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Communication

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# Water-Soluble Phosphorescent Ruthenium Complex with a Fluorescent Coumarin Unit for Ratiometric Sensing of Oxygen Levels in Living Cells

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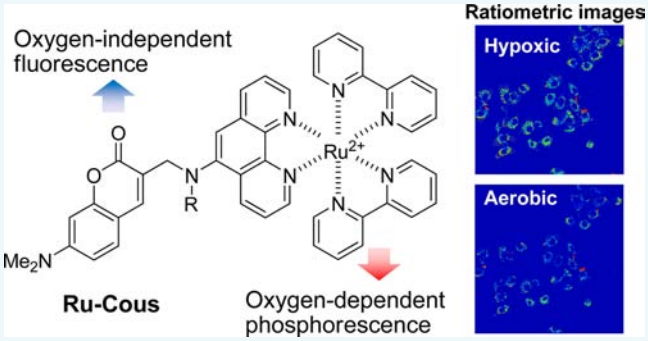
Supporting Information

**ABSTRACT:** Dual emission was applied to a molecular probe for the ratiometric sensing of oxygen concentration in a living system. We prepared ruthenium complexes possessing a coumarin unit (Ru−Cou), in which the $^3$MLCT phosphorescence of the ruthenium complex was efficiently quenched by molecular oxygen, whereas the coumarin unit emitted constant fluorescence independent of the oxygen concentration. The oxygen status could be determined precisely from the ratio of phosphorescence to fluorescence. We achieved the molecular imaging of cellular oxygen levels using Ru−Cou possessing an alkyl chain, which provided appropriate lipophilicity to increase cellular uptake.




Molecular oxygen is imperative for maintaining the vital activity in almost all living cells.[1] Cells produce the energy needed for various cellular functions by consumption of oxygen. Intracellular oxygen status reflects both cellular activities and pathological conditions,[2−7] and thus technologies to track oxygen levels in cells are desired. Especially, molecular probes that respond to oxygen, such as luminescent molecules[8−14] and polymers[15,16] and radioisotope-labeled compounds,[17,18] have attracted much attention because of their biological usability.

We and others have developed various phosphorescent metal complexes as molecular probes that track oxygen fluctuations in cells and tissues.[19−26] Energy transfer between the triplet excited state of the probe and the triplet ground state of oxygen generally causes phosphorescence quenching. Strong phosphorescence can be observed under hypoxic conditions selectively, and the addition of oxygen decreases the phosphorescence. By contrast, removal of oxygen leads to recovery of the phosphorescence emission. Thus, the phosphorescence probes show reversible emission that responds to changes in oxygen levels. Recently, several reports showed that ratiometric imaging[27,28] and lifetime measurement[29−32] using a phosphorescence probe were useful for monitoring oxygen status. Very recently, Tobita and co-workers reported on a ratiometric sensor that consisted of a phosphorescent iridium complex.[33] However, their application to biological imaging was limited because the probes had some drawbacks such as low water solubility and cellular permeability.

These research contexts have prompted us to prepare a water-soluble phosphorescent probe using ruthenium complexes[26] to monitor oxygen levels in living cells. We also employed a ratiometric imaging method because it permits precise analysis without the need for specialized instrumentation. Herein, we prepared phosphorescent ruthenium complexes possessing a fluorescent coumarin molecule at ligand (Ru−Cou) as molecular probes for sensing oxygen levels in living cells. To achieve a high cellular uptake, a hydrophobic alkyl chain was introduced into the ruthenium complex to form Ru−Cou 1 (Figure 1) in accordance with our previous reports.[26] Ru−Cou provided precise information about the oxygen levels on the basis of the ratio of phosphorescence to fluorescence intensity. We discuss the synthesis of Ru−Cou and the characterization of their emission properties in living cells.

Initially, we prepared a ruthenium complex possessing a coumarin unit (Scheme S1). Coupling of 1,10-phenanthroline-5-amine with a coumarin derivative possessing a formyl group[34] gave 2. Alkylation and subsequent complexation with Ru(bpy)$_2$ formed Ru−Cou 1. A control ruthenium complex without an alkyl group (Ru−Cou 2) was synthesized in a similar manner using phenanthroline derivative 2.

We measured the changes in the emission spectrum of Ru−Cou 1 in aqueous solution using excitation at 405 nm. As shown in Figure 2A, under anoxic conditions (0% $O_2$), we observed two emissions at 467 and 611 nm, which were attributed to the fluorescence of the coumarin unit (467 nm) and the $^3$MLCT phosphorescence of the ruthenium complex (611 nm).[35−37] Increasing the oxygen concentration led to a decrease in phosphorescence intensity at 611 nm due to the quenching effect of oxygen, whereas the fluorescence intensity

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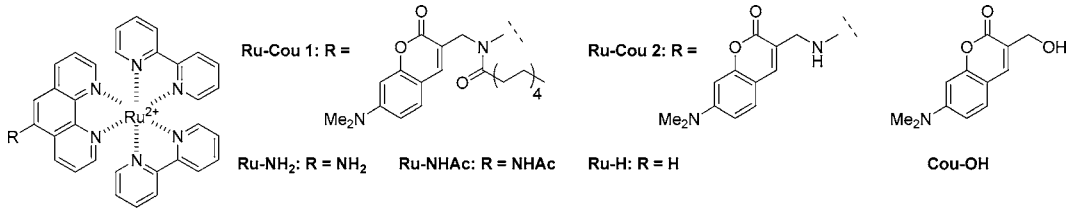


**Figure 1.** Chemical structures of ruthenium complexes and a coumarin derivative used in this study.

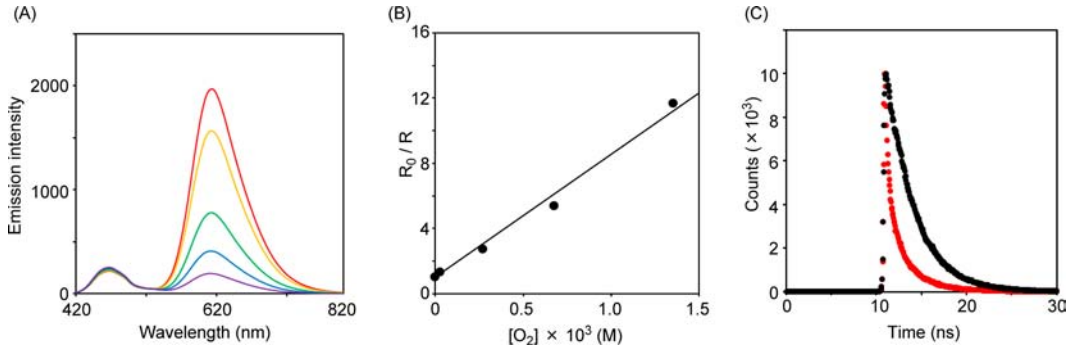


**Figure 2.** (A) Emission spectra of Ru−Cou 1 (20 $\mu$M) in Dulbecco's phosphate buffered saline (DPBS) under different oxygen concentrations (0% $O_2$: red line, 2% $O_2$: orange line, 20% $O_2$: green line, 50% $O_2$: blue line, 100% $O_2$: purple line). The spectra were measured with excitation at 405 nm. (B) Stern−Volmer plot of relative ratio of phosphorescence to fluorescence ($R_0/R$) in the presence of oxygen as a quencher. The relative ratio derived from emission intensity of Ru−Cou 1 (20 $\mu$M) was plotted against oxygen concentration [$O_2$]. (C) Time dependence of the fluorescence intensity (470 nm) of Cou−OH (black, 10 $\mu$M) and Ru−Cou 1 (red, 10 $\mu$M). Both samples were excited at 375 nm.

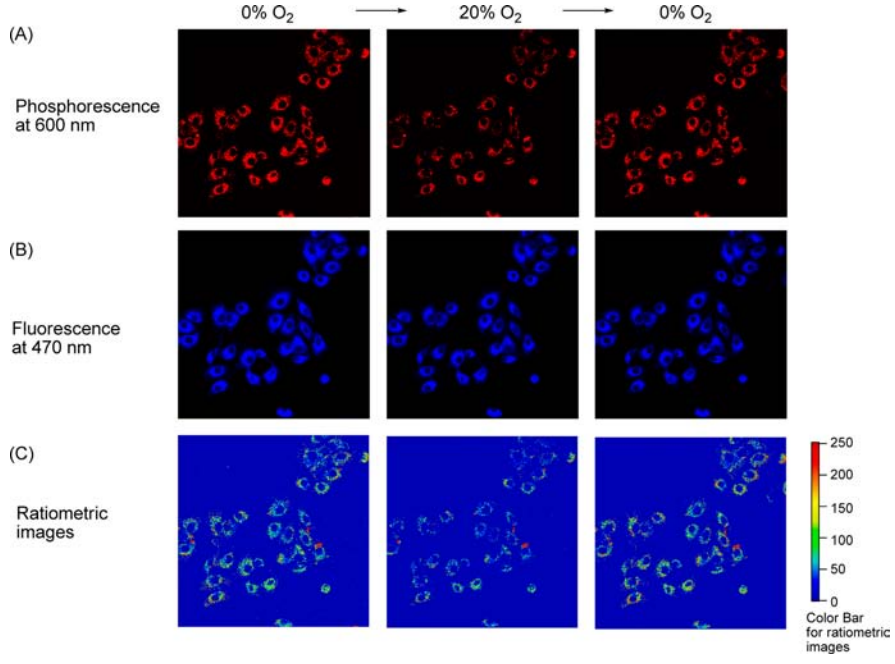


**Figure 3.** Emission images of A549 cells as incubated with Ru−Cou 1. After the cells were incubated with Ru−Cou 1 (100 $\mu$M) for 4 h at 37 °C, the emission was monitored by microscope. The oxygen concentration was set at 0% (left images) and then increased to 20% (middle images). Subsequently, the concentration was decreased to 0% (right images). The oxygen concentration change was conducted by replacement of PBS, which were kept under the designated oxygen concentrations. (A) Excitation at 458 nm and emission at 580−700 nm ($I_{600\ nm}$), (B) excitation at 405 nm and emission at 411−480 nm ($I_{470\ nm}$), and (C) ratiometric images ($I_{600\ nm}/I_{470\ nm}$).

at 467 nm was almost constant even under varying oxygen concentrations. The quenched phosphorescence emission of Ru−Cou 1 under aerobic conditions was recovered to the intrinsic level after purge of oxygen via argon gas blowing (Figure S1). These results demonstrate that the phosphorescence emission of Ru−Cou 1 was regulated reversibly according to changes in the oxygen concentration, while its fluorescence was oxygen-independent. The quantum yield ($\Phi_p$) of phosphorescent emission of Ru−Cou 1 under hypoxic conditions was estimated to be 0.111.[38]

The responses of phosphorescence to oxygen obeyed the Stern−Volmer relationship. As shown in Figure 2B, the oxygen


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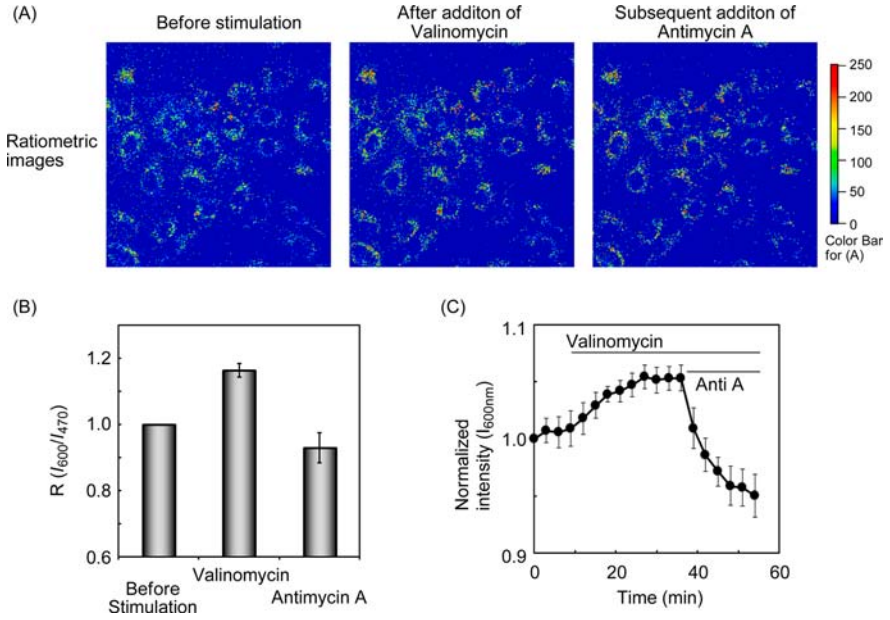


**Figure 4.** Ratiometric imaging of oxygenation and deoxygenation of A549 cells, which was induced by mitochondrial uncoupler (valinomycin, 1 $\mu$M) and inhibitor (antimycin A, 10 $\mu$M) stimulation. The cells were loaded Ru−Cou 1 (50 $\mu$M) and then stimulated. (A) Ratiometric images of the cells before stimulation (left), after stimulation by valinomycin (middle), and subsequent stimulation by antimycin A (right). (B) Ratio between phosphorescence intensity at 600 nm ($I_{600}$) and fluorescence intensity at 470 nm ($I_{470}$) before and after stimulation. (C) Profiles of phosphorescence intensity at 600 nm for the basal condition, stimulation by valinomycin and subsequent stimulation by antimycin A.

concentration was proportional to the ratiometric relationship ($R_0/R$, $R_0$: ratio of phosphorescence intensity at 611 nm/fluorescence intensity at 467 nm under anoxic conditions (0% $O_2$ concentration), $R$: ratio in the presence of the indicated concentration of oxygen), indicating that the change in phosphorescence was based on collisional quenching by oxygen. The Stern−Volmer coefficient ($K_{sv}$) of Ru−Cou 1 was estimated at 7531 $M^{-1}$, as assessed using the equation $R_0/R = 1 + K_{sv}[O_2]$.

The control compound of coumarin (Cou−OH) showed strong fluorescence upon excitation at 405 nm, while the emission of the coumarin unit in Ru−Cou 1 decreased significantly (Figure S2), indicating that the energy transfer or electron transfer process occurred between the coumarin unit and the ruthenium complex in Ru−Cou 1 to quench the fluorescence emission of coumarin unit. Thus, we next measured the emission lifetimes of Cou−OH and Ru−Cou 1 to examine the relaxation mechanism of the excited Ru−Cou 1 (Figure 2C). The fluorescence decay profile of Ru−Cou 1 monitored at 470 nm could be fitted by two exponential functions with lifetimes of 0.98 (26%) and 2.84 ns (73%). On the other hand, the fluorescence lifetime of Cou−OH was lengthened (3.05 ns). This result supports the presence of the energy transfer process in Ru−Cou 1. In order to evaluate the occurrence of the electron transfer process, we also measured the fluorescence spectra of Cou−OH in the presence of several ruthenium complexes such as Ru−$NH_2$, Ru−NHAc, and Ru−H, which have different redox potentials due to their substituents on the phenanthroline ligand (Figure 1). While the absorption spectra of these complexes were almost identical, the fluorescence of Cou−OH in the presence of Ru−$NH_2$ was brighter than that in the presence of Ru−NHAc and Ru−H (Figure S3). It is most likely that the fluorescence emission of Cou−OH was quenched by electron-accepting ruthenium complexes, indicating occurrence of the electron transfer process. Thus, both energy transfer and the electron transfer process seem to contribute to quenching of fluorescence of the coumarin unit in Ru−Cou 1.

In light of the optical properties of Ru−Cou 1, we tried to apply Ru−Cou 1 to the cellular imaging of a human lung adenocarcinoma cell line, A549. A549 cells grown in DMEM culture medium were incubated with Ru−Cou 1 for 4 h at 37 °C under hypoxic or aerobic conditions. The cells were then washed and subjected to microscopy (Figure 3). Upon excitation at 405 nm, blue fluorescence (detection at 411−480 nm) was observed from both hypoxic and aerobic cells. The intensity of the fluorescence from cells was similar in both conditions. Thus, Ru−Cou 1 emitted fluorescence in an oxygen-independent manner, which was consistent with the results of the in vitro experiments. It is striking that bright phosphorescence (detection at 580−700 nm) was observed from the cytoplasm of cells incubated under hypoxic conditions (0% $O_2$), whereas considerably weaker phosphorescence emission was observed from similarly cultured cells under aerobic conditions (20% $O_2$). We also confirmed that the phosphorescence from cells recovered as the concentration of oxygen decreased and that the emission changed immediately as a function of the oxygen conditions. These results strongly indicate that the phosphorescence emission reflected the real-time change in the intracellular oxygen concentration. In a control experiment, cells were cultured in the presence of Ru−Cou 2 under hypoxic or aerobic conditions (Figure S4). However, we observed negligible emission of Ru−Cou 2 from the cells. The evidence that the partition coefficients (log Po/w) between 1-octanol and water of Ru−Cou 1 and Ru−Cou 2 were estimated to be 0.42 and −0.19 indicates that lipophilicity of probes is critical for their cellular uptake and emission in the cells, which is consistent with the previous reports about ruthenium complexes.[26,39]

In order to assess whether Ru−Cou 1 could respond dynamically to changes in cellular respiration, A549 cells were loaded with Ru−Cou 1 and stimulated by eminent mitochon-


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drial uncouplers and inhibitors.[15] After measurement of the basal emission intensity from cells for 10 min, the cells was stimulated by these reagents. Upon cell stimulation by the addition of valinomycin, evident enhancement of emission at 600 nm was observed in the cells (Figure 4C). Stimulation by valinomycin increased oxygen consumption because it uncouples the respiratory chain and oxidative phosphorylation in the mitochondria to increase the rate of respiration. This pharmacological effect induces a deeper deoxygenation of the cells. The subsequent administration of antimycin A, which inhibits complex III of the electron transport chain and blocks cellular respiration, resulted in a decrease in the emission intensity because of an increase in oxygen concentration up to the air-saturated level. Then, Ru−Cou 1 allowed us to monitor oxygen fluctuation by ratiometric imaging. As shown in Figure 4A and 4B, the relative oxygenation and respiratory responses of A549 cells to stimulation were monitored. We confirmed that the signal (R: $I_{600}/I_{470}$) obtained from ratiometric imaging increased when the cells were stimulated by valinomycin and decreased when the cells were later treated with antimycin A. Thus, our study shows that ratiometric imaging using Ru−Cou 1 can clearly monitor oxygenation and deoxygenation in living cells.

In conclusion, we synthesized ruthenium complexes possessing a coumarin unit (Ru−Cou) and characterized their phosphorescence and fluorescence emission properties. The phosphorescence of Ru−Cous was suppressed significantly by the quenching function of molecular oxygen, while their fluorescence was unperturbed by changes in oxygen concentration. The introduction of an alkyl chain into Ru−Cou to form Ru−Cou 1 improved its cellular uptake and allowed us to visualize oxygen levels in living A549 cells. We observed oxygen-dependent phosphorescence of the ruthenium complex with concomitant oxygen-independent fluorescence emission of the coumarin unit in the cells. We successfully applied Ru−Cou 1 to the visualization of oxygenation and deoxygenation, which were induced by stimulation with valinomycin and antimycin A, respectively, in the cells. Thus, we could monitor the oxygen fluctuation by ratiometric imaging. Further improvement of the probes for optical in vivo imaging of oxygen levels is in progress.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

Experimental details, emission spectra, and emission properties of the ruthenium complexes and coumarin derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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## ■ REFERENCES

(1) Aragonés, J., Fraisl, P., Baes, M., and Carmeliet, P. (2009) Oxygen sensors at the crossroad of metabolism. *Cell Metab. 9*, 11−22.
(2) Semenza, G. L. (2003) Angiogenesis in ischemic and neoplastic disorders. *Annu. Rev. Med. 54*, 17−28.
(3) Semenza, G. L. (2001) Hypoxia-inducible factor 1: oxygen homeostasis and disease pathophysiology. *Trends Mol. Med. 7*, 345−350.
(4) Semenza, G. L. (2001) Hypoxia-inducible factor 1: control of oxygen homeostasis in health and disease. *Pediatr. Res. 49*, 614−617.
(5) Mardoch, C., Muthana, M., and Lewis, C. E. (2005) Hypoxia regulates macrophage functions in inflammation. *J. Immunol. 175*, 6257−6263.
(6) Harris, A. L. (2002) Hypoxia — a key regulatory factor in tumour growth. *Nat. Rev. Cancer 2*, 38−47.
(7) Vaupel, P., Kallinowski, F., and Okunieff, P. (1989) Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: a review. *Cancer Res. 49*, 6449−6465.
(8) Liu, Y., Xu, Y. F., Qian, X. H., Liu, J. W., Shen, L. Y., Li, J. H., and Zhang, Y. X. (2006) Novel fluorescent markers for hypoxic cells of naphthalimides with two heterocyclic side chains for bioreductive binding. *Bioorg. Med. Chem. 14*, 2935−2941.
(9) Xu, K., Wang, F., Pan, X., Liu, R., Ma, J., Kong, F., and Tang, B. (2013) High selectivity imaging of nitroreductase using a near-infrared fluorescence probe in hypoxic tumor. *Chem. Commun. 49*, 2554−2556.
(10) Nakata, E., Yukimachi, Y., Kariyazono, H., Im, S., Abe, C., Uto, Y., Maezawa, H., Hashimoto, T., Okamoto, Y., and Hori, H. (2009) Design of a bioreductively-activated fluorescent pH probe for tumor hypoxia imaging. *Bioorg. Med. Chem. 17*, 6952−6958.
(11) Okuda, K., Okabe, Y., Kadonosono, T., Ueno, T., Youssif, B. G. M., Kizaka-Kondoh, S., and Nagasawa, H. (2012) 2-Nitroimidazole-tricarbocyanine conjugate as a near-infrared fluorescent probe for *in vivo* imaging of tumor hypoxia. *Bioconjugate Chem. 23*, 324−329.
(12) Kiyose, K., Hanaoka, K., Oushiki, D., Nakamura, T., Kajimura, M., Suematsu, M., Nishimatsu, H., Yamane, T., Terai, T., Nagano, T., et al. (2010) Hypoxia-sensitive fluorescent probes for *in vivo* real-time fluorescence imaging of acute ischemia. *J. Am. Chem. Soc. 132*, 15846−15848.
(13) Komatsu, H., Harada, H., Tanabe, K., Hiraoka, M., and Nishimoto, S. (2010) Indolequinone-rhodol conjugate as a fluorescent probe for hypoxic cells: enzymatic activation and fluorescence properties. *MedChemComm 1*, 50−53.
(14) Tanabe, K., Hirata, N., Harada, H., Hiraoka, M., and Nishimoto, S. (2008) Emission under hypoxia: one-electron reduction and fluorescence characteristics of an indolequinone-coumarin conjugate. *ChemBioChem 9*, 426−432.
(15) Fercher, A., Borisov, S. M., Zhdanov, A. V., Klimant, I., and Papkovsky, D. B. (2011) Intracellular $O_2$ sensing probe based on cell-penetrating phosphorescent nanoparticles. *ACS Nano 5*, 5499−5508 2011.
(16) Ceroni, P., Lebedev, A. Y., Marchi, E., Yuan, M., Esipova, T. V., Bergamini, G., Wilson, D. F., Busch, T. M., and Vinogradov, S. A. (2011) Evaluation of phototoxicity of dendritic porphyrin-based phosphorescent oxygen probes: an *in vitro* study. *Photochem. Photobiol. Sci. 10*, 1056−1065.
(17) Fujibayashi, Y., Taniuchi, H., Yonekura, Y., Ohtani, H., Konishi, J., and Yokoyama, A. (1997) Copper-62-ATSM: a new hypoxia imaging agent with high membrane permeability and low redox potential. *J. Nucl. Med. 38*, 1155−1160.
(18) Rasey, J., Koh, W.-J., Grierson, J. R., Grunbaum, Z., and Krohn, K. A. (1989) Radiolabeled fluoromisonidazole as an imaging agent for tumor hypoxia. *Int. J. Radiat. Oncol., Biol., Phys. 17*, 985−991.
(19) Lo, K. K.-W., and Li, S. P.-Y. (2014) Utilization of the photophysical and photochemical properties of phosphorescent transition metal complexes in the development of photofunctional


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*Bioconjugate Chem.* 2015, 26, 645−649

cellular sensors, imaging reagents, and cytotoxic agents. *RSC Adv. 4*, 10560−10585.
(20) Dmitriev, R. I., Zhdanov, A. V., Jasionek, G., and Papkovsky, D. B. (2012) Assessment of cellular oxygen gradients with a panel of phosphorescent oxygen-sensitive probes. *Anal. Chem. 84*, 2930−2938.
(21) Choi, N. W., Verbridge, S. S., Williams, R. M., Chen, J., Kim, J.-Y., Schmehl, R., Farnum, C. E., Zipfel, W. R., Fischbach, C., and Stroock, A. D. (2012) Phosphorescent nanoparticles for quantitative measurements of oxygen profiles *in vitro* and *in vivo*. *Biomaterials 33*, 2710−2722.
(22) Lincoln, R., Kohler, L., Monro, S., Yin, H., Stephenson, M., Zong, R., Chouai, A., Dorsey, C., Hennigar, R., McFarland, S. A., et al. (2013) Exploitation of long-Lived $^3$IL excited states for metal−organic photodynamic therapy: verification in a metastatic melanoma model. *J. Am. Chem. Soc. 135*, 17161−17175.
(23) Ziemer, L. S., Lee, W. M. F., Vinogradov, S. A., Sehgal, C., and Wilson, D. F. (2005) Oxygen distribution in murine tumors: characterization using oxygen-dependent quenching of phosphorescence. *J. Appl. Physiol. 98*, 1503−1510.
(24) Zhang, S., Hosaka, M., Yoshihara, T., Negishi, K., Iida, Y., Tobita, S., and Takeuchi, T. (2010) Phosphorescent light-emitting iridium complexes serve as a hypoxia-sensing probe for tumor imaging in living animals. *Cancer Res. 70*, 4490−4498.
(25) Son, A., Kawasaki, A., Hara, D., Ito, T., and Tanabe, K. (2015) Phosphorescent ruthenium complexes with nitroimidazole unit that image oxygen fluctuation in tumor tissue. *Chem.—Eur. J. 21*, 2527−2536.
(26) Komatsu, H., Yoshihara, K., Yamada, H., Kimura, Y., Son, A., Nishimoto, S., and Tanabe, K. (2013) Ruthenium complexes with hydrophobic ligands that are key factors for optical imaging of physiological hypoxia. *Chem.—Eur. J. 19*, 1971−1977.
(27) Kozhevnikov, D. N., Kozhevnikov, V. N., Shafikov, M. Z., Prokhorov, A. M., Bruce, D. N., and Williams, J. A. G. (2011) Phosphorescence vs fluorescence in cyclometalated platinum(II) and iridium(III) complexes of (oligo)thienylpyridines. *Inorg. Chem. 50*, 3804−3815.
(28) Liu, Y., Guo, H., and Zhao, J. (2011) Ratiometric luminescent molecular oxygen sensors based on uni-luminophores of C-N Pt(II)(acac) complexes that show intense visible-light absorption and balanced fluorescence/phosphorescence dual emission. *Chem. Commun. 47*, 11471−11473.
(29) Guo, H., Ji, S., Wu, W., Wu, W., Shao, J., and Zhao, J. (2010) Long-lived emissive intra-ligand triplet excited states ($^3$IL): next generation luminescent oxygen sensing scheme and a case study with red phosphorescent diimine Pt(II) bis(acetylide) complexes containing ethynylated naphthalimide or pyrene subunits. *Analyst 135*, 2832−2840.
(30) Wu, W., Sun, J., Ji, S., Wu, W., Zhao, J., and Guo, H. (2011) Tuning the emissive triplet excited states of platinum(II) Schiff base complexes with pyrene, and application for luminescent oxygen sensing and triplet−triplet-annihilation based upconversions. *Dalton Trans. 40*, 11550−11561.
(31) Smith, C. S., and Mann, K. R. (2012) Exceptionally long-lived luminescence from $[Cu(I)(isocyanide)_2(phen)]^+$ complexes in nanoporous crystals enables remarkable oxygen gas sensing. *J. Am. Chem. Soc. 134*, 8786−8789.
(32) Zhao, J., Ji, S., Wu, W., Wu, W., Guo, H., Sun, J., Sun, H., Liu, Y., Li, Q., and Huang, L. (2012) Transition metal complexes with strong absorption of visible light and long-lived triplet excited states: from molecular design to applications. *RSC Adv. 2*, 1712−1728.
(33) Yoshihara, T., Yamaguchi, Y., Hosaka, M., Takeuchi, T., and Tobita, S. (2012) Ratiometric molecular sensor for monitoring oxygen levels in living cells. *Angew. Chem., Int. Ed. 51*, 4148−4151.
(34) Flašík, R., Stankovičová, H., Gáplovský, A., and Donovalový, J. (2009) Synthesis and study of novel coumarin derivatives potentially utilizable as memory media. *Molecules 14*, 4838−4848.
(35) Ji, S., Wu, W., Wu, W., Song, P., Han, K., Wang, Z., Liu, S., Guo, H., and Zhao, J. (2010) Tuning the luminescence lifetimes of ruthenium(II) polypyridine complexes and its application in luminescent oxygen sensing. *J. Mater. Chem. 20*, 1953−1963.
(36) Ji, J., Rosenzweig, N., Jones, I., and Rosenzweig, Z. (2002) Novel fluorescent oxygen indicator for intracellular oxygen measurements. *J. Biomed. Opt. 7*, 404−409.
(37) Fernández-Moreira, V., Thorp-Greenwood, F. L., and Coogan, M. P. (2010) Application of $d^6$ transition metal complexes in fluorescence cell imaging. *Chem. Commun. 46*, 186−202.
(38) Ishida, H., Tobita, S., Hasegawa, Y., Katoh, R., and Nozaki, K. (2010) Recent advances in instrumentation for absolute emission quantum yield measurements. *Coord. Chem. Rev. 254*, 2449−2458.
(39) Horobin, R. W., Stockert, J. C., and Rashid-Doubell, F. (2006) Fluorescent cationic probes for nuclei of living cells: why are they selective? A quantitative structure−activity relations analysis. *Histochem. Cell Biol. 126*, 165−175.


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