# CPAC Summer Institute

June 15-17, 2008

## *Real Time Biosensor Systems & Protein Production*


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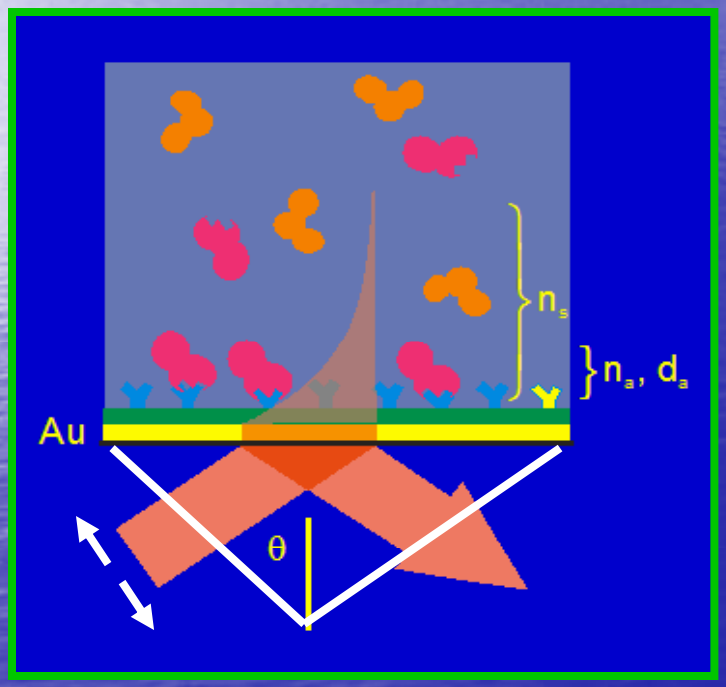
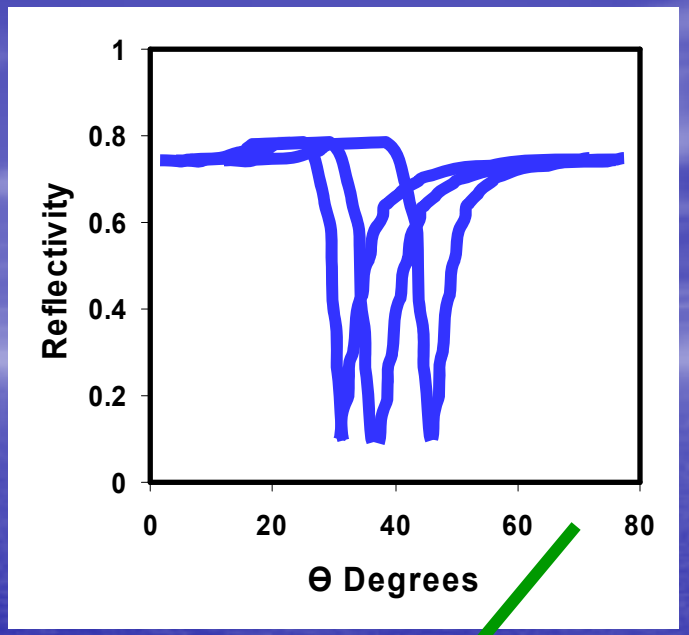
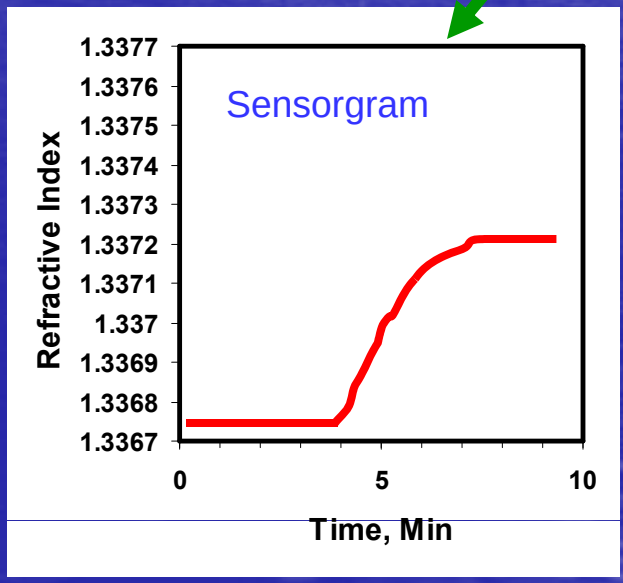
*Departments of Medicine*
*(Div. Medical Genetics) & Genome Sciences*

# Fundamentals of Surface Plasmon Resonance

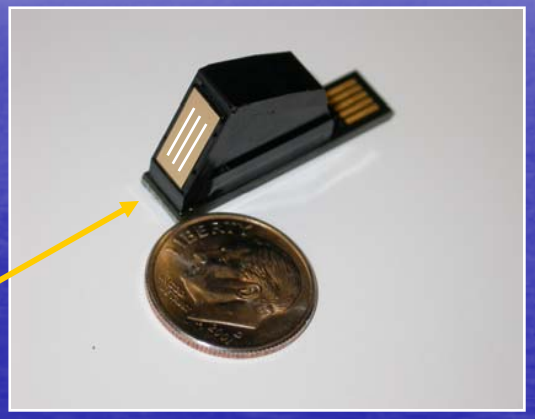



System software



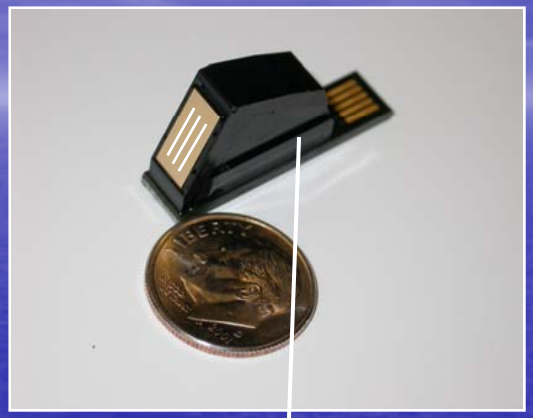
Our goal is to reduce the size and cost of SPR technology from a ~$300,000 desk top device to a portable system that costs under $30,000



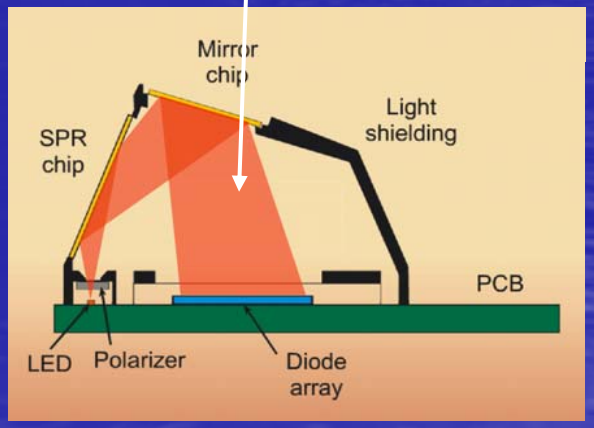
*This system is designed around the miniature TI-SPR chip, designed by José Melendez and team.*

# Spreeta sensing components

- **Spreeta SPR components developed in collaboration with UW with TI**
- **Miniaturized, robust, high performance devices.**
- **Inexpensive in large quantity**
- **Excellent manufacturing capabilities and quality control**



**Each Spreeta chip contains all of the optical components needed for sensitive SPR measurement of biomolecular interactions**

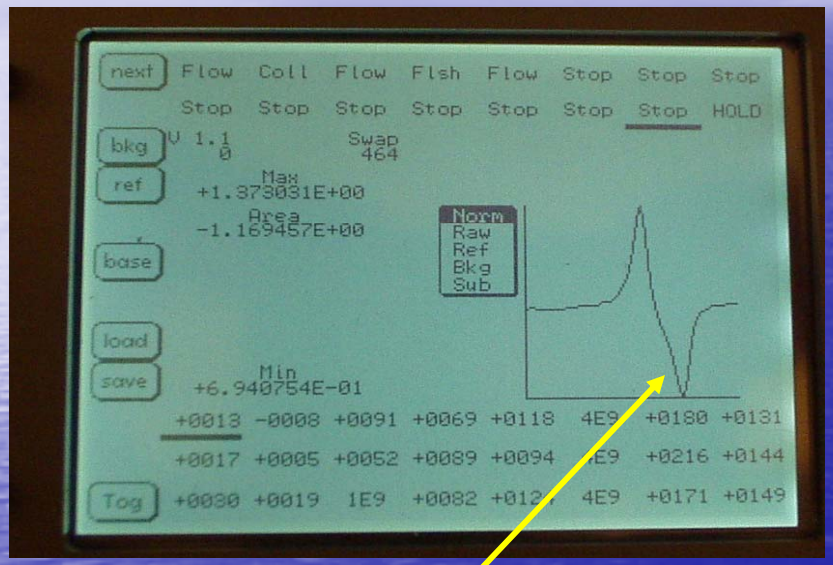


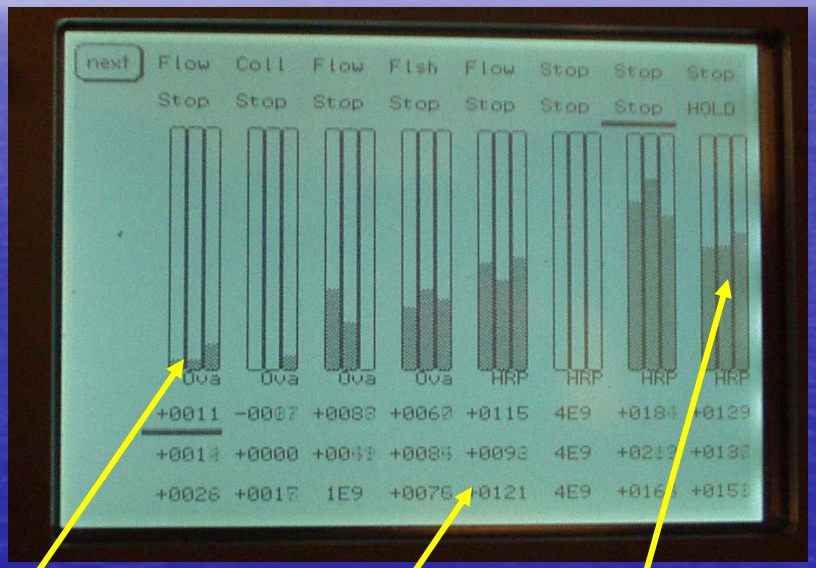
# The SPIRIT system

(Surface Plasmon Instrumentation for the Rapid Identification of Toxins)

- Compact, lightweight (lunchbox size, 6 lb.)
- High performance
- 24 simultaneous measurements
- Low power (5W) allows portable operation
- Semi-Automated

# Touchscreen data display



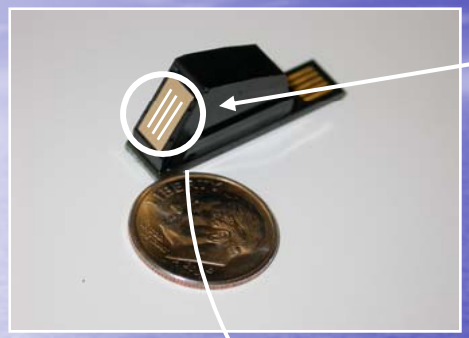

Selected SPR curve

Sensor channel

Detected levels (bargraph)

Detected levels (numeric)

# Sensor surface chemistry



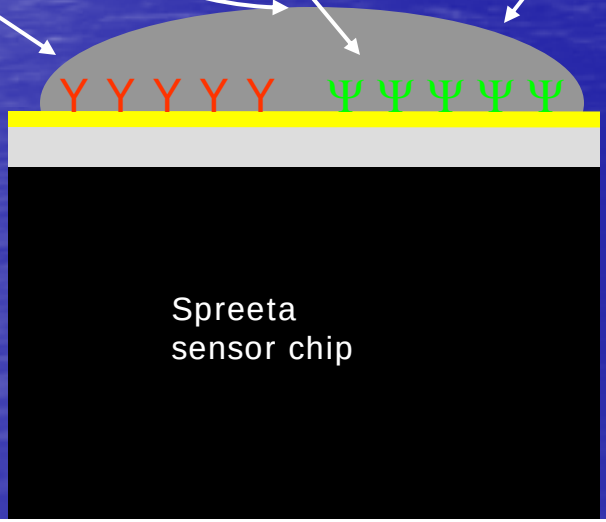
Each Spreeta chip has 3 useable channels

Soluble protective coating (dextran/trehalose) allows long-term dry storage at room temperature

Control receptors (usually antibodies) Designed NOT to respond to that agent

Target receptors: (usually antibodies) Designed to capture a specific agent or analyte e.g.:

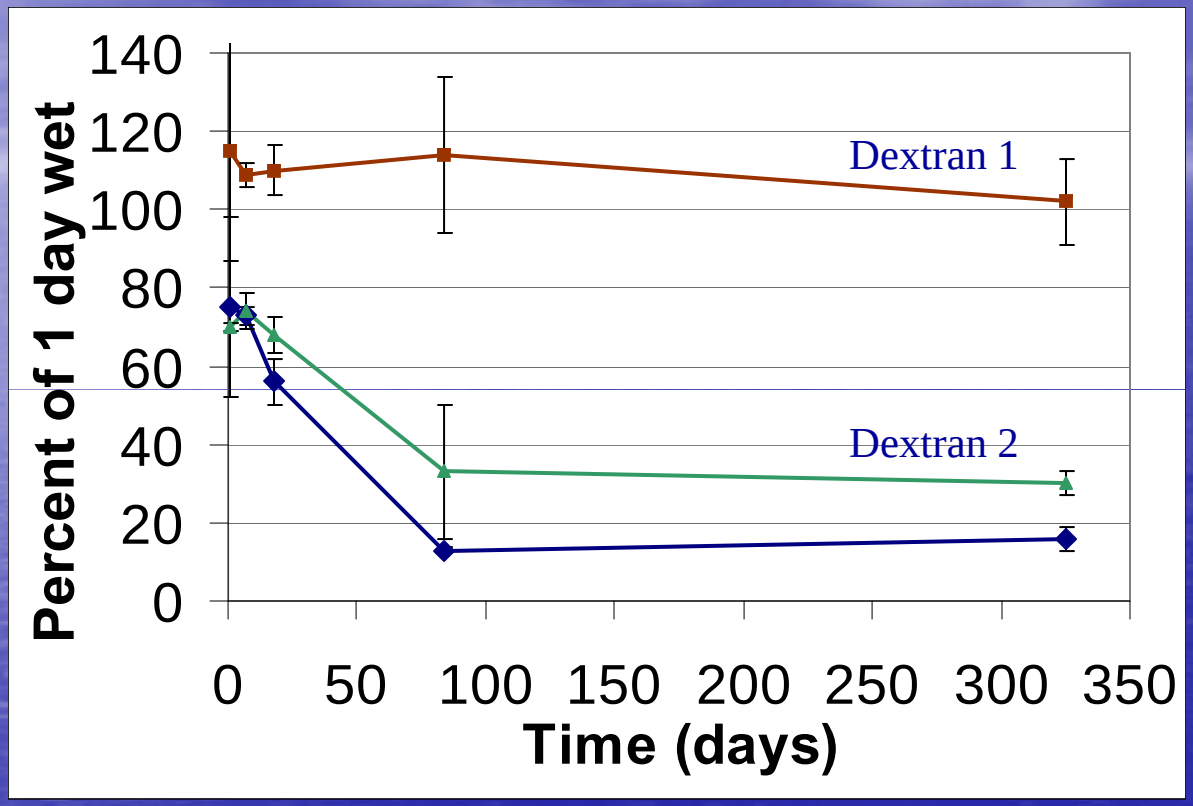
- Toxins
- Viruses
- Spores
- Bacteria




Gold layer (50 nM)

Glass substrate

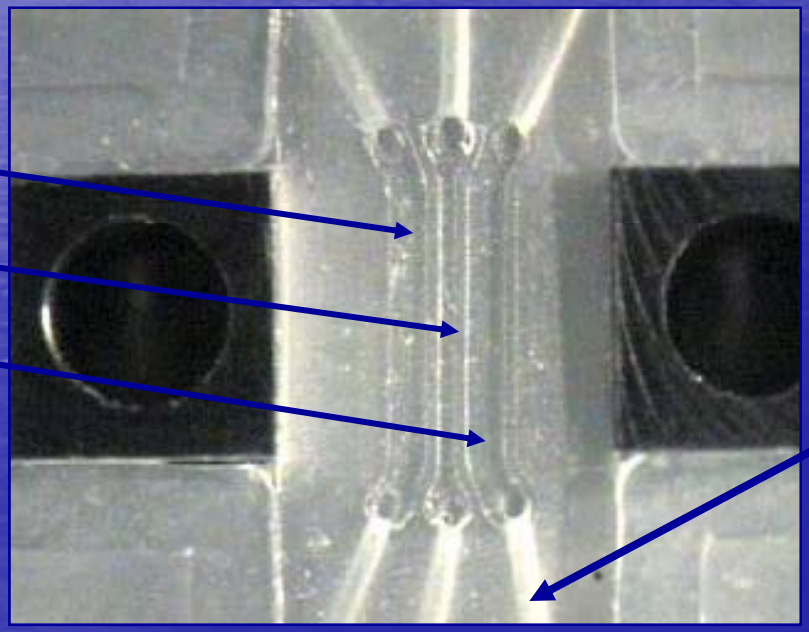
# Storage of Gold Slides




Anti-Alkaline-phosphatase(AP)-antibody-coated slides were dried with a thin layer of 10 mM Tris, pH 8.0, 2.5% trehalose and 2.5% dextran. After extended storage, slides were wetted, exposed to AP and analyzed for AP activity.

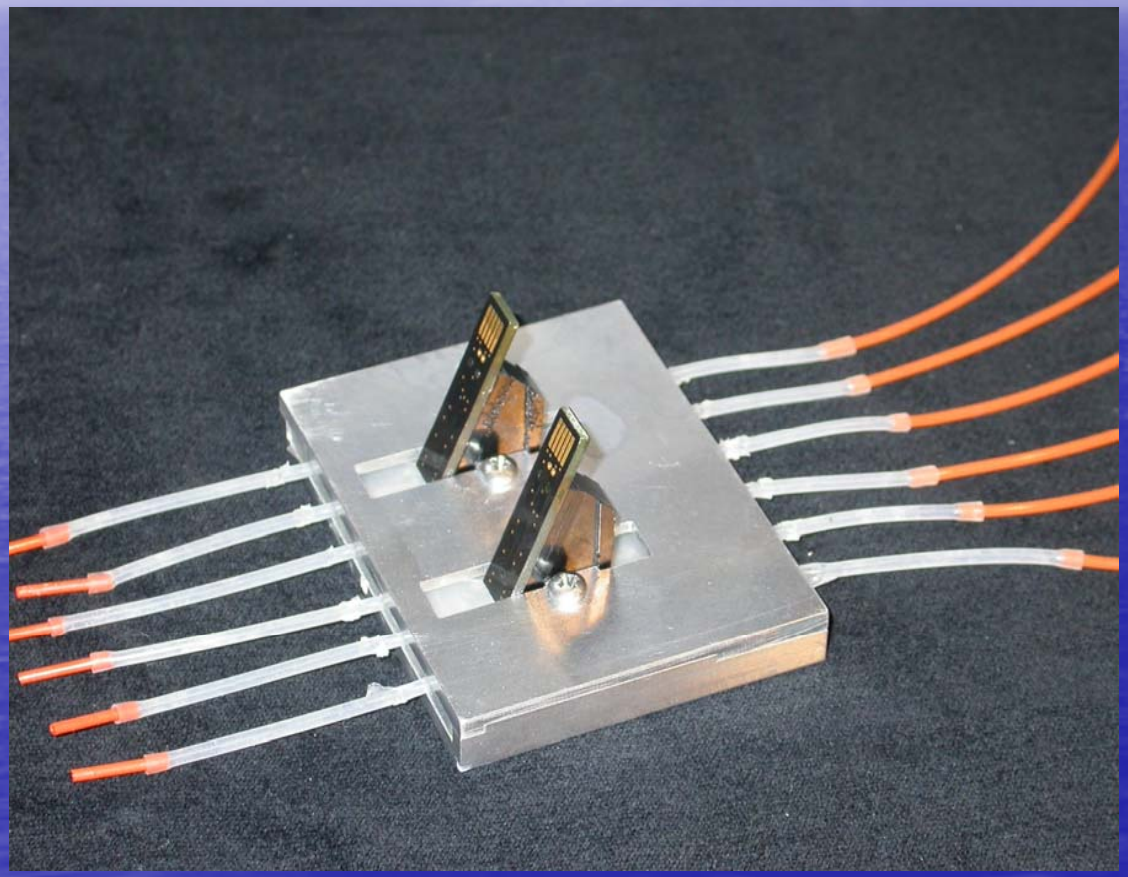
# Silicone molding

- Versatile technique for production of precision flowcells & other fluidic components

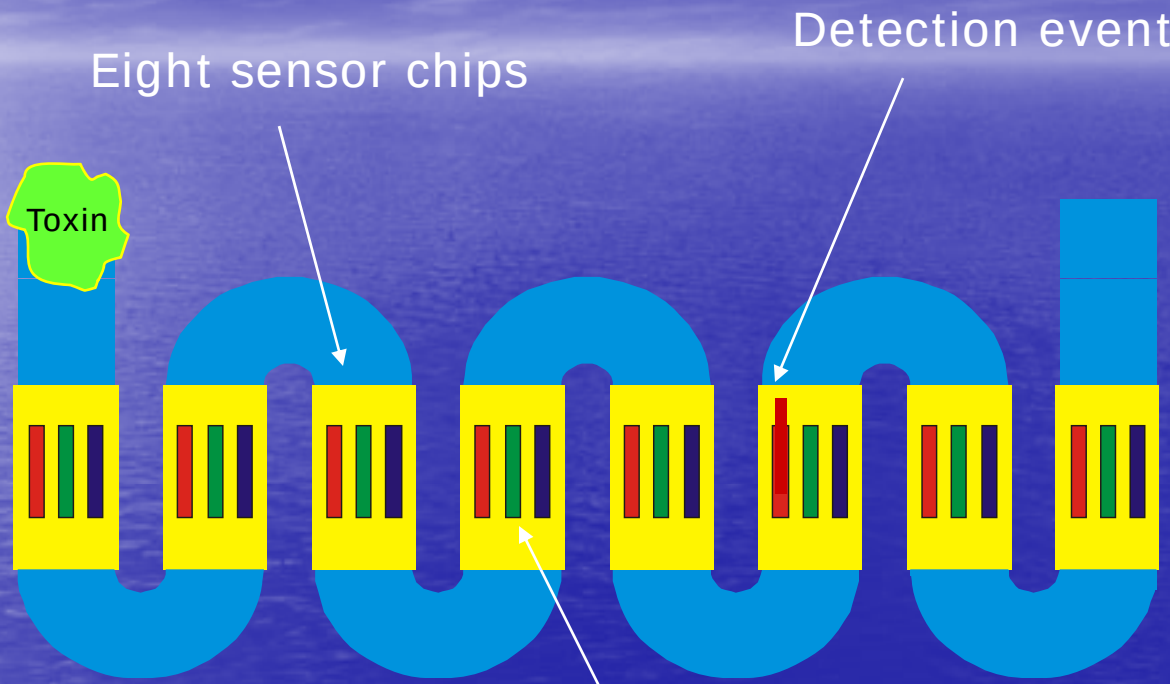



Flowcell cast from mold

# Fixture for derivatizing individual channels



# SPIRIT performs 24 simultaneous measurements of antibody binding



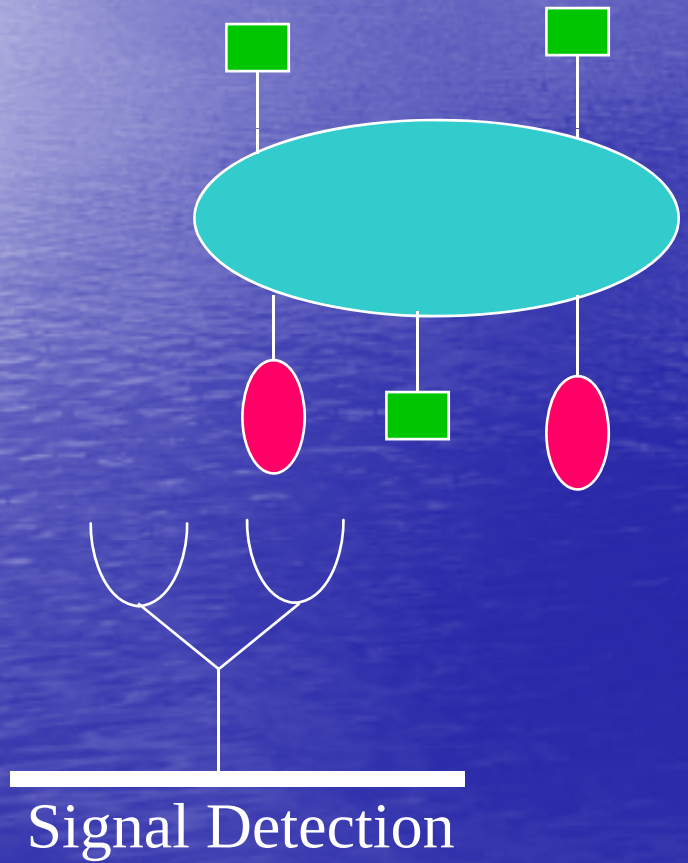
# Examples of Assays Possible with SPR

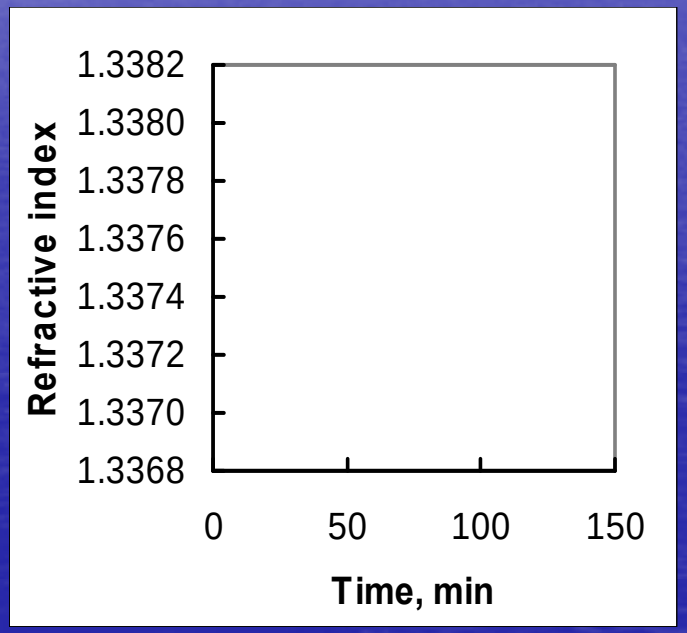
- Whole microbial cells
  -(*F.tularensis, E. coli, Y. pestis*)
- Spores
  -(e.g., anthrax)
- Viruses with or without amplification
  -(e.g. Norwalk, flu)
- Proteins by direct detection with or without amplification/verification
  -(protein toxins, industrial proteins, therapeutics)
- Small molecular weight analytes using displacement or competition assays
  -(e.g., domoic acid, cortisol, insecticides, toxic chemicals, TNT & other small organics)

# Detection of Larger Analytes

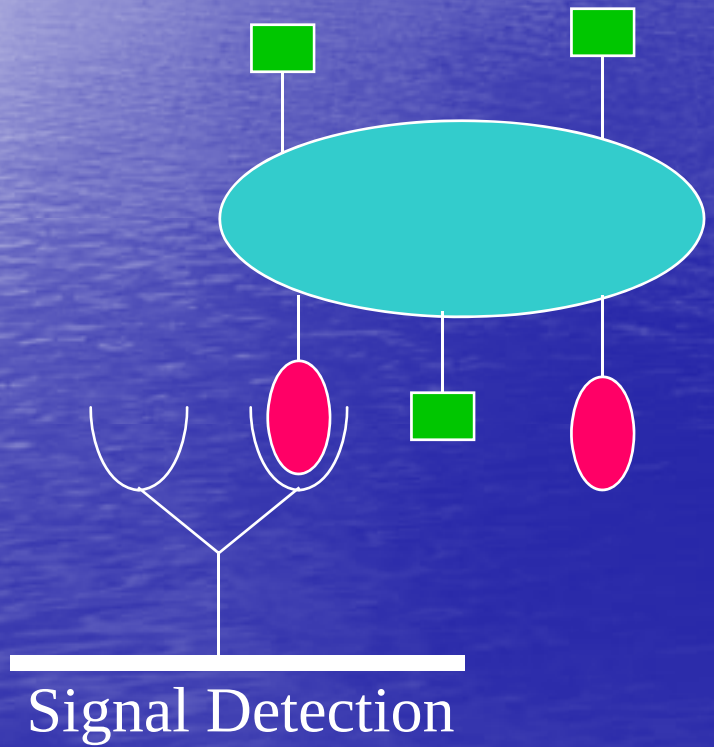
- Microbes
- Spores
- Viruses
- Proteins/Toxic Proteins
- Small molecule toxins

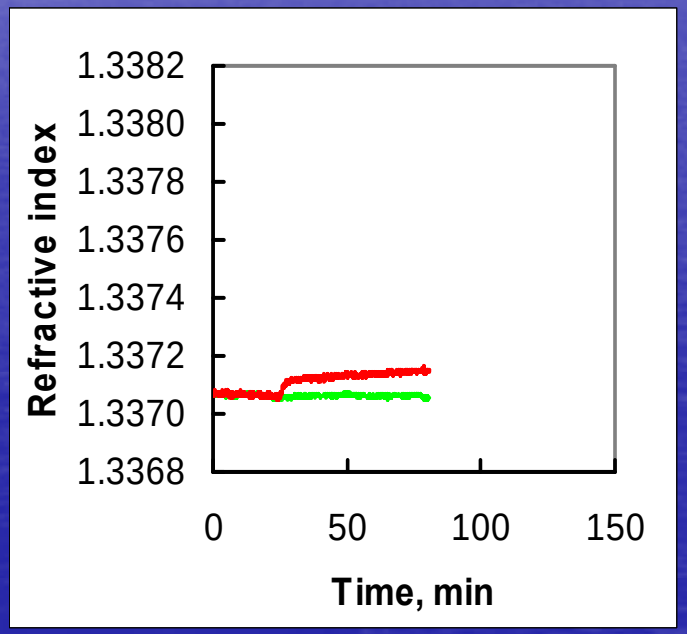
# Analyte Detection and Signal Amplification



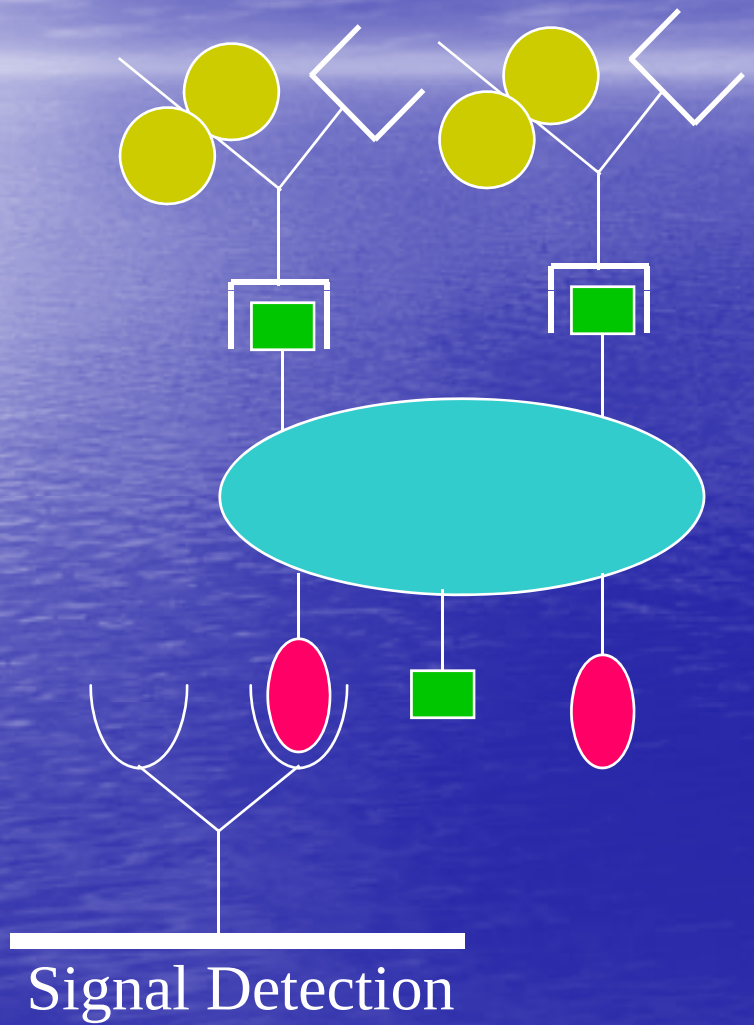



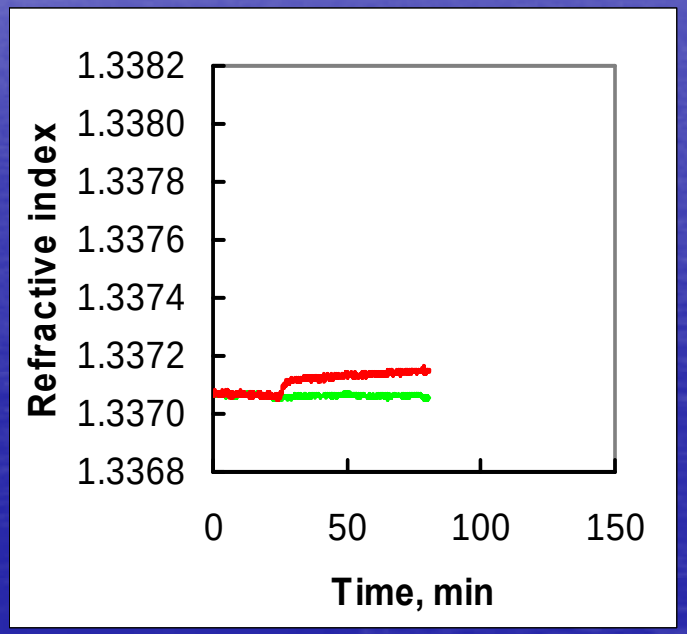
# Analyte Detection and Signal Amplification



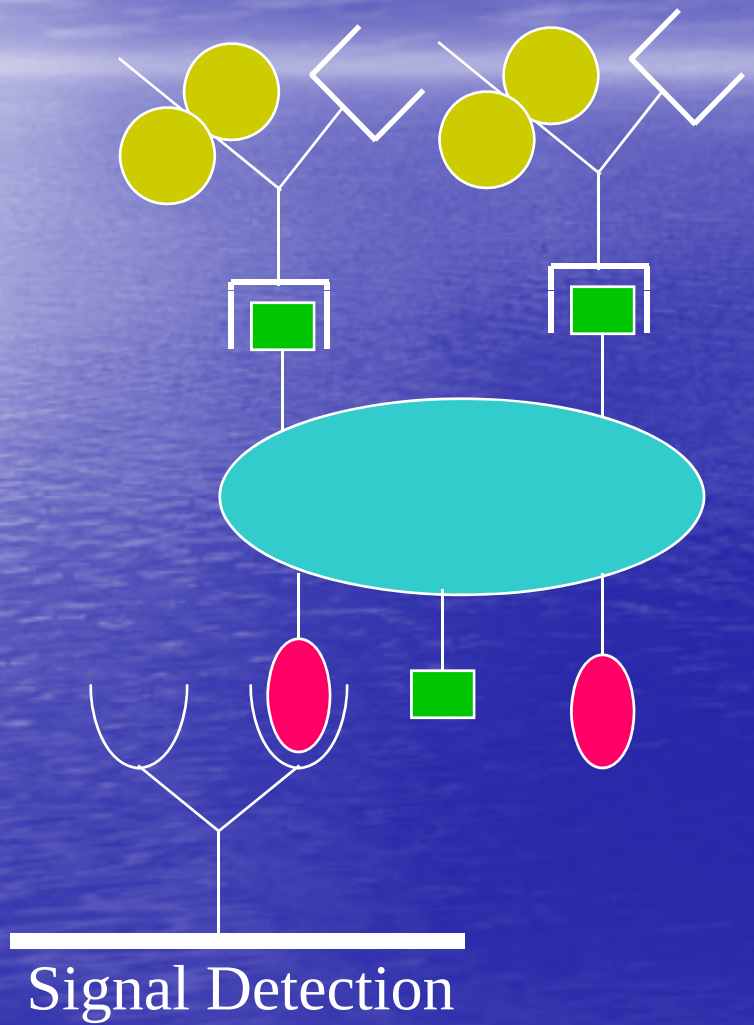



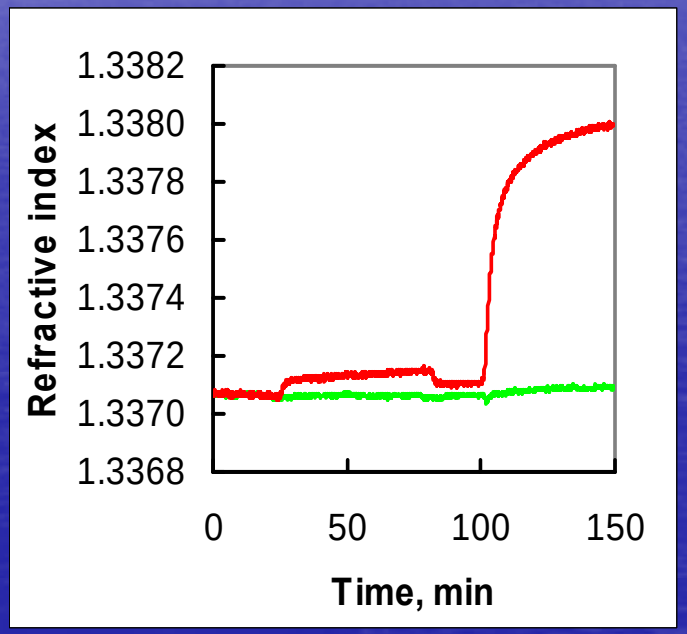
# Analyte Detection and Signal Amplification



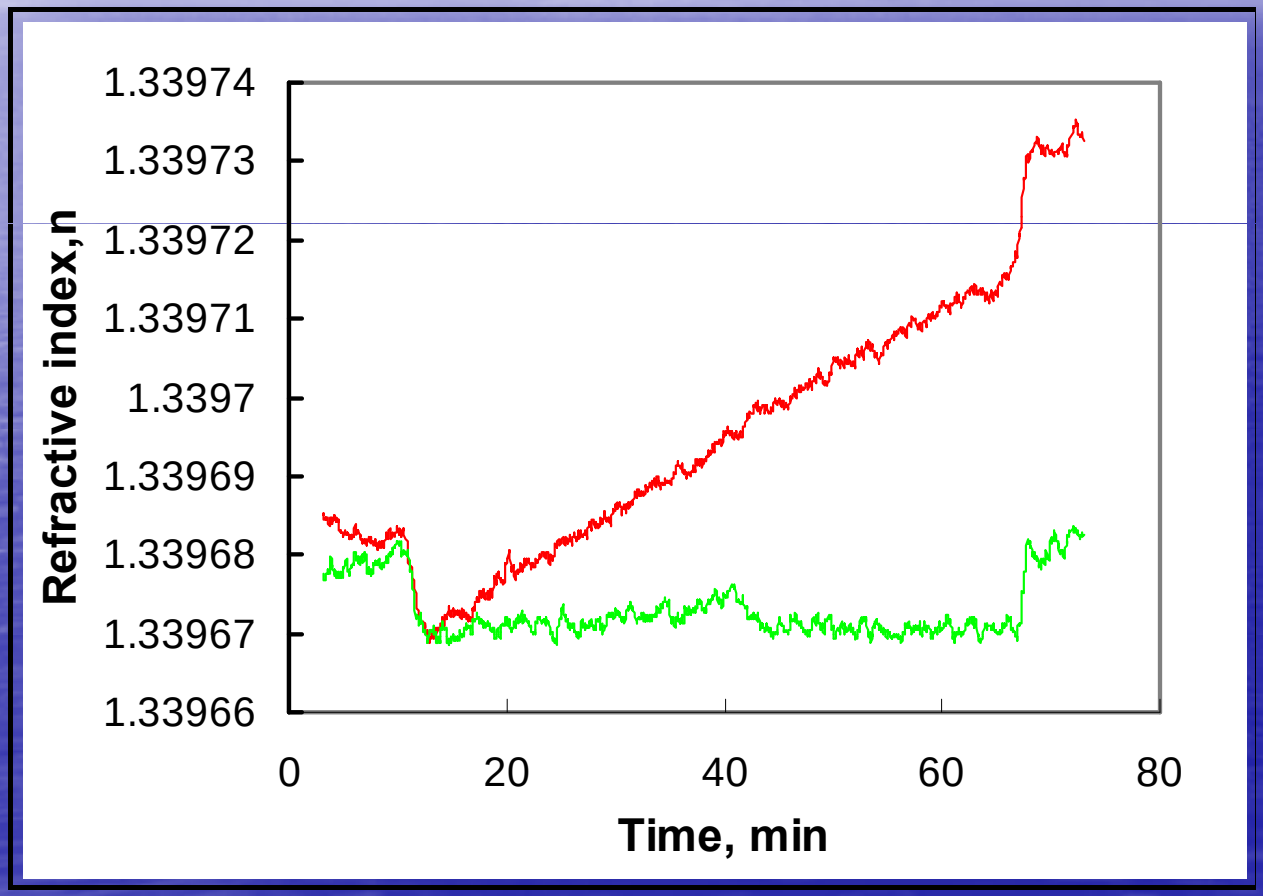



# Analyte Detection and Signal Amplification

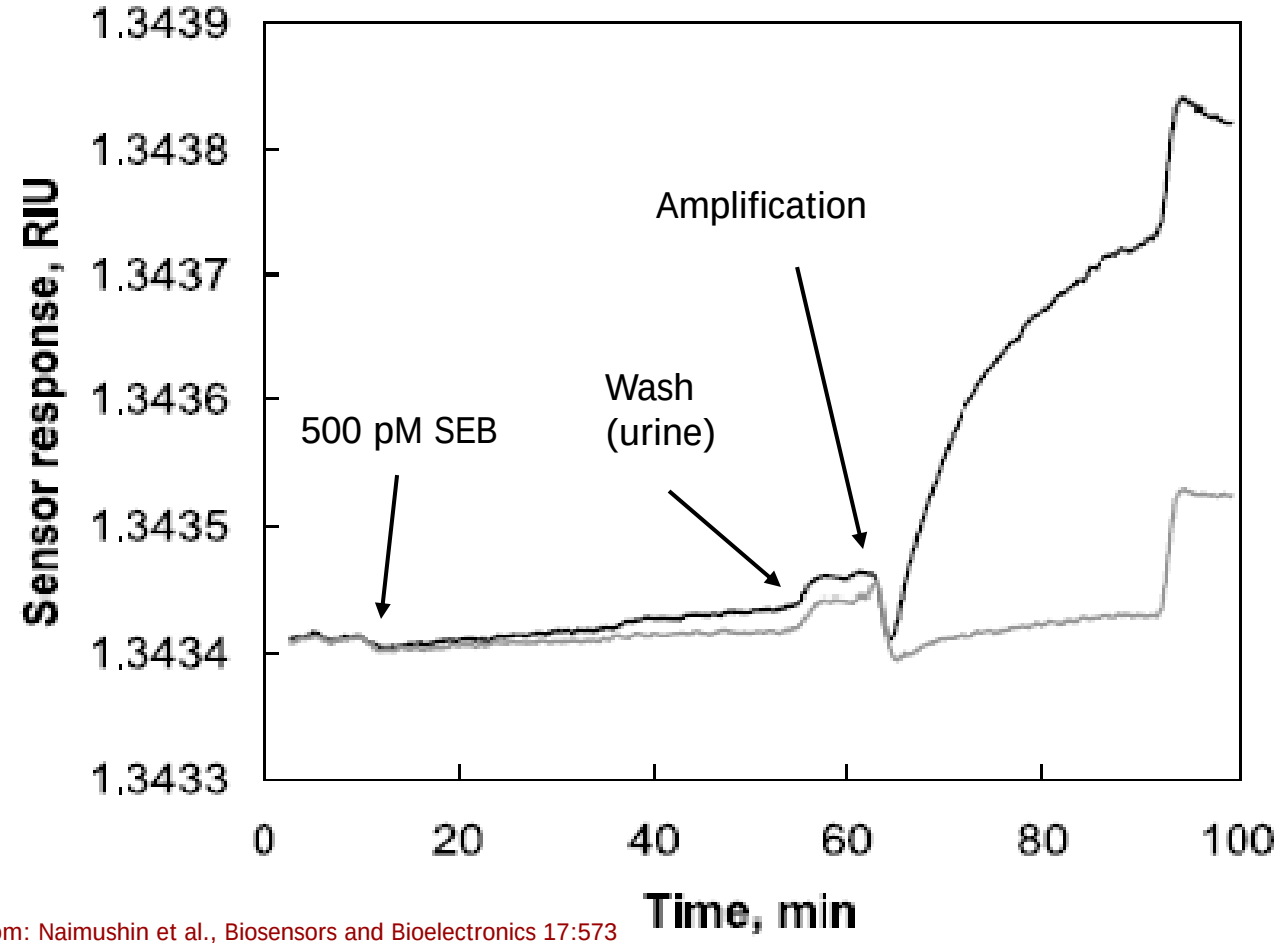


# Detection of 1 nM (28 ppb) SEB in seawater



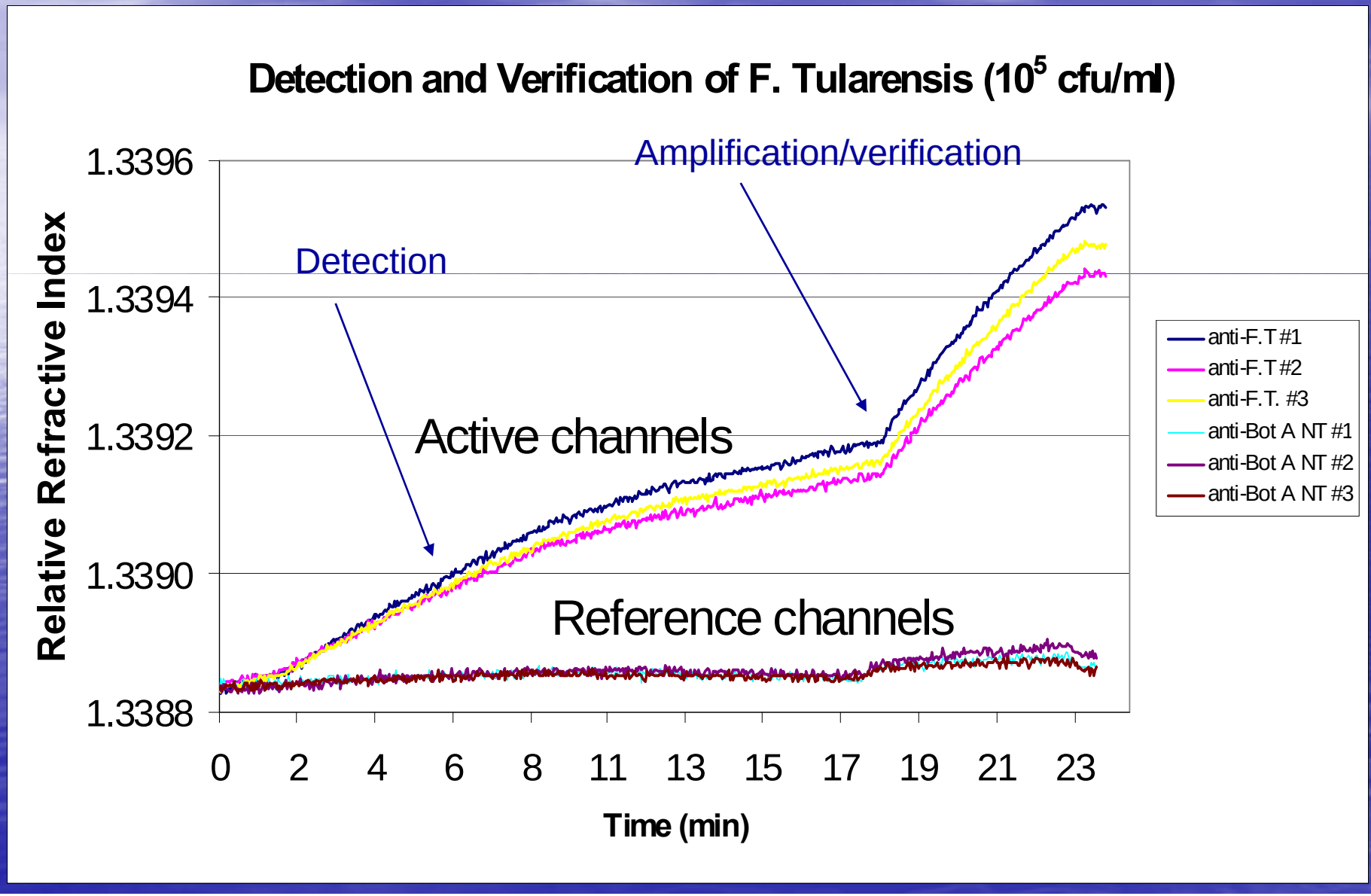
# Detection of 500 pM (14 ppb) SEB in urine



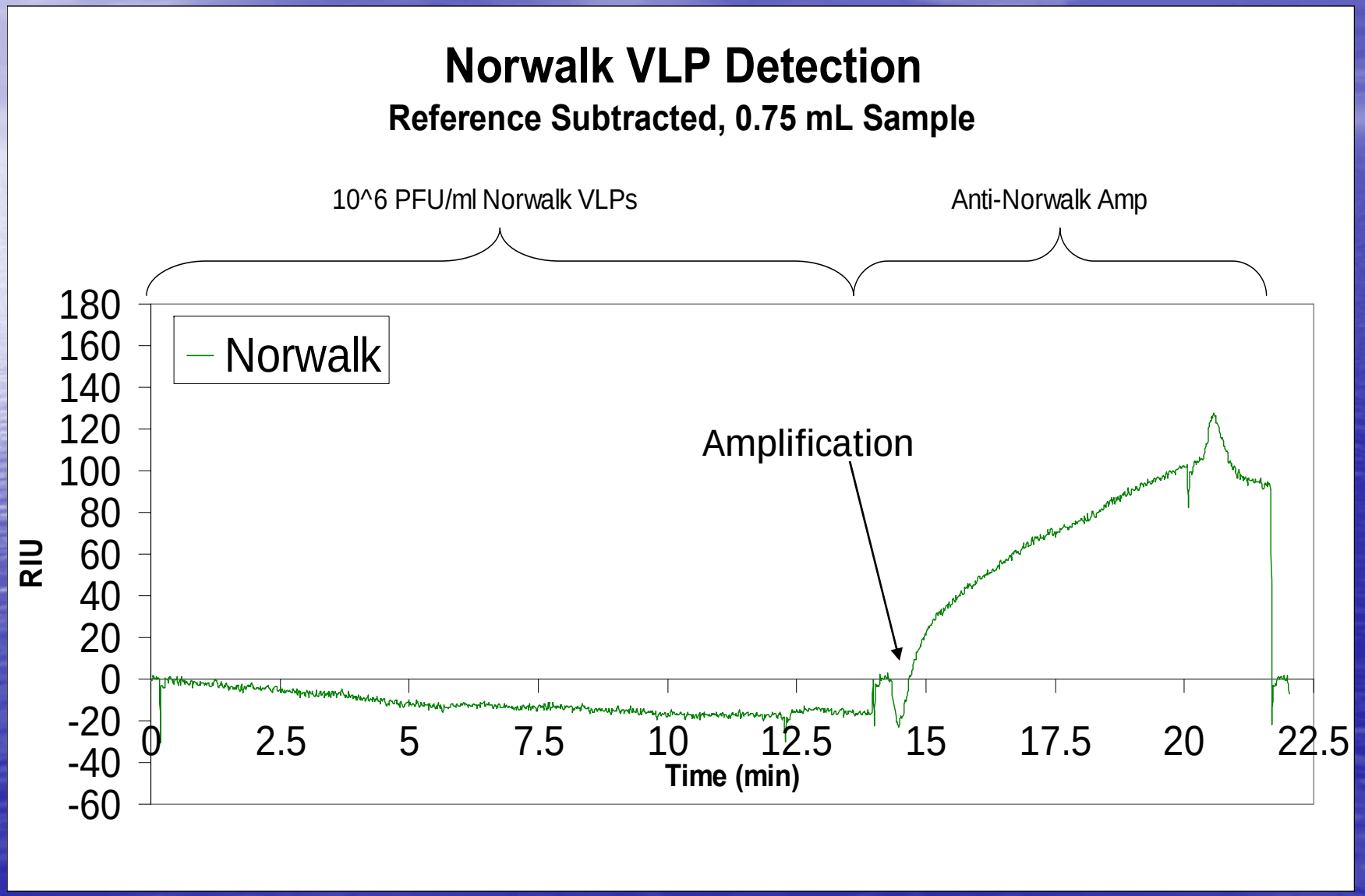

From: Naimushin et al., Biosensors and Bioelectronics 17:573

# Detection of Microbes

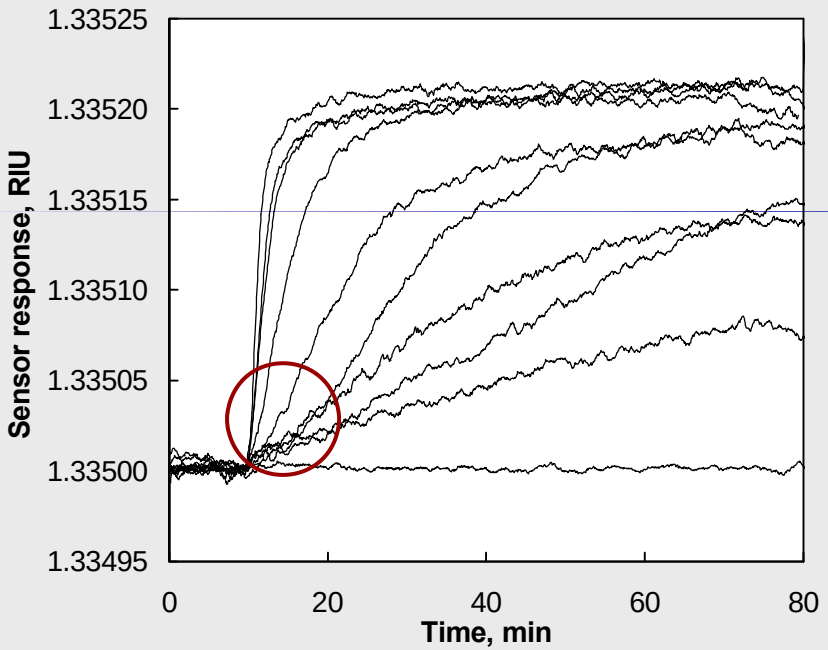
**Current Detection Limit: $10^3$ cfu / ml**

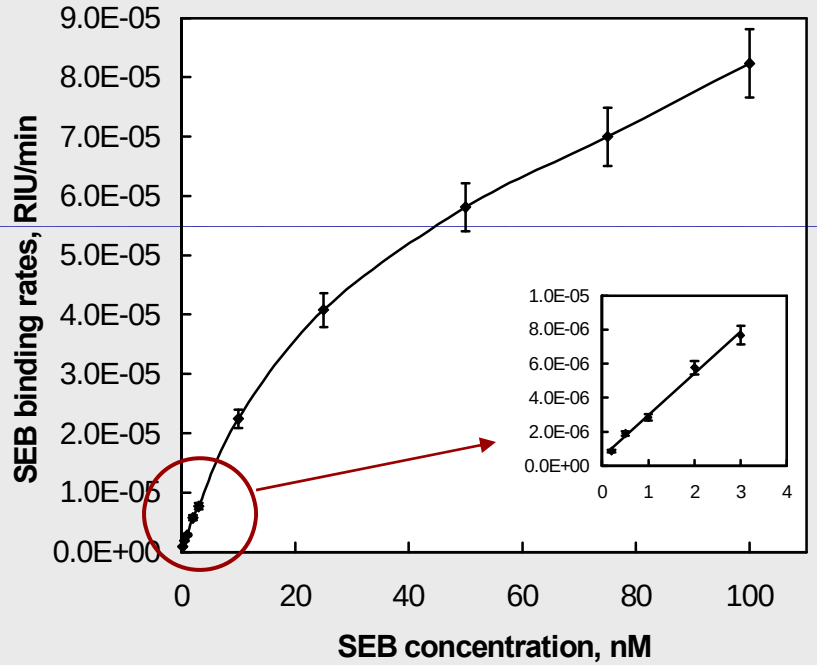


# Virus Detection

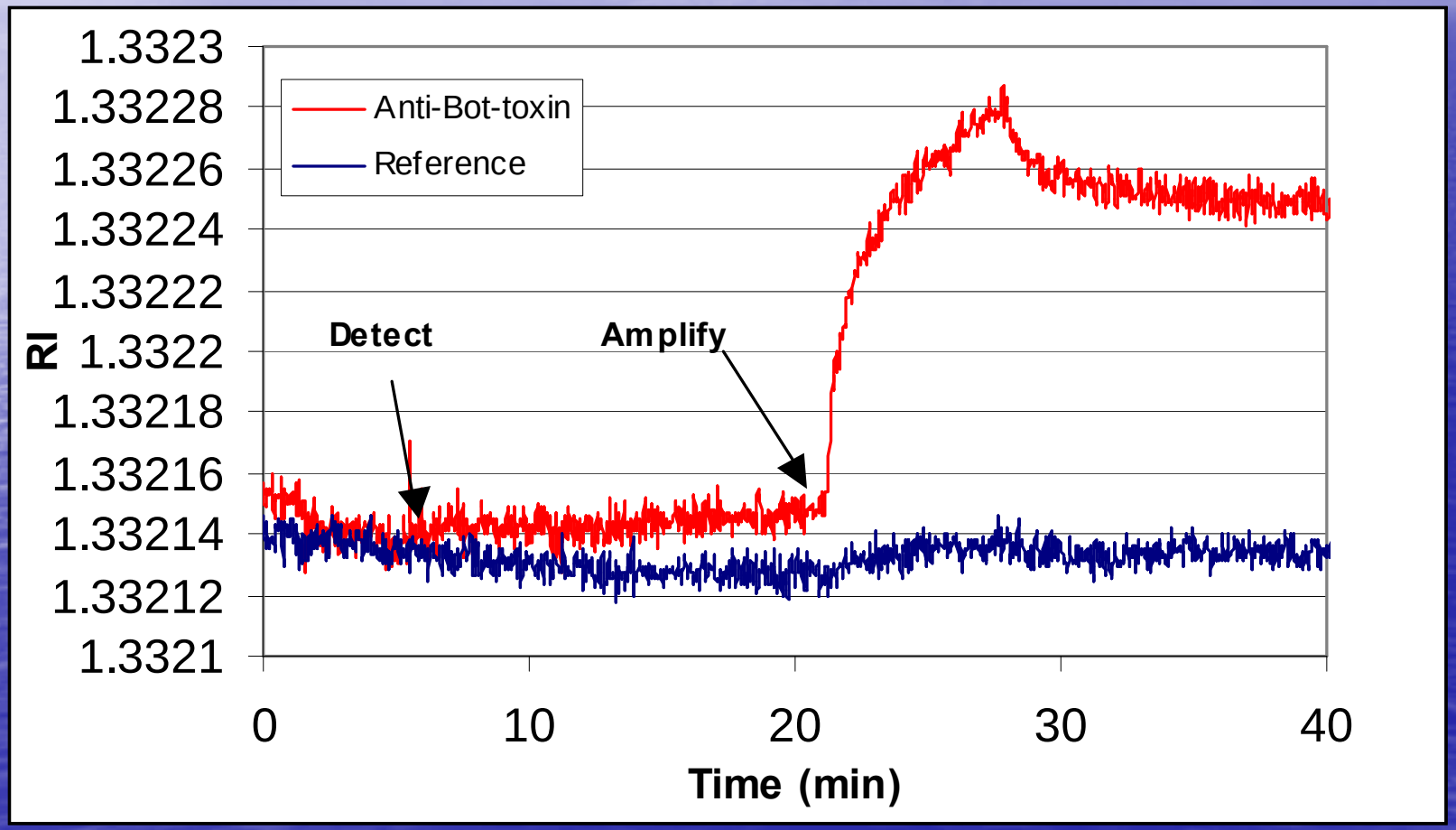


# Quantitative Detection of Staphylococcal Enterotoxin B

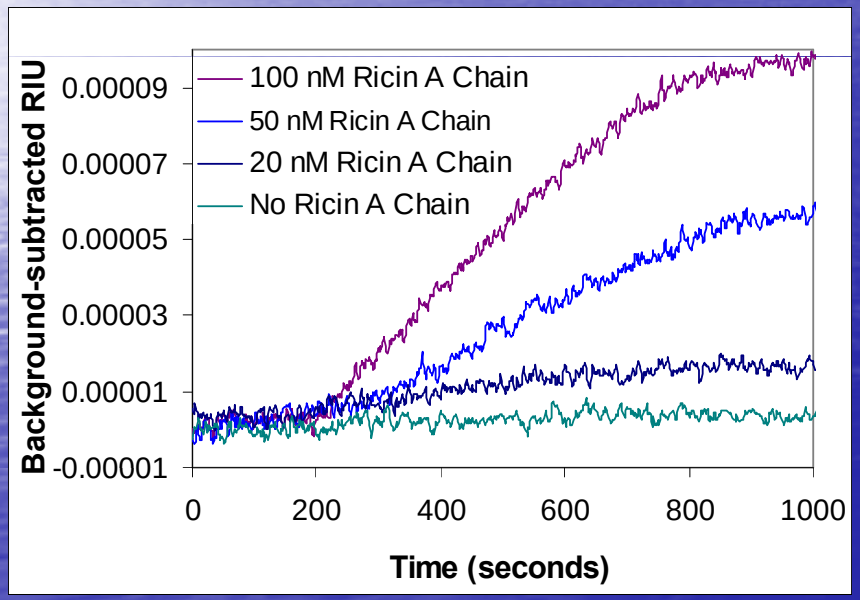


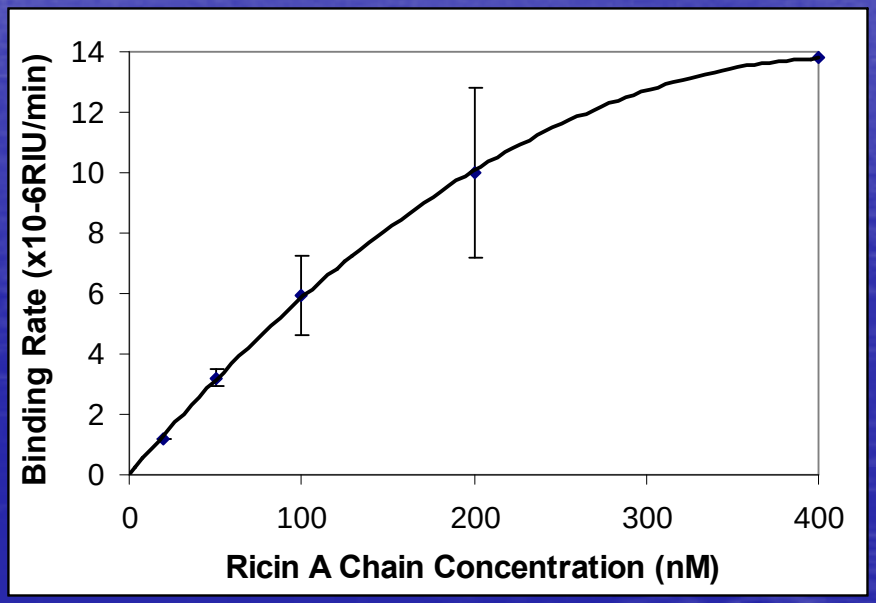



# Detection of 5 ng/mL (5 ppb; 33pM) BotNT (denatured botulinum toxin)



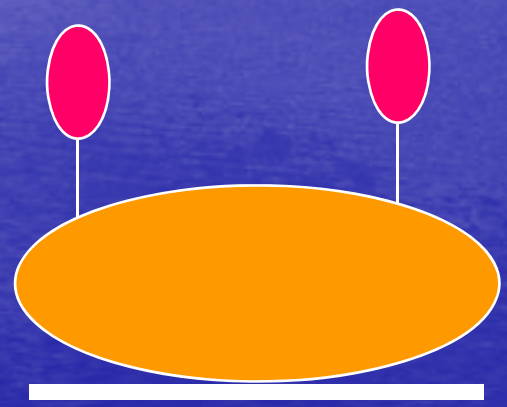
# Direct Detection of Ricin A Chain (64 ppb-320 ppb)



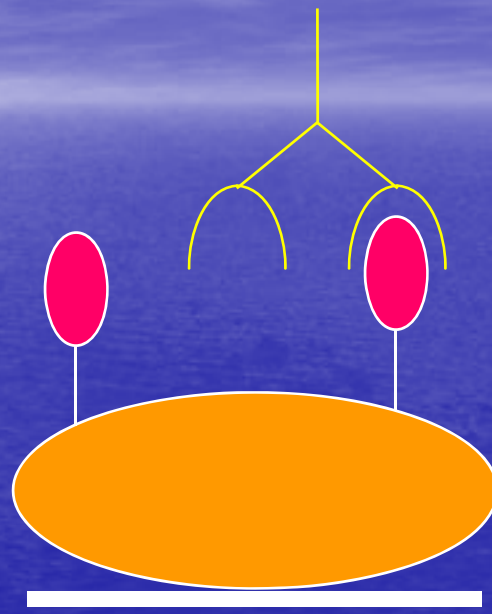



Detection of small molecule toxins by SPR is a more difficult task

# Displacement Assay

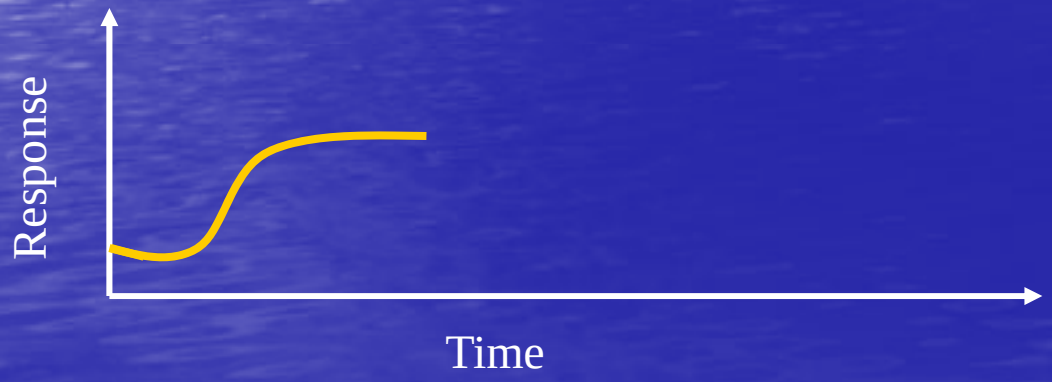


# Displacement Assay



Sensor Ready Condition

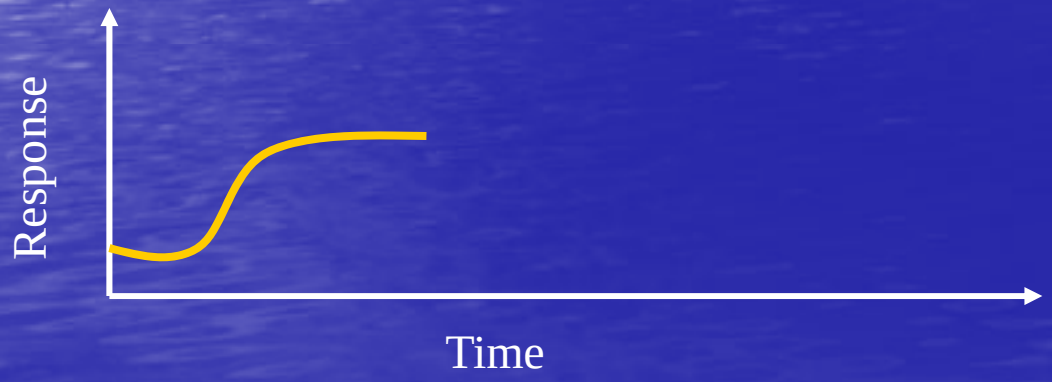
**Antibody Loading**



# Displacement Assay

**Introduction of Target**

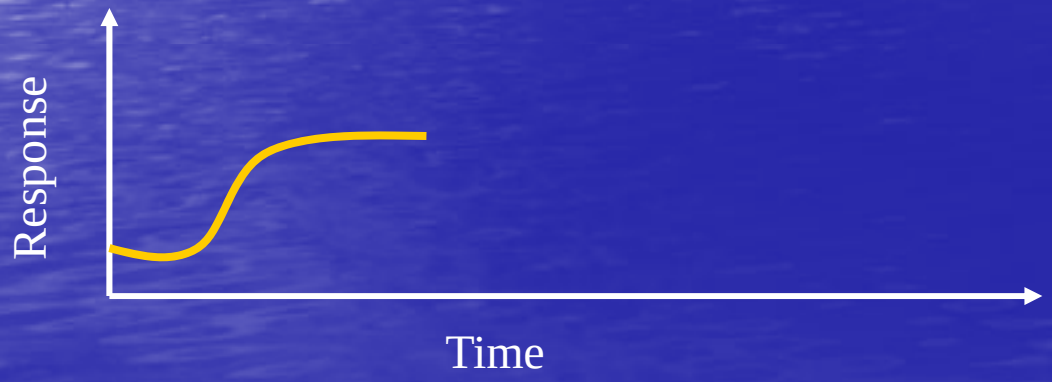
Sample Detection Mode



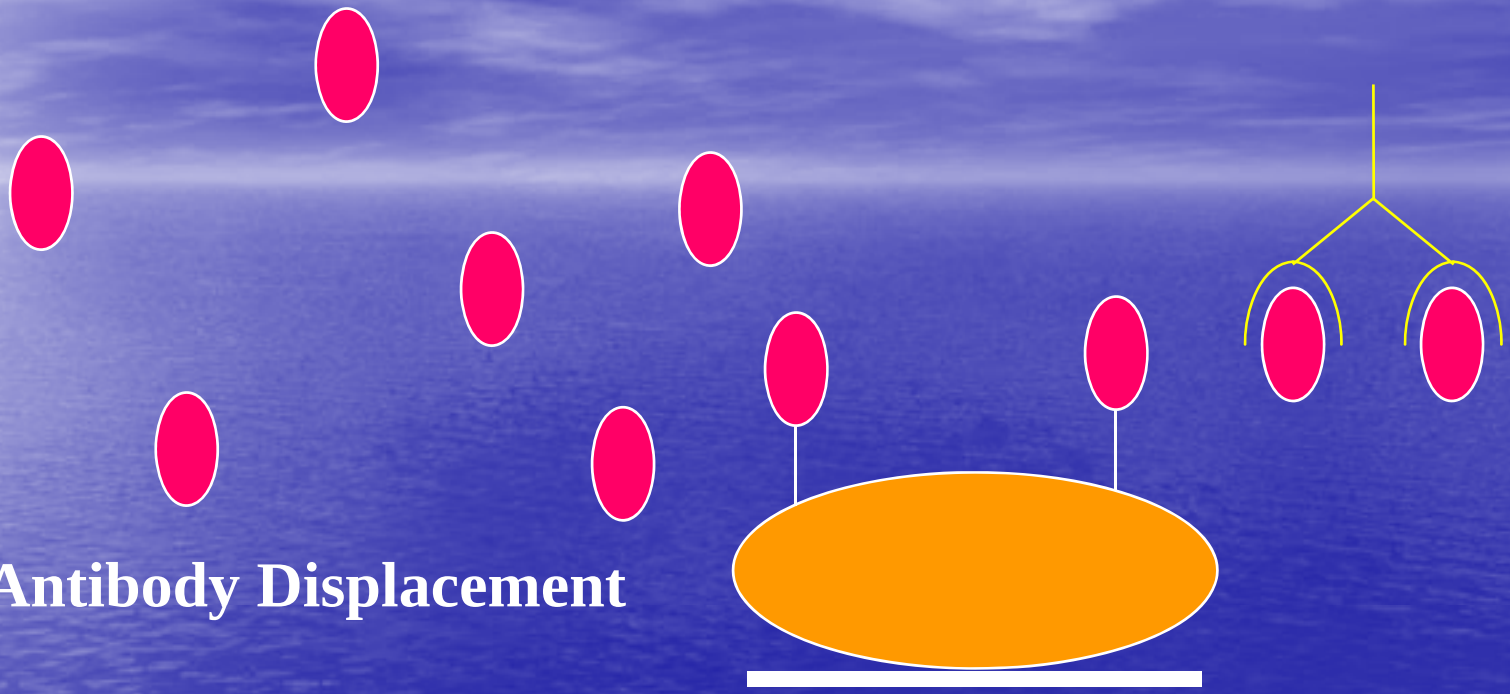
# Displacement Assay

**Introduction of Target**

Sample Detection Mode

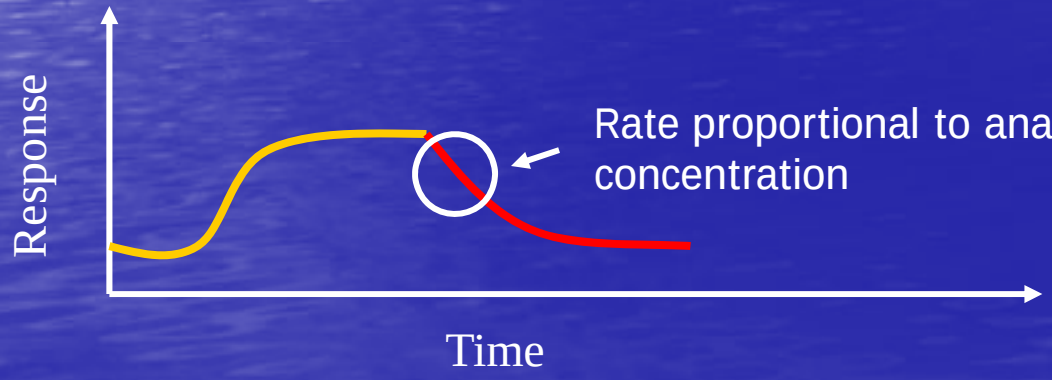


# Displacement Assay



**Antibody Displacement**

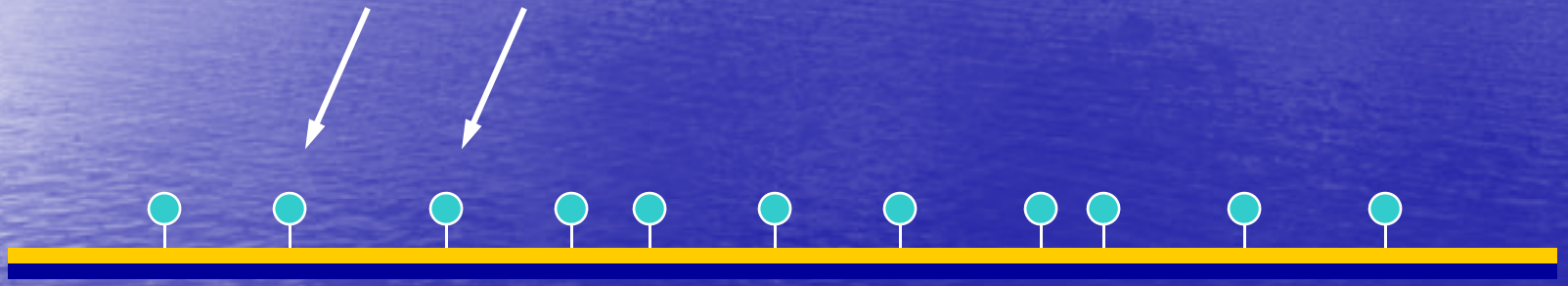
Displacement of Target Analog



The main advantage of the displacement assay is that the expensive components of the assay (antibodies or receptors) can be retained under a membrane and reused many times.

# Competition Assay

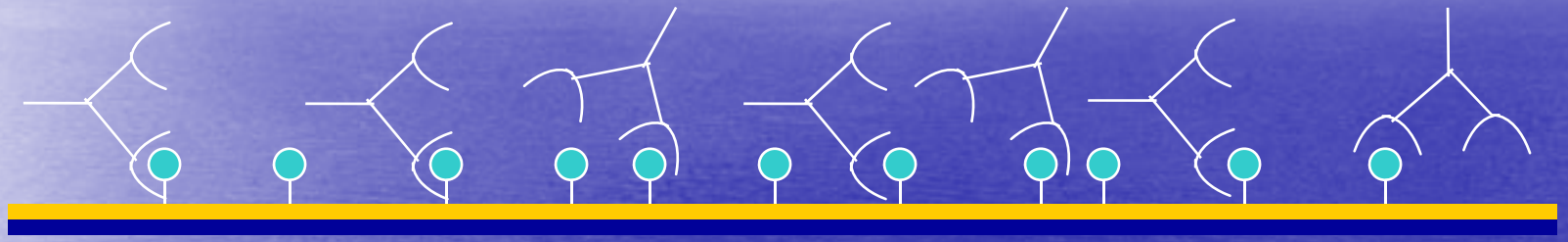
ANALYTE ATTACHED TO THE SURFACE

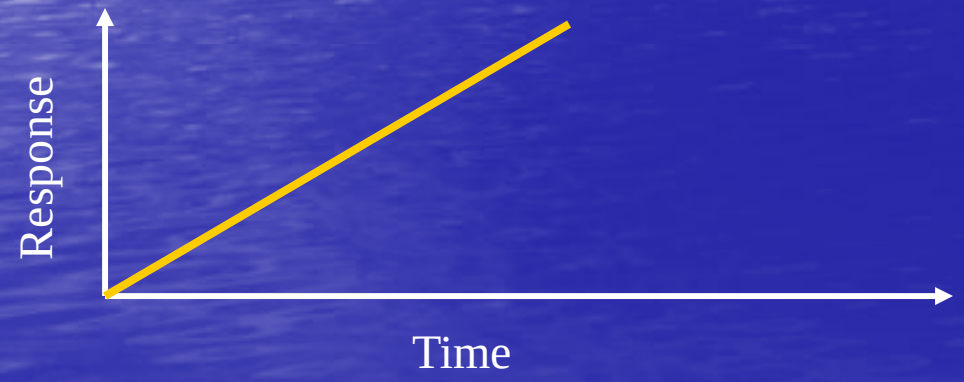


- **Small Analytes:**
- **Estriol, Cortisol, Domoate…**
- **Same analyte on the surface**

# Competition Assay

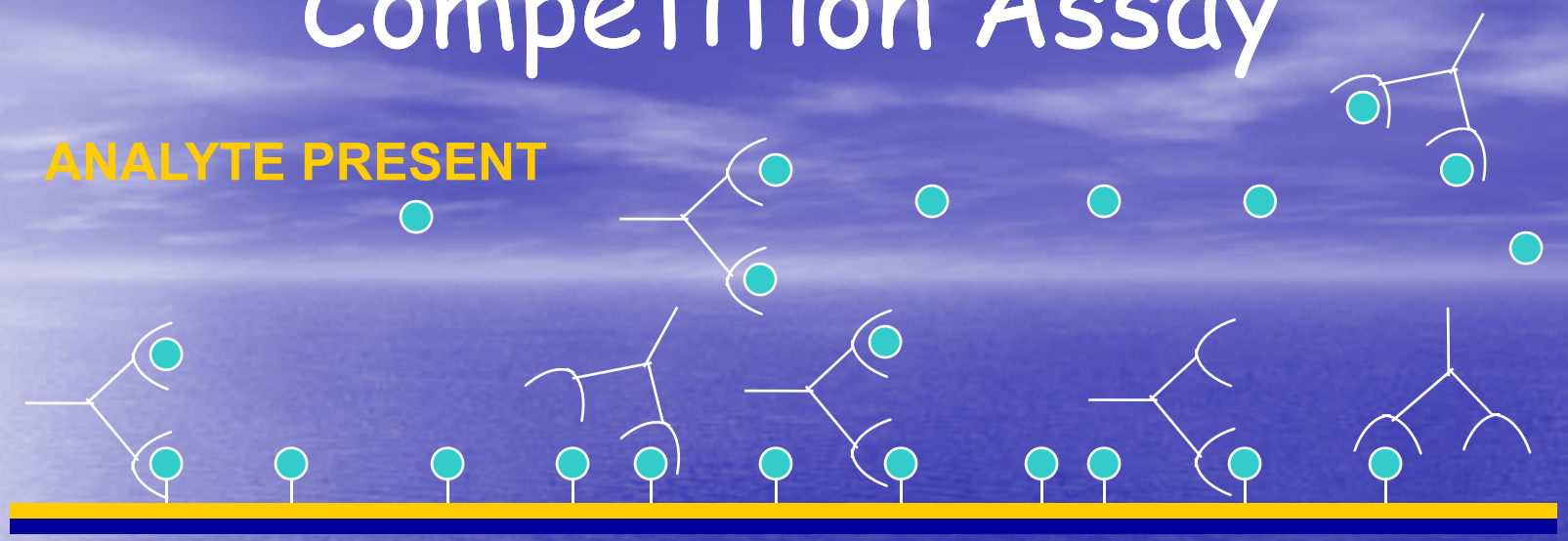
**NO ANALYTE PRESENT**

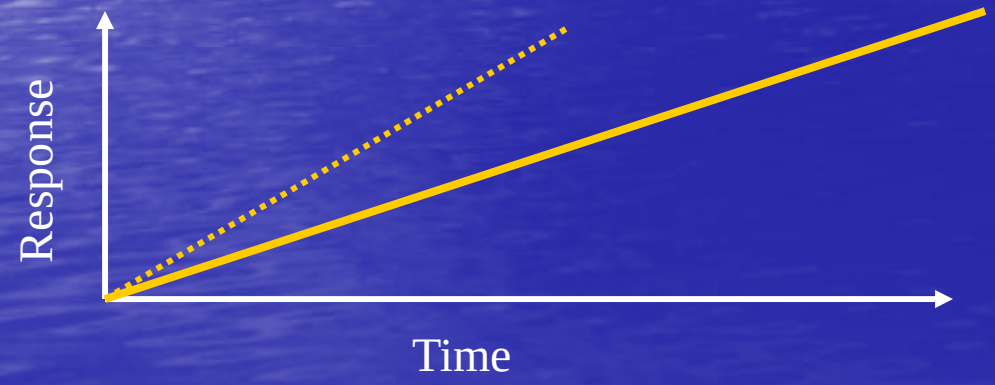




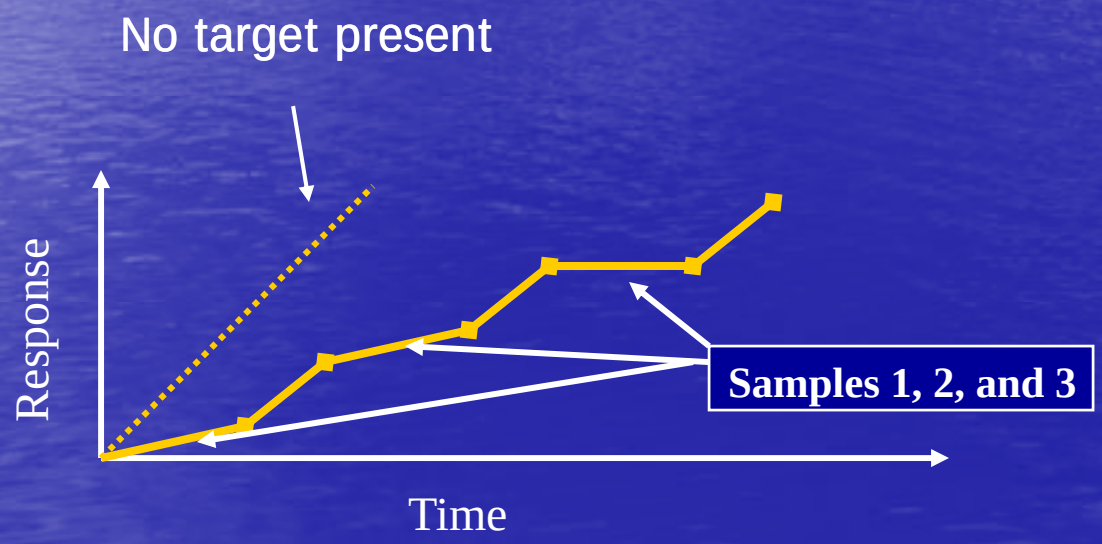
# Competition Assay

ANALYTE PRESENT

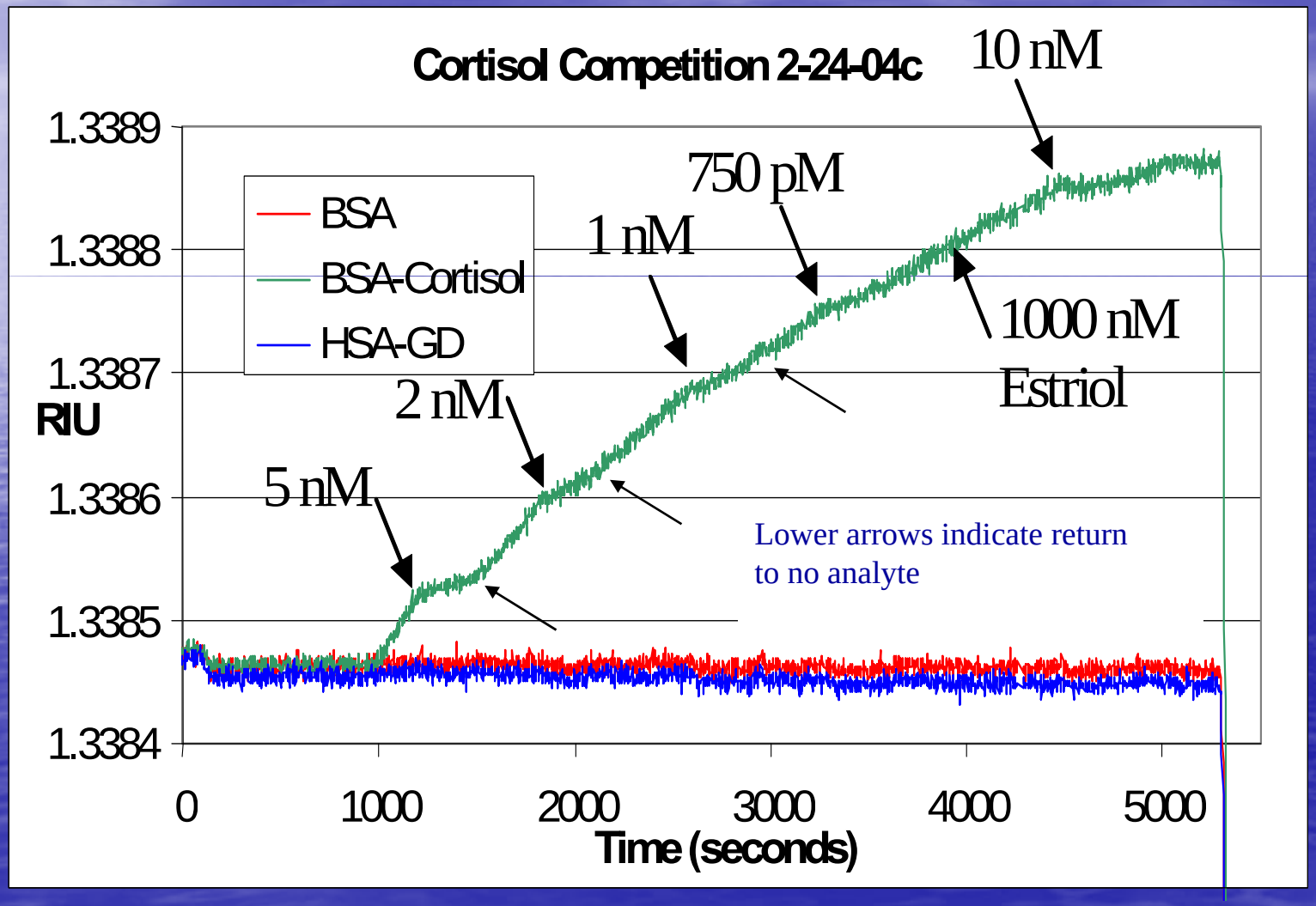




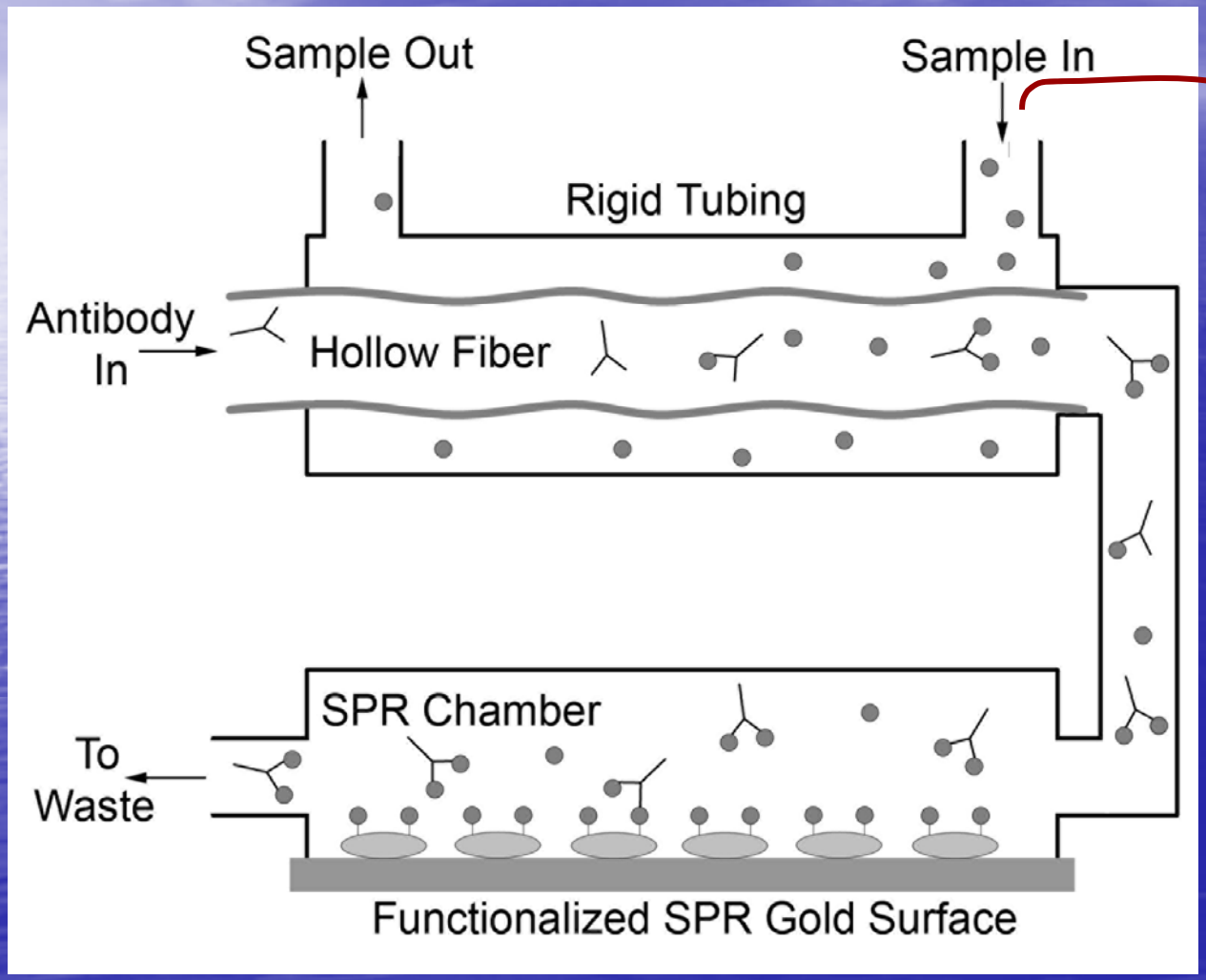
# Competition Assay

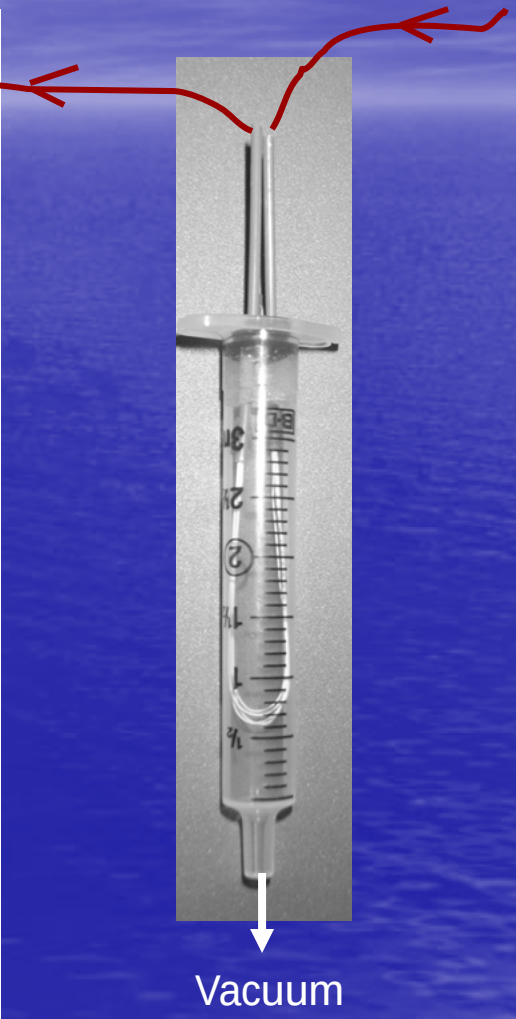


# Detection of Cortisol by Competition Assay

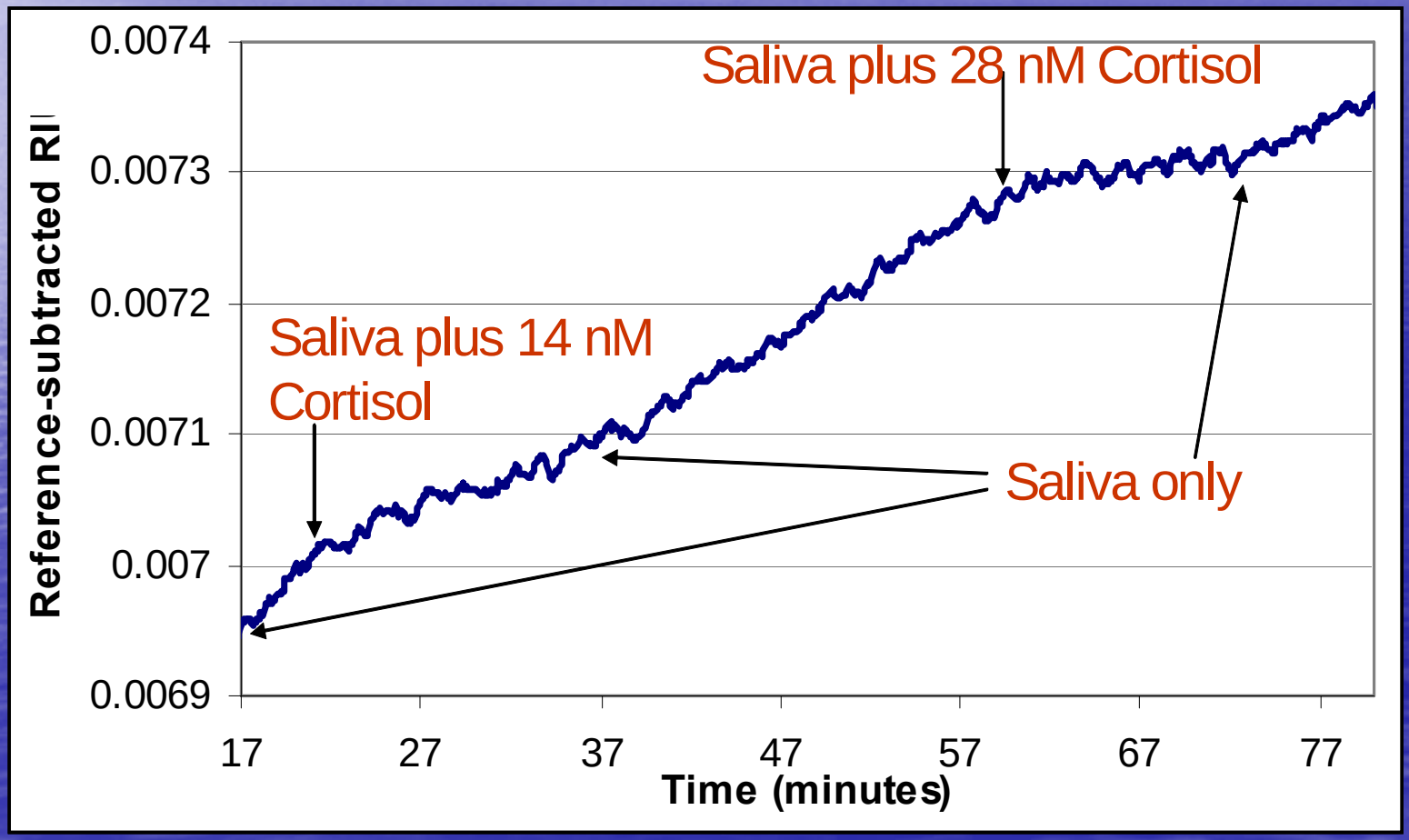


# External Compound Flow Cell

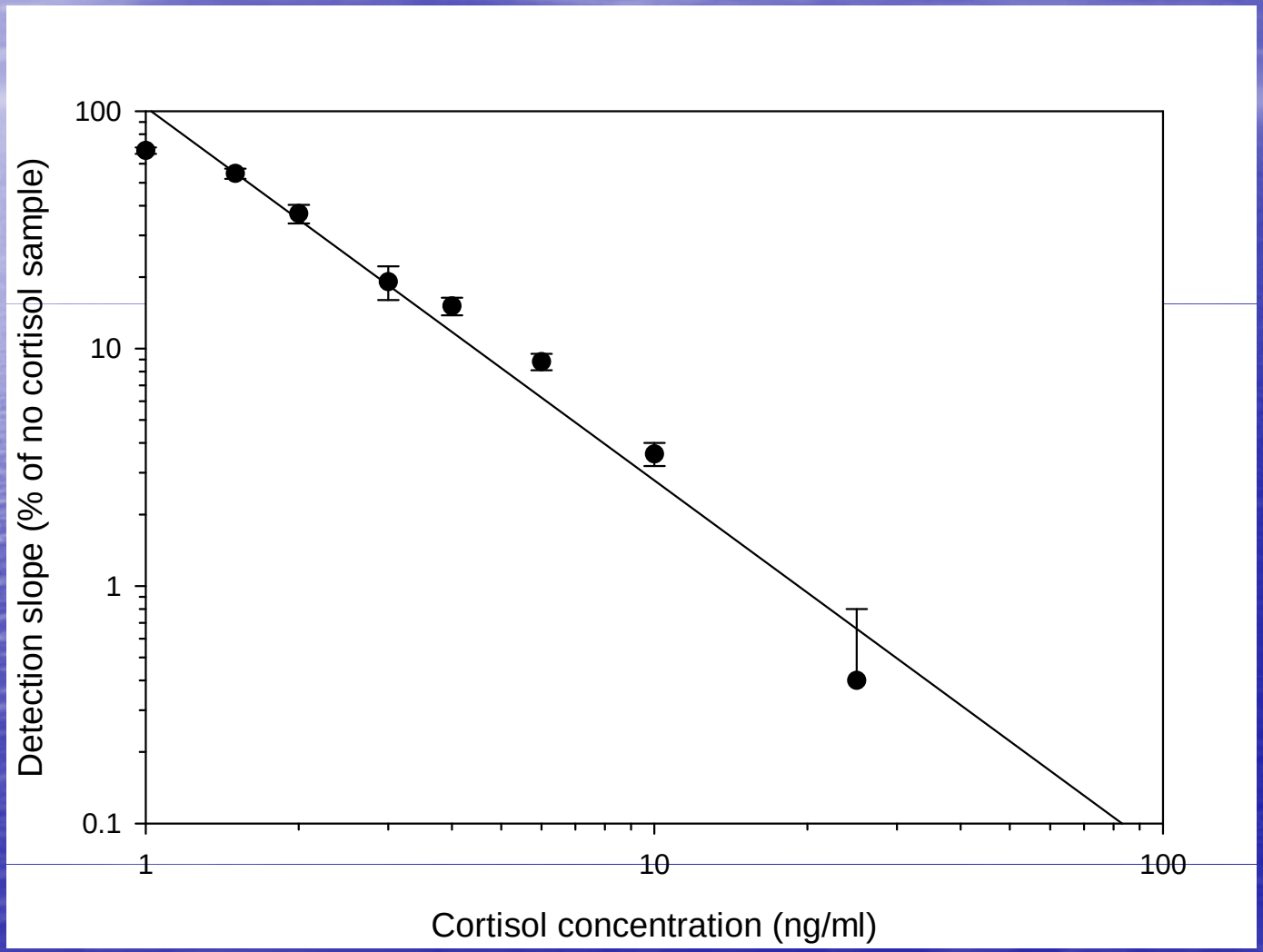


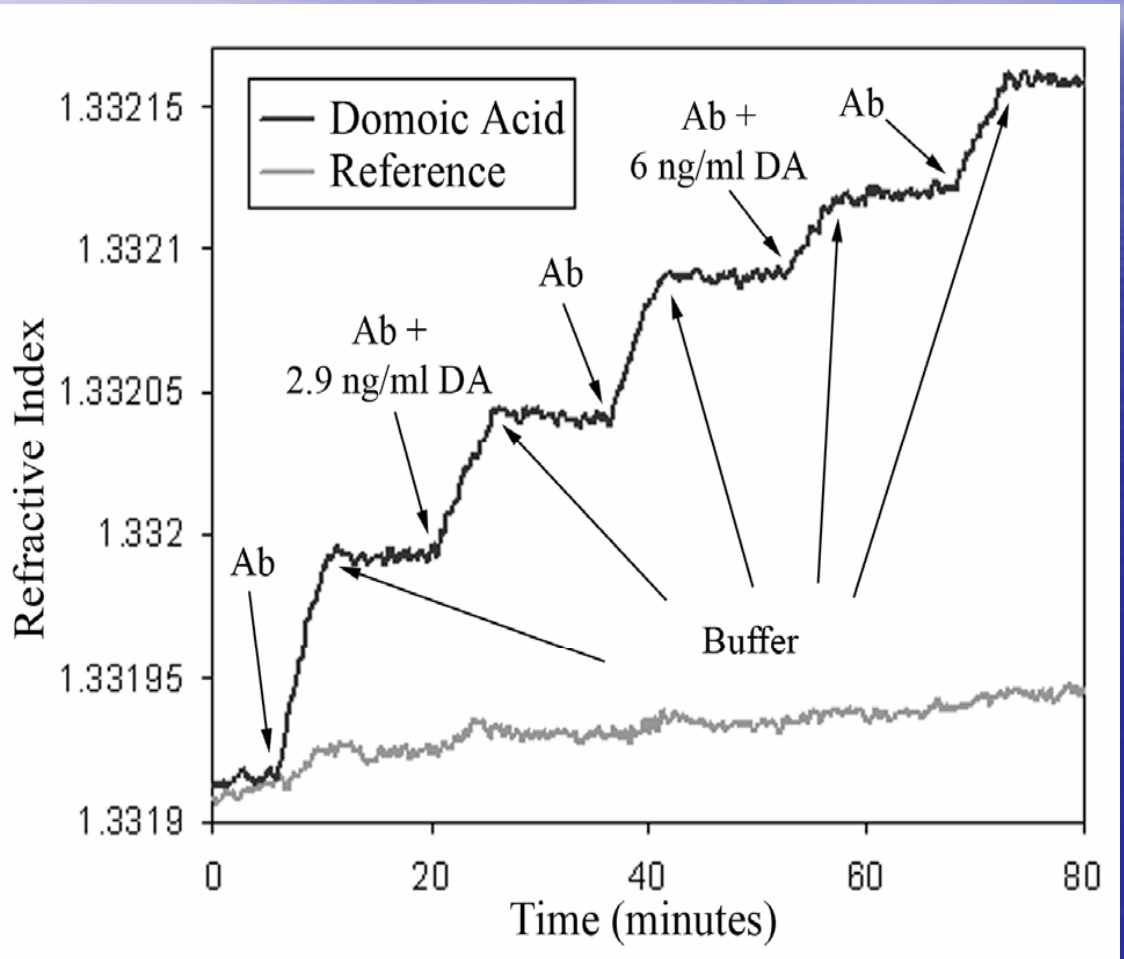
# Detection of cortisol in saliva using the compound flow cell

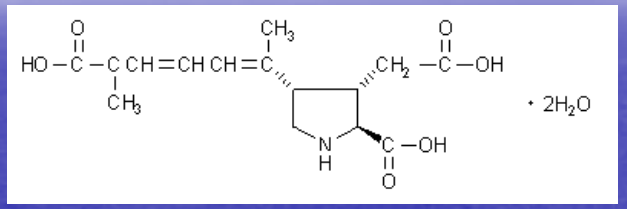


# Quantification of Cortisol



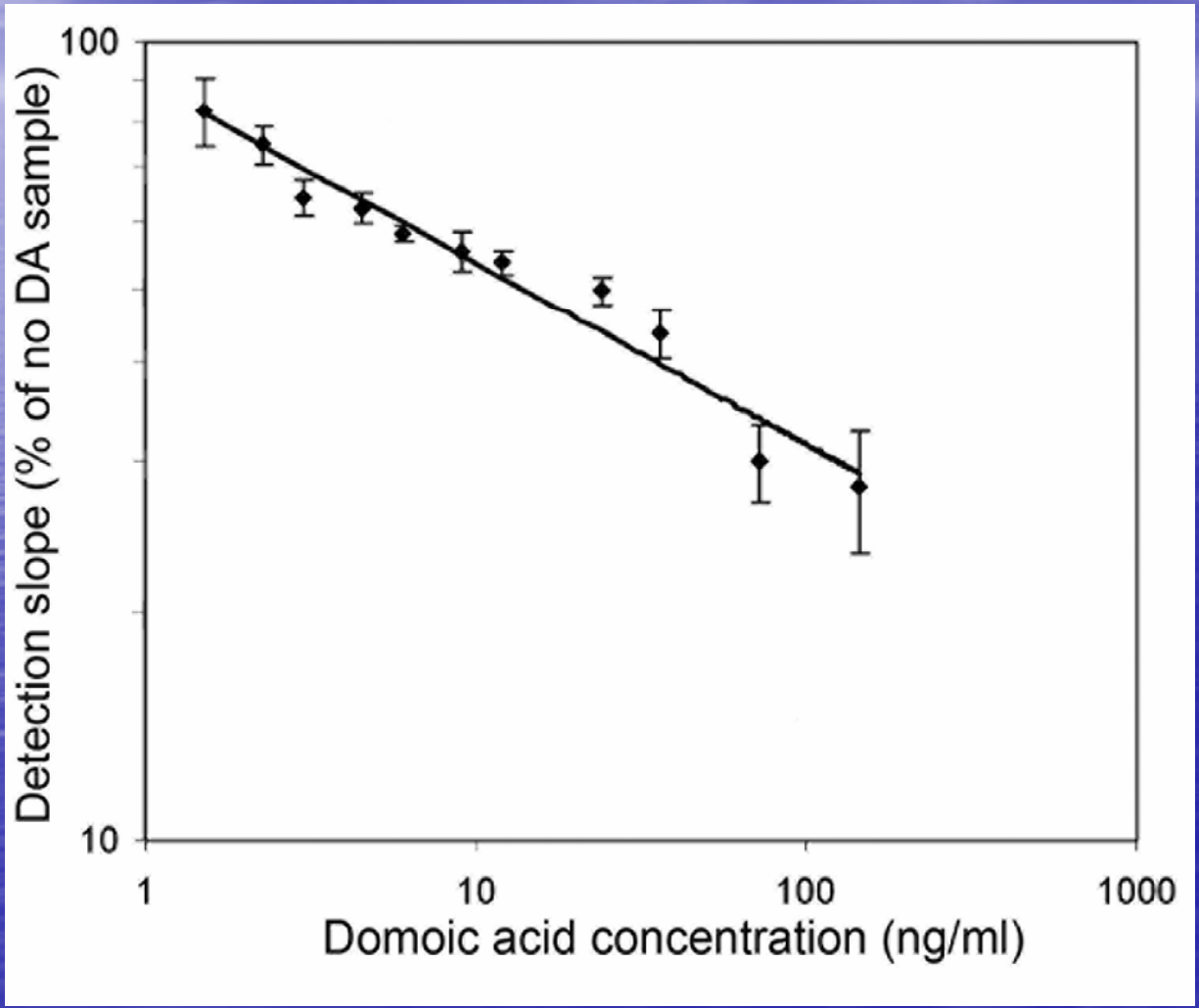
# Detection of Domoic Acid by Competition Assay



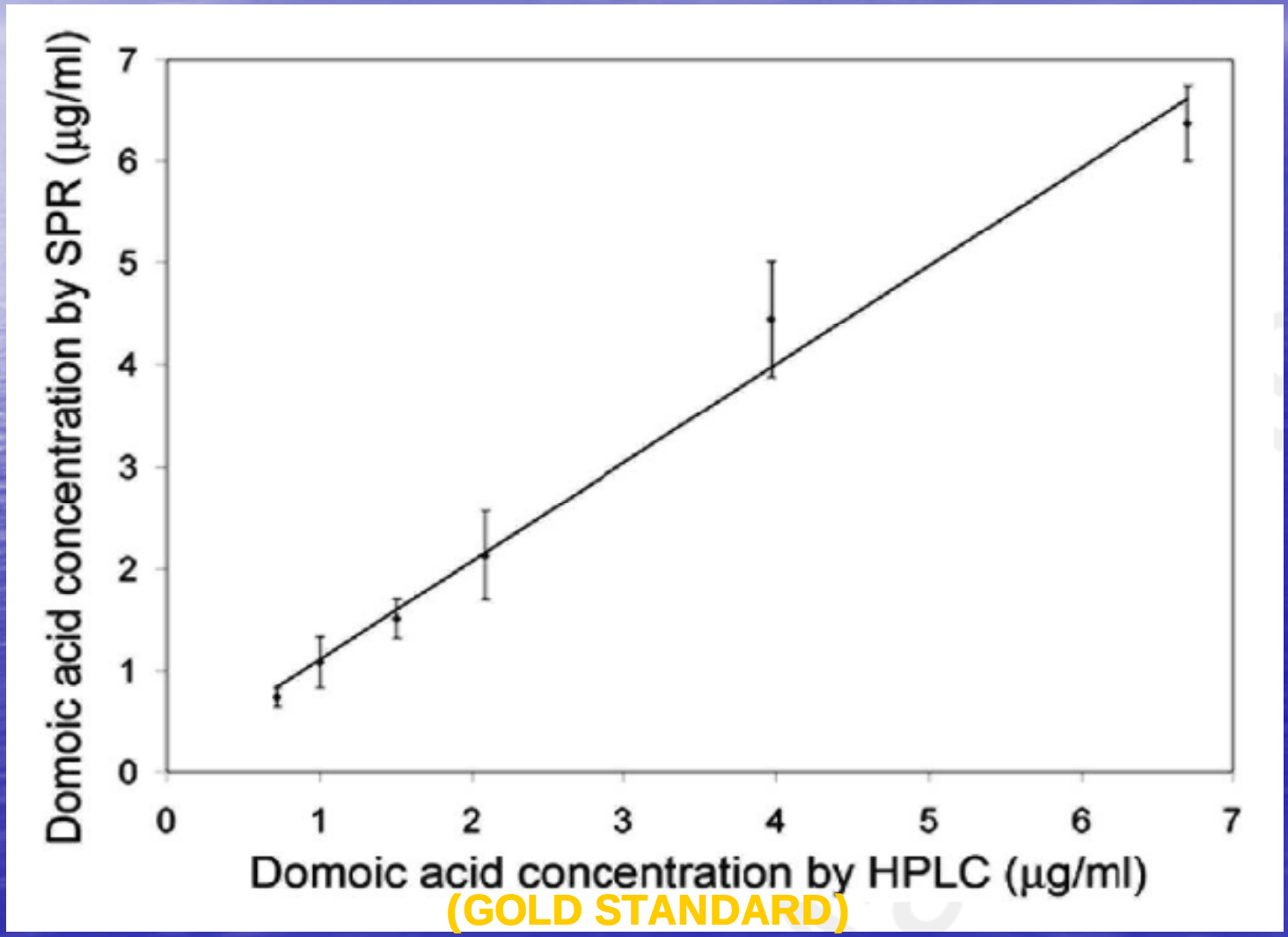

Collaboration with Dr. Vera Trainer's team at NOAA

# Standard Domoic Acid Concentration Curve in Clam Extracts

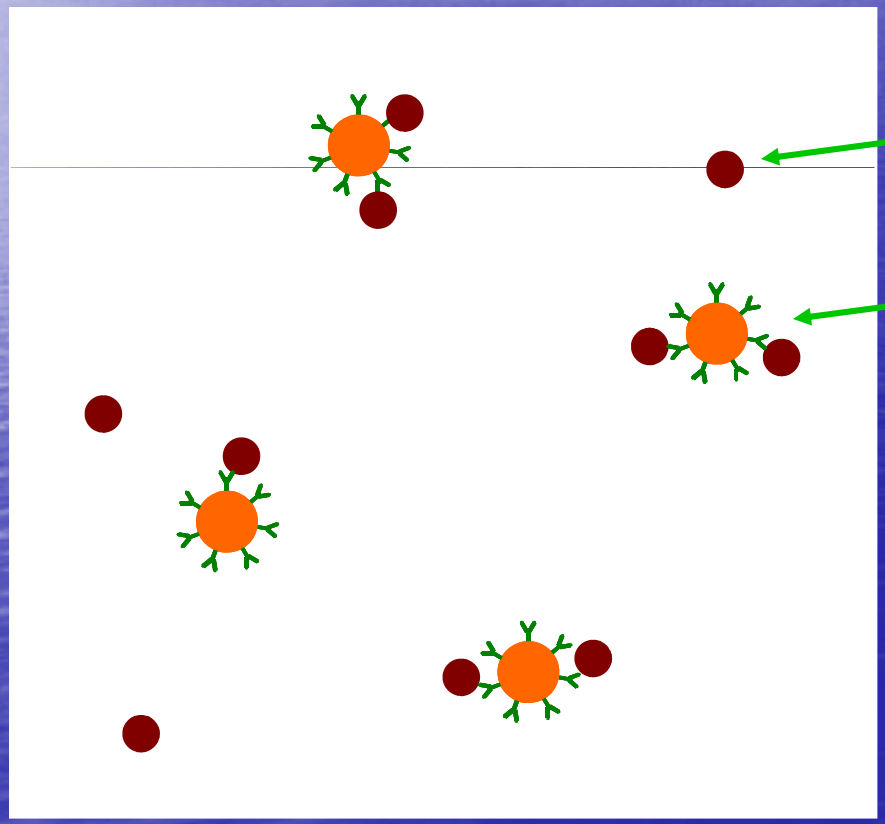



Stevens et al. Harmful Algae

# Comparison of HPLC/SPR Assays



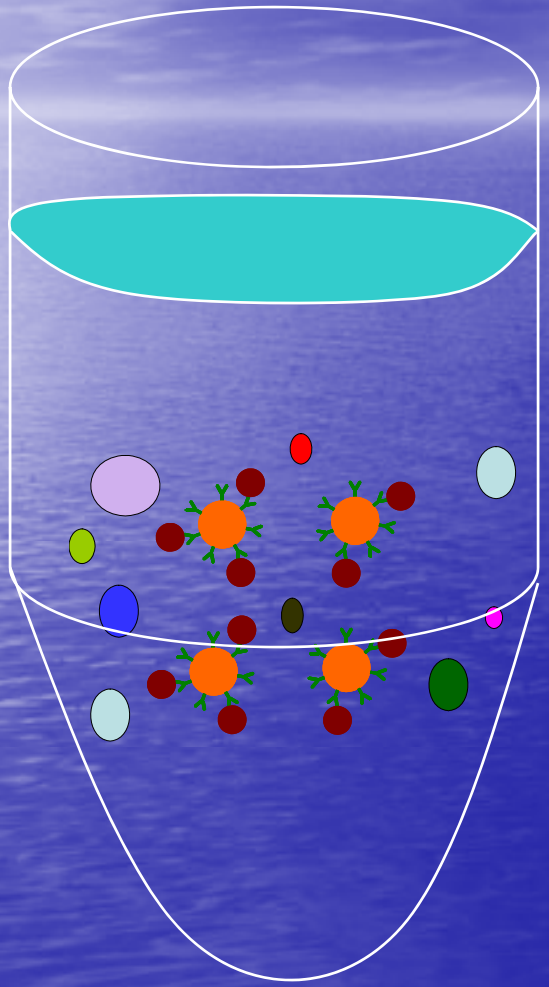
# Rapid Sample Clean Up-Concentration

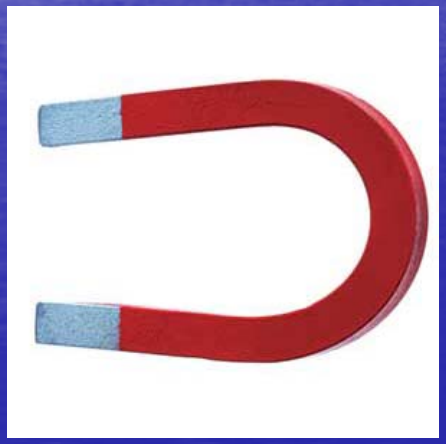


Analyte

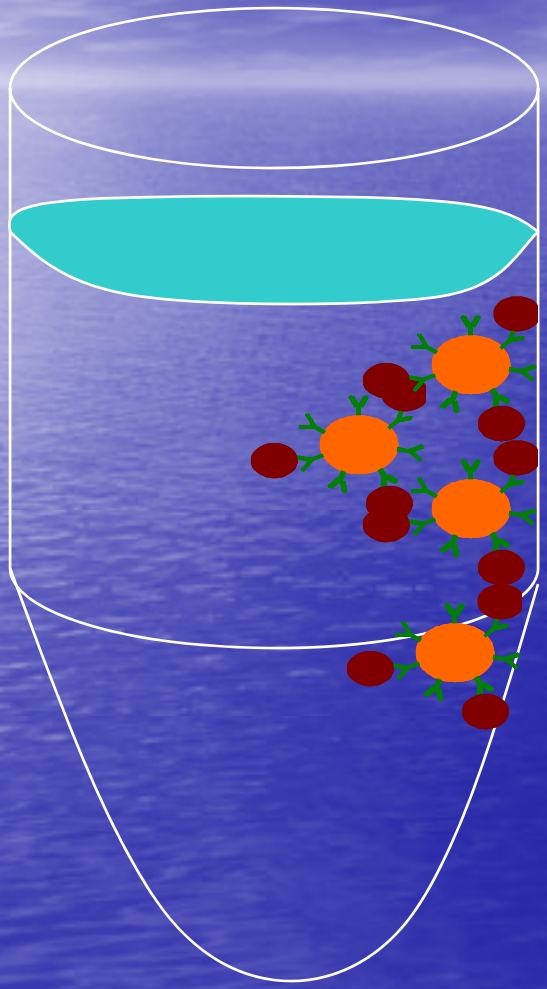
Analyte-immuno magnetic bead complex

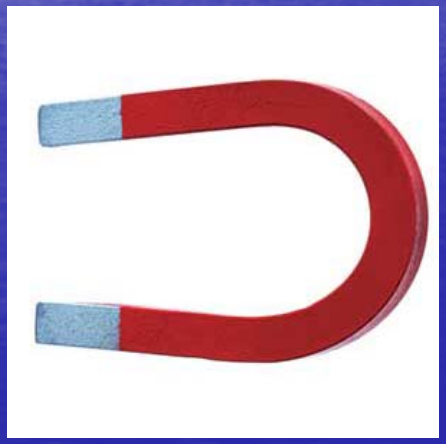
# Rapid Immuno Magnetic Bead Separation (IMS)

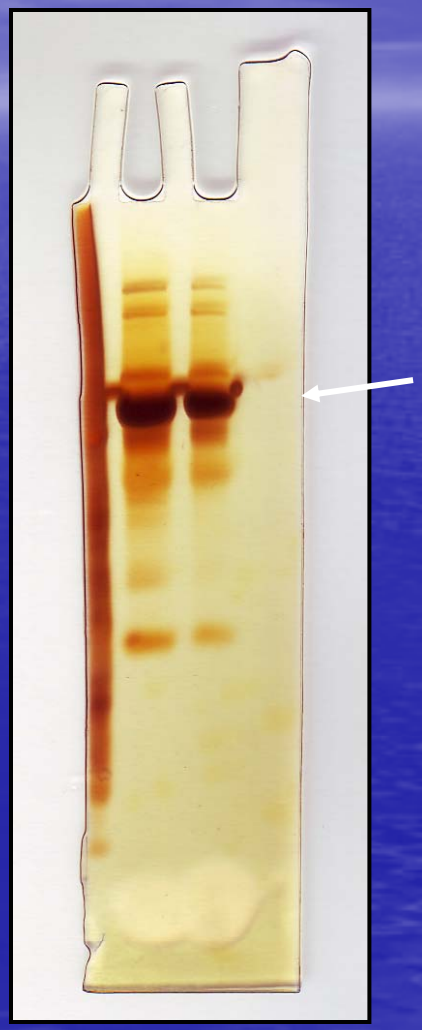




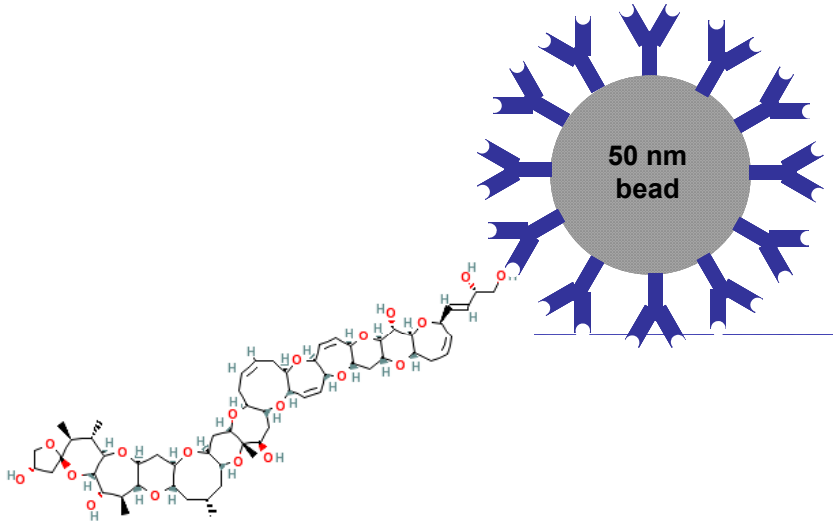
# Rapid Immuno Magnetic Bead Separation (IMS)



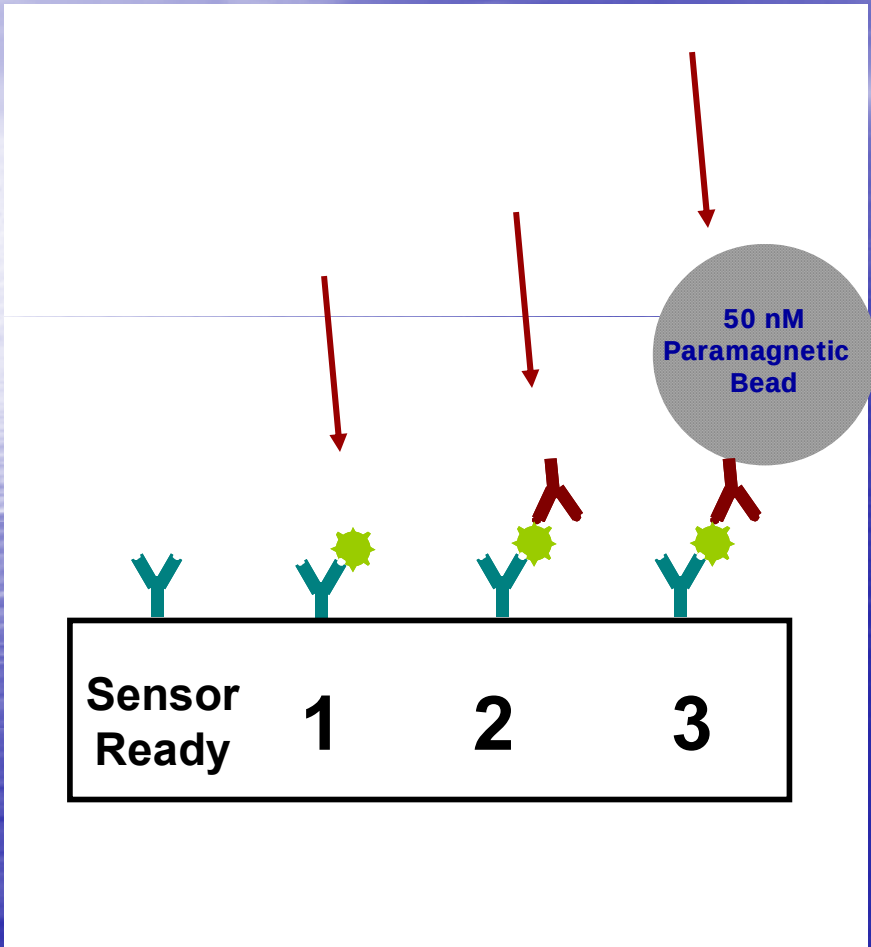


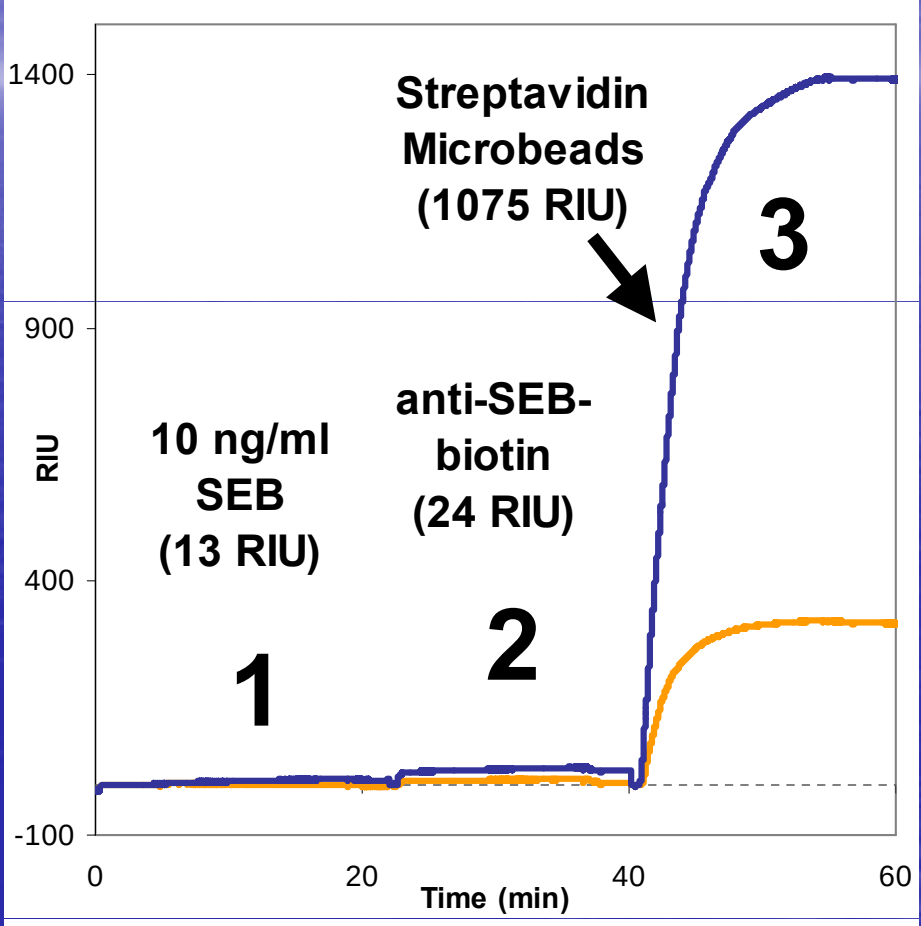


# Design for Ciguatoxin Detection Protocol

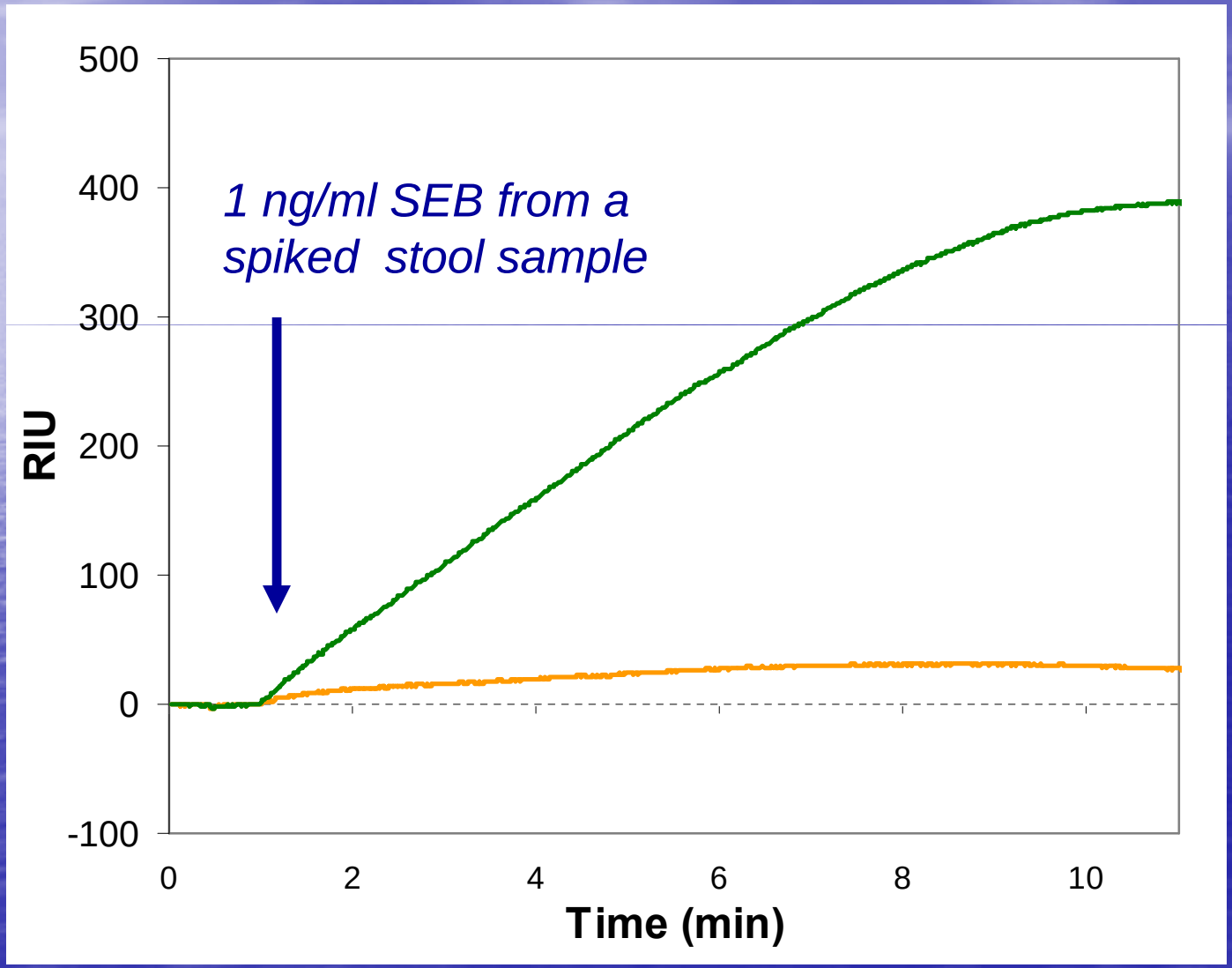



# Concentration, Verification, Amplification Protocol



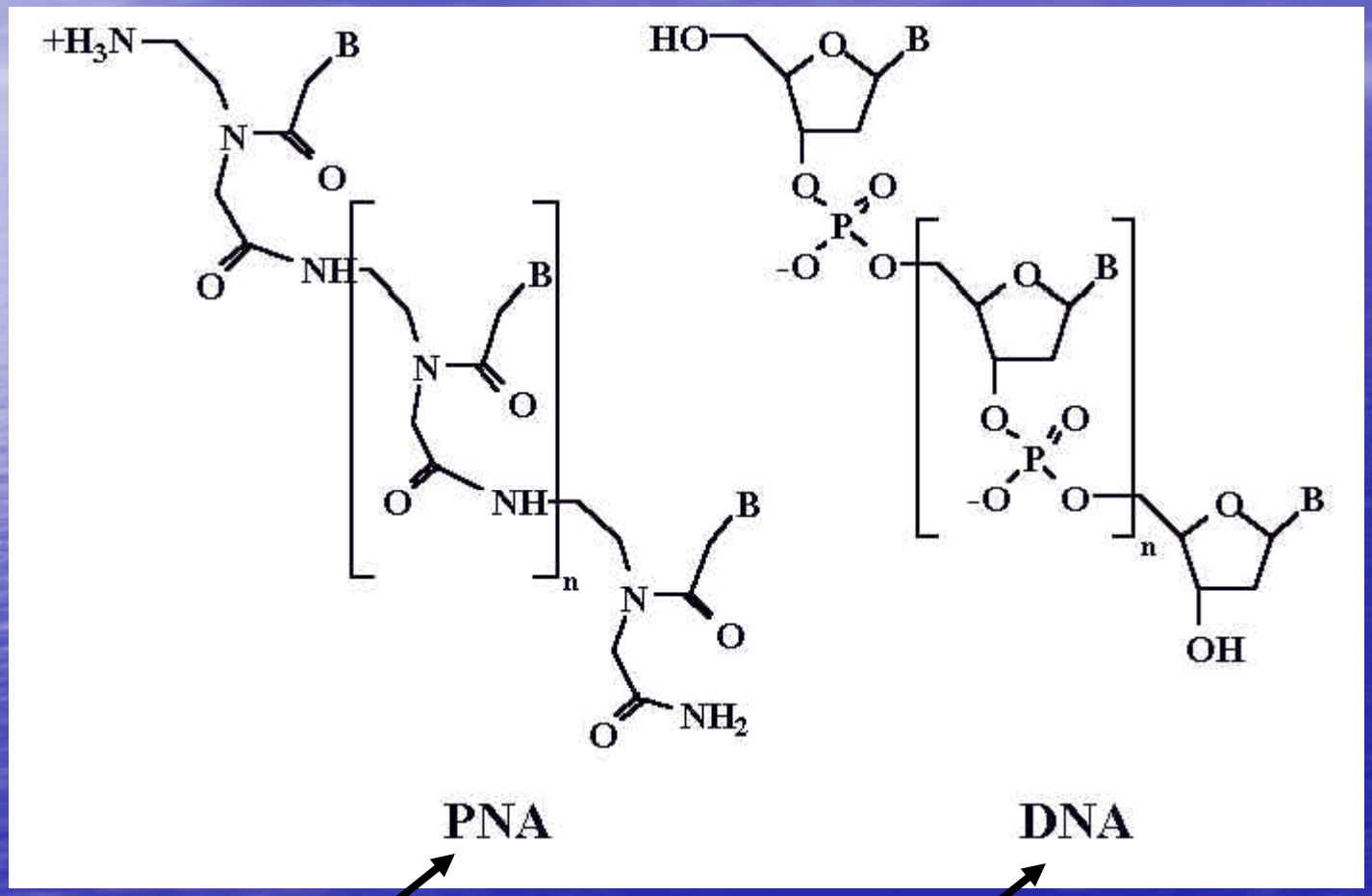
# Concentration, Amplification and Verification of SEB from a Stool Sample



# 1/03 – 6/03: Airborne SPR Sensing Test Flights

Variviggen instrumented with SPR sensors

# Protein Nucleic Acids as Recognition Elements for DNA/RNA

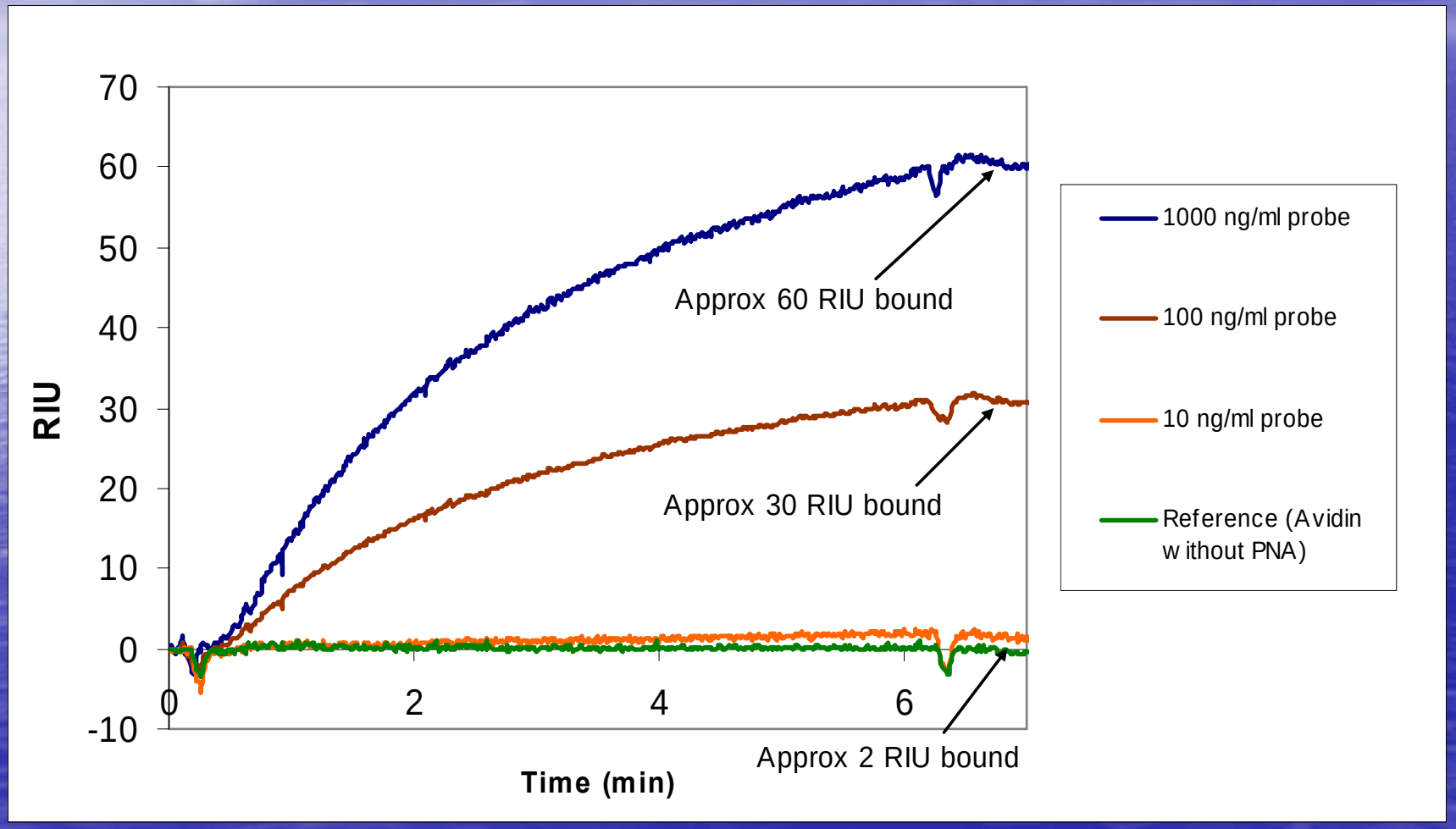



Very stable receptor on chip

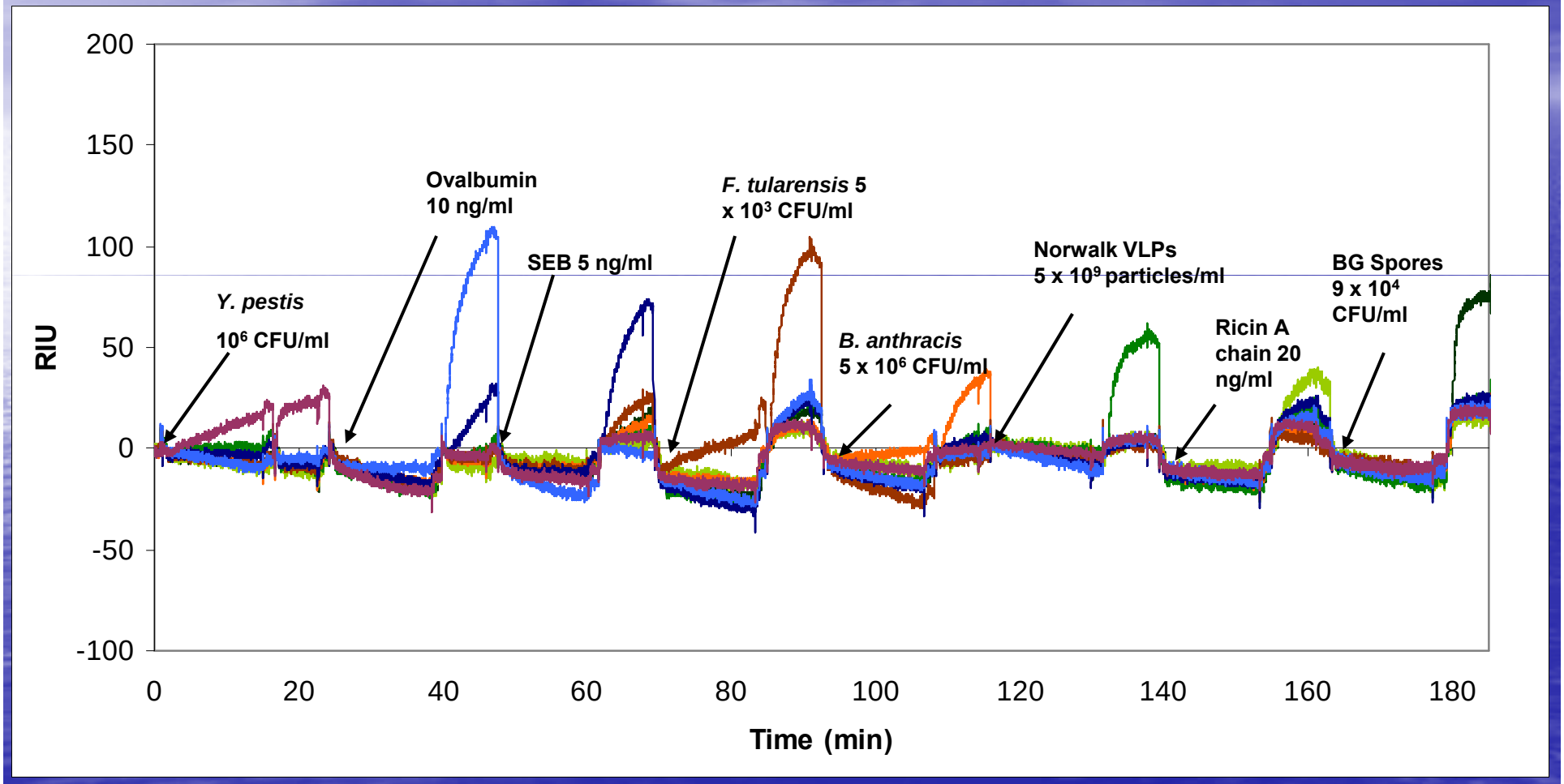
(Protein Nucleic Acid)

Allows detection of target

# Binding of a 79 bp DNA Probe to a Complementary PNA 16 mer on the Sensor Surface

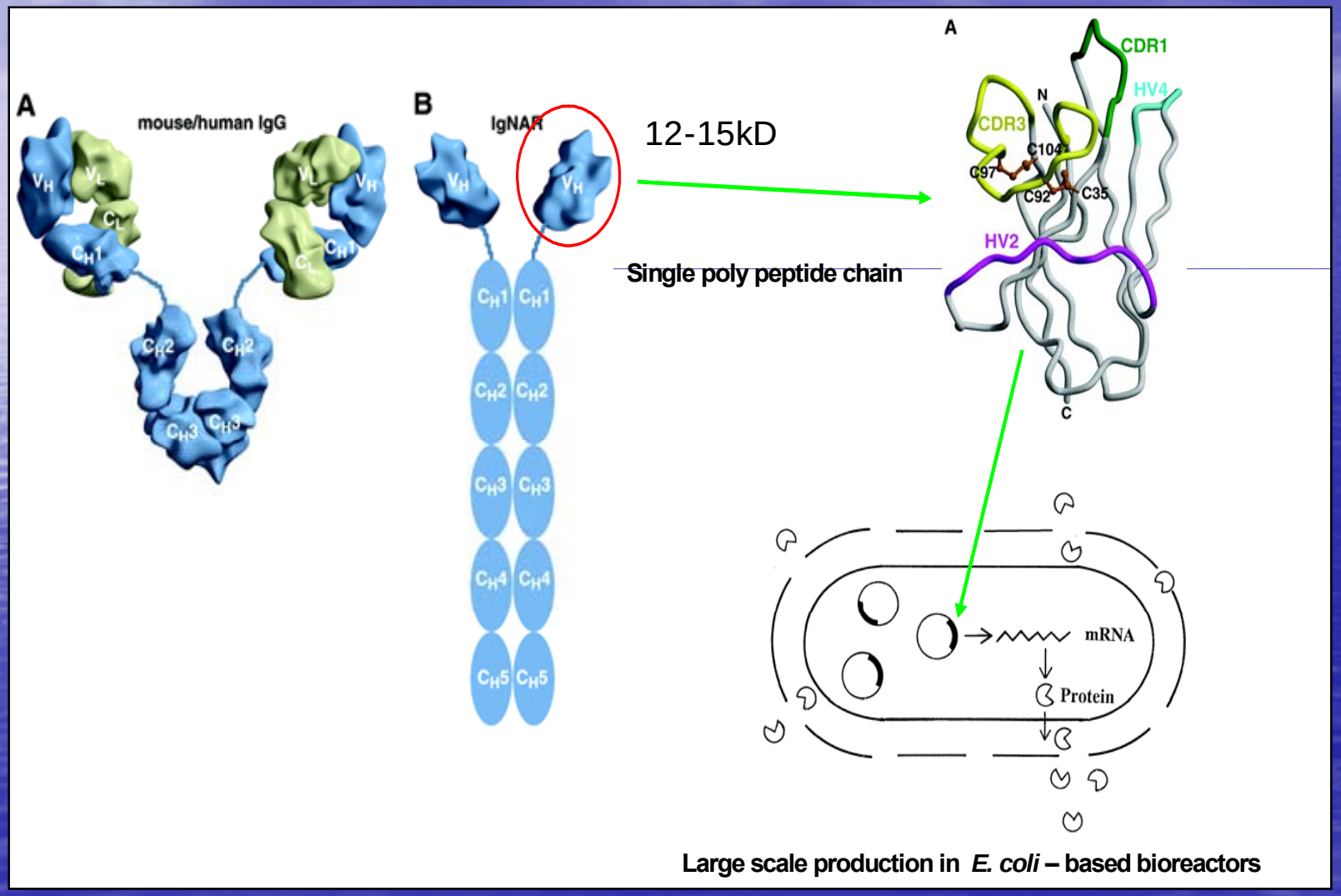


# Sequential Detection of 8 Analytes

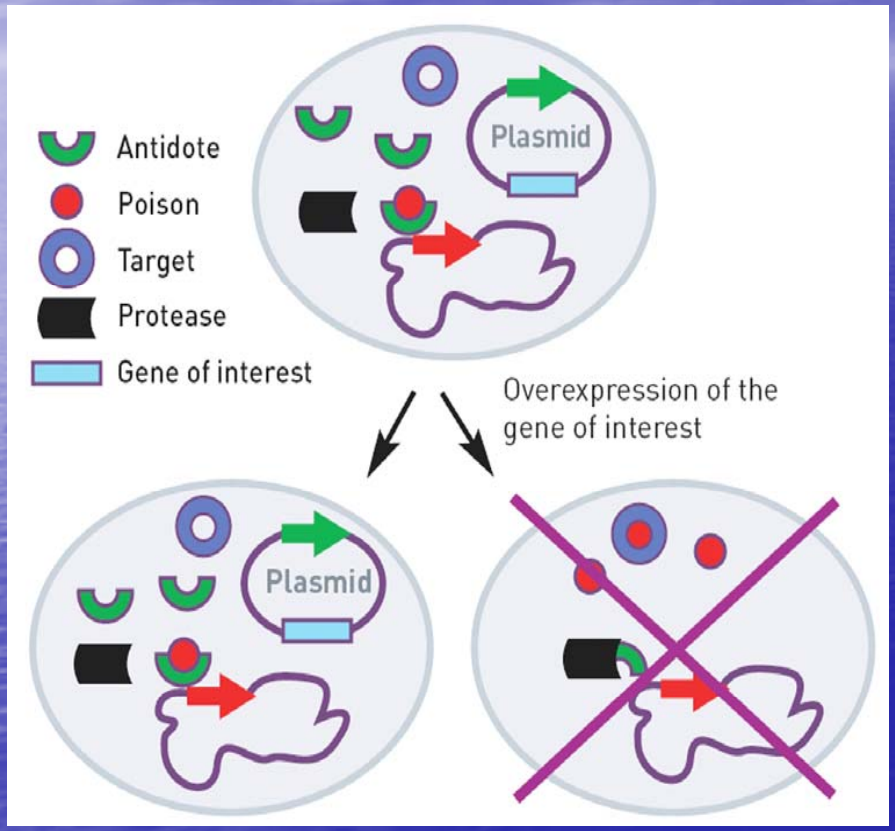


# Inexpensive Production of Antibodies

# Advantages of Shark IgNARs



# Staby Expression System



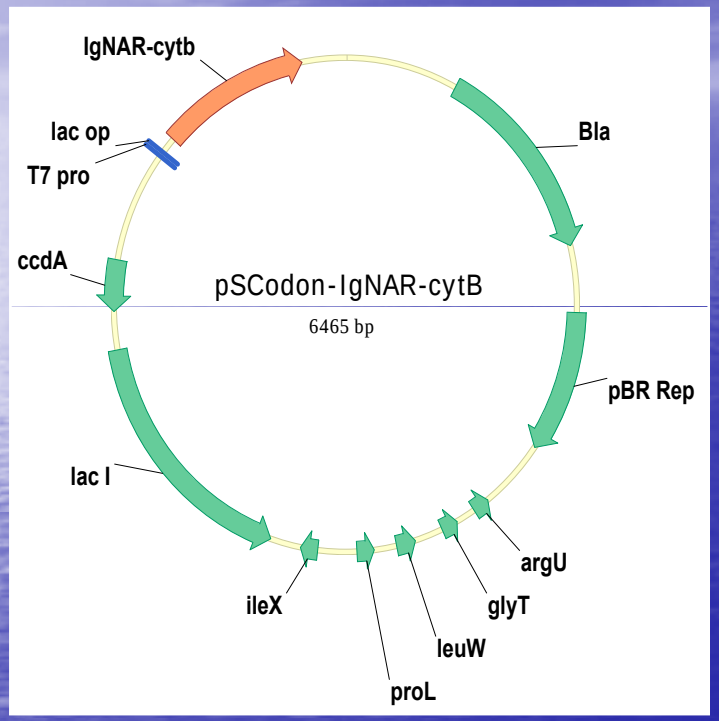

Poison = CcdB

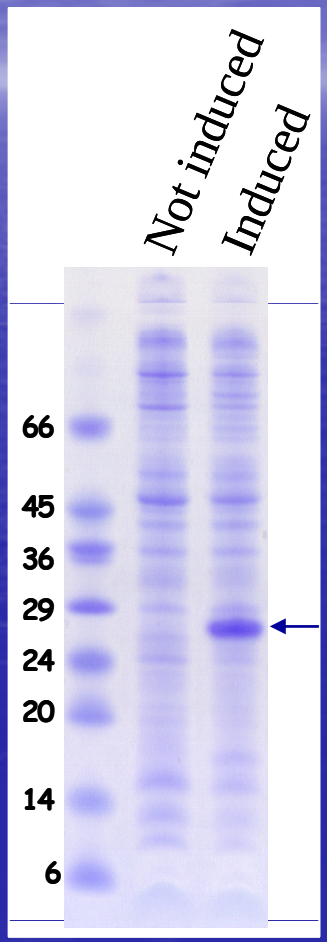
Antidote = CcdA

Target = DNA

From Eurogentec Staby manual

# Expression of IgNAR-cytb fusion

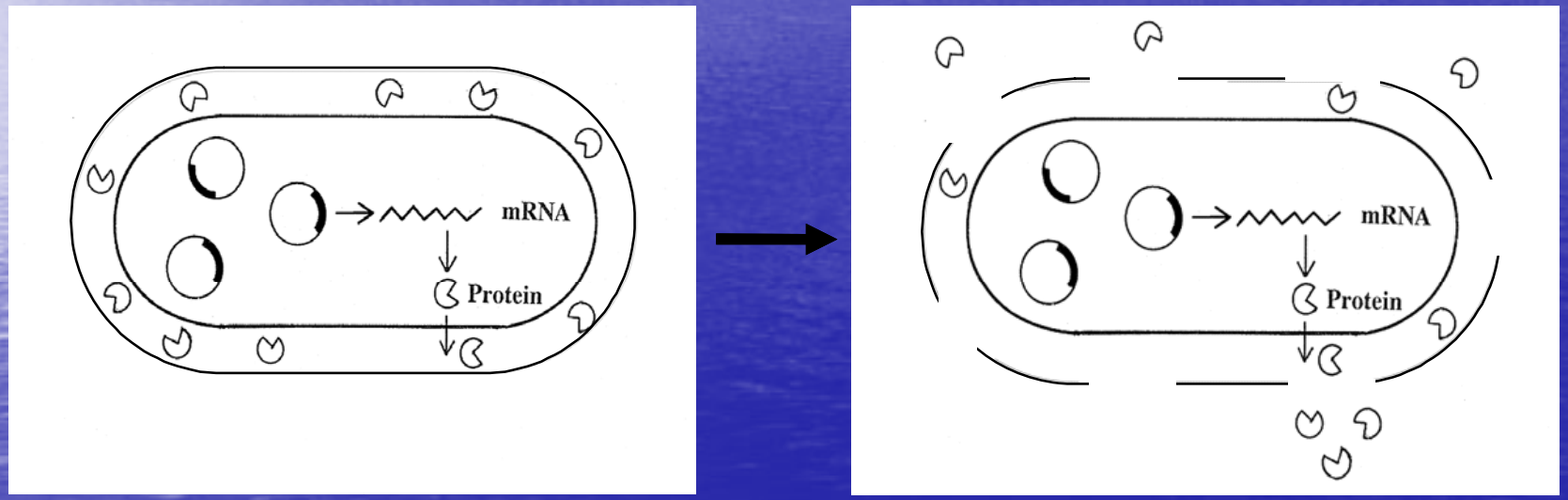


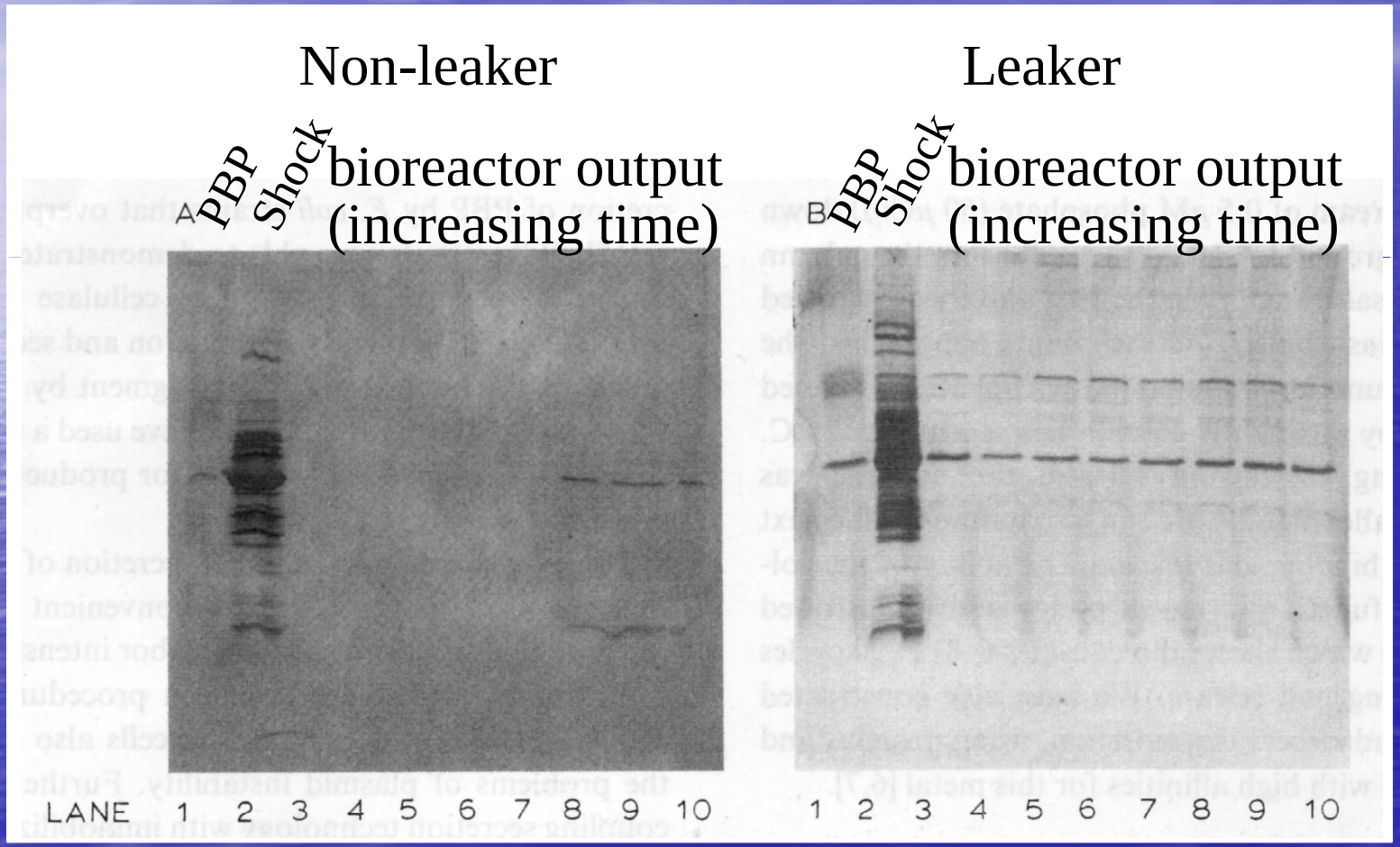


The heme-binding domain of mouse cytochrome b5 has a red color allowing visualization of expression.

# Generation of Leaker Strains

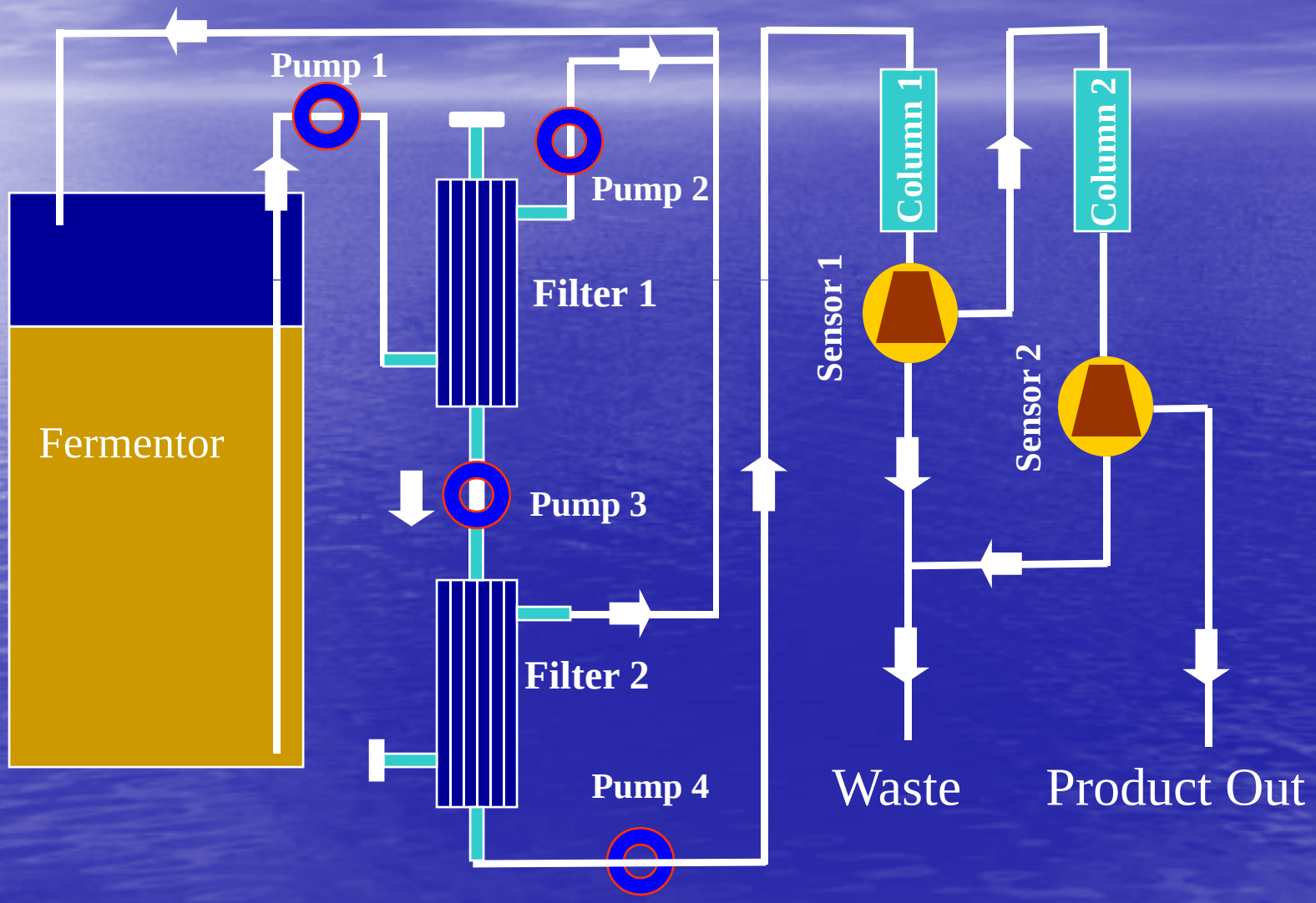


# Phosphate binding protein production




Furlong and Sundstrom, J. Indus. Microbio. 30: 141-148

# Protein purification system



# Conclusion

The portable SPR sensing system can provide near real-time monitoring for many different applications in environmental monitoring, the food industry, pharmaceutical industry, medical diagnostics and general research needs.

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  - Joshua Probert
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  - **Dwight Bartholomew**
  - **John Quinn**
- **Seattle Sensor Systems**
  - **Nathaneal Swanson**
  - **Dr. Paul Baker**

**Financial Interests:**

CEF, SDS, PK & PB hold stock in Seattle Sensor Systems

**Sponsors:**

DOD
Texas Instruments
Center for Process Analytical Chemistry (CPAC), UW, Seattle
Washington State Sea Grant
NSF/NIEHS NW Center for Human Health and Ocean Studies