



Rapidly optimizing an aptamer based BoNT sensor by feedback system control (FSC) scheme

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ABSTRACT

The sensitivity and detection time of an aptamer based biosensor for detecting botulinum neurotoxin (BoNT) depend upon the formation of proper tertiary architecture of aptamer, which closely correlates with the combinatorial effects of multiple types of ions and their concentrations presented in the buffer. Finding the optimal conditions for four different ions at 12 different concentrations, 20,736 possible combinations, by brute force is an extremely laborious and time-consuming task. Here, we introduce a feedback system control (FSC) scheme that can rapidly identify the best combination of components to form the optimal aptamer structure binding to a target molecule. In this study, rapid identification of optimized ionic combinations for electrochemical aptasensor of BoNT type A (BoNT/A) detection has been achieved. Only about 10 iterations with about 50 tests in each iteration are needed to identify the optimal ionic concentration out of the 20,736 possibilities. The most exciting finding was that a very short detection time and high sensitivity could be achieved with the optimized combinational ion buffer. Only a 5-min detection time, compared with hours or even days, was needed for aptamer-based BoNT/A detection with a limit of detection of 40 pg/ml. The methodologies described here can be applied to other multi-parameter chemical systems, which should significantly improve the rate of parameter optimization.

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1. Introduction

Optimization of multiple-components system is a general and important topic in parametric optimization during the biosensing/regulation process (Gouda et al., 2001; Qavi et al., 2009). Due to the complexity of many systems, this optimization process is labor-intensive and difficult to achieve by experiment alone. Increasingly, computer simulation along with experimental trials has been applied (Mehta et al., 1998; Todd, 2005).

Aptasensors have recently shown high efficiency in bio-sensing applications (Chiu and Huang, 2009; Jayasena, 1999; Liao and Cui, 2007; Lubin and Plaxco, 2010; Mairal et al., 2008; Wei and Ho, 2009; Willner and Zayats, 2007; Xiao et al., 2005). The bio-sensing capability of an aptamer depends on the folding process of the nucleic acid sequence and the docking process with its target proteins, which is very sensitive to environmental conditions and usually

results in multiple quasi-stable states. Changes in environment may cause the aptamer to fold into a conformation that is different from the pre-designed structure, consequently affecting the molecular recognition process. Parameters affecting both the folding and docking structure and time include temperature, ion concentrations, electrochemical/mechanical fields, and other parameters (Shim et al., 2009; Xiao et al., 2009). Among these factors, metal ions play an important role in aptamer folding and recognition (Buck et al., 2010; Shim et al., 2009; Wei and Ho, 2009). Several metal cations have been observed to either stabilize or mediate conformational change in aptamers (Draper, 2008; Draper et al., 2005; Noeske et al., 2007; Woodson, 2005). Alkali and earth alkali metals commonly exist in biological systems. The potassium ion has a diameter that fits well into the cavities between guanine tetrads and potassium ion concentration correlates highly with the folding and docking of aptamers (Radi and O'Sullivan, 2006). Calcium and magnesium are both important in enzymatically-induced protein–protein reactions. Sodium can regulate the ionic strength of an entire liquid system, which also contributes to aptamer folding and recognition. The synergistic interactions of the above four ions are important for the performance of aptasensors. However, previous research has been limited to the optimization of a single

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ion concentration at a time because the study of the combinational effects of all four ions at various concentrations results in a very large parameter space.

Botulinum neurotoxin (BoNT) is among the most toxic substances known (Kostrzewa and Segura-Aguilar, 2007). Rapid and sensitive detection of BoNT is highly desirable for addressing biosafety needs. Current methods to detect BoNT include mouse bioassay, enzyme-linked immunosorbent assay (ELISA), and aptasensors. Although the mouse bioassay and ELISA have excellent limit-of-detection (LOD) (2–60 pg/ml) (Sharma et al., 2006; Stanker et al., 2008), the time consuming (48 h and 3 h, respectively) and labor-intensive processes limit the application for rapid detection. The aptasensor, based on a high-affinity single stranded DNA aptamer, is facile and capable of detecting BoNT with a LOD of about 40 pg/ml in 24 h (Wei and Ho, 2009). Sensitivity and specificity of aptamer binding correlate highly with the combinational ion buffer (Wei and Ho, 2009), and a much shorter detection time could be obtained with an optimized buffer system (Lillehoj et al., 2010).

Identifying the optimal combination and concentration of the four ions mentioned above is important in controlling the biosensing process for a short detection time and high sensitivity. Since all the effects of the parameters are non-linearly correlated, optimization is not a straightforward process. In addition, N parameters with M different concentrations will result in M^N possibilities; if $N=4$ and $M=12$, the total number of cases would be 20,736. Testing all combinations becomes prohibitive due to intensive labor and time requirements. Hence, a rapid optimization method is necessary.

The feedback system control (FSC) technique has been developed to rapidly identify optimal combinations for therapeutic purposes (Sun et al., 2009; Tsutsui et al., 2011; Wong et al., 2008). In this work, FSC was applied for determining the best combination of ions and their concentrations for rapid detection at high aptamer sensitivity. Four different ions, Na^+ , K^+ , Ca^{2+} , and Mg^{2+} , with 12 different concentrations each, have been used to optimize aptamer-based BoNT detection. We have found that the convergence to optimal condition with high signal-to-background ratio (SBR) can be very fast. Only several iterations are needed to reach the optimal combination of ion concentrations. The detection time was only 5 min and sensitivity was 40 pg/ml. Extremely time-consuming titration tests of one or more chemicals commonly encountered in numerous chemistry analyses were avoided.

2. Materials and methods

2.1. Aptamer and BoNT/A toxoid

HPLC-purified aptamer oligonucleotides and 3'-labeled fluorescein were custom-synthesized (Operon Inc., Huntsville, AL, USA). The fluorescein label allowed for binding of anti-fluorescein-horseradish peroxidase (HRP) to amplify the electrochemical current signal.

The 76-bp aptamer sequence for BoNT/A detection was as follows (Tok and Fischer, 2008):

5'-ATACCAGCTTATTCAATT GAC ATG ACT GGG ATT TTT GGC GAA ATC GAA GGA AGC GGA GAGATAGTAAGTGCAATCT-3'.

BoNT/A toxoid (toxin inactivated by formaldehyde treatment) with a molecular weight of 150 kDa was purchased from Metabio-logs, Inc. (Madison, WI, USA).

2.2. FSC scheme

The FSC based search process started the first iteration with a set of several parallel tests. Each test has arbitrarily-decided concentrations of the ions. The aptamer reacted to the combination of ions and then conformed accordingly. Usually the system objective function of the aptasensor, such as SBR, does not meet the expectation in the first iteration, a search algorithm, the differential evolution (DE) algorithm in this paper, was used to suggest a new group of ion concentrations in the next iteration. This information was fed back to alter the aptamer conformation. The iterations continue until the system objective function achieved the desired state. With this approach, the laborious procedure of completely mapping out all the possible combinations was eliminated. FSC will “self-guide” and search for optimal objective functions in a small number of feedback iterations.

2.3. The differential evolution (DE) algorithm

The DE search algorithm, one of the genetic searching algorithms (Storn and Price, 1997) was used in this study and was coded by using MatLab (MathWorks, Natick, MA, USA) (Fig. 1a). Based on the ion concentrations of all the parallel tests in the previous iteration, the DE algorithm applies three procedures (mutation, crossover, and selection) to determine the new ion concentrations in each parallel test in the next iteration. The new ion concentrations are calculated by these three procedures, which will guide the system toward the objective function.

2.3.1. Mutation

Four combinations of the four chosen ions in the buffer are parallel tested to examine their effects on the aptamer folding. The four combinations are noted as c_i^G ($i = 1, 2, 3, 4$), where G refers to the G th iteration, and i refers to the i th parallel test in this iteration. The system objective function, I_i^G ($i = 1, 2, 3, 4$), is the output signal of the electrochemical currents, of each combination and of the blank control were measured. The mutation procedure will generate a new ion combinations, v_i^G , based on the ion concentrations, c_i^G ,

$$v_i^G = c_{r_1}^G + F \cdot (c_{r_2}^G - c_{r_3}^G) \quad (1)$$

$c_{r_1}^G, c_{r_2}^G, c_{r_3}^G$ are the three combinations other than i th combination in the G th iteration. Mutation factor (F) is one of the parameters determining the changes of ion concentrations from one iteration to the next. Two values of the mutation factor, $F=0.3$ and $F=0.5$, were investigated in this study.

2.3.2. Crossover

In order to increase the diversity of the combinations, crossover procedure is applied to generate another new ion combination, u_i^G , which is determined as follows,

$$u_i^G = \begin{cases} v_i^G & \text{if}(\text{randb} \leq \text{CR}) \\ c_i^G & \text{if}(\text{randb} > \text{CR}) \end{cases} \quad (2)$$

randb is a random number generator with the value between 0 and 1. CR is the crossover constant that was set as 0.5 in this research. randb is randomly chosen to ensure that the crossover gets at least one parameter from the mutation vector.

2.3.3. Selection

The last step of the DE algorithm, selection, is to make sure that the ion combinations, c_i^{G+1} , of $G+1$ st iteration have better or at least equal performances of the G th iteration. The system objective function $I_{i,u}^G$ ($i = 1, 2, 3, 4$), i.e. the sensor signal output, measured under the ion concentration of u_i^G is compared with I_i^G ($i =$

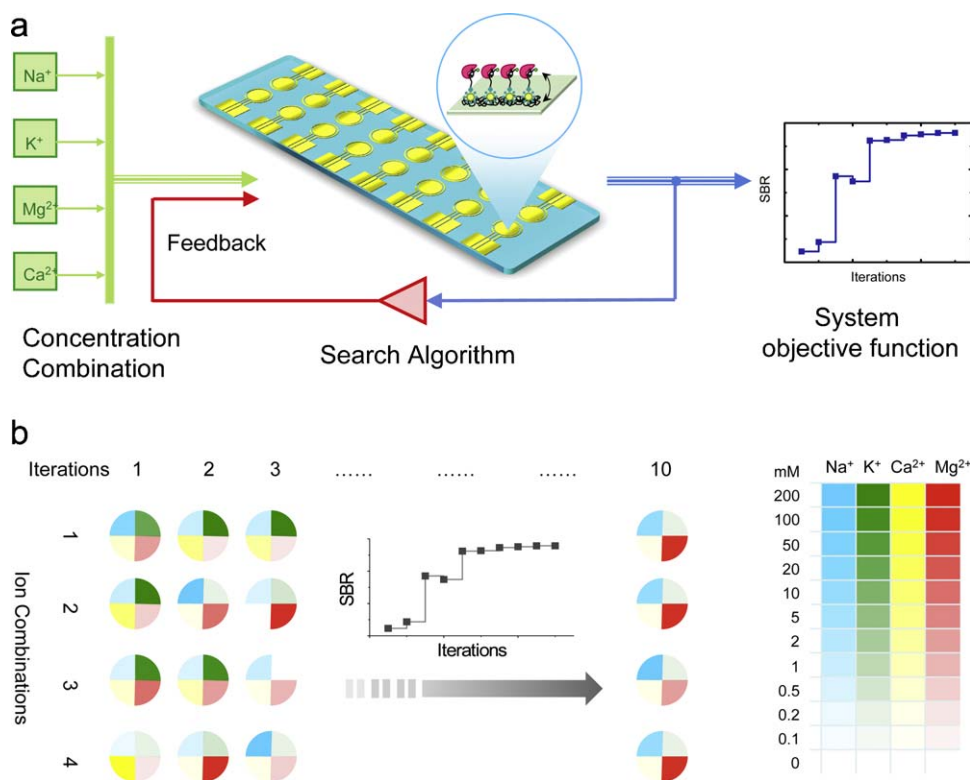


Fig. 1. Feedback system controlled optimization of the four-ion buffer for the BoNT/A aptasensor. (a) Illustration of the systematic optimization of the electrochemical aptasensor for BoNT/A based on closed-loop control. (b) Each combination is presented as a pie chart with four colors: blue, Na⁺; green, K⁺; yellow, Ca²⁺; red, Mg²⁺. The concentration scale bar is listed in the right panel. (For interpretation of the references to color in the figure caption, the reader is referred to the web version of the article.)

1, 2, 3, 4). If $I_{i,u}^G (i = 1, 2, 3, 4) > I_i^G (i = 1, 2, 3, 4)$, then c_i^{G+1} is set to u_i^G ; otherwise, the old value c_i^G is retained.

The above three procedures are repeated until there is no significant difference between $I_i^G (i = 1, 2, 3, 4)$ and $I_i^{G-1} (i = 1, 2, 3, 4)$, which means the system has reached the asymptotic state. The final combination c_i^G is the optimized combinations.

Two system-objective functions were used in this investigation. One is based on the SBR, signal/background, in BoNT recognition. The other objective function is the difference between the signal and background, $|\log_2 \text{Signal} - \log_2 \text{background}| = |\log_2 \text{SBR}|$, which was used to analyze the multiple possibilities for aptamer folding and recognition (Dudoit et al., 2002; Yang et al., 2002).

2.4. Combinational ion buffer

Four metal ions, Na⁺, K⁺, Ca²⁺, Mg²⁺, were chosen for the combined buffer. Concentrations higher than 200 mM ion are not suitable for biological process, so the 12 concentrations for each ion chosen for the search space were: 0 mM, 0.1 mM, 0.2 mM, 0.5 mM, 1 mM, 2 mM, 5 mM, 10 mM, 20 mM, 50 mM, 100 mM, and 200 mM. In the first iteration, four parallel test conditions of the four ions with randomly decided concentrations were used. After the first iteration, changes in the ion combinations were made based on suggested values of the DE search algorithm. The iteration continued until reaching the optimal aptamer conformation for satisfying the objective function of BoNT/A detection. The entire process was illustrated in Fig. 1b.

2.5. Electrochemical detection

The electrochemical detection of BoNT/A was based on the specific amplification via steric hindrance of aptamer. The details of the aptamer sensors are described in [supplementary data](#). For

the FSC process, four different combinations of ion buffers with 1 µg/ml BoNT/A toxoid in volumes of 50 µl were loaded onto the aptamer-coated electrode. In the concentration calibration, different concentrations of toxoid were prepared in the optimized ion buffers. The combinations of four ion buffers were based on the differential evolution selection, except the first run with random ion combinations. For each combination, both the electrochemical current from sample and blank control were measured in the array. The SBR is defined as the ratio between current of sample and current of the blank control. The electrical field was applied to achieve effective binding and folding of the aptamer with 20 cycles of 9 s at −300 mV and 1 s at +200 mV, followed by washing and drying. To generate amplification of signal specific for BoNT/A, HRP conjugated to anti-fluorescein antibody (Roche, Indianapolis, IN, USA) in casein/PBS buffer (50 µl) (Pierce, Rockford, IL, USA) was incubated with the electrodes for 5 min followed by washing and drying of the chip. All the ion combinations and the respective electrochemical aptasensor results were provided in [supplementary materials](#).

3. Results

3.1. FSC-based technique for optimizing the SBR

The combinational ion buffers for BoNT/A toxoid detection were optimized by the FSC scheme. Four parallel tests of ion combinations of Na⁺, K⁺, Ca²⁺, and Mg²⁺ were applied in each iteration for searching the optimal SBR, which was set as the objective function. Signal is generated by toxoid recognized by the aptamer structure, while background is derived from non-specific bindings, including those from non-optimal aptamer conformations. For the tests in the following iteration, DE algorithm in the feedback loop generated new combinational ions providing better recognition between the aptamer and the target BoNT/A toxoid to obtain higher SBR.

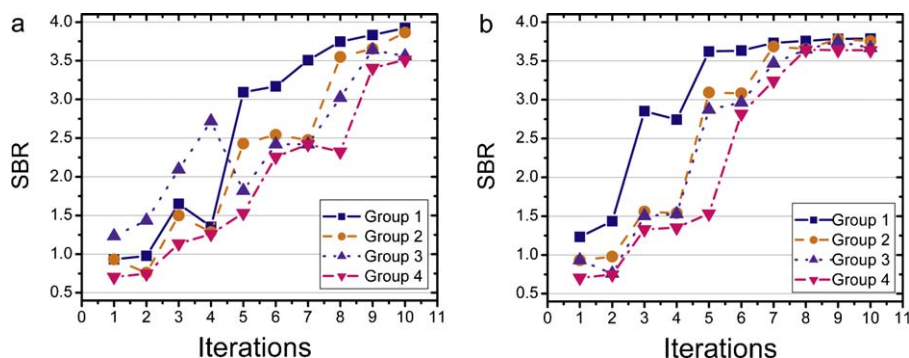


Fig. 2. Differential evolution based on SBR in feedback control for BoNT/A aptamer detection with (a) $F=0.3$ and (b) $F=0.5$. In each iteration, four groups of combinations were investigated.

In optimizing aptamer to meet better objective function, ion concentrations can affect the stability and characteristics of the aptamer under conformation changes. A high mutation constant, F , introduces more variation of the ion concentrations while low F retains less change of the ion concentrations from the last iteration (see [supplementary materials](#)). The effects of F values between 0 and 1 were investigated. Two F values, $F=0.3$ and $F=0.5$ were used. The results are illustrated in [Fig. 2](#) and the combinations with the best SBR in each iteration are listed in [Table 1S in the supplementary data](#). The first combinatorial buffer is generated randomly and then the differential evolution algorithm suggests the potential improved one for the next iteration. In the first iteration, the SBR is nearly one for all the combinations, which indicates the aptamer remains in the closed state without binding to the target toxoid. After several iterations, both groups converged to an asymptotic state, $SBR \approx 3.8$, within 10 iterations. The optimized SBR for both groups was approximately 3.8 with $1 \mu\text{g/ml}$ BoNT/A toxoid. Of the 20,736 possible combinations, we only tested $4 \times 12 = 48$ combinations and could identify the optimal combinatorial concentrations of four ions to reach a $SBR \approx 3.8$.

Both F groups show generally increasing trends along the SBR vs. iteration curves. With $F=0.5$, giving more differential variance of the ion concentrations from one iteration to the next, the curves approach the asymptotic value around iteration 7 or 8. With $F=0.3$, which allows less variance and more carry over from the previous iteration, the curves reach the asymptotic value almost at the last iteration. A fast approaching asymptotic value is preferred, especially for those systems that require a longer time to accomplish the objective of each iteration.

In the asymptotic state, a high concentration of calcium was needed, and both groups showed calcium optimized at 200 mM (iterations 8–10, [Table 1S](#)). However, the concentrations of the other three ions can have different values for optimal SBR. For example, one case has sodium concentration of approximately 20 mM and a very low potassium concentration of 0.5 mM. The other case has optimized SBR with a sodium concentration of approximately 2 mM and a low potassium concentration of 5 mM. The conformations of aptamer are results of non-linear interactions among four ions and the aptamer. In a non-linear system, there is a risk of no optimum. On the other hand, it is not surprising to see more than one optimal combination. The existence of a unique solution is expected only in a linear system.

3.2. FSC based scheme for selecting the large difference between signal and background

In the previous section, we presented the optimization process for selecting a high SBR, which indicates well-bonded aptamer

structures to BoNT/A toxoid. We examined another objective function, based on the difference between signal and background. In the FSC, the objective function depends on the goal for the optimization. As the biosensing driven goal, SBR is obviously a good criterion for the searching direction. On the other hand, in terms of understanding the mechanism of aptamer binding, the folding structure of the aptamer is important, too. Investigations have been carried out in order to identify whether the structure with the perfect binding to the BoNT is the most stable conformation. The hypothesis is that there may exist several folding conformations which are more stable than the one bind with the BoNT targets. Therefore we bring up a secondary objective function as $|\log_2(SBR)|$ to further understand the BoNT aptamer recognition. Since the value of F does not affect the search for optimal in this system as being indicated in [Fig. 2](#), only one value of mutation parameter, $F=0.5$, was used here.

From the results shown in [Fig. 3a](#) and [Table 2S](#), the selection based on $|\log_2(SBR)|$ showed similar trends as those in selections based on SBR. The first iteration with random four-ion concentration exhibited no obvious difference between signal and background, $|\log_2(SBR)| \approx 1$. After several iterations, all four combinations in each iteration reach the asymptotic value of $|\log_2(SBR)| \approx 0.6$. An interesting result was that, although the $|\log_2(SBR)|$ vs. iteration plot increased monotonically, the SBR vs. iteration plot showed different patterns. Two branches appear immediately after the very early iteration. One branch goes to the high SBR values near 1.6 and the other branch drops to the low SBR values around 0.6. Even for the high SBR branch, the optimized SBR is much lower than the $SBR \approx 3.8$ obtained for the optimizations based on SBR. The phenomena that searching based of the optimized $|\log_2(SBR)|$ ends up in a dual-stable state indicates that there are multiple quasi-stable intermediates during the reaction. Therefore, the endpoint of the whole optimization could be controlled by different objective function. To achieve the ultimate stable structures in the whole reaction system, large sampling size (the sample numbers of each iteration in this work) and small searching grid (more concentrations than the previous 12 concentrations in this work) may be implanted.

The final ion compositions in the selection based on $|\log_2(SBR)|$ are different from those based on the high SBR selection. All data are listed in [Table 2S in the supplementary data](#). Again, multiple optimal ion concentration combinations are observed as a result of nonlinear interactions among the four ions and aptamer. In most cases, a high Na^+ concentration helped to achieve high $|\log_2(SBR)|$ and SBR. High Mg^{2+} concentration (100 mM) resulted in low SBR and high $|\log_2(SBR)|$. The fourth case in iteration nine, was an exception, showing high Mg^{2+} concentration (100 mM) and high Na^+ concentration (200 mM) could reach high values for both SBR and $|\log_2(SBR)|$.

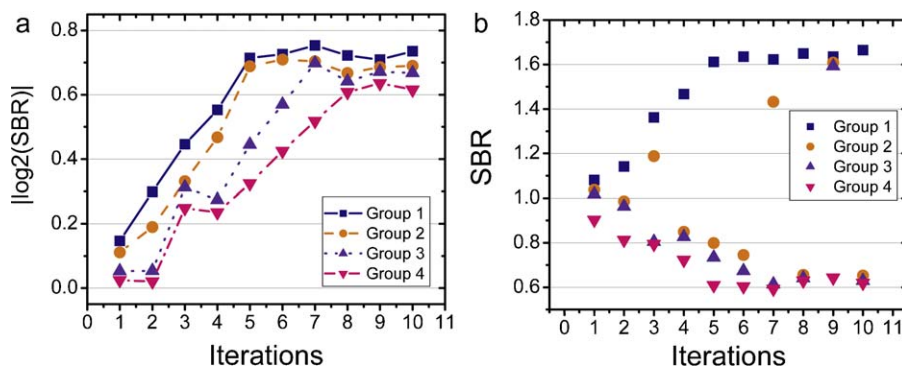


Fig. 3. Differential evolution based on $|\log_2(\text{SBR})|$ in feedback control for BoNT/A aptamer detection with $F=0.5$ in (a) $|\log_2(\text{SBR})|$ and (b) SBR representation. For each iteration, four groups of combinations were investigated.

3.3. Comparison of output sensitivity

From the three groups of FSC-based optimization for combinational ion buffers, the optimized combination was found to be (20, 0.5, 200, and 2) for 1 $\mu\text{g/ml}$ toxoid (Table 1S: $F=0.3$ iteration 10, $\text{SBR}=3.9$). Under these ion concentrations, the LOD of the aptamer sensor was studied. The random combinations, such as (20, 100, 0.2, 0.2, $\text{SBR}=1.2$), (50, 1, 100, 5, $\text{SBR}=0.9$), (200, 20, 10, 1, $\text{SBR}=0.7$) were selected as controls (Fig. 4). With the optimized combination, the LOD is around 40 pg/ml BoNT at a 2 standard deviation (SD) cutoff. The dynamic range covers from 1 $\mu\text{g/ml}$ to 40 pg/ml. Under the non-optimized conditions, there were no significant differences from 0 to 1 $\mu\text{g/ml}$ and the LOD were only approximately 1 $\mu\text{g/ml}$.

4. Discussion

An aptamer has a nucleic acid sequence that can perform both DNA and protein functions. The bi-functional character of an aptamer derives from the multiple folding conformations of the nucleic acid sequence, which is very sensitive to environmental conditions, such as buffer. The docking efficiency of folded aptamer determines the performance of an aptamer-based BoNT/A sensor. The folding process and the final configuration of the 3D aptamer structure have complex non-linear dependencies on the type and concentration of ions present in the buffer. Identifying the optimal aptamer structure, or the ion buffer leading toward the desired structure, is the key to obtaining a high performance

BoNT/A sensor. The challenge of finding the optimal buffer is that N ions, e.g., 4, with M concentrations, e.g., 12, such as in the current study, will have M^N combinations, or $12^4=20,736$ tests. This is also a typical problem in many chemistry studies involving titrations. In this study, we can identify the optimal ion combinations in only 50 tests with the help of FSC scheme.

4.1. Time of detection

When we started this work, the goal was to apply the FSC technique to rapidly explore the large parameter space to further improve the limit of detection (LOD), 40 pg/ml. Hence, SBR and $|\log_2(\text{SBR})|$ were used as the objective functions. The best LOD, 40 pg/ml, in the current four ion buffer was found to have the same value that we found in our previous study (Wei and Ho, 2009). According to the dissociation constant between the aptamer and the BoNT/A toxoid ($K_{d5}=0.003 \mu\text{M}$) (Tok and Fischer, 2008), the sensitivity of 40 pg/ml is reached for this aptamer. To further improve the sensitivity, new aptamer with better affinity to the toxoid is needed. The surprising result was that the detection time was only 5 min and not 24 h with the optimized combinational ion buffers; the reason may derive from the thermodynamics of the recognition process based on the folding process (Buck et al., 2010; Draper, 2008). The optimized combinational ions efficiently guided the folding process of BoNT aptamer toward the best recognition structure. Therefore, the recognition process was completed very quickly.

4.2. Complex interplay among ions in buffer

The efficiency of target recognition depends on many factors related to aptamer folding, such as the correlation between the ion size and the aptamer-folding cavity. In addition, the dielectric property and hydrated radius of a single ion are also important (Noeske et al., 2007). Therefore, it is extremely complicated to decode the effects of varying concentration of a single ion in buffer containing multiple ions. Because it is prohibitive to test a very large number of possibilities involving multi-ion combinations, conventional studies of aptamer-based detection usually only examine the effects of concentration variation of a single ion. Studies based on varying a single ion will not be able to sort out the intricate non-linear synergistic or anti-synergistic interaction effects of multiple ions.

To achieve high SBR, high Ca^{2+} concentration and relative low concentrations of other three ions were needed for both group 1 ($F=0.3$) and group 2 ($F=0.5$) tests, Table 1S, for inducing the optimal folding and docking of aptamer with the BoNT/A toxoid. Therefore, Ca^{2+} is an important ion for correct folding and docking of aptamer with the BoNT/A toxoid. The concentrations of Na^+ , K^+ and

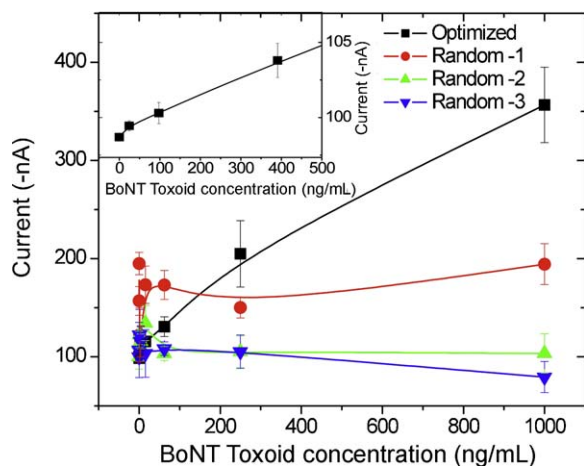


Fig. 4. Concentration profile of aptamer-based BoNT/A detection with optimized four-ion buffer combination and three random combinations. For each concentration, three experiments were carried out. The inset illustrated the signals at low concentration range.

Mg²⁺ were from 0.2 to 20 mM, and higher concentrations of these ions decreased the SBR. The three ions other than Ca²⁺ are needed to provide optimal aptamer folding. However, higher than needed concentrations may lead to an unfavorable folding conformation, which is not suitable for targeting the toxoid.

The group 3 tests (Fig. 3 and Table 2S) indicate that a high concentration of Mg²⁺ is needed, in general, for achieving the objective of high $|\log_2(\text{SBR})|$. A high concentration of Na⁺ is helpful for obtaining the higher SBR, 1.6, in group 3. This result suggests that this combination has the effect of closing the aptamer tightly when no target is present and opening the structure in the presence of the target toxoid. In some cases, increasing the concentration of certain ions will result in higher SBR. However, in other combinations, the results could be lower or no change in SBR. Several examples are shown here. The Ca²⁺ concentration in the combination (50, 0.1, 0.2, 100), [SBR = 0.7, Table 2S, iteration 10], is lower than in the combination (50, 0.1, 0.5, 100), [SBR = 0.6, Table 2S, iteration 10], but the SBR has only a difference of 0.1. The lower Ca²⁺ calcium concentration in combination (200, 0.2, 0.1, 20), [SBR = 1.4, Table 2S, iteration 7], than that in combination (200, 0.2, 1, 20), [SBR = 0.7, Table 2S, iteration 6], can result in a much higher SBR. The non-linear interactions among the ions and the aptamer impose extremely complex functional dependencies upon the desired objective functions.

5. Conclusion

The performance of an aptamer-based BoNT/A toxoid sensor is sensitive to the combinatorial concentration of multiple ions, which determine the folding structure and binding of aptamer to the toxoid. In this work, four ions, Na⁺, K⁺, Ca²⁺, and Mg²⁺. With approximately 10 iterations and a total of 50 test conditions, the FSC method can efficiently identify the best ion concentration combinations for satisfying the system objective function, SBR, or $|\log_2(\text{SBR})|$. The most exciting finding was that a very short detection time and high sensitivity could be achieved with the optimized combinational ion buffer. Only a 5-min detection time, compared with hours or even days, was needed for aptamer-based BoNT/A detection with a limit of detection of 40 pg/ml.

Traditional experimental design techniques first survey the system response in a coarse grid and then homes in a narrower region to find the optimal conditions for satisfying the objective function. On the contrary, FSC scheme starts at one or several parallel initial test conditions (combinatorial ion concentrations) and searches for the path toward the optimal condition. Each time, FSC does not need to test a large number of conditions in the survey grid as in neuralnet or experimental design techniques and therefore can accomplish the task in much less amount of time and labor. In principle, the significant time and labor saving provided by the FSC technique can be applied to a general class of experiments involving large numbers of titrations (Tsutsui et al., 2010; Wong et al., 2008). It eliminates the need for individual optimization of each parameter. Instead, it directly provides the optimized combination of all parameters. FSC offers an innovative method for the study of complex chemical and biological systems with a large parameter space.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.09.014.

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