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Light-Driven Artificial Enzymes for Selective Oxidation of Guanosine Triphosphate Using Water-Soluble POSS Network Polymers

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The light-driven artificial enzymes were constructed to realize unnatural reactions concerning with bio-significant molecules. In this manuscript, the guanosine triphosphate (GTP)-selective oxidation is reported using the network polymers composed of polyhedral oligomeric silsesquioxane (POSS). We synthesized the water-soluble POSS network polymer containing the naphthyridine ligands to capture GTP inside the networks and the ruthenium complexes to oxidize the captured GTP under light irradiation. Initially, the binding affinities of the guanosine nucleosides to the naphthyridine ligand inside the POSS network polymer were evaluated from the emission quenching experiments. Accordingly, it was observed that the naphthyridine ligand can form the stable complex only with GTP (K_a = 5.5 × 10^6 M^{-1}). These results indicate that only GTP can be captured by the network polymer. Next, the photo-catalytic activity of the ruthenium complex-modified POSS network polymer was investigated. Finally, it was revealed that the network polymer can decompose GTP efficiently under light irradiation. This is the first example, to the best of our knowledge, to offer not only the GTP-selective host polymers but also the light-driven artificial enzyme for GTP oxidation.

Introduction

The development of artificial enzymes is the topic with high relevance in biotechnology. Many researchers have aimed to establish the systems not only for emulating the biological systems but also for realizing unnatural reactions concerning with biomolecules and biological events.[1] These sophisticated systems are applied in various fields such for energy generation, sensors, and molecular machines.[1] Based on the bottom-up approach, the functional units are organized according to the preprogrammed design, resulting in the cooperative functions with each part. Therefore, the development of the parts and the assembling protocol is of significance. In addition, by incorporating the light-drive mechanism into the systems[2], we can receive several advantages: The time- and site-specificity can be readily received by modulating the timing and spot of light irradiation. Moreover, photo-activation can be achieved without further artifacts. Thus, light-driven artificial enzymes are promised to be an efficient tool to control the biological events more precisely.

Guanosine nucleosides, particularly guanosine triphosphate (GTP) concerns wide various significant biological events such as signal transduction, metabolism, and enzymatic reactions. The recognition of guanine derivatives is applicable for developing biosensors, drugs, and biotechnological tools for monitoring these significant biological events.[3] In particular, the modulation of the local concentration of guanosine nucleosides is a key technology for the regulation of biological events. For example, since GTP is one of the substrates of RNA polymerases and DNA primases, the suppression of the GTP concentration can be applied for the inhibition of nucleic acid productions.[4] Such system can be used for obtaining anti-cancer or -virus drugs. In vital bodies, the concentrations of GTP are regulated restrictedly by the series of GTP-related enzymes.[5] There are classes of enzymes that can cause both of the GTP degradation and production. Moreover, most of the GTP degradation enzyme can catalyze the hydrolysis at the triphosphate moiety to convert to nucleoside, monophosphate, or other phosphate ester species.[5] Thereby, even if we can overexpress these GTP degradation enzymes via hydrolysis in the cells, it is difficult to induce irreversible suppression of the GTP level at the local sites because of the homeostatic regulation by the GTP synthetic enzymes. We proposed the strategies for reducing the GTP concentrations at the local site via the direct decomposition to the guanine base irreversibly.

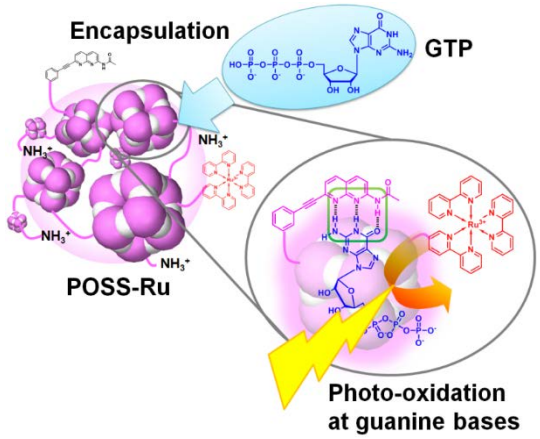


Fig. 1 Schematic illustration of the photo-triggered oxidation to the guanine derivative in the POSS network polymer. Initially, GTP is captured by the POSS network polymer (POSS-Ru), and the complex formation between the naphthyridine ligand and the guanine base occurred via hydrogen bonds. By the photo irradiation, the guanine moiety can be decomposed in the photo-oxidation with the Ru complex inside the network polymer.

It has been reported that a lot of biomolecules such as proteins, fatty acids, and nucleic acids produce the crowding circumstances in the cell.[6] In such environments called as molecular crowding, various significant differences in the molecular recognition are observed from the buffer solutions; the enhancement of the binding affinity of the complexes via the hydrogen bonds.[6] By mimicking molecular crowding with artificial systems and by applying to the molecular recognition system, we have recently established the selective encapsulation of GTP into the polyhedral oligomeric silsesquioxane (POSS)-core dendrimer via the complex formation with the naphthyridine derivatives.[7] At the surface of the hydrophobic POSS core[8] inside dendrimer, the complex stability via hydrogen bonds[9] between the guanine moiety of GTP and the naphthyridine derivative was significantly improved.[7] In addition, the negatively-charged compounds such as triphosphates made a strong interaction with the ammonium groups at the surface dendrimer. As a result, it was observed that only GTP can form the stable complex via hydrogen bonds with the naphthyridine derivative[10] inside the POSS-core dendrimer. Finally, we observed the selective encapsulation of GTP by the naphthyridine–POSS-core dendrimer complex.

We were inspired by the application of the selective encapsulation with the POSS-based polymeric materials as a recognition unit for the artificial enzyme. Herein, we report the light-driven artificial enzyme for GTP oxidation utilizing the water-soluble POSS network polymer (Fig. 1). The POSS-based water-soluble network polymer involving the naphthyridine ligand and the Ru complex was synthesized. Initially, from the titration experiments with the series of guanosine nucleosides, the selectivity in the encapsulation for each guanosine nucleoside by the POSS network polymer in the buffer solution was evaluated from the magnitude of binding affinity to the naphthyridine ligand. Accordingly, it was shown that GTP and guanosine diphosphate (GDP) can form the complex with the naphthyridine ligand, and the binding affinity with GTP was 10^4-times larger than those with GDP. Next, the photo-triggered guanine oxidation was performed in the presence of the POSS network polymer containing the Ru complex[11]. From the evaluation of the consumption rates of guanosine derivatives by the photo irradiation, we found that only GTP can be efficiently decomposed by the POSS network polymer. This is the first example, to the best of our knowledge, not only to offer the polymeric host materials for GTP-selective recognition but also to construct the light-driven artificial enzyme for guanine oxidation.

Experimentals

Generals. ^{1}H NMR and ^{13}C NMR spectra were measured with a JEOL EX–400 (400 MHz for ^{1}H and 10 MHz for ^{13}C) spectrometer. ^{29}Si NMR spectra were measured with a JEOL JNM-A400 (80 MHz) spectrometer. Coupling constants (J value) are reported in Hertz. The chemical shifts are expressed in ppm downfield from tetramethylsilane, using residual chloroform (δ = 7.24 in ^{1}H NMR, δ = 77.0 in ^{13}C NMR) or residual DMSO (δ = 2.49 in ^{1}H NMR, δ = 39.5 in ^{13}C NMR) as an internal standard. MASS spectra were obtained on a JEOL JMS–SX102A. Emission from the samples was monitored using a Perkin Elmer LS50B at 25 °C using 1 cm path length cell. MASS spectra were obtained on a JEOL JMS–SX102A. The detailed procedures and characterization data of the materials are shown in Supporting Information.

Encapsulation of nucleoside derivatives.[7] The general procedure for the complexation of guanosine nucleosides by the POSS network polymers is described here. The stock solutions of the POSS network polymers (×10) and each guanosine nucleoside (×10) were mixed at room temperature, and then the 500 μL of the samples were prepared by adding the PBS buffer (pH 7.4) solution. The complexation with POSS-Ru was also prepared with the same procedure in PBS buffer solution containing potassium ferricyanide ($K_3[Fe(CN)_6]$) as an oxidizer for the Ru complex.[11]

Fluorescence measurements of the complexes. The fluorescence emission of the naphthyridine ligand (20 μM with POSS-N and 10 μM with POSS-Ru) in the presence and absence of the guanosine nucleosides with the excitation light at 424 nm were monitored using a Perkin Elmer LS50B at 25 °C using 1 cm path length cell. The excitation and the emission bandwidth were 1 nm. The quantum yields were determined as an absolute value with an integral sphere.

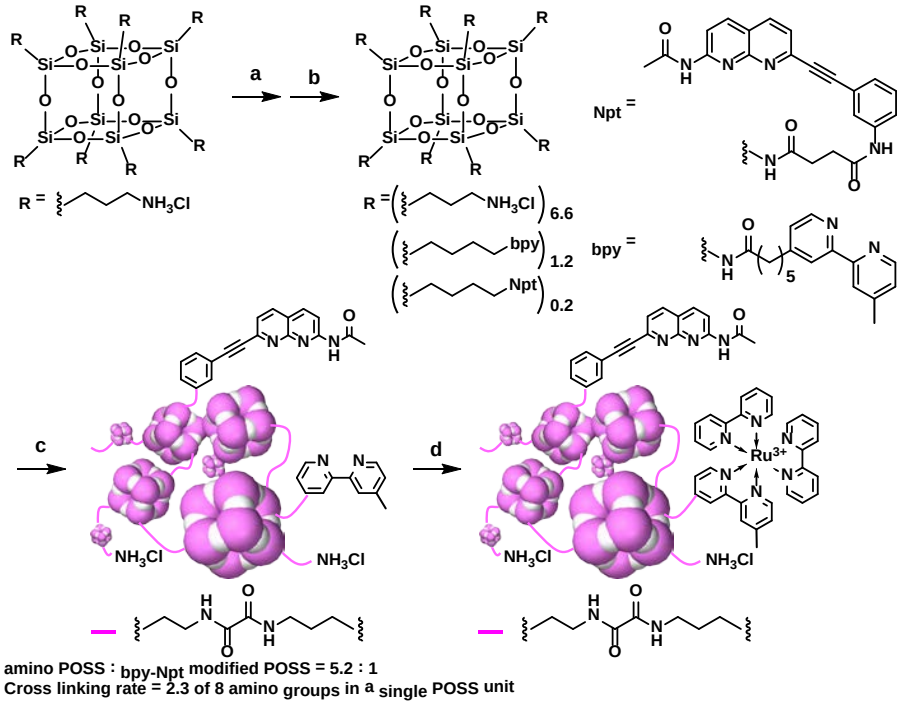
Stern–Volmer plots.[7] The data were analyzed with the Stern–Volmer equation:

$$\frac{I_0}{I} = 1+K_{SV}[Q] \qquad (1)$$

The emission intensities were plotted according to a Stern–Volmer equation, reporting I_0/I versus the concentrations of nucleosides [Q], where I_0 is the intensity in the absence of nucleosides and I is the intensity in the presence of a nucleoside concentration. K_{SV} is the Stern–Volmer quenching constant. A plot of I_0/I versus [Q] yields an intercept of one and a slope equals to K_{SV}.

Binding constants calculation.[7] According to the non-emissive complex formation between the naphthyridine ligand and guanosine nucleosides, the binding constant (K_A) can be calculated with the number of the binding molecules (n) from the following

Scheme 1. Synthesis of the POSS network polymers[a]



[a]Reagents and conditions: (a) bipyridine ligand, DMT-MM, triethylamine, MeOH, r.t., 1 d, 79%; (b) Npt ligand, DMT-MM, triethylamine, DMSO, r.t., 1 d, 43%; (c) octaammnium-POSS, oxalic acid, MeOH, r.t., 1 d, 57%; (d) $Ru^{II}(bpy)_2Cl_2$, water, reflux, 12 h, 93%.

equation:

$$\log\frac{I_0-I}{I} = \log K_A + n\log[Q] \qquad (2)$$

Fig. 2 in the main text represents the plots for evaluating the K_A values of the naphthyridine ligands to GDP and GTP.

Results and discussions

The schematic illustration of the chemical structure of the POSS network polymers and the photo-triggered GTP decomposition is shown in Fig. 1. The network polymer is composed of the combination of the four components: The naphthyridine ligand is employed for the recognition to the guanine moiety in GTP.[7,10] The ammonium groups are introduced to maintain the water-solubility and discriminate the triphosphate unit via the electrostatic interaction. The POSS core is expected to provide hydrophobic spaces inside each of POSS unit in the networks, leading to the enhancement of binding affinity of the naphthyridine moiety to the guanosine nucleosides via hydrogen bonding in aqueous solution.[8] The Ru complex was used as a photo-catalyst in the guanine oxidation under light irradiation.[2] To induce the recognition and the oxidation tandemly, the naphthyridine ligands and the Ru complexes were introduced into the same POSS core. In previous reports, the increase of the crosslinking ratios which can be modulated by the feed ration of the cross linker caused the decrease of the water-solubility and the increase of the encapsulation ability.[8] Thus, we maximized the cross-linking rate without loss of the water-solubility.

Synthesis of the POSS network polymers is presented in Scheme 1. The naphthyridine ligand and the bipyridine ligand which is scaffold for the Ru complexation were introduced into ocata-amino POSS to gather the recognition and the decomposition units. The numbers of the tethered molecules were determined from the integration ratios at the propyl chains of octa-amino POSS in ^{1}H NMR spectra (POSS core : bipyridine : naphthyridine = 1 : 1.2 : 0.2). In the presence of the modified POSS, octa-ammonium POSS, the condensation reagent and the cross-linker, the network formation proceeded (octa-amino POSS : modified POSS = 5.2 : 1). It was estimated that the 2.3 of amino groups in 8 vertices of a single POSS core was cross-linked by the linkers in the network. The products were obtained as an orange powder from the reprecipitation in acetonitrile containing 0.1% hydrochloric acid. To compare the encapsulation ability of the POSS network polymers, we also prepared the polymer without the Ru complex. All products showed good solubility in the PBS buffer as well as in water. Precipitation or color changes were hardly observed after two weeks at room temperature in the dark. Hence, we conclude that the POSS network polymers used in this study can have enough stability for the further measurements.

Initially, the encapsulation behaviors of the POSS network polymer with nucleosides were investigated according to the Cywinski's method.[10] We measured the changes of fluorescence emission from the naphthyridine ligand in POSS-N by adding the series of the guanosine nucleosides (Fig. 2). In previous reports, it was demonstrated that the fluorescence emission of the naphthyridine ligand can be quenched by the complexation with the nucleobases via the hydrogen bonds.[7,10] The emission spectra were monitored in the PBS buffer solution (pH 7.4) containing 20 μM the naphthyridine ligand in POSS-N and various

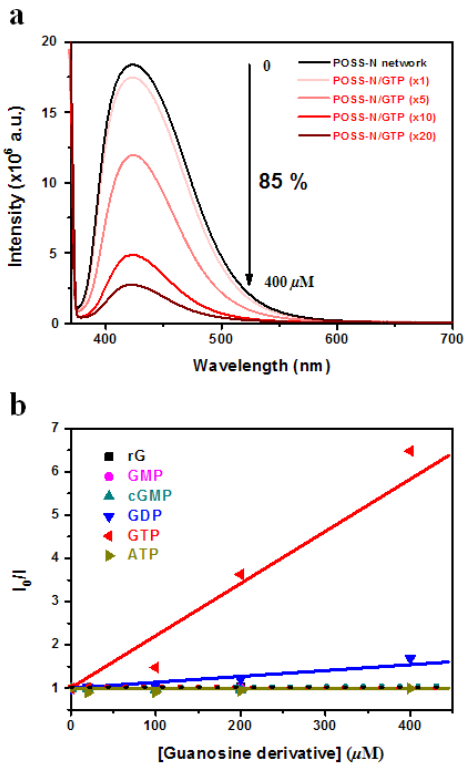


Fig. 2 (a) Emission changes of 20 μM naphthyridine ligands in POSS-N by adding GTP (0 to 400 μM) in PBS buffer solution (pH 7.4) at 25 °C. Excitation wavelength was 364 nm. (b) Stern–Volmer plots with the solution containing 20 μM naphthyridine ligand in POSS-N by adding various kinds of guanosine nucleosides.

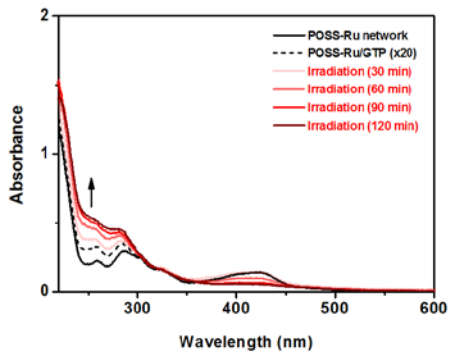


Fig. 3 UV–vis spectral changes of 10 μM naphthyridine ligand in POSS-Ru by adding 200 μM GTP with 100 μM $K_3[Fe(CN)_6]$ in PBS buffer solution (pH 7.4) at 25 °C. Photolysis was performed for 120 min at 25 °C with a 4 W UV-Lamp (365 nm).

concentrations of guanosine nucleosides (0 to 400 μM) with the excitation light at 364 nm (Fig. 2a). Correspondingly, the strong emission with the peak around 424 nm was observed from the naphthyridine ligand in POSS-N in the absence of guanine nucleosides. The addition of GTP induced the significant quenching of the emission from the naphthyridine ligand. In addition, the emission of the naphthyridine ligand gradually decreased by the addition of up to 400 μM GTP. Finally, the emission was mostly quenched to 15% by adding 20 eq. of GTP. These data represent that the naphthyridine ligand can recognize GTP by the complexation via the hydrogen bonds.

Next, to investigate the selectivity of the complexation, the titration was performed with other nucleosides. It was found that the additions of guanosine (rG), guanosine monophosphate (GMP), and cyclic guanosine monophosphate (cGMP) can slightly influence on the emission property of the naphthyridine ligand.[11] Even in the presence of the excess amounts of these nucleosides (20 eq.), less significant changes were observed in the emission spectra. In contrast, the addition of GDP (20 eq.) induced the quenching of the emission by 41%. These results indicate that only GDP can form the complex with the naphthyridine ligand in POSS-N. In other words, GTP and GDP can be encapsulated into the POSS network polymer. Furthermore, it is also indicated that the numbers of the phosphate groups can significantly affect to the complex formation of POSS-N with guanosine nucleosides. Since the ammonium groups in POSS-N can make an electrostatic interaction with the phosphate groups, GDP and GTP can be retained in the networks. We carried out the same experiment with adenosine triphosphate (ATP), and a slight emission change was observed.[12] It was confirmed that the hydrogen bonding should be essential for the encapsulation into the POSS network polymer.

Table 1. Optical properties and the binding constants of the ligand **1**[a]

	Φ[b]	$K_{SV}\times10^3$ [mol L^{-1}][c]	n[d]	K_A [M^{-1}][d]	TON [h^{-1}][f]
none	0.35	n.d.[e]	n.d.[e]	n.d.[e]	3.2±1.3
rG	0.35	n.d.[e]	n.d.[e]	n.d.[e]	1.0±0.6
GMP	0.35	n.d.[e]	n.d.[e]	n.d.[e]	2.7±1.0
cGMP	0.34	n.d.[e]	n.d.[e]	n.d.[e]	2.3±0.7
GDP	0.29	1.33±0.18	0.91±0.22	580	2.2±0.9
GTP	0.22	12.05±1.07	1.83±0.19	5.5×10^6	7.6±0.7

[a]Procedures and conditions are described in the Experimental Section.
[b]Determined as an absolute value.
[c]Quenching constants were determined with the slopes of the fitting line in the Stern–Volmer plots. The errors mean a standard error.
[d]Calculated according to the reference 7.
[e]n.d. = not determined because of too weak interaction.
[f]Determined from the slopes of the consumption rates. The errors mean a standard error.

We prepared the Stern–Volmer plots with guanosine nucleosides as a quencher (Fig. 2b). Based on the formation of a non-emissive complex model, the binding constants were calculated.[7] The optical properties, quenching constants, and the binding constants are listed in Table 1. All plots obtained from the titration of GDP and GTP were fitted on the line, and the binding constants can be determined.[7] It was found that POSS-N can enhance the binding affinity with GTP approximately 10^4-times larger than that with GDP. According to our previous results, these results clearly indicate that the POSS network polymer can greatly enhance not only the binding affinity but also the selectivity for the GTP recognition.[7] The multiple POSS cores in the networks should generate the stronger hydrophobic spaces[8], leading to the larger enhancement effects of the encapsulation than the POSS-core dendrimer.

Photoreactions for the oxidation with the guanosine nucleosides were performed with POSS-Ru. The sample solution containing POSS-Ru network (10 μM the naphthyridine concentration), 100 μM potassium ferricyanide ($K_3[Fe(CN)_6]$) in PBS buffer solution (pH 7.4) was added to 200 μM each guanosine nucleoside, and the UV light (365 nm) from the transilluminator was irradiated to the samples at room temperature. The relatively-weak emission of POSS-Ru than that of POSS-N was observed around 424 nm assigned as the fluorescence from the naphthyridine ligand in the absence of GTP (Fig. 3). The addition of 200 μM GTP immediately

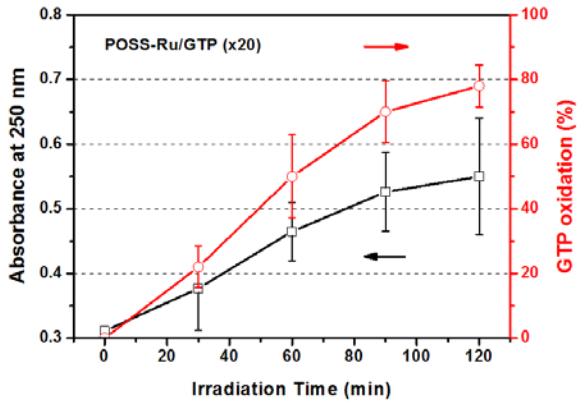


Fig. 4 The rates of the GTP oxidation with POSS-Ru. The amount of the oxidized GTP was calculated from the normalized values determined with the HPLC analyses with the sample containing $Ru^{II}(bpy)_3$ and GTP by monitoring the absorption change at 250 nm.

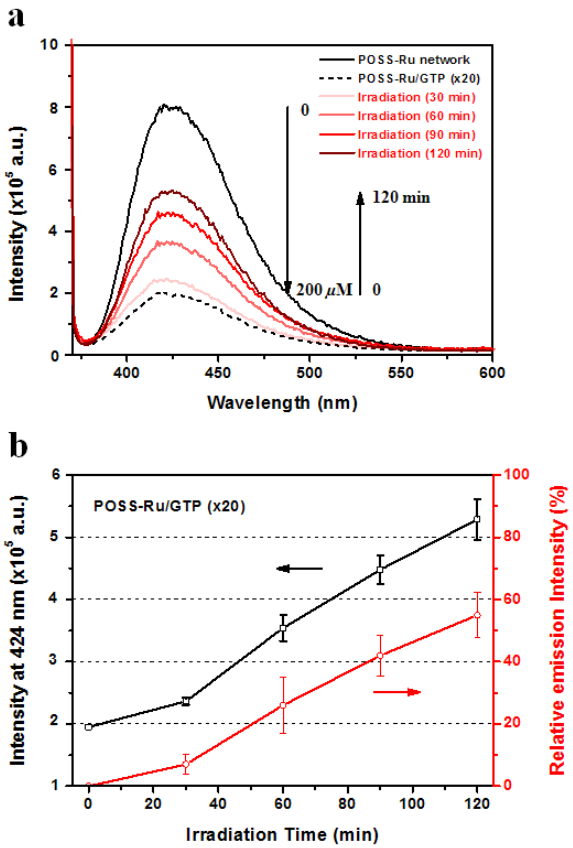


Fig. 5 (a) Emission changes of 10 μM naphthyridine ligands in POSS-Ru by adding 200 μM GTP with 100 μM $K_3[Fe(CN)_6]$ in PBS buffer solution (pH 7.4) at 25 °C. Excitation wavelength was 364 nm. Photoreaction was performed for 120 min at 25 °C with a 4 W UV-lamp (365 nm). (b) The profile was obtained in PBS buffer solution for GTP in the POSS-Ru/GTP complex under UV irradiation.

induced the quenching of the emission. Corresponded to the above results with POSS-N, the POSS-Ru network polymer should encapsulate GTP. Since the emission decreased to the similar extent to 25% with POSS-N after adding GTP, it can be said that the Ru complexes should cause less significant influence on the complexation of the naphthyridine ligand. These data mean that the naphthyridine ligand can recruit GTP near the Ru complexes inside POSS-Ru.

To determine the reaction rate, the consumptions of guanosine nucleosides were monitored by UV–vis absorption measurements. Before testing with the POSS network polymers, for the evaluation of the amount of the oxidized GTP quantitatively, we prepared the sample containing GTP and $Ru^{II}(bpy)_3$ in the absence of POSS-Ru, and the reactions were monitored by the HPLC analysis. From the ratios of the peak areas in the HPLC profiles, the reaction yields were calculated. Accordingly, 55% of GTP in the mixture with free $Ru^{II}(bpy)_3$ was oxidized by UV irradiation for 2 h in the absence of POSS-Ru. This sample showed the significant changes in the absorption spectra.[13] The absorption bands around 250 nm continuously increased by the UV irradiation. These changes were used as a standard to estimate the oxidative decomposition of guanosine nucleosides. Fig. 4 shows the changes of absorbance obtained from the complexes of POSS-Ru with GTP by the UV irradiation. Similarly to the $Ru^{II}(bpy)_3$-containing samples, the absorption bands were enhanced after the photoreaction. These results indicate that GTP should be decomposed by POSS-Ru. The oxidation of GTP gradually proceeded corresponding with the increase of the irradiation time, and finally it was found that 78% of the GTP was consumed after irradiation for 2 h. These data clearly indicate that the Ru-complexes in POSS-Ru should cause the oxidation of GTP. Same photoreactions were performed with other nucleosides such as rG, GMP, cGMP, and GDP. The oxidation was observed with lower ratios than that with GTP.[14] These results also support that the Ru-complexes can selectively oxidize GTP in POSS-Ru. Complexation between the naphthyridine ligand and GTP contributes to the increase of the local concentration of the substrate around the Ru complex, resulting in the efficient oxidation.

It is proposed that the fluorescence emission of the naphthyridine ligand should be recovered by the decomposition of the guanine base in the complex. Based on this idea, the emission properties of the naphthyridine ligands in POSS-Ru with the peak at 424 nm were investigated. Fig. 5 shows the change of emission intensity observed from the naphthyridine ligands at 424 nm by different intervals of time. The emission intensity gradually increased by increasing the UV irradiation time. This result suggests two issues: Firstly, the guanine moiety should be decomposed by the photoreactions. Secondly, the decomposed products can no longer form the complex with the naphthyridine ligand. The amount of the decomposed GTP was calculated from the change of the emission intensity before and after the UV irradiation. Accordingly, 55% of GTP was oxidized by POSS-Ru after UV irradiation for 2 h.

Turnover numbers per hour (TON) in the photoreactions were calculated from the consumption rates of each nucleoside (Table 1). The TON with GTP in the presence of POSS-Ru showed the largest value (7.6 h^{-1}) of the other nucleosides. In particular, the TON can be improved by adding POSS-N to the sample. Even with GDP which can be encapsulated into POSS-N, the TON value was similar to other guanosine nucleosides. These results clearly indicate that POSS-Ru can be a light-driven catalyst for GTP oxidation. Interestingly, the decomposed products should be released during the reaction because the turnover can be observed. Moreover, comparing to the TON value in the absence of POSS-

Ru (3.2 h^{-1}), the other values except with GTP can be suppressed. This fact represents that the Ru complexes can induce the reaction mainly inside the network polymers. According to these data, we conclude that POSS-Ru should act similarly as an enzyme: The target can be recognized with high selectivity and can react only inside the POSS network polymer. Finally, the products should be released after the reaction. Furthermore, these tandem reactions can proceed only under the light irradiation. To quantitatively monitor the reaction with UV−vis absorption spectra, we performed the decomposition reaction with the minimum amount of $K_3[Fe(CN)_6]$ because of the intrinsic light absorption. By increasing the concentration of $K_3[Fe(CN)_6]$, TON could be enhanced.

Conclusions

We demonstrate the GTP-selective oxidation using the network polymers containing the POSS units which can create the hydrophobic spaces inside polymers like the molecular crowding state. The water-soluble POSS network polymers modified with the naphthyridine ligands to capture GTP inside the networks and the ruthenium complexes to oxidize the captured GTP under light irradiation were prepared. Initially, the naphthyridine-modified POSS network polymer showed highly selective binding to GTP in the series of the guanosine derivatives. Accordingly, the complex with GTP showed the significant large binding affinity (K_a = 5.5 × 10^6 M^{-1}) with the naphthyridine ligands into the network polymers. Next, the photo-catalytic activity of the complexes with GTP and the Ru complex-modified POSS network polymers were investigated. Consequently, it was observed that the modified network polymers can decompose GTP efficiently under the light irradiation. By applying the special spaces created by the POSS core in water for the molecular recognition, we can offer not only the host polymers for the selective capturing to the significant biomolecule with high affinity but also the light-driven artificial enzymes for unnatural bioreaction, GTP oxidation.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See http://dx.doi.org/10.1039/b000000x/

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11 See Figure S1 in the Supporting Information.

12 See Figure S2 in the Supporting Information.

13 See Figure S3 in the Supporting Information.

14 See Figure S4 in the Supporting Information.