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Title: Enhancing the longevity of enzymatic sensors for continuous biochemical monitoring

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Enhancing the longevity of enzymatic sensors for continuous biochemical monitoring

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Jerzy Chlistunoff (MPA-11/LANL)
Yash Kapoor (Alcon®/Novartis)

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@ Texas A&M Univ.
National Institute of Health (RO1 EB000739)
Texas Engineering and Experiment Station

@ LANL
LANSCE-LC, Los Alamos National Laboratory
DOE office of Basic Energy and Sciences

Academic & Research Background

- **Instrument Assistant for SPEAR beamline:** state-of-the-art Neutron Reflectometer at Lujan Center, LANL

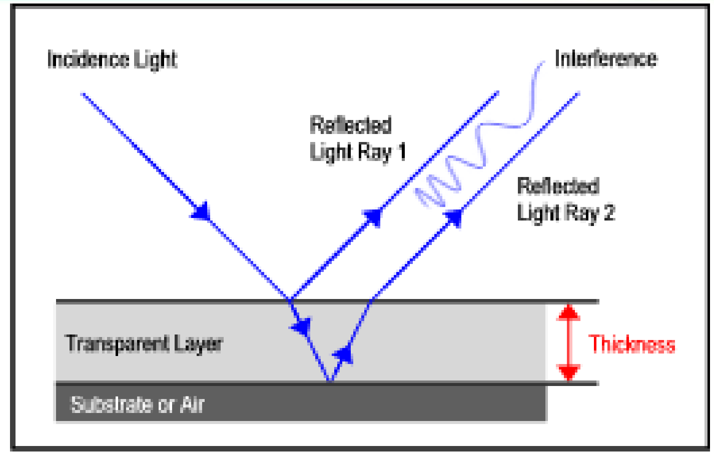
  - Development of stimuli responsive lipid membranes for biomedical applications

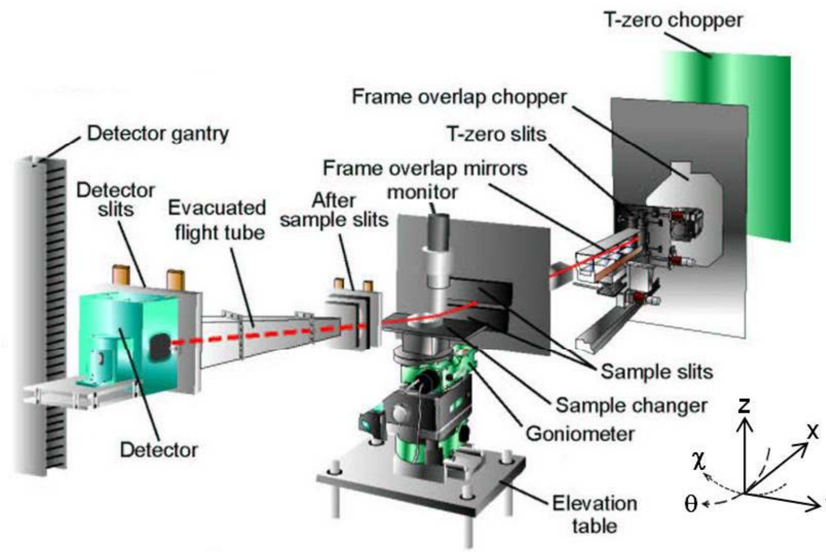
  - Neutron Reflectivity to investigate the effect of fluidic shear on polymeric thin films

  - NR characterization of LbL-deposited polymeric films

  - Collaboration with LANL weapons experiments division to study interfaces in PBX-9501

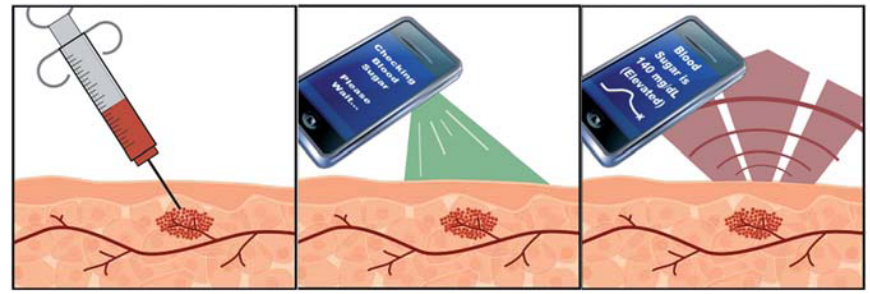
  - Collaboration with Alcon® eye care division





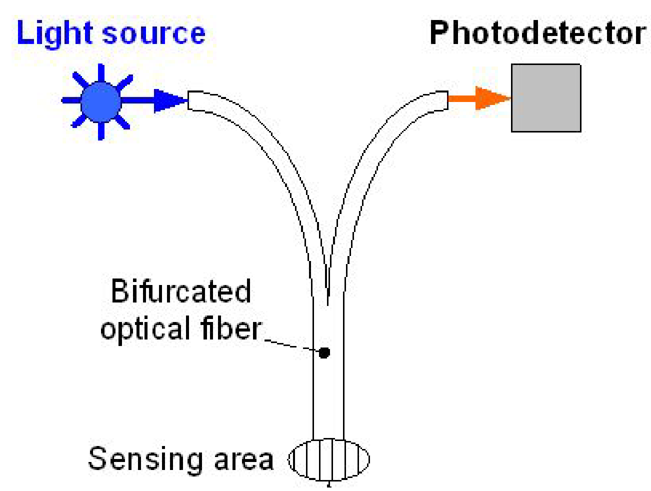
Academic & Research Background

- **Ph.D. in Biomedical Engineering @**
  Texas A&M University
  - Design and development of microparticle-based glucose sensors for continuous monitoring



M. McShane et. al., Microcapsules as optical biosensors. *J. Mater. Chem.* ***2010, 20 (38), 8189-8193.***

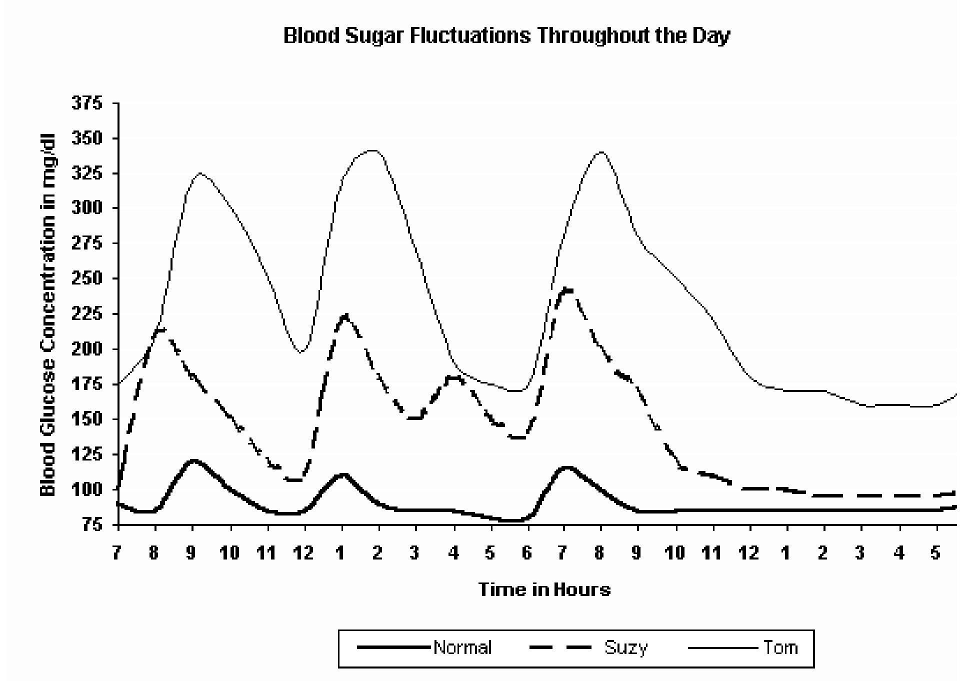
- **M.S. in Chemical Engineering @**
  Louisiana Tech University
  - Development of optical fiber based oxygen sensors and SPR sensors



- **B.S. in Chemical Engineering @**
  National Institute of Technology
  Raipur, India

Background & Motivation

- Diseases are often manifested in the form of biochemical imbalances
- A prime example is glucose/insulin imbalance, which is associated with diabetics
- Point-in-time tests can miss fluctuations in biochemical analytes that are vital to accurate diagnosis
- Excessive pain leads to incompliance – need for minimally invasive continuous monitoring



Current Technology

- CGMS (Guardian® RT, Freestyle™ Navigator, STS ® Seven System) have been introduced for the continuous monitoring of glucose levels.
- Consists of an enzymatic electrode that is inserted into the dermis and is connected to a transmitter.
- The transmitter wirelessly communicates with the receiver.

$$\text{Glucose} + O_2 \xrightarrow{\text{GOx}} \delta - \text{gluconolactone} + H_2O_2$$

Problems?

1. Short life (maximum 7 days)
2. Slow response
3. Potential infection pathway
4. Requires frequent calibration

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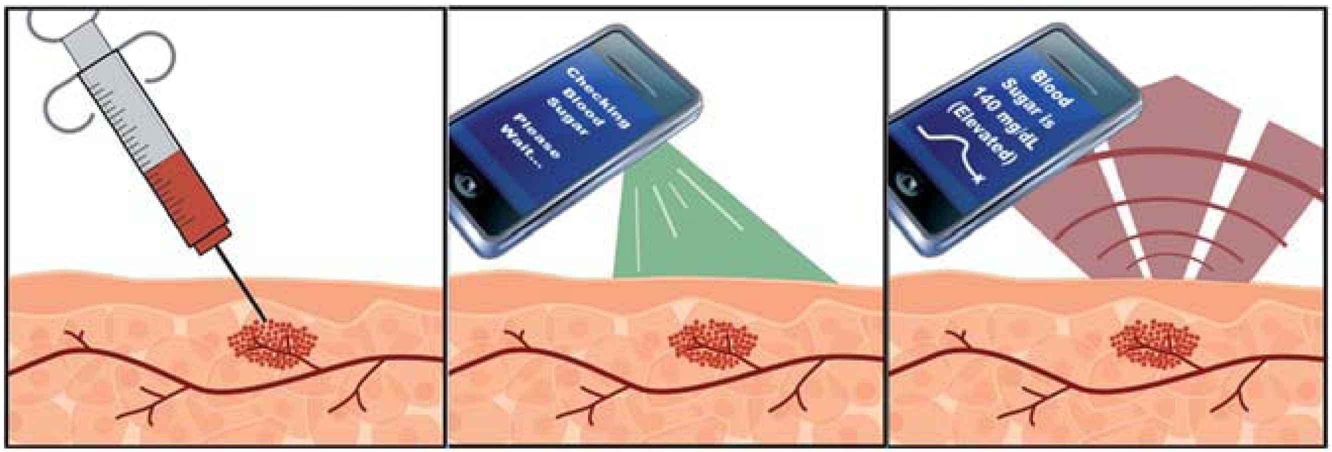
Proposed Technology

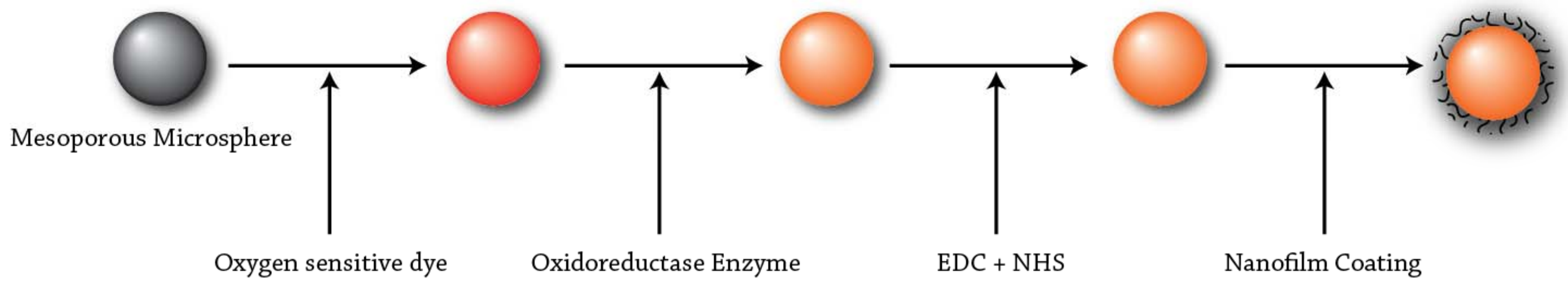
Luminescent microparticle-based sensors can address the problems associated with currently available CGMS



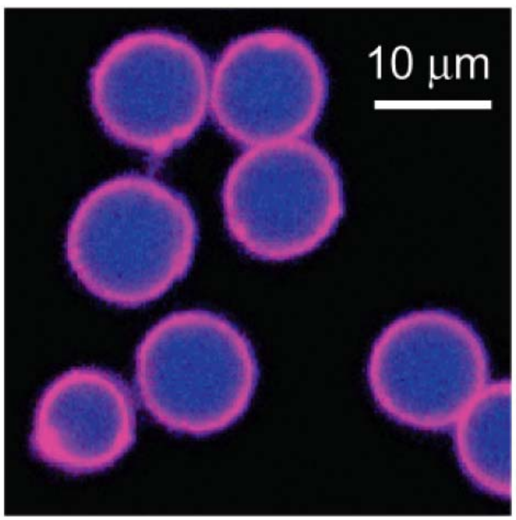
Schematic of microparticle sensor concept: implantation, interrogation, and readout.

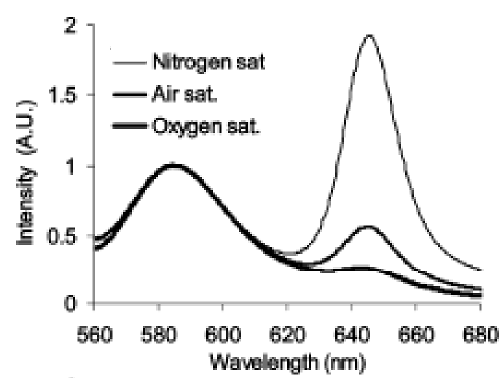
M. McShane et. al., Microcapsules as optical biosensors. *J. Mater. Chem.* ***2010, 20 (38), 8189-8193.***

Synthesis of Microparticle-based Sensors



$$\text{Glucose} + O_2 \xrightarrow{\text{GOx}} \delta - \text{gluconolactone} + H_2O_2$$



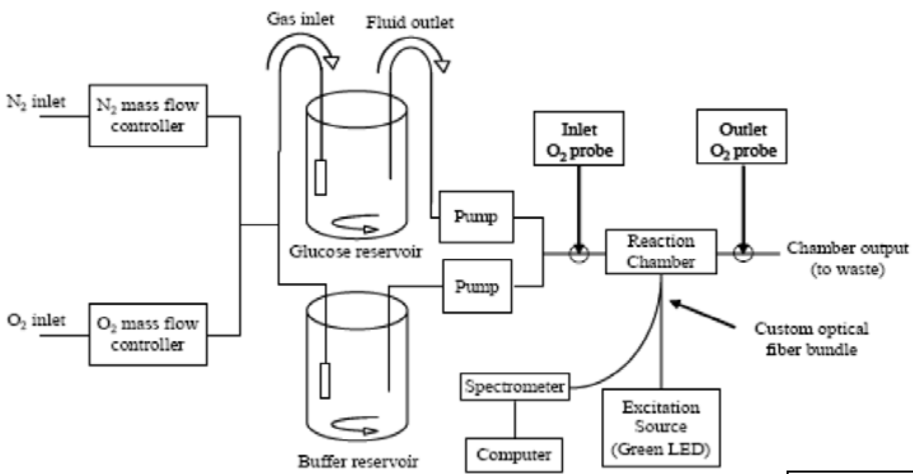


$$\frac{F_o}{F} = 1 + K_{sv}[O_2]$$

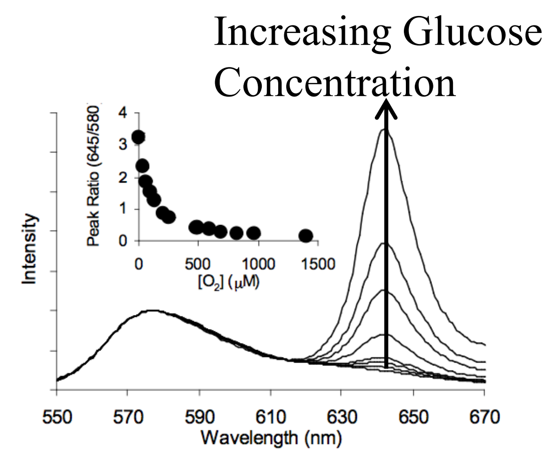
Stern-Volmer equation

E. W. Stein; **S. Singh**; M. J. McShane,. *Anal. Chem.* ***2008****, 80 (5), 1408-1417.*

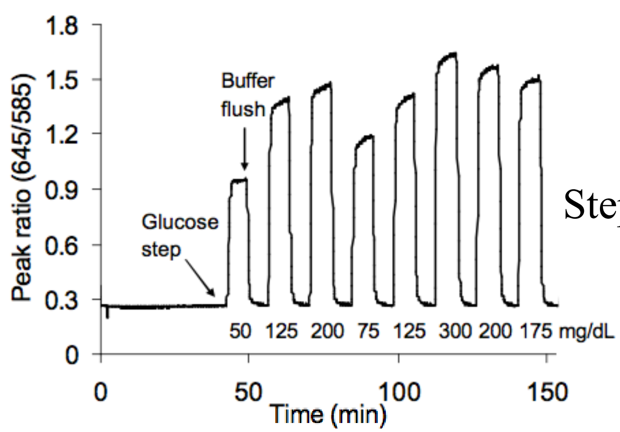
Testing Setup and Response to Glucose



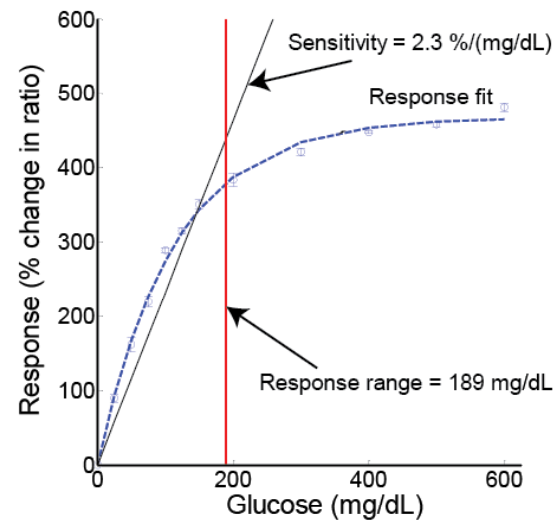
Schematic of sensor testing setup



Fit type: $I = I_{max}(1-e^{-k[glucose]})$



Step Response



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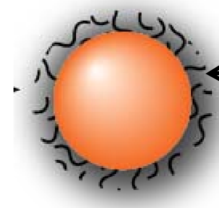
Challenge?

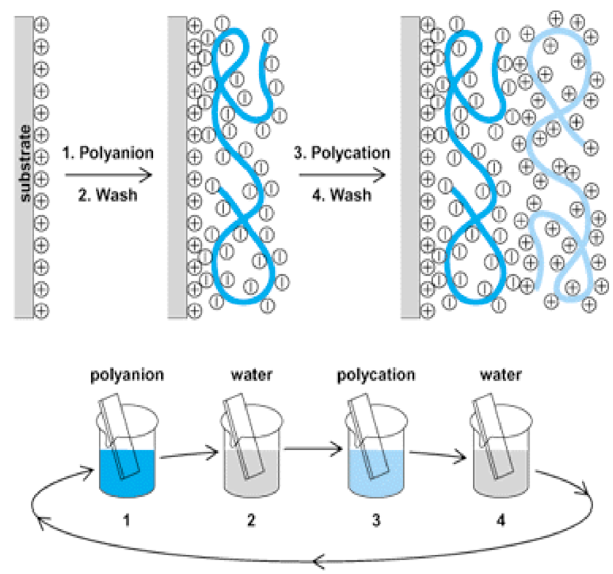
- Lower response range (< 175 mg/dL) due to lower blood oxygen level (90-270 μM) as compared to glucose level (normoglycemia: 5.5 mM)
- Short life due to deactivation of GOx (< 2days)
  - Spontaneous deactivation (half life = 90 days)
  - Peroxide-mediated deactivation

Solution

- Layer-by-layer (LbL) ultrathin films can be employed to *lower the glucose flux* without substantially affecting the oxygen flux
- Another route of decreasing the glucose flux relative to oxygen flux is to *increase the porosity of the microparticle.*
- *Increasing GOx loading*
- *Incorporation of catalase*



LbL-deposited coating



Schematic depicting the LbL process

Model

Reaction-diffusion equation

$$\frac{\partial C_i}{\partial t} + \nabla \cdot (-D_i \nabla C_i) = R_i$$

Reaction-diffusion equation in spherical coordinates

$$\frac{\partial C_i}{\partial t} + \frac{1}{r^2}\frac{\partial}{\partial r}\left(-D_i r^2 \frac{\partial C_i}{\partial r}\right) = R_i$$

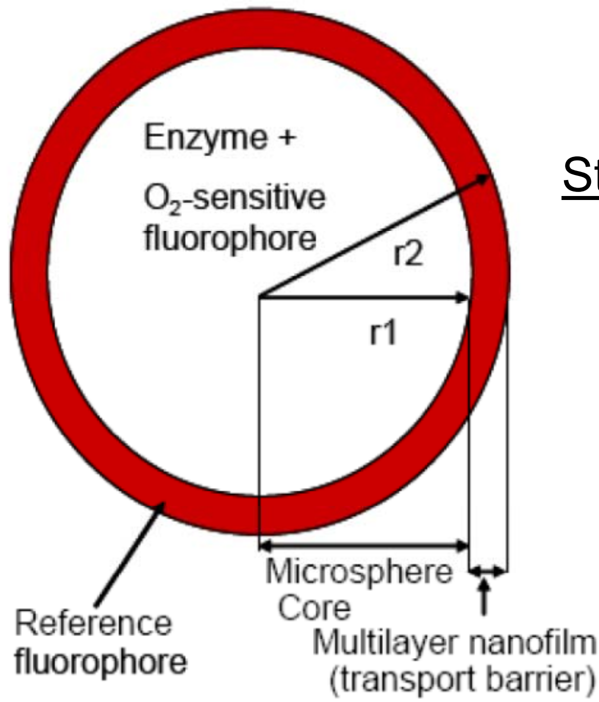
Reaction scheme

$$E_o + G \xrightarrow{k_1} X_1 \xrightarrow{k_2} E_r + P$$

$$E_r + O_2 \xrightarrow{k_3} X_2 \xrightarrow{k_4} E_o + H_2O_2$$

$$H_2O_2 + CAT \xrightarrow{k_5} I + H_2O$$

$$H_2O_2 + I \xrightarrow{k_6} H_2O + O_2$$



Stern-Volmer equation

$$\frac{F_o}{F} = 1 + K_{sv}[O_2]$$

S. Singh; M. McShane, *Biosens. Bioelctron.* ***2011,*** *26 (5), 2478-2483.*
S. Singh; M. McShane, *Biosens. Bioelctron.* **2010,** *25* (5), 1075-1081.

Model Cont.

Chemical Specie (i)	D_i (m^2/s) Alginate Matrix and NF	R_i	IC Alginate Matrix and NF	BC Center ($r=0$) Surface ($r=R+t$)
Glucose	**1.97×10^{-12}** **9.87×10^{-14}**	$-k_1[E_{ox}][G]$	C_G [a]	No flux C_G
O_2	**1.00×10^{-11}** **2.52×10^{-11}**	$-k_3[E_{red}][O_2]+k_6[H_2O_2][I]$	277 μM	No flux 140 μM
H_2O_2	7.00×10^{-12} 1.05×10^{-11}	$k_4[E_{ox}P_2]-k_5[CAT][H_2O_2]-k_6[I][H_2O_2]$	0	No flux 0
E_{ox}	0 0	$-k_1[E_{ox}][G]+k_4[E_{ox}P_2]-k_{sGOx}[E_{ox}]$	E_{GT} [b]	No flux 0
$E_{red}P_1$	0 0	$k_1[E_{ox}][G]-k_2[E_{red}P_1]-k_7[E_{red}P_1][H_2O_2]-k_{sGOx}[E_{red}P_1]$	0	No flux 0
E_{red}	0 0	$k_2[E_{red}P_1]-k_3[E_{red}][O_2]-k_8[E_{red}][H_2O_2]-k_{sGOx}[E_{red}]$	0	No flux 0
$E_{ox}P_2$	0 0	$k_3[E_{red}][O_2]-k_4[E_{ox}P_2]-k_7[E_{ox}P_2][H_2O_2]-k_{sGOx}[E_{ox}P_2]$	0	No flux 0
CAT	0 0	$-k_5[CAT][H_2O_2]+k_6[I][H_2O_2]-k_9[CAT]-k_{sCAT}[CAT]$	E_{CT} [c]	No flux 0
I	0 0	$k_5[CAT][H_2O_2]-k_6[I][H_2O_2]-k_9[I]-k_{sCAT}[I]$	0	No flux 0

[a] C_G = 5.5 mM for longevity simulations; For sensor response profile simulations, C_G was varied from 0 to 33 mM at increments of 1.5 mM.

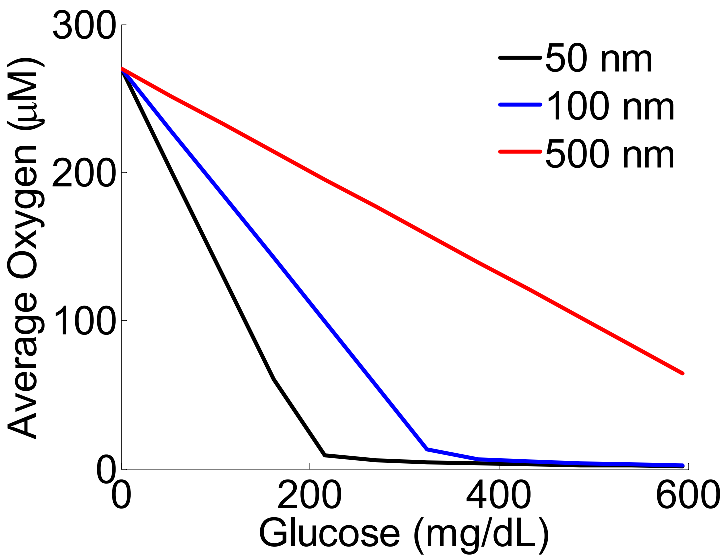
[b] E_{GT} = Concentration of total active GOx.

[c] E_{CT} = Concentration of total active CAT.

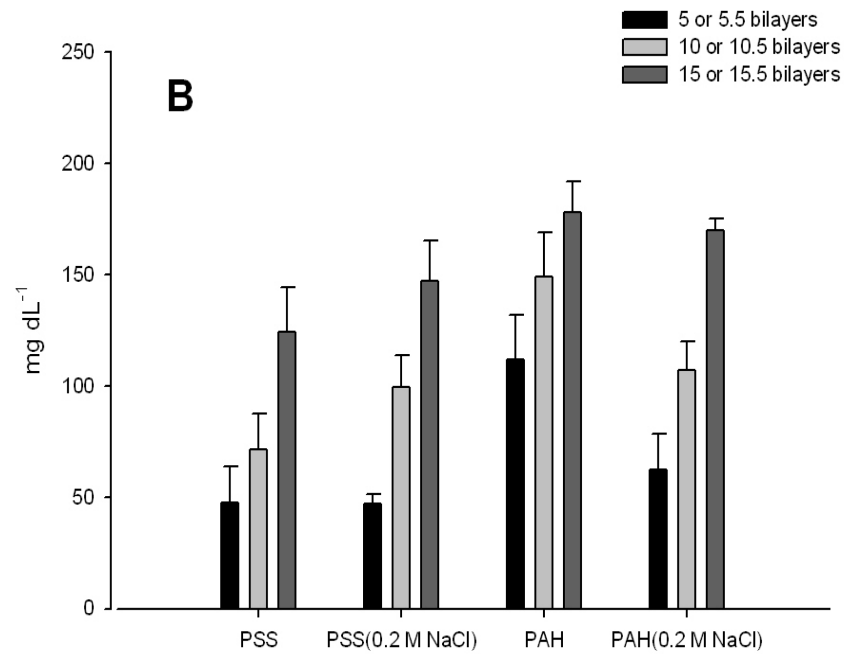
S. Singh; M. McShane, *Biosens. Bioelctron.* **2010,** *25* (5), 1075-1081.

Effect of Film Thickness – Range enhancement

Theoretical Prediction



Experimental Data



NOTE: Enzyme loading was determined using BCA protein assay

E. W. Stein; **S. Singh**; M. J. McShane,. *Anal. Chem.* ***2008****, 80 (5), 1408-1417.*

Key Findings

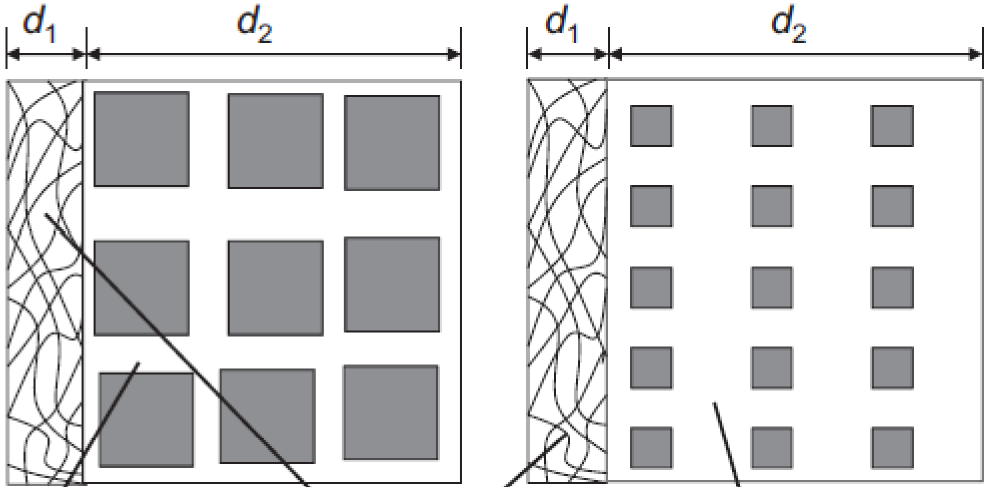
- Response range can be tuned by varying the nanofilm thickness, composition, and the capping layer.

- Nanofilm comprising of more than 60 bilayers of PAH/PSS (0.2 M NaCl) will be required to obtain a linear range up to 600 mg/dL.

- Other effective means of increasing the response range must be investigated.

Effect of Matrix Porosity

Theoretical prediction



$$D_{e,i} = \frac{d_1 + d_2}{(d_1/D_{1,i}) + (d_2/D_{2,i})}$$

Laminate 1 (α = 0.005)

$D_{eff,\ glu} = 1.4 \times 10^{-8}\ cm^2/s$

$D_{eff,\ oxy} = 1.01 \times 10^{-7}\ cm^2/s$

$D_{eff,\ glu} / D_{eff,\ oxy} = 0.14$

Laminate 2 (α = 0.6)

$D_{eff,\ glu} = 4.55 \times 10^{-8}\ cm^2/s$

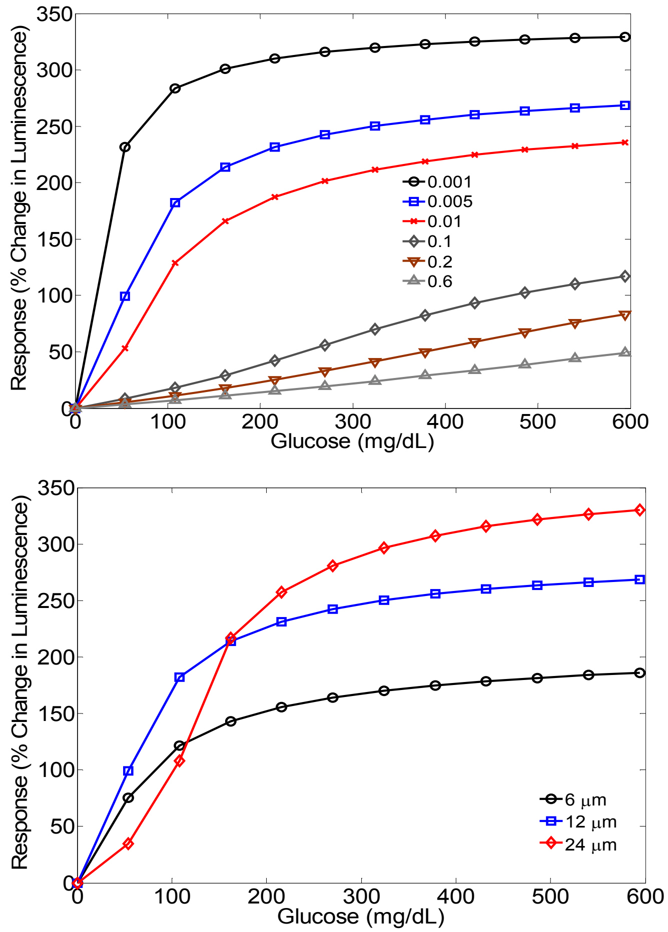
$D_{eff,\ oxy} = 5.94 \times 10^{-6}\ cm^2/s$

$D_{eff,\ glu} / D_{eff,\ oxy} = 0.0075$

Increase in the porosity of matrix resulted in *ca.* 59X increase in the effective diffusivity of oxygen, and only *ca.* 3X increase for glucose

S. Singh; M. McShane, *Biosens. Bioelctron.* ***2011,*** *26 (5), 2478-2483.*

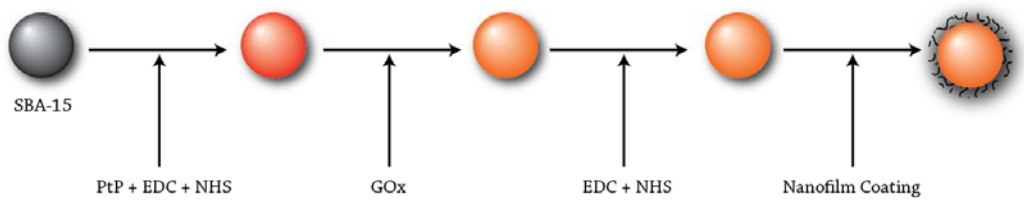
Effect of porosity on response range



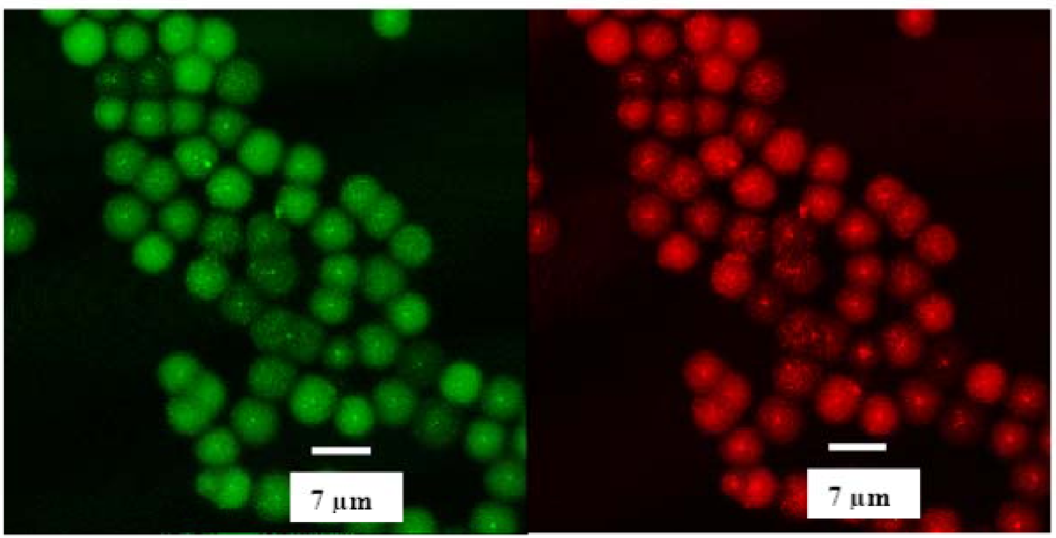
- α = pore volume/total volume
- α = 0.005 (Algilica); 0.38 m^2/g; 18.9 nm
- α = 0.6 (Zorbax™); 256 m^2/g; 8.8 nm
- Film thickness = 100 nm
- **Higher porosity should result in an increased response range and lower sensitivity**

S. Singh; M. McShane, *Biosens. Bioelctron.* ***2011,*** *26 (5), 2478-2483.*

SBA15-based Sensors



- Average particle size = ~7 µm
- Surface area = 256 m^2/g
- Porosity = 0.6
- Mean pore size = 8.8 nm
- GOx concentration = 1 mM



Confocal micrographs of Zorbax® microparticles loaded with GOx-RITC (left) and PtP (right).

S. Singh; M. McShane, *Biosens. Bioelctron.* ***2011,*** *26 (5), 2478-2483.*

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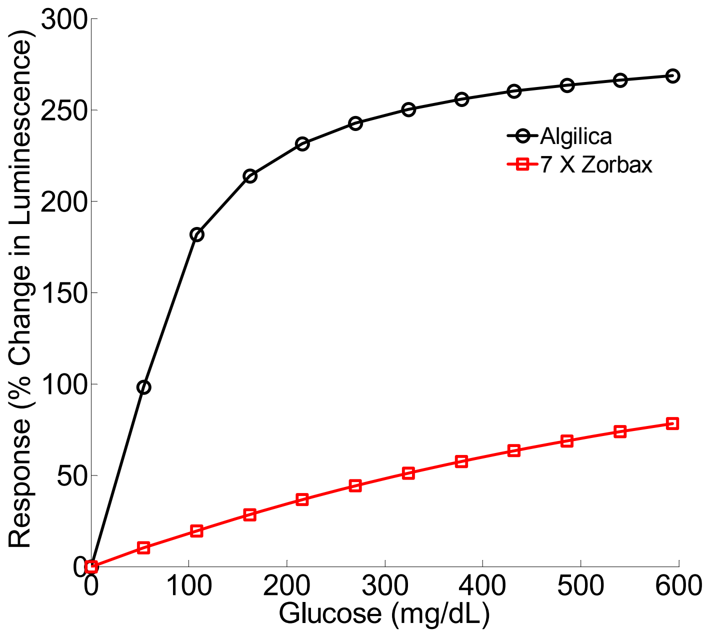
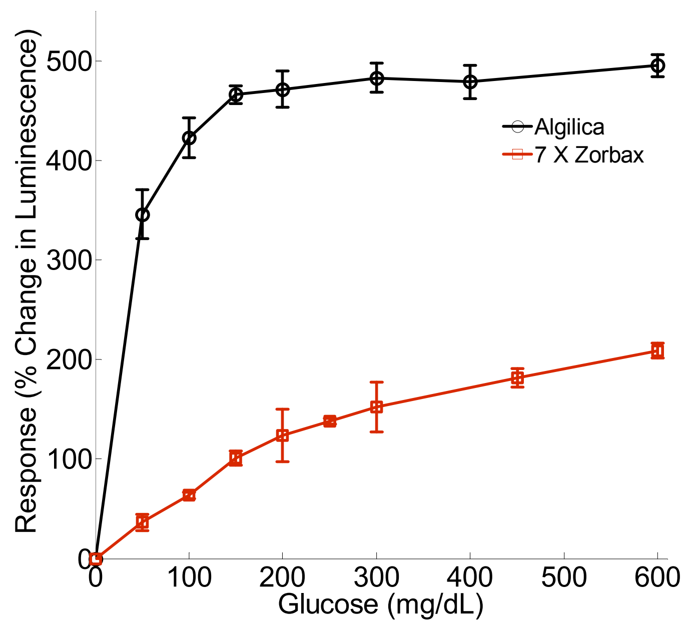
Effect of Matrix Porosity – Range Enhancement

Theoretical prediction



Experimental data



NOTE: Enzyme loading was determined using BCA protein assay

S. Singh; M. McShane, *Biosens. Bioelctron.* ***2011,*** *26 (5), 2478-2483.*

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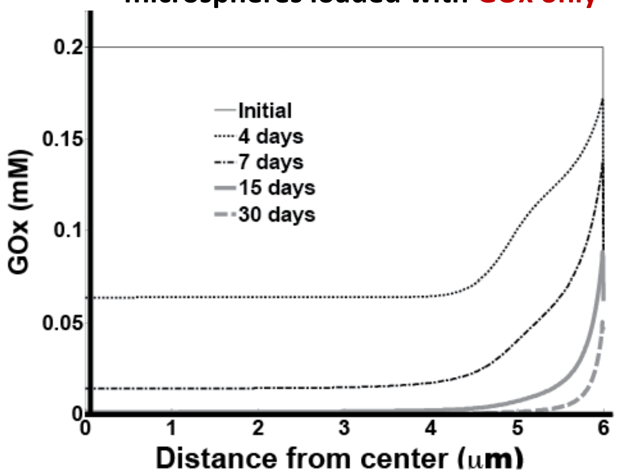
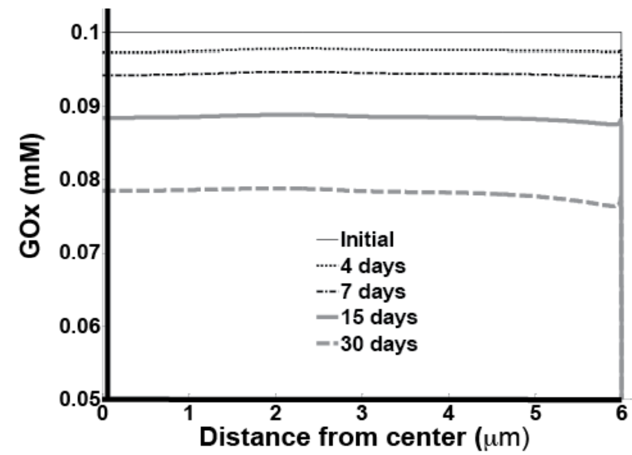
Incorporation of CAT – Longevity Enhancement

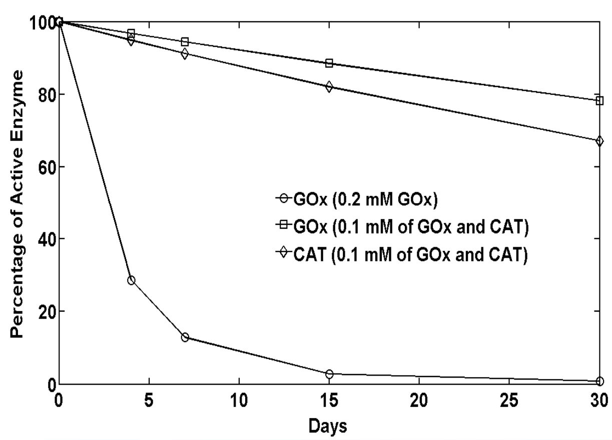
Modeling

Predicted active GOx concentration for microspheres loaded with GOx only



Predicted active GOx concentration for microspheres loaded with both GOx and CAT





Predicted volume average of the active enzyme concentration during constant operation

Reaction scheme

$$E_o + G \xrightarrow{k_1} X_1 \xrightarrow{k_2} E_r + P$$

$$E_r + O_2 \xrightarrow{k_3} X_2 \xrightarrow{k_4} E_o + H_2O_2$$

$$H_2O_2 + CAT \xrightarrow{k_5} I + H_2O$$

$$H_2O_2 + I \xrightarrow{k_6} H_2O + O_2$$

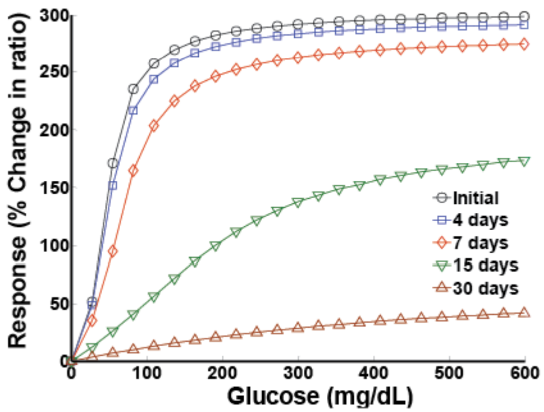
S. Singh; M. McShane, *Biosens. Bioelctron.* **2010,** *25* (5), 1075-1081.

Incorporation of CAT – Longevity Enhancement

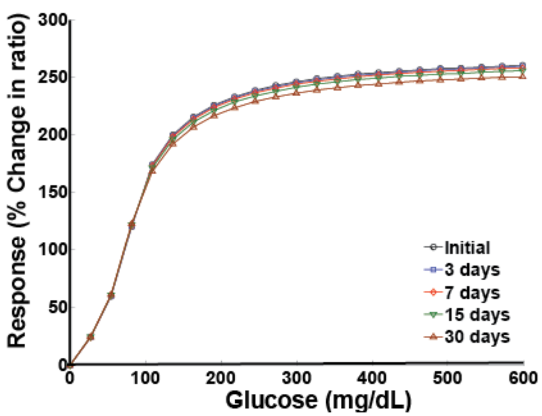
Model

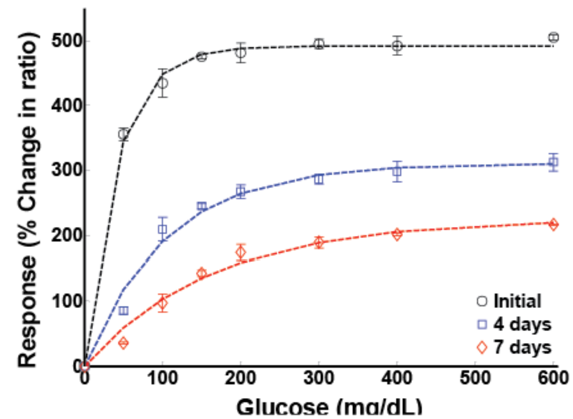
Experiment

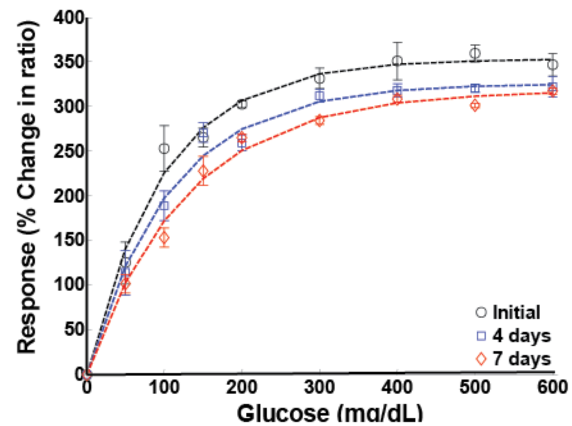
Fit type: $I = I_{max}(1\text{-}e^{-k[glucose]})$



$$A_{280} = 2.0068C_{CAT} + 1.8517C_{GOD}$$
$$A_{405} = 1.7408C_{CAT} + 0.1965C_{GOD}$$



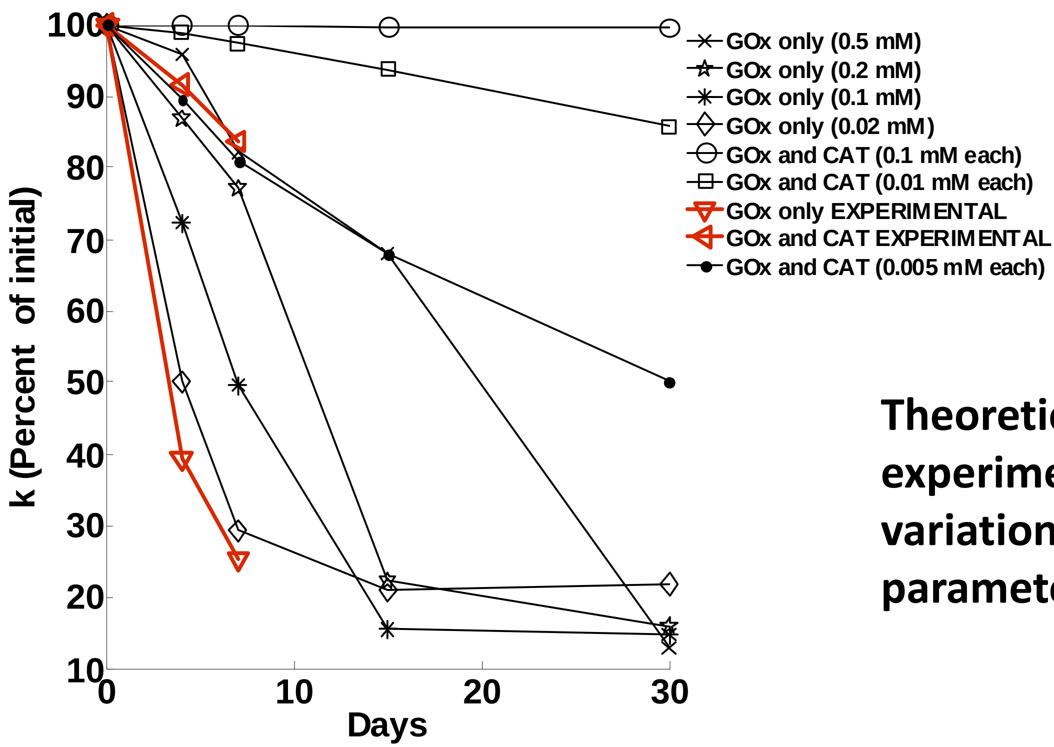




S. Singh; M. McShane, *Biosens. Bioelctron.* **2010,** *25* (5), 1075-1081.

Longevity Enhancement

Fit type: $I = I_{max}(1\text{-}e^{-k[glucose]})$



Theoretically predicted and experimentally determined variation in the curve fit parameter, *k*, with respect to time

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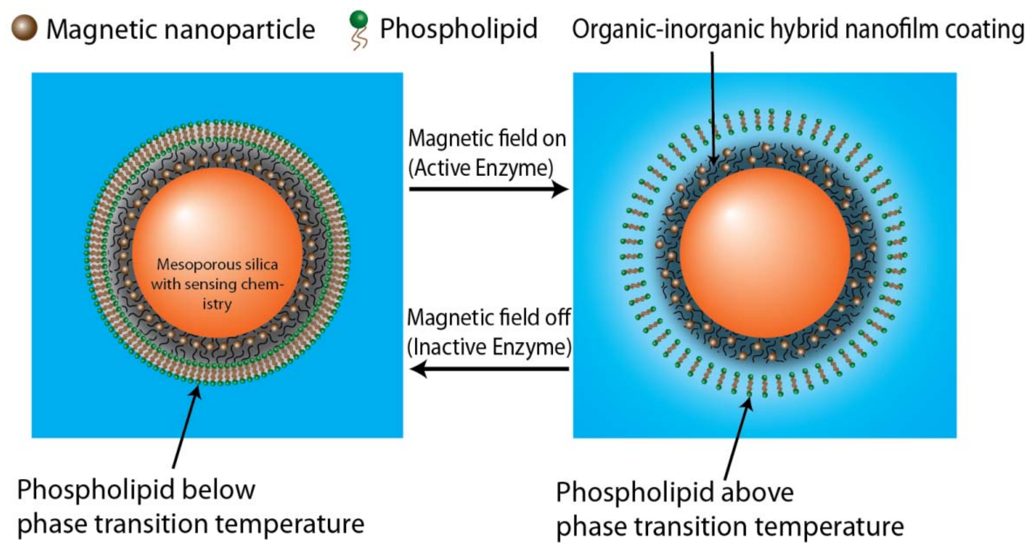
Summary

- Developed parameterized mathematical model.
- Response range can be tuned by varying the nanofilm thickness, composition, and the capping layer.
- Response range can be increased by increasing the porosity of the matrix.
- Longevity of sensors was enhanced by incorporating Catalase.

Smart biosensing systems for the future

1. Smart biosensing systems using organic-inorganic hybrid nanofilms, which will bring a paradigm shift in enzyme-based biosensing.

2. Microsphere-based sensors will be coated with nanocomposite films comprising of polymers and magnetic nanoparticles.



3. The resulting structure will be capped with a lipid bilayer.

4. Such sensors can be turned on and off via magnetic field, which will vastly increase the longevity of sensors.

5. If 12 measurements are taken every day, and each measurement takes less than 10 minutes, then such sensors will last for more than 1 year.

Key research objectives

- Accelerated testing to determine the long term stability of organic-inorganic hybrid nanofilms in physiological conditions

    - Fluorescence microscopy

    - QCM-D measurements

    - Low Q diffraction/Small Angle Neutron Scattering

- Thermal stability of GOx at different temperatures.

- Utilize multiphysics modeling tools (*e.g.* COMSOL) to predict enzyme deactivation

- Design optimization

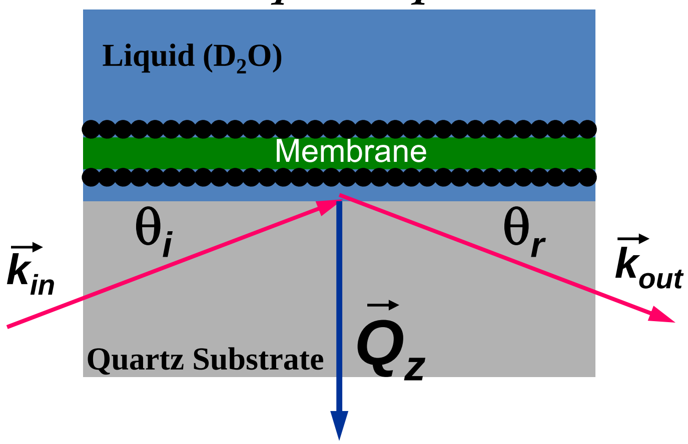
- Development of smart sensors

Funding?

- Nano-Biosensing program - National Science Foundation (NSF)
- Exploratory/Developmental Bioengineering Research Grants (R21) - National Institute of Health (NIH)
- Early Career Research Program – DOE Office of Science
- NSF-NIST interaction in basic and applied science research in Bio, and Eng – National Science Foundation (NSF)
- DOE Office of Science (DOE SCGF) and NSF Fellowships to support Graduate Students

Neutron Reflectometry?

$\theta_i = \theta_r$



$Q_z = k_{out} - k_{in} = 4\pi \sin\theta / \lambda$

$R = I_{out}/I_{in}$

Measures:

average density structure normal to the interface.

-thickness, density, roughness

$$R(Q) \approx 16 \frac{\Pi^2}{Q^4} \left| \int_{-\infty}^{\infty} \frac{d\beta}{dz} e^{-izQ} \, dz \right|^2$$

$n = 1 - \lambda^2 \beta / 2\pi$

β - scattering length density (SLD) of material

Advantages:

Sensitivity to light elements

Deuteration ⇒ Contrast

Buried interfaces (low absorption)

Non-destructive

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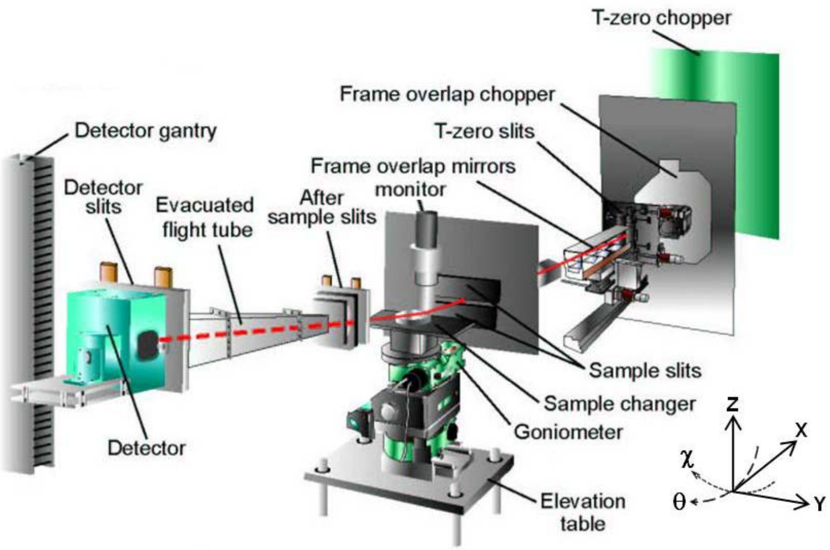
T-zero chopper
Frame overlap chopper
Detector gantry
T-zero slits
Frame overlap mirrors
Detector slits
Evacuated flight tube
After sample slits
monitor
Sample slits
Sample changer
Goniometer
Detector
Elevation table
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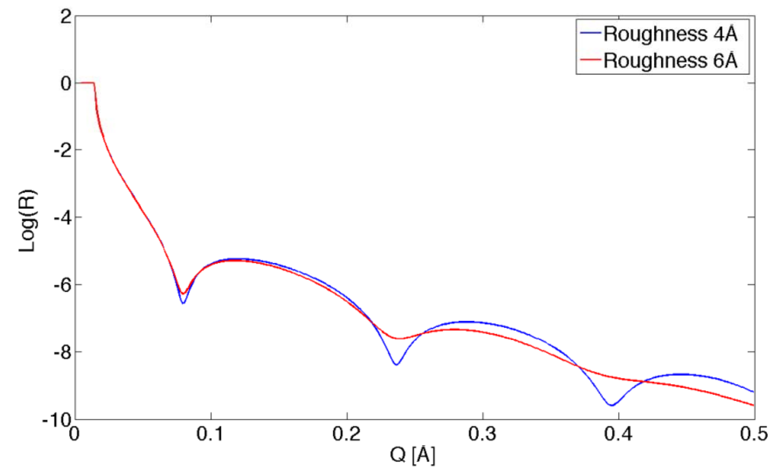
Roughness 4Å
Roughness 6Å
Log(R)
Q [Å]
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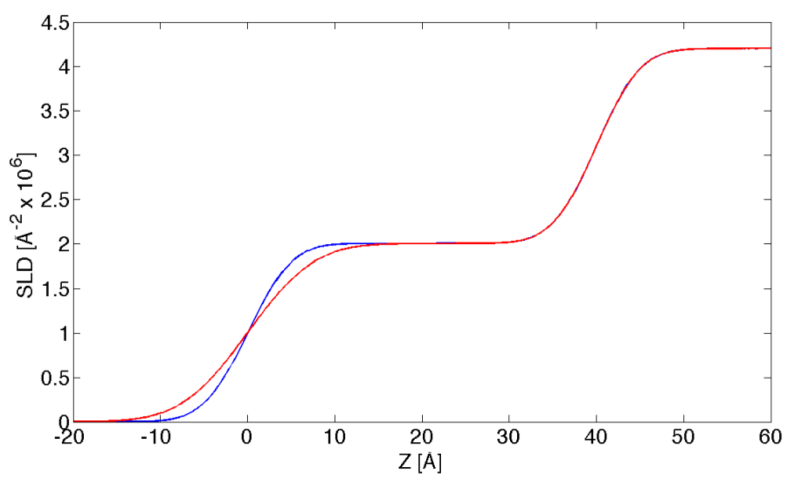
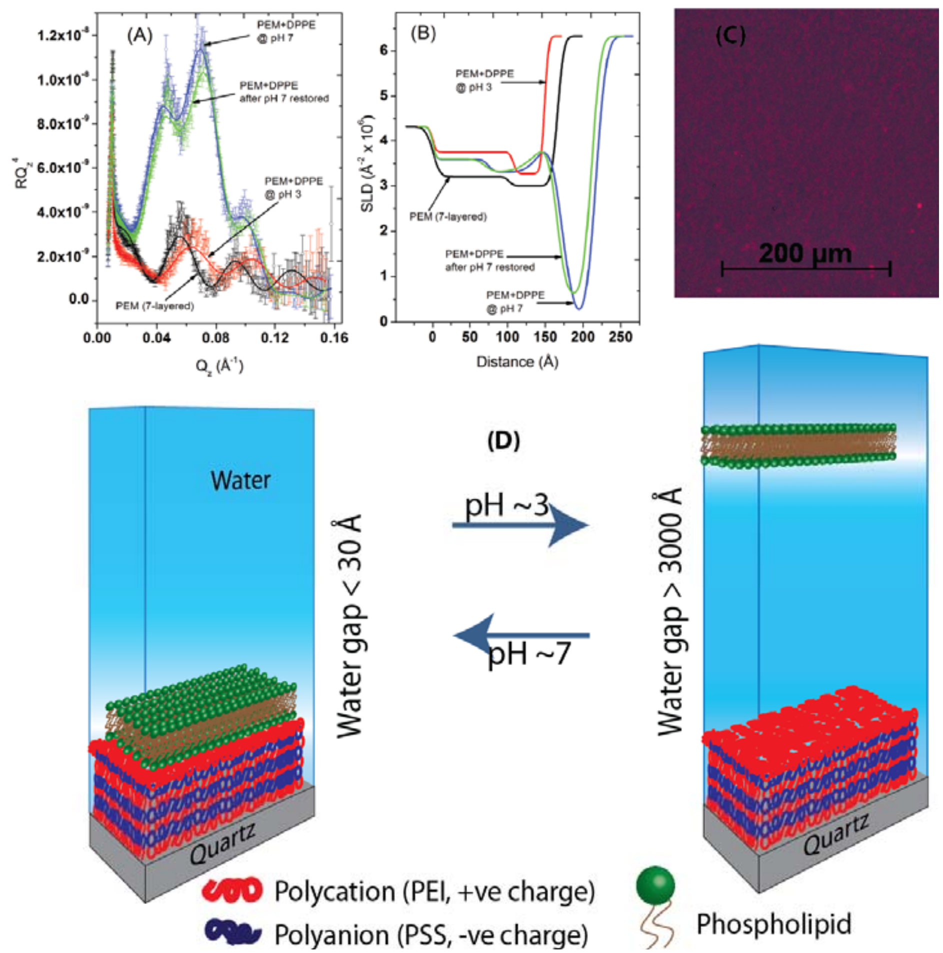
-4
-6
-8
-10
0
0.1
0.2
0.3
0.4
0.5


SLD [Å^{-2} x 10^{6}]
Z [Å]
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-20
-10
0
10
20
30
40
50
60

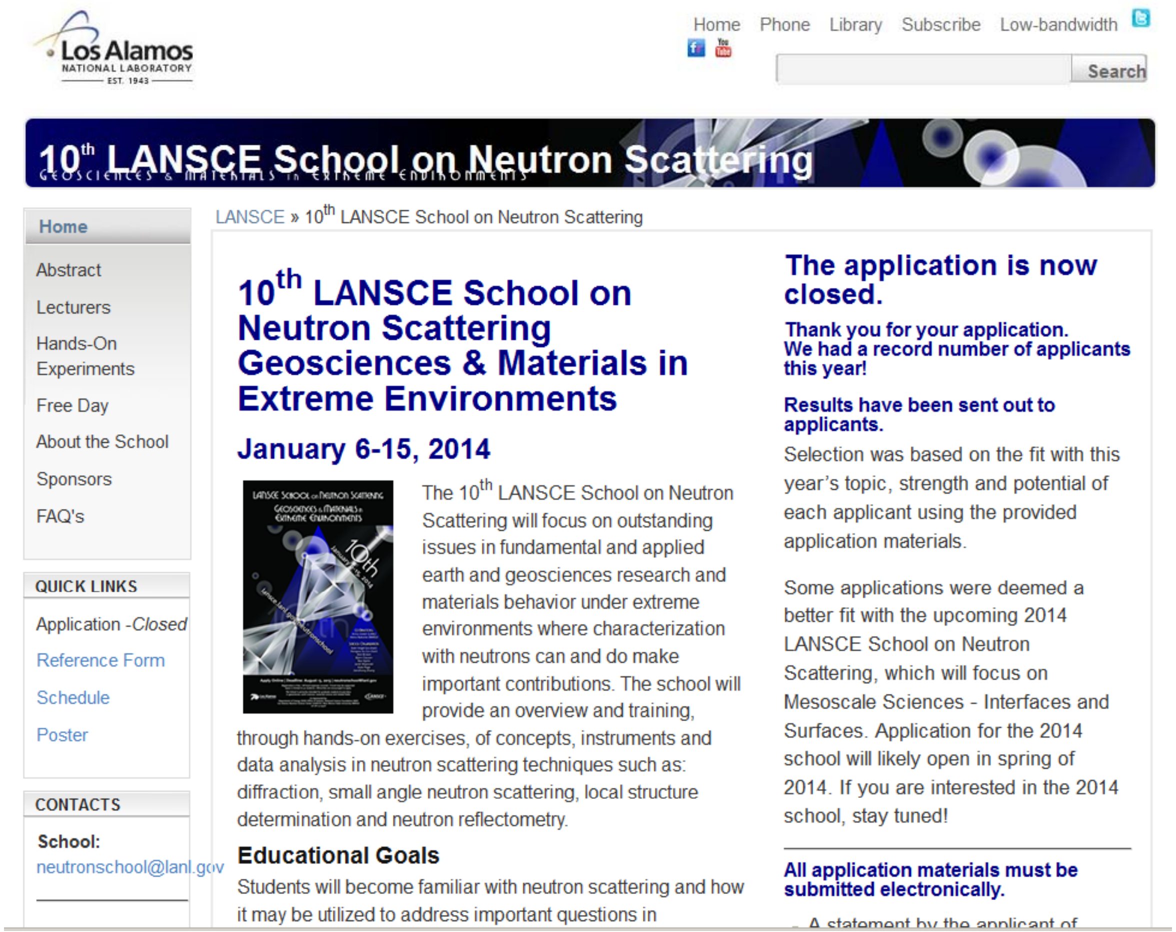
Postdoctoral work at LANL

- Structural characterization of layer-by-layer (LbL) deposited thin films using Neutron Reflectometry (NR).
- Development of stimuli responsive lipid bilayers.
- Lipid bilayers serve as a model for cellular membranes.
  - Fundamental biophysical studies
  - Applications: drug delivery, drug screening, biosensing, etc.
- Example of a pH-responsive lipid membrane is shown in the figure. NR and Electrical Impedance Spectroscopy were used to characterize this system.



S. Singh et al., Soft Matter **2013**, 9 (37), 8938-8948.

LANSCE Neutron School



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10th LANSCE School on Neutron Scattering

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CONTACTS

School:

neutronschool@lanl.gov

LANSCE » 10th LANSCE School on Neutron Scattering

10th LANSCE School on Neutron Scattering Geosciences & Materials in Extreme Environments

January 6-15, 2014

The 10th LANSCE School on Neutron Scattering will focus on outstanding issues in fundamental and applied earth and geosciences research and materials behavior under extreme environments where characterization with neutrons can and do make important contributions. The school will provide an overview and training, through hands-on exercises, of concepts, instruments and data analysis in neutron scattering techniques such as: diffraction, small angle neutron scattering, local structure determination and neutron reflectometry.

Educational Goals

Students will become familiar with neutron scattering and how it may be utilized to address important questions in

The application is now closed.

Thank you for your application. We had a record number of applicants this year!

Results have been sent out to applicants.

Selection was based on the fit with this year's topic, strength and potential of each applicant using the provided application materials.

Some applications were deemed a better fit with the upcoming 2014 LANSCE School on Neutron Scattering, which will focus on Mesoscale Sciences - Interfaces and Surfaces. Application for the 2014 school will likely open in spring of 2014. If you are interested in the 2014 school, stay tuned!

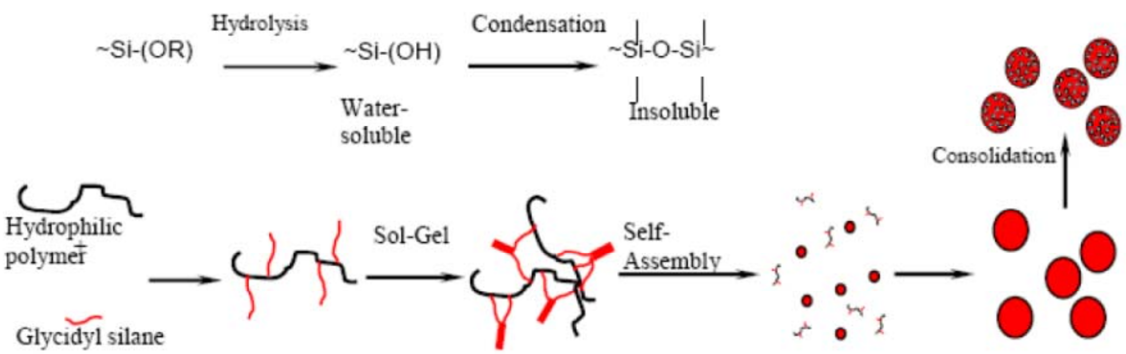
All application materials must be submitted electronically.

http://lansce.lanl.gov/neutronschool/index.shtml

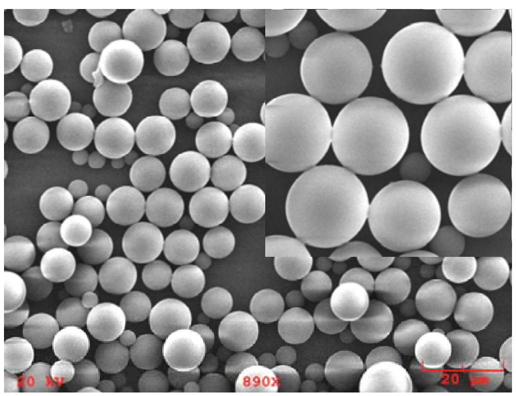
THANK YOU

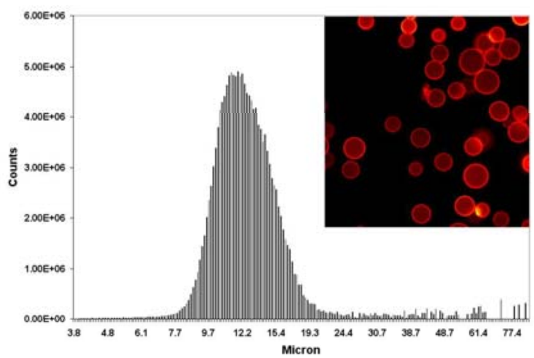
ENGINEERING TEXAS A&M UNIVERSITY · LUJAN CENTER · Los Alamos NATIONAL LABORATORY · U.S. DEPARTMENT OF ENERGY Office of Science

Synthesis of "Algilica" Microparticles



Scheme for particle fabrication, where chemical properties on the top line correspond to structures on the bottom line.





(left) SEM image of algilica particles, (right) size distribution of typical algilica particles.

Surface area (0.38 m^2/g), porosity (0.005), and pore size (18.9 nm) were determined using BET method.

Stein, E. W.; Grant, P. S.; Zhu, H.; McShane, M. J. *Analytical Chemistry* **2007**, *79*, 1339-1348.