**Biosensor-Embedded Digital Microfluidic Device for Lab-on-a-chip Platform**

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**Detailed description of fabrication process**

![Fabrication steps](image)

**Figure S1.** Detailed description of fabrication process: (a) Fabrication steps (b)-(h) Cross-sectional schematics of our proposed device across an embedded biosensor at each fabrication step (b) Fabricated underlap field-effect transistor biosensor (c) Deposition and patterning of inter-layer dielectric (ILD) silicon dioxide (200 nm in thickness) to make contact holes (d) Deposition and patterning of n⁺ poly-Si to form probing pads, droplet-driving electrodes, and a pre-charging electrode (not shown in the schematic) (e) Deposition and patterning of silicon oxide (50 nm in thickness) and silicon nitride (150 nm in thickness) to form a passivation layer (f) Thermal oxidation of exposed silicon surface (approximately 5 nm in thickness) (g) Patterning of CYTOPTM film (40 nm in thickness) on the passivation layer by lift-off process (h) Complete device with a sample droplet of anti-AI after immobilization of SBP-AIa
Immobilization of avian influenza antigens linked with silica-binding proteins on device surface

Silica-binding proteins (SBP) are special types of proteins which strongly bind on a silicon dioxide surface at one end and are fused with bio-receptor molecules, avian influenza (AI) in our case, at the other end by recombinant DNA technology. Immobilization of the AI antigen (AIa) molecules on the silicon dioxide surface is possible with these special types of proteins without any surface modification\textsuperscript{17}. SBP fused with AIa (SBP-AIa) are immobilized on the silicon dioxide surface prior to bio-experiments to detect a specific antibody against AI (anti-AI). The fabricated device was submerged in SBP-AIa sample solution with a concentration of 25 µg/ml diluted in phosphate-buffered saline (PBS, pH 7.4) at 25°C for 1 hour. After the immersion of the device in the SBP-AIa solution, the device is rinsed with deionized water for 10 minutes to remove non-bound bio-molecules on the device surface. The device was then blown dry under a stream of nitrogen.