Trapping endoplasmic reticulum with amphiphilic AIE-active sensor via specific interaction of ATP-sensitive potassium (K\textsubscript{ATP})

Zhirong Zhu\textsuperscript{1}, Qi Wang\textsuperscript{1,*}, Hongze Liao\textsuperscript{2}, Ming Liu\textsuperscript{1}, Zhenxing Liu\textsuperscript{1}, Youheng Zhang\textsuperscript{1} and Wei-Hong Zhu\textsuperscript{1,*}

\textsuperscript{1}Shanghai Key Laboratory of Functional Materials Chemistry, Key Laboratory for Advanced Materials and Institute of Fine Chemicals, Joint International Research Laboratory of Precision Chemistry and Molecular Engineering, Feringa Nobel Prize Scientist Joint Research Center, Frontiers Science Center for Materiobiology and Dynamic Chemistry, School of Chemistry and Molecular Engineering, East China University of Science and Technology, Shanghai 200237, China.
\textsuperscript{2}Research Center for Marine Drugs, State Key Laboratory of Oncogene and Related Genes, Department of Pharmacy, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200127, China.

*Corresponding authors. E-mail: whzhu@ecust.edu.cn; wangqi@ecust.edu.cn

ABSTRACT

The current aggregation-induced emission luminogens (AIEgens) sometimes suffer from poor targeting selectivity due to undesirable aggregation in hydrophilic biosystem with “always-on” fluorescence or unspecific aggregation in lipophilic organelle with premature activated fluorescence. Herein, we report an unprecedented “amphiphilic AIEgen” sensor QM-SO\textsubscript{3}-ER based on the AIE building block of quinoline-malononitrile (QM). The introduced hydrophilic sulfonate group can well control the specific solubility in hydrophilic system with desirable initial “fluorescence-off” state. Moreover, the incorporated \textit{p}-toluenesulfonamide group plays two roles: enhancing the lipophilic dispersity, and behaving as binding receptor to the ATP-sensitive potassium (K\textsubscript{ATP}) on endoplasmic reticulum (ER) membrane to generate the docking assay confinement effect with targetable AIE signal. The \textit{amphiphilic} AIEgen has for the first time well settled down the predicament of unexpected “always-on” fluorescence in aqueous system and untargetable aggregation signal in lipophilic organelle before binding to ER, thus successfully overcoming the
bottleneck of AIEgen targetability.

**Keywords:** aggregation-induced emission, *amphiphilic* AIEgens, endoplasmic reticulum, targeting specificity, high-fidelity tracking

**INTRODUCTION**

Endoplasmic reticulum (ER) is an important organelle in the cell responsible for protein synthesis, transport and balance regulation of calcium ion [1,2], and ER stress is one of the prerequisites for modern immunotherapy [3]. *In situ* ER tracking and bioimaging are of increasing importance for real-time observing of dynamic intracellular process to take a deep insight into the pathogenesis of some metabolic diseases [4,5], like diabetes [6,7]. However, most of commercially available ER sensors, such as ER-tracker Red and Green, are developed from aggregation-caused quenching (ACQ) fluorophores [8,9] that suffer from the inherent defects such as inaccurate feedback on ER information, especially bringing up inevitable noises from “always-on” pattern, and signal-loss from poor photostability (Fig. 1A).

In contrast with ACQ fluorophore, aggregation-induced emission (AIE) sensors [10-13] bestow distinct advantages on bioimaging [13-19], especially in lighting up organelles with targeting events through aggregation process. However, the current AIEgen sensors are still not ideal due to the limitation of the poor targeting specificity with activated fluorescence during undesirable aggregation before AIEgens binding to the specific receptor. Given that biological researches are always conducted in aqueous media, the majorities of available AIE sensors are structurally modified to tune the aqueous solubility for initial disperse state, such as introducing ionic group to the AIE building blocks, whereas most of them underscore the invalid aggregation in lipophilic organelle [20-23].

To overcome the traditional AIE bottleneck, herein we have for the first time proposed a completely novel strategy of “*amphiphilic* AIEgen” to realize good dispersity in both hydrophilic and lipophilic environments. The specific *amphiphilic* characteristic could not only prevent aggregation in aqueous biological environment, but also keep good disperse state once entering the lipophilic organelle to avoid false signals. It could remain extinguished “fluorescence-off” state during
cytomembrane transport until it encounters the specific receptor action for restriction of intramolecular motion (RIM) to produce activated luminescence \[10\], thereby achieving the desirably selective “off-on” fluorescence with lighting-up signal when responding to targeting events.

In the unique strategy of this amphiphilic AIEgen sensor (Fig. 1B), we exploited our established AIE building block of quinoline-malononitrile (QM) \[24-30\], and utilized the hydrophilic sulfonate group to modulate the specific solubility in hydrophilic system with desirably initial “fluorescence-off” state. Furthermore, the incorporated \(p\)-toluenesulfonamide group could play dual roles for realizing the amphiphilic characteristic, such as enhancing the lipophilic dispersity and behaving as binding receptor to the ATP-sensitive potassium (\(K_{\text{ATP}}\)) on ER membrane. By virtue of harnessing this strategy, the elaborated amphiphilic AIEgen sensor QM-SO\(_3\)-ER exhibits superior dispersity in both hydrophilic and lipophilic systems with initial “fluorescence-off” state when compared with the lipophilic QM-ER that aggregates tightly in hydrophilic system with “always-on” fluorescence (Fig. 1B). Particularly, the docking assay of QM-SO\(_3\)-ER with \(K_{\text{ATP}}\) channel protein, wherein the subunit of sulfonylurea receptor 1 (SUR1) locates on ER, can further address the targeting mechanism.

The amphiphilic AIEgen sensor QM-SO\(_3\)-ER always stays good dispersity in either hydrophilic or lipophilic system, thereby strongly eliminating the background fluorescence from unexpected AIE signals caused by uncontrollable polarity change. The targeting interaction between QM-SO\(_3\)-ER and \(K_{\text{ATP}}\) can exert the specific responsiveness to ER through molecular accumulation along with AIE lighting-up signal based on RIM mechanism (Fig. 1B). In addition, the fluorescence “off-on” amphiphilic AIE-active property of QM-SO\(_3\)-ER can facilitate the cell staining procedure with wash-free property and good photostability for long-time imaging. To the best of our knowledge, this is the first report about the amphiphilic AIE-active sensor with excellent targeting ability to overcome the bottleneck of traditional AIE fluorophore, expanding the promising toolboxes to achieve high targeting response via the interaction of AIEgens and specific protein for in situ and in vivo tracking.
RESULT AND DISCUSSION

Revealing *amphiphilicity* of AIEgen sensor with synergetic interaction of sulfonate and sulfonamide groups

AIE sensors bestow distinct advantages [31-33] on lighting up organelles through aggregation process. However, previously most AIEgens can only disperse well in either hydrophilic or lipophilic system [34,35] that always lead to uncontrollable molecular aggregation in the complicated physiological environment. To keep good dispersity in both hydrophilic and lipophilic environments, we propose a novel and ideal strategy so-called “*amphiphilic* AIEgen” to solve the traditional AIE bottleneck, that is, avoiding undesirable aggregations with “fluorescence-off” state during cytmembrane and organelle transport. Previously, our group has reported a novel AIE building block of QM, which replaces the oxygen in the traditional dicyanomethylene-4H-pyran (DCM) chromophore. The AIEgen derivatives of QM have been broadly explored for fluorescent sensors, bioimaging agents, optical waveguides, and drug delivery applications [24-30]. In this work, the design strategy of an ideal QM-based *amphiphilic* AIEgen is depicted in Fig. 1.

Firstly, the AIE building block of QM was utilized as core structure to overcome the enrichment quenching effect, then the π-conjugated backbone was used to extend the long emission wavelength, and the hydroxyl group was introduced for further modification to afford the intermediate QM-OH (Supplementary Scheme S1). However, as a typical AIEgen biosensor, QM-OH prefers to aggregate as AIE dye aggregates in aqueous environment, and causes “false” positive signal with low signal/noise (S/N) from fluorescence “always on” states. Subsequently, the ethyl group of QM-OH was replaced with hydrophilic propylsulfonate group to obtain QM-SO$_3$-OH, which could well control the specific solubility in hydrophilic system with desirably initial “fluorescence-off” state (Supplementary Fig. S1). Finally, the $p$-toluenesulfonamide group was grafted on the hydroxyl group of QM-SO$_3$-OH by ethyl linker to give the desirable sensor QM-SO$_3$-ER. It is anticipated that the incorporated sulfonamide group could play two roles in the *amphiphilic* AIEgen, that is, enhancing the lipophilic dispersity and behaving as the specifically binding receptor to K$_{ATP}$ on ER membrane. In this regard, it is the synergetic interaction of both the hydrophilic sulfonate group and the binding receptor of sulfonamide group that can result in the desirable *amphiphilicity* with AIE-active sensor. The chemical structures of QM-SO$_3$-OH and intermediates were well confirmed by $^1$H and $^{13}$C NMR,
and high-resolution mass spectroscopy (HRMS) in Supplementary data.

**Minimizing undesirable aggregation with superior dispersity of amphiphilic QM-SO$_3$-ER**

There exist two major obstacles in the reported AIEgen sensors: (i) the tight aggregation in aqueous biosystem with “always-on” fluorescence, and (ii) the improper unexpected aggregation in lipophilic organelle with activated fluorescence. Here the specific *amphiphilicity* of AIE-active QM-SO$_3$-ER is anticipated to eliminate the undesirable fluorescence lighting-up with superior dispersity or solubility in both aqueous and lipophilic systems before binding to the targeting receptor, thereby overcoming the bottleneck of AIEgens targetability.

The AIE properties of QM-ER were evaluated in THF-water mixtures with different fractions of water ($f_w$). In contrast to the ACQ characteristics of commercial sensors such as ER-tracker Red (Supplementary Fig. S2), QM-ER possess the classical AIE characteristic with solving the engaged quenching problem. Specifically, the emission of QM-ER became increased quickly and monotonously when the $f_w$ was higher than 70%, yielding a luminous orange signal with an emission peak at 589 nm (Fig. 2A and 2E). The resulting fluorescence is a typical AIE behavior, which is highly relative to the formation of QM-ER aggregates with RIM mechanism in water environment [10,27,36]. It was further confirmed by dynamic light scattering (DLS, Fig. 2I) and transmission electron microscope (TEM, Supplementary Fig. S3A). Although QM-ER has solved the ACQ problem, the poor aqueous solubility limits its further application in aqueous biological imaging.

To validate our proposal that the *amphiphilic* AIEgen QM-SO$_3$-ER could generate non-susceptible initial “fluorescence-off” signal, a series of spectral properties were conducted. As expected, QM-SO$_3$-ER didn’t emit any fluorescence signal in THF-water (Fig. 2B and 2F), ethanol (EtOH)-water (Fig. 2C and 2G) and DMSO-water system (Supplementary Fig. S4 and S5) at any water fractions, or in the liposomes (Supplementary Fig. S6), suggestive of the specific *amphiphilicity* of QM-SO$_3$-ER with free intramolecular motions in good disperse state [10]. As a direct evidence, the results of TEM (Supplementary Fig. S3B-D) and DLS (Fig. 2J-L and Supplementary Fig. S7) indicated that QM-SO$_3$-ER dispersed well in water phase with undetectable hydrodynamic diameter. Moreover, the Partition-coefficient ($\text{Log}P_{\text{o/w}}$) of QM-SO$_3$-ER (1.61) is bigger than QM-ER (1.23), suggested of better hydrophilicity. The unique *amphiphilicity* could be
ascribed to the synergetic contribution: (i) the hydrophilic sulfonate group increases the aqueous solubility, and (ii) the grafted \(\rho\)-toluenesulfonamide group enhances the dispersity in lipophilic system \([26,43]\).

Furthermore, the intrinsic AIE behavior of QM-SO\(_3\)-ER was demonstrated with increasing viscosity, that is, upon increasing the fraction of glycerin \((f_g)\), the increasingly enhanced viscosity in the glycerin-water system can recover the lighting-up AIE behavior of QM-based AIEgen via eliminating non-radiative channel with the specific RIM mechanism \([24,27]\). In the high viscosity system, the free motion of QM-SO\(_3\)-ER is restricted to release the excited state energy as a form of radiative transition. Specifically, the fluorescence intensity of QM-SO\(_3\)-ER at \(f_g = 95\%\) could reach 49-folds than its initial intensity (Fig. 2D and 2H), which is exactly corresponding to the solid state fluorescence (Supplementary Fig. S8). Here we expect that the \textit{amphiphilicity} of QM-SO\(_3\)-ER might make an innovated breakthrough that keeps good disperse state in a wide range of hydrophilic-lipophilic environments \([37]\). It is highly desirable that the \textit{amphiphilic} QM-SO\(_3\)-ER could avoid undesirable aggregation with “fluorescence-off” state during cytomembrane transport until it encounters the specific receptor action to restrict the intramolecular motion with the specific “\textit{off-on}” activatable fluorescence response, thus possibly achieving the high mapping feedback with overcoming the bottleneck to AIEgens targetability.

\textbf{Amphiphilic AIEgen: targeting mechanism with molecular docking}

The SUR1 domain (subunit of K\(_{\text{ATP}}\) channel locating on ER membrane) is the typical binding site of sulfonamide moiety \([38]\), wherein the commercial ER-tracker Red could bind to the ER membrane with the assistance of glibenclamide (a type 2 diabetes drug). Hence, we envisaged that sensor QM-SO\(_3\)-ER might have the similar interaction with ER organelle. To figure out the targeting mechanism of QM-SO\(_3\)-ER to K\(_{\text{ATP}}\) channel protein, the molecular docking assay was exploited to gain insight into the intrinsic binding sites (Supplementary Fig. S9). As expected, sensor QM-SO\(_3\)-ER could specifically bind to the SUR1 with the similar mode as glibenclamide (Fig. 3A). Specifically, the ER-targeting sulfonamide unit in sensor QM-SO\(_3\)-ER intimately interacts with the residues in the pocket of TMD0 in SUR1 domain, wherein the residues F589, F588, F437, F434,
F433, F337 and F592 can form a well-tailored pocket to accommodate the targeting moiety and conjugated benzene ring. The conventional hydrogen bond, π-σ, alkyl, and π-alkyl are the predominate interactions to assistant the docking position. The binding site F433 is found to overlap with that of glibenclamide, which further confirms the irreplaceable role of ER-targeting moiety. Surprisingly, another hydrophilic sulfonate group also has interaction with this binding site through the Salt Bridge and Attractive Charge, thus resulting in a stronger interaction with high bonding affinity. Furthermore, the AIE building block of QM could also interact with residues F1241 through π-alkyl interaction.

The molecular docking study results indicate that both sulfonamide and the sulfonate group could bind to SUR1 domain specifically. In this regard, the targeting interaction between QM-SO$_3$-ER and SUR1 domain (subunit of $K_{\text{ATP}}$ channel locating on ER membrane) ensures the specific responsiveness to ER from two aspects that, confinement effect caused by SUR1 domain as confinement-induced emission (CIE) and targeting-caused molecular aggregation with AIE emission, thereby resulting in the docking assay on the restricted intramolecular motion in limited space. As a consequence, the ER-tracking process could be proposed as follows: the amphiphilic AIEgen QM-SO$_3$-ER crosses through cell membrane unimpededly, transports in the cytoplasm without any unexpected fluorescence owing to the good dispersity or solubility with unique amphiphilicity, then targets to SUR1 on ER membrane with the assistance of ER-targeting moiety (Fig. 1B). Followed by the enrichment in ER, the intramolecular motion is restricted from the docking assay with the interaction between QM-SO$_3$-ER and the subunit of $K_{\text{ATP}}$ channel locating on ER membrane, thereby anticipating to achieve a targetable response with active fluorescence.

**Improving transmembrane efficiency and ER-targeting ability**

The transmembrane abilities of QM-OH, QM-ER, QM-SO$_3$-OH and QM-SO$_3$-ER were evaluated by incubating them with HeLa cells when compared with a commercial ER-specific fluorescent dye (ER-tracker Red, Fig. 1). As shown in Fig. 4C, the water-insoluble fluorophore QM-OH was mostly retained in extracellular matrix even when washing with PBS for three times, along with “always on” fluorescence background interference. It was suggestive that QM-OH might predominately
accumulate outside HeLa cells as AIE dye aggregates, in exact line with the aforementioned spectral property (Supplementary Fig. S1). Its poor Pearson’s correlation coefficient (0.0461) also reflected the undesirable co-localization result (Fig. 4A). For another water-insoluble QM-ER, the poor penetration efficiency was also observed with the similar low Pearson’s correlation coefficient (0.2902, Fig. 4C). In contrast, the hydrophilic QM-SO$_3$-OH (Fig. 4B) and amphiphilic QM-SO$_3$-ER (Fig. 4D) can easily penetrate the cell membrane owing to the dispersive molecular state in aqueous bio-environment. Here, the transmembrane ability and targeting effect are the obvious preconditions to map ER accurately. However, most traditional AIE sensors tend to aggregate in aqueous biological system [21,22] during the transmembrane process because of the poor solubility in hydrophilic system, such as QM-OH and QM-ER.

For achieving the specific targeting ability, the amphiphilicity of AIEgen sensor QM-SO$_3$-ER is expected to ensure cellular internalization owing to the good dispersity in hydrophilic and lipophilic systems. As demonstrated with reference to ER-tracker Red in HeLa cells (Fig. 3A), the amphiphilic QM-SO$_3$-ER was co-localized very well with high Pearson’s correlation coefficient of 0.9520, indicative of high desirable ER-targeting ability. However, the hydrophilic QM-SO$_3$-OH exhibited the much poorer Pearson’s correlation coefficient of 0.5505. The ER targeting ability of QM-SO$_3$-ER was further confirmed with high Pearson’s correlation coefficient (Supplementary Fig. S10) in pancreatic cancer cells (PANC-1), human adenocarcinoma cell (A549), and human hepatocellular carcinoma cell (7701). Benefiting from the molecular dispersion in aqueous and lipophilic systems, the amphiphilicity guarantees that the AIE-active sensor of QM-SO$_3$-ER can not only get across the cell membrane with high internalization ability and inactive “fluorescence-off” signal, but also enhance the lipophilic dispersity to well behave as binding receptor to K$_{ATP}$ on ER membrane (Fig. 3), wherein the AIE lighting-up response can be activated on base of the RIM mechanism through the specific confinement effect of molecular docking [10]. In this regard, it is the first time to well settle down the predicament of unexpected “always-on” fluorescence signal from undesirable aggregation in aqueous biology system and unexpected aggregation signal in lipophilic organelle before binding to ER, thus making a breakthrough to achieve the high mapping feedback with overcoming the bottleneck to AIEgen targetability.
Wash-free behavior and ER trapping with ultra-high signal to noise (S/N) ratio

The ER staining images of HeLa cells were further recorded with QM-SO$_3$-ER upon incubating with ER-tracker Red and QM-SO$_3$-ER for 10, 20, 30 and 60 min, respectively. Obviously, ER-tracker Red could enter cells and stain on the ER within 10 min, but the obvious background interference around the cells interrupted the merged images (Fig. 5B and 5C). In contrast, QM-SO$_3$-ER could gradually enter the cell in a time dependent manner without any extracellular background fluorescence, and gave more clear information in the merged images (Fig. 5E and 5F) with wash-free behavior. It took 30-60 min to light up the whole ER part with QM-SO$_3$-ER due to the AIEgen RIM effect with molecular docking confinement. Moreover, the S/N ratio of amphiphilic QM-SO$_3$-ER increased from 18.32 (10 min) to 15436.33 (60 min, Fig. 5G), much larger than the commercially available ER-tracker Red (in the range between 5.36 and 7.28) in the same time period. Indeed, the amphiphilic AIEgen QM-SO$_3$-ER is indicative of the minimal background interference and ultra-high sensitivity, especially for minimal background interference and ultra high S/N ratio from both free dye and bio-substrate auto-fluorescence [34,39-42].

Long-time tracking with excellent intra- and extra-cellular photostability

Photostability is an important parameter for bioluminescent imaging agents [43-47]. In particular, there exists the serious photobleaching and image distortion especially for multiple tracking acquisition cycles and prolonged light exposure of sensors. Structurally, the bonding force of fluoroboron coordination bonds in ER-tracker Red is much weaker than covalent bonds in QM-SO$_3$-ER, thus generating poor photostability. Here we compared the photostability of ER-tracker Red, QM-ER and QM-SO$_3$-ER upon continuous light irradiation for 20 minutes. As shown in the time-dependent absorbance, the A/A$_0$ values of QM-ER and QM-SO$_3$-ER remained above 80% while that of ER-tracker Red fell down below 50% (Supplementary Fig. S11). Also the photostability of QM-SO$_3$-ER and ER-tracker Red were conducted in intra-cellular environments. After sequentially scanning for 4 min in living HeLa cells, to the naked eyes, the fluorescent signal of QM-SO$_3$-ER decreased slightly from 0 to 2 min and then keep stable (Fig. 6A), which is in line with the normalized intensity (Fig. 6C). However, ER-tracker Red exhibited poorer behavior, and decreased
the signal sharply to almost invisible (Fig. 6B) with about 70% signal lose (Fig. 6C). Obviously, QM-SO$_3$-ER exhibits better photostability than commercial sensor ER-tracker Red. Meantime, upon incubating HeLa cells, amphiphilic AIEgen QM-SO$_3$-ER showed similar cell viability when compared with control group (fresh DMEM medium) after 24 h, suggestive of negligible toxicity during the ER trapping process (Fig. 6D). The HeLa cells maintained good cell viability even at concentration of QM-SO$_3$-ER as high as 15 μM, which was five times higher than that used for cell imaging study. Taken together, QM-SO$_3$-ER exhibits the long-time ER bioimaging with excellent photostability and low toxicity, serving as an alternative to the commercially available sensor ER-tracker Red.

CONCLUSIONS

We have for the first time proposed the unique strategy of “amphiphilic AIEgen” with good solubility and dispersity in both hydrophilic and lipophilic systems, for the sake of solving the traditional AIE bottleneck of poor specific targeting with in vivo high-fidelity trapping of ER. Comparing with the commercially available ER-tracker Red, the unprecedented amphiphilic AIEgen QM-SO$_3$-ER exhibited superior targeting capability from three aspects: (i) the AIE building block QM could overcome the ACQ effect with enhancing photostability; (ii) the grafted sulfonate group can well control the specific solubility in hydrophilic system for initial “fluorescence-off” state, and the incorporated $p$-toluenesulfonamide group increases the lipophilic dispersity to avoid untargetable aggregation in organelle; and (iii) the specific amphiphilicity can guarantee the superior solubility or dispersion in both hydrophilic and lipophilic systems, thereby achieving the superior targeting through binding receptor to K$_{ATP}$ on ER membrane to generate the docking assay confinement effect on the restriction of intramolecular motion, along with recovering the specific AIE-active lighting-up fluorescence signal. It is the specific amphiphilicity of QM-SO$_3$-ER that well settles down the predicament of unexpected “always-on” fluorescence signal from undesirable aggregation in hydrophilic bioimaging system and unexpected aggregation signal in cell organelle before binding to ER, and strongly eliminates the background fluorescence from unexpected AIE signals caused by uncontrollable polarity change, thereby achieving the high fidelity mapping feedback with
overcoming the bottleneck to AIEgens targetability. Both the cell co-localization experiment and
docking study provide evidences on the accurate feedback of in situ mapping ER with extraordinary
features, such as beneficial wash-free behavior, ultra-high time-dependent S/N in sensitivity, as well
high intrinsic photostability and low cytotoxicity. In regard with the ACQ effect and fluorescence
“always on” pattern of the commercial ER-tracker Red, the AIE-active sensor with the amphiphilic
strategy can pave a novel and straightforward pathway to build up high-fidelity AIE trapping sensor
with avoiding false signal from undesirable aggregation before binding to the specific receptor,
especially making a breakthrough to overcome the traditional AIE bottleneck to targeting capability,
along with high selectivity via the specific receptor interaction.

SUPPLEMENTARY DATA
Supplementary data are available at NSR online.

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AUTHOR CONTRIBUTIONS
W.-H.Z. proposed and supervised the project. Z.Z. and Q.W. carried out the synthesis and
experiments. H.L. carried out the docking simulation. Z.L. Liu and Y.Z. performed the cell imaging.
Z.Z., Q.W. and M.L. co-wrote the manuscript. All authors discussed the results and participated in
analyzing the experimental results.
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Figure 1. Proposing “amphiphilic AIEgen” strategy for in situ high fidelity mapping of ER. (A) Commercially-available senor ER-tracker Red based on ACQ chromophore and fluorescence “always-on” pattern. (B) Tracking ER with amphiphilic AIE-active sensor. (a) Molecular structures of QM-ER and QM-SO$_3$-ER, wherein the sulfonate group is assembled into QM-ER to give QM-SO$_3$-ER, the $p$-toluenesulfonamide group serves as a moiety binding to the specific K$_{ATP}$ on ER membrane. (b) Amphiphilic QM-SO$_3$-ER with superior solubility in both hydrophilic and lipophilic conditions to realize efficient cell uptake on good disperse state, thereby guaranteeing the amphiphilic AIE-active “off-on” signal to ER enrichment through targeting group of K$_{ATP}$ along with RIM mechanism of AIE.
Figure 2. AIE properties of QM-ER and QM-SO$_3$-ER with different water fractions ($f_w$) in various solvents. (A) Emission spectra of QM-ER (10 μM) in a mixture of THF-water system ($\lambda_{ex} = 447$ nm). (B-D) Emission spectra of QM-SO$_3$-ER (10 μM) in different solvents-water systems ($\lambda_{ex} = 447$ nm). (E) $I/I_0$ plots of QM-ER and (F-H) QM-SO$_3$-ER. $I$ is the fluorescence intensity of fluorophore in 95% water at 589 nm for E-G, and 95% glycerin at 540 nm for H, $I_0$ is the fluorescence intensity of fluorophore in 0% water for E-G, and 0% glycerin for H. (I) Hydrodynamic diameter of QM-ER (10 μM) in a mixture of DMSO/water (v/v = 1/95), and (J-L) Hydrodynamic diameter of QM-SO$_3$-ER (10 μM) in a mixture of DMSO/THF (v/v = 1/95), DMSO/EtOH (v/v = 1/95) and DMSO/water (v/v = 1/95), respectively, obtained from dynamic light scattering (DLS).
Figure 3. Molecular docking of QM-SO$_3$-ER with K$_{ATP}$ channel. (A) Structural model of the K$_{ATP}$ channel binding to QM-SO$_3$-ER in boxes viewing from side position. (B) View of the model from the extracellular side. (C and D) Close-up of QM-SO$_3$-ER binding site in SUR1. (E) The specific interactions between QM-SO$_3$-ER and SUR1. PDB ID of K$_{ATP}$ is 6BAA.
Figure 4. Efficient transmembrane and excellent ER-targeting ability of QM-SO$_3$-ER with HeLa cells. (A1-D1) Bright channels incubated with QM-OH, QM-SO$_3$-OH, QM-ER and QM-SO$_3$-ER using a concentration of 3 μM for 2 h, and followed by co-staining with ER-tracker Red (1 μM) for 30 min. (A2-D2) Green channels obtained from QM-OH, QM-SO$_3$-OH, QM-ER and QM-SO$_3$-ER (λ$_{ex}$ = 405 nm, λ$_{em}$ = 550-630 nm). (A3-D3) Red channels obtained from ER-tracker Red (λ$_{ex}$ = 561 nm, λ$_{em}$ = 580-630 nm). (A4-D4) Merged of green, red and bright channels. (A5-D5) Intensity scatter plots of QM-OH, QM-SO$_3$-OH, QM-ER and QM-SO$_3$-ER with ER-tracker Red, respectively. Insert: Pearson’s correlation coefficient. (E-H) The intensity profile of the linear region of interest (ROI) cross the cells in A4-D4.
Figure 5. Non-fluorescence in the aqueous system of QM-SO$_3$-ER guaranteeing the wash-free behavior for ER imaging. HeLa cells incubated with (A-C) ER-tracker Red (1 μM) and (D-F) QM-SO$_3$-ER (3 μM) for different time, green channels obtained from QM-SO$_3$-ER (λ$_{ex}$ = 405 nm, λ$_{em}$ = 550-630 nm), and red channels obtained from ER-tracker Red (λ$_{ex}$ = 561 nm, λ$_{em}$ = 580-630 nm). (G and H) The S/N ratios of ER-tracker Red and QM-SO$_3$-ER at different times. Note: Sensor QM-SO$_3$-ER is lighting-up feature with low background (F1-F4), while ER-tracker is fluorescence “always-on” feature with obvious background around the HeLa cells (C1-C4). The S/N value of QM-SO$_3$-ER increases with the incubating time in contrast with that of ER-tracker Red.
Figure 6. Excellent intracellular photostability of QM-SO$_3$-ER. Confocal images of HeLa cells stained with ER-tracker Red (A1-A5, $\lambda_{ex}$ = 561 nm, $\lambda_{em}$ = 580-630 nm) and QM-SO$_3$-ER (B1-B5, $\lambda_{ex}$ = 405 nm, $\lambda_{em}$ = 550-630 nm) at different scan times. (C) Normalized fluorescence signal loss of QM-SO$_3$-ER and ER-tracker Red. (D) MTT assays of QM-ER and QM-SO$_3$-ER at different concentrations. Note: the cell viability of QM-ER and QM-SO$_3$-ER is not obviously different when compared with the control group (n = 3, data expressed as average ± standard error; statistical significance: p values, *represents $p < 0.05$, **represents $p < 0.01$ and ***represents $p < 0.001$ were calculated with the Student’s T-test).