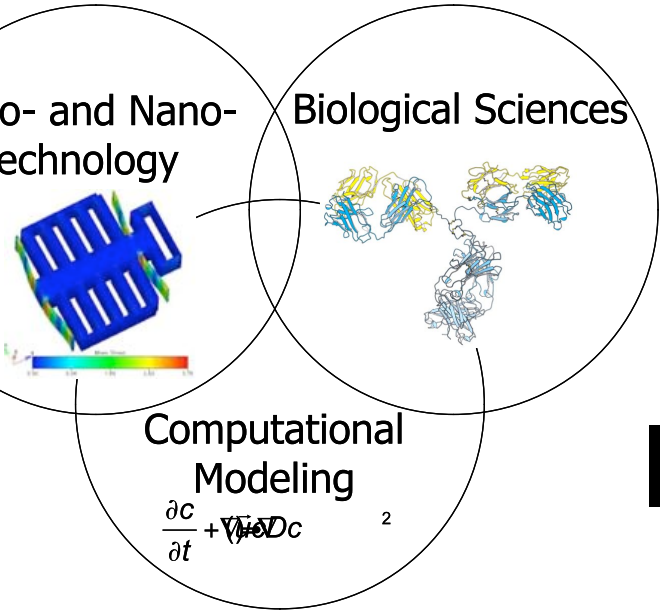
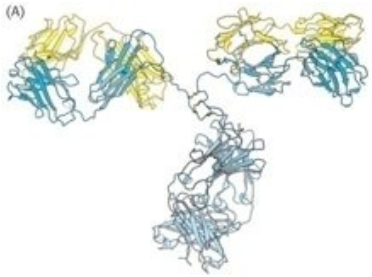


Immunosensors

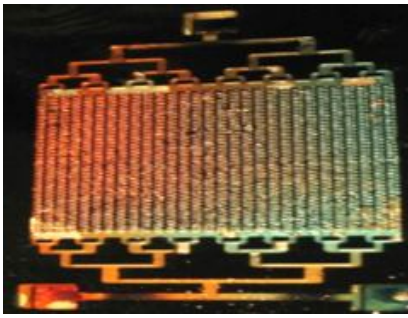
Ricardo Cortez, Mehnaaz Ali,
Thomas Bishop, Kate Hamlington,
Jerina Pillert, and Mangilal Agarwal

Antibody-based Biosensor

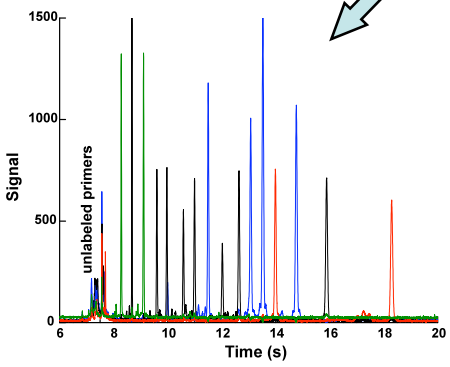
stem will be composed
ofluidic and
osensing elements
dies) targeted for the
s of biological or chemical
. **Components**:
uidic elements for sample
ocessing, nanoporous
anes for target pre-
on and carbon printed
t.



Biochemistry



Fluid flow / reactions i
micromixer



Signal

Antibody-based Biosensor

Tulane	LaTech IfM	Xavier	UNO
Jerina Pillert Kate Hamlington Amit Jain Mehnaaz Ali Hank Ashbaugh Tom Bishop Diane Blake Ricardo Cortez Lisa Fauci Don Gaver	Senaka Kanakamedala Jie Liu Mangilal Agarwal Mark DeCoster Ji Fang Yuri Lvov	Robert Blake	Steven Rick

Experiments: characterization of antibodies, determination of assay parameters, preparation and reactivation of Apo-glucose oxidase, synthesis.

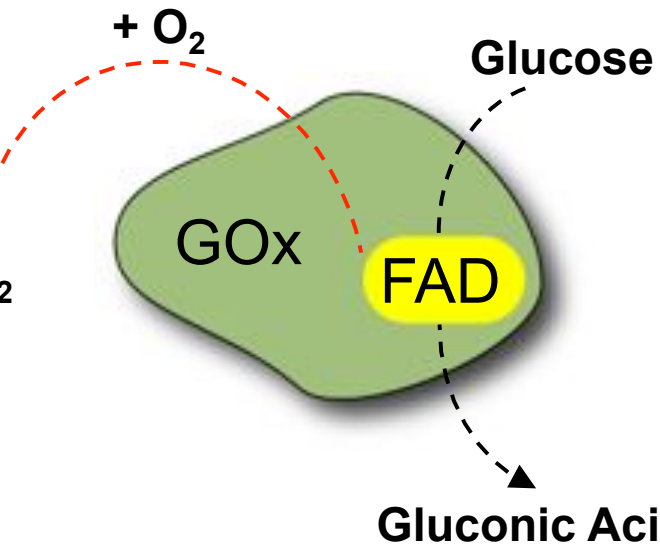
MD Simulations: antigens binding to antibody, energy minimization, loop structures, sequence alignment.

CFD Simulations: flows in microchannels, complex geometry, property optimization, reaction-diffusion-transport of concentrations, parallelization.

Manufacturing: microsensor layer fabrication, micromixer fabrication and evaluation, nanoporous membrane.

The immunosensor will use GOx mediated glucose oxidation for signal transduction

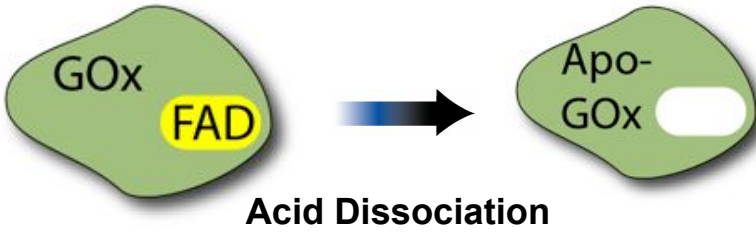
Glucose Oxidation



cose oxidase requires the cofactor for the catalysis of glucose to onic acid. This process involves initial reduction of FAD to $FADH_2$ consequent oxidation by ecular O_2 generating H_2O_2

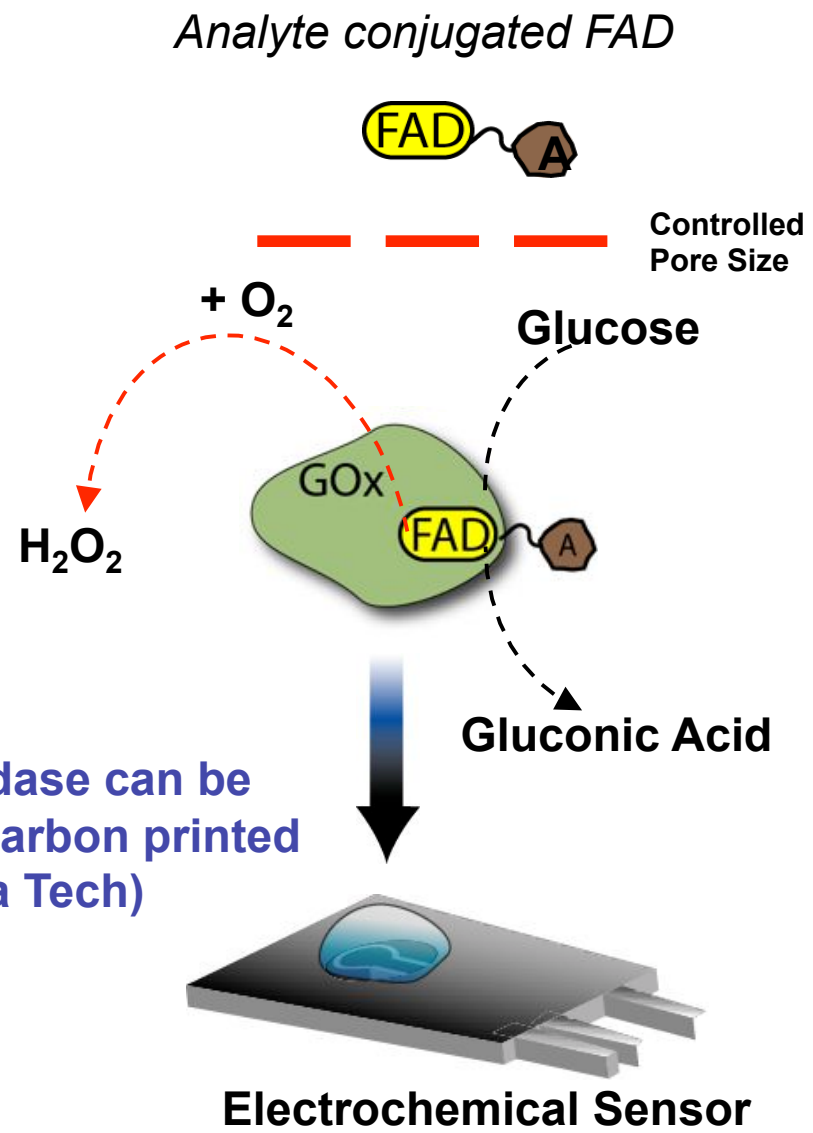
E-Chem Sensor

Enzyme activity can be modulated the removal and introduction of th cofactor FAD. The cofactor can be efficiently dissociated under acidic conditions to yield apo glucose oxidase.



Thus the cofactor FAD can be conjugated to an analyte and utilized to modulate enzyme activity.

General Strategy for E-chem Immunoassa



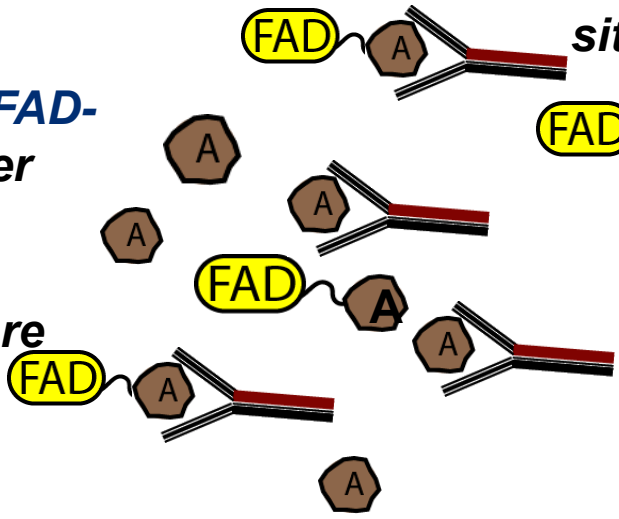
FAD-analyte mediate
reactivation of the Ap
GOx in the presence
glucose.

-Glucose oxidase can be
ted onto the carbon printed
trode (IFM, La Tech)

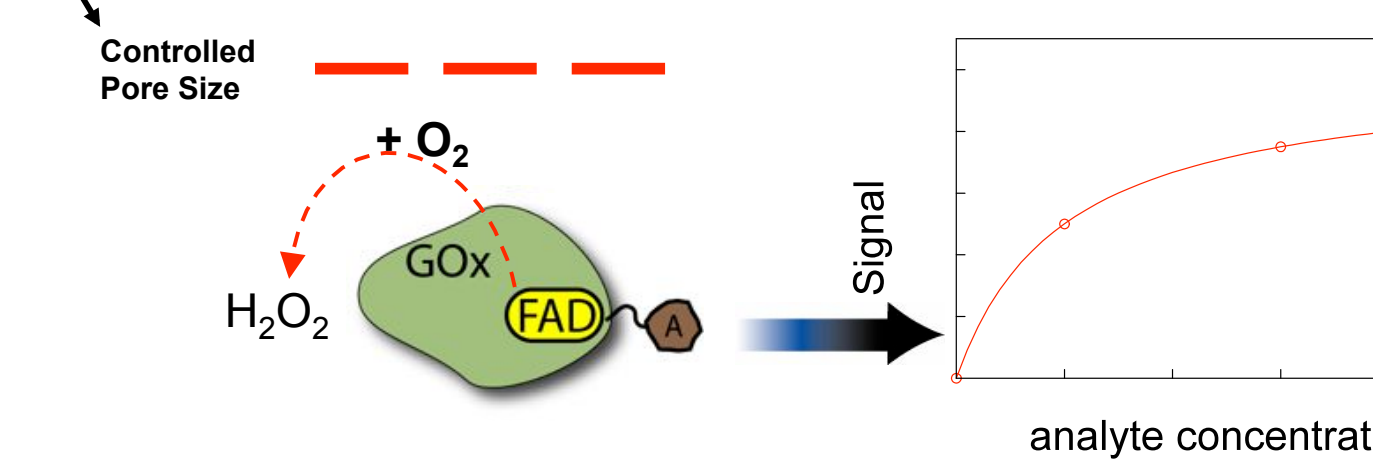
E-Chemical Immunoassay

Addition of analyte from se
or environmental sample
competes with the FAD-an
conjugate for antibody bin
sites…

body is mixed with FAD-
yte conjugate. Larger
body-analyte-FAD
ugate is not able to
etrate the control pore
layer.

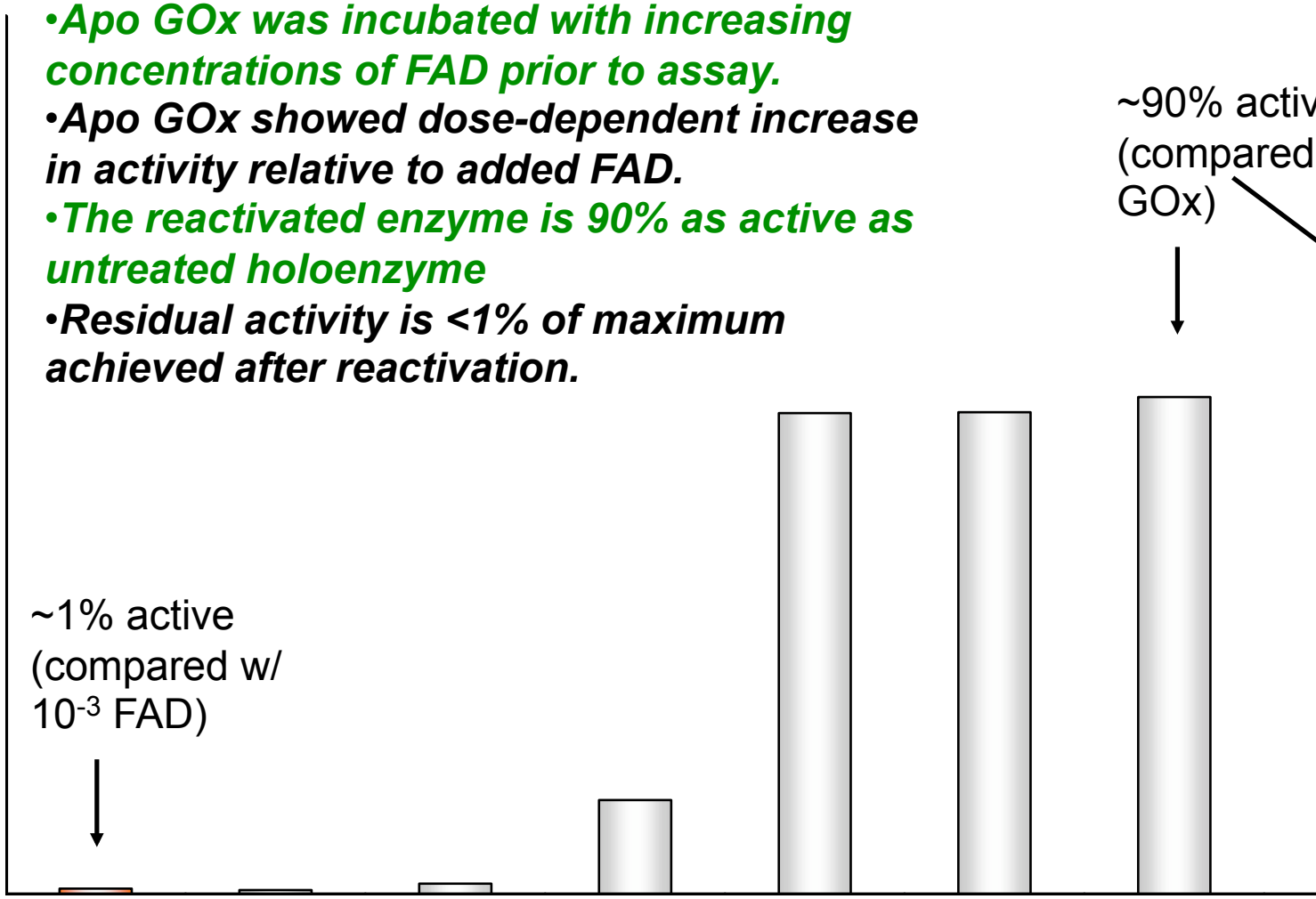


…and thus releases
the conjugate. This
FAD conjugate is sm
enough to enter pore
and activate the enzy



Reactivation of Apo-GOx

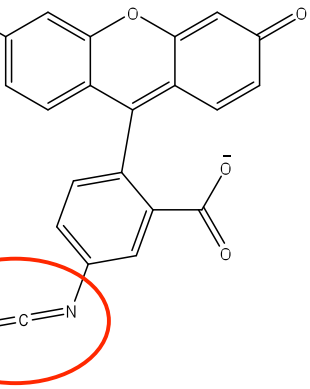
- ***Apo GOx was incubated with increasing concentrations of FAD prior to assay.***
- ***Apo GOx showed dose-dependent increase in activity relative to added FAD.***
- ***The reactivated enzyme is 90% as active as untreated holoenzyme***
- ***Residual activity is <1% of maximum achieved after reactivation.***



Antibody – Analyte Selection

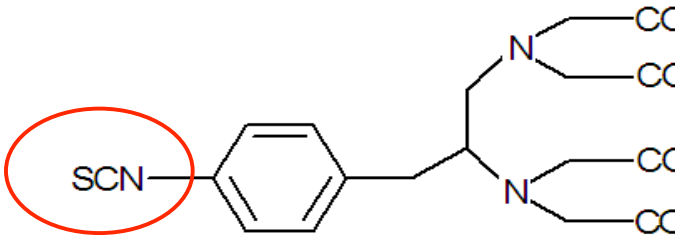
ne Number	Ligand	K_d (M)	Availability
20	Fluorescein	1.5×10^{-9}	Invitrogen
0209	Fluorescein	3.6×10^{-9}	Fitzgerald International
6	2,9-dicarboxyl-1,10 phenanthroline (DCP)	7.5×10^{-7}	Blake et al., (2004) *Bioconj. Chem.* **15**:1125.
6	UO_2^{2+}-DCP	9.1×10^{-10}	Ibid
3	EDTA	1.3×10^{-8}	Blake lab
3	Cu^{2+}-EDTA	2.2×10^{-9}	Blake lab

Analytes to be coupled to FAD



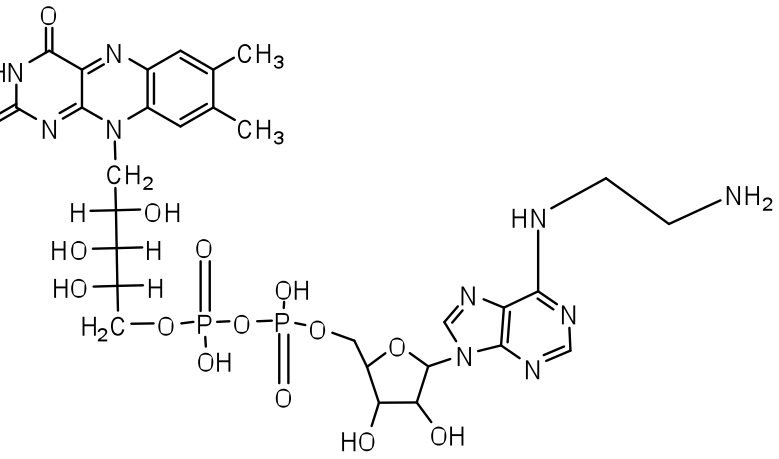
FITC

DCP



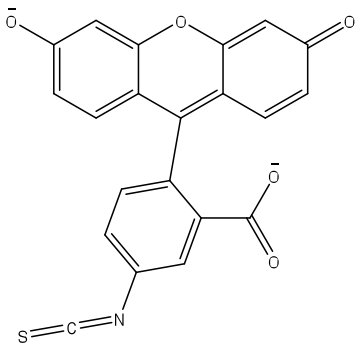
EDTA

Synthesis of FAD Conjugate

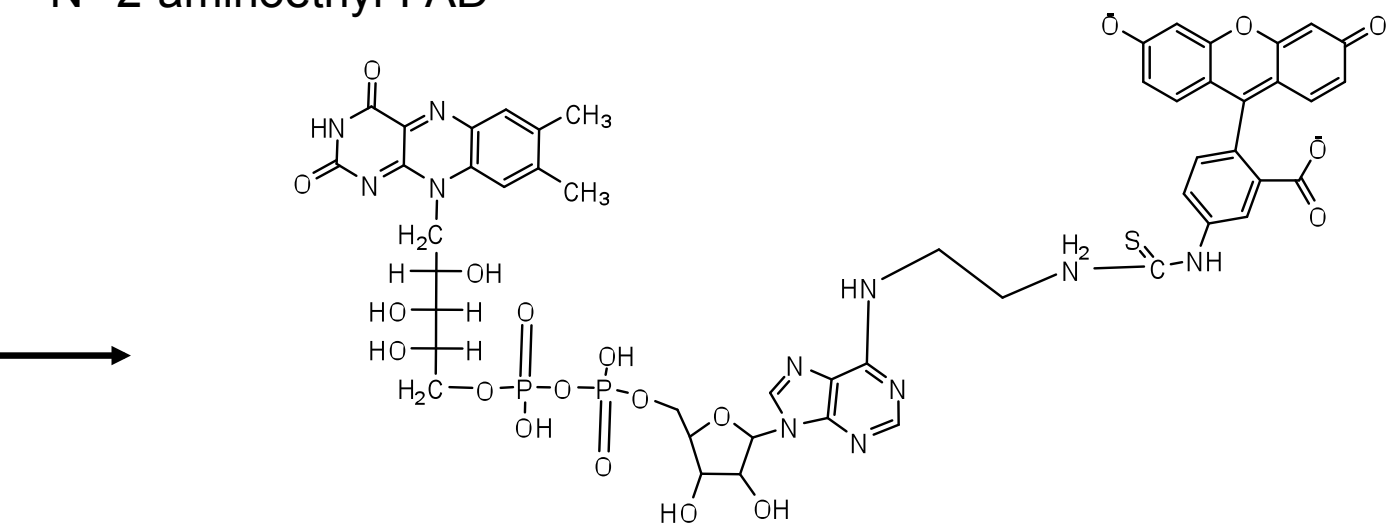


N^6-2-aminoethyl-FAD

+



FITC



FAD-FITC conjugate

Summary

election and Characterization of Antibodies

Commercial and in-house antibodies have been characterized.

po-Glucose Oxidase has been prepared

Change in UV-VIS Spectra >300nm confirmed removal of FAD.
Purification has been optimized to yield high quantity with low residual signal; storage conditions have been developed.
Apo GOx has been transferred to LATech for sensor fabrication.

AD mediated Reactivation of Apo-Glucose Oxidase

Reactivation of Apo GOx was dependent on FAD concentration.
Reactivated enzyme showed kinetics identical to native GO.
Enzyme activity was not affected by components of the immunoassa

ynthesis of primary amine terminated FAD

N^6-2-aminoethyl FAD has been synthesized and characterized.
This intermediate was used to synthesize FAD-analyte conjugates.
The apo enzyme could be reactivated with the FAD-FITC conjugate.
A newly synthesized bifunctional crosslinker is also being tested for the preparation of FAD conjugates.

Antibody Bootcamp for Modelers

perimental Rotation in Blake Lab

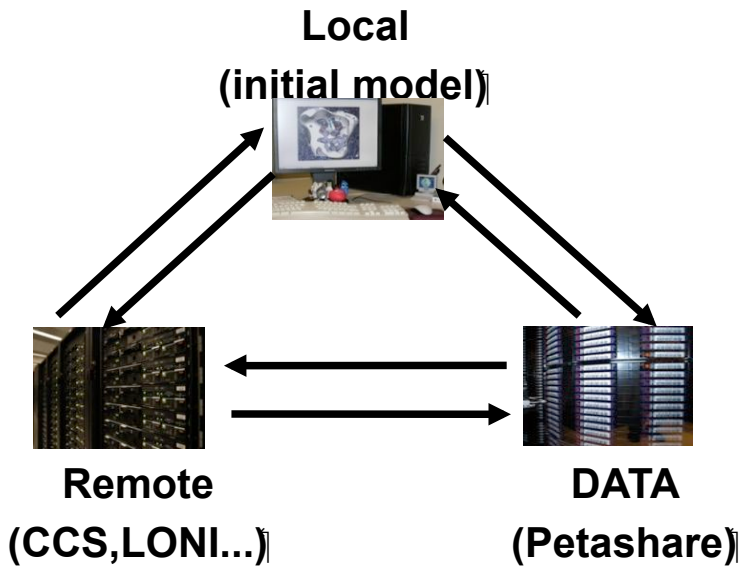
Ashbaugh graduate student (Jain) spent one and half wee
in Blake lab learning experimental protocols for antibody
sensing.

Titer experiments performed to measure concentrations of
antibody 5B2, Pb^{2+}-DTPA-benzyl-BSA conjugate, metal
chelator (DTPA), and Pb^{2+}-DTPA.

Enzyme-Linked ImmunoSorbant Assay (ELISA) used for
titer of monoclonal antibody 5B2 and Pb^{2+} conjugate.
Competitive inhibition ELISA used to infer the ability of
DTPA and Pb^{2+} to bind to 5B2.

he Molecular Modeling Requires

)Creation of putative antibody models based on sequence
Modeler)
)Parameterization of the analytes that bind to the antibodie
Gaussian)
)Docking analytes in different potential antibody binding
oops; (PackMol)
)Optimization of the antibody-analyte interaction by *in silic*
oint mutations (Methods under development, ***REDS****)*



osensors: Computational Aspects MD

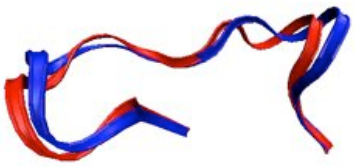
ulations of 5B2 loop region (Test Cases)

nding of antigens to antibody occurs in loop domain. Aim to identify us
mulations side chains in loop region that contribute to binding specifici
uide antibody engineering.

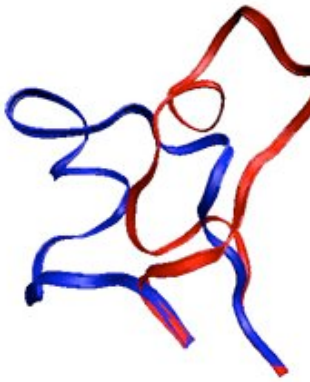
vacuo energy minimizations of 5B2 LC and HC loops confirm previou
entification of metal binding residue Lys^{58}.

eplica Exchange Molecular Dynamic performed of 5B2 in vacuo and
plicit solvent to generate families of loop structures for minimization to
etermine robustness of predictions and identify spatial and dynamic
rrelations between key binding residues

itial findings: HC3 loop has more varied and
xible structure than the other
e antibody loops



LC1



HC3

osensors: Computational Aspects MD

Simulations of 5B2 loop region (continued II)

- Replica Exchange (REMD):

  replica: several simultaneous simulations

  2 levels of parallelization

  exchange: simulations swap information

Simulation Characteristics

Loops

~1000 atoms => 2CPUS/sim

10ns run time => Gb's data;

Full REMD in 24hrs 64CPUS

Full System

~10,000atoms => 4CPUS/sim

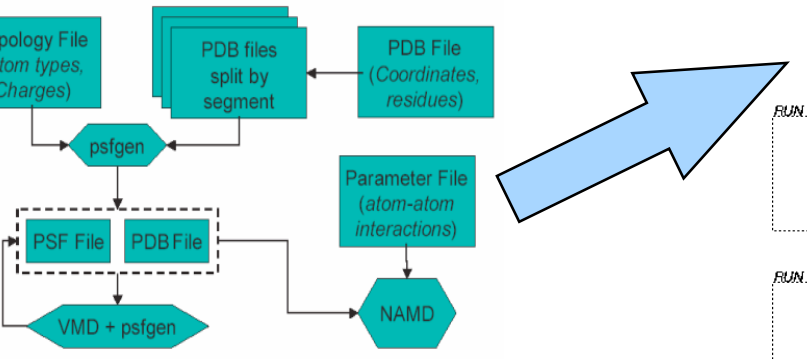
10ns run time => 10-100Gb data;

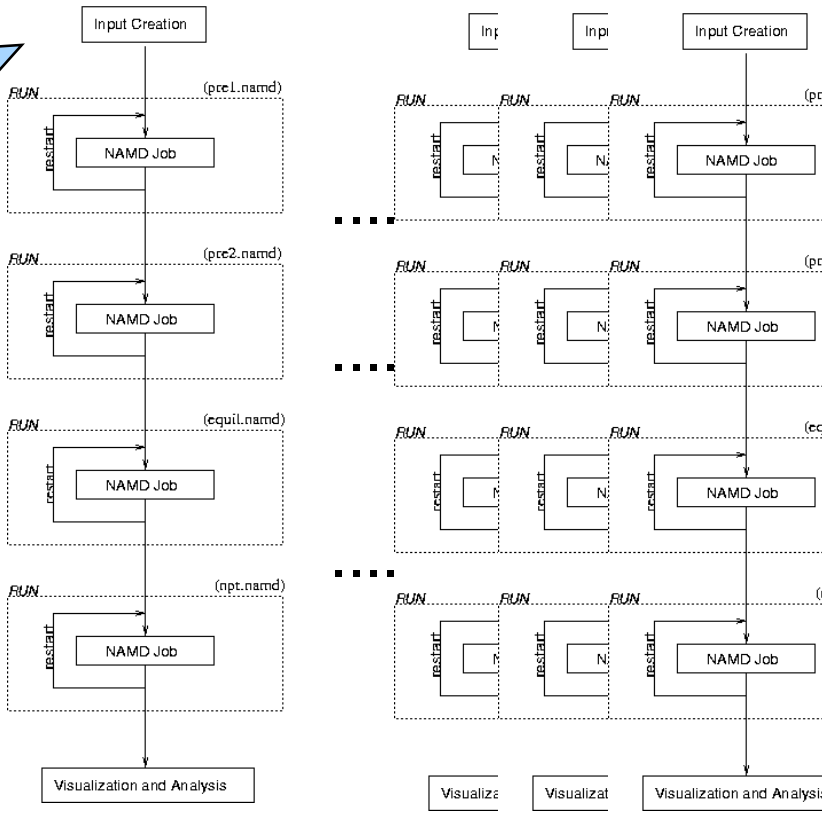
Full REMD in 2wks 64CPUS

Biosensors: MD Fast Track Study

high throughput simulation workflow

Bishop (CCS @ TU)
Emir Embahsi & Tevik Kosar (CCT @ LSU)





yberTools Connections

P 1: Scheduling and Data Services.

e details of integrating our Molecular Modeling packages into WP 1 ar
ng addressed by Drs. Thomas Bishop (Tulane) and Tevfik Kosar (LSU

P 2: Information Services and Portals.

s. Thomas Bishop and Tevfik Kosar are collaborating to bring Bishop's
A folding simulations on-line. The Workflow resulting from this effort c
readily modified to investigate the antibody and analyte interactions.

P 3: Visualization Services.

rk is in progress to create modules that will permit all scientists involv
project to visualize molecular models and other results via a commo
er interface without the necessity of transferring data or installing softw
local lab computers.

P 4: Application Services and Toolkits.

s. Steven Rick (UNO) and Henry Ashbaugh are developing replica
ulation techniques that will enable this group to efficiently identify anti
p sequences that optimize the antibody-analyte interactions.

Fluid Mechanics and Transport

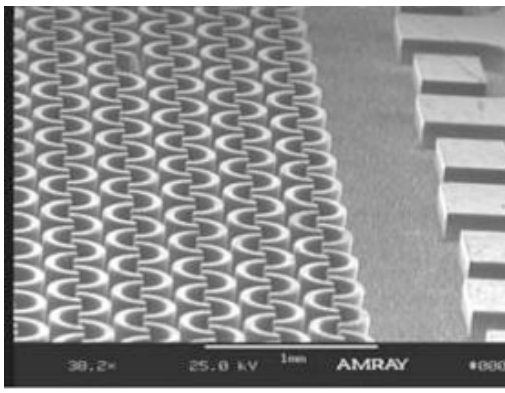
GOAL ➔ *Computationally determine the optimal geometric configuration of the omega channel network to enhance mixing of two species.*

Laminar flow field governed by continuity & Stokes equations:

$$\nabla P = \mu \nabla^2 \mathbf{u}$$

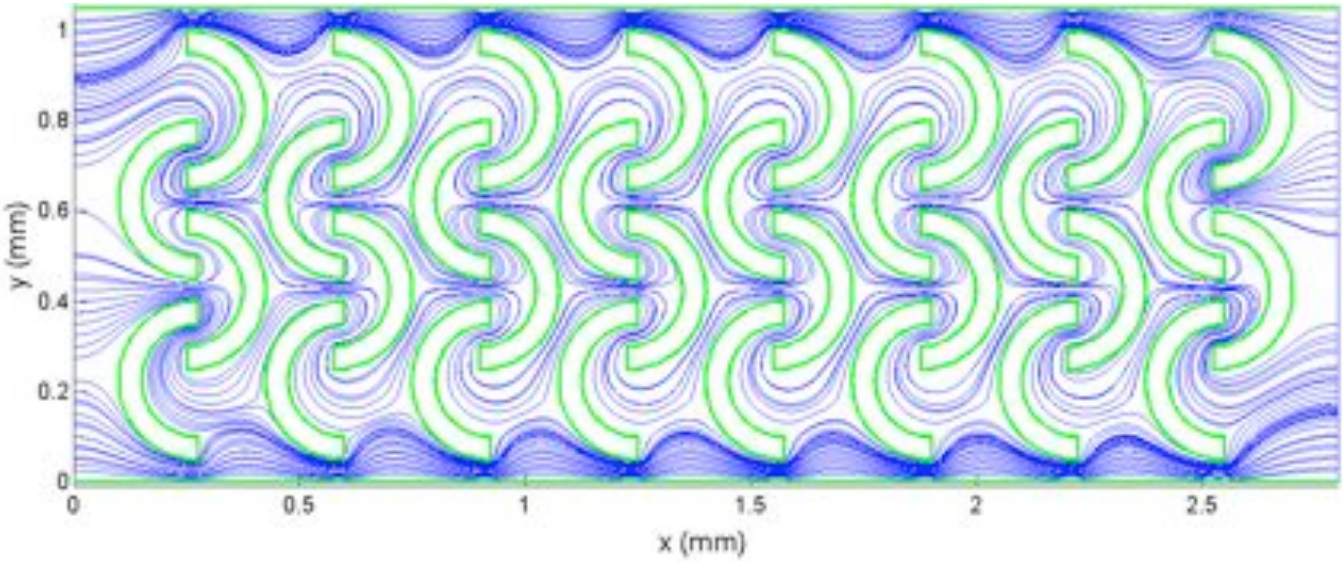
$$\nabla \cdot \mathbf{u} = 0$$

Boundary Element Method determines velocities and surface stresses

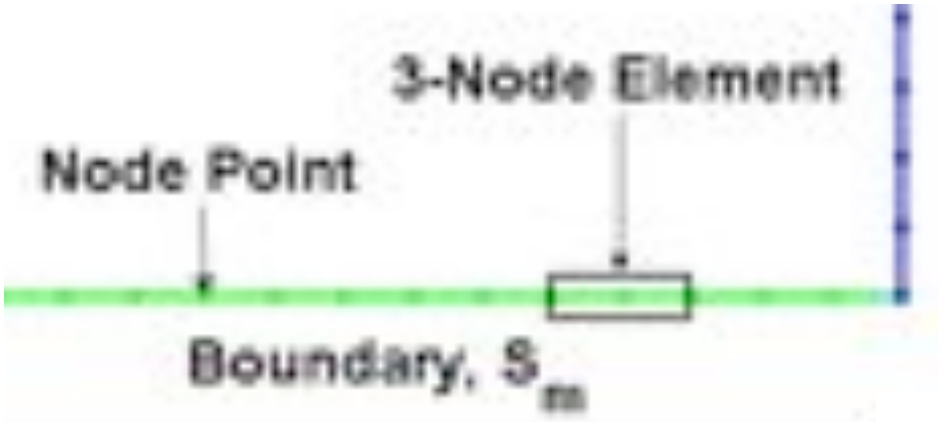


Omega channels developed by IfM

Streamlines resulting from constant pressure drop across model channel



Boundary Element Method



- Velocity **u** and stress τ are approximated as quadratic polynomials, and at each node point, satisfy

$$\mathbf{C}_{ki}u_i(\mathbf{x}) + \sum_{m=1}^{N}\int_{s_m}\mathbf{T}_{ik}(\mathbf{x},\mathbf{y})u_i(\mathbf{y})dS_m = \frac{1}{\mu}\sum_{m=1}^{N}\int_{s_m}\mathbf{U}_{ik}(\mathbf{x},\mathbf{y})\tau_i(\mathbf{y})dS_m$$

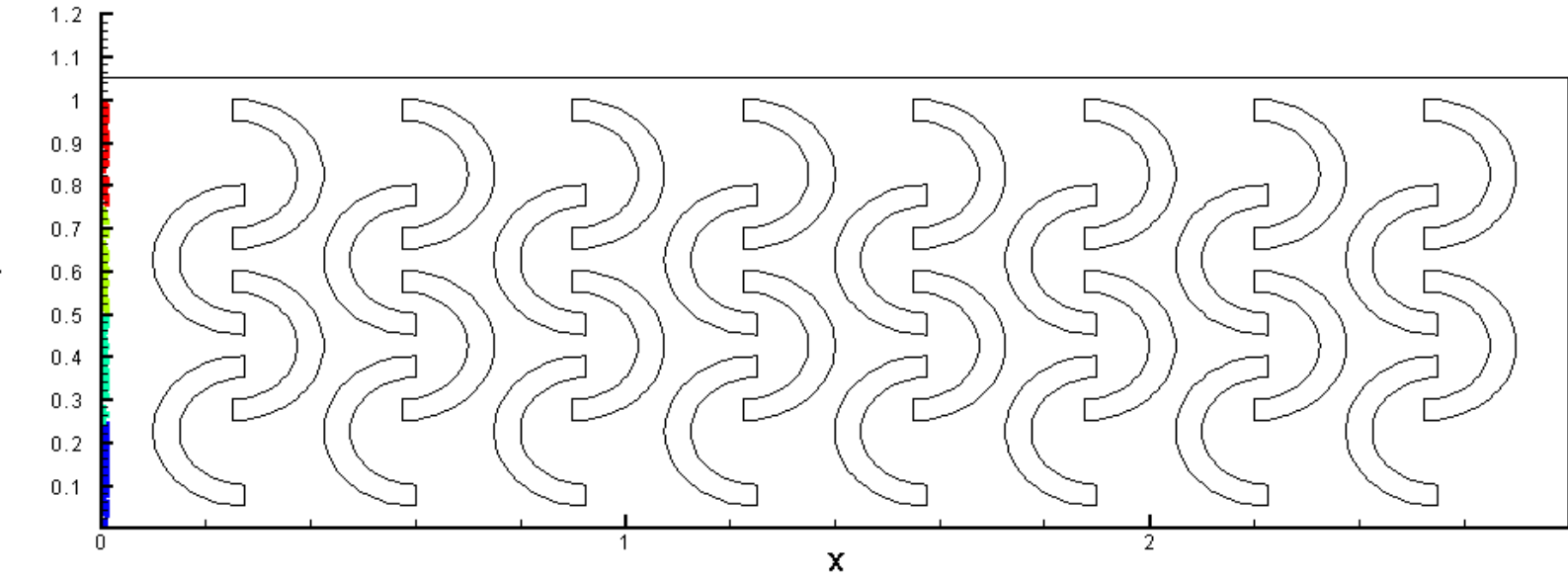
- Integral equation is expressed as system of linear equations:

$$H\mathbf{u} = G\tau$$

- Elements of H and G computed using Gaussian quadrature rules
- Optimization of simulation is being developed in conjunction with WP4 and will create a general purpose *CyberTool*.

Results: Particle Trajectory

For more info see our poster!



Particles initially positioned along y-axis at x = 0

Path of each particle traced as it flows through the domain

Note inner particles travel more slowly than outer particles that migrate quickly across channel along outer walls

Results suggest domain modification is important to improve mixing

licrosensor Mixing and Transpor

- Analyte-FAD conjugate and analyte from serum compete to bind with antibody

- Binding and release occur spontaneously as analytes and antibody are transported by fluid motion

ach analyte/antibody satisfy a reaction-diffusion equation:

$$\frac{\partial C_i}{\partial t} + \nabla \cdot (\mathbf{u} C_i) = D_i \nabla^2 C_i + R_i$$

$\mathbf{u}$ – fluid velocity

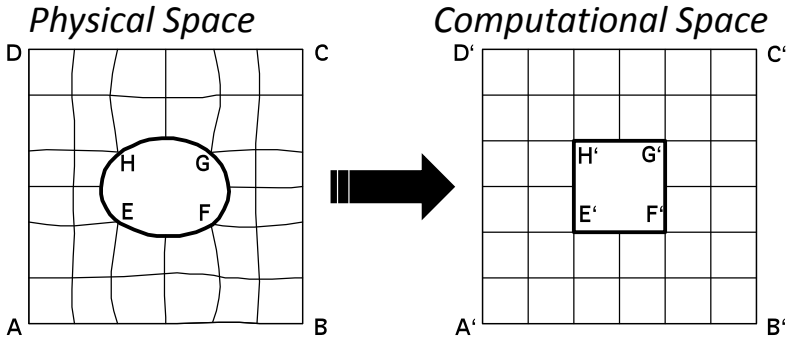
C_i – concentration of each species

D_i – diffusion coefficient

$R_i(C)$ – reaction term

Transport Methodology

ansform equations into a boundary-fitted coordinate system



e the Finite Volume Method to solve for *concentration*

ote: velocity field obtained from BEM code

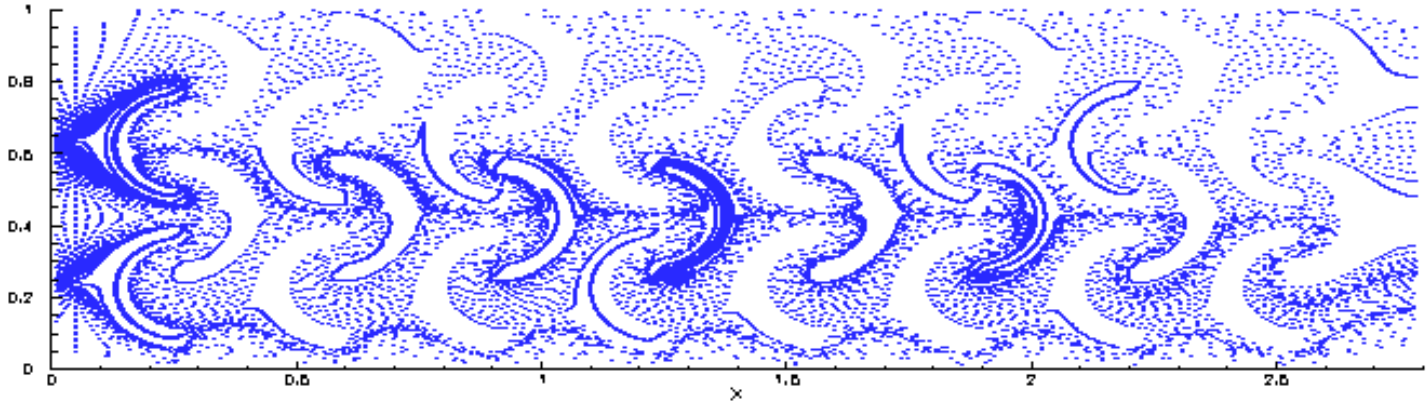
ultiblock approach for omega channels

orking with Dr. Blake for reaction/diffusion rates

CyberTools Connections

Current Work:

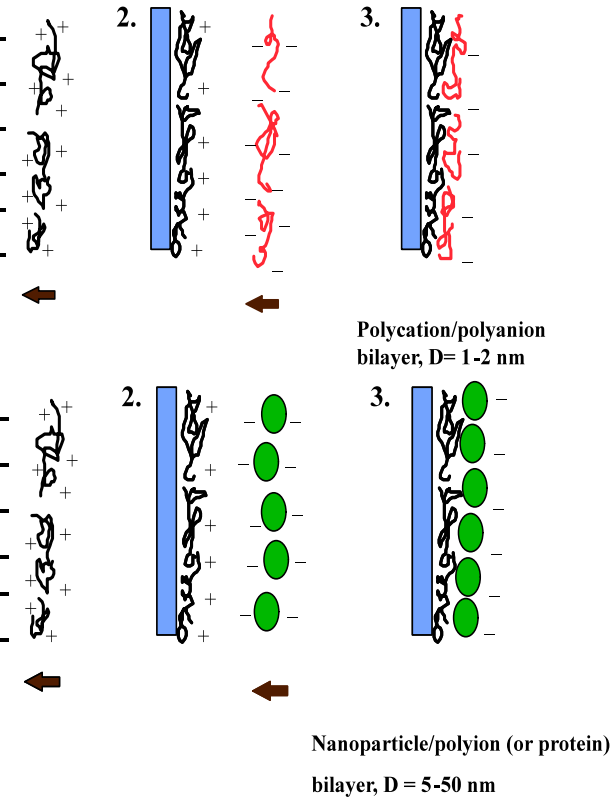
- Parallelization of Stokes flow problem for use in the HPC environment (WP4: Mayank Tyagi, Shantenu Jha, Sanjay Kodiyalam)
  - *OpenMP*
- Visualization of model problem using TecPlot (with WP3)
- Generalization of code to develop a *CyberTool* package that solves Stokes flow equations



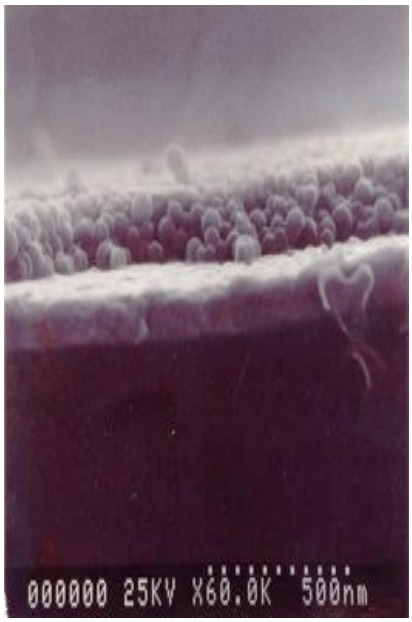
Future Work:

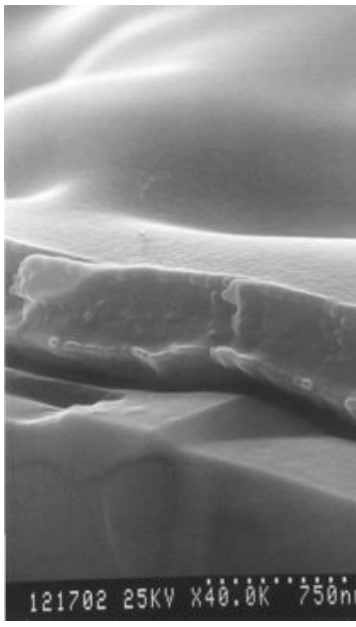
- Parallelization of source code including transport

Layer-by-Layer (LbL) Nanoporous Membrane for Immunoassay (sensor technology for enzyme deposition



eme of the layer-by-layer
passembly by alternate
orption of polycations and
anions or nanoparticles





SEM cross-section images of (glucos
oxidase/PAH)$_{22}$ multilayer on quartz (lef
and (40 nm silica/PAH)$_6$ film on silv
electrode (right).

Polymer-based Electronic Microsensor Fabrication

Silicon wafer with oxide layer

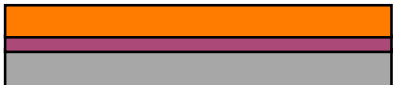
Spin coat PR 1813 resist layer

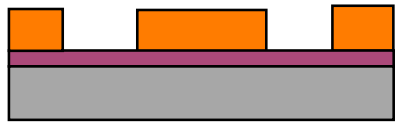
Pattern PR 1813 resist

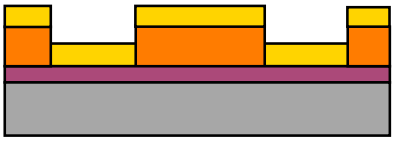
Sputter gold electrodes

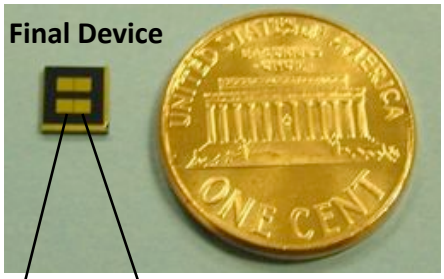
Lift-off PR 1813 resist

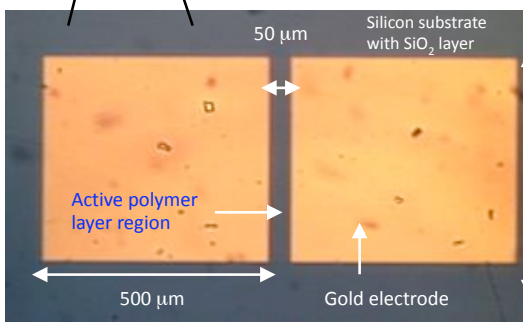
Final Device

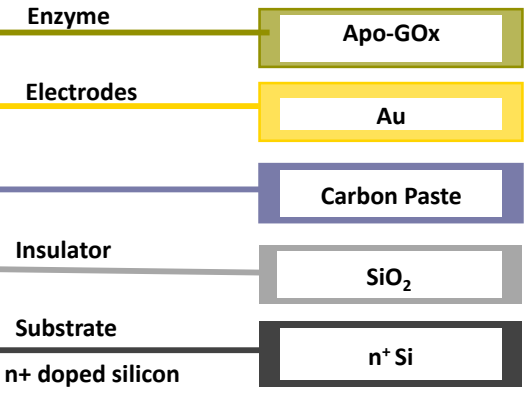












Micromixer Fabrication

n wafer
oxide layer

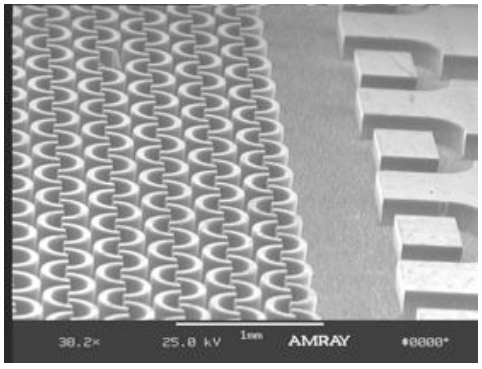
coat
313 resist layer

ern
813 resist

SiO_2

Silion

O2 & Bond
ass Substrate



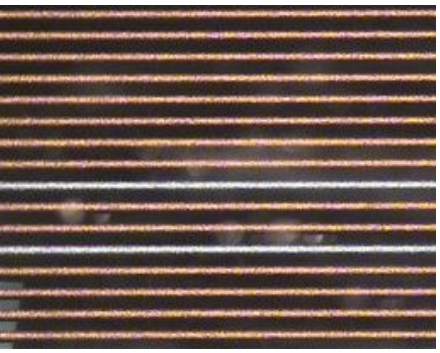
SEM → Omega Channel Micromixer

Fabrication
- Lithography
- ICP
- Bonding

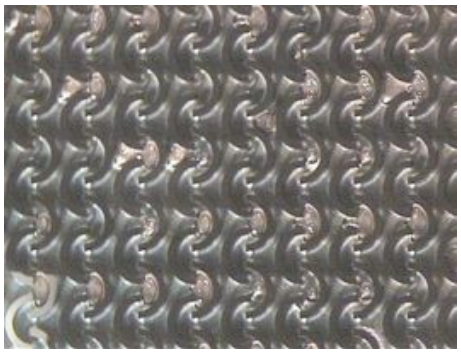
Challenges
- Connectors

Modifications
- New set of connectors from Upchurch Scientific are being tested and evaluated

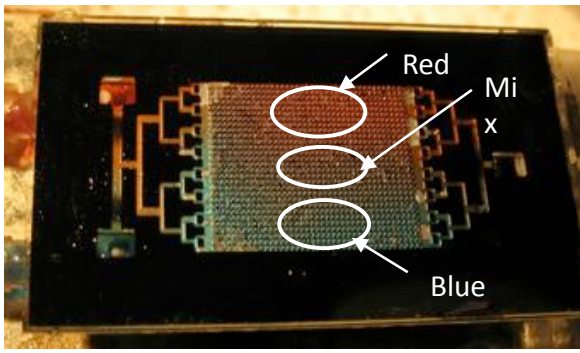
Micromixer Evaluation



Straight Channel



Omega Channel



Micromixer

allenges

aminar Flow

Mixing only at the center of the device

odifications

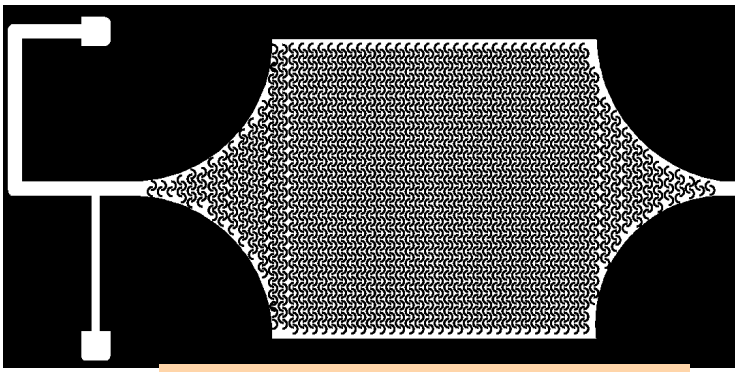
esigned ‘T’ shape inlet and outlet for

itial mixing

antification

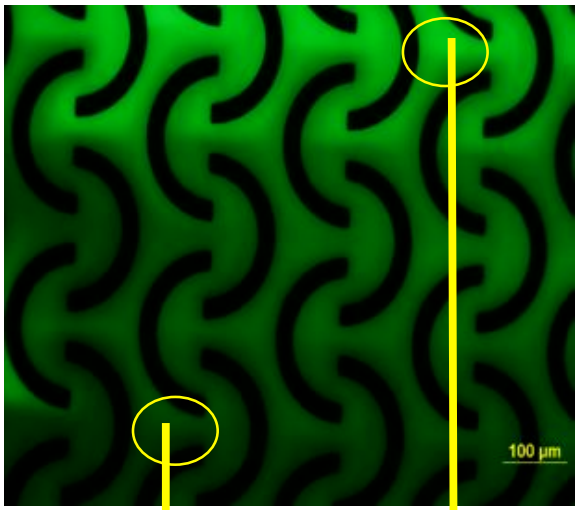
mage analysis software

sing fluorescent dyes for better signal/noise



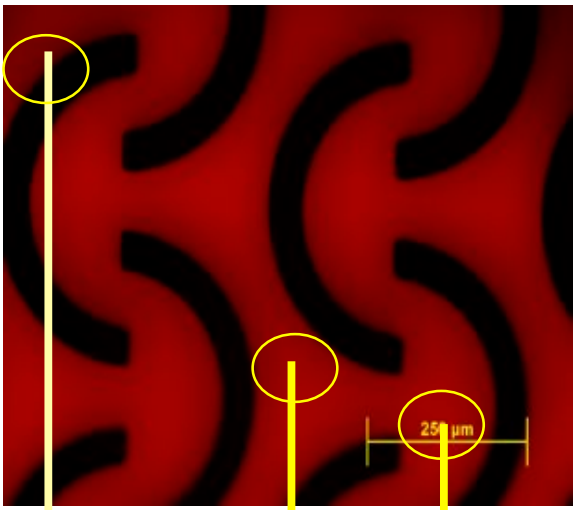
New Micromixer Design

Fluorescent dyes in the omega channels:
VISUALIZED WITH MICROSCOPY AND
DIGITAL CAMERA



Water FITC

flow of fluids



Water Rhodamine

Mixture of two

Summary and Conclusions

Microfluidic Component

- Fabricated and evaluated two sets of micromixers
- Designed new micromixer based on the results obtained (fabricated)

Nanoporous Membrane

- LbL nanoassembly is being evaluated for fabricating nanoporous membrane; ***New:*** in collaboration with Dr. Scott Gold (Louisiana Tech.) for modeling of flow through porous membrane.

Reproducibility

- Evaluating PEDOT and carbon nanotube based microsensor for reproducibility, Selectivity, an Life Time

Testing Procedures

- Currently testing florescent dyes and particles (proposed) for evaluating micromixers.
- Currently evaluating microscale sensor system based on carbon nanotubes

• Carbon-based Electrodes

- Under testing and fabrication

• *CyberTools* Connection

- Access Grid (AG) video conference with Tulane (23 July 2008): simulations and experimenta data. Evaluation of Cybertools link to visualization software: VisIt 1.9.1 (Windows version, DeCoster) thru the **Cactus Code** Link.

- *VISIT OUR GRADUATE STUDENT POSTER! -- SENAKA KANAKAMEDALA-*

Final Remarks

eractions between the laboratory and the molecular dynamics groups

rovided new models of protein structure that allow testing of hypotheses in sili
or to time-consuming laboratory experiments.

alidated *in silico* predictions based on laboratory experiments.

entified methodological refinements based on laboratory results.

eractions between the laboratory and the micromanufacturing groups

roadened the kinds of hardware and electronics that can used to construct the
nsors.

rompted micromanufacturers to examine paradigms used to validate their dev
g. they have modified the molecular identity and concentration ranges of reag
ed to test their microscale mixers).

eractions between the fluid mechanics and the micromanufacturing gro

rovided data to determine boundary conditions used in the simulations.

stablished realistic geometries for fluid flow domains.

elineated a plan to reduce the number of fabrication trials needed to optimize
al device.