Supporting Information

Photooxidation Activity Control of Dimethylaminophenyl-tris-(N-methyl-4-pyridinio)porphyrin by pH

Kazutaka Hirakawa*,§, Syunsuke Takai§, Hiroaki Horiuchi†, and Shigetoshi Okazaki‡

§Applied Chemistry and Biochemical Engineering Course, Department of Engineering, Graduate School of Integrated Science and Technology, Shizuoka University, Johoku 3-5-1, Naka-ku, Hamamatsu 432-8561, Japan
†Department of Optoelectronics and Nanostructure Science, Graduate School of Science and Technology, Shizuoka University, Johoku 3-5-1, Naka-ku, Hamamatsu 432-8561, Japan
‖Division of Molecular Science, Graduate School of Science and Technology, Gunma University, Tenjin-cho 1-5-1, Kiryu 376-8515, Japan
‡Preeminent Medical Photonics Education & Research Center, Hamamatsu University School of Medicine, Handayama 1-20-1, Higashi-ku, Hamamatsu 431-3192, Japan

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1. Synthesis of DMATPyP and DMATMPyP

Meso-(N’,N’-dimethyl-4-aminophenyl)-tris(N-methyl-p-pyridinio)porphyrin (DMATPyP) was obtained by the methylation of meso-(N,N-dimethyl-4-aminophenyl)-tris(p-pyridyl)porphyrin (DMATPyP). DMATPyP was synthesized according to the literatures [S1]. 0.75 g (5 mmol) of p-dimethylaminobenzaldehyde (FUJIFILM Wako Pure Chemical Co., Osaka, Japan) and 1.44 mL (15 mmol) of 4-pyridinecarboxaldehyde (KANTO Chemical Co. Inc., Tokyo, Japan) were dissolved in 50 mL of propionic acid (FUJIFILM Wako Pure Chemical Co.), and 1.34 mL (20 mmol) of pyrrole (FUJIFILM Wako Pure Chemical Co.) was added to reflux for 1.5 h. After this reaction, sodium acetate trihydrate (13 g) was dissolved in water (140 mL) and added to the reaction mixture and stirred to bring the pH to approximately 3.0. To
allow the porphyrin to settle, the mixture was left overnight. The resulting purple precipitate was collected by Büchner filtration and washed with N,N-dimethylformamide (DMF) and methanol. The crude product was purified by column chromatography on silica gel with chloroform-methanol (95/5, vol/vol) as an eluent five times, the result being a pure product. $^1$H-

**Figure S1** $^1$H-NMR spectra of DMATPyP and DMATMPyP (A), the list of their chemical shifts (B), and MS spectrum of DMATMPyP (C).
NMR (CDCl$_3$, TMS, 300 MHz) $\delta$= 9.06–9.03 (m, 8H, $\beta$H), 8.84–8.79 (m, 6H, 10,15,20-$m$-pyridine-H), 8.18–8.15 (m, 6H, 10,15,20-$o$-pyridine-H), 8.08 (d, 2H, $J_{H-H}$= 9.0 Hz, $m$-aniline-H), 7.12 (d, 2H, $J_{H-H}$= 9.0 Hz, $o$-aniline-H), 3.26 (s, 6H, aniline-CH$_3$), -2.80 (s, 2H, porphyrin center NH).

To obtain DMATMPyP, the methylation of DMATPyP was carried out according to the literatures [S2]. The selective methylation of the pyridine moieties was controlled by the reaction time. 10 mg of DMATPyP (15 $\mu$mol) was methylated in 4 mL of DMF with 1 mL (16 mmol) of methyl iodide (KANTO Chemical Co. Inc., Tokyo, Japan) for 4 h at room temperature. The methyl iodide was removed under vacuum. The residue in DMF was recrystallized with diethyl ether (DMF: diethyl ether = 1:4, vol/vol). The brown powder was washed with diethyl ether and dried, the result being a brown product. $^1$H-NMR (DMSO-$d_6$, TMS, 300 MHz) $\delta$ = 9.43 (broad s, 8H, $\beta$H), 9.16–9.08 (m, 4H, 10,20-$m$-pyridine-H), 9.02–8.97 (m, 4H, 10,20-$o$-pyridine-H), 8.46 (d, 2H, 6 Hz, 15-$m$-pyridine-H), 8.29 (d, 2H, 6 Hz, 15-$o$-pyridine-H), 8.04 (d, 2H, 6 Hz, $m$-aniline-H), 7.20 (d, 2H, 6 Hz, $o$-aniline-H), 4.70 (s, 9H, pyridine-CH$_3$), 3.92 (s, 6H, aniline-CH$_3$), -2.90 (s, 2H, porphyrin center NH). Exact mass (ESI-HR TOF-MS) calcd. for C$_{46}$H$_{41}$N$_8$ [M$^+$]: 705.3438. Found: 705.3505. These spectra are shown in Figure S1.

2. DFT calculation

The optimized structure and energy of DMATMPyP and those of the protonated form were calculated by the density functional theory (DFT) at the $\omega$B97X-D/6-31G* level utilizing the Spartan 18’ (Wavefunction Inc., CA, USA) (Figure S2). The highest occupied molecular orbital (HOMO) of DMATMPyP is located on the DMA moiety (-11.5 eV, in vacuum). The $S_1$ excitation ($S_0$→$S_1$ transition) of porphyrin ring was assigned to the electron transition from the HOMO-1 (-12.9 eV) to the LUMO (-7.3 eV) of DMATMPyP. In the case of the protonated form of DMATMPyP (H$^+$-DMATMPyP), the HOMO is located on the porphyrin ring (-14.9 eV). The highest occupied orbital of the DMA moiety became the HOMO-4 of DMATMPyP (-17.4 eV).

3. Calculation of the driving force of electron transfer

The energy level of the CT state ($E_{CT}$), formed by the intramolecular electron transfer, was calculated using the following equation based on the solvation and electrostatic repulsion (Figure S3):

$$E_{CT} = e(E_{red} - E_{ox}) + \frac{e}{8\pi\varepsilon_0}\left(\frac{2}{r_P} + \frac{1}{r_D}\right)\left(\frac{1}{\varepsilon_W} - \frac{1}{\varepsilon_A}\right) - \frac{2e}{4\pi\varepsilon_0\varepsilon_W d} \quad (S1)$$
where $e$ is the elementary charge ($1.602 \times 10^{-19} \text{ C}$), $E_{\text{red}}$ is the redox potential of one-electron reduction of the porphyrin ring, $E_{\text{ox}}$ is that of one-electron oxidation of the DMA moiety, $r_P$ is...
the radius of porphyrin ring \((6.8 \text{ Å})\) calculated by the DFT method (Figure S3), \(r_D\) is that of the DMA moiety \((3.0 \text{ Å})\), \(d\) is the center-to-center distance of the porphyrin ring and the DMA moiety \((6.8 \text{ Å})\), \(\varepsilon_w\) is the dielectric constant of water (80), and \(\varepsilon_A\) is that of acetonitrile (36). A cyclic voltammogram was measured with a potentiostat/galvanostat (HA-301, Hokuto Denko Corporation, Tokyo, Japan), a function generator (DF1906, NF Corporation, Yokohama, Japan), and a data logger (midi LOGGER, GL900-4, Graphtec Co., Yokohama, Japan) using a platinum working electrode, a platinum counter electrode, and a saturated calomel electrode (SCE). The sample solution for this measurement contained 1 mg DMATMPyP and 100 mM tetrabutylammonium hexafluorophosphate (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) in acetonitrile (FUJIFILM Wako Pure Chemical Co.). The \(E_{\text{red}}\) and \(E_{\text{ox}}\) were -0.67 V and +0.62 V vs. SCE, respectively. The \(\Delta G_{\text{iet}}\) of electron transfer from the DMA moiety to the \(S_1\) state of porphyrin ring is expressed as follows:

\[
\Delta G_{\text{iet}} = E_{\text{CT}} - E_{0-0} \quad (S2),
\]

where \(E_{0-0}\) is the \(S_1\) energy \((1.90 \text{ eV})\) calculated from the fluorescence maximum. The obtained value of \(\Delta G_{\text{iet}}\) was -0.73 eV.

4. Analysis of the \(pK_a\) values of DMATMPyP

Protonation of the DMA moiety and the central pyrrole nitrogen of porphyrin ring are shown in Figure S4. Acid dissociation of the protonated form of DMATMPyP, \(H^+\)-DMATMPyP, can be expressed as follows:

\[
\text{DMA-}H^+ \leftrightarrow \text{DMA} + H^+ \quad (S3),
\]

where DMA-\(H^+\) is the protonated DMA moiety of DMATMPyP and DMA is the nonprotonated DMA moiety. The acid dissociation constant, \(K_a\), and \(pK_a\) can be obtained via analysis of the relationship between the absorbance of DMATMPyP (Soret band region, at 440 nm) and the pH. These constants can be represented using the following equations [S3]:

\[
K_a = \frac{[\text{DMA}]\text{[H}^+]\text{]}{[\text{DMA-}\text{H}^+]} = \frac{R_{\text{DMA}[H^+]}}{R_{\text{DMAH}}} \quad \text{and} \quad pK_a = -\log K_a \quad (S4),
\]

where \(R_{\text{DMA}}\) is the ratio of the nonprotonated DMA moiety and \(R_{\text{DMAH}}\) is that of the protonated form \((R_{\text{DMA}} + R_{\text{DMAH}} = 1)\). The relationship between these constants and the observed absorbance (\(Abs\)) are as follows:
\[ Abs = Abs_{DMA} \times R_{DMA} + Abs_{DMAH} \times R_{DMAH} = (Abs_{DMA} - Abs_{DMAH}) \frac{K_a}{[H^+] + K_a} + Abs_{DMAH} \]

\[ = (Abs_{DMA} - Abs_{DMAH}) \frac{10^{-pK_a}}{10^{-pH} + 10^{-pK_a}} + Abs_{DMAH} \quad (S5) \]

where \( Abs_{DMA} \) is the absorbance of the nonprotonated form (completely nonprotonated case) and \( Abs_{DMAH} \) is that of the protonated form (completely protonated case). \( Abs_{DMA} \) and \( Abs_{DMAH} \) were estimated from the extrapolation of the curve in Figure 3 (main document), and the p\( K_a \) was estimated using a least squares curve fitting using equation (S5).

Similarly, the acid dissociation of the protonated central pyrrole of DMATMPyP can be expressed as follows:

\[ P-\text{H}^+ \leftrightarrow P + \text{H}^+ \quad (S6), \]

where \( P-\text{H}^+ \) is the protonated central pyrrole nitrogen of DMATMPyP and \( P \) is the nonprotonated central nitrogen. The apparent acid dissociation constant, \( K_{aP} \), and \( pK_{aP} \) can be obtained via analysis of the relationship between the absorbance of DMATMPyP and the pH. These constants are represented using the following equations:

\[ K_{aP} = \frac{[P][\text{H}^+]}{[P-\text{H}^+]} = \frac{R_P[\text{H}^+]}{R_{PH}} \quad \text{and} \quad pK_{aP} = -\log K_{aP} \quad (S7), \]

where \( R_P \) is the ratio of the nonprotonated pyrrole and \( R_{PH} \) is that of the protonated form \( (R_P + R_{PH} = 1) \). The relationship between these constants and the observed absorbance \( (Abs') \) are as follows:

\[ Abs' = Abs_P \times R_P + Abs_{PH} \times R_{PH} = (Abs_P - Abs_{PH}) \frac{K_{aP}}{[H^+] + K_{aP}} + Abs_{PH} \]

\[ = (Abs_P - Abs_{PH}) \frac{10^{-pK_{aP}}}{10^{-pH} + 10^{-pK_{aP}}} + Abs_{PH} \quad (S8), \]

where \( Abs_P \) is the absorbance of the nonprotonated form (completely nonprotonated case) and \( Abs_{PH} \) is that of the protonated form (completely protonated case). The \( Abs_P \) is same as the \( Abs_{DMAH} \), and \( Abs_{PH} \) can be estimated from the extrapolation of the curve in Figure 3 (main document), and the p\( K_{aP} \) was estimated using a least squares curve fitting using the equation (S8).
5. Transient absorption spectra of DMATMPyP

Transient absorption spectra of DMATMPyP (Figure S5) were measured with a time-resolved transient absorption spectrum system (Nanosecond system, Hamamatsu Photonics K.K., Hamamatsu, Japan) using Nd:YAG laser (SureliteI-20, Continuum, CA, USA). The observed peak at around 470 nm was assigned to the T-T absorption. The relative efficiency of T₁ formation at pH 3.2 was 2.4-times larger than that of pH 7.6. The lifetimes of T₁ state at pH 3.2 and 7.6 were about 1 μs, respectively.

![Figure S4 Protonation of DMATMPyP.](image)

6. Photosensitized singlet oxygen production by DMATMPyP

Near infrared emission at around 1270 nm demonstrated the formation of \(^1\)O₂. The time profile of \(^1\)O₂ emission is shown in Figure S6. The emission intensity of \(^1\)O₂ as a function of time, \(I(t)\), can be expressed using the following equation [S4]:

\[
I(t) = I_0 \left\{ exp \left( -\frac{t}{\tau_d} \right) - exp \left( -\frac{t}{\tau_r} \right) \right\}
\]  

\( \text{(S9)} \)
where $I_0$ is the pre-exponential factor, $\tau_d$ is the decay time constant of the emission, and $\tau_r$ is the rise time constant of this emission. When the lifetime of $^1\text{O}_2$ ($\tau_\Lambda$) is longer than $\tau_r$, $\tau_d$ corresponds to $\tau_\Lambda$. The analyzed values of $\tau_\Lambda$ at pH 7.6 and pH 3.2 were 3.8 $\mu$s and 3.9 $\mu$s, respectively.

**Figure S6** Time profile of $^1\text{O}_2$ emission produced by the photoirradiation of DMATMPyP. The sample solution contained 10 $\mu$M DMATMPyP in a 10 mM sodium phosphate buffer (indicated pH). The excitation wavelength was 532 nm.

### 7. Analysis of the interaction between DMATMPyP and HSA

The apparent association constant between DMATMPyP and HSA ($K_{bc}$) was estimated with the following equation [S5]:

$$K_{bc} = \frac{[\text{DMATMPyP-HSA}]}{[\text{DMATMPyP}][\text{HSA}]} \quad \text{(S10)},$$

where $[\text{DMATMPyP}]$ is the concentration of free DMATMPyP, $[\text{HSA}]$ is the concentration of free HSA, and $[\text{DMATMPyP-HSA}]$ is the concentration of HSA-binding DMATMPyP. The $K_{bc}$ was estimated by the following analysis. The observed absorbance of DMATMPyP with HSA ($Abs''$) can be expressed using the following equation:

$$Abs'' = Abs_0x + Abs_0(1 - x) \quad \text{(S11)},$$

where $Abs_0$ and $Abs_0$ correspond to the absorbance of DMATMPyP without HSA and that of HSA-binding DMATMPyP, respectively, $x$ is the ratio of DMATMPyP binding to HSA. The equation (S10) can be represented using this relationship (S11) as follows:

$$K_{bc} = \frac{[\text{DMATMPyP}]_0x}{[\text{DMATMPyP}]_0(1-x)[\text{HSA}]_0-[\text{DMATMPyP}]_0x} \quad \text{(S12)},$$

S8/S11
where [DMATMPyP]₀ and [HSA]₀ are the initial concentrations of DMATMPyP and HSA, respectively. The $K_{bc}$ and $x$ could be numerically obtained by the analysis of the relationship between the absorbance of DMATMPyP and [HSA]₀ using the equations S11 and S12 by the least-squares method. The observed absorbance and the calculated curves are shown in Figure S7. The obtained $K_{bc}$ values are as follows: $3.3 \times 10^6$ M$^{-1}$ and $2.1 \times 10^5$ M$^{-1}$ for pH 7.6 and pH 3.2, respectively.

![Figure S7. Relationship between the absorbance of DMATMPyP at 440 nm and the initial HSA concentration. The curves were calculated by the analysis using the equations S11 and S12. The sample solution contained 5 μM DMATMPyP and the indicated concentration of HSA in a 10 mM sodium phosphate buffer (pH 7.6 or pH 3.2).](image)

8. Analysis of binding position by FRET method

The binding position of DMATMPyP was analyzed by the Förster resonance energy transfer (FRET) method based on the Förster mechanism [S6]. The distance between the tryptophan residue of HSA and DMATMPyP (center-to-center distance) $(R)$ can be calculated using the following relationship:

$$\left(\frac{R_0}{R}\right)^6 = \frac{\tau_0}{\tau} - 1$$  \hspace{1cm} (S13)$$

where $R_0$ is the critical distance of energy transfer (described below), $\tau_0$ is the fluorescence lifetime of tryptophan without DMATMPyP, and $\tau$ is that with DMATMPyP. The $R_0$ is expressed as follows:

$$R_0^6 = \frac{9000c^4 \chi^2 \Phi_f \ln 10}{128\pi^7 n^4 N_A} \int f(v)\varepsilon(v) \frac{dv}{v^4}$$  \hspace{1cm} (S14)$$

where $c$ is light velocity, $\chi$ is the orientational factor ($\chi^2 = 2/3$, random orientation), $\Phi_f$ is
fluorescence quantum yield of tryptophan residue in HSA (0.14) [S7], $n$ is refractive index (1.3342, water), $N_A$ is the Avogadro constant, $f(\nu)$ is fluorescence spectrum of tryptophan, $\varepsilon(\nu)$ is absorption spectrum of DMATMPyP, and $\nu$ is light frequency. The obtained values of $R_0$ are 27.6 Å and 28.3 Å at pH 7.6 and 3.2, respectively. To analyze the ratio, $\tau_0/\tau$, the time profile of fluorescence intensity was measured using a time-correlated single-photon counting method with Fluorescence Lifetime System TemPro (HORIBA, Kyoto, Japan). Laser excitation at 294 nm was achieved by using a diode laser (NanoLED-295, HORIBA) at a repetition rate of 1.0 MHz. The fluorescence decay could be analyzed by double (pH 7.6) or triple (pH 3.2) exponential and the analyzed values are listed in Table S1. The average lifetime ($\tau^{\text{av}}$) can be calculated as follows:

$$
\tau^{\text{av}} = A_1 \tau_1 + A_2 \tau_2 + A_3 \tau_3
$$

(S15),

where $\tau_1$, $\tau_2$, and $\tau_3$ are the analyzed fluorescence lifetimes, and $A_1$, $A_2$, and $A_3$ are the corresponding relative amplitudes, respectively ($A_1 + A_2 + A_3 = 1$). In the case of pH 7.6, the $A_1 \tau_1$ term was ignored. The ratio of $\tau^{\text{av}}$ values with and without DMATMPyP was used for the $\tau_0/\tau$ term in the equation (S13). The obtained $R$ values were 57 Å and 55 Å at pH 7.6 and pH 3.2, respectively.

**Table S1. Fluorescence Lifetimes of HSA with or without DMATMPyP**

| pH   | DMATMPyP | $\tau_1$/ns | $A_1$ | $\tau_2$/ns | $A_2$ | $\tau_3$/ns | $A_3$ |
|------|----------|--------------|-------|--------------|-------|--------------|-------|
| 7.6  | without  | 2.45         | 0.32  | 7.48         | 0.68  |              |       |
| 7.6  | with     | 2.27         | 0.31  | 7.38         | 0.69  |              |       |
| 3.2  | without  | 0.99         | 0.12  | 3.61         | 0.53  | 7.48         | 0.35  |
| 3.2  | with     | 0.95         | 0.12  | 3.61         | 0.55  | 7.47         | 0.33  |

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