**Supporting Information**

# Effect of Microtubules Polymerization on Photoinduced Hydrogen Generation

Kosuke Okeyoshi[1], Ryuzo Kawamura[1], Ryo Yoshida[2], and Yoshihito Osada[1,*]

[1]RIKEN Advanced Science Institute

2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan

[2]Department of Materials Engineering, Graduate School of Engineering, The University of Tokyo

7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan

*To whom correspondence should be addressed

Phone: +81-48-467-2816 Fax: +81-48-467-9300 E-mail: osadayoshi@riken.jp

## Experimental

***Preparation of $Ru(bpy)_3^{2+}$-Tubulin.*** To remove MT-associated proteins, tubulin was purified from porcine brain using a high concentrations of PIPES buffer (1 M PIPES, 20 mM ethyleneglycol-bis(*β*-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), 10 mM $MgCl_2$, pH adjusted to 6.8 using HCl).[1] The tubulin concentration was determined by measuring the absorbance at 280 nm using an extinction coefficient of 115,000. $Ru(bpy)_3^{2+}$-conjugated tubulin was prepared using Bis(2,2'-bipyridine)-4'-methyl-4-carboxybipyridine-ruthenium *N*-succinimidyl ester-bis(hexafluorophosphate) by amine coupling according to previously published studies.[2-3] The $Ru(bpy)_3^{2+}$-tubulin stoichiometry, *i.e.*, $Ru(bpy)_3^{2+}$ molecules/tubulin was estimated to be 3.1 ± 0.1/2.0 on average. The efficiency of labeling by the amine coupling was 31%.

***Microscopic Observation of the MTs.*** To confirm the MTs formation, the samples were negatively-stained by uranyl acetate, and were observed on a collodion-coated grid by TEM (H-7000, Hitachi). To confirm the MTs formation in given suspensions, they were observed under a fluorescence microscope (IX71, Olympus) through a filter cube (AW078000 BP460–490 nm, LP663100 DM570 nm, AW098200 575 nm IF, U-MF2 Olympus) with an objective lens (Nikon, Plan Apo 100X Oil/1.45) and an EM-CCD camera (Andor iXon+, AndorTechnology plc., Belfast, Northern Ireland).

***Measurement of Fluorescence Spectra.*** The fluorescence spectra were measured with a spectrofluorometer (HITACHI, F-2500) with a thermal control unit. The wavelength of excitation was 460 ± 10 nm. Samples were put in a quartz cell (1 mm x 1 mm x ~50 mm). The spectra were measured at given temperature after 15 min stabilizing the temperature.

***Preparation of Tiopronin-Pt Nanoparticles.*** Tiopronin-modified Pt NPs were prepared by an alcohol reduction method with chloroplatinic acid (Wako Pure Chemical Industries, Co., Ltd., JAPAN) (68 mg), *N*-(2-mercaptopropionyl)glycine (tiopronin, Sigma-Aldrich) (12 mg) as a protector, and ethanol/water mixed solvent (60 mL/60 mL). After dialysis, the colloidal solution was concentrated and dispersed in water (6.7 mM, 12 mL). Pt concentration was determined by inductively coupled plasma mass spectrometry.

***Visible Light Irradiation.*** The samples for visible light irradiation was prepared by using a mixture of the $Ru(bpy)_3^{2+}$-conjugated tubulin suspension ([tubulin] = ~110 $\mu$M) and non-conjugated tubulin suspension ([tubulin] = ~400 $\mu$M). Firstly, 6-thio-GTP (Jena Bioscience) was mixed to keep as tubulin, and GpCpp (Jena Bioscience) was mixed to form stable MTs in BRB80 buffer (80 mM PIPES; 1 mM EGTA; 5 mM $MgCl_2$, pH6.8) for 1 day. Next, the tubulin suspension or the MTs suspension were mixed with the Pt colloidal solution and they were soaked in a mixture of EDTA/$MV^{2+}$. The final tubulin concentration was adjusted to ~210 $\mu$M. The suspensions in the reaction cell (width: 10 mm, depth: 1 mm, height: ~50 mm) were irradiated with visible light by using a 100 W halogen lamp with a flat-surface light source (Techno Light KTS-100RSV, Kenko). At given times, the absorption spectra were measured by a UV-vis spectrophotometer (UV-2500PC, SHIMADZU), and the generated gas was collected and analyzed by gas chromatography (GC-8APT, SHIMADZU).

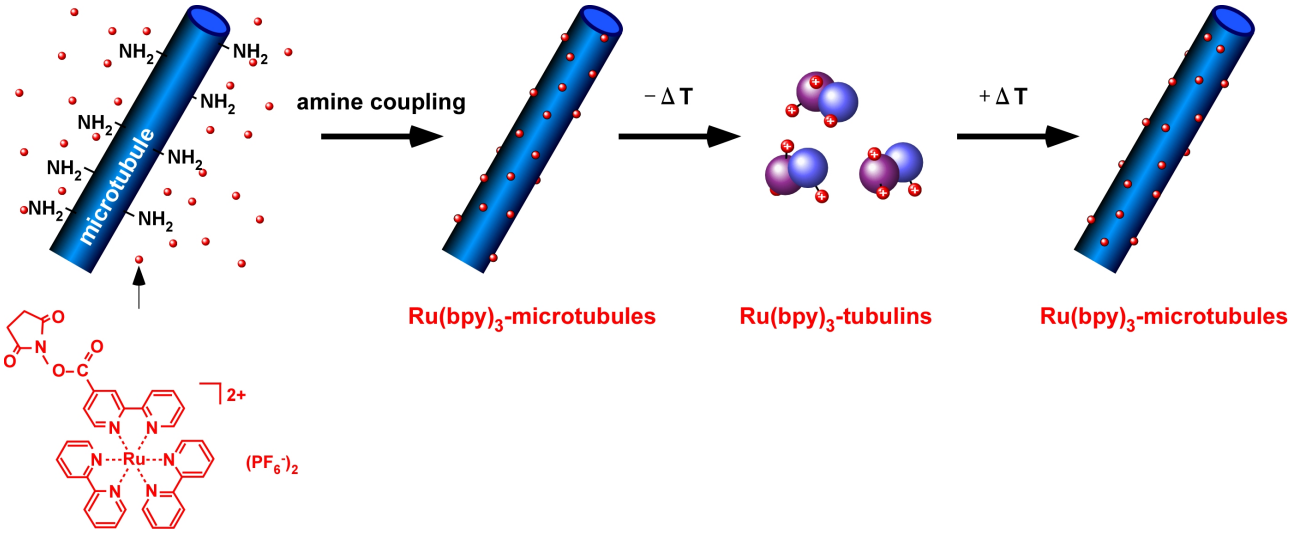



***Figure S1.*** Preparation of $Ru(bpy)_3^{2+}$-conjugated tubulin by amine coupling.

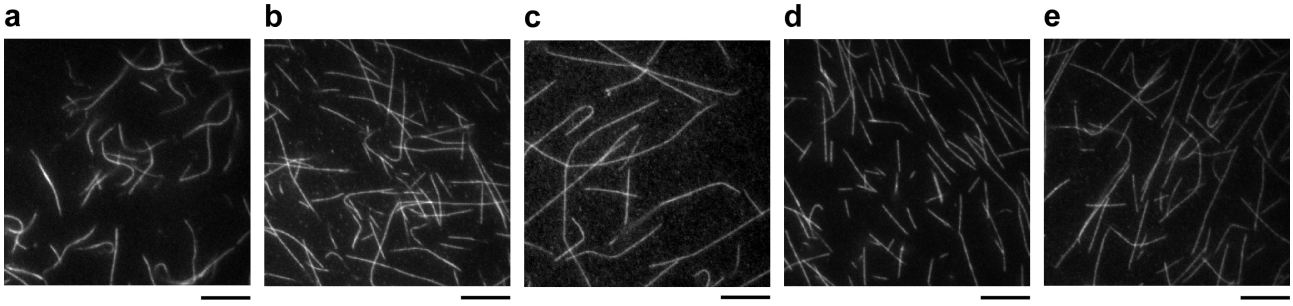



***Figure S2.*** Fluorescence microscopy images of MTs composed of rhodamine-conjugated tubulin and non-conjugated tubulin in given suspensions: 50 mM EDTA **(a)**, 200 $\mu$M $Ru(bpy)_3^{2+}$ **(b)**, 5 mM $MV^{2+}$ **(c)**, 100 $\mu$M Pt **(d)**, control **(e)**. BRB80 buffer, 1 mM GTP. Scale bars: 10 $\mu$m.

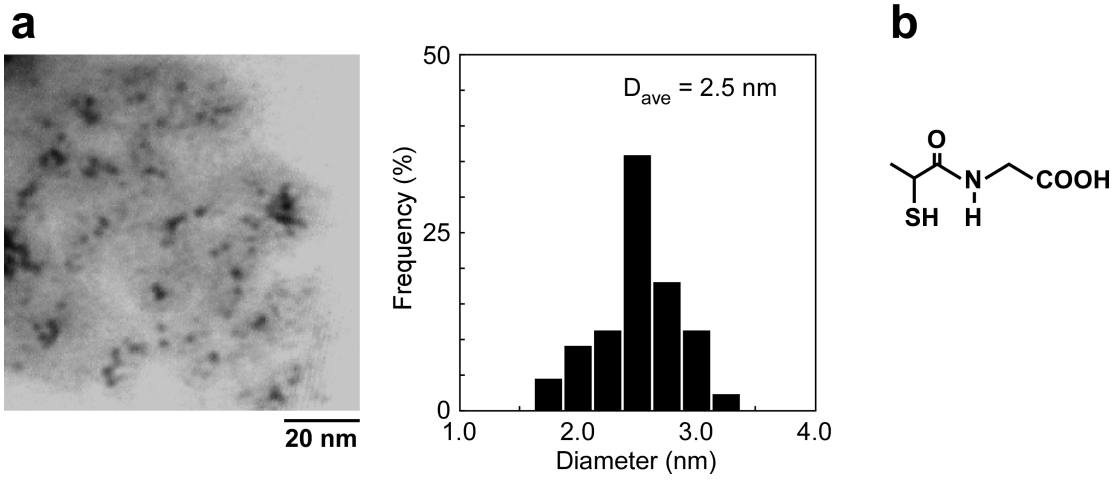



***Figure S3.*** TEM image of tiopronin-Pt NPs (a), and chemical structure of tiopronin (b).

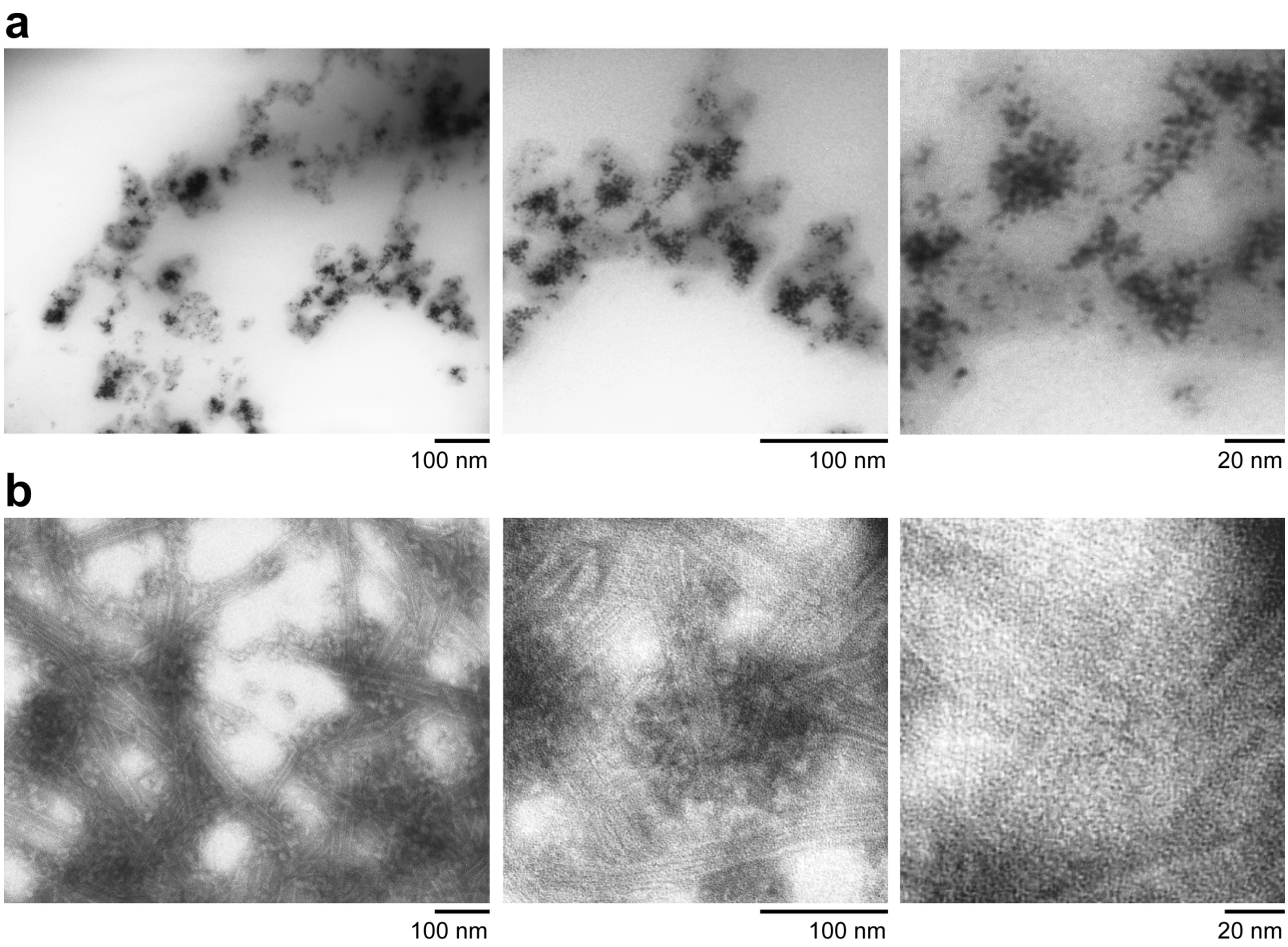



***Figure S4.*** TEM images for the Pt NPs (a) and the mixture of the Pt NPs and $Ru(bpy)_3^{2+}$-conjugated MTs.

During the sample preparation and the observation for TEM, the existence of the MTs was effective for the Pt NPs. In case that the Pt NPs colloidal solution were dried, the Pt NPs were observed in aggregated state. On the other hand, in case that the mixture of the Pt NP colloidal solution and the MT suspensions were dried, it was difficult to find such aggregation in large area.

**Theoretical analysis for fluorescence lifetime and quenching effect.**

To verify the effects of the excited lifetime and the quenchers on the fluorescence intensity of the $Ru(bpy)_3^{2+}$-tubulin/MTs, the Stern-Volmer relation should be clarified.

$$\frac{F}{F\prime} = 1 + K_{sv}[q] \qquad (1)$$

$$K_{sv} = k_q\tau \qquad (2)$$

$F$: fluorescence intensity without quencher
$F'$: fluorescence intensity with quencher
$K_{sv}$: Stern-Volmer constant
$[q]$: concentration of quencher
$k_q$: kinetic constant of quencher
$\tau$ : the lifetime of the excited state without quencher

To obtain these values ($F/F'$, $k_q$, $\tau$ ) for $Ru(bpy)_3^{2+}$-tubulin/MTs, the experiments should be held without quenchers ($[q] = 0$) involving compounds of buffer solutions, tubulin, and GTP. However, it is technically difficult, considering that these compounds are necessary for the tubulin formation and the MTs formation.
Here, anticipating the $Ru(bpy)_3^{2+}$-tubulin/MTs formation which depends on temperature, the Stern-Volmer relation with extended conditions is theoretically discussed by case analysis. At low temperature, $T_1$ and high temperature $T_2$, the factors are given as follows.

$$\frac{F}{F\prime} \approx \frac{\tau}{\tau\prime} \qquad (3)$$

Temperature $T_1$: $\frac{\tau_1}{\tau_{1}\prime} = 1 + k_{q1}\tau_1[q]$ (4)

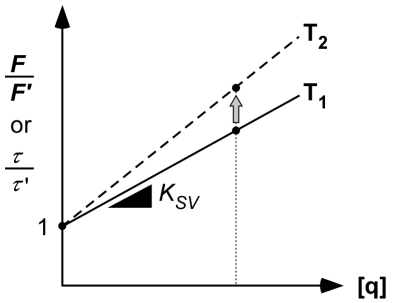
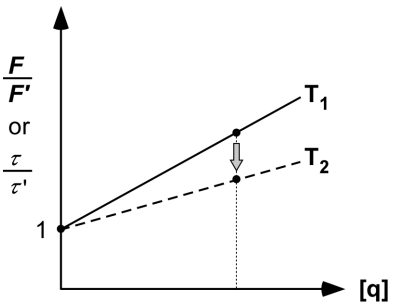
Temperature $T_2$: $\frac{\tau_2}{\tau_{2}\prime} = 1 + k_{q2}\tau_2[q]$ (5)

$\tau$ ': the lifetime of the excited state with quencher molecules

Using the equations (4) and (5), the correlation of $\tau_1$ and $\tau_2$ is as follows.

$$\frac{k_{q2}\tau_2}{k_{q1}\tau_1} = \frac{\frac{\tau_2}{\tau_2'}-1}{\frac{\tau_1}{\tau_1'}-1} = \frac{\frac{F_2}{F_2'}-1}{\frac{F_1}{F_1'}-1} \qquad i.e., \quad \frac{\tau_2}{\tau_1} = \frac{k_{q2}}{k_{q1}}\left(\frac{\frac{F_2}{F_2'}-1}{\frac{F_1}{F_1'}-1}\right) \qquad (6)$$

This correlation is used in the case analysis for $K_{SV}$ as a following table. Considering the micro-environmental changes from $Ru(bpy)_3^{2+}$-tubulin and $Ru(bpy)_3^{2+}$-MTs, the conventional properties of $\tau$ is extended.

| | $\tau_1 > \tau_2$ | $\tau_1 < \tau_2$ |
|---|---|---|
| $K_{SV1} < K_{SV2}$<br>$(k_{q1}\ \tau_1 < k_{q2}\ \tau_2)$<br>  <br> | (I)<br>$\frac{k_{q2}}{k_{q1}} > \left(\frac{\frac{F_2}{F_2'}-1}{\frac{F_1}{F_1'}-1}\right)$ | (II)<br>$\frac{k_{q2}}{k_{q1}} < \left(\frac{\frac{F_2}{F_2'}-1}{\frac{F_1}{F_1'}-1}\right)$ |
| $K_{SV1} > K_{SV2}$<br>$(k_{q1}\ \tau_1 > k_{q2}\ \tau_2)$<br>  <br> | (III)<br>$\frac{k_{q2}}{k_{q1}} < \left(\frac{\frac{F_2}{F_2'}-1}{\frac{F_1}{F_1'}-1}\right)$ | (IV)<br>$\frac{k_{q2}}{k_{q1}} > \left(\frac{\frac{F_2}{F_2'}-1}{\frac{F_1}{F_1'}-1}\right)$ |

**(I) and (IV)** : $\frac{k_{q2}}{k_{q1}} > \left(\frac{\frac{F_2}{F_2'}-1}{\frac{F_1}{F_1'}-1}\right)$ **(7)**

**(A)** In case of $\frac{k_{q2}}{k_{q1}} > 1$, there are further two cases, **(a)** and **(b)** as follows.

**(A-a)** $\left(\frac{\frac{F_2}{F_2'}-1}{\frac{F_1}{F_1'}-1}\right) < 1 < \frac{k_{q2}}{k_{q1}}$

This derives, $\frac{F_1}{F_1'} - 1 < \frac{F_2}{F_2'} - 1$ , *i.e.*, $\frac{F_1}{F_1'} < \frac{F_2}{F_2'}$ (8)

This relation is corresponding to the case **(I)**.

Besides, by involving the magnitude relation of the quenching, $\frac{F_2\prime}{F_2} < \frac{F_1\prime}{F_1} < 1$,

$$\frac{F_1 - F_1'}{F_1} < \frac{F_2 - F_2'}{F_2} \quad (9)$$

The quenching influence to the fluorescence intensity at higher temperature is larger. This

temperature dependency is a general case for a fluorescence substance in solution, also sawn in the free $Ru(bpy)_3^{2+}$ solution. Contrary to this, the temperature dependency of the $Ru(bpy)_3^{2+}$-tubulin/MTs obtained from experiment is inverse that the other relation of the factors should be considered.

**(A-b)** $1 < \left( \frac{\frac{F_2}{F_2'} - 1}{\frac{F_1}{F_1'} - 1} \right) < \frac{k_{q2}}{k_{q1}}$

This derives, $\frac{F_2}{F_2'} < \frac{F_1}{F_1'}$ (10)

This relation corresponds to to the case **(IV)**.
Besides, by involving the magnitude relation of the quenching,

$\frac{F_2 - F_2'}{F_2} < \frac{F_1 - F_1'}{F_1}$ (11)

The quenching influence to the fluorescence intensity at higher temperature is smaller.

**(B)** In case of $\frac{k_{q2}}{k_{q1}} < 1$, *i.e.*, $k_{q2} < k_{q1}$, the following relation is derived with equation (7).

$\frac{F_1}{F_1'} - 1 < \frac{F_2}{F_2'} - 1$ *i.e.*, $\frac{F_1}{F_1'} < \frac{F_2}{F_2'}$ (12)

These relations correspond to the case **(I)**.
Comparing with the experimental data of the Ru(bpy)-tub/MTs with an increase in temperature, the $k_{q2} < k_{q1}$ suggests that the MT formation induces GTP-binding which results the decrease of $k_q$.

**(II) and (III) :** $\boldsymbol{\frac{k_{q2}}{k_{q1}} < \left( \frac{\frac{F_2}{F_2'} - 1}{\frac{F_1}{F_1'} - 1} \right)}$ **(13)**

**(C)** In case of $\frac{k_{q2}}{k_{q1}} > 1$, *i.e.*, $k_{q2} < k_{q1}$, the following relation is derived with the equation (13).

$\frac{F_1}{F_1'} - 1 > \frac{F_2}{F_2'} - 1$ *i.e.*, $\frac{F_1}{F_1'} > \frac{F_2}{F_2'}$ (14)

This relation corresponds to the case **(III)**.

Besides, by involving the magnitude relation of quenching, $\frac{F_1'}{F_1} < \frac{F_2'}{F_2} < 1$,

$\frac{F_1 - F_1'}{F_1} < \frac{F_2 - F_2'}{F_2}$ (15)

The quenching influence to the fluorescence intensity at higher temperature is smaller.

**(D)** In case of $\frac{k_{q2}}{k_{q1}} < 1$, *i.e.*, $k_{q2} < k_{q1}$.

Comparing with the experimental data of the Ru(bpy)-tubulin/MTs with an increase in temperature, the $k_{q2} < k_{q1}$ suggests that the MT formation induces GTP-binding which results the decrease of $k_q$.

Comparing with the experimental data of fluorescence intensity peak, the case of **(A-a)** corresponds to the free $Ru(bpy)_3^{2+}$ solution but not to the $Ru(bpy)_3^{2+}$-tubulin/MTs suspension. The possible cases corresponding to the $Ru(bpy)_3^{2+}$-tubulin/MTs suspension are (**A-b**), **(B)**, **(C)**, and (**D**). In any cases, the theoretical analysis suggested the decrease of the quenching influence with an increase of temperature, such as decrease ratio of fluorescence intensity and decrease of $k_q$.

**References**

[1] M. Castoldi, A. V. Popov, *Protein Expression Purif.* **2003**, *32*, 83.
[2] J. Peloquin, Y. Komarova, G. Borisy, *Nat. Methods* **2005**, *2*, 299.
[3] K. Okeyoshi, R. Kawamura, R. Yoshida, Y. Osada, *J. Mater. Chem. B,* **2014**, *2*, 41.