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Molecular Computing

Sugar-based Molecular Computing by Material Implication**

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Abstract: *A method to integrate an (in principle) unlimited number of molecular logic gates to construct complex circuits is presented. Logic circuits, such as half- or full-adders, can be reinterpreted by using the functional completeness of the implication function (IMP) and the trivial FALSE operation. The molecular gate IMP is represented by a fluorescent boronic acid sugar probe. An external wiring algorithm translates the fluorescent output from one gate into a chemical input for the next gate on microtiter plates. This process is demonstrated on a four-bit full adder.*

Molecules capable of performing Boolean operations can be used today for information processing at a level of higher complexity.[1–4] Since the first molecular AND function was introduced,[5] molecular logic has reached the stage where larger circuits execute arithmetic calculations[6–11] or even play "tic-tac-toe", where preloaded multiwell plates were used for game-playing based on molecular logic with DNA strands.[12,13] Recently, the idea of using chemical sensors as molecular logic gates in medical diagnostics has given the field an important boost.[14] Physicians often evaluate clinical parameters by binary reading: they are not primarily interested in the exact numerical values. They combine YES/NOT information with logic functions such as AND or OR and come up with a first diagnosis. A good example is given by the determination of human chorionic gonadotropin (hCG) in pregnancy tests. If more analytes have to be processed, the binary responses of the different sensors can be combined to receive a final diagnostic value. Thus, the "lab-on-a-molecule"[15] or more complex circuits have to be realized.[14,16] Similar to electronics, logic gates have to be connected by physical wires (concatenation). However, integration of molecular logic into complex circuits faces several challenges: 1) computation in solution is limited, because the degree of spatial organization is low; 2) the nature of the upstream output makes it difficult to feed it into a downstream gate; 3) most real-world circuits utilize a combination of different types of logic gates (i.e., AND, XOR, and OR in case of a full-adder). Most molecular logic gates utilize different chemical species as input and produce an even broader range of outputs, which makes it very difficult to interconnect the gates into a complex circuit; 4) The accumulation of chemical in- and outputs prevents arithmetic systems from resetting.[17] Thus, only few concatenated systems with chemical logic gates have been published, and these circuits are mostly limited to a small number of integrated logic gates per array.[2,8,14,18,19] Although the problem of input/output inhomogeneity has been cleverly addressed with all-photonic gates,[14] the challenges arising from integration of molecular logic into complex circuits cannot be tackled by chemists alone.[8,20] Consequently, combined approaches from chemistry and computer science for logic-gate integration are desirable. Herein we show an interconnection of the logic gates "material implication" (IMP) and FALSE on microtiter plates for constructing a chemical platform for complex logic circuits (Figure 1). Our approach combines an algorithm with chemical input and fluorescence output signals. The IMP gate comprises a fluorescent two-component sensor for sugars under physiological conditions.[21] This concept provides universal concatenation in molecular logic.

The recent discipline "chemical computing" in the field of computer sciences is attempting to process matter and information in parallel:[11,22] it combines chemical arrays and computer algorithms to achieve control over complex information processing. This offers new applications on the interface of matter and information. The idea of their parallel flow can in particular address the concatenation problem of molecular logic gates. The algorithm-driven approach presented herein overcomes most of the practical problems outlined previously (Figure 1 a).[14] It transforms the fluorescent output into chemical input using the preserved input-output consistency over all included logic gates. Microtiter plates offer a high degree of spatial organization by separating every gate (well) from each other.[3] In this study, only one type of non-trivial logic gate IMP is used; molecular logic functions with different chemistries are not necessary. It utilizes two chemical inputs and produces one fluorescence output. We have previously demonstrated the potential of the Singaram/Wessling-type sugar probe[23,24] as an IMP logic gate using fluorescence correlation spectroscopy at the level of a few molecules of the reporting dye.[21] The sensing system can be described as an allosteric indicator displacement assay (AIDA) in aqueous buffer solution.[25] The anionic reporter dye (8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt, HPTS) can be quenched by a boronic acid-appended bipyridinium salt (BBV, input *x*, Figure 1). By the addition of a diol-containing compound (input *y*), the boronic acid forms a negatively charged boronate ester, partially neutralizing the positive pyridinium charge. As a consequence, the

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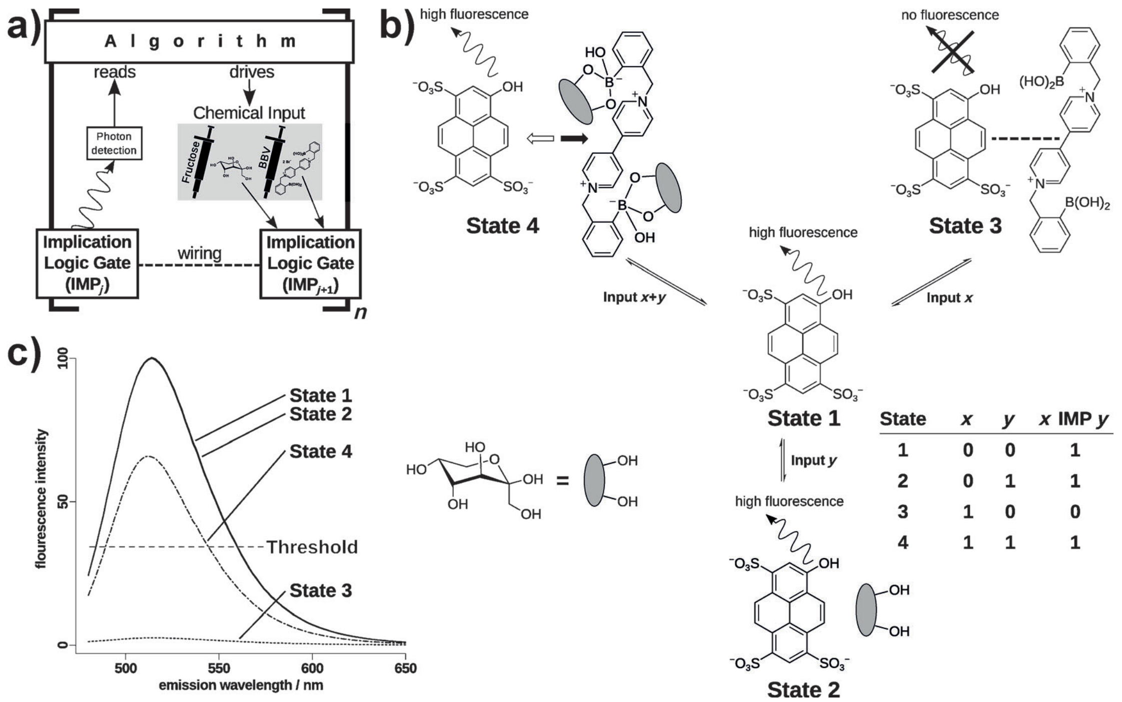

Figure 1. Implication with a molecular logic device. a) Representation of the working principle of the algorithm-driven wiring of multiple IMP gates in microtiter plates. b) Two-component saccharide probe with logic capability. The combination of a boronic acid-appended viologen (input *x*) and a fluorescent dye (HPTS) performs an IMP logic function. The second input is a sugar, for example, fructose (input *y*). c) Typical emission spectra of the states mentioned in (b), excitation wavelength $\lambda = 460$ nm, with a robust threshold to distinguish unambiguously between "0" and "1" (Figure S2). The final concentration of HPTS is 4 $\mu mol L^{-1}$ in all cases; the inputs refer to concentrations of 360 $\mu mol L^{-1}$ for BBV and 2.6 $mmol L^{-1}$ for fructose. All measurements were carried out in 0.01 $mol L^{-1}$ HEPES buffer solution (pH 7.4) and at room temperature.

quenching efficacy is drastically reduced. HPTS and fructose show no interaction under these conditions. This input/output behavior yields in an IMP logic gate: the Boolean function provides the output "1" in all circumstances, except the case $x = 1$ and $y = 0$ (Figure 1b, State 3).[21] Only very few examples of chemical IMP gates have been reported.[4,14,21,26–28] However, the IMP function recently attracted considerable attention in memory research in which memristive switches performed stateful logic operations by IMP.[29] Moreover, IMP provides an outstanding attribute in Boolean logic which has not yet been exploited in molecular logic; it contributes to "functional completeness".[30] A set of Boolean operators is called functionally complete if all possible functions with their truth tables can be expressed by the combination of the members of the set. Digital electronics utilize the completeness of the NAND gate widely.[31] Every two-input logic gate can be realized as a network of NAND gates. Only two single logic functions, such as NAND and NOR, can build a complete set by themselves. Nine complete (and non-redundant) sets can be constructed from a set of two logic functions. Seven of them contain the IMP function, the inverse implication (NIMP, also referred to the inhibit function INH) or both of them. Thus, IMP plays a major role in functional completeness. The remaining two (AND and NOT as well as OR and NOT) are equivalent to NAND and NOR.[30]

In this study, we construct a four-bit adder as a complex chemical logic array from the complete set of an IMP and FALSE function. Every elementary logic gate, such as AND, OR or XOR for half- and full-adders, can be rewritten by combinations of IMP and FALSE gates (Figure S1 and Table S1 in the Supporting Information). The FALSE gate yields the output "0" in all input cases. For this trivial case, no chemical logic gate is needed: only a "wiring" (connection of information) in the logic gate network that says "do nothing" is necessary (and this is intrinsically trivial, because no wiring is required).[32] In fact, all logic work is done by the IMP function, comparable to the NAND gates in electronic engineering.[31] It is important to note that de Silva et al. demonstrated also complex logic gate arrays with simple functions, such as PASS 0 and 1, YES and NOT.[33]

For technical applications, it is necessary to find a robust way to distinguish between "0" and "1" both at the input and output side of the logic gate. For the photonic output (in this case the fluorescence intensity), a threshold is defined. Fluorescence intensities beneath the threshold value will be interpreted as "0" and intensities above as "1" (Figure 1c, Figure S2 and S3). Resulting from the robust technique of micro pipettes, the reproducible addition of the chemical inputs in microtiter plates can be accomplished. However, human errors can occur in the manual procedures involved in pipetting.

For the construction of a four-bit adder, the major challenge lies in the concatenation of the chemical logic gates. Thus, the "wiring algorithm" reads: the output of a first IMP gate *j* is detected by measuring the fluorescence

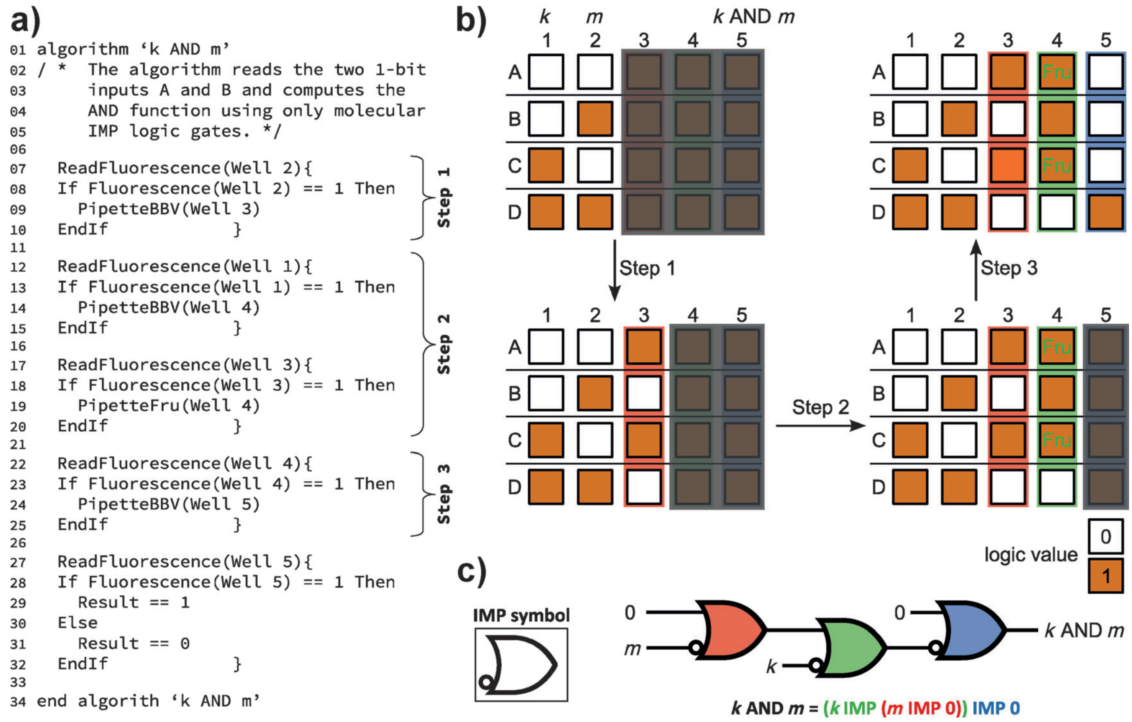

Figure 2. AND logic gate by a network of IMP and FALSE functions. a) Code representation (pipetting protocol) for a wiring algorithm yielding an AND gate from three IMP gates. b) Schematic microtiter plate representation for all four input patterns in the AND wiring algorithm. Each line (A–D) shows one possible input combination and the stepwise result generation according to the wiring algorithm in (a). c) Representation of an AND logic gate by a network of IMP and FALSE gates.

intensity. This output is interpreted digitally and represents the input for the following IMP gate ($j+1$; see Figure 1a). Transfer of the information from one gate to another is realized by a pipetting protocol. This protocol is created by using IF THEN constructions from a typical program pseudocode (Figure 2a). It is important to note that the wiring algorithm is not biasing the logic operations: it only controls the information flow (i.e., the order of the logic gates). As an illustrative example, we now show in detail the construction of an AND gate from interconnected IMP and FALSE gates on a microtiter plate. The function can be rewritten as k AND $m=(k$ IMP $(m$ IMP 0)) IMP 0 (Table S1).[30] The order of execution of the IMP gates is given by evaluating bracketed expressions first. A representation of the circuit diagram is shown in Figure 2c. The chemical inputs for the information transfer are defined with the addition of BBV (input = 1) and fructose (input = 1). The plate is preloaded with HPTS; every well is set to “1” in the beginning. The addition of BBV enables to change the well into “0”. Upon addition of fructose into the same well, the binary value can be reset to “1” again. The AND gate consists of three IMP gates and also three algorithmic steps are necessary. IMP is not commutative; thus, the two inputs must be distinguishable from each other. The first input will be realized by the addition of BBV and is always marked with the circle (Figure 2c). The second input uses fructose for the information transfer. However, input “0” means “do nothing”. Thus, the term “x IMP 0” in the AND gate construction inverts the input x (0 IMP 0 = 1, 1 IMP 0 = 0; equivalent to NOT). Fructose is only necessary as input for the green gate (Figure 2c).

An illustration of all four possible input combinations on a microtiter plate is shown in Figure 2b. The first step transfers input m to the red IMP gate (column 3). BBV is added to the destination well in case of a true (“1”) input from column 2, otherwise nothing is done. After completing step 1, the red IMP gate in column 3 stays untouched for the rest of the operation. Step 2 transfers input k to the green IMP gate by adding BBV into column 4 in case of a true value (“1”) in column 1. The second input to the green gate just has been generated in step 1; step 2 transfers the result from the red IMP gate (column 3) to the green one (column 4). In case of a true value (“1”) in column 3, fructose is also added to the green IMP gate (column 4). The third and last step transfers the result from the green to the blue IMP gate (column 5). In case of a true value (“1”) in the green gate, BBV is added to the blue IMP gate (column 5). The input of “0” in the red and blue IMP gate represents the FALSE gate (Figure 2c). No operation is needed to realize the FALSE gate on the plate.

A four-bit adder comprises one half and three full adders (Figure 3a). The elementary logic functions AND, OR, and XOR have been rewritten with IMP and FALSE gates (Table S1).[30] A 384-well microtiter plate was used to conduct the addition of two four-bit numbers. As an example, we chose the addition of the decimal numbers 10 (binary form: 1010) and 15 (binary form: 1111, see Figure 3b and Supporting Information). It is important to note that any four-bit number can be chosen (see Excel file in Supporting Informa-

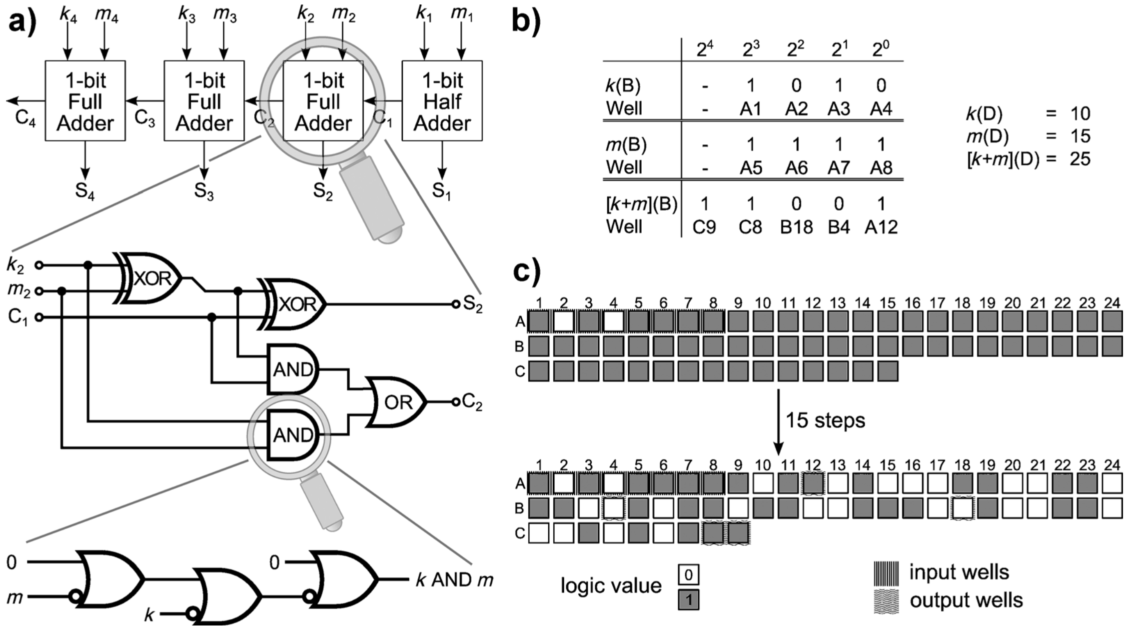

Figure 3. Operation Scheme of a molecular four-bit adder on microtiter plate. a) 4-bit adder consists of three full adders and one half adder. Every elementary logic function (AND, OR, and XOR) can be rewritten by IMP and FALSE gates. b) Transformation of the decimal(D) input in binary(B) numbers and reverse for the output. c) Upper part shows the bit pattern of the input sequence (vertical lines) and the initial state of the microtiter plate. The lower part shows the same wells after all pipetting steps and the resulting calculated sum (horizontal waves).

tion). Depending on the size of the four-bit numbers, the whole addition consisted of up to 15 detection and addition steps and required 40 min on average. On a 384-well microtiter plate, the addition of two numbers with each maximum 12 bits can be performed.

This work is one of the first demonstrations of the combination of information and material processing. The addition of natural numbers demonstrates the powerful approach to control inputs to chemical logic gates with an external algorithm. This facilitates the concatenation in a way that input–output homogeneity of a single logic gate is not necessary. Algebraic operations of natural numbers with a logic gate network point towards new applications: the outlined strategy could be adapted to a wide range of other molecular logic gates and is able to construct digital circuits of enormous complexity. This will be of interest in the field of multi-analyte diagnostics.[4]

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