SHORT COMMUNICATION

Immobilization of Polyrotaxane on a Solid Substrate
as the Design of Dynamic SurfaceBy Dae Hyeok YANG,¹ Ryo KATOONO,^{1,2} Jun YAMAGUCHI,^{1,2} Yoshiko MIURA,^{1,2} and Nobuhiko YUI^{1,2,*}

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The dynamism of proteins and lipids in cells, such as the lateral diffusion of plasma proteins and the fluidity of lipid membranes, is directly related to signal transduction and amplification across the plasma membrane in the course of intracellular metabolism.¹ It has been suggested that modulation of cellular and tissue metabolisms *via* their dynamic interfaces with biomaterials is one of the goals in development of advanced medicine and therapy.² In the last few decades, we have proposed the importance of molecular mobility in the design of biomaterials, and demonstrated that polyrotaxanes are a promising tool in performing a variety of biomedical functions.³ A polyrotaxane is a class of molecular assemblies in which cyclic compounds are threaded onto a linear polymeric chain capped with bulky end-groups at both terminals.⁴ One can easily imagine that the cyclic compounds can be freely sliding and rotating along the chain in a polyrotaxane architecture if non-covalent interactions among the components are eliminated. In this context, we envisaged that a dynamic surface would be provided by immobilizing polyrotaxanes onto a solid substrate in a looped mode,⁵ that is, both terminals of a polyrotaxane are fixed to form polyrotaxane loops on the surface (Figure 1). We describe here the preparation of a water-soluble polyrotaxane **1** consisting of α -cyclodextrin (α -CD) and poly(ethylene glycol) (PEG) using a thiol-containing terminal stopper as an anchoring group, and demonstrate the immobilization of **1** onto a gold substrate through Au-S bond formation⁶ in various conditions.

EXPERIMENTAL⁷

Fourier transform infrared reflection-absorption spectroscopy (FT/IR-RAS) and X-ray photoelectron spectroscopy (XPS) measurements were carried out in order to confirm the existence of the polyrotaxane **1** on the substrate. Quartz crystal microbalance (QCM) and ellipsometry measurements were carried out in order to examine the mass and thickness of the polyrotaxane **1** immobilized on a substrate.

RESULTS AND DISCUSSION

The water-soluble polyrotaxane **1** for immobilization onto a gold substrate was prepared as follows. The tyrosine derivative **5** was prepared as an end-capping molecule tethering an acetyl-protected thiol group (Scheme S1A), and applied to the following condensation (Scheme S1B).⁷ Mixing the poly(ethylene glycol) derivative **4** attached to amino groups at both terminals and α -CD in water gave the pseudopolyrotaxane **3**,⁸ which was then end-capped with **5** through a condensation reaction to give the polyrotaxane **2** as a water-insoluble solid.⁷ Treatment of **2** with sodium hydride and iodomethane in DMSO⁹ led to the water-soluble polyrotaxane **1** that possesses 19 molecules of nonakis(methylated) α -CD, which was determined by ¹H NMR spectroscopy (Figure S1).⁷

Figure 1. (a) Illustration of a dynamic surface based on polyrotaxane loops on a gold substrate. (b) Chemical structure of water-soluble polyrotaxane **1** with thiol-containing anchoring groups.

Table I. Change in mass (Δm) upon immobilization of **1** onto the substrates (**1a–f**), measured by QCM under a nitrogen atmosphere, and thickness of the substrates **1a–d**, and **1f** measured by ellipsometry

Substrate	[1] ($\times 10^{-5}$ M)	Δm ($\times 10^2$ ng/cm ²)	# of 1 (/100 nm ²)	Thickness (nm)
1a	0.50	0.90	2.2	2
1b	1.0	2.0	4.9	5
1c	1.5	4.3	11	10
1d	2.0	8.2	20	18
1e	2.5	8.2	20	—
1f	3.5	8.2	20	18

Immobilization of **1** through Au-S bond formation was carried out by immersing a gold substrate in a series of polyrotaxane solutions with six different concentrations of **1** in 1N NaOH aq. to control the amount of polyrotaxane on the substrate (**1a–f**) (Table I). In the basic solution, the acetyl group on the sulfur atom underwent hydrolysis to produce an intermediary thiol group.

The immobilization was first investigated by IR measurements (Figure S2).⁷ An absorbance attributed to CH stretching ($3000\text{--}2800\text{ cm}^{-1}$) was observed in both spectra measured by IR-RAS for the polyrotaxane-immobilized substrate **1f** and measured in a KBr pellet for **1**, indicating the existence of **1** on the gold substrate. Also in the region for OH stretching ($3700\text{--}3000\text{ cm}^{-1}$), a broad and intense band was found in both spectra.

In order to obtain further information on the immobilization of **1**, wide-scanned XPS spectra at a pass energy of 187.8 eV were qualitatively

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measured for the substrates **1a–f** from 0 to 600 eV (Figure S3).⁷ A signal from S2p was observed at the binding energy of 162 eV for all the substrates, and indicated a possibility of the existence of a sulfur atom on the substrates even with the difficulty in considering the intensity due to the fact that there exist sulfur atoms in the polyrotaxane only 0.12% theoretically (Table SI).⁷ These wide-scanned spectra show remarkable peaks for the C1s and O1s at the binding energies of 286 and 532 eV, respectively, as expected.

Upon the immobilization of **1** onto a QCM gold electrode, the frequency change (ΔF) was measured with QCM under a nitrogen atmosphere in order to calculate the mass change (Δm) of the electrode according to the Sauerbrey equation (Table I).¹⁰ An increase in the mass was observed up to a solution concentration of 2.0×10^{-5} M (**1a–d**), corresponding to that in the number of polyrotaxane molecules from 2 to 20 per 100 nm². For the solutions with a higher concentration (**1e,f**), the Δm did almost not vary.

In addition to the increase in the number of polyrotaxane molecules on the substrates, thickness of the substrates **1a–f** also varied with the solution concentration from 2 to 18 nm in ellipsometry (Table I).

The above-described large number and thickness for **1d–f** gave the opportunity to guess the presence of grafted polyrotaxane on the substrates, which was confirmed by the following experiment.

Upon the conjugation of colloidal silvers to remaining SH groups on the substrates, qualitative measurements of ΔF were examined with QCM under an ambient air atmosphere, since the SH group can remain at the terminal when a polyrotaxane was immobilized in a grafted mode. The substrates **1a–f** were immersed in the colloidal solution containing 1×10^{-5} M of silvers for 5 h. The frequency for the substrates **1c–f** obviously decreased, whereas the change was marginal for **1a,b** with a small number of polyrotaxane molecules on the substrates (Figure 2). Considering this result, a substrate with a small number of polyrotaxane molecules seems to be suitable for loop formation.

Immobilization mode was investigated by high-resolution XPS analyses of the sulfur atoms.¹¹ The core-level spectra of **1b** and **1f** showed the co-existence of bound (Au–S) and unbound (SH, and its oxidative product SO₃H) sulfurs, even though the remaining SH was ambiguous for **1a,b** in the above QCM measurements. Unequal amounts of bound and unbound sulfurs, roughly estimated by area ratios in the S2p spectra (Table SII),⁷ indicate the loop formation as well as the graft on a substrate. No change in the spectral shape for both substrates **1b** and **1f** was found during incubation of the substrates in distilled water for 7 d (Figure S4).⁷

In conclusion, the water-soluble polyrotaxane **1** with anchoring groups was immobilized on a gold substrate for the design of a dynamic surface. IR-RAS and wide-scanned XPS measurements indicated the polyrotaxane **1** existed on a gold substrate. The amount of **1** immobilized on the substrate was controlled by the concentration of polyrotaxane solution, and was determined by QCM measurements. The loop formation was indicated by high-resolution XPS analyses of the sulfur atoms which exist as bound and unbound forms on a substrate in unequal amounts. We are now further studying a dynamic surface with polyrotaxanes based on SAMs and hydrogels for biological application such as creation of a bio-inert surface which resists non-specific protein adsorption and cell attachment, and modulation of a specific interaction on the surface.

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Figure 2. Change in frequency (ΔF) upon conjugation of colloidal silvers (10 μ M) to the terminal thiol group of **1** remaining on the QCM gold electrodes **1a–f**, measured under an ambient air atmosphere.

Electronic Supporting Information Available: EXPERIMENTAL, SUPPLEMENTARY SCHEME, TABLES, FIGURES, and REFERENCES. These materials are available via the Internet at <http://www.spsj.or.jp/c5/pj/pj.htm>

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