

Development of Aldehyde Dehydrogenase-Responsive Turn-on Fluorescent Probe for Cancer Stem Cell Imaging

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Aldehyde dehydrogenase 1A1 (ALDH1A1) is a crucial biomarker for identifying cancer stem cells (CSCs), which are responsible for the proliferation, metastasis, and chemoresistance displayed by cancer.¹ Development of ALDH1A1-responsive turn-on fluorescence probes that can be used to visualize CSCs is highly desirable. However, it remains a challenge because the enzymatic transformation of a formyl group into a carboxylate has little effect on the electronic property of fluorescence dyes. This research focused on anionic property of carboxylate and we have developed ALDH1A1-activatable probe **C5SA** for CSC imaging. To our knowledge, **C5SA** is the first example of an ALDH-responsive near-infrared (NIR) probe.

We previously found that enzyme-mediated generation of carboxylate promoted the detachment of the quenching mercapto group from cyanine-type fluorophore (Cy5), which leads to the fluorescence activation.² Based on this discovery, we have designed and prepared an ALDH-responsive turn-on probe **C5SA** (Figure 1a). In a buffered solution (pH 7.4), ALDH1A1 transformed the formyl group of **C5SA** to carboxylate, leading to the NIR fluorescence increment, which is probably caused by thermodynamic stabilization of cationic Cy5 by anionic carboxylate. Confocal images of SUIT-2 cells indicate that **C5SA** successfully visualized CSCs among normal cancer cells more clearly than ALDEFLUOR,³ one of the best reagents available for identifying CSCs¹ (Figure 1b-c). In addition, these brightly emitting cells were isolated by fluorescence-activated cell sorting using a 640 nm red laser, indicating that **C5SA** can be used to isolate CSCs for analytical and clinical purposes.

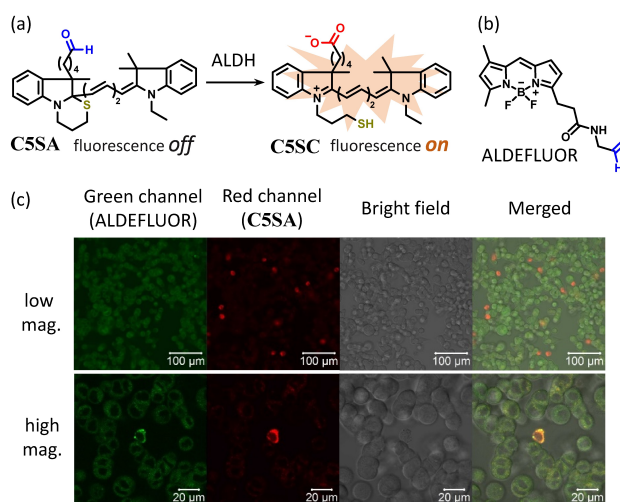


Figure 1. (a) ALDH-responsive turn-on probe **C5SA**. (b) The structure of ALDEFLUOR ($\lambda_{\text{em}} = 500\text{--}550\text{ nm}$). (c) Confocal images of SUIT-2 cells incubated with ALDEFLUOR and **C5SA**.

1) a) Ma, I.; Allan, A. L. *Stem Cell Rev. Rep.* **2011**, 7, 292–306. b) Rodriguez-Torres, M.; Allan, A. L. *Clin. Exp. Metastasis* **2016**, 33, 97–113. 2) Oe, M.; Miki, K.; Ohe, K. *Org. Biomol. Chem.* **2020**, 18, 8620–8624. 3) Storms, R. W. et al. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, 96, 9118–9123.