Exploiting molecular probes to perform near-infrared fluorescence-guided surgery

Yifan Wu  Fan Zhang

Department of Chemistry, State Key Laboratory of Molecular Engineering of Polymers, Shanghai Key Laboratory of Molecular Catalysis and Innovative Materials and iChem, Fudan University, Shanghai, China

Correspondence
Fan Zhang, Department of Chemistry, State Key Laboratory of Molecular Engineering of Polymers, Shanghai Key Laboratory of Molecular Catalysis and Innovative Materials and iChem, Fudan University, Shanghai 200433, China.
Email: zhang_fan@fudan.edu.cn

Funding information
National Key R & D Program of China, Grant/Award Number: 2017YFA0207303; National Natural Science Fund for Distinguished Young Scholars, Grant/Award Number: 21725502; Key Basic Research Program of Science and Technology Commission of Shanghai Municipality, Grant/Award Number: 17JC1400100

Abstract
Near-infrared (NIR) fluorescence imaging can provide real-time navigation for surgeons to discriminate boundaries between lesions and healthy tissue, which serves as a promising tool to enhance precise diagnosis and accurate excision during surgery. Molecular probes with NIR fluorescence can visualize diseased tissue in deep penetration with improved signal-to-noise ratio, which considerably encourages the active participation of NIR fluorescence-guided surgery in the operating room. Although a great quantity of fluorescent probes has been employed in clinical trials, the U.S. Food and Drug Administration only approves an extremely narrow number of contrast agents for clinical use so far. Currently, there remain two significant problems to be addressed in surgical resection: accurate identification of diseased tissue and the preservation of adjacent vital structures. Here, molecular probes with NIR fluorescence are systematically reviewed to offer possible solutions to these two problems. Targeting strategies of fluorescent probes are introduced, where the strengths and weaknesses of each strategy are presented. Advances in fluorescent probes for the imaging of vital structures, such as nerve and ureter, are also summarized in this review. It is convinced that NIR fluorescence-guided surgery has the potential to improve current surgical resection and ameliorate the postoperative outcomes of diverse diseases.

KEYWORDS
imaging of vital structures, molecular probes, NIR fluorescence-guided surgery, targeting strategies

1 | INTRODUCTION

Recent years have witnessed the accomplishment of imaging modalities utilized in clinical settings. Preoperative magnetic resonance imaging (MRI) and computed tomography (CT) have served as significant tools to improve the interventional decision-making of surgeons. Different from MRI and CT, near-infrared (NIR) fluorescence imaging can provide real-time feedback for surgeons to access tumor margins in a wide-field manner, making it a promising technique for intraoperative navigation. NIR window (typically 700-1700 nm in wavelength), particularly the second NIR window (NIR-II, 1000-1700 nm), has distinguished itself from the traditional visible spectrum employed in fluorescence imaging by negligible autofluorescence and relatively low scattering in deep tissue.
### TABLE 1  
FDA-approved contrast agents for intraoperative fluorescent imaging

| Contrast agent                        | Initial approval | Absorption/emission wavelength | Indication and usage                                                                 | Clinical trials                                      |
|---------------------------------------|------------------|--------------------------------|--------------------------------------------------------------------------------------|------------------------------------------------------|
| IC-GREEN (indocyanine green, ICG)     | 1959             | 807/822 nm in plasma           | Determine cardiac output, hepatic function and liver blood flow; ophthalmic angiography | Sentinel lymph node mapping; imaging of solid tumors; visualization of nerve and ureter |
| FLUORESCITE (fluorescein sodium)      | 1976             | 492/515 nm in water; 492/518 nm in plasma | Diagnostic fluorescein angiography or angioscopy of the retina and iris vasculature | Conjugated to target moieties to realize tumor-targeting imaging; visualization of nerve and ureter |
| GLEOLAN (5-aminolevulinic acid hydrochloride, converted to protoporphyrin IX) | 1999             | 375-440/620-710 nm           | Optical imaging agent in patients with glioma as an adjunct for the visualization of malignant tissue during surgery | Photodynamic therapy                                  |
| PROVAYBLUE (methylene blue)           | 2016             | 670/690 nm in water           | Treatment of pediatric and adult patients with acquired methemoglobinemia            | Diagnostic angiography; visualization of the ureter; photodynamic therapy |

Contrast agents with NIR fluorescence extend the penetration depth of imaging to several millimeters and enable the visualization of nodules as small as sub-millimeter in diameter, which substantially raises the sensitivity and signal-to-noise ratio (SNR) of fluorescence imaging.

Although a considerable number of inorganic materials, such as rare-earth nanoparticles, quantum dots, and single-walled carbon nanotubes, have demonstrated their potential for NIR fluorescence imaging, concerns about excessive immune uptake and relatively slow clearance confine them to clinical translation. Molecular probes with rationally designed chemical structures provide NIR fluorescence imaging with new alternatives because of their fast clearance from major organs after administration and the superior biocompatibility and biosafety. Since the U.S. Food and Drug Administration (FDA) approved the first NIR fluorophore indocyanine green (ICG) to determine cardiac output, hepatic function, and liver blood flow, and for ophthalmic angiography in 1959, NIR fluorescence imaging has had a voice in imaging-guided surgery. By intravenous injection, nearly 98% of ICG will immediately bind to blood protein. The increase of the hydrodynamic diameter of ICG makes it possible to preferentially accumulate in lymph nodes and tumors, which allows for its wide application in clinical trials of sentinel lymph node mapping and imaging of solid tumors, as well as guiding oncologic reconstructive surgery and neurosurgery. Especially, excretion to bile also allows ICG to visualize the anatomical structure of the biliary tree, which may minimize the iatrogenic injury of bile ducts in cholecystectomy and other hepatobiliary surgery.

Despite the boost of molecular probes with NIR fluorescence for clinical translation, however, only a limited number of contrast agents have been approved by the U.S. FDA for clinical use (Table 1). The gap between surgery demand and translational research calls for a retrospect of clinical needs to strengthen the development of NIR fluorescent probes. In most cases, surgeons still rely on palpation and their vision to differentiate lesions from healthy tissue, either by direct eyesight or through endoscopic, laparoscopic, and robotic technologies. Human vision makes it difficult to realize the complete removal of diseased tissue, leading to the unwarranted resection and positive margins (the residual of diseased tissue) after surgery. Given that positive margin has a strong association with increased rates of recurrence and decreased survival in the treatment of multiple diseases, there is tremendous demand for accurate identification of diseased tissue. Additionally, the adjacent vital structures of diseased tissue, such as nerves, blood vessels, ureters, and bile ducts, require the special attention of surgeons to prevent unexpected damage in surgery. As invisible NIR fluorescence can avoid the inaccuracy and uncertainty of human vision, it allows surgeons to evaluate lesions both from white light reflectance and fluorescence images, which helps surgeons perform excision more precisely during surgery. Nonetheless, there are still two critical problems to be improved: (a) how to further increase the accuracy of surgical resection of diseased tissue, and (b) how to avoid the unexpected damage to vital structures during surgery, especially those that hide under the tissue and become unseen to surgeons. In this review,
molecular probes with NIR fluorescence are systematically summarized to provide possible solutions toward these two questions. Herein, this review will first introduce the targeting strategies of fluorescent probes to enhance the understanding of accurate identification of diseased tissue, followed by the advances in highlighting vital structures during surgery by fluorescence imaging, in order to provide crucial insights for fluorescence-guided surgery in the future.

2 | IDENTIFICATION OF DISEASED TISSUE: TARGETING STRATEGIES

For the complete and safe removal of diseased tissue, it is a necessity to define boundaries between diseased tissue and healthy tissue at the cellular level, which demands small-molecule, fluorescent probes with the targeted ability for diseased tissue and rapid clearance from healthy tissue.

2.1 | Passive accumulation by particle-mediated delivery

Macromolecules of a certain size range can preferentially enrich in the hypervascular tissue that displays enhanced vascular permeability and poor lymphatic clearance. It does not involve any targeting moiety in the specific accumulation and is therefore defined as passive targeting in this review.

There are hundreds to thousands of articles that reported the specific accumulation of molecular probes in lymph nodes and solid tumors by means of passive targeting. Typically, their probes are linked to polyethylene glycol (PEG) or other amphiphilic polymers to form micelles or liposomes (Figure 1A), which provides molecular probes with the ability to passively accumulate in a certain region, as well as protects them from enzymatic or environmental degradation through circulation. Notably, although the aggregation of fluorophores in particles may cause emission quenching, the aggregation-induced emission luminogens demonstrate high quantum yield in aggregates, which contributes to the development of bright organic fluorophore-based particles for image-guided surgery.

In addition, the spontaneous binding of probes to plasma proteins is another way to take advantage of passive targeting. Antaris et al found that CH-4T (a NIR-II fluorophore derived from NIR-II dye CH1055) can form supramolecular assemblies with plasma proteins (e.g., fetal bovine serum [FBS]), which can realize a notable difference in fluorescence brightness of 110-fold ex vivo compared to CH-4T in phosphate-buffered solution (PBS), as well as an approximately eightfold increase in brightness in NIR-II lymph node imaging compared to free CH-4T in vivo (Figure 1C-E). The ultra-bright property of the pre-made CH-4T-FBS complexes makes it possible to illuminate mouse femoral vasculature at 2 s postinjection and perform deep lymph node imaging at a depth of ~5-8 mm in both the NIR-I and NIR-II window in murine models.

Afterward, several NIR-II molecular probes were synthesized and proved to have similar binding affinity to plasma proteins. The increasing hydrodynamic diameter of probe-protein complexes affords molecular probes with the passively targeted ability and prolongs their residence time in the tissue of interest, thus extending the time window for fluorescence imaging. For instance, Qu et al employed Q8PNA (a NIR-II molecular probe)-FBS complexes to perform imaging-guided surgery and the tumor remained visible from 7 to 48 h after administration. Besides, the quantum yield and the luminescence intensity of these NIR-II molecular probes are greatly strengthened, which further makes them suitable choices for fluorescence-guided surgery.

Although employing the passive targeting of fluorescent probes to realize the preferential enrichment in diseased tissue has made great progress in recent years, there remains controversy on the mechanism of nanoparticle transport, which may influence its clinical translation. Moreover, biosafety is another concern toward the potential of particle-mediated delivery in clinical use. Consequently, this review will pay more attention to fluorescent probes that can actively target the diseased tissue.

2.2 | Actively targeted fluorescence probes

Typically, molecular probes with “active targeting” ability refer to those that consist of a disease-target moiety, a linker, and a fluorophore (Figure 1B). The target moiety can be antibodies, peptides, or small molecules that can recognize the specific sites of diseased tissue. A complete list of targeted fluorescent probes that are currently under evaluation in clinical trials has recently been reviewed and will not be repeated here. Notably, conjugation to target moiety may change the pharmacokinetics and biodistribution of the fluorophore, as in vivo properties of molecular probes often fit the profile of larger units in them. Besides, although an intracellular target requires probes to cross the cell membrane and interact with the target, target that localized extracellularly may result in signal attenuation because of diffusion. Therefore, the selection of target moiety is of paramount significance.
FIGURE 1  Passively and actively targeted fluorescent probes. (A-B) Schematic illustration of passively (A) and actively (B) targeted fluorescent probe. IgG, immunoglobulin G; V_H and V_L, variable region of heavy (V_H) and light (V_L) chain; V_hH, the V_H domain of heavy chain antibodies. Figures of antibodies are reproduced with permission. Copyright 2005, Nature Publishing Group. (C-E) Popliteal (PO) and sacral (SC) lymph nodes imaging with approximately 30 min postinjection of 50 mM ICG (D, 50 ms exposure, color bar ranges from 4000 to 40 000), CH-4T/PBS (E, left foot, 100 ms exposure, color bar ranges from 4000 to 55 000), and ultra-bright CH-4Tprotein complexes (E, right foot, CH-4T/HSA-HT; HSA, human serum albumin; HT, complexes were heated to 70°C for 10 min). (C) Photograph of the nude mouse in prone
2.2.1 Antibody targeting

Due to the two binding sites on the molecule, antibody, especially monoclonal antibody, has high specificity and binding affinity to receptors of interest on cell surface among other target moieties, which allows for lower administration dosing of antibody-conjugated contrast agents and thus reduces the systemic toxicity of probes. However, long circulation time of large-size antibody (~150 kDa) will cause relatively slow extravasation of conjugated fluorescent probes to diseased tissue and decrease the SNR of imaging. Also, the fragment crystallizable (Fc) domain of antibody can result in the non-specific deposition in healthy tissue through Fc receptors, particularly on macrophages, leading to the unnecessary liver and spleen uptake and increased rates of unexpected immunogenicity.

To overcome the aforementioned problems, antibody fragments (Fabs) of smaller size, such as single-chain Fv fragments (scFv, ~25 kDa), dimers of scFv (diabodies, ~50 kDa), and minibodies (~75 kDa), are developed to exclude the Fc domain and manipulate specific binding sites by advanced protein engineering. Antibody fragments of lower molecular weight display improved accumulation in diseased tissue, which serves to provide fluorescence guidance more feasibly. Further, nanobodies (~12-15 kDa) are produced as the smallest naturally derived antibody fragment to realize the preferential accumulation in diseased tissue and rapid clearance from blood and healthy tissue, but the small size may also bring the problems of easily changed in vivo properties after chemical conjugation to fluorophores and impediment of complete binding toward all the receptors of interest before diffusion.

In addition to binding affinity, pharmacokinetics, and biodistribution of antibody-labeled fluorescent probes, conjugation strategy is another key factor to influence imaging quality. Debie et al linked the anti-HER2 nanobody 2Rs15d to NIR dyes IRDye800CW and IRDye680RD, respectively, by random or site-specific conjugation, and proved that site-specifically labeled nanobodies showed expected biodistribution and superior tumor-targeting potential compared to randomly labeled ones. This demonstrates the requirement for site-specifically labeled antibodies. Design and engineering of antibody fragments allow for the genetic introduction of novel amino acid residues to specific sites, which manages to raise the certainty of the location and stoichiometry in the conjugation process and contributes to more uniform products.

Similar to the particle-mediated delivery mentioned before, molecular probes that conjugated to antibody have a larger size, making their preferential enrichment in target tissue more favorable and thus extending the time window for fluorescence imaging. For example, Zeng et al employed a Cetuximab-conjugated molecular probe (H2a-4T@Cetuximab) to visualize the tumor in murine models, in which the tumor can be discriminated from healthy tissue from 2 to 20 h postinjection (Figure 1F-I). Also, they observed much lower fluorescence signals in nude mice that were injected with free H2a-4T, which demonstrated the superior targeting ability of antibody-conjugated molecular probes.

2.2.2 Peptide targeting

Cell surface markers are also available for tissue targeting. Different forms of arginine-glycine-aspartic acid (RGD) sequence, such as cyclic RGD peptides (cRGD), were designed to mimic cell adhesion proteins to recognize the highly expressed integrins on the cell membrane of abnormal cells. It is convenient to conjugate RGD peptides to molecular probes with NIR fluorescence because of the small size of tripeptides, making it a favorable targeting ligand of actively targeted fluorescent probes.
Gastrin-releasing peptide receptor (GRPR) is another commonly used target that is overexpressed in multiple tumors with bombesin (BBN) as its natural ligand. Li et al devised a dual-modality imaging probe (68Ga-IRDye800-BBN) that contained a bombesin peptide derivative moiety and employed the probe to successfully perform the first fluorescence-guided surgery of glioblastoma multiforme in human (Figure 1J). Similarly, Pagoto et al modified Sulfo-Cy5.5 with a bombesin-like peptide and used it to visualize prostate carcinoma in murine models.

Additionally, other cell surface receptors, including the unexplored, have the potential to serve as targets in specific tissue imaging as well. Kurbergovic et al conjugated a urokinase plasminogen activator receptor targeting peptide (AE105) to the NIR-II fluorophore CH1055 and proved its plasma stability up to 5 days. Wang et al screened to synthesize a novel targeting peptide toward an overexpressed cell-surface receptor (CD133) in different types of carcinomas and modified it to a NIR-II fluorophore to perform tumor imaging in murine models. Zhang et al newly designed a glypican-3-targeting peptide that can direct fluorophores specifically to hepatocellular carcinomas by structure-based virtual stimulation, which indicates the possible contribution of creation and selection of targeting ligands to the development of targeted contrast agents.

### 2.2.3 Small molecule targeting

Although folate receptor-α (FR-α) has narrow distribution in healthy tissue and is predominantly expressed on the apical surface of epithelial cells, it is overexpressed in several malignant tumors, such as nonmucinous adenocarcinomas of ovary, uterus, and cervix. Therefore, it is difficult for intravenously administrated, FR-α-targeting contrast agents to reach the healthy tissue, which instead provides opportunities for their specific enrichment in tumors, as intercellular junctions in healthy tissue will disappear and FR-α will be randomly exposed to the entire cell surface during tumorigenesis. The first-in-human fluorescence-guided surgical trial using a tumor-specific molecular probe (EC-17) was conducted by van Dam et al, which attached folate to fluorescein to provide intraoperative guidance in an exploratory laparotomy for suspected ovarian cancer (Figure 1K). Since then, folate has been confirmed as an effective target ligand and EC-17 was further investigated to visualize various kinds of tumors in the operating room. Given that fluorescein emits the visible light and thus exhibits restricted SNR in vivo, folate was then conjugated to a NIR fluorophore (OTL-38) to navigate surgical resections by targeting tumors that overexpress FR-α, as well as identify renal tumors in partial nephrectomy by labeling normal parenchyma.

As small-molecule targeting ligands, folate possesses high binding affinity for FR-α even after conjugation to contrast agents and can easily be modified to fluorescent dyes by chemical conjugation, making it a preferable ligand compared to target moieties of other categories. However, it can only identify tumors that have high FR-α expression and thus requires preoperative determination of FR-α expression level in each patient to ensure the feasibility of fluorescence guidance, which becomes its intrinsic defect for future intraoperative application owing to the heterogeneity of cancer patients. Other small molecules that can specifically bind to receptors of abnormal cells, such as luteinizing hormone releasing hormone receptor antagonist, have also displayed potential for tumor-targeting fluorescence imaging, but the same issue as folate limits their wide use as well.

Except for targeting receptors on the cell membrane, enzymes that are excreted out of the abnormal cells are also available as targets in tumor-specific fluorescence imaging, including matrix metalloproteinases, cysteine cathepsins, and other enzymes upregulating the diseased tissue. The first two targets will be discussed in “activatable fluorescent probes.” Miller et al conjugated silicon rhodamine carboxylate to a receptor tyrosine kinase (Mer) inhibitor and employed the probe (MERi-SiR) to selectively label Mer-expressing tumor-associated macrophages in murine models. Tang et al modified the NIR fluorophore IRDye800CW with a histone deacetylase inhibitor to highlight hepatocellular carcinoma in murine models and obtained distinct tumor visualization from 6 to 24 h postinjection. It is noted that a great number of enzyme inhibitors that are approved for the treatment of different cancers by the FDA can be repurposed to enhance the specificity of molecular tracers in fluorescence-guided surgery, which offers both safety and efficiency to speed up the clinical translation of molecular probes.

The history of antibody epitomizes the development of clinical demands. As every coin has two sides, several key factors should be thoroughly taken into consideration to select the proper target moiety, including binding affinity, target selectivity, pharmacokinetics, biodistribution, conjugation strategy, and other in vivo properties. The commercialization of fluorescent dyes brings new opportunities to the evaluation of different target moieties. For instance, IRDye800CW was linked to antibodies (monoclonal antibodies, antibody fragments, and nanobody), peptides and small molecules to perform targeted fluorescence imaging of diseased tissue, which serves as a practical tool to redeploy the function of clinically approved drugs, investigate their targeting ability, and indicate the feasibility of newly developed targets for intraoperative navigation.
2.3 Activatable fluorescent probes

Although actively targeted fluorescent probes demonstrate enhanced specificity to discriminate tumors from healthy tissue, the background signal relies on the clearance of probes from normal tissue to diminish to a satisfying degree for fluorescence-guided surgery, due to the “always on” property of these probes. To improve this problem, molecular tracers are developed that only turn on their fluorescent light in response to disease-specific metabolisms, pathways, and microenvironment, while remaining silent in normal tissue. These are what we call activatable fluorescent probes. The “turn on” ability of activatable probes eliminates the waiting time for complete clearance of contrast agents from nonspecific deposition and optimizes the SNR in the meantime.

Typically, an activatable fluorescent probe contains three parts: (a) a recognition moiety that either covalently reacts with the active sites of the target enzyme or can be cleaved by enzymes that are upregulated in diseased tissue; (b) a fluorescent moiety; and (c) a quenching moiety that guarantees the “turn off” state of probes in nontargeted tissue. The probe enzyme recognition will lift the restriction of the quenching moiety and recover the fluorescence of the probe (Figure 2A).

Activatable probes that covalently bind to the enzyme of interest can immobilize the fluorescence to the target enzyme, but the signal amplification is not available due to the inactivation of reacted enzymes, which restricts its application in image-guided surgery and will not be discussed in this review. On the contrary, probes that can liberate the fluorescent fragments can avoid the aforementioned problem and realize signal amplification by continuously reacting with the enzyme of interest, making it a promising tool for intraoperative discrimination of diseased tissue.

Matrix metalloproteinases (MMPs) and cysteine cathepsins are widely utilized as the targets of activatable fluorescent probes. Activated in extracellular space, MMPs play a predominant role in extracellular matrix degradation and neoplastic progression. Although cathepsins are mostly found intracellularly in lysosomes to participate in proteolysis, they are also excreted out of cells and have a strong correlation with multiple human diseases, such as cancer and immune defects. A great quantity of activatable fluorescent probes based on these enzymes have been developed owing to the overexpression of MMPs and cysteine cathepsins in abnormal tissue, which opens up a new diagnostic window for image-guided surgery.

In 1999, Weissleder et al first exploited a clinically used graft copolymer, poly-L-lysine, as the backbone of the fluorescent probe and attached 11 Cy5.5 molecules to each copolymer to fabricate a fluorescent probe that depends on the unmodified lysines on the backbone to respond to protease with lysine-lysine specificity. This research unveils the potential of protease-activated fluorescent probes in intraoperative identification of diseased tissue, in which Cy5.5 molecules are auto-quenching because of the close spatial proximity to avoid the excessive modification of a quenching moiety. Afterward, they placed peptide substrates of cathepsin D or MMP-2 between the fluorophore and backbone and employed the probes to detect the enzyme activity in murine models. Although cathepsin D-activated probes can be internalized into cells by pinocytosis, MMP-2-activated probes release fluorescence extracellularly, leading to the diffusion of fluorophores away from the targets of interest.

Cell-penetrating peptides are introduced to address the problem of rapid diffusion and signal loss (Figure 2B). Jiang et al of R. Y. Tsien’s group exploited polyarginine-conjugated cell-penetrating peptides (CPPs) that fused to polyamionic sequences by cleavable linkers in response to MMP-2 and MMP-9 as the carrier to deliver Cy5 into cells to highlight human tumors xenografted in murine models. Afterward, they expanded the application of activatable CPPs (ACPPs) to a wide variety of cancer (Figure 2C), which proved its versatility to realize signal amplification for fluorescent-guided surgery. Savariar et al of this group developed a ratiometric fluorescent probe using Cy5-conjugated ACPPs linked to Cy7-conjugated polyamionic sequences to identify primary tumors and metastases to liver and lymph nodes in vivo, in which Cy5 and Cy7 formed a fluorescence resonance energy transfer donor-acceptor pair. The ratiometric ACPP (AVB-620)-based strategy further increases the SNR of fluorescence imaging, making it possible for the accurate discrimination of different kinds of tumors in fluorescence-guided surgery (Figure 2D). Additionally, Ofori et al exploited the latent cationic lysosomotropic effect to increase the accumulation of fluorescent fragments in lysosomes, in which fluorescent moieties attached to the N-terminus of cathepsin D will resident in lysosomes for a longer period owing to protonation of free amine group. They exchanged the position of quenching moiety and fluorescent moiety in conventional probes and employed the probe (6QC-NIR) to guide surgical resection of breast, colon, and lung tumors using the FDA-approved da Vinci surgical system for fluorescence-guided surgery. Yim et al of this group then changed the fluorophore to ICG (6QC-ICG) and obtained an enhanced fluorescent signal in surgical navigation.

Except for MMPs and cysteine cathepsins, β-galactosidase is another important target for cancer diagnosis. Urano et al developed a series of activatable fluorescence probes to indicate the enzyme activity of β-galactosidase, in which the quenching mechanism
FIGURE 2 Activatable fluorescent probes. (A) Schematic illustration of activatable fluorescent probes. The example of photo-induced electron transfer is reproduced with permission. Copyright 2007, American Chemical Society. (B) Schematic illustration of activatable cell-penetrating peptides (ACPPs). (C) ACPPs delineate MDA-MB 435, GFP-positive human melanoma at the margin of resection. White light, GFP, Cy5, and overlay fluorescence images of tumors are shown on the top line. While the tumor bed (*) appears to be free of tumor in white light and GFP fluorescence images after the unguided tumor excision (middle); imaging of the Cy5 signal demonstrates a residual fluorescence signal (middle, arrowhead) in surrounding remaining tissue (the excised tumor has been laid next to the mouse). Using the Cy5 fluorescence to guide exploration (bottom), a small piece of residual tumor is identified buried underneath the pectoralis muscle (bottom, arrowhead). Once the residual tumor has been resected, the GFP signal confirming the presence of tumor cells can be visualized along with the Cy5 free ACPP signal (insets in the bottom: the excised tumor magnified and brightened 5×). Reproduced with permission. (D) White light (i), ratiometric fluorescence (ii), and the overlay (iii) images of intraoperative fluorescence imaging of a tumor-bearing mouse by ratiometric ACPP. White dashed lines: pancreas (P), tumor (T), and spleen (S). Reproduced with permission. Copyright 2014, Society of Surgical Oncology. (E) Fluorescence images of peritoneal metastasis of several mouse models at 1 h postinjection of HMReβgal. Arrowheads: metastases in the OVK18 images. Scale bars, 5 mm. Reproduced with permission. Copyright 2015, Nature Publishing Group. (F-K) Detection of metastatic liver lesions and
was based on photo-induced electron transfer (Figure 2A). They then employed the β-galactosidase-targeting probe (HMRef-βgal) to successfully identify peritoneal metastases of human ovarian cancer (less than 1 mm in diameter) in murine models (Figure 2E).76 However, their probes can only emit visible light, which failed to obtain images from deep tissue. Gu et al synthesized a ratiometric probe based on the classical dicyanomethylene-benzopyran dye (DCM-βgal) that can respond to β-galactosidase and realized real-time imaging of colorectal tumor in nude mice in the NIR-I window.77 Afterward, a ratiometric probe based on 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) dyes was also developed by their group to detect the activity of β-galactosidase in tumor-bearing mice in the NIR-I window.78 Moreover, Chen et al conjugated a BODIPY derivative to the β-galactose residue (BOD-M-βgal) and obtained a β-galactosidase-targeting probe with NIR-II fluorescence, which offered an alternative to imaging of ovarian tumors in deep tissue.79

The dependence of abnormal cells on specific metabolic pathways determines the success of targeting strategies.80 Therefore, the discovery and utilization of these dependencies are crucial for generating effective ways to realize the precise excision of diseased tissue. The abnormal tumor microenvironment, such as structural and functional alteration of the vasculature, low pH, and hypoxia, has a profound influence on the metabolic phenotype of cancer cells and responses to the genetic change of tumor metabolisms as well.81 For example, an enormous number of pH-sensitive fluorescent probes that have “off/on” ability have been developed to perform specific tumor imaging. However, many of them rely on an extra targeting ligand to reach the tumors of interest, which may need a much smarter rationale for their structure to perform better. Zheng et al reported a molecular imaging probe (PEG-conjugated iridium complex; Figure 2F) that responded to acidity and hypoxia successively to allow for impressive signal amplification (SNR ∼103) in recognition of tumors and metastatic lesions (as small as 1 mm in diameter) in murine models (Figure 2G-K).82 Though it depended on passive targeting to specifically enrich in diseased tissue, the two-step coupled stimulus guaranteed the suppression of background signal to acquire high SNR in vivo, which inspires us with novel ideas of tumor-targeting strategies.

2.4 Structure-inherent targeted fluorescent probes

Targeted fluorescent probes mentioned above mostly have relatively large size due to the existence of targeting ligands, which retards their preferential diffusion and accumulation in diseased tissue. Surprisingly, fluorescent probes without additional modification of targeting moieties have been developed to specifically enrich in lesions or vital structures (Figure 3A), because of their intrinsic targeting ability toward certain tissue or organs. Structure-inherent targeted fluorescent probes draw on the strengths of molecular tracers immensely, which will surely play an important role in future fluorescence-guided surgery.

Zhang et al first found that IR-780 iodide, a fluorescent heptamethine dye, can specifically accumulate in tumor cells, owing to the higher mitochondrial membrane potential of tumor cells compared to normal cells.83 They proved that organic-anion-transporting polypeptides are responsible for the intracellular accumulation of IR-780 iodide,83 and further developed a series of heptamethine dyes that have similar properties as IR-780, including IR-783,84 IR-808,85 IR-58,86 and IR-34,87 which reveals the potential of structure-inherent targeted fluorescent probes in surgical navigation. Guo et al conducted a preliminary structure-activity relationship (SAR) study to indicate requirements for heptamethine indocyanine dyes to have the intrinsic tumor-targeting ability.88 However, a more detailed analysis should be performed to confirm the necessary structures of heptamethine dyes with intrinsic targeting ability and their substrate-binding sites in mitochondrial membranes.

From 2014, Hyun et al prepared a NIR fluorophore library to screen for NIR fluorescent probes that possess tissue specificity by their inherent chemical structures. At first, they modified phosphonate groups to a pentamethine core and a heptamethine core, respectively, for 700 nm and 800 nm fluorescence, to obtain fluorescent probes (P700 and P800; Figure 3B) that can preferentially bind to biologically important calcium salts hydroxyapatite (HA), whereas they found the extra binding affinity of probes to calcium phosphate (CP) by additionally modifying their probes with sulfonate groups in the meantime.89 Afterward, they developed pentamethine and heptamethine fluorescent probes that target cartilage (C700 and C800;...
FIGURE 3 Structure-inherent targeted fluorescent probes. (A) Summary of structure-inherent targeted fluorescent probes. (B) Dual-channel NIR fluorescence images of cartilage and bone tissue with C700-OMe and P800SO3 in the same animal. C700-OMe and P800SO3 were co-injected intravenously into either 25-g CD-1 mice (top, 0.4 mg/kg) or 35-kg Yorkshire pigs (bottom, 0.02 mg/kg), 4 h before imaging. Images are representative of three independent experiments. Reproduced with permission.90 Copyright 2015, Wiley-VCH. (C) Dual-channel NIR fluorescence images of parathyroid and thyroid glands with both T700-F and T800-F in a pig. T800-F (5 μmol, 0.06 mg/kg) was injected intravenously into a 35-kg Yorkshire pig 4 h before dual imaging, followed by 5 μmol of T700-F injected 2 h later. La, larynx; PG, parathyroid glands (arrowheads); TG, thyroid gland (arrows). Reproduced with permission. 92 Copyright 2015, Nature Publishing Group. All NIR fluorescence images for each condition have identical exposure times and normalizations. Scale bars, 1 cm. Pseudocolored red and green colors were used for 700-nm and 800-nm channel images, respectively, in the color-NIR merged image.

Figure 3B,90 adrenal glands (ESNF20 and ESNF31),91 as well as parathyroid and thyroid glands (T700-F and T800-F; Figure 3C),92 making significant progress in native tissue-targeting fluorescent probes. Owens et al also performed SAR analysis to investigate the effect of halogenation on pentamethine cyanines for endocrine and exocrine tissue targeting and indicated that central halogenation reduced the targeting ability to the thyroid and salivary glands, whereas adrenal glands were tolerant to the modification of meso-halogen.93 Moreover, in 2018, Park et al developed novel NIR fluorophores (PM700-CA and PM800-CA) and found that they can specifically bind to calcium oxalate dihydrate (CO) crystal, as well as HA and CP crystals, which provided promising tools for the assessment of the type of breast microcalcifications at the early stage.94

The discovery of structure-inherent targeted fluorescent probes compensates for the long-existing issues of large molecular size of commonly used targeted probes. Notably, employing these fluorescent probes to image bone, cartilage, and multiple glands will contribute to the identification and preservation of vital tissue in the surgical resection of diseased tissue and improve the prognosis of relevant patients, which will make a great impact on the improvement of fluorescence-guided surgery.
Nerve injury during resection of diseased tissue can result in postsurgical chronic pain, which may lead to abnormal neural function and drastically decline the life quality of patients. Iatrogenic ureteric injuries that mostly occur in gynecologic and colorectal surgery may develop urinomas, fistula, and permanent renal compromise, which demands accurate identification of ureters to decrease the risk of complications. Therefore, it is crucial to highlight the vital structures close to the resected lesions, such as nerves, blood vessels, ureters, and bile ducts, to provide guidance for surgeons to circumvent the iatrogenic damage as much as possible. Among all the current imaging agents, fluorescence probes that target these vital structures can serve as real-time reminders for surgeons to perform vital structure-sparing surgery, which is proved to effectively mitigate poor prognosis after surgery.

3.1 Nerve imaging by fluorescence probes

Currently, most cases of intraoperative peripheral nerve identification in the operating room depend on electromyography (EMG), which places electrodes in muscles to record tonic muscle activity and the compound muscle action potential by stimulating the nerves of interest. Although EMG monitoring eliminates the rates of iatrogenic nerve injury in some operations of surgical resection, it requires the placement of electrodes right in the muscles that are innervated by relatively exposed peripheral nerves, fails to obtain signals from nerves that already lose their sensory function, and even shows false-positive results to surgeons in some cases.

Fluorescence-guided surgery can overcome the pitfalls of EMG and highlight peripheral nerves that are difficult to be discriminated by human vision. Neural tracers, such as NeuroTrace, Dio, Fluorogold, and Fast Blue, are the earliest fluorescent contrast agents in the identification of nerves. Because they rely on axonal transport mechanism to travel through nerves in a retrograde or anterograde manner, local administration and incubation in advance are necessary (Dio: 2 days; Fluorogold: 3–5 days) for the light-up process, which limits their application in surgical navigation.

Several FDA-approved molecular dyes for angiography can also serve as adjuvants to provide guidance for the differentiation of nerves from diseased tissue. For instance, He et al successfully employed ICG to perform real-time imaging of thoracic sympathetic nerves in patients with pulmonary nodules. ICG was also utilized to access phrenic nerve involvement and identify nodules in anterior mediastinal tumor resection. Although such fluorescent dyes are trapped in blood vessels, they not only visualize tumors that are normally undetectable by human vision, but can also indicate the peripheral nerves due to the presence of vasa nervorum. Despite the attractive priority of these dyes, however, they do not have the targeting ability direct to nerves and will be devoid of sensitivity in regions of fewer blood vessels.

With the development of tumor-targeting fluorescent probes, there have been several probes that can bind to nerve-specific components to preferentially label nerves (Figure 4A). Myelin, the lipid-rich substance insulating nerves, is one of the most important targets in nerve imaging. Typically, nerve-targeting fluorescent probes can be mainly categorized into four classes: distyrylbenzene derivatives, coumarin derivatives, oxazine derivatives, and fluorophore-labeled peptides.

In 2006, Stankoff et al and Wu et al, respectively, reported 1,4-bis(p-aminostyryl)-2-methoxy benzene (BMB) and (E,E)-1,4-bis(49-aminostyryl)-2-dimethoxy benzene (BDB) that can selectively stain white matter in animals and human by binding to myelin. Since then, a series of distyrylbenzene derivatives have been developed to highlight myelinated nerves, including GE 3082, GE3111, and GE3126. Notably, Liu et al employed a BMB fluorophore to visualize the whole spinal cord in rabbit via epidural administration, which can serve to indicate iatrogenic spinal cord injury during surgery.

Though the aqueous solubility and lipophilicity of distyrylbenzene derivatives are constantly optimized to reduce the nonspecific fluorescence from adipose tissue, imaging quality still cannot satisfy the need for fluorescence-guided surgery, owing to the relatively high uptake of adipose tissue, emission of visible light, and their ability to cross the blood-brain barrier. Park et al synthesized a series of oxazine derivatives that have NIR fluorescence to increase the penetration depth of nerve imaging. Afterward, Bath et al devised a topical administration strategy to co-visualize the luminescence from Nile Red and Oxazine 4 to decrease the signal from adipose tissue and improve SNR during nerve-sparing radical prostatectomy. This strategy was suitable to be integrated into the normal workflow of radical prostatectomy and obtained better outcomes compared to the previous fluorescent probes of distyrylbenzene derivatives.

However, the direct injection of fluorescent dyes requires additional washing steps to remove the excessive probes, which may bring extra damage to surgical sites. Whitney et al discovered a peptide (NP-41) by phage display that can preferentially bind to nerves, and succeeded to highlight the peripheral nerve after intravenous
FIGURE 4  Fluorescent probes for nerve imaging. (A) Summary of fluorescent probes for nerve imaging. Images of white light reflectance (B), Cy5 fluorescence overlaid on reflectance (C), and YFP fluorescence overlaid on reflectance (D) of the right facial nerve and its arborizations in a thy1-YFP mouse treated with Cy5-NP41. Short arrow: a nerve branch visible by all three imaging modes. Arrowheads: branches that are difficult to differentiate from muscle fascia in reflectance, but distinguishable in both fluorescence images. Long arrow: a deeply buried branch visible only by Cy5-NP41 due to the better penetration of far-red wavelengths. Images of white light reflectance (E), FAM fluorescence overlaid on reflectance (F, 2 hours after intravenous injection of NP41, 150 nmol), and Cy5 fluorescence overlaid on reflectance (G) of the left sciatic nerve (arrow) and its arborization in a mouse with a syngeneic 8119 mammary tumor graft. Large arrowheads in (E) and (F): a nerve branch buried under tumor, visible only by FAM fluorescence. Small arrowheads in (G): tumor. Reproduced with permission.112 Copyright 2011, Nature Publishing Group. In vivo fluorescence images of sciatic nerves from 6-month-old SKH1 mice with FAM-HNP401 (H, intravenous injection, 450 nmol, ~48.4 mg/kg) or FAM-NP41 (I, intravenous injection, 450 nmol, ~39 mg/kg). (J) In vivo fluorescence image of rat sciatic nerve with FAM-HNP401 (5 hours after intravenous injection, 2 μmol, ~54 mg/kg). (K) Image of rat prostate nerve using a real-time custom surgical imaging system, 5 hours after intravenous injection of FAMHNP401 (2 μmol). Reproduced with permission.116 Copyright 2018, Ivyspring International Publisher

administration of fluorophore-conjugated peptides (Figure 4B-G).112 They demonstrated that NP-41 cannot cross the blood-brain barrier and displayed rapid clearance from blood within 24 h, which may eliminate the side effect of such an agent.112 They also utilized NP-41 to improve facial nerve identification during parotidectomy113 and visualize the chronically degenerated facial nerve branches that were lack of myelin during surgical repair in murine models.114 Glasgow et al identified laminin-421 and -211 as the binding targets of NP-41, which provided
theoretical evidence for its unusual specificity toward highly degenerated nerves devoid of myelin and axons but containing laminin-rich extracellular matrix. Hingorani et al further described a novel peptide HNP-41 that preferentially bound the perineurium, with superior ability to NP-41, and employed it to realize the visualization of the neurovascular bundle, as well as the cavernosal nerve within the prostate gland (Figure 4H-K).

Discovery of targets always promotes the development of targeted fluorescent probes. Gonzales et al found that the expression of voltage-gated sodium channel Nav1.7 can be a novel marker for the peripheral nervous system and isolated a targeting peptide (Hspla) to highlight mouse sciatic nerves in vivo by chemical conjugation to a fluorophore, which broadens our view of nerve-targeting imaging.

3.2 Fluorescence imaging of ureter and other vital structures

Although the iatrogenic ureteric injury is relatively rare compared to nerve injury, it is difficult to accurately recognize the injury postoperatively, leading to delayed diagnosis and treatment of ureter repair. Prophylactic ureteric stents are utilized in some cases to help identify ureters, but this invasive method remains controversial among surgeons, because the insertion of the catheter may lead to the ureteric injury itself and increase the risk of postoperative urinary complications. Fluorescence-guided discrimination of ureters makes the advantage of minimal invasion, which may contribute to the prevention of iatrogenic ureteric injury.

For now, there are two FDA-approved fluorescent dyes, ICG and methylene blue, as well as eight experimental dyes to be employed in clinical trials of identifying ureters during surgery. Slooter et al has summarized the development of currently available and experimental NIR fluorescent dyes for intraoperative ureter imaging, where interested readers can find a detailed introduction. To conclude, fluorescent probes that can navigate the identification of ureters share the similarity. It is a necessity for them to be excreted by kidney and finally accumulate in urine. Harnessing the pharmacokinetic properties of fluorescent probes to realize tissue targeting does not need any specific targeting moiety and we can expect to witness its clinical application to reduce iatrogenic ureteric injury in the future.

Because the unexpected injury in surgery may bring lifelong issues of tissue dysfunction and high healthcare costs, preservation of other vital structures is equally important. As aforementioned, the clearance route of ICG in vivo allows it to visualize biliary tree, which can provide intraoperative guidance for surgeons to minimize the iatrogenic injury of bile ducts. Recently, Luciano et al reported a novel heptamethine cyanine (BL760) that displayed higher brightness and SNR in the biliary system for 2 h compared to ICG, which had the potential to ameliorate the bile duct injury in future fluorescence-guided surgery.

4 DISCUSSION AND OUTLOOK

Surgery is pivotal in the treatment of diverse diseases including solid tumors and neurological disorders. Although high volume surgeons can decrease rates of positive margin and improve surgical outcomes by their extensive experience and meticulous attention in some cases, identifying lesions mostly by palpation and human vision still brings obstacles for intraoperative decision-making. The advances of fluorescence imaging have opened up a new era for precise diagnosis. NIR fluorescence-guided surgery can provide real-time navigation in the operating room, which has tremendous potential in pinpointing diseased tissue and thus can improve the accuracy of excision during surgery. Nevertheless, the comprehensive consideration of several key factors is indispensable for the successful translation of fluorescent probes to clinical settings.

First, wavelengths and dyes. As aforementioned, imaging in the NIR window has extraordinary advantages over visible imaging. Especially, NIR-II imaging offers an unparalleled view for surgeons to identify the tissue of interest, because the quality of photon attenuation, tissue autofluorescence, and scattering are all greatly mitigated along with the increasing wavelength. Therefore, exploiting NIR-II dyes to perform imaging will further improve the SNR in fluorescence-guided surgery. Recently, several NIR-I cyanine dyes, including ICG and IRDye800CW, have been found to have off-peak emission in the NIR-II window. It is noted that the off-peak emission of NIR-I cyanine dyes can afford superior contrast in the NIR-II window to perform deep tissue NIR-II imaging, which suggested a new strategy to accelerate the translational research by simply replacing the silicon-based cameras with InGaAs sensors to repurpose NIR-I cyanine dyes for NIR-II fluorescence imaging. Inspired by the discovery, Hu et al established a multispectral imaging system and employed ICG to realize the fluorescence-guided surgical resection of primary and metastatic liver tumors in 23 patients with the aid of both NIR-I and NIR-II fluorescence (Figure 5A-K). Similarly, Wu et al employed ICG to visualize the bile duct injury in the NIR-II window and obtained better SNR with deep penetration (Figure 5L-M). Except for commercial fluorescent dyes, Sun et al obtained FD-1080 J-aggregates by self-assembly of their previously synthesized FD-1080...
FIGURE 5 Redeployment of current fluorescent dyes to improve NIR-II fluorescence-guided surgery. (A) Schematic illustration of the integrated visible and NIR-I/II multispectral imaging instrument. (B) The customized visible and NIR-I/II multispectral imaging instrument placed in the operating room. Intraoperative NIR-I/II fluorescence-guided tumor resection. White light image (C), NIR-I fluorescence image (E), and NIR-II fluorescence image (D) of a lesion in the liver tissue are shown. Although it appears to be free of tumor under the guidance of ultrasonography and white light image (F), NIR-I (H) and NIR-II (G) imaging demonstrate a residual fluorescence signal in surrounding remaining tissue (red arrows). Using the NIR-I image to guide the further resection, white light image (I), NIR-I fluorescence image (K), and NIR-II fluorescence image (J) all indicate that there was no residual tumor ultimately. Reproduced with permission. Copyright 2019, Nature Publishing Group. White light (left), NIR-I (middle), and NIR-II (right) images of transected common bile duct with (M) or without (L) tissue covered superficially. Scale bar: 1 cm. Reproduced with permission. Copyright 2020, Ivyspring International Publisher. (N) Schematic illustration of the formation of FD-1080 J-aggregates. (O) Fluorescence images of brain and hindlimb vessels by FD-1080 J-aggregates in vivo. Reproduced with permission.

(a NIR-II fluorophore) and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) via film dispersion, which can be used in brain and hindlimb vascular imaging beyond 1500 nm (Figure 5N-O). To conclude, we can expect that the redeployment of previously developed fluorescent dyes can enlighten novel properties and further improve NIR-II fluorescence-guided surgery.

Second, targeting ability. Accurate identification of diseased tissue requires the preferential accumulation of probes to eliminate the background signal. There are mainly four types of targeting strategies: (a) passive targeting; (b) active targeting by conjugating to specific targeting ligands; (c) activatable targeting by responding to certain activity of upregulated enzymes in diseased tissue; and (d) structure-inherent targeting. The selection of targeting strategies should take binding affinity and specificity, pharmacokinetics, biodistribution, and other in vivo properties into account to ensure the optimal performance of targeted
fluorescent probes. Although the mechanical combination of different scientific results to produce a “1 + 1 = 2” outcome does no good to innovation and exploration, adding targeting moiety to fluorophores in clinical trials seems to act well in tissue-targeting imaging. Nonetheless, we strongly encourage the smart and rational design of chemical structures to fabricate novel molecules with intrinsic targeting ability in clinical translation. Given that modern science has made great progress in deciphering multiple important biomarkers in different kinds of diseases, interdisciplinary research should be conducted to merge the advantages from all walks of science and engineering. Third, stability and time window for fluorescence imaging. One of the most crucial problems in fluorescence-guided surgery is the appropriate time window for fluorescence imaging, which significantly depends on the stability of fluorescent contrast agents, mostly chemical stability and photostability. Though ICG has been widely employed in clinical use, it has relatively low photostability compared to newly developed NIR molecular probes, making it insufficient for prolonged exposure need in more complicated and time-consuming surgery. Consequently, there is an urgent demand for anti-quenching molecular probes with NIR fluorescence. Wang et al developed a series of NIR-II fluorophores (benzothiopyrylium pentamethine cyanines [BTCs]) that demonstrated anti-quenching performance including high brightness and superior photostability in aqueous solution. They compared the photobleaching of ICG and BTC1070 in vivo by continuous laser irradiation and proved that BTC1070 can visualize the lymph nodes and vessels clearly, whereas ICG showed distinct photobleaching after laser irradiation. As the stability of molecular probes guarantees the possibly extended imaging time, we anticipated the development of anti-quenching NIR molecular probes in the future.

Fourth, dosing and administration. Local administration can avoid the systemic metabolism, but contrast agents may be difficult to reach the deep tissue due to poor diffusion, whereas systemic administration requires preoperative injection for contrast agents to fully accumulate in the tissue of interest with the concern of systemic side effects. Therefore, dosing of contrast agents should not only satisfy the need for fluorescence imaging, but also be adapted to the administration routes to ensure the safety of patients.

There have been a great number of targeted fluorescence probes engaging in clinical trials to ameliorate the clumsiness of the current situation. The update on imaging systems has also expedited the integration of fluorescence imaging into minimally invasive surgeries. To be noted, protecting vital structures surrounding the resected tissue during surgery is of equal prominence to the identification of diseased tissue. Only by attaching importance to both of the aspects can the surgical intervention of diseases introduce more patients to life-long benefits. In all, through joint efforts of scientists, engineers, and doctors, we can anticipate the intraoperative involvement of NIR fluorescence imaging in the near future.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

ACKNOWLEDGMENTS
This work was supported by the National Key R & D Program of China (2017YFA0207303), National Natural Science Fund for Distinguished Young Scholars (21725502), and Key Basic Research Program of Science and Technology Commission of Shanghai Municipality (17JC1400100).

ORCID
Fan Zhang https://orcid.org/0000-0001-7886-6144

REFERENCES

1. M. A. Stammes, S. L. Bughy, T. Porta, K. Pierzchalski, T. Devling, C. Otto, J. Dijkstra, A. L. Vahrmeijer, L. F. de Guss-Oei, J. S. D. Mieog, Br. J. Surg. 2018, 105, e69.
2. A. L. Vahrmeijer, M. Hutzeman, J. R. van der Vorst, C. J. van der Velde, J. V. Frangioni, Nat. Rev. Clin. Oncol. 2013, 10, 507.
3. G. Hong, A. L. Antaris, H. Dai, Nat. Biomed. Eng. 2017, 1, 22.
4. a) F. Wang, X. Liu, J. Am. Chem. Soc. 2008, 130, 5642; b) D. J. Naczynski, M. C. Tan, M. Zevon, B. Wall, J. Kohl, A. Kulesa, S. Chen, C. M. Roth, R. E. Riman, P. V. Moghe, Nat. Commun. 2013, 4, 2199; c) Y. I. Park, K. T. Lee, Y. D. Suh, T. Hyeon, Chem. Soc. Rev. 2015, 44, 1302; d) Y. Fan, P. Wang, Y. Lu, R. Wang, L. Zhou, X. Zheng, X. Li, J. A. Piper, F. Zhang, Nat. Nanotechnol. 2018, 13, 941; e) P. Wang, Y. Fan, L. Lu, L. Liu, L. Fan, M. Zhao, Y. Xie, C. Xu, F. Zhang, Nat. Commun. 2018, 9, 2898.
5. a) I. L. Medintz, H. T. Uyeda, E. R. Goldman, H. Mattoussi, Nat. Mater. 2005, 4, 435; b) S. J. Zhu, J. H. Zhang, S. J. Tang, C. Y. Qiao, L. Wang, H. Y. Wang, X. Liu, B. Li, Y. F. Li, W. L. Yu, X. F. Wang, H. C. Sun, B. Yang, Adv. Funct. Mater. 2012, 22, 4732; c) H. Ding, S. B. Yu, J. S. Wei, H. M. Xiong, ACS Nano 2016, 10, 484; d) O. T. Bruns, T. S. Bischof, D. K. Harris, D. Franke, Y. Shi, L. Riedemann, A. Bartelt, F. B. Joworski, J. A. Carr, C. J. Rowlands, M. W. B. Wilson, O. Chen, H. Wei, G. W. Hwang, D. M. Montana, I. Coropceanu, O. B. Achorn, J. Kloppeer, J. Heeren, P. T. C. So, D. Fukumura, K. F. Jensen, R. K. Jain, M. G. Bawendi, Nat. Biomed. Eng. 2017, 1, 0056; e) J. Li, K. Pu, Chem. Soc. Rev. 2019, 48, 38.
6. a) Z. Liu, S. Tabakman, K. Welscher, H. Dai, Nano Res. 2009, 2, 85; b) K. Welscher, S. P. SHERLOCK, H. Dai, Proc. Natl. Acad. Sci. USA 2011, 108, 8943; c) D. Ghosh, A. F. Bagley, Y. J. Na, M. J. Birrer, S. N. Bhatia, A. M. Belcher, Proc. Natl. Acad. Sci. USA 2014, 111, 13948; d) G. Hong, S. Diao, J. Chang, A. L. Antaris, C. Chen, B. Zhang, S. Zhao, D. N. Atochin, P. L. Huang, K. I. Andresson, C. J. Kuo, H. Dai, Nat. Photonics 2014, 8, 723; e) Y. Yamogida, T. Tanaka, M. Zhang, M. Yudasaka, X. Wei, H. Kataura, Nat. Commun. 2016, 7, 12056.
7. a) M. A. Dobrovolskaia, P. Aggarwal, J. B. Hall, S. E. McNeill, Mol. Pharm. 2008, 5, 487; b) S. Wilhelm, A. J. Tavares, Q. Dai,
113. M. A. Whitney, J. L. Crisp, L. T. Nguyen, B. Friedman, L. A. Gross, P. Steinbach, R. Y. Tsien, Q. T. Nguyen, Nat. Biotechnol. 2011, 29, 352.

114. T. Hussain, L. T. Nguyen, M. Whitney, J. Hasselmann, Q. T. Nguyen, Laryngoscope 2016, 126, 2711.

115. T. Hussain, M. B. Mastrodimos, S. C. Raju, H. L. Glasgow, M. Whitney, B. Friedman, J. D. Moore, D. Kleinfeld, P. Steinbach, K. Messer, M. Pu, R. Y. Tsien, Q. T. Nguyen, PLoS One 2015, 10, e0191600.

116. H. L. Glasgow, M. A. Whitney, L. A. Gross, B. Friedman, S. R. Adams, J. L. Crisp, T. Hussain, A. P. Frei, K. Novy, B. Wollscheid, Q. T. Nguyen, Proc. Natl. Acad. Sci. USA 2016, 113, 12774.

117. D. V. Hingorani, M. A. Whitney, B. Friedman, J. K. Kwon, J. L. Crisp, Q. Xiong, L. Gross, C. J. Kane, R. Y. Tsien, Q. T. Nguyen, Theranostics 2018, 8, 4226.

118. J. Gonzales, P. Demetrio de Souza Franca, Y. Jiang, G. Pirovano, S. Kossatz, N. Guru, D. Yarilin, A. J. Agwa, C. I. Schroeder, S. G. Patel, I. Ganly, G. F. King, T. Reiner, Bioconjug. Chem. 2019, 30, 2879.

119. S. Brandes, M. Coburn, N. Armenakas, J. McAninch, BJU Int. 2004, 94, 277.

120. G. da Silva, M. Boutros, S. D. Wexner, Asian. J. Endosc. Surg. 2012, 5, 105.

121. M. D. Slooter, A. Janssen, W. A. Benelman, P. J. Tanis, R. Hompes, Tech. Coloproctol. 2019, 23, 305.

122. M. P. Luciano, J. M. Namgoong, R. R. Nani, S. H. Nam, C. Lee, I. H. Shin, M. J. Schnerrmann, J. Cha, Mol. Pharm. 2019, 16, 3253.

123. J. A. Eastham, M. W. Kattan, E. Riedel, C. B. Begg, T. M. Wheeler, C. Gerikg, M. Gonen, V. Reuter, P. T. Scardino, J. Urol. 2003, 170, 2292.

124. J. A. Carr, D. Franke, J. R. Caram, C. F. Perkinson, M. Saif, V. Askoxylakis, M. Datta, D. Fukumura, R. K. Jain, M. G. Bawendi, O. T. Bruns, Proc. Natl. Acad. Sci. USA 2018, 115, 4465; b) S. Zhu, Z. Hu, R. Tian, B. C. Yung, Q. Yang, S. Zhao, D. O. Kiesewetter, G. Niu, H. Sun, A. L. Antaris, X. Chen, Adv. Mater. 2018, 30, e1802546.

How to cite this article: Wu Y, Zhang F. Exploiting molecular probes to perform near-infrared fluorescence-guided surgery. VIEW. 2020;1:20200068. https://doi.org/10.1002/VIW.20200068