Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Label-free biosensors based on *in situ* formed and functionalized microwires in microfluidic devices

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1. Detailed experimental procedures

1.1 Materials

SU-8 and developers for the resists were purchased from Microchem (Newton, MA, USA). 1H,1H,2H,2H-perfluorodecyl-dimethylchlorosilane was purchased from ABCR (Karlsruhe, Germany), and poly- (dimethylsiloxane) (PDMS) (Sylgard 184) was obtained from Dow Corning (Midland, MI, USA). Acetonitrile (CH_3CN, 99.8+%), 4-Aminothiophenol (4-ATPh, 97%), 6-Aminofluorescein (95%), Dimethylsulfoxid (DMSO, 99.8+%), DL-Norepinephrine hydrochloride, (>=97% TLC), Dopamine hydrochloride, Ethanol (99.8+%), Gold (III) chloride trihydrate ($HAuCl_4$, 99+% trace metal basis), Isoprenaline hydrochloride, L-Epinephrine, L-Phenylalanine (>=99%), L-Tyrosine (>99%), N-Hydroxysuccinimide (NHS, 98%), Phosphate buffered saline (PBS) tablet and Sodium cyanoborohydride ($NaCNBH_3$, 95%) were all purchased from Sigma–Aldrich (Buchs, Switzerland). 2-Aminoethanethiol hydrochloride (Cysteamine hydrochloride, 98%) were obtained from Acros Organics (Basel, Switzerland). Glutaraldehyde (GA, 50% in water solution) was purchased from Fluka (Buchs, Switzerland). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 98%) and Tetrathiafulvalene (TTF, 99+%) were obtained from TCI (Eschborn, Germany). Lissamine rhodamine B sulfonyl chloride (mixed isomers) and PBS solution (pH 7.40, 10 mM) were obtained from Invitrogen (Lucerne, Switerland). FITC-labeled human IgG antibody (0.5 mg/mL), Human IgG antibody (mAb mouse, 0.5 mg/mL) and Human IgG protein (4 mg/mL) were purchased from GenScript (Piscataway, NJ, USA).

1.2 Chip design and fabrication of the multilayer microchips

For the multilayer microfluidic chip, layers were prepared separately by casting PDMS from the respective master forms. Alignment, assembly and bonding of both layers resulted in the final device. The master forms for preparing the PDMS layers were fabricated by spin-coating the photoresist SU-8 to a height of 20 μm on a 4-inch silicon wafer. After a soft bake and exposure with UV light (intensity 150 mJ/cm^2, measured at 365 nm) in a mask aligner, the photoresist was baked again and developed using SU-8 developer. The master form for the control layer of the microchip was designed to be 1.6% larger to account for shrinkage of PDMS after casting (experimentally determined value). After a hard bake at 200°C for 3 hours, the heights of the SU-8 features were confirmed with a step profiler. All master forms were salinized overnight under vacuum using 1H,1H,2H,2H-perfluorodecyldimethylchlorosilane.

For preparation of the control layer, PDMS oligomer and curing agent were mixed at a ratio of 10:1, degassed, poured onto the wafer bearing the respective features and degassed again, then heated in oven at 80°C for 3 h. After this, the layer was removed from the wafer and holes were punched with a biopsy puncher (1 mm diameter, Miltex, York PA). For the fluid layer, softer PDMS (mixing ratio elastomer: curing agent of 15: 1) was spin-coated at 2000 rpm on the structured four-inch fluidic master mold wafer to create a 23 μm-high membrane, and then the wafer was heated at 80 °C for 1 h. Both layers were activated in oxygen plasma for 45 s and afterwards aligned under a Multizoom AZ100M microscope (Nikon Corporation, Switzerland). The assembly was cured for 2 h at 80°C to form a permanent bond between both layers.

Afterwards, the device was removed from the master form and access holes for the tubing (1.5 mm diameter, Miltex) were punched. Finally, the assembled chip were treated in oxygen plasma again, and then carefully aligned and bonded to a clean glass slide (Thermo Scientific, Menzel-Gläser, Nr. 1). Finally, the well-assembled chip was heated at 90 °C for 30 min to confirm the permanent bonding.

1.3 Chip operation

Custom-made metal connectors and silicone tubing were used to introduce nitrogen gas supply to the chip. Control channels were filled with water by pressurization before the experiments. The control layer was pressurized up to 3 bars for closing the donuts and immobilizing the wires, and then released to 2 bars in functionalization section. For the fluid layer, the reagents were loaded into plastic syringes and supplied through teflon tubings into the chip using a syringe-pump system (NanoJet, ChemyxInc, Germany). Colored images were recorded on a stereo-microscope (AZ-100M, Nikon) equipped with a digital camera (Digital sight DS-Fi1, Nikon). Other optical and fluorescent images were taken on an inverted microscope (IX71, Olympus) equipped with a digital camera (UK1117, ABS) and optical filters.

1.4 Hybrid wire characterization

The SEM images and the energy dispersive X-ray spectroscopy were obtained using a FEI Quanta 200 FEG.

1.5 Data processing

Fluorescent images were recorded, false-colored and background-corrected by Image J. Raman spectra were recorded using WiTec Project and analyzed with Origin Pro 9.1 (Academic, Originlab). We used the implemented peak analyzer tool of Origin Pro 9.1 to get background-corrected spectra, peak position and intensity (Fig. S10).

2. Supplementary Figures

S1 Movie

The real time video shows a bonded chip filled with food dye, then washed with PBS buffer at 5 μL/min.

S2 Movie

The video (×4 sped up) showed TTF-Au wires formed inside a microchip by diffusion and trapped by a donut structure.

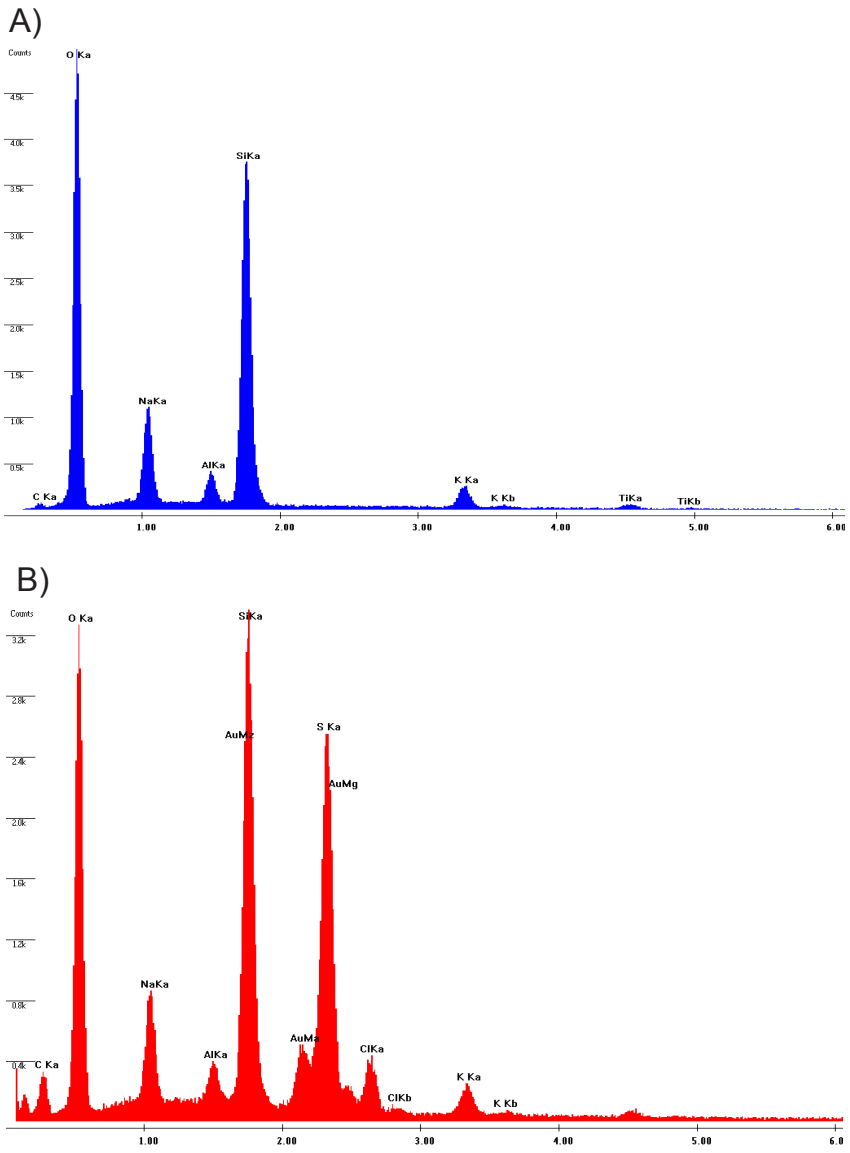

Fig. S3 Energy-dispersive X-ray (EDX) spectra of (A) the glass slide and (B) a TTF-Au wire produced by the diffusion technique. The spectrum in (B) clearly indicates the presence of S and Au in the TTF-Au hybrid wire.

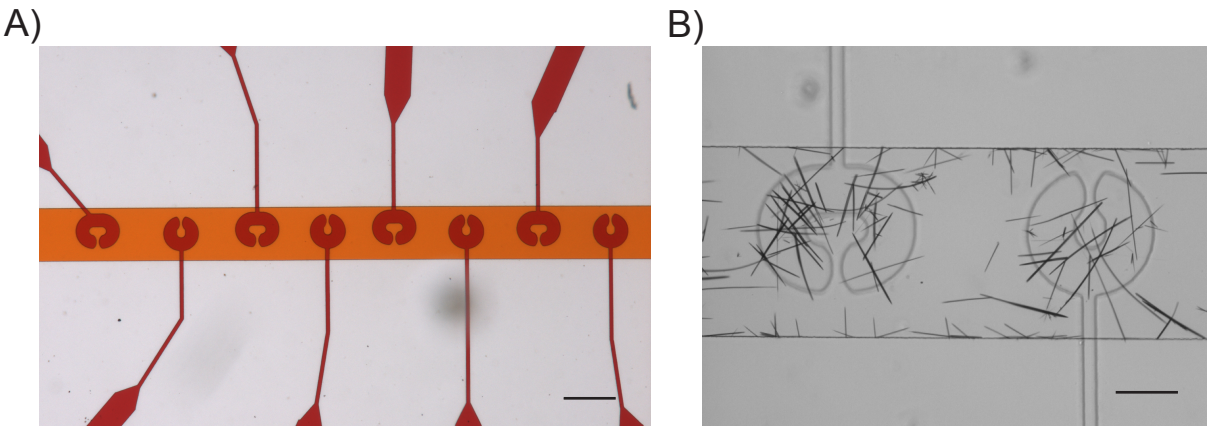

Fig. S4 A micrograph of the micro-channel and open-donuts. Scale bar: 300 µm.

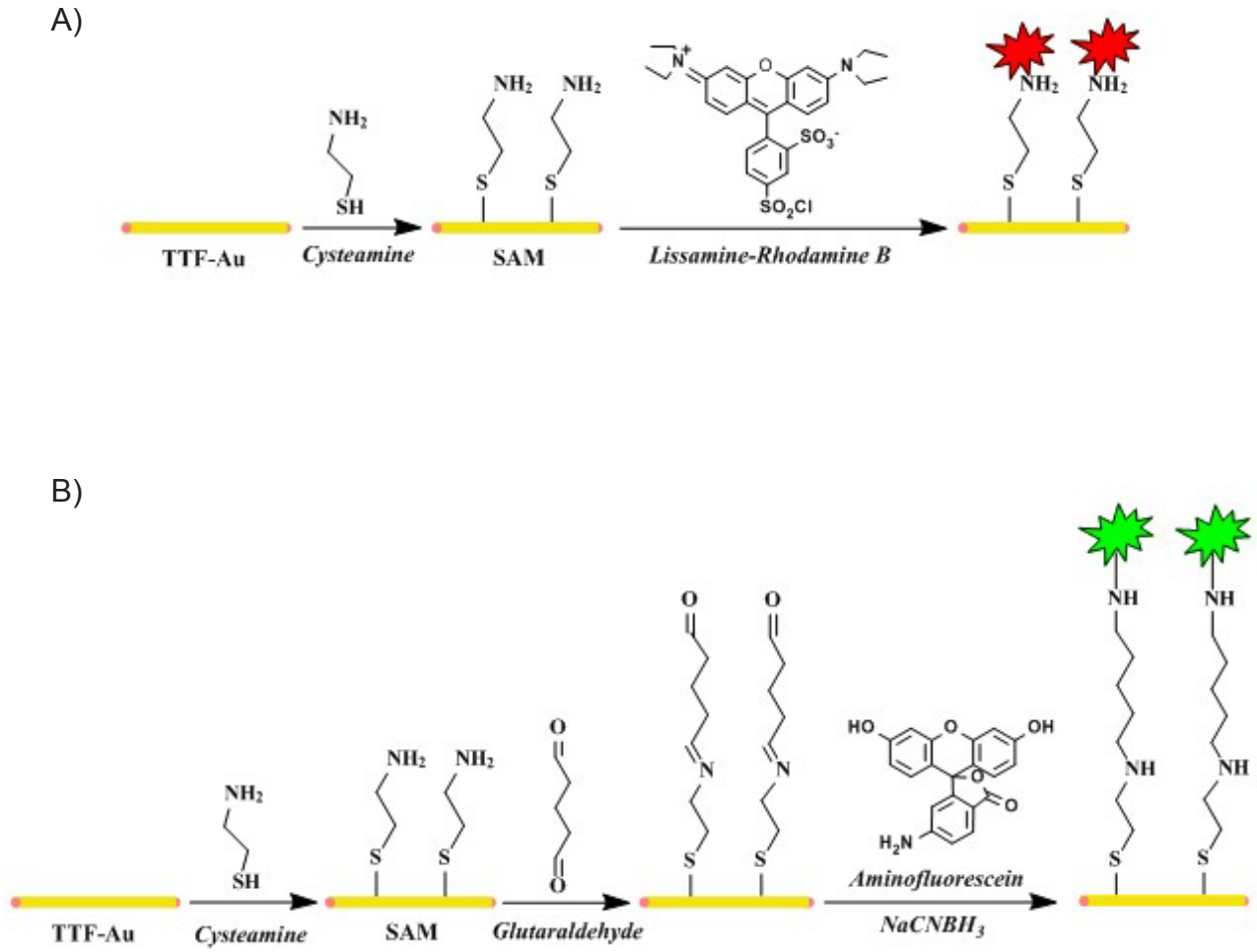

Fig. S5 Reaction schemes for the binding of a fluorophore to image by fluorescence microscopy the formation of (A) the SAM of CEA (B) and the binding of GA.

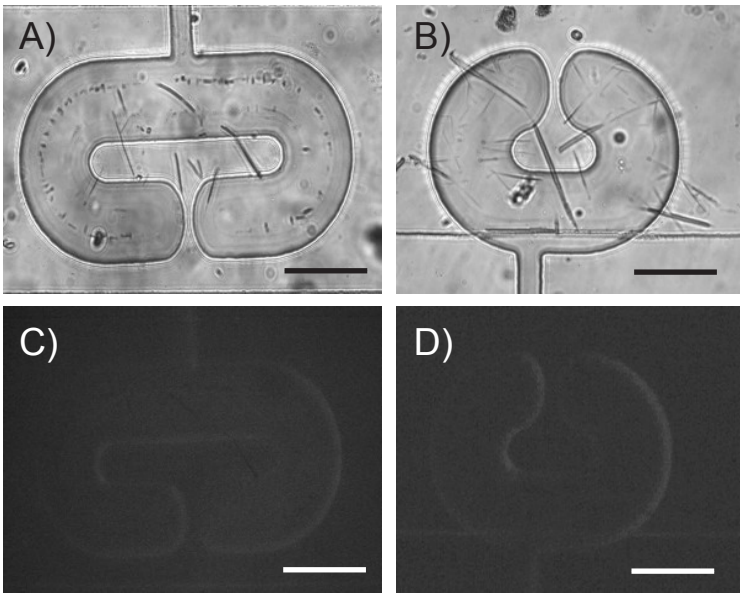

Fig. S6 (A) and (B) are the bright field images of nanowires immobilized by different donuts. (C) and (D) are the fluorescent images after incubation with CEA (10 mM) separately, before supply of the fluorophores. Scale bars: 100 μm.

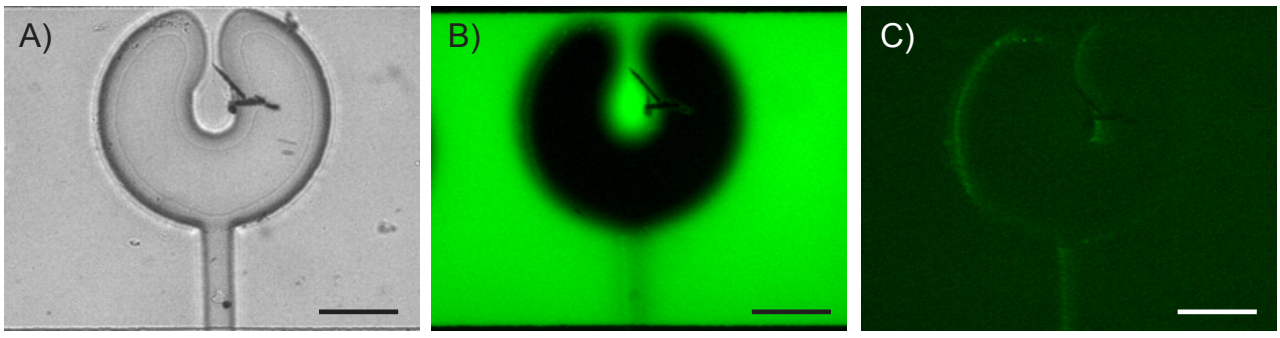

Fig. S7 Control experiment without GA solution. (A) is the bright field image of nanowire immobilized by donut. (B) is the false-colored fluorescent image after incubation with CEA (10 mM) and 6-aminofluorescein (100 μM). (C) is the false-colored fluorescent image after following supply of $NaCNBH_3$ (1 mM) and PBS washing. Blue light excitation was used for fluorescent images. Scale bars: 100 μm.

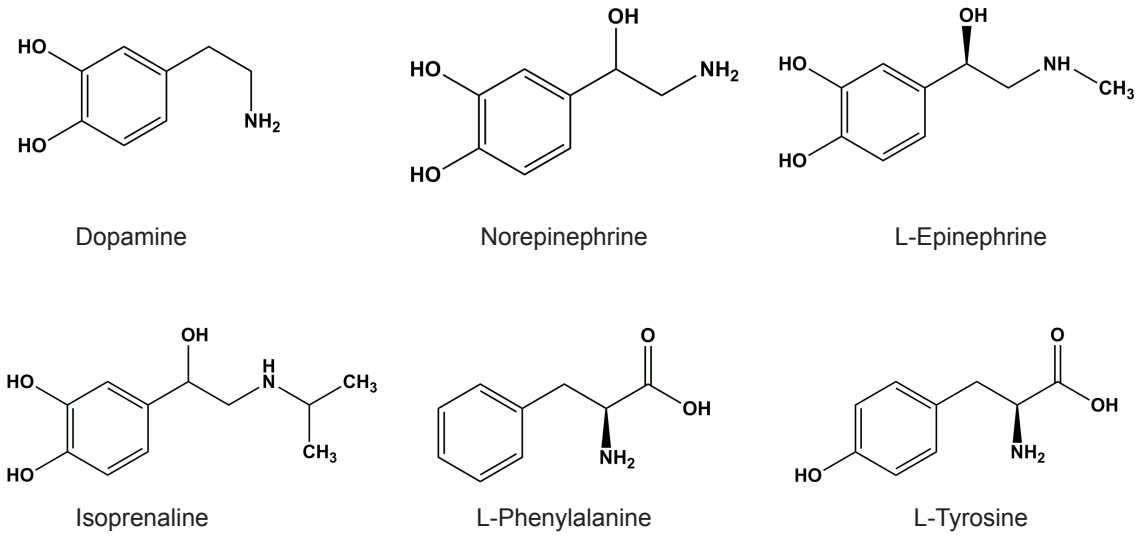

Fig. S8 Structures of catecholamines including Dopamine (DA), Norepinephrine (NE), L-Epinephrine (EPI) and Isoprenaline (ISP) and aromatic amino acids including L-Phenylalanine (Phe) and L-Tyrosine (Tyr).

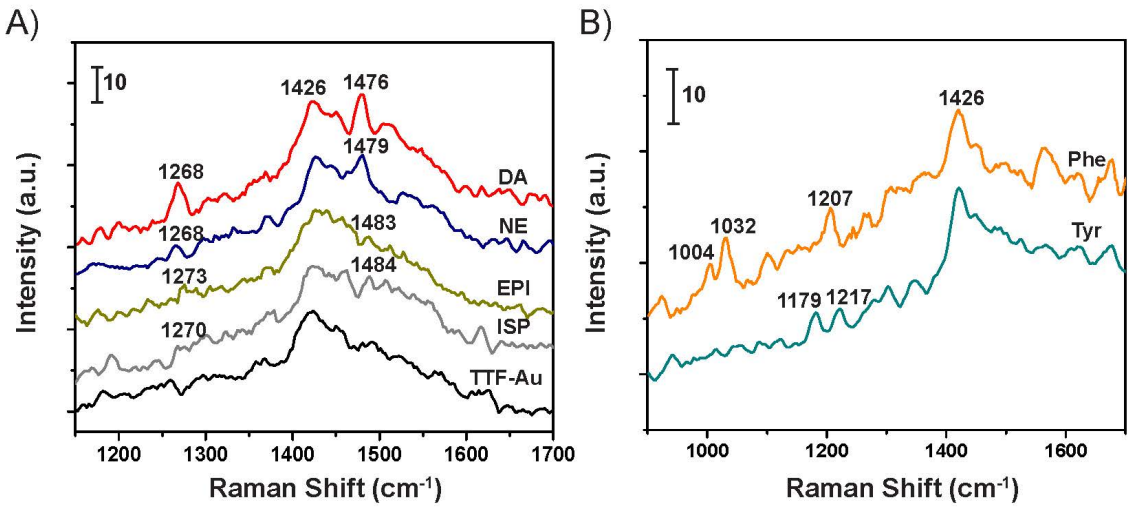

Fig. S9 (A) Comparison of the Raman spectra of single TTF-Au wire and after separate bonding of 100 µM different catecholamines including DA (1268 cm^{-1} and 1476 cm^{-1}), NE (1268 cm^{-1} and 1479 cm^{-1}), EPI (1273 cm^{-1} and 1483 cm^{-1}) and ISP (1270 cm^{-1} and 1484 cm^{-1}). (B) the Raman spectra of TTF-Au wires after bonding of 100 µM aromatic amino acids including Phe (1004 cm^{-1} , 1032 cm^{-1} and 1207cm^{-1}) and Tyr (1179 cm^{-1} and 1217 cm^{-1}) separately.

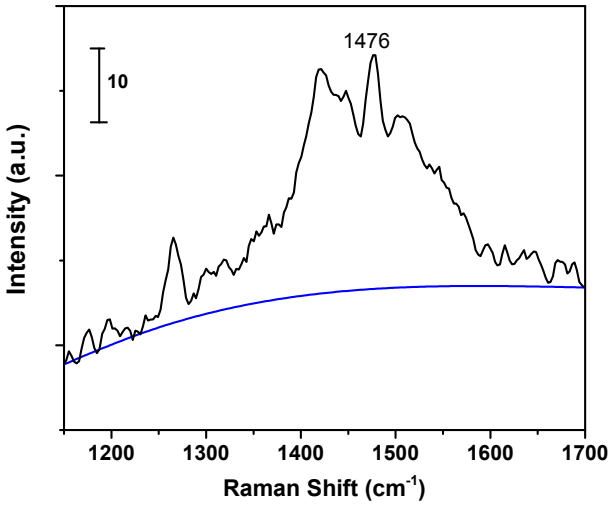

Fig. S10 The Raman spectra of TTF-Au/CEA/GA/DA (black) and the modeled baseline by Origin Pro 9.1 (Academic) program (blue).

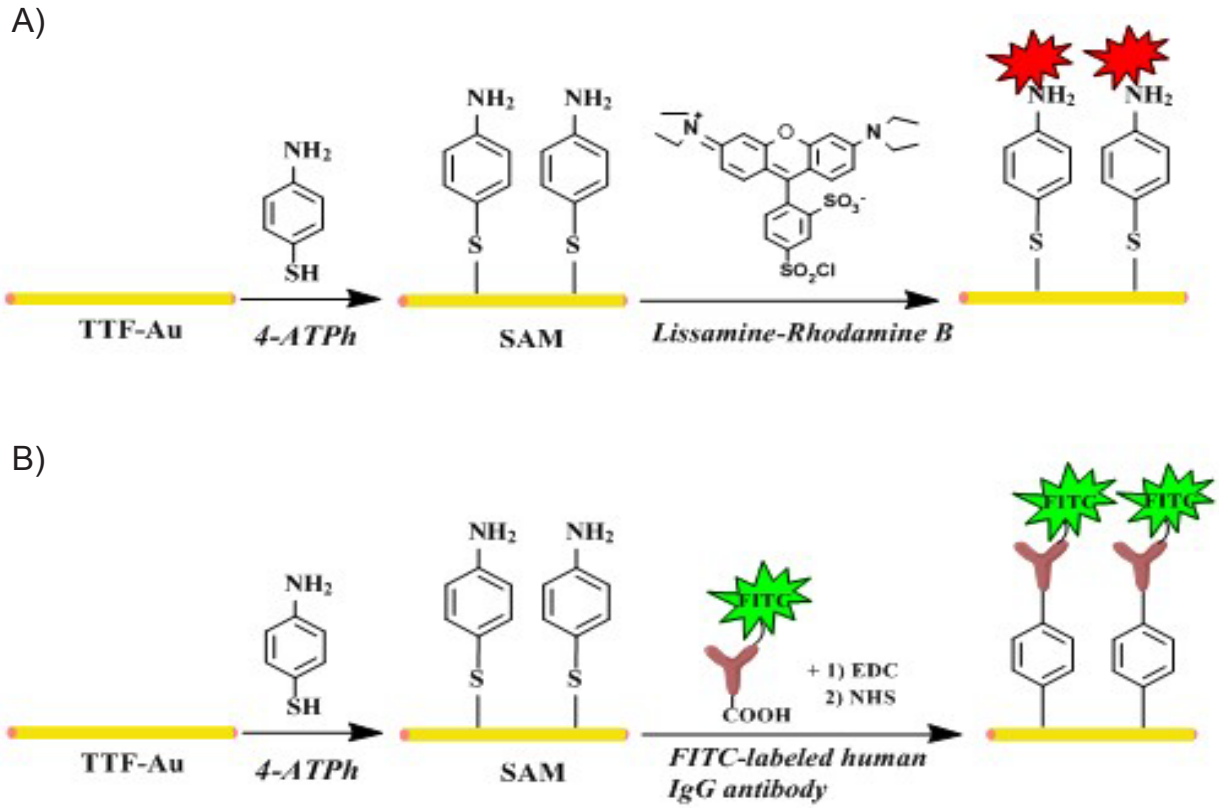

Fig. S11 Reaction mechanisms for binding of fluorophors to visualize by fluorescence micrsocopy (A) the SAM of 4-ATPh and (B) the binding of FITC-labeled antibody.

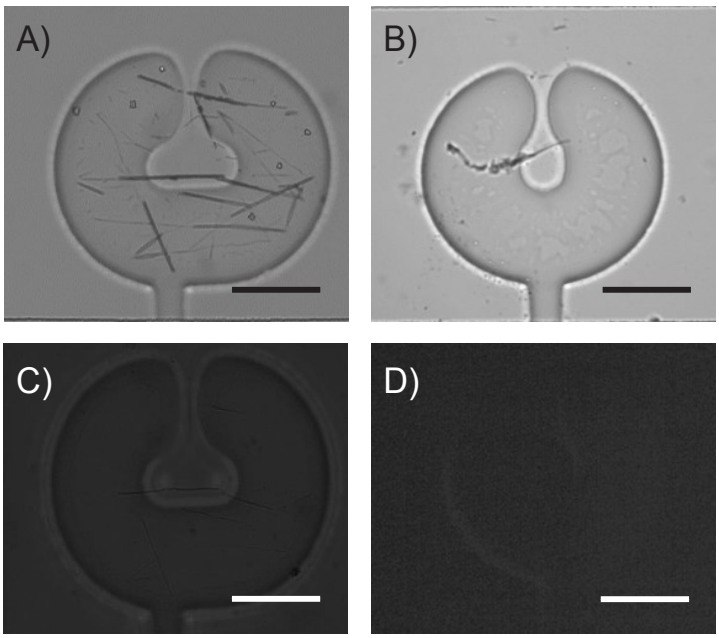

Fig. S12 (A) and (B) are the bright field images of the trapped wires inside the donuts. (C) is the fluorescent image after reaction with 4-ATPh (15 mM) but without Lissamine rhodamine B sulfonylchloride (Green light excitation). (D) is the fluorescent image after reaction with 4-ATPh (15 mM), but without FITC-labeled human IgG (Blue light excitation).