Electronic Supplementary Information

Multiplexed Detection of Lung Cancer Cells at the Single-Molecule Level

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Experimental Section

Materials. All oligonucleotides (Table S1) were synthesized and HPLC purified by Sangon Biotechnology Co. Ltd. (Shanghai, China). Dynabeads® M-280 Streptavidin and Yeast tRNA were obtained from Invitrogen (USA). DL-Dithiothreitol (DTT) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich, Inc (USA). The A549 and H69 cells were obtained from Cell Resource Center, IBMS, CAMS / PUMC (Beijin, China). The H23 cells were provided by Dr. Ke Ding at Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences (Guangzhou, China). The washing buffer contains 4.5 g/L glucose, 5 mM $MgCl_2$ in Dulbecco's PBS (pH 7.3), and the binding buffer contains 1 mg/mL tRNA and 1 mg/mL BSA in the washing buffer.[1,2]

Table S1. Sequence of the Oligonucleotides.

note	sequence (5'-3')
capture probe 1	biotin – TTT TTT TTT TGG TTG CAT GCC GTG GGG AGG GGG GTG GGT TTT ATA GCG TAC TCA G
reporter probe 1	Alexa Fluor 488 – GCT ATC TTA TGG AAA TTT CGT GTA GGG TTT GGT GTG GCG GGG CTA
capture probe 2	biotin – TTT TTT TTT TGA AGA CGA GCG GCG AGT GTT ATT ACG CTT GGA AAC AAC CCC
reporter probe 2	Alexa Fluor 647 – GAG CCC TAT CTC ACA CCG CAC CCG CAA ACT ATC ATC CTAC ATG

Multiplexed Detection of Cancer Cells at the Single-Molecule Level. All cancer cells were cultured in RPMI 1640 culture medium (Hyclone, USA) with 10% fetal bovine serum (FBS, Invitrogen, USA) and 1% penicillin-streptomycin (Invitrogen, USA) at 37 ℃ under 5% CO_2 atmosphere. Before incubation with the capture and the reporter probes, the target cancer cells (A549 and H23 cells) and nonspecific cells were grown to 90% confluence in 100-mm culture dishes and washed three times with PBS. Then the adhesive cells were scraped off and dispersed in 4 mL of PBS, and centrifuged at 800 rpm for 5 min. Finally, the cells were redispersed in the washing buffer. The cell amount was determined with a hemocytometer.

The mixture of capture and reporter probes was denatured at 95 ℃ for 10 min, followed by cooling on ice for 10 min. The cells were added to 100 μL of solution containing the mixture of capture and reporter probes, 1 mg/mL tRNA, 1 mg/mL BSA, and 20% FBS. The final

concentration of each probe was 500 nM. The cells were incubated at 4 ℃ for 1 h, and then centrifuged at 2000 rpm for 10 min to remove the upper solution. After the addition of streptavidin-coated magnetic beads (~1.2×10^8 beads/mL), the cells were incubated at room temperature for 30 min, followed by magnetic separation for 2-3 min and washing three times with the washing buffer. The cells bound by the magnetic beads were dissolved in 300 mM DTT and heated at 95 ℃ for 10 min to release both the capture and the reporter probes from the cells and the magnetic beads,[1-3] followed by centrifuging at 14,000 rpm to remove the cell debris. The obtained 80 μL of supernatant solution was subject to single-molecule detection.

The images of single molecules were obtained by total internal reflection fluorescence microscopy (TIRF) using an oil immersion objective (Olympus, 100×, NA 1.45), and imaging onto the two halves of an Andor Ixon DU897 EMCCD. To measure the fluorescence signals, 20 μL of supernatant solution was spread on a glass coverslip and imaged with an exposure time of 200 ms at the excitation wavelength of 488 nm with an emission filter of 520 ± 17.5 nm for Alexa Fluor 488, and at the excitation wavelength of 640 nm with an emission filter of 692 ± 20 nm for Alexa Fluor 647, respectively. For data analysis, the number of molecules from ten frames are counted, with an imaging region of 256 × 512 pixels (0.16 μm/pixel, 41 × 82 μm^2) being counted in each frame.[4]

Measurement of Probes Density on the Cell Surface. The probes density on the cell surface was calculated by quantification of both DNA probes and cells. The fluorescence intensity of reporter probes bound by the target cells was measured by fluorescence spectrum. Based on the fluorescence intensity calibration curves of reporter probes, the number of reporter probes 1 bound

by A549 cells was calculated to be 3.55×10^{-12} mol, and the number of reporter probe 2 bound by H23 cells was calculated to be 1.51×10^{-12} mol. In addition, the number of added A549 cells was measured to be 5×10^{5} cells (8.31×10^{-19} mol), and the number of added H23 cells was measured to be 5×10^{5} cells (8.31×10^{-19} mol). Accordingly, the average number of reporter probe 1 per A549 cell and the average number of reporter probe 2 per H23 cell was calculated to be 4.27×10^{6} and 1.81×10^{6}, respectively.

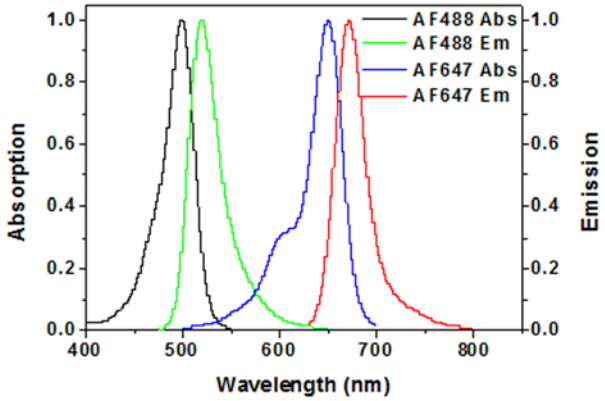

Figure S1. The normalized absorption (Abs) and emission spectra (Em) of Alexa Fluor 488 (AF488) and Alexa Fluor 647 (AF647). Black line, absorption spectrum of Alexa Fluor 488; green line, emission spectrum of Alexa Fluor 488; Blue line, absorption spectrum of Alexa Fluor 647; red line, emission spectrum of Alexa Fluor 647.

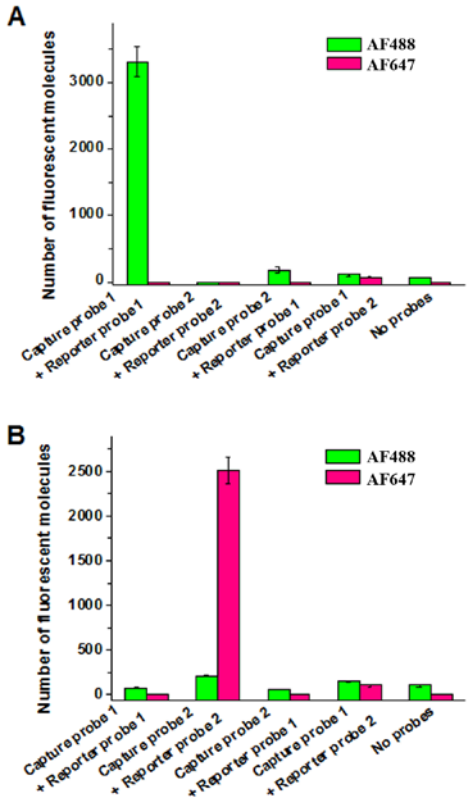

Figure S2. Specificity of designed probes for the detection of A549 (A) and H23 cells (B). The AF488 fluorescence signals are shown in green, and the AF647 fluorescence signals are shown in red. All the measurements were performed in the presence of 10^5 cells. The concentration of each probe is 500 nM. Error bars show the standard deviation of three experiments.

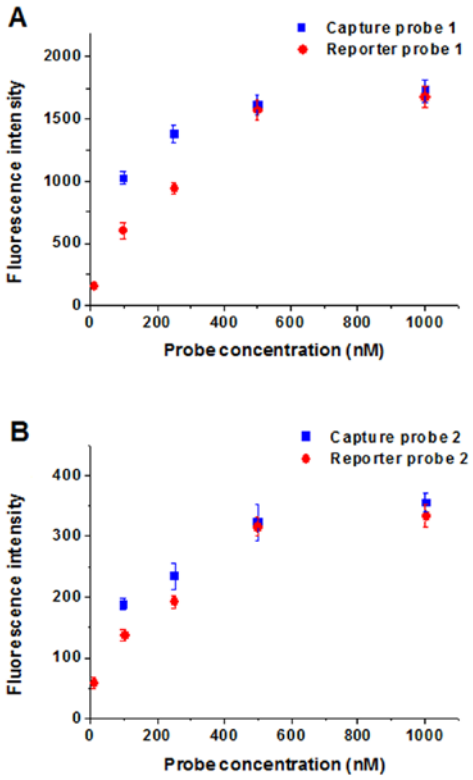

Figure S3. Optimization of the concentrations of capture probe (blue square) and reporter probe (red circle) in the presence of A549 (A) and H23 cells (B). When the concentration of one probe is optimized, the concentration of another probe is fixed at 500 nM. The amount of each type of cells is 10^5 cells/100 μL. Error bars show the standard deviation of three experiments.

References

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