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**PEGylated meso-arylporpholactone metal complexes as optical cyanide sensors in water**†

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The colorimetric cyanide sensing ability of free base porpholactone, a pyrrole-modified porphyrin in which a porphyrin β,β'-double bond was replaced by a lactone functionality, and its zinc(II), platinum(II), and gallium(III) complexes in aqueous solution are reported. Water-solubility of the parent meso-pentafluorophenyl-derivatized porphyrinoids was assured by PEGylation of the p-aryl position using a nucleophilic aromatic substitution reaction with thiol-terminated PEG chain. A central metal-dependent sensing mechanism was revealed: While the CN⁻ adds to the zinc(II) complex as an axial ligand, resulting in a minor response in its UV-vis spectrum, it undergoes a nucleophilic addition to the lactone moiety in the platinum(II) and gallium(III) complexes, leading to a much more prominent optical response. Nonetheless, these chemosensors are less sensitive than many other sensors reported previously, with detection limits at pH 7 for the zinc, gallium, and platinum complexes of 2 mM (50 ppm), 240 μM (6 ppm), and 4 mM (100 ppm), respectively. The gallium(III) complex is weakly fluorescent (φ = 0.8%) and cyanide addition leads to fluorescence intensity quenching; the cyanide adduct responds with a fluorescence switch-on response but the signal is weak (φ < 10⁻²%). Lastly, we report on the fabrication of a unique optical cyanide-sensing membrane. The PEGylated gallium-complex was incorporated into a Nafion® membrane (on a PTFE carrier film). It was shown to be stable over extended periods of time and exhibiting a reversible and selective response within minutes to cyanide, with a 5 mM (130 ppm) detection limit. This largely fundamental study on the ability to utilize the once rare but now readily available class of pyrrole-modified porphyrins as chemosensors highlights the multiple principle ways this chromophore platform can be modified and utilized.

**Introduction**

The high toxicity of cyanide (CN⁻), combined with its widespread technical use, urge the development of sensing methods for this anion.¹ As little as 0.5-3.5 mg cyanide per kg of body weight are lethal to humans.¹, ² Cyanide inhibits cellular respiration. The primary toxic effect of cyanide lies in its ability to strongly bind to iron in heme proteins, whereby the crucial target in the mammalian body is cytochrome c oxidase.

Cyanides (typically in the form of cyanogenic glycosides) are widespread in nature. They occur in certain seeds and fruit stones, such as those of apple, mango, peach, and bitter almonds, and in cassava, bamboo shoots, and linseed.¹, ² Large quantities of cyanides are used in industry, e.g., in the synthesis of plastics, inks, fertilizers, nylon, fishing, tanning, gold mining, for the case hardening of steel, and in metal electroplating.¹, ² Its wide-spread use raises the risk for accidental or intentional release into the water stream. The WHO recommended limit for cyanide in drinking water is 0.07 mg/L.²

Optical chemosensors hold much appeal for the simple and rapid chemoselective detection of many analytes,³, ⁴ including cyanide. The current state of the art of colorimetric and fluorometric cyanide chemosensing methods was recently reviewed.³ Some of the detection methods rely on hydrogen bonding interactions but they frequently suffer from poor selectivity with respect to other anions.⁵, ⁸ Reaction-based sensors (or non-reversible chemodosimeters) take advantage of the nucleophilicity of cyanide toward electron-poor carbonyl groups (or derivatives) attached to a chromophore,⁵, ¹⁵ the strong affinity of cyanide for metal ions,¹⁶-²⁰ the ability of cyanide to form Lewis acid-base pairs with triarylboranes,²¹ or the ring-opening of a pyrylium moiety. The resulting changes of the optical spectra or luminescence lifetimes upon cyanide addition were tracked.¹, ¹⁸ Even though real life cyanide detection is, in most cases, expected to take place in water, most sensors reported to date require organic solvents or a mixture of an organic solvent with some water.¹, ⁶, ⁸, ¹⁰, ¹³, ²¹ Only a few sensors were reported to operate in a purely aqueous system.¹⁶, ¹⁷, ¹⁹, ²⁰
For their strong colors, fluorescence, electrochemical activity, ability to coordinate to a variety of metals, and chemical stability, porphyrins and many other porphyrinoids are well suited for use in sensing applications. \cite{22-27} Metalloporphyrins can act as cyanide sensors because of the intrinsic affinity of cyanide for some metals coordinated to the porphyrin, such as zinc, rhodium, copper, silver and gold, thus exchanging or adding axial ligands. \cite{28-33} In this fashion, [meso-tetraphenylporphyrinato]zinc(II) (1Zn), \cite{29,33} [meso-tetrakis(4-carboxyphenyl)porphyrinato]copper(II), \cite{30} or [meso-tetrakis(4-sulfonatophenyl)porphyrinato]rhodium(III) (2Rh) \cite{33} were used for the optical detection of cyanide (Figure 1). One refined approach utilized a two-point recognition of sodium or potassium cyanide by zinc porphyrin crown ether conjugate 3Zn. \cite{31,32} However, the coordination of the cyanide to the central metal generally elicits only modest changes to the optical spectra of the metalloporphyrins, diminishing their practicality as sensors (see also below). \cite{25-33}

Porpholactones 5 are derived from porphyrins 4 by replacement of a β,β’-double bond with a lactone functionality; in effect, a pyrrole moiety was exchanged for an oxazoline. \cite{26,34-37} Though known for some time, \cite{34,35} they have only more recently become readily available along two complementary routes: The one-step Ru-catalyzed direct oxidation of a porphyrin, \cite{38} or a two-step route involving the OsO₄-mediated dihydroxylation of a porphyrin, followed by MnO₂-oxidation of the intermediate dihydroxychlorin (Scheme 1). \cite{26,37,39,40} Both pathways produce the porpholactones 5 and 6 in good yields.

![Scheme 1](image)

**Scheme 1.** Formation of tetraarylporpholactone 5 and 6 by oxidation of the corresponding porphyrins 4. **Reaction Conditions:** (i) For R = Ph: \cite{26,41} 1. OsO₄, pyridine, CHCl₃, r.t., up to 7 d; 2. H₂S, followed by column chromatography; 3. Cetyltrimethylammoniumpermanganate (CTAP), CH₃CO₂H, r.t., 12 h, followed by column chromatography. (ii) For R = C₆F₅: \cite{39} 1. OsO₄, pyridine, CHCl₃, r.t., 24 h, then removal of ~50% solvent by rotary evaporation; 2. Addition of a saturated MeOH/H₂O (1:1) solution of NaHSO₃ to the reaction mixture, r.t., up to 2 d, followed by column chromatography; 3. CTAP, CH₃CO₂H, r.t., ~3 h, followed by column chromatography.

The introduction of the external lactone functionality into the chromophore influences its electronic properties. \cite{35,37} The lactone functionality can also serve as a synthetic handle toward the generation of other porphyrinoids. \cite{27,37,42,43} Most important in the current context, the lactone functionality at the periphery of the macrocycle is electronically strongly coupled to the chromophore. This renders porpholactones to be excellent sensor platforms: The lactone moiety serves as the key functionality in the use of 6Pt, and related derivatives, as high pH indicators. \cite{26,44} At pH values above 11.5, hydroxide attacks the lactone carbonyl group, forming a cyclic orthoester anion. This sp²→sp³ conversion of the lactone β-carbon induces a ~100 nm red-shift of the optical spectrum. Interestingly, the presence of the lactone moiety also indirectly influences the sensing ability of the chromophore as the [porpholactonato]platinum(II) complex 6Pt proved to be a more effective oxygen-sensing dye in pressure-sensing paints than the corresponding porphyrin platinum complex. \cite{45-47} We have recently also demonstrated that the porpholactone chromophore can be modulated by conversion to a thionolactone. \cite{27} The hypochlorite-selective conversion of the non-fluorescent thionolactone to the fluorescent lactone forms the basis for the use of thionoporpholactones as fluorescence-based chemodosimeters for hypochlorite. \cite{27}

Herein we report the evaluation of a number of metalloporpholactones as colorimetric and fluorimetric cyanide sensors, whereby we directly compare the effectiveness of cyanide sensing by coordination to a central metal (in the case of the Zn(II) complexes) or by nucleophilic attack on the lactone carbonyl group (in the free base and Pt(II) and Ga(III)Cl complexes). Importantly, the particular meso-pentafluorophenyl-substituted porpholactones utilized are readily susceptible to conversion to novel water-soluble derivatives. Thus, we report on the selective and sensitive detection of cyanide in pure water. Lastly, we report on the incorporation of the sensitive [porphyrinato]gallium(III) chloride-based sensor dye into a Nafion® membrane, resulting in the formation of chemically robust optical cyanide sensor membranes.

**Results and Discussion**

**Syntheses of Metalloporpholactones 6M**

The metalloporpholactones, known 6Zn, 6Pt \cite{26,44} and novel derivative 6Ga, were synthesized by metatation of free base meso-tetrakis(pentafluorophenyl)porpholactone 6 using standard reaction conditions (in case of 6Ga, microwave heating) (Scheme 2). \cite{48-50} The novel gallium(III) complex showed all the expected spectroscopic and analytical properties (see ESI). It was prepared as the square pyramidal complex carrying an axial chloride ligand, thus forming an overall neutral complex.
Synthesis of PEGylated Metalloporpholactones 6M-MET/6M-PEG1000

meso-Pentafluorophenyl groups are susceptible to a nucleophilic aromatic substitution of the $p$-F atom with soft nucleophiles, such as sulfides. This reaction was frequently utilized to functionalize porphyrins.\textsuperscript{51-57} We have also shown that meso-pentafluorophenyl-substituted oxazolochlorins are susceptible to this reaction, allowing the preparation of water-soluble derivatives when using thiol-terminated PEG chains as nucleophiles.\textsuperscript{58} As expected, the related meso-pentafluorophenyl-substituted metalloporpholactones 6M are also susceptible to this reaction. Thus, reaction of 6M with at least a 4-fold molar excess of 2-(2-methoxyethoxy)ethanethiol (MET-H) under basic conditions (33% NET$_3$ in DMF) provided 6M-MET in excellent yields (Scheme 2).

The clean and diagnostic $^1$H NMR spectra of these compounds confirmed the presence of four short MET chains. However, full water-solubility could not be achieved with the short chains. We therefore reacted 6M with a longer thiol-terminated PEG of average molecular weight of 1000 amu, PEG$_{1000}$. However, the molecular weight heterogeneity of the longer PEG chains made the characterization of the resulting less homogenous products more difficult. ESI-MS allowed only the detection of peak clusters around a mass that suggested tetra-substitution had taken place and the $^1$H NMR of the compounds was dominated by the large number of methylene protons. On the other hand, the products 6M-PEG$_{1000}$ proved to be freely water-soluble. They also possessed optical spectra that were comparable to those of the parent compounds in organic solvents (see below). In the sensing experiments described in the following, we use only the PEG$_{1000}$ derivatives in purely aqueous solutions (or aqueous phosphate buffers).

**Optical Properties of 6M and their PEGylated Derivatives**

As described previously, the UV-vis spectra of free base porpholactones in organic solvents are porphyrin-like, and those of their metal complexes possess characteristics of metallochlorins.\textsuperscript{35, 37} The introduction of the thiol PEG chains to the meso-aryl groups of the porpholactones affects their optical spectra only to a minor degree: The spectra do not change their characteristic shapes but blue-shift their absorption peak maximia a few nm. The compounds are subject to larger, but overall still small, solvochrome shifts. Taken together, the high-energy bands in the UV-vis spectrum of 6-PEG$_{1000}$ in water ($\lambda_{\text{max}}$ values at 417, 514, 552, 589, and 643 nm; Figure 1A) are up to 11 nm bathochromically shifted compared to the corresponding bands of the parent compound 6 in CH$_2$Cl$_2$ ($\lambda_{\text{max}}$ values at 406, 508, 542, 590, and 644 nm);\textsuperscript{35} of note is that the longest wavelength transition is essentially unchanged. The metallochlorin-like spectra of the PEGylated platinum(II) complexes are, except for smaller shifts, likewise as previously described for the platinum porpholactone complexes, respectively (Figures 1B).\textsuperscript{36} The spectra of the novel gallium metallochlorin complexes are also as expected,\textsuperscript{36-40} and resemble those of the zinc complex (cf. Figure 1C and Figure 2A), that itself is as described previously.\textsuperscript{36}

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Scheme 2. Formation of metalloporpholactones 6M by insertion of metal ions into free base lactone 6 and formation of the PEGylated derivatives. Reaction Conditions: (i) Zn(OAc)$_2$/MeOH, CH$_2$Cl$_2$, reflux; (ii) $\text{PhNC}_2$, reflux; (iii) GaCl$_3$, NaOAc, 3H$_2$O, CH$_3$CO$_2$H, MW; (iv) H-MET or H-PEG$_{1000}$, DMF, NET$_3$ (2:1); reflux.
The addition of cyanide (in the form of an aqueous solution of NaCN), but their response profiles vary from each other upon addition of cyanide (Figure 1C). The optical response to cyanide is qualitatively equivalent, albeit slower, to that observed for 6Pt (and other metal complexes) when exposed to pH values above 11.5. Thus, we interpret the cyanide-induced shift of the optical spectrum correspondingly, namely as a nucleophilic attack of CN⁻ onto the lactone. This converts the sp²-hybridized lactone carbonyl to an sp³-hybridized cyanohydrin-like β-carbon (Scheme 3). Chemical reduction of the lactone moiety also leads to large optical changes in the resulting chromophore. The anionic charge of the cyanohydrin-like addition species is likely stabilized by interaction with the adjacent pentafluorophenyl moiety. The design of multiple other cyanide sensors also relied on the ability of cyanide to attack carbonyl groups (or vinylogous carbonyl groups or carbonyl derivatives). Some of the resulting cyanohydrin adducts were also stabilized through intramolecular interactions, mainly hydrogen bonds.

Colorimetric Cyanide Sensing

**Free Base, Pt-, and Ga-Complexes.** Free base porpholactone 6-PEG₁₀₀₀, as well as the two metal complexes 6Pt-PEG₁₀₀₀, 6Zn-PEG₁₀₀₀, and 6GaCl-PEG₁₀₀₀ showed an optical response upon addition of cyanide (in the form of an aqueous solution of NaCN), but their response profiles vary from each other (Figures 1A through 1C; the zinc complex is discussed below, Figure 2). Addition of cyanide to unmetallated 6-PEG₁₀₀₀ caused a color change from purple-orange to yellow-brown (Figure 1A). The pink color of 6Pt-PEG₁₀₀₀ switched to light pink with an orange tinge upon cyanide addition (Figure 1B). The addition of cyanide led to a decrease of the Soret band, the regular metallo-chlorin-type side bands diminished in intensity, and a single new, broad, and much red-shifted band appeared (λ_max = 703 nm). In µM dye solutions, the color changes require at ambient temperature several minutes to fully develop for the platinum 6Pt-PEG₁₀₀₀ (not shown) and gallium 6GaCl-PEG₁₀₀₀ complexes (Figure 3).

The optical response to cyanide is qualitatively equivalent, albeit slower, to that observed for 6Pt (and other metal complexes) when exposed to pH values above 11.5. Thus, we interpret the cyanide-induced shift of the optical spectrum correspondingly, namely as a nucleophilic attack of CN⁻ onto the lactone. This converts the sp²-hybridized lactone carbonyl to an sp³-hybridized cyanohydrin-like β-carbon (Scheme 3). Chemical reduction of the lactone moiety also leads to large optical changes in the resulting chromophore. The anionic charge of the cyanohydrin-like addition species is likely stabilized by interaction with the adjacent pentafluorophenyl moiety. The design of multiple other cyanide sensors also relied on the ability of cyanide to attack carbonyl groups (or vinylogous carbonyl groups or carbonyl derivatives). Some of the resulting cyanohydrin adducts were also stabilized through intramolecular interactions, mainly hydrogen bonds.

**Scheme 3.** Proposed cyanide sensing mechanism of 6Pt-PEG₁₀₀₀ and 6Zn-PEG₁₀₀₀.

Free base porpholactone 6-PEG₁₀₀₀ showed a qualitatively similar response (Figure 1A) to the corresponding platinum complex 6Pt-PEG₁₀₀₀ that we therefore also explain with a
nucleophilic CN\(^{-}\) attack onto the lactone. This is interesting as exposure of free base porpholactone 6 to more basic—though less nucleophilic—hydroxide ions merely deprotonated the inner nitrogen(s).\(^{26}\) The response of the platinum complex 6Pt-PEG\(_{1000}\) toward cyanide under identical [CN\(^{-}\)] is significantly stronger than that of the free base 6-PEG\(_{1000}\) (cf. Fig. 1A and 1B), a finding that does not surprise. The presence of a dication of a strongly electronegative element in the center of the porphyrin is expected to increase the susceptibility of the ligand to nucleophilic attack.\(^{24}\) At pH 7, we determined a detection limit for CN\(^{-}\) using 6Pt-PEG\(_{1000}\) of 4.0 mM; at a higher pH of 8.0, of 0.9 mM (all detection limits and K\(_d\) values are summarized in Table 1). Cyanide is the conjugated base of the weak acid HCN (with a pK\(_a\) value of 9.2). Thus, the higher pH value increases the effective cyanide concentration. Free base base 6-PEG\(_{1000}\) possesses a detection limit of 1.5 mM.

<table>
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<th>Table 1. Cyanide sensing data</th>
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<td><strong>Cyanide Detection Limit</strong></td>
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<td><strong>Concentration</strong></td>
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<td>6-PEG(_{1000})^{a}</td>
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\(^{a}\) at pH 7.0 \(^{b}\) Determined as 3σ/m, with σ = standard deviation, and m = slope of the response at the lower linear limit, ΔA/Δ[CN\(^{-}\)]\(^{10}\)

The green color of 6Ga-PEG\(_{1000}\) switched to yellow upon cyanide addition. The response profile of the gallium complex 6Ga-PEG\(_{1000}\) to cyanide is similar to that of the platinum complex 6Pt-PEG\(_{1000}\) and thus also suggestive of a nucleophilic attack onto the lactone (Figure 1C). Surprisingly, however, the response is significantly more sensitive compared to the platinum complex, with a detection limit of 240 \(\mu\)M CN\(^{-}\). The increased sensitivity is possibly a reflection of the higher charge of the central metal. We could not find any indication that the hard gallium(III) center has any affinity for cyanide as an axial ligand (in comparison, see for the sensing mechanism of the zinc complexes described previously,\(^{29,31,33}\) and below).

The addition of cyanide to the lactone moiety could be demonstrated to be, as expected, reversible (see also below for the demonstration of reversibility of the dye embedded into a membrane). Thus, addition of molar excess Zn(II) chloride to a solution of 6Ga-PEG\(_{1000}\) and cyanide in pH 7 buffer removes the cyanide from the lactone-cyanohydrin equilibrium by precipitation as Zn(CN)\(_2\). Consequently, the native UV-vis spectrum of the sensor is restored (after the removal of the precipitate by filtration; see ESI).

**Zn-Complexes.** The optical response of the zinc complex 6Zn-PEG\(_{1000}\) to cyanide differs fundamentally from that of the free base, platinum, and gallium complexes. Addition of cyanide causes the green color of the metallochlorin-type spectrum to take on a yellowish tone and only a modest (~10 nm) bathochromic shifts of the Soret and Q bands is observed (Figure 2A). This response is comparable of the shifts of the metalloporphyrin spectra of the metalloporphyrin [mesotetakis(pentafluorophenyl)porphyrinato]zinc(II) (8Zn) lacking the lactone functionality (Figure 2B; water-soluble zinc porphyrin 8Zn was prepared from zinc porphyrin 7Zn analogous to the water-soluble porpholactones (Scheme 4)) and other zinc porphyrin-based sensors,\(^{29,31,33}\) although the visible color change from purple to yellow is more significant. It thus can be attributed to axial ligation of the cyanide to the central metal (Scheme 3). The formation of square pyramidal complexes for the zinc porphyrinoids is well known.\(^{60}\) Evidently, the association constant for this process is significantly higher than for the nucleophilic attack of cyanide to the lactone moiety of 6Zn-PEG\(_{1000}\), for which we find no evidence.

**Scheme 4.** Formation of [porpholactonato]Zn(II) 8Zn-PEG\(_{1000}\). Reaction Conditions: (i) H-PEG\(_{1000}\), DMF, NEt\(_3\) (2:1), reflux.
the zinc lactone complex for CN\(^-\) is high, with a detection limit of 2.0 mM, i.e., in the same order of magnitude as the platinum complex, and lower than for the parent zinc porphyrin 8Zn-PEG\(_{1000}\) (3.4 mM). We attribute this to the generally decreased Lewis basicity of the oxidized porpholactone compared to the porphyrin;\(^{26}\) this renders the coordinated metal ion to be a better Lewis acid,\(^{61}\) with a corresponding higher binding affinity for cyanide (with a K\(_d\) of 14 mM for porpholactone complex 6Zn-PEG\(_{1000}\) compared to 45 mM for the porphyrin complex 8Zn-PEG\(_{1000}\)).

Selectivity of Cyanide Sensing in Aqueous Solutions
In the pH range between 5 and 9, the selectivity of the response for cyanide in water is excellent. At solutions buffered at pH 7 (phosphate buffer) or in water at ambient conditions, no other commonly occurring anion (F\(^-\), Cl\(^-\), Br\(^-\), I\(^-\), AcO\(^-\), ClO\(_4^-\), NO\(_3^-\), NO\(_2^-\), H\(_2\)PO\(_4^-\), N\(_3^-\), SCN\(^-\), or OCN\(^-\)), applied as their Na\(^+\) or K\(^+\) salts elicited a colorimetric response with 6Ga-PEG\(_{1000}\) even in the presence of large (50 molar equiv) excess of the anions (Figure 4). The gallium complex was also suitable to sense mM quantities of CN\(^-\) in the presence of 2 M Cl\(^-\) ions (30-fold molar excess, in the form of NaCl or KCl). Alas, at pH values well below 2, the free base porphyrin will become protonated and change its UV-vis spectrum;\(^{26}\) at pH values above 10, the platinum complex can act, as described previously, as a high pH sensor.\(^{61}\) The ability of the sensor to selectively detect cyanide even in high ionic strength solutions over a broad pH range may make this sensor particularly useful.

Fluorimetric Cyanide Sensing
Fluorimetric sensing modes are generally more sensitive than comparable colorimetric modes.\(^{53}\) The porpholactone zinc complex 6Zn-PEG\(_{1000}\) is, like most zinc porphyrins, highly fluorescent but this compound was deemed not to possess a large enough signal separation to be an attractive cyanide sensor (Figure 2A). Porphyrin platinum complexes are strongly luminescent but this luminescence is partial oxygen pressure-dependent.\(^{47, 64}\) We thus also did not consider this compound to be a practical fluorimetric sensor for cyanide. Gallium porphyrin complexes are chemically robust with respect to the metal oxidation state and their resistance toward demetalation.\(^{65}\) And, like all closed-shell metalloporphyrins, they are fluorescent. These characteristics provided the impetus to prepare and study the gallium(III) complex 6Ga and its PEGylated derivatives 6Ga-MET and 6Ga-PEG\(_{1000}\) in the first place.

Indeed, the porpholactone gallium complex 6Ga-PEG\(_{1000}\) in water is fluorescent. The two-band emission spectrum (\(\lambda_{\text{max}} = 610\) nm) is metallochlorin-like (Figure 5), with a relatively low fluorescence yield \(\phi\) of 0.8%. Addition of cyanide reduces, as expected, the fluorescence intensity by this fluorescent species. In turn, the fluorescence of the cyanide adduct increases (at \(\lambda_{\text{max}} = 745\) nm; note the weak emission in the main graph of Figure 5, amplified in the insert). Most damaging to the potential utility of 6Ga-PEG\(_{1000}\) as a switch-on fluorescent cyanide sensor is that fact, however, that the absolute fluorescence intensity of the cyanohydrin anion species (cf. Scheme 3) is very weak (less than 1% of the intensity of 6Ga-PEG\(_{1000}\), with an estimated \(\phi\) of less than 0.01%). We suspect that the electron-rich cyanohydrin anion species leads to fluorescence quenching through a PET mechanism.\(^{66}\)

![Fig. 4. Relative absorbance intensity response at \(\lambda = 712\) nm of 6Ga-PEG\(_{1000}\) (5.10 \(\times\) 10\(^{-4}\) M) in pH 7 buffer or water upon the addition of various anions (50 equiv, unless noted otherwise).](image1)

![Fig. 5. Fluorescence emission titration of 6Ga-PEG\(_{1000}\) (5.10 \(\times\) 10\(^{-7}\) M) in H\(_2\)O with 0-40 mM aq. NaCN; \(\lambda_{\text{exc}} = \lambda_{\text{em}} = 418\) nm. Insert: Emission intensity in the region between 700 and 800 nm recorded with amplified sensitivity.](image2)

An Optode Membrane for Optical Cyanide-sensing
Optical solid chemosensors (optodes) are based on a substrate-specific change of the absorbance or luminescence of a solid into which a sensor dye is chemically or physically immobilized. Optodes overcome several limitations of solution-based sensing methods. For instance, they are suitable to continuously monitor solution streams and readily allow remote sensing.\(^{67, 68}\) The requirements for an ideal optode are well established and include a strong color change, rapid response time, reversibility, and long-term stability.\(^{67, 68}\) As we will demonstrate, the optode material prepared largely fulfills these requirements.

Water-soluble porphyrins (generally, meso-tetraakis(4-sulphonatophenyl)-substituted derivatives) have been incorporated into Nafion® (a sulfonated polytetrafluoroethylene polymer) before.\(^{59}\) We opted for the incorporation of the most cyanide-sensitive dye, the PEG-derivatized gallium porpholactone complex 6Ga-PEG\(_{1000}\), into a Nafion® membrane. The dye was added to the precursor Nafion® solutions from which the membranes were fabricated (as Nafion®-0.018 mm (0.7 mil) PTFE film–Nafion® sandwich of overall minimum thickness of 0.025 mm (~1-2 mil)) according
to the literature. The Nafion® matrix was chosen for its transparency, high chemical and thermal stability, strength, hydrophilicity, and known ion conduction properties.

Unlike other examples of porphyrins embedded into Nafion® membranes, 6Ga-PEG\textsubscript{1000} does not display any altered optical absorption spectra as compared to the compound in aqueous solution (Figure 6; cf. to Figure 1C). This indicates that it does not form aggregate structures and further highlights the excellent solubilization properties of the PEG chains. Addition of cyanide to a pH 7 buffer solution in which the membrane is mechanically suspended elicits an optical response much similar to that of the dye in solution, with a reduced—but still respectable—sensitivity (detection limit = 5 mM CN\textsuperscript{-}, 130 ppm). We rationalize the abated sensitivity of the dye to its location within an anionic polymer matrix. The reduced sensitivity of this sensor may be of utility in the determination of high cyanide concentrations frequently used in industry.

![Fig. 6. Spectrophotometric CN\textsuperscript{-} titration of the PEGlated porpholactone 6Ga-PEG\textsubscript{1000} in Nafion® membrane in pH 7 phosphate buffer and CN\textsuperscript{-} in the form of an aqueous NaCN solution.](image)

Importantly, the optode material showed a reversible response. Upon exchanging the cyanide solution with cyanide-free pH 7 buffer, the optical response reverted nearly completely within 40 min (Figure 7). The intensity fluctuations observed from one cyanide signal maximum to the next are attributed to slight position changes of the flexible membrane in the solution (for the setup, see ESI). Nonetheless, some increase of the baseline signal was noted. The membrane remained stable and transparent even after days in aqueous solutions.

![Fig. 7. UV-vis signal of the 6Ga-PEG\textsubscript{1000}-containing Nafion® membrane upon repeated exposure to 0.5 M NaCN (in pH 7 buffer) for 10 min, followed by pH 7 buffer for 60 min each.](image)

### Conclusions

We have shown that a class of pyrrole-modified porphyrins carrying a lactone functionality at the chromophore periphery can be utilized as an optical cyanide sensors, albeit these sensors possess only modest sensitivities. The sensing mechanism relies on a nucleophilic attack of the cyanide onto the lactone moiety. The induced change in the hybridization of the carbonyl carbon that is electronically strongly coupled to the chromophore elicits the expression of a dramatically (~100 nm) red-shifted new band and a reduction of the Soret band intensity upon cyanide addition, thus enabling the ratiometric sensing of cyanide. Further, the pentafluorophenyl-substituted derivatives utilized allowed the conversion to freely water-soluble derivatives through PEGylation, thus allowing the cyanide sensing in purely aqueous solutions. We also demonstrated the influence of the presence and nature of the central metal on the sensing profile.

From a practical colorimetric assay point of view, the platinum and gallium complexes are the best sensors. The more costly platinum complex 6Pt-PEG\textsubscript{1000} possesses a larger colorimetric response (i.e. larger relative increase of the intensity of the band at 703 nm), while the less costly gallium complex 6Ga-PEG\textsubscript{1000} possesses the highest sensitivity. The ~100 nm peak separation between the spectra of the ‘off’ and ‘on’ stages of both sensors are similar and equally attractive, making these sensors suitable for ratiometric sensing methods, and distinguish these sensors from their zinc analogue and most other known cyanide sensors.

The gallium(III) complex is also a fluorescence switch-on sensor for cyanide but the signal is very weak. Only weakly fluorescent in water in its native form, its cyanide adduct is further quenched.

Beyond the introduction of a highly selective sensor for cyanide in water suitable to detecting high µM to single digit mM cyanide concentrations, the value of this work lies in the demonstration that the functionalities of suitably pyrrole-modified porphyrinoids can be readily utilized for analyte capture and signal transmission to the porphyrinic chromophore. Once rarities, porpholactones (and closely related derivatives) are now readily accessible. We hope that this third example in which a porpholactone or porpholactone derivative was utilized as a chemosensor (or chemodosimeter), the demonstration of their robustness and strong light absorption qualities, and their ability to be converted to water-soluble derivatives, will inspired further work on the intriguing class of pyrrole-modified porphyrins.

### Experimental Section

All solvents and reagents (Aldrich, Acros) were used as received. For the materials used in the fabrication of the membranes, see below. Flash column silica gel (premium...
grade, 60 Å, 32-63 μm) was provided by Sorbent Technologies, Atlanta, GA. Porpholactone 6 and metallorpholactones 6Zn and 6Pt were prepared as described previously.26, 36, 44 1H and 13C NMR spectra were recorded on a Bruker Avance II 400 instrument in the solvents indicated. UV-vis spectra were recorded on a Cary 50 and fluorescence spectra on a Cary Eclipse spectrophotometer, both Varian Inc. The fluorescence quantum yield (φ) of 6Ga-PEG1000 was determined relative to that of meso-tetraphenylporphyrin (φ = 0.11 in benzene),71 calculated to be 0.08 in CH2Cl2; λex (nm) = 640, High and low resolution mass spectra were provided by the Mass Spectrometry Facilities at the Department of Chemistry, University of Connecticut.

**meso-Tetrakis(4-[2-(methoxyethoxy)ethene][thioxy-2,3,5,6-tetrafluorophenyl]porpholactone](6-MET).**

Free base porpholactone 6 (20 mg, 2.0 × 10⁻⁵ mol) was dissolved in DMF:NET3 (2:1, 10.5 mL). 2-(Methoxyethoxy)-ethanol (4 equiv, 11 μL) was added and the solution heated to reflux for 1 h. The resulting solution was dried by rotary evaporation and purified by column chromatography (silica gel, CH2Cl2/5% MeOH) to provide 6-MET in near-quantitative yield (29 mg). 1H NMR (400 MHz, CDCl3, δ): 8.68-8.70 (m, 1H), 8.34 (d, J = 4.7 Hz, 1H), 8.64 (d, J = 4.7 Hz, 1H), 3.95-3.98 (m, 4H), 3.41-3.46 (m, 4H), 3.30-3.33 (m, 4H), 3.10 (t, J = 3.0 Hz, 1H), 1.07-1.09 (m, 2H) ppm. UV-vis λmax (CH2Cl2) (log ε): 408 (3.10), 502 (3.75), 538 (3.60), 596 (3.55), 634 (3.60) nm. HR-MS (ESI+) calc’d. for C63H54F50N4O3S4 [MH]+: 1457.2389, obs. 1457.2427.

**meso-Tetrakis(4-PEG1000[thiocytoxy-2,3,5,6-fluorophenyl]porpholactone](6-PEG1000).**

Synthesized using 6 (50 mg, 5.0 × 10⁻⁵ mol) and poly(ethylene glycol) methyl ether thiol (average Mn ~ 1,000) (~4 equiv, 202 mg) as described for the synthesis of 6-MET. 1H NMR (400 MHz, CDCl3, δ): 8.68-8.70 (m, 1H), 8.34-8.36 (m, 1H), 8.33-8.35 (m, 4H), 8.29 (s, 1H), 3.40-3.49 (m, >400 Hz), -1.77 (s, 1H), -2.03 (s, 1H) ppm. UV-vis λmax (H2O) (log ε): 417 (1.0), 514 (0.07), 552 (0.05), 589 (0.04), 643 (0.05) nm. HR-MS (ESI+) ionization, 0.5% formic acid in CH3CN, with Q-TOF detection: clusters of peaks corresponding to different degrees of protonation, all indicative of the quadruple addition of PEG1000-S-chains (with n = 18-21).

**[meso-Tetrakis(4-PEG1000(thiocytoxy-2,3,5,6-fluorophenyl)]porpholactonato[platinum(II)](6Pt-PEG1000).**

Prepared from 6Pt (25 mg, 2.1 × 10⁻⁵ mol) dissolved in DMF:Et3N (2:1, 10.5 mL). 2-(Methoxyethoxy)ethane (4 equiv, 12 μL) was added and the solution was heated to reflux for 3 h. The resulting solution was evaporated to dryness by rotary evaporation and purified by column chromatography (silica, CH2Cl2/3% MeOH) to provide 6Pt-MET in near-quantitative yield (31 mg). 1H NMR (400 MHz, CDCl3, δ): 8.71 (t, J = 2.5 Hz, 2H), 8.75 (s, 4H), 3.97-3.90 (m, 8H), 3.76-3.75 (m, 7H), 3.66-3.65 (m, 7H), 3.59-3.56 (m, 1H), 3.48-3.41 (m, 20H), 2.92 (t, J = 6.8 Hz, 1H) ppm. UV-vis (CH3Cl2) (log ε): 396 (5.25), 533 (4.20), 573 (4.79) nm. HR-MS (ESI+) calc’d. for C63H54F50N4O3S4Pt [M+H2O]+: 1667.1908, obs. 1667.3102.

**[meso-Tetrakis(4-PEG1000(thiocytoxy-2,3,5,6-tetrafluorophenyl)]porpholactonato[platinum(II)](6Pt-PEG1000).**

Prepared from 6Pt (50 mg, 4.2 × 10⁻⁵ mol), poly(ethylene glycol) methyl ether thiol (average Mn = 1,000) (~4 equiv, 169 mg) in DMF:Et3N (2:1; 19.5 mL) as described for the synthesis of 6Pt-MET. 1H NMR (400 MHz, CDCl3, δ): 8.76-8.66 (m, 6H), 3.91-3.35 (m, >400 H) ppm. UV-vis (H2O) (log ε): 396 (5.3), 534 (4.2), 573 (4.8) nm. HR-MS (ESI+) ionization, 0.5% formic acid in CH3CN, with Q-TOF detection: clusters of peaks corresponding to different degrees of protonation, all indicative of the quadruple addition of PEG1000-S-chains (with n = 18-21).

**[meso-Tetrakis(4-PEG1000[bis(2,3,5,6-tetrafluorophenyl)]porpholactonato][zinc(II)](6Zn-PEG1000).**

Prepared from 6Zn (30 mg, 2.8 × 10⁻⁵ mol), poly(ethylene glycol) methyl ether thiol (average Mn ~ 1,000) (~4 equiv, 114 mg) in DMF:Et3N (2:1, 10.5 mL) as described for the synthesis of 6Pt-MET. 1H NMR (400 MHz, CDCl3, δ): 8.78-8.75 (m, 1H), 8.72-8.67 (m, 3H), 8.66-8.58 (m, 2H) 3.72-3.32 (m, >400 H) ppm. UV-vis (H2O) (log ε): 424 (5.4), 563 (4.0), 609 (4.4) nm. HR-MS (ESI+) ionization, 0.5% formic acid in CH3CN, with Q-TOF detection: clusters of peaks corresponding to different degrees of protonation, all indicative of the quadruple addition of PEG1000-S-chains (with n = 18-21).

**[meso-Tetrakis(4-PEG1000[bis(2,3,5,6-tetrafluorophenyl)]porpholactonato][zinc(II)](8Zn-PEG1000).**

Prepared from 7Zn (30 mg, 6.05 × 10⁻⁶ mol), poly(ethylene glycol) methyl ether thiol (average Mn ~ 1,000) (~4 equiv, 120 mg) in DMF:Et3N (2:1, 10 mL) as described for the synthesis of 6Pt-MET. 1H NMR (400 MHz, CDCl3, δ): 8.96 (br s, 1H), 3.95 (t, J = 6.4 Hz, 1H), 3.75 (overlapping t, 2H) 3.6-3.4 (m, 47 H; 44 H is theoretical value) ppm. UV-vis (H2O) (log ε): 422 (5.4), 554 (4.3) nm. HR-MS (ESI+) ionization, in CH3CN, with Q-TOF detection: clusters of peaks corresponding to different degrees of protonation, all indicative of the quadruple addition of PEG1000-S-chains (with n = 18-21).

**Chloro-[meso-tetrakis(pentafluorophenyl)]porpholactonato[ gallium(III)](6Ga).**

In a 10 mL thick-walled tube containing a stir bar were placed free base meso-tetrakis(pentafluorophenyl)porpholactone 6 (50 mg, 5.04 × 10⁻⁴ mol), GaCl3 (44 mg, 5 equiv), NaOAc:3H2O (45 mg, 3.14 × 10⁻⁴ mol) and acetic acid (5 mL). The vessel was sealed with a septum and placed in a microwave cavity (CEM Discover Scientific Microwave). Using an initial
microwave power of 200 W, the reaction mixture was heated to 180°C and held at that temperature for 20 min, then cooled to 25°C. The reaction mixture was evaporated to dryness using rotary evaporation and the resulting crude product was purified by column chromatography (silica, CH₂Cl₂/50% hexane) to give 6Ga (22 mg) in 40% yield, with 55% recovery of 6. Rf (silica, CH₂Cl₂/50% hexane) = 0.39. UV-vis λmax (CH₂Cl₂) (log ε): 418 (5.25), 564 (3.80), 611 (4.42) nm. IR: v = 1782.6 (C=O) cm. Fluorescence (CH₂Cl₂, λex = 418) (Rel. Int.) 614 (1.0), 673 (0.09) nm, φ = 0.008. 1H NMR (400 MHz, CDCl₃, δ): 9.00 (t, J = 5.3 Hz, 1H), 8.93 (dq, J = 16, 4.3 Hz, 3H), 8.90 (dt, J = 8.9, 4.6 Hz, 2H), 1H ppm. 13C NMR (100 MHz, CDCl₃, δ): 170.9, 168.9, 163.2, 154.1, 153.1, 150.9, 150.5, 150.0, 147.2, 147.1, 145.1, 144.2, 141.7, 139.7, 139.53, 139.51, 137.2, 137.1, 137.0, 136.9, 134.8, 133.0, 132.9, 132.9, 131.8, 131.2, 131.1, 130.1, 129.1, 126.3, 114.3, 111.0, 110.4, 106.6, 104.4, 86.9, 66.5, 66.4, 64.6, 56.1, 53.7, 32.2, 31.1, 29.9, 24.0, 22.8, 20.5, 14.4 ppm. HR-MS (ESI⁺ ionization, 0.5% formic acid as solvent) for C₅₅H₆₁Cl₆Ga₁₀N₁₀O₉S₄, [M+Cl⁺]**: calc'd. 1058.9427, obs. 1058.9401; for C₄₃H₅₀F₂₄Ga₆N₂₀₂₃, [M-Cl+H⁺]**: calc'd. 1076.9534, obs. 1076.9499.

Chloro-meso-tetakis(4-[2-(methoxyethoxy)ethane]thioxotetra-2,3,5,6-fluorophenyl)porphaloncaton[gallium(II) (2Ga-MET)]

Prepared from complex 6Ga (22 mg, 2.0 × 10⁻⁵ mol) dissolved in DMF:Et₃N (2:1, 10.5 mL) 2-(ethoxyethoxy)ethanethiol (4 equiv, 1 mL) as described for the preparation of 6Pt-MET. Chromatography (silica gel, CH₂Cl₂:3% MeOH) provided 6Ga-MET in near-quantitative yield (29 mg). 1H NMR (400 MHz, CDCl₃, δ): 9.04-8.84 (m, 6H), 3.99-3.90 (m, 8H), 3.78-3.75 (m, 7H), 3.65 (d, J = 3.2 Hz, 7H), 3.49-3.39 (m, 22H) ppm. UV-vis λmax (CH₂Cl₂) (log ε): 408 (5.20), 560 (3.60), 607 (4.30) nm. HR-MS (ESI⁺) calc'd. for C₅₀H₅₀Cl₁₆Ga₁₀N₁₆O₆S₄, [M+Cl⁺]**: 1523.1405, obs. 1523.1370.

Chloro-meso-tetakis(4-PEG₁₀₀₀(thioxotetra-2,3,5,6-fluorophenyl)porphaloncaton[gallium(III) (6Ga-PEG₁₀₀₀)]

This compound was prepared from the gallium complex 6Ga (20 mg, 1.8 × 10⁻⁵ mol), poly(ethylene glycol) methyl ether thiol (average Mn, 1,000) (4 equiv, 73 mg) in DMF:Et₃N (2:1, 10.5 mL) as described for the synthesis of 6Pt-PEG₁₀₀₀. 1H NMR (400 MHz, CDCl₃, δ): 9.01-8.79 (m, 6H), 3.75-3.38 (m, 400 H) ppm. UV-vis λmax (H₂O) (log ε): 419 (5.4), 563 (4.0), 611 (4.5) nm. Fluorescence (CH₂Cl₂, λex = 418) 745 nm, φ = < 1.0 × 10⁻³. 008. HR-MS (ESI⁺ ionization, 0.5% formic acid in CH₃CN, with Q-TOF detection): clusters of peaks of corresponding to different degrees of protonation, all indicative of the quadruple addition of PEG₁₀₀₀-S-chains (with n = 18-21).

UV-Vis and Fluorescence Spectroscopic Titrations

All titrations were carried out in standard 1 cm path length methacrylate cuvettes (Fisher) in H₂O or the buffers indicated at ambient temperature. The PEGylated dyes 6-PEG₁₀₀₀, 6Pt-PEG₁₀₀₀ and 6Zn-PEG₁₀₀₀ were dissolved in H₂O and diluted to 10⁻⁶ M concentrations. At pH 7, 5.0 M sodium cyanide (NaCN) stock solution in H₂O was used as the source of CN⁻. The absorbance was measured as aliquots of the aqueous CN⁻ solution were added by micropipette to 3 mL of the sensor dye solutions (the absolute quantity of the additions ranged typically below 100 µL). The concentration of 6Ga-PEG₁₀₀₀ in the UV-vis titrations as well as in the fluorescence measurements was 5.10 × 10⁻⁶ M and 1.0 × 10⁻⁵ M sodium cyanide stock solution was used as the source of CN⁻. All spectra are uncorrected.

Two 1.5 cm diameter stainless steel washers were braised parallel to each other to the ends of a pair of stainless steel curved tweezers. A ~1.6 cm diameter piece of the sensor membrane was held between the washers and the tweezers were held clamped shut using a metal clip. The washers holding the membrane were submerged in a 5 × 5 × 1 cm glass cuvette containing the desired aqueous solutions, with the 1 cm path length of the cuvette perpendicular to the washer, and the hole in the washer carefully placed into the center of the beam of the UV-vis spectrometer.

Sensing Membrane

The Nafion® membranes were prepared as Nafion®-PTFE-Nafion® sandwiches using Tetratex PTFE Film (0.7 mil) and using Nafion® solutions (Ion Power, Inc., 5% DuPont™ D521 Nafion® solution in H₂O/PrOH, EtOH) according to methods adopted from the literature.⁷⁰

The dye 6Ga-PEG₁₀₀₀ (4 mg, 7.9 × 10⁻⁷ mol) was dissolved in 13 g (~14 mL) of the commercial Nafion® solution and the solution was magnetically stirred at ambient temperature for ~15 min. Then 11 mL of EtOH and 3 mL of DMF were added and once again stirred for ~15 min. In a fume hood, a large stone tile was precisely leveled and a box was fitted over it that allowed a nitrogen stream to flow through it (and to block the light). A sheet of PTFE film was stretched tightly onto a 5 × 5 clean glass plate with polished edges; excess film was rolled up to make sure none is underneath the plate. The glass was placed on the leveled tile and a ~1.6 cm diameter piece of the sensor film sticking out was then transferred from the edge of the first water were placed on the membrane surface and a second, clean tile was precisely leveled onto the first glass was placed on top. The plate was pushed down to make sure none is underneath the plate. T-shaped tweezers were held clamped parallel to each other to the ends of a pair of stainless steel curved tweezers. A ~1.6 cm diameter piece of the sensor membrane was held between the washers and the tweezers were held clamped shut using a metal clip. The washers holding the membrane were submerged in a 5 × 5 × 1 cm glass cuvette containing the desired aqueous solutions, with the 1 cm path length of the cuvette perpendicular to the washer, and the hole in the washer carefully placed into the center of the beam of the UV-vis spectrometer.

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plate to edge of the new plate. The plates were then pulled apart carefully while adding drops of DI water into the small gap forming between the old glass plate and the membrane (this may require the use of four hands). The membrane thus transferred was then stretched evenly along the new plate by hand to smooth out any wrinkles (nitrile gloves). The membrane was dried of any remaining DI water using a dust-free paper wipe. The second half of the solution was added onto the flipside of the membrane using the procedure described above, except that the EtOH priming stage was omitted. After 24 h drying time, the plate was moved into a vacuum oven and drying at 150 °C and 25 torr. The membrane was peeled off carefully with the help of DI water between the membrane and the glass plate. After air drying, the membranes were, cut into smaller pieces, and used as is.

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Notes and references

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† Electronic Supplementary Information (ESI) available: A reproduction of key 1H, 13C NMR, IR, UV-vis, and fluorescence spectra of the novel compound described herein, including images of the setup and a membrane. See DOI: 10.1039/b000000x/

G. Sancenon, and P. Gavina, (correction of systemic typesetting errors).

