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ARTICLE

Lanthanide-doped upconversion nanoparticles as nanoprobes for bioimaging

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Upconversion nanoparticles (UCNPs) are a class of nanomaterials composed of lanthanide ions with great potential for paraclinical, especially the laboratory and imaging sciences. UCNPs have tunable optical properties and the ability to convert long-wavelength (low energy) excitation light into short-wavelength (high energy) emission in the UV-visible and near-infrared (NIR) spectral region. The core-shell structure of UCNPs can be customized by chemical synthesis to meet the needs of different applications. The surface of UCNPs can also be tailored with conjugation of small molecules and or targeting ligands to achieve high specificity and selectivity, which are indispensable elements in biomedical applications. Specifically, coatings can enhance the water dispersion, biocompatibility, and efficiency of UCNPs, thereby optimizing their desired function and boosting their performance. In this context, multimodal imaging can provide more accurate *in vivo* revealing information when combined with nuclear imaging. This article intends to provide a comprehensive review of the core structure, structure optimization, surface modification, and various recent applications of UCNPs in biomolecular detection, cell imaging, tumor diagnosis, and deep tissue imaging. We also present and discuss some of their critical challenges, limitations, and potential future directions.

1. Introduction

Rare earth (RE) elements are a group of 17 metal chemical elements that include Scandium (Sc), Yttrium (Y), and fifteen Lanthanides (Lns), which have 4f electronic configuration and usually undergo f-f electronic transition. Ln elements numbered 57 to 71 in the periodic table have similar chemical properties. Neodymium (Nd), holmium (Ho), erbium (Er), thulium (Tm), and ytterbium (Yb) are commonly used to synthesize Ln-doped upconversion nanoparticles (UCNPs), which can be converted to programmable UV-visible emission by a nonlinear process of near-infrared (NIR) excitation. During upconversion processes, photons emitted by the UCNPs have a higher energy than those absorbed. This energy difference is known as the anti-Stokes shift.¹

UCNPs are typically composed of three parts: a host matrix, activators, and sensitizers. The host matrix is the material that forms the bulk of the nanoparticle.² Examples are NaREF₄ (RE = rare earth), CaF₂, and Y₂O₃. The host matrix provides a lattice structure for the activator and sensitizer ions.³ Activators, such as Er³⁺, Yb³⁺, and Tm³⁺ are embedded in the host matrix absorbing low-energy photons, and emitting high-energy photons through upconversion. Sensitizers are often co-doped into UCNP to absorb excited radiation and transfer the energy to the activator. It is known that during the process of synthesis, the concentration of activator ions in host lattices must be carefully controlled to avoid cross-relaxation and maintain high upconversion efficiency. Increasing the sensitizer ion concentration in UCNP can improve the photon absorption capacity, thus enhancing the upconversion luminescence (UCL).⁴ However, beyond

a certain threshold (1 ~ 5 mol%), any further increase of sensitizer will result in a significant decrease in the luminescence intensity.⁵ This phenomenon is commonly known as "concentration quenching".⁶ In addition, increasing the concentration of lanthanide ion doping in UCNP particles can result in a more intricate energy transfer process within the particles, leading to a higher dissipation of energy towards the surface, this phenomenon is commonly referred to as surface quenching. The concentration quenching effect is also closely coupled to the surface quenching.⁵ Due to surface quenching and concentration quenching, the quantum yield (QY) of UCNP is low. Nevertheless, different core-shell structures are designed to improve both the UCL intensity and the QY of UCNPs. Inert shells, such as NaYF₄, NaGdF₄, or CaF₂, can passivate the surface defects and reduce surface quenching. On the other hand, active shells could be constructed to disperse higher sensitizer concentrations in different layers and reduce concentration quenching.^{7,8} Concurrently, constructing core-shell structures is also a way to enhance their biological applications. For instance, through typical Stober-based surface modification, the direct introduction of primary amine groups on the surface of UCNPs enables their modifications by water-soluble targeting ligands.⁹

The species and dopant concentration ratio of guest ions are also crucial in controlling the wavelength and intensity of the emitted light in UCNPs. Usually, Yb³⁺ is added as a sensitizer in these combinations to absorb the pump photon produced by a 980 nm laser and transfer it to the activators, such as Er³⁺, Eu³⁺, Tm³⁺, or Ho³⁺, which emit light with the stipulated desired wavelength (Fig. 1).¹⁰ Another commonly used sensitizer is Nd³⁺, with an efficient absorption band at ~808 nm. Compared with the Yb³⁺ ion sensitization system, Nd³⁺ uses an 808 nm laser as the excitation light source, which reduces the photothermal undesired effect of 980 nm laser, a practical consideration that is critical to improve the feasibility of biological applications.¹¹ In addition, dye-sensitized UCNP is an excellent strategy for improving its quantum yield and upconversion efficiency.

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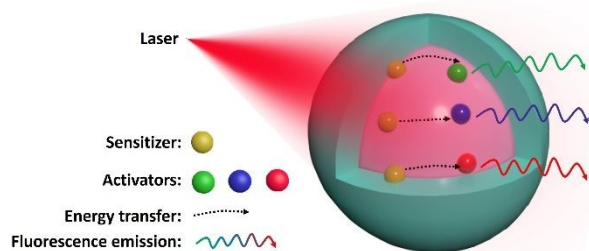


Fig. 1 Schematic of UCNP design shows the NIR light absorbed by the sensitizer and energy transferred to an activator, emitting luminescence with a specific wavelength.

This is because common industrial NIR-dyes have a high extinction coefficient. For example, the extinction coefficient of the near-infrared dye IR-806 is about $\sim 5 \times 10^6$ times than that of NaYF₄:Yb/Er. By using near-infrared dyes as "antennas", the near-infrared photons are cleverly absorbed and transferred to the excited state of lanthanide ions through a resonance energy transfer process, thus enhancing the emission intensity.¹²

Organic fluorescent probes have the advantages of a wide range of excitation and emission wavelengths, structural diversity, and strong flexibility, making them ideal fluorescent probes for biological imaging. However, traditional organic fluorescent probes are limited in their applications *in vivo* primarily because they typically need to be excited by relatively short wavelength (such as ultraviolet) to emit longer wavelength light (such as visible light). This optical property restricts the application of organic fluorescent probes in deep tissue imaging because the penetration depth of ultraviolet and visible light is relatively shallow. Moreover, long-term exposure to the short UV excitation wavelengths could cause irreversible cell damage.¹³ Due to their outstanding optical properties, quantum dots (QDs) have become a potential bioimaging technology. Including metal sulfide QDs and metal halide perovskite quantum dots. However, the inherent heavy metal content and toxicity of quantum dots limit their biological applications. Although inorganic shell or polymer coating can be introduced on the core material to protect it from slow degradation, the toxic metal ions released by these nanoprobe still seriously threaten the physiological environment due to photooxidation or poor stability.¹⁴

UCNP-based nanoprobe have shown a higher potential in bioimaging than traditional fluorescent materials due to their superior physicochemical properties, such as a large anti-Stokes shift, excellent photostability, and long excited state lifetime.¹⁵ Such nanoprobe can be functionalized to detect small organic molecule biomarkers, practically making them an ideal bio-sensors.¹⁶ UCNP are widely used in super-resolution microscopy as fluorescent probes, and their small size and high emission intensity enable them to observe subcellular structures and biomolecular dynamics.¹⁷ UCNP are also used for high-resolution imaging to visualize and track cellular structures and functions.¹⁸ For cancer diagnosis, UCNPs provides a powerful platform for tumor cell detection with the ability to regulate size through synthesis, reduce non-specific binding through surface modification. In addition, selective UCNPs surface modification with antibodies or ligands enables high-contrast imaging of tumor cells.^{15,19} Previous studies have shown that UCNPs can be used to detect blood-based biomarkers, offering potential applications for cancer diagnosis.²⁰ UCNP can also be developed for multiple applications, including diagnostics and therapy. In photodynamic therapy, UCNPs can act as a photosensitizer, absorbing external light excitation and transferring energy to produce reactive

oxygen species that could destroy tumor cells or other diseased tissues. They can also be used as drug carriers for bioimaging therapeutics or genes (such as doxorubicin, siRNA, DNA, and microRNA) or photosensitizers.²¹

The long wavelength excitation light of UCNP avoids tissue autofluorescence interference, allowing for up to a few centimeters deep tissue penetration, enable visualization of living organisms' structures and functions by imaging technique.^{17,22,23} Understandably, UCNPs probes for multimodal imaging can be constructed.²⁴ Yb³⁺ and Gd³⁺-doped UCNP can be used as contrast agents for computed tomography (CT) imaging due to their high atomic number and strong X-ray attenuation properties.²⁵ Alike, Gd-based UCNP are suitable for magnetic resonance imaging (MRI) applications. This is attributed to the unpaired 4f electrons in the Gd³⁺ ion. These unpaired electrons generate strong magnetic moments that can interact with nearby water protons, accelerating the relaxation of water protons and leading to contrast enhancement in T1-weighted MRI scans.²⁶ This approach offers high accuracy, high resolution, and low invasiveness, enabling comprehensive acquisition of imaging signals (both structural and functional) at the molecular levels.^{27,28} Due to their NIR excitation properties, UCNP could effectively and selectively be excited even in the deeper structures of the biological tissues, allowing for deep tissue imaging. This in turn will translate in clinically improving diagnostic accuracy, a critical step in early tumor detection.^{12,29} In neuro-imaging applications, peptides on the surface of UCNPs could be modified to enhance their blood-brain barrier (BBB) permeability, allowing them even to target specific neurons in the brain.³⁰ Customized UCNP were shown to act as optogenetic actuators of transcranial near-infrared light to stimulate deep brain neurons.³¹ UCNPs research in the field of bioimaging has been gradually translated into clinical applications. By using the real-time signal provided by UCNP, the upconversion emission with visible intensity is designed to realize the application in clinical operations such as guided surgical resection. This technology can help doctors pinpoint lesions and improve surgical accuracy, thereby improving patient outcomes and survival rates. As the research on UCNPs in bioimaging and clinical applications continues to deepen, it is believed that UCNPs will play a broader role in the medical field and bring more innovation and progress to clinical practice.³²

This paper reviews the application of UCNPs in bioimaging for disease diagnosis at the micro and macro levels. At the micro level, UCNPs can be used for biomolecular detection and cell imaging, helping researchers observe the biomolecular level and cell structure within cells, such as the detection and analysis of cancer cells. At the macro level, UCNP can also be employed for deep tissue imaging, penetrating deep into tissues to observe the structure and function of entire organs, aiding in the diagnosis of organ diseases such as tumors, vascular diseases, and central nervous system diseases, etc.

2. Upconversion mechanisms

In contrast to Stokes luminescence, where low energy (long wavelength) light is emitted upon absorption of high energy (short wavelength) light, UCNP convert low energy excitation to high energy emission. For instance, Ln-doped UCNP involve an Ln ion absorbing two or more photons for transition to an excited (E) state, which then relaxes to the ground (G) state and generates a photon with higher energy.³³ The basic upconversion emission mechanism can be divided into five categories: (a) Excited state absorption (ESA), (b) Energy transfer upconversion (ETU), (c) Photon avalanche (PA), (d) Cooperative sensitization upconversion (CSU), and (e) Energy migration-mediated upconversion (EMU) (**Fig. 2**).



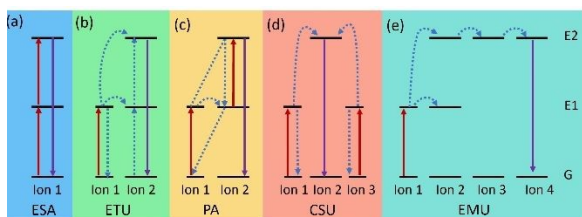


Fig. 2. Principal upconversion processes for lanthanide-doped UCNPs: (a) excited-state absorption (ESA), (b) energy transfer upconversion (ETU), (c) photon avalanche (PA), (d) cooperative sensitization upconversion (CSU), (e) energy migration-mediated upconversion (EMU). The red, broken blue, and violet lines represent photon excitation, energy migration, and emission processes respectively.

As the most basic mechanism in the upconversion emission, in ESA process, the Ln ion absorbs two photons in succession and gradually elevates to the E state ($G \rightarrow E1 \rightarrow E2$), followed by radiation relaxation back to the G state to achieve upconversion emission (Fig. 2a). Some Ln ions with ladder-level structures, such as Er^{3+} , Tm^{3+} , Ho^{3+} , and Nd^{3+} , exhibit efficient ESA, which can be excited by NIR lasers at 980 or 808 nm.³⁴ Unlike ESA, the energy transfer process of the ETU mechanism involves two adjacent Ln ions. In ETU, ion1 as a sensitizer is excited ($G \rightarrow E1$) by absorbing pump photons. The harvested energy is then transferred to its G state and activates the activator (ion2). Sustained energy transfer brings ion2 into its E2 state. Then the radiation returns to the G state, releasing higher energy photons (Fig. 2b). All UCNPs with different ion combinations, such as Yb^{3+}/Er^{3+} , Yb^{3+}/Tm^{3+} , and Yb^{3+}/Ho^{3+} , follow the ETU mechanism.³⁵ However, in the PA mechanism, an excited ion relaxes back to the G state by radiation, which excites a neighboring ion to the E2 state. These excited ions again relax back to the G state by radiation while will excite more ions. This process is repeated, causing more and more ions to participate in the upconversion emission, resulting in an exponential increase in the number of photons emitted (Fig. 2c). The PA-based upconversion process provides the highest upconversion efficiency. Tm^{3+} -doped UCNPs have been successfully used in super-resolution imaging.³⁶ While the CSU mechanism usually involves two ions (ion1 and ion3) of the same species absorbing pump photons, then cooperatively transferring harvested energy to ion2 and exciting ion2 to E2. The relaxation of ion2 from E2 to the G state implements the upconversion (Fig. 2d). And distinctively, the EMU process requires a core-shell structure with four types of lanthanide ions: sensitizer, accumulator, migrator, and activator (Fig. 2e). In this case, sequential energy transfers from the accumulator to the activator, producing UCL. Obviously, by adjusting the composition and concentration of dopant ions, the relative intensity of different emission peaks could be well-controlled and thus the emission wavelength of UCNPs will be manageable.

3. Bioimaging Applications

Ln-doped UCNPs show high potential in biological imaging due to their advantages, such as narrow emission bandwidth, deep tissue penetration, and biocompatibility.

The applications of UCNPs in bioimaging are mainly divided into four parts:

1) Biomolecular detection: by utilizing the unique luminescent mechanism and surface modification technology of UCNPs, that can detect biomolecules (such as proteins, DNA, H_2S , metal ions, etc.) with high sensitivity

2) Cell imaging: after UCNPs are modified to active uptake by cells,

non-invasive imaging of the structure and function in cells can be performed

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3) Cancer diagnosis and treatment: by exploiting the advantages of UCNPs in photonics and thermal applications, it can be used for early diagnosis, localization, and even in some cases, cancer treatment

4) Deep tissue imaging: by exploiting multimodal imaging techniques such as MRI and PET combination, UCNPs are modified and transplanted *in vivo*, enabling highly sensitive deep tissue imaging.^{37,38}

3.1 Biomolecular detection

Recently, many small molecular biomarkers have been identified as indicators of physiological and pathological states *in vitro* and *in vivo*. For instance, glucose levels are markers for diabetes, cardiovascular disease, and cerebrovascular disease;¹⁶ hydrogen sulfide (H_2S) is used to diagnose inflammatory diseases;³⁹ hydroxyl radical ($\cdot OH$) reflects the acid-base balance in the body and can be used to evaluate renal function and metabolism;⁴⁰ and hypochlorous acid (HOCl) is a reliable indicator of neuroinflammation.⁴¹ Monitoring small molecular biomarkers is essential for early diagnosis, understanding disease progression, and evaluating the efficacy of ongoing treatment. Thanks to their unique optical properties, UCNPs have emerged as promising tools for monitoring diseases with small molecules as markers. In addition, UCNPs can also be used to detect drug residues, providing detection tools for food safety and environmental protection.⁴²

Glucose is a vital biomolecule, used mainly for energy production and as an intermediary in many metabolism processes.^{43,44} Therefore, accurate measurement of glucose concentration is crucial for clinical diagnosis. Shuai Zha et al. studied an "off-on" UCNP-nanoprobe $NaGdF_4:Yb,Er@Ag$ anchored with glucose oxidase (GOx) for glucose detection. The fluorescence of the $NaGdF_4:Yb,Er$ core is suppressed by the encapsulation of a thin argentine (Ag) shell. When GOx catalyzes glucose, it produces hydrogen peroxide (H_2O_2), which causes the dense Ag shell to disintegrate and release the fluorescent signal. This phenomenon is known as "fluorescence switching." The nanoprobe exhibits high sensitivity to even deficient glucose concentrations *in vitro*, with detection as low as $1.77 \mu M$.¹⁶

Similarly, hydrogen sulfide (H_2S) is an important signaling molecule with various physiological and pathological effects in several diseases due to its antioxidant and anti-inflammatory effects. Accurate measurement of H_2S concentration can serve as a biomarker for evaluating human health status and predicting disease risk.^{45,46} Liu's group developed two types of dye-UCNP probes for H_2S detection based on the luminescence resonance energy transfer (LRET) phenomenon. In this process, energy is transferred from a photosensitive upconversion material (the donor) to another organic dye (the acceptor) through non-radiative dipole-dipole interactions, resulting in quenching of the UCL of UCNPs. Upon reacting with H_2S , the dye absorption decreases, inhibiting the LRET process, which leads to changes in the fluorescence wavelength or intensity that can be observed and measured. This approach can achieve high sensitivity and selectivity detection, making it a promising tool in biomedical research and clinical diagnostics. Practically, they developed a core-shell structured UCNP with $NaYbF_4:Gd@NaYF_4:Yb,Tm$ as the energy donor and covalently coupled fluorescein derivative (FI-1) on its surface as the energy acceptor for H_2S detection. H_2S can react with FI-1, transforming its five-membered ring into the six-membered ring (FI-2), inhibiting the LRET process and enhancing the UCL. This novel UCNP was successfully tested to determine the concentration of H_2S *in vitro* and *in vivo* (Zebrafish and mouse models). As a result, they can be



implemented in diagnosing arthritis and H₂S-associated tumors.³⁹

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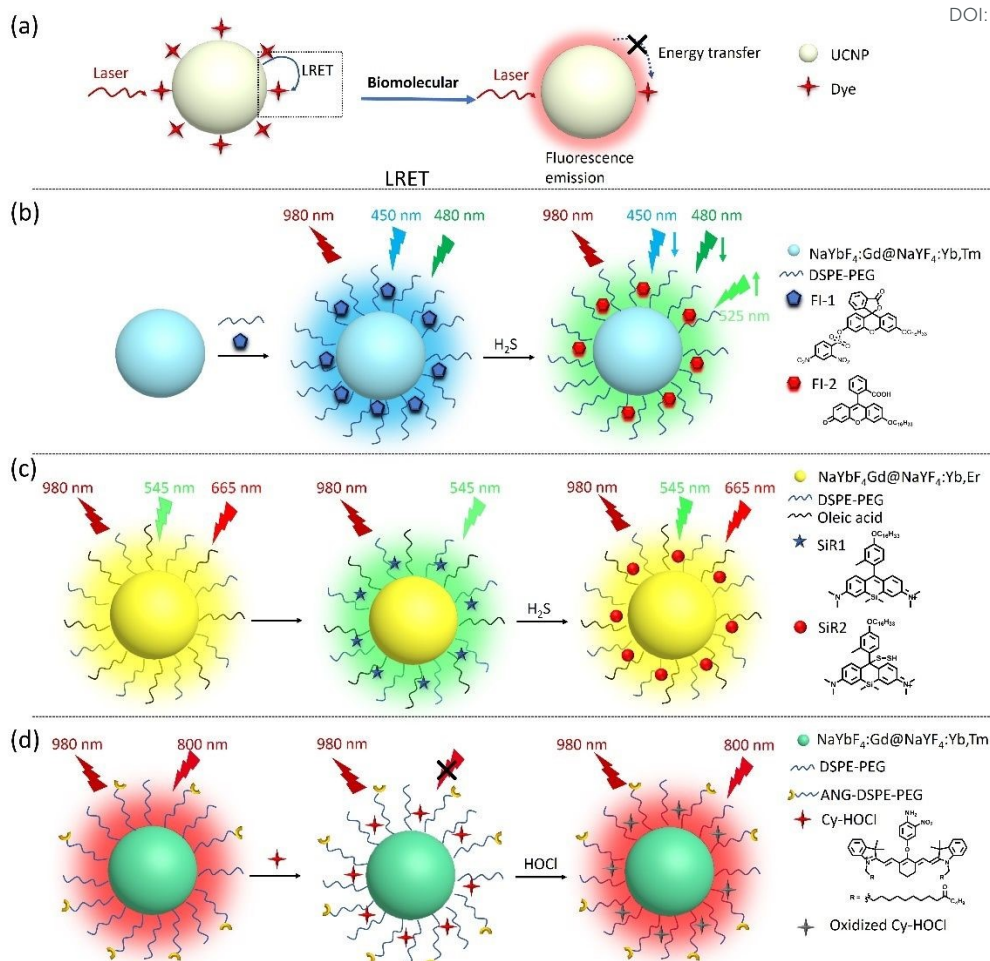


Fig. 3. (a) Schematic illustration of Dye-UCNP probes based on the luminescence resonance energy transfer; (b) the construction of FI-1-UCNP probe and its response mechanism to H₂S, the change in fluorescence signal is due to the transition of the organic dye FI-1 on the surface of UCNP to FI-2 after reacting with H₂S; (c) constructed SIR1-UCNP probe for the reversible detection of H₂S, the fluorescence signal of NaYbF₄Gd@NaYF₄:Yb,Er at 665nm was recovered after the reaction of SIR1 with H₂S; (d) illustration of the Cy-HOCl-UCNP probe for HOCl detection. ANG peptide on the surface of the UCNP determines their binding ability to brain endothelial cells, conferring their BBB permeability. Oxidation of Cy-HOCl destroys the spectral overlap between it and UCNP, restoring UCL intensity at 800 nm.

Table 1. UCNP-based nanoprobes for biomolecular detection

Nanoprobe	UCNP	Biomarker	Detection limit	Disease	Ref.
NaGdF ₄ :Yb ³⁺ ,Er ³⁺ @Ag-GOx	NaGdF ₄ :Yb,Er	Glucose	1.77 μM	High blood pressure, Diabetes	16
FI-1-UCNPs	NaYbF ₄ :Gd@NaYF ₄ :Yb,Tm	H ₂ S	0.741 μM	Arthritis, Tumor/HCT116 cell	39
CMS-UCNPs@azo	NaYF ₄ @NaYF ₄ :Yb,Tm @NaYF ₄ :Yb,Er@NaYF ₄	-OH	0.10 fM	Tumor/HeLa cells	40
CyH-UCNPs	NaYbF ₄ :Gd@NaYF ₄ :Yb/Tm	HOCl	0.9 μM	Cerebral stroke	41
NaYF ₄ :Yb,Er	NaYF ₄ :Yb,Er	Blood glucose	1.0 μM	Diabetes mellitus, Hypoglycemia	43
NaErF ₄ :Tm@NaGdF ₄ :Yb	NaErF ₄ :Tm@NaGdF ₄ :Yb	Glucose, GSH in serum	0.03 μM, 0.075 μM	Diabetes, Neurological diseases	44
SIR1-UCNPs	NaYbF ₄ :Gd@NaYF ₄ :Yb,Er	H ₂ S _n	0.486 μM	Liver injury	47



Similarly, Liu's group assembled the dyes (SiR1) and UCNPs (NaYbF₄:Gd@NaYF₄:Yb,Er) via hydrophobic-hydrophobic interactions to form SiR1-UCNPs nanoprobe for hydrogen polysulfide (H₂S_n) detection, which derived from H₂S. This nanoprobe can dynamically monitor the concentration changes of H₂S in living cells (*in vitro*) and mouse models (*in vivo*) with liver inflammation or damage.⁴⁷ These developments have potential applications in diagnosing and monitoring various diseases associated with H₂S, such as colorectal cancer, neurodegenerative diseases such as Alzheimer's Disease, and hypertension.⁴⁸

In addition, Liu's group developed an HOCl-activatable UCNPs-nanoprobe for real-time detection of inflammation in the nervous system. HOCl is a reactive oxygen species (ROS) produced by activated microglia, which plays a crucial role in neuro-inflammation. This unique UCNPs-based nanoprobe can provide specific and sensitive imaging of neuroinflammation by detecting HOCl. Upon reaction with HOCl, the emission intensity of the nanoprobe is significantly enhanced, allowing for susceptible and specific detection of neuro-inflammation. They are designed to be BBB-permeable. They are also sensible and applicable in diagnosis of lipopolysaccharide-induced neuro-inflammation. These nano probes can be used to monitor the neuro-inflammation post-cerebral stroke.⁴¹ Fig. 3 and Table 1 show the applications of UCNPs for biomolecular detection.

3.2 Cell imaging

Cell imaging is challenging when monitoring proteins or organelles inside cells is necessary to observe and analyze their structure, function, and dynamic processes without affecting cells' biological functions. Newly developed UCNPs technology provides researchers with invaluable insight into the complex mechanisms inside cells, enhancing their understanding of biological processes, and making it a promising basic research and clinical tool.⁴⁹ Therefore, it is crucial to evaluate their safety, biocompatibility, and toxicity comprehensively. For example, Bin Hu's group evaluated the cytotoxicity, uptake, and elimination process of UCNPs using HepG2 cells. They found that the cytotoxicity of UCNPs was by mitochondria-mediated generation of ROS and induction of apoptosis by oxidative stress, while being time- and dose-dependent.⁵⁰ Particularly, the cellular associations and heterogeneous interactions of UCNPs with the human immune cells are critical. Z. Gerelkhuu et al. conducted a study using high-throughput single-cell techniques to investigate the complex interaction of Yb³⁺/Er³⁺-doped NaYF₄ UCNPs with human peripheral blood mononuclear cells (hPBMCs). Their study explored the affinity of different immune cells to UCNPs, describing the heterogeneous interactions between traditional nanomaterials and the complex human immune system, facilitating the design and application of nanomaterials to ensure their safety in biomedical fields.⁵¹

By monitoring the direction of cell differentiation, early diagnosis of related diseases can be achieved (Fig. 4). For example, macrophages can be polarized into M1 macrophages with pro-inflammatory characteristics and M2 macrophages with anti-inflammatory characteristics. M1-type macrophages usually produce a series of cytokines and chemicals, such as ROS, which promote inflammatory responses, leading to tissue damage and plaque formation in atherosclerosis. M2 macrophages are in an anti-inflammatory and repair state. They suppress inflammation by secreting cytokines, such as interleukin-10 (IL-10) and promote tissue repair and healing. Feng Cao et al. developed a Gd-doped UCNPs

probe functionalized with MARCO-targeting peptide. Macrophage receptor with collagenous structure (MARCO) is a receptor expressed explicitly on the surface of pro-inflammatory macrophages (M1 macrophages), which plays a vital role in the occurrence and development of atherosclerotic plaques. Their novel UCNPs emit green light under 980 nm laser excitation, enabling high-resolution visualization of M1 macrophages, while MRI provides detailed anatomical information about the plaque and surrounding tissue. Studying M1-type macrophages polarization in atherosclerotic plaques contribute to a better understanding of plaque progression and aids in developing new diagnostic and therapeutic strategies for atherosclerosis.⁵²

Proteins are essential components of cells, and their unique characteristics determine biochemical reactions, tissue structure, and cell fate. Proteins are closely related to developmental regulation, homeostasis maintenance, and pathological progression. Albumin is a protein synthesized and secreted by hepatocytes in the liver. The differentiation status of liver organoids can be determined by monitoring albumin's expression and secretion levels. Guofeng Liu et al. developed a NIR-responsive nanoprobe called UCNPs-Ab-RBHA (UCAR) for quantitatively detecting albumin. This probe can distinguish the differentiation process of hepatobiliary duct-derived progenitor cells under different conditions, including their differentiation into organoids and hepatocytes. They presented dynamic changes in proteins and cell functions in complex biological environments, which could be used in studying hepatocyte cell development and the mechanisms involved in hepatocyte-related diseases.¹⁸

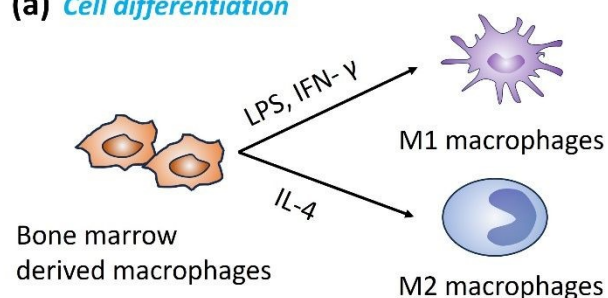
Neurological diseases are complex and involve abnormalities in nerve cell structure, function, neurotransmitters, or chemical process and transmission. These can impact bodily functions such as movement, sensation, cognition, emotions, and other behavioral and physiological processes.²⁹ UCNPs technology has been applied in neurotransmitter imaging,⁵³ neuroinflammation imaging,⁵⁴ and neural activity imaging.⁵⁵ By modifying the surface of UCNPs with targeting ligands, selective neurons can be tagged and tracked both *in vitro* and *in vivo*, allowing for a more comprehensive understanding of neurological diseases. Highly specialized UCNPs technology can also be employed to investigate the neuronal networks, their temporal and spatial activity characteristics, and the mechanisms of neuronal apoptosis. This is of utmost mechanisms of internalization of nanoparticles in the central nervous system (CNS) and their effects on tissue morphology is important for developing new cell therapies and designing controlled nanoparticles for brain therapy.^{31,56} Karolina Zajdel et al. evaluated the internalization mechanism, biodistribution, and neurotoxicity of UCNPs in rat hippocampal slices using an organotypic hippocampal slice culture (OHSCs) model. They found that UCNPs are readily internalized by hippocampal cells and co-localize with specific organelles within neurons and astrocytes, endosomes, and lysosomes. UCNPs enter neurons through clathrin- and caveolae-mediated endocytosis. In addition, UCNPs are present in cells in early and late endosomes, lysosomes, and large autolysosomes. Higher concentrations of UCNPs may induce the autophagy process but do not affect the cellular response. These findings have important implications for understanding the interaction of nanomaterials with tissues and for developing therapeutic strategies at micro (cellular) and macro (brain) levels.³⁰



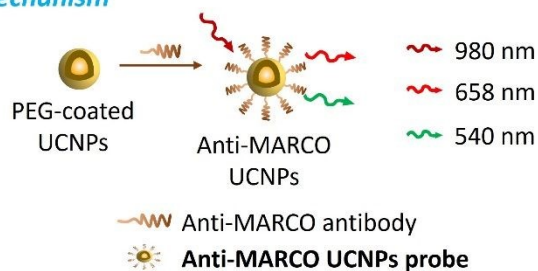
Motor-proteins are a class of proteins that use ATP energy to drive intracellular material transport by interacting with cytoskeletal proteins. Dynein-driven retrograde axonal transport moves intracellular cargos from axonal terminals to the soma. This process

is essential for many physiological functions of neurons, including nutrient supply, molecular signaling, and organelle migration. Retrograde transport disorders are closely related to the

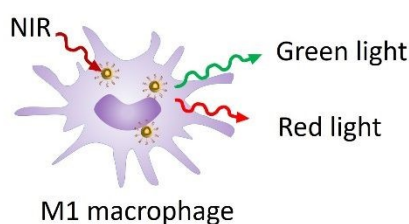
(a) Cell differentiation



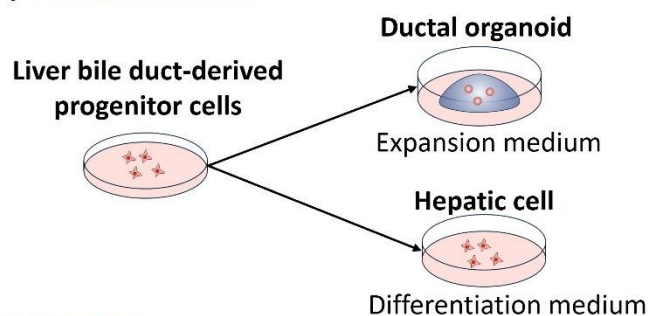
Mechanism



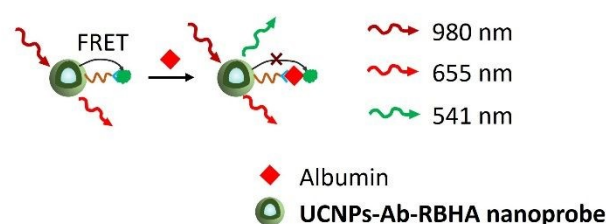
M1 macrophage cell imaging



(b) Cell differentiation



Mechanism



Differentiated hepatocytes and organoids imaging

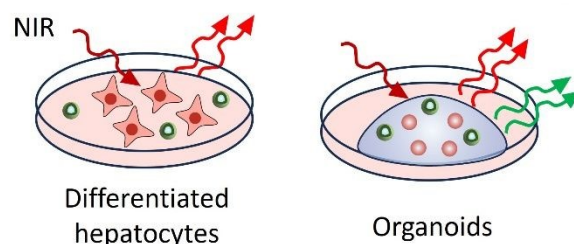


Fig. 4. Application of UCNPs in the early diagnosis of cell differentiation-related diseases. (a) Differentiation of bone marrow derived macrophages; Anti-MARCO antibody modified UCNPs were used to target M1 macrophages; application of anti-MARCO UCNPs probe in M1 macrophage imaging for the diagnosis of atherosclerotic disease. (b) Differentiation of liver bile duct-derived progenitor cells; the response mechanism among UCNPs-Ab-RBHA nanoprobe and organoids; UCNPs-Ab-RBHA nanoprobe recognition of differentiated hepatocytes and organoids imaging for hepatocyte-related diseases research.

Table 2. UCNP-based nanoprobe for cell imaging

Nanoprobe	UCNP	Modification	Cell line	Ref.
PAA-UCNPs	NaYF ₄ :Er, Yb	Poly(acrylic acid)	HepG2 cell	50
UCNP@POEGMEA-b-PMEAP	NaYF ₄ :Er, Yb	Oligo ethylene glycol methyl ether acrylate (OEGMEA), Block-bearing phosphate group (MAEP) diblock copolymer	hPBMCs	51
Anti-MARCO UCNPs	NaGdF ₄ :Yb, Er@NaGdF ₄	PEG, Polyclonal MARCO antibody	M1 macrophage	52
UCAR	NaYF ₄ :Yb, Er@NaYF ₄	PEI, antibody, Bovineserum albumin (BSA), Rose bengal hexanoic acid (RBHA)	Hepatocyte organoids	18
NaYF ₄ :Yb, Tm, Gd	NaYF ₄ :Yb, Tm, Gd	Dipicrylamine, Glutathione	HEK293 cells	55
NaYF ₄ :Yb, Er	NaYF ₄ :Yb, Er	SiO ₂	OHSCs	30



NaLuF₄:Yb,ErNaLuF₄:Yb,ErSiO₂Dorsal root ganglion
neurons
Article Online
DOI: 10.1039/D4BM00774C

development of neurodegenerative diseases. Xiao Zeng and colleagues used UCNP to label motor proteins, observe and track the movement of individual motor proteins in real time, and visualize axonal transport processes. The feasibility of UCNP probes as potential tools for early screening of neurodegenerative diseases was verified by constructing a cell model of Rett syndrome. This high-resolution imaging and tracking approach contribute to a better understanding of intracellular transport mechanisms. It could also provide new targets and strategies for treating neurodegenerative diseases.⁵⁷ The applications of UCNP based nanoprobe for cell imaging are summarized in **Table 2**.

3.3 Cancer diagnosis

With the continuous progress of nanotechnology, UCNP have emerged as a promising tool for tumor diagnosis due to their non-invasive, non-destructive, high sensitivity, super-resolution, and real-time imaging capabilities.²² Synthetic UCNP typically involve hydrophobic ligands, such as oleic acid (OA) or oleamide (OAm), which do not provide colloidal stability in aqueous media. In order to provide water-soluble UCNP, several approaches can be employed, but the single particle brightness may be affected by the technical performance. For example, ligand exchange to replace the original hydrophobic ligands (3% quenching modified with NOBF₄, 84% quenching treated by HCl), coated through inorganic silica shell deposition (21% quenching modified with SiO₂, 71% quenching modified with mSiO₂), and amphiphilic polymer self-assembly via hydrophobic-hydrophobic interaction (8% quenching coated with DSPE-PEG).⁵⁸ There are also other limitations, such as cytotoxicity, lack of selectivity for specific tumor cells, limitation of imaging depth, and poor biocompatibility, that need to be considered as well.^{59,60} Surface functionalization is necessary to overcome some of these challenges, and appropriate method selection will be more conducive to expanding its application.⁶¹ Commonly used UCNP surface modification methods include polymer coating, biomacromolecule modification, metal ion doping, nucleotide modification, cell membrane coating, etc.⁶²⁻⁶⁵ Surface modification of UCNP can be tailored according to specific application requirements to achieve targeting modification, biocompatibility improvement, drug packaging, treatment, and other functions.^{66,67}

Polyethylene glycol (PEG) is one of the most used polymers for surface modification of UCNP, which enhances the water dispersion, biocompatibility, and protein adsorption resistance of UCNP. Additionally, the terminal group of PEG is easily modified to facilitate the functionalization of UCNP.^{68,69} Studies have shown that the length and graft density of the polymer coating can affect the physical, chemical, and biological properties of UCNP. For instance, UCNP with the longest polymer coating exhibited the highest tumor cell uptake prospect and the longest cycle time at lower graft density. However, when the graft density is too high, the polymer begins to fall away from the UCNP surface, leading to decreased stability of UCNP and instability of drug release.⁶² PEG-modified UCNP have been used for targeted imaging of liver cancer cells by attaching glycyrrhetic acid (GA) to the PEG coating, which enhances the uptake of UCNP by liver cancer cells and allows for precise imaging under NIR light excitation.⁷⁰ Additionally, glycosylated pegylated phospholipid (DSPE-PEG-Glu) modified UCNP have been developed for detecting non-muscle invasive bladder cancer (NMIBC). The DSPE-PEG-Glu coating enhances UCNP targeting glucose transport

proteins (GLUTs), which are overexpressed in most cancer cells, thereby enhancing its aggregation in NMIBC.⁶³

Cyclodextrins (CD) are polysaccharides with amphiphilic structures that can also be used for surface modification of UCNP. CD modification is mainly used to enhance stability, increase the *in vivo* circulation time, improve water solubility and biocompatibility of UCNP, and reduce toxic side effects.⁷¹ β -galactosidase is a glycoside hydrolase, and its abnormal accumulation is closely related to the cellular aging process and various malignancies, such as primary ovarian cancer. Detao Jiang et al. developed a ratiometric nanoprobe based on LRET for β -galactosidase (β -gal) detection. α -CD on the surface of UCNP was assembled with β -galactosidase modified pyranitronitrile dye (DCM- β -gal) through hydrophobic interaction to form DCM- β -Gal-UCNP nanoprobes. After the addition of β -gal, the C-O bond of DCM- β -gal was broken by the enzymatic reaction, releasing DCM- β -Gal-OH with a strong intramolecular charge transfer (ICT). The maximum absorption of DCM- β -gal-UCNP was redshifted from 440 nm to 543 nm, triggering the LRET process, and the near infrared UCL emission centered at 800 nm had no significant change. The UCL intensity ratio (UCL543 nm/UCL 800 nm) was used as the output signal to realize the highly sensitive detection of β -gal activity (low limit detection of 3.1×10^{-4} U/mL). In addition, DCM- β -gal-UCNP can be effectively used to monitor β -gal in living cells and zebrafish tumor models.¹⁹

Researchers often use metal doping to enhance the luminescence efficiency of UCNP and create nanoprobes for multimodal imaging.^{26,72} Gold (Au) nanoparticles deposited on UCNP (Au-UCNP) have been widely used in CT, MRI Fluorescence imaging (FL), and photoacoustic imaging (PAI) of tumors due to their excellent imaging ability and photothermal effect.⁷³ Au-UCNP specifically targets tumor cells through surface modification of targeted ligands to achieve highly accurate imaging and photothermal therapy (PTT). In PTT, Au-UCNP absorbs light energy under near-infrared light irradiation and converts it to heat by photothermal conversion. This locally generated heat can lead to thermal damage and cell death of tumor cells without affecting the surrounding healthy tissues.⁷⁴ Zeng's group developed a core-shell UCNP nanoplatfor for NIR-II/MRI dual-modality imaging. The uniformly coated MnO₂ shell enhanced the T1-weighted MRI imaging and improved ROS release in the acidic tumor microenvironment.⁷⁵ Najim Akhtar et al. reported an upconversion nanoformulation UCNP@Tf-^{99m}Tc that enables dual-targeting (passive and active) and five-modality (infrared thermal imaging 'IRTI', UCL, MRI, SPECT, and CT) cancer imaging. The preparation is covalently conjugated to transferrin (Tf) to achieve active tumor targeting and stealth to the immune system. It also binds to Technetium-99 m (^{99m}Tc) to achieve stability and radiolabeling efficiency. This nano-formulation has good biocompatibility with adequate renal clearance, which can be considered an effective 4T1 tumor imaging agent.⁷⁶

Surface functionalization of UCNP using cell membranes is one of the research hotspots. This method directly binds cell membranes to the surface of UCNP, inheriting the unique surface physicochemical properties derived from cells. Compared with the traditional preparation methods, cell membranes can endow UCNP with a 'stealth function'. This function, which is a result of the cell membrane coating, effectively reduces the adsorption of non-specific proteins, prevents autoimmune attacks, and makes



nanomaterials safer and more biocompatible.⁶⁴ Mengting Li and colleagues designed UCNPs coated with red blood cell (RBC) membranes (RBC-UCNPs) by binding 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[folate(polyethyleneglycol)-2000] (DSPE-PEG-FA) to the cell membrane, enhancing the targeting ability of tumor cells. RBC-UCNPs have good biocompatibility *in vivo* with prolonged circulation time (~36 h), which can be used for multimodal imaging (FL/PET/CT/MR) for 4T1 breast cancer.⁶⁵

UCNPs have also been used for microRNA detection, as some miRNAs are overexpressed in various forms of tumors and have been

widely used as biomarkers for cancer detection and prognosis. Li's group reported functionalized UCNPs with complementary capture template-aDNA3 complexes (CPT-aDNA3) and nucleic acid amplicons (BHQ1-tagged 8-17 DNAzyme) for miRNA detection (Fig. 5). The UCNPs act as energy donors, BHQ1 as energy acceptor, the UCL is quenched due to LRET, and CPT-aDNA3 as a DNA trap. When targeting miR-21, it binds with CPT to release aDNA3, which can unlock 8-17 DNAzyme-BHQ1 to form aDNA3-DNAzyme-BHQ1 duplex. The green

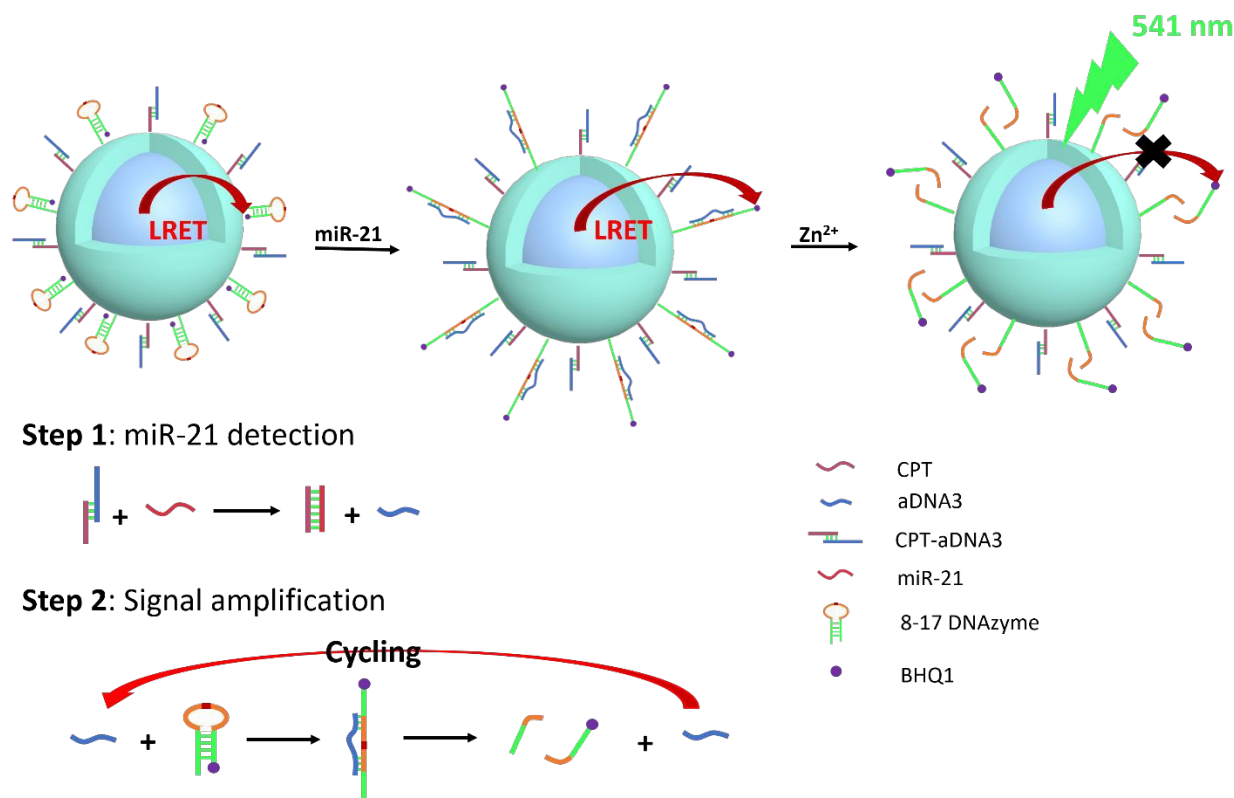


Fig. 5. Schematic representation of UCNPs@DNAzyme-BHQ1/CPT-aDNA for miR-21 detection. Ultra-low detection limit (31 fM) test performed by signal amplification.

Table 3. UCNPs-based nanoprobe for tumor diagnosis

UCNP	Modification	Targeting ligand	Imaging method	Tumor/tumor cells	Ref.
UCNP@P80-1	PPEGMEM	-	UCL	MCF-7 cells	62
GA-CSS UCNPs	PEG	GA	UCL/MR	HepG2 cells	70
UCNP-G100	DSPE-PEG-Glu	DSPE-PEG-Glu	UCL	Non-muscle invasive bladder cancer	63
DCM- β -gal-UCNPs	CD	DCM- β -gal	UCL	Ovarian cancer	19
Au-UCNPs-DSPE-PEG2K	PEG	-	PA/MRI/CT	HeLa cell	74
UCNP@Tf- ^{99m} Tc	Tf	Tf	IRTI/UCL/MRI/SPE CT//CT	4T1 cell	76



RBC-UCNPs	RBC membranes	DSPE-PEG-FA	FL/PET/CT/MR	4T1 breast cancer	View Article Online DOI: 10.1039/D4BM00774C
UCNP@DNAzyme-BHQ1/CPT-aDNA	-	CPT-aDNA3	UPL	HeLa cell	77

UCL signal of UCNPs can be recovered after the "-rA-" site of this duplex is cleaved by Zn²⁺ ions, releasing BHQ1 from the DNAzyme terminal from the surface of UCNPs. Free aDNA3 triggers the next cycle to further amplify the signal of miR-21. The optimized UCNPs nano-amplicon platform demonstrated the specificity and sensitivity of microRNA (detection limit as low as 31 fM) *in vitro* and *in vivo*. This nano-amplicon enables *in situ* imaging of miRNA expression in living animals using fluorescence imaging technology and enables the detection of changes in miRNA expression levels after drug treatment.⁷⁷ The applications of UCNPs for tumor diagnosis are summarized in Table 3.

3.4 Deep tissue imaging

Several challenges must be addressed before UCNPs can be applied for *in vivo* imaging. First, the solubility and permeability of smaller-size UCNPs are higher than those of larger sizes, and this is one of the causes that render small-size nanoparticles more cytotoxic within biological environments. The size and surface chemistry of the nanoprobe needs to be optimized to achieve the lowest toxicity and highest biocompatibility.⁷⁸ Second, reducing the non-specific binding of UCNPs to non-target tissues is necessary to achieve a relatively high signal-to-noise ratio. By ligand modification on its surface, UCNPs

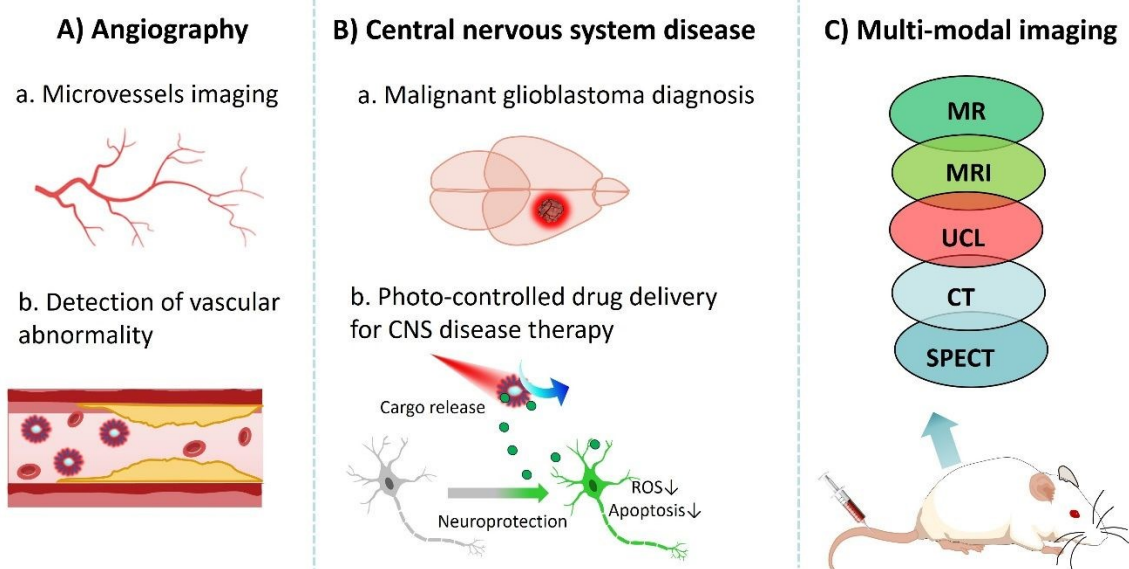


Fig. 6. Schematic representation of UCNPs for *in vivo* imaging. A) Angiography: a) Neovascularization imaging, b) Vascular inflammation diagnosis; B) Central nervous system (CNS) diseases: a) Malignant glioblastoma diagnosis, b) Photo-controlled drug delivery for CNS disease therapy; C) *In vivo* multimodal imaging.

Table 4. UCNPs-based nanoprobe for *in vivo* imaging

Nanoprobe	UCNP	Modification	Imaging method	Disease	Ref.
NaYF ₄ :Er, Yb	NaYF ₄ :Er, Yb	Janus-dendrimers	UCL	Brain vasculature	83
UCNP-CA	NaYF ₄ :Yb,Tm@NaYF ₄	Colominic acid	UCL	Inflammation	84
PDA@UCNPs	NaGdF ₄ :Er, Yb	PEG	UCL	Mouse melanoma	85
Biotin/PEG-UCNPs	KMnF ₃ :Yb,Er	Biotin/PEG	MR/UCL	Glioblastomas	86
Lf-UZSP	NaYF ₄ :Yb,Tm@NaYF ₄	Polyvinylpyrrolidone, zeolitic imidazolate framework-8	UCL	Parkinson's disease	54



UCNP-PS	NaGdF ₄ :Yb,Er	transferrin protein	MRI/UCL	4T1 breast cancer	View Article Online 1039/D000774C 87
ZnAl ₂ O ₄ : ^{171/169} Er	ZnAl ₂ O ₄ : ^{171/169} Er	-	SPECT/UCL	Mouse melanoma	88
Gd ₂ O ₂ S:Yb,Tm	Gd ₂ O ₂ S:Yb,Tm	-	CT/MR/UCL	-	89

can contribute to site-specific aggregation, thereby reducing background signal interference.⁷⁹ Third, to overcome the limitations of fluorescence imaging in terms of tissue penetration, UCNP shall be designed as a near-infrared (NIR) fluorescent probe. NIR light has a longer wavelength with lower absorption and scattering in biological tissues and hence, it penetrates deeper and less perturbed in the biological transparent window (650 - 1350 nm).⁸⁰ Thus, non-invasive *in vivo* imaging can be achieved to improve the depth and resolution of imaging.¹¹

In the following section, we discuss the application of UCNP in vascular imaging, CNS disease diagnosis and treatment, and multimodal imaging (Fig. 6).

UCNPs with high emission light intensity and low background signal can provide high-contrast images of blood vessels and detect the presence and formation of micro-vessels and abnormal vasculatures. Angiogenesis could be associated with some diseases, like vascular inflammation. Under the stimulation of inflammatory response, vascular endothelial cells release proangiogenic factors, such as vascular endothelial growth factor (VEGF) and basic fiber growth factor (bFGF), which promote the proliferation and migration of endothelial cells, thereby promoting the formation of new blood vessels.⁸¹ Tumor cells also release proangiogenic factors that stimulate the formation of new vascular networks in their surrounding tissues. Such formation of neovascularization in relation to the presence of tumors provides sufficient oxygen and nutrients for tumor cells. It also plays an important role in tumor growth and metastasis. Therefore, targeting neovascularization has become increasingly one of the critical characteristics for diagnosis and treatment monitoring.⁸² As a consequence, the surface functionalization of UCNP is required to ensure chemical and colloidal stability of UCNP in a buffer system, prolonged circulation time, deep tissue penetration, low nonspecific adsorption by/from/to blood proteins, with great sensitivity and selectivity. Shane Plunkett and co-workers used a Janus-type dendrimer as a surface ligand with multiple carboxyl groups attached to UCNP on one "surface" and polyethylene glycol (PEG) on the other "surface" to confer water solubility to UCNP. UCNP/Janus-dendritic molecules have shown excellent function as vascular markers. They were successfully used to map the depth resolution of a single capillary in the mouse brain at the remarkable depth of 1000 μm under continuous wave excitation with a power of no more than 20 mW. Compared to the conventional two-photon microscopy using red fluorescent dyes such as Alexa 680, the required time-averaged power is significantly reduced, typically around 100 mW. This optimization allowed high-quality image acquisition at a lower power level while reducing the potential risk of damage to the sample. Extraordinary high-resolution images were obtained even at high scanning speeds.⁸³ Polina A. Demina et al. used Carboxylic Acid (CA) to coat UCNP, thus obtaining nanoparticles with "invisible" properties. CA is a highly hydrophilic homopolymer composed of sialic acid residues, with no known toxicity and immune reactions, which also has the property of low nonspecific blood protein

adsorption. This allows the CA-coated UCNP to have a long circulation time *in vivo*. They can also be safely accumulated at the microvascular remodeling in the inflammatory site. By using lauro macroic acid modified UCNP, inflammation imaging in systemic mode can be achieved, and local visualization of blood vessels *in vivo* can be used to diagnose and monitor inflammatory disease treatment.⁸⁴

Upconversion nanomaterials can be implanted in deeper tissue, which can play an essential role in diagnosing and treating central nervous system diseases.⁸⁵ Malignant glioblastoma is one of the most common primary brain tumors. Its occult nature and location (confined within the skull) make early diagnosis difficult. Due to its highly aggressive nature and heterogeneity, malignant glioblastomas can deeply invade the adjacent brain parenchyma, resulting further difficulties in distinguishing the boundary of tumor from healthy tissue. Furthermore, even with surgical resection, recurrence is inevitable due to residual glioblastoma cells within the neighboring brain parenchyma. The study by Miaomiao Cheng et al. used a pegylated functionalized UCNP (KMnF₃:Yb,Er) nanoprobe coupled with biotin to achieve precise imaging of glioblastoma by targeting sodium-dependent multivitamin transporter (SMVT) overexpressed on the surface of cancer cells. The KMnF₃ master lattice endows the nanoprobe with MR imaging capabilities. Biotin /PEG-UCNPs have good biocompatibility and low *in vivo* toxicity, which is expected to be an MR/UCL imaging agent for the invasion area of glioblastoma to guide potentially a much better, near complete surgical resection. This translational research trend has the potential to become the clinically acceptable norm to improve the accuracy of early diagnosis and surgical resection of glioblastoma.⁸⁶

Parkinson's disease (PD) is a neurodegenerative disease that leads to disability in the elderly, though its etiology and pathogenesis are not fully understood. It is generally believed that oxidative stress and neuroinflammation play important roles in the development of PD. Non-invasive delivery of drugs for the treatment of PD needs to cross the blood-brain barrier (BBB), reduce oxidative stress, and inhibit neuroinflammation and apoptosis. Photo-controlled drug delivery uses photosensitive molecules or nanomaterials to trigger the release or activation of drugs when they are irradiated by light of a specific wavelength or intensity. Therefore, they could realize the spatiotemporal regulation of drug release and improve the accuracy and effect of treatment. The application of the UCNP system in photo-controlled drug delivery for PD treatment has shown excellent properties due to the strong tissue penetration of excited NIR light, controllable emission light, small size, and desired surface modification. Binbin Hu et al. constructed a nanosystem using UCNPs as carriers for photo-controlled delivery of S-nitroso-N-acetyl-DL-penicillamine (SNAP) and paeoniflorin (Pae). By modifying lactoferrin (Lf) on the surface of UCNPs, which could combine with the highly expressed lactoferrin receptor on the surface of cerebrovascular endothelial cells, UCNPs can cross the BBB and be accumulated in the microenvironment of PD brain tissue. Under NIR light irradiation, UCNPs can convert NIR light to UV light, stimulating the release of



NO from SNAP and promoting the release of Pae from the nanocarrier. Released NO and Pae can jointly remove ROS from the microenvironment, inhibit neuroinflammation, reduce the loss of dopaminergic (DA) neurons in the substantia nigra and striatum, and improve motor behavioral related symptoms in patients suffering from PD.⁵⁴

Combining UCL and nuclear imaging to construct multimodal imaging is one of the innovative ways to address poor tissue penetration of fluorescence imaging.^{87,88} Mauricot's group reported a new type of UCNPs, $Gd_2O_2S:Yb^{3+}/Tm^{3+}$, which exhibits strong fluorescence emission at 802 nm when excited by 980 nm laser. This enables them to reach deeper structures of the CNS and reduce or eliminate the effect of autofluorescence. Consequently, a higher signal-to-noise ratio and deeper tissue penetration are possible. In addition, a large amount of notably paramagnetic Gd in these UCNPs also brings interesting contrast properties in MRI and CT, which can be exploited in multimodal *in vivo* imaging. With a customized imaging system, the fluorescence signals of UCNPs can be detected in different tissues such as skin, muscle, kidney, liver, and brain, and at different depths for a relatively longer time after *in situ* injection. Results show that the nanoparticles are suitable for deep tissue imaging, and the data with high resolution and high sensitivity information can be obtained by multimodal imaging.⁸⁹ Examples of UCNP for deep *in vivo* imaging are summarized in **Table 4**.

UCNP's programmable light emission at a specific wavelength has been considered a powerful tool to facilitate optogenetics applications, enabling precise temporal and spatial manipulation of the neuronal activity.^{57,90,91} In many recent studies, UCNPs implanted near opsin-expressing neurons were used to convert NIR illumination into a spectrum of visible emissions, effectively regulating ChR light-gated ion channels. Today, implementing various forms of UCNPs in biological systems is becoming a valuable research tool for scientists and researchers studying neurodegenerative diseases.^{31,56,92,93}

4. Discussion

UCNPs have emerged as versatile tools with immense potential for revolutionizing biomedical imaging applications. Their ability to convert near-infrared light into visible and ultraviolet emissions makes them highly attractive for various biological imaging techniques, including *in vitro* and *in vivo* molecular sensing, cellular tracking, tumor diagnosis, and tissue imaging.

Looking forward, significant advances are anticipated in UCNP research. Responsively, the surface of UCNP can be modified with biological macromolecules, fluorescent markers, and targeted molecules to achieve binding to the targeted molecules and improve the sensitivities, specificities, and resolution of imaging. Highly sensitive off-on nanoprobe were synthesized using the LRET mechanism by introducing organic dyes as energy acceptors. Surface modification could also increase the intracellular stability and uptake performance of UCNP, thereby improving its application potential in cellular imaging. In tumor diagnosis, UCNP can achieve high specific recognition in tumor cell imaging by modifying their targeted ligands. This application can improve the sensitivity and resolution of tumor imaging and is expected to become a valuable tool for tumor diagnosis and treatment in the near future. In addition, UCNP can also be used for neuronal imaging and diagnosis in the CNS. Since the BBB crossing of most drugs is problematic, there are still significant challenges to developing and implementing safe and effective medical treatments for the CNS diseases. Introducing the concept of various forms and constructs of the UCNPs could overcome some of these obstacles. UCNP can be integrated into multimodal imaging to

obtain precise imaging signals and enhance the accuracy of the diagnostics in the CNS pathologies and even treatments *via* neuromodulation.

Although utilizing UCNPs in biological systems brings many advantages for diagnosis, imaging, and treatments, other restrictive and critical disadvantages must also be considered and overcome. These limitations include potential low luminescence efficiency due to radiation loss during energy transfer, high energy laser, which may cause photodamage or photothermal effect on biological tissues; further biocompatibility, cytotoxicity, and stability that still need to be investigated and improved; the time and complexity of their preparation, limitations of their mass production, cost, and large-scale application. Each one of these limitations and challenges would be an opportunity to improve and optimize the application of UCNPs in the biological system. The synthesis of UCNP with high quantum yields faces essential challenges, including maintaining high quantum yields in physiological environments, extreme pH, abnormal salinity, and abnormal oxidation or reduction conditions. In an aqueous solution, the oxygen and hydrogen ions in water molecules may transfer non-radiative energy with the excited state levels in lanthanide ions, resulting in attenuation or quenching of UCNP fluorescence. This phenomenon limits the quantum yield of UCNP in aqueous solutions, thus limiting its potential for clinical applications. In addition, the upconversion efficiency of UCNP is also affected by many factors, such as lanthanide selection, doping ratio, UCNP size, surface modification, and so on. A passivation shell is a crucial choice to enhance the fluorescence intensity of UCNP, but inappropriate surface passivation can lead to a reduction or even loss of luminous efficiency. Moreover, LRET-based small molecule testing requires controlling the passivation shell's thickness, as the detection accuracy depends on the distance between the small molecule and the lanthanide ions in the UCNP. A comprehensive evaluation of the biocompatibility of UCNP nanoprobe is essential, especially for their use in clinical applications. While numerous studies have demonstrated the safety of UCNPs for bioimaging, the long-term biosafety remains to be determined. For instance, in studies on UCNPs for diagnosing central nervous system diseases, UCNPs are injected intravenously and travel through the blood circulation to the brain vessels, then cross the blood-brain barrier into the brain. However, little has been reported about how the brain clears it after the work is done and whether it causes latent harm. This underscores the need for further research to ensure the safe use of UCNPs in clinical applications.

Despite these challenges, potential breakthroughs are on the horizon. Future research efforts may focus on integrating UCNPs with advanced imaging technologies such as photoacoustic imaging and magnetic resonance imaging (MRI), thereby expanding their utility in multimodal imaging approaches. Moreover, the development of UCNP-based theranostic platforms holds promise for simultaneous imaging and targeted therapy, paving the way for personalized medicine applications. In addition, the applications of UCNPs are poised to extend beyond traditional imaging. They are expected to play pivotal roles in precise surgical navigation, real-time monitoring of therapeutic responses, and even in the emerging field of optogenetics, where light-sensitive UCNPs can enable controlled cellular activities.

Author Contributions

Writing-original draft preparation, Hengde Li and Haitao Liu; Review & editing, Ka-Leung Wong and Angelo H. All; Supervision, Angelo H.



All; Funding support, Angelo H. All. All authors have read and given approval to the final version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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- No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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