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-Methylnaphthalene substituted Si-TMR (Si-Me, Figure S3) was synthesized according to the previous report by Koide et al., 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-nuclear 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 Ca2+ and Mg2+ (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 (λ = 0.8000 Å) at the BL38B1 in the SPring-8 with approval of JASRI (2014A1252). The cell refinements were performed with HKL2000 software. Direct method (SIR-2008) was used for the structure solution. Calculation was performed with the observed reflections [I > 2σ(I)] with the program CrystalStructure crystallographic software packages, except for refinement, which was performed using SHELXL-2013. 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.

C39H37ClN2O5Si, \( M_w = 677.27 \), \( a = 9.8459(5) \), \( b = 10.5371(10) \), \( c = 19.8151(17) \), \( α = 94.748(5)° \), \( β = 98.528(5)° \), \( γ = 110.714(5)° \), \( V = 1881.2(3) \) Å³, \( T = 93 \) K, triclinic, space group P-1 (No. 2), \( Z = 2 \), \( ρ_{calc} = 1.196 \) g cm⁻³, 6548 unique reflections, the final \( R_1 \) and \( wR_2 \) values 0.0958 (\( I > 2.0σ(I) \)) 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. 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 Rf of thin layer chromatography (TLC) (Figure S9b) and ¹H-NMR spectra especially in the case of NaBPh₄ (data not shown). From Rf 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 (τᵣf) and quantum yield (Φᵣf)**, τᵣf'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 τᵣf, 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 Φᵣf of TMR-An and SiR-An, rhodamine B (Φᵣf = 0.43) and Si-Me (Φᵣf = 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, Φᵣf was determined by comparing the slope of the linear plots with references.
S2. Total synthesis scheme

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

\[
\begin{align*}
\text{OH} & \quad \text{Cl} \\
1 & \quad \text{DMF, } 130^\circ\text{C, overnight} \\
\text{O}_2 & \quad \text{N} \\
2 & \quad \text{conc. H}_2\text{SO}_4, 130^\circ\text{C, 1 hr} \\
\text{O} & \quad \text{N} \\
3 & \quad \text{SnCl}_2, \text{EtOH, reflux overnight} \\
\text{O} & \quad \text{N} \\
4 & \quad \text{CH}_3, \text{K}_2\text{CO}_3, \text{DMF, } 100^\circ\text{C, overnight} \\
\end{align*}
\]

(2) Synthesis of Si-xanthone

\[
\begin{align*}
\text{Br} & \quad \text{Br} \\
5 & \quad \text{AcOH, 60^\circC} \\
\text{N} & \quad \text{HCHO} \\
6 & \quad \text{sec-BuLi/THF, -78^\circC to RT} \\
\text{O} & \quad \text{KMnO}_4, \text{acetone, -15^\circC} \\
\text{Si-xanthone} & \\
\end{align*}
\]

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

\[
\begin{align*}
\text{HO-} & \quad \text{B(OH)}_2 \\
\text{Br} & \quad \text{I} \\
7 & \quad 2\text{ M K}_2\text{CO}_3, \text{Pd(PPh}_3)_4, \text{THF, Overnight} \\
\end{align*}
\]
(4) Synthesis of TMR-An and SiR-An

Br
\[ \text{7} \]

1) sec-BuLi, THF, -78°C
2) -78°C to RT
3) 2N HCl, RT

\[ \text{TMR-An} \]

Br
\[ \text{7} \]

1) sec-BuLi, THF, -78°C
2) -78°C to RT
3) 2N HCl, RT

\[ \text{SiR-An} \]
(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).¹⁰H-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, 135.13, 135.20, 141.20, 157.60, 158.00. HRMS (ESI) = m/z calculated for C₃₇H₃₁N₂O₅ 519.2531; found 519.2530.

**SiR-An:** obtained as a blue solid (37% yield).¹⁰H-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.
**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 ($\lambda_{ex} = 485$ 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 ($\tau = 4.7$ ns, 2% amplitude) might originate from impurity.
Figure S3. Chemical structures of Si-Me$^1$ and Si-DMA.$^{12}$
Table S1. Comparison among optical properties of TMR-An/SiR-An and reference dyes (rhodamine B and Si-Me).

<table>
<thead>
<tr>
<th></th>
<th>λ_{abs} / nm</th>
<th>λ_{fl} / nm</th>
<th>Φ_{fl}</th>
<th>τ_{fl} / ns</th>
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<tbody>
<tr>
<td>Rhodamine B</td>
<td>545</td>
<td>568</td>
<td>0.43</td>
<td>2.24</td>
</tr>
<tr>
<td>TMR-An</td>
<td>551</td>
<td>575</td>
<td>0.05</td>
<td>0.44</td>
</tr>
<tr>
<td>Si-Me</td>
<td>647</td>
<td>665</td>
<td>0.32</td>
<td>3.56</td>
</tr>
<tr>
<td>SiR-An</td>
<td>648</td>
<td>667</td>
<td>0.05</td>
<td>0.75</td>
</tr>
</tbody>
</table>

†All values were measured in methanol. ‡Two lifetimes were found for the TMR-An sample (Figure S2). The longer one (τ = 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 far-red 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 6-fold 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] ≈ 0 mM), and PBS buffer ([NaCl] ≈ 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.](image-url)
Figure S5. Color of (a) TMR-An and (b) SiR-An solutions in methanol, MilliQ ([NaCl] ≈ 0 mM), and the PBS buffer ([NaCl] ≈ 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 μM, pathlength: 0.2 cm, and λex = 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 µM 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 (PF₆⁻) and tetraphenylborate (BPh₄⁻). (b) TLC results of SiR-An with BPh₄⁻ (right), PF₆⁻ (two spots on the center) and Cl⁻ (left). Eluent: dichloromethane:MeOH = 95:5. (c and d) Absorption spectra of SiR-An with PF₆⁻ and BPh₄⁻ in the absence and presence of additional salts after 1 hr incubation. In MilliQ, both absorption spectra of SiR-An with PF₆⁻ and BPh₄⁻ (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 PF₆⁻ 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 µM 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 µm 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