

Electronic Supplementary Information (ESI)

Interfacial electron transfer dynamics in dye-modified graphene oxide nanosheets studied by single-molecule fluorescence spectroscopy

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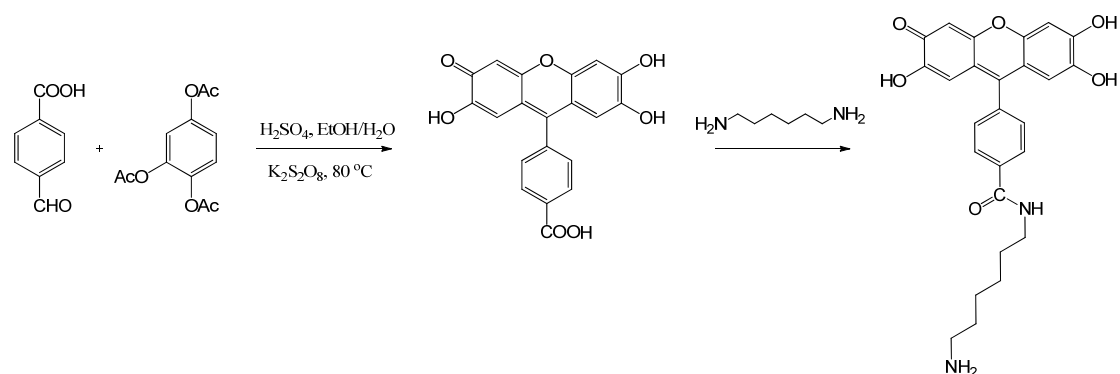
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Experimental methods

Synthesis of GO. Stable aqueous dispersions of exfoliated graphene oxide (GO) sheets were prepared by a modification of Hummers method.^{S1} In a typical synthesis, graphite powder (Bay Carbon, SP-1) (1 g) was added to a mixture of H₂SO₄ (98%, 40 mL) and NaNO₃ (2.4 g), and stirred for 1 h. K₂MnO₄ (6.0 g) was then added slowly (about 2 h) at 0 °C. The mixture was heated to 80 °C for 4 h. After completion of the reaction, distilled water (200 mL) and 30% H₂O₂ aqueous solution (6 mL) were sequentially added to the mixture. The GO was washed with distilled water 4 times and collected by centrifugation. GO was dispersed in methanol (0.5 g/L). The size of GO is fairly polydispersed with lateral dimension ranging from nanometers to micrometers.

Synthesis of 9-carboxyphenyl-2,3,7-trihydroxy-6-fluorone (PF-COOH). H₂SO₄ (98%, 3 mL) was added to a solution of 1,2,4-triacetoxybenzene (5.0 g) in 1:1 EtOH/H₂O (75 mL) and the mixture was heated to reflux. To this boiling mixture was added dropwise 4-carboxyphenyl benzaldehyde (2.5 g) within 2 min. The stirred mixture was refluxed for 1 h. Then, K₂S₂O₈ (2.70 g, 10 mmol) was slowly added at 80 °C (1 h). The contents were brought to reflux for 20 min and then poured onto ice-cold water (1 L). The crystals were collected by filtration under suction and thoroughly washed with distilled water (100 mL). After desiccation, deep red crystals (3.1 g) were obtained (yield: 60%). ¹H NMR (400 MHz, D₂O): δ 5.61 (s, 2 H), 6.11 (s, 2 H), 6.70 (d, *J* = 8.0 Hz, 2 H), 7.60 (d, *J* = 8.0 Hz, 2 H). ESI⁺-MS: calcd for [M+H]⁺, 363.051; found, 363.002.

Scheme 1. Synthetic procedure of amine-modified PF



Synthesis of GO-PF. PF-COOH (80 mg, 0.2 mmol), 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDAC, 40 mg, 0.4 mmol), and TSTU (*O*-(*N*-succinimidyl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate) (96 mL, 0.4 mmol) were dissolved in distilled water (10 mL) and stirred for 2 h. To obtain an amine-modified PF, 1,6-diaminehexane (24 μL , 0.2 mmol) was added to the mixture and stirred for 24 h. The reaction mixture was concentrated and purified by extracting impurities with CHCl_3 . GO (100 mg), EDAC (50 mg, 0.5 mmol), and TSTU (0.1 mL, 0.5 mmol) were dissolved in distilled water (10 mL) and stirred for 2 h. The amine-modified PF was added into the mixture and stirred for 24 h. The PF-modified GO (GO-PF) was collected by filtering and washed thoroughly with distilled water to remove free PF molecules. The average number of modified PF molecules per one GO nanosheet is roughly estimated to be ~ 20 . The nonspecific adsorption of PF molecules on the surface of GO nanosheets was examined by UV–visible absorption spectroscopy. Without any coupling reagents (EDAC and TSTU), it was found that about 15% of all PF molecules was nonspecifically adsorbed on the GO surface prior to purification by filtration and washing.

The molar absorption coefficient (ϵ) was determined to be $5.3 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ at 550 nm. The fluorescence quantum yield (ϕ_{fl}) of PF-COOH in methanol was estimated to be 8.4%, where rhodamine 6G in methanol was used as reference dye.

Instruments. NMR spectra were recorded on a JEOL JNM-ECS400. Mass spectra (MS) were measured with a Thermo Scientific LTQ Orbitrap XL hybrid FTMS (Fourier-transform mass spectrometer) for ESI. Steady-state UV–visible absorption spectra were measured by a Shimadzu UV-3100 UV–visible–NIR spectrophotometer. Steady-state fluorescence spectra were measured by a HORIBA FluoroMax-4 fluorescence spectrophotometer. Fourier transform infrared (FT-IR) spectra were recorded on a Jasco 4100 FT-IR spectrophotometer equipped with an attenuated total reflectance (ATR) accessory. AFM images were obtained using a SPA400 (Seiko Instruments) or an MFP-3D-BIO (Asylum Research) mounted on the Olympus IX71 inverted fluorescence microscope. All of the images were obtained using a tapping mode with silicon cantilevers (SII NanoTechnology, SI-DF20S or Olympus, OMCL-AC200TS). Thermogravimetric analysis (TGA) of graphite, GO, and GO-PF

was carried out under N₂ flow using Rigaku Thermo plus EVO II/TG-DTA (TG8120). Cyclic voltammetry (CV) experiments were carried out at room temperature using an electrochemical analyzer (ALS, model 660A) with a standard three-electrode configuration, which was composed of a Pt working electrode, a Pt wire counter electrode, and an Ag/Ag⁺ non-aqueous reference electrode. Sample solutions were prepared by dissolving the substrates in *N,N*-dimethylformamide (DMF) containing 0.1 M tetrabutylammonium tetrafluoroborate (TBATFB) as the electrolyte. All potentials were calibrated using ferrocene (Fc) (1.0 mM) as an internal standard (+0.677 V vs NHE for the Fc⁺/Fc couple).^{S2}

Fluorescence lifetime measurements. The time-resolved fluorescence spectra and decays were measured by the time-correlated single photon counting (TCSPC) using a streak scope (Hamamatsu Photonics, C4334-01) equipped with a polychromator (Acton Research, SpectraPro150). The second harmonic oscillation (400 nm) of the output of the femtosecond laser (Spectra-Physics, Tsunami 3941-M1BB; full width at half-maximum (fwhm) 80 fs; 800 nm) pumped by a diode-pumped solid-state laser (Spectra-Physics, Millennia VIIIs) was used to excite the sample in a quartz cell.

The multiexponential decay curves were fitted using a nonlinear least-squares method with a multi-component decay law given by $I(t) = a_1\exp(-t/\tau_1) + a_2\exp(-t/\tau_2) + \dots + a_n\exp(-t/\tau_n)$. The instrument response function (IRF) was also obtained by measuring the scattered laser light in order to analyze the temporal profile. The observed temporal emission profiles were well fitted by two exponential functions. All measurements were carried out at room temperature.

Picosecond transient absorption measurements. The picosecond transient absorption spectra were measured by the pump and probe method using a regeneratively amplified titanium sapphire laser (Spectra-Physics, Spitfire Pro F, 1 kHz) pumped by a Nd:YLF laser (Spectra-Physics, Empower 15). The seed pulse was generated by a titanium sapphire laser (Spectra-Physics, Tsunami 3941-M1BB; fwhm 80 fs) pumped with a diode-pumped solid state laser (Spectra-Physics, Millennia VIIIs). An optical parametric amplifier (Spectra Physics, OPA-800CF-1) was used to produce the excitation pulse (500 nm). A white light continuum pulse, which was generated by focusing the residual of the fundamental light on a sapphire crystal after the computer-controlled optical delay, was divided into two parts and used as the probe and the reference lights, of which the latter was used to compensate the laser fluctuation. Both the probe and reference lights were directed to the rotating sample cell with a 1.5-mm optical path and detected by the CCD detector equipped with the polychromator (Solar, MS3504). The pump pulse was chopped by the mechanical chopper synchronized to one half of the laser repetition rate, resulting in a pair of spectra with and without the pump, from which the absorption change induced by the pump pulse was estimated. All measurements were carried out at room temperature.

Sample preparation for single-molecule fluorescence experiments. The quartz cover glasses were purchased from DAICO MFG CO., Ltd. (Japan) and cleaned by

sonication in a 20% detergent solution (As One, Cleanace) for 6 h, followed by repeated washings with warm running water for 30 min. Finally, the cover glasses were washed again with Milli-Q ultrapure water (Millipore). Well-dispersed methanol suspensions of GO-PF sheets were spin-coated on the cleaned cover glasses.

Single-molecule fluorescence measurements with confocal microscopy. Confocal fluorescence images were taken on an objective-scanning confocal microscope system (PicoQuant, MicroTime 200) coupled to an Olympus IX71 inverted fluorescence microscope. The samples were excited through an oil objective (Olympus, UAPON 150XOTIRF; 1.45 NA, 150x) with a 485-nm pulsed laser (PicoQuant, LDH-D-C-485) 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 and 100CT) through (Chroma, z405/488rpc-xt), longpass filter (Chroma, HQ510LP), and 75- μm pinhole for spatial filtering to reject out-of-focus signals. The single-molecule polarization anisotropy experiments were performed to examine molecular motion. The emitted photons were separated by a polarizing beam splitter cube to two different directions, one vertical (V) and the other horizontal (H) to the in-plane x-axis of the optics under irradiation with a circular-polarized laser. These components were individually detected by two detectors. 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, which were used for the single-molecule analysis. FLIM images use the average arrival time of fluorescence photons after the excitation pulse as a measure for the average lifetime. The average lifetimes were calculated on the base of the average TCSPC channel of each pixel.

For the spectroscopy, only the emission that passed through a slit entered the imaging spectrograph (ActonResearch, SP-2356) that was equipped with an EM-CCD camera (Princeton Instruments, ProEM). The spectra were typically integrated for 5 s. The spectrum detected by the EM-CCD camera was stored and analyzed by using a personal computer. All the experimental data were obtained at room temperature.

Raman scattering measurements with wide-field microscopy. The experimental setup is based on an Olympus IX71 inverted fluorescence microscope. The position of the GO sheets was determined by the transmission image obtained using illumination provided by a 100-W halogen lamp (Olympus, U-LH100L-3) placed above the sample and the fluorescence image. A 532 nm CW laser light (Photop Suwtech, DPGL-2050F; 50 mW) passing through an objective lens (Olympus, UPlanSApo, 1.40 NA, 100 \times) after reflection at a dichroic mirror (Semrock, Di01-R532) was used to excite the sheets. The scattered light as well as a background emission was collected using the same objective, passed through an emission filter (Semrock, BLP01-532R-25) to remove scattered excitation light and a slit, and entered the imaging spectrograph (ActonResearch, SP-2356) that was equipped with an EM-CCD camera (Princeton Instruments, PhotonMAX:512B). The spectra were typically integrated for 3 min. The spectrum detected by the EM-CCD camera was stored and

analyzed by using a personal computer. All the experimental data were obtained at room temperature.

Experimental results

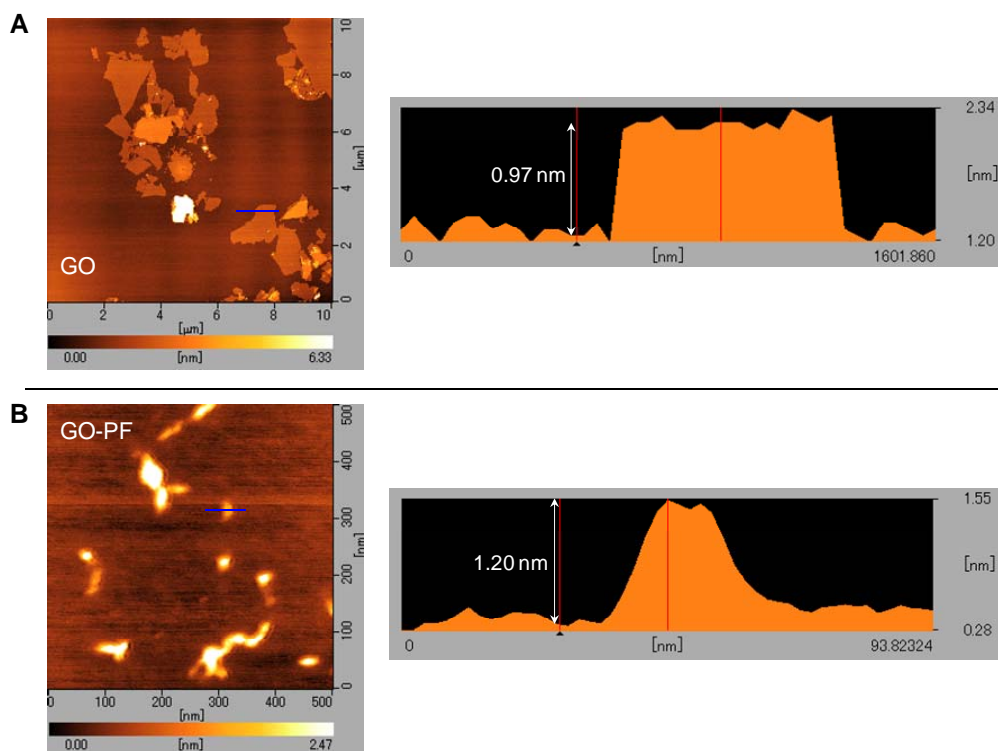


Fig. S1. AFM images of GO and GO-PF sheets. The thicknesses of isolated GO and GO-PF sheets are 0.8–1.0 nm and 1.2–1.5 nm, respectively. Considering the thickness of a single GO sheet and the molecular size of PF dye (0.5–1.0 nm), the obtained images are representative of one- or two-layer GO-PF sheets. The size of GO is fairly polydispersed with the lateral dimension ranging from nanometers to micrometers. The average size of isolated GO-PF nanosheets is 20–100 nm.

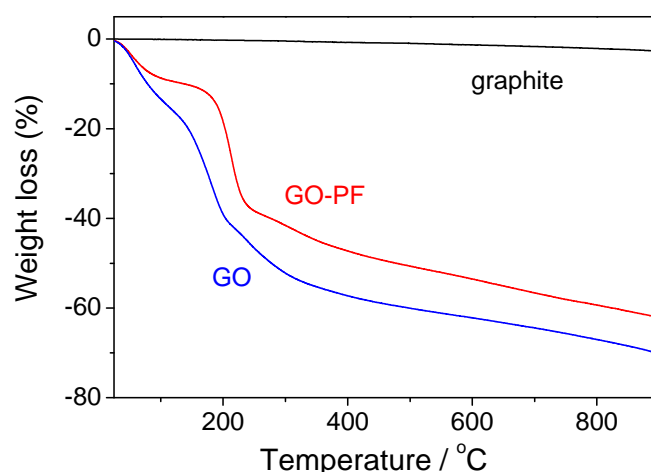


Fig. S2. TGA curves of graphite, GO, and GO-PF. The samples were heated from room temperature to 900 °C at 10 °C/min. As expected, graphite was highly stable up to 900 °C. GO shows slight mass decrease from room temperature to 150 °C and significant decrease from 150 °C to 200 °C, which caused by pyrolysis of the oxygen-containing functional groups. The mass of graphene oxide slowly further decreased up to 900 °C. The TGA curves did not show a clear weight loss corresponding to the presence of PF molecules. This is probably due to the small amount of modified PF molecules on the GO surface (1~2 % of total weight, calculated from the absorption spectrum).

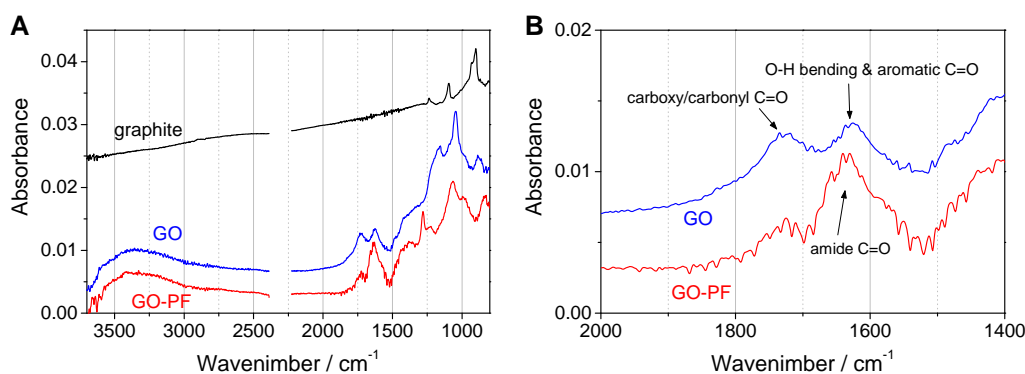


Fig. S3. FT-IR spectra observed for graphite, GO, and GO-PF. For GO, the characteristic features are the broad band at $\sim 3400\text{ cm}^{-1}$ (O-H stretching) and the bands at 1731 cm^{-1} (C=O stretching from carbonyl and carboxylic groups), 1624 cm^{-1} (O-H bending and aromatic C=C stretching), 1421 cm^{-1} (O-H deformation vibration), $\sim 1220\text{ cm}^{-1}$ (C-OH stretching), 1160 cm^{-1} (C-O stretching), and 1047 cm^{-1} (C-O stretching).^{S3-S5} Upon modification with PF, the C=O stretching vibration at 1733 cm^{-1} in GO becomes obscured. A new band at 1640 cm^{-1} can be assigned to an amide carbonyl-stretching mode.^{S5}

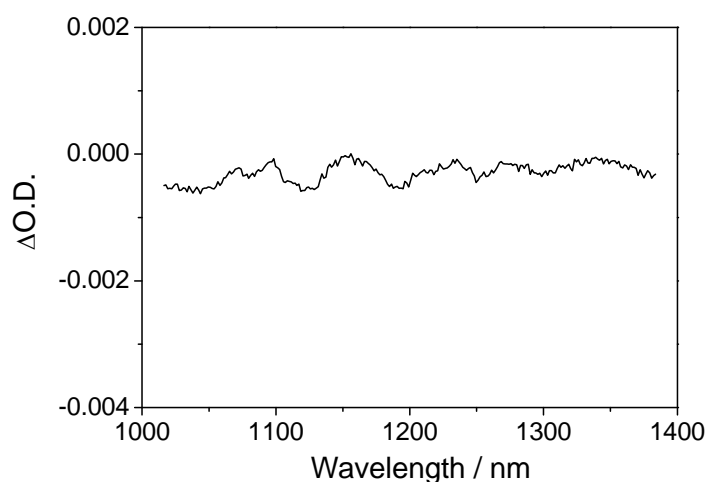


Fig. S4. Transient absorption spectrum observed at 50 ps after the laser flash during the 500-nm laser photolysis of the deuterated methanol (CD_3OD) solution of GO-PF.

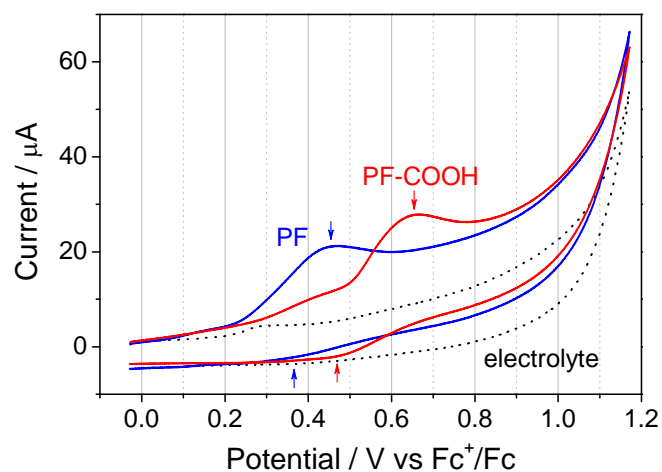


Fig. S5. Cyclic voltammograms of electrolyte solutions (0.1 M TBATFB in DMF) of PF (Tokyo Chemical Industry) (0.4 mM) and PF-COOH (0.2 mM). The oxidation potentials (E_{ox}) of PF and PF-CCOH were determined to be +1.04 and +1.24 V vs NHE, respectively. These values are close to the reported one (+1.14 V vs NHE) for PF in acetonitrile.^{S6}

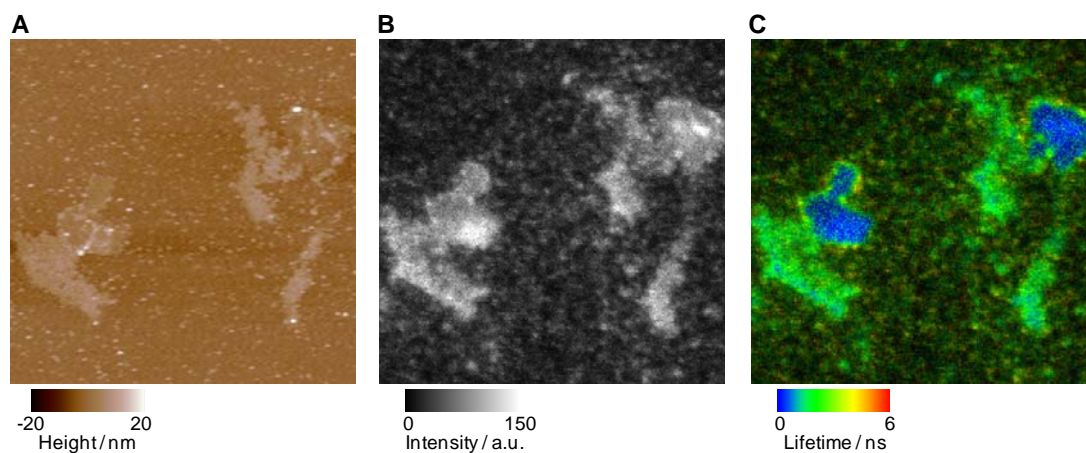


Fig. S6. AFM (A), fluorescence intensity (B), and fluorescence lifetime (C) images captured for GO-PF sheets on the glass surface in ambient air. The image size is $12\ \mu\text{m} \times 12\ \mu\text{m}$.

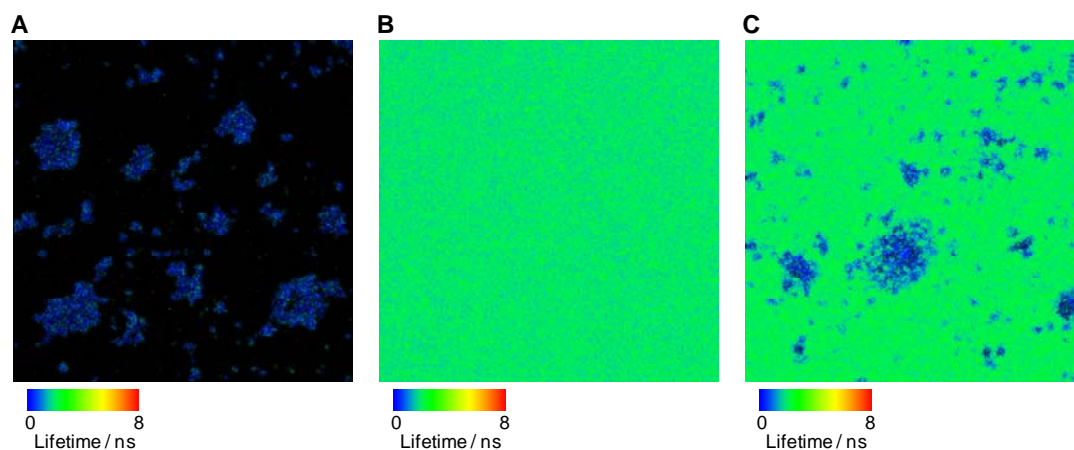


Fig. S7. Fluorescence lifetime images for GO sheets spin-coated on the glass surface (A) and PF-COOH molecules spin-coated on the glass surface (B) and on the GO-precoated glass surface (C). The image size is $25\ \mu\text{m} \times 25\ \mu\text{m}$. Strong fluorescence quenching was confirmed only for PF molecules on the GO sheets.

References

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