

Review

Porphyrins as Theranostic Agents from Prehistoric to Modern Times

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Abstract

Long before humans roamed the planet, porphyrins in blood were serving not only as indispensable oxygen carriers, but also as the bright red contrast agent that unmistakably indicates injury sites. They have proven valuable as whole body imaging modalities have emerged, with endogenous hemoglobin porphyrins being used for new approaches such as functional magnetic resonance imaging and photoacoustic imaging. With the capability for both near infrared fluorescence imaging and phototherapy, porphyrins were the first exogenous agents that were employed with intrinsic multimodal theranostic character. Porphyrins have been used as tumor-specific diagnostic fluorescence imaging agents since 1924, as positron emission agents since 1951, and as magnetic resonance (MR) contrast agents since 1987. Exogenous porphyrins remain in clinical use for photodynamic therapy. Because they can chelate a wide range of metals, exogenous porphyrins have demonstrated potential for use in radiotherapy and multimodal imaging modalities. Going forward, intrinsic porphyrin biocompatibility and multimodality will keep new applications of this class of molecules at the forefront of theranostic research.

Key words: Porphyrins, theranostics

1. Hundreds of millions of years of theranostics

Porphyrins exist abundantly in plants, animals and rocks [1] and have even been found in lunar dust [2]. They existed by the time the first chlorophyll-containing photosynthetic organisms appeared some 3 billion years ago to initiate the creation of a new atmosphere, rich with life-supporting oxygen [3]. Our present ecosystem relies extensively on porphyrins for various vital roles ranging from photosynthesis to oxygen transport, in the form of chlorophyll and heme. Iron, which is chelated in the center of the heme group, may have been selected instead of another metal for evolutionary reasons, due to its abundance in the crust of earth [4]. Hemoglobin and myoglobin

are believed to have emerged more than 600 million years ago [5]. Structurally, hemoglobin consists of four protein subunits, with each subunit associated with a heme group chelated with an iron atom in the center (**Fig 1a**) [6]. Its evolution permitted animals to develop complex circulatory systems, which in turn permitted larger organism sizes and higher functions. Hemoglobin is packed into red blood cells (RBCs), which are the most abundant cells in blood and can be traced back to ancient times (for e.g., a probable 65 million year old dinosaur RBC is shown in **Fig. 1b**) [7]. RBCs have a characteristic biconcave shape 7-8 μm in diameter and play a major role in human physiology

[8]. RBCs are produced at an astounding rate of approximately 2×10^6 per second and there are $2\text{--}3 \times 10^{13}$ in circulation at any given moment in a human adult body. Oxygen delivery is mediated by the nearly 300 million hemoglobin molecules in each RBC, which

comprise some 3×10^{22} total heme porphyrin group in the body [9] (Fig 1a). In addition, a wide range of other porphyrins is found in prosthetic groups within cells (e.g. cytochromes and vitamin B12).

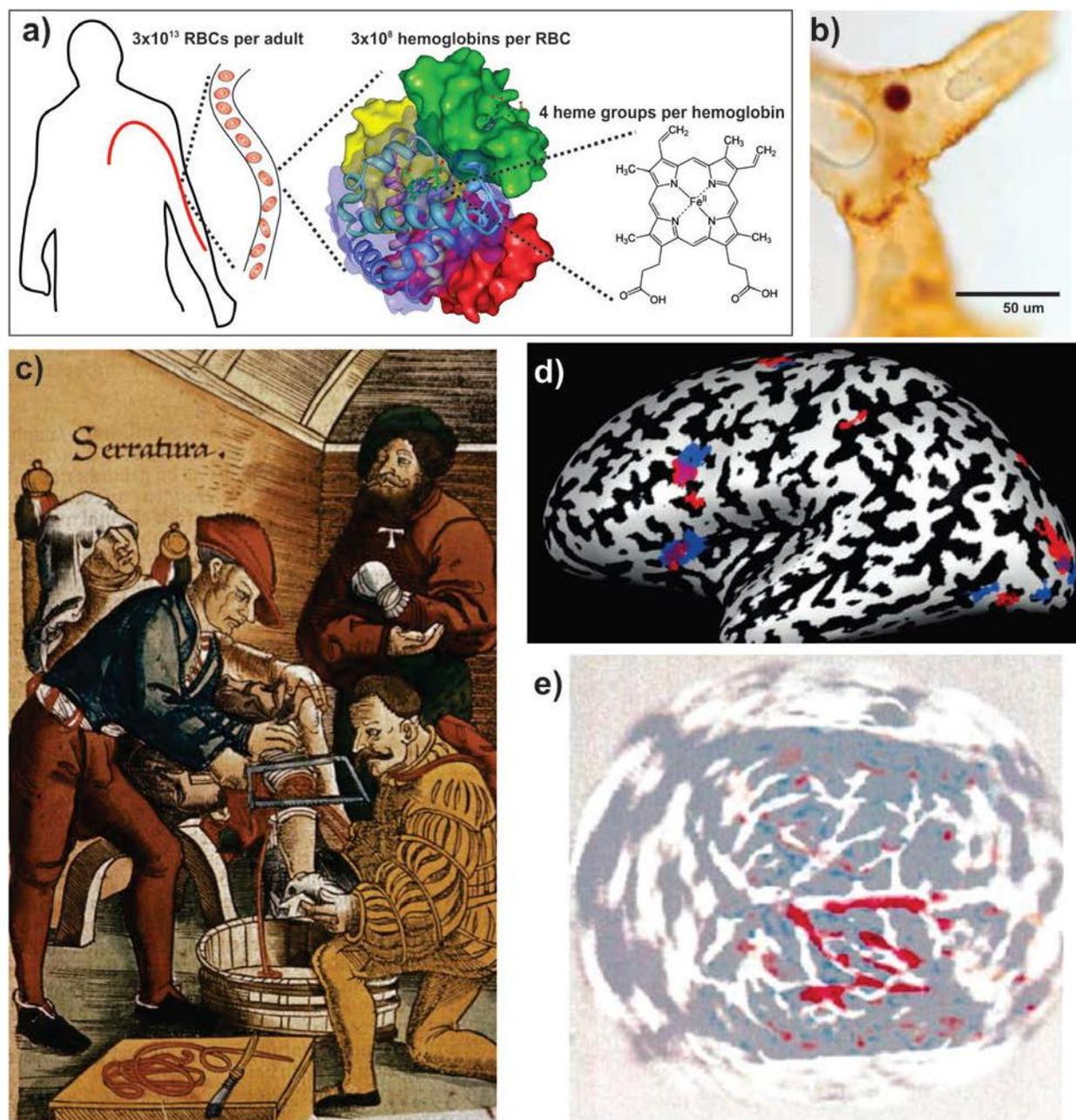


Figure 1: Endogenous porphyrins. **a)** Schematic organization of heme in the body [6,9]. **b)** A possible heme-containing red blood cell identified in 65 million year old dinosaur tissue [7]. **c)** Surgical illustration of an amputation from circa 1500 AD. Excessive bleeding can readily be observed due to the bright red heme to guide tourniquet application (Archives & Special Collections, Columbia University Health Sciences Library). **d)** BOLD MR imaging using heme oxygenation. In this case neural differences between English and Hebrew speech patterns are shown. Blue and red regions are involved in morphological processing in Hebrew and English, respectively and regions of overlap are shown in purple [22]. **e)** Non-invasive, rat brain transcranial photoacoustic imaging following right-side whisker stimulation [26]. Reproduced with permission from the publishers of corresponding references.

Hemoglobin was first isolated by Hunefeld in 1840 [5,10] and its molecular structure was revealed over one hundred years later using X-ray crystallography [11]. The first analytical applications using porphyrins originate much earlier, in the era of the first blood-bearing creatures. Heme has long been recognized as the red pigment of our blood, even if the molecular details were not known. Porphyrins in different forms can be found in many different colors. Among them, the bright red color of blood, visible under natural light, has been providing health guidance for millions of years already. For instance, an injury that causes bleeding can be detected by eye by both an injured animal and other parties who wish to provide assistance (or possibly prey on the injured). **Fig 1c** shows a 16th century painting of an amputation operation from one of the earliest recorded medical textbooks, illustrating how visual detection of blood has long provided optical feedback for guiding medical procedures. The evolutionary importance of heme is reflected in human responses to even the sight of the color red. Immersion in red rooms is associated with generating active and excited psychological states and it has been shown that some muscular responses are over 10 percent faster than normal in red light [12]. Red stimulates tension and restlessness; physiologically, the sight of the color can even increase blood pressure, pulse and respiration [13]. Thus, the intrinsic importance of heme as an indicator for bleeding is reflected in its role through human evolution to invoke response and reaction to avoid and deal with bleeding. Today, blood still maintains its theranostic legacy in millions of medical procedures performed each day as an endogenous and highly visible optical contrast agent.

In the past decades, technological advances in medical imaging have emerged concurrently and co-operatively with theranostic medicine [14]. Heme serves as an important endogenous contrast agent in at least two imaging techniques; functional magnetic resonance imaging (fMRI), and photoacoustic tomography (PAT). Hemoglobin in the form of oxyhemoglobin carries molecular oxygen to cells for cellular respiration and following oxygen delivery, returns to the lungs in the form of deoxyhemoglobin. The iron chelated in the center of hemoglobin can change its valency between Fe^{2+} and Fe^{3+} to help enable precise oxygen delivery [15]. The differences between oxy and deoxyhemoglobin can be detected by optical and MR methods.

Understanding neural activities via functional brain mapping is challenging. Fluctuating electrical signals on the scalp have been able to provide some information about electrical activity in the outer cor-

tex of the brain, but cannot provide a deep or detailed three-dimensional volumetric map of brain activity. The birth of fMRI ushered in a new era in the study of the working brain and serves as a central focus in modern neuroimaging since it enables indirect measurement of metabolic activity and blood changes caused by neural activity. fMRI was developed on the basis of nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI). NMR, discovered in 1946, is based on the phenomenon of nuclear spin, resulting in resonance radiofrequency absorption in the presence of an extrinsic magnetic field [16]. About 30 years later, MRI emerged based on tissue-specific differences in transverse and longitudinal relaxation rates among different tissues [17]. The focus of fMRI, compared to the conventional MRI, is to investigate metabolic activities through its sensitivity to relative levels of oxygen in blood. This technique, which has been gaining much attention since 1991, offers an indirect interpretation of neural activity based on the changes of blood volume, blood flow and blood oxygenation (also collectively referred to as hemodynamic oxygenation) [18–20]. It has been shown that when diamagnetic oxyhemoglobin releases its oxygen, the deoxyhemoglobin becomes paramagnetic, which influences the transverse relaxation rates of water proton spins in the immediate vicinity of vessels, resulting in the blood oxygenation level dependent (BOLD) contrast [21]. When a certain area of brain is active, oxygen metabolic rate increases, but the blood flow increases as well so the overall outcome is the oxygen extraction fraction drops, leading to more oxygenated venous blood, ultimately resulting in locally increasing MR signals. Even when the brain is given only somewhat different tasks, for instance, using either English or Hebrew speech patterns, remarkably precise activation maps of the brain can be obtained by fMRI (**Fig. 1d**) [22].

PAT is an emerging and high resolution optical imaging technique based on the photoacoustic effect [23,24]. When biological tissues absorb a pulse of optical energy, the acoustic transient pressure excited by thermoelastic expansion will produce ultrasonic waves, referred to as photoacoustic waves. These waves are detected by an ultrasonic transducer and converted into electric signals which are used to generate images [24,25]. Photoacoustic imaging can be used to non-invasively obtain functional images based on differences in optical absorption between oxy- and deoxy hemoglobin contained in blood. Photoacoustic methods can provide information on hemoglobin concentration and oxygen saturation, indicating tumor angiogenesis, hypoxia or hypermetabolism, which are important parameters of cancer char-

acterization. Meanwhile, based on the relation of neural activity and cerebral blood parameters (e.g. hemodynamics), PAT of cerebral activity can be obtained noninvasively and infer neural activity. As shown in **Fig 1e**, PAT can be used for functional imaging of cerebral hemodynamic changes induced by whisker stimulation in a rat [26]. Also, increasing focus has been placed on clinical applications in humans. For example, it was shown that PAT can be used to obtain images of breast tumors [27] and subcutaneous microvasculature skin in humans [28]. There still remains some challenges for applying PAT in the human brain due to the thickness of human skull [29]. As imaging methods improve, fMRI and PAT will continue to be prominent functional imaging modalities based on oxy and deoxy heme.

2. The original exogenous agents for imaging and therapy

Endogenous porphyrins play a central and historic role in theranostic medicine, but exogenous porphyrins are also of considerable significance. Photodynamic therapy (PDT) is a clinical and minimally invasive method to treat cancers and other diseases. It involves three elements: a photosensitizer, light and oxygen. Porphyrin and porphyrin-related compounds are the most commonly used photosensitizers. After administration and delivery of a photosensitizer to a tumor site and upon light irradiation, it will generate reactive singlet oxygen (1O_2), leading to cell death and tumor destruction [30–35]. **Fig. 2a** shows the efficacy of PDT using hexyloxyethyl devinylpyroporphorbide-a (HPPH or Photochlor) in destroying esophageal cancer [36]. PDT also has been clinically successful in treating other diseases such as age-related macular degeneration (AMD) and acne [37,38]. Singlet oxygen (1O_2) has a small diffusion range less than the diameter of a cell, therefore restricting damage only to the treatment site [39]. As a variant of PDT, photothermal therapy (PTT) was proposed as another method for cancer treatment using porphyrins at least as early as 1999 [40,41]. Generally, in the promotion of photothermal sensitized processes, photosensitized species can generate electronic excitation energy upon irradiation, leading to local temperature rises and to the destruction of cancer cells [42], even in the absence of oxygen [43]. As shown in **Fig 2b**, when porphyrins (nanoparticles formed by the conjugation of porphyrin to a phospholipid) were administered to a tumor bearing mouse and irradiated by a 658 nm laser outputting 750 mW with a power density of 1.9 W/cm² for 1 min, the tumor temperature rapidly increased to 60 °C while the tumors in control experiment with PBS in-

jected did not increase in temperature beyond 40 °C [44]. Light absorbing species can include metallic nanoparticles (e.g. Au, Ag) [45], cyanine dyes [46], azo-dyes [47], porphyrins [43,48], naphthalocyanines [41] and many others.

In the early 1900s, a number of photosensitizers were investigated for treatments of certain cancers and skin diseases [49–51]. Oscar Raab was perplexed by some inconsistent data that were generated during a heavy lightning storm; he started to wonder about the effect of light and discovered photodynamic reactions in the process [51–53]. Oxygen was soon determined to be an important mediator for photosensitization and the term “photodynamic action” was coined in 1907 [51,54,55]. Hematoporphyrin, usually extracted from bovine blood, has been used since this time. This porphyrin was first isolated from dried blood in 1841 [56,57]; its fluorescence properties were observed in 1867 [57,58] and its photobiological properties were studied in mice in 1911 [57,59]. When administered hematoporphyrin and irradiated with light, mice exhibited skin photosensitivity and phototoxicity. Around the same time, a German doctor boldly injected hematoporphyrin into himself and demonstrated how exogenous porphyrins were sunlight photosensitizers in humans [51,60]. Another early and important discovery is that the concentration of tetratraphenylporphinesulfonate (TPPS) detected in tumors of rats was higher than that using hematoporphyrins, indicating better tumor localizing ability of TPPS [61]. These pioneering works of PDT occurred mostly in Europe and were reported in non-English languages, but the rich history of PDT has been summarized by several comprehensive literature reviews [51,57,62].

Porphyrins, their derivatives or porphyrin-inducing drugs are by far the most commonly used photosensitizers in PDT. One milestone of PDT occurred when Diamond *et al.* reported favorable results of cancer treatment in rats in 1972, using crude hematoporphyrins as a photosensitizer combined with white light from fluorescent lamps [63]. When rats bearing subcutaneous glioma cell tumors were intravenously injected with hematoporphyrin and irradiated by white light a day after injection, tumors showed dramatic shrinking. In control experiments, hematoporphyrin or light alone did not have anti-tumor effects. Building on this work, in 1975 Dougherty and coworkers from Roswell Park Cancer Institute in Buffalo demonstrated effective tumor destruction in more advanced animal models [64]. Mice with mammary tumors were administered a hematoporphyrin derivative (HpD) and were exposed to red light. Half the transplanted mouse tumors were

cured and favorable results were also obtained in the case of the rats bearing chemically-induced tumors given higher doses of HpD. At about the same time, it was found that mice with carcinomas transplanted from human bladder cancers were destroyed using HpD combined with local exposure to white light [65]. Subsequently, phthalocyanines were introduced as photosensitizers, motivated by their typically higher absorption and longer wavelengths [66]. There have been many porphyrin-related compounds that have had success or show promise for clinical or preclinical applications in PDT. Photofrin, a HpD, has been the most historic and commonly used photosensitizer, approved for the treatment of many cancers including lung, bladder, gastric and cervical. But hematoporphyrin derivatives have two disadvantages: 1) after administration, drugs are retained and taken up by skin so that patients are required to avoid bright light for long time; 2) the limited penetration depth around 630 nm limits the size of tumors treated effectively [67,68]. Therefore, other porphyrin-based photosensitizers have been developed including Verteporfin, Photolon, and others. Other reviews comprehensively describe the clinical state of PDT and clinical trials using photosensitizers [33–35,38,51,69]. Challenges like sunlight toxicity and lack of clinically proven targeting still exist, which have slowed the clinical application of PDT. In the method of vascular photosensitizer targeting, light placement can effectively destroy the vasculature and endothelium around the tumor, which is a promising clinical approach for the treatment of prostate cancer and age-related macular degeneration (AMD) [70,71]. Recently, porphyrins and PDT have been widely employed to treat AMD. This medical condition is caused by choroidal neovascularization (CNV), leading to the damage of retina, loss of vision at macula, even to blindness eventually. Widespread clinical efforts have used verteporfin (trade name Visudyne) with PDT to prevent visual loss [72–75]. PDT using porphyrins has also been applied to treat acne vulgaris, a common disease linked with the bacteria *Propionibacterium acnes* and the sebaceous glands [76]. Conventionally, antimicrobial and anti-inflammatory drugs and hormones were used topically or orally to fight against the pathogenetic factors [77,78]. PDT has emerged as a highly effective alternative method in skin clinics [79,80]. Most acne treatments induce protoporphyrin IX (PpIX) using aminolevulinic acid (ALA) or ALA derivatives and with appropriate light irradiation, PDT can cure inflammatory acne lesions with high efficacy [81,82].

Radiotherapy is another therapeutic modality for cancer treatment that can be mediated by porphyrins.

Generally, aqueous radiolysis can generate free radicals which interact with biomolecules such as DNA, RNA, protein and membrane, resulting in the cell dysfunction and destruction [83]. Due to their preferential uptake in tumors, some porphyrins have been used in targeted tumor radiotherapy when labeled with therapeutic radionuclides [84]. For example, meso-tetrakis [3,4-bis(carboxymethyleneoxy)phenyl] porphyrin (T_{3,4}CPP) was labeled with ¹⁸⁸Re and used for targeted radiotherapy in mice [85]. It was shown that in different tumor bearing mice 24 h postinjection, the tumor/muscle, tumor/blood, and tumor/liver ratio for ¹⁸⁸Re-T_{3,4}CPP was as high as 19, 9.6 and 4, respectively. **Fig. 2c** shows melanoma imaging of the ¹⁸⁸Re porphyrin administered in tumor-bearing nude mice, showing the potential for radiotherapy and imaging. A review of ¹¹¹In-tetrphenylporphyrins [86], ⁵⁴Mn-HpD [87], ¹⁰⁹Pb-Porphyrins [88] and others summarizes radioporphyrin complexes comprehensively [89].

Not only do porphyrins have therapeutic functions, but they also emit fluorescence in the red or near infrared (NIR) and are useful for *in vivo* imaging. The first observation of porphyrin fluorescence from tumors was reported by Policard in 1924 [57,90]. The red fluorescence from hematoporphyrin was observed in a rat sarcoma during illumination with ultraviolet light but was mistakenly attributed to bacterial infection. Seven years later, similar results in breast carcinomas were observed and the possibility of bacteria was ruled out, confirming porphyrin imaging utility [91]. In 1942, Auler and Banzer studied the localization of exogenously administered hematoporphyrins in tumors and found they favorably accumulated in tumor and lymph nodes in rats [92]. Subsequent studies in the 1940s and 1950s further demonstrated that porphyrins exhibit affinity for neoplastic tissue [93–95]. Thus, well over 50 years prior to the popularization of commercial fluorescence animal imaging systems [96], the fluorescence of porphyrins was observed to differentiate normal tissues and tumors in optical imaging studies. Porphyrin imaging can be used to predict the success or failure of photodynamic treatment [97,98] and also for fluorescence-guided surgical tumor resection [99]. Fluorescence-guided surgery has emerged as an important research area especially for the treatment of the brain tumors. Generally, 5-aminolevulinic acid (5-ALA) is a metabolic precursor inducing the synthesis of PpIX, which leads to the accumulation the fluorescence in malignant glioma tissues, thus providing identification for guided resection of tumors [100–104]. **Fig 2d** and **Fig. 2e** show *in vivo* tumor fluorescence in mice 48 hours post injection of porphyrins [44] and image

guided resection of brain tumors in rabbit (guided by the fluorescence of PpIX) [105], respectively. Exogenous porphyrins have also been widely used in other optical imaging techniques such as photoacoustic

imaging. **Fig. 2f** shows the photoacoustic imaging of lymphatic mapping in rats after injection of 2.3 pmol porphyrins.

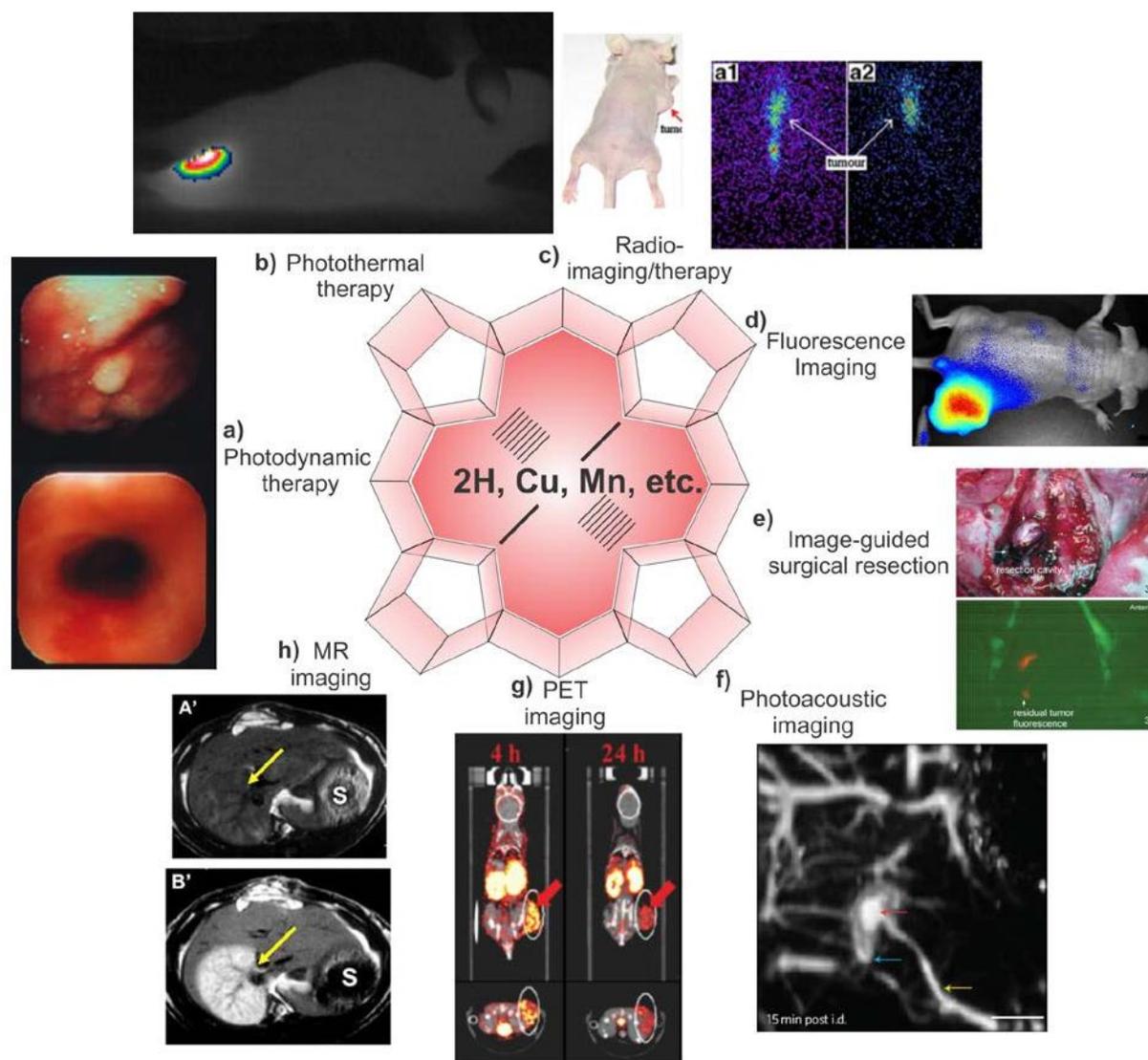


Figure 2: Multimodal theranostic capabilities of exogenous porphyrins. **a)** Human esophageal cancer successfully treated with PDT [36]. **b)** Photothermal image of a xenograft bearing mouse injected with porphyrins and then irradiated by laser for 1 min showing tumor temperature rapidly rising above 60 °C [44]. **c)** Radiotherapy: Melanoma imaging of $^{188}\text{Re-T}_{3,4}$ CPP in tumor bearing mice. Scintigraphic images were collected at 8h (a1) and 24 h (a2), showing porphyrin potential in radiotherapy and imaging [85]. **d)** Near infrared fluorescence imaging of porphyrins activation in a KB xenograft bearing mouse [44]. **e)** Fluorescence Guided Resection (FGR): the surgical cavity after white light resection of brain tumor in rabbit (top), fluorescence imaging of PpIX showing tumor margins. Additional FGR can improve the accuracy of resection [105]. **f)** Photoacoustic image of rat lymphatics mapped following intradermal injection of porphyrins in rats [44]. **g)** PET imaging showing clear delineation between the tumor and other tissues by PET was obtained at 4, 24 h after intravenous injection of a targeted ^{64}Cu porphyrin [116]. **h)** MR imaging: In the top precontrast T1 weighted image, the infarcted right liver lobe (arrow) was barely detected whereas 24 h after injection of Gadophrin-2 at 0.05 mmol/kg the infarcted liver lobe was strongly enhanced (bottom) [125]. Reproduced with permission from the publishers of corresponding references.

Porphyrins and related macrocycles, typically formed with a tetrapyrrole skeleton, can form metalloporphyrin complexes chelated with an incredibly diverse range of metals (e.g. Li, Be, Na, Mg, Al, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Ge, Rb, Sr, Ru, Rh, Pd, Ag, Cd, In, Sn, Sb, Cs, Ba, Pt, Au, Hg, Tl, Pb, Bi, Th, Y, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu) [89,106–108]. Just as porphyrins led the way for fluorescence imaging and detection of tumors [90], in 1951, ^{64}Cu -porphyrins were first used as positron emission radioisotopes for detection of brain injury and tumor [109]. In this experiment, ^{64}Cu phthalocyanine was synthesized successfully and doses of 100 mg/kg were given to rabbits, mice, guinea pigs, cats and dogs, then biodistribution and excretion were studied in rabbits. Although the positron emission tomography (PET) phenomenon has long been known, it has only recently been recognized as a valuable clinical and theranostic imaging modality in the last decades [110,111]. ^{64}Cu -porphyrins have been explored as promising probes [112,113] considering their favorable properties including excellent resistance to demetallation [109], minimal toxicity [114], the 12 hour half-life of ^{64}Cu , and the pharmacokinetics of porphyrins [115]. Recently, ^{64}Cu was incorporated into targeted peptide consisting of pyropheophorbide- α , a peptide linker and folate (PPF). After 4 and 24h post-injection, preferred tumor/background ratio and clear delineation of tumor and other tissues was detectable by PET (**Fig 2g**). In this work, porphyrin accumulation in tumor at 4h was larger than that at 24h post-injection, which could be explained by the active targeting component of the PPF [116]. ^{48}V labeled pheophorbide has also been examined for PET imaging [117]. In addition to PET, other diagnostic imaging modalities can be used combined such as computed tomography (CT), single photon emission computed tomography (SPECT), ultrasound, and MRI. For instance, PET images offer information on contrast targets with a high sensitivity, while CT and MRI stand out for their high resolutions. Hence, the combination of different imaging strategies (e.g. PET/CT, PET/MRI) compensate for disadvantages of each modality and obtain more reliable and detailed results with high spatial resolution and sensitivity [118,119].

Metalloporphyrins can be also used for enhancing MRI of tumors. MRI can offer information on tissue structure with high sensitivity based on physico-chemical and physiological properties [120,121]. However, MRI can have difficulty in distinguishing between neoplastic and normal tissues [122]. Paramagnetic metals can be used as contrast agents that decrease these relaxation times, including the use of

metal-chelated porphyrins [123,124]. Paramagnetic metals (e.g. Mn^{2+} , Gd^{3+}) are chelated in metalloporphyrins and used as contrast agents which can cause a detectable relaxation in the surrounding water [125]. In 1987, a water soluble metalloporphyrin, manganese (III) tetra-(4-sulfonatophenyl) porphyrin, Mn(III)TPPS_4 was investigated as a contrast agent for MRI. Effective proton T1 and T2 relaxations induced by Mn(III)TPPS_4 were obtained and good MRI enhancement using Mn(III)TPPS was observed in carcinomas, fibrosarcomas and lymphomas [126]. Subsequently, metalloporphyrins of manganese tetrasodium-meso-tetra (4-sulfonatophenyl)-porphine (MnTPPS), manganese meso-tetra-4-pyridylporphine and gadolinium meso-tetra-4-pyridylporphine have been used as tumor specific MR imaging contrast agents in animal models [127]. Paramagnetic metalloporphyrins show good chelate stability, low toxicity and high relaxivities. Gd^{3+} has more unpaired electron spins and is more paramagnetic than Mn^{2+} ; Gd -chelated porphyrins such as Gadophrin-2 (bis- Gd -DTPA-mesoporphyrin), Gadophrin-3 (a copper inserted at the core to improve stability and safety), and texaphyrins have been explored as MRI contrast agents [128–130]. MRI enhancement in necrotic tissue using Gadophrin-2 is clearly shown in **Fig 2h**. Compared to precontrast T1-weighted MRI, where an infarcted right liver lobe was almost undetectable, 24 hours after a 0.05 mmol/kg Gadophrin injection, the infarcted lobe was clearly demarcated [125]. It is also worth mentioning that while some degree of tumor specific accumulation of porphyrins may be assumed, this is not always the case. For example, two metalloporphyrins, gadolinium mesoporphyrin (Gd-MP) and manganese tetraphenylporphyrin (Mn-TPP) did not prove to preferentially accumulate in viable tumors tissue of hepatomas [131].

3. Looking forward

Porphyrins have great potential to further advance the development of imaging and therapeutic approaches. Endogenous porphyrins will continue to prove their utility as improvements in instrumentation permit higher spatial and temporal resolution for techniques such as fMRI and photoacoustic imaging. Exogenous porphyrins have seen intensive activity in recent decades, resulting in many clinical approvals (e.g. Photofrin, Photolon, Visudyne, Foscan). Some novel porphyrins may continue to take on altered forms that enhance functionality, such as the development of photosensitizers with long wavelength absorptions to increase the penetration depth of light [36,132]. Another strategy is the development of activatable photosensitizers to achieve local activation

and minimize damage to adjacent tissues for PDT [38,133–135]. New porphyrin nanocarriers have been explored, including polymers, micelles, dendrimers, liposomes, along with targeting strategies to better deliver the cargo [136,137].

Polymers are good carriers for porphyrin or phthalocyanine delivery. For instance, tetrakis (meso-hydroxyphenyl) porphyrin (mTHPP) was formulated into polymeric micelles made of poly-(ethylene glycol)-co-poly (D, L-Lactic acid) (PEG-PLA) via a simple solvent evaporation method resulting in high loading efficiency (85%) and desirable micelle size (~30 nm) [138]. Dendrimers have gained much attention in biomedical applications [139]. Dendritic porphyrins (consisting of a focal porphyrin surrounded by poly benzyl ether dendrons) have been created and demonstrated to selectively accumulate in choroidal neovascularization (CNV) lesions [140]. Liposomes are another commonly explored material for porphyrin delivery [141]. One example is Visudyne, a health regulatory agency approved liposomal formulation of verteporfin [71]. Dipalmitoylphosphatidylcholine (DPPC) liposomes have been used to encapsulate Photofrin in order to enhance photodynamic effect on tumors [142]. While the enhanced permeability and retention effect enables nanoparticles to accumulate in tumor passively, active targeting strategy studies have also attracted much attention to improve specificity. Various targeting agents, showing affinity for a marker that is overexpressed on tumor cells, have been explored for targeting porphyrins, such as antibodies, aptamers, folate, growth factors, transferrin, and lipoproteins [143–147]. Additionally, it is worth mentioning that some new carriers with triggered release mechanisms also hold potential for controlled porphyrin delivery, based on changes in temperature, pH, or light, leading to the carrier breakdown and local drug release [141,148–150]. There are also new experimental emerging areas that feature quite different approaches to porphyrin theranostics. Among them, the porphosome is a recent discovery of an organic nanoparticle formed by the conjugation of porphyrin to a phospholipid [44]. These nanovesicles exhibited large and tunable extinction coefficients and other unique nanoscale biophotonic properties for multimodal therapeutic applications. Porphosomes are also one of the first nanoparticles exhibiting enzymatic biodegradation *in vivo* [151].

In summary, porphyrins have existed universally since prehistoric times and have contributed, have evolved in step with life as we know it for billions of years, and continue to be prominent in biological, chemical and medical fields. Porphyrins facilitate optical and magnetic detection in the red heme in

blood, serving as a natural and excellent theranostic agent. Exogenous porphyrins have also played indispensable and defining roles in modern theranostics, with new agents enabling multimodal imaging and multimodal therapy. With concerted research efforts, porphyrins will continue to add new chapters to their theranostic legacy into the coming decades and beyond.

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Competing Interests

The authors have declared that no competing interest exists.

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