

**Development of Stimuli-Responsive Metal-Phthalocyanine
Derivatives with Controlled Solubility**

Kohei Nogita

2025

**Development of Stimuli-Responsive Metal-Phthalocyanine
Derivatives with Controlled Solubility**

Kohei Nogita

2025

Contents

General Introduction	1
Chapter 1 MMP-2-Activatable Photoacoustic Tumor-Imaging Probes Based on Al- and Si-Naphthalocyanines	20
Chapter 2 Photoacoustic Signal Enhancement of Al- and Si-Phthalocyanines Caused by Photoinduced Cleavage of Water-Soluble Axial Ligand	52
Chapter 3 Reductively Convertible Nickel Phthalocyanine Precursor as a Biological Thiol-Responsive Turn-on Photoacoustic Contrast Agent	90
Chapter 4 A Reductively Activatable Phototheranostic Agent Based on a Water-Soluble Nickel Naphthalocyanine Precursor	116
Chapter 5 Synthesis and Reductive Aromatization of Perfluoro Nickel Phthalocyanine Precursor Having Two Methoxy Groups on Adjacent Pyrrole Rings	154
General Conclusion	183
List of Publications	186
Acknowledgement	189

General Introduction

Porphyryns and phthalocyanines

Organic dyes, which absorb and emit photons, are vital in making Earth's nature vibrant and colorful. These compounds are essential for life on Earth, and their molecular structures and functions have been elucidated through the efforts of chemists. Land plants rely on green chlorophyll to absorb sunlight for photosynthesis (Figure 1a), and mammals use red heme to transport oxygen throughout their body (Figure 1b).¹ Methylene-bridged tetrapyrrole macrocycles are known as porphyrin (Por, Figure 1c), and similar compounds are called porphyrinoids. In the last century, many artificial porphyrinoids have been developed based on organic chemistry and are used in various fields. For example, phthalocyanine (Pc, Figure 1d), an artificial dye with four isoindole units bridged by nitrogen, has long been used as a heat- and light-resistant blue pigment.² Porphyrinoids have been extensively studied due to their remarkable heat and light stability, as well as their unique physical properties. The absorption spectra of porphyrinoids typically feature strong Soret-band transitions ($S_0 \rightarrow S_2$) and weak Q-band transitions ($S_0 \rightarrow S_1$) (Figure 2a).³ These spectral

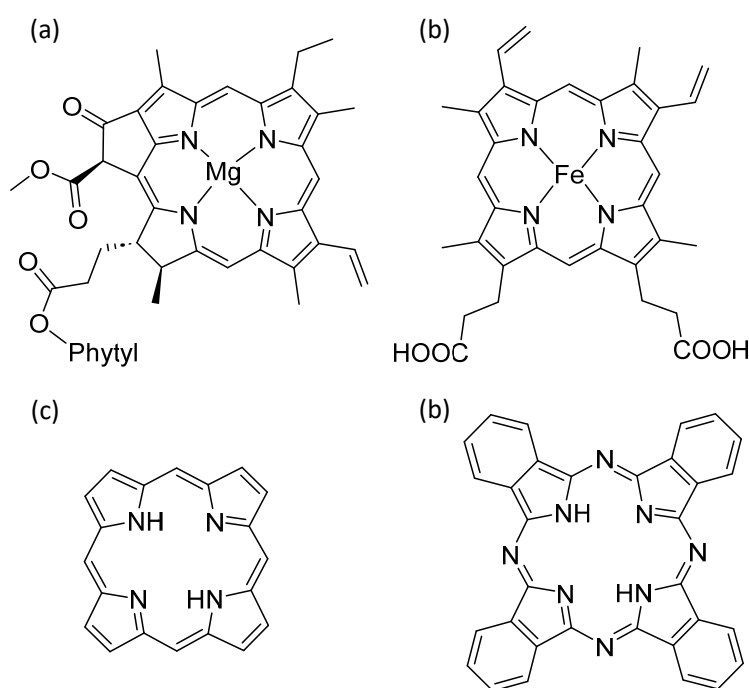


Figure 1. Structures of (a) chlorophyll a, (b) heme B, (c) porphyrin (Por), and (d) phthalocyanine (Pc).

characteristics are explained by the Gouterman four-orbital model, which describes the correlation between absorption properties (wavelengths and intensities) and the HOMO-1, HOMO, LUMO, and LUMO+1 energy levels.⁴ Since the HOMO (a_{1u} orbital) and HOMO-1 (a_{2u} orbital) of Por have similar energy levels, transitions from these orbitals to the degenerated LUMO (e_{gx} , e_{gy}) are mixed by the configurational interaction. As a result, the strong transition dipole moment for the $S_0 \rightarrow S_2$ transition causes intense Soret-band absorption, while the Q-band absorption derived from the $S_0 \rightarrow S_1$ transition is weak. In Pc, where nitrogen atoms replace the *meso*-position carbons, the a_{2u} orbital is stabilized by the high electronegativity of the nitrogen atoms at the *meso*-position, weakening the configurational interaction (Figure 2b). As a result, the Q-band absorption of Pc, which corresponds to the transition from HOMO to LUMO, has a significant molar absorption coefficient. Pc derivatives further extend the π -electron conjugation system via benzene ring fusion, shifting the Q-band absorption into the near-infrared (NIR) region (>650 nm) with a high molar absorption coefficient.

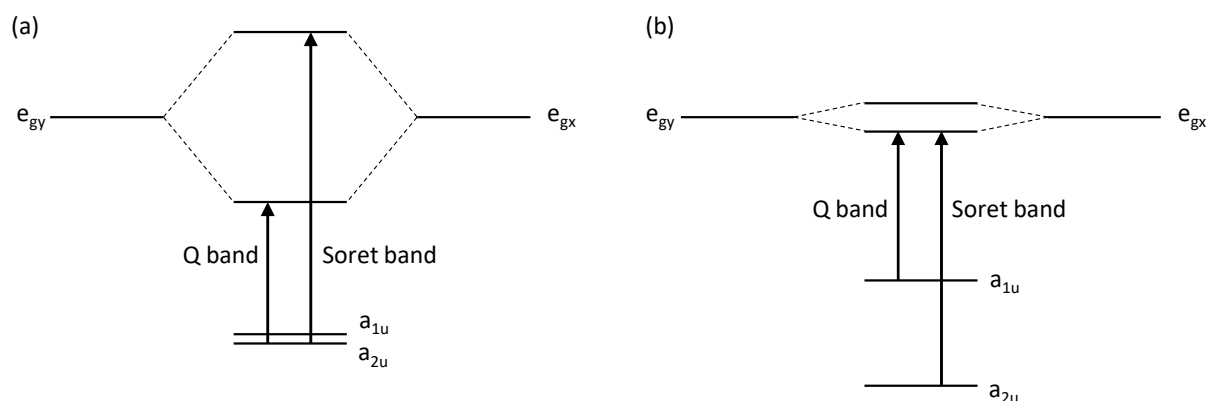


Figure 2. Frontier molecular orbital diagrams of Por and Pc with configuration interactions.

Synthesis and application of phthalocyanine

Since the beginning of the 20th century, when the synthesis of metal-coordinated Pc (MPc) was first reported,⁵ MPc has been industrially produced worldwide, mainly as a blue-colored dye. Several known synthetic methods for MPc rings are used depending on the scale and purpose. In industrial production, inexpensive phthalic acid derivatives, particularly phthalic anhydride, are mainly used to synthesize Pc (Figure 3).⁶ In this process, urea is used as a nitrogen source and ammonium molybdate $((\text{NH}_4)_2\text{MoO}_4)$ is used as a

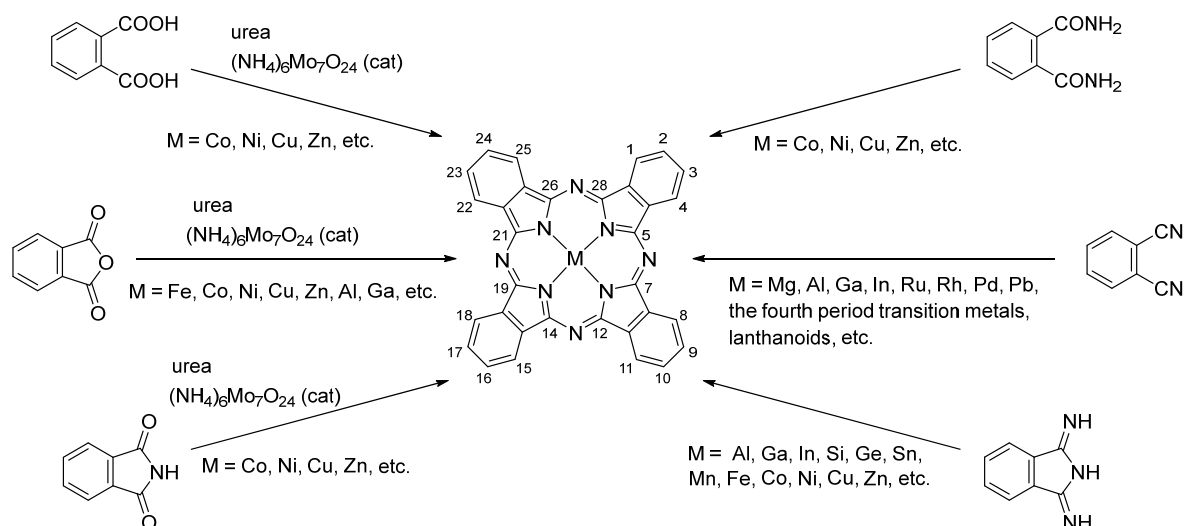


Figure 3. Precursors for MPc synthesis and MPc ring numbering.

catalyst. The key step in this complex cyclization-condensation reaction is thought to be the formation of 1,3-diiminoisoindoline. 1,3-Diiminoisoindoline is used as a substrate for MPc synthesis on a laboratory scale, but the synthesis of derivatives bearing substituents is a formidable task in many cases. Consequently, phthalonitrile and its derivatives are commonly used as precursors for synthesizing MPc compounds on a laboratory scale.

In the past few decades, MPcs have attracted significant attention due to their unique optical and electronic properties derived from the delocalization of π -electrons in the molecular structure.⁷ MPcs are known as one of the organic dyes with high stability against light and heat, which finds broad applications. Hence, wide varieties of MPcs have been intensively investigated for their potential as photosensitizers for photodynamic and photothermal therapy,⁸ organic semiconductors,⁹ and catalysts.¹⁰ The physical properties of MPcs can be tuned by modifying the peripheral benzene ring (α - and β -positions) or altering the central metals, leading to the active development of MPcs with precisely controlled optical and electronic properties (Figure 4).¹¹ Since the low solubility of MPcs hampers their applications, particularly in biological applications, chemical modification with bulky substituents that inhibits π - π stacking is limited to the peripheral benzene rings or axial ligands. Although Pc is known to incorporate a variety of metals as a core metal, MPc with a central metal from the third or fourth period, which have particularly high stability, are mostly common.

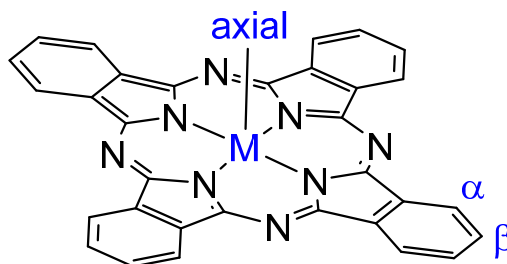


Figure 4. The substitutable positions within the molecular structure of MPc.

Diagnosis and treatment of diseases using organic dyes

In recent years, technologies that use light energy to visualize internal biological processes and treat diseases have garnered increasing attention in next-generation diagnostic and therapeutic areas.¹² The irradiated light energy is absorbed by the endogenous or exogenous dyes inside the body and converted into other forms of energy. Imaging techniques such as fluorescence imaging (FI) and photoacoustic imaging (PAI) can visualize molecular dynamics inside the body and lesion sites by converting the irradiated light energy into emitted light of different wavelengths or heat (Figures 5a, b). In the cancer treatment photodynamic therapy (PDT) and photothermal therapy (PTT) use irradiated light energy to generate reactive oxygen species (ROS) or heat, offering minimally invasive alternatives to conventional surgery, radiotherapy, and chemotherapy (Figures 5c, d). For photoirradiation inside the body, near-infrared (NIR) light (650–1400 nm) within the biological window is used because of its high tissue permeability and minimal absorption by water and hemoglobin (Figure 5e).¹³ Consequently, exogenous dyes need to be designed to efficiently absorb NIR light for diagnosis and therapeutic purposes. Carbon materials such as fullerenes and inorganic nanoparticles capable of NIR light absorption have been explored as contrast agents and photosensitizers.¹⁴ Since photosensitizers based on NIR-absorbing organic dyes can gain various functions through chemical modification, extensive research efforts have been made. To date, many NIR-absorbing dyes with narrow HOMO-LUMO energy gaps ($\Delta E_{\text{HOMO-LUMO}}$) have been developed, but their formidable tasks in synthesis often pose challenges. To make medical advancements using these dyes beneficial and easily accessible to a broader population, it is required to develop dyes that are not only highly functionalized but also cost-effective and simple to synthesize.

Dyes excited by photoirradiation within the body release the absorbed energy through three main pathways (Figure 5f): (1) **emission of photons**, resulting in fluorescence or phosphorescence; (2) **generation of heat**, which converts energy into heat; and (3) **generation of ROS**, which results from electron transfer or energy transfer via intersystem crossing (ISC). The energy release pathways play distinct roles in biomedical applications. Pathways (1) and (2) are used for *in vivo* imaging with FI and PAI, respectively. Pathways (2) and (3) are employed in cancer treatment with PTT leveraging heat generation and PDT relying on ROS production, respectively. These radiative and nonradiative processes are in a trade-off relationship, where the dominant pathway might be determined by the structure design of dyes.¹⁵ Therefore, it is crucial to develop

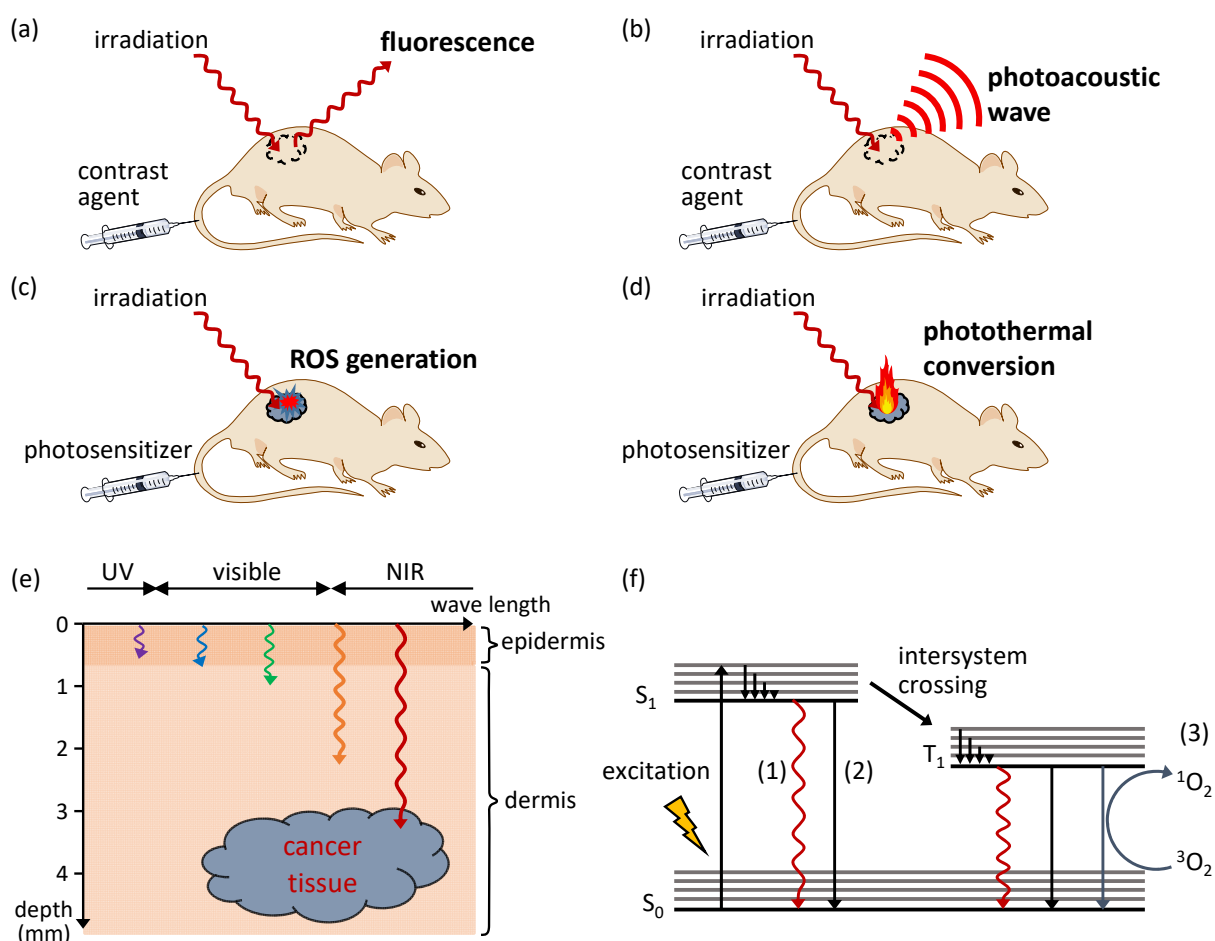


Figure 5. Schematic illustration of (a) fluorescence imaging (FI), (b) photoacoustic imaging (PAI), (c) photodynamic therapy (PDT), (d) photothermal therapy (PTT), and (e) penetration of NIR light through biological tissue. (f) Consumption of excitation energy through (1) radiative process, (2) photothermal conversion, and (3) singlet oxygen generation.

the appropriate chromophore which controls and optimizes their energy release pathways for specific applications.

NIR-based imaging technology for cancer diagnosis

Cancer ranks the leading causes of death worldwide, and the mortality rate increases with metastasis. This underscores the urgent need for advanced techniques to detect cancer at an early stage.¹⁶ Although various techniques are currently developed to diagnose cancer, many imaging methods focus on the observation of morphological information in cancer tissue.¹⁷ However, there is a growing need for the development of the technologies to acquire functional images that provide more detailed insights into tumor behavior. Magnetic resonance imaging (MRI), computed tomography (CT) and positron emission tomography (PET), commonly used in medical screening,¹⁸ come with inherent risks of radiation exposure, making them unsuitable for long-term monitoring (Table 1). In contrast, optical imaging techniques such as FI and PAI can visualize *in vivo* information with high resolution and offer the potential to detect even tiny cancer lesions that may elude conventional methods. Therefore, these minimally invasive diagnostic methods are desired for next-generation cancer screening technology in medicinal area.

In FI, NIR dyes that efficiently convert light energy into long-wavelength fluorescence are utilized as chemical probes. Due to its extremely high sensitivity and spatial resolution, FI shows great promise for real-time imaging during surgical operations, enabling precise visualization of lesion sites.¹⁹ However, FI faces

Table 1. Various imaging modalities for cancer diagnosis.

imaging modalities	spatial resolution	tissue penetration	sensitivity	cost	other disadvantages
MRI	1 mm	whole body	low	high	time-consuming
PET	5 mm	whole body	high	high	radiation risk
CT	1 mm	whole body	low	middle	radiation risk
fluorescence imaging	1-10 μ m	mm	high	low	
photoacoustic imaging	0.1 mm	cm	low	middle	

limitations in visualizing tumor tissues located deeper than several millimeters beneath the epidermis, as fluorescent signals become attenuated in biological tissues.²⁰ In contrast, PAI employs NIR dyes with low fluorescence quantum yields that efficiently convert light energy into heat. PAI can visualize tissues at depths of several centimeters because of the deep tissue penetration of photoacoustic signals.²¹ Nevertheless, there are limited dyes that can generate sufficient intensity of PA signals in response to pulsed laser irradiation for cancer diagnosis. Therefore, the development of dyes with a high molar absorption coefficient in the NIR region and high photothermal conversion efficiency is essential.

Although various organic dye scaffolds have been developed for *in vivo* imaging, some dyes suffer from low photostability, limiting their use under strong light exposure conditions required for PAI.²² When dyes with low photostability are used for long-term imaging, the signal will decay, making it challenged to identify tiny lesion sites and potentially reducing diagnostic accuracy. Additionally, there are concerns that degradation products from unstable dyes may harm the body. Therefore, remarkably photostable dyes are highly desired to ensure accurate imaging and safety in living systems.

Phototherapy and phototheranostics for cancer treatment

The light energy absorbed by dyes accumulating in tumor tissues can also be utilized for cancer treatment. Phototherapies such as PDT and PTT are effective only in the irradiated area. This makes them less invasive than conventional cancer treatments using medicines, being particularly advantageous for treating small cancers.²³ Additionally, these therapies can be repeated because they do not cause a decrease in efficacy due to drug resistance, which is a fatal flaw of conventional anticancer drugs.²⁴ Since phototherapies can be continuously applied to patients whose physical condition has deteriorated to be unable to continue conventional treatments, they are expected to improve the survival rates of elderly cancer patients.

PDT utilizing ROS to damage cells can be classified into two types: type 1, which generates radical species, and type 2, which generates singlet oxygen ($^1\text{O}_2$) (Figure 6).²⁵ Some ROS generated through type 1 PDT have relatively long lifetimes and can oxidize extracellular molecules, potentially causing harm. Therefore, to improve the therapeutic efficacy of PDT, it is necessary to increase the $^1\text{O}_2$ production efficiency by optimizing

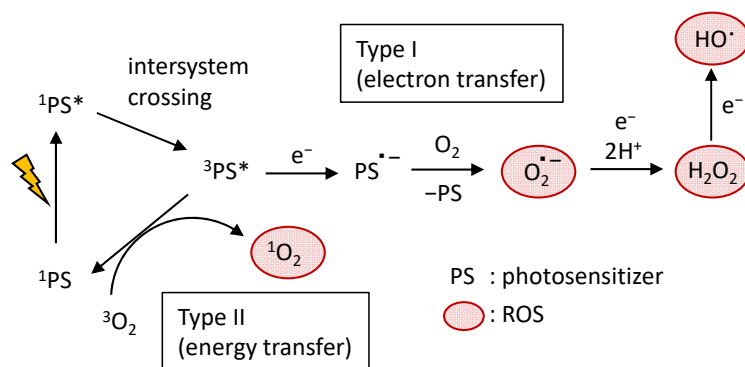


Figure 6. Mechanism of ROS generation.

the energy transfer from the triplet excited state of the photosensitizer to oxygen molecules. However, PDT suffers an inevitable limitation in the hypoxic regions of tumors, where the scarcity of oxygen molecules hampers 1O_2 generation.²⁶ Since photosensitizers in PDT can be activated by weak light, the ROS generation in non-targeted normal tissues may cause serious side effects. For this reason, patients often need to avoid continuous light exposure during treatment.²⁷ Although PTT causes cancer cell damage by elevating the local temperature, its therapeutic effectiveness remains intact even in hypoxic tumor regions.²⁸ However, achieving the necessary local temperature increase to kill cancer cells requires stronger light than that used in PDT. Therefore, it is crucial to design PTT photosensitizers with high photostability to endure prolonged or intense light exposure.

Theranostics (therapy + diagnostics), which uses a single drug for diagnosis and treatment, allows seamless integration of diagnostic findings and therapeutic strategies.²⁹ The development of tumor theranostics strategies is highly demanded and represents one of the most advanced cancer treatments. A dye utilized in both cancer imaging and phototherapy can conduct phototherapy immediately after the cancer detection. This approach significantly enhances therapeutic efficacy while minimizing invasiveness. While PAI/PTT tumor theranostics make significant progress, the discovery and development of materials with excellent photothermal conversion efficiency are highly desired.³⁰

Diagnosis and treatment of cancer using metallophthalocyanine

In recent years, MPcs have attracted attention as promising dyes for PAI contrast agents and photosensitizers for PDT or PTT due to the more suitable optical properties. In order to apply MPcs *in vivo*, it is necessary to improve their solubility in water by introducing water-soluble substituents or loading them into nanoparticles. There have been many reports on the biological applications of water-soluble MPcs with anionic or cationic subunits, MPc-loaded nanoparticles, and MPcs modified with amphiphilic polymers such as polyethylene glycol (PEG) have been widely explored.³¹ The deactivation process of excited MPc in water can also be controlled by chemical modification, including altering the central metal ion. SiPc and ZnPc derivatives as photosensitizers for PDT are intensively studied because they are prone to intersystem crossing to the triplet excited state.³² Conversely, NiPc with an insufficient energy gap between S_0 and T_1 to generate singlet oxygen generally shows high photothermal conversion efficiency, making it promising as a photosensitizer for PAI and PTT.³³

Since the Q-band absorption of MPcs overlaps with that of hemoglobin, it is possible to improve their performance as contrast agents or photosensitizers by a bathochromic shift of the Q-band absorption. Metal naphthalocyanines (MNC) with extended conjugated systems of the isoindole units of MPc absorb approximately 100 nm longer wavelengths of light than MPc.³⁴ MNC can absorb NIR light despite the hypsochromic shift in the absorption spectrum due to aggregation.

Stimuli-responsive dyes as activatable photosensitizers

Only a small amount of the dye accumulates in the lesion site when dyes are administered into the body for diagnosis and treatment.³⁵ This limitation poses challenges when using “always-on” dyes, which act as photosensitizers wherever they are exposed to light. These dyes generate signals in both lesion and regular sites in imaging, making diagnosis inaccurate. Because phototherapy using “always-on” dyes involves a risk of damage to normal cells, carefulness to avoid exposing healthy tissue to excitation light is required. To address these issues, the development of stimuli-responsive photosensitizers to enhance the activity of the photosensitizer at lesion sites in response to specific cancer biomarkers is essential.³⁶

Two main design strategies guide the development of activatable dyes: (1) controlling molecular aggregation and (2) controlling electronic properties of molecules to shift absorption wavelengths. Because many organic dyes with a large π -electron conjugation exhibit hypochromic or bathochromic shifts in their absorption spectra depending on aggregation, fluorescence and $^1\text{O}_2$ generation are quenched, resulting in photothermal conversion efficiency increase.³⁷ Dye aggregation enhancing PA signals at lesion sites makes it possible to visualize cancer cells more clearly or heat cancer tissues effectively. Although these dyes exhibit increased PA signals upon aggregation, they also generate signals before aggregation that act as noise and degrade the image quality. To detect tiny tumor tissues with high resolution in PAI, suppressing signal generation in normal cells and minimizing background noise is necessary.

The optical properties of dyes are closely related to the structure of their π -electron conjugated system. Therefore, modulating the π -electron conjugation length is a powerful method to control their optical properties. When the sp^2 -hybridized carbons in the π -conjugated framework of chromophore are chemically converted into sp^3 -hybridized carbons, the absorption peaks shift to shorter wavelengths and the solubility of a chromophore is improved by π - π stacking suppression.³⁸ The absorption spectra of these dyes change significantly when the π -conjugated structure is reformed, enabling them to be applied as photosensitizers that can work only at the lesions.

Core-modified MPc

Most stimuli-responsive MPcs developed so far rely on aggregation control but fail to suppress their activity before activation. There have been no previous examples of the MPc-based turn-on type contrast agent for PAI and photosensitizer for PTT.

The optical and electronic properties of MPc are closely related to the 18π -electron conjugated system of the Pc core structure. Chemical modifications of sp^2 -hybridized carbon atoms at the α -position of pyrroles can lead to the loss of the 18π -electron aromaticity. For example, in Ti and Nb complexes with two distorted Pc rings sandwiching the central metal, the chemical modification disrupts the 18π -electron conjugated system, leading to the absence of NIR absorption.³⁹ Other than this structure having eight isoindole units surrounding

the central metal, the penta-isindole 'helmet'-type CoPc derivative can be converted to a planar CoPc by heating or electrochemical reduction (Figure 7a).⁴⁰ MPc derivatives with two alkoxy groups on the diagonal pyrroles have been isolated as intermediates during alcohol-mediated synthesis of MPc (M = Ni, Cu) under mild conditions (Figure 7b).⁴¹ The alkoxy groups on the MPc core can be removed through heating or reduction, converting them into planar MPcs. These highly distorted Pc macrocycles with two sp^3 -hybridized carbons in the Pc core structure have significantly improved solubility in organic solvents compared to planar MPcs. For example, Kobayashi and co-workers prepared CuPc thin films by annealing core-alkoxylated CuPc derivatives and applying them to photovoltaic devices. Building on these findings, the author hypothesized that water-soluble core-alkoxylated NiPc derivatives could serve as a turn-on PAI agent to visualize glutathione (GSH)-overexpressed cancer cells. Upon reduction by GSH, the core-alkoxylated NiPc derivatives would be converted to planar NiPc, enabling enhanced PAI through its restored 18π -electron conjugated system.

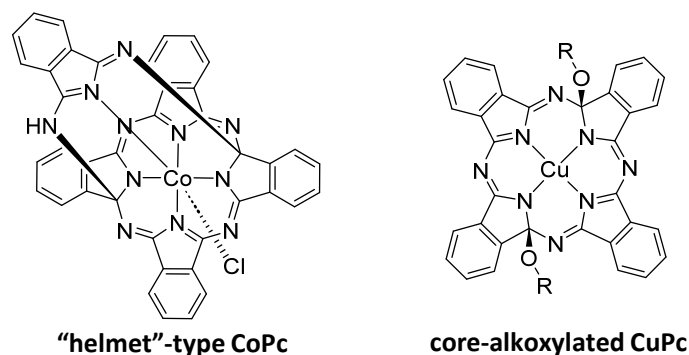


Figure 7. Structures of "'helmet'-type CoPc and core-alkoxylated CuPc.

Overview of this thesis

In this study, the author has developed MPc derivatives designed to undergo structural changes in response to external stimuli, resulting in solubility and absorption spectra changes (Figure 8). Upon stimulus-triggered conversion from highly soluble MPcs to less soluble MPcs, MPc aggregation occurs, enhancing photothermal conversion efficiency and generating strong PA signals. First, the author has clarified that the PA signal intensity of MPcs or metal-coordinated naphthalocyanines (MNcs) with water-soluble axial ligands increases upon cleavage of these ligands (Chapters 1 and 2). Next, the author has developed core-alkoxylated NiPc and NiNc derivatives that are converted to NiPc and NiNc under mild conditions via reductive aromatization,

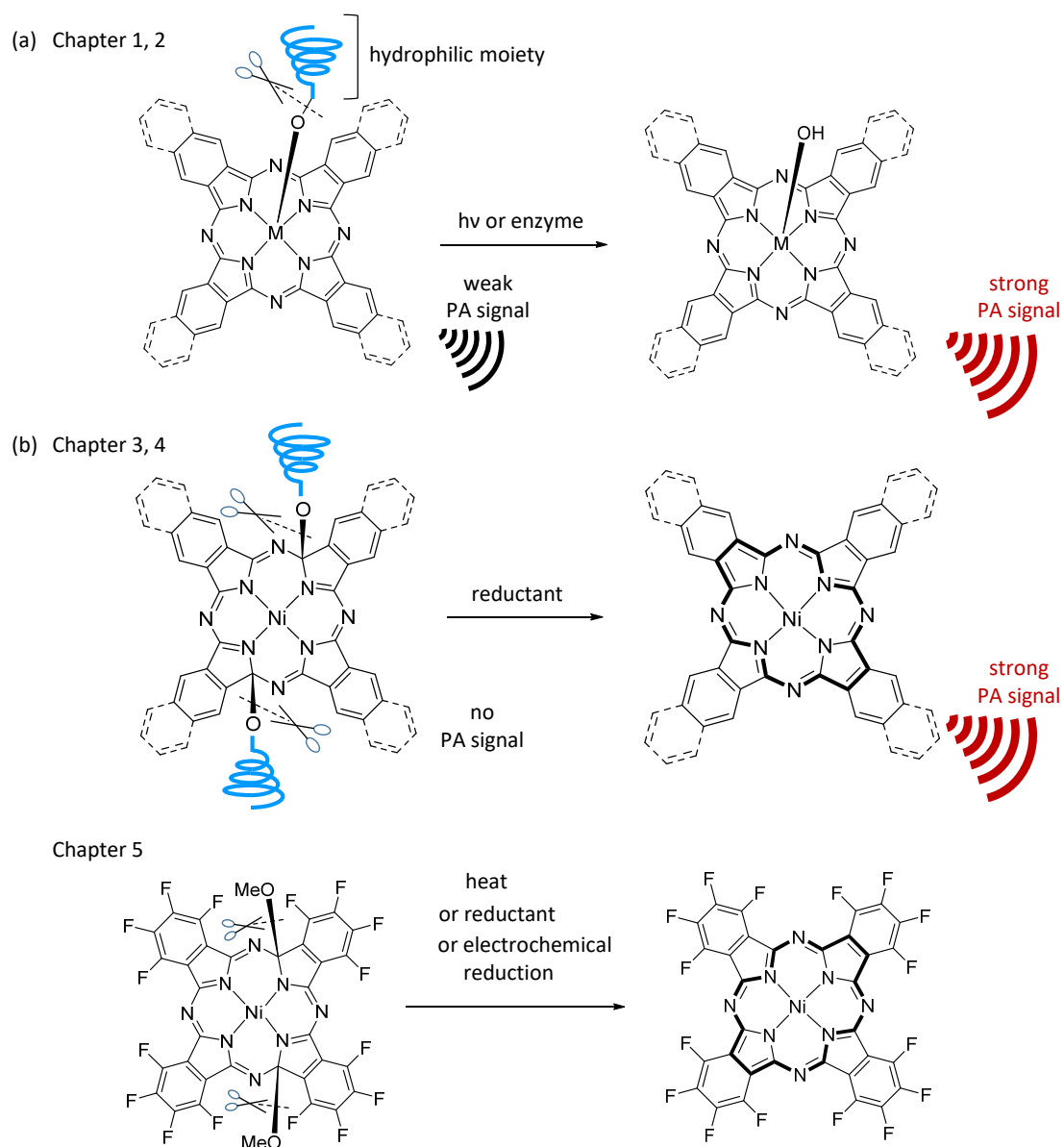


Figure 8. Overview of this thesis.

respectively. Since the PA signal intensity increases in response to reducing agents such as GSH, conversion proceeded rapidly in cancer cells overexpressing GSH (Chapters 3 and 4). Finally, the author synthesized perfluorinated NiPc (NiPcF₁₆) derivatives with alkoxyated core-structures, yielding isomers with different alkoxy group positions (Chapter 5).

In Chapter 1, the author describes the development of a PA contrast agent for tumor imaging using activatable MNcs with water-soluble axial ligands cleaved by an enzyme (Figure 9). Aluminum and silicon naphthalocyanines (AlNc and SiNc) conjugated with matrix metalloprotease-2 (MMP-2)-responsive peptide (PLGLAG) and PEG on axial ligands were designed and synthesized. Cleavage of the peptide chains by MMP-2 led to MNC aggregation, increasing PA signals from the aggregates while decreasing those from monomers. By monitoring the ratio of PA signal intensity, MMP-2-overexpressing tumor cells were visualized both *in vitro* and *in vivo*.

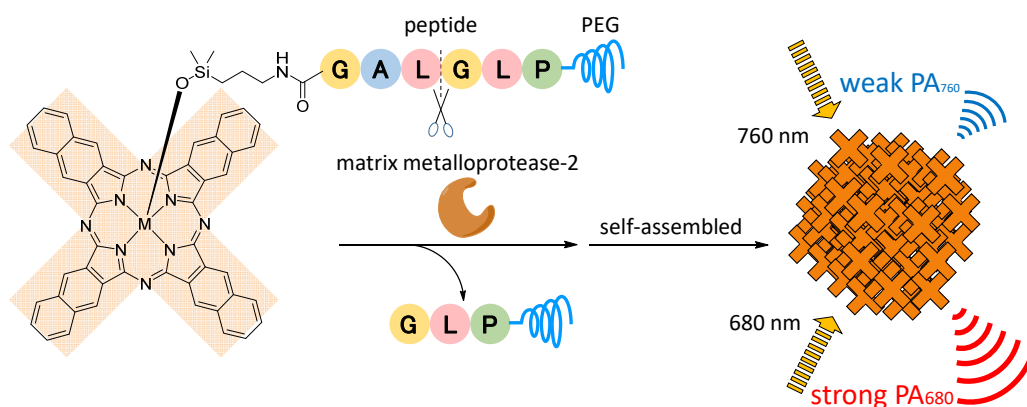


Figure 9. Enzymatic cleavage of the peptide moiety of MNC-pep-PEG.

In Chapter 2, the author describes the development of AlPc and SiPc derivatives with PEGylated axial ligands (AlPc-PEG and SiPc-PEG), which undergo the elimination of axial ligands under photoirradiation (Figure 10). The resulting MPc aggregation enhanced the PA signal intensity. The author proposes reaction mechanism through the formation of radical anions through one-electron transfer between closely located MPc molecules upon photoirradiation.

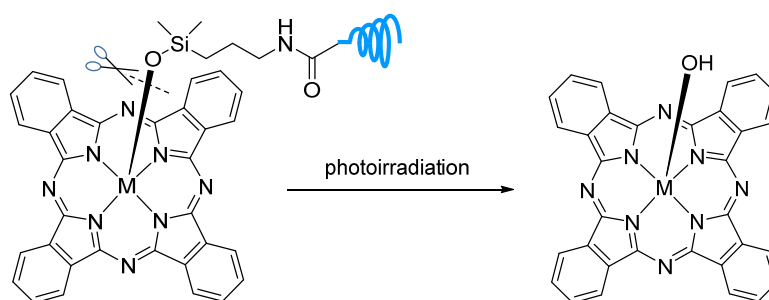


Figure 10. Photoinduced axial ligand cleavage of **MPc-PEG**.

In Chapter 3, PA cancer imaging using core-alkoxylated NiPc is described (Figure 11). The author found that the conversion of core-alkoxylated NiPc derivatives (**ox-NiPcs**) could proceed at room temperature using reductants. An amphiphilic **ox-NiPc** derivative with PEG side chains (**ox-NiPc-PEG**) was converted to NiPc in the presence of GSH in water. In GSH-overexpressing cancer cells, this conversion proceeded more efficiently than in normal cells, significantly increasing PA signal.

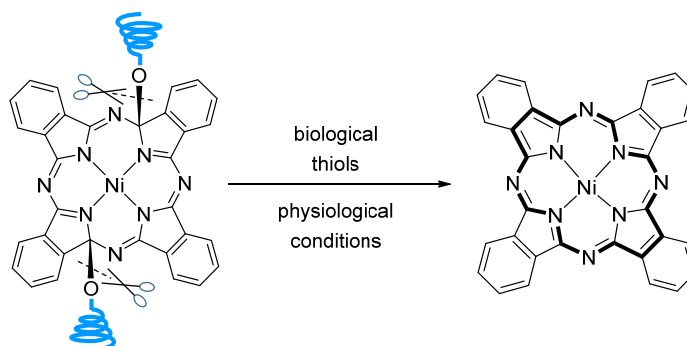


Figure 11. Reductive aromatization of **ox-NiPc-PEG**.

In Chapter 4, the author describes the development of core-alkoxylated NiNc derivatives that can be used as contrast agents for PA cancer imaging (Figure 12). A core-alkoxylated NiNc derivative having PEG side chains (**ox-NiNc-PEG**) could be converted to NiNc in response to GSH under mild conditions, enhancing PA signal intensity. NiNc exhibits a significant absorption signal in the NIR region even after aggregation in water, allowing for efficient excitation by NIR laser irradiation. In GSH-overexpressing cancer cells treated

with **ox-NiPc-PEG**, NIR laser irradiation induced significant cell death, its potential as a theranostic agent being confirmed.

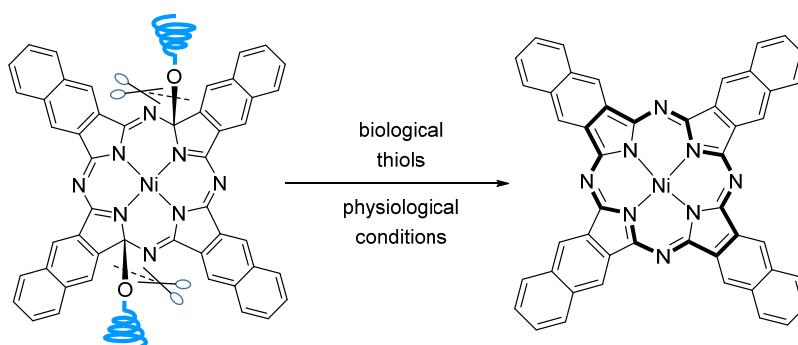


Figure 12. Reductive aromatization of **ox-NiPc-PEG**.

In Chapter 5, the author describes the synthesis of alkoxyated perfluorinated NiPc derivatives (**(RO)₂NiPcF₁₆**) (Figure 13). In the case of **ox-NiPc**, in which benzene rings are not modified, only the 5, 19-alkoxyated isomer is obtained. On the other hand, two isomers were obtained simultaneously in the synthesis of **(RO)₂NiPcF₁₆**; the major component in which the alkoxy groups were attached to the adjacent pyrrole rings (**adj-(MeO)₂NiPcF₁₆**) and the minor one in which the alkoxy groups were attached to the diagonally opposite pyrrole rings (**opp-(MeO)₂NiPcF₁₆**). **adj-(MeO)₂NiPcF₁₆** was converted to NiPcF₁₆ by heat or reduction. The conversion to NiPcF₁₆ proceeded quantitatively by reductive aromatization using H₂SnCl₄.

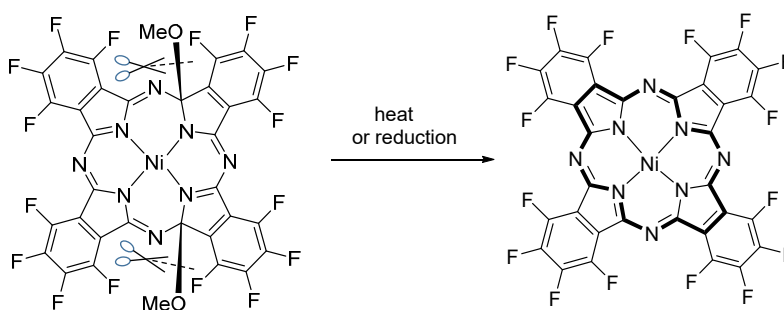


Figure 13. Reductive aromatization of **adj-(MeO)₂NiPcF₁₆**.

References

- (1) (a) M. Tahoun, C. T. Gee, V. E. McCoy, P. M. Sander, C. E. Müller, *RSC Adv.* **2021**, *11*, 7552–7563. (b) M. O. Senge, N. N. Sergeeva, K. J. Hale, *Chem. Soc. Rev.* **2021**, *50*, 4730–4789. (c) T. L. Poulos, *Chem. Rev.* **2014**, *114*, 3919–3962.
- (2) T. Nyokong, *Coord. Chem. Rev.* **2007**, *251*, 1707–1722.
- (3) G. de la Torre, P. Vázquez, F. Agulló-López, T. Torres, *Chem. Rev.* **2004**, *104*, 3723–3750.
- (4) (a) M. Gouterman, *J. Mol. Spectrosc.* **1961**, *6*, 138–163. (b) M. Gouterman, G. H. Wagniere, L. C. Snyder, *J. Mol. Spectrosc.* **1963**, *11*, 108–127. (c) A. M. Schaffer, M. Gouterman, E. R. Davidson, *Theor. Chim. Acta* **1973**, *30*, 9–30. (d) M. Wang, K. Ishii, *Coord. Chem. Rev.* **2022**, *468*, 214626.
- (5) (a) G. T. Byrne, R. P. Linstead, A. R. Lowe, *J. Chem. Soc.* **1934**, 1017–1022. (b) R. P. Linstead, A. R. Lowe, *J. Chem. Soc.* **1934**, 1022–1027.
- (6) G. Zanotti, F. Palmeri, V. Raglione, *Chem. -Eur. J.* **2024**, *30*, e20240908.
- (7) C. G. Claessens, U. Hahn, T. Torres, *Chem. Rec.* **2008**, *8*, 75–97.
- (8) (a) D. Li, S. Cai, P. Wang, H. Cheng, B. Cheng, Y. Zhang, G. Liu, *Adv. Healthcare Mater.* **2023**, *12*, 2300263. (b) B.-D. Zheng, Q.-X. He, X. Li, J. Yoon, J.-D. Huang, *Coord. Chem. Rev.* **2021**, *426*, 213548. (c) K. Janas, E. Boniewska-Bernacha, G. Dyrda, R. Słota, *Bioorg. Med. Chem.* **2021**, *30*, 115926. (d) A. Galstyan, *Chem. -Eur. J.* **2021**, *27*, 1903–1920.
- (9) (a) A. Kumar, R. Meunier-Prest, M. Bouvet, *Sensors* **2020**, *20*, 4700. (b) D. Klyamer, R. Shutilov, T. Basova, *Sensors* **2021**, *22*, 895. (c) W. Zhou, N. J. Yutronkie, B. H. Lessard, J. L. Brusso, *Mater. Adv.* **2021**, *2*, 165–185.
- (10) (a) A. B. Sorokin, *Chem. Rev.* **2013**, *113*, 8152–8191. (b) M. S. Thakur, N. Singh, A. Sharma, R. Rana, A. R. Abdul Syukor, M. Naushad, S. Kumar, M. Kumar, L. Singh, *Corrd. Chem. Rev.* **2022**, *471*, 214739. (c) S. Yang, Y. Yu, X. Gao, Z. Zhang, F. Wang, *Chem. Soc. Rev.* **2021**, *50*, 12985–13011. (d) E. Mikoloudakis, I. López-Duarte, G. Charalambidis, K. Ladomenou, M. Ince, A. G. Coutsolelos, *Chem. Soc. Rev.* **2022**, *51*, 6965–7045.
- (11) A. M. Schmidt, M. J. E. Calvete, *Molecules* **2021**, *26*, 2823.

- (12) K. K. Ng, G. Zheng, *Chem. Rev.* **2015**, *115*, 11012–11042.
- (13) P. Juzenas, A. Juzeniene, O. Kaalhus, V. Iani, J. Moan, *Photochem. Photobiol. Sci.* **2002**, *1*, 745–748.
- (14) Y. Cai, W. Si, W. Huang, P. Chen, J. Shao, X. Dong, *Small* **2018**, *14*, 1704247.
- (15) (a) J. Liu, W. Bu, J. Shi, *Chem. Rev.* **2017**, *117*, 6160–6224. (b) G. Feng, G.-Q. Zhang, D. Ding, *Chem. Soc. Rev.* **2020**, *49*, 8179–8234.
- (16) (a) D. Crosby, S. Bhatia, K. M. Brindle, L. M. Coussens, C. Dive, M. Emberton, S. Esener, R. C. Fitzgerald, S. S. Gambhir, P. Kuhn, T. R. Rebbeck, S. Balasubramanian, *Science* **2022**, *375*, 1244. (b) R. C. Fitzgerald, A. C. Antoniou, L. Fruk, N. Rosendeld, *Nat. Med.* **2022**, *28*, 666–677.
- (17) T. Hussain, Q. T. Nguyen, *Adv. Drug Deliv. Rev.* **2014**, *66*, 90–100.
- (18) X. Han, K. Xu, O. Taratula, K. Farsad, *Nanoscale* **2019**, *11*, 799–819.
- (19) Y. Zhang, G. Zhang, Z. Zeng, K. Pu, *Chem. Soc. Rev.* **2022**, *51*, 566–593.
- (20) F. Leblond, S. C. Davis, P. A. Valdés, B. W. Pogue, *J. Photochem. Photobiol. B-Biol.* **2010**, *98*, 77–94.
- (21) W. Choi, B. Park, S. Choi, D. Oh, J. Kim, C. Kim, *Chem. Rev.* **2023**, *123*, 7379–7419.
- (22) C. Li, C. Liu, Y. Fan, X. Ma, Y. Zhan, X. Lu, Y. Sun, *RSC Chem. Bio.* **2021**, *2*, 743–758.
- (23) A.-G. Niculescu, A. M. Grumezescu, *Appl. Sci.* **2021**, *11*, 3626.
- (24) G. S. Attar, M. Kumar, V. Bhalla, *Chem. Commun.* **2024**, *60*, 11610–11624.
- (25) (a) Z. Zhou, J. Song, L. Nie, X. Chen, *Chem. Soc. Rev.* **2016**, *45*, 6597–6626. (b) T. K. L. Rezende, H. P. Barbosa, L. F. dos Santos, K. de O. Lima, P. A. de Matos, T. M. Tsubone, R. R. Gonçalves, J. L. Ferrari, *Front. Chem.* **2022**, *10*, 1035449.
- (26) (a) G. Gunaydin, M. E. Gedik, S. Ayan, *Front. Chem.* **2021**, *9*, 691697. (b) X. Li, N. Kwon, T. Guo, Z. Liu, J. Yoon, *Angew. Chem. Int. Ed.* **2018**, *57*, 11522–11531. (c) Y. Wan, L.-H. Fu, C. Li, J. Lin, P. Huang, *Adv. Mater.* **2021**, *33*, 2103978.
- (27) (a) X. Li, J. F. Lovell, J. Yoon, X. Chen, *Nat. Rev. Clin. Oncol.* **2020**, *17*, 657–674. (b) J. Li, W. Zhang, W. Ji, J. Wang, N. Wang, W. Wu, Q. Wu, X. Hou, W. Hu, L. Li, *J. Mater. Chem. B* **2021**, *9*, 7909–7926.
- (28) M. Overchuk, R. A. Weersink, B. C. Wilson, G. Zheng, *ACS Nano* **2023**, *17*, 7979–8003.
- (29) (a) S. S. Kelkar, T. M. Reineke, *Bioconjugate Chem.* **2011**, *22*, 1879–1903. (b) P. Sarbadhikary, B. P.

- George, H. Abrahamse, *Theranostics* **2011**, *11*, 9054–9088.
- (30) Y. Liu, P. Bhattarai, Z. Dai, X. Chen, *Chem. Soc. Rev.* **2019**, *48*, 2053–2108.
- (31) F. Dumoulin, M. Durmuş, V. Ahsen, T. Nyokong, *Coord. Chem. Rev.* **2010**, *254*, 2792–2847.
- (32) P.-C. Lo, M. S. Rodríguez-Morgade, R. K. Pandey, D. K. P. Ng, T. Torres, F. Dumoulin, *Chem. Soc. Rev.* **2020**, *49*, 1041–1056.
- (33) G. F. M. Pereira, T. T. Tasso, *Inorg. Chim. Acta* **2021**, *519*, 120271.
- (34) A. V. Soldatova, J. Kim, X. Peng, A. Rosa, G. Ricciardi, M. E. Kenney, M. J. Rodgers, *Inorg. Chem.* **2007**, *46*, 2080–2093.
- (35) S. Luo, E. Zhang, Y. Su, T. Cheng, C. Shi, *Biomaterials* **2011**, *32*, 7127–7138.
- (36) (a) W. Wang, Y. Jin, X. Liu, F. Chen, X. Zheng, T. Liu, Y. Yang, H. Yu, *Adv. Funct. Mater.* **2021**, *31*, 2100386. (b) Y. Wang, Y. Hu, D. Ye, *Angew. Chem. Int. Ed.* **2022**, *61*, e202209512. (c) Y. Zeng, T. Dou, L. Ma, J. Ma, *Adv. Sci.* **2022**, *9*, 2202384.
- (37) Z. Zhuang, J. Li, P. Shen, Z. Zhao, B. Z. Tang, *Aggregate* **2024**, *5*, e540.
- (38) (a) S. Uno, M. Kamiya, T. Yoshihara, K. Sugawara, K. Okabe, M. C. Tarhan, H. Fujita, T. Funatsu, Y. Okada, S. Tobita, Y. Urano, *Nat. Chem.* **2014**, *6*, 681–689. (b) J. Li, X. Li, J. Jia, X. Chen, Y. Lv, Y. Guo, J. Li, *Dyes Pigm.* **2019**, *166*, 433–442. (c) M. Mandel, D. Banik, A. Karak, S. K. Manna, A. K. Mahapatra, *ACS Omega* **2022**, *7*, 36988–37007. (d) W. Song, H. Liu, S. Wang, X. Zhi, Z. Shen, *J. Photochem. Photobiol. A-Chem.* **2023**, *435*, 114319. (e) A. Martin, P. Rivera-Fuentes, *Curr. Opin. Chem. Biol.* **2024**, *80*, 102444.
- (39) (a) C. Ercolani, A. M. Paoletti, G. Pennesi, G. Rossi, Chiesi-Villa, C. Rizzoli, *J. Chem. Soc., Dalton Trans.* **1990**, 1971–1977. (b) M. P. Donzello, C. Ercolani, P. J. Lukes, *Inorg. Chem. Acta*, **1997**, *256*, 171–172. (c) E. M. Bauer, M. P. Donzello, C. Ercolani, E. Masetti, S. Panero, G. Ricciardi, A. Rosa, A. Chiesi-Villa, C. Rizzoli, *Inorg. Chem.* **2003**, *42*, 238–293. (d) J. Janczak, R. Kubisk, *Polyhedron* **2003**, *22*, 313–322. (e) M. Sumimoto, Y. Kawashima, K. Hori, H. Fujimoto, *Phys. Chem. Chem. Phys.* **2015**, *17*, 6478–6483.
- (40) (a) H. M. Kieler, M. J. Bierman, I. A. Guzei, P. J. Liska, R. W. McGaff, *Chem. Commun.* **2006**, 3326–

3328. (b) I. Y. Skobelov, E. V. Kudrik, O. V. Zalomaeva, F. Albrieux, P. Afanasiev, O. A. Kholdeeva, A. B. Sorokin, *Chem. Commun.* **2013**, 49, 5577–5579. (c) R. K. Al-Shewiki, S. Weheabby, N. Uhlig, M. Korb, T. Pester, S. Zahn, S. Grecchi, P. R. Mussini, T. Rüffer, H. Lang, *Dalton Trans.* **2024**, 53, 3836–3854.
- (41) (a) C. D. Molek, J. A. Halfen, J. C. Loe and R. W. McGaff, *Chem. Commun.* **2001**, 2644–2645. (b) Y. Kikukawa, T. Fukuda, A. Fuyuhiko, N. Ishikawa, N. Kobayashi, *Chem. Commun.* **2011**, 8518–8520. (c) T. Fukuda, Y. Kikukawa, R. Tsuruya, A. Fuyuhiko, N. Ishikawa, N. Kobayashi, *Inorg. Chem.* **2011**, 50, 11832–11837. (d) T. Fukuda, N. Ishikawa, *Dyes Pigm.* **2014**, 109, 151–154. (e) Y. Y. Karabach, M. N. Kopylovich, K. V. Luzyanin, M. F. C. Guedes da Silva, V. Yu. Kukushkin, A. J. L. Pombeiro, *Inorg. Chem. Acta* **2017**, 455, 696–700.

Chapter 1

MMP-2-Activatable Photoacoustic Tumor-Imaging Probes Based on Al- and Si-Naphthalocyanines

Abstract

Enzyme-activatable photoacoustic probes are powerful contrast agents to visualize diseases in which a specific enzyme is overexpressed. In this study, aluminum and silicon naphthalocyanines (AlNc and SiNc, respectively) conjugated with matrix metalloprotease-2 (MMP-2)-responsive PLGLAG peptide sequence and poly(ethylene glycol) (PEG) as an axial ligand were designed and synthesized. AlNc-based conjugate **AlNc-pep-PEG** formed dimeric species interacting with each other through face-to-face *H*-aggregation in water, while SiNc-based conjugate **SiNc-pep-PEG** hardly interacted with each other because of the two bulky hydrophilic axial ligands. Both conjugates formed spherical nanometer-sized self-assemblies in water, generating photoacoustic waves under near-infrared photoirradiation. The treatment of MNc-peptide-PEG conjugates (M = Al, Si) with MMP-2 smoothly induced the cleavage of the PLGLAG sequence to release the hydrophilic PEG moiety, resulting in the aggregation of MNcs. By comparing the PA signal intensity changes at 680 nm and 760 nm, the photoacoustic signal intensity ratios were shown to be enhanced by 3–5 times after incubation with MMP-2. The author demonstrated that MNc-peptide-PEG conjugates (M = Al, Si) could work as activatable photoacoustic probes in the *in vitro* experiment of MMP-2-overexpressed cell line HT-1080 as well as the *in vivo* photoacoustic imaging of HT-1080-bearing mice.

Introduction

Photoacoustic (PA) imaging is one of the most powerful modalities to visualize disease by utilizing less-invasive near-infrared (NIR) pulse laser irradiation.¹⁻³ In the visualization of target tissue, disease, and biological activity, PA contrast agents that efficiently absorb photoenergy in the tissue-permeable NIR region and generate PA waves through nonradiative decay have been intensively investigated.^{4,5} To obtain high-contrast PA images, there are three important requirements for PA contrast agents based on organic dyes. First, NIR nonemissive dyes with high molar extinction coefficients (ϵ) are required for strong PA wave generation.^{1,6} Second, to assess precisely the pharmacokinetics and therapeutic efficacy of drugs, photostable PA contrast agents that can be tolerated under continuous pulse laser irradiation for repeated diagnosis and biological assessment are preferred.⁷⁻⁹ Third, to visualize the location of accumulated probes, activatable PA contrast agents that generate PA waves by responding to biological stimuli, especially enzymes, in cells are desired.¹⁰⁻¹² In light of these requirements, the development of activatable PA probes based on a photostable NIR dye is most desirable.

Metallonaphthalocyanine (MNC) is one of the photostable NIR dyes with high ϵ value ($\sim 1 \times 10^5$), thereby being expected to be a good candidate as a photosensitizer for PA imaging in the NIR region.^{13,14} Despite their blue-shifted absorbance of smaller π -conjugated system, phthalocyanines have widely investigated as a PA contrast agent.^{15,16} In contrast, there are a limited number of water-soluble MNCs applying to PA imaging, probably because their low solubility both in organic solvents and water has hampered their functionalization. Lovell and co-workers reported tin naphthalocyanine (SnNC) conjugated with poly(ethylene glycol) (PEG) and its application to PA vascular imaging;¹⁷ however, the “always-on” property of MNCs may preclude their application to PA imaging of target tissues without background noise. Although the “turn-on” property of probes is known to improve the visualization of target tissues dramatically, to the author’s knowledge, there have been no reports involving activatable PA contrast agents based on water-soluble MNCs.

Recently, the research team of the author reported self-assemblies consisting of NIR dye- and PEG-grafted hyaluronates, in which the aggregated NIR dyes efficiently increased the PA signal intensity in water.^{18,19} Based on this finding, the author envisioned that the detachment of sterically demanding water-soluble

substituents from MNcs would lead to the aggregation of MNcs, resulting in PA signal enhancement (Figure 1). Peripheral aromatics of π -system and axial ligands on a center metal are potential modification sites in MNcs. Although peripheral functionalization is accessible, the aggregation of MNcs cannot be prevented effectively.^{20,21} The author assumed that conjugation of sterically demanding axial ligands can suppress the aggregation of MNcs, and the removal of axial ligands can lead to significant PA signal enhancement induced by MNc aggregation. Aiming at detaching the water-soluble ligand in specific cells, the author designed MNc-peptide-PEG conjugates consisting of aluminum naphthalocyanine (AlNc) or silicon naphthalocyanine (SiNc), PEG, and a PLGLAG oligopeptide sequence that is cleaved under neutral conditions by matrix metalloprotease-2 (MMP-2) as an overexpressed protease related to tumor metastasis and angiogenesis.^{22–24} While photosensitizers of the reported MMP-2-activatable PA contrast agent are NIR cyanine dyes with photobleaching disadvantage,^{25–28} no MMP-2-responsive PA contrast agents based on photostable MNcs have been reported. Here, the author describes newly developed MNc-peptide-PEG conjugates (**MNc-pep-PEG**), which smoothly respond to MMP-2, affording MNc aggregates with strong PA wave generation. MMP-2-activatable MNc conjugates were found to increase the relative PA signal intensity ratio up to five times in MMP-2-overexpressed cell line HT-1080 and were successfully applied to *in vivo* PA tumor imaging of HT-1080-bearing mice.

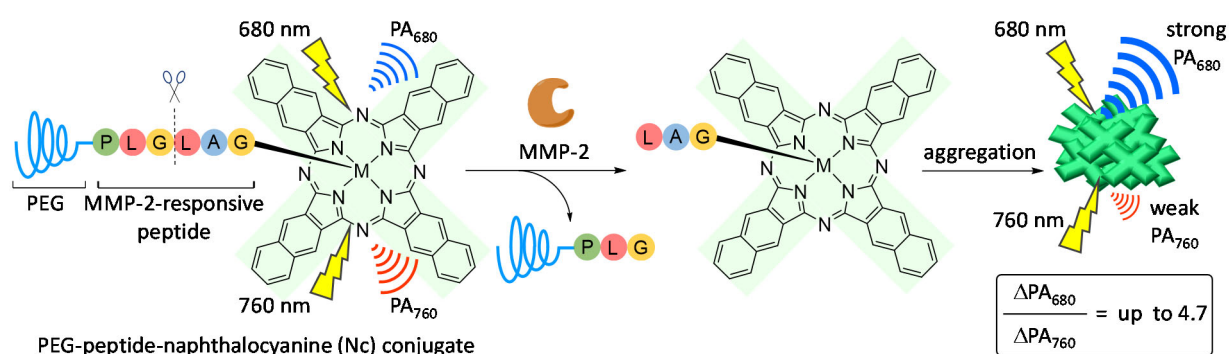


Figure 1. MMP-2-induced MNc-aggregation for PA signal enhancement. AlNc (M = Al) and SiNc (M = Si) were used. In the case of SiNc, two PEG-peptide axial ligands were incorporated on the Si atom, but one of two ligands is omitted for clarity in the figure. P: proline, L: leucine, G: glycine, A: alanine.

Results and discussion

Synthesis of MNC-*pep*-PEG conjugates

For grafting MMP-2-responsive hydrophilic functionality, **AlNc-OH**,²⁹ which has one hydroxy group as an axial ligand, and **SiNc-OH**,²⁹ which has two hydroxy groups as axial ligands, were chosen as a core structure of activatable PA probes (Figure 2). The treatment of **AlNc-OH** and **SiNc-OH** with 3-aminopropylethoxydimethylsilane afforded **AlNc-NH₂** and **SiNc-NH₂** in moderate yields.³⁰ **AlNc-*pep*-PEG** and **SiNc-*pep*-PEG** were prepared using a condensation reaction with peptide-PEG conjugate **2** having a free C-terminal, followed by dialysis and lyophilization. The parent signals of **AlNc-*pep*-PEG** and **SiNc-*pep*-PEG** were observed in MALDI-TOF mass spectra. Because the conjugation of PEGs whose average molecular weight was less than 1 kDa led to low water solubility of MNC conjugates, PEG (MW = 2 kDa) as a hydrophilic axial ligand was used. **AlNc-PEG** and **SiNc-PEG** without MMP-2 responsiveness were synthesized by grafting **1** to MNCs in a similar manner.

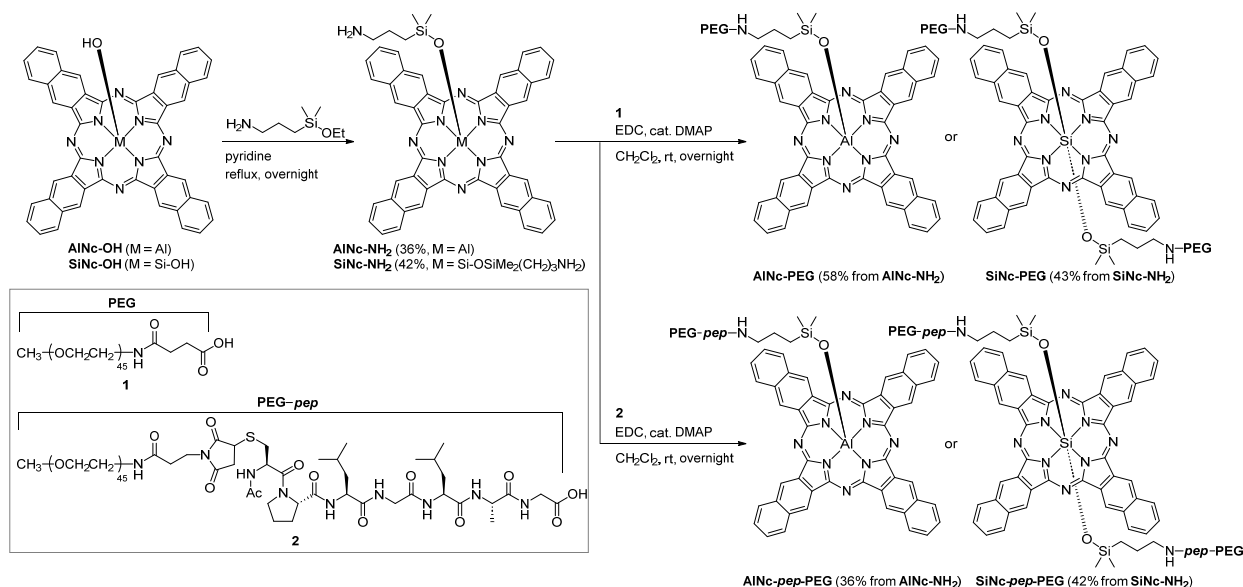


Figure 2. Synthesis of AlNc and SiNc bearing hydrophilic axial ligands. EDC·HCl: 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride, DMAP: 4-dimethylaminopyridine.

Photophysical properties of Nc-pep-PEG conjugates

The absorption maxima of **AlNc-pep-PEG** and **SiNc-pep-PEG** in *N,N*-dimethylformamide (DMF) were detected at 788 nm ($\epsilon = 1.0 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$) and 781 nm ($\epsilon = 1.3 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$), respectively, which were attributable to the Q-band (orange lines in Figures 3a and 3b).²⁹ The sharp peak in the NIR region indicates that both conjugates are well-dispersed in DMF as a monomeric form without any intermolecular interaction.

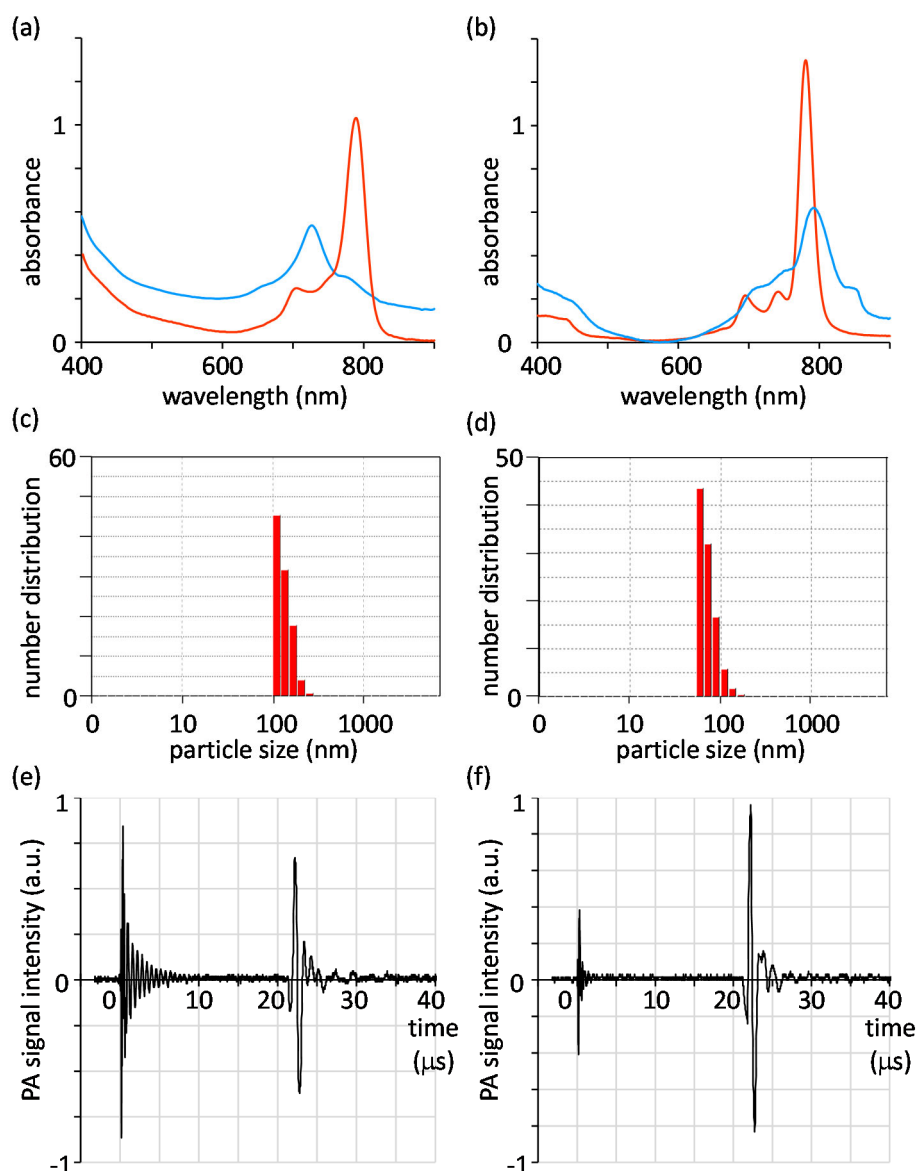
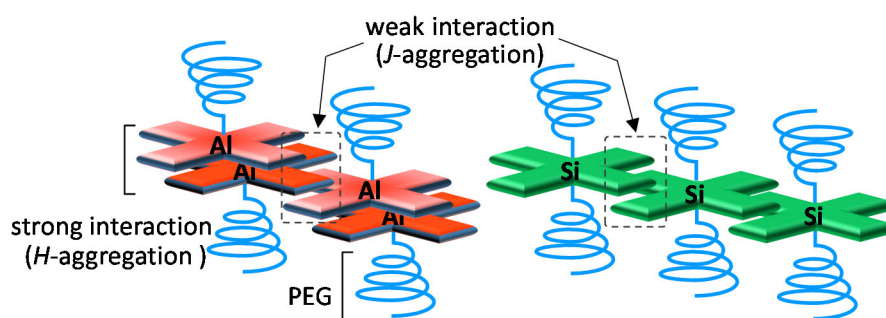


Figure 3. UV-vis absorption spectra of (a) **AlNc-pep-PEG** and (b) **SiNc-pep-PEG** in DMF (orange) and H₂O (blue). Concentration: 10 μM. Hydrodynamic diameters of self-assemblies consisting of (c) **AlNc-pep-PEG** and (d) **SiNc-pep-PEG** in H₂O measured by DLS. PA signal of (e) **AlNc-pep-PEG** and (f) **SiNc-pep-PEG** in H₂O (at 760 nm). Signals were detected at ca. 20 μs after detection of a trigger signal.

Table 1. Molar extinction coefficient of MNcs.

	molar extinction coefficient ϵ ($M^{-1} \text{ cm}^{-1}$) [$\lambda_{\text{abs,max}}$ (nm)]	
	DMF	H ₂ O
AlNc-<i>pep</i>-PEG	1.0×10^5 [788]	5.4×10^4 [726]
SiNc-<i>pep</i>-PEG	1.3×10^5 [781]	6.6×10^4 [791]
AlNc-PEG	9.6×10^4 [785]	5.9×10^4 [740]
SiNc-PEG	1.1×10^5 [779]	4.8×10^4 [784]

In contrast, the absorption maximum of **AlNc-*pep*-PEG** in water was observed at 726 nm (blue line in Figure 3a and Table 1). Because the absorbance of MNC *H*-aggregates is known to be observed around 710 nm,^{31,32} two **AlNc-*pep*-PEG** molecules are considered to form dimeric face-to-face *H*-aggregates with interaction between their concave faces in water (Figure 4, left). Nagamura and co-workers reported that pentacoordinated oxovanadium naphthalocyanine (O=VNc) interacted with each other through both *H*-aggregation ($\lambda_{\text{abs}} = 745 \text{ nm}$) and *J*-aggregation ($\lambda_{\text{abs}} = 850\text{--}950 \text{ nm}$).³³ Because no obvious absorption signal of *J*-aggregates of AlNcs was observed, the *J*-aggregation of AlNcs shown in Figure 4 seems to be a minor form probably due to the steric repulsion between the bulky PEG moieties. In the case of **SiNc-*pep*-PEG**, the absorbance of a monomeric form was observed at 791 nm as a sharp signal, although broadened (blue line in Figure 3b and Table 1). This suggests that the intermolecular interaction of **SiNc-*pep*-PEG** is weak due to the steric bulkiness of the axial ligands (Figure 4, right). **AlNc-PEG** and **SiNc-PEG** showed quite similar absorption spectra to **AlNc-*pep*-PEG** and **SiNc-*pep*-PEG**, respectively, indicating that an MMP-2-responsive peptide does not interact intermolecularly through hydrogen bonds (Figure 5 and Table 1).

**Figure 4.** Plausible aggregation modes (*H*- and *J*-aggregates) of **AlNc-*pep*-PEG** (left) and **SiNc-*pep*-PEG** (right).

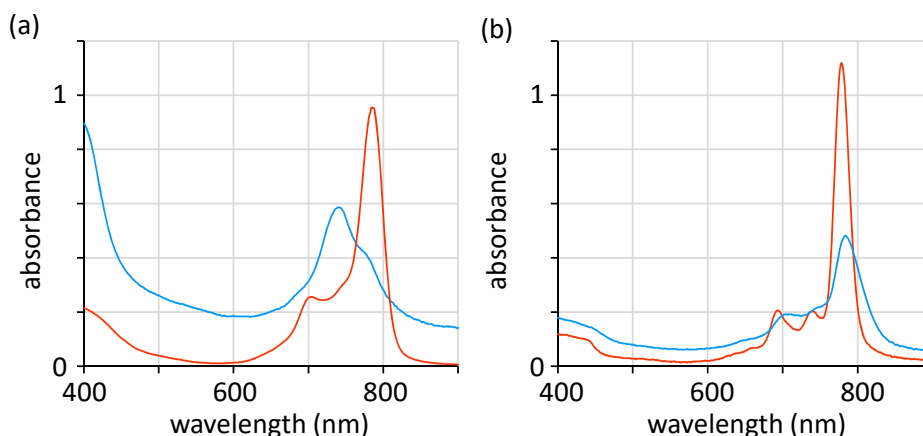


Figure 5. UV-vis absorption spectra of (a) **AlNc-PEG** and **SiNc-PEG** in DMF (orange) and H₂O (blue).

Dynamic light scattering (DLS) measurements suggest that **AlNc-pep-PEG** and **SiNc-pep-PEG** self-assembled to form nanoparticles with diameters of 121 ± 26 and 66 ± 17 nm, respectively (Figures 3c and 3d). From transmission electron microscope (TEM) measurements, small spherical nanoparticles (diameter = 6–7 nm) and slightly larger nanoparticles (diameter = 25–32 nm) were observed in both cases, which might be a mixture of micelles and multimicellar aggregates (Figure 6 and Table 2). The author measured the PA waves of **AlNc-pep-PEG** and **SiNc-pep-PEG** self-assemblies in water to investigate the effect of MNc

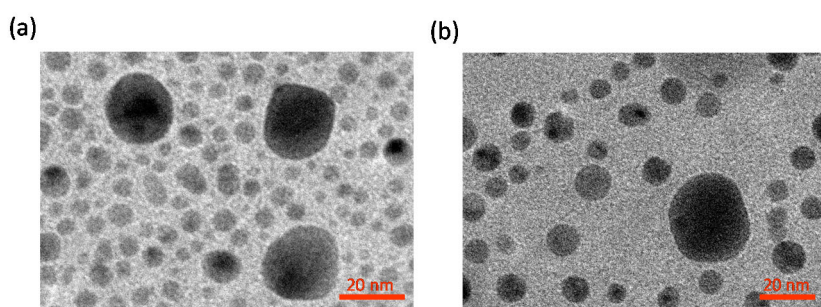


Figure 6. Representative TEM images of (a) **AlNc-pep-PEG** and (b) **SiNc-pep-PEG**. Scale (red bar): 20 nm.

Table 2. Diameters of MNcs estimated by TEM images.

	diameter (nm)	
AlNc-pep-PEG	6 ± 1	32 ± 13
SiNc-pep-PEG	7 ± 2	25 ± 6

Average values with standard deviation, $n > 50$.

aggregation on PA signal intensity (Figures 3e and 3f). Under photoirradiation with a pulse laser at 760 nm, PA signal intensity (denoted as PA_{760}) of **SiNc-pep-PEG** was 2.6 times stronger than PA_{760} of **AlNc-pep-PEG**. The two axial ligands of monomeric **SiNc-pep-PEG** might convert the excited photoenergy more efficiently through a nonradiative deactivation pathway, thereby generating stronger PA waves. By contrast, the PA_{680} of **AlNc-pep-PEG** and **SiNc-pep-PEG** under photoirradiation at 680 nm were comparable (**AlNc-pep-PEG** : **SiNc-pep-PEG** = 1 : 0.96). By considering that the photoenergy at 680 nm is mainly absorbed by AlNc *H*-aggregates, the author concluded that PA waves can be generated by dye aggregation. To check the influence of pH, the author measured PA signal intensities of probes under neutral and slightly acidic conditions. The author confirmed that PA signal intensities PA_{680} and PA_{760} as well as the ratios of PA_{680}/PA_{760} are almost constant in pH range from 5.0 to 7.7 (Figure 7).

The photostability under NIR photoirradiation was examined by monitoring time-dependent decrement of the maximum absorbance (Figure 8). Seventy percent of indocyanine green (ICG),^{34,35} which is an approved contrast agent for NIR optical imaging under photoirradiation was bleached after photoirradiation for 2 h, whereas no absorbance decrease of **AlNc-pep-PEG** was observed. This property is advantageous for PA imaging under continuous photoirradiation and preferable to lower the limit of detection. **SiNc-pep-PEG** was found to be less stable than **AlNc-pep-PEG** and 15% of **SiNc-pep-PEG** underwent bleaching after 2 h photoirradiation. Because the photoinduced cleavage of the axial ligand from silicon phthalocyanine has

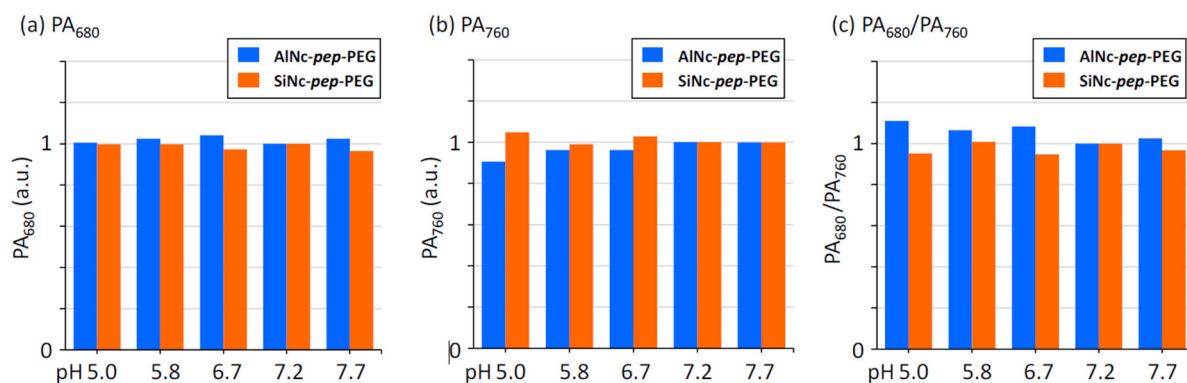


Figure 7. PA signal intensities at 680 nm and 760 nm and PA signal intensity ratio (PA_{680}/PA_{760}) of **AlNc-pep-PEG** (blue) and **SiNc-pep-PEG** (orange) normalized by the values at pH 7.2.

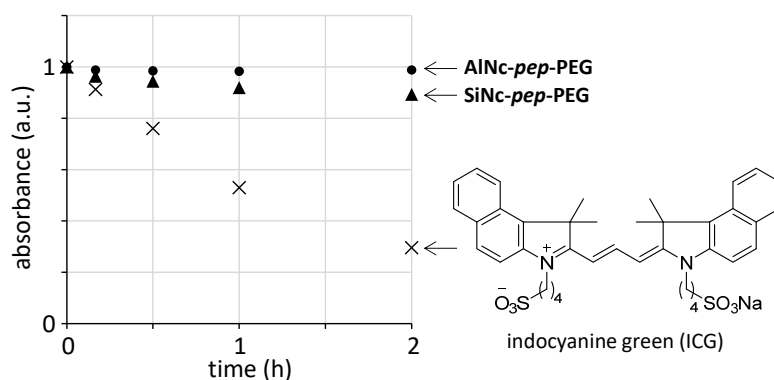


Figure 8. Photobleaching test of AINc-pep-PEG (circle), SiNc-pep-PEG (triangle), and indocyanine green (cross) under LED photoirradiation. LED: $\lambda_{\text{max}} = 737$ nm, half-bandwidth = 20 nm, power = 25 mW (at the sample level). Absorbance corresponds to the mean value of two independent experiments.

been reported,³⁰ a similar removal of axial ligands is assumed to proceed to form aggregates, resulting in the absorbance decrease.

MMP-2-responsiveness of MNc-peptide-PEG conjugates

With photostable MNc conjugates in hand, the author next examined their MMP-2-responsiveness. After treating MNc conjugates with MMP-2, time-dependent change of absorbance was monitored (Figure 9).

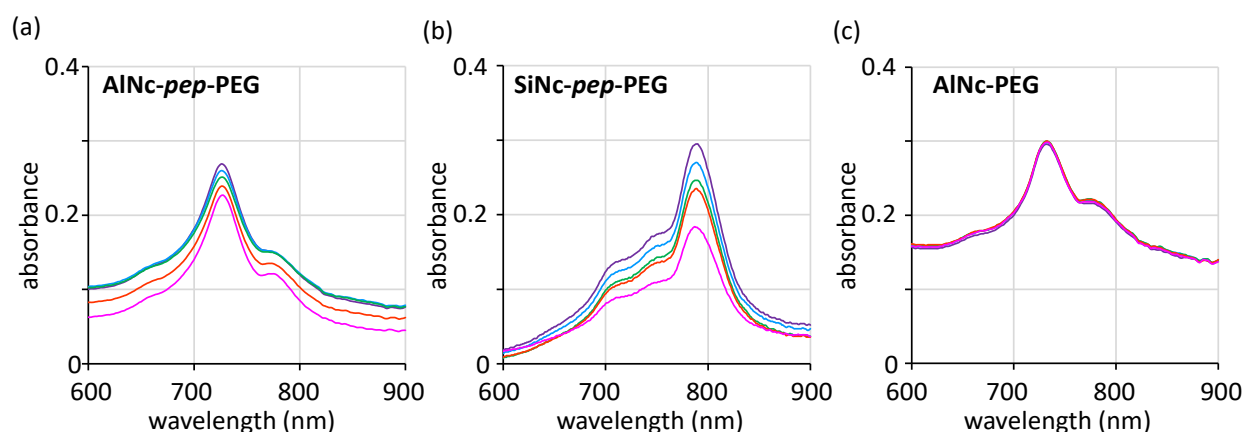


Figure 9. Time-dependent absorbance change of (a) AINc-pep-PEG, (b) SiNc-pep-PEG, and (c) AINc-PEG (5.0 μM , pH = 7.2) during incubation with MMP-2 (5.0 nM). Just after (purple), 15 min (blue), 30 min (green), 60 min (red), and 120 min (pink).

After 2 h incubation with MMP-2, **AlNc-PEG** which does not contain MMP-2-responsive peptide, showed no change of absorption spectra. The treatment of **AlNc-pep-PEG** and **SiNc-pep-PEG** with MMP-2 led to a decrease in absorbance intensity without obvious spectrum shape change. After 2 h incubation, the hydrodynamic diameters of self-assemblies in solution increased in both cases (Figure 10 and Table 3). These results indicate that MMP-2 gradually hydrolyzed the peptide sequence, inducing the aggregation of MNcs. To investigate the aggregation-induced PA signal enhancement, PA signal intensities were measured before and after incubation of **AlNc-pep-PEG** and **SiNc-pep-PEG** with MMP-2 for 2 h. By dividing PA signal intensity measured after incubation by that before incubation, the PA signal intensity change (denoted as ΔPA) was calculated. ΔPA_{680} of **AlNc-pep-PEG** and **SiNc-pep-PEG** increased to 2.0 and 2.5 after incubation for 2 h, respectively, whereas ΔPA_{760} decreased to 0.6 and 0.5, respectively (Figure 11, Table 4). Accordingly, both AlNc- and SiNc-conjugates were found to act as activatable probes for MMP-2 detection by monitoring

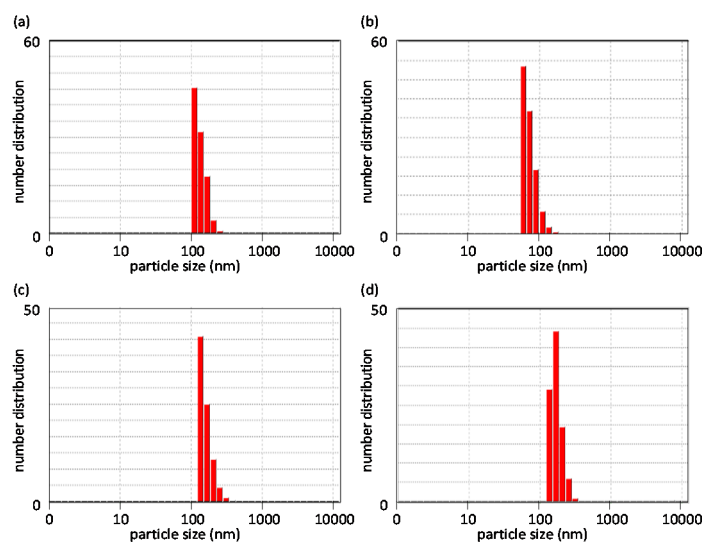


Figure 10. Hydrodynamic diameters of (a,c) **AlNc-pep-PEG** and (b,d) **SiNc-pep-PEG** in buffer solution (pH = 7.2) (a,b) before and (c,d) after treated with MMP-2 (5 nM) for 2 h.

Table 3. Hydrodynamic diameters of MNcs before/after MMP-2 treatment.

	Hydrodynamic diameter [nm]	
	before treatment	after treatment
AlNc-pep-PEG	121 ± 26	148 ± 35
SiNc-pep-PEG	66 ± 17	157 ± 32

PA₆₈₀. By focusing on the increase in ΔPA_{680} and the decrease in ΔPA_{760} after incubation, the author assumed that the relative PA signal intensity ratio $\Delta\text{PA}_{680}/\Delta\text{PA}_{760}$ could more clearly track the MMP-2 activity. Indeed, the ratios $\Delta\text{PA}_{680}/\Delta\text{PA}_{760}$ were 3.1 for **AlNc-pep-PEG** and 4.7 for **SiNc-pep-PEG** after incubation for 2 h (Table 4), supporting that the change in $\Delta\text{PA}_{680}/\Delta\text{PA}_{760}$ is more accurate for MMP-2 detection than that in ΔPA_{680} . To evaluate the enzyme specificity, **AlNc-pep-PEG** and **SiNc-pep-PEG** were treated with other enzymes including other MMPs and MMP-2 in the presence of its inhibitor GM6001 (Figure 11).³⁶ The $\Delta\text{PA}_{680}/\Delta\text{PA}_{760}$

Table 4. PA intensity ratios ΔPA_{680} , ΔPA_{760} , and $\Delta\text{PA}_{680}/\Delta\text{PA}_{760}$.

MNc conjugate		MMP-2	MMP-2 ^a	MMP-9	MMP-7	PLE ^b	lipase ^c
AlNc-pep-PEG	ΔPA_{680}	2.0	0.9	1.1	1.2	1.0	1.2
	ΔPA_{760}	0.6	0.9	0.9	0.9	0.8	0.9
	$\Delta\text{PA}_{680}/\Delta\text{PA}_{760}$	3.1	1.0	1.3	1.3	1.2	1.3
SiNc-pep-PEG	ΔPA_{680}	2.5	0.9	0.8	0.6	1.1	0.9
	ΔPA_{760}	0.5	1.3	1.0	0.9	1.0	1.0
	$\Delta\text{PA}_{680}/\Delta\text{PA}_{760}$	4.7	0.7	0.8	0.7	1.1	0.9

Enzymes (5 nM) were treated with probes (30 μM) in buffered solution (pH = 7.2) at 37 °C for 2 h.

^aGM6001 (inhibitor, 100 μM). ^bPorcine liver esterase. ^cLipase AK Amano.

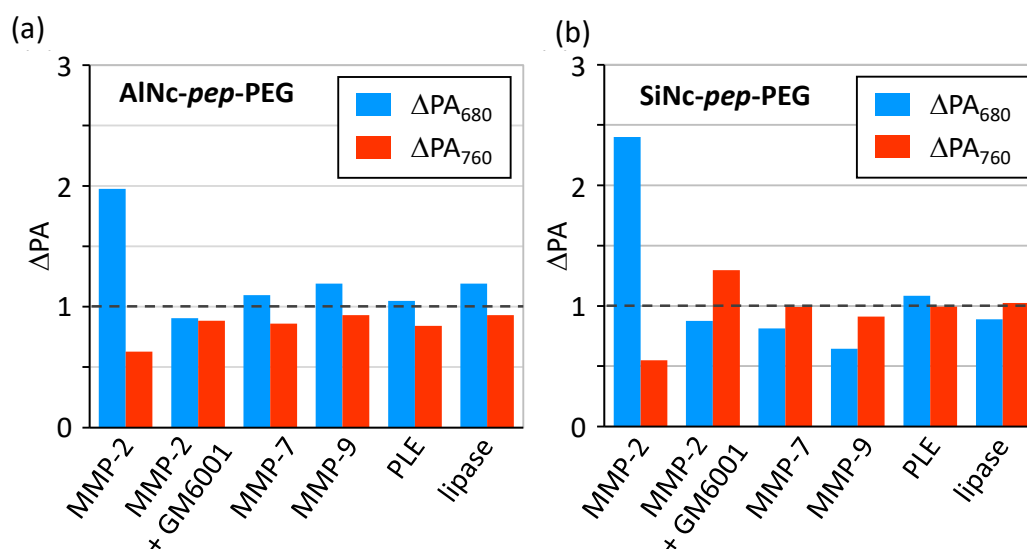


Figure 11. PA signal intensity change (ΔPA) of (a) **AlNc-pep-PEG** and (b) **SiNc-pep-PEG** (30 μM , pH = 7.2) after incubation with enzyme (0.32 $\mu\text{g}/\text{mL}$) for 2 h. Values correspond to the mean value of two independent experiments. PLE: porcine liver esterase, lipase: lipase AK Amano, GM6001: inhibitor of MMP-2.

of **AlNc-pep-PEG** and **SiNc-pep-PEG** did not significantly change, indicating the MMP-2-specific response of the probes. PA signal intensities PA_{680} and PA_{760} were affected by the concentration of MMP-2 loaded (Figure 12a and 12b). At low concentration of MMP-2 ($<5 \mu\text{M}$), PA_{680} after 2 h incubation linearly increased as the concentration increased. In contrast, PA_{760} decreased linearly, therefore, the ratio $\Delta PA_{680}/\Delta PA_{760}$ gradually increased (Figure 12c). PA signal intensities reached to a plateau when the initial concentration of MMP-2 was more than $5 \mu\text{M}$, indicating that both probes are potentially applicable as a “turn-on”-type ratiometric probe for MMP-2 activity at low concentration. To examine the concentration dependence of the enzymatic reaction, enzymatic kinetic parameters (Michaelis constant K_m , turnover number k_{cat} , and k_{cat}/K_m) of MMP-2 hydrolysis of MNc-peptide-PEG conjugates were measured according to Michaelis-Menten analysis (Figure 13 and Table 5). Those parameters for hydrolysis of both **AlNc-pep-PEG** ($K_m = 22 \mu\text{M}$ and $k_{cat} = 0.0023 \text{ s}^{-1}$, $k_{cat}/K_m = 2.5 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$) and **SiNc-pep-PEG** ($K_m = 27 \mu\text{M}$ and $k_{cat} = 0.0033 \text{ s}^{-1}$, $k_{cat}/K_m = 3.0 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$) were obtained. Because the kinetic parameters are comparable to those of the reported MMP-2-responsive optical probes,^{37–39} the enzyme approach to the peptide moiety was found to be not blocked by the bulky PEG group and MNc moiety.

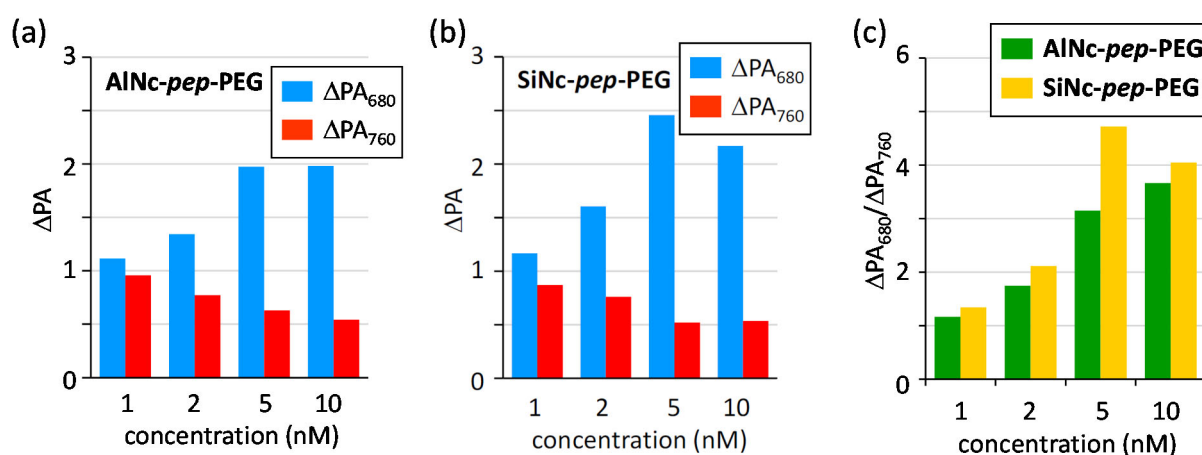


Figure 12. PA signal intensity changes (ΔPA) of (a) **AlNc-pep-PEG** and (b) **SiNc-pep-PEG** ($30 \mu\text{M}$, $\text{pH} = 7.2$) after incubation with MMP-2 for 2 h. (c) Ratio ($\Delta PA_{680}/\Delta PA_{760}$) of PA signal intensity changes of **AlNc-pep-PEG** and **SiNc-pep-PEG** ($30 \mu\text{M}$, $\text{pH} = 7.2$) after incubation with MMP-2 for 2 h.

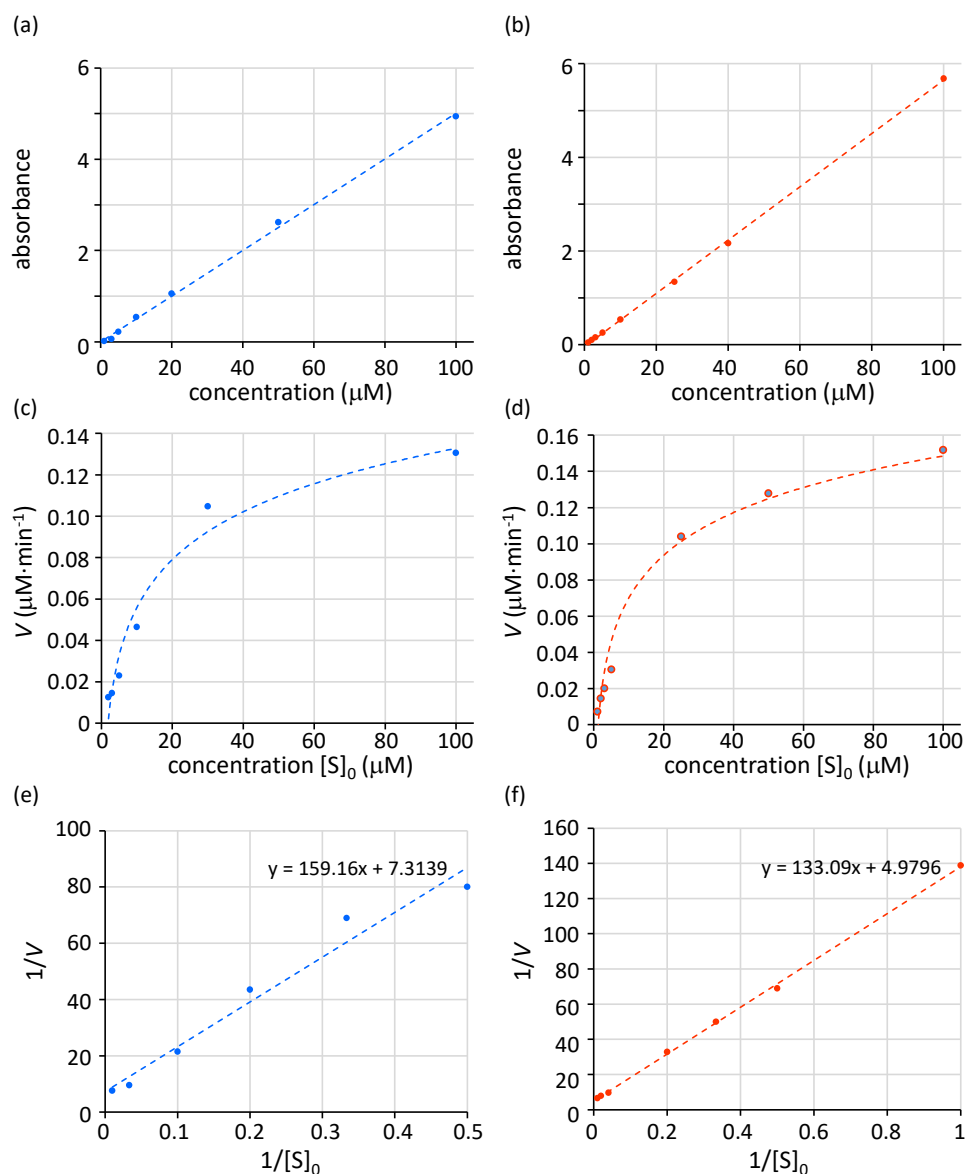


Figure 13. Concentration-dependent absorbance change of (a) AINc-pep-PEG and (b) SiNc-pep-PEG at absorption maximum. Michaelis-Menten plots of (c) AINc-pep-PEG and (d) SiNc-pep-PEG in response to MMP-2 (4.2 nM). Lineweaver-Burk plots of (e) AINc-pep-PEG and (f) SiNc-pep-PEG.

Table 5. Michaelis constant K_m , maximum rate $V_{\max}$ and turnover number k_{cat} .

	K_m [μM]	$V_{\max}$ [$\mu\text{M min}^{-1}$]	k_{cat} [min^{-1}]	k_{cat} / K_m [$\text{M}^{-1} \text{s}^{-1}$]
AINc-pep-PEG	21.8	0.14	34	2.6×10^4
SiNc-pep-PEG	26.7	0.20	48	3.0×10^4

Because the MMP-2 responsiveness of probes utilizing the PA signal output was proven, the author next evaluated the *in vitro* sensing ability of probes in MMP-2-overexpressed human sarcoma cell line HT-1080.^{28,40} Before cell experiments, the author confirmed the low cytotoxicity of probes using an MTT assay (Figure 14). After the treatment of HT-1080 with **AlNc-pep-PEG** and **SiNc-pep-PEG** for 4 h, ΔPA_{680} gradually increased to 4.3 and 2.6, respectively (Figure 15a). Moreover, the values of $\Delta PA_{680}/\Delta PA_{760}$ after 4 h were 5.4 for **AlNc-pep-PEG** and 3.9 for **SiNc-pep-PEG** (Figure 15b). The incubation with inhibitor GM6001 efficiently suppressed the MMP-2 activity in cells, the PA signal intensities remaining unchanged. The treatment of probes with human cervical cancer cell line HeLa, which is characterized with low MMP-2 expression level,³⁹ showed a negligible change in PA signal intensities. These results demonstrate that **AlNc-pep-PEG** and **SiNc-pep-PEG** can work as activatable probes for MMP-2 activity in a living system.

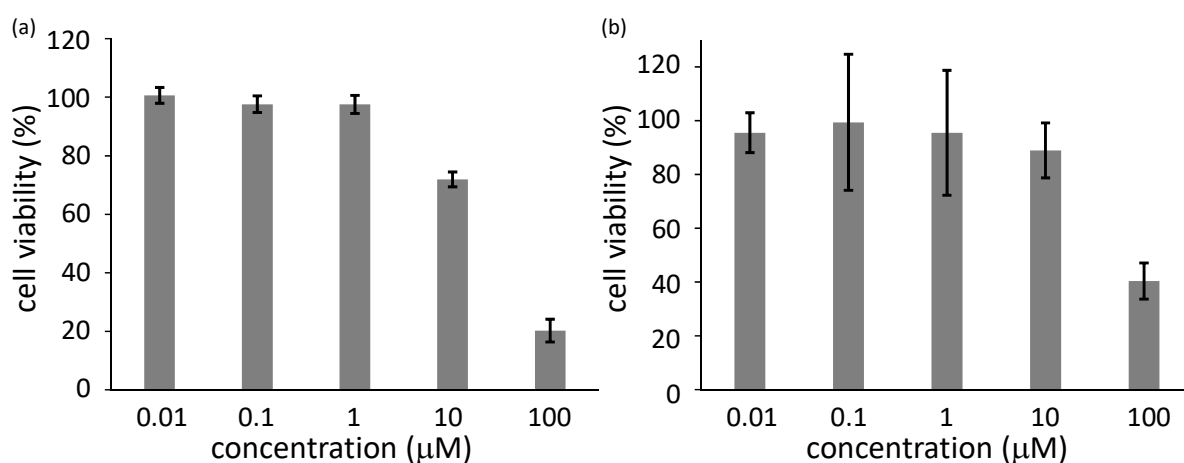


Figure 14. MTT assay of the viability of HT-1080 cells after treated with (a) **AlNc-pep-PEG** and (b) **SiNc-pep-PEG** for 24 h. Mean values and standard deviation (n = 6).

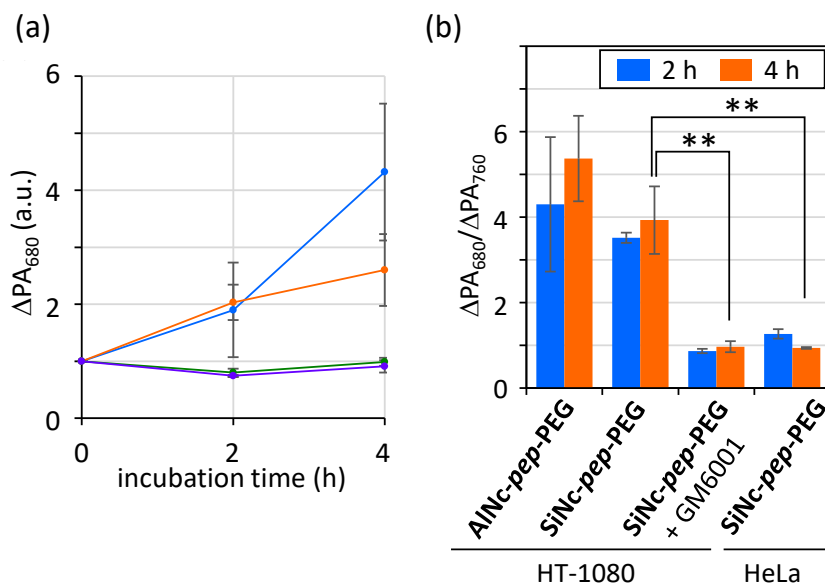


Figure 15 (a) PA signal intensity change at 680 nm (ΔPA_{680}) during the incubation of HT-1080 with AINc-pep-PEG (blue), SiNc-pep-PEG (orange), SiNc-pep-PEG and GM6001 (green), and the incubation of HeLa with SiNc-pep-PEG (purple). Dyes (30 μ M) and GM6001 (100 μ M). (b) Ratio of PA intensity change ($\Delta PA_{680}/\Delta PA_{760}$) under the same incubation conditions shown in (a). Averages and standard deviations ($n = 3$) for cell experiments are shown. Two-tailed Student's t -test: ** $P < 0.05$.

***In vivo* PA imaging**

To verify the MMP-2-responsive enhancement of PA signal intensity *in vivo*, the author then carried out *in vivo* PA imaging experiments using nude mice bearing HT-1080 in the right leg. Because AINc-pep-PEG showed a slightly better $\Delta PA_{680}/\Delta PA_{760}$ value than SiNc-pep-PEG in the cell experiments, the author selected AINc-pep-PEG for *in vivo* PA imaging experiments. After intravenous (*i.v.*) injection of AINc-pep-PEG or AINc-PEG as a control contrast agent, PA images were obtained under photoirradiation at 680 nm and 760 nm (Figures 16, and 17). To reduce the effect of background PA signal intensities mainly generated from hemoglobin, the PA signal intensity at 850 nm was subtracted from those at 680 nm and 760 nm and PA signal intensities $PA_{680,vivo}$ and $PA_{760,vivo}$ of probes at the tumor site were estimated. In the case of AINc-PEG, both $PA_{680,vivo}$ and $PA_{760,vivo}$ slightly increased as the probe accumulated through the enhanced permeation and retention effect.⁴¹ The PA signal intensity ratios ($PA_{680,vivo}/PA_{760,vivo}$) of AINc-PEG at 1 h and 6 h after administration were 1.8 ± 0.4 and 1.9 ± 0.3 , respectively (Figure 16c and Table 6). Because these

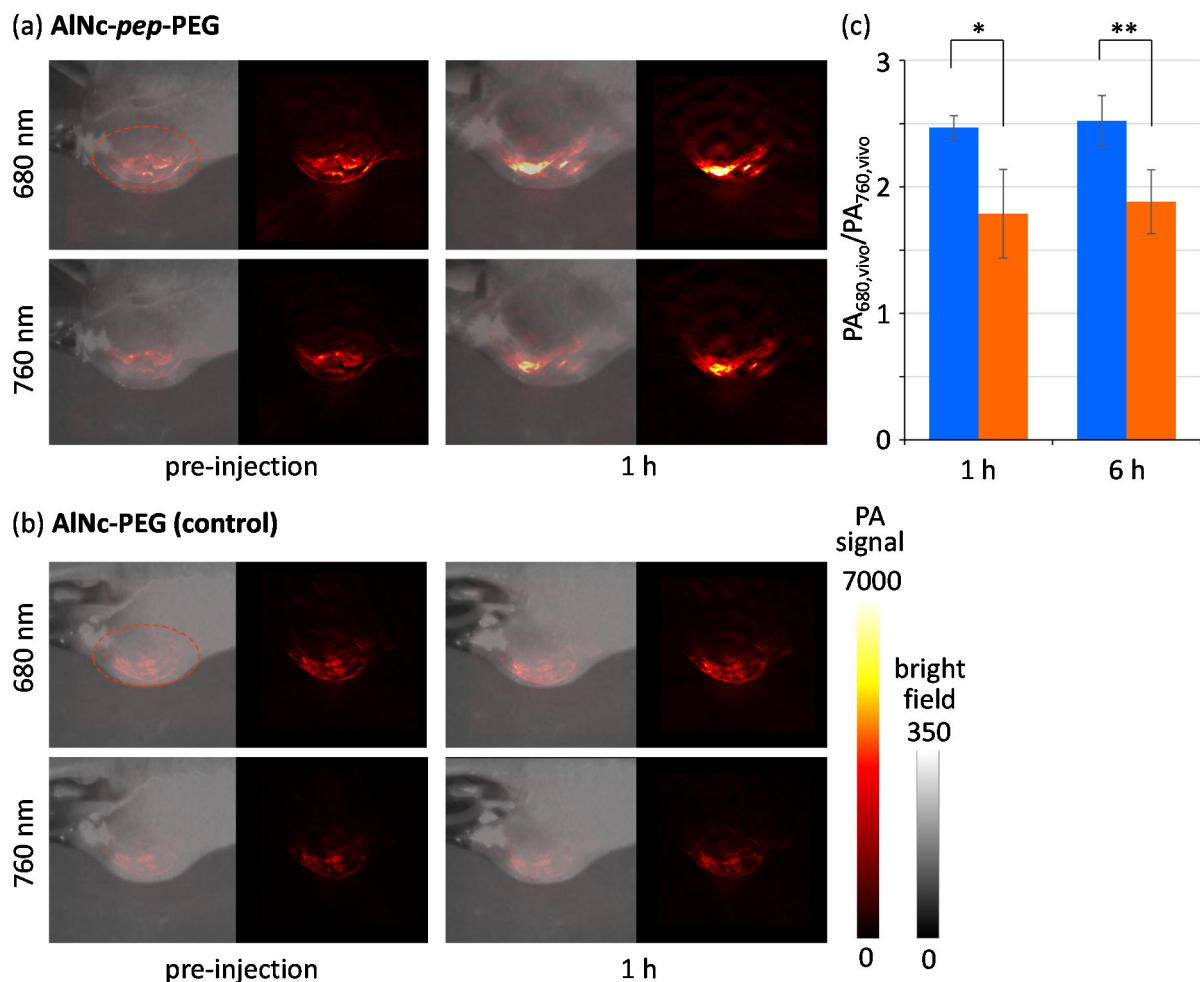


Figure 16. Representative *in vivo* PA images (tumor site) of HT-1080-bearing mice before and 1 h after *i.v.* injection of (a) **AINc-pep-PEG** and (b) **AINc-PEG** (100 μ M in saline, dose: 200 μ L). PA signal image (right) and merged image of PA signal image with bright field image (left). Red dotted circle: tumor site. Photoirradiation wavelength: 680 nm and 760 nm. (c) PA signal intensity ratio ($PA_{680,vivo}/PA_{760,vivo}$) at the tumor site of HT-1080-bearing mice 1 h and 6 h after *i.v.* injection of **AINc-pep-PEG** (blue) and **AINc-PEG** (orange). Averages and standard deviations ($n = 3$) are shown. Two-tailed Student's *t*-test: * $p < 0.1$, ** $p < 0.05$.

Table 6. $PA_{680,vivo}/PA_{760,vivo}$ for *in vivo* PA imaging.

	1 h	6 h
AINc-pep-PEG	2.5 ± 0.1	2.5 ± 0.2
AINc-PEG	1.8 ± 0.4	1.9 ± 0.3

PA signal intensity measured in the region of interest (ellipse shape, 10 mm $\times$ 14 mm axes).

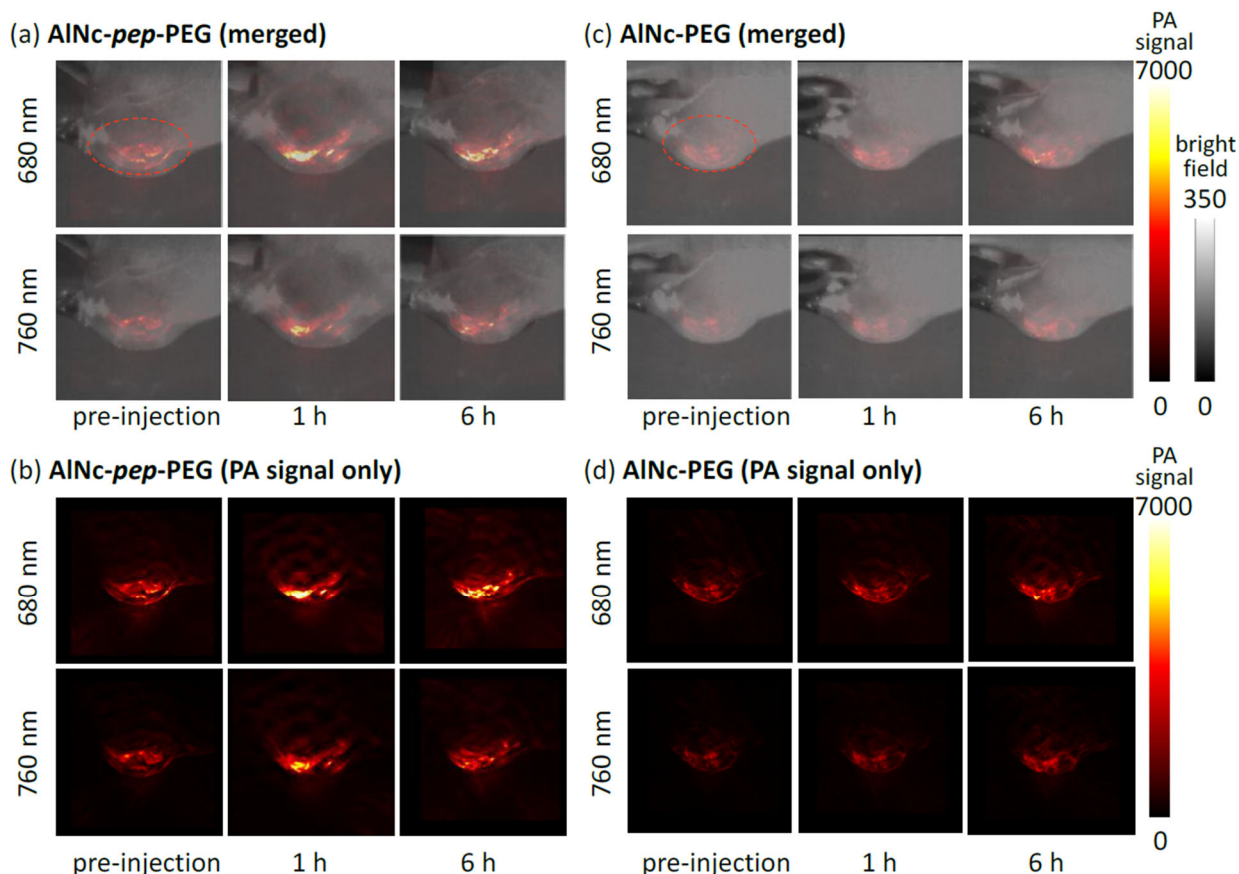


Figure 17. The representative *in vivo* PA images of HT-1080-bearing mice before and after *i.v.* injection of (a) **AINc-pep-PEG** and (b) **AIpc-PEG** (100 μM in saline, 200 μL) for 1 h and 6 h. Photoirradiation wavelength: 680 nm and 760 nm.

$\text{PA}_{680,\text{vivo}}/\text{PA}_{760,\text{vivo}}$ values are comparable to the PA signal intensity ratio ($\text{PA}_{680}/\text{PA}_{760} = 1.3$) of **AINc-PEG** in water, **AINc-PEG** was hardly decomposed in the MMP-2-overexpressed tumor site. On the other hand, the ratio of $\text{PA}_{680,\text{vivo}}/\text{PA}_{760,\text{vivo}}$ for **AINc-pep-PEG** increased to be 2.5 ± 0.1 as early as 1 h after administration and was retained until 6 h after administration ($\text{PA}_{680,\text{vivo}}/\text{PA}_{760,\text{vivo}} = 2.5 \pm 0.2$). These results clearly indicate that PA imaging using **AINc-pep-PEG** can visualize the MMP-2-overexpressed tumor site more clearly than probes without MMP-2 responsiveness. In addition, by considering that the cumulative photoirradiation for 18 min (3 min irradiation at each wavelength) did not lead to the significant decrement of PA signal intensities, **AINc-pep-PEG** and **SiNc-pep-PEG** are good candidates for repeated imaging or diagnosis under continuous photoirradiation *in vivo*.

Conclusion

The author has developed the first example of an enzyme-activatable PA probe based on AlNc and SiNc. AlNc-peptide-PEG conjugate **AlNc-pep-PEG** forms *H*-aggregates through π - π interaction between their concave faces, whereas SiNc-based conjugate **SiNc-pep-PEG** shows weak intermolecular interaction even in water. After the treatment of probes with MMP-2, the aggregation of the resulting MNc fragments led to an increase in PA₆₈₀, whereas a decrease in PA₇₆₀ was observed. Kinetic experiments supported that both **AlNc-pep-PEG** and **SiNc-pep-PEG** respond smoothly to MMP-2. By monitoring the ratio of $\Delta\text{PA}_{680}/\Delta\text{PA}_{760}$, the MMP-2-specific response of probes was confirmed through both enzymatic reactions and *in vitro* cell experiments. Furthermore, at low concentration of MMP-2 (<5 μM), the author confirmed that **AlNc-pep-PEG** and **SiNc-pep-PEG** are applicable as potential “turn-on”-type ratiometric probes for MMP-2 activity. In *in vivo* PA imaging experiments, the author confirmed that the MMP-2-activatable probe **AlNc-pep-PEG** successfully visualized the MMP-2-overexpressed tumor tissues. By assuming their activatable property as well as photostability, MNc-peptide-PEG conjugates will be applicable as PA contrast agents for diagnosis and pharmacokinetic evaluation under continuous photoirradiation.

Experimental

General

Materials and Methods

(3-Aminopropyl)dimethylethoxysilane, 4-dimethylaminopyridine (DMAP), copper sulfate, dichloromethane (CH_2Cl_2), pyridine, *N,N*-dimethylformamide (DMF) Dulbecco's modified Eagle's medium (DMEM), Eagle's minimum essential medium (EMEM), fetal bovine serum (FBS), and 1% penicillin/streptomycin (P/S) were purchased from Fujifilm-Wako Pure Chemicals Industries, Ltd. (Japan). Indocyanine green (ICG) and (3-(4,5-dimethylthazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Tokyo Chemical Industry Co., Ltd. (Japan). 1-Ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) was purchased from Watanabe Chemical Industries, Ltd. (Japan). Sodium chloride (NaCl), dimethylsulfoxide (DMSO), and phosphate buffered saline (PBS) were purchased from nacalai tesque (Japan). Dialysis membrane, Spectra/Por 6 (molecular weight cutoff (MWCO): 3.5K and 25K) was purchased from Spectrum Laboratories Inc. (Rancho Dominguez, CA, USA). Before use, pyridine was distilled over CaH_2 . Other reagents and solvents were used as received. **AcNc-OH** and **SiNc-OH** were prepared according to the reported methods.³⁶

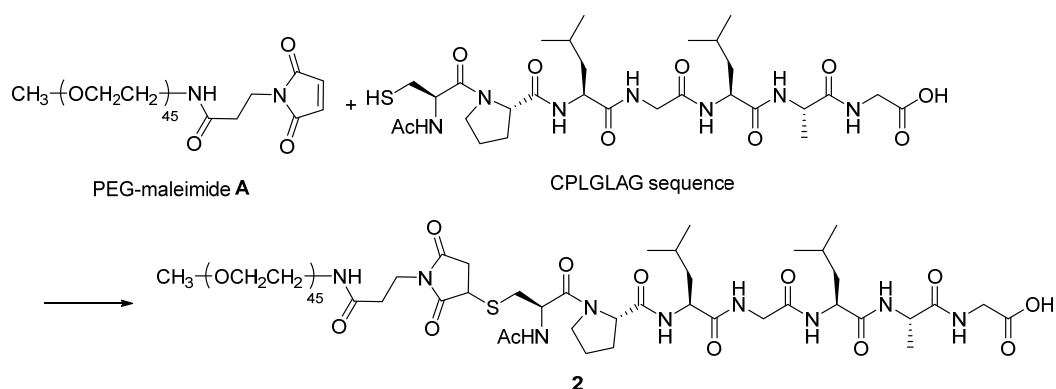
UV-vis absorption spectra were recorded by UH5300, Hitachi High Technologies, Japan. Mass spectra were measured by Ultraflex III (MALDI-TOF, Bruker Co., USA). Dynamic light scattering was measured by FPAR-1000 (Otsuka Electronics Co., Ltd., Japan). At least 3 h before measurement, the sample solutions were diluted in water (Milli-Q, 200 μM) and filtered (syringe filter, 0.45 μm pore size, PVDF). The measurements were performed at scattering angles of 90° at 25°C and the number distribution was summarized.

Photoacoustic (PA) signals of samples in water (Milli-Q, concentration: 3.0×10^{-5} M (dye)) or cell suspension were measured in borosilicate glass cell (volume: ca. 200 μL , length: 1 mm, width: 10 mm) according to the reported procedure.¹⁵ By irradiating a sample solution with pulse laser light, the ultrasonic wave generated from the sample was detected with a piezoelectric element. The PA signal intensity was determined by amplifying the detected signal with a high-speed preamplifier, followed by acquiring the amplified signal with a digital oscilloscope. A pulse laser (nitrogen laser (GL-3300)-dye laser (GL-301),

Photon Technology International, USA, 3-ethyl-2-[7-(3-ethyl-2(3H)-benzoxazolylidene)-1,3,5-heptatrienyl]benzoxazolium iodide (DOTC) for 760 nm and oxazine 170 perchlorate for 680 nm) as a light source, an ultrasonic transducer (Panametrics-NDT V303, Olympus, Japan), an ultrasonic preamplifier (Model 5682, Olympus, Japan), and digital phosphor oscilloscopes (DPO4104, Tektronix, USA) were used for measurement. The measurement conditions are as follows: trigger, detection of PA signal with a photodiode; data acquisition, 128 times (128 pulses). For measurement of dye solutions, the effect of absorbance difference was eliminated by normalizing the PA signal intensity by the absorbance of MNcs and the power of pulse laser.

Synthesis of AlNc-PEG, SiNc-PEG, AlNc-pep-PEG, and SiNc-pep-PEG

Synthesis of peptide-PEG conjugate **2**



PEG derivative **A** having a maleimide moiety was prepared from poly(ethylene glycol) monomethyl ether (MW = 2000, Sigma-Aldrich (USA)) according to the reported method.²⁵ Peptide sequence CPLGLAG having acetyl cap at the *N* terminus was prepared by solid-phase synthesis. A solution of PEG derivative **A** (62 mg, 30 μmol), peptide sequence CPLGLAG (20 mg, 30 μmol), tris(2-carboxyethyl)phosphine hydrochloride (9 mg, 30 μmol , Tokyo Chemical Industry Co., Ltd. (Japan)) in dry MeOH (10 mL) was stirred at room temperature overnight. The organic solvent was removed under reduced pressure and the residue was dissolved in H₂O (5 mL). The aqueous solution was dialyzed against H₂O for 1 day by using Spectra/Por 6 (MWCO = 3.5K). PEG-peptide conjugate **2** (72 mg, 26 μmol , 88%) was obtained as a white solid after lyophilization. In MALDI-TOF mass measurement, the *m/z* distribution center of **2** was around 2000–2100

mass unit (m.u.), ca. 300 m.u. larger than that of PEG (average MW = 2K, distribution center was 1700–1800) (Figure 18). This result points out that the conjugation of PEG-maleimide **A** with larger molecular weight and a peptide sequence did not efficiently proceed under the reaction conditions. Since the unreacted PEG-maleimide **A** cannot react with **Nc-NH₂**, the author used the prepared **2** with a small amount of unreacted PEG-maleimide for the next conjugation reaction.

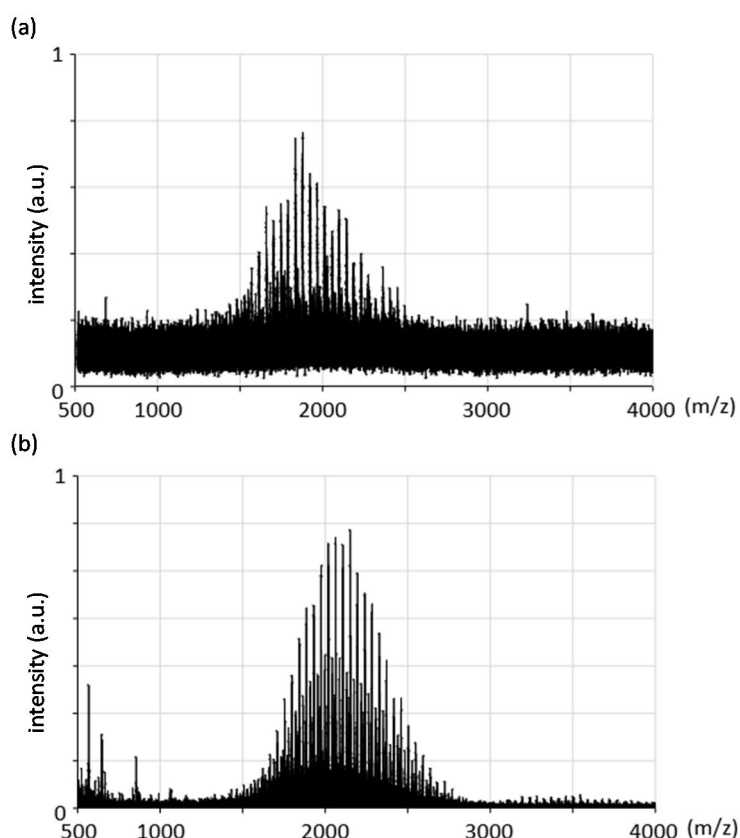


Figure 18. MALDI-TOF mass spectra of PEG-maleimide **A** and peptide-PEG conjugate **2**. Matrix: α -cyano-4-hydroxycinnamic acid (CHCA).

*Synthesis of **AlNc-NH₂** and **SiNc-NH₂***

In 200 mL flame-dried two-necked round-bottom flask, a solution of **AlNc-OH** (30 mg, 40 μ mol) and (3-aminopropyl)dimethylethoxysilane (38 μ L, 0.20 mmol) in pyridine (100 mL) was refluxed under N₂ atmosphere overnight. After removal of solvent under reduced pressure, the residue was dissolved in small amount of CH₂Cl₂. The organic layer was washed with CuSO₄ aqueous solution (5 mL) and dried over

MgSO₄. After removal of solvent under reduced pressure, **AlNc-NH₂** (crude product, 12 mg, ~36%) was obtained as a green solid. Further transformation was carried out without purification, because **AlNc-NH₂** stored in a freezer gradually decomposed and aggregated to form insoluble materials. Signals were not observed in NMR measurements due to aggregation. The parent peak of **AlNc-NH₂** was observed by MALDI-TOF mass spectroscopy (matrix: 2,5-dihydroxybenzoic acid (DHB)), together with inseparable dimeric byproduct (Figure 19). **SiNc-NH₂** was prepared by the similar synthetic method for **SiNc-OH** (~42% yield, a green solid) and used for further transformation without purification. The parent signal of **SiNc-NH₂** was not detected in MALDI-TOF mass spectrum, and signals were not observed in NMR measurements due to aggregation. Because of their low solubility in common solvents, UV-vis absorption spectra were not measured.

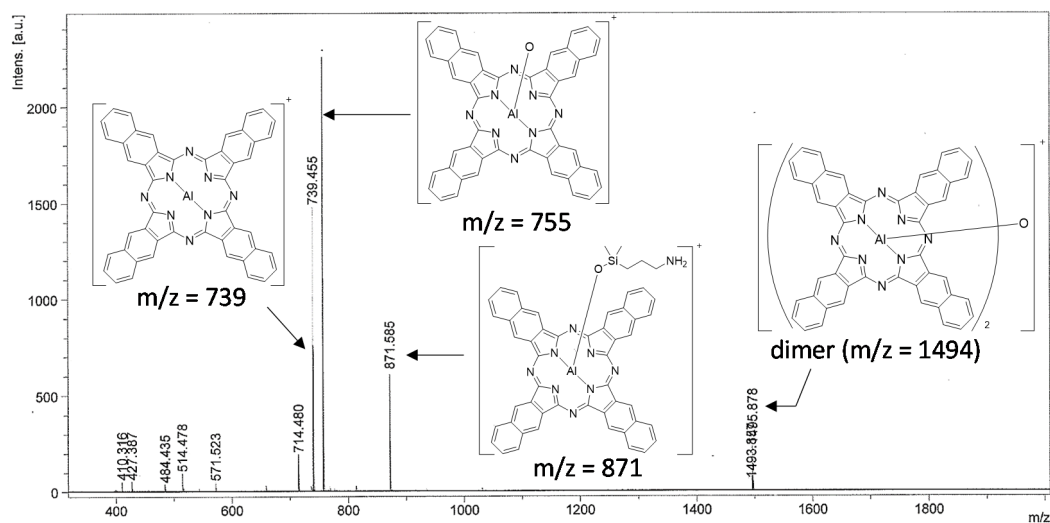


Figure 19. MALDI-TOF mass spectrum of **AlNc-NH₂**. Matrix: 2,5-dihydroxybenzoic acid (DHB).

Synthesis of AlNc-PEG, SiNc-PEG, AlNc-pep-PEG, and SiNc-pep-PEG

In 50 mL flame-dried two-necked round-bottom flask, dry CH₂Cl₂ (5 mL), compound **1**⁴² (23 mg, 11 μmol), EDC·HCl (9 mg, 44 μmol), a solution of DMAP (0.3 mg, 2.5 μmol) in CH₂Cl₂ (100 μL) were successively added and the resulting mixture was stirred at room temperature for 1 h. To this solution was added **AlNc-NH₂** (10 mg, 11 μmol) and the resulting mixture was stirred at room temperature overnight. The organic solvent was removed under reduced pressure and the residue was dissolved in water (5 mL). The

suspension was centrifuged and the supernatant was dialyzed against water for 1 day by using Spectra/Por 6 (MWCO = 25K). **AlNc-PEG** (15 mg, 5.1 μmol , 46%) was obtained as a green solid after lyophilization. **SiNc-PEG** (51% yield, a green solid) was prepared from **SiNc-NH₂** by the similar synthetic method for **AlNc-PEG**. **AlNc-pep-PEG** and **SiNc-pep-PEG** were obtained as a green solid in 58% and 43% yields, respectively, by the similar synthetic method for **AlNc-PEG** and **SiNc-PEG**, in which compound **2** was used instead of **1**. The parent signals of MNC-PEG conjugates and MNC-peptide-PEG conjugates were detected by MALDI-TOF mass spectrometry (Figures 20 and 21). Although PEG signals were strongly detected in ¹H NMR, the signals of MNC skeleton were not observed in CDCl₃, d₆-DMSO, and D₂O, probably because of their amphiphilicity and aggregation.

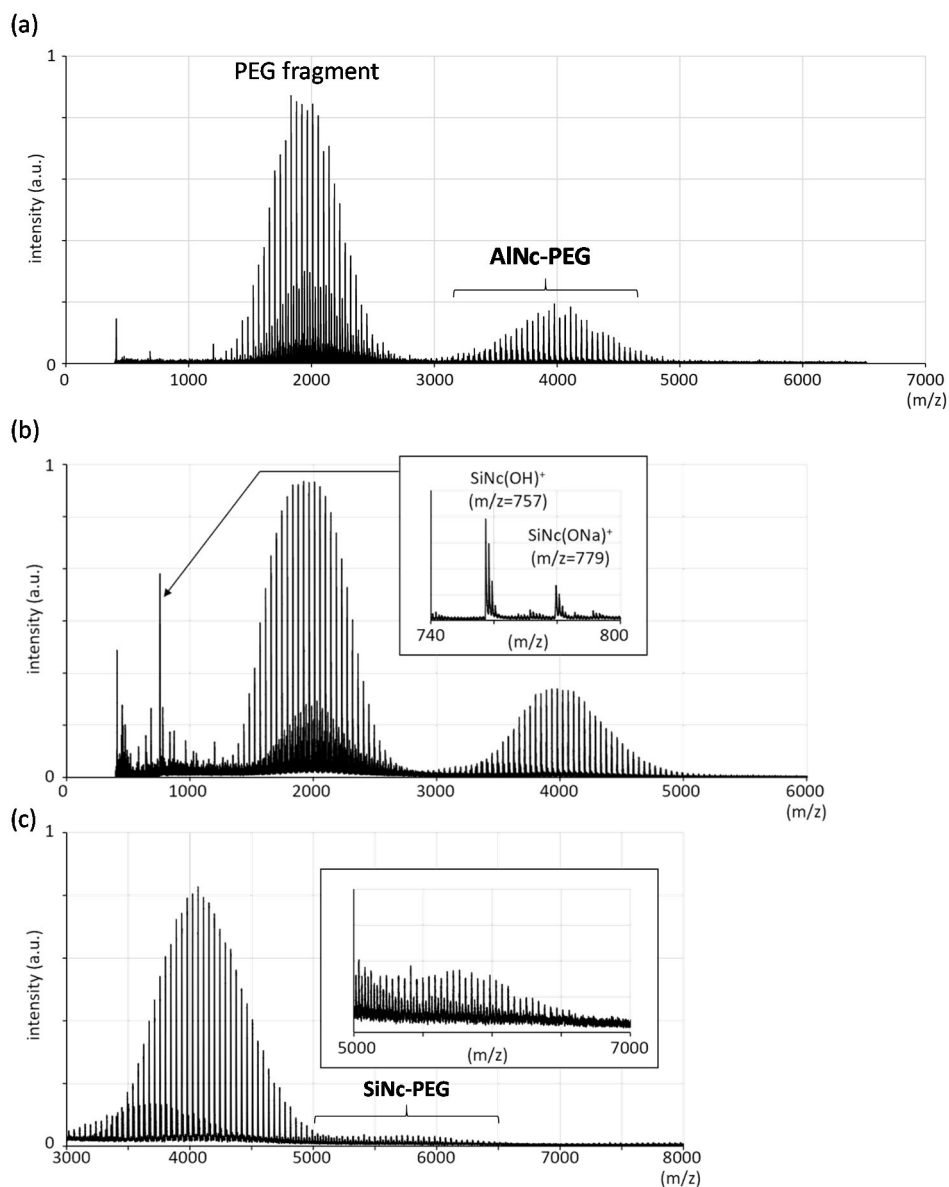


Figure 20. MALDI-TOF mass spectra of (a) AlNc-PEG and (b) SiNc-PEG. Matrix: 2,5-dihydroxybenzoic acid (DHB).

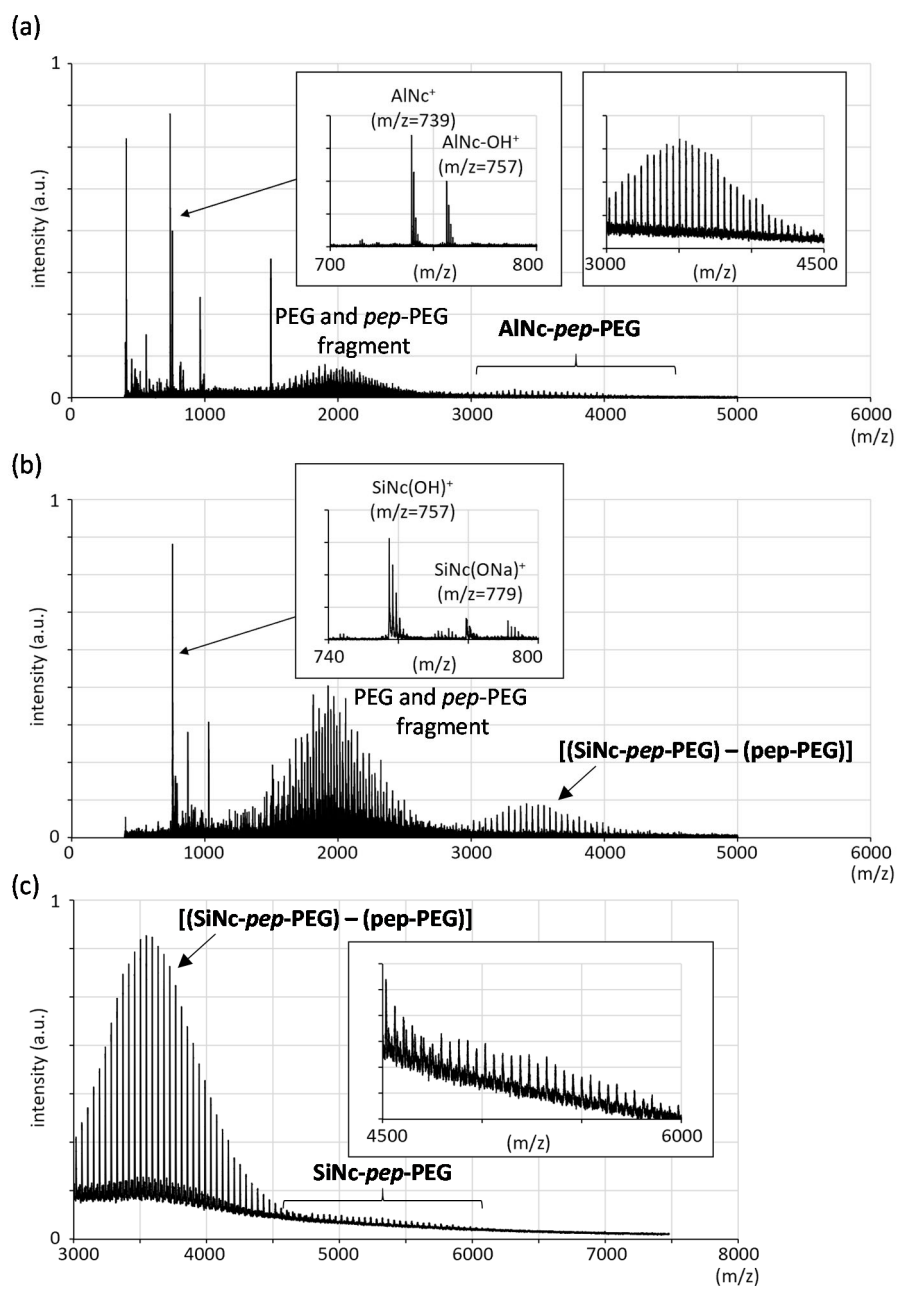


Figure 21. MALDI-TOF mass spectra of (a) **AlNc-pep-PEG** and (b) **SiNc-pep-PEG**. Matrix: 2,5-dihydroxybenzoic acid (DHB).

Photostability of AlNc-*pep*-PEG and SiNc-*pep*-PEG

AlNc-*pep*-PEG, SiNc-*pep*-PEG, and ICG were dissolved in phosphate buffer solution (pH = 7.2, 3 mL, 15 μ M). To minimize the influence of the background signal, the absorbance of all solutions was adjusted to approximately 1.0. All solutions were transferred into quartz cells and irradiated using a LED photoirradiation apparatus (CL-1501, Asahi Spectra Co., Ltd., Japan) equipped with LED head unit (CL-H1-730-9-1, λ_{max} = 737 nm, half-bandwidth = 20 nm, power = 25 mW at the sample level). The time-dependent photobleaching was monitored by measuring the absorbance at the maximum.

Enzyme responsiveness

Recombinant human MMP-2 (3 μ L, 5.0 μ M, PeproTech Inc., USA) in Milli-Q was added to a solution of dyes (5.0 μ M, 3 mL) in PBS (pH = 7.2) and the resulting mixture ([MMP-2] = 5.0 nM) was incubated at 37 °C. The enzymatic reaction was monitored by measurement of absorbance. For evaluation of enzyme-specificity of dyes, dyes (30 μ M, 3 mL) in PBS (pH = 7.2) were incubated with enzyme (3 μ L, 5.0 μ M) at 37 °C. Before and after 2 h incubation, PA signal intensities were measured at 680 nm and 760 nm. Recombinant human MMP-7 (BioLegend Inc., USA), MMP-9 (RayBiotech Inc., USA), PLE (porcine liver esterase, Roche, Switzerland), lipase AK Amano (Fujifilm-Wako Pure Chemicals Industries, Ltd., Japan), and GM6001 (inhibitor, 100 μ M, Cayman Chemical, USA) were used. For evaluation of enzyme kinetics, see the Supporting Information.

Cell experiments

Cell culture

Human cervical epithelial adenocarcinoma cell line (HeLa) and Human sarcoma cell line (HT-1080) were purchased from American Type Culture Collection and National Institutes of Biomedical Innovation, Health and Nutrition, respectively. HeLa and HT-1080 cells were cultured in DMEM and EMEM supplemented with 10% FBS and 1% P/S, respectively. The cells were cultured well-humidified incubator with 5% CO₂ and 95% air at 37 °C.

Cell experiment

HeLa and HT-1080 cells were seeded into 12-well plate at a density of 5×10^4 cells/well. The cells were incubated at 37 °C for 24 h for cell attachment, and incubated with dyes (30 μ M, 1 mL) in DMEM (for HeLa) and EMEM (for HT-1080) with 10% FBS and 1% P/S at 37 °C for 2 h or 4 h. After incubation, cells were detached by cell scraper and the cell suspension was used for PA signal measurement. For inhibition experiment, cells were incubated with **SiNc-pep-PEG** (30 μ M) and GM6001 (100 μ M).

In vivo PA imaging

Female BALB/c mice in 6–9 weeks of age were purchased from Japan SLC, Inc., Japan. The mice were housed at the Institute of Laboratory Animals at Graduate School of Medicine of Kyoto University. All animal experiments were approved by the Animal Research Committee of Kyoto University and carried out with its guidelines.

Female BALB/c mice were chosen to prepare the tumor-bearing mice by injection of HT-1080 cells (1.0 – 1.3×10^6 cells) in the right leg of each mouse. The tumor size was carefully monitored in the Radiation Biology Center, Kyoto University. The tumor-bearing mice were treated with **AlNc-pep-PEG** and **AlNc-PEG** (100 μ M in 200 μ L saline) through *i.v.* injection. *In vivo* PA imaging was performed on Nexus 128 scanner manufactured by Endra Life Sciences (Ann Arbor, MI, USA). PA images were obtained under photoirradiation at 680 nm, 760 nm, and 850 nm at indicated time points. The PA intensities at 680 nm, 760 nm, and 850 nm in the region of interest (ellipse shape, 10 mm $\times$ 14 mm axes) were calculated by OsiriX according to the instruction. To omit the effect of PA signal from hemoglobin, the author calculated the PA signal intensities $PA_{680,vivo}$ at 680 nm and $PA_{760,vivo}$ at 760 nm by the subtraction of PA signal intensity at 850 nm from PA signal intensities at 680 nm and 760 nm, respectively. For each probe, two sets of three mice were conducted to confirm the reproducibility.

TEM observation

Transmission electron microscopy (TEM, JEM-1400, JEOL Ltd., Japan) was used to visualize the morphology of dried self-assemblies. Sample solution was dropped onto a TEM copper grid covered with a carbon film (200 mesh, Nisshin EM, Japan) and dried for 3 h.

Dynamic Light Scattering

Hydrodynamic diameters of MNcs before/after MMP-2 treatment were measured by DLS and are summarized in Figure 10 and Table 3. Conditions: MNC-peptide-PEG conjugate conc. $[S]_0 = 200 \mu\text{M}$, MMP-2 conc. $[E]_0 = 5 \text{ nM}$, temp. = 37°C .

Enzyme responsiveness and Michaelis-Menten kinetics

Michaelis constant K_m and maximum rate $V_{\max}$ were determined by monitoring the time-dependent decrement of absorbance of MNC-peptide-PEG conjugates (Figure 13). The absorbance of both **AlNC-pep-PEG** and **SiNC-pep-PEG** conjugates increased proportionally in the range of 0–100 μM (Figures 13a and 13b). Based on this property, the author estimated the conversion of MNC-peptide-PEG conjugates during the MMP-2 treatment. The initial rate V ($\mu\text{M min}^{-1}$) of MMP-2-mediated reaction was determined from the absorption decrement (MMP-2 conc.: $[E]_0 = 4.2 \text{ nM}$, temp.: 37°C). The absorbance decrement was monitored every 1 min for 10 min and the initial reaction rate V was obtained from the slope of the linear fitting (conc. (μM) vs time (min)). The relationship between the initial reaction rate V and the substrate concentration ($[S]_0$, μM) was plotted in Figures 13c and 13d. From the Lineweaver-Burk plot (Figures 13e and 13f, $1/V$ vs $1/[S]_0$), Michaelis constant K_m and maximum rate $V_{\max}$ were calculated. The turnover number k_{cat} was determined from $[E]_0$ and $V_{\max}$.

MTT assay

HT-1080 cells were seeded into a 96-well microtiter plate at a density of 5.0×10^4 cells/well. The cells were incubated at 37°C for 24 h for cell attachment, and for another 24 h with different concentration of dyes (0,

0.01, 0.1, 1, 10, 100 μM) in EMEM with 10% FBS and 1% P/S. The cells were washed with PBS ($\text{pH} = 7.2$) twice. A solution of MTT (200 μL , 0.5 mg/mL) in EMEM with 10% FBS and 1% P/S was added to each well. After incubation at 37 $^{\circ}\text{C}$ for 4 h, the medium was carefully removed and the formazan crystals were dissolved in DMSO (200 μL) with gentle agitation for 5 min. The absorbance at 550 nm was measured using 800TSTM Absorbance Microplate Reader (BioTek Instruments, USA) to evaluate cell viability. IC_{50} of MNC-peptide-PEG conjugates are around 30–70 μM .

References

- (1) M. Maturi, E. Locatelli, I. Monaco, M. C. Franchini, *Biomater. Sci.* **2019**, 7, 1746–1775.
- (2) J. Weber, P. Beard, S. E. Bohndiek, *Nat. Methods* **2015**, 13, 639–650.
- (3) L. V. Wang, S. Hu, *Science* **2013**, 335, 1458–1462.
- (4) S. Wang, X. Zhang, *ChemBioChem* **2021**, 22, 308–316.
- (5) V. Gujrati, A. Mishra, V. Ntziachristos, *Chem. Commun.* **2017**, 53, 4653–4672.
- (6) H.-B. Cheng, Y. Li, B. Z. Tang, J. and Yoon, *Chem. Soc. Rev.* **2020**, 49, 21–31.
- (7) C. Ji, W. Cheng, Q. Yuan, K. Müllen, M. Yin, *Acc. Chem. Res.* **2019**, 52, 2266–2277.
- (8) X. Wang, Z. Geng, H. Cong, Y. Shen, B. Yu, *ChemBioChem* **2019**, 20, 1628–1636.
- (9) J. Li, J. Rao, K. Pu, *Biomaterials* **2018**, 155, 217–235.
- (10) P. Cheng, K. Pu, *ACS Appl. Mater. Inter.* **2020**, 12, 5286–5299.
- (11) H. J. Knox, J. Chan, *Acc. Chem. Res.* **2018**, 51, 2897–2905.
- (12) Q. Miao, K. Pu, *E Bioconjugate Chem.* **2016**, 27, 2808–2823.
- (13) H. D. Lu, T. L. Lim, S. Javitt, A. Heinmiller, R. K. Prud’homme, *ACS Comb. Sci.* **2017**, 19, 397–406.
- (14) Y. Zhang, M. Jeon, L. J. Rich, H. Hong, J. Geng, Y. Zhang, S. Shi, T. E. Barnhart, P. Alexandridis, J. D. Huizinga, M. Seshadri, W. Cai, C. Kim, J. F. Lovell, *Nat. Nanotech.* **2014**, 9, 631–638.
- (15) B.-D. Zheng, Q.-X. He, X. Li, J. Yoon, J.-D. Huang, *Coord. Chem. Rev.* **2021**, 426, 213648.
- (16) X. Li, B.-D. Zheng, X.-H. Peng, S.-Z. Li, J.-W. Ying, Y. Zhao, J.-D. Huang, J. Yoon, *Coord. Chem. Rev.* **2019**, 379, 147–160.
- (17) H. Huang, D. Wang, Y. Zhang, Y. Zhou, J. Geng, U. Chitgupi, T. R. Cook, J. Xia, J. F. Lovell, *Bioconjugate Chem.* **2016**, 27, 1574–1578.
- (18) K. Miki, T. Inoue, Y. Kobayashi, K. Nakano, H. Matsuoka, F. Yamauchi, T. Yano, K. Ohe, *Biomacromolecules* **2015**, 16, 219–227.
- (19) K. Miki, A. Enomoto, T. Inout, T. Nabeshima, S. Saino, S. Shimizu, H. Matsuoka, K. Ohe, *Biomacromolecules* **2017**, 18, 249–256.
- (20) M. T. M. Choi, P. P. S. Li, D. K. P. Ng, *Tetrahedron* **2000**, 56, 3881–3887.

- (21) S. Tai, N. Hayashi, *J. Chem. Soc. Perkin Trans. 2* **1991**, 1275–1279.
- (22) Y. Yang, G. A. Rosenberg, *Brain Res.* **2015**, *1623*, 30–38.
- (23) G. B. Fields, *Front. Immunol.* **2019**, *10*, 1278.
- (24) C. E. Brinckerhoff, L. M. Matrisian, *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 207–214.
- (25) L. Yin, H. Sun, H. Zhang, L. He, L. Qiu, J. Lin, H. Xia, Y. Zhang, S. Ji, H. Shi, M. Gao, *J. Am. Chem. Soc.* **2019**, *141*, 3265–3273.
- (26) J. Peng, Q. Yang, Y. Xiao, K. Shi, Q. Liu, Y. Hao, F. Yang, R. Han, Z. Qian, *Adv. Funct. Mater.* **2019**, *29*, 190004.
- (27) J. Levi, S.-R. Kothapalli, S. Bohndiek, J.-K. Yoon, A. Dragulescu-Andrasi, C. Nielsen, A. Tisma, S. Bodapati, G. Goweishankar, X. Yan, C. Chan, D. Starcevic, S. S. Gambhir, *Clin. Cancer Res.* **2013**, *19*, 1494–1502.
- (28) J. Levi, S. R. Kothapalli, T.-J. Ma, K. Hartman, B. T. Khuri-Yakub, S. S. Gambhir, *J. Am. Chem. Soc.* **2010**, *132*, 11264–11269.
- (29) W. E. Ford, M. A. J. Rodgers, L. A. Schechtman, J. R. Sounik, B. D. Rihter, M. E. Kenney, *Inorg. Chem.* **1992**, *31*, 3371–3377.
- (30) K. Sato, K. Ando, S. Okuyama, S. Mariguchi, T. Ogura, S. Totoki, H. Hanaoka, T. Nagaya, R. Kokawa, H. Takakura, M. Nishimura, Y. Hasegawa, P. L. Choyke, M. Ogawa, H. Kobayashi, *ACS Cent. Sci.* **2018**, *4*, 1559–1569.
- (31) K.-H. Song, M. Gu, M.-S. Kim, H.-J. Kwon, H. Rhee, H. Han, M. Cho, *J. Phys. Chem. Lett.* **2015**, *6*, 4314–4318.
- (32) L. Luan, J. Chen, L. Ding, J. Wang, X. Cheng, Q. Ren, W. Liu, *Chem. Lett.* **2012**, *41*, 1012–1014.
- (33) F. Ito, T. Inoue, D. Tomita, T. Nagamura, *J. Phys. Chem. B* **2009**, *113*, 5458–5463.
- (34) H. Wang, X. Li, B. W.-C. Tse, H. Yang, C. A. Thorling, Y. Liu, M. Touraud, J. B. Chouane, X. Liu, M. S. Roberts, X. Liang, *Theranostics* **2018**, *8*, 1227–1242.
- (35) B. E. Schaafsma, J. S. D. Mieog, M. Hutteman, J. R. van der Vorst, P. J. K. Kuppen, C. W. G. M. Löwik, J. V. Frangioni, C. J. H. van de Velde, A. L. Vahrmeijer, *J. Surg. Oncol.* **2011**, *104*, 323–332.

- (36) A. F. Prado, L. Pernomian, A. Azevedo, R. A. P. Costa, E. Rizzi, J. Ramos, A. F. Paes Leme, L. M. Bendhack, J. E. Tanus-Santos, *Redox Biol.* **2018**, *18*, 181–190.
- (37) W. J. Akers, B. Xu, H. Lee, G. P. Sudlow, G. B. Fields, S. Achilefu, W. B. Edwards, *Bioconjugate Chem.* **2012**, *23*, 656–663.
- (38) X. Zhang, J. Bresee, G. B. Fields, W. B. Edwards, *Bioorg. Med. Chem. Lett.* **2014**, *24*, 3786–3790.
- (39) Y. Wu, A. Wang, X. Ding, F.-J. Xu, *ACS Appl. Mater. Inter.* **2017**, *9*, 127–135.
- (40) T. A. Giambernardi, G. M. Grant, G. P. Taylor, R. J. Hay, V. M. Maher, J. J. McCormick, R. J. Klebe, *Matrix Biol.* **1998**, *16*, 483–496.
- (41) Y. Matsumura, H. Maeda, *Cancer. Res.* **1986**, *46*, 6387–6392.
- (42) H. Huang, T. Yue, Y. Xu, K. Xu, H. Xu, S. Liu, J. Yu, J. Huang, *J. Appl. Polym. Sci.* **2015**, *132*, 42360.

Chapter 2

Photoacoustic Signal Enhancement of Al- and Si-Phthalocyanines Caused by Photoinduced Cleavage of Water-Soluble Axial Ligand

Abstract

Although a few examples of activatable photoacoustic (PA) contrast agents based on MPcs have been reported, their “turn-on” properties are considered to be affected by the concentration of endogenous biological and chemical substances. To avoid such influences, PA signal enhancement triggered by controllable external stimuli such as photoirradiation is required. Aluminum and silicon phthalocyanines derivatives bearing water-soluble poly(ethylene glycol) as axial ligands, which formed vesicles in water, released their axial ligands under continuous photoirradiation. The photoinduced cleavage of axial ligands in water caused the aggregation of these phthalocyanines, resulting in enhancement of PA signal intensities.

Introduction

Metallophthalocyanine (MPc), one of cyclic tetrapyrrole family, is a photostable near-infrared (NIR) dye with high molar extinction coefficient (ϵ) ($\sim 1.0 \times 10^5$) and low photoluminescence quantum yield (Φ) (< 0.10), converting the excited energy into strong photoacoustic (PA) wave efficiently.¹⁻³ Because of these properties, many researchers focused their attentions on MPcs as a promising NIR photosensitizer for practical PA imaging.⁴⁻⁶ Although the reported MPc-containing PA contrast agents can generate strong PA wave, their “always-on” properties may cause the strong background signals from non-targeted tissues and blood vessels. Hence, activatable PA contrast agents based on MPcs whose PA signal intensity can be enhanced by biological stimuli have been developed.^{2a,7,8} Pu and co-workers developed MPcs bearing oxidant-responsive water-soluble side chains as an activatable PA contrast agent.⁹ Yoon and co-workers prepared protein-responsive MPc-based photosensitizers for tumor theranostics.¹⁰ Toriumi, Uchiyama, and co-workers reported benzipthalocyanines which change PA signal intensities through tautomerization, as a potent activatable PA contrast agent.¹¹ In Chapter 1, the author has developed naphthalocyanines bearing enzyme-responsive peptide and water-soluble poly(ethylene glycol) (PEG) as an axial ligand and demonstrated their application as enzyme-activatable contrast agents to *in vivo* PA tumor imaging.¹² However, the turn-on properties of these photosensitizers should be affected by the concentration of endogenous oxidant, protein, and enzyme in the target tissues. To avoid the influence of endogenous biological and chemical substances, the PA signal enhancement triggered by controllable external stimuli such as photoirradiation is ideal. In the course of the author’s continuous investigation of MPcs bearing a water-soluble axial ligands, the author found that photoirradiation induced the cleavage of axial PEG ligands in aluminum phthalocyanine (AlPc) and silicon phthalocyanine (SiPc) derivatives, resulting in the aggregation of MPcs to enhance PA signal intensity (Figure 1). Because bulky axial ligands grafted on MPcs can more efficiently suppress their aggregation compared with the peripheral decoration of MPcs, AlPc and SiPc were selected as a backbone of activatable photosensitizers.¹³ Although some of “always-on”-type water-soluble MPcs were applied to tumor therapy under photoirradiation,¹⁴ there is no example of activatable PA probes consisting of PEG and MPcs under photoirradiation. Here, the author describes the preparation of self-assemblies consisting of PEG-grafted

MPc derivatives ($M = \text{Al}, \text{Si}$) and the evaluation of photoactivatable enhancement of their PA signal intensity. Based on the theoretical calculation and the isolation of cleaved axial ligands, the author proposes that the photoinduced cleavage of axial ligands proceeds through the intermolecular electron transfer between two MPcs, followed by hydrolysis.

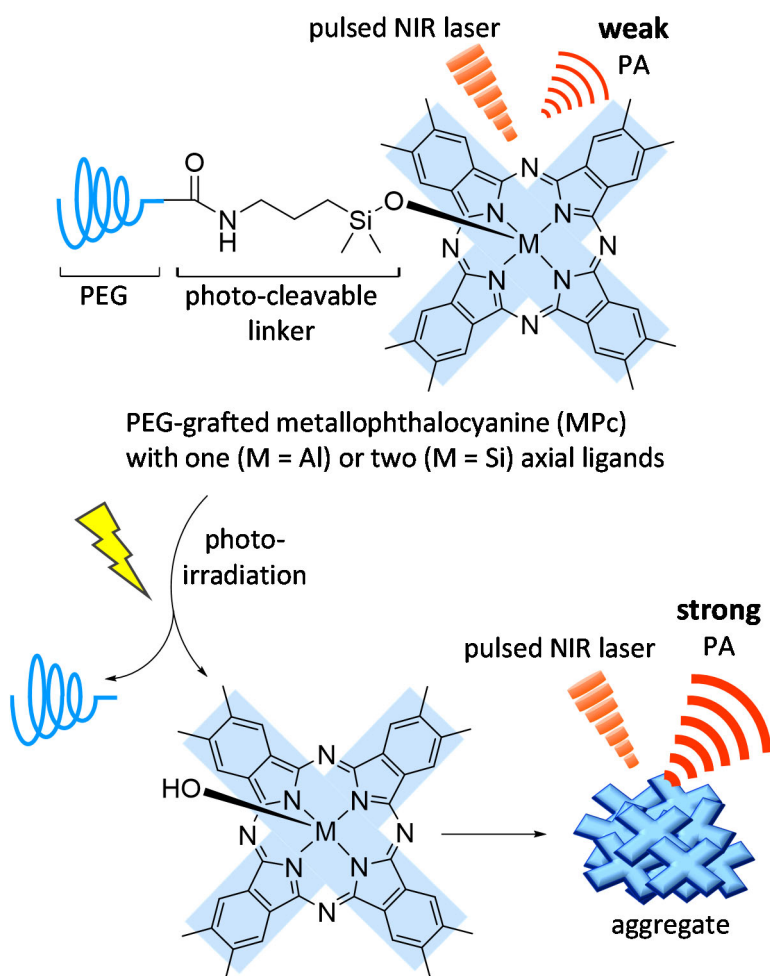
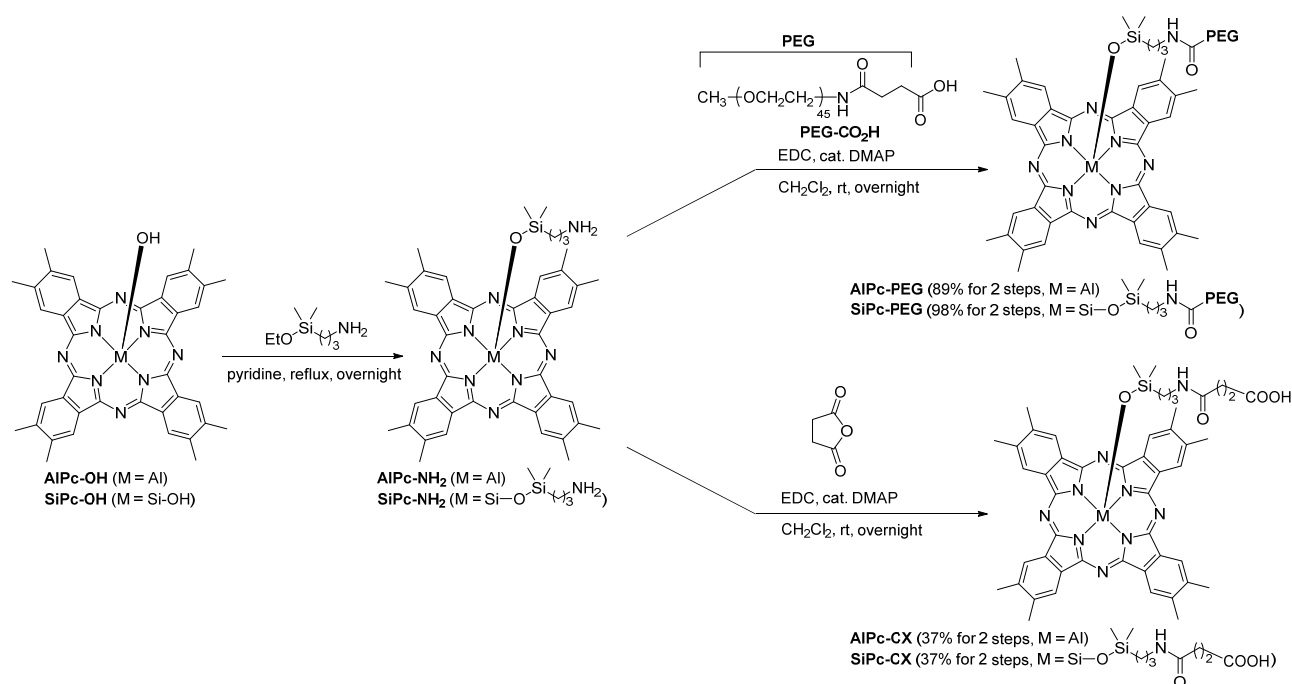


Figure 1. Photoinduced MPc aggregation for PA signal enhancement. AlPc ($M = \text{Al}$) and SiPc ($M = \text{Si}$) were used. Two PEG axial ligands were incorporated in SiPc, but one of two ligands is omitted for clarity.

Results and discussion

Synthesis of PEGylated Pcs.

Water-soluble MPc derivatives **AlPc-PEG** and **SiPc-(PEG)₂** were synthesized from metalloctamethylphthalocyanine **MPc-OH** bearing hydroxy group(s) (M = Al and Si) (Scheme 1). According to the conventional phthalocyanine synthesis,¹⁵ **MPc-OH** was prepared from phthalonitrile or diiminoisoindoline species. **AlPc-CX** and **SiPc-CX** without PEG chains were also synthesized from **MPc-OH**.



Scheme 1. Synthesis of water-soluble MPc derivatives from **MPc-OH**.

Preparation of self-assemblies of MPc-PEG.

Self-assemblies of **AlPc-PEG** were prepared as follows (Figure 2).¹⁶

Method A: Powdered **AlPc-PEG** (1.0 mg) was dissolved in Milli-Q water (3.5 mL) under sonication (100 W, 28 kHz). The resulting solution was filtered by using syringe filter (pore size: 0.45 μm) and the filtrate (**A-AlPc**) was kept in the dark at room temperature for 3 h before measurement.

Method B: Powdered **AlPc-PEG** (1.0 mg) was dissolved in CH_2Cl_2 (2.0 mL) in a 25 mL round-bottom

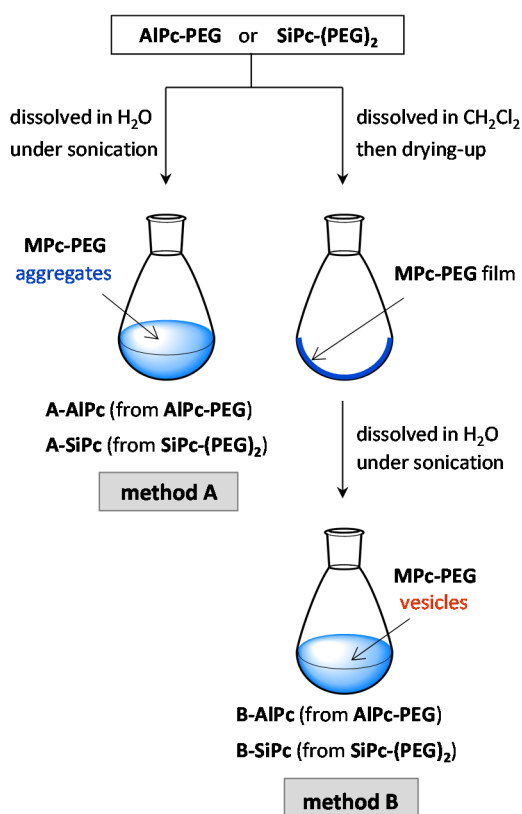


Figure 2. Preparation of **MPc-PEG** self-assemblies through **methods A and B**.

flask. The solvent was removed by gentle blowing of nitrogen gas to prepare **AlPc-PEG** film. To this flask, Milli-Q water (3.5 mL) was added and the resulting mixture was sonicated (100 W, 28 kHz). The resulting solution was filtered by using syringe filter (pore size: 0.45 μm) and the filtrate (**B-AlPc**) was kept in the dark at room temperature for 3 h before measurement.

Self-assemblies **A-SiPc** and **B-SiPc** were similarly prepared from **SiPc-(PEG)₂**. The average particle size of self-assemblies is summarized in Table 1.

In UV-vis absorption spectra of **AlPc-PEG** and **SiPc-(PEG)₂** in DMF, strong signals attributed to the Q-band

Table 1. Particle size distribution and morphology of self-assemblies composed of **MPc-PEG**.

self-assembly	method	D _{DLS} (nm)	D _{TEM} (nm)	morphology
A-AlPc	method A	301 $\pm$ 71	143 $\pm$ 35	aggregate
B-AlPc	method B	307 $\pm$ 67	312 $\pm$ 149	vesicle
A-SiPc	method A	221 $\pm$ 44	139 $\pm$ 27	aggregate
B-SiPc	method B	321 $\pm$ 61	572 $\pm$ 193	vesicle

of their monomeric forms were observed at 679 nm ($\epsilon = 2.4 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) and 678 nm ($\epsilon = 8.0 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$), respectively (Figures 3a and 3b). In the case of **AlPc-PEG**, a broadened signal around 750 nm was observed besides Q-band, which indicated that a small part of **AlPc-PEG** form aggregates even in DMF solution. In regard to UV-vis spectra of **A-AlPc** and **A-SiPc** prepared according to **method A**, the absorption signals of monomeric MPc decreased and new signals which are assigned as *J*-aggregates¹⁷ were observed at 722 nm

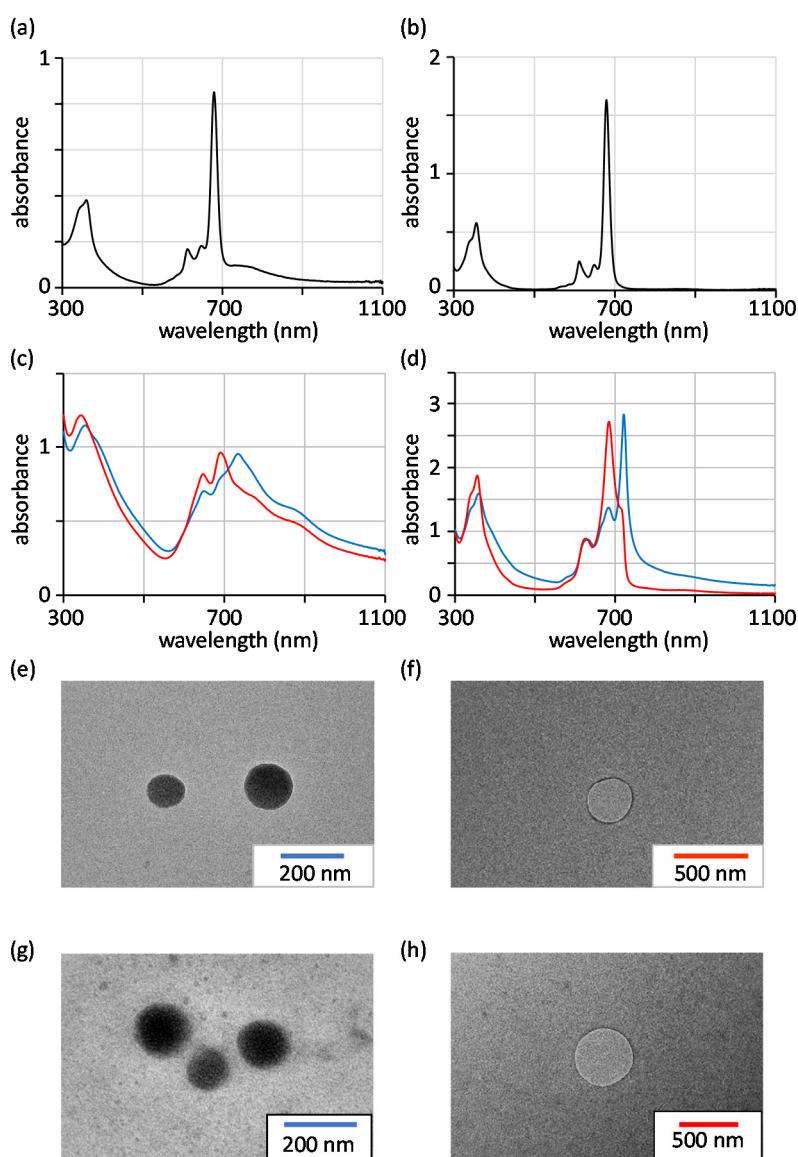


Figure 3. UV-vis absorption spectra of (a) **AlPc-PEG** ($3.5 \times 10^{-5} \text{ M}$) and (b) **SiPc-(PEG)₂** ($2.0 \times 10^{-5} \text{ M}$) in DMF. UV-vis absorption spectra of (c) **A-AlPc** (blue) and **B-AlPc** (red) ($1.8 \times 10^{-4} \text{ M}$) and (d) **A-SiPc** (blue) and **B-SiPc** (red) ($1.0 \times 10^{-4} \text{ M}$) in water. TEM images of self-assemblies of (e) **A-AlPc**, (f) **B-AlPc**, (g) **A-SiPc** and (h) **B-SiPc**.

and 733 nm, respectively (Figures 3c and 3d, blue lines). Interestingly, in UV-vis spectra of **B-AIPc** and **B-SiPc**, which were prepared according to **method B** shown in above, the absorbance of *J*-aggregates decreased and the sharp monomeric signal (*Q*-band) increased. In transmission electron microscope (TEM) observation, whereas **A-AIPc** and **A-SiPc** formed spherical nanometer-size aggregates (Figures 3e and 3g), it was observed that **B-AIPc** and **B-SiPc** formed vesicles (Figures 3f and 3h). From dynamic light scattering (DLS) measurement, hydrodynamic diameters of self-assemblies prepared by **methods A** and **B** were estimated to be 220–320 nm (Table 1).

Crystallographic analyses of AlPcs were reported; however, their intermolecular interaction was not discussed in detail.¹⁸ Mizuguchi and co-workers reported the bowl-shaped titanium(IV) phthalocyanine oxide having a small axial ligand.¹⁹ In the crystal structure, Ti(O)Pcs were packed through the concave-concave interaction (*H*-aggregation) and the convex-convex interaction (*J*-aggregation). Considering Mizuguchi's observation and UV-vis spectra shown in Figures 3c and 3d, it was assumed that **AlPc-PEG** which is closed to bowl-shape favors random *H*- and *J*-aggregation²⁰ in **A-AIPc**, whereas **B-AIPc** forms a layer of AlPc dimers with concave-concave interaction (Figure 4a). In contrast, **A-SiPc** favors *J*-aggregate forms with partial overlap of Pcs (*J*-aggregation) because of two large hydrophilic axial ligands. The hydrophilic-hydrophobic-hydrophilic structure of **SiPc-(PEG)₂** facilitates forming vesicles composed of a SiPc monolayer in **B-SiPc** (Figure 4b).

The photostability of self-assemblies was compared with indocyanine green (ICG), which is an approved

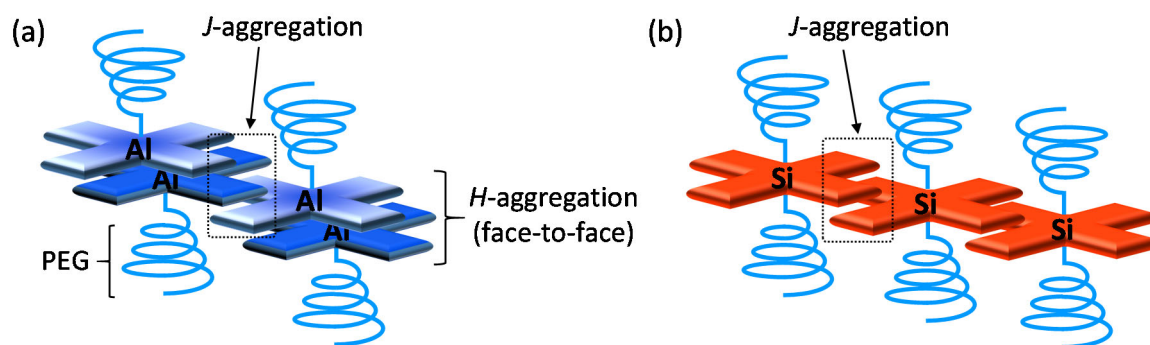


Figure 4. Plausible aggregation modes (*H*- and *J*-aggregates) in vesicles composed of (a) **B-AIPc** and (b) **B-SiPc**.

NIR dye for practical diagnosis.²¹ Under continuous photoirradiation using light-emitting diode (LED) at 25 mW ($\lambda = 730$ nm) for 1 h, the absorbance decrement of ICG was 45%, whereas the absorbance decrement of **A-AIPc** and **A-SiPc** were 3% and 1%, respectively (Figure 5 and Table 2). In contrast, the absorbance decrement of **B-AIPc** and **B-SiPc** were 9% and 12% for 1 h under the identical photoirradiation conditions, respectively, the precipitate being gradually generated for several hours. In MALDI-TOF mass spectra of precipitates, the parent mass numbers of **MPc-PEG** were not observed, but the corresponding fragment mass numbers of **AIPc-OH** and **SiPc-OH** were detected (Figure 6). These results indicate that the axial ligands of **AIPc-PEG** and **SiPc-(PEG)₂** were cleaved by continuous photoirradiation, where **MPc-OH** precipitated due to the low water solubility. Next, the PA signal intensity were monitored before/after continuous photoirradiation.²² As expected, no change of the PA signal intensity of **A-AIPc** and **A-SiPc** was observed,

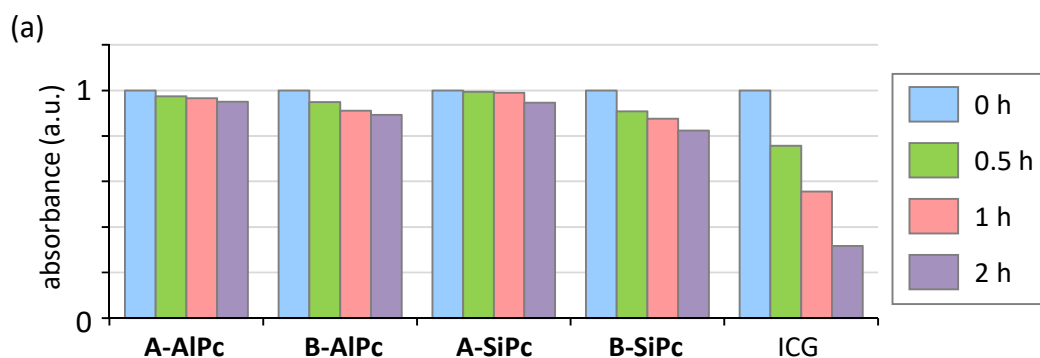


Figure 5. Photostability of ICG and self-assemblies of **A-AIPc**, **B-AIPc**, **A-SiPc**, and **B-SiPc** under continuous photoirradiation at 25 mW ($\lambda = 730$ nm).

Table 2. UV-vis absorbance of MPc self-assembly or ICG under photoirradiation.^a

	photoirradiation time (h)		
	0.5	1	2
A-AIPc	97%	97%	95%
B-AIPc	95%	91%	89%
A-SiPc	99%	99%	95%
B-SiPc	91%	88%	82%
ICG	76%	55%	32%

^aAbsorbance after photoirradiation was normalized by that before photoirradiation and shown as a percentage.

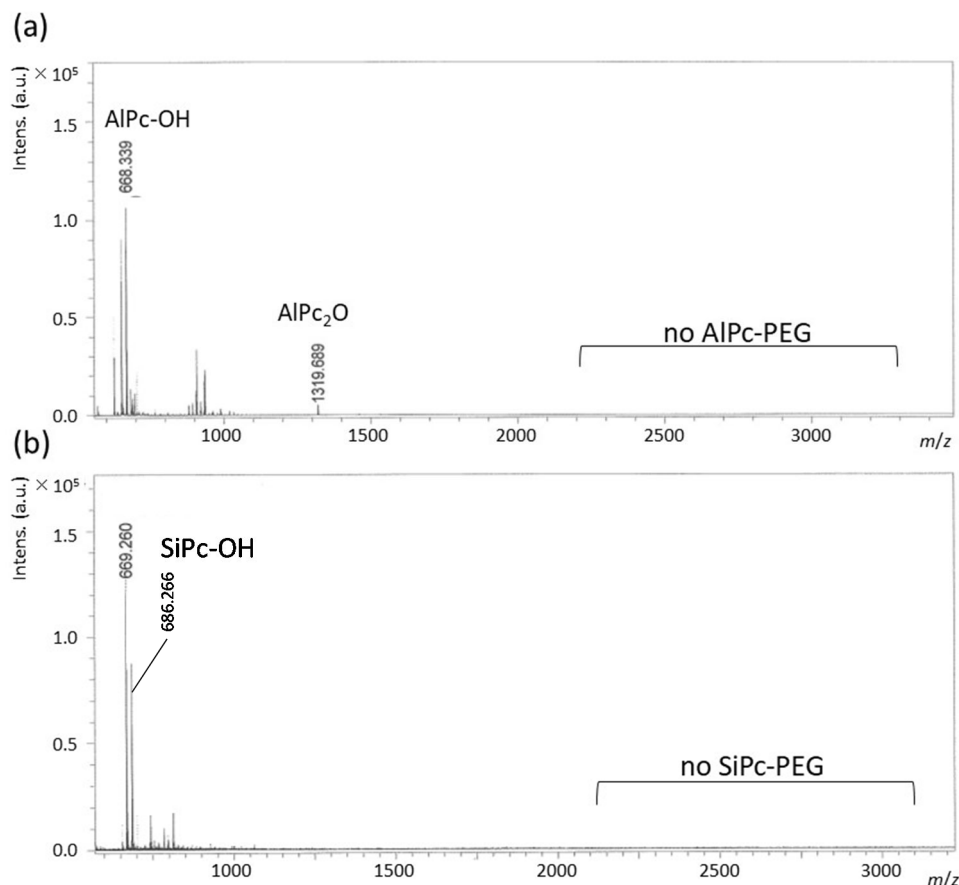


Figure 6. MALDI-TOF spectra of precipitates obtained after photoirradiation ($\lambda = 730$ nm, 25 mW, 6 h) of (a) **AlPc-PEG** and (b) **SiPc-(PEG)₂**. Matrix: 2,2':5',2''-terthiophene.

whereas PA signal intensity of vesicles **B-AlPc** and **B-SiPc** gradually increased during photoirradiation for 10–20 min and kept constant after then (Figure 7). The DLS measurement of the irradiated solutions points out that the hydrodynamic diameters of self-assemblies of **B-AlPc** gradually increased, whereas those of **A-AlPc** showed no significant change (Figure 8). In contrast, PA signal intensities of **B-AlPc** and **B-SiPc** were not changed in the dark for 48 h (Figure 9). These results support that **AlPc-OH** generated from **B-AlPc** under photoirradiation gradually aggregated to form larger nanoparticles, enhancing PA signal intensity. It is well-accepted that the aggregation of π -conjugated dyes enhances the PA signal intensity due to fluorescence quenching.^{4e,6c,23} Although Pc-based probes utilizing aggregation-induced PA signal intensity enhancement have been reported,⁹ to the author's knowledge, this is the first example of photo-activatable PA signal intensity enhancement of MPcs.

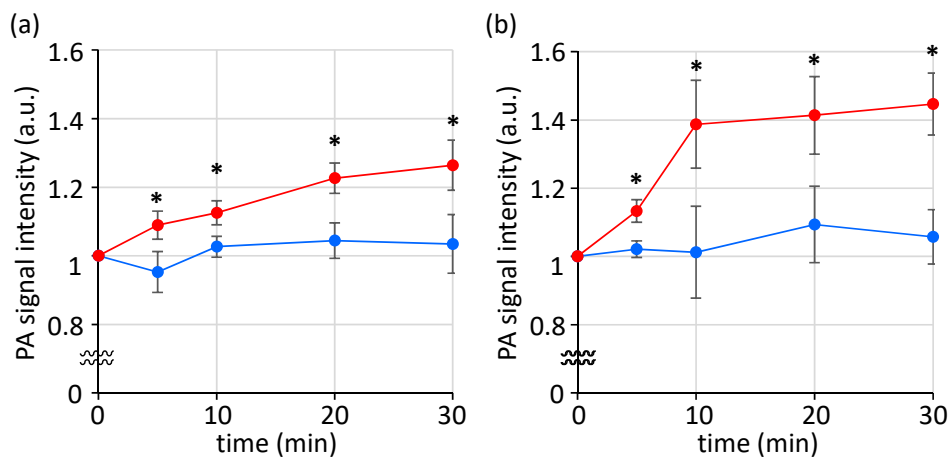


Figure 7. Time-dependent PA signal intensity change of (b) **A-AlPc** (blue) and **B-AlPc** (red), and (c) **A-SiPc** (blue) and **B-SiPc** (red) during continuous photoirradiation at 25 mW ($\lambda = 730$ nm). PA signal intensities were measured using pulsed laser ($\lambda_{\text{ex}} = 680$ nm). Conc.: 1.0×10^{-4} M. The statistical significances were determined using a two-tailed t -test, $*p \leq 0.05$.

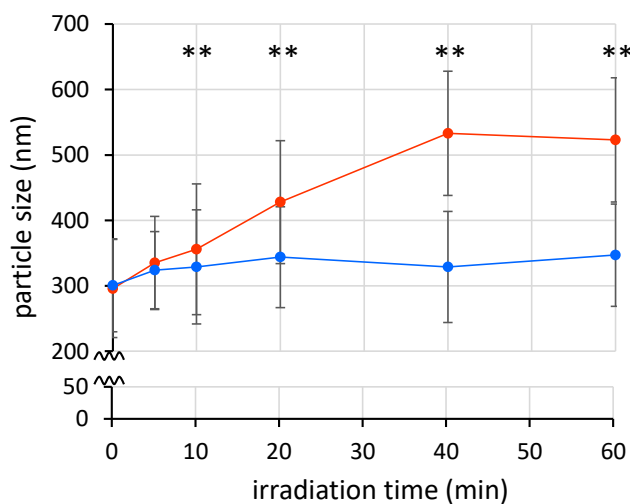


Figure 8. Time-dependent change of nanoparticle size of **A-AlPc** (red) and **B-AlPc** (blue) under photoirradiation monitored by DLS.

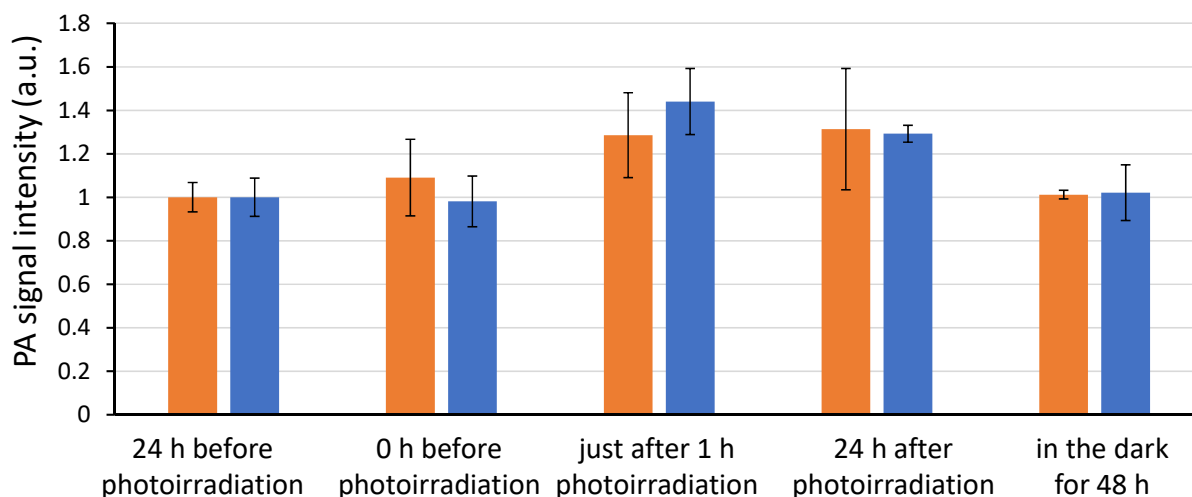


Figure 9. Time-dependent PA signal intensity change of **B-AlPc** (orange) and **B-SiPc** (blue).

The efficiency of photoinduced cleavage of axial ligands in **MPc-PEG** was next examined by measuring UV-vis absorbance. In the case of **AlPc-PEG**, the yields of AlPc aggregates collected by centrifugation after continuous photoirradiation were 18% (for 10 min), 32% (for 30 min), and 34% (for 60 min), respectively (Figure 10a). These results as well as time-dependent PA signal intensity change (Figure 7) and size distribution change (Figure 8) accord to that the cleavage efficiency of axial ligand in **AlPc-PEG** was up to 30% under continuous photoirradiation for 30 min. In contrast, the yields of SiPc precipitates generated from **SiPc-(PEG)₂** were 6% (for 10 min), 6% (for 30 min), and 9% (for 60 min), respectively (Figure 10b).

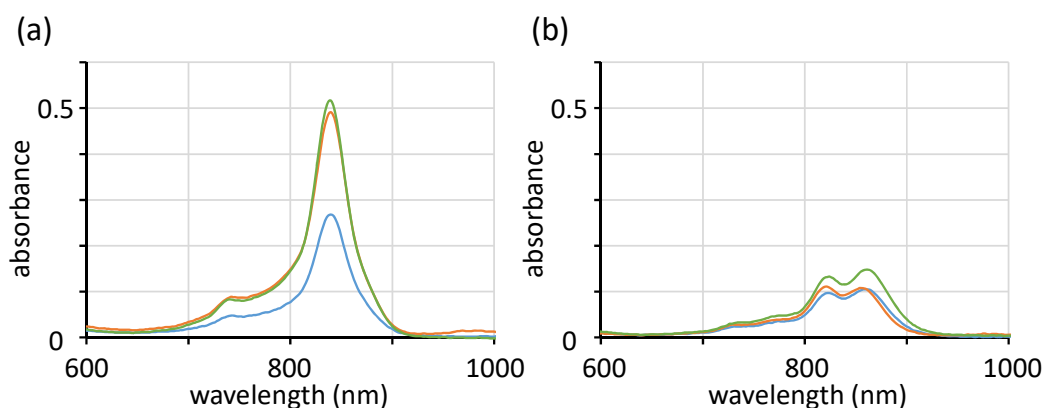


Figure 10. Time-dependent absorbance change of the precipitates (in H_2SO_4) generated from aqueous solutions of (a) **B-AlPc** and (b) **B-SiPc** after continuous photoirradiation for 10 min (blue), 30 min (orange), 60 min (green).

Because of the water solubility of **SiPc-PEG** bearing an unreacted PEG ligand, the yields of SiPc aggregates are much lower than those of AlPc aggregates. It is not clear that the yields of AlPc and SiPc aggregates were saturated at about 30% and 10%, but it may be reasoned that the partial transformation of **MPc-PEG** leads to the morphology change from vesicles to non-photoresponsive MPc aggregates.

To clarify the mechanism for photoinduced cleavage of axial ligands, the author carried out the continuous photoirradiation of their aqueous solutions of water-soluble **AlPc-CX** and **SiPc-CX** for 2 h and measured high-resolution mass spectrometry to detect photodegraded fragments of axial ligands (Figures 11 and 12). The detection of $[\text{C}_9\text{H}_{18}\text{NO}_4\text{Si}]^-$ and $[\text{C}_9\text{H}_{16}\text{NO}_3\text{Si}]^-$ ions in negative ion detection mode supports that the M-O-Si moiety of **AlPc-CX** and **SiPc-CX** was hydrolyzed. Although trialkylsilanol was not observed, the detection of siloxane **A** is sufficient evidence to support the photoinduced cleavage of axial ligands (Figures 13 and 14).

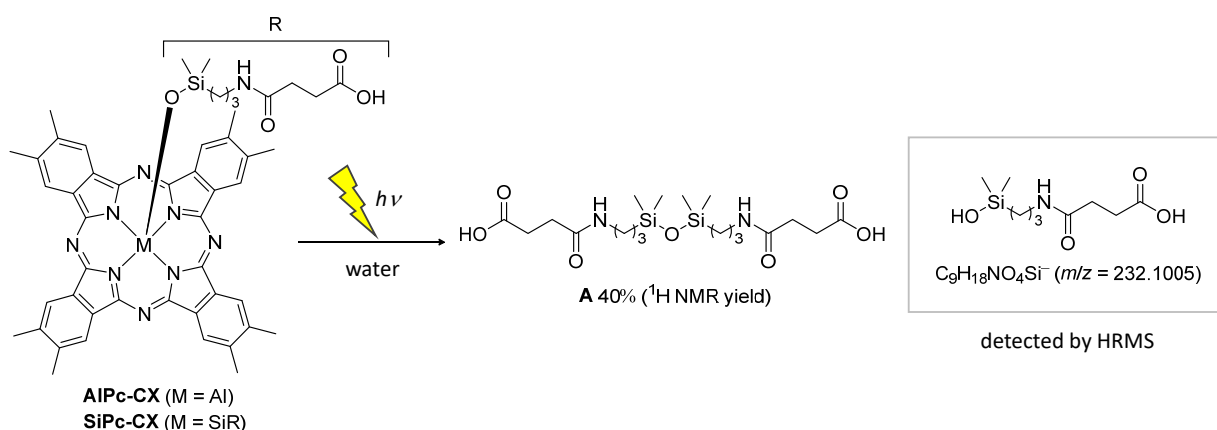


Figure 11. Photoinduced cleavage of **MPc-CX**.

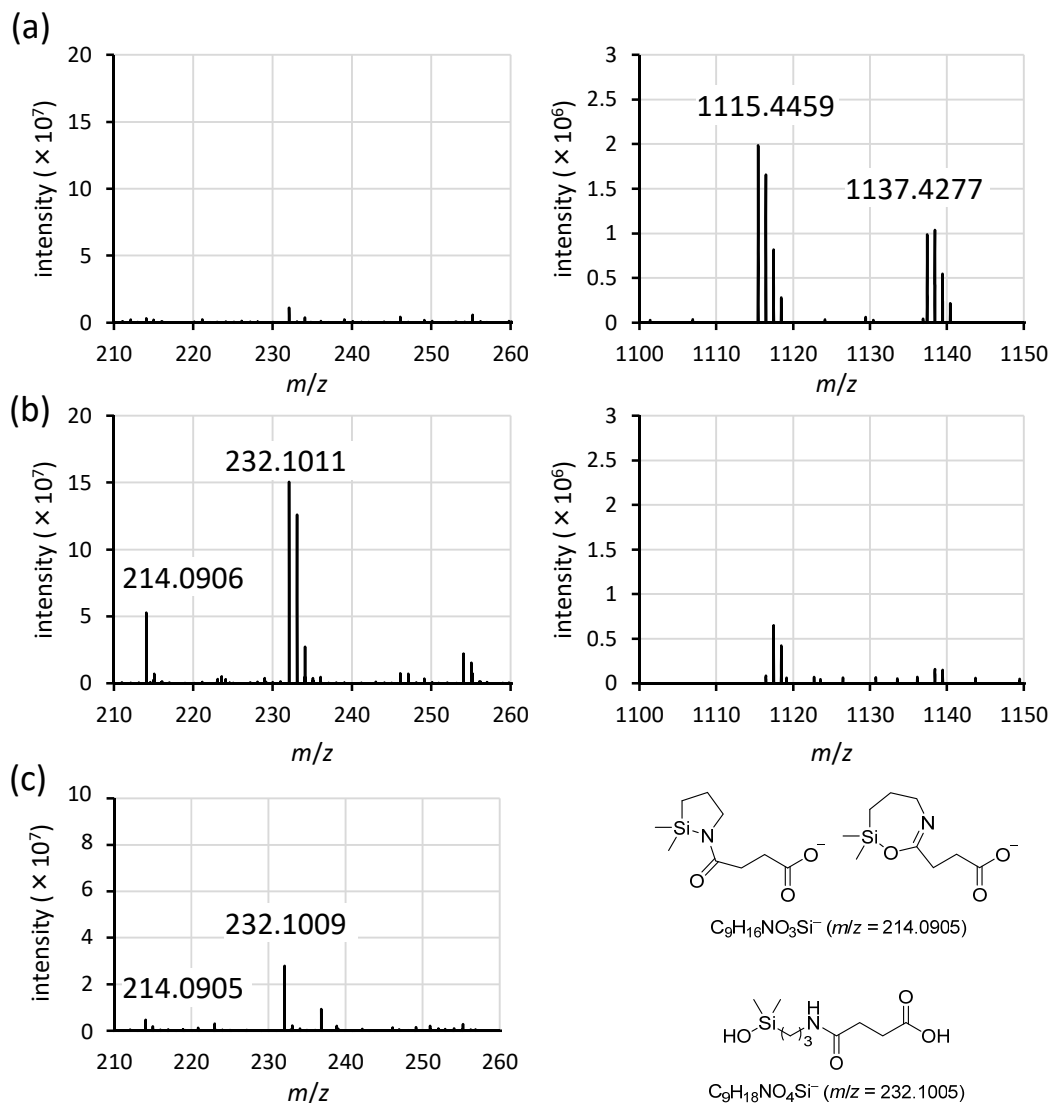


Figure 12. ESI mass spectrometry of a basic solution of **SiPc-CX** ($C_{58}H_{67}N_{10}O_8Si_3^-$: m/z ($[M-H]^-$) = 1115.4451, $C_{58}H_{66}N_{10}NaO_8Si_3^-$: m/z ($[M-2H+Na]^-$) = 1137.4271) (a) before and (b) after photoirradiation. (c) Same signals of axial ligand fragments at 214 ($C_9H_{16}NO_3Si^-$) and 232 ($C_9H_{18}NO_4Si^-$) (m/z) were detected in the resulting mixture of photoirradiated **AlPc-CX** sample.

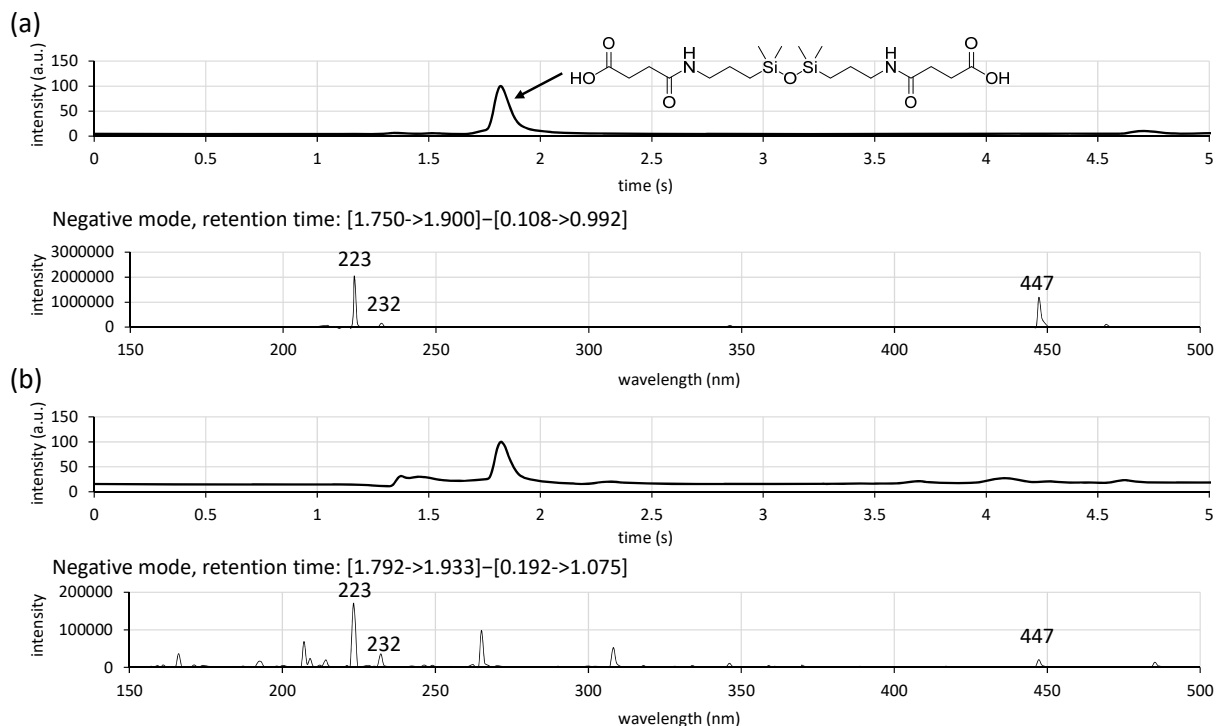


Figure 13. HPLC-MS analyses of (a) authentic siloxane **A** and (b) axial ligand fragment generated from **AlPc-CX**. Upper chart: LC; lower chart: MS (negative ion mode).

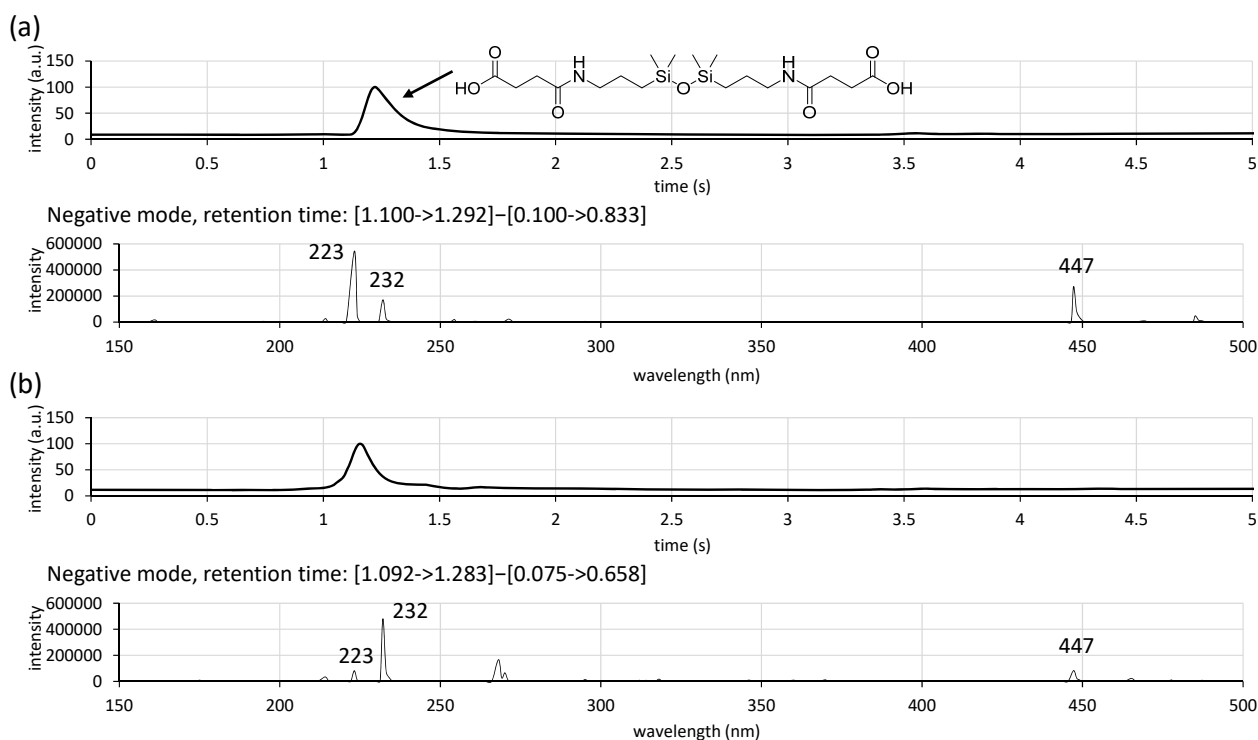


Figure 14. HPLC-MS analyses of (a) authentic siloxane **A** and (b) axial ligand fragment generated from **SiPc-CX**. Upper chart: LC; lower chart: MS (negative ion mode).

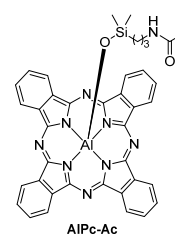
Recently, Burda, Basilion, and co-workers reported that SiPc bearing $\text{OSiMe}_2(\text{CH}_2)_3\text{NH}_2$ and $\text{OSiMe}_2(\text{CH}_2)_3\text{NMe}_2$ as an axial ligand showed photoinduced decomposition.²⁴ Kobayashi and co-workers reported that the axial ligand $\text{OSiMe}_2(\text{CH}_2)_3\text{N}^+\text{R}_3$ in SiPc was gradually hydrolyzed by photoirradiation in the presence of one electron-donating molecules under hypoxic conditions and no cleavage occurred in the absence of one electron-donating molecules, such as sodium ascorbate and L-cysteine, or under normoxic conditions.²⁵ They pointed out that SiPc radical anion was generated through one electron transfer from electron-donating molecules, and then an axial ligand was cleaved by H_2O .²⁶ Because **MPc-PEG** undergoes the photoinduced cleavage in the absence of any external electron donors, the author assumed that MPc radical anion might be generated from **MPc-PEG** through the intermolecular electron transfer between two MPc molecules or the intramolecular electron transfer from an amide moiety to an MPc core. Considering that the vesicle formation is essential for the photoinduced cleavage of axial ligands, the author assumed the intermolecular electron transfer between two **MPc-PEGs** is likely. The plausibility of the intermolecular electron transfer was supported by the density functional theory (DFT) calculation with consideration of the energy-minimized structures of the ground state, the excited state, the radical cation, and radical anion states of an MPc derivative. Consequently, the sum of Gibbs free energies of the ground and excited states is larger than the sum of radical cation and radical anion states (Table 3), indicating that the closely located MPcs in vesicle membrane can lead

Table 3. Gibbs free energy of energy-minimized structures of **AlPc-Ac**^[a].

AlPc-Ac	E_{elec} (hartree)	ΔE_{elec} (eV)	G (hartree)	ΔG (eV)
ground state	-2680.1680203	0	-2679.598945	0
excited state ^[b]	-2680.0470194	+3.29	-2679.479871	+3.24
radical cation state	-2679.9702796	+5.38	-2679.400531	+5.40
radical anion state	-2680.3127757	-3.94	-2679.742092	-3.90

[a] AlPc derivatives bearing acetyl group instead of PEG chain on axial ligand.

[b] The HOMO-LUMO gap was estimated at 0.10465 hartree.

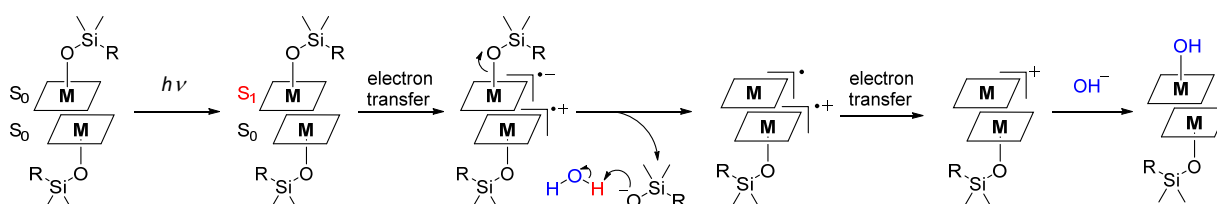


to the intermolecular electron transfer to form radical cation and radical anion pairs under photoirradiation. It has been reported that triplet MPcs ($M = \text{Al}$ and Si) form excimers via triplet-triplet annihilation reaction and then excimers disproportionate to give MPc radical anion and MPc radical cation in protic solvents.²⁷ Although it is not clear whether the photoinduced electron transfer proceeds through photoredox reaction or excimer disproportionation, considering the measured redox potential of **MPc-PEG** (Table 4), the author concludes that the generation of radical anion species through the intermolecular electron transfer is plausible pathway for the photoinduced cleavage of an axial ligand.

The plausible reaction mechanism is depicted in Scheme 2. One of MPcs in a vesicle membrane is excited by photoirradiation. One electron is transferred between closely located two MPc molecules to form a pair of radical cation and radical anion. The axial ligand as an siloxide anion from radical anion is released to generate MPc radical.²⁶ One electron is transferred from the MPc radical to radical cation and the resulting cation species reacts with H_2O or hydroxide to generate water insoluble **MPc-OH**. It is noted that the cleavage of axial ligands in **MPc-PEG** proceeded under both hypoxic and normoxic conditions, although the PA signal generation was slightly suppressed under normoxic conditions (Figure 15). This result also indicates that the intermolecular one electron transfer of **MPc-PEG** is less hampered by oxygen molecules.

Table 4. Redox potentials of **AlPc-PEG** and **SiPc-(PEG)₂** in the ground state or excited state.

	E_{ox}	E_{red}	$E_{0,0}$	E^*_{ox}	E^*_{red}
	(V vs. Fc^+/Fc)		(eV)	(V vs. Fc^+/Fc)	
AlPcPEG	0.782	-0.694	1.776	-0.994	1.082
SiPcPEG	0.343	-1.330	1.805	-1.462	0.475



Scheme 2. Plausible mechanism of photoinduced cleavage of axial ligands.

This result and high photostability of MPcs suggests that the singlet oxygen species ($^1\text{O}_2$)-mediated cleavage of an axial ligand is negligible, although MPc is known to be a photosensitizer for $^1\text{O}_2$ generation (Figure 16).²⁸ Because the photoirradiation efficiently enhanced the PA signal intensity in the absence of additional electron donors, MPcs having a photocleavable axial ligand in this study are one of good candidates as a photoactivatable photosensitizer in tumor theranostics.

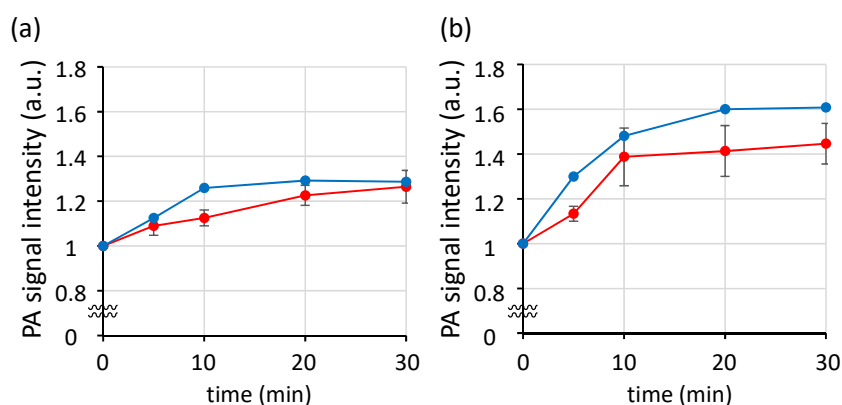


Figure 15. Time-dependent PA signal intensity change of (a) **B-AlPc** and (b) **B-SiPc** under hypoxic (blue) and normoxic (red) conditions during continuous photoirradiation at 25 mW ($\lambda = 730$ nm).

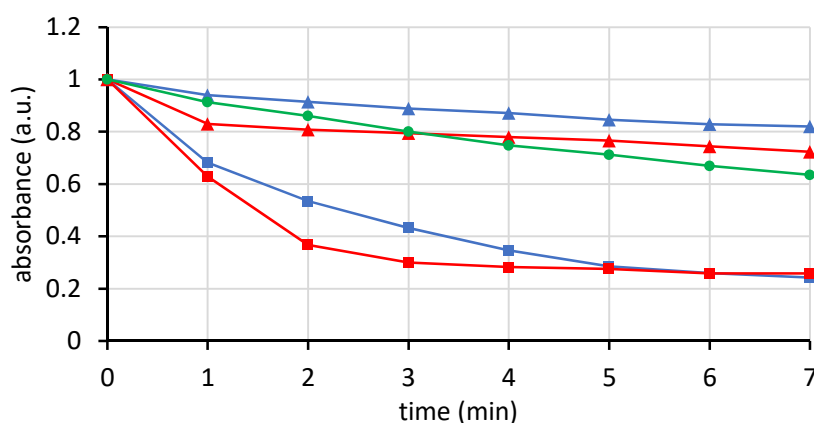


Figure 16. Photobleaching of ADPA in H₂O under irradiation with 2.7 mW at 680 nm. **AlPc-PEG** (triangle) or **SiPc-(PEG)₂** (square) aqueous solutions prepared with **method A** (blue line) or **method B** (red line) were used. Methylene blue (green circle) was used as a control.

Conclusion

In summary, the author has developed phthalocyanine derivatives **AlPc-PEG** and **SiPc-(PEG)₂** bearing water-soluble PEG moieties, which exhibit photoinduced enhancement of PA signal intensities via the cleavage of axial ligands. Vesicles of **MPc-PEG** prepared by **method B** gradually released axial ligand under photoirradiation to generate water insoluble **MPc-OH**, which aggregated to form larger nanoparticles in water, enhancing their PA signal intensities up to 1.4 times. The cleavage of axial ligands in *J*-aggregates of **MPc-PEG** prepared by **method A** was not observed, resulting in no enhancement of PA signal intensity. The most plausible mechanism of the photoinduced cleavage of axial ligands involves the generation of a pair of MPc radical anion and redical cation through the intermolecular one electron transfer between closely located two MPcs, followed by the hydration of formed cation species. Because an amide moiety in an axial ligand is widely applicable to biological research, further modification using photocleavable axial ligand having tumor-targeting molecules will provide highly tumor-specific photoactivatable PA contrast agents in near future.

Experimental

General

Materials and Methods

Poly(ethylene glycol) monomethyl ether (MW = 2000) was purchased from Sigma-Aldrich (USA). 1-Ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) was purchased from Watanabe Chemical Industries, Ltd. (Japan). 1-Pentanol, aluminum chloride (AlCl₃), triethylamine, (3-aminopropyl)dimethylethoxysilane, 4-dimethylaminopyridine (DMAP), and anhydrous dichloromethane (CH₂Cl₂) were purchased from Fujifilm Wako Pure Chemicals Industries, Ltd. (Japan). 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), pyridine, potassium carbonate (K₂CO₃), *N,N*-dimethylformamide (DMF), methanol (MeOH), and succinic anhydride were purchased from Nacalai Tesque (Japan). Dichloromethane (CH₂Cl₂) and diethyl ether (Et₂O) was purchased from Kishida Chemical Co., Ltd. (Japan). Tetrachlorosilane (SiCl₄), quinoline, and indocyanine green (ICG) were purchased from Tokyo Chemical Industry Co., Ltd. (Japan). Dialysis membrane Spectra/Por 6 (molecular weight cutoff (MWCO): 25 kDa) was purchased from Spectrum Laboratories Inc. (Rancho Dominguez, CA, USA). Before use as a reaction solvent, pyridine and DMF were distilled over CaH₂, and MeOH was distilled over Mg. Other reagents and solvents were used as received.

UV-vis absorption spectra were recorded by UV-vis spectrophotometer (UH5300, Hitachi High Technologies, Japan).

Dynamic light scattering (DLS) (FPAR-1000, Otsuka Electronics Co., Ltd., Japan) measurements were performed at scattering angles of 90° at 25 °C.

PA signals of samples in H₂O (3.0×10^{-5} M) were measured according to the reported procedure^{12b} or measured by Nexus 128 (ENDRA Life Sciences Inc., USA). The sample solutions were kept in the dark at room temperature for more than 3 h before measurement. For measurement using Nexus128 apparatus, a sample solution was placed in 2 mL microtube.

Mass spectra were measured by Exactive Plus Orbitrap (ESI, Thermo Fisher Scientific Inc., USA) and

Ultraflex III (MALDI, Bruker Co., USA).

Synthesis of PEGylated Pcs

Synthesis of AlPc-OH and SiPc-OH

In a flame-dried Schlenk tube, a mixture of 4,5-dimethylphthalonitrile²⁹ (0.11 g, 0.67 mmol) and AlCl₃ (23 mg, 0.17 mmol) in 1-pentanol (1.5 mL) and DBU (0.20 mL) was stirred at 140 °C. After stirring for 23 h, the organic solvents were removed under reduced pressure. The residue was washed by MeOH (10 mL) and Et₂O (5 mL × 3), and then dried under reduced pressure to afford crude solid. A mixture of the crude solid and K₂CO₃ (0.12 g, 0.90 mmol) in H₂O (0.50 mL) and DMF (4.5 mL) was stirred at 80 °C. After stirring for 23 h, the resulting mixture was dispersed in H₂O (5.0 mL) and the blue precipitate was collected by centrifugation. The solid was washed with MeOH (10 mL) and Et₂O (5 mL × 3), and then dried under reduced pressure to afford **AlPc-OH** (60 mg, 90 μmol, 54 %) as a blue solid. No peak was observed in ¹H NMR spectrum because of aggregation. **AlPc-OH**: mp: >250 °C; IR (neat) 502, 508, 519, 548, 556, 579, 719, 735, 758, 813, 878, 992, 1030, 1086, 1179, 1244, 1313, 1342, 1381, 1409, 1432, 1468, 1515, 1618, 2853, 2920, 3048, 3417 cm⁻¹; HRMS (MALDI-TOF) calcd for C₄₀H₃₃AlN₈O (M⁺): 668.2593, found: 668.2594.

In a flame-dried Schlenk tube, a mixture of 5,6-dimethyl-1,3-diiminoisoindoline³⁰ (86 mg, 0.50 mmol) and SiCl₄ (68 μL, 0.60 mmol) in quinoline (1.0 mL) was stirred at 200 °C. After stirring for 3.5 h, the organic solvent was removed under reduced pressure. The residue was washed by MeOH (10 mL) and Et₂O (5 mL × 3), and then dried under reduced pressure to afford crude solid (82 mg). A mixture of the crude solid (34 mg) and K₂CO₃ (29 mg, 0.20 mmol) in H₂O (0.50 mL) and DMF (4.5 mL) was stirred at 80 °C. After stirring for 23 h, the resulting mixture was dispersed with H₂O (5.0 mL) and the bluish green precipitate was collected by centrifugation. The solid was washed with MeOH (10 mL) and Et₂O (5 mL × 3), and then dried under reduced pressure to afford **SiPc-OH** (28 mg, 40 μmol, 80 %) as a bluish green solid. No peak was observed in ¹H NMR spectrum because of aggregation. **SiPc-OH**: mp: >250 °C; IR (neat) 503, 519, 524, 562, 584, 595, 617, 724, 738, 753, 771, 812, 880, 1020, 1040, 1082, 1135, 1180, 1206, 1315, 1345, 1378, 1411, 1433,

1471, 1520, 1619, 1657, 1738, 2938, 2971, 3046, 3455 cm^{-1} ; HRMS (MALDI-TOF) calcd for $\text{C}_{40}\text{H}_{35}\text{N}_8\text{O}_2\text{Si}$ (M^+): 686.2574, found: 686.2568.

Synthesis of AlPc-NH₂ and SiPc-NH₂

Aluminum octamethylphthalocyanine **AlPc-NH₂** bearing an amino group at the end of an axial ligand was synthesized from **AlPc-OH** bearing a hydroxy group as an axial ligand according to the reported method (Scheme 1).³¹ Silicon octamethylphthalocyanine **SiPc-NH₂** was synthesized from **SiPc-OH** in the similar manner.

In a 200 mL flame-dried two-necked round-bottom flask, a mixture of **AlPc-OH** (15 mg, 23 μmol) and 3-(aminopropyl)dimethylethoxysilane (50 μL , 0.20 mmol) in dry pyridine (65 mL) was stirred at 100 $^{\circ}\text{C}$ overnight under N_2 atmosphere. After stirring for 12 h, the organic solvent was removed under reduced pressure ($< 40^{\circ}\text{C}$). The residue was dissolved in MeOH and an insoluble solid was removed by centrifugation. After removal of solvents under reduced pressure, the residue was washed with Et_2O (5 mL $\times$ 3) and then dried under reduced pressure to afford **AlPc-NH₂** (12 mg, 14 μmol , 36%) as a blue solid. No peak was observed in ^1H NMR spectrum because of aggregation. **AlPc-NH₂** was used for the next reaction without further purification. **SiPc-NH₂** (~42% yield, bluish green solid) was prepared according to the similar synthetic procedure from **SiPc-OH**. 3-(Aminopropyl)dimethylethoxysilane (20 equiv) was used. The crude **SiPc-NH₂** was used in the next step without purification. **SiPc-NH₂**: ^1H NMR (400 MHz, CDCl_3 , 25 $^{\circ}\text{C}$): δ = 9.40 (s, 8H), 2.94 (s, 24H), 1.15 (t, J = 7.3 Hz, 4H), -1.18 to -1.26 (m, 4H), -2.28 (t, J = 8.2 Hz, 4H), -2.86 (s, 12H).

Synthesis of AlPc-PEG

In a 50 mL flame-dried two-necked round-bottom flask, a mixture of **PEG-COOH**³² (70 mg, 35 μmol , average molecular weight: 2000) and EDC $\cdot$ HCl (9.0 mg, 44 μmol) in dry CH_2Cl_2 (5.0 mL) was stirred at room temperature for 30 min. This solution was added to a solution of DMAP (2.0 mg, 25 μmol) and **AlPc-NH₂**

(20 mg, 23 μmol) in dry CH_2Cl_2 (5.0 mL) under N_2 atmosphere, and the resulting mixture was stirred at room temperature overnight. After removal of the solvent under reduced pressure, the residue was dissolved in pure water (10 mL) and the mixture was centrifuged. The blue supernatant was dialyzed against water (MWCO: 25 kDa) for 24 h and freeze-dried to afford **AlPc-PEG** (blue solid, 58 mg, 20 μmol , 89% (assuming as a pure material), probably containing a small amount of free **PEG-COOH**) as a blue solid. **AlPc-PEG**: mp: 51–52 $^\circ\text{C}$; IR (neat) 534, 548, 585, 667, 701, 737, 759, 796, 841, 962, 1060, 1102, 1147, 1241, 1342, 1360, 1410, 1467, 1548, 1645, 2742, 2883, 3295 cm^{-1} . Although signals of the PEG fragment were observed in ^1H NMR spectrum (solvent: D_2O , CDCl_3 or CD_3OD), no signal of AlPc was observed because of aggregation. In MALDI-TOF mass spectrum of **AlPc-PEG**, the weak parent signal was observed along with signals of PEG fragments.

Synthesis of SiPc-(PEG)₂

A mixture of **PEG-COOH** (0.14 g, 70 μmol) and $\text{EDC}\cdot\text{HCl}$ (20 mg, 0.10 mmol) in dry CH_2Cl_2 (10 mL) was stirred at room temperature for 30 min. This solution was added to a solution of DMAP (2.0 mg, 25 μmol) and **SiPc-NH₂** (20 mg, 22 μmol) in dry CH_2Cl_2 (5 mL) under nitrogen atmosphere, and the resulting mixture was stirred at room temperature overnight. After removal of the solvent under reduced pressure, the residue was dissolved in pure water (10 mL) and the mixture was centrifuged. The blue supernatant was dialyzed against water (MWCO: 25 kDa) for 24 h and freeze-dried to afford **SiPc-(PEG)₂** (blue solid, 0.11 g, 22 μmol , 98% (assuming as a pure material), probably containing a small amount of free **PEG-COOH**) as a bluish green solid. **SiPc-(PEG)₂**: a bluish green solid; mp: 50–51 $^\circ\text{C}$; IR (neat) 506, 516, 521, 534, 546, 570, 584, 738, 759, 795, 841, 962, 1060, 1103, 1147, 1241, 1279, 1342, 1359, 1467, 1547, 1647, 2882, 3293 cm^{-1} . Although signals of the PEG fragment were observed in ^1H NMR spectrum (solvent: D_2O , CDCl_3 or CD_3OD), no signal of SiPc was observed because of aggregation. In MALDI-TOF mass spectrum of **SiPc-(PEG)₂**, the fragment peaks of SiPc bearing one PEG axial ligand and peaks of PEG fragments were observed together with the parent peaks.

Synthesis of AlPc-PEG and SiPc-(PEG)₂

To a mixture of succinic anhydride (12 mg, 0.12 mmol) and triethylamine (25 mg, 0.25 mmol) in dry CH₂Cl₂ (5 mL) was added **AlPc-NH₂** (13 mg, 17 μmol). After stirring for 12 h, the resulting solution was washed with water and the organic layer was dried over MgSO₄. After the organic solvent was removed under reduced pressure, the residue was dispersed in water (10 mL) and the blue precipitate was collected by centrifugation. The crude solid was washed with Et₂O (5.0 mL × 3), and then dried under reduced pressure to afford **AlPc-CX** (5.6 mg, 6.0 μmol, 37%) as a blue solid. **AlPc-CX**: mp: >250 °C; IR (neat) 518, 526, 531, 543, 578, 598, 610, 722, 735, 757, 794, 839, 878, 1029, 1085, 1217, 1229, 1313, 1366, 1619, 1739, 2856, 2928, 2970, 3017, 3456 cm⁻¹; HRMS (MALDI-TOF) calcd for C₄₉H₄₉AlN₉O₄Si [M+H⁺]: 884.3649, found: 884.3631.

SiPc-CX was synthesized from **SiPc-NH₂** (23 mg, 26 μmol) in a similar manner of **AlPc-CX**. Succinic anhydride (36 mg, 0.36 mmol) and triethylamine (75 mg, 0.75 mmol) were used. **SiPc-CX**: a bluish green solid (11 mg, 9.4 μmol, 37% yield); mp: >250 °C; IR (neat) 509, 515, 527, 541, 561, 578, 723, 737, 758, 773, 830, 879, 1038, 1091, 1206, 1217, 1229, 1316, 1354, 1366, 1412, 1435, 1521, 1619, 1739, 2863, 2946, 2971, 3017, 3456 cm⁻¹; ¹H NMR (400 MHz, CDCl₃, 25 °C): δ = 9.39 (s, 8H), 2.93 (s, 24H), 2.91 to 2.87 (m, 8H), 1.16 (t, *J* = 7.2, 4H), -1.18 to -1.26 (m, 4H), -2.28 (t, *J* = 8.2 Hz, 4H), -2.86 (s, 12H); HRMS (ESI) calcd for C₅₈H₆₇N₁₀O₈Si₃ [M-H⁺]⁻: 1115.4457, found: 1115.4459.

Preparation of self-assemblies of MPc-PEG.

Method A: Powdered **AlPc-PEG** (1.0 mg) was dissolved in Milli-Q water (3.5 mL) under sonication (100 W, 28 kHz). The resulting solution was filtered by using syringe filter (pore size: 0.45 μm) and the filtrate (**A-AlPc**) was kept in the dark at room temperature for 3 h before measurement.

Method B: Powdered **AlPc-PEG** (1.0 mg) was dissolved in CH₂Cl₂ (2.0 mL) in a 25 mL round-bottom flask. The solvent was removed by gentle blowing of nitrogen gas to prepare **AlPc-PEG** film. To this flask, Milli-Q water (3.5 mL) was added and the resulting mixture was sonicated (100 W, 28 kHz). The resulting

solution was filtered by using syringe filter (pore size: 0.45 μm) and the filtrate (**B-AIPc**) was kept in the dark at room temperature for 3 h before measurement.

TEM observation

Transmission electron microscopy (TEM, JEM-1400, JEOL Ltd., Japan) was used to visualize the morphology of dried self-assemblies (Figures 17, 18, and 19). Sample solution was dropped onto a TEM copper grid covered with a carbon film (200 mesh, Nisshin EM, Japan) and dried for 3 h.

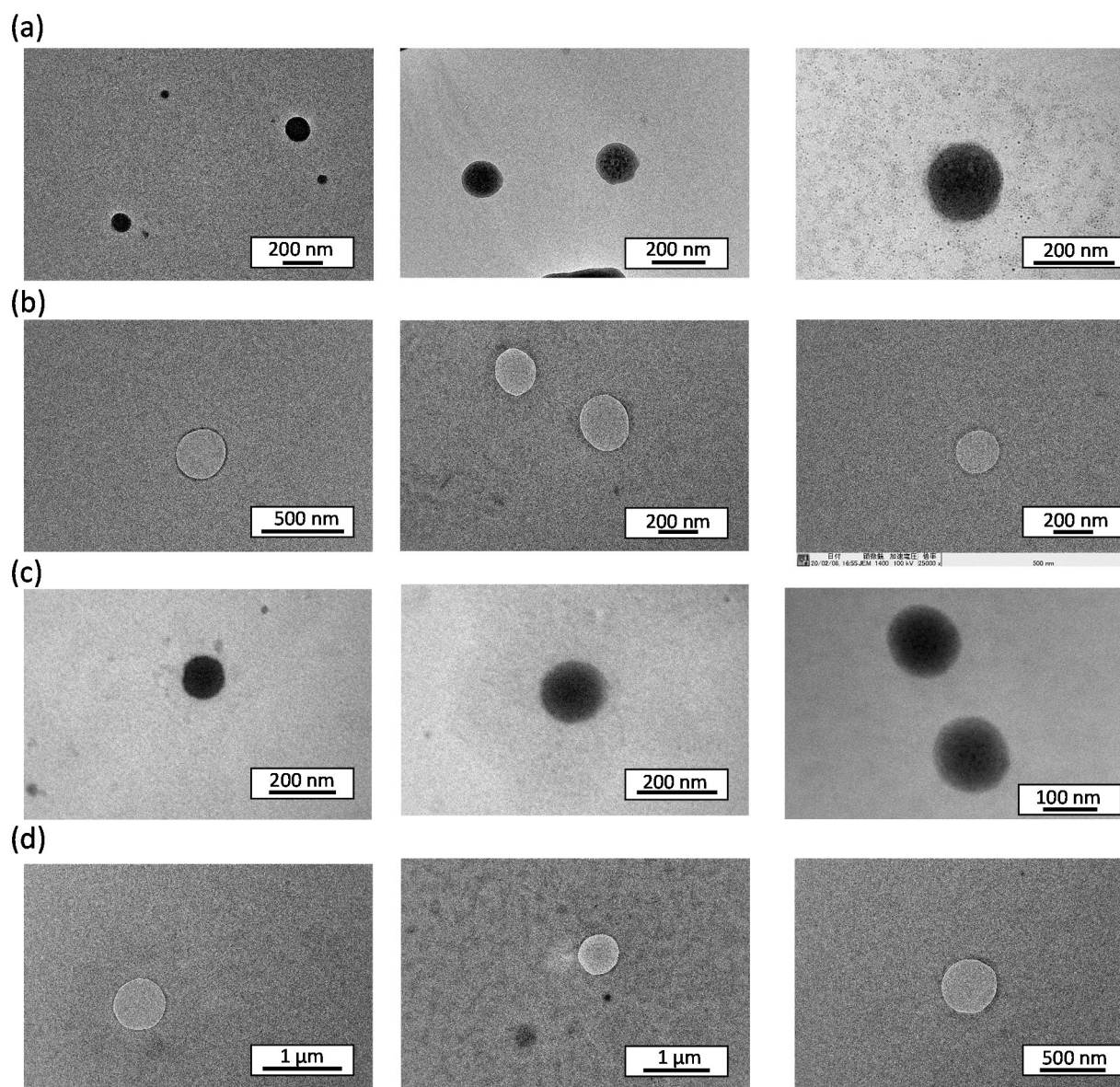


Figure 17. Representative enlarged TEM images of self-assemblies of (a) **A-AIPc**, (b) **B-AIPc**, (c) **A-SiPc**, and (d) **B-SiPc**.

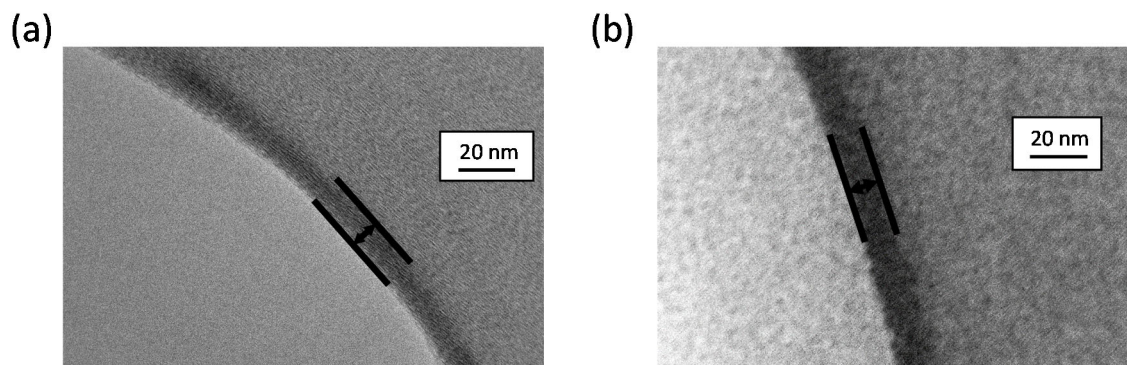


Figure 18. Representative enlarged TEM images of self-assemblies of (a) **B-AlPc** and (b) **B-SiPc**. Both of the thickness were around 10–11 nm.

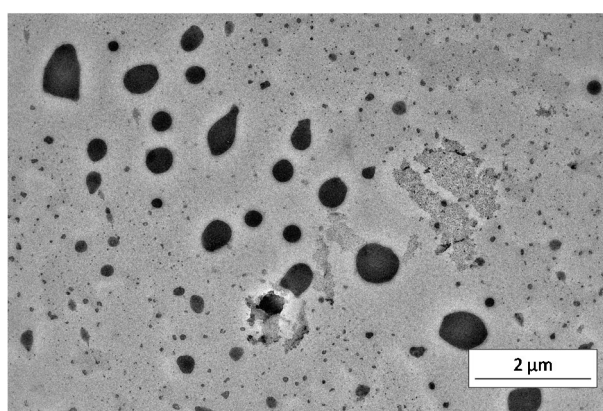


Figure 19. Representative TEM images of self-assemblies of **B-AlPc** after photoirradiation for 30 min.

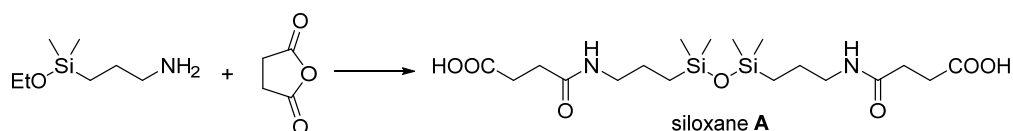
Photoinduced cleavage of axial ligands in water-soluble AlPc and SiPc.

Photostability of AlPc-PEG and SiPc-(PEG)₂

Each solution of **A-AlPc**, **B-AlPc**, **A-SiPc**, or **B-SiPc** in water (0.50 mg/mL) was prepared. ICG was dissolved in Milli-Q water (2.5 μM). Each solution was transferred into a quartz cuvette and irradiated using a LED photoirradiation apparatus (CL-1501, Asahi Spectra Co., Ltd., Japan) equipped with LED head unit (CL-H1-730-9-1, $\lambda_{\text{max}} = 737$ nm, half-bandwidth = 20 nm, illuminance: 25 mW ($\lambda = 730$ nm) at the sample level). The time-dependent photobleaching was monitored by measuring the absorbance at the maximum, and nanoparticle size change was monitored by TEM and DLS. The precipitate was collected by centrifugation and washed several times with Milli-Q water for MALDI-TOF mass spectrometry.

Preparation and characterization of authentic siloxane A

The authentic compound **A** was prepared as follows. In a 100 mL flame-dried two-necked round-bottom flask, 3-(aminopropyl)dimethylethoxysilane (0.60 mL, 3.2 mmol) was dissolved in dry THF (10 mL). Under N₂ atmosphere, a solution of succinic anhydride (0.40 g, 4 mmol) in dry THF (1 mL) was added. After stirring at room temperature for 20 h, the organic solvent was removed under reduced pressure and the residue was dissolved in CH₂Cl₂ (20 mL). The organic solution was washed with water (30 mL × 3) and saturated NaCl aq (30 mL) and the organic layer was dried over MgSO₄. The organic solvents were removed under reduced pressure to afford siloxane **A** (0.45 g, 1.0 mmol) as a white solid. mp: 65–66 °C; IR (ATR) 504, 517, 543, 559, 576, 640, 663, 709, 776, 792, 839, 930, 1043, 1183, 1205, 1252, 1303, 1334, 1366, 1384, 1417, 1428, 1549, 1645, 1693, 2874, 2929, 2954, 3085, 3300 cm⁻¹; ¹H NMR (400 MHz, CDCl₃, 25 °C): δ = 3.20 (dd, *J* = 13.3, 7.3 Hz, 4H), 2.66 (t, *J* = 6.4 Hz, 4H), 2.52 (t, *J* = 6.6 Hz, 4H), 1.53 (tt, *J* = 8.2, 7.6 Hz, 4H), 0.49 (t, *J* = 8.4 Hz, 4H), 0.05 (s, 12H). ¹³C NMR (100 MHz, CDCl₃, 25 °C): 176.1, 172.7, 42.5, 30.5, 29.8, 23.0, 15.2, 0.05. HRMS (ESI) calcd for C₁₈H₃₅N₂O₇Si₂ [M–H]⁻: 447.1988, found: 447.1982.

*Photoinduced cleavage of axial ligands in water-soluble AlPc and SiPc*

Aqueous solutions of **A-AlPc**, **B-AlPc**, **A-SiPc**, or **B-SiPc** (0.50 mg/mL) were prepared. Each solution was transferred into quartz cuvettes and irradiated using a LED photoirradiation apparatus (CL-1501, Asahi Spectra Co., Ltd., Japan) equipped with LED head unit (CL-H1-730-9-1, λ_{max} = 737 nm, half-bandwidth = 20 nm, illuminance: 25 mW (λ = 730 nm) at the sample level). For hypoxic conditions, nitrogen bubbling was conducted for 15 min and the cuvettes were sealed during irradiation. The precipitates were collected by centrifugation. The precipitates were dissolved in conc. H₂SO₄ and the amounts of MPc derivatives were quantified by UV-vis absorbance utilizing the linear relationship as shown in Figure 20.

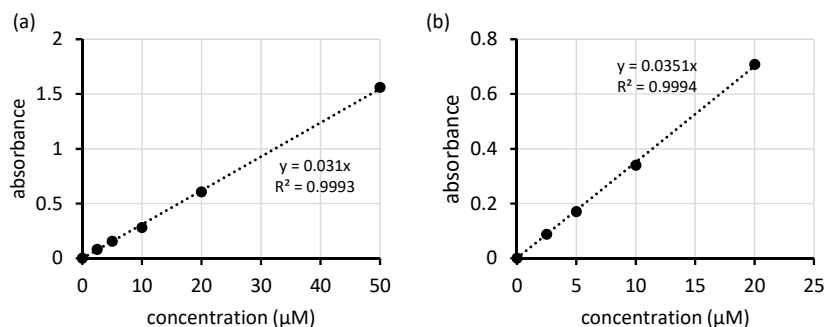


Figure 20. Maximum absorbance of (a) **AlPc-PEG** and (b) **SiPc-(PEG)₂** in H_2SO_4 .

The fragment generated from the axial ligand under photoirradiation of **MPc-CX** was corrected as follows. **MPc-CX** (1.0 mg) was dissolved in water (10 mL) with K_2CO_3 (2.0 mg). Part of the resulting aqueous solution in a quartz cuvette (optical path length: 10 mm) was irradiated for 2 h using a Xenon light source device (MAX-303, Asahi Spectra Co., Ltd., Japan) equipped with a visible mirror module and a rod lens (RLQL80-1, illuminance: 25 mW ($\lambda = 680$ nm) at the sample level). After photoirradiation, the precipitate formed was removed by centrifugation and ESI high-resolution mass spectra of supernatant were measured to confirm the consumption of **MPc-CX** and the generation of fragments of the axial ligand (Figure 12).

For isolation of the axial ligand fragment, in a 100 mL round-bottom flask, a mixture of **MPc-CX** (4.0 mg) and K_2CO_3 (8.0 mg) in water (40 mL) was irradiated for 24 h using the same photoirradiation apparatus. To the resulting solution was added dichloromethane (20 mL) and the organic layer was separated. After neutralization with HCl aq., the organic solution was concentrated under reduced pressure to afford a white solid. ^1H NMR and liquid chromatography-tandem mass spectrometer (LC-MS) measurements (Figures 13 and 14) of this solid were carried out. Nexera X2 UHPLC liquid chromatograph system (LC-30AD, Shimadzu Industrial System Co., Ltd. Japan) with column (InertSustain C18 (2 μm , for SiPc-CX) or InertSustainSwift C18 (1.9 μm , for AlPc-CX), 2.1 I. D. $\times$ 150 mm, GL Sciences Inc., Japan) and PDA detector (SPD M30A) was used for LC-MS/MS measurements. The mobile phase consisted of H_2O (phase A) and acetonitrile (phase B). The gradient program was set as follows, 0–3 min, 30% B; 3–5 min, 30–50% B. The flow rate was 0.3 mL/min with a pump (LC-30AD, Shimadzu Industrial System Co., Ltd. Japan). Detection was performed on a triple quadrupole mass spectrometer (LCMS-8030, Shimadzu Industrial System Co., Ltd.,

Japan) with an electrospray ionization (ESI) source. The parameters of the source were set under a N₂ generator (AT 10NP5NSC). The MS was operated in the negative ionization mode with the data acquisition mode of selective ion monitoring (SIM).

Theoretical Calculation

To reduce the calculation cost, methyl groups on phthalocyanine were omitted and the substituent on the nitrogen atom was replaced by an acetyl group. The density functional theory (DFT) calculation was performed for the geometry optimization of the ground state, the excited state, the radical cation state, and the radical anion state of **AlPc-Ac** having acetyl-capped siloxy axial ligand at the UM06-2X/6-31+G(d,p),³³ scrf(cpcm, solvent = water)³⁴ level by using Gaussian 16 package.³⁵ The energy-minimized structures estimated are visualized by Avogadro software³⁶ and summarized in Figure 21. Their electronic and Gibbs free energies are summarized in Table 3. The Cartesian coordinates of the optimized structures are summarized at the end of experimental section.

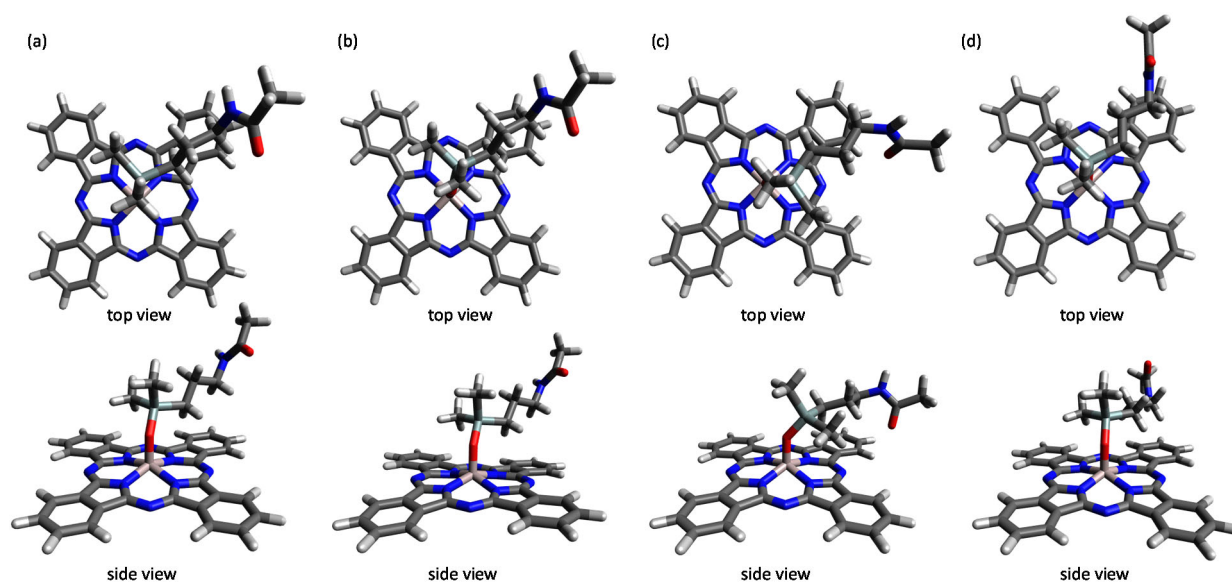


Figure 21. Energy-minimized structures of (a) the ground state, (b) the excited state, (c) the radical cation state, and (d) the radical anion state of **AlPc-Ac** at UM06-2X/6-31+G(d,p), scrf(cpcm, solvent = water) level visualized by Avogadro software.

The electrochemical data of MPc-PEGs

A solution of **AlPc-PEG** (5.0×10^{-5} M) or **SiPc-(PEG)₂** (2.5×10^{-5} M) and tetra-*n*-butylammonium perchlorate (0.10 M) in CH₂Cl₂ was prepared. Pt electrode (counter), glassy carbon electrode (working), and Ag/AgNO₃ electrode (reference) were used. The cyclic voltammogram (scan rate: 0.5 V/s) and differential pulse voltammogram were measured by electrochemical analyzer (610C-S, ALS Co., Ltd., Japan). The ferrocene/ferrocenium couple (Fc⁺/Fc) was also measured in the same electrochemical system. The one-electron reduction potential (E_{red} [V]) and one-electron oxidation potential (E_{ox} [V]) of probes in the ground state was determined by differential pulse voltammetry because the clear reversible signal was not detected in cyclic voltammetry (Figure 22). The one-electron reduction potential (E_{red}^* [V]) and one-electron oxidation potential (E_{ox}^* [V]) of **MPc-PEGs** in the excited state were estimated according to the equation:

$$E_{\text{red}}^* = E_{\text{red}} + E_{0,0}$$

$$E_{\text{ox}}^* = E_{\text{ox}} - E_{0,0}$$

$E_{0,0}$ is estimated by calculating the energy of the wavelength at crossing point of the normalized UV-vis absorption and emission spectra of **MPc-PEGs**.

$E_{0,0} = 1240/x$ (eV) (x: wavelength of crossing point of normalized UV-vis absorption and emission spectra.)

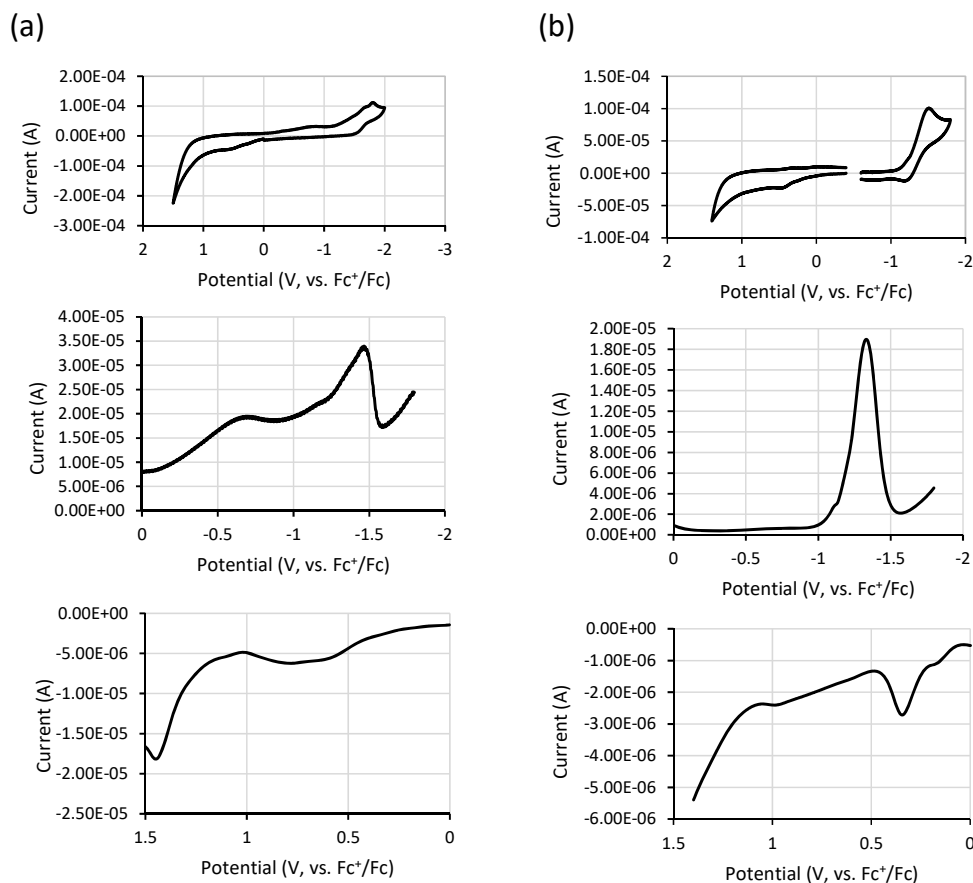


Figure 22. Cyclic voltammograms (upper) and differential pulse voltammograms (middle, bottom) of (a) **AlPc-PEG** and (b) **SiPc-(PEG)₂**.

Effect of singlet oxygen on PA signal enhancement.

Time-dependent PA signal intensity change of **B-AlPc** and **B-SiPc** under hypoxic and normoxic conditions were monitored under continuous photoirradiation at 25 mW ($\lambda = 730$ nm) (Figure 15).

Detection of singlet oxygen.

9,10-Anthracenedipropionic acid (ADPA) sodium salt was prepared according to the reported procedure.³⁷ Aqueous solutions of **AlPc-PEG**, **SiPc-(PEG)₂**, and methylene blue (standard, Waldeck GmbH & Co. KG, Germany) (15 μ M) and ADPA (sodium salt, 25 μ M) in a quartz cuvette (optical path length: 10 mm) were photoirradiated using a Xenon light source device (MAX-303, Asahi Spectra Co., Ltd., Japan) equipped with

VIS mirror module and rod lens (RLQL80-1, illuminance: 2.7 mW ($\lambda = 680$ nm) at the sample level).

Absorbance of ADPA at 400 nm were monitored during photoirradiation (Figure 16).

Cartesian coordinates of energy-minimized structures

AlPc-Ac (ground state)

C	-4.6917364958	1.9930319109	-0.9783181090
C	-4.1268199784	3.1318887981	-0.3981707688
C	-2.8581858836	2.7101866863	0.1767783341
N	-2.6619105204	1.3699862222	-0.0464736651
C	-3.7555709319	0.9053443577	-0.7367399995
C	-2.4281393567	-3.4807966031	-0.7038128904
C	-3.4784047011	-2.7067741956	-1.2059105083
C	-3.1816101985	-1.3311881082	-0.8384364236
N	-1.9953424170	-1.2717600741	-0.1516144982
C	-1.5179750532	-2.5554865437	-0.0481656969
N	-3.9945193882	-0.3309931697	-1.1288027862
C	2.2448767178	-1.3965811307	2.2997526592
C	1.6443021825	-2.5488932996	1.7832900541
C	0.4136847983	-2.1139185868	1.1423032503
N	0.2745381607	-0.7522792859	1.2644625364
C	1.3595233762	-0.2926436179	1.9682164929
N	-0.4036036207	-2.9546751908	0.5354197131
C	-0.0671679522	4.0603186762	2.1096347161
C	1.0191368704	3.2986940726	2.5487936437
C	0.7862802531	1.9436818521	2.0718959338
N	-0.3904475115	1.8896297795	1.3637224397
C	-0.9299994991	3.1509074113	1.3705476265
N	-2.0622309935	3.5452768422	0.8176633378
N	1.6135032928	0.9510255774	2.3347057871
C	3.4689645946	-1.4493416180	2.9685275729
C	4.0700550138	-2.6965052662	3.0959504053
C	3.4646640403	-3.8585260633	2.5753429029
C	2.2433702621	-3.8031131886	1.9131653636
C	-2.3967065942	-4.8655996749	-0.8749902381
C	-3.4537467724	-5.4441643732	-1.5687082954
C	-4.5137738465	-4.6639553126	-2.0732626017
C	-4.5436991981	-3.2850123690	-1.8978670005
C	-0.1708248795	5.4201004435	2.4048711416
C	0.8551174348	5.9878726851	3.1525947514
C	1.9502268813	5.2196555540	3.5959144394
C	2.0478039750	3.8635704762	3.3034135580
C	-5.9295790003	2.0398986279	-1.6205200247
C	-6.5764519110	3.2702305504	-1.6640757392
C	-6.0067594198	4.4186825289	-1.0789759495
C	-4.7749632120	4.3669220931	-0.4355213964
Al	-0.9658131585	0.3846481836	0.2113895630
O	-0.1142893570	0.6727254571	-1.2799051203
Si	1.0006833314	-0.0530720079	-2.2496524081
C	0.3032820680	-1.6536809688	-2.9577285007
C	1.4474690998	1.1139677170	-3.6511359355
C	2.5418152963	-0.4636589284	-1.2373565524
C	3.7796327828	-0.8620693241	-2.0458564501

C	4.9195250762	-1.3072805820	-1.1277706611
N	6.1279354125	-1.6766774010	-1.8488576744
C	7.0258088837	-0.7595794329	-2.2680148513
C	8.2108285115	-1.2817394587	-3.0479401447
O	6.8985318241	0.4443333329	-2.0243352155
H	9.1256513644	-0.9782993967	-2.5343297553
H	-3.4676055008	-6.5176160696	-1.7265013585
H	-5.3208018336	-5.1538046333	-2.6081613265
H	-7.5398408884	3.3519740950	-2.1566310830
H	-6.5437565232	5.3599459327	-1.1333898439
H	0.8153405180	7.0429651330	3.4026799706
H	2.7301094120	5.6999255808	4.1776718494
H	5.0247802574	-2.7820682959	3.6045430595
H	3.9665348044	-4.8130968475	2.6949450008
H	3.9310702612	-0.5515831290	3.3656960123
H	1.7748182719	-4.6943516174	1.5090941351
H	-1.5798017723	-5.4620039095	-0.4824036873
H	-5.3581401061	-2.6803809833	-2.2828463328
H	-1.0172461143	6.0065877120	2.0632500494
H	2.8873441758	3.2670067175	3.6445664711
H	-6.3651335361	1.1522018323	-2.0670550462
H	-4.3333447452	5.2476505033	0.0187236749
H	0.9519255327	-2.0572131584	-3.7431465485
H	0.2079291747	-2.4196324955	-2.1789656534
H	-0.6893717279	-1.4857209870	-3.3910855428
H	2.1591670581	0.6457373324	-4.3396584614
H	1.9020510028	2.0329556747	-3.2659202182
H	0.5556852233	1.3892278095	-4.2239742019
H	2.7787247587	0.3954209982	-0.5916891315
H	2.2846475151	-1.2921736838	-0.5618608138
H	3.5424042558	-1.6827955895	-2.7359989660
H	4.1297551871	-0.0217094382	-2.6569575636
H	5.1738604971	-0.4973882351	-0.4368792789
H	4.6053392749	-2.1700197861	-0.5313316254
H	6.2604694791	-2.6423036691	-2.1122122641
H	8.2056972701	-2.3664335526	-3.1636006499
H	8.2100090667	-0.8148398437	-4.0354429922

AlPc-Ac (excited state)

C	5.1083667539	-0.2089591920	0.7705241238
C	4.9905322485	1.1919787242	0.7144694263
C	3.6362013319	1.4624641634	0.3223520786
N	2.9504333142	0.2907526912	0.1403921107
C	3.8219356094	-0.7311489920	0.4073079215
C	0.9863761695	-4.0850305101	-0.9550908094
C	2.2073403651	-3.9630619865	-0.3124521144
C	2.4690569284	-2.5077155697	-0.1681305597
N	1.3980411018	-1.7821594093	-0.6902887442

Chapter 2

C	0.4820830919	-2.7061445453	-1.1811259820
N	3.5644633987	-2.0756769157	0.3502263535
C	-2.5605227655	0.4394973713	-2.4905281193
C	-2.3915810739	-0.9561302143	-2.5473746346
C	-1.1338905041	-1.2312043999	-1.9109431691
N	-0.5502846111	-0.0663024940	-1.4864088232
C	-1.3943606428	0.9548820511	-1.8301620007
N	-0.6609167746	-2.5029078512	-1.7416193324
C	1.4323263337	4.3116176699	-0.4461333456
C	0.1530612258	4.1814172033	-0.9608657011
C	-0.0940168390	2.7260210454	-1.1283498604
N	1.0221661184	2.0071441903	-0.7074011015
C	1.9695549239	2.9359549183	-0.2837319411
N	3.1523422696	2.7348160607	0.1835317616
N	-1.2057993211	2.2931553092	-1.6140037522
C	-3.7060022465	1.0537060284	-3.0065835100
C	-4.6723225256	0.2302214930	-3.5724377901
C	-4.5039392609	-1.1700809832	-3.6273942155
C	-3.3649827527	-1.7825631894	-3.1179059464
C	0.4394264095	-5.3179921839	-1.2587611818
C	1.1783825211	-6.4504982734	-0.8850635546
C	2.4096902862	-6.3277595994	-0.2383423931
C	2.9509268411	-5.0678912001	0.0590184574
C	1.9896858224	5.5480959987	-0.1775871323
C	1.1981240557	6.6745234642	-0.4474476465
C	-0.0915466340	6.5432585080	-0.9660605869
C	-0.6395120502	5.2803669500	-1.2362837778
C	6.3145240184	-0.8224160887	1.1245149480
C	7.3899394177	0.0065371900	1.4204673776
C	7.2714448434	1.4120171481	1.3652773894
C	6.0745934167	2.0239652331	1.0123457531
Al	1.0108385989	0.0968253257	-0.2543799371
O	0.2841121881	0.0420607252	1.3363872746
Si	-0.9957556396	-0.6730384449	2.0791047234
C	-0.8133213670	-2.5501402755	2.0577217844
C	-1.0819604426	-0.0782662505	3.8591166015
C	-2.5944356652	-0.2184314439	1.1770089746
C	-3.8871424802	-0.5261843676	1.9381722729
C	-5.1202373169	-0.2403200503	1.0791476501
N	-6.3742931811	-0.4921790671	1.7726581354
C	-6.9305301301	0.4116311741	2.6072085195
C	-8.2091972468	-0.0042154163	3.2980908285
O	-6.4325188331	1.5257808690	2.7962635801
H	-8.9873455046	0.7227186183	3.0556009791
H	0.7873179767	-7.4390947862	-1.1013388128
H	2.9567401817	-7.2229070318	0.0382258642
H	8.3424822932	-0.4312279838	1.7003388998
H	8.1355662151	2.0232863754	1.6039916081
H	1.5939699619	7.6652698170	-0.2505090703
H	-0.6785358148	7.4338862105	-1.1641373736
H	-5.5771833811	0.6684231384	-3.9805522083
H	-5.2832899668	-1.7771638514	-4.0761968477
H	-3.8347217338	2.1301054348	-2.9604557128
H	-3.2344362800	-2.8590296123	-3.1562602318
H	-0.5178758172	-5.4039277304	-1.7619490399
H	3.9079252797	-4.9644540286	0.5594967558
H	2.9933401804	5.6416004234	0.2234669343
H	-1.6395938641	5.1694530807	-1.6418929208

H	6.4029503525	-1.9030108303	1.1667552923
H	5.9810735015	3.1041205867	0.9694194332
H	-1.5271923968	-3.0250037259	2.7400587376
H	-0.9962493400	-2.9550542678	1.0553209919
H	0.1961707524	-2.8463778776	2.3661040038
H	-1.8982692861	-0.5693646935	4.3996979960
H	-1.2477022169	1.0035330504	3.9035263735
H	-0.1475675061	-0.3009976071	4.3851275765
H	-2.5605809535	0.8509543359	0.9185192653
H	-2.6108512702	-0.7629553866	0.2214985575
H	-3.9108221594	-1.5794620626	2.2487018878
H	-3.9502432453	0.0772458854	2.8515110917
H	-5.1144656559	0.8076409657	0.7634891087
H	-5.1027915579	-0.8620896220	0.1784835207
H	-6.8015839502	-1.4030315869	1.6882524313
H	-8.5471103988	-1.0017210511	3.0139020208
H	-8.0465921568	0.0261846669	4.3778292964

AlPc-Ac (radical cation state)

C	5.0469576488	0.9547496611	0.3405747640
C	4.4884200757	2.2280134305	0.2247974185
C	3.0737666789	2.0257698940	-0.0779681987
N	2.7853984223	0.6834413593	-0.1280603903
C	3.9566244538	0.0100272490	0.1103935566
C	2.1549624550	-4.1551179358	-0.4991549971
C	3.3897138427	-3.5849863924	-0.1879253931
C	3.1697198908	-2.1418585331	-0.1347155404
N	1.8559234533	-1.8503739748	-0.4004549995
C	1.2155922646	-3.0441662908	-0.6302671066
N	4.1484101574	-1.2953825660	0.1248045809
C	-2.8227122931	-1.1500967163	-1.6730497402
C	-2.2734502858	-2.4248604548	-1.5156312545
C	-0.8758344089	-2.2221672804	-1.1464462845
N	-0.5923715430	-0.8819206279	-1.0700604012
C	-1.7449434039	-0.2073149757	-1.3930878962
N	-0.0518249042	-3.2336401216	-0.9398533289
C	0.1061666151	3.9600965126	-0.9636935798
C	-1.1152469124	3.3898194720	-1.3250618389
C	-0.9383381488	1.9451887622	-1.2114719595
N	0.3400929987	1.6523972245	-0.8044749046
C	0.9964090539	2.8466868054	-0.6483749022
N	2.2531029550	3.0360240933	-0.2886706999
N	-1.9207473297	1.0994513817	-1.4586982280
C	-4.1560988127	-0.9631231392	-2.0102380364
C	-4.9272428641	-2.1166936981	-2.1851022793
C	-4.3760740809	-3.3961040808	-2.0313266397
C	-3.0307219822	-3.5743959771	-1.6925334725
C	1.9972632418	-5.5269973386	-0.6392990822
C	3.1360947865	-6.3149878543	-0.4484393673
C	4.3767432135	-5.7419749463	-0.1354443455
C	4.5267940119	-4.3585706480	-0.0011011072
C	0.2971342096	5.3351690857	-0.9551142322
C	-0.7960808878	6.1254863883	-1.3214392304
C	-2.0237386817	5.5522763379	-1.6830725321
C	-2.2057457972	4.1664369280	-1.6925209650
C	6.3951223536	0.7694125119	0.6136316571
C	7.1698987387	1.9220256612	0.7739006156
C	6.6084929757	3.2014411389	0.6578822040

Chapter 2

C	5.2506216679	3.3780276207	0.3764061964	C	-2.4718723734	2.0009644989	-1.6147049781
Al	0.9733552233	-0.1029663606	-0.1375430498	C	-2.8645359770	0.6735378763	-1.7801926380
O	0.5104008506	-0.1142551486	1.5313515799	C	-1.6918703862	-0.1509981108	-1.4827637215
Si	-0.7469053543	0.3186794694	2.5096115168	N	-0.6247941612	0.6602098600	-1.1405007098
C	-0.2370711597	0.0957748599	4.3000970036	C	-1.0606772352	1.9726627179	-1.2225877806
C	-1.1963365775	2.1272228837	2.2238309250	N	-1.7250852980	-1.4521679317	-1.5609203826
C	-2.2441433020	-0.7795312235	2.1291346463	C	3.0204045907	3.7831967866	-0.2098545467
C	-3.6072098725	-0.0941805982	2.2703212253	C	1.7189593447	4.2142487433	-0.5408665755
C	-4.7521323108	-1.0171506401	1.8508793257	C	0.9264373838	3.0298819000	-0.6805574379
N	-6.0596374387	-0.3858971734	1.9564605788	N	1.6996701447	1.9160450694	-0.4446752278
C	-6.5287543617	0.4634306131	1.0176993581	C	2.9691814846	2.3528823772	-0.1569343100
C	-7.8824534356	1.0821862318	1.2822870706	N	4.0455930978	1.5978816366	0.1365505500
O	-5.8950790855	0.7199697379	-0.0109153124	N	-0.3777387762	3.0616064871	-1.0106322124
H	-8.5447656483	0.8387336026	0.4487065726	C	-3.3661094135	3.0522860510	-1.8144144096
H	3.0608426660	-7.3927038453	-0.5448462134	C	-4.6696112166	2.7265312902	-2.1824421871
H	5.2370182992	-6.3876545757	0.0040395302	C	-5.0641208476	1.3862740333	-2.3546163345
H	8.2282222219	1.8268246705	0.9914374747	C	-4.1649897211	0.3401076085	-2.1562153173
H	7.2435090140	4.0710240992	0.7883356531	C	-1.6365565170	-4.5575507274	-1.7828931653
H	-0.6952180325	7.2054031802	-1.3266003774	C	-1.3068428559	-5.9006645770	-1.7610290911
H	-2.8480389974	6.2001740835	-1.9608542987	C	-0.0163563363	-6.3358774199	-1.3594662070
H	-5.9775516256	-2.0203533654	-2.4391017636	C	0.9577749364	-5.4331194337	-0.9733570229
H	-5.0089195181	-4.2653626876	-2.1745426546	C	4.0596343495	4.7094350642	-0.0087167167
H	-4.5872911348	0.0266503344	-2.1019376061	C	3.7622406468	6.0531695163	-0.1436858210
H	-2.5998064889	-4.5620036593	-1.5693038145	C	2.4520535942	6.4874118938	-0.4775649530
H	1.0357774298	-5.9650150640	-0.8841429451	C	1.4257269083	5.5830665163	-0.6800969454
H	5.4847268231	-3.9097831174	0.2380703968	C	5.6402374906	-2.9124173932	0.5290874629
H	1.2488167437	5.7735267132	-0.6750594442	C	6.9551322239	-2.5898434348	0.8586244054
H	-3.1528122250	3.7174523218	-1.9717463993	C	7.3700564985	-1.2505407939	0.9670550791
H	6.8252722241	-0.2225443366	0.6988035895	C	6.4798816913	-0.2009218107	0.7492419353
H	4.8118699248	4.3653579681	0.2818672764	Al	1.0167616871	0.0639330317	-0.2056613384
H	-1.0818794534	0.3064378320	4.9654239187	O	0.5441635885	0.0343480306	1.4867855440
H	0.0918164738	-0.9319694882	4.4853508158	Si	-0.7516145528	-0.4013924606	2.3972626044
H	0.5812388176	0.7723935144	4.5668056578	C	-1.2177541073	-2.2019865998	2.0862236597
H	-1.8541689057	2.4904964352	3.0214128890	C	-0.2961415996	-0.2177454749	4.2133768260
H	-1.7217751506	2.2877748546	1.2758040915	C	-2.2379960542	0.7256322046	2.0218869867
H	-0.2928414228	2.7487350970	2.2242546284	C	-3.5334271368	0.0098232479	1.6229275063
H	-2.1410819654	-1.1590494722	1.1032526735	C	-4.6561600692	0.9996564990	1.3112321470
H	-2.2023369455	-1.6675931077	2.7754543621	N	-5.8937029006	0.3488772783	0.9075702992
H	-3.7700625254	0.2311275351	3.3061550837	C	-6.7936478639	-0.1201119941	1.7968869730
H	-3.6492442884	0.8044513519	1.6429018422	C	-8.0052335173	-0.8205980845	1.2245322684
H	-4.6086132966	-1.3263500007	0.8089475537	O	-6.6507363867	0.0183679058	3.0160787483
H	-4.7647347880	-1.9169323178	2.4721317354	H	-8.9025896633	-0.3052326802	1.5738677222
H	-6.6001344539	-0.5266957978	2.7975950559	H	-2.0438388591	-6.6411813672	-2.0559015538
H	-8.3361542528	0.7418712474	2.2140701848	H	0.2035653460	-7.3989289409	-1.3578226525
H	-7.7682765235	2.1681153772	1.3138416039	H	7.6745659581	-3.3832096896	1.0344321413
AlPc-Ac (radical anion state)				H	8.4021310991	-1.0347548930	1.2242882771
C	4.7545257013	-1.8580119645	0.3166261573	H	4.5404703094	6.7950282725	0.0060815280
C	5.1659827824	-0.5307786942	0.4241556924	H	2.2593055951	7.5512836318	-0.5751249301
C	3.9920855044	0.2968295653	0.1312749635	H	-5.3962027836	3.5166330564	-2.3422033297
N	2.9048701732	-0.5146964296	-0.1442927720	H	-6.0865403006	1.1692686268	-2.6494561325
C	3.3350002813	-1.8277406591	-0.0480364250	H	-3.0568345115	4.0841869058	-1.6824632817
C	-0.6515373013	-3.6334593720	-1.3911790125	H	-4.4609839536	-0.6965413594	-2.2870725227
C	0.6284107594	-4.0658285445	-0.9872588961	H	-2.6222407400	-4.2226918976	-2.0913793220
C	1.3670561746	-2.8829937760	-0.6597623733	H	1.9450614840	-5.7644669871	-0.6663559414
N	0.5825527561	-1.7705677671	-0.8472865017	H	5.0611530073	4.3751365213	0.2442902460
C	-0.6438827329	-2.2050160683	-1.2894555958	H	0.4244327353	5.9140525210	-0.9380808291
N	2.6525094908	-2.9166133312	-0.2597642883	H	5.3154137356	-3.9442624213	0.4414375226
				H	6.7942098493	0.8347968506	0.8293677760

Chapter 2

H	-1.9350111945	-2.5553111375	2.8359298613
H	-1.6653406419	-2.3473011509	1.0973640027
H	-0.3235404077	-2.8342678088	2.1444990881
H	-1.1503899439	-0.4684543703	4.8524137902
H	0.0023475895	0.8119313980	4.4383991687
H	0.5339309209	-0.8799677823	4.4817701465
H	-2.4288333852	1.3577681120	2.9009712422
H	-1.9406632472	1.4132969358	1.2166974462
H	-3.3664153933	-0.6187022855	0.7381404021
H	-3.8691382981	-0.6587475752	2.4261135290
H	-4.8702625140	1.6127822914	2.1920761214
H	-4.3519396019	1.6680900834	0.4985856023
H	-6.0402791480	0.1717302373	-0.0763818749
H	-8.0089355948	-0.8509297670	0.1340282532
H	-8.0323553939	-1.8412881668	1.6127245891

References

- (1) (a) J. Weber, P. C. Beard, S. E. Bohndiek, *Nat. Methods* **2016**, *16*, 639–650. (b) R. E. Borg, J. Rochford, *Photochem. Photobiol.* **2018**, *94*, 1175–1209.
- (2) (a) J. Huang, K. Pu, *Angew. Chem. Int. Ed.* **2020**, *59*, 11717–11731. (b) C. J. Reinhardt, J. Chan, *Biochemistry* **2018**, *57*, 194–199. (c) H. J. Knox, J. Chan, *Acc. Chem. Res.* **2018**, *51*, 2897–2905.
- (3) (a) X. Li, B.-D. Zheng, X.-H. Peng, S.-Z. Li, J.-W. Ying, Y. Zhao, J.-D. Huang, J. Yoon, *Coord. Chem. Rev.* **2019**, *379*, 147–160. (b) K. Mitra, M. C. T. Hartman, *Org. Biomol. Chem.* **2021**, *19*, 1168–1190.
- (4) (a) H. Pan, S. Li, J. L. Kan, L. Gong, C. Lin, W. Liu, D. Qi, K. Wang, X. Yan, J. Jiang, *Chem. Sci.* **2019**, *10*, 8246–8252. (b) H.-G. Jin, W. Zhong, S. Yin, X. Zhang, Y.-H. Zhao, Y. Wang, L. Yuan, X.-B. Zhang, *ACS Appl. Mater. Inter.* **2019**, *11*, 3800–3808. (c) H. Ding, Y. Cai, J. Chen, T. Lu, W. Wen, G. Nie, X. Wang, *Microchim. Acta* **2019**, *186*, 237. (d) R. Chen, S. Huang, T. Lin, H. Ma, W. Shan, F. Duan, J. Lv, J. Zhang, L. Ren, L. Nie, *Nat. Nanotechnol.* **2021**, *16*, 455–465. (e) F. Wu, L. Yue, K. Cheng, J. Chen, K.-L. Wong, W.-K. Wong, X. Zhu, *ACS Biomater. Sci. Eng.* **2020**, *6*, 5230–5239. (f) H. D. Lu, T. L. Lim, S. Javitt, A. Heinmiller, R. K. Prud'homme, *ACS Comb. Sci.* **2017**, *19*, 397–406.
- (5) (a) D. Gao, P.-C. Lo, *J. Control. Release* **2018**, *282*, 46–61. (b) K. Stokov, A. H. Schäfer, U. Dobrindt, A. Galstyan, *ACS Appl. Bio Mater.* **2020**, *3*, 3751–3760.
- (6) (a) S. Li, L. Zhao, R. Chang, R. Xing, X. Yan, *Chem. Eur. J.* **2019**, *25*, 13429–13435. (b) F. Wu, L. Chen, L. Yue, K. Wang, K. Cheng, J. Chen, X. Luo, T. Zhang, *ACS Appl. Mater. Inter.* **2019**, *11*, 21408–21416. (c) X. Li, E.-Y. Park, Y. Kang, N. Kwon, M. Yang, S. Lee, W. J. Kim, C. Kim, J. Yoon, *Angew. Chem. Int. Ed.* **2020**, *59*, 8630–8634.
- (7) S. Xu, H.-W. Liu, S.-Y. Huan, L. Yuan, X.-B. Zhang, *Mater. Chem. Front.* **2021**, *5*, 1076–1089.
- (8) J. Morales, R. H. Pawle, N. Akkilic, Y. Luo, M. Xavierselvan, R. Albokhari, I. A. C. Calderon, S. Selfridge, R. Minns, L. Takiff, S. Mallidi, H. A. Clark, *ACS Sens.* **2019**, *4*, 1313–1322.
- (9) C. Xie, X. Zhen, Y. Lyu, K. Pu, *Adv. Mater.* **2017**, *29*, 1703693.
- (10) (a) X. Li, C. Kim, S. Lee, D. Lee, H.-M. Chung, G. Kim, S.-H. Heo, C. Kim, K.-S. Hong, J. Yoon, *J. Am.*

- Chem. Soc.* **2017**, *139*, 10880–10886. (b) X. Li, S. Yu, Y. Lee, T. Guo, N. Kwon, D. Lee, S. C. Yeom, Y. Cho, G. Kim, J.-D. Huang, S. Choi, K. T. Nam, J. Yoon, *J. Am. Chem. Soc.* **2019**, *141*, 1366–1372.
- (11) N. Toriumi, N. Asano, T. Ikeno, A. Muranaka, K. Hanaoka, Y. Urano, M. Uchiyama, *Angew. Chem. Int. Ed.* **2019**, *58*, 7788–7791.
- (12) (a) K. Miki, N. Imaizumi, K. Nogita, M. Oe, H. Mu, W. Huo, H. Harada, K. Ohe, *Bioconjugate Chem.* **2021**, *32*, 1773–1781. (b) K. Miki, N. Imaizumi, K. Nogita, M. Oe, H. Mu, W. Huo, K. Ohe, *Methods Enzymol.* **2021**, *657*, 89–109.
- (13) J.-W. Hofman, F. van Zeeland, S. Turker, H. Talsma, S. A. G. Lambrechts, D. V. Sakharov, W. E. Hennink, C. F. von Nostrum, *J. Med. Chem.* **2007**, *50*, 1485–1494.
- (14) (a) N. Brasseur, R. Ouellet, C. La Madeleine, J. E. van Lier, *Br. J. Cancer* **1999**, *80*, 1533–1541. (b) J.-J. Chen, Y.-Z. Huang, M.-R. Song, Z.-H. Zhang, J.-P. Xue, *ChemMedChem* **2017**, *12*, 1504–1511. (c) K. Li, W. Dong, Q. Liu, G. Lv, M. Xie, X. Sun, L. Qiu, J. Lin, *J. Photochem. Photobiol., B* **2019**, *190*, 1–7.
- (15) (a) J. E. Owen, M. E. Kenney, *Inorg. Chem.* **1962**, *1*, 331–333. (b) C. W. Dirk, T. Inabe, K. F. Schoch Jr., T. J. Marks, *J. Am. Chem. Soc.* **1983**, *105*, 1539–1550.
- (16) F. Zhou, B. Feng, H. Yu, D. Wang, T. Wang, Y. Ma, S. Wang, Y. Li, *Adv. Mater.* **2019**, *31*, 1805888.
- (17) K. J. Wynne, *Inorg. Chem.* **1984**, *23*, 4658–4663.
- (18) (a) T. Doane, A. Chomas, S. Srinivasan, C. Burda, *Chem. Eur. J.* **2014**, *20*, 8030–8039. (b) X.-F. Zhang, Q. Xi, J. Zhao, *J. Mater. Chem.* **2010**, *20*, 6726–6733.
- (19) J. Mizuguchi, G. Rihs, H. R. Karfunkel, *J. Phys. Chem.* **1995**, *99*, 16217–16227.
- (20) (a) N. B. Sul'timova, P. P. Levin, A. V. Lobanov, A. M. Muzafarov, *High Ener. Chem.* **2013**, *47*, 98–102. (b) P. Juzenas, A. Juzeniene, R. Rotomskis, J. Moan, *J. Photochem. Photobiol., B* **2004**, *75*, 107–110. (c) S. Dhami, D. Phillips, *J. Photochem. Photobiol., A* **1996**, *100*, 77–84.
- (21) (a) T. Namikawa, J. Iwabuchi, M. Munekage, S. Uemura, H. Maeda, H. Kitagawa, T. Nakayama, K. Inoue, T. Sato, M. Kobayashi, K. Hanazaki, *Surg. Today* **2020**, *50*, 821–831. (b) C. Shi, J. B. Wu, D. Pan, *J. Biomed. Opt.* **2016**, *21*, 050901.

- (22) K. Miki, T. Inoue, Y. Kobayashi, K. Nakano, H. Matsuoka, F. Yamauchi, T. Yano, K. Ohe, *Biomacromolecules* **2015**, *16*, 219–227.
- (23) (a) S. Li, X. Yan, J. Zhang, X. Guo, Y. Zhang, M. Su, C. Yang, H. Zhang, C. Li, *Adv. Funct. Mater.* **2021**, 2105189. (b) H. Mu, K. Miki, H. Harada, K. Tanaka, K. Nogita, K. Ohe, *ACS Sens.* **2021**, *6*, 123–129. (c) D. Yao, S. Yang, Y. Wang, K. Bian, W. Yang, D. Wang, B. Zhang, *Nanoscale* **2019**, *11*, 6307–6314.
- (24) D. Luo, X. Wang, E. Walker, J. Wang, S. Springer, J. Lou, G. Ramamurthy, C. Burda, J. P. Babilion, *ACS Nano* **2020**, *14*, 15193–15203.
- (25) K. Sato, K. Ando, S. Okuyama, S. Moriguchi, T. Ogura, S. Totoki, H. Hanaoka, T. Nagaya, R. Kokawa, H. Takakura, M. Nishimura, Y. Hasegawa, P. L. Choyke, M. Ogawa, H. Kobayashi, *ACS Cent. Sci.* **2018**, *4*, 1559–1569.
- (26) (a) M. Kobayashi, M. Harada, H. Takakura, K. Ando, Y. Goto, T. Tsuneda, M. Ogawa, T. Taketsugu, *ChemPlusChem* **2020**, *85*, 1959–1963. (b) E. D. Anderson, S. Sova, J. Ivancic, L. Kelly, M. J. Schnermann, *Phys. Chem. Chem. Phys.* **2018**, *20*, 19030–19036.
- (27) M. E. Frink, D. K. Geiger, G. J. Ferraudi, *J. Phys. Chem.* **1986**, *90*, 1924–1927.
- (28) P.-C. Lo, M. S. Rodríguez-Morgade, R. K. Pandey, D. K. P. Ng, T. Torres, F. Dumoulin, *Chem. Soc. Rev.* **2020**, *49*, 1041–1056.
- (29) J. Metz, O. Schneider, M. Hanack, *Inorg. Chem.* **1984**, *23*, 1065–1071.
- (30) A. Weitemeyer, H. Kliesch, D. Wöhrle, *J. Org. Chem.* **1995**, *60*, 4900–4904.
- (31) A. Galstyan, D. Block, S. Niemann, M. C. Grüner, S. Abbruzzetti, M. Oneto, C. G. Daniliuc, S. Hermann, C. Viappiani, M. Schäfers, B. Löffler, C. A. Strassert, A. Faust, *Chem. Eur. J.* **2016**, *22*, 5243–5252.
- (32) M. E. Ourailidou, P. Dockerty, M. Witte, G. J. Poelarends, F. J. Dekker, *Org. Biomol. Chem.* **2015**, *13*, 3648–3653.
- (33) Y. Zhao, D. G. Truhlar, *Theor. Chem. Acc.* **2008**, *120*, 215.
- (34) (a) M. Cossi, N. Rega, G. Scalmani, V. Barone, *J. Comput. Chem.* **2003**, *24*, 669–681. (b) V. Barone, M. Cossi, *J. Phys. Chem. A* **1998**, *102*, 1995–2001.

- (35) Gaussian 16, Revision B.01, 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. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2016.
- (36) M. D. Hanwell, D. E. Curtis, D. C. Lonie, T. Vandermeersch, E. Zurek, G. R. J. Hutchison, *Cheminformatics* **2012**, 4, 17.
- (37) B. A. Lindig, M. A. J. Rodgers, A. P. Schaap, *J. Am. Chem. Soc.* **1980**, 102, 5590–5593.

Chapter 3

Reductively Convertible Nickel Phthalocyanine Precursor as a Biological Thiol-Responsive Turn-on Photoacoustic Contrast Agent

Abstract

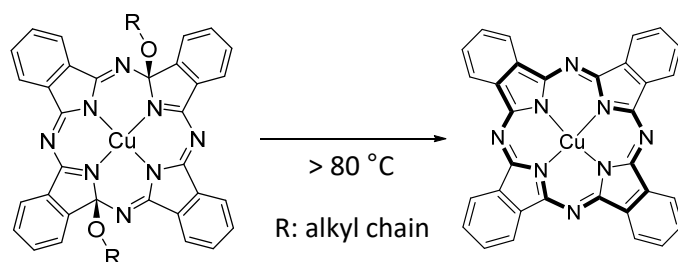
Metallophthalocyanines can generate strong photoacoustic (PA) waves under near-infrared (NIR) pulsed laser irradiation. However, avoiding PA signal generation in non-targeted areas is necessary to visualize cancer tissue clearly when using them as contrast agents for PA cancer imaging. The author prepared a nickel phthalocyanine (NiPc) precursor bearing poly(ethylene glycol) as a turn-on contrast agent for photoacoustic imaging. This precursor generates PA signals only after conversion to NiPc. The water-soluble polymeric chains were smoothly eliminated through thiol-mediated reductive aromatization in cancer cells, enabling the detection of endogenous biological thiols *in vitro* and *in vivo*.

Introduction

Photoacoustic (PA) imaging has attracted attention as a reliable method to visualize cancer tissues *in vivo* because of its advantages, such as high spatial resolution, deeper tissue visualization, and low invasiveness.¹ Metallophthalocyanine (MPc) is a photostable near-infrared (NIR)-absorbing dye and is considered one of the most promising photosensitizers for PA imaging because of its high molar extinction coefficient (ϵ) and excellent photothermal conversion efficiency.² Although MPc-containing nanoparticles³ and water-soluble MPcs⁴ have been used for PA cancer imaging, they generate PA signals as background noise in normal tissues. Including the author's previous works shown in Chapter 1, several 'activatable' contrast agents based on MPc, which can enhance PA signal intensity in response to tumor-specific biological compounds, have been developed to visualize cancer tissue more clearly.⁵ Despite the activatable nature of such MPc agents, generating background PA signals by unreacted MPcs still needs to be suppressed for efficient imaging with high contrast. Therefore, a 'turn-on' PA photosensitizer that generates PA signals only in cancer tissues is ideal;⁶ however, to the author's knowledge, there is no example of a turn-on PA contrast agent based on MPc. Modified MPcs with two alkoxy substituents at the α -position of pyrroles (ox-MPc) were reported as thermally convertible MPc precursors (Figure 1a).⁷ Considering that the transformation of ox-MPc to MPc is a formal reduction reaction, reductive aromatization is a more suitable conversion method.⁸ The author envisioned that biological thiols such as glutathione (GSH) overexpressed in many cancer cell lines can be utilized for the reductive aromatization of ox-MPcs under physiological conditions.⁹

Herein, the author reports the turn-on PA contrast agent **ox-NiPc-PEG** consisting of ox-NiPc and water-soluble poly(ethylene glycol) (PEG) (Figure 1b). Because the introduction of two alkoxy groups on the 18π aromatic core structure of NiPc causes a hypsochromic shift in the absorption peak of **ox-NiPc-PEG**, no PA signal is generated under NIR pulsed laser irradiation for PA imaging. The conversion of **ox-NiPc-PEG** to NiPc by GSH proceeded under physiological conditions, resulting in strong PA signal generation. PA imaging using **ox-NiPc-PEG** enabled the detection of GSH in xenograft tumor-bearing mice.

(a) thermal conversion of ox-CuPc



(b) reductive conversion of ox-NiPc, this work

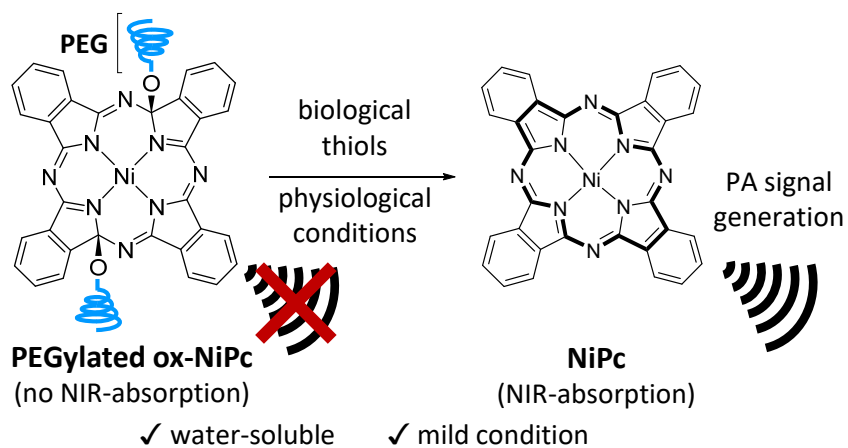
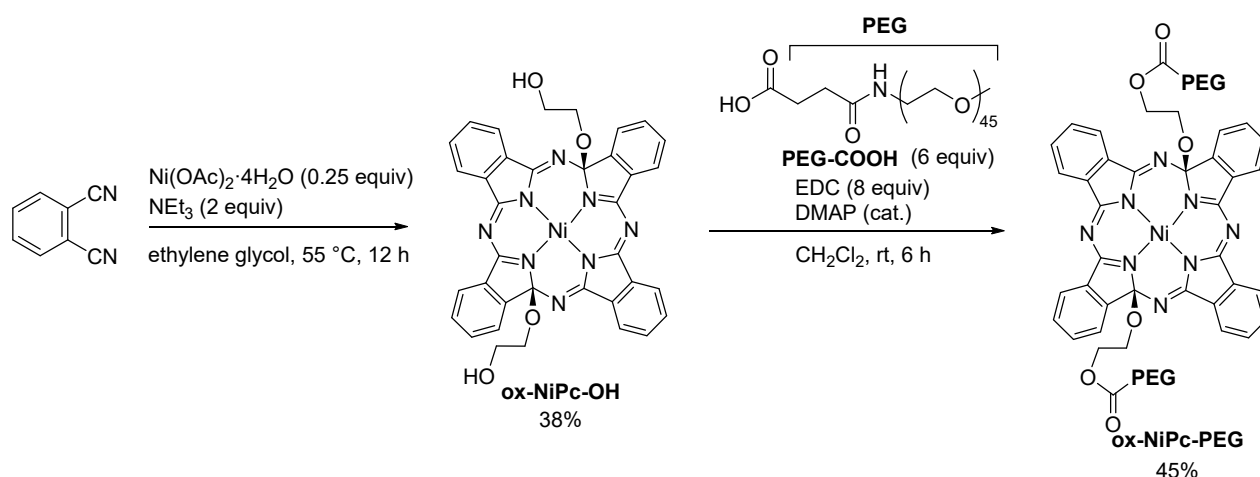


Figure 1. (a) Thermal conversion of ox-CuPc and (b) reductive-conversion of ox-NiPc.

Results and discussion

The water-soluble NiPc precursor **ox-NiPc-PEG** was synthesized from phthalonitrile in two steps via **ox-NiPc-OH** with two hydroxy groups at the side chain terminal (Scheme 1). The structure of **ox-NiPc-OH** was confirmed by X-ray crystallographic analysis (Figure 2). The crystal structure clarified that two hydroxyethoxy groups on the α -carbons of diagonal pyrrole extended to the convex face of the significantly distorted NiPc macrocycle having sp^3 -hybridized carbons.^{7,10} The formation of the thermodynamically less stable *trans*-isomer was not observed (Figure 3). **ox-NiPc-OH** with a distorted structure could dissolve well in common organic solvents, such as toluene, THF, acetone, CH_2Cl_2 , and $CHCl_3$. To enhance the water solubility of ox-NiPc, **ox-NiPc-PEG** was synthesized from **ox-NiPc-OH** by condensation with PEG derivative



Scheme 1. Synthesis of **ox-NiPc-PEG**.

PEG-COOH¹¹ in 45% yield. **ox-NiPc-PEG** dissolved in water at the concentration of 500 μ M or below (Figure 4).

Next, the author examined the reactivity of ox-NiPcs with reductants. The reaction of **ox-NiPc-PEG** with GSH in phosphate buffer solution (PBS) at room temperature gave a blue solid. This solid was identified as NiPc by MALDI-TOF mass spectrometry, IR absorption spectrometry, and elemental analysis. When **ox-NiPc-OH** was treated with GSH at room temperature for 24 h, NiPc was obtained in 98% isolated yield. The UV-vis absorption spectrum of an aqueous solution of **ox-NiPc-PEG** showed no absorption peaks in the NIR region because of the non-aromatic nature of the distorted NiPc core (Figure 5a, red line). After the

reaction of **ox-NiPc-PEG** with GSH in aqueous solution, a new absorption peak at 606 nm appeared (Figure 5a, blue line). The absorption of the Q band of NiPc, which is generally observed at approximately 670 nm, was broadened and blue-shifted, indicating that NiPc formed *H*-aggregates in water. These results showed that ox-NiPc can be quantitatively transformed to NiPc by reduction and utilized as a biological thiol-responsive photosensitizing molecule under physiological conditions.

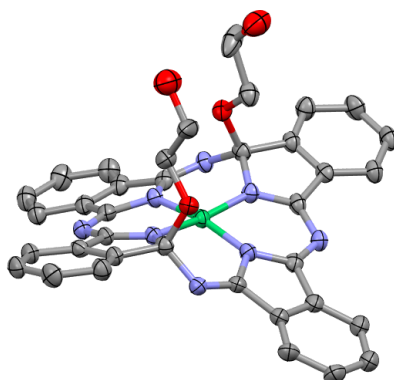


Figure 2. ORTEP drawing of **ox-NiPc-OH** (50% ellipsoids). Hydrogen atoms and solvent molecules are omitted for clarity.

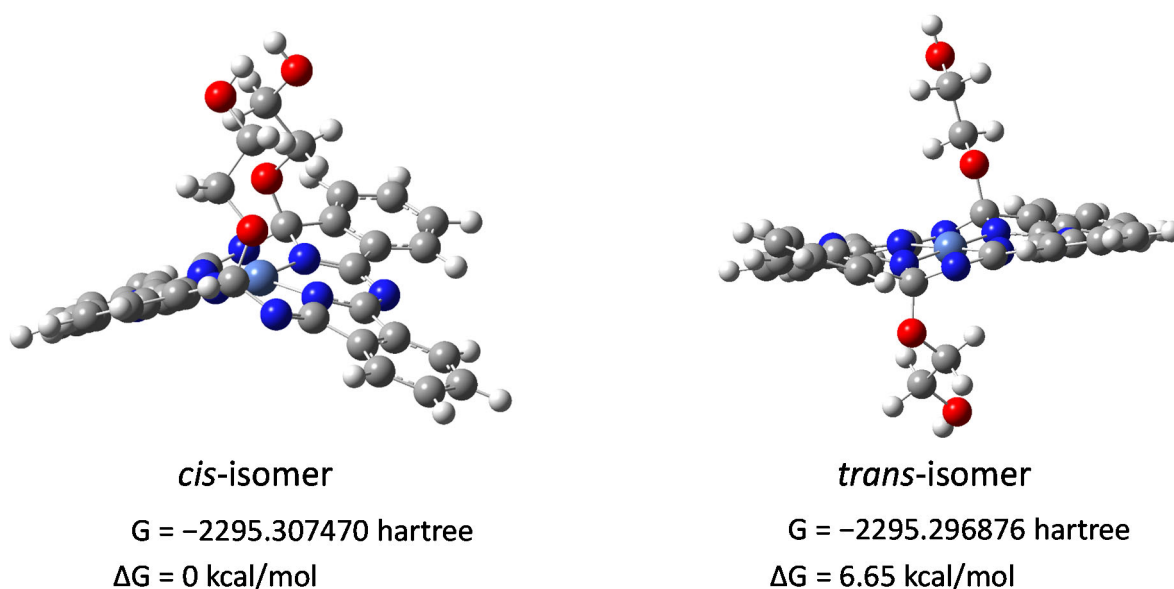


Figure 3. Energy-minimized structures and Gibbs free energy of two isomers of **ox-NiPc-OH** at the B3LYP/LANL2DZ level for Ni and the B3LYP/6-31G (d, p) level for C, H, N, O.

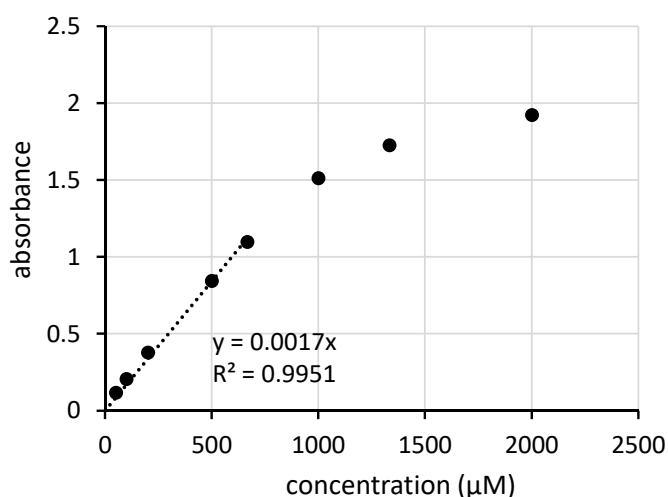


Figure 4. Concentration-dependent absorbance change of **ox-NiPc-PEG** in water at 459 nm.

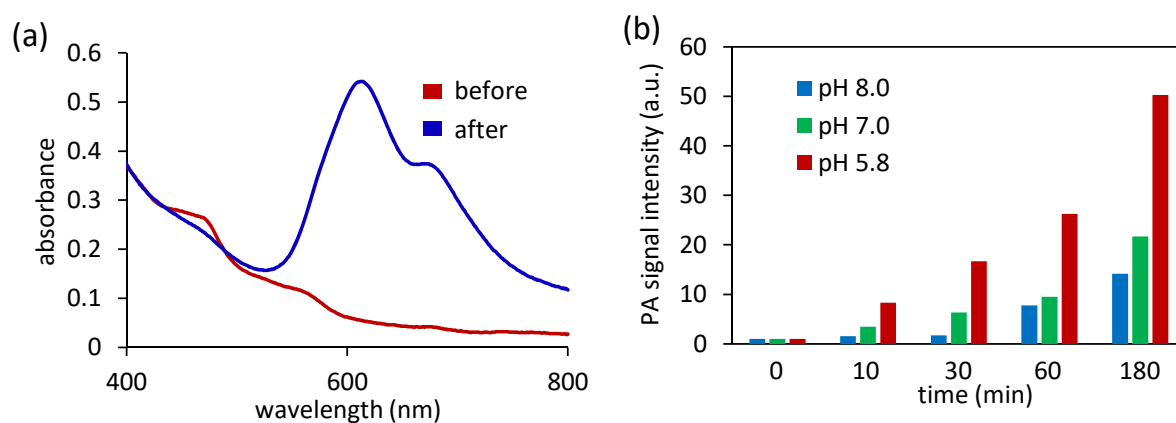
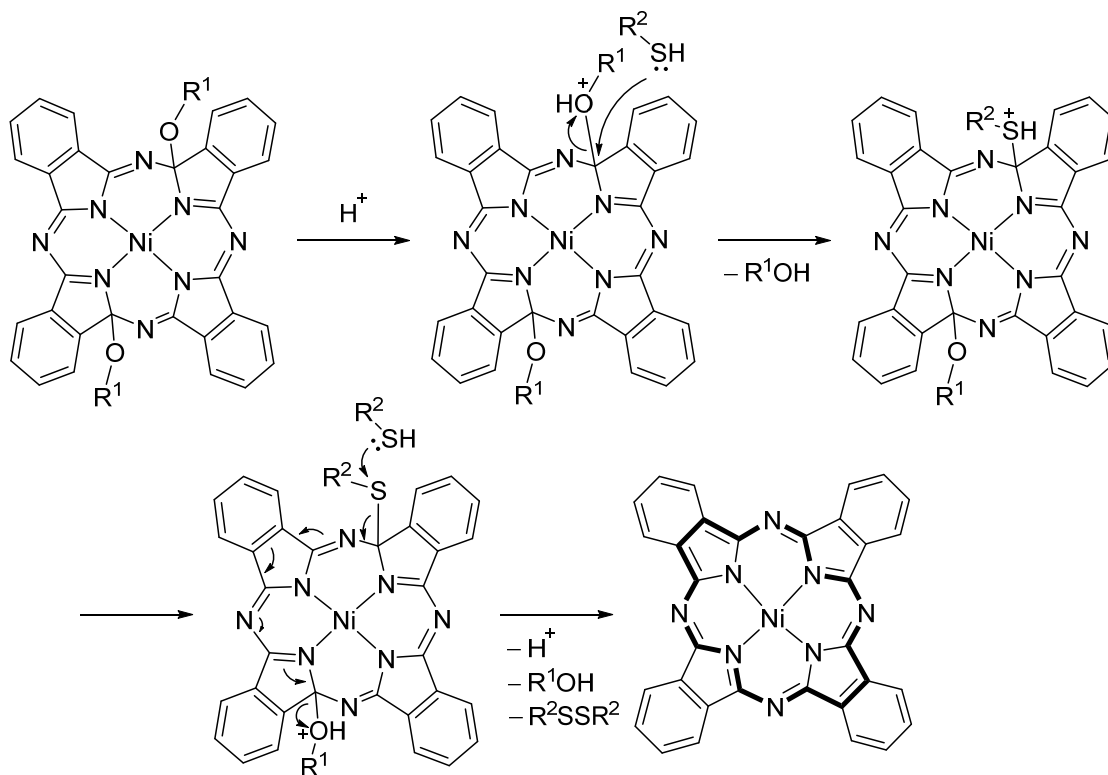


Figure 5. (a) UV-vis absorption spectra of **ox-NiPc-PEG** (50 μM) in water before (red line) and after (blue line) GSH treatment. (b) Time-dependent PA signal intensity change of **ox-NiPc-PEG** in PBS at various pH (blue: pH 8.0, green: pH 7.0, red: pH 5.8) under GSH treatment (1 mM). Excitation wavelength is 680 nm.

Next, the author examined the dependency of pH on the GSH-mediated reduction of **ox-NiPc-PEG**. The conversion of **ox-NiPc-PEG** to NiPc was accelerated in acidic PBS, and the resulting NiPc aggregates generated strong PA signals under NIR pulsed laser irradiation (Figure 5b). This result suggests that the protonation of the alkoxy groups attached to pyrrole α -carbons is crucial in the reductive aromatization of **ox-NiPc-PEG**.⁸ A plausible reaction mechanism is shown in Scheme 2. Upon protonation, the alcohol is



Scheme 2. Plausible mechanism of biological thiol-mediated reductive aromatization.

eliminated by a nucleophilic attack of biological thiols on the sp^3 -hybridized carbon. The subsequent nucleophilic attack of biological thiol to thioether affords an 18π aromatic structure of the NiPc core together with a disulfide and alcohols.

To evaluate the GSH-mediated turn-on of the PA signal, the author investigated the increase in the PA signal intensity of **ox-NiPc-PEG** under various GSH concentrations (Figure 6a). The PA signal intensity was dependent on the GSH concentration. By contrast, the PA signal intensity of **ox-NiPc-PEG** scarcely increased in the absence of GSH. When **ox-NiPc-PEG** was treated with GSH at the intracellular concentrations, the PA signal intensity was 4.9- and 6.3-fold higher than when treated with Cys and Hcy, respectively (Figure 6b). These results indicate that NiPc was produced more effectively upon treatment with GSH at the intracellular concentration. The author confirmed that no 1O_2 was generated from NiPc, which is produced from **ox-NiPc-PEG** under NIR photoirradiation.¹² Therefore, the oxidative stress to normal cells by 1O_2 is expected to be avoidable during PA imaging using the NiPc precursor (Figure 7).

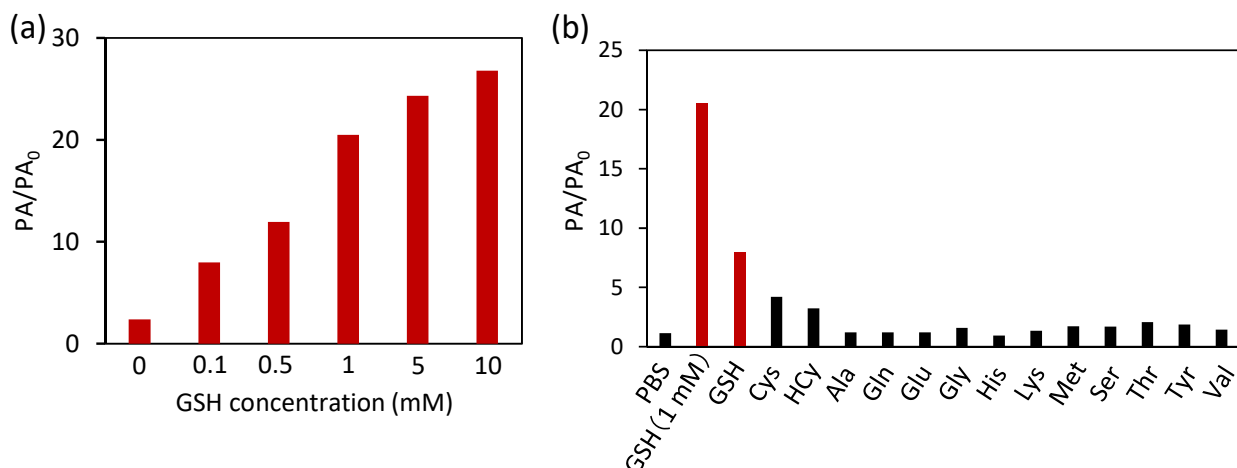


Figure 6. (a) GSH concentration-dependent PA signal intensity of **ox-NiPc-PEG** (0.25 mM) in PBS (pH = 5.8) after 30 min. (b) PA signal intensity of **ox-NiPc-PEG** after incubation with GSH (1.0 mM or 0.10 mM, red) or amino acids (0.10 mM, black) in PBS (pH = 5.8) for 1 h at 37 °C.

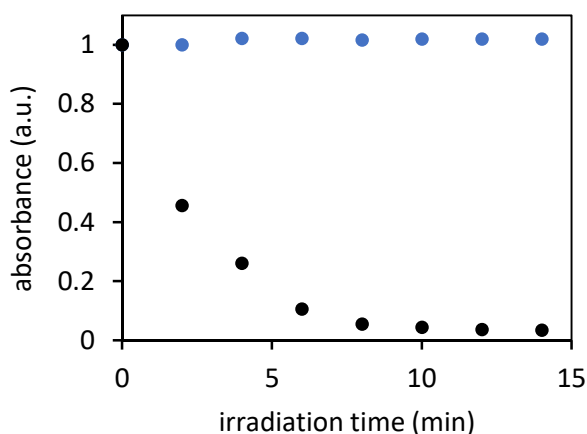


Figure 7. Time-dependent change of absorbance of ADPA at 400 nm in H₂O in the presence of NiPc obtained from **ox-NiPc-PEG** under photoirradiation (blue). Methylene blue (black) was used as a positive control.

Having confirmed that the reduction of **ox-NiPc-PEG** can be monitored by the PA signal output, the author next evaluated the PA signal intensity of **ox-NiPc-PEG** in GSH-overexpressing human lung cancer cell line A549.¹³ After incubation with **ox-NiPc-PEG** (10 μM), the PA signal intensity was significantly increased in A549 cells. After 4 h, the PA signal intensity in A549 cells was 4.6-fold stronger than that in the normal cell line HEK293 (Figure 8, red and blue). By a control experiment using *N*-ethylmaleimide (NEM) as a trapping agent of biological thiols in A549 cells, the author confirmed that the increase in PA signal intensity was due

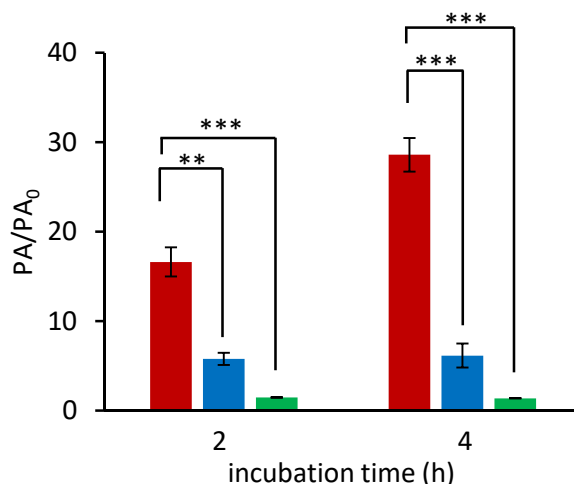


Figure 8. Time-dependent PA signal intensity change during the incubation of A549 (red) and HEK293 (blue) with **ox-NiPc-PEG** (10 μ M), and the incubation of A549 with **ox-NiPc-PEG** (10 μ M) after incubation with NEM (green). Excitation wavelength is 680 nm. Two-tailed Student's *t*-test: ** $p < 0.01$, *** $p < 0.001$.

to the formation of NiPc (Figure 8, green). It was confirmed that there was no cytotoxicity of **ox-NiPc-PEG** for cancer cells and normal cells when the concentration of **ox-NiPc-PEG** was less than 50 μ M, using an MTT assay (Figure 9). These results indicate that **ox-NiPc-PEG** is suitable for use as a PA contrast agent to visualize the reductive environment of the tumor site *in vivo*.

Finally, the author conducted PA imaging in living mice to verify the applicability of **ox-NiPc-PEG** as a biological thiol-responsive PA contrast agent *in vivo* (Figure 10). A549 cells were xenografted into the right

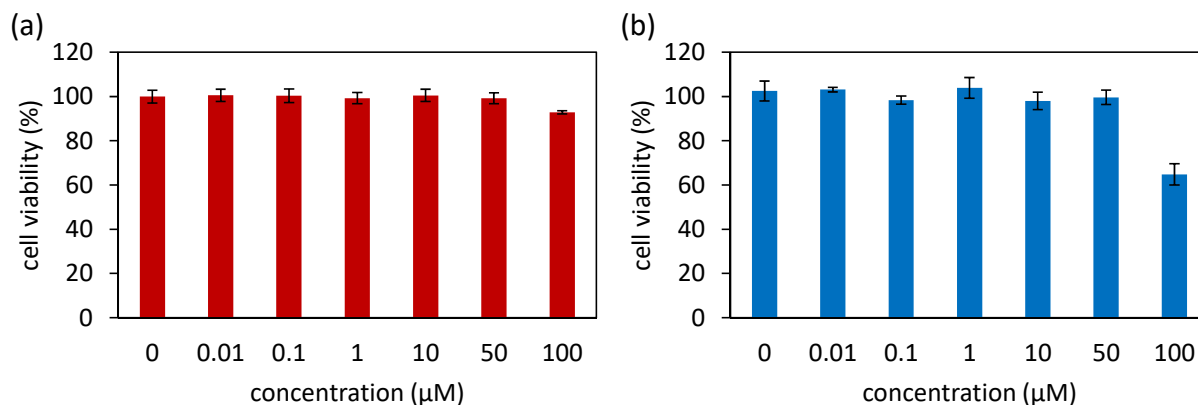


Figure 9. Cell viabilities of (a) A549 and (b) HEK293 cells *in vitro* after incubation with **ox-NiPc-PEG** for 24 h. Mean values and standard deviation ($n = 8$).

legs of nude mice to prepare tumor-bearing mice. Eight prepared mice were divided into two groups for *in vivo* experiments through intratumoral administration. For the first group, saline (30 μ L) was pre-treated at the tumor site in the right leg and the normal muscle in the left leg. After 30 min pre-treatment, **ox-NiPc-PEG** was administered intratumorally in the right leg and subcutaneously in the left leg, respectively. For the second group, a pre-treatment of NEM (2.0 mM in 30 μ L saline) was conducted in tumor sites before the intratumoral administration of **ox-NiPc-PEG**. The PA tomography system was used to obtain horizontal and vertical view images at the indicated time points of post-injection of **ox-NiPc-PEG** (Figure 11), and the PA signal intensities at the administration sites were evaluated under NIR pulsed laser irradiation (Figure 12). After the injection of **ox-NiPc-PEG**, PA signal intensity in the tumor site significantly increased in three hours ($p < 0.05$) in good contrast to the non-tumor site, demonstrating its sensitivity to distinguish tumor sites from normal muscle. Furthermore, the NEM-pretreated tumors exhibited weak PA signals before and after injection, suggesting that **ox-NiPc-PEG** can selectively detect biological thiols in living mice.

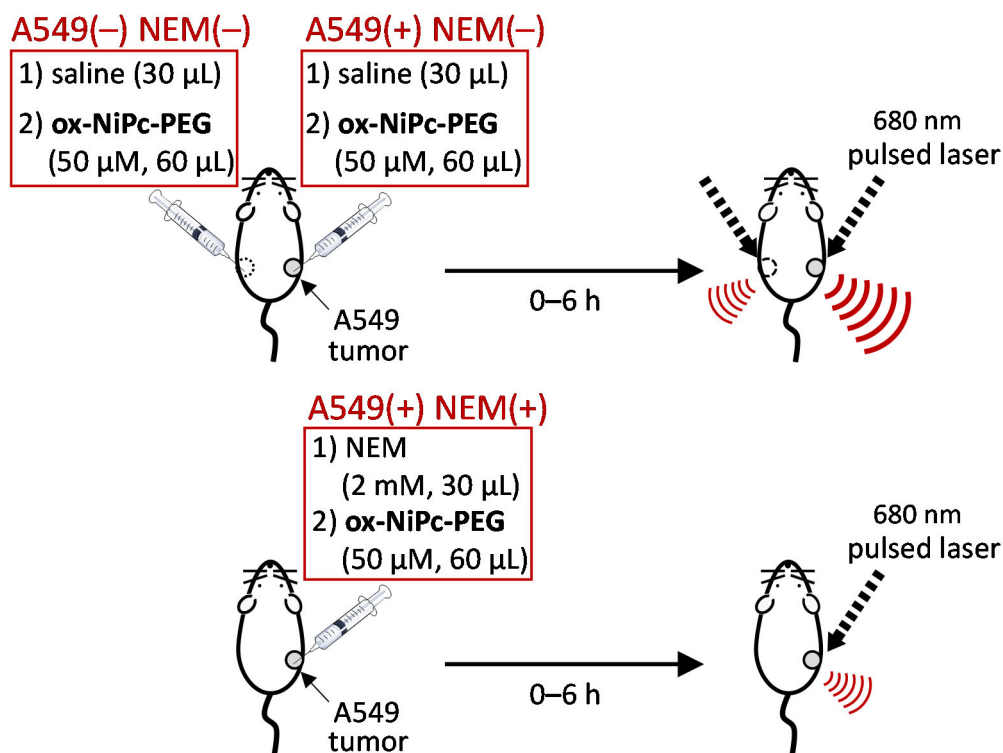


Figure 10. Schematic illustration of *in vivo* experiment.

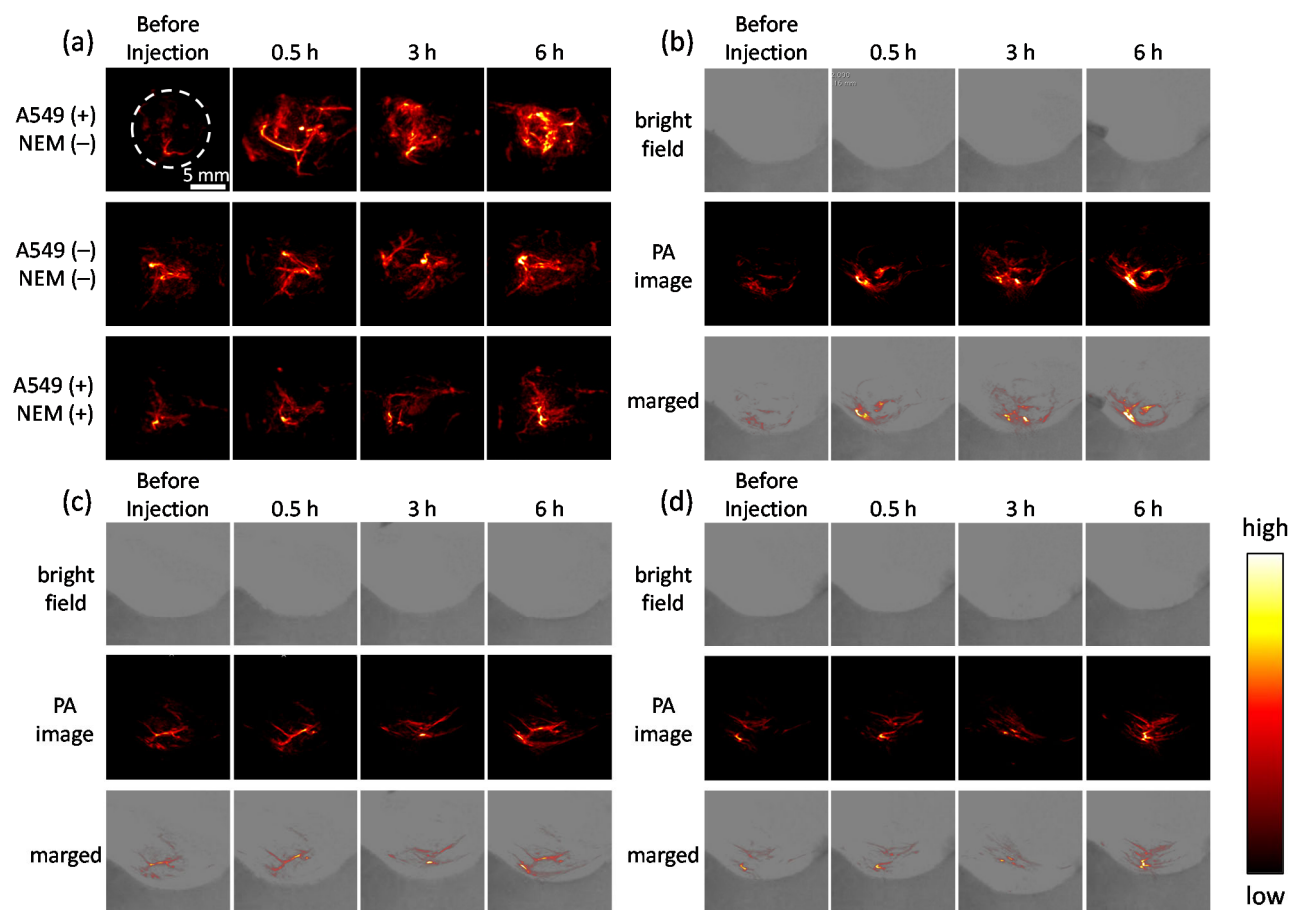


Figure 11. (a) *In vivo* PA top-view images of administrated sites before and 0.5, 3, 6 hours after injection. (A549(+): tumor site, A549(-): normal muscle) (b) *In vivo* PA side-view images of (b) A549(+) NEM(-), (c) A549(-) NEM(-), and (d) A549(+) NEM(+) region before and 0.5, 3, 6 h after injection (top: bright field, middle: PA image, bottom: merged).

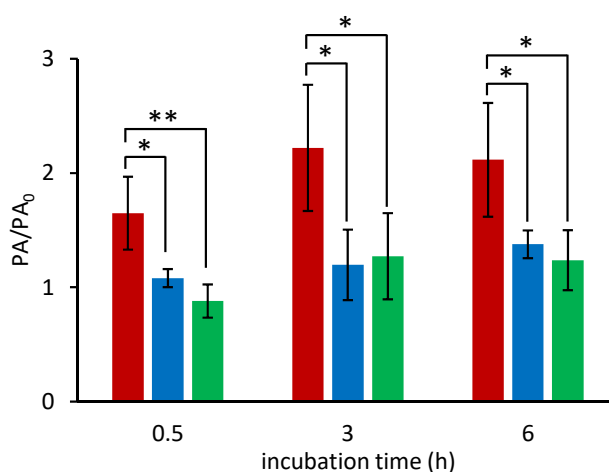


Figure 12. Normalized PA signal intensity at the tumor sites (red: NEM(-), green: NEM(+)) and normal muscle (blue) at 0.5, 3, 6 h after intratumoral or subcutaneously injection of **ox-NiPc-PEG** (Sample size: $n = 4$). Excitation wavelength is 680 nm. Two-tailed Student's t -test: * $p < 0.05$, ** $p < 0.01$.

Conclusion

In conclusion, the author has developed a turn-on PA contrast agent based on reductive aromatization for cancer imaging. A water-soluble NiPc precursor **ox-NiPc-PEG** was converted to NiPc by biological thiols, generating strong PA signals under NIR pulsed laser irradiation. Because the biological thiol-mediated transformation proceeded smoothly under acidic conditions, the protonation of the alkoxy groups was considered to be crucial in the reductive aromatization. The results obtained *in vitro* and *in vivo* demonstrated that the increase in PA signal intensity was mainly due to the reduction of **ox-NiPc-PEG** by GSH overexpressed in cancer cells. Because biological thiol-mediated reductive aromatization proceeds under mild physiological conditions, it is suitable for constructing a π -conjugated system of photosensitizer *in vivo*.

Experimental

General

Materials and methods

1-Ethyl-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC·HCl) was purchased from Watanabe Chemical Industries, Ltd. (Japan). Triethylamine, ethylene glycol, 4-dimethylaminopyridine (DMAP), dichloromethane (CH₂Cl₂), Dulbecco's modified Eagle's medium (DMEM), and 1% penicillin/streptomycin (P/S) were purchased from Fujifilm-Wako Pure Chemicals Industries, Ltd. (Japan). Methylene blue was purchased from Waldeck GmbH & Co. KG (Germany). (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), glycine (Gly), alanine (Ala), valine (Val), lysine (Lys), and phosphate buffer solution (pH 7.2, 0.1 M) were purchased from Tokyo Chemical Industry Co., Ltd. (Japan). Nickel acetate tetrahydrate, dimethyl sulfoxide (DMSO), *N*-ethylmaleimide (NEM), tyrosine (Tyr), histidine (His), serine (Ser), threonine (Thr), methionine (Met), glutamine (Gln), glutamic acid (Glu), cysteine (Cys), glutathione (GSH, reduced), and methanol (MeOH) were purchased from nacalai tesque (Japan). MgSO₄ was purchased from Kishida Chemical Co., Ltd. (Japan). Phthalonitrile and homocysteine (Hcy) were purchased from Aldrich Chemical Co. (USA). Isoflurane was purchased from Viatris Inc. (USA). Silica gel (SiO₂, 230–400 mesh) for column chromatography was purchased from Silicycle (Canada).

PEG-COOH was prepared from poly(ethylene glycol) monomethyl ether (MW = 2000) according to the reported method.¹¹

Buffered solutions (pH 5.8–8.0) were prepared by dissolving sodium dihydrogenphosphate (NaH₂PO₄) and disodium hydrogenphosphate (Na₂HPO₄) in water (MilliQ).

UV-vis absorption spectra were recorded by UV-vis spectrophotometer (UH5300, Hitachi High Technologies, Japan).

PA signals were measured by Nexus 128 (ENDRA Life Sciences Inc., USA). The sample solutions were kept in dark at room temperature for more than 3 h before measurement.

Mass spectra were measured by Exactive Plus Orbitrap (ESI, Thermo Fisher Scientific Inc., USA) and Ultraflex III (MALDI, Bruker Co., USA).

IR absorption spectra were measured by Spectrum Two (FT-IR, PerkinElmer Inc., USA).

The absorbance at 550 nm for MTT cytotoxicity assay was measured using 800TSTM Absorbance Microplate Reader (BioTek Instruments, USA).

Intensity data for X-ray crystallography were collected on a RIGAKU Saturn 724 HG CCD system with VariMax Mo Optic using MoK α radiation.

Synthesis

Synthesis of ox-NiPc-OH

In a 100 mL two-necked round-bottom flask, phthalonitrile (0.22 g, 1.8 mmol), nickel acetate tetrahydrate (0.11 g, 0.43 mmol), and triethylamine (0.50 mL, 3.6 mmol) in ethylene glycol (2 mL) were stirred at 55 °C under nitrogen atmosphere. After stirring for 12 h, the dark-green suspension was diluted with CH₂Cl₂ (30 mL) and filtered to remove the blue solid. The resulting red crude solution was washed with H₂O (30 mL $\times$ 3) and the organic layer was dried over MgSO₄. After the organic solvent was removed under reduced pressure, the crude product was purified by column chromatography on SiO₂ (eluent: CH₂Cl₂/MeOH, v:v = 1:0 to 0:1) to afford **ox-NiPc-OH** (0.11 g, 0.16 mmol, 38%) as a reddish-brown solid. When triethylamine was not added to the reaction solution, the isolated yield was 11% after the reaction for 24 h.

ox-NiPc-OH: IR (neat) 3323, 3061, 2934, 2871, 1640, 1614, 1568, 1535, 1488, 1455, 1407, 1326, 1292, 1248, 1195, 1166, 1148, 1128, 1093, 1064, 1035, 1004, 957, 923, 884, 845, 773, 727, 678, 659, 623, 603, 589, 581, 567, 542, 533, 525, 513 cm⁻¹. UV-vis (CHCl₃) λ_{max} (log ϵ) = 561 (3.30), 468 (3.65), 409 (3.53). ¹H NMR (400 MHz, CDCl₃, 25 °C) δ = 3.36–3.46 (m, 2H), 3.80–3.90 (m, 6H), 7.51–7.58 (m, 6H), 7.60–7.64 (m, 2H), 7.85–7.87 (m, 4H), 7.96–7.99 (m, 4H). ¹³C NMR (100 MHz, CDCl₃, 25 °C) δ = 61.7, 65.7, 100.4, 122.0, 122.3, 122.7, 123.3, 130.0, 131.7, 131.8, 135.7, 138.1, 147.0, 161.6, 165.2, 168.1. HRMS (ESI) calcd for C₃₆H₂₆N₈NaO₄ ([M+Na]⁺) 715.1323, found 715.1324.

Synthesis of **ox-NiPc-PEG**

In a 50 mL Schlenk tube, EDC·HCl (0.18 g, 0.91 mmol), DMAP (0.15 mg, 1.2 μ mol), **PEG-COOH** (1.1 g, 0.51 mmol), and CH₂Cl₂ (3.0 mL) were stirred at room temperature for 20 min. After addition of **ox-NiPc-OH** (0.10 g, 0.15 mmol), the solution was stirred at room temperature for 6 h. The resulting solution was washed with H₂O (50 mL $\times$ 3) and the organic solvent was removed under reduced pressure. The crude red solid was dissolved in water. The aqueous solution was filtered to remove unreacted **ox-NiPc-OH**. The filtrate was freeze-dried and **ox-NiPc-PEG** (45%, containing a small amount of free **PEG-COOH**) as a red solid was obtained. In MALDI-TOF mass spectrum of **ox-NiPc-PEG**, the fragment peaks of ox-NiPc bearing one PEG chain and peaks of PEG fragments were observed together with the parent peaks (Figure 13).

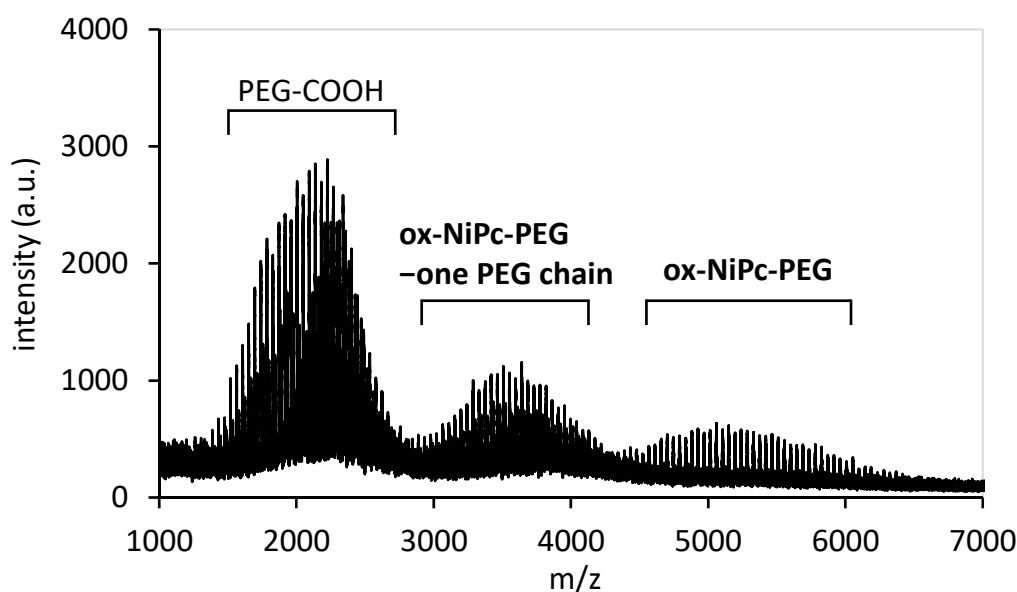


Figure 13. MALDI-TOF mass spectrum of **ox-NiPc-PEG**. Matrix: dithranol.

Theoretical calculation

The density functional theory (DFT) calculations were performed for the geometry optimization of two isomers of **ox-NiPc-OH** having two hydroxyethoxy groups at the B3LYP/LANL2DZ level for Ni and the B3LYP/6-31G(d, p) level for C, H, N, O by using Gaussian 16 package.^{14,15} Their electronic and Gibbs free

energies are summarized in Table 1. The cartesian coordinates of the optimized structures are summarized at the end of the experimental section.

Reduction by GSH

Reduction of ox-NiPc-OH to NiPc by GSH

In a 50 mL test tube, GSH (2.1 g, 6.7 mmol) was completely dissolved in PBS (pH 5.8, 20 mL). Then, the solution of **ox-NiPc-OH** (1.0 g, 1.5 mmol) in acetone (20 mL) was added dropwise and stirred at room temperature for 24 h. The reaction mixture was centrifuged to remove the supernatant. After washing with water (30 mL $\times$ 3) and acetone (30 mL $\times$ 3), and the precipitate was dried under reduced pressure to give NiPc (0.83 g, 1.5 mmol, 98%).

Reduction of ox-NiPc-PEG to NiPc by GSH

In a 50 mL test tube, GSH (1.0 g, 3.3 mmol), **ox-NiPc-PEG** (0.15 g, 0.22 mmol), and water (10 mL) were stirred at room temperature for 3 d. The reaction mixture was centrifuged to remove the supernatant. After washing with water (30 mL $\times$ 5) and acetone (30 mL $\times$ 5), and the precipitate was dried under reduced pressure to obtain NiPc. NiPc was identified by MALDI-TOF mass spectrum (Matrix: dithranol), IR absorption spectrum, and elemental analysis.

NiPc: MS (MALDI) calcd for $C_{32}H_{16}N_8Ni$ ($[M]^+$) $m/z = 570$, found 570. IR (neat) 3048, 3015, 2934, 2887, 2798, 2671, 2640, 2595, 2422, 2278, 2261, 2201, 2102, 1895, 1818, 1645, 1613, 1599, 1533, 1494, 1472, 1428, 1349, 1333, 1289, 1200, 1165, 1120, 1090, 1006, 980, 948, 940, 916, 869, 862, 806, 771, 775, 721, 643, 573, 540, 530 cm^{-1} . Elemental analysis: calcd for $C_{32}H_{16}N_8Ni$ (NiPc): C, 67.29; H, 2.82; N, 19.62%, found C, 67.10; H, 2.83; N, 19.81%.

In vitro PA signal measurement

In 1.5 mL Eppendorf tubes, the PBS (pH 5.8) solution of 0.20–20 mM GSH (50 μ L) or 0.20 mM amino

acids (50 μ L) were added to the 500 μ M **ox-NiPc-PEG** solution (50 μ L) and incubated at 37 °C for 30 min, respectively. After incubation, the PA signal intensities of these samples were measured under 680 nm pulsed laser irradiation (5 mJ/cm²).

Detection of singlet oxygen

A water-soluble 9,10-anthracenedipropionic acid (ADPA) sodium salt was prepared according to the reported procedure.¹⁷ **ox-NiPc-PEG** (10 μ M) was incubated with GSH (1 mM) in water for 24 h. A solution of ADPA sodium salt (0.10 mM) was added to the mixture in a quartz cuvette (optical path length: 10 mm). The mixture was photoirradiated using a Xenon light source device (MAX-303, Asahi Spectra Co., Ltd., Japan) equipped with VIS mirror module, rod lens [RLQL80-1, illuminance: 2.7 mW (λ = 680 nm) at the sample level], and long pass filter (λ > 590 nm). For positive control experiment, methylene blue (10 μ M) was used instead of **ox-NiPc-PEG** and GSH. During photoirradiation, absorbance of ADPA at 400 nm were monitored every two minutes (Figure 6c).

Cell experiment

Cell culture

The human lung carcinoma cell line, A549, was purchased from American Type Culture Collection (Manassas, VA). Normal cell line, Human Embryonic Kidney cells (HEK293), were purchased from Japanese Collection of Research Bioresources Cell Bank (JCRB Cell Bank), Japan. A549 cells and HEK293 cells were cultured in DMEM with 10% fetal vobine serum (FBS). Cells were cultured in a well-humidified incubator with 5% CO₂ and 95% air at 37 °C.

MTT cytotoxicity assay

The cell viability was evaluated by monitoring the reduction of MTT to formazan crystals using mitochondrial dehydrogenases. A549 cells were seeded into a 96-well microtiter plate at a density of 5×10^4

cells/well. The cells were incubated for 24 h for cell attachment. A549 cells were incubated at 37 °C for an additional 24 h with various concentrations of **ox-NiPc-PEG** (0, 0.01, 0.1, 1, 10, 50, and 100 μ M). After washing the cells with PBS, MTT (0.20 mL, 0.5 mg/mL) was added to each well. After incubation at 37 °C for 4 h, the remaining MTT solution was removed, and the formazan crystals were dissolved in 200 μ L of DMSO with gentle agitation for 5 min. For the evaluation of viability of HEK293, HEK293 cells were used instead of A549 cells. IC_{50} of **ox-NiPc-PEG** was over 100 μ M.

PA signal increment caused by endogenous GSH

A549 and HEK293 cells were seeded into 12-well plates at a density of 1×10^5 cells/well and incubated at 37 °C for 24 h for cell attachment. For the thiol-trapping experiment, A549 cells were precultured with *N*-ethylmaleimide (NEM, 2.0 mM) for 30 min. The aqueous solution of **ox-NiPc-PEG** was diluted with DMEM with 10% FBS and 1% P/S (final concentration: 10 μ M). The cells were incubated with the **ox-NiPc-PEG** solution (1 mL) at 37 °C for 2 or 4 h. After incubation, the cells were washed with PBS and detached by trypsin/EDTA solution (0.10 mL). After adding PBS (0.90 mL), the cell suspension was transferred to 1.5 mL Eppendorf tube. After centrifugation, the supernatant was removed and PBS (1.0 mL) was added. The PA signal intensities were measured under 680 nm pulsed laser irradiation (5 mJ/cm²).

***In vivo* experiment**

A549 xenograft model

Female BALB/c mice in 9 weeks of age were purchased from Japan SLC, Inc., Japan. The mice were housed at the Institute of Laboratory Animals at Graduate School of Medicine of Kyoto University. All of animal experiments were approved by the Animal Research Committee of Kyoto University and carried out within its guidelines. To prepare the tumor-bearing mice, the injection of A549 cells (5×10^6 cells) in the right leg of each mouse was carried out.

In vivo PA signal intensity measurement

The A549 tumor-bearing mice were randomly divided into two groups ($n = 4$). For the first group, saline (30 μ L) was pre-treated at the tumor site in the right leg and at the normal muscle in the left leg, respectively. After 30 min pretreatment, **ox-NiPc-PEG** (50 μ M in 60 μ L saline) was administered intratumorally in the right leg and subcutaneously in the left leg, respectively. For the second group, a pre-treatment of NEM (2.0 mM in 30 μ L saline) was conducted in tumor sites before the intratumoral administration of **ox-NiPc-PEG** (50 μ M in 60 μ L saline). The PA tomography system was used to obtain from horizontal and vertical view images under pulsed laser irradiation ($\lambda = 680$ nm, 5 mJ/cm²). PA images of administrated sites were recorded before and post-injection at different time points (30 min, 3 h, and 6 h). The PA intensities of regions of interest (ROI: muscle or tumor sites) were detected from each mouse in the same size (diameter of circle = 1.13 cm). The represent intensities were calculated in the mean of independent experiments ($n = 4$) with standard deviation (s.d.).

X-ray crystallographic data

Single crystal of **ox-NiPc-OH** was obtained from recrystallization in CH₂Cl₂/hexane at room temperature. Crystal data are summarized in Table 1. The structures were solved by a direct method (SHELXT)¹⁸ and refined by a full-matrix least square method on *F*² for all reflections (SHELXL-2016).¹⁹ All hydrogen atoms were placed using AFIX instructions, while all other atoms were refined anisotropically. The residual scattered electron densities, originating from disordered solvents, were eliminated by the SQUEEZE protocol equipped in PLATON.²⁰ The separation of the disorders on the hydroxyethoxy moiety is difficult, resulting in the Alerts B in the checkCIF/PLATON report. However, this problem does not affect the whole structure of the molecule. The molecular structure was visualized by Mercury.²¹

Table 1. Crystallographic data of **ox-NiPc-OH**.

CCDC number	2303413
Empirical formula	C ₃₆ H ₂₆ N ₈ NiO ₄
Formula weight	693.36
Temperature/K	143(2)
Crystal size/mm ³	0.1 × 0.1 × 0.1
Radiation	Mo K α (λ = 0.71075)
Crystal color	red
Crystal system	orthorhombic
Space group	<i>P b c n</i>
<i>a</i> (Å)	12.655(3)
<i>b</i> (Å)	15.538(3)
<i>c</i> (Å)	17.066(3)
α (°)	90
β (°)	90
γ (°)	90
Volume (Å ³)	3355.7(12)
<i>Z</i>	4
<i>D</i> _{calc} (g/cm ³)	1.372
μ (mm ⁻¹)	0.63

$F(000)$	1432
2θ range for data collection ($^{\circ}$)	3.164 to 27.481
Index ranges	$-16 \leq h \leq 14$, $-20 \leq k \leq 19$, $-22 \leq l \leq 22$
Reflections collected	25539
Independent reflections	3849
Data/restraints/parameters	3849/0/223
Goodness-of-fit on F^2	1.006
Final R indexes [$I \geq 2\sigma(I)$]	$R_1 = 0.0620$, $wR_2 = 0.1136$
Final R indexes [all data]	$R_1 = 0.0716$, $wR_2 = 0.1177$
Largest diff. peak/hole ($e \text{ \AA}^{-3}$)	0.579/-0.56

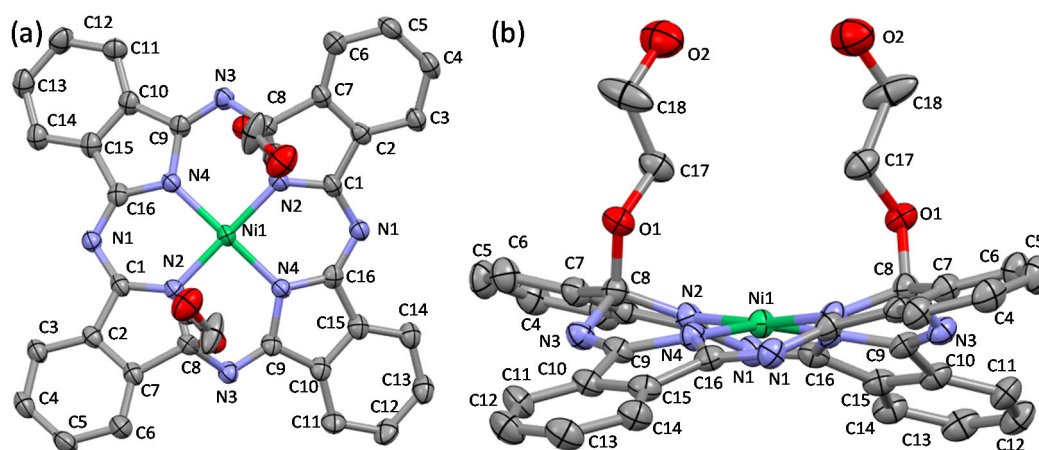


Figure 14. (a) Top-view and (b) side-view of **ox-NiPc-OH** (50% ellipsoids) with labels. Hydrogen atoms and solvent molecules are omitted for clarity.

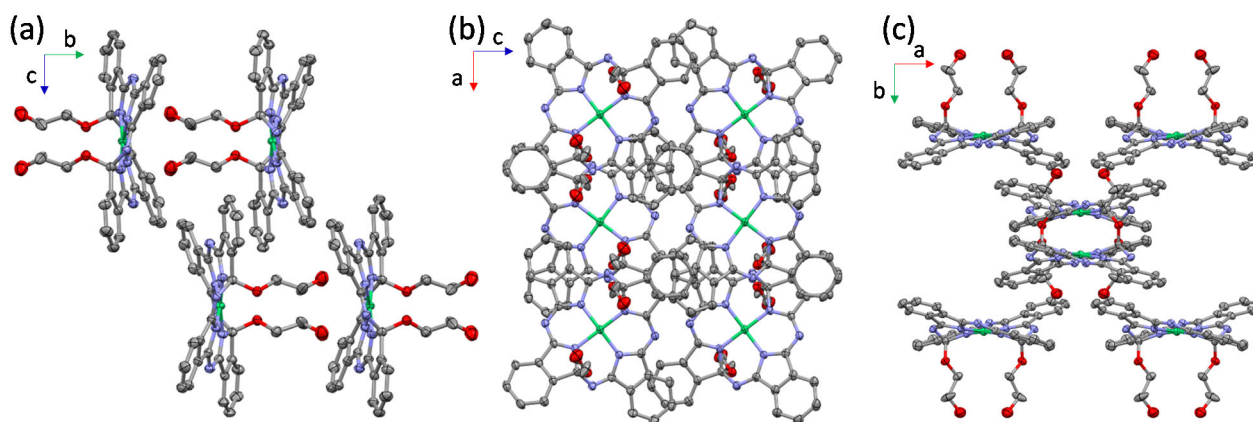


Figure 15. Packing structure of **ox-NiPc-OH** (50% ellipsoids) viewed from the (a) a axis, (b) b axis, and (c) c axis. Hydrogen atoms and solvent molecules are omitted for clarity.

Table 2. Selected (a) bond lengths and (b) angles for **ox-NiPc-OH**.

(a)	Length (Å)	(b)	Angle (deg)
Ni1—N2	1.845	N2—Ni1—N4	90.6
Ni1—N4	1.871	N2—Ni1—N4	90.3
O1—C8	1.434(4)	C1—N2—C8	110.0(2)
O1—C17	1.427(4)	C1—N1—C16	118.1(3)
N2—C1	1.328(4)	C8—N3—C9	116.2(2)
N2—C8	1.474(4)	C16—N4—C9	107.9(2)
N3—C8	1.448(4)	N2—C1—N1	127.0(3)
N3—C9	1.290(4)	N2—C1—C2	110.8(3)
N1—C1	1.352(4)	O1—C8—N2	111.1(2)
N1—C16	1.310(4)	O1—C8—N3	103.7(2)
N4—C16	1.365(4)	O1—C8—C7	113.4(2)
N4—C9	1.410(4)	N2—C8—N3	112.7(2)
O2—C18	1.393(6)	N2—C8—C7	103.3(2)
C1—C2	1.471(4)	N4—C16—C15	109.5(3)
C7—C2	1.377(4)	N4—C16—N1	128.6(3)
C7—C8	1.520(4)	N3—C9—N4	127.6(3)
C7—C6	1.378(5)	N4—C9—C10	108.6(2)
C15—C10	1.386(5)		
C15—C16	1.473(5)		
C10—C9	1.472(4)		

Cartesian coordinates of energy-minimized structures**cis-isomer**

Ni	-0.000000	-0.000000	0.359586	H	5.518740	-0.217909	2.069136
O	0.454115	2.604034	-1.600607	C	5.846518	1.909511	2.392604
N	-0.766380	1.699958	0.246331	H	6.846492	1.782313	2.796918
N	1.207937	3.134359	0.464721	C	4.026152	3.407785	1.759862
N	-3.005218	1.242923	0.969480	H	3.603538	4.402274	1.662514
N	1.718570	0.763356	0.585954	C	1.958162	2.139819	0.770438
O	-0.644199	1.503251	-4.840079	C	-2.001596	6.160342	-0.332571
H	-0.127602	1.249416	-5.613803	H	-1.930267	7.218412	-0.568221
C	-2.014119	2.038921	0.524193	C	-0.878630	5.338183	-0.501492
C	-1.008977	3.995045	-0.184695	H	0.064222	5.739035	-0.859596
C	-2.217928	3.480870	0.283450	C	-0.513388	2.301692	-2.604319
C	-3.215205	5.642179	0.138432	H	-1.133605	3.176722	-2.838322
H	-4.067493	6.304185	0.260286	H	-1.170427	1.482309	-2.289259
C	-3.339796	4.285661	0.454773	C	5.310126	3.199988	2.279043
H	-4.270000	3.866443	0.823556	H	5.902723	4.052170	2.599004
C	3.839016	1.001525	1.484362	C	0.287104	1.874141	-3.826511
C	3.302996	2.287451	1.373837	H	0.926177	2.709592	-4.148680
C	0.000000	2.855925	-0.277489	H	0.937940	1.032174	-3.557781
C	2.809527	0.064898	0.989857	N	0.766380	-1.699958	0.246331
C	5.114652	0.785854	1.991248	O	-0.454115	-2.604034	-1.600607
				N	-1.207937	-3.134359	0.464721

Chapter 3

N	3.005218	-1.242923	0.969480	C	-2.238965	-1.602328	-0.497730
N	-1.718570	-0.763356	0.585954	N	-3.104828	-0.660422	-0.543273
O	0.644199	-1.503251	-4.840079	N	0.768712	-3.190808	0.435263
H	0.127602	-1.249416	-5.613803	C	3.571935	-3.869490	1.504163
C	2.014119	-2.038921	0.524193	C	4.927060	-3.932271	1.843186
C	1.008977	-3.995045	-0.184695	C	5.785347	-2.857985	1.571583
C	2.217928	-3.480870	0.283450	C	5.316170	-1.693705	0.947580
C	3.215205	-5.642179	0.138432	C	4.188521	3.712174	0.860724
H	4.067493	-6.304185	0.260286	C	4.208795	5.110048	0.930622
C	3.339796	-4.285661	0.454773	C	3.058038	5.867298	0.664718
H	4.270000	-3.866443	0.823556	C	1.850384	5.249180	0.323295
C	-3.839016	-1.001525	1.484362	C	-3.735570	-3.631526	-1.218757
C	-3.302996	-2.287451	1.373837	C	-3.741794	-5.029986	-1.289586
C	-0.000000	-2.855925	-0.277489	C	-2.624697	-5.781695	-0.897047
C	-2.809527	-0.064898	0.989857	C	-1.464046	-5.157689	-0.425660
C	-5.114652	-0.785854	1.991248	C	-3.356328	4.084537	0.976523
H	-5.518740	0.217909	2.069136	C	-4.733739	4.168583	-1.207409
C	-5.846518	-1.909511	2.392604	C	-5.552848	3.042633	-1.061904
H	-6.846492	-1.782313	2.796918	C	-5.023631	1.804599	-0.666701
C	-4.026152	-3.407785	1.759862	Ni	0.173444	0.045587	-0.033915
H	-3.603538	-4.402274	1.662514	O	3.594680	-0.729998	-1.464439
C	-1.958162	-2.139819	0.770438	O	-2.868785	0.467994	1.498521
C	2.001596	-6.160342	-0.332571	O	1.588084	-0.110257	-4.420905
H	1.930267	-7.218412	-0.568221	C	2.140562	-0.912014	-3.384004
C	0.878630	-5.338183	-0.501492	C	2.938015	0.026568	-2.485465
H	-0.064222	-5.739035	-0.859596	C	-3.891019	-0.105465	3.546218
C	0.513388	-2.301692	-2.604319	O	-5.131700	-0.517291	4.104528
H	1.133605	-3.176722	-2.838322	C	-4.109927	0.030100	2.046473
H	1.170427	-1.482309	-2.289259	H	2.893304	-4.692785	1.700999
C	-5.310126	-3.199988	2.279043	H	5.321583	-4.822955	2.323443
H	-5.902723	-4.052170	2.599004	H	6.833414	-2.931638	1.847953
C	-0.287104	-1.874141	-3.826511	H	5.977478	-0.862955	0.725773
H	-0.926177	-2.709592	-4.148680	H	5.074276	3.116817	1.055534
H	-0.937940	-1.032174	-3.557781	H	5.131435	5.619778	1.192613
trans-isomer				H	3.108699	6.950567	0.723942
C	-2.844975	2.849414	-0.595530	H	0.953463	5.821098	0.110889
C	-3.659346	1.732723	-0.426382	H	-4.598324	-3.041632	-1.510083
C	-2.777828	0.594804	0.069815	H	-4.627581	-5.543731	-1.651670
N	-1.400770	1.108822	-0.124454	H	-2.663970	-6.865241	-0.959300
C	-1.458830	2.415612	-0.348307	H	-0.594647	-5.726517	-0.113454
C	2.982606	3.107373	0.531272	H	-2.700591	4.939861	-1.100491
C	1.839632	3.860255	0.264724	H	-5.172440	5.113346	-1.514705
C	0.759460	2.901673	-0.033657	H	-6.616866	3.127808	-1.263653
N	1.229873	1.631378	0.038137	H	-5.658369	0.929680	-0.574437
C	2.613769	1.688292	0.323895	H	1.044575	-0.676097	-4.981591
N	-0.461549	3.313096	-0.314922	H	1.362210	-1.407080	-2.786223
C	3.115700	-2.698635	0.906250	H	2.811775	-1.690361	-3.778603
C	3.968638	-1.636086	0.633396	H	3.710694	0.531472	-3.075541
C	3.171539	-0.575801	-0.108949	H	2.272564	0.788648	-2.075342
N	1.755871	-1.032500	0.044486	H	-3.556093	0.861940	3.949852
C	1.770102	-2.295974	0.464092	H	-3.091154	-0.839045	3.728124
N	3.481940	0.750960	0.330618	H	-5.013410	-0.611377	5.056964
C	-2.575444	-3.020318	-0.761934	H	-4.907656	0.760448	1.858461
C	-1.464255	-3.769038	-0.369709	H	-4.416604	-0.934282	1.626417
C	-0.423730	-2.802379	0.031887				
N	-0.893284	-1.537192	-0.088675				

References

- (1) (a) J. Weber, P. C. Beard, S. E. Bohndiek, *Nat. Methods* **2016**, *16*, 639–650. (b) R. E. Borg, J. Rochford, *Photochem. Photobiol.* **2018**, *94*, 1175–1209. (c) W. Choi, B. Park, S. Choi, D. Oh, J. Kim, C. Kim, *Chem. Rev.* **2023**, *123*, 7379–7419.
- (2) (a) E.-Y. Park, D. Oh, S. Park, W. Kim, C. Kim, *APL Bioeng.* **2021**, *5*, 031510. (b) D. Li, S. Cai, P. Wang, H. Cheng, B. Cheng, Y. Zhang, G. Liu, *Adv. Healthcare Mater.* **2023**, 2300263. (c) B.-D. Zheng, J. Ye, Y.-Y. Huang, M. T. Xiao, *Biomater. Sci.* **2021**, *9*, 7811–7825.
- (3) (a) K. Strokov, A. H. Schäfer, U. Dobrindt, A. Galstyan, *ACS Appl. Bio Mater.* **2020**, *3*, 3751–3760. (b) X. Li, S. Yu, Y. Lee, T. Guo, N. Kwon, D. Lee, S. C. Yeom, Y. Cho, G. Kim, J.-D. Huang, S. Choi, K. T. Nam, J. Yoon, *J. Am. Chem. Soc.* **2019**, *141*, 1366–1372. (c) A. Shaukat, E. Anaya-Plaza, S. Julin, V. Linko, T. Torres, A. de la Escosura, M. A. Kostianinen, *Chem. Commun.* **2020**, *56*, 7341–7344.
- (4) (a) X. Li, E.-Y. Park, Y. Kang, N. Kwon, M. Yang, S. Lee, W. J. Kim, C. Kim, J. Yoon, *Angew. Chem. Int. Ed.* **2020**, *59*, 8630–8634. (b) S. Li, L. Zhao, R. Chang, R. Xing, X. Yan, *Chem. Eur. J.* **2019**, *25*, 13429–13435. (c) X. Li, C. Kim, S. Lee, D. Lee, H.-M. Chung, G. Kim, S.-H. Heo, C. Kim, K.-S. Hong, J. Yoon, *J. Am. Chem. Soc.* **2017**, *139*, 10880–10886.
- (5) (a) C. Xie, X. Zhen, Y. Lyu, K. Pu, *Adv. Mater.* **2017**, *29*, 1703693. (b) K. Miki, N. Imaizumi, K. Nogita, M. Oe, H. Mu, W. Huo, H. Harada, K. Ohe, *Bioconjugate Chem.* **2021**, *32*, 1773–1781. (c) K. Nogita, K. Miki, N. Imaizumi, M. Oe, H. Mu, K. Ohe, *J. Photochem. Photobiol. A* **2023**, *438*, 114547.
- (6) (a) Z. Zhao, C. B. Swartchick, J. Chen, *Chem. Soc. Rev.* **2022**, *51*, 829–868. (b) J. Huang, K. Pu, *Angew. Chem. Int. Ed.* **2020**, *59*, 11717–11731. (c) Y. Liu, L. Teng, B. Yin, H. Meng, X. Yin, S. Huan, G. Song, X.-B. Zhang, *Chem. Rev.* **2022**, *122*, 6850–6918. (d) Y. Zeng, T. Dou, L. Ma, J. Ma, *Adv. Sci.* **2022**, *9*, 2202384.
- (7) (a) Y. Kikukawa, T. Fukuda, A. Fuyuhiko, N. Ishikawa, N. Kobayashi, *Chem. Commun.* **2011**, 8518–8520. (b) T. Fukuda, Y. Kikukawa, R. Tsuruya, A. Fuyuhiko, N. Ishikawa, N. Kobayashi, *Inorg. Chem.* **2011**, *50*, 11832–11837. (c) T. Fukuda, N. Ishikawa, *Dyes Pigm.* **2014**, *109*, 151–154.
- (8) J. L. Marshall, D. Lehnher, B. D. Lindner, R. R. Tykwinski, *ChemPlusChem* **2017**, *82*, 967–1001.

- (9) (a) R. Kaushik, N. Nehra, V. Novakova, P. Zimcik, *ACS Omega* **2023**, 8, 98–126. (b) W. Xie, J. Jiang, D. Shu, Y. Zhang, S. Yang, K. Zhang, *Molecules* **2023**, 28, 4252. (c) J. Dai, C. Ma, P. Zhang, Y. Fu, B. Shen, *Dyes Pigm.* **2020**, 177, 108321. (d) H. S. Jung, X. Chen, K. Seung, J. Yoon, *Chem. Soc. Rev.* **2013**, 42, 6019–6031.
- (10) (a) C. D. Molek, J. A. Halfen, J. C. Loe and R. W. McGaff, *Chem. Commun.* **2001**, 2644–2645. (b) Y. Y. Karabach, M. N. Kopylovich, K. V. Luzyanin, M. F. C. Guedes da Silva, V. Yu. Kukushkin, A. J. L. Pombeiro, *Inorg. Chem. Acta* **2017**, 455, 696–700.
- (11) M. E. Ourailidou, P. Dockerty, M. Witte, G. J. Poelarends, F. J. Dekker, *Org. Biomol. Chem.* **2015**, 13, 3648–3653.
- (12) G. F. M. Pereira, T. T. Tasso, *Inorg. Chim. Acta* **2021**, 519, 120271.
- (13) K. Umezawa, M. Yoshida, M. Kamiya, T. Yamasoba, Y. Urano, *Nat. Chem.* **2017**, 9, 279–286.
- (14) (a) A. D. Becke, *J. Chem. Phys.* **1993**, 98, 5648–5652. (b) C. Lee, W. Yang, R. G. Parr, *Phys. Rev. B* **1988**, 37, 785–789.
- (15) Gaussian 16, Revision B.01, 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. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, and D. J. Fox, Gaussian, Inc., Wallingford CT, **2016**.
- (16) H. Tomoda, S. Saito, S. Shiraishi, *Chem. Lett.* **1983**, 12, 313–316.
- (17) B. A. Lindig, M. A. J. Rodgers, A. P. Schaap, *J. Am. Chem. Soc.* **1980**, 102, 5590–5593.

(18) G. M. Sheldrick, *Acta Crystallogr. Sect. A* **2015**, 71, 3–8.

(19) G. M. Sheldrick, *Acta Crystallogr. Sect. C* **2015**, 71, 3–8.

(20) A. L. Spek, *Acta Crystallogr. Sect. C* **2015**, 71, 9–18.

(21) Provided by The Cambridge Crystallographic Data Centre (CCDC):

<https://www.ccdc.cam.ac.uk/solutions/csd-system/components/mercury/>

Chapter 4

A Reductively Activatable Phototheranostic Agent Based on a Water-Soluble Nickel Naphthalocyanine Precursor

Abstract

Metallonaphthalocyanines (MNCs) are among the most promising near-infrared (NIR) dyes to serve as photosensitizers for phototheranostics, combining photoacoustic imaging (PAI) and photothermal therapy (PTT). However, the extremely low solubility of MNCs has limited their practical application as theranostic agents. In this study, we synthesized NiNc precursors (**ox-NiNc**) that exhibit significantly improved solubility by installing two alkoxy groups on the α -carbons of the diagonal pyrroles. We also prepared an amphiphilic derivative (**ox-NiNc-PEG**) and investigated its conversion into NiNc through reductive aromatization. We demonstrate that **ox-NiNc-PEG** efficiently converted into NiNc in cancer cells with elevated glutathione levels, resulting in enhanced photoacoustic signals and increased phototoxicity based on photothermal conversion under NIR light irradiation. The target-oriented reductive aromatization that occurs in response to the reducing environment in cancer cells offers an important strategy for developing PAI/PTT theranostic agents.

Introduction

Metallo naphthalocyanines (MNC), a class of tetrapyrrole macrocyclic compounds, are near-infrared (NIR) light-absorbing dyes renowned for their exceptional photo- and thermal stability.^{1,2} Because of their high molar extinction coefficient (ϵ) in the NIR region and robustness under physiological conditions, MNCs have garnered significant attention as photosensitizers for *in vivo* phototheranostics, particularly in applications combining photoacoustic imaging (PAI) and photothermal therapy (PTT).^{3–7} However, MNCs suffer from poor solubility in water and common organic solvents compared to other NIR-absorbing dyes, such as cyanine,⁸ which limits their chemical modification and hinders the development of highly functionalized MNC-based theranostic agents. Furthermore, most of the previous MNC-based theranostic agents, which are “always-on” types, act as photosensitizers whenever they are exposed to light. These photosensitizers cause unavoidable photoacoustic (PA) signals and phototoxicity in normal tissues, making them difficult to use as selective phototheranostic agents.^{9,10} To address these challenges, in Chapter 1, the author developed ratiometric PAI probes based on amphiphilic MNC-peptide conjugates that change their absorption wavelength in tumor tissue through aggregation triggered by enzymatic cleavage of the hydrophilic moiety.¹¹ Although this approach enabled tumor-specific activation of photosensitizers, it failed to completely suppress background signals arising from spectral overlap before and after activation. An ideal solution would involve constructing the π -conjugated structure of the NIR-absorbing photosensitizer under physiological conditions, ensuring that PA signals and phototoxicity are induced exclusively at the lesion site.

As the optical properties of MNCs mainly arise from their 18π -electron aromatic structure, it is plausible that their absorption wavelength can be modulated by the π -conjugation length. Kobayashi and coworkers reported the synthesis of copper naphthalocyanine (CuNc) derivative bearing two methoxy groups on the α -carbons of diagonal pyrroles in its core structure (**ox-CuNc-OMe**), which lacks absorption in the NIR region. Although its thermal solid-state transformation into CuNc was demonstrated at 180 °C, the harsh conditions are unsuitable for biological applications (Figure 1a).¹² In Chapter 3, the author dealt with core-alkoxylated nickel phthalocyanine (**ox-NiPc**), which has a distorted macrocyclic structure similar to that of **ox-CuNc-OMe**, undergoing the reductive transformation with glutathione (GSH) under physiological conditions to give the

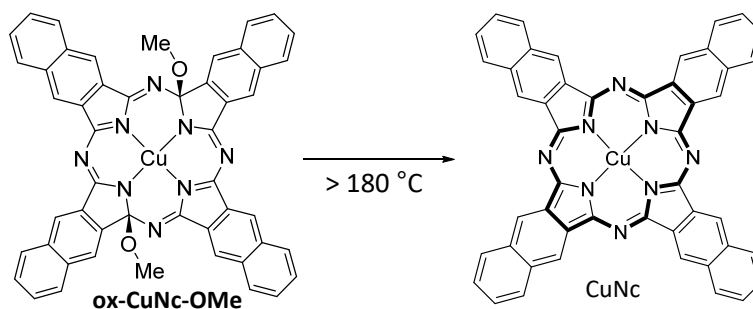
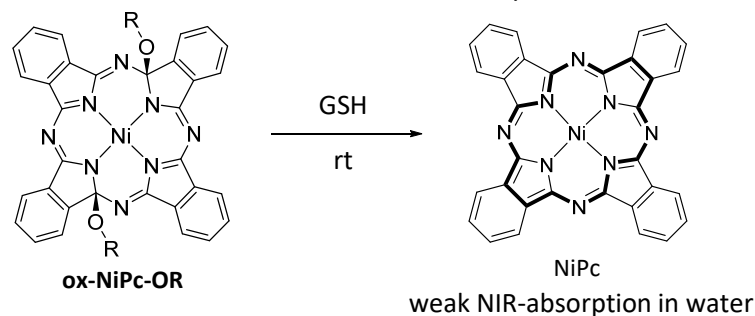
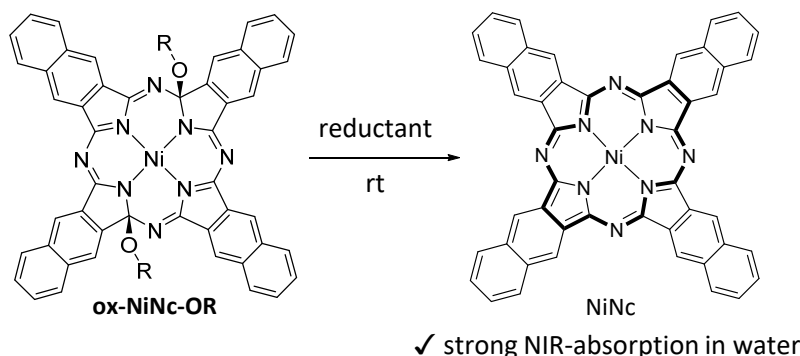
(a) thermal conversion of **ox-CuNc-OMe**(b) reductive conversion of **ox-NiPc-OR**, our previous work(c) reductive conversion of **ox-NiNc-OR**, this work

Figure 1. (a) Thermal conversion of **ox-CuNc-OMe**, (b) reductive-conversion of **ox-NiPc-OR**, and (c) reductive-conversion of **ox-NiNc-OR**.

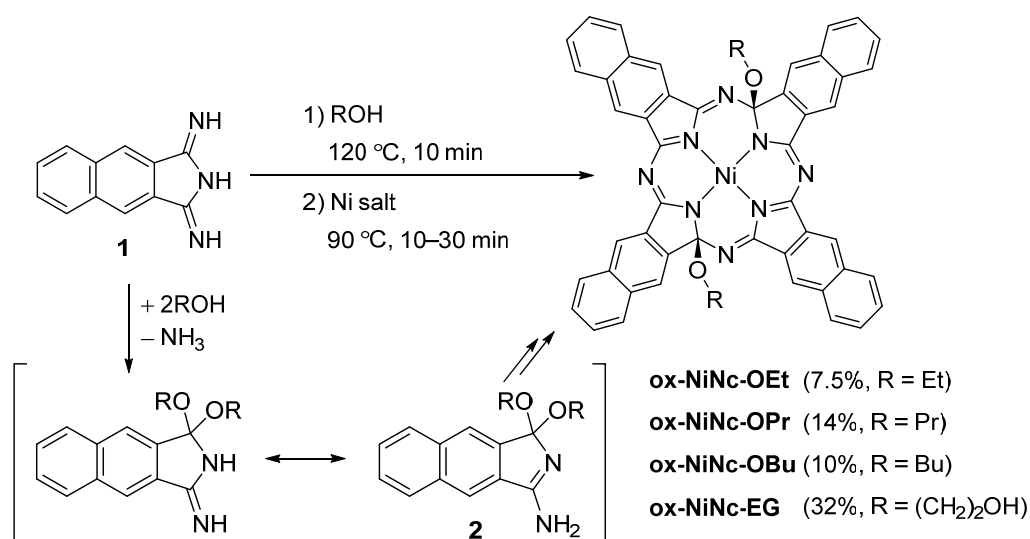
corresponding NiPc (Figure 1b).¹³ Notably, a water-soluble derivative **ox-NiPc-PEG**, bearing poly(ethylene glycol) (PEG) chains on R groups, underwent smooth GSH-mediated reductive aromatization both *in vitro* and *in vivo*, making it an efficient “turn-on” PA contrast agent. However, the aggregation of the resulting NiPc caused a hypsochromic shift of its absorption wavelength, limiting its photothermal conversion in the NIR region. To overcome these limitations, the author hypothesized that water-soluble core-alkoxylated nickel naphthalocyanine (**ox-NiNc**) derivatives, which can generate more π -expanded NiNc frameworks compared to NiPc, would serve as more efficient “turn-on” photosensitizers in NIR photoirradiation-based theranostics. The author herein reports the synthesis of **ox-NiNc** derivatives and the application of amphiphilic **ox-NiNc-**

PEG, bearing PEG chains, for PAI and PTT (Figure 1c). The water-soluble **ox-NiNc-PEG** was converted into NiNc in the presence of biological thiols under physiological conditions. In GSH-overexpressing cancer cells,¹⁴ this transformation proceeded efficiently, resulting in enhanced PA signal intensity, which enabled clear discrimination between cancer cells and normal cells. Furthermore, the heat generation by NiNc significantly reduced cancer cell viability under NIR laser irradiation, demonstrating that **ox-NiNc-PEG** can act as a turn-on-type photosensitizer for PAI/PTT theranostics.

Results and discussion

Kobayashi and coworkers reported the synthesis of **ox-CuNc-OMe** by the reaction of 2,3-dicyanonaphthalene with lithium methoxide in methanol at 70 °C, followed by the addition of a copper salt. A similar reaction using nickel(II) salt instead of copper salt was attempted; however, the core-modified **ox-NiNc** derivative was not obtained. To increase the reactivity of the starting compound, 1*H*-benzo[*f*]1,3-diiminoisoindoline (**1**)¹⁵ was prepared from 2,3-dicyanonaphthalene. Although the reaction of **1** and nickel salt in refluxing ethanol or *n*-propanol failed to yield the corresponding products, **ox-NiNc-OEt** and **ox-NiNc-OPr** were obtained by heating **1** in a mixed solvent of ethanol/DMF or *n*-propanol/DMF at 120 °C, followed by the addition of nickel(II) chloride (Scheme 1). Attempts to use lithium alkoxide in the reaction were unsuccessful, because the product decomposed at high temperatures in the presence of a base. In reactions using *n*-butanol and ethylene glycol, which have higher boiling points, the reaction proceeded without DMF, affording the corresponding **ox-NiNc-OBu** and **ox-NiNc-EG** in 10% and 32% yields, respectively. Notably, no core-alkoxylated naphthalocyanine was obtained in the absence of nickel salt, indicating the essential role of the nickel atom as a template for the macrocycle formation.

The structures of the **ox-NiNcs** were unambiguously determined by single-crystal X-ray structure analysis (Figure 2). The two alkoxy groups on the α -carbons of diagonal pyrroles in the distorted NiNc core structure



Scheme 1. Synthesis of **ox-NiNcs**.

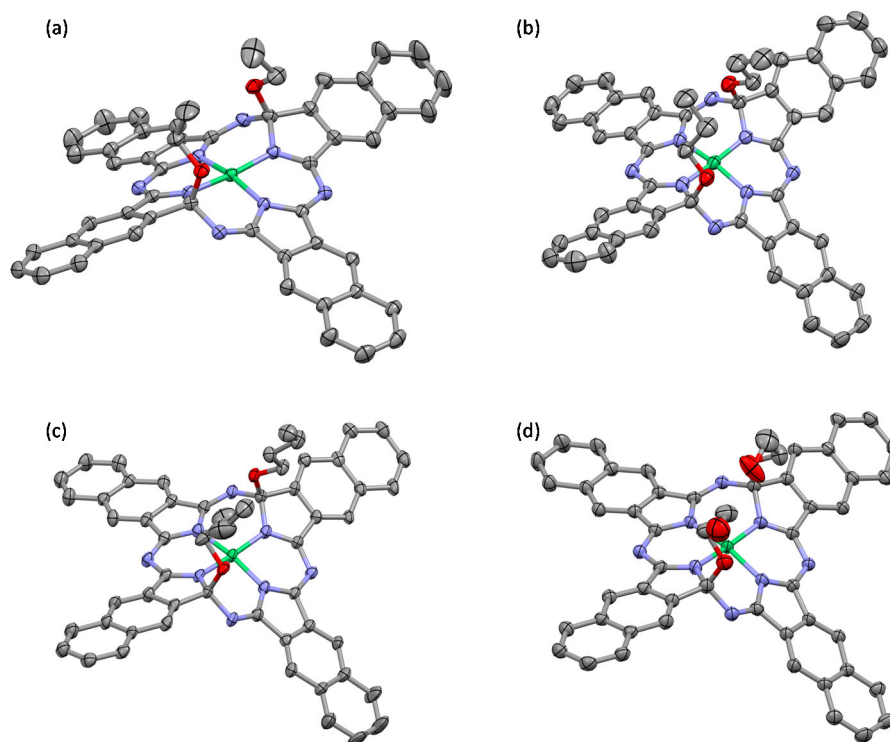


Figure 2. ORTEP drawing of (a) **ox-NiNc-OEt**, (b) **ox-NiNc-OPr**, (c) **ox-NiNc-OBu**, and (d) **ox-NiNc-EG** (50% ellipsoids). Hydrogen atoms and solvent molecules are omitted for clarity. Detailed crystallographic data and packing structures are summarized in the Experimental Section.

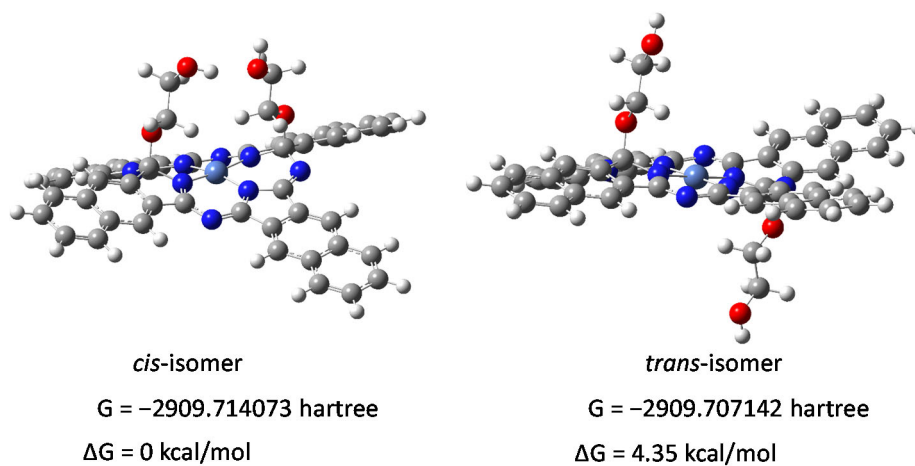


Figure 3. Energy-minimized structures and Gibbs free energy of two isomers of **ox-NiNc-EG** at the B3LYP/LANL2DZ level for Ni and the B3LYP/6-31G (d, p) level for C, H, N, O.

were located on the same side of the macrocycle.^{13,16–18} Density functional theory calculations suggested that the *cis*-isomer, bearing both alkoxy groups on the same side of the macrocycle, is more stable than the *trans* isomer (Figure 3). The distorted macrocycle of **ox-NiNcs** rendered them soluble in common organic solvents such as acetone, CH_2Cl_2 , and CHCl_3 , whereas planar NiNc was poorly soluble in these solvents. The high

solubility of **ox-NiNcs** facilitated their purification by column chromatography. The sp^2 -hybridized carbon atoms in the 18π -electron conjugated framework of the NiNc were partially replaced with sp^3 -hybridized carbon atoms, resulting in the absence of absorption in the NIR region above 700 nm, which is the typical absorption of MNc (Figure 4).^{19–21}

To utilize **ox-NiNc** derivatives under physiological conditions, the author explored the conjugation of **ox-NiNc** with PEG chains. A water-soluble **ox-NiNc-PEG** was synthesized in 38% yield via the condensation of **ox-NiNc-EG** with **PEG-COOH**²² (Scheme 2); notably, **ox-NiNc-PEG** dissolved in water at the concentration up to 500 μ M (Figure 5).

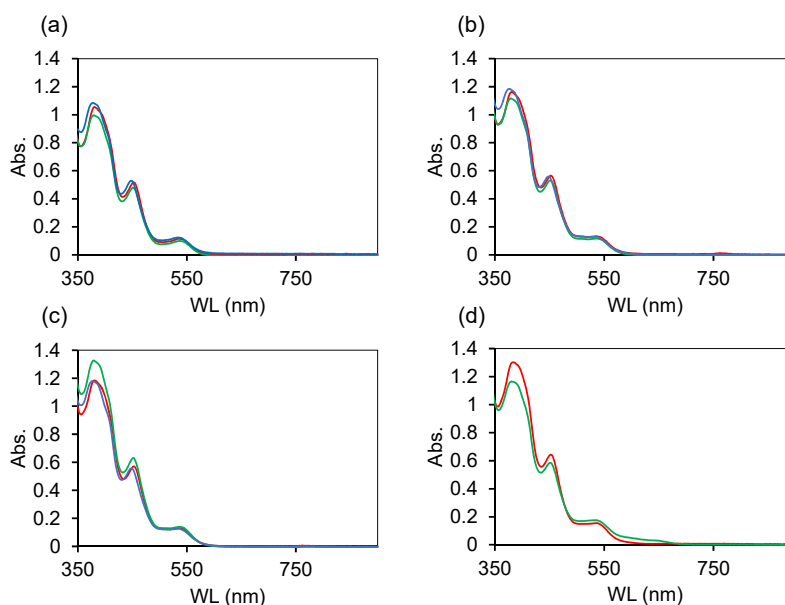
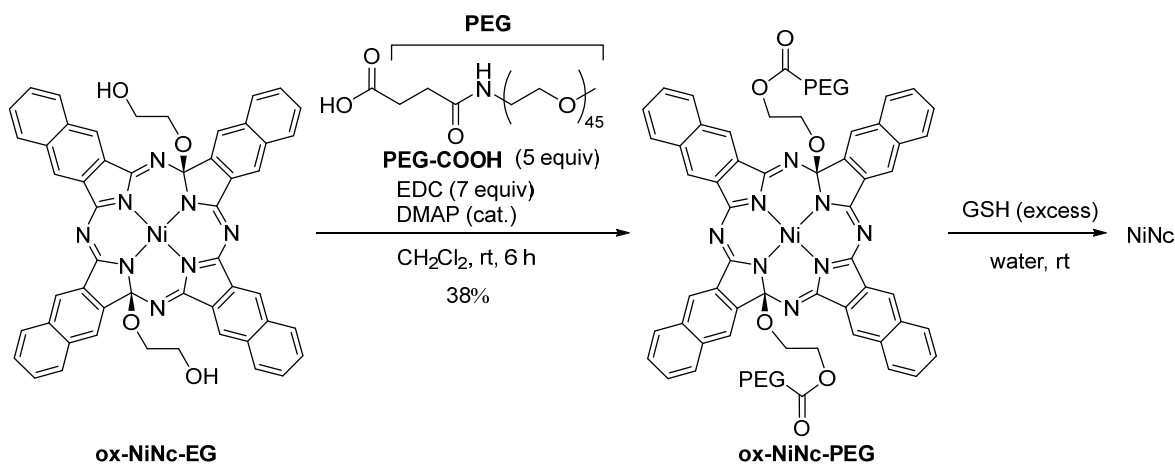


Figure 4. UV-vis absorption spectra of (a) **ox-NiNc-OEt**, (b) **ox-NiNc-OPr**, and (c) **ox-NiNc-OBu** in CHCl_3 (red), CH_2Cl_2 (green), and acetone (blue) (concentration: 100 μ M).



Scheme 2. Synthesis and reductive aromatization of **ox-NiNc-PEG** with GSH.

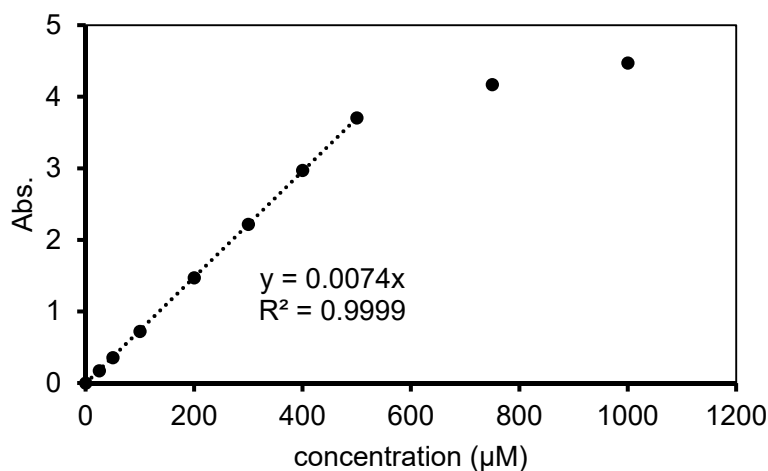


Figure 5. Concentration-dependent absorbance change of **ox-NiNc-PEG** in water at 543 nm.

The reactivity of **ox-NiNc** with reducing agents in aqueous solution was then examined. The reduction of **ox-NiNc-EG** with GSH (5 mM) at room temperature yielded a green solid, which was identified as NiNc by high-resolution mass spectrometry and elemental analysis. The isolated yield of NiNc from the reduction of **ox-NiNc-EG** was 91%, suggesting that reductive aromatization proceeded quantitatively. The UV-vis absorption spectral changes of an aqueous solution of **ox-NiNc-PEG** in the presence of GSH showed an increment in signal intensity in the NIR region (600–850 nm), attributed to NiNc (Figure 6). This observation confirmed the conversion of **ox-NiNc-PEG** into NiNc in response to GSH in phosphate buffer solution (PBS) at room temperature. The absorption spectrum of NiNc produced by GSH-mediated conversion in PBS at room temperature exhibited two absorption maxima at 784 nm (derived from the monomer) and 710 nm (derived from the *H*-aggregate). These results suggest that NiNc forms *H*-aggregates in aqueous solution, reflecting its low water solubility. As NiNc in PBS exhibits negligible fluorescence and singlet oxygen ($^1\text{O}_2$) generation under NIR light irradiation (Figure 7), non-radiative relaxation pathways are supposed to be predominant, resulting in high photothermal conversion efficiency.²³ Thus, **ox-NiNc**, which can be converted into water-insoluble NiNc, holds potential as a biological thiol-responsive turn-on PA contrast agent and photosensitizer for PTT.

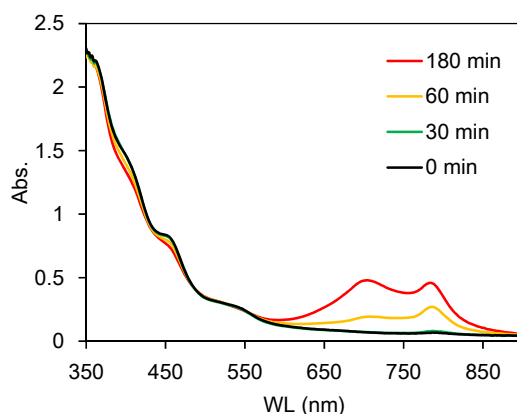


Figure 6. Absorbance spectra of **ox-NiNc-PEG** (50 μ M) in PBS (pH = 5.8) under GSH (5.0 mM) treatment.

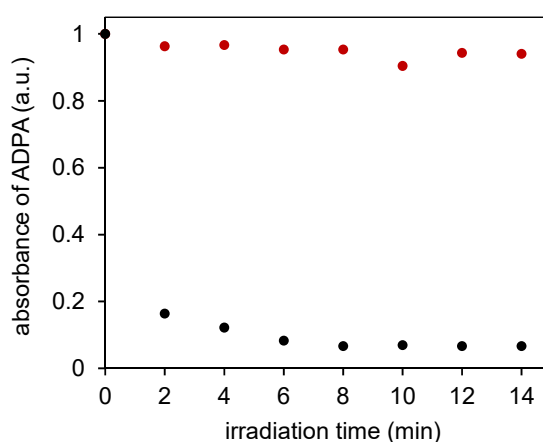


Figure 7. Time-dependent change of absorbance of ADPA at 400 nm in H₂O in the presence of NiNc obtained from **ox-NiNc-PEG** under photoirradiation (red). Methylene blue (black) was used as a positive control.

The PA signal generation after the GSH-mediated reduction of **ox-NiNc-PEG** was next examined in PBS with a range of pH values (Figure 8a). Stronger PA signals were detected under NIR pulsed laser irradiation as the pH decreased, indicating that the conversion of **ox-NiNc-PEG** into NiNc is facilitated under acidic conditions. The results suggest that protonation is a crucial step in the reductive aromatization of **ox-NiNc**, which is consistent with the results of the study on **ox-NiPc**.¹³ The PA signal intensity increased in a GSH concentration-dependent manner (Figure 8b), whereas negligible changes were observed in the absence of GSH. When **ox-NiNc-PEG** was treated with GSH at intracellular concentrations (1 mM), the PA signal intensity increased 10-fold and 6.2-fold compared to treatments with cysteine (Cys) and homocysteine (Hcy), respectively (Figure 8c), although no significant difference was observed between GSH and HCy at

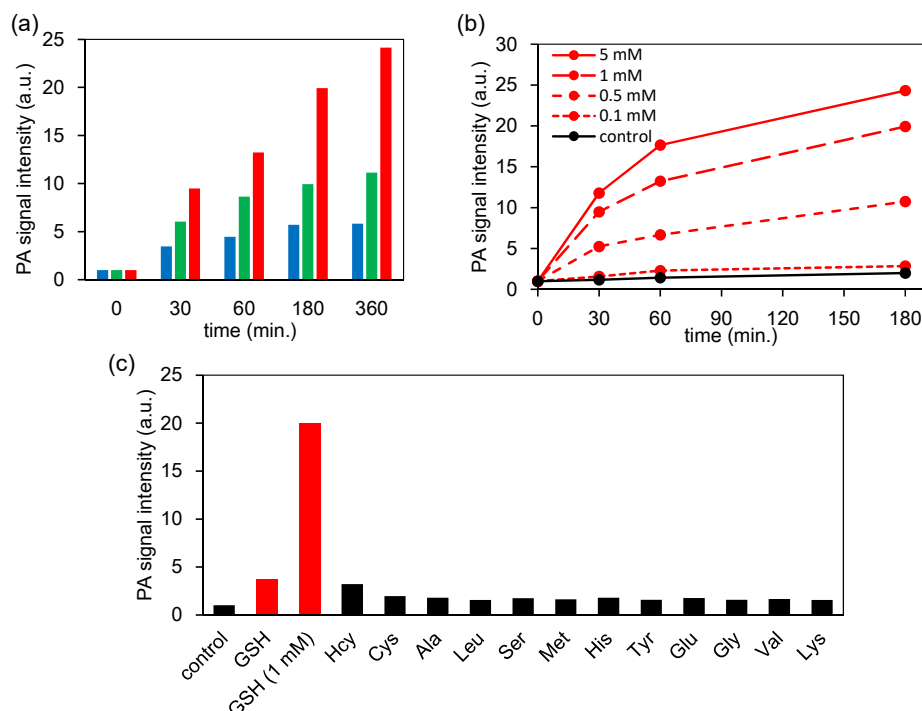


Figure 8. (a) Time-dependent PA signal intensities change of **ox-NiNc-PEG** (250 μ M) in PBS (pH = 8.0 (blue), 7.0 (green), 5.8 (red)) under GSH (1.0 mM) treatment. (b) GSH concentration-dependent PA signal intensity of **ox-NiNc-PEG** in PBS. (c) PA signal intensity of **ox-NiNc-PEG** after incubation with GSH (1.0 mM or 0.10 mM, red) or amino acids (0.10 mM, black) in PBS (pH = 5.8) for 1 h at 37 °C.

concentrations of 0.1 mM.^{24–26} Considering higher level of GSH than that of Hcy, **ox-NiNc-PEG** can serve as a chemical probe to discriminate between cancer cells with elevated GSH levels and normal cells.

The author then examined the photothermal conversion of an **ox-NiNc-PEG** in aqueous solution under continuous NIR laser irradiation after GSH treatment. The increase in temperature depended on both the laser intensity and the concentration of **ox-NiNc-PEG**, indicating that NiNc effectively elevated the temperature of the solution through photothermal conversion (Figures 9a and 9b). NiNc exhibited good thermal stability under photoirradiation without decomposition under repeated NIR laser irradiation, and the system presented consistent and repeatable temperature rises (Figure 10a). In contrast, the FDA-approved NIR dye indocyanine green (ICG) decomposed rapidly under the same photoirradiation conditions. The photothermal conversion efficiency (η) was calculated to be 40.7% (Figure 10b),^{27–29} which is comparable to those of previously reported PTT agents based on phthalocyanines and other organic dyes.³⁰ These results show that **ox-NiNc-PEG** is converted into robust NiNc with high photothermal conversion efficiency in

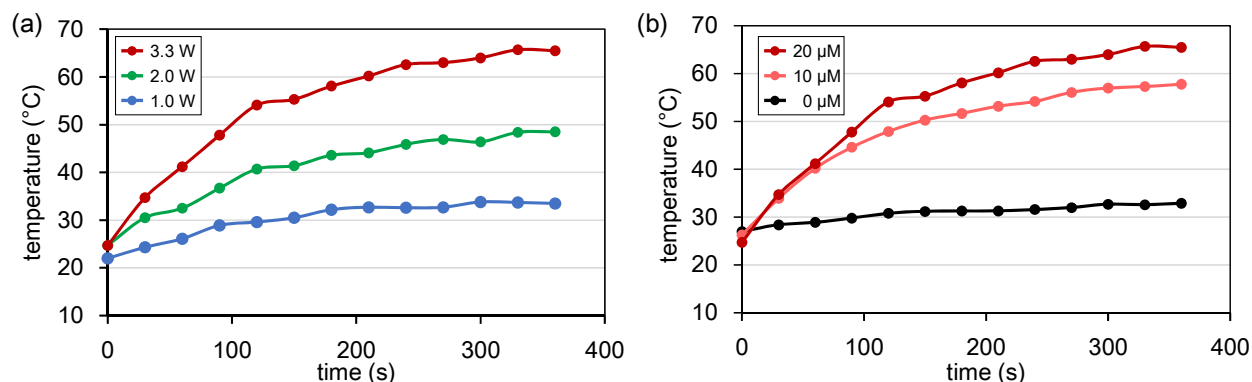


Figure 9. Time-dependent temperature change under NIR laser irradiation to **ox-NiNc-PEG** after incubation with GSH for 24 h in PBS ((a) The laser intensities: (blue) 1.0, (green) 2.0, or (red) 3.3 W, [**ox-NiNc-PEG**]: 20 μM . (b) The laser intensities: 3.3 W, [**ox-NiNc-PEG**]: (black) 0, (pink) 10, (red) 20 μM .)).

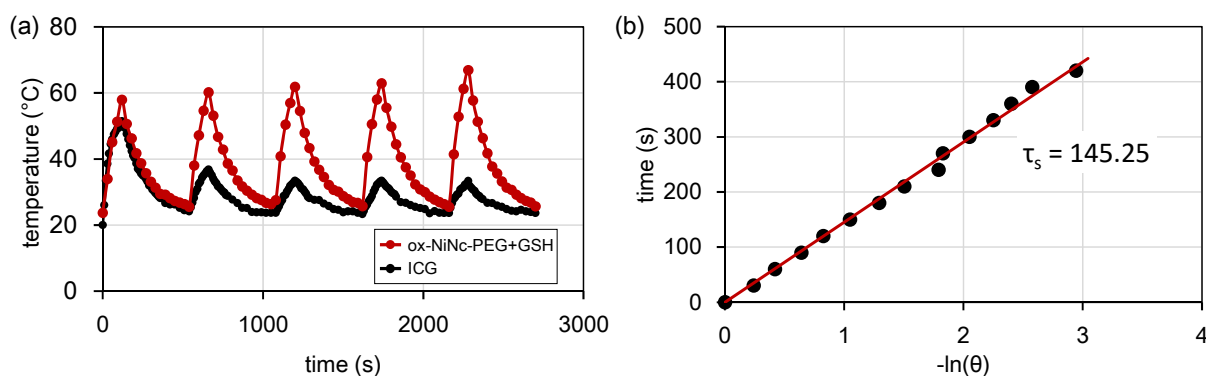


Figure 10. (a) Temperature change of samples during five ON/OFF cycles of 808nm laser irradiation (3.3 W) to **ox-NiNc-PEG** (20 μM , red) incubated with GSH for 24 h. ICG (black) was used for comparison as a photodegradable dye. (b) Linear time data versus $-\ln(\theta)$ of NiNc obtained from the first cooling period of (a).

response to GSH, highlighting the significant potential of **ox-NiNc-PEG** as a turn-on photosensitizer for PTT.

To evaluate the endogenous GSH-mediated conversion of **ox-NiNc-PEG** in cancer cells, we measured the PA signal of **ox-NiNc-PEG** in the GSH-overexpressing human lung cancer cell line A549.³¹ Prior to the cell experiments, a WST assay confirmed the low cytotoxicity of **ox-NiNc-PEG** for both A549 and the normal cell line HEK293 (Figure 11). After incubating A549 cells with **ox-NiNc-PEG** (10 μM) for 4 h, the PA signal intensity caused by excitation at 700 nm and 780 nm increased significantly (Figures 12a and 12b). When A549 cells were pretreated with the biological thiols scavenger *N*-ethylmaleimide (NEM) to inhibit the

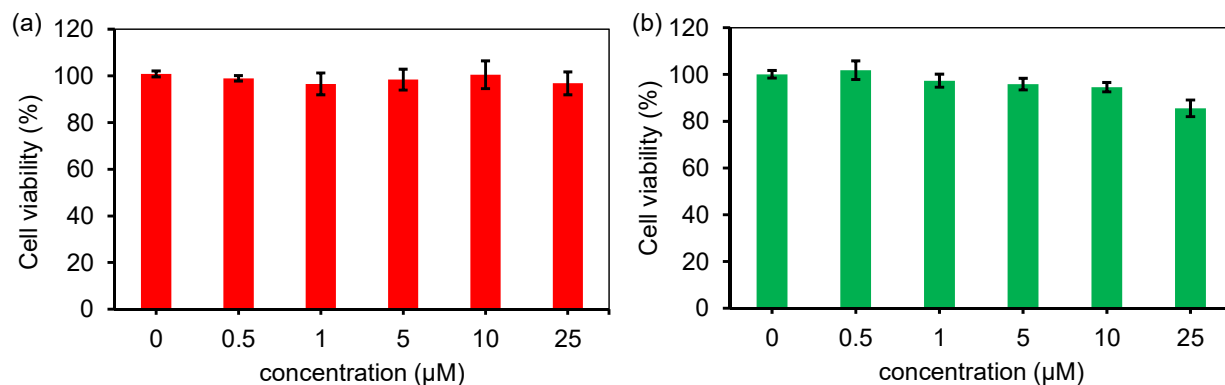


Figure 11. Viabilities of (a) A549 and (b) HEK293 cells after incubation with **ox-NiNc-PEG** for 24 h. Mean values and standard deviation ($n = 8$).

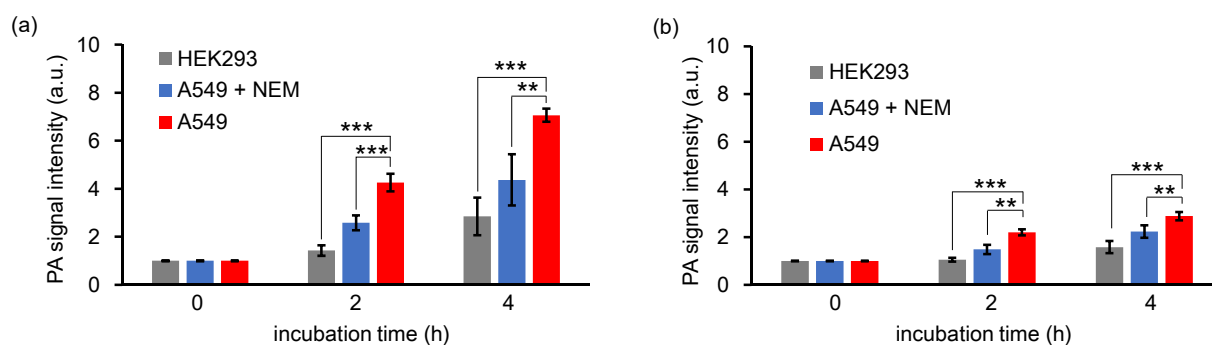


Figure 12. Time-dependent PA signal intensity changes, excited at (a) 700 nm and (b) 780 nm, during the incubation of HEK293 (black) with **ox-NiNc-PEG** (10 μM) and A549 with **ox-NiNc-PEG** (10 μM) after incubation with (blue) or without (red) NEM. Two-tailed Student's t -test: ** $p < 0.05$, *** $p < 0.005$.

conversion of **ox-NiNc-PEG** into NiNc, the PA signal enhancement of cells was suppressed. These results support the conclusion that the PA signal enhancement could be caused by the conversion of **ox-NiNc-PEG** to NiNc in response to endogenous biological thiols. The higher levels of intracellular GSH in A549 than in HEK293 led to efficient conversion of **ox-NiNc-PEG** and resulted in the PA signal intensity for A549 being 2.5 fold stronger than that of HEK293 after incubation for 4 h ($\lambda_{\text{ex}} = 700$ nm). These results confirm that **ox-NiNc-PEG** could serve as a PA contrast agent to distinguish cancer cells from normal cells.

Finally, the author investigated the photothermal-induced cytotoxicity of GSH-overexpressing cancer cells, which are resistant to photodynamic therapy due to the scavenging of reactive oxygen species by GSH.³² After NIR laser irradiation ($\lambda = 808$ nm), the cell viability of A549 incubated with **ox-NiNc-PEG** for 4 h

decreased with increasing laser power intensity (Figure 5c and d). As no $^1\text{O}_2$ is generated from NiNc under NIR photoirradiation, it is reasonable to conclude that cell death was induced by a temperature increase resulting from the photothermal conversion of NiNc. The photothermal therapeutic efficiency of **ox-NiPc-PEG**^[13] and **ox-NiNc-PEG** was compared by evaluating the cell viability of A549 cells under NIR photoirradiation. **ox-NiPc-PEG** did not induce cell death under NIR laser irradiation because its weak absorbance ($\epsilon = 3860$ at 808 nm) in the NIR region is insufficient for effective photothermal conversion. In contrast, the extended π -conjugated system and strong NIR absorption of NiNc increased the cytotoxicity of **ox-NiNc-PEG** under NIR laser irradiation (2.0 and 3.3 W power) dramatically. The results demonstrate that PTT using **ox-NiNc-PEG** is highly effective in causing damage to GSH-overexpressing cancer cells through temperature elevation.

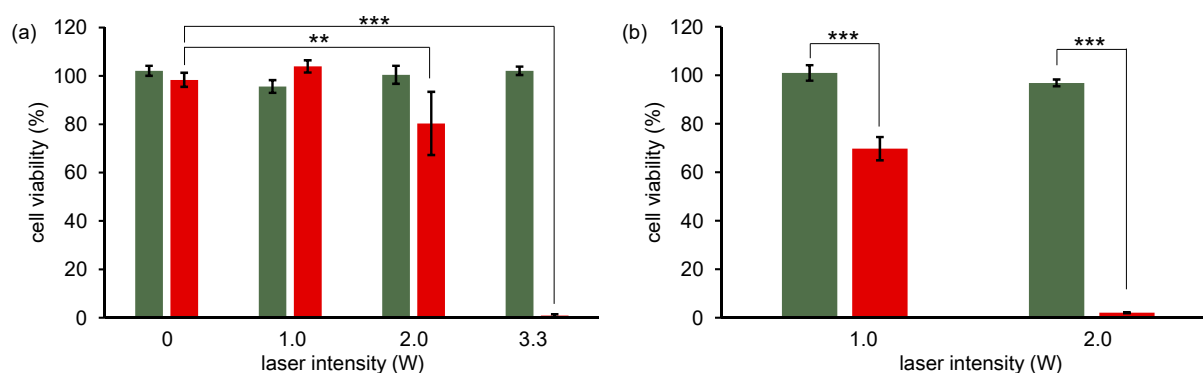


Figure 13. (a) Cell viability of A549 incubated with **ox-NiNc-PEG** (20 μM , red) after 5 minutes of NIR laser irradiation of different light power. (b) Cell viability of A549 incubated with **ox-NiPc-PEG** (20 μM) or **ox-NiNc-PEG** (20 μM) after NIR laser irradiation (1.0 W, red) for 20 min or no irradiation (black).

Conclusion

The author developed a synthetic method to access **ox-NiNc** derivatives using 1*H*-benzo[*f*]1,3-diiminoisoindoline (**1**) and primary alcohols. The amphiphilic **ox-NiNc-PEG** was then evaluated as a PAI/PTT theranostic agent. **ox-NiNc-PEG** was smoothly converted into NiNc in the presence of GSH under ambient conditions, triggering PA signal generation under NIR pulsed laser irradiation and a temperature increase under continuous NIR laser irradiation. This GSH-responsive conversion enables **ox-NiNc-PEG** to function as a PA contrast agent to distinguish GSH-overexpressing cancer cells from normal cells and as a photosensitizer to induce cancer cell death through PTT. Given the absence of NIR absorption prior to reduction, **ox-NiNc-PEG** serves as a highly effective turn-on theranostic agent for PAI/PTT applications.

Experimental

General

Materials and Methods

1-Ethyl-3-(dimethylamino)propyl carbodiimide hydrochloride (EDC·HCl) was purchased from Watanabe Chemical Industries, Ltd. (Japan). Ethylene glycol, *n*-propanol, *n*-butanol, 4-dimethylaminopyridine (DMAP), dichloromethane (CH₂Cl₂), Dulbecco's modified Eagle's medium (DMEM), and nickel(II) chloride hexahydrate (NiCl₂·6H₂O) were purchased from Fujifilm-Wako Pure Chemicals Industries, Ltd. (Japan). Methylene blue was purchased from Waldeck GmbH & Co. KG (Germany). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), glycine (Gly), alanine (Ala), valine (Val), lysine (Lys), and phosphate buffer solution (pH 7.2, 0.1 M), and 2,3-dicyanonaphthalene were purchased from Tokyo Chemical Industry Co., Ltd. (Japan). Formamide, dimethyl sulfoxide (DMSO), *N*-ethylmaleimide (NEM), tyrosine (Tyr), serine (Ser), methionine (Met), glutamine (Gln), glutamic acid (Glu), cysteine (Cys), glutathione (GSH, reduced), methanol (MeOH), and ethanol (EtOH) were purchased from nacalai tesque (Japan). MgSO₄ was purchased from Kishida Chemical Co., Ltd. (Japan). Phthalonitrile and homocysteine (Hcy) were purchased from Aldrich Chemical Co. (USA). Silica gel (SiO₂, 230–400 mesh) for column chromatography was purchased from Silicycle (Canada).

PEG-COOH was prepared from poly(ethylene glycol) monomethyl ether (MW = 2000) according to the reported method.²² 2,3-Dicyanonaphthalene³³ and **ox-NiPc-PEG**¹³ were prepared according to the reported method.

Buffered solutions (pH 5.8–8.0) were prepared by dissolving sodium dihydrogenphosphate (NaH₂PO₄) and disodium hydrogenphosphate (Na₂HPO₄) in water (Milli-Q).

UV-vis absorption spectra were recorded by UV-vis spectrophotometer (UH5300, Hitachi High Technologies, Japan).

PA signals were measured by Nexus 128 (ENDRA Life Sciences Inc., USA). The sample solutions were kept in dark at room temperature for more than 3 h before measurement.

Mass spectra were measured by Exactive Plus Orbitrap (ESI, Thermo Fisher Scientific Inc., USA) and

Ultraflex III (MALDI, Bruker Co., USA).

IR absorption spectra were measured by Spectrum Two (FT-IR, PerkinElmer Inc., USA).

The cell viability was estimated using a Cell Counting Kit-8 (CCK-8, Dojindo, Japan). The absorbance at 450 nm was measured by 800TS Absorbance Microplate Reader (BioTek Instruments, USA).

Synthesis of ox-NiNcs

*Synthesis of **1***¹⁵

A mixture of 2,3-dicyanonaphtalene (4.08 g, 23 mmol), NaOH (3.5 g, 88 mmol), formamide (90 mL, 2.3 mmol), and THF (100 mL) was stirred at 50 °C. After stirring for 24 hours, water (200 mL) was poured into the reaction mixture and the white precipitate was collected by filtration. The organic solvent was removed under reduced pressure to afford **1** (4.15 g, 21 mmol, 93%) as a white solid.

Synthesis of ox-NiNc-OEt

1 (0.25 g, 1.3 mmol) in ethanol (3 mL) and DMF (3 mL) was stirred at 120 °C. After stirring for 10 min, the reaction temperature was reduced to 90 °C and NiCl₂·6H₂O (76 mg, 0.3 mmol) was added to the reaction solution. After stirring for additional 20 min, solvents were removed under reduced pressure. The resulting solid was dissolved in CH₂Cl₂ and filtered to obtain red crude solution. After the organic solvent was removed under reduced pressure, the crude product was purified by silica gel column chromatography (hexane/CH₂Cl₂/Et₃N = 20/20/1) to afford **ox-NiNc-OEt** (20 mg, 24 μmol, 7.5%) as a red solid.

ox-NiNc-OEt: IR (neat) 3057, 2968, 2869, 1650, 1601, 1538, 1486, 1446, 1422, 1381, 1352, 1337, 1270, 1247, 1218, 1173, 1139, 1120, 1108, 1078, 1054, 1019, 985, 953, 923, 894, 887, 850, 811, 799, 788, 766, 746, 701, 622, 605, 583, 567, 544, 508, 497, 485, 477, 468, 457 cm⁻¹; ¹H NMR (400 MHz, CDCl₃, 25 °C) δ = 1.41 (t, *J* = 7.0 Hz, 6H), 3.33 (dq, *J* = 8.7, 7.2 Hz, 2H), 4.13 (dq, *J* = 8.7, 7.2 Hz, 2H), 7.60–7.70 (m, 8H), 7.98–8.11 (m, 8H), 8.39 (s, 2H), 8.42 (s, 2H), 8.56 (s, 4H); ¹³C NMR (100 MHz, CDCl₃, 25 °C) δ = 15.4, 30.9, 59.8, 100.7, 122.4, 122.8, 123.0, 127.1, 127.9, 128.1, 128.1, 129.2, 129.7, 129.9, 130.0, 133.5, 134.1, 134.2, 134.2, 134.9, 134.9, 135.3, 142.4, 160.8, 164.6, 167.6. HRMS (ESI) calcd for C₅₂H₃₄N₈NaNiO₂ ([M+Na]⁺) 883.2050, found

883.2049.

Synthesis of ox-NiNc-OPr

1 (0.10 g, 0.51 mmol) in *n*-propanol (2.0 mL) and DMF (2.0 mL) was stirred at 120 °C. After stirring for 10 min, the reaction mixture was cooled to 90 °C and NiCl₂·6H₂O (30 mg, 0.13 mmol) was added to the reaction solution. After stirring for additional 15 min, solvents were removed under reduced pressure. The resulting solids were dissolved in CH₂Cl₂ and filtered to obtain red crude solution. After the organic solvent was removed under reduced pressure, the crude product was purified by silica gel column chromatography (hexane/CH₂Cl₂/Et₃N = 20/20/1) to afford **ox-NiNc-OPr** (15 mg, 16 μmol, 14%) as a red solid.

ox-NiNc-OPr: IR (neat) 3056, 2958, 2872, 1636, 1600, 1537, 1446, 1481, 1380, 1338, 1268, 1217, 1245, 1137, 1117, 1054, 1021, 1082, 981, 953, 891, 848, 800, 787, 747, 695, 621, 581, 567, 547, 532, 510, 493, 474, 481, 464 cm⁻¹; ¹H NMR (400 MHz, CDCl₃, 25 °C) δ = 0.89 (t, *J* = 7.4 Hz, 6H), 1.59–1.89 (m, 4H), 3.12 (td, *J* = 8.0, 7.8 Hz, 2H), 4.04 (td, *J* = 8.2, 6.0 Hz, 2H), 7.59–7.69 (m, 8H), 7.97–8.11 (m, 8H), 8.36 (s, 2H), 8.42 (s, 2H), 8.56 (s, 4H); ¹³C NMR (100 MHz, CDCl₃, 25 °C) δ = 10.5, 23.2, 66.0, 100.7, 122.3, 122.7, 122.8, 123.0, 127.1, 127.9, 128.1, 129.2, 129.7, 129.9, 129.9, 133.6, 134.2, 134.2, 134.9, 134.9, 135.3, 142.5, 160.8, 164.6, 167.7. HRMS (ESI) calcd for C₅₄H₃₈N₈NaNiO₂ ([M+Na]⁺) 911.2363, found 911.2369.

Synthesis of ox-NiNc-OBu

1 (0.10 g, 0.51 mmol) in *n*-butanol (4.0 mL) was stirred at 120 °C. After stirring for 10 min, the reaction mixture was cooled to 90 °C and NiCl₂·6H₂O (30 mg, 0.13 mmol) was added to the reaction solution. After stirring for additional 10 min, *n*-hexane (30 mL) was added to the reaction mixture and the precipitates were corrected by filtration. The resulting solids were dissolved in CH₂Cl₂ and filtered to obtain red crude solution. After the organic solvent was removed under reduced pressure, the crude product was purified by silica gel column chromatography (hexane/CH₂Cl₂/Et₃N = 20/20/1) to afford **ox-NiNc-OBu** (12 mg, 13 μmol, 10%) as a red solid.

ox-NiNc-OBu: IR (neat) 3055, 2955, 2930, 2869, 1640, 1600, 1537, 1481, 1444, 1422, 1378, 1337, 1246,

1218, 1270, 1171, 1137, 1118, 1081, 1019, 981, 952, 890, 848, 800, 788, 701, 746, 655, 621, 608, 587, 567, 546, 497, 485, 472, 457 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3 , 25°C) δ = 0.78 (t, J = 7.3 Hz, 6H), 1.28–1.43 (m, 4H), 1.74 (tt, J = 7.2, 7.2 Hz, 4H), 3.20 (dt, J = 8.7, 7.3 Hz, 2H), 4.13 (dt, J = 8.7, 6.4 Hz, 2H), 7.59–7.70 (m, 8H), 7.98–8.11 (m, 8H), 8.37 (s, 2H), 8.42 (s, 2H), 8.56 (s, 4H); ^{13}C NMR (100 MHz, CDCl_3 , 25°C) δ = 13.8, 19.3, 32.0, 64.1, 100.6, 122.3, 122.7, 122.7, 122.9, 127.0, 127.9, 128.1, 129.2, 129.7, 129.9, 133.6, 134.1, 134.1, 134.2, 134.9, 135.2, 142.5, 160.7, 164.5, 167.6. HRMS (ESI) calcd for $\text{C}_{56}\text{H}_{42}\text{NaN}_8\text{NiO}_2$ ($[\text{M}+\text{Na}]^+$) 939.2676, found 939.2676.

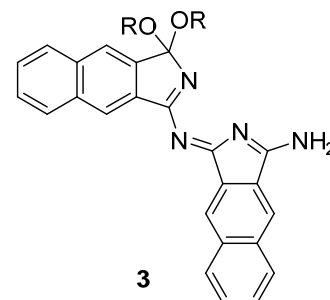
Synthesis of **ox-NiNc-EG**

1 (0.51 g, 2.9 mmol) in ethylene glycol (20 mL) was stirred at 120°C for 15 min, the reaction mixture was cooled to 90°C and $\text{NiCl}_2\cdot 6\text{H}_2\text{O}$ (0.18 g, 0.76 mmol) was added to the reaction solution. After stirring for 30 min, the dark-green suspension was diluted with CHCl_3 (300 mL) and the resulting mixture was filtered to remove green solid. The resulting red crude solution was washed with H_2O ($150\text{ mL}\times 3$) and the organic layer was dried over MgSO_4 . After the organic solvent was removed under reduced pressure, the crude product was purified by column chromatography on SiO_2 (hexane/ $\text{CH}_2\text{Cl}_2/\text{Et}_3\text{N}$ = 30/10/1) to afford **ox-NiNc-EG** (0.19g, 0.21 mmol, 32%) as a red solid.

ox-NiNc-EG: IR (neat) 3277, 3055, 2925, 1717, 1638, 1600, 1538, 1481, 1446, 1423, 1378, 1338, 1270, 1219, 1171, 1137, 1081, 1019, 986, 955, 892, 849, 787, 748, 701, 604, 567, 546, 534, 514, 497, 480, 460, 456 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3 , 25°C) δ = 3.47–3.52 (m, 2H), 3.78–3.83 (m, 2H), 3.88–3.93 (m, 2H), 4.01–4.06 (m, 2H), 7.61–7.71 (m, 8H), 7.99–8.11 (m, 8H), 8.36 (s, 2H), 8.41 (s, 2H), 8.57 (s, 2H), 8.58 (s, 2H); ^{13}C NMR (100 MHz, CDCl_3 , 25°C) δ = 46.1, 61.7, 65.7, 100.3, 122.6, 122.8, 123.0, 123.3, 127.3, 128.0, 128.3, 128.3, 129.2, 129.8, 129.9, 130.0, 133.3, 133.8, 134.0, 134.1, 135.0, 135.1, 141.6, 161.0, 164.8, 167.8. HRMS (ESI) calcd for $\text{C}_{52}\text{H}_{34}\text{NaN}_8\text{NiO}_4$ ($[\text{M}+\text{Na}]^+$) 915.1949, found 915.1948.

Pre-heating step in synthesis of ox-NiNcs

Dialkoxylated isoindoline derivative **2** and dimerized species **3-OR** were formed when **1** was pre-heated in alcohols at high temperatures, which were analysed by ESI-MS. In contrast, **2** and **3-OR** were barely detected when heated at 90 °C. These findings suggest that the pre-heating step, which forms ketal, is crucial for preparation of **ox-NiNcs**. After the pre-heating step, the reaction mixture needed to be cooled to avoid the thermal decomposition of **ox-NiNcs**.



2-OEt (R=Et): HRMS (ESI) calcd for $C_{16}H_{18}N_2O_2$ ($[M+Na]^+$) 325.1159, found 325.1152.

2-OPr (R=Pr): HRMS (ESI) calcd for $C_{18}H_{23}N_2O_2$ ($[M+H]^+$) 299.1755, found 299.1742.

2-OBu (R=Bu): HRMS (ESI) calcd for $C_{20}H_{27}N_2O_2$ ($[M+H]^+$) 327.2068, found 327.2064.

2-EG (R=(CH₂)₂OH): HRMS (ESI) calcd for $C_{16}H_{18}N_2O_4Na$ ($[M+Na]^+$) 325.1159, found 325.1152.

3-OEt (R=Et): HRMS (ESI) calcd for $C_{28}H_{23}N_4O_2$ ($[M+H]^+$) 449.1973, found 449.1972.

3-OPr (R=Pr): HRMS (ESI) calcd for $C_{30}H_{29}N_4O_2$ ($[M+H]^+$) 477.2286, found 477.2267, calcd for $C_{30}H_{28}N_4NaO_2$ ($[M+Na]^+$) 499.2105, found 499.2083.

3-OBu (R=Bu): HRMS (ESI) calcd for $C_{32}H_{33}N_4O_2$ ($[M+H]^+$) 505.2599, found 505.2593.

3-EG (R=(CH₂)₂OH): HRMS (ESI) calcd for $C_{26}H_{25}N_4O_4$ ($[M+H]^+$) 481.1871, found 481.1862.

Synthesis of ox-NiNc-PEG

EDC·HCl (40 mg, 0.21 mmol), DMAP (7.2 mg, 0.06 mmol), **PEG-COOH** (0.27 g, 0.14 mmol), and CH₂Cl₂ (20 mL) were stirred at room temperature for 30 min. After addition of **ox-NiNc-EG** (28 mg, 0.03 mmol), the solution was stirred at room temperature. After stirring for 6 h, the reaction solution was diluted with CH₂Cl₂ (20 mL) and the resulting mixture was filtered to remove green solid. The filtrate was washed with H₂O (50 mL × 3) and the organic solvent was removed under reduced pressure. The crude red solid was dissolved in water and insoluble solid was removed by filtration. The filtrate was freeze-dried to afford **ox-NiNc-PEG** (38%, containing a small amount of free **PEG-COOH**) as a red solid. In MALDI-TOF mass

spectrum of **ox-NiNc-PEG**, the fragment peaks of ox-NiNc bearing one PEG chain were observed together with the parent peaks (Figure 14).

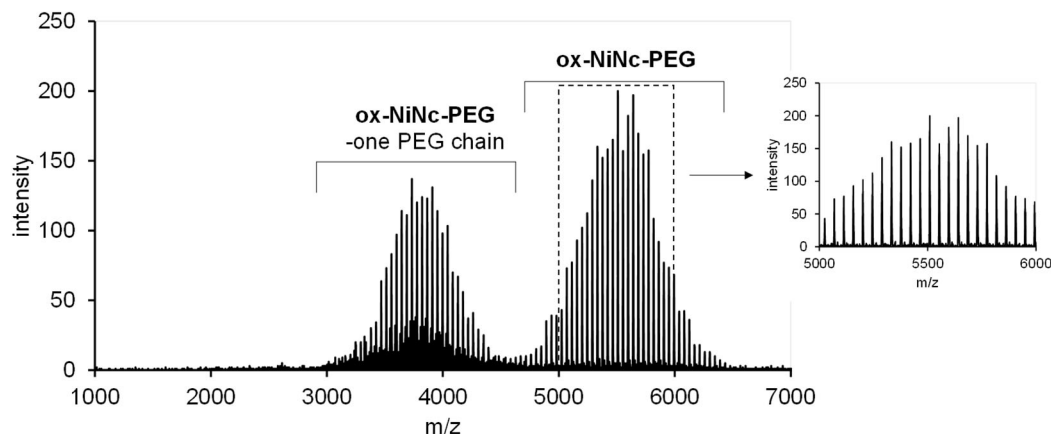


Figure 14. MALDI-TOF mass spectrum of **ox-NiNc-PEG**. Matrix: dithranol.

X-ray crystallographic data

Single crystals were obtained from recrystallization in MeOH/ CHCl_3 (for **1**) or CHCl_3 /hexane (for **ox-NiNcs**) at room temperature. Intensity data were collected on a RIGAKU Saturn 724 HG CCD system with VariMax Mo Optic using $\text{MoK}\alpha$ radiation (for **1**, **ox-NiNc-OEt**, **ox-NiNc-OPr**, and **ox-NiNc-OBu**) or a Rigaku XtaLAB Synergy-R diffractometer equipped with PhotonJet-R generator and HyPix-6000HE photon counting detector with $\text{CuK}\alpha$ radiation (for **ox-NiNc-EG**). Crystal data are summarized in Table 1 and Table 2. The structures were solved by a direct method (SHELXT)³⁴ and refined by a full-matrix least square method on F^2 for all reflections (SHELXL-2016).³⁵ All hydrogen atoms were placed using AFIX instructions, while all other atoms were refined anisotropically. The residual scattered electron densities, originating from disordered solvents, were eliminated by the SQUEEZE protocol equipped in PLATON for **ox-NiNc-OEt**, **ox-NiNc-OBu**, and **ox-NiNc-EG**.³⁶ The separation of the disorders on the alkoxy moiety is difficult, resulting in the Alerts B in the checkCIF/PLATON report. However, this problem does not affect the whole structure of the molecule. The molecular structure was visualized by Mercury.³⁷

Table 1. Crystallographic data of **1**.

CCDC number	2409310
Empirical formula	C ₁₂ H ₉ N ₃
Formula weight	195.22
Temperature/K	143(2)
Crystal size/mm ³	0.2 × 0.2 × 0.15
Radiation	Mo K α (λ = 0.71075)
Crystal color	yellow
Crystal system	monoclinic
Space group	<i>P</i> 2 ₁ /c
<i>a</i> (Å)	7.129(2)
<i>b</i> (Å)	9.534(3)
<i>c</i> (Å)	14.008(5)
β (°)	95.604(5)
Volume (Å ³)	947.5(5)
<i>Z</i>	4
<i>D</i> _{calc} (g/cm ³)	1.368
μ (mm ⁻¹)	0.085
<i>F</i> (000)	408
2 θ range for data collection (°)	3.580 to 27.489
Index ranges	$-8 \leq h \leq 9$, $-12 \leq k \leq 12$, $-17 \leq l \leq 18$
Reflections collected	7242
Independent reflections	2161
Data/restraints/parameters	2161/0/166
Goodness-of-fit on <i>F</i> ²	1.000
Final <i>R</i> indexes [<i>I</i> ≥ 2 σ (<i>I</i>)]	<i>R</i> ₁ = 0.0401, <i>wR</i> ₂ = 0.0867
Final <i>R</i> indexes [all data]	<i>R</i> ₁ = 0.0475, <i>wR</i> ₂ = 0.0888
Largest diff. peak/hole (e Å ⁻³)	0.287/−0.182

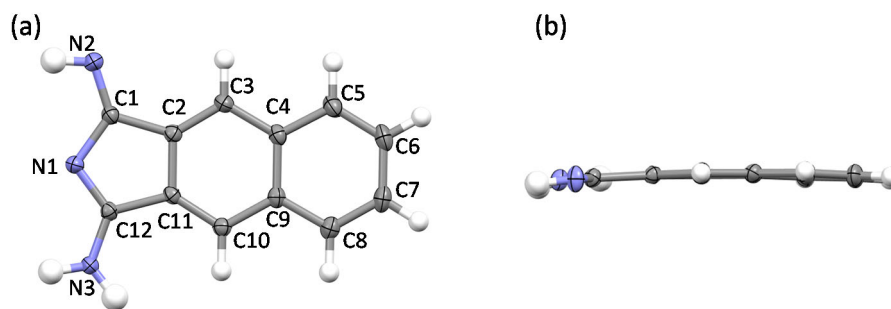


Figure 15. (a) Top-view and (b) side-view of **1** (50% ellipsoids) with labels.

Table 2. Crystallographic data of **ox-NiNcs**.

	ox-NiNc-OEt	ox-NiNc-OPr	ox-NiNc-OBu	ox-NiNc-EG
CCDC number	2409312	2409309	2409313	2409311
Chemical formula	C ₅₂ H ₃₄ N ₈ NiO ₂	C ₅₄ H ₃₈ N ₈ NiO ₂	C ₅₆ H ₄₂ N ₈ NiO ₂	C ₅₂ H ₃₄ N ₈ NiO ₄
Formula weight	861.58	889.63	917.68	893.58
Crystal size/mm ³	0.20×0.20×0.05	0.15×0.10×0.10	0.15×0.10×0.05	0.07×0.02×0.01
Crystal color	red	red	red	orange
Crystal system	triclinic	monoclinic	triclinic	triclinic
Space group	<i>P</i> -1	<i>C</i> 2/c	<i>P</i> -1	<i>P</i> -1
<i>a</i> (Å)	7.4733(11)	25.267(12)	13.611(9)	13.6855(7)
<i>b</i> (Å)	17.110(3)	12.160(5)	14.492(9)	13.7142(3)
<i>c</i> (Å)	19.482(3)	14.706(7)	14.492(9)	14.1611(2)
α (°)	93.581(6)	90	104.015(6)	69.8619(17)
β (°)	97.212(6)	114.555(7)	107.716(4)	86.123(4)
γ (°)	100.449(8)	90	110.892(5)	64.528(4)
Volume (Å ³)	2421.1(7)	4110(3)	2338(3)	2242.63(15)
<i>Z</i>	2	4	2	2
Temperature (K)	143(2)	143(2)	143(2)	100.00(10)
Radiation source	Mo K α	Mo K α	Mo K α	Cu K α
Wavelength (Å)	0.71075	0.71075	0.71075	1.54184
<i>D</i> _{calc} (g/cm ³)	1.182	1.438	1.303	1.323
μ (mm ⁻¹)	0.447	0.529	0.467	1.075
<i>F</i> (000)	892	1848	956	924
2 θ range for data collection/°	3.080 to 27.469	3.046 to 27.486	3.026 to 27.477	3.339 to 74.503

Index ranges	$-6 \leq h \leq 9,$ $-22 \leq k \leq 22,$ $-25 \leq l \leq 23$	$-32 \leq h \leq 29,$ $-12 \leq k \leq 15,$ $-18 \leq l \leq 19$	$-17 \leq h \leq 17,$ $-18 \leq k \leq 18,$ $-18 \leq l \leq 18$	$-17 \leq h \leq 17,$ $-17 \leq k \leq 17,$ $-14 \leq l \leq 17$
Reflections collected	19709	16136	18733	43547
Independent reflections	10616	4694	10164	9148
Data/restraints/parameters	10616/0/654	4694/0/295	10164/0/606	9148/0/588
Goodness-of-fit on F2	1.000	1.000	1.003	1.000
Final R indexes [$I \geq 2\sigma(I)$]	$R_1 = 0.0381,$ $wR_2 = 0.0668$	$R_1 = 0.0959,$ $wR_2 = 0.1750$	$R_1 = 0.0968,$ $wR_2 = 0.1647$	$R_1 = 0.0718,$ $wR_2 = 0.1597$
Final R indexes [all data]	$R_1 = 0.0510,$ $wR_2 = 0.0693$	$R_1 = 0.1430,$ $wR_2 = 0.1990$	$R_1 = 0.1495,$ $wR_2 = 0.1900$	$R_1 = 0.0816,$ $wR_2 = 0.1645$
Largest diff. peak/hole ($e \text{ \AA}^{-3}$)	0.310 / -0.340	0.906 / -0.353	0.915 / -0.956	1.066 / -0.715

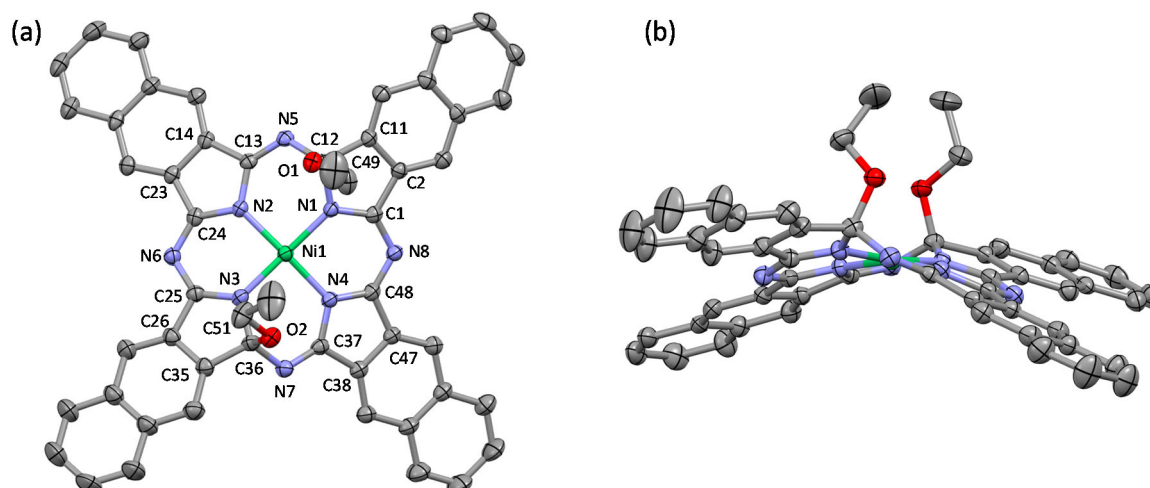


Figure 16. (a) Top-view and (b) side-view of **ox-NiNc-OEt** (50% ellipsoids) with labels. Hydrogen atoms and solvent molecules are omitted for clarity.

Table 3. Selected (a) bond lengths and (b) angles for **ox-NiNc-OEt**.

(a)	Length (Å)	(b)	Angle (deg)
Ni1—N1	1.855(1)	N1—Ni1—N2	90.76(6)
Ni1—N2	1.875(1)	N2—Ni1—N3	90.09(6)
Ni1—N3	1.856(1)	N3—Ni1—N4	90.94(6)
Ni1—N4	1.869(1)	N4—Ni1—N1	89.89(6)
O1—C12	1.416(2)	C1—N1—C12	110.7(1)
O2—C36	1.416(2)	C13—N2—C24	108.6(1)
N1—C1	1.322(2)	C25—N3—C36	110.9(1)
N1—C12	1.482(2)	C37—N4—C48	108.3(1)
N2—C13	1.405(2)	C12—N5—C13	117.3(1)
N2—C24	1.360(2)	C24—N6—C25	117.8(1)
N3—C25	1.318(2)	C36—N7—C37	118.2(1)
N3—C36	1.489(2)	C1—N8—C48	117.6(1)
N4—C37	1.415(2)	N1—C1—N8	127.9(2)
N4—C48	1.362(2)	N1—C12—N5	113.3(1)
N5—C12	1.447(5)	N2—C13—N5	128.9(2)
N5—C13	1.288(4)	N2—C24—N6	128.6(2)
N6—C24	1.318(5)	N3—C25—N6	128.4(2)
N6—C25	1.347(4)	N3—C36—N7	114.0(1)
N7—C36	1.447(4)	N4—C37—N7	129.0(2)
N7—C37	1.278(5)	N4—C48—N8	128.6(2)
N8—C1	1.350(4)	O1—C12—N1	110.0(1)
N8—C48	1.324(4)	O1—C12—N5	105.2(1)
C1—C2	1.474(2)	O1—C12—C11	114.5(1)
C11—C12	1.512(3)	O2—C36—N3	110.7(1)
C13—C14	1.477(2)	O2—C36—N7	104.7(1)
C23—C24	1.475(3)	O2—C36—C35	113.8(1)
C25—C26	1.473(2)		
C35—C36	1.525(2)		
C37—C38	1.479(3)		
C47—C48	1.470(3)		

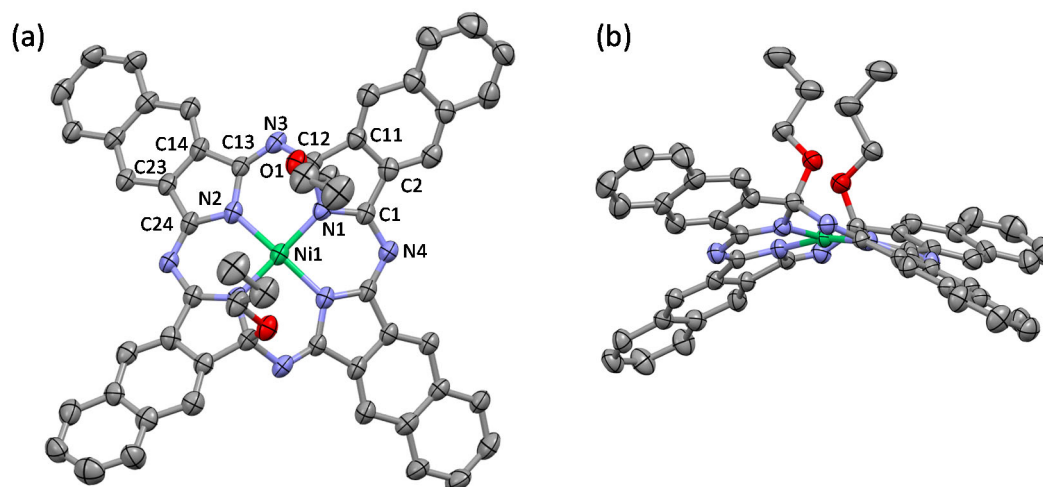


Figure 17. (a) Top-view and (b) side-view of **ox-NiNc-OPr** (50% ellipsoids) with labels. Hydrogen atoms and solvent molecules are omitted for clarity.

Table 4. Selected (a) bond lengths and (b) angles for **ox-NiNc-OPr**.

(a)	Length (Å)	(b)	Angle (deg)
Ni1—N1	1.844	N1—Ni1—N2	91.2
Ni1—N2	1.879	N2—Ni1—N1	89.6
O1—C12	1.438(6)	C1—N1—C12	109.8(4)
N1—C1	1.316(8)	C13—N2—C24	108.8(4)
N1—C12	1.486(6)	C12—N3—C13	117.4(5)
N2—C13	1.421(6)	C24—N4—C1	117.8(5)
N2—C24	1.366(8)	N1—C1—N4	127.5(5)
N3—C12	1.457(8)	N1—C12—N3	112.6(4)
N3—C13	1.285(8)	N2—C13—N3	128.7(5)
N4—C1	1.365(7)	N2—C24—N4	127.9(5)
N4—C24	1.320(6)	O1—C12—N1	109.5(4)
C1—C2	1.475(7)	O1—C12—N3	104.7(4)
C11—C12	1.504(9)	O1—C12—C11	112.9(4)
C13—C14	1.483(9)		
C23—C24	1.481(7)		

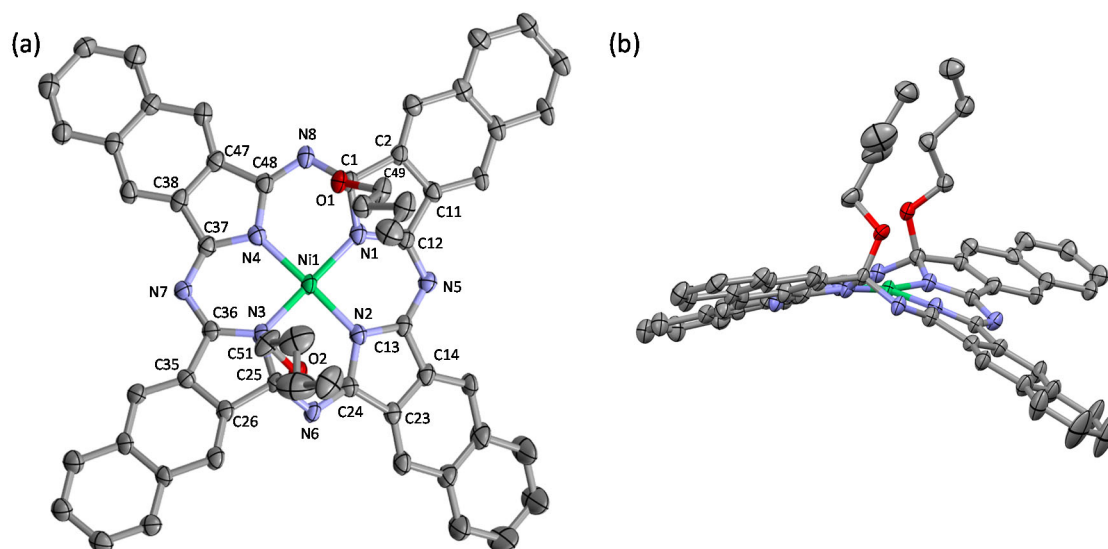


Figure 18. (a) Top-view and (b) side-view of **ox-NiNc-OBu** (50% ellipsoids) with labels. Hydrogen atoms and solvent molecules are omitted for clarity.

Table 5. Selected (a) bond lengths and (b) angles for **ox-NiNc-OBu**.

(a)	Length (Å)	(b)	Angle (deg)
Ni1—N1	1.857(6)	N1—Ni1—N2	90.76(6)
Ni1—N2	1.873(5)	N2—Ni1—N3	90.09(6)
Ni1—N3	1.863(5)	N3—Ni1—N4	90.94(6)
Ni1—N4	1.882(5)	N4—Ni1—N1	89.89(6)
O1—C1	1.422(6)	C1—N1—C12	110.7(1)
O2—C25	1.433(7)	C13—N2—C24	108.6(1)
N1—C1	1.481(9)	C25—N3—C36	110.9(1)
N1—C12	1.339(9)	C37—N4—C48	108.3(1)
N2—C13	1.371(8)	C12—N5—C13	117.3(1)
N2—C24	1.398(7)	C24—N6—C25	117.8(1)
N3—C25	1.473(6)	C36—N7—C37	118.2(1)
N3—C36	1.32(1)	C1—N8—C48	117.6(1)
N4—C37	1.359(7)	N1—C1—N8	127.9(2)
N4—C48	1.401(9)	N1—C12—N5	113.3(1)
N5—C12	1.355(9)	N2—C13—N5	128.9(2)
N5—C13	1.320(8)	N2—C24—N6	128.6(2)
N6—C24	1.287(8)	N3—C25—N6	128.4(2)
N6—C25	1.457(8)	N3—C36—N7	114.0(1)
N7—C36	1.359(8)	N4—C37—N7	129.0(2)
N7—C37	1.329(8)	N4—C48—N8	128.6(2)
N8—C1	1.443(9)	O1—C12—N1	110.0(1)
N8—C48	1.30(1)	O1—C12—N5	105.2(1)
C1—C2	1.536(2)	O1—C12—C11	114.5(1)
C11—C12	1.440(9)	O2—C36—N3	110.7(1)
C13—C14	1.484(9)	O2—C36—N7	104.7(1)
C23—C24	1.498(9)	O2—C36—C35	113.8(1)
C25—C26	1.52(1)		
C35—C36	1.467(8)		
C37—C38	1.466(8)		
C47—C48	1.49(1)		

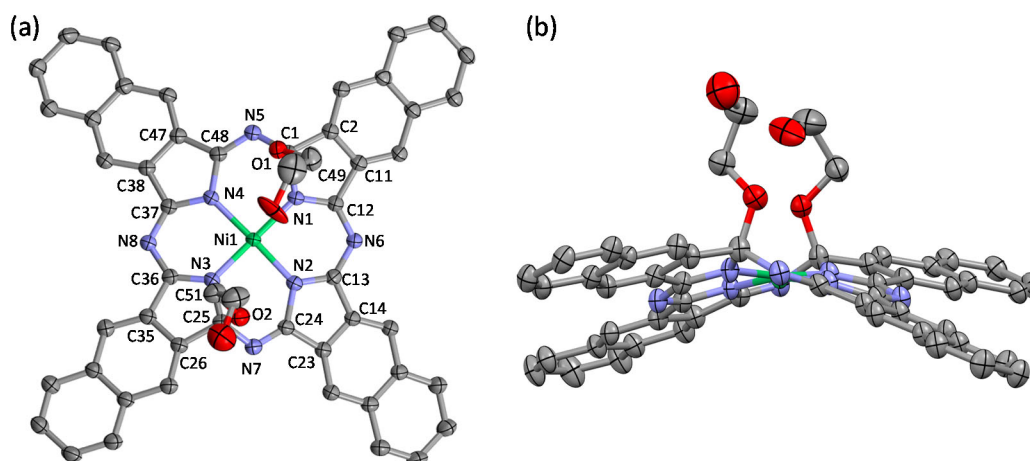


Figure 19. (a) Top-view and (b) side-view of **ox-NiNc-EG** (50% ellipsoids) with labels. Hydrogen atoms and solvent molecules are omitted for clarity.

Table 6. Selected (a) bond lengths and (b) angles for **ox-NiNc-EG**.

(a)	Length (Å)	(b)	Angle (deg)
Ni1—N1	1.871(2)	N1—Ni1—N2	90.4(1)
Ni1—N2	1.887(2)	N2—Ni1—N3	90.5(1)
Ni1—N3	1.857(2)	N3—Ni1—N4	90.6(1)
Ni1—N4	1.880(3)	N4—Ni1—N1	90.3(1)
O1—C1	1.430(6)	C1—N1—C12	110.6(3)
O2—C25	1.440(6)	C13—N2—C24	108.8(3)
N1—C1	1.487(5)	C25—N3—C36	110.0 (3)
N1—C12	1.308(4)	C37—N4—C48	108.4(3)
N2—C13	1.356(4)	C48—N5—C1	118.8(3)
N2—C24	1.404(4)	C12—N6—C13	118.7(3)
N3—C25	1.485(5)	C24—N7—C25	118.7(3)
N3—C36	1.327(4)	C36—N8—C37	118.3(2)
N4—C37	1.363(4)	N1—C1—N5	114.2(3)
N4—C48	1.415(4)	N1—C12—N6	127.9(4)
N5—C1	1.437(5)	N2—C13—N6	128.4(4)
N5—C48	1.281(4)	N2—C24—N7	128.9(4)
N6—C12	1.356(5)	N3—C25—N7	114.5(3)
N6—C13	1.323(4)	N3—C36—N8	128.2(4)
N7—C24	1.281(4)	N4—C37—N8	128.9(4)
N7—C25	1.437(5)	N4—C48—N5	127.9(4)
N8—C36	1.347(4)	O1—C1—N1	109.8(3)
N8—C37	1.317(4)	O1—C1—N5	104.4(3)
C1—C2	1.513(4)	O1—C1—C2	104.0(3)
C11—C12	1.462(4)	O2—C25—N3	111.2(3)
C13—C14	1.467(6)	O2—C25—N7	103.2(3)
C23—C24	1.477(6)	O2—C25—C26	113.8(3)
C25—C26	1.515(4)		
C35—C36	1.467(5)		
C37—C38	1.468(6)		
C47—C48	1.459(6)		

Theoretical calculation

The density functional theory (DFT) calculations were performed for the geometry optimization of two isomers of **ox-NiNc-EG** having two hydroxyethoxy groups at the B3LYP/LANL2DZ level for Ni and the B3LYP/6-31G(d, p) level for C, H, N, O by using Gaussian 16 package.^{38,39} Their electronic and Gibbs free energies are summarized in Table 1. The Cartesian coordinates of the optimized structures are summarized at the end of the experimental section.

Reduction by GSH

Reduction of ox-NiNc-EG to NiNc by GSH

In a 50 mL test tube, GSH (0.29 g, 0.93 mmol) was completely dissolved in water (20 mL). Then, the solution of **ox-NiNc-EG** (0.16 g, 0.18 mmol) in acetone (20 mL) was added dropwise and stirred at room temperature for 24 h. The reaction mixture was centrifuged to remove the supernatant. After washing with water (30 mL $\times$ 3) and acetone (30 mL $\times$ 3), and the precipitate was dried under reduced pressure to give NiNc (0.13 g, 0.14 mmol, 91%) as dark-green solid. NiNc was identified by high-resolution mass spectrometry and elemental analysis.

HRMS (MALDI): calcd for C₄₈H₂₄N₈Ni ([M]⁺) 770.1477, found 770.1469.

elemental analysis: calcd for C₄₈H₃₂N₈O₄Ni (NiNc·4H₂O): C, 68.35; H, 3.82; N, 13.28%, found C, 68.53; H, 3.44; N, 13.25%

Reduction of ox-NiNc-PEG to NiNc by GSH

In a 100 mL test tube, GSH (1.9 g, 6.2 mmol), **ox-NiNc-PEG** (0.37 g, 75 μ mol), and water (35 mL) were stirred at room temperature for 3 d. The reaction mixture was centrifuged to remove the supernatant. After washing with water (30 mL $\times$ 5) and acetone (30 mL $\times$ 5), and the precipitate was dried under reduced pressure to obtain NiNc as dark-green solid. NiNc was identified by MALDI-TOF mass spectrum. For UV-vis absorption spectra measurements, **ox-NiNc-PEG** (50 μ M) was reduced by GSH (1.0 mM) in PBS (Figures 6 and 24).

MS (MALDI): calcd for $C_{48}H_{24}N_8Ni$ ($[M]^+$) 770, found 770.

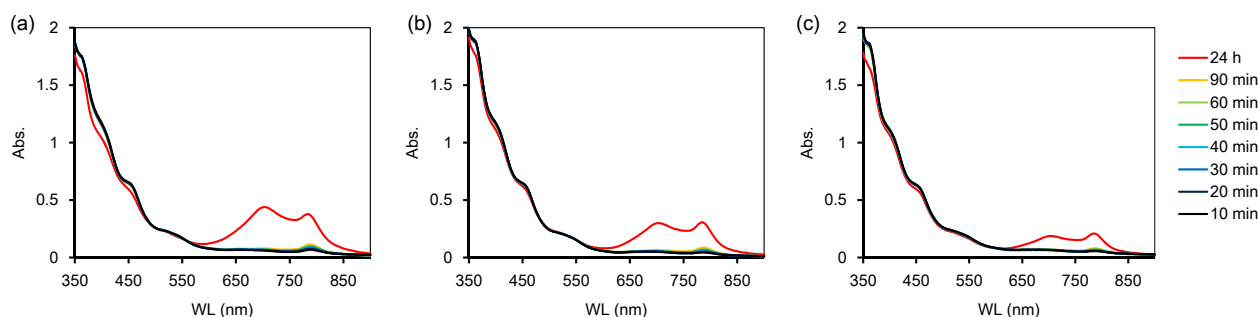


Figure 24. Absorbance spectra of **ox-NiNc-PEG** (50 μ M) in PBS (pH = (a) 5.8, (b) 7.4, (c) 8.0) under GSH (1.0 mM) treatment.

Detection of singlet oxygen

A water-soluble 9,10-anthracenedipropionic acid (ADPA) sodium salt was prepared according to the reported procedure.⁴⁰ **ox-NiNc-PEG** (10 μ M) was incubated with GSH (1 mM) in water for 24 h. A solution of ADPA sodium salt (0.10 mM) was added to the mixture in a quartz cuvette (optical path length: 10 mm). The mixture was irradiated using a Xenon light source device (MAX-303, Asahi Spectra Co., Ltd., Japan) equipped with VIS mirror module, rod lens (RLQL80-1, illuminance: 2.7 mW ($\lambda = 680$ nm) at the sample level), and long pass filter ($\lambda > 590$ nm). For positive control experiment, methylene blue (10 μ M) was used instead of **ox-NiNc-PEG** and GSH. During photoirradiation, absorbance of ADPA at 400 nm were monitored every two minutes (Figure 7).

In vitro PA Signal Measurement

In 1.5 mL Eppendorf tubes, the PBS (pH 5.8) solution of 0.20–20 mM GSH (50 μ L) or 0.20 mM amino acids (50 μ L) were added to the 500 μ M **ox-NiNc-PEG** solution (50 μ L) and incubated at 37 $^{\circ}$ C for 30 min, respectively. After incubation, the PA signal intensities of these samples were measured under 680 nm pulsed laser irradiation (Figure 8, 5 mJ/cm²).

In 1.5 mL Eppendorf tubes, the PBS (pH 5.8) solution of 2.0 mM GSH (50 μ L) were added to the 500 μ M

ox-NiNc-PEG solution (50 μL) for 2 days. After incubation, the PA signal intensities of these samples were measured under 680–880 nm pulsed laser irradiation (Figure 25, 5 mJ/cm^2).

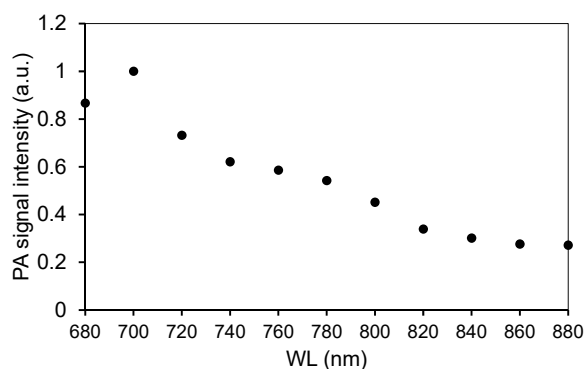


Figure 25. Photoacoustic signal intensity of **ox-NiNc-PEG** (250 μM) at various excitation wavelengths after incubation with GSH for 24 h in water.

Photothermal test

In 1.5 mL Eppendorf tubes, an aqueous solution of GSH (100 μL , 2 mM) was added to an aqueous solution of **ox-NiNc-PEG** (100 μL , 40 μM), and the resulting mixture was shaken at room temperature for 1 day. In a control experiment, an aqueous solution of **ox-NiNc-PEG** without treatment of GSH was similarly shaken for 1 day. These tubes were exposed to an NIR light source (1.0 W/cm^2 , $\lambda = 808$ nm, OPC-B015-808-FCPS, P9802-368, Opto Power Corp.) for 20 min. The time-dependent temperature change was monitored using an IR thermographic camera (FLIR Cx-Series C5; FLIR, Inc.).

Photostability of ox-NiNc-PEG

An aqueous solution of **ox-NiNc-PEG** (50 μM) placed in a quartz cuvette (10×10×40 mm) was irradiated with NIR laser (2.0 W/cm^2 , $\lambda = 808$ nm, OPC-B015-808-FCPS, P9802-368, Opto Power Corp.). UV-vis absorbance spectra were monitored after laser irradiation for 0, 5, and 10 minutes.

Calculation of the photothermal conversion efficiency

In 1.5 mL Eppendorf tubes, an aqueous solution of GSH (100 μL , 2 mM) was added to an aqueous solution of **ox-NiNc-PEG** (100 μL , 40 μM), and the resulting mixture was shaken at room temperature for 1 day.

These tubes are exposed to an NIR light source (3.3 W/cm², $\lambda = 808$ nm, OPC-B015-808-FCPS, P9802-368, Opto Power Corp.) for 2 min, followed by cooling without NIR laser irradiation for 7 min. The time-dependent temperature change was monitored using an IR thermographic camera.

The photothermal conversion efficiency of the NiNc was determined according to the reported method.^{28,29}

The photothermal conversion efficiency (η) is defined by the following equation 1.

$$\eta = \frac{hS(T_{\text{Max}} - T_{\text{Surr}}) - Q_{\text{Dis}}}{I(1 - 10^{-A_{808}})} \quad (1)$$

The maximum steady-state temperature (T_{Max}) of the **ox-NiNc-PEG** solution was 57.9 °C and environmental temperature (T_{Surr}) was 23.7 °C. So, the temperature change ($T_{\text{Max}} - T_{\text{Surr}}$) of the **ox-NiNc-PEG** solution was 34.2 °C. The laser power (I) was 3.3 W. The absorbance of NiNc at 808 nm (A_{808}) was calculated to be 0.0575 from Figure 23b. Q_{Dis} expresses heat dissipated from the light absorbed by the solvent and container, and it was measured independently to be 31.212 mW. The heat transfer coefficient (h) and the surface area (S) of the container were defined by the following equation 2.

$$hS = \frac{m_D C_D}{\tau_s} \quad (2)$$

, in which weight of water (m_D) and the specific heat capacity of water (C_D) are 0.2 g and 4.2 J/g °C, respectively.

In order to determine hS , a dimensionless parameter θ , known as the driving force temperature, was introduced as follows,

$$\theta = \frac{T - T_{\text{Surr}}}{T_{\text{Max}} - T_{\text{Surr}}} \quad (3)$$

A sample system time constant (τ_s) can be determined to be 145.25 s according to equation 4 and the slope shown in Figure 9d.

$$t = -\tau_s \ln(\theta) \quad (4)$$

From equation 2–4, hS could be calculated to be 5.78 mW/°C. As a result, by introducing these values into equation 1, η was calculated to be 40.7%.

Cell experiment

Cell Culture

The human lung carcinoma cell line, A549, was purchased from American Type Culture Collection (Manassas, VA). Normal cell line, Human Embryonic Kidney cells (HEK293), were purchased from Japanese Collection of Research Bioresources Cell Bank (JCRB Cell Bank), Japan. A549 cells and HEK293 cells were cultured in DMEM with 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin (P/S). Before seeding of HEK293, a 96-well microtiter plate was treated with poly-L-lysine-solution (20 μ M) and washed with PBS for each well. Cells were cultured in a well-humidified incubator with 5% CO₂ and 95% air at 37 °C.

Cytotoxicity Assay

A549 cells were seeded into a 96-well microtiter plate at a density of 5×10^4 cells/well. The cells were incubated for 24 h for cell attachment. A549 cells were incubated at 37 °C for an additional 24 h with various concentrations of **ox-NiNc-PEG** (0, 0.5, 1, 5, 10, 25 μ M). After washing the cells with PBS, DMEM/CCK-8 (0.10 mL, 10/1) was added to each well. After incubation at 37 °C for 2 h, the absorbance at 450 nm was measured. The cell viability of HEK293 was estimated using the same method as for A549.

PA signal increment caused by endogenous GSH

A549 and HEK293 cells were seeded into 12-well plates at a density of 1×10^5 cells/well and incubated at 37 °C for 24 h for cell attachment. For the thiol-trapping experiment, A549 cells were precultured with *N*-ethylmaleimide (NEM, 2.0 mM) for 30 min. The aqueous solution of **ox-NiNc-PEG** was diluted with DMEM with 10% FBS and 1% P/S (final concentration: 10 μ M). The cells were incubated with an **ox-NiNc-PEG** solution (1 mL) at 37 °C for 2 or 4 h. After incubation, the cells were washed with PBS and detached by trypsin/EDTA solution (0.10 mL). After adding PBS (0.90 mL), the cell suspension was transferred to 1.5 mL Eppendorf tube. After centrifugation, the supernatant was removed and the cells were

suspended in PBS (1.0 mL). The PA signal intensities were measured under 680 nm pulsed laser irradiation (5 mJ/cm²).

*Phototoxicity of **ox-NiNc-PEG** and **ox-NiPc-PEG***

A549 cells were seeded into 96-well plates at a density of 8×10^4 cells/well and incubated at 37 °C for 24 h for cell attachment. The aqueous solution of **ox-NiNc-PEG** or **ox-NiPc-PEG** was diluted with DMEM with 10 % FBS and 1% P/S (final concentration: 20 μM, water/DMEM = 1/99). The cells were incubated with an **ox-NiNc-PEG** or **ox-NiPc-PEG** solution (100 μL) at 37 °C for 4 h. Then, the photoirradiation (808 nm laser, 1 W/cm²) to the 96-well plate on a hotplate (37 °C) was carried out for 20 min. After washing the cells with PBS, DMEM/CCK-8 (0.10 mL, 10/1) was added to each well. After incubation at 37 °C for 2 h, the absorbance at 450 nm was measured.

Cartesian coordinates of energy-minimized structures							
ox-NiNc-EG				C	-7.765611	-1.143924	0.471432
<i>cis-isomer</i>				C	-3.691765	6.804488	-1.757565
C	-3.672857	-1.762389	-0.278567	C	-3.377832	8.082462	-2.166410
C	-4.101398	-0.553767	0.315192	C	-2.025690	8.486591	-2.272218
C	-2.874474	0.310354	0.584887	C	-1.011688	7.605159	-1.967094
N	-1.770676	-0.504203	0.008482	C	-2.508915	-0.570689	2.831962
C	-2.209707	-1.677014	-0.416719	C	-2.104856	-0.032091	4.205844
C	2.014025	-3.429430	-0.891837	O	-1.826681	-1.085623	5.105215
C	0.681736	-3.792834	-1.202652	C	1.772701	-1.053858	2.753716
C	-0.170291	-2.663820	-0.789469	C	1.966041	-0.973896	4.260818
N	0.607196	-1.670078	-0.279828	O	0.954331	-1.787931	4.863232
C	1.954909	-2.077742	-0.290828	H	4.238617	3.931810	-0.559178
N	-1.485210	-2.707888	-0.896943	H	5.772670	-0.583652	1.018734
C	3.699534	1.951597	0.057881	H	4.083264	-3.982937	-0.898365
C	4.124940	0.682865	0.503096	H	-0.652119	-5.268736	-1.999902
C	2.889259	-0.166230	0.761406	H	-4.021423	4.225288	-0.919832
N	1.780521	0.691243	0.254726	H	0.784684	5.657013	-1.297675
C	2.234043	1.898510	-0.058323	H	-4.201143	-3.679676	-1.078780
N	2.991313	-1.460438	0.144300	H	-5.753502	0.610135	1.025135
C	-1.957186	3.690118	-0.719605	H	6.621745	4.680364	-0.657485
C	-0.605113	4.095718	-0.824405	H	9.032020	4.212441	-0.365596
C	0.213708	2.931649	-0.442707	H	9.785969	1.969026	0.410151
N	-0.594560	1.893498	-0.111735	H	8.132825	0.197798	0.893519
C	-1.942536	2.289038	-0.245080	H	4.860519	-6.174814	-1.796803
N	-3.007504	1.619243	-0.001347	H	4.375446	-8.366453	-2.830686
N	1.534361	2.977112	-0.453541	H	2.031228	-9.004127	-3.377134
C	4.586738	2.962413	-0.216041	H	0.173184	-7.448681	-2.889359
C	5.976729	2.708028	-0.048265	H	-6.570494	-4.468806	-1.160078
C	6.412483	1.410575	0.400796	H	-8.973749	-4.093577	-0.715855
C	5.448133	0.395679	0.680355	H	-9.735693	-1.964741	0.328094
C	3.065204	-4.270220	-1.143608	H	-8.097737	-0.214837	0.927110
C	2.795843	-5.536469	-1.740956	H	-4.729637	6.492782	-1.675749
C	1.438809	-5.905507	-2.057802	H	-4.169364	8.785384	-2.409126
C	0.375155	-5.002041	-1.771180	H	-1.790038	9.496259	-2.595512
C	-2.987403	4.544510	-1.008845	H	0.027478	7.913019	-2.047765
C	-2.674083	5.868757	-1.434527	H	-3.451365	-1.128334	2.903912
C	-1.296790	6.280288	-1.542763	H	-1.731568	-1.255590	2.467617
C	-0.256982	5.359932	-1.224047	H	-1.248428	0.648186	4.078038
C	-4.551827	-2.758985	-0.622520	H	-2.926229	0.557791	4.626298
C	-5.937942	-2.556632	-0.371977	H	-0.937148	-1.418869	4.892784
C	-6.378323	-1.325331	0.231857	H	1.818720	-2.101949	2.433936
C	-5.422793	-0.321157	0.574586	H	0.790117	-0.649221	2.479806
Ni	0.005069	0.100990	0.028967	H	1.890880	0.071367	4.589247
O	2.825489	-0.286369	2.183083	H	2.968136	-1.342768	4.515790
O	-2.637218	0.555901	1.963211	H	1.090368	-1.781894	5.819087
C	6.954736	3.702951	-0.318190	<i>trans-isomer</i>			
C	8.296930	3.440995	-0.155721	C	3.690097	-1.939571	-0.530180
C	8.725100	2.165774	0.285041	C	4.183646	-0.703469	-0.067830
C	7.804127	1.176953	0.555093	C	3.014763	0.057327	0.533035
C	3.837894	-6.454609	-2.035526	N	1.824096	-0.705437	0.068520
C	3.565930	-7.676223	-2.612171	C	2.228958	-1.907952	-0.347258
C	2.233643	-8.038731	-2.922770	C	-1.907472	-3.717295	0.332290
C	1.197032	-7.172744	-2.651345	C	-0.591817	-4.161961	0.056862
C	-6.907018	-3.541257	-0.704335	C	0.197593	-2.961837	-0.266821
C	-8.245242	-3.330970	-0.456634	N	-0.597473	-1.866642	-0.249103
C	-8.677998	-2.120839	0.136710				

Chapter 4

C	-1.925853	-2.267981	0.048998	H	-3.906540	-4.240211	0.900617
N	1.497553	-3.019465	-0.499359	H	0.773874	-5.810463	-0.067058
C	-3.659203	1.848144	0.097096	H	4.082656	4.202663	0.040723
C	-4.127401	0.575171	-0.282122	H	-0.715218	5.684563	0.320525
C	-2.922058	-0.286386	-0.627765	H	4.120863	-3.857334	-1.388966
N	-1.762659	0.566628	-0.243974	H	5.886811	0.555637	0.242691
C	-2.188196	1.788627	0.064817	H	-6.487402	4.618379	1.006779
N	-2.996536	-1.575305	-0.004972	H	-8.917318	4.164582	0.937048
C	2.017164	3.635952	0.114628	H	-9.751279	1.912984	0.275666
C	0.671162	4.054283	0.197309	H	-8.158907	0.118718	-0.315829
C	-0.149202	2.830879	0.186095	H	-4.555585	-6.584844	1.436056
N	0.641977	1.732904	0.081438	H	-3.981826	-8.979895	1.636859
C	1.996118	2.155859	0.127225	H	-1.668850	-9.760887	1.146280
N	3.060356	1.457205	0.246597	H	0.069548	-8.145363	0.453387
N	-1.463540	2.896858	0.264491	H	6.441826	-4.521521	-2.031884
C	-4.510501	2.875581	0.421001	H	8.856927	-4.012750	-2.181838
C	-5.911399	2.628805	0.383194	H	9.726087	-1.820608	-1.380123
C	-6.393512	1.326248	-0.000097	H	8.182436	-0.141071	-0.430124
C	-5.464592	0.297882	-0.343746	H	4.794707	6.591414	0.041455
C	-2.897673	-4.588923	0.702535	H	4.239689	8.997863	0.126737
C	-2.573541	-5.970993	0.827958	H	1.864794	9.733177	0.269417
C	-1.233911	-6.423240	0.544786	H	0.045537	8.060155	0.327274
C	-0.238317	-5.484051	0.150571	H	2.105217	1.484794	2.724391
C	3.050206	4.534867	0.091681	H	1.196159	-0.040921	2.602718
C	2.739917	5.925355	0.136388	H	2.700475	-1.060073	4.309097
C	1.364970	6.351389	0.219525	H	3.597863	0.468974	4.428624
C	0.324427	5.379705	0.252446	H	1.873817	0.349236	6.007872
C	4.516056	-2.905058	-1.048502	H	-3.704491	1.131232	-2.844471
C	5.909261	-2.624541	-1.140015	H	-1.924066	1.105111	-2.794453
C	6.411798	-1.356466	-0.676540	H	-1.896586	-0.763811	-4.422423
C	5.512578	-0.396331	-0.121003	H	-3.673644	-0.770311	-4.461537
Ni	0.024314	-0.067133	-0.088285	H	-2.631701	0.583753	-6.139921
O	-2.857049	-0.598424	-2.023123				
O	3.178196	-0.133994	1.945999				
C	-6.855594	3.637544	0.717509				
C	-8.208407	3.383444	0.678850				
C	-8.682239	2.103033	0.302858				
C	-7.795518	1.101534	-0.027375				
C	-3.546504	-6.927379	1.222939				
C	-3.225298	-8.262043	1.334329				
C	-1.910276	-8.706203	1.055087				
C	-0.940147	-7.806825	0.670060				
C	6.824997	-3.566416	-1.682129				
C	8.169978	-3.281668	-1.765805				
C	8.663647	-2.035373	-1.310380				
C	7.803987	-1.097823	-0.780434				
C	3.758885	6.914081	0.104417				
C	3.447913	8.255108	0.151893				
C	2.097728	8.673290	0.233106				
C	1.082889	7.742094	0.266246				
C	2.180149	0.394284	2.814343				
C	2.607375	0.033384	4.229449				
O	1.611122	0.549281	5.101828				
C	-2.819000	0.489429	-2.942412				
C	-2.789425	-0.127356	-4.332686				
O	-2.774404	0.950167	-5.259454				
H	-4.127141	3.850855	0.704922				
H	-5.824102	-0.684712	-0.634547				

References and Notes

- (1) C. G. Claessens, U. Hahn, T. Torres, *Chem. Rec.* **2008**, *8*, 75–97.
- (2) N. Sekkat, H. van den Bergh, T. Nyokong, N. Lange, *Molecules* **2012**, *17*, 98–144.
- (3) Y. Zhang, J. F. Lovell, *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **2017**, *9*, e1420.
- (4) X. Li, B.-D. Zheng, X.-H. Peng, S.-Z. Li, J.-W. Ying, Y. Zhao, J.-D. Huang, J. Yoon, *Coord. Chem. Rev.* **2019**, *379*, 147–160.
- (5) E.-Y Park, D. Oh, S. Park, W. Kim, C. Kim, *APL Bioeng.* **2021**, *5*, 031510.
- (6) D. Li, S. Cai, P. Wang, H. Cheng, B. Cheng, Y. Zhang, G. Liu, *Adv. Healthcare Mater.* **2023**, *12*, 2300263.
- (7) Y. Tian, N. Carrillo-Malani, K. Feng, J. Miller, T. M. Busch, K. M. Sundaram, Z. Cheng, A. Amirshaghghi, A. Tsourkas, *Nanotheranostics* **2024**, *8*, 100–111.
- (8) S. Lee, S. Min, G. Kim, S. Lee, *Coord. Chem. Rev.* **2024**, *506*, 215719.
- (9) J. F. Lovell, T. W. B. Liu, J. Chen, G. Zhang, *Chem. Rev.* **2010**, *110*, 2839–2857.
- (10) P. Cheng, K. Pu, *ACS Appl. Mater. Interfaces* **2020**, *12*, 5286–5299.
- (11) K. Miki, N. Imaizumi, K. Nogita, M. Oe, H. Mu, W. Huo, H. Harada, K. Ohe, *Bioconjugate Chem.* **2021**, *32*, 1773–1781.
- (12) Y. Kikukawa, T. Fukuda, A. Fuyuhiko, N. Ishikawa, N. Kobayashi, *Chem. Commun.* **2011**, 8518–8520.
- (13) K. Nogita, T. Sugahara, K. Miki, H. Mu, M. Kobayashi, H. Harada, K. Ohe, *Chem. Commun.* **2024**, *60*, 1472–1475.
- (14) G. K. Balendiran, R. Dabur, D. Fraser, *Cell Biochem. Funct.* **2004**, *22*, 343–352.
- (15) Y. Igarashi, Y. Okada, A. Muranaka, M. Suzuki, N. Kobayashi, *J. Porphyr. Phthalocyanines* **2023**, *27*, 423–433.
- (16) C. D. Molek, J. A. Halfen, J. C. Loe, R. W. McGaff, *Chem. Commun.* **2001**, 2644–2645.
- (17) T. Fukuda, Y. Kikukawa, R. Tsuruya, A. Fuyuhiko, N. Ishikawa, N. Kobayashi, *Inorg. Chem.* **2011**, *50*, 11832–11837.
- (18) Y. Y. Karabach, M. N. Kopylovich, K. V. Luzyanin, M. F. C. Guedes da Silva, V. Yu. Kukushkin, A. J. L. Pombeiro, *Inorg. Chem. Acta* **2017**, *455*, 696–700.

- (19) A. V. Soldatova, J. Kim, X. Peng, A. Rosa, G. Ricciardi, M. E. Kenney, M. J. Rodgers, *Inorg. Chem.* **2007**, *46*, 2080–2093.
- (20) R. Fukuda, M. Ehara, H. Nakatsuji, *J. Chem. Phys.* **2010**, *133*, 144316.
- (21) T. F. M. de Souza, A. O. Ribeiro, *J. Photochem. Photobiol. A* **2017**, *340*, 1–7.
- (22) M. E. Ourailidou, P. Dockerty, M. Witte, G. J. Poelarends, F. J. Dekker, *Org. Biomol. Chem.* **2015**, *13*, 3648–3653.
- (23) M. J. Duffy, O. Planas, A. Faust, T. Vogl, S. Hermann, M. Schäfers, S. Nonell, C. A. Strassert, *Photoacoustics* **2018**, *9*, 49–61.
- (24) M. Tian, F. Guo, Y. Sun, W. Zhang, F. Miao, Y. Liu, G. Song, C.-L. Ho, X. Yu, J. Z. Sun, W.-Y. Wong, *Org. Biomol. Chem.* **2014**, *12*, 6128–6133.
- (25) B. Guo, X. Pan, Y. Liu, L. Nie, H. Zhao, Y. Liu, J. Jing, X. Zhang, *Sens. Actuator B-Chem.* **2018**, *256*, 632–638.
- (26) M. Ye, H. Li, H. Luo, Y. Zhou, W. Luo, Z. Lin, *Antioxidants* **2023**, *12*, 202.
- (27) Q. Tian, F. Jiang, R. Zou, Q. Liu, Z. Chen, M. Zhu, S. Yang, J. Wang, J. Wang, J. Hu, *ACS Nano* **2011**, *5*, 9761–9771.
- (28) C. M. Hessel, V. P. Pattani, M. Rasch, M. G. Panthani, B. Koo, J. W. Tunnell, B. A. Korgel, *Nano Lett.* **2011**, *11*, 2560–2566.
- (29) X. Liu, B. Li, F. Fu, K. Xu, R. Zou, Q. Wang, B. Zhang, Z. Chen, J. Hu, *Dalton Trans.* **2014**, *43*, 11709–11715.
- (30) X.-L. Weng, J.-Y. Liu, *Drug Discovery Today* **2021**, *26*, 2045–2052.
- (31) K. Umezawa, M. Yoshida, M. Kamiya, T. Yamasoba, Y. Urano, *Nat. Chem.* **2017**, *9*, 279–286.
- (32) Y. Xiong, C. Xiao, Z. Li, X. Yang, *Chem. Soc. Rev.* **2021**, *50*, 6013–6041.
- (33) B. L. Wheeler, G. Nagasubramanian, A. J. Bard, L. A. Schechtman, M. E. Kenney, *J. Am. Chem. Soc.* **1984**, *106*, 7404–7410.
- (34) G. M. Sheldrick, *Acta Crystallogr. Sect. A* **2015**, *71*, 3–8.
- (35) G. M. Sheldrick, *Acta Crystallogr. Sect. C* **2015**, *71*, 3–8.

- (36) A. L. Spek, *Acta Crystallogr. Sect. C* **2015**, *71*, 9–18.
- (37) Provided by The Cambridge Crystallographic Data Centre (CCDC):
<https://www.ccdc.cam.ac.uk/solutions/csd-system/components/mercury/>
- (38) (a) A. D. Becke, *J. Chem. Phys.* **1993**, *98*, 5648–5652. (b) C. Lee, W. Yang, R. G. Parr, *Phys. Rev. B*, **1988**, *37*, 785–789.
- (39) Gaussian 16, Revision B.01, 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. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, D. J. Fox, Gaussian, Inc., Wallingford CT, 2016.
- (40) B. A. Lindig, M. A. J. Rodgers, A. P. Schaap, *J. Am. Chem. Soc.* **1980**, *102*, 5590–5593.

Chapter 5

Synthesis and Reductive Aromatization of Perfluoro Nickel Phthalocyanine Precursor Having Two Methoxy Groups on Adjacent Pyrrole Rings

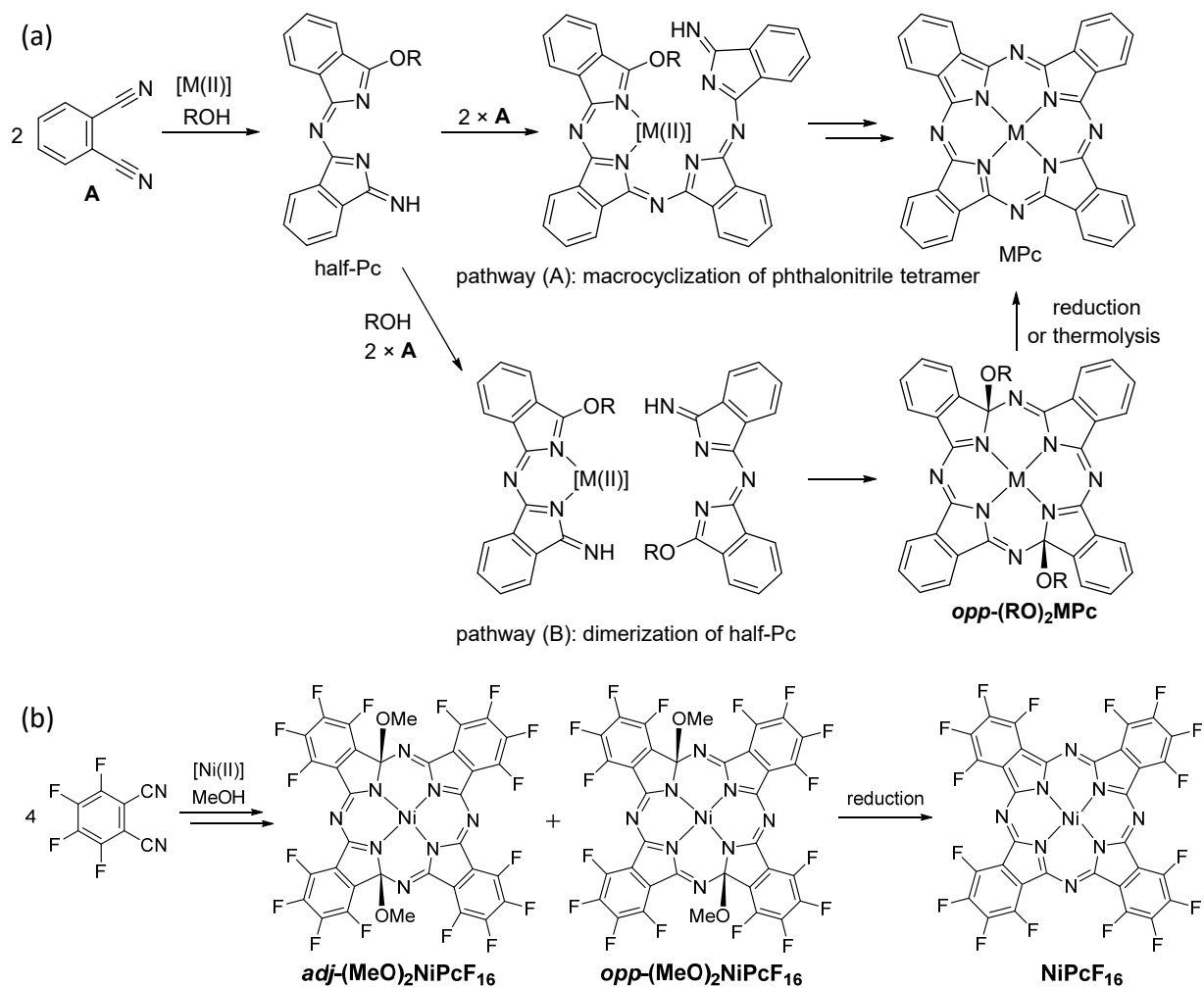
Abstract

Methanol-mediated tetramerization of 3,4,5,6-tetrafluorophthalonitrile with nickel(II) chloride yielded the perfluoro nickel phthalocyanine (NiPcF_{16}) derivative ***adj*-(MeO)₂NiPcF₁₆**, in which two methoxy groups are bound to adjacent pyrrole rings in an 18π -electron backbone, together with ***opp*-(MeO)₂NiPcF₁₆** as a minor product, in which two methoxy groups are in diagonal position. The NiPcF_{16} derivative ***adj*-(MeO)₂NiPcF₁₆** dissolved in organic solvents was converted to NiPcF_{16} by electrochemical reduction, chemical reduction, or thermal conversion. A transparent glass electrode coated with an *H*-aggregate of NiPcF_{16} was successfully prepared by utilizing electrochemical reduction. While thermal conversion of ***adj*-(MeO)₂NiPcF₁₆** was inefficient, the tin(II)-mediated reduction quantitatively afforded the corresponding NiPcF_{16} , indicating that this mild transformation provides a practical method for converting alkoxyated precursors into the corresponding metallophthalocyanines.

Introduction

Since its discovery in the early 20th century, metallophthalocyanine (MPc), one of the tetrapyrrole macrocycles, has been produced and used on a large scale (worldwide) as a heat- and light-resistant blue colorant.¹ Over the past several decades, MPcs have garnered significant attention due to their unique optical and electronic properties derived from the extended delocalization of π -electrons in the molecules.²⁻⁴ MPc derivatives with modified isoindoles and/or various central metal atoms have been intensively investigated for potential applications as photosensitizers in photodynamic or photothermal therapy,⁵⁻⁸ organic semiconductors,⁹⁻¹¹ and catalysts.¹²⁻¹⁵ Synthetic methods of MPcs involving the reaction of phthalic anhydride with metal salts at high temperatures (200–250 °C) have been industrially utilized.¹⁶ On the other hand, the alcohol-mediated tetramerization of phthalonitrile or its derivatives, suitable for the synthesis of functionalized MPcs at lower temperatures (~150 °C), has been employed as a milder synthetic method at the laboratory scale.¹⁷⁻¹⁹

The formation of the MPc ring was thought to proceed primarily via the macrocyclization of a tetramer derived from four equivalents of phthalonitrile (pathway (A) in Scheme 1a).²⁰⁻²⁶ However, the isolation of core-modified phthalocyanines with two alkoxy groups on the α -carbons of diagonal pyrrole rings (***opp*-(RO)₂MPc**) and their subsequent transformation into MPcs suggested that core-modified phthalocyanines could serve as key intermediates in alcohol-mediated phthalocyanine synthesis (pathway (B) in Scheme 1a).²⁷⁻³¹ During the author's investigation of perfluoro nickel phthalocyanine (NiPcF₁₆) synthesis via the alcohol-mediated tetramerization of perfluorinated phthalonitrile,³²⁻³⁶ the author was pleased to find the formation of NiPc derivatives, in which the two alkoxy groups are bound to adjacent pyrrole rings (***adj*-(MeO)₂NiPcF₁₆**) (Scheme 1b, NiPcF₁₆: perfluoro nickel phthalocyanine). Furthermore, the author demonstrated that ***adj*-(MeO)₂NiPcF₁₆** undergoes direct conversion to the corresponding MPc through electrochemical reduction, chemical reduction, or thermal conversion. Herein, the author describe the synthesis of perfluoro phthalocyanine derivative ***adj*-(MeO)₂NiPcF₁₆** through the methanol-mediated tetramerization of 3,4,5,6-tetrafluorophthalonitrile and its transformation into NiPcF₁₆ under reductive conditions.



Scheme 1. Plausible reaction mechanisms of (a) alcohol-mediated MPc synthesis through cyclotetramerization of phthalonitriles and through $opp-(RO)_2MPc$ and (b) MeOH-mediated $NiPcF_{16}$ synthesis through $adj-(MeO)_2MPcF_{16}$ and $opp-(MeO)_2MPcF_{16}$.

Results and discussion

Synthesis and structures of (MeO)₂NiPcF₁₆

The reaction of 3,4,5,6-tetrafluorophthalonitrile with nickel(II) chloride in methanol at 55 °C for 10 days afforded *adj*-(MeO)₂NiPcF₁₆ and *opp*-(MeO)₂NiPcF₁₆ in 10.7% and 0.5% NMR yields, respectively (Figure 1a). While *adj*-(MeO)₂NiPcF₁₆ could be purified by SiO₂ column chromatography, purification of *opp*-(MeO)₂NiPcF₁₆ by column chromatography was unsuccessful. As the NMR yield of *opp*-(MeO)₂NiPcF₁₆ decreased as the reaction time increased (the NMR yields of *adj*-(MeO)₂NiPcF₁₆ and *opp*-(MeO)₂NiPcF₁₆ after the reaction for 24 hours were 4.1% and 1.0%, respectively), this might be due to the isomerization of *opp*-(MeO)₂NiPcF₁₆ into *adj*-(MeO)₂NiPcF₁₆ (*vide infra*). The X-ray crystallographic analysis of (MeO)₂NiPcF₁₆ clearly shows that two methoxy groups are bound to adjacent or diagonal pyrroles (Figures 1b and 1c). In both cases, the two methoxy groups at the α -position of pyrroles are on the same side of the

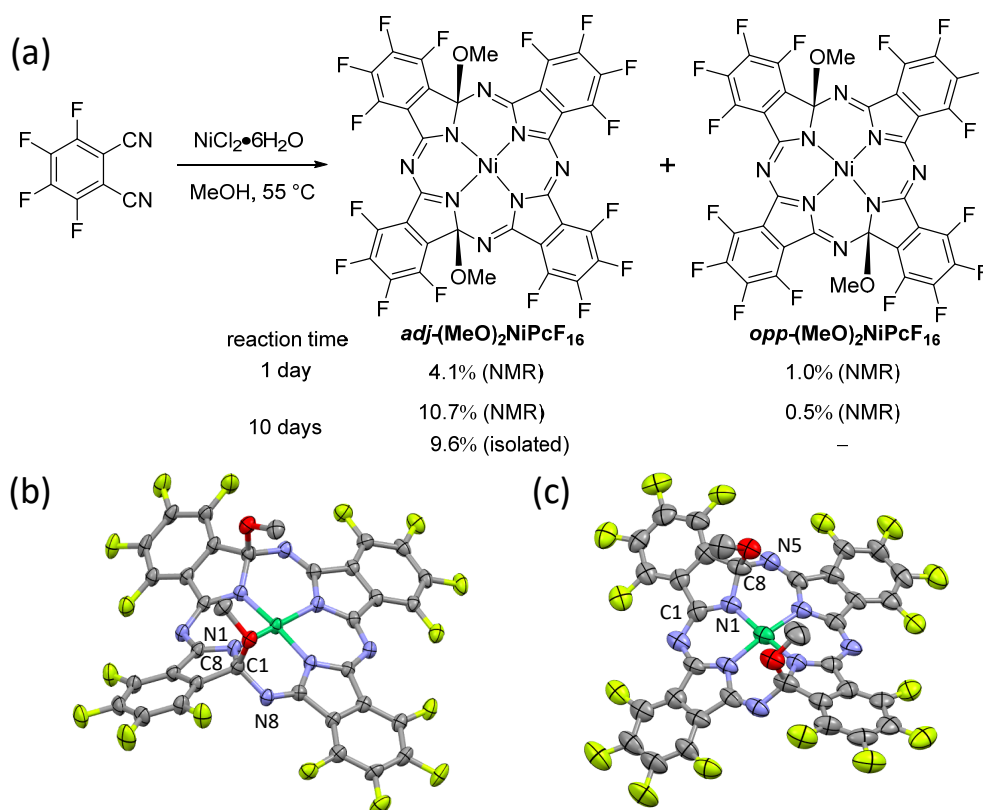


Figure 1. (a) Synthesis of *adj*-(MeO)₂NiPcF₁₆ and *opp*-(MeO)₂NiPcF₁₆. ORTEP drawings of (b) *adj*-(MeO)₂NiPcF₁₆ and (c) *opp*-(MeO)₂NiPcF₁₆ (50% ellipsoids). Hydrogen atoms and solvent molecules are omitted for clarity.

macrocycle. The presence of two sp^3 -hybridized methoxylated carbon atoms caused a significant distortion of the macrocyclic structure.^{9,10,12} The distortion of the macrocycle resulted in a loss of the D_{4h} symmetry of NiPc. The twisted structures of *opp*-(MeO)₂NiPcF₁₆ exhibit C_2 symmetry and chirality,^{9,10} whereas the bent structure of *adj*-(MeO)₂NiPcF₁₆ exhibits C_s symmetry and is achiral. The distances between the nickel atom and the nitrogen atoms in the methoxylated pyrroles (1.85–1.87 Å) are slightly shorter than those in the non-methoxylated pyrroles (1.87–1.88 Å). The dihedral angles of $N_{meso}-C(sp^3)-N-C$ reflect the distorted macrocyclic structures, with values of 124°–134° for *adj*-(MeO)₂NiPcF₁₆ and 129°–130° for *opp*-(MeO)₂NiPcF₁₆.

Reaction mechanism for *adj*-(RO)₂NiPc formation

Considering that *opp*-(MeO)₂NiPcF₁₆ isomerizes into *adj*-(MeO)₂NiPcF₁₆ during purification, it can be inferred that the activation energy for the isomerization from *opp*-(MeO)₂NiPcF₁₆ to *adj*-(MeO)₂NiPcF₁₆ is relatively low. Given that HCl and the solvent methanol are present in the reaction, an intrinsic reaction

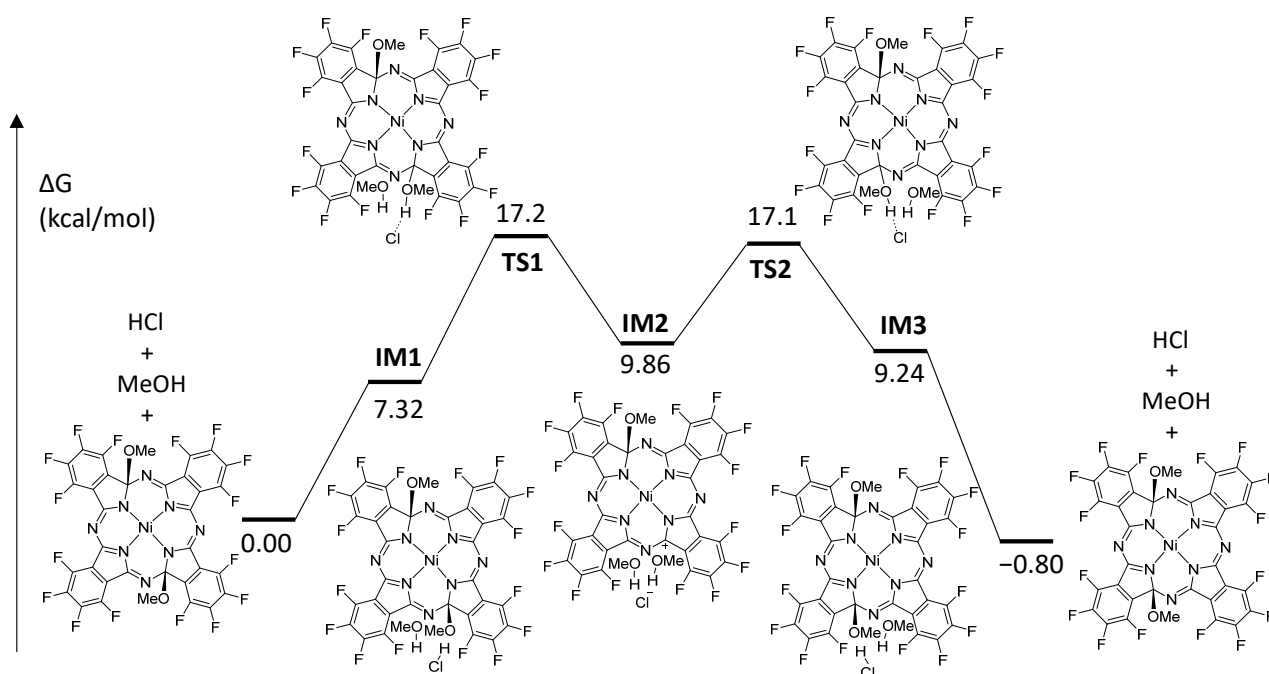


Figure 2. The energy profile for the formation of *adj*-(MeO)₂NiPcF₁₆ from *opp*-(MeO)₂NiPcF₁₆, along with the relative free energies.

Table 1. Gibbs free energy of energy-minimized structures of core-methoxylated NiPcs at ω B97X-D/6-31++G(d,p), scrf (solvent = MeOH) level.

	solvent	G (hartree)	ΔG (kcal/mol)
<i>opp</i> -(MeO) ₂ NiPcF ₁₆	MeOH	-3653.186469	0
<i>adj</i> -(MeO) ₂ NiPcF ₁₆	MeOH	-3653.187737	-0.80

coordinate (IRC) calculation was performed for *opp*-(MeO)₂NiPcF₁₆ and *adj*-(MeO)₂NiPcF₁₆ (Figure 2). The results revealed that the isomerization from *opp*-(MeO)₂NiPcF₁₆ to *adj*-(MeO)₂NiPcF₁₆ involves two energy barriers, which were calculated to be 17.2 kcal/mol and 7.26 kcal/mol, respectively, suggesting that isomerization can occur even at room temperature. Similarly, for the reverse reaction in which *adj*-(MeO)₂NiPcF₁₆ converts back to *opp*-(MeO)₂NiPcF₁₆, two energy barriers were also identified, with activation energies of 17.9 kcal/mol and 7.38 kcal/mol, respectively. Since the energy barrier for the reverse reaction is higher than that of the forward reaction, and *adj*-(MeO)₂NiPcF₁₆ is thermodynamically more stable than *opp*-(MeO)₂NiPcF₁₆ (Table 1), it is reasonable that *adj*-(MeO)₂NiPcF₁₆ was preferentially obtained.

Optical and electrochemical properties of *adj*-(MeO)₂NiPcF₁₆

The optical properties of core-methoxylated MPc with sp³-hybridized carbons in the core structure significantly differ from those of planar MPcs. The distortion of the macrocycle prevented π - π stacking between the Pc rings, resulting in improved solubility of *adj*-(MeO)₂NiPcF₁₆ in various organic solvents, such

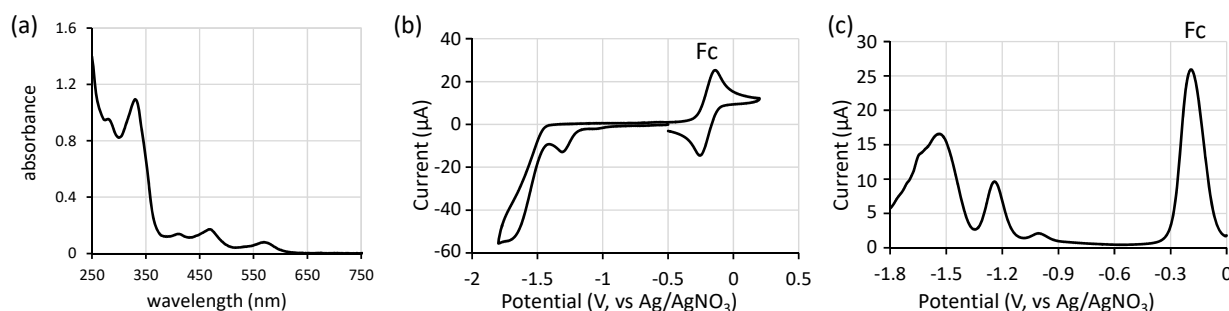


Figure 3. (a) UV-vis absorption spectrum of *adj*-(MeO)₂NiPcF₁₆ (25 μ M in CHCl₃). (b) Cyclic voltammograms of 0.5 mM of *adj*-(MeO)₂NiPcF₁₆ with 1.0 mM of ferrocene at a scan rate 0.05 V/s. (c) Differential pulse voltammograms of 0.5 mM of *adj*-(MeO)₂NiPcF₁₆ with 1.0 mM of ferrocene.

as toluene, diethyl ether, acetone, dimethylformamide, dichloromethane, chloroform, and 1,2-dichloroethane, although NiPcF₁₆ is barely soluble in these solvents. The loss of the 18 π -electron conjugated system of the NiPc resulted in a significant hypsochromic shift of the absorption spectra and no absorbance in the NIR region (> 650 nm) (Figure 3a).

Because the methoxylated core structure can be considered to be an oxidized form of phthalocyanine, the author next performed cyclic voltammetry (CV) measurements of *adj*-(MeO)₂NiPcF₁₆ in dichloromethane to investigate its reduction potential (Figure 3b). In a cyclic voltammogram, *adj*-(MeO)₂NiPcF₁₆ exhibited two reduction peaks: at -1.3 and -1.7 V. The current values obtained from differential pulse voltammetry (DPV) showed two reduction steps in the irreversible reduction of *adj*-(MeO)₂NiPcF₁₆, indicating that the electrolytic conversion proceeded via radical anion states (Figure S2). Density functional theory (DFT) calculation of the ground state showed that the lowest unoccupied molecular orbital (LUMO) of *adj*-(MeO)₂NiPcF₁₆ was mainly localized in the macrocyclic skeleton (Figures 3c and S4). Furthermore, the spin distribution analysis of the radical anion showed that the nickel atoms contribute minimally to the spin density (Figure S5). Results suggest that the first reduction step involves a one-electron reduction localized within the macrocycle.

Since the electrochemical properties of *adj*-(MeO)₂NiPcF₁₆ was clarified, the author next investigated the electrochemical reduction of *adj*-(MeO)₂NiPcF₁₆. At a constant potential of -1.5 V, *adj*-(MeO)₂NiPcF₁₆ was successfully converted to NiPcF₁₆ in 85% isolated yield. Utilizing electrochemical transformation, the author

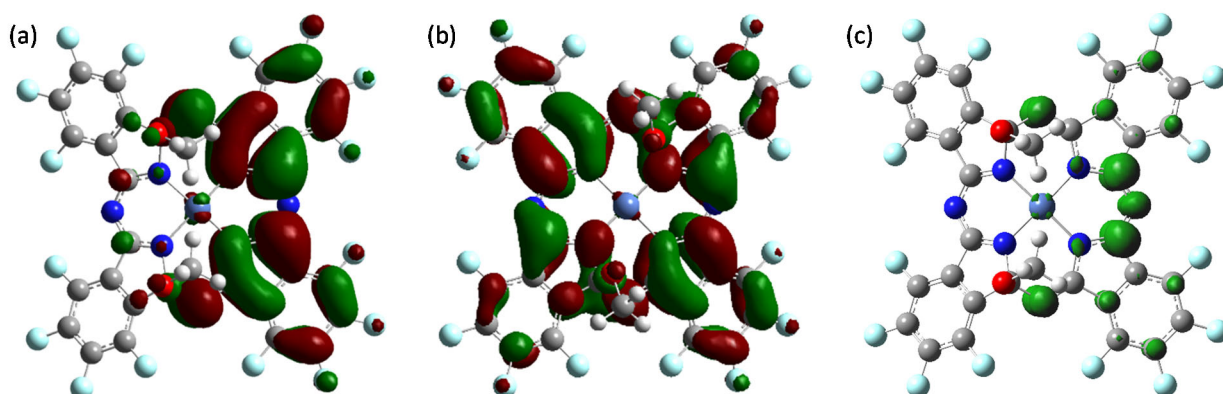


Figure 4. Isosurfaces for the LUMO orbitals of (a) *adj*-(MeO)₂NiPcF₁₆ and (b) *opp*-(MeO)₂NiPcF₁₆. (c) Isosurface (0.005 a.u.) plots of Mulliken spin population for the one-electron reduced species of *adj*-(MeO)₂NiPcF₁₆.

attempted to fabricate an electrode coated with NiPcF_{16} . When the electrochemical reduction of *adj*-(MeO) $_2\text{NiPcF}_{16}$ was conducted at a constant potential of -1.5 V on a transparent indium-tin-oxide (ITO) glass electrode as cathode, a blue solid adhered to the electrode surface. Spectroscopic analysis of the coated electrode revealed an absorption signal at 595 nm (Figure 3d), assignable to an *H*-aggregate of NiPcF_{16} .^[41,42] This result indicated that the electrochemical transformation of *adj*-(MeO) $_2\text{NiPcF}_{16}$ provides a useful method for preparing NiPcF_{16} -coated materials without the need for the sublimation of insoluble NiPcF_{16} .

Thermal conversion of *adj*-(MeO) $_2\text{NiPcF}_{16}$

To assess the potential for the thermal conversion of *adj*-(MeO) $_2\text{NiPcF}_{16}$ to planar NiPcF_{16} in the solid state,⁴³ differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) were performed (Figure 6a). Upon heating *adj*-(MeO) $_2\text{NiPcF}_{16}$, noticeable weight loss occurred at 270 °C. The weight loss at 200–300 °C was 6.7%, corresponding to the elimination of the methoxy groups (comparable to a theoretical value of 6.7%). After heating *adj*-(MeO) $_2\text{NiPcF}_{16}$ followed by washing the resulting solid with chloroform and methanol, a dark blue solid with low solubility was obtained. It was identified as NiPcF_{16} by MALDI-TOF

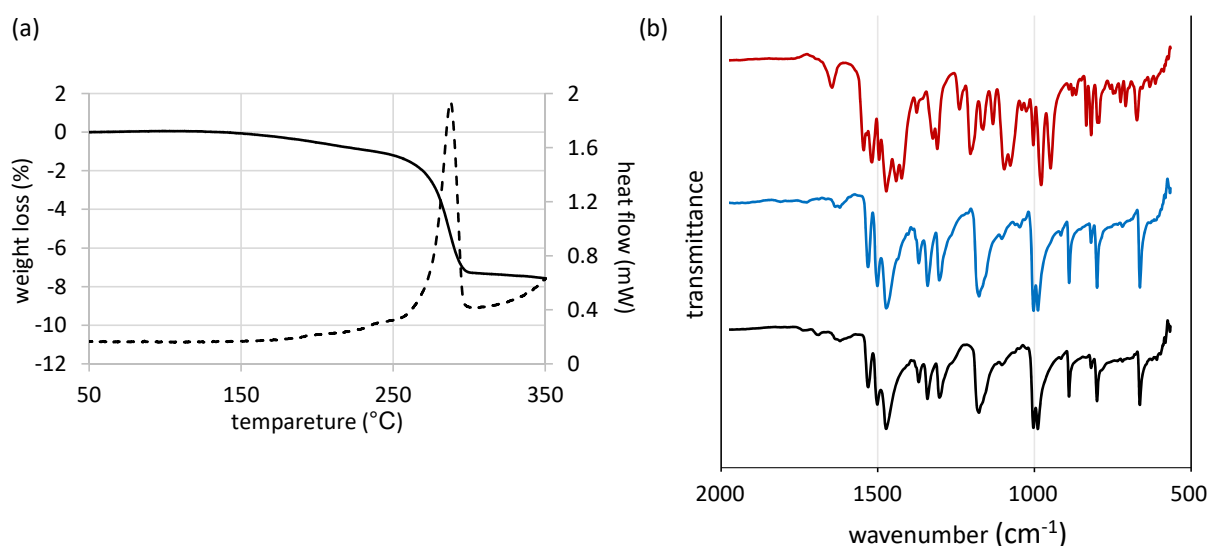


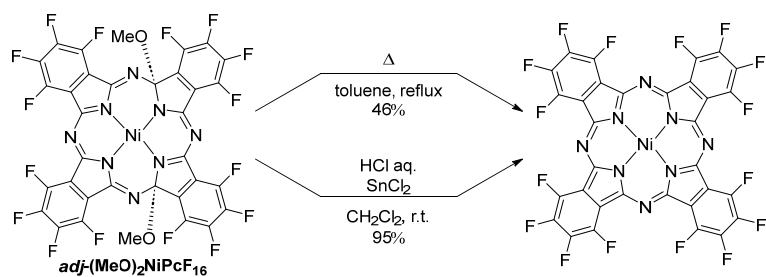
Figure 6. (a) TGA (solid line) and DSC (dashed line) curves of *adj*-(MeO) $_2\text{NiPcF}_{16}$ during the course of thermal elevation. Scan rate = 5.0 K min⁻¹. (b) IR spectra of *adj*-(MeO) $_2\text{NiPcF}_{16}$ (red), *adj*-(MeO) $_2\text{NiPcF}_{16}$ after thermal conversion (blue), and authentic sample of NiPcF_{16} (black).¹³

mass spectrometry and IR absorption spectrometry (Figure 6b). These results show that ***adj*-(MeO)₂NiPcF₁₆** can be thermally converted to NiPcF₁₆.

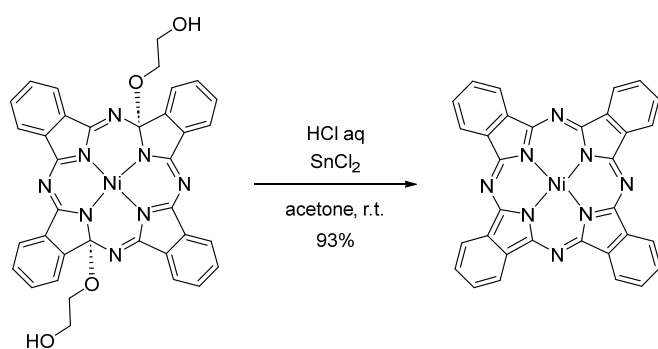
The thermal conversion of ***adj*-(MeO)₂NiPcF₁₆** was further examined in organic solvents. After refluxing a toluene solution of ***adj*-(MeO)₂NiPcF₁₆** for 36 h, the red solution became colorless, and NiPcF₁₆ was precipitated as an insoluble blue solid (Scheme 2). This thermal conversion of ***adj*-(MeO)₂NiPcF₁₆** afforded NiPcF₁₆ in 46% yield. Despite the complete consumption of these precursors during thermal conversion, the yields of NiPcF₁₆ did not exceed 50%. In the absence of external reducing agents, this is probably because half of the core-alkoxylated NiPcF₁₆ acted as a sacrificial two-electron reducing agent to facilitate the formation of NiPcF₁₆. Because of the potential contamination by impurities generated during sacrificial reduction, the thermal conversions of core-alkoxylated MPcs involving ***adj*-(MeO)₂NiPcF₁₆** are unsuitable for practical applications, including the preparation of pure MPc thin films.^[30] Given the higher MPc yield of electrochemical conversion, this method could be more suitable for various applications than thermal conversion.

Reductive aromatization of ***adj*-(MeO)₂NiPcF₁₆** with SnCl₂

Previously, the author had demonstrated that water-soluble *opp*-isomers of core-alkoxylated MPcs could be quantitatively converted to MPcs in water using glutathione as a reducing agent.³¹ However, water-insoluble ***adj*-(MeO)₂NiPcF₁₆** could not be applied to reduction with glutathione. Among the various reducing agents for reductive aromatization, H₂SnCl₄^{44,45} prepared from tin(II) chloride and hydrochloric acid was applicable. When ***adj*-(MeO)₂NiPcF₁₆** was treated with H₂SnCl₄ in CH₂Cl₂, NiPcF₁₆ was obtained in 95% isolated yield (Scheme 2). The tin(II)-mediated reductive aromatization approach was also applied to the *opp*-isomer of non-fluorinated core-alkoxylated MPc (Scheme S1, Figure S3). These findings demonstrate that the tin(II)-mediated reductive aromatization is a highly efficient and practical method to convert both *adj*- and *opp*-isomers into the corresponding MPcs, regardless of peripheral benzene ring modification.



Scheme 2. Thermal and Reductive conversion of *adj*-(MeO)₂NiPcF₁₆ to NiPcF₁₆.



Scheme 3. Reduction of *opp*-(OCH₂CH₂OH)₂NiPc to NiPc by SnCl₂

Conclusion

In conclusion, the author prepared the NiPcF_{16} precursor ***adj*-(MeO)₂NiPcF₁₆** with two methoxy groups located on the α -carbons of adjacent pyrrole rings through methanol-mediated tetramerization of 3,4,5,6-tetrafluorophthalonitrile with nickel(II) chloride. Indications are that it is essential to consider ***adj*-(RO)₂MPc** as a synthetic intermediate of MPcs alongside the previously recognized linear tetramer and ***opp*-(RO)₂MPc**. The conversion of ***adj*-(MeO)₂NiPcF₁₆** to NiPcF_{16} was successfully achieved under both thermal and reductive conditions. By utilizing electrochemical reduction, a NiPcF_{16} -coated ITO electrode was successfully prepared. The reductive transformation of ***adj*-(MeO)₂NiPcF₁₆** using H_2SnCl_4 proceeded to afford NiPcF_{16} in a quantitative yield. Given that both electrochemical and chemical reduction of core-alkoxylated MPcs with good solubility affords MPcs under mild conditions, these transformations represent promising complementary methods for preparing MPc-coated materials and MPc films.

Experimental

General

Materials and methods

Dichloromethane (CH_2Cl_2), chloroform (CHCl_3), toluene, 1,2-dichloroethane, hexane, and tin chloride dehydrate ($\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$) were purchased from Fujifilm-Wako Pure Chemicals Industries, Ltd. (Japan). Nickel dichloride hexahydrate ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$), tetra-*n*-butylammonium perchlorate, methanol (MeOH) and ethanol (EtOH) were purchased from nacalai tesque (Japan). 3,4,5,6-Tetrafluorophthalonitrile was purchased from BLD Pharmatech Ltd. (China). Silica gel (SiO_2 , 230–400 mesh) for column chromatography was purchased from Silicycle (Canada).

UV-vis absorption spectra were recorded by UV-vis spectrophotometer (UH5300, Hitachi High-Tech, Japan). Mass spectra were measured by Exactive Plus Orbitrap (ESI, Thermo Fisher Scientific Inc., USA) and Ultraflex III (MALDI, Bruker Co., USA).

IR absorption spectra were measured by Spectrum Two (FT-IR, PerkinElmer Inc., USA).

The cyclic voltammogram (CV) (scan rate: 0.1 V/s) and differential pulse voltammogram (DPV) were measured by electrochemical analyzer (610C-S, ALS Co., Ltd., Japan).

TG analysis was performed on TG/DTA6300 (Hitachi High-Tech, Japan) analyzer under a nitrogen atmosphere at a heating rate of $5\text{ }^\circ\text{C min}^{-1}$.

Differential Scanning Calorimetry was performed on Thermoplus Evo DSC 8230 (Rigaku, Japan).

Synthesis of core-alkoxylated NiPcF₁₆

*Synthesis of **adj-(MeO)₂NiPcF₁₆** and **opp-(MeO)₂NiPcF₁₆***

In a 50 mL Schlenk tube, 3,4,5,6-tetrafluorophthalonitrile (1.0 g, 5.0 mmol), $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (0.30 g, 1.25 mmol), and methanol (4.0 mL) were mixed and stirred at $55\text{ }^\circ\text{C}$. After stirring for 10 days, the dark-green suspension was diluted with methanol (30 mL) and filtered through celite pad. The organic solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and filtered to remove the blue solid. After the

organic solvent was removed under reduced pressure, the crude product was purified by column chromatography on SiO₂ (eluent: hexane/CH₂Cl₂, v:v = 3:1) to afford ***adj*-(MeO)₂NiPcF₁₆** (0.11 g, 0.12 mmol, 9.6%) as a reddish-brown solid together with a mixture of ***adj*-(MeO)₂NiPcF₁₆** and ***opp*-(MeO)₂NiPcF₁₆** (18 mg, 20 μmol, 1.6% total yield, ***adj*-(MeO)₂NiPcF₁₆** : ***opp*-(MeO)₂NiPcF₁₆** = 69:31 determined by NMR (Figure S10)). Single crystal of ***adj*-(MeO)₂NiPcF₁₆** and ***opp*-(MeO)₂NiPcF₁₆** were obtained from recrystallization in 1,2-dichloroethane/hexane at room temperature. Due to its low solubility, no obvious signals of ***adj*-(MeO)₂NiPcF₁₆** was not detected in ¹³C NMR (Figure S8).

***adj*-(MeO)₂NiPcF₁₆**: IR (neat) 3287, 3005, 2937, 2834, 2630, 2450, 1653, 1545, 1534, 1517, 1492, 1468, 1434, 1416, 1364, 1309, 1294, 1219, 1181, 1138, 1105, 1066, 1046, 1006, 991, 967, 940, 908, 845, 834, 822, 803, 786, 770, 749, 743, 704, 694, 688, 670, 653, 614, 596, 571, 551, 527, 518, 508 cm⁻¹; ¹H NMR (400 MHz, CDCl₃, 25 °C) δ = 3.59 (s, 4H). HRMS (MALDI-TOF) calcd for C₃₄H₇N₈O₂F₁₆Ni ([M+H]⁺) 920.9785, found 920.9777.

***opp*-(MeO)₂NiPcF₁₆**: ¹H NMR (400 MHz, CDCl₃, 25 °C) δ = 3.37 (s, 4H).

Heating of NiPcF₁₆ in methanol

NiPcF₁₆ (10 mg) was dispersed in methanol (5 mL) and stirred at 60°C. After heating and stirring for 12 hours, MeOH was removed under reduced pressure. MALDI-TOF mass spectrometry confirmed that **(MeO)₂NiPcF₁₆** did not form.

Electrochemical data of ***adj*-(MeO)₂NiPcF₁₆**

***adj*-(MeO)₂NiPcF₁₆** (5.0×10⁻⁴ M), ferrocene (1.0×10⁻³ M), and tetra-*n*-butylammonium perchlorate (0.10 M) were dissolved into CH₂Cl₂. Pt electrode (counter), glassy carbon electrode (working), and Ag/AgNO₃ electrode (reference) were used.

Electrochemical reduction of ***adj*-(MeO)₂NiPcF₁₆**

***adj*-(MeO)₂NiPcF₁₆** (31 mg, 33 μmol) and tetra-*n*-butylammonium perchlorate (0.10 M) were dissolved in

CH₂Cl₂ (50 mL). Pt electrode (counter), glassy carbon (working), and Ag/AgNO₃ electrode (reference) were used. The electrochemical reduction of **adj-(MeO)₂NiPcF₁₆** was performed at a constant potential of -1.5 V. The blue precipitate was collected by centrifugation and washed with acetone and water to obtain NiPcF₁₆ (24 mg, 28 μmol, 85%).

IR (neat) 1643, 1625, 1531, 1498, 1467, 1391, 1355, 1327, 1286, 1153, 1140, 1075, 1030, 1015, 965, 952, 845, 770, 753, 700, 663, 604, 577 cm⁻¹; LRMS (MALDI-TOF): calcd for C₃₂N₈F₁₆Ni ([M]⁺) 858, found 858.

Thermal Conversion at solid state

DSC analysis was performed by heating **adj-(MeO)₂NiPcF₁₆** (2.8 mg) on an aluminum dish to 350 °C at a rate of 5.0 K/min⁻¹. TGA was performed by heating **adj-(MeO)₂NiPcF₁₆** (3.46 mg) on an aluminum dish to 350 °C at a rate of 5.0 K/min⁻¹ in a nitrogen atmosphere. Alumina was used as the reference material for the DSC and TGA measurements. After DSC analysis, the solid sample was washed with CHCl₃ (3 mL × 3) and methanol (4 mL) to obtain NiPcF₁₆.

IR (neat) 1642, 1626, 1530, 1498, 1468, 1393, 1357, 1327, 1287, 1152, 1074, 1029, 1014, 966, 951, 845, 771, 750, 664, 604, 569 cm⁻¹; LRMS (MALDI-TOF): calcd for C₃₂N₈F₁₆Ni ([M]⁺) 858, found 858.

Thermal Conversion at solution state

Thermal Conversion of adj-(MeO)₂NiPcF₁₆ in toluene

In a 50 mL flask, **adj-(MeO)₂NiPcF₁₆** (72 mg, 78 μmol) in toluene (10 mL) was refluxed for 36 h. The reaction mixture was centrifuged to remove the supernatant. After washing with CHCl₃ (4 mL × 3) and methanol (4 mL), the solid was dried under reduced pressure. The resulting solid was sublimed to afford NiPcF₁₆ (32 mg, 37 μmol, 46%).

IR (neat) 1642, 1626, 1530, 1500, 1471, 1393, 1358, 1329, 1289, 1158, 1075, 1031, 1013, 968, 953, 846, 771, 750, 664, 605, 568 cm⁻¹; MS (MALDI): calcd for C₃₂N₈F₁₆Ni ([M]⁺) 858, found 858.

Reduction with SnCl₂

*Reduction of **adj**-(MeO)₂NiPcF₁₆ to NiPcF₁₆ with SnCl₂*

In a 50 mL flask, **adj**-(MeO)₂NiPcF₁₆ (18 mg, 20 μ mol), SnCl₂·2H₂O (40 mg), 10% HCl aqueous solution (0.5 mL), CH₂Cl₂ (12 mL), and methanol (6.0 mL) were mixed and stirred at room temperature for 36 h. The reaction mixture was centrifuged to remove the supernatant. After washing with water (4 mL $\times$ 3) and acetone (4 mL $\times$ 5), and the solid was dried under reduced pressure. The resulting solid was sublimed to afford NiPcF₁₆ (16 mg, 19 μ mol, 98%).

IR (neat) 1644, 1625, 1532, 1499, 1470, 1391, 1355, 1328, 1287, 1153, 1141, 1075, 1031, 1015, 966, 954, 846, 770, 753, 702, 664, 605, 568 cm⁻¹; MS (MALDI): calcd for C₃₂N₈F₁₆Ni ([M]⁺) 858, found 858.

*Reduction of **opp**-(OCH₂CH₂OH)₂NiPc to NiPc by SnCl₂*

In a 50 mL flask, **opp**-(OCH₂CH₂OH)₂NiPc³¹ (21 mg, 30 μ mol), SnCl₂·2H₂O (60 mg), 10% HCl aqueous solution (0.5 mL), and acetone (6.0 mL) were mixed and stirred at room temperature for 36 h. The reaction mixture was centrifuged to remove the supernatant. After washing with water (4 mL $\times$ 3), methanol (4 mL $\times$ 3) and acetone (4 mL $\times$ 5), the solid was dried under reduced pressure. The resulting solid was sublimed to afford NiPc¹⁷ (16 mg, 28 μ mol, 93%).

IR (neat) 2671, 2595, 2422, 2203, 1612, 1599, 1531, 1492, 1471, 1428, 1333, 1290, 1202, 1120, 1089, 1078, 1022, 1006, 952, 866, 805, 773, 755, 722, 644 cm⁻¹; MS (MALDI): calcd for C₃₂H₁₆N₈Ni ([M]⁺) 570, found 570.

Theoretical calculation

The density functional theory (DFT) calculations were performed for the geometry optimization of the ground state and radical anion state of **adj**-(MeO)₂NiPcF₁₆ and **opp**-(MeO)₂NiPcF₁₆ at the ω B97X-D/LANL2DZ level for Ni, the ω B97X-D/6-31++G(d, p) level for C, H, N, O, F, and scrf(solvent = MeOH) level by using Gaussian 16 package.^{47,48} The molecular geometries for the transition states were first estimated by Reaction plus Pro (ver. 2) software package (Software to optimize reaction paths along the user's expected ones, HPC Systems

Inc., <http://www.hpc.co.jp/chem/react2.html> (written in Japanese)), based on the nudged elastic band (NEB) method,^{49,50} and were subsequently re-optimized at ω B97X-D/6-31G(d,p) level for C, H, N, O, F, and scrf(solvent = MeOH) by using Gaussian 16 package. Single point energies were calculated at ω B97X-D/6-31++G(d, p) level for C, H, N, O, F, and scrf(solvent = MeOH) level by using Gaussian 16 package. The cartesian coordinates of the optimized structures are summarized at the end of the ESI.

X-ray crystallographic data

Intensity data were collected on a RIGAKU Saturn 724 HG CCD system with VariMax Mo Optic using MoK α radiation. The structures were solved by a direct method (SHELXT)⁵¹ and refined by a full-matrix least square method on F^2 for all reflections (SHELXL-2016).⁵² All hydrogen atoms were placed using AFIX instructions, while all other atoms were refined anisotropically. The residual scattered electron densities, originating from disordered solvents, were eliminated by the SQUEEZE protocol equipped in PLATON.⁵³ Crystal data are summarized in Table 2. The separation of the disorders on the alkoxy moiety is difficult, resulting in the Alerts B in the checkCIF/PLATON report. However, this problem does not affect the whole structure of the molecule. The molecular structure was visualized by Mercury, which is provided by The Cambridge Crystallographic Data Centre (CCDC): <https://www.ccdc.cam.ac.uk/solutions/csd-system/components/mercury/>.

Table 2. Crystallographic data of *adj*-(MeO)₂NiPcF₁₆ and *opp*-(MeO)₂NiPcF₁₆.

	<i>adj</i> -(MeO) ₂ NiPcF ₁₆	<i>opp</i> -(MeO) ₂ NiPcF ₁₆
CCDC number	2401667	2401666
Chemical formula	C ₃₄ H ₆ F ₁₆ N ₈ NiO ₂	C ₃₄ H ₆ F ₁₆ N ₈ NiO ₂
Formula weight	921.18	921.18
Crystal size/mm ³	0.30×0.20×0.15	0.25×0.02×0.15
Crystal color	red	red
Crystal system	triclinic	triclinic
Space group	<i>P</i> -1	<i>P</i> -1

a (Å)	14.7434(19)	12.382(7)
b (Å)	15.676(2)	15.713(7)
c (Å)	15.7752(15)	21.192(12)
α (°)	71.164(10)	72.772(18)
β (°)	63.852(8)	89.62(2)
γ (°)	79.860(10)	78.897(19)
Volume (Å ³)	3095.1(7)	3859(4)
Z	4	4
Temperature (K)	143(2)	143(2)
Radiation source	Mo K α	Mo K α
Wavelength (Å)	0.71075	0.71075
D_{calc} (g/cm ³)	1.977	1.586
μ (mm ⁻¹)	0.777	0.623
$F(000)$	1816	1816
2θ range for data collection/°	3.062 to 27.480	3.062 to 27.480
	$-19 \leq h \leq 18$	$-16 \leq h \leq 15$
Index ranges	$-20 \leq k \leq 20$	$-15 \leq k \leq 20$
	$-20 \leq l \leq 17$	$-27 \leq l \leq 27$
Reflections collected	25118	30630
Independent reflections	13534	16624
Data/restraints/parameters	13534/0/1103	16624/0/1103
Goodness-of-fit on F^2	1.001	1.001
Final R indexes [$I \geq 2\sigma(I)$]	$R_1 = 0.0395$ $wR_2 = 0.0777$	$R_1 = 0.0780$ $wR_2 = 0.1054$
Final R indexes [all data]	$R_1 = 0.0583$ $wR_2 = 0.0827$	$R_1 = 0.1598$ $wR_2 = 0.1268$
Largest diff. peak/hole (e Å ⁻³)	0.450 / -0.443	0.834 / -0.582

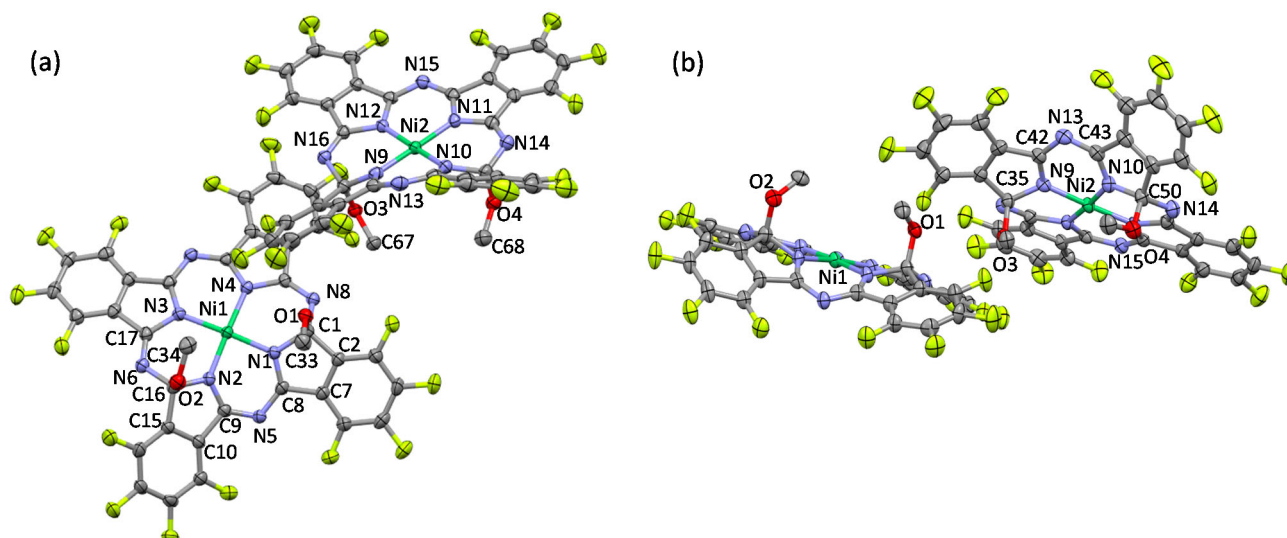


Figure 7. (a) Top-view and (b) side-view of *adj*-(MeO)₂NiPcF₁₆ (50% ellipsoids) with labels. Hydrogen atoms and solvent molecules are omitted for clarity.

Table 3. Selected (a) bond lengths, (b) angles, and (c) torsion for *adj*-(MeO)₂NiPcF₁₆.

(a)	Length (Å)	(b)		Angle (deg)	
Ni1—N1	1.857(2)	N1—Ni1—N2	87.8(1)	N9—Ni2—N10	88.9(1)
Ni1—N2	1.853(3)	N2—Ni1—N3	90.5(1)	N10—Ni2—N11	90.3(1)
Ni1—N3	1.874(2)	N3—Ni1—N4	91.2(1)	N11—Ni2—N12	91.2(1)
Ni1—N4	1.872(3)	N4—Ni1—N1	90.2(1)	N12—Ni2—N9	89.5(1)
Ni2—N9	1.851(3)	O1—C1—N1	111.1(2)	O3—C35—N9	110.5(2)
Ni2—N10	1.853(2)	O1—C1—N8	103.7(2)	O3—C35—N16	103.7(2)
Ni2—N11	1.875(3)	O1—C1—C2	114.4(2)	O3—C35—C36	113.2(2)
Ni2—N12	1.880(2)	O2—C16—N2	111.2(2)	O4—C50—N10	109.3(2)
C1—O1	1.411(3)	O2—C16—N6	109.1(2)	O4—C50—N14	102.2(2)
C1—N1	1.470(5)	O2—C16—C15	105.6(2)	O4—C50—C49	114.1(2)
C1—N8	1.448(4)	N1—C1—N8	114.6(2)	N9—C35—N16	114.1(2)
C1—C2	1.524(4)	N1—C1—C2	102.8(2)	N9—C35—C15	103.5(2)
C16—O2	1.416(3)	N2—C16—N6	115.8(2)	N10—C50—N14	117.4(2)
C16—N2	1.473(4)	N2—C16—C15	103.9(2)	N10—C50—C49	103.1(2)
C16—N6	1.444(3)				
C16—C15	1.514(5)				
C35—O3	1.404(3)	(c) Torsion (deg)			
C35—N9	1.478(4)	N8—C1—N1—C8	124.3(3)		
C35—N16	1.448(4)	N6—C16—N2—C9	−130.8(3)		
C35—C36	1.516(4)	N16—C35—N9—C42	128.1(3)		
C50—O4	1.419(4)	N14—C50—N10—C43	−134.2(3)		
C50—N10	1.483(4)				
C50—N14	1.437(4)				
C50—C49	1.514(4)				

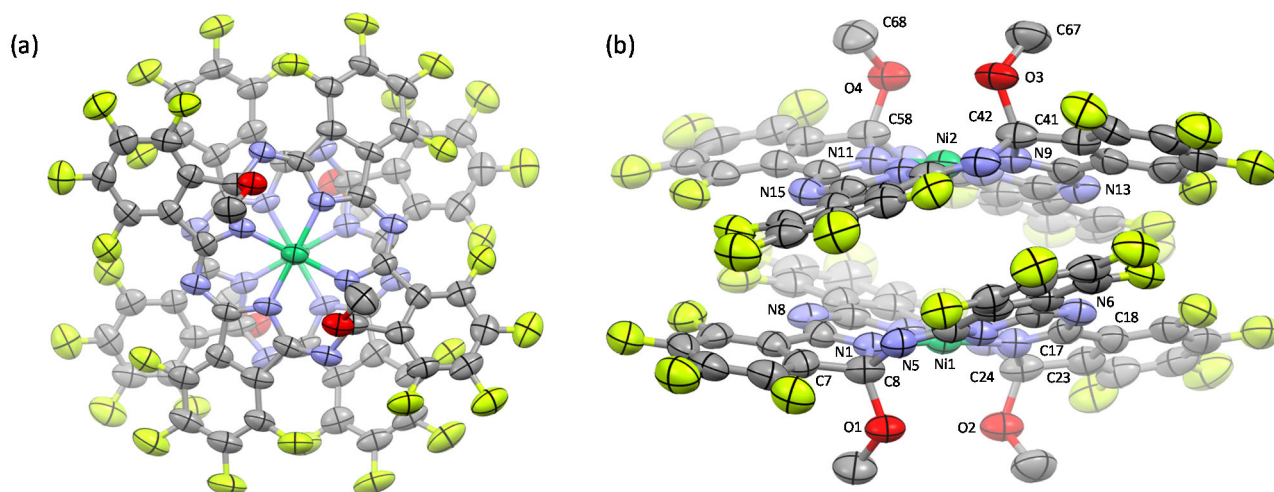


Figure 8. (a) Top-view and (b) side-view of *opp*-(MeO)₂NiPcF₁₆ (50% ellipsoids) with labels. Hydrogen atoms and solvent molecules are omitted for clarity.

Table 4. Selected (a) bond lengths, (b) angles, and (c) torsion for *opp*-(MeO)₂NiPcF₁₆.

(a)	Length (Å)	(b)	Angle (deg)		Angle (deg)
Ni1—N1	1.862(5)	N1—Ni1—N2	90.8(2)	N9—Ni2—N10	90.9(2)
Ni1—N2	1.883(4)	N2—Ni1—N3	90.0(2)	N10—Ni2—N11	89.9(2)
Ni1—N3	1.863(5)	N3—Ni1—N4	90.5(2)	N11—Ni2—N12	90.7(2)
Ni1—N4	1.879(4)	N4—Ni1—N1	89.5(2)	N12—Ni2—N9	89.6(2)
Ni2—N9	1.871(4)	O1—C8—N1	109.6(4)	O3—C42—N9	109.0(4)
Ni2—N10	1.884(5)	O1—C8—N5	102.4(4)	O3—C42—N14	103.1(4)
Ni2—N11	1.854(4)	O1—C8—C7	114.6(5)	O3—C42—C41	114.0(5)
Ni2—N12	1.884(6)	O2—C24—N3	109.9(4)	O4—C58—N11	110.0(4)
C8—O1	1.449(7)	O2—C24—N7	102.8(4)	O4—C58—N16	102.3(4)
C8—N1	1.504(7)	O2—C24—C23	114.3(5)	O4—C58—C57	115.2(5)
C8—N5	1.439(7)	N1—C8—N5	115.3(5)	N9—C42—N14	115.0(5)
C8—C7	1.50(1)	N1—C8—C7	103.0(4)	N9—C42—C41	103.0(4)
C24—O2	1.439(6)	N3—C24—N7	113.5(5)	N11—C58—N16	113.6(5)
C24—N3	1.503(8)	N3—C24—C23	103.6(4)	N11—C58—C57	104.1(4)
C24—N7	1.445(7)				
C24—C23	1.52(1)				
C42—O3	1.446(7)				
C42—N9	1.512(8)				
C42—N14	1.440(8)				
C42—C41	1.525(9)				
C58—O4	1.436(6)				
C58—N11	1.507(6)				
C58—N16	1.466(9)				
C58—C57	1.508(9)				

(c)	Torsion (deg)	
N5—C8—N1—C1	-129.4(5)	
N7—C24—N3—C17	-130.4(5)	
N14—C42—N9—C35	-129.6(5)	
N16—C58—N11—C51	-130.2(5)	

Cartesian coordinates of energy-minimized structures

adj-(MeO)₂NiPcF₁₆ (neutral)

C	2.4274898	-3.1756757	-0.3419898
C	3.3799077	-2.3855185	0.2899298
C	2.6878611	-1.1658532	0.8667390
N	1.3085345	-1.2998558	0.3732978
C	1.1338614	-2.4750400	-0.2148085
C	-3.3799324	-2.3854812	0.2899472
C	-2.4275259	-3.1756510	-0.3419735
C	-1.1338899	-2.4750274	-0.2148026
N	-1.3085475	-1.2998397	0.3733002
C	-2.6878722	-1.1658177	0.8667440
N	-0.0000182	-3.0383059	-0.6190479
C	-2.4334153	3.3582517	-0.2119040
C	-3.4287740	2.3903406	-0.0710752
C	-2.7407695	1.1146918	0.2289796
N	-1.3500582	1.3668174	0.2240015
C	-1.1456476	2.6629054	-0.0324511
N	-3.3674836	0.0395486	0.4725718
C	3.4288042	2.3903052	-0.0710516
C	2.4334563	3.3582267	-0.2118848
C	1.1456803	2.6628931	-0.0324431
N	1.3500755	1.3668025	0.2240075
C	2.7407847	1.1146621	0.2289928
N	3.3674857	0.0395105	0.4725800
N	0.0000200	3.3173482	-0.1488759
C	-2.7496284	4.6765579	-0.4844037
C	-4.0993850	5.0141704	-0.6128703
C	-5.0919328	4.0480222	-0.4726336
C	-4.7637251	2.7171797	-0.1956459
C	-4.6963120	-2.7806487	0.3720556
C	-5.0598057	-3.9926611	-0.2195199
C	-4.1101960	-4.7838427	-0.8602690
C	-2.7751607	-4.3834956	-0.9226223
C	4.7637594	2.7171314	-0.1956121
C	5.0919822	4.0479714	-0.4725936
C	4.0994451	5.0141299	-0.6128347
C	2.7496843	4.6765305	-0.4843787
C	2.7751094	-4.3835204	-0.9226473
C	4.1101413	-4.7838807	-0.8603045
C	5.0597624	-3.9927118	-0.2195567
C	4.6962840	-2.7806993	0.3720281
Ni	0.0000013	0.0414052	0.3640218
F	-6.3233708	-4.4023622	-0.1756837
F	-4.4887031	-5.9314428	-1.4165829
F	-4.4473401	6.2686205	-0.8734453
F	-6.3635414	4.4026139	-0.6036990
F	6.3635952	4.4025509	-0.6036489
F	4.4474146	6.2685772	-0.8734037
F	4.4886340	-5.9314811	-1.4166275
F	6.3233239	-4.4024251	-0.1757307
F	-5.7417562	1.8326724	-0.0711181
F	-1.8400710	5.6291867	-0.6252365
F	1.8401372	5.6291686	-0.6252155
F	5.7417808	1.8326139	-0.0710801
F	5.6299542	-2.0622992	0.9871035

F	1.9029964	-5.1699762	-1.5394507
F	-1.9030590	-5.1699634	-1.5394263
F	-5.6299714	-2.0622356	0.9871325
O	2.7547700	-1.3176774	2.2604534
C	2.1844034	-0.2947658	3.0549257
O	-2.7547778	-1.3176287	2.2604598
C	-2.1844545	-0.2946866	3.0549245
H	2.3199817	-0.6151791	4.0877997
H	2.6939920	0.6643232	2.9075296
H	1.1135458	-0.1789747	2.8508334
H	-1.1136073	-0.1788301	2.8508143
H	-2.6941021	0.6643725	2.9075398
H	-2.3199946	-0.6151114	4.0877999

adj-(MeO)₂NiPcF₁₆ (radical anion)

C	2.4279299	-3.1659189	-0.3583502
C	3.3724605	-2.3880833	0.3001416
C	2.6840447	-1.1524674	0.8569277
N	1.3070838	-1.2884212	0.3432906
C	1.1327482	-2.4560072	-0.2470650
C	-3.3724922	-2.3880383	0.3001423
C	-2.4279713	-3.1658898	-0.3583444
C	-1.1327812	-2.4559929	-0.2470642
N	-1.3071021	-1.2884020	0.3432845
C	-2.6840629	-1.1524254	0.8569186
N	-0.0000199	-3.0218412	-0.6618816
C	-2.4304773	3.3723410	-0.2111561
C	-3.4168789	2.3859376	-0.0613761
C	-2.7171421	1.1229207	0.2380299
N	-1.3630723	1.3785062	0.2429462
C	-1.1499755	2.7106952	-0.0303287
N	-3.3696756	0.0305130	0.4715261
C	3.4169142	2.3858952	-0.0613290
C	2.4305272	3.3723116	-0.2111171
C	1.1500140	2.7106807	-0.0303112
N	1.3630904	1.3784877	0.2429602
C	2.7171573	1.1228850	0.2380605
N	3.3696741	0.0304678	0.4715550
N	0.0000245	3.3455238	-0.1503093
C	-2.7861823	4.6882968	-0.4899209
C	-4.1323624	4.9997347	-0.6152272
C	-5.1139942	4.0157214	-0.4672488
C	-4.7617990	2.7007812	-0.1871994
C	-4.6839347	-2.7980243	0.4049418
C	-5.0507867	-4.0047326	-0.1905297
C	-4.1099731	-4.7804042	-0.8610736
C	-2.7815515	-4.3689567	-0.9461256
C	4.7618399	2.7007228	-0.1871319
C	5.1140552	4.0156605	-0.4671685
C	4.1324378	4.9996868	-0.6151548
C	2.7862520	4.6882645	-0.4898695
C	2.7814951	-4.3689864	-0.9461390
C	4.1099117	-4.7804507	-0.8610901
C	5.0507350	-4.0047952	-0.1905412
C	4.6838979	-2.7980863	0.4049385

Chapter 5

Ni	0.0000003	0.0636042	0.3480496	C	-3.3563000	4.1192200	-0.9319210
F	-6.3148084	-4.4312934	-0.1226031	C	-3.1552600	5.4102530	-1.4239460
F	-4.4951312	-5.9300603	-1.4248953	C	-1.8707660	5.9194670	-1.5742750
F	-4.5122595	6.2543760	-0.8825223	C	-0.7550470	5.1502120	-1.2389650
F	-6.4012799	4.3594287	-0.5977062	C	3.3563160	-4.1192230	-0.9318270
F	6.4013470	4.3593527	-0.5976055	C	3.1552810	-5.4102460	-1.4238900
F	4.5123540	6.2543250	-0.8824370	C	1.8707880	-5.9194260	-1.5743340
F	4.4950557	-5.9301076	-1.4249198	C	0.7550640	-5.1501430	-1.2391010
F	6.3147515	-4.4313719	-0.1226180	C	4.3391120	3.2490280	0.0693720
F	-5.7340183	1.7996747	-0.0558304	C	5.7062770	3.0952560	0.2925210
F	-1.8889959	5.6633220	-0.6394504	C	6.2149210	1.8759550	0.7211840
F	1.8890797	5.6633014	-0.6394061	C	5.3662880	0.7900830	0.9460950
F	5.7340463	1.7996038	-0.0557546	Ni	-0.0000220	0.0000190	0.2772570
F	5.6151058	-2.0996679	1.0512929	F	-4.1983230	6.1661100	-1.7538940
F	1.9182919	-5.1478795	-1.5944290	F	-1.7102730	7.1535320	-2.0441780
F	-1.9183579	-5.1478646	-1.5944106	F	-6.5372740	-4.1144750	0.0817300
F	-5.6151336	-2.0995898	1.0512917	F	-7.5251580	-1.7462100	0.9112530
O	2.6959939	-1.3400897	2.2616293	F	4.1983520	-6.1661200	-1.7537670
C	2.2014952	-0.2715211	3.0435339	F	1.7103040	-7.1534760	-2.0442770
O	-2.6960169	-1.3400340	2.2616222	F	6.5373190	4.1143610	0.0815690
C	-2.2015586	-0.2714444	3.0435247	F	7.5251740	1.7460820	0.9110940
H	2.2801258	-0.6012524	4.0808059	F	-5.9130280	0.3648640	1.3353030
H	2.7985416	0.6367860	2.9048647	F	-3.8963820	-4.4259470	-0.3663070
H	1.1518268	-0.0534446	2.8119688	F	-0.4520760	-5.6845390	-1.3971210
H	-1.1519093	-0.0533024	2.8119357	F	4.6031260	-3.6747570	-0.8021290
H	-2.7986609	0.6368295	2.9048786	F	5.9129930	-0.3649220	1.3353120
H	-2.2801441	-0.6011910	4.0807952	F	3.8964180	4.4259170	-0.3663090

opp-(MeO)₂NiPcF₁₆ (neutral)

C	3.5078080	2.1678800	0.2955190	F	-4.1983230	6.1661100	-1.7538940
C	4.0138000	0.9502860	0.7477890	F	-1.7102730	7.1535320	-2.0441780
C	2.8385010	-0.0097590	0.9214170	F	-6.5372740	-4.1144750	0.0817300
N	1.7144790	0.7476770	0.3625940	F	-7.5251580	-1.7462100	0.9112530
C	2.0584490	1.9815990	0.0904990	F	4.1983520	-6.1661200	-1.7537670
C	-2.2507140	3.3618450	-0.6092090	F	1.7103040	-7.1534760	-2.0442770
C	-0.9606860	3.8727380	-0.7578540	F	6.5373190	4.1143610	0.0815690
C	-0.0321190	2.8092450	-0.3180570	F	7.5251740	1.7460820	0.9110940
N	-0.7375500	1.7334410	0.0843720	F	-5.9130280	0.3648640	1.3353030
C	-2.1047600	1.9997150	-0.0488540	F	-3.8963820	-4.4259470	-0.3663070
N	1.2705800	2.9842120	-0.3346550	F	-0.4520760	-5.6845390	-1.3971210
C	-3.5078150	-2.1678850	0.2954620	F	4.6031260	-3.6747570	-0.8021290
C	-4.0138200	-0.9502980	0.7477400	F	5.9129930	-0.3649220	1.3353120
C	-2.8385350	0.0097780	0.9213130	F	3.8964180	4.4259170	-0.3663090
N	-1.7145300	-0.7476380	0.3624490	F	0.4520840	5.6846630	-1.3968690
C	-2.0584620	-1.9815680	0.0903870	F	-4.6031110	3.6747170	-0.8023330
N	-3.0986380	1.2623390	0.2610800	O	-2.4852510	0.1977510	2.2689250
C	2.2507280	-3.3618240	-0.6091930	C	-3.4165120	0.8870690	3.0993000
C	0.9607000	-3.8726850	-0.7579470	O	2.4852440	-0.1977160	2.2690370
C	0.0321140	-2.8091970	-0.3181850	C	3.4164830	-0.8870670	3.0993960
N	0.7375400	-1.7334130	0.0843140	H	-3.7222060	1.8379620	2.6549080
C	2.1047550	-1.9996920	-0.0488300	H	-4.2974620	0.2687500	3.2997820
N	3.0986040	-1.2623340	0.2612000	H	-2.8900710	1.0723460	4.0350850
N	-1.2705850	-2.9841610	-0.3348220	H	2.8901120	-1.0721400	4.0352620
C	-4.3390940	-3.2490690	0.0693820	H	3.7219870	-1.8380670	2.6551000
C	-5.7062510	-3.0953400	0.2926090	H	4.2975540	-0.2688670	3.2997190
C	-6.2149100	-1.8760450	0.7212750				
C	-5.3663060	-0.7901370	0.9461040				

opp-(MeO)₂NiPcF₁₆ (radical anion)

C	-3.5303029	2.1700875	0.2871786
C	-4.0379880	0.9546011	0.7433751
C	-2.8743777	-0.0299724	0.8894153
N	-1.7347746	0.7558033	0.3757359
C	-2.0776164	1.9802378	0.0900091
C	2.2344671	3.3685958	-0.5967162
C	0.9437624	3.8765620	-0.7572028
C	0.0116208	2.8108018	-0.3148169
N	0.7221441	1.7415433	0.0979958
C	2.0858331	2.0051241	-0.0287551
N	-1.2869910	2.9871828	-0.3433622
C	3.5135529	-2.1732605	0.3115571
C	4.0118387	-0.9403636	0.7359664
C	2.8231192	0.0025933	0.9263268
N	1.7137473	-0.7450937	0.3930087
C	2.0550197	-2.0223010	0.1392272

Chapter 5

N	3.0815280	1.2733963	0.2831954	C	-2.0057780	-5.6635240	-2.0191900
C	-2.2492775	-3.3395995	-0.6307542	C	-2.4783280	-4.4902010	-1.4253560
C	-0.9448877	-3.8752879	-0.7390598	C	-1.5602780	-3.5244110	-1.0748770
C	-0.0400998	-2.8548251	-0.2878532	C	-1.7049410	-2.2128040	-0.4088900
N	-0.7667198	-1.7512293	0.0930144	N	-0.4316910	-1.6561820	-0.2745170
C	-2.1098687	-1.9964647	-0.0867574	N	-2.8299040	-1.7471880	-0.0191340
N	-3.1346400	-1.2235462	0.1905519	C	-2.8232780	-0.5185810	0.7194490
N	1.2919561	-3.0183271	-0.2351282	C	-4.1749800	0.1722650	0.6738440
C	4.3670349	-3.2410531	0.0854253	C	-5.4204260	-0.2892450	1.0262290
C	5.7350769	-3.0564188	0.2759610	C	-6.5067850	0.5812070	0.9102800
C	6.2333562	-1.8218571	0.6757007	C	-6.3263740	1.8844570	0.4602960
C	5.3678802	-0.7525137	0.9030345	C	-5.0572610	2.3493220	0.1148490
C	3.3422665	4.1276000	-0.9194698	C	-3.9903360	1.4780470	0.2286530
C	3.1397446	5.4129388	-1.4234875	C	-2.5475480	1.6382930	-0.0343800
C	1.8537338	5.9185969	-1.5864549	N	-1.9193770	0.5057460	0.1885010
C	0.7372871	5.1511210	-1.2520121	N	-2.0247770	2.8065390	-0.4326760
C	-3.3582231	-4.1007019	-0.9923246	C	-0.7134910	2.9335130	-0.4366560
C	-3.1632640	-5.3857392	-1.4658879	C	-0.0605970	4.1923950	-0.8452260
C	-1.8678015	-5.9198816	-1.5747805	C	-0.5658150	5.4027130	-1.2737230
C	-0.7606385	-5.1775909	-1.2145623	C	0.3396970	6.4171460	-1.5942280
C	-4.3568781	3.2606495	0.0747279	C	1.7091600	6.2102130	-1.4800630
C	-5.7193746	3.1189851	0.3263833	C	2.2131930	4.9832280	-1.0401620
C	-6.2284028	1.9066304	0.7791986	C	1.3153660	3.9838000	-0.7349300
C	-5.3862499	0.8149847	0.9903200	C	1.4963720	2.6068400	-0.2282850
Ni	-0.0127481	-0.0150129	0.3051449	N	0.2245830	2.0335470	-0.0848110
F	4.1841100	6.1743651	-1.7548594	N	2.6358510	2.0962300	0.0322970
F	1.6972556	7.1529028	-2.0693414	C	2.6578770	0.7930030	0.6491300
F	6.5868170	-4.0651211	0.0612233	C	4.0126460	0.1158850	0.4642090
F	7.5526741	-1.6673227	0.8374516	C	5.2923550	0.5431630	0.7345380
F	-4.2074250	-6.1474128	-1.8288212	C	6.3555250	-0.3311960	0.5015790
F	-1.7276996	-7.1705652	-2.0396738	C	6.1289430	-1.6024580	-0.0124870
F	-6.5514552	4.1479190	0.1331841	C	4.8344220	-2.0232550	-0.3127410
F	-7.5388086	1.7989530	1.0135866	C	3.7916840	-1.1491460	-0.0757550
F	5.9047023	0.4184027	1.2708633	C	2.3431900	-1.2742780	-0.3022750
F	3.9456024	-4.4364498	-0.3218279	N	1.7344930	-0.1675260	0.0419300
F	0.4494004	-5.7309441	-1.3354970	N	1.7912150	-2.3838970	-0.8275800
F	-4.6076689	-3.6363097	-0.9040027	F	1.5668860	-5.1013650	-2.0918000
F	-5.9338921	-0.3205444	1.4329620	F	-0.2293620	-6.9794730	-2.8010030
F	-3.9137895	4.4376313	-0.3648635	F	-2.8601170	-6.6123650	-2.3801970
F	-0.4681491	5.6834840	-1.4231976	F	-3.7829610	-4.3544330	-1.2302190
F	4.5906652	3.6916821	-0.7787089	F	-5.6267410	-1.5240050	1.4725840
O	2.5241223	0.2074509	2.3015296	F	-7.7253790	0.1683990	1.2337370
C	3.4766897	0.9010759	3.0836725	F	-7.3780420	2.6896210	0.3603260
O	-2.5524853	-0.2010117	2.2674197	F	-4.9287570	3.6001610	-0.3072810
C	-3.1500882	-1.3007174	2.9310638	F	-1.8629820	5.6480400	-1.3931650
H	2.9820726	1.1118381	4.0338132	F	-0.1070860	7.5944540	-2.0133940
H	4.3652005	0.2859530	3.2751595	F	2.5430370	7.1932170	-1.7935900
H	3.7867444	1.8408207	2.6145428	F	3.5270450	4.8312810	-0.9431470
H	-2.7931486	-2.2555005	2.5316336	F	5.5650420	1.7576180	1.2120080
H	-2.8402661	-1.2069505	3.9739320	F	7.5992650	0.0532700	0.7633760
H	-4.2432174	-1.2772735	2.8679967	F	7.1605030	-2.4119530	-0.2296850
IM1				F	4.6605970	-3.2350490	-0.8236380
C	0.4885630	-2.5101880	-0.7805180	Ni	-0.0947360	0.1747950	0.0225830
C	-0.1945630	-3.7150280	-1.2906740	O	-2.4489750	-0.8662050	2.0428530
C	0.2800040	-4.8735460	-1.8699820	C	-2.3708410	0.1958340	2.9962020
C	-0.6464050	-5.8526850	-2.2371810	H	-2.0855890	-0.2738170	3.9366000
				H	-1.6132430	0.9282720	2.7052760

Chapter 5

H	-3.3425500	0.6832070	3.1135470
O	2.2509520	0.8605260	1.9931960
C	2.9988070	1.7124080	2.8535820
H	3.9866710	1.2913780	3.0656390
H	3.1097460	2.7140930	2.4308320
H	2.4281970	1.7670560	3.7800840
O	2.6984050	-2.9490230	2.1161450
C	3.3694410	-2.1730540	3.0896660
H	1.7521560	-2.8886990	2.2889690
H	3.2743710	-2.5983900	4.0978600
H	4.4298550	-2.1641760	2.8213300
H	3.0119280	-1.1373400	3.1038690
H	-1.3897900	-2.1587800	2.4201310
Cl	-0.6552230	-3.1545810	2.8843630

TS1

C	0.6409730	-2.5381130	-0.8483120
C	0.0269030	-3.8184430	-1.2399710
C	0.5592510	-4.9912800	-1.7273370
C	-0.3138300	-6.0591530	-1.9651050
C	-1.6729860	-5.9391050	-1.7068130
C	-2.2045240	-4.7461430	-1.2040650
C	-1.3394530	-3.6981970	-0.9792680
C	-1.5445910	-2.3378650	-0.4542260
N	-0.3428610	-1.6904300	-0.4075020
N	-2.7172970	-1.9014100	-0.1039010
C	-2.8435480	-0.6190820	0.3298400
C	-4.2000070	-0.0174830	0.4178330
C	-5.4257350	-0.5625960	0.7161260
C	-6.5342770	0.2915150	0.7321760
C	-6.3932030	1.6485120	0.4673860
C	-5.1400060	2.1947500	0.1754930
C	-4.0531500	1.3446380	0.1578290
C	-2.6215830	1.5693270	-0.0988690
N	-1.9598530	0.3983410	-0.0238050
N	-2.1358620	2.7594350	-0.3750660
C	-0.8144900	2.9226540	-0.4435680
C	-0.2266590	4.2192020	-0.8121330
C	-0.7908610	5.4303620	-1.1572940
C	0.0680840	6.4904010	-1.4602850
C	1.4472690	6.3253080	-1.4112130
C	2.0107210	5.0963050	-1.0529090
C	1.1590000	4.0531120	-0.7669850
C	1.4017970	2.6693360	-0.3167220
N	0.1446950	2.0340900	-0.1841180
N	2.5524920	2.1985500	-0.0526970
C	2.6099820	0.8921150	0.5599180
C	3.9948990	0.2739470	0.4199160
C	5.2462650	0.7558840	0.7260370
C	6.3497600	-0.0798450	0.5397780
C	6.1892700	-1.3670650	0.0408930
C	4.9225190	-1.8436570	-0.2946400
C	3.8410970	-1.0048350	-0.1093210
C	2.4097230	-1.1844690	-0.3803910
N	1.7433770	-0.1024130	-0.0841530
N	1.9207900	-2.3364490	-0.8987190

F	1.8477760	-5.1532120	-1.9828750
F	0.1588440	-7.2021030	-2.4411830
F	-2.4732210	-6.9692440	-1.9416980
F	-3.5051850	-4.6776840	-0.9686100
F	-5.5984780	-1.8460290	0.9896430
F	-7.7349030	-0.1941740	1.0079600
F	-7.4636350	2.4297360	0.4911010
F	-5.0489460	3.4912500	-0.0715100
F	-2.0977770	5.6336290	-1.2112330
F	-0.4333610	7.6696050	-1.8009310
F	2.2339620	7.3495930	-1.7071280
F	3.3299420	4.9841070	-1.0127290
F	5.4534330	1.9849790	1.1958680
F	7.5674790	0.3566100	0.8354390
F	7.2569780	-2.1390450	-0.1274870
F	4.8112860	-3.0701450	-0.7859540
Ni	-0.1029470	0.1551040	-0.1388340
O	-2.5614440	-0.8497170	2.1626730
C	-2.2102430	0.3127950	2.9145470
H	-2.0365280	0.0069550	3.9475780
H	-1.3112190	0.7898430	2.5144560
H	-3.0545110	1.0025740	2.8783040
O	2.1509700	0.9397570	1.8823160
C	2.8685080	1.7704610	2.7884840
H	3.8473830	1.3418960	3.0251810
H	2.9933450	2.7825300	2.3949500
H	2.2648620	1.8019660	3.6946940
O	2.5254400	-2.6121490	2.0256710
C	3.1538520	-1.9691540	3.1109650
H	1.5795880	-2.6951600	2.2398130
H	3.2234490	-2.6178060	3.9958800
H	4.1718530	-1.7105690	2.8008810
H	2.6431060	-1.0417140	3.3955310
H	-1.8501790	-1.5549660	2.3056050
Cl	-0.5358140	-2.8544290	2.8714160

IM2

C	-1.1414620	-2.5740760	-0.6863310
C	-2.4368980	-3.1527300	-1.0662060
C	-2.8191990	-4.4289110	-1.4082310
C	-4.1772230	-4.6587610	-1.6711600
C	-5.1047630	-3.6311590	-1.5759000
C	-4.7066270	-2.3360660	-1.2175710
C	-3.3706460	-2.1190130	-0.9704740
C	-2.6218140	-0.9298560	-0.5526220
N	-1.3128720	-1.2263540	-0.4273970
N	-3.2119920	0.2350880	-0.3614450
C	-2.4800650	1.3172840	-0.1999350
C	-3.0717930	2.6399150	0.0251020
C	-4.3640900	3.0531270	0.2578800
C	-4.5893210	4.4242040	0.4388700
C	-3.5411130	5.3322460	0.3777210
C	-2.2293120	4.9018740	0.1363390
C	-2.0180300	3.5549060	-0.0400430
C	-0.8035160	2.7672210	-0.2782150
N	-1.1366860	1.4348070	-0.3274110
N	0.3586140	3.2999020	-0.5238030

Chapter 5

C	1.4399040	2.5176840	-0.5902600	C	-0.5932510	-2.6458640	-0.7448690
C	2.7474740	3.0540800	-0.9860750	C	-1.8011370	-3.4686310	-0.9425640
C	3.1517380	4.3062310	-1.4019700	C	-1.9828520	-4.7683270	-1.3640220
C	4.5046310	4.4902810	-1.7020840	C	-3.2871790	-5.2732290	-1.3885980
C	5.4101050	3.4433660	-1.5767840	C	-4.3625600	-4.4894950	-0.9892990
C	4.9932260	2.1800960	-1.1424260	C	-4.1683670	-3.1736270	-0.5543980
C	3.6576100	2.0020890	-0.8622850	C	-2.8831840	-2.6823140	-0.5477920
C	2.8974630	0.8514180	-0.3405840	C	-2.3264870	-1.3719190	-0.1330330
N	1.5364830	1.2323370	-0.2647000	N	-0.9630460	-1.3998430	-0.3473060
N	3.4268760	-0.2391330	0.0373070	N	-3.1169050	-0.2672540	-0.2370380
C	2.5863190	-1.1934990	0.7157700	C	-2.5877630	0.9082570	-0.2840850
C	3.2143920	-2.5804470	0.6851070	C	-3.4072230	2.1255390	-0.3819780
C	4.4748630	-3.0182580	1.0140930	C	-4.7673460	2.3284400	-0.3881440
C	4.7608390	-4.3815470	0.8961540	C	-5.2313310	3.6482350	-0.4634010
C	3.7976160	-5.2749170	0.4431470	C	-4.3432520	4.7131280	-0.5241310
C	2.5262740	-4.8248920	0.0879870	C	-2.9599630	4.4955060	-0.5111440
C	2.2563790	-3.4741520	0.2113300	C	-2.5139760	3.1948590	-0.4378820
C	1.0727510	-2.6803730	-0.1357440	C	-1.1683410	2.6089010	-0.3810530
N	1.2786180	-1.4090250	0.0696570	N	-1.2532910	1.2615770	-0.2998290
N	-0.0578490	-3.2734460	-0.5993980	N	-0.0917460	3.3580180	-0.4364590
F	-1.9726270	-5.4399050	-1.5026470	C	1.1119390	2.7943770	-0.3437250
F	-4.5825000	-5.8721370	-2.0124700	C	2.3299640	3.5993310	-0.5098980
F	-6.3810750	-3.8799680	-1.8260530	C	2.5299510	4.9413170	-0.7642210
F	-5.6265040	-1.3905340	-1.1205890	C	3.8466920	5.3963700	-0.8736110
F	-5.3899590	2.2231760	0.3255340	C	4.9169730	4.5211990	-0.7293040
F	-5.8168480	4.8624870	0.6720100	C	4.7045970	3.1641640	-0.4660840
F	-3.7864890	6.6210310	0.5553180	C	3.4045870	2.7211630	-0.3683310
F	-1.2602120	5.7993130	0.0905980	C	2.8275770	1.3981230	-0.0630600
F	2.3242440	5.3305220	-1.5278990	N	1.4185990	1.5204230	-0.1011030
F	4.9340180	5.6747970	-2.1127870	N	3.5300420	0.3811870	0.2262240
F	6.6844160	3.6495500	-1.8717380	C	2.8738630	-0.8111470	0.6916360
F	5.8906440	1.2136370	-1.0287980	C	3.7483920	-2.0333100	0.4370820
F	5.4371990	-2.2022640	1.4373380	C	5.0734630	-2.2800560	0.7086010
F	5.9663420	-4.8316080	1.2128130	C	5.6045880	-3.5277690	0.3706310
F	4.1003630	-6.5628600	0.3372860	C	4.8149600	-4.4957410	-0.2377000
F	1.6408940	-5.7009010	-0.3632270	C	3.4760900	-4.2360740	-0.5292460
Ni	0.0848860	0.0117310	-0.1979400	C	2.9637690	-2.9974800	-0.1906830
O	-0.4212150	2.8928380	2.3531830	C	1.6380370	-2.3974110	-0.3992710
C	0.8514810	2.4609110	2.7834820	N	1.6233980	-1.1498570	-0.0028110
H	0.9660850	2.5401260	3.8738950	N	0.6104130	-3.1305580	-0.8805210
H	1.0643290	1.4267360	2.4837930	F	-0.9882740	-5.5508510	-1.7505770
H	1.6014750	3.1098330	2.3193770	F	-3.5049020	-6.5143400	-1.7983560
O	2.2506010	-0.7657290	2.0035520	F	-5.5848230	-4.9985760	-1.0205430
C	3.3250020	-0.4769600	2.8942560	F	-5.2158510	-2.4657800	-0.1634280
H	3.8570500	-1.3884020	3.1813240	F	-5.6510100	1.3455200	-0.3273580
H	4.0268370	0.2384650	2.4580220	F	-6.5346910	3.8866170	-0.4764500
H	2.8582830	-0.0393310	3.7757680	F	-4.8138820	5.9504520	-0.5941540
O	-2.8256640	-2.2376950	1.9510900	F	-2.1509860	5.5414340	-0.5653720
C	-4.2047340	-2.4346350	2.1644430	F	1.5417180	5.8105060	-0.9042350
H	-2.6030660	-1.3586640	2.3140630	F	4.0842900	6.6773980	-1.1195800
H	-4.8167970	-1.6720800	1.6603920	F	6.1531600	4.9845920	-0.8418200
H	-4.4718640	-3.4124150	1.7528280	F	5.7536140	2.3668500	-0.3315110
H	-4.4675570	-2.4296050	3.2309480	F	5.8760910	-1.3834070	1.2779420
H	-1.0672440	2.2092270	2.6181450	F	6.8784470	-3.7938060	0.6256610
Cl	-2.4061380	0.6320000	3.0852120	F	5.3480040	-5.6707140	-0.5518300
				F	2.7603790	-5.1762540	-1.1304330
				Ni	0.2005290	0.0648880	-0.1478610

TS2

Chapter 5

O	-2.4217860	1.8177230	2.4476090
C	-1.2057750	1.3516570	2.9908000
H	-1.1923900	1.4055960	4.0884550
H	-0.9893930	0.3183550	2.6905540
H	-0.4042960	1.9925750	2.6108640
O	2.4821250	-0.6777990	2.0301820
C	3.5051860	-0.3895760	2.9785330
H	4.1753970	-1.2444210	3.1105720
H	4.0853700	0.4904130	2.6894600
H	2.9857720	-0.1938710	3.9156380
O	-2.3582480	-1.5081430	1.7450080
C	-1.9465720	-2.7208340	2.3666100
H	-3.2275280	-1.1782880	2.1384250
H	-2.6085120	-3.5520140	2.1064570
H	-0.9270330	-2.9318670	2.0397850
H	-1.9485140	-2.5696870	3.4478610
H	-3.1280730	1.2096330	2.7268880
Cl	-4.6423860	-0.3886300	3.1881680

IM3

C	-0.4989460	-2.6918850	-0.5911560
C	-1.6619330	-3.6007830	-0.6799120
C	-1.8032370	-4.8876730	-1.1633060
C	-3.0523400	-5.4993820	-1.0544460
C	-4.1203940	-4.8350560	-0.4610890
C	-3.9637500	-3.5390630	0.0336490
C	-2.7351060	-2.9361480	-0.0969380
C	-2.2623880	-1.5700310	0.3654440
N	-0.8818790	-1.5244890	-0.0943070
N	-3.1344530	-0.5345620	-0.1029750
C	-2.6739710	0.6360250	-0.2979270
C	-3.5448220	1.7888180	-0.6029090
C	-4.9086720	1.9033170	-0.7555510
C	-5.4393620	3.1763400	-0.9833890
C	-4.6128930	4.2919300	-1.0393170
C	-3.2309180	4.1682590	-0.8726380
C	-2.7161220	2.9081100	-0.6538930
C	-1.3461920	2.4212750	-0.4229660
N	-1.3497910	1.0978750	-0.2323560
N	-0.3158080	3.2526850	-0.4564380
C	0.9107060	2.7844720	-0.2806380
C	2.0797290	3.6756850	-0.3778550
C	2.1997070	5.0304120	-0.6139280
C	3.4844900	5.5770200	-0.6565520
C	4.6051570	4.7766230	-0.4680540
C	4.4745610	3.4059310	-0.2281930
C	3.2049460	2.8727940	-0.1921420
C	2.7101170	1.5025390	0.0569510
N	1.3042130	1.5318430	-0.0260600
N	3.4815700	0.5282610	0.3206770

C	2.9329220	-0.7464340	0.6998620
C	3.8734280	-1.8725390	0.2776060
C	5.2301770	-2.0369830	0.4371950
C	5.8268310	-3.2028920	-0.0481010
C	5.0674250	-4.1746790	-0.6867290
C	3.6949050	-3.9987240	-0.8606650
C	3.1166410	-2.8395560	-0.3797070
C	1.7303800	-2.3365290	-0.4405160
N	1.6724620	-1.1179080	0.0623480
N	0.7299790	-3.0958800	-0.8945790
F	-0.8108860	-5.5624400	-1.7301450
F	-3.2304980	-6.7317200	-1.5183750
F	-5.2950610	-5.4460220	-0.3670170
F	-4.9985480	-2.9370910	0.6126590
F	-5.7348850	0.8678660	-0.7017330
F	-6.7475520	3.3275580	-1.1452680
F	-5.1484380	5.4874520	-1.2530650
F	-2.4843100	5.2623510	-0.9238970
F	1.1615320	5.8328060	-0.7972250
F	3.6436850	6.8753930	-0.8801610
F	5.8109860	5.3268120	-0.5159760
F	5.5708910	2.6811660	-0.0530380
F	6.0066100	-1.1314690	1.0314670
F	7.1343390	-3.3862730	0.0940760
F	5.6611380	-5.2725950	-1.1438050
F	3.0092870	-4.9424260	-1.4945120
Ni	0.1817300	0.0077750	-0.0146230
O	-2.1756130	2.5306830	2.4031480
C	-1.1447480	1.7289090	2.9475840
H	-1.1301910	1.7623670	4.0452500
H	-1.2242570	0.6823480	2.6274200
H	-0.1948060	2.1241200	2.5782890
O	2.6393730	-0.7601060	2.0750090
C	3.7107520	-0.4816440	2.9687350
H	4.4456720	-1.2930230	2.9738620
H	4.2086170	0.4593650	2.7211590
H	3.2566020	-0.4063300	3.9562940
O	-2.3073470	-1.4300440	1.7798320
C	-1.5437600	-2.3519640	2.5571820
H	-3.6256580	-0.5966270	2.4653910
H	-1.8522400	-3.3835940	2.3628030
H	-0.4770090	-2.2306470	2.3528260
H	-1.7500190	-2.0990460	3.5962290
H	-3.0155130	2.1263690	2.6484300
Cl	-4.5305610	0.1415700	3.0849060

References

- (1) R. P. Linstead, *J. Chem. Soc.* **1934**, 1016.
- (2) G. Bottari, G. de la Torre, D. M. Guldi, T. Torres, *Coord. Chem. Rev.* **2021**, 428, 213605.
- (3) D. Gounden, N. Nombona, W. E. van Zyl, *Coord. Chem. Rev.* **2020**, 420, 213359.
- (4) T. Nyokong, *Coord. Chem. Rev.* **2007**, 251, 1707–1722.
- (5) D. Li, S. Cai, P. Wang, H. Cheng, B. Cheng, Y. Zhang, G. Liu, *Adv. Healthcare Mater.* **2023**, 12, 2300263.
- (6) B.-D. Zheng, Q.-X. He, X. Li, J. Yoon, J.-D. Huang, *Coord. Chem. Rev.* **2021**, 426, 213548.
- (7) K. Janas, E. Boniewska-Bernacha, G. Dyrda, R. Słota, *Bioorg. Med. Chem.* **2021**, 30, 115926.
- (8) A. Galstyan, *Chem. –Eur. J.* **2021**, 27, 1903–1920.
- (9) A. Kumar, R. Meunier-Prest, M. Bouvet, *Sensors* **2020**, 20, 4700.
- (10) D. Klyamer, R. Shutilov, T. Basova, *Sensors* **2021**, 22, 895.
- (11) W. Zhou, N. J. Yutronkie, B. H. Lessard, J. L. Brusso, *Mater. Adv.* **2021**, 2, 165–185.
- (12) S. Yang, Y. Yu, X. Gao, Z. Zhang, F. Wang, *Chem. Soc. Rev.* **2021**, 50, 12985–13011.
- (13) E. Mikoloudakis, I. López-Duarte, G. Charalambidis, K. Ladomenou, M. Ince, A. G. Coutsolelos, *Chem. Soc. Rev.* **2022**, 51, 6965–7045.
- (14) J.-H. Wu, J.-W. Wang, B. M. Aramburu-Trošelj, F.-J. Niu, L.-J. Guo, G. Ouyang, *Nanoscale* **2024**, 16, 11496–11512.
- (15) Y.-C. Feng, X. Wang, D. Wang, *Mater. Chem. Front.* **2024**, 8, 228–247.
- (16) G. Zanotti, F. Palmeri, V. Raglione, *Chem. –Eur. J.* **2024**, 30, e20240908.
- (17) G. de la Torre, C. G. Claessens, T. Torres, *Eur. J. Org. Chem.* **2000**, 2821–2830.
- (18) D. Langerreiter, M. A. Kostianen, S. Kaabel, E. Anaya-Plaza, *Angew. Chem. Int. Ed.* **2022**, 61, e202209033.
- (19) B. I. Kharisov, U. O. Méndez, J. L. A. Garza, J. R. A. Rodríguez, *New J. Chem.* **2005**, 29, 686–692.
- (20) R. P. Linstead, A. R. Lowe, *J. Chem. Soc.* **1934**, 1017–1022.
- (21) T. J. Hurley, M. A. Robinson, S. I. Trotz, *Inorg. Chem.* **1967**, 6, 389–392.

- (22) C. H. Yang, C.-T. Chang, *J. Chem. Soc. Dalton Trans.* **1982**, 2539–2540.
- (23) S. W. Oliver, T. D. Smith, *J. Chem. Soc. Perkin Trans.* **1987**, 1579–1582.
- (24) R. M. Christie, D. D. Deans, *J. Chem. Soc. Perkin Trans.* **1989**, 193–198.
- (25) C. Wang, X. Chen, D. Qi, S. Bi, J. Jiang, *Inorg. Chem. Commun.* **2017**, 85, 9–15.
- (26) I. Paramio, T. Torres, G. de la Torre, *Org. Chem. Front.* **2024**, 11, 60–66.
- (27) C. D. Molek, J. A. Halfen, J. C. Loe, R. W. McGaff, *Chem. Commun.* **2001**, 2644–2645.
- (28) Y. Y. Karabach, M. N. Kopylovich, K. V. Luzyanin, M. F. C. Guedes da Silva, V. Yu. Kukushkin, A. J. L. Pombeiro, *Inorg. Chem. Acta* **2017**, 455, 696–700.
- (29) Y. Kikukawa, T. Fukuda, A. Fuyuhiko, N. Ishikawa, N. Kobayashi, *Chem. Commun.* **2011**, 8518–8520.
- (30) H. Wang, T. Fukuda, N. Ishikawa, Y. Matsuo, *Org. Electron.* **2014**, 15, 139–143.
- (31) K. Nogita, T. Sugahara, K. Miki, H. Mu, M. Kobayashi, H. Harada, K. Ohe, *Chem. Commun.* **2024**, 60, 1472–1475.
- (32) Z. Bao, A. J. Lovinger, J. Brown, *J. Am. Chem. Soc.* **1998**, 120, 207–208.
- (33) N. V. S. D. K. Bhupathiraju, W. Rizvi, J. D. Batteas, C. M. Drain, *Org. Biomol. Chem.* **2016**, 14, 389–408.
- (34) E. A. Kuzmina, T. V. Dubinina, L. G. Tomilova, *New J. Chem.* **2019**, 43, 9314–9327.
- (35) Q. Zhou, Z.-F. Liu, T. J. Marks, P. Darancet, *J. Phys. Chem. A* **2021**, 125, 4055–4061.
- (36) B. King, M. C. Vebber, R. Ewenike, M. Dupuy, C. French, J. L. Brusso, B. H. Lessard, *Chem. Mater.* **2023**, 35, 8517–8528.
- (37) T. Fukuda, Y. Ogi, N. Kobayashi, *Chem. Commun.* **2006**, 159–161.
- (38) J. R. Robinson, K. A. Bahr, M. J. Bierman, I. A. Guzei, H. M. Kieler-Ferguson, A. M. McCoy, R. W. McGaff, *Dalton trans.* **2011**, 40, 11809–11814.
- (39) F. M. Kolomeychuk, E. A. Safonova, M. A. Polovkova, A. A. Sinelshchikova, A. G. Martynov, A. V. Shokurov, G. A. Kirakosyan, N. N. Efimov, A. Yu. Tsivadze, Y. G. Gorbunova, *J. Am. Chem. Soc.* **2021**, 143, 14053–14058.

- (40) R. K. Al-Shewiki, S. Weheabby, N. Uhlig, M. Korb, T. Pester, S. Zahn, S. Grecchi, P. R. Mussini, T. Rüffer, H. Lang, *Dalton Trans.* **2024**, 53, 3836–3854.
- (41) J. Spadavecchia, G. Ciccarella, R. Rella, S. Capone, P. Siciliano, *Sens. Actuators B-Chem.* **2003**, 96, 489–497.
- (42) D. Peng, Y. Zhang, X.-Q. Liu, H. Shang, G. Lin, H.-Y. Jin, X.-F. Liu, R. He, Y.-H. Xu, S.-P. Luo, *Mol. Catal.* **2022**, 525, 112342.
- (43) T. Fukuda, Y. Kikukawa, R. Tsuruya, A. Fuyuhiko, N. Ishikawa, N. Kobayashi, *Inorg. Chem.* **2011**, 50, 11832–11837.
- (44) V. K. Patel, E. Kayahara, S. Yamago, *Chem. -Eur. J.* **2015**, 21, 5742–5749.
- (45) K. Miki, K. Ohe, *Chem. -Eur. J.* **2020**, 26, 2529–2575.
- (46) H. Tomoda, S. Saito, S. Shiraishi, *Chem. Lett.* **1983**, 12, 313–316.
- (47) J.-D. Chai, M. Head-Gordon, *Phys. Chem. Chem. Phys.* **2008**, 10, 6615–6620.
- (48) Gaussian 16, Revision B.01, 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. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, D. J. Fox, Gaussian, Inc., Wallingford CT, 2016.
- (49) H. Jónsson, G. Mills, K. W. Jacobsen, *Classical and Quantum Dynamics in Condensed Phase Simulations* **1998**, 385–404.

- (50) G. Henkelman, H. Jónsson, *J. Chem. Phys.* **2000**, *113*, 9978.
- (51) G. M. Sheldrick, *Acta Crystallogr. Sect. A* **2015**, *71*, 3–8.
- (52) G. M. Sheldrick, *Acta Crystallogr. Sect. C* **2015**, *71*, 3–8.
- (53) A. L. Spek, *Acta Crystallogr. Sect. C* **2015**, *71*, 9–18.

General Conclusion

In this thesis, the author has developed stimuli-responsive MPc and MNc derivatives that form MPc and MNc aggregates by eliminating axial ligands or alkoxy groups on the core-structure. The following two approaches have been conducted for axial ligand dissociation in these derivatives. The first one involves axial ligands containing a peptide sequence that can be cleaved by enzymes highly expressed in tumor cells. The second one involves cleavage between the central metal and the axial ligand through photoinduced intermolecular electron transfer. The following three methods have been demonstrated for the dissociation of alkoxy groups on the core structure: (1) a thermal conversion reaction using the central metal of another molecule as a sacrificial reductant, (2) an electrochemical two-electron reduction reaction, and (3) a reductive aromatization via external reductants such as glutathione or tin(II) chloride. The author has also elucidated the structures and photophysical properties of all MPc and MNc precursors prepared. The author has achieved tumor theranostics and thin-film fabrication by employing methods that enable stimuli-responsive conversion into MPc and MNc. These findings are summarized in the following five chapters.

Chapter 1 described **MNc-pep-PEG** conjugates in which the peptide linker connecting MNc and PEG is cleaved in response to MMP-2. After enzyme-responsive cleavage of PEG moieties, the water solubility of MNc decreases, resulting in a change in PA signal intensity and the PA signal intensity ratio because of MNc aggregation. **MNc-pep-PEG** was found to act as an activatable PA probe *in vitro* and *in vivo*, demonstrating that the absorption wavelength shift caused by MNc aggregation can serve as a design principle for activatable PA probes.

Chapter 2 described the photoinduced cleavage of PEGylated axial ligands of MPc. The photolysis of the hydrophilic axial ligand was found to occur through the generation of a pair of MPc radical anion and radical cation caused by intermolecular electron transfer. This reaction mechanism suggested photoimmunotherapy can be applied to lesions by using amphiphilic phthalocyanines that form self-assemblies under non-reductive conditions.

Chapter 3 dealt with GSH-responsive reductive aromatization of core-alkoxylated NiPc and its PA signal enhancement. NiPcs having alkoxy groups on their α -carbons of the pyrroles exhibited remarkably high solubility because of their distorted macrocycles due to the replacement of sp^2 -hybridized carbons with sp^3 -hybridized carbons in the macrocyclic framework. They showed no absorption in the NIR region above 700 nm, causing only negligible PA signal generation before conversion to NiPc during PA cancer imaging. Since core-alkoxylated NiPc is converted to water-insoluble NiPc through GSH-mediated reductive aromatization *in vivo*, it can serve as a turn-on PA contrast agent.

Chapter 4 described the synthesis of core-alkoxylated NiNc and its GSH-responsive reductive aromatization. Although the NIR photothermal conversion of NiPc in water was inefficient due to a hypsochromic shift of the absorption spectrum caused by aggregation, NiNc with extended π -conjugated structure showed high NIR photothermal conversion efficiency enough to induce cell death of cancer cells, which will find application in photothermal therapy (PTT). PA signal intensity of core-alkoxylated NiNc was increased in response to its conversion to NiNc, showing the potential as a PAI/PTT theranostic agent.

Chapter 5 dealt with two isomers of core-alkoxylated perfluorinated $(RO)_2NiPcF_{16}$. The author demonstrated that $(RO)_2NiPcF_{16}$ derivative with two methoxy groups on α -carbons of the adjacent pyrroles was isolated and its transformation to planar $NiPcF_{16}$ by heat, electrochemical reduction, and reaction with a reducing agent. These transformation was applied to prepare MPc-coated materials and MPc films.

MPcs and MNcs possess optical properties that make them suitable as photosensitizers for photoacoustic imaging and phototherapy for cancer; however, their low solubility has posed a challenge in their development. This thesis demonstrated that modifying the core structure with axial ligands or alkoxy groups bound to the α -carbon of pyrrole units effectively suppresses π - π stacking, thereby enhancing the solubility of MPcs and MNcs in water or organic solvent. Furthermore, several methods were developed to convert these core-modified MPcs and MNcs into their corresponding MPcs and MNcs under mild conditions. Notably, core-modified MNcs undergo reductive aromatization by glutathione (GSH) in a biological environment, leading to the formation of MNcs that absorb near-infrared (NIR) light. This property enables their application

General Conclusion

as turn-on photosensitizers for photoacoustic imaging and photothermal therapy. These findings provide significant insights into the development of drugs that facilitate minimally invasive cancer theranostics. The development of core-alkoxylated MPcs with substituents on the peripheral benzene ring is expected to simplify the fabrication of functionalized MPc thin films, thereby promoting the advancement of high-performance organic semiconductors.

List of Publication

Chapter 1 “MMP-2-Activatable Photoacoustic Tumor Imaging Probes Based on Al- and Si-Naphthalocyanines”

Koji Miki, Naoto Imaizumi, Kohei Nogita, Masahiro Oe, Huiying Mu, Wenting Huo, Hiroshi Harada, and Kouichi Ohe

Bioconjugate Chem. **2021**, 8, 1773-1781.

Chapter 1 is the unedited author's version of a submitted work that was subsequently accepted for publication in *Bioconjugate Chemistry*, copyright © American Chemical Society after review.

To access the final edited and published work, see the following website:

<https://doi.org/10.1021/acs.bioconjchem.1c00266>

Chapter 2 “Photoacoustic Signal Enhancement of Al- and Si-Phthalocyanines Caused by Photoinduced Cleavage of Water-Soluble Axial Ligand”

Kohei Nogita, Koji Miki, Naoto Imaizumi, Masahiro Oe, Huiying Mu, and Kouichi Ohe

J. Photochem. Photobiol. A-Chem. **2023**, 438, 114547.

Chapter 2 is the unedited author's version of a submitted work that was subsequently accepted for publication in *Journal of Photochemistry and Photobiology A: Chemistry*, copyright © Elsevier after review. To access the final edited and published work, see the following website:

To access the final edited and published work, see the following website:

<https://doi.org/10.1016/j.jphotochem.2023.114547>

Chapter 3 “A reductively convertible nickel phthalocyanine precursor as a biological thiol-responsive turn-on photoacoustic contrast agent”

Kohei Nogita, Takaya Sugahara, Koji Miki, Huiying Mu, Minoru Kobayashi, Hiroshi Harada, and Kouichi Ohe

Chem. Commun. **2024**, 60, 1472–1475.

Chapter 3 is the unedited author's version of a submitted work that was subsequently accepted for publication in *Chemical Communication*, copyright © Royal Society of Chemistry after review.

To access the final edited and published work, see the following website:

<https://doi.org/10.1039/D3CC05628G>

Chapter 4 A reductively Activatable Phototheranostic Agent Based on a Water-Soluble Nickel Naphthalocyanine Precursor

List of Publication

Kohei Nogita, Yusuke Kataoka, Takaya Sugahara, Koji Miki, Huiying Mu, and Kouichi Ohe

In preparation.

Chapter 5 Synthesis and reductive aromatization of perfluoro nickel phthalocyanine precursor having two methoxy groups on adjacent pyrrole rings

Kohei Nogita, Koji Miki, Huiying Mu, and Kouichi Ohe

Asian J. Org. Chem. **2025**, accepted.

Other Publications Not Included in This Thesis

1. pH-Activatable Cyanine Dyes for Selective Tumor Imaging Using Near-infrared Fluorescence and Photoacoustic Modalities
Huiying Mu, Koji Miki, Hiroshi Harada, Kouki Tanaka, Kohei Nogita, and Kouichi Ohe
ACS Sens. **2021**, 6, 123-129.
<https://doi.org/10.1021/acssensors.0c01926>
2. Aluminum naphthalocyanine conjugate as an MMP-2-activatable photoacoustic probe for in vivo tumor imaging
Koji Miki, Naoto Imaizumi, Kohei Nogita, Masahiro Oe, Huiying Mu, Wenting Huo, Hiroshi Harada, and Kouichi Ohe
Methods Enzymol. **2021**, 657, 89-109.
<https://doi.org/10.1016/bs.mie.2021.07.001>
3. Amphiphilic γ -cyclodextrin-fullerene complexes with photodynamic activity
Koji Miki, Zi Dan Zhang, Kaho Kaneko, Yui Kakiuchi, Kentaro Kojima, Akane Enomoto, Masahiro Oe, Kohei Nogita, Yasujiro Murata, Hiroshi Harada, and Kouichi Ohe
Mater. Adv. **2022**, 3, 312-317.
<https://doi.org/10.1039/D1MA00743B>
4. An activator-induced quencher-detachment-based turn-on probe with a cationic substrate moiety for acetylcholinesterase
Masahiro Oe, Koji Miki, Akito Masuda, Kohei Nogita, and Kouichi Ohe
Chem. Commun. **2022**, 58, 1510-1513.
<https://doi.org/10.1039/D1CC05132F>
5. AIE-ESIPT Photoluminescent Probe Based on 3-(3-Hydroxypyridin-2-yl)isoquinolin-4-ol for the Detection of Intracellular Hydrogen Peroxide
Wenting Huo, Kohei Takayama, Koji Miki, Kohei Nogita, Shuai Shao, Ayako Suzuki, Takashi Morimoto, Huiying Mu, and Kouichi Ohe
Chem. Eur. J. **2024**, 30, e202401451.
<https://doi.org/10.1002/chem.202401451>

Acknowledgement

The studies described in this thesis have been carried out under the direction of Professor Kouichi Ohe from April 2018 to May 2025 at Department of Energy and Hydrocarbon Chemistry, Graduate School of Engineering, Kyoto University.

First and foremost, the author wishes to express sincere gratitude to the university researchers whose guidance and support made this doctoral study possible. The knowledge and insights gained through discussions with experienced researchers have been invaluable in shaping scientific perspectives and refining problem-solving approaches. The author extends special appreciation to the professors for fostering scientific thinking, providing continuous encouragement, and offering constructive feedback that strengthened this research. Sincere gratitude is also extended to research collaborators, whose valuable insights broadened perspectives and refined experimental methodologies. Furthermore, the author is grateful for the financial support from the Japan Science and Technology Agency (JST) and the Research Institute of Synthetic Organic Chemistry which made this research possible. Additionally, the author thanks the administrative staff for their indispensable assistance in ensuring a smooth and efficient research process.

The author is especially indebted to Professor Ohe, Associate Professor Koji Miki, and Assistant Professor Huiying Mu for their unwavering support and guidance. Professor Ohe provided insightful advice on academic writing, refining the author's ability to convey complex ideas with clarity and precision. His keen observations and constructive feedback significantly improved the structure and coherence of this dissertation. The knowledge and writing skills developed under his mentorship will be invaluable for the author's future work. Associate Professor Miki offered guidance on navigating life in the laboratory, providing practical advice on conducting research efficiently while maintaining a balanced approach. His insights into research management and collaboration within the group were instrumental in shaping the author's ability to work effectively in a research environment. His guidance extended beyond research techniques, fostering a professional mindset crucial for a successful career in science. Assistant Professor Mu offered crucial technical guidance, particularly in cell experiments, enabling mastery of key experimental techniques. Her expertise and patience

in teaching complex procedures helped the author gain confidence in handling challenging experimental setups.

The author is also sincerely grateful to all laboratory members for their warm support and collaboration. Countless formal and informal discussions enhanced critical thinking and fostered intellectual growth. The welcoming and collaborative environment encouraged the open exchange of ideas, constructive feedback, and continuous learning. Laboratory seminars provided invaluable opportunities to refine research ideas and receive feedback, fostering intellectual growth and camaraderie. Beyond research, casual conversations and shared moments of laughter enriched the lab environment, reinforcing professional relationships and honing essential communication and teamwork skills. The insights and experiences from these interactions will continue to guide the author in future research and professional endeavors.

In particular, heartfelt appreciation is extended to the members of the research team. Working alongside such talented and highly motivated colleagues has been an enriching experience. Special thanks are due to Mr. Naoto Imaizumi for his insightful advice and unwavering support. His expertise and guidance provided valuable perspectives on research challenges, while his encouragement helped foster the author's growth as a researcher. Appreciation is also extended to Mr. Takaya Sugahara, Mr. Yusuke Kataoka, and Mr. Taichi Sawamura, whose curiosity and enthusiasm greatly contributed to the research. Their dedication and fresh perspectives highlighted the importance of cultivating a collaborative and supportive research environment.

Finally, the author expresses heartfelt gratitude to everyone who has contributed to this academic journey. The knowledge, experiences, and relationships cultivated throughout this research will remain invaluable in future endeavors. The shared dedication of all involved has made this journey meaningful and fulfilling. Lastly, the author is deeply grateful to his parents for their unwavering support and encouragement throughout this endeavor.

May 2025

Kohei Nogita

