NIR-II emissive AIEgen photosensitizers enable ultrasensitive imaging-guided surgery and phototherapy to fully inhibit orthotopic hepatic tumors

Ruizhen Jia1†, Han Xu1†, Chenlu Wang2†, Lichao Su2, Jinpeng Jing1, Shuyu Xu1, Yu Zhou3, Wenjing Sun1, Jibin Song2, Xiaoyuan Chen4,5 and Hongmin Chen1*

Abstract
Accurate diagnosis and effective treatment of primary liver tumors are of great significance, and optical imaging has been widely employed in clinical imaging-guided surgery for liver tumors. The second near-infrared window (NIR-II) emissive AIEgen photosensitizers have attracted a lot of attention with higher-resolution bioimaging and deeper penetration. NIR-II aggregation-induced emission-based luminogen (AIEgen) photosensitizers have better phototherapeutic effects and accuracy of the image-guided surgery/phototherapy. Herein, an NIR-II AIEgen phototheranostic dot was proposed for NIR-II imaging-guided resection surgery and phototherapy for orthotopic hepatic tumors. Compared with indocyanine green (ICG), the AIEgen dots showed bright and sharp NIR-II emission at 1250 nm, which extended to 1600 nm with high photostability. Moreover, the AIEgen dots efficiently generated reactive oxygen species (ROS) for photodynamic therapy. Investigations of orthotopic liver tumors in vitro and in vivo demonstrated that AIEgen dots could be employed both for imaging-guided tumor surgery of early-stage tumors and for ‘downstaging’ intention to reduce the size. Moreover, the therapeutic strategy induced complete inhibition of orthotopic tumors without recurrence and with few side effects.

Keywords: NIR-II imaging, AIE, Imaging-guided surgery, Phototherapy, Orthotopic hepatic tumors
**Introduction**

Liver cancer is globally the seventh most frequent cancer and the third leading cause of cancer-related death [1]. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer [2]. Surgical resection is one of the highly effective approaches for liver cancer [3]. Clear visualization of the tumor margin during surgery is of utmost importance for the real-time surgical guidance [4, 5].

As a sensitive, noninvasive, and radiation-free technology, the first near-infrared window (NIR-I) fluorescence imaging has been widely employed in clinical imaging-guided surgery for liver tumors and retinal angiography [6, 7]. However, the following disadvantages limit further applications of NIR dyes: (1) autofluorescence of tissues, which decreases the sensitivity and signal-to-noise ratio; (2) photobleaching; (3) aggregation-caused quenching (ACQ) effect. Therefore, much effort has been made to develop new luminescent agents to achieve excellent imaging ability [8–13]. In 2001, Tang group found an optical property of aggregation-induced emission (AIE) based on the restriction of intramolecular motion mechanism [14]. Moreover, AIE could be extended to the second near-infrared window (NIR-II, 900–1700 nm) [15–20]. NIR-II allows for higher-resolution bioimaging with deeper penetration (ca. 5–20 mm) compared with the visible and the NIR-I bands (ca. 1–3 mm) [21–23]. These advantages make NIR-II fluorescent agents suitable for broad applications in whole-body angiography, organ visualization, and tumor diagnosis and imaging-guided therapy [24–28].

Metal-containing inorganic NIR-II dots have been shown to have higher luminescence than the NIR-I equivalents; however, one major concern is their potential toxicity after decomposition within the body [29–31]. As an alternative, NIR-II organic dyes have attracted more attention because of their relatively low toxicity, good biocompatibility and pharmacokinetics, as well as their well-defined structure [32–35]. Although many NIR-II fluorophores have been explored as imaging agents, the emission is easily quenched due to the dominant nonradiative decay caused by intense intermolecular π–π interactions [35–40]. Furthermore, most NIR-II fluorescent dyes have a single imaging function and need to be combined with NIR-II activated photosensitizers to realize phototherapy [41–47]. Recent studies have shown that NIR-II agents could achieve both fluorescence and photothermal (PTT) or photodynamic (PDT) processes,
thereby boosting NIR-II imaging-guided surgery to achieve optimal effect [48–56]. Surgical resection is the treatment option for a small number (<30%) of patients with early-stage liver cancers who have normal liver function [57]. Given that patients with large or multiple HCC cannot undergo surgery, ‘downstaging’ pretreatment may be required to reduce the size or number of active tumors [57–60]. Therefore, it is urgent to design and synthesize NIR-II emissive agents with both fluorescence imaging and phototherapeutic ability in their aggregation state.

Herein, we designed and successfully synthesized a novel AIE-based NIR-II photosensitizer with donor–acceptor–donor (D–A–D) structure (Scheme 1). We compared the optical properties of the photosensitizer with clinically used indocyanine green (ICG) and evaluated its ability to generate reactive oxygen species (ROS). Furthermore, we investigated the efficacy of imaging-guided surgical resection of orthotopic early-stage liver tumor and ‘downstaging’ intention of large HCC.

Results and discussion

Synthesis and characterization of PTZ-TQ dye

A Suzuki cross-coupling reaction between compound 3 and compound 6 formed the final dye with a D–A–D architecture, 7,7’-(6,7-diphenyl-[1,2,5]thiadiazolo[3,4-g]quinoxaline-4,9-diyl)bis(10-octyl-10H-phenothiazine) (termed PTZ-TQ) (Fig. 1a). The total synthesis route is shown in Additional file 1: Fig. S1. The D–A–D structure was confirmed by nuclear magnetic resonance (NMR) spectroscopy and ESI−MS analysis (Additional file 1: Figs. S2–S12). The D–A–D structure exhibited excellent NIR absorption property at 650 nm in THF and showed enhanced absorption at 660 nm, with the absorption tail extending to 900 nm in THF–water mixtures with water volume fractions ($f_w$) of 95% (Fig. 1b). The photoluminescence (PL) spectra of PTZ-TQ in THF–H$_2$O were measured in 0–95% $f_w$. As indicated, under 808-nm excitation, the PTZ-TQ solution emitted almost no luminescence even after increasing the $f_w$ up to 40%. Then, the emission of PTZ-TQ enhanced dramatically when the $f_w$ exceeded 50% (Fig. 1c). Together with the plot of emission intensity at 1000 nm vs. $f_w$, the higher $f_w$ and the stronger emission indicate typical AIE characteristics (Fig. 1d) [14].

The emission of PTZ-TQ was measured in the THF–water mixture with an $f_w$ of 95%. Under 808-nm excitation, the PTZ-TQ solution exhibited significant NIR-II fluorescence signals at 1050 nm and 1150 nm (Fig. 1e). Then, the penetration depth of fluorescence at 1050 nm...
was studied using 1% intralipid as the mimic of tissue. Compared with commercial ICG dye, edges were clearly visualized up to a depth of 6 mm in PTZ-TQ (Fig. 1f). Density functional theory (DFT) calculations were carried out to explore the relationship between the structure and the emission property of the PTZ-TQ. Obviously, the highest occupied molecular orbitals (HOMOs) were delocalized along the whole backbone, while the lowest unoccupied molecular orbitals (LUMOs) were mainly distributed on electron acceptor moieties, indicating the intramolecular charge transfer of the fluorophore [61]. It is generally believed that stronger D–A effect is associated with smaller singlet–triplet energy gap (Fig. 1g). The smaller energy band gap (1.73 eV) would endow PTZ-TQ with longer absorption and would greatly promote the generation of ROS [62, 63]. These features are remarkably beneficial for PTZ-TQ to achieve NIR-II AIE imaging and therapy.

**Preparation and characterization of PTZ-TQ-AIE dots**

PTZ-TQ-AIE dots were prepared by a nanoprecipitation method using 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-3400] (DSPE-PEG$_{3400}$-NH$_2$) as the encapsulation matrix. The size of the PTZ-TQ-AIE dots was about 80 nm as measured by transmission electron microscopy (TEM) and 255 nm as measured by dynamic light scattering (DLS) analysis (Fig. 2a, b). After loading into DSPE-mPEG$_{3400}$-NH$_2$, PTZ-TQ-AIE dots showed a positive surface charge (about 7 mV) and had high stability in buffer and biological fluids (PBS, DMEM, and FBS solution) (Additional file 1: Figs. S13–S16). The UV–vis-NIR absorption and NIR-II fluorescence emission spectra of the PTZ-TQ-AIE dots in water showed that the absorption peak at 675 nm was extended to 900 nm, and the fluorescence emission peak located at 1150 nm with emission tail was extended to close to 1600 nm (Fig. 2c). The quantum yield (QY) of
PTZ-TQ-AIE dots in aqueous solution was 0.3% under 808-nm laser excitation, using dye 4-(7-(2-phenyl4H-1-benzothiopyran-4-ylidene)-4-chloro-3,5-trimethylenyl1,3,5-heptatrienyl)-2-phenyl-1-benzothiopyrylium perchlorate (IR26) in dichloroethane (DCE) (QY = 0.5%) as the reference [64]. Importantly, the PTZ-TQ-AIE dots showed superior photostability in media (PBS, FBS and DMEM). No obvious changes were observed in the absorption and emission spectra of the PTZ-TQ-AIE dots when the mixture was irradiated continuously with a 808-nm laser at a power density of 0.25 W/cm² for up to 30 min (Fig. 2d, e). Furthermore, the luminescence intensities of PTZ-TQ-AIE dots in phosphate-buffered saline (PBS), Dulbecco's modified Eagle medium (DMEM), and fetal bovine serum (FBS) showed no changes after continuous irradiation for 60 min under the same density (Fig. 2f). The photostability was far better than that of the commercial ICG dye, indicating that PTZ-TQ-AIE dots have an excellent potential for long-term in vivo fluorescent imaging.

Figure 2c shows that PTZ-TQ-AIE dots emitted luminescence in the NIR-II region (1400–1700 nm); so, we investigated the fluorescence properties of PTZ-TQ-AIE dots in NIR-IIb. The NIR-IIb fluorescence intensity was measured by different band-pass filters. PTZ-TQ-AIE dots exhibited better NIR-IIa (1000–1300 nm) and NIR-IIb (1400–1700 nm) fluorescence signals than PTZ-TQ (fₚ = 95%) (Fig. 2g). Furthermore, the penetration depths of fluorescence at 1050 nm indicated that PTZ-TQ-AIE dots resolved sharper edges of the capillary at a depth of up to 8 mm; under the same conditions, ICG showed similar resolution only at a depth of 4 mm (Fig. 2h).

The ROS generation ability of the PTZ-TQ-AIE dots was assessed using dichlorodi-hydrofluorescein diacetate
(DCFH-DA) as the indicator. Fluorescence of DCFH-DA increased sharply with increasing irradiation time in the presence of PTZ-TQ-AIE dots (808 nm, 0.25 W/cm²) (Additional file 1: Fig. S18). For further verification, electron paramagnetic resonance (EPR) was applied to verify the generation of ROS. After incubating 5 mM of an ROS indicator, 4-oxo-2,2,6,6-tetramethylpiperidinooxy (TEMPONE), with PTZ-TQ-AIE dots in solution, followed by irradiation for 5 min for EPR measurements, the EPR signal decreased when β-carotene was added (Fig. 3a), demonstrating the efficient production of \(^{1}\text{O}_2\).

To understand the generated species, singlet oxygen sensor green (SOSG) was firstly used to assess the \(^{1}\text{O}_2\) generation. Under the irradiation (808 nm, 0.25 W/cm²) in the presence of PTZ-TQ-AIE dots, the fluorescence of SOSG increased sharply with the increase in irradiation time, confirming that \(^{1}\text{O}_2\) was the predominant ROS (Fig. 3b, c). Subsequently, the \(^{1}\text{O}_2\) quantum yield of the PTZ-TQ-AIE dots was calculated as 10% using ICG as a reference (12%) (Additional file 1: Fig. S19).

As PTZ-TQ-AIE dots have excellent NIR-II emission and ROS generation ability, the potential of such AIE dots in cancer diagnostics and therapeutics was investigated. As shown in Fig. 3d, under irradiation (808 nm, 0.25 W/cm²), bright green fluorescence of DCFH-DA was displayed in the group of PTZ-TQ-AIE dots plus laser, indicating that ROS generation was efficiently induced, and the intracellular ROS generation was quantified by flow cytometry (Additional file 1: Fig. S20). The efficient generation of ROS resulted in cell death (Fig. 3e). However, only irradiation or PTZ-TQ-AIE dots showed negligible red fluorescence, implying the good biocompatibility of the PTZ-TQ-AIE dots and limited harm of the laser irradiation alone (Fig. 3e). Using hepatic LO2 normal cells as a control, in vitro cytotoxicity studies showed that PTZ-TQ-AIE dots had almost no obvious toxic effects on both normal cells and cancer cells at concentrations as high as 100 μg/mL (Fig. 3f). Additionally, PTZ-TQ-AIE dots and irradiation resulted in a significant decrease in cell viabilities, demonstrating in vitro photodynamic therapy.
(PDT) efficacy with minor side effects of the PTZ-TQ-AIE dots themselves.

**In vivo imaging and therapy**

To further investigate the capabilities of NIR-II imaging and photodynamic therapy, the biocompatibility and biodistribution of PTZ-TQ-AIE dots were first evaluated in vivo. After intravenous injection of PTZ-TQ-AIE dots into healthy BALB/c mice (200 µL, 500 µg/mL), blood samples of the mice were collected, and serum chemistry and blood cells were analyzed. No significant changes in the main blood cell counts (Additional file 1: Fig. S21) were seen at day 1 and day 7 post-injection (p.i.), suggesting high biocompatibility of PTZ-TQ-AIE dots. Furthermore, a biodistribution study of PTZ-TQ-AIE dots in vitro was also carried out to evaluate their distribution in major organs at 168 h after injection (Additional file 1: Fig. S22). High accumulation was found in the liver and spleen, indicating that the clearance pathway of PTZ-TQ-AIE dots was through the hepatobiliary system. Compared with the commercial ICG, PTZ-TQ-AIE dots exhibited a relatively long blood-circulation half-life of 61 ± 21 min (Additional file 1: Fig. S23) [65, 66]. After confirming its good biocompatibility, the capability of vasculature imaging using the PTZ-TQ-AIE dots was then investigated. The vasculature was clearly visualized through NIR-II imaging after 2, 5, and 15 min post-injection (Fig. 4a). Furthermore, the resolution (711.8 μm) of the hind limb vasculature was reached via measuring the Gaussian-fitted full width at half maximum (FWHM) (Fig. 4b), which would be adequate for surgical operation [6]. To evaluate the imaging and therapy for deep tissues, an orthotopic liver tumor model was established in nude mice in line with our previous procedure [67]. Human hepatocellular carcinoma HepG2 cells were transfected with firefly luciferase and then inoculated into the right liver lobe by laparotomy. At day 10 after the operation, bioluminescence imaging (BLI) was carried out to monitor the tumor growth. After confirming the successful establishment of the orthotopic liver tumor, the PTZ-TQ-AIE dots (200 µL, 500 µg/mL) were intravenously injected into tumor-bearing mice. As observed, NIR-II fluorescence in both normal liver and the tumor sites was continuously recorded under the excitation of an 808-nm laser. At 2 h post-injection, ex vivo results indicated NIR-II fluorescence signals at the tumor site, which were lower than those at normal liver tissues; importantly, the lower NIR-II signals in the tumors clearly identified the boundary between the tumor and normal liver organs (Additional file 1: Fig. S24). Notably, the accumulation of PTZ-TQ-AIE dots in the tumors reached the maximum at 48 h post-injection (Fig. 4c, Additional file 1: Fig. S25), and bright and sharp NIR-II signals were clearly visualized in the tumor regions at 48 h post-injection. The signal to noise ratio (SNR) gradually increased with time and reached the maxima at 48 h for NIR-II fluorescence imaging (Additional file 1: Fig. S26). To further confirm the tumor position, the whole liver tissue was photographed at 96 h post-injection (Fig. 4d); the location was in accordance with that observed on NIR-II images in vivo (circles in Fig. 4c). These results demonstrate that the boundary between the tumor and normal liver organs can be clearly identified at 2 h post-injection by negative enhancement or at 48 h post-injection by positive enhancement. So, the PTZ-TQ-AIE dots can be employed as imaging-guided surgery both at 2 h and 48 h post-injection, which can provide personalized treatment choice according to the size of tumors.

**NIR-II optical imaging surgical guidance**

Delineating the tumor margin is the key step for precise tumor resection and is essential for tumor curation [4, 68, 69]. As indicated, the tumor margins were clearly delineated, and surgical resection of the tumors was then performed at 2 h or 48 h post-injection of PTZ-TQ-AIE dots using the NIR-II imaging system (Additional file 2: Videos S1 and S2). The resected tissues showed that most of the tumor was removed from the liver, which was confirmed by the bright NIR-II fluorescence signals from the isolated tumor and the hematoxylin and eosin (H&E) results. The boundary between the tumor and normal tissue was clearly visualized, confirming the successful tumor resection under the guidance of NIR-II optical imaging (Figs. 4e, f, Additional file 1: Fig. S27).

**Surgery/PDT on the orthotopic hepatic tumor**

In clinical setting, the tumor removal operation sometimes cannot be performed because of the presence of...
Fig. 4 (See legend on previous page.)
multiple smaller tumors or a very large tumor [70, 71]. Multiple treatments, a common methodology in the clinical setting, is a good choice for patients with large or multiple HCC [72]. Given that PTZ-TQ-AIE dots are strong ROS generators, we investigated the feasibility of PTZ-TQ-AIE dots in photodynamic therapy to reduce the tumor size for further surgical resection and prevent the recurrence of tumors.

Thus, we applied different therapy treatments: for large tumors, we first applied PDT treatments to reduce the tumor size, followed by surgery to minimize the injury of the liver in the therapeutic process (named as PDT + Surgery); for small tumors, we first applied surgery and then PDT treatment for the residual tumors (named as Surgery + PDT). The therapeutic processes were monitored by BLI (Additional file 1: Fig. S28a). The tumor growth with various treatments was summarized using the total BLI intensity and relative tumor growth. As indicated, the BLI signals in control groups (PBS, PBS + Laser, and PTZ-TQ-AIE dots) increased rapidly (Fig. 4g), indicating a high tumor growth rate. Importantly, single therapeutic process (PDT or surgery) induced significant decrease of BLI signals, demonstrating the efficacy of single imaging-guided surgery or PDT in tumor management. However, the tumors in both single therapeutic processes relapsed over time (green and pink curves in Fig. 4g). The therapy in the early stage achieved therapeutic efficacies similar to that of the single therapeutic process but without tumor recurrence during the observation period (purple and dark blue curves in Fig. 4g).

‘Downstaging’ intention for orthotopic hepatic tumors

Notably, we simulated a large HCC model that was not suitable for surgery [73–75] (purple curve in Fig. 4g). In the clinical setting, ‘downstaging’ intention involves pre-treatment to reduce the size or number of active tumors. In our protocol, we first employed PDT to reduce the size. After PDT treatment, the BLI signals decreased sharply at day 7 post-treatment, and then, surgery was conducted to achieve almost complete tumor inhibition (purple curve in Fig. 4g). Magnetic resonance imaging (MRI) of the livers in vivo and photographs ex vivo confirmed that there were almost no tumor nodules in the multiple treatments groups (Fig. 4h, Additional file 1: Fig. S29). Moreover, PTZ-TQ-AIE dots showed good biocompatibility, as indicated by the assessment of body weight, histological analysis of organs, and blood analysis (Additional file 1: Figs. S28b, S30).

Conclusions

In summary, biocompatible NIR-II emissive AIEgen photosensitizers enable ultrasensitive imaging-guided surgery and phototherapy to fully inhibit orthotopic hepatic tumors. Compared with ICG, the PTZ-TQ-AIE dots showed bright and sharp NIR-II emission at 1250 nm, which extended to 1600 nm with high photostability. Moreover, the PTZ-TQ-AIE dots were able to efficiently generate ROS for photodynamic therapy. Investigations of orthotopic liver tumors in vitro and in vivo demonstrated that PTZ-TQ-AIE dots could be employed both for imaging-guided tumor surgery of early-stage tumors and for ‘downstaging’ intention to reduce the tumor size. Moreover, the current therapy achieved full inhibition of orthotopic tumors without recurrence.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12951-021-01168-w.
of orthotopic liver cancer mice treated with PBS, PBS+Laser, PTZ-TQ-AIE dots, PDT, Surgery, Surgery+PDT, PDT+Surgery. Before PDT, AT after PDT. BS: Before surgery, AS: after surgery. (b) body weight curves after different treatments (n=3). Figure S29. MR imaging of orthotopic liver cancer mice treated with PBS, PBS+Laser, PTZ-TQ-AIE dots, PDT, Surgery, Surgery+PDT, PDT+Surgery after therapy. Figure S30. H&E staining of heart, liver, spleen, lung, and kidney tissue slices for different groups after treatments: (a) PBS, (b) PBS+Laser, (c) PTZ-TQ-AIE dots, (d) PDT, (e) Surgery, (f) Surgery+PDT, (g) PDT+Surgery.

Additional file 2. Video of the NIR-II imaging guided surgery in orthotopic liver cancer model after tail vein injection of the PTZ-TQ-AIE dots 2 h and 48 h.

Acknowledgements
We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

Authors’ contributions
RJ, HK and CW contributed equally to this work. RJ, HK and CW proposed the concepts, conceived the experiments, and analyzed data. LS, JJ, SX and WS conducted parts of the synthesis and performed in vitro and in vivo experiments. YZ calculated HOMOs and LOMOs. RJ, WS, JS, XC, and HC discussed the results. RJ, WS, XC and HC wrote the original draft. WS, XC and HC revised the manuscript and offered funding. HC supervised the work. All authors read and approved the final manuscript.

Funding
The work was supported by the National Science Foundation of China (81771977, 82172007, 82001956), the Science Fund for Distinguished Young Scholars of Fujian Province (2021J06007), the National Postdoctoral Program (BX2020196), the National University of Singapore (NUS) School of Medicine Nanomedicine Translational Research Program (NUHSRO/2020/133/Startupt/08), the NUS School of Medicine Kickstart Initiative (NUHSRO/2021/044/Kickstart/09/LOA), the Singapore National Medical Research Council (NMRC), the National University of Singapore Start-up Grant (NUHSRO/2020/133/Startup/08), the National University of Singapore Nanomedicine Translational Research Programmes (NUHSRO/2021/034/TRP/09/Nanomedicine), and the NUS School of Medicine Kickstart Initiative (NUHSRO/2021/044/Kickstart/09/LOA).

Availability of data and materials
All data analyzed during this study are included in this published article and its additional file.

Declarations

Ethics approval and consent to participate
All animal experiments were approved by the Animal Management and Ethics Committee of the Xiamen University.

Consent for publication
All authors agree to be published.

Competing interests
The authors declare no conflict of interests.

Author details
1 State Key Laboratory of Molecular Vaccinology and Molecular Diagnostics & Center for Molecular Imaging and Translational Medicine, School of Public Health, Xiamen University, Xiamen 361102, China. 2 MOE Key Laboratory for Analytical Science of Food Safety and Biology, College of Chemistry, Fuzhou University, Fuzhou 350108, China. 3 State Key Laboratory of Physical Chemistry of Solid Surfaces, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China. 4 Departments of Diagnostic Radiology and Surgery, Clinical Imaging Research Centre, Centre for Translational Medicine, Nanomedicine Translational Research Program, NUS Center for Nanomedicine, Yong Loo Lin School of Medicine, Singapore, Singapore. 5 Departments of Chemical and Biomolecular Engineering, and Biomedical Engineering, Faculty of Engineering, National University of Singapore, Singapore, Singapore.

Received: 23 September 2021 Accepted: 28 November 2021
Published online: 13 December 2021

References
1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jamal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209–49.
2. Bruix J, Reig M, Sherman M. Evidence-based diagnosis, staging, and treatment of patients with hepatocellular carcinoma. Gastroenterology. 2016;150(4):835–53.
3. Kulik L, Heimbach JK, Zaiem F, Almasri J, Prokop LJ, Wang Z, Murad MH, Mohammed K. Therapies for patients with hepatocellular carcinoma awaiting liver transplantation: a systematic review and meta-analysis. Hepatology. 2018;67(1):381–400.
4. Wang P, Fan Y, Lu L, Liu L, Fan L, Zhao M, Xie Y, Xu C, Zhang F. NIR-II nanoprobes in vivo assembly to improve image-guided surgery for metastatic ovarian cancer. Nat Commun. 2019;10(1):2898.
5. Zhan Y, Ling S, Huang H, Zhang Y, Chen G, Huang S, Li C, Guo W, Wang Q. Rapid unperturbed-tissue analysis for intraoperative cancer diagnosis using an enzyme-activated NIR-II nanoprobe. Angew Chem Int Ed. 2021;113(5):2669–74.
6. Hu Z, Fang C, Li B, Zhang Z, Cao C, Cai M, Su S, Sun X, Shi X, Li C. First-in-human liver-tumour surgery guided by multispectral fluorescence imaging in the visible and near-infrared-II windows. Nat Biomed Eng. 2020;4(3):259–71.
7. Novotny HR, Alvis DL. A method of photographing fluorescence in circulating blood in the human retina. Circulation. 1961;24(1):82–6.
8. Hong G, Antaris AL, Dai H. Near-infrared fluorophores for biomedical imaging. Nat Biomed Eng. 2017;1(1):0010.
9. Younis MR, Wang C, An R, Wang S, Younis MA, Li Z-Q, Wang Y, Ihsan A, Ye D, Xia X-H. Low-power single-laser activated synergistic cancer phototherapy using photosensitizer functionalized dual plasmonic photothermal nanoagents. ACS Nano. 2019;13(2):2544–57.
10. Li X, Lovell JF, Yoon J, Chen X. Clinical development and potential of photothermal and photodynamic therapies for cancer. Nat Rev Clin Oncol. 2020;17(11):657–74.
11. Zheng J, Liu Y, Song F. A nitroreductase-activatable near-infrared theranostic photosensitizer for photodynamic therapy under mild hypoxia. Chem Commun. 2020;56(43):5819–22.
12. Li T, Liu L, Xu P. Multifunctional nanotheranostic agent for NIR-II imaging-guided synergistic photothermal/photodynamic therapy. Adv Ther. 2021;41(3):2000240.
13. Zhao J, Yan K, Xu G. An Ir(III) complex bearing a donor–acceptor–donor type ligand for NIR-triggered dual phototherapy. Adv Funct Mater. 2021;31(11):2008325.
14. Luo J, Xie Z, Lam JW, Cheng L, Chen H, Qu C, Kwok HS, Zhan X, Liu Y, Zhu D. Aggregation-induced emission of 1-methyl-1, 2, 3, 4, 5-pentaphenylsilole. Chem Commun. 2001;18:1740–1.
15. Gao S, Wei G, Zhang S, Zheng B, Xu J, Chen G, Li M, Song S, Fu W, Xiao Z. Albumin tailoring fluorescence and photothermal conversion effect of near-infrared-II fluorophore with aggregation-induced emission characteristics. Nat Commun. 2019;10(1):2206.
16. Deng G, Peng X, Sun Z, Zheng W, Yu J, Du L, Chen H, Gong P, Zhang P, Cai L. Natural-killer-cell-inspired nanorobots with aggregation-induced emission characteristics for near-infrared-II fluorescence-guided glioma theranostics. ACS Nano. 2020;14(9):11452–62.
17. Alfuso N, Zebibula A, Qi J, Zhang H, Sun C, Yu X, Xue D, Lam JW, Li G, Qian J. Single-molecule near-infrared-II theranostics: ultrasensitive aggregation-induced emission nanoparticles for long-term tracing and efficient photothermal therapy. ACS Nano. 2018;12(11):11282–93.
18. Li Y, Cai Z, Liu S, Zhang H, Wong ST, Lam JW, Kwok RT, Qian J, Tang BZ. Design of AIEgens for near-infrared IIb imaging through structural modulation at molecular and morphological levels. Nat Commun. 2020;11(1):1255.
Gao S, Yu S, Zhang Y. Molecular engineering of near-infrared-II photosensitizers with sterically-hindrance effect for image-guided cancer photodynamic therapy. Adv Funct Mater. 2021;31(14):2008356.

Zhang L, Xue S, Ren F. An atherosclerotic plaque-targeted single-chain antibody for MR/NIR-II imaging of atherosclerosis and anti-atherosclerosis therapy. J Nanobiotechnol. 2021;19:296.

Smith AM, Mancini MC, Nie S. Second window for in vivo imaging. Nat Nanotechnol. 2009;4(11):710–1.

Haque A, Faizi MSH, Rather JA, Khan MS. Next generation NIR fluorophors for tumor imaging and fluorescence-guided surgery: a review. Biomed J. 2017;35(7):17–34.

Zhang N, Lu C, Chen M. Recent advances in near-infrared II imaging technology for biological detection. J Nanobiotechnol. 2021;19:132.

Hong G, Lee JC, Robinson JT, Raaz U, Xie L, Huang NF, Cooke JP, Dai H. Multifunctional in vivo vascular imaging using near-infrared II fluorescence. Nat Med. 2012;18(12):1841–6.

He S, Song J, Qu J, Cheng Z. Crucial breakthrough of second near-infrared biological window fluorophores: design and synthesis toward multimodal imaging and theranostics. Chem Soc Rev. 2018;47(12):4258–78.

Hong G, Diao S, Chang J, Antaris AL, Chen C, Zhang B, Zhao S, Atochin DN, Huang PL, Andresson KI. Through-skin fluorescence imaging of the brain in a new near-infrared window. Nat Photon. 2014;8(8):723–30.

Xu F, Hu L, Yu C. Unsymmetrical cyanine dye via in vivo hitchhiking endogenous albumin affords high-performance NIR-I/NIR-II multimodal imaging and photothermal therapy. J Nanobiotechnol. 2021;19:334.

Guo B, Chen J, Chen N. High-resolution 3D NIR-II photocoating imaging of cerebral and tumor vasculatures using conjugated polymer nanoparticles as contrast agent. Adv Mater. 2019;31(25):1803555.

Naczynski D, Tan M, Zevon M, Wall B, Kohl J, Kulesa A, Chen S, Roth C, Riman R, Moghe P. Rare-earth-doped biological composites as contrast agent. Adv Mater. 2019;31(15):1803558.

Franko D, Harris DK, Chen O, Bruns OT, Carr JA, Wilson MW, Bawendi MG. Continuous injection synthesis of indium arsenide quantum dots emissive in the short-wavelength infrared. Nat Commun. 2016;7(1):12759.

Antaris AL, Robinson YT, Yaghi OK, Hong G, Diao S, Luong R, Dai H. Ultra-low doses of chirality sorted (5, 5) carbon nanotubes for simultaneous tumor imaging and photothermal therapy. ACS Nano. 2021;15(4):6445–57.

Guo B, Feng Z, Hu D. Precise deciphering of brain vasculatures and microscopic tumors with dual NIR-II fluorescence and photocoating imaging. Adv Mater. 2019;31(30):1902504.

Guo B, Sheng Z, Hu D. Through scalp and skull NIR-II photothermal therapy of deep orthotopic brain tumors with precise photocoating imaging guidance. Adv Mater. 2018;30(35):1802591.

Yang Q, Ma H, Liang Y, Dai H. Rational design of high brightness NIR-II organic dyes with SDDAS structure. Adv Mater. 2021;33(13):1902504.

Lei Z, Zhang F. Molecular engineering of NIR-II fluorophores for improved biomedical detection. Chem Soc Rev. 2021;30(13):16430–44.

Ren TB, Wang ZY, Xiang Z, Lu P, Dai HH, Yuan L, Zhang XB, Tan W. A general strategy for development of activatable NIR-II fluorophoric probes for in vivo high-contrast bioimaging. Angew Chem Int Ed. 2021;133(2):813–8.

Liu S, Li Y, Kwok RT, Lam JW, Tang BZ. Structural and process controls of AIEgens for NIR-II theranostics. Chem Sci. 2021;12(10):3427–36.

Zhong J-H, Liu B. Strategies to overcome the limitations of AIEgens in photodynamic therapy of orthotopic brain tumors. Adv Mater. 2018;30(29):1800766.

Yu W, Guo B, Zhang H. NIR-II fluorescence in vivo confocal microscopy with aggregation-induced emission dots. Sci Bull. 2019;64(6):410–17.

Raza A, Sood GK. Hepatocellular carcinoma review: current treatment, and evidence-based medicine. World J Gastroenterol. 2014;20(15):4115–27.

Zhong J-H, Lu S-D, Wang Y-Y, Ma L, Li-L-Q. Intermediate-stage HCC—upfront resection can be feasible. Nat Rev Clin Oncol. 2015;12(5):2585049.

Lin C-C, Cheng Y-T, Lin S-M. The effectiveness of multiple electrode radiofrequency ablation in patients with hepatocellular carcinoma with lesions more than 3 cm in size. Asian J Gastroenterol. 2020;12(18):20281–6.

Liu KH, Ruo L, Shia J, Padela A, Jarnagin WR, Fong Y, Angelica MI, Blumgart LH, DeMatteo RP. Outcome of partial hepatectomy for large (> 10 cm) hepatocellular carcinoma. Cancer. 2005;104(9):1948–55.

Li S, Yin C, Wang R, Fan Q, Wu W, Jiang X. Second near-infrared aggregation-induced emission fluorophores with phenoazine derivatives as the donor and 6, 7-diphenyl[1, 2, 3] thiadiazolo[3, 4-q] quinoloxa-line as the acceptor for in vivo imaging. ACS Appl Mater Interfaces. 2015;7(18):10452–61.

Shi H, Liu J, Geng J, Tang BZ, Liu B. Specific detection of integrin αvβ3 by light-up bioprobe with aggregation-induced emission characteristics. J Am Chem Soc. 2012;134(23):9569–72.

Lucky SS, Soo KC, Zhang Y. Nanoparticles in photodynamic therapy. Chem Rev. 2015;115(4):1900–2042.

Hu W, He T, Zhao H, Tao H, Chen R, Jin L, Li J, Fan Q, Huang W, Bae A. Stimuli-responsive reversible switching of intersystem crossing in pure organic material for smart photodynamic therapy. Angew Chem Int Ed. 2019;58(32):11105–11.

Liu J, Huang J, Ao Y, Li S, Miao Y, Yu Z, Zhu L, Lan X, Zhu Y, Zhang Y. Synergistic upconversion nanoparticles and photodynamic sensitizers with hyperbolic oxygen to remodel the extracellular matrix for enhanced photodynamic cancer therapy. ACS Appl Mater Interfaces. 2018;10(27):22985–96.

Xu W, Wang D, Tang BZ. NIR-II AIEgens: a win-win integration towards bioapplications. Angew Chem Int Ed. 2021;133(14):7552–63.

Shinn J, Lee S, Lee HK, Ahn J, Lee SA, Lee S. Recent progress and development of applications of second near-infrared (NIR-II) nanobubbles. Acc Chem Res. 2021;44:165–81.

Cai Y, Si W, Huang W, Chen P, Shao J, Dong X. Organic dye based nanoparticles for cancer phototheranostics. Small. 2018;14(25):1704247.

Liu W, Xiang H, Tan M, Chen Q, Jiang Q, Yang L, Cao Y, Wang Z, Ran H, Chen Y. Nanomedicine enables drug-potency activation with tumor sensitivity and hyperthermia synergy in the second near-infrared biowindow. Adv Funct Mater. 2021;31(14):2007026.

Guo S, Yu S, Zhang Y, Wu A, Zhang S, Wei G, Wang H, Xiao Z, Lu W. Molecular engineering of near-infrared-II photosensitizers with sterically-hindrance effect for image-guided cancer photodynamic therapy. Adv Funct Mater. 2021;31(14):2008356.

Hu X, Chen Z, Jin AJ, Yang Z, Gan D, Wu A, Ao H, Huang W, Fan Q. Rational design of all-organic nanoplatform for highly efficient NIR-II/NIR-I guided cancer phototheranostics. Small. 2021;17(12):2007566.

Zheng Z, Guo B, Hu D. Bright aggregation-induced-emission dots for targeted synergistic NIR-II fluorescence and NIR-I photocoating imaging of orthotopic brain tumors. Adv Mater. 2018;30(29):1800766.

Yu W, Guo B, Zhang H. NIR-II fluorescence in vivo confocal microscopy with aggregation-induced emission dots. Sci Bull. 2019;64(6):410–16.
blood–brain barrier for imaging and therapy of brain tumors. Adv Funct Mater. 2020;30(14):1909369.

67. Shi T, Sun W, Qin R, Li D, Feng Y, Chen L, Liu G, Chen X, Chen H. X-ray-induced persistent luminescence promotes ultrasensitive imaging and effective inhibition of orthotopic hepatic tumors. Adv Funct Mater. 2020;30(24):2001166.

68. Andreou C, Neuschmelting V, Tschaharganeh D-F, Huang C-H, Oseledchyk A, Iacono P, Karabeber H, Colen RR, Mannelli L, Lowe SW. Imaging of liver tumors using surface-enhanced Raman scattering nanoparticles. ACS Nano. 2016;10(5):5015–26.

69. Colby AH, Berry SM, Moran AM, Pasion KA, Liu R, Colson YL, Ruiz-Opazo N, Grinstaff MW, Herrera VJ. Highly specific and sensitive fluorescent nanoprobe for image-guided resection of sub-millimeter peritoneal tumors. ACS Nano. 2017;11(2):1466–77.

70. Yang L, Xu J, Ou D, Wu W, Zeng Z. Hepatectomy for huge hepatocellular carcinoma: single institute’s experience. World J Surg. 2013;37(9):2189–96.

71. Tsai T-J, Chau C-Y, Lui W-Y, Tsay S-H, King K-L, Loong C-C, Hsia C-Y, Wu C-W. Clinical significance of microscopic tumor venous invasion in patients with resectable hepatocellular carcinoma. Surgery. 2000;127(6):603–8.

72. Romero D. Combination set to transform HCC therapy. Nat Rev Clin Oncol. 2020;17(7):389.

73. Luo J, Peng Z-W, Guo R-P, Zhang Y-Q, Li J-Q, Chen M-S, Shi M. Hepatic resection versus transarterial lipiodol chemoembolization as the initial treatment for large, multiple, and resectable hepatocellular carcinomas: a prospective nonrandomized analysis. Radiology. 2011;259(1):286–95.

74. Renne SL, Sarcognato S, Sacchi D, Guido M, Roncalli M, Terracciano L, Di Tommaso L. Hepatocellular carcinoma: a clinical and pathological overview. Pathologica. 2021;113(3):203–17.

75. Parikh ND, Cuneo K, Mendiratta-Lala M. Radiation therapies for the treatment of hepatocellular carcinoma. Clin Liver Dis. 2021;17(5):341–6.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:
- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.
Learn more biomedcentral.com/submissions