

Extreme Stabilization and Redox Switching of Organic Anions and Radical Anions by Large-Cavity, CH Hydrogen-Bonding Cyanostar Macrocycles

Christopher R. Benson,[†] Elisabeth M. Fatila,[†] Semin Lee,[‡] Matthew G. Marzo,[†] Maren Pink,[†] Michelle B. Mills,[§] Kathryn E. Preuss,[§] and Amar H. Flood^{*,†}

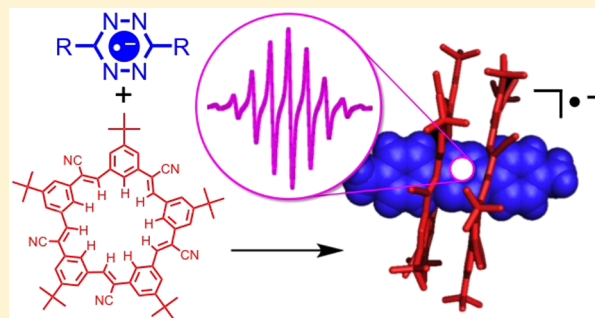
[†]Department of Chemistry, Indiana University, 800 East Kirkwood Avenue, Bloomington, Indiana 47405, United States

[‡]The Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana–Champaign, Urbana, Illinois 61801, United States

[§]Department of Chemistry, University of Guelph, Guelph, Ontario N1G 2W1, Canada

Supporting Information

ABSTRACT: Encapsulation of unstable guests is a powerful way to enhance their stability. The lifetimes of organic anions and their radicals produced by reduction are typically short on account of reactivity with oxygen while their larger sizes preclude use of traditional anion receptors. Here we demonstrate the encapsulation and noncovalent stabilization of organic radical anions by C–H hydrogen bonding in π -stacked pairs of cyanostar macrocycles having large cavities. Using electrogenerated tetrazine radical anions, we observe significant extension of their lifetimes, facile molecular switching, and extremely large stabilization energies. The guests form threaded pseudorotaxanes. Complexation extends the radical lifetimes from 2 h to over 20 days without altering its electronic structure. Electrochemical studies show tetrazines thread inside a pair of cyanostar macrocycles following voltage-driven reduction ($+e^-$) of the tetrazine at -1.00 V and that the complex disassembles after reoxidation ($-e^-$) at -0.05 V. This reoxidation is shifted 830 mV relative to the free tetrazine radical indicating it is stabilized by an unexpectedly large -80 kJ mol $^{-1}$. The stabilization is general as shown using a dithiadiazolyl anion. This finding opens up a new approach to capturing and studying unstable anions and a radical anions when encapsulated by size-complementary anion receptors.



INTRODUCTION

An attractive feature of receptors is their ability to encapsulate chemically unstable guests to prolong their life.¹ The first of these was Cram's incarceration of cyclobutadiene (C₄H₄).² More recently, Nitschke and co-workers³ stabilized highly pyrophoric white phosphorus (P₄) by capturing it within a cage complex, while Cummins and Nocera⁴ were able to stabilize a peroxide dianion (O₂²⁻) inside a cryptand. The strategies employed to engage unstable species include the deployment of noncovalent contacts, e.g., strong hydrogen bonds,⁴ π contacts,⁵ and metal-ion coordination,^{6–8} as well as entrapment by molecular encapsulation^{2,3} and in some cases by formation of mechanically interlocked molecules.^{9–12} When utilized in combination, these strategies can be very effective, but there remain several classes of unstable guest that have not yet been addressed.

Radical anions are highly unstable; they can react by electron transfer,¹³ H atom abstraction,^{14–16} and nucleophilic attack^{17,18} in thermodynamically favored and kinetically rapid processes,¹⁹ particularly for nitrogen-centered radicals.^{20,21} This reactivity makes them difficult to isolate and analyze. While organic radical anion^{22–25} can be stabilized by metal coordination⁷ for

studying catalysis and redox noninnocent complexes,^{26,27} their encapsulation chemistry has not been investigated. Other new anionic species,^{28,29} e.g., the P₂N₃⁻ aromatic anion and the cyclopentazole anion (N₅⁻), are of particular interest owing to their novel bonding and potential for new reactivity but are also difficult to isolate and stabilize. Radical–radical interactions, also known as pancake bonding,^{30–32} may be available for stabilization but has not been generalized beyond a few cases^{5,33–36} that show π -stacked association. Other modes involve NH hydrogen-bonding anion receptors,^{37–43} but most anion receptors are too small for organic molecules. Thus, we present a new and general strategy to stabilize electrochemically generated radical anions in a threaded binding mode by using an anion receptor large enough to host organic molecules.

We reasoned that macrocyclic receptors that are both large enough to host organic molecules and lacking reactive sites could bind and stabilize electrogenerated radical anions. There is significant and growing interest in large macrocycles^{44–55} for larger anions. Our candidate receptor, cyanostar (CS, Figure

Received: September 12, 2016

Published: October 19, 2016

1a),^{51–55} provides a macrocyclic array of 10 low-acidity C–H hydrogen-bond donors that form an electropositive cavity.

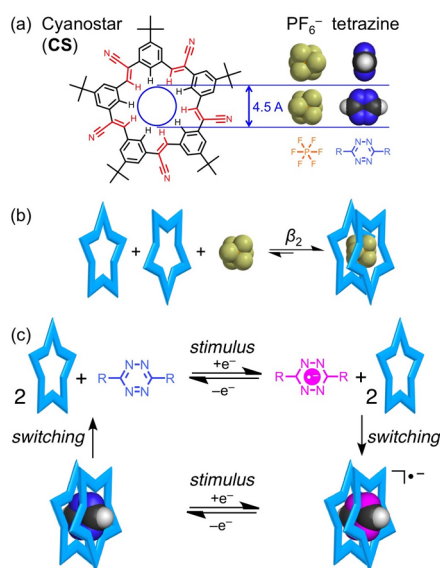


Figure 1. (a) Dimensions of cyanostar's binding pocket match the size of PF₆⁻ and tetrazine. (b) Equilibrium reaction showing formation of the 2:1 sandwich complex with PF₆⁻. (c) Square scheme showing a thermodynamic cycle representing the redox-driven switching of tetrazine (blue) following reduction to a radical anion (magenta) and then threading through a pair of cyanostars.

Cyanostars have a preference for binding larger anions⁵² stabilized as high-fidelity 2:1 sandwich complexes. The highest affinity anion is PF₆⁻ ($\beta_2 = 10^{12} \text{ M}^{-2}$), which is size-matched to the cavity formed by a π -stacked pair of macrocycles (Figure 1b). Inspired by size matching, we proposed that organic species of comparable size could serve as guests after being reduced to anions (depicted as a thermodynamic cycle or “square scheme” in Figure 1c). Further, we believed that the stabilization afforded by cyanostar's binding pocket would help attenuate the radical anions' reactivity to extend their lifetimes.

Herein, we examine tetrazine (Tz) heterocycles that undergo facile one-electron reduction chemistry at ca. -1 V versus Ag/AgCl, to form tetrazine radical anions, Tz^{•-} (Figure 2).

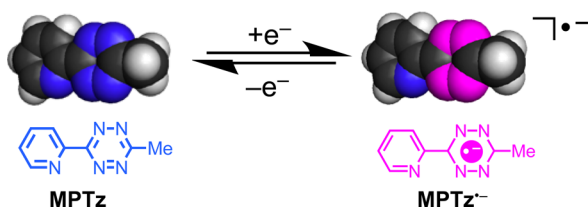


Figure 2. Structure and redox chemistry of 3-methyl-6-(pyridin-2-yl)-1,2,4,5-tetrazine, MPTz, the model compound for this study.

Tetrazines serve as redox noninnocent ligands in coordination compounds;^{33,35,36} energetic materials for propellants, explosives, and pyrotechnic ingredients;^{56–59} and reagents for bio-orthogonal click chemistry.^{60,61} When considered as guests for cyanostars, the tetrazine's space-filling volume (75 Å³) matches PF₆⁻ (73 Å³) even though its disc-shape is mismatched to the spherical binding pocket.

In this work, we demonstrate that cyanostar macrocycles can stabilize radical anions in the form of size-matched tetrazines.

We confirm the morphology of a threaded 2:1 CS₂·Tz complex by X-ray crystallography. With electron paramagnetic resonance (EPR) spectroscopy, we show this threaded complex, CS₂·MPTz^{•-}, substantially stabilizes the bound tetrazine radicals, extending its lifetime in solution by at least a factor of 360 and in the solid state by more than 30 days. Electrochemistry is used to show that tetrazines bind reversibly to the cyanostars and undergo molecular switching.^{62–65} Surprisingly, the stabilization provided by the two neutral cyanostars (ca. -80 kJ mol⁻¹) outperforms the Coulombic stabilization imparted to tetrazine ligands by a single Cu⁺ cation. Finally, we verify the generality of the approach showing that cyanostars can stabilize a variety of other reduced tetrazine radicals as well as an anionic dithiadiazolide guest. This study opens up a new approach to stabilize and study unstable anions and their radicals that are size-matched to cavities of complementary hosts.

RESULTS AND DISCUSSION

Stabilized and Long-Lived Radicals. Addition of cyanostar to solutions of reduced tetrazine radicals greatly extends their lifetimes while leaving their spectral properties intact. The radical of MPTz (Figure 2) was produced by reduction with 4 equiv⁶⁶ of cobaltocene⁶⁷ (Figure 3a) in dry

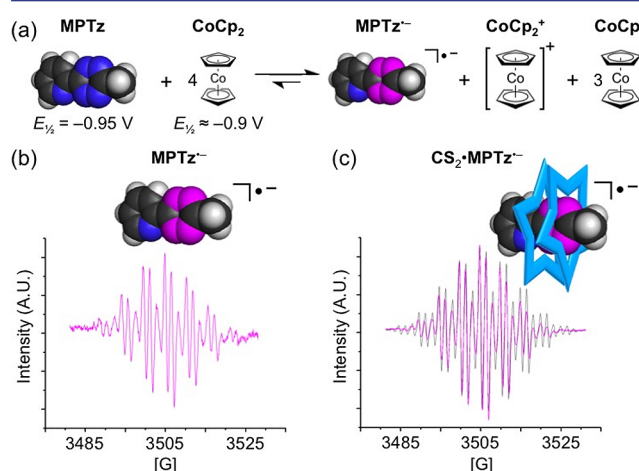


Figure 3. EPR experiments with MPTz. (a) Scheme showing the preparation of MPTz^{•-} by reduction of MPTz with CoCp₂. X-band EPR spectra of (a) MPTz^{•-} and (b) MPTz^{•-} with 2 equiv of CS added. The simulated EPR spectrum of the CS₂:MPTz^{•-} complex is overlaid in gray. 1 mM MPTz, 4 mM CoCp₂, THF, N₂, 298 K. Spectrometer settings: microwave (MW) frequency 9.855 GHz; MW power, 2.002 mW; modulation amplitude, 0.50 G; time constant, 20.960 ms; 20.42 s scan time.

tetrahydrofuran (THF) under an inert atmosphere (N₂) in a gastight tube (J. Young). The reduced tetrazine produces a distinctive EPR spectrum³⁶ (Figure 3b). The well-resolved nine-line pattern shows a typical resonance for tetrazine radicals at $g = 2.0094$ with four coupled nitrogen atoms ($A_N = 5.150 \text{ G}$) and additional hyperfine coupling to the three substituent methyl protons ($A_H = 1.730 \text{ G}$, see Supporting Information). Interestingly, upon addition of cyanostar, none of the spectral properties change (Figure 3c): The number of lines, the g -values (referenced against 2,2,6,6-tetramethylpiperidine-1-oxyl, TEMPO), the observed hyperfine coupling, and their associated coupling constants remain identical to those of the free ligand. At this point we also examined another tetrazine to ascertain the generality of the result. Similar results were

obtained with BPTz (Figure 4, inset), as reduced and complexed versions, $\text{CS}_2\cdot\text{BPTz}^{\bullet-}$ (see SI). This equivalence

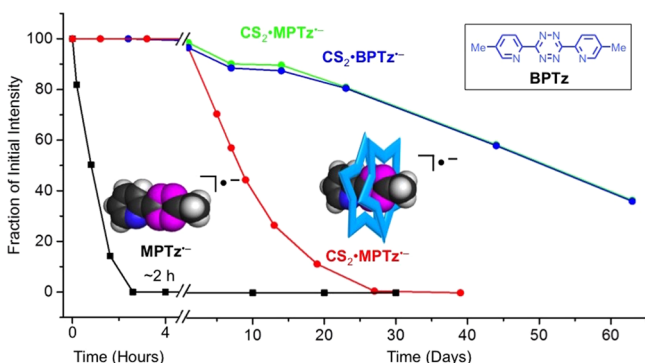


Figure 4. Lifetime of $\text{MPTz}^{\bullet-}$ radicals in the presence (red traces) or absence (black traces) of cyanostar stabilization as determined by EPR spectroscopy. The J. Young tubes containing the samples were stored at room temperature in low-light conditions. Flame-sealed EPR tubes were also examined: $\text{CS}_2\cdot\text{MPTz}^{\bullet-}$ (green trace) and $\text{CS}_2\cdot\text{BPTz}^{\bullet-}$ (blue trace). Inset: Structure of BPTz (3,6-bis(5-methyl-2-pyridine)-1,2,4,5-tetrazine). Sample and analysis conditions are identical to Figure 3. Conditions: (red trace) 1 mM MPTz , 4 mM CoCp_2 , 2 mM CS ; (black trace) plus an additional 6 mM TBAPF_6 .

is somewhat surprising. Several groups^{68–71} have studied the impact of hydrogen bonding on the spectral parameters of radicals, and routinely observed changes in g -value, splitting constants, or coupling constants. However, in each of these cases, the hydrogen-bond donor is a traditional “strong” donor, e.g., amide NH , ammonium NH , alcohol OH , whereas our system features less polar CH hydrogen bonds. This finding may also result from a host–guest shape mismatch, i.e., cyanostar’s spherical pocket binding a discoid tetrazine.

The lifetime of the radical is significantly enhanced in the presence of cyanostar despite the lack of spectral changes. For the uncomplexed tetrazine radical, it is short-lived and starts to decay during the time required to prepare and analyze the sealed sample (Figure 4), consistent with 10–30 min half-lives seen previously.³⁶ By contrast, addition of cyanostar extends the lifetime to 27 days.

The essential role of cyanostar in prolonging the lifetime of the tetrazinyl radical was determined using a competition experiment. First, a solution of $\text{CS}_2\cdot\text{MPTz}^{\bullet-}$ was prepared by adding 4 equiv of cobaltocene to nitrogen-degassed THF solution containing 2 equiv of cyanostar and 1 equiv of MPTz . The solution was then split into two fractions. To the first, 6 equiv of the PF_6^- anion was added to competitively eject the reduced tetrazine from the macrocycle while the second was left untouched. Both solutions were then placed in sealed tubes and their EPR signals monitored for several hours. In the solution that is poisoned by addition of PF_6^- , the tetrazine radical has limited access to the cavity of cyanostar, and the radical is seen to completely decompose within 130 min (Figure 4, black line). When this is compared to the ~30 days (Figure 4, red line) seen for the solution stabilized by cyanostar, there is an astonishing 360-fold increase in the lifetime. These results show that access of the tetrazine radicals to the cyanostar’s cavity is responsible for the extended lifetime.

Inert storage conditions result in substantially longer lifetimes. When the previous experiment was repeated using flame-sealed EPR vials, complexes of $\text{CS}_2\cdot\text{MPTz}^{\bullet-}$ and of $\text{CS}_2\cdot\text{BPTz}^{\bullet-}$ both only lost 65% of the initial signal intensity over 2

months. We estimate the radical should persist in solution approximately ~85 days. Without the cyanostar, sealed vials of the ligand radicals did not show an appreciable increase in lifetime (~200 min).

Lifetime of the Complexed Radical in the Solid State.

In order to study the radical’s stability as a complex in the solid state and to investigate its lifetime, samples were prepared using either precipitation or crystallization. In both cases, a concentrated solution of tetrazine (MPTz or BPTz) was mixed with 2 equiv of cyanostar and 4 equiv of cobaltocene in dry THF under an inert N_2 atmosphere. Direct addition of dry pentane to this concentrated mixture resulted in precipitation of the $\text{CS}_2\cdot\text{Tz}^{\bullet-}$ complex (with a putative cobaltocenium counteranion) as a charcoal-colored powder. Vapor diffusion of pentane into the concentrated mixture resulted in the growth of a black crystalline solid. EPR spectra of both the powder and crystalline samples suffered from poor resolution, showing only a single broad line. However, dissolution of these solid-state samples in THF returned the nine-line signature of the tetrazine radical.

The lifetime of the encapsulated radical in the solid state is enhanced even further than in solution. The powder and crystal samples show that EPR activity remains strong after the 30 days seen for the solution sample, losing only 1% intensity over 30 days (in contrast to 100% over 30 days in solution). The solid samples were stored under N_2 or in flame-sealed glass tubes. The lifetimes of the radicals were verified by EPR measurements of the solids or of samples dissolved in THF. To probe the limits of this stabilization, the solid samples were exposed to air. They are seen to completely lose radical character over approximately 200 min and change their color from black to a red-brown color, which we attribute to oxidation by O_2 . This is in stark contrast to the solutions; in those cases, the EPR activity was lost over the course of minutes when the samples are exposed to air. The radical character also disappears rapidly when solids are suspended in water, showing immediate loss of the single-line EPR signal. Overall, the solid state permits an increase in lifetime. Relative to the solution phase, we attribute this outcome to a decrease in the probability of dethreading in the solid state.

X-ray Crystal Structure of the Threaded Tetrazine [3]Pseudorotaxane.

Crystallographic data provides structural evidence that tetrazine and cyanostar form threaded [3]-pseudorotaxane complexes (Figure 5). We grew crystals of the reduced tetrazine complexed with CS by adding excess cobaltocene to a mixture of DTTz and CS in THF. Slow diffusion of pentane resulted in dark brown crystals. Upon X-ray crystallographic analysis, the crystal was determined to be a 2:1 complex between cyanostars and DTTz with a threaded configuration. The crystal structure analysis shows whole molecule disorder consistent with the existence of M and P bowl chirality in the cyanostars that pair up into 67.5% meso MP and 32.5% chiral MM (or PP) diastereomers in the solid state as seen previously.^{51–55} We observe the central tetrazine ring is disordered over two rotamers either 23° (66.4%) or approximately coplanar (33.6%) with the two outer phenyls. Out of the 20 CH donors, we observe 3–4 $\text{CH}\cdots\text{N}$ hydrogen bonds per nitrogen with bond lengths ranging 2.5–2.9 Å. The other ca. 4 CH donors per cyanostar are almost perpendicular to the plane of the ring.

We were not able to determine the charge state of the tetrazine in this complex with complete confidence from the crystal structure alone. The $\text{N}-\text{N}$ bond length is 1.376 Å,

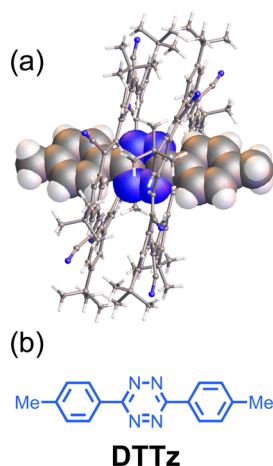


Figure 5. (a) Crystal structure showing DTTz threaded within a pair of π -stacked cyanostars. (b) Structure of DTTz (3,6-di-*p*-tolyl-1,2,4,5-tetrazine).

which is 0.03 Å longer than neutral tetrazines and is consistent with the one-electron reduced radical species.⁷² If it is a radical anion, we would expect to locate a balancing cation. We observed high electron density in the difference map; however, it does not readily resolve into a chemically reasonable pattern. Analysis of the solvent accessible area suggests that either disordered solvent or a cobalt species is also present. Nevertheless, the key outcome of the crystal structure analysis is unambiguous confirmation of the threaded [3]-pseudorotaxane configuration.

Voltage Gated Switching of Cyanostar–Tetrazine Complexes. We performed cyclic voltammetry (CV) experiments on MPTz in the presence of cyanostar to test for the ability to reversibly switch the reduced tetrazine in and out of the cyanostars. The CV experiments required a significant modification from standard procedures on account of the strong anion-capture capabilities of the cyanostar. First, we used a large noncoordinating anion, tetrabutylborate, BPh₄[−], as the 100-mM supporting electrolyte. Second, we had to exclude all other sources of anions. Thus, we were not able to use the traditional Ag/AgCl reference electrode and instead made use of a Ag wire as a quasireference electrode. Consequently, there

were minor variations (± 50 mV) in potential values between experiments. Therefore, the tetrazine's cathodic reduction peak served as an internal standard set to -1.00 V on account of the fact that tetrazine reduction potentials are largely insensitive to substitution.^{33,36,63,73}

We see clear evidence of switching from the CVs (Figure 6). The solution of MPTz alone (Figure 6a) shows a reversible one-electron reduction process typical of tetrazines ($E_{1/2} = -0.94$ V). When CS is added into solution, the corresponding reoxidation peak at -0.88 V disappears (Figure 6b) and is replaced by a new reoxidation peak at -0.05 V. This represents a stabilization of 830 mV.⁷⁴ We observed a range of peak shifts ranging from 800 to 1000 mV depending upon experimental conditions (*vide supra*). Cyanostar itself has no redox properties in the electrochemical window we examined. The new CV (Figure 6b) matches the profile of a traditional electrochemistry–chemistry (EC) mechanism (Figure 1c);^{75,76} therein, electrochemical (E) reduction of free tetrazine drives production of a new chemical (C) species, CS₂·MPTz^{•−}. It is the reoxidation of the new complex that we see at -0.05 V. Only one new reoxidation peak is observed during addition of 0–2 equiv and 2–4 equiv of cyanostar into the tetrazine solution. No evidence for intermediates, such as a 1:1 species expected at 1 equiv, was observed. This finding indicates formation of the new 2:1 complex proceeds with a high degree of positive cooperativity, consistent with cyanostar's binding preferences with other anions.⁵²

Control studies show that molecular switching is the only explanation for these CV signatures. The retention of the reduction peak at -1.00 V in the outward sweep indicates that free tetrazine is in solution and that complexation only occurs after reduction. Consistently, ¹H NMR spectroscopy experiments show that addition of 1 equiv of Me₂Tz (Figure 7), the smallest tetrazine, to a solution of cyanostar (CD₂Cl₂) produces no discernible shifts in cyanostar proton resonances. This is consistent with negligible binding in the neutral state. At 10 equiv of the neutral tetrazine, small shifts may be observed indicating weak binding and an affinity of less than -5 kJ mol^{−1}. It is not possible to achieve saturation by titration, nor to determine if the shifts are associated with a 1:1 or 2:1 CS:Me₂Tz binding stoichiometry.

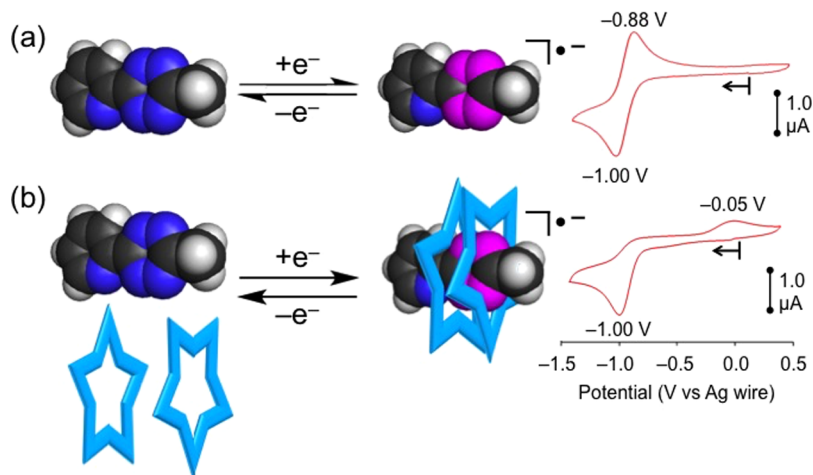


Figure 6. CVs of MPTz (a) before and (b) after the addition of 2 equiv of cyanostar. Conditions: 1.0 mM MPTz, 0.1 M TBABPh₄, CH₂Cl₂ N₂, 200 mV s^{−1} scan rate, glassy carbon wire working electrode, Pt wire counter electrode, Ag wire quasireference, 298 K, referenced to E_{pc} MPTz = -1.00 V.

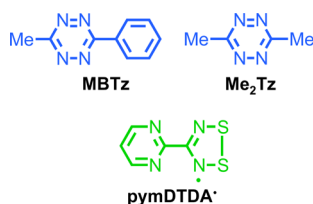


Figure 7. List of other reducible organic compounds investigated in this study: **MBTz** (3-methyl-6-phenyl-1,2,4,5-tetrazine), **Me₂Tz** (3,6-dimethyl-1,2,4,5-tetrazine), and **pymDTDA•** (4-(2'-pyrimidyl)-1,2,3,5-dithiadiazolyl).

The large anodic shift (~ 830 mV) for the reoxidation of the complexed tetrazine radical corresponds to a significant thermodynamic stabilization of -80 kJ mol⁻¹. An alternative explanation is that encapsulation may significantly slow the rate of heterogeneous electron transfer, as was seen with metallocenes inside basket compounds.^{77,78} Varying the scan rate (25 – 200 mV s⁻¹) showed no changes in the position or shape of the reoxidation peak. This observation suggests that any quasireversible or irreversible electron-transfer kinetics cannot account for the large 830-mV peak shift. The peak's shifted position could also be indicative of some other type of chemical species formed by a cyanostar-mediated decomposition of the radical anion. However, repeated cycling of the same solution showed the process to be fully reversible, and therefore not chemically destructive, which is perfectly consistent with the EPR data (Figure 3).

The peak intensity for the reoxidation peak at -0.05 V is much lower than that of the parent tetrazine. This intensity loss largely arises from a lower diffusion coefficient for the complex: Tetrazines and the representative tetrazine complexes^{62,64} have diffusion coefficients of ca. 1×10^{-5} and 0.5×10^{-5} cm² s⁻¹, respectively. Simulations of switching CVs were undertaken to evaluate the mechanisms by which the observed peak intensity and shape could be produced. Good correspondence to experiment is seen for **MPTz** by altering the electron-transfer kinetics that slightly decrease the peak intensity and broadening the peak width while having a negligible impact on peak position (e.g., α values between 0.5 and 0.7 and/or electron-transfer kinetics 2 orders of magnitude less than free tetrazine). We also experimentally observed variations in the reoxidation peak's shape and intensity with changes in solution resistance (a consequence of electrolyte concentration and solubility) and electrode geometry.

Generality of Redox Switching. The stabilization and the switching imparted by cyanostar are broadly applicable to other radical anions and unstable anions as a multiple number of redox-active guests (Figures 2, 4, 5, and 7) showed similar electrochemical profiles. These include symmetric and non-symmetric pyridyl- and phenyl-based tetrazines (**MPTz**, **BPTz**, **DTTz**, **MBTz**), and dimethyltetrazine (**Me₂Tz**), as well as pyrimidyl dithiadiazolyls (**pymDTDA•**).^{79–83} The redox-active moieties of these guests are all comparable in size. They also show a similar degree of stabilization inside the pair of cyanostars. As a consequence, the binding energies for tetrazine guests, **Tz•⁻**, ranging from -75 to -85 kJ mol⁻¹, are all similar and all strong, while **pymDTDA⁻** shows a lower binding energy of -60 kJ mol⁻¹.

It came as some surprise that the stabilities of the tetrazines are all about the same. We expected some degree of variation to stem from the structural variations. For example, the pyridyl nitrogens in the **MPTz** or **BPTz** have no apparent

thermodynamic impact relative to the CH groups present in the phenyl groups in **MBTz** and **DTTz**. It seems that the size match between the cavity and the redox-active moiety influences the thermodynamics of association for the examined tetrazines.

To broaden the scope and generality of reversible association of short-lived organic anions, we examined 4-(2'-pyrimidyl)-1,2,3,5-dithiadiazolyl, **pymDTDA•**.⁸³ 1,2,3,5-Dithiadiazolyls are stable, isolable, neutral radicals but are sensitive to decomposition by acidic protons.^{81,84–86} Typical 1,2,3,5-dithiadiazolyls radicals (**DTDA•**) are capable of accepting one electron to form a closed-shell monoanion, which can be observed electrochemically but has never been conclusively isolated⁸⁶ because the corresponding dithiadiazolide anions are known to rapidly decompose in both solution and solid state under inert atmosphere.⁸⁴ We measured the reduction properties of **pymDTDA•** by CV employing conditions identical to those used for tetrazines and observed a reversible wave at $E_{1/2} = -0.95$ V (vs platinum wire quasireference) as expected for dithiadiazolyls.^{84,86} As with tetrazine, redox cycling of **pymDTDA•** in the presence of cyanostar shows a significant anodic shift of the reoxidation by ~ 630 mV. This observation is compatible with a -60 kJ mol⁻¹ stabilization of the **pymDTDA⁻** anion by association with two cyanostars. Subsequent addition of PF₆⁻ provides a competing anion for the cyanostar cavity and regenerates a fully reversible reduction process assigned to the free **pymDTDA•**. The stabilization of the **pymDTDA⁻** anion reinforces the generality of cyanostar as a host for electrogenerated organic anions.

A further set of experiments was done to determine whether cyanostar could slow decomposition and effect longer lifetimes for **pymDTDA⁻** as was seen with tetrazines. This idea was tested by monitoring whether the five-line EPR signal characteristic of the neutral **pymDTDA•** radical⁸⁵ could be recovered after redox cycling. In the presence of cyanostar, reduction of **pymDTDA•** by sodium metal for 30 min followed by reoxidation by stirring the solution under a stream of O₂ for 15 min unambiguously returns the five-line pattern at 10% intensity. Conversely, in the absence of cyanostar, the same procedure results in a very different EPR signal of much reduced intensity that is inconsistent with oxidative reformation of **pymDTDA•**. Using a different experimental setup yielded similar results: Reduction of **pymDTDA•** with 3 equiv of cobaltocene for 1 h followed by oxidation with nitrosonium hexafluorophosphate (NOPF₆) returns the radical's original EPR spectrum at 50% intensity, but only in the presence of cyanostar. The corresponding experiment in the absence of cyanostar results in a complete loss of signal. From these observations, we postulate that cyanostar impairs the spontaneous decomposition of the **pymDTDA⁻** anion by its encapsulation. Thus, we believe that unstable anionic species that are size-matched to cyanostars may all see a corresponding enhancement in their lifetimes (See SI).

Confirmation of Thermodynamic Stabilization of Reduced Tetrazines. The stabilization of tetrazine radicals is estimated from the electrochemistry (using Faraday's constant) to be $\Delta G = -80$ kJ mol⁻¹ inside the 2:1 complex. We did not expect such a large value. This free energy is on the same order of magnitude as the inorganic anion, PF₆⁻, which was measured in 60:40 CH₂Cl₂:MeOH to be approximately -70 kJ mol⁻¹.⁵² Despite the different solvents, we still expect the affinity for PF₆⁻ to have a similar order of magnitude in CH₂Cl₂. The EPR experiment (Figure 4) had already provided

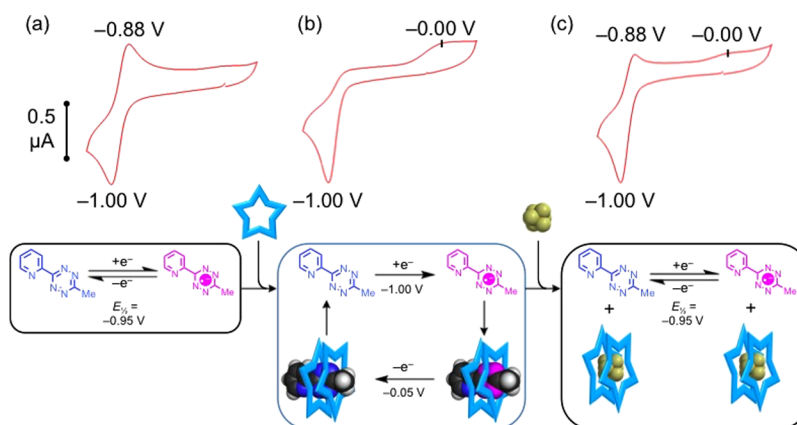


Figure 8. CV of MPTz (a) before and (b) after addition of 2 equiv of cyanostar. (c) CV of MPTz with 2 equiv of cyanostar plus 1 equiv of PF_6^- (1.0 mM MPTz, 0.1 M TBABPh₄, CH_2Cl_2 , N_2 , 200 mV s^{-1} scan rate, glassy carbon working electrode, Pt wire counter electrode, and Ag wire quasireference electrode, 298 K, referenced to $E_{\text{pc}} \text{Tz} = -1.00 \text{ V}$).

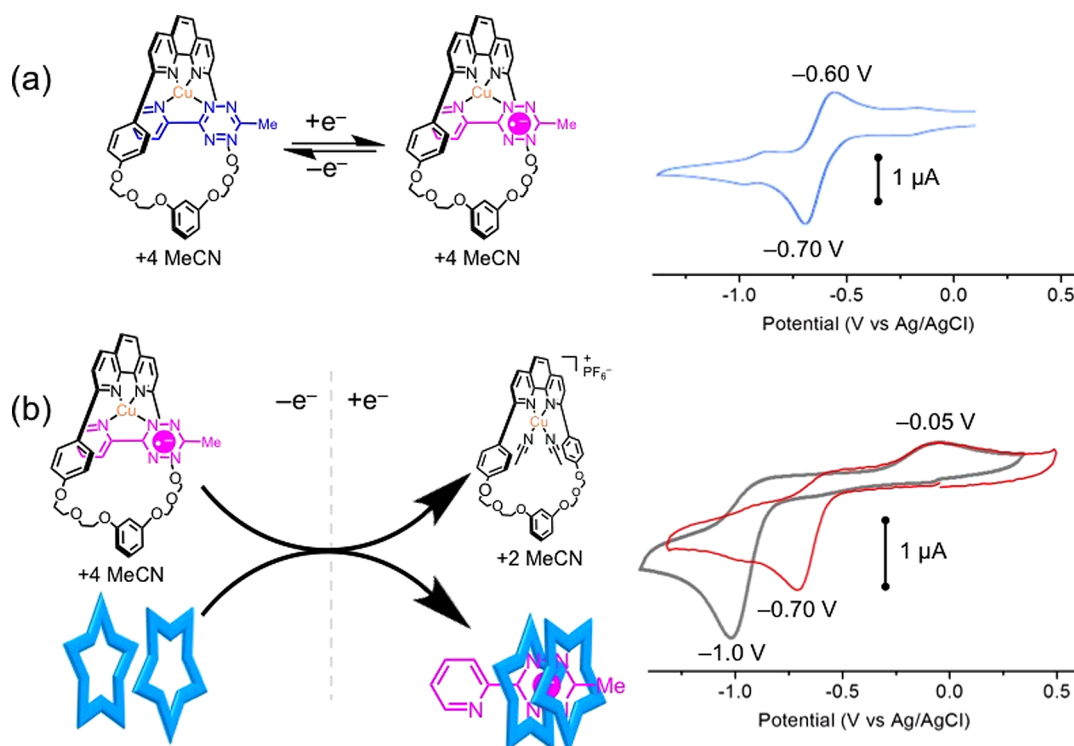


Figure 9. (a) CV of the copper-templated [2]pseudorotaxane derived from MPTz (1 mM). (b) CV of the copper-templated [2]pseudorotaxane (1 mM) in the presence of 4 equiv of cyanostar (red trace) overlaid on the CV (gray trace) of MPTz with 2 equiv of cyanostar. Conditions: 1:1:1 mixture (1 mM) of MPTz, MC, $\text{Cu}(\text{MeCN})_4^+\text{PF}_6^-$, 0.1 M TBABPh₄, CH_2Cl_2 , N_2 , 200 mV s^{-1} , glassy carbon working electrode, Pt wire counter electrode, Ag wire quasireference electrode, 298 K, referenced to $E_{\text{pc}} \text{Tz} = -1.00 \text{ V}$.

evidence that 6 equiv of PF_6^- can interfere with association of the reduced tetrazine, and additional evidence was sought.

We conducted an anion competition experiment to provide independent verification of the stabilization energy experienced by the tetrazine radical. Specifically, the CV shows that addition of PF_6^- can displace the reduced MPTz^{•-} from the cyanostars. As a reference, the CV of MPTz alone has a simple one-electron wave at $E_{1/2} = -0.94 \text{ V}$ (Figure 8a). Addition of 2 equiv of cyanostar generates a CV signature of switching (Figure 8b) with a peak for the complex around 0.00 V. When 1 equiv of PF_6^- is added to the solution, we see that the CV resembles the signature of the free tetrazine (Figure 8c). The reoxidation peak at -0.88 V regains intensity on account of the

fact that reduced MPTz^{•-} must compete for binding against the comparably stable CS_2PF_6^- complex. Adding 2 further equivalents of cyanostar restores the switching CV. This cycle can be repeated several times. In order to completely exclude MPTz^{•-} association with cyanostar, 5–6 equiv of PF_6^- is required.

The competition experiment has two critical implications for understanding the stabilization of tetrazine radical anions. First, it corroborates our estimate of the large association constant for reduced tetrazines with cyanostars by its close relation to PF_6^- affinity. Second, this finding supports the explanation that the large shift in the reoxidation potential of the $\text{CS}_2\text{Tz}^{\bullet-}$ complex

comes as a result of thermodynamic stabilization instead of slow heterogeneous electron-transfer kinetics.

Switching between Metal Coordination and Cyanostar Encapsulation. Having established that the association between reduced tetrazines and cyanostars is sufficient for the electroreduced tetrazine to compete with other anions, we wanted to examine whether the neutral cyanostar was better than metal cations for the stabilization of tetrazines. Our prior work on tetrazine switches^{62–65} made use of the complexation between a neutral tetrazine that is threaded inside a copper(I)-macrocycle⁸⁷ (Figure 9a). This stabilization increases by ~ 30 kJ mol⁻¹ upon reduction to the tetrazine radical anion. In contrast, the neutral tetrazine only binds weakly to cyanostars. Consequently, the neutral MPTz should favor the copper(I)-macrocycle binding partner until it is reduced whereupon it should switch reversibly over to the two cyanostars.

Our experiments demonstrate that the planned exchange between cyanostar and a copper(I)-tetrazine complex follows thermodynamic expectations. The copper-based [2]-pseudorotaxane shows a reversible reduction centered at $E_{1/2} = -0.65$ V⁸⁸ (Figure 9a). Upon addition of 4 equiv of cyanostar, we see production of the peak for the CS₂·MPTz^{•-} species (Figure 9b) at -0.05 V after reduction of the copper-based [2]pseudorotaxane indicative of switching. We needed to add 4 equiv of cyanostar; the first two to sequester the PF₆⁻ counteranions that came along with the copper salt leaving the other 2 equiv for switching.

While the observed switching is expected from thermodynamic arguments, the fact that a pair of neutral macrocycles provides more stability to an anion than a cationic metal ion is unusual. What we instead observe is the single ion–ion contact (Cu⁺...Tz^{•-}) and the supporting metal–ligand bonds are outweighed by 20 ion-dipole contacts (hydrogen bonds) and ideal size matching. This example is illustrative of the myriad factors that contribute to molecular recognition, and underscores that many weak interactions may overwhelm a single strong interaction.

CONCLUSIONS

We have demonstrated a new and general strategy to stabilize electrochemically generated organic radical anions and organic anions using size-matched, anion-binding cyanostar macrocycles. This approach may benefit from a combination of encapsulation and the many low-polarity CH hydrogen bonds. Competition experiments between the organic radical anions and inorganic anions confirms that the thermodynamic stabilization energies are upward of -80 kJ mol⁻¹, which confers substantially longer lifetimes to the bound anions without changing their underlying electronic properties. The strength of this association exceeds the Coulombic stabilization imparted by metal cations despite the individually weak neutral hydrogen bonds. Finally, the reversible redox-responsive activity of the host-guest complex constitutes an elementary molecular switch. These findings provide a basis for the development of interlocked molecular machines as well as a general strategy for stabilizing organic radicals and anions.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b09459.

Experimental details, additional EPR experiments, discussion of cyclic voltammetry, voltammetry control studies, and full report on the cyanostar-tetrazine crystal structure with analysis (PDF)
Crystallographic details (CIF)

AUTHOR INFORMATION

Corresponding Author

*aflood@indiana.edu

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

C.R.B., E.M.F., S.L., M.G.M., and A.H.F. acknowledge support from the National Science Foundation (CHE 1412401), Brian J. Cook and Ken G. Caulton for technical support, and Don Garvin for construction of custom EPR glassware for lifetime experiments. K.E.P. acknowledges the Natural Sciences and Engineering Research Council (NSERC) of Canada for a Discovery Grant (DG) and the government of Canada for a Tier II Canada Research Chair (Chemistry of Molecular Materials).

DEDICATION

This manuscript is dedicated to Professors Jean-Pierre Sauvage, J. Fraser Stoddart, and Bernard (Ben) Feringa on the occasion of their receipt of the 2016 Nobel Prize in Chemistry.

REFERENCES

- (1) Galan, A.; Ballester, P. *Chem. Soc. Rev.* **2016**, *45*, 1720–1737.
- (2) Cram, D. J.; Tanner, M. E.; Thomas, R. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1024–1027.
- (3) Mal, P.; Breiner, B.; Rissanen, K.; Nitschke, J. R. *Science* **2009**, *324*, 1697–1699.
- (4) Lopez, N.; Graham, D. J.; McGuire, R.; Alliger, G. E.; Shao-Horn, Y.; Cummins, C. C.; Nocera, D. G. *Science* **2012**, *335*, 450–453.
- (5) Barnes, J. C.; Fahrenbach, A. C.; Cao, D.; Dyar, S. M.; Frascioni, M.; Giesener, M. A.; Benítez, D.; Tkatchouk, E.; Chernyashvskyy, O.; Shin, W. H.; Li, H.; Sampath, S.; Stern, C. L.; Sarjeant, A. A.; Hartlieb, K. J.; Liu, Z.; Carmieli, R.; Botros, Y. Y.; Choi, J. W.; Slawin, A. M. Z.; Ketterson, J. B.; Wasielewski, M. R.; Goddard, W. A.; Stoddart, J. F. *Science* **2013**, *339*, 429–433.
- (6) Schwach, M.; Hausen, H.-D.; Kaim, W. *Inorg. Chem.* **1999**, *38*, 2242–2243.
- (7) Ulas, G.; Lemmin, T.; Wu, Y.; Gassner, G. T.; DeGrado, W. F. *Nat. Chem.* **2016**, *8*, 354–359.
- (8) Shuvaev, K. V.; Sproules, S.; Rautiainen, J. M.; McInnes, E. J. L.; Collison, D.; Anson, C. E.; Powell, A. K. *Dalton Trans.* **2013**, *42*, 2371–2381.
- (9) Parham, A. H.; Windisch, B.; Vögtle, F. *Eur. J. Org. Chem.* **1999**, *1999*, 1233–1238.
- (10) Sugiyama, J.; Tomita, I. *Eur. J. Org. Chem.* **2007**, *2007*, 4651–4653.
- (11) Arunkumar, E.; Forbes, C. C.; Noll, B. C.; Smith, B. D. *J. Am. Chem. Soc.* **2005**, *127*, 3288–3289.
- (12) Oku, T.; Furusho, Y.; Takata, T. *Org. Lett.* **2003**, *5*, 4923–4925.
- (13) Donoghue, P. J.; Wiest, O. *Chem. - Eur. J.* **2006**, *12*, 7018–7026.
- (14) Moorthy, P. N.; Hayon, E. *J. Phys. Chem.* **1974**, *78*, 2615–2620.
- (15) Ratera, I.; Veciana, J. *Chem. Soc. Rev.* **2012**, *41*, 303–349.
- (16) Staley, P. A.; Lopez, E. M.; Clare, L. A.; Smith, D. K. *J. Phys. Chem. C* **2015**, *119*, 20319–20327.
- (17) Danen, W. C.; Warner, R. J.; Arudi, R. L. Nucleophilic Reactions of Superoxide Anion Radical. In *Organic Free Radicals*; American Chemical Society, 1978; Vol. 69, pp 244–257.

- (18) De Vleeschouwer, F.; Chankisjijev, A.; Geerlings, P.; De Proft, F. *Eur. J. Org. Chem.* **2015**, 2015, 506–513.
- (19) Hicks, R. G. *Org. Biomol. Chem.* **2006**, 5, 1321–1338.
- (20) Mas-Torrent, M.; Crivillers, N.; Rovira, C.; Veciana, J. *Chem. Rev.* **2012**, 112, 2506–2527.
- (21) Suarez, A. I. O.; Lyaskovskyy, V.; Reek, J. N. H.; van der Vlugt, J. I.; de Bruin, B. *Angew. Chem., Int. Ed.* **2013**, 52, 12510–12529.
- (22) D'Souza, F.; Gadde, S.; Schumacher, A. L.; Zandler, M. E.; Sandanayaka, A. S. D.; Araki, Y.; Ito, O. *J. Phys. Chem. C* **2007**, 111, 11123–11130.
- (23) Bhosale, S. V.; Jani, C. H.; Langford, S. J. *Chem. Soc. Rev.* **2008**, 37, 331–342.
- (24) Song, Q.; Li, F.; Wang, Z.; Zhang, X. *Chem. Sci.* **2015**, 6, 3342–3346.
- (25) Jiao, Y.; Liu, K.; Wang, G.; Wang, Y.; Zhang, X. *Chem. Sci.* **2015**, 6, 3975–3980.
- (26) Kaim, W. *Inorg. Chem.* **2011**, 50, 9752–9765.
- (27) Lyaskovskyy, V.; de Bruin, B. *ACS Catal.* **2012**, 2, 270–279.
- (28) Velian, A.; Cummins, C. C. *Science* **2015**, 348, 1001.
- (29) Bazanov, B.; Geiger, U.; Carmieli, R.; Grinstein, D.; Welner, S.; Haas, Y. *Angew. Chem., Int. Ed.* **2016**, 55, 13233–13235.
- (30) Beneberu, H. Z.; Tian, Y.-H.; Kertesz, M. *Phys. Chem. Chem. Phys.* **2012**, 14, 10713–10725.
- (31) Preuss, K. E. *Polyhedron* **2014**, 79, 1–15.
- (32) Cui, Z.-h.; Lischka, H.; Beneberu, H. Z.; Kertesz, M. *J. Am. Chem. Soc.* **2014**, 136, 12958–12965.
- (33) Kaim, W. *Coord. Chem. Rev.* **2002**, 230, 127–139.
- (34) Trabolzi, A.; Khashab, N.; Fahrenbach, A. C.; Friedman, D. C.; Colvin, M. T.; Cotí, K. K.; Benítez, D.; Tkatchouk, E.; Olsen, J.-C.; Belowich, M. E.; Carmielli, R.; Khatib, H. A.; Goddard, W. A.; Wasielewski, M. R.; Stoddart, J. F. *Nat. Chem.* **2010**, 2, 42–49.
- (35) Chifotides, H. T.; Dunbar, K. R. *Acc. Chem. Res.* **2013**, 46, 894–906.
- (36) Benson, C. R.; Hui, A. K.; Parimal, K.; Cook, B. J.; Chen, C.-H.; Lord, R. L.; Flood, A. H.; Caulton, K. G. *Dalton Trans.* **2014**, 43, 6513–6524.
- (37) Breinlinger, E.; Niemz, A.; Rotello, V. M. *J. Am. Chem. Soc.* **1995**, 117, 5379–5380.
- (38) Ge, Y.; Lillenthal, R. R.; Smith, D. K. *J. Am. Chem. Soc.* **1996**, 118, 3976–3977.
- (39) Ge, Y.; Miller, L.; Ouimet, T.; Smith, D. K. *J. Org. Chem.* **2000**, 65, 8831–8838.
- (40) Bu, J.; Lillenthal, N. D.; Woods, J. E.; Nohrden, C. E.; Hoang, K. T.; Truong, D.; Smith, D. K. *J. Am. Chem. Soc.* **2005**, 127, 6423–6429.
- (41) Jordan, B. J.; Pollier, M. A.; Miller, L. A.; Tiernan, C.; Clavier, G.; Audebert, P.; Rotello, V. M. *Org. Lett.* **2007**, 9, 2835–2838.
- (42) Peng, B.; McNew, S. R.; Li, Q.-S.; Xie, Y.; Schaefer, H. F. *Chem. Phys. Lett.* **2012**, 523, 120–123.
- (43) Baggerman, J.; Haraszkiwicz, N.; Wiering, P. G.; Fioravanti, G.; Marcaccio, M.; Paolucci, F.; Kay, E. R.; Leigh, D. A.; Brouwer, A. M. *Chem. - Eur. J.* **2013**, 19, 5566–5577.
- (44) Gong, H.-Y.; Rambo, B. M.; Karnas, E.; Lynch, V. M.; Sessler, J. L. *Nat. Chem.* **2010**, 2, 406–409.
- (45) Kolesnikov, G. V.; German, K. E.; Kirakosyan, G.; Tananaev, I. G.; Ustynuk, Y. A.; Khrustalev, V. N.; Katayev, E. A. *Org. Biomol. Chem.* **2011**, 9, 7358–7364.
- (46) Schaly, A.; Belda, R.; García-España, E.; Kubik, S. *Org. Lett.* **2013**, 15, 6238–6241.
- (47) Valderrey, V.; Escudero-Adán, E. C.; Ballester, P. *Angew. Chem., Int. Ed.* **2013**, 52, 6898–6902.
- (48) Kim, M. J.; Choi, Y. R.; Jeon, H.-G.; Kang, P.; Choi, M.-G.; Jeong, K.-S. *Chem. Commun.* **2013**, 49, 11412–11414.
- (49) Yawer, M. A.; Havel, V.; Sindelar, V. *Angew. Chem., Int. Ed.* **2015**, 54, 276–279.
- (50) Lee, S.; Hirsch, B. E.; Liu, Y.; Dobscha, J. R.; Burke, D. W.; Tait, S. L.; Flood, A. H. *Chem. - Eur. J.* **2016**, 22, 560–569.
- (51) Qiao, B.; Anderson, J. R.; Pink, M.; Flood, A. H. *Chem. Commun.* **2016**, 52, 8683–8686.
- (52) Lee, S.; Chen, C.-H.; Flood, A. H. *Nat. Chem.* **2013**, 5, 704–710.
- (53) Hirsch, B. E.; Lee, S.; Qiao, B.; Chen, C.-H.; McDonald, K. P.; Tait, S. L.; Flood, A. H. *Chem. Commun.* **2014**, 50, 9827–9830.
- (54) Singharoy, A.; Venkatakrishnan, B.; Liu, Y.; Mayne, C. G.; Lee, S.; Chen, C.-H.; Zlotnick, A.; Schulten, K.; Flood, A. H. *J. Am. Chem. Soc.* **2015**, 137, 8810–8818.
- (55) Liu, Y.; Singharoy, A.; Mayne, C. G.; Sengupta, A.; Raghavachari, K.; Schulten, K.; Flood, A. H. *J. Am. Chem. Soc.* **2016**, 138, 4843–4851.
- (56) Chavez, D. E.; Hiskey, M. A.; Naud, D. L. *Propellants, Explos., Pyrotech.* **2004**, 29, 209–215.
- (57) Chavez, D. E.; Hiskey, M. A.; Gilardi, R. D. *Angew. Chem., Int. Ed.* **2000**, 39, 1791–1793.
- (58) Chavez, D. E.; Tappan, B. C.; Hiskey, M. A.; Son, S. F.; Harry, H.; Montoya, D.; Hagelberg, S. *Propellants, Explos., Pyrotech.* **2005**, 30, 412–417.
- (59) Huynh, M. H. V.; Hiskey, M. A.; Chavez, D. E.; Naud, D. L.; Gilardi, R. D. *J. Am. Chem. Soc.* **2005**, 127, 12537–12543.
- (60) Devaraj, N. K.; Weissleder, R.; Hilderbrand, S. A. *Bioconjugate Chem.* **2008**, 19, 2297–2299.
- (61) Blackman, M. L.; Royzen, M.; Fox, J. M. *J. Am. Chem. Soc.* **2008**, 130, 13518–13519.
- (62) McNitt, K. A.; Parimal, K.; Share, A. I.; Fahrenbach, A. C.; Witlicki, E. H.; Pink, M.; Bediako, D. K.; Plaisier, C. L.; Le, N.; Heeringa, L. P.; Vander Griend, D. A.; Flood, A. H. *J. Am. Chem. Soc.* **2009**, 131, 1305–1313.
- (63) Share, A. I.; Parimal, K.; Flood, A. H. *J. Am. Chem. Soc.* **2010**, 132, 1665–1675.
- (64) Parimal, K.; Witlicki, E. H.; Flood, A. H. *Angew. Chem., Int. Ed.* **2010**, 49, 4628–4632.
- (65) Benson, C. R.; Share, A. I.; Marzo, M. G.; Flood, A. H. *Inorg. Chem.* **2016**, 55, 3767–3776.
- (66) This was necessary to push the equilibrium of the reduction fully towards reduced tetrazine, see Geiger et al. in next citation.
- (67) Connelly, N. G.; Geiger, W. E. *Chem. Rev.* **1996**, 96, 877–910.
- (68) Kubota, T.; Oishi, Y.; Nishikida, K.; Miyazaki, H. *Bull. Chem. Soc. Jpn.* **1970**, 43, 1622–1634.
- (69) Franchi, P.; Lucarini, M.; Pedrielli, P.; Pedulli, G. F. *ChemPhysChem* **2002**, 3, 789–793.
- (70) Fukuzumi, S.; Okamoto, K.; Yoshida, Y.; Imahori, H.; Araki, Y.; Ito, O. *J. Am. Chem. Soc.* **2003**, 125, 1007–1013.
- (71) Yuasa, J.; Yamada, S.; Fukuzumi, S. *Angew. Chem., Int. Ed.* **2007**, 46, 3553–3555.
- (72) Parimal, K.; Vyas, S.; Chen, C.-H.; Hadad, C. M.; Flood, A. H. *Inorg. Chim. Acta* **2011**, 374, 620–626.
- (73) McGrane, S. D.; Bolme, C. A.; Greenfield, M. T.; Chavez, D. E.; Hanson, S. K.; Scharff, R. J. *J. Phys. Chem. A* **2016**, 120, 895–902.
- (74) The window for analysis is -2.5 to $+0.7$ V, which is limited by electrolysis of the electrolyte (TBABPh₄) rather than methylene chloride solvent.
- (75) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods: Fundamentals and Applications*, 2nd ed.; John Wiley & Sons: Hoboken, NJ, 2001.
- (76) Flood, A. H.; Kaifer, A. E., *Supramolecular Electrochemistry*. In *Supramolecular Chemistry: From Molecules to Nanomaterials*; Steed, J. W., Gale, P. A., Eds.; John Wiley & Sons, Ltd., 2012; Vol. 1, pp 451–472.
- (77) Jeon, W. S.; Moon, K.; Park, S. H.; Chun, H.; Ko, Y. H.; Lee, J. Y.; Lee, E. S.; Samal, S.; Selvapalam, N.; Rekharsky, M. V.; Sindelar, V.; Sobransingh, D.; Inoue, Y.; Kaifer, A. E.; Kim, K. *J. Am. Chem. Soc.* **2005**, 127, 12984–12989.
- (78) Gadde, S.; Kaifer, E. A. *Curr. Org. Chem.* **2011**, 15, 27–38.
- (79) Alange, G. G.; Banister, A. J.; Bell, B.; Millen, P. W. *Inorg. Nucl. Chem. Lett.* **1977**, 13, 143–144.
- (80) Vegas, A.; Perezsalazar, A.; Banister, A. J.; Hey, R. G. *J. Chem. Soc., Dalton Trans.* **1980**, 1812–1815.
- (81) Banister, A. J.; Smith, N. R. M.; Hey, R. G. *J. Chem. Soc., Perkin Trans. 1* **1983**, 1181–1186.

- (82) Rawson, J. M.; Banister, A. J.; Lavender, I. *Adv. Heterocycl. Chem.* **1995**, *62*, 137–247.
- (83) Jennings, M.; Preuss, K. E.; Wu, J. *Chem. Commun.* **2006**, 341–343.
- (84) Aherne, C. M.; Banister, A. J.; Gorrell, I. B.; Hansford, M. I.; Hauptman, Z. V.; Luke, A. W.; Rawson, J. M. *J. Chem. Soc., Dalton Trans.* **1993**, 967–972.
- (85) Boere, R. T.; Oakley, R. T.; Reed, R. W.; Westwood, N. P. C. *J. Am. Chem. Soc.* **1989**, *111*, 1180–1185.
- (86) Boere, R. T.; Roemmele, T. L. *Coord. Chem. Rev.* **2000**, *210*, 369–445.
- (87) Xue, Z.; Mayer, M. F. *J. Am. Chem. Soc.* **2010**, *132*, 3274–3276.
- (88) Benson, C. R.; Share, A. I.; Marzo, M. G.; Flood, A. H. *Inorg. Chem.* **2016**, *55*, 3767–3776.