Supporting Information

Different EDC/NHS Activation Mechanisms between PAA and PMAA Brushes and the Following Amidation Reactions

Cuie Wang, Qin Yan, Hong-Bo Liu, Xiao-Hui Zhou, Shou-Jun Xiao*

State Key Laboratory of Coordination Chemistry, School of Chemistry and Chemical Engineering, Nanjing National Laboratory of Microstructures, Nanjing University, Nanjing 210093, Jiangsu, China

*E-mail: sjxiao@nju.edu.cn (Shou-Jun Xiao)

EDC/NHS Activation of PAA and PMAA in Aqueous Solution

Materials

PAA (MW: ~3000) was purchased from Aladdin Aladdin Reagent Company, sodium polymethacrylate, 40 wt. % in H₂O (MW: 4000~6000), was from Sigma-Aldrich, glutaric acid, glutaric anhydride, and 1-acetylurea were from J&K Chemical Ltd.

EDC/NHS Activation

PMAA (MW: ~4000) (~0.2 g) was prepared from 1 g sodium polymethacrylate (40 wt. % in H₂O) after stirred with 5 mL 1 M HCl solution overnight, 30 mL acetone added, white solid precipitated and dried in vacuum.

PAA, 24 mg, 120 mg, 240 mg in 4 mL water (2 μM, 10 μM, 20 μM) was added with 76 mg EDC (0.1 M) and 92 mg NHS (0.2 M) respectively.
The solution was stirred at room temperature for 1h. After incubation, CH$_2$Cl$_2$ (10 mL) was added to extract PAA-derived products 3 times. The organic layers were combined, washed with water thoroughly, dried over anhydrous Na$_2$SO$_4$, and evaporated in vacuum, and finally white powder was obtained.

PMAA, 32 mg, 160 mg, 320 mg in 4 mL water (2 μM, 10 μM, 20 μM) was added with 76 mg EDC (0.1 M) and 92 mg NHS (0.2 M) respectively. The solution was stirred at room temperature for 1h. After incubation, CH$_2$Cl$_2$ (10 mL) was added to extract PMAA-derived products 3 times. The organic layers were combined, washed with water thoroughly, dried over anhydrous Na$_2$SO$_4$, and evaporated in vacuum, and finally white powder was obtained.

The EDC/NHS activation products of PAA (or PMAA) under different concentrations in solution show the same infrared spectra. A good yield of PAA-derived NHS-ester was obtained in PAA solution, indicated in Figure S1-(1). In contrast, PMAA-derived anhydride (plus part of N-acylurea), but not NHS-ester, was obtained in PMAA solution, indicated in Figure S1-(2).
Figure S1. Infrared spectra of (1) EDC/NHS activated PAA and (2) EDC/NHS activated PMAA. PAA (average molecular weight ~3000) or PMAA (average molecular weight ~4000) in 4 mL water (2, 10, 20 μM) was activated by 0.1 M EDC and 0.2 M NHS for 1h, then extracted by CH$_2$Cl$_2$, washed with water, and finally dried under vacuum.

Influence of pH for EDC/NHS Activation of PAA and PMAA Brushes

The optimum pH was checked with 2 mL 0.2M MES buffer. The PAA (or PMAA) porous silicon chip was placed in 2 mL MES buffer with 0.1 M EDC and 0.2 M NHS at pH 3, 4, 5, and 6 for 1h, then rinsed with water and dried under a stream of nitrogen.

Figure S2-a shows the infrared spectra of surface products after activation of PAA brushes in 0.1 M EDC and 0.2 M NHS for 1h at pH 3, 4, 5, and 6. When pH = 3 and 4, most of the acids, indicated by the acid
band at 1710 cm\(^{-1}\), still remain unreacted. At pH = 5 and 6, infrared spectra indicate the appearance of NHS-ester. Since EDC can only work at pH 3~7, pure water which has pH ~6 is the right media for EDC/NHS activation. Figure S2-b shows the infrared spectra of PMAA brushes after EDC/NHS activation in 0.1 M EDC and 0.2 M NHS for 1h at pH 3, 4, 5, and 6. Anhydride appeared even at pH = 3, and 4. The yield of anhydride was improved with increasing pH to 5 and 6. Both Figures of a and b indicate that pH 6 is the best for EDC/NHS activation. That is why we chose the pure water as the reaction media.

**Figure S2.** Infrared spectra of (a) EDC/NHS activated PAA brushes at pH 3, 4, 5, and 6 and (b) EDC/NHS activated PMAA brushes at pH 3, 4, 5, and 6 in 0.1 M EDC and 0.2 M NHS MES (0.2M) buffer for 1h.

**Determination of Extinction Coefficient Relationships of Acid, Anhydride, N-Acylurea, and NHS-ester**

The infrared spectra of 4 monomers (glutaric acid, glutaric anhydride, acetic acid N-hydroxysuccinimide ester, and 1-acetylurea) were measured as follows: mixing and grinding 26 nmol monomer, 3.4 mg glutaric acid,
3 mg glutaric anhydride, 4 mg acetic acid N-hydroxysuccinimide ester, and 2.7 mg 1-acetylurea, with 1g potassium bromide respectively, then 100 mg mixture was taken out and pressed as a pellet and its IR spectrum was recorded with 32 scans at a 7.5 kHz velocity and a resolution of 4 cm$^{-1}$. Their extinction coefficients were related as follows according to their largest peak heights, where glutaric was divided by 2:

$$\varepsilon_{\text{NHS-ester}} : \varepsilon_{\text{anhydride}} : \varepsilon_{\text{N-acylurea}} : \varepsilon_{\text{acid}} = 2 : 1.5 : 1.5 : 1$$

**Thickness of PAA (or PMAA) Brushes by SEM Cross Section Imaging**

*Figure S3.* The thickness of PAA (or PMAA) brushes after staining with 1% acetate uranium for 5 min was assessed at around 200 nm by SEM (Hitachi S-4800, Japan) cross section imaging.