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Using T–Hg–T and C–Ag–T: a four-input dual-core molecular logic gate and its new application in cryptography†

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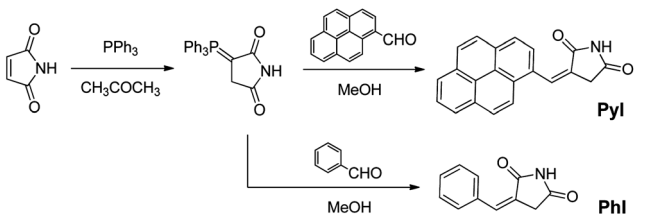
A simple four-input dual-core (thymine & cytosine) logic gate was successfully developed that utilized a succinic imide labelled pyrene probe as the signal responser. Moreover, this molecular logic gate could be made into fluorescent paper and applied in the field of cryptography.

Molecular logic gates are an emerging interdiscipline, utilizing integrated circuits in electronics on a molecular scale.[1] With the rapid development of logic gates, complex molecular circuits with more inputs, outputs and gate symbols are now arousing more and more interest.[2] Although a variety of them have been manufactured, logic gates with more than three-inputs are still rare. The reason is that multiple-inputs make the molecular circuits more complicated and difficult to synthesize. One effective strategy is introducing DNA into the logic system. Many specific structures such as C–Ag–C, T–Hg–T and G-quadruplexes have been used for the construction of complex logic gates.[3] Among them, thymine and cytosine are two important elements in many DNA logic systems with metal ion inputs. Besides, many groups set out to develop the application of these complex logic gates. These molecular logic gates have been applied in biosensing and diagnostics, prodrug activation and drug delivery/release, intelligent materials and molecular computers.[4] Meanwhile, many groups have stepped into the field of cryptography and developed many new nano or other chemical code systems.[5] However, most of them rely on the use of DNA, enzymes and specialist equipment. Thus, these encryptions and decryptions seemed tedious and time-consuming. In this paper, we tried to integrate the functions of thymine and cytosine in one small organic molecule. Then we used this logic gate to develop a more user-friendly code system.

In recent years, simulating the interaction between thymine and Hg^{2+}, "T–Hg–T"-like fluorescent probes based on imide structures have been developed and applied in the sensing of Hg^{2+}. However, only perylene bisimide and naphthalimide were reported.[6] Both could bind to Hg^{2+} in a "T–Hg–T"-like style only. Therefore, they were only used in Hg^{2+}-sensors and not made into molecular logic gates.

In medicinal chemistry, a novel and efficient method was found to connect an aromatic group with a succinic imide. The products synthesized by this method have been applied in drug research.[7] Meanwhile, as examples of important fluorescent probes, pyrene derivatives have been widely used as sensors of pH, DNA, metal ions and so on.[8] Herein, we chose pyrene as the fluorescent unit and synthesized **PyI** through a one-pot step without column chromatography (Scheme 1). Such a simple synthetic route is one of its advantages. For comparison, compound **PhI** was also synthesized. Unlike perylene bisimide and naphthalimide, the structure of **PyI** is not planar. So the intermolecular π–π stacking of **PyI** is not as easy and its aggregation-caused quenching effect was not significant. Actually, the fluorescence of **PyI** in an EtOH–H_2O mixture (1 : 4, v/v) was obvious (Fig. S1†). In addition, it had excellent solid fluorescence (Fig. S2†). Consequently in Fig. 1, it was found that its fluorescence could be quenched by Hg^{2+} in a PBS buffer of pH 6.0, or by Hg^{2+} or Ag^{+} in a PBS buffer of pH 8.0. Meanwhile, there were no changes when other metal ions (K^{+}, Na^{+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Co^{3+}, Al^{3+}, Cr^{3+}) were added under both pH conditions. Then the same



Scheme 1 Synthesis of **PyI** and **PhI**.

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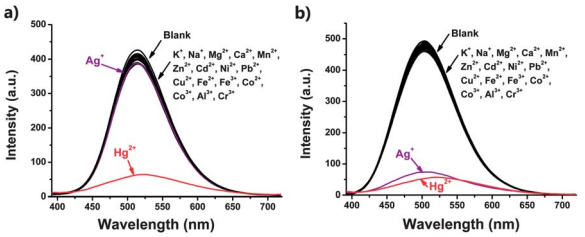
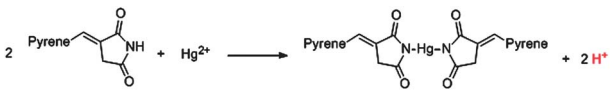


Fig. 1 Fluorescence changes of **PyI** (5.0 μM) upon the addition of 1 equiv. of various metal ions (λ_{ex} = 375 nm) in EtOH–PBS buffer (5 mM) (1 : 4, v/v) (a) pH = 6.0; (b) pH = 8.0. (λ_{ex} = 375 nm).

experiment was performed in a PBS buffer of pH 6.13–7.89. In Fig. S3,† we observed a different quenching effect by Hg^{2+} and Ag^{+} under different pH conditions.

Fig. S4 and S5† show the fluorescence spectra of 5 μM **PyI** after adding different concentrations of Hg^{2+} or Ag^{+}. In the spectra, there is a rapid decrease in intensity at 500 nm before the addition of 0.5 equiv. Hg^{2+} or Ag^{+}. The intensity was generally stable at even higher concentrations of Hg^{2+} or Ag^{+}. Thus, we suggest that Hg^{2+} or Ag^{+} and **PyI** form mainly 1 : 2 complexes. It was also proved by a Job's plot experiment (Fig. S6†). However, complete quenching required more Hg^{2+} at pH 6.0. The possible reason is that the binding process between Hg^{2+} and **PyI** occurs as shown in Scheme 2. Clearly, H^{+} was an obstacle to this reaction and more Hg^{2+} was needed to form this "T–Hg–T"-like complex.

These above experiments were conducted for our next molecular logic gate. We chose Na_2S as the inhibitor for Hg^{2+} and Ag^{+}. Taking into account the complete quenching and HS^{-} existing in an acidic environment, we decided to use 5 μM **PyI**, 5 μM $Hg(NO_3)_2$, 5 μM $AgNO_3$ and 15 μM Na_2S as the inputs of our logic gate. For the inputs of Ag^{+}, Hg^{2+} or S^{2-}, we defined their presence as 1 and absence as 0. For the input of H^{+}, we defined pH 6.0 as 1 and pH 8.0 as 0. The fluorescence intensity at 500 nm (demarcation line: 200) was defined as the output (1 or 0). Fig. 2a displays the possible input and output combinations. Its logic circuit is given in Fig. 2b. The results show that our molecular logic gate can do the same as a DNA logic system can. Besides, it introduced H^{+} as the inhibitor for Ag^{+}. With 16 kinds of input combinations and 3 different kinds of gate symbols (INHIBITOR, OR and IMPLICATION), our logic gate is brief but not simple. In addition, our logic gate (only needs 5 μM Hg^{2+} or Ag^{+}) can be easily seen by the naked eye (Fig. 2c). We also studied the reversibility of this logic gate. Fig. S8† shows the repeated switching behavior with alternating addition of (a) Hg^{2+} and S^{2-} at pH 6.0; (b) Hg^{2+} and S^{2-} at pH 8.0; (c) Ag^{+} and S^{2-} at pH 8.0; (d) dilute HNO_3 and NaOH in the presence of Ag^{+}.



Scheme 2 The binding process between Hg^{2+} and **PyI**.

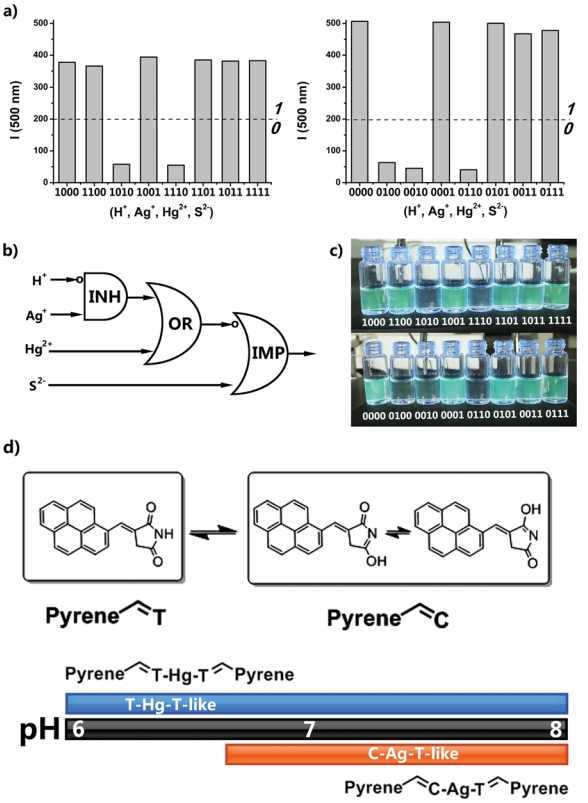


Fig. 2 (a) Fluorescence intensity of **PyI** (5 μM) at 500 nm in the presence of different inputs, (λ_{ex} = 375 nm); (b) the logic circuit of the system (INH = INHIBIT; IMP = IMPLICATION); (c) photos of the logic gate **PyI** (5 μM) in solution under a UV lamp (365 nm); (d) the conversion between cytosine-like and thymine-like structures and the stability of the T–Hg–T-like and the C–Ag–T-like structures at pH 6–8.

Next, we also needed to study the mechanism of the logic gate **PyI**. Firstly, the changes in the absorption spectra reflected the aggregation of fluorescent molecules. In Fig. S9,† adding Hg^{2+} at pH 6.0 and adding Ag^{+} or Hg^{2+} at pH 8.0 could cause the reduction in its UV-Vis absorption peaks. Then the apparent association constants K_{app} for Hg^{2+} and Ag^{+} were calculated using linear regression of the curves in Fig. S10† by the Hill equation. Positive cooperative binding (n > 1) was observed, which indicated that their binding strength to Ag^{+} or Hg^{2+} became stronger as another **PyI** binded.[9]

According to previous literature, it was obvious in our research that a succinic imide could also form a "T–Hg–T"-like structure. This structure proved to be stable between pH 6.0–8.0. However, different from the previous imide probes, it was very unusual that the same quenching was observed after adding Ag^{+} at pH 6.8–8.0. Based on the electrospray ionization-MS (ESI-MS) analysis, we chose **PhI** to study the interaction between this kind of imide structure and Ag^{+}. In Fig. S20,† the molecular ion peak at m/z 479.0179 [2(**PhI** − H^{+}) + Ag^{+}] could be found and suggested the formation of 1 : 2 complexes of Ag^{+} with this kind of imide. In the ^{1}H NMR spectra of the complex of **PhI-Ag-PhI**, the imido proton peak (11.4 ppm) disappeared completely (Fig. S21†). We supposed that this kind of imide structure might

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change into another structure to capture Ag^+. Akira Ono's group reported that 5-fluorouracil could shift from a thymine structure to a cytosine structure and form the stable "C–Ag–T" structure under basic conditions.[10] Due to the molecular conjugative effect, **PyI** could also become a cytosine-like structure (Fig. 2d). This structure was preferably present in a basic environment (Fig. S11 and Scheme S1†). In an acidic environment, it was so difficult to combine Ag^+ with thymine-like **PyI** that the fluorescence quenching by Ag^+ could not be observed.

In our previous work, we have studied the addition of nitro compounds to open unsaturated double bonds.[11] In this paper, there was also an unsaturated double bond in **PyI**. Ag^+ or Hg^{2+} could tie two **PyI**s together and the fluorescence quenching was mainly caused by heavy atom enhanced inter-system crossing/spin–orbit coupling.[12] What's more, this quenching effect might be transmitted to the pyrene unit through the conjugated double bond. To prove our hypothesis, we added 10 μL CH_3NO_2 into the system. Because the double bond was opened *via* a Michael reaction, this quenching effect was partially hindered. This caused enhanced fluorescence (Fig. 3a and b). However, due to the molecular conjugative effect, the cytosine-like structure was actually not a good substrate for a Michael reaction (Fig. 3c). In Fig. 3b, as expected, the change in the fluorescence intensity after adding CH_3NO_2 in the presence of Ag^+ was about half of that in the presence of Hg^{2+}. Different fluorescence lifetimes (t(**PyI** + CH_3NO_2) ≈ t(**PyI** + Hg^{2+} + CH_3NO_2) > t(**PyI** + Ag^+ + CH_3NO_2)) also reflected the difference between these two reactions (Fig. S12†). With the help of the Michael reaction, we verified the quenching mechanism.

In addition, different from other common fluorescence logic gates, its solid fluorescence is so good that it could be made into fluorescent paper. Thus, we could use the fluorescent paper as the molecular logic gate. Meanwhile, we found that 16 kinds of inputs could lead to two outputs by **PyI**. The input could be represented by hexadecimal (0 ~ F). We defined the function of the logic gate **PyI** as $y = f_{PyI}(x)$ (Fig. S13†) and had a molecular code book as shown in Table 1. Therefore, it could be considered to be applied in the field of cryptography. Different from previous code systems, it looks more like asymmetric encryption. We could encrypt a message using the code book and decrypt it using chemical input tubes (Fig. S14 and Table S4†). For example, a spy wrote a secret letter (ABCD 2346 EAF) on **PyI** fluorescent paper using the code book (Fig. 4). From it, the receiver could decrypt the message (0111 0100 001) using chemical input tubes (detailed steps in Table S3†). Then decrypted using the Morse alphabet (Fig. S16†), the letter really meant "JLU". Thus, all the spy needed was the special paper to write on using the code book, without knowledge of the fluorescent dye in the paper. All the receiver needed to do was to decrypt using chemical input tubes without being clear about the code book and which chemicals were in the input tubes. By changing the chemical input tubes (Fig. S15†), we could get 384 kinds of code systems. Compared with traditional encryption methods, this new molecular method is also very secure and reliable. Like never before, our logic gate **PyI** doesn't require cryptographic computations, complicated operations, biochemical knowledge or specialist equipment. More importantly, the logic gate paper is easy to prepare and visible to the naked eye. This is the first report of its kind and could be a novel development direction for the application of molecular logic gates.

In summary, with the idea of using T–Hg–T and C–Ag–T, we successfully synthesized a novel molecular logic gate based on succinic imide for H^+, Ag^+, Hg^{2+} and S^{2-}. Owing to its molecular conjugative effect, the functions of cytosine and thymine were

Table 1 Molecular code book of the logic gate **PyI**

Hexadecimal input[a]	Binary output
2, 4, 6, A, E	0
0, 1, 3, 5, 7, 8, 9, B, C, D, F	1

[a] Transcoded from the inputs of Fig. 2a.

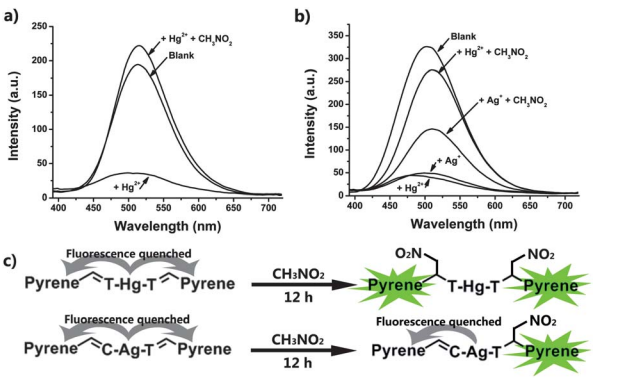

Fig. 3 Fluorescence changes of **PyI** (5.0 μM) upon the addition of 1 equiv. Hg^{2+} or Ag^+ (λ_{ex} = 375 nm) with or without 10 μL CH_3NO_2 : EtOH–PBS buffer (5 mM) (1 : 4, v/v) (a) pH = 6.0; (b) pH = 8.0; (c) the reaction scheme between **PyI**–Hg–**PyI** or **PyI**–Ag–**PyI** with CH_3NO_2.

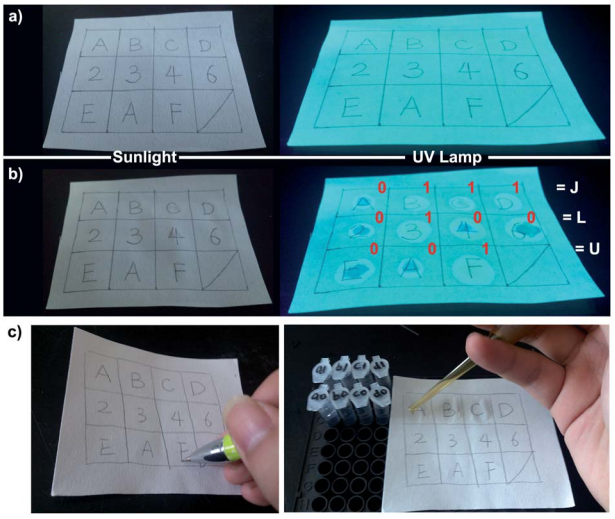

Fig. 4 (a) Encrypted using the code book (Table 1); (b) decrypted using chemical input tubes; (c) the details of the encryption and decryption.

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integrated into one system. Different from common molecular logic gates with several binding sites, one of this dual-core (thymine and cytosine) logic system's advantages is its simple structure and synthesis. With these two cores present, this new dual-core logic gate can handle both Ag^{+} and Hg^{2+} without interference from other metal ions. In addition, the logic gate is visible to the naked eye and can be made into useful and portable fluorescent paper. Then taking advantage of its hexadecimal inputs and binary outputs, we used the molecular logic gate **PyI** in the field of cryptography. We believe that the sensitive, recycled, visualized and simple logic gate has broad prospects in cryptographic applications.

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