Electronic Supplementary Information

Precisely Controlled Molecular Imprinting of Glutathione-s-Transferase by Orientated Template Immobilization Using Specific Interaction with an Anchored Ligand on a Gold Substrate

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XRR measurements

X-ray reflectivity profiles of each substrate were obtained as shown in Figure S1(a)-(d). X-ray reflectivity is represented as $|R_{n,n+1}|^2 = \frac{I}{I_0}$. Reflectivity ($R_{j,j+1}$) of each layer was obtained the following formulas.

$$R_{j,j+1} = \frac{(R_{j-1,j} + F_{j,j+1})}{R_{j-1,j} \times F_{j,j+1} + 1} \times {a_{j+1}}^4$$

$$F_{j,j+1} = \frac{g_{j+1}-g_j}{g_{j+1}+g_j} \times \exp\left(\frac{-8\pi^2 g_j g_{j+1} {\sigma_j}^2}{\lambda^2}\right),$$

$$a_{j+1} = \exp(-i\pi\, g_{j+1} d_{j+1}/\lambda),$$

$$g_j = \sqrt{n_j^{*2} - \cos^2\theta}$$

θ is the incidence angle of X-ray, λ is the X-ray wavelength, I_0 is the incident intensity, I is the reflected intensity, n^*_i is the refraction index of each layer, calculated according to the formula n^*_i:1-δ_i-i β_i, where δ_i and β_i are shifts from the refraction index (=1). δ_i and β_i are defined as follows:

$$\delta_i = r_e \lambda^2 \rho N_A / 2\pi \frac{\Sigma[x_j\{Z_j + f'_j(\lambda)\}]}{\Sigma[x_j A_j]},\ \beta_i = r_e \lambda^2 \rho N_A / 2\pi \frac{\Sigma[x_j\{Z_j f'_j(\lambda)\}]}{\Sigma[x_j A_j]}.$$

σ_j is the interfacial roughness of each layer and d_i is the thickness of each layer. The optimal values of these four parameters (δ_i, β_i, d_i and σ_i) were calculated by minimizing χ^2 and reliability factor (R(%)). χ^2 represents logarithmic error sum of the squares between the experimental value and calculated value via non-linear least-squares method.

$$\chi^2 = \sum_{i=1}^{N_p} \left[\log\{I_{exp}(\alpha_i)\} - \log\{I_{cal}(\alpha_i)\}\right]^2$$

$$R\,(\%) = \sqrt{\frac{\chi^2}{\sum_{i=1}^{N_p}\left[\log\{I_{exp}(\alpha_i)\}\right]^2}} \times 100$$

The thicknesses of the polymer thin films were estimated by fitting an analysis curve to each X-ray reflective profile. The starting values for fitting the experimental curves were as follows: substrate (element): first layer Au was 50 nm at 19.3 g/cm^3, second layer Cr was 40 nm at 7.19 g/cm^3, third layer SiO_2 was 0.0 nm at 2.20 g/cm^3. SiO_2 was carrier having 1.15~1.20 mm as thickness and it was too thick for X-ray reflection, therefore, the thickness was negligible for the curve fitting; element ratios of the mixed SAM and the polymer thin films were assumed as follows: mixed SAM as C: O: H: S: Br = 47: 9: 91: 3: 2 and polymer thin film as C: O: H: N = 30 : 1 : 15 : 50.

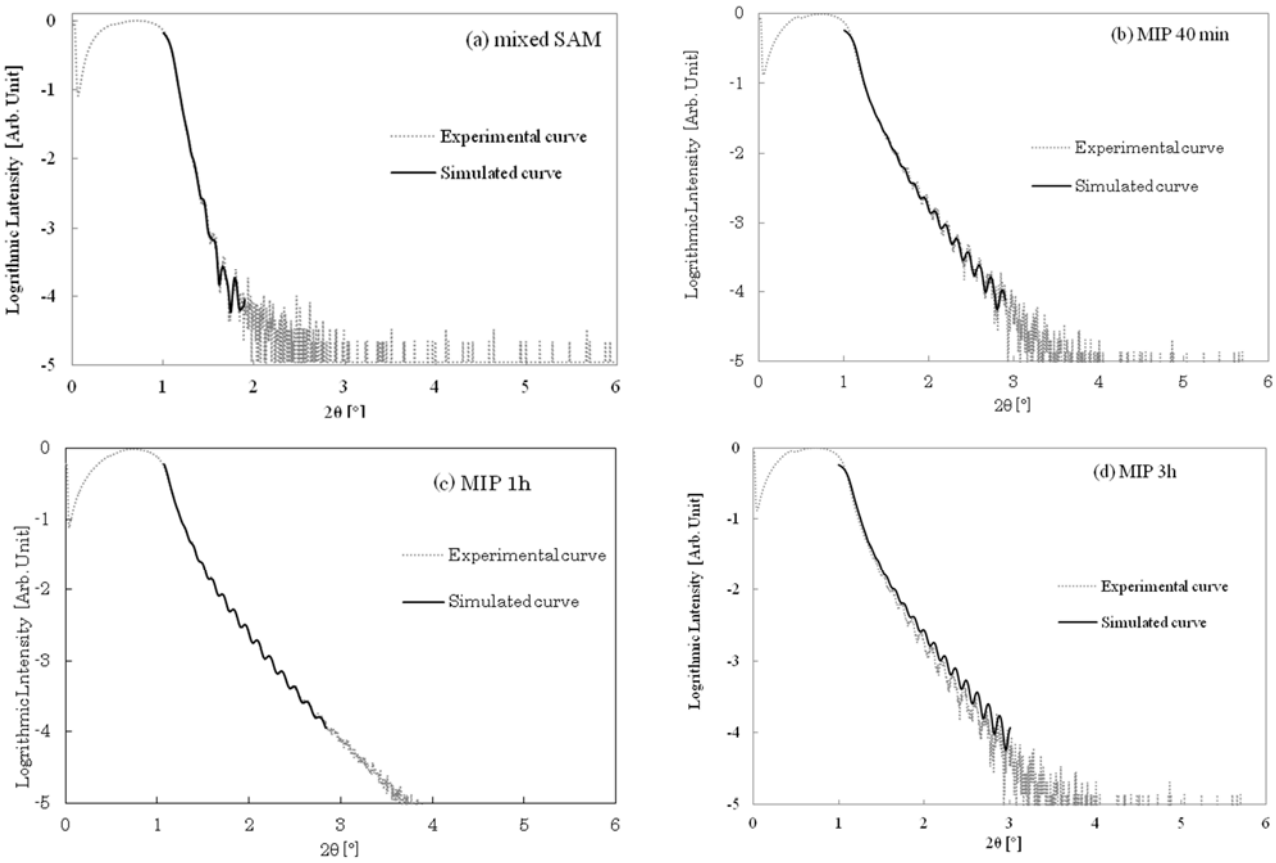


Figure S1 XRR experimental and simulated curves; (a) mixed SAM, (b) MIP 40 min, (c) MIP1h and (d) MIP 3h.

Table S1 XRR parameters obtained by fitting analysis (a) mixed SAM, (b) MIP 40 min, (c) MIP 1h and (d) MIP 3h

(a) mixed SAM

Layer Name	Density [g/cm^3]	Thickness [nm]	Roughness [nm]
mixed SAM	2.1	2.1	0.5
Au	19.5	51.0	2.2
Cr	13.0	42.0	1.3
SiO_2	2.2 (const.)	0.0 (const.)	1.7

(b) MIP 40 min

Layer Name	Density [g/cm^3]	Thickness [nm]	Roughness [nm]
polymer	3.9	12.7	2.1
Au	20.8	58.6	0.8
Cr	14.9	38.1	0.0
SiO_2	2.2 (const.)	0.0 (const.)	21.6 (const.)

(c) MIP 1h

Layer Name	Density [g/cm^3]	Thickness [nm]	Roughness [nm]
polymer	3.4	14.5	2.4
Au	19.2	56.8	0.8
Cr	9.1	38.9	1.4
SiO_2	2.2 (const.)	0.0(const.)	21.6 (const.)

(d) MIP 3h

Layer Name	Density [g/cm^3]	Thickness [nm]	Roughness [nm]
polymer	2.3	38.6	2.4
Au	20.5	61.7	0.8
Cr	14.2	40	0.0
SiO_2	2.2(const.)	0.0(const.)	21.6(const.)

Scatchard analysis

The association constant K_a toward GST-π was calculated from the Scatchard equation: $B/F = -BK_a + B_{max}K_a$. B is the amount of protein bound on MIP calculated by $B = \Delta RU / M_w$ [fmol/mm^2] and F is the concentration of free protein [μM], calculated according to this formula; $F = C_0 - [(\Delta RU \times S) / (M_w \times V)]$, where C_0 is the initial concentration of protein, V is the injection volume (20 μL) and S is the response area in a flow-cell (1.2 mm^2). K_a is the association constant [M^{-1}] and B_{max} is the maximum amount of bound proteins [fmol/mm^2].

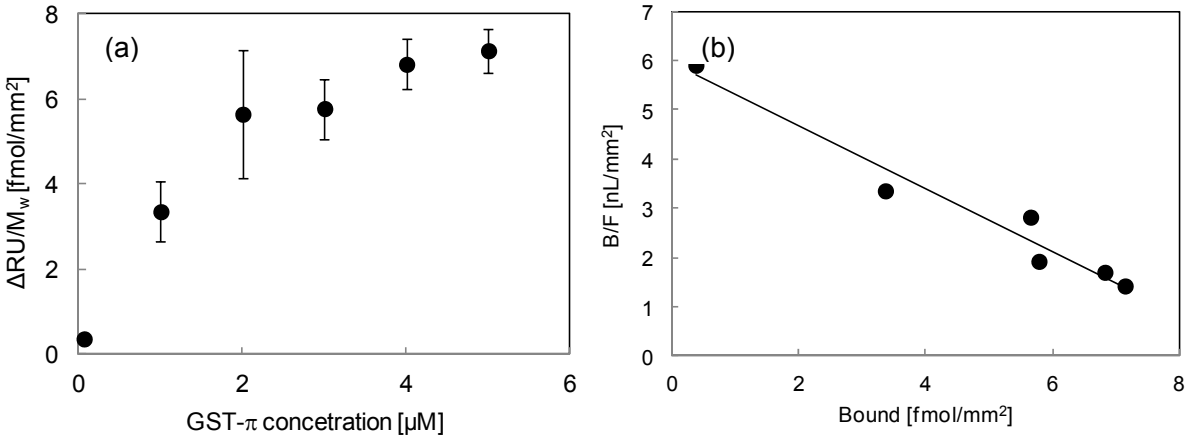


Figure S2. Binding isotherm (a) and Scatchard plot (b) of GST-π toward GSH-anchored gold substrate in 15 mM PBS buffer pH 7.4. An association constant and a maximum amount of immobilized GST-π were estimated to be 6.3×10^3 M^{-1} and 9.4 fmol/mm^2, respectively.

FT-IR spectra

FT-IR measurements were carried out by Reflection Absorption Spectroscopy (RAS) method using Varian 660 KU-IR (Agilent Inc., California, USA).

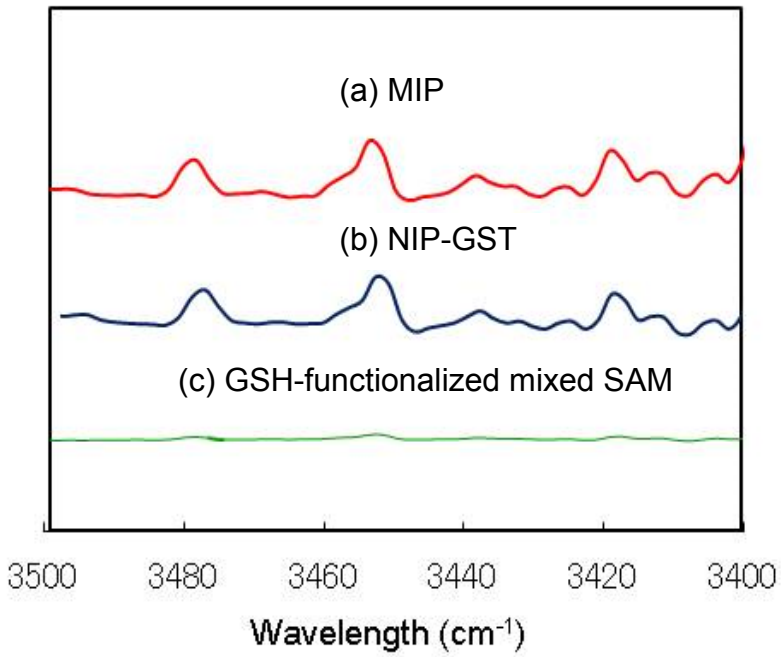
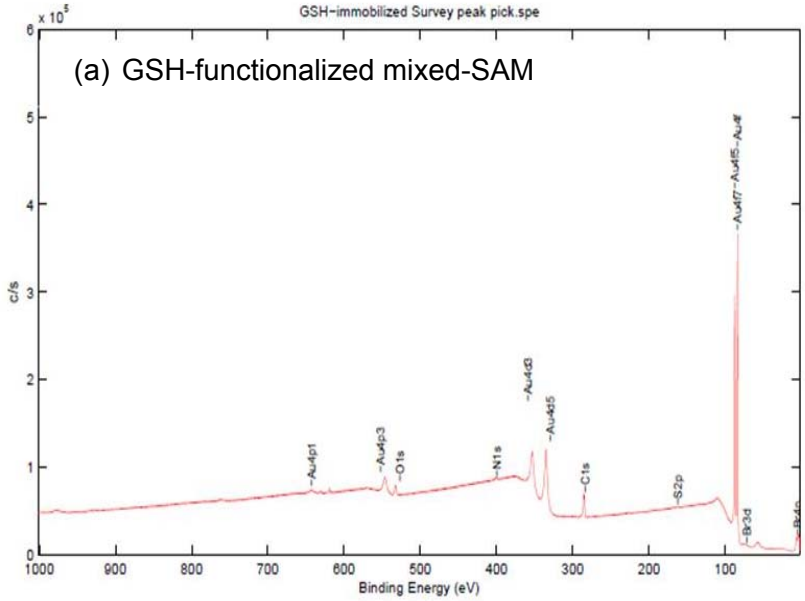
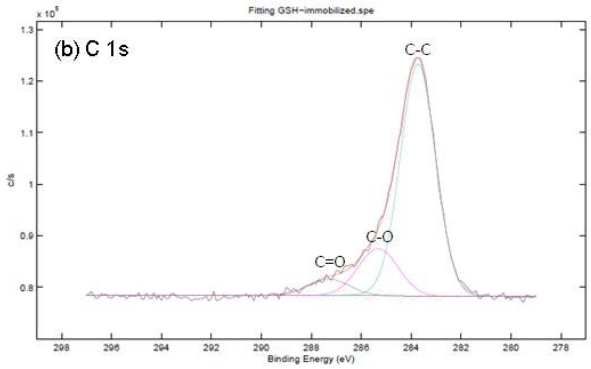
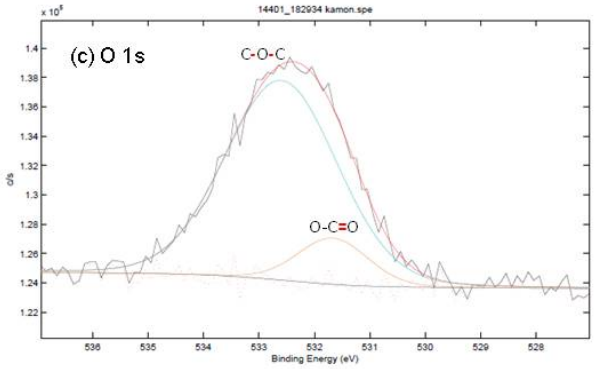


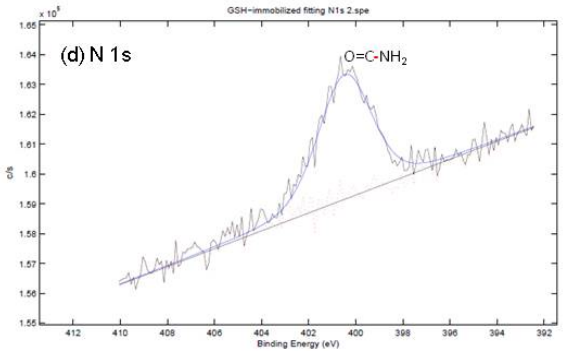
Figure S3 FT-IR spectra (RAS method) of MIP thin film (a), NIP-GSH thin film (b), and GSH-functionalized mixed SAM (c) and prepared on the gold substrate

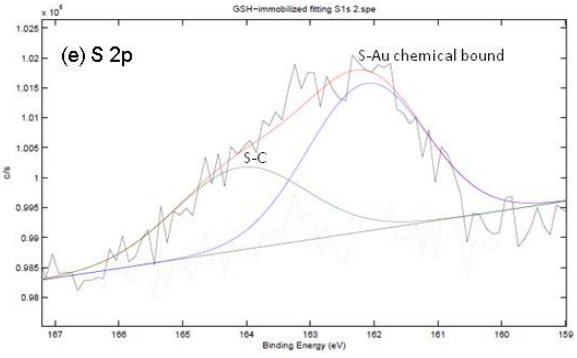
XPS spectra

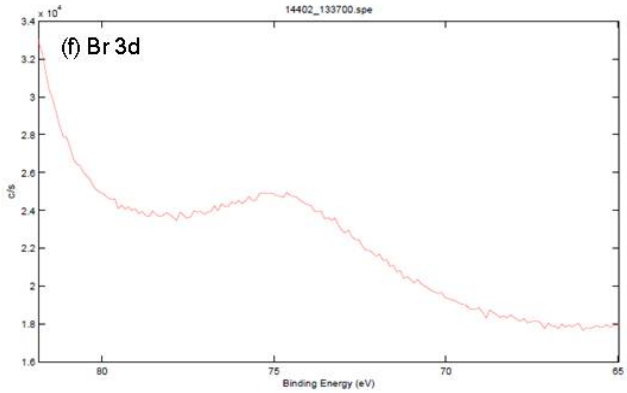

GSH-immobilized Survey peak pick.spe
(a) GSH-functionalized mixed-SAM
c/s
Binding Energy (eV)
Au4p1
Au4p3
O1s
N1s
Au4d3
Au4d5
C1s
S2p
Au4f7
Au4f5
Au4f
Br3d


Fitting GSH-immobilized.spe
(b) C 1s
C-C
C-O
C=O
c/s
Binding Energy (eV)


14401_182934 kamon.spe
(c) O 1s
C-O-C
O-C=O
c/s
Binding Energy (eV)


GSH-immobilized fitting N1s 2.spe
(d) N 1s
O=C-NH2
c/s
Binding Energy (eV)


GSH-immobilized fitting S1s 2.spe
(e) S 2p
S-Au chemical bound
S-C
c/s
Binding Energy (eV)


14402_133700.spe
(f) Br 3d
c/s
Binding Energy (eV)

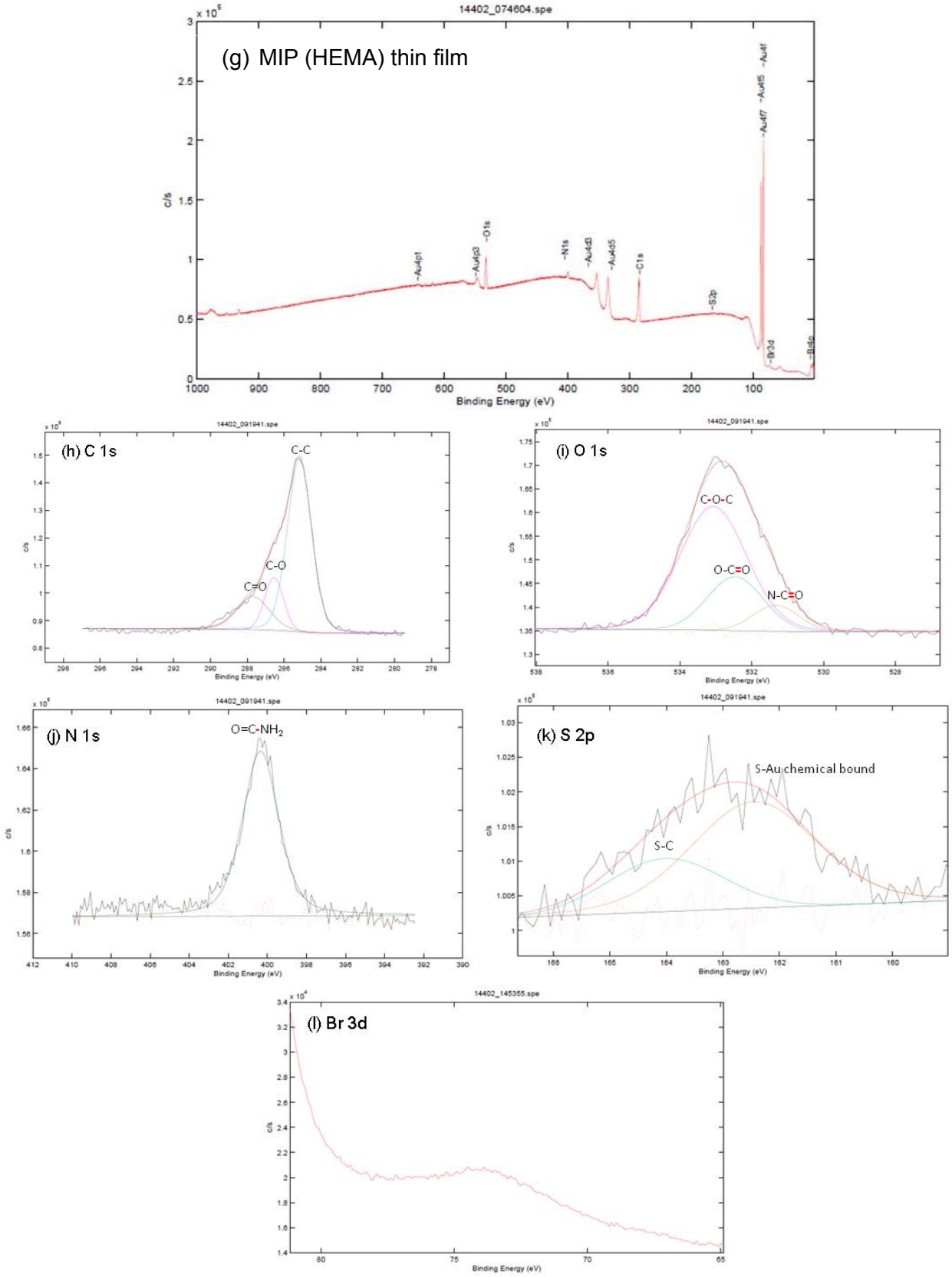


Figure S4 Survey (a,g) and narrow(b-f, h-l) XPS spectra of GSH-functionalized mixed SAM (a~f) and MIP (HEMA) thin film (g~l) prepared on the gold substrate.

Binding experiments

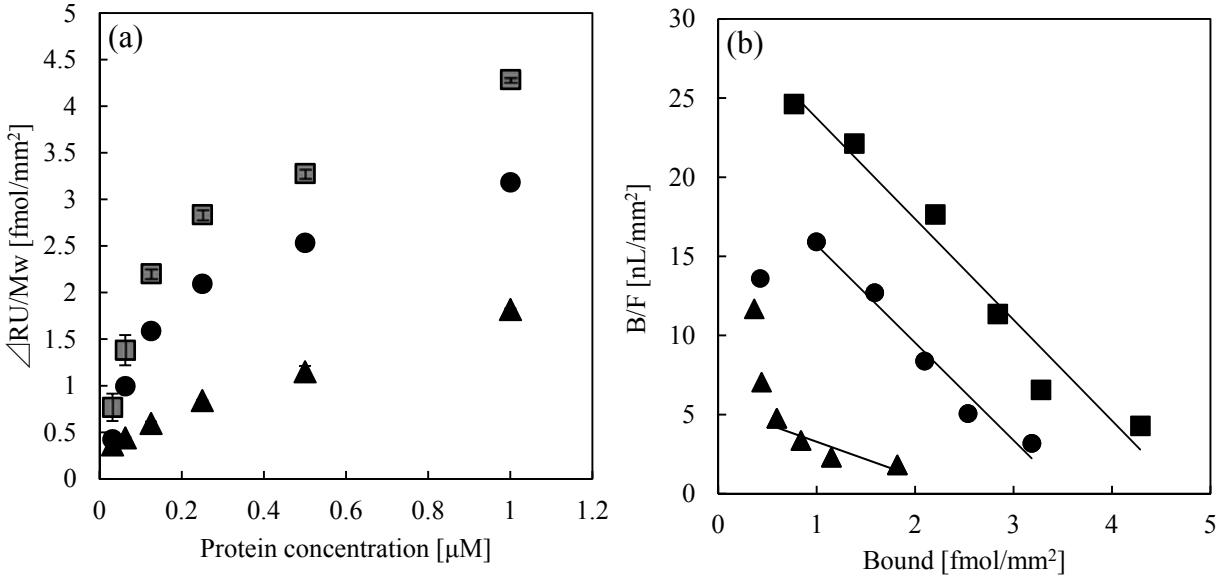


Figure S5 (a) Binding isotherms and (b) Scatchard plot of GST-π toward MIP 1h in three types of running buffers as follows; 10 mM Tris-HCl pH 7.4 (■), 10 mM HEPES buffer pH 7.4 (●) and 10 mM Phosphate buffer pH 7.4 (▲).

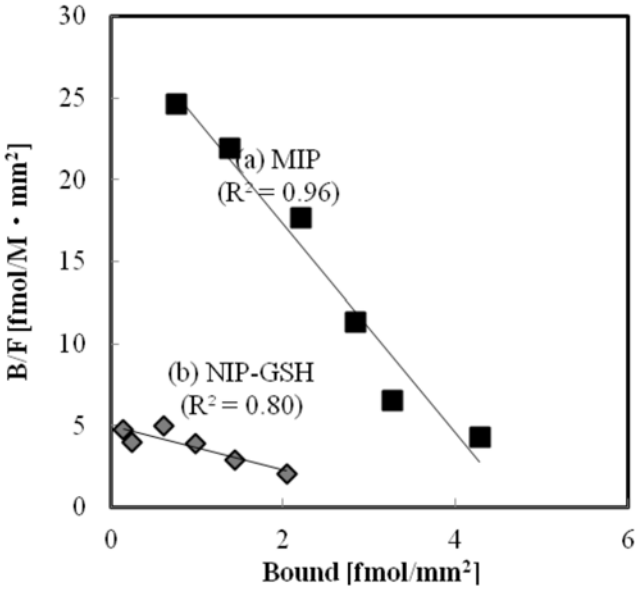


Figure. S6 Scatchard plots of GST-π binding towards (a) MIP and (b) NIP-GSH prepared with HEMA as a co-monomer in10 mM Tris-HCl buffer pH 7.4. Association constants were estimated to be 6.4 ×1 0^6 M^{-1} for MIP and 1.4 ×10^6 M^{-1} for NIP-GSH, respectively. The amounts of maximum binding cavities for MIP and NIP were estimated to be 4.7 fmol/mm^2 and 3.7 fmol/mm^2, respectively.

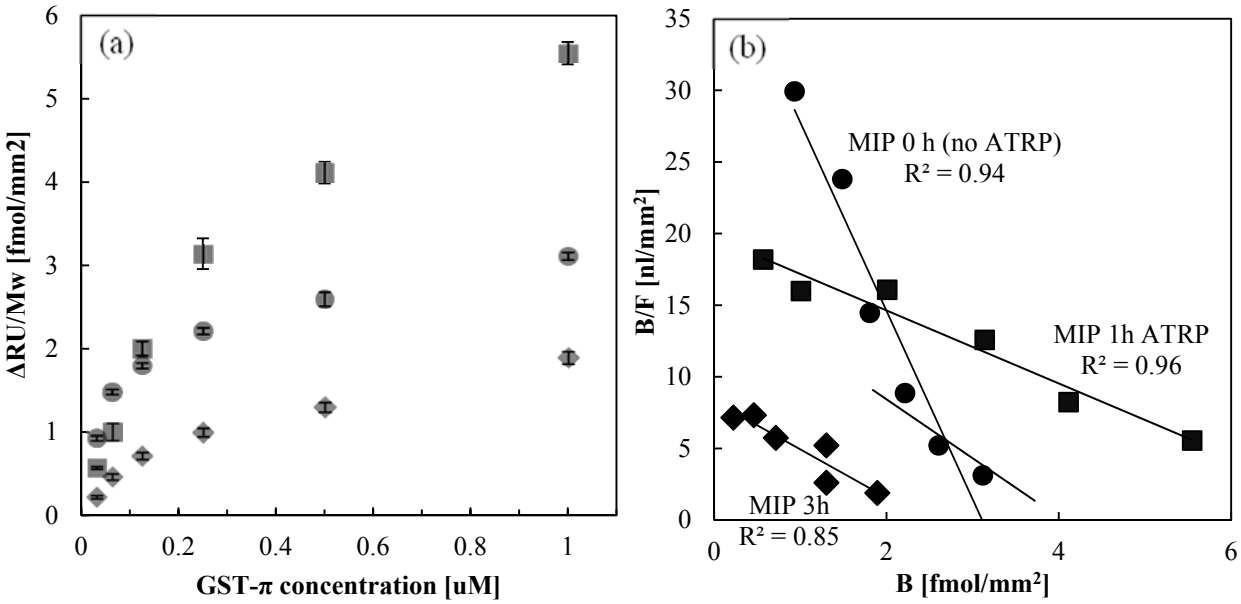


Figure S7 (a) Binding isotherms and (b) Scatchard plots of GST-π in 10 mM Tris-HCl buffer pH 7.4 containing 140 mM NaCl for GSH-immobilized substrate (no ATRT, 0 h) (●), MIP 1h (■) and MIP 3h (◆).

Contact angles

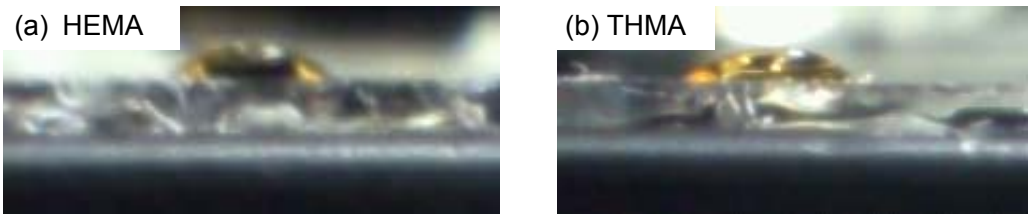


Figure S8. Water contact angles of MIPs prepared by SI-AGET ATRP using HEMA (a) and THMA (b) as co-monomers.

Binding experiments for THMA-based polymers

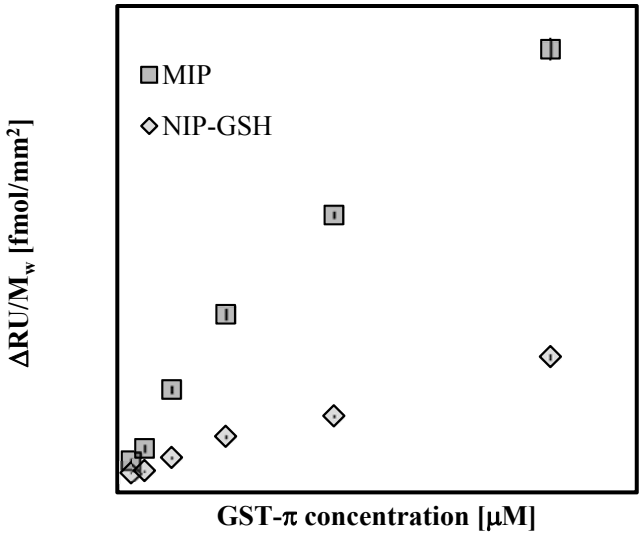


Figure S9. Binding isotherms of GST-π in 10 mM Tris-HCl buffer pH7.4 containing 140 mM NaCl for MIP (■), NIP-GSH (◆) prepared with HMA as a co-monomer.

Effect of polymer hydrophilicity on the selectivity

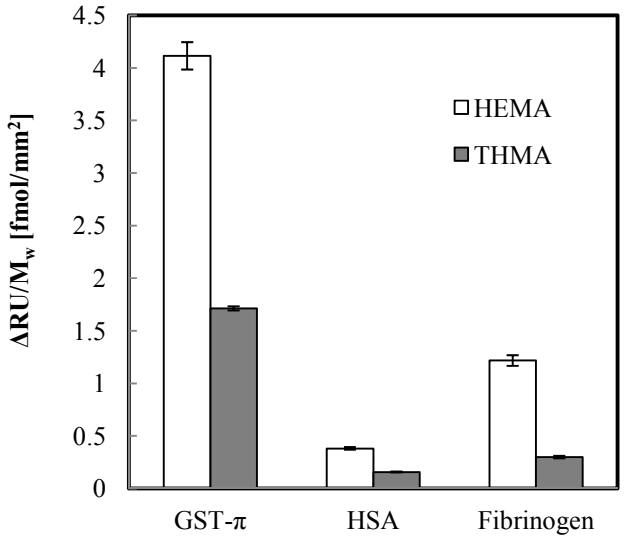


Figure S10. Influence of hydrophilicity of co-monomers on the protein binding selectivity of MIPs prepared with HEMA (white) and THMA (gray) as co-monomers in 10 mM Tris-HCl buffer pH 7.4 containing 140 mM NaCl (n=3).