Electronic Supplementary Material (ESI) for Materials Horizons.


# Supplementary Information

**Molecular logic operations from complex coacervation with aggregation-induced emission characteristics**

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## Materials

Dextran from *Leuconostoc* spp. (*ca.* 40 kDa and 2000 kDa), PEG 8000 (as the crowding agent) and N, N-dimethylformamide (DMF) were purchased from Sigma-Aldrich (USA). Dextran T500 (as the crowding agent) was purchased from Pharmacosmos (Denmark). Peptides were synthesized via solid-phase peptide synthesis method and purified (purity>95%) with high-performance liquid chromatography (HPLC) by Sangon Biotech (China). The identities of peptides were double confirmed by mass spectroscopy using mass spectrometer (Bruker Daltonics UltrafleXtreme MALDI TOF/TOF Mass-spectrometer, USA). All the other chemicals were purchased from Sigma-Aldrich (USA) unless otherwise specified.

## Methods

### Synthesis of BSBOTPE

The AIEgen, namely, BSBOTPE, was synthesized with the procedures reported previously[1], and characterized by proton nuclear magnetic resonance ($^1$H NMR) spectroscopy (Figure S16) and high-resolution mass spectrometry (HRMS) (Figure S17).

$^1$H NMR (400 MHz, DMSO-$d_6$), δ (TMS, ppm): 7.17-7.06 (6H, m), 6.99-6.94 (4H, m), 6.87-6.81 (4H, m), 6.73-6.66 (4H, m), 4.89-3.83 (4H, m), 2.47-2.42 (4H, m), 1.72-1.67 (8H, m).

HRMS (TOF-MS), m/z: calcd. for $C_{34}H_{34}O_8S_2^{2-}$: 317.0853, found: $[M-2Na]^{2-}$: 317.0854.

### Synthesis of DCH

Dextran was firstly functionalized with vinylsulfone (VS) group, followed by conjugation of CGGRGG peptide, purification by dialysis and characterization as has been reported previously[2]. The degree of modification of CGGRGG peptide used is 37.9 %.

### Complexation coacervation of DCH and BSBOTPE

Unless otherwise specified, DCH stock solution (*ca.* 30x, in $H_2O$) was heated to 70 °C and incubated for 2 min in sealed centrifuge tubes, followed by dilution in 25 °C intracellular

physiological-mimicking buffer (IPM buffer, containing 150 mM NaCl, 10 mM HEPES, 10 wt% PEG 8k, pH=7.4) at certain concentration, brief (*ca.* 2s) vortex mixing and 15-min incubation at 25 °C for maturation. The BSBOTPE was then incorporated by inserting stock solution (*ca.* 35x, in a mixture of 50 % $H_2O$ and 50 % DMF), brief (*ca.* 2s) vortex mixing and 15-min incubation at 25 °C for maturation. The scale of preparation is 200 μL.

Note that IPM buffer was used as the default solvent in this paper except solvents of NAND logic operation (150 mM NaCl, 10 wt% PEG 8000, pH=7.4) and XOR logic operation (10 mM HEPES, 10 wt% PEG 8000, pH=7.4).

**Confocal laser scanning microscopy (CLSM)**

Imaging was performed on a confocal laser scanning microscope (Zeiss LSM710, Germany) at 60 x magnification under 25 °C. 6 μL solution was applied to a confocal dish for imaging. All dishes were passivated with Pluronic F-127 (10 wt%) for 1 h prior the imaging to prevent wetting of droplets on a glass surface.

**Microplate reading**

Absorbance (at 600 nm, as the indicator of turbidity) and fluorescence were measured using transparent and black 384-well plate, respectively, on a Varioskan LUX Multimode Microplate Reader (Thermo Fisher Scientific, USA). 50-μL samples were loaded to microplate, followed by absorbance or fluorescence measurement with background corrected. Dark condition was maintained for the whole process.

**Solidification of logic gates in hydrogels**

Dextran 2000 kDa was modified with vinylsulfone[3] and thiol (SH)[4] groups at the degree of modification of 1.28 % and 4 %, respectively. The synthesized Dex2000VS and Dex2000SH were used as scaffold materials of hydrogels.

DCH stock solution (*ca.* 30x, in $H_2O$) was heated to 70 °C and incubated for 2 min in sealed centrifuge tubes, followed by dilution in 25 °C buffer (containing 150 mM NaCl, 10 mM HEPES, 5 wt% Dextran T500, pH=7.4) at 4.90 mg/mL (*ca.* 48 μM), brief (*ca.* 2s) vortex mixing

and 15-min incubation at 25 °C for maturation. The BSBOTPE was then incorporated by inserting stock solution (*ca.* 35x, in a mixture of 50 % $H_2O$ and 50 % DMF) at 141 μM, brief (*ca.* 2s) vortex mixing and 15-min incubation at 25 °C for maturation. The synthesized Dex2000VS and Dex2000SH were then incorporated at 3.3 wt % and 1.7 wt %, respectively, followed by rapid vortex mixing until complete dissolution. The hydrogel precursor was then pipetted onto parafilm surface, followed by 30-min maturation for curing. The scale of hydrogel prepared is 200 μL.

## Supplementary Figures

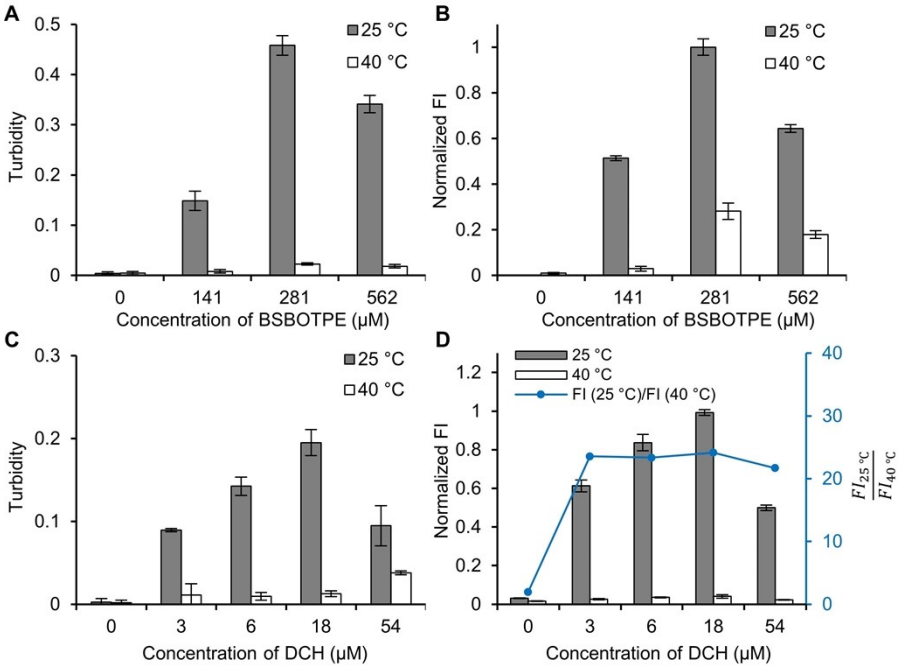


**Fig. S1**. Formation of complex coacervation and aggregation-induced emission from DCH and BSBOTPE. A, B) BSBOTPE-dependent turbidity (A) and normalized FI (B) complexed with DCH (6 μM). C, D) DCH-dependent turbidity (C) and normalized FI (D) complexed with BSBOTPE (141 μM). Excitation and emission wavelengths were set as 350 nm and 471 nm for fluorescence experiments, respectively. n=3.

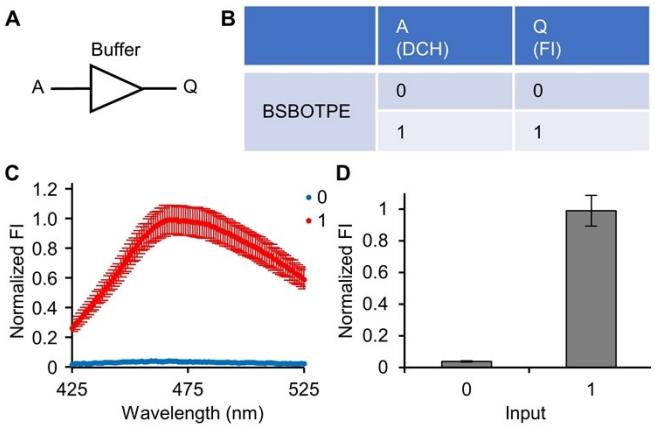


**Fig. S2**. Buffer (2) logic operation. A) Symbol of logic gate. B) Truth table. C) Normalized fluorescence spectra of logic operations with different inputs. D) Normalized FI at 471 nm. The concentrations of DCH and BSBOTPE used are 6 μM and 141 μM, respectively. Excitation wavelength was set at 350 nm. n=3.

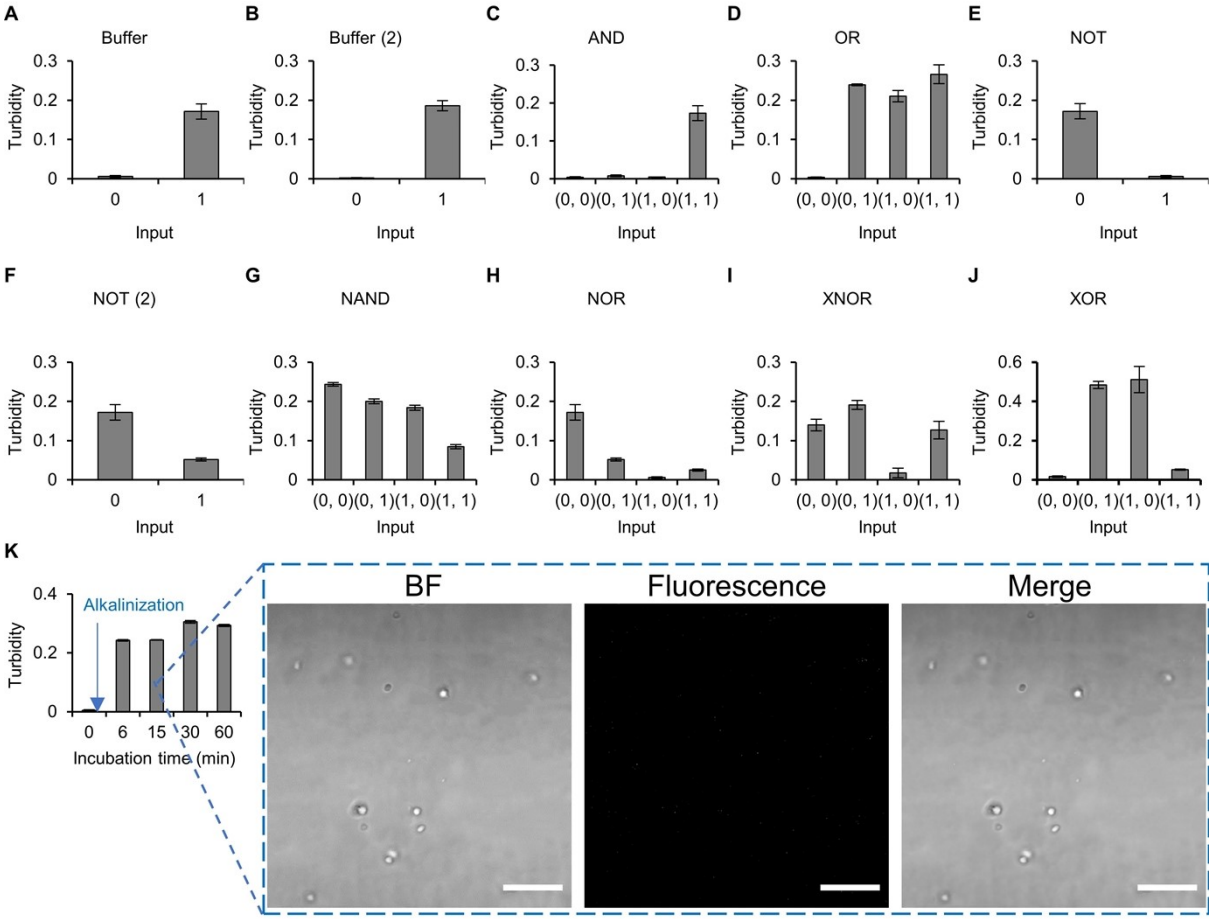


**Fig. S3**. Turbidimetry of logic operation. (K) represents the alkalinization of DCH (48 μM) alone in IPM buffer from 7.4 to 11.4, leading to the presence of turbidity and coacervation. Scale bars, 20 μm. n=3.

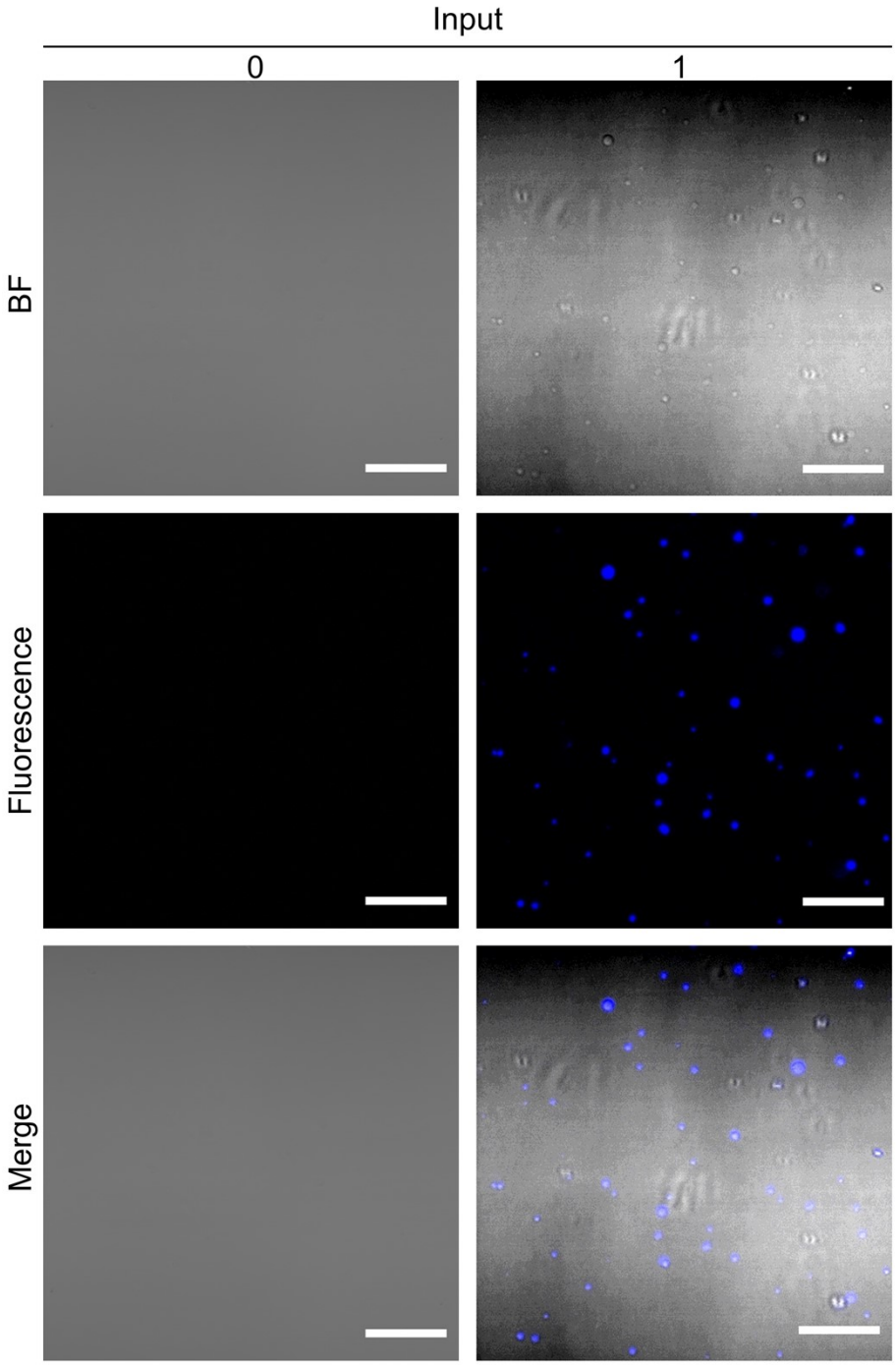


**Fig. S4**. Confocal laser scanning microscopy of ‘Buffer’ logic operation. Scale bars, 20 μm.

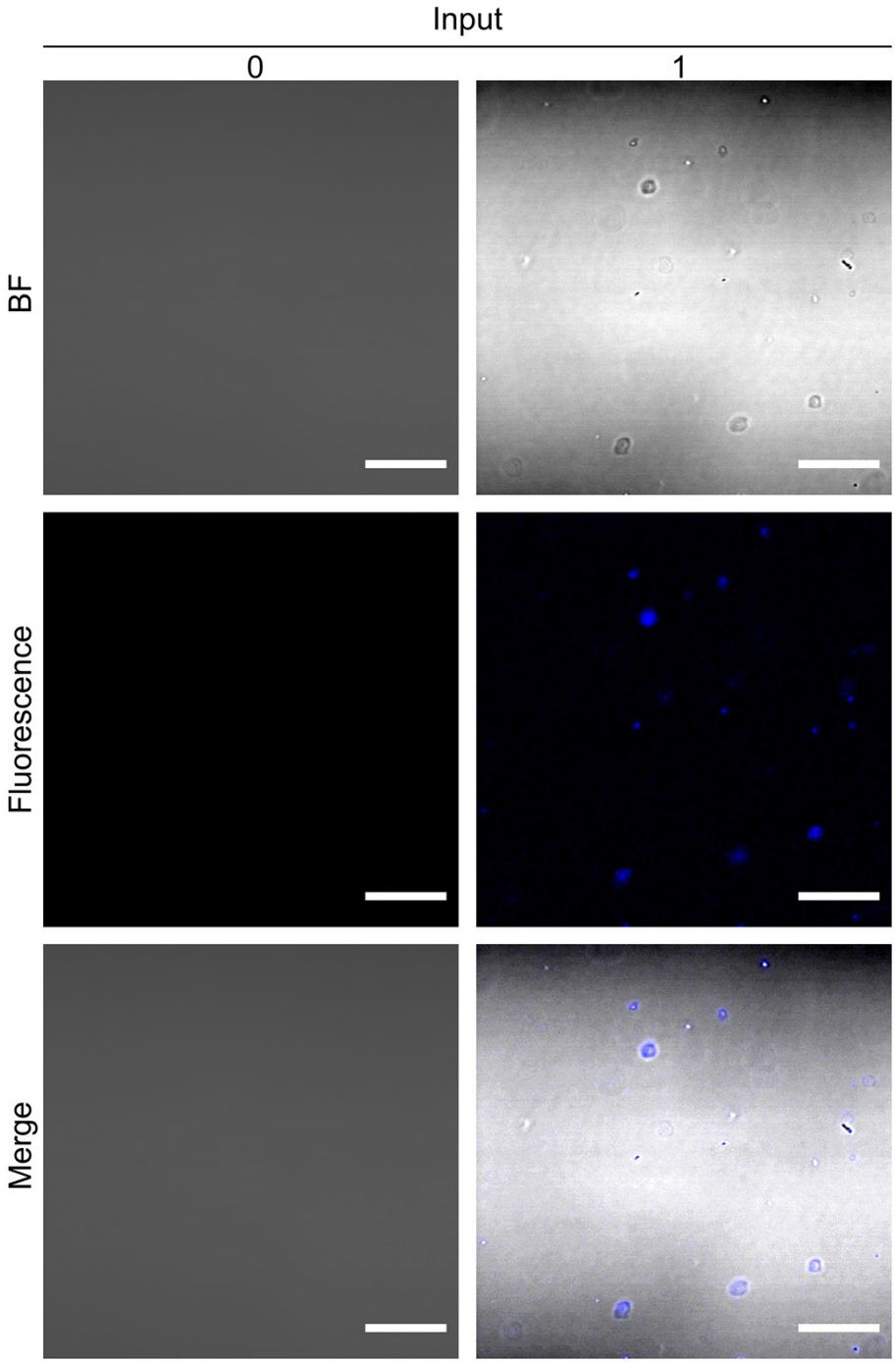


**Fig. S5**. Confocal laser scanning microscopy of ‘Buffer (2)’ logic operation. Scale bars, 20 μm.

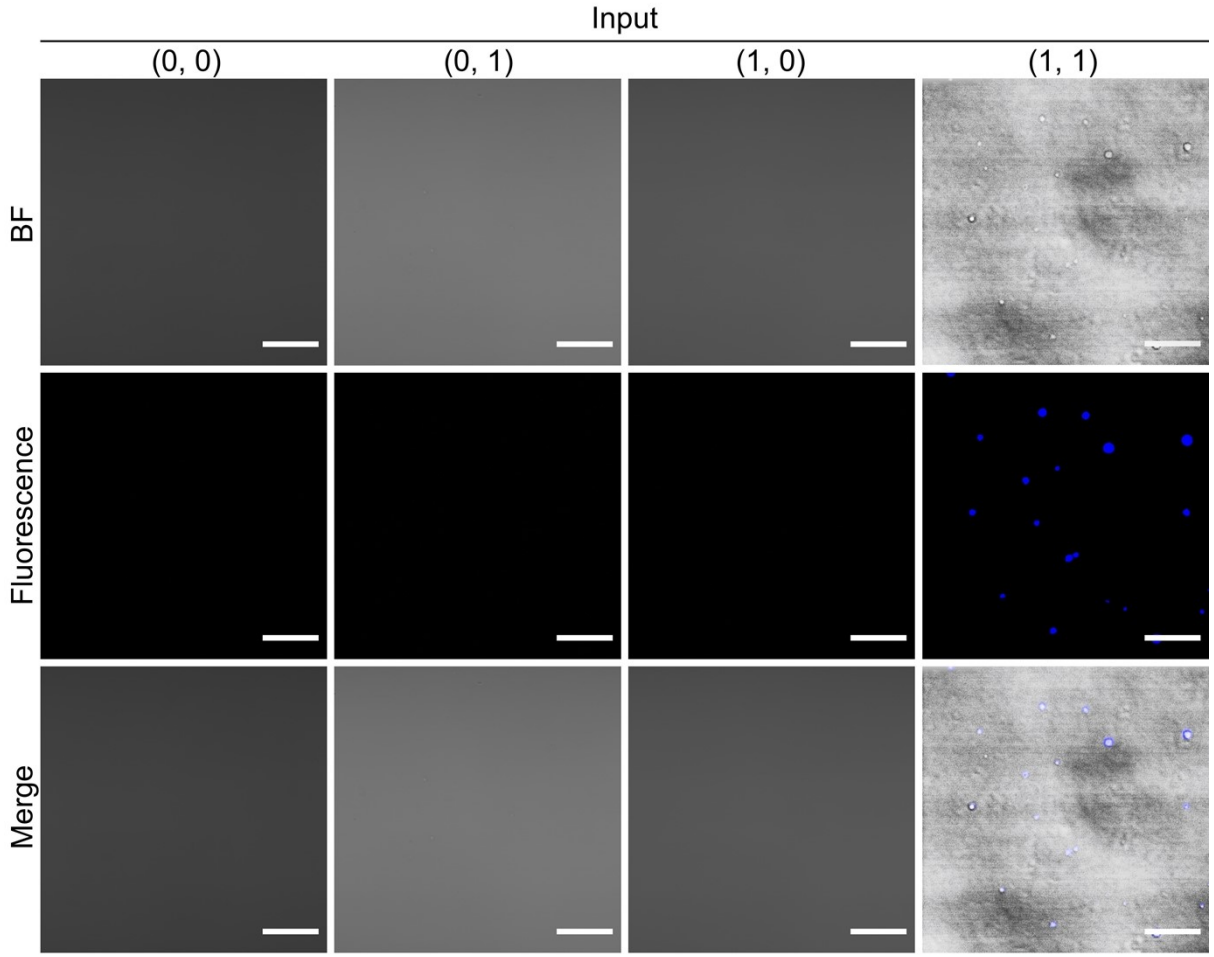


**Fig. S6**. Confocal laser scanning microscopy of 'AND' logic operation. Scale bars, 20 μm.

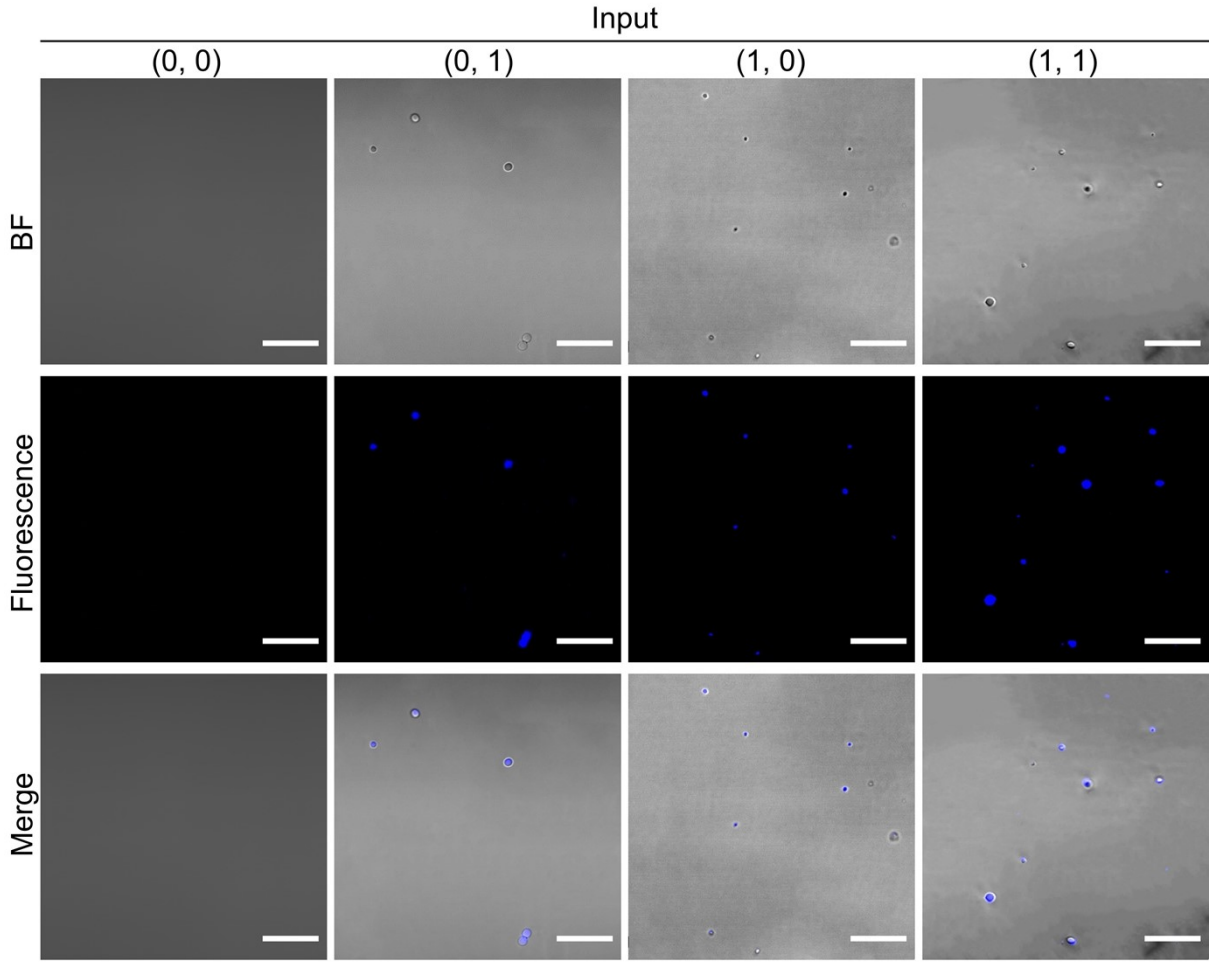


**Fig. S7**. Confocal laser scanning microscopy of ‘OR’ logic operation. Scale bars, 20 μm.

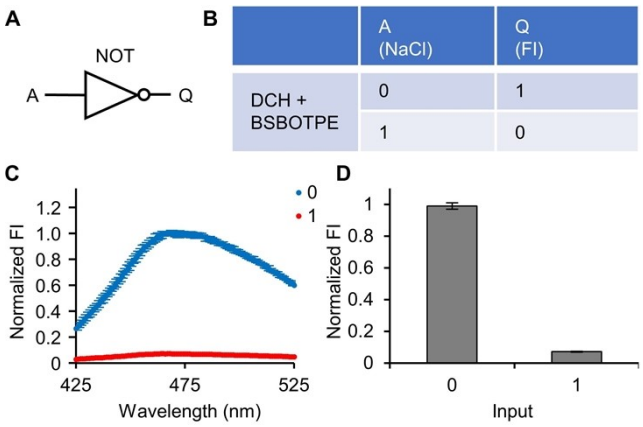


**Fig. S8**. NOT (2) logic operation. A) Symbol of logic gate. B) Truth table. C) Normalized fluorescence spectra of logic operation with different inputs. D) Normalized FI at 471 nm. The concentrations of DCH and BSBOTPE used are 6 μM and 141 μM, respectively. Excitation wavelength was set at 350 nm. The 'NaCl' input is increasing NaCl concentration from 150 mM to 600 mM, followed by 2-min incubation. n=3.

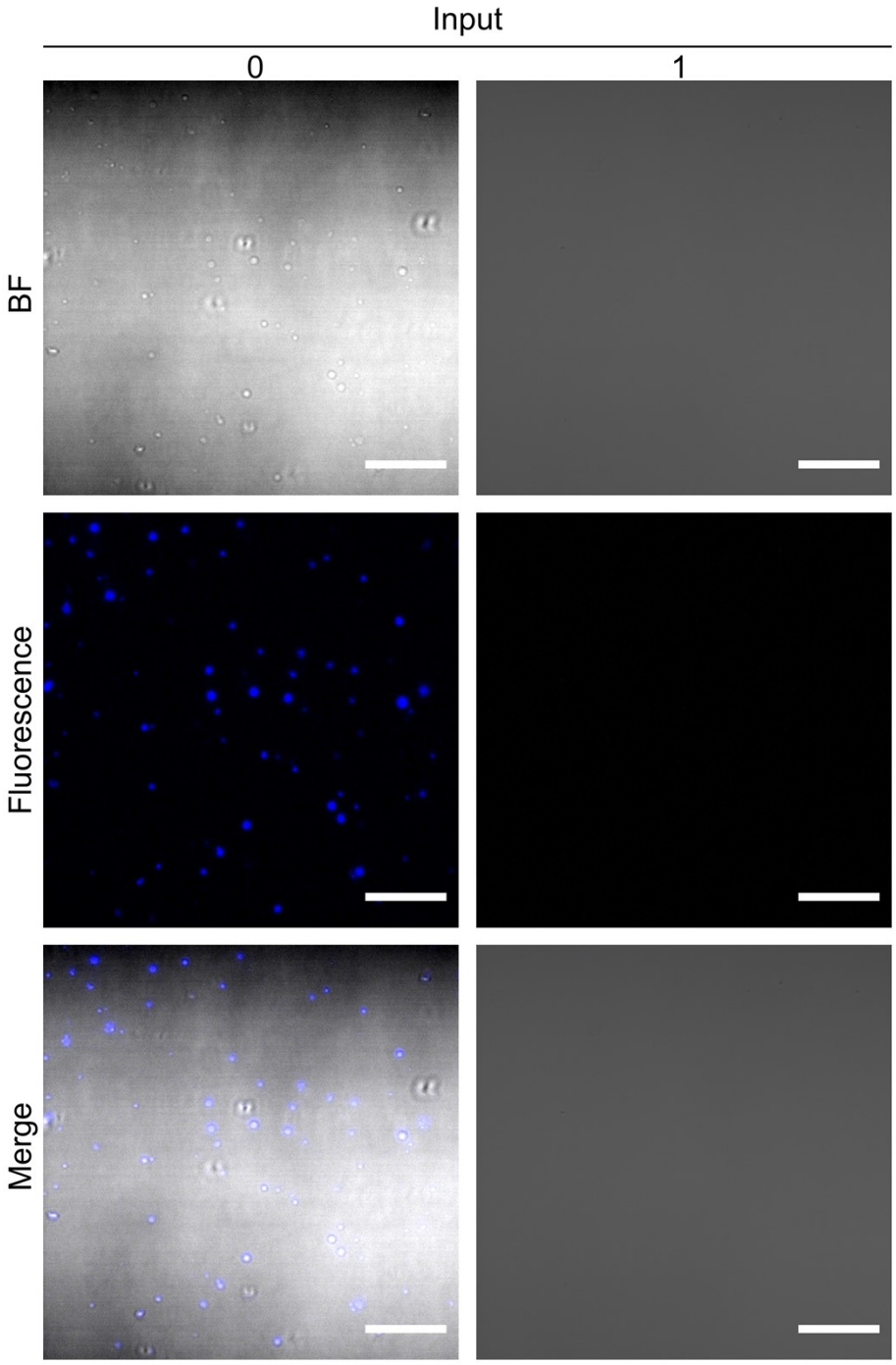


**Fig. S9**. Confocal laser scanning microscopy of ‘NOT’ logic operation. Scale bars, 20 μm.

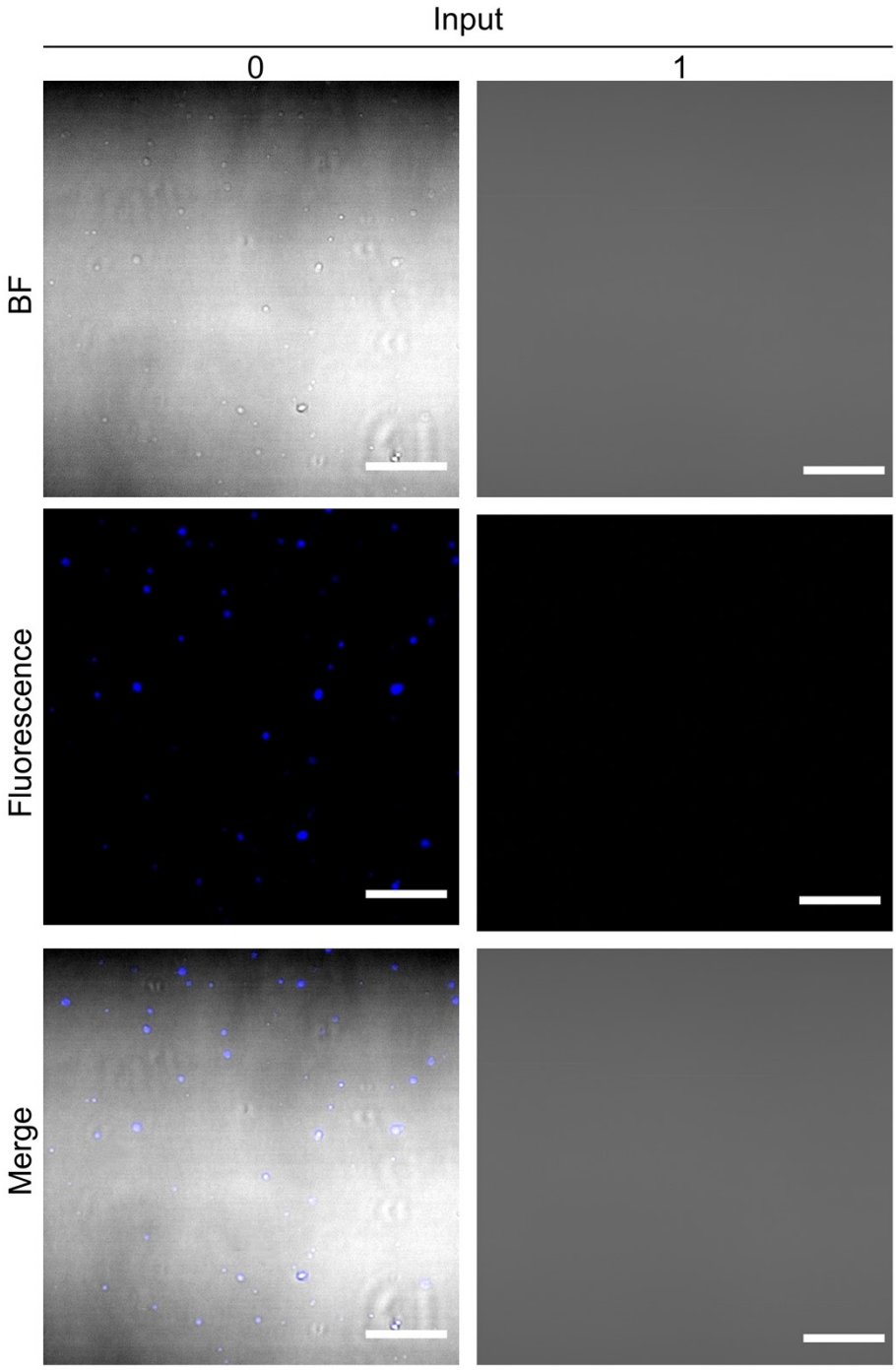


**Fig. S10**. Confocal laser scanning microscopy of 'NOT (2)' logic operation. Scale bars, 20 μm.

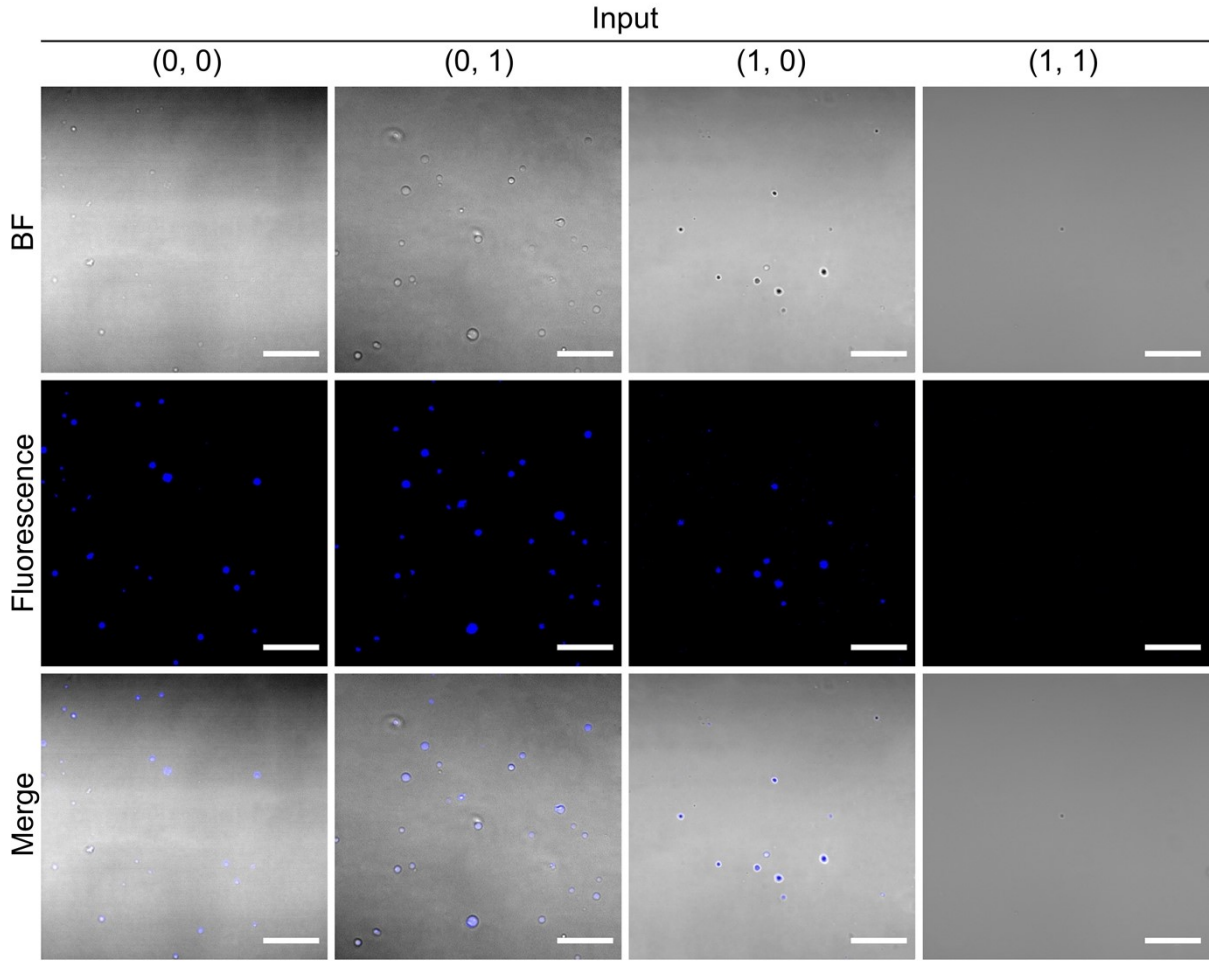


**Fig. S11**. Confocal laser scanning microscopy of 'NAND' logic operation. Scale bars, 20 μm.

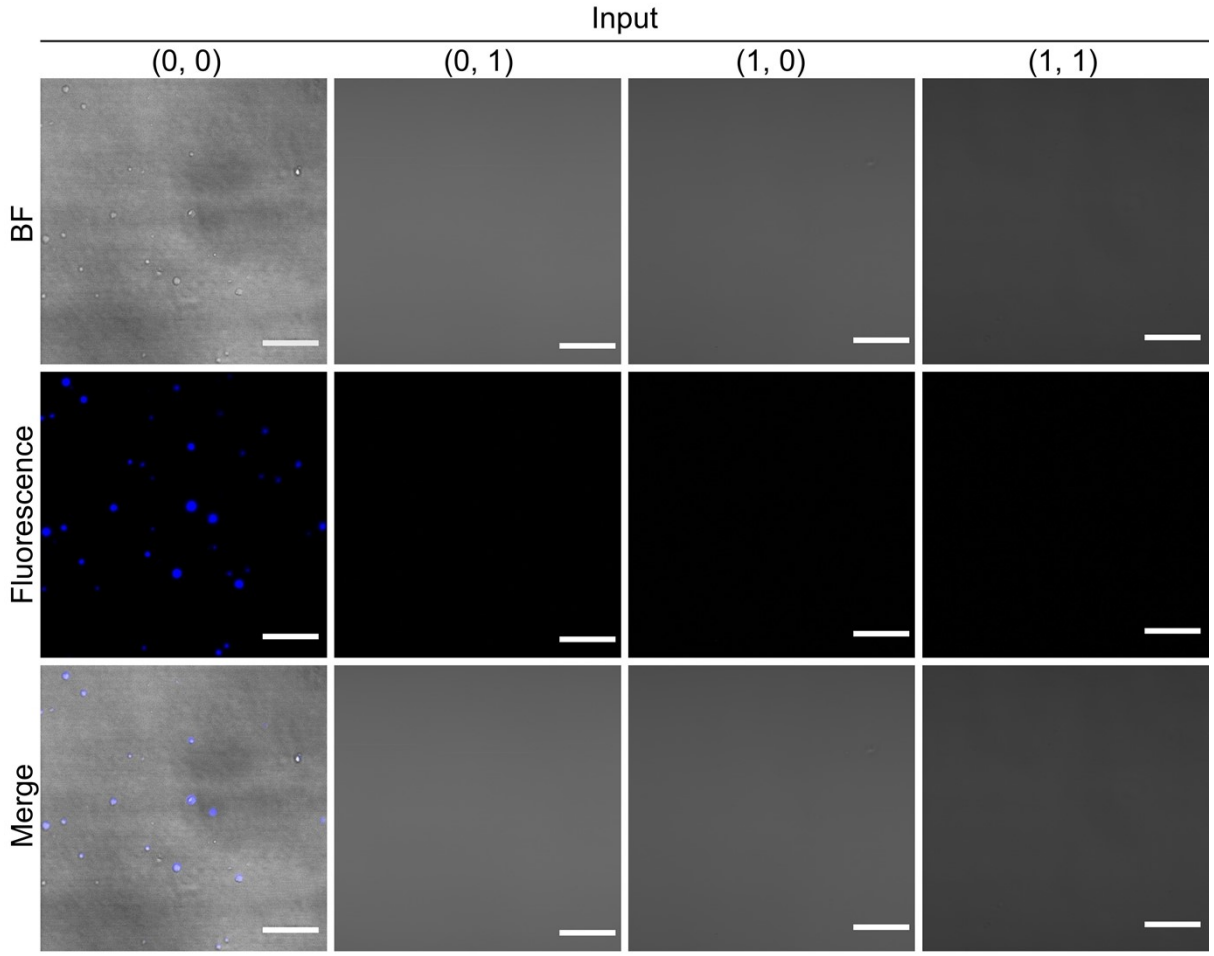


**Fig. S12**. Confocal laser scanning microscopy of 'NOR' logic operation. Scale bars, 20 μm.

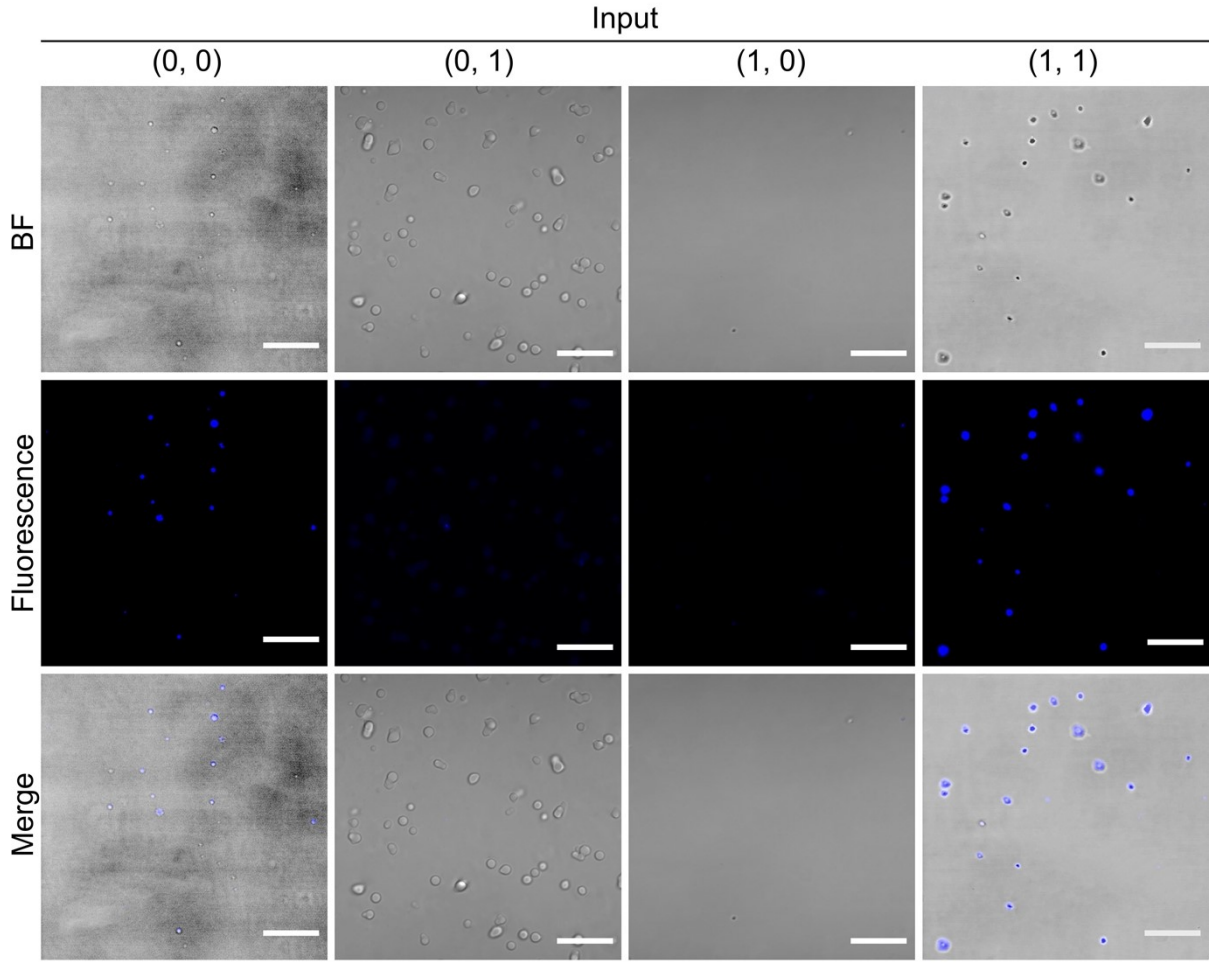


**Fig. S13**. Confocal laser scanning microscopy of 'XNOR' logic operation. Scale bars, 20 μm.

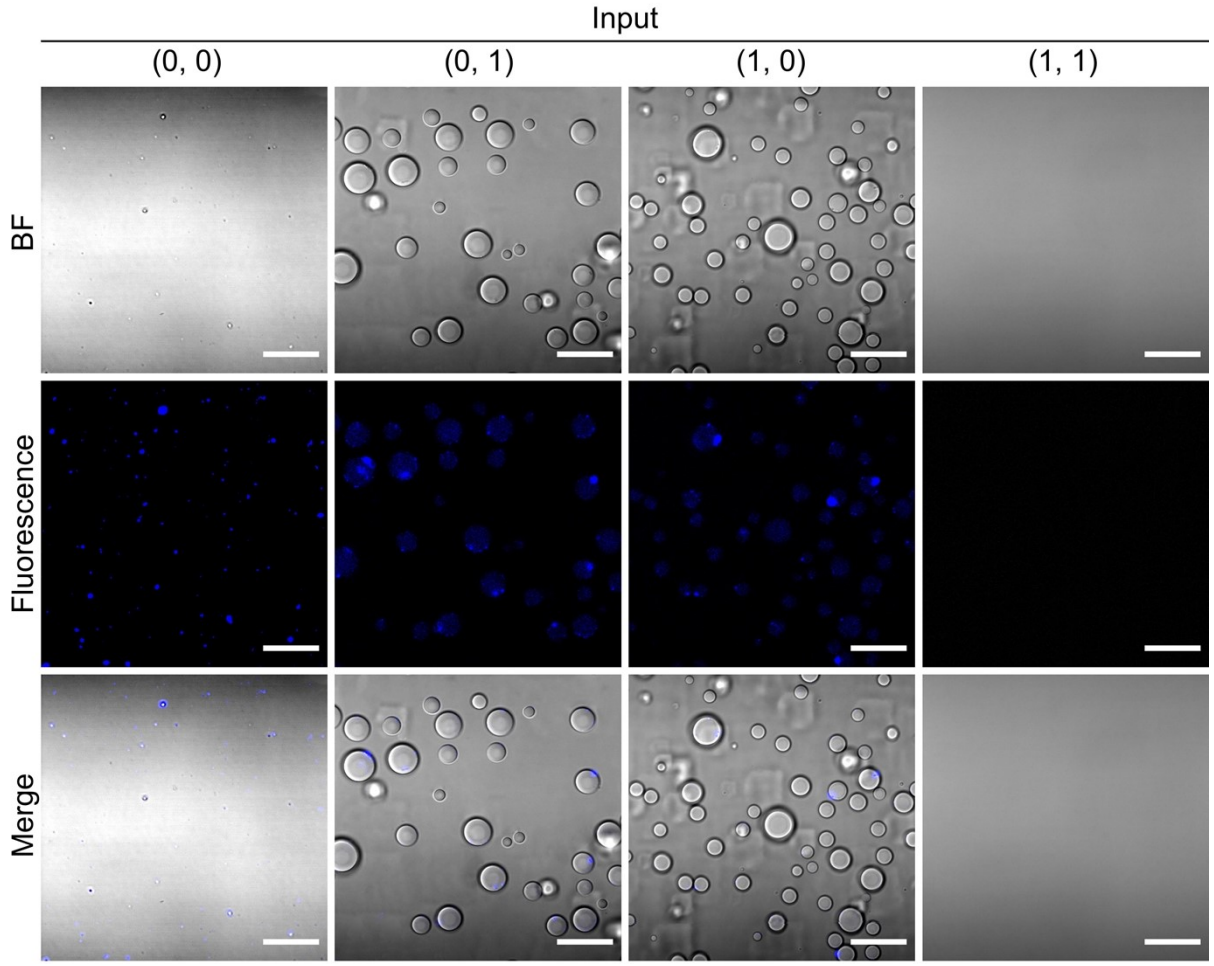


**Fig. S14**. Confocal laser scanning microscopy of 'XOR' logic operation. Scale bars, 20 μm.

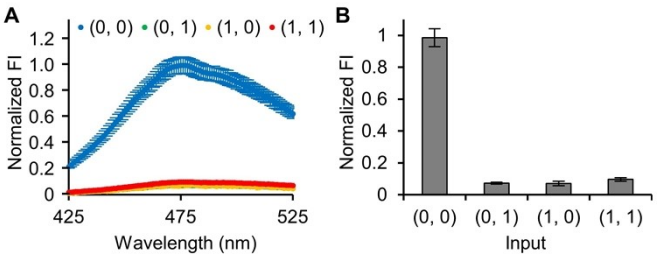


**Fig. S15**. Fluorescence measurement of 'XOR' logic operation. A) Normalized fluorescence spectra of logic gates with different inputs. B) Normalized FI at 471 nm. Excitation wavelength was set at 350 nm. n=3.

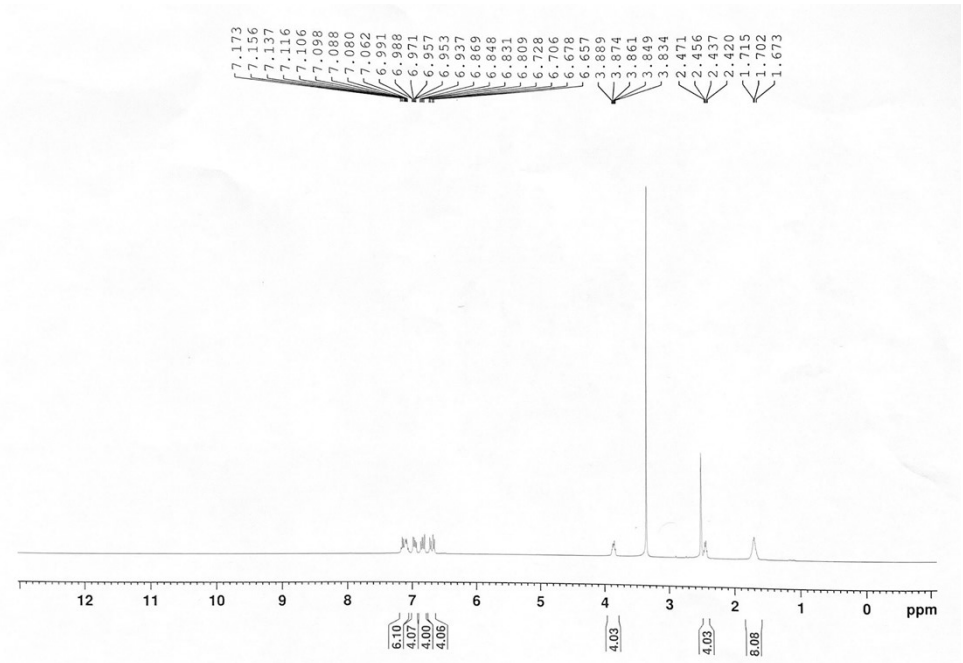


**Fig. S16**. $^{1}$H NMR of BSBOTPE.

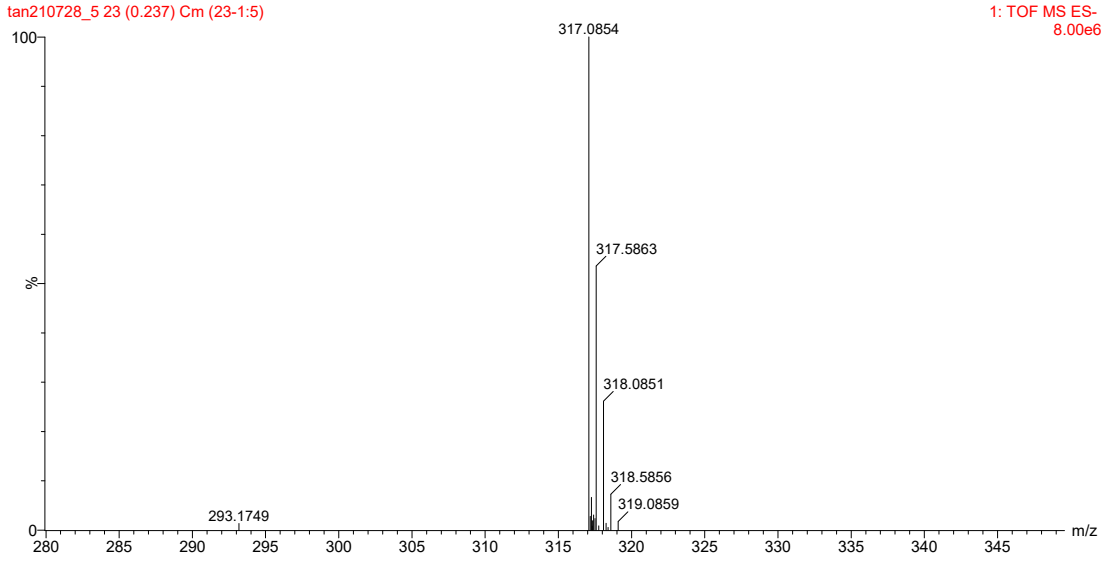


**Fig. S17**. HRMS of BSBOTPE.

## References


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4 C. M. L. Lau, G. Jahanmir and Y. Chau, *Acta Biomater.*, 2020, **101**, 219–226.