Multi-plex biomolecular detection using Bio-nanotransduction

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ABSTRACT

Bio-nanotransduction is a biological detection scheme that is based on biological recognition and the production of biological nano-signals (Bio) which allows the change of recognition events into universal signal molecules (nanotransduction). A single sample can be converted into a pool of nano-signals specific for each target. Detection and differentiation of the nano-signals are correlated to the presence of targets in the original sample. It is possible to link various biological recognition events to platforms (such as biosensors) capable of detecting nano-signals. This allows the application of new target recognition while using a universal platform previously optimized for nano-signal detection. In this paper we explored the utility of the Bio-nanotransduction for the detection of biomolecular targets in a single sample using three detection platforms for nano-signal profile recording. We also demonstrated the flexibility of the system adapting to the detection of new targets.

Keywords: multi-plex assay, biosensors

1 INTRODUCTION

Specific and sensitive detection of target analytes is crucial to many areas of the applied biosciences[1, 2]. Biosensor research is a key component in this process. Biosensor technologies allow biological detection to move from the laboratory and into the field. The development of low-cost, easy to use, portable and accurate detection platforms will continue to change the way in which detection information is collected, disseminated and applied. Nanotechnology and nanoscience provide the building blocks on which new biosensor technologies can be developed. Nano based biosensors have been shown to increase sensitivity[3, 4] and decrease assay times[5] as compared to traditional approaches. However, the ultimate utility of emerging technologies will be judged by the time frame and effectiveness of moving these biosensors into the applications for which they are intended. In order for new sensors to be applied to current and emerging targets in a timely fashion, novel paradigms, effective collaborations and systematic approaches are required.

An emerging concept in the development of new biological detection schemes is the linkage of specific biological recognition molecules with nucleic acid probes[3], primers[6] or templates[7]. Standard molecular biology techniques can then be used produce a specific nucleic acid signal related to the original recognition event. An advantage to these techniques is that nucleic acid manipulation and detection are well understood, applicable to nanotechnology and can be used to transform disparate recognition molecules into a single and more uniform biological molecule.

We use the term Bio-nanotransduction to describe the concept above as it applies to nanotechnology and biosensor development. Bio-nanotransduction is a name that helps describe our research orientation and provides a term that can be conceptually understood by multiple disciplines. In general, a biological input signal (specific biological recognition) is transformed into a biological output nano-signal (specific nucleic acid). The biological output (nano-signal) is so named because it is a nanometer sized molecule that can be designed and manipulated to function as an intimate part of a nanotechnology based biosensor.

To hone Bio-nanotransduction as a tool that can be used to effectively tie new technologies with meaningful biological detection, we have selected a T7 polymerase (enzymatic RNA production from a DNA template) based approach. In brief, biological recognition elements (BREs) are linked to DNA templates capable of producing RNA nano-signals through an enzymatic reaction. A pool of nano-signals can be produced from a single sample of targets through the addition of several BREs each linked to a DNA template coding for a specific RNA signal. A detection platform is used to record a nano-signal profile and is correlated back to the presence of target in the original sample. Essentially, molecular linkages and biological machinery are used to transform disparate interactions into a universal biological nano-signal. As a result, the requirements of the biosensor platform are limited to the detection and differentiation of RNA sequences. At the same time, specific characteristics of RNA molecules including hybridization[8], charge detection[9], peptide/ protein interactions[10], and novel nanotechnology interfaces such as molecular electronics[11, 12] can be used to improve biosensor characteristics. Additionally, molecular linkages between BREs and DNA templates can be exchanged for different targets and target types in a straightforward manner.

In this paper we demonstrate the flexibility of the Bio-nanotransduction system and potential applications to biosensor development. Initially, we show that three different detection platforms can be used to develop a nano-signal profile and the data can be used to determine the presence of targets in the original sample. Secondly, we show that new targets can be detected by changing

BREs without requiring changes in the detection platforms.

2 RESULTS and DISCUSSION

2.1 Bio-nanotransduction Model System

The general detection approach used in this paper is based on immuno-detection amplified by T7 RNA polymerase (IDAT)[7]. However, our system has been developed to move the ideas set forth in the original work forward to biosensor applications including both multi-target antibody and non-antibody based detection. The current model system we have developed to explore the utility of Bio-nanotransduction is shown in Figure 1. The system consists of three steps: 1. Target capture/ Biological Recognition, 2. Nano-signal Production, and 3. Nano-signal Detection.

The DNA templates used in this work were carefully designed to produce RNA nano-signals that could be easily detected and differentiated under standard hybridization conditions at room temperature. Table 1 shows the four nano-signal sequences. Each nano-signal consists of a conserved 5' region (italics) and a variable 3' region (bold). The conserved region was used to recognize a biotinylated DNA probe for detection while the variable region was used to direct the nano-signal to its specific physical location.

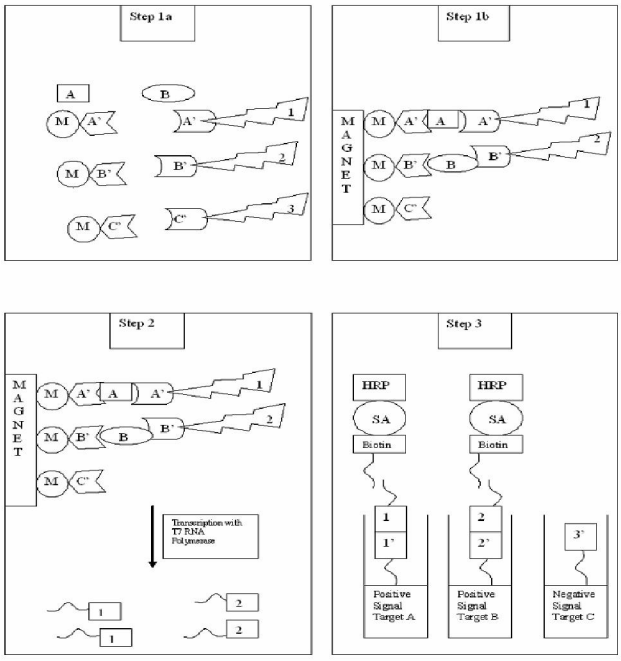

Figure 1: The three steps of Bio-nanotransduction.

Table 1 RNA NANO-SIGNALS

Signal 1
5'*GGGAACACUUUACUU***AUUCUAUUCUAUUCUAUUCUAUUCUA** 3'

Signal 2
5'*GGGAACACUUUACUU***UACUUUACUUUACUUUACUUUACUUU** 3'

Signal 3
5'*GGGAACACUUUACUU***AUCUUAUCUUAUCUUAUCUUAUCUUA** 3'

Signal 4
5'*GGGAACACUUUACUU***CAUUUUCAUUUUCAUUUUCAUUUUCA** 3'

In order to anticipate the use of other recognition molecules in the future we have also included an RNA/ peptide and RNA/ DNA detection construct in our initial studies. A single RNA molecule was designed to represent both interactions. The 5' end of the molecule contained a sequence known to interact with a specific peptide (P22 anti-termination complex), while the 3' end of the RNA was designed to hybridize to a synthesized DNA probe sequence.

2.2 Multi-analyte Detection on Fluorescent and Luminescent Platforms

Three biological targets were used in the initial studies. The targets included two protein targets (Chicken IgG and Mouse IgG) and the RNA construct described above. Goat anti-species specific IgG polyclonal antibodies were used for both linkage to magnetic beads and conjugated to theDNA template for the protein targets. RNA construct detection was accomplished by functionalizing magnetic beads with the P22 N21 peptide and designing a DNA template with a single stranded overhang specific for the RNA sequence. Each target was represented by a specific nano-signal sequence (Chicken IgG= signal 1, Mouse IgG= signal 2, RNA construct= signal 3). The fourth nano-signal in all cases represents a fixed amount of DNA template 4 which was attached to magnetic beads.

Each assay was carried out by mixing a set amount of magnetic beads in a fixed sample volume containing variable amounts of biological targets. Following magnetic bead capture and a brief rinse, biological recognition element/ DNA template conjugates were allowed to react with the sample. Magnetic beads were again collected, exhaustively washed and transferred into a T7 *in vitro* transcription reaction. The reaction was allowed to proceed for a previously determined optimum time (10 minutes) and then transferred into a hybridization solution containing a biotinylated detector probe. For each sample, the 400 ul hybridization mix was split evenly across 4 wells of a 96 well plate. Each of the 4 wells was modified to contain a complementary DNA probe to one of the 4 nano-signals. The sample profile of the 4 nano-signals was ultimately developed using streptavidin horseradish peroxidase (fluorescent) or streptavidin alkaline phosphatase (luminescent), the appropriate substrate buffer and read using a plate reader.

Table 2 reports the average nano-signal intensity plus or minus the standard error of the mean using fluorescent and luminescent detection platforms. Specific nano-signal results when target was in the test sample were recorded and averaged as signal intensity while specific nano-signal results in the absence of target were recorded as background intensity. The results reflect the ability to specifically detect all three targets at the levels used in the assay. Interestingly, although two different enzymes and two different detection platforms were used, the results were very similar when signal to background on the two platforms is compared. This lends credence to the fact that it is possible to interchange the ultimate detection event as needed without significantly affecting detection results. At the same time, it seems likely that optimization of the system may be required to take advantage of a particular detection platform.

Table 2

		Signal Intensity (RFU or RLU)	Background Intensity (RFU or RLU)
Chicken IgG (nano-signal 1)	Fluorescent	16500±1500	4330±503
	Luminescent	970±25	300±20
Mouse IgG (nano-signal 2)	Fluorescent	9250±720	2380±365
	Luminescent	700±23	245±10
RNA Construct (nano-signal 3)	Fluorescent	28000±3700	3125±325
	Luminescent	1420±40	230±12

2.3 Multi-analyte Detection on Electrochemical Biosensor Platform

In order to address the application of Bio-nanotransduction to biosensor development we designed a gold electrode array for use with an electrochemical ELONA. The electrode array was built with 9 gold 1mm diameter electrodes integrated into a PEEK block. The electrodes were individually addressable from the back side of the array. Removable PEEK containers were fabricated to connect to the array to hold solutions. The PEEK containers were removable to allow for cleaning and polishing of the electrode surfaces. Current signals from 8 electrodes were simultaneously read using a Palm Sense Electrochemical analyzer with an 8 channel multiplexer attachment. For these experiments, a thiol terminated DNA probe complementary to 1 of the 4 nano-signals was bound to a single electrode. Therefore, each array had an electrode representing each of the 4 nano-signals and 4 electrodes with no DNA modification. After initial modifications, the whole array was blocked for non-specific binding using mercaptohexanol followed by a bovine serum albumin solution.

The same targets and protocol were followed as in subsection 2.2 with the following change. Instead of splitting the hybridization solution, the entire 400 ul was placed on the array. Nano-signals were hybridized to the array, washed and then mixed with streptavidin horseradish peroxidase. The nano-signal profile was recorded using a pre-made TMB peroxidase substrate buffer and chronoamperometric detection protocol. An external platinum electrode was used to set the voltage (-0.350 V vs. platinum) of the solution and all measurements were made for 60 seconds. The current at 60 seconds was used for nano-signal profile determination in all experiments.

Results for the multi-plex detection are shown in figure 2. Current signals were subtracted from the averaged background on an individual array and then individual nano-signal results were averaged across arrays. It is clear that individual samples can be interrogated for the presence of the three target molecules and successfully detected using this Bio-nanotransduction approach in combination with an electrochemical biosensor. One limitation to the biosensor compared to the 96 well plate assays is the increase in the standard error of the mean for averaged measurements across arrays. The subtraction of the background signal from individual arrays improved the results slightly, but we are continuing to search for physical and statistical methods for normalizing nano-signal profiles for multiple arrays treated under the same conditions. Even with the need for refinement, there is a definite indication that this approach shows promise for use with biosensors.

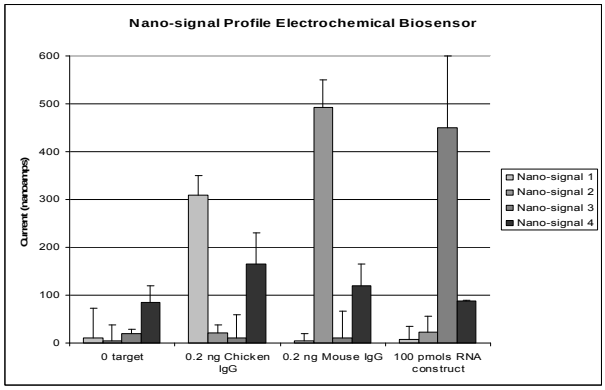

Figure 2: Nano-signal profile recorded on an electrochemical array biosensor. Nano-signal intensity is recorded as the current reading at 60 seconds subtracted from background and averaged across arrays. Error bars represent ± S.E.M..

2.4 Application of Bio-nanotransduction to New Targets

The initial sections of this paper set the general framework for the use of Bio-nanotransduction for biological detection. This final section demonstrates flexibility of the scheme for applications to new targets.

For this testing we chose to continue using the RNA construct as one target molecule. However, for the other two targets we selected two different strategies. First we chose to detect *Salmonella* serovar Typhimurium using a direct antibody approach. Goat polyclonal antibodies specific for *Salmonella* were used for both capture and recognition. Next, we selected *E. coli* O157. In this case a polyclonal goat anti-*E. coli* O157 antibody was used for capture while an unlabeled mouse monoclonal anti- *E. coli* O157 antibody was used for pathogen recognition. Finally, a goat polyclonal anti-mouse IgG conjugated to a DNA template was used for nano-signal production. Nano-signals 1, 2, 3 and 4 correspond to E. coli, Salmonella, RNA construct, and a fixed amount of DNA template 4 attached to magnetic beads respectively.

Target levels were fixed at 10^6 cells/ml of heat killed cells (*Salmonella* Typhimurium and *E. coli* O157) and 100 picomoles RNA construct. Samples were mixed with capture magnetic beads for each target along with the anti-*E. coli* monoclonal antibody. Beads were then collected, washed and placed in a 100ul solution containing the biological recognition element/ DNA template specific for each target. Nano-signal profiles for each sample were recorded using the electrochemical biosensor as described in subsection 2.3. The results for this set of experiments are summarized in figure 3. Nano-signal profiles as reported in current measurements from the samples reflect an increase in specific nano-signal in relationship to the presence of the target in the original sample. These results are very encouraging as this reports our first attempt at the detection of target pathogens using our biosensor design.

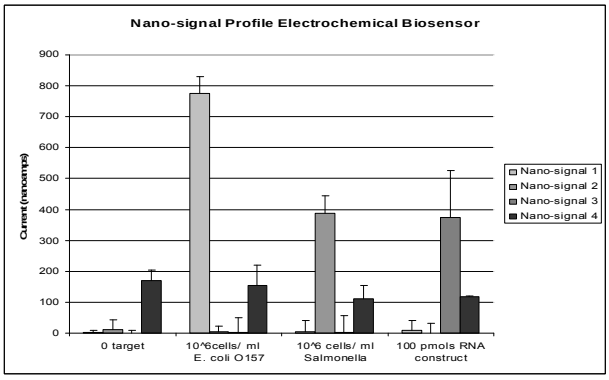

Figure 3: Nano-signal profile recorded on an electrochemical array biosensor. Nano-signal intensity is recorded as the current reading at 60 seconds subtracted from background and averaged across arrays. Error bars represent ±S.E.M.

3 CONCLUSIONS

Bio-nanotransduction provides a tool that can be used for biosensor development through nano-technology. Additionally, the conceptual framework of Bio-nanotransduction illuminates a path through rigorous multi-disciplinary demands of biosensor technology design. This paper provides a cursory examination of the principles and how they might be applied to meaningful detection of target biomolecules on biosensor platforms. Mirkin[3] and co-workers have set the ground work for applications of DNA signal linked biological recognition. We believe our adaptable approach and selection of a tunable nano-signal (RNA) will provide additional insights and technologies to biological detection especially at the nano-scale.

4 AKNOWLEDGEMENTS

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