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TECHNICAL NOTE

Base Pair Biotechnologies Aptamers Combined with Vista Therapeutic's NanoBioSensors™ for Biomarker Measurement in Human Serum

INTRODUCTION

Aptamer development has historically been limited to one-target at a time. **Base Pair Biotechnologies** has addressed this severe limitation by successfully multiplexing the conventional aptamer selection process. Our platform technology is a patented approach to aptamer discovery that allows us to offer de novo aptamer discovery services at unprecedented speed and throughput. Our expertise in aptamer and related assay development allows us to support our customers in a wide range of novel applications.

Vista Therapeutics, Inc. and Base Pair Biotechnologies, Inc. are collaborating to create a powerful collection of Nanowire-Aptamer probes whose sensitivity and ease of use is currently unmatched. Using Vista's proprietary 'Universal Linker' system, aptamers can be readily attached to Vista's nanowires without modification. Vista has demonstrated that Base Pair's aptamers can be used quite successfully with nanowires as probes for protein analytes even in human blood serum. Because aptamers are small, have a single attachment site, and because they are linearized prior to covalently attaching them, they coat the nanowire surface much more thoroughly than antibodies. This increases signal strength and greatly improves the signal:noise ratio. In addition, aptamer-coated nanowires can be dried and rehydrated many times without loss of signal. And since aptamers are simply DNA strands, Vista and Base Pair can easily create Nanowire-Aptamer probes to DNA, mRNA, microRNA. Using Nanowire-Aptamer probes, the end user can measure combinations of transcripts, proteins, microRNA's and DNA sequences in the same reaction. We present specific detection of Heat Shock Protein 27 (HSP27) and fibronectin in PBS and blood serum by aptamers selected by Base Pair and immobilized on Vista nanowires.

***Figure 1**: Vista Therapeutics Inc. NanoBioSensors™ instruments are based on detection of analytes via nanowires, microscopic wires whose conductance varies (with great sensitivity) as the concentration of target molecules passing over the wires changes. Consumable products to date include NanoCards, Functionalization Kits, and Sample Preparation Kits.*

VISTA

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BACKGROUND

Aptamer selection

Aptamers are single-stranded DNA or RNA oligonucleotides selected to have unique three dimensional folding structure for binding to a variety of targets such as proteins, peptides, and even small molecules with affinity and specificity rivaling that of antibodies. They are typically selected in vitro by a process commonly referred to as "SELEX" [1,2] as depicted in ***Figure 2***. Using a proprietary variant of this process, Base Pair Biotechnologies is developing aptamers to multiple targets simultaneously.

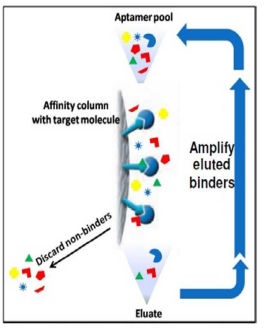

***Figure 2**: Overview of SELEX for Production of DNA Aptamers. A randomized library, flanked by two constant regions for PCR priming is constructed. The library is allowed to bind with the target and partitioned from the non-binding population. Following repeated rounds of selection and enrichment, high affinity DNA ligands are cloned and sequenced.*

Aptamer target for this study

HSP27 is a small heat shock protein that is regulated both transcriptionally and posttranslationally and modulates actin polymerization and reorganization. Its expression level increases several-fold in response to stress and is phosphorylated by MAPKAP kinase 2.[3] Fibronectin is a large, cell surface and plasma protein that exhibits structural and adhesive properties in cell-associated fibrillar matrices. Fibronectin is one of the primary cell adhesion molecules. [4] Following several rounds of aptamer selection, aptamer clones to both proteins were evaluated as described below.

Vista Therapeutics Inc., NanoBiosensor™

The technology underlying Vista's NanoBioSensor involves microscopic nanowires embedded into a "chip" about an inch square. The chip is then functionalized by attaching "capture" molecules to the various wires within the chip. The chip is placed in the NanoBioSensor reader unit and one injects a solution containing "target" molecules across the chip. When the target molecules flow over nanowires coated with their complementary molecule, they bind to the wire, changing the conductivity of the wire. Changes in conductivity induce more or less electrical current to flow through the wire (in correlation with the concentration of the particular target molecule within the fluid). The amount of current flowing through each wire is measured in real time and displayed on a PC attached to the NanoBioSensor. It should be noted that a typical molecular binding only lasts about seven seconds, but as more target molecules float by, they replace the previous ones, maintaining the same electrical flow through the wire.

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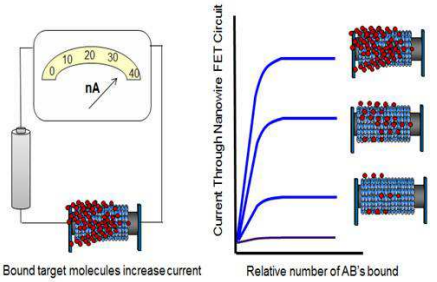
BACKGROUND CONTINUED

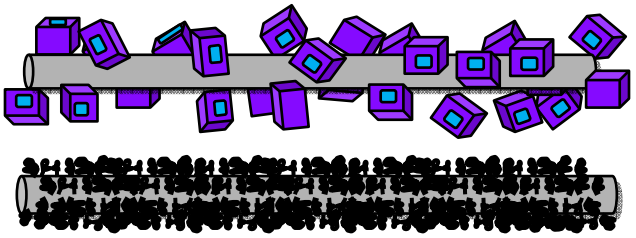
The only factor that changes the current and its subsequent measurement and display would be a change in concentration of the target molecule within the incoming solution. Thus, real-time monitoring is possible, given a fluid containing the molecule whose concentration one is interested in measuring.

Figure 3: *Transconductance is proportional to target molecule concentration.*



The smaller size advantage of aptamers over other binding molecules such as antibodies improves nanowire coverage reducing access to non specific targets and presenting a uniform distance to the target. Aptamers are also immobilized through a single binding site where antibodies utilize multiple lysines creating a more random binding surface.

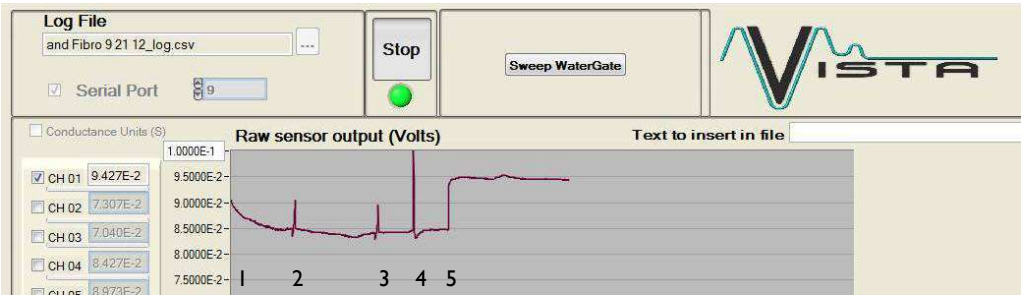
Figure 4: *Schematic comparison of nanowires immobilized with antibodies (A) and aptamers. The compact binding f the aptamers conveys maultiple advantages in detecting binding.*



RESULTS

Specificity for HSP27 by functionalized anti HSP27 aptamers and not for fibronectin is shown in ***Figure 5***:. At time point 1 the aptamers are being rehydrated with 0.01X PBS pH 7.6, both HSP27 and fibronectin aptamers had been dried and rehydrated multiple times during these experiments. Point 2 and point 3 are applied voltage controls. Point 4 is addition of 0.6pg/ml of fibronectin, note the voltage spike but no sustained binding. Point 5 is where 1pg/ml HSP27 is applied and does register a sustained voltage change.

Figure 5*: HSP27 specific response in Nanowire coated with anti-HSP27 aptamer. NanoCard has been dried and re-hydrated four times.*



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RESULTS

Specificity for fibronectin by functionalized anti fibronectin aptamers and not for HSP27is shown in ***Figure 6***:. At time point 1 the aptamers are being rehydrated with 0.01X PBS pH 6.6. Point 2 is an applied voltage control. Point 3 is switch to 0.01X PBS pH 7.6 which lowers transductance. Point 4 is addition of 0.6pg/ml of fibronectin, note the sustained binding. Point 5 is addition of 1pg/ml HSP27 and there is no sustained voltage change.

***Figure 6**: Fibronectin specific response in Nanowire coated with anti-fibronectin aptamer. NanoCard has been dried and re-hydrated four times.*

Fibronectin is a highly abundant protein in all human serum. We diluted human blood serum in 0.1x DPBS (no Ca or Mg). In order not to saturate the capture aptamers so that the final fibronectin concentration was approximately 10 ng/ml. Point 1 in ***Figure 7*** is 0.1x DPBS control added, 0.01% BSA was added at point 2. Point 3 is where the diluted serum was applied and a sustained charge was detected. We next increased the concentration to approximately 16 ng/ml of purified fibronectin. This increase in concentration corresponds to an increase in voltage change at point 4. Point 5 is a return to 0.1x DPBS

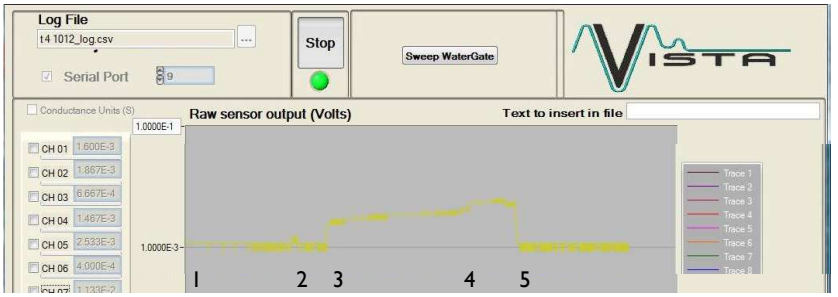

***Figure 7**: Fibronectin specific response in Nanowire coated with anti-fibronectin aptamer in Human Serum and DPBS.*

CONCLUSION

Preliminary experiments with Vista Therapeutics NanoBiosensors with Base Pair Biotechnologies custom selected aptamers show sensitivity and specificity for low concentrations of their respective proteins HSP27 and fibronectin even in human sera. The aptamers were also subjected to multiple rehydration cycles with no affect on response, data not shown. These results show the feasibility of aptamers as detection reagents in the NanoBiosensor system.

References:
1. Tuerk C, Gold L: Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science 1990,249:505-10.
2. Ellington AD, Szostak JW: In vitro selection of RNA molecules that bind specific ligands. Nature 1990, 346:818-22.
3. http://www.phosphosite.org/proteinAction.do?id=989
4. http://products.invitrogen.com/ivgn/product/33010018

Information on Vista Therapeutics Inc, products and services can be found at http://www.vistatherapeutics.org/index.html or contact by email at **info@vistatherapeutics.org** or 505-474-3143