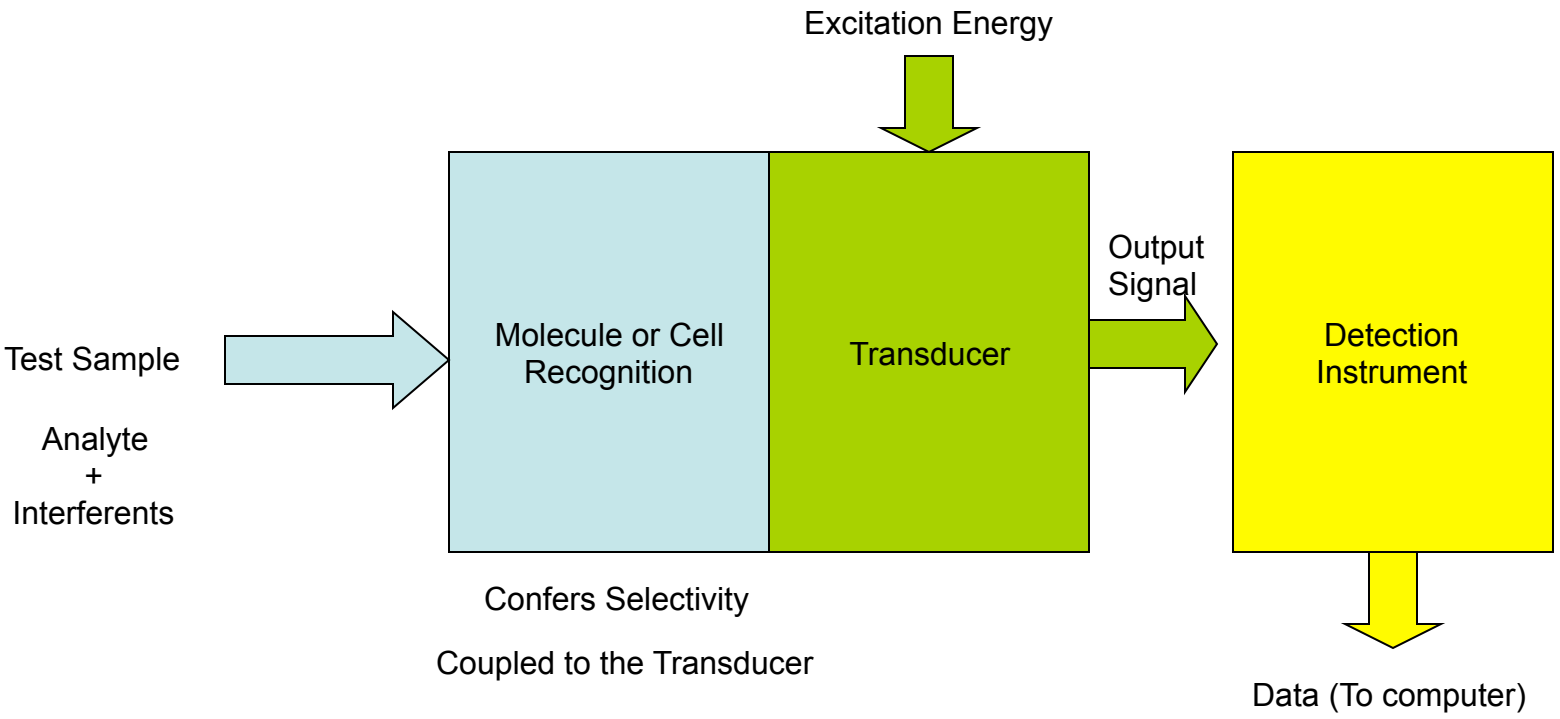
Introduction to Biosensors

Lecture 2

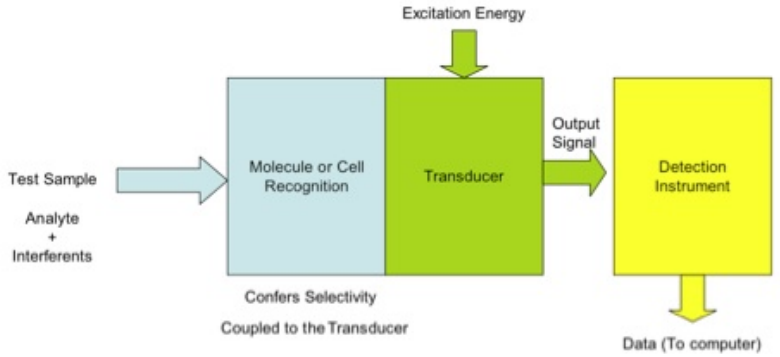
Biosensor Language

Definition of a Biosensor

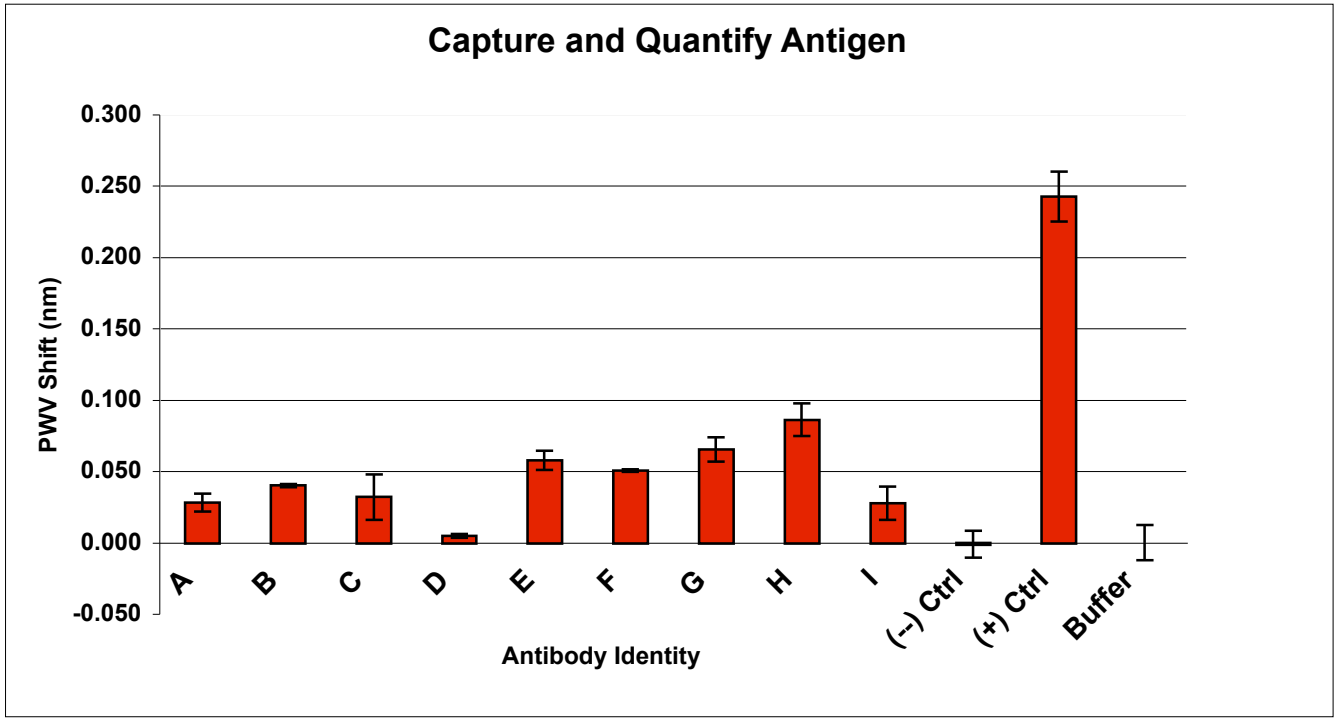


Test Sample

- Simple
  - Contains analyte in buffer solution without any additional materials
  - Examples
- Complex
  - Contains analyte mixed with a variety of other materials
  - Examples

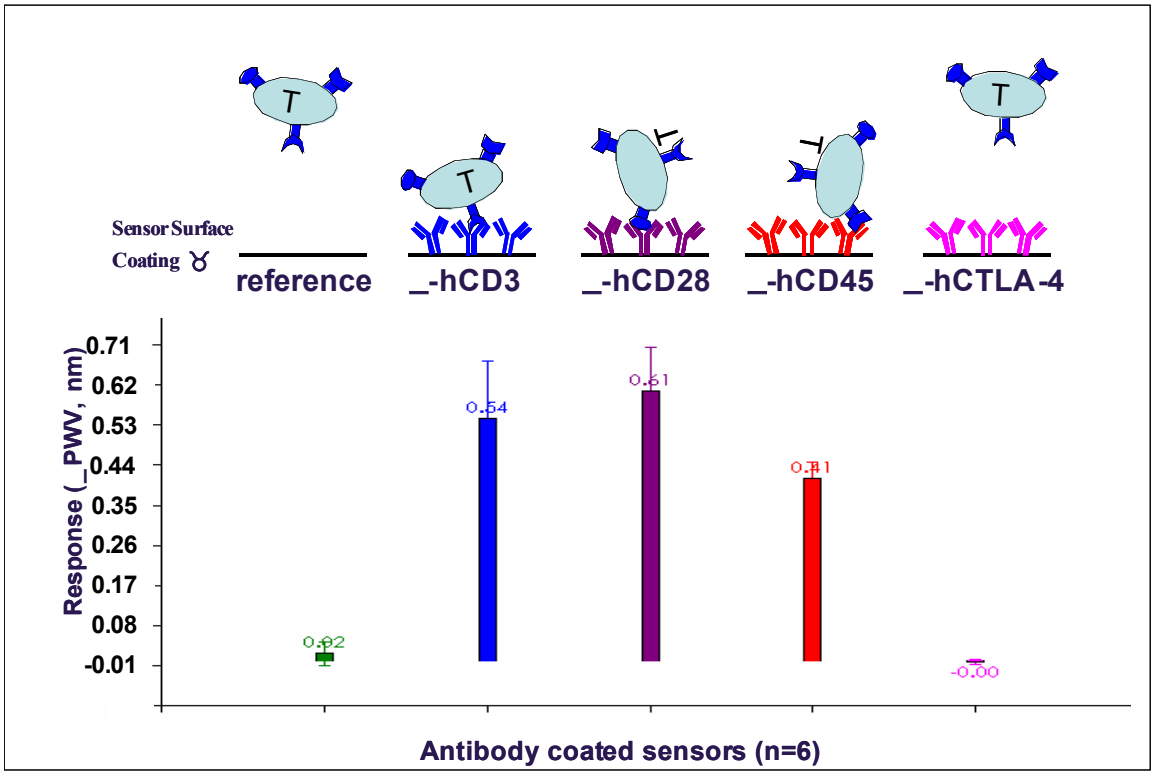


Simple test sample - proteins



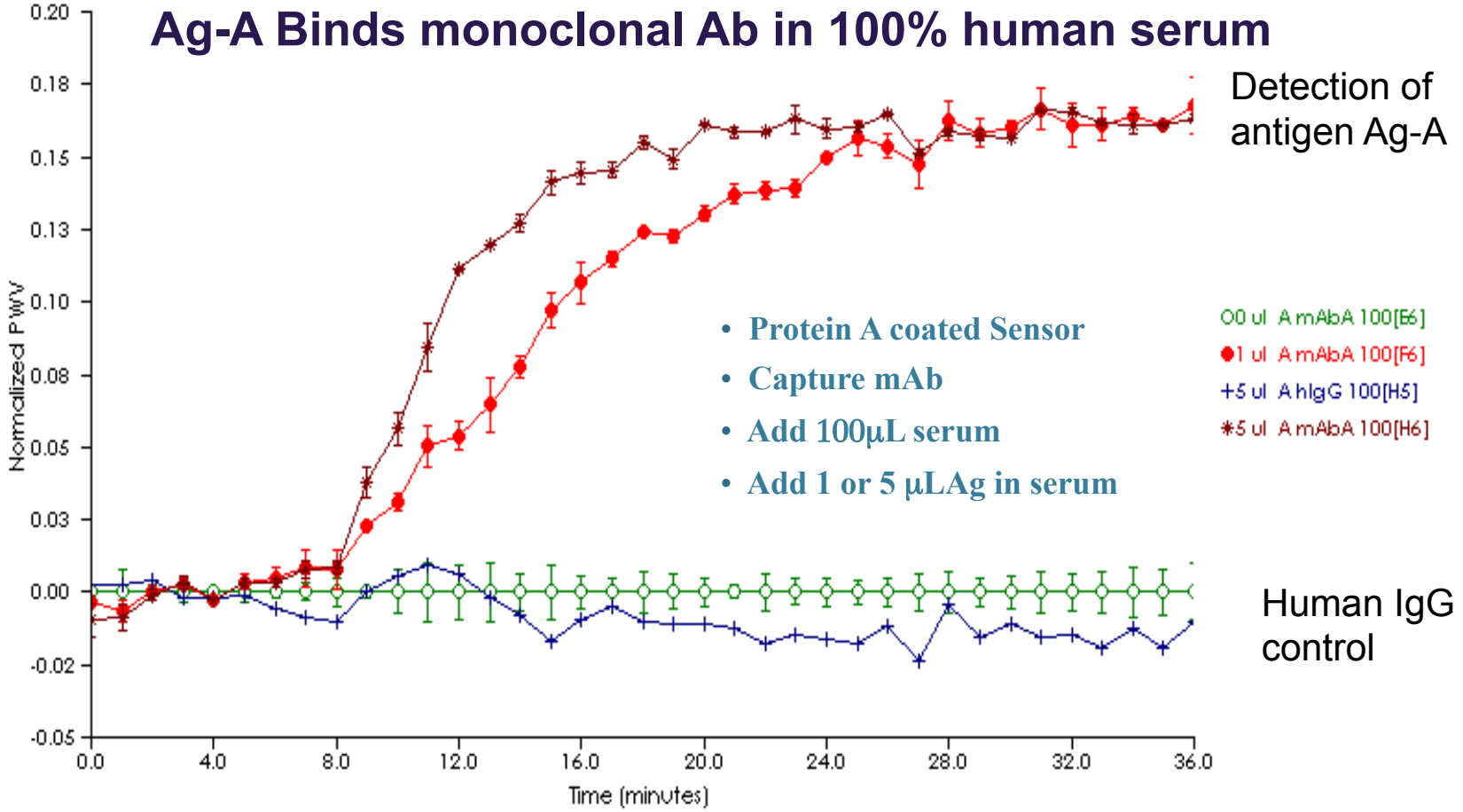
Capture and quantification of the antigen. Antigen (M_r >10,000) was added to the nine antibodies that were captured in different wells in the previous step. The PWV immobilization level is directly related to the amount of antigen that is captured in each well. The controls are performing exactly as expected and each of the nine antibodies is capturing different amounts of antigen.

Simple sample - cells

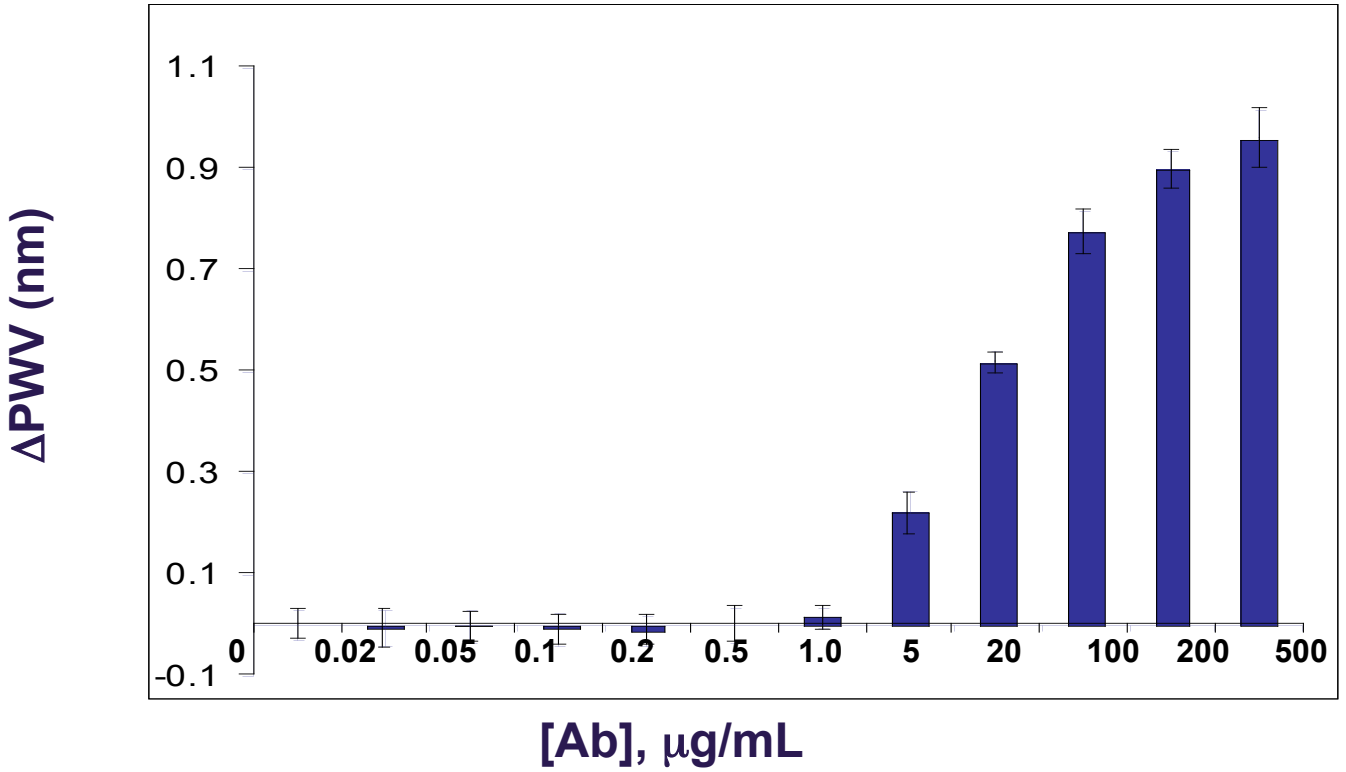


Non-adherent cells can be identified and quantified for specific native cell surface protein production. The endpoint data from a BIND label-free experiment shown above demonstrates the capability for the system to identify, capture, and quantify cells expressing specific proteins on their surface. For the experiment, the sensor was coated with an antibody (M_r ~150,000) that recognizes the protein present on the cell surface. Unbound antibody is removed from the well and subsequently about 10,000 to 100,000 Jurkat cells are added to the antibody-coated wells. Where no antibody was coated or antibody for a non-existent cell surface protein is coated, no signal is obtained when the Jurkat cells are added to the well. The same type of experiment can also be performed with adherent cells.

Complex sample: Serum



Direct Ab Binding From 100% Human Plasma



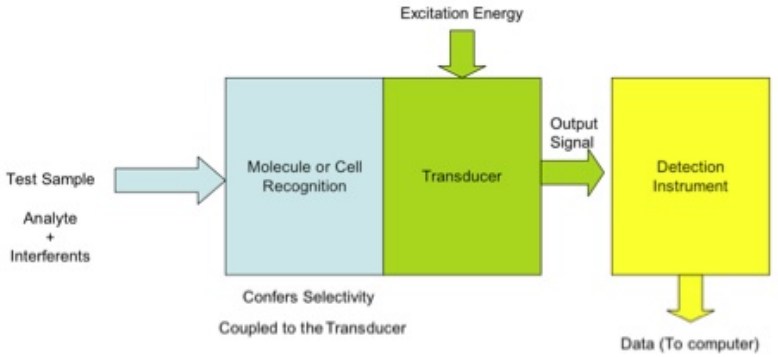
- **Ab binding to immobilized antigen on the sensor plate**

Analyte and Interferents

- Analyte
  - The substance in the test sample that we are trying to detect
  - Try to determine
    - Presence
    - Concentration
    - Biochemical "affinity" with molecular recognition layer attached to the transducer
  - Examples
- Interferents
  - Things that are in the test sample along with the analyte that we do not wish to detect
  - The biosensor cannot tell the difference between analytes and interferents unless the "recognition" element only allows the analyte to transduce a signal
  - Biosensor signal output that is caused by detection of interfering material is called "NONSPECIFIC"
  - Examples

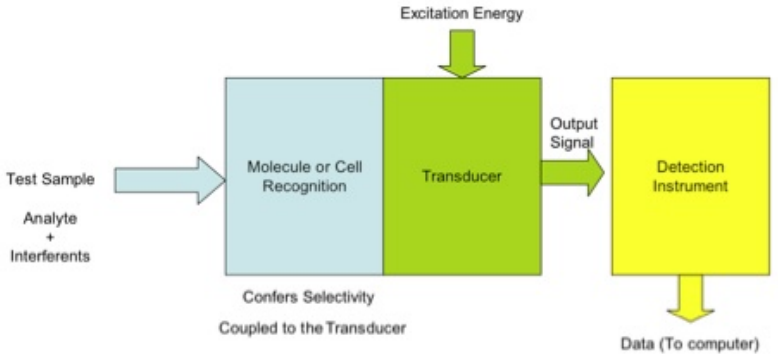
Molecule and Cell Recognition

- A layer of material applied to the surface of the transducer
- Selected to form a strong chemical association specifically with a particular analyte
- Examples
- Material applied to the transducer is "IMMOBILIZED" on the sensor surface
  - Often called an IMMOBILIZED LIGAND
- We want the detection to be
  - SPECIFIC
  - SELECTIVE



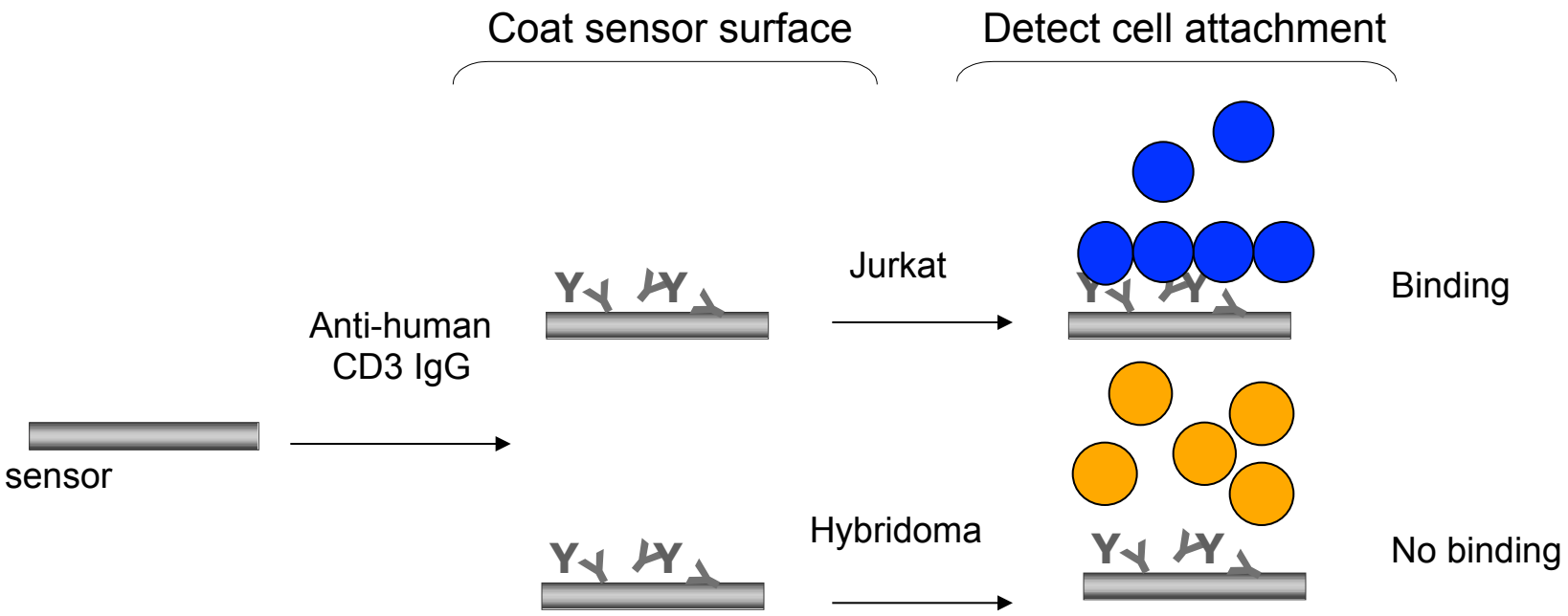
Specificity/Selectivity

- Ability to detect the analyte while not detecting the interfering materials
  - DNA-DNA binding
  - Protein-Protein binding
- Possible problem situations:
  - Low concentration of analyte and high concentration of interferents
  - Many possible interferents present at the same time at nearly the same concentration as the analyte



Specific Cell Detection

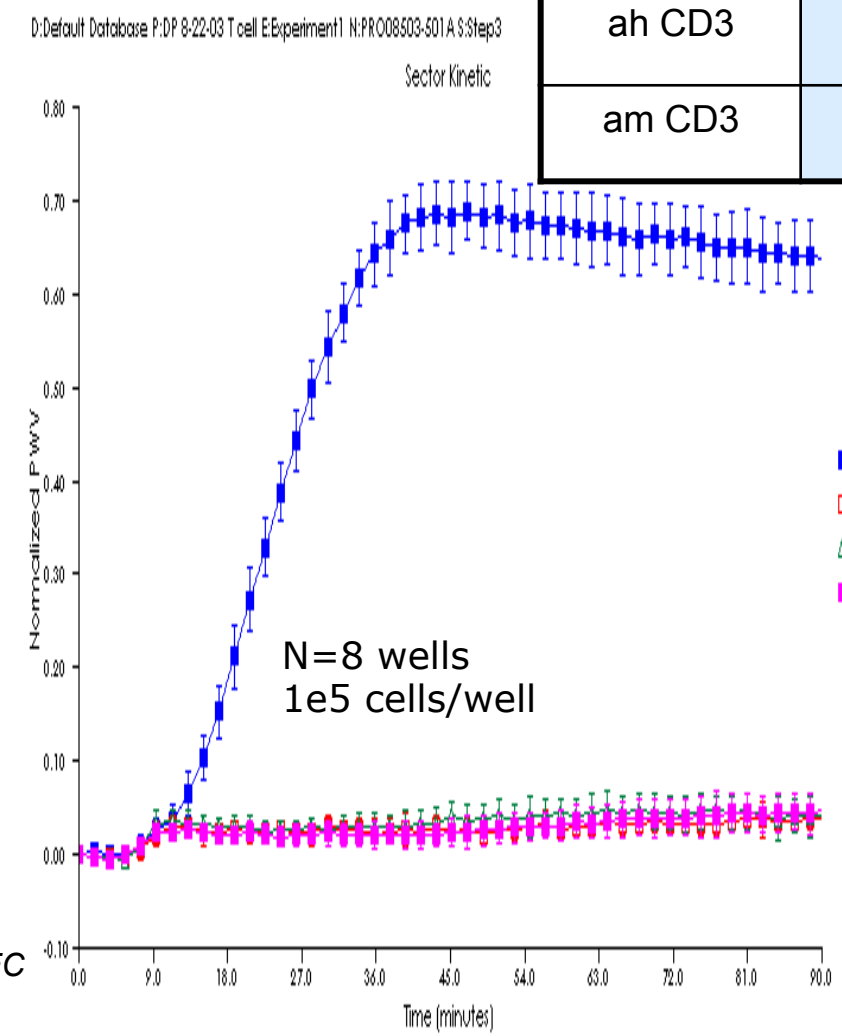
- Differentiate/Identify cells by proteins expressed on outer surface
- Simple two-step process
  - Apply protein antibody to sensor surface
  - Expose to test sample containing cells

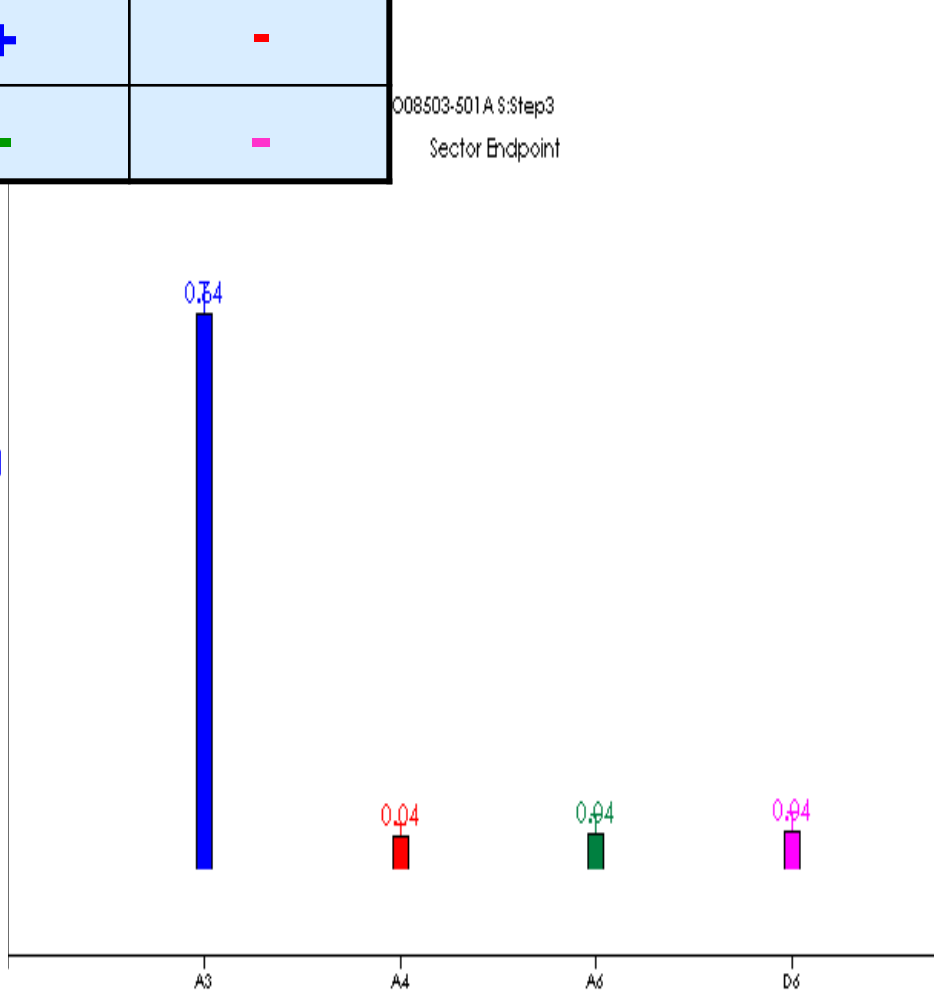


Specific Cell Detection

- Jurkat cells specifically binding to anti-human CD3 sensor surface

	Jurkat	Hybridoma
ah CD3	+	-
am CD3	-	-





EC

Coupling to the Transducer

- How to attach immobilized ligands to the transducer?
- Not as simple as it sounds!
- Requirements
  - Do not alter the FUNCTIONALITY of the molecule
  - Cannot easily be rinsed away from the transducer
  - Present at high density
- Many possible methods - selection will depend on the specific ligand and the specific transducer

Transducer

- Device that can translate the presence of the analyte into a physically measurable signal

Unique physical properties	Tags	General Physical Properties
Electrochemical Cell (redox potential)	Fluorescent Tag	Acoustic Sensor (mass)
Mass Spectrometer (charge/mass ratio)	Radioactive Tag	Optical Sensor (dielectric permitivity)
Raman Spectrometer (excited electronic transitions)	Nanoparticle Tag	Height Sensor (size)
		Electrical Sensor (impedance)

RED = Covered in 416

Transducer Selection

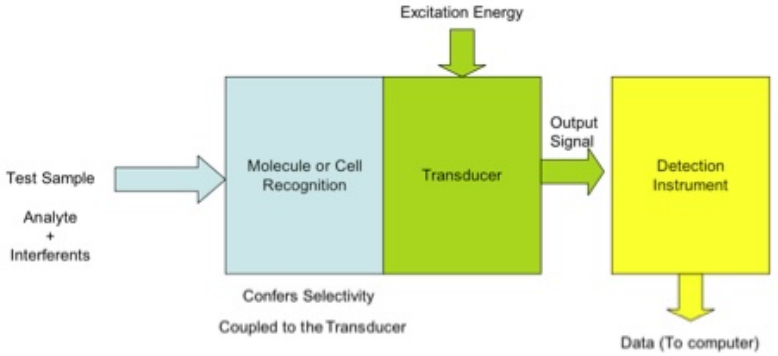
- Depends on requirements of the application

Applications	Requirements
Single-use Disposable	Sensitivity
Disease Diagnosis by Protein Detection	Cost/assay
Need to perform 500,000 assays/day	Throughput
Need to gather continuous data over time	Kinetics
Sensors implanted in the body	Size

Signal

The output of the transducer is collected by some type of instrument that can report/record/process the measurement

- Simple
  - Voltmeter
  - Optical intensity detector (photodiode or photomultiplier tube)
  - Geiger counter
- More complex
  - Acoustic resonator circuit
  - Atomic force microscope
  - Optical Spectrometer
- Even more complex
  - Imaging instruments
    - Scanners
    - CCD imagers
  - Mass spectrometer



Figures of Merit

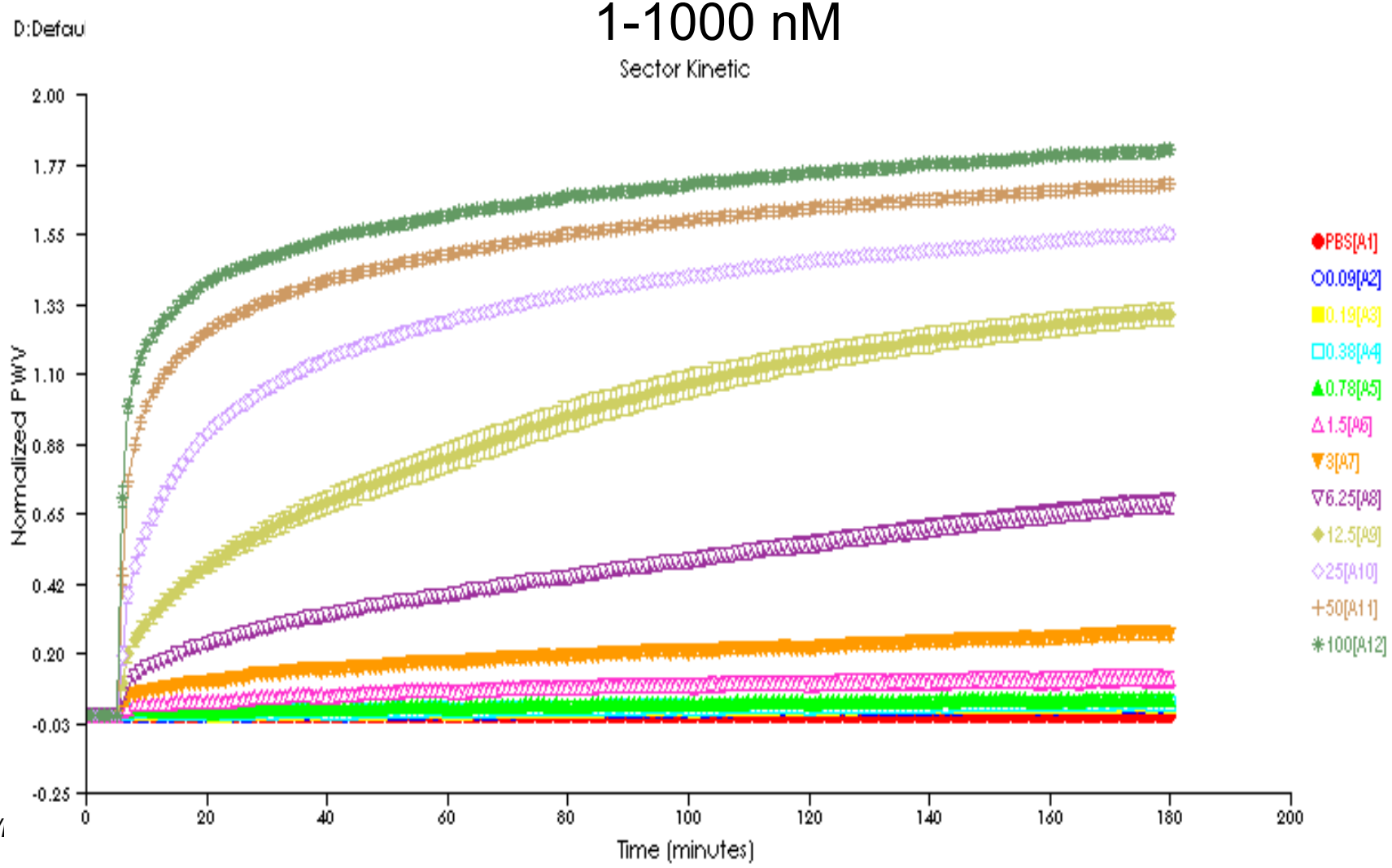
How can you tell if a sensor is good enough for your application, and how would you compare it to another type of sensor?

- Two components of every measurement
  - Signal
    - How much does the output change in response to a known analyte concentration
  - Noise
    - Any variable in the output of the transducer that is NOT due to the analyte
    - RESONATORS help reduce random noise
      - What's a resonator?

Protein-Protein: Pig-IgG on Protein-A

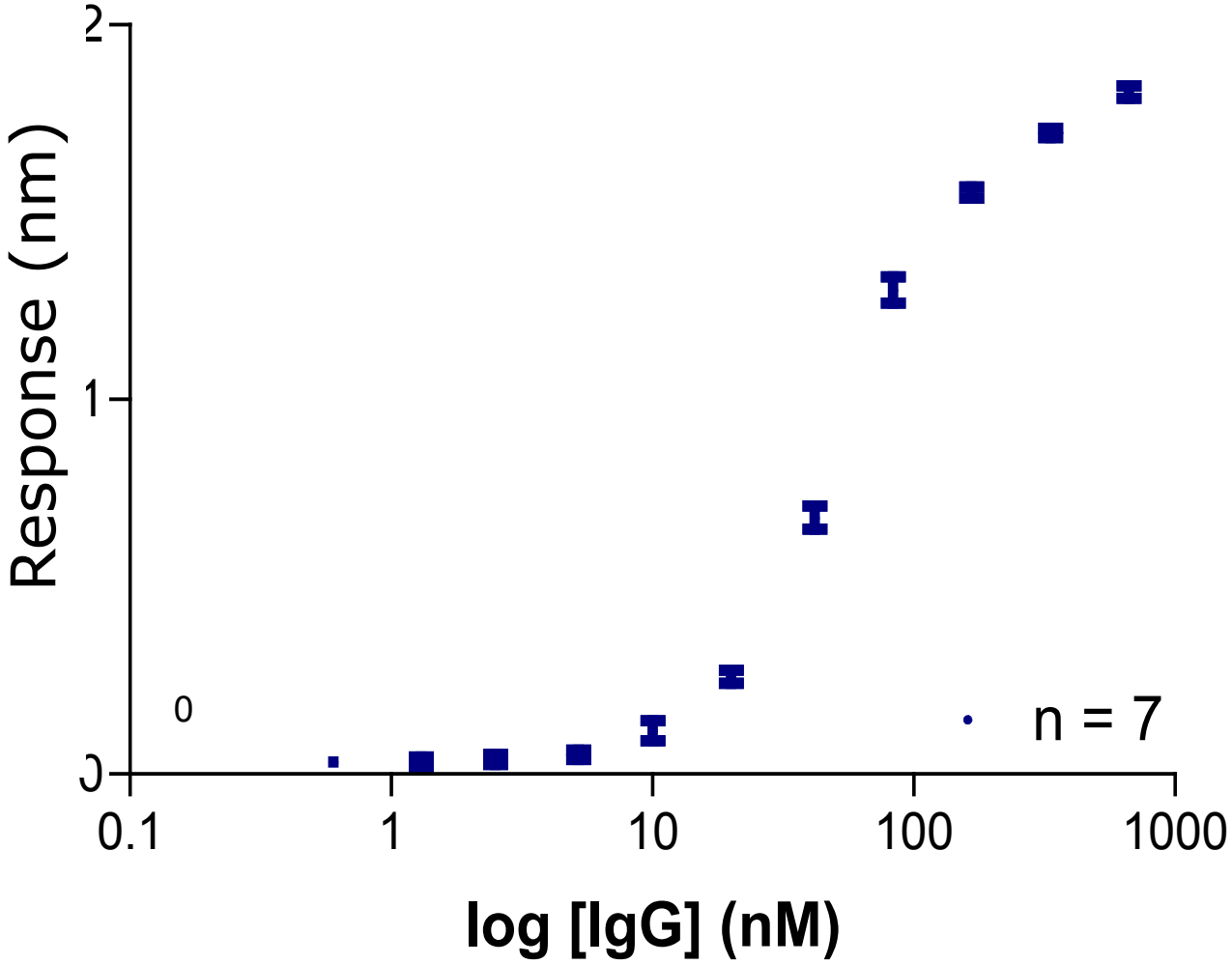
Exposure to concentration series of Pig IgG

1-1000 nM

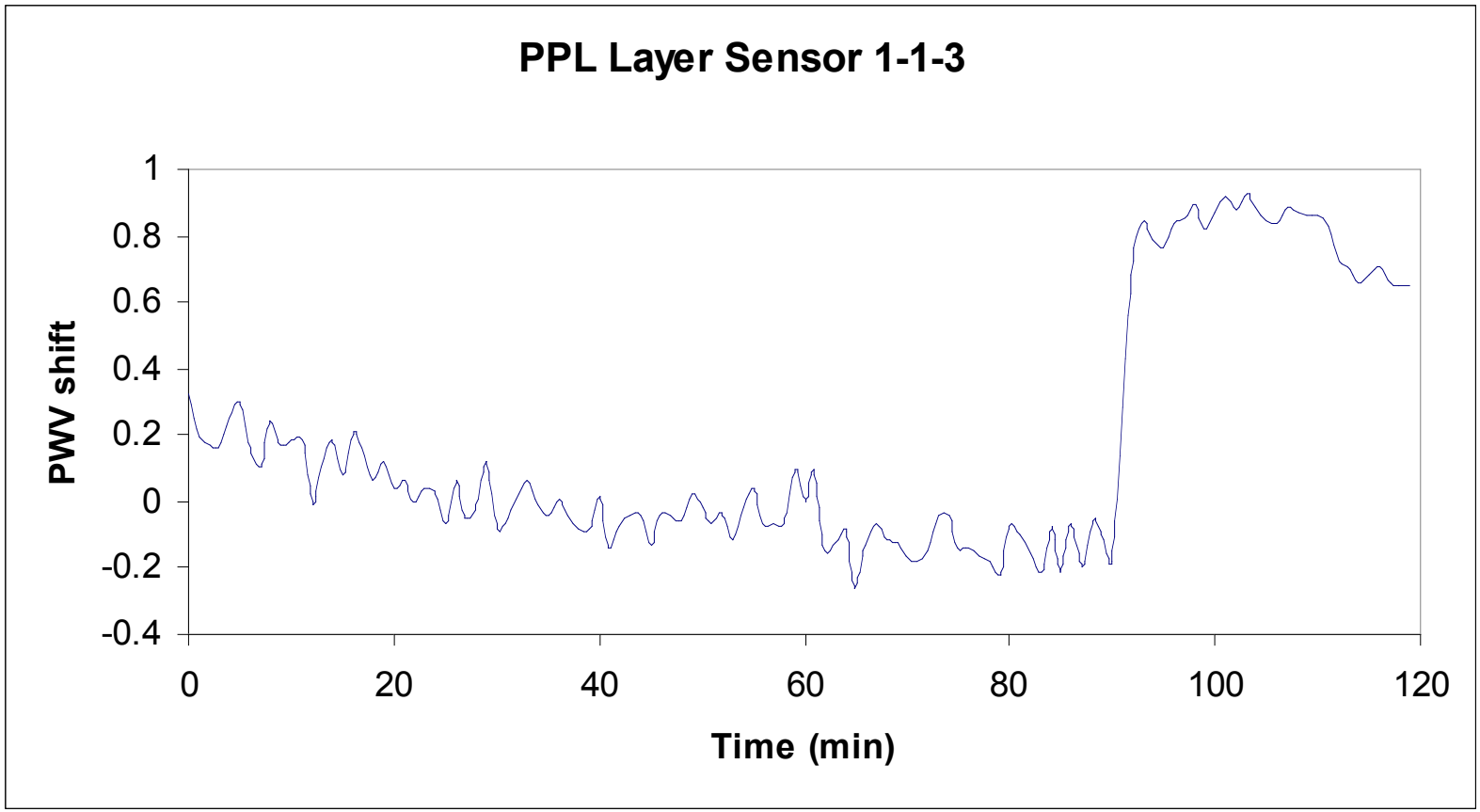


Protein-Protein: Pig-IgG on Protein-A

Endpoint plot response vs. concentration.



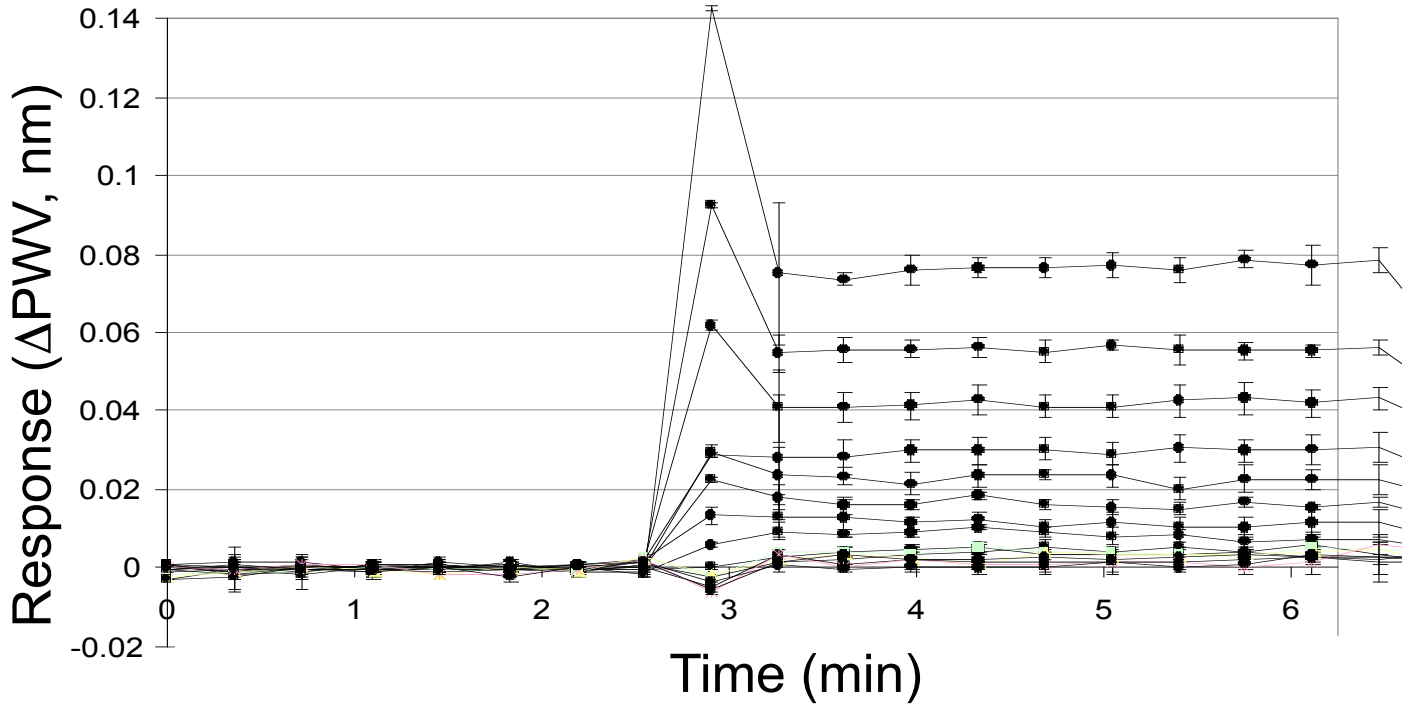
Random Noise



Random Noise

Same type of sensor, lower noise. HOW?

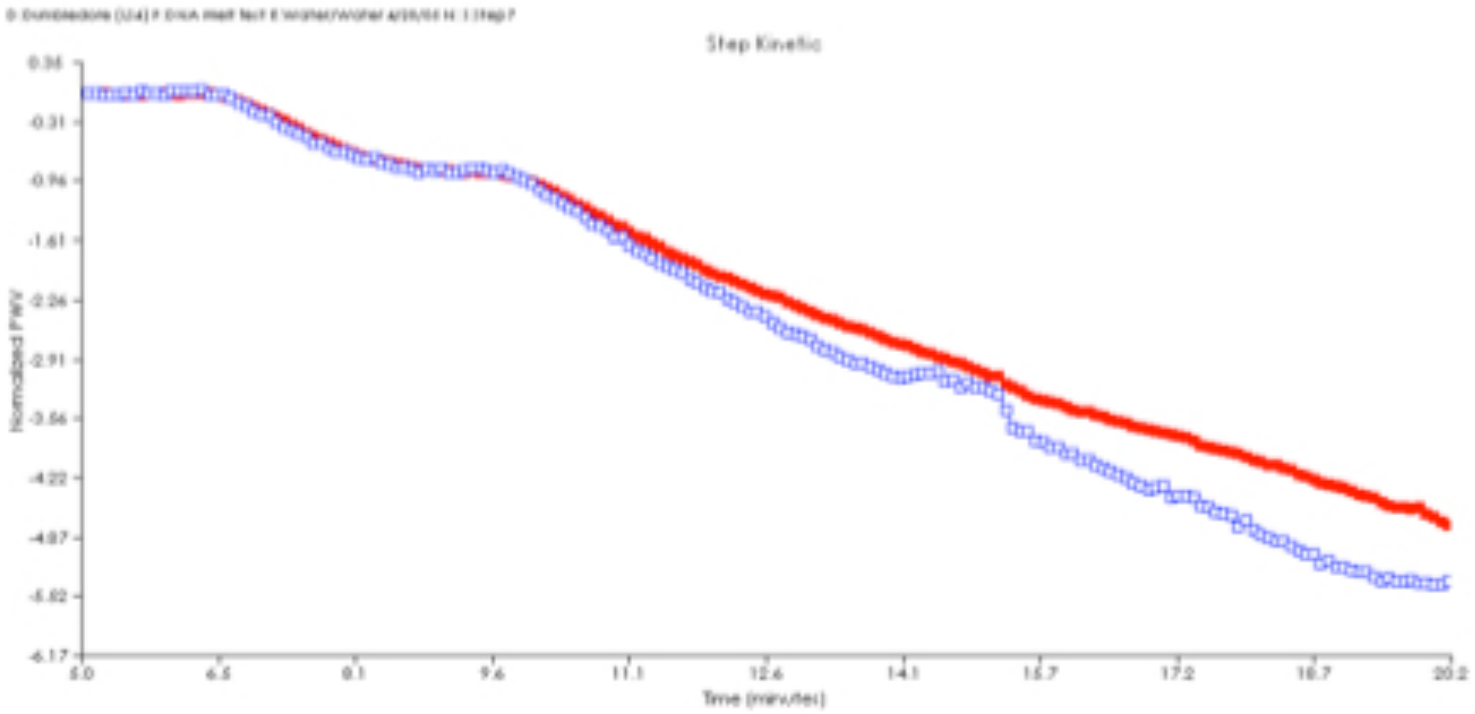
Detection of 240 Da Molecular Weight Molecule



Sources of Noise

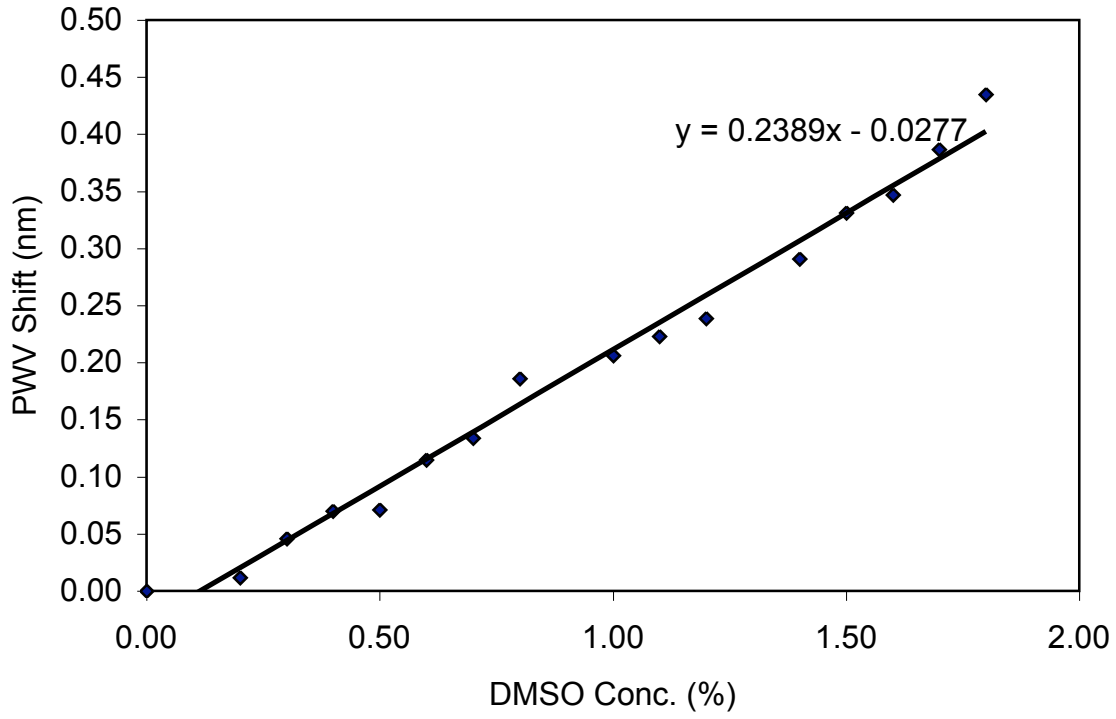
- Interferents/nonspecific binding
- Environment
  - Temperature
- Properties of the test sample
  - Bulk refractive index (optical sensors)
  - Viscosity (acoustic sensors)
  - Autofluorescence (fluorescence sensors)
- Drift
  - Rate of signal change as a function of time not due to analyte detection
  - Sources of signal drift

Temperature

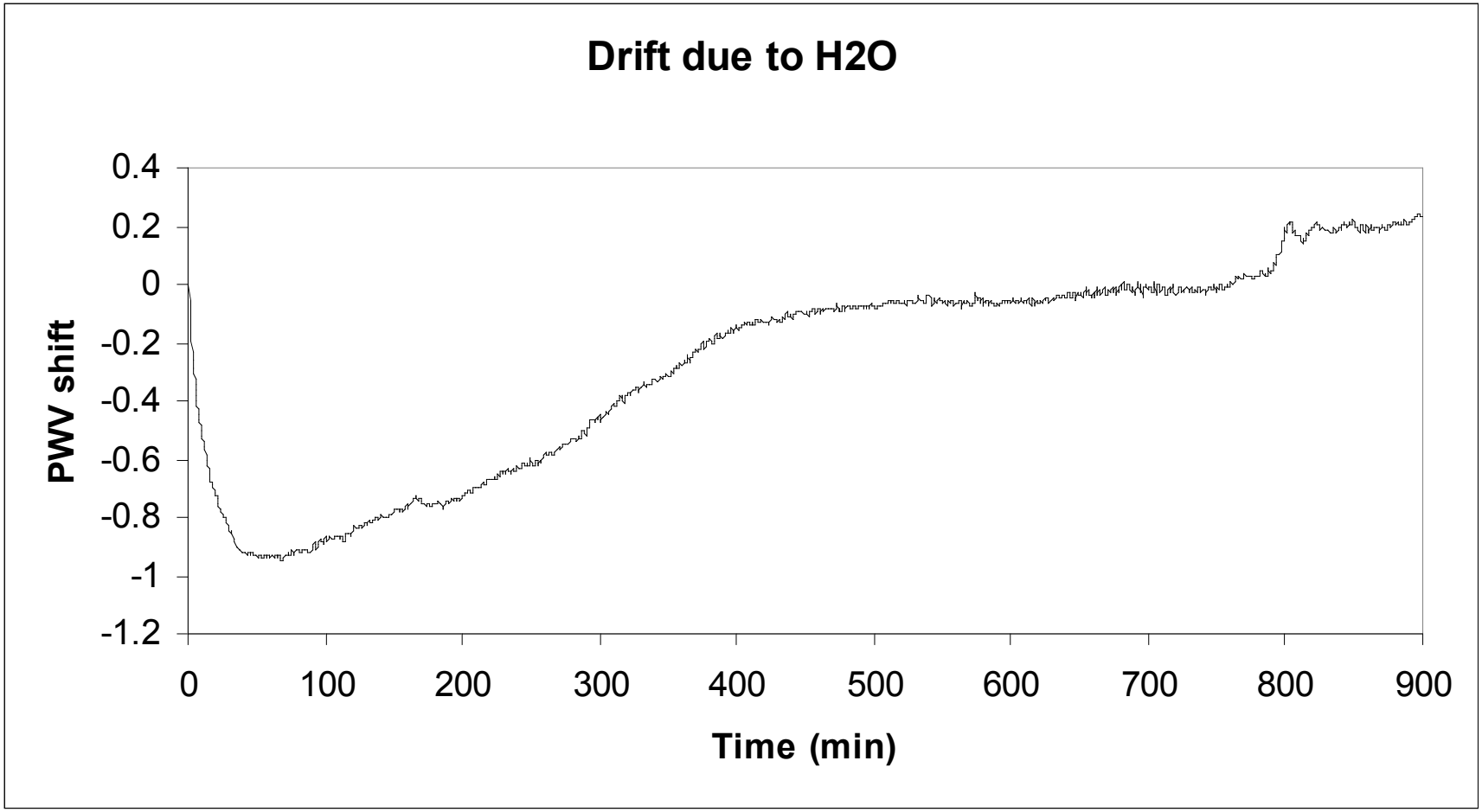


Graph 5: Kinetic Plot showing heating

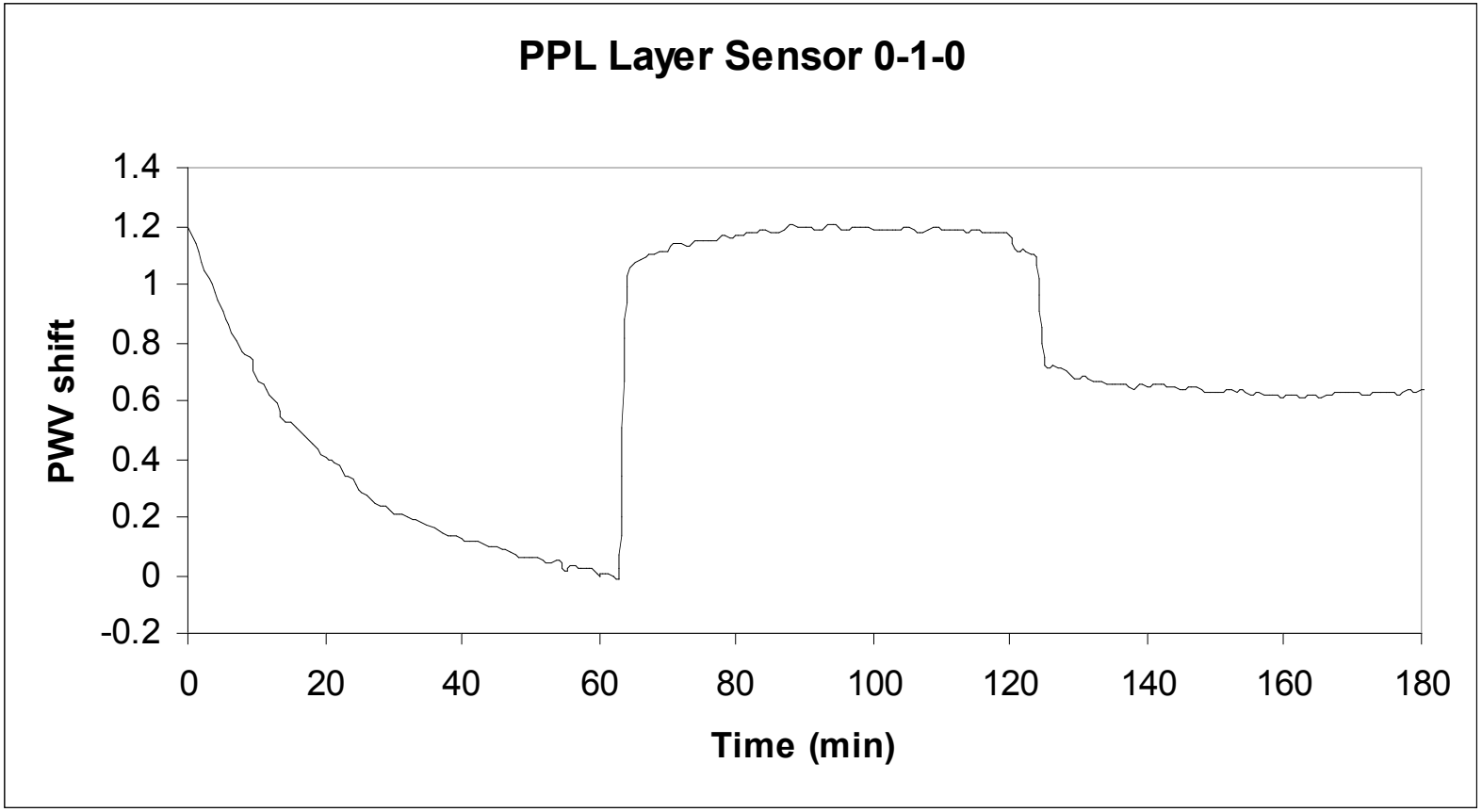
Bulk Refractive Index



Drift


Drift due to H2O
PWV shift
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
0
100
200
300
400
500
600
700
800
900
Time (min)

Drift


PPL Layer Sensor 0-1-0
PWV shift
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
20
40
60
80
100
120
140
160
180
Time (min)

More Noise Sources

- Random noise
  - Noise due to lowest limit of signal change determination
- Nonuniformity of Response
  - For imaging-based approaches OR method requiring comparison of measurements taken from multiple sensors.
    - Nonuniformity within a multi-element sensor
    - Nonuniformity between batches of sensors
  - Causes of nonuniformity

Referencing

Incorporate method for subtraction of certain noise sources into the detection system

- Example - Referencing of thermal drift

Sensitivity & Resolution

$$Sensitivity = \frac{\Delta Signal}{\Delta Analyte}$$

Does not depend on the amount of noise in the system

Resolution = Smallest DETECTABLE ΔAnalyte

"Detectable" usually means signal is 3x greater than standard deviation of all the uncorrectable noise

Example