## CONJUGATED POLYMERS AS FLUORESCENCE-BASED CHEMICAL SENSORS

Reported by Jay Wackerly November 1, 2004

### INTRODUCTION

A chemical sensor is a molecule that interacts with another molecule giving a measurable signal in response. Chemical sensors based on conjugated polymers have received a great deal of attention due to their ability to detect analytes at low concentrations.[1] Fluorescence quenching[2] provides an effective means for detection because of the extreme sensitivity and simple on/off detection.[3] When the advantages of each of these are combined, fast, simple, and accurate chemical sensors based on fluorescence quenching are obtained.

Chemical sensors based on conjugated polymers detect a variety of analytes (*vide infra*) but they all have similar detection mechanisms. Conjugated polymers contain a chromophore requiring low energy for excitation of an electron. Because it is highly delocalized, the excited electron (exiton) can travel along the polymer until fluorescence (or quenching) occurs. Each chemical sensor contains a receptor that binds to the analyte causing a change in the fluorescence. A conjugated polymer-based chemical sensor has one receptor site for every repeat unit, and since the exiton can travel across the polymer it samples many receptor sites causing it to be more sensitive than a non-polymeric system. A fluorescence-based chemical sensor has two modes of action. The fluorescence can be quenched in the presence of the analyte producing a "turn-off" chemical sensor, (Figure 1) or its fluorescence quenching is supressed by the presence of the analyte producing a "turn-on" chemical sensor.

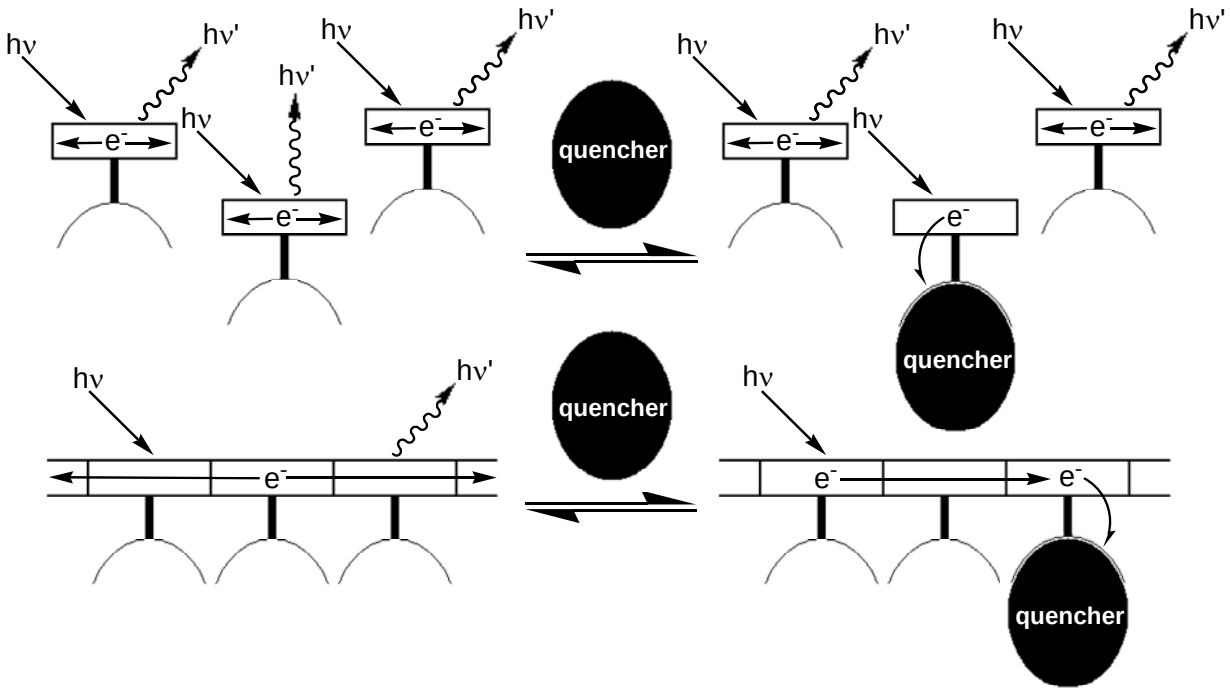
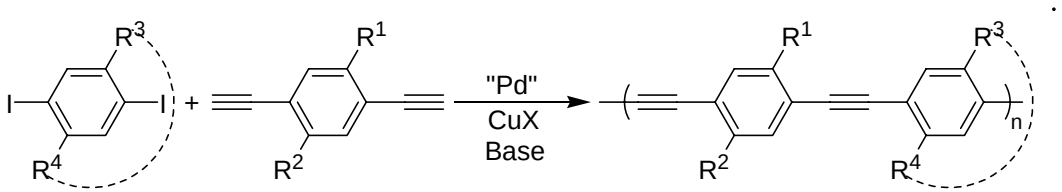



**Figure 1.** (Top) Traditional fluorescence quenching "turn-off" chemical sensor. (Bottom) A chemical sensor with a delocalized exiton quenching the fluorescence of the whole polymer.

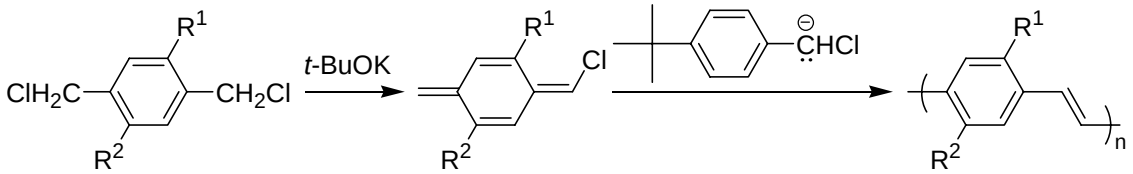
This report will review the use of conjugated polymers as fluorescence-quenching-based chemical sensors. This coverage will include a discussion of the synthesis of the conjugated polymer sensors, the detection of organic molecules, and selective detection of biological building blocks.

## CONJUGATED POLYMERS

Two types of conjugated polymers are commonly used as fluorescence chemical sensors. The first type, poly(*p*-phenyleneethynylene) (PPE), is prepared through Sonogashira step-growth polymerization of a nearly equimolar amount of *p*-diethynylbenzene and 1,4-diiodobenzene that yields molecular weights ($M_n$) of >100 kDa and with a polydispersity index (PDI) of approximately 2 (Scheme 1).[4] The second type of polymer, poly(*p*-phenylenevinylene) (PPV), can be prepared via chain-growth polymerization giving $M_n$ as high as 70 kDa and PDI >5 (Scheme 2).[5] Examples of PPE (**1**-**5**) and PPV (**6**-**7**) polymers made through these methods are shown in Chart 1. Other conjugated polymers such as polyacetylene[6] and polyfluorene[7] have uses as fluorescence chemical sensors, however they will not be discussed in this report.




**Scheme 1.** Sonogashira polymerization of phenyldiacetylene and diiodobenzene.




**Scheme 2.** Activation of p-xylene species to form p-xylylene that polymerizes to give PPV.

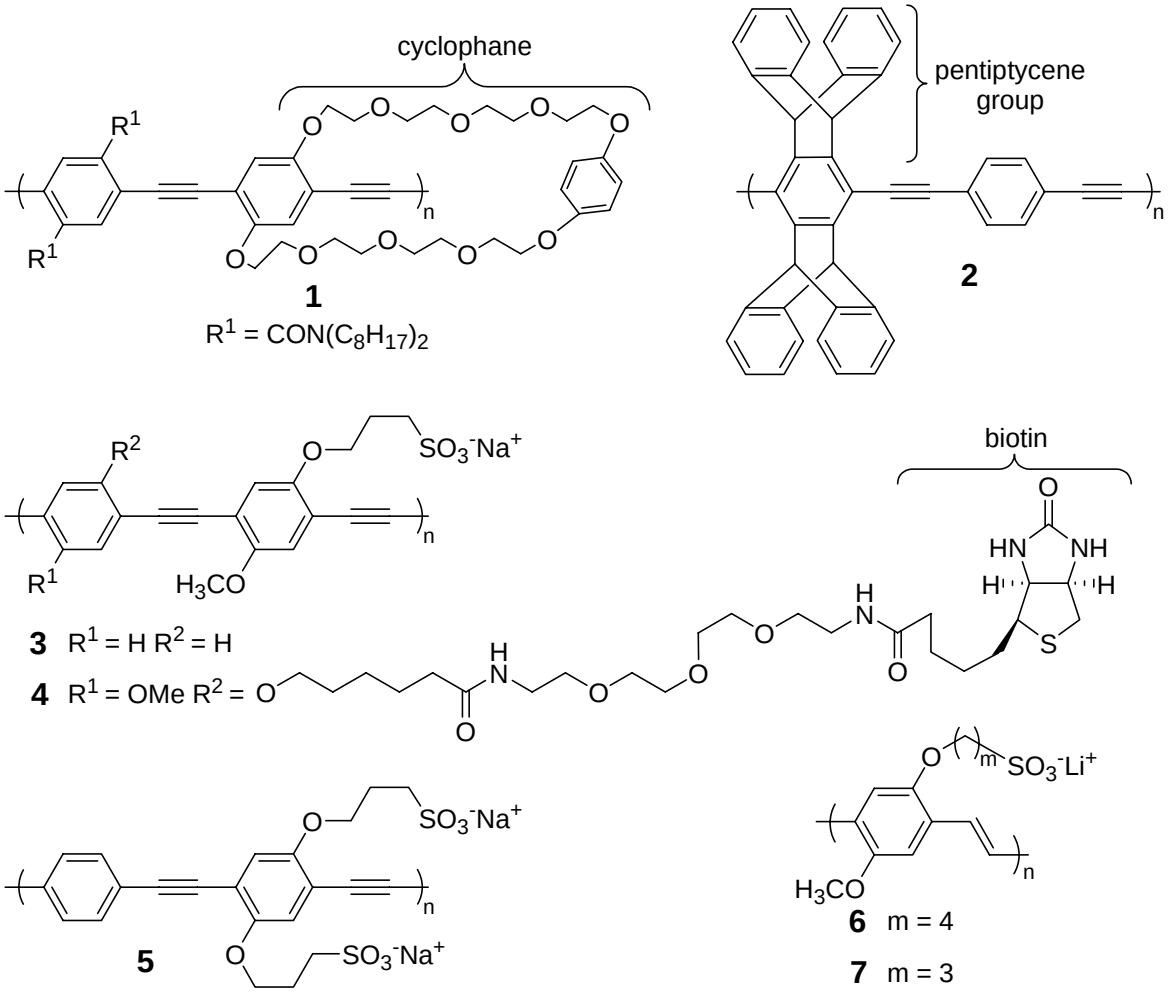
## ORGANIC MOLECULE SENSORS

The ability to quickly detect trace amounts of specific organic molecules in the gas phase or in solution is important in such areas as forensics, industrial processing, food packaging and distribution, etc. Often accurate detection of small organic molecules requires purification (HPLC, column chromatography, etc.) followed by the use of specialized instruments (MS, NMR, IR, etc.). Conjugated polymer based sensors posses the ability to perform this function by utilizing "turn-off" fluorescence detection of viologen and explosive nitroaromatic containing compounds.

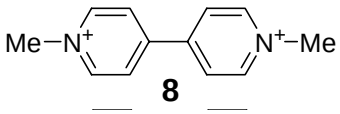
### Viologen Sensors

The first conjugated polymer-based fluorescence sensor was developed by Swager and Zhou[8,9] and utilized a PPE polymer containing a receptor cyclophane (**1**, Chart 1). Because Stoddard and coworkers[10] showed that cyclophanes reversibly bind methyl viologen (MV, **8**) and that MV is an effective fluorescence

**Chart 1.**




quencher, MV is the analyte of interest for these studies. A 60-fold increase in fluorescence quenching of the conjugated polymer is obtained in comparison to an analogous compound containing only one cyclophane per molecule (i.e. monomer of **1**). From the reported molecular weight, the PPE polymer has an average of 53 repeat units which is a greater than one fold increase in sensitivity per monomer unit. The sensitivity does not increase as more repeat units are added above this weight. The authors propose that this weight (length of the polymer chain) is the threshold for the length an exition can travel during its lifetime ($6.4 \times 10^{10}$ s) in the excited state.[8,9]




In efforts to expand the applicability of these sensors to other media, Heeger and coworkers designed a water soluble polymer by placing anionic side chains on PPV (**6**, Chart 1).[11] This PPV polymer shows over 100-fold greater sensitivity to MV compared to the previous PPE system.[12] It is proposed that this is due to the electrostatic interactions between the dicationic MV and the anionic side chains. To determine the role of the electrostatic interactions between the analyte and the PPV, Heeger and coworkers synthesized viologen species with varying charges.[13] A dicationic viologen analyte has 100-fold greater ability to quench fluorescence over a

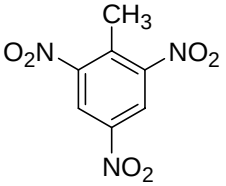
neutral viologen species, and a monocationic viologen species has 10-fold greater sensitivity over a neutral species. Thus a dicationic species is the optimal analyte.[12]

These studies demonstrate the ability for detection of viologen by using conjugated polymers as fluorescence quenching chemical sensors. These model studies show the capability of conjugated polymers to detect analytes at low concentrations. Because the analyte-receptor binding is the important factor in these chemical sensor systems, new systems can be designed to detect other analytes without varying the polymer or the sensitivity of the system.

**Nitroaromatic Sensors**

According to the United Nations, there are 120 million active landmines buried in over 70 countries.[14] Detection of these landmines is a major problem because many countries cannot afford the equipment and specialists to find them. The most common method for locating buried landmines in these countries involves the use of a metal detector and a long stick, to prod the ground to find the exact location of the mine.[15] This is obviously a crude and time consuming method that could be improved upon by the utilization of a TNT chemical sensor that can detect the chemical constituents of the explosive.

Swager and Yang designed a chemical sensor that can detect 2,4,6-trinitrotoluene (TNT) (present in 80%-90% of landmines[13]) vapors within seconds.[16,17] This sensor is a PPE polymer functionalized with pentiptycene groups (**2**, Chart 1) that when cast as a film is pourous and exhibits the same absorption and fluorescence as in solution. Upon exposure to TNT (in the gas phase at 10 ppb) fluorescence quenching is observed. The authors propose that this is due to π-π interactions between the π-electron rich polymer backbone and the π-electron poor TNT (Figure 2). In support of this theory, π-electron rich analytes with higher vapor pressures (e.g. benzoquinone) are found to not quench fluorescence as readily as TNT. Polymer film thickness has a profound effect on how well the fluorescence is quenched by TNT, 25 Å films, the thinnest tested, are the most effective at fluorescence quenching. The authors attribute this to the difficulty with which TNT vapor traverses through thick films in short amounts of time. Thus fewer polymers are quenched because the TNT could not reach the polymers on the inside of the film. Ultimately these studies find that 25 Å PPE films quench 75% of



**TNT**

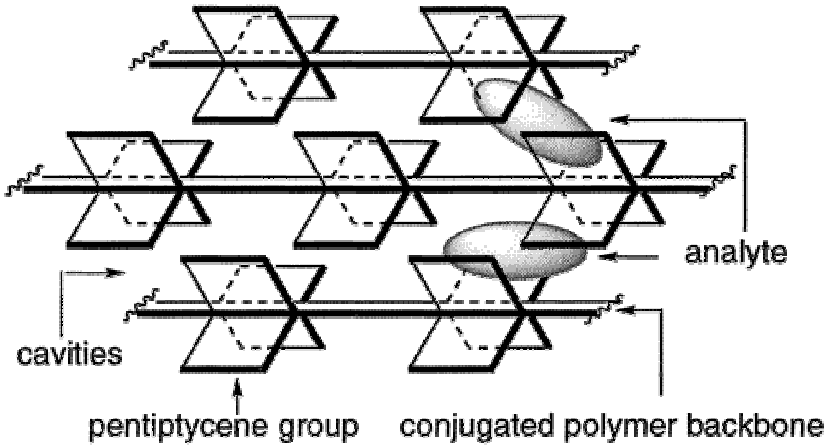


**Figure 2.**[15] Representation of TNT (analyte) binding to the pentiptycene groups and PPE (conjugated polymer backbone).

fluorescence within 60 s of exposure to TNT. Other nitroaromatics with higher vapor pressures such as 2,4-dinitrotoluene (DNT) quench fluorescence by >90% in 10 s using 25 Å films.

Nomadics, Inc. has developed a portable landmine detector based on this technology.[18,19] They report the ability to detect femtogram quantities (56 fg of TNT/mL of air, 6 ppt) of nitroaromatic explosive compounds in the air in near real-time (1 s). Field studies show that this detector is more likely to detect a landmine and less likely to give a false detection than a TNT sniffing dog and team (the current best method for explosives detection in the United States).

## BIOLOGICAL SENSORS

Detection of biological molecules by fluorescence-based conjugated-polymer chemical sensors has become a rapidly developing field since its inception in 1999.[20] High sensitivity, shorter assay times, and high throughput analysis of biological molecules provides for more efficient and less expensive analysis. Biological molecule (i.e. proteins and carbohydrates) detection exemplifying the advantages of conjugated polymers and fluorescence quenching is discussed below.

### Protein Sensors

Enhanced detection of selected proteins would be tremendously beneficial to the medical and biological communities because of its potential to identify diseases, pregnancies, injuries, etc. Whitten and coworkers were the first to show that conjugated polymers could be used as fluorescence based chemical sensors for biological molecules.[21] This particular system detects avidin, and although the use of avidin is a proof of concept, the method shows a great deal of promise for uses in other proteins. The avidin sensor is based on tethering a biotin group (known to bind very tightly in the active site of avidin) to a viologen to produce an MV-

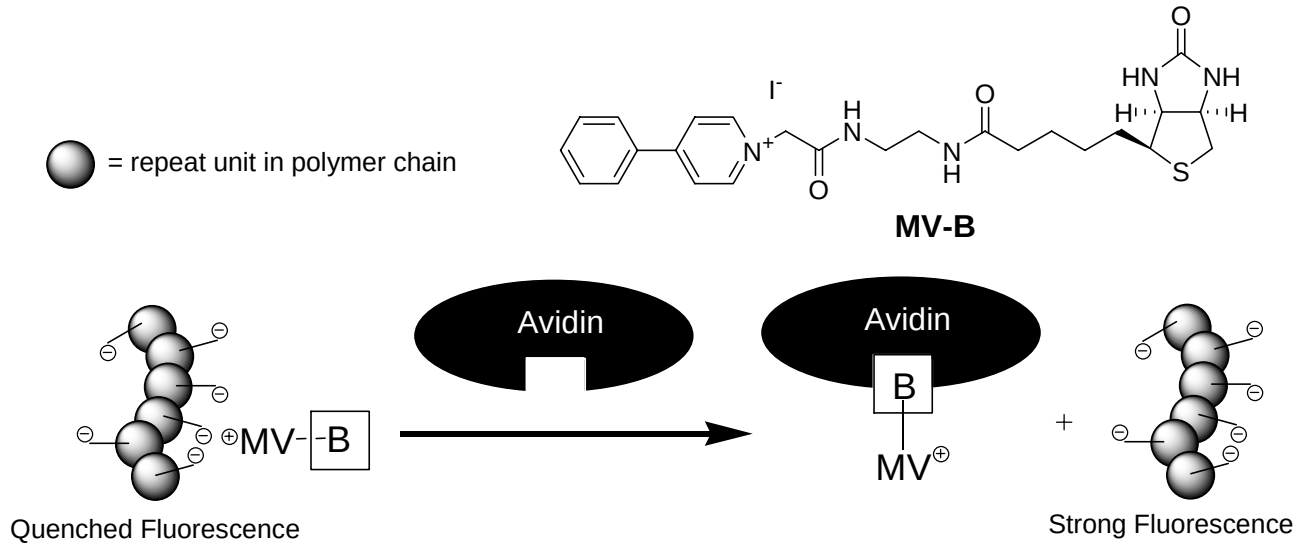



**Figure 3.** Selective binding of biotin to avidin over methyl viologen to the PPE polymer.

B hybrid (Figure 3). Fluorescence quenching is observed due to the electrostatic interactions between the cationic MV-B adduct and the anionic polymer PPV (**7**, Chart 1) in aqueous solution. Upon addition of avidin, the MV-B is selectively complexed because the high association constant, thus restoring the fluorescence. When a different protein (choleratoxin) that does not bind biotin is employed, fluorescence is not restored. Also a monocationic viologen without a tether to biotin is tested in the presence of avidin and again fluorescence is not restored.[20] This "turn-on" fluorescence sensor demonstrates the ability to selectively detect the protein avidin.

Protease enzymes play important roles in regulating biological systems such as blood coagulation (thrombin), extracellular disposition of insoluble amyloid plague (β-secretase), and apoptosis (caspace), among many other functions.[22] Because of the importance of these enzymes, both Schanze and Pinto[23] as well as Whitten and coworkers[24] developed chemical sensors for detection of protease activity. Both methods involve ion pairing of an anionic PPE polymer (**5** and a 3:1 distribution of **3**:**4** respectively, Chart 1) with a cationic peptide chain. The end of each chain is functionalized with a quencher, so that initially, fluorescence is quenched. When a protease is introduced that can cleave a specific bond in the peptide chain, the quencher is released into solution and fluorescence is restored (Figure 4). In this manner "turn-on" fluorescence sensing can monitor protease activity. Sensors for both systems are able to detect nanomolar concentrations of proteases (specifically thrombin, β-secretase, and caspace) in five minutes or less.

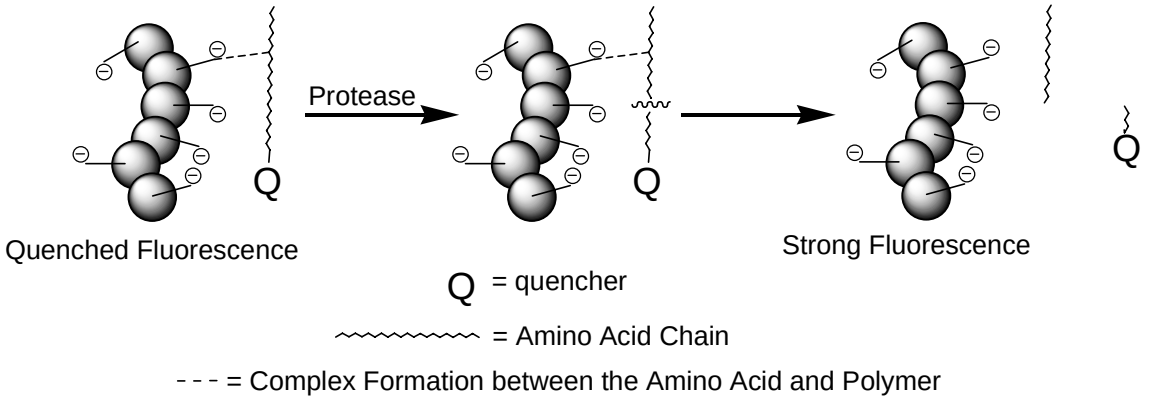



**Figure 4.** A fluorescence "turn-on" protease sensor. The protease cleaves the polymer releasing the quencher into solution.

**Saccharide Sensors**

Sugar (saccharide) sensors are extremely important for detection of blood-sugar levels in persons having diabetes or hypoglycemia. Although the field for fluorescence detection of sugars was developed some time ago,[3] interest in using conjugated polymer sensors is in its infancy. Traditional methods use boronic acids that bond monosacchrides resulting in a negative charge on the boron.[25] Lakowicz and coworkers show that if no sugar is present in an aqueous solution then a dicationic boronic acid-viologen species quenches the fluorescence of a PPE polymer (**5**, Chart 1) by electrostatic interactions.[26] However, upon the addition of a

sugar (specifically D-fructose, D-galactose, or D-glucose) the boronic acid-viologen species becomes neutral and does not have an affinity for the anionic polymer chain; hence fluorescence is "turned-on" (Figure 6). The use of a conjugated polymer backbone allows the researchers to observe a 10-fold increase in fluorescence intensity over other fluorescence-based sugar sensors. Though the detection limit for this sensor is not reported, if a sensor can be made for the detection of human blood-sugar levels, then a real-time monitoring of blood sugar is a distinct possibility with this technology.

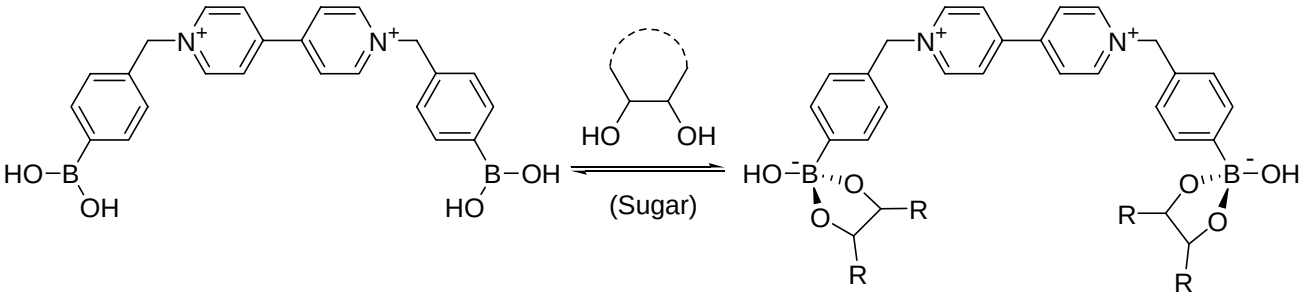



**Figure 6.** The dicationic viologen quencher containing boronic acid groups becomes neutral in the presence of a sugar.

## CONCLUSIONS

Conjugated polymers as fluorescence-based chemical sensors have been developed to quickly detect important organic (TNT) and biological (sugars, thrombin, β-secretase, and caspace) molecules at low concentrations. These sensors are not without their individual problems, such as false positives (the sensor detecting an undesired substance as the analyte). Though the groundwork in this field as been laid, there is still considerable potential for this field to evolve by both overcoming the current sensor problems and through the ability to detect a larger number of molecules. In the future, enhanced detection of proteins and sacchrarides should become more developed allowing for reversible sensors for the analysis of biological samples, and small molecules such as drugs or chemical warfare agents show great potential to be detected through this system also.

## REFERENCES


(1) McQuade, T. D.; Pullen, A. E.; Swager, T. M. *Chem. Rev.* **2000,** *100*, 2537-2574.

(2) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*. Plenum Press: New York, 1983.

(3) Czarnik, A. W. *Fluorescent Chemosensors for Ion and Molecule Recognition*. American Chemical Society: Washington, 1993.

(4) Neenan, T. X.; Whitesides, G. M. *J. Org. Chem.* **1988**, *53*, 2489-2496.

(5) Hsieh, B. R.; Yu, Y.; VanLaeken, A. C.; Lee, H., *Macromolecules* **1997,** *30*, 8094-8095.

(6) For a recent example see: Liu, Y.; Mills, R. C.; Boncella, J. M.; Schanze, K. S., *Langmuir* **2001,** *17*, 7452-7455.

(7) For a recent example see: Gaylord, B. S.; Heeger, A. J.; Bazan, G. C. *J. Am. Chem. Soc.* **2003**, *125*, 896-900.

(8) Zhou, Q.; Swager, T. M. *J. Am. Chem. Soc.* **1995,** *117*, 7017-7018.

(9) Zhou, Q.; Swager, T. M. *J. Am. Chem. Soc.* **1995,** *117*, 12593-12602.

(10) Alwood, B. L.; Spencer, N.; Shahriari-Zavareh, H.; Stoddart, J. F. *Chem. Commun.* **1987**, 1064-1066.

(11) Wang, J.; Wang, D.; Miller, E. K.; Moses, D.; Bazan, G. C.; Heeger, A. J. *Macromolecules* **2000**, *33*, 5153-5158.

(12) Wang, J.; Wang, D.; Miller, E. K.; Moses, D.; Heeger, A. J. *Synth. Met.* **2001**, *119*, 591-592.

(13) Wang, D.; Wang, J.; Moses, D.; Bazan, G. C.; Heeger, A. J. *Langmuir* **2001,** *17*, 1262-1266.

(14) Yinon, J. *Anal. Chem.* **2003**, 99A-105A.

(15) Rouhi, A. M. *Chem. Eng. News* **1997**, 14-22.

(16) Yang, J.-S.; Swager, T. M. *J. Am. Chem. Soc.* **1998,** *120*, 5321-5322.

(17) Yang, J.-S.; Swager, T. M. *J. Am. Chem. Soc.* **1998,** *120*, 11864-11873.

(18) Cumming, C. J.; Aker, C.; Fisher, M.; Fox, M.; La Grone, M.; Reust, D.; Rockley, M.; Swager, T. M.; Towers, E.; Williams, V. *IEEE Transactions on Geoscience and Remote Sensing* **2001,** *39*, 1119-1128.

(19) La Grone, M.; Cumming, C.; Fisher, M.; Fox, M.; Jacob, S.; Reust, D.; Rockley, M.; Towers, E. *Proceedings of the SPIE, Detection and Remediation Technologies for Mines and Minelike Targets V* **2000,** *4038*, 553-562.

(20) Wosnick, J. H.; Swager, T. M. *Curr. Opin. Chem. Biol.* **2000,** *4*, 715-720.

(21) Chen, L.; McBranch, D. W.; Wang, H.-L.; Helgeson, R.; Wudl, F.; Whitten, D. G. *Proc. Natl. Acad. Sci. U.S.A.* **1999,** *96*, 12287-12292.

(22) Barrett, A. J.; Rawlings, N. D.; Woessner, J. F. *Handbook of Proteolytic Enzymes*. Academic Press: San Diego, 1998.

(23) Pinto, M. R.; Schanze, K. S. *Proc. Natl. Acad. Sci. U.S.A.* **2004,** *101*, 7505-7510.

(24) Kumaraswamy, S.; Bergstedt, T.; Shi, X.; Rininsland, F.; Kushon, S.; Xia, W.; Ley, K.; Achyuthan, K.; McBranch, D.; Whitten, D. *Proc. Natl. Acad. Sci. U.S.A.* **2004,** *101*, 7511-7515.

(25) James, T. D.; Sandanayake, K. R. A. S.; Iguchi, R.; Shinkai, S. *J. Am. Chem. Soc.* **1995,** *117*, 8982-8987.

(26) DiCesare, N.; Pinto, M. R.; Schanze, K. S.; Lakowicz, J. R. *Langmuir* **2002,** *18*, 7785-7787.