Organic Molecules: Desirable Candidates for NIR-II Window Bioimaging

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Abstract. The second near infrared (NIR-II) window in optical wavelength of 1000–1700 nm have become a hot topic in bioimaging, especially in vivo imaging. Compared to the first near infrared (NIR-I) window (700-900 nm), NIR-II fluorescence imaging has advantages including low auto-fluorescence, high signal-to-background ratio, deep penetration, and high resolution. Among many fluorescence imaging constructing agents, organic probe has benefits of low toxicity, easy structure tunability, high excretion rate, and good biocompatibility. Meanwhile, conjugated polymer nanoparticles can also be used in photoacoustic imaging. This review focuses on recent progress in development of NIR-II probe in optical imaging and photoacoustic imaging.

1. Introduction

Near-infrared (NIR) technology, which is known as the transmitted or reflected spectra of electromagnetic with the wave ranges from 800 to 2500 nm [1]. It has been used in many fields, such as nondestructive measurement and material assessment. Compared with other imaging methods (i.e. NIR-I imaging), the NIR-II imaging has the advantage of higher penetrating ability, higher resolution and higher stability. Thus, it has been used more and more widely in various fields. The NIR-II imaging also shows great potential in biological imaging since many recent studies have explored its improvement and application in that field. Previous studies have identified different types of materials for NIR-II imaging, which include metal sulfides, noble metals and two-dimensional (2D) materials[2–8]. Metal sulfides, especially copper and bismuth, possess a small carrier effective mass, small band overlap energy, and long Fermi wavelength, extending the absorption wavelength to the NIR-II region. Nobel metals show considerable potential for use in photoacoustic imaging (PAI) and photothermal therapy (PTT) because of their superior light absorption abilities, and the tunable localized surface plasmon resonance (LSPR) properties as well. However, it is difficult to ensure their bio-safety.
materials, such as mxenes and 2D vanadium carbide quantum dots (V2C QDs), have long circulation time and excellent biocompatibility.

In the current paper, we reviewed recent research that focuses on the biological imaging of organic materials, including polymers and small organic molecules, and discussed the target molecules’ and synthesis. In the process of improving and assembling the targeting bio-nano, organic molecules are expected to be formed. A higher absorbance value would be preferred, while brighter imaging could be shown. Moreover, the different donor units would also affect the molecular structure, further affecting the interaction between molecules and the final result of imaging. Besides, since the target product will be used for biological imaging, low biological toxicity and compatibility are also required to ensure that it does not cause harm the imaging object.

Overall, this review article introduced NIR research that aims at biological imaging of organic polymers and small organic molecules, most of which have been published in the latter half of the 2010s.

2. Small molecules applied for NIR-II imaging

Nowadays, fluorescence imaging technology is widely used in many biomedical research. Compared to the commonly used visible light and NIR-I (750-900 nm), the NIR-II fluorescence imaging technology has the advantage of a longer emission wavelength. With that said, the NIR-II can reduce the light scattering autofluorescence effect significantly. Thus, a deeper detection depth and higher resolution higher could be achieved. Recently, Zhu et al. reported a molecular engineering approach based on fused-ring acceptor (FRA) molecules [9]. Among all the reported FRA, the molecules(CPTIC-4F) illustrated in Fig. 1b has one of the longest wavelength absorption (up to 1100 nm). During building up the structures, the 3-EHOT is taken place by the donor unit of PDOT-C8 since the larger number of branched size can decrease the intermolecular aggregation. Meanwhile, the structure could also protect the molecules from interaction within the water. Such a structure, as shown in Fig. 1a, could efficiently inhibit the quenching effects for CPTIC-4F.

Indicated in Fig. 1b are the absorption and emission spectra of the nanofluorophores, which were measured in phosphate buffer saline (PBS). Compared to the molecular in toluene, the phenomenons of bathochromic shift could be observed for both CBTIC-4F nanofluorophores and CPTIC-4F nanofluorophores, with the absorption peaks of 949/869 nm and 876/810 nm. For COTIC-4F nanofluorophores, CBTIC-4F nanofluorophores and CPTIC-4F nanofluorophores, the peak absorption coefficients are $8.70 \times 10^{-4}$, $8.80 \times 10^{-4}$ and $14.5 \times 10^{-4} \text{ M}^{-1} \cdot \text{cm}^{-1}$, respectively. According to the similar absorption coefficients of the first two molecules, it can be found that lengthening alkyl chains of 3-AOT has a negligible effect on the coefficient. Furthermore, the PDOT-C8 donor is superior to the other two donors since it was able to cause higher brightness in the NIR-II imaging [2].

![Fig 1. (a) Structures of the FRA molecules. (b) Absorption (concentration: 0.01 mg·mL−1) and fluorescence spectra (optical density: 0.08 at 808 nm) of COTIC-4F NFs, CBTIC-4F NFs and CPTIC-](image-url)
4F NFs in PBS (pH = 7.4). QY values calculated at 900–1,000 nm and 1,000 nm regions are presented in the emission spectra.

Moreover, the biological effects are shown as the imaging performance of CPTIC-4F nanofluorophores. It shows a significantly higher intensity than ICG in the same PBS concentration. Since its future of high brightness and extended emission, the sort of nanofluorophores can obtain the high SBR imaging of the whole body. The imaging of cerebral vessels could be conducted by CPTIC-4F nanofluorophores as well (Fig 2a). Thus, tissue scattering and autofluorescence were inhibited efficiently. Moreover, the nano molecules have the advantages of high stability. Compared to the vessel imaging of ICG, the imaging with CPTIC-4F molecules is much more precise and time-enduring.

Fig 2. (a) (b) Imaging performance of COTIC-4F NFs, CPTIC-4F-NFs and ICG with same concentration (50 μg·mL−1) in PBS at 1,000, 1,200 and 1,300 nm LP filter, respectively. (c) Non-invasive in vivo NIR-II imaging of whole body vessels of CPTIC-4F NFs and ICG in mice at 1,300 nm LP filter. The red line represents the cross-section of the vessels.

Another research from Benhao Li also shows a nanomolecular fluorophore [10], FD-1080, with both excitation and emission in the NIR-II region for vivo imaging. The molecule has a superior imaging resolution compared to the NIR excitation from 650-980 nm. The synthesis of FD-1080 is shown in Fig. 3a, and the yield is usually around 60%. The products have the features of high stability and higher water solubility, and it would turn bright under the serum proteins (Fig. 3b). In this situation, the quantum yield is around 5.94%, and the excitation of fluorescence is 1064 nm.

Fig 3. (a) Synthetic route of FD-1080. (b) Absorbance and fluorescent emission spectra of FD-1080 dye with an absorbance peak at about 1046 nm and an emission peak at about 1080 nm. (c) Reported
NIR-II agents with varied excitation wavelengths. (d) Photostability of FD-1080 and ICG complexes in a variety of biological media under continuous 1064 nm and 808 nm exposure for about 80 min at a power density of 0.33 W cm\(^{-2}\), respectively.

The biological imaging of FD-1080-FBS is used at the left hindlimb vasculature and abdomen vessel of the mouse under the excitation wavelength from 655 nm to 1064 nm, as shown in Fig. 4a. Due to the lack of light absorption, scattering and autofluorescence of tissues, the imaging of left hindlimb vasculature are under higher NIR-II excitation was superior to that under shorter ones (Fig. 4b). Fig. 4c and Fig. 4d show the SBR and FWHM of the imaging of vessels under 1064 nm NIR-II excitation.

Further research about the imaging performance of FD-1080-FBS with NIR-II excitation is based on the imaging of mouse’s vivo cerebrovascular, which can be seen in Fig. 4e. Similarly, a 1064 nm NIR-II excitation was used to obtain the sharpest images with the highest cerebrovascular solution (Fig. 4f). Meanwhile, the excitation wavelength dependence could be explained by the extinction spectra of the scalp skin and the cranial bone. A phenomenon of bathochromic shift can be observed from NIR-I to NIR-II (Fig. 4g).

![Fig 4](image_url)

**Fig 4.** (a) Comparison of the fluorescence images of FD-1080-FBS complexes in mouse left hindlimb vasculature under different excitation as indicated (1300 nm long-pass filter). (b) The fluorescence intensity profiles across a red line of interest with different excitation wavelengths. (c) The corresponding signal background ratio (SBR) analysis with intensity across a red line of interest. (d) The fluorescence intensity profiles (black dots) and Gaussian fit across a red line of interest under 1064 nm excitation. (e) The penetration of NIR-II optical imaging through brain tissue scalp and skull. (f) Extinction spectra of scalp skin (black) and cranial bone (red). (g) The Gaussian fit fluorescence intensity profiles across a red line of interest with 808 and 1064 nm excitation wavelengths, respectively.

Furthermore, Yu et al’s study also provided a general summary of NIR-II fluorescence imaging [11]. According to Yu, NIR-II fluorescence imaging has played a vital role in vivo imaging due to the
features of high temporal resolutions and sensitivities. In order to overcome the defects of organic NIR-II fluorophores, some engineering approaches have been applied to improve the imaging properties.

In the area of photothermal therapy and photoacoustic imaging, fluorophores are usually used for the photothermal conversion capability. In order to improve the energy conversion efficiency, some approaches were taken to make the molecules in the aggregation state. A method is to add a long-chain alkane structure to the thiophene of a D-A-D fluorophore. Thus, a novel molecule named NIRb14 is formed, and it would be wrapped in a nanoparticle (Fig. 5a-5b). This nanoparticle could achieve a much higher aqueous solution temperature than traditional gold-based nanoparticles under the 808 nm laser irradiation (Fig. 5c-5d).

![Fig 5. (a), (b) Structures and schemes of modified groups on thiophene of a D-A-D molecule to realize intermolecular rotation. (c), (d) Intentionally enhanced photothermal conversion efficiency (compared with GNR) based on principles of (a) and (b).](image)

Even though the NIR-II fluorophores are hard to apply to the traditional molecular response design, some other methods could be used due to organic NIR-II fluorophores' properties. A non-fluorescent dye precursor, Hydro-1080, could be produced by replacing the end of ET-1080 with its reduction product to change the conjugation and planar structure (Fig. 6a-6b). The Hydro-1080 would be oxidized into Et-1080 again once it exposes to hydroxyl radicals. The feature of fluorescence would be restored. It can be seen that the fluorescence of NIR-II disappears because of the intermolecular structure when PDF combines with ITTC to form SPNP (Fig. 6c).
Lin et al. reported a novel NIR-II fluorophore for in vivo molecular tumor imaging utilizing a CH1055 derived small molecule H2b with two modifiable carboxyl groups. Conjugated with an osteosarcoma-specific oligopeptide PT-7, a novel small-molecule fluorescence probe H2b-PT-7 (Fig. 7a) with osteosarcoma specificity was successfully synthesized[12]. The fluorescence intensity peak validated the FIR-II emission of H2b-PT-7 at 1,100 nm wavelength (Fig. 7b). Furthermore, in vivo real-time fluorescence imaging of orthotopic osteosarcoma bearing mouse has shown that the H2b-PT-7 fluorescence probe can specifically bind to the tumour tissue, and its intensity is adequate for optical imaging (Fig. 7c). The fluorescence signal was optically visible for at least 12 hours after the injection. The osteosarcoma was indicated by white arrows in Figure 7c and was confirmed by the histological section (Fig. 7e). In the NIR II imaging performed by Lin et al., non-specific signals (Fig. 7c, d) were also presented in the hepatobiliary system of the mouse as part of the breakdown route of H2b-PT-7. As the location of targeted tissue has no conflict with the histological system, H2b-PT-7 yields a promising fluorescence probe for guiding osteosarcoma surgery.
osteosarcoma nude mouse model at 6 h, 9 h, 12 h, and 29 h after tail vein injection of H2b-PT-7 (0.2 mL, 10 mg kg⁻¹). (d) The ex-biodistribution of H2b-PT-7 in orthotopic 143B tumor bearing mice at 29 h under 808 nm excitation. (e) The tumor cells in the resected specimens.

Same as H2b-PT-7, many of the recently developed FIR-II fluorescence probes accumulate in the liver tissue, making them impractical to use in hepatocellular carcinoma (HCC) surgery. Tang C. et al. reported a novel near-infrared probe for HCC by targeting overexpressed histone deacetylases (HDACs) in HCC[13]. Suberoylanilide hydroxamic acid (SAHA) was used to target HDAC, and was labelled with fluorescein isothiocyanate (FITC) and IRDye800CW for in vitro and in vivo imaging respectively (Fig. 8a). Tang demonstrated the specificity of FITC-SAHA by contrasting cellular uptake of HCC tumor cell Bel-7402 and normal liver cell LO2, as shown in Figure 8b. In Figure 8d to e, in vivo FIR imaging from 0 to 48 hour after injection of IRDye800CW-SAHA was performed, as well as the blocking group with excess unlabeled SAHA. The fluorescence signal is clearly visible as indicated by the dotted circle in Figure 8e with high tumor-to-background ratio (TBR) as shown in Figure 8f. The tumor uptake was further verified by ex vivo NIR imaging (Fig. 8g) and significant difference in FMI radiant efficiency of the imaging group and the blocking group.

By introducing Se atoms and amino groups to D-A-D fluorophore benzo[1,2-c:4,5-c']bis[1,2,5]thiadiazole (BBTD), Fang et al. developed a novel small molecule organic fluorophore FM1210 (Fig. 9a), which increased the emission wavelength more than 200 nm comparing to BBTD-based fluorophores (800-1000 nm)[14]. As shown in Figure 9b and c, the peak wavelength emission is 1210 nm while the analogue CF1065 is around 1100 nm. The increase in emission wavelength allows FM1210 to produce higher resolution and signal-to-background ratio. The in vivo imaging of BALB/c nude mice have demonstrated that FM1210 yields higher resolution than CF1065 (Fig. 9d, e). This advancement opened the door for future specific molecular fluorescence probes in the NIR-II window.

![Fig 8.](image-url)
or FITC treatment. (d) In vivo fluorescence images of Bel-7402 subcutaneous tumor-bearing mice before and after IRDye800CW-SAHA administration. (e) Fluorescence images with excess SAHA blocking. (f) The TBR of FMI profiles for the imaging and blocking groups. (g) Ex vivo fluorescence imaging (Labels: T tumor, H heart, L liver, S spleen, L lung, K kidney, I intestines), (h) Quantitative analysis of fluorescence intensity of the dissected tumors and organs. *P < 0.05.

Fig 9. (a) Structures of NIR-II Fluorophores FM1210 and CF1065 (Control). (b) Fluorescence spectra of FM1210 and (c) CF1065 under the excitation of 980 and 808 nm in different solvents. (d) Representative NIR-II fluorescence images of BALB/c nude mice (n = 3) with FM1210 and (e) CF1065 after intravenous injection with FM1210 and CF1065 500 (μM) in 150 μL saline solution.

3. Polymers applied for NIR-II imaging

Based on a donor-acceptor (D-A) structure, conjugated polymers and semiconducting polymers have received more and more attention in recent years [15–21]. Thanks to the sizeable conjugated backbone, the excitation of small organic fluorophores are turned to a long wavelength window. Due to the strong electron-withdrawing abilities, the bandgap was lowered and an absorption peak has resulted in the NIR-II window. Therefore, they often exhibit excellent penetration depth, desirable in vivo imaging stability and advanced photothermal conversion efficiency. They are good candidates especially for photoacoustic imaging (PAI) and photothermal therapy (PTT).

For example, Huang et al. developed a series of quinoid polymers which can control the intramolecular charge transfer (ICT), thus maintaining the low band gap and improving the NIR-II fluorescence intensity (Fig. 10a) [22]. They found that the electron-withdrawing groups' density decreased as the thiophene chain length increased from TT-T to TT-3T, leading to brighter NIR-II fluorescence (Fig. 10b). TT-3T polymer probes (CPs) showed the brightest NIR-II fluorescent signals among the three quinoid polymer probes after the absorption spectrum and fluorescence emission spectrum examinations. To further confirm the long-lasting stability of TT-3T NPs, in vivo cell tracking with TT-3T CPs was conducted in live mice. As shown in Fig. 10c, the injected region could be clearly differentiated through bright NIR-II fluorescence even after ten days, which sheds light on physiological imaging, and images of the blood vascular system and lymphatic drainage supports the excellent in vivo NIR-II imaging quality of TT-3T CPs. Moreover, aimed at weakening the ICT to produce bright fluorescence signals, their "dilute electron-withdrawing side chains" strategy provided a convenient and effective approach for designing high-performance NIR-II conjugated polymer probes.

In addition, Pu et al. reported the first broadband absorbing semiconducting polymer nanoparticles (SPN-II) which can excite PA signals in both NIR-I and NIR-II windows, and analyzed its applications in PA imaging in NIR-II window (Fig. 11a-11c) [23]. They first compared PA images between NIR-I and NIR-II regions and reported that NIR-II photoacoustic imaging (PAI) merits weaker background
signals, higher signal-to-noise ratio (SNR), and better imaging depth. When comparing at the imaging depth of 3 cm, the SNR of SPN-II resulted in PA images at 1064 nm can be 1.4-times higher than that at 750 nm. (Fig. 11e-11f). Following in vivo imaging in brain vasculature of living rats using proof-of-concept in NIR-II PA imaging showed 1.5-times higher SNR than NIR-I PA imaging (Fig. 11g-11h).

Fig 10. (a) Chemical structures of the conjugated polymer. (b) NIR-II fluorescence intensity of 4T1 breast cancer cells after incubation with 0.1 mg mL−1 TT-3T CPs at 37 °C for 1, 6, and 12 h. (c) In vivo fluorescence images of a mouse at 0, 3, 6, and 10 days after subcutaneous injection with 1 × 10^6 of 4T1 cancer cells cultured with a 0.1 mg mL−1 solution of the TT-3T CPs (white arrows point to the injection region). The scale bar in b indicates 10 mm. (d) Absorption spectra and (e) fluorescence emission spectra of the three conjugated polymers at the same concentration in THF.

Fig 11. (a) Synthetic route of SP2. (b) Chemical structure of SP1. (c) Schematic illustration for preparation of SPN-II via nanoprecipitation method. (d) absorption spectra of SPN-I and SPN-II, (e)
PA images of an agar gel phantom containing SPN-II solutions at different depths, (f) SNR at 1064 or 750 nm as a function of chicken breast tissue depth, (g) PA images of brain vasculature at different wavelengths and (h) SNR of the NIR-I and NIR-II windows. (d–h) were reproduced from ref.23 with permission from the American Chemical Society, Copyright 2017.

Recently, Pu et al. designed a sequence of SPN-based NIR-II PA probes which can be metabolized (named SPN-PT, SPN-OT, and SPN-DT) for deep brain vasculature and subcutaneous tumors imaging(Fig. 12a) [24]. The nanoprobes with spherical morphology (Fig. 9a) had a broad-band absorption ranging from 700 nm to 1350 nm (Fig. 9b-9c), and SPN-PT among them showed the highest photothermal conversion efficiencies (53%), which was used in following in vitro and in vivo experiments. After that, they tested the PA amplitude of the brain cortex, fresh blood, muscles, and skin(Fig. 12d) and found that the PA signal was intensified after intravenous injection and culminated at 8 hours later. Obviously, at 2 min after injection, the superior sagittal sinus (i in Fig. 12e), transverse sinus (ii in Fig. 12e), vascular branches (iii in Fig. 12e), and middle cerebral arteries (iv in Fig. 12e) could be observed. However, what was surprising was that they could still be seen after three hours because of the long blood circulation of SPN-PT, which verified its long-time stability and implied its application in PAI and PTT.

Furthermore, Liu et al. developed a HA-decorated semiconducting nanoparticle based on thienoisoindigo derivative-based Donor-Acceptor (D-A) polymer (BTPBFDTTS), which successfully shifted the absorption range from NIR-I to NIR-II window and showed intense NIR-II absorption, manifesting excellent absorption characteristics (Fig. 13a)[25]. After that, examinations on photothermal properties of NPsBTPBFDTTS @HA nanoparticles and cellular uptake and photothermal effect of NPs in vitro showed better penetrability under 1064 nm than 808 nm and excellent targeting effects. Cytotoxicity tests suggested that the as-prepared NPBTPBFDTTS@HA NPs showed little toxicity for Hela cells (Fig 13b-13d) in darkness, and in vivo toxicity evaluation, the organs of all groups exhibited...
negligible damages, indicating excellent biocompatibility of the NPs in vivo (Fig. 14a-14c). The NPs exhibited excellent stability, photothermal conversion ability and photoacoustic imaging effect in the second near-infrared window, thus indicating it as a potential photothermal agent for in vivo photothermal therapy and anti-cancer use.

In order to increase the photothermal conversion efficiency, Fan et al. designed a triblock conjugate polymer based on diketopyrrolopyrrole named POEGMA-b-PDPP-b-POEGMA, which showed highly water solubility and greater dispersion in aqueous condition owing to the decoration of the water-soluble polymer POEGMA (Fig. 15a-15b) [26]. The polymer showed an extremely high quantum yield(QY) of 1.0%, exceeding most NIR-II probes which have been reported, and the NIR-II images showed a great imaging depth of nearly 7 mm (Fig. 16a). The photothermal conversion efficiency was calculated to reach 52%, exceeding most reported PTT agents. This exhibited excellent thermal ablation ability during cancer treatment, which was verified by in vitro and in vivo examinations on Hep G2 cells (Fig. 15c,16b). Finally, the cytotoxicity test exhibited only slight damage caused by POEGMA-b-PDPP-b-POEGMA, indicating its low cytotoxicity and high biocompatibility. This was also confirmed by the in vivo tumor inhibition experiment on Hep G2 tumor-bearing mice. Meanwhile, this polymer could turn into a broad range of water-soluble polymers through other monomers modifications and broaden its expectation for biomedical applications.

Fig 13. (a) Synthetic routes of BTPBFDT. (b)Relative viability of Hela cells under different treating conditions. (c) Fluorescent microscopy images of live/dead staining and (d) Cell death mechanism evaluation of Hela cells cultured with different materials without or with 1064 nm laser irradiation (0.8 W/cm2 ,10 min).
Figure 14. (a) H&E staining of major organs of mice after indicated 3 treatments for 16 days. (b) Tumor volume change curves of every group of mice after 1064 nm laser treatment. (c) Confocal fluorescent microscopy images of Hela cells cultured with NPsBTPBFDT S@HA, HA+NPsBTPBFDT S@HA and NPsBTPBFDT S for 6 h. Scale bar is 20μm.

Fig 15. (a) Synthesis procedures for POEGMA-b-PDPP-b-POEGMA. (b) Fluorescence (FL) spectra of POEGMA-b-PDPP-b-POEGMA in H2O. (c) Cell viabilities of Hep G2 cells incubated with POEGMA-b-PDPP-b POEGMA (0.10–0.50 mg mL−1) for 4 h without or with laser irradiation.
Fig 16. (a) NIR-II images of POEGMA-b-PDPP-b-POEGMA at different depths (3, 5, and 7 mm). (b) Thermal images of Hep G2 tumor-bearing tumor tissue samples from mice injected intravenously with POEGMA-b-PDPPb-POEGMA. (c) Quantitative analysis of temperature varies in the tumor area at different post-injection times. The black line represents POEGMA-b-PDPP-b-POEGMA and the red one represents saline.

Furthermore, Xie et al. synthesized a narrow bandgap D-A conjugated polymer (TBDOPV-DT) used as photothermal material, while 2,2-bithiophene worked as the donor and thiophene-fused benzodifurandione-based oligo(p-phenylenevinylene) as the acceptor (Fig. 17a-17b) [27]. They found that TPDOPV-DT not only possess excellent photoacoustic imaging (PAI), but photothermal therapy (PTT) ability as well. TPDOPV-DT showed an excellent photothermal conversion efficiency of 50% and exhibit great photostability (Fig 17c). Comparisons between NIR-I and NIR-II lasers used chicken breasts exhibited that TPDOPV-DT irradiated by laser in the NIR-II window can achieve much stronger tissue penetration and less decrease of photothermal heating than that in NIR-I window (Fig 17d). Simultaneously, cytotoxicity was examined to discover its extremely low risk and outstanding PTT efficacy of TBDOPV-DT NPs with 1064 nm laser irradiation. And following in vitro and in vivo tests based on HepG2 and HeLa cells verified obvious efficient in tumor elimination with little side effect (Fig. 17e-17g). The results showed that TBDOPV-DT NPs can be an excellent candidate for imaging-guided cancer therapy, and worked as an example to shed light on other polymer photothermal agents in NIR-II optical window.
Fig 17. (a) The preparation of TBDOPV-DT NPs. (b) Absorption spectra and picture (inset) of TBDOPV-DT (THF, left) and TBDOPV-DT NPs (water, right). (c) The photothermal response of TBDOPV-DT NPs under 1064 nm laser irradiation (0.90 W cm⁻²) and 10 min later laser was shut off. (d) Relative temperature elevations of IR780 NPs and TBDOPV-DT NPs in water upon exposure to 808 and 1064 nm laser under chicken breast muscles of varying thicknesses (0, 1, 2, 3, 4, 6 and 8 mm).

Cytotoxicity against cancer cells. (e) Cell viabilities of HepG2 cells under incubation of TBDOPV-DT NPs without or with 1064 nm laser irradiation. In vivo PTT of tumors. The whole-body IR images of mice after injection of TBDOPV-DT NPs (f) intratumorally (0.56 mg kg⁻¹) and irradiated by 1064 nm laser (0.90 W cm⁻²) or (g) intravenously (1.94 mg kg⁻¹) and irradiated by laser (1.30 W cm⁻²).

There are some other relevant researches that contributed to the application of NIR-II imaging. For example, Parmanik et al. designed novel SPNs based on poly (thienoisoindigo-alt diketopyrrolepyrrole) (PIGD) with high structural planarity through the nanoprecipitation method, which had an absorption in the NIR-II window (Fig 18b) [18]. PIGD possesses extraordinary PA imaging abilities and physiological stability, thus can be used for deep-tissue contrast-enhancement in vivo PAI in the NIR-II window (Fig. 18a) [28, p.]. Moreover, Liu et al. designed NIR-II window D–A CP (P1) which was highly biocompatible along with low cytotoxicity, good PA stability, broad-band absorption, and imaging ability in both the NIR-I and NIR-II windows through D–A selection. Such D-A CP has been applied to NIR-II PA imaging of an orthotopic brain tumor (Fig. 18c) [29]. PA imaging showed lowered background interference at 1064 nm, and in vivo imaging revealed an extremely low PA background signal (Fig. 18d). PA signal examination exhibited excellent penetration properties of P1 probe. This was the first time that NIR-II PAI of orthotopic brain tumors were excellently performed using organic molecules. Subsequently, Liu et al. developed CP NPs with wide-band absorption in both NIR-I and NIR-II windows through the nanoprecipitation method. Such NPs was soon modified with cRGD (an active targeting ligand) for targeted PAI and imaging-guided PTT of brain tumors (Fig. 19a-19b) [30]. The modification with c-RGD NPs exhibited some merits, such as the 2-fold higher PA signal of the probe group at 3h post-injection and 1.85-fold signal/background ratio at 24h post-injection (Fig. 
More importantly, during the study of orthotopic brain tumor models, the tumor was specifically detected by PA imaging, while the skull, areas near the tumor, blood, and tissue around the tumor were not detected, showing the superiority of the 1064 nm laser for PA imaging of tumor (Fig. 19e). Last but not least, Bian et al. reported a positively charged OSPN+ nanoprobe which exhibited excellent PA stability (Fig. 20a-20b) [31]. OSPN+ -labeled cell pellets showed 3 times higher PA intensities than that of the OSPNs-labeled cell pellets. Besides, compared to the NIR-I window, it expressed higher imaging capacity and a higher SNR in the NIR-II window (Fig. 20c-20d). It also exhibited great increasing NIR-II PAI in in vivo tests (Fig. 20e-20f). The properties mentioned can indicate its applications in stem-cell imaging, labeling and tracking.

Fig 18. (a) Proposed mechanism of NIR-II absorbing NPs based on PIGD. (b) Schematic illustration of the fabrication of P1RGD nanoparticles (NPs) and their application in brain tumor treatment. (c) absorption spectra of P1 NPs, (d) PA scanning of brain of tumor-bearing mice at different excitation wavelengths. (a) was reproduced from ref. 28 with permission from the Society of Photo-Optical Instrumentation Engineers, Copyright 2019, (b–d) were reproduced from ref. 29 with permission from the Royal Society of Chemistry, Copyright 2017.
Fig 19. (a) Schematic illustration of the fabrication of P1RGD nanoparticles (NPs) and their application in brain tumor treatment. (b) absorption spectrum of P1 NPs and P1RGD NPs, (c) quantitative results of brain tumor PA intensity, (d) in vivo PA imaging of the subcutaneous U87 tumor model at different time points. (e) PA imaging of a mouse brain tumor through the scalp and skull. (a-e) were reproduced from ref. 30 with permission from Wiley-VCH, Copyright 2018.

Fig 20. (a) Chemical structure of SP. (b) absorption spectra of OSPNs+, (c) PA signal intensity of the OSPNs+ under laser excitation at different wavelengths. (d) SNR of PAI as a function of PA imaging
depth, (e) combined ultrasound and PAI of subcutaneously transplanted unlabeled or OSPNs+-labeled hMSCs, and (f) merged ultrasound and PAI and SNR of brain transplanted unlabeled or OSPNs+-labeled hMSCs. (a-f) were reproduced from ref. 31 with permission from the American Chemical Society, Copyright 2018

4. Conclusions

As illustrated by numerous study findings, organic molecules, both small molecules and polymers, are desirable agents used in the NIR-II window thanks to their deeper penetrating abilities, high stability, and lower cytotoxicity. Previous studies mentioned several types of NIR-II nanomaterials such as metal sulfides, noble metals and mxenes, while our work described organic NIR-II materials in different categories in detail. Though effective, it should be noticed that organic NIR-II imaging is still an emerging field. There are challenges on the way, as the results show some uncertainty, and numerous endeavors in clinical research are still required to contribute to the development of organic NIR-II imaging. Moreover, optimizing the organic materials to overcome biosafety barriers and strengthen biocompatibility should also be focused on. Anyway, organic materials have exhibited their potential in PAI and PTT fields. We hope our article could shed light on researches about organic NIR-II imaging agents and promote innovations and applications in biomedicine.

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