Supporting Information

CHA-based DNA stochastic walker that traverses on cell membranes

Yanlei Hu and Xia Chu*

Address: State Key Laboratory of Chemo/Bio-Sensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, P. R. China

*Corresponding Author. Fax: +86-731-88821916; E-mail: xiachu@hnu.edu.cn.

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Material and methods

Reagents and Instruments

Oligonucleotides, bovine serum albumin (BSA), D-glucose, magnesium chloride ($MgCl_2$), Acryl/Bis 30% Solution, 10X PBS and 5X TBE buffer were purchased from Sangon Biotech (Shanghai, China). Ammonium persulfate (APS) and N,N,N′,N′-tetramethylethylenediamine (TMED) were obtained from Sigma-Aldrich (Darmstadt, Germany). Deionized water was prepared by Milli-Q Ultrapure Water System (Merck KGaA Darmstadt, Germany). Solutions were sterilized with 0.22 μm filters (Merck KGaA Darmstadt, Germany). Temperature was controlled with CFX96 Real-Time System (Bio-Rad Hercules, USA), Veriti 96-Well Thermo Cycler (Applied Biosystems Waltham, USA) or Mini Box-C Dry Bath (Ruicheng Hangzhou, China). Polyacrylamide gel electrophoresis (PAGE) was performed on a JY600C electrophoresis system (JUNYI Beijing, China). The gel image was captured by Tanon 4600SF (Tanon Shanghai, China). Flow cytometry data was recorded on CytoFLEX (Beckman Coulter Brea, USA). Cell images were acquired on A1 MP multiphoton confocal microscope (Nikon Tokyo, Japan).

Preparation of Hairpin H1 and H2

H1 and H2 strands were dissolved in water and diluted to 20 μM in 1×PBS buffer separately. Hairpin H1 and H2 were prepared by heating to 95 °C for 5 min, followed by slowly decreasing the temperature to 23 °C at a rate of 0.1 °C s^{-1}. Then H1 and H2 were incubated at 4°C for 30 min for further refolding.

PAGE Electrophoresis

Hairpin H1, H2 and catalyst strands (500:500:50nM) mixed in 1X PBS/$MgCl_2$ buffer (containing 5 mM $MgCl_2$ in 1X PBS). The mixtures were incubated for 30 min at 4 °C, 15°C or 37 °C separately. Then the mixtures were loaded onto pre-prepared 20% polyacrylamide gel and separated with electrophoresis. After electrophoretic separation, the gel was stained with EB and imaged on a gel imaging system.

CHA-based DNA Walker on Cell Membranes

CEM cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, and were maintained at 37 ℃ environments with 5% CO_2. Cells were diluted to 5×10^5 cells/ml in working buffer (containing 5 mM $MgCl_2$, 4.5 g/L glucose and 1 mg/mL bovine serum albumin in 1X PBS) and washed two times by centrifuging at 1500 rpm (about 200g) for 2 min. The cells were incubated with 250 nM Chol-H1 at 4 °C for 30 min and gently shaken every 10 min. Then the cells were washed three times to discard non-binding probes. After washed, the cells were incubated with catalyst-strands and 500 nM MB-H2 at 15 °C for 30 min, and gently shaken every 10 min. Cell fluorescence was recorded with flow cytometry. For imaging, cells were washed three times and imaged on a confocal microscope. For figure 2d and 2e, the cell fluorescence was measured with ImageJ. We selected the cell regions in DIC channel with Freehand selections, save the selected regions and measured the fluorescence intensity for each region in FAM channel.

CHA-based DNA Motor on Cell Membranes

CEM cells were diluted to 5×10^5 cells/ml in working buffer and washed two times. The cells were incubated with anchoring-catalyst for 30 min and washed three times. Then the cells were incubated with 250 nM Chol-H1 for 30 min. After washed, the cells were incubated in 500 nM MB-H2 at 15 °C for 30 min. Cell fluorescence was recorded with flow cytometry. For imaging, the cells were washed three times and imaged on a confocal microscope.

TC01 Sensing

CEM cells were diluted to 5×10^5 cells/ml in working buffer and washed two times. The cells were incubated with 100 nM TC01-catalyst or non-aptamer-catalyst for 30 min and washed for three times. The cells were then incubated with 250 nM Chol-H1 for 30 min. After washed, the cells were incubated in 500 nM MB-H2 at 15 °C for 30 min. Cell fluorescence was recorded with flow cytometry.

Supplementary Table

Table S1. DNA Sequences for CHA on cell membranes.

Name	**Sequences (5' to 3')**
H1	GTCAGTGAGCTAGGTTAGATGTCGCCATGTGTAGACGACATCTAACCTAGC
H2	AGATGTCGTCTACACATGGCGACATCTAACCTAGCCCATGTGTAGA
Single-catalyst	CGACATCTAACCTAGCTCACTGAC
Non-catalytic control	/6-FAM/ CGACATCTAACCTAGCTCACTGAC
Double-catalyst	CGACATCTAACCTAGCTCACTGACCATATCACATACCTCTACTCATCCACAC ATATTACACACACCGACATCTAACCTAGCTCACTGAC
anchoring-catalyst	CGACATCTAACCTAGCTCACTGACTTTTTTTTTTTTTTTTTTTTTTTT /Cholesteryl/
Chol-H1	GTCAGTGAGCTAGGTTAGATGTCGCCATGTGTAGACGACATCTAACCTAGC ATTTTTTTTTTTTTTTTTTTTTTTTTTTT /Cholesteryl/
FAM-Chol-H1	/6-FAM/GTCAGTGAGCTAGGTTAGATGTCGCCATGTGTAGACGACATCTAACCT AGCATTTTTTTTTTTTTTTTTTTTTTTTTTT /Cholesteryl/
MB-H2	AGATGTCG/iBHQ1dT/CTACACATGGCGACATCTAACCTAGCCCATGTGTAGA /6-FAM/
TC01-catalyst	CGACATCTAACCTAGCTCACTGACTTTTTTTTTTTTTTTTTTTTACCAAACAC AGATGCAACCTGACTTCTAACGTCATTTGGT
Non-aptamer-catalyst	CGACATCTAACCTAGCTCACTGACTTTTTTTTTTTTTTTTTTTTNNNNNNNNN NNNNNNNNNNNNNNNNNNNNNNNNNNNN

Supplementary Figures

Figure S1. Native PAGE analysis of CHA at different temperatures. Line 1: ladder; Line 2: 500 nM H1 alone; Line 3: 500 nM H2 alone; Line 4, 6, 8: 500 nM H1 and 500 nM H2 at the respective temperatures for 30 min; Line 5, 7, 9: 500 nM H1, 500 nM H2 and 50 nM catalyst at the respective temperatures for 30 min.

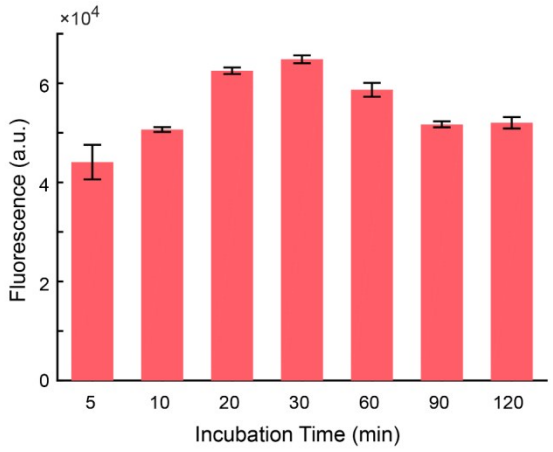

Figure S2. Cell fluorescence vs incubation time. Cells were incubated with 250 nM FAM-Chol-H1, and cell fluorescence was measured with flow cytometry at different time points (mean ± SD, n = 3 biological replicates). The gradual fluorescence loss may be related to DNase, endocytosis and equilibrium of anchoring DNA between the medium and the cell surface (Adv. Sci. 2019, 6, 1900043).

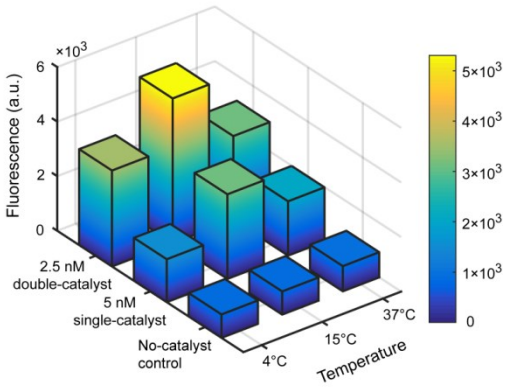

Figure S3. Flow cytometry study of CHA on cell membranes with single-catalyst and double-catalyst at different temperatures.

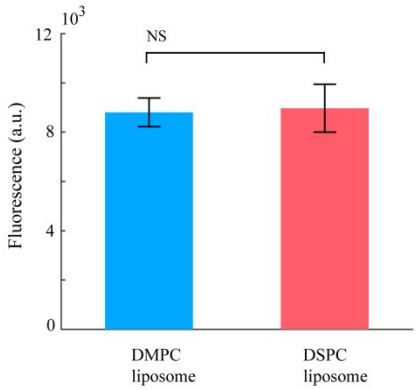

Figure S4. Flow cytometry study of CHA on liposomes with single-catalyst (mean ± SD, n = 3 biological replicates, NS: not significant, *t*-test). We prepared liposomes with different membrane fluidity by thin film dispersion method. We removed large particles with centrifugation at 2000 rpm/min, selected large liposomes with centrifugation at 5000 rpm/min, and counted the liposomes with a cell counter. Then we performed CHA on the liposomes with 20 nM single-catalyst. The composition of a membrane can affect its fluidity. Lipids with shorter chains are less stiff and less viscous. DMPC liposomes are more fluid then DSPC liposomes. However, there is no significant difference in the fluorescence signals of the two liposomes. Since the DNA strand migration is much faster compared with the membrane diffusion (Nat. nanotech. 2017, 12, 453-459. Sup. Fig. 7), the fluorescence was mainly affect by the strand displacement reaction.

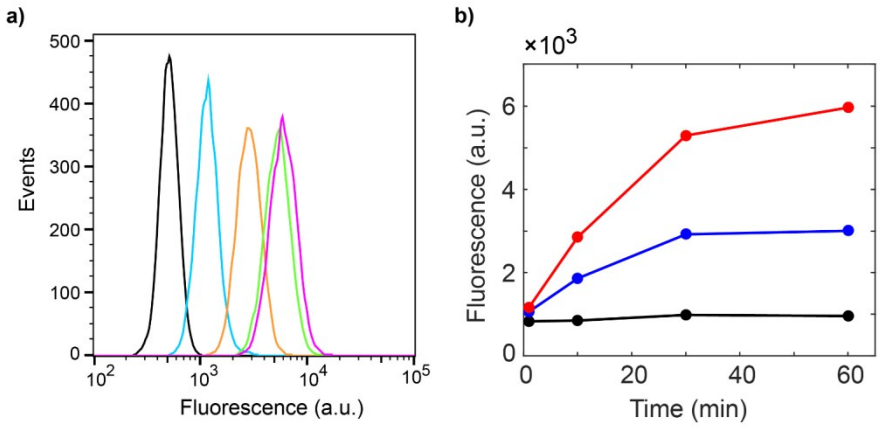

Figure S5. (a) Flow cytometry data of CHA on cell membranes with 2.5 nM double-catalyst at different time points (black line: CEM cells, light blue line: 1 min, orange line: 10 min, green line: 30 min, pink line: 60 min). (b) Fluorescence intensity of CHA on cell membranes with double-catalyst vs reaction time (black line: no-catalyst control, blue line: 1 nM double-catalyst, red line: 2.5 nM double-catalyst).

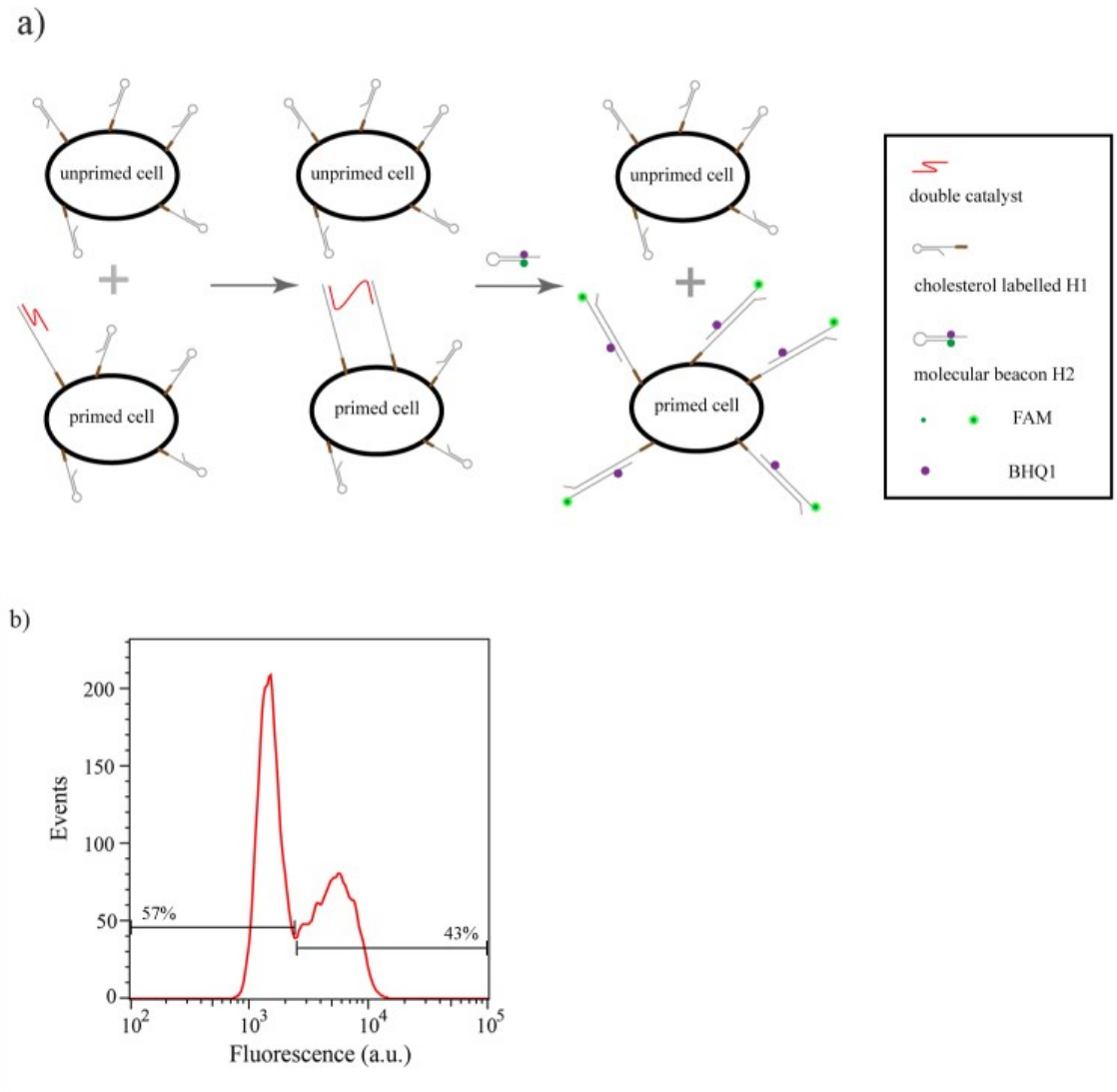

Figure S6. (a) Schematic illustration of the experiment to verify the prediction that the double-catalyst walks on cell membranes. (b) Flow cytometry data generated by cells hybridized & unhybridized with double-catalyst (50:50% ratio). We prepared two different types of probes for anchoring on cell membranes: H1 and H1 hybridized with double-catalyst. About 1% of H1 strands were pre-hybridized with double-catalyst. H1 and H1 hybridized with double-catalyst were anchored on cell membranes separately. Unprimed and primed cells were mixed in a 50:50% ratio, and incubated with MB-H2 at 15 °C for 30 min. Then cell fluorescence intensity was measured with flow cytometry.

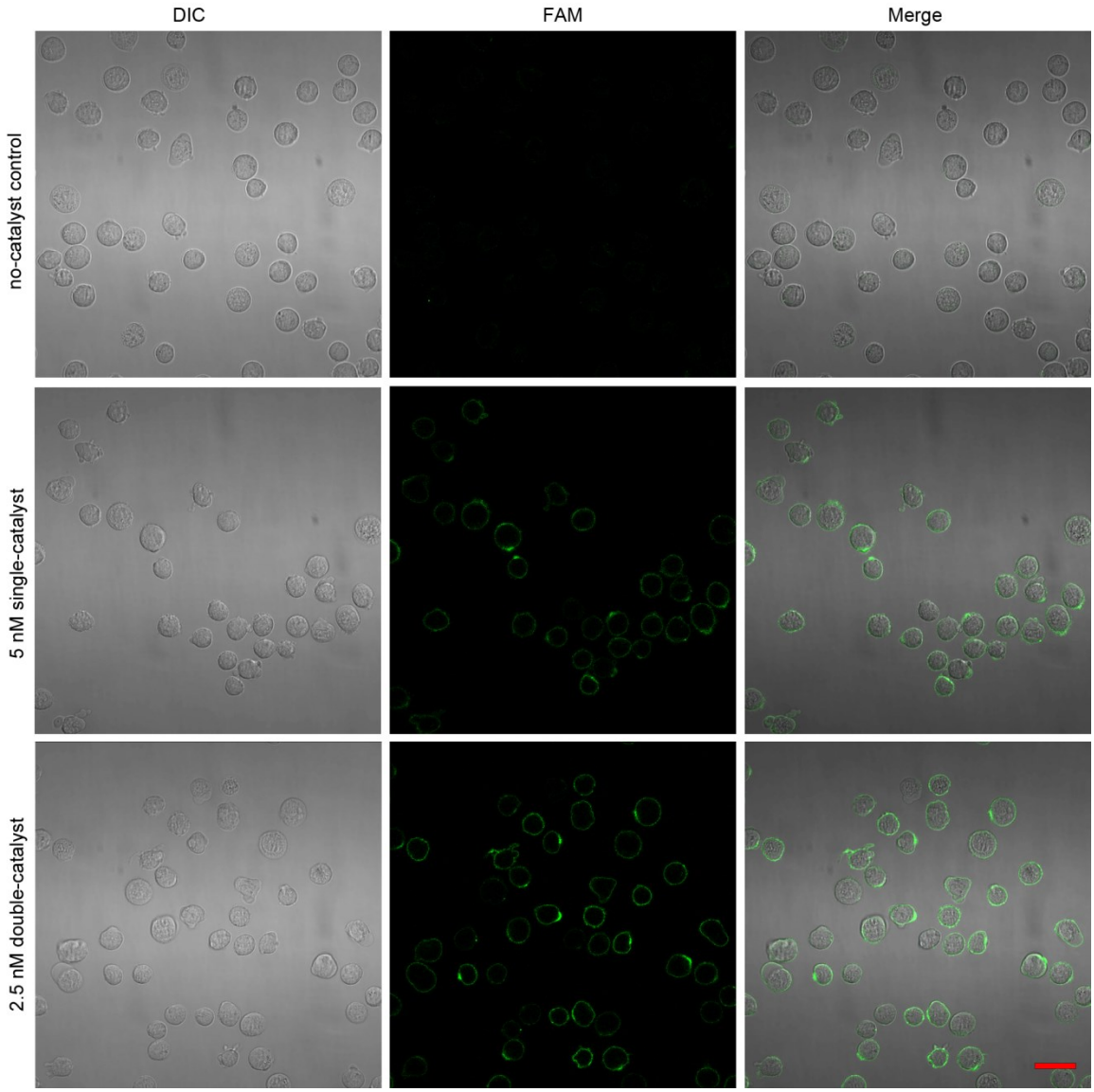

Figure S7. Images of CHA on cell membranes with single-catalyst or double-catalyst (scale bar: 25 μm).

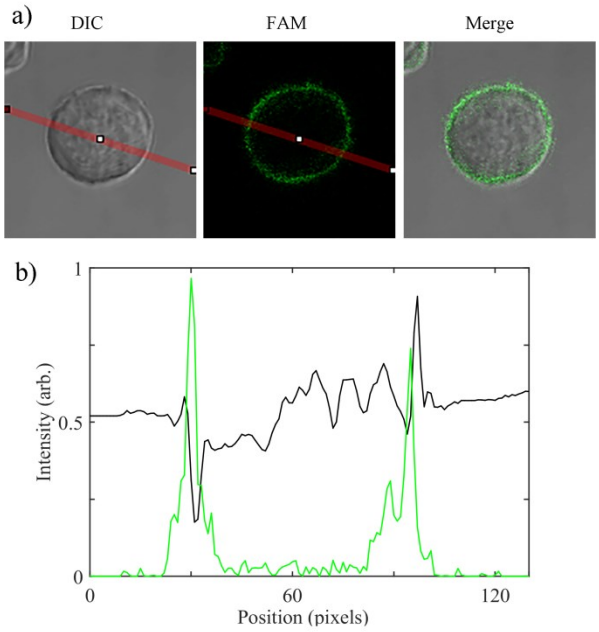

Figure S8. (a) The cell for colocalization analysis. (b) Relative intensity profile of red lines in picture a (blank line: DIC channel; green line: FAM channel). Fluorescence peaks in FAM channel are located at the position where the gray value in DIC channel sharply changed. This indicates that the CHA occurred on cell membranes.

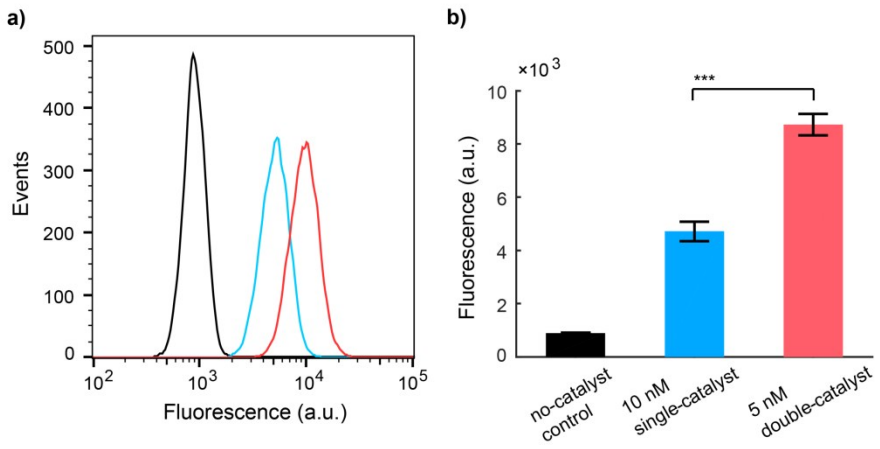

Figure S9. Comparing CHA on cell membranes catalyzed by single-catalyst with that catalyzed by double-catalyst. (a) Flow cytometry data of CHA on cell membranes (black line: no-catalyst control, blue line: 10 nM single-catalyst, red line: 5 nM double-catalyst). (b) Fluorescence intensity of CHA on cell membranes (mean ± SD, n = 3 biological replicates, ***P<0.001 *t*-test).

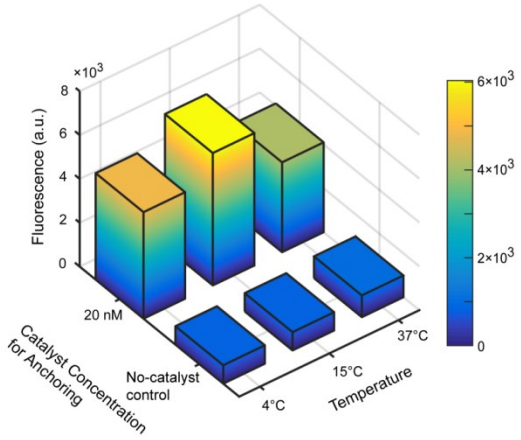

Figure S10. Flow cytometry study of CHA on cell membranes with anchoring-catalyst at different temperatures.

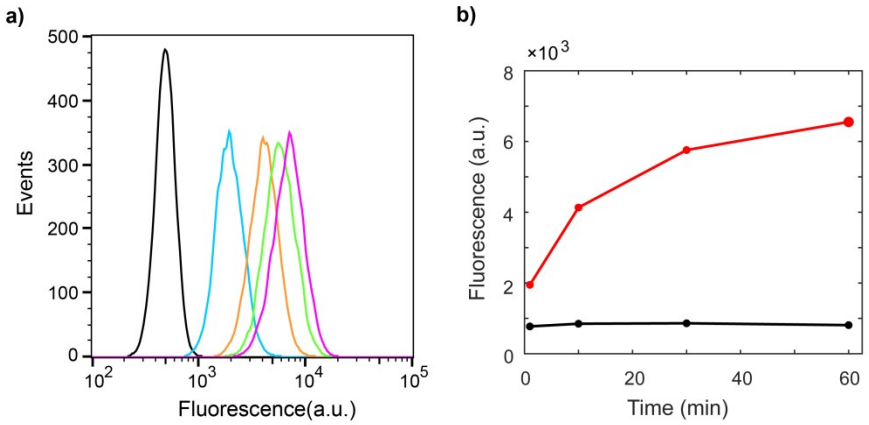

Figure S11. (a) Flow cytometry data of CHA on cell membranes with 20 nM anchoring-catalyst at different time points (black line: CEM cells, light blue line: 1 min, orange line: 10 min, green line: 30 min, pink line: 60 min). (b) Fluorescence intensity of CHA on cell membranes with anchoring-catalyst vs reaction time (black line: no-catalyst control, red line: 20 nM catalyst for anchoring).

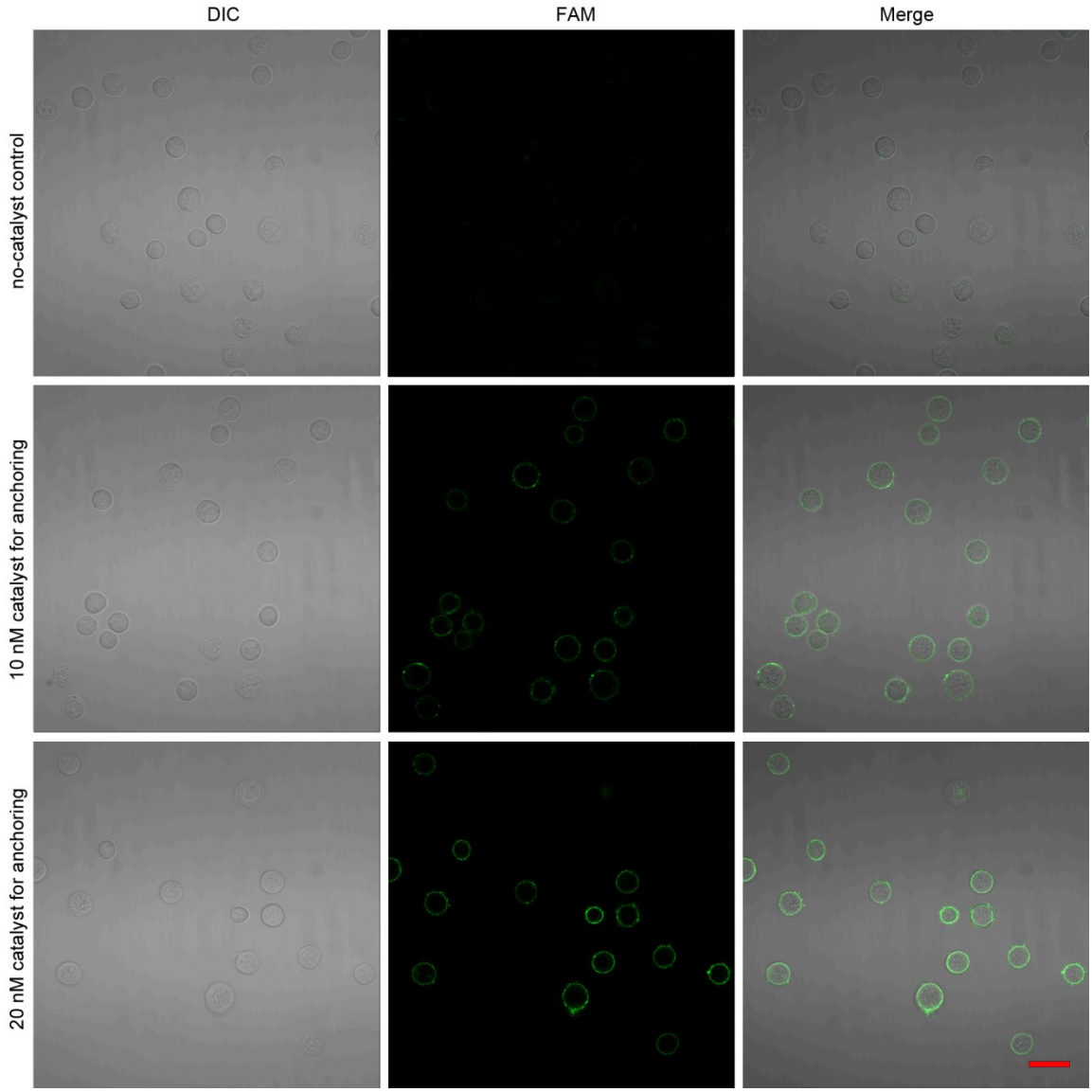

Figure S12. Images of CHA on cell membranes with anchoring-catalyst (scale bar: 25 μm).

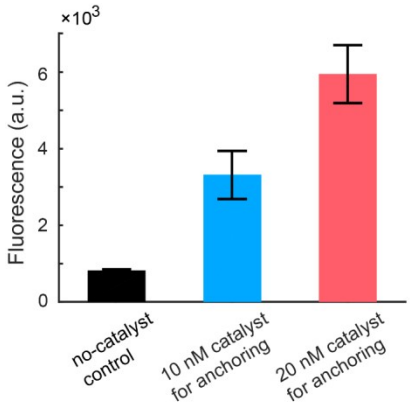

Figure S13. Fluorescence intensity of CHA on cell membranes with anchoring-catalyst (mean ± SD, n = 3 biological replicates).

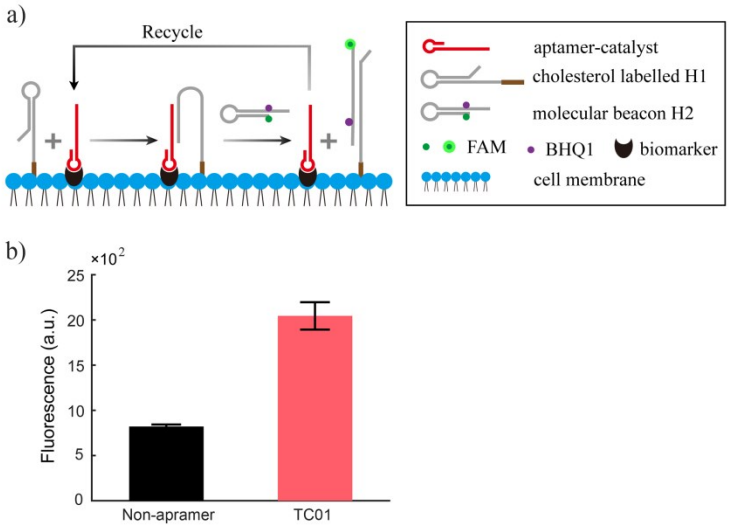

Figure S14. (a) Schematic illustration of CHA for recognizing cell membrane target. (b) Fluorescence intensity of CHA on cell membranes for TC01 sensing (mean ± SD, n = 3 biological replicates).