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Redox-Modulated Recognition of Tetrazines Using Thioureas

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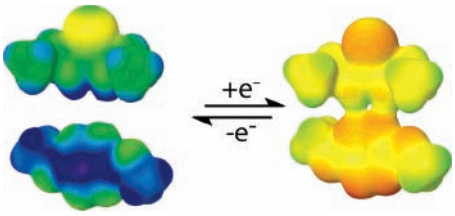
ABSTRACT



Reduced 1,2,4,5-tetrazines serve as two-point hydrogen-bonding acceptors for thiourea. This host–guest system does not exhibit significant binding in the neutral state, making the complex an electrochemical "on/off" switch.

Synthetic host−guest systems that respond to external stimuli provide supramolecular building blocks for applications in sensors,[1] molecular electronics,[2] and tunable solid-state materials.[3] Host−guest systems can utilize dynamic noncovalent interactions, such as hydrogen bonding, donor atom−π, electrostatics, and aromatic stacking, to yield reversible supramolecular complexes that are controlled via electronic,[4] photonic,[5] or thermal stimuli.[6]

Molecular recognition coupled with electrochemistry generates synthetic host−guest systems whose binding strength is modulated by the capacity of the host to readily accept or donate electrons. For example, electron-rich dialkoxyaryl derivatives bind to electron-deficient cyclophane cyclobis(paraquat-*p*-phenylene) ($CBPQT^{4+}$) and then disassemble upon reduction of the $CBPQT^{4+}$ host.[7] Other synthetic strategies employ hydrogen bonding to modulate the binding strength between host−guest systems due to the substantial electrostatic character of hydrogen bonds. Three-point hydrogen-bonding interactions between flavin and diamidopyridine derivatives[8] exhibit a 500-fold increase in binding strength upon reduction of the flavin host. Two-

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point hydrogen bonding between *o*-quinones[9] and arylureas in DMF provides an on/off redox switch where the strength of hydrogen bonding is controlled through the electrochemical conversion of the *o*-quinone to the radical anion state. More recently, nitrobenzene derivatives and arylureas display strong selective redox-dependent two-point hydrogen-bonding interactions within a host−guest system.[10]

1,2,4,5-Tetrazine derivatives serve as potential candidates for redox modulation via two-point hydrogen bonding (Figure 1). Tetrazines are electron-deficient C−N heterocycles that

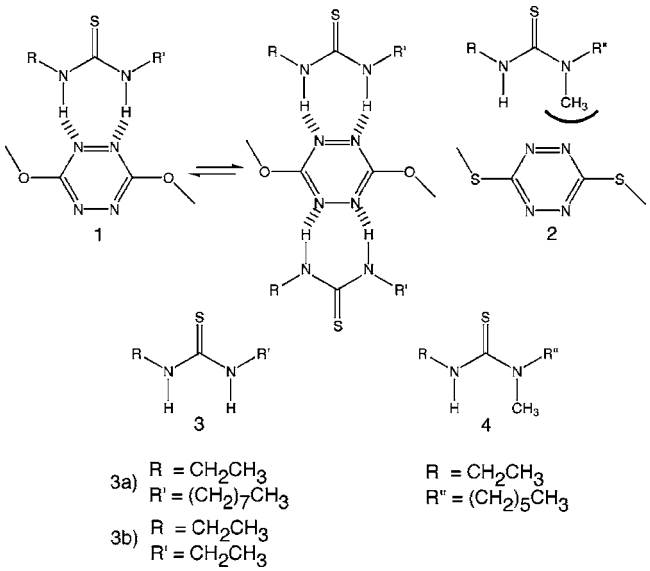


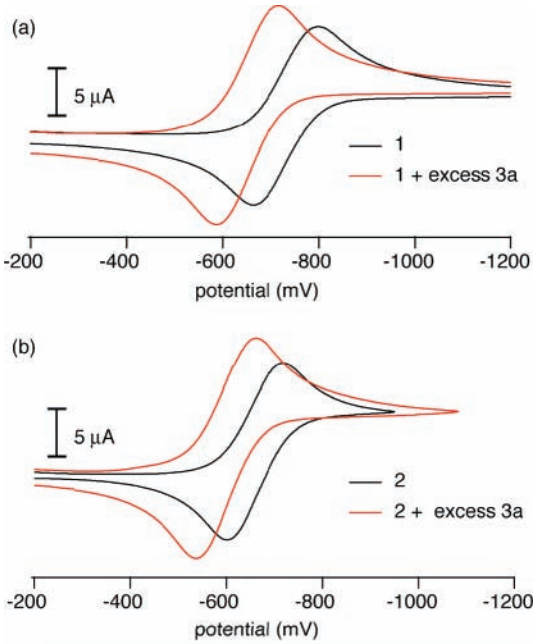
Figure 1. Representative tetrazine complexes displaying both 1:1 and 1:2 possible binding modes. Diethyl thiourea **3b** was used for computational analysis to minimize steric effects. Trialkyl thiourea **4** was used as a control to prevent two-point hydrogen bonding.

exhibit reversible electrochemical behavior at relatively high negative potentials.[11] Initial studies of these systems have focused on optical and reversible electrochemical properties of various substituted heteroatom tetrazine derivatives.[12]

Examination of the nitrogen framework of the ring system suggests that tetrazines should be capable of two-point hydrogen bonding. To this end, we explored the redox modulation of 3,6-bis(methoxy)-1,2,4,5-tetrazine (**1**) and 3,6-bis(methylthio)-1,2,4,5-tetrazine (**2**) by a dialkyl thiourea guest (**3a**). The reduction of the tetrazine derivatives affords essentially an electrochemical "on/off" switch where the addition of an electron turns "on" the hydrogen bonding between tetrazine and thiourea.

Dialkyl thiourea **3a** was used to assess the capacity of the tetrazine derivatives to form hydrogen-bound host−guest complexes due to its strong hydrogen-bonding capabilities and limited proton transfer.[9,13] The lack of association between tetrazine derivatives in the oxidized (neutral) state and dialkyl thiourea **3a** was determined via ^{1}H NMR titrations in $CDCl_3$. Aliquots of tetrazine were added to a constant concentration of **3a** while monitoring the resultant chemical shift of the urea proton (Supporting Information). Only a minor chemical shift was recorded in the presence of the tetrazine neutral forms indicating a negligible association to dialkyl thiourea **3a**. Additional fluorescence titrations confirmed that there was no appreciable complex formation between tetrazine derivatives in the oxidized state and dialkyl thiourea **3a** (Supporting Information).

Cyclic voltammetry (CV) was used to probe the extent of hydrogen bonding within the host−guest complex upon reduction of the tetrazine. Voltammetric studies were performed in CH_2Cl_2 with *n*-$Bu_4N^+ClO_4^-$ as a supporting electrolyte. Tetrazines **1** and **2** exhibited a reversible one-electron reduction with a half-wave potential ($E_{1/2}$) of −741 mV and −660 mV, respectively, relative to ferrocene (Figure 2). The methoxy substituent (i.e., electron-donating group)



Tetrazine	$E_{1/2}$(u)	$E_{1/2}$(b)	$\Delta E_{1/2}$
1	-741	-661	+80
2	-660	-600	+60

Figure 2. CV traces of 1 mM tetrazine **1** (a) and **2** (b) in the presence of 50 mM excess thiourea **3a** in CH_2Cl_2. Shifts in reduction potential ($E_{1/2}$) demonstrate stabilization of reduced tetrazines via hydrogen bonding. Peak heights vary due to solvent loss upon purging.

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on tetrazine **1** enhanced the negative charge on the otherwise deficient tetrazine core through efficient molecular overlap. This electronic contribution lead to a more negative potential relative to tetrazine **2** which suggests that **1** is harder to reduce. Upon addition of excess dialkyl thiourea **3a** (50 equiv), there was a significant shift to the less negative potential for both tetrazine derivatives. Tetrazine **1** displayed a +80 mV shift while tetrazine **2** exhibited a +60 mV shift in its half-wave potential. The difference in half-wave potentials indicated that the net negative charge on tetrazine radical anion $\mathbf{1}^{\bullet-}$ was more stabilized by hydrogen bonding than tetrazine $\mathbf{2}^{\bullet-}$. The more electron rich tetrazine $\mathbf{1}^{\bullet-}$ acts as a better hydrogen bond acceptor resulting in the enhanced stabilization of the complex.

The induced hydrogen bonding between tetrazines and dialkyl thiourea **3a** promoted the formation of supramolecular complexes. The stoichiometry of the complex was assumed to be 1:1, though it is quite possible that 1:2 binding also occurs.[9] The complex formation was considered an "on" state since hydrogen bonding between the tetrazines and dialkyl thiourea **3a** was turned "on" upon addition of an electron. Furthermore, no detectable shift in reduction potential was observed in the presence of the control trialkyl thiourea **4** which cannot participate in two-point hydrogen bonding (Supporting Information).

The stabilization of both $\mathbf{1}^{\bullet-}$ and $\mathbf{2}^{\bullet-}$ by dialkyl thiourea **3a** was quantified by constructing thermodynamic cycles for the [tetrazine/thiourea] host−guest systems. The relative change ($\Delta\Delta G$) in free energy was determined from the observed decreases in reduction potential in the bound states (Figure 3).[14] Only the *relative* binding equilibria (assuming

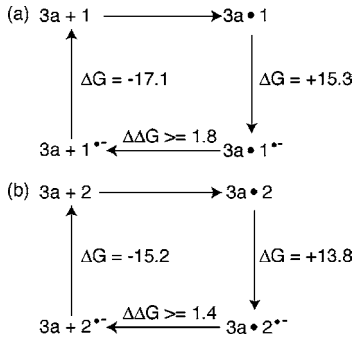

Figure 3. Thermodynamic cycles used to quantify the redox-modulated binding between hydrogen-bound complexes of (a) tetrazine **1** and thiourea **3a** and (b) tetrazine **2** and thiourea **3a**. All energies are calculated in kcal/mol.

1:1 complex) can be determined since the association between the neutral tetrazine derivatives and dialkyl thiourea **3a** was not measurable. The transitions that occur from left to right depict the *relative* binding equilibria between the specific tetrazine host and dialkyl thiourea **3a**, while the top to bottom transitions represent the introduction of one electron into the tetrazine core. Both tetrazine $\mathbf{1}^{\bullet-}$ and $\mathbf{2}^{\bullet-}$ demonstrated a moderate increase in the ΔG association when bound to dialkyl thiourea **3a**. Tetrazine $\mathbf{1}^{\bullet-}$ displayed greater than ~1.8 kcal/mol stabilization resulting in a ~23 fold enhancement in binding over its netural counterpart, while tetrazine $\mathbf{2}^{\bullet-}$ exhibited greater than ~1.4 kcal/mol stabilization corresponding to a ~10 fold increase in binding over tetrazine **2**. These values are reported as lower limits since the strong binding that is present in only one oxidation state underestimates the true binding of the complex.[15] The apparent binding enhancement shifts the equilibrium toward the bound complex, indicating that addition of an electron turns "on" binding. The difference in stabilization and hence larger association constant for tetrazine $\mathbf{1}^{\bullet-}$ was attributed to the ability of $\mathbf{1}^{\bullet-}$ to effectively strengthen its hydrogen-bonding interactions with dialkyl thiourea **3a** upon addition of an electron.[16]

Computational analysis was used to simulate the [tetrazine/thiourea] host−guest complex upon reduction of the tetrazine. The minimum binding energies were calculated for the complex using a simplified diethyl thiourea (**3b**) to minimize steric effects. The diethyl thiourea **3b** was preoptimized yielding a cis conformation of ethyl groups as the energy minima. The complex was optimized at HF 6-31G*//DFT B3LYP 6-31G* over various distances, binding modes, and molecular orientations to achieve a resultant relative binding minimum. The hybrid level of theory was chosen as a result of previous successful calculations for similar radical anion species.[17] The calculations supported the electrochemical data demonstrating a stabilization of both tetrazine radical anions via complexation with **3b**. Tetrazine $\mathbf{1}^{\bullet-}$ displayed a significant increase in stabilization ($\Delta E_{calc} = -23.4$ kcal/mol), while tetrazine $\mathbf{2}^{\bullet-}$ exhibited a slightly lower stabilization ($\Delta E_{calc} = -22.3$ kcal/mol) consistent with the trend from the thermodynamic cycles. The overestimated stabilization predicted for both tetrazines was acceptable when one considers that the calculations do not explicitly account for entropic effects or subtle energy differences that may occur to desolvation of the interacting species.[18]

The electrostatic potential maps indicated that the electrons in the reduced tetrazines are delocalized but maintain a significant negative charge residing on the electronegative nitrogen atoms in the ring system (Figure 4).

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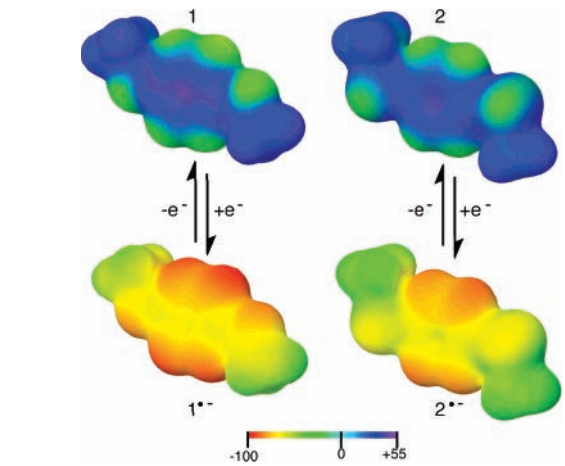

Figure 4. Electrostatic potential maps for **1** (left) and **2** (right) in their neutral (top) and radical anion (bottom) forms. Energies are relative to the neutral form (kcal/mol).

The "on/off" switch consisting of tetrazines and **3b** binding was evident in the electrostatic maps for the entire complex. The neutral tetrazine species demonstrated a relatively weak single hydrogen bond to **3b**, but reduction of the tetrazines led to the formation of two hydrogen bonds (Figure 5). The reduced tetrazines provided a significant negative charge on the nitrogen acceptor atoms in the tetrazine core leading to stronger interactions with the partially positively charged **3b** hydrogen donor atoms which in turn enhanced the hydrogen bond strength in the overall complex.

In summary, we have demonstrated that redox-modulated binding between tetrazines and thiourea stabilizes the tetrazine radical anion. The hydrogen-bound complex serves as an "on/off" switch where both tetrazines exhibit no appreciable binding in the oxidized state and substantial binding upon reduction of the tetrazine core. Computational simulations offer a qualitative agreement with experimental results suggesting that the electronic character of the hydrogen-bonding acceptors on the tetrazine influence the binding affinity of the complex. Future work utilizing this "on/off" switch in material applications is presently underway and will be reported in due course.

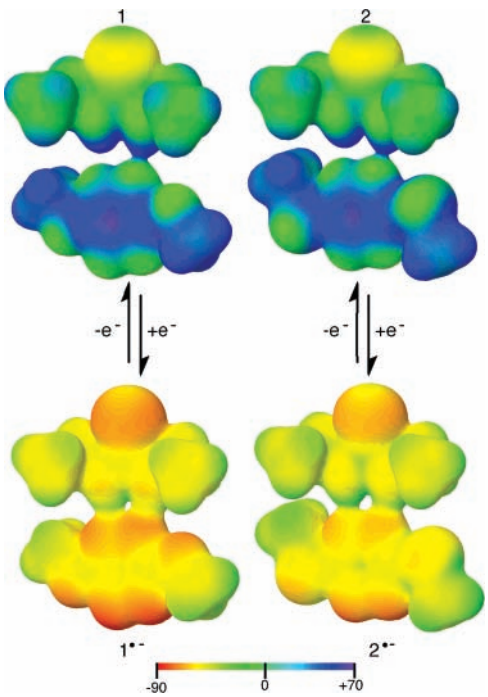

Figure 5. Electrostatic potential maps of **1** (left) and **2** (right) bound to **3b** in their neutral (top) and radical anion (bottom) forms. Energies are relative to the neutral form (kcal/mol).

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Supporting Information Available: ^{1}H NMR and fluorescence titrations, cyclic voltammetry, and computational methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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