

## Supporting Information

### **Bio-inspired immobilization of low-fouling phospholipid polymers via a simple dipping process: a comparative study of phenol, catechol and gallol as tethering groups**

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## Materials

Methyl 4-hydroxybenzoate, methyl 3,4-dihydroxybenzoate, methacryloyl chloride, methyl gallate, N,N-diisopropylethylamine, and chloromethyl methyl ether were purchased from Tokyo Chemical Industry and used as received. Lithium aluminum hydride, anhydrous tetrahydrofuran (THF), sodium hydroxide, triethylamine (TEA), hydrochloric acid (6 N), N,N,N,N-ethylenediaminetetraacetic acid (EDTA) and other organic solvents were purchased from Wako Pure Chemical Industry and used as received. Anti-bovine albumin rabbit immunoglobulin (IgG) (#B1520), antirabbit IgG goat IgG labeled with a 10-nm gold colloid (#G7402), and ovalbumin (#A5503) were obtained from Sigma-Aldrich, St. Louis, MO, USA. Fetal bovine serum (FBS) for cell culture medium (Gibco®) was purchased from Thermo Fisher Scientific Inc., IL, USA.

## Synthesis of gallol-functionalized methacrylate monomer, 3,4,5-Tris(methoxymethoxy)benzyl methacrylate (TMBM)

### ·Step 1: Synthesis of methyl 3,4,5-tris(methoxymethoxy)benzoate (MTMB)

Under an N<sub>2</sub> atmosphere at 0 °C, chloromethyl methyl ether (19.20 g, 240 mmol) was added to a solution of methyl gallate (7.40 g, 40 mmol) and N,N-Diisopropylethylamine (DIEA) (15.60 g, 120 mmol) in 100 mL of THF. After reacting for 24 h at 40 °C, the reaction mass was filtered, concentrated under vacuum, and extracted with 50 mL of ether three times. The organic layer was washed with 50 mL of brine three times, dried over MgSO<sub>4</sub>, filtered, and evaporated to yield colorless oil without further purification. The crude product was directly used for the next reaction.

### ·Step 2: Synthesis of 3,4,5-tris(methoxymethoxy)benzyl alcohol (TMBA)

Under an N<sub>2</sub> atmosphere at 0 °C, the crude product of MTMB obtained in Step 1 was added dropwise to a suspension of LiAlH<sub>4</sub> (1.90 g, 50 mmol) in 150 mL anhydrous THF in an ice bath. After reacting for 2 h at room temperature, the reaction mass was quenched very slowly by 2 mL DI-water, 2 mL 15% NaOH, and 6 mL DI-water. The mixture was stirred for 15 min with MgSO<sub>4</sub> and filtered to remove solids. The filtrate was concentrated in vacuum to yield a white crystal (70% in two steps) without further purification.

### ·Step 3: Synthesis of 3,4,5-tris(methoxymethoxy)benzyl methacrylate (TMBM)

Under an argon atmosphere at 0 °C, methacryloyl chloride (1.57 g, 15 mmol) was added dropwise to a solution of TMBA (2.88 g, 10 mmol) and triethylamine (TEA) (1.32 g, 11 mmol) in 50 mL of THF. After reacting for 24 h at room temperature, the reaction mass was filtered, concentrated under vacuum, and extracted with 50 mL of ether. The organic layer was washed with 50 mL of brine three times, dried over MgSO<sub>4</sub>, filtered, and evaporated to yield pale-yellow oil. The crude product was subjected to flash chromatography (hexane : ethyl acetate = 7 : 3, R<sub>f</sub> = 0.35) to obtain a white crystal (66%). <sup>1</sup>H NMR (400 MHz, 400 MHz, CDCl<sub>3</sub>, Figure S1): (δ, ppm)

6.75 (s, 2H), 6.15 (s, 1H), 5.57 (s, 1H), 5.16 (s, 4H), 5.01 (s, 2H), 4.99 (s, 2H), 3.49 (s, 3H), 3.38 (s, 6H), 1.96 (s, 3H)

### **Synthesis of catechol-functionalized methacrylate monomer, 3,4-di(methoxymethoxy)benzyl methacrylate (DMBM)**

#### **·Step 1: Synthesis of methyl 3,4-di(methoxymethoxy)benzoate (MDMB)**

Under an N<sub>2</sub> atmosphere at 0 °C, chloromethyl methyl ether (12.80 g, 160 mmol) was added to a solution of methyl 3,4-dihydroxybenzoate (6.72 g, 40 mmol) and N,N-diisopropylethylamine (DIEA) (10.40 g, 80 mmol) in 100 mL of THF. After reacting for 24 h at 40 °C, the reaction mass was filtered, concentrated under vacuum, and extracted with 50 mL of ether three times. The organic layer was washed with 50 mL of brine three times, dried over MgSO<sub>4</sub>, filtered, and evaporated to yield colorless oil without further purification. The crude product was directly used for the next reaction.

#### **·Step 2: Synthesis of 3,4-di(methoxymethoxy)benzyl alcohol (DMBA)**

Under an N<sub>2</sub> atmosphere at 0 °C, the crude product of MDMB obtained in Step 1 was added dropwise to a suspension of LiAlH<sub>4</sub> (1.90 g, 50 mmol) in 150 mL anhydrous THF in an ice bath. After reacting for 2 h at room temperature, the reaction mass was quenched very slowly by 2 mL DI-water, 2 mL 15% NaOH, and 6 mL DI-water. The mixture was stirred for 15 min with MgSO<sub>4</sub> and was then filtered to remove solids. The filtrate was concentrated in vacuum to yield a white crystal (80% in two steps) without further purification.

#### **·Step 3: Synthesis of 3,4-di(methoxymethoxy)benzyl methacrylate (DMBM)**

Under an N<sub>2</sub> atmosphere at 0 °C, methacryloyl chloride (1.57g, 15 mmol) was added dropwise to a solution of DMBA (2.28 g, 10 mmol) and triethylamine (TEA) (1.32 g, 11 mmol) in 50 mL of THF. After reacting for 24 h at room temperature, the reaction mass was filtered, concentrated under vacuum, and extracted with 50 mL of ether. The organic layer was washed with 50 mL of brine three times, dried over MgSO<sub>4</sub>, filtered, and evaporated to yield pale-yellow oil. The crude product was subjected to flash chromatography (hexane : ethyl acetate = 7 : 3, R<sub>f</sub> = 0.45) to obtain a white crystal (70%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, Figure S2): (δ, ppm) 7.19 (d, 1H), 7.12 (d, 1H), 6.99 (dd, 1H), 6.15 (s, 1H), 5.57 (s, 1H), 5.25 (d, 4H), 5.12 (s, 2H), 3.49 (s, 6H), 1.96 (s, 3H)

### **Synthesis of phenol-functionalized methacrylate monomer, 4-methoxymethoxybenzyl methacrylate (MMBM)**

#### **·Step 1: Synthesis of methyl 4-methoxymethoxy benzoate (MMMB)**

Under an N<sub>2</sub> atmosphere at 0 °C, chloromethyl methyl ether (6.40 g, 80 mmol) was added to a solution of methyl 4-hydroxybenzoate (6.08 g, 40 mmol) and N,N-diisopropylethylamine (DIEA) (5.20 g, 40 mmol) in 100 mL of THF. After reacting for 24 h at 40 °C, the reaction mass was filtered, concentrated under vacuum, and

extracted with 50 mL of ether three times. The organic layer was washed with 50 mL of brine three times, dried over  $\text{MgSO}_4$ , filtered, and evaporated to yield colorless oil without further purification. The crude product was directly used in the next reaction.

·Step 2: Synthesis of 4-methoxymethoxybenzyl alcohol (MMBA)

Under an  $\text{N}_2$  atmosphere at 0 °C, the crude product of MMBB obtained in Step 1 was added dropwise to a suspension of  $\text{LiAlH}_4$  (1.90 g, 50 mmol) in 150 mL anhydrous THF in an ice bath. After reacting for 2 h at room temperature, the reaction mass was quenched very slowly by 2 mL DI-water, 2 mL 15% NaOH, and 6 mL DI-water. The mixture was stirred for 15 min with  $\text{MgSO}_4$  and filtered to remove solids. The filtrate was concentrated in vacuum to yield a white crystal (70% in two steps) without further purification.

·Step 3: Synthesis of 4-Methoxymethoxybenzyl methacrylate (MMBM)

Under an  $\text{N}_2$  atmosphere at 0 °C, methacryloyl chloride (1.57g, 15 mmol) was added dropwise to a solution of DMBA (1.68 g, 10 mmol) and triethylamine (TEA) (1.32 g, 11 mmol) in 50 mL of THF. After reacting for 24 h at room temperature, the reaction mass was filtered, concentrated under vacuum, and extracted with 50 mL of ether. The organic layer was washed with 50 mL of brine three times, dried over  $\text{MgSO}_4$ , filtered, and evaporated to yield pale-yellow oil. The crude product was subjected to flash chromatography (hexane : ethyl acetate = 7 : 3,  $R_f$  = 0.65) to obtain a colorless oil (70%).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , Figure S3): ( $\delta$ , ppm) 7.25 (t, 2H), 6.99 (t, 2H), 6.15 (s, 1H), 5.90 (dd, 2H), 5.54(s, 1H), 5.16 (s, 2H), 4.99(s, 2H), 3.46 (s, 3H), 1.96 (s, 3H)

**Synthesis of phospholipid polymer with different tethering groups**

·Synthesis of P(MPC-co-TMBM)

In a test tube, phospholipid monomer 2-methacryloyloxyethyl phosphorylcholine polymer (MPC) (742 mg, 2.5 mmol), TMBM (890 mg, 2.5 mmol), and an initiator 2,2'-azobis(2-methylpropionitrile) (AIBN) (8.2 mg, 0.05 mmol) were dissolved in a 5 mL mixed solvent of 1,4-dioxane and methanol (v/v=1:1). The polymerization was conducted at 65 °C under an  $\text{N}_2$  atmosphere for 24 h. The precipitate of the copolymers from hexane was isolated by centrifugation and dried in vacuum (Figure S4).

·Synthesis of P(MPC-co-DMBM)

In a test tube, MPC (742 mg, 2.5 mmol), DMBM (740 mg, 2.5 mmol), and an initiator 2,2'-azobis(2-methylpropionitrile) (AIBN) (8.2 mg, 0.05 mmol) were dissolved in a 5 mL mixed solvent of 1,4-dioxane and methanol (v/v = 1/1). The polymerization was conducted at 65 °C under an  $\text{N}_2$  atmosphere for 24 h. The precipitate of the copolymers from hexane was isolated by centrifugation and dried in vacuum (Figure S5).

#### ·Synthesis of P(MPC-co-MMBM)

In a test tube, MPC (742 mg, 2.5 mmol), MMBM (590 mg, 2.5 mmol), and an initiator 2,2'-azobis(2-methylpropionitrile) (AIBN) (8.2 mg, 0.05 mmol) were dissolved in 5 mL of ethanol. The polymerization was conducted at 65 °C under an N<sub>2</sub> atmosphere for 24 h. The precipitate of the copolymers from the hexane was isolated by centrifugation and dried in vacuum (Figure S6).

#### ·Synthesis of P(MPC-co-MBz)

In a test tube, MPC (742 mg, 2.5 mmol), benzyl methacrylate (MBz) (440 mg, 2.5 mmol), and an initiator 2,2'-azobis(2-methylpropionitrile) (AIBN) (8.2 mg, 0.05 mmol) were dissolved in the mixture of 4 mL of ethanol and 1 mL DMSO. The polymerization was conducted at 65 °C under an N<sub>2</sub> atmosphere for 24 h. The precipitate of the copolymers from hexane was isolated by centrifugation and dried in vacuum.

#### ·Synthesis of P(MPC-co-BMA)

In a test tube, MPC (742 mg, 2.5 mmol), n-butyl methacrylate (BMA) (355 mg, 2.5 mmol), and an initiator 2,2'-azobis(2-methylpropionitrile) (AIBN) (8.2 mg, 0.05 mmol) were dissolved in 5 mL of ethanol. The polymerization was conducted at 65 °C under an N<sub>2</sub> atmosphere for 24 h. The precipitate of the copolymers from acetone was isolated by centrifugation and dried in vacuum.

### **Molecular weight, compositions and PDIs of copolymers**

The number-average molecular weights (M<sub>n</sub>) and PDI of the copolymers were measured by gel permeation chromatography (GPC) (JASCO, Tokyo, Japan) in water/methanol (v/v = 3/7) containing 10 mmol/L of lithium bromide. The compositions of copolymers were determined by the ratio of peak areas in the <sup>1</sup>H NMR spectrums of the polymers (Table S1).

### **Deprotection of phenolic polymers**

As a typical example of de-protection, P(MPC-co-TMBM) (500 mg) was dissolved in 10 mL of DI-water in a 100 mL round-bottom flask equipped with a magnetic stirring bar under a nitrogen atmosphere. The solution was stirred for 1 h before 167 μL of an HCl solution (6 N) was added dropwise and the reaction was left to stir for 72 h while monitored by <sup>1</sup>H NMR. The resulting polymer solution was directly diluted to the desired concentration (0.5 wt%). P(MPC-co-DMBM) and P(MPC-co-MMBM) were de-protected under the same conditions (Figure S9).

### **Surface characterization by ATR/FT-IR spectroscopy**

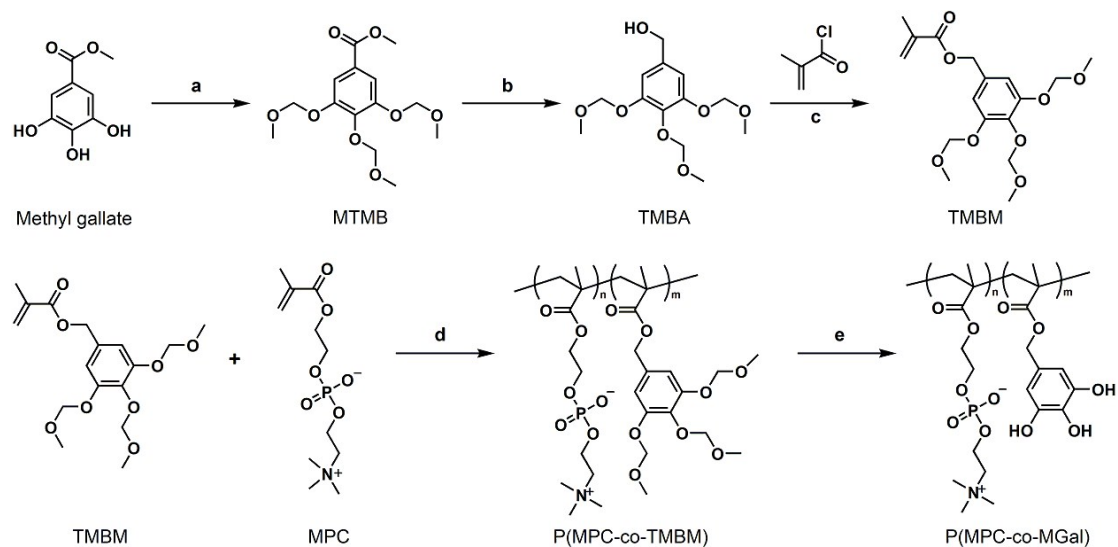
ATR/FT-IR (IMV-4000, JASCO Ltd., Tokyo, Japan) were used to characterize the coated surfaces. Five points were obtained with the ATR probe. The FT-IR spectra were examined in 128 scans over the range of 650–4000 cm<sup>-1</sup> at a resolution of 8.0 cm<sup>-1</sup>.

### **Surface characterization by X-ray Photoelectron Spectroscopy**

The elemental compositions of the surfaces were determined by XPS (AXIS-His, Shimadzu/Kratos Co. Ltd., Kyoto, Japan) with an MgK $\alpha$  (12 kV) radiation source at the anode. The take-off angle of the photoelectron was fixed at 90°. The signals attributed to C<sub>1s</sub>, N<sub>1s</sub>, O<sub>1s</sub>, and P<sub>2p</sub> were characterized. All spectra were referred to C<sub>1s</sub> peak at 285.0 eV of the binding energy.

### **Visualization of the protein adsorbed at the plasma-contacting surface**

Densities of albumin adsorbed on the plasma-contacting surface of the substrates were examined using the gold colloid-labeled immunoassay method. Substrates were placed in a 24-well tissue culture plate. To equilibrate the substrate surface, these substrates were treated with PBS for 1 h. Then, PBS was removed and FBS was poured into each well and the wells were incubated at 37°C for 1 h. The substrates were sufficiently rinsed with PBS and treated overnight with 15 wt% non-fat milk solution containing 2 mM N,N,N,N-ethylenediaminetetraacetic acid at 4°C. The substrates were rinsed with PBS and incubated in PBS solution containing 0.02 mg/mL of a primary antibody (anti-bovine albumin rabbit IgG) and 1.0 wt% of ovalbumin for 1 h at 37°C. The substrates were rinsed with PBS and incubated overnight with 2.5 wt% of glutaraldehyde in PBS at 4°C. The substrates were rinsed with PBS and subjected to the secondary antibody (anti-rabbit IgG goat IgG labeled with 10-nm gold colloid) treatment for 60 min at 37°C. The size of the gold colloid retained after rinsing increased to 200–300 nm, as detected using a silver enhancer kit (SE-100, Sigma-Aldrich, St. Louis, USA). The surface of the substrate was observed using scanning electron microscopy (SEM, SM-200, TOPCON, Tokyo, Japan) after being freeze-dried and sputtered with gold. The amount of gold particle-labeled proteins on the surface was calculated based on SEM images. Briefly, the number of gold particles in the images was counted using a software, ImageJ2x (Rawak Software, Inc. Germany). Densities of gold particles were determined by dividing the total number of gold particles by the actual area of SEM images.



Scheme S1. Synthetic route of the gallol-functionalized copolymer P(MPC-co-MGal): (a) DIEA,  $\text{CH}_3\text{OCH}_2\text{Cl}$ , THF,  $45^\circ\text{C}$ , 24 h; (b)  $\text{LiAlH}_4$ , THF,  $0^\circ\text{C}$  to r.t., 12 h; (c) TEA, THF, r.t., 24 h; (d) AIBN, 1,4-Dioxane/MeOH (1:1),  $65^\circ\text{C}$ , 24 h; (e) 0.5 mM HCl, 72 h.

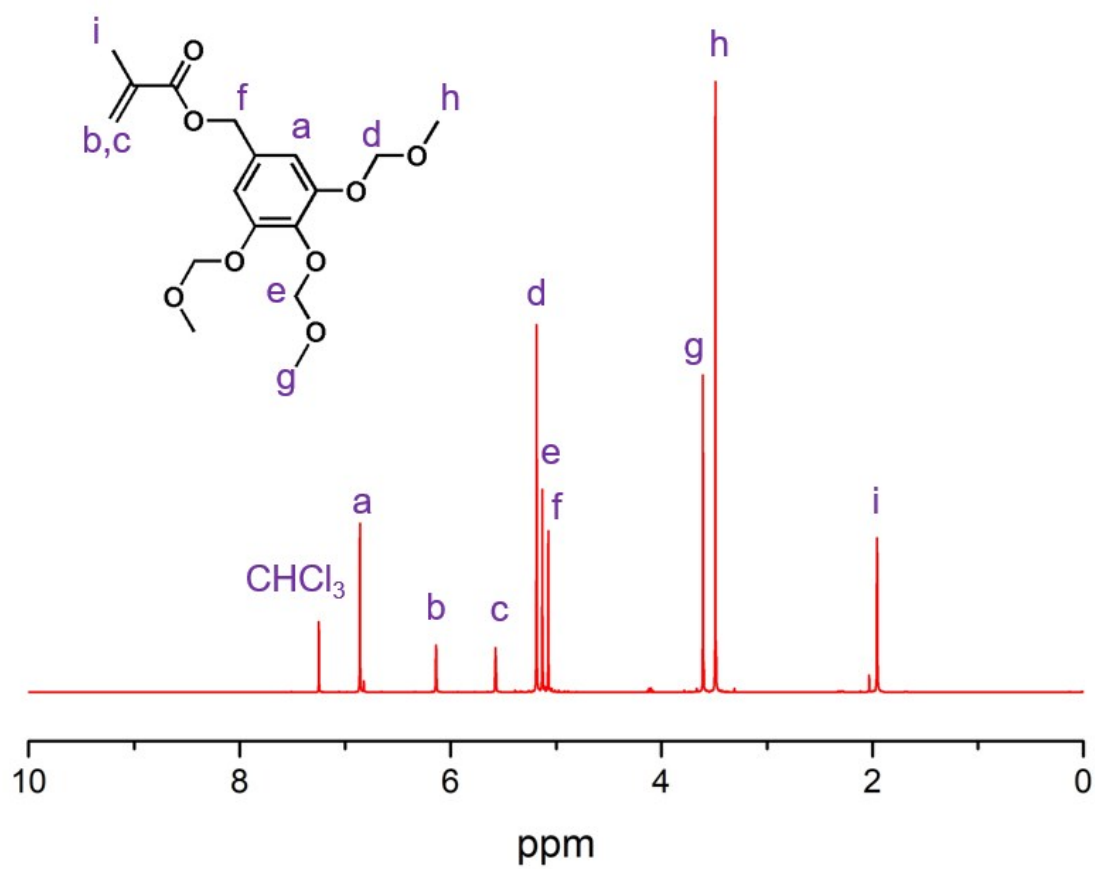


Figure S1.  $^1\text{H}$  NMR of 3,4,5-Tris(methoxymethoxy)benzyl methacrylate (TMBM) (400 MHz,  $\text{CDCl}_3$ )



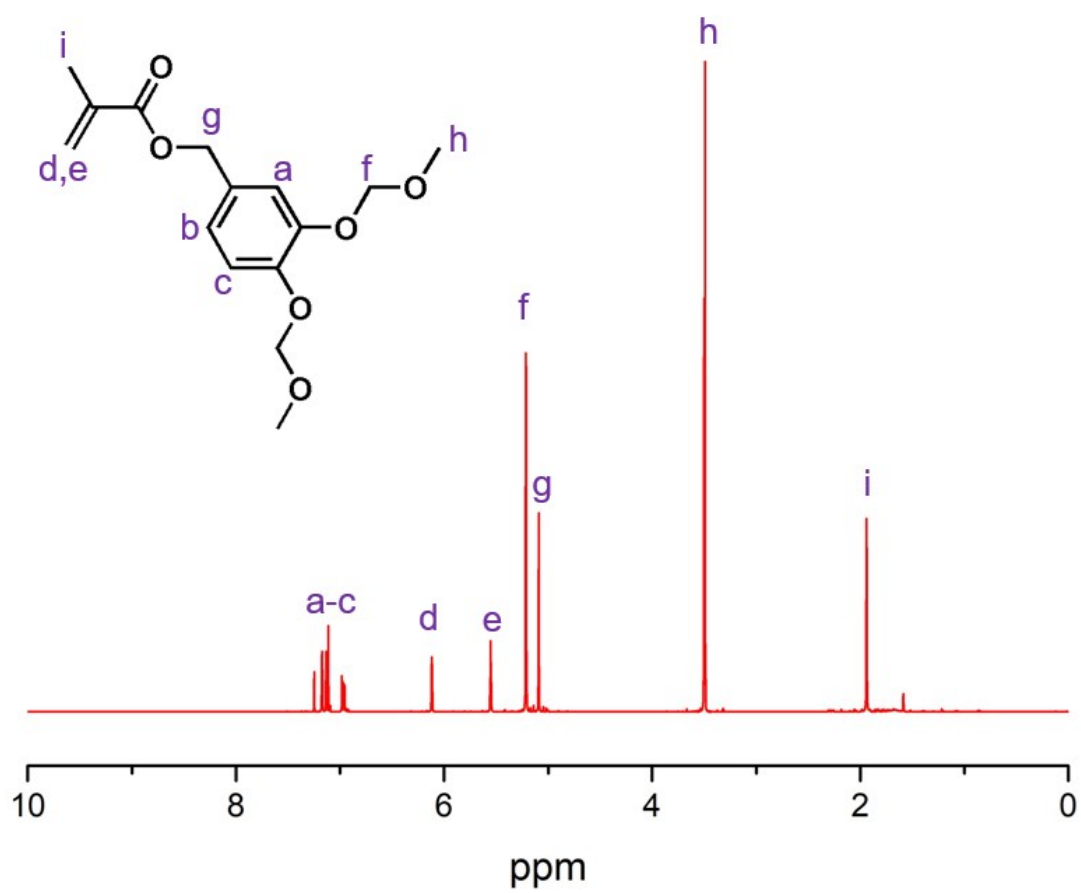


Figure S2. <sup>1</sup>H NMR of 3,4-Di(methoxymethoxy)benzyl methacrylate (DMBM) (400 MHz, CDCl<sub>3</sub>)

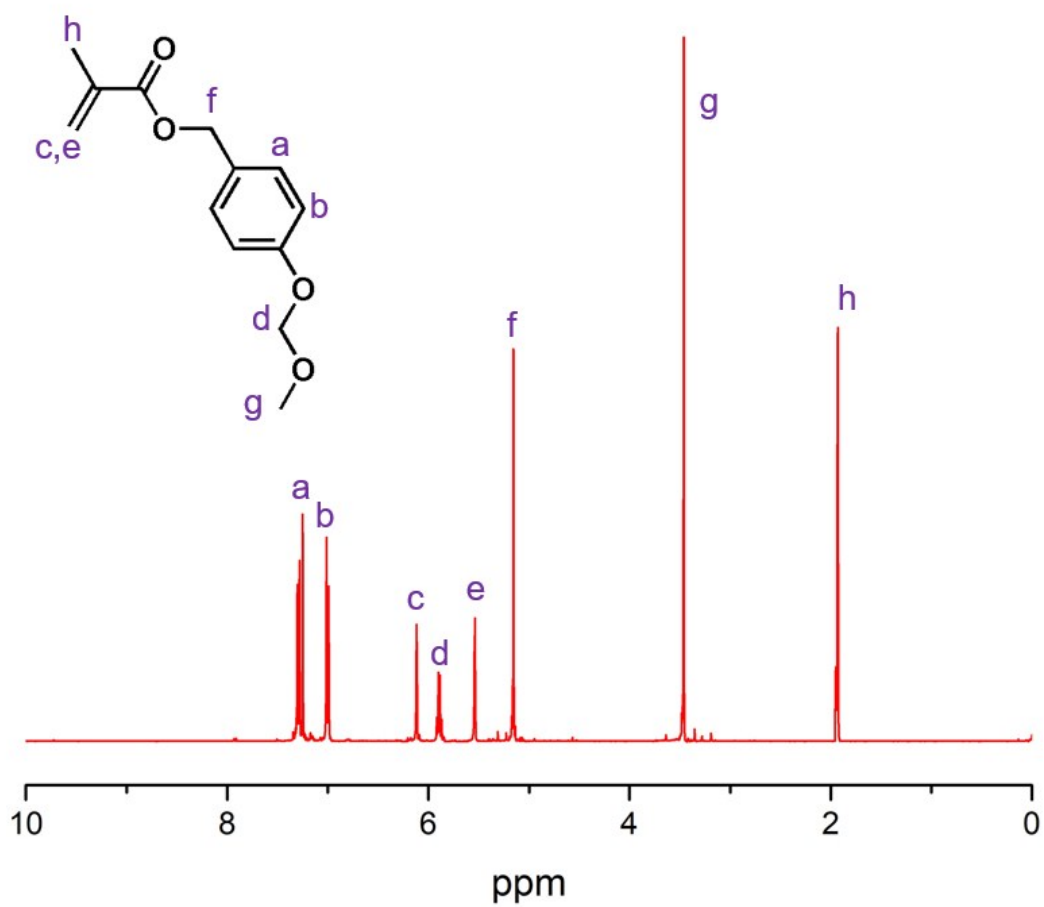


Figure S3.  $^1\text{H}$  NMR of 4-Methoxymethoxybenzyl methacrylate (MMBM) (400 MHz,  $\text{CDCl}_3$ )

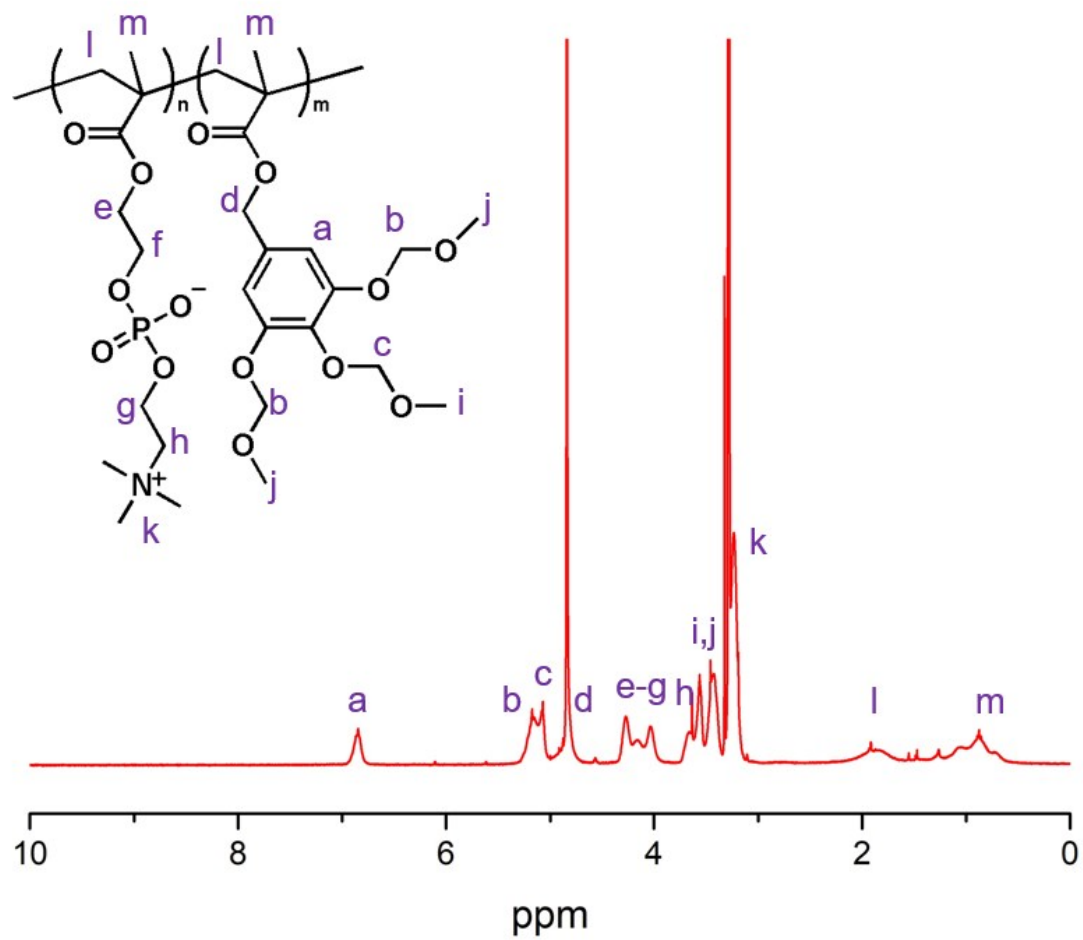


Figure S4.  $^1\text{H}$  NMR of P(MPC-co-TMBM) (400 MHz,  $\text{CD}_3\text{OD}$ )

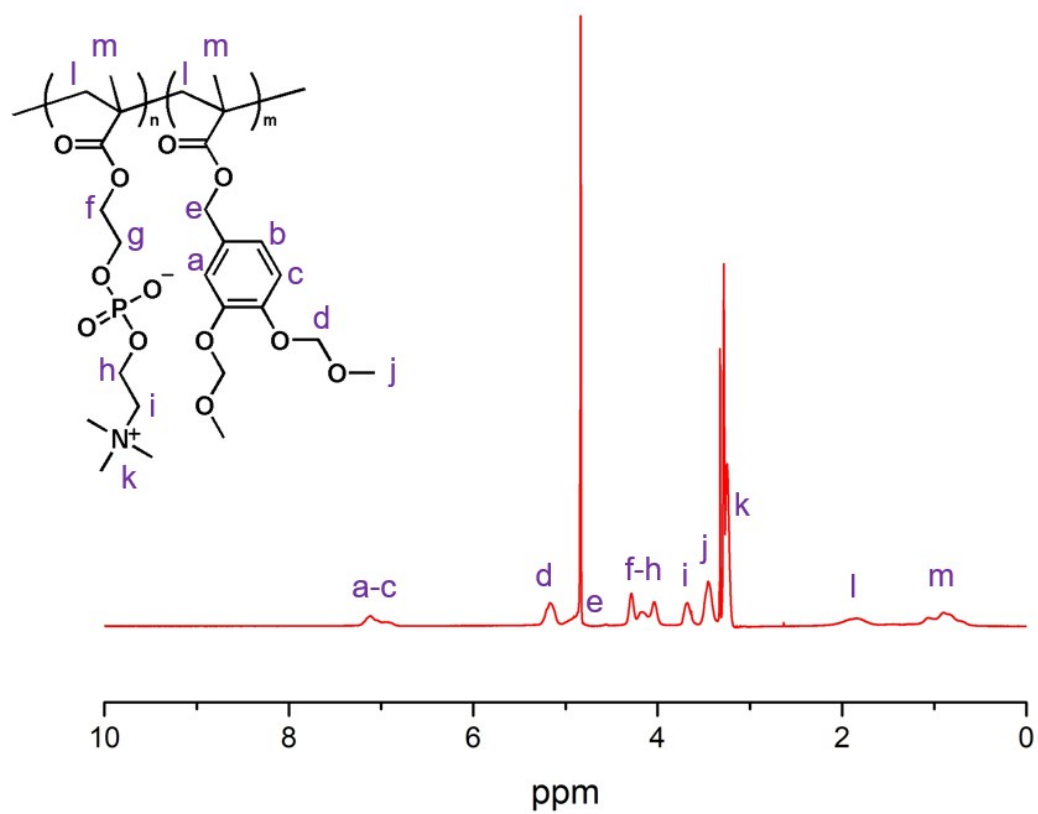


Figure S5.  $^1\text{H}$  NMR of P(MPC-co-DMBM) (400 MHz,  $\text{CD}_3\text{OD}$ )

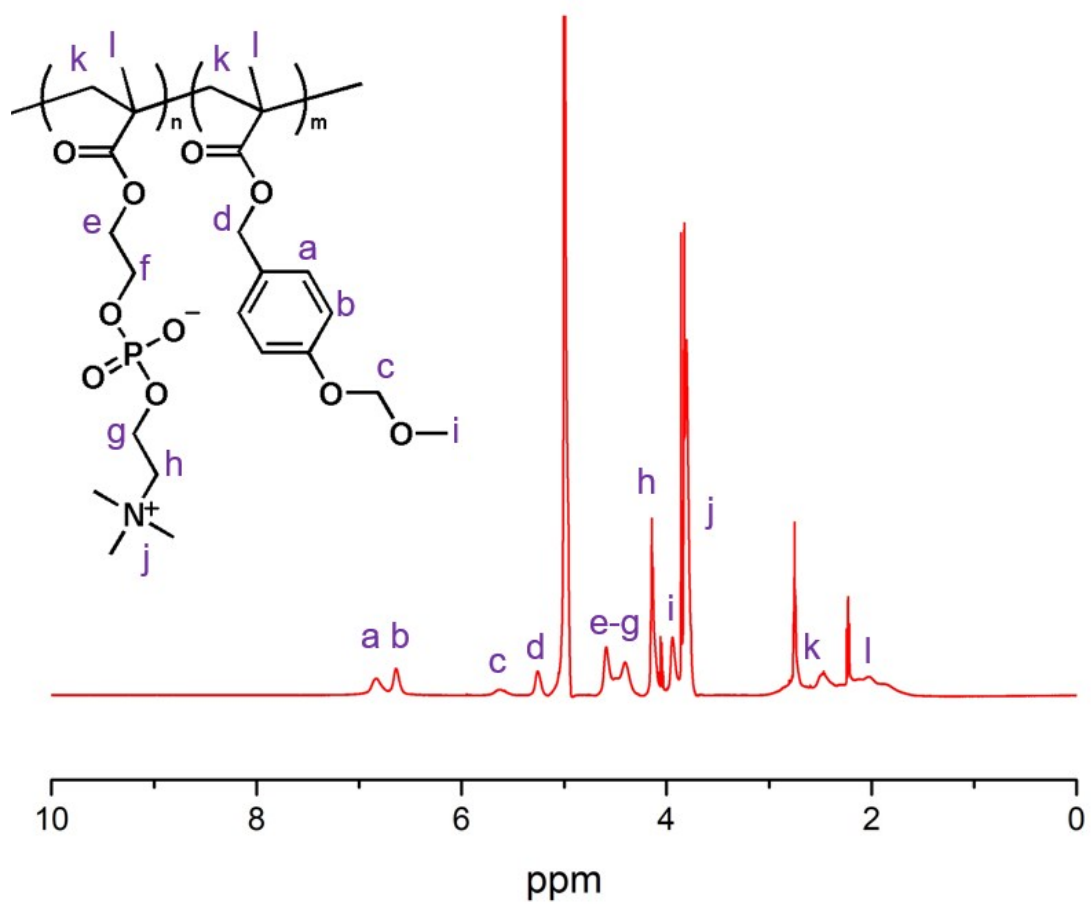


Figure S6. <sup>1</sup>H NMR of P(MPC-co-MMBM) (400 MHz, CD<sub>3</sub>OD)

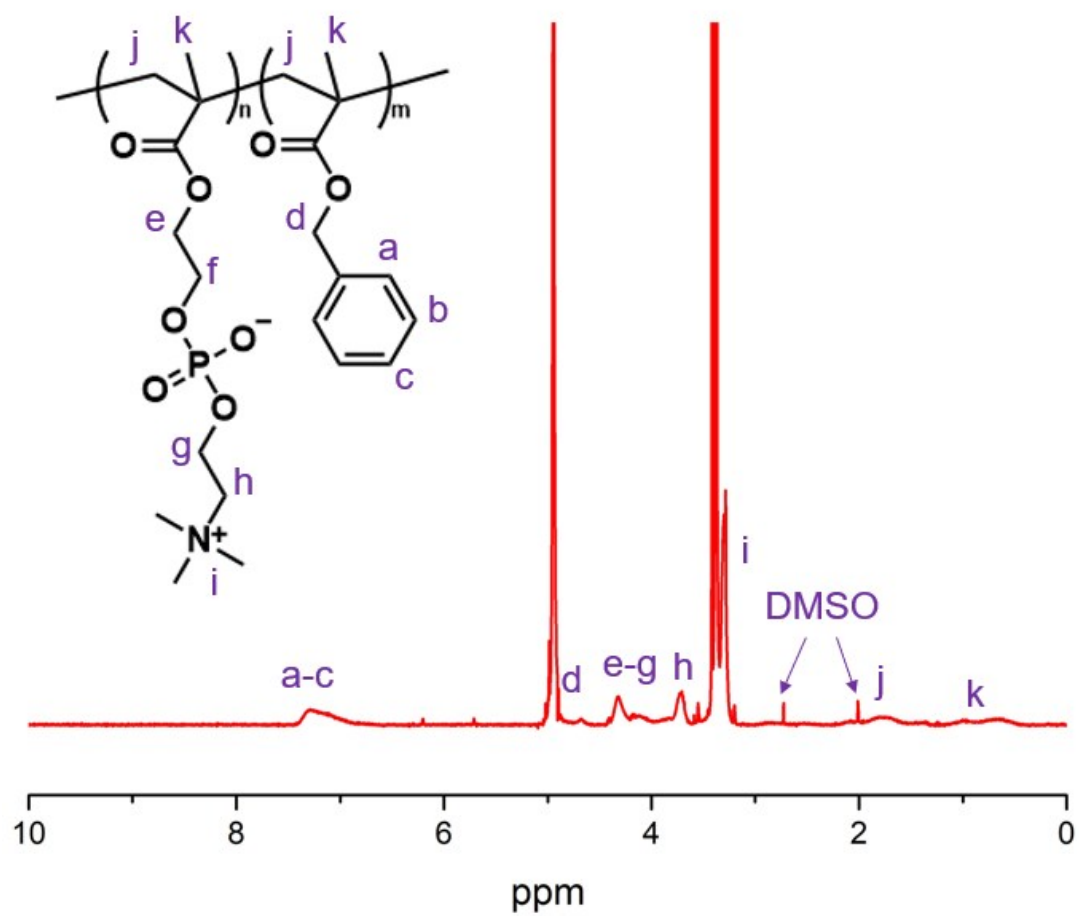


Figure S7.  $^1\text{H}$  NMR of P(MPC-co-MBz) (400 MHz,  $\text{CD}_3\text{OD}$ )

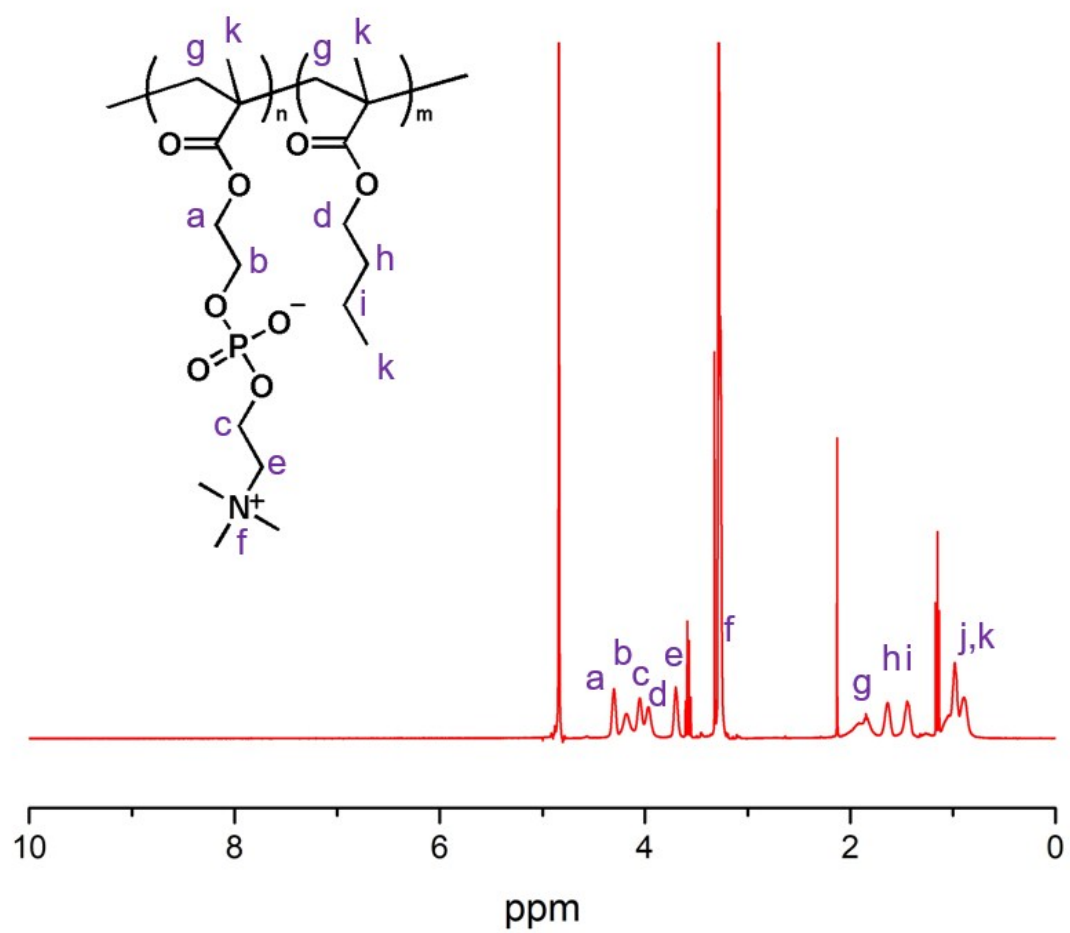


Figure S8.  $^1\text{H}$  NMR of P(MPC-co-BMA) (400 MHz,  $\text{CD}_3\text{OD}$ )

Table S1. Molecular weights, compositions, and PDIs of copolymers

	$M_n (\times 10^4)^*$	PDI*	Composition of MPC units**	Composition of tethering units**
P(MPC-co-TMBM)	2.2	2.0	48	52
P(MPC-co-DMBM)	2.5	1.9	49	51
P(MPC-co-MMBM)	2.4	1.9	50	50
P(MPC-co-MBz)	1.8	1.5	50	50
P(MPC-co-BMA)	1.7	1.8	50	50

\* Characterized by GPC

\*\* Calculated by  $^1\text{H}$  NMR



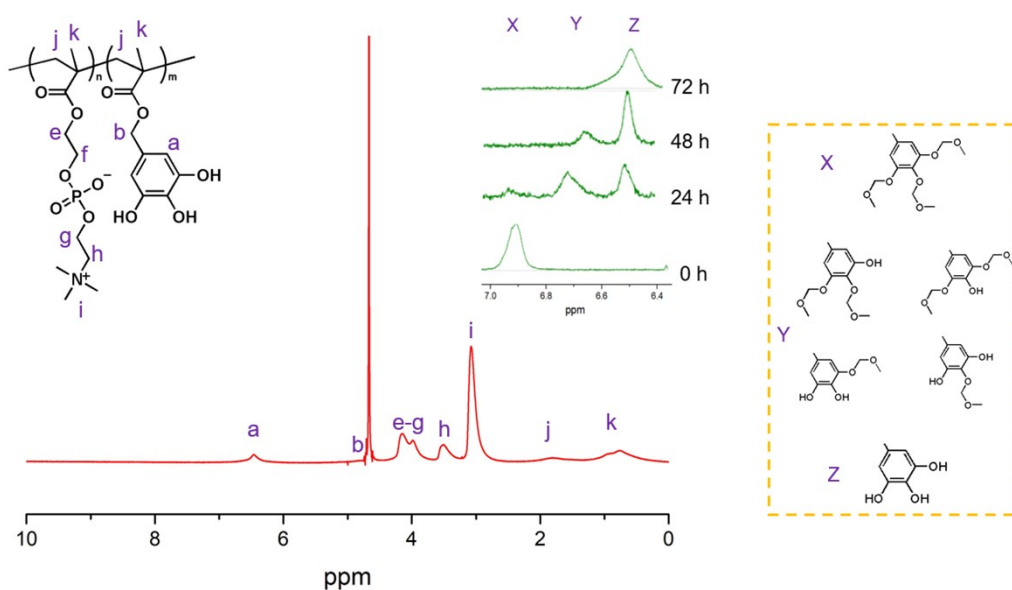


Figure S9. De-protection of gallol-functionalized polymer monitored by  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ). The completion of de-protection can be proven by the location shift of peak “a” and the disappearance of the MOM groups in the proximity of 5.2 ppm and 3.5 ppm. The de-protection of other phenolic polymers was carried out under the same conditions.

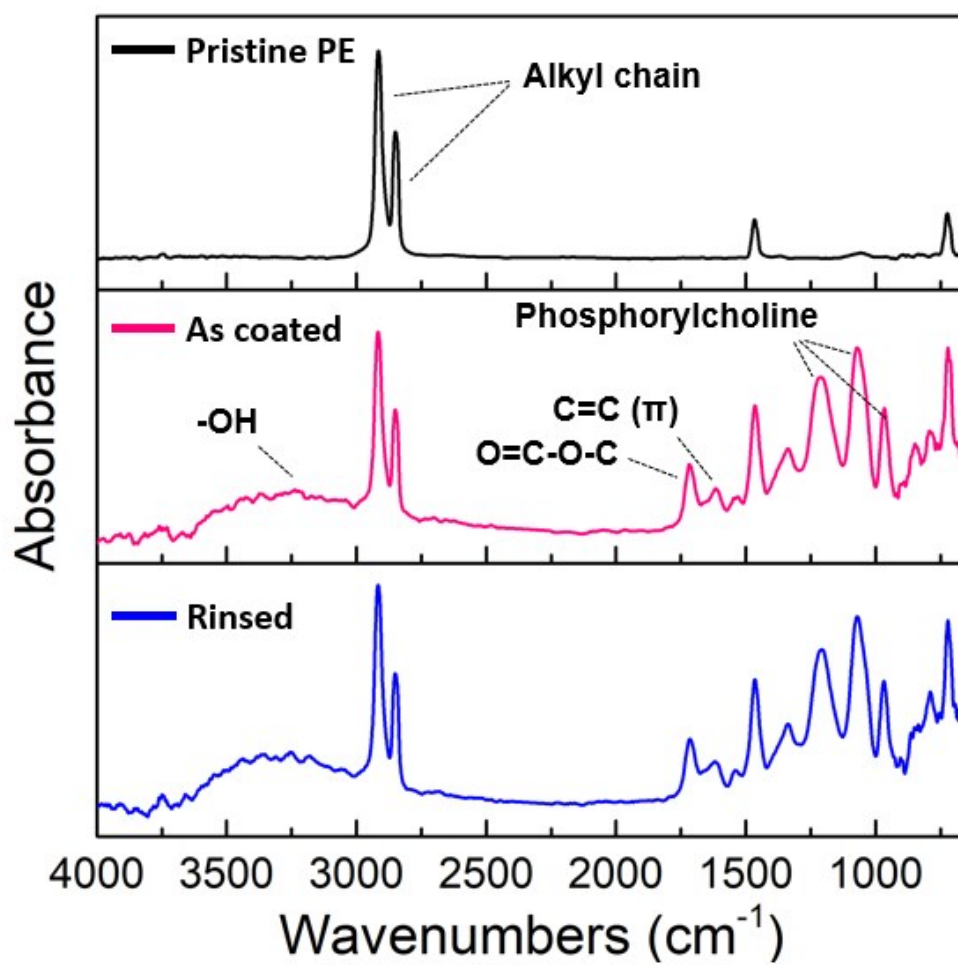


Figure S10. ATR-FTIR spectra of P(MPC<sub>48</sub>-co-MGal<sub>52</sub>) coated polyethylene (PE)

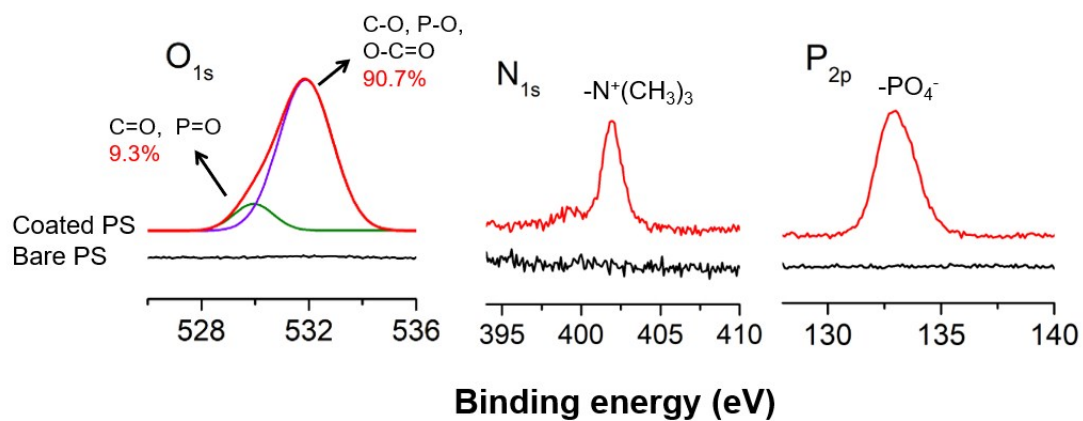


Figure S11. XPS spectra of P(MPC<sub>48</sub>-co-MGal<sub>52</sub>) coated polystyrene (PS)

Table S2. Atomic concentration (%) of surfaces determined by XPS

	PS (Theoretical)	PS (measured)	P(MPC <sub>48</sub> %-co-MGal <sub>52</sub> %) coated PS (Theoretical)	P(MPC <sub>48</sub> %-co-MGal <sub>52</sub> %) coated PS (measured)
C <sub>1s</sub>	100%	99.97%	63.89%	65.39%
O <sub>1s</sub>	0.00%	0.02%	30.56%	28.97%
N <sub>1s</sub>	0.00%	0.00%	2.78%	3.10%
P <sub>2p</sub>	0.00%	0.01%	2.78%	2.54%

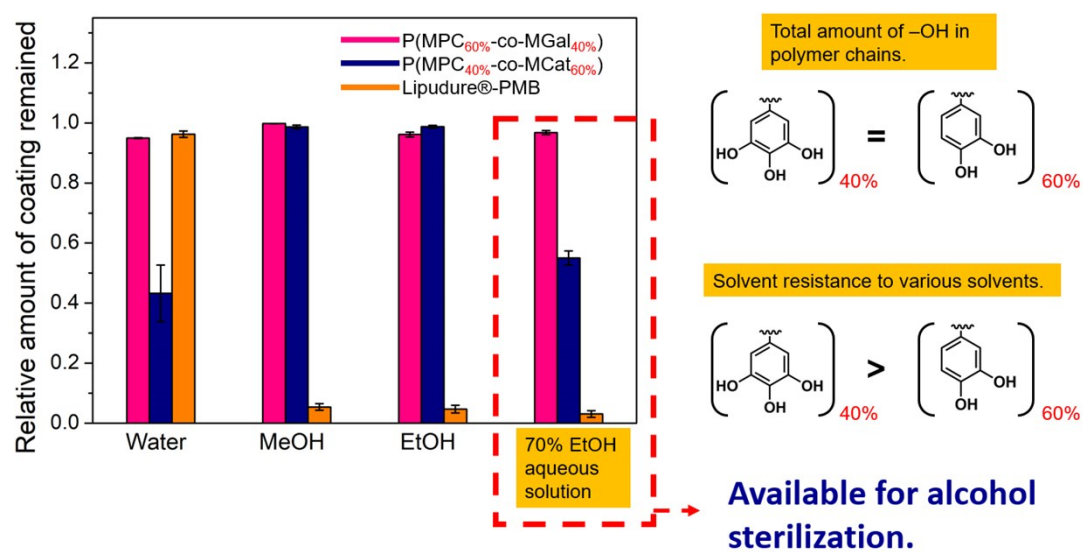


Figure S12. Relative amounts of polymers remaining on Au surfaces (the original amount is 1.0). The amounts were calculated by recording the frequency shift of Au QCM sensors after rinsing.

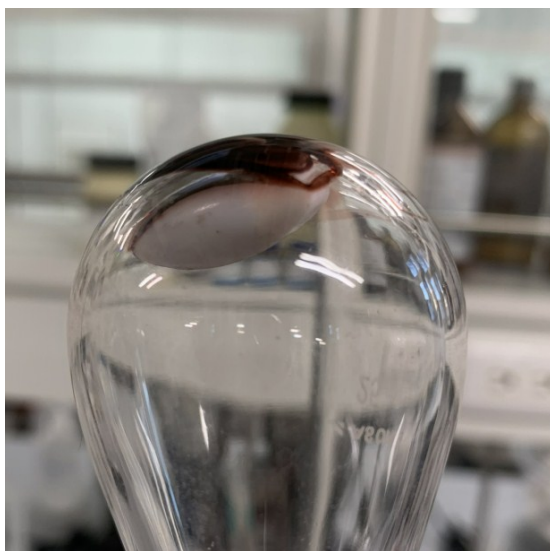


Figure S13. Irreversible precipitation during the de-protection of P(TMBM).

Table S3. Hydrophilicity of uncoated and coated surfaces

	Bare Au substrate	LIPIDURE®-PMB coated Au	P(MPC <sub>48%</sub> -co-MGal <sub>52%</sub> ) coated Au
SCA (Water drop in air, $\theta^\circ$ )	$66.3 \pm 0.7$	$104.3 \pm 4.9$	$51.4 \pm 4.7$
SCA (Air drop in water, $180-\theta^\circ$ )	$55.6 \pm 6.6$	$24.3 \pm 5.0$	$15.5 \pm 5.5$

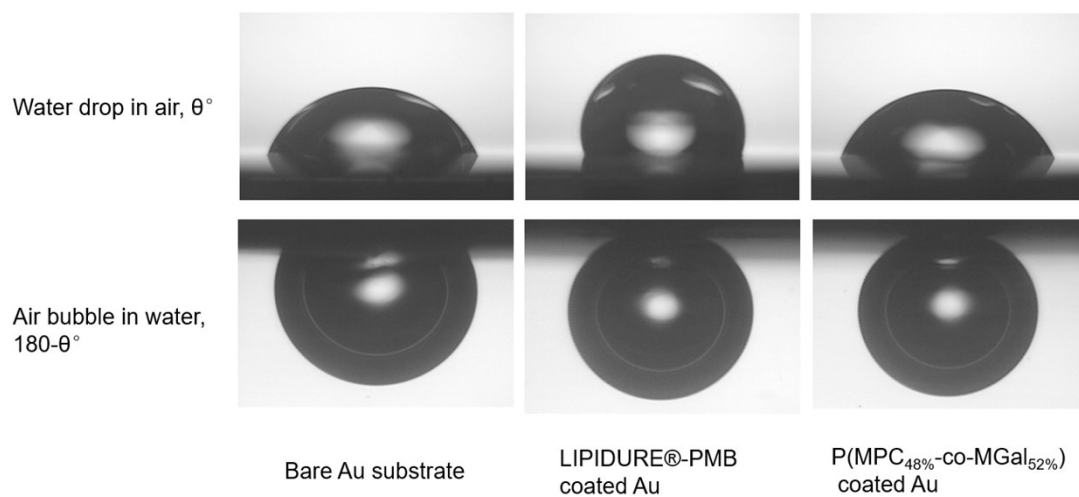


Figure S14. Photos of water droplets or air bubbles on bare and coated surfaces.

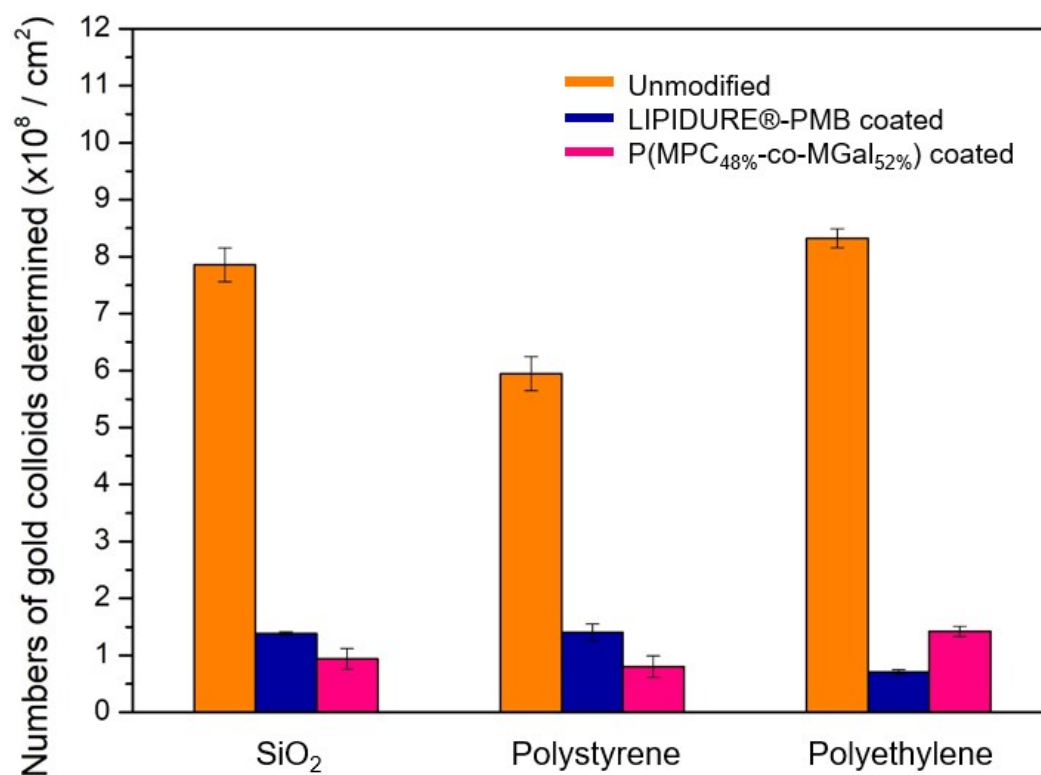


Figure S15. Amount of albumin at the FBS-contacting SiO<sub>2</sub>, polystyrene and polyethylene surfaces. The amounts were determined by calculating the numbers of gold colloids observed on the surfaces.



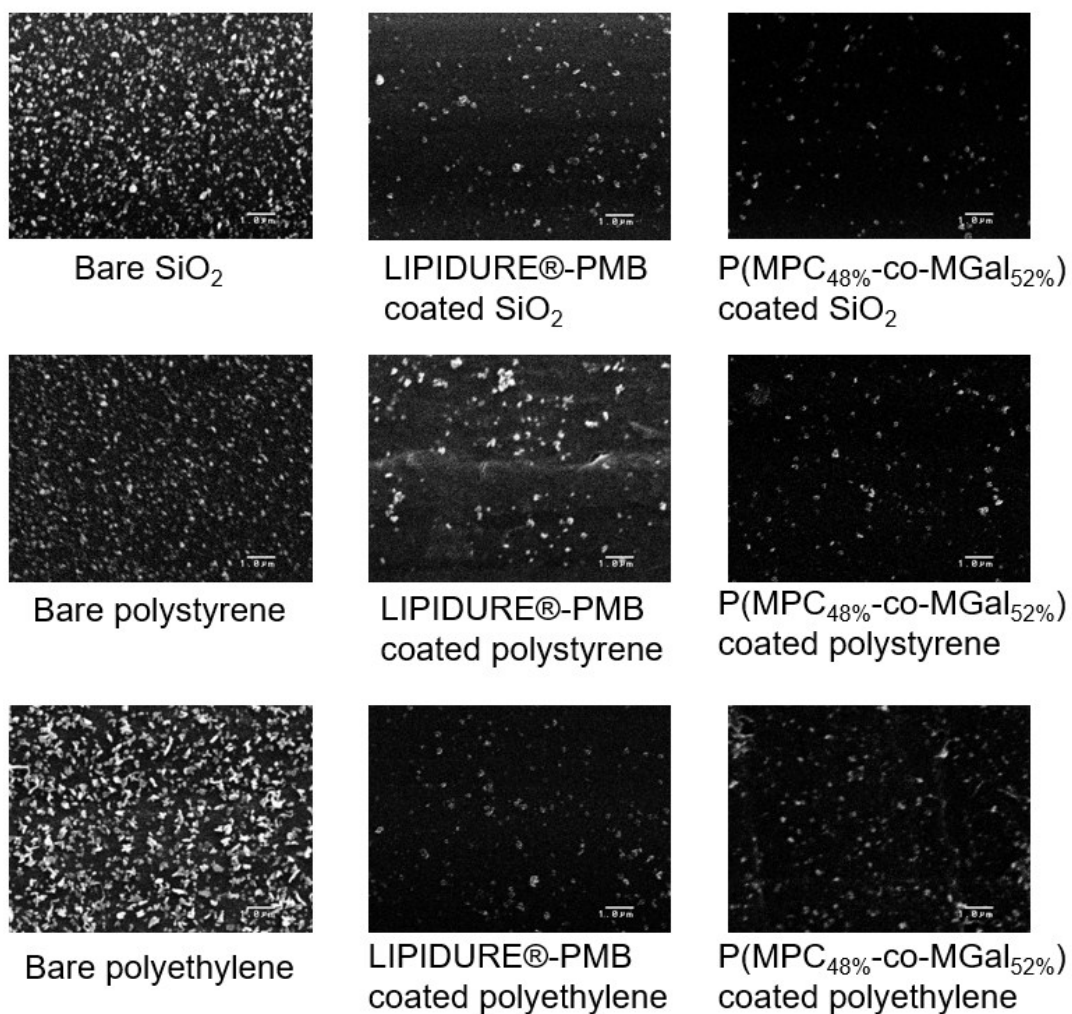


Figure S16. SEM photographs of gold colloids adsorbed on the FBS-contacting SiO<sub>2</sub>, polystyrene and polyethylene surfaces.