The Awesome Power of Small

Vista Therapeutics, Inc. now offers a fully integrated NanoBioSensor™ platform for the rapid, ultrasensitive detection and measurement of low-abundance biomarkers directly from bodily fluids, tissue cultures, environmental sources and many other fluids. The NanoBioSensor™ System ('NBS') uses SiO nanoscale semiconductor sensors to quantify biomarkers on a label-free basis. Furthermore, it does so continuously on a real-time basis. The NBS provides detailed information about bi-molecular binding kinetics and thermodynamics. Vista's NBS monitors temperatures of binding reactions and can optionally manipulate temperatures for label-free, real-time PCR. It also provides water-gate and back-gate normalization tools. Options include built-in pump(s), gated valves, temperature monitoring and control, all of which are controlled by Vista's software. The NBS System empowers scientists from any field to use the tools of nanotechnology to address biological questions.

The need for more versatile bioassays continues to grow unabated. Label free assays that provide real-time, continuous multiplex results over several logs and several months, detect single molecules, provide kinetic and thermodynamic data, allow automated binder molecule segregation and permit repeated use would be ideal. If such a platform would perform all of the above and could perform label-free PCR, were inexpensive and easy to use this would represent a powerful biosensing platform indeed. Vista has developed NanoBioSensor™ technology that performs all of the above. The NanoBioSensor (NBS) uses capture molecules specific for a given biomarker to coat the surface of semi-conducting nanoscale constructs that serve as field-effect transistors (FETs). These constructs control the conductance of highly amplified electric currents where that conductance is directly proportional with the target molecule concentration…at every moment, as illustrated in **Fig. 1**.

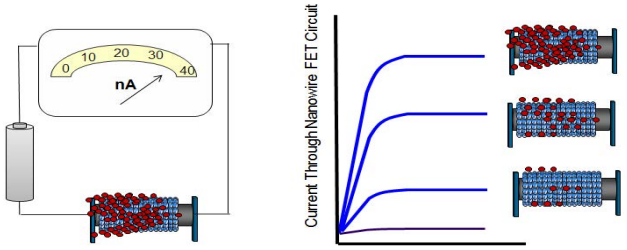

Figure 1. Left illustration shows nanowire coated with capture molecule (blue) binding target protein (red) results in an increase in current flowing through the sensor circuit. Right illustration shows that the amount of current measured is dependent on the amount of target protein bound at any given instant.

Vista has developed the NBS as a complete platform that provides all components required by the user to coat nanoscale constructs within re-usable 'NanoCards™' with capture molecules of their choice for detection, amplification or segregation of a target biomarkers (**Fig.2)**. Capture molecules can be receptors, antibodies, antibody fragments, enzymes, DNA or PNA. Conversely, the target biomarker can be tethered to the nanowire to detect and segregate capture molecules with desired binding properties such as desired on-rate, off-rate and K_d. The system has a software-controlled reversible micro-pump and specialized NanoCards that are temperature controlled for thermodynamic studies and for label-free, real-time PCR analysis.

Figure 2. NanoBioSensor System includes all of the components needed for detecting biomarkers.

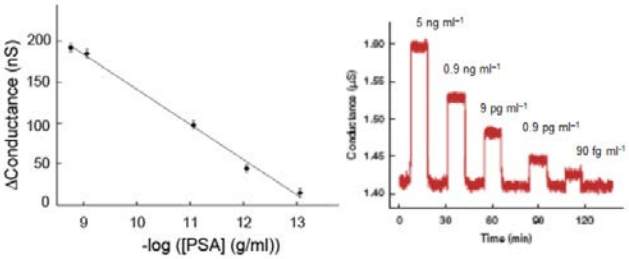

Figure 3. Binding cycle measured using the NanoBioSensor platform for the detection of prostate specific antigen (PSA). The signal is linear for up to five logs in concentration. The system uses small sample volumes and the sample can be recovered unchanged. (Data courtesy of Charles Lieber of Harvard).

Additional features include functionalization kits for coating all nanostructures with capture/target molecules, front- or back-gate sweeps for normalization and temperature control for hypersensitive, label-free PCR. The gates are designed to be equidistant from the nanosensors so there is no correction needed for distance between gates and sensors. The NBS system includes software that runs every function, and collects and analyzes data in numerous ways. For instance, the software will build standard curves, determine ΔG, on- and off-rates, calculates dissociation constants under

different conditions and, monitor concentrations over days or weeks. The system can also monitor cell motility. Additional options include the ability to adjust the signal frequency and filters to avoid EMI. The system also allows one to adjust the frequency with which samples are measured from 1 Hz to hours. The 'Dip-Chip™'option allows placement of nanosensors into culture wells, toilets or water streams and measure or monitor multiple biomarkers at a distance of up to a meter away from the NBS unit itself. The combined technology can detect the presence of biomarker(s) directly from 5 µl of blood in less than 5 minutes over a 4-5 log range with sensitivities frequently extending into the mid-femtogram ml^{-1} range. Single viral or yeast particles can easily be detected.

Example applications of the technology include:

- Optimized capture molecule selection
- Label-free detection and sample recovery
- Kinetic and thermodynamic analysis
- Development of specific Point-of-Care tests
- Optimized nanowire development & testing
- Label-free, sensitive real-time PCR
- Continuous culture & bioreactor monitoring
- Environmental (air and water) monitoring
- microRNA analysis
- Catabolic pathway analysis
- Automated toilet waste analysis
- Real-Time cell motility
- Membrane potential

Collaboration Options.

Vista is amenable to collaborations with companies who might wish to incorporate NanoBioSensor technology into a rapid point-of-care, wearable, portable or implantable versions.

Customized Capture Molecule Development.

Vista can also provide artificial antibodies with many useful features, such as dry storage. Many such capture molecules can be stored dry and re-hydrated by addition of the sample itself. These artificial antibodies can detect many small molecules that are otherwise non-antigenic. By coating the nanowire with the biomarker itself, Vista's NanoBioSensor System can be used to screen any collection of binders for those with desired kinetic properties in the fluid in which they will ultimately be used. Vista can automate the detection and segregation process **(Fig. 4.).**

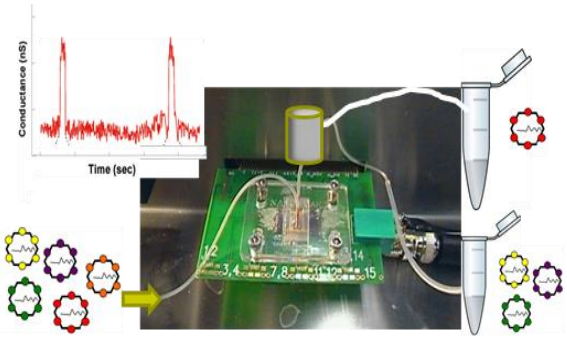

Fig.4. Use of NBS System to segregate binders with desired qualities from large pool of binders. Each iteration produces more specific binders.

Vista also provides multiple types of NanoCards™ for flow-through, spot analysis or continuous monitoring in tissue culture wells and bio-reactors. For scientists with experience creating their own nanowires and/or chips, Vista can create custom 'translator' connectors that will permit their use with Vista's NanoBioSensor and software. In addition, Vista is perfecting a process by which it can place one or more nanowires anywhere on a circuit it/they are needed. Core elements of a basic NanoBioSensor System are shown below **(Fig.5)**. Finally, Vista's close association with TEAM Engineering allows us to design and incorporate almost and additional feature or NanoCard type a customer may need including built in spectroscopy for sample normalization.

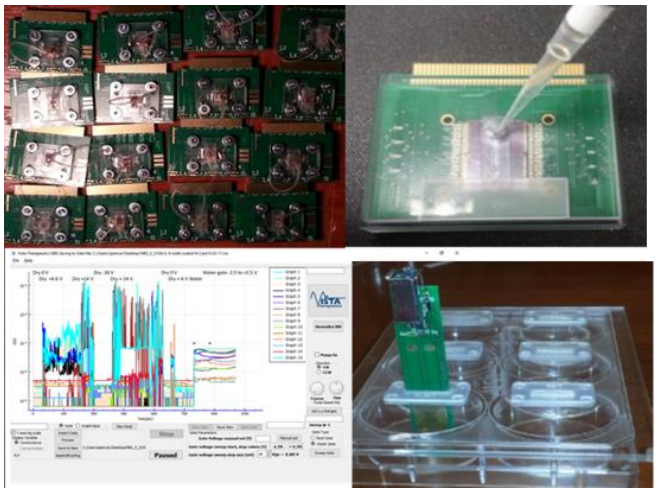

Figure 5. Vista's NanoCards, Multi-chip NanoCard, Software and Dip-Chip NanoCard.

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