Bioelectronics: Biological Charge Transfer Research at MSU

Renewable Fuels for the Future (RF^2)
January 19, 2012

Overview of presentation

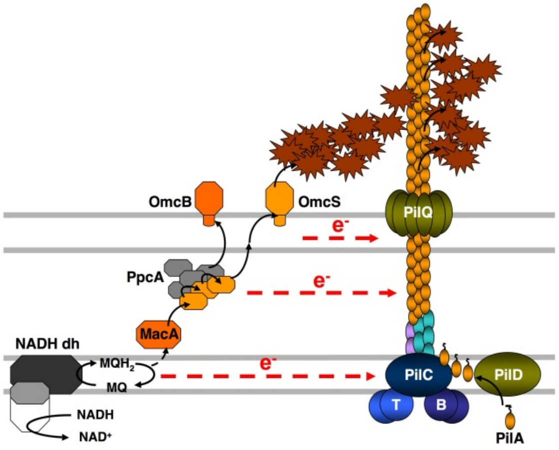
- MSU bioelectronics participants and expertise
- Example bioelectronics research efforts
    - *Microbial Redox Mechanisms*
    - *Nanostructured Biomimetic Interfaces*
    - *Biofuel Cells*
    - *Charge Transfer Across Biomembranes*
    - *Gas-Intensive Electrofuel Fermentations*
- Summary

MSU participants and expertise areas

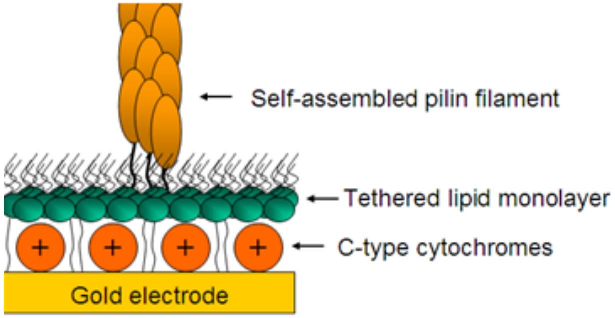
Faculty Member	Department	Focus Area(s)
Scott Calabrese Barton	Chemical Eng. & Mat. Sci.	Multiscale transport, electrochemistry
Gary Blanchard	Chemistry	Catalysis, biointerfaces with lipid bilayers
R. Michael Garavito	Biochem. & Molec. Biol.	Protein structure and function
Phil Duxbury	Physics and Astronomy	Condensed matter theory
Norbert Kaminski	Medicine	Molecular mechanisms of nanotoxicity
Andrew Mason	Electrical and Computer Eng.	Bioelectronic microsystems, microfluidics
Stuart Tessmer	Physics and Astronomy	Scanning tunneling microscopy; protein nanowires
Robert Ofoli	Chemical Eng. & Mat. Sci.	Biomimetic interfaces, catalysis
Gemma Reguera	Microbiol. & Molec. Genetics	Protein nanowires; microbial fuel cells
James Tiedje	Microbiol. & Molec. Genetics	Microbial ecology; bioremediation mechanisms
Jon Sticklen	Computer Science and Eng.	STEM education research
Claire Vieille	Microbiol. & Molec. Genetics	Redox enzymes; microbial fuel cells
Mark Worden	Chemical Eng. & Mat. Sci.	Multiscale transport, bioelectronics and biocatalysis
Tim Whitehead	Chemical Eng. & Mat. Sci.	In-silico molecular design; synthetic biology

Examples of bioelectronic systems

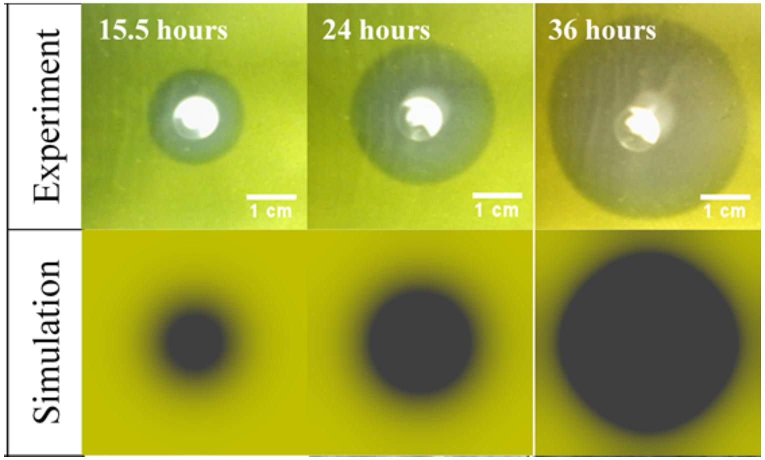
- ***Microbial Redox Mechanisms*** (Reguera, Tiedje, Tessmer, Worden, Duxbury)
- Funding: DOE, NIEHS, ED (GAANN)



Electron transfer by conductive pilin (protein nanowire)



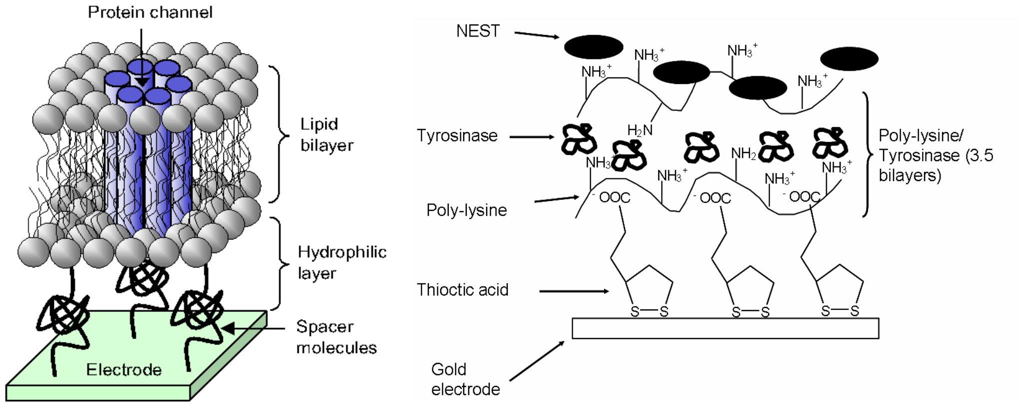
Biomimetic interface with protein nanowires produced in lab



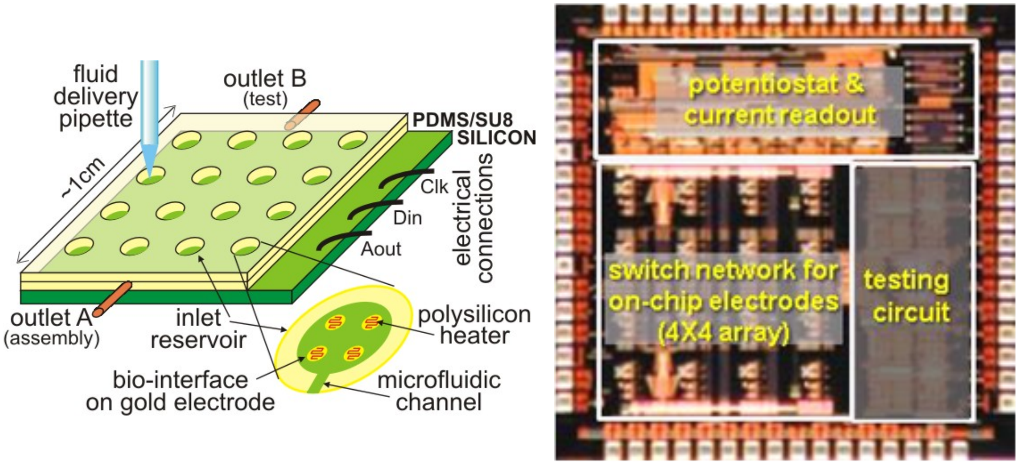
Coupled microbial transport and metal reduction

Examples of bioelectronic systems

- ***Nanostructured Biomimetic Interfaces*** (Worden, Calabrese Barton, Vieille, Garavito, Whitehead, Duxbury, Ofoli, Blanchard)
- Funding: NSF, USDA, ED (GAANN)



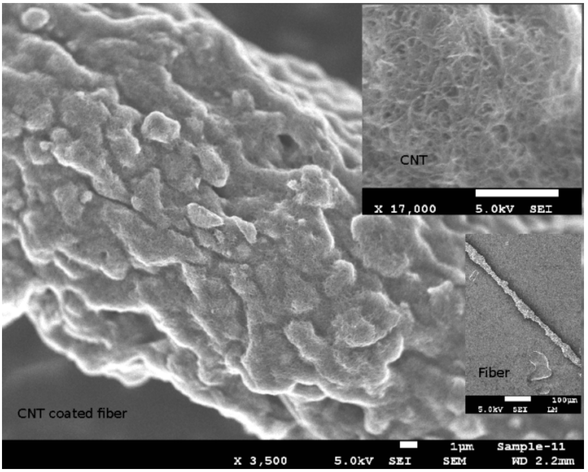
Biomimetic interfaces for ion channel protein (left) and multiple-enzyme pathway (right)



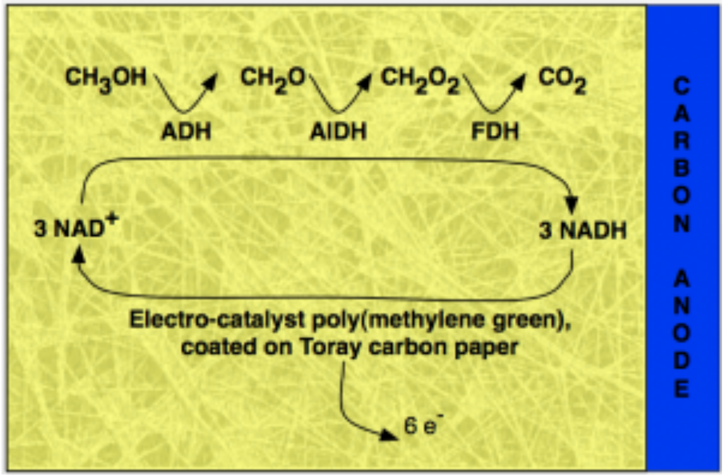
Bioelectronic MEMS devices with microfluidics and microelectronics

Examples of bioelectronic systems

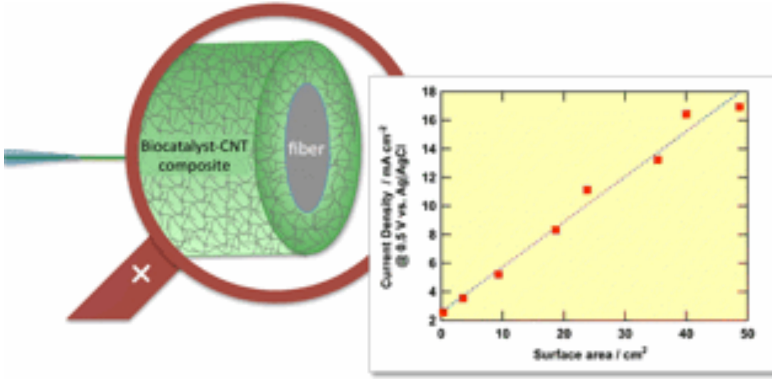
- ***Biofuel cells*** (Calabrese-Barton, Reguera, Vieille, Duxbury)
- Funding: NSF, Air Force, ED (GAANN)



Carbon nanotubes grown on carbon fiber



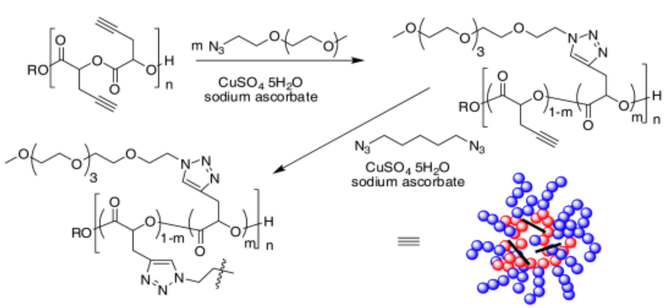
Multistep enzymatic pathway on electrode



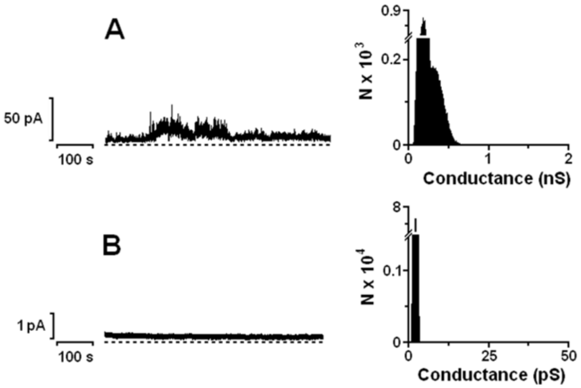
Nanotubes increase area and overall reaction rate

Examples of bioelectronic systems

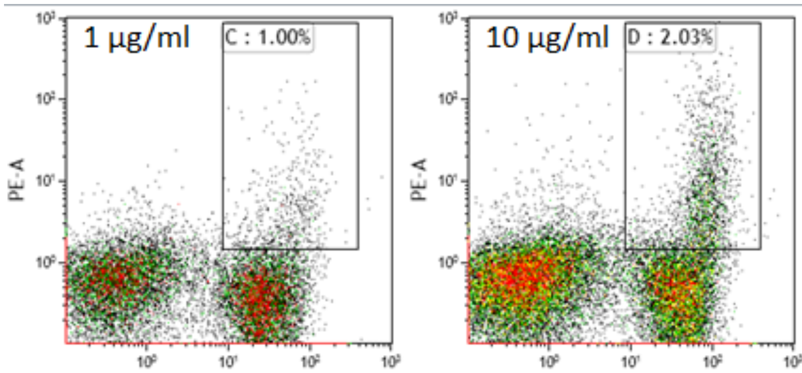
- ***Charge Transfer across Biomembranes*** (Worden, Mason, Baker, Kaminski, Duxbury)
- Funding: NIEHS



Custom nanoparticle design and synthesis



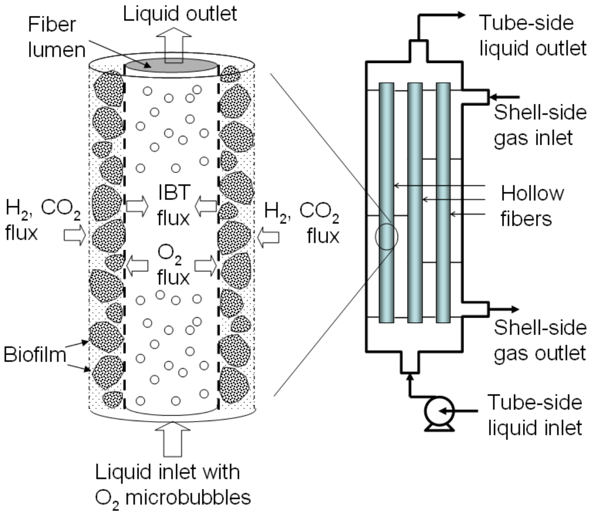
Nanoparticle-induced pores in cell membranes



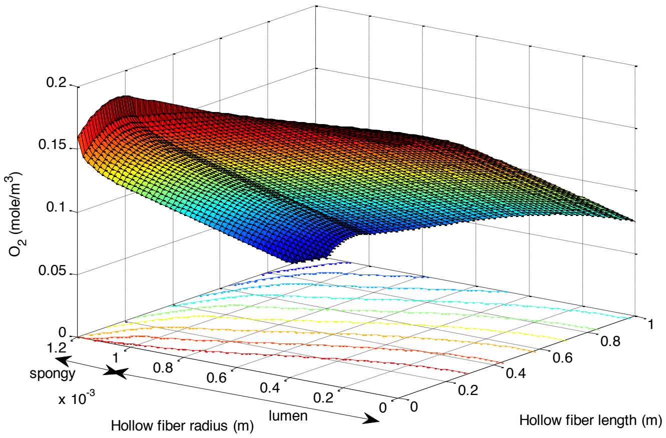
Toxicity of nanoparticles in cells and animals

Examples of bioelectronic systems

- ***Gas-Intensive Electrofuel Fermentations*** (Worden, Vieille, Reguera, Michigan Biotechnology Institute (MBI))
- Funding: DOE (Electrofuels)



Novel *Bioreactor for Incompatible Gases*

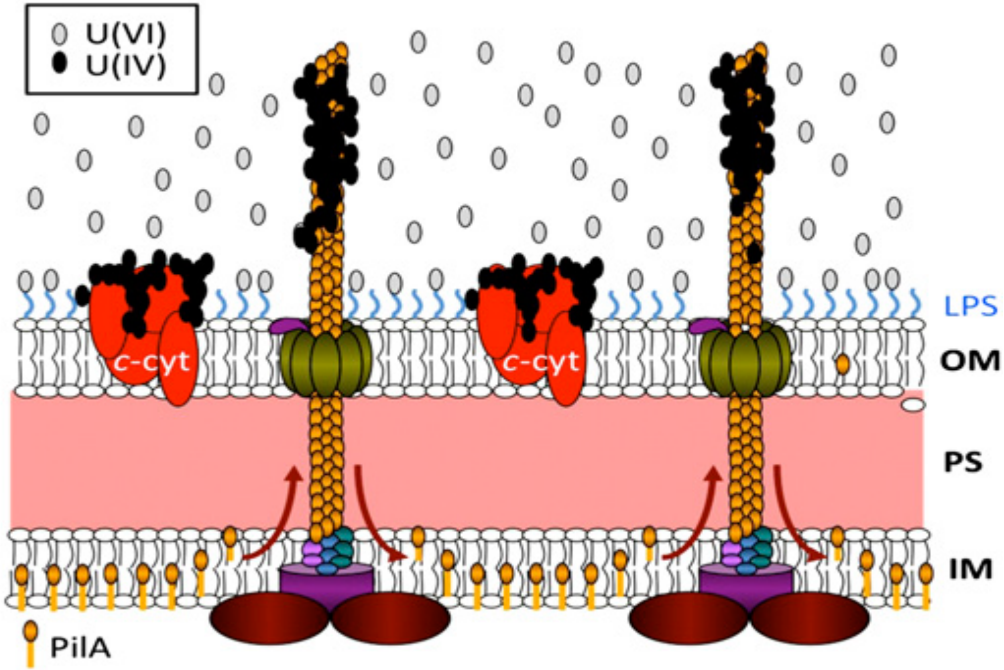


Design challenges for new bioreactor

Partnership with MBI for fermentation scaleup

Microbial redox mechanisms

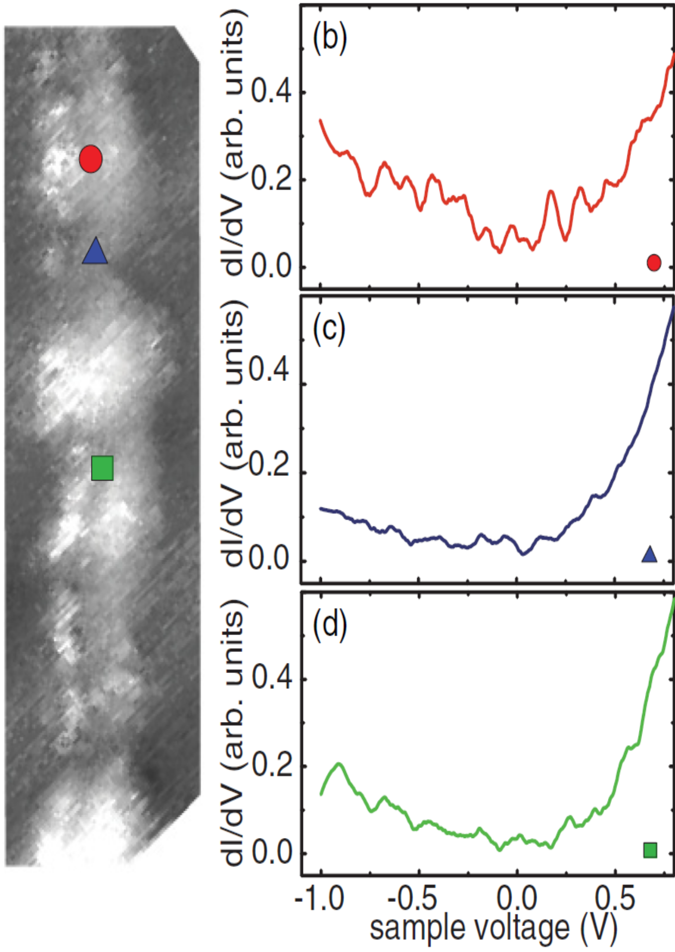
- Metal reduction by *Geobacter* PpcA
  - Elucidating role of conductive pili, cytochromes
    - Conductive pili primary mechanism for U reduction
    - Surface-bound c-cytochromes play supportive role
    - Extracellular U precipitation prevents cytotoxicity



Proc. Nat. Acad. Sci., **108**, 15248-15252 (2011)

Microbial redox mechanisms

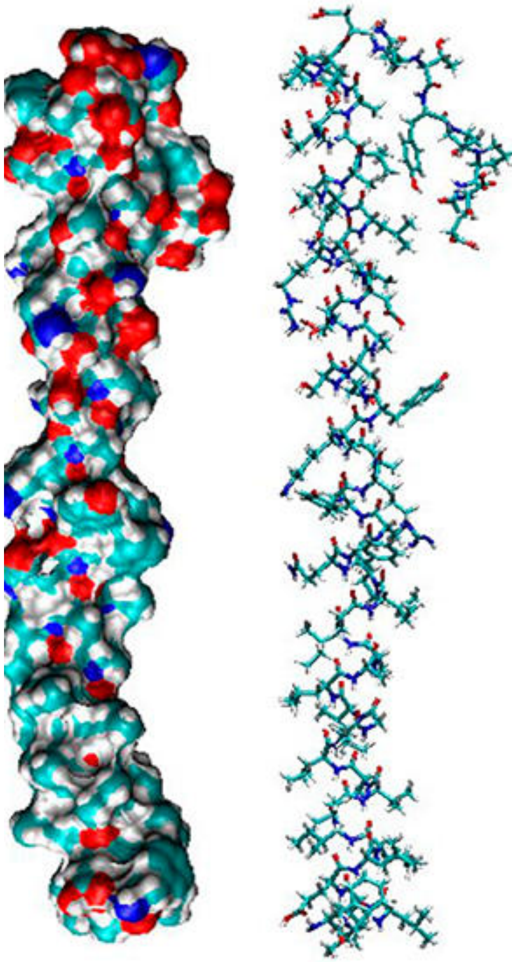
- Scanning tunneling microscopy of pilus
  - Periodic substructures along length of pilus
  - Electronic substructures
  - Electronic states
    - Vary with position in pilus
    - Some near the Fermi level
    - Consistent with conductivity
    - Not consistent with cytochromes



Phys. Rev. E, **84**, 060901 (2011)

Microbial redox mechanisms

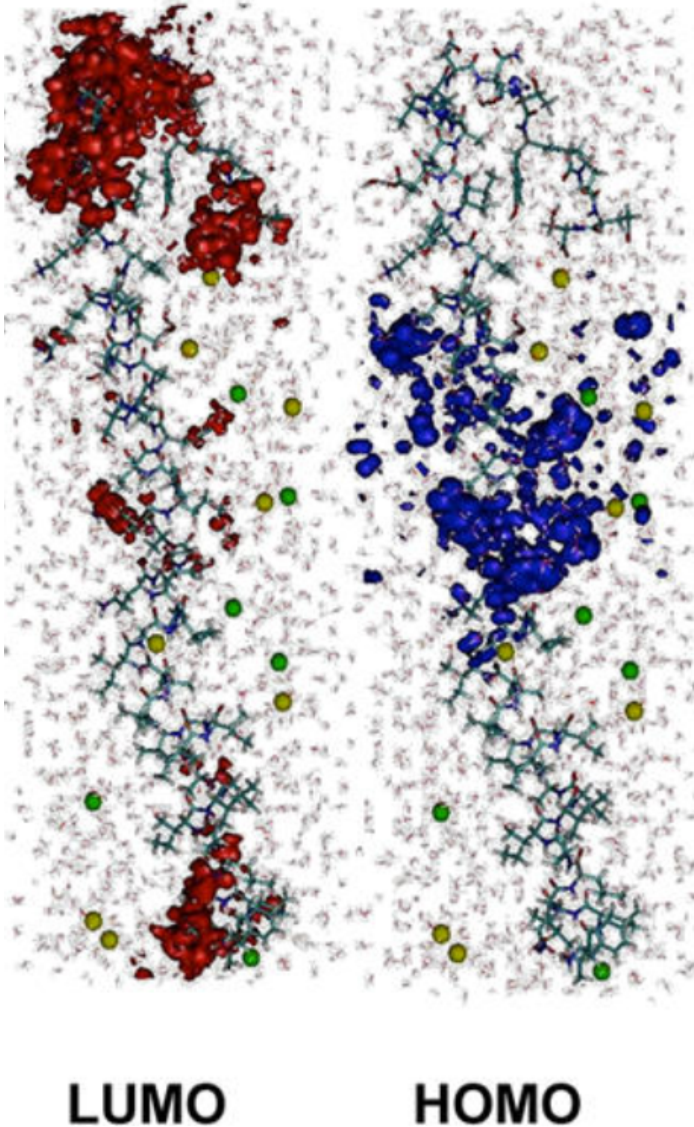
- Molecular dynamics model of pilin
  - Quantum-mechanical, first principles model
  - Density-functional theory
- Model predictions
  - N-terminus: conserved α helix
  - C-terminus: nonconserved region
  - Low HOMO-LUMO gap (band gap)
  - Orbital delocalization (aromatic AA)
  - Consistent with conductive pilin



J. Phys. Chem. A 2012, 116, 8023−8030

Microbial redox mechanisms

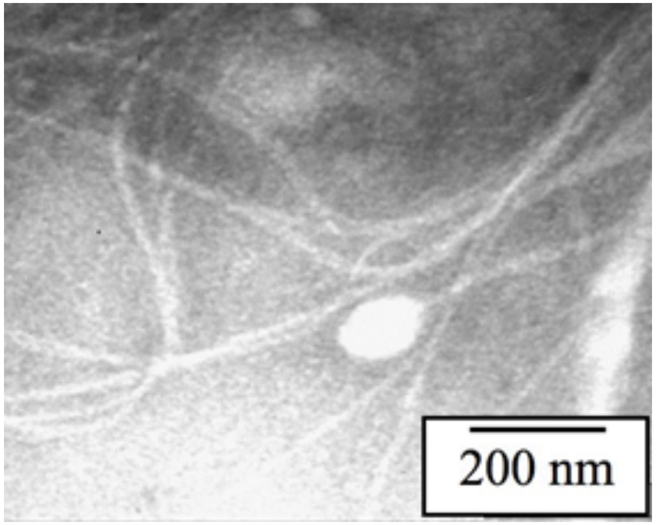
- Predicted density of states in pilin
  - Red: positive amino acids
  - Blue: negative amino acids
  - Orange: aromatic amino acids
- Biphasic charge distribution
  - LUMO: C and N terminals
  - HOMO: middle region
- Highest density of states
  - Nonconserved C terminus



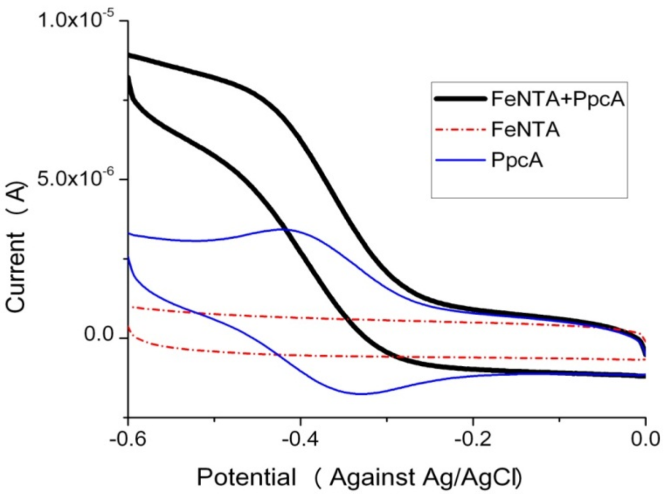
J. Phys. Chem. A 2012, 116, 8023−8030

Nanostructured biomimetic interfaces

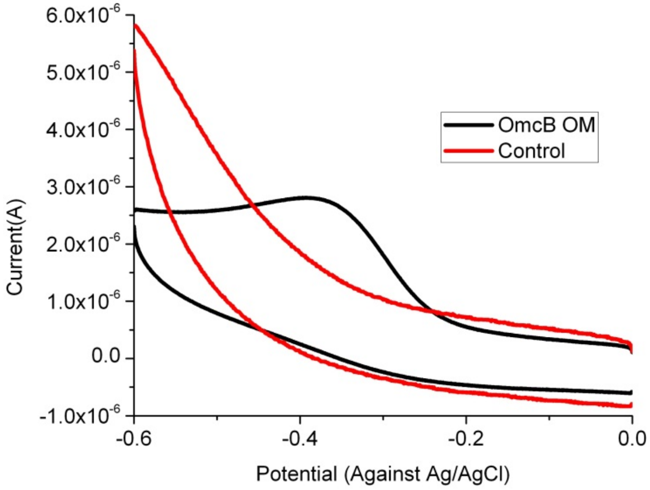
- Cloning, expression of *Geobacter* proteins
  - Pilin protein PpcA
  - Periplasmic cytochrome PpcA
  - Outer membrane cytochrome OmcB



Recombinant PilA self-assembled into pili



PpcA redox activity

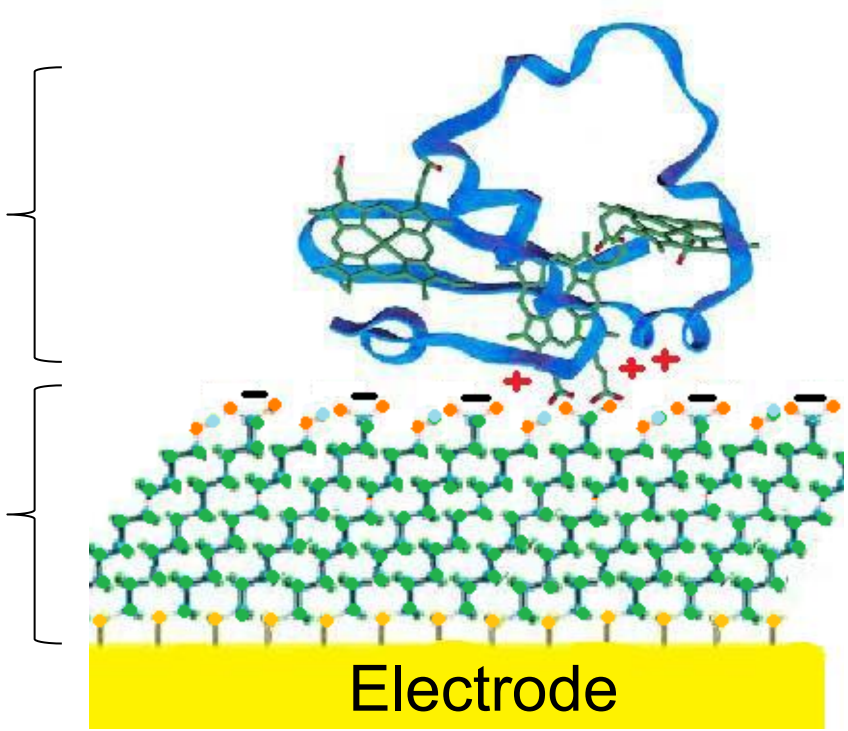


OmcB redox activity

US Patent Application: “Methods for the reductive precipitation of soluble metals and biofilms and devices related thereto”, filed 2012.

Nanostructured biomimetic interfaces

- Recombinant PpcA expressed and purified
- PpcA assembled into biomimetic interface
  - Mimics e^- transfer across *Geobacter* periplasm

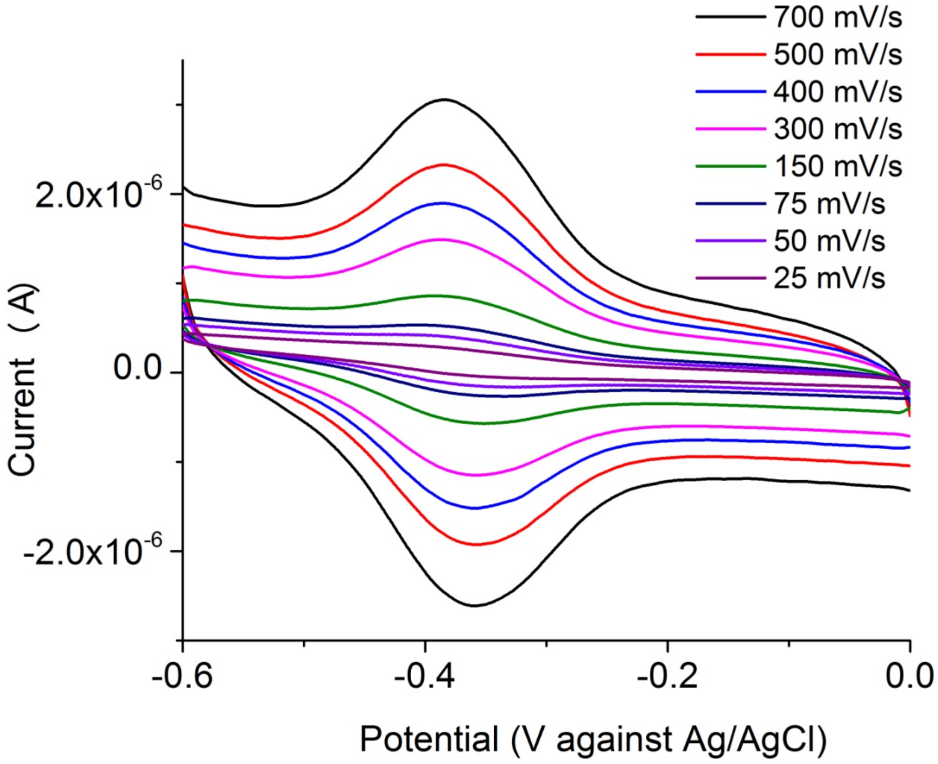


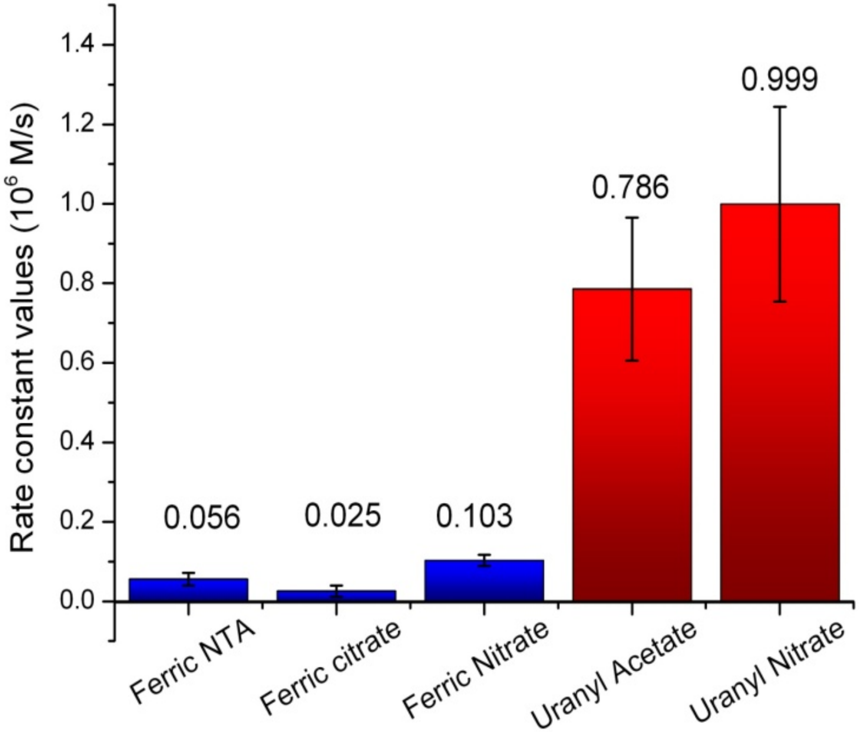
Recombinant *Geobacter* PpcA

Alkanethiol self-assembled monolayer (SAM)

Nanostructured biomimetic interfaces

- Determine rate constant for metal reduction
  - Cyclic voltammetry in presence of metal salt
  - Nicholson and Shain graphical analysis used
  - PpcA reduces uranium faster than iron



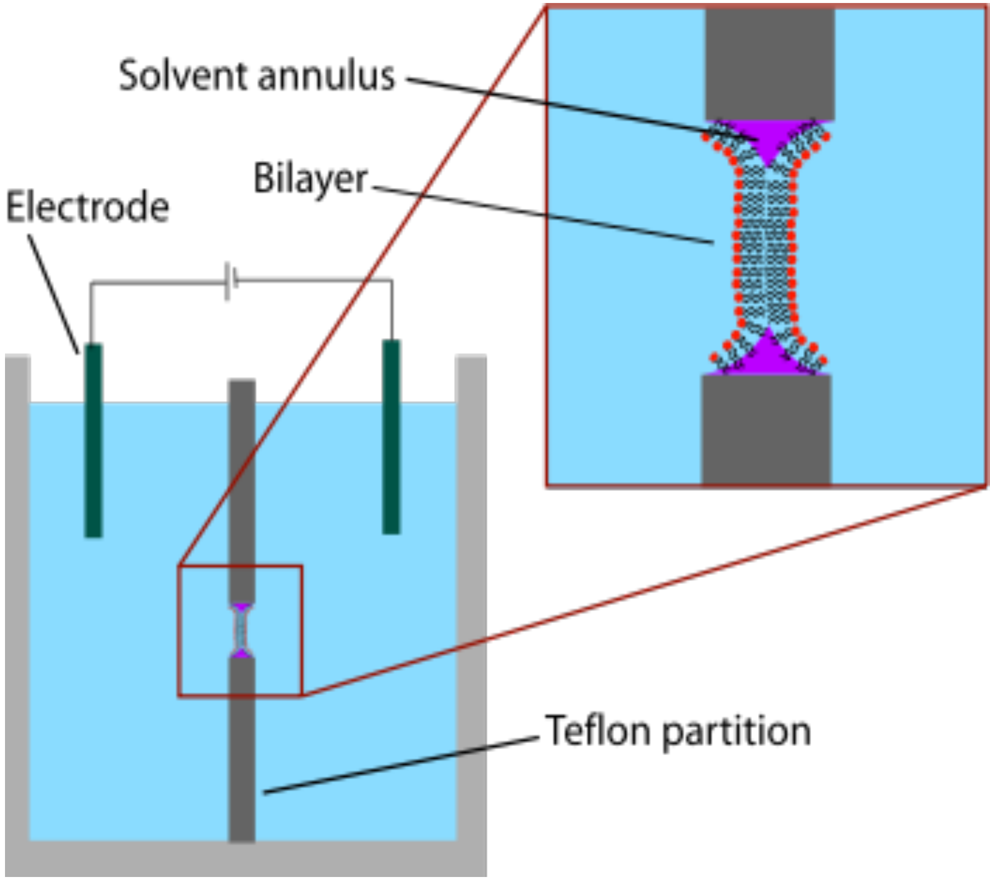


Possible redox protein projects for RF^2

- Clarify pilin conductivity mechanism
- Characterize electrical properties of pili
- Customize pili properties for applications
- Integrate pili into biomimetic interfaces
  - Protein/inorganic nanocomposites
  - Protein nanowire brushes
  - High surface area electrodes, catalysts,
- Mass produce, assemble recombinant pili
- Control self-assembly of recombinant pili
- Determine toxicity of pili as protein nanowires

Charge transfer across biomembranes

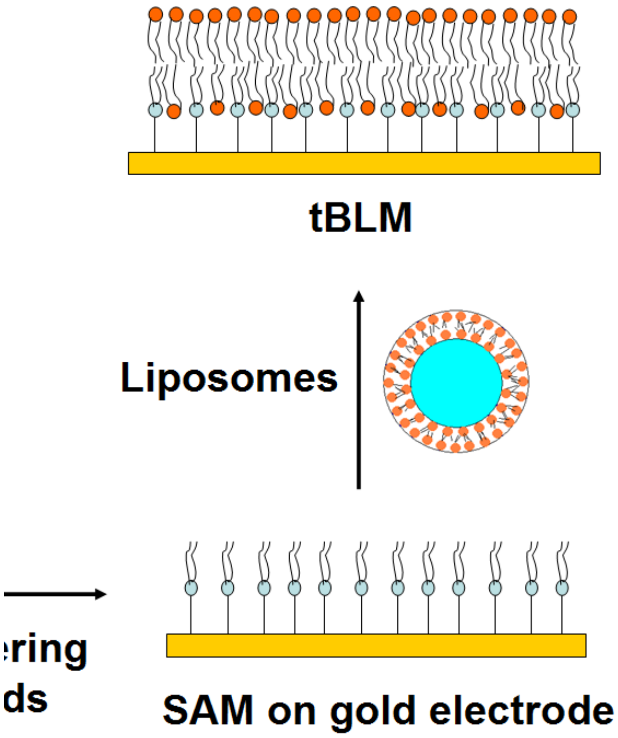
- Planar (black) bilayer lipid membrane (pBLM)
  - Well established electrophysiology methods
    - Rapid dynamics
    - High sensitivity
  - Mimics cell membrane
  - Fragile

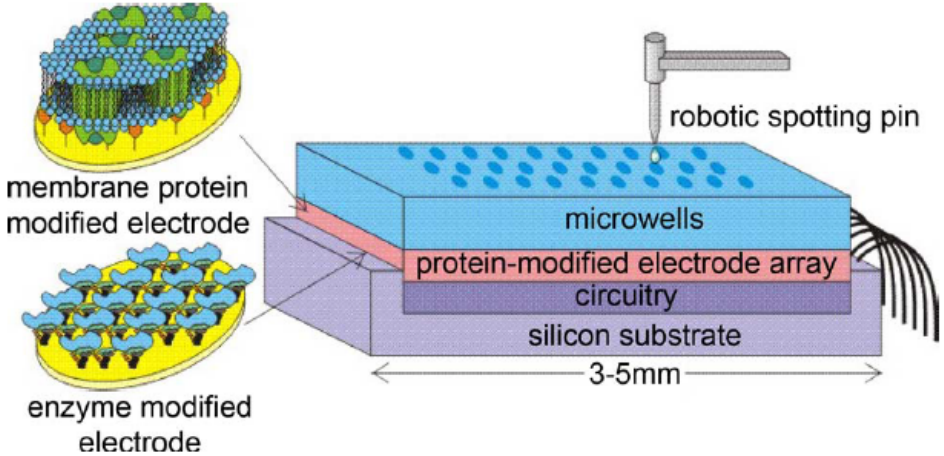


Journal of Colloid and Interface Science 390 (2013) 211–216

Charge transfer across biomembranes

- Tethered bilayer lipid membrane (tBLM)
  - More robust than planar BLM
  - Can be tethered to multiple surfaces
  - Can be self-assembled, miniaturized
  - Lower dynamic range, sensitivity
  - Adaptable to MEMS systems



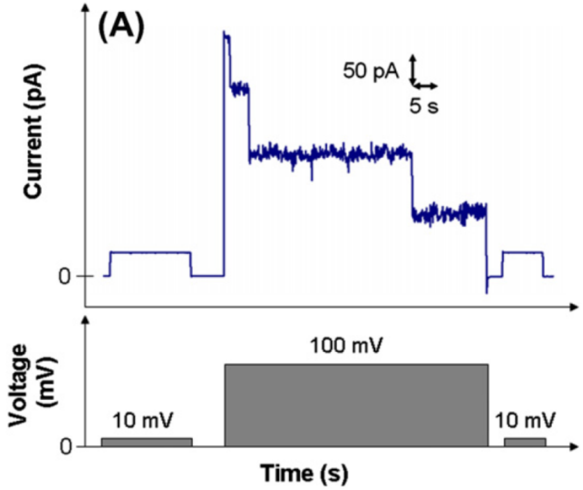


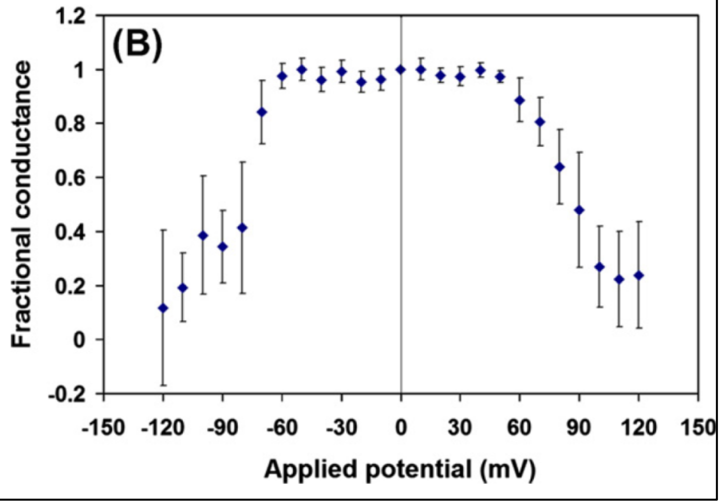
IEEE Trans. Biomed. Circuits Systems 2013, (in press) DOI, 10.1109/tbcas.2012.2195661

Charge transfer across biomembranes

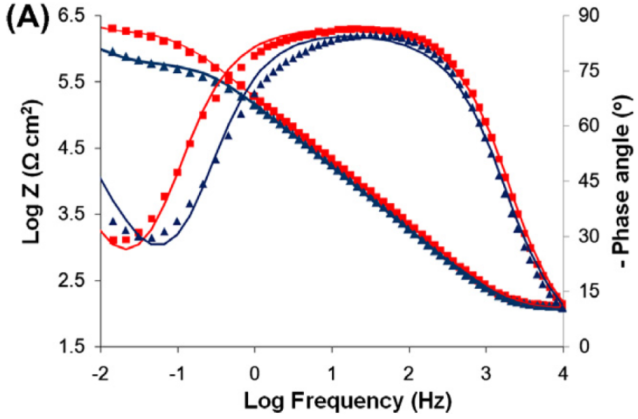
- Protein-mediated charge transfer across BLM
  - Voltage gating in PorB (Neisseria meningitidis)

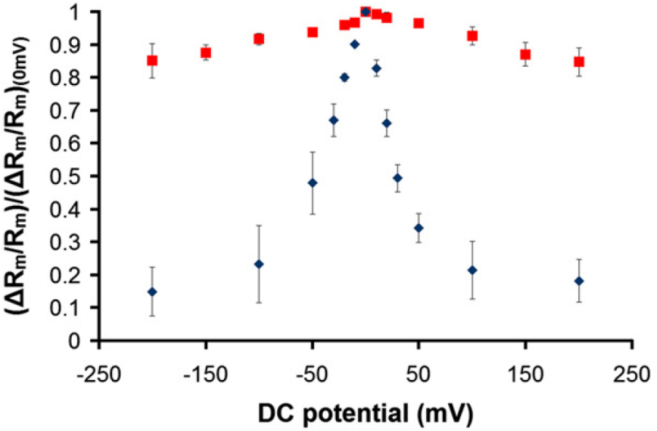
Planar
BLM





Tethered
BLM





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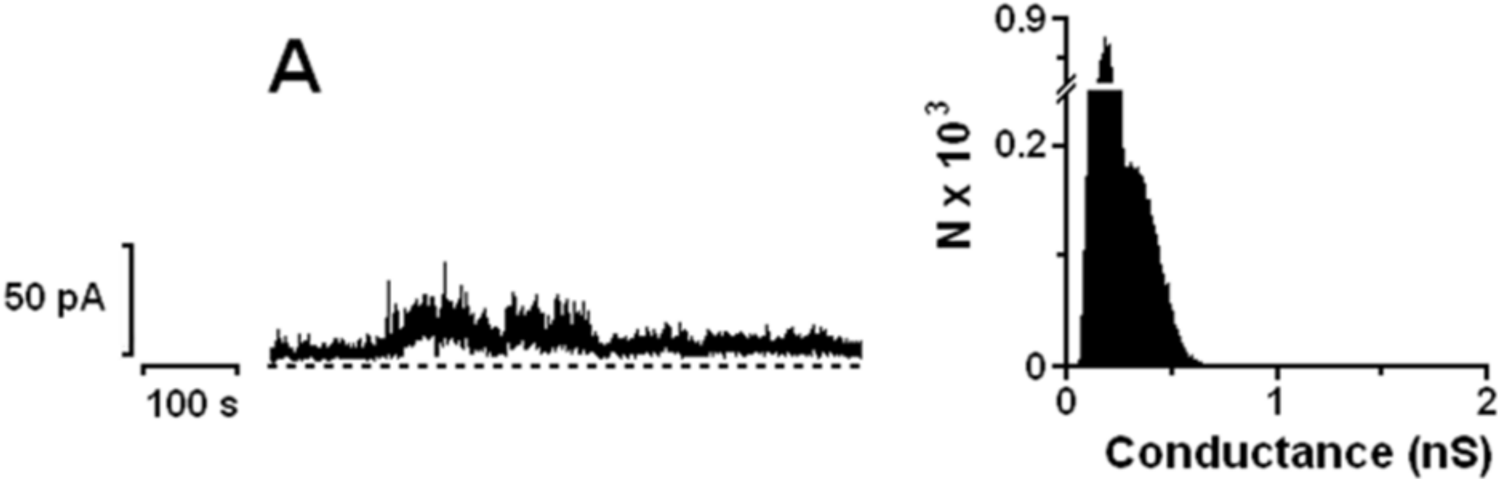
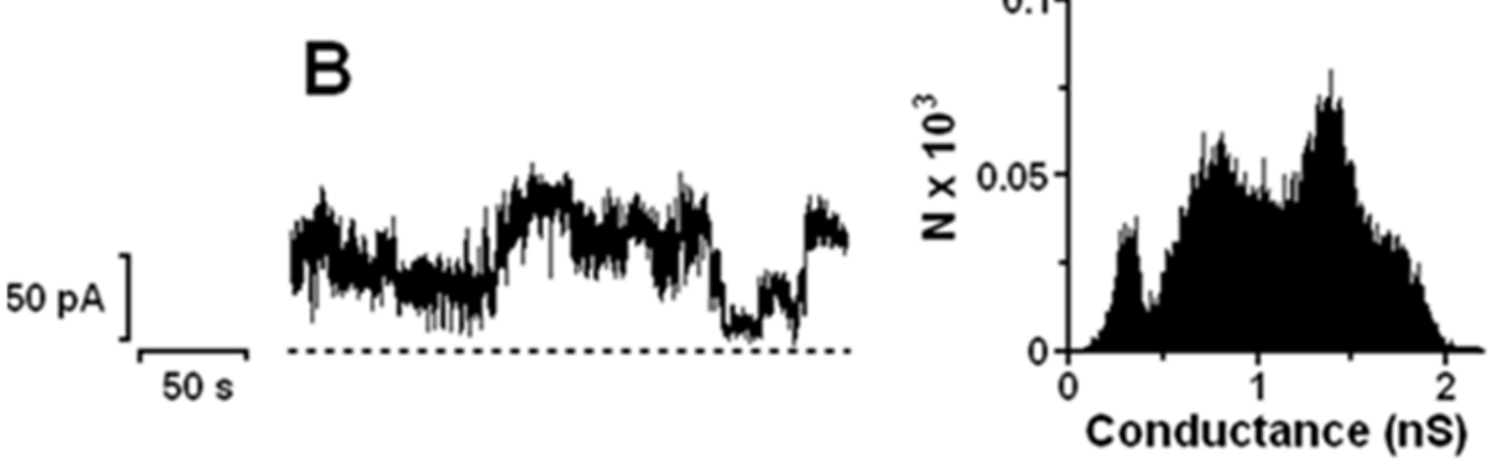
Charge transfer across biomembranes

- Nanoparticle-mediated charge transfer
  - Planar BLM currents induced by nanoparticles

0.6 µg/mL
quantum dots



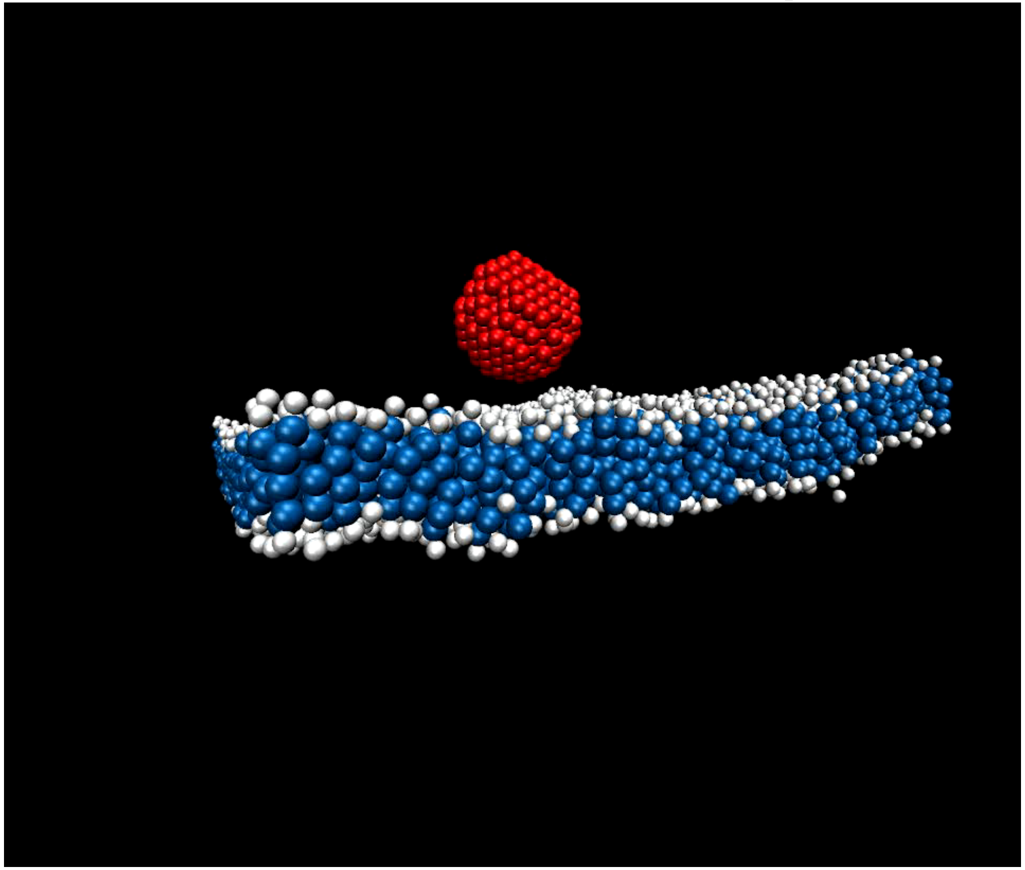
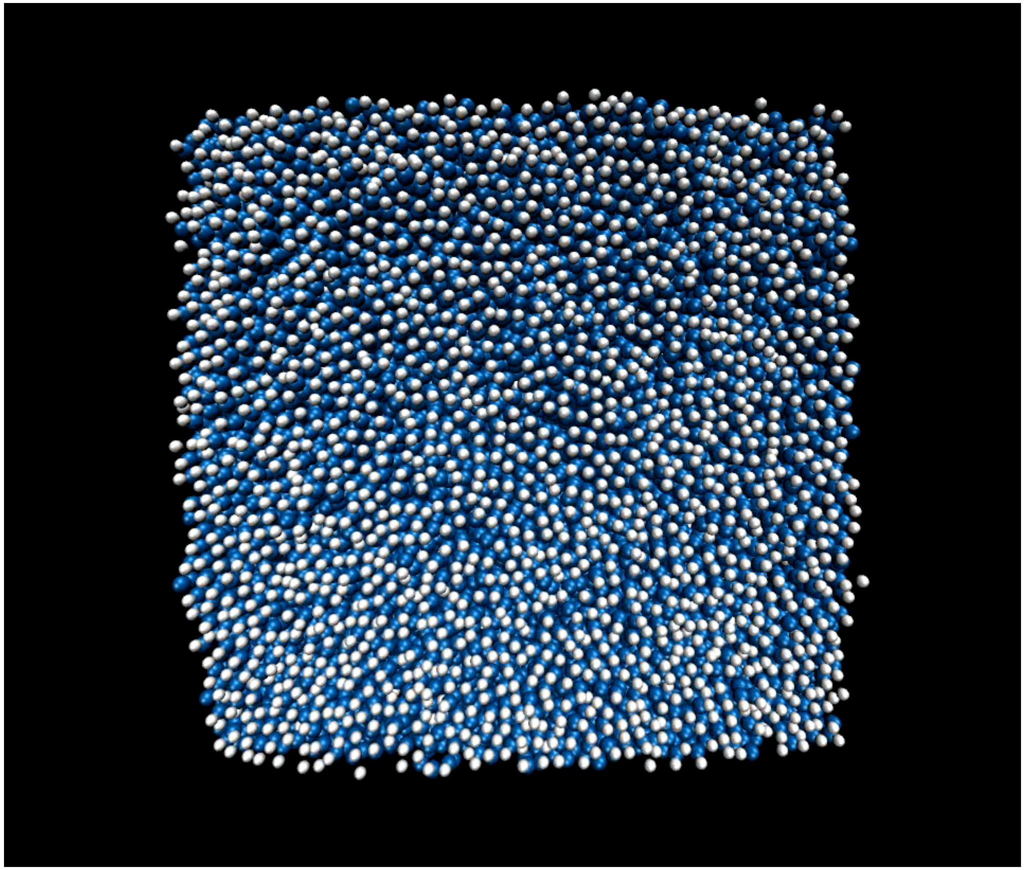
10 µg/mL carbon
nanotubes



Int. J. Biomed. Nanosci. Nanotechnol, 2013, (in press)

Charge transfer across biomembranes

- ***Molecular simulation: ENM pore formation***
  - Different modes of particle-bilayer interaction
  - Pore formation predicted

Int. J. Biomed. Nanosci. Nanotechnol, 2013, (in press)

Possible biomembrane projects for RF^2

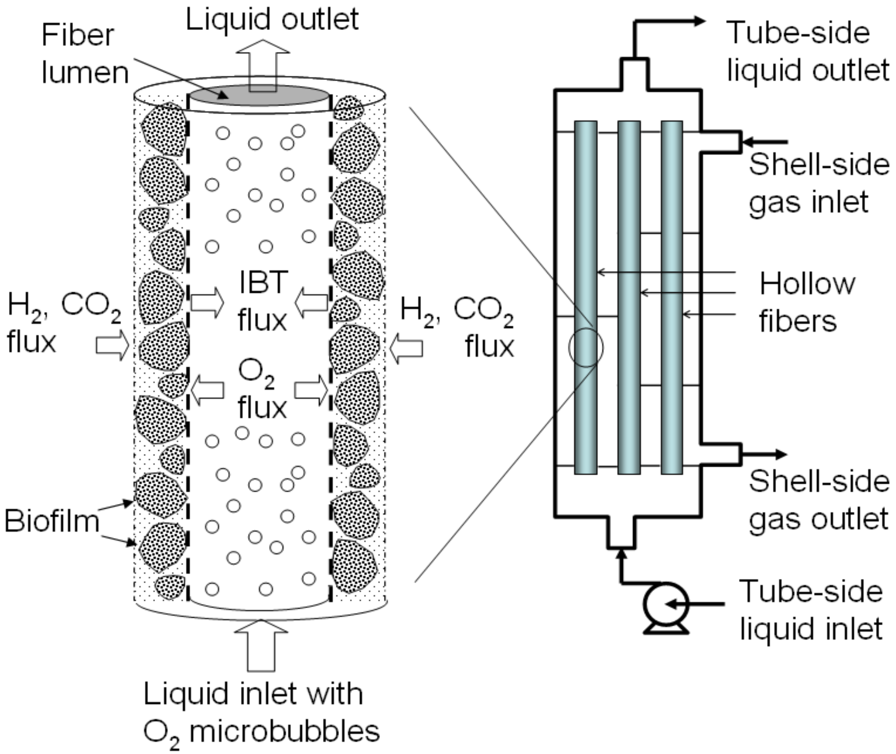
- Assemble biomimetic *Geobacter* membrane
  - Electrode, recombinant *Geobacter* cytochromes and pili, SAM and BLM to test hypotheses
- Fabricate nanomachines that mimic biomembrane structure/function motifs
- Use BLM platforms to study biomembrane charge transfer processes
- Use BLM platforms to screen energy-related nanomaterials for biomembrane interactions

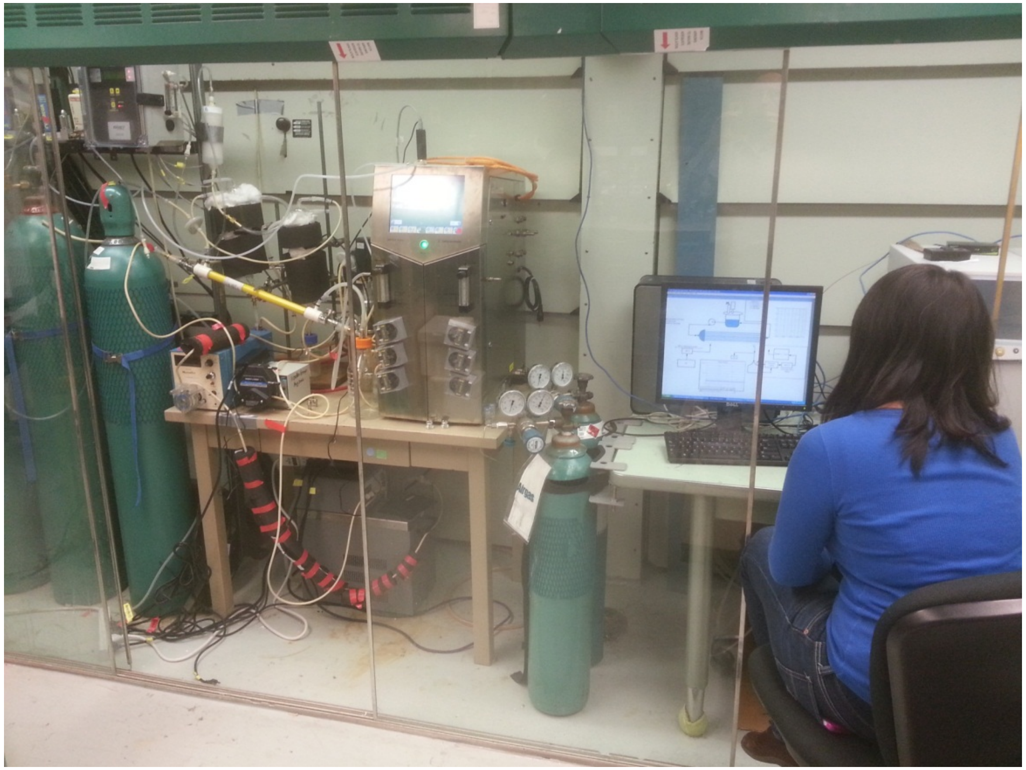
Gas-intensive electrofuel fermentations

- Electrofuel: Carbon-neutral fuel produced from solar energy without green plants
- Challenges:
  - Genetic engineering (appears to work)
  - Process engineering and scale-up difficult
    - Strongly limited by slow gas mass transfer
      - Low solubility of gaseous reactants (H_2, CH_4, O_2)
      - High molar demand for gaseous reactants
    - Safety issues using incompatible gases (H_2, O_2)

Gas-intensive electrofuel fermentations

- Bioreactor for Incompatible Gases
  - H_2, O_2 gases separated by hollow fiber wall
  - Efficient O_2 mass transfer via microbubbles
  - Efficient H_2 transfer by direct gas-cell contact

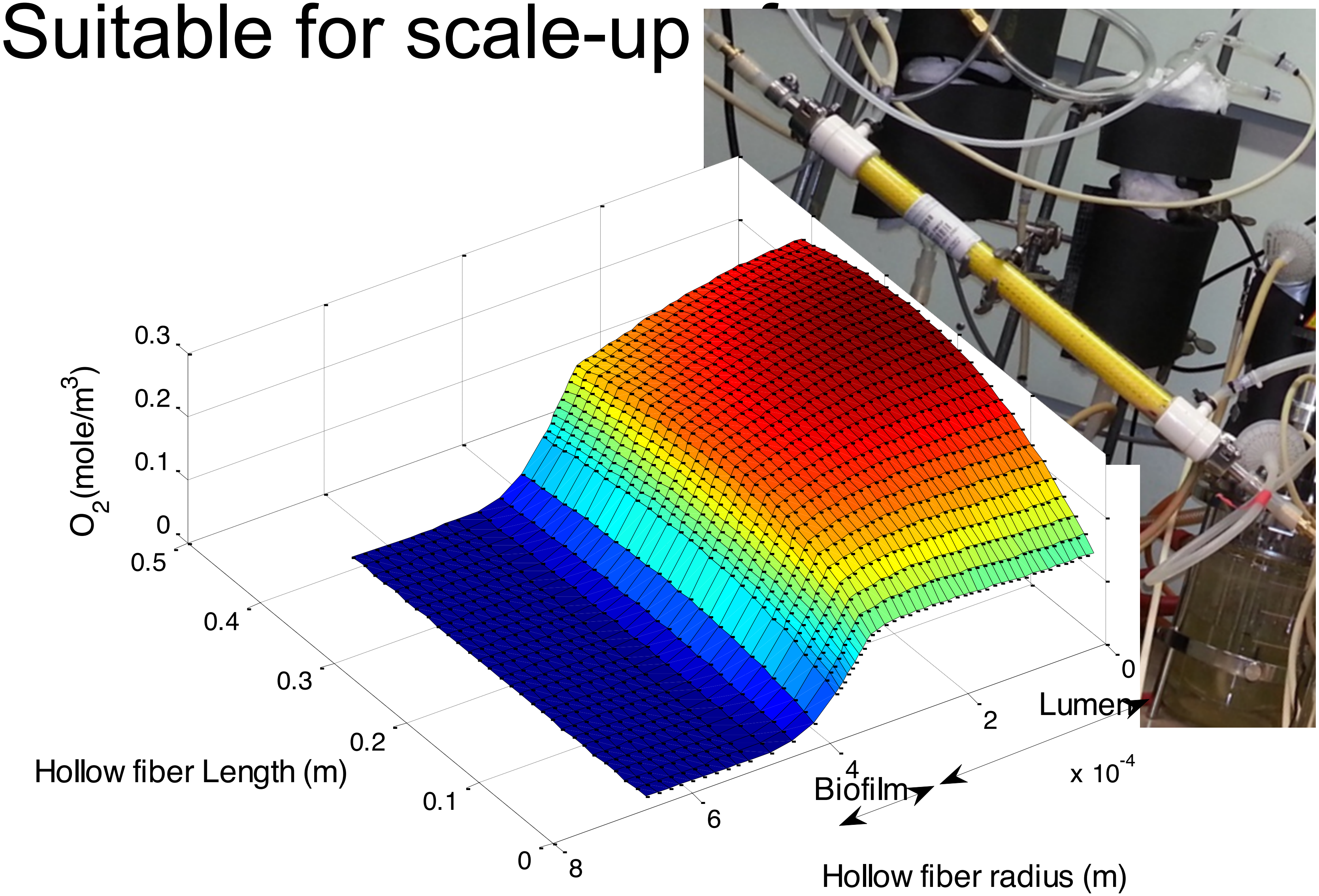




Patent Application US2012/053958, “Catalytic Bioreactors and Methods of Using Same” filed 2012

Gas-intensive electrofuel fermentations

- Mathematical model of new bioreactor
- Suitable for scale-up



Possible electrofuels projects for RF^2

- Develop infrastructure for electrofuel scaleup
- Use MBI pilot plant as national scale-up facility
- Develop new microbial biocatalysts
  - H_2, CH_4, CO as electron-carrying feedstocks
- Develop new electrofuel products
- Integrate solar H_2 production, fermentation

Summary

- MSU has strength in bioelectronics
  - *Microbial Redox Mechanisms*
  - *Nanostructured Biomimetic Interfaces*
  - *Biofuel Cells*
  - *Charge Transfer Across Biomembranes*
  - *Gas-Intensive Electrofuel Fermentations*
- Bioelectronics synergistic with other RF^2 areas

Thank you