

Conjugated Metallopolymers for Fluorescent Turn-On Detection of Nitric Oxide

Rhett C. Smith, Andrew G. Tennyson, Annie C. Won, and Stephen J. Lippard*

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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A series of eight π -conjugated polymers (CPs) composed of phenylenevinylene, phenyleneethynylene, fluorene, and thiophene derivatives have been prepared with bipyridyl or terpyridyl substituents within the π -conjugated backbone or at side-chain positions. These ligand-modified CPs serve as macromolecular scaffolds for conducting metallopolymers. The optical and photoluminescent properties of the polymers and corresponding copper(II) metallopolymers were investigated. Copper(II) is a highly efficient quencher of CP emission (75–100% quenching). CPs featuring bipyridyl units within the CP backbone are quenched more efficiently than those with terpyridyl units. The copper(II) metallopolymer undergoes reduction to the corresponding copper(I) species upon reaction with nitric oxide, with concomitant changes in integrated emission ranging from a 50% decrease to a 320% increase. The positive emission response is largest when Cu(II) was bound to the CP through bipyridyl units within the backbone, making these materials the best candidates for NO sensing by a turn-on emission mechanism.

Introduction

Nitric oxide (NO) is one of the most fascinating and enigmatic biological signaling molecules.^{1–3} The involvement of NO in the physiology and pathophysiology of neurodegenerative disease,^{4–6} cancer,^{7–9} vasodilation,^{1–3} and learning/memory formation^{10,11} has sparked the imagination of researchers. Unfortunately, conclusions drawn from these multifaceted investigations often rely upon analytical techniques that detect NO reaction products such as nitrite or nitrate rather than NO itself.^{12–17} The absence of NO-specific sensors capable of reporting biologically relevant concentra-

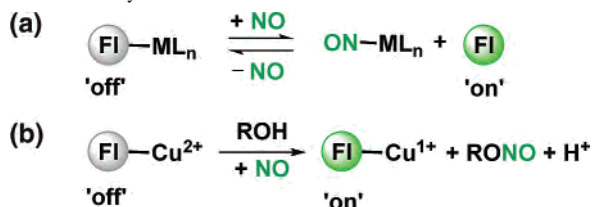
tions of NO has promulgated conflicting hypotheses.⁹ Our laboratory initiated a program to eliminate these ambiguities through the design of fluorescent turn-on sensors for NO.^{18–27} We focused on fluorescent imaging of NO using transition metal complexes incorporating fluorescent reporter ligands that are quenched in the primary coordination sphere of a paramagnetic transition metal center. Transition metal-based sensors operate by two general mechanisms. The first

* To whom correspondence should be addressed. E-mail: lippard@mit.edu.

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Scheme 1. Two Strategies for Transition Metal-Based Fluorescent Detection of Nitric Oxide: (a) Fluorophore Displacement and (b) Reductive Nitrosylation



approach involves fluorophore displacement by NO and formation of a metal nitrosyl (Scheme 1a). Dirhodium tetracarboxylates with dansyl-appended axial ligands are particularly promising candidates for this approach because they can reversibly bind NO/fluorophore in solution with a sensitivity of approximately $10 \mu\text{M}$.^{18,24} Unfortunately, water can also displace axial ligands, precluding direct use in aqueous solution. Physical separation of the sensor from an aqueous sample by encapsulation in a poly(methyl methacrylate) film allows detection of NO(aq).¹⁸

More recently, we have explored a second strategy whereby NO may undergo redox chemistry at a paramagnetic transition metal center affording a diamagnetic complex with attendant emission turn-on. Insightful fundamental studies demonstrated that, upon reaction with NO in the presence of ROH, cupric complexes are reduced to the corresponding cuprous forms, accompanied by the generation of RONO and H^+ (Scheme 1b).^{28–42} Inspired by these investigations, we prepared Cu(II) complexes of small molecule and π -conjugated polymer (CP) fluorophores. These complexes display enhanced emission intensity upon reduction of (paramagnetic, quenching) Cu(II) to (diamagnetic, non-quenching) Cu(I) by NO. Notably, a cell permeable small molecule probe (**FIAQ-Cu**, Chart 1a) operating by ligand nitrosation following Cu(II) reduction is a useful histological stain for imaging NO genesis by nitric oxide synthase (NOS) in living cells by fluorescence microscopy.^{20,43}

Small molecule strategies have been complimented by investigations using conducting metallopolymers (CMPs) as

Chart 1. (a) Small Molecule- and (b) Polymer-Based Fluorophores Used as Reporter Elements in NO Detection

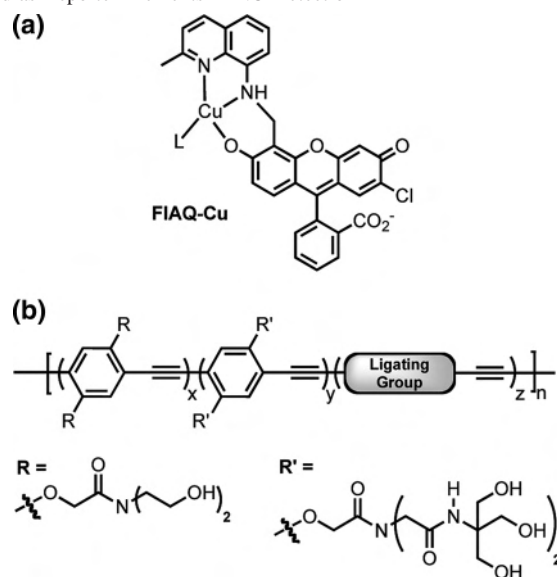
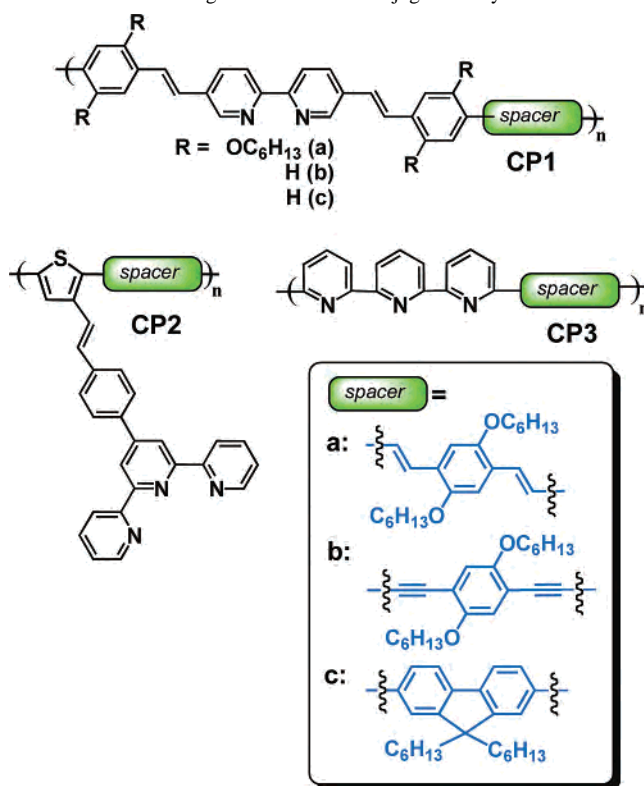


Chart 2. Series of Ligand-Substituted Conjugated Polymers Studied



reporter elements.^{21,25} We initially employed a Cu(II) complex of a bipyridyl-modified poly(*p*-phenylenevinylene) (PPV) derivative (**CP1a**, Chart 2) as the NO receptor/reporter.²⁵ In 4:1 dichloromethane/ethanol, a 2.8-fold emission enhancement was observed upon exposure to NO(g). Double integration of the EPR signal of **CP1a-Cu(II)** prior to and following treatment with excess NO(g) showed a decrease in signal intensity, confirming NO-induced reduction of Cu(II) to Cu(I). Moreover, we demonstrated that Cu(II) significantly quenches the fluorescence of **CP1a**, whereas Cu(I) produces only a slight decrease in integrated emission. NO release from *S*-nitroso-*N*-acetylpenicillamine

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(SNAP) could also be detected by **CP1a**-Cu(II) down to nanomolar concentrations. Although selective for NO over the nitrosonium ion (NO^+), the CMP responded similarly to NO and nitroxyl (HNO), another biological signaling molecule. Selectivity and biocompatibility were improved in a subsequent study using water-soluble CPs (Chart 1b).²¹ Although the emission response of the Cu(II) complexes of these CPs to NO was disappointing in water, a 3.2-fold turn-on was induced upon addition of NO to water-soluble CMPs in acetonitrile/water (95:5).

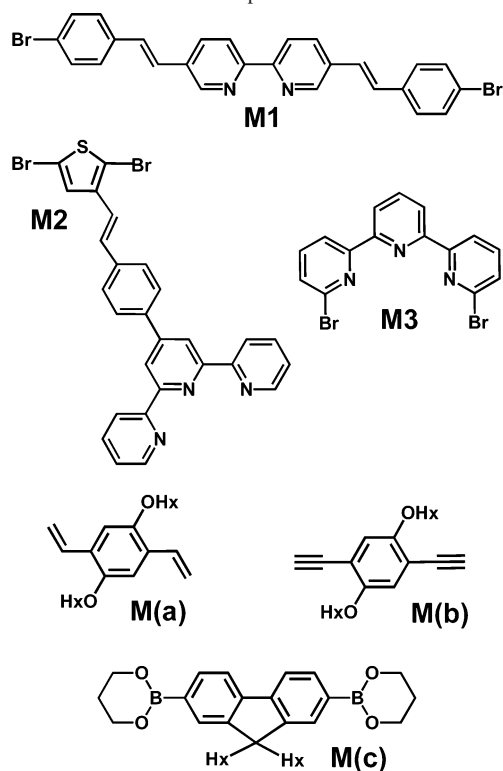
During the course of our investigations on CP-based fluorescent NO sensors, a series of CPs were prepared with the aim of assessing the influence of conjugated backbone identity and metal-ligating unit on the relative fluorescent responses of the systems (Chart 2). Some of the most studied CPs for application to sensory applications are poly(*p*-phenylenevinylene) (PPV), poly(*p*-phenyleneethynylene) (PPE), poly(thiophene) (PT), and poly(flourene) (PF). We therefore incorporated elements of these CPs into our set of potential NO sensors. **CP1a** included bipyridyls within the PPV π -conjugated backbone as the copper binding units.²⁵ In the present study, the set of metal-ligating units has been expanded to include both main- and side-chain terpyridyl groups tethered to a thiophene unit via a π -conjugated linker. Here, we detail the preparation of eight CPs and the response of corresponding Cu(II)-CP complexes to NO(g).

Experimental Section

Materials and Methods. Reagents were obtained from Strem, Aldrich Chemical Co., Mallinckrodt, or Cayman Chemical and used as received. Solvents were dried and purified by passage through alumina columns under an Ar atmosphere. Commercially unavailable compounds **M2**,⁴⁴ **M(a)**,^{45,46} **M(b)**,⁴⁷ **M(c)**⁴⁸ (Chart 3), and **CP1a**²⁵ were prepared by literature procedures. Column chromatography employed a silica gel-60 (230–400 mesh) stationary phase. NMR spectra were recorded in CDCl_3 at 22 °C on a 300 MHz Varian instrument, and chemical shifts are expressed in ppm relative to residual CHCl_3 . Number average molecular weights for new CPs were estimated using ^1H NMR spectroscopic end group analysis. The polymerization reactions were carried out in heavy-walled tubes sealed with Teflon screw caps in an atmosphere of dry nitrogen. Other air- or water-sensitive manipulations were carried out in a dry nitrogen atmosphere using standard Schlenk or glovebox techniques. Nitric oxide gas was purified following a reported procedure³⁶ by passage through an Ascarite column and a 6 ft silica gel coil at -78 °C. Manipulation and transfer of purified NO(g) was performed using gastight syringes in an inert atmosphere.

CP1b. A solution of **M(b)** (33 mg, 0.10 mmol), **M1** (52 mg, 0.10 mmol), $[\text{Pd}(\text{PPh}_3)_4]$ (42 mg, 0.036 mmol), and cuprous iodide (11 mg, 0.060 mmol) in diisopropylamine (5 mL) was heated in a Teflon screw cap-sealed heavy-walled tube at 90 °C for 24 h. The

Chart 3. Monomers Used To Prepare CPs



solution was allowed to cool to room temperature, and 50 mL of CH_2Cl_2 was added. The solution was washed with an equal volume of saturated EDTA(aq) and then water ($\times 2$), and the organic layer was collected. The organic layer was filtered to remove a small amount of precipitate and then concentrated under reduced pressure to give a brick red solid that was rinsed with 10 mL of MeOH and dried in vacuo to yield 22 mg (32%) of **CP1b**. ^1H NMR (CDCl_3): δ 0.7–1.0 (br m, 6 H), 1.1–1.7 (br m, 12 H), 1.7–1.9 (br m, 4 H), 3.9–4.1 (br m, 4 H), 6.5–7.0 (br m, 5 H), 7.1–7.9 (br m, 19 H), 8.0–9.0 (br m, 6 H). $M_n = 6100$ g/mol.

CP1c. A suspension of **M(c)** (49 mg, 0.10 mmol), **M1** (52 mg, 0.10 mmol), Cs_2CO_3 (210 mg, 0.60 mmol), and $[\text{Pd}(\text{PPh}_3)_4]$ (46 mg, 0.040 mmol) in DMF (3 mL) was heated at 100 °C for 24 h. The solution was allowed to cool to room temperature, and 50 mL of CH_2Cl_2 was added. The solution was washed sequentially with saturated EDTA(aq), water, and brine. The solution was filtered and concentrated in vacuo to produce an oil, presumed to be predominantly DMF and **CP1c**. The addition of 5 mL of water caused precipitation of a yellow solid, which was collected by filtration. The solid was washed with MeOH and dried in vacuo to yield 28 mg (41%) of **CP1c**. ^1H NMR (CDCl_3): δ 0.7–1.1 (br, 10 H), 1.1–1.4 (br m, 12 H), 1.8–2.2 (br, 4 H), 6.5–7.0 (br m, 2 H), 7.0–7.9 (br m, 18 H), 7.9–9.0 (br m, 6 H). $M_n = 4100$ g/mol.

CP2a. A suspension of **M2** (58 mg, 0.10 mmol), **M(a)** (33 mg, 0.10 mmol), Cs_2CO_3 (210 mg, 0.60 mmol), and $[\text{Pd}(\text{PPh}_3)_4]$ (42 mg, 0.036 mmol) in DMF (2 mL) was heated at 120 °C for 24 h. The solution was allowed to cool to room temperature, and 100 mL of CH_2Cl_2 was added. The solution was washed with saturated EDTA(aq), water, and brine. The organic layer was filtered and then concentrated under reduced pressure to give a violet solid. The solid was rinsed with MeOH and dried in vacuo to yield 44 mg (59%) of **CP2a**. ^1H NMR (CDCl_3): δ 0.7–1.1 (br m, 6 H), 1.1–1.7 (br m, 12 H), 1.7–2.0 (br, 4 H), 3.9–4.2 (br m, 4 H), 6.8–7.2 (br m, 4 H), 7.0–7.8 (br m, 18 H), 7.8–8.1 (br m, 3 H), 8.5–8.8 (br m, 4 H). $M_n = 5500$ g/mol.

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CP2b. A solution of **M(b)** (30 mg, 0.092 mmol), **M2** (53 mg, 0.092 mmol), [Pd(PPh₃)₄] (42 mg, 0.036 mmol), and cuprous iodide (11 mg, 0.060 mmol) in diisopropylamine (5 mL) was heated in a Teflon screw cap-sealed heavy-walled tube at 90 °C for 24 h. The solution was cooled to room temperature, and 50 mL of CH₂Cl₂ was added. The solution was washed with an equal volume of saturated EDTA(aq), and the organic layer was collected. The organic layer was filtered to remove a small amount of precipitate and then concentrated under reduced pressure to give a brick red solid that was rinsed with 10 mL of MeOH and dried in vacuo to yield 32 mg (43%) of **CP2b**. ¹H NMR (CDCl₃): δ 0.7–1.0 (br m, 6 H), 1.1–1.7 (br m, 12 H), 1.7–2.0 (br, 4 H), 3.9–4.2 (br m, 4 H), 6.8–7.2 (br m, 4 H), 7.2–7.8 (br m, 14 H), 7.8–8.1 (br m, 3 H), 8.6–8.9 (br m, 4 H). *M_n* = 5500 g/mol.

CP2c. A suspension of **M(c)** (49 mg, 0.10 mmol), **M2** (58 mg, 0.10 mmol), Na₂CO₃ (33 mg, 0.30 mmol), and [Pd(PPh₃)₄] (46 mg, 0.040 mmol) in a 1:1 DMF/toluene mixture (6 mL) was heated at 90 °C for 24 h. The solution was cooled to room temperature, and 50 mL of CH₂Cl₂ was added. The solution was washed sequentially with saturated EDTA(aq), water, and brine. The solution was filtered and concentrated in vacuo to produce a yellow-brown solid. The solid was rinsed with refluxing water and MeOH and then dried in vacuo to yield 7.2 mg (10%) of **CP2c**. ¹H NMR (CDCl₃): δ 0.4–1.2 (br m, 22 H), 1.6–2.2 (br, 4 H), 6.6–9.2 (br m, 23 H). *M_n* = 5500 g/mol.

CP3b. Compounds **M3** (0.0196 g, 0.05 mmol), 1,4-dibromo-2,5-dihydroxybenzene (0.044 g, 0.1 mmol), **M(b)** (0.033 g, 0.15 mmol), cuprous iodide (0.014 g, 0.045 mmol), and [Pd(PPh₃)₄] (0.048 g, 0.045 mmol) were stirred in 2.5 mL of diisopropylamine and 8 mL of toluene. The reaction mixture was heated in a sealed heavy-walled tube at 80 °C for 24 h. The organic layers were washed with saturated EDTA(aq), and the aqueous layers were washed with CH₂Cl₂. The organic layers were combined, and the volatiles were removed in vacuo to yield a yellow solid. The solid was washed with water and MeOH to give 9 mg (14%) of **CP3b**. ¹H NMR (CDCl₃): δ 0.7–1.0 (br m, 6 H), 1.1–2.0 (br, 16 H), 3.7–4.3 (br m, 4 H), 7.0–7.8 (br m, 4 H), 7.8–8.1 (br m, 1 H), 8.6–8.9 (br m, 2 H). *M_n* = 6000 g/mol.

CP3c. Compounds **M(c)** (0.074 g, 0.15 mmol), 2,7-dibromo-9,9-dihexylfluorene⁴⁹ (0.041 g, 0.1 mmol), **M3** (0.0196 g, 0.05 mmol), Cs₂CO₃ (0.013 g, 0.45 mmol), and [Pd(PPh₃)₄] (0.048 g, 0.045 mmol) were combined in DMF under N₂. The reaction mixture was stirred at 100 °C for 48 h. The organic layers were washed with saturated EDTA(aq), and the aqueous layers were washed with CH₂Cl₂. The organic layer was evaporated under reduced pressure to yield a yellow powder that was subsequently washed with water and MeOH. The dried product was isolated as a yellow solid (26 mg, 39%). ¹H NMR (CDCl₃): δ 0.4–1.2 (br m, 22 H), 1.4–2.6 (br, 4 H), 6.4–9.0 (br m, 14 H). *M_n* = 5000 g/mol.

Absorption and Fluorescence Spectroscopy. Absorption spectra were recorded on a Cary IE spectrophotometer. Fluorescence spectra were acquired on a Photon Technology International Quanta Master 4L fluorimeter at 22 °C. Slit widths, excitation wavelength, and integration times were kept constant for all experiments pertaining to a given CP. Samples for fluorescence had a path length of 1 cm in Spectrosil quartz cuvettes, with an optical density of <0.08 and volumes of 3 mL in degassed 4:1 dichloromethane/ethanol, unless otherwise noted. Quantum yields (Φ) were determined by using the average of three values from samples with an optical density of <0.04 in Spectrosil quartz cuvettes with a path length of 1 cm.

Table 1. Key Physical Properties of Conjugated Polymers^a

	λ _{max}	log ε	λ _{emit}	Φ _{eff}	I _o /I _{Cu(II)}	I _{NO} /I _{Cu(II)}
CP1a	462	4.64	540	0.30(6)	6.2	2.8
CP1b	380	4.88	430	0.41(4)	58	2.8
CP1c	402	4.32	485	0.44(6)	310	3.2
CP2a	328	4.34	430	0.02(0)	4.2	1.3
CP2b	430	4.40	420	0.05(1)	9.4	1.9
CP2c	285	4.28	475	0.06(0)	22	1.4
CP3b	329	4.98	486	0.17(0)	4	1.9
CP3c	389	4.63	470	0.27(1)	28	0.5

^a Properties in 4:1 dichloromethane/ethanol. Details of spectroscopic measurements are provided in the Experimental Section.

Values were referenced both to fluorescein (0.100 M NaOH, Φ = 0.92) and to NIST-issued quinine bisulfate (0.105 M perchloric acid, Φ = 0.546). Measurements involving Cu(I), NO, or RNS species were carried out in gastight, septum-equipped cuvettes under an atmosphere of nitrogen in degassed solvents as previously described.²⁵

General Procedure for Titration of CPs with Cu(OTf)₂. (See Figures S1–S7 in the Supporting Information.) A solution of CP was prepared in 4:1 dichloromethane/ethanol to give an optical density of <0.08. A 3 mL aliquot of this solution was added to a quartz cell with an optical path length of 1 cm, and the sample cell was sealed with a gastight Teflon-lined screw cap equipped with a septum. Small (5–10 μL) aliquots of a 60 μM solution of Cu(OTf)₂ in 4:1 dichloromethane/ethanol were then added to the sample cell. The cell was agitated, and a fluorescence spectrum was collected before the first aliquot was added and after each aliquot addition. The integrated fluorescence intensity was quenched by between 4- and 330-fold (Table 1).

General Procedure for Assessing Cu(II)–CP Response to NO(g). (See Figures S8–S13 in the Supporting Information.) A solution of the CP with the same concentration used for Cu(II) titrations was prepared in 4:1 dichloromethane/ethanol under an atmosphere of nitrogen. A 3 mL aliquot of this solution was added to each of two matched quartz cells. Each cell had an optical path length of 1 cm and was sealed with a gastight screw cap equipped with a septum. An aliquot of a 60 μM solution of Cu(OTf)₂ in 4:1 dichloromethane/ethanol corresponding to 1 equiv per terpyridyl or 0.5 equiv per bipyridyl was then added through the septum in each sample cell. A sample of NO (~300 equiv) was added to the other identical sample via a gastight syringe through the septum in the cell cap, and the solution was shaken briefly. The change in integrated emission is provided in Table 1.

General Procedure for Assessing CP Response to Nitrosonium Ion. Under an atmosphere of dry nitrogen, a solution of CP with the same concentration used for Cu(II) titrations was prepared in 1:1:3 CH₂Cl₂/EtOH/MeCN. A 3.0 mL aliquot of the solution was added to each of two matched quartz cuvettes. A total of 1 equiv of [Cu(MeCN)₄]BF₄ was added to one cuvette to form CP–Cu(I). An aliquot of a 3.15 mM acetonitrile solution of NOBF₄ (50 equiv) was subsequently added to each cuvette. None of the CP–Cu(II) or CP–Cu(I) complexes exhibited a significant change in the emission spectrum. The addition of 1 equiv of Cu(OTf)₂ to a CP/NOBF₄ solution resulted in a spectrum resembling that of CP1–Cu(II), indicating that nitrosonium does not interfere with metal binding if it is present prior to metal addition.

General Procedure for Assessing CP Response to Nitroxy. Under an atmosphere of dry nitrogen, a solution of the CP with the same concentration used for the Cu(II) titrations was prepared in 1:1:3 CH₂Cl₂/EtOH/MeCN. A 3.0 mL aliquot of the solution was added to each of three matched quartz cuvettes. One equivalent of Cu(OTf)₂ was added to one cuvette to form CP–Cu(II), and 1

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equiv of $[\text{Cu}(\text{MeCN})_4]\text{BF}_4$ was added to another cuvette to form $\text{CP1}-\text{Cu}(\text{I})$. The addition of 16 equiv of Angeli's Salt ($\text{Na}_2\text{N}_2\text{O}_3$, 1 M KOH) to each cuvette was followed by 10 volume equiv of pH 7.4 HEPES buffer to produce HNO from Angeli's Salt. Stocks of Angeli's Salt were prepared at pH 12 and kept frozen until just prior to use. The fluorescent response of each sample was then measured within 60 s of addition. No additional changes in integrated emission were noted up to 20 min.

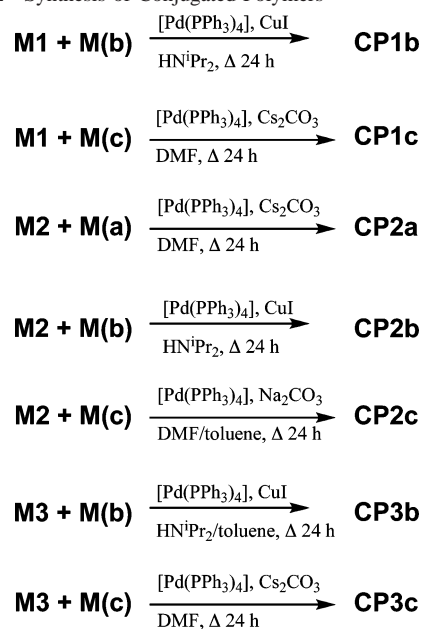
Results and Discussion

Design of Conjugated Polymers. We previously reported a PPV derivative (**CP1a**) with bipyridyl units within the π -conjugated backbone and the positive emission response of $\text{Cu}(\text{II})-\text{CP1a}$ to NO in organic solution.²⁵ The CPs designed for the current study were chosen to increase our understanding of how the CP backbone identity, the metal-ligating unit, and the ligand-CP interface impact emission quenching by $\text{Cu}(\text{II})$ and subsequent emission restoration upon exposure to $\text{NO}(\text{g})$. Bipyridyl- and terpyridyl-substituted CPs have been tested for their response to metal ions and are quenched efficiently by $\text{Cu}(\text{II})$,^{44,50-58} a necessity for turn-on sensing by reductive nitrosylation. Consequently, we selected bipyridyl (**CP1**, Chart 2) and terpyridyl (**CP2** and **CP3**, Chart 2) units as the metal-binding units. The bipyridyl-containing CPs include the previously studied **CP1a** and two similar CPs (**CP1**, Chart 2). Pioneering studies on CPs featuring terpyridyl units tethered to main chain thiophene units via a conjugated linker elucidated the influence of ligand-CP connector on metal-induced spectroscopic response.⁴⁴ Building on this work, we prepared similar CPs incorporating this connector (**CP2**, Chart 2). Two additional CPs with terpyridyl units within the main chain (**CP3**, Chart 2) were prepared for comparison to the CPs with terpyridyl side chains. Furthermore, we anticipated that redox changes and attendant perturbations of inter/intrachain interactions upon metal binding⁵⁹ could lead to greater differences in metal/quenching and analyte/restoring responses.

We employed phenylenevinylene, phenyleneethynylene, and fluorene CP spacers in our initial trials. These units have been thoroughly investigated as components of CPs and CMPs, thus providing a wide range of known systems for comparison.⁶⁰ Additionally, requisite monomers (Chart 3) for these common CP constituents are readily prepared, facilitating rapid synthesis of materials.

Syntheses. **CP1a** was prepared by Wittig condensation,²⁵ and Pd-catalyzed coupling using a modular set of monomers

Scheme 2. Synthesis of Conjugated Polymers



(Chart 3) was employed to prepare the other CPs (Scheme 2). **CP1b** and **CP2b** were prepared by Sonogashira coupling of **M(b)** with **M1** and **M2**, respectively. Suzuki coupling of **M(c)** with **M1** and **M2** produced **CP1c** and **CP2c**, respectively. Although Suzuki coupling can be problematic for the preparation of thiophene-containing CPs,⁶¹⁻⁶³ this concern only applies when thiophene boronic acids are used. We observed no decomposition or side products in the current study with the substituted 2,5-dibromothiophene monomer **M2**. Thiophenevinylene polymer **CP2a** was obtained by Heck coupling of **M2** and **M(a)**. This method is not ideal for preparing PPVs because defects are incorporated along the conjugated backbone of the polymer.^{64,65} We used this method to prepare **CP2a** because it allowed us to initiate the synthesis from significantly simpler starting materials. The likelihood that defects are present in this material must be borne in mind when assessing its physical characteristics. Attempts to prepare **CP3a** by Heck coupling of **M3** and **M(a)** produced unsatisfactorily low molecular weight material, as determined by ¹H NMR end group analysis. This material was not studied further.

Purification of the CPs was accomplished by dissolution in a water-immiscible organic solvent and rinsing of the solution with water and EDTA(aq)⁵⁴ to remove salts and coordinated metal ions such as $\text{Cu}(\text{I})$, used in the Sonogashira coupling step. Precipitation from and/or rinsing with methanol was also performed to remove low molecular weight materials.

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Spectroscopic Properties and Response to Cu(II). The CPs were isolated as yellow to brick red solids. Their optical absorption maxima in dichloromethane/ethanol (4:1) solutions are summarized in Table 1. Fluorescence emission maxima fall in a fairly narrow range from 420 to 530 nm. The fluorescence quantum yields span a wide range from 0.02 to 0.44 when measured in 4:1 dichloromethane/ethanol. We continued to use this solvent system because it was used for studies on **CP1a**²⁵ and because ROH or water is required in the reductive nitrosylation mechanism (Scheme 1b).^{28–42,66} Quantum yields are reasonably high for **CP1** and **CP3**, but disappointingly low for **CP2**. Visually, **CP2** is more fluorescent in THF or dichloromethane alone than in the mixed alcohol system (the quantum yields were not determined in these solvents), but fluorescence is still substantially less than that observed for **CP1**. Although the low quantum yields of **CP2** prohibit practical application to NO sensing, we continued our investigation for comparison of their properties to the more promising candidates.

All CP emission was efficiently quenched by Cu(II) ions. Quantification of quenching is summarized in Table 1, and individual fluorescence spectra for all titrations are provided in the Supporting Information (Figures S1–S7). The most efficient quenching occurs for main chain bipyridyl **CP1**, with 84–100% quenching in the presence of 0.5 equiv of Cu(II) per bipyridyl unit. The 2:1 ligand-to-metal ratio indicates that coordinative cross-links form, in agreement with recent studies on bipyridyl-substituted PPEs.⁵⁴ Cu(II)-induced emission quenching was also observed for **CP2**, but with less efficiency than for **CP1**. The emission of **CP3** continues to diminish as Cu(II) in excess of 1 equiv is added (Figures S6 and S7). Because significant structural changes are required to accommodate terdentate coordination in these CPs, this observation may result from geometrically enforced bidentate/monodentate terpyridyl units that interact with two Cu(II) centers.

An interesting relationship was observed between the CP emission quenching and the identity of the spacer between metal binding sites. In all cases, emission quenching efficiency follows the trend **CPXa** < **CPXb** < **CPXc** (**X** = **1**, **2**, or **3**). CPs containing alternating electron-rich/electron-poor segments have diminished effective conjugation lengths because of mismatched energy at the connection points.⁶¹ A close match between metal-based and CP-based redox energies is important for maximizing metal-CP interaction.⁶⁰ Markedly more efficient emission quenching in CPs with the less electron-donating fluorene spacers can be rationalized by their better overlap with the electron-deficient Cu(II)-bipyridyl segments.

Response of Cu(II)-CP Complexes to NO. After quantifying the emission quenching of CP by Cu(II), we examined the ability of NO(g) to restore emission under the same conditions previously reported for **CP1a**.²⁵ The NO-induced relative fluorescent turn-on is summarized in Table 1, and one set of representative spectra is shown in Figure

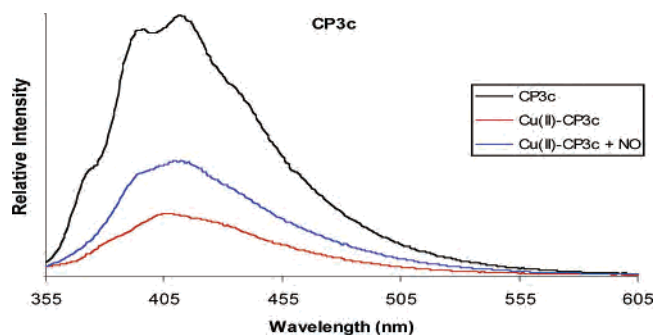


Figure 1. Emission response of **CP3c** to Cu(II) and of Cu(II)-**CP3c** to NO(g). Details are provided in the Experimental Section.

1. Spectra for all sets of related experiments are provided in the Supporting Information (Figures S8–S13). In contrast to the quenching trends, the NO-induced emission enhancement had no dependence on spacer identity and was constant within the **CP1** (approximately 300% enhancement) and **CP2** (30–90% enhancement) series. In contrast, **CP3a** emission increased by 90% upon exposure to NO(g), but **CP3b** emission decreased by 50%. The origin of this behavior is not immediately apparent, but it may derive from the reduction to Cu(I) and the large change in inter/intrachain geometry expected for this structure. Because the absorbance of Cu(II)-CP at the excitation wavelength changed by <10% upon addition of NO(g) in all cases, the observed emission trends cannot be attributed to these fluctuations.

CP1 is clearly the most promising set for practical NO sensing because of the incorporation of main-chain metal-ligating groups requiring little geometric rearrangement upon metal binding or redox changes. **CP1b** and **CP1c** were selected for additional studies, **CP1a** having already been examined in detail.²⁵ Because the Cu(I)-CP complexes would result from a reductive nitrosylation mechanism, Cu(I)-**CP1b** and Cu(I)-**CP1c** were independently prepared for comparison of their emissive properties to those of Cu(II)-CP following NO addition. As was observed for **CP1a**, the emission from Cu(II)-CP complexes was not fully restored to the level of Cu(I)-CP (Figures S8 and S9). One drawback of **CP1a** is its inability to distinguish between NO and nitroxyl (HNO), a redox derivative of NO with a potentially orthogonal biological signaling pathway. **CP1b** and **CP1c** do not respond to NO⁺ but, unfortunately, do turn on in the presence of a nitroxyl donor (Angeli's Salt, Na₂N₂O₃) in a manner similar to that for NO(g) under these conditions. Copper(II) complexes of related bipyridyl-substituted PPE derivatives (Chart 1b) exhibit ~3-fold emission enhancement in response to NO(g) in 95:5 acetonitrile/water, but no change in emission intensity was observed upon exposure to HNO.²¹ This disparity may be the result of solvent effects. Because **CP1** is not soluble in acetonitrile/water because of the hydrophobic *n*-hexyl substituents, this solvent-dependency hypothesis could not be fully tested. Future work would benefit from the preparation of additional water-soluble variations of the **CP1** series.

Summary. The families of CPs described in this research incorporate elements from some of the most studied CPs in conjunction with bipyridyl or terpyridyl units for metal

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binding. The emission of these materials is quenched by 75–100% in the presence of Cu(II) ions. The observed quenching efficiency depends on the metal-ligating group and the identity of the spacer between these units. Main-chain bipyridyl binding units permit more efficient quenching than do main- or side-chain terpyridyl units. Decreasing the electron-donating ability of spacers between binding units increases the quenching efficiency, possibly because of closer energy matching between ligating and spacer segments of the CP. Bipyridyl-functionalized CPs also provide the greatest relative emission enhancement in response to NO(g). The three bipyridyl CP derivatives respond similarly, with an approximately 3-fold emission increase induced by NO(g) and Angeli's Salt with no response to nitrosonium. NO-sensing CMPs would be optimized by the introduction of electron-

deficient spacers between main chain bipyridyl units to increase metal–CP interaction.

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Supporting Information Available: Fluorescence spectra for Cu(II) titrations and analyte response. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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