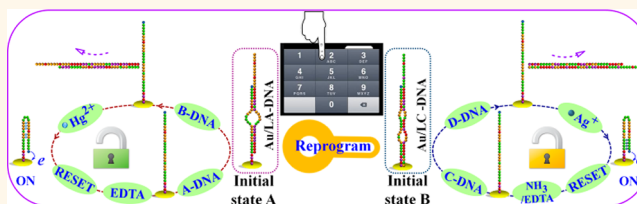


A Resettable and Reprogrammable DNA-Based Security System To Identify Multiple Users with Hierarchy

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ABSTRACT Molecular-level security devices have raised ever-increasing interest in recent years to protect data and information from illegal invasion. Prior molecular keypad locks have an output signal dependent upon not only the appropriate combination but also the exact sequence of inputs, but it cannot be reset or reprogrammed. Here, a DNA-based security system with reset and never-reported reprogram function is successfully developed in proof-of-principle, with which one can change the password in case that the system is cracked. The previous password becomes invalid in the reprogrammed security system. Interestingly, more than one password is designed to permit multiple users to access. By harnessing the intrinsic merit of the different passwords, the system can distinguish different user who is endowed with prior authority. The intelligent device is addressed on solid support and facilitates electronic processes, avoiding chemical accumulation in the system by simple removal of the electrode from the input solution and indicating a main avenue for its further development.



KEYWORDS: molecular keypad lock · DNA · molecular device · reset · reprogram

With the extensive and ever-growing investigations on molecular computing, information processing, and data storage at the molecular level, it would be of importance to develop a molecular security system to prevent data and information from illegal invasion.^{1–6} Molecular keypad lock is considered as a new approach to protect information at the molecular level.^{2–6} The output of a keypad lock system strongly depends on the exact combination and sequence of the inputs, corresponding to the “password”.^{2–6} The “lock” is opened only when the correct “password” is adopted. Otherwise, it stays at close state. Thus, the keypad lock can protect the system against those unauthorized. Considering that the password may be cracked by those unauthorized or in case that the password is disclosed, it is necessary to reprogram the system to authorize access by changing the password, which remains a great challenge for an unconventional molecular security system. Furthermore, it is of convenience and safety to define multiple passwords for multiple users. Since an administrator usually has priority over guest

users who have limited authority, it would be important to distinguish the users to access distinct databases, which can be realized by endowing them with different passwords.⁷ In spite of its fundamental importance, no such molecular security system is reported so far.

Thanks to the programmable sequence-specific recognition property and the ability to capture target molecules in a highly specific manner, DNA is considered a powerful medium for the construction of logic gates,⁸ data storage,⁹ information processing,¹⁰ and molecular computing.¹¹ The interaction of nucleic acids with metal ions, for example, Hg²⁺ ions bridge thymine bases (T-Hg²⁺-T) and Ag⁺ ions bridge cytosine bases (C-Ag⁺-C), makes the molecular-scale logic gate operations in a simple and cost-effective way.¹² Taking advantage of the properties of DNA, here, an electrochemically transduced keypad lock is conceptually realized by linkage of DNA on solid substrate. Not only can the developed security system be easily reset to the initial state after finishing operations, but it can also be reprogrammed to change password. Interestingly, the

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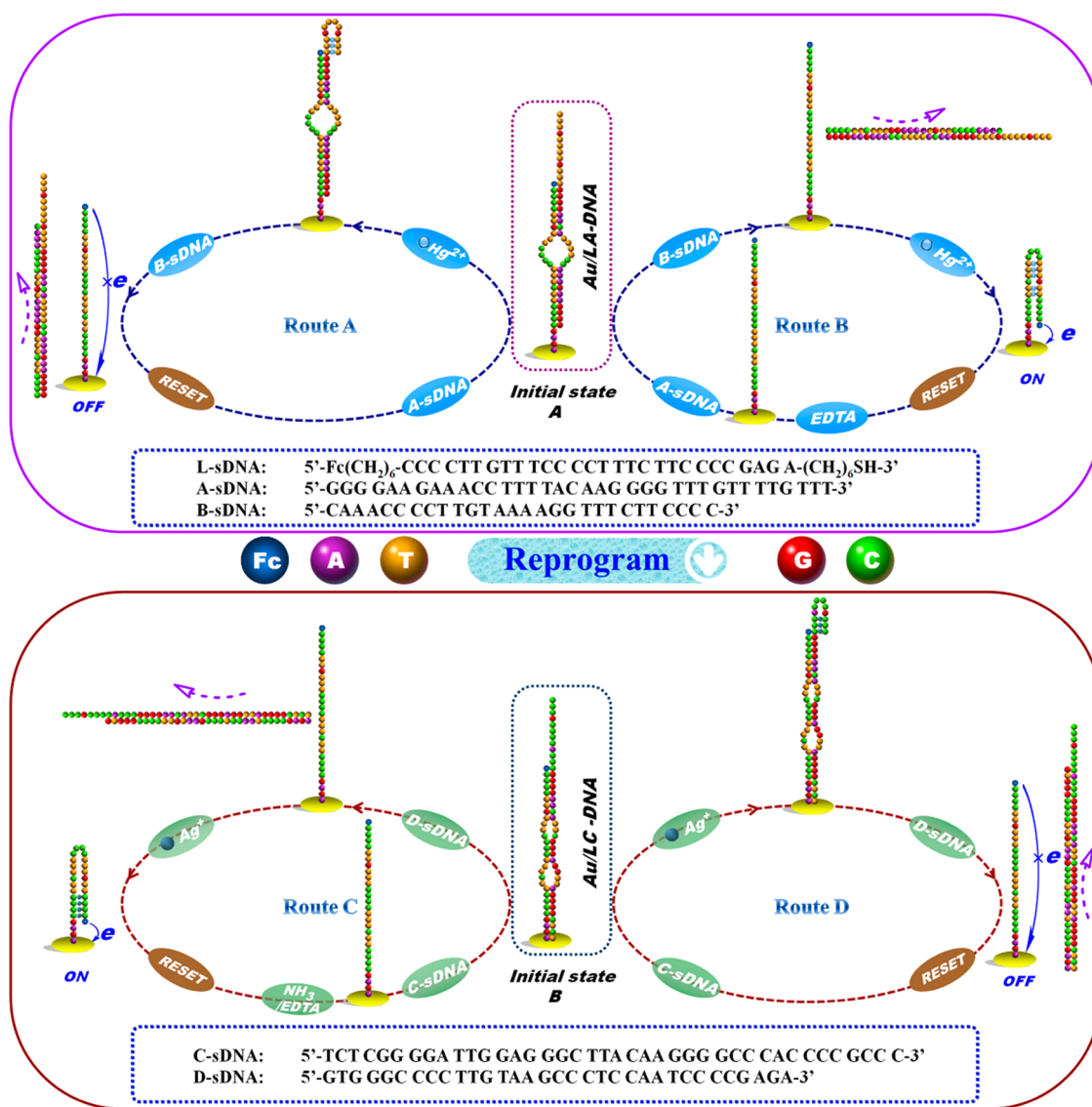
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designed system can be opened by multiple passwords, which can be further used to distinguish the users' hierarchy (an administrator or a guest). The developed system exhibits novel properties and can implement multiple functions to strengthen the security.

RESULTS AND DISCUSSION

The keypad lock system is developed by immobilizing DNA on solid substrate (all designed DNAs are single strands in the investigation). The linked single DNA (L-sDNA) is dually labeled with 3'-SH and 5'-ferrocene (Fc) and is rich in thymine (T) and cytosine (C). Through the formation of Au-S bond, L-sDNA is covalently immobilized on a gold substrate (defined as Au/L-sDNA modified electrode). The current response of the surface-tethered ferrocene is highly sensitive to the distance away from the underlying electrode⁶

(see Figure S1, Supporting Information and discussion there). The keypad lock system is designed by virtue of hybridization and displacement of DNA sequence, as well as the specific recognition between nucleic acid bases and metal ions (shown in Scheme 1). A-sDNA is partially complementary with L-sDNA to form Au/LA-DNA modified electrode, which is defined as the initial state A. B-sDNA and Hg^{2+} are used as the two inputs. B-sDNA completely complements A-sDNA, while it does not hybridize with L-sDNA. Thus, A-sDNA can be released from the electrode by forming duplex AB-DNA with B-sDNA. The interaction among the DNA strands was validated with native polyacrylamide gel electrophoresis (PAGE), as shown in Figure 1A. Lanes 1, 2, and 3 represent the L-sDNA, A-sDNA, and B-sDNA, respectively. A new band is observed at different positions in either lane 4 or lane 6, indicating the formation of duplex LA-DNA and AB-DNA,



Scheme 1. Scheme of the keypad lock operation. The keypad lock system presents different current output signals with inputs in different sequences. Reset and reprogram function could be facilely realized.

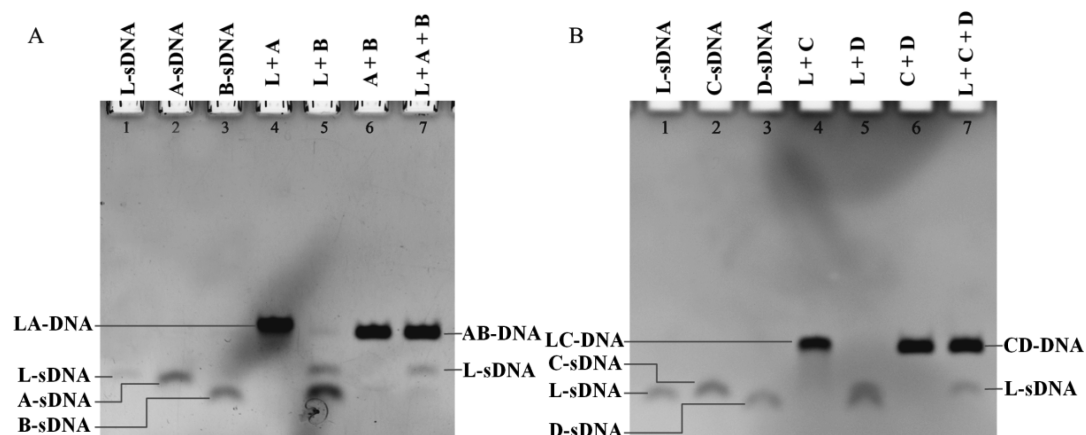


Figure 1. Native polyacrylamide gel analysis of the interactions among DNA strands. Native polyacrylamide gel (12%) analysis of the interaction among L-sDNA, A-sDNA, and B-sDNA (A) and the interaction among L-sDNA, C-sDNA, and D-sDNA (B). The sample in each lane and the identities of the main bands are indicated above and at the sides of the gel image, respectively.

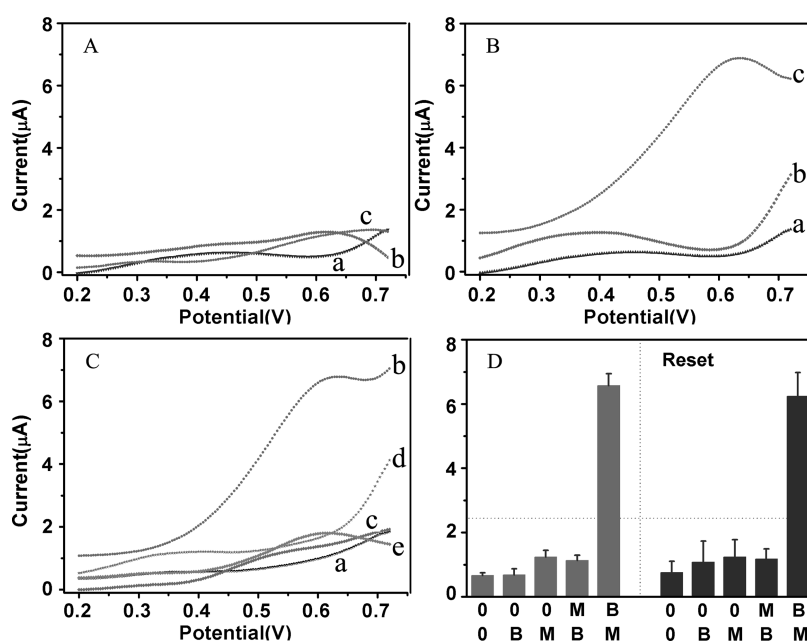


Figure 2. Electrochemical current output signal of the keypad lock system based on L-sDNA, A-sDNA, B-sDNA, and Hg^{2+} . (A) DPV responses of the Au/LA-DNA electrode (a) after being first incubated in Hg^{2+} solution (b), and then in B-sDNA solution (c). (B) DPV responses of the Au/LA-DNA electrode upon reverse inputs: first, incubation in B-sDNA solution (b), and then in Hg^{2+} solution (c). (C) The operation of the keypad system after reset (a) with different inputs: (b) first B-sDNA and then Hg^{2+} ; (c) first Hg^{2+} and then B-sDNA; (d) only B-sDNA; (e) only Hg^{2+} . (D) The histograms of the output signal of the PAND logic operation with reset function. The output signal responds to the current intensity at 0.63 V. The dashed line represents the threshold value for the authentication of the specific user. The characters 0, M, and B represent no input, Hg^{2+} input, and B-sDNA input, respectively. Only the correct combination of both inputs in exact order generates a strong signal, leading to open state of the keypad lock. The error bar represents the standard deviation of three independent experiments.

respectively. In lane 5, two distinct bands appear at the positions which are the same to the single L-sDNA and B-sDNA, respectively, confirming that L-sDNA does not hybridize with B-sDNA. The coexistence of the three DNA strands generates two bands (lane 7), which are ascribed to duplex AB-DNA and single L-sDNA strand, respectively, according to the bands' position. The result indicates that A-sDNA prefers to hybridize with B-sDNA rather than with L-sDNA since A-sDNA and B-sDNA are completely complementary to exhibit high

stability. The native PAGE results in Figure 1 confirm the successful design of the interaction among the DNA strands.

Differential pulse voltammetry (DPV) was performed to detect the current response of the distal Fc against different input sequences. At the initial state A, Au/LA-DNA, the distal Fc is far away from the underlying electrode (about 10 nm) and, thus, no obvious redox signal is observed (a in Figure 2A). The two inputs, B-sDNA and Hg^{2+} , produce two input sequences, first

Hg²⁺ followed by B-sDNA (route A in Scheme 1) and first B-sDNA followed by Hg²⁺ (route B in Scheme 1). Following route A, the initial state of Au/LA-DNA was first incubated in Hg²⁺ solution. Since the residual part of A-sDNA is T-rich after LA-DNA hybridization, the formed T-Hg²⁺-T hairpin structure is located at the outmost of the Au/LA-DNA surface, which depresses the DNA layer on certain degree, leading to a slightly increased current response (b in Figure 2A). The resultant electrode was then incubated in B-sDNA solution, forming duplex AB-DNA. The AB-DNA duplex together with Hg²⁺ is released into solution while L-sDNA stays on the electrode. The release of AB-DNA eliminates the depression of T-Hg²⁺-T to the L-sDNA, and the signal is slightly decreased. Due to the long distance separation, the distal ferrocene produces a low redox current response ("OFF" state, c in Figure 2A).

In the reversal input sequence (route B in Scheme 1), the initial state A of Au/LA-DNA was first incubated in B-sDNA solution. AB-DNA duplex is formed and released from the electrode because B-sDNA is partially complementary to L-sDNA while completely complementary to A-sDNA. L-sDNA still remains on the electrode surface, leading to an Au/L-sDNA modified electrode. The distal Fc is still far away from the underlying electrode, and thus, no obvious redox signal of the Fc can be detected (b in Figure 2B). The resultant Au/L-sDNA electrode was subsequently incubated in Hg²⁺ solution. The T-rich L-sDNA is then switched to a T-Hg²⁺-T hairpin conformation, which brings ferrocene in proximity to the electrode, producing a significantly enhanced redox current signal ("ON" state, c in Figure 2B). When the output is read at 0.63 V, the ratio of ON/OFF electrical current is 5.3.

According to the above discussions, the developed system presents an input-sequence-dependent property and performs functions of a molecular keypad lock. The inputs of B-sDNA and Hg²⁺ are shortened as "B" and "M", respectively. The presence and absence of the input are defined as "1" and "0", respectively. The output signal is defined as "1" when the oxidation current is higher than a threshold value of 2.5 μ A at 0.63 V, mimicking opening of the lock ("ON" state). Otherwise, it is defined as "0", indicating deny of the access. The keypad lock is turned to "ON" state only when it is first stimulated by the input "B" and then input "M". From a logic point of view, a two-input keypad lock is equivalent to a priority AND (PAND) gate.¹³ The system presents a low output signal when at least one input is absent. It is turned to "ON" with a high output signal only when both inputs are adopted in correct sequence. The operation of the keypad lock, *i.e.*, PAND logic operation, is illustrated in the left part of Figure 2D.

The developed keypad lock can be facily reset after operations, which is critical for multiple operations of the keypad lock. Otherwise, the system could be used

only once, greatly limiting its further development. According to the above results, the electrode modified with L-sDNA (Au/L-sDNA) stays at "OFF" state. By incubating it in A-sDNA solution (route A in Scheme 1), a reset security system of Au/LA-DNA is obtained and presents a low current response (a in Figure 2C), similar to that of the initial security system (a in Figure 2A). At "ON" state, L-sDNA settles on the electrode in a T-Hg²⁺-T-mediated hairpin conformation (route B in Scheme 1). To reset the system, the electrode was first incubated in ethylenediamine tetraacetic sodium salts (EDTA) solution. Due to the strong coordination interaction of EDTA with Hg²⁺, Hg²⁺ is released into the solution, liberating L-sDNA from T-Hg²⁺-T-mediated hairpin structure. The resultant Au/L-sDNA electrode was further incubated in A-sDNA solution, resetting to the initial state A (Au/LA-DNA). The reset security system from either "ON" or "OFF" state can perform keypad lock function again. Only the correct password "BM" can switch on the system (b in Figure 2C). Otherwise, the system stays "OFF" to deny access if the incorrect "MB" input sequence (c in Figure 2C) or only single input is adopted (d and e in Figure 2C). The keypad lock with reset operations are illustrated in Figure 2D.

In case that the password is disclosed, it would be interesting to reprogram the security system to endow the user with a different password. Here, the reprogram function was experimentally achieved on the molecular level for the first time. To validate the reprogram function, the following experiments are performed on the basis of the Au/L-sDNA system that resulted from the first round of experiments involving Hg²⁺, as shown in Scheme 1. We designed two single DNA strands, C-sDNA and D-sDNA. C-sDNA performs the same function as A-sDNA to partly complement L-sDNA, fabricating the initial state B of the reprogrammed security system (Au/LC-DNA). It presents a low current response (a in Figure 3A). The residual part of C-sDNA after hybridization with L-sDNA is C-rich. D-sDNA and Ag⁺ are used as the two inputs. D-sDNA does not hybridize with L-sDNA while it completely complements C-sDNA. The interaction among L-sDNA, C-sDNA, and D-sDNA is confirmed by native PAGE analysis, as shown in Figure 1B. By comparing the bands in different lanes, we can draw a conclusion that duplexes LC-DNA (lane 4) and CD-DNA (lane 6) are formed from the corresponding single strands, while L-sDNA and D-sDNA cannot form duplex (lane 5). However, the mixture of L-sDNA, C-sDNA, and D-sDNA prefers to form duplex CD-DNA, leaving L-sDNA alone (lane 7). Following route C in Scheme 1, the reprogrammed Au/LC-DNA system was first incubated in D-sDNA solution. The formed duplex CD-DNA is released into solution. L-sDNA still stays on the substrate, generating an Au/L-sDNA modified electrode and showing a low current response (b in Figure 3A).

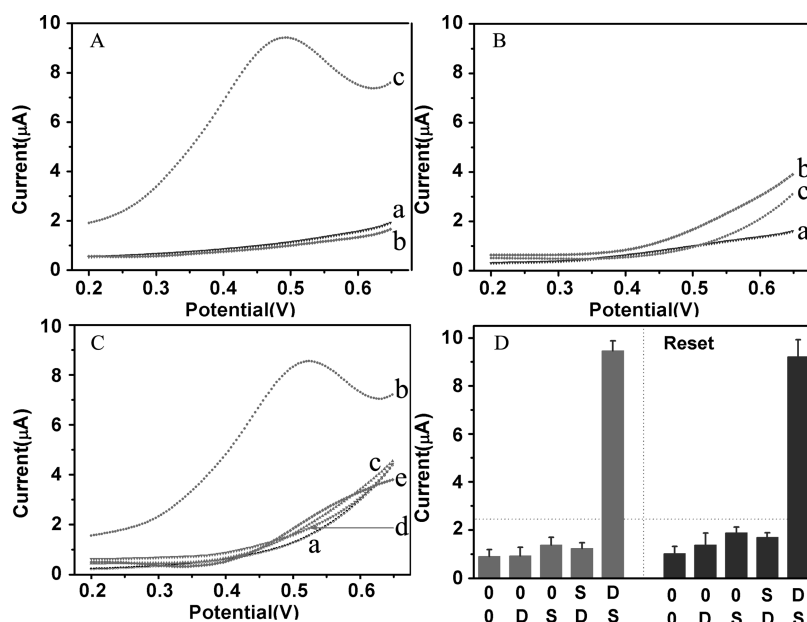


Figure 3. Electrochemical current output signal of the reprogrammed keypad system based on L-sDNA, C-sDNA, D-sDNA, and Ag^+ . (A) DPV responses of Au/LC-DNA modified electrode (a) after being first incubated in D-sDNA solution (b), and then in Ag^+ solution (c). (B) DPV responses of Au/LC-DNA modified electrode (a) upon reverse inputs: first incubation in Ag^+ solution (b), and then in D-sDNA solution (c). (C) The operation of the keypad system after reset (a) with different inputs: (b) first D-sDNA + Ag^+ ; (c) first Ag^+ + D-sDNA; (d) only D-sDNA; (e) only Ag^+ . (D) The histograms of the output signal of the reprogrammed PAND logic operation before and after reset. The output signal responds to the current intensity at about 0.52 V. The dashed line represents the threshold value for the authentication of the specific user. The characters 0, S, and D represent no input, Ag^+ input, and D-sDNA input, respectively. Only the correct combination of both inputs in exact order generates a strong signal, leading to open state of the keypad lock. The error bar represents the standard deviation of three independent experiments.

The resultant Au/L-sDNA electrode was then incubated in Ag^+ solution. The distal Fc approaches the underlying electrode due to the formation of a C- Ag^+ -C-bridged hairpin structure, which triggers the system to "ON" state with a high redox current response (c in Figure 3A). In the reverse input sequence (route D in Scheme 1), the Au/LC-DNA was first incubated in Ag^+ solution. The residual part of C-sDNA in LC-DNA duplex can form a C- Ag^+ -C-bridged hairpin structure, compressing the DNA layer on the electrode and yielding a slightly intensified current response (b in Figure 3B). After further incubation in D-sDNA solution, the duplex CD-DNA, together with Ag^+ , is released into the solution. The resultant Au/L-sDNA presents low current response due to the long distance between Fc and electrode ("OFF" state, c in Figure 3B). The results confirm that the output of the reprogrammed security system is also input-sequence-dependent. The combination of D-sDNA (abbreviated as "D") and Ag^+ (abbreviated as "S") in correct sequence "DS" function as the new password to open the security system. To reset the system from "OFF" state, Au/L-sDNA, the electrode was incubated in C-sDNA solution to recover the initial state B (Au/LC-DNA). At the "ON" state, L-sDNA is situated on the electrode with a C- Ag^+ -C-bridged hairpin structure. To reset the system, the resultant electrode was first incubated in $\text{NH}_3 \cdot \text{H}_2\text{O}$ /EDTA solution to remove Ag^+ and then incubated in

C-sDNA solution to generate the reprogrammed initial state B (Au/LC-DNA). The reset system still works for the next operations. It is opened only by the exact password "DS" (b in Figure 3C) and stays mute in other cases (a and c–e in Figure 3C). The operations of the keypad lock with reset function can be directly visualized in Figure 3D. It should be noted that the peak current is slightly shifted to around 0.52 V in comparison with Hg^{2+} -involved security system, which may be ascribed to the microenvironment difference of the electrode surface. The microenvironment also leads to a higher Fc potential in this work than expected.⁶

Once the security system is reprogrammed, the previous password should not work any more. The reprogrammed system can be opened with the exact password "DS" (Figure 4A) with a strong current response, while keeping close upon introduction of the previous "BM" password (Figure 4B). The results validate that the security system is successfully reprogrammed. The key point of the successful design is that the B-sDNA does not displace L-sDNA from duplex LC-DNA during the fixed reaction time, which can guarantee the reprogrammed keypad lock cannot be opened by the previous password. According to the above results, the developed system can continuously perform keypad lock function four times, including the two reset processes and one reprogram process. The system presents high stability and reproducibility

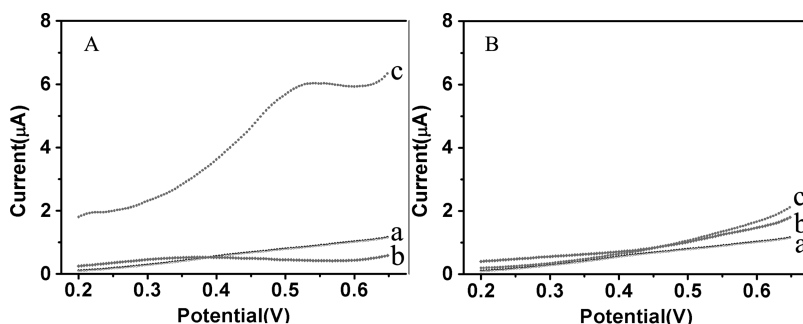


Figure 4. Cross inputs on the reprogrammed security system. DPV responses of Au/LC-DNA electrode under different conditions. (A) DPV responses of Au/LC-DNA electrode (a) after being first incubated in D-sDNA solution (b), and then in Ag^+ solution (c). (B) DPV responses of Au/LC-DNA electrode (a) after being first incubated in B-sDNA solution (b), and then in Hg^{2+} solution (c).

and can perform the keypad lock function within the standard deviation range, obtained from three independent experiments (Figures 2D and 3D).

It is common that multiple users share one information database, which usually requires multiple ways to activate the security system. It is noted that the system can be opened once T-Hg^{2+} -T or C-Ag^+ -C turns L-sDNA into a hairpin structure on the electrode. Therefore, both “BM” and “BS” can work as passwords for the first security system. For the reprogrammed security system, “DS” and “DM” can act as authorized passwords. Thus, more than one password can be defined in the developed system. For the database users, the administrator has prior authority over a guest, and only part of service is available for a guest. Interestingly, the developed system can distinguish the users' hierarchy according to the endowed password and its inherent merits. Here, the Au/LC-DNA was used as a model to confirm the identification function (Figure 5). After opening the system with “DS” (c in Figure 5), the system was subjected to continuous potential scan. A new oxidation peak appeared at about 0.21 V (d in Figure 5) and disappeared in the next scan (e in Figure 5). The phenomenon is not observed when Hg^{2+} acts as one of the inputs. The possible reason is investigated by recording cyclic voltammogram of the related modified electrode (Figure S2, Supporting Information and discussion there). It is found that the surface-tethered silver ions are reduced to silver atoms at 0.1 V, which is influenced by the surface microenvironment.^{14,15} During the positive potential scan, the produced silver clusters are oxidized to silver ions and then stripped off the electrode surface.^{14–16} Therefore, no oxidation peak was observed in the following potential scan. Under these circumstances, the Au/L-sDNA modified electrode was then recovered from the C-Ag⁺-C-bridged hairpin structure and the distal ferrocene was far away from the underlying electrode again. The results inspired us to consider that the oxidation of the silver cluster can be another way to reset the security system. To confirm this, the resultant Au/L-sDNA electrode was reimmersed into

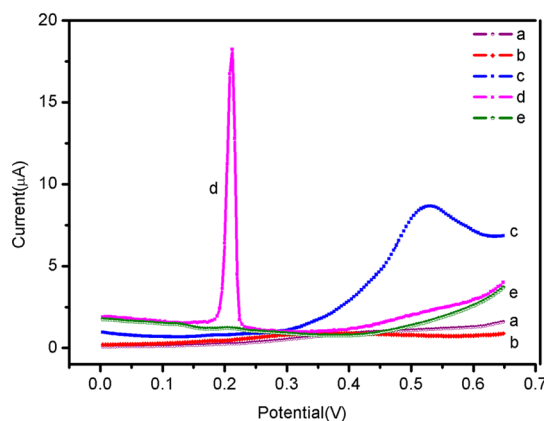


Figure 5. Characteristic DPV responses of Ag^+ -involved correct input sequences. DPV responses of Au/LC-DNA electrode (a) after being first incubated in D-sDNA solution (b), and then in Ag^+ solution (c). After the introduction of the inputs in correct order, the electrode was further subjected to a second potential scan (d) and third potential scan (e).

C-sDNA solution to form the reprogrammed initial state B (Au/LC-DNA). As with the previous investigations, the reset system still can perform keypad lock functions and is opened by the “DS” password. An oxidation peak of silver cluster was again observed in the second potential scan after correct inputs (Figure S3, Supporting Information). Interestingly, this phenomenon was not observed when Hg^{2+} was used as one of the inputs within the investigated potential range. It is reported that the reduction potentials of Hg^{2+} to Hg^+ and then to Hg are at 0.6 and -0.3 V versus Ag/AgCl , respectively.¹⁷ Since the reduction of Hg^{2+} may be influenced by the electrode surface microenvironment, in our investigations, Hg^{2+} is bound to the electrode surface through T-Hg^{2+} -T, which presents high binding ability to Hg^{2+} and might inhibit the reduction of Hg^{2+} . Therefore, no oxidation peak of Hg is observed correspondingly. According to the results, the security system can distinguish the status of the users by monitoring whether there is an oxidation current peak at 0.21 V, if the users are authorized as administrator and guests with the passwords “DS” and “DM”, respectively. This function can be set as a

verification procedure to further protect the system. If one wants to enter the system, he must know the password and the verification information.

The novel functions of the developed security system, including endowing multiple users with hierarchy (administrator and guests), reprogram and reset, make it more complex and difficult for successful design and implementation. This is the reason we first develop a two-input system to experimentally validate that the above novel functions can be achieved on the molecular level. To visualize this sequence-dependent phenomenon directly, a password entry can be constructed using the two inputs B and M (or D and S). We take the two inputs B and M as an example. The output signal above the threshold value $2.5 \mu\text{A}$ at the oxidation peak potential of ferrocene is defined as "P" (high electrical intensity, "ON" state). The input "BM" generates the state of "P", which can serve as a hidden signal and in turn verify whether the inputs are correct. Therefore, the exactly correct input sequence creates a secret code "BMP" to permit access, as shown in Figure S4, Supporting Information. In contrast, the reverse input sequence gives the wrong password entry "MBN" ("OFF" state, designated as the character "N") to deny illegal invasion. This means that an authorized person can use "BMP" security code to open the lock. In a two-digit password, there are 650 different combinations with the use of individual letters from A to Z, which is more than 90 different combinations obtained by using numerical digits from 0 to 9. By designating different ions or DNA sequence to the other letters, the only one correct access password out of all the 650 two-digit combinations could be made possible through inputs starting from B and ending with M. If a two-digit password contains both a character and a number, there are 1260 different

combinations with the use of letters from A to Z and numbers from 0 to 9. For unauthorized users, the case would be more complex since they have no idea of the digits and there are much more other combinations to try. To further enhance the security level, we are working on a five-input keypad lock system, which requires a massive amount of work for the design and experiments.

CONCLUSIONS

In conclusion, a resettable and reprogrammable DNA-based security system with high repeatability and stability is successfully developed as a proof of principle. Thanks to the reset function, multiple operations are experimentally validated with the developed security system. The placement of the system on solid substrate with electrochemical current as output signals provides great advantages for avoiding chemical accumulation in the system. The developed system presents several novel properties, which never have been reported: (1) The security system is endowed with reprogram function. The previous password does not work in the reprogrammed system, further protecting the database in case that the previous system is attacked or the password is disclosed. (2) The developed security system can be opened by more than one password to allow different users to access. (3) The security system can distinguish an administrator from a guest who is endowed with different authority. Though the developed system has great advantages, the investigation is fundamental research and is still in experimental stages. Great challenges still exist, especially how to reduce operation time, which should be addressed in future work. There is a long road ahead to fulfill the requirement for a ready-to-use device.

EXPERIMENTAL SECTION

Materials. All chemicals used were of analytical grade and were used without further purification. The synthesized oligonucleotides were purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). The water used throughout all experiments was purified through a Millipore system. The stock DNA solution was prepared with 25 mM Tris-HCl buffer (pH 7.0). DNA concentration was estimated by measuring the absorbance at 260 nm. The desired DNA concentration in the following experiments was achieved by diluting the stock solution with 25 mM HEPES buffer containing 500 mM NaNO_3 , 30 mM KNO_3 , 0.5 g/L Triton X-100, and 20 mM $\text{Mg}(\text{NO}_3)_2$ (pH 7.8). The desired Ag^+ or Hg^{2+} solution was obtained by diluting concentrated AgNO_3 (1 mM) or $\text{Hg}(\text{OAc})_2$ (1 mM) solution with HEPES buffer (25 mM HEPES, 500 mM NaNO_3 , 30 mM KNO_3 , 0.5 g/L Triton X-100, pH 7.8), respectively.

Preparation of L-sDNA-Modified Gold Bead Electrode. A gold bead electrode with 0.11 cm^2 was first annealed with a hydrogen flame and then cooled to room temperature gradually. Afterward, the electrode was rinsed with Milli-Q water. Subsequently, the electrode was immersed into 500 μL of 3.6 μM L-sDNA solution, annealing from 95 to 30 $^\circ\text{C}$ over a period of 8 h.

Keypad Operation of Hg^{2+} -Involved Security System. The initial state, Au/LA-DNA electrode, was prepared by immersing Au/L-sDNA electrode into 500 μL of 2.9 μM A-sDNA solution to allow hybridization for 5 h. Then the electrode was rinsed with water before inputs. In general, the time for the first input was about 2 h and the second input needed about 6.5 h. The electrode was rinsed with water before and after each input. In the correct input sequence, Au/LA-DNA electrode was first incubated in 500 μL of 2.0 μM B-sDNA solution for about 2 h. After potential scan, the electrode was further incubated in 500 μL of 2.0 μM Hg^{2+} solution for 6.5 h and then the electrochemical signal detection was performed. The resultant electrode at "ON" can be reset to initial Au/AB-DNA state after first incubation in 25 mM EDTA solution for 5 h and then incubation in 500 μL of 2.9 μM A-sDNA solution for 5 h.

In the reverse input sequence, Au/LA-DNA electrode was first incubated in 500 μL of 2.0 μM Hg^{2+} solution for about 2 h. After potential scan, the electrode was incubated in 500 μL of 2.0 μM B-sDNA solution for 6.5 h and the potential scan was performed. To reset from this "OFF" state, the resultant electrode was immersed in 500 μL of 2.9 μM A-sDNA solution for 5 h.

Keypad Operation of Ag^+ -Involved Security System. The initial state, Au/LC-DNA electrode, was prepared by immersing

Au/L-sDNA electrode into 500 μL of 2.2 μM C-sDNA solution to allow hybridization for 5 h. Then the electrode was rinsed with water before inputs. As with the Hg^{2+} -involved security system, the time for the first input was about 2 h and the second input needed about 6.5 h. The electrode was rinsed with water before and after each input. In the correct input sequence, Au/LC-DNA electrode was first incubated in 500 μL of 2.5 μM D-sDNA solution for about 2 h. After potential scan, the electrode was further incubated in 500 μL of 35 μM Ag^+ solution for 6.5 h and then the electrochemical signal detection was performed. The resultant electrode at "ON" can be reset to initial Au/LC-DNA state after first incubation in 5 mM EDTA and 1 M $\text{NH}_3 \cdot \text{H}_2\text{O}$ solution for 7 h, and then incubation in 500 μL of 2.2 μM C-sDNA solution for 5 h.

In the reverse input sequence, Au/LC-DNA electrode was first incubated in 500 μL of 35 μM Ag^+ solution for about 2 h. After potential scan, the electrode was incubated in 500 μL of 2.5 μM D-sDNA solution for 6.5 h and the potential scan was performed. To reset from this "OFF" state, the resultant electrode was immersed in 500 μL of 2.2 μM C-sDNA solution for 5 h.

Native Polyacrylamide Gel Electrophoresis. Polyacrylamide gel (12%) was prepared with $1 \times$ TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3). In each sample, the concentration of each DNA strand is 2 μM in $1 \times$ TBE buffer containing 12 mM Mg^{2+} . Each sample was subjected to annealing started from 95 $^\circ\text{C}$ and terminated at about 25 $^\circ\text{C}$. Twenty microliters of each sample was mixed with 2 μL of Gel-Dye Super Buffer Mix before loading into the gels. The gel was run under a constant voltage of 100 V over a period of about 2 h. Then the gel was immersed in 0.5 $\mu\text{g}/\text{mL}$ EB solution for about 1 h, washed with water twice, and photographed under UV light using a fluorescence imaging system (Vilber Lourmat, Marne laVallee, France).

Electrochemical Experiments. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed in a classical three-electrode cell. Platinum foil and a saturated calomel electrode (SCE) worked as counter and reference electrodes, respectively. HEPES buffer (25 mM HEPES, 500 mM NaNO_3 , 30 mM KNO_3 , 0.5 g/L Triton X-100, pH 7.8) containing 1 M NaClO_4 was used as supporting electrolyte. The differential pulse voltammograms were recorded with CHI 900 (Co. Chenhua, China). The parameters of DPVs: amplitude, 50 mV; pulse width, 0.05 s; pulse period, 0.2 s.

Conflict of Interest: The authors declare no competing financial interest.

Supporting Information Available: Figures and related discussion of DPV responses of Au/L-DNA modified electrode, cyclic voltammograms of Au/LC-DNA electrode, and DPV responses of Au/LC-DNA electrode, all under different conditions. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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