

SUPPLEMENTAL INFORMATION

Interfacial Nano-biosensing in Microfluidic Droplets for High-sensitivity Detection of Low-solubility Molecules

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EXPERIMENTAL SECTION

Chemicals and materials

17 β -estradiol and ethyl acetate were purchased from Sigma (St. Louis, MO). Graphene oxide was purchased from Graphene Laboratories (Calverton, NY). Polydimethylsiloxane (PDMS, Sylgard 184) was obtained from Dow Corning (Midland, MI). All other chemicals were purchased from Sigma (St. Louis, MO) and used without further purification, unless stated otherwise. Unless otherwise noted, all solutions were prepared with ultrapure Milli-Q water (18.2 M Ω .cm) from a Millipore Milli-Q system (Bedford, MA).

The sequence of the cy3 fluorescence labeled estradiol aptamer (Integrated DNA technologies, Coralville, IA) was listed as following (76 mer, 5'-3'):¹

Cy3-

GCTTCCAGCTTATTGAATTACACGCAGAGGGTAGCGGCTCTGCGCATTCAATTGCTG
CGCGCTGAAGCGCGGAAGC

Microfluidic system fabrication

PDMS microfluidic devices were moulded through a Silicon master. Briefly, we used a thin layer of chrome (50 nm, RF sputtered) as a mask on a 4" wafer. Then the design was lithographically transferred using 1813 PR (photoresist), after developing the PR and 100-second etching Cr with Chrome etchants. Using a Plasmalab-100 System from Oxford Instruments, a DRIE BOSCH process was used to etch Silicon by 45 microns. The DRIE process used 150 steps to etch through Silicon, and each step etched 30 nm of silicon each 12 seconds.

PDMS films were prepared following standard soft lithography procedures.² Firstly, the

liquid PDMS base and the curing agent were mixed at a weight ratio of 10:1. Then the PDMS precursor mixture was poured onto the silicon wafer, degassed in a vacuum desiccator for ~30 minutes, and incubated at 95 °C for 2 hours. The total length of the microchannel is 190.85 mm. Inlet reservoirs in the top PDMS layer and outlet reservoirs were excised using biopsy punches. After 30 seconds exposure in an oxidizing air Plasma Cleaner (Ithaca, NY), PDMS films and the glass slide were face-to-face sandwiched to bond irreversibly. Thus, the biochip became ready for use.

Aptamer-GO preparation

The GO functionalized aptamer solution was prepared with 50 mM Tris-HCl buffer (pH 7.4 with 5 mM KCl, 100 mM NaCl and 1.0 mM MgCl₂), with a final GO concentration of 0.04 mg/mL. The aptamer-functionalized GO was incubated for 15 minutes to quench the fluorescence of the aptamer.

Droplet generation

Droplets were generated by using a T-junction method. Aptamer-GO was used as the water phase and estradiol in the ethyl acetate solvent was used as the oil phase with flow speed of 0.6 μL/min and 2.4 μL/min, respectively. The volume of each droplet was ~0.125 nL. The droplet generation process was demonstrated in **Supplementary Video V-1**, by using the Cy3-labelled aptamer. After 30 min incubation at room temperature inside the outlet, droplets were detected at the outlet region with a cover slip on its top by a Nikon Ti-E microscope (Melville, NY) with appropriate Cy3 optical filters.

Supplementary Table S-1 Estradiol assay sensitivity and assay time by using commercial estradiol ELISA kits

Company	Assay Sensitivity (pg/mL)	Assay Time (hours)
Sigma-Aldrich	5.0	2.5
ThermoFisher	5.0	> 2.5
Eagle Biosciences	8.68	> 2.5
Abcam	8.68	> 2
ALPCO	10.0	> 1.5
R&D Systems	12.1	3.5
Cayman Chemical	15.0	> 2
Abnova	28.5	> 2
Enzo	28.5	3

Note: Information is obtained from the product information provided by related companies.

References

- 1 Y. S. Kim, H. S. Jung, T. Matsuura, H. Y. Lee, T. Kawai and M. B. Gu, *Biosensors and Bioelectronics*, 2007, **22**, 2525.
- 2 A. Y. Xia and G.M. Whitesides, *Rev. Mater. Sci.*, 1998, **1998**, 31.