



Integrity ★ Service ★ Excellence

Bioenergy

06 MAR 2012

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AFOSR/RSL**

Air Force Research Laboratory

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2012 AFOSR Spring Review Portfolio Overview



NAME: Patrick O. Bradshaw, Ph.D.

BRIEF DESCRIPTION OF PORTFOLIO:

- **Bioenergy** is a program that characterizes, models and explains the structural features, metabolic functions and gene regulatory mechanisms utilized by various biological systems to capture, transfer, convert, or store energy for the purpose of producing renewable biofuels and improving the power output of biofuel cells. (~80% of portfolio)

Sub-Areas: (1) BioSolar Hydrogen, (2) Algal Oil (3) Artificial Photosynthesis, and (4) Biofuel Cells (Microbial and Enzymatic)

- **Photo-Electro-Magnetic Stimulation of Biological Responses** is a beginning program that characterizes, models and explains the stimulatory and inhibitory responses of biological systems to low-level exposures of photo-electro-magnetic stimuli. Potential long-term benefits may include accelerated recovery from mental fatigue and drowsiness, enhanced learning and training, and noninvasive treatment of traumatic brain injuries. (~20% of portfolio)



Visionary Transformational AF Capabilities



Bioenergy:

- **Biofuel Produced from CO₂, H₂O and Sunlight:**
 - Algal systems biology data used to bioengineer lipid biosynthetic pathways in microbes or to create novel synthetic pathways in artificial solar fuel systems
- **Portable H₂ Fuel Generated from H₂O or Cellulose:**
 - Cheap, self-healing inorganic catalysts split water into H₂ and O₂
 - Engineered photosynthetic microbes produce H₂ fuel
- **Compact Power from Ambient Biomass:**
 - Efficient electron transport coupled with unique electrode architectures enhance power and energy densities of biofuel cells

Photo-electro-magnetic Stimulation of Bio-Responses:

- **Electromagnetically Enhanced Cognition, Protection and Healing:**
 - low-level exposure with photo-electro-magnetic stimuli enhance cognitive functions, bio-molecular repair and bio-resiliency



Overview of Topic Areas 3003P



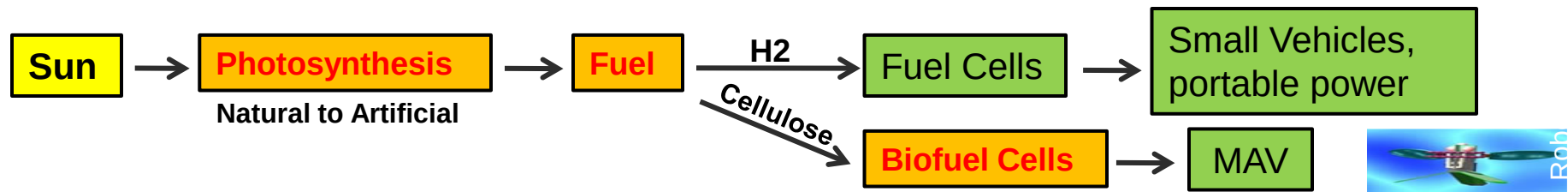
Bioenergy: Alternative Energy

• Biofuels—Macro-scale Energy

- ➔ • Biosolar Hydrogen
- ➔ • Algal Oil for Jet Fuel
- ➔ • Artificial Biology

• Biofuel Cells—Micro-scale Energy

- ➔ • Enzymatic Fuel Cells
- ➔ • Microbial Fuel Cells
- ➔ • Artificial Photosynthesis



Future Direction

- ➔ • Photo-Electro-Magnetic Stimulation of Biosystems
- ➔ • Biomarkers, Physiological responses and toxicology
- ➔ • Artificial Biology – explore non coding genetic information



Bioenergy: A Progressive Research Strategy



Generation	1 st	2 nd	3 rd	4 th
System Type	<u>Natural Biosystems</u>	Optimized Natural Biosystems	Hybrid Systems	<u>Artificial Systems</u>
Basic Research Type	Characterization Mechanisms Models	Metabolic/ Protein Engineering	Synthetic Biology	Chemistry & Materials Science
Disciplinary Inputs	Biology Math Physics Chemistry Engineering			



Challenges, Opportunities and Breakthrough Examples



Natural Systems Research:

Challenge: Explain gene regulatory mechanisms of metabolic pathways and networks

Payoffs: - potentially economical viable biofuels
- enhanced energy density of microbial fuel cells (MFC)

Challenge: Understand mechanisms and kinetics of enzyme-catalyzed reactions

Payoffs: - enhanced energy density of enzymatic fuel cells (EFC)
- sustained oxygen-tolerant hydrogen production by photosynthetic microbes

Artificial Systems Research:

Challenge: Discover/fabricate cheap, durable synthetic materials that mimic the enzymatic or structural functions in natural energy systems

Payoffs: - cheap water-splitting catalysts as platinum replacements in H₂-generating devices
- enhanced power and energy densities for EFC

Challenge: Integrate and assemble nano-scale inorganic/organic/bio-materials

Payoffs: - ordered enzyme alignments for enhanced power densities in EFC
- enhanced electron transport and power density in biofuel cells
- light is harvested and split in artificial photosynthetic solar fuel generator



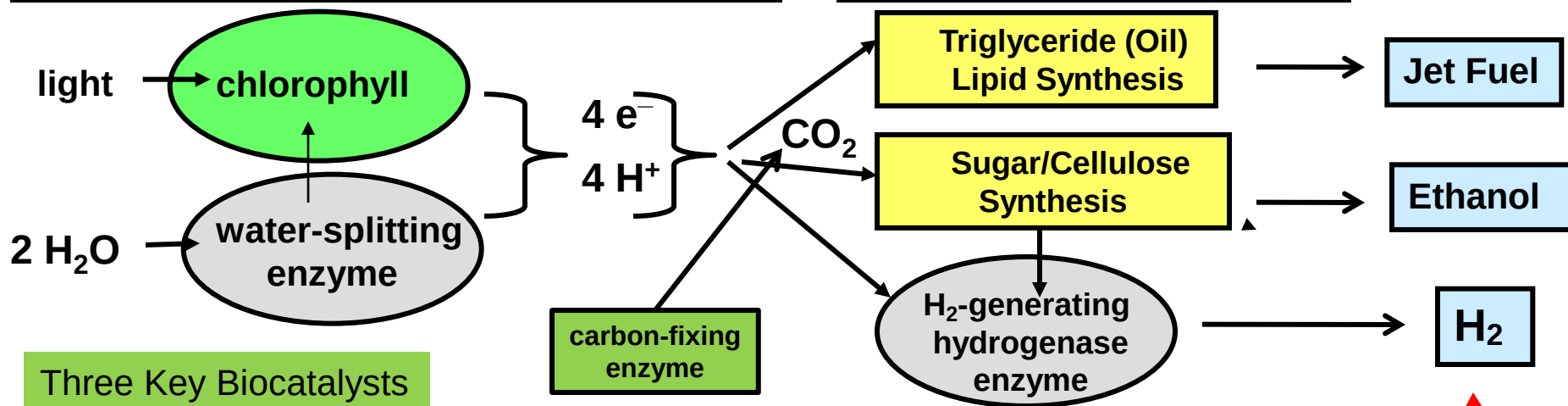
Photosynthesis, Systems Biology and Metabolic Engineering for the Production of Biofuels



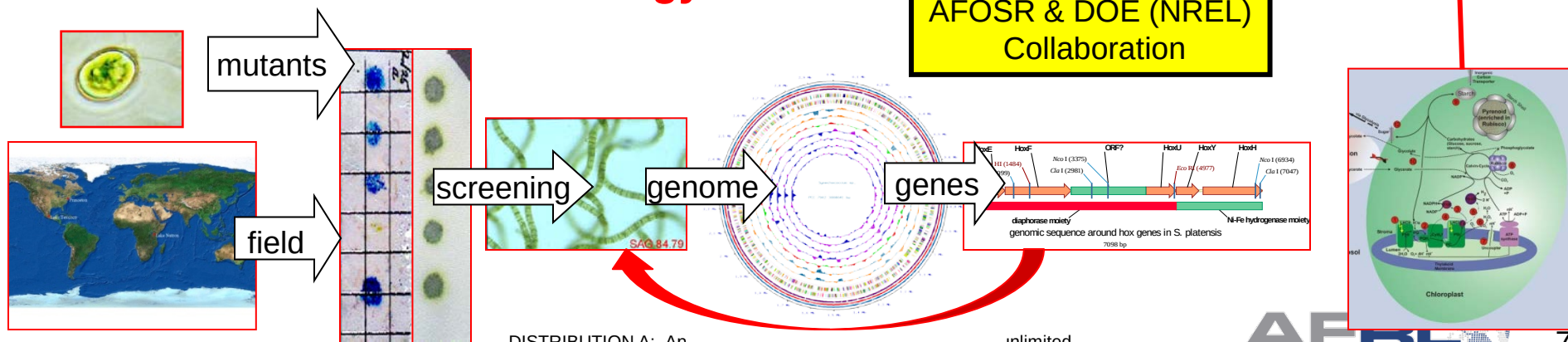
Microalgae & Cyanobacteria Make Hydrogen, Lipids & Sugars

Light Reactions PSI and PSII

Dark Reactions



Overview of Research Strategy





2012 AFOSR Spring Review: Bioenergy (3003P)



Biosolar Hydrogen (MURI and Core Funding)



Bio-Solar Hydrogen Production

Eight Labs Including AFRL & DOE



Objective:



- Obtain knowledge of the basic scientific principles governing H_2 production in microalgae and cyanobacteria
- Genetically engineer pathways to improve the H_2 producing capacity of these phototrophs

H_2 Detectors



- Electrode consumes H_2
- Extended spectral range
- Increased light source intensity 500X with LED

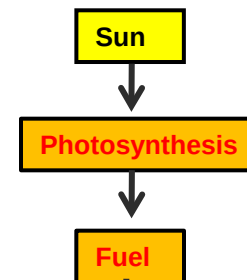
Technical Approaches:

- Bio-prospecting new strains & species
- New H_2 detection & analytical methods
- Stress responses and H_2 production
- Systems biology and pathway analyses
- Genetic engineering of pathways

Accomplishments:

- Developed techniques for high throughput screening of H_2 -producing phototrophs
- Identified physiological factors for increasing rates & yields of cellular H_2 production
- Engineered metabolic pathways with increased production of H_2

DoD Benefit:



1. Stable fuel supply & price
2. Energy independence
3. Carbon neutral
4. Anti-climate change

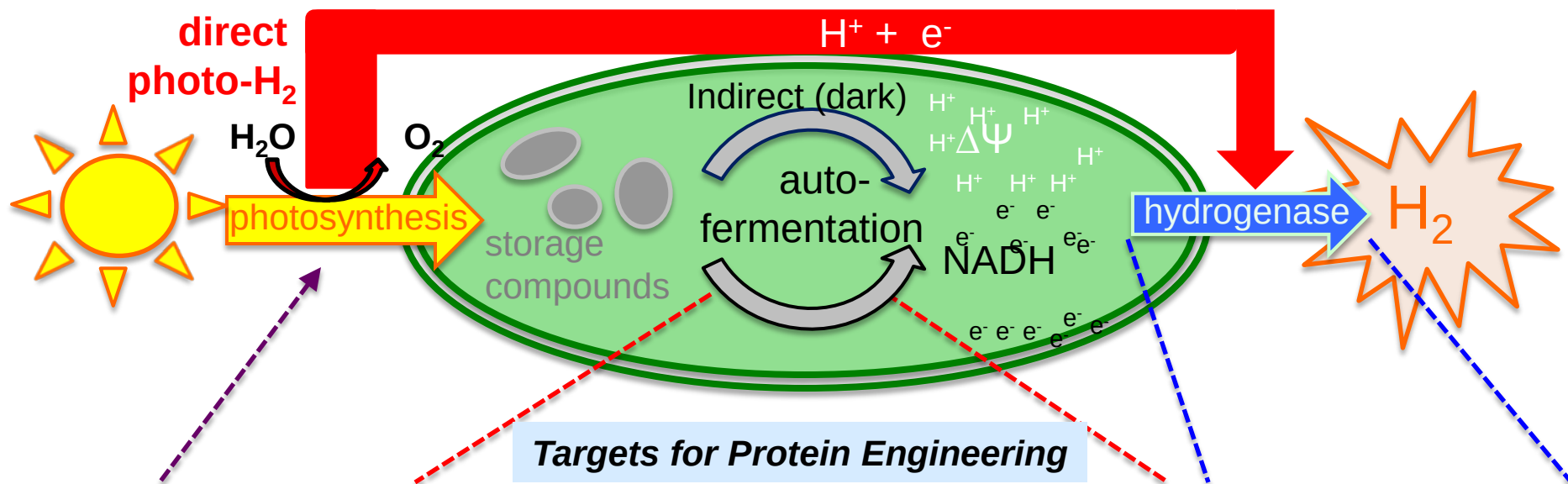




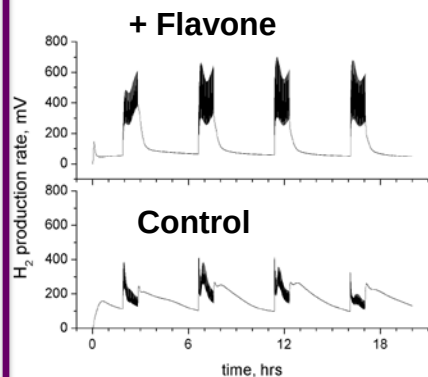
BioSolar H₂ Cyanobacterial Metabolism

Improving Cellular Fuel Production Efficiency

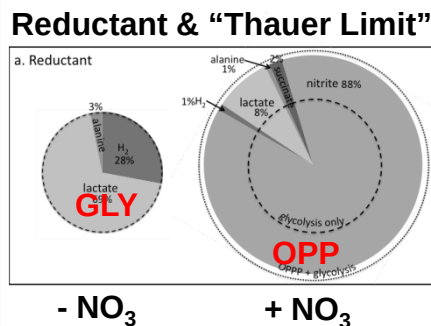
Dismukes (Rutgers)



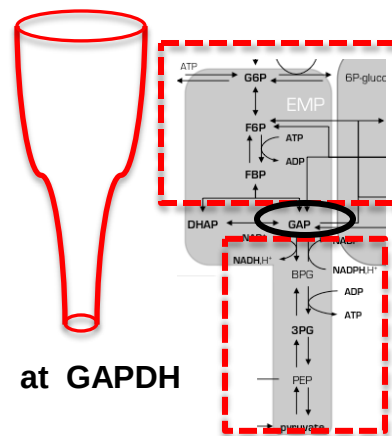
Channeling reductant flux through one of two NADH enzymes increases photo-H₂



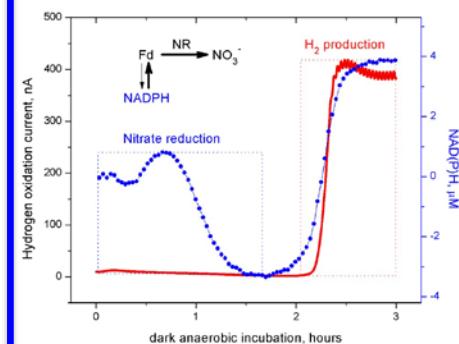
Revealed NO₃⁻ master switch between glycolysis (GLY) & oxidative pentose phosphate (OPP)



Identified the metabolic bottleneck in glycogen fermentation



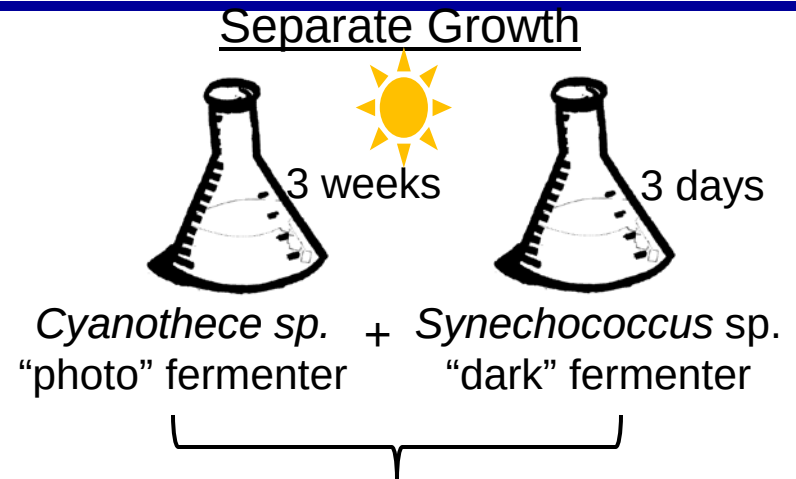
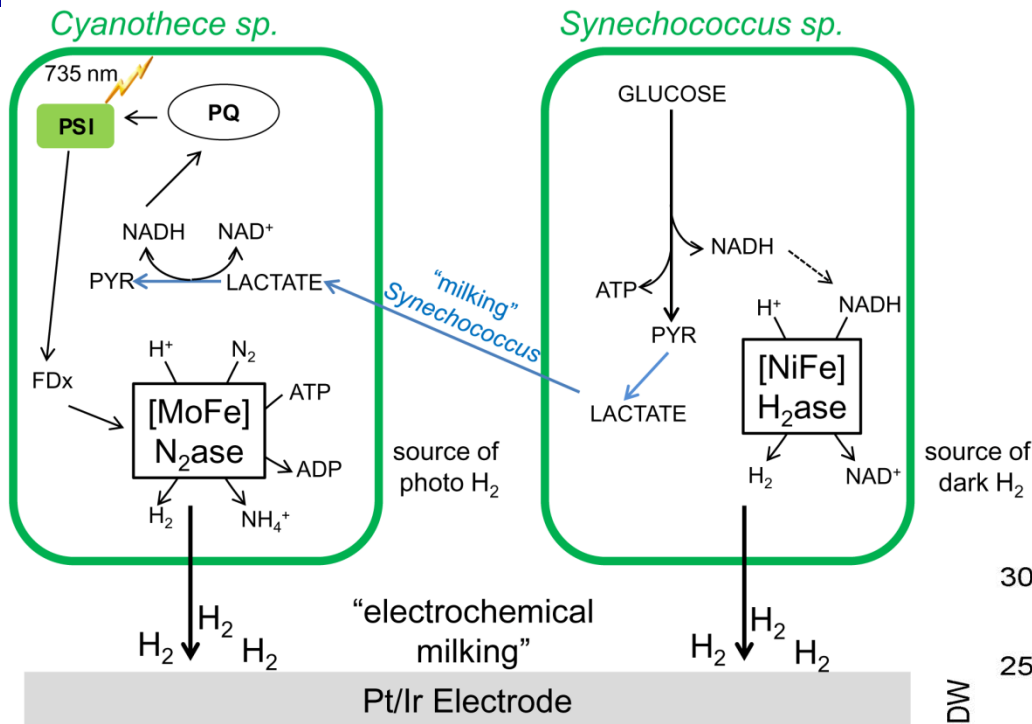
NADH is reductant for phase II H₂ and NAD⁺ is feedback inhibitor of hydrogenase



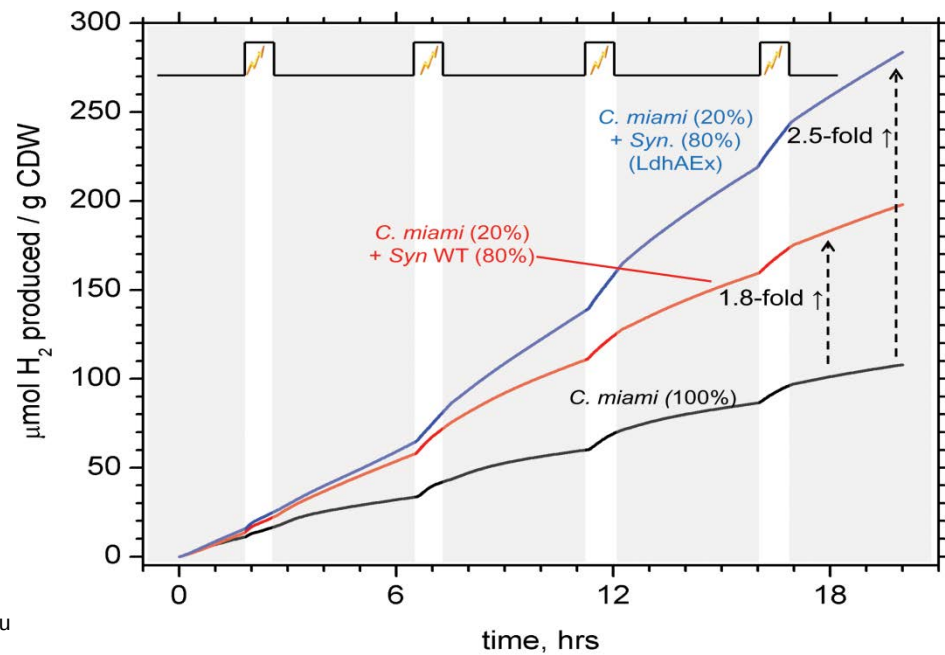


“Milking” More H₂ by Co-Fermentation

PI: G. C. Dismukes Spring Review FY12



Co-Fermentation



*Rate of Dark+Photo H₂ ↑ from *Cyanothecce* is limited by intracellular reductant glycogen

**Syn. WT* excretes reductant as lactate which stimulates 2x H₂ from mixed cultures with *Cyanothecce*

**SynLdhAEx* Over-expression strain excretes more lactate than *Syn WT* and stimulates H₂ even more by 2.5x



2012 AFOSR Spring Review: Bioenergy (2308C)



Algal Oil

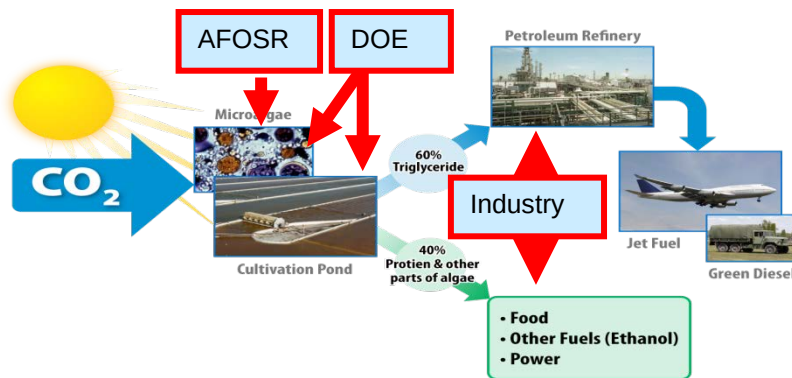


Algal Oil

Ten Labs Including DOE and USAFA



Objective: Gain knowledge of basic algal biology needed to engineer and enhance photosynthetic and lipid biosynthetic pathways



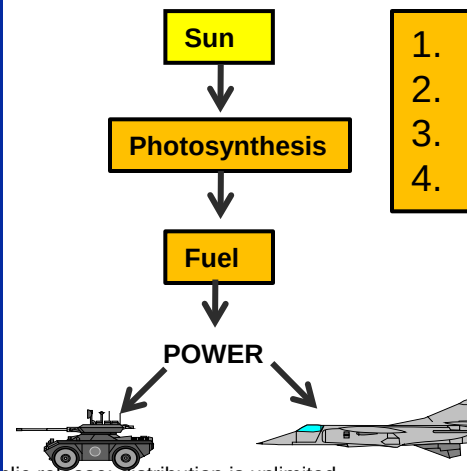
Technical Approach:

- Partner with DOE's National Renewable Energy Lab
- Bioprospect for new lipid-producing algal strains
- Optimize light capture and photosynthetic efficiency
- Optimize environmental factors for lipid biosynthesis
- Use systems biology ("omics") to map lipid pathways
- Identify genetic targets and model metabolism
- Build genetic tools for enabling algal bioengineering

Accomplishments:

- Screened 1200 algal strains for oil yield and identified 50 candidate strains for future studies
- High pH raises oil yields further in NO_3 -stressed cells
- Transformed carbonic anhydrase into algal genome, resulting in CO_2 availability and enhanced growth rate
- Cell cycle arrest or silica starvation elevates lipid production in brown algae (diatoms)
- Identified proteins involved in forming intracellular lipid droplets and in controlling their storage capacity

AF Benefit:



1. Stable fuel supply & price
2. Oil independence
3. Carbon-neutral
4. Anti-climate change

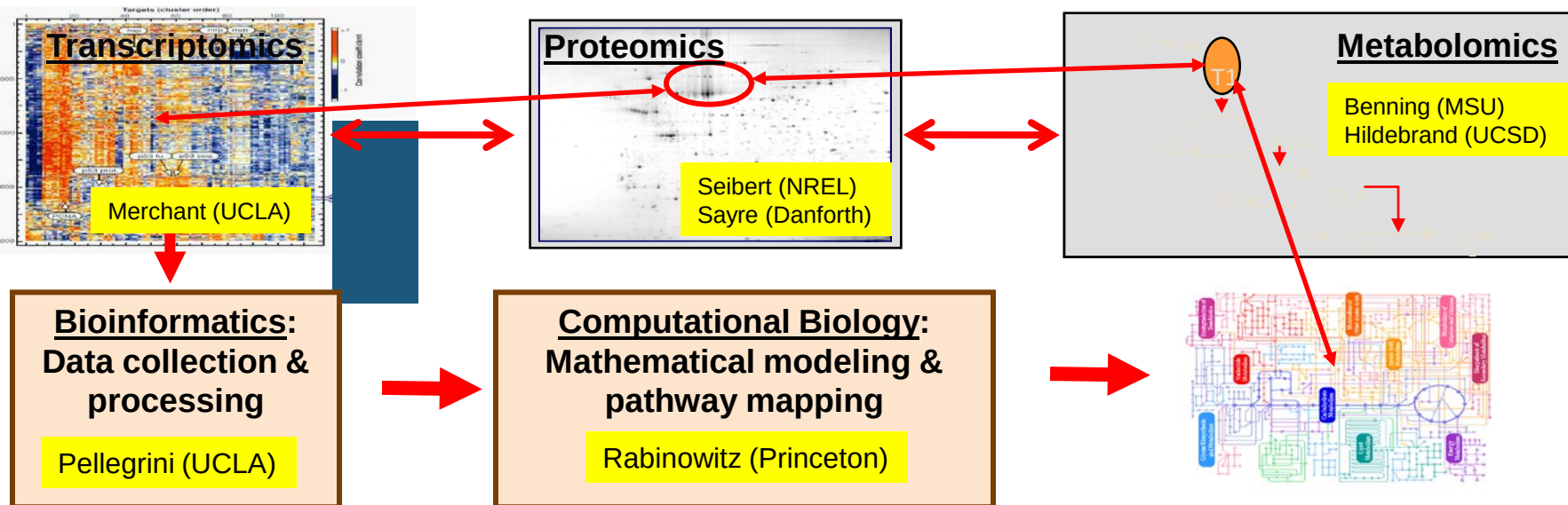


Systems Biology for Algal Lipid Pathway Analyses: A 7 Lab Collaboration



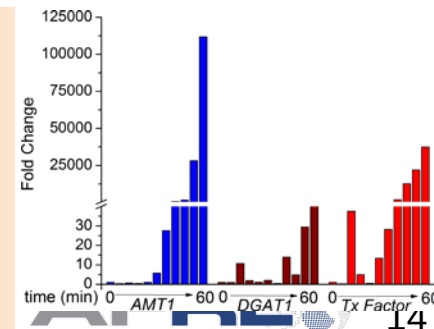
Objectives: Next generation RNA Sequencing technologies are used to compare gene expression profiles in lipid- and non-lipid-producing algae

APPROACH



Recent Findings:

- 3 time-course experiments analyzed by RNA-Sequencing: from 0 to 48 h
- *DGAT1*, triglyceride synthesis enzyme, is induced early in the time course
- A transcription factor, *NRTF1*, is co-expressed with *DGAT1*
- Developed a web-based protein function annotation tool for algal genomes (<http://pathways.mcdb.ucla.edu/chlamy/>)





Enhanced Photosynthetic Efficiency & Algal Growth by Optimizing Light Harvesting Antennae Size



Richard Sayre (Danforth Plant Science Center)

Transgenic algae with reduced Chl b have:

- 1) Reduced antennae size
- 2) Reduced steady state fluorescence

FACT:

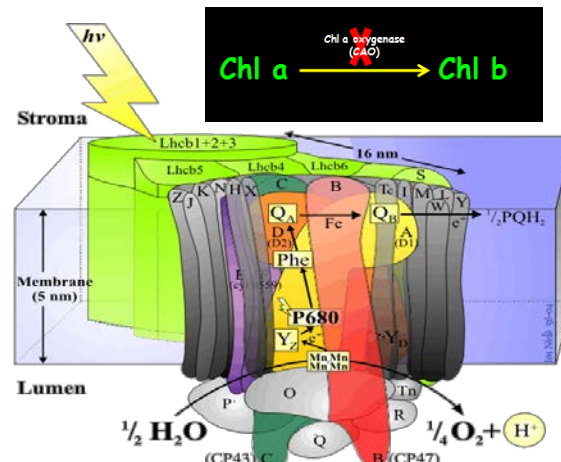
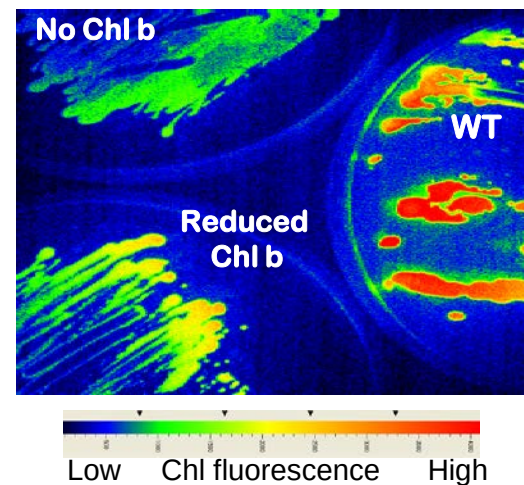
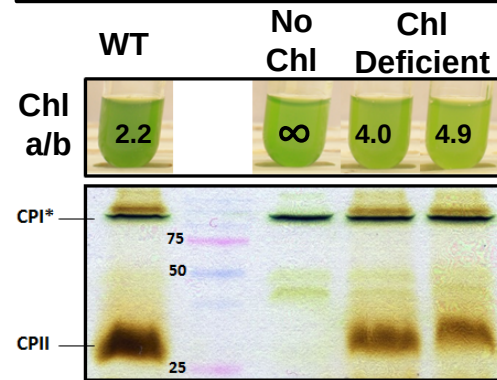
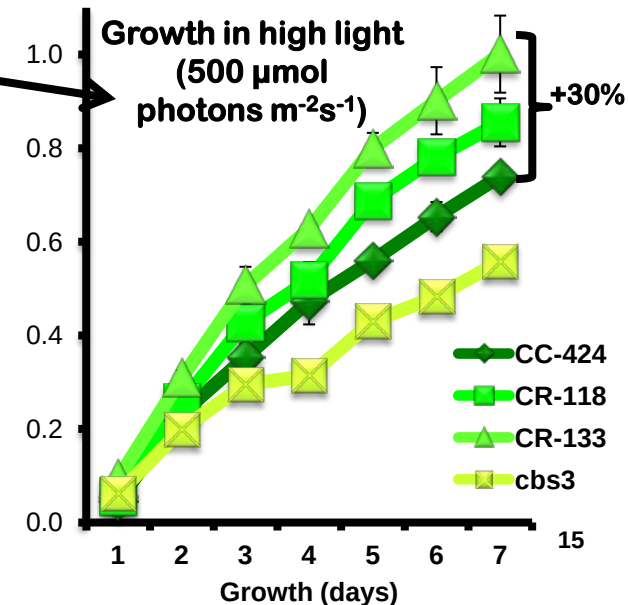
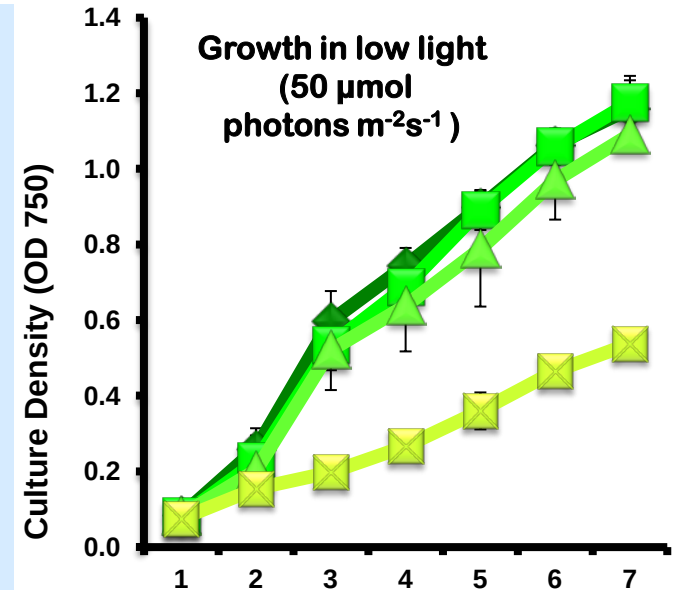
At full sunlight 75% of the captured energy is given off as fluorescence or heat.

HYPOTHESIS:

Reducing the antennae size optimizes energy transfer between the antennae and reactions centers

RESULT:

Reductions in Chl b levels reduced the antennae size resulting in a 30% increase in biomass yield at high light intensities relative to wild type





2012 AFOSR Spring Review: Bioenergy (3003P)



Enzymatic Fuel Cell

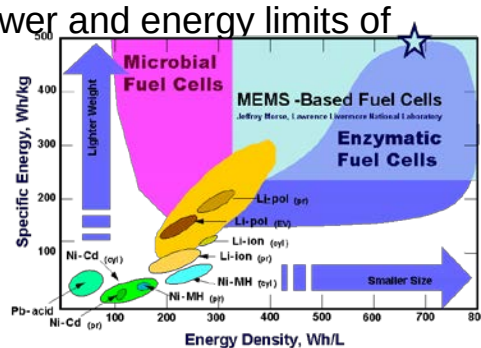


Fundamentals and Bioengineering of Enzymatic Fuel Cells: Seven Labs Including AFRL



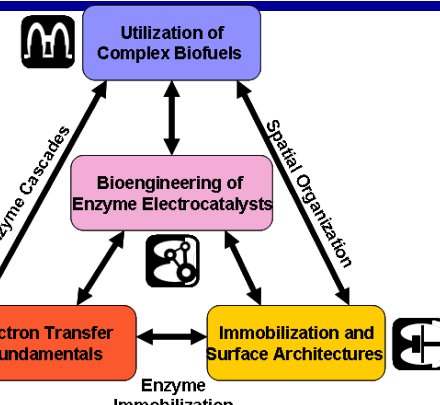
Objectives:

- (1) Exploit biochemical reactions for converting chemical to electrical energy and for generating power from fuels readily available in the environment.
- (2) Estimate the specific power and energy limits of enzyme fuel cells to define potential powering uses
- (3) Transition technology towards sub-miniature sustainable mobile power sources



Technical Approach:

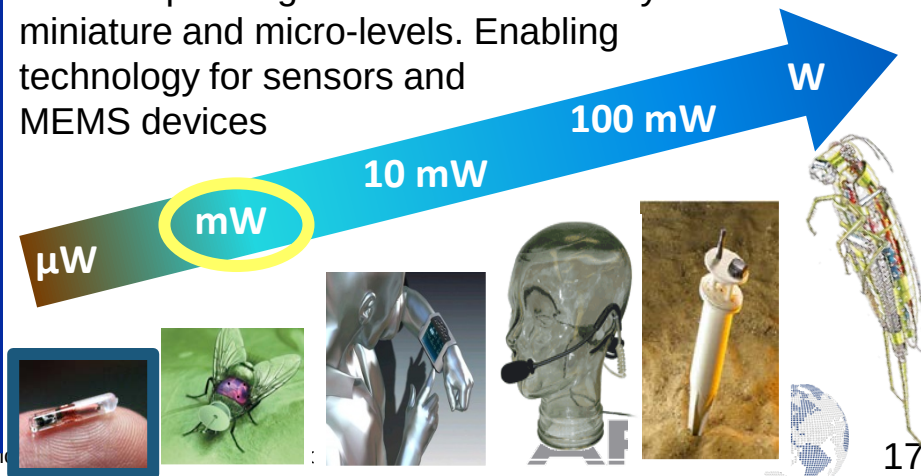
- Provide multi-enzyme cascades for full utilization of complex biofuels
- Protein engineering of enzymes to improve bioelectrocatalysts
- Establish mechanisms of electron transfer
- Design and fabricate novel electrode architectures for enhanced performance



Accomplishments:

- Developed multi-enzyme cascades for complete oxidation of biofuels, enhancing energy density
- Modeling identified major obstacles in multi-step enzyme catalysis—electrode surface area and co-factor (NAD) instability
- Engineered enzymes to self-assemble into conducting hydro-gels and broadened their specificity to accept both NAD & NADP
- Determined O₂ binding site in multi-copper oxidases

DoD Benefit: Energy technology platform for scalable power generation. Particularly useful at miniature and micro-levels. Enabling technology for sensors and MEMS devices





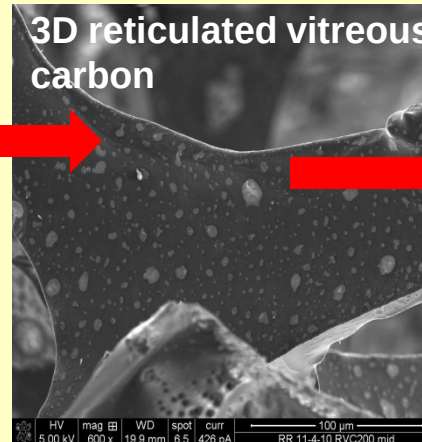
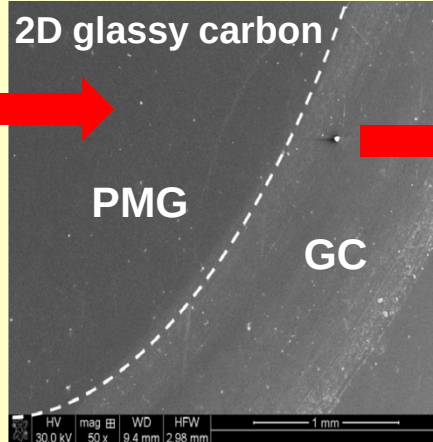
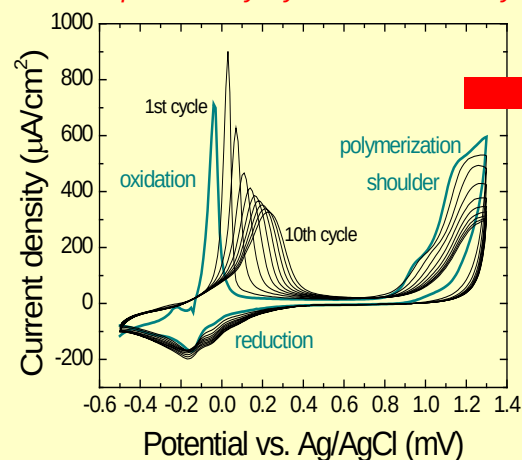
Integrated Enzymatic Biofuel Cell

Atanassov (UNM)

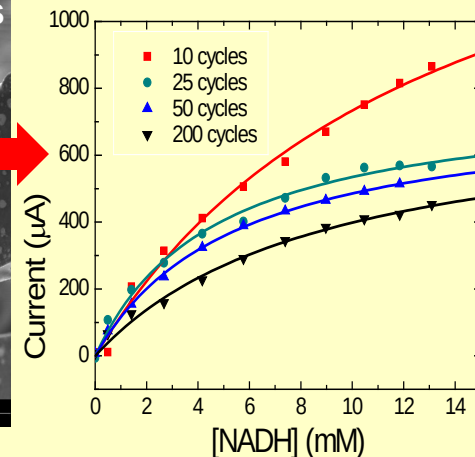


Deposition and characterization of poly-(methylene green) catalysts for NADH oxidation

Deposition by cyclic voltammetry

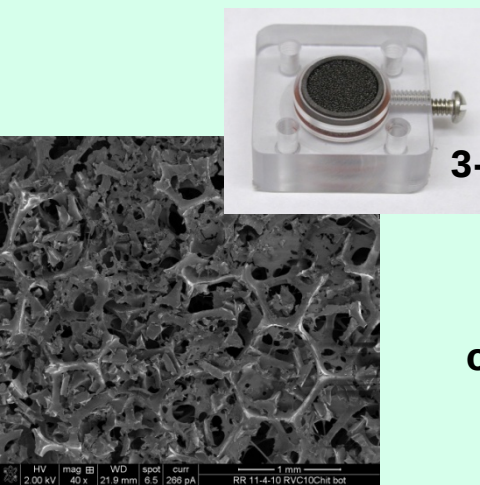


Electrochemical characterization



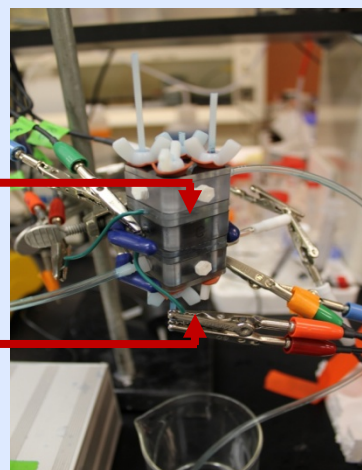
Integration of poly-(MG) modified RVC with NAD^+ -dependent enzymes immobilized in chitosan/CNTs composite scaffold

Integration with laccase-based bio-cathode in a flow-through membrane-less biofuel cell



3-D Anode

Cathode open to air

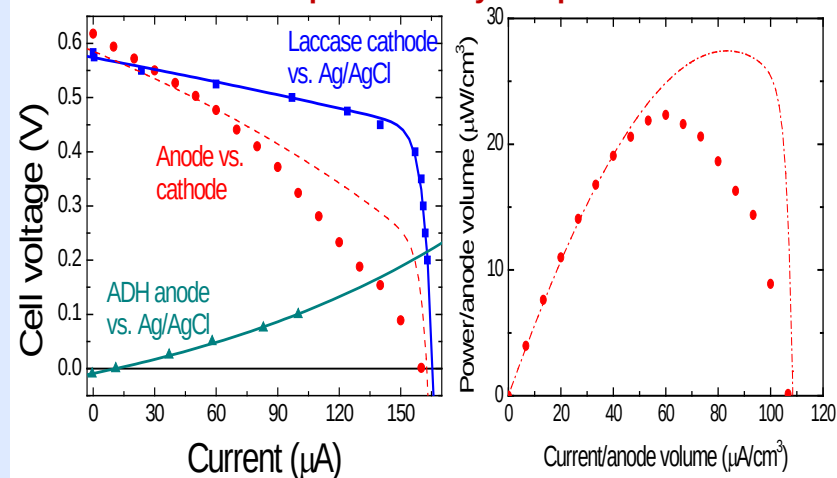


Polarization and power curves in 475 ethanol

$$E_{0\text{ cell}} = 0.618 \text{ V, pH} = 6.3$$

$$\text{Limiting current} = 160 \mu\text{A}$$

$$\text{Maximum power density} = 27 \mu\text{W}/\text{cm}^3$$





88 Personnel Involved in the Research: June 1, 2011

51 Supported by the MURI Program

6 University PIs and
8 Collaborators
88 Researchers involved

+ and 3 more...



51 of them supported fully or in part by the MURI

5 Research Faculty / Senior Researchers
18 Postdoctoral Fellows
34 Graduate Students
31 Undergraduate Students and 2 High School Students



36 Female

42 Male



11 Hispanics



2 African American

1 Native American

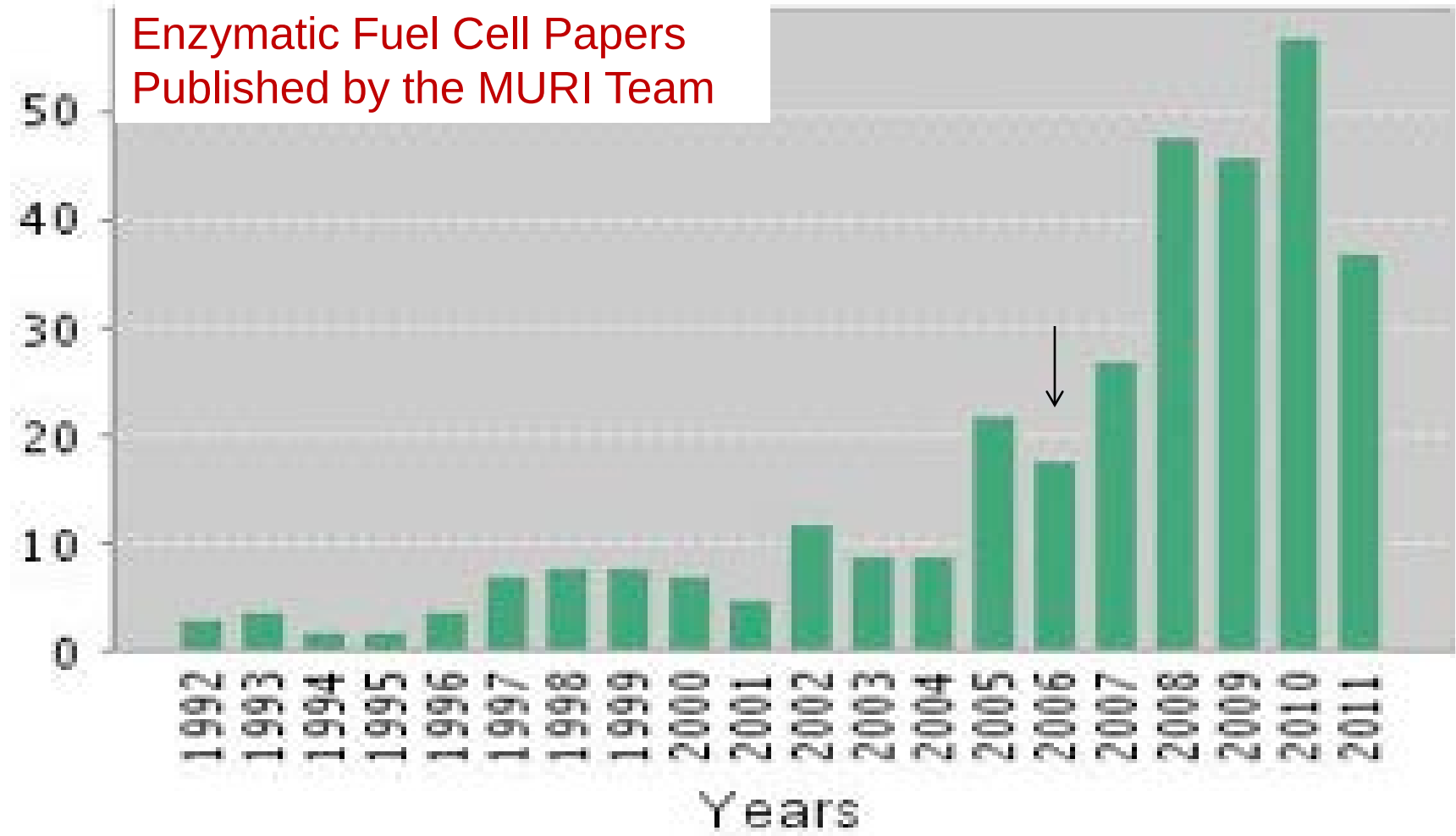




ISI Publication Record on Enzymatic Fuel Cells: 1992 - 2011

AFOSR MURI: Fundamentals & Bioengineering of Enzyme Fuel Cells

Published Items in Each Year





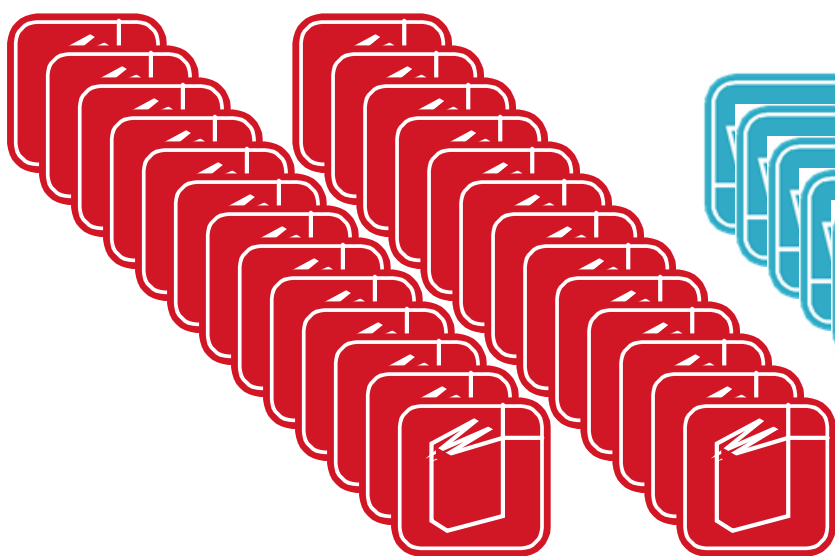
Peer-Reviewed Journal Publications: June 1, 2011

99 Publications & Book Chapters and 6 Patent Applications

74 Published

16 Submitted or
In Press

9 In
Prep.



2010 Special Issue of
Electroanalysis on Biofuel Cells



3 US Patent Applications

~ 215 Presentations at Conferences,
With abstracts published in the
Conference Proceedings,
Including ~80 invited talks.

~ 75 Department Seminars,
Press Releases,
Interface article (ECS)
Media Coverage,
Issue Guest Editing.





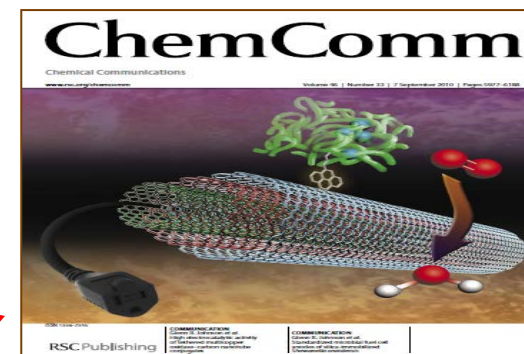
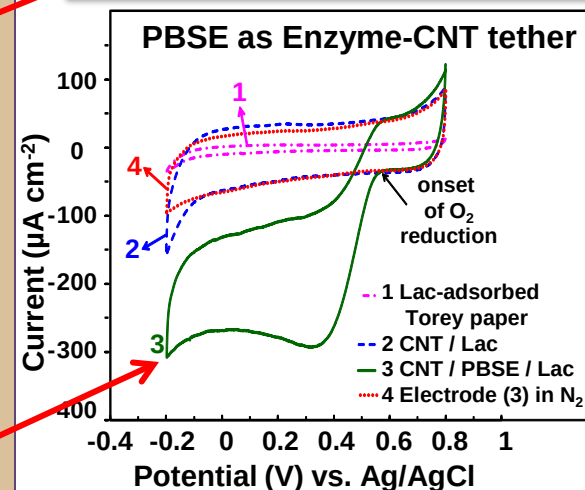
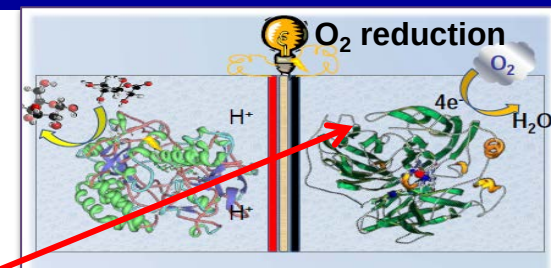
Controlling Direct Electron Transfer (DET) Between Electrodes and Conductive Materials

Johnson & Pachter (AFRL) & Atanasov (UNM)



Objectives: Devise means to characterize and organize the interface between redox-active enzymes and nanomaterials

- Background:** DET requires an electronic interface for electrons to “hop” from enzyme to the electrode surface.
Multi-copper containing oxidases (MCO) serve as model bioelectrocatalysts for fuel cell cathode, accepting electrons from electrode and then catalyzing O_2 reduction.
- Approach:** Various MCO were linked to carbon nanotubes (CNT) using a chemical “tethering” reagent (1-pyrene butanoic acid, succinimidyl ester (**PBSE**)). The method conjugates the enzyme and CNT without changing material conductivity.
- Results:** Electrochemical potential and kinetics of O_2 reduction reaction approach theoretical optima (+600 mV vs. Ag/AgCl)
High-potential maintained under increased current density, <100 mV decrease @ 50 mA cm⁻²
Bioelectrodes provided exceptional DET.
- Conclusion:** Materials and processing approach accommodates various biocatalysts and is potentially scalable
→ significant advance over previous literature reports → key steps toward application. Cover feature on Chem Comm

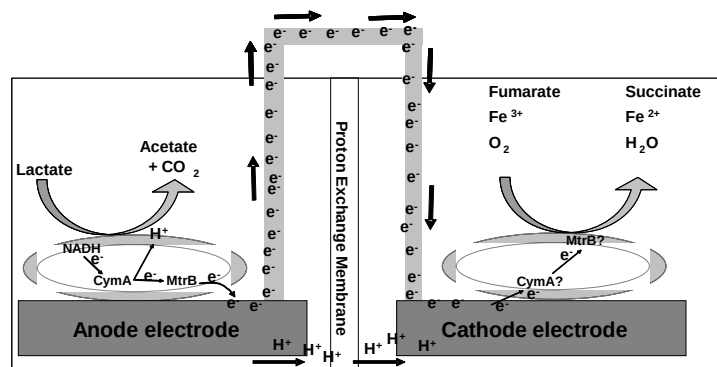




2012 AFOSR Spring Review: Bioenergy (3003P)



Microbial Fuel Cells (MURI and Core Funding)



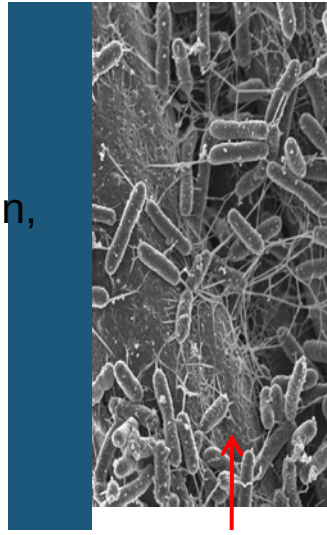


Optimizing Microbial Fuel Cells via Genetics, Modeling and Nanofabrication: Seven Labs



Objective:

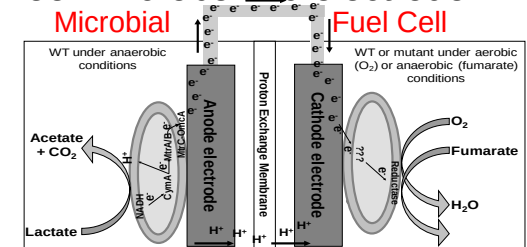
To understand the mechanism(s) involved in microbial current production, and to utilize multi-scale modeling to exploit this understanding in order to optimize microbes and microbial communities for microbial fuel cells.



Current transfer by nanowires...

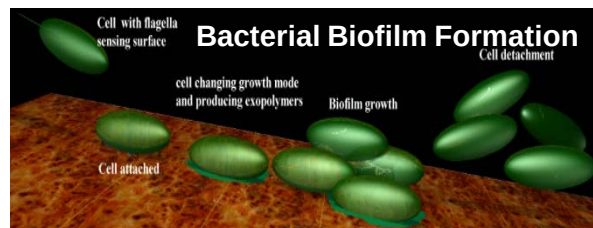
Technical Approach:

- Identification & regulation of the genes, molecular machines and structures used to produce and transfer current between microbe and electrode
- Modeling & bioengineering
- Development & exploitation of microbial consortia with the ability to utilize a wide range of energy sources
- Modeling, fabrication & testing of miniaturized MFCs

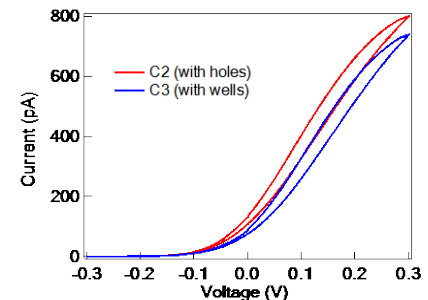
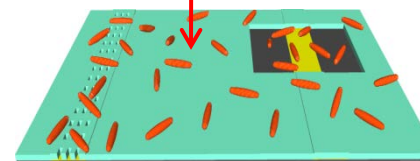


Accomplishments:

- Identified current associated genes in *Shewanella*
- Developed novel vertical scanning interferometry for interfacial analysis at electrode surface
- Characterized the bacterial behavior of electrokinesis
- Showed the value of bacterial biofilms in current production



...and/or soluble mediators?



DoD Benefit:

This project may enable high performance microbial fuel cells as power sources. The ability to use multiple complex fuels under changing physical and chemical conditions may enhance capabilities.



Molecular Identification of Bacterial Nanowires and Their Role in Microbial Fuel Cells: Ringeisen (NRL) Spring Review FY2012



Objective: Use a variety of microbial fuel cell (MFC) platforms to correlate structure and function of extracellular nanofilaments with rate of extracellular electron transfer (current generation). Measure conductivity and protein identification of bacterial nanofilaments.

Technology Platforms Used for Protein ID of *Shewanella oneidensis* MR-1 Nanowires



- Miniature MFCs
- Direct Write Nanoelectrodes
- Immunolabeling and Transmission Electron Microscopy (TEM)
- Liquid Chromatography/Mass Spectrometry/Mass Spectrometry (LC/MS/MS)
- Temperature-Controlled Probe Station

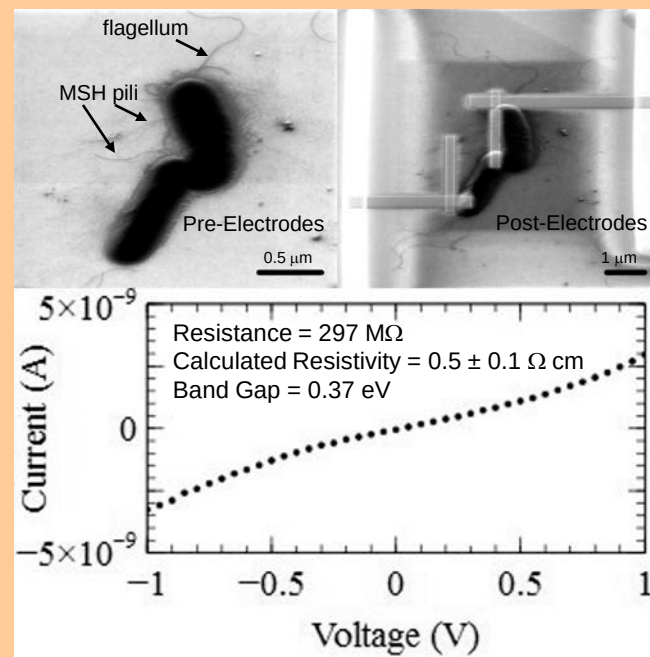
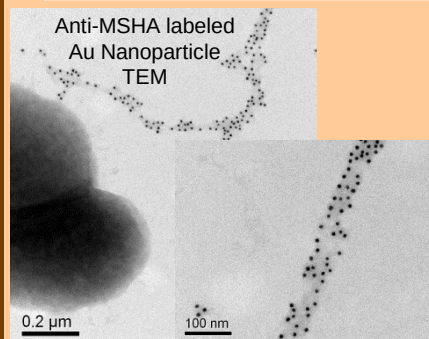
Analysis of *S. oneidensis* nanofilaments has determined that a previously unsuspected protein (mannose sensitive haemagglutinin, MSH) is involved in extracellular electron transfer (EET) in microbial nanowires

Extracellular Protein ID in Nanofilament Preps via LC/MS/MS

MSHA

MSHB

Flagellin





2012 AFOSR Spring Review 3003P Portfolio



Photo-Electro-Magnetic Stimulation of Biological Responses (Core Funding)

Photo-Electro-Magnetic Stimulation of Biological Responses is a beginning program that characterizes, models and explains the stimulatory and inhibitory responses of biological systems to low-level exposures of photo-electro-magnetic stimuli. Potential long-term benefits may include accelerated recovery from mental fatigue and drowsiness, enhanced learning and training, and noninvasive treatment of traumatic brain injuries. (~20% of portfolio)

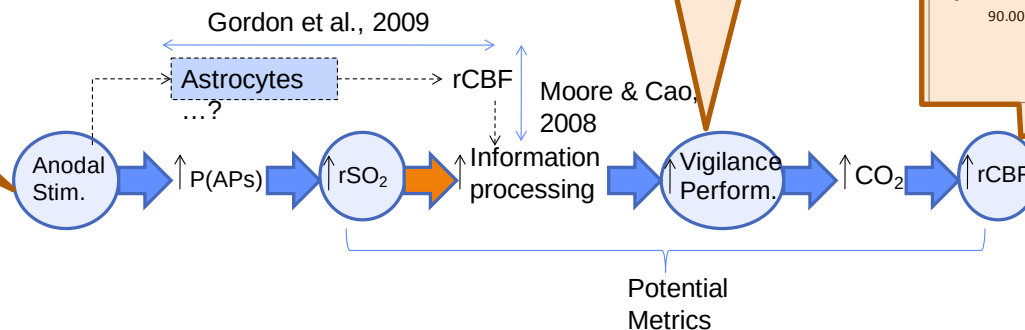
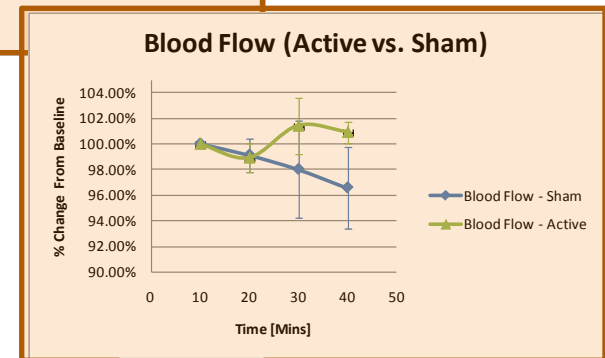
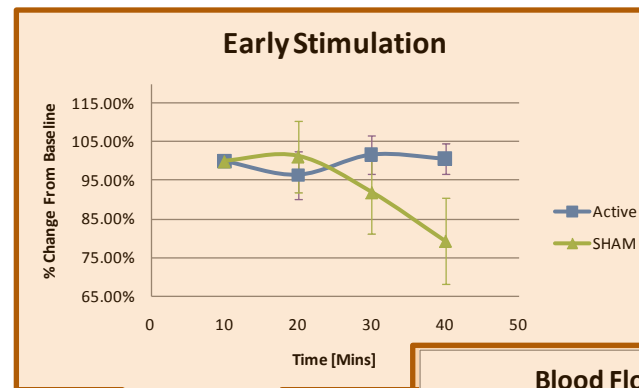
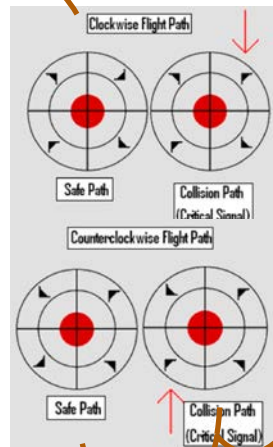


Electric Stimulation of the Brain, Hemodynamics and Sustained Attention: McKinley (AFRL/RH)



Objective: Quantify effects on human vigilance and hemodynamics due to non-invasive stimulation of the brain by low levels of direct current (1 mA).

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2011**



Merzagora et al., 2010

Helton et al., 2010
DISTRIBUTION A: Approved for public release; distribution is unlimited.

Hellige, 1993 & Warm et al., 2009





Coupling Terahertz Radiation to Biomolecules for Controlling Cell Response: Wilmink (AFRL/RHDR)

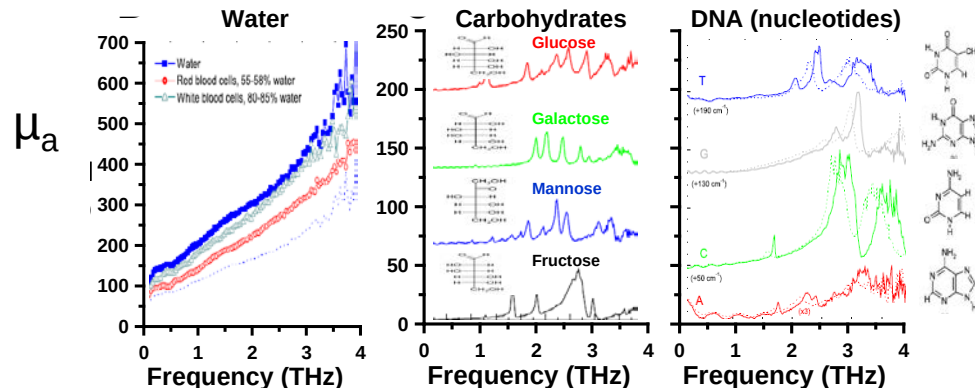


Terahertz (THz) Radiation:

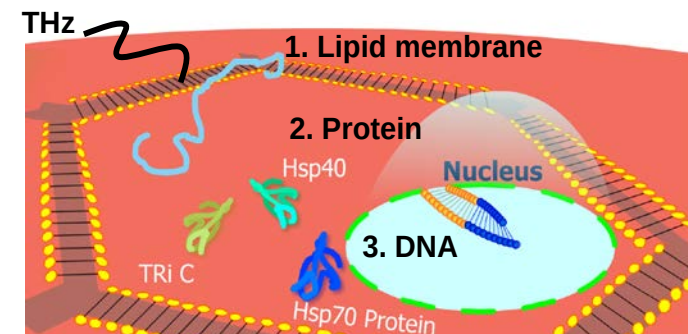
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- Alters lipid membranes and modulates neuronal action potentials.
- Oscillates in the same ps time-scale as breathing modes of DNA & proteins (~40 ps).

Biomolecules display unique spectra in THz region



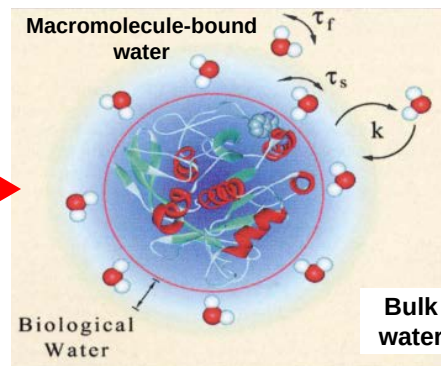
THz energy couples to biomolecules



Objectives: Investigate coupling mechanism and exploit the understanding to activate adaptive responses and modify cellular behaviors

Working Hypothesis:

THz-coupling is mediated via macromolecule-bound water on the surface of membranes and biomolecules



Testing Hypothesis:

- THz exposure system on a microscope
- Raman & THz spectroscopy
- Fluorescence & atomic force microscopy
- DNA mutation assays

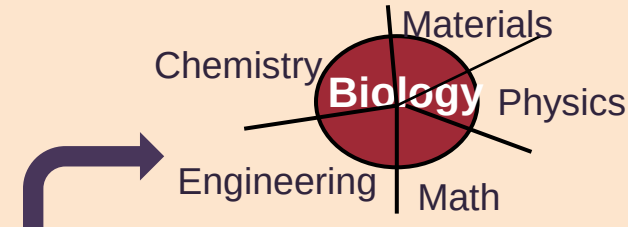


Related Research Funded by Other Agencies



Funding Criteria:

1. Basic research of high quality and relevant to the AF
2. Unique or complementary, but non-duplicative—finds a “niche”
3. Leverages research in other agencies
4. Critical mass or team of collaborators with focused, multi-disciplinary research objectives



Algal Oil: DOE and DARPA research application oriented; NSF funds mostly individual grants of smaller size that are not based on a coordinated, multi-disciplinary team approach; USDA interested in farming aquaculture; EPA interested in regulation. AFOSR niche is lipid biosynthesis via systems biology. AFOSR has collaborated with DOE-NREL since 2006 and coordinates research as member of emerging Algal Interagency Working Group.

Biosolar Hydrogen: DOE and NSF fund mostly individual grants of smaller size that are not based on a coordinated, multi-disciplinary team approach. AFOSR niche is systems biology and bioengineering for enhanced H₂ production. AFOSR has collaborated with DOE-NREL since 2003.

Biofuel Cells: ONR funds only microbial fuel cell (MFC) research for dissolved nutrients in the marine sediment environment. AFOSR funds enzymatic and MFC research for solid substrates in terrestrial environments and coordinates research via ONR reviews and direct personal contact.

Artificial Photosynthesis: This topic is biologically oriented and part of a 2009 AFOSR Initiative “***Catalysts for Solar Fuels***” with PMs Berman and Curcic, whose topics are chemically and physically oriented. To our knowledge there are no initiative counterparts at other agencies.

BioResponse to Photo-electromagnetic Stimulation: Complementary to other funded research.