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14. ABSTRACT
A technical Exchange meeting was held at The Frontier –Research Triangle Park on September 15-16, 2015. The purpose of the meeting was to assess the current state and emerging opportunities in studies of intracellular and extracellular bacterial electron transfer. One specific goal was to establish interdisciplinary discussions between scientists and engineers with different expertise, ranging from the microbiology of electroactive multispecies communities to emerging theoretical descriptions of charge carrier transport in soft matter conductors and

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## Report Title

Final Report: Technical Meeting of Biological Electron Transport

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# Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

### (a) Papers published in peer-reviewed journals (N/A for none)

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**Number of Papers published in peer-reviewed journals:**

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<u>Received</u> <u>Paper</u>

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### (c) Presentations

**Number of Presentations:** 0.00

## Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

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**Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):**

## Peer-Reviewed Conference Proceeding publications (other than abstracts):

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## Patents Submitted

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## Graduate Students

| NAME | PERCENT_SUPPORTED |
|---|---|
| **FTE Equivalent:** | |
| **Total Number:** | |

## Names of Post Doctorates

| NAME | PERCENT_SUPPORTED |
|---|---|
| Yan, Hengjing | 0.00 |
| **FTE Equivalent:** | **0.00** |
| **Total Number:** | **1** |

## Names of Faculty Supported

| NAME | PERCENT_SUPPORTED | National Academy Member |
|---|---|---|
| Bazan, Guillermo C. | 0.00 | No |
| Bond, Daniel | 0.00 | No |
| Ista, Linnea | 0.00 | No |
| Kumar, Manish | 0.00 | No |
| Lovley, Derk | 0.00 | |
| Nealson, Kenneth | 0.00 | |
| Regan, John M. | 0.00 | |
| Reguera, Gemma | 0.00 | |
| Schuler, Andrew | 0.00 | |
| **FTE Equivalent:** | **0.00** | |
| **Total Number:** | **9** | |

## Names of Under Graduate students supported

| NAME | PERCENT_SUPPORTED |
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## Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: ...... 0.00

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Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):...... 0.00

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## Names of Personnel receiving masters degrees

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## Names of personnel receiving PHDs

| NAME |
|---|
| **Total Number:** |

## Names of other research staff

| NAME | PERCENT_SUPPORTED |
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## Inventions (DD882)

## Scientific Progress

See Attachment.

# Technology Transfer

# Report on the Technical Exchange Meeting on Biological Electron Transfer

"Life is nothing but an electron looking for a place to rest."

--Albert Szent-Gyorgi

*Summary*

A technical Exchange meeting was held at The Frontier –Research Triangle Park on September 15-16, 2015. The purpose of the meeting was to assess the current state and emerging opportunities in studies of intracellular and extracellular bacterial electron transfer. One specific goal was to establish interdisciplinary discussions between scientists and engineers with different expertise, ranging from the microbiology of electroactive multispecies communities to emerging theoretical descriptions of charge carrier transport in soft matter conductors and semiconductors. A series of presentations was provided on the first day, as listed in Appendix 1. Relevant summary comments are provided in Appendix 2. Attendants included scientists and engineers from academia and DoD laboratories. The second day of the Meeting focused on drawing conclusions and perspectives that summarize some of the most critical knowledge gaps and relevant opportunities for technological implementation.

*Motivation for the workshop*

Specific species of microbes are able to exchange electrons with charge collecting surfaces. This electrogenic capability occurs in only a select number of naturally occurring microorganisms, is critical for the survival of certain species under anaerobic conditions, and has been exploited in a variety of bioelectrochemical technology platforms, including microbial fuel cells and bioelectrosynthesis devices. Emerging evidence, however, implicates very long-range (i.e. cm range) electron transfer in sediments and within synergistic microbial communities. Electron injection from working electrodes to drive microbial metabolism has also received considerable attention recently, particularly within the context of new technologies for bioremediation and bioelectrochemical synthesis of fuels and chemicals derived from carbon dioxide.

Mechanistic studies of the different mechanisms by which microbes participate in extracellular electron extraction/injection are under considerable lively debate. It is unclear whether similar processes are operative with respect to the relationship of the microbe relative to an electrode or members of a biofilm. Microbe fragments thought to be important for charge transport, for example the pili from *G. sulfurreducens*, have been claimed to exhibit metallic-like conductivity. A proper description of the arrangements of electroactive units in such a protein assembly and a calculation of rate constants from first principles is lacking, despite claims of similarity to organic semiconducting and conducting materials. Needs have also been identified for establishing the rational design and engineering of synergistic communities of bacterial species and the design and characterization of novel synthetic and chemical enhancements that can improve the electron transport capacity of non-electrogenic microbes.

As a result of intrinsic biological complexity, diverse microbial systems, and opportunities for new applications that require interfacing with device systems, the understanding and application of extracellular electron transfer in microbial systems requires coalescing the expertise of different branches of science and engineering. The ideas and methodologies exchanged at the meeting provide a unique opportunity to define opportunities mutually-reinforcing transdisciplinary research needed to make significant strides in the understanding and the application extracellular electron transfer. Bringing together mutually-complementary expertise for tackling this problem has not been actively pursued in the past.

*Summary of Critical Questions to Address and Emerging Opportunities*

Participants met on September 16 to discuss fundamental scientific challenges and emerging technological opportunities. This discussion was accomplished by first separating the participants into three independent subgroups. Subsequently, the findings from the three subgroups were discussed with all participants present.

The questions that reached closest to complete consensus are detailed below and are divided into subsections that coalesce into broader topics.

*i) Electronic Materials Structure, Function and Design*

Mechanisms of charge transport are likely to determine how far we can engineer function and thereby define applications. Therefore, what are the mechanisms of charge carrier transport in "electronic" protein building blocks? We need to bridge the structure/function/atomistic theory continuum to take advantage of physics-based understanding of conductivity. To what extent are analogies to existing materials appropriate, and to what extent are there mechanisms unique to biological systems? How different are in vivo and in vitro functions? Are there intrinsic barriers or limitations to charge transport? It should be possible with this information to design *a priori* protein structures with electronic properties that exceed those evolved by living microorganisms. Conversely, what are truly synthetic systems that derive their structure and functionality from natural systems? There are excellent opportunities for synthetic biology/engineering to increase conductivity of microbial systems that already generate conductive secretions (i.e., EPS of *Geobacter*) to impart novel electronic/biological properties to these microbes and to start tackling the practical problem of scale up for device fabrication.

*ii) Diversity of Electrogenic Microbes and Community Behavior*

There is a diversity of Bacteria and Archaea in hand that have remarkable electron transfer abilities: Gram-positive, salt-tolerant, thermophilic, capable of metabolizing compounds not accessible by the *Geobacter* and *Shewanella* systems. Each of these has solved issues of interfacial and interspecies electron transfer. Genetic systems, genomics, and biochemistry in each of these is in its infancy.

There is considerable interest in the enrichment and isolation, identification and metabolic characterization of as of yet unknown microorganisms that may function even better than our current workhorse study subjects (*Shewanella* and *Geobacter*). What are unexplored extreme environments to be mined? There are excellent opportunities to explore electron transfer by diverse organisms, either to find more effective strains or to establish the uniqueness of *Shewanella* and *Geobacter.* However, there are some concerns regarding timescale of these experiments and the necessary resources needed for a widespread survey. These counterbalancing factors need to be further considered.

Is the extracellular matrix in multispecies community inert, or does it influence organization of electronically active units to help collective behavior? What is the molecular level organization that aids in electron transport within a biofilm?

There is a need to understand EET in membrane bound proteins by using reasonable methods that give structural insight when the organisms from which they are derived are challenging or impossible to culture. Such information could yield structural, rather than sequence, characteristics useful for understanding and mining for novel anode and cathode organisms.

*iii) Chemical Transformations by Bacterial Electron Transfer*

Mixed communities for concomitant C and N removal can be envisioned and have been experimentally verified, yet there remains a lack of tools for understanding how partners function together to achieve this goal. What are specific new chemical synthesis that can be accomplished via electron transfer? What selectivity improvements are afforded by direct electron transfer in contrast to diffusion based counterparts? What is the maximum length scale for macroscopic electron transfer? There is interest in engineering interactions in biofilms used in multi-chambered devices to achieve greater productivity and selectivity. Part of the problem is the absence of a coherent model that explains community behavior.

*iv) Emerging Technological Opportunities*

- Decentralized and portable biodegradation opportunities. For example, food degradation and human waste. Electrode/microbe synergy for bioremediation via reduction, i.e. Cr and Se.
- Biomaterials derived from conductive proteins.
- Biodegradable electronics.
- Manufacturing of nanoscale materials through electron transfer.
- Corrosion sensing and environmental sentinels.
- Electronic recording of microorganism metabolism to determine cell viability.
- Hybrid power generating systems for unattended sensors.

## Appendix 1. *Agenda*

**Biological Electron Transfer Meeting**
The Frontier - Research Triangle Park
800 Park Offices Dr, Research Triangle Park, NC 27709
September 15-16, 2015

### Tuesday, September 15

**0845-0900** Introduction
Robert Kokoska, US Army Research Office; James Sumner, US Army Research Laboratory; Guillermo Bazan, University of California, Santa Barbara

***Applications to environmental and waste water remediation***

**0900-0925** *Current barriers to implementation of bioelectrochemical systems in energy and resource recovery from wastes*
John (Jay) M. Regan, Pennsylvania State University

**0925-0950** *BES's for removal of critical contaminants by mixed culture biofilms: Opportunities in electrode and reactor design*
Andrew Schuler, University of New Mexico

**Status of applications for energy generation and electromicrobiological synthesis of novel compounds and the use of carbon dioxide as a starting material**

**0950-1015** *Rewiring the synergy of the microbial world*
Gemma Reguera, Michigan State University

**1015-1040** *Synthetic Electromicrobiology to Improve Microbe-Electrode and Microbe-Microbe Electron Exchange for Bioenergy and Biosensing*
Derek Lovley, University of Massachusetts

**1040-1100** Break

**Mechanism of electron transport and exchange between microbes and external surfaces or within microbial communities**

**1100-1125** *Crossing the Geobacter inner and outer membrane: how pathways change from metals to electrodes*
Daniel Bond, University of Minnesota

**1125-1150** *Bacterial responses to changes in surface potential and redox conditions: a few knowns, and many unknowns*
Kenneth Nealson, University of Southern California

**1150-1215** *Microbial Electrochemistry vs. Electromicrobiology*
Lenny Tender, Naval Research Laboratory

**1215-1315** Lunch

**1315-1340** *Integrating 'omics', microscopy, and electrochemistry to understand biologically mediated electron transfer at the biocathode*
Sarah Glaven, Naval Research Laboratory

**Structural basis for charge transport in biological materials**

**1340-1405** *First principle calculations of electronic transport in organic and bio-systems using atomistic structures*
Sergei Tretiak, Los Alamos National Laboratory

**Interfacial layers to promote charge transport across the biotic/abiotic interface**

**1405-1430** *Interfacial interactions in bacterial physiology: motion, biofilm formation and charge transfer*
Linnea Ista, University of New Mexico

**1430-1455** *Packing membrane proteins at high densities in biomimetic membranes and interfacing them with electrodes*
Manish Kumar, Pennsylvania State University

**1455-1520** Break

**1520-1545** *Improved electron transfer across biotic/abiotic interfaces: new materials and membrane modifiers*
Hengjing Yan, University of California, Santa Barbara

**1545-1610** *Conjugated oligoelectrolytes: mechanism of function*
Rick Dahlquist, University of California, Santa Barbara

**1610-1700** Organization for next day's breakout session
Open discussion

**Biological Electron Transfer Meeting**
The Frontier - Research Triangle Park
800 Park Offices Dr, Research Triangle Park, NC 27709
September 15-16, 2015

## Wednesday, September 16, 2015

**0900** Start

**0900-1015** Breakout Sessions
Specific participants to be determined at the meeting.
(a) Fundamental Scientific Challenges
(b) Emerging Technological Opportunities

**1015-1030** Coffee Break

**1030-1200** Open Discussion
Draft of a summary on research opportunities for the next 5-10 years

Appendix 2. *Summary Notes from Presentations*

**Talk 1: *Current barriers to implementation of bioelectrochemical systems in energy and resource recovery from wastes* -John M. Regan, Pennsylvania State Univeristy**

- Bioelectrochemical systems (BESs) are emerging technologies with potential for the following benefits in wastewater treatment:
    - Require no aeration energy
    - Result in less sludge
    - Are energy positive
- Commercialization of BESs is just beginning
- Challenges associate with BES technology:
    - Nitrogen removal (passive to avoid aeration energy, but impacts of nitrates)
    - Costs:
        - Electrodes
        - Separator
        - Membranes
        - Power management
        - Gas/product separation
    - To be economically practical, costs must be less than $100/m$^2$ and obtain currents greater than 500-100 A/m$^3$
        - Technology using membraneless systems is close to cost metric but not the current density
- Overview of system performances
    - Most studies have used:
        - high acetate concentration
        - Significant buffering
        - Moderate conductivity
        - Stable temperatures
    - Few studies have been performed in real conditions, including:
        - Low substrate concentrations
        - Range of kinetics
        - Low conductivity
        - Less buffering
        - Variable composition and temperature
    - Implications of wastewater properties:
        - Poor COD removal
        - Lower power/current index
        - AC cathode fouling?

- Some pilot scale studies have been performed (breweries, wineries, municipal wastes)
    - Two of these studies had high waste concentrations, setting them up for initial success. . However, problems with:
        - Low conductivity
        - Fouling of cathode
        - Production of methane instead of hydrogen
    - Municipal wastewater treatment had great COD removal along with:
        - Low power densities
        - Low coulombic efficiency, clogging
- No real trends were observed in wastewater fed power densities as a function of volume
    - The cathodes were undersized as reactor sizes increased
- Efforts have been made to incorporate nitrogen removal
    - Most rely on side stream aeration followed by denitrifying the biocathode
        - Drawbacks: ammonia crossover to cathode, requires aeration energy
        - Strategies to mitigate: pre-enrichment of a nitrifying cathode biofilm followed by MFC
            - Ammonia removal rate and efficiency increased with increasing gas diffusion
        - Cell voltage increased due to higher cathode potential (maybe pH related)

    - Anode vs Nitrate competition
        - Nitrate availability controls reactions, not anode potential
            - The flux of nitrate into the biofilm is key
                - Nitrate is preferentially used over anode
            - Nitrate concentration can change the flow of electrons (anodic vs cathodic) and is dependent on initial electrode potentials
- Can a low-pH biocathode from AMD inoculum be developed?
    - Low pH biocathodes from AMD induce higher currents than abiotic controls
    - Pyrosequencing showed presence of *Acidithiobacilus ferroxidans*

**Talk 2: *BESs for removal of critical contaminants by mixed culture biofilms: Opportunities in electrode and reactor design-* Andrew Schuler, University of New Mexico**

- Focused on wastewater treatment

    - Removal of organics, nutrients, and reduction of heat and electrical energy
    - Listed “newer” goals (found on slides)
- Biofilms have been increasingly used for wastewater treatment
    - The biggest challenge is nutrient removal
    - Autotrophic denitrifiers are enriched on the biocathode
    - External nitrification cells are attached to the systems
    - Little work has been done on phosphorus removals
        - Struvite precipitation is the most common method (Zhang, 2012)
    - Have been tested for trace organics removal, including:
        - Pharmaceuticals
        - Pesticides
        - Industrial chemicals
        - Natural and synthetic hormones
- Surface modification of electrodes has been studied
    - Electrode modification using SAMs has been studied
        - SAMs and pure cultures showed high surface energy surfaces favored nitrifier attachment
            - Surface energy correlated with specific nitrification rate
                - Higher biomass
                - Provided higher hormone removal rate
        - Studied surface energy effects on MFC electrodes (*Shewenella*)
            - Highest surface energy gave highest current
        - AFM images revealed different biofilm structures on different SAMs
    - Looked at mixed cultures (sludge)
        - Saw similar results; higher surface energy gave higher current
        - Showed PCA data of bacteria species vs surface chemistry
    - Looked at many other surface modifications
- Effects of electrode physical features (macro scale)
    - Carbon felt, panels (thin and flat), graphite fiber
        - Spacing between electrodes can be critical
    - Roughness changes did not correlate with electrode performance
    - Skewness did affect the performance
    - Activated carbon cathode (effects of temperature and pressure)
        - Saw best performance at 200°C
- Studied millimeter-scale features via 3D printing
    - The depth of channels had an effect; observed lower ammonia flux drop as the channel depth increased
    - Looked at different geometries via micro fabrication

**Talk 3: *Rewiring the synergy of the microbial world* - Gemma Reguera, Michigan State University**

- Life is all about electron transfer
  - Goal is to tailor organisms for efficient electron transfer reactions
- Geobacter
  - Capable of interspecies electron transfer
    - Metal traps for cations along wires
  - Would like to build biomimetic systems that replace the organism
    - Would remove biological noise
  - For protein filaments – need to understand how aromatic amino acids show electrons a proper pathway for travel
- Synergy using Geobacter
  - Mix a fermentative microbe with a Geobacter b
    - Rationale – in any fermentation you will have waste byproducts; if you remove the byproducts by genetic engineering, you can increase efficiency of fermentation, and thus increase the power produced by the system
  - Looked at conversion of cellobiose to ethanol
  - Looked at microbial electrochemical reactors for the clean-up of human urine
  - Looked at the conversion of glycerin to ethanol and propane diol
    - Evolved strains to tolerate glycerin and ethanol/propane diol
    - Currently scaling to 500L fermenters to increase efficiency of the reaction
  - How far can we push this idea?
    - What about conditions with oxygen?
      - Cyanobacteria produces oxygen as a byproduct and produces hydrogen
        - Can the hydrogen be used to create a bioelectrode?
      - First, they need to isolate/evolve oxygen-tolerant mutants of Geobacter
        - Idea is that they would get more hydrogen production when these Geobacter strains were added to cyanobacteria
      - Synthetic synergistic interaction
        - When Geobacter was added to cyanobacteria, they grew in a filamentous form

**Talk 4: *Synthetic Electromicrobiology to Improve Microbe-Electrode and Microbe-Microbe Electron Exchange for Bioenergy and Biosensing* – Derek Lovley, UMass Amherst**

- Interested on syn bio approaches to improve processes
    - Microbial electrosynthesis to produce fuels
    - Biological computing – potential for electrical input/output instead of chemical input/output
    - Corrosion – the uptake of electrons from metals may be important for corrosion
    - Potential for new electronic materials (wires, transistors, etc.)
- What is the mechanism of long range electron transport in Geobacter?
    - Hypothesis – electrically conductive pili (e-pili)
        - Cytochromes are decorated along the length of the pili, but they are spaced too far apart for electron hopping between proteins (100-fold too far apart)
        - If cytochromes are not mediating electron transport along pili, what is?
            - Hypothesis – metallic-like conductivity (like polyaniline) mediated by overlapping pi-pi orbitals
                - If they mutate aromatic residues to alanine, conductivity is removed, suggesting that pi-pi orbitals are important
                - If they engineer pili from another strain into Geobacter, they do not get conductive pili, also suggesting that sequence is important and thus pi-pi orbitals from aromatic residues are critical for electron transport
- E-pili are like carbon nanotubes which transport electrons by metallic-like conductivity
    - The packing of individual pili proteins allows for the pi-pi stacking that mediates electron transport
- If cytochromes are involved in electron transport, what is their role?
    - Cytochromes are important for iron reduction
- Using the metallic-like conductivity model to engineer pili leads to a million-fold improvement in electron transport
- If you remove or mutate the cytochromes, the results are not consistent with cytochromes being involved in electron transport
- The e-pili model makes good predictions for syn bio improvements
- Direct interspecies electron transport (DIET)
    - Pili are required
    - Can use synthetic analogues of pili to increase anaerobic digestion
- MURI topic concept
    - Green electrical components
        - Would require microbiologists and material scientists

**Talk 5:** ***Crossing the Geobacter inner and outer membrane: how pathways change from metals to electrodes*** **– Daniel Bond, University of Minnesota**

"The membrane is where energy is made."
The cell membrane represents two hydrophobic barriers for electron transport. This is a very challenging barrier and cells try to extract a benefit from electron transport.

In order to gain more insight to this process the Bond lab has been working to expand the Geobacter genome database beyond pure isolates. They are using Tn-Seq to identify essential proteins at different redox potentials. They discovered several novel cytochromes associated with the inner membrane that are essential for growth at low redox potentials. They have found that the potential is the driving force that determines which pathway the to transport electrons.
Experiments performed with Geobacter that was grown on 17 different metal oxides showed that Geobacter was able to sense the redox potential and use the best pathway. At low potential ChcL was the predominant pathway for electron transfer while at high potential ImcH was the predominant pathway.

They have also been looking at outer membrane electron conduits. Knocking out the multi-heme cytochrome OmcB did not affect electron transport although they are necessary for Fe(III) and Mn(IV) reduction.
They identified a new extABCD pathway for electron transfer that is not involved in Fe(III) and Mn(IV) reduction. In contrast chemosensory gene knockouts don't effect Fe reduction but does effect electrode attachment. From these lines of evidence they hypothesized that electrodes are treated as "friends".

The team is now looking at multi-species interactions with engineered consortia and are labeling biofilms to determine where cells are active.
Some conclusions that have been attained:

-Geobacter adjusts output redox potential
-Geobacter shifts pathways during reduction
-Geobacter senses the difference between metals and "friends"

More work is needed to identify additional cytochromes
Still many unknowns regarding outer membrane electron conduits

**Talk 6:** ***Bacterial responses to changes in surface potential and redox conditions: a few knowns, and many unknowns*** **– Ken Nealson, University of Southern California**

The presentation described the importance of surface potential for electric bacteria. The long-term effects of surface potential include attachment and biofilm formation.

The short term effects include sensing and responding to changes in surface potential.
The work primarily focused on *Shewenella*.

Recent work has focused on isolating novel species from sediments using a poised electrode. So far his team has isolated 14 new uncultured genera from 10 phyla. All isolates had different potentials than *Shewenella* and did not contain any known cytochromes. Most were not from genera known to be involved in extracellular electron transfer and they appear to only grow on the electrode.

Either an anode or cathode can be used for enrichment and the populations are distinctly different at different surface charges. The community that develops is very stable long term (3 years). The community is also capable of short-term responses to changes in potential. They found that 60-100 gene groups changed in response to a 100 mV change in potential in 1 hr. Microbes in the community that are not known to be associated with electron transfer had the best response. These could represent new mechanisms of electron transfer.

Outstanding questions remain. How do they take up electrons in the absence of known pathways? What are the roles of nanowires or excreted flavins? This work has implications for fields as diverse as medicine and corrosion control.

**Talk 7: *Microbial Electrochemistry vs Electromicrobiology* – Lenny Tender, Naval Research Laboratory**

An excellent overview on the relevance of cyclic voltammetry (CV) and related electrochemical characterization techniques was provided. The NRL team is interested in bio-electron transport from proteins to electrodes and the redox conductivity of electrons through soft materials.

The CV of biofilms exhibits Nerstian behavior. The magnitude of the CV curve is driven by the microbe and the concentration and spacing of the electron transporter.

Electrochemical gating experiments that push electrons from one electrode to the other have shown that this transfer is not dependent on the metabolic state of the cells.

Confocal Raman spectroscopy of biofilms has showed that cytochromes are denser near the electrode. This insight suggests they play a role in mediating the transfer of electrons between cells and the electrode.

**Talk 8: *Integrating 'omics', microscopy, and electrochemistry to understand biologically mediated electron transfer at the biocathode* – Sara Glaven, Naval Research Laboratory**

This work has focused on integrating an "omics" understanding of a microbial consortium. One of the challenges faced in the field is that direct electron transfer at the cathode is not well understood.

The team's strategy has been to use a MFC for enrichment of microorganisms from environmental samples and characterized the community on the cathode. Both photosynthetic and dark reactors were tested. The dark reactor was selected for further characterization because it had significantly higher current. They were able to stably cultivate it on a cathode.

The community was sequenced and showed high diversity. Following additional enrichment there was less diversity but many of the same organisms were there. Alpha and gamma proteobacteria were the predominant organisms.
DNA, RNA, and protein sequencing were performed along with electrochemistry to characterize the community. Members of the family Chromatiaceae were implicated in electron transfer and an electron transfer pathway was proposed. Intensity mapping of the biofilm provided some insight into the biofilm surface characteristics.

This work is particularly meaningful in that it demonstrates that cathodes provide a mechanism for stably cultivating previously uncultivable organisms and a means of monitoring the community. However, several outstanding challenges remain. Efforts have been unsuccessful in getting pure cultures from the cathode. There are unknown benefits for the organisms by being part of the consortia. More work is also needed on the metabolome and surface chemistry.

**Talk 9: *First principle calculations of electronic transport in organic and bio-systems using atomistic structures*– Sergei Tretiak, Los Alamos National Laboratories**

This presentation provided an overview of theoretical approaches used in determining the charge transport properties of organic semiconductors, particularly on crystalline solids in which the exact orientation and distances between aromatic fragments is fully understood through information obtained by single crystal x-ray diffraction studies.

Approaches to modeling charge transport through Geobacter pili were also presented. These methods are similar to modeling organic photovoltaics which are composed of amorphous and crystalline sections randomly organized.

For accurate modeling of electron transport more structural information is needed. Of particular interest is to determine if electron transport in pili are due to band vs. hopping mechanisms. For wire like properties hopping transfer is needed. Marcus theory for charge transport models requires the wave function overlaps to be understood.

Discussed different versions of models:

-1 The initial model looked at aromatic residues and their location and indicated there would be no coupling and no conductivity

-2 The second generation model used a refined pilA structure which indicated aromatic residues were in closer proximity than previously thought. Was not sufficient to show conductance but there was some charge hopping. Not metallic like indicating disparity between this particular model and experiment.

-3 A third structure was used with even closer association of the aromatic residues. Simulation showed conductivity similar to structure 2. Still hopping but closet to metallic like.
Structure fluctuations may be responsible for metallic properties but to determine this better structural information is needed. Also molecular dynamics may be needed in the modeling to account for movement of aromatic fragments.

**Talk 10:** ***Interfacial interactions in bacterial physiology: motion, biofilm formation and charge transfer*** **– Linnea Ista, University of New Mexico**

- Effect of biofilms on EET
- A discussion of type iV pili important in S. Oneidensis revealed uncertainty on what structural units are required for achieving conductivity.
- Need to understand the effects of biofilm formation and structure on EET.
- The % coverage verus pil mutants, dflg-still biofilm, all others dramatically reduced
- SAM microscopy experiments shown on planar substrates.
- Tracking twitching and swarming.
- Orientation of cell binding versus SAM head group tracked.
- Nanoparticle work summary.
- Aritificial Biofilms for Sanitary / Hygenic Interface Technology – Gates foundation SEED grant, Engineered consortia from pit toilet remediation.
- DTRA – interested in motility on SAMS.

**Talk 11:** ***Packing membrane proteins at high densities in biomimetic membranes and interfacing them with electrodes*****– Manish Kumar, Pennsylvania State University**

- Artificial Membranes with proteins to build systems (lipids and block co-polymers) that can be integrated into energy generating systems.
- Glucose powered enzymatic fuel cell example.

- Problems: protein orientation, electronic coupling, denaturation / lifetime
- Works with PSI (cyt c fused) and future PSII.
- The application of conjugated oligoelectrolytes modified PLBLs as the electrode modifier and achieved significant power enhancement of Phtosystem I two dimensional crystals.
- There is interest in increasing the diversity of synthetic materials to both help the self-assembly of the devices and to promote improvements in power generation. An example includes the integration of block co-polymers.

- **Talk 12: *Improved electron transfer across biotic/abiotic interfaces: new materials and membrane modifiers*– Hengjin Yan, University of California, Santa Barbara**

- There are a variety of approaches being investigated toward promoting electron transport across biotic/abiotic interfaces by using artificial materials. These techniques typically include synthetic materials that promote adhesion to electrode surfaces and/or that try to improve the bioelectrochemical/bioelectronics features of charge extraction or injection into microbial systems.
- Conjugated oligoelectrolytes (COEs) were introduced as molecular systems with structural attributes that facilitate membrane intercalation and optical features that allow the speed of insertion into microbes together with confirmation of the orientation of the molecular insertion relative to the membrane structure.
- COEs allow increases of charge extraction from a variety of microorganisms, including yeast and E coli, as determined by using a microbial fuel cell setup as the test bed for examining relevant electronic behavior.
- One finds that COEs can actually promote the establizhing electrogenic biofilms with the existing microbial population present in wastewater.
- COEs can also be used to perturb the selectivity of microbial electrosynthesis biofilms by represing the formation of methane generation, while allowing the generation of hydrogen to be unperturbed.
- Mechanistic studies with Shewanella indicate the COEs need to couple their function with the available electron transport multiprotein structures available in the microbe. Less is certain on how COEs improve the charge extraction from yeast and E coli, for which no such previous information is available.
- There are excellent opportunities to explore the function of COEs to modify the behavior of microbial biofilms, particularly within the context of interspecies electron transport.