

### Supplementary Information

Visualizing dopamine released from living cells using a nanoplasmonic probe

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## Contents

1. Experimental Section .....	S2
1.1 Materials.....	S2
1.2 DFM Imaging and Scattering Spectroscopy Measurements.....	S2
1.3 DA-induced Seeded-growth of AuNPs.....	S2
1.4 Imaging and In Situ Detection of Released DA from ATP-stimulated PC12 cells.....	S2
1.5 Fluorescence Imaging of PC12 Cells Staining with Fluo Calcium Indicator.....	S2
2. Supplementary table and figures .....	S3
2.1 Table S1.....	S3
2.2 Figure S1.....	S4
2.3 Figure S2.....	S4
2.4 Figure S3.....	S5
2.5 Figure S4.....	S6

## 1. Experimental Section

### 1.1 Materials

Differentiated rat pheochromocytoma (PC12) cell line was purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China).  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ , dopamine, catechol (CT), phenethylamine (PEA), tyrosine (TR), epinephrine (EP) and norepinephrine (NE) were purchased from Sigma-Aldrich. Fluo calcium indicator, fluo-4, was purchased from Invitrogen. All other chemicals were of analytical grade and used without further purification. All solutions were prepared with Milli-Q water ( $18.2 \text{ M}\Omega \cdot \text{cm}$ ) from a Millipore system

### 1.2 DFM Imaging and Scattering Spectroscopy Measurements

The dark-field measurements were carried out on an inverted microscope (Olympus IX73, Japan) equipped with a dark-field condenser and a 60x objective lens ( $\text{NA}=0.8$ ). The sample slides are immobilized on the microscopy platform, and a 100 W halogen lamp provided white light source to excite the AuNPs to generate plasmon resonance scattering light. The scattered light was collected by a true-color digital camera (Olympus DP70, Japan) and was also splitted by a monochromator (Acton SP2300i, PI, USA) which was equipped with a grating (grating density: 300 lines/mm; blazed wavelength: 500 nm) and recorded by a spectrograph CCD (CASCADE 512B, Roper Scientific, PI, USA) to obtain the scattering spectra. LSPR spectrum of Au NPs of an individual nanoparticle during the growth process was recorded with a time-interval of 1 min.

### 1.3 DA-induced Seeded-growth of AuNPs

Au NPs with average diameter of 50 nm was synthesized according to a seed-mediated growth method with slight modification. The as-prepared Au NPs was then attached on silanized glass slides. Briefly, a microscopy slide was thoroughly rinsed with ethanol and blow-dried with  $\text{N}_2$ . Then the slide was immersed into an ethanol solution of 1% (3-aminopropyl)trimethoxysilane (V/V) for 1h, followed by successive cleaning with ethanol, blow-drying with  $\text{N}_2$  and heating at  $110 \text{ }^\circ\text{C}$  in an oven for 30 min. AuNPs was drop-cast on the silanized glass slides and then incubated for 3 min. The resulting AuNPs-modified slides were rinsed with water and dried with  $\text{N}_2$ . Zeta potential of the as-prepared Au NPs was measured with a zeta sizer (Nano-zs90, Malvern). The zeta potential was obtained as  $-36.2 \text{ mV}$ , indicating the surface of Au NPs was negatively charged. The DA-induced seeded growth of Au NPs was carried out by drop-cast a growth solution containing  $0.2 \text{ mM HAuCl}_4$  and different concentrations of DA in  $0.01 \text{ M}$  phosphate buffer saline (PBS) ( $\text{pH } 7.2$ ) on the Au NPs-modified slides.

### 1.4 Imaging and In Situ Detection of Released DA from ATP-stimulated PC12 cells

PC12 cells were cultured in DMEM, supplemented with 10% heat-inactivated FBS and antibiotics ( $100 \text{ }\mu\text{g/mL}$  of streptomycin and  $100 \text{ U/mL}$  of penicillin) at  $37 \text{ }^\circ\text{C}$  in the humidified atmosphere with 5%  $\text{CO}_2$ . The cells in a 4-well plate were transferred to a single well and incubated overnight before experiment. Then Au NPs ( $10 \text{ pM}$ ) was added to the medium and incubated for 0.5 h. Before stimulation, the suspended medium was decanted and  $1 \times \text{PBS}$  was added. The slide was then immediately mounted on microscope for experiment. Then a stimulation solution containing  $2 \text{ mM CaCl}_2$  and  $100 \text{ }\mu\text{M}$  ATP was cast on the slide. After 2 min of incubation,  $0.2 \text{ mM HAuCl}_4$  was added and allowed to reaction for 1 min. Then the reaction medium was removed, and  $1 \times \text{PBS}$  was added for dark-field image.

### 1.5 Fluorescence Imaging of PC12 Cells Staining with Fluo Calcium Indicator

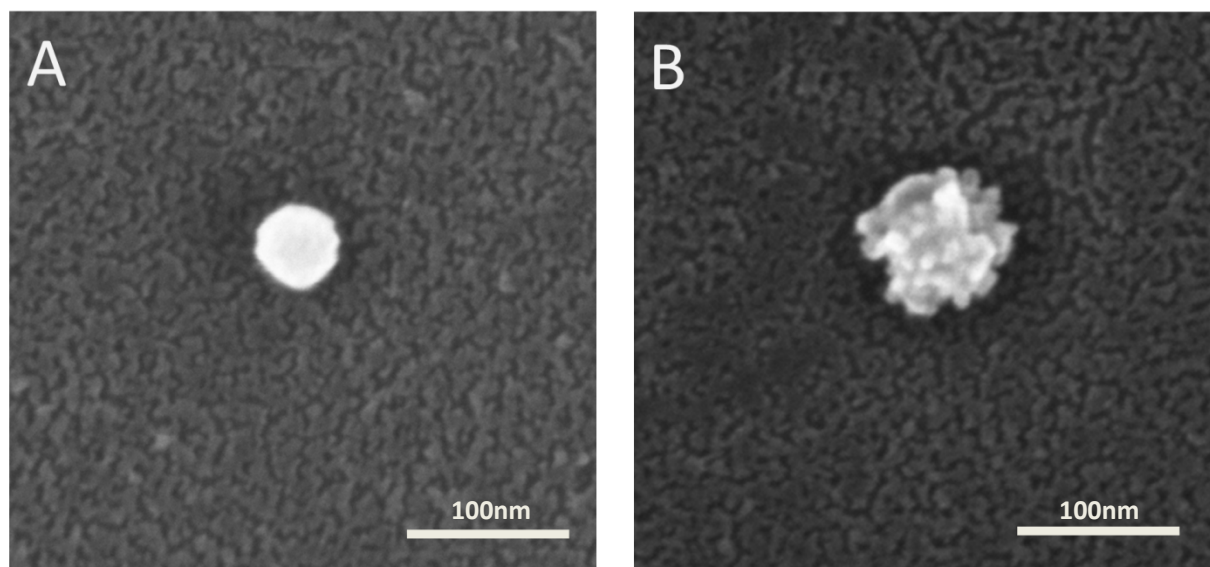
A Fluo calcium indicator, fluo-4, was used to stain PC12 cells to indicate the influx of  $\text{Ca}^{2+}$  ions. Living PC12 cells were plated onto poly-L-lysine-coated glass coverslips and incubated with fluo-4 (with a final concentration of  $5 \text{ }\mu\text{M}$ ) at  $37^\circ\text{C}$  for 30 min, and the glass coverslips were washed with  $1 \times \text{PBS}$  to remove the excess of fluo-4. Then the fluorescence images of PC12 cells before and after ATP stimulation were recorded with a confocal laser scanning microscopy (Leica, TCS SP5) in  $1 \times \text{PBS}$  medium. The fluorescent signals from  $\text{Ca}^{2+}$  binding to fluo-4 were recorded at  $516 \text{ nm}$  with excitation of  $488 \text{ nm}$ . Captured images were off-line analyzed with Image J.

## 2. Supplementary table and figures

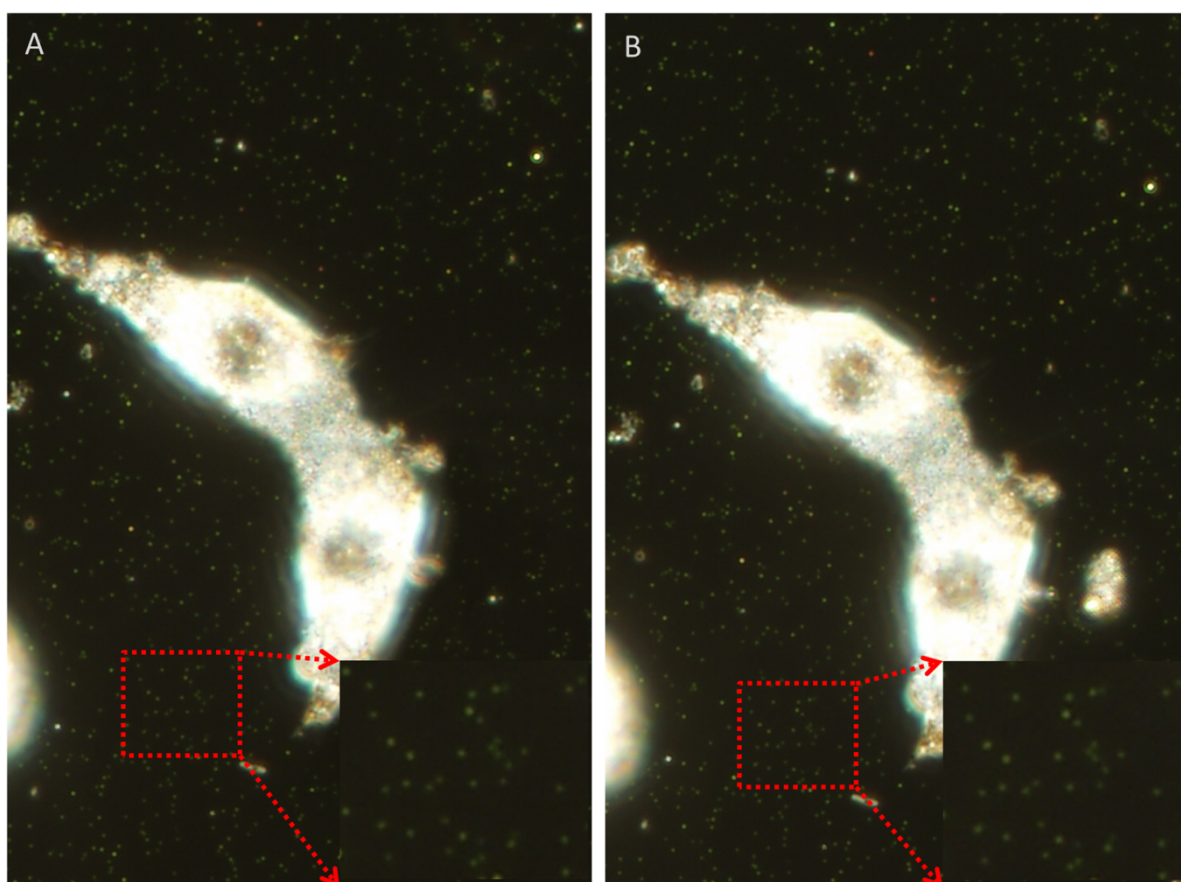
2.1 Table S1. Comparison of the detection limits of various DA biosensors

Biosensing technique	Materials	Linear working Range(M)	Detection limit(M)	Ref.
Field-effect transistor	SiNW	$10^{-11} - 10^{-8}$	$10^{-11}$	[S1] <sup>1</sup>
Colorimetric detection	MBA-DSP-AuNPs	$10^{-9} - 10^{-7}$	$10^{-9}$	[S2] <sup>2</sup>
	AHMP-AuNPs	$10^{-6} - 10^{-5}$	$10^{-8}$	[S3] <sup>3</sup>
	HAuCl <sub>4</sub>	$10^{-6} - 10^{-5}$	$10^{-6}$	[S4] <sup>4</sup>
Fluorescence detection		$10^{-7} - 10^{-5}$	$10^{-8}$	[S5] <sup>5</sup>
Electrochemiluminescence detection	CdSe	$10^{-8} - 10^{-6}$	$10^{-9}$	[S6] <sup>6</sup>
Electrochemical detection	Hydrogenated carbon surface	$10^{-10} - 10^{-9}$	$10^{-10}$	[S7] <sup>7</sup>
	PET	$10^{-7} - 10^{-4}$	$10^{-8}$	[S8] <sup>8</sup>
	GO	$10^{-7} - 10^{-4}$	$10^{-8}$	[S9] <sup>9</sup>
	Overoxidized Plmox and GO	$10^{-5} - 10^{-4}$	$10^{-7}$	[S10] <sup>10</sup>
	Graphene	$10^{-8} - 10^{-5}$	$10^{-8}$	[S11] <sup>11</sup>
	Glassy carbon	$10^{-7} - 10^{-5}$	$10^{-7}$	[S12] <sup>12</sup>
	AuNPs	$10^{-6} - 10^{-5}$	$10^{-6}$	[S13] <sup>13</sup>
	OPPD-coated carbon fiber	$10^{-8} - 10^{-5}$	$10^{-8}$	[S14] <sup>14</sup>
	Au/ITO	$10^{-9} - 10^{-4}$	$10^{-9}$	[S15] <sup>15</sup>
	AuNPs/PMT	$10^{-8} - 10^{-6}$	$10^{-8}$	[S16] <sup>16</sup>
Plasmonic detection	AuNPs	$10^{-12} - 10^{-6}$	$10^{-13}$	This work

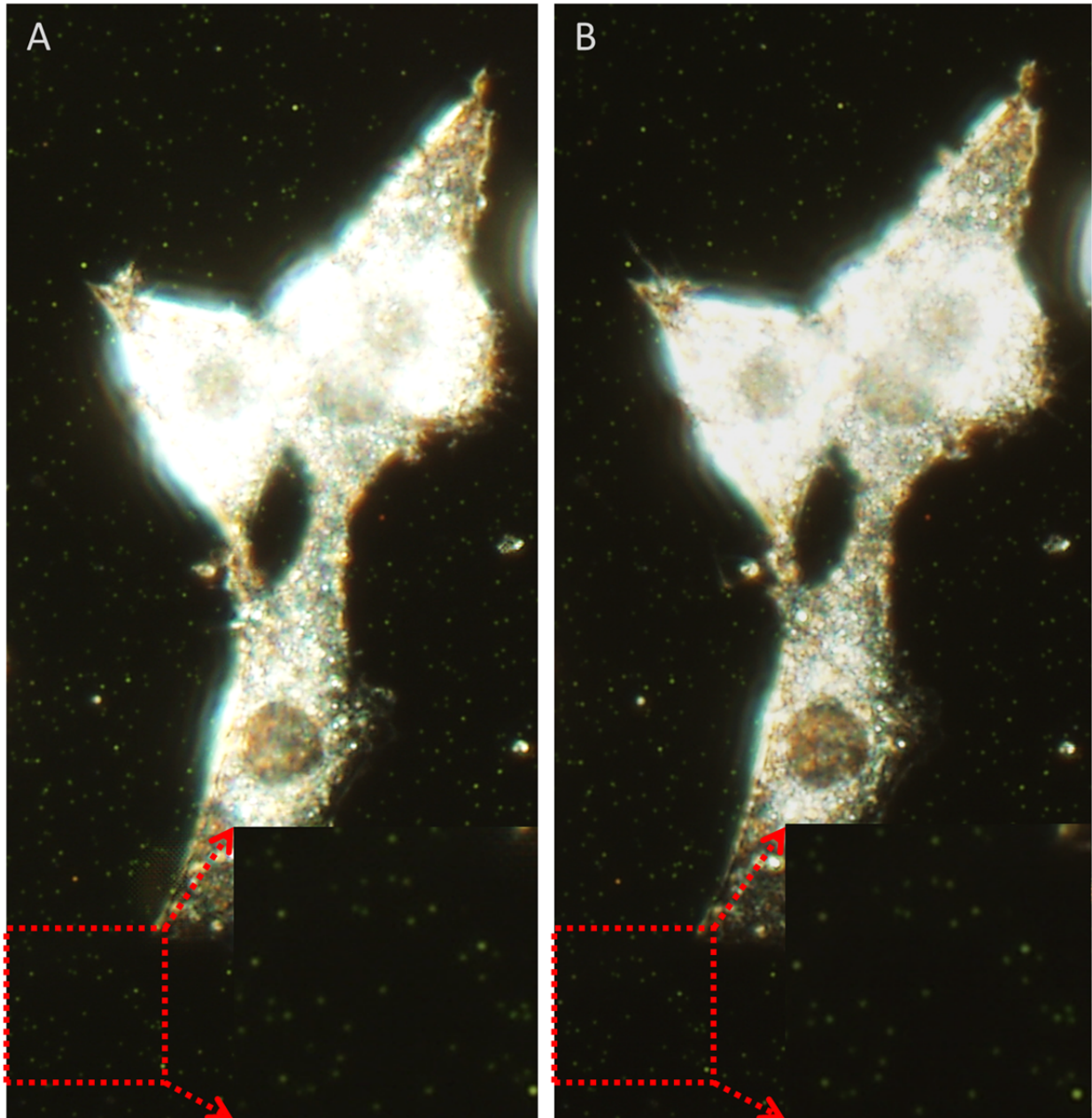
2.2 Figure S1. SEM images of Au NPs before (A) and after (B) enlargement by DA.



2.3 Figure S2. (A) Dark-field images of the nanoplasmonic probes incubated with living PC12 cells (B) Dark-field images of the nanoplasmonic probes incubated with living PC12 cells after the addition of 0.2 mM HAuCl<sub>4</sub> in the presence of 2 mM CaCl<sub>2</sub> without ATP stimulation. Insets of (A) and (B) are magnified dark-field images of the representative areas in the red square respectively.

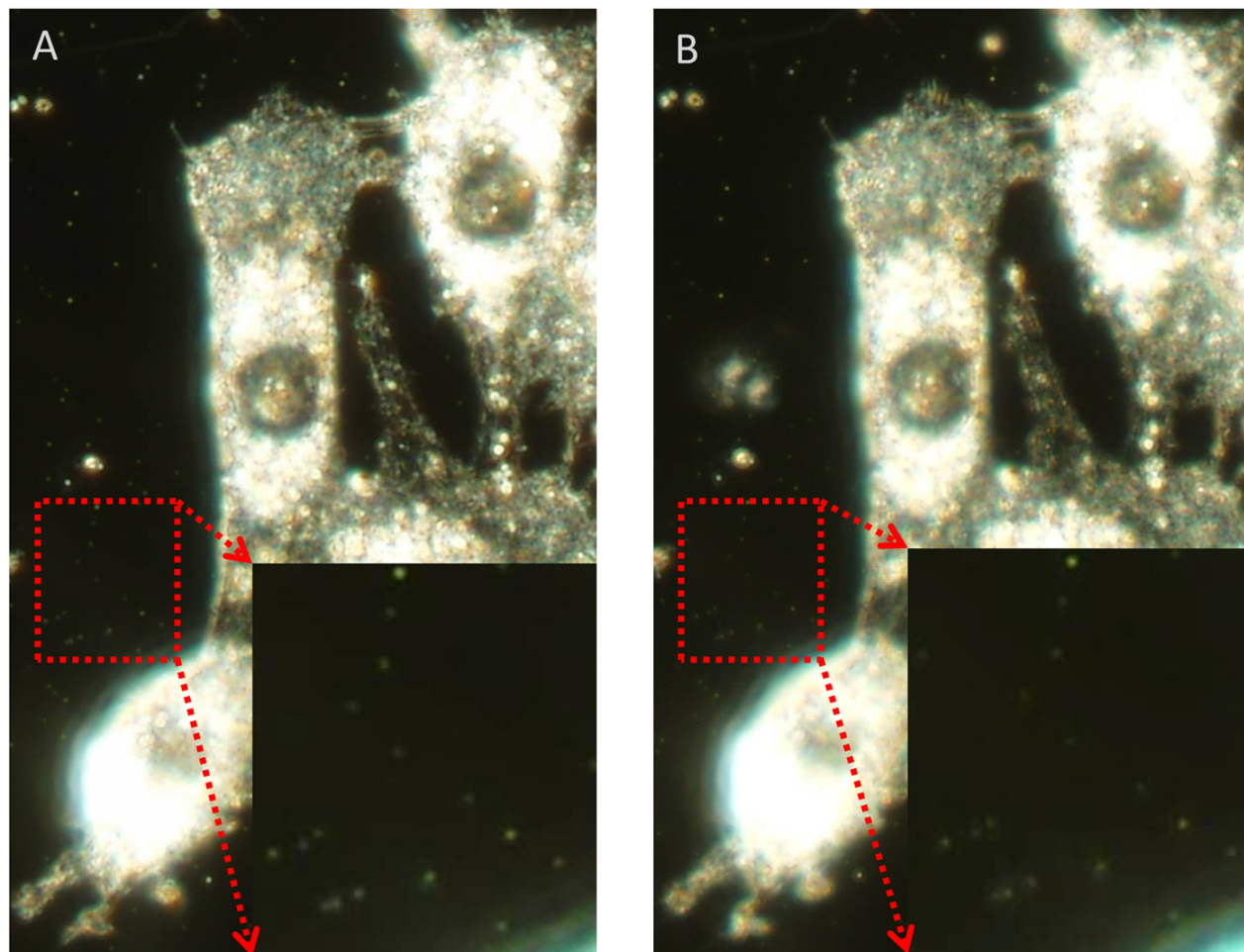


2.4 Figure S3. (A) Dark-field images of the nanoplasmonic probes incubated with living PC12 cells (B) Dark-field images of the nanoplasmonic probes incubated with living PC12 cells after ATP stimulation and the addition of 0.2 mM  $\text{HAuCl}_4$  in the absence of 2 mM  $\text{CaCl}_2$ . Insets of (A) and (B) are magnified dark-field images of the representative areas in the red square respectively.





2.5 Figure S4. (A) Dark-field images of the nanoplasmonic probes incubated with living PC12 cells (B) Dark-field images of the nanoplasmonic probes incubated with living PC12 cells after ATP stimulation in the presence of 2 mM CaCl<sub>2</sub> without the addition of 0.2 mM HAuCl<sub>4</sub>. Insets of (A) and (B) are magnified dark-field images of the representative areas in the red square respectively.



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