Collaborative Research for the Design and Fabrication of Microscale Devices

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What is a Biosensor?

- An analytical tool consisting of biologically active material used in close conjunction with a device that will convert a biochemical signal into a quantifiable electrical signal.

- A type of biomolecular probe that measures the presence or concentration of an analyte by translating a biological recognition event at the probe surface into a quantifiable physical signal such as light or an electric pulse.

Antibodies have advantages as the biological recognition unit of biosensors

- Antibodies are relatively stable.
- Antibodies are well-characterized.
- There are many commercially available reagents that permit the coupling of an antibody binding event to quantifiable signal.
- Antibodies can be generated for almost any type of ligand.

Antibody-based assays: Effective analytical tools for environmental analysis

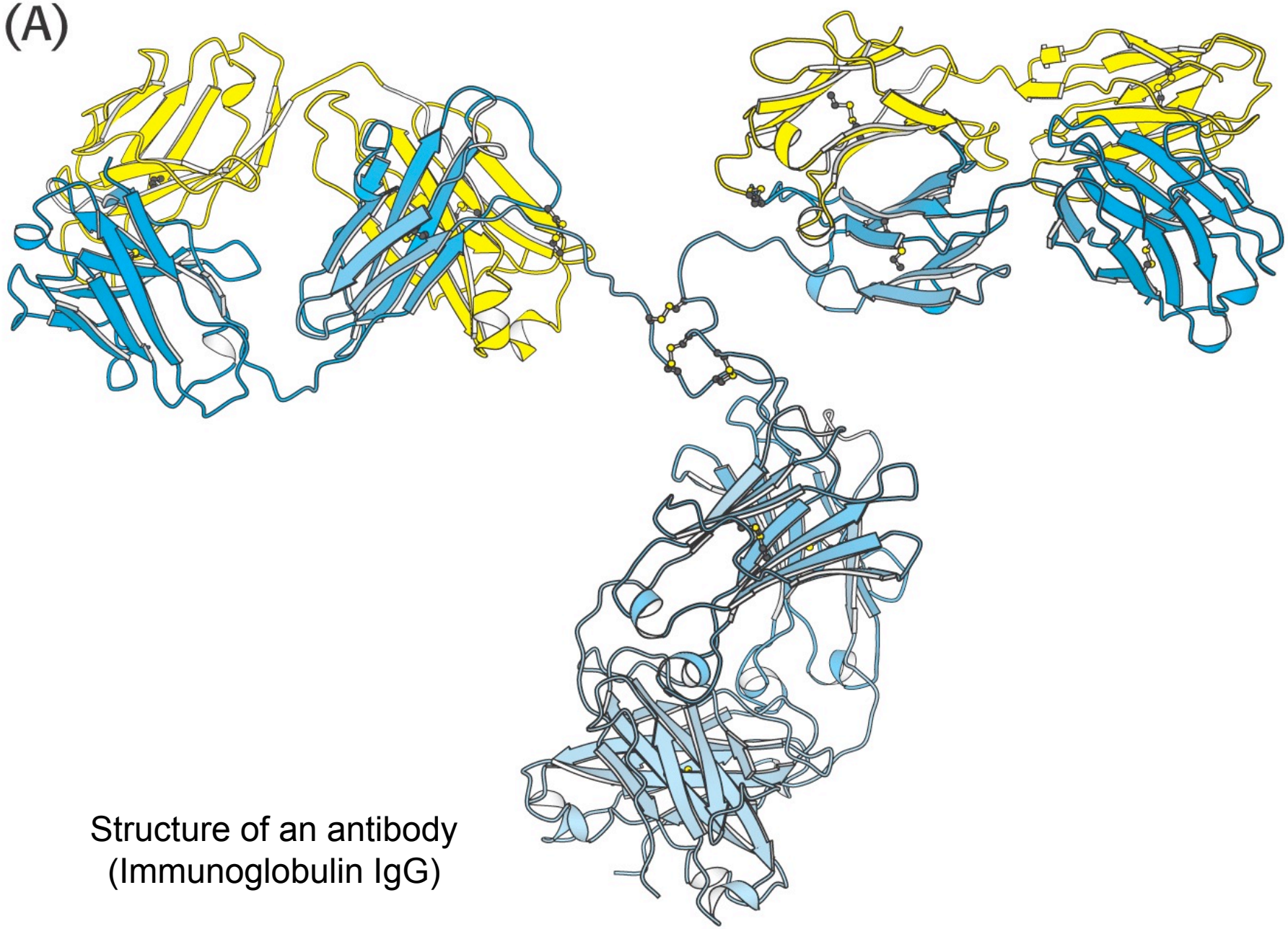
- Provide rapid results at or near the site of contamination
- Used to map contaminated sites and to quickly monitor the effectiveness of remediation and containment efforts
- Antibody-based assays can reduce analysis costs by 50% or more
- Assays do not require expensive instrumentation or highly skilled analysts

Antibody-based assays have been used for decades in clinical diagnostics and preventative medicine

- Can be used at the point-of-care to gather information required for medical decisions.
- Can be used by epidemiologists and public health workers to track infectious outbreaks or identify at-risk populations.
- Sales of antibody-based research and diagnostic products are estimated at $3 billion annually and are projected to increase to $15 billion by 2010.

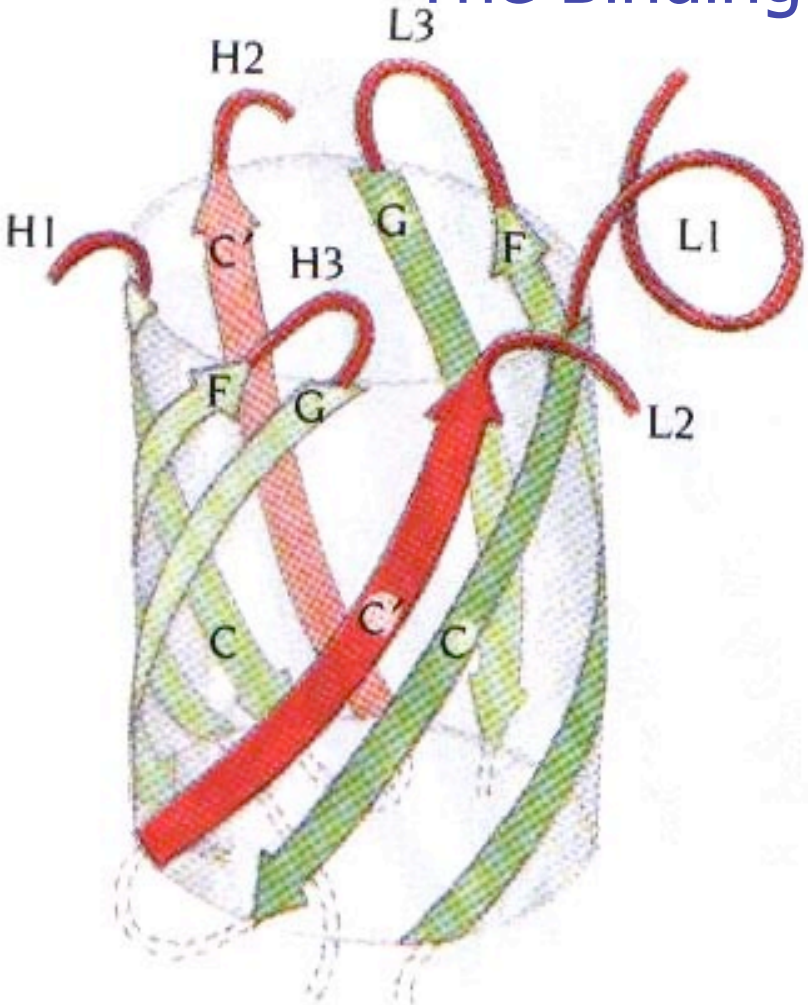
Detailed Knowledge of Antibody Structure and Binding Is Important for Biosensor Development

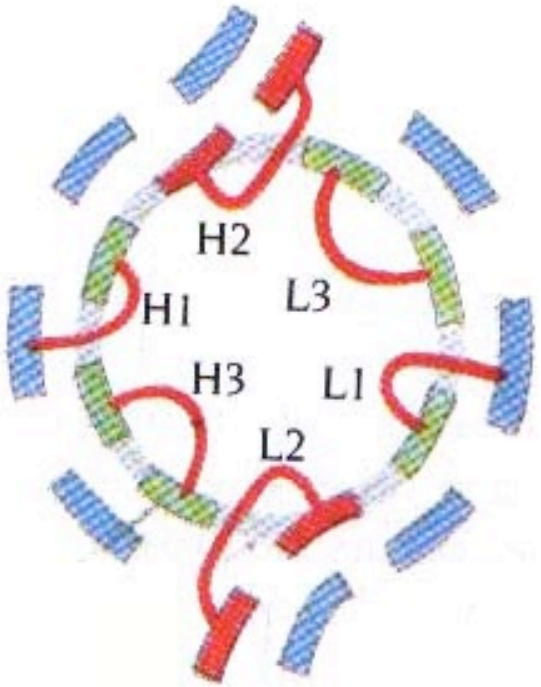
- Assay sensitivity is directly related to antibody affinity; the tighter the binding of the antibody to the antigen, the more sensitive the assay
- Assay selectivity is directly related to the binding specificity of the antibody
- Assay performance is directly related to the kinetic properties of the antibody binding reaction
- Detailed knowledge of the structure of the antibody binding site can lead to “engineered” antibodies with better performance in the biosensor format



Structure of an antibody
(Immunoglobulin IgG)

The Binding Site of an Antibody



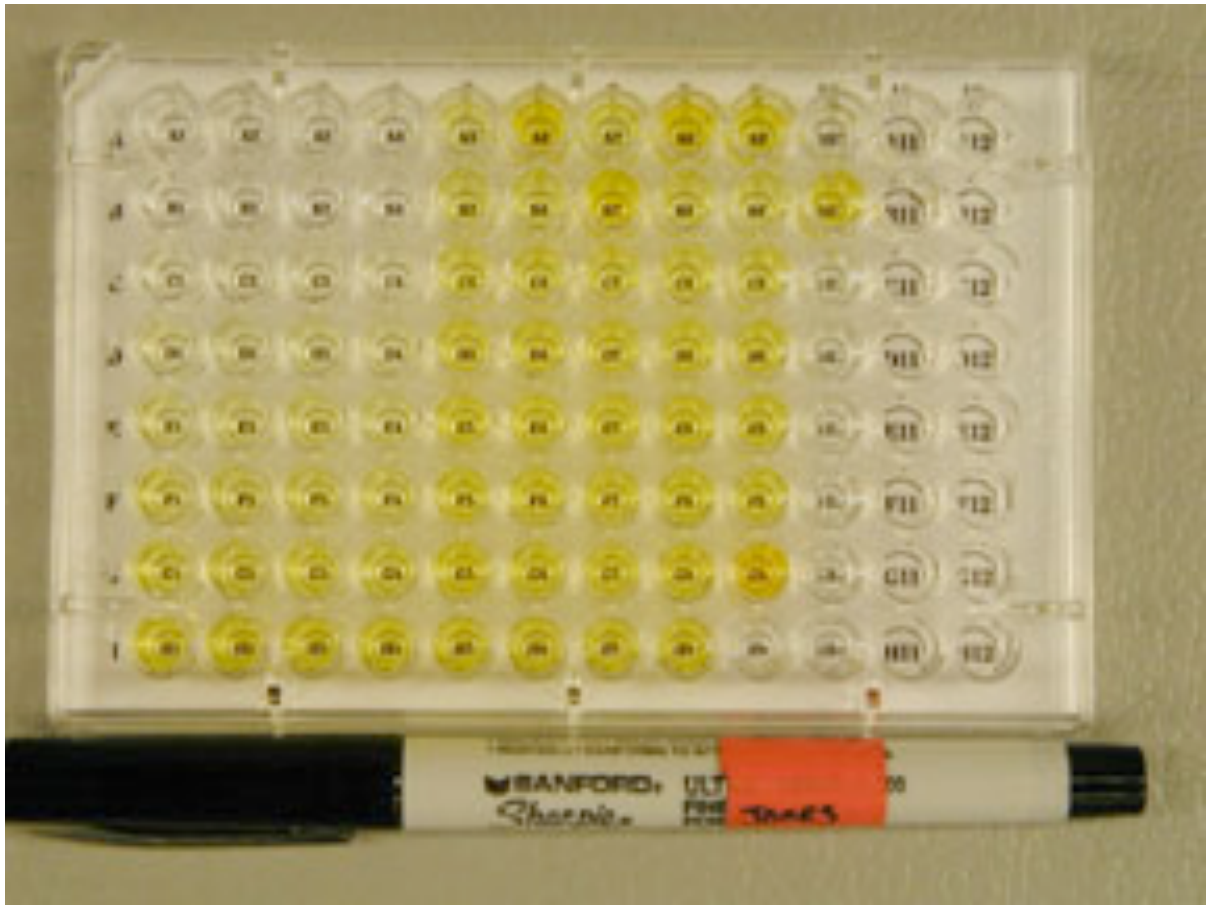


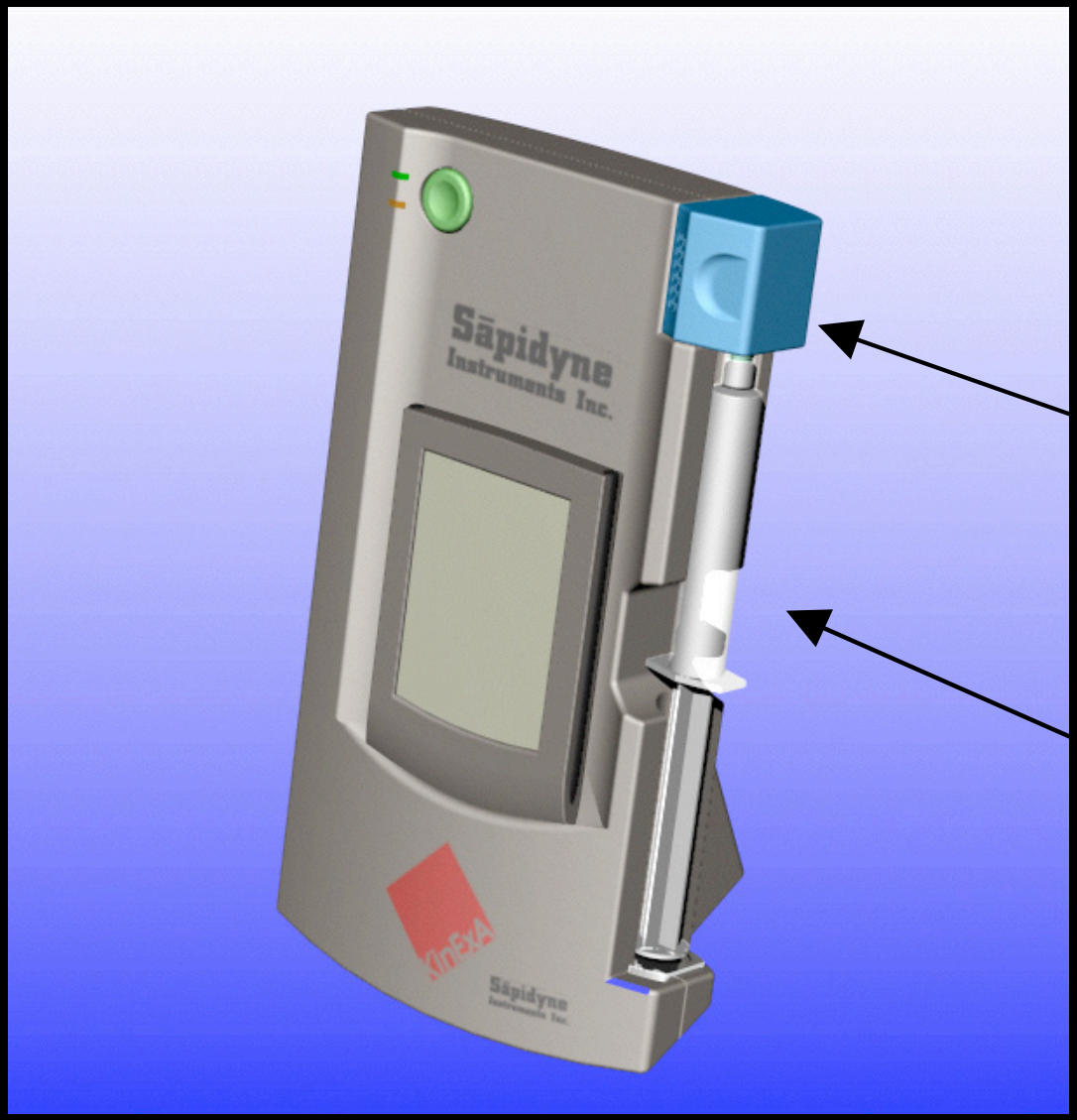
From Branden and Tooze, *Introduction to Protein Structure*

Tom's Modeling stuff here?

- Tom, I have some data showing that we improved the binding of the 12F6 antibody after modeling and subsequent site-directed mutagenesis, if you want to use it here.

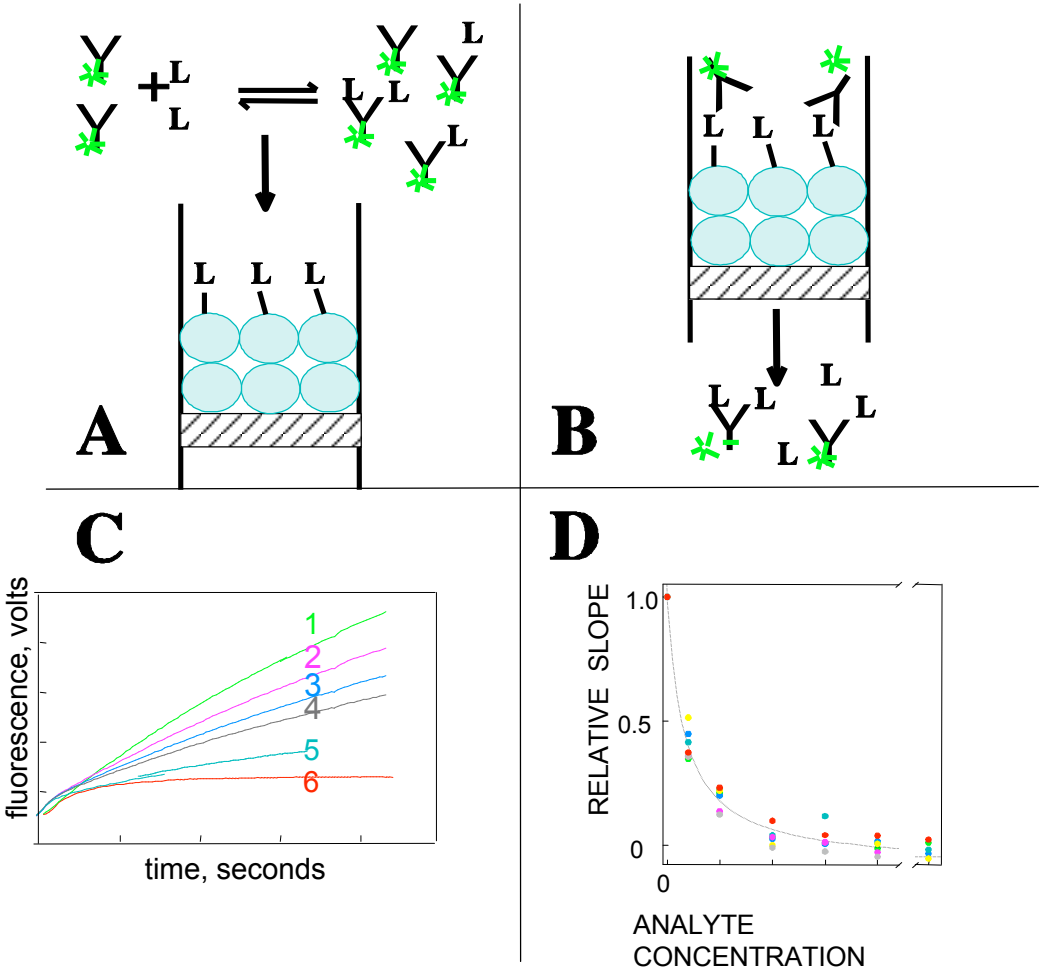
Microwell plates: a relatively slow format (1-2 hours) for antibody-based assays



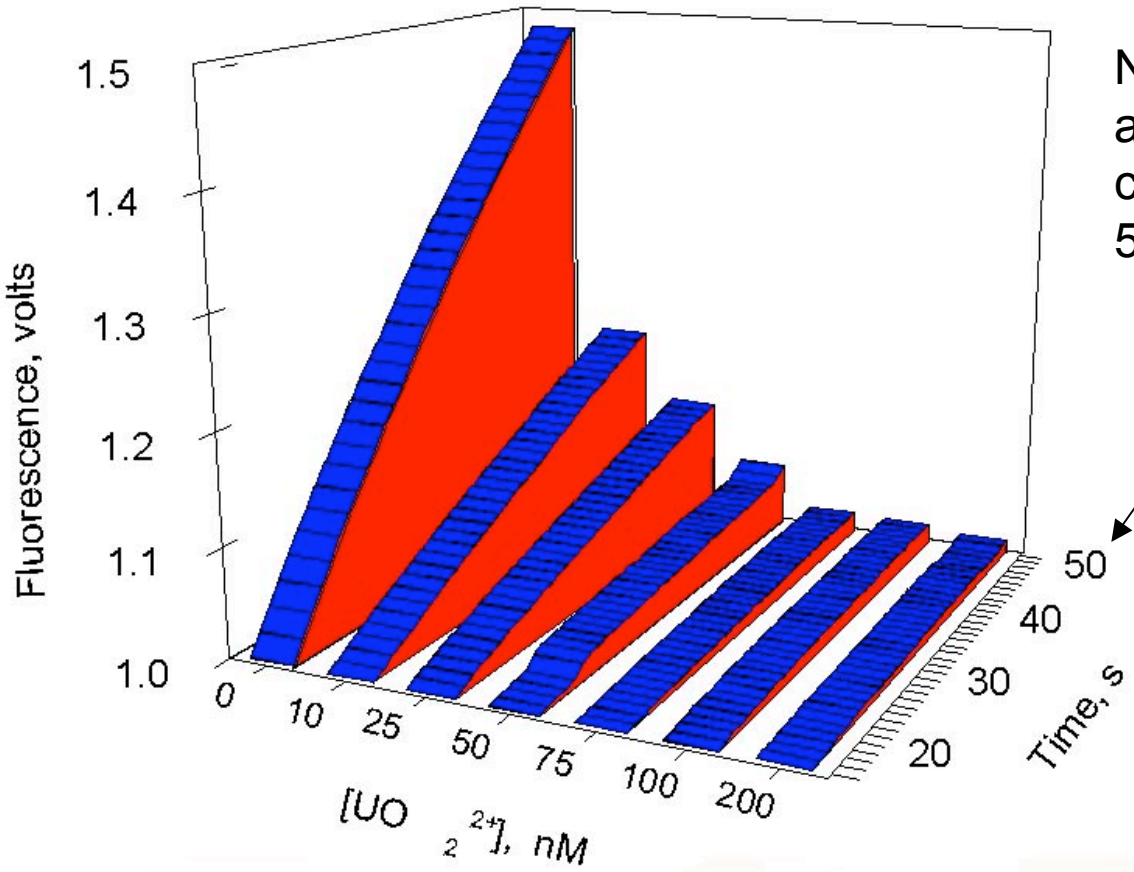


- ❖ Palm Pilot adaptable design allows easy interface with PC
- ❖ Disposable cassette for sample analysis and optics
- ❖ Reagents mixed in disposable syringe
- ❖ Final dimensions 10x5x2 inches

Principle of sensor operation



Raw data from prototype sensor

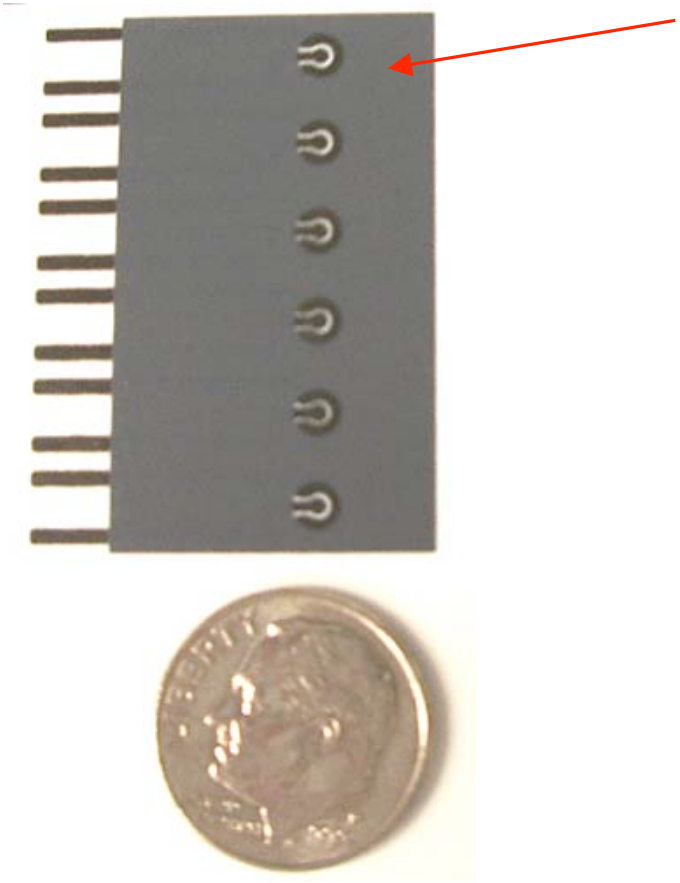


Note: these assay are complete in 50 seconds.

How can we make our sensors smaller/faster/better?

- Replace pumps for liquid handling with microfabricated devices
- Replace lamps and LED's with carbon-printed electrodes
- Replace wash steps with engineered coatings.

Measuring low molecular weight analytes using antibody binding and an electrochemical detection system

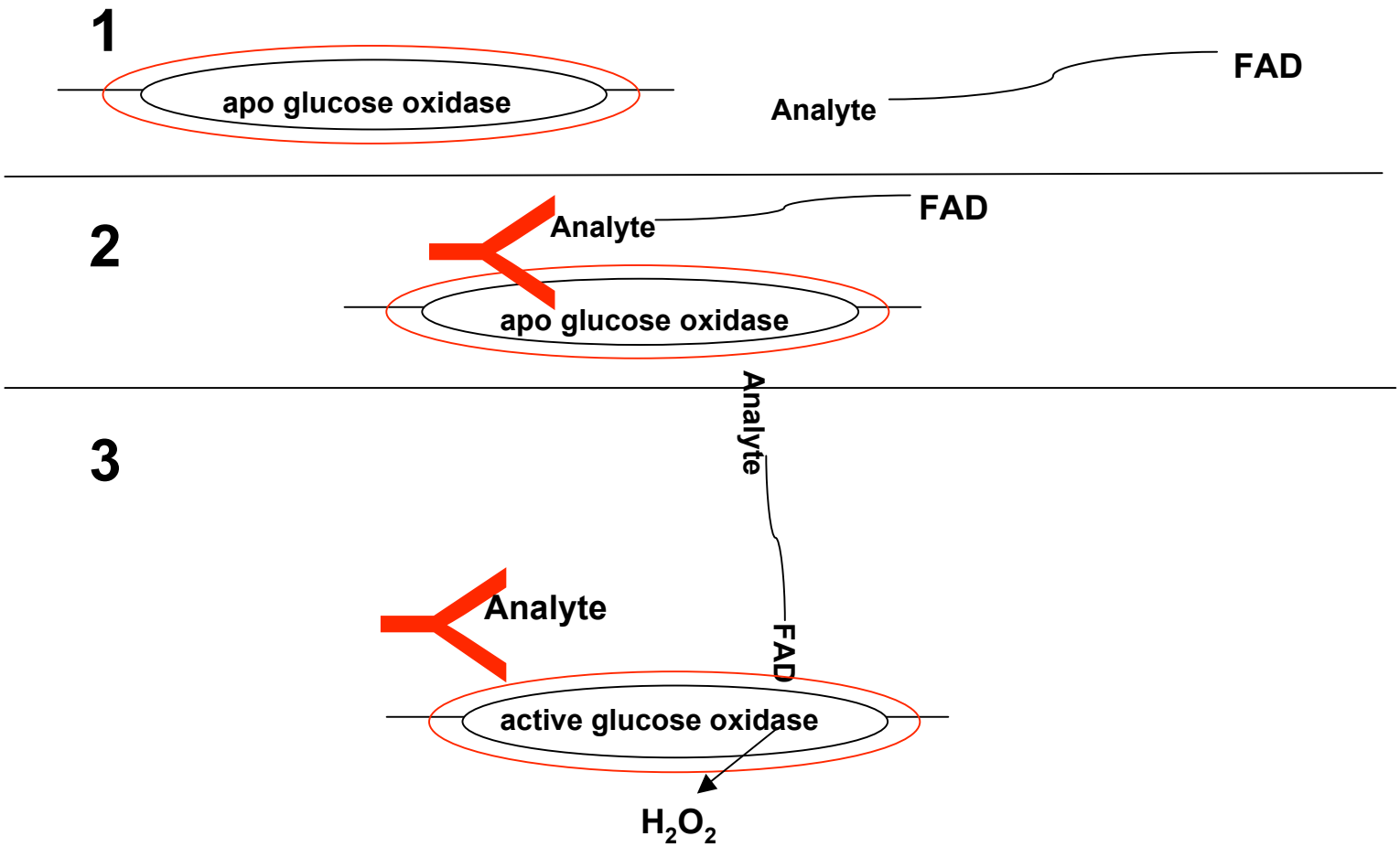


Apo glucose oxidase will be applied to the electrode surface and coated with a microporous material with a controlled pore size.

The antibody-analyte mixture will be applied to the device, and the signal generated will be proportional to the amount of analyte in the mixture. (see details of the method on subsequent slides).

These microelectrodes can be made even smaller and incorporated into microfabricated devices for automated delivery of assay components from reservoirs.

General procedure for electrochemical immunoassay of low molecular weight analytes



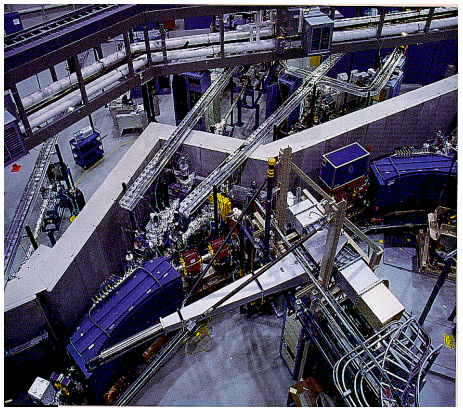
General procedure for electrochemical immunoassy of low molecular weight analytes

- Carbon-printed electrode containing apo glucose oxidase is surrounded by a coating with a controlled pore size (La Tech). Analyte is covalently conjugated to FAD (Tulane Biochem).

- Antibody is mixed with the analyte-FAD conjugate and applied to the electrode. The antibody-conjugate complex is too big to penetrate the coating surrounding the apo glucose oxidase, and glucose oxidase remains in the inactive form.

3. When soluble analyte is present, it competes with the analyte-FAD conjugate for the antibody binding sites, and some of the analyte-FAD conjugate is released. This conjugate is small enough to enter through the controlled pore size coating and activate the apo glucose oxidase. The H_2O_2 produced by the reaction is detected electrochemically.

Institute for Micromanufacturing
65,000 sq ft of R & D Facilities



- 41,000 sq ft R & D Building
- 20,000 sq ft Tech Transfer Center
- X-ray Beam Lines at CAMD
- 5,000 sq ft Modular Cleanrooms
- Processing Laboratories
- Testing Laboratories
- Instructional Laboratories
- Auditorium/Classrooms
- Faculty/Staff Offices

Micromanufacturing Capabilities at the Institute

Lithographic Techniques

- Optical Lithography
- X-ray Lithography
- E-beam Lithography

Material Deposition Techniques

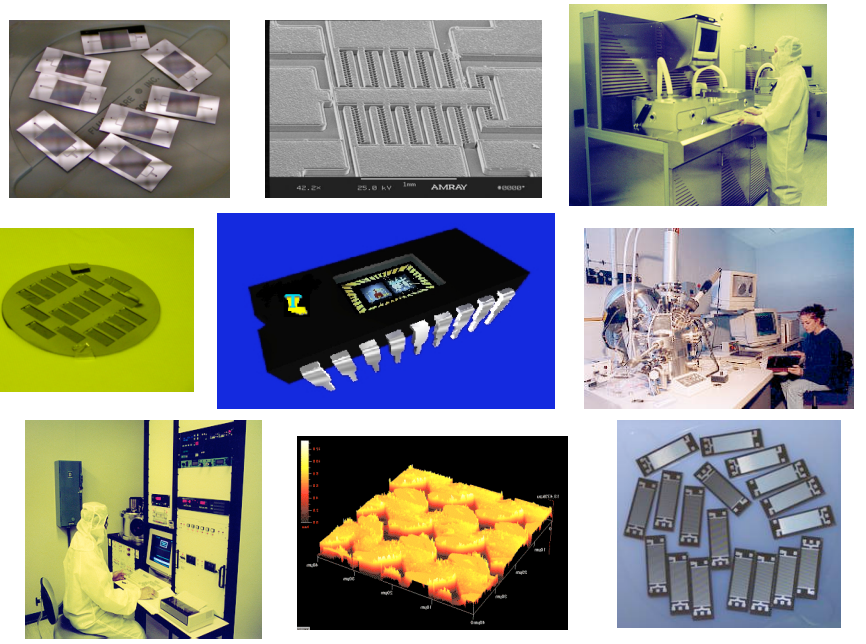
- LPCVD
- PECVD
- Sputtering
- E-beam Evaporator
- Electro/Electroless Plating

Material Etching Techniques

- RIE
- ICP
- Wet Etching

Material Doping, Oxidation & Annealing Techniques

- Thermal Diffusion
- Thermal Oxidation
- Rapid Thermal Processing

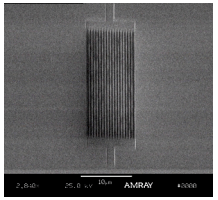
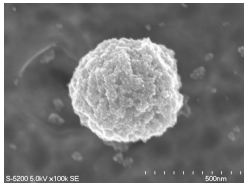
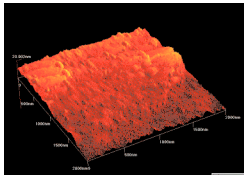
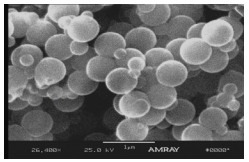


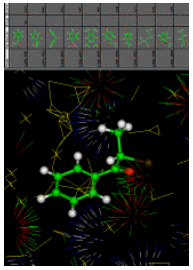
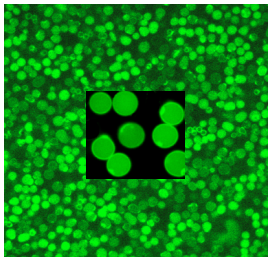
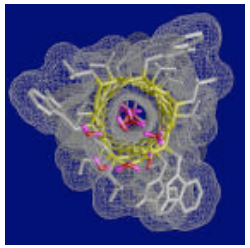
Alternative Microfabrication Techniques

- Ink-Jet Printing
- Hot Embossing
- Micromilling
- Focused Ion Beam

Nanomanufacturing Capabilities at the Institute

Nanoassembly Techniques
Layer-by-Layer Assembly
Molecular Recognition-Based Self-Assembly
Self-Assembled Monolayers (SAM)
Nanoassembly by Step-Wise Polymerization
Nanopatterning Techniques
X-ray Lithography
E-beam Lithography
Nanoimprint Lithography
Molecular Imprinting
Electroless Deposition Techniques
With Charged Nanoparticles
Measurement and Characterization Tools
TEM, AFM, SEM, XPS, Confocal Microscope, Nanoparticle Analyzer, Other!

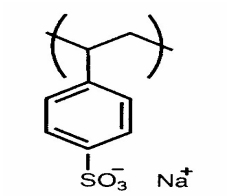





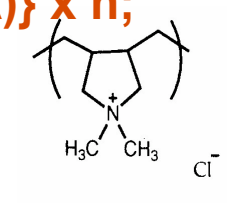





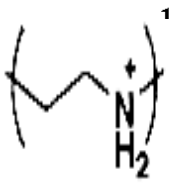
Scheme of Layer-by-Layer Assembly by Alternate Adsorption of Oppositely Charged Linear Polyions and Nanoparticles or Proteins

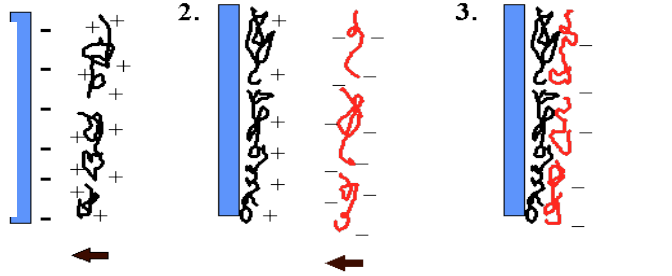
The LbL-assembly regimes for more than 50 compounds were established at IfM (dipping automate).

{PSS/PDDA)} x n;
n = 1 – 200.





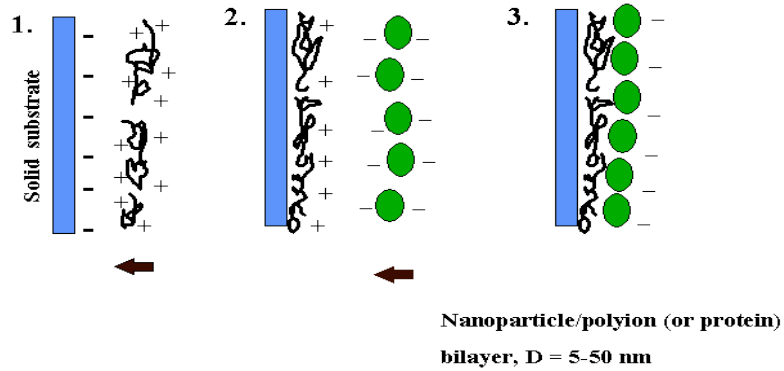




PSS **PDDA** **PEI**

1992-1999
G.Decher,
H.Moehwald,
Y.Lvov

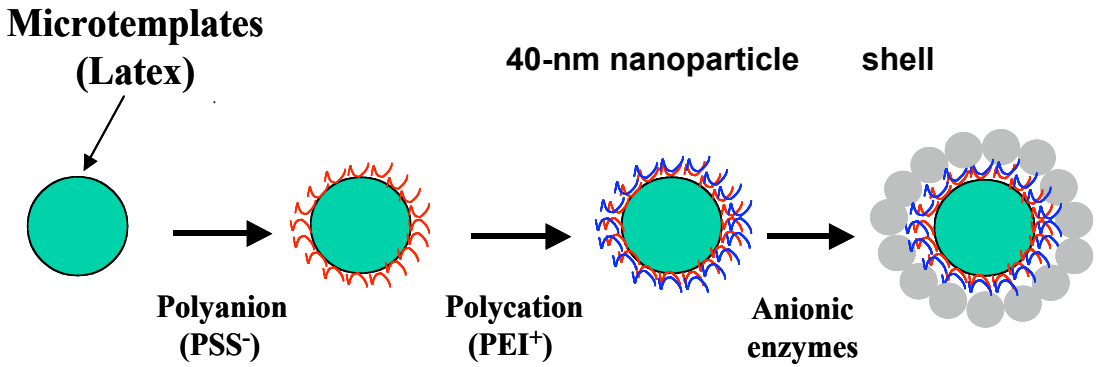
{Glucose oxidase / PEI} x n
or
{20-nm Silica / PEI} x n
- 20 different enzymes were assembled

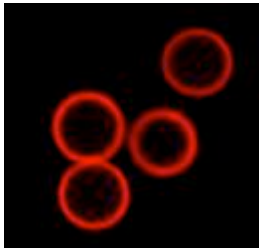


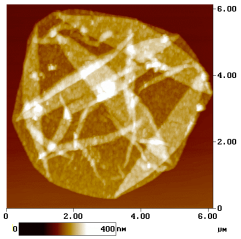
G. Decher *Science*, 1997, v.227, 1232 "Fuzzy nanoassemblies: Toward layered multicomposites"

Y. Lvov, K. Ariga, T. Kunitake, *J. Am. Chem. Soc.*, 1995, v.117, 6117-6122 "Assembly of multicomponent protein films by means of electrostatic layer-by-layer adsorption"

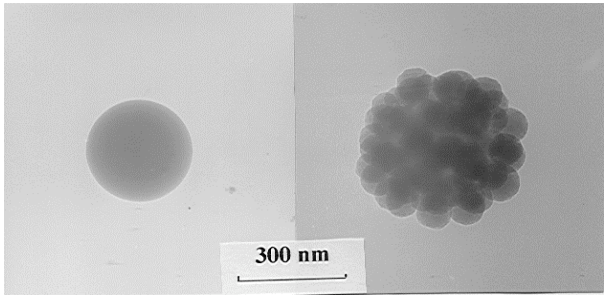
Nano-Assembly on Microtemplates. Nanocapsules and Microshells

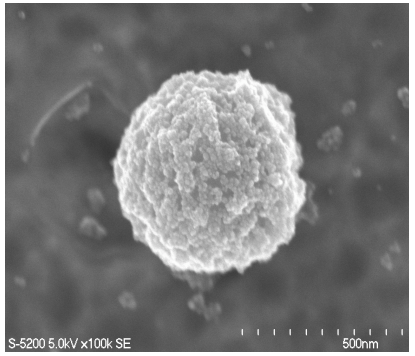






Ordered 40-nm diameter silica shells on 250-nm diameter latex.

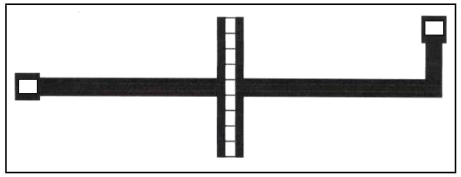


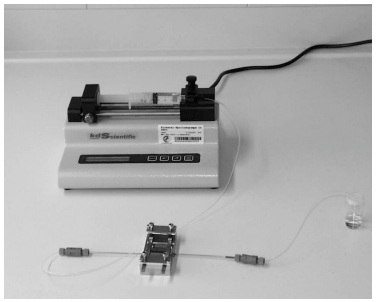


Y. Lvov, R. Price, *Colloids and Surfaces: Biointerfaces*, 2002, v.23, 251-256

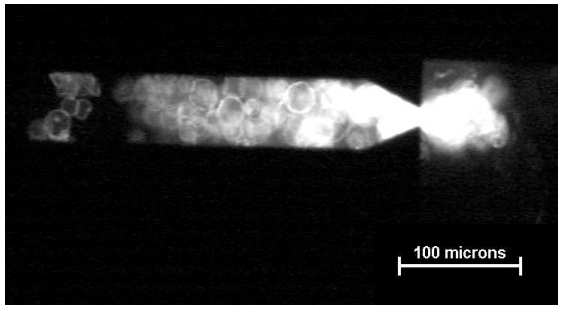
Y. Lvov, R. Price, A. Singh, J. Selinger, M. Spector, J. Schnur, *Langmuir*, 2000, v.16, 5932-5935, "Nanoscale patterning on biologically derived microstructures"

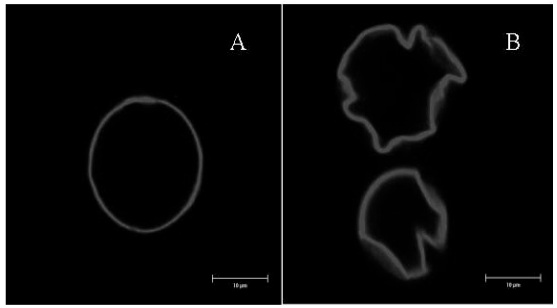
Microfluidic Device for Microshell Studies





Layout of the silicon chip and injection system with all the components.



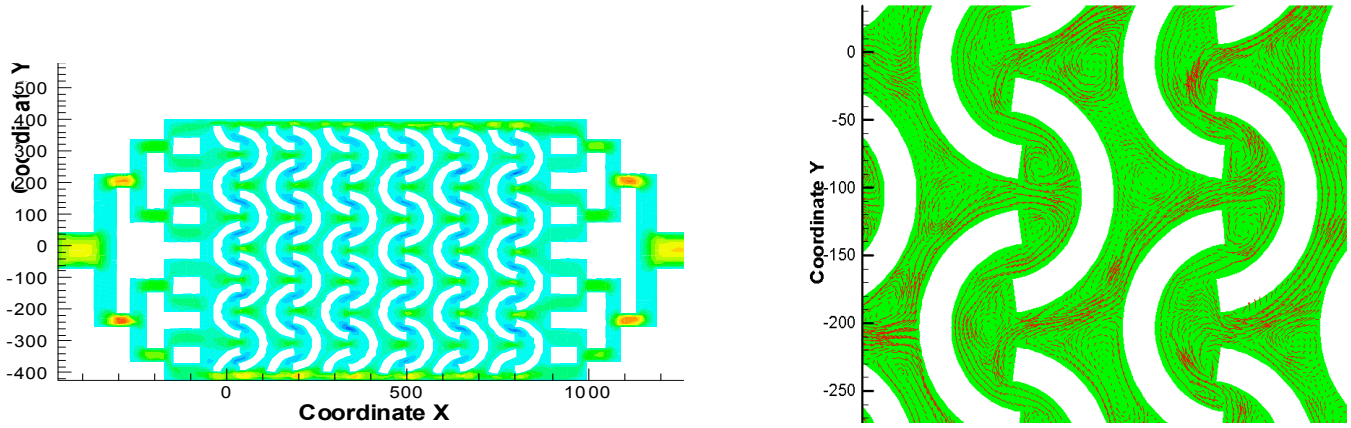


Channel at the edge of the chip demonstrating polyelectrolyte microcapsule that flowed through. Microcapsules of 10 μm diameter with (PSS/PAH)4 wall composition were used. Confocal microscope images.

Microfluidics Simulation

- Microfluidics simulations will help us to identify important criteria for transport and mixing of analytes and antibody as well as the reaction rates between different species.
- Simulation will help guide us towards optimal design criteria to miniaturize sensors.

Convection and Mixing



a. b.

(a) 2-D Top View of Velocity Contours in an Omega Channel Microfluidic System;

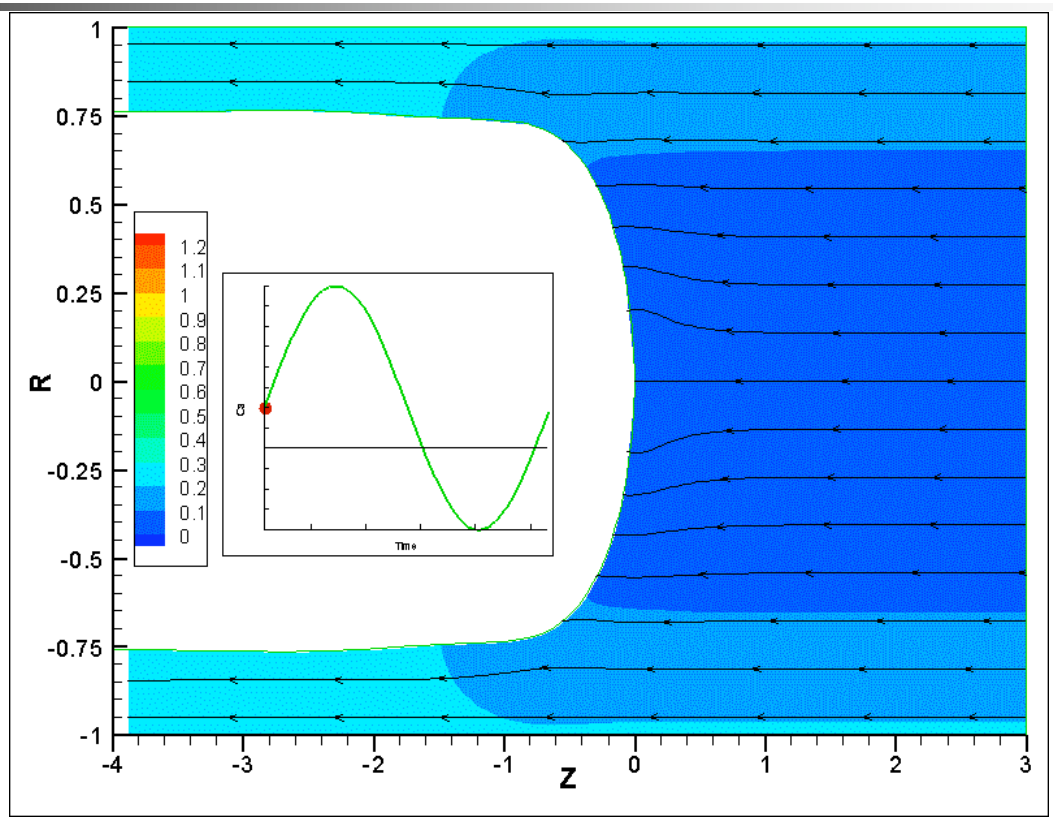
(b) (b) asymmetrical flow displaying vortices.

Simulations will be conducted based upon Stokes Flow approximations

$$\nabla P = \mu \nabla^2 \vec{u}, \ \nabla \cdot \vec{u} = 0$$

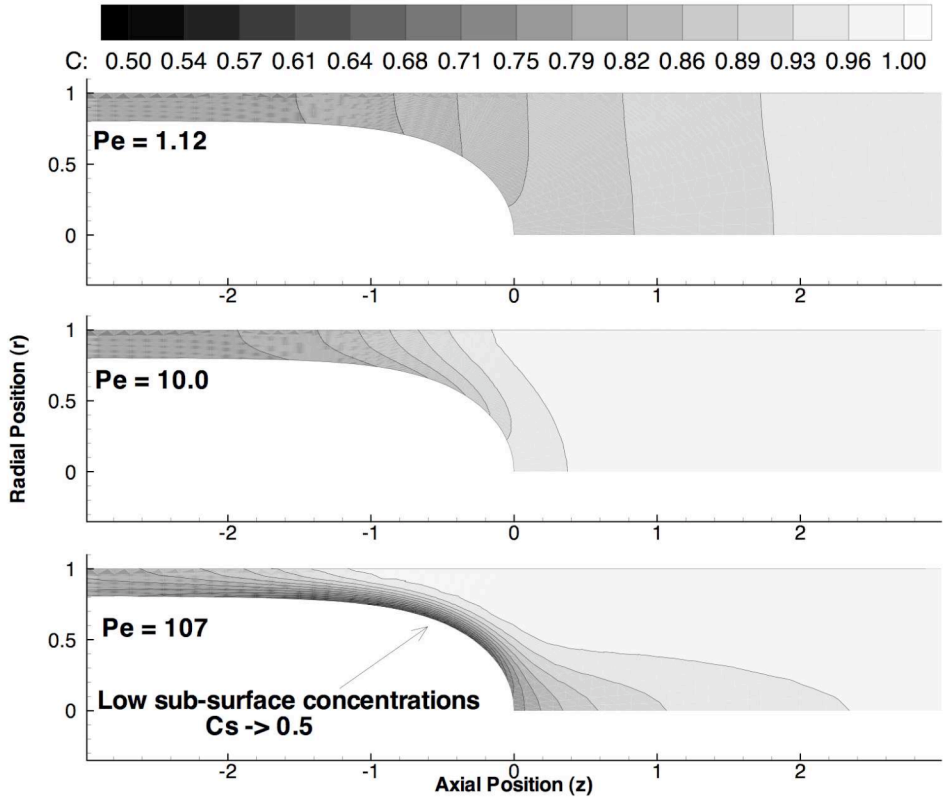
using the boundary element method, immersed boundary method and method of regularized Stokeslets (as appropriate). Members of the Fluids Group are experts in these techniques of scientific computation.

Time-dependent behavior



Mixing can be strongly influenced by time-dependent behavior. Our computations are capable of accurately simulating these complex flows with complex free-surface boundaries.

Transport



Surfactant transport dependence on the relationship between convection and diffusion, Pe = UL/D_{mol}

Convection-Diffusion Interactions

$$\frac{\partial c}{\partial t} + (\vec{u}\cdot\nabla)c = D\nabla^2 c$$

The interaction between convection and diffusion can play an extremely large role in transport behavior.

Reactions

Reactions are determined

$$\frac{\partial \varphi}{\partial t} + \vec{u} \cdot \nabla \varphi = D \Delta \varphi + R$$

$$R_{as} = K_s^b \varphi_s \varphi_a - K_s^r \varphi_{as}$$

$$R_s = K_s^r \varphi_{as} - K_s^b \varphi_s \varphi_a$$