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Electrochemical response of metal complexes in homogeneous solution under photoirradiation

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ELECTROCHEMISTRYArisa Fukatsu^{1,2}, Mio Kondo^{1,2,3,4}, Masaya Okamura^{1,2}, Masaki Yoshida¹ & Shigeyuki Masaoka^{1,2,3}Received
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requests for materials
should be addressed to
S.M. (masaoka@ims.
ac.jp)

¹Department of Life and Coordination-Complex Molecular Science, Institute for Molecular Science, 5-1, Higashiyama, Myodaiji, Okazaki, Aichi 444-8787 Japan, ²The Graduate University for Advanced Studies [SOKENDAI], Shonan Village, Hayama, Kanagawa 240-0193 Japan, ³Research Center of Integrative Molecular Systems (CIMoS), Institute for Molecular Science, 38, Nishigo-naka, Myodaiji, Okazaki, Aichi 444-8585 Japan, ⁴ACT-C, Japan Science and Technology Agency (JST), 4-1-8, Honcho, Kawaguchi, Saitama 332-0012 Japan.

The electrochemical detection of metal complexes in the photoexcited state is important for understanding photoinduced electron transfer (PET) processes, which play a central role in photo-energy conversion systems. In general, however, the redox potentials of excited states have been indirectly estimated by a combination of spectroscopic properties and ground-state redox potentials. To establish a simple method for directly determining the redox potentials of the photoexcited states of metal complexes, electrochemical measurements under several conditions were performed. The electrochemical response was largely influenced not only by the generation of photoexcited molecules but also by the convection induced by photoirradiation, even when the global temperature of the sample solution was unchanged. The suppression of these unfavourable electrochemical responses was successfully achieved by adopting well-established electrochemical techniques. Furthermore, as an initial demonstration, the photoexcited state of a Ru-based metal complex was directly detected, and its redox potential was determined using a thin layer electrochemical method.

Photoinduced electron transfer (PET) is a key process in reactions that convert light energy to electrical or chemical energy, both in natural^{1,2} and artificial systems^{3–5}. The efficiency of PET, which largely affects the performance of these systems, is correlated with the redox properties of the photoexcited molecule, which transfers electrons or holes during the PET reaction. Hence, determining the redox potentials of photoexcited molecules is of great significance not only for understanding the mechanisms of PET reactions but also for achieving highly efficient light-energy conversion systems. Electrochemical analysis under photoirradiation should enable the measurement of the redox potentials of excited species. However, reports of the direct electrochemical detection of photoexcited molecules have been limited to only a few examples in which specialised photoelectrochemical instrumentation was required^{6,7}. This limitation may be due to the difficulty to avoid the complication of voltammogram profiles that arises from the unintended side effects of photoirradiation, such as temperature increases and enhanced mass transfer. Thus, the redox potentials of excited states have more commonly been *indirectly* estimated using the 0-0 transition energy (E_{00})⁸ or the quenching rate constant (k_q)^{9,10}. Therefore, the establishment of a versatile methodology for electrochemical measurements under photoirradiation will provide new insights into PET phenomena.

In this contribution, we report simple and convenient methods for determining the redox potentials of metal complexes under photoirradiation. The current response arising from the side effects induced by photoirradiation was effectively suppressed by adopting several electrochemical techniques. Moreover, the direct electrochemical detection of a metal complex in a photoexcited state was also achieved.

Results

Electrochemical measurements were performed using a custom-made electrochemical cell with coolant to maintain the temperature of the sample solution during the measurement (Fig. 1). Ferrocene (Fc), which exhibits reversible redox behaviour in various electrochemical conditions¹¹ and is a photochemically inactive metal complex, was adopted as a redox probe. The electrochemical properties of Fc are expected to be unchanged upon photoirradiation because the lifetime of the photoexcited triplet state of Fc is quite short (0.6 ns)¹². Thus, the

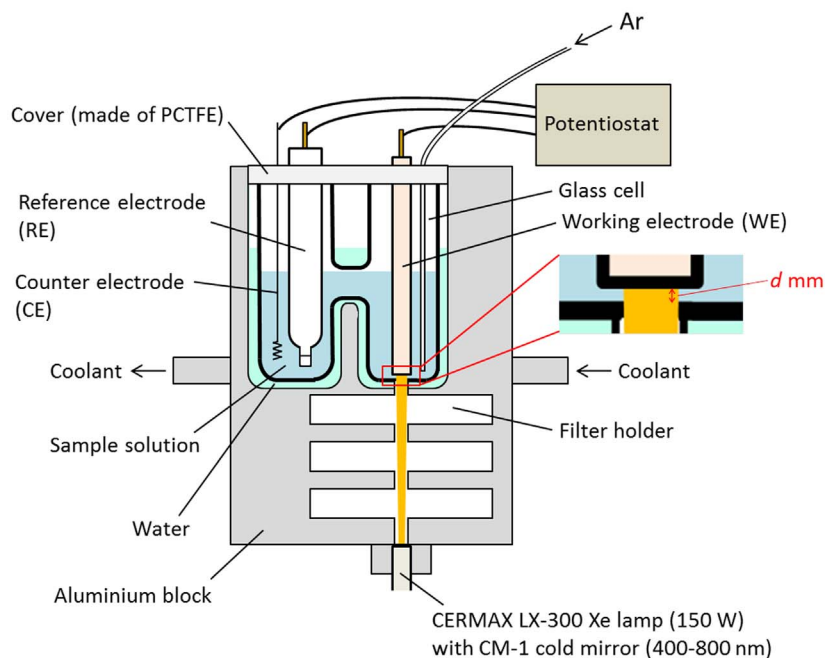


Figure 1 | Custom-made photoelectrochemical cell. Schematic illustration (left) and a photograph (right) are shown. The coolant was set to 20 °C to maintain a constant sample temperature during measurement. d indicates the thickness of the solution layer.

influence of the side effects induced by photoirradiation can be extracted from the electrochemical measurements of Fc under photoirradiation.

The electrochemical behaviour of Fc in acetonitrile under photoirradiation by a Xe lamp with a CM-1 cold mirror (400–800 nm) was investigated by cyclic voltammetry (CV) and chronoamperometry. Figure 2a shows the cyclic voltammograms of Fc with and without light irradiation. The reversible wave attributed to the redox process between Fc and ferrocenium (Fc^+) was observed without light irradiation. In contrast, under photoirradiation, the peak shape became sigmoidal, and the limiting current was observed. In chronoamperometry at +0.2 V (*vs.* Fc^+/Fc), an increase in current was observed repeatedly upon photoirradiation (Fig. 2b).

To identify the origin of this phenomenon, the temperature of the sample solution was measured. However, a large elevation in the temperature of the solution was not observed within the timescale of the electrochemical measurements performed in this study (Supplementary Figure 1 in the Supplementary Information). Note that small increases in temperature do not affect the electrochemical response of Fc because the reversibility of the redox process is maintained at 248.15–298.15 K¹³. Therefore, a change in the global temperature of the sample solution was not the cause of the electrochemical response under photoirradiation. Subsequently, CV was performed using light that cannot be absorbed by Fc ($\lambda > 570$ nm, Fig. 2c). Even in this case, a sigmoidal voltammogram was also obtained, as depicted in Figure 2d, suggesting that the changes in the cyclic voltammograms were not related to the photochemical properties of Fc. The sigmoidal shape observed in CV under photoirradiation indicated that the diffusion layer was affected by the mass transfer induced by convection¹⁴. These results suggested that the photoirradiation may have generated convection due to local increases in temperature, and the mass transfer subsequently induced by this convection affected the electrochemical responses.

Based on the above results, we aimed to decrease the current change under photoirradiation. First, measurements were performed using a rotating disk electrode (RDE)¹⁵ as the working electrode. RDEs can generate an extremely thin diffusion layer by forcing convection via the rotation of the electrode. In fact, upon increasing the

rotating speed of the working electrode, the cyclic voltammograms under photoirradiation gradually became similar in appearance to those in the absence of photoirradiation (Fig. 3). This result indicates that the convection induced by photoirradiation was negated by the forced convection of the RDE electrode.

Next, the effect of the scan rate on the electrochemical response under photoirradiation was examined to identify more easily accessible conditions for suppressing convection. As shown in Figure 4, although a relatively fast scan rate, which is not suitable for detecting redox species that exhibit slow electron transfer, was required, the current change was effectively suppressed by increasing the scan rate. In general, the thickness of the diffusion layer (μ) changes with time (t), and μ is on the order of $\mu = (Dt)^{1/2}$ (D : diffusion coefficient)¹⁶. Therefore, as the scan rate increases, μ becomes small. In this situation, the mass transfer of Fc and Fc^+ around the electrode is predominantly determined by the electrochemical reaction, and the effect of the convection induced by photoirradiation becomes negligible.

Finally, thin layer cyclic voltammetry¹⁷ (TLCV) was used as a simple and versatile technique to evaluate electrochemical properties under photoirradiation. This technique allows us to perform electrochemical measurements without considering the convection within a cell, and thus the effect of convection is expected to be excluded. To assess the validity of our strategy, the thickness of the solution layer (d , Fig. 1) was changed by adjusting the position of the working electrode. The sigmoidal-shaped voltammogram gradually changed to a reversible shape with reducing d , and the reversible redox wave of Fc was observed even at slow scan rates (5 mV s^{-1}) when d was smaller than 0.25 mm (Fig. 5). The similar results were observed in various solutions with the thin layer technique (Supplementary Figure 2 in the Supplementary Information).

The results described above encouraged us to detect photoexcited species. In this experiment, the TLCV technique was employed because this simple technique allows both the precise determination of redox potentials and the detection of redox species that exhibit slow electron transfer (*vide supra*). A well-known metal complex-based dye, tris(2,2'-bipyridyl)ruthenium(II) ($\text{Ru}(\text{bpy})_3^{2+}$), which is known to have an excited state ($^3\text{MLCT}$) with a long lifetime ($\tau =$

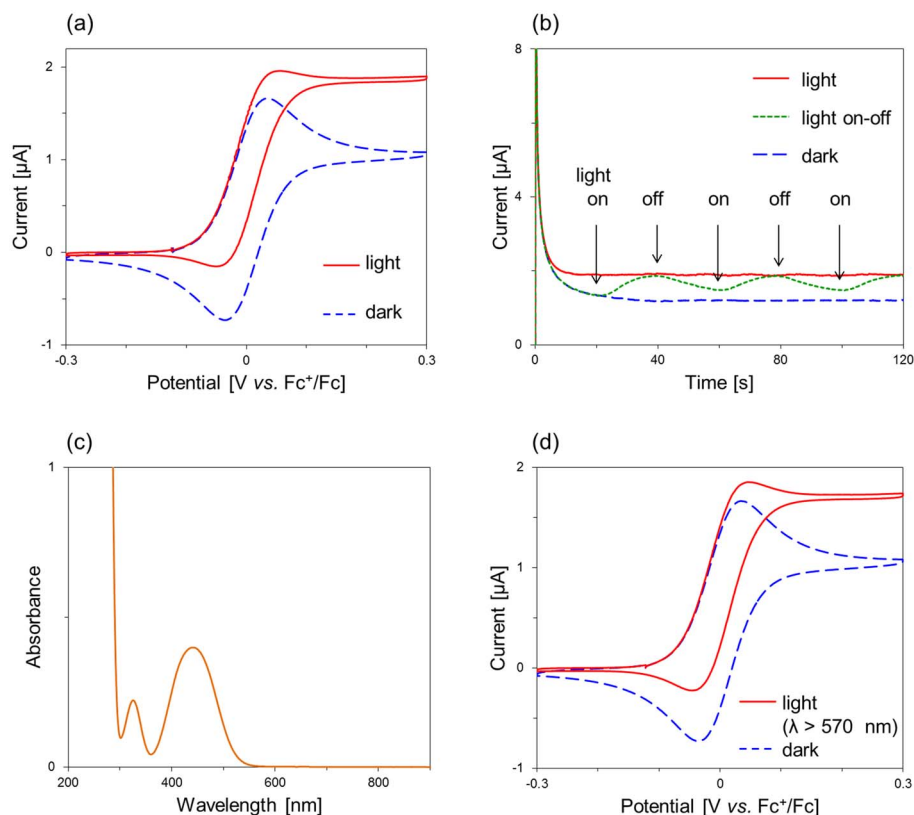


Figure 2 | Relationship between the optical properties of Fc and the electrochemical response. (a) Cyclic voltammograms of Fc (0.2 mM) in 0.1 M TBAP acetonitrile solution with photoirradiation (red, $400 < \lambda < 800$ nm) and without photoirradiation (blue) under an Ar atmosphere (WE: GC; CE: Pt wire; RE: Ag^+/Ag ; scan rate: 5 mV s^{-1}). (b) Current responses to photoirradiation ($400 < \lambda < 800$ nm) in the chronoamperograms (0.2 V vs. Fc^+/Fc) of Fc (0.2 mM) in 0.1 M TBAP acetonitrile solution under an Ar atmosphere (WE: GC; CE: Pt wire; RE: Ag^+/Ag). (c) UV-Vis absorption spectrum of Fc (4 mM) in acetonitrile. (d) Cyclic voltammograms of Fc (0.2 mM) in a 0.1 M TBAP acetonitrile solution under photoirradiation (red, $570 < \lambda < 800$ nm) and in the dark (blue) under an Ar atmosphere (WE: GC; CE: Pt wire; RE: Ag^+/Ag ; scan rate: 5 mV s^{-1}). The shapes of the cyclic voltammograms dramatically changed upon photoirradiation regardless of the irradiation wavelength.

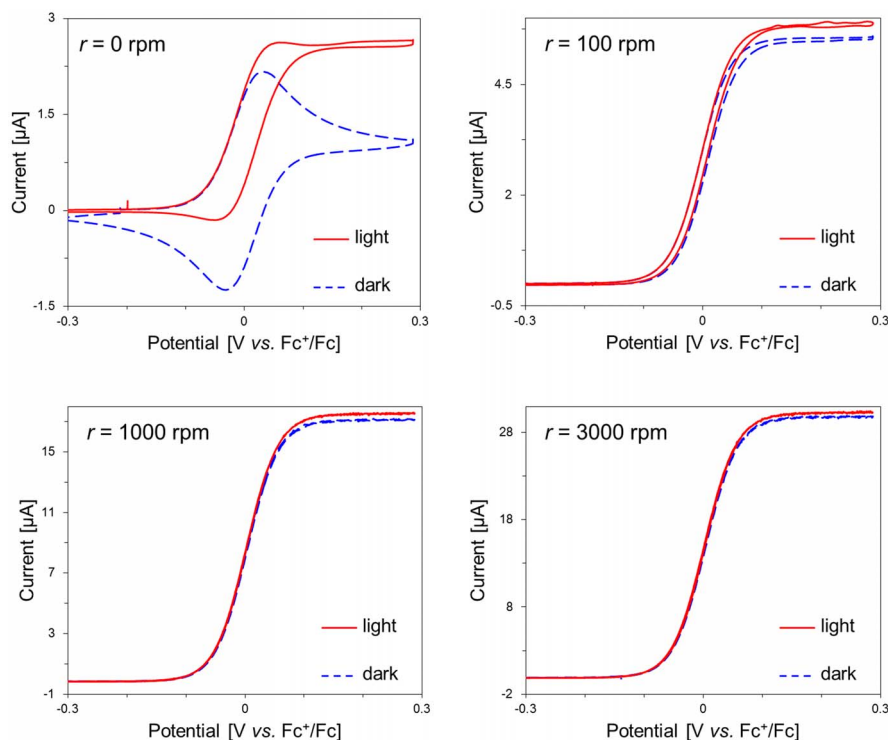


Figure 3 | Cyclic voltammograms of Fc with various rotation speeds of RDE with and without photoirradiation. Measurements were performed in a 0.1 M TBAP acetonitrile solution under an Ar atmosphere, and the concentration of Fc was adjusted to 0.2 mM. The red and blue lines are the cyclic voltammograms under photoirradiation ($400 < \lambda < 800$ nm) and in the dark, respectively. r indicates the rotation speeds of the working electrode. (WE: GC; CE: Pt wire; RE: Ag^+/Ag ; scan rate: 10 mV s^{-1}).

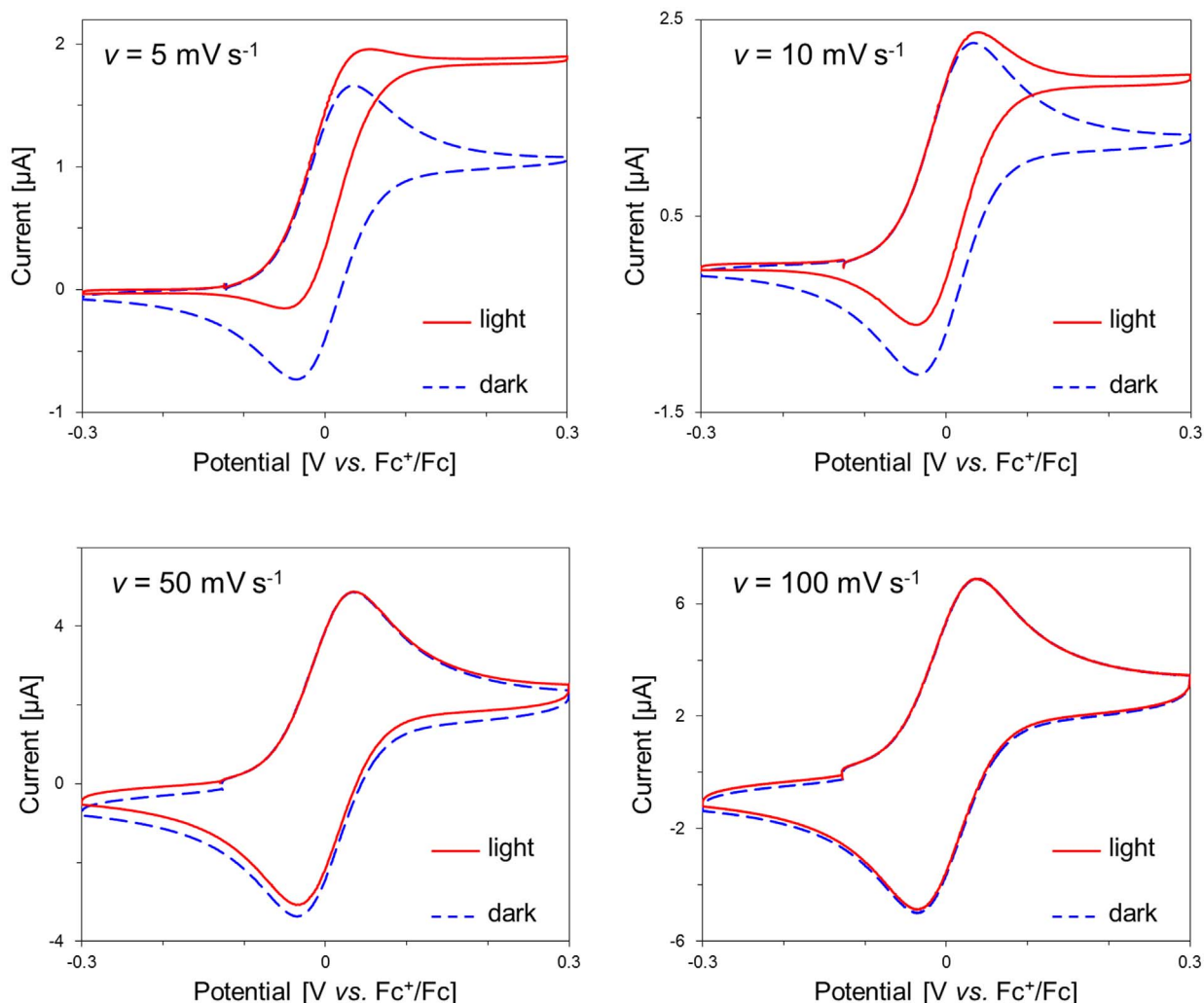


Figure 4 | Dependence of the cyclic voltammograms of Fc on the scan rate with and without photoirradiation. Measurements were performed in a 0.1 M TBAP acetonitrile solution under an Ar atmosphere, and the concentration of Fc was adjusted to 0.2 mM. The red and blue lines are the cyclic voltammograms under photoirradiation ($400 < \lambda < 800$ nm) and in the dark, respectively. v indicates the scan rate of each measurement. (WE: GC; CE: Pt wire; RE: Ag⁺/Ag).

0.64 μs in water (20°C)¹⁸), was adopted as the redox probe in this experiment. As shown in Figure 6a, one reversible peak, attributed to the Ru^{III}/Ru^{II} redox couple, was observed in the measurement without photoirradiation. Upon photoirradiation, the redox wave attributed to the oxidation of Ru^{II} to Ru^{III} changed to an irreversible peak, and a new broad peak appeared at approximately +0.8 V (vs. SCE). Note that these changes upon photoirradiation occurred only when Ru(bpy)₃²⁺ absorbed the irradiated light (Fig. 6), which implies that the generation of the photoexcited species of Ru(bpy)₃²⁺, [Ru(bpy)₃²⁺]*, was related to these photo responses. Further analysis of this phenomenon was performed by square wave voltammetry (SWV), which can minimise the contribution from the capacitive charging current and provide voltammograms with low background. Similar to the results of CV, a new peak appeared at +0.79 V vs. SCE only when the wavelength of the light overlapped with the absorption band of Ru(bpy)₃²⁺ (Fig. 7a). Furthermore, the peak appeared repeatedly in conjunction with photoirradiation (Fig. 7b). It was also confirmed that the peak disappeared almost completely under an O₂ atmosphere¹⁹, and then reproduced after Ar bubbling (Supplementary Figure 3 in the Supplementary Information).

Discussion

The electrochemical measurement of homogeneous solutions under photoirradiation is difficult for numerous reasons. In cyclic voltam-

metry, which is most frequently used technique to determine redox potentials, the oxidation/reduction of electroactive molecule creates a concentration gradient at the electrode surface. Subsequently, the current which is proportional to concentration gradient near the electrode surface flows if the electron transfer is fast enough¹⁶. The redox potentials of molecules can be precisely evaluated when the concentration gradient is mainly determined by the electrochemical reaction. However, as described in the previous section, photoirradiation induces convection, and this convection generates the concentration gradient that disturbs the molecular diffusion. As a result, cyclic voltammograms become sigmoidal in shape because the electrochemical signal originating from convection, which is essentially unrelated to the properties of the molecules, dominates, and the determination of the redox potential is not possible.

To overcome this problem, the RDE technique and measurements using fast scan rates were adopted. As shown in Figures 3 and 4, we succeeded in suppressing the disturbance of mass transfer induced by convection and in collecting substantial data of electrochemical measurements under photoirradiation by thinning the diffusion layer. Additionally, the TLCV technique was also employed, and the influence of photoirradiation was found to become negligible (Fig. 5) when limiting the thickness of the solution layer to less than 0.25 mm. These results led us to investigate the photoelectrochemical properties of Ru(bpy)₃²⁺, and electrochemical signals attributed

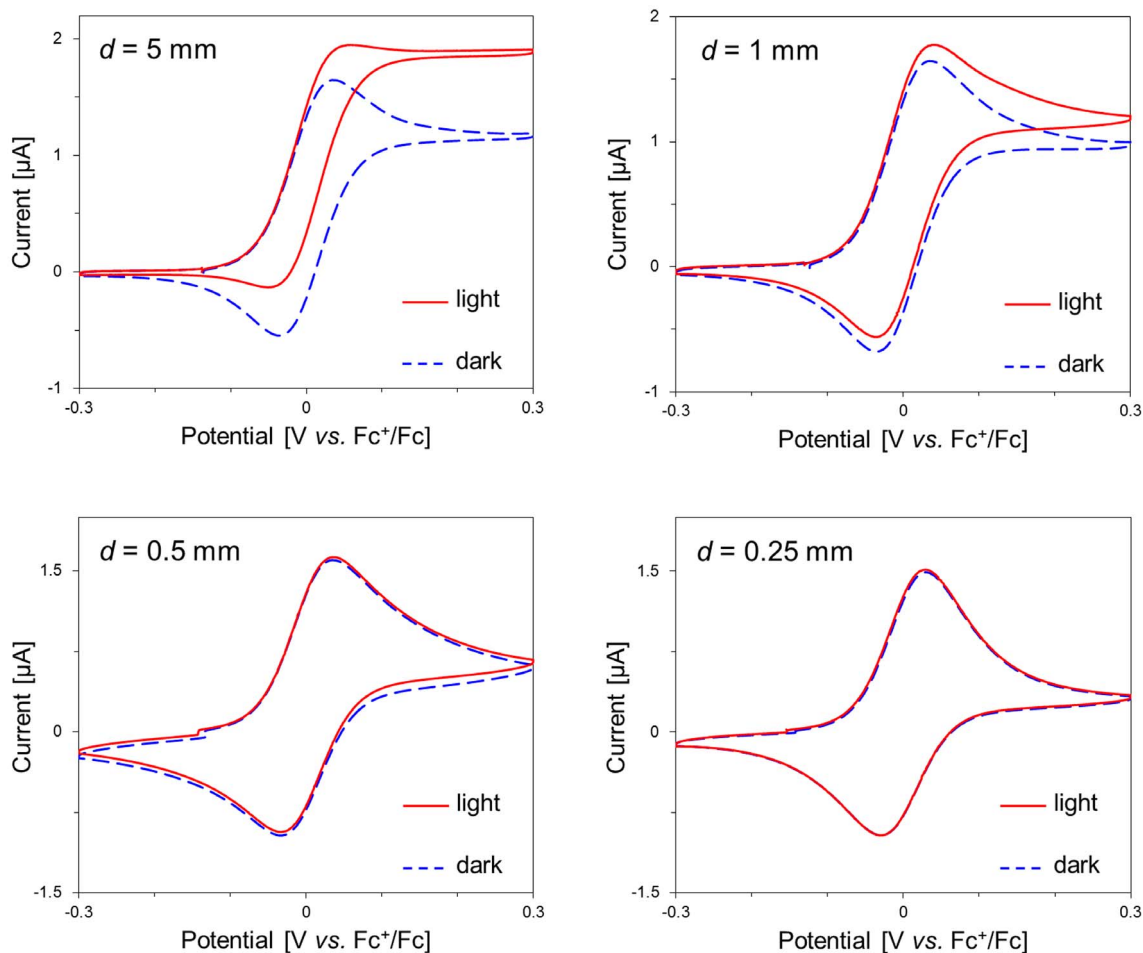


Figure 5 | Dependence of the cyclic voltammograms of Fc on the layer thickness with and without photoirradiation. Measurements were performed in a 0.1 M TBAP acetonitrile solution under an Ar atmosphere, and the concentration of Fc was adjusted to 0.2 mM. The red and blue lines are the cyclic voltammograms under photoirradiation ($400 < \lambda < 800$ nm) and in the dark, respectively. d indicates the thickness of the solution layer. (WE: GC; CE: Pt wire; RE: Ag^+/Ag ; scan rate: 5 mV s^{-1}).

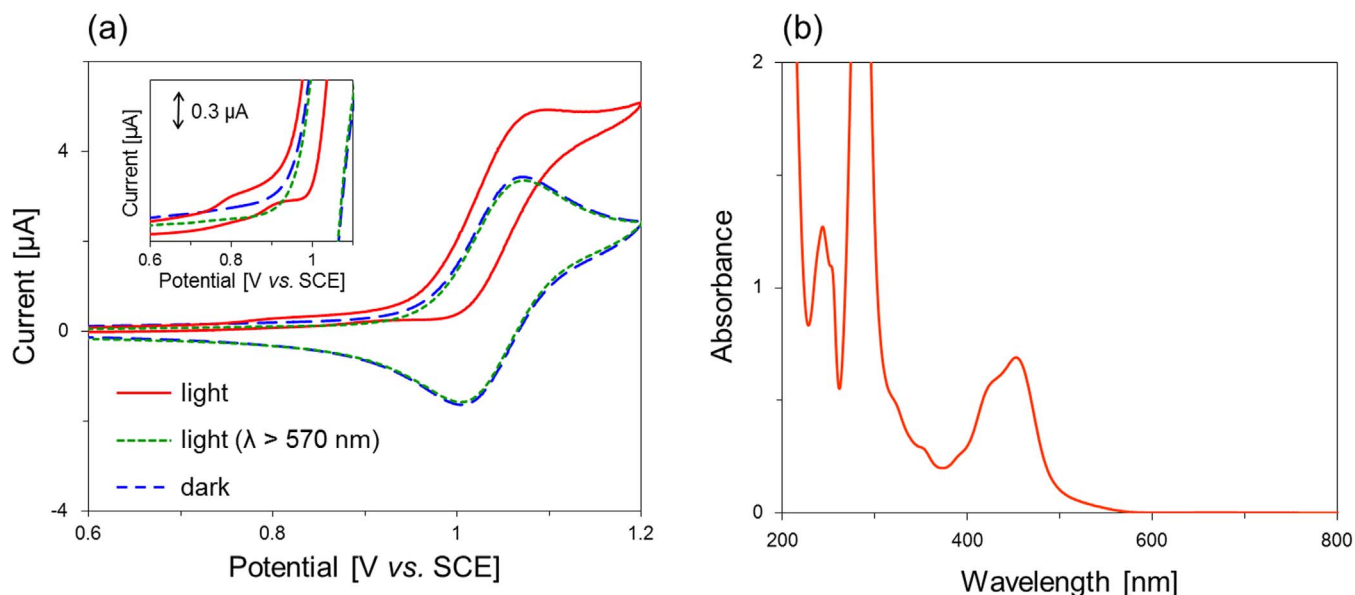


Figure 6 | Relationship between the optical properties of $[\text{Ru}(\text{bpy})_3](\text{NO}_3)_2 \cdot 5\text{H}_2\text{O}$ and the electrochemical response. (a) Thin layer cyclic voltammograms of $[\text{Ru}(\text{bpy})_3](\text{NO}_3)_2 \cdot 5\text{H}_2\text{O}$ (1 mM) in 0.1 M Na_2SO_4 aqueous solution under photoirradiation (red, $400 \text{ nm} < \lambda < 800 \text{ nm}$; green, $570 \text{ nm} < \lambda < 800 \text{ nm}$) and in the dark (blue) under an Ar atmosphere (WE: GC; CE: Pt wire; RE: SCE; scan rate: 5 mV s^{-1}). (b) UV-Vis absorption spectrum of $[\text{Ru}(\text{bpy})_3](\text{NO}_3)_2 \cdot 5\text{H}_2\text{O}$ (0.05 mM) in water. A drastic change in the shape of the cyclic voltammogram was observed when the solution was irradiated with visible light (400–800 nm).

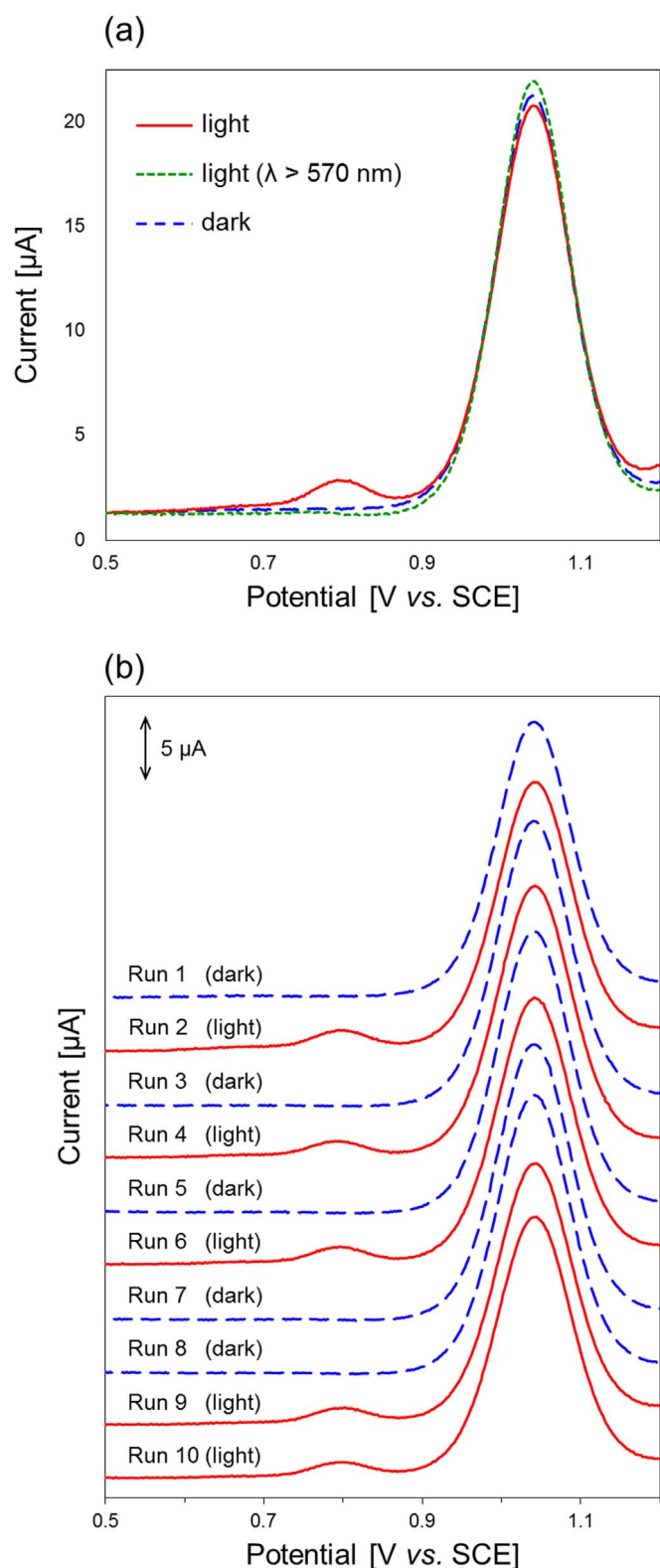


Figure 7 | Square wave voltammograms of $[\text{Ru}(\text{bpy})_3](\text{NO}_3)_2$ with and without photoirradiation. (a) Thin layer square wave voltammograms of $[\text{Ru}(\text{bpy})_3](\text{NO}_3)_2 \cdot 5\text{H}_2\text{O}$ (1 mM) in a 0.1 M Na_2SO_4 aqueous solution under photoirradiation (red, $400 \text{ nm} < \lambda < 800 \text{ nm}$; green, $570 \text{ nm} < \lambda < 800 \text{ nm}$) and in the dark (blue) under an Ar atmosphere (WE: GC; CE: Pt wire; RE: SCE; scan rate: 5 mV s^{-1}). Similar to the results of CV, a new peak appeared when the sample was irradiated with visible light (400–800 nm). (b) Thin layer square wave voltammograms of $[\text{Ru}(\text{bpy})_3](\text{NO}_3)_2 \cdot 5\text{H}_2\text{O}$ (1 mM) in a 0.1 M Na_2SO_4 aqueous solution

under photoirradiation (red, $400 \text{ nm} < \lambda < 800 \text{ nm}$) and in the dark (blue) under an Ar atmosphere (WE: GC; CE: Pt wire; RE: SCE; scan rate: 5 mV s^{-1}). The peak at approximately 0.79 V (vs. SCE) repeatedly appeared only when light was irradiated.

to the photochemical reaction were successfully detected both by CV and SWV under thin layer conditions (Figs. 6 and 7, and Supplementary Figure 3 in the Supplementary Information). A detailed analysis of the phenomena by SWV under the thin layer technique indicated that the peak originated from the oxidation of $[\text{Ru}(\text{bpy})_3^{2+}]^*$ (For details, see Supplementary Figure 4 in the Supplementary Information). In addition, the reduction potential of $[\text{Ru}(\text{bpy})_3^{2+}]^*$ was determined from the SWV measurements and was found to be similar to the indirectly estimated values⁸. The simple method presented here enables the evaluation of the electronic states of photoexcited molecules. The electrochemical properties of several photoexcited metal complexes by thin layer techniques are currently being investigated.

Methods

Materials. Ferrocene (Fc) and Na_2SO_4 were purchased from Wako Pure Chemical Industries, Ltd. Acetonitrile (HPLC grade) was purchased from the Kanto Chemical Co., Inc. Tetra(*n*-butyl)ammonium perchlorate (TBAP) was purchased from Tokyo Chemical Industry Co., Ltd. $[\text{Ru}(\text{bpy})_3](\text{NO}_3)_2 \cdot 5\text{H}_2\text{O}$ was prepared as previously described²⁰. All solvents and reagents were of the highest quality available and were used as received, except for TBAP. TBAP was recrystallised from absolute ethanol and dried in vacuo. H_2O was purified using a Millipore MilliQ purifier.

Measurements. All electrochemical measurements were conducted under argon. A three-electrode configuration was employed in conjunction with a Biologic SP-50 potentiostat interfaced to a computer with Biologic EC-Lab software. The measurements were performed using the system shown in Figure 1, with the exception of the rotating disk electrode (RDE) measurements. The RDE measurements were conducted using a BAS RRDE-3A at ambient temperature, 20°C . The photoirradiation was performed using an ILC Technology CERMAX LX-300 Xe lamp (operated at 150 W) equipped with a CM-1 cold mirror ($400 \text{ nm} < \lambda < 800 \text{ nm}$). In some experiments, an OG570 longpass filter ($\lambda > 570 \text{ nm}$, from SCHOTT) was used to select a specific wavelength region for photoirradiation. In all cases, a platinum wire counter electrode (diameter 0.5 mm, from BAS) and a GC disk working electrode (diameter 3 mm, from BAS) were used. The working electrode was treated between scans by means of polishing with $0.05 \mu\text{m}$ alumina paste (from BAS) and washing with purified H_2O . For the measurements in acetonitrile solutions, an Ag^+/Ag reference electrode was used. The supporting electrolyte was 0.1 M TBAP. The potentials reported within these measurements were referenced to the Fc^+/Fc couple at 0 V. In the aqueous conditions, a saturated calomel electrode (SCE) was used for the reference electrode. The supporting electrolyte was 0.1 M Na_2SO_4 . UV-visible absorption spectra were recorded on a SHIMADZU UV-1800 UV/Visible spectrophotometer.

- Dau, H. & Zaharieva, I. Principles, efficiency, and blueprint character of solar-energy conversion in photosynthetic water oxidation. *Acc. Chem. Res.* **42**, 1861–1870 (2009).
- Brettel, K. & Leibl, W. Electron transfer in photosystem I. *Biochim. Biophys. Acta.* **1507**, 100–114 (2001).
- Nocera, D. G. The artificial leaf. *Acc. Chem. Res.* **45**, 767–776 (2012).
- Magnuson, A. *et al.* Biomimetic and microbial approaches to solar fuel generation. *Acc. Chem. Res.* **42**, 1899–1909 (2009).
- Grätzel, M. Conversion of sunlight to electric power by nanocrystalline dye-sensitized solar cells. *J. Photochem. Photobiol. A.* **164**, 3–14 (2004).
- Jones, W. E. & Fox, M. A. Determination of excited-state redox potentials by phase-modulated voltammetry. *J. Phys. Chem.* **98**, 5095–5099 (1994).
- Oda, N., Tsuji, K. & Ichimura, A. Voltammetric measurements of redox potentials of photo-excited species. *Anal. Sci.* **17**, i375–i378 (2001).
- Juris, A. *et al.* Ru(II) polypyridine complexes: photophysics, photochemistry, electrochemistry, and chemiluminescence. *Coord. Chem. Rev.* **84**, 85–277 (1988).
- Bock, C. R. *et al.* Estimation of excited-state redox potentials by electron-transfer quenching: application of electron-transfer theory to excited-state redox processes. *J. Am. Chem. Soc.* **101**, 4815–4824 (1979).
- Ballardini, R., Varani, G., Indelli, M. T., Scandola, F. & Balzani, V. Free energy correlation of rate constants for electron transfer quenching of excited transition metal complexes. *J. Am. Chem. Soc.* **100**, 7219–7223 (1978).
- Gritzner, G. & Kuta, J. Recommendations on reporting electrode potentials in nonaqueous solvents. *J. Pure Appl. Chem.* **56**, 461–466 (1984).
- Maciejewska, A., Jaworska-Augustyniaka, A., Szelugaa, Z., Wojtczaka, J. & Karolczak, J. Determination of ferrocene triplet lifetime by measuring $T_1 \rightarrow T_1$



- energy transfer to phenylsazone-D-glucose. *Chem. Phys. Lett.* **153**, 227–232 (1988).
13. Tsierkezos, N. G. Cyclic voltammetric studies of ferrocene in nonaqueous solvents in the temperature range from 248.15 to 298.15 K. *J. Solution Chem.* **36**, 289–302 (2007).
 14. Renault, C., Anderson, M. J. & Crooks, R. M. Electrochemistry in hollow-channel paper analytical devices. *J. Am. Chem. Soc.* **136**, 4616–4623 (2014).
 15. Opekar, F. & Beran, P. Rotating disk electrodes. *J. Electroanal. Chem.* **69**, 1–105 (1976).
 16. Bard, A. J. & Faulkner, L. R. *Electrochemical methods: fundamentals and applications*. 2nd ed. (Wiley & Sons, New York, 2001).
 17. Hubbard, A. T. Study of the kinetics of electrochemical reactions by thin-layer voltammetry: I. theory. *J. Electroanal. Chem.* **22**, 165–174 (1969).
 18. Van Houten, J. & Watts, R. J. Temperature dependence of the photophysical and photochemical properties of the tris(2,2'-bipyridyl)ruthenium(II) ion in aqueous solution. *J. Am. Chem. Soc.* **98**, 4853–4858 (1976).
 19. Quenching of triplet excited state of $\text{Ru}(\text{bpy})_3^{2+}$ is known to proceed effectively by O_2 . See the following reference, Kalyanasundaram, K. Photophysics, photochemistry and solar energyconversion with tris(bipyridyl)ruthenium(II) and its analogues. *Coord. Chem. Rev.* **46**, 159–244 (1982).
 20. Palmer, R. A. & Piper, T. S. 2,2'-bipyridine complexes. I. polarized crystal spectra of tris(2,2'-bipyridine)copper(II), -nickel(II), -cobalt(II), -iron(II), and -ruthenium(II). *Inorg. Chem.* **5**, 864–878 (1966).

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Author contributions

A.F., M.K., M.O. and S.M. conceived the project. A.F., M.K., M.O., M.Y. and S.M. designed the experiments. A.F. performed all electrochemical experiments. A.F., M.K. and S.M. analysed the data and co-wrote the manuscript. All authors discussed the results and commented on the manuscript.

Additional information

Supplementary information accompanies this paper at <http://www.nature.com/scientificreports>

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