

Supporting Information

Bio-inspired Strategy for Controlled Dopamine Polymerization in Basic Solutions

*Xin Du, Linxian Li, Farid Behboodi-Sadabad, Alexander Welle, Junsheng Li, Stefan Heissler, Huijie Zhang, Nicolas Plumeré, Pavel A. Levkin**

Experimental Section

Dopamine hydrochloride, mercaptoethanol, pyrogallol, were purchased from Sigma-Aldrich (Germany). Sodium ascorbate (SA), Na₂S₂O₃, formaldehyde, acetone and the other solvents were obtained from Merck KGaA (Germany). The glass plates used in the experiment were Nexterion B glass from Schott AG (Germany). Silicon wafers (CZ-Si-wafer 4 inch) were obtained from MicroChem GmbH (Germany). Gold substrate was obtained by sputtering 100 nm thick gold layer on the silicon wafer. An OAI model 30 deep-UV collimated light source (San Jose, CA, USA) fitted with a 500W HgXe lamp was used for UV irradiation. The lamp was calibrated to 7.5 mW cm⁻² at 260 nm with the OAI 306 UV power meter. For the electrochemistry tests, a neoLab-UV Inspection Lamp Type 6 with 14 mW cm⁻² at 365 nm was used for UV irradiation. UV-Vis spectroscopy was performed with a HR2000+ high resolution spectrometer (Ocean Optics Inc., USA) with DH-2000-BAL light source (Mikropack GmbH, Germany).

AFM measurements were taken in the Institute of Nanotechnology (INT) at the Karlsruhe Institute of Technology (KIT). Our data were recorded by a Bruker Dimension Icon AFM, using a cantilever with platinum silicide probe (NanoAndMore GmbH).

UV-Vis spectrometry measurement

Time dependent UV-Vis absorbance measurement: dopamine (2 mg/mL) Tris buffer solution (10 mM, pH 8.5, with 0%/0.1%/0.2%/0.4%/1% wt. SA with respect to dopamine) was filled into cuvettes and placed in a dark environment for 2h, UV-Vis absorption spectra (300-900 nm, air as reference) were taken at the following time points: 1 min, 2 min, 5 min, 10 min, 15 min, 30 min, 60 min, 90 min and 120 min. The same experiments were also done under UV irradiation. The same process was also applied on dopamine solutions at pH 7.4 and pH 9.5. For the 0%, 0.4% and 1% wt. test, Na₂S₂O₃, formaldehyde, mercaptoethanol and pyrogallol were also tested.

UV-Dark ON-OFF experiment: dopamine solution (2 mg/mL, Tris buffer pH 8.5, 10 mM, with 1% wt. SA with respect to dopamine) was kept in the dark for 30 min before recording the solution's UV-Vis absorption spectra. After UV irradiation, the solution was placed in the dark and the solution's UV-Vis spectrum was recorded every 30 min for 3h. Similar experiments were done on dopamine solutions with 2 min UV irradiation at different time points (30 min, 60 min, 90 min, 120 min) and the UV-Vis spectra of the solutions were recorded every 30 min.

Electroanalytical methods

Electrochemical experiments were carried out by using a Reference 600 potentiostat (Gamry Instruments, Warminster, PA). All electrochemical measurements were performed in a three-electrode cell with a coiled Pt wire as the counter electrode, a Ag/AgCl (3M KCl) reference electrode.

The working electrodes for the monitoring of SA by cyclic voltammetry (CV) measurements were 3 mm GCEs (BASi, West Lafayette, IN). The electrodes were polished in alumina slurry with 1 μm , 0.3 μm and 0.05 μm size and rinsed with deionized water after ultrasonic 5min in the mixture of EtOH and water.

The working electrodes for the monitoring of SA in SA-DA mixtures by differential pulse voltammetry (DPV) measurements were specifically activated glassy carbon electrodes prepared as described in the literature.¹ The low buffer concentration does not provide sufficient ionic strength for the DPV measurements. Therefore, 0.1 M KCl was added to the solution before measurement. The DPV was measured from -0.2V to 0.2V with 25 mV pulse size and the frequency is 5 Hz.

Long-term control experiment

5 mL dopamine solution (2 mg/mL, in 10 mM Tris buffer pH 8.5, with 5% wt. SA with respect to dopamine) was added into a 20 mL glass vial and the vial was sealed by a plastic cap. We placed three such vials in the dark for 30h and took photos at the time points of 10h, 24h, 30h to check the condition of dopamine polymerization. For two of the vials, 6 min UV irradiation was applied at different time points. A dopamine solution without SA was used as the control sample.

Experiment on PD deposition

Polydopamine deposition on glass and gold substrates: 20 mL dopamine solution (2 mg/mL, in 10 mM Tris buffer pH 8.5, with 1% wt SA with respect to dopamine) was added into a Petri dish and the glass or gold substrate was immersed in the solution.

The coating procedure was allowed to take 24h, and then the substrate was washed

with water and acetone, and dried by nitrogen. For UV-SA assisted deposition, 3% wt. SA (with respect to dopamine) was added in the buffer solution. After the addition of dopamine and glass/gold substrate, dopamine polymerization was inhibited for 6h, followed by 5 min UV irradiation to consume the SA and start the polymerization. The PD deposition was allowed to take 24h and then the substrate was washed with water and acetone, and dried by nitrogen. To pattern on the glass surface, we used the same setup as in our previous report.^[2] After filling the dopamine solution (10 mg/mL, in 50 mM Tris buffer pH 8.5, containing 1% wt. SA according to dopamine), a photomask was placed onto the cover glass and the sample was irradiated for 30 min. Then the device was opened and the sample was rinsed with water and acetone and dried with nitrogen. To compare UV-SA method with our previous reported method, PD surface with 2h UV irradiation were prepared for both methods (pH 8.5 with 1% wt SA with respect to dopamine, or pH 6.0), and the PD surfaces were tested with AFM.

Polydopamine deposition with stirring: 80 mL 2 mg/mL dopamine solution (10 mM Tris buffer pH 8.5, with 1% wt. SA with respect to dopamine) was added to a 100 mL beaker, a glass slide was perpendicular immersed into the solution and a magnetic stirrer was added to trigger the stirring (300 rpm). After 3h the beaker was irradiated under UV for 6 min and then placed in dark again. The whole coating process was allowed to last for 24h. Then the glass slide was washed with water and acetone and dried. A sample without SA and UV irradiation (standard process) was prepared as comparasion.

Secondary modification on PD: For secondary modification of PD surface by dodecanethiol, a PD coated glass slide was added to the modification solution (30 mL DCM, 200 μ L dodecanethiol, 50 μ L Triethylamine) in a 50 mL Falcon tube, the tube was then placed on a shaker for 24h, and washed with water and acetone and dried. For the secondary modification by AgNO₃, the patterned substrates were immersed in a 1 mg/mL AgNO₃ aqueous solution for 24h, followed by washing with water and acetone and drying with nitrogen.

Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) measurement: Images were obtained with TOF-SIMS 5 machine (ION-TOF GmbH, Münster, Germany). The analysis chamber was held at $\sim 8 \cdot 10^{-9}$ mbar during the experiment. A pulse of 25 keV Bi₁⁺ primary ion beam was used for all image and spectral data acquisition. All data were collected in high mass resolution bunched mode. Care was taken to use a new sample area for each analysis.

SEM test on PD surfaces: SEM images were obtained using the LEO 1530 Gemini scanning electron microscope (Zeiss, Germany) at the Institute of Nanotechnology (INT), KIT. The samples were sputtered with a 30 nm gold layer using a Cressington 108 auto sputter coater (INT, KIT) before measuring.

Infrared Reflection-Absorption Spectroscopy (IRRAS) measurement: The IR absorption spectra were obtained using a Bruker VERTEX 80 FTIR spectrometer (Bruker Optics, Ettlingen, Germany), equipped with a liquid nitrogen cooled mercury cadmium telluride (MCT) detector and a grazing incidence external reflection accessory, at an 80° incidence angle relative to the surface with a spectral resolution

of 2 cm^{-1} . The PD layers were deposited on gold substrates. Perdeuterated hexadecanethiol SAMs on a gold substrate was used as reference. Dry air was purged continuously through the spectrometer and the sample compartment. Samples were measured when the water absorption bands from ambient air disappeared. The data were processed using the Bruker OPUS[®] software version 7.2.

Supplementary Figures

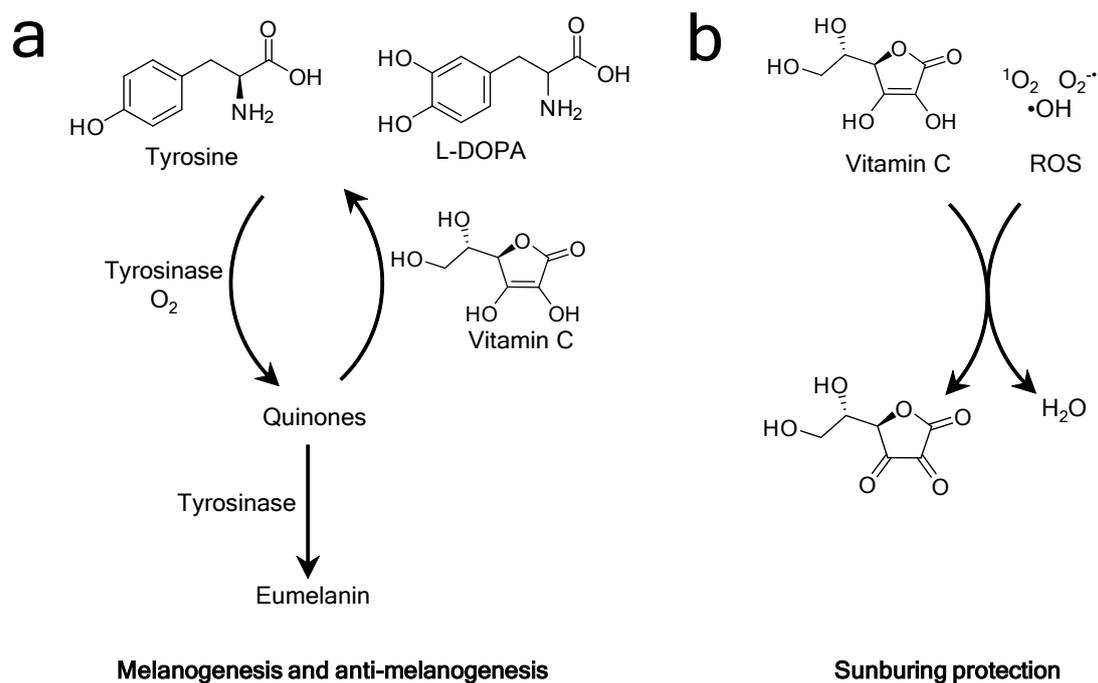


Fig. S1 The role of Vitamin C in natural processes in human body. (a) Role of Vitamin C in the treatment of skin pigment disorder. Vitamin C reduced quinones back to phenols and catechols, therefore inhibit the first step of melanin formation. (b) Role of Vitamin C in sunburing protection. Vitamin C react with the ROS formed under sunlight, therefore prevent the possible damage in cells.

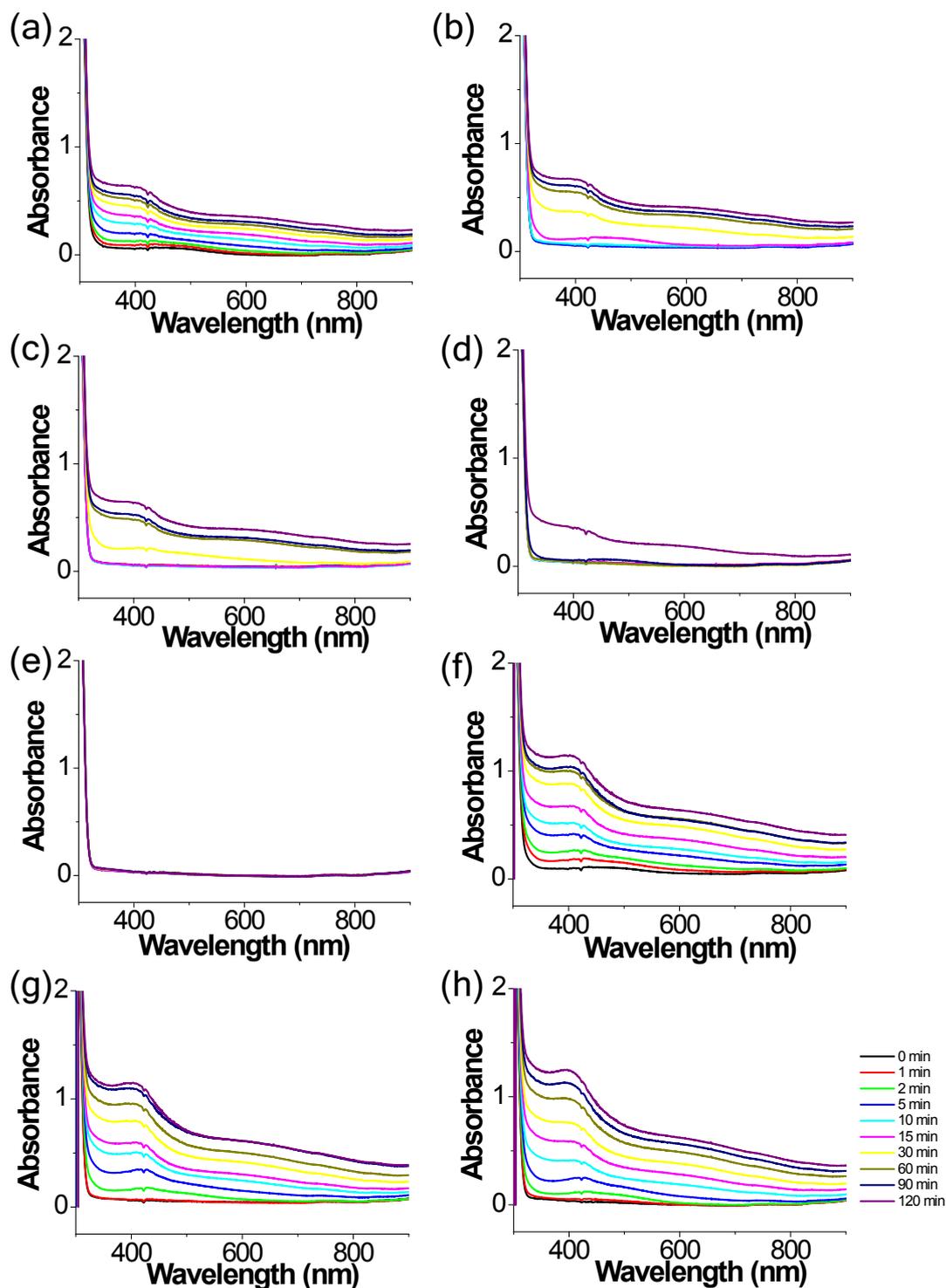


Fig. S2 UV-Vis spectra of dopamine solutions (with different SA concentrations with respect to dopamine) at different time points. (a)-(e) in dark, (f)-(h) under UV. (a) without SA. (b) 0.1% wt. SA. (c) 0.2% wt. SA. (d) 0.4% wt. SA. (e) 1% wt. SA. (f) without SA. (g) 0.1% wt. SA. (h) 1% wt. SA. Dopamine solutions (2 mg/mL in 10 mM Tris buffer pH 8.5, with 0-1% wt. SA with respect to dopamine, no deoxygen process was applied to the solutions) was placed in dark or under UV irradiation and the UV-Vis curve of the solutions were tested for different time points (0 min, 1 min, 2 min, 5 min, 10 min, 15 min, 30 min, 60 min, 90 min, 120 min).

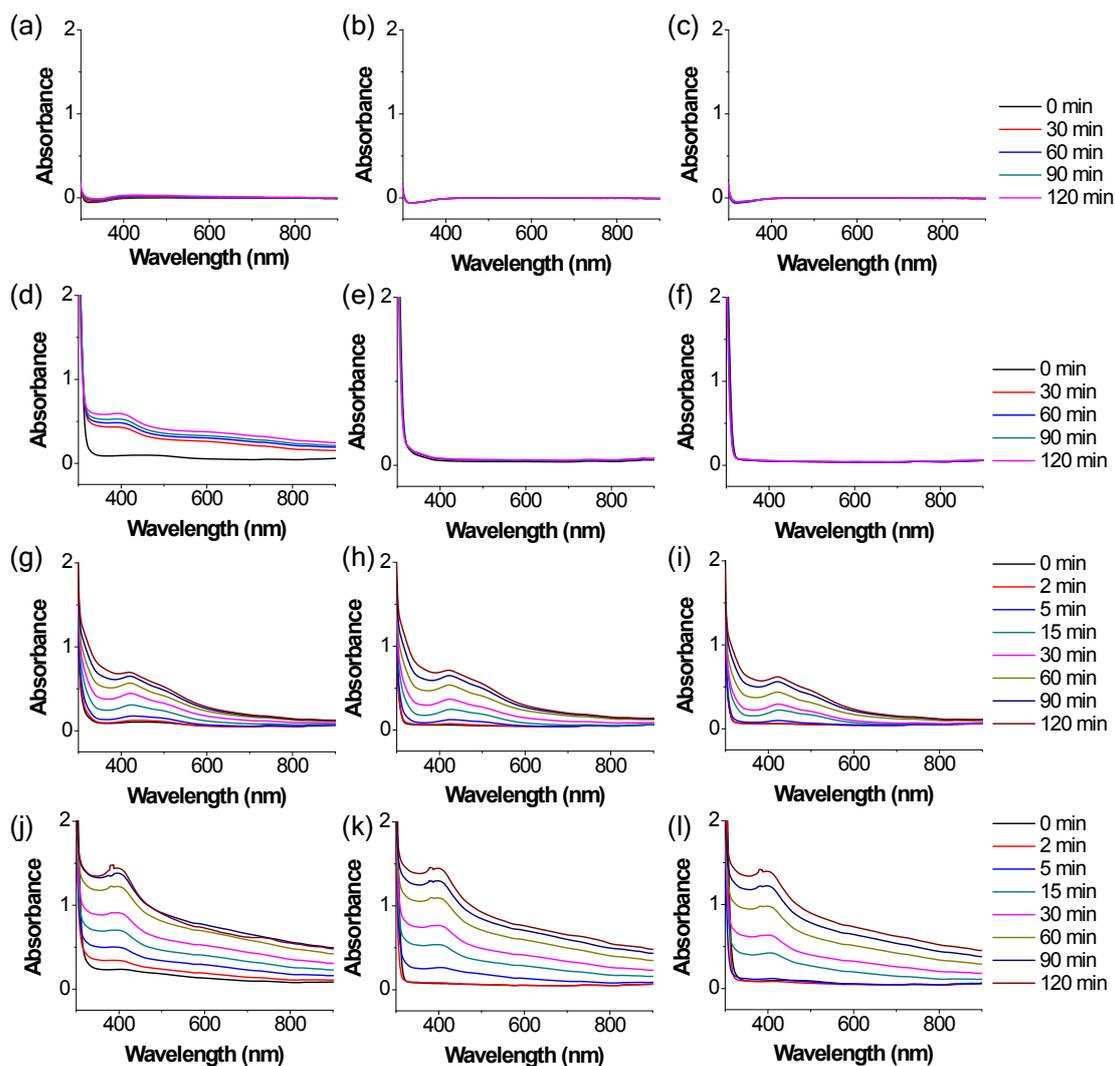


Fig. S3 UV-Vis spectra of different dopamine solutions (2mg/mL) at pH 7.4 and pH 9.5, in dark and under UV. (a)-(c): Dopamine solution at pH 7.4 in dark, with 0%, 0.4% and 1% SA, respectively (wt., with respect to dopamine). (d)-(f): Dopamine solution at pH 9.4 in dark, with 0%, 0.4% and 1% SA, respectively (wt., with respect to dopamine). (g)-(i): Dopamine solution at pH 7.4 under UV, with 0%, 0.4% and 1% SA, respectively (wt., with respect to dopamine). (j)-(l): Dopamine solution at pH 9.5 under UV, with 0%, 0.4% and 1% SA, respectively (wt., with respect to dopamine).

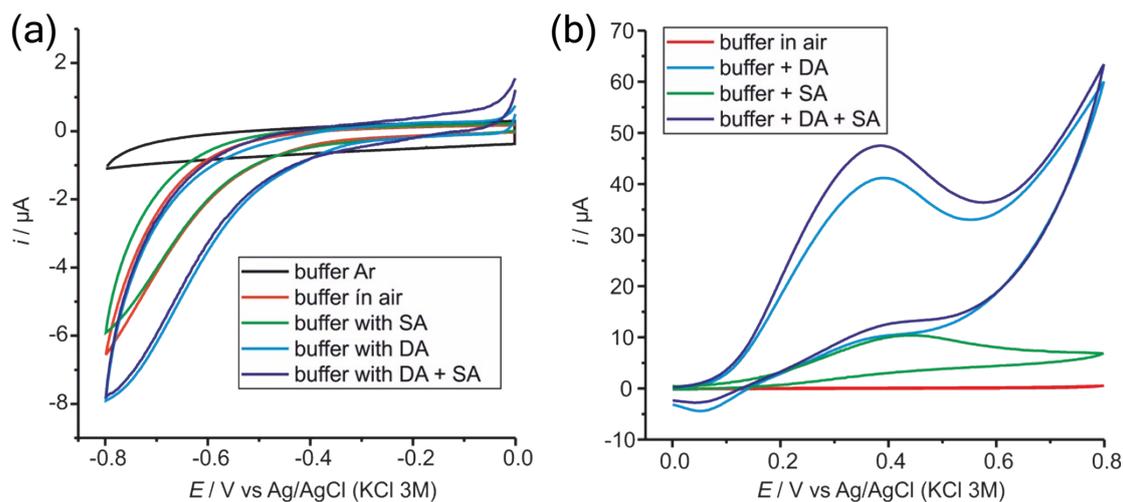


Fig. S4 (a) O_2 reduction current for solutions of Ar purged buffer (black), buffer under air (red), buffer with 5% wt. SA in air immediately after preparation (green solid line) and after one hour under ambient air (green dashed line). (b) Oxidation currents for solutions of buffer under air (red), buffer with 5% wt. SA in air (green), buffer with DA (2 mg/mL) in air (light blue), buffer with DA (2 mg/mL) and 5% wt. SA in air (dark blue).

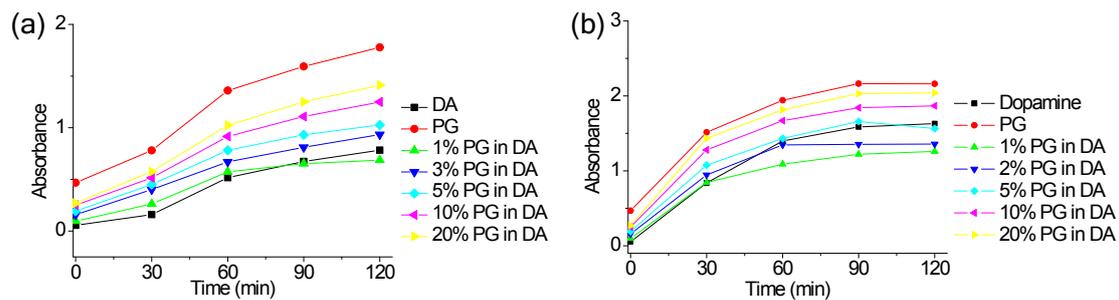


Fig. S5 Time dependent absorbance change of dopamine or pyrogallol (PG) solutions at 400 nm. 2 mg/mL dopamine or PG was dissolved in 10 mM Tris buffer pH 8.5, and the obtained solutions were (a) placed in dark or (b) irradiated under 7.5 mW/cm² UV light at 265 nm. The UV-Vis curve of the solution was recorded each 30 min. The results indicate that PG does not show any inhibition effect in dark, however, it can to some extent inhibit dopamine polymerization under UV, if the concentration of PG is low.

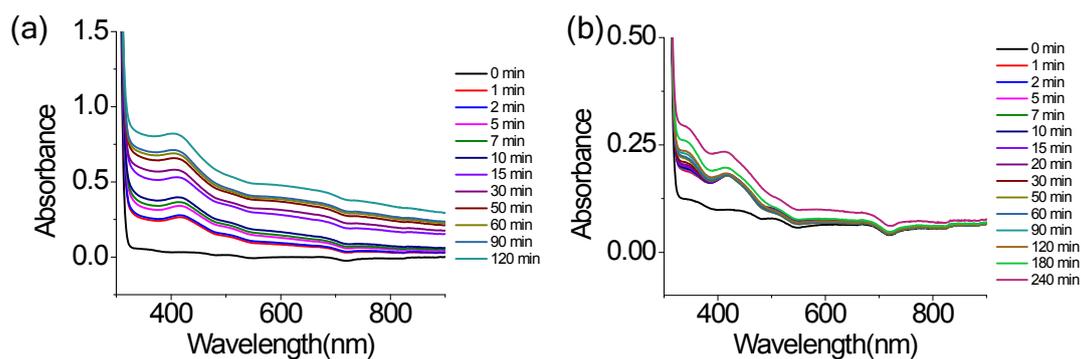


Fig. S6 UV-Vis spectra of dopamine solutions with different SA concentrations at different time points. (a) 1% wt. SA with respect to dopamine, (b) 5% wt. SA with respect to dopamine. The samples were first irradiated with 14 mW cm^{-2} 365 nm UV light for 15 min, then placed in dark for 2 or 4h. All measurements were performed in Tris buffer (10 mM pH 8.5) containing dopamine (2 mg/mL) under ambient air.

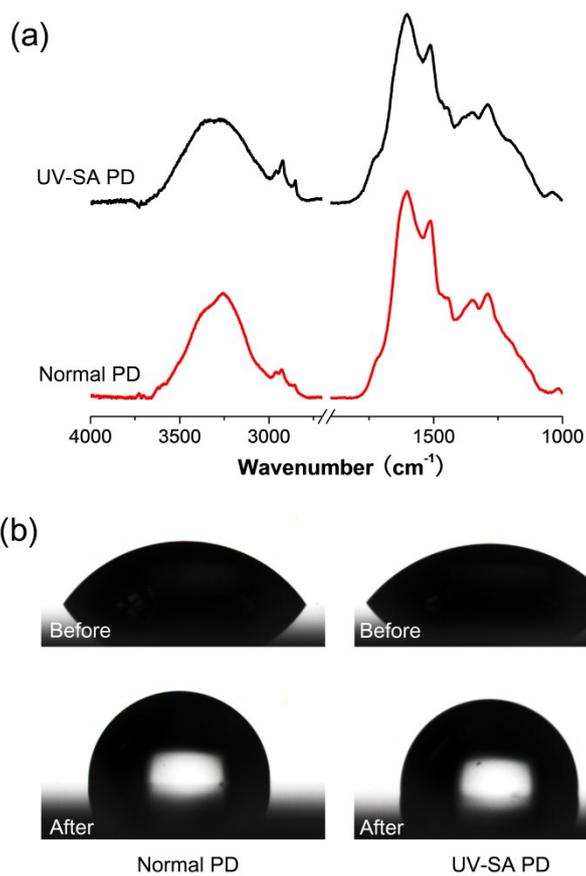


Fig. S7 (a) IR spectra of the PD layer on gold substrate. The red curve refers to the PD coating produced using the common base-induced polydopamine deposition (without UV and SA). The black curve refers to the PD coated by UV-SA assisted dopamine polymerization as described in the experimental part. (b) WCA test of normal PD and UV-SA PD before and after secondary modification with dodecanethiol.

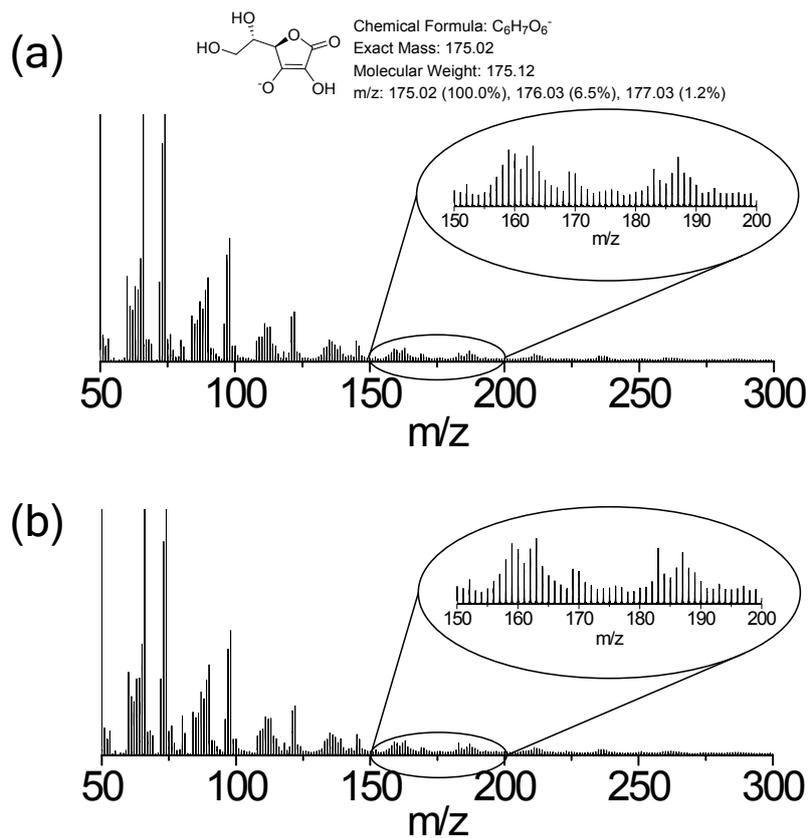


Fig. S8 ToF-SIMS spectra (negative mode) of the PD layers deposited by (a) UV-SA method and (b) standard method. The spectra of two PD surfaces are very similar, and no difference can be observed at $m/z=175$, indicating no SA is incorporated in the PD layer deposited by UV-SA method.

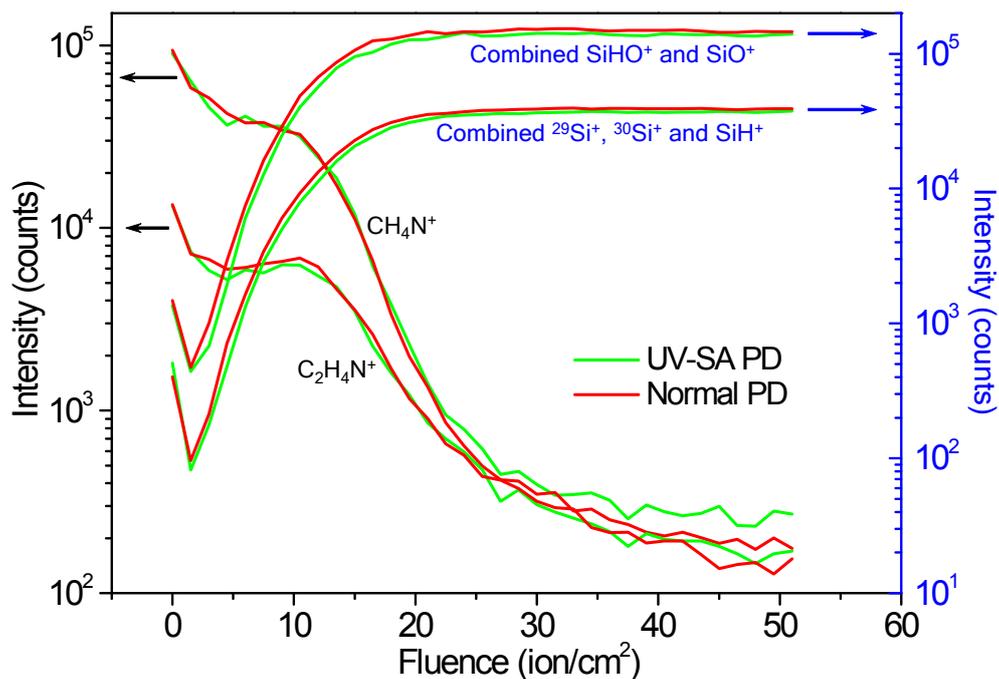


Fig. S9 Dynamic SIMS test on PD layers. The image shows the ion intensity change of CH_4N^+ , $\text{C}_2\text{H}_4\text{N}^+$ (from PD layer) and SiHO^+ , SiO^+ , $^{29}\text{Si}^+$, $^{30}\text{Si}^+$, SiH^+ (from glass substrate) from the ToF-SIMS spectra of PD layers (deposit with or without SA) during the Argon cluster erosion. As the erosion goes on, the PD layer is gradually corroded and therefore a decrease of PD characteristic ion intensity (CH_4N^+ and $\text{C}_2\text{H}_4\text{N}^+$) and an increase of the substrate ion intensity (Si derivated ions) would be observed. The tendencies of ion intensity change on normal PD and UV-SA PD are almost the same, indicating that the thickness of the two PD layer are very close. Preparation of the PD layer: for PD without SA, a standard process was applied: 2 mg/mL dopamine in 10mM Tris buffer pH 8.5, glass as substrate, dark for 24h. For PD with SA: 2 mg/mL dopamine in 10mM Tris buffer pH 8.5, with 1% wt. SA with respect to dopamine (0.06 mg/mL), glass as substrate, and dark for 24h.

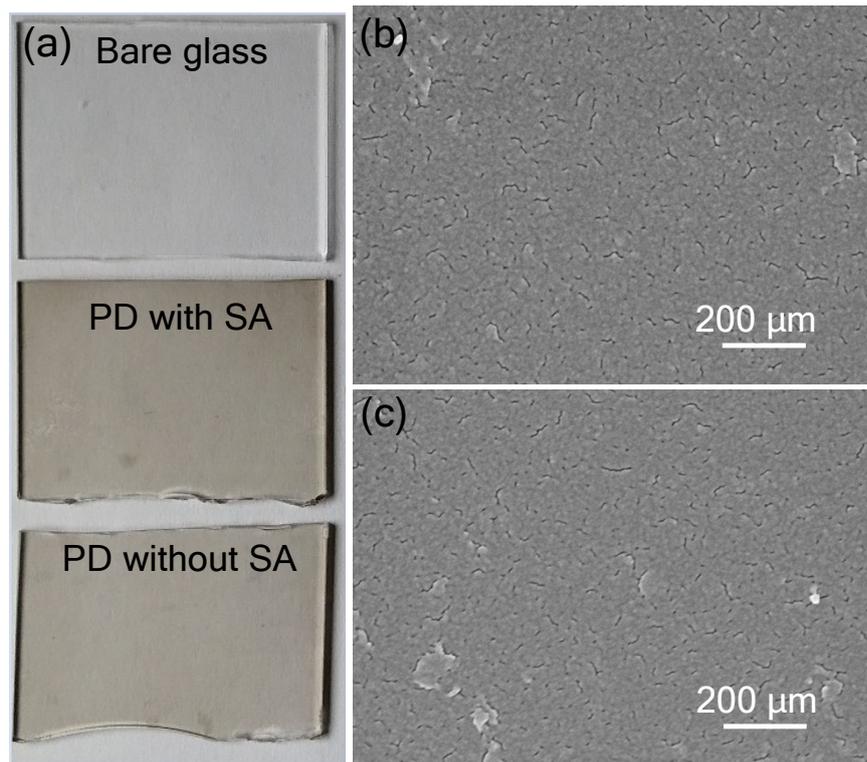


Fig. S10 PD prepared by a standard method (2 mg/mL DA, 10 mM Tris pH 8.5 buffer, 300 rpm stirring, dark for 24h) and the UV-SA method (2 mg/mL DA, 10 mM Tris pH 8.5 buffer, 3% wt. SA with respect to dopamine, 300 rpm stirring, polymerization inhibited for 3h and then started by 6 min UV irradiation and then dark for 24h), accompanied by rigorous stirring. (a) Photograph of bare glass, PD deposited by the standard method and PD deposited by the UV-SA method. No difference could be observed by naked eyes. The SEM images on the two PD surface also indicated that no difference in morphology could be observed on normal PD (b) and UV-SA PD (c).

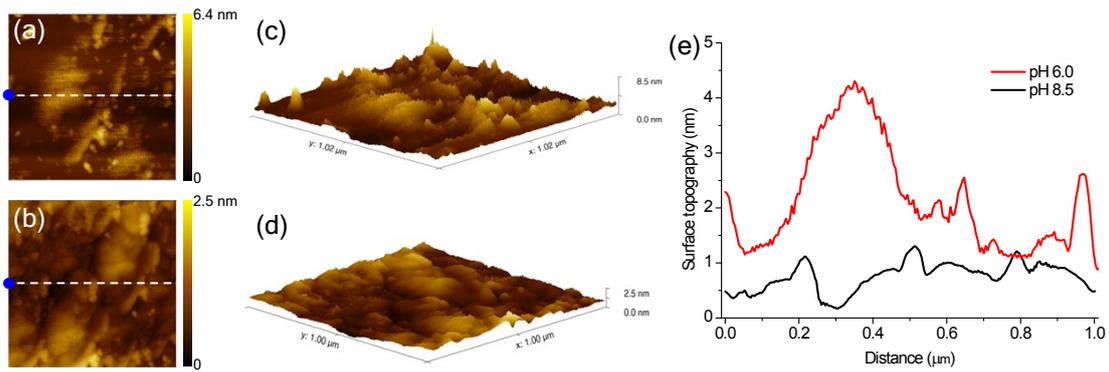


Fig. S11 AFM test on PD surfaces deposited by UV-assisted dopamine polymerization. To achieve the control on dopamine polymerization, pH 6.0 buffer ((a) and (c)) or 1% wt. SA in pH 8.5 buffer ((b) and (d)) were used. The obtained images indicated that the PD layer coated in pH 8.5 is smoother than the PD layer coated at pH 6.0. (e) Surface topographies measured along the dash line in (a) and (b).

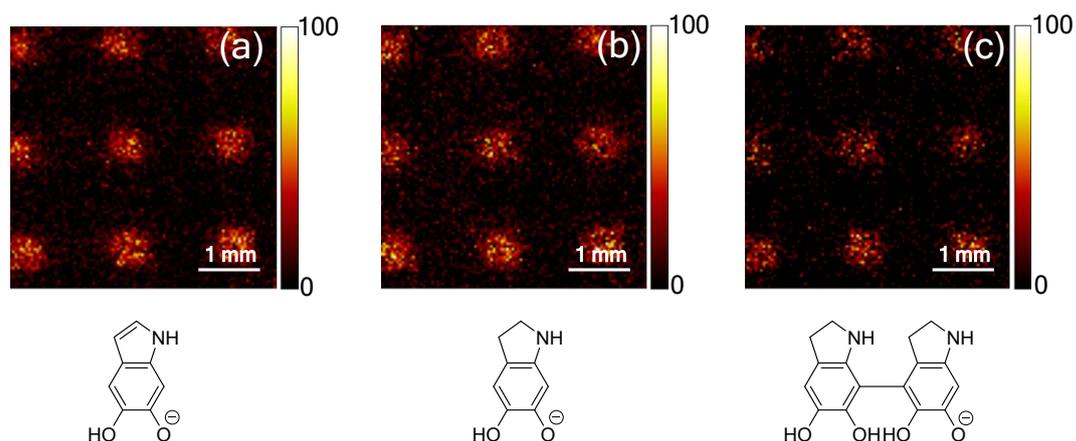


Fig. S12 Fragments of the repeat units found in the ToF-SIMS results corresponding to Figure 4d. (a) $m/z=148$, corresponds to 5,6-dihydroxy indole. (b) $m/z=150$ corresponds to another common repeat unit in PD (see structure above). (c) $m/z=299$, corresponds to a dimer of the fragment in (b).

References

- [1] S. Thiagarajan, T. H. Tsai, S. M. Chen, *Biosensors and Bioelectronics*, **2009**, 24, 2712–2715.
- [2] X. Du, L. Li, J. Li, C. Yang, N. Frenkel, A. Welle, S. Heissler, A. Nefedov, M. Grunze, P. A. Levkin, *Adv. Mater.* **2014**, 47, 8029.