide applications ranging from environmental hazard assessment to biosensing and medical diagnostics. The use of UCNP probes for a particular sensing application depends on several factors including their fluorescence, detection platform, compatibility to the reaction system, and detection method. In addition to this, different UCNPs-based codes (color, shape, etc.) can be developed for simultaneous detection of multiple analytes. To achieve a specific and selective detection, UCNPs are functionalized with biological and chemical elements for target recognitions via typical antigen-antibody pairing, hybridization between complementary base pairs of nucleic acid, ionic interactions, etc. This section discusses the assays developed for environment, food, biosensing and medical diagnostics using UCNPs-based nanoprobe in the past two years.
4.1 Environmental hazard and food assays

Hazardous chemical and biological pollutants in environmental and food samples are a matter of global concern due to their detrimental impact on the ecological systems. The build-up of these substances could be attributed to the residual medical (pharmaceuticals pollutants), agricultural (pesticides), biological (pathogens and the toxins released by them), and industrial (chemical solvents, industrial by-products, etc.) wastes at a level deemed unsafe for human health. To control these contaminants, it is critical to precisely detect and treat them effectively. With regards to these, myriad UCNP-based nanoprobes have been prepared to develop sensitive, accurate, rapid, and cost-effective assays for spiked and real environmental and food samples.

4.1.1 Pesticides

Use of pesticides including herbicide, insecticide, fungicides, etc. are prevalent everywhere to control the pests and disease-causing vectors. Excessive use of pesticides often leads to their residual build up in environmental samples, livestocks, aquaculture, and plant-based food items with a threat of causing antibacterial resistance as well as diseases like cancer. Varied assays have been developed using UCNP-based sensors. Hu et al. utilized NaYF₄:Yb/Ho@SiO₂ UCNPs as donor and AuNPs as acceptors for FRET-based detection of acetamiprid, a pesticide used widely in agriculture and gardening. The detection was performed in adulterated tea samples and the limit of detection (LOD) was determined to be 3.2 nM.⁹⁷ FRET principle was also used by Mei and coworkers to detect pesticide thiram in spiked apple juices on a filter paper using Cu²⁺-modified UCNPs.⁸⁷ The readout was done using smartphone integrated with the detection platform with the LOD of 0.1 µM, thereby
exhibiting the potential of the assay for performing point-of-care (POC) diagnostics. UCNPs-AuNPs system was also used for IFE-based detection of neonicotinoid insecticide imidaclothiz (LOD = 2.1 ng/mL) and imidacloprid (LOD = 0.79 ng/mL) in different spiked samples (rice, cabbage, pakchoi, apple, tomato, pear, soil water and paddy water) and real samples (paddy water and pear).  

4.1.2 Drugs

Clinical drugs and antibiotics used in healthcare and agricultural settings have negative impact on human health owing to their abuse and accumulation in environmental and food samples. Jianrong’s group adopted double recognition method by using aptamer-molecularly imprinted polymer (MIP)-modified UCNPs for the detection of enrofloxacin (ENR), an antibacterial agent. The MIP grafting was done by the polymerization of methyl methacrylate monomer in the presence of crosslinkers with target ENR acting as the template. Upon sample addition, the fluorescence intensity was found to decrease proportionally with the increase in target concentration bound to nanosensor. The ENR detection was demonstrated in different fish samples and the LOD was determined to be 0.04 ng/mL. Since the emission spectrum of nanoprobe did not overlap with the absorption spectrum of target molecules, fluorescence quenching was ascribed to photo-induced electron transfer between the functional groups of target and UCNPs@MIP nanoprobe. The same group employed this principle for ENR detection in fish sample but omitted the aptamer and developed MIP-modified UCNPs via photoexcitation. In a simplified process, they used 980 nm radiation to excite UCNPs and the visible light emitted in turn led to the photopolymerization of the monomer, thus, generating the MIP shell around UCNPs (Figure 7A). The thickness of
this MIP shell was found to be tunable by altering the time of photopolymerization. In the
presence of target ENR, probe’s fluorescence decreased with increasing analyte concentration
and the LOD was determined to be 8 ng/mL. A sensitive platform using UCNPs-based probes
was developed for anti-inflammatory drug Diclofenac (DCF) detection by indirect
competitive upconversion-linked immunosorbent assay (ULISA). In this work, UCNPs
were used as signaling probes, where they were bound to the reporter molecules against the
target. DCF-bovine serum albumin (BSA) conjugate was coated onto the microtiter plate and
the competitive assay was performed after the addition of anti-DCF mouse antibody, whose
attachment to the target DCF was detected using anti-mouse IgG-UCNPs conjugate as
signaling probe (Figure 7B). Compared to conventional ELISA, ULISA did not require
enzyme-based amplification of signal, thereby reducing the assay steps and complexity. The
detection limit for DCF was reported as 0.05 ng/mL. Later, the same group reported direct
competitive DCF detection with LOD reduced to 0.02 ng/mL by carefully designing the tracer
comprised of carboxylated silica-modified NaYF₄:Yb/Er UCNPs (~90 nm in diameter) coated
with DCF-modified bovine-γ-globulin.

4.1.3 Pathogens

Pathogens and the toxins released by them are a matter of grave concern due to their
detrimental health impacts. Accumulation of pathogens leads to several diseases that spreads
further via environmental samples. Poultry and livestocks get contaminated with the
pathogens that can spread to other animals via feedstocks. Jin et al. developed NaYF₄:Yb/Er
UCNPs-based FRET aptasensor for sensing E.coli in tap and pond water. Hybridization
between aptamers conjugated onto AuNPs (acceptor) and the corresponding complementary
DNA (cDNA) attached onto the UCNPs (donor), led to the FRET-based quenching of the UCNPs fluorescence. Upon sample addition, the aptamers on AuNPs bound preferably to the target bacteria, resulting to their dissociation from the UCNPs and subsequent recovery of the fluorescence. The assay was single step, rapid (within 20 minutes), with a detection range and LOD of 5-10^6 cfu/mL and 3 cfu/mL, respectively. Wang and group grafted the aptamer-functionalized NaYF\textsubscript{4}:Yb/Tm@NaYF\textsubscript{4}:Yb core-shell UCNPs on the surface of MoS\textsubscript{2} nanosheets via π-π interaction for the detection of MC-LR. Due to the spectral overlap between the upconversion fluorescence and the absorption spectrum of MoS\textsubscript{2}, the UCNPs fluorescence got quenched. The fluorescence, however, was recovered in the presence of MC-LR to which the aptamer bound preferentially (Figure 7C). The nanoprobe was deployed for MC-LR detection in real samples (tap and lake water), and the LOD was determined to be 0.002 ng/mL. Dai 	extit{et al.} used aptamer-modified NaYF\textsubscript{4}: Yb/Er UCNPs as donors and cDNA-functionalized AuNRs as acceptor for mycotoxin Ochratoxin A (OTA) detection. The hybridization between donor and acceptor molecules led to the quenching of UCNPs fluorescence, which recovered in the presence of OTA due to the competitive binding. The OTA detection was performed in beer with LOD of 27 pg/mL. Recently, OTA contamination in red wine, grape juice, and beer was demonstrated using FRET aptasensor, where Mn\textsuperscript{2+}-doped NaYF\textsubscript{4}:Yb/Er UCNPs (red emission) was used as the energy donor and black hole quencher 3 (BHQ3) was the energy acceptor. The UCNPs were modified with quencher-labeled aptamer via adenine linker. Upon addition, the target OTA in the food sample bound to the aptamer with the formation of a folded BHQ3-aptamer complex, bringing it closer to the UCNPs nanoprobe, thereby causing the FRET-based fluorescence quenching.
quenching (Figure 7D).\textsuperscript{41} The quenching efficiency was found to be controllable via modulating the linker length. The nanoprobe demonstrated selective mycotoxin detection in colored food samples and the assay was rapid (<10 min) without involving multiple steps.

4.1.4 Ions

Ions play an important role as trace nutrients for cellular processes. However, an excessive exposure to ions can cause notorious health hazards. Conventional methods of ions detection including ion chromatography, ion-selective electrodes, nuclear magnetic resonance spectroscopy, absorption spectroscopy, etc. have drawbacks such as low sensitivity, high cost, and complex operation procedures requiring skilled personnel. Therefore, UCNPs-based fluorescent detection methods have gained impetus over the traditional techniques. NaYF\textsubscript{4}:Yb,Tm,Er UCNPs probes were used in combination with natural chemical curcumin for sensing the fluoride ions (F\textsuperscript{-}) in tap water and milk.\textsuperscript{106} In the presence of the F\textsuperscript{-}, a curcumin-F\textsuperscript{-} complex is formed accompanied with a shift in the UV absorption peak maxima of curcumin as well as fluorescence quenching of UCNPs via the IFE process. Based on the different target concentrations, the absorption and fluorescence changes were monitored for the F\textsuperscript{-} detection. The LOD via colorimetric and fluorescence measurements was determined to be 0.48 ppm and 0.10 ppm, respectively. The detection system was found to be highly selective to F\textsuperscript{-} ions regardless of the presence of other test ions in the sample. A nanoprobe composed of UCNPs with N,N-diethyl-p-phenylenediamine (EPA) as acceptor was used for the detection of ferric ion (Fe\textsuperscript{3+}) owing to the contamination of water and soil samples in their excessive amount. The NaYF\textsubscript{4}:Yb,Gd,Ho used in the study had emission peaks at 546, 657, 758 and 812 nm under 980 nm excitation. The addition of target Fe\textsuperscript{3+} led to the EPA
oxidation, generating EPA oxide with 552 nm UV absorption peak. Consequently, this led to a reduction in 546 nm peak of UCNPs fluorescence due to the IFE process, while the other peaks remained unchanged (Figure 7E). Based on this, the target ferric ion concentration was quantified by ratiometric measurement of fluorescence at 546 and 758 nm peaks, with the LOD of 0.25 µM.\textsuperscript{107}

**Figure 7.** UCNPs nanoprobes for environmental hazards and food assays. (A) Aptamer-MIP@UCNPs for enrofloxacin (ENR) detection in different fish samples. (a) Preparation of the nanoprobe, and (b) graph showing decreasing fluorescence spectra with increasing target ENR concentration. Adapted from Tang, Y., Li, M., Gao, X., Liu, X., Gao, J., Ma, T. and Li, J. *Microchim. Acta* 2017, 184, 3469-3475 (ref 100). Copyright 2017 Springer. (B) Indirect competitive upconversion-linked immunosorbent assay (ULISA) with UCNPs as signaling probes for pharmaceutical micropollutant diclofenac (DCF) sensing in water samples. Reproduced from Hlaváček, A., Farka, Z., Hübner, M., Horňáková, V., Němeček, D., Niessner, R., Skládal, P., Knopp, D. and Gorris, H. H. *Anal. Chem.* 2016, 88, 6011-6017 (ref
To further improve the UCNPs nanoprobes-based sensor, a dual colorimetric and fluorometric nanoprobe was synthesized for the detection of Cu\(^{2+}\) ions, that are detrimental to kidneys and liver in high levels.\(^{108}\) Here, ligand-free UCNPs were grafted with porphyrin hydrate (tetraphenylporphyrin tetrathiosulfonic acid hydrate, TPPS), causing a reduced fluorescence from the UCNPs and green color fluorescence from the TPPS due to the FRET. With Cu\(^{2+}\) addition, the color of UCNPs/TPPS-Cu\(^{2+}\) complex displayed pink color attributed to the inhibition of FRET process. This system was used with color scanning app conjugated smartphone for in-situ and real-time qualitative and quantitative Cu\(^{2+}\) detection. LOD from the colorimetric and fluorescence approaches was determined to be 0.13 µM and 0.32 µM, respectively. The method demonstrated the bright future of utilizing UCNPs nanoprobes for
developing POC diagnostic platforms.

4.2 Biological analysis

Sensing of dynamic localizations of biomolecules and biochemical pathways for understanding the normal and pathological processes is critical for wide areas including disease diagnostics, therapy, proteomics, genomics, etc. Cellular investigations have been performed both in living cells outside the organism as well as inside it (in vivo). Additionally, cell extracts serving as storehouse of important biomarkers are often utilized for detection using nanoprobes. Conventional techniques such as mass spectrometry, polymerase chain reaction, etc. require complex, bulky, and costly instruments. On the other hand, the conventional fluorescent methods have limitations associated with the use of organic dyes. Moreover, the techniques developed so far are unable to assess the expression levels of nucleic acid or perform multiplexed detection. To circumvent these challenges, UCNPs have been extensively explored to develop cells- and cell extracts-based assays. The choice of UCNPs-based nanoprobes is based on certain parameters including surface functionalization, size and shape, and their toxicity. After administration to the site, nanoprobes get aggregated with biomolecules forming a protein corona, that in turn, reduces the targeting efficiency of the nanoprobe. Therefore, surface functionalization of UCNPs is critical to confer biocompatibility and biostability along with maintaining or improving their specific targeting efficiency. For establishing intracellular assays, the nanoscale dimension of probes is highly beneficial for the uptake by cells. Additionally, the shapes of nanoprobes also play a critical role in ensuring minimum damage to cell membrane and cellular activities. Lastly, for sensing involving in vivo conditions, the nanoprobes must be assessed for their cytotoxicity to avoid
the non-specific cell death.

4.2.1 Intracellular assays

To develop the intracellular assays, UCNPs nanoprobes have been used as labeling agents in order to tag the biomolecules for exploring their structures, locations, and involvement in cellular pathways. Prior to their use in the in vivo conditions, nanoprobes are tested in living cells outside the living organisms to detect the cellular pathways and specific biomolecules. Detection of cytochrome c (Cyt c), an early stage biomarker of apoptosis, was performed in human liver carcinoma cell line using polydopamine (PDA)-modified core-shell UCNPs (as internal reference) conjugated with cyanine-labelled Cyt c aptamers (for ratiometric measurement). The cells were subjected to etoposide (apoptosis inducing drug) for specific release of Cyt c from mitochondria. Upon cellular uptake of the nanoprobe, the attached Cyt c aptamer bound to target intracellular Cyt c, thereby leading to their dissociation from the nanoprobe and subsequent activation of Cy3 fluorescence (Figure 8A). This fluorescence was observed to enhance with the increasing concentration of Cyt c, thereby allowing the quantitative detection of Cyt c. The developed assay showed low LOD of 20 nM. Furthermore, the UCNPs nanoprobes were highly selective towards sensing the Cyt c despite the presence of various interfering molecules. This study provided promising application of UCNPs for the cell screening of apoptosis-inducing drugs. Core-shell UCNPs nanoprobes were used for resolving the cytoplasmic protein structure in living cells. UCNPs were tuned down to 30 nm size to assist their easy mobility in the cytoplasm as well as achieve minimum biasness of cellular function induced by the engineered UCNPs-based nanoprobes. UCNPs were functionalized with anti-
fluorescent protein (GFP) nanobody for the specific detection of GFP-tagged fusion proteins in the outer mitochondrial membrane via FRET process. Xu’s group, with a great contribution in developing chiral nanoscale structures for ultrasensitive detection of nucleic acids and cancer biomarkers, fabricated a DNA-driven Au-UCNP pyramids for the sensing of intracellular microRNA (miRNA) in living cells. The developed nanopyramid had dual signaling capability based on the strong plasmonic circular dichroism (CD) and upconversion fluorescence. Upon the addition of target miRNA, CD was found to be decreasing while fluorescence intensity increased. The nanoprobes showed LOD of 0.03 fmol/10 µgRNA and 0.12 fmol/10 µgRNA for CD and fluorescence intensity, respectively. Later, Xu’s group also performed simultaneous detection of telomerase enzyme and miRNA in living cells by combining upconversion signal with surface-enhanced Raman scattering. To achieve this, plasmonic AuNRs and NaGdF$_4$:Yb/Er UCNPs were prepared and the LOD was found to be 0.011 amol/ngRNA and $3.2 \times 10^{-13}$ IU for miR-21 and telomerase, respectively. Gu et al. used thiazole derivative-conjugated UCNPs for the FRET-based detection of toxic Hg$^{2+}$ in living cells. Thiazole derivative dye is responsive towards the Hg$^{2+}$ and the target detection was done based on 540 nm to 803 nm emission intensity ratio of UCNPs that showed increasing green emission band with the increasing amount of target Hg$^{2+}$, with the LOD of 0.063 µM.

Several advances have been made in the UCNP nanoprobes-based in vivo sensing. A UCNP-based nanosensor was fabricated using UV light-activatable DNA aptamer probe with NaGdF$_4$:70%Yb,1%Tm@NaGdF$_4$ UCNPs for the detection of ATP in nude mice with HeLa xenograft tumors. The light-controlled UCNP-based nanosensor was found to effectively deliver the aptamer as well as offer temporal control over the ATP sensing efficiency.
nanoprobe consisting of UCNPs linked with peptide (Cys-Arg-Gly-Asp) and differentiation-inducing molecule (kartogenin) by a photocaged linker on their surface was prepared, and demonstrated to remotely control the human stem cells differentiation as well perform the prolonged \textit{in vivo} tracking of the migration of the implanted stem cells.\textsuperscript{119} Notably, differentiation of human stem cells is critical for several physiological and pathological processes. Therefore, this study could be helpful in designing UCNPs-based stem cell therapy for a wide spectrum of diseases. Apart from sensing the biomolecules, UCNPs-based probes were also designed for the \textit{in vivo} detection of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that drive the onset of many diseases via irreversible oxidative damage to the biomolecules. Owing to their short half-life, the ROS and RNS detection is a challenge, signifying the need for the on-site detection. Amongst the different types of ROS, hydroxyl radical (\textsuperscript{.}OH) has been reported to be the most potent oxidant. Song \textit{et al.} developed a nanoprobe comprising core-multishell NaYF\textsubscript{4}@NaYF\textsubscript{4}:Yb/Tm@NaYF\textsubscript{4}:Yb/Er@NaYF\textsubscript{4} UCNPs (donor) modified by azo dye (recognition element) for the FRET-based \textsuperscript{.}OH (acceptor) detection in mice models.\textsuperscript{120} The same group also prepared 4-amino salicylic acid-iron(II) complex-modified NaLuF\textsubscript{4}:Yb/Er/Tm for real-time sensing of \textsuperscript{.}OH induced by titanium oxide nanomaterial.\textsuperscript{121} An overdose of commonly used paracetamol drug can induce hepatotoxicity by generating RNS in the liver. For detection, core-shell UCNPs (donor) were coated with PEI and Cy7 chromophore (RNS responsive energy acceptor) (Figure 8B). In the presence of the RNS, an emission at 800 nm was observed due to the energy transfer from the UCNPs to Cy7. The real-time monitoring of the hepatotoxicity was demonstrated in nude mice, showing the
promising potential of the developed nanoprobe for screening the hepatotoxicity of synthetic drugs.\textsuperscript{122}

### 4.2.2 Cell extract-based analysis

Cell lysates, after extraction process, are used as test samples for sensing the target biomolecules of interest. $\beta$-NaYF$_4$:Yb/Er/Gd nanorods, with emissions at 545 and 659 nm, were demonstrated for Cu$^{2+}$ detection in HeLa cell lysates using FRET principle.\textsuperscript{113} UCNPs were modified with meso-tetra(4-sulfonatophenyl)porphine dihydrochlorid (TSPP) that had strong affinity for Cu$^{2+}$. Binding of the target Cu$^{2+}$ to TSPP caused a shift of TSPP absorption maxima from 515 to 545 nm that overlapped with UCNPs emission at 545 nm, thereby resulting in the quenching of emission at 545 nm. With the unaffected 659 nm emission of UCNPs in the presence of Cu$^{2+}$, the quantification was achieved based on the ratio of the upconversion emission between 545 nm and 659 nm. The assay was very rapid (10 s) and the LOD was determined to be 2.16 $\mu$M. Exosomes extracted from human liver cells were quantified using UCNPs and AuNPs-based FRET system on a filter paper.\textsuperscript{110} The targeting was done for the surface protein of exosomes called CD63 and therefore, the nanoparticles were modified with CD63 aptamer as recognition unit (Figure 8C). The aptamer sequence was split into two fragments, out of which, one fragment (CP) was fixed onto the surface of sodium periodate oxidated filter paper along with the PEI-modified UCNPs. The other fragment (DP) was modified with thiol groups at 3’ end followed by their conjugation to AuNRs. The DP-AuNR conjugate was mixed with test sample and added to the filter paper. In the presence of target exosomes, the CD63 proteins of exosomes were observed to bind the CP and DP fragments. This subsequently reduced the distance between the AuNRs and
UCNPs, thereby leading to FRET-based fluorescence quenching. The rate of quenching was found to be proportional to the exosome concentration, and the LOD of exosome detection was found to be $1.1 \times 10^3$ particles/µL. Blood cell lysate was used for the detection mRNA biomarker BACE-1 relevant to Alzheimer’s disease. To achieve this, UCNPs coated with target-specific complementary sequence and GO flakes as energy acceptors were subsequently added to the test solution. The LOD of BACE-1 was determined to be as low as 500 fM.\textsuperscript{123} The study also showed the detection of mRNA biomarker PCA3 for prostate cancer in human serum sample using the developed assay. Recently, Zhu’s group demonstrated tumor-related noncoding RNA (ncRNA) detection using UCNP-based nanoprobe.\textsuperscript{124} UCNPs (energy donors) were first modified with linker DNA followed by their conjugation to one hairpin DNA (Hp)-modified AuNPs (energy acceptors) via hybridization. This led to FRET-based reduction of upconversion emission at 543 nm, which was recovered upon the addition of a single DNA (SDNA) sequence that could specifically open the Hp. By using multifunctional Hp (mHp) DNA with ncRNA as the recognition sequence, the SDNA sequence identified the ncRNA as well as induced exonuclease III assisted removal of mHp and release of ncRNA and SDNA. The ncRNA thus released, bound to another mHp to further release the SDNA, thereby improving the sensitivity of ncRNA detection. Varying the recognition portion of mHp, the nanoprobe was also used to detect the miRNA-21 expression levels in the cells lysate of MCF-7. Furthermore, the detection limit was determined to be in the sub-femtomolar range. Telomerase enzymes were detected in HeLa cells extracts using a TS primer functionalized cellulose paper microzone plate (Figure 8D).\textsuperscript{125} UCNPs were modified with TC oligonucleotide enabling their conjugation to the long single DNA
produced by TS primer via telomerase reaction. This in turn led to a strong signal, which was observed to be proportional to the amount of single DNA, thereby signifying the telomerase activity. By replacing UCNPs with methylene blue, the developed strategy was demonstrated to perform colorimetric detection. The assay required only 5 µL of test sample and was found to be stable at room temperature. The work also showed the potential to distinguish telomerase levels in the different cell lines.

**Figure 8.** *In vivo* imaging using UCNPs-based nanoprobes. (A) Detection of Cyt c in living cells. (a) Fabrication of aptamer-modified PDA-coated UCNPs nanoprobe. (b) FRET-based intracellular detection of Cyt c. Reprinted from Ma, L., Liu, F., Lei, Z. and Wang, Z. *Biosen. Bioelectron.* 2017, 87, 638-645 (ref 112). Copyright 2017, with permission from Elsevier. (B) (a) Design of PEI and Cy7-loaded UCNPs for sensing hepatotoxicity *in vivo.* (b) Energy transfer-based sensing mechanism of the probe wherein Cy7 (green star) energy acceptor was degraded by the RNS. (c) Cy7 UV/Vis spectra in the absence and presence of RNS, and
Medical diagnostics involve the detection of biomolecules in human samples (blood, urine, saliva, etc.), and have gained enormous attention for disease diagnostics owing to their several benefits. Compared to the detection done directly in the host body, these medical diagnostic strategies are non-invasive in nature, and are done outside the body. The detection is relatively quicker and simpler, with the potential to be developed as POC diagnostic platforms. Thus far, medical diagnostics using UCNPs-based nanosensors have been applied to detect proteins, nucleic acid, pH, exosomes, pathogens, toxic ions, etc. Prior to applications, the UCNPs nanosensor must be analyzed for their stability in bodily samples with varying pH, salt compositions, etc. to ensure the successful detection in the real complex samples.

### 4.3.1 Blood

Blood is the most used sample for developing the medical diagnostic devices. Zhang’s group developed a FRET system with UCNPs (donor) and AuNPs (acceptors) for the whole blood immunoassay on a glass substrate as solid support. The blood sample was spiked...
with goat IgG and the LOD of IgG in 20-fold diluted sample was found to be 0.51 µg/mL.

The in-situ solid substrate-based approach required low sample and was free of steps such as washing and pre-separation. This offered the potential of UCNPs nanoprobes to develop POC diagnostics. PDA-coated UCNPs-based nanoprobes were employed for the sensing of glucose in whole blood sample as well as serum. To achieve this, ligand-free UCNPs were mixed with dopamine and glucose oxidase (GOx). Due to its absorbance in visible spectral region, PDA acted as an energy quencher. The quenching, however, was reduced by the H$_2$O$_2$ that was generated by the enzymatic reaction between GOx and glucose (Figure 9A). Based on the UCNPs luminescence quenching inhibition-based response, the LOD in serum and whole blood was determined to be 1.2 µM. Blood and urine sample was assayed to detect the homogeneous glycated hemoglobin (HbA1c) via FRET, with NaYF$_4$:Yb/Er UCNPs as the donor. The acceptor was the target molecule itself, with its 541 nm absorption overlapping with the UCNPs emission. The detection was cost-effective as well as time-saving due to the intrinsic absorbance of target analytes. Numerous medical diagnostics have been carried out using human serum samples. For instance, UCNPs-based nanoprobes were used to detect small oligonucleotides such as miRNA in human serum samples. NaYF$_4$:Yb/Er@SiO$_2$ UCNPs grafted with single stranded (ssDNA) was prepared and captured on the streptavidin-coated wells. The azide group presented at the 3’ end of the ssDNA-UCNPs underwent interstand ligand interaction with the second probe ssDNA-biotin in solution via click reaction that was induced by the target sequence. The fluorescence from the system was found to be directly proportional to the target sequence concentration with the LOD of 10$^{-17}$ moles per well (100 fM). Furthermore, the detection system was able to distinguish between
the full complementary sequences and those with mismatches or noncomplementary sequences, wherein the latter displayed significantly smaller fluorescence. A proof-of-concept study was performed for the detection of Ebola virus oligonucleotide using BaGdF$_5$:Yb/Er UCNPs and AuNPs via energy transfer.\textsuperscript{130} In comparison to the picomolar detection level of homogeneous format, the LOD of heterogeneous assay was found to be in femtomolar level. This improved LOD was due to the use of nanoporous alumina membrane in the heterogeneous assay. The nanopore walls of the membrane enabled an increased light-matter interaction resulting in the improved LOD. Dual signal probe (luminescence and SERS) comprised of Au-Au-UCNPs trimers was prepared by self-assembly to detect two cancer biomarkers namely, Alpha-fetoprotein (AFP) and mucoprotein1 in serum sample.\textsuperscript{131} Here, FRET between AuNPs and UCNPs resulted in the quenching of UCNPs fluorescence. Whereas, with the conjugation of SERS reporter (4-aminothiophenol) to the trimer, the strong plasmonic coupling between the two AuNPs subsequently enhanced SERS signal. It was observed that the presence of mucoprotein1 (LOD = 4.1 aM) led to a decrement in the SERS intensity, while the presence of the AFP (LOD = 0.059 aM) led to an increment in the fluorescence. AFP was also detected via ultrasensitive photoelectrochemical enzyme immunoassay using nanosphere made by sandwiching Au layer between UCNPs and cadmium sulfide (CdS) semiconductor (UCNP@Au@CdS).\textsuperscript{132} The nanospheres were coupled with glucose oxidase (GOx) and polyclonal antihuman AFP antibody (Ab$_2$)-conjugated AuNPs (Ab$_2$-AuNP-GOx) and added to the monoclonal mouse antihuman AFP antibody (Ab$_1$)-coated microplate. The assay developed using this strategy comprised of two panels including enzyme immunoreaction and NIR light-activated photocurrent measurement. The
GOx detection occurred by typical sandwich assay using Ab\textsubscript{1}-coated microplate as capture system and Ab\textsubscript{2}-AuNP-GOx anti-AFP as secondary antibody. Moreover, the GOx present in the system oxidized glucose substrate to generate H\textsubscript{2}O\textsubscript{2} that further enhanced the photocurrent of UCNP@Au@CdS nanosphere via fluorescence- and plasma-resonance energy transfer. Remarkably, the plasmonic Au layer acted as both energy harvesters of NIR light as well as energy conveyers from UCNPs to CdS. The AFP detection limit in serum samples was 5.3 pg/mL. Cancer biomarker PSA was detected by using FRET between anti-PSA detection antibodies-modified UCNPs (donor) and anti-PSA capture antibodies-modified AuNPs via sandwich-type single-particle enumeration (SPE) immunoassay.\textsuperscript{133} The detection was performed on a glass slide surface using human serum samples. UCNPs and AuNPs were brought in close proximity in the presence of target PSA, consequently quenching the UCNPs fluorescence. The particle-particle interaction was counted based on the change of fluorescence and the LOD was determined to be 1.0 pM. In another study utilizing the benefits of ULISA, UCNPs were used as labels for PSA biomarker single molecules counting. The single molecule ULISA detection yielded a LOD of 1.2 pg/mL (42 fM) for PSA in blood serum, which was reported to be ten times more sensitive than conventional ELISAs.\textsuperscript{134} UCNPs with green and blue fluorescence were employed for the simultaneous detection of antigens associated with heart failure (BNP and ST2). The assay was performed using human serum samples and the detection platform was comprised of smartphone integrated multiplexed upconversion fluorescent lateral flow strip (Figure 9B).\textsuperscript{90} In the presence of the antigens, the fluorescence from dual color UCNPs were located in two test lines that would be absent in control line otherwise. The system had the LOD of 17.46 pg/mL and 29.92 ng/mL
for BNP and ST2, respectively, and offered potential to be used for risk monitoring by patients at home. Serum IgG and IgM antibodies for influenza A and human adenoviruses were detected by antihuman IgG-coated green (NaYF$_4$:Yb/Er) and antihuman IgM-coated blue (NaYF$_4$:Yb/Tm) emitting UCNP$_s$ via a dual-mode multiplexed array.$^{85}$ The detection was performed in microtiter wells using human serum samples obtained 3 weeks post-vaccination and assessed spectrally and spatially. The spectral overlap and cross-reactivity were observed to be negligible in the dual color UCNP$_s$-based assay. Instead of using conventional acceptors, Ping et al. employed carbon nanoparticles as energy acceptors with NaYF$_4$: Yb/Er UCNP$_s$ as donors. The FRET pair was used for sensing the total IgE in human serum.$^{135}$

Physiologically significant small amino acids, if present in abnormal levels can lead to a series of impairment, thereby highlighting the need to detect them. Tyrosine was detected in human serum sample by enzymatically-induced upconversion photoinduced electron transfer and the LOD was found to be 1.1 µM.$^{136}$ Ratiometric UCNP$_s$-based nanoprobes were designed to detect cysteine (Cys). To achieve this, fluorescein-based fluorescent probe was loaded in the pores of mesoporous silica layer on NaYF$_4$:Yb/Tm UCNP$_s$.$^{137}$ It was observed that with increasing Cys concentrations, the green and blue emissions of UCNP$_s$ ($I_{518}/I_{475}$) grew exponentially and the color change was also recognizable by the naked eye. The developed system could detect Cys in human serum sample showing their potential to develop medical diagnostic platforms. Ratiometric UCNP$_s$-based nanoprobes utilizing fluorescence emissions at 540 and 656 nm were also employed for small molecules sensing like nitric oxide (NO) via FRET process. The mesoporous silica-coated UNCP$_s$ (donor) were loaded with reactive rhodamine B-derived molecules (acceptor), followed with the conjugation of
β-cyclodextrin layer on the UCNP surface (Figure 9C). The developed assay was successfully employed for NO detection in human serum and mouse models-derived liver tissue slices.

**Figure 9.** UCNPs nanoprobes-based medical diagnostics. (A) PDA-coated UCNPs for H$_2$O$_2$ and glucose detection in whole blood and serum samples. Adapted from Liu, Y., Tu, D., Zheng, W., Lu, L., You, W., Zhou, S., Huang, P., Li, R. and Chen, X. *Nano Research* 2018, 11, 3164-3174 (ref 127). Copyright 2018 Springer. (B) Upconversion fluorescent for prognosis of heart failure. (a) Smartphone integrated portable platform. (b) Lateral flow strip platform with dual color UCNPs. (c) UCNPs captured in two test lines in the presence (top) and in control line in the absence (bottom) of the targets. (d) setup of the smartphone-based reader. Reproduced from You, M., Lin, M., Gong, Y., Wang, S., Li, A., Ji, L., Zhao, H., Ling, K., Wen, T., Huang, Y. and Gao, D. *ACS nano* 2017, 11, 6261-6270 (ref 85). Copyright 2017 American
4.3.2 Urine

UCNPs nanosensor have also been utilized for urine sample assays for different biological as well as chemical elements. Earlier, the UCNPs/TPPS complex used to detect Cu$^{2+}$ in tap water was also employed to detect the ions in urine sample that was assessed by the smartphone integrated to the system.\textsuperscript{108} In a sequential detection approach, branched PEI-capped NaGdF$_4$:Yb/Tm UCNPs-based nanoprobe was developed for the assay of Cu$^{2+}$ in HeLa cells, pyrophosphate (PPi) in urine, and alkaline phosphatase (ALP) in serum via FRET process (Figure 9D).\textsuperscript{139} Coordination between PEI and Cu$^{2+}$ led to the fluorescence quenching of UCNPs. However, the binding between Cu$^{2+}$ and PPi, led to the detachment of the former complex, thereby restoring the UCNPs fluorescence. The Cu$^{2+}$/PPi complex got further dissociated by the ALP-mediated hydrolysis of PPi, followed by the re-binding of Cu$^{2+}$ with PEI with the subsequent fluorescence quenching. The LOD was determined to be 57.8 nM, 184 nM, and 0.019 U/mL for Cu$^{2+}$, PPi, and ALP, respectively.

4.3.3 Saliva

Portable paper device with UCNPs-based FRET nanosensor was used to demonstrate the
detection of cocaine in human saliva samples (Figure 4E). For the assay, the anticocaine aptamers (ACA) were cut into two flexible ssDNA viz. ACA-1 and ACA-2. UCNPs (energy donors) were modified with ACA-1 and deposited onto the surface of the cellulose fiber, whereas ACA-2 was conjugated to the AuNPs (acceptors). Upon the addition of target cocaine, the UCNPs and AuNPs bearing respective ssDNA assemble to form the complex, resulting in FRET-based quenching of UCNPs fluorescence (Figure 9E). The LOD for cocaine detection in human saliva was found to be 50 nM. The assay was monitored using smartphone with simple data analysis, further contributing to the affordability and portability of the system important to develop POC diagnostics.

5 Conclusions and perspectives

By taking advantages of abundant energy levels of lanthanide ions, UCNPs exhibit superior optical properties. Several new strategies were developed to synthesize UCNPs with specific requirements, including heavy doping, orthogonal emission, organic dyes modified UCNPs, and so on. After surface modifications, a myriad of upconversion nanosensors were designed for environmental analysis, food assays, cell-based analysis, and medical diagnostics. Although great progresses have been made, there remains a huge room for the improvement before they can be translated from the bench studies to real world applications.

Several challenges exist that must be addressed before UCNPs’ practical applications. First, the low luminescence efficiency of UCNPs always limits their final applications, such as ultra-trace analysis and high-resolution imaging. Coating of an inert shell or optimizing the multi-shell structured UCNPs to manipulate the local environment of doping activators is a promising strategy to enhance luminescent intensities. Because of the great absorption effect
of aqueous solutions under 980 nm laser light, another alternative strategy to enhance the luminescence intensities of UCNPs-based nanosensors is shifting the excitation wavelengths to 808 nm or 1532 nm or developing paper-based upconversion detection. Second, in the UCNPs-based detection systems, the energy transfer efficiency is very low. Except the excellent overlap between emission spectra of UCNPs and absorption spectra of energy acceptors, the distance between luminescent activators and energy acceptors is also crucial for improving energy transfer efficiency. In a typical core structured UCNPs, luminescent activators are homogeneously doped in whole nanoparticles so that the distance between luminescent centers and energy acceptors is much more than the effective radius for energy transfer. Heavy doping of activators or doping of the activators in the outside shell of UCNPs may be an effective strategy to enhance the luminescent energy transfer efficiency. Last but not the least, surface modification, the dispersibility, chemical stability, and biocompatibility also are important issues that need to be well addressed in the design of UCNPs-based nanosensors. Taken all together, the upconversion nanosensors are developing at a very fast pace. There are still plenty of scopes for further innovative studies, such as using orthogonal emissive UCNPs for detection, exploring novel analysis formats, and so on, which requires multidisciplinary collaborative research and with more focus it is expected to realize the practical applications.

Author information

Corresponding authors

lilyjzhang@shu.edu.cn (Jing Zhang)
bmeqsmei@gmail.com (Qingsong Mei)
biezy@nus.edu.sg (Yong Zhang)

Notes
The authors declare no competing financial interest.

Biographies

Zhiming Zhang received his bachelor degree in Applied Chemistry from Anhui Polytechnic University (2016). He is now doing his master study at Shanghai University. His current research focuses on the synthesis of rare-earth doped upconversion nanomaterials.

Swati Shikha completed her bachelor degree in Biotechnology from Vellore Institute of Technology, India in 2011 and received her Master degree in Biotechnology from Indian Institute of Technology, Kharagpur, India in 2013. She received her Ph.D. degree at National University of Singapore (NUS) in 2018. Her research focused on the use of encoded beads for multiplexed bio-detection. Currently, she is working as a research fellow at NUS.

Jinliang Liu received his bachelor degree (2003) and master degree (2006) from Qufu Normal University, and Ph.D. degree (2009) from Tongji University. He worked as a postdoctoral researcher at Fudan University from 2009 to 2011, and then at National University of Singapore from 2011 to 2012. He is now working as an Associate Professor at Shanghai University since 2013. His current research interests involve organic probes and upconversion luminescent materials for sensing and bioimaging.

Jing Zhang received her Ph.D. degree from National University of Singapore (NUS) in 2009. After several years’ post-doctoral training at NUS, she joined Shanghai University as a Lecturer in 2016. Her research interests include nanomaterials, biomaterials, drug delivery, and pharmacology.
Qingsong Mei received his Ph.D. degree in 2012 from University of Science and Technology of China. After a short research period at the Institute of Intelligent Machines, Chinese Academy of Sciences, he moved to Hefei University of Technology as an associate professor in 2013. He is now conducting a postdoctoral research work with Prof. Yong Zhang at National University of Singapore (NUS). His research interests focus on the design and preparation of novel upconversion nanosensors for chemo/biosensing and imaging.

Yong Zhang is a Provost’s Chair Professor of the Department of Biomedical Engineering, National University of Singapore (NUS), and a senior member of NUS Graduate School for Integrative Sciences and Engineering (NGS). His current research interests include nanobiophotonics, nanomedicine, biomaterials and biomedical microdevices.

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