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Supporting Information

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

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Experimental Section

General. ¹H NMR and ¹³C NMR spectra were measured with a JEOL EX–400 (400 MHz for ¹H and 10 MHz for ¹³C) spectrometer. ²⁹Si 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 ($\delta = 7.24$ in ¹H NMR, $\delta = 77.0$ in ¹³C NMR) or residual DMSO ($\delta = 2.49$ in ¹H NMR, $\delta = 39.5$ in ¹³C 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. Synthesis was carried out according to Schemes S1 and S2.

Synthesis of the naphthyridine ligands, 5.

The compound 1. A reaction mixture containing 9.07 g of malic acid (67.6 mmol) and 6.67 g of 2,6-diaminopyridine (61.1 mmol) was slowly added to 40 mL of 95% H₂SO₄ at 0 °C. After stirring at 110 °C for 3 h, the solution was neutralized with NH₄OH, and the product was yielded as a precipitate. Compound 1 was separated by the filtration as a white powder (9.05 g, 92%). ¹H NMR (DMSO- d_6) δ 11.85 (br, 2H), 7.64 (d, 1H, J = 9.6 Hz), 7.63 (d, 1H, J = 9.2 Hz), 6.32 (d, 1H, J = 9.2 Hz), 6.01 (d, 1H, J = 8.9 Hz).

LRMS (NBA) $[(M+H)^+]$ calcd. 162, found 162.

The compound 2. A suspension of compound 1 (30.0 g, 93.2 mmol) in 300 mL of acetic anhydride was heated at reflux for 3 h. The resulting mixture was cooled to room temperature. The precipitate was collected by vacuum filtration, washed with diethyl ether, and air-dried to give 32 g (85%) of 2 as a yellow powder. ¹H NMR (DMSO- d_6) δ 11.90 (s, 1H), 10.50 (s, 1H), 8.02 (d, 1H, J = 8.4 Hz), 7.90 (d, 1H, J = 8.4 Hz), 7.82 (d, 1H, J = 9.2 Hz), 6.40 (d, 1H, J = 9.2 Hz), 2.12 (s, 3H, CH₃). HRMS (NBA) [(M+H)⁺] calcd. 203.0695, found 203.0694.

The compound 3. A mixture of compound **2** (20.0 g, 98.5 mmol) and POCl₃ (350 mL) was heated at 90–95 °C for 2 h. The resulting solution was cooled to room temperature, and excess POCl₃ was removed by Kugelrohr distillation. The residue was dissolved in ice water, and the solution was adjusted under the basic condition (pH = 8) with concentrated ammonium hydroxide, which prompted formation of a brownish green precipitate. The solid was collected by vacuum filtration, air-dried, and continuously extracted (Soxhlet extraction) with chloroform for 12 h. The solvent was removed *in vacuo*, and the crude product was purified by column chromatography (CH₃OH/CH₂Cl₂,

1 : 9 v/v) to give 13 g (60%) of **3** as metallic yellow needles. ¹H NMR (DMSO- d_6) δ 11.13 (s, 1H), 8.40 (m, 3H), 7.54 (d, 1H, J = 8.5 Hz), 2.16 (s, 3H). ¹³C NMR (DMSO- d_6) δ 170.41, 155.13, 154.18, 152.76, 140.42, 139.66, 121.46, 118.99, 115.13, 24.25. Anal. Calcd for C₁₀H₈N₃ClO: C, 54.19; H, 3.46; N, 18.96. Found: C, 54.07; H, 3.64; N, 18.88.

The compound 4. In a 25-mL round-bottom flask, 310 mg of compound 3 (1.4 mmol), 35 mg of PdCl₂(PPh₃)₂ (cat.) and 7.8 mg of CuI (cat.) were stirred in 10 mL of dry THF under argon. To the solution, 2 mL of triethylamine was slowly added through a syringe. The mixture was stirred at room temperature for 30 min and then 0.2 mL of 3-ethynylaniline (1.9 mmol) were added dropwise. The reaction was stirred at room temperature overnight. By removing the volatiles under reduced pressure, the residue was dissolved in dichloromethane, passed through a plug of celite and then washed with ethyl acetate. The solvents were removed under reduced pressure, and the crude product was dried under *vacuo*. Column chromatography in silica gel with an increasing gradient of methanol in dichloromethane (CH₂Cl₂/CH₃OH from 20:1 to 10:1) yielded 180 mg (31%) of **4**. ¹H NMR (DMSO-*d*₆) δ 11.02 (s, 1H), 8.43 (m, 3H), 7.65 (m, 1H), 7.11 (t, 1H, *J* = 7.8 Hz), 6.84 (s, 1H), 6.79 (d, 1H, *J* = 7.8 Hz), 6.69 (d, 1H, *J* = 7.8 Hz),

5.32 (s, 2H, NH₂), 2.20 (s, 3H, CH₃). ¹³C NMR (DMSO-*d*₆) δ 170.8, 155.4, 155.1, 149.4, 146.3, 139.7, 138.0, 136.9, 129.9, 124.2, 121.7, 119.8, 116.9, 116.1, 115.7, 91.8, 88.8, 24.7.

The compound 5. Compound 4 (4.5 g, 15 mmol) was dissolved in 100 mL of dry methanol, and then succinic anhydride (4.0 g, 40 mmol) was added. The reaction mixture was stirred at room temperature for 6 h. After the reaction, the precipitate was collected by vacuum filtration, and the chloroform solution of the products was washed with methanol and dried over Na₂SO₄. After removing volatiles by evaporation, **5** was obtained as a brown powder (4.04 g, 67%). ¹H NMR (DMSO-*d*₆) δ 11.07 (s, 1H), 10.15 (s, 1H), 8.43 (m, 3H), 7.99 (s, 1H), 7.72 (m, 1H), 7.60 (m, 1H), 7.42 (m, 1H), 7.33 (m, 1H), 2.58 (m, 4H) 2.18 (s, 3H). ¹³C NMR (DMSO-*d*₆) δ 177.3, 173.7, 168.9, 157.6, 150.0, 146.3, 143.6, 138.1, 136.9, 133.8, 128.6, 127.9, 126.4, 124.5, 122.9, 121.3, 115.8, 90.3, 86.5, 32.3, 30.7, 22.2. FABMS (NBA/CHCl₃) *m/z* 266 ([(M+H)⁺]), HRMS (NBA) [(M+H)⁺] calcd. 266.1657, found 266.1657.

4-(5-Cyanopentyl)-4'-methyl-2,2'-bipyridine, 6. To a solution of diisopropylamine (4.6 mL, 33 mmol) in THF (100 mL), *n*-butyllithium (1.6 M solution in hexane, 18.4

mL, 29.4 mmol) was added, and the reaction mixture was stirred at -78 °C for 15 min. After adding 4,4'-dimethyl-2,2'-bipyridine (5.16 g, 28 mmol) dissolved in THF (100 mL), the reaction mixture was stirred additionally for 1 h at -78 °C. 5-Bromovaleronitrile (3.6 mL, 31 mmol) in THF (5 mL) was subsequently added, and the reaction mixture was stirred for 2 h at 0 °C. The resulting mixture was diluted with water (100 mL), neutralized with 1 N HCl and extracted with ethyl acetate. The organic phase was washed with brine, dried over MgSO₄ and concentrated in vacuo. The crude product was purified by silica gel column chromatography (hexane : ethyl acetate = 10:1) to give 7.4 g (99%) of the title compound as a brown powder. ¹H NMR (CDCl₃) δ 8.51 (dd, 1H, J = 0.7, 5.1 Hz), 8.49 (dd, 1H, J = 0.5, 4.9 Hz), 8.19–8.18 (m, 2H), 7.09– 7.06 (m, 2H), 2.66 (t, 2H, J = 7.7 Hz), 2.33 (s, 3H), 2.28 (t, 2H, J = 7.0 Hz), 1.73–1.60 (m, 4H), 1.50–1.44 (m, 2H). ¹³C NMR (CDCl₃) δ 155.9, 155.6, 151.6, 148.8, 148.6, 147.8, 124.4, 123.5, 121.7, 120.8, 119.3, 34.8, 29.2, 27.9, 24.9, 20.8, 16.7. FABMS $(NBA/CHCl_3) m/z 266 ([(M+H)^+]), HRMS (NBA) [(M+H)^+] calcd. 266.1657, found$ 266.1657.

6-(4'-Methyl-2,2'-bipyridin-4-yl)hexanoic acid, 7. A mixture of bipyridine 6 (7.40 g, 27.9 mmol) and conc. HCl (35–37 %, 50 mL) was stirred at 100 °C overnight. The

resulting mixture was adjusted the pH value to 4.0–4.5 with 6 *N* NaOH and extracted with chloroform. The organic phase was washed with brine, dried over MgSO₄ and concentrated *in vacuo* to yield bipyridine 7 (7.70 g, 97%) as a magenta powder. ¹H NMR (CDCl₃) δ 8.57 (d, 1H, *J* = 0.7 Hz), 8.56 (d, 1H, *J* = 0.7 Hz), 8.21 (s, 1H), 8.18 (d, 1H, *J* = 0.7 Hz), 7.17 (ddd, 1H, *J* = 0.7, 1.6, 5.1 Hz), 7.14 (dd, 1H, *J* = 1.8, 5.1 Hz), 2.72 (t, 2H, *J* = 7.7 Hz), 2.45 (s, 3H), 2.36 (t, 2H, *J* = 7.3 Hz), 1.77–1.67 (m, 4H), 1.46–1.40 (m, 2H). ¹³C NMR (CDCl₃) δ 177.7, 155.5, 152.8, 148.7, 148.6, 148.5, 148.4 124.7, 124.0, 122.6, 121.8, 35.1, 34.0, 29.7, 28.5, 24.5, 21.1. FABMS (NBA/CHCl₃) *m/z* 285 [(M+H)⁺], HRMS (NBA) [(M+H)⁺] calcd. 285.1603, found 285.1612.

Preparationofdichlorobis(4,4'-dimethyl-2,2'-bipyridine)ruthenium(II), $Ru^{II}(bpy)_2$ -Cl2. A mixture containing 4,4'-dimethyl-2,2'-bipyridine (1.417 g, 7.69mmol), RuCl3·3H2O (1.0 g, 3.82 mmol), and lithium chloride (1.1 g, 25.9 mmol) inDMF (10 mL) was refluxed for 7 h. After the reaction, the resulting solution was pouredinto acetone (25 mL) and stored at 5 °C overnight to form the precipitate. The productswere removed by filtration and washed with water (3 × 25 mL) and diethyl ether (3 × 25 mL) before drying in *vacuo* to yield the title compound as a black solid (1.72 g, 83%).The product was used directly in the next step.

Octaammonium POSS, POSS-[NH₃Cl]₈, 8. (3-Aminopropyl)triethoxysilane (100 mL, 0.427 mol) and conc. hydrochloric acid (35–37 %, 135 mL) in methanol (800 mL) produced **8** as a white precipitate after 5 days at room temperature. The product was obtained after filtration, washing with cold methanol, and drying. The POSS-[NH₃Cl]₈ was spectroscopically pure in 30% yield (18.8 g). ¹H NMR (DMSO-*d*₆) δ 8.23 (s, 24H), 2.76 (t, 16H), 1.71 (m, 16H), 0.72 (t, 16H). ¹³C NMR (DMSO-*d*₆) δ 40.53, 20.13, and 7.96. ²⁹Si NMR (DMSO-*d*₆) δ –66.4 (s). MALDI–TOF [(M+H)⁺] calcd. 880.41, found 879.42.

Compound 9. In a 200-mL round-bottom flask, 14 g of bipyridine 7 (49 mmol), 7 mL of triethylamine (49 mmol) and 14 g of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM, 90%) (56 mmol) were stirred in 100 mL of dry methanol until the solid had completely dissolved. To the mixture solution, 20 g of POSS-[NH₃Cl]₈ **8** (17 mmol) was added. The mixture was stirred at room temperature under argon. After stirring for 24 h, the resulting mixture was evaporated to 20 mL and poured into acetonitrile containing 0.1% HCl. The precipitate was washed with acetonitrile. The POSS derivative containing the bipyridine lignad was obtained to yield

(79%) as a yellowish powder after drying *in vacuo*. ¹H NMR (DMSO-*d₆*) δ 8.65 (br, 1.2H), 8.55 (br, 0.6H), 8.30 (br, 2.4H), 7.64 (br, 0.6H), 2.94 (br, 1.2H), 2.75 (br, 2H), 2.49 (br, 0.6H), 2.06 (br, 0.6H), 1.71 (br, 2.6H), 1.45 (br, 1.2H), 1.22 (br, 0.6H), 0.70 (s, 2H). ¹³C NMR (DMSO-*d₆*) δ 172.4, 157.1, 154.7, 147.9, 146.7, 146.1, 145.8, 127.5, 126.6, 124.3, 123.4, 99.4, 89.8, 59.8, 41.1, 35.1, 34.6, 29.2, 28.2, 25.1, 20.6, 8.47, and 2.50. ²⁹Si NMR (DMSO-*d₆*) δ -66.5 (s). MALDI–TOF [(M+H)⁺] calcd. 1414.17, found 1412.56.

Compound 10. 1,8-Naphthyridine ligand **5** (9.9 g, 24.5 mmol), triethylamine (3.5 mL, 24.5 mmol) and DMT-MM (8.9 g, 37 mmol) were stirred in 50 mL of dimethyl sulfoxide (DMSO) until the solid had completely dissolved. To the mixture solution, 23 g of POSS derivative **9** (17 mmol) was added. The mixture was stirred at room temperature under argon. After stirring for 24 h, the resulting mixture was poured into acetonitrile containing 0.1% HCl. The precipitate was collected by vacuum filtration, and washed with acetonitrile. The crude product was dissolved in water and filtrated, and the solvent was removed by a rotary evaporator. The POSS derivative containing the bipyridine and the naphthyridine ligands was obtained as a darkish brown powder (43%). ¹H NMR (DMSO-*d*₆) δ 11.10 (s, 1H), 10.46 (s, 1H), 9.91 (s, 1H), 8.62 (br, 32H),

8.25 (br, 98H), 7.64 (br, 32H), 7.21 (br, 11H), 2.74 (br, 86H), 2.45 (br, 51H), 2.06 (br, 22H), 1.83 (br, 28H), 1.66 (br, 66H), 1.44 (br, 43H), 1.21 (br, 22H), 0.63 (br, 72H). ¹³C
NMR (DMSO-*d*₆) δ 172.10, 155.27, 150.01, 147.16, 134.46, 123.61, 120.99, 103.00, 90.55, 82.20, 67.72, 60.92, 52.37, 41.31, 35.25, 34.68, 29.28, 28.24, 25.03, 22.59, 21.30, 20.77, 17.76, and 9.05. ²⁹Si NMR (DMSO-*d*₆) δ –66.6.

POSS-N. A typical protocol for the polymerization reaction is described here. To a solution of octa-ammonium POSS **8** (13.2 g, 11.2 mmol) and POSS **10** (4 g, 2.2 mmol) in 20 mL of dry methanol, the premixed solution containing 4.03 g of oxalic acid (44.8 mmol), 12.5 mL of triethylamine (89.6 mmol), 24.1 g of DMT-MM (0.1 mol) in dry methanol (100 mL) was added, and the reaction mixture was stirred at room temperature. After stirring for 24 h, the resulting mixture was poured into acetonitrile containing 0.1% HCl, and the precipitate was washed with acetonitrile. The crude product was dissolved in water and filtrated, and the solvent was removed by a rotary evaporator. The title compound was obtained as a darkish red powder (57%) after drying *in vacuo*. ¹H NMR (DMSO-*d*₆) δ 11.12 (br, 1H), 10.99 (br, 2H), 10.45 (br, 4H), 8.22 (br, 135H), 7.10 (br, 10H), 3.75 (br, 10H), 3.19 (br, 20H), 2.75 (br, 73H), 2.34 (br, 12H), 2.15 (br, 5H), 2.04 (br, 6H), 1.68 (br, 104H), 1.52 (br, 10H), 1.25 (br, 5H), 0.68

(br, 104H). ¹³C NMR (DMSO-*d*₆) δ 172.41, 161.67, 161.39, 156.51, 155.43, 150.04, 131.36, 127.68, 59.36, 42.45. 41.04, 33.59, 31.91, 28.32, 26.84, 25.29, 22.13, 21.08, 20.65, 10.01, 9.31, 8.52, and 2.83. ²⁹Si NMR (DMSO-*d*₆) δ –66.5.

POSS-Ru. A mixture containing 1g of POSS-N network polymer (bipyridine unit; 14.5 mg, 0.0508 mmol) and Ru^{II}(bpy)₂Cl₂ (27.4 mg, 0.0508 mmol) was heated at reflux in water (20 mL) for 24 h. The resulting solution was poured into acetonitrile containing 0.1% HCl, and the precipitate was collected by vacuum filtration, washed with acetonitrile. The crude product was dissolved in water and filtrated, and the solvent was removed by a rotary evaporator. The title compound was obtained as a blackish powder (93%). ¹H NMR (D₂O) δ 8.86 (br, 1H), 8.42 (br, 3H), 8.05 (br, 1H), 7.73 (br, 11H), 7.25 (br, 2H), 3.58 (br, 6H), 3.14 (br, 74H), 2.80 (s, 1H), 2.72 (s, 1H), 2.54 (br, 2H), 2.36 (br, 1H), 1.91 (br, 63H), 1.71 (br, 2H), 1.47 (br, 2H), 0.91 (br, 63H). ²⁹Si NMR (D₂O) δ –65.3.

Encapsulation of nucleoside derivatives. General procedure for the complexation of guanosine nucleosides by the POSS network polymers is described here. The stock solutions of the POSS-N network polymers (x10) and guanosine nucleosides (x10) were

mixed at room temperature, and then the 500 μ L of the samples were prepared by adding the PBS buffer (pH 7.4) solution. The complexation with POSS-Ru network polymers was also prepared with the same procedure in PBS buffer solution containing potassium ferricyanide (K₃[Fe(CN)₆]).

Fluorescence measurements of the complexes. The fluorescence emissions of the naphthyridine ligand 5 (20 μ M naphthyridine in the POSS-N network polymer and 10 μ M the naphthyridine in POSS-Ru network polymer) 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. The data were analyzed with the Stern–Volmer equation:

$$\frac{\mathbf{I}_0}{\mathbf{I}} = 1 + K_{\rm SV} \left[\mathbf{Q} \right]$$

The emission intensities were plotted according to a Stern–Volmer equation, reporting I_0/I versus the concentrations of guanosine nucleosides [Q], where I_0 is the intensity in the absence of quencher and I is the intensity in the presence of a quencher

concentration. K_{SV} is the Stern–Volmer quenching constant. A plot of I₀/I versus [Q] yields an intercept of one and a slope equals to K_{SV} .

Binding constants calculation. According to the non-emissive complex formation between the 1,8-naphthyridine receptor and guanosine nucleosides, the binding constant (K_A) can be calculated with the number of the binding molecules (*n*) from the following equation:

$$\log \frac{\text{Io-I}}{\text{I}} = \log K_{\text{A}} + n \log[\text{Q}]$$

Figure 2 in the main text represents the plots for evaluating the K_A values of the 1,8naphthyridine ligands to GDP and GTP.



Chart 1. ¹H NMR spectrum of the Npt ligand 5.



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Chart 2. ¹H NMR spectrum of the bpy ligand 7.



Chart 3. ¹H NMR spectrum of the bpy modified POSS 9.



Chart 4. ¹H NMR spectrum of the Npt-bpy modified POSS 10.



Chart 5. ¹H NMR spectrum of **POSS-N**.



Chart 6. ²⁹Si NMR spectrum of **POSS-N**.



Chart 7. ¹H NMR spectrum of **POSS-Ru**.

Scheme S1. Synthesis of the ligands^{*a*}



^{*a*}Reagents and conditions: (a) Malic acid, 95% H₂SO₄, 110 °C, 3 h, 92%; (b) acetic anhydride, reflux, 3 h, 85%; (c) POCl₃, 95 °C, 2 h, 60%; (d) 3-ethynylaniline, PdCl₂(PPh₃)₂, CuI, triethylamine, THF, r.t., 16 h, 31%; (e) succinic anhydride, MeOH, r.t., 12 h, 67%.; (f) (i) LDA, THF, -78 °C, 1 h, (ii) 5-bromovaleronitrile, 0 °C, 2 h, 99%; (g) conc. HCl, 100 °C, 18 h, 97%.



Scheme S2. Synthesis of the network polymers^{*a*}

^aReagents and conditions: (h) conc. HCl, MeOH, r.t., 5 d, 33%; (j) bipyridine ligand, DMT-MM, triethylamine, MeOH, r.t., 1 d, 79%; (k) Npt ligand, DMT-MM, triethylamine, DMSO, r.t., 1 d, 43%; (l) octaammnium-POSS, oxalic acid, MeOH, r.t., 1 d, 57%; (m) Ru^{II}(bpy)₂Cl₂, water, reflux, 12 h, 93%.



Figure S1. Emission changes of 20 μ M the naphthyridine ligand in POSS-N by adding each guanosine nucleoside (0 μ M to 400 μ M) in PBS buffer solution (pH 7.4) at 25 °C. Excitation wavelength was 364 nm.



Figure S2. Emission change of 20 μ M the naphthyridine ligand in POSS-N by adding 400 μ M adenosine triphosphate (ATP) in PBS buffer solution (pH 7.4) at 25 °C. Excitation wavelength was 364 nm.

Figure S3. (a) UV–vis spectra of 10 μ M Ru^{II}(bpy)₃·6H₂O by adding 200 μ M GTP with 100 μ M K₃[Fe(CN)₆] in PBS buffer solution (pH 7.4) at 25 °C. Photoreactions were performed for 120 min at 25 °C with a 4 W UV-lamp (365 nm). (b) Oxidation ability of Ru^{II}(bpy)₃ to GTP was evaluated from the results of HPLC analyses (CH₃CN/H₂O form 19:1 to 0:100) (I_G; intensity of GTP, I_T; intensity of thymidine, total concentration of GTP and thymidine was fixed).

Figure S4. Changes in UV–vis absorption spectra of the solutions containing 10 μ M naphthyridine ligands in POSS-Ru by adding 200 μ M (a) rG , (b) GMP, (c) cGMP, and (d) GDP with 100 μ M K₃[Fe(CN)₆] in PBS buffer solution (pH 7.4) at 25 °C. Photoreactions were performed for 120 min at 25 °C with a 4 W UV-Lamp (365 nm).