

Supporting Information

Factors for the emission enhancement of dimidium in the specific medium such as DNA and clay surface

Ryosuke Nakazato,[†] Keito Sano,[†] Hiroyuki Ichihara,[†] Tamao Ishida,^{†,††,§} Tetsuya Shimada,^{†,††} Shinsuke Takagi^{*,†,††}

[†] Department of Applied Chemistry for Environment, Graduate School of Urban Environmental Sciences, Tokyo Metropolitan University, 1-1 Minami-ohsawa, Hachioji-shi, Tokyo 192-0397, Japan

^{††} Research Center for Hydrogen Energy-based Society (ReHES), Tokyo Metropolitan University, 1-1 Minami-ohsawa, Hachioji-shi, Tokyo 192-0397, Japan

[§] Research Center for Gold Chemistry, Tokyo Metropolitan University, 1-1 Minami-ohsawa, Hachioji-shi, Tokyo 192-0397, Japan

〈Fluorescence self-quenching of dimidium (NH₂PhP) on the clay surface〉

It is known that most of guest molecules on the clay surface exhibit a decay of fluorescence intensity without a change in spectral shape as increasing the adsorption density. This is called fluorescence self-quenching. This phenomenon is interpreted as a result of the collision among guest molecules, which have a certain translational mobility on the clay surface. [Y. Ishida *et al.*, *J. Phys. Chem. A*, **2012**, *116*, 12065] In order to obtain the intrinsic fluorescence intensity, it is necessary to perform the measurement at the loading level where fluorescence self-quenching does not occur. (a) Fluorescence spectra of NH₂PhP (dimidium) with clay in aqueous solution (excited at 530 nm) and (b) fluorescence intensity decay at 613 nm as increasing loading level were shown in Fig. S1. The conditions are [NH₂PhP] = 2.5 × 10⁻⁸ M (Loading level = 0.0038–0.10% vs. CEC) and [Clay] = 6.7 × 10⁻⁴ - 2.5 × 10⁻⁵ equiv. L⁻¹. As shown in Fig. S1 (b), the decay of fluorescence intensity without a change in spectral shape was observed in the loading level of more than 0.03% vs. CEC. On the other hand, no decay of fluorescence intensity was observed in the loading level of less than 0.01% vs. CEC. In this range of the loading level, self-fluorescence quenching does not take place, thus, intrinsic fluorescence

intensity can be observed.

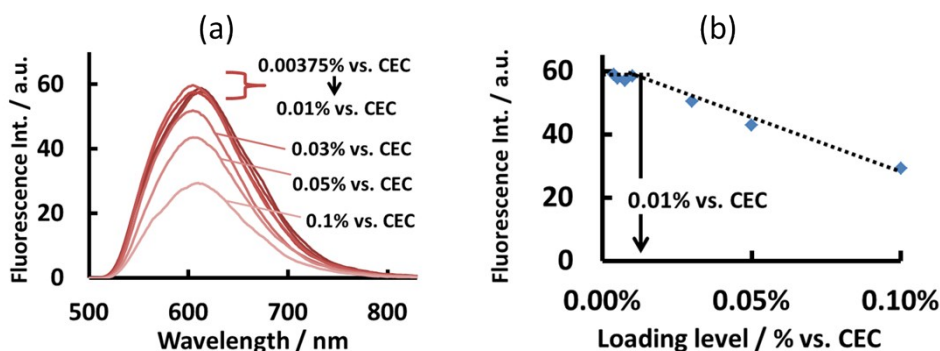


Fig. S1. (a) Fluorescence spectra of NH_2PhP (dimidium) with clay in aqueous solution (excited at 530 nm) and (b) fluorescence intensity decay at 613 nm with increasing loading level. $[\text{NH}_2\text{PhP}] = 2.5 \times 10^{-8} \text{ M}$ (Loading level = 0.0038–0.10 vs. CEC) and $[\text{Clay}] = 6.7 \times 10^{-4} - 2.5 \times 10^{-5} \text{ equiv. L}^{-1}$.

〈Synthesis〉

3,8-diamino-6-phenylphenanthridine and phenanthridine were purchased from Sigma-Aldrich Co. LLC., and used without further purification. Iodomethane was purchased from Tokyo Chemical Industry Co., Ltd., and used without further purification. Sodium carbonate, sodium nitrite, sulfamic acid and hypophosphorous acid were purchased from Nacalai Tesque, Inc., and used without further purification. Methanol, acetonitrile, hydrochloric acid and sodium hydroxide were purchased from Kanto Chemical Co., Inc., and used without further purification. Water was purified by an ion exchange system (Organo, BB-5A (PF filter \times 2 + G-10 column)). All syntheses were carried out under nitrogen atmosphere. $^1\text{H-NMR}$ spectra in D_2O and DMSO-d_6 were recorded on a Bruker B-500. Elemental analyses were performed on Exeter Analytical CE-440F. UV-vis absorption spectra were obtained on Shimadzu UV-3150 spectrophotometer.

Synthesis of 3,8-bisdimethylamino-5-methyl-6-phenylphenanthridinium chloride (NMe_2PhP chloride)

3,8-bisdimethylamino-5-methyl-6-phenylphenanthridinium chloride (NMe_2PhP chloride) was synthesized as follows. The synthetic procedure of NMe_2PhP is adapted from the method by L. P. Walls *et al.* [*J. Chem. Soc.*, **1950**, 41.] Iodomethane (6 mL), water (3.6 mL) and sodium carbonate (2705 mg) was added to an methanol solution (15

mL) of 3,8-diamino-6-phenylphenanthridine (1510 mg). The solution was refluxed at 50 °C for 8 h under stirring. The reaction was checked by TLC (SiO₂, methylene chloride : methanol = 4 : 1). The obtained solution was filtered and washed with methanol. The obtained white solid of (6-phenylphenanthridine-3,8-bistrimethylammonium iodide) (1080 mg) was heated at 180 °C for 15 minutes. After standing to cool, Greenish black solid was observed. The Greenish black solid was added into toluene heated at 80 °C. The obtained solution was filtered and washed with toluene. The black residue was added into methylene chloride. The obtained black solution containing white solid (inorganic salts) was filtered, and the filtrate was evaporated to the dryness. The reaction was checked by TLC (SiO₂, methylene chloride : methanol = 9 : 1). To a methylene chloride solution (200 mL) of the obtained orange solid (220.8 mg), sodium sulfate (4 g) was added. The solution was maintained under stirring for 3 h at room temperature. The orange solid was reprecipitated from toluene (2 L). It was added into the sufficient amount of water to perfectly dissolve at 50 °C. The solution was evaporated, and the reprecipitated orange solid of NMe₂PhP iodide was collected. After ¹H-NMR measurement, its counter anion (I⁻) was exchanged for Cl⁻ with ion-exchange resin (Organo, Amberlite Resin IRA-400) and hydrochloric acid. ¹H NMR (DMSO-d₆ / ppm): δ2.37 (6H, s, N-CH₃), δ3.22 (6H, s, N-CH₃), δ4.16 (3H, s, ⁺N-CH₃), δ6.26 (1H, s, Benzene), δ7.15 (1H, s, Benzene), δ7.61 (1H, d, Benzene), δ7.73 (2H, multi, Phenyl), δ7.79 (3H, multi, Phenyl), δ7.85 (1H, d, Benzene), δ8.81 (1H, d, Benzene), δ8.84 (1H, d, Benzene). (Fig. S2) Elemental Anal. Calcd for C₂₄H₂₆I₁N₃ · 0.2H₂O C, 59.09; H, 5.34; N, 8.49.

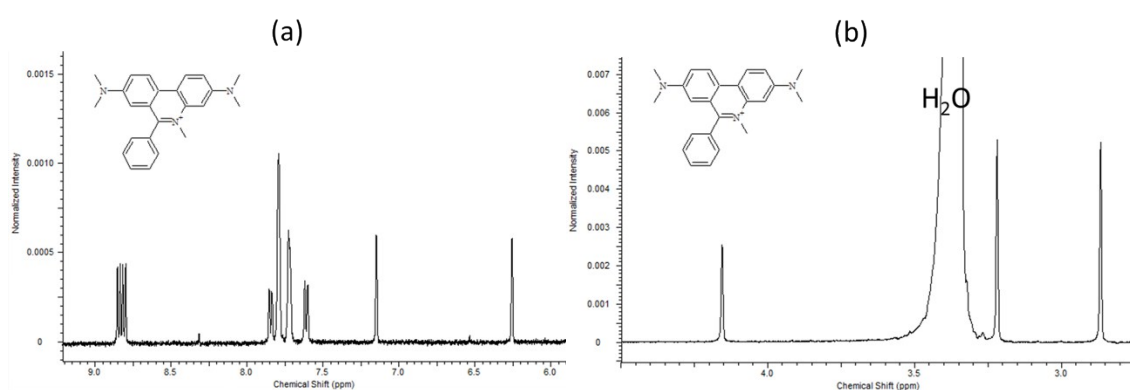


Fig. S2. ¹H-NMR spectrum of NMe₂PhP iodide in a range of (a) high magnetic field and (b) low magnetic field.

Synthesis of 5-methyl-6-phenylphenanthridinium chloride (PhP chloride)

5-methyl-6-phenylphenanthridinium chloride (PhP chloride) was synthesized as follows. The synthetic procedure of PhP is adapted from the method by W. J. Firth, III *et al.* [*J. Heterocyclic Chem.*, **1983**, 20, 759] 50% Hypophosphorous acid (0.4 mL) was added to a solution of dimidium bromide (107.2 mg) in 0.1M hydrochloric acid (8 mL) under cooling with iced water. To the solution, a solution of sodium nitrite (182.0 mg) in 0.1M hydrochloric acid (2 mL) cooled with iced water was titrated. The mixture was cooled under stirring for 23 h with iced water. Sodium hydroxide (38.00 mg) and sulfamic acid (250 mg) dissolved the sufficient amount of water were added. Water (150 mL) and methylene chloride (150 mL) were added to the solution, and the organic phase was collected after liquid-liquid extraction procedure and its repetition. The solution was evaporated to the dryness. PhP chloride was isolated with a silica column (methylene chloride : methanol = 8 : 2). ¹H NMR (D₂O / ppm): δ 4.33 (3H, s, ⁺N-CH₃), δ 7.57 (1H, d, Benzene), δ 7.58 (1H, multi, Phenyl), δ 7.74 (1H, t, Benzene), δ 7.79 (4H, multi, Phenyl), δ 8.05 (1H, t, Benzene), δ 8.08 (1H, t, Benzene), δ 8.23 (3H, multi, Benzene), δ 8.44 (1H, d, Benzene), δ 8.97 (1H, d, Benzene), δ 9.01 (1H, d, Benzene). (Fig. S3)

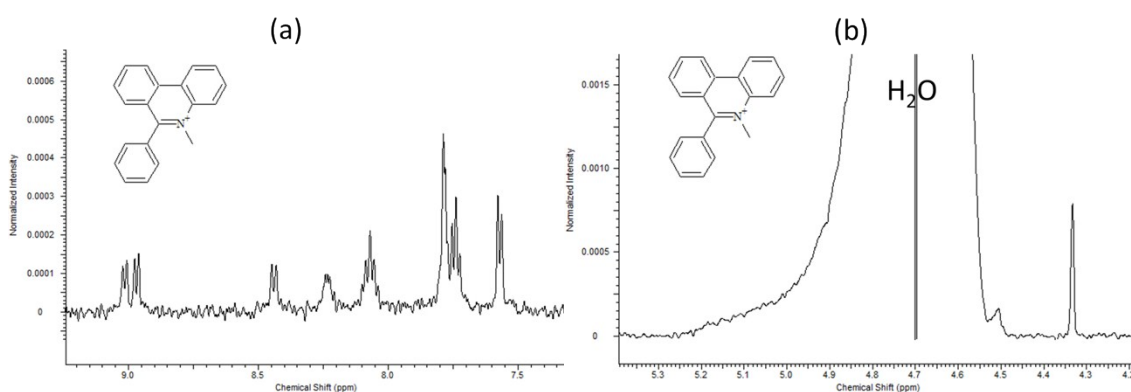


Fig. S3. ¹H-NMR spectrum of PhP chloride in a range of (a) high magnetic field and (b) low magnetic field.

Synthesis of 5-methylphenanthridinium chloride (P chloride)

□5-methylphenanthridinium chloride (P chloride) was synthesized as follows. Iodomethane (4 mL) was added to an acetonitrile solution (6 mL) of phenanthridine (107.2 mg). The reaction mixture was maintained under stirring for 23 h at room temperature. After this procedure, yellow solid of P iodide was observed. The precipitate

was filtered and washed with hexane. Its counter anion (I^-) was exchanged for Cl^- with ion-exchange resin (Organo, Amberlite Resin IRA-400) and hydrochloric acid. 1H NMR (D_2O / ppm): δ 4.74 (3H, s, $^+N-CH_3$), δ 8.09 (1H, t, Benzene), δ 8.13 (1H, t, Benzene), δ 8.18 (1H, t, Benzene), δ 8.39 (1H, t, Benzene), δ 8.46 (1H, d, Benzene), δ 8.53(1H, d, Benzene), δ 8.98 (1H, d, Benzene), δ 9.05 (1H, d, Benzene), δ 9.90 (1H, s, Pyridinium). (Fig. S4) Elemental Anal. Calcd for $C_{14}H_{12}Cl_1N_1 \cdot 1.12H_2O$ C, 67.02; H, 5.59; N, 5.57.

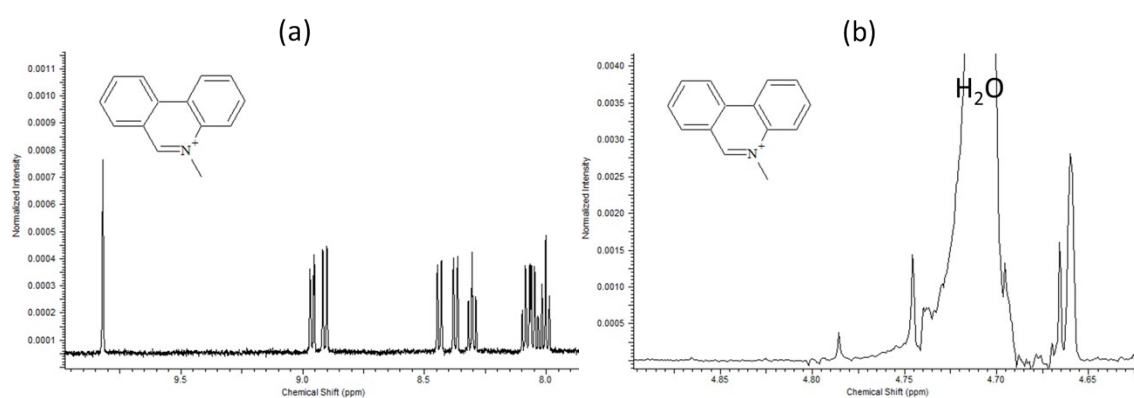


Fig. S4. 1H -NMR spectrum of P chloride in a range of (a) high magnetic field and (b) low magnetic field.

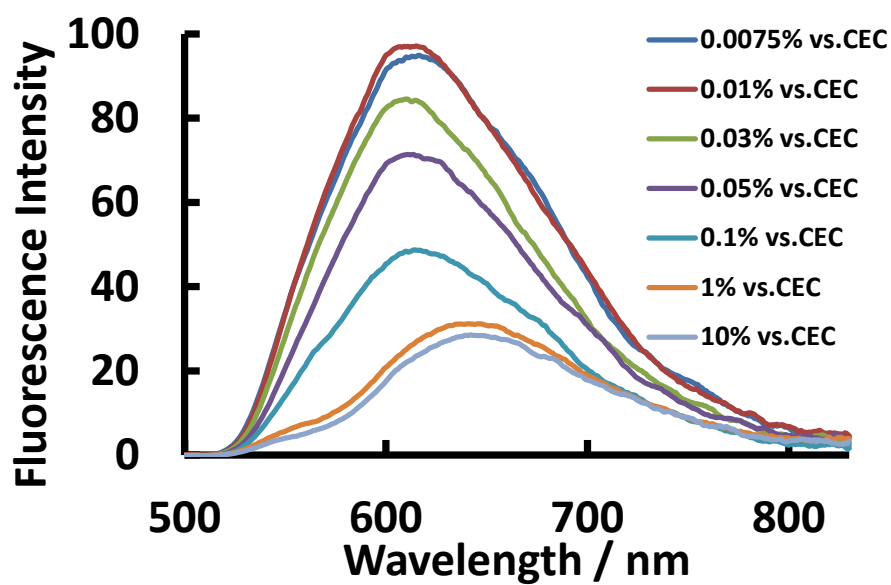


Fig. S5. The fluorescence spectra of NH₂PhP on the Clay. The loading level of NH₂PhP was 0.0075, 0.01, 0.03, 0.05, 0.1, 1, and 10% vs CEC of the Clay.

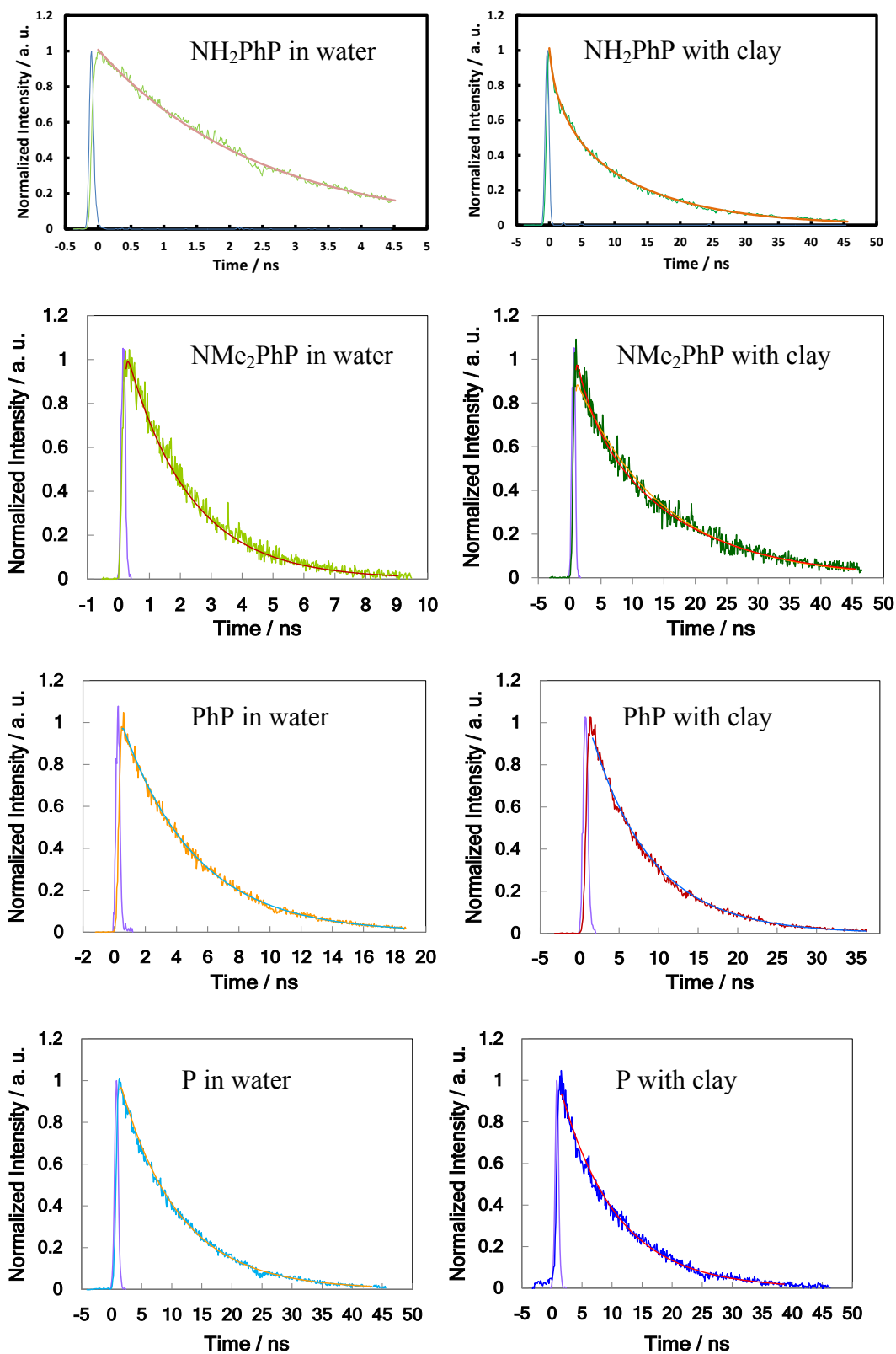


Fig. S6. Fluorescence decay curve for our PDs (NH₂PhP, NMe₂PhP, PhP, P) without and with clay. Fitting curve is also shown in the graph.