



VISTA NanoBioSensors™

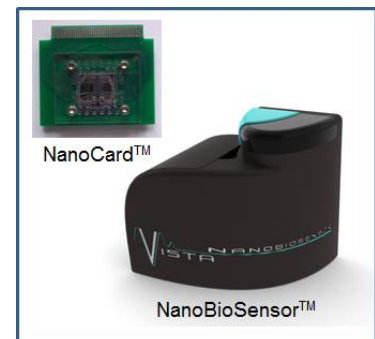
An entirely new way to measure biomarkers.

We all know of the power of ELISA assays to provide a one-time, good approximation of the concentration of certain protein biomarkers within a few hours of blood sample collection. Wouldn't it be great if one could go beyond ELISA's and do any, or possibly all, of the following:

- a. *Conduct real-time, continuous biomarker measurement?*
- b. *Obtain biomarker concentrations starting with 200 μ l or less of whole blood.*
- c. *For such 'snap-shot' experiments, obtain results within five minutes?*
- d. *Be able to use ear-nick, tail nick or finger stick to obtain abundance of sample?*
- e. *Enjoy a five-log linear range with sensitivity usually in the low picomolar and as low as attomolar range?*
- f. *Be able to measure dozens of biomarkers in the same reaction?*
- g. *Be able to measure proteins and transcripts in the same reaction with the same tool?*
- h. *Obtain k_{on} , k_{off} , K_D , ΔG , ΔH and ΔS within minutes?*
- i. *Use mAB's, aptamers, oligo's, fAB's, receptors and enzymes as capture molecules?*
- j. *Conduct all of the above with a single instrument?*
- k. *Benefit from techniques so simple most beginning technicians can run them?*
- l. *Be able to perform all of the above at costs lower than ELISA'?*

Of course it would be great....but on what world would such capabilities exist?

Many of these capabilities exist and others will soon exist using an entirely new way to measure target biomarkers, namely Field Effect Transfer-based biomarker sensing. Vista, Inc. will soon introduce the world's first commercially available NanoBioSensor™ System for FET-based nanobiosensing. There is a path by which your organization can obtain early access to Vista's NanoBioSensor™ technology. The following describes the core technology and strategic co-development opportunities sought by Vista.





VISTA NanoBioSensors™.

An entirely new way to measure biomarkers.

*Introduction to Nanowire Field Effect Transistor-Based
Continuous, Label-Free, Real-Time, Multiplex Biomarker
Measurement for the Pharmaceutical Industry*

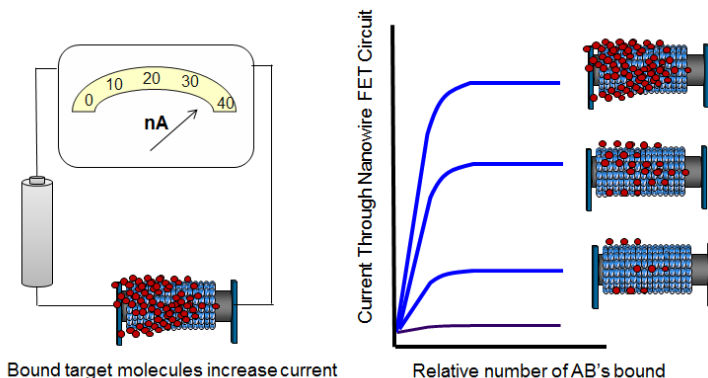
Issues in drug development that Vista's NanoBioSensor™ System can address:

New biomarkers are constantly being developed and validated as both pre-clinical and clinical indicators of drug efficacy and safety. Innumerable studies have demonstrated their utility as predictors of organ damage prior to the development of gross pathology. As powerful as biomarkers are, the methods by which they are currently measured limit their actual utility: This is especially true for blood protein biomarkers. One major limitation derives from the volume of blood required for their detection, usually multiple milliliters. In human and large animal studies, venipuncture is required. Venipuncture is painful and is usually limited to three times per 24 hours or fewer in most hospital or clinical settings. For early animal studies using mice and often rats, one animal may be sacrificed for each dose-time point. A second and significant limitation of current biomarker measurement systems is the time required to obtain results; the fastest of which requires three hours. Many physiological responses to candidate compounds are highly dynamic and blood levels change rapidly. Today's methods are virtually useless for assessing biomarker changes that occur in single digit hours, much less minutes (the time course relevant to many acute disorders/responses such as seizures, shock, stroke, infarct, etc.). It is entirely understandable that the predictive value of biomarkers as currently measured is limited to chronic and/or genetic disorders. Potentially informative biomarkers for highly dynamic responses are either not recognized as such or are under-utilized. The inability to monitor biomarkers associated with highly dynamics processes – which encompass 80% of all emergency and ICU conditions and drugs for acute conditions – represents a huge gap in scientific understanding, drug development and patient care. Besides their inutility for acute disorders, conventional biomarker measurement tools such as ELISA assays, transcript analysis and western blots, also yield little or no information about the kinetics and thermodynamics of the interaction(s) between the target biomarkers and the molecules used to capture them. These limitations are inherent in ELISA-type assays and will never be surmounted despite improvements in multiplexing and ease of use. It will require an entirely different approach in order to utilize biomarkers for immediate analysis and intervention. Vista's NanoBioSensor™ technology, based upon a radically different measurement system overcomes these limitations and provides additional insights that simply cannot be made by conventional methods of biomarker measurement. The fol-

Following section outlines the scientific basis of Vista's NanoBioSensor™ System, first invented by Professor Charles Lieber, a distinguished Professor of Chemistry at Harvard and a co-founder of Vista.

Vista's Technology:

Vista's NanoBioSensor™ overcomes the limitations of ELISA-type assays through the innovative integration of nanotechnology, biotechnology and materials science. More specifically, antibodies and other capture molecules such as receptors, oligonucleotides and aptamers are used to covalently coat silicon nanowire transistors which serve as 'gates' in Field Effect Transistors (FET's) and conduct current based upon changes in the electric



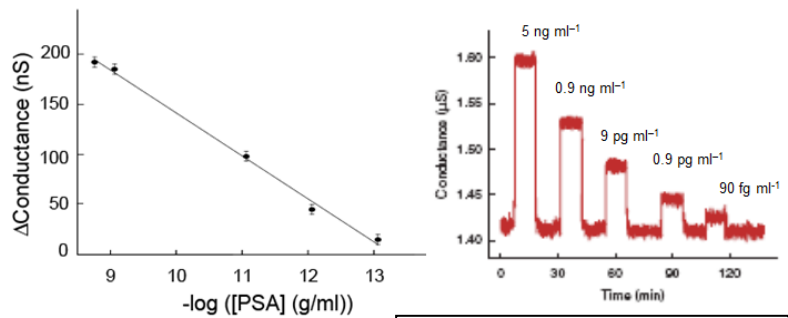
field near their surface. The vast majority of protein biomarkers in the blood are soluble because they are charged at physiological pH. When they are brought in close proximity to FET sensors, they create/alter the electric field near the sensor and thus change the conductance through the FET-controlled electronic circuit. The minute diameter of an antibody-coated FET – approximately 50 nm or less – imparts unprecedented sensitivity to electric circuits they control. Because such transistor-controlled gates can be configured as amplifiers, the ratio of gate to circuit current can be 1 to 1,000,000, rendering the circuits easily measurable by conventional electronic means. Lieber and his colleagues have demonstrated the ability to detect single molecules under ideal conditions. In more typical lab settings, detection of target biomarkers is in the mid-picomolar range. For some biomarkers, the detection limit is in the attomolar level. Because of the dimensions of the nanowire circuitry, hundreds of individual circuits – thus potentially hundreds of distinct biomarkers – can be measured on a surface of 1 square millimeter. Hundreds of distinct biomarkers could be measured in a volume of a few microliters. Future chip versions will incorporate sensing surfaces in triangular, rectangular and higher dimension surfaces to allow thousands of biomarkers to be monitored simultaneously in only a few microliters of sample fluid.

While Vista's initial products will be more modest in the number of distinct molecules they can monitor, each circuit behaves independently, hence several biomarkers can be measured in the current design. As noted, soluble protein biomarkers have a net charge at physiological pH. The greater the number of a particular biomarker molecule attached to a given nanowire, via binding to antibodies (or other capture molecules), the greater the conductance of current through that circuit. Measured in units of transduction (Siemens), the strength of the signal is directly proportional to the ratio of

occupied to unoccupied capture molecules. In cases where the binding constant is around 10^{-10} M, a typical value for most commercially available antibodies, any one particular binding event lasts about 7 seconds. This factor imparts a second remarkable advantage to Vista's method of biomarker measurement: it permits continuous, real-time monitoring, since the signal is directly proportional to the biomarker concentration at that moment; as the concentration changes the signal changes...and that signal is detected instantly and continuously. No other commercially available system has this capability. The quantum mechanical features of the FET NanoBioSensorTM permits target detection at two to three logs lower concentration than ELISA's and, it has a much broader linear range, often up to five logs, versus the two to three log range for ELISA assays.

Advantages of Nanowire FET-Based Biomarker Measurement:

There are several additional advantages of Vista's nanowire-FET based bio-sensing compared to other biomarker detection systems. Besides the ability to multiplex measure multiple protein biomarkers on a continuous basis, Vista's NanoBioSensorTM can also measure oligonucleotides, viral particles, ligands for many receptors and many small molecules. Vista's NanoBioSensorTM System may well permit different classes of molecules to be measured or continuously monitored in the same reaction (Vista is looking for the appropriate partner with whom to confirm this supposition). A further advantage of biomarker measurement using Vista's NanoBioSensorTM System for a single snap-shot measurement of biomarker concentration is its speed. For a single time point, as little as 50 μ l of blood can be used. Thus a finger stick, ear nick or tail nick provides more than enough sample. It only takes a trained technician one minute or less to collect such samples. Since such samples must first be rid of cells and the salt concentration lowered, Vista has developed a sample collection kit that allows blood sample collection and preparation in about three minutes. The prepared sample or a dilution series therefrom, is injected into the sensor's injection port. Depending upon the pump speed chosen by the end-user, results are available and displayed on the computer monitor in about 60 seconds. That means that one can quantitatively determine the level of one or more blood biomarkers within about six minutes from the time of finger prick or tail nick. Even the most dynamic of physiological responses becomes amenable to quantitative analysis using Vista's regime. Furthermore, since such low sample volumes are required, and since binding and release of biomarkers can be observed in real-time, it becomes realistic to perform pharmaco- and toxico-kinetic evaluation of a candidate compound using a single mouse (per dose). Vista's NanoBioSensorTM technology could reduce the number of mice required for exploratory studies by 80% and up to



Data courtesy of Charles Lieber

50% for regulatory studies. It can also greatly reduce the number of small animals needed per compound, and it allows time-course experiments to be conducted in mice (and other rodents). Studies usually applicable to larger animals could be conducted in rodents, saving the number of large animals, the number of rodents, the amount of time per analysis and the amount of compound synthesized. Furthermore, kinetic and thermodynamic information is provided at no additional cost and the quality of data is improved since more time-course experiments can be conducted in rodents (versus one rodent per dose per time point required by existing assays). Inter-animal variability is greatly reduced using our regime. The savings in animals, animal treatment time, lab personnel time, supplies, animal care, phylogenic lowering and drug candidate synthesis will vastly exceed the cost of the NanoBioSensor™ System itself. Indeed, Vista is so confident that we can save your company money on the above expenses, we are willing to be remunerated *on the basis of a percentage of the savings you enjoy*.



NanoBioSensor™



NanoCard™

In addition to the aforementioned technical advantages, cost per data point from our System and ease of use are also compelling factors. Vista's NanoBioSensor™ System consists of two major components (not including the optional Sample Prep Kits). These are the easy-to-use NanoBioSensors™ themselves and the disposable NanoCards™ that accompany them (as shown). The NanoCards™ contain “functionalized” (with capture molecules) nanowires, circuitry and inlet/outlet tubes. The

NanoCard™ is plugged into the NanoBioSensor™ and the tubes connected to ports in the Sensor. Once the sample has been collected and prepared, which takes about three minutes using Vista's Sample Prep kit, it is injected into a port on the Sensor. Another advantage is that Vista's intuitive software queries the end-user for certain data, such as sample description, date, user name, etc. Vista's software permits some analysis of certain elements including concentration, trends, AUC and other statistics. The data is also easily exported to most of the more sophisticated analysis programs such as Excel, Matlab, Spotfire and others. Vista has written a number of sub-routines in Excel to perform kinetic, thermodynamic and predictive analyses. An example of how Vista's NanoBioSensor can conduct rapid kinetic analysis is provided in Appendix 1 to this paper in the form of a Vignette. *A Vista Vignette is a short presentation on how Vista's NanoBioSensor™ System can be used for a specific purpose.* A List of currently thought-through Vignettes is provided in Appendix 2, and we anticipate our clients can come up with many others.

Status of the NanoBioSensor™ Technology and Opportunities for Early Access:

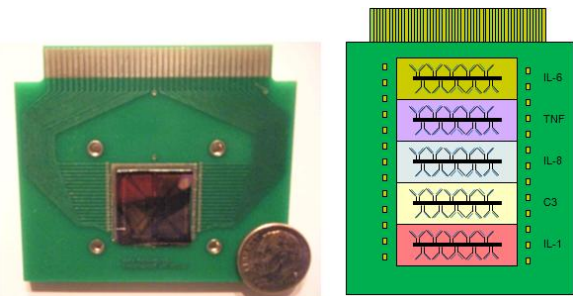
Please note that while we have hard data demonstrating our system's capabilities, as described above, Vista has, at the time of this writing, not yet officially marketed the NanoBioSensor™ System. We first seek to optimize the system, through actual in-the-

field use, before we release our product on the open market. We assume that if you are reading this, you or your company uses, or has an interest in the use of, biomarkers for drug development or individualized patient treatment. We hope you will seriously consider working with Vista in our initial deployment, to both of our advantage. Vista seeks cooperative development with people and organizations with expertise in one or more of the specific areas described below. Although we will entertain any meaningful prospect, Vista is most interested in potential collaborators/early adopters whose priorities and expertise lie within our current goals.

For collaborators, Vista will provide early access to our NanoBioSensor™ instruments and NanoCards™ in exchange for technical, marketing and other considerations. Because we are attempting to retain control of our own fate, we will also request a project-dependent up-front payment (quite modest) and payment upon mutually defined milestones. Co-development partners will receive early access to our technology, but will also receive *very substantial, ongoing discounts* once we make the resultant product widely available. The types of specific technical partnerships/collaborations that we seek are as follows:

Strategic partnership opportunities to help optimize Vista’s NanoBioSensor™ System for drug development and patient treatment:

- i) **Development of NanoCards™ with antibodies against additional biomarkers.** Currently, although Vista’s NanoCards™ contain almost 200 individual circuits, it is difficult to individually functionalize circuits with different probes. Vista’s first release will incorporate sets of chips which have been differentially functionalized and then assembled as the last step. As the number of antibody functionalized chip sets increase, partners will be able to obtain NanoCards™ with an ever-increasing permutation of options. Before offering new probes, however, Vista must confirm that those antibodies retain the sensitivity and specificity to their cognate antigens after covalent attachment to nanowires. Potential partners who need to measure specific biomarkers against which Vista has not yet validated antibodies can move their preferred antibodies to the front of the line by providing antibodies and supporting the cost of antibody validation. This process is straight-forward and takes about two months. Vista can also create custom NanoCards™ containing proprietary antibodies. Vista can even work with antibodies and antigens that are blinded



Vista’s current NanoCard and ‘Mix-and-Match’ version in development.

to Vista to maintain secrecy about the partner's research. The above image shows a current NanoCard™ and an illustration of Vista's next generation of NanoCards™ that can contain up to 16 probe sets. A detailed account of the steps involved in validation of a new antibody for NanoCard™ use is provided in Appendix 3.

Creation and use of custom NanoCards™ is an excellent approach for monitoring biomarkers that have rapid induction and resolution profiles, such as many inflammatory cytokines. If a drug candidate is itself an antibody or protein, Vista's technology can be used to conduct pharmacokinetic studies. If your organization or lab needs to measure biomarkers more rapidly, frequently and less expensively, contact Vista for a realistic time frame and cost for development of a NanoCard™ that suits your needs.

- ii) **Development of sample preparation methods for CSF, urine, saliva, tears and condensed breath.** Biomarkers of drug efficacy, metabolism and toxicity have been found in all bodily fluids, including breath condensate. Vista's System works best when particulates have been removed and salt concentrations have been optimized. For certain target proteins, the maximum charge (thus 'detectability') will occur at non-physiological pH. If multiple target biomarkers are to be measured, the pH will have to be optimized for that fluid and those markers. Vista would like to allow customers to use existing NanoCards™ or create new NanoCards™ to measure biomarkers in other bodily fluids or extracted from tissues such as breast and liver biopsy samples. If your organization can use the power of Vista's NanoBioSensor™ System to measure biomarkers in fluids and tissues other than blood plasma, please contact Vista.

- iii) **Continuous biomarker measurement of plasma proteins using intravenous line.** It would revolutionize drug development and patient treatment if relevant biomarkers could be monitored on a real-time, continuous basis. Vista's technology has the very strong potential for reaching that goal. In order to continuously monitor blood serum biomarkers in animals and humans, it is first necessary to continuously discard cells and reduce salt concentration. Given the small sample requirements, it would be possible to monitor blood plasma from larger test animals for up to 24 hours using less than 25 ml of blood by a combination of 2-fold dilution and filtration combined with tube-within-a-tube filtration. Vista is exploring continuous cell removal and salt reduction by a variety of other means as well, including acoustic separation, continuous filtration and 'bump-post' field techniques. We believe that working in collaboration with the right partners, we can have a working, continuous biomarker monitoring system for animals within six months of project initiation. If your organization is working to develop drugs for highly dynamic biomarker changes (seizures, complement

cascade, inflammation, sleep disorders, ischemia, myocardial infarct, stroke, etc.), or are developing protein or mAB-based drugs with rapid clearance, continuous, real-time monitoring would provide new insights of tremendous power. Continuous monitoring also allows transiently expressed disease components to become ‘drugable.’ For example, the complement cascade is composed of about 13 proteins that are expressed sequentially. The cascade is required and cannot be shut down entirely. Over-expression can lead to lethal consequences such as organ failure. It becomes feasible to fine-tune the complement cascade with modulating drugs only if the status of the cascade can be monitored continuously and on a real-time basis. Vista’s NanoBioSensor™ System has that capability. The same system could be used to optimize patient treatment in a myriad of ways. Individuals vary dramatically in their tolerance to the toxic side effects of drugs due to genetics, age, gender, overall health, other drugs, etc. For example, several aminoglycoside-type antibiotics are dose-limited by kidney damage. Ideally, the physician would titrate up the dose of such antibiotics to the point where early markers of kidney damage begin to rise in the blood such as Kim1 and Gal1 and back off that dose by a small amount. This is possible only if the physician easily accessible data showing ongoing levels of plasma Kim1 and/or Gal1. Vista seeks a variety of partners who can help expedite continuous blood preparation or who wish to continuously monitor certain biomarkers. Please contact Vista if you wish to co-develop these capabilities.

- iv) **Development of NanoBioSensor™ System for tissue culture or bio-reactor applications.** If components of a tissue culture or bio-reactor can be detected by mAB’s (or certain other capture molecules), they can probably be continuously monitored by Vista’s NanoBioSensor™. Because the output of Vista’s detection system is a continuous change in the current running from the source to drain electrodes of a FET transistor, it easily lends itself to directing feedback control systems that maintain one or more tissue culture or bio-reactor constituents within allowed ranges. Such feedback systems could also be incorporated into patient or animal treatment to maintain homeostasis. If your organization runs bio-reactors or tissue cultures where continuous monitoring or feedback control would be beneficial, Vista would like to work with you to develop such systems. We would also like to work with groups who would benefit from feedback systems in animals right now.

- v) **Development of kinetic and thermodynamic tools for drug candidate analysis.** As described in the ‘Vignette’ attached as an Appendix, Vista’s NanoBioSensor™ System has the ability to provide powerful kinetics and thermodynamics information almost immediately. Vista has only scratched the surface of this enormous potential. We have looked mainly at antibody antigen interactions and have not explored ligand:receptor or enzyme:substrate interactions.

The work we have done on antibody:antigen interactions, albeit limited, clearly shows how this technology could be used to differentiate between multiple mAB's that bind the same target molecule, to study competing agonists and antagonists much more rapidly. It could also be used in certain enzymatic reactions to characterize interactions with various substrates and inhibitors. One obvious application is the rapid assessment of multiple competing mAB's and fAB's that might be used as drugs. Vista would like to work with groups who are creating mAB's to be used as drugs. We would also like to work with software companies that provide enzyme analysis and/or SAR capabilities to help us maximize the enormous potential of our System.

- vi) **Development of transcript and protein co-measurement capabilities of proteins and transcripts, or proteins and DNA.** As noted above, there are no obvious reasons why gene transcripts and proteins, or proteins and viral particles cannot be measured in the same sample fluid. The ability to correlate transcription with translation from the same sample would provide powerful evidence for the validity of transcription changes being directly related to protein changes. Vista seeks partners who have a vested interest in showing the direct relevance of transcription to outcomes.
- vii) **Optimization of Kidney Dialysis.** The typical hemodialysis patient is dialyzed 3 to 5 hours, 3 times a week. At present, the 'adequacy' of each session is defined grossly as a urea reduction (predialysis BUN – postdialysis BUN) and patients suffer from treatment-limiting side effects with enormous variability. Nephrologists agree that if tools were available to continuously monitor biomarkers of adequacy and toxicity on a real-time basis, this would represent a critical advancement in patient care. Vista's technology is ideal for monitoring biomarkers of efficacy and toxicity during dialysis.

General terms for early access to Vista's technology:

As indicated above, Vista's founders, principals and shareholders believe that the most effective way to realize the vast potential of our FET-based biosensing is by working with a limited number of strategic partners who bring expertise to the arrangement. Ultimately, Vista will make fully developed products widely available and the price will reflect the true value of the mature product. In the meantime, Vista will make its technology available to early adopters who help Vista develop mature products (available at a very steep and ongoing discount). Under certain conditions, co-marketing and limited exclusive agreements are possible. Because Vista needs capital to participate in these joint-development projects, Vista will generally expect a small 'down payment' and then success-based milestone payments. During the collaboration, Vista will provide NanoBioSensorTM instruments, NanoCardsTM and sample preparation kits to the partner at Vista's costs.

The biomarker detection technology underlying Vista's system offers dramatic new possibilities, and it's just a matter of time before mature measurement systems become available. We invite you to be amongst the first to explore and benefit from what Vista's system can do. (Check out the included appendices for more specific potential "vignettes" and information.)

If you or someone in your organization would like to learn more about Vista's technology and business development opportunities, please contact Vista or visit our website. Thanks ...

Spencer Farr, Ph.D., Chief Scientific Officer

Vista Therapeutics Inc.
3900 Paseo del Sol
Santa Fe, NM 87507

505-474-3143
505-690-6711 (direct)

spencer@vistatherapeutics.org
spencefarr@aol.com

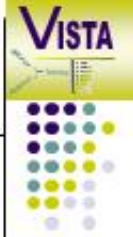
www.vistatherapeutics.org

Appendix 1

Vignette 1: Kinetic and Thermodynamic Studies Using Vista's NanoBioSensor™



Support Materials



Vista Therapeutics, Inc.

Vignette # 1: Using Vista NanoBioSensor to Determine Antibody:Antigen Kinetics, Including k_{on} , k_{off} , K_d and ΔG .

Vignette: Antibody Kinetics

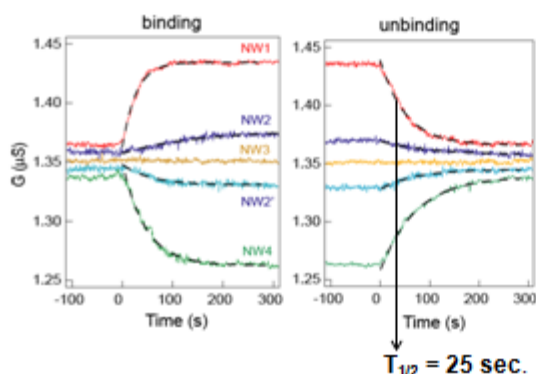
Vista Therapeutics, Inc.

Vista Therapeutics, Inc. is commercializing the world's first nanowire-based biomarker and drug sensing devices. There are several extremely powerful uses of NanoWire (NW) FET-based sensors. This vignette illustrates the ease with which the end-user can determine multiple parameters of any particular antibody (AB) : antigen (AG) pair. With one measurement of transduction over a brief period of time where antigen is first added, then removed from the reaction chamber, the researcher can determine such powerful factors as k_{on} , k_{off} , and K_d . If one conducts the experiment at two temperatures, such as 25°C and 37°C, one can also quickly determine ΔG , ΔH and ΔS . The actual 'hands-on' time is no more than one hour maximum. Vista's software makes these calculations easy. These tools are incredibly powerful for identifying the best candidate if the drug is an mAB, or which candidate molecule is optimal if the target is an AB. No other regime permits such rapid and cost-effective studies. Very similar steps allow one to determine analogous enzyme kinetics.

Spencer Farr, Ph.D.
Chief Science Officer

CONFIDENTIAL

Kinetics Analysis using NW's



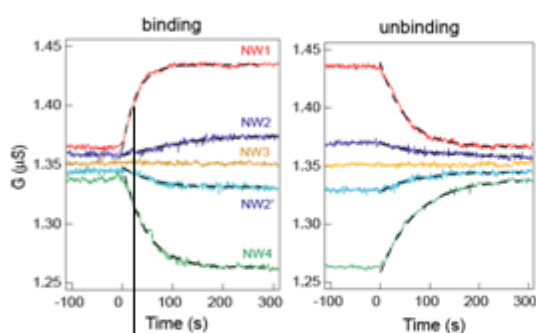
- NW1 – NW4, modified with GT1b, GM2, GM3 and GM1, respectively, with points as (1) 62 nM BTA; (2) 620 pM BTA; (3) 62 nM CTB; (4) 620 pM CTB.
- Note: theoretical half-life ($T_{1/2}$) for typical mAB with binding constant of 10^{-10} M is 7 seconds. Experimental $T_{1/2}$ is about 25 seconds, and k_{off} is 0.027 sec^{-1} close to theoretical value.

$$T_{1/2} = \ln 2 / k_{off} = 0.69 / X \text{ sec}^{-1} = 25 \text{ sec}$$

$$k_{off} = 0.027 \text{ sec}^{-1}.$$

CONFIDENTIAL
Zheng, Patolsky & Lieber

Kinetics Analysis using NW's



Note: theoretical half-life ($T_{1/2}$) for typical mAB with binding constant of 10^{-10} M is 7 seconds. Experimental $T_{1/2}$ k_{off} is about 25 seconds, and k_{off} is quite close to theoretical value!

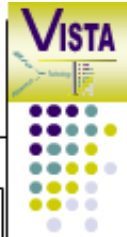
Note: since $K_{eq} = \frac{k_{on}}{k_{off}}$ both of which are derived in minutes using Vista's NBS System, one can quickly identify best mAB or target in drug discovery.

$$T_{1/2} \sim 15 \text{ sec.}$$

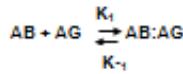
$$K_{eq} = \frac{k_{on}}{k_{off}}$$

CONFIDENTIAL
Zheng, Patolsky & Lieber

Relationship between Biomarker Concentration [AG] and Signal



AB:AG complexes form and dissociate continuously.



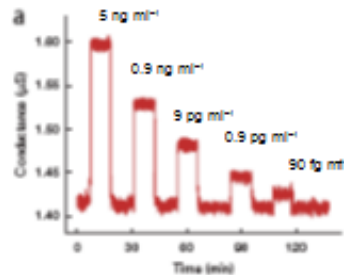
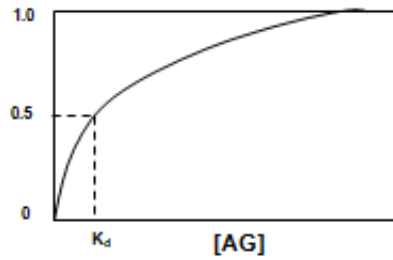
$$\frac{[AB:AG]}{[AB_T]}$$

At equilibrium K_d is the ligand concentration where half the total antibodies (AB_T) are occupied.

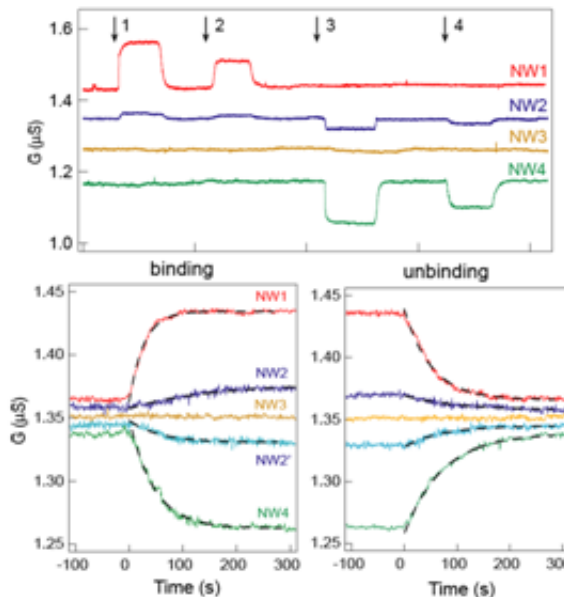
Typical K_d values for most AB:AG complexes are in the range of 10^{-10} M. In a diffusion-limited bi-molecular interaction, K_{off} will be around 0.1 sec^{-1} and thus the typical half-life for an AB:AG complex is given by

$$T_{1/2} = \ln 2 / K_{off} = 0.69 / 0.1 \text{ sec}^{-1} = 6.9 \text{ seconds.}$$

Because AB:AG complex formation is rapidly reversible, there is no accumulation of signal over time. Conductance varies only with [AG]. The figure to the right shows how conductance varies with AG concentration (human PSA).



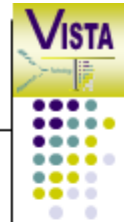
Multiplexed Kinetics Analysis



- NW1 – NW4, modified with GT1b, GM2, GM3 and GM1, respectively, with points as (1) 62 nM BTA; (2) 620 pM BTA; (3) 62 nM CTB; (4) 620 pM CTB.
- k_a , k_d , and K_D are consistent with values from the literature, when available.
- Sensitivity of nanowire devices enables kinetics in previously inaccessible regimes!

CONFIDENTIAL
Zheng, Patolsky & Lieber

Summary: Antibody Kinetics



Vista Therapeutics, Inc.

By using Vista's NBS System, the investigator interested in identifying lead drug candidates where the drug is either an antibody (mAB), or a compound that inhibits an antibody, the end-user can determine multiple parameters of any particular antibody (AB): antigen (AG) pair. This same analysis can be performed with certain ligand:receptor and enzyme:substrate pairs. In addition k_{on} , k_{off} , and K_d if one conducts the same experiment at two temperatures, such as 25°C and 37°C, one can also quickly determine ΔG , ΔH and ΔS . The actual 'hands-on' time is no more than several minutes and the quantity of reagents can be in the nanograms or picograms, depending upon the affinity and charge of the target molecule. In addition, one can also quickly determine the isoelectric points for both the antibody and the target molecule. These tools are quite powerful for identifying the best candidate if the drug is an mAB, or which candidate molecule is optimal if the target is an AB. No other regime permits such rapid and cost-effective studies.


Spencer Farr, Ph.D.
Chief Science Officer

CONFIDENTIAL

Appendix 2

List of Available Vignettes

Support Materials





Vista Therapeutics, Inc.

Vignette # 2: Using Nanowires to Determine the Number of Factors Involved in a Given Blood Biomarker Level

Spencer Farr, Ph.D., Chief Scientific Officer

...remember where you got this idea.





Support Materials

Vista Therapeutics, Inc.

Vignette # 3: Use of Nanowire Sensors in Feedback Loops to Maintain Constant level of Biomarker or drug in a Human, Animal, or Reaction or Fermentation Chamber

Spencer Farr, Ph.D., Chief Scientific Officer

...remember where you got this idea.





Support Materials

Vista Therapeutics, Inc.

Vignette # 4: Use of fAB Fragments, Aptamers, Oligo's, Receptors, Ligands, Enzymes and Substrates as Capture Molecules.

Spencer Farr, Ph.D., Chief Scientific Officer

...remember where you got this idea.






Support Materials


Vista Therapeutics, Inc.

Vignette # 5: Relationship between the Diameters and Isoelectric Points of both the mAB and the Target Biomarker: Optimizing pH to Maximize Signal.

Spencer Farr, Ph.D., Chief Scientific Officer

...remember where you got this idea.





Support Materials

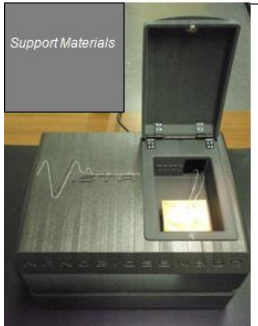
Vista Therapeutics, Inc.

Vignette # 6: Use of pH Scan to Verify and Quantify mAB Attachment to Nanowire.

Spencer Farr, Ph.D., Chief Scientific Officer

...remember where you got this idea.






Support Materials


Vista Therapeutics, Inc.

Vignette # 7: Use of mAB's Against Key Enzymes as a Control to Verify Nanowire Probe Sensitivity and Specificity.

Spencer Farr, Ph.D., Chief Scientific Officer

...remember where you got this idea.







**Vignette # 8:
Incorporation of
NanoWire Sensors in
Non-Denaturing Protein
Gels to Locate Target
Molecules.**

Spencer Farr, Ph.D., Chief Scientific Officer
...remember where you got this idea.



**Vignette # 9: Use of
Secondary Epitope on
Target Biomarker
Coupled with
Secondary Antibody to
greatly Enhance Signal.**

Spencer Farr, Ph.D., Chief Scientific Officer
...remember where you got this idea.



**Vignette # 10:
Simultaneous
Measurement of Protein
and Transcript Level
from the Same Gene:
Relationship between
Transcript and Protein
Levels.**

Spencer Farr, Ph.D., Chief Scientific Officer
...remember where you got this idea.

Appendix 3

Steps involved in validation of a new antibody for NanoCard™ use.

General Steps for Creating New NanoCards™

Suggested Steps.

1. Can we bind fAB and detect target under ideal conditions.

The first step would be to confirm that we can attach the fAB to the nanowire and that it retains its binding affinity to the target molecule. Using relatively high doses, we would conduct the detection experiment under best-guess pH. Just to confirm that target molecule does not naturally bind to nanowires, with or without the capture molecule, we would run the target molecule over ‘functionalized’ (antibody attached) and un-functionalized nanowires just to exclude or at worst, subtract non-specific binding. Once we know we have specific binding, we would repeat the process with functionalized nanowires while continuously varying the pH from about 5.5 to about 8.5 in order to empirically identify pH where signal to noise ratio is highest. Finally, we conduct a dilution series to see what the level of detection is and to determine the linear range of the assay.

2. Confirm specificity in increasingly complex mixtures.

Once we know the linear range under ideal conditions (best pH and low salt), we next start adding back into the solution increasing concentrations of plasma. Given the size of the target molecule, it is sometimes worth filtering out proteins larger than the target. If the concentration is quite low, we can use a filter lower than the target size and re-suspend the precipitate in NBS buffer. This too will improve signal to noise since one can get rid of antibody-binding proteins like complement factors. We can also try it using increasing concentrations of whole plasma if size exclusion does not add anything to the detection.

3. Determine if de-salting is necessary.

Depending upon the efficiency of binding the fAB to the nanowire and subsequent blocking, this determines the effect of salt on the noise level. If the target is in the mid nM range, we can often just dilute the sample up to 1000 fold in low salt buffer. If the detection range does not permit such dilution, we might have to desalt. To desalt we use a filter to get rid of the bulk of the plasma, then re-suspend in appropriate buffer. It adds about two minutes to the process. It may not be necessary if the mAB or fAB fragments pack onto the NW so tightly as exclude many of the noise-generating ions.

This is the process we go through with any antibody. If one’s budget allows, Vista can create custom NanoCards that allow the customer to monitor a combination of targets simultaneously.

Assuming there is nothing unusual about the target(s) or the mAB(s), it takes us about two months to get to a working custom NanoCard with up to four new capture molecules.

Before we provide estimated prices, Vista needs to obtain an estimate of the number of samples the client would want to monitor per unit time and over what time span.