Sensors

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Bright, "Clickable" Porphyrins for the Visualization of Oxygenation under Ambient Light

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Abstract: *A new group of "clickable" and brightly emissive metalloporphyrins has been developed for the visualization of oxygenation under ambient light with the naked eye. These alkynyl-terminated compounds permit the rapid and facile synthesis of oxygen-sensing dendrimers through azide–alkyne click chemistry. With absorption maxima overlapping with the wavelengths of common commercial laser sources, they are readily applicable to biomedical imaging of tissue oxygenation. An efficient synthetic methodology, featuring the stable trimethylacetyl (pivaloyl) protecting group, is described for their preparation. A paint-on liquid bandage containing a new, click-synthesized porphyrin dendrimer has been used to map oxygenation across an ex vivo porcine skin burn model.*

Proper oxygen supply and consumption are essential to cellular function; thus, the measurement of oxygen partial pressures (pO_2) is of great importance in the study of disease pathophysiology. Molecular oxygen has a known, quantifiable effect on the phosphorescence lifetime and intensity of certain molecules, as described by the Stern–Volmer equation.[1,2] Optical methods based on oxygen-dependent phosphorescence quenching are under active development because of their ability to map oxygen concentrations with high spatial resolution.[3–6] Out of the many known oxygen-responsive materials,[7–11] metalloporphyrins have been extensively explored as oxygen sensors[10,12–18] because of their room-temperature phosphorescence, structural versatility, and tunable photophysical properties.

Nonetheless, the phosphorescence of most porphyrins is very weak and thus hard to detect under ambient light conditions. In addition, their absorption maxima often lie far from the wavelengths of common laser sources, thereby requiring complex optical setups for both excitation and detection. Lastly, bare porphyrins tend to exhibit poor behavior in biological systems, necessitating modification with biocompatible moieties. As a result, there is an ongoing need for bright, readily functionalizable oxygen-sensing porphyrins that can be excited by commercial laser lines and LED sources.

To address these needs, we report the development of a new group of metalated, meso-unsubstituted porphyrins and benzoporphyrins bearing surface alkyne groups. The terminal alkynes encourage modification through the Huisgen 1,3-dipolar cycloaddition, a fast and highly efficient azide–alkyne "click" reaction.[19] These new compounds, named "Clickaphors" following the convention of Wilson and colleagues,[20] allow for the rapid, modular synthesis of a vast array of brightly emitting dendritic oxygen sensors.

The Clickaphors were designed from meso-unsubstituted porphyrins,[21] which are generally brighter than their bulkier, meso-substituted analogues. As a result, the cyclohexenyl metalloporphyrins described in this work exhibit phosphorescence so strong that they are visible with the naked eye, and were evaluated in the mapping of transdermal oxygenation in an ex vivo porcine skin burn model.

The synthesis of oxygen-sensing metalloporphyrins **7** through **12**, presented in Scheme 1, was achieved from the common intermediate **6**, whose precursors have been synthesized by adapting previously developed chemistry.[22,23] Briefly, the intermediate **6** was prepared by a Lindsey-type condensation of the pivaloyl-protected tetrahydroisoindole **5**. Insertion of platinum or palladium yielded the metalated cyclohexenyl porphyrins **7** and **8**. Subsequent aromatization led to the Pt or Pd benzoporphyrins **9** and **10**, respectively. After removal of the pivaloyl groups, a Williamson-type alkylation resulted in the final alkynyl-terminated clickable metalloporphyrins **11** and **12**, hereafter referred to as "Clickaphor Red" and "Clickaphor Blue," based on the colors of the respective purified solids. The route to free-base porphyrin **6** has been successfully reproduced by an outside manufacturer on a multigram scale. Its high yield of approximately 20% is the product of extensive optimization. The use of the pivaloyl protecting group provided stability that allowed the synthesis of substituted tetrahydroisoindole **5** with a yield of 52%, even though isoindole derivatives are known to be unstable,[21] Furthermore, our use of the reported optimal concentration of 10^{-3} M in the Lindsey-type condensation[24] to form **6** gave a yield of 69%. We found that metal insertion prior to aromatization afforded significantly higher yields and required shorter reaction times than when the metal was inserted into the poorly soluble benzoporphyrins, though the two steps are transposable. Attempts to forgo the use of protecting groups resulted in unwanted polymerization products when an alkynyl-terminated free-base porphyrin was subjected to metal insertion or aromatization conditions.

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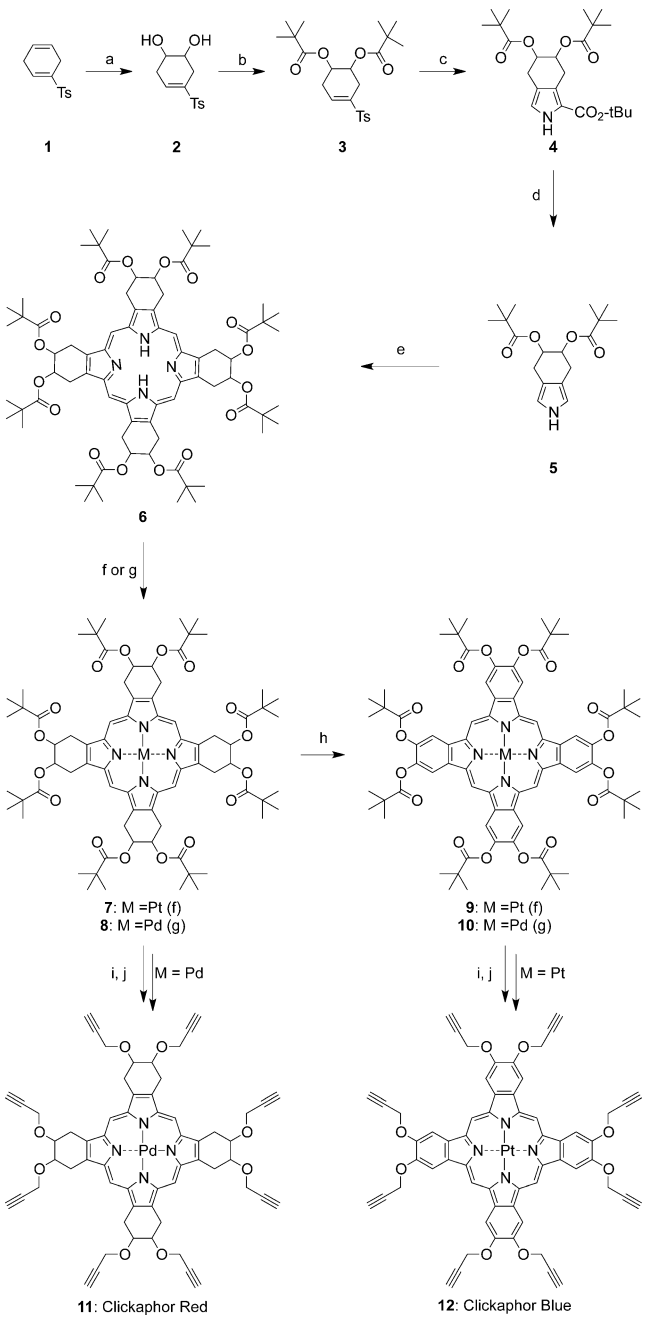

Scheme 1. Synthesis of alkynyl-terminated porphyrins. Reagents and conditions: a) K_2OsO_4, $K_3Fe(CN)_6$, K_2CO_3, $CH_3SO_2NH_2$, *t*BuOH/H_2O (1:1), r.t., overnight, 78%; b) $(CH_3)_3CCOCl$, 4-dimethylaminopyridine, pyridine, CH_2Cl_2, r.t., 18 h, 85%; c) $CNCH_2CO_2tBu$, *t*BuOK, THF, r.t., 4 h, 81%; d) TFA, r.t. 30 min, 52%; e) *p*-TsOH (monohydrate), formaldehyde (37% in water), benzene, reflux (Dean–Stark apparatus), 8 h, 69%; f) $PtCl_2$, benzonitrile, reflux, 6–8 h, 65%; g) $PdCl_2$, benzonitrile, reflux, 15–20 min, 85%; h) DDQ, dry 1,4-dioxane, reflux, 8–10 h, 60%; i) DIBAL-H, dry CH_2Cl_2, −78 °C→r.t., 18 h; j) NaH (60%), $BrCH_2CCH$, dry DMF, 18 h, 0 °C→r.t., 35% (yield over two steps).

Prior efforts to aromatize cyclohexenyl porphyrins with acetyl protecting groups using DDQ proceeded only after elimination of four of the eight acetyl groups.[23] The pivaloyl groups used in our synthesis, on the other hand, tolerated these conditions and permitted formation of the octapivaloxy, metalated benzoporphyrins **9** and **10**. The presence of eight attachment points enables the synthesis of dendrimer layers denser than typical Oxyphor-type dendrimers. Limited elimination and aromatization byproducts, formed during both the metal insertion and aromatization steps, were easily removed by column chromatography.

Since benzoporphyrins **9** and **10** dissolve appreciably only in dichloromethane, the less common, dichloromethane-soluble diisobutylaluminum hydride (DIBAL-H) was found to be ideal for the deprotection of the pivaloyl esters, offering near-quantitative yields with all four porphyrins.

The four pivaloyl-protected metalloporphyrins have strong and distinctive absorption bands throughout the UV and visible regions (Figure 1). They exhibit bright room-temperature phosphorescence with high quantum yields in the absence of oxygen. Most importantly, the quantum yields

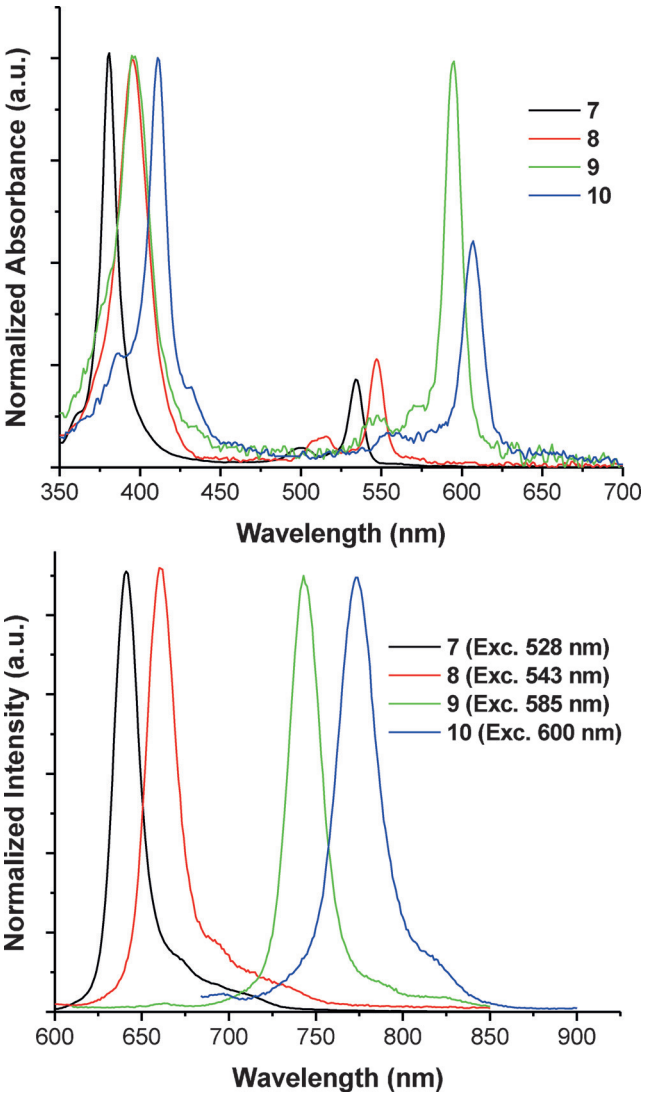

Figure 1. Absorption (top) and emission (bottom) spectra of pivaloyl-protected porphyrins **7** and **8** (in DMF as solvent) and benzoporphyrins **9** and **10** (in dichloromethane). Spectral profiles have been normalized to display similar absorbances at the Soret bands and similar emission intensities.

Table 1: Photophysical properties of porphyrins **7**–**12**.[a]

Compound	Absorption λ_{max} [nm] (log ε)	Emission λ_{max} [nm]	τ [μs]	ϕ	"Brightness" (Soret; Q)
7	377 (5.40), 531 (4.74)	645	108	0.41	102,000; 22,000
8	392 (4.98), 543 (4.38)	661	484	0.17	16,000; 4,000
9	396 (4.98), 592 (4.95)	744	99	0.20	20,000; 19,000
10	410 (5.18), 605 (4.98)	774	412	0.10	15,000; 9,500
11	392 (4.97), 544 (4.26)	660		0.15	14,000; 3,000
12	398 (4.95), 595 (4.88)	745		0.20	18,000; 15,000

[a] Absorption and emission spectra acquired in *N*,*N*-dimethylformamide for **7**, **8** and **11**, and dichloromethane for **9**, **10**, and **12**. Quantum yields were measured in the corresponding deoxygenated solution, purged with argon gas. For detailed protocols and the instrumentation used for photophysical measurements and lifetime determination, see the Supporting Information. The "brightness" metric is defined as the product of the molar absorption coefficient and the quantum yield. As a comparison, the "brightness" metric for the commercially available phosphor Oxyphor G2 is 6000 at the Q-band.

of the alkynyl derivatives were found identical to those of their parent, pivaloyl-protected porphyrins (Table 1). The lower quantum yields of **9** and **10** may be due to aggregation-induced self-quenching under the measurement conditions.

These phosphors, with their substituents linked to the central porphyrin via an oxygen instead of a carbon atom, were designed such that their absorption peaks exist at or very near the wavelengths of common commercial laser sources, including the helium–neon laser lines at 543 and 594 nm, as well as the doubled output of most Nd-based lasers at 532 nm.

To demonstrate the visible response of the sensor to oxygenation changes, the phosphorescence of a deoxygenated solution of platinum porphyrin **7** was captured under ambient light with a regular smartphone camera (Figure 2a). Phosphor **8** was embedded in a liquid bandage formulation, along with Coumarin 500 ($\lambda_{exc,max} = 392$ nm; $\lambda_{em,max} = 495$ nm), a green, oxygen-insensitive fluorophore. The intensity of the phosphor's red emission, visible under ambient light, increases upon deoxygenation, turning the apparent color of the film from green to red (Figure 2b).

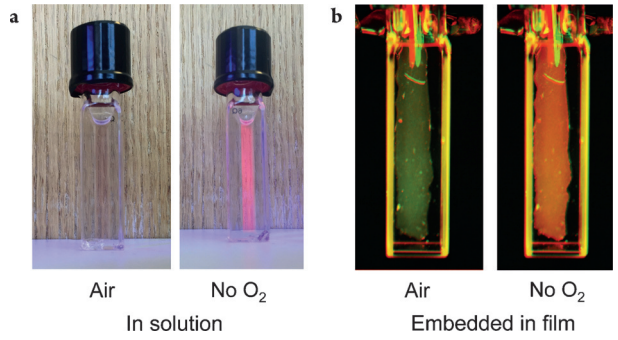

Figure 2. Sensor response to different oxygen levels. a) Solution of platinum porphyrin **7** in dichloromethane in air (left) and in the absence of oxygen (right); the porphyrin was excited with a handheld LED flashlight. Image was acquired using a smartphone camera. b) Palladium porphyrin **8** was embedded in a liquid bandage formulation along with Coumarin 500, and the film's emission was recorded with a commodity color camera equipped with filters, in air (left) and in the absence of oxygen (right).

To show the ease of dendrimer synthesis, a glutamic dendrimer was built by simply clicking eight azido-terminated, second-generation glutamic dendrons onto Clickaphor Red. The resulting dendritic oxygen sensor, Clickaphor Red G2 (see Supporting Information for structure), and Coumarin 500 were embedded in a liquid bandage matrix formulation. Once applied to a wound, the bandage dries into a thin, solid, airtight film that conforms to the skin.

Following the procedure of Li et al.,[18] this bandage was used to visualize and quantify tissue oxygenation of full thickness burns in fresh ex vivo porcine skin. The brightness of Clickaphor Red G2 allowed for the rapid acquisition of tissue oxygen tension images using a custom camera (see the Supporting Information). Collected in both intensity and lifetime modes, the oxygen consumption maps obtained with the Clickaphor Red G2 bandage (Figure 3) clearly show the extent of burned skin, which exhibits reduced oxygen consumption arising from necrosis. The burn can be readily identified as a clear, dark, non-O_2-consuming region within the tissue.

The "clickability" and bright emission of this new group of oxygen sensors enable both the modular assembly of bio-

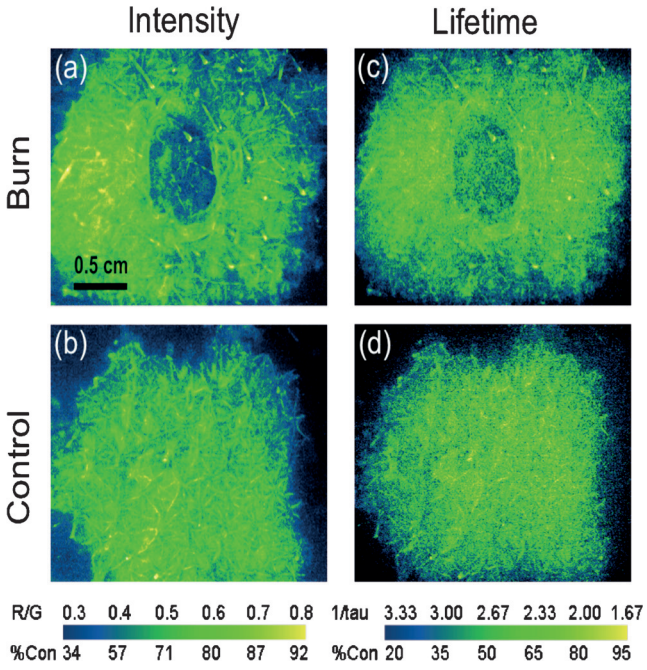

Figure 3. Oxygen consumption maps, expressed as normalized percentage oxygen consumption, obtained with Clickaphor Red G2 in burned tissue on freshly excised porcine skin. Images shown for a, b) circular burn and control skin imaged using the intensity-based ratiometric approach; c, d) circular burn and control skin imaged using the lifetime approach; R/G: red-to-green ratio; %Con: percentage oxygen consumption. The information presented in these images was obtained from Matlab analysis of the raw data acquired with a custom-modified camera.[18]

compatible, oxygen-sensing dendrimers and, more importantly, the straightforward detection of pO_2 by naked eye under ordinary room lighting conditions. The synthetic route reported here, a marked improvement upon earlier porphyrin syntheses, provides eight sites for functionalization. The convenient absorption and emission profiles of these probes eliminate the need for complex optical setups. Lastly, the ability to mass produce both the porphyrin sensors and the bandage formulation should make large-scale oxygen-sensing applications, such as the noninvasive monitoring of wounds and grafts, readily available to a broad pool of clinicians and researchers.

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