

# Extraction, Concentration and Detection of Toxins

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# Objectives

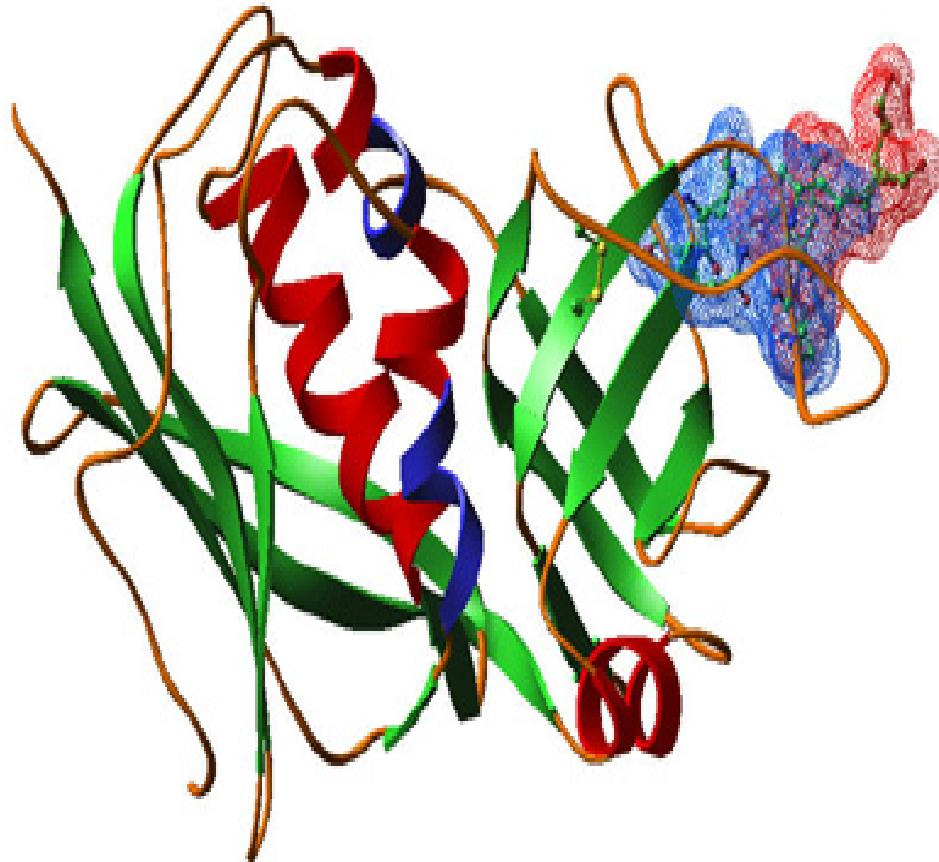
- Fabricate hydrogel imprinted polymers for concentration of *Staphylococcus aureus* enterotoxin B
- Construct impedimetric immuno-sensors to detect enterotoxin B
- Develop a pathogenicity biosensor

# Staphylococcal Enterotoxin B

- Bacterial toxin
  - *Staphylococcus aureus*
- Easily soluble in water
- Very resistant to temperature fluctuations
  - Withstands boiling for several minutes
  - Freeze dried remains active for one year

# Enterotoxin B

- Mw 28 kDa
- 240 Amino Acids
- LD<sub>50</sub> 30ng/Kg
- Detection limits 1ng



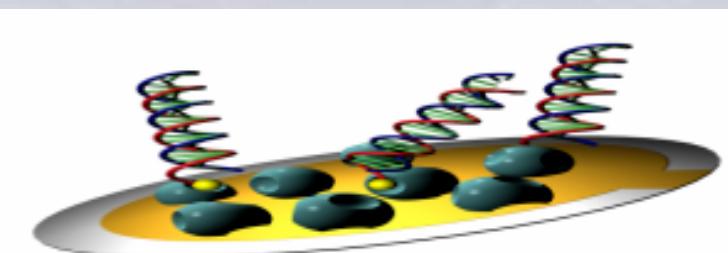


# *Development of Hydrogel-based Molecularly Imprinted Polymers (HydroMIPs) for Protein Recognition*

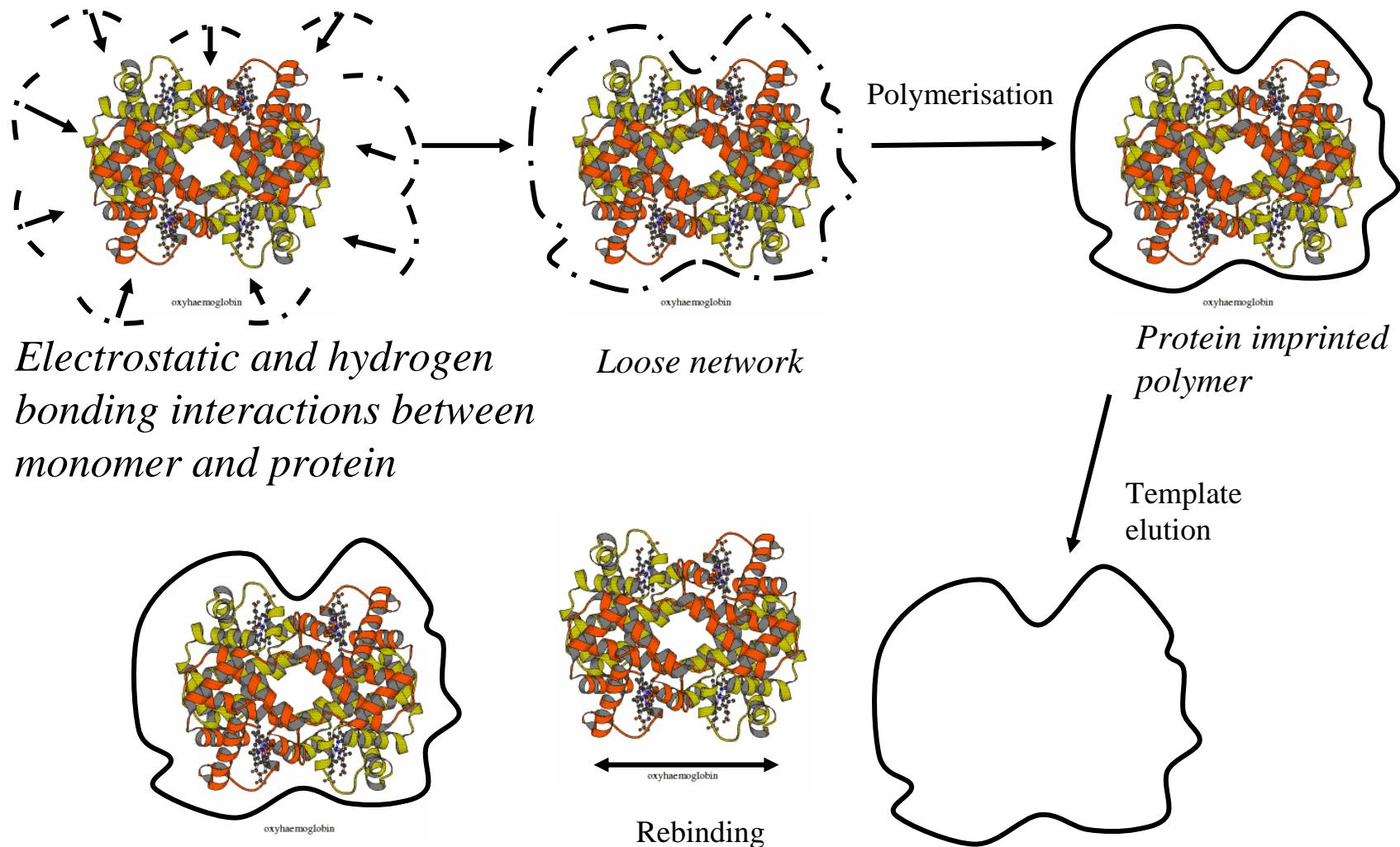
Dr Sub Reddy and Quan Phan

School of Biomedical and Molecular Sciences

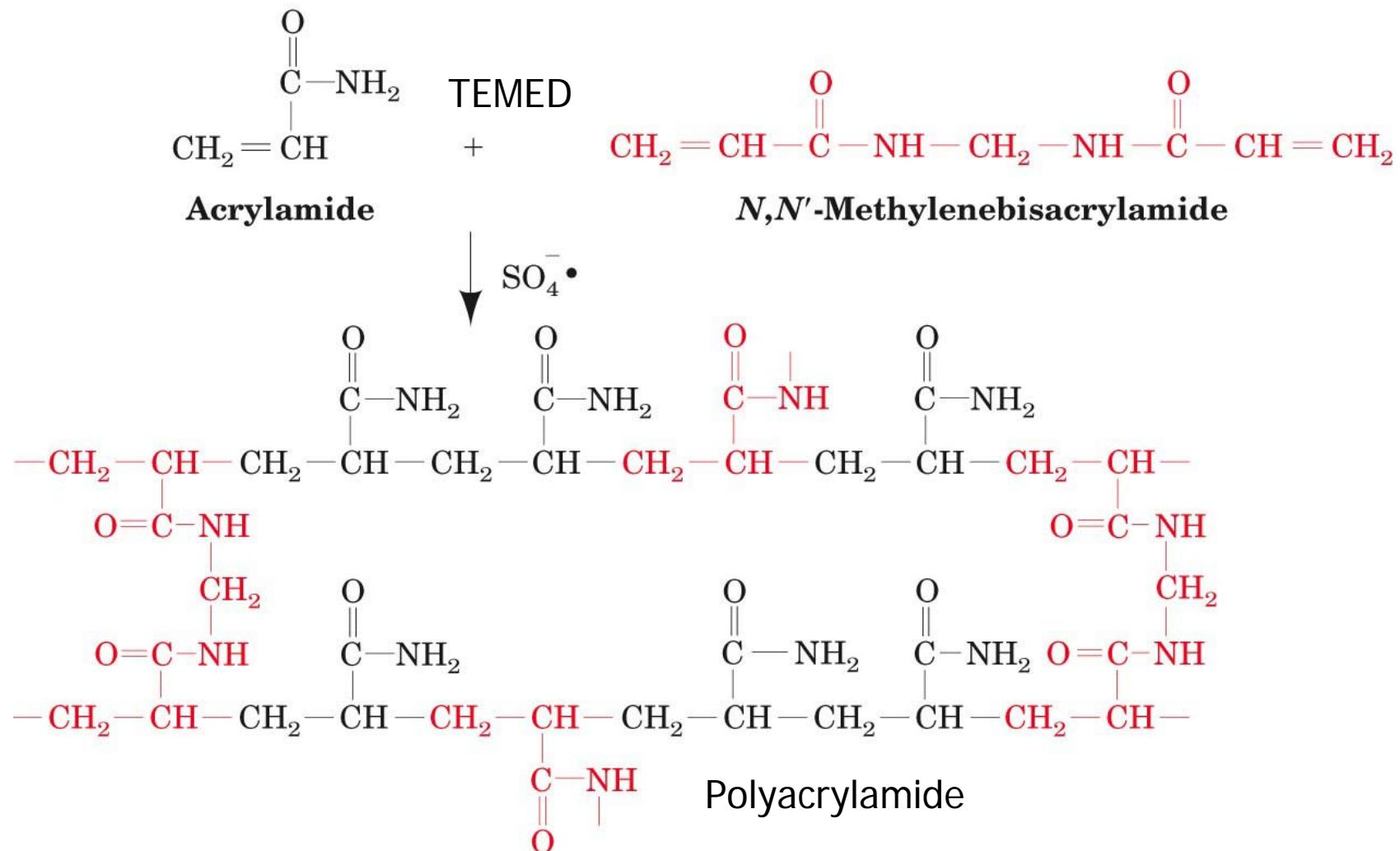
University of Surrey, UK



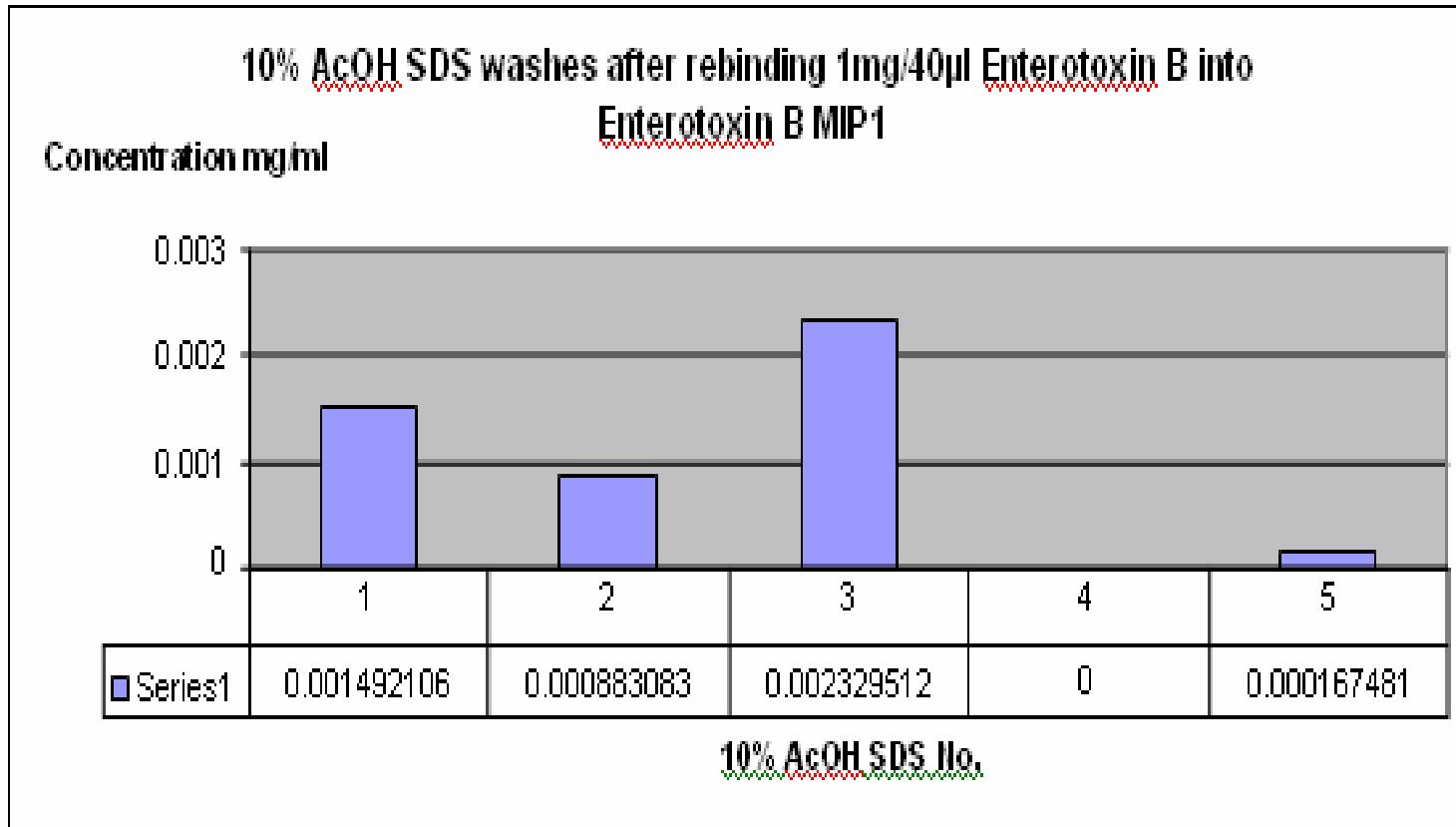
# Protein Imprinted Polymers



# Molecularly Imprinted Polymer Hydrogels: 'Nano-play-dough!'



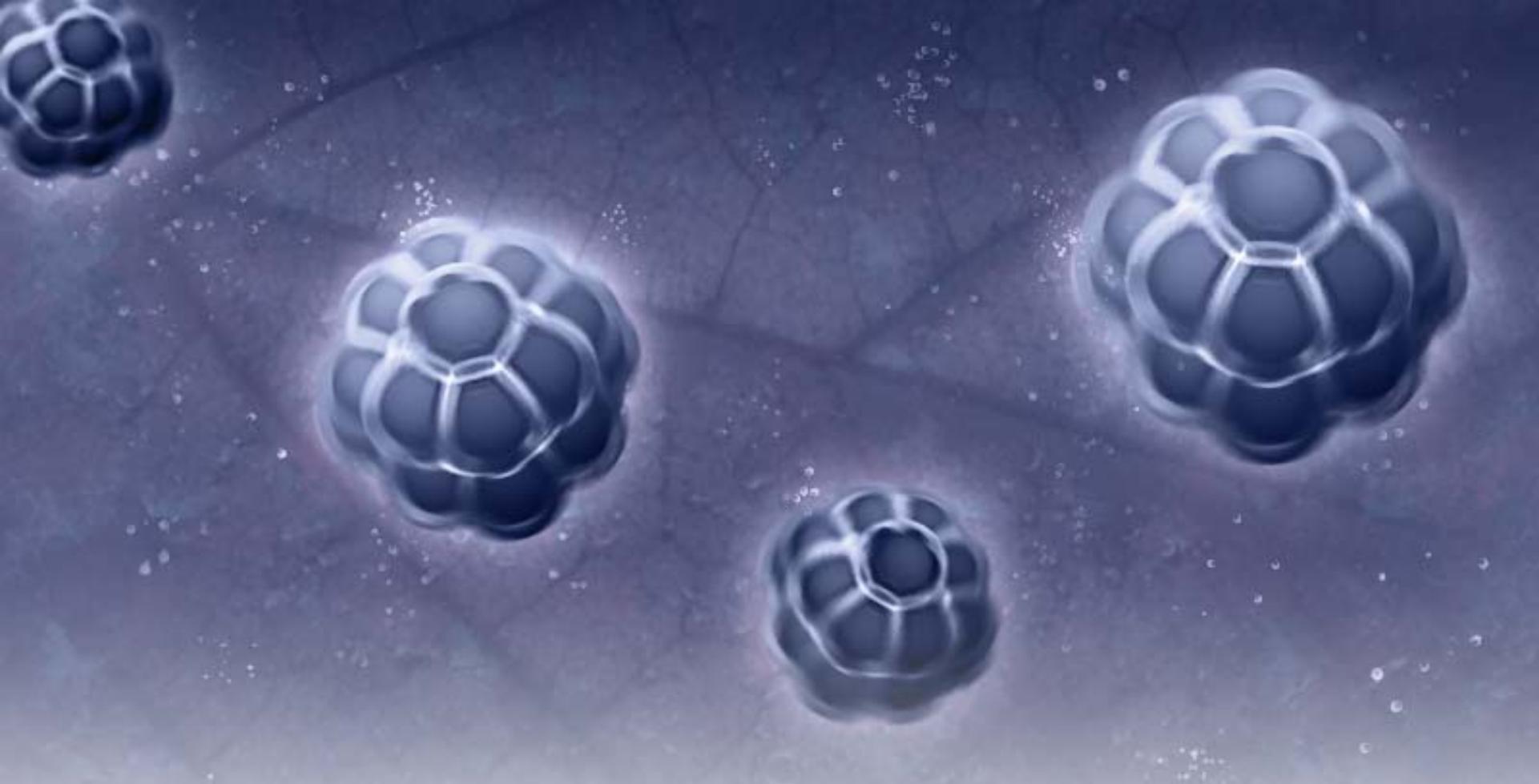
# Retention of enterotoxin by polymer



10% AcOH SDS washes after 1mg/40 $\mu$ l enterotoxin B reloaded into enterotoxin B MIP (10% cross linking)

# Points to consider

- Effect of crosslinking density on SEB imprinting
- Effect of pH of imprinting
- Effect of co-polymerisation using other functional monomers (hydroxy-acrylamide; hydroxyethylmethacrylate (HEMA))
- Optimised method of eluting SEB
- Method of quantifying eluted SEB from HydroMIP matrix.



# Impedimetric Immuno-assay based on Modified Conducting Polymer Electrodes

Keith Warriner

University of Guelph

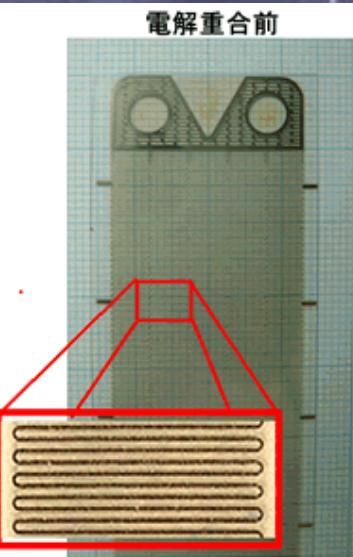
# Conducting Polymer Electrodes

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- Free standing conducting polymer films
- Low cost (<\$0.2) per electrode
- Disposable
- Compatible with mass production

# What are conducting polymers?

- Polyconjugated polymers with high electrical conductivities, low ionization potentials, high electronic affinities
- Applications
  - Stealth (Radar Absorbing)
  - LED display
  - Fuel cell
  - Organic computers
  - Sensors

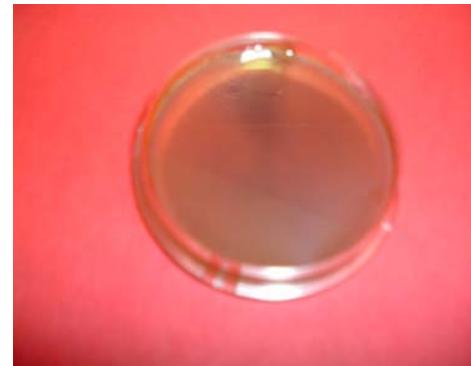


# Formation of conducting polymer electrode



Microporous polycarbonate membrane  
(45mm ,10 $\mu$ m pore size)

Preconditioning:  
Pyrrole(0.5M) & SDS(1mM)0



Polymerization:  
 $\text{FeCl}_3$ (0.4M) & lactic acid (0.1M)

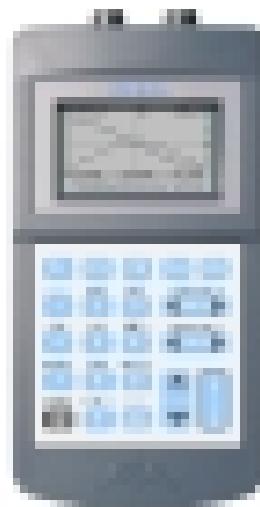


Polymer electrode

# Electrochemical characterization of polypyrrole electrodes

- Cyclic voltammetry

- Impedance spectroscopy

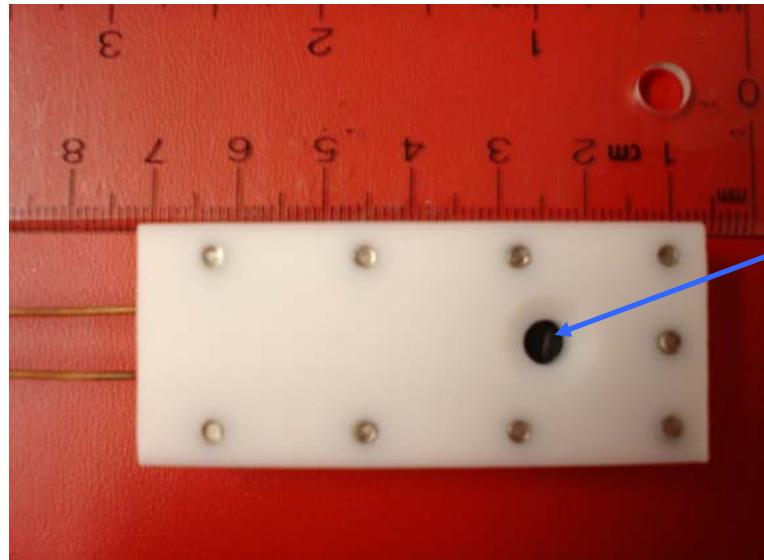


Handheld FRA

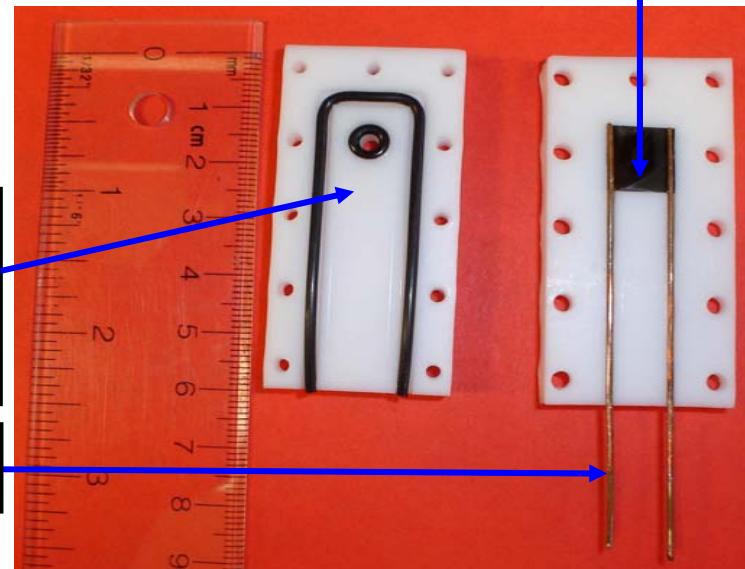


Solartron Impedance Analyser

# Reusable Electrode assembly



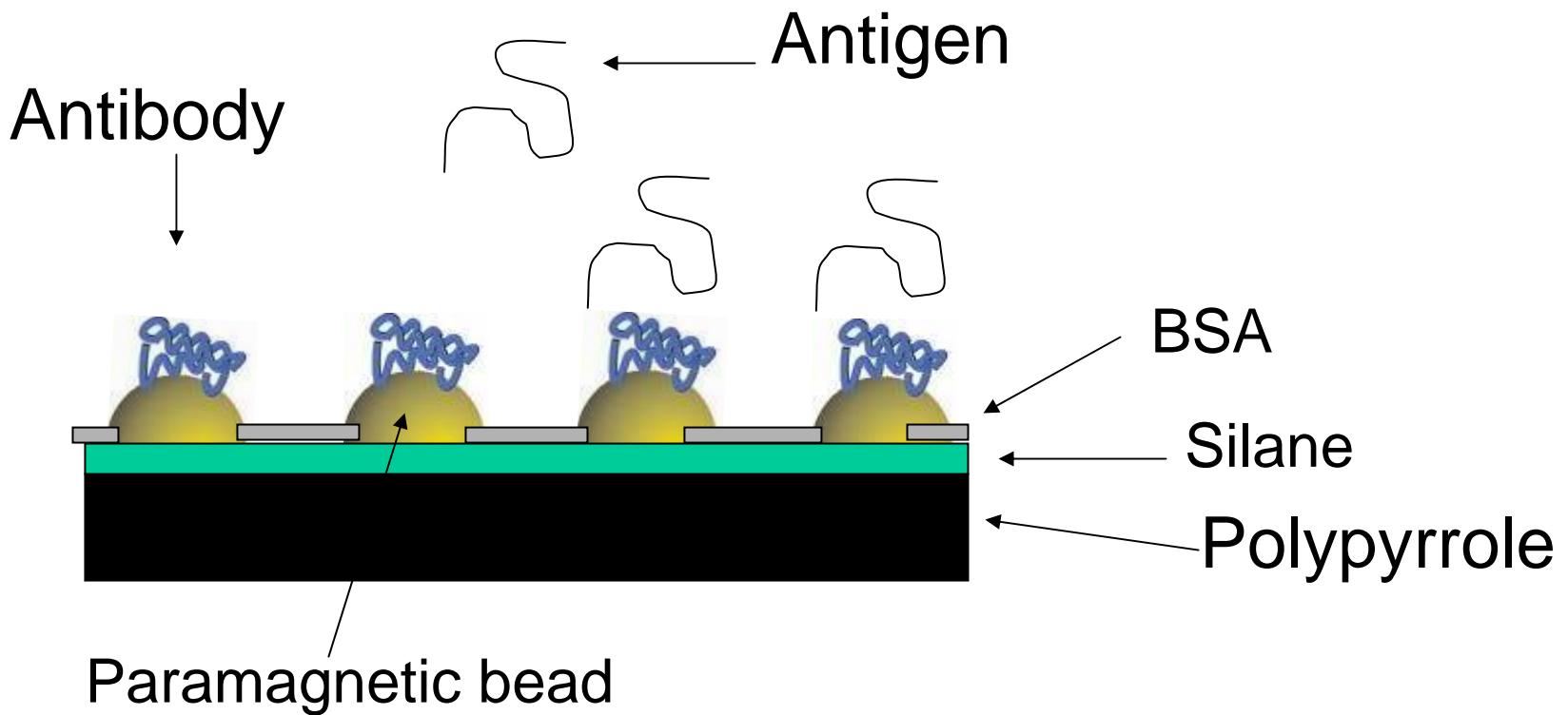
Reaction well



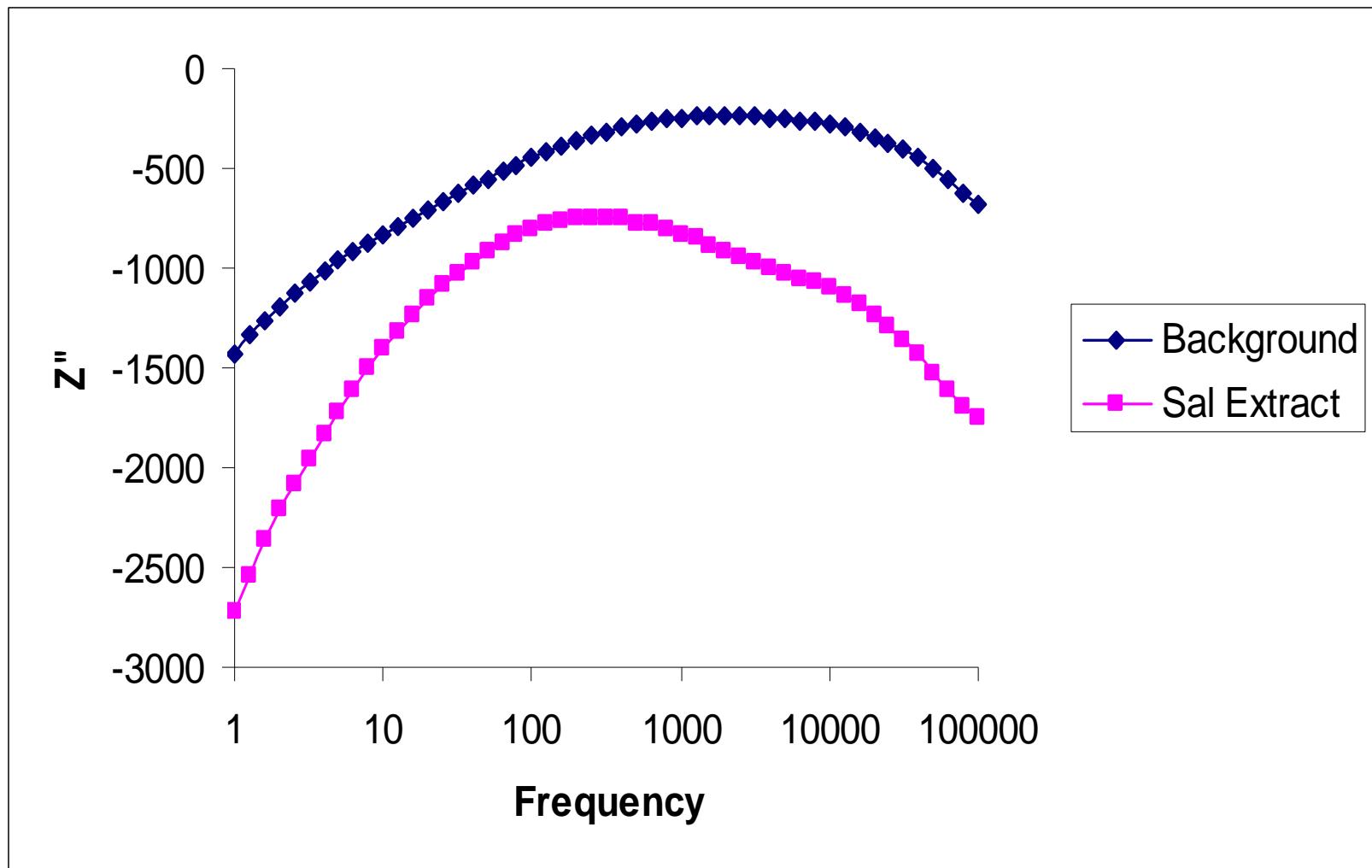
Top plate with  
reaction well  
(inside view)

Copper connections

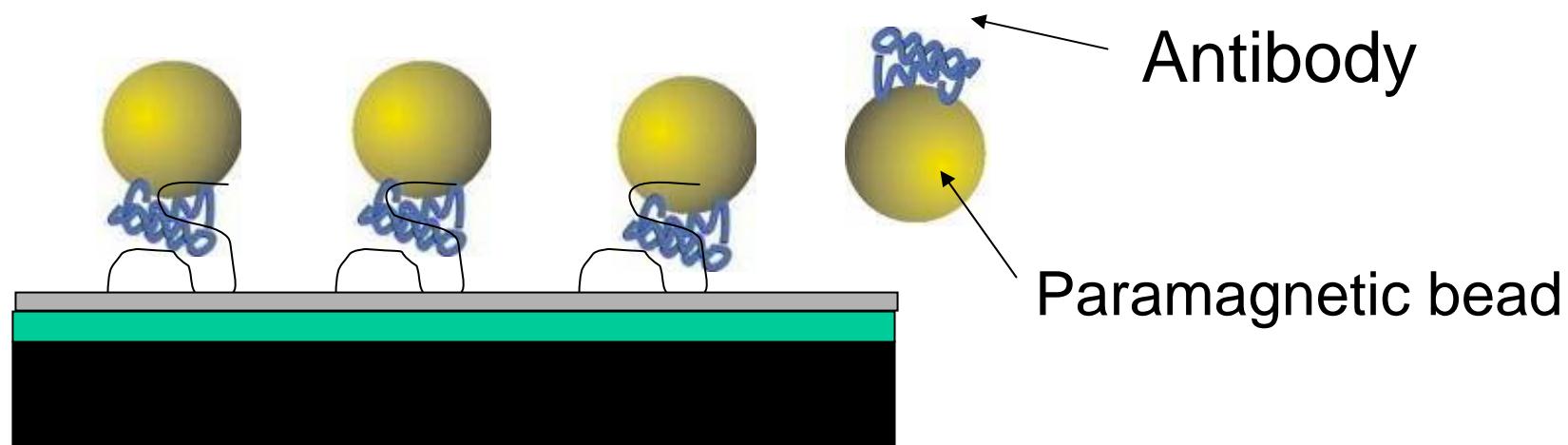
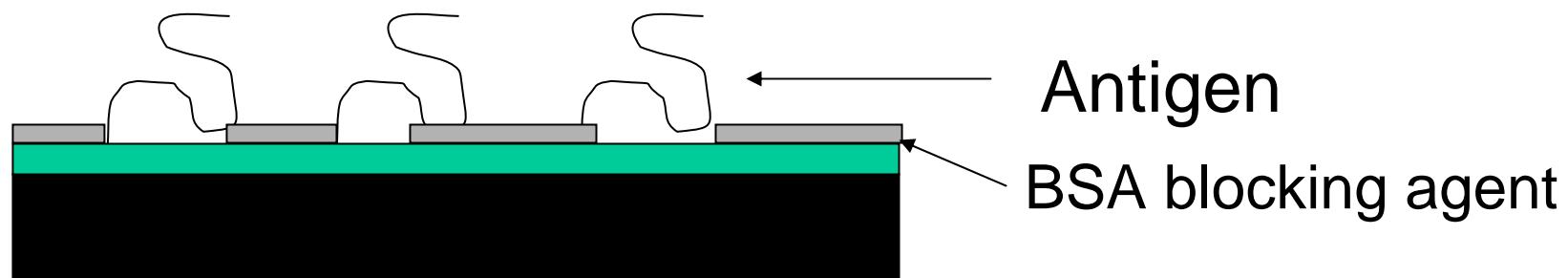
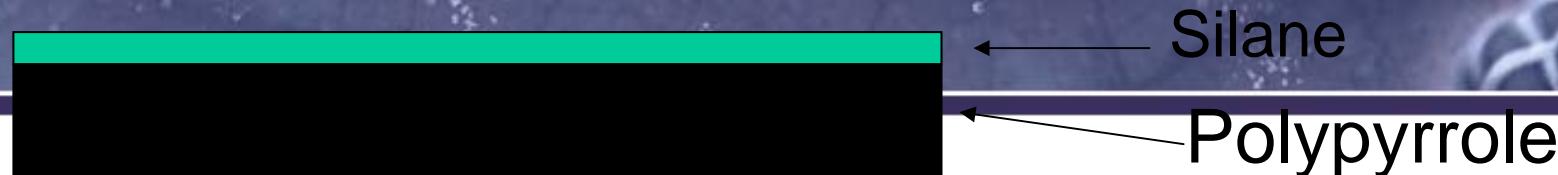
# Principle



# Sensor Response to *Salmonella*

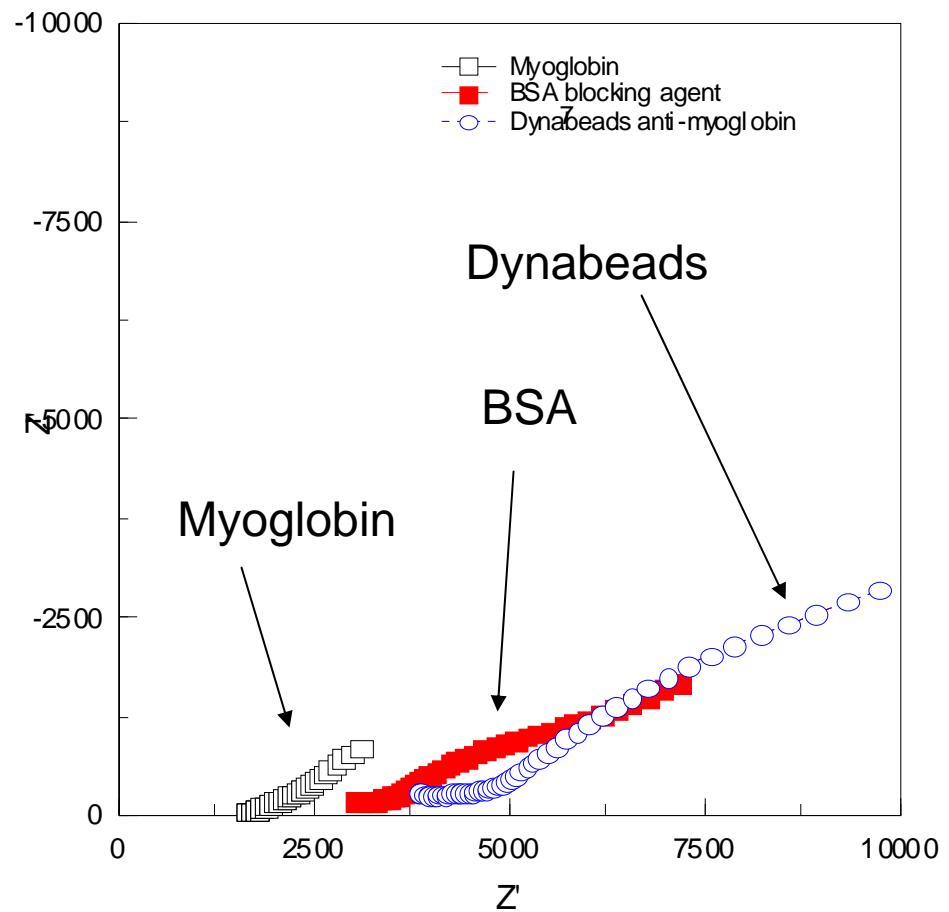


## Detection Strategy



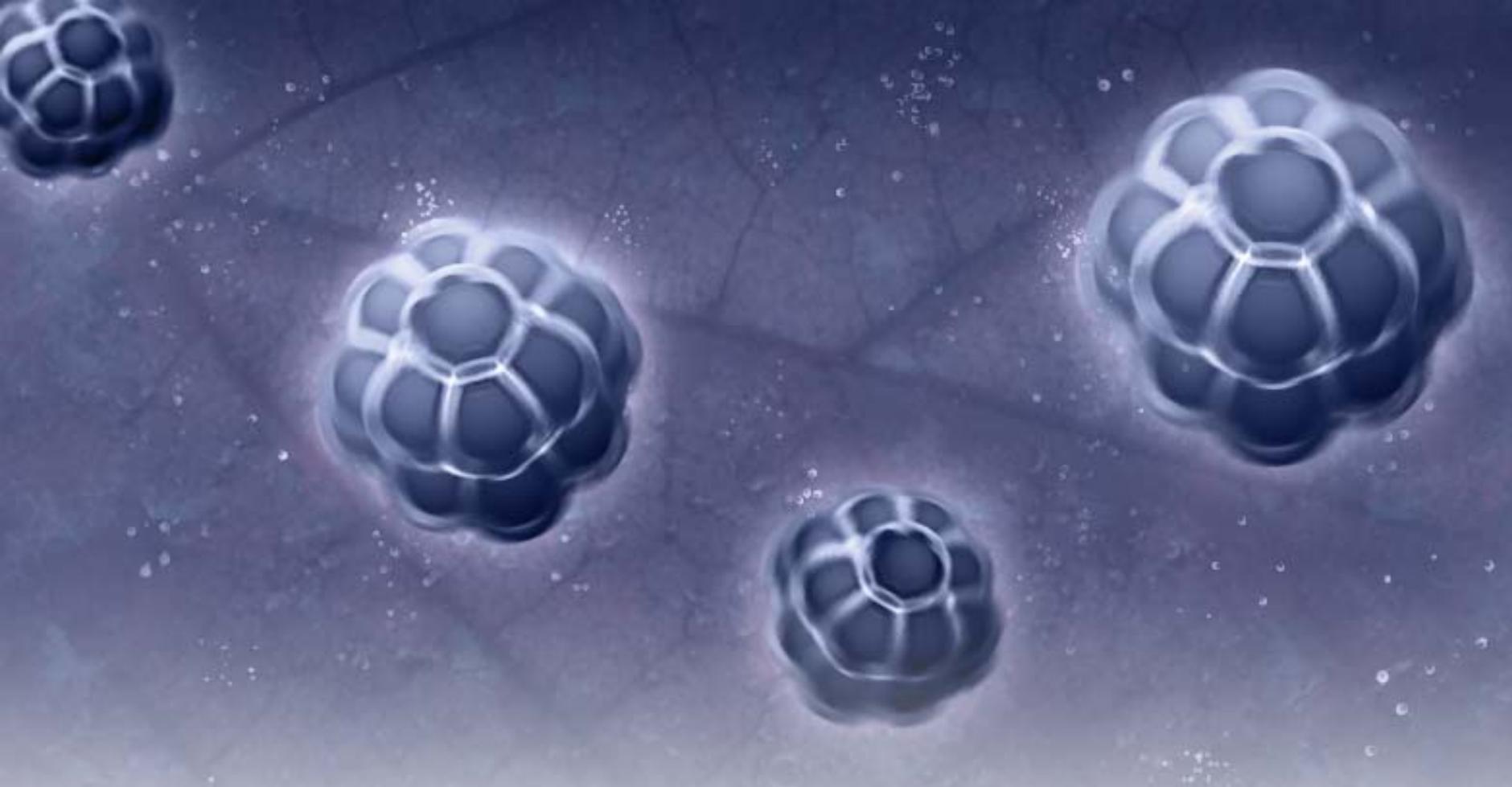
# Myoglobin Detection

- Myoglobin model for SEB
- 100ng Myoglobin
- BSA blocking agent
- Anti-myoglobin dynabeads



# Future Work

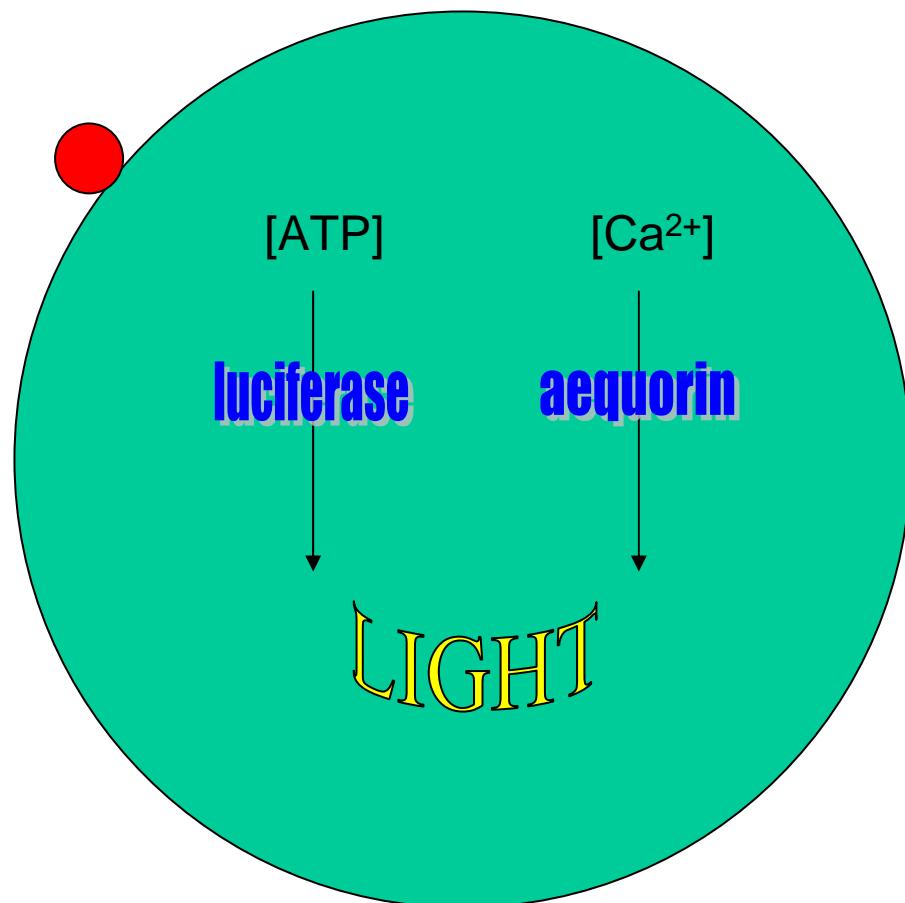
- Further optimize myoglobin detection protocol.
- Repeat experiments with SEB
- Integrate hydrogel MIP with detection platform



# Cell-based Biosensors

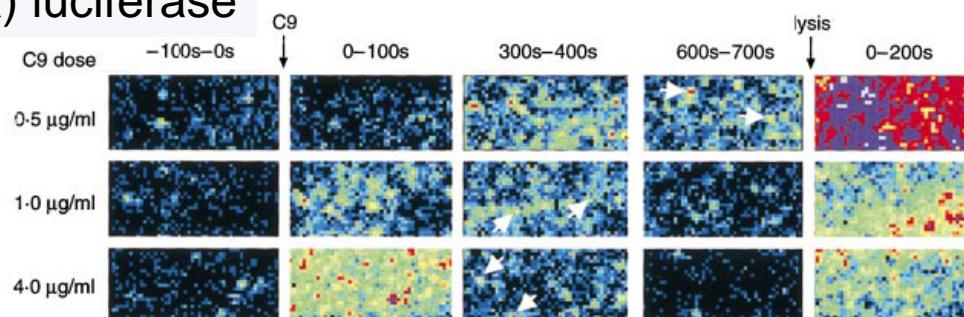
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# Principle of toxicity biosensor

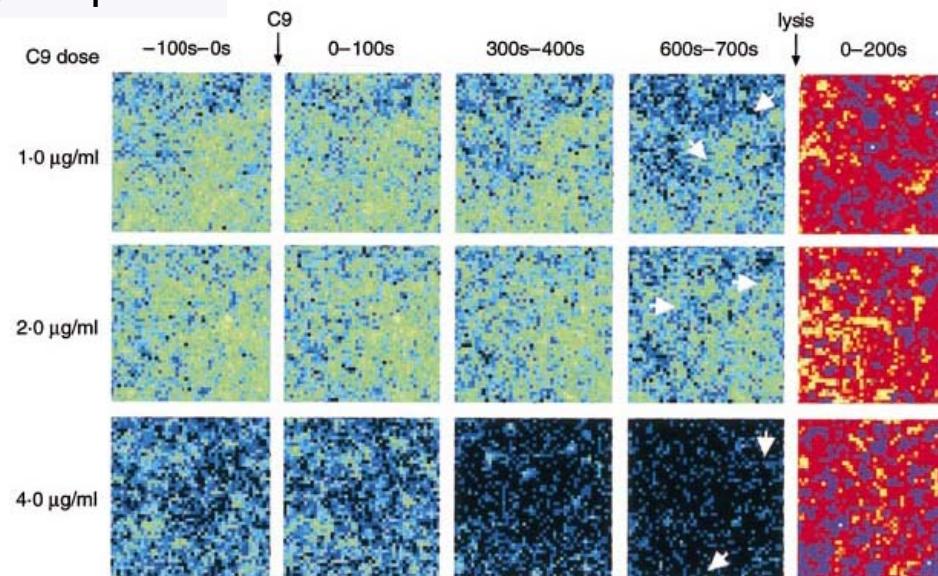


# Expression of luciferase and aequorin genes in HeLa cells in the presence of human complement component C9

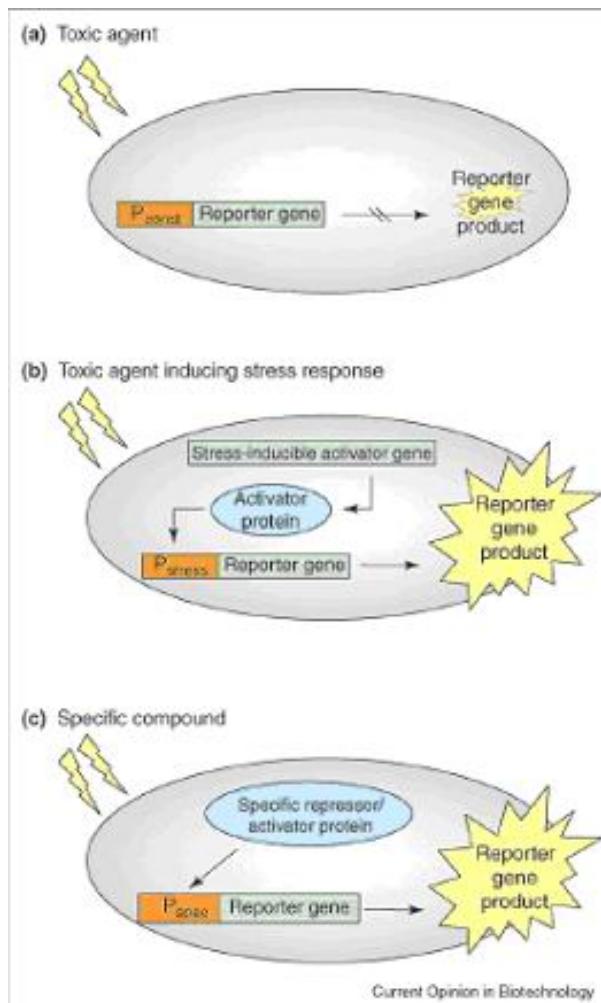
a) luciferase



b) aequorin



# Other luminescent toxicity biosensors





The future looks bright