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Review Article

Medical Diagnostics using Designed Molecules with Sense and Logic

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Abstract. Luminescent molecules responsive to cations, anions and even small molecules can be designed with the appropriate selectivity and sensitivity for monitoring physiological and pathological levels of analytes. We highlight some recent examples of designed molecules that can sense for a specific analyte or a combination of analytes in blood and in living cells. Furthermore, we demonstrate how molecules can be designed with built-in algorithms according to principles of Boolean logic to perform information processing. The potential future application of molecular systems able to perform multi-analyte sensing as 'lab-on-a-molecule' systems for medical and environmental diagnostics is also presented.

Keywords Metal ions; Chemosensors; Photoinduced electron transfer; Molecular logic gates; Lab-on-a-molecule; Biomedical diagnostics

1 Introduction

Fluorescent chemosensors are molecules that selectively bind an analyte resulting in an alteration in the photophysical properties of the system via an optical signal (Valeur et al. 2012). These types of sensors are advantageous as they can be designed with good selectivity and sensitivity besides having a rapid response. Furthermore, they require only simple instrumentation for their use: a hand-held UV lamp is enough for qualitative studies. For quantitative studies, the traditional tools are absorption and fluorescence spectrometers.

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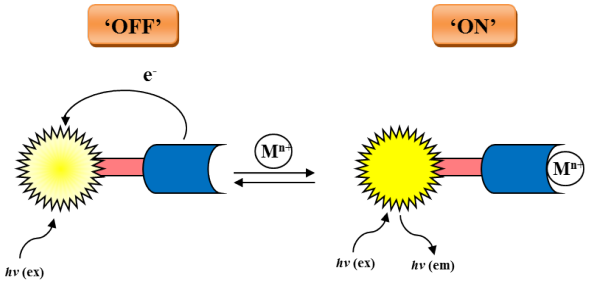

Figure 1: Diagram showing the design format (fluorophore (yellow), spacer (pink), and receptor (blue)) and 'off' and 'on' states of a PET chemosensor.

For real-life applications, fluorescent chemosensors must meet several requirements (Lakowicz 2009). Foremost, they must be selective for a specific targeted metal ion even in the presence of other metals ions found at higher concentration levels and should also be sensitive to the pathological concentration range of the analyte (Burtis et al. 2001). The rational design of such selective chemosensors owes a lot to the principles governing guest-host and coordination chemistry (Cotton 1999). The sensors must also be compatible with the biological matrix and generally water-soluble (Domaille et al. 2008). Typically, a 'turn-on' emission response or a wavelength shift is preferred over a 'turn-off' emission quenching response for maximizing spatial resolution, notably with a light microscope. In cell biology studies, a higher fluorescence brightness is advantageous as less of the intrusive indicator is needed, thus minimizing the possibility of toxicity and altering the cellular environment (Que, 2008). Many applications use fluorophores that emit in the visible region ($400 - 650\,\text{nm}$); however, those emitting at even longer wavelengths in the near infra-red region ($650 - 900\,\text{nm}$) are needed for biomedical uses (Johnson et al. 2010). This range is useful in penetrating deep within the tissue without causing photo-damage to samples, and without interference from background autofluorescence of cellular com-

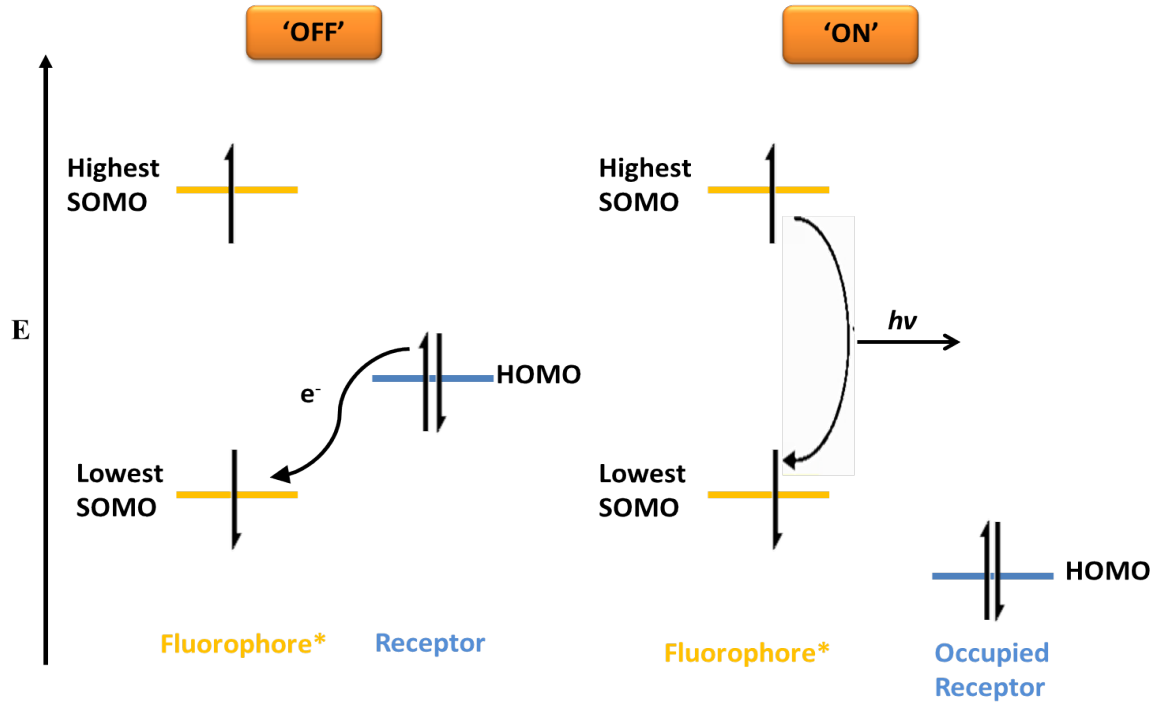

Figure 2: Molecular orbital energy diagrams showing the relative energy of the frontier orbitals of receptor and fluorophore in the "off" and "on" states during the PET process.

ponents. This problem can also be compensated for by using chemosensors with a high extinction coefficient and high fluorescence quantum yield.

This review highlights some examples of fluorescent molecules that have been used for sensing and diagnosing purposes. Some notable examples have been selected with proven application in clinical chemistry or cell imaging. We have included representative examples of molecules able to sense for various species including protons, metal cations and anions, and examples of molecular logic gates that can detect for a combination of analytes (Callan et al. 2005; Magri et al. 2007; de Silva et al. 1997; Prodi et al. 2000; Formica et al. 2012 and Jeong et al. 2012).

2 Photoinduced Electron Transfer (PET)

The basic design of an 'off-on' PET sensor involves a 'fluorophore-spacer-receptor' format as illustrated in Figure 1 (de Silva 2011). They operate based on the competition between photoinduced electron transfer and fluorescence. Such chemosensors have three components: (i) a luminescent component (fluorophore), (ii) a receptor for binding the analyte and (iii) a spacer connecting (i) and (ii). In the 'off' state, upon irradiation of the fluorophore, electron transfer occurs from the unbound receptor to the fluorophore resulting in essentially no fluorescence. However, in the 'on' state, the analyte is bound by the receptor preventing electron transfer and resulting in fluorescence. The ideal PET probe causes an 'off-on' switching of the fluorescence intensity only with no change in the wavelength.

Figure 2 illustrates simplified molecular orbital diagrams for the 'off' and 'on' states. Upon excitation of the fluorophore, an electron from the highest occupied molecular orbital (HOMO) is promoted to the lowest unoccupied molecular orbital (LUMO) of the fluorophore, which become singly occupied molecular orbitals (lowest SOMO). When the lone electron pair in the HOMO of the unbound receptor has a slightly higher energy than the SOMO of the fluorophore, a fast intramolecular PET occurs resulting in quenching of the fluorescence (Kavamos 1993). However, when the receptor binds to the analyte, the oxidation potential of the donor is increased so that the HOMO of the bound receptor becomes lower in energy than the lowest SOMO of the fluorophore. The intramolecular PET process is now not feasible and quenching is suppressed, representing the 'on' stage of the fluorescent sensor. It is the competition between electron transfer and fluorescence that is the basis of PET chemosensors (de Silva 2008).

Having a design criterion is common in engineering, but rare in chemistry (Bissell 1992). Hence, the design of fluorescent PET sensors is a rare example of molecular engineering (de Silva 2009). The key parameter in the overall design is the binding constant β (which is the inverse of the dissociation constant, K_d), which relates

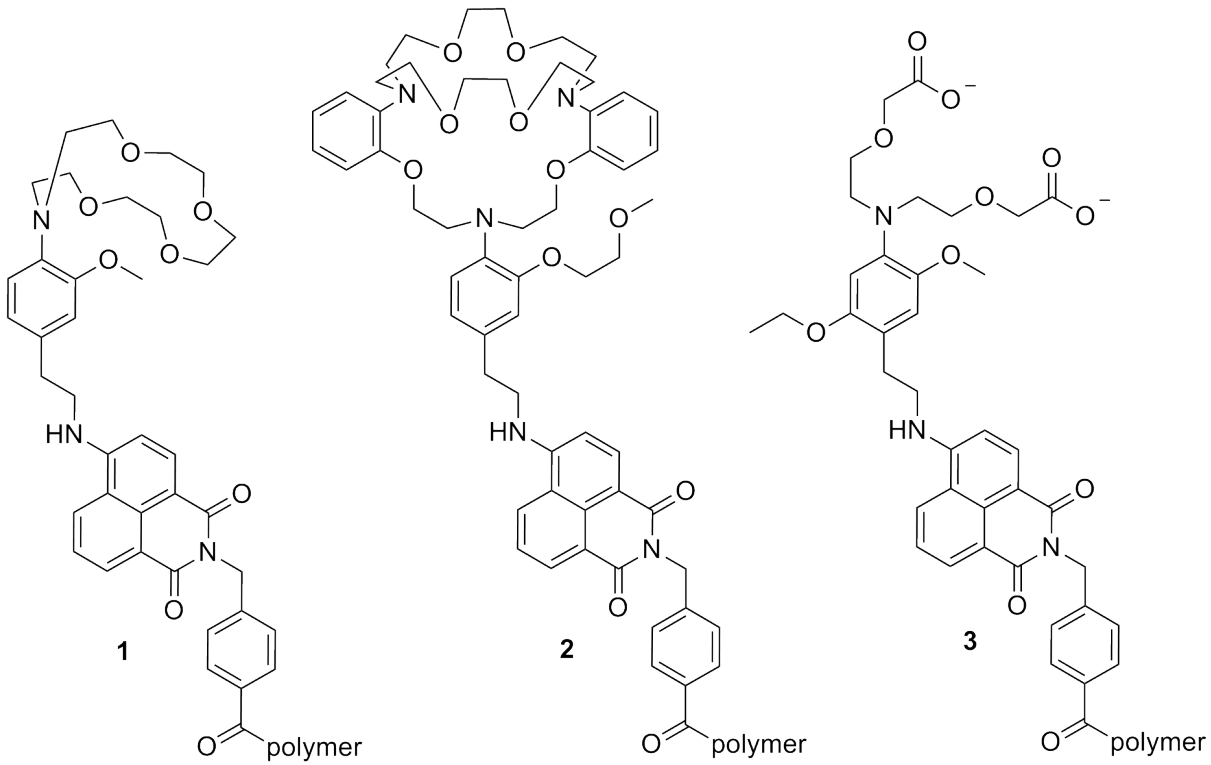

Figure 3: The chemosensor for Na^+ (1), K^+ (2) and Ca^{2+} (3) ions used in the OPTI disposable cassettes.

the fluorescence signal to the ion concentration. The reciprocal of the binding constant for the receptor-analyte interaction determines the average analyte concentration to be sensed. As mentioned, at the design stage the selectivity of the receptor towards the targeted analyte in the presence of elevated levels of other analytes is an important consideration.

3 The State-of-the-Art in Point-of-Care Technology

The Osmetech OPTI® system, consisting of a disposable cassette with incorporated molecular chemosensors, and the critical care analyzer, measure the analyte concentration of a patient from a tiny sample of blood. The device can simultaneously measure six critical care analytes at a time on a 120 µL sample of whole blood in about 2 minutes. There are five types of OPTI disposable cassettes available: a standard model measures pH, pCO_2 and tHb, Na^+, K^+, Ca^{2+} and Cl^- levels (Tusa et al. 2005). The pH and CO2/tHb sensor exploits the physicochemical properties of pyranine (HPTS), a water soluble pyrene-based indicator (Han et al. 2010). The measuring of Cl^- concentration is based on a collisional quenching mechanism (Bissell et al. 1992). The sensors for Na^+, K^+ and Ca^{2+} on the disposable cassette, as shown in Figure 3, are based on the design principle of photoinduced electron transfer (PET) (He et al., 2003a). Selective recognition of Na^+, K^+ and Ca^{2+} is achieved using carefully designed chemosensors consisting of a naphthalimide fluorophore attached to an aza-crown ether for Na^+, a cryptand for K^+ and a chelator for Ca^{2+} attached at one end of the fluorophore and to the other end to a polymer support (He et al. 2003b).

The chemosensors on the OPTI cassette are designed with a linker for immobilization to a hydrophilic polymer (Tusa et al. 2005). In water or blood serum, 1 with an aza-15-crown-5 ether reversibly binds Na^+ with a dissociation constant of 119 mM or a log Na^+ of 0.92 at a near-neutral of pH 7.4, physiological temperature of 37°C and ionic strength of 160 mM. Similarly, 2 reversibly binds K^+ at concentrations about 17 mM and 3 binds Ca^{2+} at concentrations about 1.1 mM. Excitation with a blue LED causes a green fluorescence signal from the 4-aminonaphthalimide fluorophore in all three cases. The modified iminodiacetic moiety in the Ca^{2+} sensor is reminiscent of the BAPTA receptor popularized in cell biology studies (Tsien, 1980). The OPTI device, shown in Figure 4, is the first commercial success to exploit the switching phenomena between PET and fluorescence for medical diagnostic applications.

Figure 4: A disposable cassette (left) and OPTI critical care analyser (right). The black spots (left picture) are organic polymer fibre mats attached to the appropriate sensor molecules for: Na^+, K^+, Ca^{2+}, pH and CO_2. The orange spot is the sensor for O_2. Adapted from reference 20 and reproduced with permission of the Royal Society of Chemistry (RSC).

4 Alkali and Alkaline Earth Cation Chemosensors

The monitoring of monovalent and divalent metal ions, notably Na^+, K^+, Mg^{2+} and Ca^{2+}, in blood and urine are of major medical diagnostic importance (Burtis et al. 2001). Table 1 lists typically concentration levels for various metal cations in blood including upper and lower limits, known as the critical values, which are indicative of a potential life-threatening condition. Sodium is the most abundant cation in whole blood at levels of about 140 mM. The normal concentration range of these analytes in various biological fluids can be significantly different, particularly for potassium, which has a concentration of 4 mM in blood and 70 mM in urine. Monitoring of sodium and potassium blood serum levels is routinely done for patients with high blood pressure; monitoring of sodium and potassium in urine is generally a concern for kidney problems.

Table 1. Typical concentration levels of metal cations in blood.

Cation	Blood Serum[a]/mM	Lower Limit/mM	Upper Limit/mM
Na^+	140	120	160
K^+	4.0	2.8	6.2
Ca^{2+}	1.2	1.0	3.2
Mg^{2+}	0.8	0.4	1.9
Fe^{3+}	19	9.0	31
Zn^{2+}	15	11	19
Cu^{2+}	16	11	22

[a]At physiological pH between 7.35 – 7.42.

5 Heavy Metal Cation Chemosensors

Many heavy metal ions are essential to the composition of the human body and in living organisms. The most abundant biologically important heavy metals are iron, copper and zinc. Their average concentration and critical values in blood are included in Table 1. Iron (Fe^2+ and Fe^{3+}) is ubiquitous in cells and plays a key role in oxygen transport (Sahoo 2012). Zinc (Zn^{2+}) is an integral part of hundreds of enzymes for the synthesis of genetic material and proteins and for growth and reproduction (Xu and Yoon, 2010). Copper (Cu^{2+} or Cu^+), mainly associated with many metalloproteins, is required for proper iron metabolism (Lippard and Berg, 1994). Other metals ions, including vanadium, chromium, manganese, cobalt, nickel and molybdenum are currently thought to be required in trace amounts for normal health (Burtis and Ashwood, 2001). Many diseases, such as Alzheimer's disease and Parkinson's disease, have been linked to an improper balance of metal ions in the body. As the most abundant heavy metal ions in living beings, Fe^{+3}, Zn^{2+} and Cu^+ are of interest in blood medical diagnostics. Many other metal ions are not involved in essential biological reactions; however, some of these (arsenic, aluminum, lead, mercury, and tin) are toxic. Consequently, from a medical diagnostics point-of-view, early detection of these species is highly desirable. Hence, there is considerable motivation for developing novel fluorescent chemosensors for trace-level environmental and clinical detection (Dutta et al. 2012; Kaur et al. 2012).

A number of reviews specifically on fluorescent chemosensors for heavy metals ions have recently appeared in the literature (Formica et al. 2012; Jeong et al. 2012; Kim et al. 2012): we highlight a few interesting examples in Figure 5. The pyrazoline-based probe **4** with an azatetrathiacrown receptor was used for visualizing labile copper Cu^+ in cellular systems (Thomas Morgan 2011). The four hydroxymethyl groups and the sulfonated triarylpyrazoline fluorophore provide water solubility (de Silva et al. 1993). On capturing Cu^+, **4** displays a 65-fold fluorescence enhancement at 508 nm with an observed quantum yield of 0.083 and a negligible background fluorescence of 0.002. A rare example of a Pb^{2+} chemosensor used for living cell imaging is 5 (He et al. 2006). The xanthenone-based probe has a pseudo crown-ether with two different carboxylate ligands. In the absence of Pb^{2+}, **5** has a background fluorescence with a negligible quantum yield of 0.001. Under tested physiological conditions, an 18-fold fluorescence enhancement is observed. The probe responds to changes of Pb^{2+} in the cytosol of living mammalian cells in a similar fashion. The chemosensor **6** for Zn^{2+} is comprised of a 4-amino-1,8-naphthalimide fluorophore and a phenyl iminodiacetate ligand as the binding site (Parkesh et al. 2007). The compound shows an absorption peak at 450 nm and emits a green fluorescence at 550 nm on binding Zn^{2+}. The low pK_a value of 3.2 is advantageous for monitoring Zn^{2+} in the physiological pH range of 7.4. Sensor **6** has a fluorescence quantum yield of 0.004 in water in the absence of Zn^{2+}, which increases to 0.21 in the presence of 5 µM Zn^{2+} with a 56-fold fluorescence enhancement.

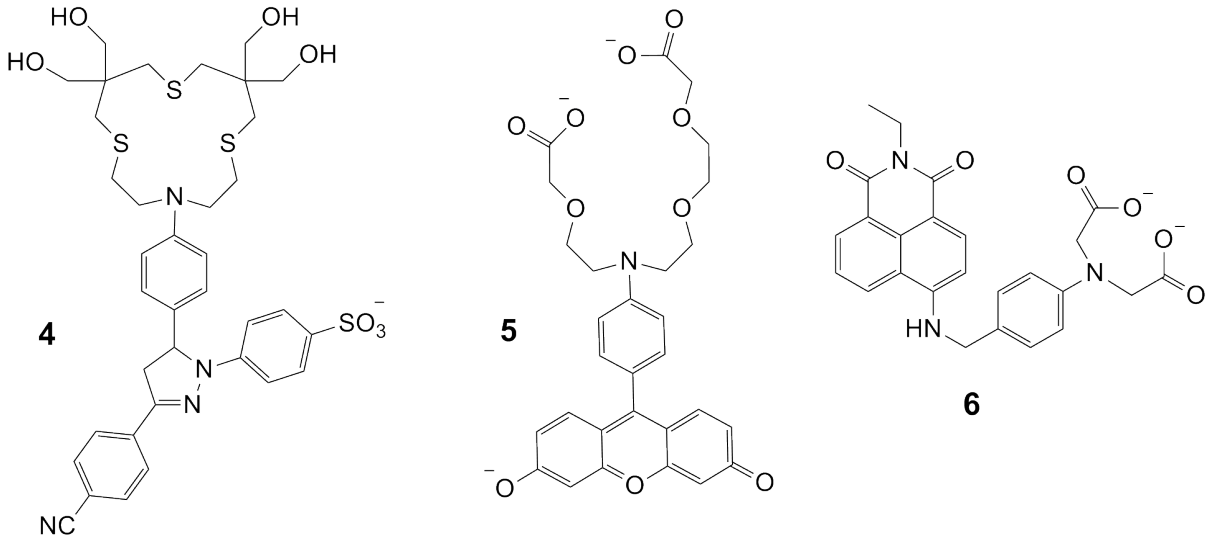

Figure 5: Representative examples of water-soluble chemosensors for Cu^{+} (4), Pb^{2+} (5) and Zn^{2+} (6).

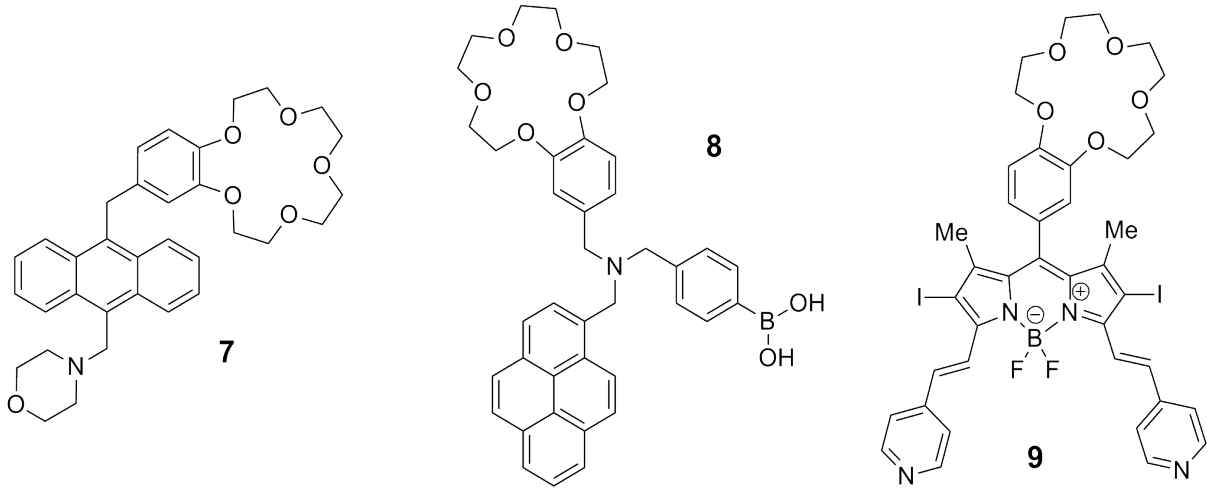

Figure 6: Representative examples of two-input AND logic gates.

6 Two-Input AND Logic Gates

Sensing for more than one analyte within a single molecule has been around for two decades with the demonstration of the first two-input molecular AND logic gate (de Silva et al. 1993). Since then many two-input logic gates (de Silva et al. 2004; de Silva et al. 2007; Szacilowski 2008) and many three and four-input logic gate arrays (de Silva, 2011) have been demonstrated to perform information processing at the molecular level (Magri 2012).

One of the earliest examples of a PET fluorescent sensor with two receptors 7, shown in Figure 6, consists of an anthracene fluorophore, a tertiary amine for binding protons and a benzo-15-crown-5 ether for complexing Na^{+} (de Silva et al. 1997). The molecule responds to H^{+} and Na^{+} inputs and fluorescence as the output according to an AND Boolean algorithm. When one or both of these analytes are in low concentration, essentially no fluorescence is observed. However, on addition of 10 mM Na^{+} and 1 mM acid, which correspond to the high input levels, 7 shows a high fluorescence output with a quantum yield of fluorescence of 0.24 as tabulated in Table 2.

Another example **8**, consisting of a pyrene fluorophore with a tertiary amine, a crown ether and a boronic acid group, is an example of a molecule that can detect for an ion pair (Koskela et al. 2005). A fluorescent enhancement was observed on addition of 6 mM potassium fluoride to the solution. In comparison, no enhancement was observed on the addition of potassium chloride or potassium bromide.

The chemosensor **9** is another AND logic gate with H^{+} and Na^{+} as the inputs, but with the generation of singlet oxygen as the output (Ozlem et al. 2009). Unlike **7** and **8**, where the fluorescence signal is the output, **9** harvests the fluorescence output signal to convert triplet oxygen (the form readily available in the atmosphere) to

singlet oxygen, which is used as a cytotoxic agent against cancer cells in photodynamic therapy (PDT). A current issue during PDT therapy is that many healthy cells are killed by singlet oxygen along with cancerous cells. However, it is known that certain types of cancer cells have higher levels of H^+ and Na^+ ion levels in the lysosomes compared to those of healthy normal cells (Iessi et al. 2008). By designing molecules, like **9**, it may be possible to have chemosensors that only generate single oxygen on excitation with a laser on detecting high levels of both H^+ (pH below 4) and Na^+ ions in specific cells according to an AND logic algorithm. The implications could be improved recovery times for patients after PDT treatment.

Table 2 Truth table for two-input AND logic gate 10.

$Input_1$	$Input_2$	Output
Na^+ [a]	H^+ [b]	Fluorescence [c]
0 (low)	0 (low)	0 (low, 0.003)
0 (low)	1 (high)	0 (low, 0.006)
1 (high)	0 (low)	0 (low, 0.005)
1 (high)	1 (high)	1 (low, 0.24)

[a]High input level $10^{-2.0}$ M sodium methanesulfonate. Low input level maintained with no added sodium salt. [b]High and low input levels correspond to $10^{-3.0}$ M and no added methanesulfonic acid. [c]Fluorescence quantum yields in methanol.

7 Three-Input AND Logic Gates: 'Lab-on-a-Molecule' Systems

The first reported illustration of the potential cross-fertilization between Boolean algebra and biomedical sensing was reported for a 'lab-on-a-molecule' - a three-input AND logic gate **10** based on a competition between PET and fluorescence (Magri et al. 2006). Three receptors are incorporated within a single molecule: a benzo-15-crown-5 ether for Na^+, a tertiary amine for H^+, and a phenyliminodiacetate for Zn^{2+}. In the absence of one, or two or all three analytes, the fluorescence emission in water of **10** is low due to PET from a vacant receptor to the excited state fluorophore. However, when all three analytes are bound in excess threshold concentrations, a high fluorescent signal is observed. The modular arrangement of the receptors, spacers and fluorophore facilitates a cooperative sensing algorithm as seen from Table 3 (Magri and de Silva 2010).

Another 'lab-on-a-molecule' **11** is based on the highly fluorescent boron-dipyrromethene dye (Bozdemir et al 2010). Combining both internal charge transfer and PET processes **11** incorporates three selective receptors that simultaneous detect Ca^{2+}, Hg^{2+} and Zn^{2+}. Advantageously, **11** uses a visible wavelength for excitation at 620 nm, with a fluorescence emission maximum at 656 nm and a significant fluorescence quantum yield of 0.266 when all three analytes are present at elevated concentrations.

Table 3 Truth table for three-input AND logic gate 11.

$Input_1$	$Input_2$	$Input_3$	Output
Na^+ [a]	H^+ [b]	Zn^{2+} [c]	Fluorescence [d]
0 (low)	0 (low)	0 (low)	0 (low, 0.001)
0 (low)	1 (high)	0 (low)	0 (low, 0.001)
0 (low)	0 (low)	1 (high)	0 (low, 0.002)
0 (low)	1 (high)	1 (high)	0 (low, 0.003)
1 (high)	0 (low)	0 (low)	0 (low, 0.006)
1 (high)	0 (low)	1 (high)	0 (low, 0.006)
1 (high)	1 (high)	0 (low)	0 (low, 0.007)
1 (high)	1 (high)	1 (high)	1 (high, 0.020)

[a]High input level 5 M sodium methanesulfonate. Low input level maintained with no added sodium salt. [b]High and low input levels correspond to $10^{-6.0}$ M and $10^{-9.5}$ M protons adjusted with methanesulfonic acid and tetramethylammonium hydroxide. [c]High input level corresponds to pZn = 3.1 at pH 6.0 and pZn = 4.8 at pH8.0. [d]Fluorescence quantum yields in water.

8 Conclusions

The current paradigm is to measure the blood ions such as Na^+, K^+ and Ca^{2+} using a specific molecule for a single analyte on a one-for-one basis. In the future, we envision the development of engineered molecules that could detect for many analytes simultaneously. The targeted analytes could also include anions and neutral molecules in addition to the above mentioned cations. These 'lab-on-a-molecule' systems with built-in algorithms would be able to sense for specific disease conditions by testing for numerous medically relevant parameters simultaneously, and make an intelligent diagnosis autonomously (Konry et al. 2009). They could even communicate a 'yes' or 'no' decision on a disease condition. Such technology could increase productivity at hospitals and clinical laboratories by saving time, especially in emergency point-of-care situations where every second is precious.

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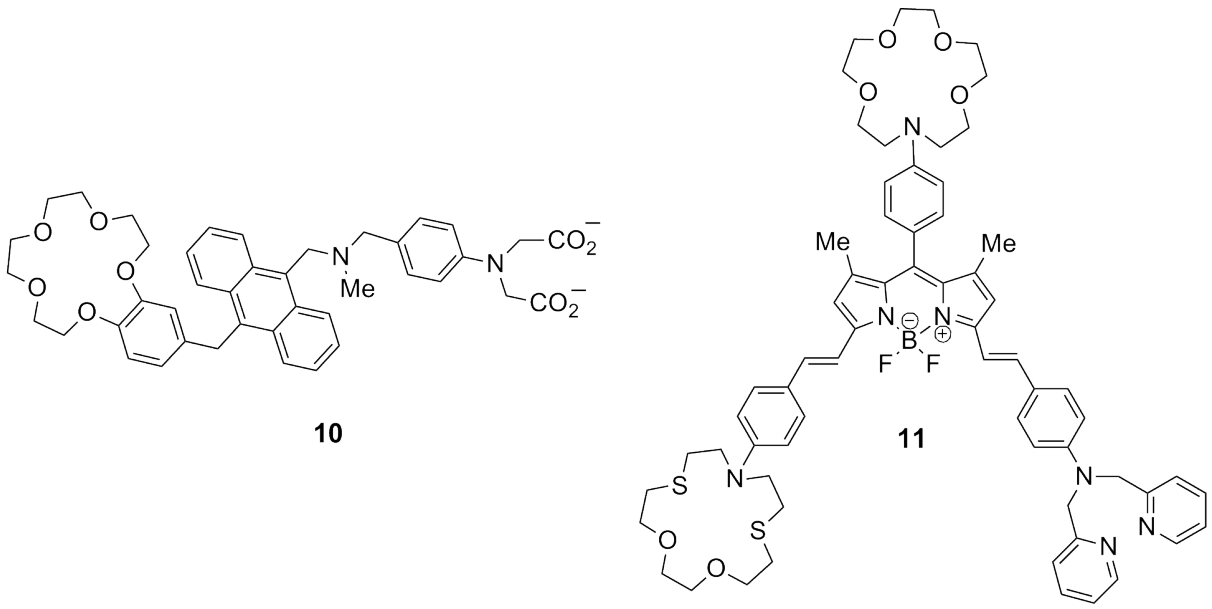

Figure 7: Examples of three-input AND logic gates.

Abela and Ray Scicluna.

References

Bissell R. A., de Silva A. P., Gunaratne H.Q.N., Lynch P.L.M., Maguire G.E.M., Sandanayake K.R.A.S. (1992) Molecular Fluorescent Signalling with 'Fluor-Spacer-Receptor' Systems: Approaches to Sensing and Switching Devices via Supramolecular Photophysics. *Chem. Soc. Rev.* 21, 187-195.

Bozdemir O. A., Guliyev R., Buyukcakir O., Selcuk S., Koleman S., Gulseren G., Nalbantoglu T., Boyaci H., Akkaya E. U. (2010) Selective Manipulation of ICT and PET Processes in Styryl-Bodipy Derivatives: Applications in Molecular Logic and Fluorescence Sensing of Metal Ions. *J. Am. Chem. Soc.* 132, 8029-8036.

Burtis C. A., Ashwood E. R. (2001) Tietz Fundamentals of Clinic Chemistry. 5th ed. W. B. Saunders Company, Philadelphia, Pennsylvania, USA.

Callan J. F., de Silva A. P., Magri D. C. (2005) Luminescent sensors and switches in the early 21st century. *Tetrahedron* 61, 8551-8588.

Cotton F. A. (1999) Advanced Inorganic Chemistry. 6th ed.Wiley-VCH, New York, New York, USA.

de Silva A. P. (2011) Luminescent Photoinduced Electron Transfer (PET) Molecules for Sensing and Logic Operations. *J. Phys. Chem. Lett.* 2, 2865-2871.

de Silva A. P. (2011) Molecular Logic Gate Arrays. *Chem. Asian. J.* 6, 750-766.

de Silva A. P., Gunaratne H. Q. N., Gunnlaugsson T., Huxley A. J. M, McCoy C. P., Rademacher J. T, Rice T. E. (1997) Signaling Recognition Events with Fluorescent Sensors and Switches. *Chem. Rev.* 97, 1515-1566.

de Silva A. P., Gunaratne H. Q. N., McCoy C. P. (1997) Molecular Photoionic AND Logic Gates with Bright Fluorescence and "Off-On" Digital Action. *J. Am. Chem. Soc.* 119, 7891-7892.

de Silva A. P., Gunaratne H. Q. N., McCoy, C. P. (1993) A molecular photoionic AND gate based on fluorescent signalling. *Nature* 364, 42-44.

de Silva A. P., McClenaghan N. D. (2004) Molecular-Scale Logic Gates. *Chem. Eur. J.* 10, 574-586.

de Silva A. P., Moody T. S., Wright G. D. (2009) Fluorescent PET (Photoinduced Electron Transfer) sensors as potent analytical tools. *Analyst* 134, 2385-2393.

de Silva A. P., Uchiyama S. (2007) Molecular logic and computing. *Nat. Nanotechnol.* 2, 399-410.

de Silva A. P., Vance T. P., West M. E. S., Wright G. D. (2008) Bright molecules with sense, logic, numeracy and utility. *Org. Biomol. Chem.* 6, 2468-2481.

Domaille D. W., Que E.L., Chang C. J. (2008) Synthetic fluorescent sensors for studying the cell biology of metals. *Nature Chem. Biol.* 15, 168-175.

Dutta M., Das D. (2012) Recent developments in fluorescent sensors for trace-level determination of toxic-metal ions. *Trends Anal. Chem.* 32, 113-131.

Formica M., Fusi V., Giorgi L., Micheloni M. (2012) New fluorescent chemosensors for metal ions in solution. *Coord. Chem. Rev.* 256, 170-192.

Han J., Burgess K. (2010) Fluorescent Indicators for Intracellular pH. *Chem. Rev.* 110, 2709-2728.

He H., Mortellaro M. A., Leiner M. J. P, Fraatz, R.

J., Tusa, J. (2003b) A fluorescent sensor with high selectivity and sensitivity for potassium in water. *J. Am. Chem. Soc.* 125, 1468-1469.

He H., Mortellaro M. A., Leiner M. J. P, Young S. T., Fraatz R. J., Tusa, J. K. (2003a) A Fluorescent Chemosensor for Sodium Based on Photoinduced Electron Transfer. *Anal. Chem.* 75, 549-555.

He Q. W., Miller E. W., Wong A. P., Chang C. J. (2006). A selective fluorescent sensor for detecting lead in living cells. *J. Am. Chem. Soc.* 128, 9316-9317.

Iessi E., Marino M. L., Lozupone F., Fais S., De Milito, A. (2008) Tumor acidity and malignancy: novel aspects in the design of anti-tumor therapy. *Cancer Therapy* 6, 55-66.

Jeong Y., Yoon J. (2012) Recent progress on fluorescent chemosensors for metal ions. *Inorg. Chem. Acta.* 381, 2-14.

Johnson I., Spence M. T. Z. (2010) The Molecular Probes Handbook: A Guide to Fluorescent Probes and Labeling Technologies. 11th ed., Life Technologies Corporation, USA.

Kaur K, Saini R., Kumar A., Luxami V., Kaur N., Singh P., Kumar S. (2012) Chemodosimeters: An approach for detection and estimation of biologically and medically relevant metal ions, anions and thiols. *Coord. Chem. Rev.* 256, 1992-2028.

Kavarnos G. J. (1993) Fundamentals of Photoinduced Electron Transfer, VCH-Wiley Weinheim, Germany.

Kim H. N., Ren W. X., Kim J. S., Yoon J. (2012) Fluorescent and colorimetric sensors for detection of lead, cadmium, and mercury ions. *Chem. Soc. Rev.* 41, 3210-3244.

Konry T., Walt D. R. (2009) Intelligent Medical Diagnostics via Molecular Logic. *J. Am. Chem. Soc.* 131, 13232-13233.

Koskela S. J. M., Fyles T. M., James T. D. (2005) A ditopic fluorescent sensor for potassium fluoride. *Chem. Commun.* 945-947.

Lakowicz J. R. (2009) Principles of Fluorescence Spectroscopy. 3rd ed., Plenum: New York, New York, USA.

Lippard S. J., Berg J. M. (1994) Principles of Bioinorganic Chemistry, University Science Books, Mill Valley, CA. USA.

Magri D. C. (2012) in Molecular and Supramolecular Information Processing: From Molecular Switches to Logic Systems. ed. E. Katz, Wiley-VCH Verlag, Weinheim, Germany. p.79-98.

Magri D. C., Brown G. J., McClean G. D., de Silva A. P. (2006) Communicating Chemical Congregation: A Molecular AND Logic Gate with Three Chemical Inputs as a "Lab-on-a-Molecule" Prototype. *J. Am. Chem. Soc.* 128, 4950-4951.

Magri D. C., de Silva A. P. (2010) From PASS 1 to YES to AND logic: building parallel processing into molecular logic gates by sequential addition of receptors. *New J. Chem.* 34, 476-481.

Magri D. C., Vance T. P., de Silva A. P. (2007) From Complexation to Computation: Recent Progress in Molecular Logic. *Inorg. Chim. Acta* 360, 751-764.

Ostemetch. http://www.cedarinteractive.com/demos/Osmetech/start.html. Iessi E., Marino M. L., Lozupone F., Fais S., De Milito, A. (2008) Tumor acidity and malignancy: novel aspects in the design of anti-tumor therapy. *Cancer Therapy* 6, 55-66.

Ozlem S., Akkaya E. U. (2009) Thinking Outside the Silicon Box: Molecular AND Logic as an Additional Layer of Selectivity in Singlet Oxygen Generation for Photodynamic Therapy. *J. Am. Chem. Soc.* 131, 48-49.

Parkesh R., Clive Lee T., Gunnlaugsson T. (2007) Highly selective 4-amino-1,8-naphthalimide based fluorescent photoinduced electron transfer (PET) chemosensors for Zn (II) under physiological pH conditions Org. *Biomol. Chem.* 5, 310-317.

Prodi L., Bolletta F., Montalti M., Zaccheroni N. (2000) Luminescent chemosensors for transition metal ions. *Coord. Chem. Rev.* 205, 59-83.

Que E. L., Domaille D. W., Chang C. J. (2008) Metals in Neurobiology: Probing Their Chemistry and Biology with Molecular Imaging. *Chem. Rev.* 108, 1517-1549.

Sahoo S. K., Sharma D., Bera R. K. Crisponi G., Callan, J. F. (2012) Iron(III) selective molecular and supramolecular fluorescent probes. *Chem. Soc. Rev.* 41, 7195-7227.

Szacilowski K., Digital Information Processing in Molecular Systems. (2008) *Chem. Rev.* 108, 3481-3548.

Morgan M. T, Bagchi P., Fahrni C. J. (2011) Designed To Dissolve: Suppression of Colloidal Aggregation of Cu(I)Selective Fluorescent Probes in Aqueous Buffer and In-Gel Detection of a Metallochaperone. *J. Am. Chem. Soc.* 133, 15906-15910.

Tsien R. Y. (1980) New Calcium Indicators and Buffers with High Selectivity against Magnesium and Protons: Design, Synthesis, and Properties of Prototype Structures. *Biochemistry* 19, 2396-2404.

Tusa J. K., He H. (2005) Critical care analyzer with fluorescent optical chemosensors for blood analytes. *J. Mater. Chem.* 15, 2640-2647.

Valeur B., Berberan-Santos M. N. (2012) Molecular Fluorescence. 2nd ed.Wiley-VCH, Weinheim, Germany.

Xu Z., Yoon J., Spring D. R. (2010) Fluorescent chemosensors for Zn^{2+}. *Chem. Soc. Rev.* 39, 1996-2006.