Electronic Supporting Information

Revealing the formation mechanism of insoluble polydopamine by a simplified model system

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Experimental details

Materials

2-(4-Methoxy-3-methylphenyl)ethylamine (MOE) is purchased from J&K Scientific Co, Ltd. (3aminopropyl)trimethoxysilane (APTES), 3-hydroxytyramine hydrochloride (DA), and cysteamine are purchased from Sigma-Aldrich. Ammonium persulphate (APS) and potassium persulphate (KPS) are purchased from Kelong Chemical Ltd. The quartz purchased from Changchun Jixiang photoelectric instrument Co., Ltd. (China) and Au-coated substrates (80 nm Au with a 5 nm chrome adhesion layer on quartz, by thermal evaporation in vacuum, Suzhou Hongrong Biotechnology Co., Ltd. China) are used as substrates in this work. Deionized (DI) water (>15 M Ω •cm) and ethanol are used as solvent and rinsing solution. All chemicals are analytically pure and utilized without any further treatment.

Substrates preparation

The quartz slides (hereafter referred as NH₂-SiO₂ substrate) are firstly immersed in the hot piranha solution (a mixture solvent of 98% H₂SO₄ and 30% H₂O₂ at the ratio of 7:3) for 30 min. The cleaned quartz slides are immersed in the APTES solution (2mM, CH₂Cl₂ solution) at room temperature for 4 h. During the silanization, containers are sealed and placed in a dark box. The quartz slides are then rinsed in an ultrasonic bath with CH₂Cl₂, ethanol and DI water for 5 min, respectively. The silanized quartz substrates are finally blown dry for further treatment. The Au-slides (hereafter referred as NH₂-Au substrate) are treated by immersing in an ethanol solution of cysteamine (26 mM, 2 mg/mL) at room temperature for 20 min. Then, the amino-modified Au-slides (hereafter referred as NH₂-substrate) are soaked in ethanol solution for 20 min. Before use, the samples are rinsed with ethanol and DI water for several times, respectively, then blown dry for further use.

Control experiments of MOE

To verify the possibility of physisoprtion of MOE, MOE is dissolved in DI water to a concentration of 2 mM. The NH₂-SiO₂ substrate is immersed in the solution. Then, UV-Vis spectra are used to measure the above sample, before and after the immersion in DI water for 20 min to remove the physisorbed substance. The NH₂-SiO₂ samples after SIP reaction for 20 min under acidic condition is immersed in acidic (pH 1, adjusted by HCl), alkaline (pH 12, adjusted by NaOH), NaCl solutions (1 M) and potassium persulphate (KPS, 0.2 M) for 20 min, respectively. UV-Vis spectra are also used to measure these samples before and after the immersion.

Cross-linked film of polydopamine (PDA) and MOE under acidic condition

The NH₂-SiO₂ slides are treated by immersion in a 10 mL mixed DI water solution of 2 mM DA and 1 mM APS (pH 4.0) and a 10 mL mixed DI water solution of 2 mM MOE and 1 mM APS (pH 4.0), respectively. After 18 h of incubation, the cross-linked PDA can be found but cross-linked PMOE cannot be found.

SIP of DA

Acidic condition: The NH₂-SiO₂ slide is treated by immersion in a 10 mL mixed DI water solution of 2 mM DA and 1 mM APS (pH 4.0). After 10 and 20 min incubation, the samples are rinsed by DI water for further treatment.

Alkaline condition: The NH₂-SiO₂ slide is treated by immersion in a 10 mL mixed DI water solution of 2 mM DA and 1 mM APS (pH is adjusted by NaOH to 9.0). After 10 and 20 min incubation, the samples are rinsed by DI water for further treatment.

Static contact angle (CA) measurements

Water contact angles are measured using a constant volume drop of the DI water (5 μ L) and recorded by a drop shape analysis system (DSA 100, Kruss) at room temperature. More than 8 measurements are carried out for each NH₂-SiO₂ substrate sample and the resulting values are averaged.

Ellipsometry measurements

The ellipsometric characterizations were carried out using a Mueller Matrix Ellipsomerter (Wuhan Eoptics Technology Co., Ltd). The NH₂-Au substrates after SIP reaction for 1 h was used as the sample to measure the thickness of the surface immobilized polymer.



Fig. S1 The previously reported oxidation mechanism of the formation of PDA.¹



Fig. S2 The relationship between R_{DA} and R_{MOE} due to the different number of active sites (possible active sites are denoted by stars). R_{DA} and R_{MOE} represent the possible reactions in the polymerization of DA and MOE, respectively.



Fig. S3 The possible SIP process of MOE via free radical polymerization.

Control experiments are carried out to validate polymerization in the presence of a polymerization initiator, APS, under acidic condition (pH 4.0). According to the UV-Vis spectra in Fig. S4a, it can be observed that MOE alone (without APS) can hardly initiate the SIP reaction on the starting substrate. After 20 min immersion in DI water, UV-absorbance peaks of MOE are leveling off and the absorbance at 198 nm (A₁₉₈) of the residues on surface is only 13.7 % left. It indicates that most of the physically adsorbed MOE can be removed easily from the substrate by rinsing. Only if APS is also introduced in the system, the SIP reaction can be initiated.

As for evaluations under harsh conditions, the NH₂-SiO₂ samples after SIP reaction have been immersed in HCl (pH 1.0), NaOH (pH 12.0), NaCl (1.0 M) and potassium persulphate (KPS, 0.2 M) solutions for 20 min, respectively. According to the UV-Vis spectra of A₁₉₉ (Fig. S4b, c, d, e), we can observe that under the harsh conditions, 96.8%, 93.9%, 97.2% and 95.7% of the original amount of polymers are remained on the sample surfaces, respectively. It further demonstrates that the polymers (PMOE) are covalently tethered to the substrate.²



Fig. S4 UV-Vis spectra of the NH₂-SiO₂ samples in control experiments. (a) UV-Vis spectra of a NH₂-SiO₂ substrate immersed in a 2 mM MOE solution (no APS) for 20 min, and then immersed and rinsed in DI water for another 20 min. UV-Vis spectra of the samples (SIP reaction of MOE (2 mM) for 30 min) that treated by immersion in (b) HCl solution (pH 1.0), (c) NaOH solution (pH 12.0), (d) NaCl solution (1 M) and (e) potassium persulphate (KPS, 0.2 M) for 20 min, respectively.



Fig. S5 UV-Vis spectra of MOE in DI water. MOE has three peaks of UV-absorbance in DI water, i.e., 198 nm, 227 nm and 278 nm, respectively.

Morphology and CA analysis under acidic condition



Fig. S6 AFM morphological images ($10 \times 10 \mu m$) of (a) the starting substrate NH₂-SiO₂ slide and (b) PMOE grafted substrate after 10 min SIP reaction.



Fig. S7 Contact angles of the NH₂-SiO₂ samples before and after SIP reaction of MOE.

FTIR and XPS analysis under acidic condition

The NH₂-Au substrate is used as the initial substrate. The sample is measured before and after the SIP reaction for 1 h.

For the initial NH₂-Au substrate, the peaks around 3500 and 1600 cm⁻¹ are assigned to the stretching vibration of NH₂-CH₂- and one peak around 650 cm⁻¹ is attributed to the stretching vibration of -C-S. After SIP reaction, new peaks appeared around 3500 cm⁻¹ and 1200 cm⁻¹ (Fig. 2a), which can be assigned to the stretching vibration of NH₂-CH₂- and the vibration of Ph-O-CH₃, respectively. A new peak around 1500 cm⁻¹ (Fig. 2b) is attributed to the isolated C-H vibration of benzene rings. Another peak around 1100 cm⁻¹ (Fig. 2b) is assigned to the stretching vibration of Ph-NH-CH₂-.

XPS spectra of C 1s and O 1s (Fig. S8) also verify the formation of PMOE. As shown in C 1s spectra (Fig. S8a, b), a shift of peaks from 284.9 and 287.5 eV (-CH₂-CH₂- and NH₂-CH₂-) to 284.8, 286.15 and 288.5 eV (carbon atoms of benzene ring, Ph-O-CH₃ and NH₂-CH₂- from PMOE) can be observed. As for O 1s XPS spectra (Fig. S8c), the emerging peaks of 531.15 and 532.25 eV (Ph-O-CH₃ from PMOE) can be observed.



Fig. S8 C 1s XPS spectra of (a) initial, (b) PMOE grafted substrate and (c) O 1s XPS spectra of the PMOE grafted substrate that prepared under acidic condition (pH 4.0).

SMFS analysis of cross-linked PDA and PMOE under acidic condition



Fig. S9 Typical F-E curves of (a) cross-linked PDA film (the zoomed-in F-E curve is shown in the insets), and (b) the initial silanized substrate (i.e., before the SIP process).

PDA can deposit on the NH₂-SiO₂ surface, resulting in a cross-linked film. By SMFS analysis, we can obtain the F-E curves of the cross-linked PDA, see Fig. S9a. Due to the dense cross-linking points in the PDA film, only slight deformation of the film can be observed (~15 nm). However, we cannot obtain any F-E curves of PMOE that is similar to the cross-linked one shown in Fig. S9a after reaction even for a

long time (18 h). Only F-E curves corresponding to single-chain elongation can be obtained, see Fig. 3a. Fig. S9b shows a typical F-E curve obtained on an initial silanized substrate.



UV-Vis spectra analysis under alkaline condition

Fig. S10 UV-Vis spectra of the sample after SIP of MOE in the presence of a polymerization initiator, APS, under alkaline condition (pH 8.5). The inset shows the absorbance at 199 nm *vs* reaction time.

After SIP reaction under alkaline condition (pH 8.5), A₁₉₉ is monitored *via* UV-Vis spectrometer, which increases rapidly as reaction time prolongs (Fig. S10). These results present a similar tendency as those obtained under acidic condition (pH 4.0).

FT-IR and XPS analysis under alkaline condition



Fig. S11 (a) FT-IR spectra of NH_2 -Au substrate (black curve) and PMOE grafted from the substrate *via* SIP reaction under alkaline condition (pH 8.5) after 1 h reaction (red curve). (b) The partial FT-IR spectra of (a) from 600 to 1650 cm⁻¹.

The NH₂-Au substrate is measured before and after the SIP reaction for 1 h under alkaline condition (pH 8.5). The results of FT-IR spectra also confirm the free radical polymerization of MOE under alkaline condition in the presence of a polymerization initiator, APS. For the initial NH₂-Au substrate, the peaks around 3500 and 1600 cm⁻¹ are assigned to the stretching vibration of NH₂-CH₂- and one peak around 650 cm⁻¹ is attributed to the stretching vibration of -C-S. After the SIP reaction, new FT-IR peaks that appeared around 3400 and 1200 cm⁻¹ (Fig. S11) can be assigned to the stretching vibration of NH₂-CH₂- and the vibration of Ph-O-CH₃, respectively. A new peak around 1500 cm⁻¹ (Fig. S11b) is attributed to the stretching vibration of benzene rings. Another peak around 1100 cm⁻¹ (Fig. S11b) is assigned to the stretching vibration of Ph-NH-CH₂-. The new characteristic peaks for secondary amine (~ 1100 cm⁻¹), methoxyl (1250 cm⁻¹) and isolated C-H vibration of aromatic ring (1500 cm⁻¹) all support the formation of PMOE on the sample surface under alkaline condition.

The XPS results under alkaline condition (pH 8.5) are in accordance with those under acidic condition (pH 4.0). XPS spectra of N1s, C 1s and O 1s (Fig. S12) also verify the formation of PMOE. In N 1s XPS spectra (Fig. S12a, b), a new emerging peak at 401.65 eV indicates the formation of Ph-NH-CH₂- group. As shown in C 1s XPS spectra (Fig. S12c, d), a shift of peaks from 284.85 and 287.5 eV (-CH₂-CH₂- and NH₂-CH₂-) to 284.8, 286.2 and 288.05 eV (carbon atoms of benzene ring, Ph-O-CH₃ and Ph-NH-CH₂- from PMOE) can be observed. As for O 1s XPS spectra (Fig. S12e), the emerging peaks of 532.4 and 531.15 eV (Ph-O-CH₃ from PMOE) can be observed. These results of N1s, C1s and O1s XPS spectra demonstrate that PMOE has been grafted from the surface *via* SIP reaction.



Fig. S12 N 1s XPS spectra of (a) initial NH₂-Au substrate, (b) PMOE grafted substrate. C 1s XPS spectra of (c) initial substrate, (d) PMOE grafted substrate. O 1s XPS spectra of (e) PMOE grafted substrate. All samples are prepared under alkaline condition (pH 8.5).

Ellipsometry analysis under alkaline condition

The thickness of the NH₂-Au substrate surface after the SIP reaction for 1 h is measured to be 45.16 \pm 3.58 nm.



Fig. S13 (a) Typical F-E curves of linear polymers after SIP reaction of MOE for 20 min under alkaline condition (pH 8.5). (b) The normalized F-E curves of those shown in (a).

By SMFS analysis after 10 and 20 min reaction respectively, the F-E curves with different chain lengths are superposed well after normalization corresponding to the extension of the same force (e.g. 200 pN).³



Fig. S14 Statistical histograms of apparent polymer contour length and molecular weight after reaction under alkaline condition (pH 8.5) for (a) 10 min and (b) 20 min. The red dash lines are Gaussian fit of the histograms.

On the bases of the F-E curves, the apparent contour lengths and molecular weights are also studied under alkaline condition. The Gaussian fit of chain length after 10 and 20 min SIP reaction are mainly distributed around 141 and 265 nm (Fig. S14), respectively. The apparent molecular weight (MW) can be calculated from the apparent contour length (*L*) by the following equation:

$$MW = L (nm) / 0.59 (nm) \times 163.2 (g mol^{-1})$$
(1)

where 163.2 g mol⁻¹ is the molecular weight of the repeating unit. The apparent molecular weights are

calculated to be distributed around 39.0×10^3 and 73.3×10^3 g mol⁻¹.

Fig. S15 (a) Typical F-E curves of linear polymer obtained from SIP reactions of PMOE under acidic (pH 4.0) and alkaline (pH 8.5) conditions. (b) The normalized F-E curves of those shown in (a).

Furthermore, some F-E curves obtained under acidic (pH 4.0) and alkaline (pH 8.5) conditions are presented in Fig. S15a. After normalization to the same extension, the F-E curves obtained from acidic and alkaline conditions are superposed well (Fig. S15b). This result indicates that the same species of linear polymer is grafted from the surface.⁴

Reference

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