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Communication

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Molecular Logic with a Saccharide Probe on the Few-Molecules Level

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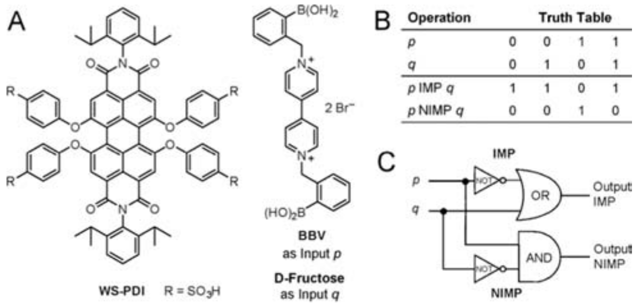
S *Supporting Information*

ABSTRACT: In this Communication we describe a two-component saccharide probe with logic capability. The combination of a boronic acid-appended viologen and perylene diimide was able to perform a complementary implication/not implication logic function. Fluorescence quenching and recovery with fructose was analyzed with fluorescence correlation spectroscopy on the level of a few molecules of the reporting dye.

Molecular logic gates involve chemical and/or optical inputs and outputs. Prasanna de Silva et al. showed for the first time that molecular fluorescent probes for ions can function as logic gates.[1] pH is often used as one input to implement molecular sensors into logic circuits. In physiological media, however, more complex inputs have to be found such as carbohydrates, oligonucleotides, oligopeptides, proteins, and metal ions. In combination with suitable receptors, fascinating examples in molecular digital analysis have been shown.[2−13] Novel applications of these molecular logic gates can be found in the design of smart materials,[14−16] in the delivery/activation of drugs,[17,18] and in clinical diagnostics.[19−21] However, only a few molecular logic gates with the exceptional implication function (IMP) have been reported.[22−28] IMP was introduced by the philosophers Whitehead and Russell together with three fundamental logic operations: AND, OR, and NOT.[29] The last three built the fundamental of our computer age.[28] Nevertheless, Russell regarded IMP as particularly powerful and named it "material implication": in the IMP function, either input p implies input q or vice versa. Moreover, IMP and FALSE operations (where the FALSE operation always yields the logic value 0) build a computationally complete logic basis.[30] This means that all 16 distinct binary Boolean operations on two logic values can be defined by IMP and FALSE.[31]

Here it is demonstrated that a complementary IMP/NIMP function is naturally realized in a supramolecular saccharide probe (NIMP, not implication, equal to INH, inhibit).[3,32−34] Fluorescence correlation spectroscopy (FCS)[35] was used to investigate the combination of *N*,*N*′-4,4′-bis(benzyl-2-boronic acid)bipyridinium dibromide (BBV, a receptor for diols)[3,36] and 1,6,7,12-tetrakis(4-sulfonylphenoxy)-*N*,*N*′-(2,6-diisopropylphenyl)perylene-3,4:9,10-tetracarboxidiimide (WS-PDI, the reporting dye)[37,38] in aqueous buffer solution (Figure 1A).



Operation	Truth Table			
p	0	0	1	1
q	0	1	0	1
p IMP *q*	1	1	0	1
p NIMP *q*	0	0	1	0

Figure 1. (A) Supramolecular saccharide sensing system: water-soluble perylenediimide (WS-PDI) and boronic acid-appended viologen (BBV). (B) Truth table of IMP and NIMP. (C) Schematic representation of an IMP/NIMP logic circuit.

The complexity of sugar chemistry in biological systems competes with that of nucleic acids and proteins.[39] Thus, sugars will be important targets as biocodes.[40] Supramolecular probes for saccharides from Singaram et al. are excellent receptors for possible encoding.[41,42] Investigation of supramolecular dynamics of a single or a few molecules has been already performed by FCS.[35,43,44] Although a few FCS-based sensors have been reported,[45,46] the technique has not been applied in saccharide sensors nor in molecular logic gates.

The two-component saccharide probe in the IMP logic function follows the principle of an allosteric indicator displacement assay (AIDA, Scheme 1).[47,48] The trivial case of WS-PDI alone represents the inputs $p(\text{BBV}) = q(\text{fructose}) = 0$ and the fluorescence intensity as output = 1 (Figure 1B). The combination of the WS-PDI and the sugar retains the fluorescence ($p = 0$, $q = 1$, output = 1). Ground-state complex formation between BBV and WS-PDI results in static quenching of the fluorescent dye ($p = 1$, $q = 0$, output = 0). When BBV and fructose are added to the dye ($p = q = 1$), the boronic acids are converted into anionic fructoboronate esters, partially neutralizing the net charge of the cationic bipyridinium salt. This reduces the quenching efficacy of the viologen (allosteric site) and increases the fluorescence intensity of WS-PDI (output = 1, Figure 1B).

Quenching characteristics of BBV and WS-PDI were established by FCS. Figure 2 shows photon bursts of diffusing WS-PDI molecules (60 nM) as fluorescence transients. Only 1 s out of 100 s is shown. The average count rate of the transients

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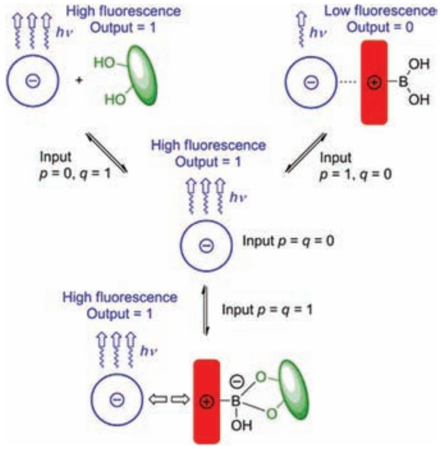
Scheme 1. Saccharide Probe Performs IMP Logic by an Allosteric Indicator Displacement Assay (AIDA)[a]



[a] Blue circle, fluorescent dye WS-PDI; red rectangle, saccharide receptor and quencher BBV; green ellipse, fructose.

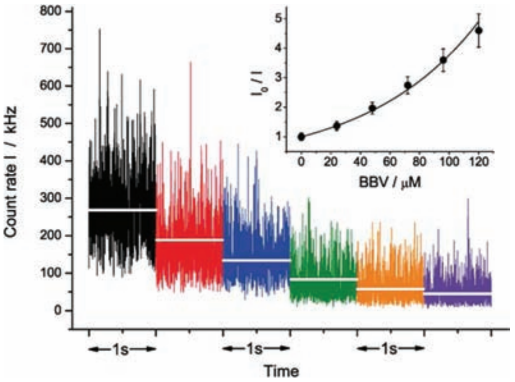


Figure 2. Fluorescence transients of WS-PDI (60 nM) at different concentrations of BBV (0 μM, black; 24 μM, red; 48 μM, blue; 72 μM, green; 96 μM, orange; 120 μM, violet). The average count rate is shown by the white line. Inset: Stern–Volmer plot with static Perrin model fit.[31]

is given by a white line. By increasing the concentration of BBV, the average count rate is reduced. This can be explained by increased quenching of fluorescent WS-PDI molecules. Thus, the number of fluorescent WS-PDI molecules in the confocal volume is reduced. The corresponding Stern–Volmer plot was treated with the static Perrin quenching model (Figure 2, inset).[31]

Autocorrelation curves of WS-PDI at different BBV concentrations revealed no significant changes in the diffusion time ($\tau_D = 57 \pm 2\ \mu s$) and triplet relaxation time ($\tau_T = 0.76 \pm 0.07\ \mu s$).[49] Thus, increasing concentrations of BBV do not influence the diffusion behavior of the fluorescent WS-PDI molecules or the triplet relaxation.[31]

An FCS titration was performed in an aqueous buffer solution of BBV and WS-PDI with fructose (0–2 mM). WS-PDI was unquenched with increasing concentrations of fructose. The average count rate of WS-PDI with no BBV present was recovered with 2 mM fructose and 120 μM BBV.[31] The detection limit of BBV against fructose of about 0.5 mM was confirmed.[36]

We have shown that the fluorescence intensity of 60 nM WS-PDI can be switched from the quenched state to the unquenched state and vice versa with BBV and fructose. The reagent inputs p(BBV) and q(Fru) at concentrations of 120 μM and 2 mM, respectively, were implemented into a complementary IMP/NIMP logic function (Figure 3). In the case of the IMP logic gate, the average count rate of the fluorescence transients represented the output. The average count rate corresponds to the number of fluorescent molecules in the confocal volume (Figure 3A). The IMP gate produced high fluorescence output under all circumstances except in the presence of only q(BBV). Fluorescence transients were also used to calculate autocorrelation functions $G(\tau)$. The $G(0)$ intercept was used as the output for a NIMP logic function, as it is indirectly proportional to the mean number of fluorescent molecules (Figure 3B). The NIMP gate produced a low $G(0)$ intercept under all circumstances except in the presence of only q(BBV). However, threshold values have to be introduced into the complementary IMP/NIMP logic gate to distinguish between outputs 0 and 1. The threshold for the IMP gate was fixed at a count rate of 150 kHz. Some single fluorescent events do not match with the output (Figure 3A). The average count rate over 100 s and five replicates leads to the correctly assigned output values. In contrast, the threshold of the NIMP gate was set to $G(0) = 2.2$. Here the output values can be unambiguously assigned, as the $G(0)$ intercept is very sensitive to the concentration of the fluorescent WS-PDI molecules.[35] Calculation of the autocorrelation curves delivers $G(0)$ intercept values in a very short time frame. Thus, distinct decisions between 0 and 1 can be made within 1 s.

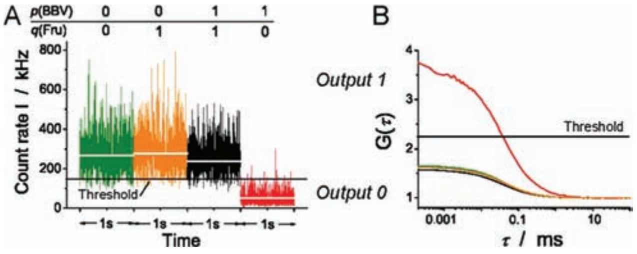


Figure 3. Fluorescence transients and corresponding autocorrelation functions from WS-PDI and the inputs p(BBV) and q(Fru): green (input 0,0), orange (0,1), black (1,1), and red (1,0). (A) IMP logic gate; threshold, count rate of 150 kHz. (B) NIMP logic gate with $G(0)$ intercept as output; threshold, $G(0) = 2.2$.

In summary, we combined fluorescence correlation spectroscopy with a supramolecular saccharide probe to detect fructose at a nanomolar dye concentration. Digital analysis revealed a complementary IMP/NIMP logic function. As a general perspective, this work demonstrates ways by which Boolean logic can process information in the field of sugar diagnostics.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) de Silva, A. P.; Gunaratne, N. H. Q.; McCoy, C. P. *Nature* **1993**, *364*, 42.

(2) Pischel, U.; Andreasson, J. *New J. Chem.* **2010**, *34*, 2701.

(3) Kikkeri, R.; Grünstein, D.; Seeberger, P. H. *J. Am. Chem. Soc.* **2010**, *132*, 10230.

(4) Amelia, M.; Zou, L.; Credi, A. *Coord. Chem. Rev.* **2010**, *254*, 2267.

(5) Ran, T.; Kaplan, S.; Shapiro, E. *Nat. Nano* **2009**, *4*, 642.

(6) Szaciłowski, K. *Chem. Rev.* **2008**, *108*, 3481.

(7) de Silva, A. P. *Nature* **2008**, *454*, 417.

(8) Credi, A. *Nat. Nano* **2008**, *3*, 529.

(9) Bozdemir, O. A.; Guliyev, R.; Buyukcakir, O.; Selcuk, S.; Kolemen, S.; Gulseren, G.; Nalbantoglu, T.; Boyaci, H.; Akkaya, E. U. *J. Am. Chem. Soc.* **2010**, *132*, 8029.

(10) Guo, Z.; Zhu, W.; Shen, L.; Tian, H. *Angew. Chem., Int. Ed.* **2007**, *46*, 5549.

(11) Guliyev, R.; Ozturk, S.; Kostereli, Z.; Akkaya, E. U. *Angew. Chem., Int. Ed.* **2011**, *50*, 9826.

(12) de Silva, A. P. *J. Phys. Chem. Lett.* **2011**, *2*, 2865.

(13) de Silva, A. P.; Uchiyama, S. *Top. Curr. Chem.* **2011**, *300*, 1.

(14) de Silva, A. P.; James, M. R.; McKinney, B. O. F.; Pears, D. A.; Weir, S. M. *Nat. Mater.* **2006**, *5*, 787.

(15) Angelos, S.; Yang, Y.-W.; Khashab, N. M.; Stoddart, J. F.; Zink, J. I. *J. Am. Chem. Soc.* **2009**, *131*, 11344.

(16) Tokarev, I.; Gopishetty, V.; Zhou, J.; Pita, M.; Motornov, M.; Katz, E.; Minko, S. *ACS Appl. Mater. Interfaces* **2009**, *1*, 532.

(17) Amir, R. J.; Popkov, M.; Lerner, R. A.; Barbas, C. F.; Shabat, D. *Angew. Chem., Int. Ed.* **2005**, *44*, 4378.

(18) Privman, M.; Tam, T. K.; Bocharova, V.; Halámek, J.; Wang, J.; Katz, E. *ACS Appl. Mater. Interfaces* **2011**, *3*, 1620.

(19) Halámek, J.; Zhou, J.; Halámková, L.; Bocharova, V.; Privman, V.; Wang, J.; Katz, E. *Anal. Chem.* **2011**, *83*, 8383.

(20) Konry, T.; Walt, D. R. *J. Am. Chem. Soc.* **2009**, *131*, 13232.

(21) Margulies, D.; Hamilton, A. D. *J. Am. Chem. Soc.* **2009**, *131*, 9142.

(22) Pischel, U.; Heller, B. *New J. Chem.* **2008**, *32*, 395.

(23) Xu, C.-H.; Sun, W.; Zheng, Y.-R.; Fang, C.-J.; Zhou, C.; Jin, J.-Y.; Yan, C.-H. *New J. Chem.* **2009**, *33*, 838.

(24) Gupta, T.; van der Boom, M. E. *Angew. Chem., Int. Ed.* **2008**, *47*, 5322.

(25) Upadhyay, K. K.; Kumar, A.; Mishra, R. K.; Fyles, T. M.; Upadhyay, S.; Thapliyal, K. *New J. Chem.* **2010**, *34*, 1862.

(26) Kaur, P.; Kaur, S.; Singh, K. *Org. Biomol. Chem.* **2012**, *10*, 1497.

(27) de Silva, A. P.; McClenaghan, N. D. *Chem.—Eur. J.* **2002**, *8*, 4935.

(28) Rurack, K.; Trieflinger, C.; Koval'chuck, A.; Daub, J. *Chem.—Eur. J.* **2007**, *13*, 8998.

(29) Whitehead, A. N.; Russell, B. *Principia Mathematica*; Cambridge University Press: Cambridge, UK, 1910; Vol. *1*.

(30) Borghetti, J.; Snider, G. S.; Kuekes, P. J.; Yang, J. J.; Stewart, D. R.; Williams, R. S. *Nature* **2010**, *464*, 873.

(31) See the Supporting Information.

(32) Straight, S. D.; Andréasson, J.; Kodis, G.; Bandyopadhyay, S.; Mitchell, R. H.; Moore, T. A.; Moore, A. L.; Gust, D. *J. Am. Chem. Soc.* **2005**, *127*, 9403.

(33) Gunnlaugsson, T.; Mac Donail, D. A.; Parker, D. *Chem. Commun.* **2000**, 93.

(34) de Silva, A. P.; Dixon, I. M.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Maxwell, P. R. S.; Rice, T. E. *J. Am. Chem. Soc.* **1999**, *121*, 1393.

(35) Haustein, E.; Schwille, P. *Annu. Rev. Biophys. Biomol. Struct.* **2007**, *36*, 151.

(36) Gamsey, S.; Miller, A.; Olmstead, M. M.; Beavers, C. M.; Hirayama, L. C.; Pradhan, S.; Wessling, R. A.; Singaram, B. *J. Am. Chem. Soc.* **2007**, *129*, 1278.

(37) Weil, T.; Vosch, T.; Hofkens, J.; Peneva, K.; Müllen, K. *Angew. Chem., Int. Ed.* **2010**, *49*, 9068.

(38) Zang, L.; Liu, R.; Holman, M. W.; Nguyen, K. T.; Adams, D. M. *J. Am. Chem. Soc.* **2002**, *124*, 10640.

(39) *The Sugar Code*; Gabius, H.-J., Ed.; Wiley-VCH: Weinheim, 2009.

(40) Pasparakis, G.; Vamvakaki, M.; Krasnogor, N.; Alexander, C. *Soft Matter* **2009**, *5*, 3839.

(41) Vilozny, B.; Schiller, A.; Wessling, R. A.; Singaram, B. *Anal. Chim. Acta* **2009**, *649*, 246.

(42) Schiller, A.; Wessling, R. A.; Singaram, B. *Angew. Chem., Int. Ed.* **2007**, *46*, 6457.

(43) Al-Soufi, W.; Reija, B.; Felekyan, S.; Seidel, C. A. M.; Novo, M. *ChemPhysChem* **2008**, *9*, 1819.

(44) Hellerer, T.; Schiller, A.; Jung, G.; Zumbusch, A. *ChemPhysChem* **2002**, *3*, 630.

(45) Powe, A. M.; Fletcher, K. A., St.; Luce, N. N.; Lowry, M.; Neal, S.; McCarroll, M. E.; Oldham, P. B.; McGown, L. B.; Warner, I. M. *Anal. Chem.* **2004**, *76*, 4614.

(46) Opitz, N.; Rothwell, P. J.; Oeke, B.; Schwille, P. S. *Sens. Actuators B: Chem.* **2003**, *96*, 460.

(47) Schiller, A. In *Molecules at Work. Selfassembly, Nanomaterials, Molecular Machinery*; Pignataro, B., Ed.; Wiley-VCH: Weinheim, 2012; p 315.

(48) Anslyn, E. V. *J. Org. Chem.* **2007**, *72*, 687.

(49) Margineanu, A.; Hofkens, J.; Cotlet, M.; Habuchi, S.; Stefan, A.; Qu, J.; Kohl, C.; Müllen, K.; Vercammen, J.; Engelborghs, Y.; Gensch, T.; De Schryver, F. C. *J. Phys. Chem. B* **2004**, *108*, 12242.