New contrast agents for photoacoustic imaging and theranostics: Recent 5-year overview on phthalocyanine/naphthalocyanine-based nanoparticles

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ABSTRACT
The phthalocyanine (Pc) and naphthalocyanine (Nc) nanoagents have drawn much attention as contrast agents for photoacoustic (PA) imaging due to their large extinction coefficients and long absorption wavelengths in the near-infrared region. Many investigations have been conducted to enhance Pc/Ncs’ photophysical properties and address their poor solubility in an aqueous solution. Many diverse strategies have been adopted, including centric metal chelation, structure modification, and peripheral substitution. This review highlights recent advances on Pc/Nc-based PA agents and their extended use for multiplexed biomedical imaging, multimodal diagnostic imaging, and image-guided phototherapy.

I. INTRODUCTION
Photoacoustic (PA) imaging is a hybrid imaging modality based on the PA effect that exploits both rich optical contrast and high ultrasonic spatial resolution in deep tissues.1,11 It visualizes optical chromophores in biological tissues where ultrasound (US) signals are generated from light absorption and subsequent thermoelastic expansions. In the last decades, PA imaging has been studied extensively, with many advantages for biomedical imaging. First, unlike traditional optical imaging techniques where imaging depth is limited to few millimeters due to round trip optical attenuation in biological tissues, PA imaging can reach up to a few centimeters of depth in biological tissues, thanks to one-way photon propagation and acoustic detection which suffers far much less attenuation and scattering than optical detection.5-9 Second, the spatial resolution and achievable imaging depth are scalable based on the US detector frequency, thus covering many applications ranging from organelles and cells to small animals and humans.10-16 Third, PA imaging is inherently compatible with many complementary imaging modalities, e.g., conventional US imaging and optical imaging modalities, and thus are highly suitable for multiplexed and multimodal imaging.17-22

The PA imaging exploits both endogenous and exogenous contrast agents to image biological tissues. The endogenous agents include DNA/RNA,23,24 melanin,25-28 oxygenated-/deoxygenated-hemoglobin,29-31 lipid,32-34 and water.35 These agents provide functional, metabolic, and histopathologic information such as blood oxygenation, oxygen metabolism, and vascularity.36,37 A large variety of exogenous contrast agents have been investigated, including inorganic nanomaterials (gold nanostructures, carbon nanotubes, etc.), organic dyes (porphyrine-/cyanine-based dyes), and fluorescent proteins [green/red fluorescent protein (GFP/RFP)].38-42 Gold nanomaterials are one of the most widely investigated PA agents thanks to their surface plasmon resonance properties and biocompatibilities. The plasmonic peaks can be tuned from the visible to the near-infrared (NIR) range depending on their shapes and provide optical contrast,43 but still has limitations for clinical translation due to photoinstability.44 Fluorescent proteins have drawn much attention in live-cell optical
imaging. GFP, however, has a lower absorption peak wavelength, high fluorescent quantum yield (i.e., low heat conversion efficiency), and shallow imaging depth for in vivo applications.

The phthalocyanine (Pc) and naphthalocyanine (Nc) are organic dyes that have been explored widely for optical imaging and phototherapy due to their strong extinction coefficients and long absorption wavelengths in the NIR region. This region is also known as an optical/biological/therapeutic window where light can penetrate deeply in tissues with low phototoxicity. The Pc/Ncs also have attractive traits of good photostability and tunable optical properties through facile chemical modifications. Low aqueous solubility is the main challenge for Pc/Ncs as a contrast agent, and many attempts have been successfully made to solubilize Pc/Ncs using biocompatible and biodegradable coating (e.g., surfactant modification, micelle/liposome carrier). Several Pc/Nc derivatives, including aluminum Pc, silicon Pc, and zinc Pc, have either already been through the FDA approval process or are under clinical trials.

In this review, we summarize the recent advances in Pc/Nc-based nanomaterials as PA imaging agents and synergistic applications for using multimodal imaging and theranostics. First, we present design strategies and processes to enhance the functionality of Pc/Ncs, including photophysical properties and targeting ability. We then focus on their biomedical applications, including contrast-enhanced PA imaging, biosensing of tumor and other biomolecules, and cancer theranostics. Finally, we will discuss future directions for Pc/Nc-based PA agents.

II. FUNCTIONALIZING PCS AND NCS AS A PHOTOACOUSTIC CONTRAST AGENT

A. Photophysical property

The two important photophysical properties of nanoparticle for considering them as potential PA contrast agent are: (1) does the peak absorption wavelength ensures sufficient penetrating depth and (2) how the absorbed energy efficiently transfers to thermal expansion, which is directly proportional to photoacoustic signal intensity. These aspects connote two strategies for engineering the nanoparticles; how to tune the wavelength and how to increase the given particle’s absorption yield.

1. Absorption spectrum shift

For most biomedical imaging techniques, the allowed penetration depth is under the boundary of the optical ballistic region (< 1 mm). However, the PA imaging crosses over the boundary because the PA technique involves a one-way optical propagation and, independent of scattering, it responds with an acoustic signal. For reaching the maximum penetration depth in tissue, the wavelength of the optical source is generally selected in the specific range of NIR zone (650–1350 nm), named as “NIR window.” In such wavelength range, the photon gets absorbed less by blood than shorter wavelengths and bypasses the absorption by water, compared to longer wavelengths. In addition, determining the absorption wavelength carefully can lead to accurate multiplex imaging or combinatorial therapy such as photodynamic therapy (PDT) or photothermal therapy (PTT).

Even though the free-base Pc and Nc have their absorption peak around 670 nm and 750 nm in DMF solution, respectively, centric ion chelation, at the center of the Pc/Ncs molecule, enables the tuning of the optical absorption band of the agent through the metal type. Depending on the metal, Pc/Nc's absorption peak can be varied through chelation in the range of 650–950 nm. In addition to wavelength tuning, chelation with appropriate diamagnetic metallic ions increases the absorption yield of Pc/Ncs and stabilizes the charge balance, and, consequently, lengthens the molecules’ lifetime.

Currently, metallic ions such as zinc (Zn), copper (Cu), iron (Fe), and indium (In) are widely favored in research studies with a corresponding peak absorption of 700, 850, 680, and 840 nm, respectively.

Duffy et al. showed through a comprehensive study that Nc particle’s central ion change results in maximum PA intensity wavelength shift at 850, 848, 774, and 808 nm, for copper, nickel, silicon, and vanadium ion, respectively. The study further identified that central ion change also affected singlet oxygen photoproduction and fluorescence pattern.

In addition to metallic ions, variation with nonmetallic ion chelation can also be used for engineering the nanoparticle’s optical response. Chelation with semiconducting elements, such as silicon (Si), exhibits good photothermal conversion efficiency and is capable of both PDT and PTT effect, which enables the use of such material as single-agent theranostic nanoprobes. Choice of peak absorption to
NIR-II window (1000–1700 nm) instead of NIR-I window is advantageous from both aspects: penetration depth and maximum permissible exposure (MPE). Hence, contrast agents with its peak absorption at NIR-II window enlarges the scope of imaging target to deeper organs, which is an important factor to translate from preclinical studies to future clinical application toward human. A number of studies already present the superiority of NIR-II PAI with multi-centimeter penetration depth compared to NIR-I PAI. Together with chicken breast phantom, rodent mammary tumor models, lymphatic mapping, and ultimately with 2.6–5.1-cm-thick compressed human breast, transcendent depth penetrating ability of NIR-II PAI was well validated as well as giving proof to its applicability. Having deep penetration depth as its major strength, a number of precedent studies eagerly expect its potential to extend barrier of PAI application toward gastrointestinal tract, lymphatic system, and excretory system.\(^7\)

The electron-deficient group 15 elements (P, As, and Sb) and the electron-rich group 16 elements (S, Se, and Te) can also be used as central substituents, which expands the applicable optical band beyond 1000 nm.\(^7\) For example, Zhou et al. observed intense absorption at 1000 nm with phosphorous Pc to target deeper imaging depth with PACT.\(^7\) Without using a central inorganic semiconducting element or polymer, Pan et al. synthesized NIR-II (1064 nm) agent by arranging four ZnPc molecules into cruciform pentad.\(^6\)

The representative publications for absorption spectrum shift are summarized in Table I.

### 2. Increased absorption yield

Due to the hydrophobic nature, arising from their \(\pi\)-conjugated planar arrangement, Pc and Nc exhibit high self-aggregation in aqueous solution. During the supramolecular self-assembly phase, collective aggregation induces complete FL self-quenching. It blocks radical oxygen species (ROS) generation pathways, leading to a favorable increase in nonradiative thermal relaxation in its excited phase [Fig. 1(b)].\(^7\)\(^9\) Thus, supramolecular self-assembly can effectively increase PA signal yield compared to its monomolecular application. Liu et al. introduced a polymeric nanosystem for near-infrared multispectral PA imaging, which augmented the self-assembly by using hydrophobized ZnPc and oxazoline block copolymer complex (H-PcZn), to efficiently encapsulate the micelles by self-assembly.\(^8\)\(^1\)\(^2\)\(^7\)\(^9\) Li et al. reported a molecular recognition-based supramolecular approach between Pcs\(_4\) and Pcs\(_6\), the two water-soluble Pc derivatives, and observed completely quenched fluorescence (FL) and reduced singlet oxygen generation.\(^2\)

The representative publications for increasing absorption yield are summarized in Table II.

### B. Passive delivery

1. **Enhanced solubility**

   Low water solubility is a frequently mentioned demerit for Pc and Nc nanoparticles for using them as a PA contrast agent. The administered pharmaceutics dissolve in body fluids, e.g., blood, and get delivered to the destination tissue through the circulatory system’s capillary network. Therefore, for diagnostic/therapeutic application, low water solubility is a big hurdle for tissue permeability and poses threats to stricture or stenosis. For enhancing the solubility, hydrophobic molecules are mostly coated with hydrophilic functional substances or reprocessed into amphiphilic nanocarriers with biologically suitable surfactants [Fig. 2(a)].\(^2\)\(^5\)\(^6\)

   When the molecular complex unit is composed of the hydrophobic part and hydrophilic part, it forms a spherical supramolecular assembly called micelle when dissolved into water. The polarity of the solvent and intermolecular interaction forces grants “packing behavior,” arranging hydrophilic parts of the monomer to face the exterior surface of the assembly, and hydrophobic parts cluster toward the center of the assembly.\(^6\)\(^5\)\(^7\)\(^6\) The aggregates form a colloidal suspension, which leads to a better solubility of the assembly. Pluronic F127, also known as poloxamer 407, is a hydrophilic nonionic block copolymer surfactant. In pharmaceutics, pluronic copolymer micelles are commonly used as a passive drug container to improve the delivery of the hydrophobic drug.\(^7\)\(^6\) To enhance the stability of the resulting multifunctional nanodroplets, Choi et al. conjugated their Nc particles with...
the in particular, developed form of Pluronic F127 and crosslinked with the primary amine of six-arm PEG. By similar means, to create a lipophilic or amphipathic PC-carrying micelle, Rizvi et al. applied tomamine, another type of commercial surfactant. They identified an induced Pc distortion, which enabled photothermal processes for PA imaging and uncomplicated surface-enhanced resonance Raman spectroscopy. To accommodate higher loading of Nc/Pc, Zhang et al. introduced novel low-temperature processing called "surfactant-stripped frozen micelle." Through the removal of free or unbound solubilizing surfactant, this processing increased the drug to surfactant ratio by 2–3 orders of magnitude higher than the conventional method. Zhang et al. named their surfactant-stripped high loading Nc micelles as "nanonaPS" and introduced its use for multifunctional cancer theranostics.

Phospholipids are the representative nature-derived amphiphilic surfactant, having hydrophilic phosphate head and two hydrophobic long fatty acid tails. The Pc/Nc enclosed with a monolayer of phospholipid forms phospholipid micelle, and the assembly is reinforced with PEG chains. Wei et al. reported the effective diagnostic nano agents for PA imaging, PTT, and PDT combination therapy. The liposome is another variation of a phospholipid-complex vesicle. Contrary to phospholipid micelles, liposomes consist of a lipid bilayer membrane, which can be loaded with hydrophilic nutrients or pharmaceutical drugs in its most inner void. Using such an approach, Ma et al. complexed ZnPc with soybean phospholipid using hydrogen bond and intermolecular interaction. The self-assembled liposomal nanoparticles were loaded with doxorubicin to achieve additional chemotherapy.

Conjugating short hydrophilic peptide onto Pc/Nc particle also increases the water solubility by inducing the supramolecular self-assembly. Furthermore, short peptides may increase cellular uptake during their interaction with cell membranes due to their excellent biocompatibility. Li et al. suggested spatiotemporally coupled tumor phototheranostic nanoparticles, composed of easy Pc-peptide conjugation and switchable photoactivity, triggered by cellular membrane interaction.

### TABLE II. Summary of the representative publications for increasing absorption yield of phthalocyanine/naphthalocyanine nanoparticles. \( \lambda_{\text{max}} \), maximum absorption wavelength; \( \epsilon \), molar extinction coefficient; O.D., optical density; \( \zeta \), zeta potential; FL, fluorescence.

| Group          | Nanomaterial                      | \( \lambda_{\text{max}} \) (nm) | Monomer/assembly size (nm) | \( \epsilon \) [O.D.] | Major features                                                                 |
|----------------|-----------------------------------|---------------------------------|---------------------------|----------------------|--------------------------------------------------------------------------------|
| Liu et al.81   | Hydrophobized zinc Pc complex      | 680                             | –/20–100                  | 8.9 (P-NP);          | • Hydrophobizing characterization with oxazoline block copolymer,              |
|                |                                   |                                 |                           | 7.4 (N-NP)           | • \( \zeta = P-Pc: +29 \text{ mV} \); N-Pc: –43.8 \text{ mV}                  |
| Li et al.54    | PcS4-PcN4 complex                  | 694                             | 0.5–3/30–100              | ··                   | • Total FL self-quenching identified at 610 nm                                |

**FIG. 2.** Functionalizing phthalocyanine/naphthalocyanine nanoparticles for passive and active delivery.
The representative publications for nanocarriers enhancing aqueous solubility are summarized in Table III.

2. Prolonged circulation

The capillary network delivers the injected nanoparticles into cells via the reticuloendothelial system using the principle of diffusion. The difference between hydraulic pressure inside the capillary wall and intracellular pressure of the recipient cell transfers oxygen and nutrients essential for metabolic events of the cell. The enhanced permeability and retention effect (EPR) is a generally accepted phenomenon in vessels surrounding rapidly growing tumor cells. These tumor cells have greater cellular uptake through large fenestrations between endothelial cells and allow nanoparticles to reach the matrix easily than normal cells. Thus, the prolonged circulation of the nanoparticle increases the possibility of them reaching the peritumor site, and is necessary for efficient passive delivery of the nanoparticle.

Many methods such as cross-linking with polyethylene glycol (PEG) classes and variants, or PEGylation, are adopted to extend the circulation time of the injected nanoparticles by growing the volume (PEG) classes and variants, or PEGylation, are adopted to extend the necessary for efficient passive delivery of the nanoparticle.

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Table III. Summary of the representative publications for nanocarriers enhancing aqueous solubility of phthalocyanine/naphthalocyanine nanoparticles. α, mean diameter; PEG, polyethylene glycol; ω, maximum absorption wavelength; ε, molar extinction coefficient; Φ<sub>ε</sub>, fluorescence quantum yield; Φ<sub>g</sub>, singlet oxygen generation yield; Φ<sub>ν</sub>, singlet oxygen generation quantum yield; SERS, Surface-enhanced Raman spectroscopy; η, photothermal conversion coefficient; PDT, photodynamic therapy; PTT, photothermal therapy.

| Group          | Nanomaterial                  | Carrier type                  | Excipient type                  | d (nm)   | Major features                                                                 |
|----------------|-------------------------------|------------------------------|--------------------------------|----------|--------------------------------------------------------------------------------|
| Choi et al.    | Nc/PFH@PCPN                   | Block copolymer micelle      | Six-arm PEG-amino crosslinked   | 500–2500 | λ<sub>max</sub> = 850 nm, 89.4 % 14-day sustainability (37 °C)                   |
| Rizvi et al.   | ZnPc (tomamine)<sub>n</sub>  | Block copolymer micelle      | Tomamine                       | 89 ± 8;  | λ<sub>max</sub> [nm] = 774; 810; 805; 820                                    |
| Zhang et al.   | Nanonap                       | Block copolymer micelle      | Pluronic F127                  | 29.5     | Φ<sub>ε</sub> < 1, Φ<sub>g</sub> < 0.01, strong SERS signal                 |
| Wei et al.     | SiNCOH-DSPE-PEG(NH<sub>2</sub>) | phospho-lipid micelle        | PEG-conjugated phospholipid    | 160      | η = 59.8 %, 7-day stability                                                    |
| Ma et al.      | ZnPc-SPC                      | Liposome                     | ZnPc-soybean phospholipid complex | 296.9 ± 6.553 | Drug loading (doxorubicin), Folate receptor(FRα), max. 15 day stability |
| Li et al.      | Pc-petide conjugate self-assembly | Short Petide micelle        | L-phenylalanine-L-phenylalanine | 54.8 ± 17.6 | Theranostic application (PDT/PTT), pH dependent nanoparticle size |

C. Active delivery

In addition to the passive delivery, engineering the nanoparticles with targeting or stimulus-triggered release function helps to achieve optimal therapeutic and diagnostic effects [Fig. 2(b)]. The presence of closed d-shell diamagnetic ions (e.g., Zn<sup>2+</sup>, Al<sup>3+</sup>, Ga<sup>3+</sup>) strengthens Pc/Nc complexes of both high triplet yields and long lifetimes and consequently forms hydroperoxide products (type I), or yield singlet oxygen generation converted from ground-state triplet oxygen experimental results showed 4 times longer half-life and 10 times greater area under the curve. Albumin binding is also a well-preferred method to regulate the circulation time. Human serum albumin is the most abundant protein in human blood plasma; it constitutes about 50% of serum protein and is produced in the liver. Against the hydraulic pressure that diffuses the solvents to peripheral cells during capillary metabolism, albumin regulates the oncotic pressure of blood by trapping water, cations (e.g., Ca<sup>2+</sup>, Na<sup>+</sup>, K<sup>+</sup>), fatty acids, hormones, bilirubin, tyroxine, thus preventing discharge of pharmaceuticals from microvessels. The passive delivery rate of drugs is often enhanced by binding with albumin in the pharmaceutical field. Using inherent biocompatibility and reduced immunogenicity, Jia et al. fabricated a human serum albumin-iron(II) Pc nanoparticle complex as a phototheranostic agent and observed the monotonic increase in PA signal intensity at < 15 hours post-injection.

The representative publications for absorption spectrum shift are summarized in Table IV.

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**APL Bioeng.** 5, 031510 (2021); doi: 10.1063/5.0047660

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Both oxidative reactions degrade cellular components, and the damage level highly depends on the local concentration of the dye and oxygen availability. Excessive dosage into the living matter may adversely occur damage to the nontarget systemic normal cells together with targeting tumor cells and lead to pathologic response. Therefore, selective Pc/Nc delivery restricted only to the targeting tumor cells becomes important to minimize the dosage needed for the treatment. The designed nanocarriers prevent the excessive release of dosage into the bloodstream and normal tissues and therefore keep up the delivery to desired tumor tissue, resulting in high drug concentration within the tumor. From this section, we explain both concepts into the boundary of "active delivery," as the technique selectively marks the target tumor cells depending on nanoparticle-cell interaction under specific target cell conditions. The representative publications for absorption spectrum shift are summarized in Table V.

### 1. Receptor-mediated endocytosis

In active targeting, ligands bonded to the nanoparticle promote receptor-mediated endocytosis specifically to the receptor-acquainted cells. The nanoparticles engineered with active delivery are often multi-functionalized not only for the diagnostic imaging purpose but also for the therapeutic purpose. The binding biomolecule, such as biotin (or vitamin B7) or folic acid (or vitamin H), which combines overexpressed receptors on the cell membrane, is considered a promising biomarker for substantially reducing the stress effects of aggressive pharmacetics.

Studies have revealed the tendency of biotin receptors to overexpress in tumors than in normal tissues. Besides, the structure of biotin is simple and can be readily functionalized. Thus, it has the potential to be an active tumor-targeting ligand. Wu et al. produced a tumor-targeting capability of fabricated ZnPc nanodots by conjugating biotin onto the surface. Also, Ding et al. equipped tumor-specific delivering property by targeting ligand by coupling ZnPc nanodots with folic acid-conjugated PEG chains.

To overcome the intrinsic disadvantages of traditional biological molecules, such as a limited number of receptors and tumor heterogeneity, a two-step chemical tumor-targeting concept, composed of metabolic glycoeengineering and click chemistry, has been actively applied from the time it was first proposed by Koo et al. The addition of artificial chemical receptor increases the number of binding nanocarriers significantly on the surface of most tumor cells, regardless of tumor type. Such a strategy was first presented by Du et al. in the photothermal/photoacoustic synergistic therapy application.

### 2. Triggered release

The difference between receptor-mediated endocytosis and triggered release is that the latter involves a physical or chemical breakage of nanocarrier structure and is triggered by a specific cell condition. Hydrogen peroxide or H$_2$O$_2$-triggered drug release is designed for exclusive oxidative microenvironment within cancer cells. The ROS responsive nanocarrier rapidly disassembles in target cells and promotes rapid intracellular drug release. The four self-immolative PEG arms consisting of the activatable probe, developed by Xie et al., are specifically cleaved with the ROS presence. The increased hydrophobicity promotes the supramolecular regrowth around the tumor cells and gets spatiotemporally highlighted in the photoacoustic image.

Joining selective recognition of protein as a biomarker and reverse application of self-quenching monomer could lead to the novel design of switchable photoactivity. Li et al. explored biotin-receptor-responsive photoswitchable property from biotin-conjugated Pc-based nanodots. The presence of biotin-receptor-like protein triggers the nanoparticle’s partial disassembly, resulting in the generation of fluorescence and reactive oxygen species instead of photothermal conversion, inducing PA signal generation and PTT effect. The nanoparticle delivery showed high selectivity toward biotin receptor-positive cancer cells on xenograft models (A549), compared to the receptor-negative cancer cells (WI38-VA13). The switchable photoactivity from short peptide micelle was also reported in aforementioned Li et al.’s work.

### III. BIOMEDICAL APPLICATIONS OF Pc/Ncs

#### A. Biomedical Imaging

##### 1. Contrast-enhanced PA imaging

Using endogenous biological chromophores, such as oxy-/deoxy-hemoglobin and melanin, the PA imaging achieves superb optical imaging contrast, high spatial resolution, and a larger depth of penetration compared to pure optical imaging techniques. However, due to
strong light absorption and scattering in tissue, light fluence and signal-to-noise ratio (SNR) decrease exponentially with increased imaging depth. In addition, some tissues or organs that need to be imaged, such as tumor and lymphatic system, do not possess intrinsic optical contrast. Many Pc/Nc-based materials have been developed as PA imaging contrast agents to visualize such biological targets and improve imaging sensitivity, specificity, and penetration depth.78,99,117–119

For in vivo PA tumor imaging, Attia et al. investigated three tetrasulfonate phthalocyanine formulations as contrast agents: phthalocyanine tetrasulfonic acid (PcS4), Zn(II) phthalocyanine tetrasulfonic acid (ZnPcS4), and Al(III) phthalocyanine chloride tetrasulfonic acid (AlPcS4).118 They observed high PA imaging contrast in the tumor accumulation of the developed nanoparticles in the tissue was photoacoustically monitored to confirm the optimal time point of HIFU treatment.70 The intratherapy monitoring was performed by US imaging and/or US detection, the PA imaging can be easily integrated with the conventional US imaging, and achieved precise phototheranostics.70 The switchable photoactivity was observed when the NPs were disassembled by the cell membrane-interaction, which permitted spatiotemporally optimal therapeutic windows via adaptive PA/FL imaging, and achieved precise phototheranostics.

With a nature of PA imaging of combining photon excitation and US detection, the PA imaging can be easily integrated with the conventional US imaging. Choi et al. designed surface crosslinked nanodroplets encapsulating Nc and perfluorohexane for PA/US dual-modal imaging-guided high-intensity focused ultrasound (HIFU) therapy.19 The accumulation of the developed nanoparticles in the tissue was photoacoustically monitored to confirm the optimal time point of HIFU treatment. The intherrapy monitoring was performed by US imaging visualizing echogenic microbubbles generated under HIFU exposure.

2. Multimodal imaging agents

Thanks to the inherent capability of Pc/Nc for high NIR absorption and centric metal ion chelation, Pc/Ncs have great potential to be a contrast agent for complementary multimodal imaging.

The FL is one of the most widely used biological imaging modalities in visualizing living organelles. When a substance absorbs light, the excess energy dissipates through the emission of light, which is FL and/or heat, contributing to PA wave generation. Though FL and PA have competing yield, both are based on high absorbance of light, and thus, many contrast agents have been reported as a bimodal FL and PA imaging agent or FL-PA switchable imaging agent.93–119

| Group | Material name | Delivery type | Mechanism | Major features |
|-------|---------------|---------------|-----------|---------------|
| Wu et al.110 | Biotin-conjugated ZnPc nanodots | Receptor-mediated endocytosis | Biotin receptor | ● $\eta = 45.7\%$
 ● $d = 80\, \text{nm}$
 ● Max. PA signal in +8 hr post-injection
 ● Increased photoactivity with receptor-rich HeLa cell |
| Ding et al.57 | PEG-folate/ZnPc nanodots | Receptor-mediated endocytosis | Folic acid receptor | ● $d = 20\, \text{nm}$
 ● Effectively internalized to folate receptor-overexpressed CNE-2 cell |
| Du et al.113 | DBCO-ZnPc-LP | Receptor-mediated endocytosis | Bioorthogonal Metabolic Glycoengineering-Activated Tumor Targeting | ● $d = 78.82\, \text{nm}$
 ● Complete self-quenching
 ● $\eta = \sim 44.39\%$
| Xie et al.115 | PCBP | Triggered release | H2O2-triggered self-immolative PEG arms | ● $d = \max. \sim 350\, \text{nm}$
 ● $\sim 1.4$-fold PA amplitude increase |
| Li et al.116 | NanoPcTB | Triggered release | Photoswitchable protein disassembly | ● Biotin receptor-mediated switchable property (FL/PDT)
 ● L-phenylalanine-L-phenylalanine (FF)—mediated switchable photoactivity (FL/PDT) |
| Li et al.93 | Pc-peptide conjugate self-assembly | Photoswitchable protein disassembly | | |

Summary of the representative publications for active delivery of phthalocyanine/naphthalocyanine nanoparticles. $\eta$, photothermal conversion coefficient; $d$, mean diameter; PA, photoacoustic; FL, fluorescence; PDT, photodynamic therapy.
Other imaging modalities have also been explored using \( \text{Pc}\)/\( \text{Nc}\)-based PA agents. Wang et al. fabricated polydopamine (PDA)/aluminum \( \text{Pc} \) (\( \text{AlPc} \))/bovine serum albumin (BSA) coated magnetic Prussian blue nanoparticles for triple-modal FL/MR/PA cancer imaging [Fig. 4(a)]. Lu et al. compared and screened a series of porphyrin \( \text{Pc} \) and \( \text{Nc} \) dyes, encapsulated in the interior of nanoparticles through the flash nanoprecipitation (FNP) process, and revealed strong PA responses at high loadings of dyes and combined PA and FL responses at lower loadings of dyes. The incorporability of nanoparticles with MR/PET/SPEC imaging agents and therapeutic drugs was also demonstrated. Zhang et al. developed nanoformulated Ncs for \text{in vivo} \) dual-modal PA/PET imaging of lymph nodes and tumors [Fig. 4(b)].

The representative publications for biomedical imaging application are summarized in Table VI.

**B. Probing biological processes**

**1. Tumor detection**

Active targeting for anticancer is essential for delivering theragnostic agents to the region of interest while avoiding normal tissues. Several \( \text{Pc}/\text{Nc} \) derivatives have been introduced as an active cancer diagnostic probe or to deconvolute passive vs active targeting effects. As an actively targeted cancer theragnostic agent, Ma et al. presented a zinc phthalocyanine soybean phospholipid (ZnPc-SPC), which showed high sensitivity to FR\( \alpha \) over-expressed tumor cells. Lu et al. developed multiplexed PA imaging agents of cRGD-modified nanoparticles, which can bind to \( \alpha_\text{v} \beta_\text{3} \) integrin expressed in Lewis lung carcinoma tumors, and unmodified nanoparticles based on \( \text{Pc}/\text{Nc} \).
Nc macrocycles, which can internally normalize active ligand targeting effects from passive targeting such as EPR effect.\textsuperscript{123} PA imaging of the two spectrally separable nanoparticles showed the distinct distribution of agents in the tumor site [Fig. 5(a)]. Du et al. investigated tumor-targeting Pc compounded with dual-targeting effect via metabolic glycoengineering and click chemistry.\textsuperscript{113} The PA signal in the tumor region was substantially higher in the mice group injected with the proposed dual-targeting nanoagent intravenously than in the control group with a passive-targeting agent.

2. Biomolecule detection

Tracking biomolecules in the body is critically important in many fields ranging from basic science research to medical health care. The tracking helps significantly in understanding biological/physiological processes in living organisms, diagnosing diseases, or monitoring pre-/in-/post-treatment responses. Recently, Morales et al. developed a DNA-based PA nanosensor to detect interferon-gamma (IFN\textgamma) that plays a critical role in activating immunity against...
In the presence of IFNγ, the DNA structure’s arms were folded to induce stacking of Pc dye, achieving a 55% increase in PA signal [Fig. 5(c)]. Toriumi et al. reported tautomeric benziphthalocyanines, by introducing free hydroxyl group with esterase- or H2O2-labile markers, for activatable PA detection of esterase or H2O2. Xie et al. designed Pc-based semiconducting macromolecule, which first underwent ROS-induced cleavage process of PEG and then subsequently the residual Pcs self-assembled into large nanoparticles, showing enhanced PA signal [Fig. 5(b)] in the presence of ROS.

The representative publications for probing biological processes with Pc/Ncs are summarized in Table VII.

### C. Therapeutic agents

#### 1. Photothermal therapy

Since PA imaging is based on high optical absorption and local heat conversion, many Pc/Ncs investigated for PA imaging agents inherently have great potential for PTT, which is based on local heat generation at a specific excitation band. The Pc/Ncs, mainly due to strong absorbance at long wavelengths, possess attractive traits of theranostics such as deeper penetrability, lower photon energy, and much safer to adjacent normal cells/tissues. In 2020, Li et al. reported a nanostructured theranostic agent for both PA imaging and PTT through supramolecular self-assembly of two water-soluble Pc derivatives. The Pc assemblies showed completely quenched FL and strong PA/photothermal responses, resulting in high contrast PA imaging of cancer tumors and efficient anticancer PTT in vivo [Fig. 6(a)]. To further exploit the advantages of maximum permissible exposure (MPE) of laser and deep penetration in tissues, a phototheranostic agent in the second near-infrared (NIR-II) window was proposed by Pan et al. The agent was developed based on an organic molecular material, cruciform Pc pentad. The in vitro and in vivo studies demonstrated strong PA signals and PTT effect at 1064 nm. Du et al. developed a tumor-targeting nanoagent, dibenzyl cyclooctyne zinc(II)-Pc lipid-poly(ethylene glycol) (DBCO-ZnPc-LP), for multifunctional phototheranostics of PTT and PA therapy, which is a recently emerged phototherapy to eradicate tumor cells using PA shockwave.

#### 2. Photodynamic therapy

The Pc/Ncs have been widely exploited as photosensitizers in PDT due to strong absorption at long wavelengths and easily tunable photochemical properties. Ding et al. synthesized zinc(II)-phthalocyanine (ZnPc) nanodot by cryodesiccation-driven crystallization for PA image-guided cancer PDT. In addition to the PDT capability, with cytotoxic singlet oxygen generation, the nanodots showed excellent water-soluble and stealth properties with the surface modified using...
(a) Multiplexed in vivo tumor PA imaging. Representative 2D slice images exhibiting US (first row), cRGD NP PA (second row), unmodified NP PA (third row), blood PA (fourth row), and overlay signals (fifth row), and example 3D reconstruction (sixth row) of mice tumors, 48 h after tail vein inoculation with 5% cRGD NPs + 0% cRGD NPs (left column) and 25% cRGD NPs + 0% cRGD NPs (right column), respectively. Reprinted with permission from Lu et al., ACS Biomater. Sci. Eng. 3(3), 443 (2017). Copyright 2017 American Chemical Society. (b) Representative PA MAP images of tumors for BSO-pretreated and untreated mice after systemic administration of PCBP (30 μg per mouse) through tail vein. Republished with permission from Xie et al., Adv. Mater. 29(44), 1703693 (2017). Permission conveyed through Copyright 2017 John Wiley and Sons Clearance Center, Inc. (C) (i) Schematic of the nanosensor 2-step binding mechanism between IFN-c and its receptors. (ii) Response of the IFN-c nanosensor (780 nm) to buffer and 10 μM IFN-c (169 μg/ml) in buffer. Error bars represent the standard deviation of three independent trials. Reprinted with permission from Morales et al., ACS Sens. 4(5), 1313 (2019). Copyright 2019 American Chemical Society. PA, photoacoustic; US, ultrasound.

| Group          | Agent                        | Targeting/Probing strategy                  | Application                                                                 |
|----------------|------------------------------|---------------------------------------------|-----------------------------------------------------------------------------|
| Ma et al.      | ZnPc-SPC                     | FRz receptor                                | • FRz over-expressed tumor cell detection (e.g. 4T1)                         |
|                |                              |                                             | • Multiphase phototheranostics (Phase I: PTT/PA → Phase II: PDT/FL)           |
|                |                              |                                             | • pH-sensitive drug release in tumor                                          |
|                |                              |                                             | • Multiplexed PA imaging                                                     |
|                |                              |                                             | • Lewis lung carcinoma tumor detection                                       |
| Lu et al.      | PEG-coated Pc/Nc with tert-  | xvβ3 integrin                               | • PA tumor imaging                                                          |
|                | butyl or butoxy functional   |                                             | • PA image-guided PTT/PAT                                                    |
|                | groups with/without cRGD   |                                             | • IFNγ detection                                                            |
|                | surface modification        |                                             |                                                                             |
| Du et al.      | DBCO-ZnPc-LP                 | Metabolic glycoengineering + click chemistry| • Activatable PA detection of esterase or H2O2                             |
|                |                              |                                             |                                                                             |
| Morales et al.| DNA-based nanostructure     | IFNγ receptors (IFNγ R1, IFNγ R2)          | • PA detection of ROS during drug treatment                                  |
|                | conjugated with Pc and IFNγ |                                             |                                                                             |
|                | receptors                    |                                             |                                                                             |
| Toriumi et al.| BPCs derivatives             | Free hydroxyl group with esterase/H2O2-     |                                                                             |
|                |                              | labile markers                              |                                                                             |
|                |                              |                                             |                                                                             |
| Xie et al.     | Macromolecular PCBP          | Four PEG arms with ROS-responsive          |                                                                             |
|                |                              | linker, inducing self-assembling upon        |                                                                             |
|                |                              | ROS-induced cleavage of PEG                |                                                                             |
PEG2000-folate and Pluronic F127. Li et al. developed nanostructured Pc assemblies as a PDT agent, which are activatable in biotin receptor-positive cancer cells. The Pc assemblies displayed synergistic phototheranostic capability with intrinsic PA and PTT properties and cancer-specific FL and PDT properties [Fig. 6(b)]. Two-photon excited PDT (TPE-PDT) was introduced by Mauriello-Jiménez et al. for precise cancer treatment. Similar to two-photon excitation microscopy, the TPE-PDT allows deep penetration with longer wavelengths and superb spatiotemporal resolution. Bridged silsesquioxane nanoparticles were designed from tetrasilylated porphyrin and Pc, showing excellent TPE-PDT performance and strong PA imaging contrast in vivo.

The representative publications for phototherapy with Pc/Nc nanoparticles are summarized in Table VIII.

IV. CONCLUSIONS AND OUTLOOK

In this review, we summarized recent advances in nanoformulated Pc/Nc agents for PA imaging, synergistic biomedical imaging, biochemical sensing, and phototherapy. Due to strong extinction coefficients at long wavelengths ($\lambda_{\text{max}} > 10^5$ M$^{-1}$ cm$^{-1}$, $\lambda_{\text{max}} > 650$ nm), Pc/Ncs inherently possess high contrast for PA imaging in deep tissues. Diverse metal chelation can also be performed to modulate their optical properties to further increase the extinction coefficients (1) for high-sensitive PA imaging, (2) to enable multiplexing imaging by
TABLE VIII. Summary of the representative publications for phototherapy with phthalocyanine/naphthalocyanine nanoparticles. PA, photoacoustic; PTT, photothermal therapy; FL, fluorescence; NIR, near infrared; PAT, photoacoustic therapy; PDT, photodynamic therapy.

| Group                  | Agent          | Phototherapy parameters | Application                                                   | Major features                                                                 |
|------------------------|-----------------|--------------------------|---------------------------------------------------------------|---------------------------------------------------------------------------------|
| Li et al. 54           | PcS4-PcN4       | 660 nm, 2 W·cm⁻², 8 min  | PA image-guided PTT                                           | • Completely quenched FL, leading to strong PA/PTT responses                     |
| Pan et al. 56          | Zn₄H₃Pc/DP NPs  | 1064 nm, 0.6 W·cm⁻², 10 min | PA image-guided PTT                                           | • Phototheranostic agent in NIR-II window                                       |
| Du et al. 113          | DBCO-ZnPc-LP    | 808 nm, 0.1 W·cm⁻², 5 min | PA image-guided PTT/PAT                                       | • Strong heat energy transfer for PTT                                           |
| Ding et al. 57         | FA-ZnPcNDs      | 808 nm, 0.5 W·cm⁻², 3 min | PA image-guided PDT                                           | • Thermal-enhanced ultrasonic shockwave for PAT                                  |
| Li et al. 116          | NanoPcTBs       | 655 nm, 2.0 W·cm⁻², 1 min. + 0.22 W·cm⁻², 5 min | PA/FL image-guided PTT/PDT                                    | • Excellent water solubility and stealth properties                              |
| Mauriello-Jimenez et al. 122 | BSPOR, BSPHT    | 750 nm, 800 nm, 3 W, 1.57 s | PA image-guided PDT                                           | • Activatable in biotin receptor-positive cancer cells                           |
|                        |                 |                          |                                                               | • Intrinsic PA+PTT properties                                                     |
|                        |                 |                          |                                                               | • Cancer-specifically activating FL+PDT properties                               |
|                        |                 |                          |                                                               | • Two-photon excited PDT for precise treatment in deep tissues                  |

TABLE VIII. Summary of the representative publications for phototherapy with phthalocyanine/naphthalocyanine nanoparticles. PA, photoacoustic; PTT, photothermal therapy; FL, fluorescence; NIR, near infrared; PAT, photoacoustic therapy; PDT, photodynamic therapy.

 sharpening and separating the absorption peak, and (3) to explore multimodal imaging such as64Cu PET imaging. A large variety of chemical substituents have been reported in the literature, which can easily conjugate on Pc/Nc peripheries and induce targeting ability, switchable/activatable properties, enhanced stability, and improved water solubility. Many Pc/Ncs have also been used as PA image-guided phototheranostic agents in addition to their conventional use as photosensitizers in phototherapy. Rich absorption over NIR-I window has well verified its suitability as an angiographic contrast agent, and breaking its barrier to NIR-II window has overlooked its applicability to reveal unseen physiological findings from important deeply located organs as (1) gastrointestinal tract, (2) lymphatic system, and (3) excretory system. Intestinal motility disorder is one of the common factors that may deteriorate to severe bacterial infection and diabetes.125 Sentinel lymph node biopsy is a decisive process to staging of metastatic cancer and plays a critical role in future treatment and prognosis.126 Venous pyelogram is a test to check for abnormalities in kidney function, stones in the kidneys and ureters, and cancer.127 While application sites are inaccessible with noninvasive optical imaging techniques, aforementioned diagnostic protocols involve incision or cumbersome minimal invasive catheterized/endoscopic technique, otherwise heavily rely on ionizing radiation procedures as X-ray and computed tomography. As being a noninvasive, nonionizing process, NIR-II PAI possess high functional potential to innovate conventional diagnostic protocol of unreachable organs. Strong NIR absorption and photostability are their true strengths as multi-modal bioimaging and phototherapy agents. To achieve successful clinical translation and commercialization, rigorous and systemic studies must precede the approval showing further improvements in biocompatibility, solubility, clearance, and diagnostic/therapeutic efficacy. With the current emerging trends and continuous developments, we believe Pc/Ncs could serve as a vital tool for biomolecular imaging and theranostics in the near future.

AUTHORS’ CONTRIBUTIONS

E.Y.P. and D.O. contributed equally to this work.

ACKNOWLEDGMENTS

This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2020R1A6A1A03047902). It was also supported by BK21 FOUR project.

DATA AVAILABILITY

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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