NIR-II Fluorescence imaging for cerebrovascular diseases

Feng Ren | Zhilin Jiang | Mengxiao Han | Hao Zhang | Baofeng Yun | Hongqin Zhu | Zhen Li

Abstract
As the major pipeline for transporting oxygen and nutrients to living tissues, well-ordered and functional vasculature is vital to maintain the function of organisms. The cerebrovascular disorders induced by acute or chronic diseases, including traumatic brain injury (TBI), stroke, and brain tumors, could cause vascular cognitive impairment and even mortality. Due to the complication of these cerebrovascular diseases, early diagnosis and monitoring their pathological processes in real time in animal models could enlighten us with insights into early prevention and effective treatment. Over the last decade, the NIR-II fluorescence imaging technology has been well developed in both nanofluorophores and imaging systems. For cerebrovascular disorders, the collaborative use of wide-field imaging setup with NIR-II nanoprobes enable arteriovenous staging, and calculation of blood flow velocity to distinguish ischemic area in TBI and stroke. The changes in molecular and cellular levels of TBI provide the guidance on the design of targeted and activatable NIR-II nanoprobes for evaluating and monitoring the microenvironment variations. For the brain tumors, both targeted strategy and focus ultrasound sonication are efficient approaches for overcoming the blood-brain-barrier and brain-tumor-barrier for delivery of nanoprobes. Therefore, NIR-II fluorescence imaging-guided surgical navigation of brain tumors and resected lesions biopsy intraoperatively ensure the accuracy of surgery based on the precise definition of tumor margins. This review summarizes the recent advances in NIR-II fluorescence imaging technology, which is mainly inclusive of well-developed NIR-II nanofluorophores and their applications in cerebrovascular diseases with address of its challenges and great potential in these aspects.

Key words
brain diseases, cerebrovasculature, nanoprobes, NIR-II fluorescence imaging
INTRODUCTION

A well-organized and functional vasculature is indispensable to the maintenance of good health and growth of living organisms by transporting blood and nutrients to tissues and organs of the whole body. The structural and functional abnormalities of blood vessels induced by acute or chronic progressive diseases, especially in the brain, which are inclusive of traumatic brain injury (TBI), hemorrhagic and ischemic stroke, brain tumors, vascular dementia or vascular cognitive impairment, are notably high incidence and mortality. These cerebrovascular diseases involve complex processes with the inclusion of inflammation, oxidative stress, vasculopathy, central nervous system (CNS) damage. Early diagnosis of cerebrovascular diseases with precision and monitoring their pathophysiological processes in real-time can give insights into early prevention and effective treatment.

Intravital fluorescence imaging technology featured with dynamic and high spatiotemporal resolution provides a feasible method to obtain pathophysiological information on molecular, cellular, and tissue levels. Since the discovery of single-walled carbon nanotubes (SWNTs) with the fluorescence in the second near-infrared window (NIR-II window, 1000-1700 nm) in 2009, which presented excellent performance in in vivo NIR-II fluorescence imaging, probes with the similar fluorescent properties have been extensively studied and explored. In comparison with the imaging wavelength in the visible (400–650 nm) and first near-infrared regions (650–950 nm), the overall effects of reduced scattering, minimum absorption, and negligible autofluorescence in the NIR-II window endow unprecedented penetration depth and spatiotemporal resolution, despite of strong photon absorption of water near 1450 nm (Figure 1A-E).

Currently, a series of newly developed NIR-II nanoprobes (Figure 2, SWNTs, black phosphorous nanoparticles (BP NPs), gold nanoclusters (Au NCs), rare-earth nanoparticles (RE NPs), quantum dots (QDs), semiconducting polymer dots (Pdots), aggregation-induced emission luminogens (AIEgens), small molecule dyes (SMDs)-based aggregates (J-aggregates) have been reported for anatomical and functional bioimaging to reveal biological processes in living organisms. Meanwhile, the evolving NIR-II fluorophores also promote the development of NIR-II fluorescence imaging systems.
in view of different applications including broadband multiplexed in vivo imaging system and microscopy, intraoperative navigation system, time-resolved in vivo imaging system, confocal microscopy, and light-sheet microscopy.10–13

In this review, we focus on the latest progress of the well-designed inorganic- and organic-based NIR-II nanoprobes, and their applications in cerebrovascular angiography and cerebral diseases, including TBI, ischemic stroke, and brain tumors (Scheme 1), by using various imaging setups of macroscopy, microscopy, and mesoscopy.

2 | NIR-II NANOFUOROPHORES FOR CEREBROVASCULAR ANGIOGRAPHY

To noninvasively acquire cerebrovascular images with high signal-to-background ratio (SBR) and spatiotemporal resolution by in vivo NIR-II fluorescence imaging, nanoprobes should have narrow band emissions in the NIR-II biological window with the least optical interference induced by skin and cranial bone. It has been identified that the emissions of nanoprobes located in the NIR-IIa (1300-1400 nm) and NIR-IIb (1500-1700 nm) sub-windows are ideal wavelengths for imaging.6,14 Accordingly, we next depict inorganic- and organic-based NIR-II nanoprobes, respectively, with the focus on their emissions in these two subwindows and applications in the cerebrovascular imaging.

**SCHEME 1** Illustrative representation of the NIR-II nanofluorophores and their applications in cerebral diseases

**2.1 | Inorganic-based NIR-II nanoprobes**

2.1.1 | SWNTs

Inorganic NIR-II nanoprobes as the promising candidates for NIR-II fluorescence imaging typically possess the merits of controllable size and morphology, modifiable surface,
and tunable optical properties. SWNTs (Figure 3A), a type of one-dimensional (1D) carbon nanomaterials, were the first NIR-II nanoprobe reported for in vivo fluorescence imaging, which showed an emission in 1000-1300 nm and a low quantum yield (QY, 0.4%).8,15 Thereafter, the improvement of QYs and extension of their emissions to the NIR-IIb region by increasing the diameter of SWNTs make them promising for deep-tissue and high-resolution in vivo optical imaging.16

2.1.2 | BP NPs

Nanoscale black phosphorous (BP) with anisotropic structure presents an intrinsic layer-dependent bandgap tuning from 0.3 eV (bulk) to 2.0 eV (single layer), which enables its high excitation coefficient and rich optical properties for photothermal and photoluminescent applications.17 Similar to the above SWNTs, two-dimensional (2D) BP NPs showed a broadband emission across the NIR-IIa and NIR-IIb regions, as reported by Li’s group (Figure 3B).18 More importantly, water-soluble BP NPs can be easily prepared to show bright NIR-II fluorescence with a relatively high QY (8%). They can be modified with biocompatible and targeting molecules to exhibit excellent biocompatibility, biodegradability, and targeting capacity. In addition to NIR-II fluorescence imaging, they can be also used for photoacoustic imaging (PAI), photothermal therapy, and photodynamic therapy. These merits make BP NPs as a strong competitor for theranostics of cancers.18,19 Since the optical

FIGURE 3 (A) NIR fluorescence spectra of HiPCO and semiconducting LV SWNTs upon 808 nm excitation. (B) Emission spectrum of BP@lipid-PEG nanosphere aqueous solution under 808 nm excitation. Reprinted with permission from Ref. [18]. Copyright 2019, American Chemical Society. (C) Photon scattering effects in the NIR-II (1000-1700 nm), NIR-IIa (1300-1400 nm), and NIR-IIb (1500-1700 nm) regions under a piece of approximately 4 mm-thick chicken breast and the corresponding measurement of Gaussian-fitted full width at half maximum (FWHM) of the cross-sectional intensity profiles. (D) Fluorescence images of the cerebrovasculature of mice (n = 2) without craniotomy in the NIR-I, NIR-II, and NIR-IIb regions. (A), (C), and (D) Reprinted with permission from Ref. [16]. Copyright 2015, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (E) SWIR emissions tuned by selecting suitable combinations of Yb\(^{3+}\)-A\(^{3+}\) (A = Ho, Pr, Tm, Er) to enable emissions at 1185, 1310, 1475, and 1525 nm, respectively. (F) The optical efficiencies of the differently doped systems follow an order of Er > Ho > Tm > Pr, where the relative ratios are 688:12:5:1, respectively. (E) and (F) Reprinted with permission from Ref. [21]. Copyright 2013, Springer Nature. (G) Spectra of five different SWIR QDs with core–shell and core–shell–shell nanostructures: InAsCdSe\(_2\)(ZnSe)\(_3\) (QD1080); InAsCdSe\(_{3.5}\) (QD1120); InAsCdSe\(_{2}\) (QD1170); InAsCd\(_{0.9}\)Zn\(_{0.1}\)S (QD1280); InAsCdSe\(_3\) (QD1330). (H) QYs of core-shell (CS) and core-shell-shell (CSS) InAs QDs (up to 30%) dispersed in aqueous buffers. (G) and (H) Reprinted with permission from Ref. [39]. Copyright 2017, Springer Nature. (I) TEM image of CD-Au NCs. Insets: high-resolution TEM image and the size distribution diagram. (J) Absorption spectrum of CD-Au NCs in the visible, NIR-I region, and their NIR-II PL spectrum (excitation: 808 nm). (I) and (J) Reprinted with permission from Ref. [49]. Copyright 2020, Wiley-VCH GmbH
interference of narrowband emission (NIR-IIa and NIR-IIb) is always lower than broadband emission (NIR-II), the SBR of NIR-II fluorescence penetrating through 4 mm-thick chicken breast followed an order of NIR-IIb > NIR-IIa > NIR-II (Figure 3C). Further, in vivo through-skull cerebrovascular imaging also displayed the higher resolution obtained through the NIR-IIb channel than NIR-II channel (Figure 3D).

2.1.3 | RE NPs

Lanthanide-based nanoparticles are generally composed of host matrix, sensitizers, and activators. In the early researches, the well-developed RE nanoparticles from combination of Yb\(^{3+}\)-RE\(^{3+}\) or Nd\(^{3+}\)-Yb\(^{3+}\)-Er\(^{3+}\) can up-convert lower-energy photons into high-energy ones, that is, convert the near infrared light (~980 or ~808 nm) into the ultraviolet visible light.\(^{20}\) With the shift of focus to NIR-II fluorescence imaging, Naczynski et al. found that the typical upconversional Yb\(^{3+}\)-RE\(^{3+}\) (Er, Ho, Tm, Pr) combinations can also produce downconversion emissions across the NIR-II region by simply tuning the dopants (Figure 3E).\(^{21}\) They also found that the optical efficacy of this Yb\(^{3+}\)-RE\(^{3+}\) codoping system in the NIR-II region followed an order of Er\(^{3+}\) > Ho\(^{3+}\) > Tm\(^{3+}\) > Pr\(^{3+}\) with relative ratios of 688 : 12 : 5 : 1 (Figure 3F). Thereafter, Zhong et al. introduced Ce\(^{3+}\) ions into Yb\(^{3+}\)-Er\(^{3+}\) system to form NaYbF\(_4\):Er,Ce@NaYF\(_4\) nanoparticles and improve their NIR-II emission by approximately nine-fold under excitation of 980 nm laser through the nonradiative phonon-assisted cross relaxations between Ce\(^{3+}\)-Er\(^{3+}\) ions.\(^{22}\) Further doping optimized Zn\(^{2+}\) ions (10\%) into Yb\(^{3+}\)-Er\(^{3+}\)-Ce\(^{3+}\) system (NaYbF\(_4\):Er,Ce,Zn@NaYF\(_4\)) endowed the transformation of nanocrystals from hexagonal phase to cubic phase, which led to approximately 11-fold enhancements in the intensity of NIR-IIb emission (~1600 nm).\(^{23}\)

Although phonon management of Yb\(^{3+}\)-Er\(^{3+}\) system by tuning dopants and shell can boost the NIR-II emission intensity, single-state Yb\(^{3+}\) ions as sensitizers are limited to approximately 980 nm excitation. The perfect match of excitation light with the strong absorption of water could cause severe heating effect to damage biological tissues under continuous irradiation of approximately 980 nm laser, and to trigger up- and downconversion processes.\(^{24}\) In this context, approximately 800 nm laser is preferable and favorable for excitation of lanthanide-based nanoprobes to generate NIR-II fluorescence.

Since Nd\(^{3+}\) ions can simultaneously serve as sensitizers and activators, Ren et al. fabricated Nd\(^{3+}\)-doped core-shell nanostructures (NaAF\(_4\):5\%Nd@NaBF\(_4\), A and B = Y, Gd) to improve NIR-II emissions at 1060 and 1340 nm. In addition to NIR-II fluorescence, the resultant nanoparticles exhibited paramagnetism and X-ray attenuation capability for multimodal imaging of tumors.\(^{25}\)

It should be noted that the Nd\(^{3+}\)-enriched nanoparticles (NaNdF\(_4\)) possessed size-dependent photothermal conversion and luminescent properties under 808 or 793 nm irradiation, respectively, which indicates that NaNdF\(_4\) nanoparticles can be used for different purposes under the excitation of different lasers such as NIR-II imaging, photothermal therapy, and imaging-guided theranostics.\(^{26}\) However, the emission of Nd\(^{3+}\)-based nanoparticles is below 1500 nm. Wang et al. constructed a novel multishell lanthanide-doped nanocrystals (β-NaGdF\(_4\):NaGdF\(_4\):Yb,Er@NaYF\(_4\):Yb@NaNdF\(_4\):Yb), which can be excited in the biological transparent window by an 808 nm laser for the in vivo NIR-IIb fluorescence bioimaging.\(^{27}\)

A shortcoming of lanthanide-based nanoparticles is their weak fluorescence and low QY, particularly for NIR-II fluorescence, due to the narrow (~10 nm) and weak absorption (cross section of 10\(^{-20}\)/cm\(^2\), 1000-fold lower than organic dyes).\(^{28}\) To improve the fluorescence of lanthanide-based nanoparticles, Shao et al. fabricated an organic-inorganic system (Dye-Nd\(^{3+}\)-Yb\(^{3+}\)-X\(^{3+}\), X = null, Er, Ho, Tm, Pr) to enhance light-absorption at approximately 800 nm to improve the NIR-II emission intensity by approximately four folds.\(^{29}\) However, this multistep energy transfer process requires careful control of dopants, interlayer and outer shell to boost energy transfer and improve NIR-II fluorescence. Johnson et al. found that Er\(^{3+}\) ions could serve both as sensitizers and activators with multi-wavelength excitations (980, 800, 658 nm) and emissions (650, 1550 nm), which were achieved simply by constructing NaErF\(_4\):NaLuF\(_4\) core-shell nanostructures to alleviate the surface quenching effect.\(^{30}\) In view of energy transfer efficiency in excitation absorption, Ren et al. further developed Dye-Er\(^{3+}\)-Ce\(^{3+}\)-Yb\(^{3+}\) system with an efficient energy-cascaded process, which was based on emission sensitization by Dye, energy trapping by NaYbF\(_4\) active interlayer, and efficient energy cross-relaxations between Er\(^{3+}\) ions and Ce\(^{3+}\) ions.\(^{31}\) They designed a dye-brush polymer to precisely control the amount of dyes on nanoparticle’s surface for achieving the best sensitization effect, which was completely different from previously reported dye-wrapping method.

2.1.4 | QDs

QDs are a type of semiconductor nanocrystals with radii smaller than the excitation Bohr radius. Since QDs were firstly introduced in biomedical applications by groups of Alivisatos and Nie,\(^{32}\) they have witnessed the extensive
researches on engineering and application of their fluorescence from visible to near infrared regions for biodetection and diagnosis.\textsuperscript{33} Recently, the absorption and emission of QDs were extended to the NIR-II window by tuning their chemical components and size.\textsuperscript{34–36} Compared to other QDs, lead sulfide (PbS) QDs possess size-dependent emissions across the entire NIR-II biological window,\textsuperscript{30} due to their narrow bandgap and large Bohr’s radii.\textsuperscript{37} However, their sensitivity to aqueous media could dramatically reduce fluorescence and optical instability. Zhang et al. constructed core-shell PbS@CdS nanoparticles to protect the cores from reactions or degradation in aqueous media, thus, retained strong NIR-IIb emission at approximately 1600 nm.\textsuperscript{38} Since the highly toxic components, such as Pb and Cd in these QDs result in potential biotoxicity, lead-free NIR-II QDs with high biocompatibility have been developed. Following the design of core-shell nanostructures, Bruns et al. prepared InAs-based multishell QDs with narrow and size-tunable emissions in the NIR-II window (Figure 3G),\textsuperscript{39} and the QY could reach to 30% in aqueous media in comparison with PbS QDs (4%) (Figure 3H). Both of the shell coating and phospholipid surface wrapping can endow InAs-based QDs with excellent optical properties and biocompatibility simultaneously.

Another important type of NIR-II metal chalcogenides is silver sulfide (Ag₂S) QDs reported by Du et al. in 2010.\textsuperscript{40} Ag₂S QDs possessed size-tunable NIR-II fluorescence ranging from 900 to 1250 nm, and high QY (~20%).\textsuperscript{41} Due to the long blood circulation time of Ag₂S QDs (5.4 nm) nanoparticles, Li et al. could visualize the mouse cerebrovasculature down to nearly 24 μm with a high spatial resolution.\textsuperscript{42,43} They engineered the bandgaps of Ag₂E (E = S, Se, Te) QDs through manipulating chemical components, core-shell nanostructures to expand the emissions to NIR-IIa or even NIR-IIb regions.\textsuperscript{44} These chalcogenides QDs possess high chemical and photo stability with potential for bioimaging applications, whether they are used for early diagnosis, intraoperative navigation, or prognosis of treatment.

### 2.1.5 Au NCs

Au NCs have long been investigated for bioimaging applications with tunable emissions from ultraviolet to near-infrared regions.\textsuperscript{45} To further reduce optical interference from biological tissues, modulation of NIR-II fluorescence of Au NCs by using different types of surface ligands with varied stacking structures has become a hot topic.\textsuperscript{46} Chen et al. synthesized lipoic acid-based sulfobetaine-capped Au NCs with 20-50 atoms and NIR-II fluorescence.\textsuperscript{47} However, the density of surface ligands cannot red-shift emission peak and improve QY simultaneously. Liu et al. prepared Au NCs with 25 gold atoms and 18 peptide surface ligands with an emission at 1100–1350 nm. In this work, surface ligands and metal doping approaches were introduced to improve QY of Au NCs.\textsuperscript{48} The highest QY relative to IR-26 was up to 0.67% under 808 nm excitation for the sample doped with 4% Cu atoms and modified with cysteine. In view of labelling proteins or antibody, Song et al. employed macrocycle-based host-guest chemistry to prepare ultrasmall Au NCs below 2 nm (Figure 3I) with a relative QY up to approximately 0.11% and an emission peak at approximately 1050 nm (Figure 3J).\textsuperscript{49} In contrast, Wang et al. engineered a protein corona structure on the surface of Au NCs to red-shift the emission to NIR-II window.\textsuperscript{50} The resultant ribonuclease-A capped nanoclusters showed 1.9% QY relative to IR-26 at 1050 nm. Such strategies of manipulating atom stacking or surface ligands could be effective for obtaining NIR-II Au NCs with high QY and long-wavelength emission.

### 2.2 Organic-based NIR-II nanoprobes

SMDs based on cyanine and donor-acceptor-donor (D-A-D) structures are promising for fabricating NIR-II organic probes. The first NIR-II SMDs based on D-A-D structure (CH1055) was reported by Antaris et al. with an emission peak at approximately 1055 nm and 0.3% QY relative to IR-26, which had approximately 90% renal excretion within 24 h and outperformed imaging capacity compared to clinically approved indocyanine green (ICG).\textsuperscript{51} The research of NIR-II SMDs was focused on engineering of their QY, excitation and emission wavelength, water solubility, blood circulation time, and biocompatibility.\textsuperscript{52–54} A representative was cyanine-structured FD-1080 SMD designed by Li et al. with significant improvement of QY to 5.94% in fetal bovine serum (FBS).\textsuperscript{53} This supramolecular assembly of protein complex with the locked dyes could significantly improve the QY of NIR-II fluorescence and prolong the blood circulation simultaneously.\textsuperscript{55} However, rarely synthesized SMDs were reported to possess absorption or emission beyond 1500 or even 1300 nm.

#### 2.2.1 J-Aggregates

J-Aggregates composed of highly ordered assembly of organic dyes were conceived by supramolecular chemistry.\textsuperscript{56} The head-tail molecular stacking of dyes led to strong red-shifted absorption/emission and enhanced fluorescence intensity.\textsuperscript{57} Sun et al. fabricated J-aggregate nanoparticles (SQP-NPs)\textsubscript{(3J)} in aqueous solution by exploiting bispyrrole-sq-bispyrrole dye as a model.\textsuperscript{58} In
comparison with vertical alignment of SQP (SQP-NPs(II)), SQP-NPs(J) possessed 4.6-fold enhancements of emission intensity at the maximum near 1100 nm, indicating the significant effect of orientation arrangement of dye molecules on optical properties of their aggregates. Sun et al. developed a novel type of NIR-II J-aggregates from the self-assembly of FD-1080 cyanine dyes and DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine), which showed approximately 300 nm red-shift of absorption and emission compared to that of dye monomer (Figure 4A and B). The bioimaging performance of FD-1080 J-aggregates displayed the highest SBR (5.56) of NIR-II fluorescent cerebrovasculature images beyond 1500 nm among the three optical channels.

2.2.2 | AIEgens

As the common strategies of shifting the emission of SMDs to long wavelength by extending the conjugation units, the resulting large π-conjugates could induce emission quenching in the form of aggregates or nanoparticles due to the strong intramolecular π-π interactions. Since AIE characteristic was discovered by Tang group
in 2001,\textsuperscript{61} considerable effort was paid to the exploration of AIE luminogens (AIEgens) and their biomedical applications. Most recently, a series of NIR-II AIEgens have been developed through molecular engineering of electron donors and acceptors (D-A) to show large emission extension to the NIR-IIb region, high QY relative to IR-26, and photobleaching threshold.\textsuperscript{62,63} Notably, the D-A structured 2TT-oC26B molecules were wrapped with phospholipid polyethylene glycol to result in AIE dots, which displayed the maximum emission at approximately 1030 nm with a tail extension to 1600 nm, and a high QY up to 11.5\% (Figure 4C and D).\textsuperscript{63} Noninvasive through-skull macroscopic NIR-IIb imaging of cerebrovasculature was reached to approximately 71.6 μm of resolution, and further high-magnification microscopic images can finely show 10 μm-width vessel due to the substantially minimized optical interference.

2.2.3 | Pdots

Similar to the NIR-II AIEgens, the classic donor-acceptor (D-A) method was also introduced to fabricate NIR-II fluorescent polymer dots (Pdots). Hong et al. first reported D-A structured Pdots with intrinsic fluorescence located in the NIR-II biological window by copolymerization of an electron-donating monomer (benzo[1,2-b:3,4-b’]) and an electron-withdrawing monomer (fluorothieno-[3,4-b]thiophene) (Figure 4E-G).\textsuperscript{64} The fluorescence of Pdots at approximately 1050 nm exhibited a large Stokes shift (~400 nm) and a high QY (1.7\%) in aqueous solution relative to IR-26 were achieved after they were wrapped with amphiphilic polymer to render water solubility (Figure 4H). Due to the hydrophobicity of most fluorescent semiconducting polymer, Guo et al. introduced microfluidics method to fabricate biocompatible NIR-II pdots with a relative low QY (~0.1\%).\textsuperscript{65} In view of the existence of ACQ (aggregation-caused quenching) effect along with inter- and intrachain stacking interactions for Pdots, Zhang et al. manipulated AIE luminogens and different levels of steric hindrance to boost the QY to 1.7\% in an aqueous solution (Figure 4I).\textsuperscript{66} In addition, the optimal Pdots (P3c) possessed the maximum absorption at approximately 746 nm and emission in the NIR-IIa region (1300-1400 nm) with an excellent photostability under continuous NIR light excitation (808 nm, 1.0 W/cm²), compared to IR-26 and clinically approved ICG dyes (Figure 4J and K). On the basis of this strategy, Liu et al. integrated the planer twisted motifs with high solid-state QY into a copolymer (Figure 4L).\textsuperscript{67} The resulting Pdots displayed the maximum emission at approximately 1040 nm with a high relative QY up to approximately 2.2\% and an emission extended to the NIR-IIa region. These examples suggest that engineering the NIR-II AIEgens into Pdots is an efficient approach to improve the QY and emission wavelength beyond 1500 nm.

3 | NIR-II FLUORESCENCE IMAGING OF CEREBRAL DISORDERS

3.1 | NIR-II Fluorescence imaging of cerebral vasculature

Combination of high-quality NIR-II fluorophores with excellent imaging system can give full capacity of NIR-II imaging in depicting anatomical and functional of organisms at different levels. Zhong et al. deployed dynamic fluorescence imaging in the NIR-IIb biological window by using the NIR-IIb emission of RE NPs and their homemade intravital NIR-II imaging device, which was mainly composed of a liquid-nitrogen cooled, 320 × 256 pixel two-dimensional InGaAs array (Princeton Instruments, detection range 800–1700 nm) and 980 nm continuous-wave laser coupled to a collimator (F240SMA-980; Thorlabs).\textsuperscript{10,22} With the bright luminescence in the region of 1500–1700 nm and much shorter exposure time (20 ms) than previously utilized NIR-IIb nanoprobessuch as SWNTs\textsuperscript{16} and QDs,\textsuperscript{68} the RE NPs enabled excellent spatial-temporal resolution for in vivo video-rate imaging of blood flow in mouse cerebrovasculature, including confluence of sinuses at approximately 3 s, inferior cerebral veins (ICV), the superior sagittal sinus (SSS), the superficial veins (SV), and the transverse sinuses (TS) at approximately 4 s (Figure 5A-D). Based on the acquired videorate of through-skull NIR-IIb images of mouse cerebrovasculature, blood-flow velocities of ICV, SSS, SV, and TS were calculated through plotting the distance travelled by the flow front as a function of time (Figure 5C). Further arteriovenous staging was performed to distinguish venous vessels (such as the ICVs, SSS, and SV) from the arterial vessels (red color) through principle component analysis (PCA) of time-coursing images (80 frames/∼3s) (Figure 5E and F). The measured SBRs of vessels could reach to 5.3 for the ICVs and 3.1 for the SV by plotting the crossing-sectional intensity profiles (Figure 5G).

To obtain the microscopic structures, Wan et al. developed a 3D NIR-II confocal microscopy by using the one-photon fluorescence technique, which was able to decipher cerebral vessels of formalin fixed mouse brain tissue layer-by-layer with apparent widths of approximately 5-7 μm in diameter at approximately 1.3 mm imaging depth by using an organic-based nanofluorophore (p-FE) with ultra-long blood circulation half-live time (∼16 h) (Figure 5H).\textsuperscript{69} This NIR-II one-photon confocal technique combined with high-performance NIR-II fluorophore can decipher...
FIGURE 5  (A) Color photograph of a hair-shaved-off C57BL/6 mouse for NIR-IIb fluorescence imaging. (B–D) Time-course NIR-IIb brain fluorescence images present the perfusion of rare-earth NPs into various cerebral vessels. The blood-flow velocities of cerebral vessels are given in C (scale bar corresponds to (B–D): 2 mm). (E–F) Cerebral vascular image and the corresponding PCA overlaid image F showing arterial (red) and venous (blue) vessels (exposure time: 20 ms). (G) SBR analysis of NIR-IIb cerebrovascular image. (A–G) Reprinted with permission from Ref. [22]. Copyright 2017, Springer Nature. (H) Small area (200 × 200 μm, x × y, step size: 1 μm) and 3D reconstruction of vascular confocal images of a mouse brain (ex vivo, excitation: 785 nm, emission > 1100 nm). Reprinted with permission from Ref. [69]. Copyright 2018, Springer Nature. (I) A simplified schematic illustration of NIR-II LSM. (J) Fluorescence emission spectra of p-PE and PEGylated PbS/CdS CSQDs. (K) 3D rendering of vasculatures in a mouse brain labelled with PEGylated PbS/CdS CSQD (ex vivo, excitation: 1319 nm, emission: 1500–1700 nm, excitation power: approximately 1.4 mW, exposure time: 0.8 s). (L) 3D rendering of a smaller region in (K). (I–L) Reprinted with permission from Ref. [74]. Copyright 2019, Springer Nature

3D structure of in vitro biological samples (such as mouse brain and ovary) without tissue clearing. Qian’s group built the NIR-II confocal microscopy with fine adjustment to resolve the 3D cerebrovasculature of mouse, rat, and even rhesus macaques through intact skull using organic fluorophores (clinical approved ICG and AIEgens). Taking the nonhuman primate as an example, the marriage of ICG and NIR-II confocal microscopy could resolve 3D cortical cerebrovasculature up to depth of approximately 500 μm and diameter of <7 μm, along with measurement of blood flow velocities.

Since the diversity and ability of NIR-II fluorophores in excitation/emission bands and lifetime, multiplexed NIR-II imaging methods have been applied to visualizing chemotherapy and immunotherapy, resolving multiple tumor biomarkers quantitatively, deciphering immunotherapy response for tumor cells and cytotoxic T lymphocytes, and establishing relationships between biological structure and photophysical properties through imaging of awake and moving mice. On the basis of super-resolution techniques, which have revolutionized the application of fluorescence, Wang et al. developed NIR-II light-sheet microscopy (LSM), which is suitable for large-scale volumetric imaging up to single-cell resolution with an appropriate adjustment of setups. Collaborative use of NIR-II LSM and multiplexed NIR-II nanofluorophores (p-PE and PEGylated PbS/CdS CSQDs) can record cerebrovasculature images of glycerol-cleared mouse brain as a function of tissue depth in three fluorescence emission windows (850-1000 nm, p-PE emission; 1100–1200 nm, p-PE emission; 1500–1700 nm, CSQD emission) under the same 785 nm excitation (Figure 5I and J). The multiple fluorescence channels allow layer-by-layer comparison of NIR-II LSM imaging in three subregions of 850–1700 nm. When both excitation (1319 nm) and emission (1500–1700 nm) of LSM located in the NIR-II biological window, minimized scattering and maximized penetration depth (~2.5 mm) were achieved to obtain high-resolution 3D NIR-II LSM sectioning in glycerol-cleared mouse brain tissue (Figure 5K and L). Although the imaging samples are ex vivo biotissues, the well-developed NIR-II LSM could decipher deep-tissue volumetric vasculature...
and effectively prevent sectioning interpretation artifacts, which were occurred when cells were cut in cross-section by vibratome, freezing microtome, or cryostat.74,75

Although the human brain accounts for approximately 2% of total body mass, it receives approximately 20% of cardiac output for nutrient and oxygen supply to support brain functions through cerebral vasculature.76,77 Blood-brain-barrier (BBB) as a specialized cerebral vascular system is highly significant for maintaining the normal functions of the CNS.78,79 The disruption of BBB plays a key role in the occurrence and development of neurological dysfunction in acute and chronic cerebrovascular disorders, which are meanwhile accompanied by cascaded injuries, such as ischemia, oxidative stress, hypoxia, and immune responses, from tissue, molecular, to cellular level.2,80 Therefore, establishing the relationship between imaging characteristics and physiological changes of cerebrovascular diseases is of great importance for early diagnosis in real-time and effective and immediate intervention, since some of processes are stealth on nontissue level. Currently, a vast variety of nanofluorophores have been investigated for this purpose by using diverse NIR-II imaging methodologies.

3.2 NIR-II Fluorescence imaging of stroke

Stroke is a cerebrovascular accident induced by the disturbance in the blood supply to the brain, which can increase inflammatory and immune responses along with severely limited angiogenesis and neuronal growth.81 Liu et al. employed NIR-IIa Au25(SG)18 nanoclusters with rapid clearance from mouse cerebral vessels to noninvasively evaluate the structural changes and blood perfusion for ischemic stroke in real time (Figure 6A-C).48 The arteriovenous staging results obtained through PCA of the time-course NIR-IIa fluorescence images (∼30 s with 100 frameworks) clearly showed the neovascularization of arterial vessels (red) in the left cerebral hemisphere compared to the right one (Figure 6D). The average of NIR-IIa intensity in the middle of cerebral arteries can quantify the cerebral blood perfusion based on dynamic NIR-IIa fluorescence images.15 The quantitative analysis of cerebral blood perfusion rates in the arterial vessels were 0.11/s for the injured left cerebral hemisphere and 0.24/s for the right cerebral hemisphere (Figure 6E), which displayed 0.13/s in rate difference.

Unlike the block of the left middle cerebral artery using microfilament and the reopening of it in 1 h interval to build ischemic mouse stroke, Hong et al. surgically built the middle cerebral artery occlusion (MCAO) to cause the stroke of left cerebral hemisphere of mice.14 Dynamic cerebrovascular fluorescence imaging was also applied to monitor the structural variation and blood flow of the healthy and MCAO mice, respectively, after the mice were injected with NIR-IIa SWNTs-IRDye800 through their tail veins (Figure 6F). Similar to the results obtained from the ischemic stroke model, the left hemisphere with MCAO showed a prominent delay of blood flow, in comparison with the right hemisphere without MCAO. PCA of the time-course images for the healthy and MCAO mice revealed a more extended venous network (blue) in the right hemisphere than in the left one, the arterial vessels (red) showed up in the intact right hemisphere (Figure 6G and H), which presented a larger ischemic area in the brain. In addition to that, quantitative cerebral blood perfusion measurement displayed relative perfusion of approximately 1 for both hemibrain of healthy mice, and approximately 0.159 for the occluded left hemibrain of MCAO mice (Figure 6I and J). Compared to the healthy mice and the ischemic stroke mice (blood perfusion of 54%) in the above studies, MCAO mice displayed the dramatic decrease in the cerebral blood perfusion of 85% (Figure 6K) and the larger ischemic area in hemibrain. These results solidly demonstrate the capacity of dynamic NIR-II fluorescence imaging in real time to identify the ischemic area and analyze cerebral perfusion to evaluate the degree of ischemia in the mice with cerebrovascular injury.

The onset of stroke initiates an ischemic cascade involving neuroinflammation, and BBB disruption is a key hallmark in the neuroinflammation’s pathophysiology.82 Previous studies have investigated the self-repairing function of the BBB of healthy mice after focused sonication through PAI, single-photon emission computed tomography and NIR-II fluorescence imaging using high-performance exogenous probes.54,78 Although the wide-field NIR-II fluorescence imaging equipment enables to track cerebral blood flow in multiple vessels simultaneously, and two-photon microscopy as a complementary approach has also been applied in measuring mouse blood flow of one single cerebral vessel at one time through cranial window,83 it is still short of detailed imaging information of the effect of BBB. Qi et al. set up a SWIR (short-wave infrared) fluorescence microscopy to precisely monitor photothermal ischemia (PTI)-induced BBB damage of a mouse brain in real-time by utilizing TQ-BPN AIEgens as a NIR-II nanofluorophore.84 A 1040 nm femtosecond laser beam and rose bengal (RB, photosensitizer) were collaboratively used to release singlet oxygen (1O2) in cerebral vessels to damage the vascular endothelial cells (Figure 6L).85 Cerebral thrombosis was formed after approximately 30 s continuous irradiation, which led to the block of blood flow at the injured area (red circle) and the accumulation of AIEgens to show obvious signal enhancement at the obstructive site (green arrow) (Figure 6Mii). When
the irradiation time was up to 3 min, the NIR-II fluorescence of AIEgens leaked out from the cerebral vessels indicated that the produced $^{1}\text{O}_2$ by RB caused severe damage to the BBB in PTI site (Figure 6Miii-iv). These results suggest that the invention of SWIR fluorescence microscopic imaging may allow us to probe the pathogenesis of cerebralvascular diseases.

3.3 NIR-II Fluorescence imaging of TBI

While TBI has been conceptualized as a primary injury event from mechanical damage, and followed by the secondary injury due to the molecular and cellular responses to the primary injury. In term of vascular pathology as a TBI biomarker, Zhang et al. adopted noninvasive in vivo dynamic cerebral NIR-II imaging to evaluate vascular hemodynamics, which is similar to the methodology used for mouse stroke model. Analysis of dynamic NIR-II fluorescence images revealed that cerebral blood flow perfusion rate was reduced by approximately 17% in the right injured hemibrain than that in the left normal hemibrain, along with the apparent ischemic area formed in a short time. The subsequently continuous NIR-II signal increment and diffusion in the injured hemibrain indicated the leakage of probes through the damaged BBB. Since the previous studies on the ineffectiveness of anti-inflammatory drugs in the treatment of TBI patients revealed that early inflammation response could set the stages for proper tissue regeneration and recovery, it is of
great significance to acquire insights into pathological process on molecular and cellular levels. Wang et al. fabricated anti-CD11b PEGylated PbS/CdS CSQDs to label meningeal macrophages and microglia in a TBI mouse model for noninvasively monitoring the inflammatory responses. The leakage of NIR-IIb CSQDs through damaged BBB was observed after 24 h postinjection (Figure 6N). The labeled meningeal macrophages and microglia at the boundary of TBI region showed the dynamics after brain injury 24 h noninvasively monitored by 3D time-course NIR-IIb light-sheet imaging (Figure 6O-Q).

In addition, patients with mild TBI rarely show signs of injury by CT scanning, which requires accurate biomarkers for precise diagnosis and effective prognosis. From the perspective of biochemical stress, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are highly correlated with the progression of TBI. Among them, peroxynitrite (ONOO⁻) generated form the cerebrovasculature can serve as a prodromal biochemical marker for the progression of TBI. Accordingly, Li et al. constructed a dual-function NIR-II fluorescent nanoprobe (V@A@Ag₂S) with VCAM1 binding peptide to target inflamed endothelium expressing VCAM1 in TBI regions, and ONOO⁻-responsive A1094 chromophore to turn on the NIR-II fluorescence of Ag₂S QDs (Figure 7A). Compared to the targeted “always on” NIR-II nanoprobes (V@Ag₂S), this well-designed activatable V@A@Ag₂S precisely pinpointed the TBI region at the right cerebral hemisphere with a superior SBR over 10.2 within 10 min postinjection (Figure 7B and C). Since the migration of immune cells and the production of reactive species (ROS and RNS) are part of inflammatory responses in TBI, the effects of brain inflammation on BBB, as the main pipeline to transfer substances in blood to brain parenchyma, are also worthy of attention.

In the field of neuroscience, lipopolysaccharides (LPS) is widely applied to induce brain inflammation. LPS induced brain inflammation could prolong the retention of Au NCs in cerebral vessels, indicating the inflammatory response to blood perfusion. To effectively cross the
BBB to reach the inflammatory site, Liu et al. manipulated inflammatory tended immune cells (neutrophils, NE) to mediate delivery of NIR-II fluorescent AIEgens (DSPE-PEG wrapped 2Tt-oC6B) to the inflammatory site (Figure 7D). The NIR-II fluorescence images of mouse brain against time displayed a SBR value of 30.6 at inflamed area for AIEgens@NE, which was 6.5-fold stronger than that obtained from ICG@NE group at 12 h (Figure 7E), and in accordance with the distribution of NIR-II fluorescence in the isolated mice brain (Figure 7F). The results suggested the potential of cell-mediated probe delivery in theranostics of brain diseases. For LPS-induced septic mouse model, Imamura et al. utilized PbS QDs to observe pathophysiological state of cerebral vessels in sepsis through NIR-II fluorescence imaging. Prominent thromboses in cerebral vessels were visualized in the skull-removed/isolated/magnified brain from the LPS(+) group of mice, in comparison with the mice from the LPS(-) group and heparin treated LPS(+) group (Figure 7G-I), which presented a complete pathological state of cerebrovasculature in mouse brain. These results have solidly demonstrated the huge potential of NIR-II imaging in visualizing immune cells migration, free-radical generation, and BBB changes in response to brain inflammation. However, additional research is demanded to identify the critical time window when and how inflammatory cells take part in repair after TBI.

4 | NIR-II FLUORESCENCE IMAGING OF BRAIN TUMORS

4.1 Delivery methods of NIR-II nanofluorophores

Malignant brain tumors (such as glioblastoma) are featured with infiltrated growth, microvascular proliferation, and pleomorphic vessels in CNS. To reveal the vascular characteristics of medulloblastoma, Liu et al. developed a tetrafluorinated m-PBTQ4F Pdots with good photostability and an relative QY of 3.2% in aqueous solution for visualizing cerebrovascular network of wild-type and transgenic C57BL/6 mice through the NIR-II fluorescence imaging. In contrast to the evenly distributed and well-differentiated cerebral vessels for the wild-type mice, unevenly spaced and chaotic cerebrovascular networks were observed in the NIR-II fluorescence images (Figure 8A). Multiple parameters calculated from the Hessian-matrix-enhanced images, including total vessel length, vessel branches, and diameter entropy, asymmetry of vascular topography, clearly outlined the characteristics of chaotic and hyperplastic cerebral vessels for medulloblastoma (Figure 8B-E). To visualize the microstructure of cerebral vessels in tumor implanted brain, Bruns et al. used an one-photon excitation SWIR intravital microscopy to observe them through cranial window. Multiple injection of NIR-II QDs was applied to obtain time-course images, and the resulting deconvolved and color-coded images (Figure 8F) displayed the disordered cerebrovasculature induced by brain tumor growth. The existence of BBB, blood-tumor-barrier (BTB), and tumor extracellular matrix become the major obstacles for the effective delivery of theranostic drugs to brain tumors for early diagnosis, imaging guided surgery, and therapy. Shen et al. developed c-RGD conjugated TBI AIEgens for targeted NIR-II imaging of glioma. Bright fluorescence (relative QY of 6.2%) in the NIR-II window enabled the clear and noninvasive visualization of cerebrovascular network (Figure 8G and H). The formulated AIEgens could target αvβ3 integrin receptors over-expressed by glioma cells to achieve higher accumulation in the tumor and stronger NIR-II fluorescence than the nontargeted ones (Figure 8I). This covalent conjugation approach can also be expanded to other nanoprobes conjugated with different ligands, such as transferrin, angiopep-2, RI7217, COG133, for BBB transportation and tumor homing.

In contrast to conjugation with targeting molecules, the collaborative use of focused ultrasound sonication (FUS) and microbubbles provides an efficient approach for theranostic probes to cross biological barriers including BBB, BTB, and extracellular matrix. The local stimulation and reversible opening of BBB featured by FUShave been monitored by different imaging methods. The FUS-assisted delivery of NaNdF4@NaLuF4/IR-808@PEG nanoparticles to the orthotopic glioma was monitored by the NIR-II imaging (Figure 8J). These hybrid nanoprobes with large absorption cross-section and multiple emissions within near infrared enabled the fluorescence visualization of their accumulation and retention through multiplexed channels (Figure 8J), which was further confirmed by the staining results (Figure 8L). FUS-mediated opening of BBB/BTB could also efficiently transport nontargeted diagnostic agents, including small molecules and nanoparticles to the brain tumors, which were noninvasively monitored by magnetic resonance imaging (MRI) and PAI.
disadvantageous in the aspects of photostability, tissue penetration, and tumor targeting. Various strategies were used to engineer NIR-II nanoprobes for tackling these issues. Hu et al. integrated visible and NIR-I/II multispectral imaging instruments for intraoperative imaging-guided surgery of primary and metastatic liver tumors in 23 patients by using ICG as a probe, which solidly demonstrated the potential of NIR-II imaging for clinical practice in intraoperative navigation. Li et al. employed preoperative MRI detection and NIR-II fluorescence imaging guided intraoperation for orthotopic glioma by using dual-function Gd-Ag2S nanoprobes (Figure 9A).

As mentioned above, BBB/BTB are the major impediments for the orthotopic brain tumors. On the basis of previous studies of FUS-mediated delivery of therapeutic agents to brain tumors, Ren et al. collaboratively employed FUS with tumor-specific Er-based lanthanide nanoprobes for finely defining the brain tumor margins. By use of core-shell nanoparticles, which showed 675-fold enhancement of 1525 nm emission in the NIR-IIb window compared to bare core nanoparticles in aqueous solution, noninvasively through-skull imaging of deep-seated brain tumors was realized. Following the preoperative T2-weighted MRI-guided FUS and intraoperative NIR-IIb fluorescence imaging-guided surgery sequential procedures (Figure 9B and C), small orthotopic glioma was successfully resected and confirmed by the NIR-II fluorescence and corresponding tissue staining (Figure 9D-F).
Although surgery is the primary therapeutic method for most solid tumors, hematoxylin and eosin (H&E) staining in intraoperative frozen section histopathology are the golden standards to largely determine the direction of surgical operations. Since the typical tissue section procedures are time consuming and cannot fulfill the requirement for accurate intraoperative histopathological analysis, Li et al. fabricated a matrix metalloperative (MMP) 14-activated tumor-specific NIR-II nanoprobe based on Ag$_2$S QDs (A&MMP@Ag$_2$S-AF7P) for rapid and nondestructive histopathological analysis of neuroblastma in vivo and ex vivo (Figure 9G and H). The exclusive neuroblastoma cell staining only took approximately 30 min by using A&MMP@Ag$_2$S-AF7P nanoprobes, which was shorter than tissue fixing and frozen sectioning, and held great potential for cancer surgery or tissue biopsy as a complementary or alternative method.

5 | DISCUSSIONS AND PERSPECTIVES

Since the first published bioimaging application of nanofluorophore (SWNTs) in NIR-II biological window (1000-1700 nm) in 2009, the synergetic development in both NIR-II nanofluorophores and imaging systems promote the depth and width of this technology in fundamental and preclinical researches. Taking comprehensive consideration of overall optical interferences, including tissue scattering, absorption and autofluorescence, NIR-IIa (1300-1400 nm), and NIR-IIb (1500-1700 nm), are ideal windows for imaging with high spatial-temporal resolution. Although many kinds of NIR-IIa and NIR-IIb nanofluorophores have been fabricated to fulfill the need of bioimaging applications, they could still suffer from potentially biotoxicity due to long-term retention and release of toxic elements, which impeded their clinical translation. In addition, ultra-small nanoparticles,
such as Au NCs (<2 nm), with capability of renal excretion and chemical stability are promising for clinical translation, but they are facing issues of low QY and short wavelength emission in the optimized biological window.

From the perspective of fundamental research, the cooperation of NIR-II nanofluorophores with currently well-devised macroscopic, microscopic, and mesoscopic imaging systems enable the acquirement of abundant bioinformation on tissue, molecular, and cellular levels through optimized conditions. Using the wide-field NIR-II fluorescence setups to identify arteries and veins of mouse cerebral vasculature could indicate their distribution, ischemic area, and blood velocity changes for assessing the severity of cerebral vascular injury in TBI and stroke, which could even be visualized from microscopic view. Given that the potential risks of brain injury-induced neurovascular dysfunction could develop into neurodegenerative diseases, such as Alzheimer, the immune responses in the injured site might decipher the existence of maladaptive neuroinflammation deadline for guiding TBI therapy.

The cutting-edge NIR-II LSM have enabled noninvasive monitoring of the dynamics of macrophages and meningeal microglia at the boundary of TBI region, which could enlighten us with better understanding immune responses in acute and chronic TBI.

In contrast to cerebrovascular injury, brain tumors also seriously influence the cerebrovascular morphology and diameter entropy found in transgenic cerebroma mice though NIR-II fluorescence imaging. Tumor-specific ligands and FUS were used for efficient delivery of NIR-II nanofluorophores to orthotopic brain tumors. Since the finely delineated tumor margins followed by the pinpoint of preoperative MRI, NIR-II fluorescence imaging-guided surgery was deployed smoothly. As the indispensable part of precise surgery, the exclusive tumor cell staining by using activatable and targeted NIR-II nanofluorophores without tissue fixing and frozen sectioning is very promising. Recently reported intraoperative NIR-II imaging-guided surgery of liver tumors in patients with higher tumor-detection sensitivity and tumor-to-normal-liver-tissue signal ratio and enhanced tumor-detection rate showed the great potential of this technology in clinical applications. These independent researches demonstrate the great potential of NIR-II fluorescence imaging as a complementary or alternative approach for future cancer surgery or pathological tissue biopsy.

Despite the above-mentioned merits of NIR-II fluorescence imaging, the acquired dimension and depth of bioinformation by NIR-II fluorescence imaging is still limited. Therefore, it is necessary to use other complementary imaging methods to obtain the comprehensive pathophysiological information. One possible imaging method is the photoacoustic tomography (PAT), which is also based on photoconversion. The fundamentals of PAT is that the tissue is irradiated by the NIR light to cause thermal expansion-relaxation process to generate ultrasound waves, which are recorded by the ultrasound detector and reconstructed into image. PAT exhibits the merits of optical imaging (i.e., sensitivity) and ultrasound imaging (i.e., deep penetration). It is capable of in vivo imaging, the variation of hemoglobin in the cerebral vasculature of mice responding to their front-paw stimulation, and evaluating blood pulse wave velocity to characterize cardiovascular disease progression correlated with arterial stiffness without exogenous contrast agents. Although the PAT shows deeper penetration than optical imaging, it is difficult to reach the depth above 5 cm. An alternative imaging method is MRI, which has no penetration limit and enables the dynamic evaluation of cerebral ischemia from blood volume and blood flow in large animals with assistance of contrast agent (e.g., polyethylene glycol-stabilized iron oxide nanoclusters).

Since precise diagnosis and treatment would significantly reduce the mortality of different kinds of critical illness, it is of great importance to combine complementary multimodal imaging methods and therapeutic approaches. While previous studies on tumor theranostics have solidly demonstrated the significance of multimodal imaging guidance in precision therapy. The design methods of multifunctional nanoscale contrast agents could be classified into physical and chemical reaction loaded or coupled or built-in integration. Ma et al. employed the chemical coupling method to endow Fe3O4 nanoparticles with dual ratiometric photoluminescence for pinpointing the location and deciphering the MMP-9 activity and extracellular pH of tumors in vivo simultaneously. Ai et al. used physical absorption to load radicals-sensitive NIR dyes (HCy5 and Cy7) on the surface of upconverting lanthanide nanoparticles for visualizing dynamic endogenous redox biomarkers in pathophysiological progression through multispectral optoacoustic imaging and upconversion fluorescence imaging. While He et al. designed a trimodal lanthanide nanoparticle-based contrast agents with simultaneous enhancement of photoluminescence, MRI relaxivity, and CT contrast simply by integrating different components into one single core-shell nanostructure through rational tuning constituent fraction and shell thickness. Following these designing logic, the integration of multiple imaging and therapy techniques with the NIR-II fluorescence imaging not only could provide comprehensive bioinformation for early diagnosis, effective intervention, and prognosis monitoring of cerebrovascular diseases, but also show great potential in diagnosis and treatment of other diseases for maintaining good health.
CONFLICT OF INTEREST
The authors declare no conflict of interest.

ACKNOWLEDGMENTS
Z.L. acknowledges support from the National Natural Science Foundation of China (81971671), the National Key Research and Development Program of China (2018YFA0208800), the Jiangsu Provincial Key Research and Development Program (BE2019660), and Suzhou Science and Technology Bureau (N312861019). The authors also are grateful for support from the Jiangsu Provincial Key Laboratory of Radiation Medicine and Protection, the Priority Academic Development Program of Jiangsu Higher Education Institutions (PAPD).

ORCID
Zhen Li https://orcid.org/0000-0003-0333-7699

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**AUTHOR BIOGRAPHIES**

**Feng Ren** received his BS degree from Yancheng Institute of Technology in 2015 and MS degree from Soochow University in 2018. He is now a PhD candidate in the School for Radiological and Interdisciplinary Sciences at Soochow University supervised by Prof. Zhen Li. His current research interests focus on the synthesis of lanthanide-based nanomaterials for bioimaging applications.

**Prof. Zhen Li** works in the Centre of Molecular Imaging & Nuclear Medicine at the School of Radiation Medicine and Radiation Protection, Soochow University. He received his PhD degree from the Institute of Chemistry (Chinese Academy of Sciences) in 2005, and worked in world-leading universities in UK, Germany, and Australia for a few years. He joined the Soochow University in 2014. His research focuses on the molecular imaging and nanomedicine for precise diagnosis and therapy. Prof. Li has published 150 articles, and received several prestigious awards and fellowships.

**How to cite this article:** F. Ren, Z. Jiang, M. Han, H. Zhang, B. Yun, H. Zhu, Z. Li. *VIEW.* 2021, 20200128. https://doi.org/10.1002/VIW.20200128