Squaraine-Based Fluorescent Materials

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Squaraine dyes (SQs) are a peculiar class of cyanine dyes. One of the biggest structure differences between SQs and other types of cyanine dyes is that SQs contain an electron-deficient square ring at the center of the polymethine chain, leading to a quadrupolar donor-acceptor-donor (D-A-D) structure with a unique resonance-stabilized zwitterionic feature, as shown ina. In addition, the central square ring can make the polymethine chain rigid and planar to resist the photoisomerization and oxidation, and thus SQs intrinsically exhibit a greater stability over other cyanines.

Keywords: squaraine dyes ; bioimaging ; phototherapy

1. New Design Strategies of Squaraines -Based Fluorescent Materials

1.1. Squaraines with CIEE Properties

Unsymmetrical squaraine dyes (SQs) have constituted a large portion of SQ-based fluorescent materials. This is because compared to the symmetrical structure, unsymmetrical SQs exhibit much better flexibility for synthetic manipulation, making their absorption and emission properties easily tunable by modifying the end-groups [1]. More importantly, symmetry breaking can to some degree weaken the strong intermolecular interactions between SQs. For example, Gao et al. developed two piperidine-capped unsymmetrical SQs.

1.2. SQs with AIE Properties

Due to their out-of-order packing and strong dipole–dipole interaction, the AIE phenomenon is difficult to realize for SQ-based luminogens in aqueous solutions. To address this issue, it was recently developed a new series of symmetrical and unsymmetrical SQs with AIE properties using tetraphenylethylene (TPE) functionalized diarylamine derivatives as the end groups [2].

2. SQ-Based Functional Materials for Biomedical Applications

2.1. Fluorescent SQs as Biosensors

Selective and sensitive detection of a specific analyte is crucial when designing molecular probes. To date, SQs have been applied for sensing various specific analytes, such as metal ions, anions, nucleic acids, amino acid, proteins, and so on [3][4][5][6][7]. Different detection mechanisms have been developed, including metal coordination, nucleophilic addition, H-bond interaction, protonation/deprotonation, and aggregation/disaggregation and so on. [8] Here, based on these mechanisms, some representative works will be introduced.

In 2017, Lu et al. developed a “turn-on” far-red/NIR fluorescent sensor based on unsymmetrical SQ, i.e., SQ-DNBS, for selective detection of thiophenol [9].

Pang et al. prepared a symmetrical SQ, namely SQ1, as a fluorescent sensor of glutathione (GSH) [10]. Due to strong aggregation of SQ1 in borate buffer solution, it showed very weak fluorescence. Upon addition of GSH to the SQ1 borate buffer solution, the fluorescence was notably enhanced at 630 and 820 nm in the presence of *o*-phthalaldehyde (OPA, react with GSH formation of isoindole-GSH derivative at room temperature). Owing to a strong interaction between isoindole-GSH and SQ1, the SQ1 disaggregated and self-assembled with isoindole-GSH.

Würthner et al. reported a new amphiphilic core-substituted SQgl as an NIR “turn-on” fluorescent probe to detect VAV-1 (G4s, one of the G-quadruplexes, which are DNA or RNA tertiary structures) with high selectivity [11]. SQgl formed a nonfluorescent aggregate by self-assembly in buffer solution. However, upon addition of VAV-1, the fluorescent intensity at 700 nm was pronouncedly enhanced by forming a sandwich-like SQgl/G4 1:2 complex, accompanied by significantly increased Φ_{PLQY} up to 0.61 in the far-red/NIR region.

2.2. Fluorescent SQs for Bioimaging

Due to their intense deep-red and NIR emission, high photostability and low cytotoxicity, SQs have also been investigated for in vitro and in vivo bioimaging [12][13][14]. Currently there are two different methods for SQs to realize effective bioimaging: direct conjugation with a targeting ligand and nano-precipitation [15]. For example, it was demonstrated that TPE-SQ3-based NPs encapsulated by amphiphilic polymer PEG-*b*-PCL can bring bright far-red cell fluorescence with outstanding photostability when used for 4T1 cell imaging [2].

Xi et al. reported a new core-substituted SQ, i.e., MitoESq-635, by specifically connecting SQ to the membrane proteins in the mitochondria. By adopting super-resolution technology, MitoESq-635 was investigated as a bioimaging reagent to image the dynamic structures of mitochondrial cristae in living HeLa cells. The stimulated emission depletion (STED) imaging of the living Hela cells incubated with MitoESq-635 showed low saturation intensity and high photostability. Meanwhile, the time-lapse imaging of the mitochondrial inner membrane in living HeLa cells was carried out with an outstanding resolution of 35.2 nm.

Zhao et al. developed a NIR-absorbing quinoline-based SQ for in vivo fluorescence and photoacoustic imaging, namely D1 [16]. Dicyanomethylene substitution at the center of squarate bridge results in redshifted absorption and emission, and increased Φ_{PLQY}. D1 was encapsulated with the biocompatible Pluoronic F-127 to obtain NPs ($D1_{micelle}$) in aqueous conditions with low cytotoxicity. The resulting $D1_{micelle}$ not only showed high fluorescent intensity in Huh-7 cells, but also emitted strong NIR fluorescence signal in thoracic/abdominal area of the mouse. Besides, the photoacoustic imaging capability of $D1_{micelle}$ was also focused, and a high signal intensity was observed at 840 nm, showing favorable photoacoustic imaging ability.

In addition to the core-substituted SQs described above, symmetrical and unsymmetrical SQs have also been successfully explored as the bioimaging materials. Delcamp et al. recently synthesized a water-soluble NIR-absorbing SQ, namely SO_3SQ, using the indolizine derivatives as end groups [17]. SO_3SQ showed a very high Φ_{PLQY} of 58% with absorption and emission >700 nm in fetal bovine serum. Compared to FDA approved dye indocyanine green (ICG), SO_3SQ exhibited higher molecular brightness and prolonged photostability, making it an attractive potential NIR biological imaging material with low cytotoxicity.

Additionally, SQ-based conjugated polymers were also developed in bioimaging. Compared to the small molecules, they showed larger Stokes shifts and higher Φ_{PLQY}s in the NIR region [18]. For example, Chiu et al. developed a series of SQ-based, pH-responsive NIR emitters with high photostability, i.e., PFSqG0-2, by covalently incorporating SQs into the polyfluorene backbone [19]. When the concentration of SQ backbone was less than 5%, the fluorescence self-quenching of these SQ-based polymers was effectively suppressed.

Up-conversion luminescence (UCL) is a special process whereby long wavelength photons are converted into shorter wavelength photons by efficient energy-transfer processes [20]. In two-photon absorption (TPA), one molecule absorbs two photons simultaneously when excited, and the two-photon fluorescence belongs to UCL, which shows good spatial selectivity and less damage to samples compared with those of the one-photon process [21]. SQs with high TPA cross-section (δ) value were successfully designed, including extended π-systems and various conjugated D/A units [22]. Zhang et al. reported a series of symmetrical SQs for TPA bioimaging, i.e., ISD-1-7 [23]. Among the series, ISD-7 had a remarkable TPA δ value above 8000 GM at 780 nm, which is suitable for the optical window in biological tissue. Hence, ISD-7 was successfully employed in NIR fibroblast cell imaging and in vivo cerebrovascular blood fluid tracing using two-photon laser confocal scanning microscopy. Recently, Belfield and Hagan et al. also developed an unsymmetrical SQ with charged nonconjugated substituent [24], offering bright red fluorescence two-photon imaging of HeLa cells.

2.3. Fluorescent SQs for PTT and PDT

Wang et al. developed a dicyanomethylene-substituted SQ with high photostability [25]. Biocompatible supramolecular adduct SQ⊂BSA was achieved by hydrophobic and H-bonding interactions between SQ and bovine serum albumin (BSA), leading to 80-fold fluorescence enhancement. Meanwhile, to enhance the targeting ability of SQ⊂BSA, folic acid (FA) was chosen as the targeting ligand to conjugate to SQ⊂BSA. The resulting SQ⊂BSA-FA was cultivated in KB cells, and most of these were dead treated with NIR laser, resulting in a notable PTT effect. Subsequently, photothermal tumor therapy ones in vivo were carried out in KB cells xenografted nude mouse models, demonstrating SQ⊂BSA-FA as one of the potential photothermal materials for antitumor therapy under NIR window.

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