Supporting Information

DNA-Mediated Cell Surface Engineering for Multiplexed glycan profiling using MALDI-TOF Mass Spectrometry

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Experimental sections

1. Lectin-DNA conjugation

Lectins were first dissolved in pH=7.4 PBS at a concentration of 1 mg/mL. 20 mole equivalents of succinimidyl 6-hydrazinonicotinate acetone hydrazone (S-HyNic, Solulink, USA) prepared in DMF was added to the lectin solution and incubated at room temperature for 2 h. Using a 10 KD Ultra Centrifugal Filter (Merck Millipore, Germany), excess S-HyNic was removed and solution was buffer exchanged to pH=6.0 citrate buffer. Then 10-fold excess of 5'-aldehyde modified DNA (Sangon Biotech, China) was added and allowed to react overnight at room temperature. The mixture was purified with a 30 KD Ultra Centrifugal Filter (Merck Millipore, Germany) to remove the noncoupled DNA. Protein concentration was determined using Bradford protein assay (BioDee Biotechnology, China). The synthesis of lectin-DNA conjugates was verified by microchip capillary electrophoresis (MCE-202 MultiNA, Shimadzu, Japan). 1% hydroxyethyl cellulose (HEC) (w/v) in TE buffer was used as sieving matrix for separation and SYBR gold was used as chromosphere for nucleic acid staining. Results were analyzed in MultiNA Viewer software.

2. Cell culture and viability assay

MCF-7 cells and MCF-7/ADR cells (Cancer Institute & Hospital, Chinese Academy of Medical Science, Beijing, China) were cultured in RPMI 1640 medium (Gibco, USA) supplied with 10% fetal bovine serum (FBS, Hyclone, USA), 100 $U \bullet mL^{-1}$ penicillin (Gibco, USA) and 100 $U \bullet mL^{-1}$ streptomycin(Gibco, USA) at $37^{O}C$ in a humidified atmosphere of 5% CO_2. Before using in the experiment, cells were trypsinized, directly seeded onto the ITO glass and grew in a humidified atmosphere of 5% CO_2 at 37 °C for 1-2 days. The cell viability was examined by live/dead assay kit (Calcein-AM/EthD-1, Invitrogen, USA). Cells were incubated with live/dead reagent at 37 °C for 30 min. Fluorescent images were then taken by a fluorescent microscope (DMI 4000B, Leica, Germany) equipped with a cooled CCD camera. The viable cells showed green fluorescence while the dead cells showed red fluorescence.

3. Evaluation of lectin-DNA conjugates

MCF-7 cells were first cultured on the ITO glass (1cm×1 cm). After growing confluent, cells were washed three times with PBS and incubated with synthesized ConA-DNA-FITC or simple DNA- FITC in PBS containing 0.1 mM Ca^{2+} and 0.01 mM Mn^{2+} for 40 min. Cells were then washed with PBS for three times and imaged with a laser scanning confocal microscope (TCS SP5, Leica, Germany).

For flow cytometry analysis, cells were trypsinized, washed three times with PBS and incubated with synthesized ConA-DNA-FITC or simple DNA-FITC in PBS containing 0.1 mM Ca^{2+} and 0.01 mM Mn^{2+} for 40 min. After washing three times, cells were resuspended in 300 uL PBS and analyzed on a flow cytometer (FACS Calibur, BD Biosciences, USA). The FITC fluorescence was analyzed using BD Cell Quest Pro program.

Monosaccharide inhibition assay: 0.1 μM ConA-primer1 was preincubated with 100 μM free monosaccharides (D-mannose, D-glucose and D-galactose) respectively at 37 °C for 1 h. After the removal of excess monosaccharides by 30KD Ultra Centrifugal Filter, the conjugates were incubated with MCF-7 cells at 37 °C for 1 h. Cells were then washed with PBS and mixed with complementary FITC-labeled DNA probe1 for 15 min at 37 °C. After washing, cells were analyzed on a flow cytometer. The FITC fluorescence was analyzed using BD Cell Quest Pro program.

4. Cell labeling and rolling circle amplification

Cells were plated on the ITO glass (1 cm×1 cm) and grown for 1-2 days. They were washed with PBS and fixed with 4% paraformaldehyde. The lectin-primer conjugates were mixed with corresponding circular templates and T4 DNA ligase (New England Biolabs, UK) in 1× ligation buffer. This solution was incubated at 37 °C for 1 h. After washing three times with PBS containing 0.1 mM Ca^{2+} and 0.01 mM Mn^{2+}, the above solution was added to cells for 1 h at 37 °C. The cells were then washed with PBS and the rolling circle amplification solution containing 250 μM

dNTPs, 0.125 U/μL phi29 polymerase (New England Biolabs, UK) in 1× polymerase buffer [50 mM Tris·HCl, 10 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$, pH 7.5] was added for 1.5 h at 37 °C. After washing three times with PBS, solution containing 1 μg/mL Hoechst and 0.5 μM of DNA probes was added for 15 min at 37 °C. Cells were then washed three times with PBS to remove the excess DNA probes and imaged with a confocal microscope (TCS SP5, Leica, Germany).

5. MALDI matrix application

3-Hydroxypicolinic Acid (3-HPA, Sigma Aldrich, USA) was chosen as MALDI matrix for DNA detection. The matrix solution was prepared by dissolving 20 mg 3-HPA and 45 mg dihydrogen ammonium citrate (DHAC) in 1 mL mixture solution of 50% acetonitrile/50% water. A home-built inkjet printing device was developed to print an array of matrix droplets on sample. This device consisted of a piezoelectric inkjet print head for matrix solution ejection (Fuji Electrics Systems Co., Ltd, Japan) and an XY-motorized stage where the ITO glass was placed (MMU-30X, Chuo Precision Industrial Co., Ltd, Japan). A laboratory-made software was used to controll the inkjet waveform and the movement of x–y stage. ITO glass with cells was firstly washed with 100 mM ammonium acetate solution to remove non-volatile salts which might affect ionization efficiency. After air drying, a 6×6 matrix array of circular regions ~300 μm in diameter was printed on the ITO glass and imaged with fluorescent microscope (DMI 4000B, Leica, Germany). For quantitative analysis, DNA internal standard and matrix solution were loaded into different channels of the inkjet printing head. The internal standard was printed prior to the matrix on the same position.

6. MALDI-MS analysis

MALDI-MS analysis was performed in an AXIMA Performance MALDI-TOF/TOF mass spectrometer (Shimadzu Co. Ltd., Japan). This instrument was equipped with a 337 nm nitrogen laser. ITO glass with cells was attached to the stainless MALDI plate by conductive tapes. Data were acquired in a linear negative

mode and signals between m/z 3000–7000 were collected. Raster scans on cell surfaces were performed automatically using the mass spectrometry software (Shimadzu Biotech., Japan). The scan area was 200 μm × 200 μm with the sampling distance of 50 μm. For each coordinate, mass spectra resulting from 20 laser shots at 5 Hz were accumulated to obtain an average mass spectrum.

7. Tunicamycin treatment

MCF-7 cells were seeded on the ITO glass. After adhering, they were washed once with PBS and cultured in cell culture medium in the absence and presence of tunicamycin (Solarbio, China) of different concentration (1 μg/mL, 5 μg/mL, 20 μg/mL) for 24 h. Cells were treated with the above-mentioned DNA labeling and amplification procedure and analyzed by MALDI-TOF MS.

CCK-8 assay (Cell Counting Kit-8, Dojindo Molecular Technologies, Japan) was used to evaluate the cytotoxicity of tunicamycin. Cells were seeded in 96-well plate and cultured for 12 h. After washing once with PBS, cell culture medium with different concentration of tunicamycin (0, 1, 10, 50, 75, 100 μg/mL) was added and incubated for 24 h. 10 μL CCK-8 reagent (tetrazolium salt WST-8) was added into each well with 100 μL cell culture medium inside. Absorbance at 450 nm was measured by a microplate reader after 1 h incubation.

For flow cytometry analysis, cells were treated with different concentration of tunicamycin (0, 1, 5, 20 μg/mL) for 24 h. Then they were trypsinized, washed three times with PBS and incubated with 0.1 uM ConA-FITC for 1 h. After washing three times, cells were resuspended in 300 uL PBS and analyzed on a flow cytometer (FACS Calibur, BD Biosciences, USA). The FITC fluorescence was analyzed using BD Cell Quest Pro program.

8. MALDI imaging mass spectrometry

Human lung tissue was provided by the First Affiliated Hospital of Fujian Medical University in China. FFPE human lung tumor tissue section was cut at 4 μm and placed on ITO glass slide. After deparaffinization, the tissue section was labeled

by ConA-primer1, performed RCA reaction and hybridized with Texas Red modified DNA probe1. Matrix solution (20 mg/mL 3-HPA and 45 mg/mL DHAC in 50% acetonitrile/50% water) was sprayed on the tissue section homogeneously using an ImagePrep spray station (Bruker Daltonics, Germany). Mass spectra were acquired across the selected tissue section on a MALDI-TOF spectrometer (Autoflex III, Bruker Daltonics, Germany), equipped with a 200 Hz Smart Beam Nd:YAG 355 nm laser. A linear negative ion mode was used with 500 shots per raster point. The raster range was set as 200 μm. Following MS analysis, data was loaded into FlexImaging Software to generate overall average mass spectra and ion map of DNA probe1.

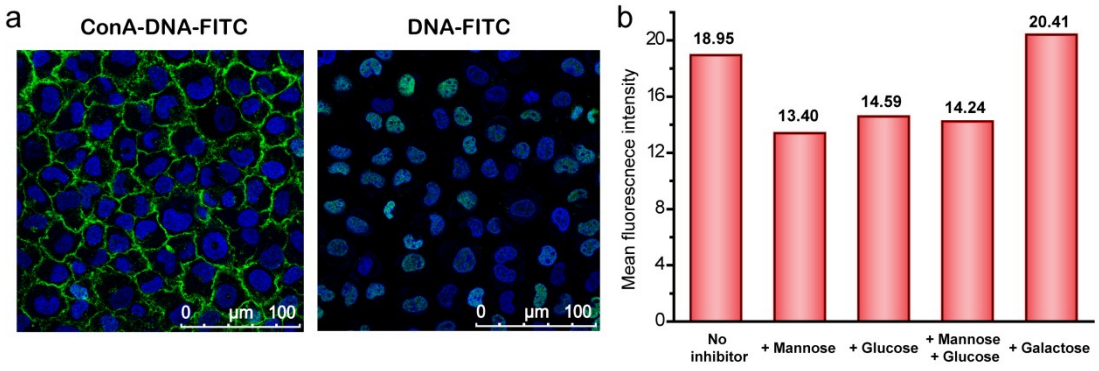

Figure S1. a) Confocal images of cells labeled by ConA-DNA-FITC (left) and DNA-FITC (right). DAPI is used as the nuclear stain (blue). The scale bars are 100 μm in all images. b) Monosaccharide inhibition assay. Flow cytometry analysis of FITC fluorescence in cells. Cells were incubated with ConA-primer1 pretreated by free monosaccharides (D-mannose, D-glucose and D-galactose) and then hybridized with complementary FITC-labeled DNA probe1.

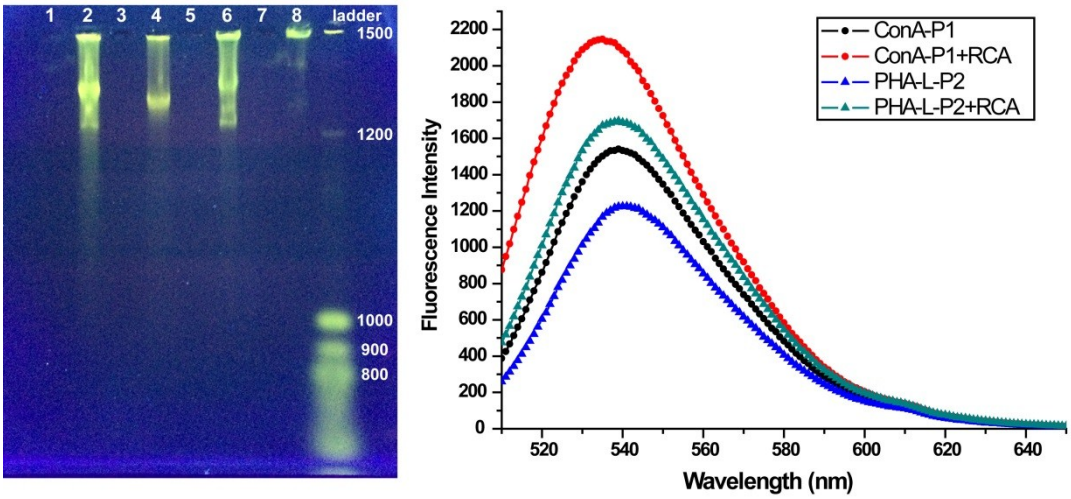

Figure S2. Verification of rolling circle amplification by 1% agarose electrophoresis and fluorescence spectrometry. a) Gel image of RCA products. lane 1: ConA-primer1 + circular template1, lane 2: ConA-primer1 + circular template1 + RCA reaction, lane 3: PHA-L-primer2 + circular template2, lane 4: PHA-L-primer2 + circular template2 + RCA reaction, lane 5: Primer1 + circular template1, lane 6: Primer1 + circular template1 + RCA reaction, lane 7: Primer2 + circular template2, lane 8: Primer2 + circular template2 + RCA reaction, lane 9: DNA ladder. b) Fluorescence spectrum before and after RCA reaction, SYBR gold was used for DNA staining. Distinct fluorescence increase can be observed after RCA reation.

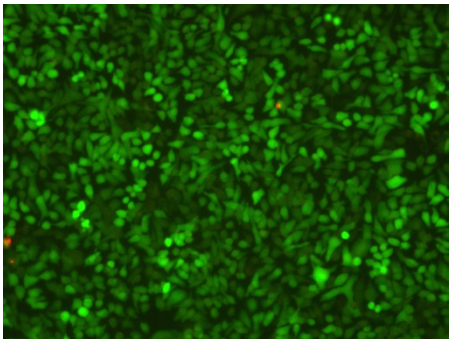

Figure S3. Cell viability evaluation by live/dead assay after culturing on the ITO glass for two days (green: live cells, red: dead cells).

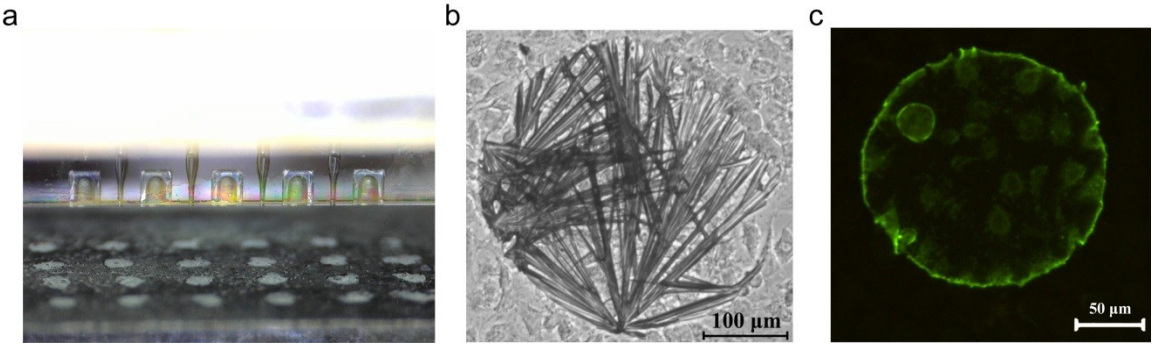

Figure S4. MALDI matrix application. a) An array of matrix droplets was produced by home-built inkjet printing device; b) Matrix crystallization on the sample surface; c) DNA internal standard printed on prior to the matrix.

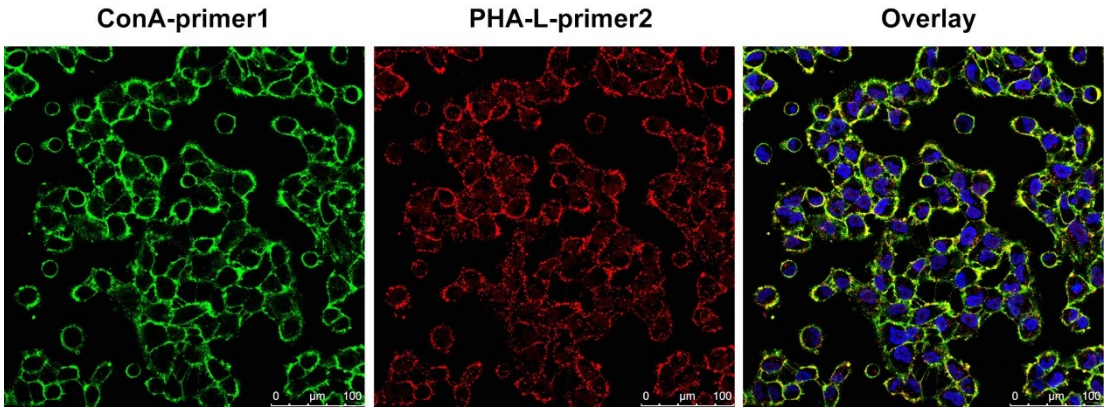

Figure S5. Confocal images of cells co-labeled with PHA-L-primer1 and ConA-primer2, perfromed RCA reaction and hybridized with FITC labeled DNA probe1 and Texas Red labeled DNA probe2. DAPI is used as the nuclear stain (blue). The scale bars are 100 µm in all images.

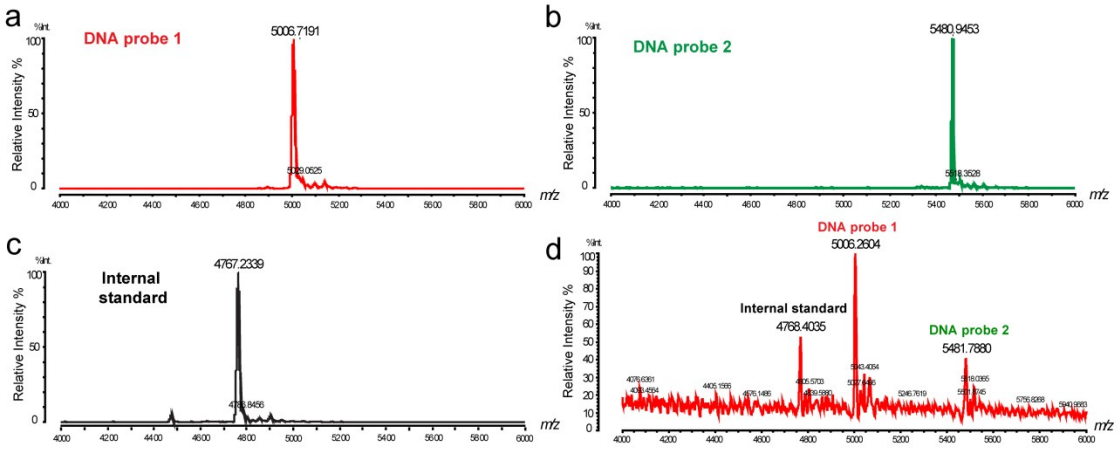

Figure S6. Mass spectra of pure samples of a) DNA probe1, b) DNA probe2 and c) internal standard. d) Mass spectrum of cells co-labeled with ConA-primer1 and PHA-L-primer2, performed RCA reaction and hybridized with DNA probe1 and DNA probe2. DNA internal standard was applied by inkjet device for semi-quantitative analysis. The peaks in cell analysis were consistent with the mass spectra of the pure samples of DNA probe 1, DNA probe 2 and internal standard, confirming the validity of detection.

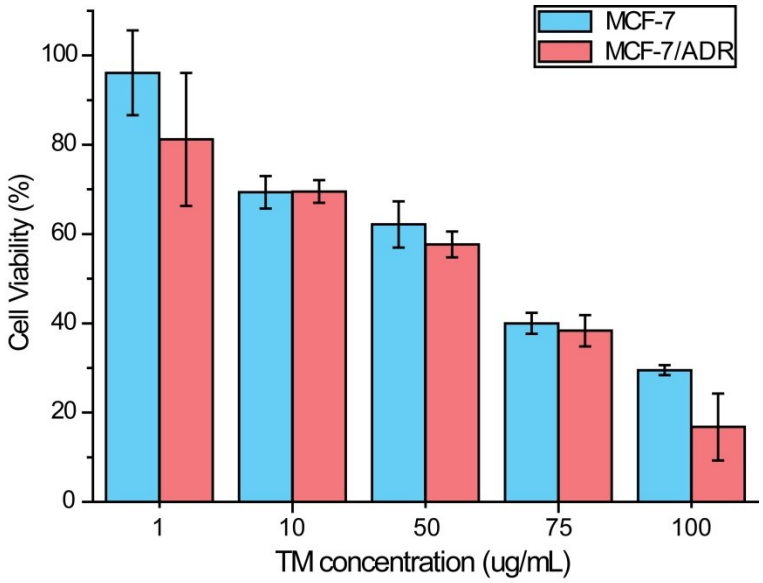

Figure S7. Cell viability at different tunicamycin concentration by CCK-8 assay.

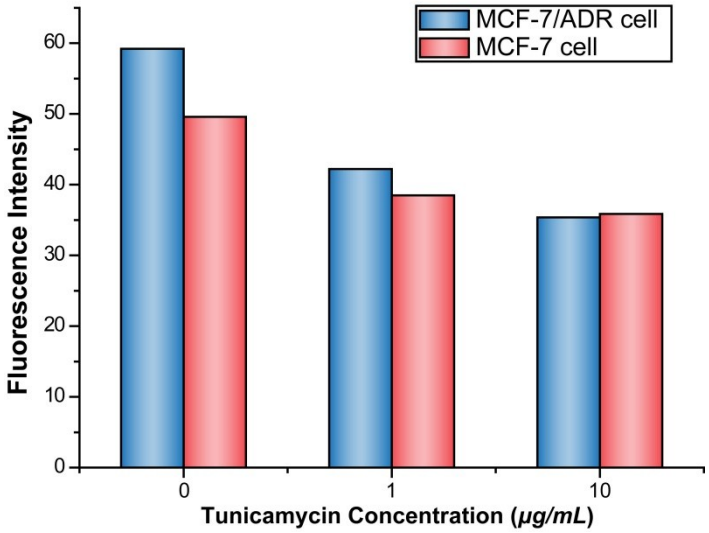

Figure S8. Flow cytometry analysis of expression of the α-mannosyl groups in cells under the stimuli of tunicamycin at different concentration.

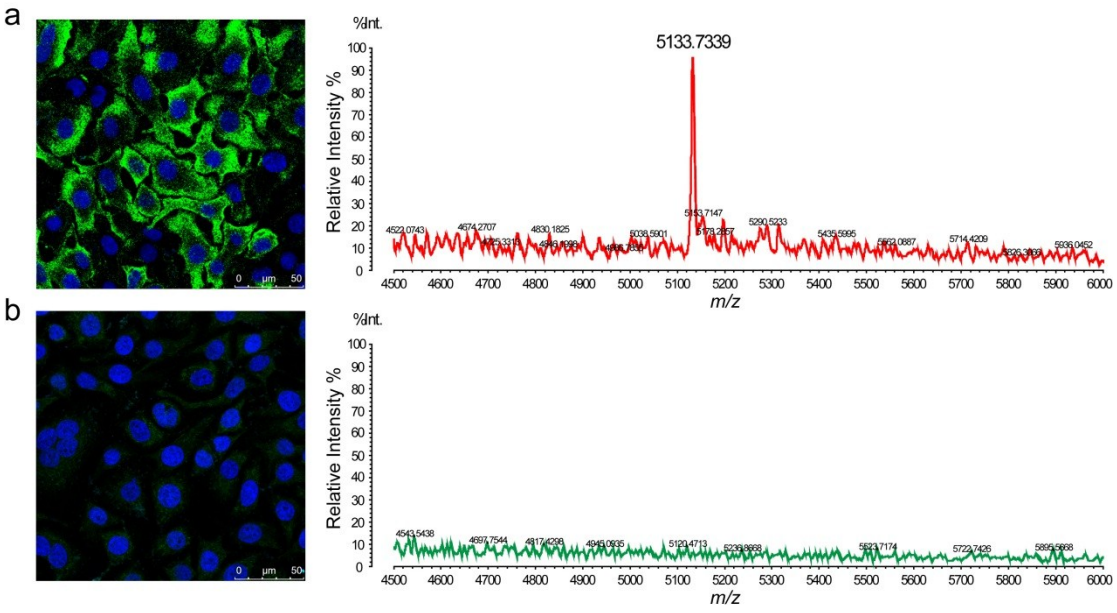

Figure S9. Analysis of actin protein in cells based on DNA labeling and signal amplification strategy. a) Confocal image (left) and MALDI-MS spectrum (right) of HepG2 cells labeled by actin antibody-primer1, performed RCA reaction and hybridized with FITC labeled short DNA probe2. b) Confocal image (left) and MALDI-MS spectrum (right) of HepG2 cells incubated with primer1, performed RCA reaction and hybridized with FITC labeled short DNA probe2. DAPI is used as the nuclear stain (blue). The scale bars are 50 μm in all images.

Table S1. Glycan groups, lectins and DNA sequences used in this work.

Glycan/lectin	α-D-mannosyl / Concanavalin A（ConA)
Glycan/lectin	β1-6GlcNAc branched N-glycan / Phaseolus vulgaris leucoagglutinin (PHA-L)
Primer1	5'-AAAAAAAAAAAAAAACACAGCTGAGGATAGGACAT-3'
Circular template1	5'-P-CTCAGCTGTGTAACAACATGAAGATTGTAGGTCAGAACTCACC TGTTAGAAACTGTGAAGATCGCTTATTATGTCCTATC-3'
DNA probe1	5'-FITC-TGTCCTATCCTCAGC-3' Mw = 5016.4 Da
Primer2	5'-AAAAAAAAAAAAAAATATGACAGAACTAGACACTCTT-3'
Circular template2	5'-P-GTTCTGTCATATTTCAGTGAATGCGAGTCCGTCTAAGAGAGTAG TACAGCAGCCGTCAAGAGTGTCTA-3'
DNA probe2	5'-Texas Red-CAGTGAATGCGAGTC-3' Mw = 5498.1 Da
Internal standard	5'-FITC-CGACTAGTCTGATC-3' Mw = 4776.3 Da

Explanation for mass deviation:

As shown in Table S1 and Figure S6, there were mass deviations between the molecular ion peaks in mass spectra and the calculated molecular weight values. The deviation is about 10 Da for DNA probe1 and internal standard, and 17 Da for DNA probe2. Explanations for these deviations was illustrated in detail below:

1. In our experiment, cells were cultured on the ITO glass. After cell surface engineering, the ITO glass with cells was affixed onto the stainless steel MALDI plate by conductive tapes. As shown in the Figure S10, compared to the calibration peptides that ionized from MALDI plate, the thickness of ITO glass (1.1 cm) shortens the distance from initial position of ions to the extraction grid when DNA probes were ionized from ITO glass (s'<s). Since the electric-field strength $E_s = V/s$, and the voltage V is the same on MALDI plate and ITO glass, the electric-field strength is enhanced between ITO glass and extraction grid $(E_s' > E_s)$.

Refering to the basic principle of Time-of-Flight Mass Spectrometry (TOF-MS), ion's mass-to-charge ratio is determined via a time measurement. Ions are accelerated by an electric field of known strength. The time (t_{TOF}) that it subsequently takes for

the particle to reach a detector at a known distance is measured. From this time and the known experimental parameters one can find the mass-to-charge ratio of the ion.

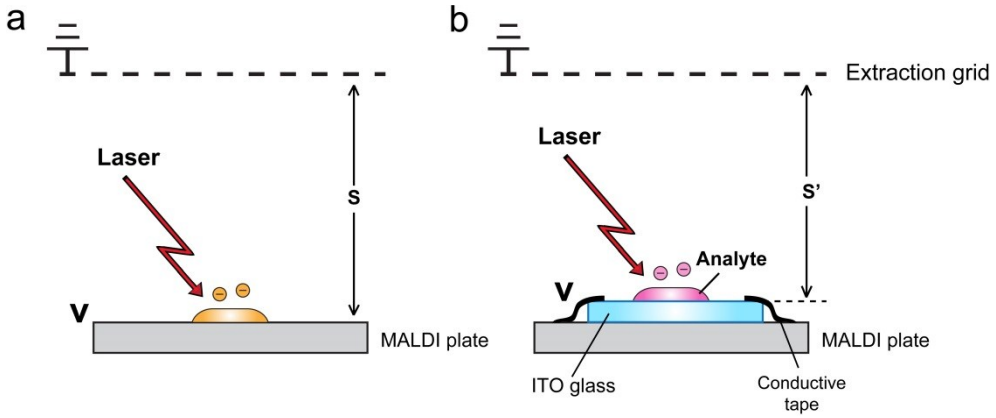

Figure S10. Schematic diagram of molecule ionization on a) MALDI plate and b) ITO glass.

The equation for the arrival time of an ion (t_{TOF}) is shown below [1, 2]:

$$t_{TOF} = t_s + t_D + t_0 = \frac{(2m)^{1/2}}{zeE_s}\left[(U_0 + zeE_s s)^{1/2} \mp U_0^{1/2}\right] + \frac{(2m)^{1/2} D}{2(U_0 + zeE_s s)^{1/2}} + t_0$$

Where t_0 = time of ion formation, t_s = time that ion is accelerating from v_0 to v_D, t_D = time in drift region, m = the mass of the ion, e = the charge on an electron, z = number of charges, E_s = electric-field strength, U_0 = initial translational energy of ion (at t_0, s_0)， s = distance in E from average s_0 (s_0 = initial position of ion) to drift region, D = drift distance, and applied voltage $V = E_s \cdot s$.

Referring to the equation, and assuming that the ion formation time (t_0) is the same in the two conditions, the enhancement of electric-field strength $(E_s' > E_s)$ reduces the time t_{TOF} ($t_{TOF}' < t_{TOF}$), leading to decrease of the observed molecular ion peak. Thus our detected molecular ion peaks were all smaller than the calculated molecular weights.

2. Owing to heterogeneity of matrix crystallization, matrix thickness, different ionization properties between analyte and calibration peptides, and so on, mass deviations always exist in MALDI-TOF MS [3-5]. For DNA analysis, the mass accuracy is estimated to be within $\pm$ 0.2% ($\pm$ 10 Da for molecular weight about 5000 Da) [6].

Reference:

1 R. J. Cotter, in *Time-of-Flight Mass Spectrometry*, ed. R. J. Cotter, ACS Symposium Series; American Chemical Society, Washington, DC, 1993, vol. 549, ch. 2, pp. 16-48.

2 M. Guilhaus, *J. Mass Spectrom.,* 1995, **30**, 1519-1532.

3 N. S. Selden, M. E. Todhunter, N. Y. Jee, J. S. Liu, K. E. Broaders and Z. J. Gartner, *J. Am. Chem. Soc.*, 2012, **134**, 765-768.

4 X. Bai, Z. Li, S. Jockusch, N. J. Turro, and J. Ju, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 409-413.

5 E. Nordhoff, C. Luebbert, G. Thiele, V. Heiser, and H. Lehrach, *Nucleic Acids Res.*, 2000, **28**, e86.

6 Mass Spectrometry Analysis of Oligonucleotide Syntheses, Integrated DNA Technologies. (https://www.idtdna.com/pages/docs/technical-reports/mass-spectrometry-analysis-of-oligonucleotide-syntheses.pdf?sfvrsn=4).