Supporting Information

Filter Beacon: A Gating-Free Architecture for Protein-Specific Glycoform Imaging on Cell Surface

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Experimental Section

Apparatus. The UV-vis absorption spectra were obtained on a UV-3600 UV-VIS-NIR spectrophotometer (Shimadzu, Japan). The fluorescence spectra were obtained on an F-7000 fluorescence spectrophotometer (Hitachi, Japan). The images of cells were acquired on a TCS SP5 confocal laser scanning microscope (CLSM) (Leica, Germany). Cell number was calculated with Countess® II Automated Cell Counter (Invitrogen, USA). Protein transfer to PVDF membranes was performed on a Trans-Blot Turbo Transfer System (Bio-Rad, USA). Native polyacrylamide gel electrophoresis (PAGE) and SDS-PAGE analysis were conducted on an Electrophoresis Analyzer (Bio-Rad, USA) and imaged on a Bio-Rad ChemDoc XRS facility (Bio-Rad, USA).

Cell Culturing. MCF-7 cells were cultured in RPMI-1640 medium supplemented with 10% FBS, penicillin (100 μg/mL), and streptomycin (100 μg/mL). T47D cells were cultured in RPMI-1640 medium supplemented with 10% FBS, 0.2 U/mL insulin, penicillin (100 μg/mL), and streptomycin (100 μg/mL). HepG2 cells were cultured with DMEM supplemented with 10% FBS, penicillin (100 μg/mL), and streptomycin (100 μg/mL). Cells were grown in a 37 °C incubator containing 5% CO₂.

Plasmid Transfection. The MCF-7 cells were seeded on the 6-well confocal dishes and cultured at 37 °C for 12 h. A mixture of 1.25 μg plasmid (shRNA or shRNA-NC) and 250 μL Opti-MEM and a mixture of 5 μL lipofectamine 3000 and 125 μL Opti-MEM were individually prepared. Then 125 μL aliquot of the solution was taken from each of the two mixtures, mixed, and incubated at r.t. for 10 min. The mixture was added into the culture medium. After thorough mixing, the cells were incubated at 37 °C for 6 h. Then RPMI-1640 medium supplemented with 10 % FBS was added into each well and the cells were incubated at 37 °C for 42 h.

Western Blot Analysis of MUC1. After the transfection of MCF-7 cells with shRNA or
shRNA-NC, the RPMI-1640 medium supplemented with 10 % FBS was discarded and the cells were gently washed with 1× PBS. A sample without plasmid transfection was used as a control. Then 250 μL cell lysis buffer containing RIPA lysis buffer, protease inhibitor cocktail, and PMSF was added into each well. The lysates were transferred into a 1.5 mL centrifuge tube. Then 16000 g centrifugation was performed at 4 °C for 20 min. The protein concentration of supernatant was determined by BCA test. A mixture of supernatant and 1× SDS-PAGE sample buffer was boiled at 100 °C for 5 min. The proteins in the sample were separated by SDS-PAGE, and transferred onto a PVDF membrane. After the transfer, the membrane was cut into two identical parts and washed three times with 1× TBST (a mixture of TBS and Tween 20). The two membranes were immersed in BSA blocking buffer and shaken at 60 rpm for 2 h. After three times washing with 1× TBST, one membrane with anti-MUC1 antibody and the other with anti-GAPDH antibody were incubated at 4 °C overnight in 3% BSA-containing 1× TBST buffer. The membranes were washed three times with 1× TBST, incubated with HRP conjugated goat anti-rabbit IgG in 3% BSA 1× TBST buffer at r.t. for 1 h, and washed three times again with 1× TBST. Then ECL luminescent working solution was evenly placed on the surface of the membranes and the membranes were imaged on a Bio-Rad ChemDoc XRS imaging system.
Supporting Table and Figures

Table S1. DNA sequence information

<table>
<thead>
<tr>
<th>Name</th>
<th>DNA Sequence (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-Apt</td>
<td>CGCTGAGGAATAAATGGAAAATGAGTAG-iSp18-GCAGTTGA</td>
</tr>
<tr>
<td></td>
<td>TCCTTTGGATAACCCCTGG</td>
</tr>
<tr>
<td>F-P-Apt</td>
<td>FAM-CGCTGAGGAATAAATGGAAAATGAGTAG-iSp18-GCAGT</td>
</tr>
<tr>
<td></td>
<td>TGATCCTTTGGATAACCCCTGG</td>
</tr>
<tr>
<td>F-P-Ran</td>
<td>FAM-CGCTGAGGAATAAATGGAAAATGAGTAG-iSp18-GAGAA</td>
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<tr>
<td></td>
<td>CCTGAGTCAGTATTGCGGAG</td>
</tr>
<tr>
<td>FB</td>
<td>CCACGGTAGTCAGTGTATTCCCTACGCCTGGTTTTTTTT</td>
</tr>
<tr>
<td>Q-FB-F</td>
<td>DABCYL-CCACGCTAGTCCTACGCCTGG-iFAMdT-TTTTTT</td>
</tr>
<tr>
<td>FB-F</td>
<td>CCACGGTAGTCAGTGTATTCCCTACGCCTGG-iFAMdT-TTTTTT</td>
</tr>
<tr>
<td>Q-FB-F-DBCO</td>
<td>DABCYL-CCACGCTAGTCCTACGCCTGG-iFAMdT-TTTTTT-DBCO</td>
</tr>
<tr>
<td>FB-DBCO</td>
<td>CCACGGTAGTCAGTGTATTCCCTACGCCTGG-iFAMdT-TTTTTT-DBCO</td>
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<tr>
<td>Q-FB-F-N3</td>
<td>DABCYL-CCACGCTAGTCCTACGCCTGG-iFAMdT-TTTTTT-N3</td>
</tr>
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</table>

Note: The bold parts in DNA sequences represent the aptamer segment; the red parts represent the fluorophore and quencher portions; the green parts represent the nicking restriction site (cleavage of CC and TCAGC); the purple parts represent the structural moiety for click reaction.
**Figure S1.** Native PAGE analysis showing partial hybridization between **P-12** (1 μM) and **FB** (1 μM), full cleavage of **FB** and complete retention of **P-12** with the nicking action from NE (1000 U/mL). Lane 1: **P-12** and **FB** (thermally annealed); 2: **P-12**; 3: **FB**; 4: **P-12** and **FB** (37 °C for 1.5 h). 5: **P-12**, **FB**, and NE (37 °C for 1.5 h); 6: DNA ladder. Buffer: 1× Cutsmart buffer.
Figure S2. Native PAGE analysis showing partial hybridization between P-Apt and FB.
Lane 1: P-Apt (1 μM); 2: FB (1 μM); 3: FB (200 nM); 1-3: 37 °C for 1.5 h; 4 and 5: P-Apt (1 μM) and FB (1 μM); 4: 4 °C for 1.5 h; 5: 37 °C for 1.5 h; 6: P-Apt (1 μM) and FB (1 μM) (thermally annealed); 7 and 8: P-Apt (1 μM) and FB (200 nM); 7: 4 °C for 1.5 h; 8: 37 °C for 1.5 h; 9: P-Apt (1 μM) and FB (200 nM) (thermally annealed); 10: DNA ladder. Buffer: 1× Cutsmart buffer.
**Figure S3.** Native PAGE analysis showing partial hybridization between F-P-Apt (5 μM) and FB (5 μM), P-Apt (5 μM) and FB-F (5 μM), full cleavage of FB-F and complete retention of F-P-Apt with the nicking action from NE (1000 U/mL) (all 37 °C, 1.5 h). Lane 1: F-P-Apt; 2: F-P-Apt and FB; 3: F-P-Apt, FB, and NE; 4: FB-F; 5: P-Apt and FB-F; 6: P-Apt, FB-F, and NE. Buffer: 1× Cutsmart buffer. The fluorescent imaging is achieved directly with FAM and without staining.
Figure S4. Fluorescence analysis showing partial hybridization between P-Apt and Q-FB-F (fixed at 100 nM). (a) 4 °C in PBS for 30 min. (b) 4 °C in 1× Cutsmart buffer for 1.5 h. (c) 37 °C in 1× Cutsmart buffer for 1.5 h.
Figure S5. CLSM imaging showing specific binding of F-P-Apt to MUC1 through aptamer segment. CLSM imaging of MUC1-positive MCF-7 cells and MUC1-negative HepG2 cells after incubation at 4 °C in PBS buffer solution of F-P-Apt (1 μM) and F-P-Ran (1 μM) for 30 min. Scale bar: 25 μm.
Figure S6. Requirement of both metabolic engineering incorporation of Sia azide and DBCO moiety for Cu(I)-free click docking of filter beacon probe. CLSM imaging of MCF-7 cells after metabolic engineering treatment with Ac₄ManNAz and incubation at 4 °C in PBS buffer solution of FB-F (10 μM) and FB-F-DBCO (10 μM) for 30 min. Scale bar: 25 μm.
Figure S7. Optimization of duration of nicking action for filter beacon imaging. (a) CLSM imaging of MCF-7 cells after metabolic engineering incorporation of Sia azide, treatments with **P-Apt** (1 μM) and **Q-FB-F-DBCO** (10 μM), and different durations of nicking action from NE (600 U/mL, 1× Cutsmart buffer, 37 °C). (b) Fluorescence signal intensity as a function of duration of nicking action. Scale bar: 25 μm. For fluorescence signal intensity measurement, 10 cells are used.
Figure S8. Optimization of NE concentration for filter beacon imaging. (a) CLSM imaging of MCF-7 cells after metabolic engineering incorporation of Sia azide, treatments with P-Apt (1 μM) and Q-FB-F-DBCO (10 μM), and different concentrations of NE (1× Cutsmart buffer, 37 °C, 1.5 h). (b) Fluorescence signal intensity as a function of NE concentration. Scale bar: 25 μm. For fluorescence signal intensity measurement, 10 cells are used.
**Figure S9.** CLSM imaging showing no direct impact on the expression level of MUC1 from BAG and TM treatments. (a) CLSM imaging of MCF-7 cells after treatment with either BAG or TM and then incubation in **F-P-Apt** (1 μM) for 30 min. Control indicates no treatment. (b) Fluorescence signal intensity obtained from (a). Scale bar: 25 μm. For fluorescence signal intensity measurement, 10 cells are used. Statistical analysis: t-test (NS: not significant).
Figure S10. CLSM imaging of MUC1-bound terminal Sia on control MCF-7 cells (as controls for Fig. 3). (a) No plasmid (as a control for plasmid transfection). (b) No NEU treatment (as a control for NEU treatment). Scale bars: 25 μm.
Figure S11. Vector map of shRNA. The vector map of shRNA-NC (control plasmid) is otherwise identical except with the insertion of shRNA-NC oligonucleotides into the pRNAT-U6.1/Neo vector.
Figure S12. CLSM imaging showing the knockdown of MUC1 with shRNA. (a) Western blot analysis of MUC1 after the transfection of either shRNA or shRNA-NC. Control indicates no treatment. (b) CLSM imaging of MCF-7 cells after the transfection of either shRNA or shRNA-NC and then incubation in \textbf{F-P-Apt} (1 μM) for 30 min. (c) Fluorescence signal intensity obtained from (b). Scale bar: 25 μm. For fluorescence signal intensity measurement, 10 cells are used. Statistical analysis: t-test (***P < 0.001; NS: not significant).