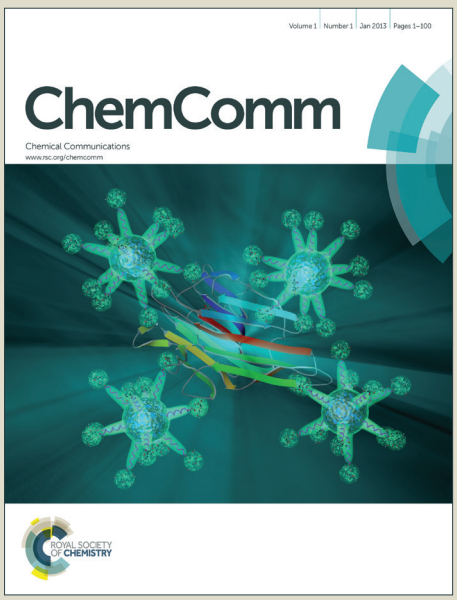
# ChemComm

Accepted Manuscript

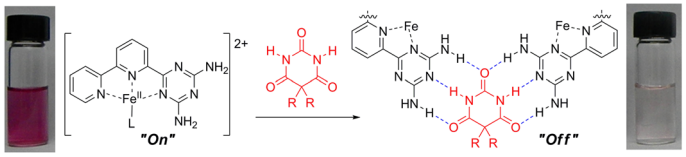




ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

**Graphical Abstract:**




Iron-based spin crossover complexes can be used as selective, visible sensors for barbiturates in the presence of other biologically relevant hydrogen bonding species.

Journal Name

RSCPublishing

COMMUNICATION

# Colorimetric Barbiturate Sensing with Hybrid Spin Crossover Assemblies

Cite this: DOI: 10.1039/x0xx00000x

Michael C. Young, Erica Liew and Richard J. Hooley*

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

**Spin crossover complexes based on either iron(II) or iron(III) give a colorimetric response upon self-assembly with barbituric acids. They can be used as visible sensors for these narcotics, selectively detecting barbiturates in the presence of other biologically-relevant hydrogen bonding species.**

Many non-covalent interactions can be exploited for selective analyte detection, but the most effective highly selective sensors (as opposed to array-based sensors and "chemical noses") rely on strong, directed coordination events between sensor and analyte. The strongest interactions exploit charge complementarity: anions[1] and cations[2] can be bound with good selectivity by designed recognition pockets. Sensing of neutral species is much more challenging, as the binding interactions are typically much weaker.[3] Shape-selective binding in a tailored pocket is known,[4] and reactive species can be sensed via formation of covalent bonds.[5] Species capable of self-complementary hydrogen bonds such as nucleotides or barbiturates are also simple targets for selective molecular recognition.[6]

The challenge in *sensing* via hydrogen-bond mediated assembly is twofold: the recognition must be target selective, and a sensor (as opposed to a host molecule) must undergo an obviously detectable change upon binding. Many host molecules are known for barbiturates, notably Hamilton's designed receptors,[7] and good selectivities and binding affinities are possible. The conversion of this recognition event into a colorimetric sensor is far less common, as the hosts tend to lack innate chromophores and there are only minimal electronic changes upon the hydrogen-bond mediated recognition event. Chemical addition of chromophores to the analyte is known,[8] as is the incorporation of a host into a liquid crystal.[9] Fluorescence spectroscopy of covalently derivatized Hamilton receptors allows analysis of barbiturate binding in aprotic solvents,[8a] but simple "on/off" visual detection of barbiturates is generally an unsolved issue. Other, more variable scaffolds are conceivable, but these suffer significant loss in selectivity. Barbiturates are a class of narcotics that pose problems for molecular sensing, in that they share some structural elements with urea and nucleotides, leading to poor selectivity.[10] Immunological methods are known to give false positives and chromatographic and mass spectrometric methods often require elaborate equipment.[11] A simple, colorimetric barbiturate sensor would provide a novel detection system for challenging neutral analytes.

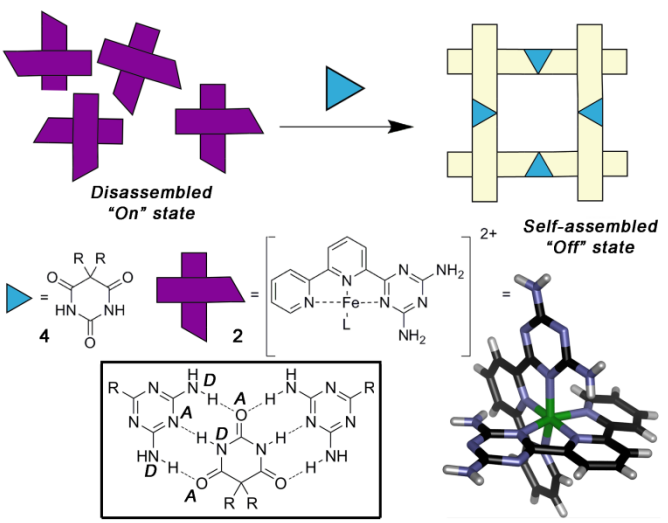



**Fig. 1.** Strategy for selective sensing of substituted barbituric acids via hybrid self-assembly, and an X-ray crystal structure of the sensor molecule **2**.[12]

Recently, we described an Fe(II) complex that showed spin crossover (SCO) behavior upon hydrogen-bond mediated self-assembly.[12] SCO complexes have previously been used for colorimetric detection of anionic analytes,[13] but our barbiturate self-assembly system does not involve any direct interaction between the metal center and analyte. The system takes advantage of the well-precedented multicomponent self-assembly of barbiturates with diaminotriazines.[14] Here we describe iron(II) and iron(III)-SCO complexes as simple, selective colorimetric sensors for barbiturates.

Dissymmetric ligand **1**[15] can be converted to the requisite sensor by treatment with metal salts. The ligand contains three metal-

coordinating sites as well as a diaminotriazine motif: this combination of metal-coordinating and hydrogen bonding groups confers both detection and recognition motifs on the sensor scaffold. When two equivalents of ligand **1** are added to $Fe(ClO_4)_2$, the cationic complex **2** is formed. This complex displays an intense purple color: the dissymmetric ligand coordinates to the metal to give a distorted octahedral complex (Figure 1). Although ligands such as 2,2′:6′,2″-terpyridyl form complexes with preference for low spin Fe(II), the dissymmetry of ligand **1** and resultant distorted coordination sphere leads to SCO behavior of the iron(II) complex **2**.[12,16] The intense purple color is the result of an absorption at 557 nm due to MLCT interactions with the Fe(II) center. Compound **2** naturally exists as a SCO complex at room temperature with $\mu_{eff}$ of 4.4, suggesting an average spin between 1 and 1½, with the low spin state dominant at lower temperatures. Upon self-assembly with dioctyl barbiturate **4b**, the band at 557 nm disappears, while a new peak grows in at 370 nm. This is consistent with a transition of the low spin portion of Fe(II) to high spin Fe(II) upon self-assembly with the barbiturate.[17]

The simple and highly distinctive loss of color upon self-assembly suggested that **2** would be a useful barbiturate sensor. To test the ability of **2** as a selective sensing motif, the complex was combined with differently substituted barbiturates in acetonitrile. Three different substrates were tested, with varying sidechains (guests **4a-c**, Figure 2). The loss of color was not immediate, but after sitting at room temperature for 1h, significant loss of color was observed for all barbiturate additives. After sitting for an additional 15h the barbiturate containing samples became completely colorless. (Figure 2). There were minor differences in the rate of decolorization: **4c** was slightly faster at decolorization of **2** off, but **4a-c** all required a few hours for completion.

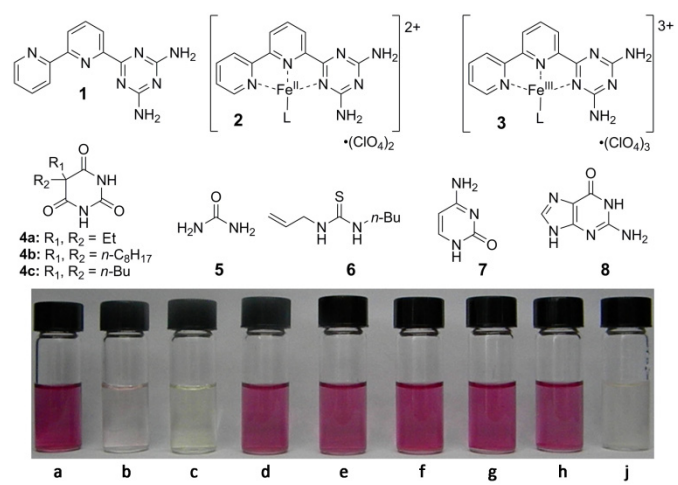



**Fig. 2.** Colorimetric detection of various analytes (10 mM, 87 eq. ea.) using **2** (MeCN, 115 uM): a) **2** only; b) **4a**; c) **4b**; d) **5**; e) **6**; f) Saturated **7**; g) Saturated **8**; h) Mixture of **5**, **6**, and **7**; j) Mixture of **4b**, **5**, **6**, and **7**.

The delayed response is inextricably linked to the selectivity of the sensor: the detection event relies on the multicomponent self-assembly properties of complex **2**. As shown in cartoon form in Figure 1, only "double-sided" complementary hydrogen bonding species such as barbiturates can trigger the self-assembly process. The exact stoichiometry of the assembly is challenging to determine,[12] but multiple molecules of **2** and **4** are assembled via H-bonding into an oligomeric aggregate. Attempts to characterize the stoichiometry of resultant high spin self-assembly by diffusion NMR and ESI-MS were unsuccessful, as were attempts at growing the co-crystalline species. This assembly was proposed to be a $\mathbf{2_4 \bullet 4_4}$ square complex (Figure 1, see ESI for modeling) as this is the smallest discrete structure that can form without significant strain. It is conceivable that the aggregate is a mixture of oligomers, however. While other small molecules that display multiple hydrogen bonding motifs are capable of weak association with **2**, the proximity-directed quenching process only occurs if two or more Fe(II) centers are brought together by barbiturate-mediated self-assembly. In this event, the exact stoichiometry of assembly is unimportant: all self-assembled structures associate multiple Fe centers. This is shown in Figure 2: other common hydrogen bonding species such as urea **5**, or the more acidic 1-allyl-3-butylthiourea **6** cause no loss of color in the solution of **2**, even at large excesses. These two-point hydrogen-bonding motifs have only weak associations with the diaminotriazine core (see ESI), but much more strongly compatible species such as nucleotides cytosine **7** and guanine **8** are also not detected by complex **2**, even though the molecules display identical hydrogen-bonding contacts as the barbiturate targets.

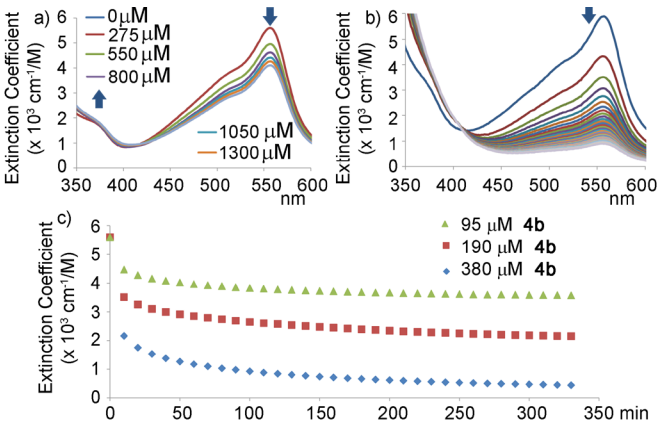



**Fig. 3** Concentration and time dependence of the response for sensor **2** in the presence of barbiturate **4b**: a) **2** treated with different concentrations of **4b** (approximately 20 s incubation time); b) **2** treated with **4b** (380 μM), measured at 10 min intervals; c) Concentration-dependent change in absorbance (557 nm) over time of **2** treated with **4b** (MeCN, 50 μM).

Most importantly, the sensing event is not disrupted by these competitive hydrogen bonding units. If equimolar amounts of urea, 1-allyl-3-butylthiourea, and cytosine (Fig 2, vial h) are added to a solution of **2b**, no color change is observed. If dioctylbarbiturate **4b** is added to the mixture (Fig 2, vial j), the **2:4b** assembly is formed with concomitant loss of color. Despite being in the presence of competitive hydrogen bonding inhibitors, assembly and visual detection of barbiturates is unaffected. There is an upper limit: the detection event does not occur in highly competitive solvents such as DMSO, but it can tolerate the presence of protic solvents in $CH_3CN$. When the system is treated with varying amounts of ethanol, the decolorization is slowed. Barbiturate detection, however, still occurs in a 4:1 $CH_3CN$:EtOH mix. If a solution containing the **2:4b** complex is left sitting, it will slowly absorb enough water to re-equilibrate and regenerate **2** in the "on" state (see ESI for full data).

While complex **2** shows simple, qualitative "on/off" detection of barbiturates, more quantitative data can be obtained by UV analysis. Analysis was performed using **4b** as hydrogen bonding partner (Figure 3). The iron(II) complex shows a decrease in MLCT band at 557 nm as barbiturate is added with a slight increase in the high spin MLCT band at 370 nm. This loss in the band at 557 nm is not complete at 26 equivalents of barbiturate over the course of 10 min.

The lack of immediacy in the detection is likely the result of the supramolecular aggregate forming through multiple equilibrating structures. While the complete self-assembly forces the iron complexes into a high spin state (thereby turning the sensor off), it takes time to reach this state. To quantify the rate of the self-assembly process, complex **2** was treated with **4b**, followed by taking UV/Vis spectra every 10 minutes (Figure 3b). Although the initial rate is proportional to the concentration of barbiturate, it becomes negligible after only a few hours regardless of the amount of barbiturate added (Figure 3c). Using this, we were able to reproducibly detect barbiturate solutions as dilute as 1 μM after a 1h incubation time using a 50 μM solution of sensor (see ESI).

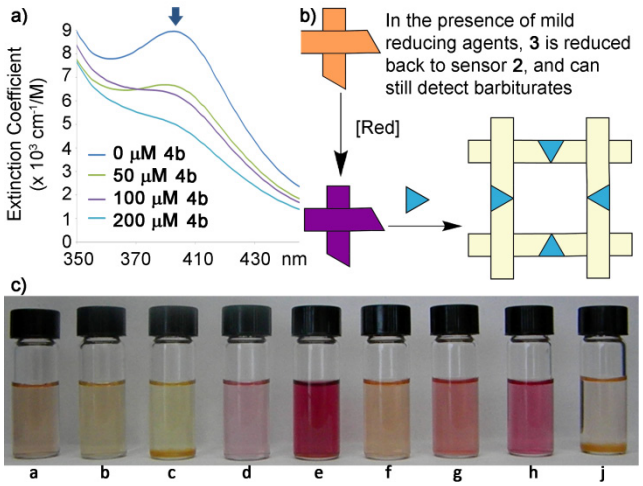



**Fig. 4.** Applications using sensor **3**: a) Titration of **4b** into a solution of **3** with an approximate incubation time of 20 s (MeCN, 115 μM); b) Reduction pathway to regenerate sensor **2**; c) Colorimetric detection of various analytes (10 mM, 23 eq. ea.) using **3** (MeCN, 460 uM): a) **3** only; b) **4a**; c) **4b**; d) **5**; e) **6**; f) Saturated **7**; g) Saturated **8**; h) **5**, **6**, and saturated **7**; j) **4b**, **5**, **6**, and saturated 7.

While complex **2** displays simple, selective on/off sensing behavior, its Fe(III) counterpart shows a more sensitive detection profile. Complex **3** was synthesized from ligand **1** and iron (III) perchlorate via the method used for the synthesis of **2**. Complex **3** is a pale orange color: the MLCT band is no longer in the visible region, but shifts to the UV region (380 nm). As a result, there is only a slight visible change between the free complex **3** and the samples containing barbital and dioctyl barbiturate (Fig 4c). The assembly event still occurs, however, and can be easily monitored by UV spectroscopy (Fig 4a): the MLCT band disappears upon assembly as before. There is again no competition or inhibition of assembly from other hydrogen bond donors, but interestingly, complex **3** is quite easily reduced to complex **2** in the presence of the extremely mild thiourea reducing agent **6**. If **6** is combined with **3**, the characteristic purple color of the Fe(II) complex **2** is observed. Although the iron(III) salt **3** can be reduced *in situ*, it is clear that even then the sensing ability for barbiturates is undiminished: addition of barbiturate **4b** decolorizes the system as usual.

In summary, the iron(II) complex **2** is able to function as a general sensor for barbituric acid derivatives. The sensor selectively recognizes barbiturates with varied chain lengths (from two to eight carbons), while showing no colorimetric response in the presence of a variety of biologically relevant hydrogen bonders. The sensor appears stable to oxidation, and even has the capacity to recognize the target analyte class in the presence of competitive but non-responsive hydrogen bonding species.

## Acknowledgements

Acknowledgement is made to the National Science Foundation (CHE-1151773) as well as UC Riverside (Dissertation Research Grant for M.C.Y.) for support of this research.

## Notes and references

[a] University of California Riverside, Department of Chemistry Riverside, CA 92521 (USA), E-mail: richard.hooley@ucr.edu

† Electronic Supplementary Information (ESI) available: See DOI: 10.1039/b000000x/

1 S. K. Kim, G. I. Vargas-Zúñiga, B. P. Hay, N. J. Young, L. H. Delmau, C. Masselin, C.-H. Lee, J. S. Kim, V. M. Lynch, B. A. Moyer and J. L. Sessler, *J. Am. Chem. Soc.*, 2012, **134**, 1782.

2 S. K. Kim, J. L. Sessler, D. E. Gross, C.-H. Lee, J. S. Kim, V. M. Lynch, L. H. Delmau and B. P. Hay, *J. Am. Chem. Soc.*, 2010, **132**, 5827.

3 G. J. Mohr, *Chem.-Eur. J.*, 2004, **10**, 1082.

4 S. Zampolli, P. Betti, I. Elmi and E. Dalcanale, *Chem. Commun.*, 2007, 2790.

5 V. Kumar and E. V. Anslyn, *Chem. Sci.*, 2013, **4**, 4292.

6 *a*) C. Bazzicalupi, A. Bencini, A. Bianchi, E. Faggi, C. Giorgi, S. Santarelli and B. Valtancoli, *J. Am. Chem. Soc.*, 2008, **130**, 2440.

7 *a*) J. M. McGrath and M. D. Pluth, *J. Org. Chem.*, 2014, **79**, 711; *b*) S.-K. Chang, D. Van Engen, E. Fan and A. D. Hamilton, *J. Am. Chem. Soc.*, 1991, **113**, 7640; *c*) S.-K. Chang and A. D. Hamilton, *J. Am. Chem. Soc.*, 1988, **110**, 1318.

8 *a*) I. Aoki, Y. Kawahara, T. Sakaki, T. Harada and S. Shinkai, *Bull. Chem. Soc. Jpn.*, 1993, **66**, 927 *b*) R. Schmidt, S. Uemura and F. Würthner, *Chem. Eur. J.*, 2010, **16**, 13706; *c*) P. Tecilla, R. P. Dixon, G. Slobodkin, D. S. Alavi, D. H. Waldeck and A. D. Hamilton, *J. Am. Chem. Soc.*, 1990, **112**, 9408.

9 R. C. Chambers, E. J. Bell, T. M. Records, A. Cherian, K. Ragan and B. Swartout, *Liq. Cryst.*, 2007, **34**, 1221.

10 J. Westwood, S. J. Coles, S. R. Collinson, G. Gasser, S. J. Green, M. B. Hursthouse, M. E. Light and J. H. R. Tucker, *Organometallics*, 2004, **23**, 946.

11 *a*) L. L. Johnson and U. Garg, *Methods Mol. Biol.*, 2010, **603**, 65; *b*) R. Meatherall, *J. Forensic Sci.*, 1997, **42**, 1160.

12 M. C. Young, E. Liew, J. Ashby, K. E. McCoy and R. J. Hooley, *Chem. Commun.*, 2013, **49**, 6331.

13 Z. Ni, A. M. McDaniel and M. P. Shores, *Chem. Sci.*, 2010, **1**, 615.

14 *a*) S. M. S. Chauhan and N. G. Giri, *Supramol. Chem.*, 2008, **20**, 743; *b*) S. Yagai, T. Karatsu and A. Kitamura, *Chem. Commun.*, 2003, 1844; *c*) A. G. Bielejewska, C. E. Marjo, L. J. Prins, P. Timmerman, F. de Jong and D. N. Reinhoudt, *J. Am. Chem. Soc.*, 2001, **123**, 7518.

15 F. H. Case, *J. Org. Chem.*, 1966, **31**, 2398.

16 J. Olguín and S. Brooker, *Coord. Chem. Rev*., 2011, **255**, 203.
17 *a*) P. D. Southon, L. Liu, E. A. Fellows, D. J. Price, G. J. Halder, K. W. Chapman, B. Moubaraki, K. S. Murray, J.-F. Letard and C. J. Kepert, *J. Am. Chem. Soc*., 2009, **131**, 10998; *b*) E. C. Constable, G. Baum, E. Bill, R. Dyson, R. van Eldik, D. Fenske, S. Kaderli, D. Morris, A. Neubrand, M. Neuburger, D. R. Smith, K. Wieghardt, M. Zehnder and A. D. Zuberbühler, *Chem.–Eur. J*., 1999, **5**, 498.

