



Near-infrared AIEgens for sulfatase imaging in breast cancer *in vivo*†

Cite this: *Nanoscale*, 2024, **16**, 11538

Received 25th March 2024,
Accepted 28th May 2024

DOI: 10.1039/d4nr01314j

rsc.li/nanoscale

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Aggregation-induced emission luminogens (AIEgens) enable highly sensitive and *in situ* visualization of sulfatase to benefit the early diagnosis of breast cancer (BC), but current sulfatase AIEgens always emit visible light (<650 nm). Herein, a near-infrared (NIR) AIEgen QMT-SFA is developed for sulfatase imaging *in vivo*. Hydrophilic QMT-SFA is cleaved by sulfatase to yield hydrophobic QMT-OH, which subsequently aggregates into nanoparticles to turn the AIE fluorescence “on”, enabling sensitive sulfatase imaging in 4T1 cells and mouse models.

Breast cancer (BC) is the most frequently diagnosed malignant tumor in women worldwide, with high morbidity and mortality rates.^{1,2} In clinical practice, mammography plays an essential role in BC screening, but it has limitations in the early diagnosis of BC due to its low sensitivity;³ tissue biopsy can help diagnose BC but is commonly used until late cancer stages.⁴ Therefore, it is of clinical importance to establish advanced methodologies for the early diagnosis of BC. To this end, some molecular or nanoprobe have been recently developed for sensitive and *in situ* detection of BC biomarkers (*e.g.*, matrix metalloproteinase, cathepsin, and sulfatase) at the molecular level, which benefits the identification of BC at early stages.⁵ Among these biomarkers, sulfatase is a highly conserved sulfate-hydrolyzing protease which is overexpressed upon the occurrence of BC.^{6,7} Thereby, sensitive imaging probes of sulfatase are highly desired for the early diagnosis of BC.

Among the various imaging modalities, fluorescence imaging shows unique advantages in biomarker detection, such as non-invasiveness and superior temporal and spatial

resolution.^{8–11} In recent years, a range of fluorescence probes have been devised for the sensitive imaging of sulfatase activity.¹² Of note, these fluorescence probes with aggregation-induced emission (AIE) characteristics (*i.e.*, AIEgens) have attracted high interest due to their small autofluorescence interference, low toxicity, and strong resistance to photobleaching.^{13–16} However, current sulfatase AIEgens always emit visible light (<650 nm), which gives rise to non-negligible autofluorescence interference and inferior tissue penetration, thus severely hindering their further biological application and clinical translation.¹⁷ In this regard, an AIEgen with a near-infrared (NIR) emission, which affords a higher signal-to-background ratio and deeper tissue penetration,^{18,19} would benefit the sensitive imaging of sulfatase *in vivo*. However, so far, as we know, such sulfatase-activated AIEgens with NIR fluorescence emissions have not been reported.

Herein, a sulfatase-activatable NIR fluorescence probe (QMT-SFA) was fabricated, which contains an AIE luminophore (QMT)¹³ and a hydrophilic sulfatase-cleavable sulfate moiety (Fig. 1). As we hypothesized, QMT-SFA exhibited an initially weak fluorescence emission under physiological conditions because the hydrophilic QMT-SFA molecules were dispersive. When the sulfate group in QMT-SFA was cleaved by sulfatase inside BC cells, the obtained hydrophobic QMT-OH aggregated into nanoparticles, which emitted bright NIR fluorescence through the AIE mechanism. In this study, QMT-SFA has been successfully applied to visualize sulfatase activity *in vivo*.

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† Electronic supplementary information (ESI) available. See DOI: <https://doi.org/10.1039/d4nr01314j>

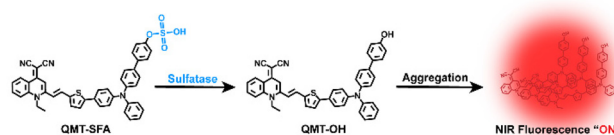


Fig. 1 Schematic illustration of the sulfatase-activatable near-infrared (NIR) aggregation-induced emission probe QMT-SFA.

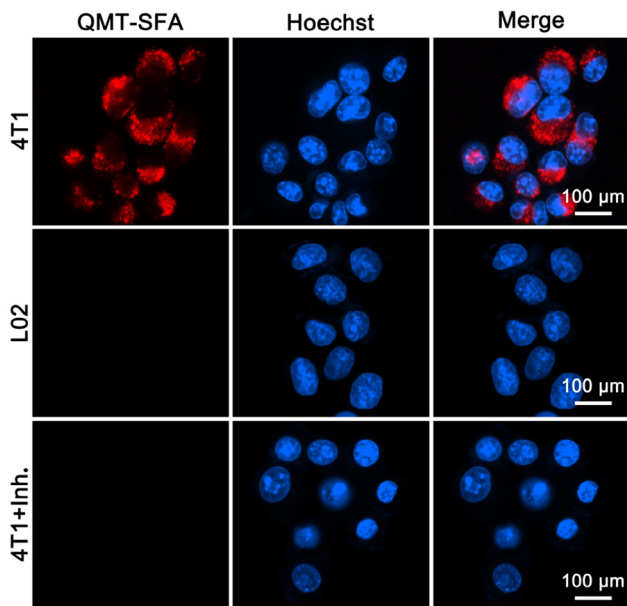


Fig. 3 Fluorescence images of 10 μM QMT-SFA-treated 4T1 cells, L02 cells, and hydroxylamine-pretreated 4T1 cells for 1 h.

“QMT-SFA + Inh.” group). The fluorescence signals of the tumor regions were monitored by a small animal imaging system. As shown in Fig. 4, for the mice in the “QMT-SFA” group, the fluorescence within the tumor region was quickly turned “on” and reached the strongest brightness at 1 h post-injection, followed by a slow decrease within 16 h (Fig. S7†). In contrast, for the mice in the “QMT-SFA + Inh.” group, a significantly low-level fluorescence profile was observed within the tumor region. The above results illustrated that QMT-SFA can be hydrolyzed by sulfatase to turn its NIR AIE fluorescence “on” in 4T1 tumor-bearing nude mice. In addition, the biodistribution of QMT-SFA in the above nude mice was further observed by *ex vivo* fluorescence imaging. As shown in Fig. S8,† fluorescence was undetectable in the major mouse organs (*i.e.*, heart, liver, spleen, lungs, and kidney) from both

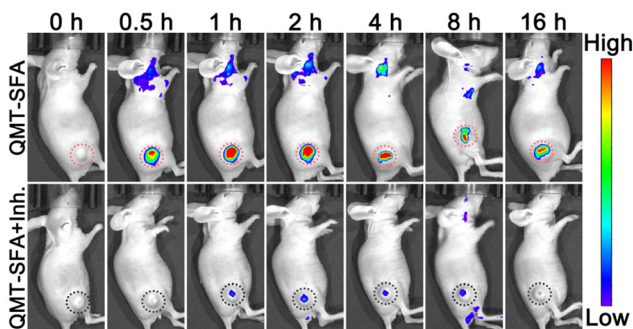


Fig. 4 Time-course fluorescence images of 4T1 tumor-bearing mice after the intratumoral injection of 0.25 mg kg^{-1} QMT-SFA. For the “QMT-SFA + Inh.” group, tumors were pretreated with 0.02 mg kg^{-1} inhibitor for 0.5 h before QMT-SFA injection. The dashed circles indicate tumor regions.

groups, as well as in the tumor from the “QMT-SFA + Inh.” group. In contrast, the tumor from the “QMT-SFA” group showed a strong NIR fluorescence. These *in vivo* results demonstrated that QMT-SFA could be applied for sulfatase imaging in tumors of living animals.

Conclusions

In summary, we have successfully designed an NIR fluorescence probe QMT-SFA for the sensitive detection of sulfatase *in vitro* and imaging sulfatase activity in tumors of living mice. QMT-SFA could be cleaved by sulfatase to yield QMT-OH, which thereby aggregated into nanoparticles to turn the NIR AIE fluorescence “on” (centered at 660 nm). The probe exhibited high sensitivity toward sulfatase with an LOD of 0.26 U mL^{-1} *in vitro*. Molecular docking calculation showed that QMT-SFA had a high binding affinity ($-6.19 \text{ kcal mol}^{-1}$) with the sulfatase docking score. In addition, QMT-SFA had been successfully employed for tracking sulfatase in 4T1 cells and 4T1 tumor-bearing nude mice. To the best of our knowledge, QMT-SFA was the first NIR AIEgen for sulfatase imaging in living cells and animals. Compared with current sulfatase AIEgens which emit visible light, QMT-SFA has higher potential in biological application owing to its improved tissue penetration and suppressed autofluorescence interference. We anticipate that this NIR AIEgen QMT-SFA could be employed for the early diagnosis of sulfatase-associated disease in clinic in near future.

Author contributions

Lingling Xu: investigation, formal analysis, methodology, validation, visualization, and writing – original draft. Yu Deng: investigation and validation. Hang Gao: validation and project administration. Yuchen Yao: validation. Xiaoyang Liu: visualization and formal analysis. Wenjun Zhan: supervision and project administration. Gaolin Liang: funding acquisition, project administration, supervision, and writing – review & editing. Xianbao Sun: conceptualization, funding acquisition, project administration, and writing – review & editing.

Ethical statement

All animal experiments were approved and performed according to the guidelines of the Animal Care and Use Committee of the Southeast University Laboratory Animal Center (No: 20240306009).

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grants 22204019 and 22234002), the Full-time Talents Program of Hebei Province (Grant 2023HBQZYCY027), and the China Postdoctoral Science Foundation funded project (Grant 2023M730577).

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