Supporting Information of
Homogenous deposition of matrix-analyte cocrystals on gold-nanobowls array for improving MALDI-MS signal reproducibility

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Materials

Sulfuric acid (H_2SO_4), hydrogen peroxide (30 %), 4-mercaptopropyl trimethoxysilane, monodisperse polystyrene (PS, 600 nm, 1 wt%), sodium dodecyl sulfate(SDS, 2wt%), $HAuCl_4$ (2 mM), strong aqua ammonia, hydrazine aqueous solution (85wt%), toluene. These chemicals were of analytical grade (J&K chemicals, China) and were used as received. 3-hydroxypicolinic acid (3-HPA, 99.0 % HPLC grade) and 2-cyano-3-(4-hydroxyphenyl) acrylic acid (CHCA, ≥98% TLC grade) were purchased from Sigma-Aldrich. The silicon wafers (n-type (100)) were obtained from Xilika Tianjin, China. The oligonucleotide 5′-TGT TGG GGT AAC-3′ (M.W.=3716.5 Da) and 5′ -ACG CAA TGA CTG TAC TGA CT-3′ (M.W.=6101 Da) were purchased from Sangon Biotech, China. An oligopeptide (ACTH fragment 18-39 (human), M.W.=2465.7 Da) was obtained from Sigma-Aldrich, and a polypeptide in a mixture, and DNA with multiple

oligonucleotides fragments were provided by BioYong Tech. Beijing, China.

Preparation of the Au-nanobowls array

The Au-nanobowls array chip on a silicon wafer (SW) was prepared at the air/liquid interface by nanosphere lithography technique (**Fig. S1**). Briefly, microscopic glass slides were treated with piranha solution to remove organic matter (*Caution: piranha is corrosive and must be handled with care*). A self-assembled monolayer of polystyrene microsphere (PSM) was obtained on a glass cover-slip (2.5×2.5 cm). The PSM was then transferred onto the surface of a $HAuCl_4$ (2 mM) aqueous solution. The $HAuCl_4$ aqueous solution with a floating PSM was subjected to gas-diffusion-reduction with a mixture of strong aqua ammonia/hydrazine (85 % aqueous solution) (3/7 mL) in a closed desiccator for 1 h at room temperature. A uniform Au film of considerable mechanical strength was deposited on the PSM. The SW (2×2 cm) soaked in the piranha solution before were placed in a toluene solution of 4-mercaptopropyltrimethoxysilane for 12 h to modify the thiol group on the SW for strong bonding of Au. The PSM-Au composite film was picked up with SW subsequently rinsed with deionized water for 10 min and etched in toluene for 10 min to dissolve the PSM. The resultant Au-nanobowls were treated with ozone for 30 min.

Sample Preparation

Oligonucleotides (5′-TGT TGG GGT AAC-3′) were dissolved in deionized water (DIW) to obtain a 100 μM stock solution. The stock solution was further diluted to a series of concentrations 0.3, 0.6, 1.6, 3.3, and 6.6 μM. The oligonucleotide solution was then mixed with matrix (3-HPA, 10 mg/mL) at the molar matrix:analyte ratios of 217878:1-to-10894:1. A 0.5 μL drop of the mixture was dropped on the Au-nanobowls array chip substrate, evaporated the solvent at 4 °C, and prone to MALDI-MS analysis. Similarly, the oligopeptide, bovine serum insulin protein in a mixture, and a sample consisting of different oligonucleotides fragments samples were prepared by dissolving analytes in DIW and mixed with matrix (for oligopeptide and bovine serum insulin the CHCA (20 mg/mL) matrix was used) at molar matrix:analyte ratio of 53000:1. The mixture was dropped on Au-nanobowls array and dried at 4 °C before analysis.

Evaluation of DNA in real sample

In order to evaluate the capability of Au-nanobowls array for real sample analysis, the oligonucleotide (5′-ACG CAA TGA CTG TAC TGA CT-3′) were dissolved in DIW and PCR-buffer. The PCR buffer consists of Tris-HCl, KCl, $MgCl_2$, EDTA, DTT, glycerinum, and Triton X. The 2 μM oligonucleotide solutions were obtained either in DIW and in PCR buffer. The sample was mixed with 3-HPA (matrix), deposited on Au-nanobowls array, and prone to MALDI-MS analysis.

Further, the sample pre-treatment of oligonucleotide solution in PCR buffer was performed using Millipore (Amnicon Ultra-0.5 Centrifugal Filter Device, normalized M.W.=3000). A 0.5 mL of oligonucleotide solution was added to Millipore and centrifuged at 12000 rpm for 25 min. The filtrate was removed and the 0.45 mL DIW was added to the concentrate. The solution was re-centrifuged at 12000 rpm for 25 min. The addition of DIW and centrifugation was performed twice. Subsequently, DIW was added to the concentrate to obtained solution, mixed with 3HPA matrix, and deposited on Au-nanobowls for MALDI-MS analysis. **Fig. S5** shows the MALDI-MS spectrum of oligonucleotide in DIW, PCR buffer, and in PCR buffer after sample pre-treatment.

Instrumentation

The morphology of the Au-nanobowls array chip before and after matrix-analyte co-crystal (MAC) deposition was observed by scanning electron microscope (SEM, SU8010, Hitachi, Japan). The MALDI-TOF MS experiment was performed in linear mode on a Clin-TOF–II spectrometer (YQ02161003, BioYong Tech., China) with a nitrogen laser light (λ=337 nm) and an acceleration voltage of 20 kV. The MAC was deposited on Au-nanobowls array chip and attached to the MALDI plate with conductive paste and sent directly to the injection chamber of the mass spectrometer for MALDI-MS analysis. For a single sample spot, the amount of analyte was 0.16, 0.33, 0.82, 1.66, and 3.33 pmol. All the freshly prepared samples were quickly spotted at room temperature and stored at 4 °C until they were crystallized on the plate/chip. 200 laser shots were averaged for each spectrum. The signal-to-noise (S/N) ratio was calculated from the signal intensity divide by the average background intensity.

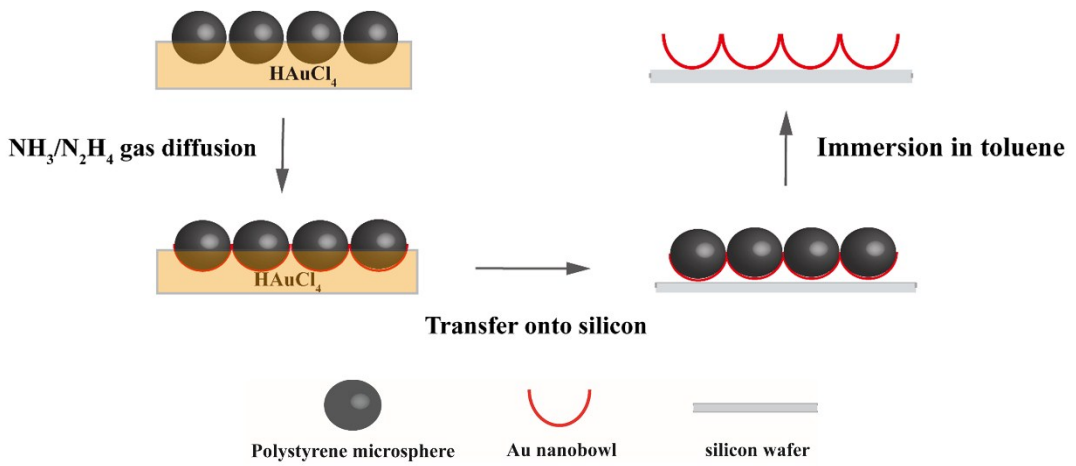

Fig. S1 Synthesis of Au-nanobowls at the air/liquid interface by nanosphere lithography technique

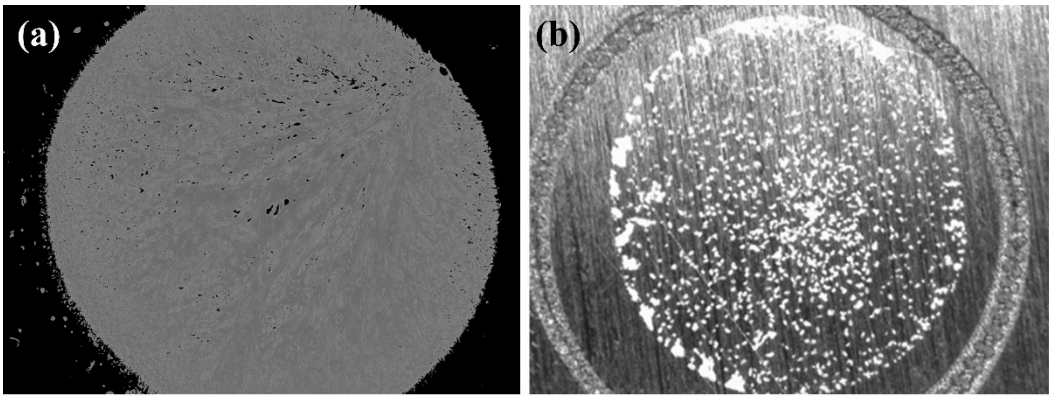

Fig. S2 Optical images of matrix-analyte cocrystals deposited on (a) Au-nanobowls, and (b) conventional MALDI plate.

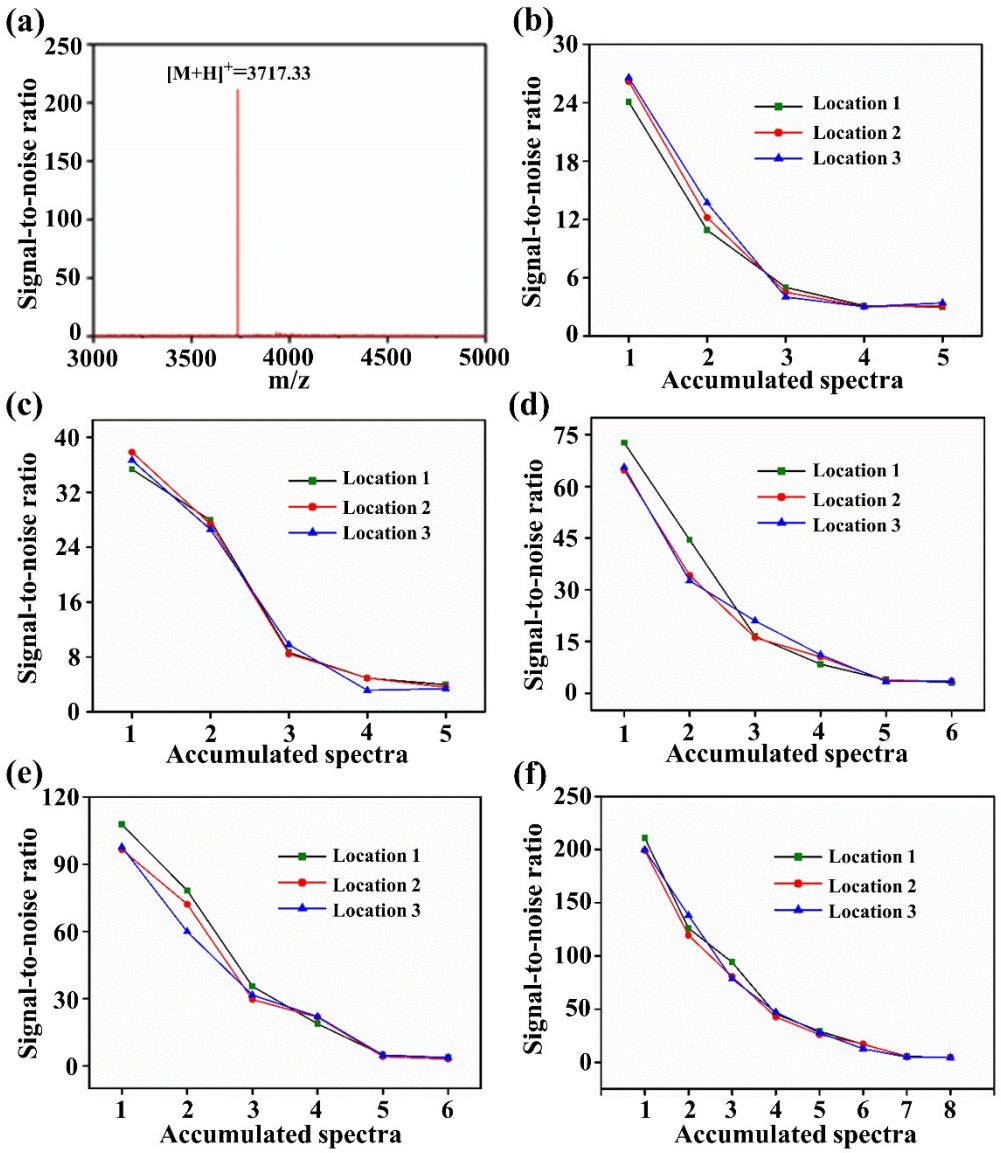

Fig. S3 (a) $[M+H]^+$ spectrum of oligonucleotide (5′-TGT TGG GGTAAC-3′) deposited on Au-nanobowl array; the plots of S/N against number of accumulated spectrum of oligonucleotide deposited on Au-nanobowls at different concentrations of (b) 0.3, (c) 0.6, (d) 1.6, (e) 3.3, and (f) 6.6 μM.

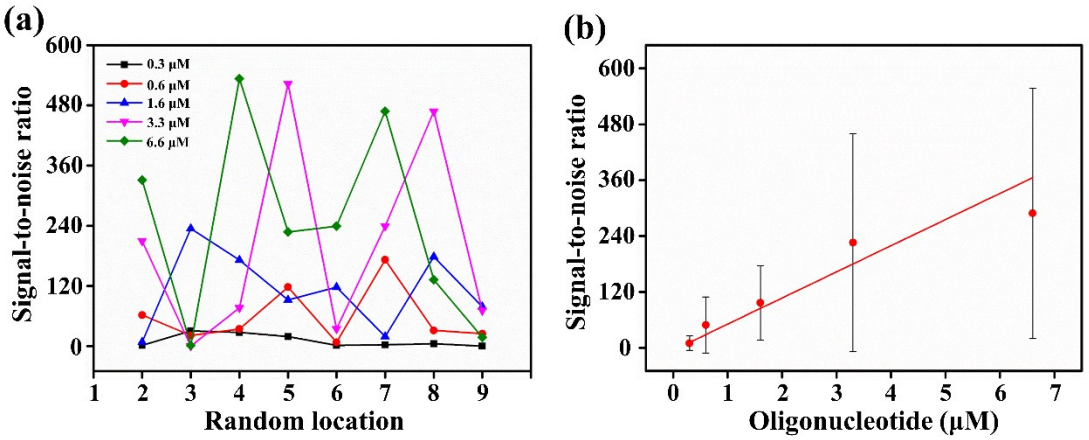

Fig. S4 (a) The S/N plot of $[M+H]^+$ peak at different locations of a single deposit and (b) S/N vs oligonucleotide concentrations of a single deposit on conventional MALDI-plate.

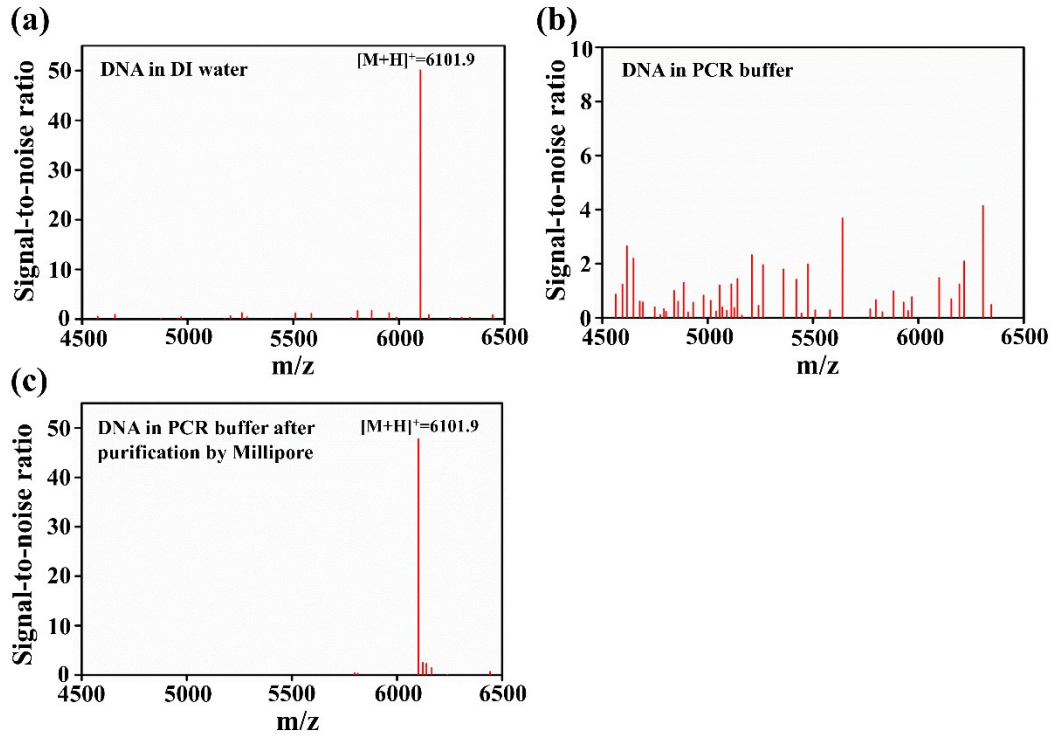

Fig. S5 $[M+H]^+$ MALDI-MS spectra of DNA solution in (a) DIW, (b) PCR buffer, and (c) PCR buffer after purification using Millipore, deposited on Au-nanobowls.

Table S1 Calculated concentration from absolute intensity of mass spectrum obtained from DNA sample consisting of multiple oligonucleotides deposited on Au-nanobowls.

Peak	m/z $[M+H]^+$	Signal to noise ratio (S/N)	Conc. (µM)	Possible nucleotide[1]
a	2922.99	11.49	0.15	CCA TCG CCT C
b	3237.61	20.82	0.28	CCA TCG CCT CA
c	3422.79	23.74	0.32	(5′dSpacer)-CCA TCG CCT CA
d	3597.41	23.07	0.31	CCA TCG CCT CA (3′Carboxy-dt)
e	3926.88	67.03	0.90	CCA TCG CCT CAG (3′-Carboxy-dt)
f	4184.17	39.53	0.53	CCA TCG CCT CAG GC
g	4297.81	65.23	0.87	(5′ Amino dT (C2)) CCA TCG CCT CAG G
h	4513.23	24.64	0.33	CCA TCG CCT CAG GAT
i	4692.32	92.93	1.24	(5′dSpacer) CCA TCG CCT CAG GAT
j	4873.55	27.41	0.37	CCA TCG CCT CAG GAT (3′ carboxyl-dt)
k	4916.42	32.41	0.43	(5′Amino dT (C2)) CCA TCG CCT CAG GAT
l	5228.50	38.34	0.51	(5′ Amino dT (C2))CCA TCG CCT CAG GAT A
m	5433.18	25.28	0.34	(5′Cynanine (CY)3.5) CCA TCG CCT CAG GAT A
n	5643.39	41.28	0.55	(5′Amino dT (C6))CCA TCG CCT CAG GAT A (Carboxyl-dT)
o	5794.08	10.21	0.14	(5′Cynanine (CY)3.5 CCA TCG CCT CAG GAT A (3′Carboxyl-dT)
p	5832.53	19.7	0.26	(5′Amino-dT(C2)) CCA TCG CCT CAG GAT ACA
q	6394.47	8.05	0.11	(5′Cynanine (CY)3.5) CCA TCG CCT CAG GAT ACA (3′Carboxyl-dt)
r	6497.54	16.63	0.22	(5′Amino dT (C2))CCA TCG CCT CAG GAT ACA T (3′Carboxyl-dT)
s	6861.79	36.9	0.49	(5′Amino dT (C2)) CCA TCG CCT CA (reverse 3′-5′) GGT AGC GGA G
t	7205.10	44.95	0.60	(5′Spacer-C12) CCA TCG CCT CA (reverse 3′-5′) GGT AGC GGA GT
u	7478.59	10.82	0.14	(5′Amino dT (C2))CCA TCG CCT CA (reverse 3′-5′) GGT AGC GGA GT
Sum of concentration (µM)			**8.74 ± 0.6**	
Original concentration (µM)			**10**	
Recovery (in %)			**87± 6**	

[1]The oligonucleotide sequence given for each $[M+H]^+$ is proposed combination. The combination of nucleotide was made using on-line database of oligonucleotide properties calculator, http://biotools.nubic.northwestern.edu/OligoCalc.html#helpIUPAC. The molecular mass obtained from MALDI-MS spectrum was matched with the oligonucleotide combination.

Movie S1 Irradiation of laser light (337 nm) on Au-nanobowls of defined geometry using CST.