

## Supporting Information for

# Synthesis of Grafted Phosphorylcholine Polymer Layers as Specific Recognition Ligands for C-Reactive Protein Focused on the Grafting Density and Thickness to Achieve Highly Sensitive Detection

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## ***S1. Experimental Section***

### ***S1-1. Materials***

Ethanol, CuBr<sub>2</sub>, L-ascorbic acid, ethylene diamine tetraacetic acid tetrasodium (EDTA-4Na), human serum albumin (HSA), pyridine, and diethyl ether were purchased from Nacalai Tesque (Kyoto, Japan). 2-bromo-2-methylpropionyl bromide was purchased from TCI Corporation (Tokyo, Japan). Dichloromethane, hexane, and ethyl acetate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). C-reactive protein was purchased from AbD Serotec (Kidlington, United Kingdom). MPC was purchased from NOF Corporation (Tokyo, Japan). Bis[2-(2-bromoisobutyryloxy)undecyl] disulfide, (11-mercaptoundecyl)tetra(ethylene glycol), tris[2-(dimethylamino)ethyl]amine (Me<sub>6</sub>TREN), and triethylene glycol monomethyl ether were purchased from Sigma-Aldrich (USA). All water used was obtained from a Millipore Milli-Q purification system. Gold-coated SPR sensor chips (superficial area used in protein binding: 120 mm<sup>2</sup>) were purchased from GE Healthcare (Tokyo, Japan). Gold substrates for X-ray photoelectron spectra (XPS) and ellipsometry measurements were purchased from JASCO Corporation (Tokyo, Japan). Alumina 90, Activated, Basic, Activity I was purchased from Merck Millipore (Darmstadt, Germany).

### ***S1-2. Apparatus***

XPS measurements were carried out using a PHI X-tool (ULVAC-PHI, Inc., Kanagawa, Japan). All thicknesses and surface roughness characterization of SAMs and polymeric layers were measured using MARY-102FM (Five Lab Co., Ltd., Saitama, Japan). SPR measurements were performed on a Biacore 3000 (GE Healthcare Japan, Tokyo, Japan).

GPC measurement was carried out using Tosoh GPC-8020 Model II high-speed liquid chromatography system (Tosoh Corporation, Tokyo, Japan).

### ***S1-3. Synthesis of water solvled ATRP initiator***

Triethylene glycol monomethyl ether (6.22 g, 37.9 mmol) and pyridine (3 mL, 37.2 mmol) were dissolved in 100 mL dried CH<sub>2</sub>Cl<sub>2</sub>, and the mix solution was cooled at 0 °C. 2-Bromo-2-methylpropionyl bromide (4.5 mL, 36.4 mmol) dissolved in 30 mL CH<sub>2</sub>Cl<sub>2</sub> was added under nitrogen atmosphere at 0 °C, and the reaction mixture was stirred over night at room temperatures. After the reaction, half volume solvent was evaporated and cold diethyl ether (15 mL) was added and kept -20 °C for overnight to precipitate white powder. After removal of the white powder with filtration, the filtrated solution with impurities was purified by silica column chromatography (eluent: Hexane→Hexane : Ethyl acetate= 1:1→Ethyl acetate), and then slightly yellow-tinged liquid oil was obtained. The chemical structure of water-soluble ATRP initiator was confirmed using <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) measurement (5.92 g, yield: 55.4 %). <sup>1</sup>H-NMR: δ 1.94 (s, 6H), 3.38 (s, 3H), 3.53-3.76 (m, 10H). 4.33 (t, 2H)

#### ***S1-4. Selection of regeneration solution in SPR measurement***

Regeneration solutions were selected appropriately from following candidates; 0.075 % SDS + 150 mM NaCl aqueous solution, 10 mM Gly-HCl pH 1.5 and 0.3% Triton-100X. The optimal regeneration solutions were injected once or twice for 15 sec, 30 sec, 60 sec or 90 sec until protein binding signals dropped and reached a steady signal value.

#### ***S1-5. XPS measurement***

The formation of the each SAM and PMPC thin layer on a gold substrate were evaluated by XPS measurement. The conditions of the XPS measurements were as follows; X-ray source: AlK $\alpha$  (20 kV, 101 W); takeoff angle: 45°; initial survey scans: 0-1000 eV binding energy. Compositional narrow scans for C 1s, O 1s, S 2p, Br 3d, N 1s, P 2p and Au 4f were carried out using detector pass energy of 112 eV.

#### ***S1-6. Ellipsometry measurement***

Each SAM and PMPC thin layer was characterized by ellipsometry measurements. The conditions of ellipsometry measurements were as follows; wave length: 632.8 nm (He-Ne beam), measurement area: 2 × 2 mm in both areas (total 25 points), step 0.5 mm, incident angle: 70°. Fitting analysis was carried out by using the parameters of  $n= 0.181- 3.068i$  for Au,  $n= 3.136- 3.312i$  for Cr and  $n= 1.457-0.000i$  for substrate glass (SiO<sub>2</sub>).

#### ***S1-7. Gel permeation chromatography (GPC) experimental***

GPC analysis was carried out at 25°C on a Tosoh GPC-8020 Model II system equipped with two 30 cm columns (Tskgel GMPW<sub>XL</sub>, exclusion limit= 50,000,000), and a differential refractometer (Tosoh RI-8020). In order to estimate grafting densities of PMPC thin layers obtained after 3h polymerization on SAM A, B, and C, we added water-soluble synthesized ATRP initiator to the pre-polymer solution with 1/200 molar ratio to MPC monomer. After polymerization, Cu (II) ions were removed from the polymer solution with Alumina 90, Activated, Basic, Activity I column ( $\Phi$  5 mm ×H 5 cm). Mw (weight-average molecular weight) and Mn (number-average molecular weight) of produced polymers without initiator molecular weight of 282 were estimated by using GPC instruments whose system were calibrated by poly(ethylene oxide) solutions of Mw 420, 4210, 11900, 21600, and 610000. And then the grafting densities ( $\sigma$ ) were estimated by using the formula:  $\sigma = h \rho N_A / M_w$ , where  $h$  is the dried PMPC thickness on SAM A, B, and C measured by ellipsometry,  $\rho$  is the bulk PMPC density (1.30 g/cm<sup>3</sup> for PMPC<sup>S3</sup>),  $N_A$  is Avogadro's number, and  $M_w$  is the weight-average molecular weight.

## ***S2. Characterization of SAMs A, B, and C, and PMPC thin layers on SAMs A, B, and C by XPS measurement***

We performed peak separation analysis of C and O using the software package MultiPak in order to estimate the approximate incorporation ratio of the ATRP initiator in SAMs A, B, and C (Figure S1). The incorporation percentages of C-C, C-O, and C=O bonds were obtained by calculating peak area ratios, as shown in Table S1.

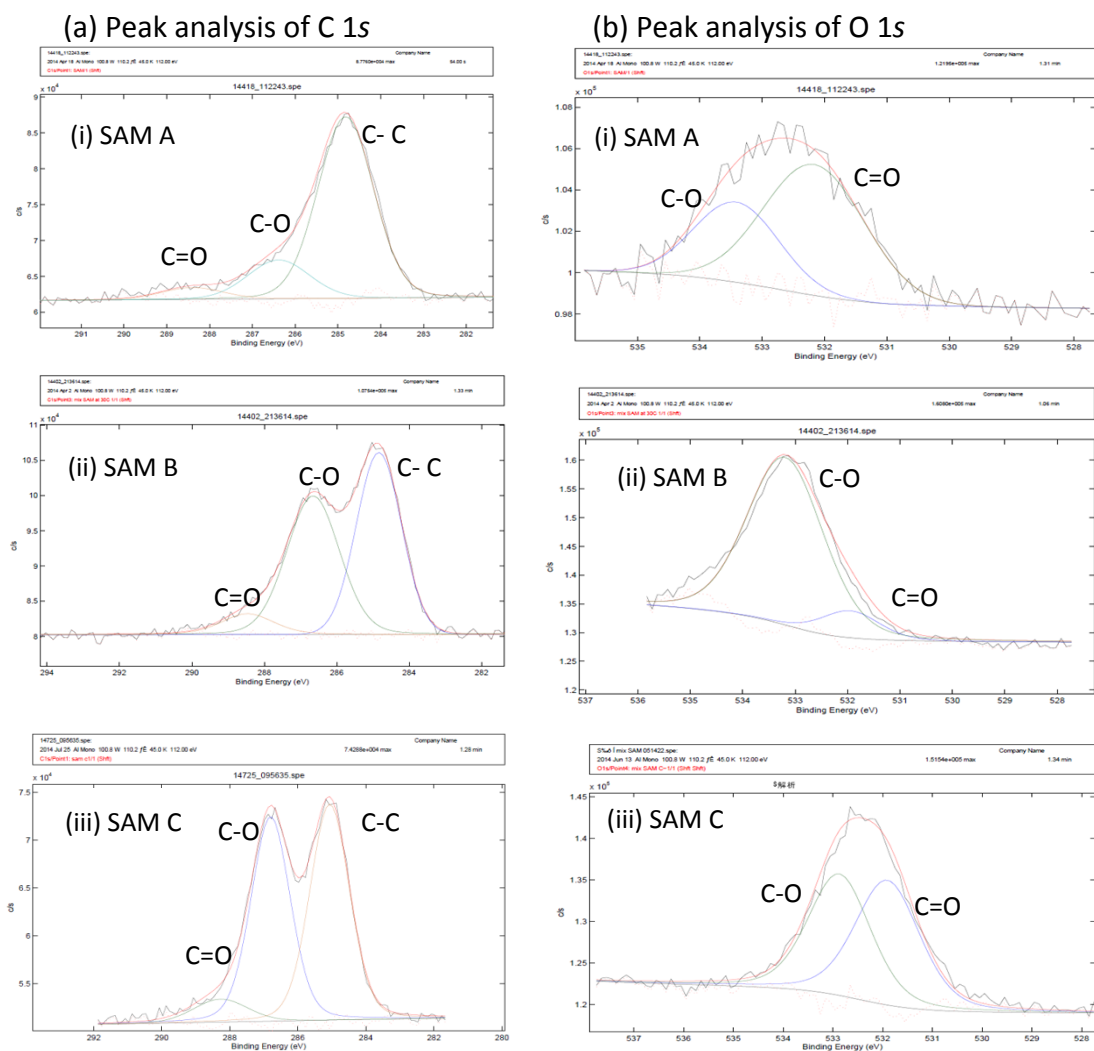


Figure S1. Peak analysis of C 1s (a) and O 1s (b) of SAMs A (i), B (ii) and C (iii).

Table S1. Comparison of element percentage of each C bound between SAMs A, B, and C.

Samples	C-C bond [%] (at 285 eV)	C-O bond [%] (at 286.5 eV)	C=O bond [%] (at 288 eV)
SAM A	73.1	17.9	9.0
SAM B	49.7	41.2	9.1
SAM C	47.9	45.5	6.7

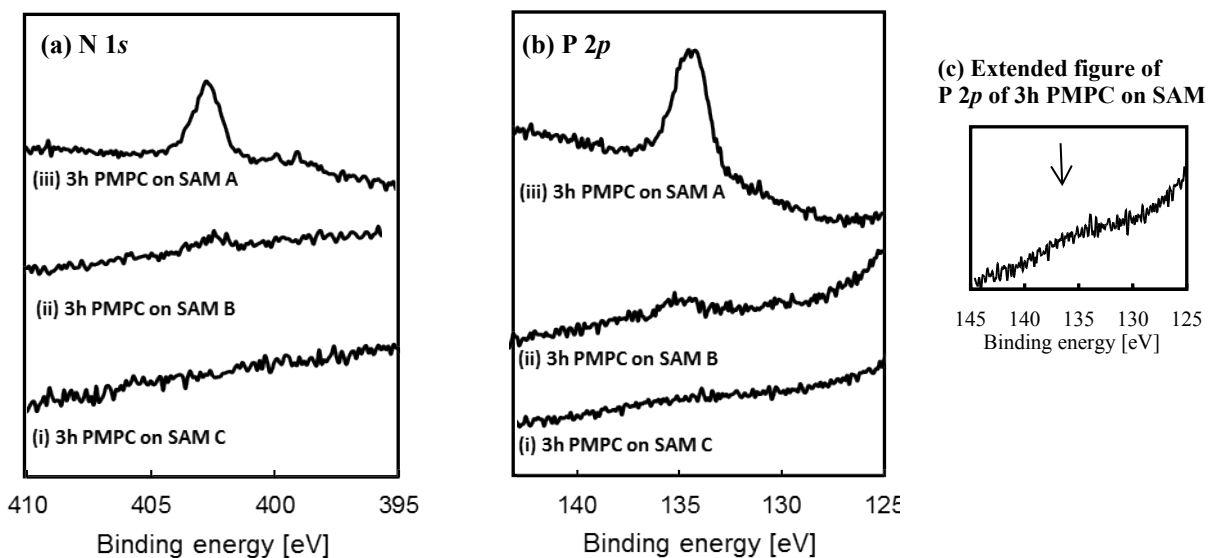


Figure S2. XPS spectra of N 1s (a) and P 2p (b) of PMPC layers obtained at 3h polymerization on SAM A, B and C, and extended figure of P 2p (c) of 3h PMPC on SAM C.

### ***S3. Characterization of thickness and roughness of SAMs and PMPC layers by ellipsometry measurements***

Ellipsometry is widely used in industry in that it allows for the contact-free measurement of the thickness and optical constants of all kinds of layers, since the measured signal depends on both thickness and material properties. Ellipsometry measures changes in polarization as incident radiation interacts with the material structure. This change is quantified by the amplitude ratio,  $\Psi$ , and the phase difference,  $\Delta$ , distinguishing both the  $p$ - ( $r_p$ ) and  $s$ -components ( $r_s$ ) of the reflected light. If the sample is a multilayer film,  $r_p$  and  $r_s$  are given as a function of film thickness ( $d$ ), incident angle ( $\varphi$ ), wavelength ( $\lambda$ ), and the refractive index ( $n = n - ik$ ) of the film and the substrate. These are referred to as the Fresnel coefficients, which are utilized in the following equation:<sup>S1</sup>

$$\rho = \tan\psi \exp(i\Delta) = \frac{r_{012,p}}{r_{012,s}} = \frac{\left[ \frac{r_{01,p} + r_{12,p} \exp(-i2\beta)}{1 + r_{01,p} r_{12,p} \exp(-i2\beta)} \right]}{\left[ \frac{r_{01,s} + r_{12,s} \exp(-i2\beta)}{1 + r_{01,s} r_{12,s} \exp(-i2\beta)} \right]} \quad (S1)$$

$$\beta = 2\pi \left( \frac{d}{\lambda} \right) n_1 \cos\theta_1$$

An ellipsometer (MARY-102 FM, FiveLab) was used, with the incident angle set to  $70^\circ$ , a fixed wavelength of 632.8 nm, and a beam spot with diameter of 0.8 mm. We carried out the ellipsometry measurements with three different substrates prepared completely same conditions. The measurement area was  $2 \text{ mm} \times 2 \text{ mm}$  which has 75 measure points. Optical constants of  $n = 1.456 - 0i$ ,  $n = 3.1 - 3.31i$ , and  $n = 0.161 - 3.37i$  were used for glass, chromium, and gold, respectively.

We used changes in the  $\Delta$  value we used changes in the  $\Delta$  value to monitor thickness and layer growth in a manner similar to how it is commonly used for metal substrates; at the initial stage of layer growth  $\Delta$  drops proportionally as layer thickness increases until 30 nm is reached (Figure S3). We assumed  $n = 1.5 - 0i$  for all calculations; this was meant to simplify calculations, as the refractive index of SAMs should change given that they are a mixture of two compounds, each of which have their own optical value.

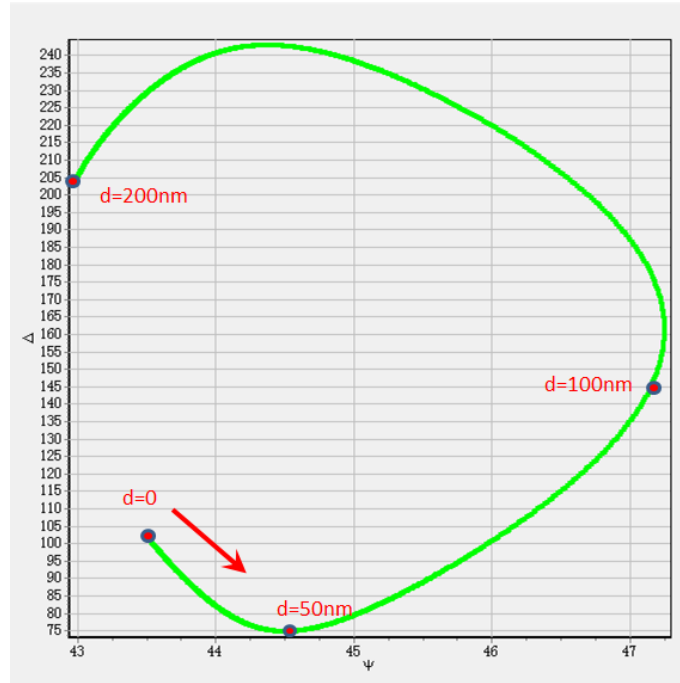


Figure S3.  $\Delta$ - $\psi$  simulation graph associated with thickness increasing of polymer films or layers whose refractive index were assumed as 1.5 on the gold substrate

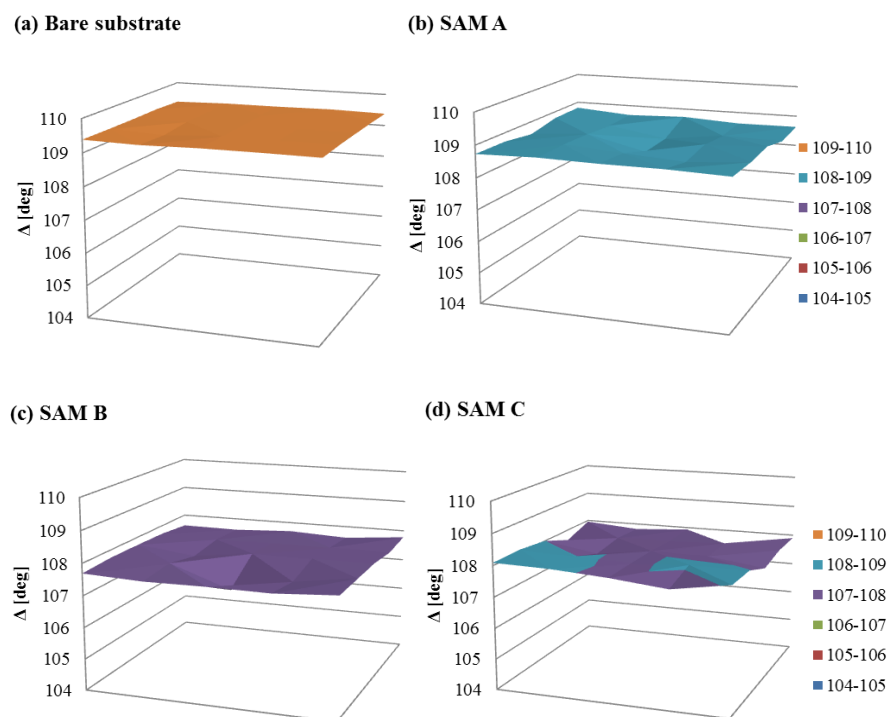


Figure S4.  $\Delta$  value mapping results (25 points in mapping area: 2 mm  $\times$  2 mm) of bare substrate (a), SAM A (b), SAM B (c) and SAM C (d).

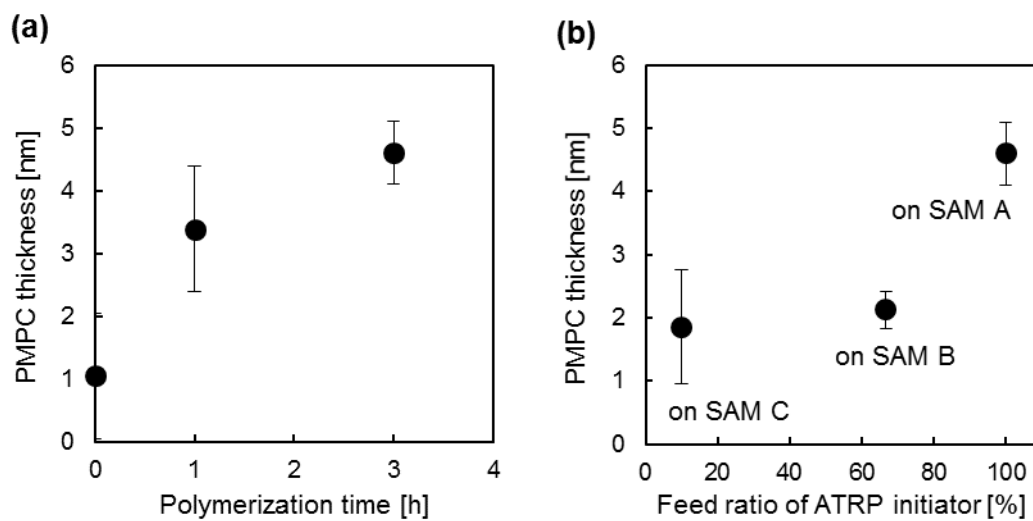


Figure S5. PMPC layer thickness obtained on SAM A vs. polymerization time (a) and PMPC layer thickness obtained after 3h polymerization vs. feed ratio of ATRP initiator (b) determined by ellipsometry. The error bars were derived from the thickness of three samples prepared by the same procedure.

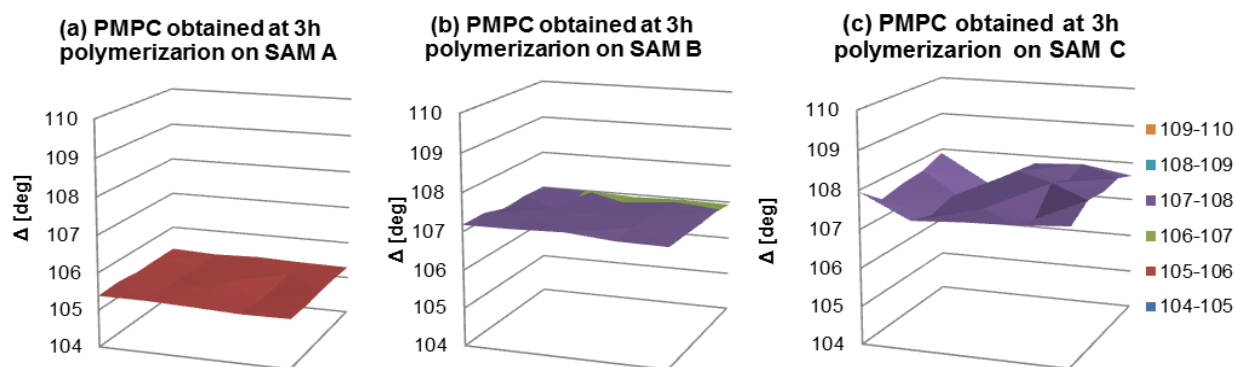


Figure S6.  $\Delta$  value mapping of PMPC thin layers obtained at 3h polymerization on SAMs A (a), B (b) and C (c) measured by ellipsometry experiments.

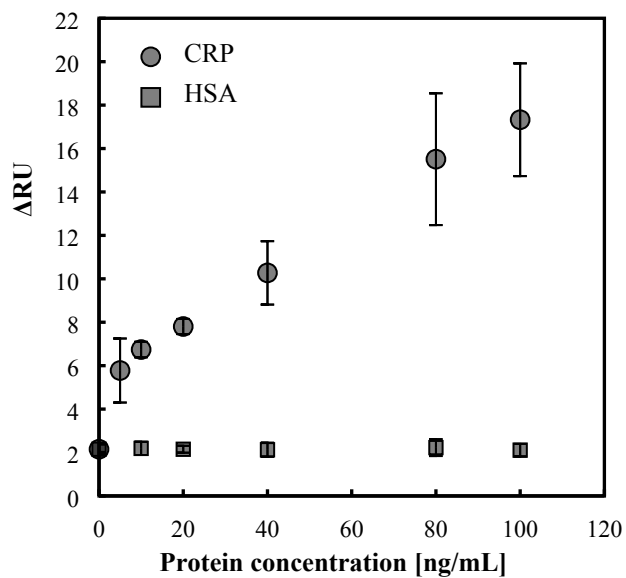


Figure S7. CRP binding selectivity toward PMPC obtained at 3h polymerization on SAM A below 100 ng/mL of protein concentration.



Table S2. Mn, Mw, PDI, Thickness, and grafting density of PMPC layers obtained at 3h polymerization on SAM A, B, and C.

	PMPC 3h on SAM A	PMPC 3h on SAM B	PMPC 3h on SAM C
Mn	2581	2644	2688
Mw	2833	2961	3027
PDI (Mw/Mn)	1.10	1.12	1.13
Thickness [nm]	4.6	2.1	1.9
Grafting density $\sigma$ [chains/nm <sup>2</sup> ]	1.27	0.56	0.48

### References

- S1. McCrackin, F. L.; Passaglia, E.; Stromberg, R. R.; Steinberg, H. L. Measurement of the Thickness and Refractive Index of Very Thin Films and the Optical Properties of Surfaces by Ellipsometry. *J. Res. Natl. Inst. Stan. Technol.* **1963**, 63, 363-377.
- S2. Fujiwara, H. *Spectroscopic ellipsometry: principles and applications*; [Online] John Wiley & Sons, 2007; Chapter 5, pp 147–207.  
<http://onlinelibrary.wiley.com/book/10.1002/9780470060193> (accessed Sep 1 2014)
- S3. R. Iwata, P. Suk-In, V. P. Hoven, A. Takahara, K. Akiyoshi and Y. Iwasaki, *Biomacromolecules*, 2004, **5**, 2308-2314.