

# Authorizing Multiple Chemical Passwords by a Combinatorial Molecular Keypad Lock

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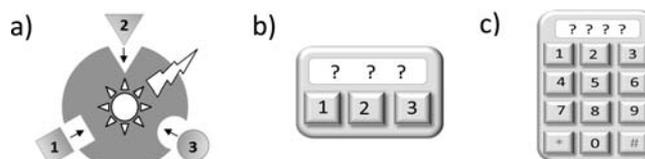
**S** Supporting Information

**ABSTRACT:** A combinatorial fluorescent molecular sensor operates as a highly efficient molecular security system. The ability of a pattern-generating molecule to process diverse sets of chemical inputs, discriminate among their concentrations, and form multivalent and kinetically stable complexes is demonstrated as a powerful tool for processing a wide range of chemical “passwords” of different lengths. This system thus indicates the potential for obtaining unbreakable combination locks at the molecular scale.

Molecular keypad locks<sup>1,2</sup> constitute a unique class of chemical logic systems<sup>3</sup> that are only activated by specific sequences of input signals, namely, by introducing the correct chemical or optical “passwords”. The potential advantages of such devices over conventional security circuits are their molecular scale, their unusual structures, and their ability to respond to unconventional input signals, which complicate their detection, forgery, and cracking, respectively.<sup>1</sup> Although a wide range of molecular-scale combination locks have recently been developed and demonstrated the versatility of the approach,<sup>1,2,4</sup> these systems are limited to entering only a single, default password. Therefore, they cannot compete with electronic keypad circuits.

Figure 1 and Table 1 highlight the main differences between a 3-input molecular keypad lock<sup>1,2,4</sup> (Figure 1a), a corresponding 3-input electronic lock (Figure 1b), and a conventional digital lock with a 10-digit keypad (Figure 1c). As shown in Table 1, common molecular keypad locks, which process three input signals, can only authorize a single 3-digit password out of a factorial number of permutations (i.e.,  $3! = 6$ ), namely, 123, 132, 213, 231, 312, and 321. An equivalent 3-input electronic lock, on the other hand, not only can process additional permutations (i.e.,  $3^3 = 27$ ) but also can distinguish among them. As a result, this device can be programmed to authorize 27 different passwords. The reason for the increase in the number of permutations is that the electronic lock can also differentiate among sequences containing repeated input keys, such as 111, 112, 121, 223, 333, etc. By increasing the password length and the number of input keys, the security level substantially increases. For example, common electronic locks that respond to 10 input keys (digits 0–9) and authorize 4-digit passwords can readily differentiate among 10 000 different permutations.

Herein, we demonstrate the feasibility of creating a novel class of molecular keypad locks that, similar to the electronic devices, can respond to diverse input keys, as well as authorize multiple



**Figure 1.** Schematic representation of (a) a 3-input molecular keypad lock (chemical inputs are denoted as 1, 2, and 3), (b) a 3-input electronic keypad lock, and (c) a conventional electronic keypad lock.

**Table 1. Properties of Molecular and Electronic Keypad Locks**

property	(a) 3-input molecular lock	(b) 3-input electronic lock	(c) conventional electronic lock
no. of input keys	3	3	10
password length	3 digits	3 digits	4 digits
no. of permutations	factorial $3! = 6$	exponential $3^3 = 27$	exponential $10^4 = 10000$
no. of recognized passwords	1	27	10000

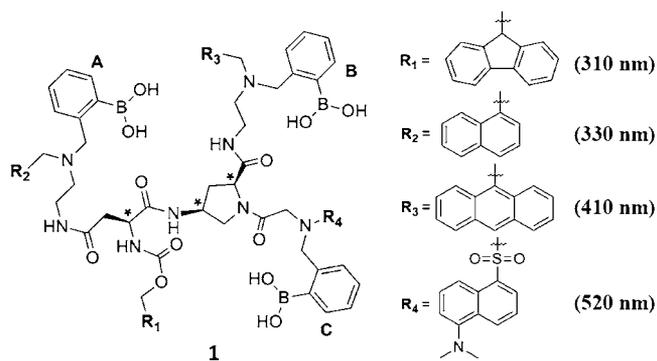
password entries that are assembled from the same set of keys. By exploiting the ability of a combinatorial fluorescent molecular sensor<sup>5</sup> to generate unique optical “fingerprints” for different chemical inputs and for different concentrations, as well as its tendency to form multivalent and kinetically stable complexes, we show that unbreakable molecular-scale combination locks could be within reach.

Our approach to device miniaturization and information processing at the molecular scale is different from the Boolean logic methodology. Rather than imitating the function of electronic logic circuits,<sup>3</sup> we have recently developed a combinatorial fluorescent molecular sensor<sup>5</sup> (Figure 2) that mimics the operation of optical cross-reactive sensor arrays<sup>6</sup> (the so-called chemical “noses/tongues”). Sensor 1 (Figure 2) integrates different nonspecific fluorescent receptors (i.e., boronic acid–dye conjugates) and utilizes photoinduced electron transfer (PET), internal charge transfer (ICT), and fluorescence resonance energy transfer (FRET) for generating distinguishable emission patterns for different carbohydrate-based drugs and their combinations.

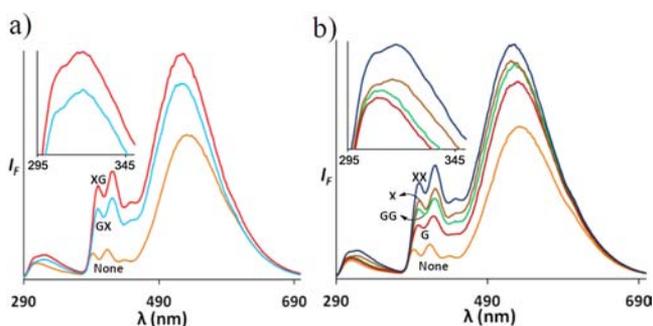
We anticipated that 1 could operate as an efficient molecular keypad lock for the following reasons: First, its ability to generate unique optical “fingerprints” for a wide range of analytes<sup>5</sup> substantially increases the number of input “keys” that can be processed by the molecular device. Second, because pattern-

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**Figure 2.** Structure of a unimolecular keypad lock **1** containing three phenyl boronic acids (A–C) and four spectrally overlapping fluorophores: fluorene (R<sub>1</sub>), naphthalene (R<sub>2</sub>), anthracene (R<sub>3</sub>), and dansyl (R<sub>4</sub>). The emission wavelength of each dye is indicated.



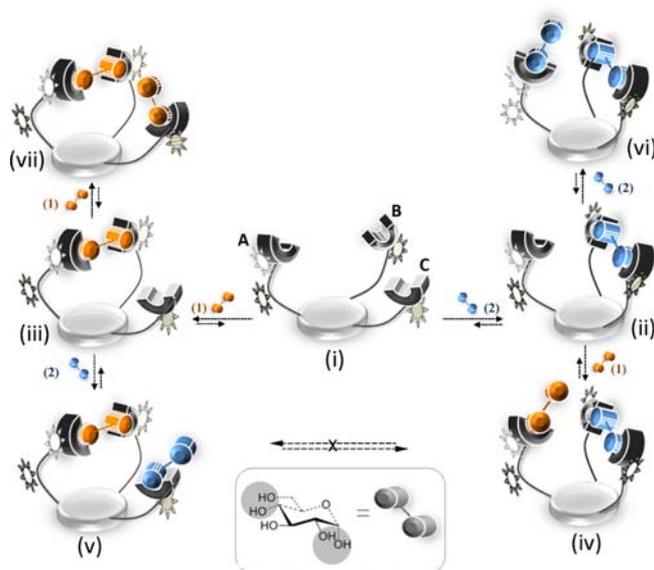
**Figure 3.** (a) Emission spectra of molecule **1** (3 μM) upon the addition of G (2.5 mM) and X (25 mM) in different orders, and (b) at different concentrations. Excitation: 270 nm.

generating systems are very efficient in discriminating among input concentrations,<sup>5,6</sup> **1** should be able to distinguish among password entries containing distinct ratios of identical inputs, for example, between 112 and 122. Finally, the tendency of multivalent receptors to exhibit binding cooperativity and conformational dynamics,<sup>7</sup> as well as their ability to be entrapped in kinetically stable states,<sup>8</sup> should allow **1** to distinguish among chemical input sequences.

These principles were first demonstrated by preparing a series of 2-input molecular keypad locks that respond to different sequences of saccharide pairs, such as D-glucose (G) and D-xylose (X), D-glucose (G) and galactose (L), as well as D-fructose (F) and maltitol (M) (Figures 3a and S1). The distinct optical signatures observed for passwords GX/XG (Figure 3a), GL/LG, and FM/MF (Figure S1) indicate that some of these sequences lead to the formation of kinetically stable states. Similar phenomena have also been observed with various molecular sequential devices,<sup>2a,9</sup> supramolecular architectures,<sup>10</sup> and during the formation of multivalent host–guest complexes.<sup>8</sup> With saccharides, kinetic intermediates can be expected owing to their ability to bind boronic acid-based receptors in distinct stoichiometries (e.g., 1:1, 1:2, or 1:3), in different binding modes (e.g., bi-, tri-, or tetravalent complexes), and in distinct isomerization states (e.g., pyranose or furanose), all of which<sup>11</sup> increase the possibility of entrapment in various local energy minima.

Scheme 1 illustrates how the strong binding of the first saccharide (i.e., saccharide 1 or 2) to two of the three boronic acids (i.e., complexes **ii** and **iii**) followed by a weaker binding of the second saccharide can result in a kinetically stable complex

### Scheme 1. Illustration of a Unimolecular Combinatorial Keypad Lock Function<sup>a</sup>

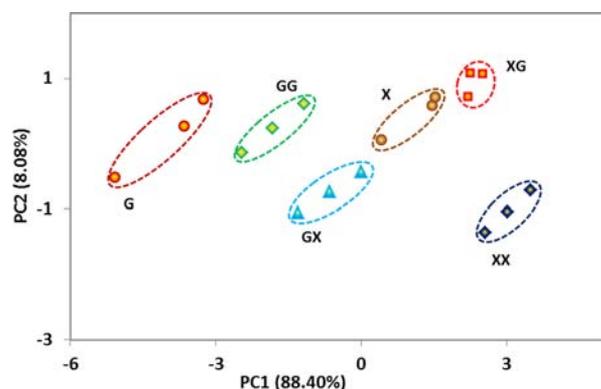


<sup>a</sup>Possible complexes that can be formed upon the addition of two distinct saccharides (1 and 2) in different orders (iv vs v) or at different concentrations (ii vs vi and iii vs vii).

(i.e., complex iv or v) whose conversion to the thermodynamic product occurs over a prolonged reaction time. Density function theory calculations (see the Supporting Information for computational details) support the feasibility of binding cooperativity and the formation of different metastable states. For example, the energies obtained for the tetravalent interaction of D-glucose (G) or D-xylose (X) with sensor **1** (Tables S1 and S2) are lower than those obtained for the bivalent complexes, indicating the preferential formation of complexes such as **ii** or **iii** (Scheme 1) in the primary interaction stage. The calculations also show that these particular saccharides favorably bind to the same site, which further slows down their displacement.

The system can also distinguish among “passwords” containing different inputs or different ratios of the same input (Figure 3b). The different emission patterns obtained upon the addition of each saccharide (X or G), followed by a second addition of the same input signal (XX or GG), demonstrate that **1** can also recognize X, G, XX, and GG as distinct code entries. Scheme 1 illustrates the way changes in the saccharide type (i.e., 1 vs 2) and/or its concentration (i.e., 1 vs 11 or 2 vs 22) could lead to the generation of distinguishable emission patterns for passwords 1, 2, 11, and 22. According to this scheme, changing the saccharide type alters the type of complexes formed (i.e., **ii** and **vi** or **iii** and **vii**), whereas changes in its concentration affect the ratio between them. Principal component analysis (PCA, Figure 4) of the complete spectral data (Figure 3a,b) shows that an individual fluorescent molecule can discriminate among all possible permutations of 1- and 2-code entries, namely, X, G, XX, GG, XG, and GX, akin to an equivalent 2-digit electronic keypad device.

Following this proof-of-concept, we set out to develop a more advanced molecular security system that will be capable of processing 3-digit passwords consisting of different permutations of 3-input keys. Although this poses the challenge of processing 23 additional permutations (Table 1, entry b), a careful inspection of the 27 code entries (Table 2) reveals that many



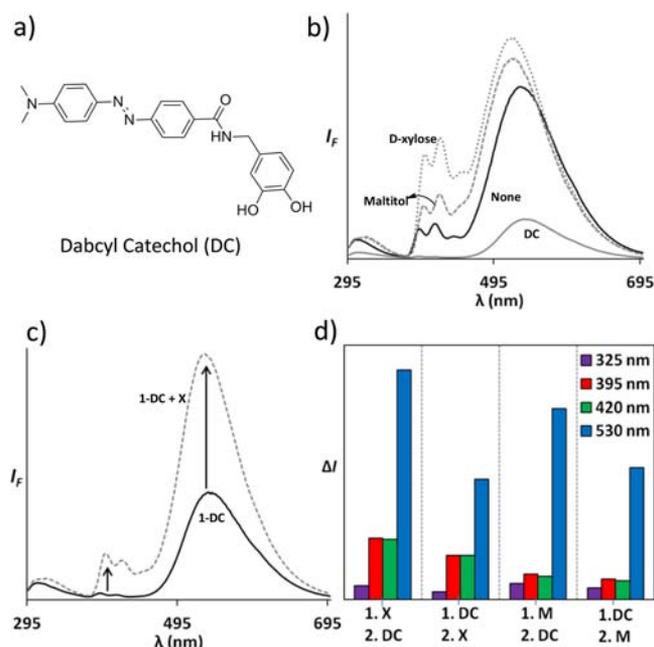
**Figure 4.** PCA mapping of emission patterns generated by **1** upon the addition of D-glucose (G) and D-xylose (X) in three replicates of different sequences and concentrations.

**Table 2.** All Possible Entry Codes of a 3-Input Keypad Lock

1 key	2-keys (1, 2)	2-keys (1, 3)	2-keys (2, 3)	3-keys (1, 2, 3)
a. 111	d. 112	e. 113	f. 223	j. 123
b. 222	121	131	232	132
c. 333	211	311	322	213
	g. 122	h. 133	i. 233	231
	212	313	323	312
	221	331	332	321

of them should be readily differentiated by the molecular device. In Table 2 each digit (1, 2, or 3) represents a different chemical input and the 27 permutations are divided into 10 password groups (groups a–j) that differ either in the type of input “keys” or in the ratio between them. Because **1** can effectively differentiate among different chemical inputs and among distinct input concentrations<sup>5</sup> (Figure 3b), many of the passwords that belong to different groups (a–j) should be distinguishable. In addition, the ability of **1** to discriminate among input sequences (Figures 3a and S1) should allow it to differentiate among passwords within each group (i.e., groups d–j). For example, passwords (11)2 and 2(11) in group d should induce the formation of distinct optical fingerprints.

To efficiently discriminate among groups a–j (Table 2), we first screened for chemical inputs and different concentrations that, individually, induce the most distinguishable changes in the emission signal (see the Supporting Information). In addition to testing 11 different saccharides (Figure S2), we also synthesized a new chemical input that integrates catechol and dabcyll functionalities (Figure 5a, DC). We expected that the strong affinity of catechol to boronic acids<sup>12</sup> and the ability of dabcyll to quench the emission of various fluorophores would enable DC to compete with the binding of various saccharides, as well as to generate markedly distinct emission patterns. As shown in Figure 5b, maltitol, D-xylose, and DC, which were selected from this screening, generated entirely different patterns, and the addition of DC indeed led to fluorescence quenching across the UV–vis spectrum. Moreover, the fluorescence emission was restored by adding a competing saccharide (Figure 5c) and the optical fingerprints were dependent on the order of addition (Figure 5d).

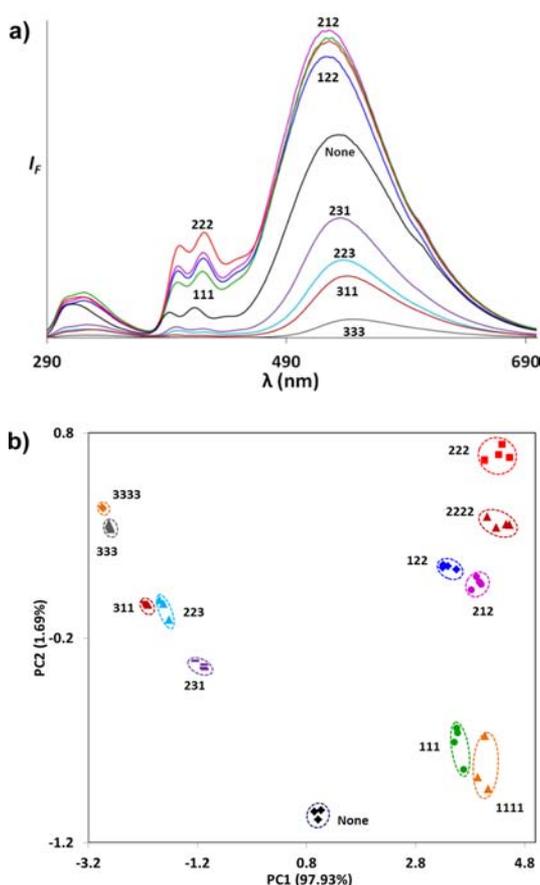


**Figure 5.** (a) Structure of DC. (b) Change in fluorescence spectrum of **1** ( $9 \mu\text{M}$ ) upon the addition of maltitol (50 mM), D-xylose (17 mM), or DC ( $125 \mu\text{M}$ ). (c) Displacement of DC by D-xylose (X). (d) Fluorescence response of **1** to different sequences of DC and D-xylose (X) or maltitol (M).

The final molecular password system (Figure 6) was created after testing all 27 possible code entries (Table 2) with a wide range of input concentrations and eliminating any group of sequences that generated overlapping spectra (Figure S3). Notably, by varying the experimental conditions, both the number and the type of passwords that could be differentiated by the molecular device were changed. The final configuration of the molecular keypad lock was determined as the one that maximizes the number of password entries. Figure 6a shows the patterns generated by different “passwords” consisting of maltitol (1), D-xylose (2), and DC (3) as input signals. Pattern analysis of all 27 permutations (Figure S3) reveals that eight 3-digit passwords can be authorized by the unimolecular security system (Figure 6b). The feasibility of distinguishing 4-digit code entries, such as 1111, 2222, and 3333, was also demonstrated (Figure 6b). Because patterns generated from repeats of identical chemical inputs are unique, these 4-digit “passwords” should also be distinguishable from the 81 possible 4-digit combination codes (i.e.,  $3^4 = 81$ ).

In conclusion, combinatorial sensing by a pattern-generating molecule is demonstrated as a powerful technique for password protection at the molecular scale. With this approach, a unimolecular security system can be used to authorize multiple users without having to change the “lock” or the “keys”. Most importantly, each user can readily change his own password or “program” the system to authorize an entirely different set of passwords simply by altering the chemical inputs. The latter can be selected from a vast library of structurally similar saccharides that are transparent in the visible region and hence, their structure and concentration levels cannot be straightforwardly determined.

Bearing in mind that fluorescence signaling can provide the system with ultimate steganography,<sup>1</sup> breaking such locks becomes exceedingly difficult. Specifically, it requires prior knowledge of the most recent code entries, as well as access to a



**Figure 6.** (a) Fluorescence spectra of **1** upon the addition of maltitol (1), D-xylose (2), and DC (3) in different combinations. (b) The corresponding PCA plot. Excitation: 270 nm.

molecular-scale security device and to colorless and randomly selected chemical inputs. An additional layer of protection comes from the fact that the system utilizes both password and pattern recognition for user authentication. Thus, unlike electronic keypad locks or biometric locks that rely on a single defense mechanism, the molecular devices can ensure that even if the combination codes or the entry “keys” are exposed, the system remains secure.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

Experimental procedures and DFT calculations. 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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