SUPPORTING INFORMATION

Gold Nanoparticle-mediated signal amplification of Liquid Crystal biosensors for dopamine

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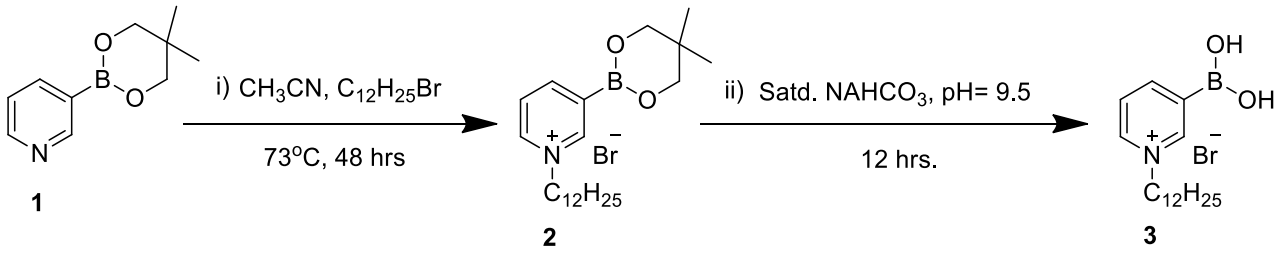
1. Experimental Details

1.1 Materials

N,N-Dimethyl-N-octadecyl-3-aminopropyltrimethoxysilyl chloride (DMOAP), 5CB, 3-Pyridinylboronic acid, Gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), sodium citrate, dopamine (DA), dithiobis [succinimidylpropionate] (DSP), ascorbic acid, glucose, epinephrine, norepinephrine and PBS buffered saline (pH 7.4) were obtained from Sigma Aldrich, India. Tyramine and Serotonin were purchased from TCI chemicals, India. Sulfuric acid and hydrogen peroxide (30% w/v) were purchased from Merck. Ethanol was obtained from Jebsen & Jenssen GmbH and Co., Germany. Fischer's Finest Premium Grade glass slides were obtained from Fischer Scientific. Deionization of a distilled water source was performed using a Milli-Q-system (Millipore, bedford, MA). Gold specimen grids (20 µm thickness, 50 µm wide bars, 282 µm grid spacing) were obtained from Electron Microscopy Sciences (Fort Washington, PA).

1.2 Synthesis of N-dodecyl pyridinium 3-boronic acid (3NPBA)



Procedure: The amphiphile compound **3** (3NPBA) was synthesized according to modified reported in published literature.[1,2] A mixture of compound **1** (500 mg, 1 eq.) and 1-bromododecane (1304.47 mg, 2 eq.) in acetonitrile (30 mL) was refluxed for 48 h under N_2. After that, acetonitrile was removed in vacuo to afford 3-borono-N-dodecylpyridinium bromide neopentyl glycol ester as yellow liquid in quantitative yield. The resulting yellow liquid obtained was washed with dry ether to remove unreacted starting materials. Then the yellow liquid was dissolved in dichloromethane followed by aqueous solution of sodium bicarbonate (9:1). The pH of the water phase was maintained at 9.5. After stirring for 12 hours at room temperature, the organic phase was decanted and the water layer was washed with an excess of dichloromethane. Organic solvent was removed in vacuo, and final product (**3**) was recrystallized from chloroform/hexane.

3-borono-1-dodecylpyridin-1-ium bromide (3):

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.05-9.03 (d, 2H, J = 5.96), 8.63 (t, 1H, J = 7.78.), 8.15 (t, 1H, J = 6.74), 4.66 (t, 2H, J = 7.58), 2.05 (m, 2H), 1.41-1.31 (m, 18H), 0.92 (m, 3H).

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 145.09, 145.06, 144.99, 128.41, 128.40, 70.86, 62.29, 32.01, 31.90, 29.58, 29.50, 29.35, 29.33, 29.06, 26.08, 22.69, 21.43, 14.14.

IR (Neat, KBr, ν_{max}/cm^{-1}): 3407.8, 3054.9, 2924.6, 2854.4, 1612.1, 1587.4, 1571.4, 1480.3, 1419.5, 1365.2, 1174.4, 1095.5, 1056.1.

ESI-MS (m/z): M^- 370.1565 (calcd. for $C_{17}H_{31}BBrNO_2$ = 370.1552).

1.3 Preparation of DMOAP coated of glass substrates

First, glass microscope slides were cleaned using 'piranha' solution [70:30 (% v/v) H_2SO_4: H_2O_2(30%)].[3] Briefly, the glass slides were immersed in a piranha bath at 100°C for 1 h and then rinsed in running deionized (DI) water for 15 min. Further, the

slides were rinsed thrice sequentially in ethanol and dried under a stream of nitrogen. The cleaned slides were stored in an oven at 100 °C for 12 h. The cleaned glass slides were dipped into 0.1% (v/v) DMOAP solution in DI water for 5 min at room temperature and were then rinsed with DI water to remove unreacted DMOAP from the surface following earlier report.[4] The DMOAP coated glass slides were dried under a stream of nitrogen gas and kept in oven at 100 ºC for 4 h to allow crosslinking of DMOAP.

1.4 Preparation of optical cells

The DMOAP coated glass slides were cut into squares (1cm x 1cm) for sensing experiment as described elsewhere.[4] Then, a gold TEM grid was placed on the slide, and approximately 0.3 µL of 5CB was dispensed onto the grid. Excess amount of LC on the grid was removed by using a capillary tube. Then LC filled TEM grid on DMOAP coated slide are immersed into 2 mL aqueous solution of interest.

1.5 Optical characterization with microscope

The orientational ordering of the LC was determined using a conventional Zeiss polarizing microscope Scope.A1 with crossed polars with 5X objective. All images were captured with an exposure time of 80 ms at pixel dimension of 2056 x 2056 and a shutter speed of 1/10 s. Textures were quantified by interpreting them through gray scale of intensities using image processing software Adobe Photoshop.

1.6 Preparation of gold nanoparticles

All glassware was thoroughly cleaned with freshly prepared aqua regia (HCl: HNO_3 =3:1), rinsed thoroughly in water and dried in air. GNPs with a diameter of 13 nm were synthesized as reported previously.[5] Briefly, 5 mL of 38.8 mM trisodium citrate was added into 50 mL of 1 mM $HAuCl_4$ boiling solution and the resulting solution was then kept continuously boiling for 30 min until a red solution was obtained. The prepared NNP solution was cooled to room temperature and then stored in a refrigerator at 4 °C for use. Modification of GNPs through the ligand-exchange reaction was performed at room temperature for 12 h by mixing a 10 mL of the as-prepared AuNPs with a 20 µl of aqueous solution of 2.5 mM DSP.

1.7 Detection of Dopamine

Different concentration of dopamine in phosphate buffer solution (pH 7.4, 10 mM) was added to the 3NPBA solution and incubated for 10 min at room temperature. Next, the DSP functionalized GNPs was introduced and incubated for 15 min. Then a LC filled TEM grid on DMOAP coated slide were immersed in the solution and LC micrograph were captured after 20 min stabilization of LC-aqueous interface.

1.8 Details of Raman spectroscopy measurement

The Raman spectra and SERS spectra were recorded with inVia Raman spectrometer from Renishaw, UK equipped with a 514.5 nm line of an Ar^+ laser, 1800 lines/mm grating and a thermoelectrically cooled charge-coupled device (CCD) detector. Before recording the spectra from the sample, the calibration of the system was checked with the 520.5 cm^{-1} line using silicon reference. The 5 mW power of 514.5 nm laser on the sample was used with acquisition time for each spectrum was 60 sec per window with accumulation of 1 scan. The incident laser beam has been focussed on the sample by a 50x short distance objective (1 μm^2 spot) attached to the Leica DM 2500M microscope. The Raman scattered light was collected in back-scattering geometry by the same objective and slit width of 50 μm has been maintained throughout the measurement. The resolution of the spectrometer is better than 1 cm^{-1} with a repeat deviation less than ±0.2 cm^{-1}. Spectrometer scanning, data collection and processing were done by a dedicated computer using Wire 4.0 software.

It is noted that GNPs with different diameters have unequal SERS enhancement effects, and maximized enhancement is achieved with GNPs of 30-80 nm.[6] However, GNPs of the size always require complicated synthesis and exhibit decreased stability. To increase enhancement of SERS signal, we have prepared 30 nm GNP and unable to functionalized with DSP due to aggregation of GNPs. In our assay, GNPs of 13 nm can be prepared easily with higher stability and desirable mono dispersity. Also DSP functionalization of 13 nm GNP can be done easily.

1.9 Details of UV-Vis instrument and dynamic light scattering experiment

The UV-Vis spectra of the compounds were recorded using Cary 500 UV-Vis-NIR spectrometer (Version 2.23). The size of nanoparticles was measured using Malvern Zetasizer Nano ZS90 instrument, Southborough, Massachusetts.

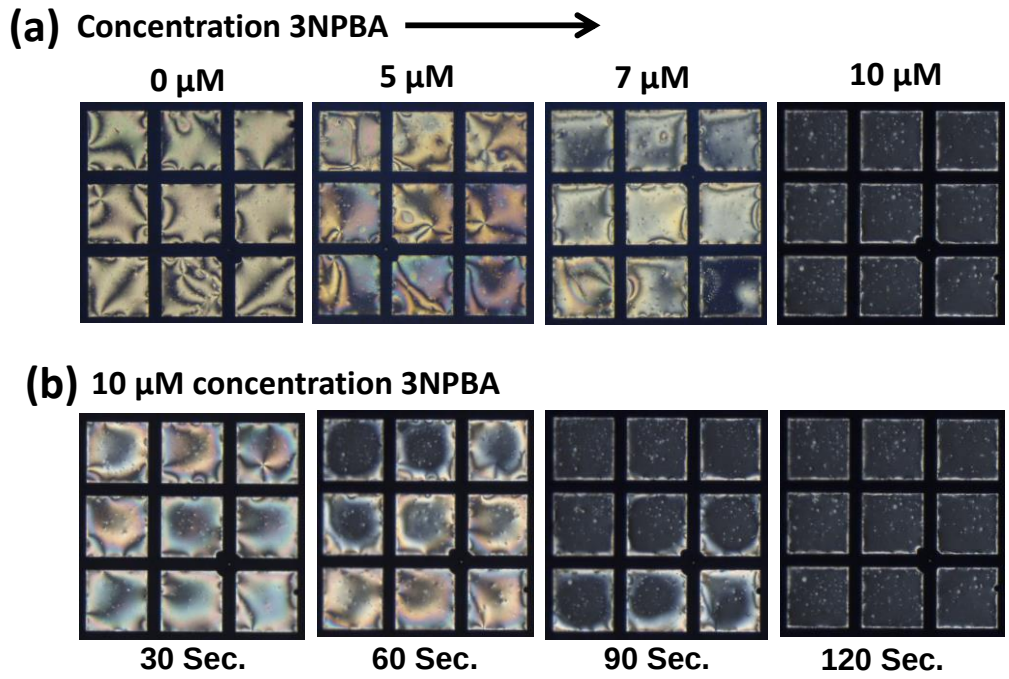


Figure S1. (a) Cross-polarized optical images of 5CB when exposed to different concentrations of 3NPBA aqueous solution. Images are taken after 5 min of stabilization of LC-aqueous interface. (b) Time lapse orientation transition of 5CB from planar (bright image) to homeotropic (black image) in contact with 10 µM of 3NPBA aqueous solution (282 µm square grid size; 20 µm depth).

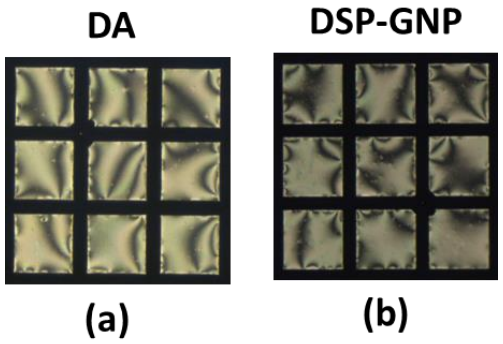


Figure S2. POM images of 5CB at LC-aqueous interface in contact with (a) dopamine of 1 mM concentration and (b) 0.17 nM concentration of DSP-GNPs. The images are taken after 20 minutes of stabilization of LC-aqueous interface (282 µm square grid size; 20 µm depth).

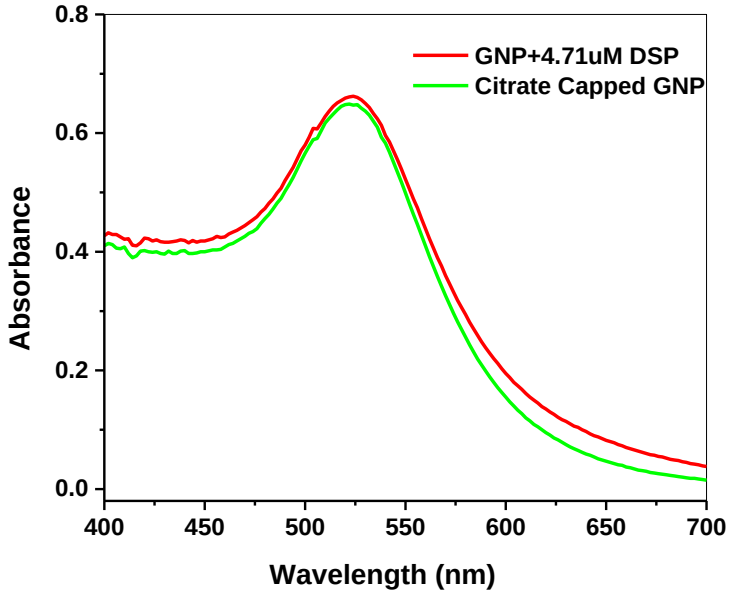


Figure S3. UV-Vis absorption spectra of 13 nm diameter GNPs (green) and DSP functionalized GNPs (DSP-GNPs) (red). The concentration of the GNPs were determined from its absorbance (ε = 2.7 x 10^8 M^{-1} cm^{-1} at λ=520 nm).

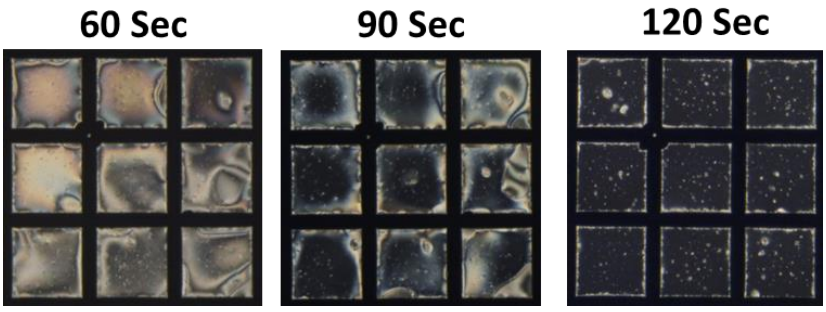


Figure S4. Time lapse orientational transition of 5CB from planar (bright image) to homeotropic (black image) in contact with 10 µM of 3NPBA and 0.17 nM of DSP-GNP aqueous solution (282 µm square grid size; 20 µm depth).

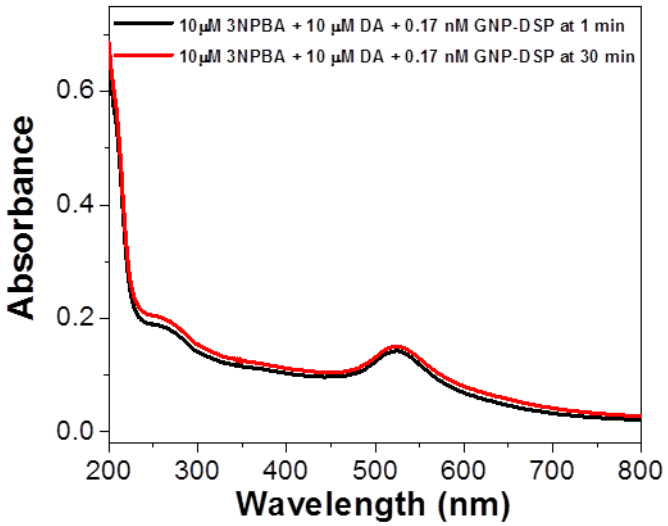


Figure S5. UV-vis absorption spectra of 0.17 nM concentration of DSP-GNPs in addition of 10 µM 3NPBA and 10 µM DA at different time. No significant changes observed in the colour as well as in surface Plasmon peak of GNPs.

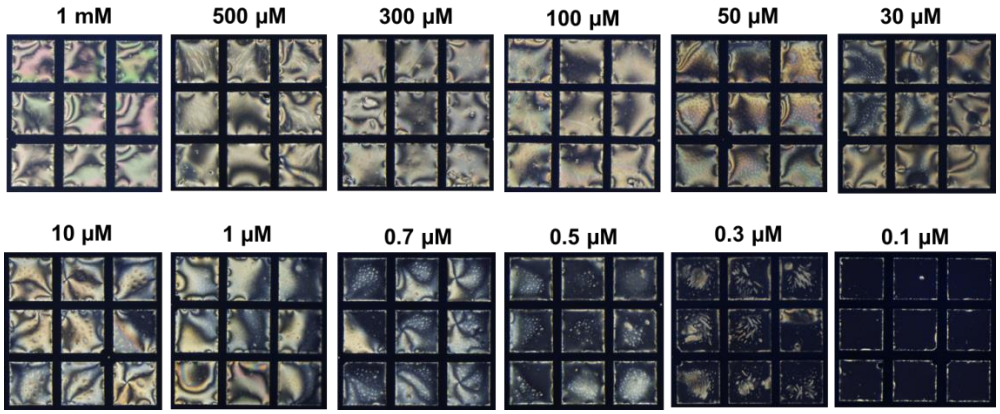


Figure S6. Polarized image of LCs immersed in the aqueous solution containing different concentration of dopamine with 10 µM of 3NPBA and 0.17 nM of DSP-GNP (282 µm square grid size; 20 µm depth). The images are taken after 20 minutes of stabilization of LC-aqueous interface.

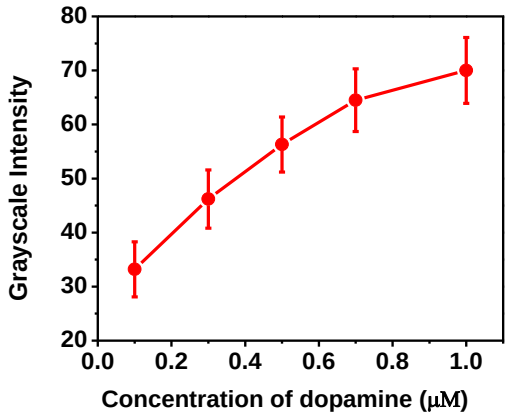


Figure S7. Grayscale intensity plot of dopamine concentration range (0.1- 1.0 μM) with 3NPBA and DSP-GNP.

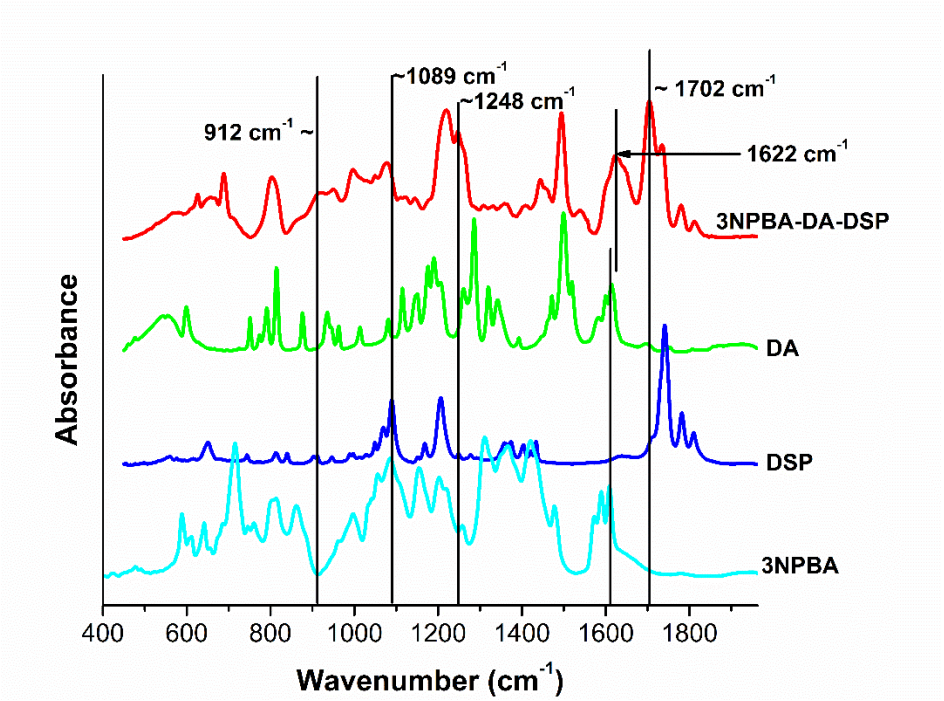


Figure S8. Comparison of FTIR spectra of 3NPBA, Dopamine (DA), DSP and their product molecule due to binding between 3NPBA, DSP and dopamine (3NPBA-DA-DSP) prepared in methanol solvent. The vibrational assignments of some bands are given in Table S1. The new band at 1702 cm^{-1} of product molecule is due to amide C=O stretching band, indicates formation of amide bond due to binding DSP and dopamine (DA). The band at 1248 cm^{-1} of product molecule is due to C-O stretching band of new five membered ring formation.

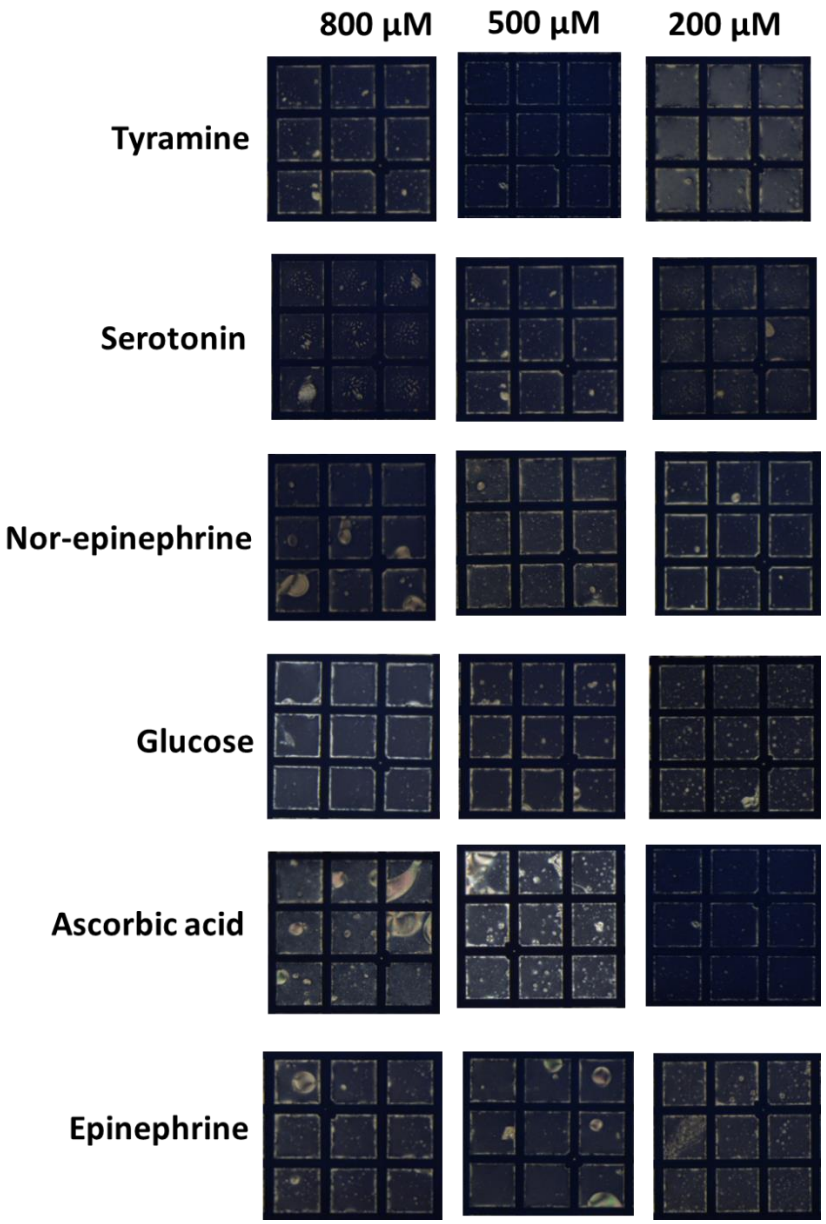


Figure S9. POM images of 5CB at LC-aqueous interface in contact with 0.17 nM concentration of DSP-GNPs and 10 µM of 3NPBA with different structurally similar molecules at different concentrations (282 µm square grid size; 20 µm depth). The images are taken after 20 minutes of stabilization of LC-aqueous interface.

Table S1. Tentative Vibrational assignment of dopamine[7]

3NPBA	DA	DSP	Dopamine–3NPBA complex	Dopamine-3NPBA-DSP Complex	Vibrational assignment
-	-	1742	-	1702	C=O stretching
1609	1612	-	1622	1622	C=C stretching of phenyl ring
1590	1600				
1570	1582				
-	-	-	1246	1248	C-O stretching of new five membered ring
-	-	1089		1079	C-S stretching
-	-	-	912	912	Ring deformation of new five membered ring

Table S2. Average particle size of gold nanoparticles

Solution	Average size of the particles (nm)
GNP	12.33 ± 0.9
DSP-GNP	12.51 ± 1.1
DSP-GNP + 3NPBA	12.64 ± 1.2

Table S3. The comparison of dynamic range and detection limit of dopamine.

Method	Dynamic range	Detection limit	Reference
Colorimetric	0.5-3.8 nM	0.05 nM	8
Fluorescence	15-50 µM	15 µM	9
Electrochemical	80–400 µM	9.6 µM	10
Liquid crystal	0.3-500 µM	0.3 µM	This work

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