solved by direct methods with SHELXS-86 and refined with SHELXL-93.[12] The program XPMA was used for the graphical processing of the data.[13] The figures were produced with WINRAY-32.[14] The refinement was carried out anisotropically against F^2, hydrogen atoms were included in calculated positions. **1**: space group $P2_1/c$, $a = 1277.4(3)$, $b = 1956.0(4)$, $c = 2436.8(5)$ pm, $\beta = 103.63(3)^\circ$, $V = 5917 \times 10^6$ pm^3, $\rho_{calcd} = 1.641$ g cm^{-3}, $2\theta_{max} = 52.1^\circ$, $Z = 4$, 71 438 measured reflections, 11 474 independent reflections, of which 5485 ($I > 2\sigma(I)$) observed, 758 refined parameters, $R = 0.070$, $Rw = 0.112$, max. residual electron density 1.54×10^{-6} e pm^{-3}. **2**: space group $P\bar{1}$, $a = 1422.2(3)$, $b = 1902.5(4)$, $c = 2609.1(5)$ pm, $\alpha = 100.58(3)$, $\beta = 103.63(3)$, $\gamma = 108.48(3)^\circ$, $V = 6490 \times 10^6$ pm^3, $\rho_{calcd} = 1.543$ g cm^{-3}, $2\theta_{max} = 55.0^\circ$, $Z = 4$, 58 282 measured reflections, 29 826 independent reflections, of which 19 004 ($I > 2\sigma(I)$) observed, 1463 refined parameters, $R = 0.077$, $Rw = 0.204$, max. residual electron density 2.39×10^{-6} e pm^{-3}. The two crystallographically independent anions **2** are the same with regard to all the important structural features. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-137594 (**1**), -137593 (**2**), and -137592 ($Na_2[\{(CO)_5Cr\}_2Pb(NO_3)_2]$). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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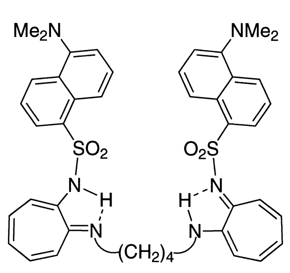
Metal-Based NO Sensing by Selective Ligand Dissociation[**]

Katherine J. Franz, Nisha Singh, and
Stephen J. Lippard[*]

The large and increasing number of biological processes for which nitric oxide is implicated necessitates the development of improved methods of NO detection. Currently available techniques often rely on the identification of NO metabolites, such as nitrite and nitrate, or lack sensitivity.[1] The application of ratiometric biosensors[2] and ultramicro amperometric sensors[3–5] affords one avenue to obtain selective, sensitive detection of NO in vivo. Fluorescent indicators also provide desirable properties that allow direct, real-time detection with both spatial and temporal resolution,[6] as has been amply demonstrated for Ca^{II} sensors.[7] Although the invention of fluorescent NO sensors is an active research area, so far these methods rely on indirect detection of more reactive NO$_x$ species,[8–10] display decreased fluorescence intensities upon NO binding,[11] or require further chemistry to provide a positive fluorescence response.[12, 13] We report herein an approach in which the formation of a transition metal nitrosyl complex triggers a positive fluorescent signal in response to NO.

The design of this NO sensor takes advantage of the fluorescence-quenching properties of transition metal ions with partly filled d shells. We prepared a ligand containing a fluorophore that is quenched by the metal center, in this case Co^{II}, in the absence of NO to give little residual signal for the "off" response. In the presence of NO, however, the formation of the metal–nitrosyl adduct selectively displaces a fluorescent ligand, thereby removing it from the quenching environment and turning the fluorescence "on". A similar approach was reported for an Fe^{II} complex of a quinoline pendant cyclam but the fluorescence intensity decreased in the presence of NO.[11] An analogous ligand-displacement strategy has also been applied for a pH-sensitive fluorescent probe.[14]



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In the newly designed ligand H_2DATI-4 (**1**), each aminotroponiminate (ATI) ring is modified with a dansyl fluorophore on one of the imine nitrogen atoms and linked through the other nitrogen by a 4-methylene chain to a second such chelating unit.[15] The yellow ligand **1** is rather in-

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[**] This work was supported by a grant from the National Science Foundation and a fellowship to N.S. from the Undergraduate Research Opportunity Program (MIT). We thank Prof. Roger Tsien for valuable discussions at the inception of this project.

soluble in organic solvents but deprotonation with KH in a THF/CH_3CN mixture affords a clear, orange solution. Addition of $[Co(CH_3CN)_4](PF_6)_2$ and work-up provides an 80% yield of [Co(DATI-4)] **2**. The single-crystal X-ray diffraction study of **2** revealed its solid-state structure (Figure 1).[16] The Co^{II} center has distorted tetrahedral geometry and the dansyl groups align in a parallel planar fashion. The two six-membered aromatic rings attached to the S atoms are stacked with an average aryl–aryl distance of 3.63(9) Å. Compound **2** displays only 9% of the fluorescence emission intensity of **1**, as revealed by their quantum yields Φ of 9×10^{-4} and 0.01, respectively.

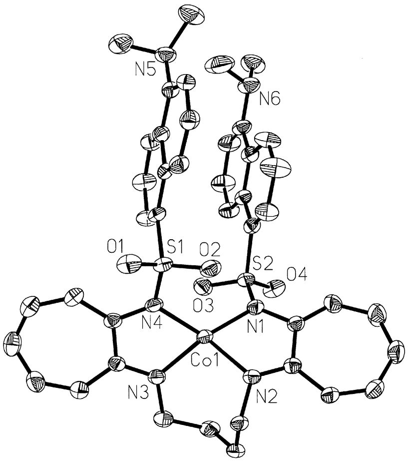
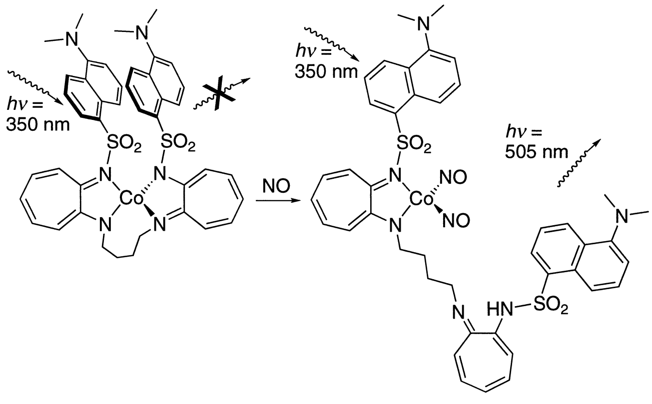


Figure 1. ORTEP diagram of [Co(DATI-4)] **2** showing 50% thermal ellipsoids and selected atom labels. Selected bond lengths [Å] and angles [°]: Co1-N1 1.986(3), Co1-N2 1.963(2), Co1-N3 1.996(3), Co1-N4 1.977(2), N1-S2 1.607(3), N4-S1 1.618(3), S1-O1 1.440(2), S1-O2 1.442(2), S2-O3 1.439(2), S2-O4 1.437(2); N1-Co1-N2 79.78(10), N2-Co1-N3 103.94(10), N3-Co1-N4 79.93(10), N4-Co1-N1 123.78(10), N2-Co1-N4 136.82(10), N1-Co1-N3 141.36(11).

Isolated **2** is air stable in both the solid state and solution, an important property for applications as a sensor that recognizes NO but not O_2.[17] Complex **2** reacts readily with NO, as indicated by two new IR bands at 1827 and 1751 cm^{-1} (KBr). These bands shift to 1793 and 1719 cm^{-1} upon isotopic labeling with ^{15}NO, in excellent agreement with the values of 1794 and 1720 cm^{-1} calculated by assuming a classic diatomic oscillator model. The IR bands indicate formation of a Co–dinitrosyl species and are at the low end of the range for tetrahedral $\{Co(NO)_2\}^{10}$ complexes, which exhibit symmetric and asymmetric NO stretching bands between 1820–1876 cm^{-1} and between 1755–1798 cm^{-1}.[18] In some cases, formation of $\{Co(NO)_2\}^{10}$ species proceeds by disproportionation of $Co^{II}L_2$ in the presence of NO to afford $[Co^{I}(NO)_2L]$ and $[Co^{III}L_3]$, where L is a bidentate ligand.[19, 20] There is no apparent evidence for a Co^{III} species in the reaction of **2** with NO and, at present, the nature of the reductant is unknown.

The formation of a Co–dinitrosyl species with a tetrahedral coordination environment around the Co center from **2** is possible if one of the DATI arms dissociates from the metal center (Scheme 1). Similar products are obtained upon the reaction of $[Co(i\mathrm{Pr}_2ATI)_2]$ with NO, in which $Hi\mathrm{Pr}_2ATI$ is released as $[Co(NO)_2(i\mathrm{Pr}_2ATI)]$ forms.[21] A crystal structure determination revealed the geometry of $[Co(NO)_2(i\mathrm{Pr}_2ATI)]$ in the solid state to be pseudotetrahedral with one bidentate $i\mathrm{Pr}_2ATI^-$ and two NO ligands. In the IR spectrum, there are two NO stretching bands at 1809 and 1730 cm^{-1}.[21] These bands are lower in energy than those in the product of **2** with NO because of the electron-withdrawing influence of the sulfonamide groups.



Scheme 1. Formation of a cobalt–dinitrosyl complex results in ligand dissociation and fluorescence enhancement.

The dissociation of a dansyl-containing ligand fragment suggests that fluorescence of the Co complex should be restored upon addition of NO. Figure 2a displays the fluorescence emission spectra of a solution of **2** in CH_2Cl_2 at 25°C under a NO atmosphere. After 3 min, the earliest time point recorded, the fluorescence intensity doubled over that of the starting complex. The intensity continued to rise at smaller increments over 6 h to afford a greater than four-fold increase in intensity over that of the starting material. In a separate experiment, aliquots of NO gas were added incrementally to a solution of **2** to gauge the lower limit of NO detection. Addition of 2 μL of NO caused only a slight fluorescence enhancement, whereas addition of 5 μL (2.9 equiv based on Co) of NO doubled the fluorescence intensity. The NO detection limit of **2** can therefore be estimated at 50–100 μM.

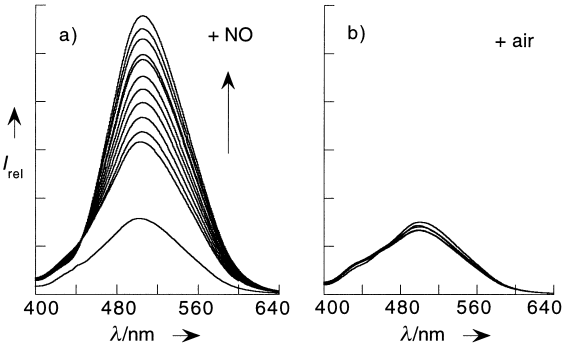


Figure 2. a) The lowest intensity spectrum is that of a 40 μM CH_2Cl_2 solution of [Co(DATI-4)] **2** under N_2 at 25°C. Residual fluorescence of the paramagnetic complex gives an emission at 505 nm (excitation at 350 nm). Within 3 minutes after purging NO gas through the headspace, the intensity of the emission at 505 nm doubles. The intensity continues to increase over the course of 6 hours to a final fourfold increase. b) A 40 μM CH_2Cl_2 solution of **2** exposed to air over 6 hours. Residual fluorescence of the paramagnetic complex gives an emission at 505 nm (excitation at 350 nm).

In addition to the dissociation of a DATI moiety, a metal-based mechanism may also contribute to the positive increase in fluorescence. Fluorescence quenching by transition metals can occur by energy or electron transfer between the excited state of the fluorophore and empty or partially filled d orbitals on the metal. If the d shell is full, such as in the d^{10} ions Zn^{II} or Cu^{I}, such an energy transfer is not accessible and fluorescence occurs.[22] Formation of a cobalt–dinitrosyl species $\{Co(NO)_2\}^{10}$ also creates a filled d^{10} shell and therefore this moiety may itself account for some of the fluorescence response. An additional fluorescence enhancement mechanism is conceivable if $\pi - \pi$ stacking of the fluorophores, as observed in the solid-state structure of **2**, creates an excimer complex that is disrupted by formation of the dinitrosyl adduct. Related $[Co(^{R}DATI)_2]$ where $^{R}DATI$ = alkyldansylaminotroponiminate, complexes that exhibit similar fluorescence responses to NO do not exhibit such $\pi - \pi$ interactions in the solid state.[15, 21]

The ability of a sensor to report only the desired analyte while ignoring competitors is crucial. Although NO rapidly reacts with O_2 to yield NO_2, the reaction is second order with respect to NO.[23] For regulatory functions such as vasodilation and neurotransmission, the supposed 0.1 to 10 μM concentrations of NO translates to a possible lifetime of up to ten minutes.[24] It is therefore essential that a sensor respond only to NO and not O_2, a situation that creates a challenge for metal-based systems. Compound **2** is stable to dioxygen indefinitely and displays no fluorescence response in the presence of dissolved O_2, as illustrated by the fluorescence spectra in Figure 2b.

In conclusion, we have demonstrated ligand dissociation from a transition metal ion to be an attractive approach to NO sensor design. The present NO sensor overcomes two major challenges: It selectively reports NO and not O_2 and it displays a *positive* fluorescence response to this important analyte. The mechanism of fluorescence enhancement involves ligand dissociation upon metal–nitrosyl binding and perhaps also involvement of $\{Co(NO_2)\}^{10}$ in which energy or electron transfer quenching is not possible. We are currently using the principles learned in this study to develop NO sensors that are more sensitive, water-soluble, display greater fluorescence intensity enhancement, and can bind NO reversibly for in vivo applications.

Experimental Section

2: A slurry of **1** (164 mg, 0.22 mmol) in a THF/CH_3CN mixture (5/1, 10 mL) was added to KH (18 mg, 0.45 mmol) under a nitrogen atmosphere. A clear, orange solution was obtained after stirring for 30 min. To this solution, 3 mL of a CH_3CN solution of $[Co(CH_3CN)_4](PF_6)_2$ (122 mg, 0.24 mmol) was added dropwise. The resulting dark amber solution was evacuated to dryness after stirring for 3 h under a dry nitrogen atmosphere. The brown powder was extracted into $CHCl_3$ and filtered through Celite. A brown, microcrystalline powder (143 mg, 80% yield) was obtained by diethyl ether diffusion into a $CHCl_3$/MeOH (4/1) solution of the complex. X-ray quality crystals were grown from the same solvent combination. FTIR (KBr): $\tilde{\nu} = 2937, 1610, 1577, 1509, 1451, 1287, 1209, 1129, 1059, 864, 788, 746, 627, 571, 499, 474\ cm^{-1}$; UV/Vis ($CH_2Cl_2$): λ_{max} (ε) = 254 (55000), 367 nm (26800 $M^{-1} cm^{-1}$); $\Phi = 9 \times 10^{-4}$; elemental analysis (%): calcd for $CoC_{42}H_{42}N_6S_2O_4$: C 61.68, H 5.18, N 10.28; found: C 61.54, H 5.29, N 10.01.

Received: February 7, 2000 [Z 14662]

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[16] Crystal data for **2**: Dark brown, diamond-shaped plates, 0.36 × 0.24 × 0.08 mm. Monoclinic, space group $P2_1/c$, $a = 13.239(3)$, $b = 20.614(8)$, $c = 14.055(5)$ Å, $\beta = 92.54(2)°$, $V = 3832(2)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.418\ Mg\,m^{-3}$. The data were collected at −85°C on a Siemens SMART diffractometer equipped with a CCD detector ($2\theta_{max} = 56.54°$) using $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å). A total of 23805 reflections were measured, of which 8811 were unique. The structure was solved by the direct methods program XS and refined by full-matrix least-squares minimization on F^2 and Fourier techniques using SHELXTL-PLUS. Absorption corrections were applied with the program SADABS. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were assigned to idealized positions and given a thermal parameter 1.2 times that of the thermal parameter of the carbon atom to which each was attached. Final residuals were $R = 0.047$ and $wR2 = 0.119$ for 5353 observed reflections with $I > 2\sigma(I)$ and 496 variable parameters. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-139962. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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