Fluorescent Molecular Logic Gates based on Photoinduced Electron Transfer (PET) Driven by a Combination of Atomic and Biomolecular Inputs

Wright, G., Yao, C., Moody, T., & De Silva, A. (2020). Fluorescent Molecular Logic Gates based on Photoinduced Electron Transfer (PET) Driven by a Combination of Atomic and Biomolecular Inputs. Chemical Communications. https://doi.org/10.1039/d0cc00478b

Published in:
Chemical Communications

Document Version:
Publisher's PDF, also known as Version of record

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

Publisher rights
Copyright 2020 the authors.
This is an open access Creative Commons Attribution-NonCommercial License (https://creativecommons.org/licenses/by-nc/4.0/), which permits use, distribution and reproduction for non-commercial purposes, provided the author and source are cited.

General rights
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.
Fluorescent molecular logic gates based on photoinduced electron transfer (PET) driven by a combination of atomic and biomolecular inputs‡

Glenn D. Wright,* Chao-Yi Yao,* Thomas S. Moody and A. Prasanna de Silva†

Molecular AND logic gates 1, 3, 5 and 7, which are designed according to principles of photoinduced electron transfer (PET) switching, respond to co-existing Candida antarctica lipase B and H+ (and Na+).

Molecular logic-based computation1–4 requires gates to process and store information. Besides its ability to operate in biocompatible micrometric spaces, the diversity of information available to molecular logic distinguishes it from its semiconductor cousin which employs voltage information only. For instance, the inputs feeding gates can take the form of physical entities (e.g. light dose,5 temperature6), chemical species (e.g. atomic,1 molecular7) and biochemical species (e.g. nucleotides,8 enzymes3). However, there are hardly any examples of combined atomic and enzyme inputs in the literature, if at all.

Fluorescent PET switches grew out of the sensing literature.9 Although atomic inputs were present from the beginning,10 protein inputs were incorporated only recently.11 Even these covered only some receptor- and transport-proteins.§ A way to incorporate hydrolase enzymes was described by Ojida et al.12 and us.13 Here, a fluorescent PET system based on a ‘fluorophore-spacer-amine’ format relied on the upward shift of the amine’s pKₐ value by ~2 pH units upon hydrolyzing a neighbouring ester into a carboxylate anion. Now we show how such systems can serve as fluorescent molecular logic gates driven by atomic ions, H+ and Na+, and a hydrolase enzyme, Candida antarctica lipase B (CALB).

Logic gate 1¶ is synthesized by nucleophilic substitution of 4-bromomethyl-7-methoxycoumarin with sarcosine ethyl ester. 1 is a typical fluorescent PET ‘off–on’ switch of the ‘fluorophore-spacer–receptor’ format8 with H+ being the input. By itself, the 7-methoxycoumarin fluorophore has no significant interaction with H+ in the pH range of our experiments since it lacks a suitable receptor.14 1’s pH-dependent fluorescence intensity (Iₑ) is analysed according to eqn (1)14 to give pKₐ = 3.6. The fluorescence quantum yields are Φₑ = 0.97 and Φₑ = 0.02.

\[
\log[(Iₑ - Iₑ)/(Iₑ - Iₑ)] = pH - pKₐ
\]

The hydrolysis product of 1, which is 2 (shown in Scheme 1 as the carboxylic form owing to the operational pH range of 6–10 for gate tests), is tested similarly and yields pKₐ = 6.7. The fluorescence quantum yields of 2 are Φₑ = 0.48 and Φₑ = 0.01. The lower Φₑ value of 2 (cf. 1) is due to the three-atom linker folding the carboxylate unit over the fluorophore15 to allow intramolecular interaction in the excited state.16 All these parameters for all compounds studied are collected in Table 1.

1 was subjected to an enzyme screen to see which enzyme would hydrolyze 1 most efficiently to 2 at pH 7 (Fig. 1). CALB was found to be the most efficient, which is gratifying since CALB is known17 to hydrolyze a variety of esters over a wide pH range. Additionally, it was found that the hydrolysis reaction could be most conveniently followed by the fluorescence emission signal (Fig. 1). Thus we realize that 1 becomes a ‘fluorophore-spacer₁–receptor–spacer₂–enzyme substrate’ system.

The fluorescence spectra of 1 at pH 6 and 10 with/without CALB exposure for 30 min are shown in Fig. 2a. The AND logic response of the fluorescence signal is clear since both H+ and

---

¶ Electronic supplementary information (ESI) available: Synthesis procedures and characterization details for all compounds. See DOI: 10.1039/d0cc00478b

† In honour of Professor Eric Anslyn’s 60th Birthday.

‡ Electronic supplementary information (ESI) available: Synthesis procedures and characterization details for all compounds. See DOI: 10.1039/d0cc00478b

§ A way to incorporate hydrolase enzymes was described by Ojida et al.12 and us.13

|||---

| R | Structure |
|---|---|
| N(Me)CH₂CO₂Et | ![](image1.png) |
| N(Me)CH₂CO₂ | ![](image2.png) |
| NH₂CH₂CO₂ | ![](image3.png) |
| NH₂CH₂CO₂ | ![](image4.png) |
| N(Me)CH₂CO₂Et | ![](image5.png) |

Scheme 1 Structures of logic gates 1, 3, 5 and 7 and their hydrolysis products 2, 4, 6 and 8 respectively.
CALB are needed to elicit a ‘high’ fluorescence response from 1. When exposed to CALB at pH 6, 1 gradually hydrolyzes to 2 and shows a gradual increase in fluorescence intensity. The 1–CALB interaction is governed by a Michaelis constant $K_M$ of $1.9 \times 10^{-5}$ M and $V_{\text{max}}$ value of $5.0 \times 10^{-10}$ M s$^{-1}$. These values are obtained by applying eqn (2) to convert the rate of change of fluorescence intensity into the rate of change of the product 2 concentration, followed by the application of eqn (3).  

$$V = \frac{d(2)}{dt} = \frac{dF}{dt} = \left( \frac{dF}{dE} \right)_{E=0} \left[ \left( \Phi_F \frac{\partial \Phi_F}{\partial E} \right) \right]$$

$$1/V = [K_M/V_{\text{max}}(1)] + 1/V_{\text{max}}$$

It is to be noted that the enzyme reaction is irreversible under our experimental conditions so that the logic device is suitable only for single-use situations. Such single-use situations are commonly present in the medical diagnostics sphere, as seen with two$^{19}$ or three$^{20}$ (Scheme 1).

Owing to the diversity available in inputs, outputs, power supplies and devices within molecular logic, several routes to reconfigurability have become available.$^3$ However, we are not aware of any cases in the primary literature where logic is reconfigured by changing molecular configuration. Since the enantiodiscrimination of enzymes is well-established, we now have an opportunity to present such an approach.

The enantiomeric pair of logic gates 3$^f$ and 5$^f$ arise from a synthesis analogous to that of 1. The hydrolysis products of these are 4 and 6 respectively, although only 6 was available for $K_M$ determination. $K_M$ values are measured for these compounds with the aid of eqn (1), as done for 1 and 2. The values obtained for 3, 5 and 6 are 4.5, 4.5 and 7.1 respectively. The $K_M$ value of 4 is expected to be the same as that found for its opposite enantiomer 6, i.e. 7.1.

As seen in Fig. 3a, the fluorescence spectra of 3 at pH 6 and 10 with/without CALB exposure for 30 min correspond to a PASS 0 logic action. On the other hand, Fig. 3b shows an AND logic action for 3. 3 and 5 differ in the configuration of the functional groups around the asymmetric carbon. Thus, logic reconfiguring is achieved by changing the molecular configuration of the device. At pH 6, $K_M$ values are not very different, i.e. $1.1 \times 10^{-5}$ and $1.6 \times 10^{-5}$ M, for 3 and 5 respectively. However, $V_{\text{max}}$ values differ significantly, i.e. $7.5 \times 10^{-9}$ and $9.7 \times 10^{-8}$ M s$^{-1}$, as a result of CALB’s enantioselectivity.

We have explored the modularity of our design by building a prototype 3-input AND gate 7$^f$ of the ‘receptor-spacer$_1$-fluorophore-spacer$_2$-receptor$_2$-spacer$_3$-enzyme substrate’ format,
which is driven by CALB, H\(^+\) and Na\(^+\). The sensitivity of logic gate 7's fluorescence to Na\(^+\), cf. that of 1, arises from the new benzox-10-crown-6 ether functional group within 7.

7 is synthesized by reacting a known anthracene-crown-ether conjugate\(^2\) with sarcosine ethyl ester. Its AND logic behaviour is shown in Fig. 4a and c. Its pK\(_\text{a}\) is 5.8 (at 1.0 M Na\(^+\)) in water: DMSO (1 : 1, v/v), 8, the hydrolysis product of 7, has pK\(_\text{a}\) of 8.8 under the same conditions. The log\(f_{\text{Na}}\) values for 7 and 8 are 0.7 and 0.8 respectively (at pH 4.5). The 7-CALB interaction is characterized at pH 7 and at 1.0 M Na\(^+\) by \(K_M = 2.2 \times 10^{-6}\) M and \(V_{\text{max}} = 7.5 \times 10^{-10}\) M s\(^{-1}\). This proof of principle study does not examine selectivity issues with respect to other metal ions.

We conclude that the fluorescent PET sensing/switching design is a useful starting point for constructing tailored molecular logic systems which employ mixed inputs from the chemical and biological spheres, especially when the latter concerns a hydrolyse enzyme. Such systems are unique when compared with previously developed AND and other logic gates.\(^3\) This approach also allows demonstration of logic reconfiguring by changing the molecular configuration of the logic device.

We acknowledge the Department of Employment and Learning, Northern Ireland and T. J. Lively for support and help.

Note added in proof: Fluorescent PET probes for some oxidoreductase proteins are also available.\(^8\)

**Conflicts of interest**

There are no conflicts to declare.

**Notes and references**

See note added in proof.

1; \(^1\)H NMR (CDCl\(_3\)): \(\delta 1.30\) (t, 3H, OCH\(_2\)CH\(_3\), \(J = 7\) Hz), 2.44 (s, 3H, NCH\(_3\)), 3.38 (s, 2H, ArCH\(_2\)N), 3.84 (s, 2H, NCH\(_2\)CO), 3.89 (s, 3H, OCH\(_3\)), 4.21 (q, 2H, OCH\(_2\)CH\(_3\), \(J = 7\) Hz), 6.35 (s, 1H, CH\(_3\)), 6.83 (m, 1H, ArH), 6.87 (m, 1H, ArH), 7.90 (d, 2H, ArH, \(J = 9\) Hz). \(^1\)C NMR (CDCl\(_3\)): 31.1, 31.7, 32.1, 41.1, 41.5, 56.8, 58.5, 100.9, 111.8, 112.7, 112.8, 126.8, 151.9, 150.0, 162.2, 163.7, 172.0. MS([ES]): 306.1341 [M + H\(^+\)] . Calculated m/z: 306.1355.

5; \(^1\)H NMR (CDCl\(_3\)): \(\delta 1.38\) (d, 3H, C(CH\(_3\))\(_3\), \(J = 7\) Hz), 1.86 (s, 1H, NH), 3.44 (q, 1H, C(CH\(_3\))\(_2\)H, \(J = 7\) Hz), 3.77 (s, 3H, CO\(_2\)CH\(_3\)), 3.87 (s, 3H, OCH\(_3\)), 3.89 (dd, 2H, CH\(_2\)NH, \(J = 16, 107\) Hz), 6.43 (s, 1H, CH\(_2\)CO), 6.82 (m, 1H, ArH), 7.58 (d, 1H, ArH, 9 Hz). \(^1\)C NMR (CDCl\(_3\)): \(\delta 17.4, 45.8, 50.1, 53.8, 54.4, 99.1, 108.5, 110.1, 110.4, 123.2, 151.6, 153.5, 159.6, 160.7, 174.0. MS([ES]): 292.1170 [M + H\(^+\)] . Calculated m/z: 292.1185.

7; \(^1\)H NMR (CDCl\(_3\)): \(\delta 1.29\) (t, 3H, CH\(_2\)CH\(_3\), \(J = 7\) Hz), 2.49 (s, 3H, NCH\(_3\)), 3.43 (s, 2H, NCH\(_3\)), 3.50–3.90 (m, 16H, CH\(_2\)O), 4.19 (q, 2H, OCH\(_2\)CH\(_3\), \(J = 7\) Hz), 4.73 (2H, s, AnthCH\(_2\)N), 4.88 (s, 2H, AnthCH\(_2\)Ar), 6.46–6.57 (3H, m, ArH), 7.48 (m, 4H, AnthH), 8.19 (d, 2H, AnthH, \(J = 9\) Hz), 8.61 (d, 2H, AnthH, \(J = 9\) Hz). \(^1\)C NMR (CDCl\(_3\)): \(\delta 14.7, 35.0, 45.1, 55.0, 60.6, 69.4, 70.0, 70.9, 71.4, 114.3, 114.9, 117.4, 121.2, 122.5, 124.0, 125.8, 127.3, 129.0, 140.9, 141.4, 171.7. MS([ES]): 588.2994 [M + H\(^+\)] . Calculated m/z: 588.2961.

1. A. P. de Silva, H. Q. N. Gunaratne and C. P. McCoy, Nature, 1993, 364, 42.

2. Molecular and Supramolecular Information Processing, ed. E. Katz, Wiley-VCH, Weinheim, 2012; Biomolecular Information Processing, ed. E. Katz, Wiley-VCH, Weinheim, 2012; Szulc, Infocomms. Wiley, Chichester, 2012; A. P. de Silva, Molecular Logic-based Computation, Royal Society of Chemistry, Cambridge, 2013; V. Balzani, A. Credi and M. Venturi, Molecular Devices and Machines, VCH, Weinheim, 2nd edn, 2008; A. P. de Silva, Y. Leydet, C. Lincheneau and N. D. McClennagham, J. Phys. Condens. Matter, 2006, 18, S1847, S. Uchiuma and A. P. de Silva, Nanotechnol., 2007, 2, 395, J. Andreasson and U. Pischel, Chem. Soc. Rev., 2015, 44, 1053; B. Dahy, L. Jing, V. A. Silvenson and A. P. de Silva, Chem. Commun., 2015, 51, 8403; S. Erbas-Calnak, S. Kolemen, 2020. This journal is © The Royal Society of Chemistry 2020
A. C. Sedgwick, T. Gunnlaugsson, T. D. James, J. Y. Yoon and E. U. Akkaya, Chem. Soc. Rev., 2018, 47, 2228; J. Andreasson and U. Pischel, Chem. Soc. Rev., 2018, 47, 2266.

3 Katz and V. Privman, Chem. Soc. Rev., 2010, 39, 1835; Enzyme-Based Computing Systems, ed. E. Katz, Wiley-VCH, Weinheim, 2019.

4 Recent examples: A. C. Sedgwick, H.-H. Han, J. E. Gardiner, S. D. Bull, X.-P. He and T. D. James, Chem. Sci., 2018, 9, 3672; C.-Y. Yao, J. Ling, L.-Y.-H. Chen and A. P. de Silva, Chem. Sci., 2019, 10, 2272; B. Daly, T. S. Moody, A. J. M. Huxley, C.-Y. Yao, B. Schazmann, A. Alves-Areias, J. F. Malone, H. Q. N. Gunaratne, P. Nockemann and A. P. de Silva, Nat. Commun., 2019, 10, 49; J.-Z. Li, Y.-H. Sun, C.-Y. Wang, Z.-Q. Guo, Y.-J. Shen and W.-H. Zhu, Anal. Chem., 2019, 91, 11946; M. V. Refalo, N. F. Farrugia, A. D. Johnson, S. Klejna, K. Szacilowski and D. C. Magri, J. Mater. Chem. C, 2019, 7, 15225; A. Ghosh, A. Patel and M. Schmittle, J. Am. Chem. Soc., 2019, 141, 18954; A. Fernandez, E. J. Thompson, J. W. Pollard, T. Kitamura and M. Vendrell, Angew. Chem., Int. Ed., 2019, 58, 16894.

5 U. Pischel and J. Andreasson, New J. Chem., 2010, 34, 2701; D. Gust, J. Andreasson, U. Pischel, T. A. Moore and A. L. Moore, Chem. Commun., 2012, 48, 1947.

6 S. Uchiyama, N. Kawai, A. P. de Silva and K. Iwai, J. Am. Chem. Soc., 2004, 126, 3032.

7 M. E. Huston, E. U. Akkaya and A. W. Czarnik, J. Am. Chem. Soc., 1989, 111, 8735; C. R. Cooper and T. D. James, Chem. Commun., 1997, 1419; C. R. Cooper and T. D. James, J. Chem. Soc., Perkin Trans. 1, 2000, 963.

8 A. Saghatelian, N. H. Volker, K. M. Guckian and M. R. Ghadiri, J. Am. Chem. Soc., 2003, 125, 346; M. N. Stojanovic, D. Stefanovic and S. Rudchenko, Acc. Chem. Res., 2014, 47, 1845; R. Lopez, R. F. Wang and G. Seelig, Nat. Chem., 2018, 10, 746.

9 R. A. Bissell, A. P. de Silva, H. Q. N. Gunaratne, P. L. M. Lynch, G. E. M. Maguire and K. R. A. S. Sandanayake, Chem. Soc. Rev., 1992, 21, 187; A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, Chem. Rev., 1997, 97, 1515; A. P. de Silva, T. P. Vance, M. E. S. West and G. D. Wright, Org. Biomol. Chem., 2008, 6, 2468; A. P. de Silva, T. S. Moody and G. D. Wright, Analyst, 2009, 134, 2385; W. Zhang, Z. Ma, L. P. Du and M. Y. Li, Analyst, 2014, 139, 2641; B. Daly, J. Ling and A. P. de Silva, Chem. Soc. Rev., 2015, 44, 4203; D. Wu, A. C. Sedgwick, T. Gunnlaugsson, E. U. Akkaya, J. Y. Yoon and T. D. James, Chem. Soc. Rev., 2017, 46, 7105.

10 Y. C. Wang and H. Morawetz, J. Am. Chem. Soc., 1976, 98, 3611; B. K. Selinger, Aust. J. Chem., 1977, 30, 2087; G. S. Beddard, R. S. Davidson and T. D. Whelan, Chem. Phys. Lett., 1978, 56, 54; H. Shirzad, M. Nakamura and T. Morita, J. Phys. Chem., 1979, 83, 2019; H. Shirzad, T. Ogiwara and E. Kimura, J. Phys. Chem., 1985, 89, 4302; J. P. Konopelski, F. Kotzby-Hibert, J.-M. Lehn, J.-P. Desvergne, F. Fages, A. Castellan and H. Bouas-Laurent, J. Chem. Soc., Chem. Commun., 1985, 433; A. P. de Silva and R. A. D. D. Rupasinghe, J. Chem. Soc., Chem. Commun., 1985, 1669; G. Grynkiewicz, M. Poiene and R. Y. Tsien, J. Biol. Chem., 1985, 266, 3440; A. P. de Silva and S. A. de Silva, J. Chem. Soc., Chem. Commun., 1986, 1709; M. E. Huston, K. W. Haider and A. W. Czarnik, J. Am. Chem. Soc., 1988, 110, 4460.

11 B. McLaughlin, E. M. Surender, G. D. Wright and A. P. de Silva, Chem. Commun., 2018, 54, 1319.

12 Y. Oshikawa and A. Ojida, Chem. Commun., 2013, 49, 11373.

13 G. D. Wright, PhD thesis, Queen’s University Belfast, 2010.

14 A. P. de Silva, H. Q. N. Gunaratne, P. L. M. Lynch, A. L. Patty and G. L. Spence, J. Chem. Soc., Perkin Trans. 2, 1993, 1611.

15 F. Hirayama, J. Chem. Phys., 1965, 42, 3163.

16 J. F. Callan, A. P. de Silva and N. D. McClenaghan, J. Chem. Soc., Chem. Commun., 2004, 2048.

17 E. M. Anderson, M. Karin and O. Kirk, Biocatal. Biotransform., 1998, 16, 181.

18 H. Lineweaver and D. Burk, J. Am. Chem. Soc., 1934, 56, 658.

19 T. Konry and D. R. Walt, J. Am. Chem. Soc., 2009, 131, 13232.

20 D. C. Magri, G. J. Brown, G. D. McClean and A. P. de Silva, J. Am. Chem. Soc., 2006, 128, 4950; G. J. Scerri, J. C. Spiteri, C. J. Mallia and D. C. Magri, Chem. Commun., 2019, 55, 4961.

21 A. P. de Silva, H. Q. N. Gunaratne and C. P. McCoy, J. Am. Chem. Soc., 1997, 119, 7891; S. A. de Silva, B. Amorelli, D. C. Isidor, K. C. Loo, K. E. Crooker and Y. E. Pena, Chem. Commun., 2002, 1360; A. P. de Silva, G. D. McClean and S. Pagliari, Chem. Commun., 2003, 1310; S. Uchiyama, E. Fukatsu, G. D. McClean and A. P. de Silva, Angew. Chem., Int. Ed., 2016, 55, 768.

22 T. Guo, L. Cui, J. N. Shen, W. P. Zhu, Y. F. Xu and X. H. Qian, Chem. Commun., 2013, 49, 10820; D. D. Li, Y. Q. Xu, N. N. Zhou, J. X. Liu, R. Wang, T. Cheng, Y. Tang, W. P. Zhu, Y. F. Xu and X. H. Qian, Dyes Pigments, 2017, 136, 627; L. Yang, J. Y. Niu, R. Sun, Y. J. Xu and J. F. Ge, Sens. Actuators, B, 2018, 259, 299; Z. J. Zhang, T. Lv, B. B. Tao, Z. F. Wen, Y. Q. Xu, H. J. Li, F. Y. Liu and S. G. Sun, Bioorg. Med. Chem., 2020, 28, 115280; X. L. Sha, X. Z. Yang, X. R. Wei, R. Sun, Y. J. Xu and J. F. Ge, Sens. Actuators, B, 2020, 307, 127653.