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

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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.<sup>1,2</sup> 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;<sup>3</sup> tissue biopsy can help diagnose BC but is commonly used until late cancer stages.<sup>4</sup> 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.<sup>5</sup> Among these biomarkers, sulfatase is a highly conserved sulfate-hydrolyzing protease which is overexpressed upon the occurrence of BC.<sup>6,7</sup> 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.<sup>8–11</sup> In recent years, a range of fluorescence probes have been devised for the sensitive imaging of sulfatase activity.<sup>12</sup> 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.<sup>13–16</sup> 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.<sup>17</sup> In this regard, an AIEgen with a near-infrared (NIR) emission, which affords a higher signal-to-background ratio and deeper tissue penetration,<sup>18,19</sup> 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.

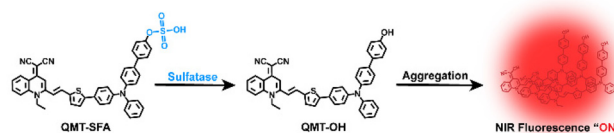
Herein, a sulfatase-activatable NIR fluorescence probe (QMT-SFA) was fabricated, which contains an AIE luminophore (QMT)<sup>13</sup> and a hydrophilic sulfatase-cleavable sulfate moiety (Fig. 1). As we hypothesized, QMT-SFA exhibited an initially weak fluorescence emission under physiological conditions 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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**Fig. 1** Schematic illustration of the sulfatase-activatable near-infrared (NIR) aggregation-induced emission probe QMT-SFA.



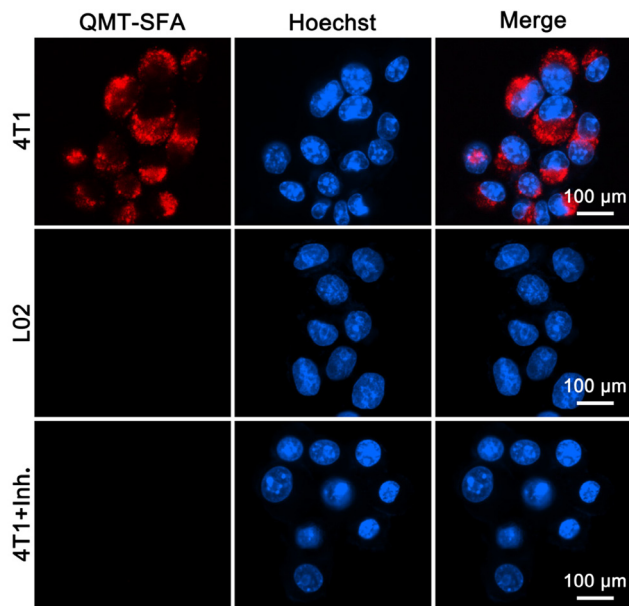


Fig. 3 Fluorescence images of 10  $\mu\text{M}$  QMT-SFA-treated 4T1 cells, L02 cells, and hydroxylamine-pretreated 4T1 cells for 1 h.

“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

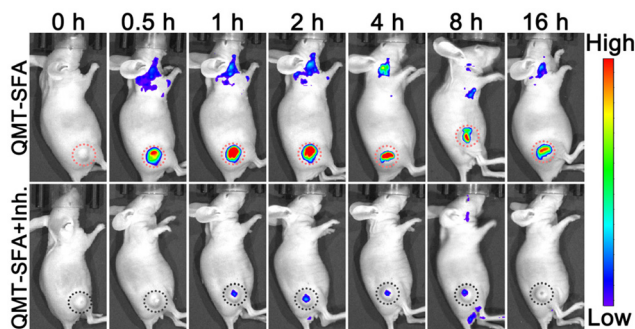


Fig. 4 Time-course fluorescence images of 4T1 tumor-bearing mice after the intratumoral injection of  $0.25 \text{ mg kg}^{-1}$  QMT-SFA. For the “QMT-SFA + Inh.” group, tumors were pretreated with  $0.02 \text{ mg kg}^{-1}$  inhibitor for 0.5 h before QMT-SFA injection. The dashed circles indicate tumor 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  $0.26 \text{ U mL}^{-1}$  *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.

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## References

- 1 S. Loibl, P. Poortmans, M. Morrow, C. Denkert and G. Curigliano, *Lancet*, 2021, **397**, 1750–1769.
- 2 A. N. Giaquinto, H. Sung, K. D. Miller, J. L. Kramer, L. A. Newman, A. Minihan, A. Jemal and R. L. Siegel, *CA Cancer J. Clin.*, 2022, **72**, 524–541.
- 3 D. Barba, A. León-Sosa, P. Lugo, D. Suquillo, F. Torres, F. Surre, L. Trojman and A. Caicedo, *Crit. Rev. Oncol. Hematol.*, 2021, **157**, 103174.
- 4 I. H. Kunkler, L. J. Williams, W. J. L. Jack, D. A. Cameron and J. M. Dixon, *N. Engl. J. Med.*, 2023, **388**, 585–594.
- 5 D. Yue, M. Wang, F. Deng, W. Yin, H. Zhao, X. Zhao and Z. Xu, *Chin. Chem. Lett.*, 2018, **29**, 648–656.
- 6 S. R. Hanson, M. D. Best and C.-H. Wong, *Angew. Chem., Int. Ed.*, 2004, **43**, 5736–5763.
- 7 R. Mashima and M. Nakanishi, *Int. J. Mol. Sci.*, 2022, **23**, 8153.
- 8 L. Xu, N. Liu, W. Zhan, Y. Deng, Z. Chen, X. Liu, G. Gao, Q. Chen, Z. Liu and G. Liang, *ACS Nano*, 2022, **16**, 19328–19334.
- 9 X. Wu, R. Wang, N. Kwon, H. Ma and J. Yoon, *Chem. Soc. Rev.*, 2022, **51**, 450–463.
- 10 S. He, P. Cheng and K. Pu, *Nat. Biomed. Eng.*, 2023, **7**, 281–297.
- 11 J. Li, H. Gao, R. Liu, C. Chen, S. Zeng, Q. Liu and D. Ding, *Sci. China: Chem.*, 2020, **63**, 1428–1434.
- 12 M. H. Xiang, Z. Y. Jiang, W. L. Zhao, E. Zhang, L. Xia, R. M. Kong, Y. Zhao, W. Kong, X. Liu, F. Qu and W. Tan, *ACS Sens.*, 2023, **8**, 2021–2029.
- 13 L. Xu, H. Gao, W. Zhan, Y. Deng, X. Liu, Q. Jiang, X. Sun, J.-J. Xu and G. Liang, *J. Am. Chem. Soc.*, 2023, **145**, 27748–27756.
- 14 Z. Zhu, Q. Wang, X. Chen, Q. Wang, C. Yan, X. Zhao, W. Zhao and W. H. Zhu, *Adv. Mater.*, 2022, **34**, e2107444.
- 15 S. Jia, Z. Gao, Z. Wu, H. Gao, H. Wang, H. Ou and D. Ding, *CCS Chem.*, 2022, **4**, 501–514.
- 16 J. Qi, H. Ou, Q. Liu and D. Ding, *Aggregate*, 2021, **2**, 95–113.
- 17 Y. Xu, M. Cui, W. Zhang, T. Liu, X. Ren, Y. Gu, C. Ran, J. Yang and P. Wang, *Chem. Eng. J.*, 2022, **428**, 132514.
- 18 L. Xu, H. Gao, Y. Deng, X. Liu, W. Zhan, X. Sun, J. J. Xu and G. Liang, *Biosens. Bioelectron.*, 2024, **255**, 116207.
- 19 H. Shen, F. Sun, X. Zhu, J. Zhang, X. Ou, J. Zhang, C. Xu, H. H. Y. Sung, I. D. Williams, S. Chen, R. T. K. Kwok, J. W. Y. Lam, J. Sun, F. Zhang and B. Z. Tang, *J. Am. Chem. Soc.*, 2022, **144**, 15391–15402.