Organic Mitoprobes based on Fluorogens with Aggregation-Induced Emission


Abstract: Fluorescence imaging of mitochondria is of great interest to understand its functions and roles in various critical bioprocesses. Conventional fluorescence reagents for mitochondrial imaging and tracking suffer from various problems such as poor photostability and high cytotoxicity. The emerging of fluorogens with aggregation-induced emission (AIE) property provides great opportunities to develop mitoprobes with high specificity, good photostability and multiple functions for mitochondrial imaging and tracking. This review summarizes the recent advance of AIE mitoprobes, including the design principle for AIE mitoprobes, the applications of AIE mitoprobes for super-resolution mitochondrial imaging and tracking, as well as the therapeutic functions of AIE mitoprobes for cancer cell ablation.

Keywords: aggregation-induced emission · mitochondria · fluorescence imaging · super-resolution imaging · image-guided therapy

1. Introduction

The origin of extant mitochondria can be traced to symbiotic arrangements involving α-proteobacteria invasion to host cells that give rise to eukaryotic cells, which is supported by genome analysis.[1,2] Although this interesting endosymbiont hypothesis has not been totally confirmed yet, mitochondria do play critical roles in eukaryotic cells. The most well-known function of mitochondria is to produce adenosine triphosphate (ATP) through citric cycle[3,4] to supply chemical energy for complicated metabolic processes.[5] Recent studies also revealed that mitochondria are also involved in reactive oxygen species (ROS) regulation,[6–8] calcium homeostasis[9–11] and cell apoptosis.[12] It is expected that the mitochondrial dysfunctions would give rise to a convenient and predictable set of defects in all tissues. However, mitochondrial dysfunction has diversiform effects in multicellular organelles.[13] As a consequence, tremendous efforts have been made to monitor and study them. In the early stage, the properties and functions of mitochondria have been elaborately studied as separated organelles.[14–16] However, mitochondria are highly dynamic organelles with fission-fusion events and frequent communication with other intracellular organelles,[17] posing severe challenges for further exploration. Fortunately, advanced microscopies allow us to visualize cellular organelles in living cells with spatial resolution of micrometer[18] or even nanometer,[19,20] which offers good opportunity to understand their functions and relationships with other cell components easily.

Amongst numerous imaging modalities, fluorescence imaging is one of the most powerful tools for visualizing and analysing the distribution and dynamics of mitochondria. It utilizes fluorescent agents to label mitochondria with real-time operation, non-invasive testing and cost-effective performance.[21] A variety of fluorescent agents, including quantum dots (QDs),[22] organometallic compounds,[23] fluorescent proteins (FPs),[24] and organic dyes,[25] and so on, have been developed and used in mitochondrial dynamism tracking and monitoring. Amongst them, QDs are highly emissive and photostable, surface-modified QDs with target peptides/proteins are effective fluorescent markers,[22] but most of them (e.g., CdSe and PbS) are inherently cytotoxic and they often blink.[26] Organometallic compounds also suffer from cytotoxicity.[27] FPs have received considerable attention because they can be genetically encoded to mitochondria,[28] but the use of FPs requires transfection processes and their labelling sometimes can disrupt normal cell functions.[29] Therefore, organic dyes are most widely used and easily accessible with good biocompatibility for mitochondria imaging. Simple combination of fluorophores with mitochondria specific ligands can lead to mitochondria probes (mitoprobes). Some other mito-probes were designed to be selectively oxidized for light-up mitochondria imaging. The absorption and emission wavelengths could also be easily regulated through altering the luminescent organic dyes.[30] However, typical organic dyes exhibit serious fluorescence quenching at high concentrations due to aggregation-caused quenching (ACQ) and show poor photostability at low concentrations. Meanwhile, most of traditional fluorophores show narrow Stokes shifts. All these disadvantages greatly limited their applications for bio-imaging.[31]

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Recently, the rapid development of fluorogens with aggregation-induced emission (AIE) characteristics (AIEgens) creates new opportunities and opportunities in the biomedical field due to their outstanding fluorescent property that is opposite to ACQ dyes. Their fluorescence is negligible in molecularly dissolved state, but booms dramatically in aggregate state or at high concentrations. This abnormal phenomenon is ascribed to the intrinsic propeller-shape structures of AIEgens. For example, tetraphenylethene (TPE) is an iconic AIEgen (Figure 1A), in which the free rotation and vibration of the phenyl rings consume energy from excited states via radiationless decay. When TPE is in aggregate state or at high concentration, its intramolecular motions are more or less inhibited, resulting in the elimination of non-radiative decay and enhanced fluorescence. In addition, some specially designed AIEgens may also exhibit excited-state intramolecular proton transfer (ESIPT) phenomenon.

For example, (2-hydroxy-4-methoxyphenyl)(phenyl)-methanone azine (HMPA) is weakly emissive in dilute solution, in which the intramolecular motions are not severely restricted. In aggregate state or at high concentrations, the formation of intramolecular H-bonds in HMPA greatly inhibit the intramolecular motions and thus promote radiative decay to give strong green fluorescence (Figure 1B). Benefiting from the strong aggregate-state fluorescence, AIEgens enable to be used at high concentrations to ensure reliable signals and high photostability. Meanwhile, AIEgens also enjoy tunable emission colors and large Stokes shift which can avoid self-absorption when used as luminescent agents. So far, AIEgens have been widely applied for chemical and biological sensing and imaging including mitochondria tracking and mitochondria-related bioprocess monitoring. In addition, the accumulation of some AIEgens on mitochondria can induce mitochondrial dysfunctions to ablate cancer cells.

Furthermore, rational design of AIEgens can endow them with singlet oxygen generation ability in the presence of oxygen upon light irradiation, which makes them qualified to serve as photosensitizers for photodynamic cancer cell ablation. Since mitochondria play an important role in cellular metabolism and are directly related to cell apoptosis, the chemo-toxicity and photo-toxicity of properly designed AIEgens to mitochondria make them highly efficient therapeutic agents for cancer cell ablation.

In this review, we summarize the applications of mitochondria-targeting probes (mitoprobes) based on AIEgen for mitochondria tracking, mitochondria-related process monitoring and cancer cell ablation. Firstly, we discuss the emission color tuning and design principles of AIE mitoprobes.

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Secondly, we demonstrate the utilization of mitoprobes for mitochondria dynamism tracking with high- and super-resolution imaging, mitochondria-related bioprocess such as mitophagy monitoring and mitochondrial membrane potential sensing. Lastly, we summarize the mitochondria-specific image-guided cancer cell ablation including chemotherapy and photodynamic therapy (PDT). Considering the importance of mitochondria in cellular metabolism, aging and disease development, the emergence of AIE mitoprobes with high performance not only helps to visualize mitochondrial structures, but also provides great opportunities for disease diagnosis and therapy.

2. Design of AIE Mitoprobes

Resulting from the redox process for ATP production, there is a constant membrane potential of around $-180 \text{ mV}$ across outer and inner mitochondrial membranes. This negative potential is much higher than that in other organelles and provides an opportunity to design positive chemical ligands for specific targeting mitochondria through electrostatic interactions. Triphenylphosphonium (TPP) and pyridinium groups bearing positive charges are generally decorated on fluorescent dyes for probing mitochondria. Meanwhile, ammonium, isoquinolinium and indolium can also serve as mitochondrial delivery vehicles following a similar mechanism. The chemical structures of these positively charged ligands are shown in Figure 2A. A number of AIE mitoprobes with high photostability and specificity have been developed by integrating AIEgens with the targeting ligands.

As the emission color of AIEgens can be fine-tuned with a wide range from blue to the second near-infrared window, colors have been developed. Among them, salicylaldehyde azine (SAA) are typical AIEgens with greenish blue and green emission colors, respectively. The red-shift in emission of AIE mitoprobes can be accomplished by introducing electron donating/withdrawing groups to AIE units. Electron donating groups can increase the highest occupied molecular orbital (HOMO) levels of AIEgens while electron withdrawing groups can decrease the lowest unoccupied molecular orbital (LUMO) levels of AIEgens, both can reduce the HOMO–LUMO gaps to result in red-shifted absorption and emission spectra. As shown in Figure 2B, TPE is greenish blue emissive with a emission peak at 460 nm. After decoration with two electron donating methoxy groups, the emission peak of obtained 2MeOTPE is slightly red-shifted to 480 nm. The decoration of electron withdrawing groups can also red shift the emissions of TPE-based AIEgens. For example, conjugation of isoquinolinium makes the emission of TPE-IQ red-shifted to green color with a peak at 502 nm. Stronger electron withdrawing groups can result in further red-shifted emission. By introducing electron-withdrawing vinyl dicyano, vinyl pyridinium, and indolium groups, the obtained TPECN, TPE-Py, and TPE-In exhibit gradually red-shifted emissions at 564 nm, 600 nm, and 650 nm, respectively. Furthermore, the integration of both electron donating and withdrawing groups can lead to more red-shifted AIEgens due to the formation of donor-acceptor structures. For example, after introducing two electron donating methoxy groups, TPE-IQ, TPECN, TPE-Py were turned to TPE-IQ-2O, TPECM, AIE-Red, respectively. And their emission peaks are red-shifted from 502 nm, 564 nm, 600 nm to 620 nm, 600 nm, 660 nm, respectively. Therefore, the emission color of AIE mitoprobes can be easily tuned from 460 nm to 660 nm, almost covering the whole visible region.
3. Mitochondrial Imaging and Tracking by AIE Mitoprobes

Based on the design principles illustrated in the previous section, numerous AIE mitoprobes with tunable emission colors have been developed, which provide great opportunities to realize polychromatic imaging and tracking of mitochondria with high and super-resolution and to achieve real-time monitoring of mitochondrial activities. In the following section, we summarize the recent development and imaging applications of AIE mitoprobes in detail with selected examples.

3.1 Polychromatic Imaging of Mitochondria

The first design and utilization of AIEgens for mitochondrial targeted imaging and tracking was reported in 2012. By incorporating the mitochondrial targeting ligand, TPP, to TPE, the first AIE mitoprobe called TPE-TPP was synthesized, showing typical AIE property with bright blue emission peaked at 466 nm in solid state. TPE-TPP could target the mitochondria of HeLa cells and light them up with bright blue fluorescence (Figure 3A). After co-staining the cells using commercial MitoTracker® Red FM, a perfect overlap could be observed with a Pearson’s correlation coefficient of 0.96, indicating the high targeting specificity of TPE-TPP to mitochondria. More importantly, TPE-TPP obviously excels MitoTracker® Red FM in terms of photostability. Under continuous laser scanning by the confocal microscope, TPE-TPP exhibits less than 20% signal loss even after 50 scans, while MitoTracker® red FM already loses more than 75% of its fluorescent signals after 6 scans. These observations indicate that AIE mitoprobes could serve as better fluorescence trackers for mitochondrial imaging and monitoring as compared to conventional dyes.

Subsequently, AIE mitoprobes with red-shifted excitation and emission wavelengths were developed, attributing to the advance in the molecular design of AIEgens, which realized the polychromatic imaging of mitochondria. In 2014, we reported a new AIE mitoprobe with green emission for mitochondrial imaging. AIE-MitoGreen-1 was obtained by incorporating SAA with two positively charged pyridinium groups as the mitochondrial targeting ligands, which exhibits typical AIE property due to both the restriction of intramolecular rotation and ESIPT in aggregate state. The emission maximum of AIE-MitoGreen-1 locates at 532 nm, yielding a large Stokes shift of 176 nm. AIE-MitoGreen-1 locates at 532 nm, yielding a large Stokes shift of 176 nm.

Figure 2. Chemical structures of different positively charged mitochondria-targeting ligands (A) and AIEgens with different emission peaks (B).
polychromatic imaging of mitochondria by AIE mitoprobes. (A) Blue; (B) Green; (C) Yellow; and (D) Red. Scale bars: 30 μm.

Although one-photon fluorescence imaging of mitochondria was successfully achieved by various AIE mitoprobes, the short excitation wavelength of these probes limits the penetration depth and could easily lead to intrinsic background autofluorescence and photo-damage to the cells. To overcome these limitations, AIE mitoprobes with multi-photon absorption, which are suitable for multi-photon fluorescence imaging of mitochondria, were developed. The AIE mitoprobe of IQ-TPA has been successfully used to realize two-photon fluorescence imaging of mitochondria. IQ-TPA has large two-photon absorption cross section of ~215 GM at 900 nm, which ensures bright two-photon fluorescence imaging of mitochondria when excited at 900 nm to achieve low autofluorescence signal generation and low photodamage. Three photon fluorescence imaging of mitochondria were also explored by He and Qian using TPE-TPP, the first AIE mitoprobe, upon 1020 nm three-photon excitation. TPE-TPP also exhibits better photostability than commercial MitoTracker® Red FM even upon 1020 nm excitation. As compared to one-photon excitation at 405 nm and two-photon excitation at 810 nm, three-photon excitation of TPE-TPP at 1020 nm could almost completely remove the autofluorescence generated by the intrinsic endogenous fluorophores inside the cells since these fluorophores have negligible multi-photon absorption compared to that of TPE-TPP, further increasing the signal to noise ratio to image and monitor the mitochondria. Meanwhile, the photodamage to cells could also be significantly reduced. Furthermore, the fact that the annoying autofluorescence problem faced by the AIE mitoprobe with absorption and emission spectra in short wavelength range could be overcome by the multi-photon imaging technique further broaden their application for mitochondrial imaging and monitoring.

3.2 Super-Resolution Imaging of Mitochondria by AIE Mitoprobes

The monitoring of mitochondrial dynamics is of particular importance to understand the function and activity of mitochondria as well as their interaction with other cellular structures. However, the resolution of visible light microscopy is limited to 200 nm due to the diffraction barrier, which could not provide precise characterization of mitochondria. The emerging of super-resolved fluorescence imaging such as photoactivated localization microscopy (PALM), stochastic optical reconstruction microscopy (STORM) could break down the barrier and lead to higher resolution up to nanometer scale. However, the operation of such super-resolution microscope relies on the photoactivatable fluorogens, while the conventional mitochondria targeting fluorogens suffer from...
high background signal, high phototoxicity and unstable photoswitching.

The first fluorescence turn-on photoactivatable AIE mitoprobe was developed in 2015.[72] The AIE mitoprobe in the pre-activated state was in a form called o-TPP3M, which contained TPE, electron-donating methoxy groups and cationic, electron-withdrawing 1-methylpyridinium unit and was non-emissive as the twisted intramolecular charge-transfer (TICT) effect quenched the light emission. Upon UV light irradiation, o-TPP3M was transformed into c-TPP3M due to the photocyclodehydrogenation reaction, which resulted in the dominance of the AIE effect over the TICT effect and the turn-on of blue fluorescence. This mitoprobe could specifically stain and light-up the mitochondria upon UV light irradiation.

Subsequently, another fluorescence turn-on photoactivatable AIE mitoprobe of o-TPE-ON+ with yellow emission was reported[73] which could be photoactivated after transforming into c-TPE-ON+ through the same mechanism as previous one (Figure 4A). The probe could efficiently light up the mitochondria in living and fixed HeLa cells upon UV light irradiation. Furthermore, the fluorescence of c-TPE-ON+ could be photobleached by applying appropriate light (Figure 4B), making this AIE mitoprobe suitable for super-resolution imaging. Furthermore, it could exhibit spontaneous blink under physiological conditions with higher photon counts, faster fluorescence switching on-off rate, and higher localization precision as compared to the widely used commercial MitoTracker® Orange CMTMRos. Attributing to these merits, real-time super-resolution imaging of mitochondrial dynamics was successfully achieved under physiological conditions on STORM without adding external additives to enhance the blink. The imaging quality could be significantly improved as indicated by the comparison between the blurry epifluorescent image and the clear STORM image of mitochondria as shown in Figure 4C, in which the resolution was increased from 697.1 nm (epifluorescent image) to 104.5 nm (STORM image). Attributing to the excellent capability of o-TPE-ON+ for super-resolution mitochondrial imaging, the fission and fusion process of mitochondria could be clearly monitored as indicated by the green and red arrows shown in Figure 4D, demonstrating the promising potential of AIE mitoprobe for super-resolution imaging applications.

3.3 Monitoring of Mitochondria Related Bioprocess by AIE Mitoprobes

As illustrated in the introduction, mitochondria are highly dynamic organelles which are involved in various bioprocess by communicating or interacting with other intracellular organelles.[17] Thus, apart from imaging mitochondria alone, one of the most important applications of AIE mitoprobe is to monitor the mitochondria related bioprocess. Recently, we reported a red-emissive AIE mitoprobe, AIE-Red (Figure 5A, λ_em = 665 nm), which could cooperate with another green-emissive AIE lysosome tracker, AIE-Green (Figure 5A, λ_em = 538 nm), to monitor the mitophagy process.[64] AIE-Red could efficiently stain mitochondria without interfering the staining of lysosome by AIE-Green, which excels the interference problem that commercial dyes sometimes encounter during co-stain of cells. Meanwhile, it exhibits extremely high photostability and low cytotoxicity towards mitochondria upon laser irradiation. Real-time monitoring of mitophagy process was achieved in HeLa cells by observing the overlap between the red fluorescence from mitochondria and the green fluorescence from lysosome (Figure 5C), which provided direct visual information with high accuracy. Tang’s group also achieved similar process monitoring using an AIE mitoprobe, TPE-Py-NCS, and the commercial LysoTracker® Red DND-99.[74] Mitochondria and lysosome of HeLa cells were stained by TPE-Py-NCS and LysoTracker® Red DND-99.
respectively. The mitophagy process were indicated by the weaker green emission from TPE-Py-NCS stained mitochondria when the mitophagy was induced by rapamycin and the mitochondria were digested by the LysoTracker® Red DND-99 labeled autophagosome. Furthermore, as reported by Tang’s group in 2016, AIE mitoprobe could also help to monitor the cell behavior during the cell population using a yellowish AIE mitoprobe TPE-PyN3 as the fluorescent indicator. TPE-PyN3 could retain in living HeLa cells to 5th generation during in vitro cell proliferation and monitor the cell morphology and behavior in living zebrafish embryos up to 60 hours, demonstrating good photostability and high biocompatibility, which is a promising candidate for non-invasive imaging of cell behavior in living organisms.

3.4 Biosensing of Mitochondria by AIE Mitoprobes

Since mitochondria play critical roles in various cellular processes, the sensing and detection of its membrane potential and the analytes inside it is highly desirable to understand its function status. TPE-Ph-In is the first non-self-quenching probe that could monitor the membrane potential change of mitochondria. TPE-Ph-In is AIE-active with an absorption peak located at ~445 nm and an emission peak located at ~670 nm (Figure 6A), which could stain the mitochondria of HeLa cells specifically with stable fluorescent signal (Figure 6B) and avoid the self-quenching problem. Since the targeting of TPE-Ph-In to mitochondria depends on its membrane potential, which promotes the accumulation of dyes in normal condition, after incubating HeLa cell with TPE-Ph-In, the membrane potential change could be monitored by its fluorescence variation when the cells were treated with oligomycin or carbonyl cyanide 3-chlorophenylhydrazone to increase or decrease the membrane potential, respectively (Figure 6C). Furthermore, light-up sensing of esterase accumulating inside the mitochondria was realized by Tong et al. by using an AIE mitoprobe named Probe 4 in 2017. Probe 4 contains an esterase responsive acetoxyl group and exhibits very weak fluorescence in aqueous solutions. The presence of esterase could cleave the acetoxyl and release the hydroxyl group, resulting in the activation of ESIPT and AIE to induce strong red fluorescence (λem = 650 nm), achieving a promising detection limit of 0.005 U/mL for in vitro esterase detection. Attributing to the donor-acceptor structure, including the SAA core, the electron-donor diethylamine and the electron-acceptor maleonitrile, the emission of ESIPT dye was firstly shifted to the red range, which is more suitable for fluorescence imaging applications. Probe 4 could specifically stain and light-up the mitochondria after reacting with esterase in living MCF-7 cells, which further extend the application of AIE mitoprobe to the analytes detection related to mitochondrial activities.

4. Theranostics with AIE Mitoprobe

As the powerhouse of cells, mitochondria dysfunctions are related to many diseases such as tumorigenesis. Therefore, mitochondria targeting has emerged as a promising strategy
for enhanced therapy outputs with minimized side effects. In the previous section, we have introduced the application of nontoxic AIE mitoprobes for visualization of mitochondria structures or related progresses. In this section, we focus on the theranostic applications of AIE mitoprobes.

4.1 Chemotherapy with AIE Mitoprobes

The pioneer work of AIE mitoprobes for theranostics was reported in 2014. AIE-2TPP was synthesized by conjugating two triphenylphosphonium (TPP) segments to an AIEgen exhibiting ESIPT feature (Figure 7A). Benefiting from the AIE and ESIPT characteristics, AIE-2TPP could induce fluorescence change through intramolecular hydrogen bonding, which further promotes high image contrast and large Stokes shift. Due to the higher plasma membrane potential and mitochondria membrane potential of cancerous cells over normal epithelial cells, AIE-2TPP exhibited selective cellular uptake towards cancer cells, where they showed predominate accumulation and specific fluorescence turn-on in mitochondria. The accumulation of the bi-armed AIE-2TPP in mitochondria could cause depolarized membrane, increased intracellular reactive oxygen species (ROS) level, and reduced ATP generation (Figure 7B), leading to specific cell apoptosis and death to cancer cells but not normal cells.

By linking positively charged pyridinium group to TPE units and with careful selection of counter anions, the resultant AIE mitoprobes also exhibited selective cytotoxicity towards cancer cells. Upon changing the counter anions from toluenesulfonate (TPE-Py-2O-1) to tetrphenyl borate (TPE-Py-2O-2) or tetra(4-chlorophenyl) borate (TPE-Py-2O-3) (Figure 7A), the resultant TPE-Py-2O-X mitoprobes showed red-shifted emission maximum from 560 to 605 and 620 nm, while the mitochondrial targeting ability remain inherited. Different from TPE-Py-2O-1, the latter two TPE-Py-2O derivatives showed selective cytotoxicity towards cancer cells via inhibiting the oxidative phosphorylation process and ATP production in mitochondria. It is further demonstrated that the cationic TPE-Py-2O contributes to the cytotoxicity, while the counter anions are used to fine-tune the selectivity, where the cationic TPE-Py-2O and tetrphenyl borate (2) or tetra(4-chlorophenyl) borate (3) worked cooperatively to selectively targeting cancer cell mitochondria and kill them. Moreover, TPE-Py-2O-2 and TPE-Py-2O-3 also demonstrated excellent therapeutic performance in in vivo tumor models with significant tumor volume shrinkage and minimal systemic toxicity.

Kim and co-workers also reported a responsive AIE mitoprobe to achieve in situ activation for selective cancer cell ablation. The AIE mitoprobe (TPE-TPP-a) contains a NAD(P)H:quinone oxidoreductase-1 (NQO1) responsive segment to quench TPE fluorescence via photon-induced electron transfer (PET) effects (Figure 8A). The fluorescence could only be activated by sodium dithionite (an enzyme free model of NQO1) among various analytes, with an enhancement factor of over 200-fold (Figure 8B). As NQO1 is overexpressed in cancer tissues or cells, TPE-TPP-a showed selective light-up mitochondria only in cancer cell with high NQO1 level. Different from TPE-TPP-b which could kill all the tested cells, TPE-TPP-a only showed cytotoxicity towards NQO1 overexpressed cancer cells such as A549 cells (Figure 8C). The selective toxicity was further confirmed with in vivo
tumor model, where TPE-TPP-a showed low therapeutic efficiency towards siNQO1-transfected tumor with NQO1 knockdown to reduce the NQO1 expression (Figure 8D). Very recently, the same group further conjugated an chlorambucil prodrug to the TPE-TPP system to achieve mitochondria targeted combinational chemotherapy, where chlorambucil could be activated by the high glutathione concentration in cancer cells and the accumulation of TPE-TPP promote their cancer cell ablation performance, which further expanded the scope of AIE mitoprobes for chemotherapy.\[80\]

4.2 Photodynamic Therapy with AIE Mitoprobes

Photodynamic therapy (PDT) as a non-invasive cancer treatment approach has attracted great research interest recently.\[81,82\] PDT relies on a photosensitizer to absorb localized
photons to produce toxic ROS to kill cancer cells. As ROS has short lifetime and small radius of action, PDT showed minimized side toxicity towards healthy tissue, which requires the precise subcellular localization and efficient intracellular ROS generation of photosensitizers to achieve maximum lesion towards cancer cells.\textsuperscript{[83,84]} Mitochondria are reported to be the primary target of ROS, whose dysfunctions always occurs as early stage of PDT.\textsuperscript{[85]} However, conventional photosensitizers exhibited largely decreased ROS production in aggregates when they were bound to mitochondria. In this regard, AIEgens with efficient ROS production in aggregate state are beneficial for the design of AIE mitoprobes for PDT.

The earliest report of AIE mitoprobes for PDT is based on TPE-IQ (Figure 9A), which exhibited absorption and emission maxima of 400 nm and 502 nm, respectively.\textsuperscript{[86]} The ROS generation ability of TPE-IQ under UV light irradiation is verified by H2DCF-DA, which emits green emission at 530 nm after reaction with ROS (Figure 9B). Together with its excellent mitochondria targeting ability, TPE-IQ could effectively kill cancer cells upon UV irradiation while showing excellent dark biocompatibility (Figure 9C). Moreover, the mitochondrial morphology changes under PDT could also be real-time visualized by its green emission, where the mitochondria networks swelled from long-tubular-like network to dispersed fragments.

Recently, two methoxy groups were introduced as the electron donating units to TPE-IQ to render TPE-IQ-2O with red-shifted absorption and emission spectra. TPE-IQ-2O showed an absorption maximum at 430 nm with a molar absorptivity of 15000 M$^{-1}$ cm$^{-1}$, and an emission maximum at 620 nm. TPE-IQ-2O could be internalized into HeLa cells and show strong fluorescence in mitochondria with sharp contrast, while it exhibited poor uptake towards normal cells, e.g. COS-7 cells (Figure 9D). Incubation with six different cancerous cells and four normal cells further demonstrate that TPE-IQ-2O could differentiate cancer cells from normal cells (Figure 9E), making it a promising fluorescent probe for cancer cell targeting. The high cancer cell uptake of TPE-IQ-2O is believed to be associated with the high plasma and mitochondrial membrane potentials of cancer cells.\textsuperscript{[87]} The efficient ROS production of TPE-IQ-2O under white light irradiation further makes it an excellent PDT reagent to selective kill cancer cells over normal cells (Figure 9F).

4.3 Combination Therapy of AIE Mitoprobes

Despite the great advances in cancer therapy, single modal treatment approach could not provide sufficient therapeutic output owing to their intrinsic drawbacks, e.g. PDT suffers...
from the low oxygen level in tumor microenvironment. There has been increasing research interests to develop combinational cancer therapy that through incorporation of two or more therapeutic modalities to achieve therapy outcome that cannot be obtained by individual therapy approach.\[48,88–90\] One simple approach is to introduce photosensitizing ability to the AIE mitoprobes that already exhibit chemotherapeutic effects. Based on this concept, Liu and coworkers synthesized TPECM-2TPP by conjugating two TPP segments to the central core of TPECM for image-guided chemo- and photodynamic combination cancer cell ablation (Figure 10A).\[63\] TPECM is a typical AIEgen, which not only showed bright emission in aggregate, but also demonstrate efficient ROS generation under light irradiation, as accessed by the ABDA absorbance changes in response to its reaction with ROS to show decreased absorbance (Figure 10B). As predicted, the TPECM-2TPP showed excellent mitochondria targeting ability. Moreover, it exhibited a very high dark toxicity (chemotherapy) towards cancer cells with a half maximum inhibition concentration (IC\(_{50}\)) of 6.31 \(\mu\)M, while TPECM-1TPP with only one TPP arm exhibited no obvious toxicity towards HeLa cells (Figure 10C). Upon light irradiation, the generated ROS at mitochondria site could quickly oxidize and damage the mitochondria membrane, leading largely improve cancer ablation effects, where IC\(_{50}\) value of TPECM-2TPP is further reduced to 3.13 \(\mu\)M (Figure 10D). The development of photosensitizing AIE mitoprobes demonstrates a simple and facile approach, where introducing mitochondria targeting ligands to AIE photosensitizers could readily generate a molecular probe with four functions: mitochondria targeting, fluorescence imaging, chemotherapy, and PDT.

Very recently, a theranostic probe composed of a photosensitizing AIE mitoprobe and a natural product of Artemisinin (ART) was reported.\[91\] TPETH-Mito-1ART consists three components: 1) an AIE core as the fluorescence reporter and ROS generator; 2) two cationic ammonium salt arms as mitochondria targeting moieties, and 3) ART on one arm (Figure 11A). The TPETH-Mito-1ART could not only provide selectivity between cancer cells and normal cells, but also aid to deliver ART and AIE photosensitizer to the mitochondria at which site the fresh produced heme could quickly active the anticancer ability of ART to generate chemotoxicity to cancer cells, while further light irradiation could promote its lesion to cancer cells by ROS generation (Figure 11A). Delivery of ART to mitochondria could largely enhance its lesion towards cancer cells, where the IC\(_{50}\) value drops from 88.8 \(\mu\)M for ART to 11.1 \(\mu\)M for TPETH-Mito-1ART, and to 3.1 \(\mu\)M for two ART armed AIE mitoprobe (TPETH-Mito-2ART). However, TPETH-Mito-2ART also exhibits severe toxicity (IC\(_{50}\) = 12.6 \(\mu\)M) to normal cells due to high ART loading. In this regard, TPETH-Mito-1ART that shows low toxicity toward normal cells (IC\(_{50}\) > 100 \(\mu\)M) represents a non-invasive probe for cancer cell ablation. Further light irradiation could reduce the IC\(_{50}\) value to 8.1 \(\mu\)M. Moreover, the light irradiation leads to more severe damage to mitochondria which not only depolarizes mitochondrial membrane potential but also inhibits cancer cell migration. The combination of ART and PDT leads to synergistic therapeutic effects.

Figure 10. (A) Chemical structure of TPECM-1TPP and TPECM-2TPP. (B) ROS generation ability of TPECM access by ABDA decomposition under light irradiation. Cell viabilities of HeLa cells after treatment with TPECM-1TPP or TPCM-2TPP under dark (C) or light irradiation (D).\[65\]
effects, which opens new opportunities of AIEgen conjugated for theranostic applications.

4. Conclusion

In summary, we have demonstrated the great advantages of AIEgen based mitochondria probes in the bioimaging and theranostic applications. Different from conventional fluorophores, AIEgens show very weak emission as isolated molecules and are able to turn on their fluorescence upon aggregate formation. This unique feature has led to design of AIE bioprobes for specific imaging of mitochondria networks or monitoring progresses in mitochondria with very high signal-to-noise ratios. Moreover, the unique light-up features and excellent photostability of AIE mitoprobes also provide opportunities for real-time visualization of the mitochondrial progresses without washing steps or concerns of photobleaching. With facile molecular design and synthesis, AIEgen based mitoprobes with different emission colors and targeting groups have been reported, which show promising imaging performance. The recent development of AIE mitoprobes with cancer cell selective cytotoxicity further expands their scopes to theranostics. When photosensitizing ability is introduced to these AIE mitoprobes, they provide excellent PDT performance as mitochondria is the main target of ROS. Moreover, drug delivery capability of AIE mitoprobes also provides new windows for combination cancer therapy. With this review, we hope to stimulate more research interests from chemistry, materials and biology to further promote the advancement of AIE mitoprobes in the biological field.

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