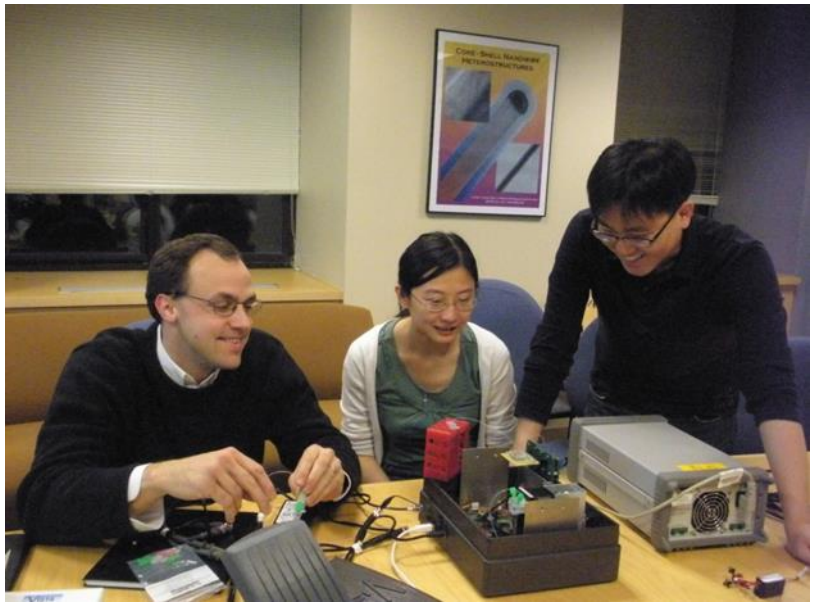
Overview of Vista NanoBioSensor™ Technology.

Vista Therapeutics, Inc. November 2018



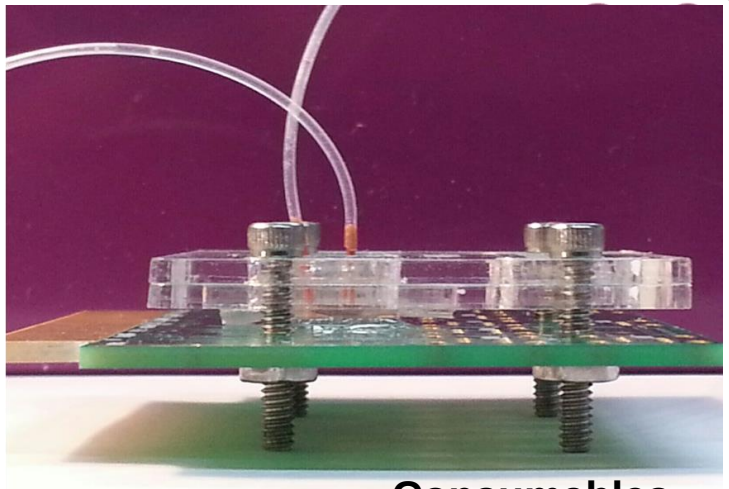
The Awesome Power of Small

The NanoBioSensor System

NanoBioSensor

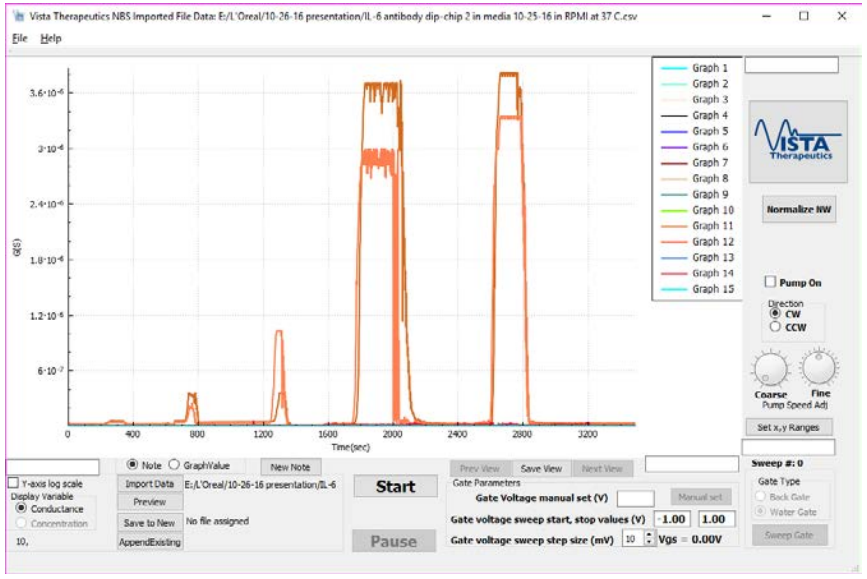
NanoBioSensor

NanoCards



NanoBioSensor System is comprised of instrumentation, NanoCards™ or DipChips™, software and consumables. Vista can also develop optimized capture molecules.

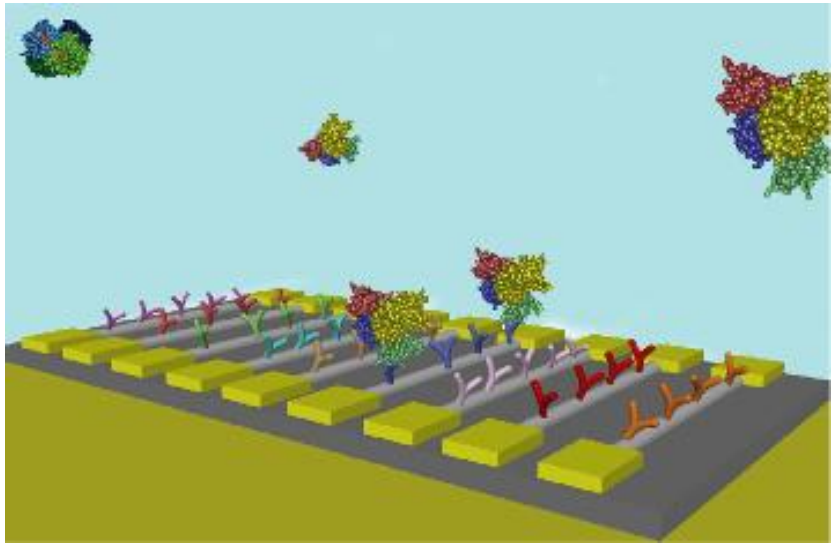
Software

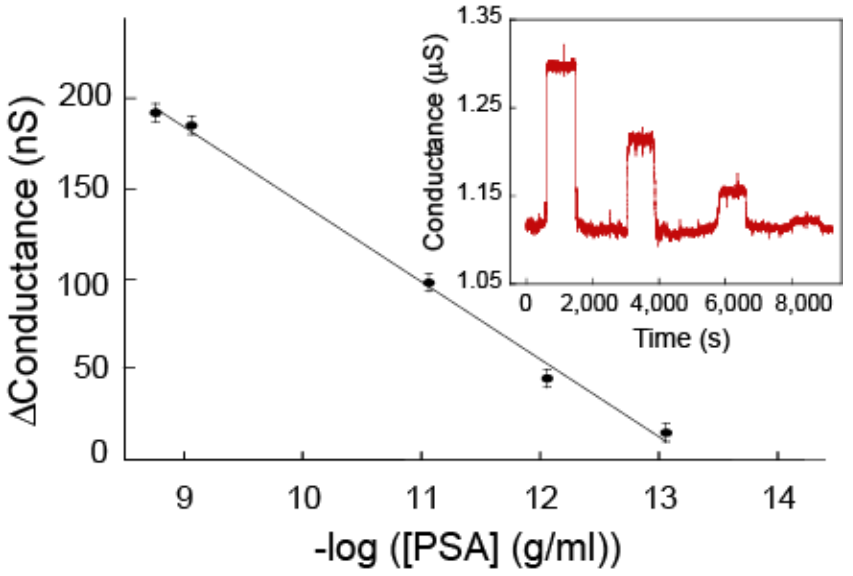


Consumables

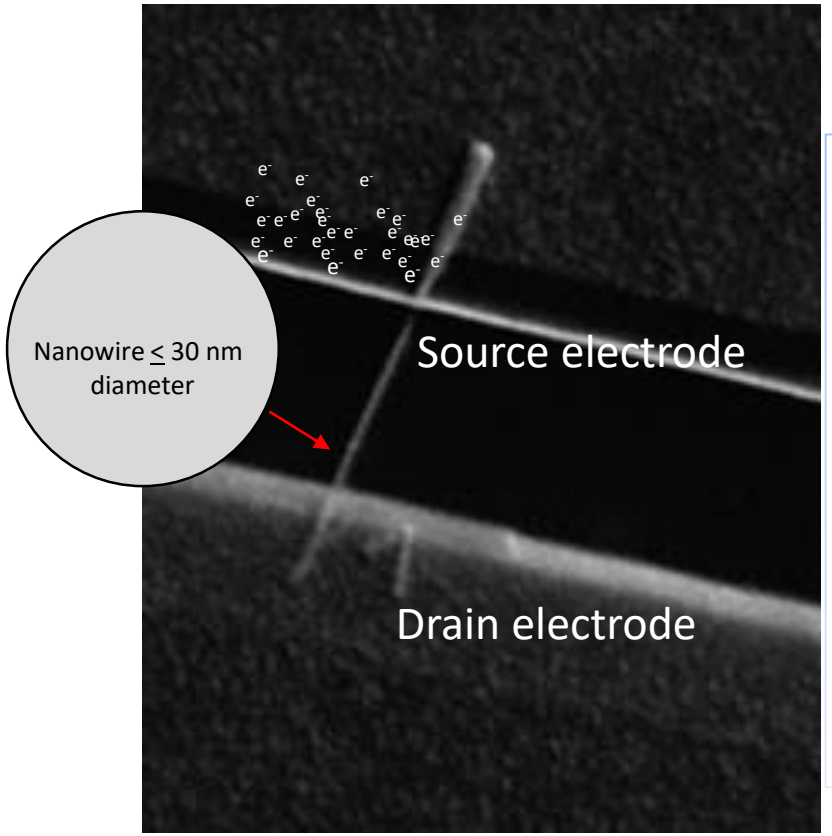
The power of the NanoBioSensor Platform.

- Extensive and True Multiplexing.
- Sensitivity: Femtograms/ml and 5 log liner range (10^3 improvement).
- Continuous measurement.
- Extensive Kinetic & Thermodynamic information provided.
- Immediate read out using only a few µl of sample.
- No labeling required.
- Cost per data-point low.

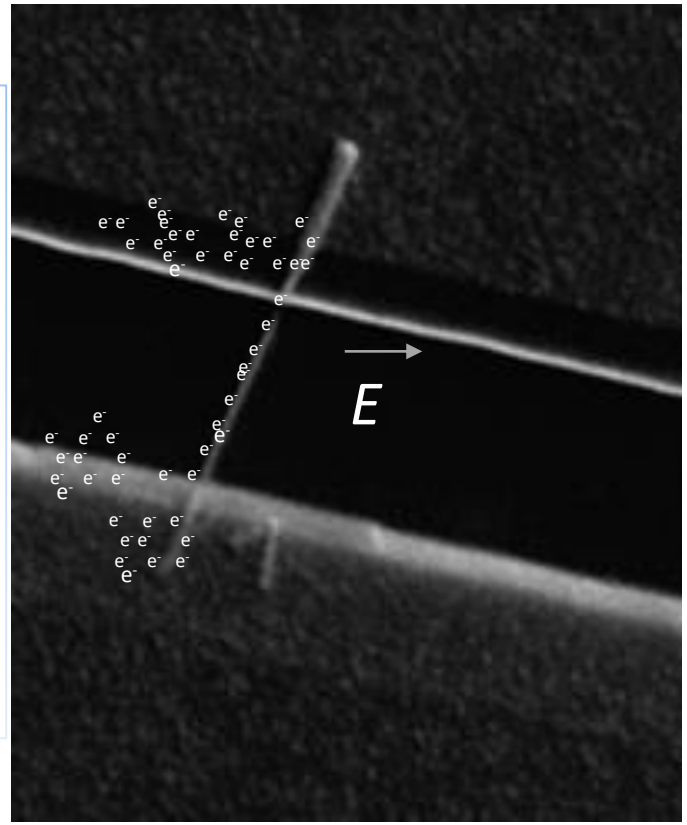




Background: What are Nanowire Field Effect Transistors (FETs).

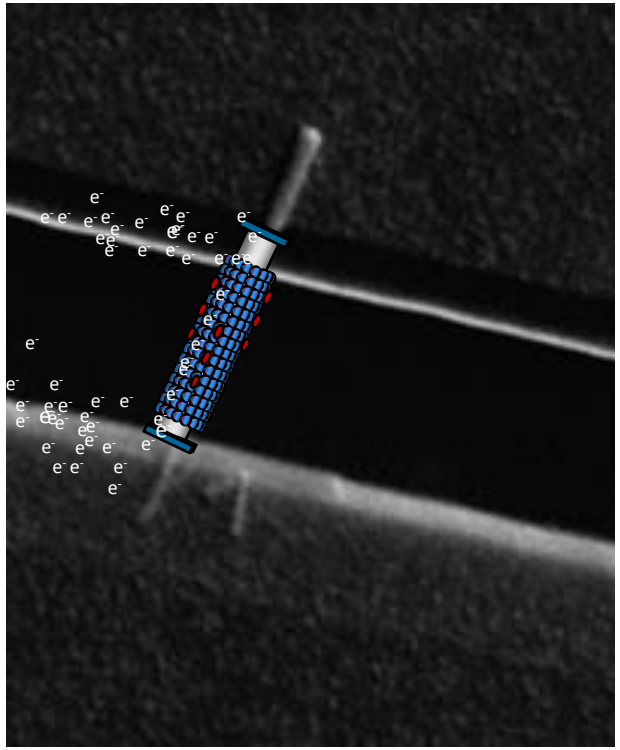


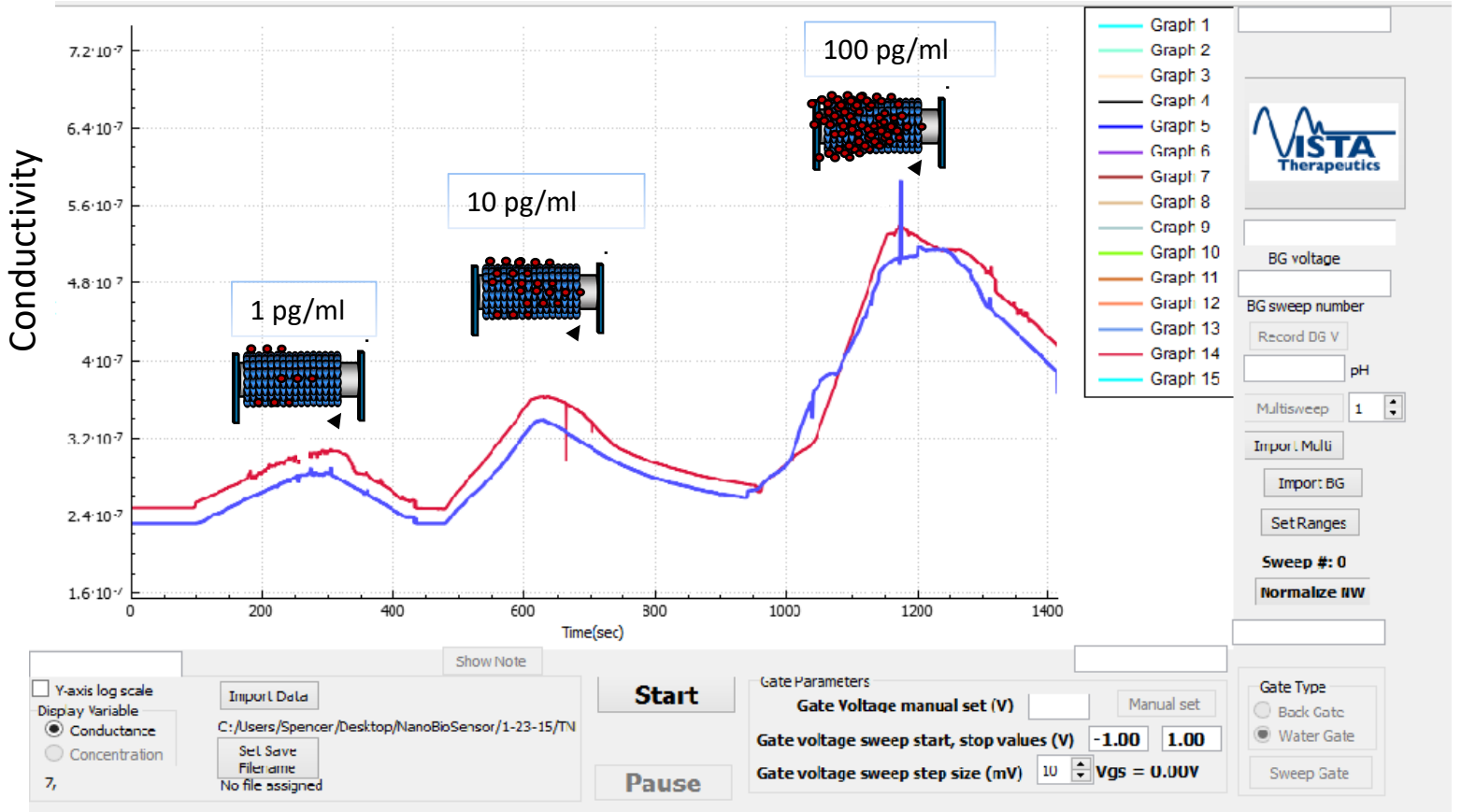
Silicon semiconducting nanowires are Field Effect Transistors (FETs) that complete an electric circuit. However, a nanowire FET poorly conducts electricity in the absence of an electric field..



In the presence of an electric field, the nanowire FET conducts electricity in proportion to the strength of that electric field.

When charged biomolecules bind to nanowire FETs, they change the FET's conductivity in direct proportion to the number of molecules bound at any given moment, which in turn, is directly related to the biomarker's concentration.

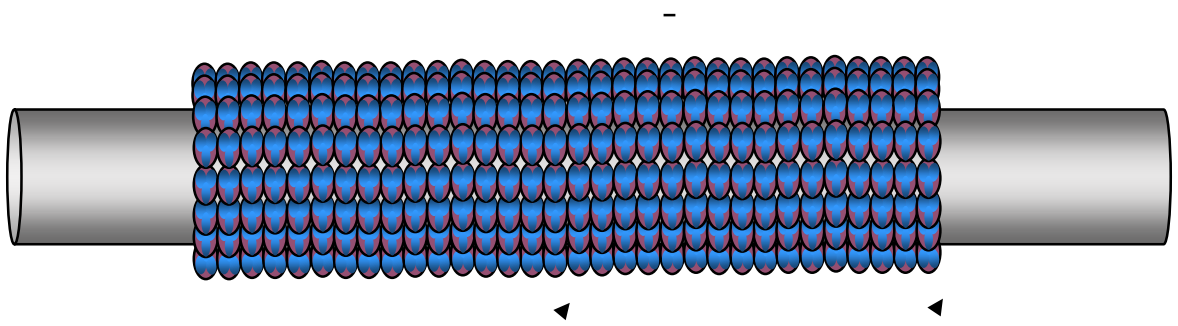


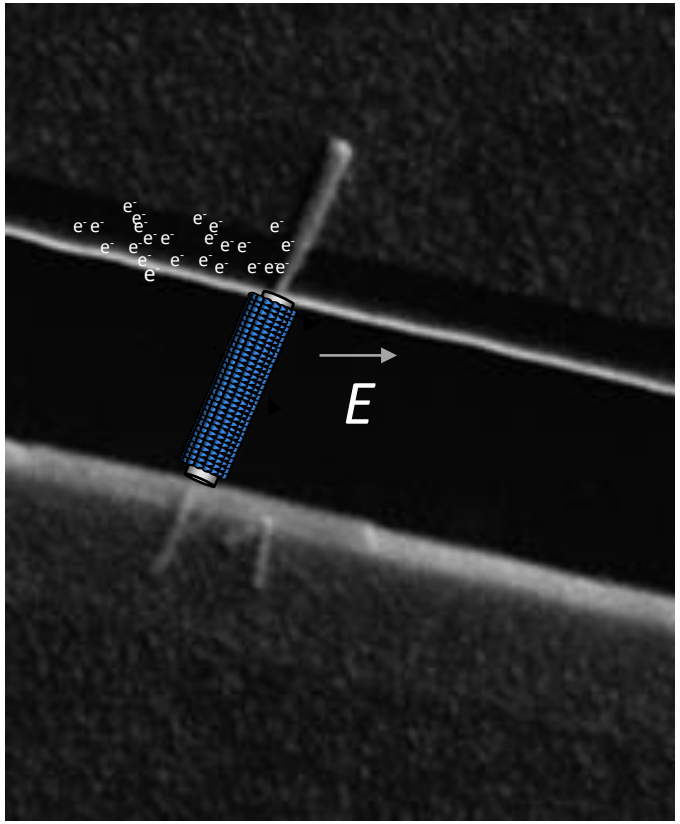


To detect specific biomolecules, we coat the nanowires with specific capture molecules.

Capture molecules can be:

- Monoclonal or polyclonal antibodies
- Oligonucleotides
- Receptors
- Enzymes
- Peptides
- Aptamers Affimers, Nanobodies and small sd mABs.





The best probe for a given target depends upon the application. There are a few important considerations in choosing capture molecules.

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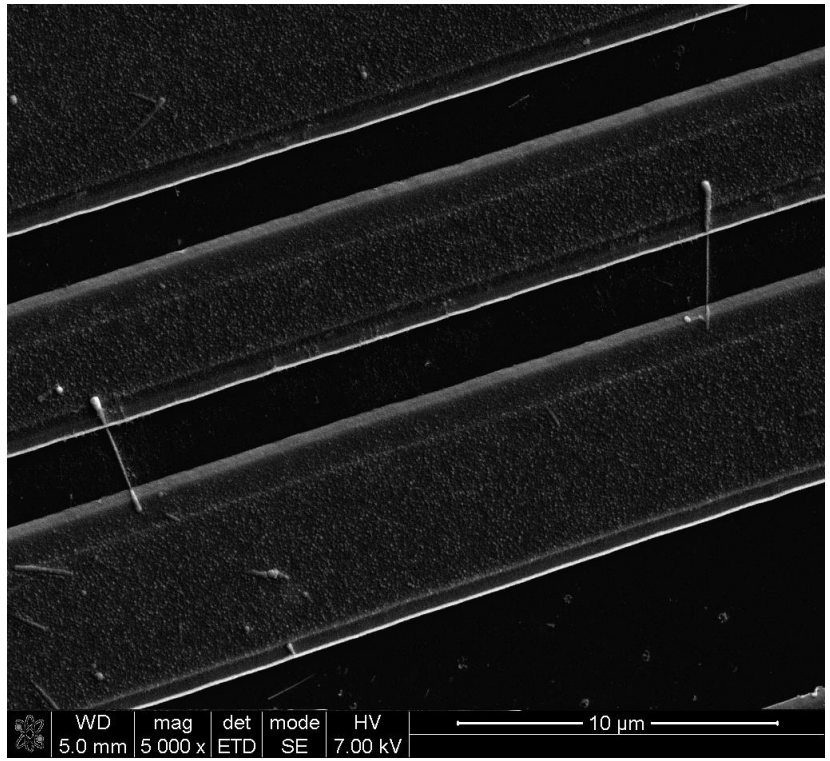
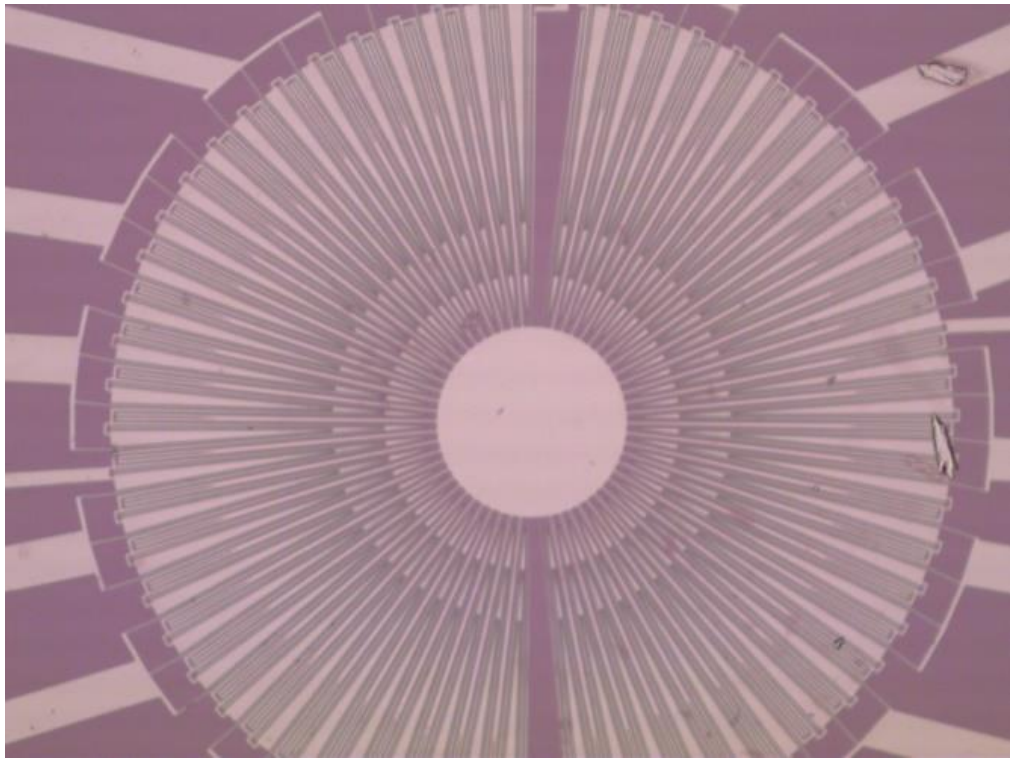
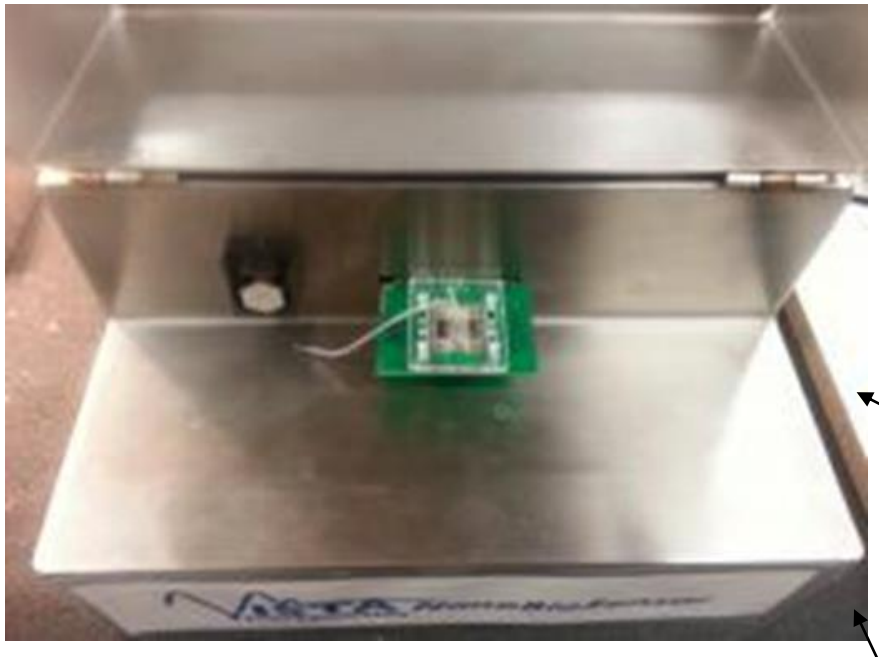
Placement of multiple NWs per circuit. Integration of multiple circuits into single circuits.



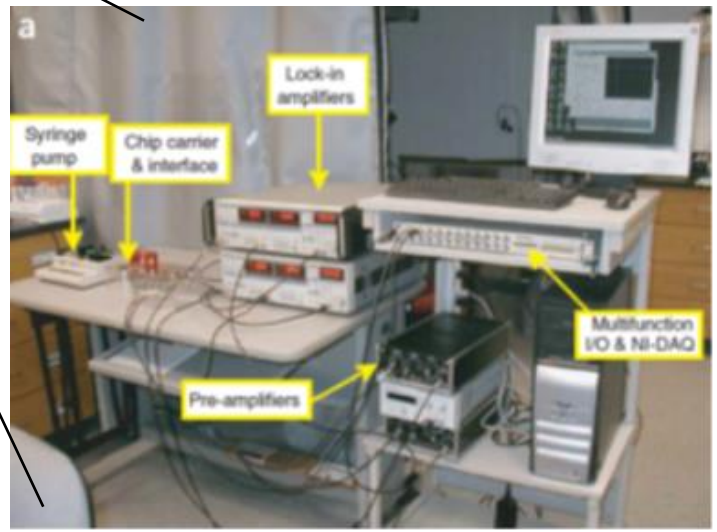


Vista proprietary FET Radial Array NanoChip 'FRANC' chip is designed to allow several circuits containing several nanowires to be combined into circuit thus increasing the sensitivity and dynamic range. In addition, the control Watergate circuit is designed to be equidistant to every nanowire thus permitting accurate inter-circuit normalization.

NanoBioSensor™ System.



Has all of the electrical components required to generate, store, and analyze field effect transistor nanowire data integrated into one benchtop unit. Stainless steel cover allows for decontamination. Benchtop unit includes software-controlled precision peristaltic pump for NanoCard activation or for conducting flow-through experiments and heater for PCR or thermodynamic studies.



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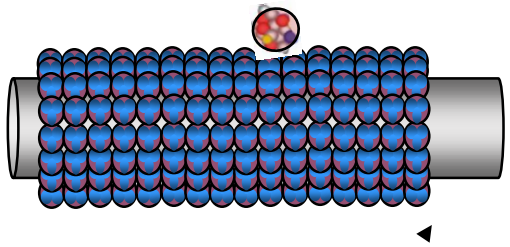
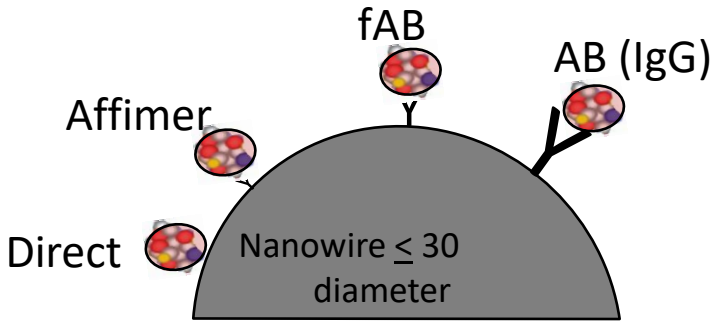
The size of the capture molecule will affect signal strength.

Electric field strength falls as the inverse square of the distance between target and NW surface. $\frac{1}{D^2}$

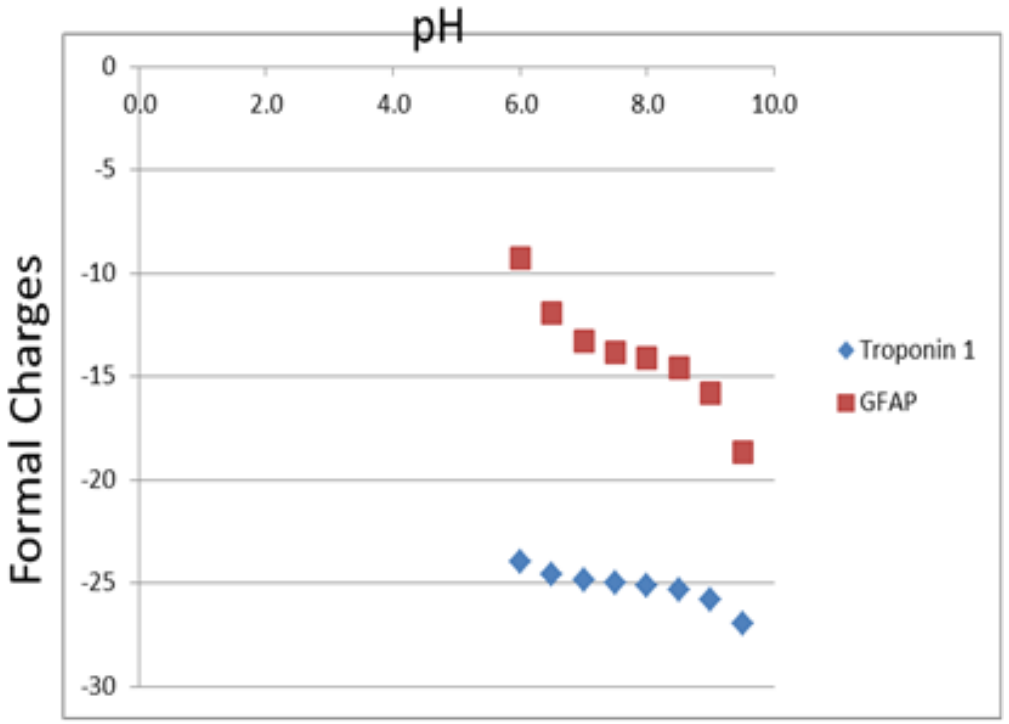




BioMarker --- Nanowire Separation (nm)		Relative Field Strength (Vm)
Direct binding	**1**	**1**
Nanobody/Affimer	**2**	**1/4**
fAB	**4**	**1/16**
AB (IgG)	**8**	**1/64**

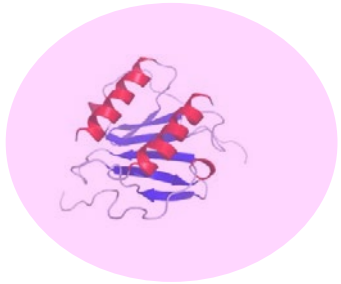
All else being equal, a Nanobody will detect the field of a target biomolecule eight times greater than an IgG antibody. The small size helps overcome Debye length limitations.

The pH of the sample fluid will directly affect the number of charges on the target biomolecule and thus signal strength.



Relationship between pH and number of formal charges on target proteins.

Relative charge density $\left[\frac{\text{\# Charges}}{\text{diameter}}\right]$ will affect signal strength.



IL-6.

miRNA

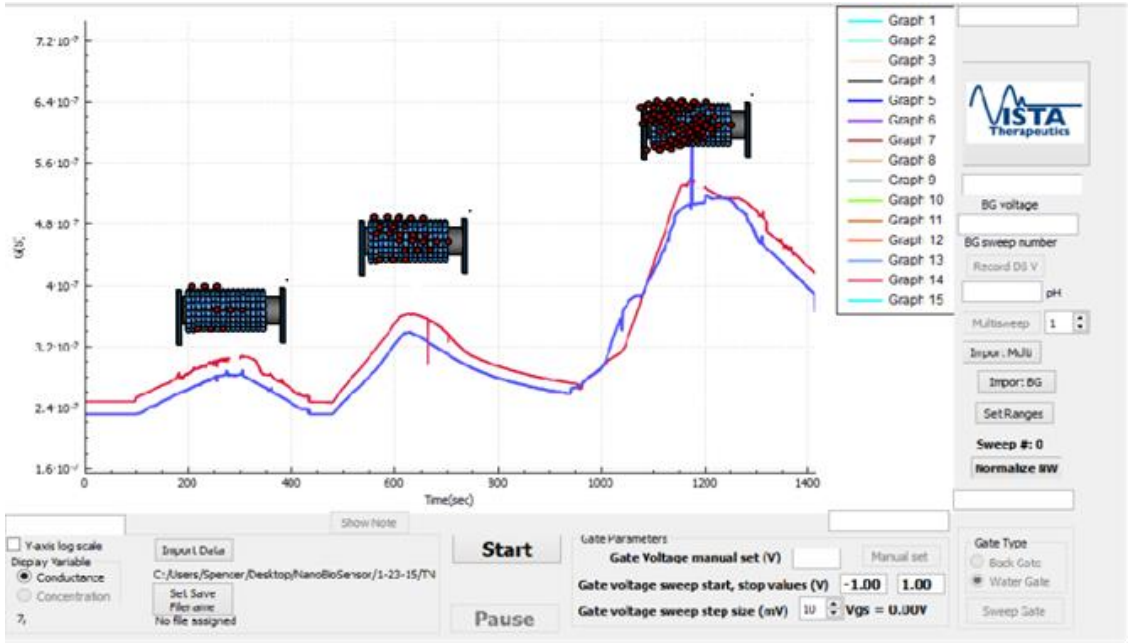
Charge density of IL-6 at pH 7.4 is 5 charges/3 nm = 1.25.

Charge density of 23-mer miRNA at pH 7.4 is 23 charges/1 nm = 23

Using these metrics microRNA has a relative charge density almost 20 times greater than that of IL-6.

Another important consideration is the relationship between the Dissociation Constant and duration of complexes.

The stronger the binding (lower K_D), the longer the biomarker-capture molecule complex lasts. If the K_D is lower than about 1 x 10^{-10} M, there will be an accumulation of signal over time. Thus for continuous monitoring the K_D is important.



Calculated \(k_{off}\) and t1/2 for binary complexes assuming diffusion-controlled \(k_{on}\)

Complex	KD (M)	\(k_{off}\) (s^{-1})	\(t_{1/2}\)
H_2	1 x 10^{-71}	1 x 10^{-63}	2 x 10^{55} yr
RtV3 : Rt'L3(a)	10^{-17}	1 x 10^{-9}	2 yr
Avidin:biotin	10^{-15}	1 x 10^{-7}	80 days
thrombin:hirudin(b)	5 x10^{-14}	5 x 10^{-6}	2 days
lacrep:DNAoper(c)	1 x 10^{-13}	1 x 10^{-5}	0.8 days
Zif268:DNA(d)	10^{-11}	1 x 10^{-3}	700 s
GroEL:r-lactalbumin(e)	10^{-9}	0.1	7 s
TBP:TATA(f)	2 x 10^{-9}	2 x 10^{-1}	3 s
TBP:TBP	4 x 10^{-9}	4 x 10^{-1}	2 s
LDH (pig): NADH(g)	7.1 x 10^{-7}(j)	7.1 x 10^{1}	10 ms
profilin: CaATP-G-actin	1.2 x 10^{-6}	1.2 x 10^{2}	6 ms
TBP: DNAnonspec(h)	5 x 10^{-6}	5 x 10^{2}	1 ms
TCR(i): cyto C peptide	7X10-5	7X10^{3}	100 ms
lacrep:DNAnonspec(h)	1 x 10-4	1 X10^{4}	70 ms
uridine-3P: RNase	1.4 x 10^{-4} (j)	1.4X10^{-4}	50 ms
Creatine Kinase: ADP	8.2 x 10^{-4} (j)	8.2X10^{4}	10 ms
Acetylcholine:Esterase	1.2 x 10^{-3}	1.2 x 10^{5}	6 ms
no interaction	4 x 10^{73}	4 x 10^{81}	-

Example applications of the NanoBioSensor System

- Rapid biomarker quantification ★
- Long-Term continuous monitoring ★
- Monitor multiple biomarkers in same NanoCard ★
- Multiple normalization tools ★
- Kinetic and thermodynamic analysis ★
- Label free, real-time PCR ★
- SNP detection ★
- Tissue culture & bioreactor monitoring ★
- Label-free detection and sample recovery ★
- Isolation of binders from non-binders ★
- microRNA analysis
- Catabolic pathway analysis
- Automated toilet waste analysis
- Real-Time cell motility
- Membrane potential
- Optimized capture molecule selection
- Environmental (air and water) monitoring

★ = To be covered

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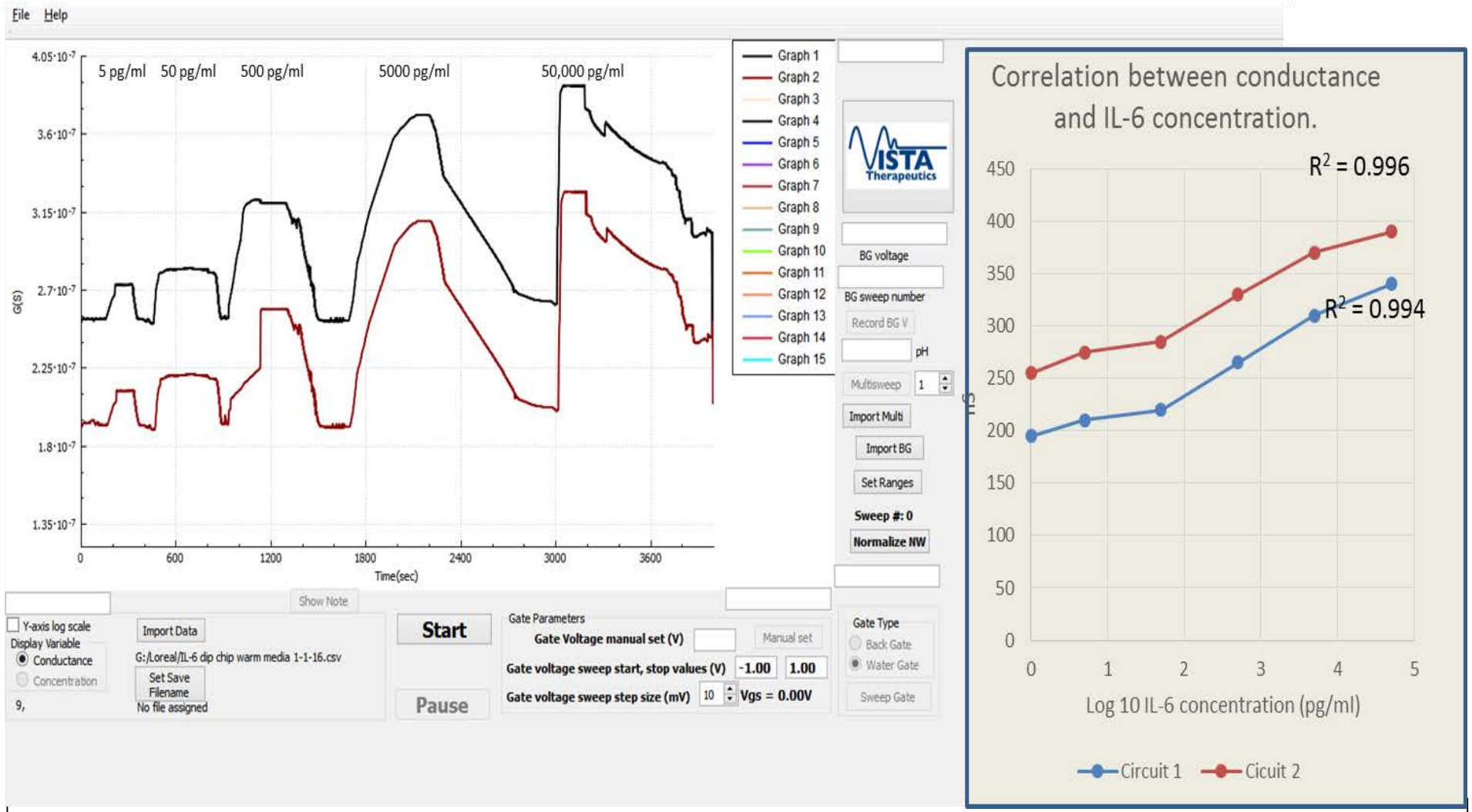
Rapid biomarker quantification.



Standard curve generated by flowing media with and without IL-6 over a Dip-Chip in increasing concentrations of IL-6 at 0, 5, 50, 500, 5000 and 50,000 pg/ml. The correlation between conductance and IL-6 concentration was very tight with R^2 values at 0.99 for both circuits.

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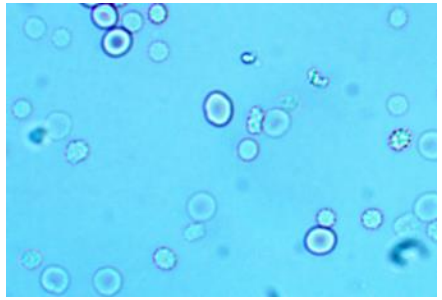
Rapid biomarker quantification: 5-10 minutes from blood stick to detection of malaria biomarker.

1

Using lancet, obtain 40 µl of whole blood.

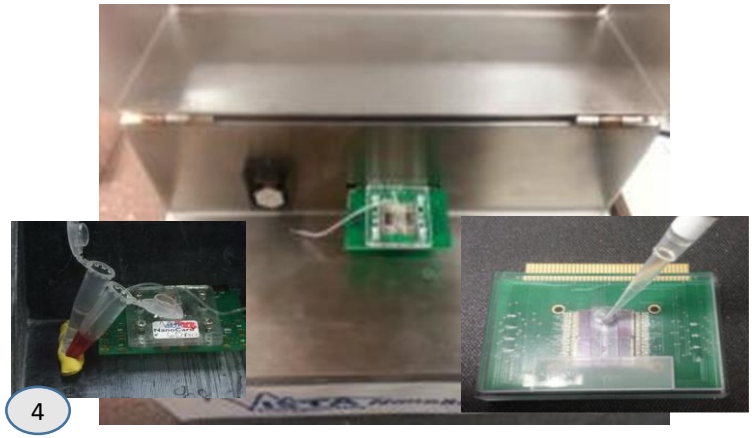
2

Quickly transfer to tube containing 360 µl ddH_2O at room temp.



3

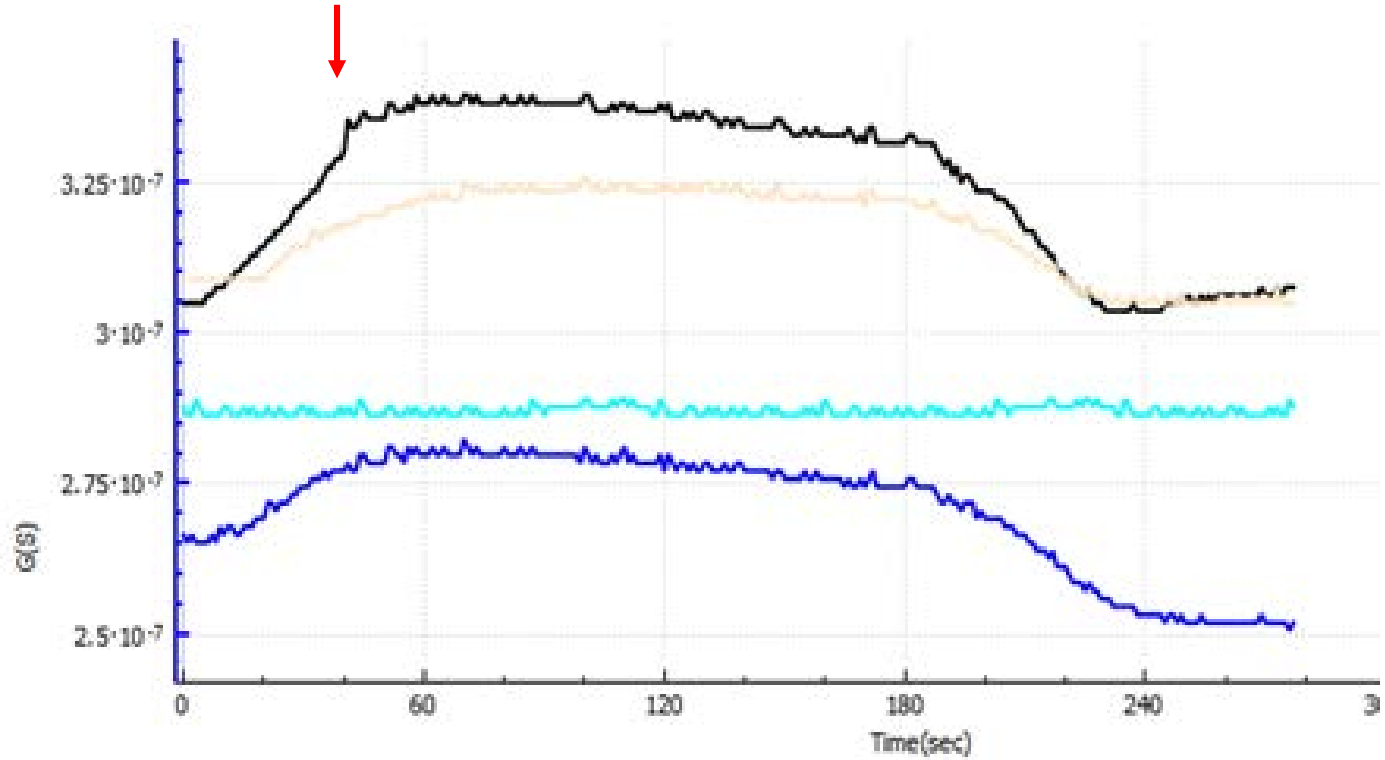
Gently agitate for 5 min to allow RBCs to leak or burst.



4

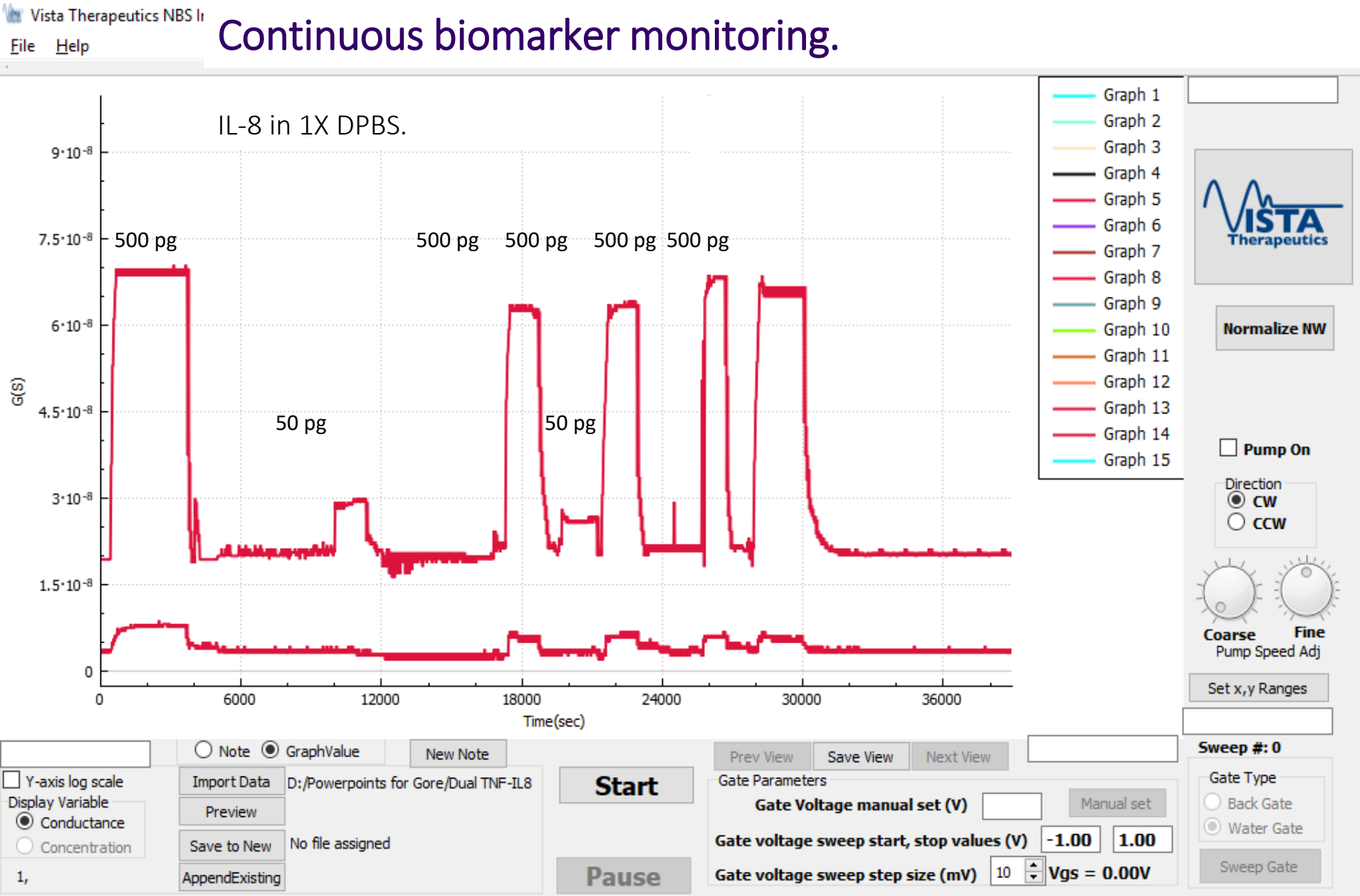
For dose-response, kinetic and thermo- dynamic information, pull sample over nanowire card functionalized with anti-HRP2-antibodies. For simple quantification, pipette sample into NanoCard port.

500 fg/ml HRP2 ($1.35x10^{-11}$ M) Three weeks before symptoms.

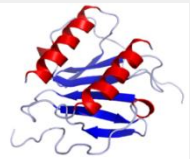


NOTE: Most of the HRP2 work was funded by the Bill and Melinda Gates Foundation.

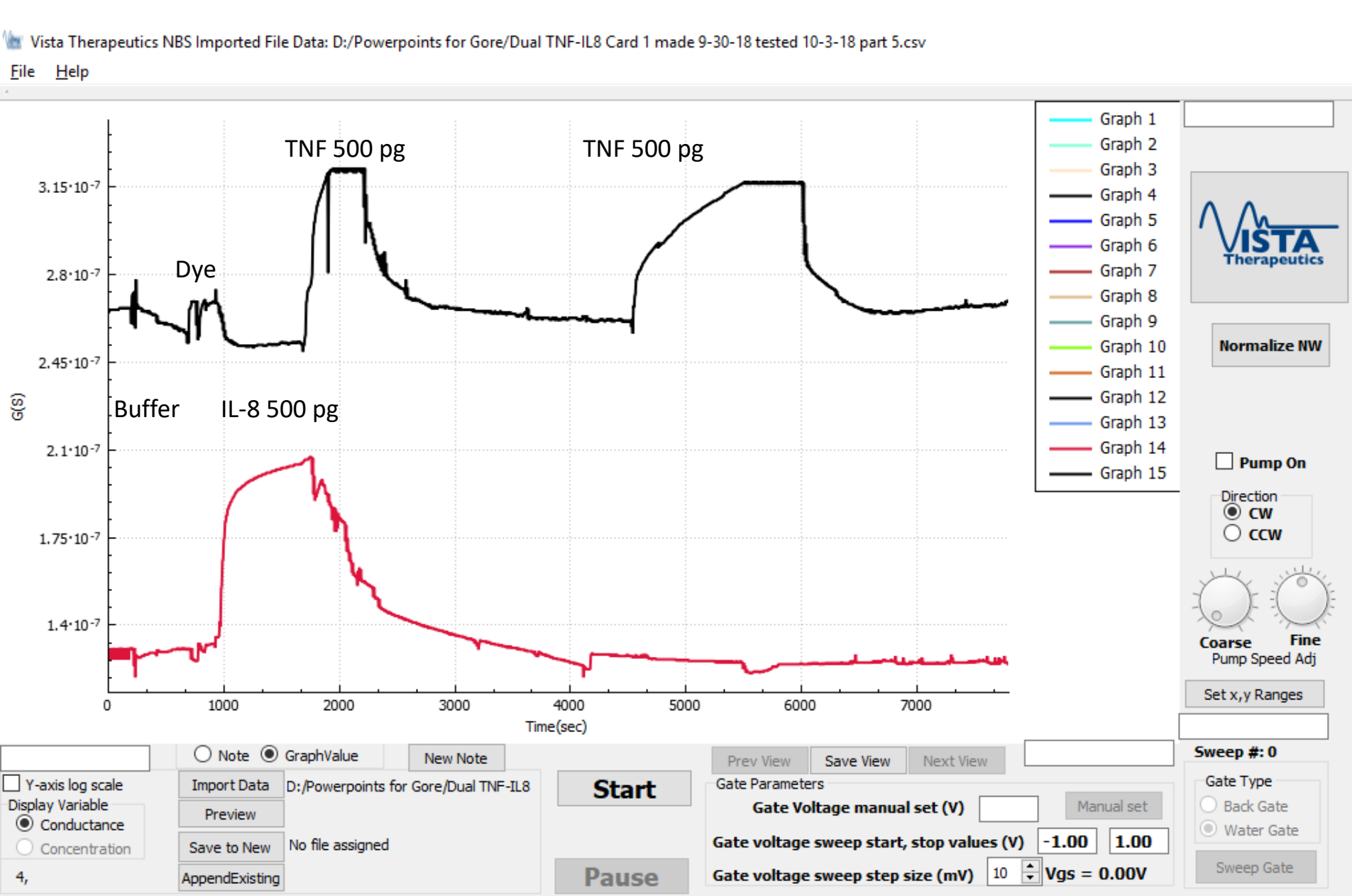
Continuous biomarker monitoring.



This N-Type NanoCard was tested continuously for seven days. Repeat exposure to the same dose of IL-8 elicited the same response after seven days of testing.



IL-8
Number of charges at pH 7.4 = +15.
IEP ~ 9.1
Capture molecule Affimer Diameter 1.5 nm.



Dual IL-8 and TNF-alpha chip exposed repeatedly to TNF and/or IL-8 at 50 and 500 pg ml-1 in 1 X DPBS.

Note no cross reactivity.

Capture molecules:

Anti-TNF Nanobody 2 nm Dia.

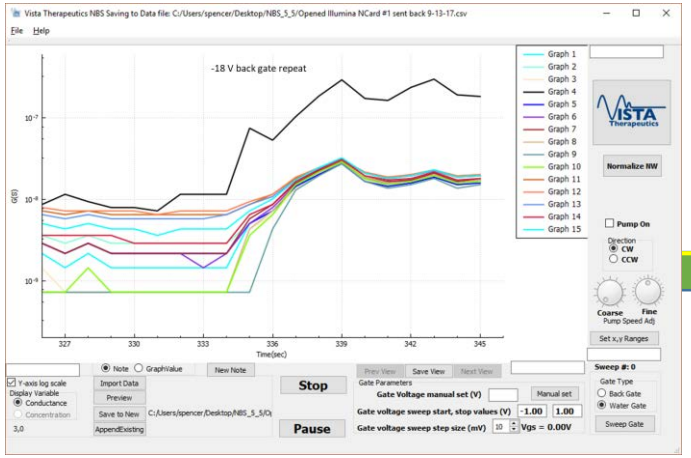
Anti-IL-8 Affimer 1.5 nm Dia.

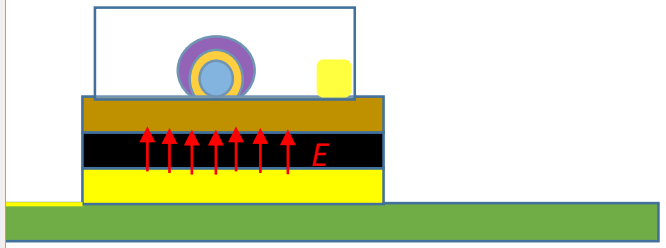
Both capture molecules can be stored dry almost indefinitely.

Multiple normalization and QC tools.

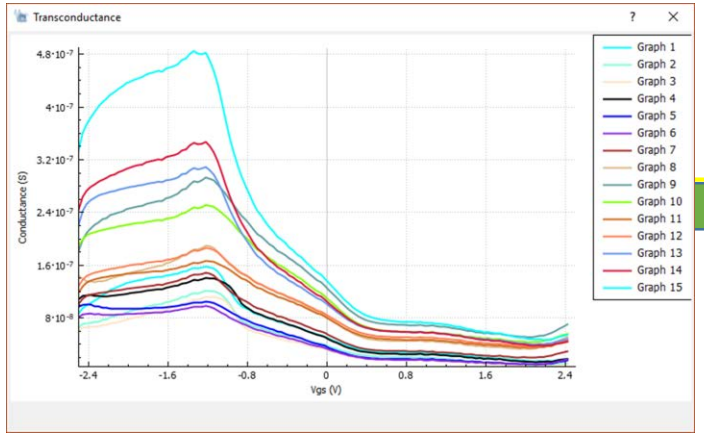
Application of applied, known voltage sweeps allow for the normalization of multiple circuits on multiple NanoCards.

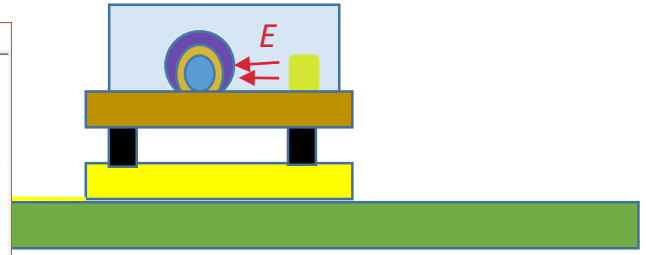
Back gate sweep applies voltage to back of each chip. Can be used dry and thus no damage to capture molecules.



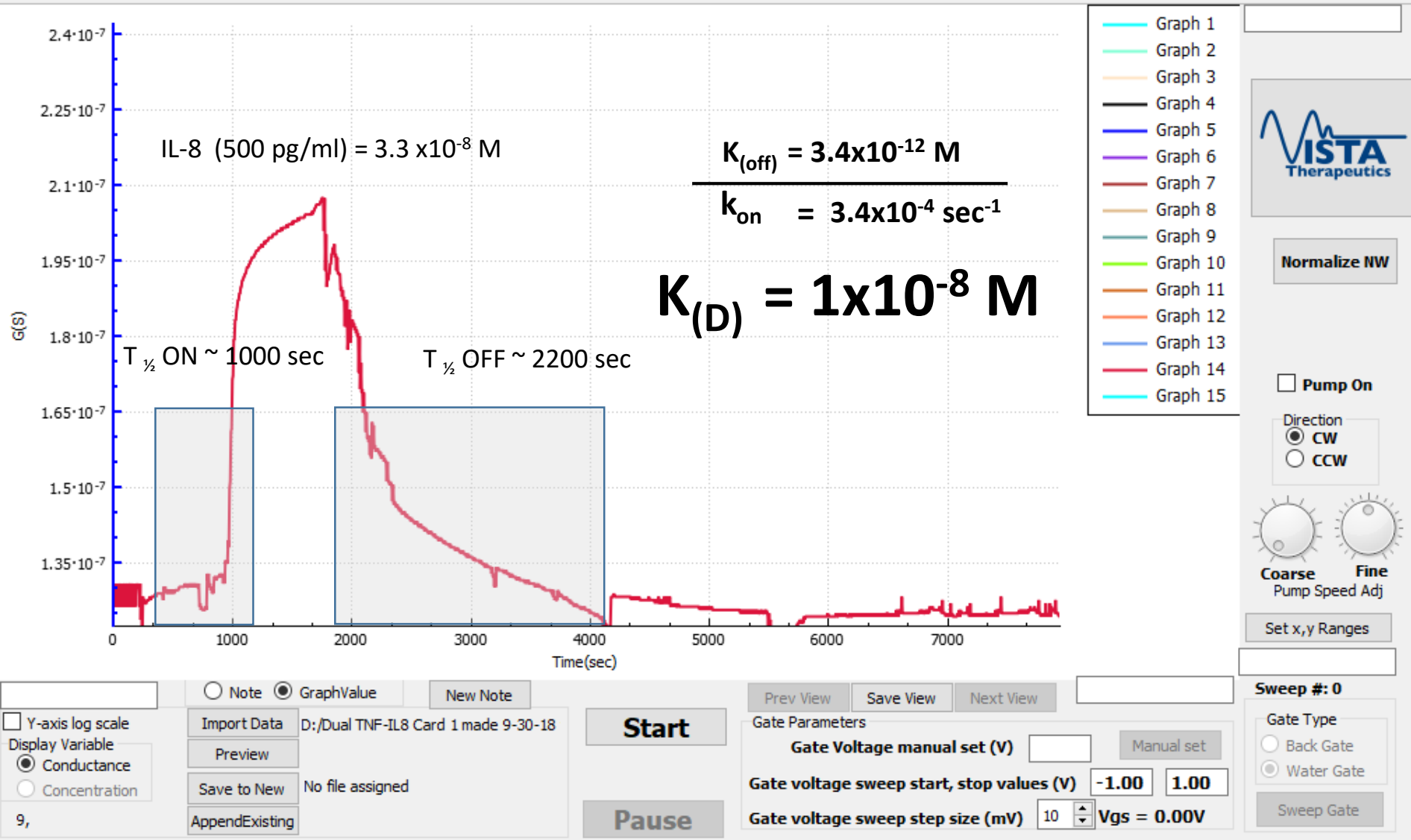


Water gate sweep applies known voltage to all circuits in water, buffer or sample solution.





Easy determination of Equilibrium Dissociation Constant (K_D).



$$K_{(off)} = 3.4\text{x}10^{-12}\ \text{M}$$

$$k_{on} = 3.4\text{x}10^{-4}\ \text{sec}^{-1}$$

$$K_{(D)} = 1\text{x}10^{-8}\ \text{M}$$

$$T_{1/2}\,(on) = \frac{1}{K_{(on)}}\left[AG\right]$$

$$K_{(on)} = \frac{\left[AG\right]}{T_{1/2}\,(on)}$$

$$K_{(on)} = \frac{\left[3.3\text{x}10^{-8}\ \text{M}\right]}{1000}$$

$$K_{(on)} = 3.4\text{x}10^{-12}\ \text{M}$$

$$T_{1/2}(off) = \frac{0.693}{k_{off}}$$

$$k_{off} = \frac{0.693}{T_{1/2}(off)}$$

$$k_{off} = \frac{0.693}{2000\ \text{sec}}$$

$$k_{off} = 3.4\text{x}10^{-4}\ \text{sec}^{-1}$$

By determining T_{on} and T_{off}, one can easily calculate the the Equilibrium Dissociation Constant (K_D).

Kinetic and thermodynamic applications Temperature at nanowire surface can be controlled and monitored.

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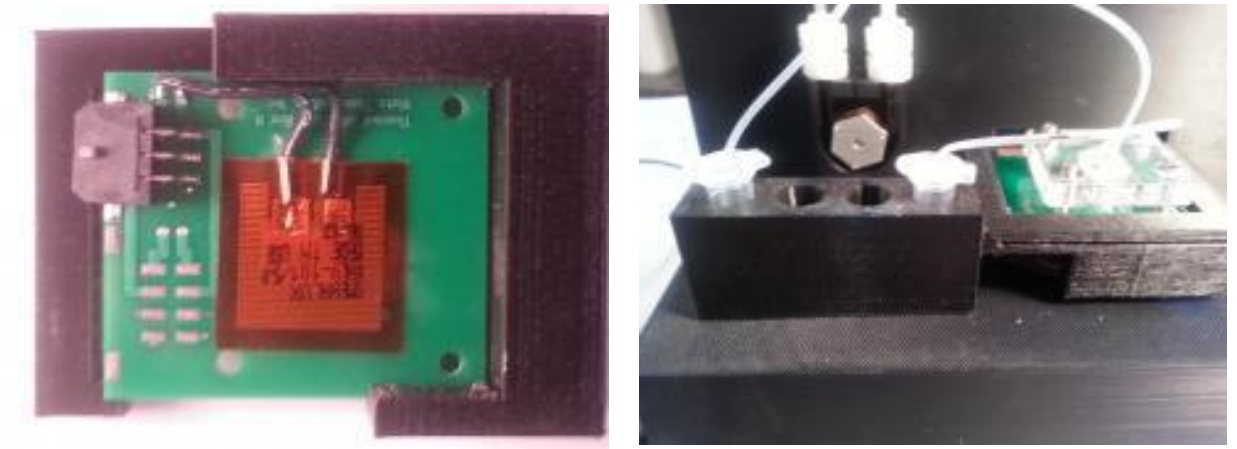
By varying temperatures we can derive the Gibbs free energy of the AB:AG complex. By varying temperature, we can confirm ΔΔG, and we can use ΔG to confirm K_D

$$\Delta G = \Delta H - T\Delta S$$
$$\Delta G = RT\ln K_d$$

ΔG- *Gibbs Free Energy, or "available energy"*
ΔH- *Enthalpy change*
T- *Temperature in Kelvin*
ΔS- *Entropy change*
R- *Gas constant, 8.314JK^{-1}mol^{-1}*
K_d- *Dissociation rate*

$$K_D = e^{(\Delta G/RT)}$$



Temperature controlling Peltier NanoCards with built-in thermistors.

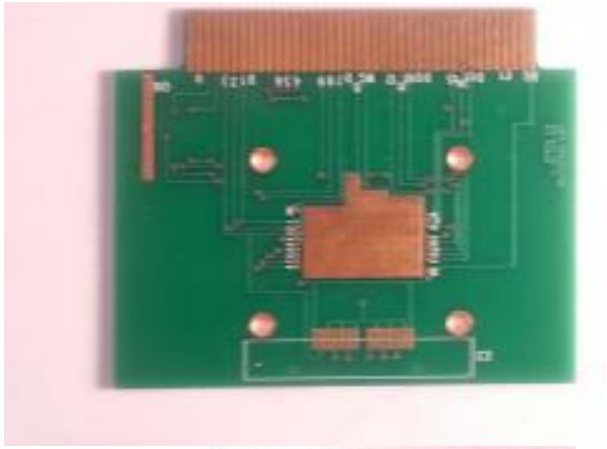
Label-free, real-time PCR

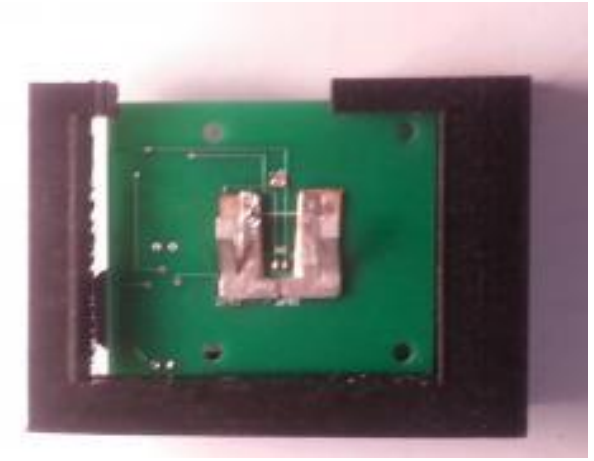
Requirements for NanoBioSensor-Based PCR:

1. Temperature controlled NanoCards
2. Electric field-neutral probes.

Conventional PCR requires labels, many tubes and only measures the amount of total DNA. Additional analysis (i.e. gel electrophoresis) is require to confirm specificity.

Vista's NanoBioSensor with PCR attachment, using just single NanoCard , one measures only the target DNA.

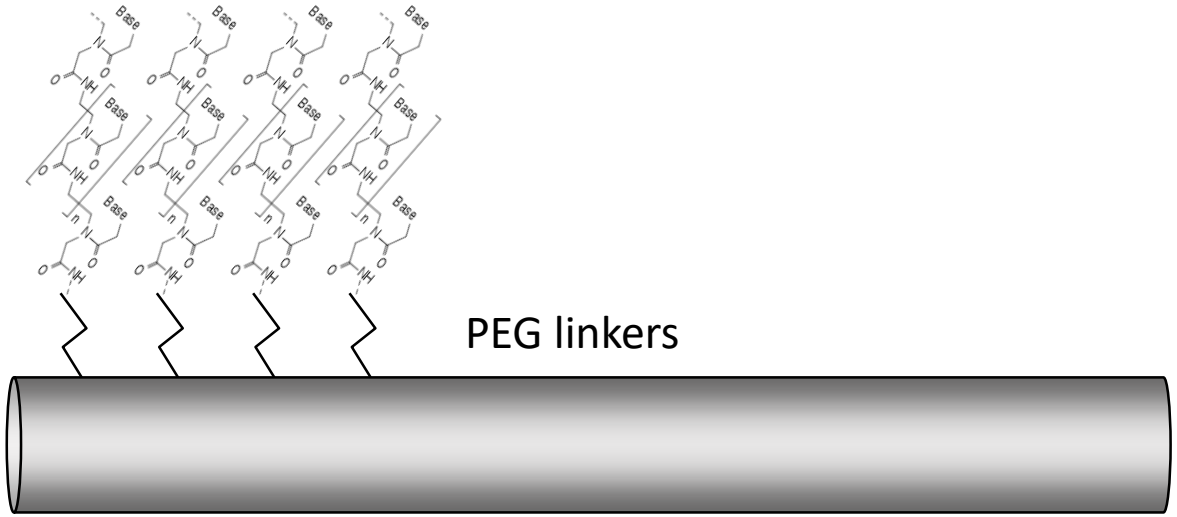


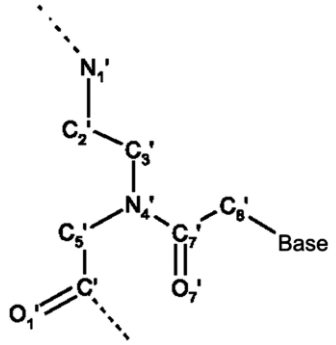


Label-free, real-time PCR

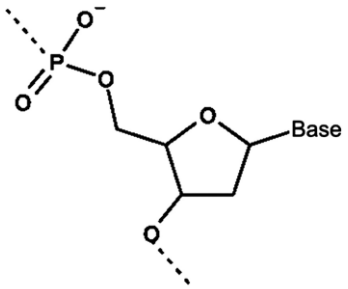
Because standard DNA probes and aptamers are highly charged and create dense and powerful electric fields of their own, they significantly reduce the signal to noise ratio. DNA aptamers and linear DNA probes as well as SOMAmers can detect target biomolecules if they have sufficient charge density.

PNA probes, which are uncharged at neutral pH and when tethered to nanowires by neutral PEG linkers, work quite well with any DNA or RNA targets.



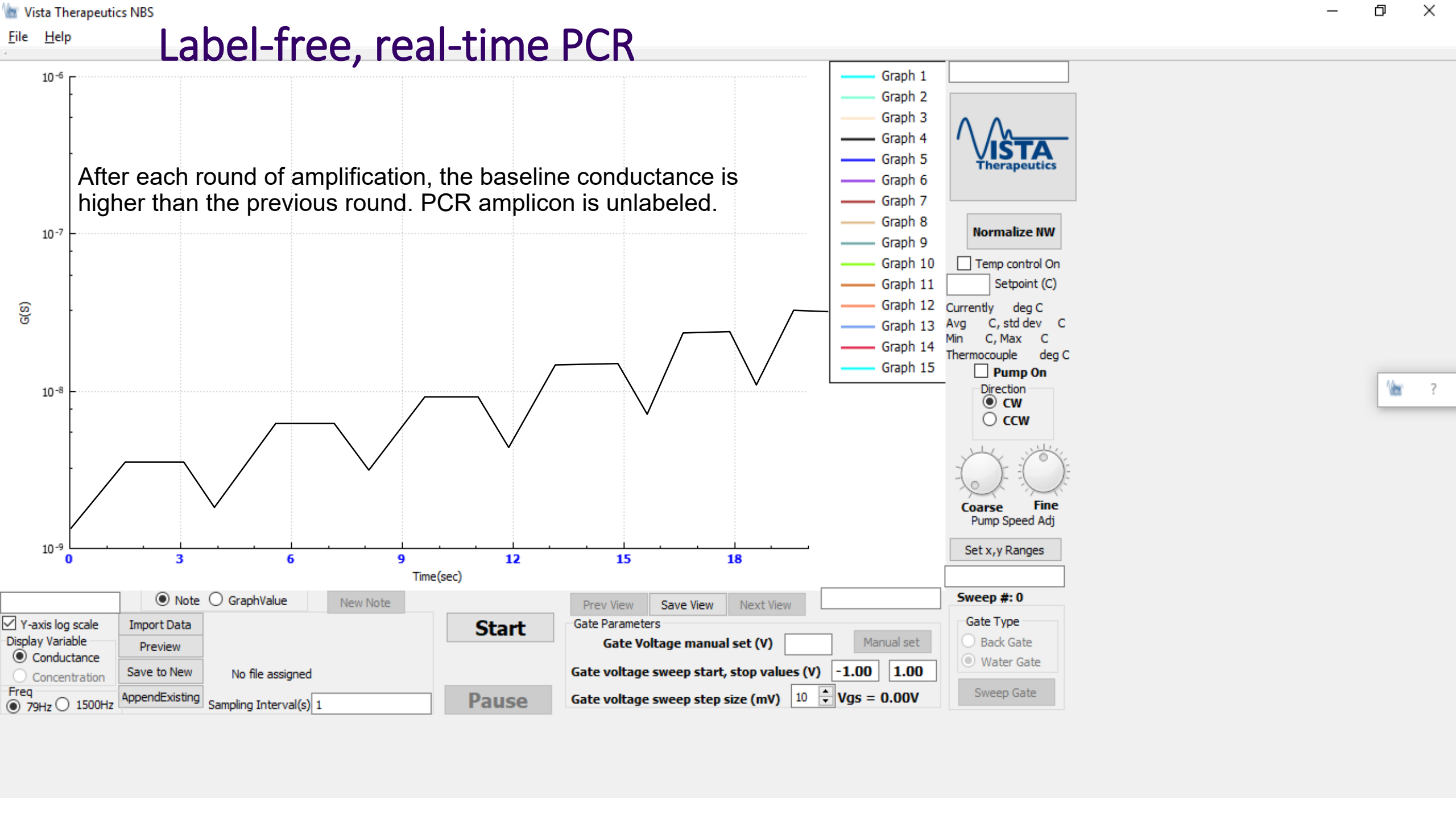


PNA

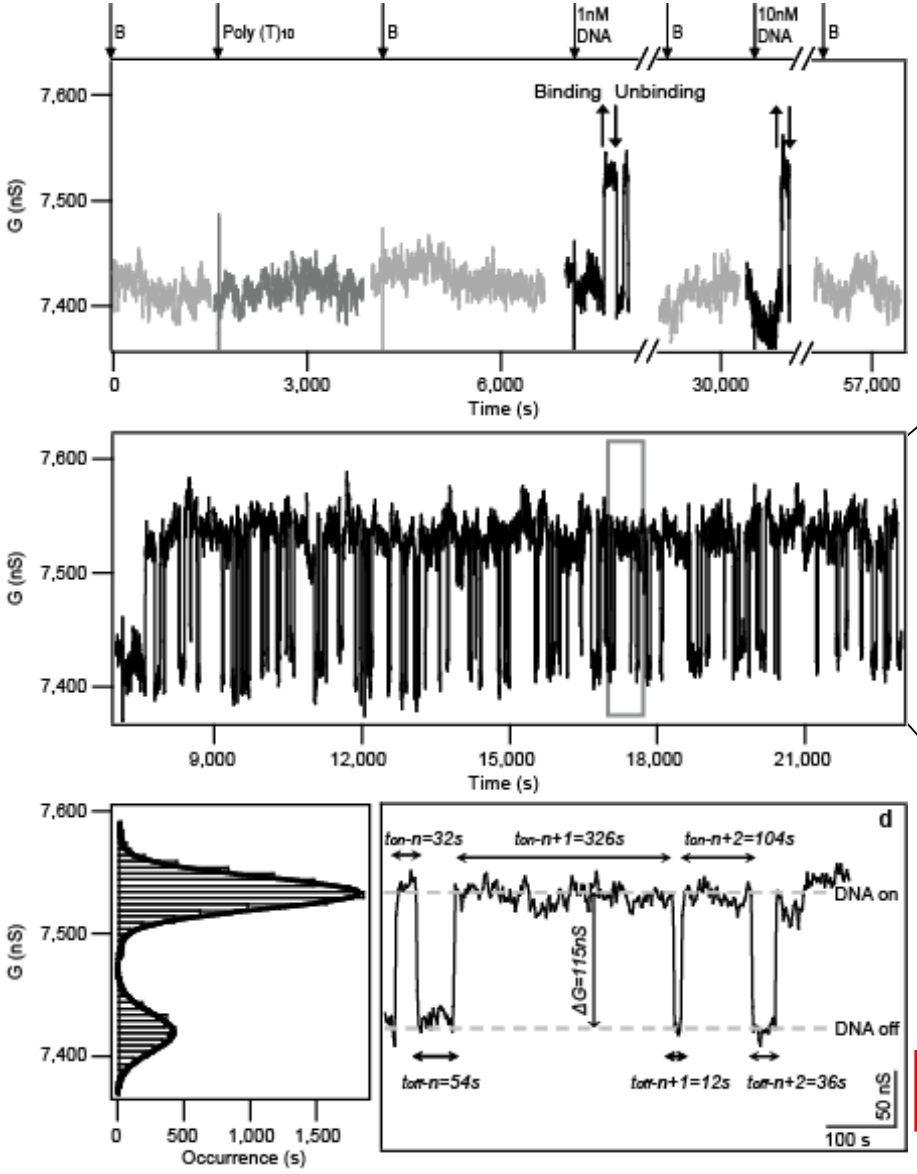


DNA

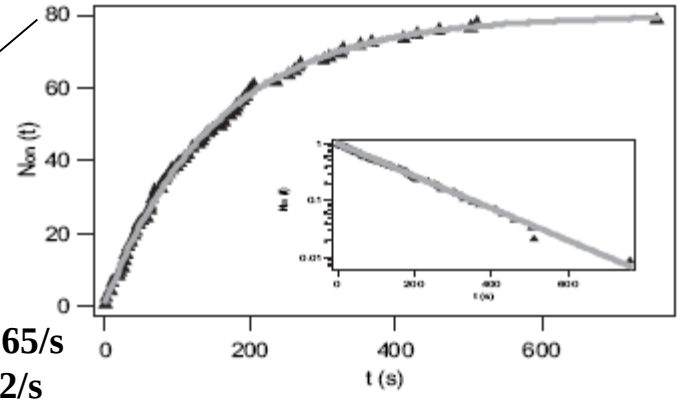
Label-free, real-time PCR



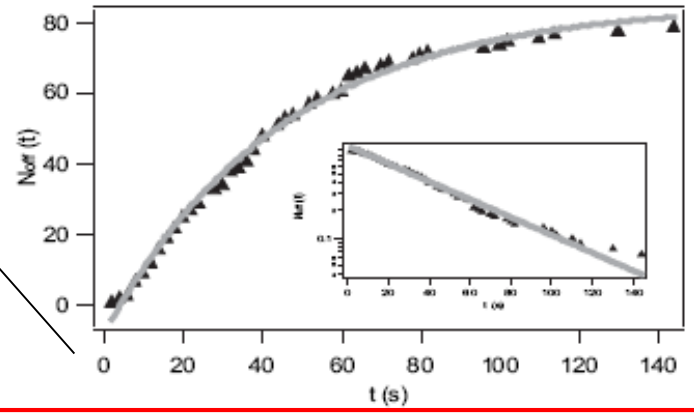
Label-free, PNA-based polymorphism detection.



PNA Receptor: ATCATCTTTG
DNA Target: GTAGAAACTA



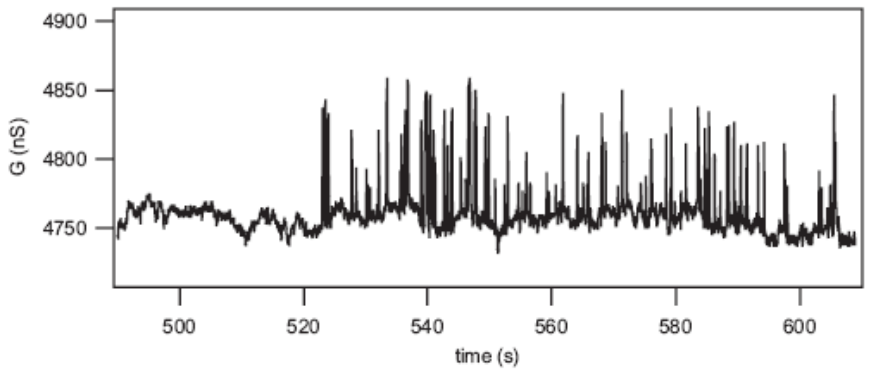
k_{off} = .0065/s
k_{on} = .022/s



ΔQ of 10-charges detected with excellent S/N

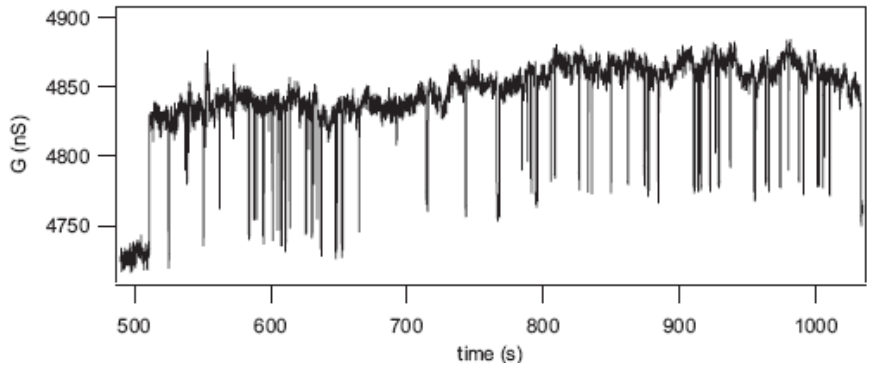
Data courtesy of Charles Lieber.

Label-free, PNA-based polymorphism detection.



PNA Receptor: ATCATCTTTG
DNA Target: TAGTCCAAAC
k_{on} = 1.2 s^{-1}
k_{off} = 14.8 s^{-1}

ΔΔG ~ 2.8 kcal/mol

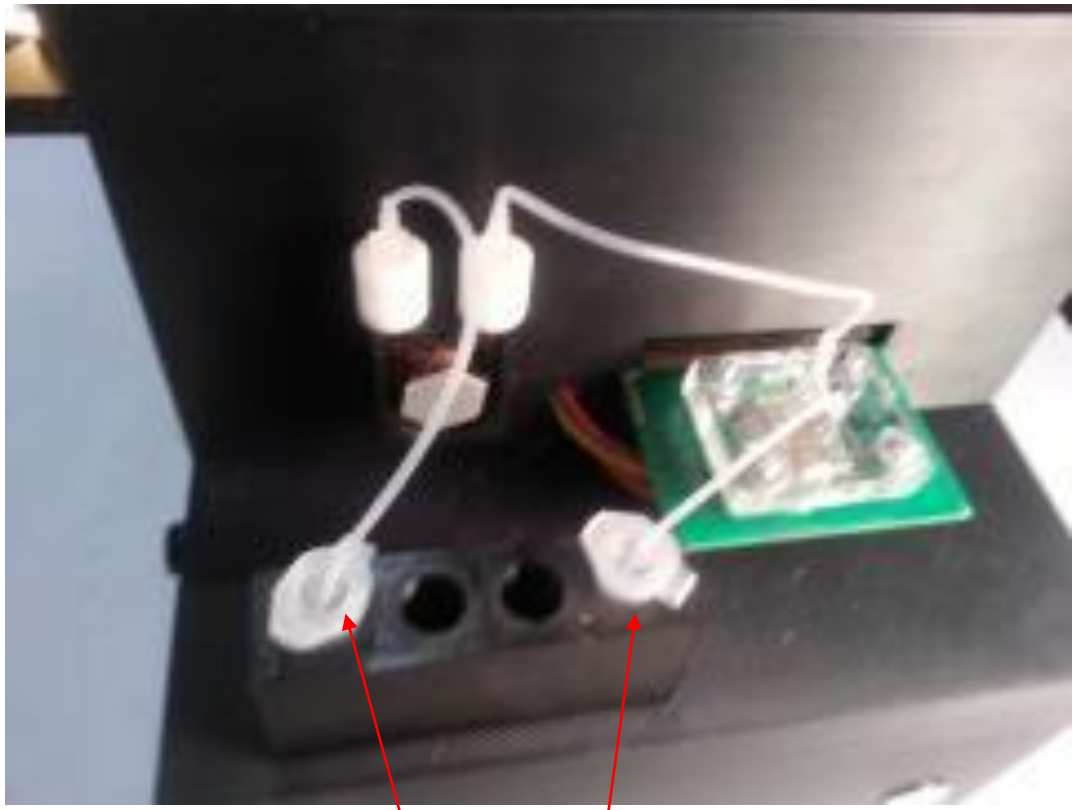


PNA Receptor: ATCATCTTTG
DNA Target: TAGTACAAAC
k_{on} = 1.7 s^{-1}
k_{off} = 0.166 s^{-1}

- Single-base mismatches readily detected through 'visualization' of fundamental binding/unbinding kinetics.
- Can enable new opportunities for rapid DNA analysis and more generally label-free single-molecules studies in biology.

Data courtesy of Charles Lieber.

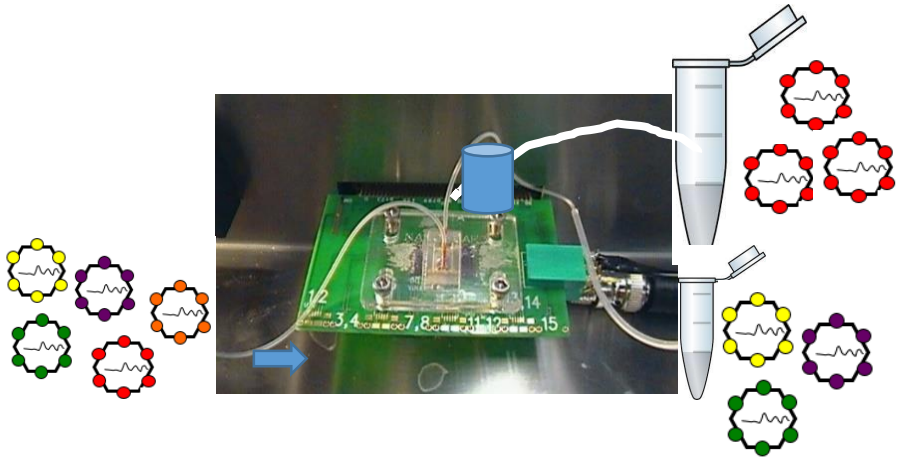
Label-free detection and sample recovery unchanged.

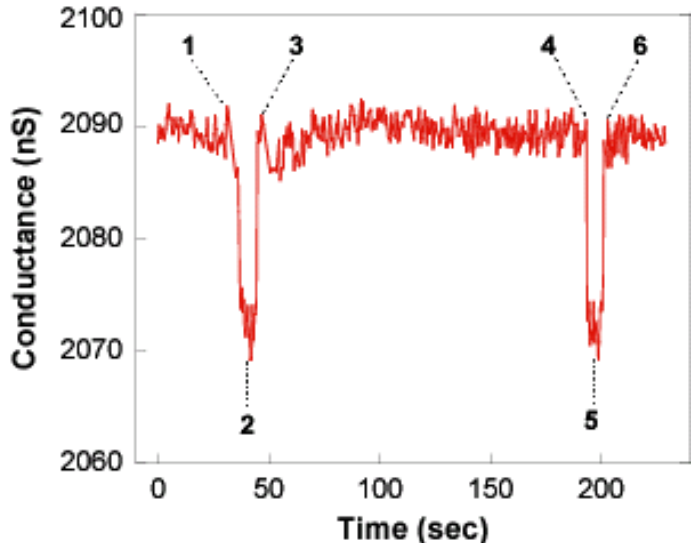


Sample is unchanged by measurement.

One of the major advantages of Vista's NanoBioSensor platform is that there is no labelling required. Samples and PCR products can be recovered unaltered.

By combining electrical detection with signal-controlled gating valves, binders can be isolated from non-binders.

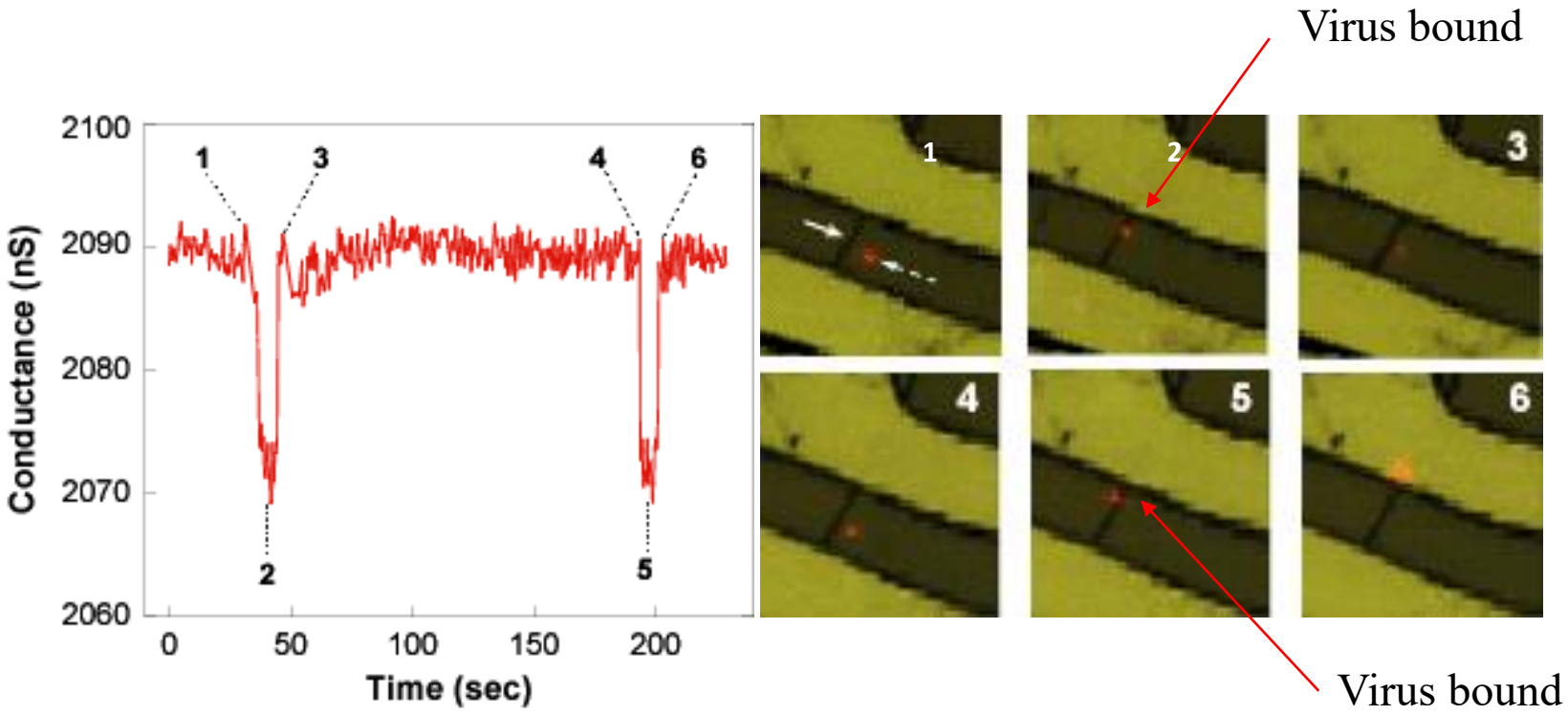




Applications:

- Detection of IgM antibodies in blood to confirm prior exposure to antigenic pathogens such as Zika, Dengue.
- Isolation of candidate antibodies from pool of antibodies.
- Isolate p450s that bind to a specific substrate(s).
- Iterative runs increase purity.
- No labelling required.

Single Viruses Detection: Confirmation through simultaneous optical-electrical measurements: Signal activated gates can isolate binders.



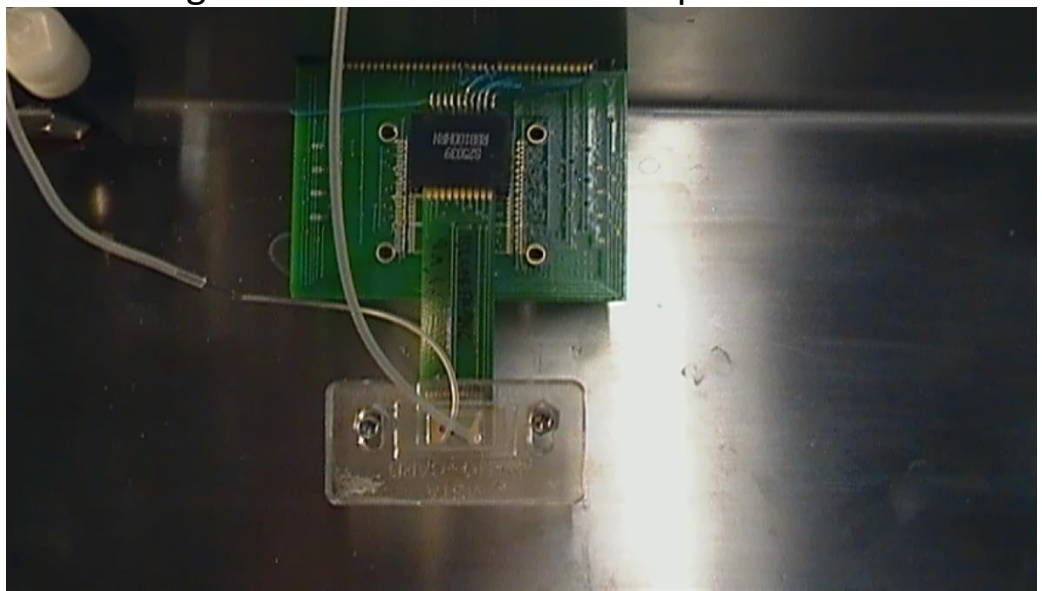
Patolsky, Zhuang, Lieber & coworkers, *PNAS* **101**, 14017 (2004)

Combined with signal-controlled pinch valves, strong binders can be isolated from non- or poor-binders.

Continuous tissue culture monitoring.

Bio-Tracker™ for continuous, flow-through or rapid field sample measurement.

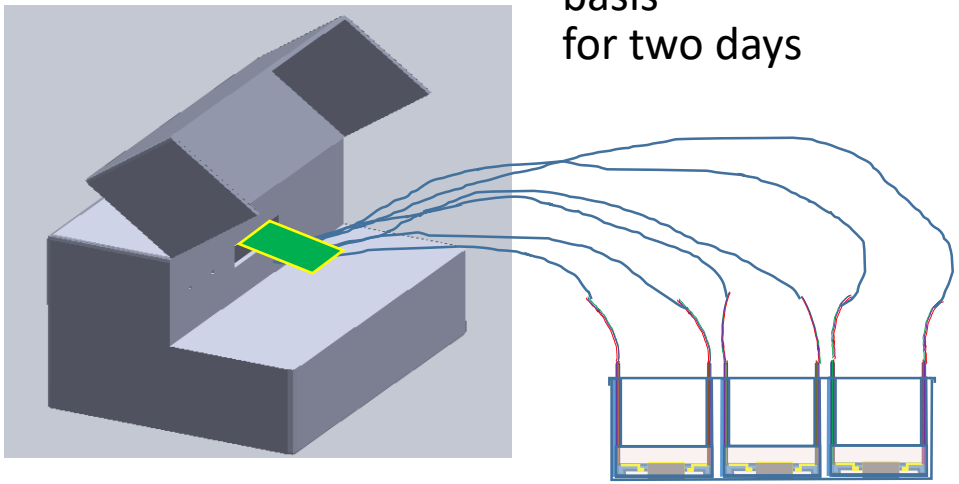
- Rapid one-time or continuous biomarker measurement
- 48-hour continuous monitoring in tissue culture wells
- Adaptable for flow-through applications.
- Can be used with 20 µl blood and urine
- Compatible with all NBS units.
- Lids and gaskets for use with six-well plates.



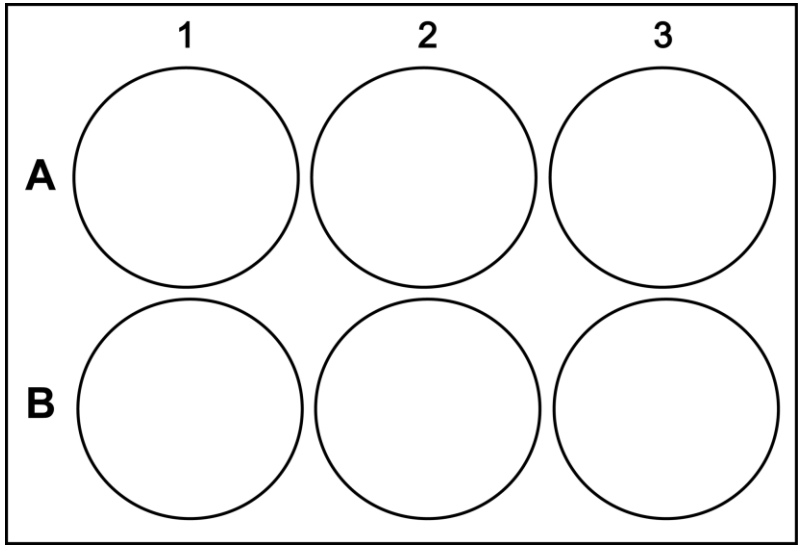
The Awesome Power of Small

Vista's 'Well-Dwell' chip for continuous cell culture monitoring.

Continuous Biomarker monitoring on real time basis
for two days



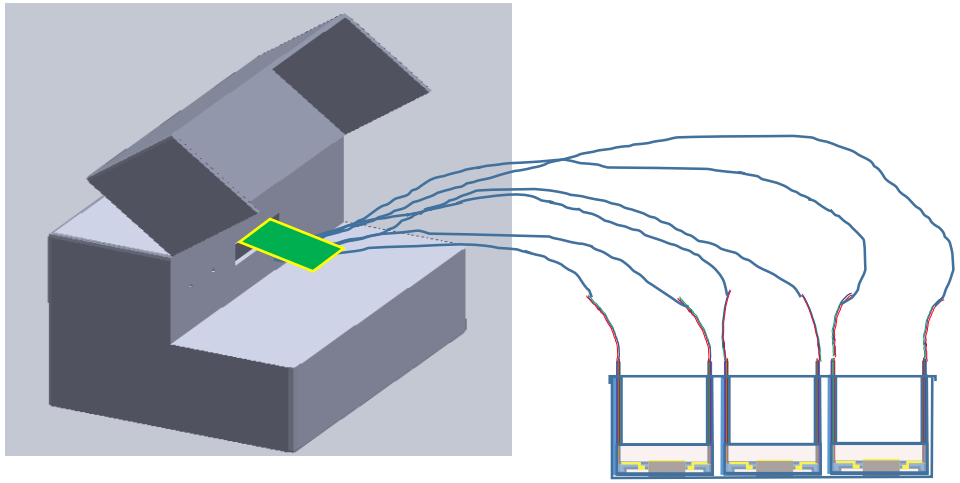
Dip-Chips sit at the base of each of well and continuously monitor the concentration of biomarkers for up to several days.



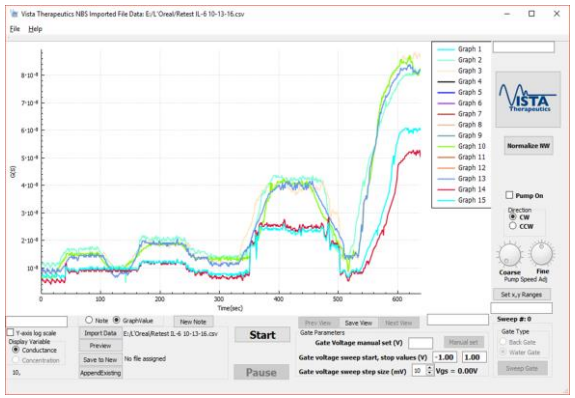
Vista's 'Well-Dwell' chip for continuous cell culture monitoring.

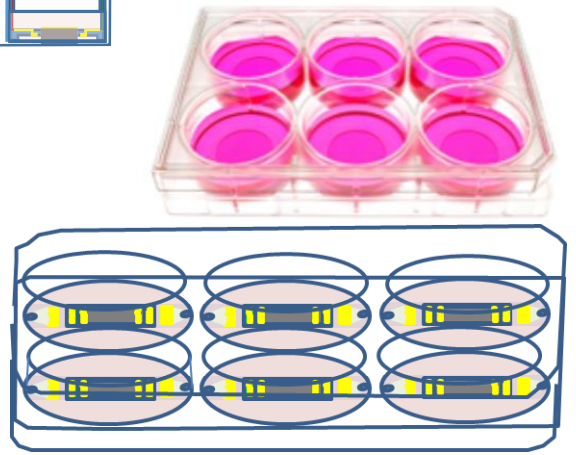
The Awesome Power of Small

Continuous Biomarker monitoring on real time basis for two days



Dip-Chips sit at the base of each of six well and continuously monitor the concentration of biomarker 'x' in each well once per second for up to two days.



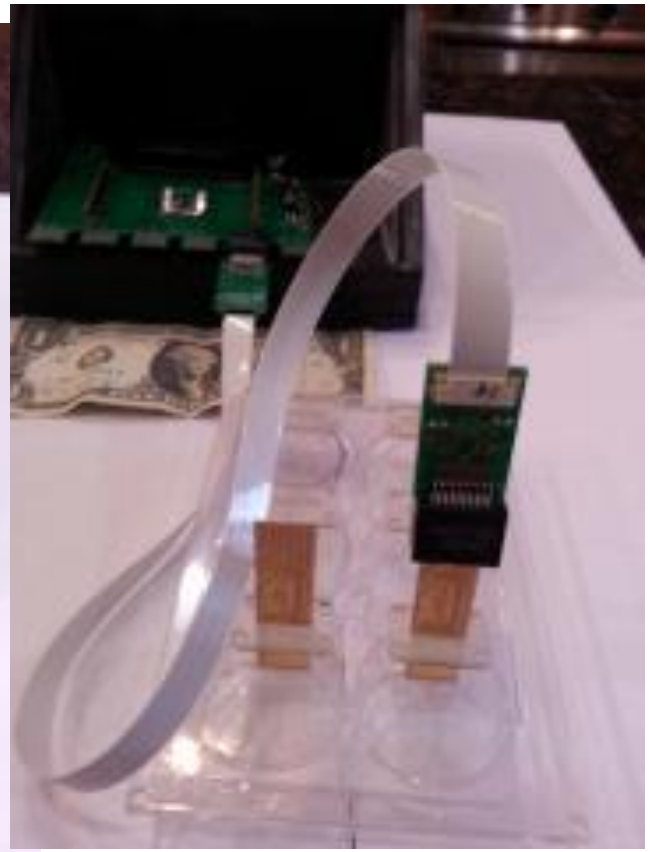


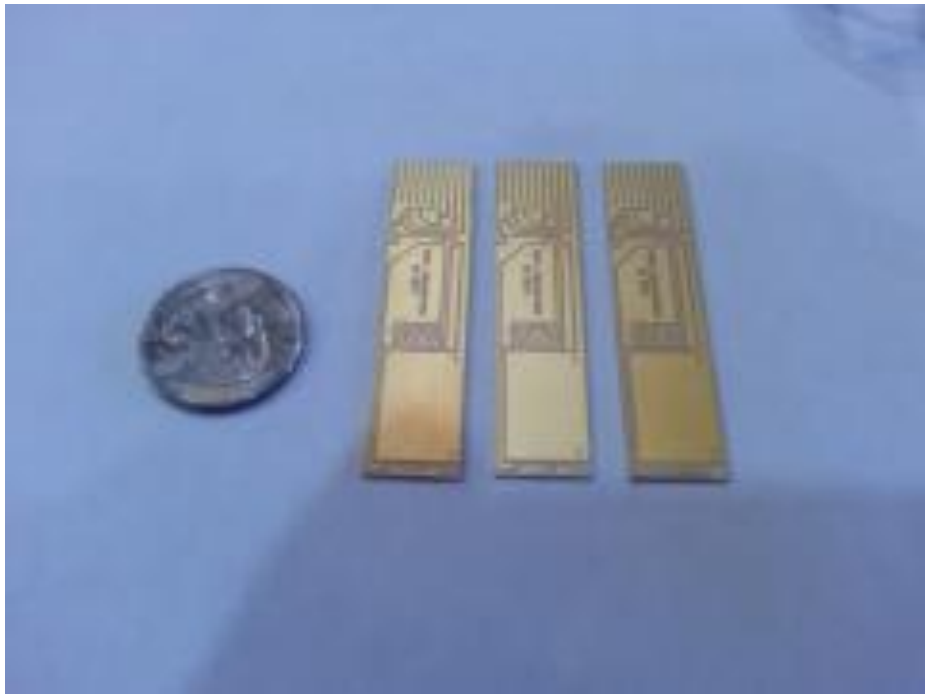
Size of NBS components.

Original form factor for NanoBioSensor. Updated Board could probably fit.

DipChip and connection to NBS unit. New NBS has all components built into motherboard.

Ceramic DipChips (no chip attached yet). Need parylene Conformal coating.

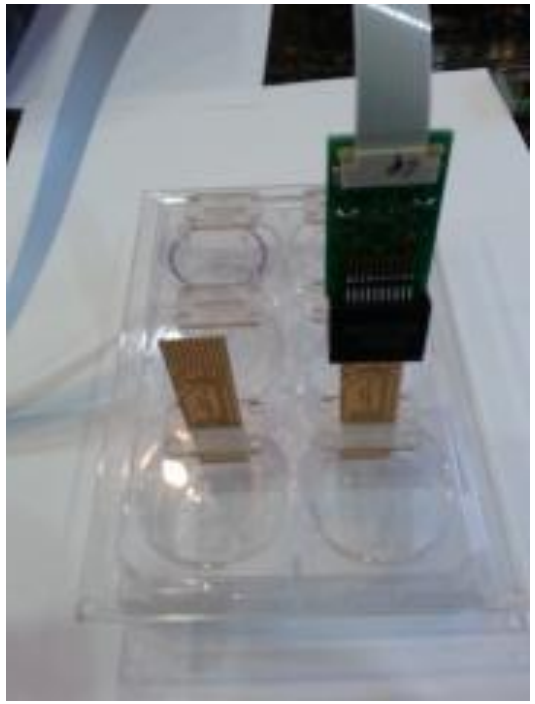




Size of NBS components.

Submersible Dip-Chip for six-well plate.

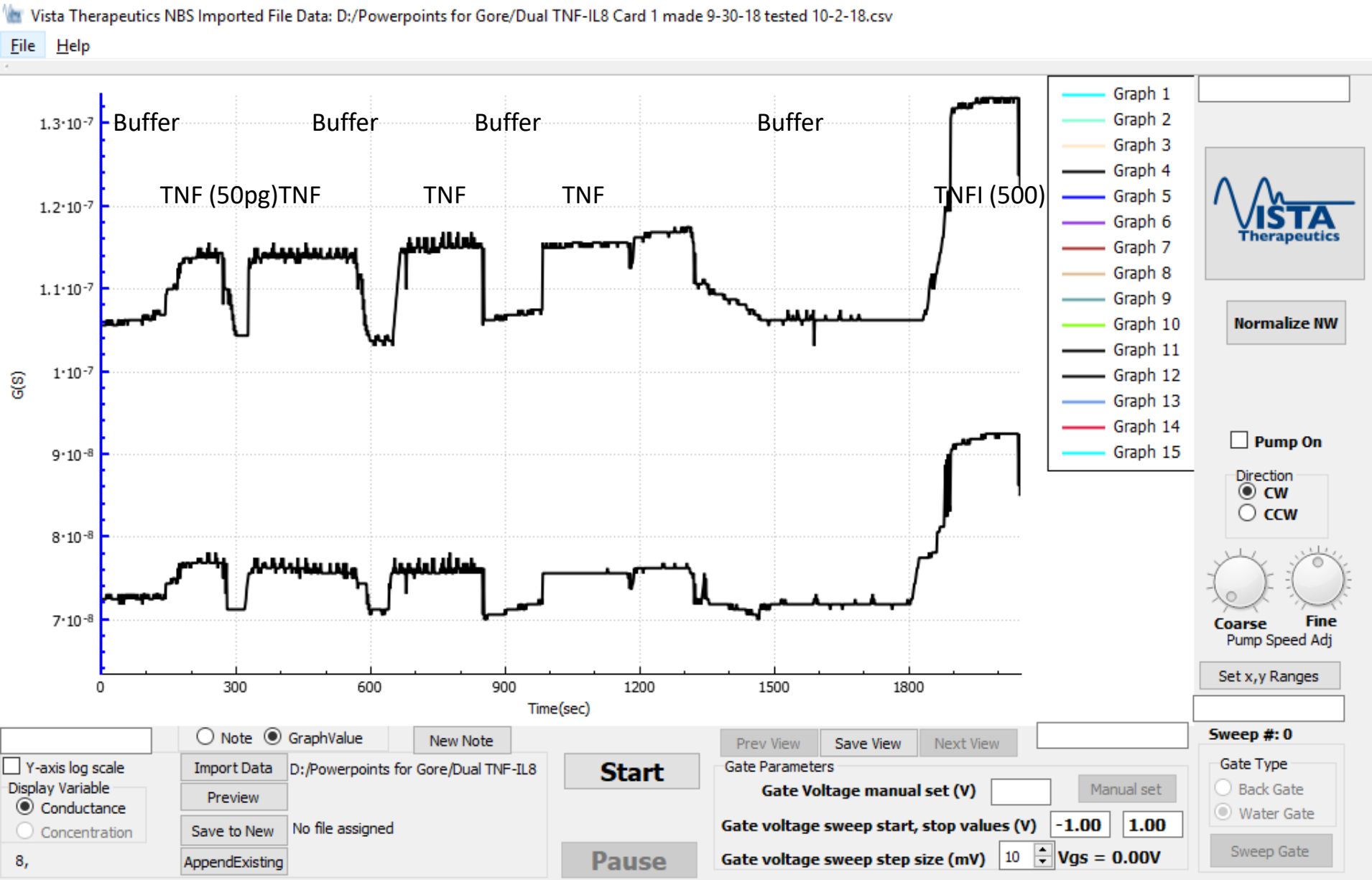
Built-in band-pass filter reduces EMI from antenna effect of ribbon wires.



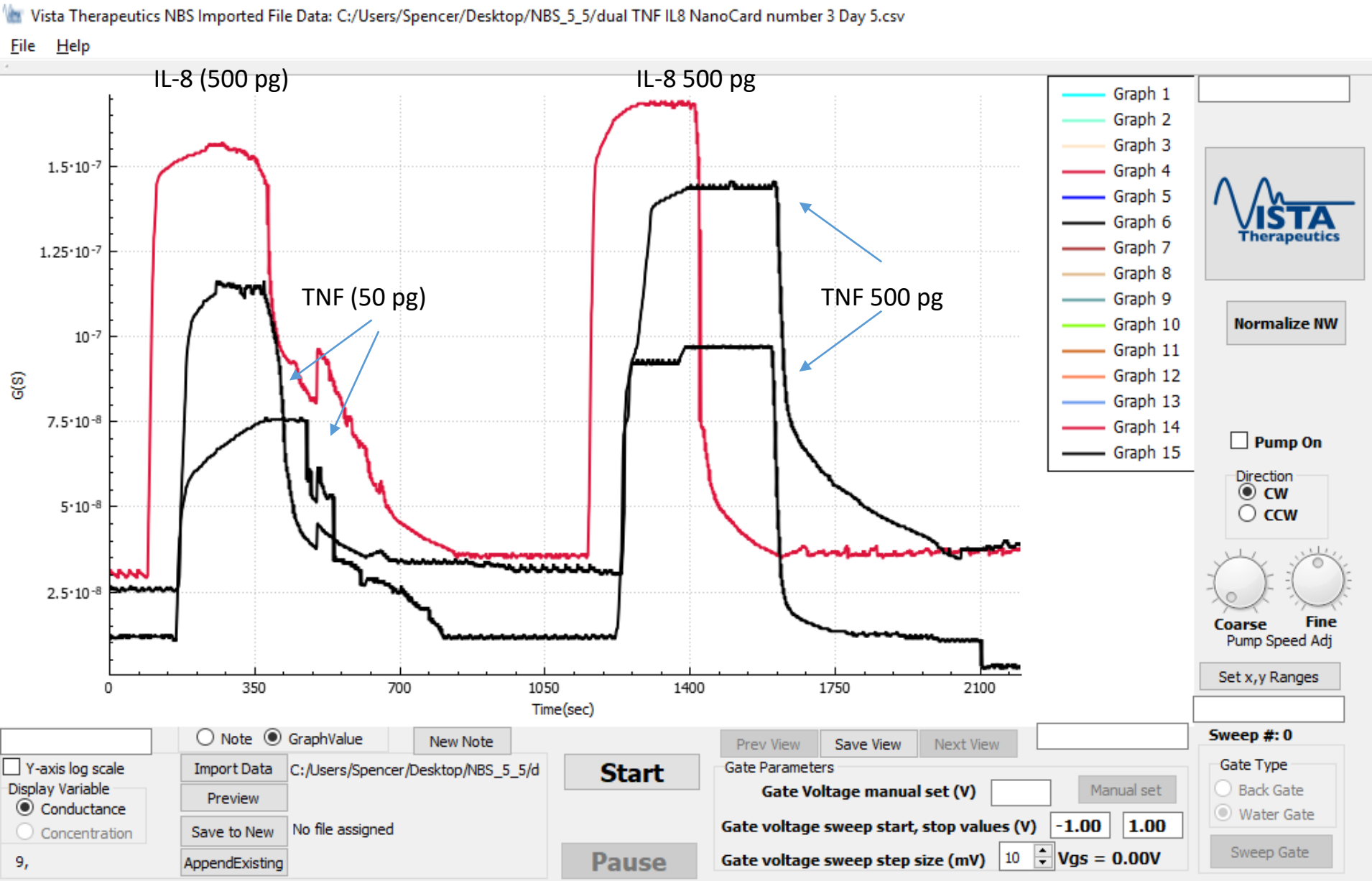
VISTA

Bio Nano Technology

The Awesome Power of Small



TNF-alpha chip exposed repeatedly to DPS-HAC buffer and TNF at either 50 or 500 pg/ml as indicated.



This was third Dual TNF-IL-8 NanoCard tested after five days of continuous flow. This was a new card made with higher concentrations of NanoBody and Affimer The sensitivity was somewhat higher at approximately 115 nS for IL-8 at 500 pg/ml and 80 nS for the two TNF circuits.

End Here