Supplementary Information

# *In vivo* fluorescence bioimaging of ascorbic acid in mice: Development of an efficient probe consisting of phthalocyanine, TEMPO, and albumin

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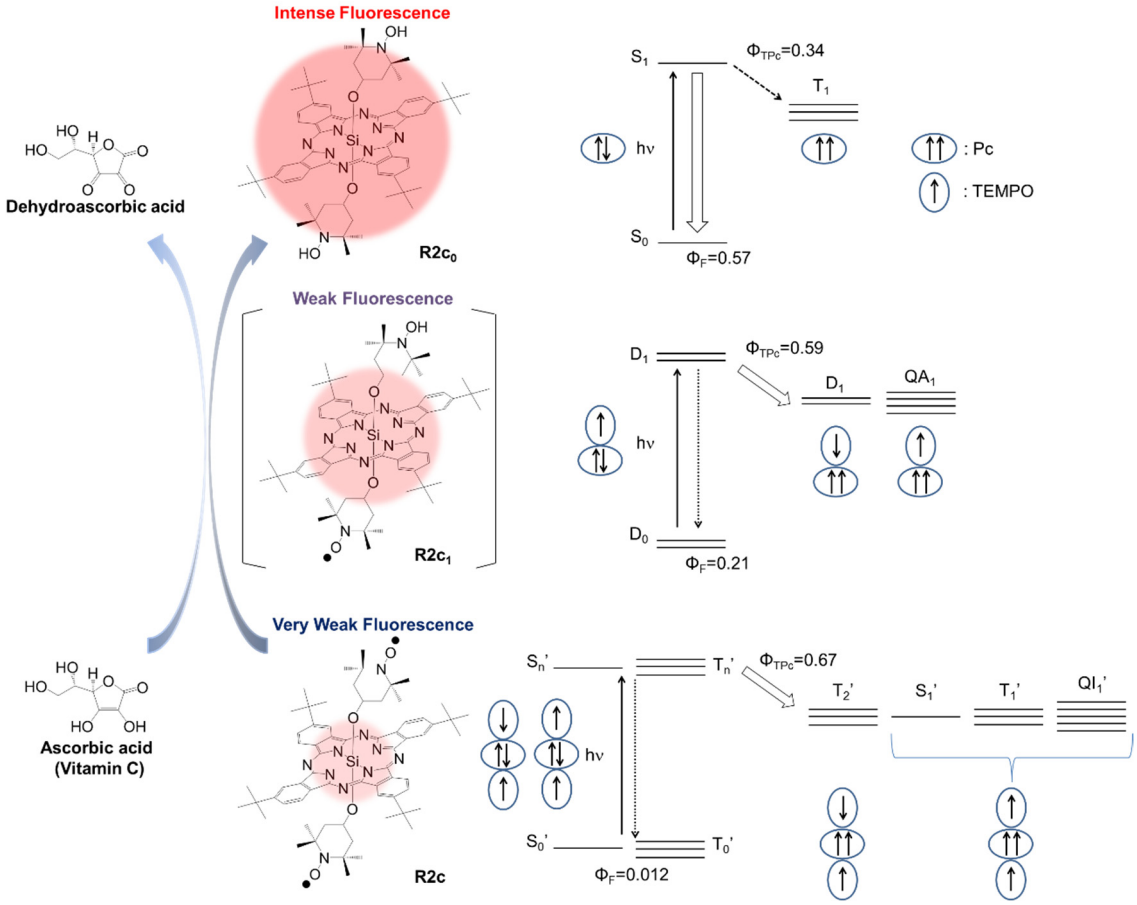
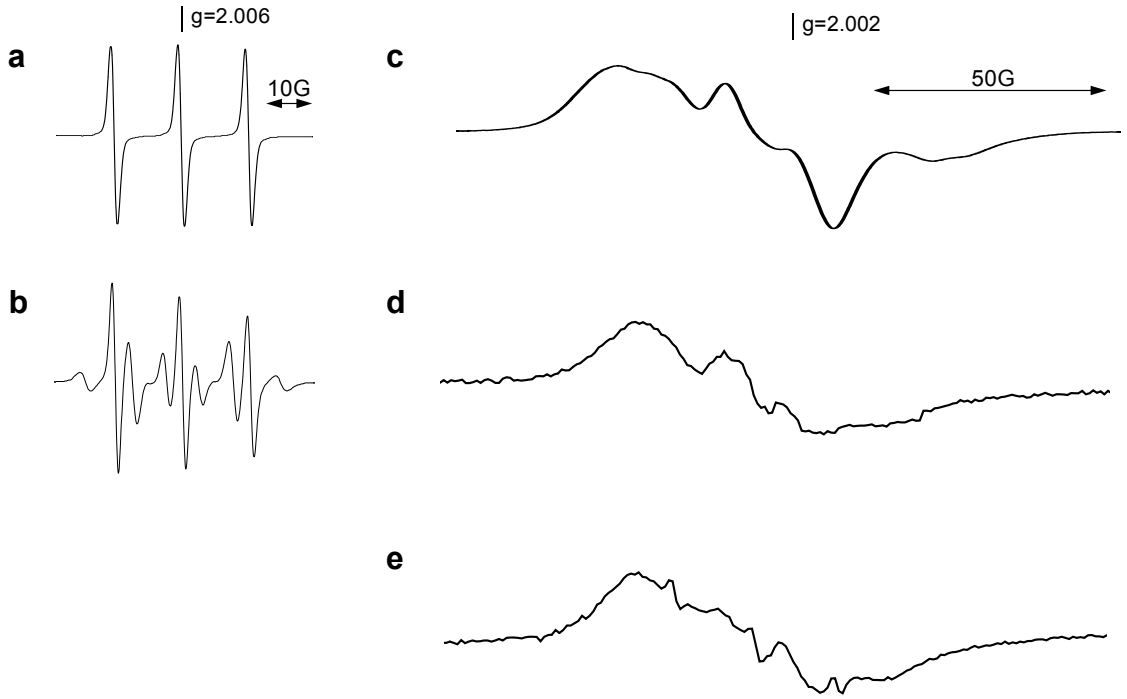



**Supplementary Figure S1. Excited-state dynamics of silicon phthalocyanine (SiPc) derivatives, quenching of SiPc fluorescence due to TEMPO radicals, and reaction between R2c and ascorbic acid.**

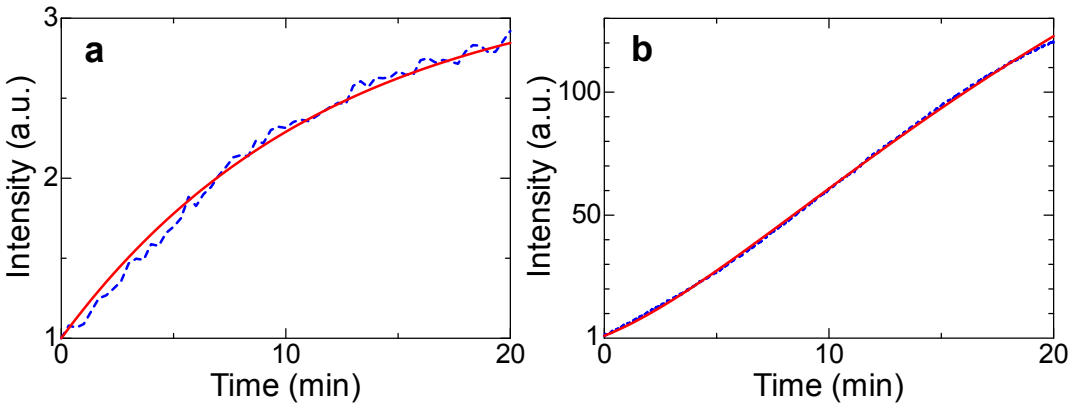
Illustrated are the excited-state properties of (dihydroxy)SiPc (R0), which are fundamentally similar to those of $R2c_0$, the two-electron reduced form of R2c. Ascorbic acid exists as an ascorbate anion when pH is >4.2. The lowest excited singlet ($S_1$) and triplet ($T_1$) states, located at ~14500 $cm^{-1}$ and ~9000 $cm^{-1}$, are nearly derived from the $^1(a_{1u}e_g)$ and $^3(a_{1u}e_g)$ configurations, respectively, with the $a_{1u}$ ($\pi$) and $e_g$ ($\pi^*$) orbitals denoting the HOMO and LUMO of SiPc. The fluorescence ($\Phi_F$) and triplet ($\Phi_{TPc}$) quantum yields are 0.57 and 0.34, respectively[1-3]. In $R2c_1$, the one-electron reduced form of R2c, the doublet ground ($D_0$) state and the excited doublet ($D_n$) state consist of TEMPO in the $D_0$ state ($^2$TEMPO) and SiPc in the singlet ($S_0$ or $S_1$) state ($^1$SiPc or $^1$SiPc$^*$). On the other hand, the lowest excited doublet ($D_1$) and quartet ($QA_1$) states are generated by the interaction between $^2$TEMPO and SiPc in the $T_1$ state ($^3$SiPc$^*$). Studies of SiPc linked to one TEMPO radical (R1c) indicated that generation of the $D_n \rightarrow D_1$ transition enhanced the $^1$SiPc$^* \rightarrow ^3$SiPc$^*$ intersystem crossing ($\Phi_{TPc}$ = 0.59), and decreased the $\Phi_F$ value to 0.21[1-3]. In R2c, the singlet ($S_0'$) and triplet ($T_0'$) ground states are generated by the

interaction between two $^{2}$TEMPO radicals. The excited singlet ($S_n'$) and triplet ($T_n'$) states are derived from $^{1}$SiPc$^{*}$ and two $^{2}$TEMPO radicals. The interactions between $^{3}$SiPc$^{*}$ and the two $^{2}$TEMPO radicals result in the lowest excited singlet ($S_1'$), triplet ($T_1'$), and quintet ($QI_1'$) states, as well as the second lowest excited triplet ($T_2'$) state. Here, the two $^{2}$TEMPO radicals exhibit triplet and singlet characters in the $T_1'$ and $T_2'$ states, respectively. As a result of interactions with the two $^{2}$TEMPO radicals, the $\Phi_F$ value significantly decreased to 0.012, because $^{3}$SiPc$^{*}$ ($\Phi_{TPc} = 0.67$) was preferably formed[1-3].



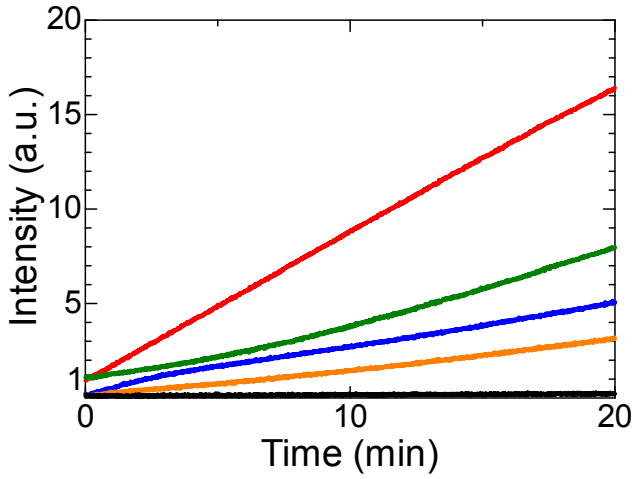

**Supplementary Figure S2. Electron spin resonance spectra.**

Steady-state electron spin resonance spectra of 4-hydroxy-TEMPO in toluene (a), R2c in toluene (b), R2c in frozen toluene (c), R2c@$(BSA)_2$ in aqueous solution (d), and R2c@TX-100 in aqueous solution (e).



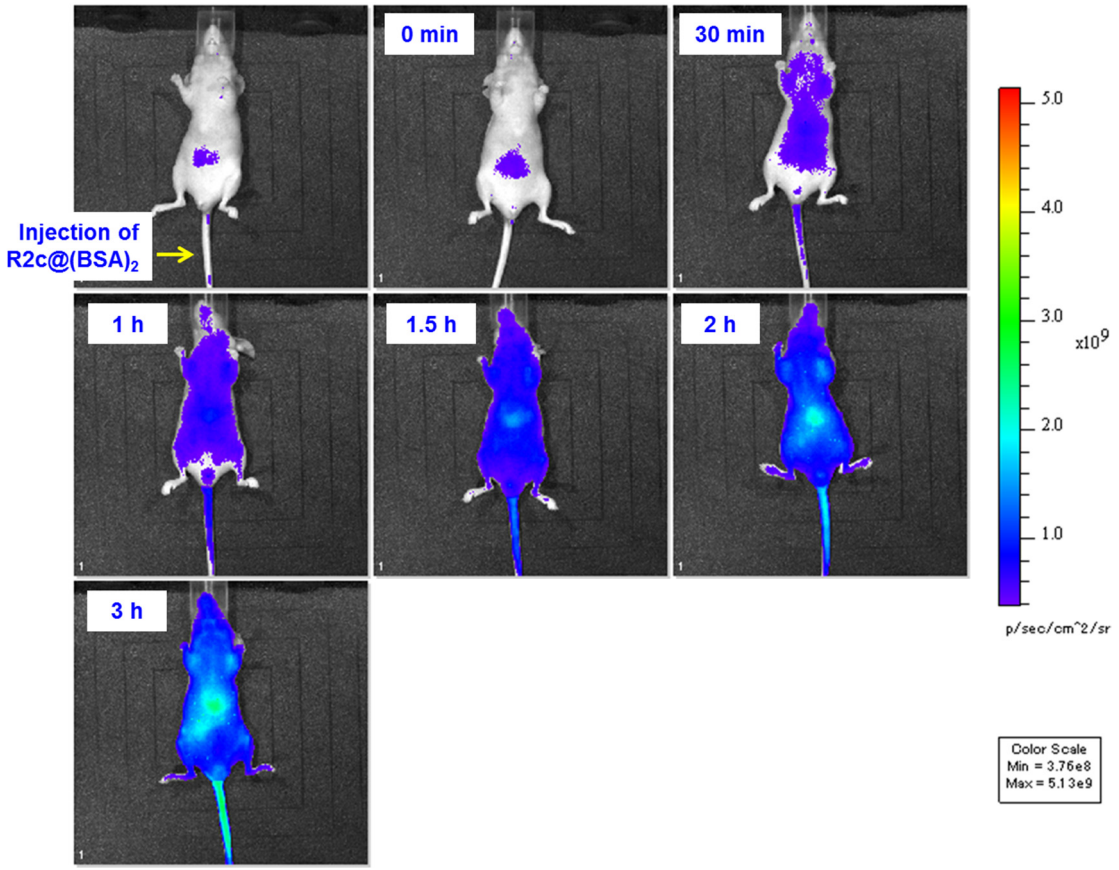

**Supplementary Figure S3. Analyses of time courses of fluorescence from liposomal R2c and R2c@(BSA)$_2$.**

Time courses (blue dashed lines) of fluorescence from liposomal R2c (a) and R2c@(BSA)$_2$ (b) in aqueous solutions at pH 7.4, which can be analysed, respectively, by a single exponential function (a, red line), and as a function based on consecutive reaction (b, red line).



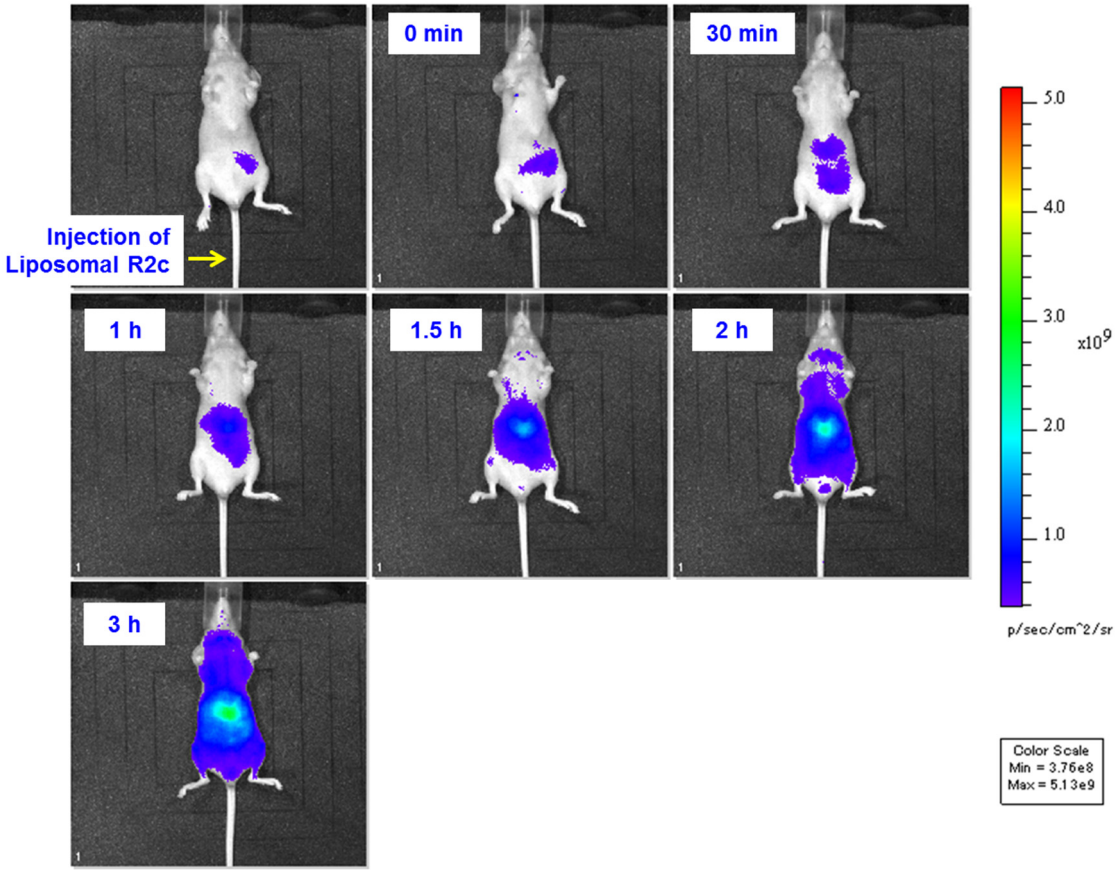

**Supplementary Figure S4. pH dependence of fluorescence.**

Time courses of R2c@$(BSA)_2$ fluorescence at pH 2 (green), pH 3 (red), pH 4 (blue), pH 5 (orange), and pH 6 (black): the differences between pH 6, 7, and 8 were negligible. Accordingly, pH 3 is the best condition at which to measure R2c@$(BSA)_2$ fluorescence due to ascorbic acid.



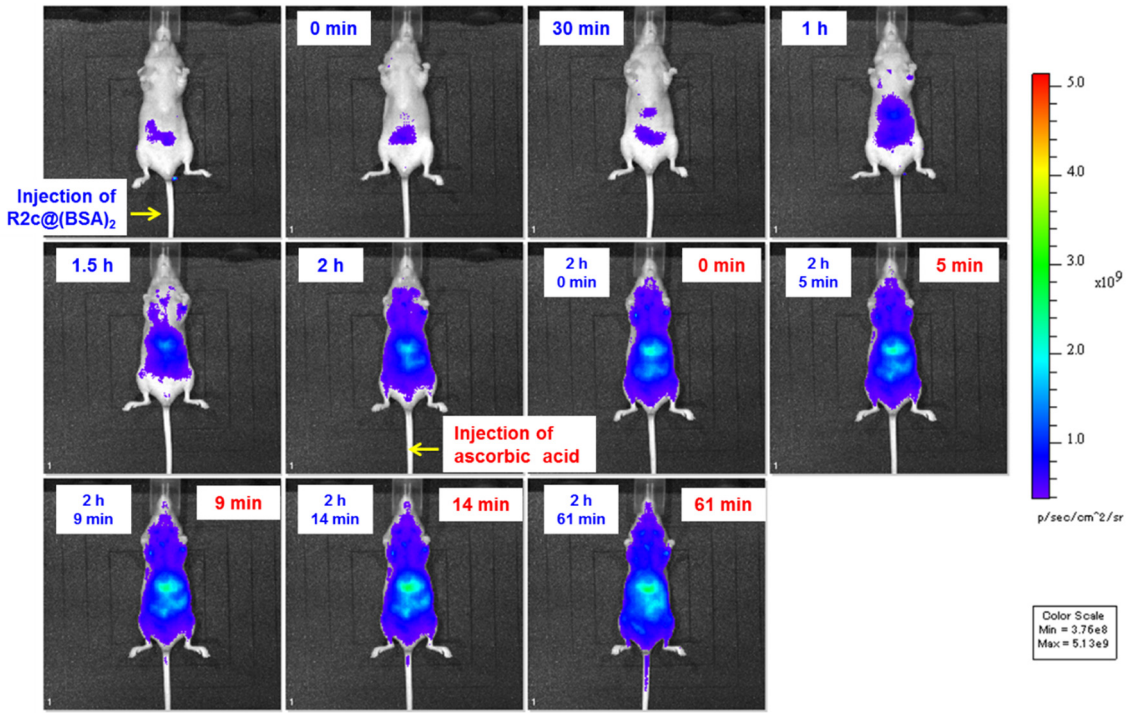

**Supplementary Figure S5. Fluorescence imaging of a mouse injected with R2c@(BSA)$_2$.**

Representative *in vivo* fluorescence imaging of a mouse injected with R2c@(BSA)$_2$, but not with ascorbic acid. Eventually, fluorescence due to R2c@(BSA)$_2$ was observed throughout the body.




**Supplementary Figure S6. Fluorescence imaging of a mouse injected with liposomal R2c.**

Representative *in vivo* imaging of a mouse injected with liposomal R2c, but not with ascorbic acid. At the end of experiment, fluorescence was observed throughout the body, but was particularly intense in the liver.




**Supplementary Figure S7. Fluorescence imaging of a mouse injected with liposomal R2c and ascorbic acid.**

Representative *in vivo* liposomal R2c-based fluorescence imaging of intravenously injected ascorbic acid in a mouse. Time elapsed after R2c@$(BSA)_2$ injection is indicated at the upper left in each image, while that after ascorbic acid injection is shown at the upper right in each image.

**Supplementary References**


1. Ishii, K., Kubo, K., Sakurada, T., Komori, K. & Sakai, Y. Pthalocyanine-based fluorescence probes for detecting ascorbic acid: phthalocyaninatosilicon covalently linked to TEMPO radicals. *Chem. Commun.* **47**, 4932-4934 (2011).
2. Ishii, K., Hirose, Y., Fujitsuka, H., Ito, O. & Kobayashi, N. Time-resolved EPR, fluorescence, and transient absorption studies on phthalocyaninatosilicon covalently linked to one or two TEMPO radicals. *J. Am. Chem. Soc.* **123**, 702-708 (2001).
3. Ishii, K., Takeuchi, S., Shimizu, S. & Kobayashi, N. A concept for controlling singlet oxygen ($^1\Delta_g$) yields using nitroxide radicals: phtalocyanninatosilicon covalenly linked to nitroxide radicals. *J. Am. Chem. Soc.* **126**, 2082-2088 (2004).