

Supporting Information

Immobilization of Polyrotaxane on a Solid Substrate as the Design of Dynamic Surface

*by Dae Hyeok YANG, Ryo KATOONO, Jun YAMAGUCHI, Yoshiko MIURA, Nobuhiko YUI**

1. EXPERIMENTAL

1.1. Materials

Poly(ethylene glycol) (PEG, M_w : 3,000) was purchased from Wako Pure Chemical Co., Ltd (Osaka, Japan). A Quartz crystal microbalance gold (111) electrode was purchased from Q-sense Inc. (Sweden). A Gold (111) substrate for X-ray photoelectron spectroscopic analysis was purchased from MORITEX Co., San Jose (U.S.A).

1.2. Analyses

Fourier transform infrared reflection-absorption spectroscopy (FTIR-RAS) measurement

An FTIR-RAS measurement was performed on a Perkin Elmer Spectrum 100 equipped with a reflector (Reflector2, (Harrick Scientific Co, USA)) with a grazing angle of 85° . The spectra were measured with a liquid nitrogen cooled mercury cadmium telluride detector under a nitrogen atmosphere. All spectra were collected with 2 cm^{-1} resolution under a nitrogen gas. To minimize the signal-to-noise ratio, 10,000 scans were accumulated.

X-ray photoelectron spectroscopy (XPS) measurement

The atomic compositions, O1s, N1s, C1s, and S2p, of the polyrotaxane-immobilized substrates **1a-f** were measured with ULVAC Phi ESCA5601 (Osaka, Japan) at a grazing angle of 90° under high vacuum ($< 3.1 \times 10^{-9}$ Torr). The excitation source was monochromatic aluminum K- α X-ray radiation

(photoelectron energy = 1486.6 eV). The wide-scanned XPS and high-resolution of S2p core-level spectra were obtained at a pass energy of 187.8 and 20 eV, respectively. The spectra were charge-referenced to the Au4f_{7/2} (84 eV) of sputtered gold. The atomic compositions were corrected by using a MultiPak Version 6.1 Spectrum software. The high-resolution of S2p spectrum showed the sulfur atom (162 eV) bound with gold and the unbound sulfur atoms (164 and 169 eV) co-existed on the substrates. The intensity at each binding energy was determined by the area ratio in the spectrum, which was fitted by using the 'Fitt' software as an XPS curve fitting program.

Quartz crystal microbalance (QCM) measurement

A QCM was measured with Q-sense D300 (Sweden) under an ambient air or a nitrogen atmosphere as necessary. All QCM measurements were performed at a controlled temperature of 25 ± 0.1 °C. The QCM analysis was carried out using the frequency change obtained from the frequency of 15 Hz.

Ellipsometric measurement

The thickness of a polyrotaxane-immobilized gold substrate was measured with DHA-FX (MIZOJIRI, Japan) using a He-Ne laser of 632.8 nm, in which the incident angle was 70° from the normal. The thickness was calculated with the refractive index of gold substrate, $n = 3.180$, and the extinction coefficient, $k = 0.166$.

1.3. Preparation

Preparation of **7**

To a solution of *trans*-1,4-dibromo-2-butene (4.97 g, 23.2 mmol) in DMF (100 mL) was added a solution of potassium thioacetate (2.41 g, 21.1 mmol) in DMF (100 mL)¹ via an additional funnel over 30 minutes at room temperature, and the mixture was stirred for a further period of 90 minutes. The mixture was diluted with 1N HCl aq., and extracted with diethyl ether. The organic layer was washed with brine, and then dried over MgSO₄. After evaporation of the solvent, chromatographic separation on SiO₂ (hexane-ethyl acetate/hexane = 1:40) gave **7** (2.50 g) as a colorless oil in 57% yield. ¹H NMR δ_H(300 MHz; CDCl₃; TMS)/ppm 5.89 (1H, dt, *J* = 7.5, 15 Hz), 5.74 (1H, dt, *J* = 6.9, 15 Hz), 3.92 (2H, d, *J* = 7.5 Hz), 3.54 (2H, d, *J* = 6.9 Hz), 2.35 (3H, s); ¹³C NMR δ_C(75 MHz; CDCl₃; TMS)/ppm 194.73, 130.12, 129.50, 31.74, 30.44, 30.38; IR (NaCl) 3024, 1688, 625 cm⁻¹; ESI-MS *m/z* 207 (M⁺, BP), 208 ([M+1]⁺, 7%); Elemental Analysis Calcd. for C₆H₉BrOS C 34.46%, H 4.34%, Found C 34.86%, H 4.47%.

Preparation of **6**

To a solution of **7** (2.32 g, 11.1 mmol) in 2-butanone (25 mL) were added Z-Tyr-OMe (1.83 g, 5.56 mmol) and K₂CO₃ (1.55 g, 11.2 mmol), and the mixture was refluxed for 50 hours. After removal of a solid by filtration, the solvent was evaporated. The residue was diluted with diethyl ether, and then washed with 1N HCl aq. and brine. The organic layer was dried over MgSO₄. After evaporation of the solvent, chromatographic separation on SiO₂ (ethyl acetate/hexane = 3:7) gave **6** (2.21 g) as a colorless

oil in 67% yield. ^1H NMR δ_{H} (300 MHz; CDCl_3 ; TMS)/ppm 7.40-7.19 (5H, m), 6.98 (2H, d, $J = 8.7$ Hz), 6.78 (2H, d, $J = 8.7$ Hz), 5.89 (1H, dt, $J = 5.1, 15$ Hz), 5.80 (1H, dt, $J = 6.3, 15$ Hz), 5.26 (1H, d, $J = 8.1$ Hz), 5.10 (1H, d, $J = 15$ Hz), 5.06 (1H, d, $J = 15$ Hz), 4.61 (1H, dt, $J = 5.7, 8.1$ Hz), 4.44 (2H, d, $J = 5.1$ Hz), 3.70 (3H, s), 3.56 (2H, d, $J = 6.3$ Hz), 3.08 (1H, dd, $J = 5.7, 14$ Hz), 3.00 (1H, dd, $J = 5.7, 14$ Hz), 2.33 (3H, s); ^{13}C NMR δ_{C} (75 MHz; CDCl_3 ; TMS)/ppm 194.93, 171.95, 157.58, 155.55, 136.20, 130.20, 128.61, 128.44, 128.09, 128.00, 127.80, 114.72, 67.66, 66.87, 54.84, 52.23, 37.28, 30.70, 30.41; IR (NaCl) 3362, 3024, 2949, 1719, 1693, 1688, 1612, 1511, 625 cm^{-1} ; ESI-MS m/z 457 (M^+ , BP), 458 ($[\text{M}+1]^+$, 26%); EI-HRMS Calcd. for $\text{C}_{24}\text{H}_{27}\text{NO}_6\text{S}$ 457.1559, Found 457.1557.

Preparation of **5**

To an ice-cooled solution of **6** (550 mg, 1.2 mmol) in THF (20 mL) was added a solution of lithium hydroxide (86 mg, 3.6 mmol) in water (10 mL), and the mixture was stirred at 0 °C for 1 hour, then acetic anhydride (0.68 mL, 7.2 mmol) was added to the mixture, followed by further stirring for 15 min at the temperature. The reaction mixture was diluted with 2N HCl aq., and extracted with ethyl acetate. The organic layer was washed with water and brine, and dried over MgSO_4 . After evaporation of the solvent, chromatographic separation on SiO_2 (ethyl acetate/hexane = 1:1) gave **5** (280 mg) as a colorless oil in 53% yield. ^1H NMR δ_{H} (300 MHz; CD_3OD)/ppm 7.35-7.20 (5H, m), 7.10 (2H, d, $J = 8.7$ Hz), 6.79 (2H, d, $J = 8.7$ Hz), 5.95-5.74 (2H, m), 5.06 (1H, d, $J = 13$ Hz), 4.99 (1H, d, $J = 13$ Hz), 4.45 (2H, d, $J = 5.1$ Hz), 4.36 (1H, dd, $J = 4.8, 9.0$ Hz), 3.55 (2H, d, $J = 6.6$ Hz), 3.12 (1H, dd, $J = 4.8, 14$ Hz), 2.80 (1H, dd, $J = 9.0, 14$ Hz), 2.30 (3H, s); ^{13}C NMR δ_{C} (75 MHz; CD_3OD)/ppm 196.63, 175.10, 158.84, 158.29,

138.18, 131.27, 130.62, 129.99, 129.43, 129.39, 128.87, 128.61, 115.70, 68.73, 67.44, 56.89, 37.78, 31.51, 30.35; IR (NaCl) 3323, 3028, 2927, 1719, 1693, 1612, 1511, 625 cm^{-1} ; ESI-MS m/z 443 (M^+ , BP), 444 ($[\text{M}+1]^+$, 25%).

Preparation of pseudopolyrotaxane **3**²

To a saturated solution of α -CD in water was added PEG-bisamine **4** (0.86 g, 0.30 mmol), which was prepared according to the literature,³ and the mixture was stirred at room temperature for 24 hours. The suspension was collected by centrifugation and repeatedly washed with water. Drying of the solid in vacuo for 1 week gave 7.5 g of pseudopolyrotaxane **3** as a white solid in 73% yield. mp > 300 °C; ¹H NMR δ_{H} (300 MHz; D₂O; TMS)/ppm 4.93 (H^1 of α -CD, d, $J = 3.3$ Hz), 3.86 (H^3 of α -CD, t, $J = 9.0$ Hz), 3.81-3.67 ($\text{H}^{5,6,6'}$ of α -CD, m), 3.58 (-CH₂CH₂O- of PEG, s), 3.51 (H^2 of α -CD, dd, $J = 3.3, 9.0$ Hz), 3.46 (H^4 of α -CD, dd, $J = 8.7, 9.0$ Hz), $\text{H}^1/(\text{H}^2+\text{H}^3+\text{H}^4+\text{H}^5+\text{H}^6+\text{H}^{6'})$ of α -CD + -CH₂CH₂O- of PEG) = 6:48.68; IR (KBr) 3390, 2928, 2874, 1637, 1365, 1249, 1155, 1078 cm^{-1} .

Preparation of polyrotaxane **2**

To a solution of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM)⁴ (177 mg, 0.640 mmol) and **5** (284 mg, 0.640 mmol) in MeOH (5 mL) was added **3** (600 mg, containing 0.03 mmol of **4**), and the reaction mixture was stirred at room temperature for 48 hours. After removal of the solvent by filtration, the remaining solid was dialyzed (MWCO : 8,000) in water, and then collected by centrifugation and lyophilization to give 91 mg of polyrotaxane **2** as a white solid in 19%

yield. mp > 300 °C; $^1\text{H NMR } \delta_{\text{H}}$ (300 MHz; D_2O containing 1 v/v% NaOD; TMS)/ppm 4.92-4.80 (H^1 of α -CD, m), 3.92-3.62 ($\text{H}^{3,5,6,6'}$ of α -CD, m), 3.55 ($-\text{CH}_2\text{CH}_2\text{O}-$ of PEG, s), 3.47-3.17 ($\text{H}^{2,4}$ of α -CD, m), $\text{H}^1/(\text{H}^2+\text{H}^3+\text{H}^4+\text{H}^5+\text{H}^6+\text{H}^{6'})$ of α -CD + $-\text{CH}_2\text{CH}_2\text{O}-$ of PEG) = 6:54.12; IR (KBr) 3390, 2928, 2875, 1713, 1642, 1515, 1363, 1244, 1156, 1079, 1032 cm^{-1} .

Preparation of water-soluble polyrotaxane **1**⁵

To a solution of **2** (89 mg, 4.0 μmol) and iodomethane (75 μL , 1.2 mmol) in DMSO (5 mL) was added 60% sodium hydride in oil (29 mg, 1.2 mmol), and the reaction mixture was stirred at room temperature for 6 hours. The reaction mixture was diluted with 1N HCl aq. and then washed with ethyl acetate. The aqueous layer was dialyzed (MWCO : 8,000) in water. After removal of a precipitate by filtration, the remaining solution was evaporated and lyophilized to give 51 mg of **1** as a white solid in 52% yield. mp > 300°C; $^1\text{H NMR } \delta_{\text{H}}$ (300 MHz; D_2O ; TMS)/ppm 5.31-4.83 (H^1 of α -CD, m), 4.05-2.98 ($\text{H}^{2,3,4,5,6,6'}$ of α -CD, m), 3.55 ($-\text{CH}_2\text{CH}_2\text{O}-$ of PEG, s), 3.45 (O(3)Me of α -CD, s), 3.28 (O(6)Me of α -CD, s), $\text{H}^1/(\text{H}^2+\text{H}^3+\text{H}^4+\text{H}^5+\text{H}^6+\text{H}^{6'})$ of α -CD + $-\text{CH}_2\text{CH}_2\text{O}-$ of PEG + O(3)Me + O(6)Me) = 6:81.76; IR (KBr) 3390, 2930, 2873, 2839, 1702, 1635, 1514, 1364, 1247, 1155, 1081, 1043 cm^{-1} ;

Immobilization of **1** onto a gold substrate

A series of polyrotaxane solutions with six different concentrations of **1** in 1N NaOH aq. were prepared by stirring for 30 minutes to produce a thiol group at both terminals by the hydrolysis of a thioacetate group. Six gold substrates were immersed in each of the six basic solutions for 24 hours to prepare the

polyrotaxane-immobilized substrates **1a-f**, followed by washing with water to remove physically adsorbed **1**, and then drying *in vacuo* prior to the QCM and XPS measurements.

It is likely that the greatest number of polyrotaxane molecules immobilized on the substrate is less than 25 per 100 nm², assuming that a grafted polyrotaxane molecule occupies a space roughly corresponding to the size of an α -CD molecule (ca. 2 nm in diameter). It is also assumed that a space of 10 nm by 10 nm can be occupied by at least two polyrotaxane molecules lying on the space in either mode, when a poly(ethylene glycol) molecule in the polyrotaxane adopts a conformation with all of the sigma bonds staggered to be ca. 25 nm long.

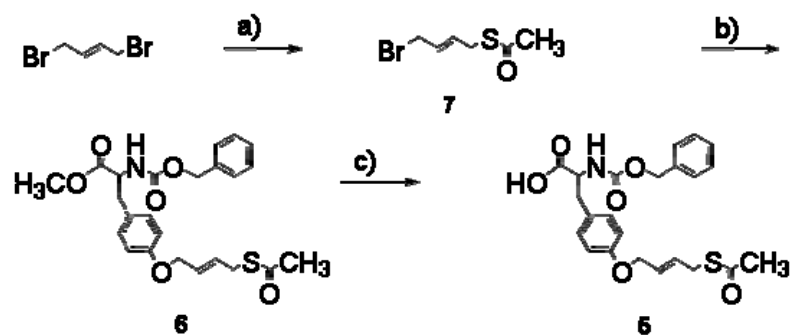
Preparation of colloidal silvers⁶

To an ice-cooled solution of sodium borohydride (19 mg, 0.50 mmol) in water (1 L) was added a solution of silver nitrate (4 mg, 0.02 mmol) in water (1 L), and then the reaction mixture was stirred for 1 hour at room temperature. In order to decompose excess sodium borohydride, the mixture was heated at 70 °C for 1 hour. After cooling the reactant, it was stored in a refrigerator (1 °C).

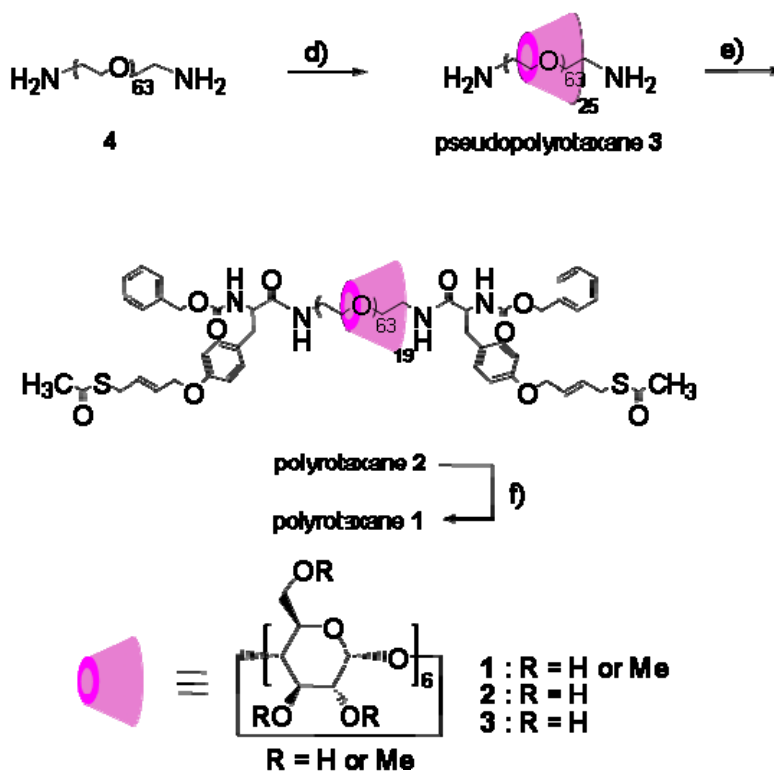
Although the concentration of silver was lowered to 10 μ M, the Ag-S bond formation can be competitive with the aggregation of colloidal silvers during the immersion, which was indicated by a slight increase in absorbance in UV-Vis spectroscopy due to the aggregation (Figure S5a). A marginal change in diameter of the colloids was observed by DLS during that time (Figure S5b,c).

2. SUPPLEMENTARY SCHEME, TABLES, AND FIGURES

A. Preparation of tyrosine derivative 5



B. Preparation of polyrotaxanes 1 and 2



Scheme 1. Reagents and yields. a) KSAc, DMF (57%); b) Z-Tyr-OMe, K_2CO_3 , 2-butanone (67%); c) LiOH, Ac_2O , THF/water (53%); d) α -CD, water (73%); e) 5, DMT-MM, MeOH (19%); f) CH_3I , NaH, DMSO (52%).

Structure of the PEG derivative **4** was characterized by not only ^1H NMR but also ESI-mass spectroscopy. The latter spectrum shows a single distribution centered at an m/z value of 2857, and provides the following formula $\text{H}_2\text{N}-(\text{CH}_2\text{CH}_2\text{O})_{63}-\text{CH}_2\text{CH}_2\text{NH}_2$, which was used to determine the number of α -CD molecules in the pseudopolyrotaxane **3** and the polyrotaxane **1** from their ^1H NMR spectra. The calculated molecular weight for **1** is $2.45 \times 10^4 \text{ g mol}^{-1}$. GPC profiles of water-soluble polyrotaxane **1**, and water-insoluble polyrotaxane **2** are shown below. For **1** (a), the GPC was measured with a system equipped with a refractive index detector (JASCO RI-930), a quaternary pump (JASCO PU-980), and two ultrahydrogel linear mixed columns (Waters) in series. Pure water containing 1M NaNO_3 was used as an eluent at a flow rate of 0.2 mL/min. For **2** (b), the GPC was measured with a system equipped with a refractive index detector (JASCO RI-2031 Plus), a quaternary pump (JASCO PU-980). DMSO was used as an eluent at a flow rate of 0.3 mL/min.

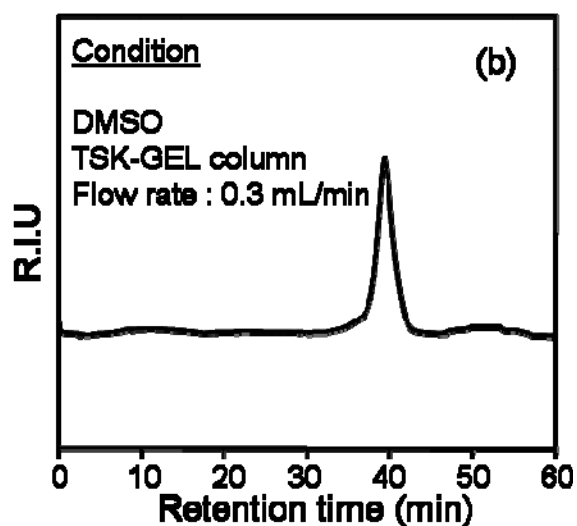
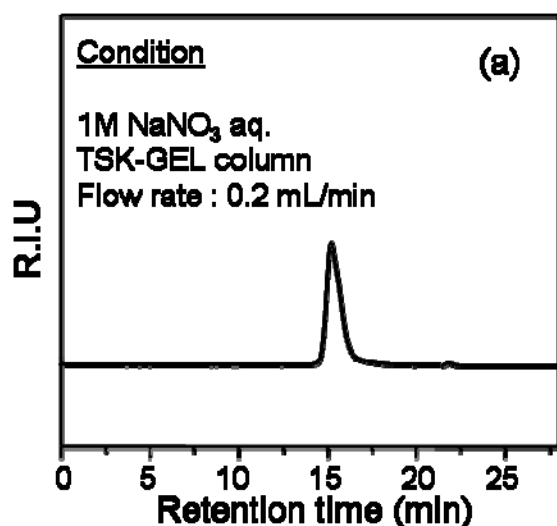


Table SI. Atomic compositions^{a)} (O1s, N1s, C1s, and S2p) on the polyrotaxane-immobilized substrates

1a-f, measured by wide-scanned XPS^{b)}

	O1s (532 eV)	N1s (400 eV)	C1s (286 eV)	S2p (162, 164, 169 eV)
Theoretical value^{c)}	38.38	0.24	61.26	0.12
1a	37	< 1	63	< 1
1b	39	< 1	60	< 1
1c	38	< 1	62	< 1
1d	38	< 1	62	< 1
1e	37	< 1	62	< 1
1f	39	< 1	61	< 1

a) The atomic compositions were corrected by using a MultiPak Version 6.1 Spectrum software. b) The measurement was carried out three times. The averaged atomic compositions lie within a relative error of 7%. c) The theoretical values of atomic compositions are calculated for the following formula, $C_{1023}H_{1780}N_4O_{641}S_2$, which was determined by 1H NMR spectroscopy.

Table SII. Immobilization mode of the polyrotaxane **1** on the substrates **1a-f**,^{a)} estimated by high-resolution XPS measurement

Substrate	Ratio ^{b)}		Immobilization mode ^{c)}	
	Free	Au-S	Loop	Graft
1a	2	8	6	4
1b	3	7	3	7
1c	4	6	3	7
1d	4	6	3	7
1e	4	6	3	7
1f	4	6	3	7

a) The immersion time for immobilization is 24 hours. b) The ratio of unbound (free) to bound (Au-S) sulfur atoms is determined by the area ratio in the S2p spectrum, fitted by using the “Fitt” software as an XPS curve fitting program. c) Immobilization mode of the polyrotaxane **1** is calculated by the ratio of sulfur atoms on the substrate.

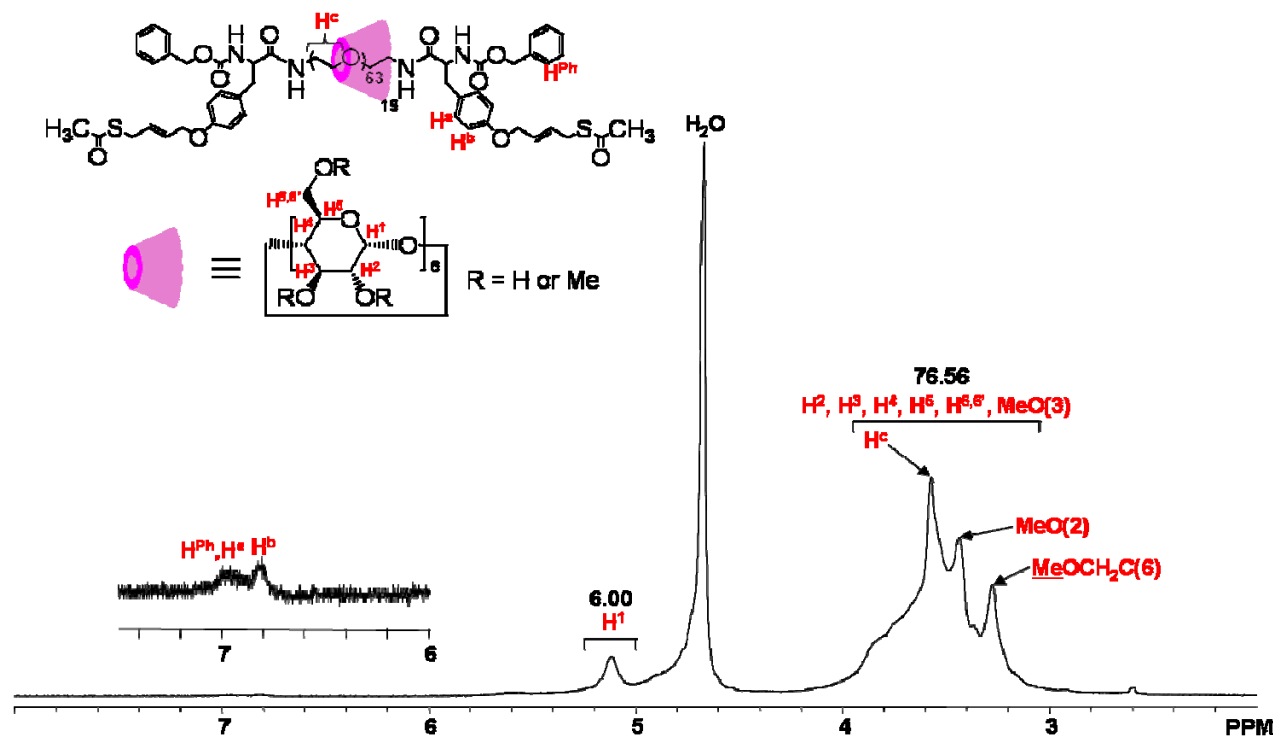


Figure S1. ^1H NMR spectrum (300 MHz) of the polyrotaxane **1** measured in D_2O .

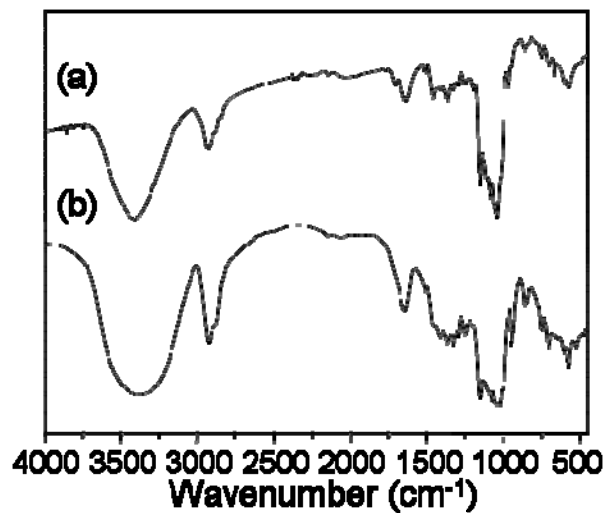


Figure S2. IR spectra of (a) the polyrotaxane **1** immobilized on the substrate **1f**, measured by IR-RAS, and (b) the polyrotaxane **1** measured in a KBr pellet. A broad and intense band centered at 3409 cm⁻¹ (spectrum a), and 3390 cm⁻¹ (spectrum b) in the OH stretching region can arise from an OH group in both the polyrotaxane and adsorbed water, whose spectral shape is dependent on their binding states.⁷

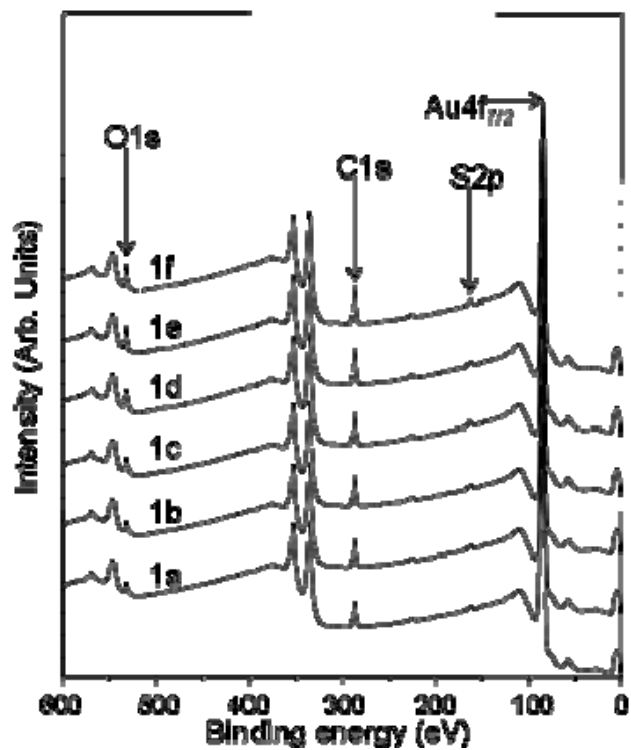


Figure S3. Wide-scanned XPS data of the polyrotaxane-immobilized gold substrates **1a-f** measured from 0 to 600 eV.

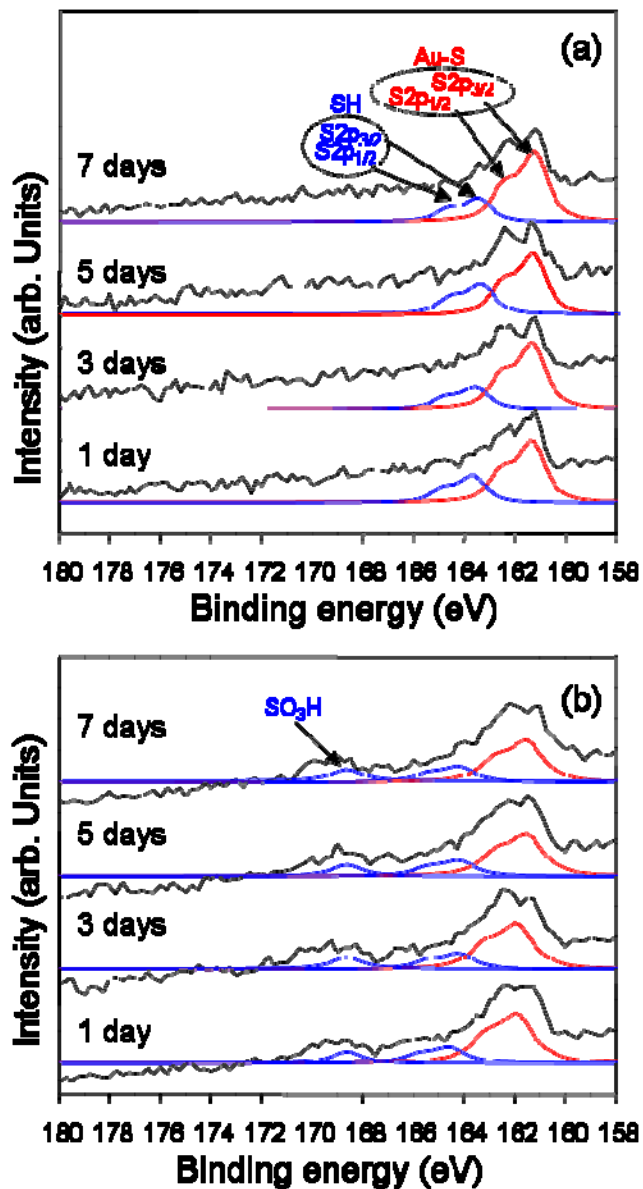


Figure S4. XPS data of the polyrotaxane-immobilized gold substrates (a) **1b** and (b) **1f** after various incubation periods of 1, 3, 5, and 7-days in water. The red and blue lines indicate the bound sulfur atom (Au-S) and the unbound sulfur atoms (SH and SO₃H), respectively. The S2p spectrum was fitted by using the “Fitt” software as an XPS curve fitting program.

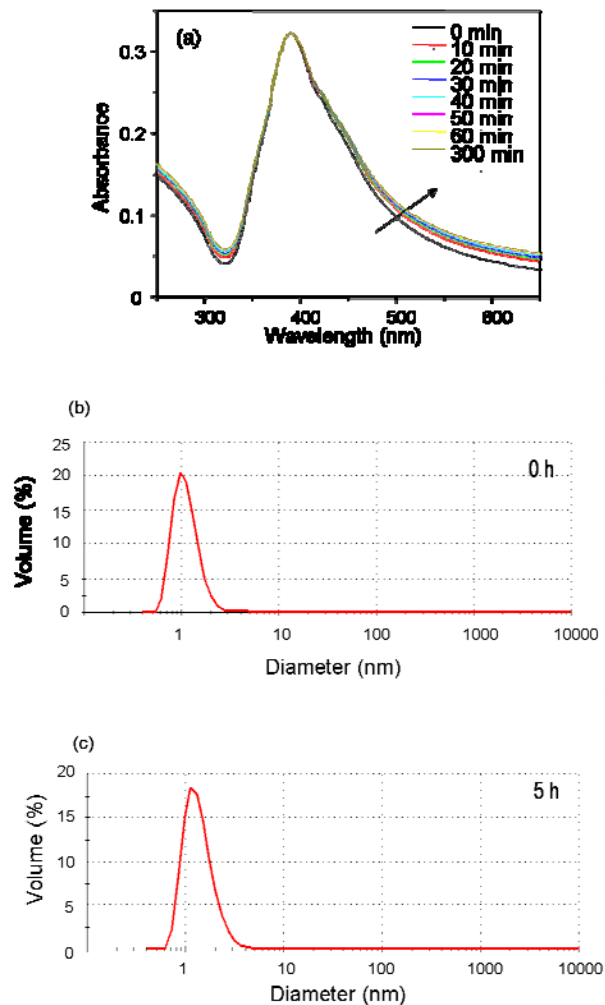


Figure S5. Time-dependent change in the UV-Vis spectrum of the colloidal silvers ($[Ag] = 1 \times 10^{-5}$ M) measured in water (a), and size distribution of colloidal silvers in water measured by DLS; (b) 0 hour refers to the time at which the reaction of colloidal silvers with sodium borohydride is terminated; (c) 5 hours refers to the storage time of colloidal silver in water in a refrigerator (1°C) after reaction termination.

3. REFERENCES

1. A. B. Northrup, and D. W. C. MacMillan, *Science*, **305**, 1752 (2004).
2. A. Harada, and M. Kamachi, *Macromolecules*, **23**, 2821 (1990).
3. a) R. V. N. Pillai, M. Mutter, E. Bayer, and I. Gatfield, *J. Org. Chem.*, **45**, 5364 (1980).
b) A. Harada, J. Li, T. Nakamitsu, and M. Kamachi, *J. Org. Chem.*, **58**, 7524 (1993).
c) A. Harada, J. Li, and M. Kamachi, *Nature*, **356**, 325 (1992).
d) A. Harada, J. Li, and M. Kamachi, *J. Am. Chem. Soc.*, **116**, 3192 (1994).
4. a) M. Kunishima, C. Kawachi, F. Iwasaki, K. Terao, and S. Tani, *Tetrahedron Lett.*, **40**, 5327 (1999).
b) M. Kunishima, C. Kawachi, J. Morita, K. Terao, F. Iwasaki, and S. Tani, *Tetrahedron*, **55**, 13159 (1999).
5. a) M. Kidowaki, C. Zhao, T. Kataoka, and K. Ito, *Chem. Commun.*, 4102 (2006).
b) T. Kataoka, M. Kidowaki, C. Zhao, H. Minamikawa, T. Shimizu, and K. Ito, *J. Phys. Chem. B*, **110**, 24377 (2006).
6. W. Wang, S. Efrima, and O. Regev, *Langmuir*, **14**, 602 (1998).
7. S. Morita, M. Tanaka, and Y. Ozaki, *Langmuir*, **23**, 3750 (2007).