



Title	Exploring photo-excited states of aromatic sulfones for efficient near-infrared-activated photothermal cancer therapy
Author(s)	Yoshida, Kazuya; Suzuki, Toshiaki; Osakada, Yasuko et al.
Citation	Bulletin of the chemical society of Japan, 98(1) https://doi.org/10.1093/bulcsj/u0ae137
Issue Date	2025-01-17
Doc URL	https://hdl.handle.net/2115/97376
Rights	This is a pre-copyedited, author-produced version of an article accepted for publication in Bulletin of the chemical society of Japan following peer review. The version of record Bulletin of the Chemical Society of Japan, Volume 98, Issue 1, January 2025, u0ae137 is available online at: 10.1093/bulcsj/u0ae137 .
Type	journal article
File Information	112466.pdf



Exploring Photo-excited States of Aromatic Sulfones for Efficient NIR-Activated Photothermal Cancer Therapy

Kazuya Yoshida¹, Toshiaki Suzuki², Yasuko Osakada^{3,4,5}, Mamoru Fujitsuka^{4,5}, Yukiko Miyatake⁶, Vasudevanpillai Biju^{1,7} and Yuta Takano^{*1,7}

¹Graduate School of Environmental Science, Hokkaido University, N10, W5, Sapporo 060-0810, Japan.

²Unisoku Co. Ltd, Hirakata, Osaka 573-0131, Japan.

³SANKEN (The Institute of Scientific and Industrial Research), Osaka University, Mihogaoka 8-1, Ibaraki, Osaka 567-0047, Japan

⁴Open and Transdisciplinary Research Initiatives (ICS-OTRI), Osaka University, 2-1 Yamadagaoka, Suita, Osaka 565-0871, Japan

⁵Institute for Advanced Co-Creation Studies, Osaka University, Yamadagaoka 1-1, Suita, Osaka 565-0871, Japan

⁶Department of Pathology, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, N15, W7, Sapporo 060-8638, Japan.

⁷Research Institute for Electronic Science, Hokkaido University, N20, W10, Sapporo 001-0020, Japan.

* Research Institute for Electronic Science, Hokkaido University, N20, W10, Sapporo 001-0020. Email: tak@es.hokudai.ac.jp



Yuta Takano

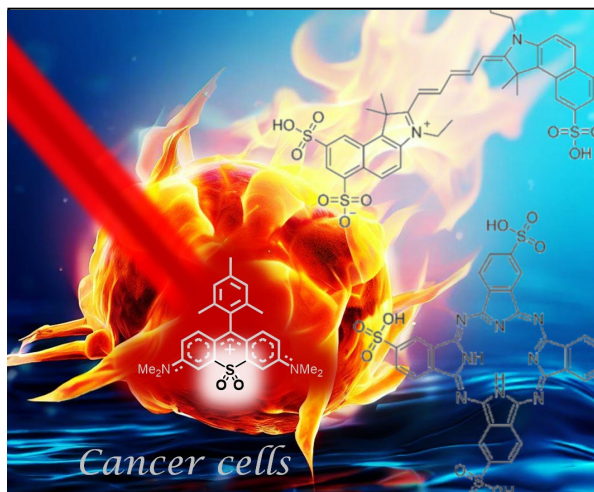
Yuta TAKANO is an associate professor at the Research Institute for Electronic Science, Hokkaido University. He obtained his Ph.D. from the University of Tsukuba in 2010. His career includes postdoctoral work at Tsukuba and a position as program-specific assistant professor at the Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University. His research focuses on photofunctional molecules, photoinduced electron transfer reactions, and controlling biological cellular functions.

Abstract

Near-infrared (NIR) light activation enhances phototherapeutic efficacy due to deep-tissue penetration and minimal invasion. While NIR-phototherapeutic agents are widely studied, conventional designs often increase molecular size, affecting pharmacokinetics. This study elucidates the photo-excited states of sulfone-containing aromatic compounds as efficient NIR-activated photothermal agents for cancer therapy. We synthesized sulfone-rosamine compounds (**3** and **4**) exhibiting strong optical absorption around 700 nm, attributed to a unique orbital in the LUMO. Compound **4**, 35% smaller than conventional NIR-dye molecules, such as Cy5.5 (0.86 nm²/molecule), enhances intracellular mobility. Upon photoexcitation, **4** undergoes rapid non-radiative relaxation, resulting in remarkable photothermal effects and photostability. Transient absorption spectroscopy and DFT calculations revealed a mechanism involving rapid non-radiative deactivation after illumination, efficiently converting light to heat. The NIR-induced photothermal effect effectively killed various cancer cell lines, including HeLa, MDA-MB-231, and PCI-55. This study provides guidelines for designing novel photodrugs with improved intracellular mobility, near-infrared light responsiveness, and photostability, and introduces the advancement of cancer treatment.

Keywords: photothermal effect, lysosome, NIR-dye molecules

Graphical abstract



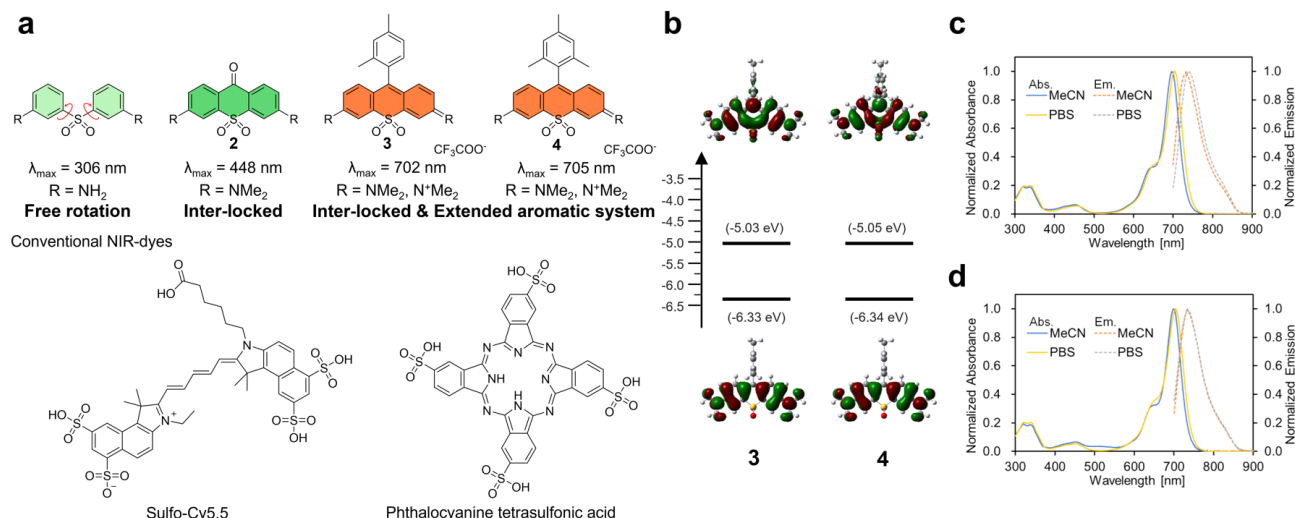


Figure 1. (a) Structures and absorption maxima of sulfone-containing aromatic compounds and conventional NIR-dyes. The λ_{max} corresponds to the absorption spectra shown in Figures 1c, 1d, and S1. (b) Calculated energy levels and orbital distributions of LUMOs and HOMOs of **3** and **4** obtained by the density functional theory (DFT) calculations at the LSDA/6-31+G(d,p) level with SCRF, where H_2O is the solvent. (c) Normalized absorption (solid line) and emission spectra (dashed line) of (c) **3** and (d) **4** in phosphate-buffered saline (PBS) or MeCN.

1. Introduction

Phototherapy is a promising approach for medical treatments^{1,2}, including cancer therapy³, immunomodulation^{4,5}, and gene expression/suppression⁶. Recently, the use of near-infrared (NIR) light in the specific region called the biological window ($700 \text{ nm} \leq \lambda \leq 1300 \text{ nm}$)⁷⁻⁹ has been recognized as essential for efficient phototherapy. This is because NIR light can penetrate deeper into tissues and cause less damage to normal cells than visible or ultraviolet light⁴.

Cancer phototherapy, which uses light to activate therapeutic reagents and induce cell death, may offer a more specific, efficient, and minimally invasive alternative to conventional therapies such as surgery, radiotherapy, and chemotherapy³. In this regard, many photo-therapeutic reagents that can utilize NIR light have been intensively reported recently¹. The general approaches to achieving efficient NIR-light harvesting include extending the π -conjugation system¹⁰⁻¹³, using an intramolecular charge transfer (ICT) process^{14,15}, or applying up-conversion^{16,17}.

Although these approaches are helpful, they tend to increase molecular sizes and hamper the flexible design and pharmacokinetics of the photodrugs¹⁸. Large molecular size is known to hamper free movement and penetration in cellular compartments such as cell membranes or the blood-brain barrier^{7,10,18,19}. Small molecules are advantageous for controlling and tuning intracellular dynamics for biological applications²⁰. Therefore, developing small-molecule NIR-light harvesting reagents remains a significant challenge in the field.²¹

In this study, we focus on aromatic sulfones among several candidates due to their accessibility, chemical

stability, and bio-compatibility. The sulfur atom in sulfone is much more chemically stable than that of sulfide or other certain hetero-atom systems because the electrons of the sulfur in sulfone are shared with adjacent atoms and the two oxygen atoms. Therefore, sulfone-containing compounds have been reported for stable drug components^{22,23} and bio-active compounds^{24,25}.

Sulfone-rosamines, including sulfone rhodamines, are a recently developed promising class of aromatic sulfones. They demonstrate excellent NIR light harvesting ability around 700 nm, showing potential as excellent NIR photolabels^{24,25}. However, the phototherapeutic effect of sulfone-rosamines has not yet been studied extensively,²⁶ and their photophysical properties, including their photoexcited states, have not been studied.

In this study, we developed and studied sulfone-rosamine molecules **3** and **4** (Figure 1a). Their molecular sizes are much smaller than conventional NIR-dye molecules, facilitating energy-independent accumulation and unique localization into cells. This smaller size potentially enhances their ability to penetrate cellular compartments and improves their pharmacokinetic properties. Photophysical properties of **4** were investigated through time-resolved absorption and fluorescence spectra and theoretical calculations. Importantly, **4** shows high photostability and a potent photothermal therapeutic effect, effectively killing various cancer types, including HeLa cells, malignant breast cancer cells (MDA-MB-231)²⁷, and aggressive pancreatic cancer cells (PCI-55)^{28,29}. Our work elucidated the photophysical properties of sulfone-rosamines and demonstrated their possibility as efficient, small-molecule NIR-activated photothermal agents for phototherapy.

2. Experimental

2.1. General information

A UV-Vis spectrophotometer (Evolution 220, ThermoFisher Scientific, USA) and a fluorescence spectrophotometer (RF-6000, Shimadzu Co., Japan) were used to record the absorption and fluorescence spectra. ^1H and ^{13}C NMR spectra were measured using a JEOL JNN-EX400 spectrometer (Japan). ESI-mass spectra were measured using a JEOL JMS-T100LC (Japan).

All chemicals used in this research were analytical grade and used as received unless otherwise stated. Solvents in reagent grade were obtained from FUJIFILM Wako Pure Chemical Corporation, Japan.

Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were obtained from Wako (Japan) and Sigma Aldrich Corp. (St. Louis, MO, USA), respectively. HeLa cells, human cervical cancer cells, were collected from Riken BRC (Japan). The premix WST-1 Cell Proliferation Assay System kit was obtained from Takara Bio Inc. (Japan).

2.2. Density functional theory (DFT) calculations

Geometries of the molecules were optimized using the Gaussian 16 Revision C.01 program with the LSDA functional in the default Gaussian 16 package³⁰. LSDA (Local spin density approximation) here is a method keyword in the Gaussian 16 that requests a Local Spin Density Approximation calculation using the Slater exchange functional and the VWN correlation functional for the DFT calculation. The 6-31+G(d,p)³¹ basis set was used. Molecular orbital analyses were performed with the natural transition orbitals (NTO).³²

Molecular sizes based on 0.001 electrons/bohr³ density were determined by the DFT calculations using the keyword "volume".

2.3. Subnanosecond transient absorption spectra

Subnanosecond laser-induced transient absorption spectra were collected by a commercially available measuring system (picoTAS; Unisoku, Japan) based on the randomly interleaved pulse train (RIPT) method developed by Nakagawa et al.³³ The pump source is a mode-locked picosecond laser (PL2210A/PG403, EKSPLA, 1 kHz, 25 ps, 355 nm/410-2300 nm). The time resolution of the system was estimated at 100 ps from the 10–90% rise time. For the laser flash photolysis experiments, deaerated MeCN solutions of the sample were excited by an Nd:YAG laser (Continuum, SLII-10, 4–6 ns FWHM) at 355 nm with a power of 10 mJ per pulse. The transient absorption measurements in the visible and near-IR region were performed with a continuous xenon lamp (75 W) as probe light and a photomultiplier (Hamamatsu R2949; 350–800 nm) and an InGaAs PIN photodiode (Hamamatsu G10899-01K; 800–1600 nm) as detectors. The output from the photodiodes and photomultiplier was recorded with a digitizing oscilloscope (HDO4022, Teledyne LeCroy, 200 MHz).

2.4. Electrochemical Measurements

Electrochemical measurements were performed using a BAS ALS 630 electrochemical analyzer. Redox potentials were determined by differential pulse voltammetry (DPV) in dried and argon-purged PBS. A glassy carbon (3 mm diameter) working electrode, Ag/AgCl (sat. KCl) for PBS reference electrodes, and Pt wire counter electrode were employed. Ferrocenium hexafluorophosphate was used as the internal standard.

2.5. Evaluation of photothermal effect

Compounds **3** and **4** in PBS were irradiated with a Xe lamp (700 nm bandpass 50 nm, 290 mW / 0.8 cm²-spot) and recorded their temperature changes. The solutions were also measured for absorption spectra before and after the irradiation. As a reference compound, Sulfo-Cyanine5.5 (Cy5.5), a NIR-absorbing dye with a cyanine moiety, was subjected to the same procedure.

2.6. Cell lines and cell culture

HeLa cells were obtained from the RIKEN cell bank, MDA-MB-231 cells were from ECACC, and PCI-55 cells were established by our group from pancreatic ductal adenocarcinoma tissues that were resected from primary sites at Hokkaido University Hospital²⁸. The cells were grown in DMEM medium supplemented with 10 % FBS (fetal bovine serum) and 1 % penicillin-streptomycin at 37 °C in a humidified environment of 5 % CO₂. The medium was changed every two days, and the cells were passaged by trypsinization after the density reached 80-90 % confluency.

Other information on the experimental details is provided in the Supplementary data.

3. Results and discussion

3.1. Molecular designing strategy and synthesis

We first investigated the correlation between structures and optical absorption properties of aromatic sulfones. A simple motif of sulfone compounds, bis(3-aminophenyl)sulfone, does not show absorption in the visible light region (Figures 1a, left, and S1). The two aromatic rings in the compound can freely rotate, potentially limiting their electronic interaction. Interlocking the aromatic rings by a carbonyl moiety in **2** shifts the absorption maxima to the blue-light region (448 nm) (Figure S1). Remarkably, **3** and **4** demonstrate a significant red-shift in absorption to over 700 nm (Figures 1c and 1d), which originates from the stabilization of the LUMO level due to the incorporation of sulfone and sulfoxide groups into π -systems,³⁴ along with a notable narrowing of HOMO-LUMO gaps (Figures 1b and S3). Our DFT calculations on **3** and **4** suggest such contributions to lower the LUMO energy, with their relative contributions depending on the specific molecular geometry and electronic environment (Figure S2). This effect results from the interlocking of aromatic rings with π -conjugation, forming an extended aromatic system.

Table 1. Optical properties (absorption and fluorescence maxima, absorption coefficients, and fluorescence quantum yield) of **3** and **4**.

	solvent	λ_{abs} [nm]	λ_{fl} [nm]	ϵ_{max} [10 ⁵ M ⁻¹ cm ⁻¹]	ϵ_{700} [10 ⁵ M ⁻¹ cm ⁻¹]	Φ_{fl}
3	PBS	702	741	1.03	1.01	0.024
	MeCN	697	738	1.05	1.04	0.096
4	PBS	705	735	1.09	1.04	0.053
	MeCN	700	734	1.14	1.14	0.160

We propose that this principle can be applied to the universal design of NIR-light harvesting molecules. For example, we can predict modification ideas for recently reported NIR-photo theranostic reagents of hemicyanine dyes reported by S. F Yao et al.³⁵ They reported a sulfide analog. According to our DFT calculation prediction (Figure S4), the sulfone analog of this compound will possess an absorption maximum at around 810 nm (1.53 eV), which is a longer wavelength than the sulfide compound they reported (760 nm in phosphate-buffered saline, PBS). Consequently, the interlocking sulfone-containing aromatic system may be predictable by DFT calculations to design molecules harvesting NIR light using unique orbitals.

For the study of phototherapeutic effects, the present study employs sulfone-rosamines possessing an electron-donating aromatic moiety (**3**: xylyl, **4**: mesityl)³⁶ connected to the xanthene moiety (Figure 1a). The molecular sizes of **3** and **4** are 0.56 nm³/molecule (based on 0.001 electrons/bohr³ density calculated by the DFT calculations), which is 35% smaller than conventional NIR-dye molecules such as sulfo-Cy5.5³⁷ (0.86 nm³/molecule) or the others (e.g. phthalocyanine tetrasulfonic acid¹³: 0.80 nm³/molecule) (Figure 1a). **3** and **4** were synthesized from bis[4-(dimethylamino)phenyl]methane, via 3,6-bis(dimethylamino)-9H-thioxanthene 10,10-dioxide (**1**), and **2**, in total three steps (see the Supplementary data for details), and characterized by spectroscopic methods, including NMR and high-resolution ESI mass spectrometers (Figure S5).

3.2. Photophysical properties of **3** and **4**

The maximum absorption wavelengths and the absorption coefficients of **3** and **4** are both around 700 nm and 1 × 10⁵ M⁻¹ cm⁻¹ (Figures 1c, 1d, and Table 1). The fluorescence quantum yields of **3** and **4** exhibited a solvent-dependent behavior, decreasing as solvent polarity increased from MeCN to PBS (Table 1). This phenomenon suggests a higher probability of photo-induced electron transfer that can be enhanced by solvent polarity³⁸ and decrease the fluorescence. Furthermore, the fluorescence quantum yields of **3** and **4** (2.4 and 5.3 % in PBS and 9.6 and 16.0 % in MeCN) are lower than those of corresponding molecules lacking a methyl group at the para-position of the

benzene ring connected to the xanthene moiety (7.3 and 9.7 % in PBS and 29.0 and 28.4 % in MeCN)²⁴. The presence of the additional methyl group in **3** and **4** would increase the electron density of the electron-donor part of the molecule, promoting photo-induced intramolecular charge separation that may compete with radiative deactivation.

To elucidate the feasibility of photo-induced electron transfer reactions, redox potential measurements were performed on **4** (Table 2). The thermodynamic feasibility was verified by the Rehm-Weller equation shown as eq. 1.

$$\Delta G \text{ (eV)} = E_{\text{ox}} - E_{\text{red}} - E_{0,0} \quad (\text{eq. 1})$$

where E_{ox} is the first oxidation potential of the electron donor part, E_{red} is the first reduction potential of the acceptor part, and $E_{0,0}$ is the zero-zero transition energy (Table 2). The negative Gibbs energy change ($\Delta G_{\text{ET}} = -0.52$ eV) indicates that photo-induced electron transfer can occur spontaneously.

Table 2. Redox potentials^[a], $E_{0,0}$ values^[b], and the free energy charge of photo-induced electron transfer (ΔG_{ET})^[c] of compound **4** in PBS.

$E_{\text{red}2}$ [V]	$E_{\text{red}1}$ [V]	$E_{\text{ox}1}$ [V]	$E_{0,0}$ [eV]	ΔG_{ET} [eV]
-0.87	-0.31	0.93 ^[d]	1.76 ^[b]	-0.52

[a] Values versus Fc0/+ couple. Conditions: The working electrode was a glassy carbon electrode, the counter electrode was a platinum wire, and Ag/Ag⁺ was a reference electrode. [b] Obtained from the offset in the absorption spectra. [c] Obtained from eq. 1. [d] Irreversible

Transient absorption spectra and DFT calculations supported the feasible photo-induced electron transfer accompanied by rapid charge recombination in **4**. First, we conducted a conventional transient absorption spectroscopy for nanosecond time-resolved absorption measurements on **4** in PBS. However, no compound-derived signals were observed beyond the instrument's lower time limit of approximately 30 ns (Figure S6).

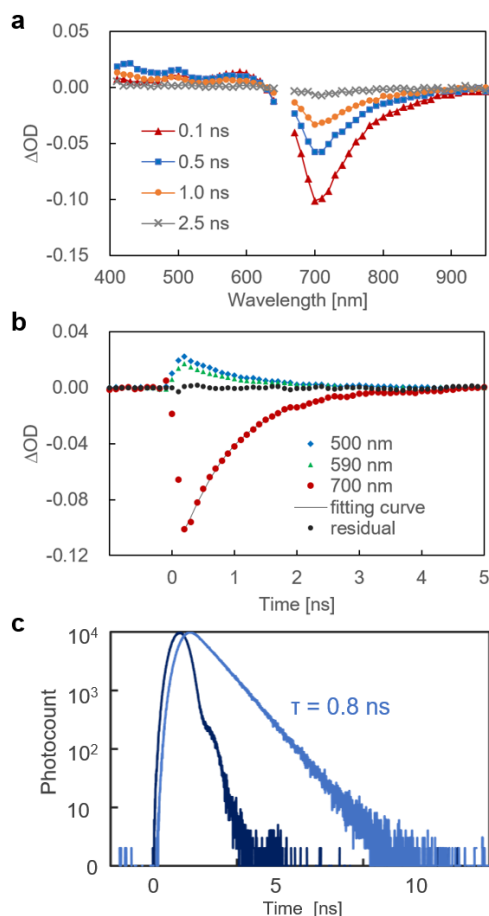


Figure 2. (a) Transient absorption spectra of **4** (WL_{ex} : 660 nm), (b) decay profiles at various wavelengths with (black line) a single exponential fitting curve and the residual signals (black dots), and (c) time-resolved fluorescence decay of **4** (WL_{ex} : 630 nm, WL_{em} : 725 nm) in PBS, and the instrumental response function (IRF) (dark blue).

To probe faster dynamics, we employed subnanosecond transient absorption (sns-TA) spectral measurements based on the randomly interleaved pulse train (RIPT) method.³³ This technique cancels emission signals, enabling precise observation of transient absorption spectra on the 1-100 ns timescale where emission from organic molecules typically occurs. The sns-TA spectra of **4** exhibited remarkable bleach signals centered at 700 nm with a rate constant of $1.3 \times 10^9 \text{ s}^{-1}$ (τ : 0.8 ns), obtained by single exponential fitting (Figures 2a and 2b). The decays at 500 and 590 nm also demonstrated the same rate constants, indicating their same origin. Correspondingly, time-resolved emission decay measurements showed a single exponential decay with a lifetime of 0.8 ns (Figure 2c), indicating that the observed decay in sns-TA spectra is attributable to the first singlet excited (S_1) state of **4**.

Remarkably, no detectable signals from other photoexcited states were observed in the sns-TA measurements. This absence of signals suggested that the primary non-radiative process in **4** does not involve the formation of triplet-excited states or long-lived charge-separated states, which typically have lifetimes exceeding several nanoseconds.¹² The remaining possible non-radiative processes may include various molecular motions that can dissipate the excited state energy as heat, such as rotational motion, conformational changes, and intramolecular movements.³⁹ Additionally, recombination from a short-lived photo-induced electron-transferred state may contribute to the non-radiative decay, although further experimental evidence would be needed to confirm this mechanism. These are preferable for efficiently and stably converting light energy to heat.

Our theoretical calculations support this hypothesis. DFT calculations with a solvent effect (using the polarizable continuum model with water) were employed to predict the electronic properties of **4**. Here, we found that the commonly used B3LYP functional often overestimated the HOMO-LUMO gaps and electron transition energies for sulfone-containing compounds (Table S2). This suggests that this functional may not be suitable for calculating electron transitions involving the unique orbitals of sulfur atoms in aromatic sulfones. As an alternative, we employed the LSDA functional because it more accurately reproduces the experimentally observed electron transition, which corresponds to the absorption maxima shown in Table 1, and the HOMO-LUMO gap (Figures 1b, S2, and S3).

To elucidate the excited states and the photo-induced transition pathway, the excitation energies and natural transition orbitals (NTOs) were analyzed (Figs. S2b and S2c). NTOs, known to describe compact frontier orbitals,³² can represent one-electron properties associated with the electronic transition.⁴⁰ The calculated energy levels of the photoexcited states suggest two types of charge-separated states (CSS) as meta-stable states, in which the mesityl moiety acts as an electron donor and the xanthene moiety as an acceptor (Figure S2). The formation of these CSS is energetically favorable via the S_1 state. The proximity of the donor and acceptor sites likely results in rapid recombination, consistent with the sns-TA measurements, which show no longer time components than the S_1 state.

Our experimental and theoretical results supported the idea that recombination from the photo-induced short-lived CSS state is the primary activation process that efficiently generates heat. These findings provide a strong rationale for exploring compounds **3** and **4** as photothermal reagents capable of efficiently converting light energy into heat.

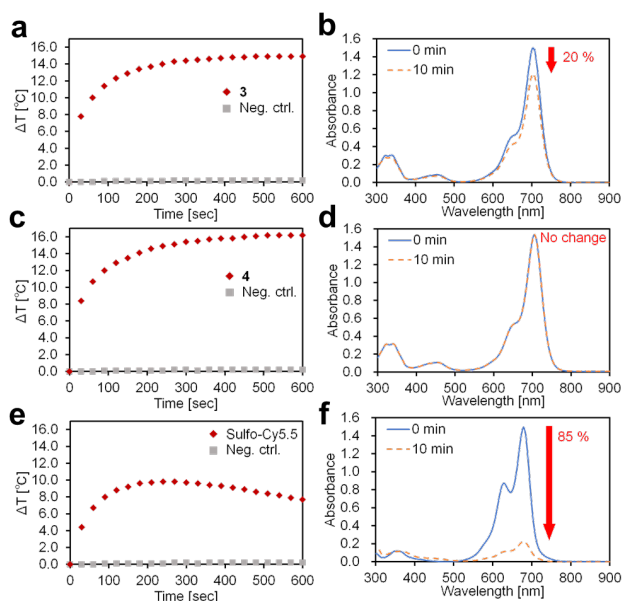


Figure 3. The temperature rises of the solutions containing 128 μM of (a) **3** and (c) **4** in PBS irradiated by a xenon lamp ($700\text{ nm} \pm 25\text{ nm}$, $290\text{ mW} / 0.8\text{ cm}^2\text{-spot}$), and absorption spectra of (b) **3** and (d) **4** before and after the irradiation. (e) The temperature rise and (f) absorption spectra of Sulfo-Cy5.5 of the solution of 158 μM Sulfo-Cy5.5, whose absorbance at 700 nm is identical to those of 128 μM **3** and **4**, in PBS irradiated by the xenon lamp. The red arrows in Figures 3b and 3f indicate the absorbance decreases after the irradiation.

3.3. Photothermal effects and photostability

Under NIR-light illumination, **3** and **4** demonstrated remarkable photothermal effects, increasing the solution temperature by $14.9\text{ }^\circ\text{C}$ and $16.2\text{ }^\circ\text{C}$ after 10 min, respectively (Figures 3a and 3c). Notably, there was a 20% decrease in absorbance of **3** after the irradiation, while **4** showed no change (Figures 3b and 3d). This difference in stability can be attributed to the structural difference, with a possible mechanism for the decrease in **3** being decomposition triggered by nucleophilic attack onto the ninth position of the xanthene moiety (Figure S7).⁴¹ In stark contrast to **4**, sulfo-Cy5.5, a reference compound with a cyanine skeleton often used in photothermal therapeutic studies³⁷, showed significant fading by 85 % and a smaller rise of the temperature ($7.7\text{ }^\circ\text{C}$) compared to **3** and **4** after irradiation (Figures 3e and 3f). These results indicate that **4** exhibits both a high photothermal effect and excellent photostability, making it the most promising candidate for a NIR light-absorbing photothermal reagent.

To further evaluate the photo-durability of **4**, we conducted repeated illumination and stationary periods (Figures 4a and 4b). **4** demonstrated the ability to increase the solution temperature repeatedly, with unchanged absorption spectra, thus verifying its high photo-durability. Furthermore, the photothermal effects

were found to be almost linearly controllable by the concentration of the compound and illuminated light intensity (Figures 4c and 4d). Using heating-cooling process analysis, the photothermal conversion efficiency (η) of **4** was determined to be 58.0% (Figure S8). This value is comparable to typical photothermal conversion reagents such as inorganic materials and semiconductor polymers reported previously (43–62.5%).^{42–45}

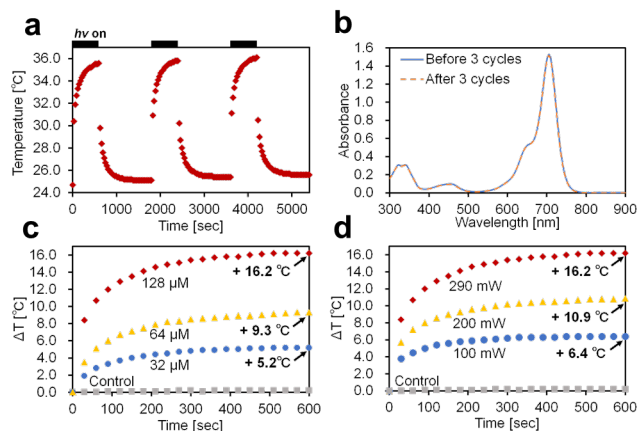


Figure 4. (a) Photothermal durability study of **4** in PBS during three cycles of heating-cooling processes, and (b) the absorption spectra before and after the heating-cooling processes. (c) Concentration-dependent temperature rise and (d) light intensity-dependent temperature rise of 128 μM of **4**.

3.4. Cancer-killing effect in various cells

To apply **4** as a photothermal therapeutic reagent, we initially investigated its stability in the presence of several biological compounds to predict if **4** would reasonably function in biological conditions. L-cysteine, reduced glutathione, and H_2O_2 were selected as representative nucleophiles and a reactive oxygen specie. The spectral changes in absorption and fluorescence of **4** ($10.0\text{ }\mu\text{M}$) were recorded with these compounds in water ($100\text{ }\mu\text{M}$), respectively (Figure S9). No remarkable decrease was observed, indicating that decomposition of **4** was not likely to occur with these biological compounds.

Then, the localization of **4** in HeLa cells was studied. Fluorescence images were obtained using confocal laser microscopic observations on HeLa cells treated with **4** and organelle-staining dyes (Figure 5); LysoTracker Green DND-26 (lysosomes), MitoTracker Green FM (mitochondria), ERSeeing (endoplasmic reticulum), or ECGreen (endosomes). A notable advantage of **4** is its sufficient fluorescence in the NIR region ($\lambda > 700\text{ nm}$), enabling recognition of the compound's location. The Pearson's correlation coefficient, which quantifies the feasibility of colocalization, was highest for **4** when co-stained with LysoTracker Green DND-26 (0.85), followed by ERSeeing (0.64), ECGreen (0.59), and MitoTracker

1 Green FM (0.54). This trend aligns with reported
2 observations using other sulfone-rosamine dyes,²⁴
3 suggesting that the compounds, including ours, are
4 mainly localized in the lysosome.

5 Our experiments with dosing at 4°C, which showed
6 a similar result to that at 37°C, indicated that the
7 accumulation occurs through direct cell membrane
8 penetration (Figure S10), rather than typical energy-
9 dependent pathways such as clathrin-mediated or
10 caveolae-mediated endocytoses^{46,47}. This behavior may
11 be attributed to the small molecular size of **4**. In
12 contrast, the larger dye molecule, sulfo-Cy5.5, mostly
13 demonstrated localization in endosomes and the outer
14 cell membrane or formed aggregates (Figure S11).

15 Regarding the intracellular distribution of **4**, it is
16 worth noting that a sulfone unit with an aromatic ring
17 structure has often been reported as a lysosome-
18 targeting reagent⁴⁸, while aminoxanthenes such as
19 rhodamine dyes are well-known mitochondria-targeting
20 compounds⁴⁹. This dual-targeting nature of **4** allows it
21 to localize predominantly to lysosomes and, to some
22 extent, other organelles, potentially enhancing its
23 efficacy in causing cytotoxicity⁵⁰.

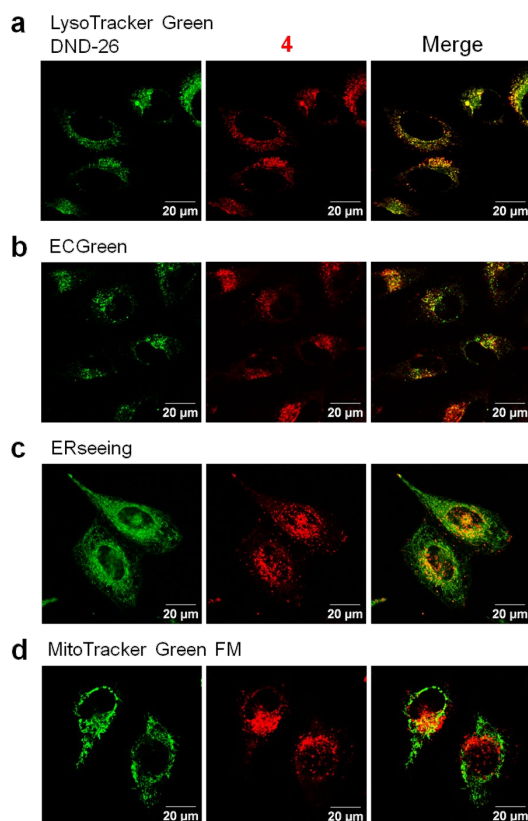


Figure 5. Confocal laser microscope images of HeLa cells stained with (green) organelle-specific dyes and (red) **4**, and their merged images. (a) LysoTracker Green for lysosomes, (b) ECGreen for endosomes, (c) ERseeing for endoplasmic reticulum, and (d) MitoTracker Green for mitochondria.

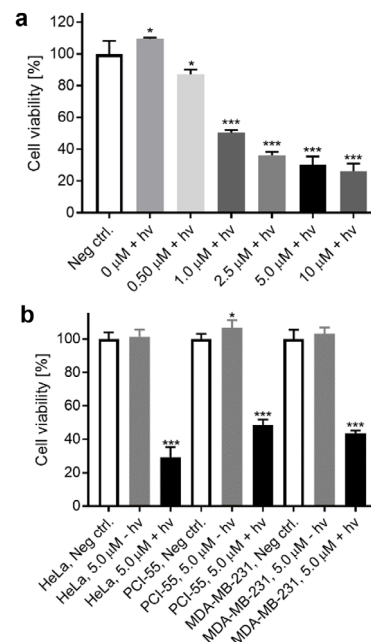


Figure 6. MTT assays to determine the cancer-killing ability of **4** (a) on HeLa cells with the NIR-illumination (700 nm ± 25 nm, 290 mW / 0.8 cm²-spot), and (b) comparison of the cancer-killing abilities of **4** among HeLa, PCI-55, and MDA-MB-231 cells. Statistically significant differences between the negative control and each condition are illustrated with asterisks (*P < 0.05, **P < 0.01, ***P < 0.001).

Photothermal-induced cancer cell death was then examined on HeLa cells (Figure 6a). Significant cell death was observed starting at 0.5 μM of **4**, while no dark toxicity was detected below 20 μM (Figures S12 and S13), despite mild light irradiation enhancing MTT signaling in the absence of **4**, presumably *via* mitochondrial activation⁶. At a concentration of 5.0 μM, **4** caused significant cell death of 70 % due to the photothermal effect. The median lethal dose (LD₅₀) was calculated to be 0.9 μM, which is comparable to or better than previously reported molecules (Table S3)⁵¹⁻⁵³.

The anticancer efficacy was also investigated using PCI-55 and MDA-MB-231 cells. These cells were chosen because they have been the subject of much attention and research for the following reasons: (i) pancreatic cancer cells, such as PCI-55, have a high mortality rate²⁹, and (ii) highly malignant cancer cells, such as MDA-MB-231, often reduce the complete cure rate of cancer. Figure 6b illustrates the photo-induced cancer-killing abilities of **4** against HeLa, PCI-55, and MDA-MB-231 cells. **4** demonstrated a significant photothermal cancer-killing effect on PCI-55 and MDA-MB-231 cells, highlighting its versatility for cancer phototherapy.

4. Conclusion

In conclusion, this study presents a universal strategy for designing and developing sulfone-containing aromatic compounds as phototherapeutic reagents. The DFT calculations predicted and supported the molecular design for efficient NIR-light harvesting using the unique orbitals of aromatic sulfones. These principles can be applied to the universal molecular design of NIR-light harvesting molecules.

The fundamental photophysical properties of **4** were elucidated through a combination of time-resolved spectroscopic techniques and theoretical calculations, revealing its efficient NIR absorption and rapid non-radiative relaxation mechanisms. The enhanced NIR absorption is crucial for its effectiveness as a NIR-activated photothermal agent. Furthermore, **4** exhibited a high photothermal conversion efficiency of 58.0%. The small molecular size of **4** facilitates cellular uptake, contributing to its efficacy in causing photocytotoxicity in various cancer cell lines. Therefore, sulfone-containing aromatic compounds show great potential as photostable small molecules for NIR light harvesting and phototherapy. Their unique combination of efficient NIR absorption, high photothermal conversion, and favorable cellular properties opens new avenues for targeted cancer therapy and other light-activated treatments.

Acknowledgement

We thank Dr. T. Nakagawa and Dr. T. Kawabata (Unisoku Co.) for their technical support on the transient absorption measurements.

Supplementary data

Supplementary material is available at *Bulletin of the Chemical Society of Japan*.

Funding

This work was supported by JSPS KAKENHI (Grant Numbers 21H01753 and 21K19036 to Y.T., JP22H04926 for ABiS), JSPS JPJSBP11202323 to Y.T., JST SPRING (JPMJSP2119 to K.Y.), the JSPS Core-to-Core Program for Advanced Research Network, and the JSPS Cooperative Research Program of "NJRC Mater. & Dev."

Conflict of interest statement. None declared.

References

- (1) Li, L.; Han, X.; Wang, M.; Li, C.; Jia, T.; Zhao, X. Recent Advances in the Development of Near-Infrared Organic Photothermal Agents. *Chem. Eng. J.* **2021**, *417*(January), 128844. <https://doi.org/10.1016/j.cej.2021.128844>.
- (2) Li, J.; Pu, K. Semiconducting Polymer Nanomaterials as Near-Infrared Photoactivatable Protherapeutics for Cancer. *Acc. Chem. Res.* **2020**, *53* (4), 752–762. <https://doi.org/10.1021/acs.accounts.9b00569>.
- (3) Kennedy, J. C.; Pottier, R. H. New Trends in Photobiology. *J. Photochem. Photobiol. B Biol.* **1992**, *14*

- (4), 275–292. [https://doi.org/10.1016/1011-1344\(92\)85108-7](https://doi.org/10.1016/1011-1344(92)85108-7).
- (4) Walski, T.; Dąbrowska, K.; Drohomirecka, A.; Jedruchiewicz, N.; Trochanowska-Pauk, N.; Witkiewicz, W.; Komorowska, M. The Effect of Red-to-near-Infrared (R/NIR) Irradiation on Inflammatory Processes. *Int. J. Radiat. Biol.* **2019**, *95* (9), 1326–1336. <https://doi.org/10.1080/09553002.2019.1625464>.
- (5) Yang, S.; Wu, G.; Li, N.; Wang, M.; Wu, P.; He, Y.; Zhou, W.; Xiao, H.; Tan, X.; Tang, L.; Yang, Q. A c Synergistic Photothermal/Photodynamic/Immune Therapy. *J. Nanobiotechnology* **2022**, *20* (1), 475. <https://doi.org/10.1186/s12951-022-01679-0>.
- (6) Nguyen, L. M. D.; Malamo, A. G.; Larkin-Kaiser, K. A.; Borsa, P. A.; Adihetty, P. J. Effect of Near-Infrared Light Exposure on Mitochondrial Signaling in C2C12 Muscle Cells. *Mitochondrion* **2014**, *14* (1), 42–48. <https://doi.org/10.1016/j.mito.2013.11.001>.
- (7) Wang, L.; Du, W.; Hu, Z.; Uvdal, K.; Li, L.; Huang, W. Hybrid Rhodamine Fluorophores in the Visible/NIR Region for Biological Imaging. *Angew. Chemie Int. Ed.* **2019**, *58* (40), 14026–14043. <https://doi.org/10.1002/anie.201901061>.
- (8) Ma, Z.; Wang, F.; Wang, W.; Zhong, Y.; Dai, H. Deep Learning for in Vivo Near-Infrared Imaging. *Proc. Natl. Acad. Sci.* **2021**, *118* (1), 1–8. <https://doi.org/10.1073/pnas.2021446118>.
- (9) Gil, H. M.; Price, T. W.; Chelani, K.; Bouillard, J.-S. G.; Calamini, S. D. J.; Stasiuk, G. J. NIR-Quantum Dots in Biomedical Imaging and Their Future. *iScience* **2021**, *24* (3), 102189. <https://doi.org/10.1016/j.isci.2021.102189>.
- (10) Lang, K.; Mosinger, J.; Wagnerová, D. M. Photophysical Properties of Porphyrinoid Sensitizers Non-Covalently Bound to Host Molecules; Models for Photodynamic Therapy. *Coord. Chem. Rev.* **2004**, *248* (3–4), 321–350. <https://doi.org/10.1016/j.ccr.2004.02.004>.
- (11) Escobedo, J. O.; Rusin, O.; Lim, S.; Strongin, R. M. NIR Dyes for Bioimaging Applications. *Curr. Opin. Chem. Biol.* **2010**, *14* (1), 64–70. <https://doi.org/10.1016/j.cbpa.2009.10.022>.
- (12) Takano, Y.; Miyake, K.; Sobhanan, J.; Biju, V.; Tkachenko, N. V.; Imahori, H. Near-Infrared Light Control of Membrane Potential by an Electron Donor–Acceptor Linked Molecule. *Chem. Commun.* **2020**, *56* (83), 12562–12565. <https://doi.org/10.1039/D0CC05326K>.
- (13) Satrialdi; Munechika, R.; Biju, V.; Takano, Y.; Harashima, H.; Yamada, Y. The Optimization of Cancer Photodynamic Therapy by Utilization of a Pi-Extended Porphyrin-Type Photosensitizer in Combination with MITO-Porter. *Chem. Commun.* **2020**, *56*(7), 1145–1148. <https://doi.org/10.1039/C9CC08563G>.
- (14) Karton-Lifshin, N.; Albertazzi, L.; Bendikov, M.; Baran, P. S.; Shabat, D. "Donor–Two-Acceptor" Dye Design: A Distinct Gateway to NIR Fluorescence. *J. Am. Chem. Soc.* **2012**, *134* (50), 20412–20420. <https://doi.org/10.1021/ja308124q>.
- (15) Tang, W.; Dai, Y.; Gu, B.; Liu, M.; Yi, Z.; Li, Z.; Zhang, Z.; He, H.; Zeng, R. A near Infrared Fluorescent Probe Based on ICT for Monitoring Mitophagy in Living Cells. *Analyst* **2020**, *145* (4), 1427–1432. <https://doi.org/10.1039/C9AN02053E>.
- (16) Hososhima, S.; Yuasa, H.; Ishizuka, T.; Hoque, M. R.; Yamashita, T.; Yamanaka, A.; Sugano, E.; Tomita, H.; Yawo, H. Near-Infrared (NIR) up-Conversion

- Optogenetics. *Sci. Rep.* **2015**, *5*, 1–10. <https://doi.org/10.1038/srep16533>.
- (17) Yanai, N.; Kimizuka, N. New Triplet Sensitization Routes for Photon Upconversion: Thermally Activated Delayed Fluorescence Molecules, Inorganic Nanocrystals, and Singlet-to-Triplet Absorption. *Acc. Chem. Res.* **2017**, *50* (10), 2487–2495. <https://doi.org/10.1021/acs.accounts.7b00235>.
- (18) Takano, Y.; Numata, T.; Fujishima, K.; Miyake, K.; Nakao, K.; Grove, W. D.; Inoue, R.; Kengaku, M.; Sakaki, S.; Mori, Y.; Murakami, T.; Imahori, H. Optical Control of Neuronal Firing via Photoinduced Electron Transfer in Donor–Acceptor Conjugates. *Chem. Sci.* **2016**, *7* (5), 3331–3337. <https://doi.org/10.1039/C5SC04135J>.
- (19) Wang, S.; Shi, H.; Wang, L.; Lored, A.; Bachilo, S. M.; Wu, W.; Tian, Z.; Chen, Y.; Weisman, R. B.; Zhang, X.; Cheng, Z.; Xiao, H. Photostable Small-Molecule NIR-II Fluorescent Scaffolds That Cross the Blood–Brain Barrier for Noninvasive Brain Imaging. *J. Am. Chem. Soc.* **2022**, *144* (51), 23668–23676. <https://doi.org/10.1021/jacs.2c11223>.
- (20) Weinstein, R.; Slanina, T.; Kand, D.; Klán, P. Visible-to-NIR-Light Activated Release: From Small Molecules to Nanomaterials. *Chem. Rev.* **2020**, *120* (24), 13135–13272. <https://doi.org/10.1021/acs.chemrev.0c00663>.
- (21) Grimm, J. B.; Tkachuk, A. N.; Xie, L.; Choi, H.; Mohar, B.; Falco, N.; Schaefer, K.; Patel, R.; Zheng, Q.; Liu, Z.; Lippincott-Schwartz, J.; Brown, T. A.; Lavis, L. D. A General Method to Optimize and Functionalize Red-Shifted Rhodamine Dyes. *Nat. Methods* **2020**, *17* (8), 815–821. <https://doi.org/10.1038/s41592-020-0909-6>.
- (22) Lee, J. H.; Kanwar, B.; Lee, C. J.; Sergi, C.; Coleman, M. D. Dapsone Is an Anticatalysis for Alzheimer's Disease Exacerbation. *iScience* **2022**, *25* (5), 104274. <https://doi.org/10.1016/j.isci.2022.104274>.
- (23) Apaydin, S.; Török, M. Sulfonamide Derivatives as Multi-Target Agents for Complex Diseases. *Bioorg. Med. Chem. Lett.* **2019**, *29* (16), 2042–2050. <https://doi.org/10.1016/j.bmcl.2019.06.041>.
- (24) Liu, J.; Sun, Y. Q.; Zhang, H.; Shi, H.; Shi, Y.; Guo, W. Sulfone-Rhodamines: A New Class of near-Infrared Fluorescent Dyes for Bioimaging. *ACS Appl. Mater. Interfaces* **2016**, *8* (35), 22953–22962. <https://doi.org/10.1021/acsami.6b08338>.
- (25) Raliski, B. K.; Mun, D. M.; Miller, E. W. Sulfone Rhodamines for Voltage Imaging. *Chem. – An Asian J.* **2022**, *17* (24), <https://doi.org/10.1002/asia.202200906>.
- (26) Si, H.; Wang, D.; Du, X.; Zhou, X. Synthesis of Photostable Near-Infrared Sulfone-Rhodamines for Photoacoustic Imaging-Guided Photothermal Therapy. *Chinese Chem. Lett.* **2024**, *35* (3), 108595. <https://doi.org/10.1016/j.ccl.2023.108595>.
- (27) Fan, J.; Fu, B. M. Quantification of Malignant Breast Cancer Cell MDA-MB-231 Transmigration Across Brain and Lung Microvascular Endothelium. *Ann. Biomed. Eng.* **2016**, *44* (7), 2189–2201. <https://doi.org/10.1007/s10439-015-1517-y>.
- (28) Kishimoto, T.; Ishikura, H.; Kimura, C.; Takahashi, T.; Kato, H.; Yoshiki, T. Phenotypes Correlating to Metastatic Properties of Pancreas Adenocarcinoma in Vivo: The Importance of Surface Sialyl Lewis X Antigen. *Int. J. Cancer* **1996**, *69* (4), 290–294. [https://doi.org/10.1002/\(SICI\)1097-0215\(19960822\)69:4<290::AID-IJC9>3.0.CO;2-S](https://doi.org/10.1002/(SICI)1097-0215(19960822)69:4<290::AID-IJC9>3.0.CO;2-S).
- (29) Hidalgo, M. Pancreatic Cancer. *N. Engl. J. Med.* **2010**, *362* (17), 1605–1617. <https://doi.org/10.1056/NEJMra0901557>.
- (30) M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V., D. J. F. *Gaussian 16, Revision C.07*; Gaussian, Inc., 2016: Wallingford CT.
- (31) Petersson, G. A.; Bennett, A.; Tensfeldt, T. G.; Al-Laham, M. A.; Shirley, W. A.; Mantzaris, J. A Complete Basis Set Model Chemistry. I. The Total Energies of Closed-shell Atoms and Hydrides of the First-row Elements. *J. Chem. Phys.* **1988**, *89* (4), 2193–2218. <https://doi.org/10.1063/1.455064>.
- (32) Martin, R. L. Natural Transition Orbitals. *J. Chem. Phys.* **2003**, *118* (11), 4775–4777. <https://doi.org/10.1063/1.1558471>.
- (33) Nakagawa, T.; Okamoto, K.; Hanada, H.; Katoh, R. Probing with Randomly Interleaved Pulse Train Bridges the Gap between Ultrafast Pump-Probe and Nanosecond Flash Photolysis. *Opt. Lett.* **2016**, *41* (7), 1498. <https://doi.org/10.1364/OL.41.001498>.
- (34) Fukazawa, A.; Oshima, H.; Shimizu, S.; Kobayashi, N.; Yamaguchi, S. Dearomatization-Induced Transannular Cyclization: Synthesis of Electron-Accepting Thiophene-S, S'-Dioxide-Fused Biphenylene. *J. Am. Chem. Soc.* **2014**, *136* (24), 8738–8745. <https://doi.org/10.1021/ja503499n>.
- (35) Yao, S.; Chen, Y.; Ding, W.; Xu, F.; Liu, Z.; Li, Y.; Wu, Y.; Li, S.; He, W.; Guo, Z. Single-Atom Engineering of Hemicyanine and Its Amphiphilic Derivative for Optimized near Infrared Phototheranostics. *Chem. Sci.* **2022**, *14* (5), 1234–1243. <https://doi.org/10.1039/d2sc05982g>.
- (36) Tsudaka, T.; Kotani, H.; Ohkubo, K.; Nakagawa, T.; Tkachenko, N. V.; Lemmetyinen, H.; Fukuzumi, S. Photoinduced Electron Transfer in 9-Substituted 10-Methylacridinium Ions. *Chem. – A Eur. J.* **2017**, *23* (6), 1306–1317. <https://doi.org/10.1002/chem.201604527>.
- (37) Lu, Z.; Huang, F. Y.; Cao, R.; Tan, G. H.; Yi, G.; He, N.; Xu, L.; Zhang, L. Intrinsic, Cancer Cell-Selective Toxicity of Organic Photothermal Nanoagent: A Simple Formulation for Combined Photothermal Chemotherapy of Cancer. *ACS Appl. Mater. Interfaces* **2018**, *10* (31), 26028–26038. <https://doi.org/10.1021/acsami.8b07801>.
- (38) Imahori, H.; Tamaki, K.; Guldí, D. M.; Luo, C.; Fujitsuka, M.; Ito, O.; Sakata, Y.; Fukuzumi, S. Modulating Charge Separation and Charge Recombination Dynamics in Porphyrin-Fullerene Linked Dyads and Triads: Marcus-Normal versus Inverted Region. *J. Am. Chem. Soc.* **2001**, *123* (11), 2607–2617. <https://doi.org/10.1021/ja003346i>.
- (39) Lv, X.; Gao, C.; Han, T.; Shi, H.; Guo, W. Improving the Quantum Yields of Fluorophores by Inhibiting Twisted Intramolecular Charge Transfer Using Electron-Withdrawing Group-Functionalized Piperidine Auxochromes. *Chem. Commun.* **2020**, *56* (5), 715–718. <https://doi.org/10.1039/C9CC09138F>.
- (40) Chen, R.; Tang, Y.; Wan, Y.; Chen, T.; Zheng, C.; Qi, Y.; Cheng, Y.; Huang, W. Promoting Singlet/Triplet Exciton Transformation in Organic Optoelectronic Molecules: Role of Excited State Transition Configuration. *Sci. Rep.* **2017**, *7* (1), 1–11. <https://doi.org/10.1038/s41598-017-05339-4>.

- (41) Vygranenko, K. V.; Poronik, Y. M.; Wrzosek, A.; Szewczyk, A.; Gryko, D. T. Red Emissive Sulfone-Rhodols as Mitochondrial Imaging Agents. *Chem. Commun.* **2021**, 57 (63), 7782–7785. <https://doi.org/10.1039/D1CC02687A>.
- (42) Sasikumar, D.; Takano, Y.; Biju, V. Photoinduced Betaine Generation for Efficient Photothermal Energy Conversion. *Chem. - A Eur. J.* **2020**, 26 (9), 2060–2066. <https://doi.org/10.1002/chem.201905030>.
- (43) Wang, Q.; Dai, Y.; Xu, J.; Cai, J.; Niu, X.; Zhang, L.; Chen, R.; Shen, Q.; Huang, W.; Fan, Q. All - in - One Phototheranostics: Single Laser Triggers NIR - II Fluorescence/Photoacoustic Imaging Guided Photothermal/Photodynamic/Chemo Combination Therapy. *Adv. Funct. Mater.* **2019**, 29 (31), 1901480. <https://doi.org/10.1002/adfm.201901480>.
- (44) Wu, C.; Huang, X.; Tang, Y.; Xiao, W.; Sun, L.; Shao, J.; Dong, X. Pyrrolopyrrole Aza-BODIPY near-Infrared Photosensitizer for Dual-Mode Imaging-Guided Photothermal Cancer Therapy. *Chem. Commun.* **2019**, 55 (6), 790–793. <https://doi.org/10.1039/C8CC07768A>.
- (45) Liang, P.; Tang, Q.; Cai, Y.; Liu, G.; Si, W.; Shao, J.; Huang, W.; Zhang, Q.; Dong, X. Self-Quenched Ferrocenyl Diketopyrrolopyrrole Organic Nanoparticles with Amplifying Photothermal Effect for Cancer Therapy. *Chem. Sci.* **2017**, 8 (11), 7457–7463. <https://doi.org/10.1039/c7sc03351f>.
- (46) Biju, V. Chemical Modifications and Bioconjugate Reactions of Nanomaterials for Sensing, Imaging, Drug Delivery and Therapy. *Chem. Soc. Rev.* **2014**, 43 (3), 744–764. <https://doi.org/10.1039/C3CS60273G>.
- (47) Yamada, Y.; Takano, Y.; Satrialdi; Abe, J.; Hibino, M.; Harashima, H. Therapeutic Strategies for Regulating Mitochondrial Oxidative Stress. *Biomolecules* **2020**, 10 (1), 83. <https://doi.org/10.3390/biom10010083>.
- (48) Lin, J.; Yang, K.; New, E. J. Strategies for Organelle Targeting of Fluorescent Probes. *Org. Biomol. Chem.* **2021**, 19 (43), 9339–9357. <https://doi.org/10.1039/d1ob01447a>.
- (49) Zhao, H.; Takano, Y.; Sasikumar, D.; Miyatake, Y.; Biju, V. Excitation - Wavelength - Dependent Functionalities of Temporally Controlled Sensing and Generation of Singlet Oxygen by a Photoexcited State Engineered Rhodamine 6G - Anthracene Conjugate. *Chem. - A Eur. J.* **2022**, 28 (71), e20220201. <https://doi.org/10.1002/chem.202202014>.
- (50) Vyas, S.; Zaganjor, E.; Haigis, M. C. Mitochondria and Cancer. *Cell* **2016**, 166 (3), 555–566. <https://doi.org/10.1016/j.cell.2016.07.002>.
- (51) Wan, G.; Cheng, Y.; Song, J.; Chen, Q.; Chen, B.; Liu, Y.; Ji, S.; Chen, H.; Wang, Y. Nucleus-Targeting near-Infrared Nanoparticles Based on TAT Peptide-Conjugated IR780 for Photo-Chemotherapy of Breast Cancer. *Chem. Eng. J.* **2020**, 380 (May 2019), 122458. <https://doi.org/10.1016/j.cej.2019.122458>.
- (52) Zou, Q.; Abbas, M.; Zhao, L.; Li, S.; Shen, G.; Yan, X. Biological Photothermal Nanodots Based on Self-Assembly of Peptide-Porphyrin Conjugates for Antitumor Therapy. *J. Am. Chem. Soc.* **2017**, 139 (5), 1921–1927. <https://doi.org/10.1021/jacs.6b11382>.
- (53) Bian, W.; Wang, Y.; Pan, Z.; Chen, N.; Li, X.; Wong, W. L.; Liu, X.; He, Y.; Zhang, K.; Lu, Y. J. Review of Functionalized Nanomaterials for Photothermal Therapy of Cancers. *ACS Applied Nano Materials*. American

Chemical Society November 2021, pp 11353–11385.
<https://doi.org/10.1021/acsnm.1c01903>.

