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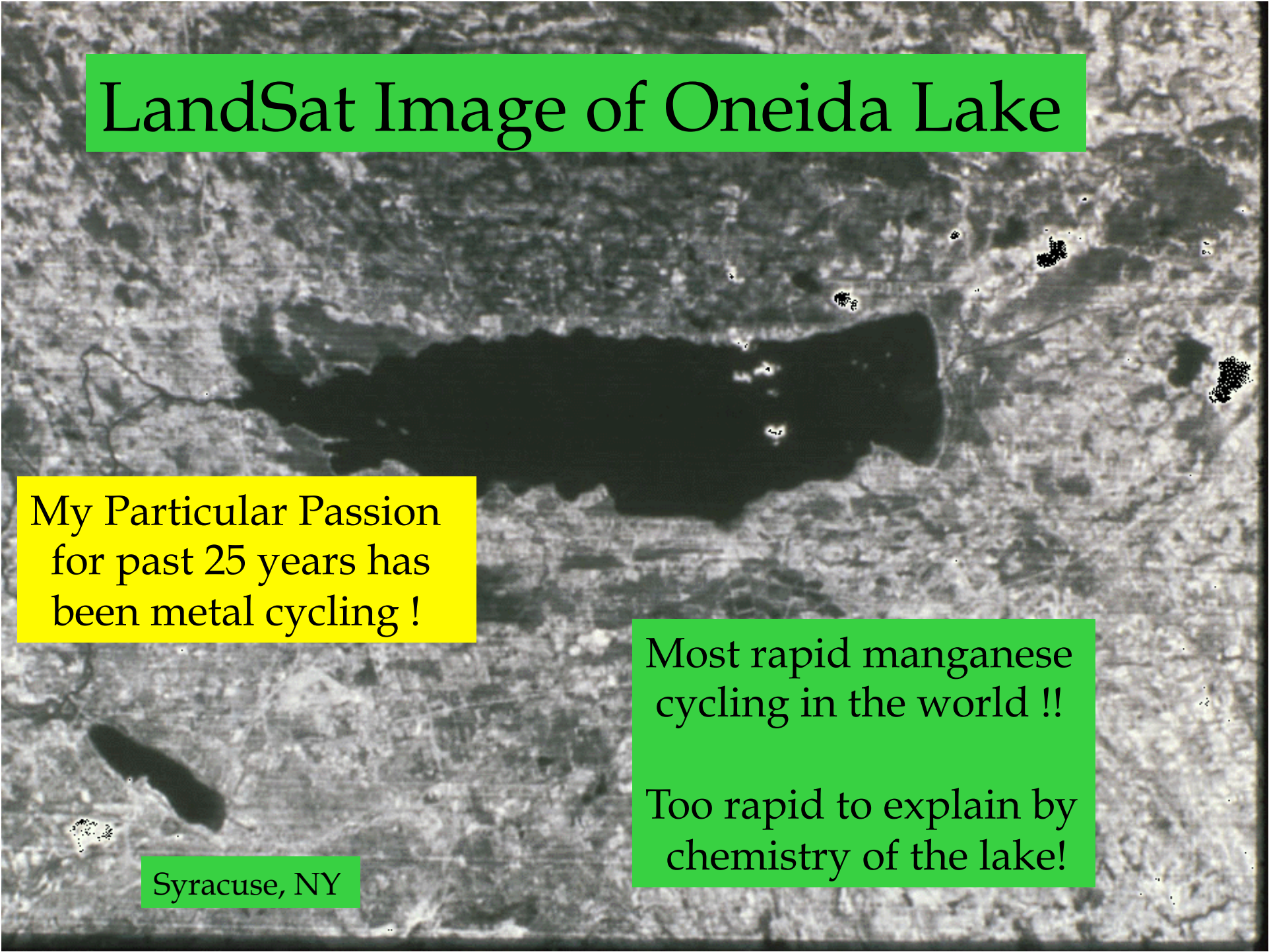
Extracellular Electron Transport:
Activities at the Microbe/Mineral Interface

Presentation at
Caltech
April 10, 2012

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Agenda for the next 30 minutes or so:

1. Discovery of *Shewanella* and EET – setting the scene
2. EET in bacteria -- overview
3. Bacterial behavior in response to surface charge
5. The future – near and far !



LandSat Image of Oneida Lake

My Particular Passion
for past 25 years has
been metal cycling !

Most rapid manganese
cycling in the world !!

Too rapid to explain by
chemistry of the lake!

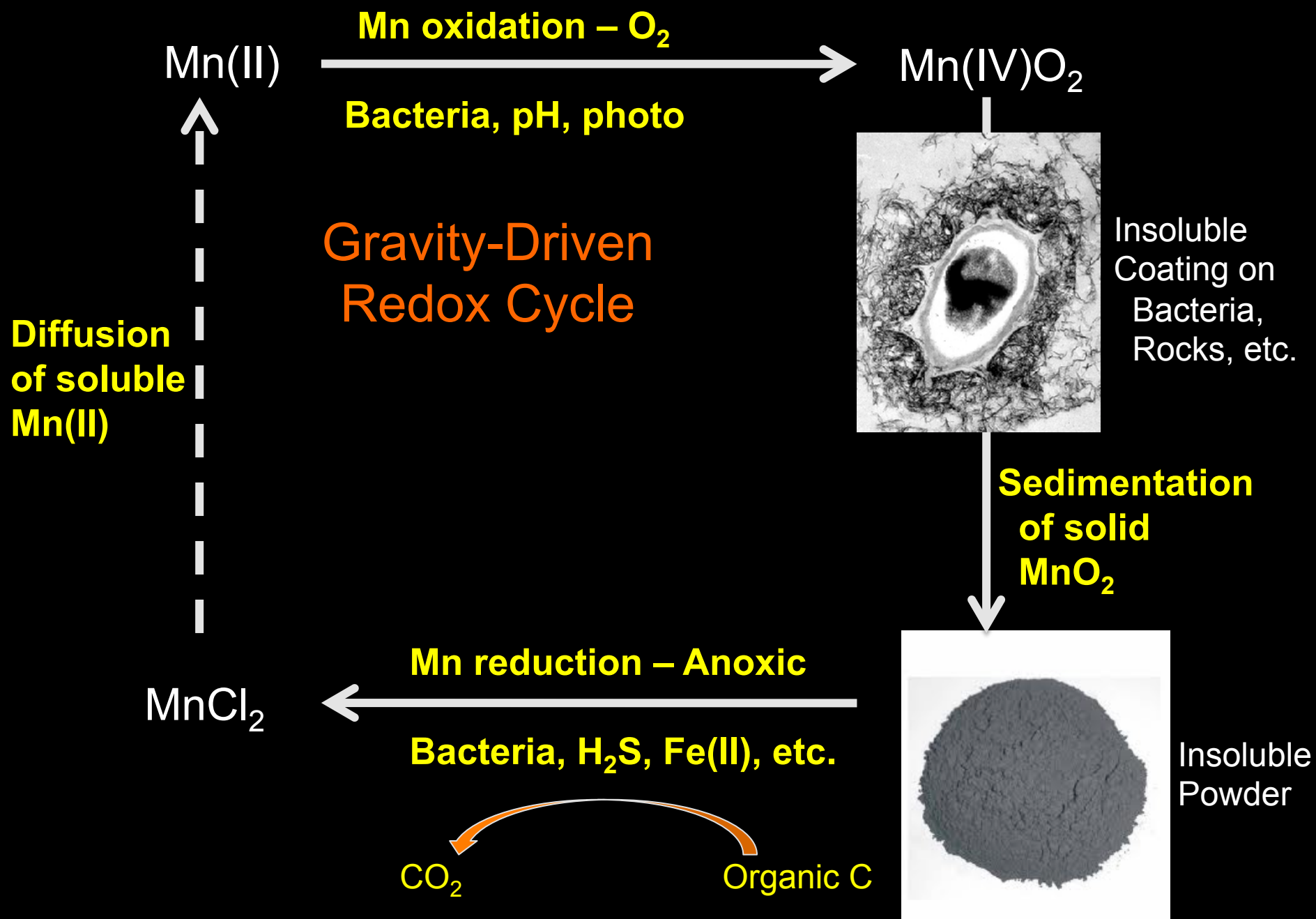
Syracuse, NY

Oneida Lake Sediment Surface

3" Core Taken From Bottom of Oneida Lake

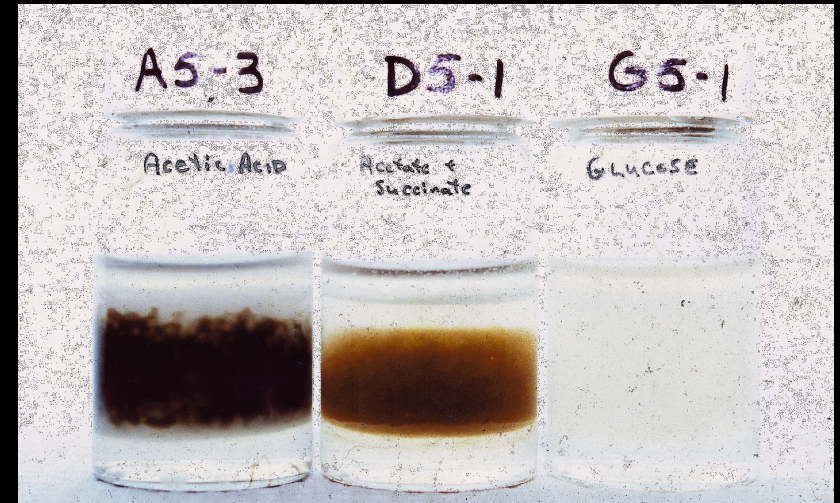
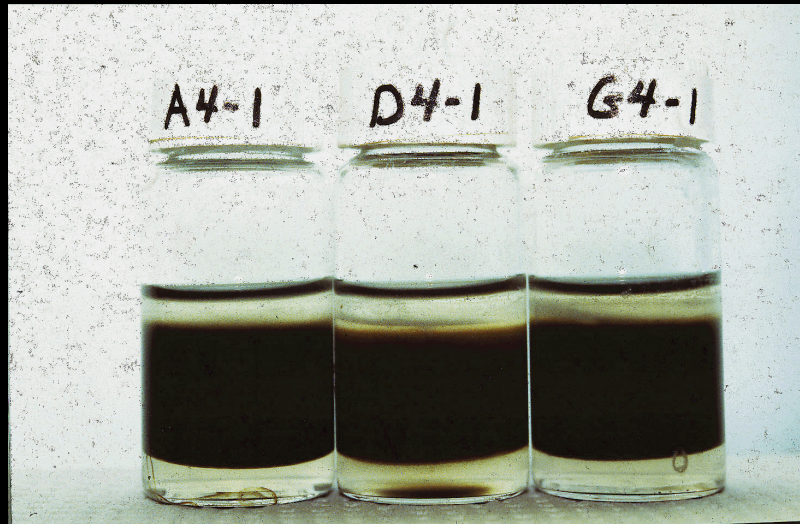
In Oneida Lake, Mn reduction accounts for 50-75% of C turnover in sediments !

FLUX OF REDUCED IRON AND MANGANESE IS ORDERS OF MAGNITUDE TOO HIGH TO BE EXPLAINED BY CHEMISTRY



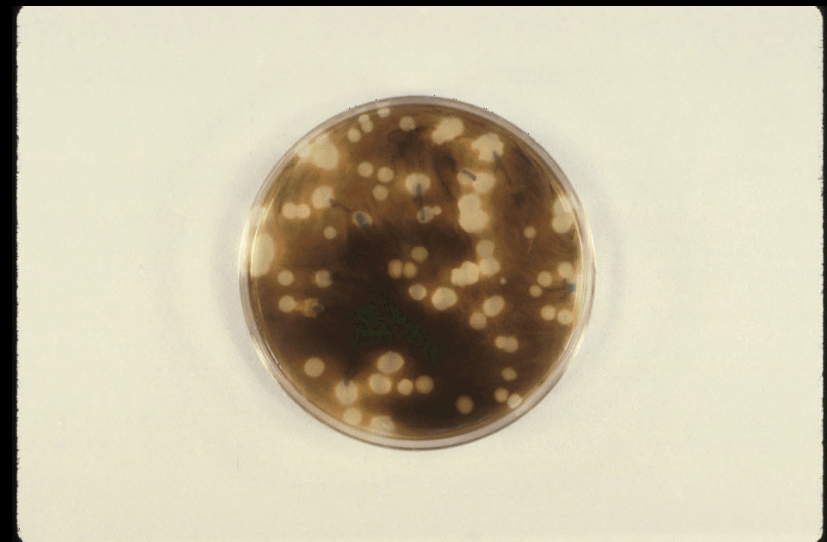
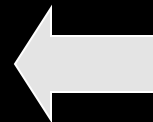
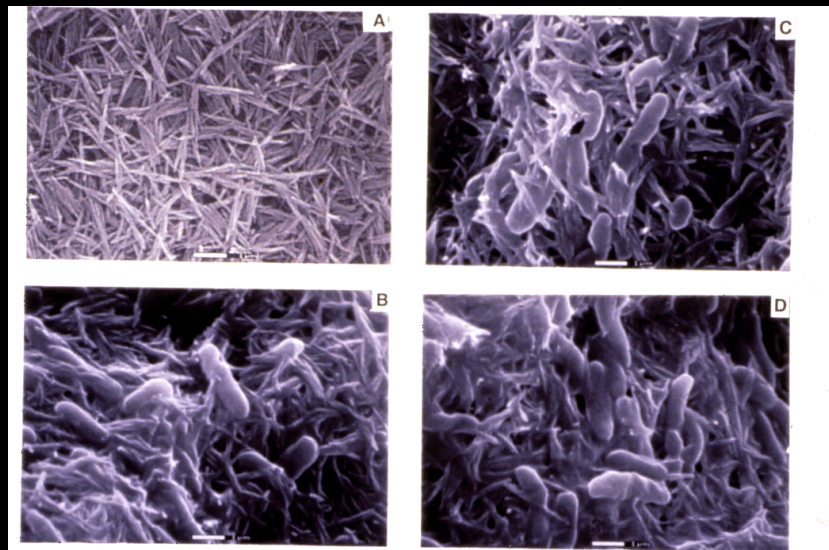
Enrichment Culture – MnO_2 in soft agar

Few days/weeks incubation



Cells on MnO_2

Dilute to soft agar overlay with MnO_2



Shewanella oneidensis – MR-1

e^- donors

Formate

Lactate

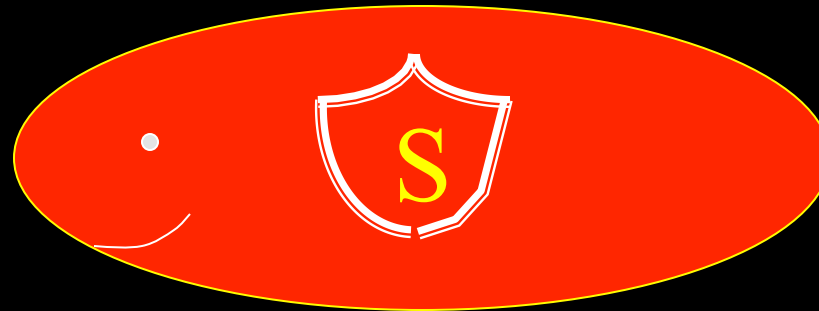
Pyruvate

Amino
Acids

H_2

Mine waste
Black Sea
Oneida Lake
Green Bay

Panama Basin
Mississippi Delta
North Sea Redox Interfaces



O_2

NO_3^- , NO_2^-

Mn(IV)

Mn(III)

Fe (III)

Fumarate

DMSO

TMAO

S^0

$S_2O_3^{2-}$

U, Cr, Tc, As, Se

I, Co^{+3}

e^- acceptors

The most versatile anaerobe on the planet is an aerobe too!

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***Science.* 3 June 1988: 240: 1319-1321**

Bacterial Manganese Reduction and Growth with Manganese Oxide as the Sole Electron Acceptor

Charles R. Myers and Kenneth H. Nealson

Ctr. Great Lakes Studies, University of Wisconsin-Milwaukee, Milwaukee, WI 53201.

***Appl. Environ. Microbiol.* 15 June, 1988: 54: 1472-1480**

Novel Mode of Microbial Energy Metabolism: Organic Carbon Oxidation Coupled to Dissimilatory Reduction of Iron or Manganese

Derek R. Lovley* and Elizabeth J. P. Phillips

U.S. Geol. Survey, Water Resources Div., 432 National Center, Reston, Virginia 22092

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The Good News:

We have two model systems, not one !

The Bad News:

We only have two model systems – need more!

From the beginning, it was clear that these organisms presented a bit of a conundrum !

How to donate electrons to an extracellular solid?

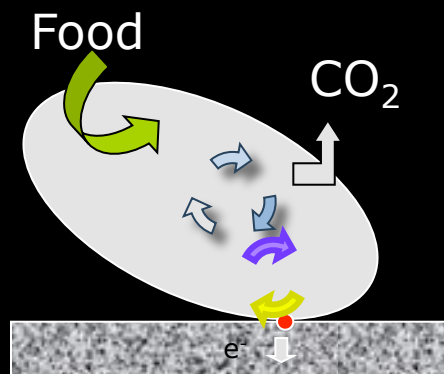
This should be difficult and biochemically challenging

I took a trip to European labs in 1989, and virtually no one was ready to believe this!!

Ensuing 25 years, a number of solutions to this problem have been hypothesized -- some are probably correct !

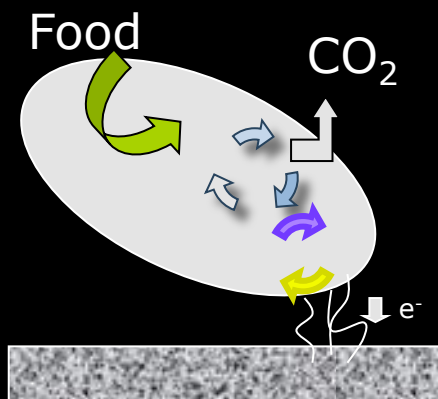
Interacting with solid substrates: the current view

Complex Enzymes
on outer cell wall

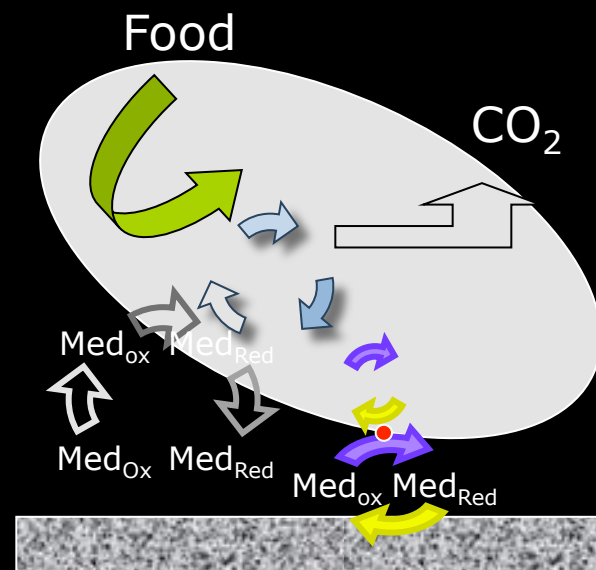


Direct Electron Transfer
(Membrane)

Conductive wires
made by bacteria



Direct Electron Transfer
(Nanowires and conductive
minerals in extracellular
Matrices).



Mediated Electron Transfer

Image adapted from: Schröder, U., 2007. *Phys. Chem. Chem. Phys.*, 9, 2619-2629

The ability to do EET is probably of central importance to many bacteria in the natural environment.

Certainly for *Shewanella* it is a matter of survival

Why not expect a number of solutions to this problem?

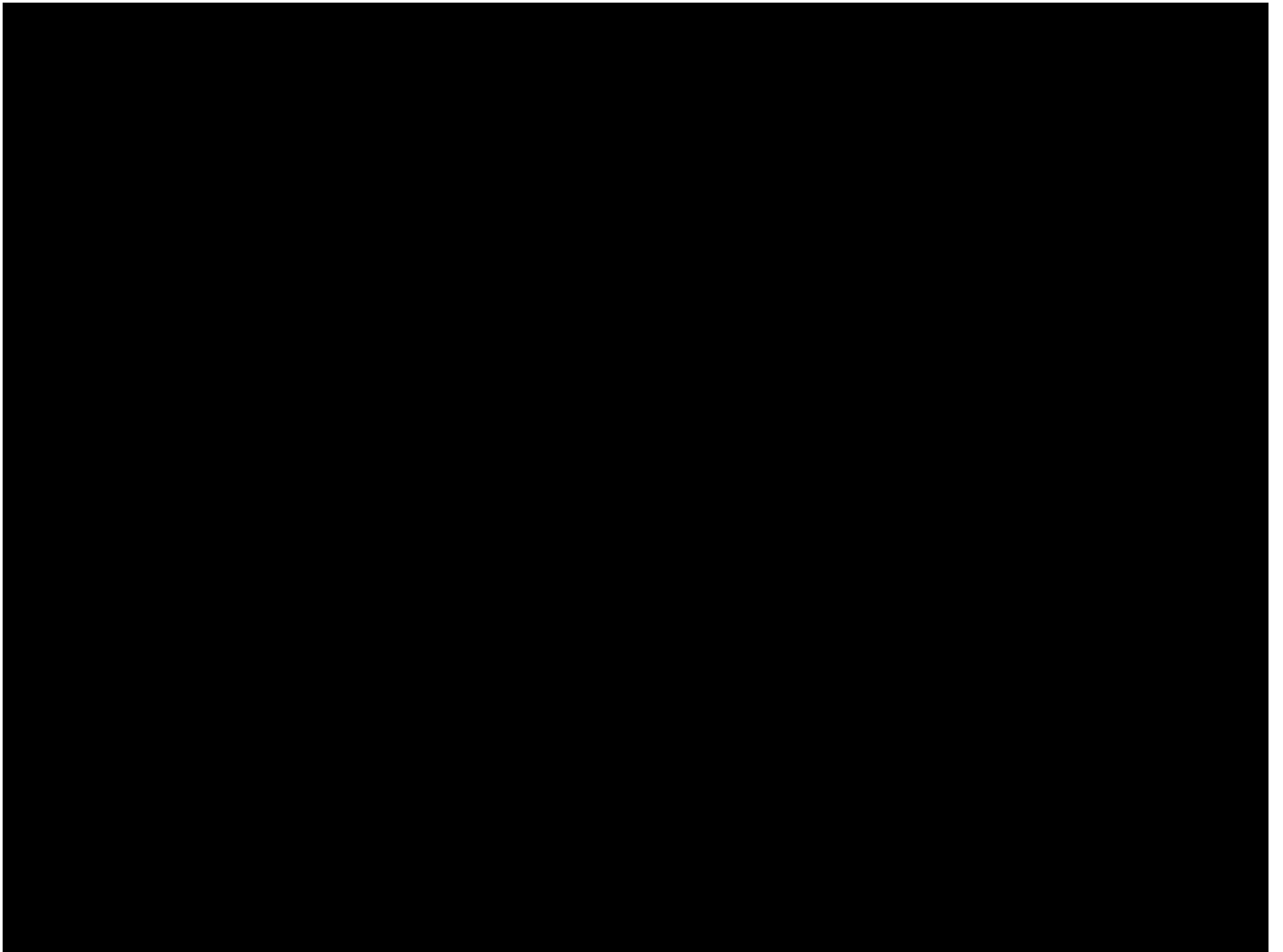
This is what we see !

I will finish my presentation with another variation on the theme -- it involves microbial behavior at the microbe mineral interface

Behavioral interactions with minerals:

First noted when we took our first movies and
really looked at the bacteria and mineral
particles

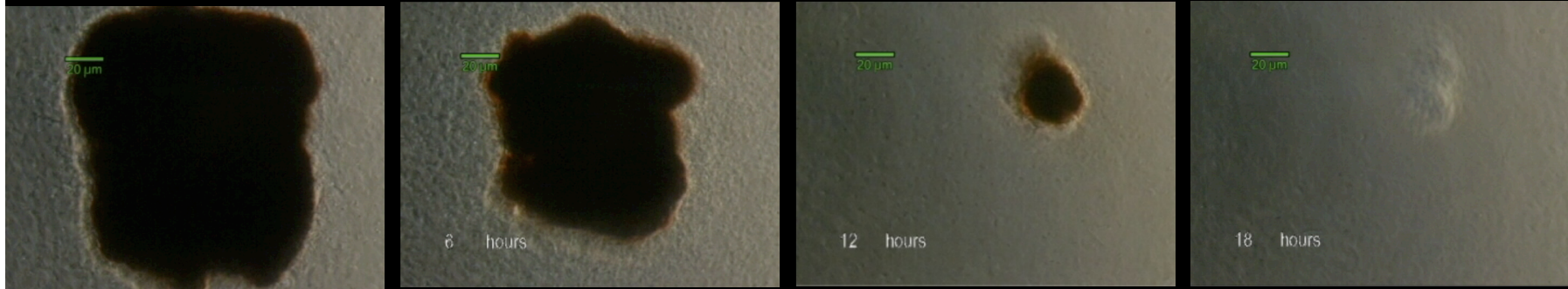
Can be easily seen in the following time-lapse
movie



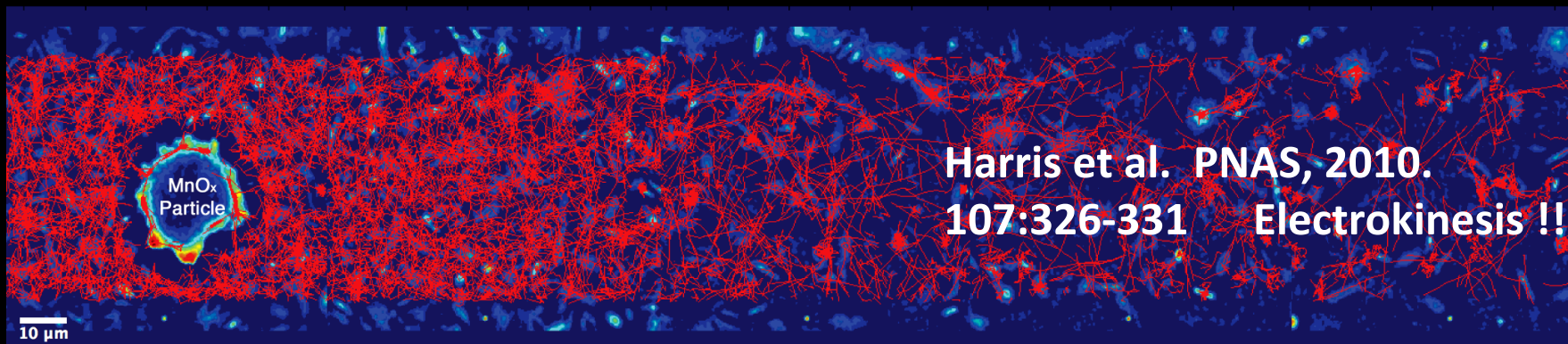
What did we learn from this movie?

- Cells are very motile when oxygen is present
- Motility is retained around MnO_2 particles
- Cells are far less motile away from particles
- When MnO_2 is gone, motility ceases
- Introduction of O_2 restores motility
- Within 48 hours cells begin to die rapidly

It is this interaction of bugs with metal oxides that we have been focusing on, first using the flat capillaries as seen in the movie

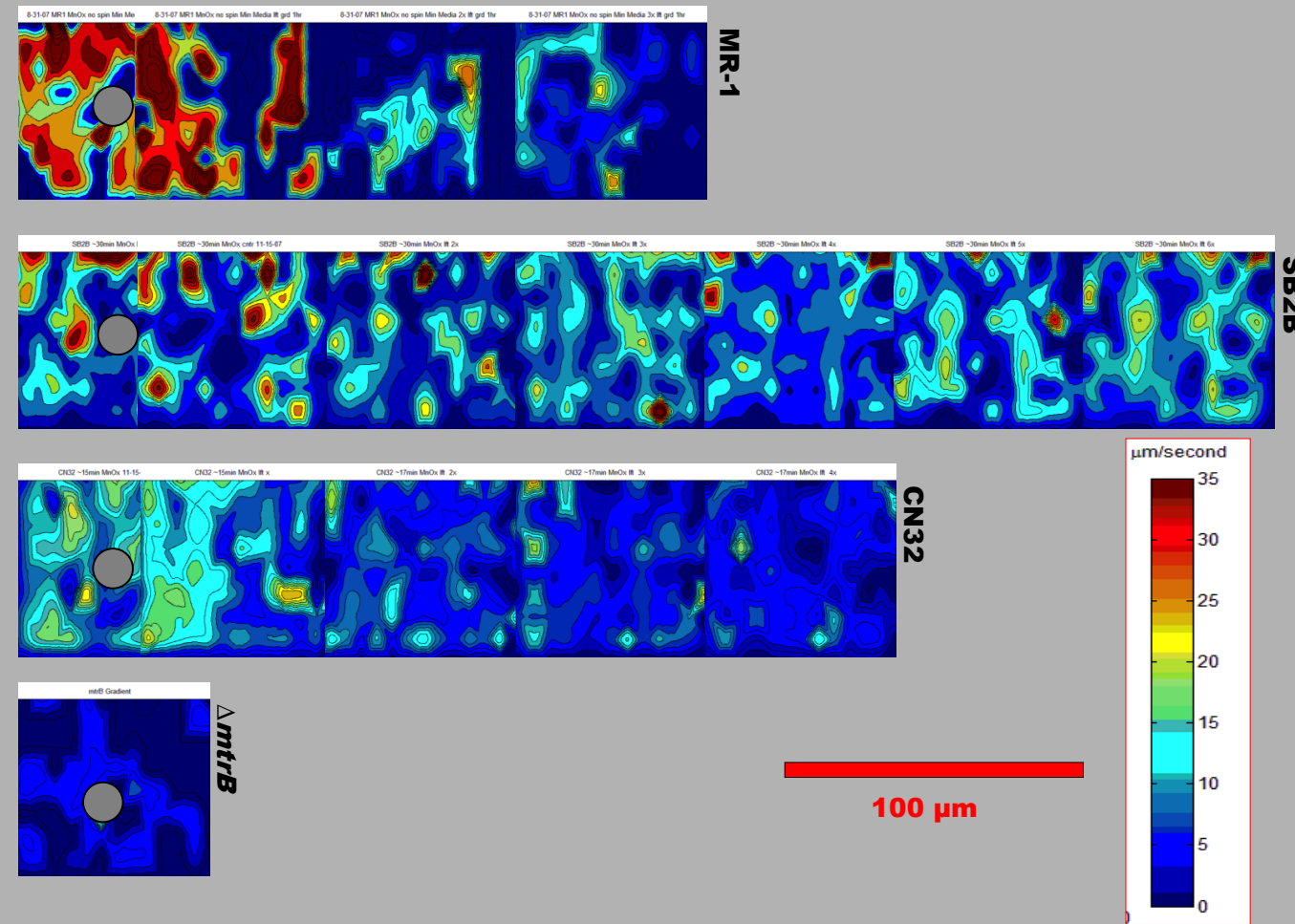


We developed digital tracking algorithms that trace the trajectories and swimming speed of individual bacteria as they reduce MnO_2 particles -- red lines below are swimming tracks -- speed is estimated by program.



Motility Response of MR-1 & other strains and Mutants!

Movement around MnO_2 particles – This is a “heat diagram showing average speed of swimming



Different strains of *Shewanella* are different with respect to swimming responses

Any mutant that inhibits EET also blocks electrokinesis

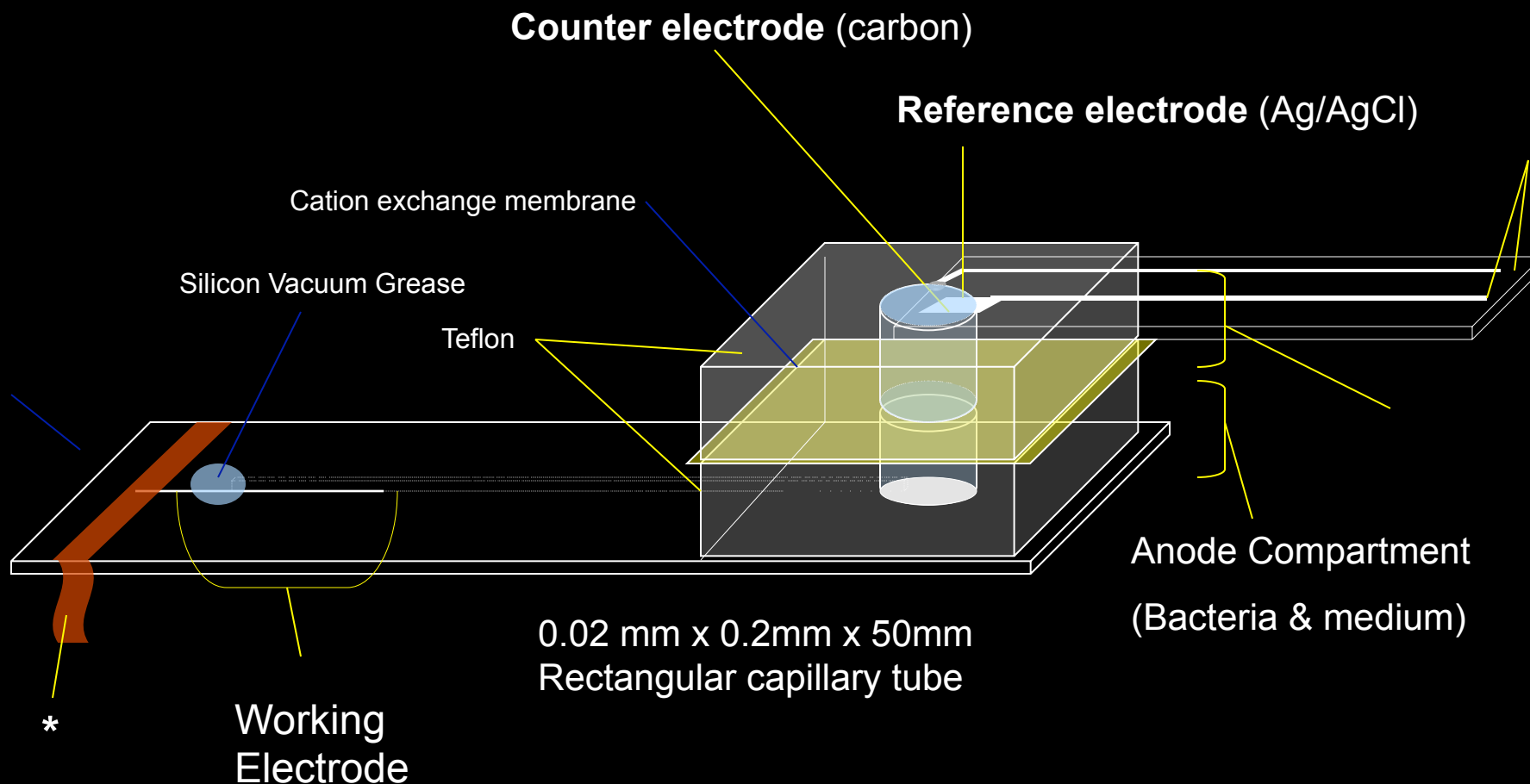
This is a pretty terrible experimental system.

Flat capillary, insertion of metal oxides, visual tracking

Really impossible as an experimental tool

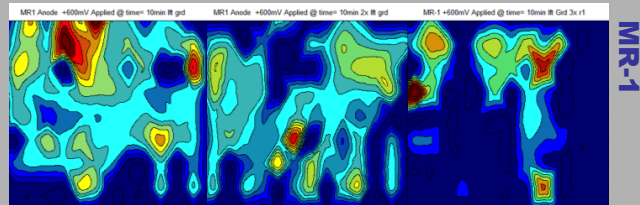
So we constructed a capillary system with a single carbon fiber electrode in it, instead of a metal oxide

Minerals difficult to work with: What about electrodes?

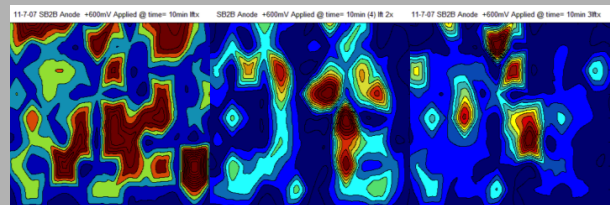


MFC on a slide, with single graphite filament as electrode
Can be run by potentiostat to control potential on electrode

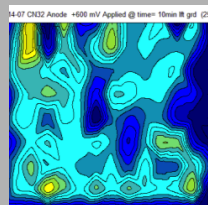
Swimming response to electrode poised at 600 mV



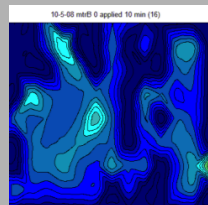
MR-1



SB2B



CN32

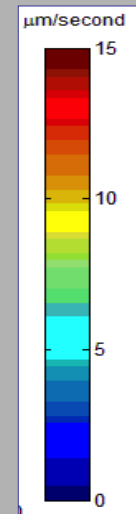


$\Delta mtrB$

Now have a real experimental tool. Can vary voltage. Can open the circuit.

Cells are responding to a potential by swimming
Different strains are different

100 μm



Different strains show different responses

Mutants used to examine the mechanism

ET mutants can not swim in response to potential

These data closely track the Mn oxide data – better system!

What did we learn from our electrode experiments?

1. Electrode poised at proper potential gives a similar response
2. Swimming speed is roughly proportional to surface charge
MnO₂ is best for MR-1, Fe oxides not as effective (slower)
3. Strains show same relative responses to charged electrodes
4. Mutants show same relative patterns
all mutants blocked in EET abolish kinetic response
5. Different strains can be very different in kinetic response

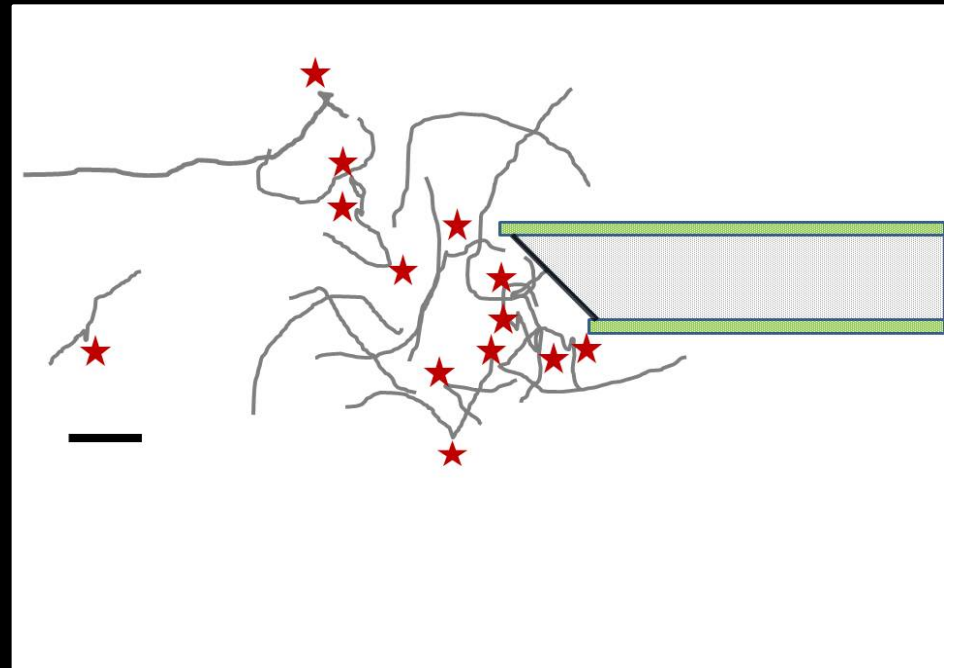
That is: kinetic effects are not enough!

Simply increasing speed will not give this response!

Saw this with Δ CheA mutant -- in *E. coli* this mutant is incapable of flagellar reversal

In *Shewanella*, increased speed is seen, but no focus of activity around the particles

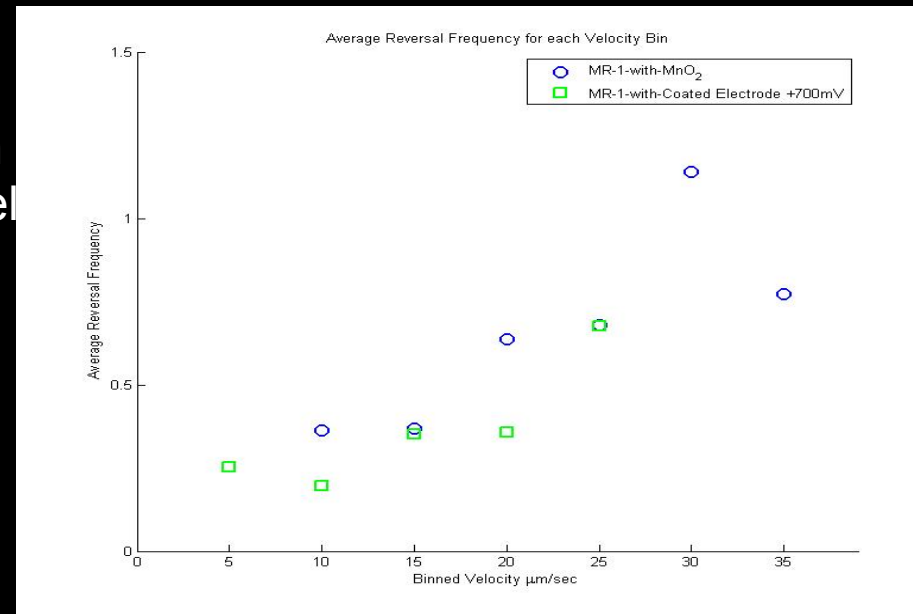
Asked the tracking program to also measure reversals as well as speed

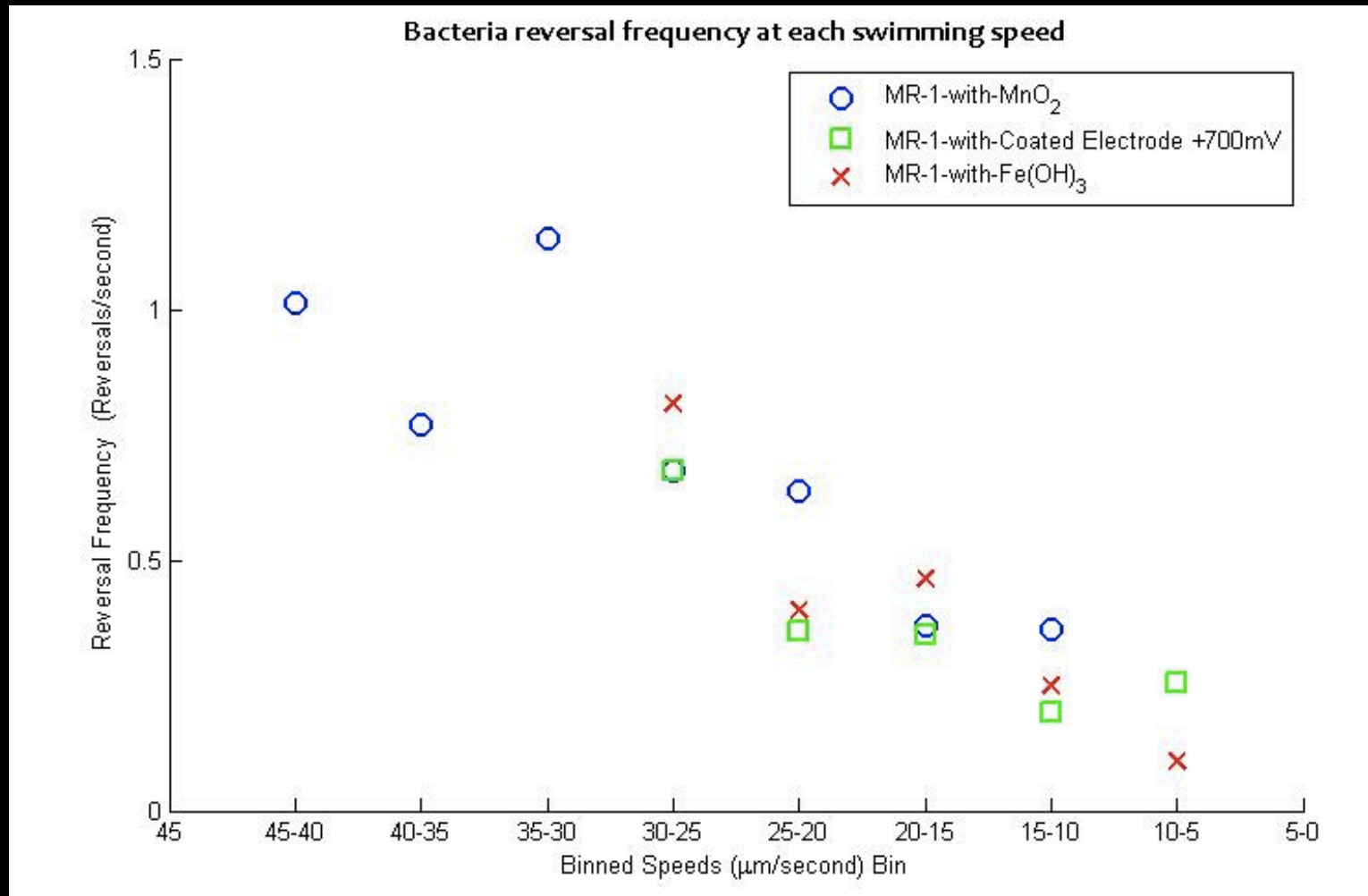


Reversals reveal an unusual phenomenon

Cells near charged surface reverse more often
 Reversal means 180 deg. change – polar flagellum
 Cells return to charged surface !

Very clever strategy





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Congregation

Hypothesis for “sensing” charged
surfaces

Wayne Harris, Mandy Ward, KHN

Hypothesis/Model to explain our results:

Call this process “Congregation” -- It is a simple strategy in which:

- I -- cells contact the electron acceptor & become energized
- II – energized cells become motile and rapid swimming
- III – rapidly swimming cells reverse flagellar rotation often
- IV - result is accumulation of cells in vicinity of electron acceptor



1. Stochastic cell contact & activation

- Cells moving by Brownian motion randomly contact solid electron acceptor
- Cells “dump” electrons and become active swimmers
- Swimming cells “randomly” contact solid electron acceptor and “dump” electrons – swimming rate increases after contact with electron acceptor

2. Flagellar reversal stimulation

- Flagellar reversal results in motion reversal, nearly 180 deg reversal due to single polar flagellum
- Rapid reversals result in accumulation of cells near electron acceptor
- Speed of swimming remains high, contacts increase in frequency
- Result is congregation of cells around electron acceptors (metal oxides or electrodes)

Why make a big deal of this?

- Congregation is mechanism whereby attachment and eventually biofilm formation is enhanced.
- See major differences between strains with regard to attachment
- These differences appear to track the differences in congregation as they do the mutants

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Sooo, I end where I began, with more questions than answers, and:

The distinct impression that somehow, EET is a very important thing, and that adaptation has occurred at nearly every level, including the behavior of the microbes participating.

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As more microbes are found that can participate in EET, I look for the behavioral responses to become an important part of the picture, impacting a number of areas, including:

Geochemistry, Geology, materials science, medicine dental science, and many more

I also expect many young scientists to try to prove this is wrong, and the fun to begin!!

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Remember: It's all about electrons!!

**Thanks for your attention
Ken Nealson**