Supplementary Information

The Unprecedented J-Aggregate Formation of Rhodamine Moieties Induced by 9-Phenylanthracenyl Substitution

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S1. Experimental procedures

Materials. All chemical reagents and solvents used for the synthesis were purchased from Sigma-Aldrich Chemical Co., Tokyo Chemical Industries, Nacalai Tesque, and Wako Pure Chemical, and used without further purification. 2-Methylbenzene substituted Si-TMR (Si-Me, Figure S3) was synthesized according to the previous report by Koide et al.¹, and **TMR-An** and **SiR-An** were synthesized as described in S2 of Supporting Information. In all spectroscopic experiments, dyes stored in dimethyl sulfoxide (DMSO) at –20°C in dark were used.

Characterizations. ¹H-, ¹³C-nucler magnetic resonance (NMR) spectra, and high resolution mass spectrum (HRMS) of Si-An were measured by a JEOL ESC400 (400 MHz), JEOL ESA600 (150 MHz), Bruker Avance III700 (175 MHz), and LTQ Orbitrap XL[™] Hybrid Ion Trap-Orbitrap Mass Spectrometer with electron spray ionization (ESI), respectively. NMR chemical shift, *J*-value and integral were calculated using Delta Ver.5 software (JEOL). Field emission scanning electron microscopy (SEM) measurements were performed using a JEOL JSM-6330FT.

Steady state measurements. Methanol at the spectroscopic grade, pH 7.5 phosphate buffered saline (PBS) solution without Ca^{2+} and Mg^{2+} (both purchased from Nacalai Tesque), or MilliQ water were used as solvent. Ground-state absorption and fluorescence emission spectra were measured using a Shimadzu UV-3100 and Horiba FluoroMax-4, respectively. Particle size of Si-An aggregates was measured using a fiber-optics particle analyzer with autosampler, FPAR-1000 (Otsuka electronics).

Single crystal diffraction. Diffraction data were collected by using the synchrotron radiation ($\lambda = 0.8000$ Å) at the BL38B1 in the SPring-8 with approval of JASRI (2014A1252). The cell refinements were performed with HKL2000 software.² Direct method (SIR-2008) was used for the structure solution.³ Calculation was performed with the observed reflections [$I > 2\sigma(I)$] with the program CrystalStructure crystallographic software packages,⁴ except for refinement, which was performed using SHELXL-2013.⁵ All non-hydrogen atoms was refined with anisotropic displacement parameters, and hydrogen atoms were placed in idealized positions and refined as rigid atoms with the relative isotropic displacement parameters.

 $C_{39}H_{37}CIN_2O_5Si$, $M_w = 677.27$, a = 9.8459(5), b = 10.5371(10), c = 19.8151(17) Å, $\alpha = 94.748(5)^\circ$, $\beta = 98.528(5)^\circ$, $\gamma = 110.714(5)^\circ$, V = 1881.2(3) Å³, T = 93 K, triclinic, space group *P*-1 (No. 2), Z = 2, $\rho_{calcd} = 1.196$ g cm⁻³, 6548 unique reflections, the final *R*1 and *wR*2 values 0.0958 ($I > 2.0\sigma(I)$) and 0.3422 (all data), respectively. Crystallographic data for this paper has been deposited at the Cambridge Crystallographic Data Centre under deposition numbers CCDC 1037986.

Exchanging counterion of SiR-An. Before the treatment, chloride ion is considered to be the counterion of **SiR-An**, which probably comes from washing with brine during the work-up process of **SiR-An** synthesis. In

order to change the counterion of Si-TMR to the bulkier one, the previously reported method for rhodamine B octadecyl ester has been followed.⁶ Briefly, 1 mg of **SiR-An** (app. 1.8 µmol) dissolved in 0.2 mL acetonitrile was added to the 0.8 mL acetonitrile solution containing 50 eq. of sodium tetraphenylborate (NaBPh₄) or sodium hexafluorophosphate (NaPF₆). Under magnetic stirring, **SiR-An** solution with excess salts were kept in dark for overnight. Next, the blue solid was obtained after reduced pressure evaporation, followed by extraction with water to remove excess amounts of salt. Exchanging of counterion from Cl⁻ to BPh₄⁻ or PF₆⁻ was confirmed by different R_f of thin layer chromatography (TLC) (Figure S9b) and ¹H-NMR spectra especially in the case of NaBPh₄ (data not shown). From R_f of TLC, exchanging of counteranion of **SiR-An** has been accomplished with nearly 100% yield although the exact yield could not be determined due to errors in weight caused by remaining salt and absolutely small amount of **SiR-An** (≤ 1 mg after extraction).

Measurements of fluorescence lifetime (τ_{fl}) **and quantum yield** (Φ_{fl}). τ_{fl} s of dyes in methanol were measured using a time-resolved fluorescence microscope with a confocal optics (MicroTime 200; PicoQuant, Berlin-Adlershof, Germany). In order to measure τ_{fl} , 70 µL of 100 nM Si-Me or 500 nM TMR-An or SiR-An methanol solutions were loaded into the micro-chamber made by a 1-inch glass cover slip and Secure-Seal (S24733, Invitrogen). The samples were excited through an oil objective (Olympus, UAPON 150XOTIRF; 1.45 NA, 150x) with a 485-nm or 640-nm pulsed laser (PicoQuant, full width at half-maximum 120 ps) controlled by a PDL-800B driver (PicoQuant). The emission was collected with the same objective and detected by a single photon avalanche photodiode (Micro Photon Devices, PDM 50CT) through a 75-µm pinhole for spatial filtering to reject out-of-focus signals and 680/40 bandpass filter (Chroma). The data collected using the PicoHarp 300 TCSPC module (PicoQuant) were stored in the time-tagged time-resolved mode (TTTR), recording every detected photon with its individual timing.

To determine $\Phi_{\rm fl}$ of **TMR-An** and **SiR-An**, rhodamine B ($\Phi_{\rm fl} = 0.43$)⁷ and Si-Me ($\Phi_{\rm fl} = 0.32$) were used as references, respectively. In order to decrease the error and prevent the intermolecular interactions, we prepared the dye samples with absorbance of approximately 0.005, 0.01, and 0.015 at 520 or 600 nm. Next, the integrated areas of fluorescence spectra of the samples were calculated and linearly fitted against the responsive absorbance. Finally, $\Phi_{\rm fl}$ was determined by comparing the slope of the linear plots with references.

S2. Total synthesis scheme

(1) Synthesis of 3,6-dimethylaminoxanthone (4)



(2) Synthesis of Si-xanthone



(3) Synthesis of 9-(4-bromophenyl)anthracene (7)



(4) Synthesis of TMR-An and SiR-An



(1) Synthesis of 4: We prepared 1, 2, 3, and 4 as previously reported by J. Cui et al.⁸ and Y.-H. Ahn et al.⁹

(2) Synthesis of Si-xanthone: We prepared **4**, **5**, and Si-xanthone as previously reported by G. Lukinavicius et al.¹⁰ and Y. Koide et al.¹

(3) Synthesis of 9-(4-bromophenyl)anthracene, **6**: We prepared 9-(4-bromophenyl)anthracene as previously reported by J. Huang et al.¹¹

(4) General procedure of TMR-An and SiR-An

To a dried flask flushed with argon, **7** (1 mmol, 330 mg) and anhydrous THF (10 mL) were added. The solution was cooled to -78° C, *sec*-BuLi (1.4 M solution in cyclohexane, 0.72 mL, 1 mmol) was added, and the mixture was stirred for 30 min. At the same temperature, **4** or Si-xanthone (0.062 mmol) dissolved in anhydrous THF (10 mL) was slowly added, the mixture was warmed to RT, and then stirred for overnight under argon purging. The reaction was quenched by an addition of 2 N HCl until the solution color turned from yellow to blue, and the mixture was stirred at RT for 10 min. Saturated NaHCO₃ was added, and the mixture was extracted with CH₂Cl₂. The organic layer was dried over Na₂SO₄ and evaporated. The resulting residue was purified twice by gradient column chromatography (silica gel, CH₂Cl₂:MeOH = 20:1 to 2:1) and subsequent gel permeation chromatography (eluent: CHCl₃) to give pure **TMR-An** or **SiR-An**.

TMR-An: obtained as a dark pink solid (15% yield).¹H-NMR (400 MHz, CD₃OD) δ : 3.40 (s, 12H), 7.10 (d, 2H, J = 2.3 Hz), 7.28 (dd, 2H, J = 2.9, 9.6 Hz), 7.48–7.58 (m, 4H), 7.74 (d, 2H, J = 9.6 Hz), 7.76–7.81 (m, 6H), 8.18 (d, 2H, J = 8.7 Hz), 8.68 (s, 1H). ¹³C-NMR (175 MHz, CD₃OD) δ : 39.53, 96.23, 113.29, 114.31, 124.93, 125.56, 125.73, 126.99, 128.32, 129.70, 129.90, 131.45, 131.47, 131.51, 135.12, 141.20, 157.60, 158.00. HRMS (ESI) = m/z calculated for C₃₇H₃₁N₂O 519.2531; found 519.2530.

SiR-An: obtained as a blue solid (37% yield). ¹H-NMR (400 MHz, CD₃OD) δ : 0.65 (s, 6H), 3.39 (s, 12H), 6.96 (dd, 2H, J = 9.9, 2.8 Hz), 7.41 (d, 2H, J = 2.8 Hz), 7.41–7.54 (m, 8H), 7.62 (d, 2H, J = 7.8 Hz), 7.75 (d, 2H, J = 8.2 Hz), 8.13 (d, 2H, J = 8.2 Hz), 8.62 (s, 1H). ¹³C-NMR (150 MHz, CD₃OD) δ : 0.00, 40.98, 115.22, 122.32, 126.38, 126.96, 127.28, 128.30, 129.13, 129.78, 130.82, 131.42, 132.27, 132.78, 132.97, 136.92, 140.03, 140.84, 143.31, 149.71, 155.80, 170.81. HRMS (ESI) = m/z calculated for C₃₅H₃₇N₂Si 561.2721; found 561.2720.

S3. Supporting Figures S1-S12 and Supporting Table



Figure S1. Normalized absorption (black) and fluorescence spectra (red) of (a) **TMR-An** and (b) **SiR-An** in MeOH.



Figure S2. Fluorescence decay profiles of Si-Me (black), **SiR-An** (red), and **TMR-An** (blue) in methanol (λ_{ex} = 485 and 640 nm for **TMR-An** and Si-Me/**SiR-An**, respectively). Two lifetimes were found for the **TMR-An** sample (Table S1). The longer one (τ = 4.7 ns, 2% amplitude) might originate from impurity.



Figure S3. Chemical structures of Si-Me¹ and Si-DMA.¹²

	λ_{abs} / nm	λ_{fl} / nm	$\Phi_{ m fl}$	$ au_{ m fl}$ / ns
Rhodamine B	545	568	0.43	2.24
TMR-An	551	575	0.05	0.44‡
Si-Me	647	665	0.32	3.56
SiR-An	648	667	0.05	0.75

Table S1. Comparison among optical properties of **TMR-An/SiR-An** and reference dyes (rhodamine B and Si-Me).[†]

[†]All values were measured in methanol. [‡]Two lifetimes were found for the **TMR-An** sample (Figure S2). The longer one ($\tau = 4.7$ ns, 2% amplitude) is considered to be impurity.

Similar to the previous rhodamine derivatives, **TMR-An** and **SiR-An** monomers exhibit green-orange and farred absorption and fluorescence, respectively (black lines in Figures 2a and 3a in the main text). However, fluorescence of both compounds was quenched as compared to their fluorophores without a phenylanthracenyl substitution. For example, fluorescence quantum yield and lifetime (Φ_{fl} and τ_{fl} , respectively) of **SiR-An** were 6fold weaker and 5 times shorter than Si-TMR without an anthracene moiety (Si-Me, Figure S3). This is because the methyl substitution at 2 position of the benzene prohibits rotations between Si-TMR and phenyl moieties in the case of Si-Me, whereas this geometric hindrance does not exist in **SiR-An**, resulting in the increased rate of internal conversion.¹³



Figure S4. Color of **SiR-An** solutions in methanol, MilliQ ([NaCl] \approx 0 mM), and PBS buffer ([NaCl] \approx 110 mM). Although there was no dramatic color change in **SiR-An** solution during aggregation, blue color became less vivid in the case of PBS solution because absorption band of **SiR-An** J-aggregate shifts to the near-infrared region.



Figure S5. Color of (a) **TMR-An** and (b) **SiR-An** solutions in methanol, MilliQ ([NaCl] \approx 0 mM), and the PBS buffer ([NaCl] \approx 110 mM) after 7 days from the initial preparation. Interestingly, both samples prepared in the PBS buffer are precipitated. On the other hand, the color of **TMR-An** solution in MilliQ turned to be pink as compared to the freshly prepared solution (Figure 2b, inset, in the main text). This is presumably due to slow and spontaneous dissociation of **TMR-An** J-aggregate under the aqueous condition without Cl⁻.



Figure S6. Fluorescence spectra of **TMR-An** in MilliQ water upon the addition of NaCl from 0 to 100 mM (green to red). A red asterisk indicates J-aggregate emission. [TMR-An] = 5 μ M, pathlength: 0.2 cm, and λ_{ex} = 553 nm.



Figure S7. (a) Extended views of the crystal structure of **SiR-An** aggregate. (b) Plausible CH- π interactions between two neighboring Si-TMR moieties of **SiR-An** shown as pink dashed lines. The distance between the nearest H and the centroid of the closest aniline ring of Si-TMR is in a range of 2.6-2.8Å. Carbon, silicon, nitrogen, hydrogen atoms, water molecule, and chloride ion are represented in gray, yellow, blue, white, red, and green, respectively.



Figure S8. (a-c) Formation of **SiR-An** J-aggregate in the presence of [NaCl], [NaBr], and [NaI] = 50 mM in MilliQ (violet to red color). Black arrows show the time dependent absorption changes, related to J-aggregate formation. [**SiR-An**] = 5 μ M and pathlength: 0.2 cm. (d) Absorbance increases at around 740 nm indicating time-dependent formation of **SiR-An** J-aggregates triggered by NaCl (black), NaBr (red), and NaI (blue). **SiR-An** J-aggregate formation was the fastest with NaBr, and NaCl, and the slowest with NaI. Since this order does not follow the ionic strength neither size of ion, we tentatively suggest that Br⁻ has the most appropriate size to gather **SiR-An** molecules into the arrangement of J-aggregate.



Figure S9. (a) Exchanging counterion of **SiR-An** with hexafluorophosphate (PF_6^-) and tetraphenylborate (BPh_4^-). (b) TLC results of **SiR-An** with BPh_4^- (right), PF_6^- (two spots on the center) and Cl^- (left). Eluent: dichloromethane:MeOH = 95:5. (c and d) Absorption spectra of **SiR-An** with PF_6^- and BPh_4^- in the absence and presence of additional salts after 1 hr incubation. In MilliQ, both absorption spectra of **SiR-An** with PF_6^- and BPh_4^- (black lines) show a monomer-like shape, indicating that no H- and J-aggregate were formed because of the steric effect on the Si-TMR moiety. Further addition of NaPF₆ and NaBPh₄ did not induce spectral changes. On the other hand, by adding [NaCl] = 50 mM to Si-An with PF_6^- in MilliQ solution, absorption band of J-aggregate could be monitored as depicted by a blue asterisk. Considering the results of Figures S4, S5, S8, and Figures 2 and 3 in the main text, the presence of halide ion is crucial for the formation of **SiR-An** J-aggregate.



Figure S10. (a) DLS results of **SiR-An** J-aggregate formation. (b) Time-dependent changes in the average diameter of two aggregates of peak 1 and peak 2 as written in Figure S10a. [**SiR-An**] = 5 μ M and [NaCl] = 50 mM in MilliQ. Before the addition of NaCl and during initial 13 min of incubation, we could not observe any measurable scattering signal by DLS. Thus, **SiR-An** H-aggregate formed in MilliQ is considered to be a few nm in size or smaller, indicating that H-dimer or oligomer is probably formed in the aqueous solution. On the other hand, there are at least two different J-aggregates in size (peak 1 and peak 2, black and red, respectively): In the case of peak 1, the monitored diameter is fluctuating between 200 and 600 nm, while peak 2 shows a gradual size increase from 2 to 18 μ m during 150 min. We tentatively assign that peak 1 and peak 2 represent a unit of **SiR-An** J-aggregate and the secondary aggregation of the J-aggregate unites, respectively.



Figure S11. Tentative molecular arrangement of two **SiR-An** molecules with H-coupling. Concerning the dimethyl group at silicon atom and two dimethylamine at both edges of Si-TMR moiety, **SiR-An** H-dimer presumably exhibits antiparallel stacking rather than parallel stacking.



Figure S12. SEM pictures of **SiR-An** microcrystals. The sample was prepared by slow evaporation of concentrated **SiR-An** methanol solution for overnight, which is the similar method to what we have prepared **SiR-An** microcrystal for X-ray crystallography. The lowest picture is the enlarged view of a black circle in the second picture. As shown clearly, **SiR-An** solid has multi-layered structure in the crystal in accordance with the result of X-ray crystallography (Figure 5 in the main text).

S4. References

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