4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) as the robust dyes have been widely applied in biomedical areas, especially for tumor imaging and phototherapy. Most of the BODIPY dyes are hydrophobic, so the nanoparticle formulations are imperative for their dispersion in physiological conditions. The nanoparticles are usually obtained in the presence of surfactants or auxiliaries. In recent years, our group and others have reported the pure BODIPY nanoparticles (BODIPY NPs), which were prepared via the self-assembly of BODIPY molecules in aqueous solution without addition of any surfactants. These BODIPY NPs possess excellent colloid stability, near 100% of BODIPY, and particular physicochemical properties, which could be potentially used in cancer treatment and diagnosis. In this review, we will summarize and highlight the reported unadulterated organic nanoparticles based on BODIPY dyes and their application in bioimaging and cancer therapy to provide a paradigm of rational design of novel BODIPY photosensitizers for biomedical applications.
hydrophobic molecules have also been reported to self-assemble into NPs based on different mechanisms, including symmetrical dimer assembly [25–29], disulfide-induced assembly [30–32] and conjugated molecule assembly [33,34]. The nanoparticle formulations could increase the water solubility of hydrophobic drugs or dyes, which is beneficial for their biological application in a wider range [20–22,35–45]. Up to now, there have been several reviews about the assembly of pure drugs, also called self-delivery systems for cancer therapy [46,47]. In addition, some reviews have been seen about BODIPY for biomedical applications [1,2,10,48–52]. However, as far as we know, there is no summary about unadulterated BODIPY NPs and their application in tumor treatment and imaging.

In this review, we will summarize the organic NPs prepared from small molecular BODIPY dyes, as well as their biomedical applications (Scheme 1). This review highlights the potential of BODIPY NPs in biomedical fields and provides important perspective for advances of BODIPY dyes. We will discuss the progress of BODIPY NPs according to their applications, including imaging, phototherapy and combination of imaging and therapy.

2. BODIPY NPs for bioimaging

Bioimaging is a highly important and powerful tool in life sciences and biological research today because it makes the process of diagnosis easy and offers a unique approach to visualize the morphological details of cells and tissues. Recently, researchers have paid much attention on using various imaging techniques, such as magnetic resonance imaging [53–55], positron emission tomography [56–58], computed tomography [59–62], single photon emission computed tomography [63–65], fluorescence imaging [66–72] and photoacoustic/ultrasound imaging [73–77]. Among these imaging techniques, fluorescence imaging possesses numerous advantages, including minimal invasiveness, high contrast, good temporal resolution, high sensitivity and ease of use. In the past decade, fluorescent proteins [78–80], quantum dots [81–84], carbon dots [85–87] and organic fluorescent dyes [88–92] have been extensively studied and used as imaging agents. However,
intrinsic drawbacks, such as poor stability, short Stokes shift, poor biocompatibility and degradation under repeated excitation have limited their application in bioimaging. Fluorescent organic nanoparticles based on organic dye molecules have acquired great promise for bioimaging due to their unique sizes, flexible preparation strategy, and abilities to be modified with biological recognition ligand. BODIPY NPs as the representative fluorescent organic nanoparticles have many other advantages, such as good biocompatibility and physiological stability, high absorption coefficients, as well as excellent photostability.

A few years ago, some organic dots with unique optical properties have been developed by Tang et al. from aggregation-induced emission (AIE) dyes and used for vasculature imaging and cell tracing, but organic solvents or lipid-PEG derivatives are required as cosolvents or encapsulation matrix for the formation of dots [93–97]. Purely organic fluorescent nanoparticles from hydrophobic dyes without any cosolvent or carrier have rarely been synthesized. In 2014, our group has reported the first unadulterated organic nanoparticle based on a BODIPY dimer (BDY-NPs), which was prepared with a nanoprecipitation method (Fig. 1a, b) [7]. The BDY-NPs formed in water after complete evaporation of tetrahydrofuran (THF) exhibit intense fluorescence, due to the attached tetraphenylethene (TPE) groups, which suppress the intermolecular π–π interaction. The BDY-NPs are in uniform spherical shapes as shown in scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images (Fig. 1b), and show high stability in water at room temperature. BDY-NPs are demonstrated to be biocompatible with living cells. The cellular uptake and imaging of the BDY-NPs by human cervical carcinoma (HeLa) cells were examined with confocal laser scanning microscopy (CLSM), and bright red fluorescence was observed when cells were incubated with 5 μg mL⁻¹ of BDY-NPs, which demonstrated the potential of the purely organic nanoparticles for biological imaging applications. Thereafter, stable nanoparticles prepared from a newly synthesized molecule containing triple-BODIPY (TBDP) by nanoprecipitation procedure were reported [98]. It is interesting that the fluorescent nanoparticles exhibit fluorescence at both 566 and 683 nm upon single excitation (500 nm), and the emission at 683 nm could be used for sensing the self-assembly behaviors. The stable nanoparticles possess good biocompatibility and can be applied to cellular imaging for cancer cells.

It has been reported that disulfide bond bridges could induce the assembly of hydrophobic molecules via insertion of the disulfide bond into the molecules to balance the intermolecular forces [41]. Our group has synthesized a fluorescent BODIPY dimer
(SNBDP) with two o-nitrobenzyl groups and one disulfide bond via one-step multi-component Passerini reaction (Fig. 2a), which could self-assemble into nanocapsules (NCs) via disulfide-induced assembly in pure water [99]. The NCs were light-responsive, reduction-sensitive, and could be served as drug carriers to deliver indocyanine green (ICG) for photothermal therapy of HeLa cells (Fig. 2b). Afterwards, to evaluate the effect of molecular structures of BODIPY dimers on the stability of the prepared organic nanoparticles, fluorescent nanoparticles were prepared from BODIPY dimers with various lengths of linkers through nanoprecipitation method [100]. The results show that the nanoaggregates from dimer with longer spacer are more stable in aqueous solution. In another work, a redox-hypersensitive BODIPY dimer (SePTX) with diselenide was synthesized, and organic NPs were prepared and used for cellular imaging [26].

On the other hand, fluorescent nanoparticles formed from BODIPY-based amphiphiles have also been reported [101–103]. For example, Wang et al. synthesized two structurally comparable BODIPY amphiphiles (1 and 2) with different emission color, which could co-self-assemble into nanoaggregates in aqueous solution (Fig. 3) [103]. The formed nanoaggregates are fully fluorescence-quenched, but can disassemble into free dye molecules with bright fluorescence at different wavelength with the increasing of organic solvent or upon cellular uptake. Accordingly, the fluorescence-quenched nanoaggregates are used for dual color and ratiometric fluorescence imaging of living cells. However, the assembly of

Table 1
Comparison of BODIPY NPs with other phototherapy agents.

<table>
<thead>
<tr>
<th>Phototherapy agents</th>
<th>Stability</th>
<th>Absorption wavelength</th>
<th>Biocompatibility</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanine dyes</td>
<td>Poor</td>
<td>Tunable</td>
<td>Moderate</td>
<td>[126]</td>
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<tr>
<td>Porphyrin derivatives</td>
<td>High</td>
<td>Restricted</td>
<td>Good</td>
<td>[127]</td>
</tr>
<tr>
<td>Semiconducting polymers</td>
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<td>Tunable</td>
<td>Potential biological toxicity</td>
<td>[130]</td>
</tr>
<tr>
<td>Carbon materials</td>
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<td>Broad</td>
<td>Potential biological toxicity</td>
<td>[134]</td>
</tr>
<tr>
<td>Metal nanostructures</td>
<td>High</td>
<td>Tunable</td>
<td>Potential biological toxicity</td>
<td>[137]</td>
</tr>
<tr>
<td>BODIPY NPs</td>
<td>High</td>
<td>Tunable</td>
<td>Good</td>
<td>[141]</td>
</tr>
</tbody>
</table>

Fig. 4. a) Chemical structures of BDP based NPs. b) Photoconversion routes of three NPs. c) Cooperative PDT and PTT treatments of tri-BDP-NPs against tumor cells under laser irradiation. d) Growth profile and e) photo of the tumors of mice treated with tri-BDP-NPs (8.0 mg kg\(^{-1}\) in the presence or absence of Vitamin C (Vc) under 785 nm laser irradiation (0.5 W cm\(^{-2}\), 5 min). *p < 0.05, **p < 0.01. [Reprinted with permission from Ref. [141]. Copyright © 2018 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.]
BODIPY in these systems is in organic solvent-containing water not in pure aqueous solution, which might limit their practical application.

Most reports utilize BODIPY for bioimaging by directly dissolving them in organic solvents, such as dimethylsulfoxide and ethanol, then diluting the solution with buffer solution or cell culture medium. Compared with free BODIPY molecules, the BODIPY NPs without organic solvents are more biocompatible, and show enhanced photostability, long circulation time in blood, as well as favorable photophysical properties in bioimaging and diagnosis.

### 3. BODIPY NPs for phototherapy

Phototherapy, mainly including photodynamic therapy (PDT) and photothermal therapy (PTT), as an emerging treatment for malignant tumors, has attracted widespread attention due to its high precision, low toxicity and negligible drug resistance [104–118]. Usually, singlet oxygen ($^{1}O_2$) is the dominating agent for killing cancer cells in PDT. $^{1}O_2$ is generated by the photosensitizers (PS) under the irradiation of low-energy light with the presence of oxygen [119–121]. With respect to PTT, light energy is converted into heat, resulting in the thermal ablation and subsequent death of tumor cells [113,122–124]. The molecular structures of photosensitizers play a crucial role in the efficacy of phototherapy. Cyanine [125,126], porphyrin derivatives [28,127–129], semiconducting polymers [105,111,116–118,130–133], carbon materials [134–136] and metal nanostructures [137–140] are widely explored as photosensitizers. However, there are some limitations hindering their bio-applications, such as the poor photostability of cyanine, the restricted absorption range of porphyrin derivatives, as well as the potential biological toxicity of semiconducting polymers, carbon materials and metal nanoparticles (Table 1). BODIPY is a type of the new and robust photosensitizers with great potentials for phototherapy. As reported, functional BODIPY NPs could generate both $^{1}O_2$ and heat for synergistic PDT and PTT of tumors, while no obvious dark toxicity was observed [113].

It was first demonstrated by Chen et al. that rational design of BODIPY (BDP)-based conjugated photosensitizers (Fig. 4a) could achieve dual cooperative phototherapy for tumor ablation upon near-infrared (NIR) light irradiation (Fig. 4b, c) [141]. The coupling of BDP monomers into dimeric or trimeric BDP (di- or tri-BDP) induces photoconversion from fluorescence to nonradiative transitions or intersystem crossing. Particularly, tri-BDP-NPs exhibit conversions into preferable photothermal effect and moderate $^{1}O_2$ generation upon 785 nm laser irradiation, thus realizing tumor ablation through both moderate early apoptosis and dominant late...
apoptosis without any regrowth (Fig. 4d, e). This is a valuable paradigm for cooperative PDT and PTT of cancer in precision medicine. Thereafter, Chen et al. synthesized a novel phototherapeutic agent aza-BDP (IABDP, Fig. 5) possessing strong near infrared absorbance [142]. The nanoparticles (IABDP NPs) prepared from IABDP via reprecipitation method show sizes of about 200 nm with high stability in water. IABDP NPs exhibit excellent PDT activity under light irradiation at low power density, while with the increase of the irradiated power, they possess significant PTT ability. Besides, the NPs could generate severe damage to cancer cells and remarkable inhibition to the tumors via cooperative PDT/PTT treatments. A more recent work also from Chen’s group reported a simple tetraethylene glycol-borondipyrromethene (TBDP, Fig. 6), which had low fluorescence quantum yield ($\Phi_f = 2\%$) and possessed prominent photodynamic and photothermal efficacy under the same irradiation [143]. TBDP could form stable nanoparticles, which also had excellent photodynamic and photothermal efficacy. Both $^{1}\text{O}_2$ and heat could be generated to kill cancer cells when the nanoparticles were internalized by cells and irradiated by 635 nm red light. The outstanding phototoxicity of the nanoparticles was also validated by the in vivo anti-tumor experiments. These works highlight the potential of organic photosensitizers in cancer treatment.

4. BODIPY-based NPs for simultaneous imaging and cancer therapy

It is interesting and imperative for therapeutic agents to have the imaging ability for guiding treatment. Imaging provides a multi-dimensional visualization for monitoring drug delivery and biodistribution. The combination of medical therapy and bioimaging diagnostics for optimizing the efficacy and safety of therapeutic regimes is kind of personalized medicine. Emerging nanotechnol-
Fig. 8. a) Chemical structure of CAP-BDP (1, red: BODIPY, blue: CAP), proposed mechanism of self-assembly and disassembly-driven fluorescence turn-on. Growth curves of PC-3 tumors after treatment with b) CAP-BDP in DMSO and c) aggregates in H$_2$O/THF (99.4: 0.6). Right: Confocal microscopy images of tumor slices. Scale bars: 100 μm. [Reprinted with permission from Ref. [150]. Copyright © 2018 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.]

Fig. 9. a) Chemical structure of PPAB and the application of PPAB NPs in PAI/NIR-FI imaging-guided PTT. b) Relative viabilities of cells incubated with different concentrations of PPAB NPs ("P < 0.01, "*P < 0.001). c) Relative tumor volumes of various groups (n = 5, "P < 0.05, "**P < 0.001). [Reprinted with permission from Ref. [151]. Copyright © 2019 Royal Society of Chemistry.]
The conjugation of BODIPY dyes with chemotherapeutic drugs will form small molecular drug-dye conjugates, which could self-assemble into nanoparticles and be used for imaging and chemotherapy. Our group has developed a paclitaxel containing amphiphilic drug-dye conjugate (PTX-Pt-BDP) by utilizing the three-component Passerini reaction (Fig. 7a) [19]. PTX-Pt-BDP could self-assemble into highly stable spherical nanoparticles (PTX-Pt-BDP NPs) in aqueous solution. The NPs could be endocytosed by cancer cells and exert imaging and cytostatic functions concurrently (Fig. 7b, c).

Moreover, the attachment of an appropriate hydrophobic dye to a chemotherapeutic drug would promote the aggregation of the drug into water-soluble \( \pi \)-assemblies [144–147], accordingly increasing the drug loading efficiency directly without using drug carriers, which may avoid the limitations of using carriers, such as low drug loading efficiency, inefficient release and side effects [148]. Capsaicin (CAP) is a natural alkaloid being assigned to the family of capsaicinoids, which has been discovered to exhibit anti-tumor activity toward a number of cell types [149,150]. However, the pungency and high needed doses to exert its activity have hampered its practical application in cancer therapy. Fernandez’s group has put forward a straightforward strategy by covalently attaching a BODIPY dye to CAP (CAP-BDP) to improve its antitumor effect based on increasing its propensity of aggregation in aqueous media [150]. CAP-BDP could self-assemble into globular nanoparticles with weak fluorescence in aqueous solutions, which would become highly emissive upon disassembly induced by cell uptake (Fig. 8a). More importantly, improved delivery to tumor tissues was realized due to the aggregation, thus sharply reducing the injection doses of CAP-based drugs in vivo antitumor application while substantial antitumor activity was retained (Fig. 8b, c). The only drawback was that 0.6% of THF was used in aqueous solution.

Besides the integration of imaging and chemotherapeutic functions, the combination of imaging and phototherapy has also been developed by Dong et al. [151]. They synthesized an efficient NIR photosensitizer in a straightforward manner, namely pyrrolopypyrrrole aza-BODIPY (PPAB). The prepared PPAB NPs could be used for photoacoustic imaging (PAI) and NIR fluorescence imaging (NIR-FI) guided PTT (Fig. 9a). The NPs were demonstrated to have excellent tumor ablation ability both in vitro and in vivo, even at a low concentration (Fig. 9b, c). This work plays an important role in promoting the clinical application of dual-modality imaging-guided PTT.

5. Concluding remarks and perspective

Great advances have been made in BODIPY NPs due to their advantages of high content of BODIPY, robust stability, flexible preparation strategy and nanoscale sizes for biomedical applications. However, the development of BODIPY NPs is in the infancy. Several key issues need to be addressed in the near future. Firstly, the mechanism of assembly of hydrophobic BODIPY in aqueous media is not clear, although we know it is the supramolecular interactions among BODIPY molecules. Secondly, the sizes and morphologies of BODIPY NPs are hard to be tuned, which is pretty important in the translational medicine. Finally, the absorption and emission of BODIPY NPs extending to longer wavelengths is imperative for better tissue penetrating and higher biosafety.

In our view, the development in BODIPY NPs should focus on the clinical translation. The BODIPY NPs with the clinical prospects in selective imaging and tumor treatment need to be considered. Similar to porphyrin derivatives, the photosensitizers based on BODIPY NPs must enter the clinical trials and go through system-atic evaluation. Much more efforts should be made to optimize the size and morphology of the NPs to ensure the prolonged blood circulation, enhanced cellular uptake, controllable cargo release and selective tumor targeting after systemic administration. The “ADME” (“absorption-distribution-metabolism-excretion”) mechanism of BODIPY NPs needs to be systematically evaluated. We believed that the BODIPY NPs will find a place in the nanoscale therapeutic agents.

Acknowledgments

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References