Electronic Supplementary Information

Rapid identification of molecular subtyping of breast cancer cell lines using multi-channel sensor array

Yuyao Jin,[†ab] Yuanjie Sun,[†a] Nan Du,[a] Wei-Tao Dou,[c] Ying Tan,[ab] Xiao-Peng He,[c] Naihan Xu [*ab] and Chunyan Tan[*ab]

[a] The State Key Laboratory of Chemical Oncogenomics, Key Laboratory of Chemical Biology, Tsinghua Shenzhen International Graduate School, Tsinghua University, Shenzhen, 518055, P. R. China

[b] Open FIESTA, Tsinghua Shenzhen International Graduate School, Shenzhen 518055, P. R. China

[c] Key Laboratory for Advanced Materials and Joint International Research Laboratory of Precision Chemistry and Molecular Engineering, Feringa Nobel Prize Scientist Joint Research Center, School of Chemistry and Molecular Engineering, Frontiers Center for Materiobiology and Dynamic Chemistry, East China University of Science and Technology, Shanghai 200237, P. R. China

Corresponding authors: C. Tan Email: tancy@sz.tsinghua.edu.cn; N. Xu Email: xu.naihan@sz.tsinghua.edu.cn

1. Experiment section

Materials.

The original probes were RGD-FITC, UNO-FITC and Gly-DCM, details were described in our previous work. RGD-CY and UNO-TAMRA were purchased from Shanghai Apeptide Co., Ltd (Shanghai, China). Dulbecco's modified Eagle's medium, Roswell Park Memorial Institute (RPMI) 1640 medium, Leibovitz's L-15 Medium (L15) were purchased from Corning. Trypsin-EDTA solution, 10 × PBS buffer, 100 × Penicillin-Streptomycin Solution and DMSO were purchased from Beyotime (Shanghai, China). Fetal bovine serum (FBS) was purchased from Gibco (Rockvile, America).

Cell Culture. All cell lines used in this study were obtained from the American Type Culture Collection (ATCC) and cultured according to ATCC recommendations. Cell lines include Hela, HepG2, SK-BR-3, MCF10A, MDAMB436, MDAMB231, BT474, T47D, and MCF7. MCF10A and BT474 were grown in RPMI 1640 supplemented with 10% FBS and 1% Penicillin-Streptomycin Solution at 37 °C in a humidified 5% CO2 incubator. Hela, HepG2, MCF7, T47D and SK-BR-3 were grown in DMEM media supplemented with 10% FBS and 1% Penicillin-Streptomycin Solution at 37 °C in a humidified 5% CO2 incubator. MDAMB231 and MDAMB436 were cultured in L15 supplemented with 10% FBS and 1% Penicillin-Streptomycin Solution at 37 °C in a 100% humidified atmosphere. Under the above culture conditions, the cells were treated with trypsinized with 1 × trypsin to passage approximately every two days.

Flow cytometry analysis. Cells were washed with 1 × PBS buffer and trypsinized with 1 × trypsin and collected in the corresponding medium. The cell suspension was counted by flow Coulter counter and then diluted to about 1×10^6 cells mL^{-1}. The probes were added into the medium for labeling separately, and the final concentration of each probe was 10uM. After incubated in the dark for 60 minutes, the cells were centrifugated at 1500 rpm for 5 min. Cells were washed three times with 1 × PBS buffer to remove free probes and resuspended in 1mL 1 × PBS buffer for flow cytometry analysis. 10000 cells were recorded and 1000 cells were displayed, single cell staining ratio was recorded. Six replicates were designed for each cell line.

Cell pretreatment for fluorescence analysis. Cells were washed with 1 × PBS buffer and trypsinized with 1 × trypsin and collected in the corresponding medium. The cell suspension was counted by flow Coulter counter and then diluted to about 2×10^5 cells mL^{-1}. 1 mL of each cell suspension was mixed with sensing solution, the working concentrations of each probe were shown in Table S1. The well-mixed mixture was incubated for an hour and then centrifugated at 1000 rpm for 5 min. Cells were washed three times with 1 × PBS buffer to remove free probes and resuspended in the complete medium to 1 mL. Next, 100 μL of cell suspension (20,000 cells) was added to each well on a black-wall 96-well plate and then incubate in the dark for 24 hours to facilitate cell adherence and then used for cell sensing studies.

Sensing Studies in multi-well analysis. The fluorescence test was performed in a microplate reader. RGD-FITC, UNO-FITC and GLY-DCM were incubated with cells in different wells in multi-well analysis. Fluorescence spectra of cells were acquired at

specific excitation wavelengths (Table S1). This process was completed for all cell lines to generate 6 replicates.

Sensing Studies in one-well analysis. Gly-DCM, RGD-Cy and UNO-TAMRA were mixed and added in one well to incubate with cells. The final working concentrations of three probes were shown in Table S2. After cell pretreatment, the one-well analysis was performed in a microplate reader. The excitation light was transformed three times, and the excitation light wavelengths were set to Gly-DCM (465 nm), RGD-Cy (600 nm), and UNO-TAMRA (520 nm) respectively. Then the fluorescence emission spectral data of the corresponding bands were collected, each cell line possesses a unique fluorescence response data with the sensor array.

Linear Discriminant Analysis. LDA was performed using the Scikit-learn machine learning library in Python (version 3.7.3). For the multi-well sensing, the raw fluorescence response data contained a matrix of 6 (replicates) × 4 (different cell lines) × 3 (channels) and a matrix of 6 (replicates) × 7 (Normal / BC cell lines) × 3 (channels). For the one-well sensing, the raw fluorescence response data contained a matrix of 8 (replicates) × 4 (different cell lines) × 3 (channels) and a matrix of 8 (replicates) × 5 (Normal / BC subtypes) × 3 (channels). The raw data were transferred to canonical scores to maximize the ratio between-class variance and minimize the within-class variance, thus best separate each target group. The first two canonical scores were plotted in 2D (n_component = 2) with 95% confidence ellipse.

2. The pathological subtypes of breast cancer cell lines.

Table S1. BC subtypes corresponding to different cell lines

Cell Lines	BC Subtype
MCF10A	Normal
MDAMB231	Triple-negative (TN)
MDAMB436	Triple-negative (TN)
BT474	Luminal B (LB)
SKBR3	HER2-enriched (HER2+)
T47D	Luminal A (LA)
MCF7	Luminal A (LA)

Footnote: Four BC subtypes are classified according to different gene expression patterns: Luminal-like tumors overexpress the estrogen receptor (ER) and could be subclassified into two subgroups, Luminal A and Luminal B. HER2-enriched tumors show high level expression of Erb-B2 oncogene. Triple-negative tumors often fail to express ER, Erb-B2 oncogene and progesterone receptor (PR)[1].

3. Photophysical parameters and microplate reader parameters of each probe

Table S2. Fluorescence measurement parameters on the microplate reader of each probe.

Probe	Working solution concentration/μM	Excitation/nm	Emission scan range/nm	Bandwidth/nm
Gly-FITC	4	440	460-700	5/5
RGD-FITC	4	440	460-700	5/5
Gly-DCM	1	465	500-800	5/5
RGD-Cy	1	600	620-820	5/5
UNO-TAMRA	1	520	540-740	5/5

4. Sensing methods and data comparison

Table S3. Comparison of fluorescence spectral analysis and flow cytometry data.

Experiment method	Experiment instrument	Data type	Data dimension
Flow cytometry analysis	Flow cytometer	Fluorescence staining ratio	3*1
Fluorescence sensing in multi-well analysis	Microreader	Fluorescence spectrum	3*41
Fluorescence sensing in one-well analysis	Microreader	Fluorescence spectrum	3*101

4. UV-visible absorption and fluorescence emission spectra of the probes

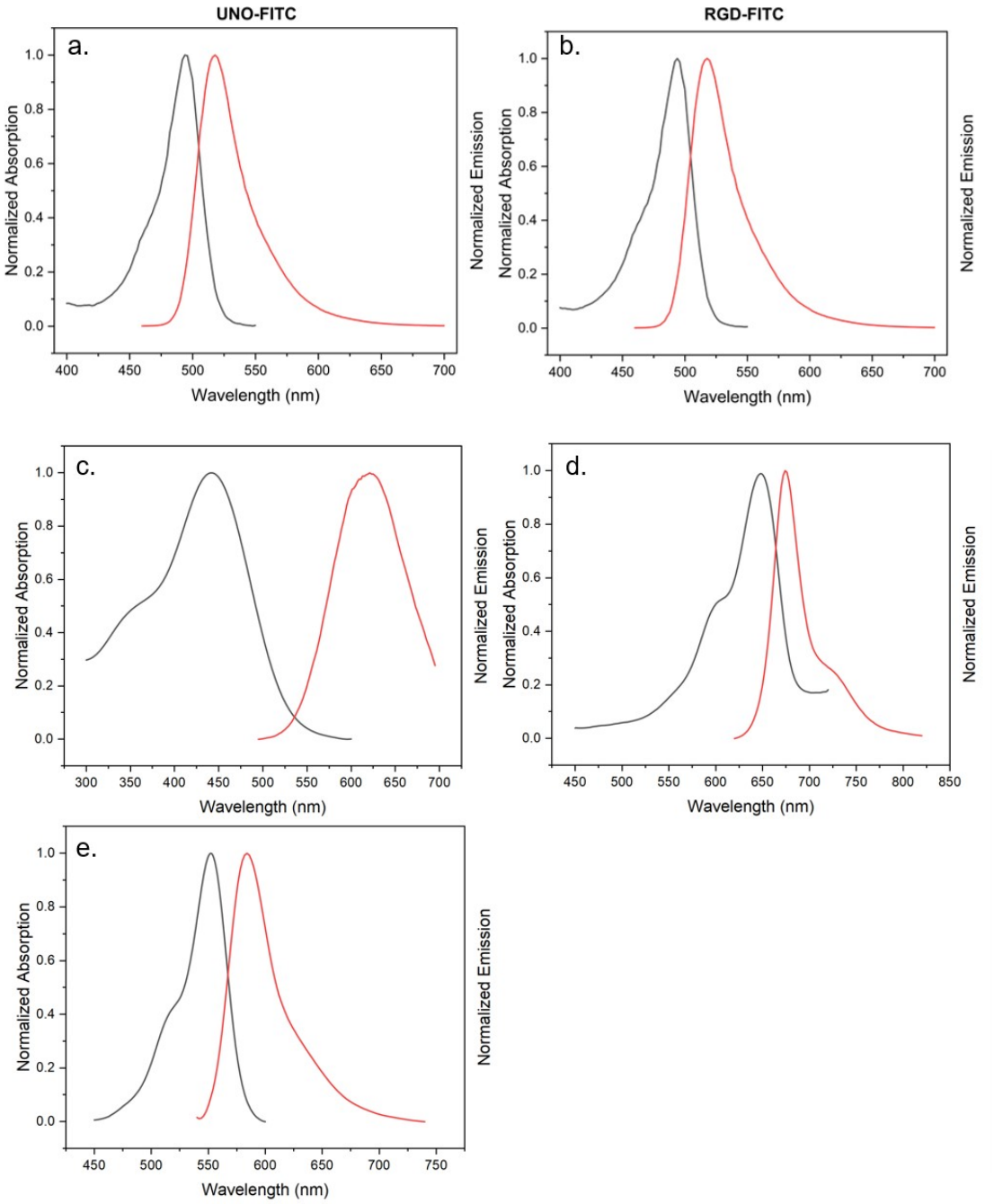

Figure S1. UV-Visible absorption (black) and fluorescence emission spectra (red) of (a) RGD-FITC, (b) UNO-FITC, (c) Gly-DCM, (d) RGD-Cy and (e) UNO-TAMRA in PBS buffer (pH=7.4). All absorbance and emission spectra were normalized.

5. Fluorescence emission of probes in mixed system

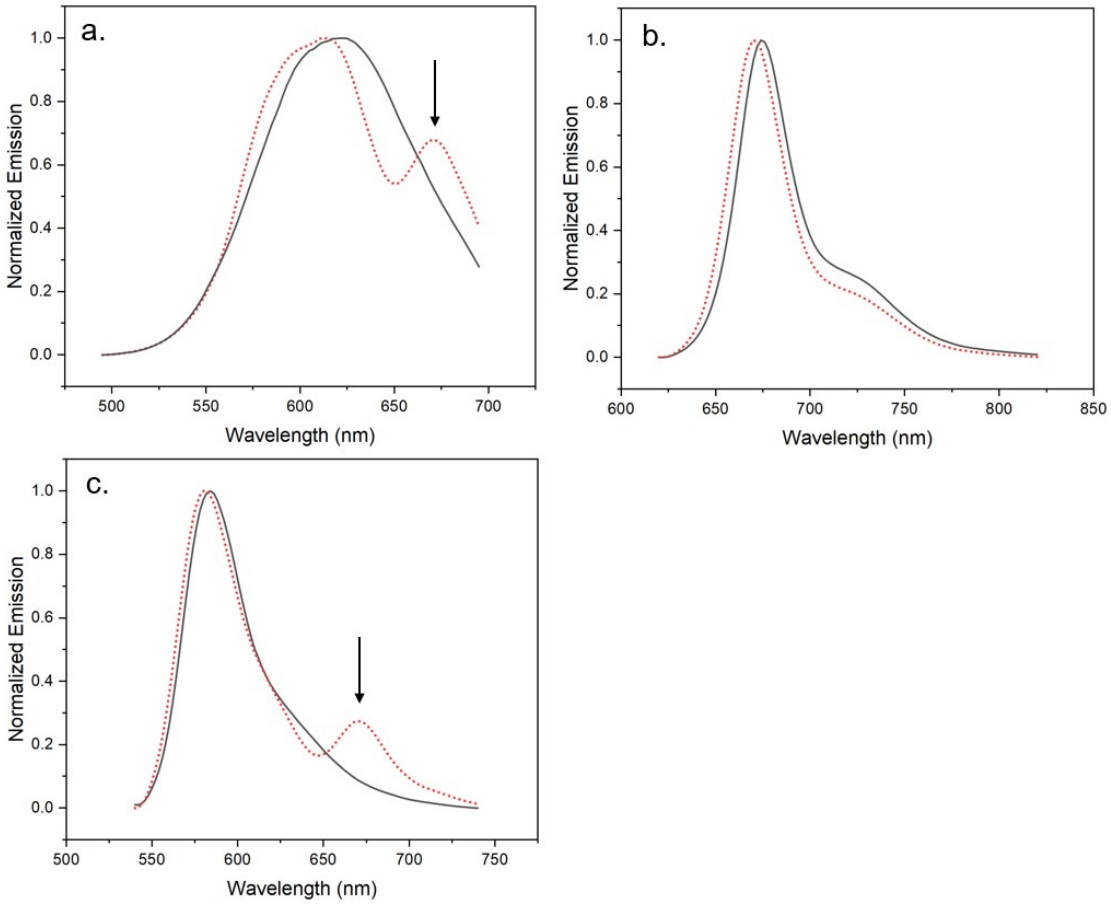

Figure S2. Normalized emission spectra generated by individual probes (Solid black line) and mixed probes (Red dotted line): (a) Gly-DCM, (b) RGD-Cy and (c) UNO-TAMRA in PBS buffer (pH=7.4), respectively. All tests were performed at a probe concentration of 1uM.

6. Principle Component Analysis (PCA) results

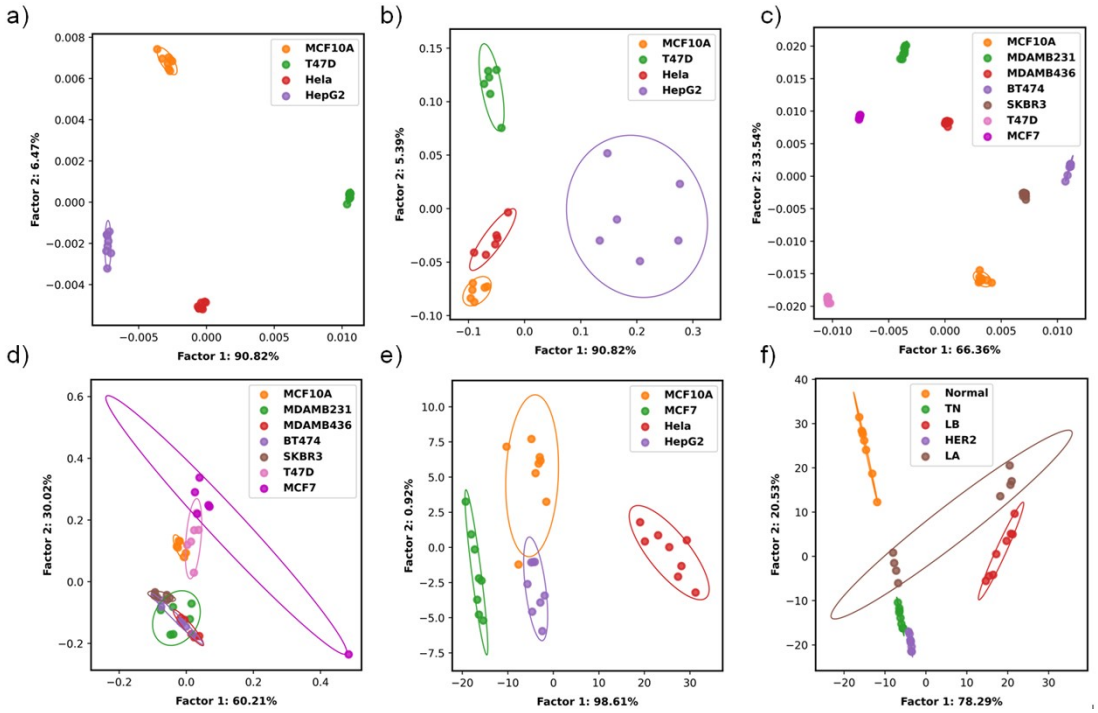

Figure S3. Principle Component Analysis (PCA) score plots: Differentiation of normal cells and cancer cells using a multi-well sensor array by (a) FCM analysis and (b) fluorescence spectral analysis. Differentiation of one normal cell line and six BC Cell lines using a multi-well sensor array through (c) FCM and (d) fluorescence spectral analysis. (e) Differentiation of a normal cell line and three cancer cell lines using the one-well sensor array by fluorescence spectra analysis. (f) Differentiation of molecular subtypes of BC Cell lines using the multi-channel sensor array by fluorescence spectral analysis.

References

1. T. Sørlie, R. Tibshirani, J. Parker, T. Hastie, J. S. Marron, A. Nobel, S. Deng, H. Johnsen, R. Pesich, S. Geisler, J. Demeter, C. M. Perou, P. E. Lønning, P. O. Brown, A.-L. Børresen-Dale and D. Botstein, *Proc. Natl. Acad. Sci.*, 2003, **100**, 8418-8423.