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Playing Tic-Tac-Toe with a Sugar-Based Molecular Computer

M. Elstner and A. Schiller*

Institute for Inorganic and Analytical Chemistry, Friedrich Schiller University Jena, Humboldtstr. 8, 07743 Jena, Germany

Supporting Information

ABSTRACT: Today, molecules can perform Boolean operations and circuits at a level of higher complexity. However, concatenation of logic gates and inhomogeneous inputs and outputs are still challenging tasks. Novel approaches for logic gate integration are possible when chemical programming and software programming are combined. Here it is shown that a molecular finite automaton based on the concatenated implication function (IMP) of a fluorescent two-component sugar probe via a wiring algorithm is able to play tic-tac-toe.

Since A. P. de Silva introduced the "molecular logic" concept in 1993,[1] it has reached the stage where larger circuits execute arithmetic calculations and work as keypad locks or error checkers.[2−14] In 2003, Stojanovic and Stefanovic[15] published their seminal study on the use of molecular logic to play the game tic-tac-toe. Originally, tic-tac-toe was the first graphical computer game. In 1952, it ran on the electronic EDSAC computer at the University of Cambridge.[16] The theoretical part of the "analytical engine" had previously been developed in 1840.[17] In the molecular version, Stojanovic and Stefanovic[15] used a deoxyribozyme-based automaton (MAYA-I) that followed a perfect strategy with DNA logic gates. The improved MAYA-II enabled a more complex gaming strategy based on 128 integrated logic gates.[18] However, the low integration density of "wet computers" compared with semiconductor circuits is still a major bottleneck in chemical computing.[3] Directing chemical reactions or signals in channels (similar to a wire in an electronic circuit) is challenging. However, concatenation between logic gates is a key step to yield circuits with possible applications, such as information storage,[19] smart materials,[20−22] delivery/activation of drugs,[23,24] clinical diagnostics,[25,26] photochemical edge detection,[27] or even playing games.[15,18]

Tic-tac-toe is a complex test case for human−computer interaction. Taking turns, each player must react to the decisions made before (Figure 1). Tic-tac-toe on a 3 × 3 field is a game of "perfect information", and an optimal strategy guarantees a win or draw for the player moving first. One player writes X's and the other writes O's until either three identical symbols are in a straight line (horizontal, vertical, or diagonal), in which case the player wins, or all nine cells are filled without a winner (in which case it is a "cat's game" or tie). A typical game proceeding is shown in Figure S1. The straightforward decision matrix of the game makes it ideal to test new computing paradigms (Figure 1).[17]

In general, decisions in tic-tac-toe follow an if−then scheme. The binary version of such if−then statements can be described by an implication logic function (IMP). However, making a move in tic-tac-toe requires the combination of IMP gates to construct OR gates.[15] We recently presented a new concept for

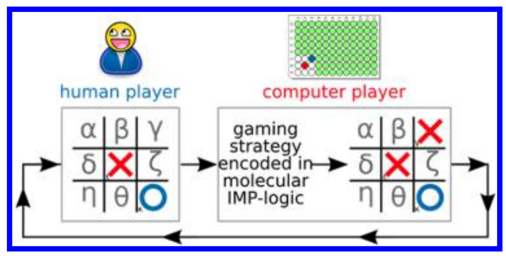

Figure 1. Implementation strategy of the molecular-logic-based tic-tac-toe automaton. Moves from the human player (marked with blue luminescent nanoparticles) are read out, and the gaming strategy (coded in algorithm-wired IMP gates; also see Figure 2) determines where to move next. The computer move is finally marked with red luminescent nanoparticles, and the turn goes again to the human player.

combining molecular IMP gates to generate all possible two-input logic functions.[28] Concatenation of hundreds of IMP functions has been demonstrated using a fluorescent sugar sensor via implementation of a wiring algorithm. With this universal concept, a serial four-bit binary adder was constructed.[28] It is important to note that our concept does not rely on exclusive molecular programming; only the combination of the IMP and FALSE functions with suitable software enabled us to integrate an (in principle) unlimited number of molecular logic gates to generate complex computing. In this study, we show that sugar-sensor-based IMP concatenation is also able to provide a gaming algorithm for playing tic-tac-toe (Figure 2).

The key idea is that every well on a microtiter plate serves as single, separated logic gate. To obtain concatenation between several gates, chemical species have to be transferred to the target well. This is done by an algorithm that drives dosing steps (e.g., a pipetting robot) depending on the fluorescence value of a given well. For example, if information is to be transferred from well A1 to well A2, the algorithm is `if`

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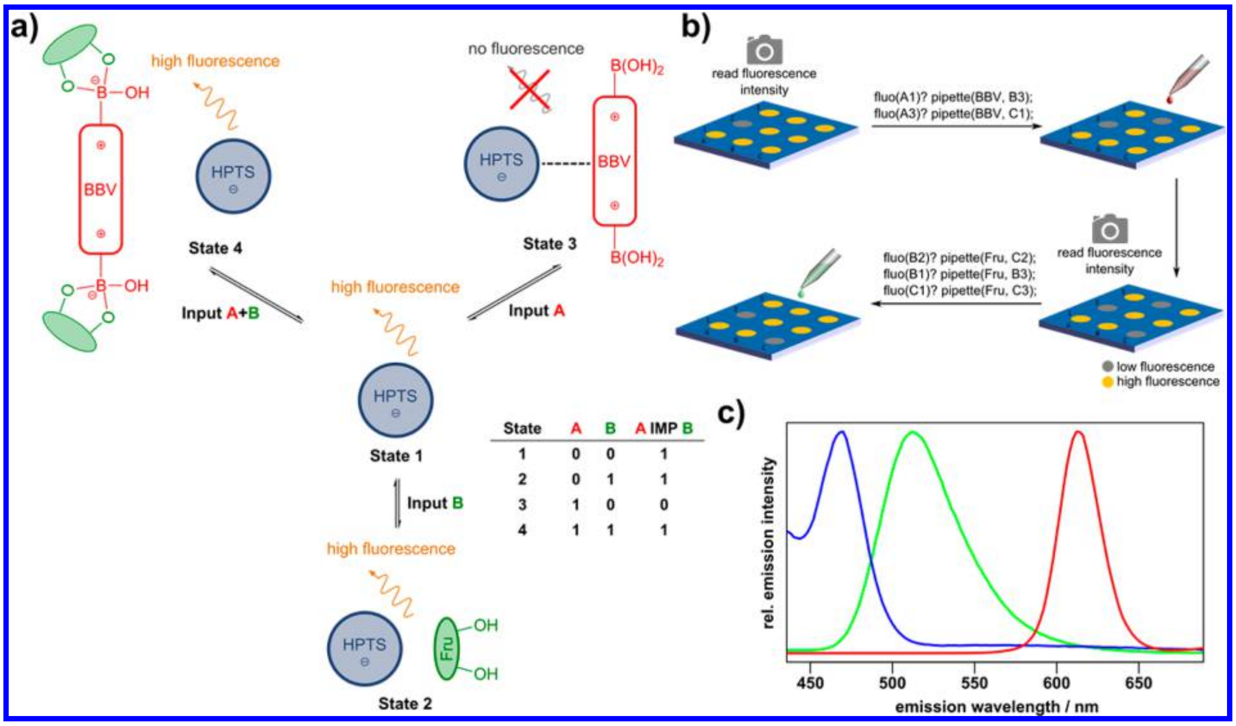

Figure 2. (a) The combination of a boronic acid-appended viologen (BBV, input *A*, red) and a fluorescent dye (8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt, HPTS, blue) performs an implication logic function (IMP). The second input is a sugar, e.g., fructose (input *B*, green). In aqueous buffered solution, HPTS fluoresces on its own ($A = 0$, $B = 0$, output = 1) and also in the presence of fructose ($A = 0$, $B = 1$, output = 1). BBV quenches that fluorescence ($A = 1$, $B = 0$, output = 0) unless fructose, which reacts with the boronic acid, is also present ($A = 1$, $B = 1$, output = 1).[29,30] (b) Logic gate concatenation starts with a readout of the input data, i.e., the fluorescence intensity is measured. On the basis of this input, the concatenation algorithm drives the dosing of chemicals. (c) Emission spectra of the luminophores used in this study (normalized): blue, blue-emitting nanoparticles; green, HPTS; red, red-emitting nanoparticles, all in HEPES buffer (pH 7.4, 0.1 mol/L), excitation wavelength = 415 nm.

`fluorescence(A1=1), then pipette(compoundX, A2)`. This instruction means that the fluorescence intensity of well A1 is measured, and in the case of high fluorescence intensity compound X is added to well A2 (see also Figure 2b).[28] The optical readout of the fluorescence signal and the subsequent addition of chemicals act as intrinsic signal amplification. Thresholding and signal restoration make our system more robust than single-solution approaches, since the effects of dilution and interference of substances are minimized. The algorithm steps also allow distinguishing between different luminescence colors. This means that we can distinguish which player occupies a field in the tic-tac-toe game. The overall concept can be described as a custom finite automaton where some parts are held chemically (IMP gates on the microtiter plate) and others are held in the software (the wiring algorithm). This hybrid approach is also used for the transition rules of the automaton, which are also distributed over the chemical space and the software space.[17]

In MAYA-I and -II, a human player has to select a certain reagent that fits to the intended move and distribute the reagent over the whole game field. For instance, to move in field 3, the human player has to dispense equal amounts of "solution 3" in all fields of the game board, and the subsequent deoxyribozyme-based reactions switch on a fluorescent signal in a specific field as the computer output.[15,18] In contrast, our concatenation protocol for universal molecular logic gate integration[28] allows us to construct a tic-tac-toe gaming strategy with only a two-component saccharide probe[29−33] as an allosteric indicator displacement assay.[34−38] Furthermore, our logic gate integration approach can be scaled up easily; thousands of interacting logic gates can be implemented on microtiter plates. Last, the wiring algorithm can be adapted to other molecular logic gates or different luminophores as input and output signals. For example, the tic-tac-toe algorithm could use other IMP gates[39−41] only via adjustment of the input signal.

In the following we describe how tic-tac-toe can be played on microtiter plates with molecular logic. A 96-well microtiter plate comprises a 3×3 gaming area (wells A1 to C3; Figure 3). We use luminophoric nanoparticles in this area to visualize inputs and outputs with the naked eye. A human player makes a move by marking a well via pipetting 300 μL of blue nanoparticle solution. It is important to note that the nanoparticles were only used in the gaming area. The rest of the microtiter plate is prefilled with HPTS solution to run the "program" that determines the moves of the gaming automaton (Figure 2).

Tic-tac-toe on a 3×3 grid seems very simple. However, the number of possible positions is unexpectedly large. Truth tables indicate positions of X's and O's by an own column for each position (numbered 1−9). There are each nine possible positions to place an X and an O, so we get a truth table with 18 columns. To cover all possible input combinations, the truth table would need 2^{18} = 262 144 rows. This number can be reduced via simplifications. Rows that contain X and O at the same position can be deleted because this is not a legal position. Also rows with four X's and no O's can be removed, as in each turn only the placement of one sign is allowed. In addition, situations with three identical symbols in line do not need to be continued because one player has won.

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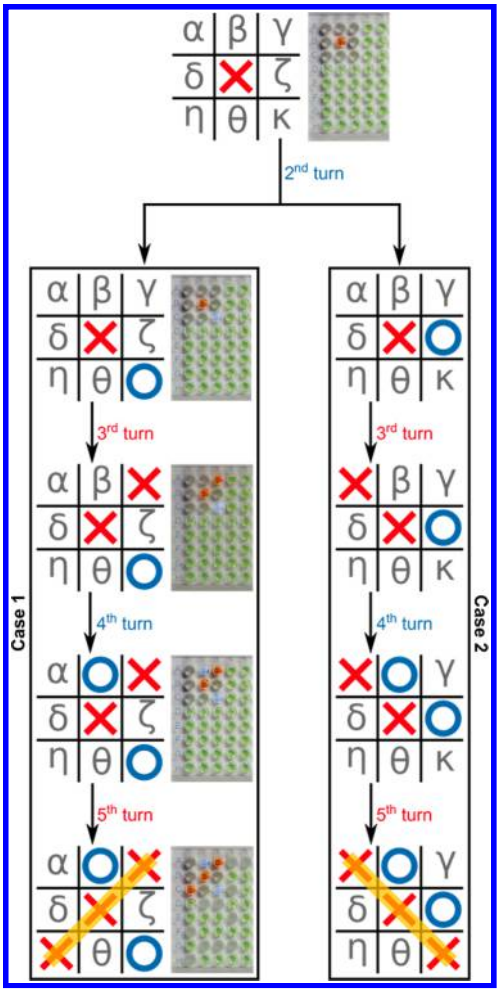

Figure 3. Game runs with nonoptimal playing by the human player. In the fourth turn, the human player did not block the computer player. This ended the game after the fifth turn with a win for the computer. For case 1, photographs of the microtiter plate are also shown; the green wells display the IMP logic gates for the wiring algorithm (also see Figure 2b).

This shrinks the truth table to 4520 rows, which is only 1.79% of the original but still far too large for it to be computed chemically on microtiter plates. Therefore, we further reduced the complexity of the game by additional restrictions. We fixed human moves to corners or edges. In addition, blocking (optimal strategy) or nonblocking by the human player is possible. In total, 28 possible game courses were implemented in our gaming algorithm. For convenient labeling of the gaming area, we chose small Greek letters (Figure 3). The computer starts always at first and occupies the middle field (ε). The human player can now select eight fields, but we handle only two different cases due to symmetry reasons. In case 1, the human player occupies field α or κ, and the device selects field γ; otherwise, field α is selected by the device (case 2). The cases were handled via an OR operation. For example, the command for the third turn is `if` (α = "blue" `or` κ = "blue"), `then` γ = "red", `else` α = "red" (Figure 3). The OR logic gate can be expressed by two IMP gates,[28] so the command reads (α = "blue" `IMP 0`) `IMP` κ = "blue". The wiring algorithm handles the different input colors and finally produces the computer move with `pipet "red" in` γ (case 1) or `pipet "red" in` α (case 2) (see the Supporting Information). In the fourth round, nonoptimal ("stupid") decisions by the human player result in losing the game. In case 1, the device has a winning chance after the third turn. If the human player does not block field η in the fourth turn, the device wins the game in the fifth turn by completing the diagonal over η. In case 2, the winning chance is the same in the symmetric diagonal. However, if the human player does not block κ in the fourth turn, the device wins the game in the fifth turn via the completed diagonal over κ. A more advanced player will end up in an additional case 3, where the player blocks field η in the fourth turn (Figure 4). If the human player follows an

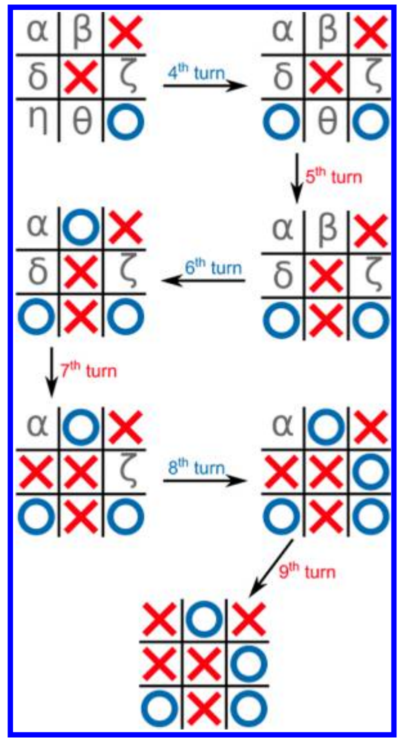

Figure 4. Game run with optimal playing by the human player based on case 1 (also see Figure 3). Herein, the human player blocks all wining possibilities of the computer (by moving to η in the fourth turn, by moving to β in the sixth turn, and by moving to ζ in the eighth turn).

optimal strategy, all winning chances of the computer are blocked. In the next turn, blocking field β is necessary, and in the eighth turn a move to field ζ prevents the computer from winning. In addition, further examples of possible game runs are given in the Supporting Information. Since the computer always starts with a first move to ε, the human player has no chance to win. An optimal game run by blocking all chances for the computer is the best achievable result (Figure 4).

In conclusion, we have demonstrated the flexibility and implementation power of the wiring algorithm approach[28] on a tic-tac-toe automaton. Besides the joyful applications in game playing, we assume that our concept for molecular logic gate integration will have great impact for biochemical or medical analytics.[42−44] Chemical information processing allows the handling of huge data or sample quantities. Together with automated liquid handling systems, such as pipetting robots and microfluidic devices, a new control level of chemical space is achievable.

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ASSOCIATED CONTENT

Supporting Information

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Additional figures and experimental details (PDF)
Wiring algorithm (XLSX)

AUTHOR INFORMATION

Corresponding Author

*E-mail: alexander.schiller@uni-jena.de.

Notes

The authors declare no competing financial interest.

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