Metal Cycling by Bacteria: Moving Electrons Around

Seminar Presented to ANL
July 7, 2005

Ken Nealson
Wrigley Professor of Geobiology
USC

First: Some Thanks & Acknowledgements:

1. **Daniel Haskins & ANL for invitation**
2. **Jim Fredrickson & Yuri Gorby (PNNL) physiology & ecology**
3. **B. Hong Kim (KIST); Margie Romine (PNNL) electrochemistry and mutagenesis**
4. **Students and colleagues from USC/JPL labs Orianna Bretschger – fuel cell work**
6. **Funding Support: DOE, NASA, NSF**
7. **Ken Kemner – synchrotron analyses and good times doing science at ANL**
8. **Brian Tonner, Klaus Pecher, Gordon Brown, John Barger, others!**

Today's Menu:

Appetizer	Metal cycling, Biominerals, Microbial metabolism Introduction to MR-1
Main Course	Metal reduction by MR-1
Dessert	Electrochemistry by MR-1
Doggy Bag	Nanowires and the future

USC *Geobiology* *Astrobiology* CENTER for LIFE DETECTION JPL

GEOBIOLOGY: INTERACTIONS OF MICROBES AND MINERALS

Rocks to Eat and Breathe:
Iron or sulfides for lunch
Metal oxides to breathe

Prokaryotes

Rocks for Protection and Structure:
Bones
Shells
Teeth
Si frust.

Eukaryotes

Planetary Chemistry

Magnetite - orientation

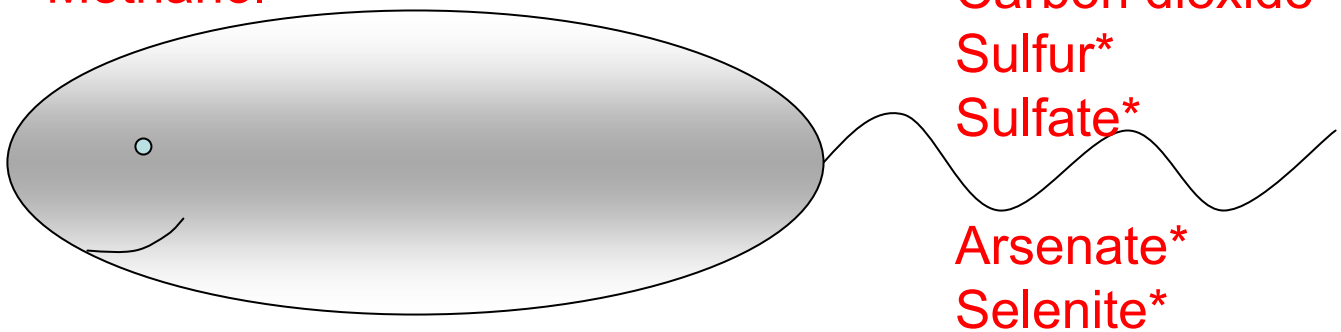
Fossil Record !
Planetary Chemistry!

Fuels (EDIBLES!!)

SUNLIGHT

ORGANICS
- **Glucose**
- **Ethanol**
- Formaldehyde
- Methanol

Hydrogen
Ammonia
Hydrogen sulfide*
Sulfur*
Iron*
Manganese*
Carbon monoxide*
Arsenite*



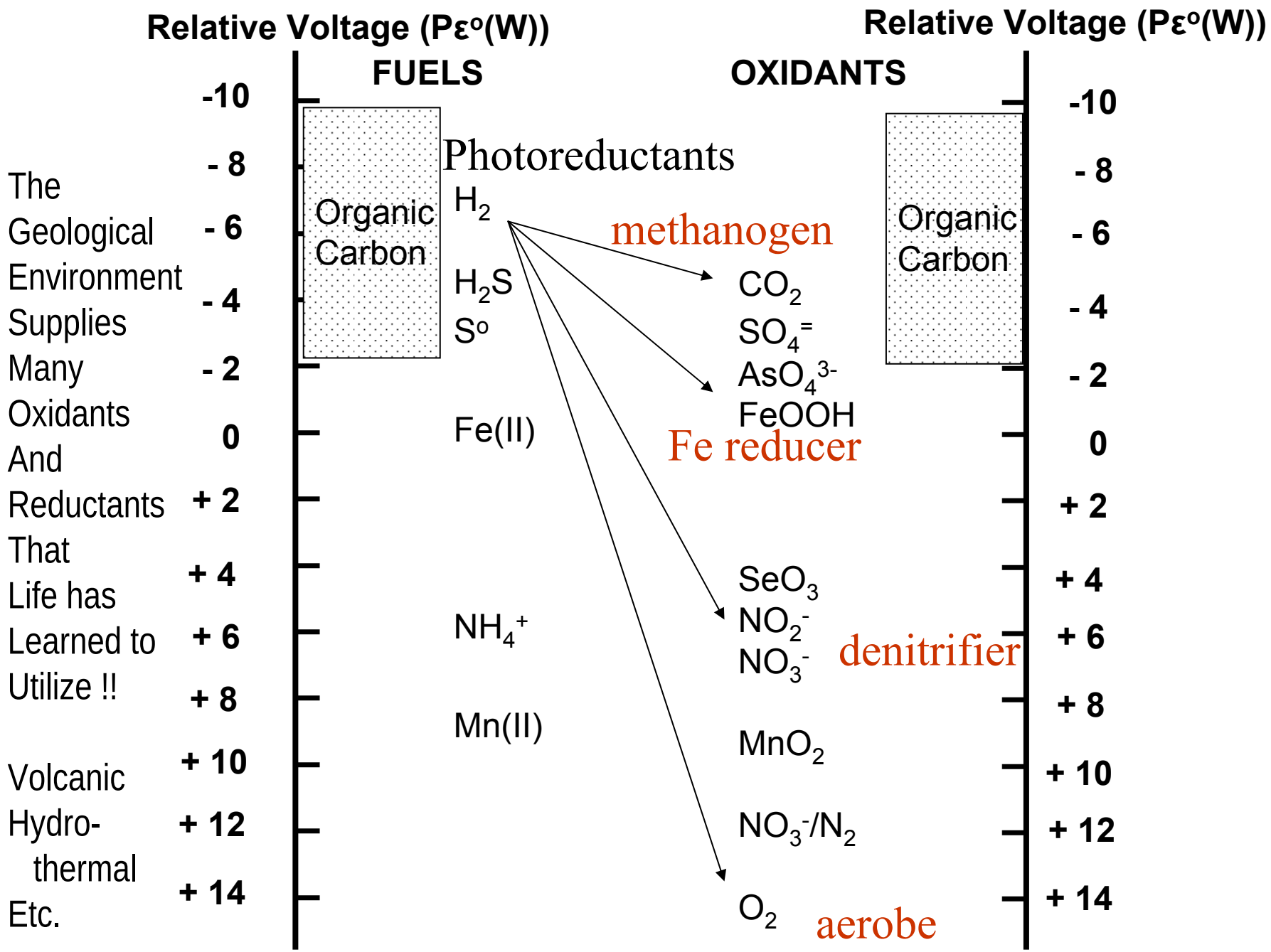
Oxidants (BREATH-ABLES !!)

ORGANICS
- fumarate, DMSO
- TMAO

Carbon dioxide*
Sulfur*
Sulfate*
Arsenate*
Selenite*
Iron*
Manganese*
Nitrate

Oxygen*

Asterisks indicate those that
End up in rocks or minerals! (N does not, many others do!)


Relative Voltage (Pε°(W))
FUELS
OXIDANTS
Relative Voltage (Pε°(W))
-10
- 8
- 6
- 4
- 2
0
+ 2
+ 4
+ 6
+ 8
+ 10
+ 12
+ 14
The Geological Environment Supplies Many Oxidants And Reductants That Life has Learned to Utilize !!
Volcanic Hydro-thermal Etc.
Organic Carbon
Photoreductants
H2
H2S
S°
Fe(II)
NH4+
Mn(II)
methanogen
CO2
SO4=
AsO43-
FeOOH
Fe reducer
SeO3
NO2-
NO3-
denitrifier
MnO2
NO3-/N2
O2
aerobe
Organic Carbon

ΔG < 0; Slow reaction at pH < 9;

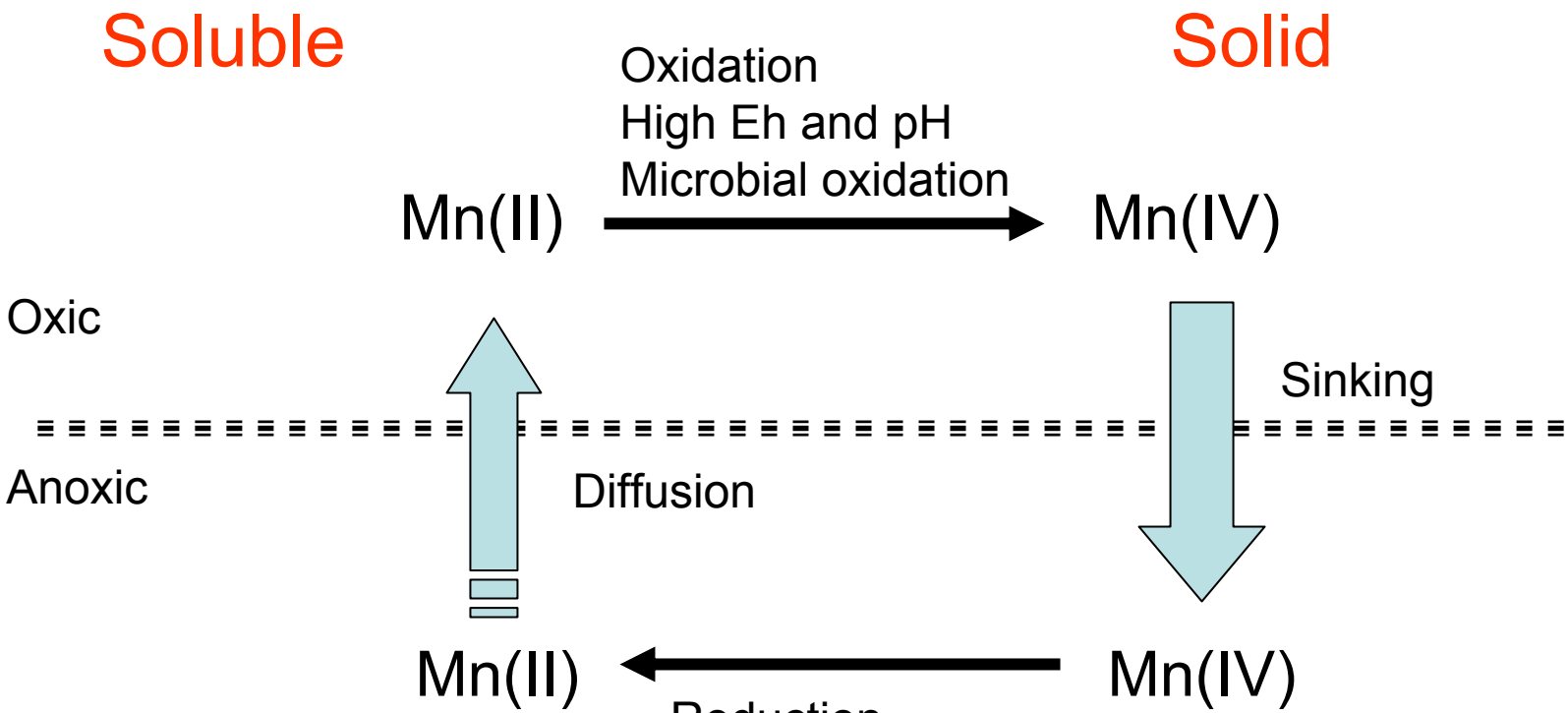
Soluble reduced forms Mn oxides and oxyhydroxides
(Mn Carbonate Mn(III) chelates Mn(IV) colloids)

Mn(II) ⟷ Mn(III) ⟷ Mn(IV)

ΔG > 0; High redox potential; Slow reaction at pH > 5

Dissimilatory metal reduction -- Using solid or dissolved metal oxides as electron acceptors

Chemical reduction is also possible:
sulfide, Fe(II), organic acids, quinones
(work of Alan Stone).



(Thamdrup; Aguilar, Kostka, Jorgensen)

Many environments where this is a major process
50-100 % of organic C
400 cycles before burial
Rates far to fast to be chemical !!!

Mn oxidizers

mechanism pretty well known; Mn(III) intermediate; Multicopper oxidases in all (synchrotron analysis of speciation!!)

No idea why!!

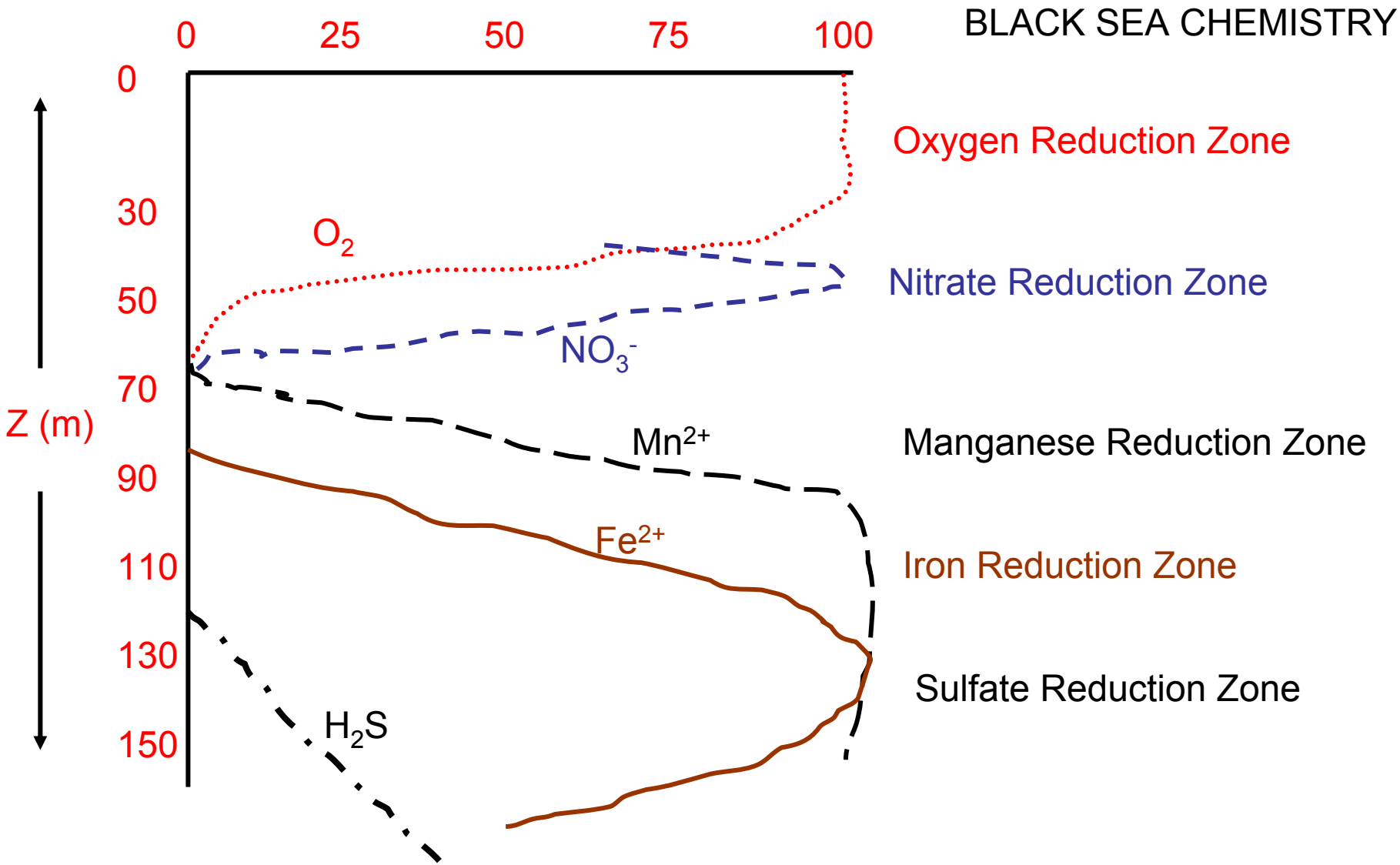
No example of energy conservation

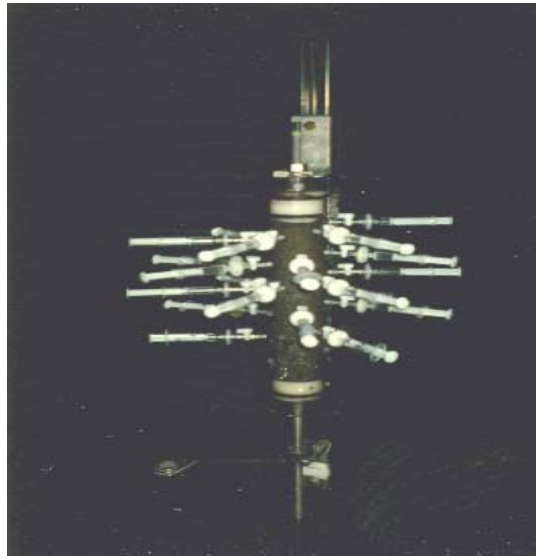
Mn reducers

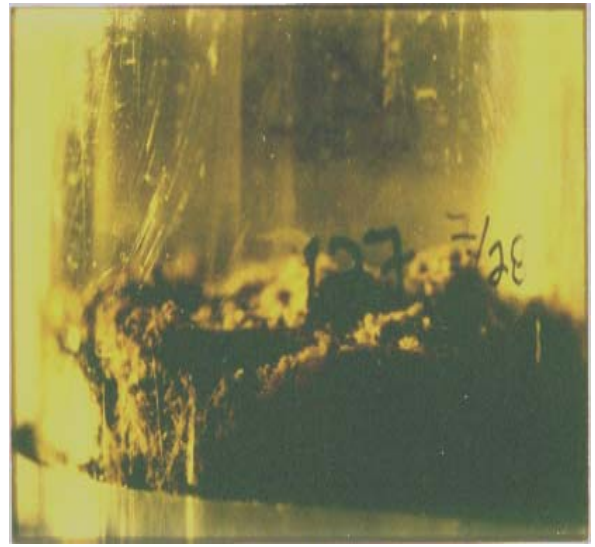
mechanism elusive

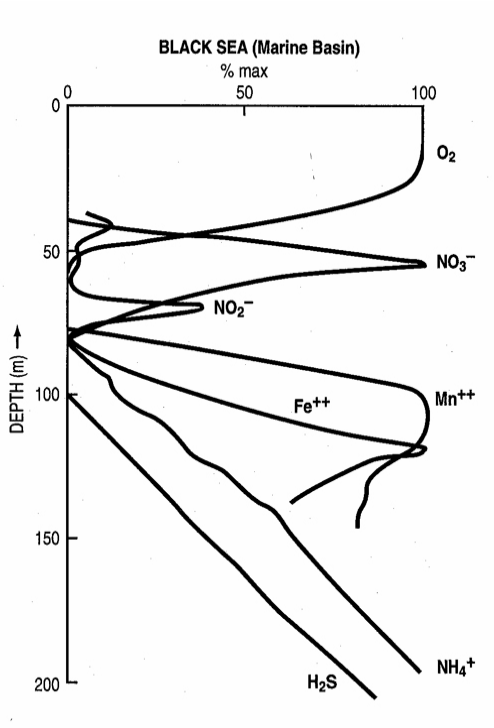
presumptive identification of genes

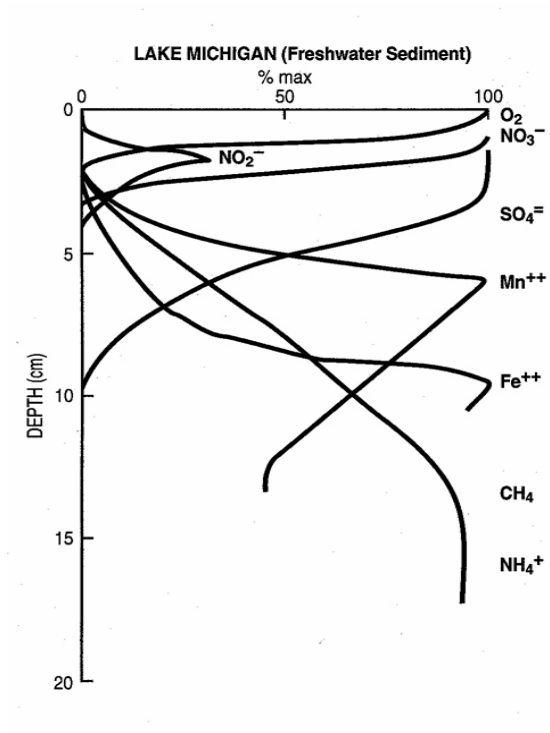
Why is obvious – Mn(IV) is good electron acceptor

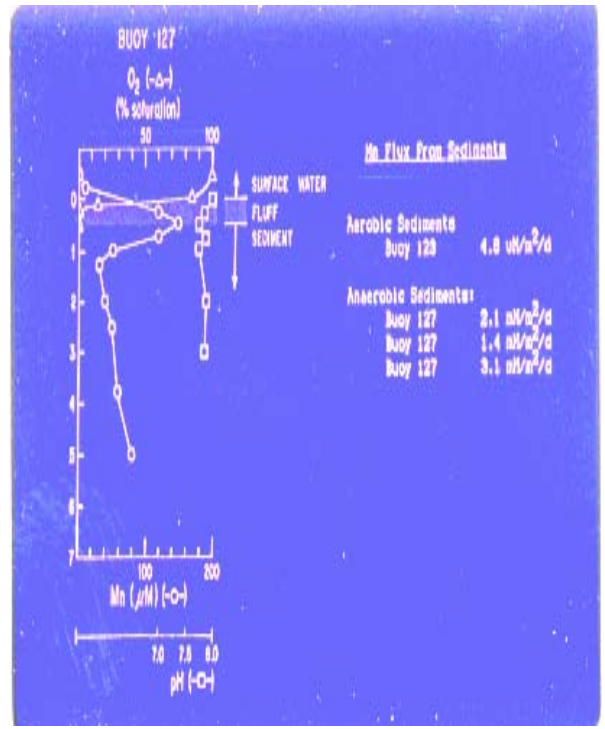

BLACK SEA CHEMISTRY
0
25
50
75
100
0
30
50
70
90
110
130
150
Z (m)
O_2
NO_3^-
Mn^{2+}
Fe^{2+}
H_2S
Oxygen Reduction Zone
Nitrate Reduction Zone
Manganese Reduction Zone
Iron Reduction Zone
Sulfate Reduction Zone






BLACK SEA (Marine Basin)
% max
0
50
100
0
50
100
150
200
DEPTH (m)
O2
NO3−
NO2−
Fe++
Mn++
NH4+
H2S


LAKE MICHIGAN (Freshwater Sediment)
% max
0
50
100
0
5
10
15
20
DEPTH (cm)
O2
NO3−
NO2−
SO4=
Mn++
Fe++
CH4
NH4+


BUOY 127
O2 (-o-)
(% saturation)
50
100
SURFACE WATER
FLUFF
SEDIMENT
Mn Flux From Sediments
Aerobic Sediments
Buoy 123
4.8 mM/m2/d
Anaerobic Sediments:
Buoy 127
2.1 mM/m2/d
Buoy 127
1.4 mM/m2/d
Buoy 127
3.1 mM/m2/d
100
200
Mn (μM) (-o-)
7.0
7.5
8.0
pH (-o-)

Enrichment Cultures for Dissimilatory Metal Reducers

Soft agar (0.75%) to suspend metal oxides

Non-fermentable carbon source (acetate,lactate,etc)

- avoid acid production
- at low pH, Mn oxides can be reduced

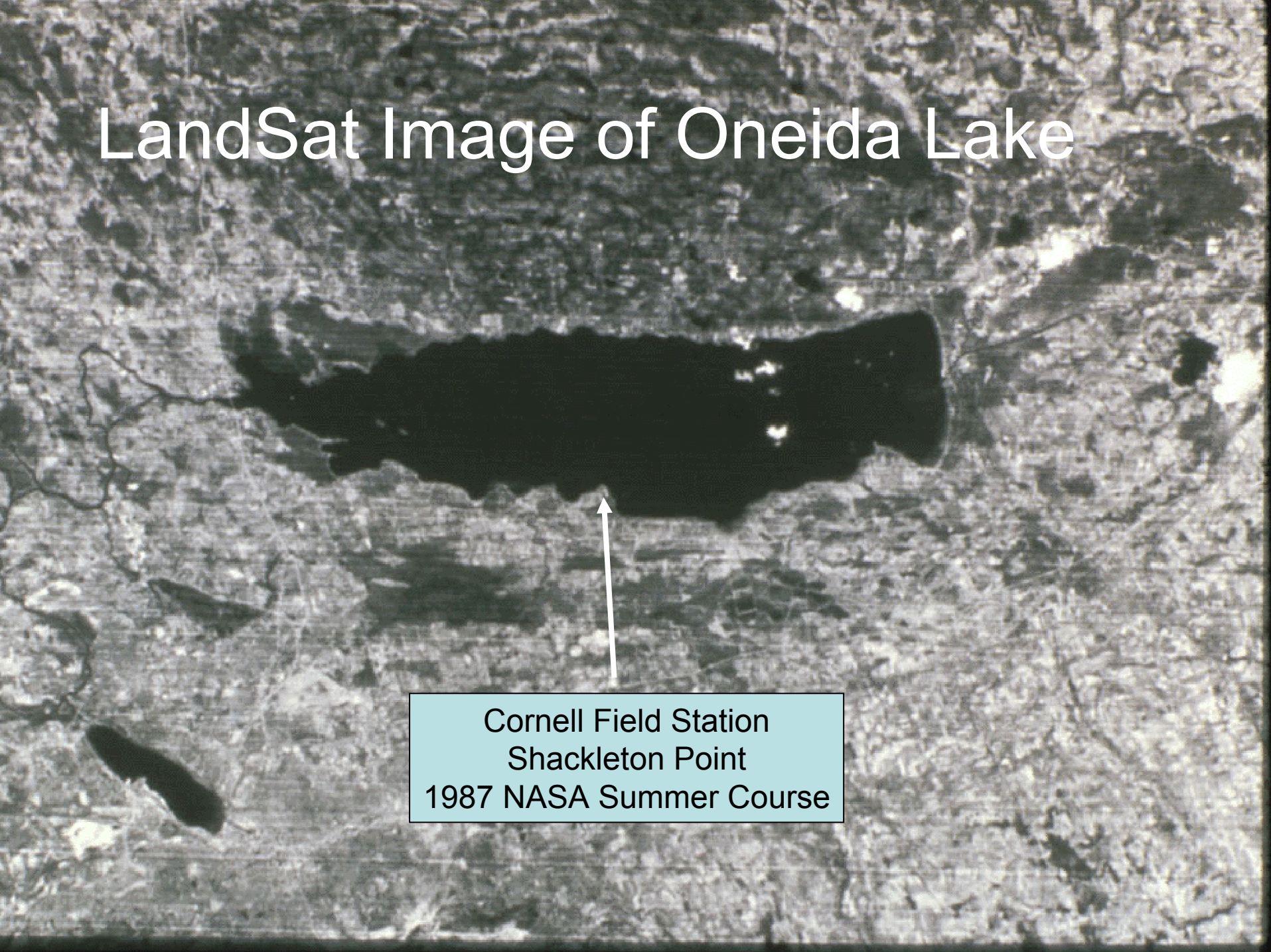
Leave out sulfate ! Sulfide reduces Fe(III) & Mn(IV)

Mn or Fe oxides as only oxidant

Incubate and look for zones of clearing

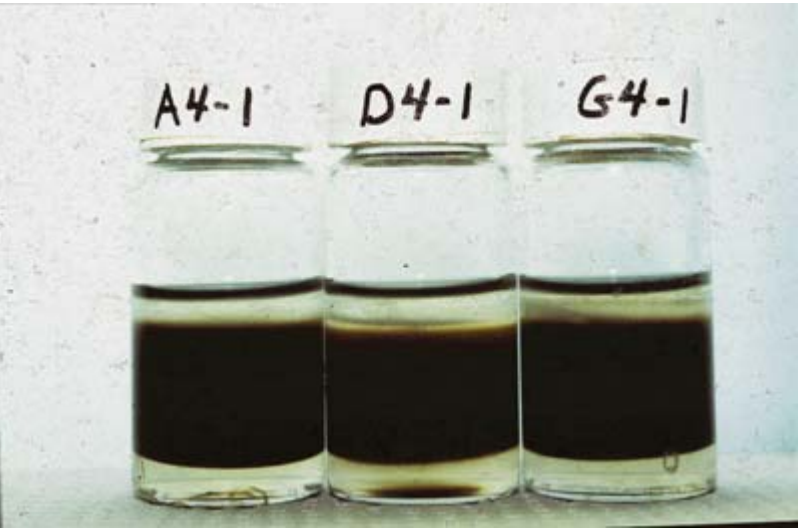
GO TO THE RIGHT PLACE !!

LandSat Image of Oneida Lake

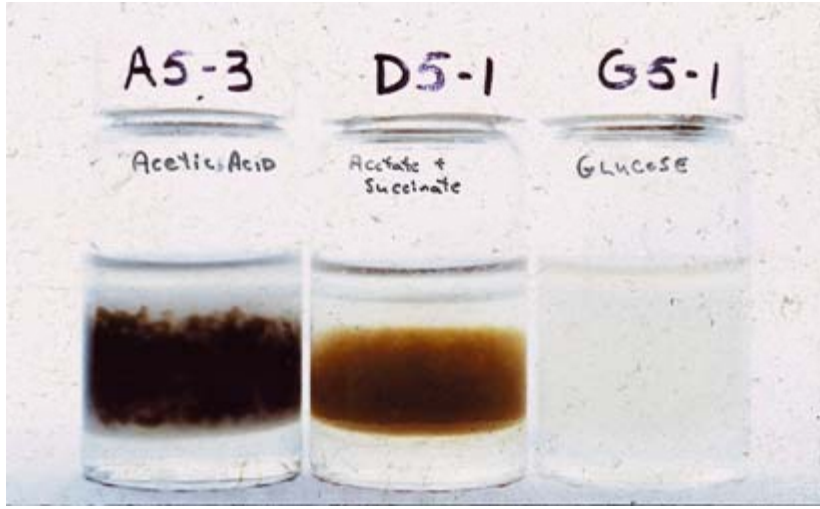



Oneida Lake Sediment Surface
3” Core Taken From Reducing Area of Lake
Highest Rates of Mn Flux Known

Enrichment Culture

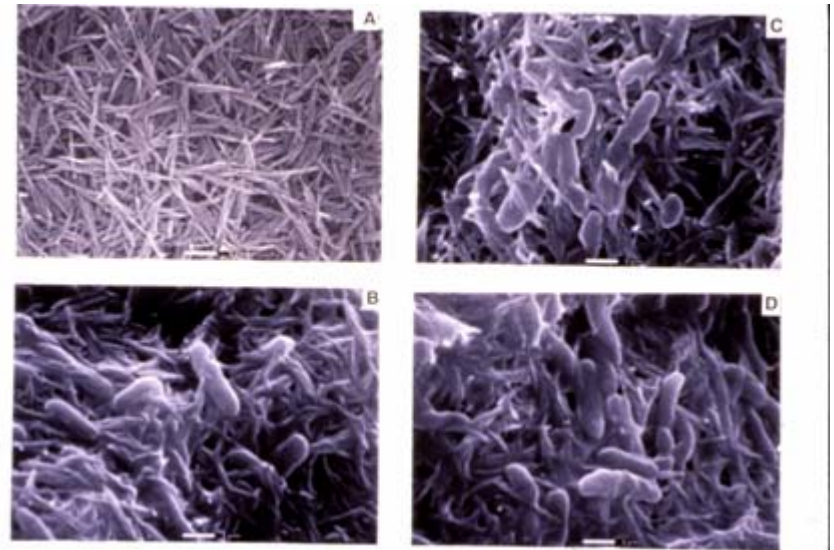


Five Days Incubation

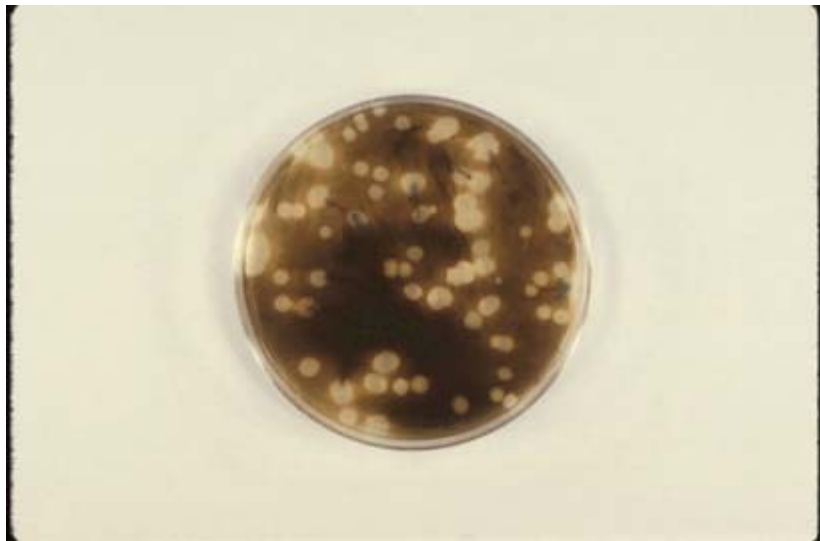


Kinetics !!
Solubility

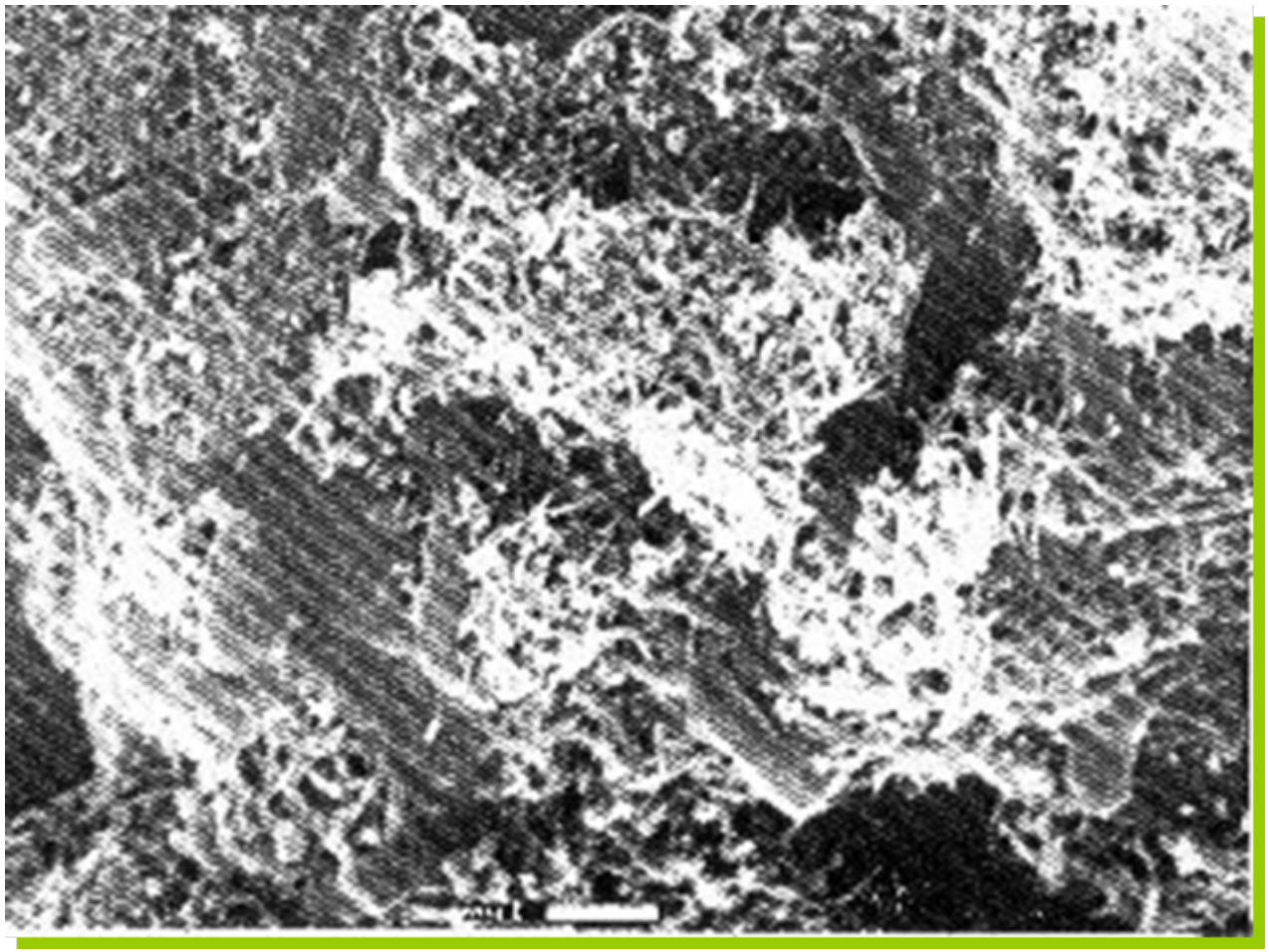
Pure Culture on MnO_2



Breathing Mn oxide!



Unusual Life Form!!



Shewanella oneidensis

Discovered in 1987

Energy source is lactic acid, hydrogen or formaldehyde

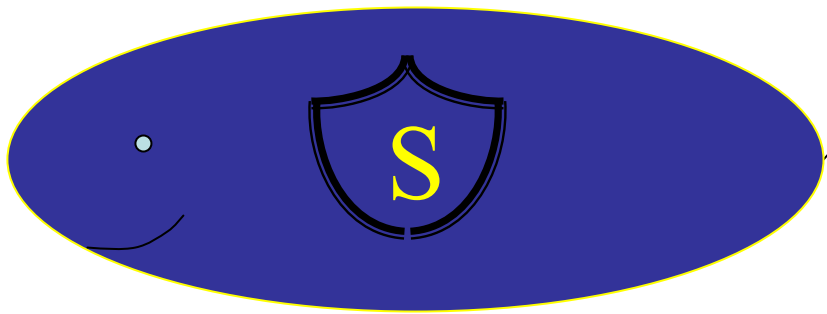
Breathes rocks! Mn oxides or Iron oxides

***Shewanella oneidensis* – MR1**

Chosen with care:

1. Grows quickly & forms single colonies
2. Grows on a defined medium
3. Reduces iron and manganese well
4. Reduces many other things in addition
5. Grows aerobically !!! – easier to do genetics

Shewanella oneidensis – MR-1



Formate

Lactate

Pyruvate

Amino Acids

H_2

Mine waste
Black Sea
Oneida Lake
Green Bay

Panama Basin
Mississippi Delta
North Sea Interfaces

O_2*

NO_3^-, NO_2^-

Mn(IV)*

Mn(III)*

Fe (III)*

Fumarate

DMSO

TMAO

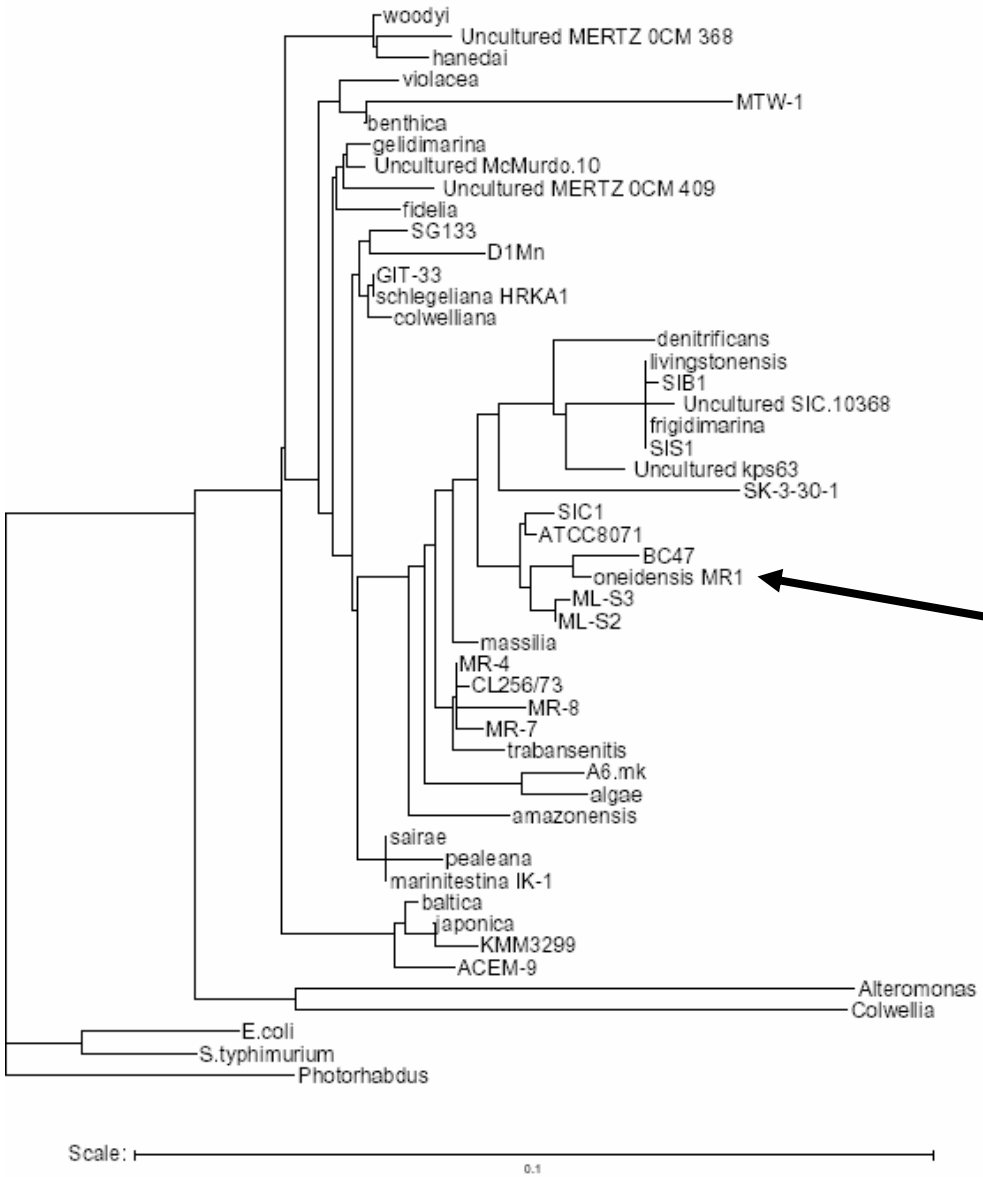
S^o *

$S_2O_3^{2-}$ *

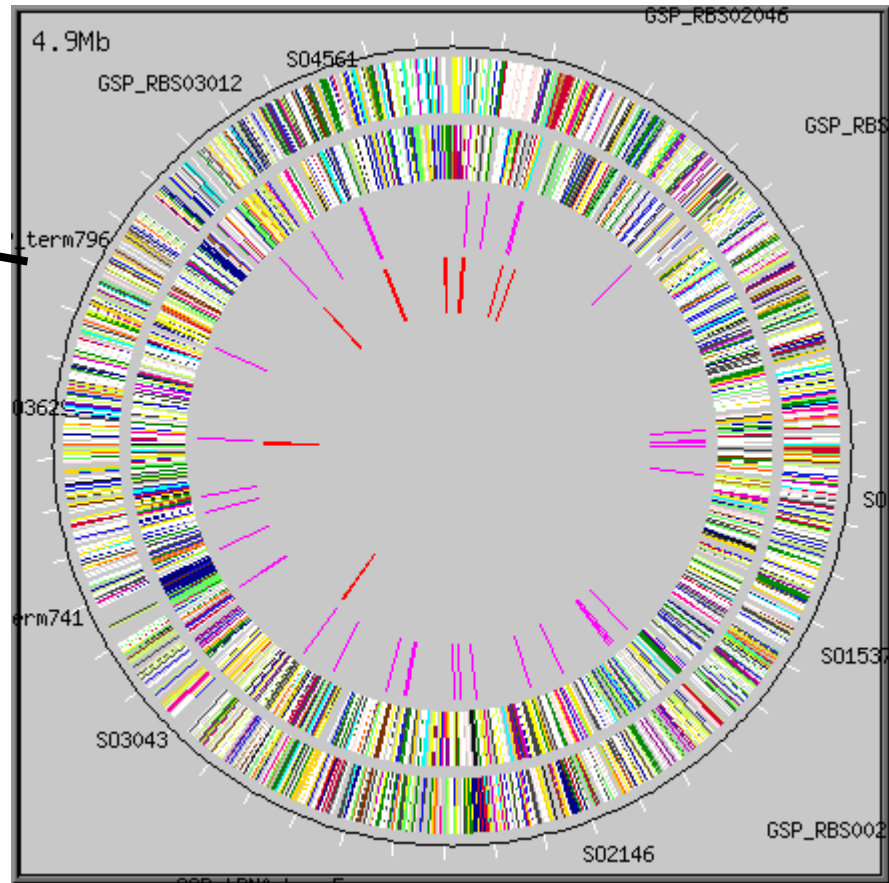
U(VI)*

Cr(VI)*, Tc*, As, Se*, I, *

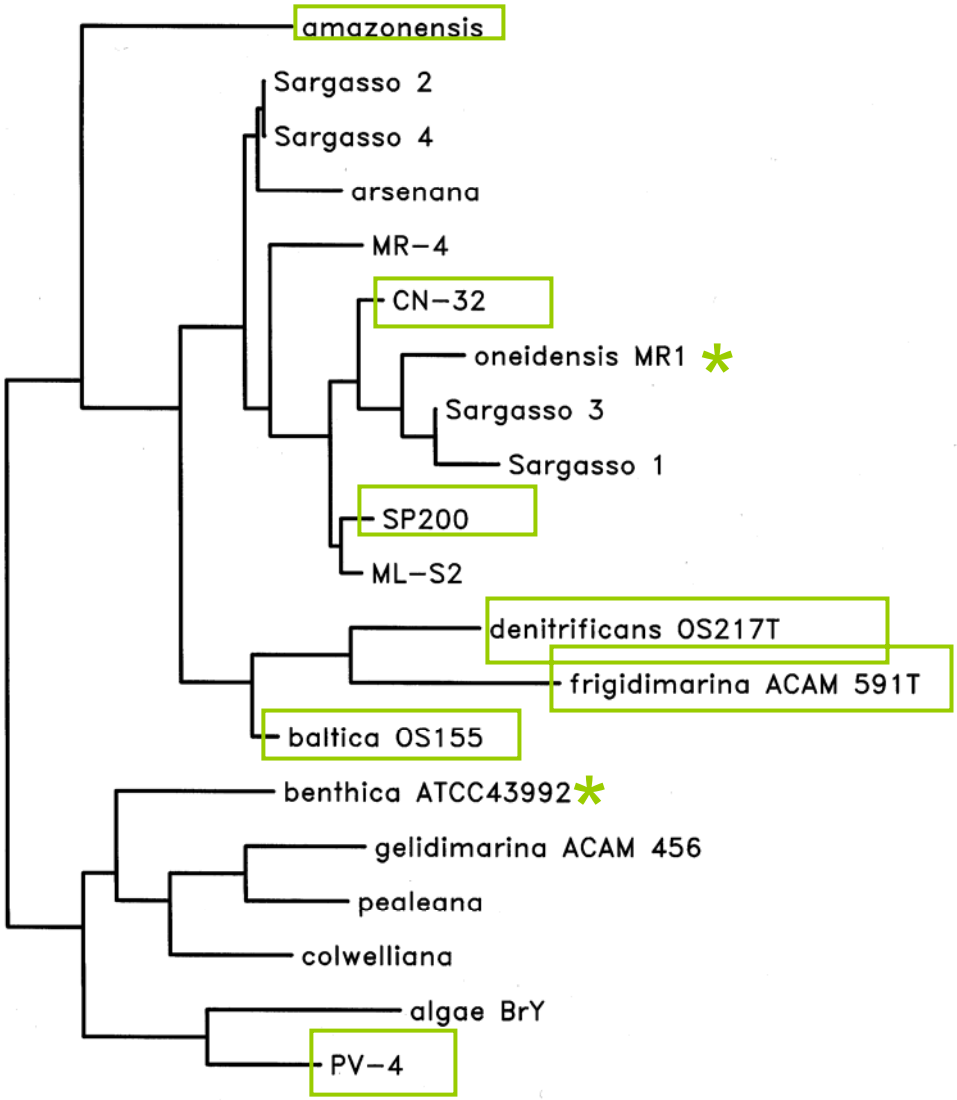
These bacteria are GREAT for teaching chemistry !!



MR-1 is one of many shewanellae



16S Tree – *Shewanella*
Lee Ann McCue



These strains chosen for sequencing.
Took three years for first strain to be sequenced
During the summer of 2004, these 7 were sequenced

With the exception of S. denitrificans, all are capable of metal ion reduction.

Now we are swimming in data!

But, how do these bugs reduce solid oxides?
Several possible explanations:

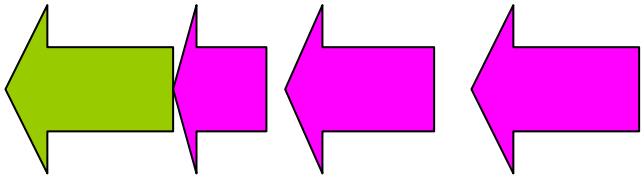
1. **Cells alter Eh/pH of environment.**
2. **Cells produce strong reductants**
3. **Cells utilize "electron shuttle" that is present in environment (humic acids)**
4. **Cells produce their own "electron shuttle"**
5. **Cells use oxidized metal ions directly as electron acceptors for metabolism**
6. **Combination of some of above!**

From the beginning:
Many hints that that surface attachment was important:

1. Separation of cells from oxides inhibit reduction of metals unless electron mediators are added
2. Process is dependent on surface area of the mineral
3. Examination of metal surfaces show a biofilm that forms on the surface
4. Enzymes involved in iron reduction are located on outer membrane of the cells (very unusual)
5. Visual evidence for appearance of these when limited for electron acceptors

S. oneidensis MR-1 Mtr Loci

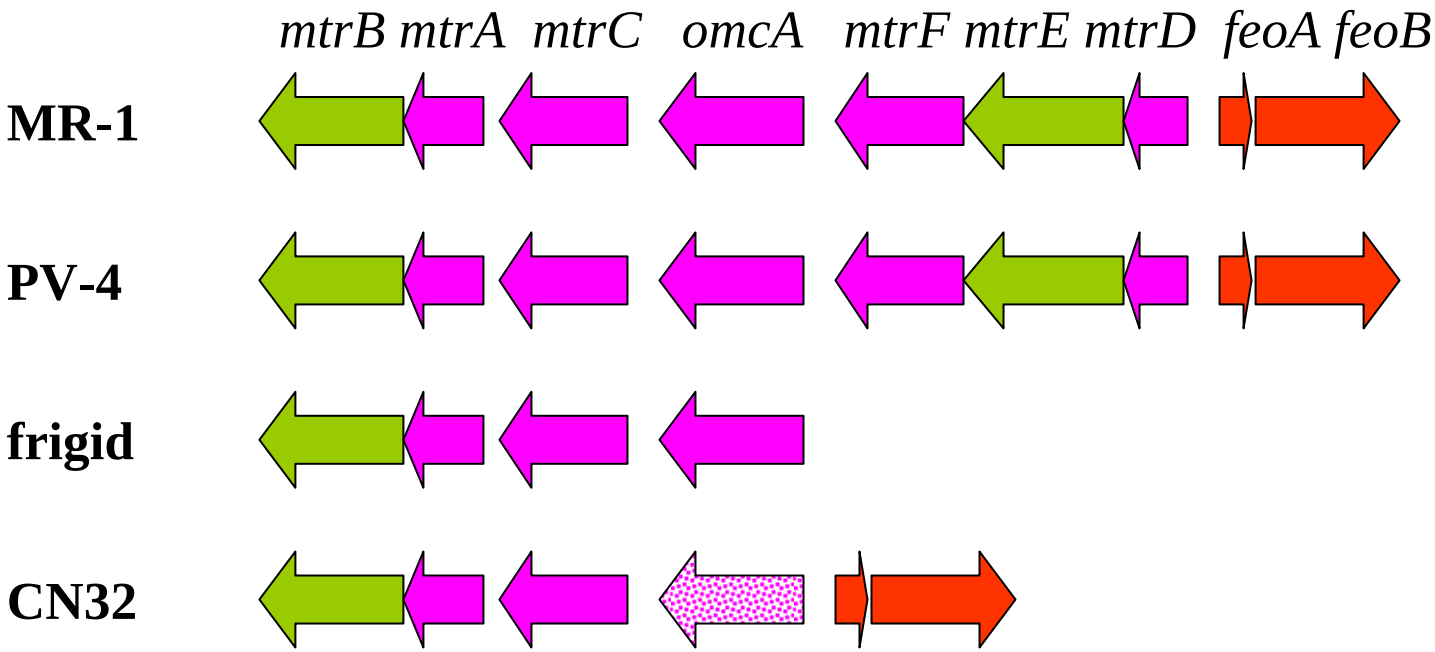
mtrB mtrA mtrC omcA



- mtrB (SO1776) – β-barrel OM protein w/ heme
- mtrA (SO1777) – periplasmic decaheme cytochrome
- mtrC (omcB) (SO1778) – OM decaheme cytochrome
- omcA (SO1779) - OM decaheme cytochrome

All mutants are defective in metal reduction although there is some variation reported between labs, likely due to differences in growth conditions & form of metal. (doubles and extras!)

Comparison of the Mtr Decaheme Loci



- Genes upstream to and downstream to this locus are conserved
- The *mtrDEF* operon is present only in PV-4 and MR-1
- CN32 also lacks *omcA*. In its place is an 11 heme cytochrome c
- The ferrous iron transporter is deleted from this locus only in *S. frigidimarina*

MR-1 cytochrome	PV4	CN32	frigid	denit
putative monoheme c, truncated (SO4570)	NA	NA	NA	NA
hypothetical protein (SO0409)	✓	✓	✓	✓
ubiquinol-monoheme c reductase PetC (SO0610)	✓	✓	✓	✓
cbb3-type diheme c oxidase, subunit III CcoP (SO2361)	✓	✓	✓	✓
cbb3-type monoheme c oxidase, subunit II CcoO	✓	✓	✓	✓
monoheme c' (SO3420)	✓	✓	✓	✓
diheme c oxidase, subunit II CyoA (SO4606)	✓	✓	✓	✓
diheme c4 CytcB (SO4666)	✓	✓	✓	✓
diheme c NapB (SO0845)	✓	✓	✓	
split-soret diheme c (SO0939)	✓	✓	✓	
fumarate reductase FccA (SO0970)	✓	✓	✓	
OmcA-like decaheme c (SO1659)	✓	✓	✓	
decaheme c MtrA (SO1777)	✓	✓	✓	
decaheme c MtrC/OmcB (SO1778)	✓	✓	✓	
small tetraheme c3 CctA (SO2727)	✓	✓	✓	
nitrite reductase NrfA (SO3980)	✓	✓	✓	
SoxA-like diheme c (SO4047)	✓	✓	✓	
diheme c4 (SO4048)	✓	✓	✓	
tetraheme c CymA (SO4591)	✓	✓	✓	
monoheme c5 ScyA/CytcA (SO0264)		✓	✓	✓
putative seven heme c (SO0479)	✓	✓		
split tetraheme flavocytochrome (SO3056)	✓	✓		
degenerate nonaheme c (SO3141)	✓	✓		
pentaheme c TorC (SO1233)	✓		✓	
decaheme c OmcA (SO1779)	✓		✓	
putative monoheme c (SO4142)	✓		✓	
octaheme c (SO4144)	✓		✓	
tetraheme flavocytochrome IfcA-1 (SO1421)		✓	✓	
MtrAD-like decaheme c (SO1427)		✓	✓	
split tetraheme flavocytochrome (SO3623)		✓	✓	
split tetraheme flavocytochrome (SO1413)	✓			
decaheme c MtrF (SO1780)	✓			
decaheme c MtrD (SO1782)	✓			
putative diheme c (SO2930)	✓			
putative diheme c (SO2931)	✓			
putative triheme c (SO4572)	✓			
hypothetical protein (SO0581)		✓		
diheme c551 peroxidase CcpA (SO2178)		✓		
monoheme c Shp (SO4484)		✓		
diheme c (SO4485)		✓		
monoheme c4 (SO0714)				
putative monoheme c (SO0716)				
monoheme c4 (SO0717)				
hypothetical protein (SO1748)				
split tetraheme flavocytochrome (SO3300)				
MtrAD-like decaheme c (SO4360)				

Occurrence of homologs to MR-1 cytochromes in other genomes

- *Shewanella denitrificans* does not reduce iron, while the others do
- Only 11 cytochromes are unique to the metal reducing group
- Nearly all of these 11 have been linked to metal reduction, but knock-outs in any one does not eliminate iron reduction!
- Note that OmcA is not on this list!

- diheme c NapB (SO0845)
- split-soret diheme c (SO0939)
- fumarate reductase FccA (SO0970)
- OmcA-like decaheme c (SO1659)
- decaheme *c* MtrA (SO1777)
- decaheme *c* MtrC/OmcB (SO1778)
- small tetraheme c_3 CctA (SO2727)
- nitrite reductase NrfA (SO3980)
- SoxA-like diheme *c* (SO4047)
- diheme *c*4 (SO4048)
- tetraheme *c* CymA (SO4591)

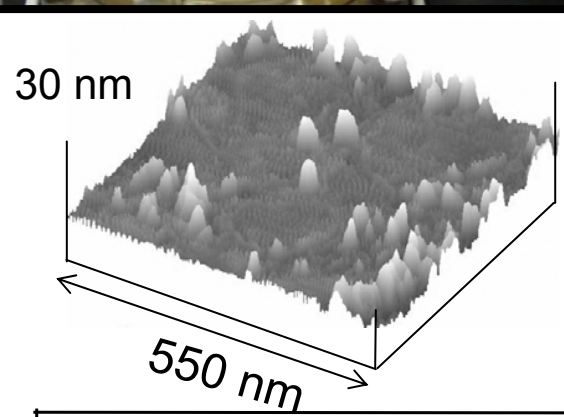
Shewanella oneidensis MR-1 Continuous Cultures

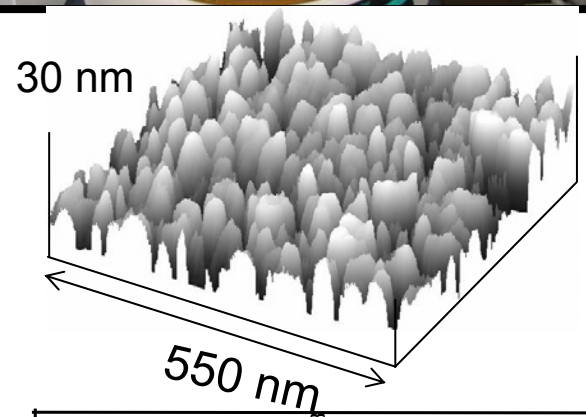
Courtesy of Y. Gorby, PNNL

Aerobic, carbon limited

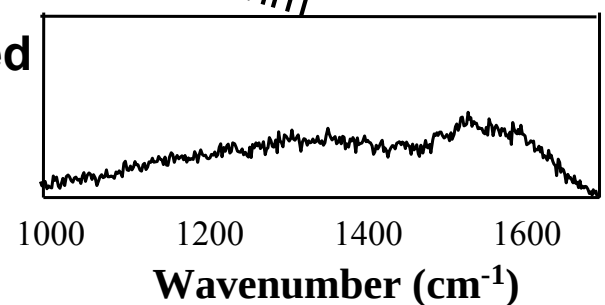
O_2 limited

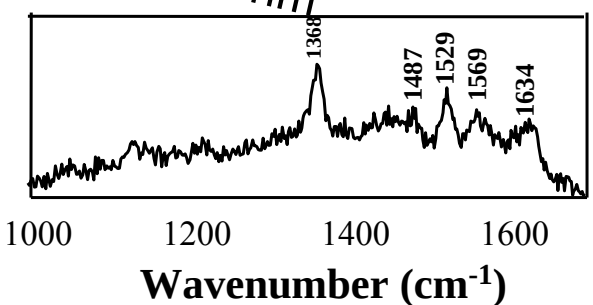
AFM of cell surface





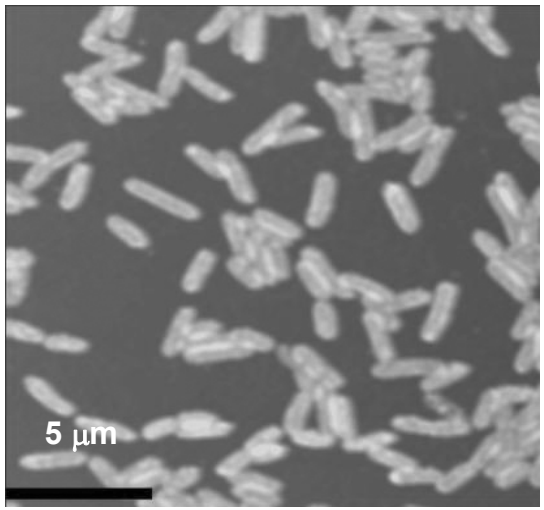
Surface enhanced Raman spectroscopy

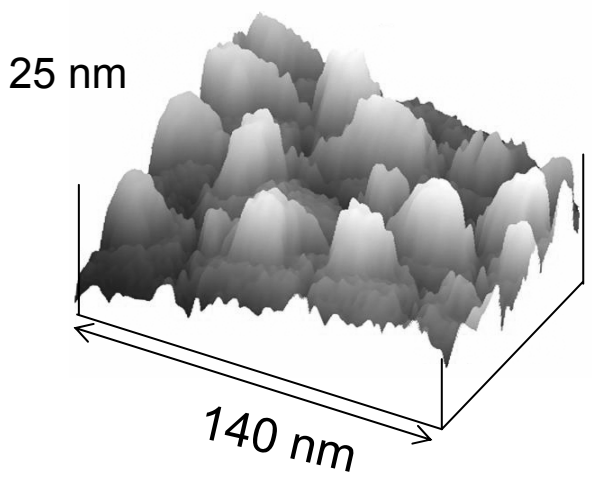




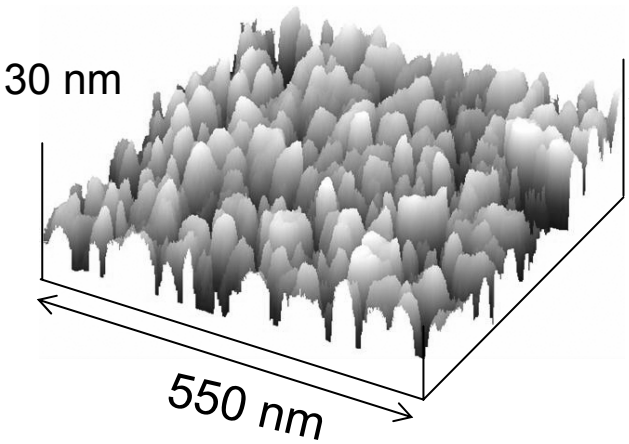
AFM Analysis

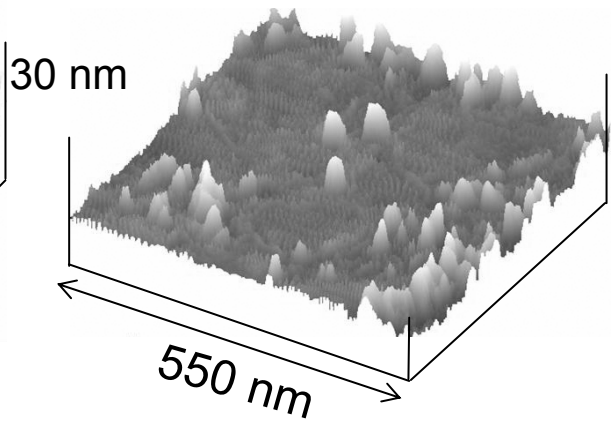
Electron acceptor limited



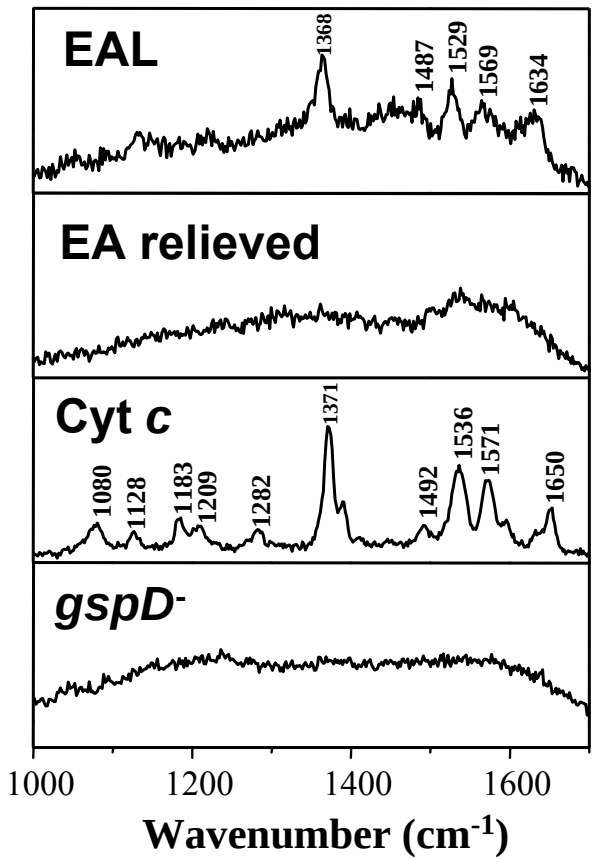


EA relieved





Surface-Enhanced Raman Spectroscopy



(Courtesy of Y. Gorby & P. Lu)

Summary of MR-1 Metal ion reduction data:

Genes for metal reduction are identified as necessary, though exact mechanism remains elusive

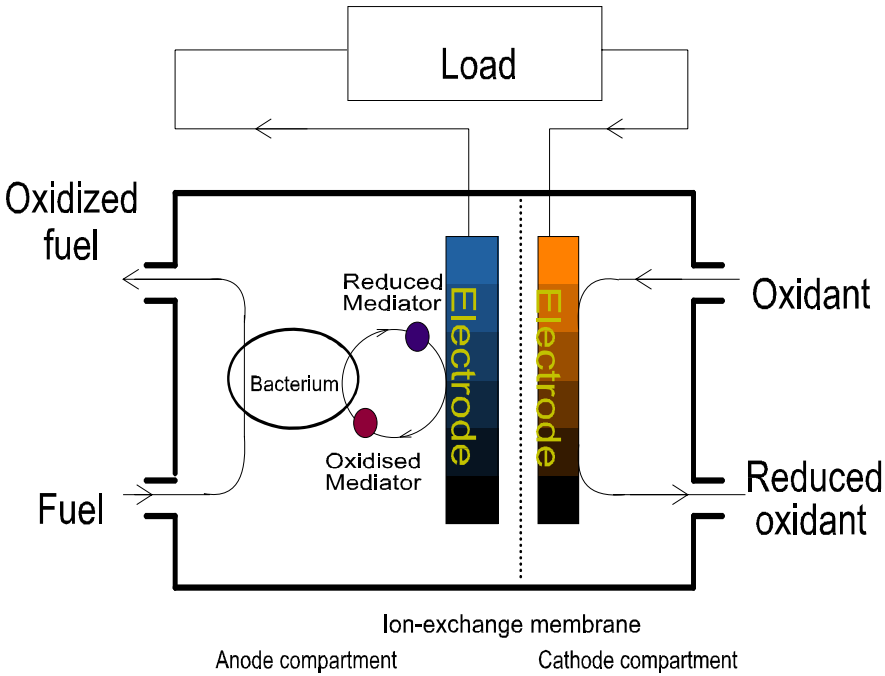
Growth under electron acceptor limitation results in formation of extracellular structures

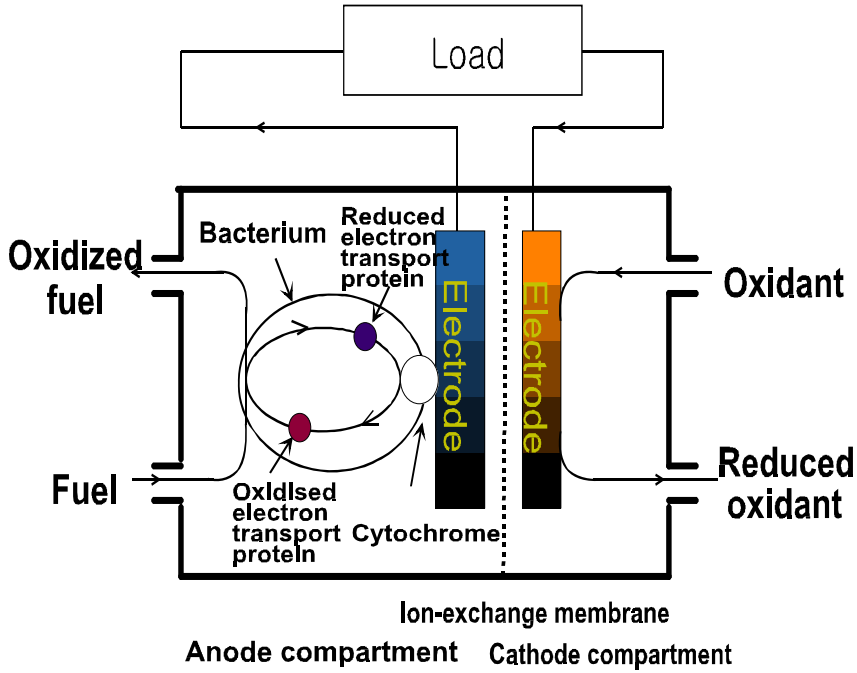
These structures look like c-type cytochromes

This happens very quickly (seconds)

Do not see the structures in gspD (secretion) mutant.

Microbial Fuel Cell (MFC)



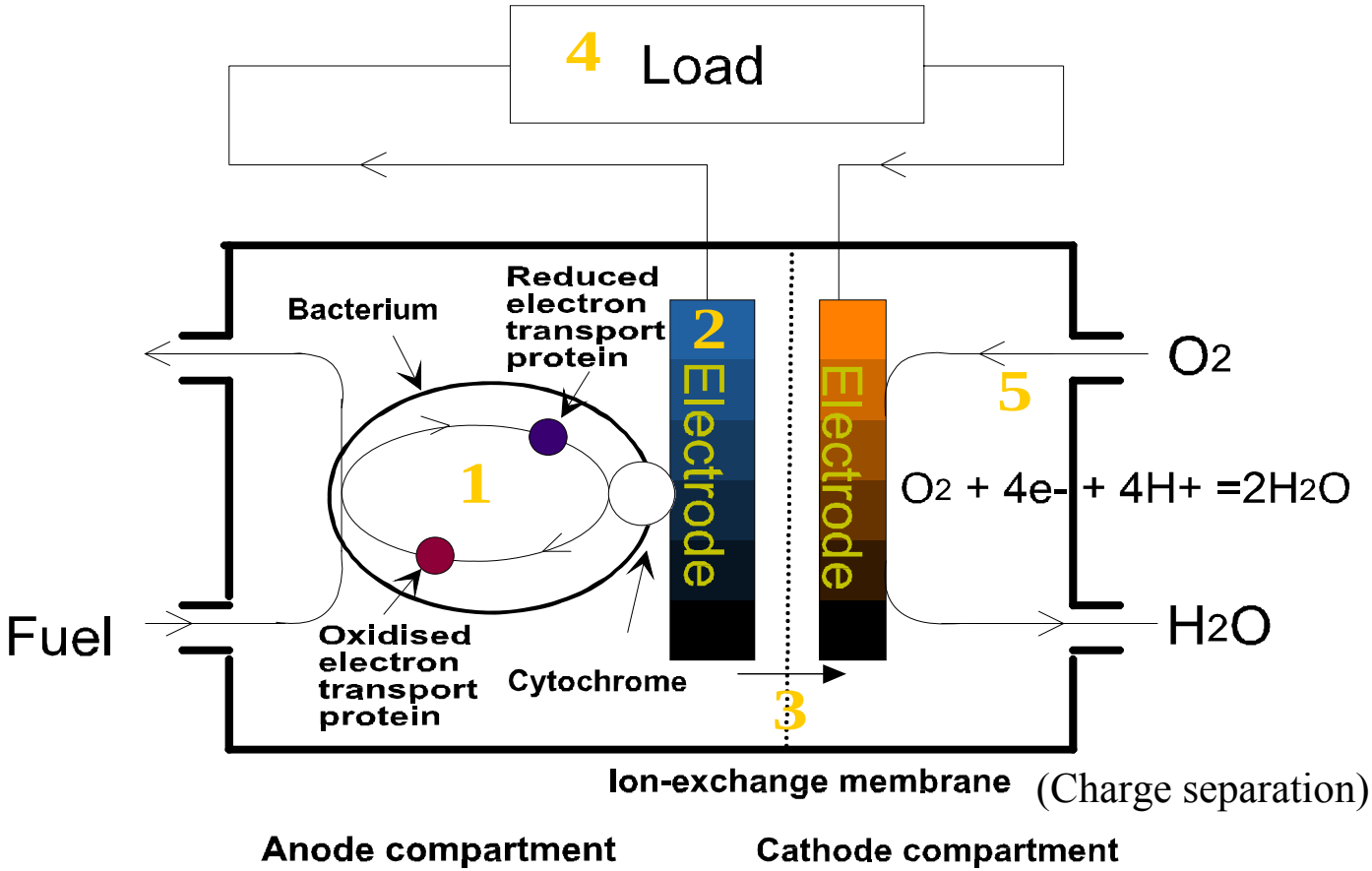


Mediated MFC

Mediator-less MFC

B.Hong Kim (KIST) – USC undergrad, Howard Harris; two new graduate students Orianna Bretschger, Ray Glover

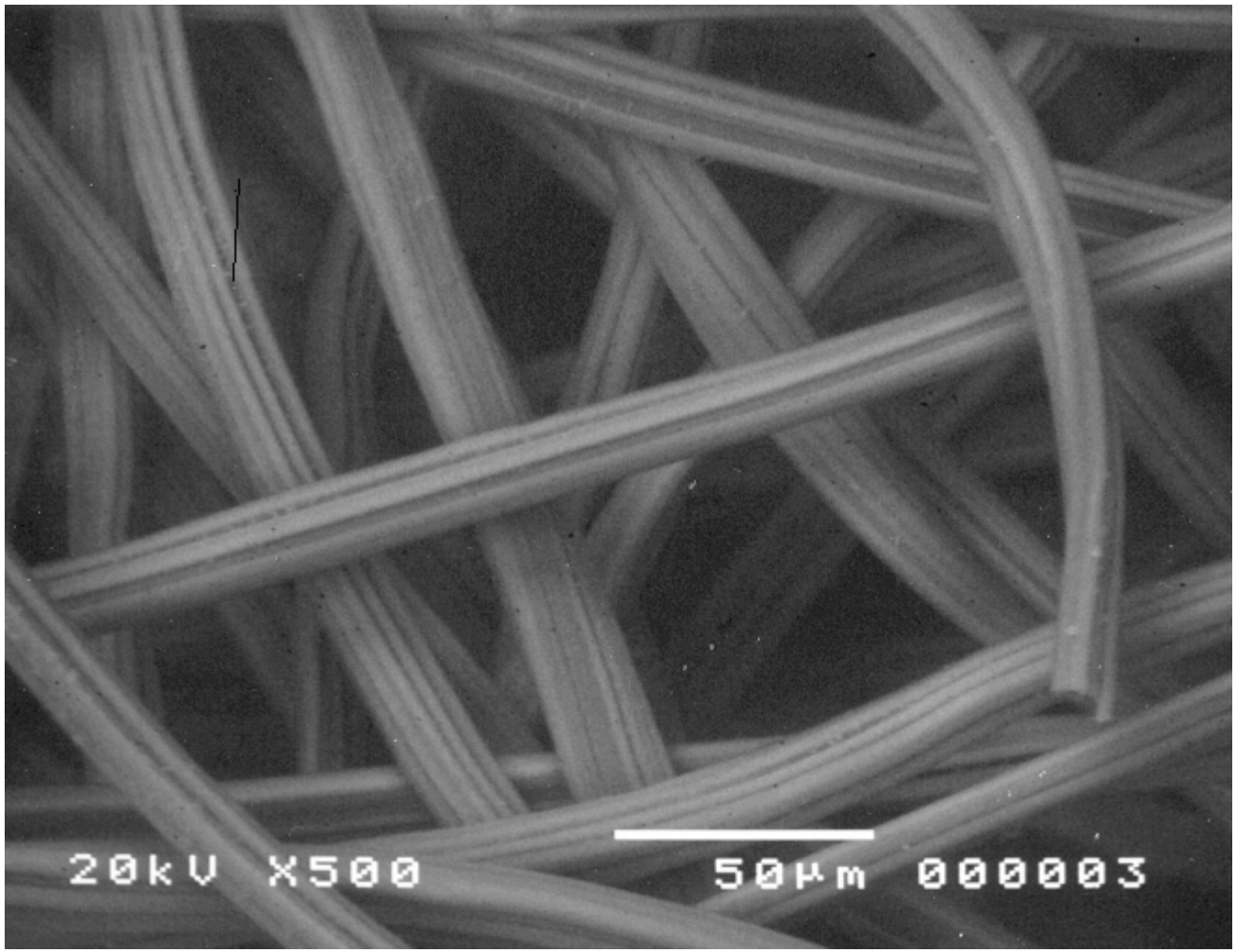
Electron transfer in MFC

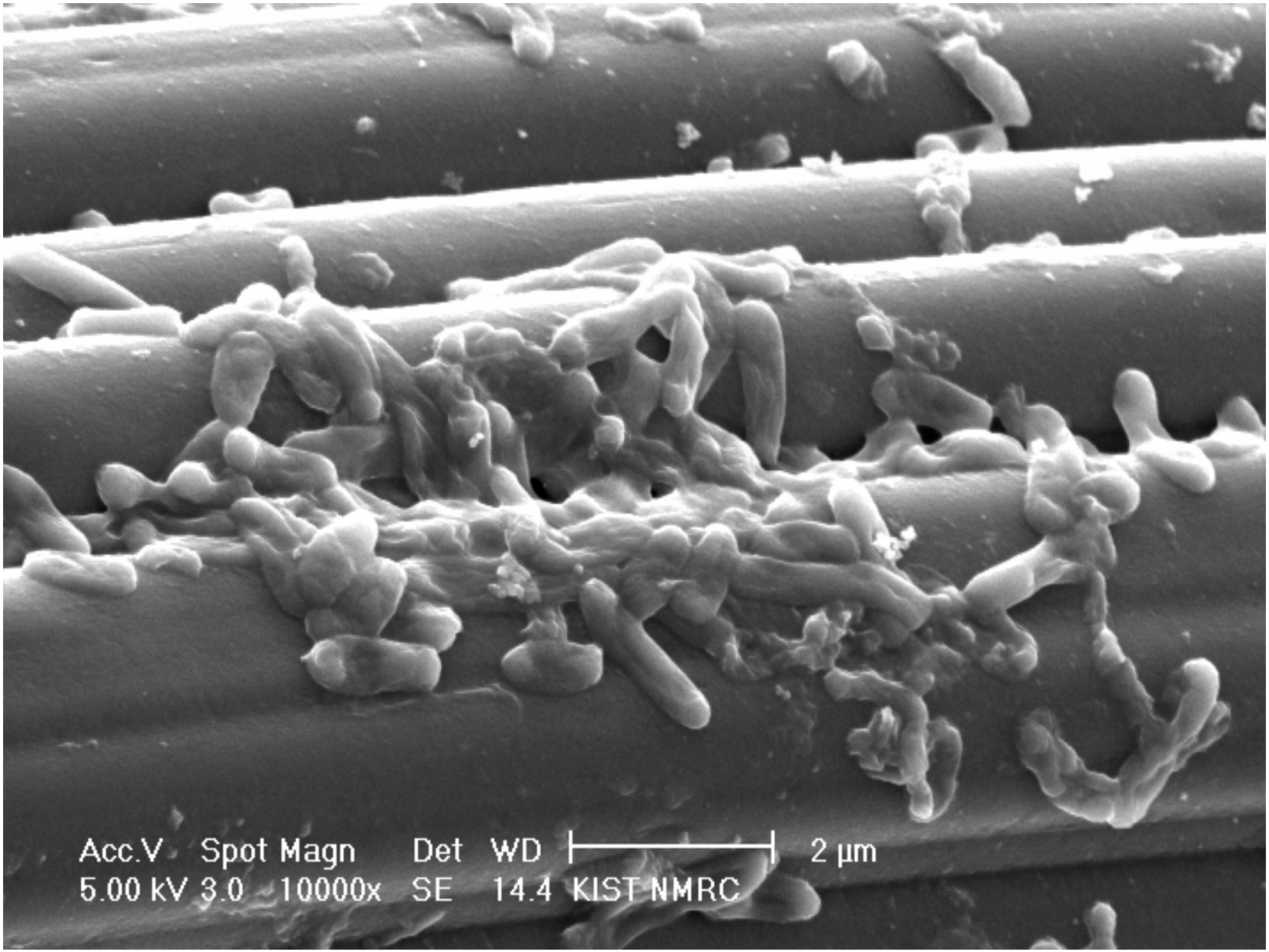


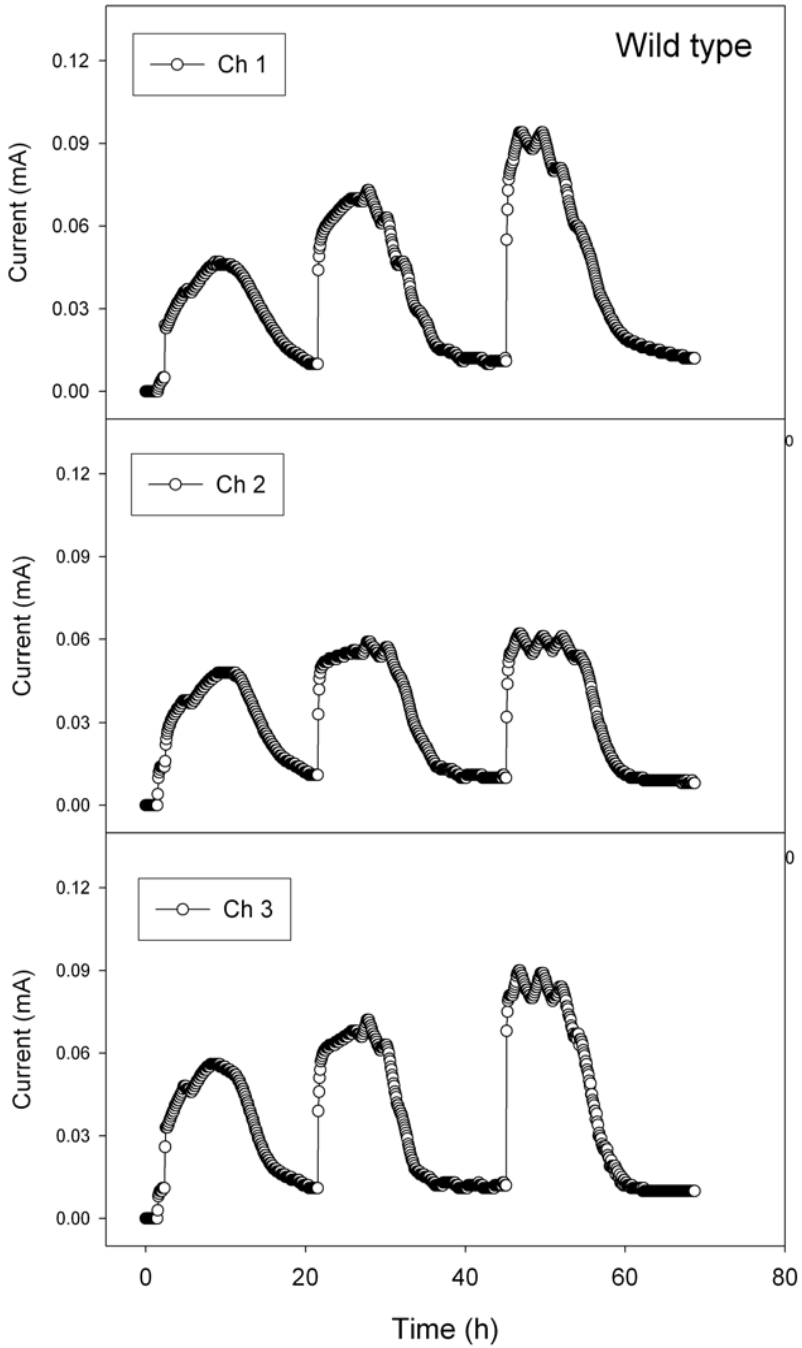
Anode reaction : $(CH_2O)n + nH_2O \rightarrow nCO_2 + 4ne^- + 4nH^+$

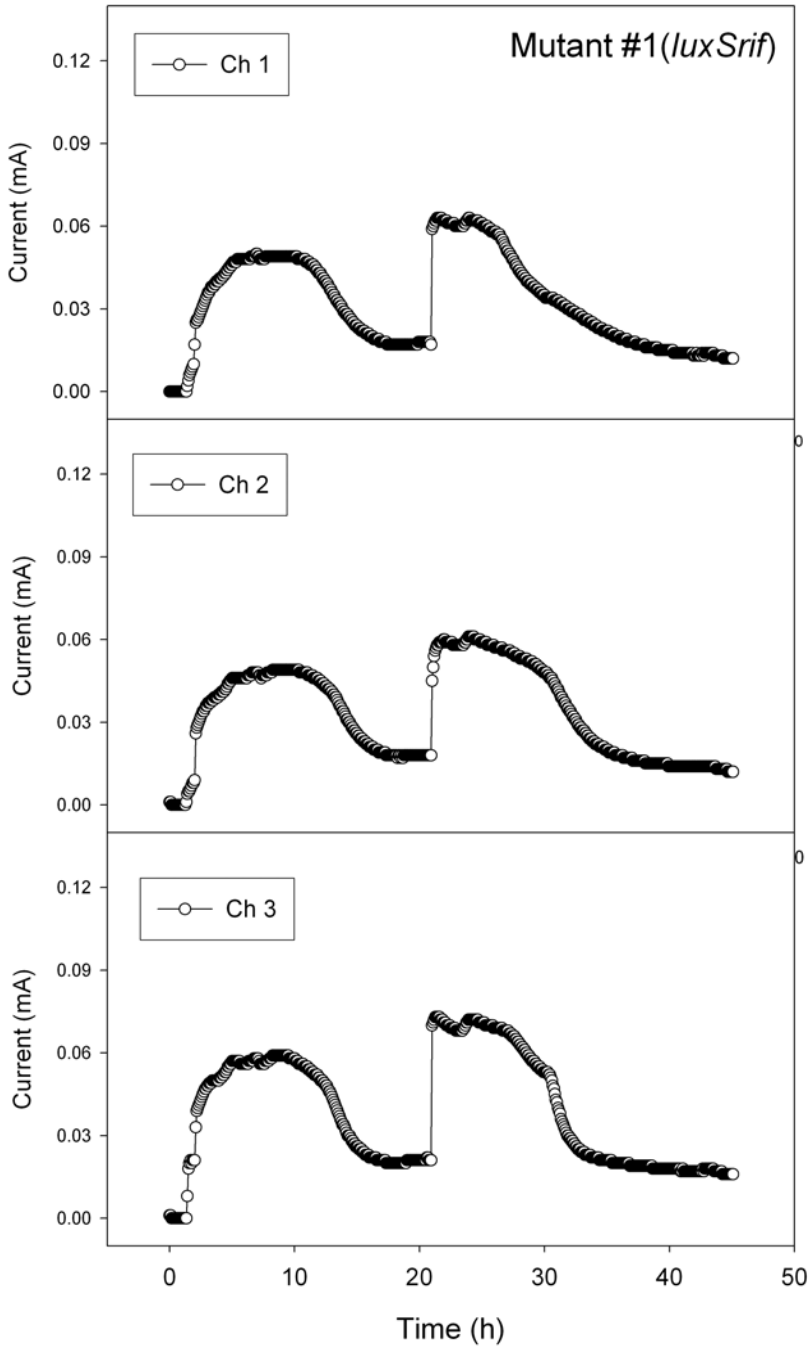
Cathode reaction : $O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$

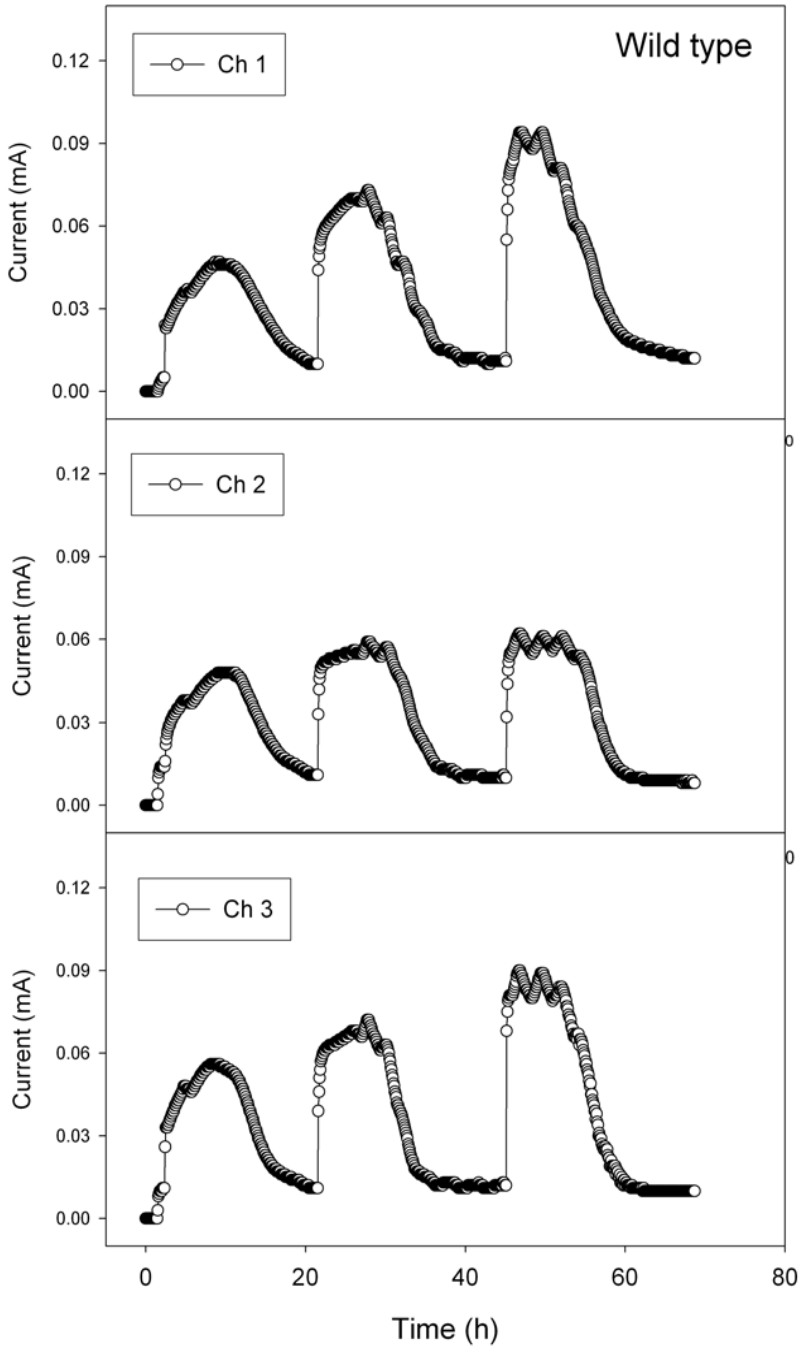
Graphite Fibers of Electrode

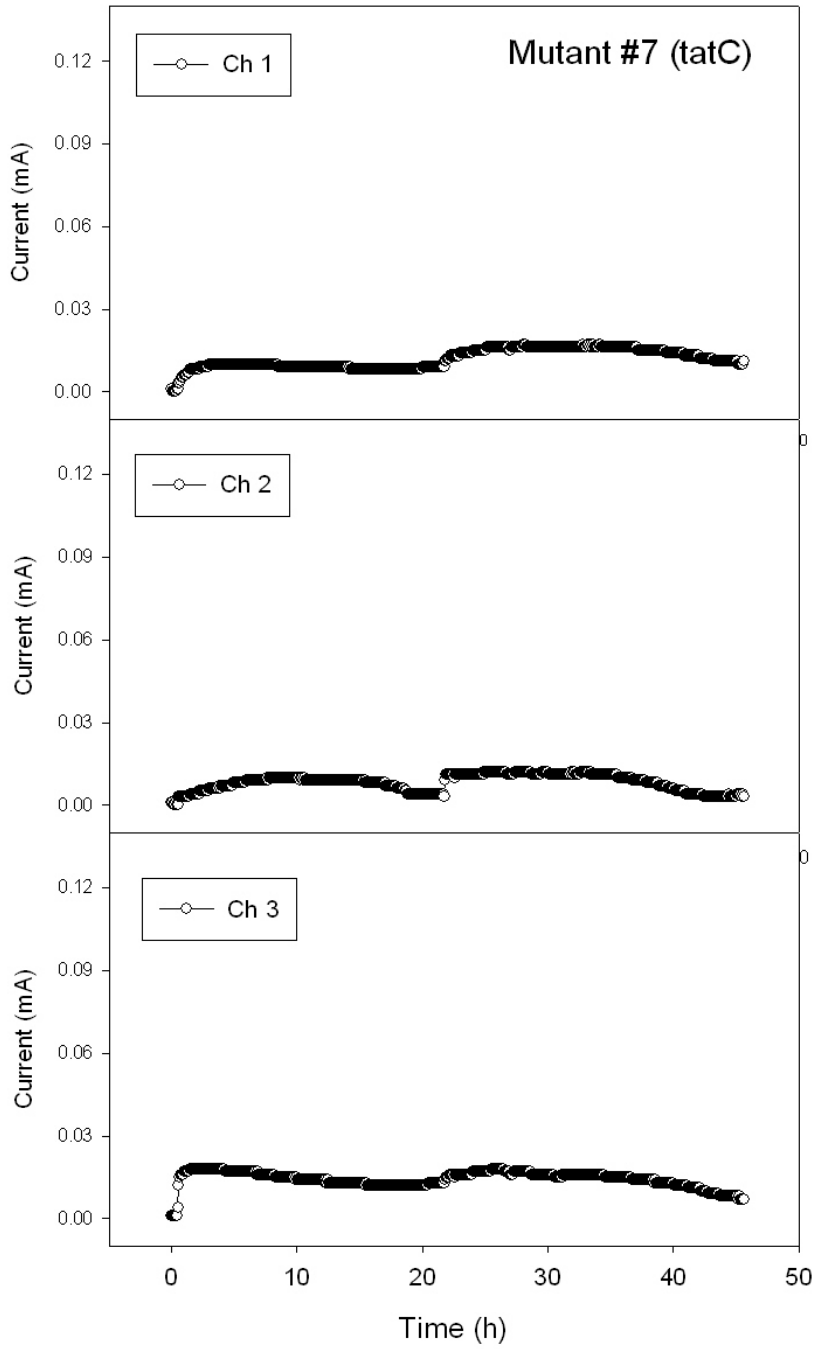


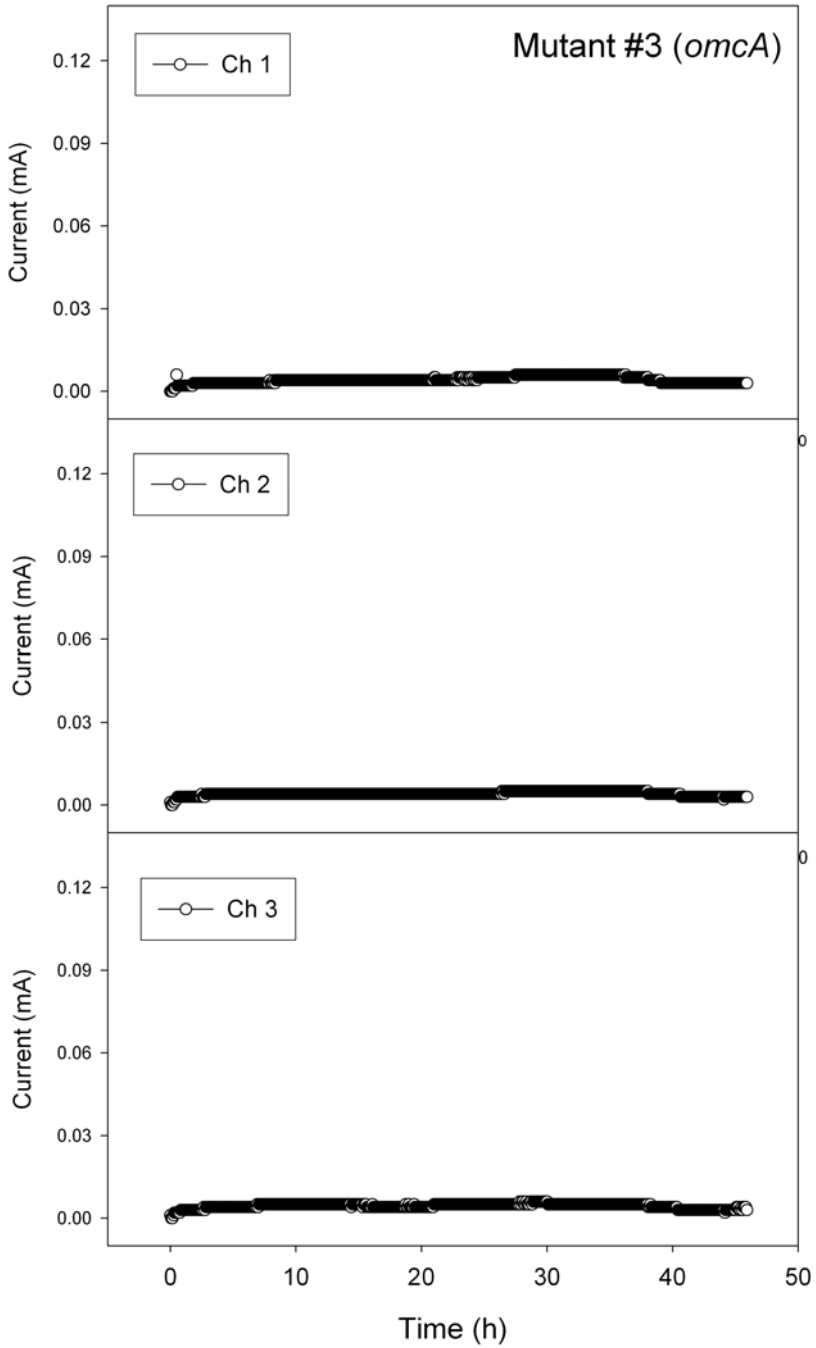

Acc.V Spot Magn Det WD 2 µm
5.00 kV 3.0 10000x SE 14.4 KIST NMRC


Wild type
Ch 1
Ch 2
Ch 3
Current (mA)
0.00
0.03
0.06
0.09
0.12
0
20
40
60
80
Time (h)


Mutant #1(luxSrif)
Ch 1
Ch 2
Ch 3
Current (mA)
0.00
0.03
0.06
0.09
0.12
0
10
20
30
40
50
Time (h)


Wild type
Ch 1
Ch 2
Ch 3
Current (mA)
0.00
0.03
0.06
0.09
0.12
0
20
40
60
80
Time (h)


Mutant #7 (tatC)
Ch 1
Ch 2
Ch 3
Current (mA)
0.00
0.03
0.06
0.09
0.12
0
10
20
30
40
50
Time (h)


Mutant #3 (omcA)
Ch 1
Ch 2
Ch 3
Current (mA)
0.12
0.09
0.06
0.03
0.00
0
10
20
30
40
50
Time (h)

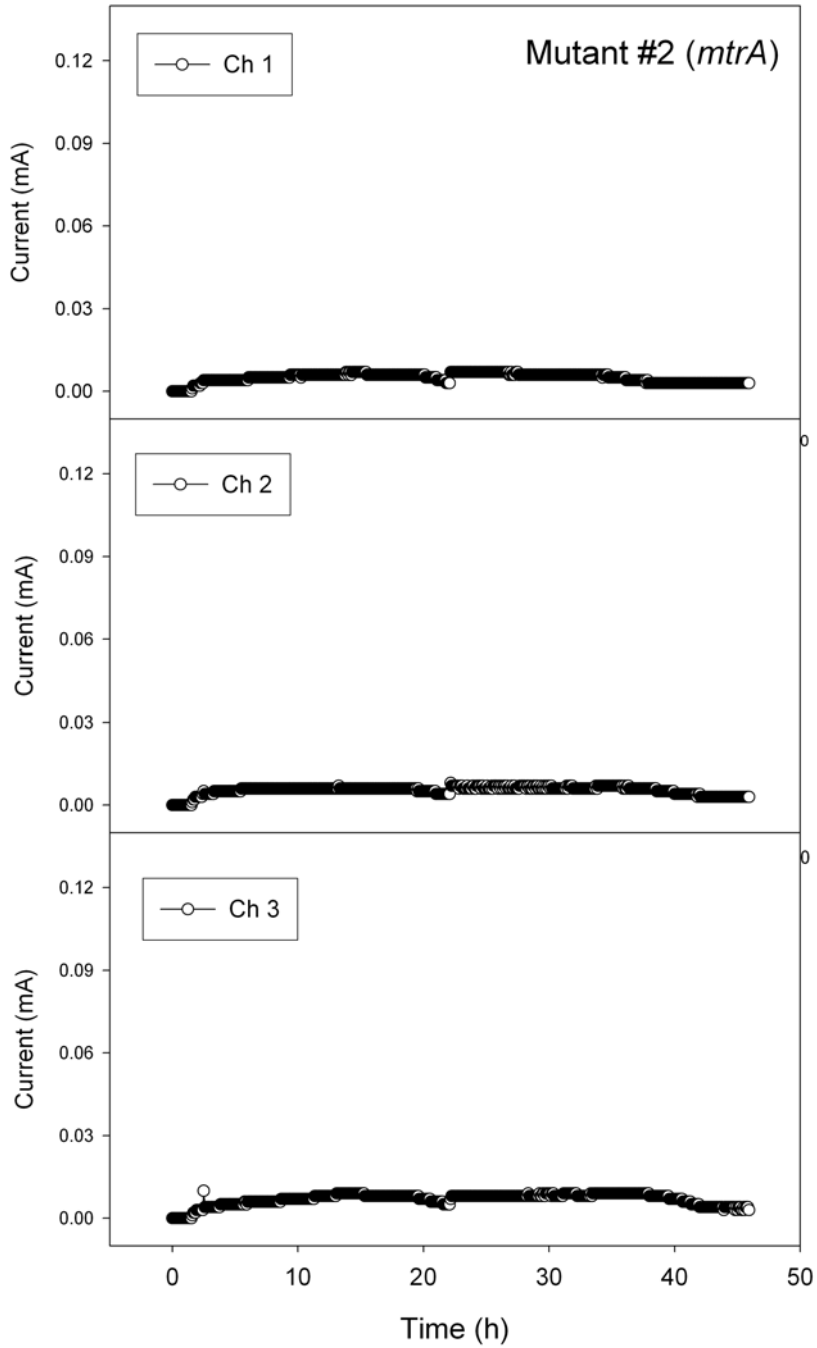

Mutant #2 (mtrA)
Ch 1
Ch 2
Ch 3
Current (mA)
0.12
0.09
0.06
0.03
0.00
0
10
20
30
40
50
Time (h)

Table 1. Electrochemical activities of *Shewanella oneidensis* MR-1 and its mutants

KIS TNo	Gene	Growth[1] on Lac/Fum	Max. current (microA)	Coulomb[3] (C)	CV[4] test
1	*Δ luxSrif*	+++	65.0±6.10	2.54±0.25	++
2	*Δ mtrA*	+++	7.33±0.58	0.432±0.09	+
3	*Δ omcA*	+++	4.67±0.58	0.32±0.01	+/-
4	*Δ hydB*	+++	61.0±15.4	2.63±0.37	+
5	*Δ hyaB and ΔhydA*	+++	66.3±17.9	2.94±0.48	+
6	*Δ hydA*	+++	54.0±	2.53±0.50	+
7	*Δ tatC*	+	15.3±2.89	0.97±0.16	++
8	*Δ mpw*	+++	48.0±7.21	2.15±0.09	+
9	*Δ fur*	++	26.0±2.00	1.30±0.25	+
10	*Δ crp*	+	19.0±6.56	1.11±0.36	+
11	*Wild type*	+++	68.0±7.81	2.56±0.18	+

2-1	*Δ mtrE (#2)*	+++	107.5±13.43	4.61±0.73	+
2-2	*Δ mtrD (#3)*	+++	65.0±2.64	3.25±1.12	+
2-3	*Δ mtrB (#9)*	+++	7.0±1.00	0.45±0.10	+/-
2-4	*Δ mtrF (#11)*	+++	52.0±1.73	2.82±0.06	+
2-5	*Δ mtrC/omcB (#21)*	+++	24.0±2.65	1.27±0.26	+
2-6	3300 (#22)	+++	57.3±9.61	3.19±0.60	+
3-9	*Δ mtrC w/ complement*	+++	43.0±2.00	2.65±0.31	+
3-10	*Δ mtrC w/ vector only*	+++	24.7±3.51	1.66±0.28	+
3-15	*Δ omcA-DC #6*	+++	62.7±6.50	3.28±0.18	+
3-31	*gspD* (BG 155)	+++	12.3±0.58	0.81±0.05	-
Ctr.	*E.coli*	+++[2]	5.0±0.1	0.36±0.10	-

Summary of Microbial fuelcell (MFC) Measurements:

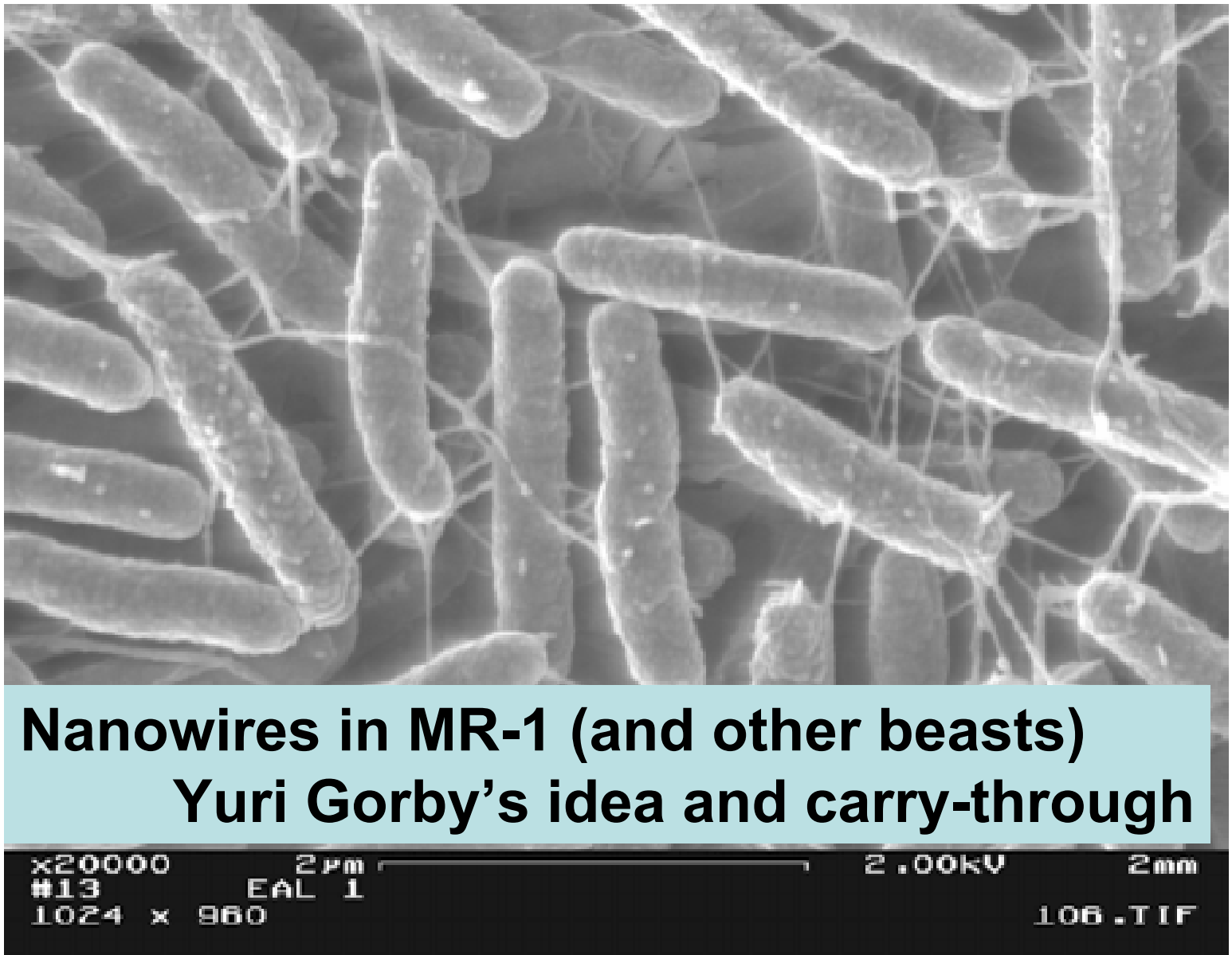
1. Almost a perfect 1:1 correlation with the ability to reduce iron & MFC activity

2. MtrC gene (suspected of being iron reductase) still has substantial activity in both

3. All negative cases share the absence of MtrA + MtrB

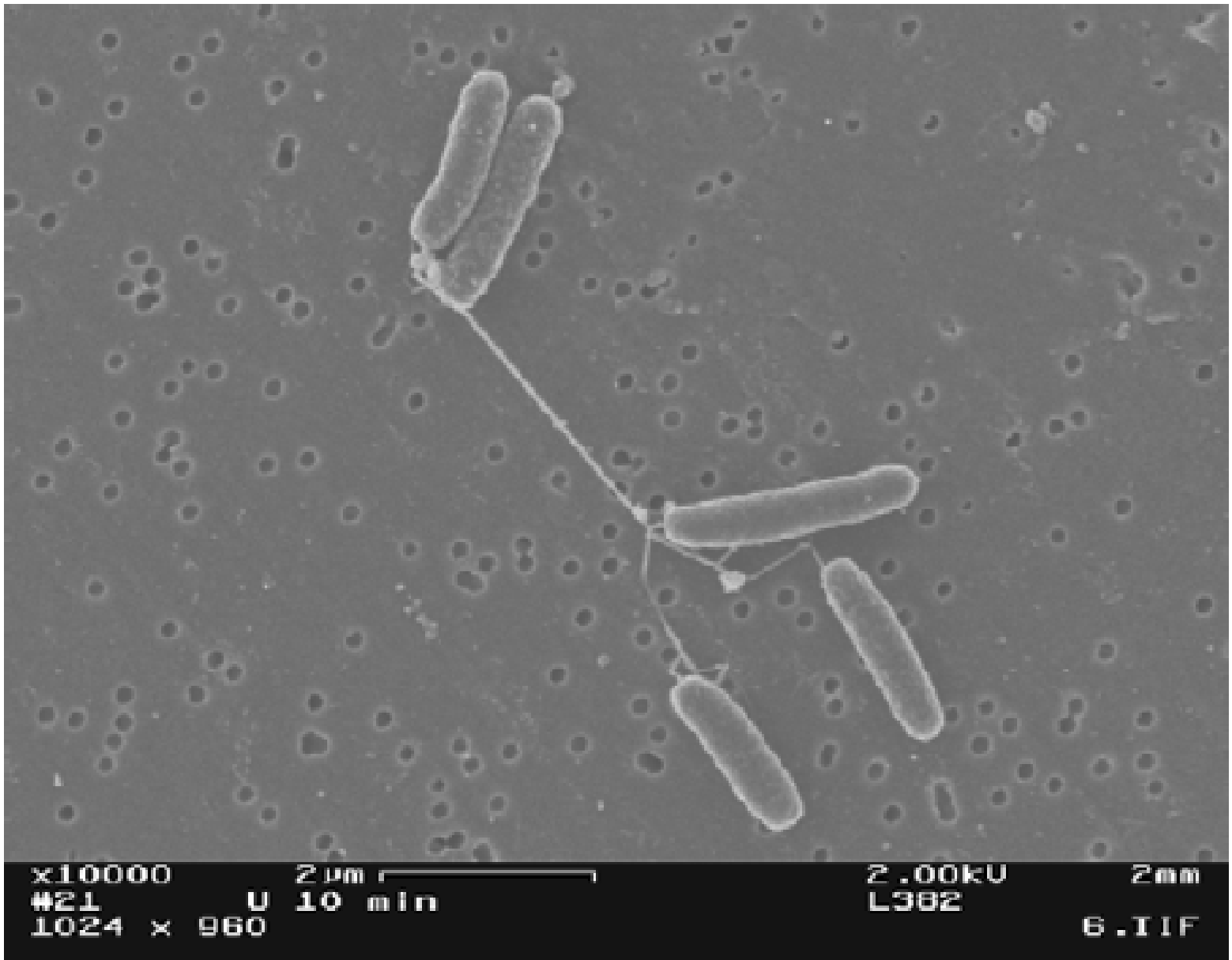
4. MtrE deletion results in 50% increase in current production as well as rate of iron reduction

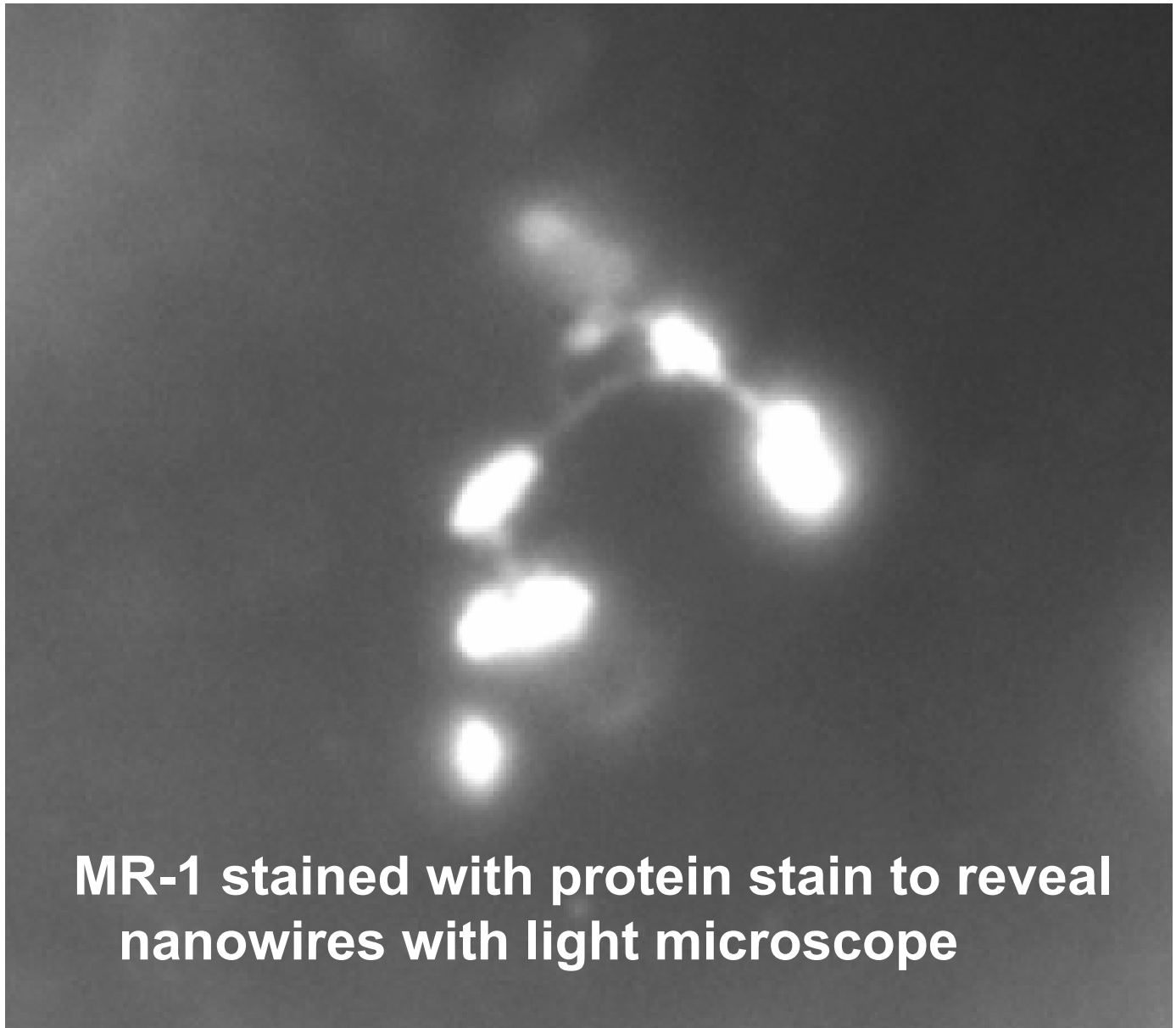
5. This is a good proxy for the ability and rate at which MR-1 can reduce Fe(III)



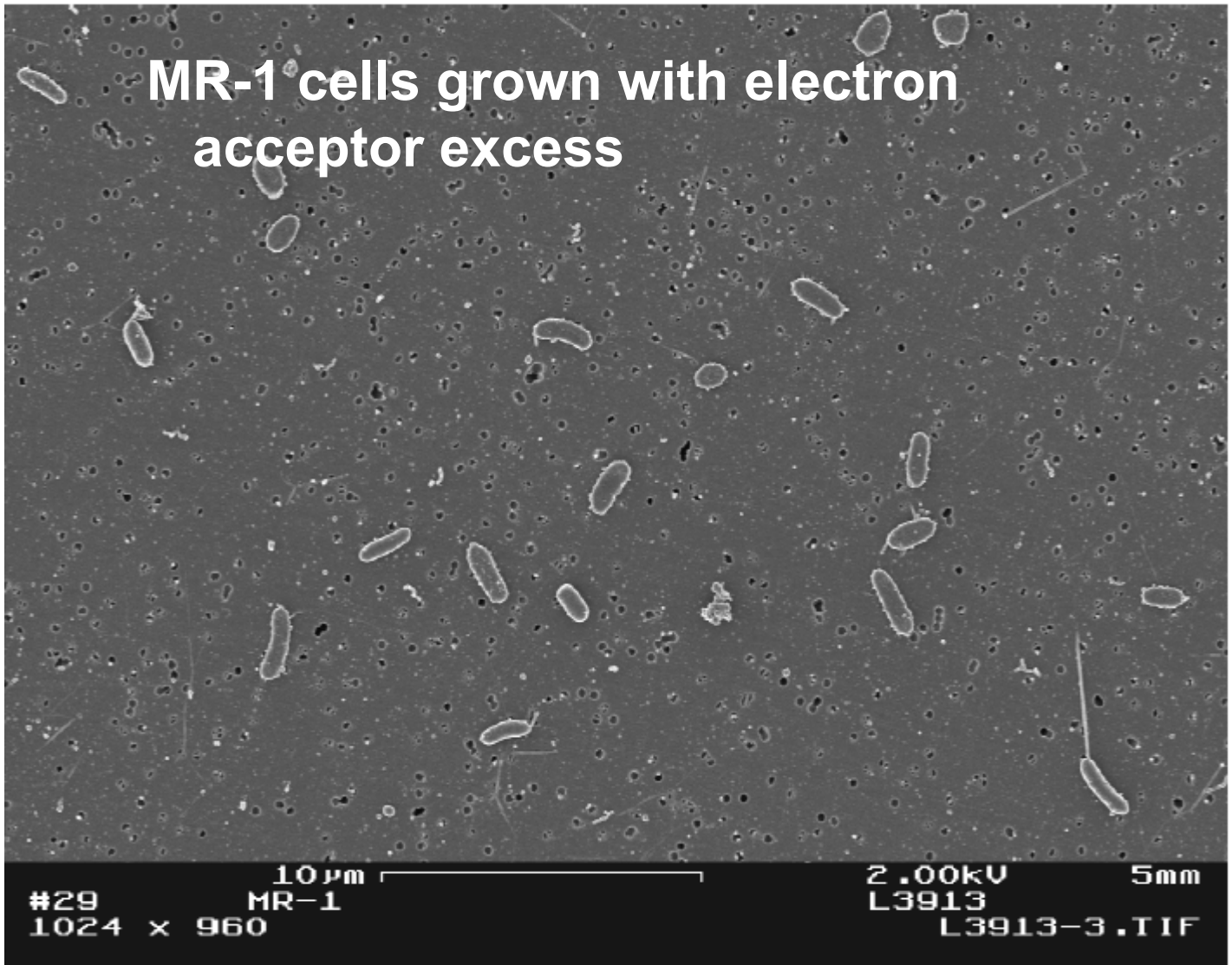
Nanowires in MR-1 (and other beasts)

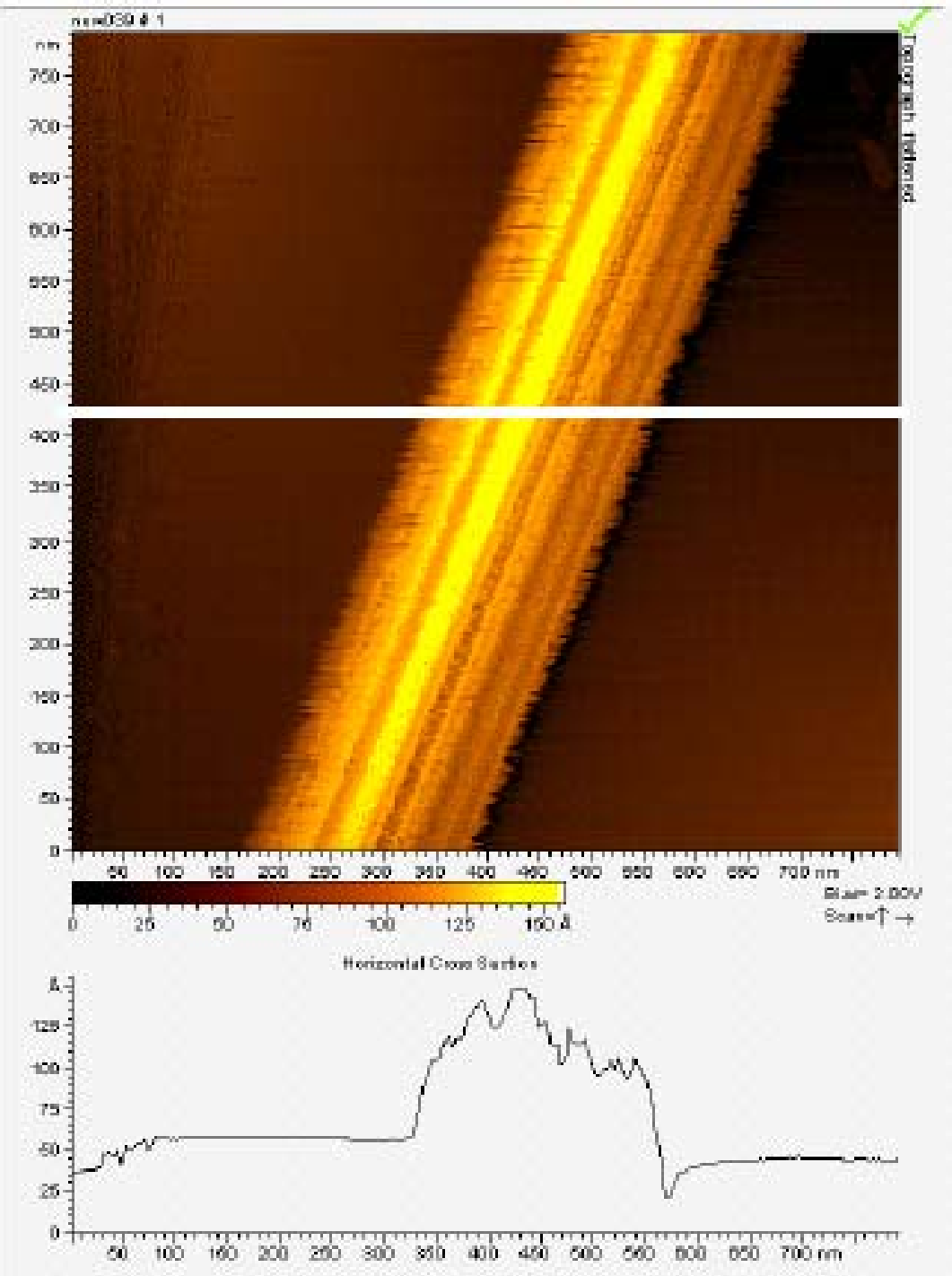
Yuri Gorby's idea and carry-through


x10000 2µm 2.00kU 2mm
#21 U 10 min L382
1024 x 960 6.TIF



MR-1 stained with protein stain to reveal nanowires with light microscope

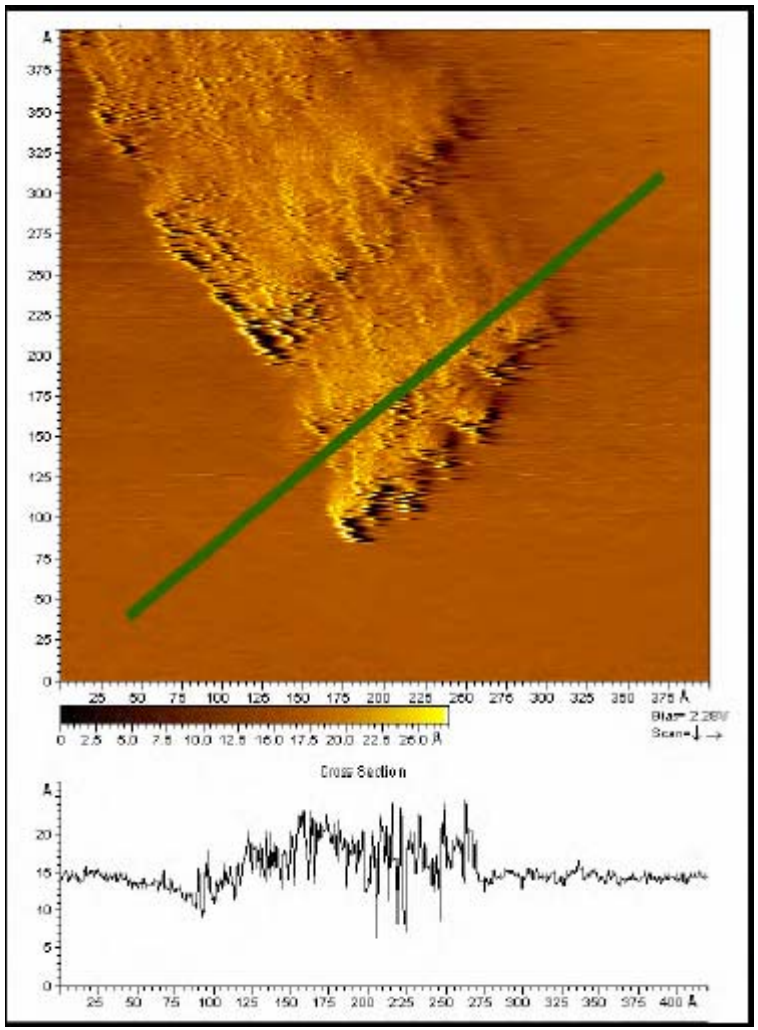

MR-1 cells grown with electron acceptor excess
10µm
2.00kV
5mm
#29
MR-1
L3913
1024 x 960
L3913-3.TIF

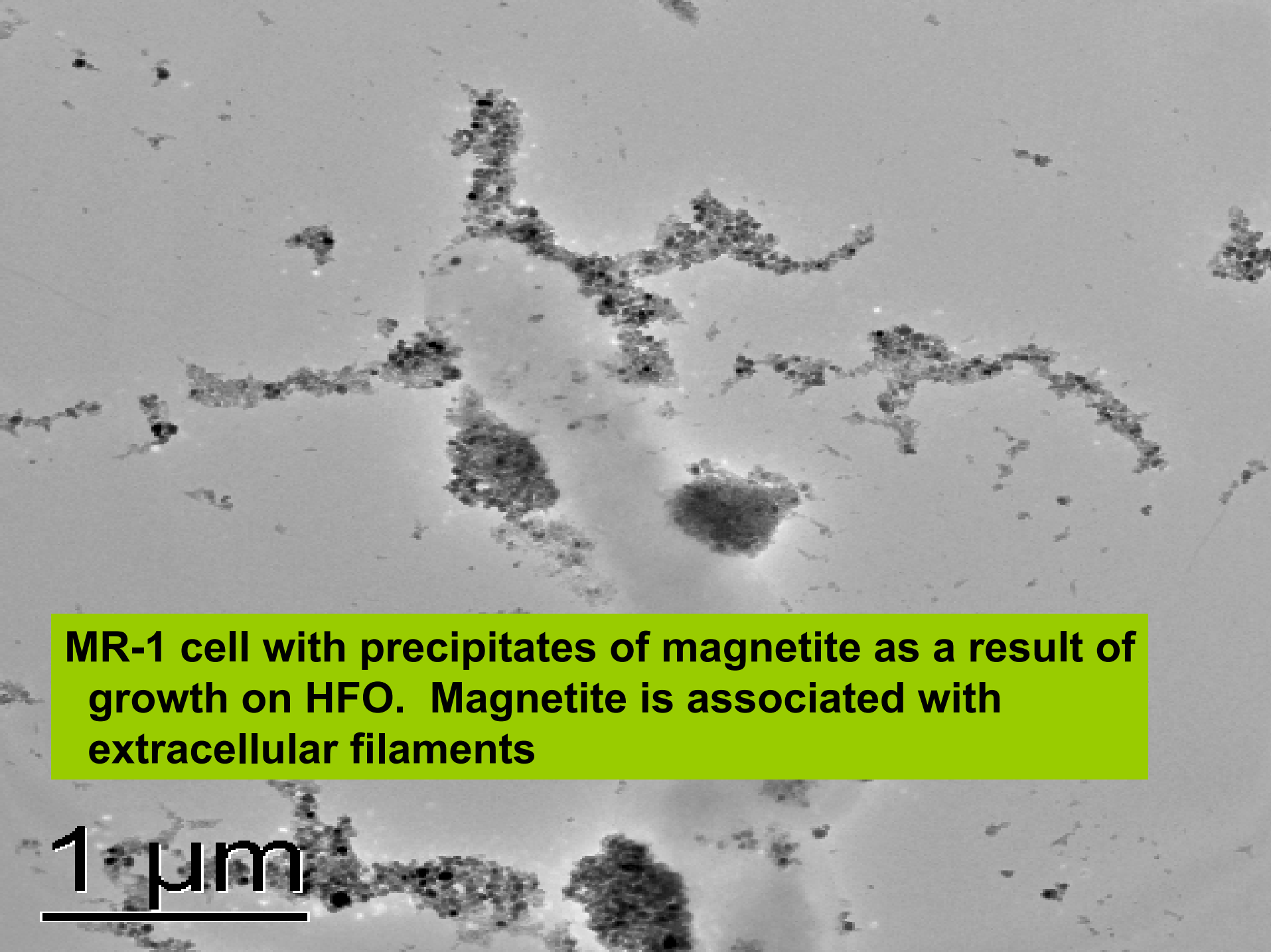


MR-1 bundle of nanowires

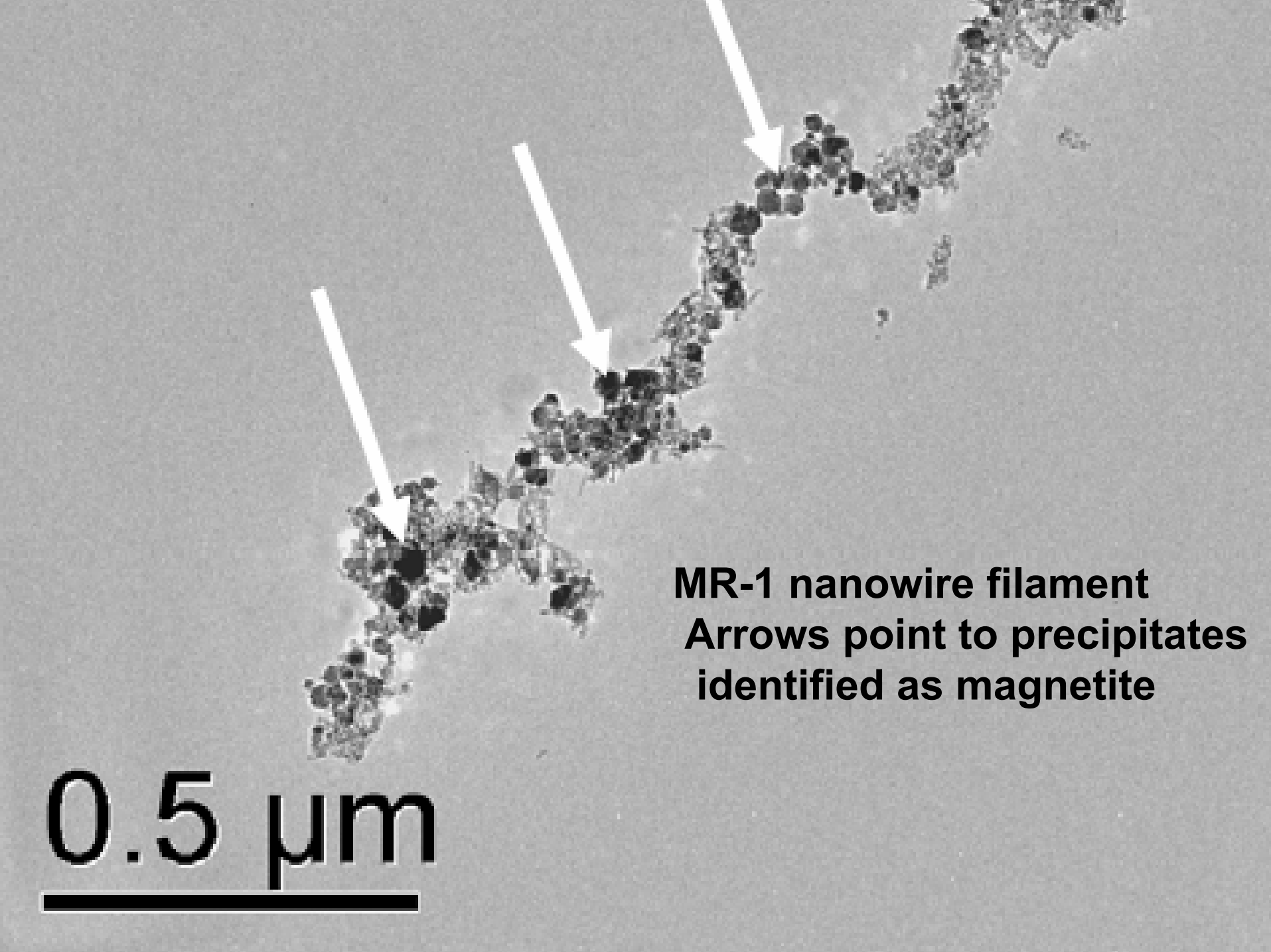
STM image with height (proxy for conductivity) shown below.

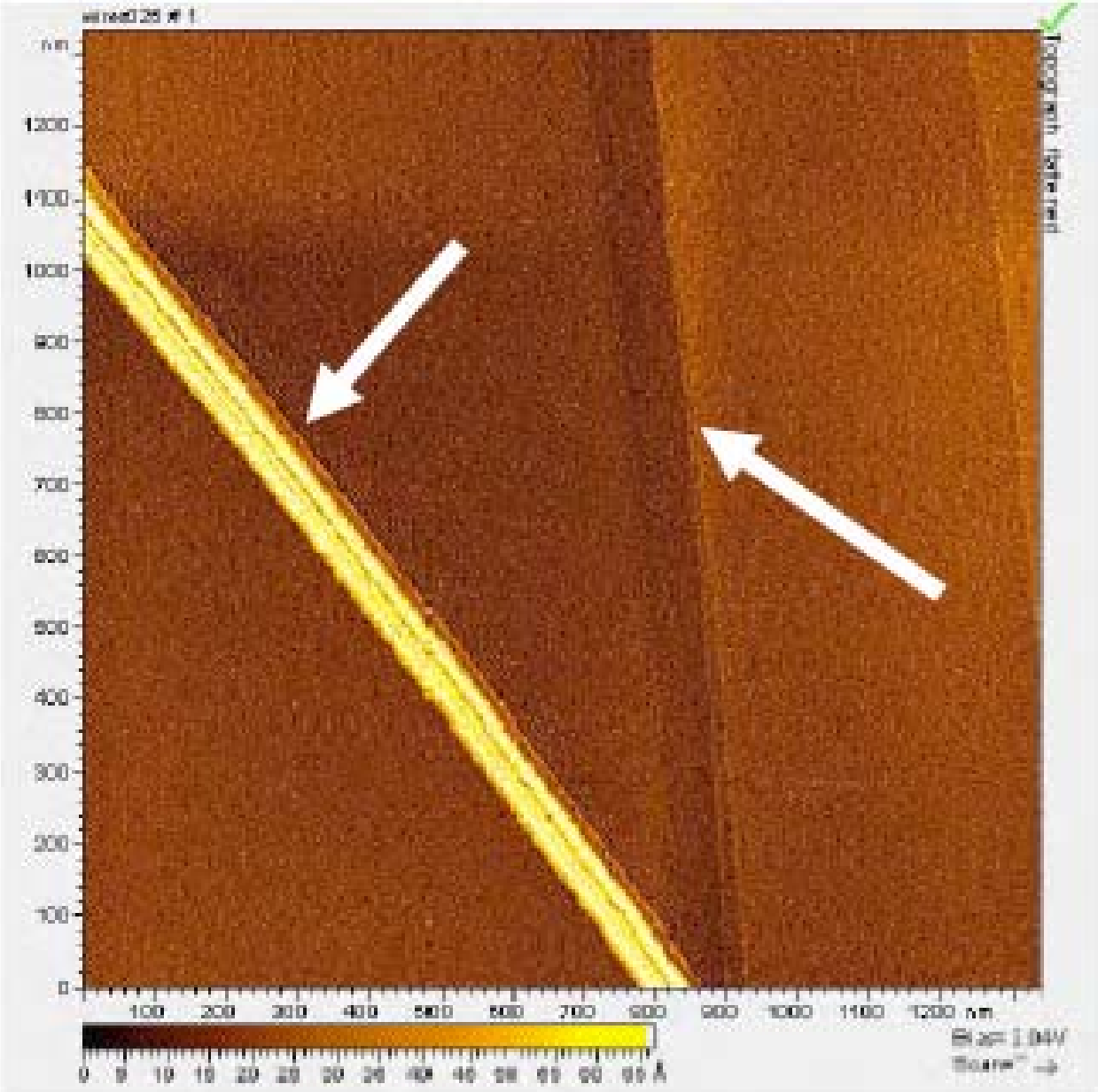
Filaments are highly conductive

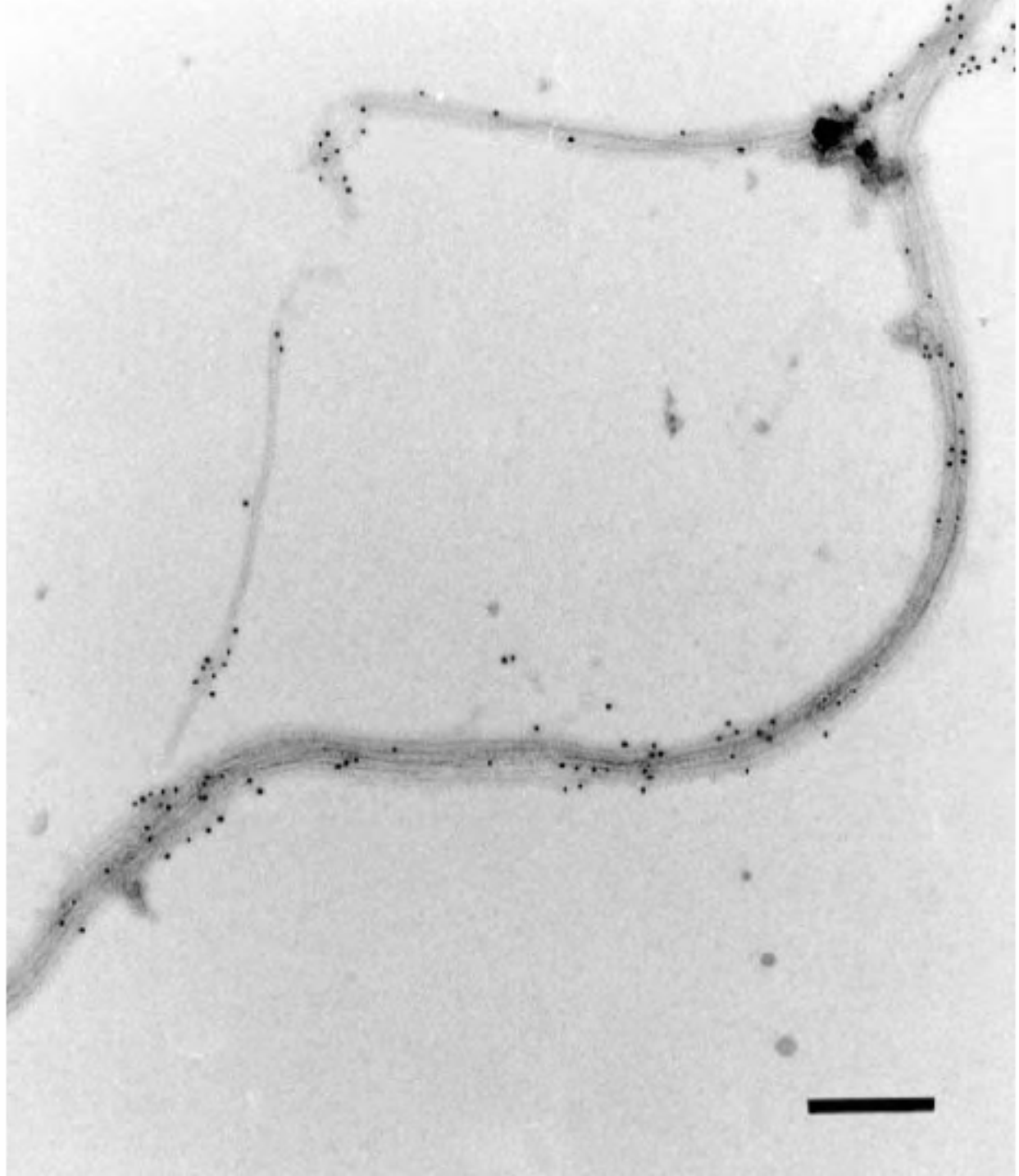

Cross Section



MR-1 cell with precipitates of magnetite as a result of growth on HFO. Magnetite is associated with extracellular filaments

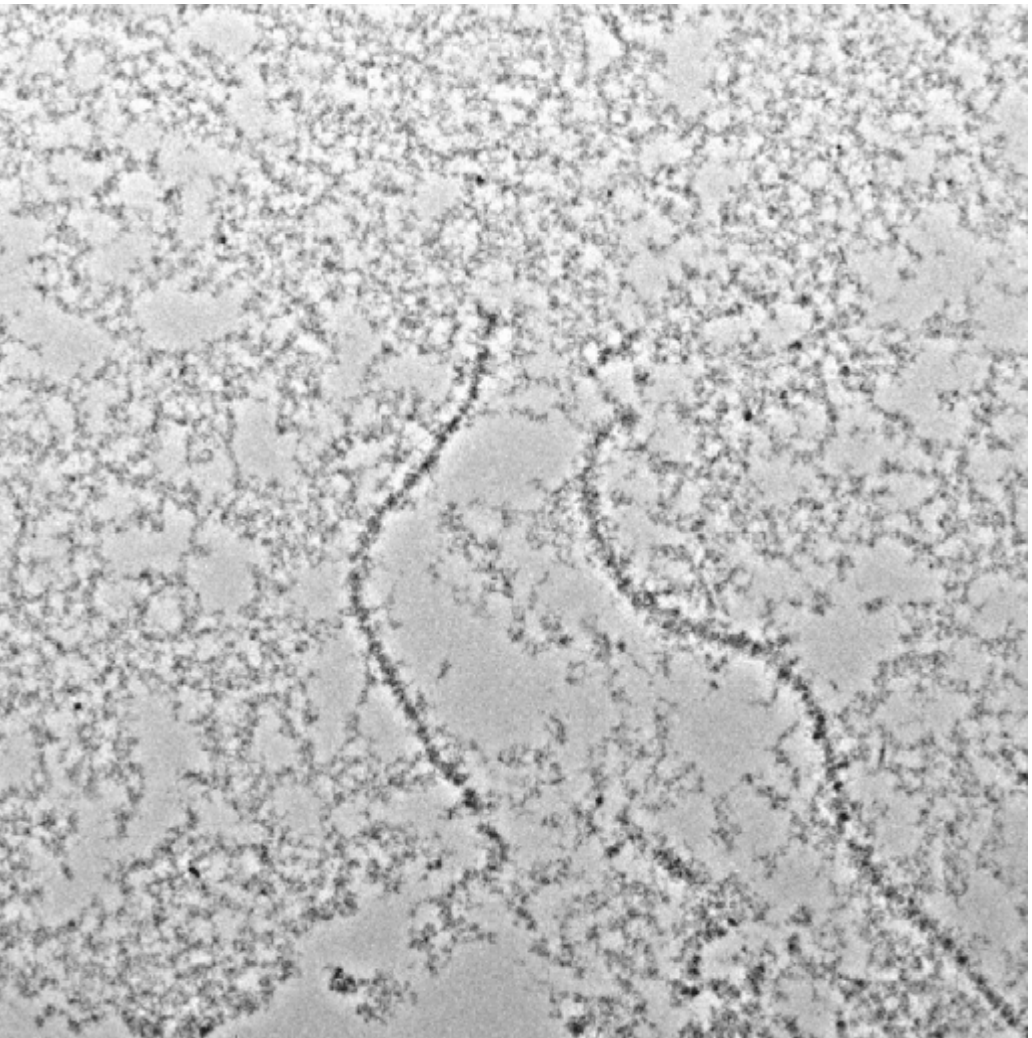

MR-1 nanowire filament
Arrows point to precipitates
identified as magnetite
0.5 µm


nm
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
100 200 300 400 500 600 700 800 900 1000 1100 1200 nm



MR-1 nanowires incubated with antibody to MtrC with gold label on the antibody

Clearly, MtrC is associated with the pilus-like filaments

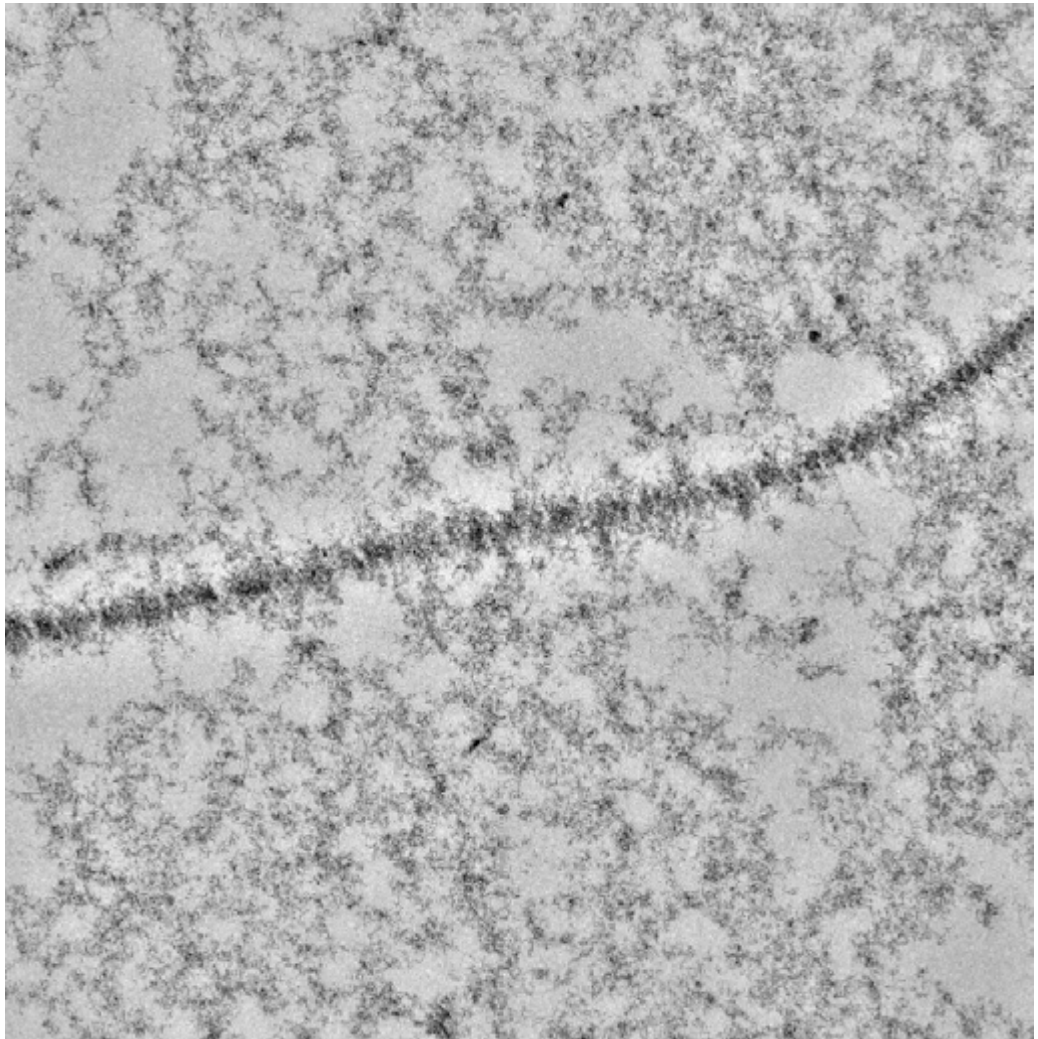


Nanowires of MR-1 grown in soft agar with EAL.

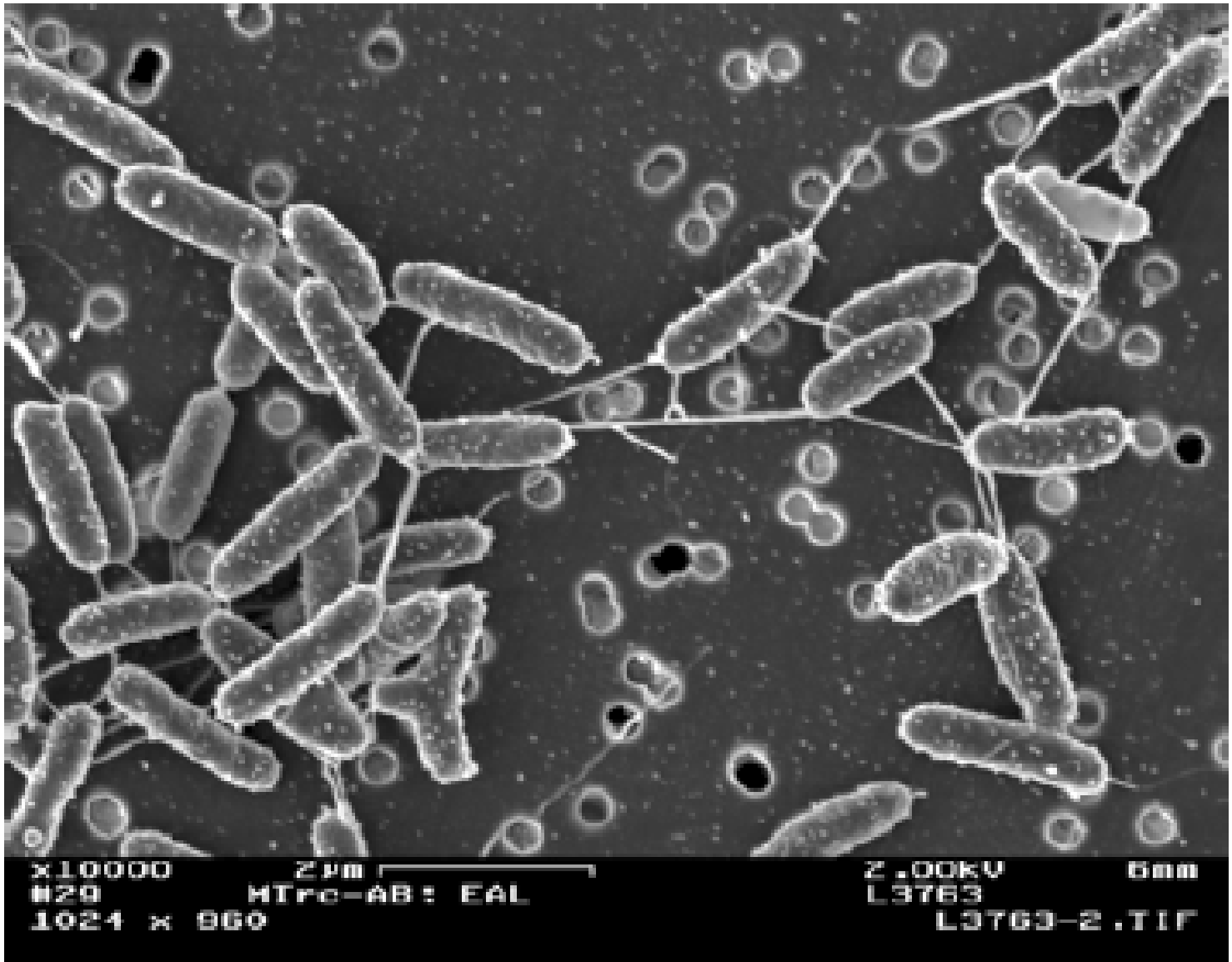
Filaments form many mm distant from the cells

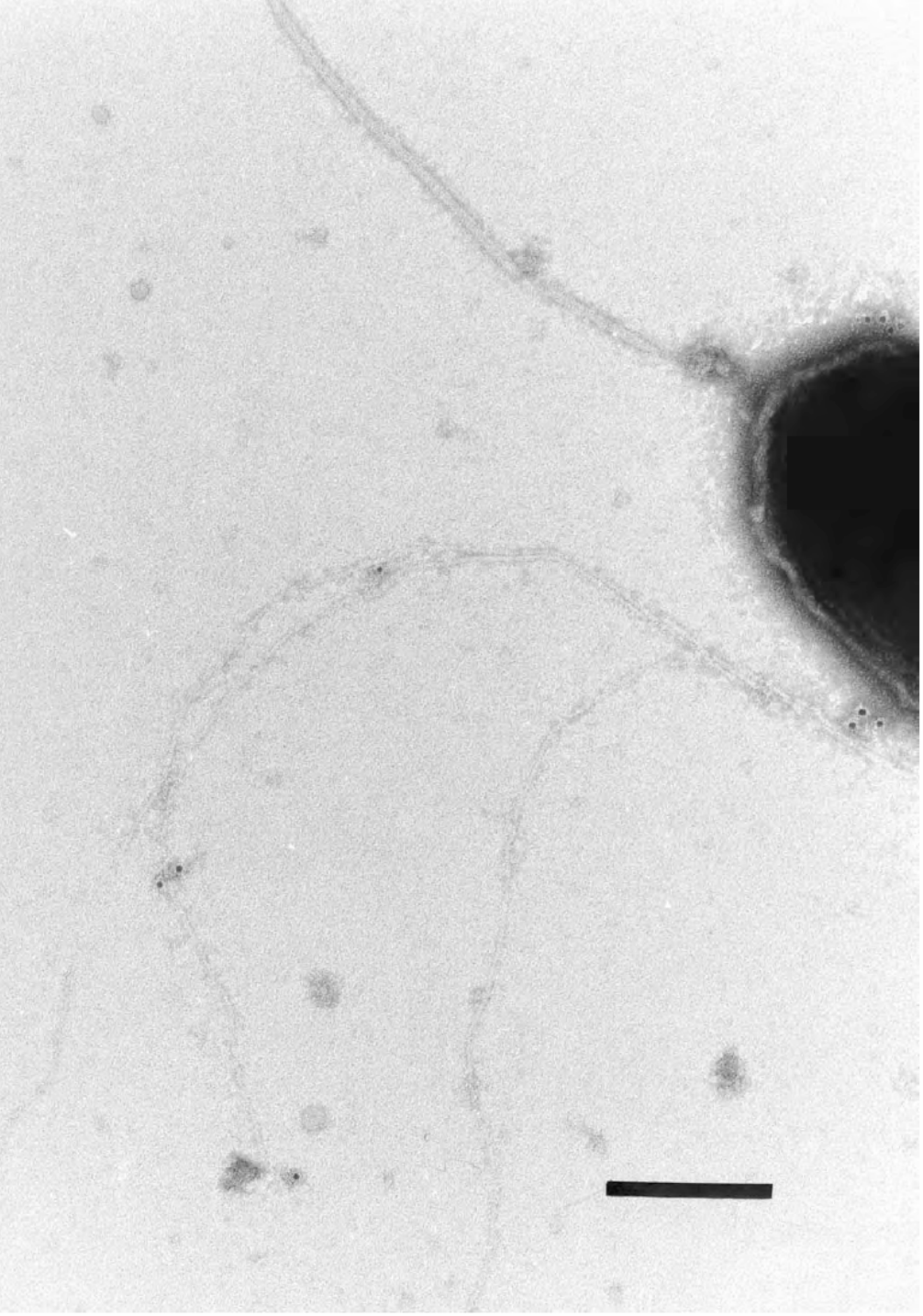
These are in the vicinity of the solid electron acceptor (HFO)

High resolution EM of wire mm's from cells!



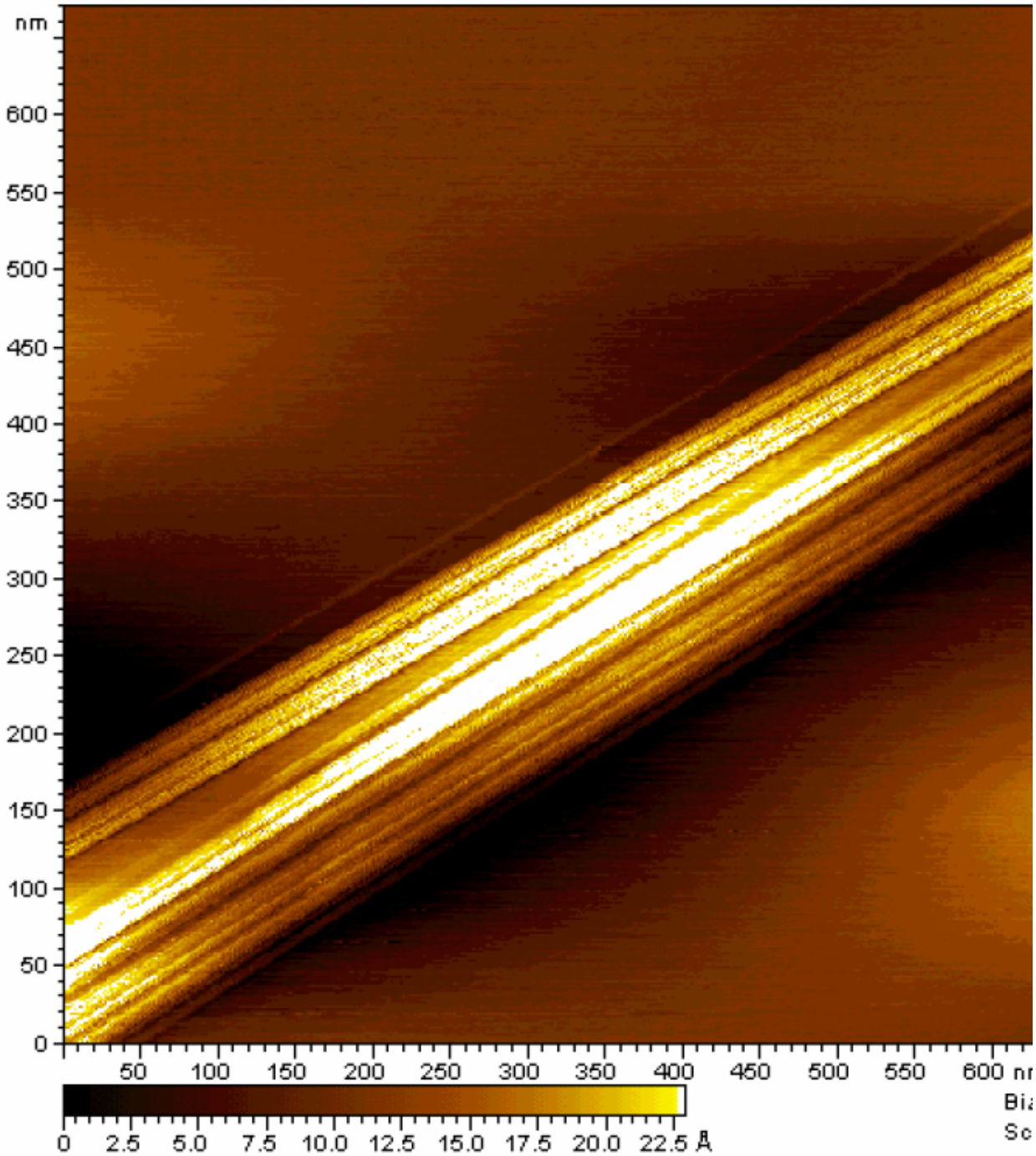
Mr-1 cells grown with EAL. These are mutants that can not produce MtrC protein. Pilus-like filaments appear normal.





High resolution picture of cells from MtrC mutant.

While pilus appears normal, no reaction is seen with peptide antibody to MtrC.



Just for fun !!

Nanowire from a cyanobacterium Synechocystis

We have observed these wires in a variety of different organisms.

Summary of nanowires:

Small electrically conductive pilus-like structures are made under conditions of EAL

These structures have MtrC and OmcA proteins attached to them, visible by gold-labeled Ab

Absence of the structures leads to iron reduction negative phenotype

Absence of MtrC leads to non-conductive wire

See similar structures in many other groups of bacteria.

Summary:

1. Mn & Fe cycling on Earth is strongly impacted by microbial activities
2. Strain MR-1 is very versatile metal reducer
3. *mtr* genes are present in all metal reducers so far tested
4. Interaction with solid metal oxides requires outer membrane enzymes
5. Microbial Fuel Cell activity is a good proxy for metal ion reduction
6. Nanowires are apparently intimately connected with the ability to reduce solid substrates

USC *Geobiology*

Major Challenges: Metal speciation

- Fe(II)/Fe(III)
- Mn(II)/Mn(III)/Mn(IV)
- Environmental distribution and flux
  - temporal variation – major species
- Laboratory distribution and flux
  - high spatial resolution
  - enzymatic catalysis

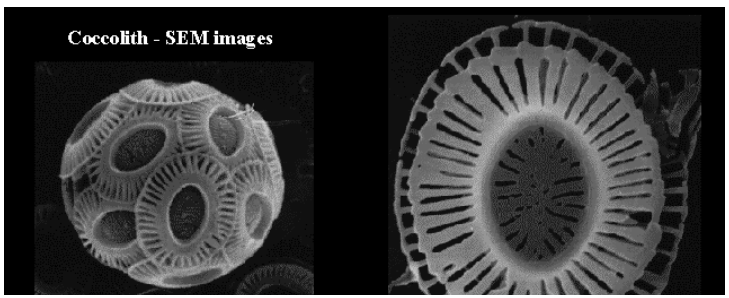
Great opportunities for synchrotron science with microbiology

Thank you for
your attention !

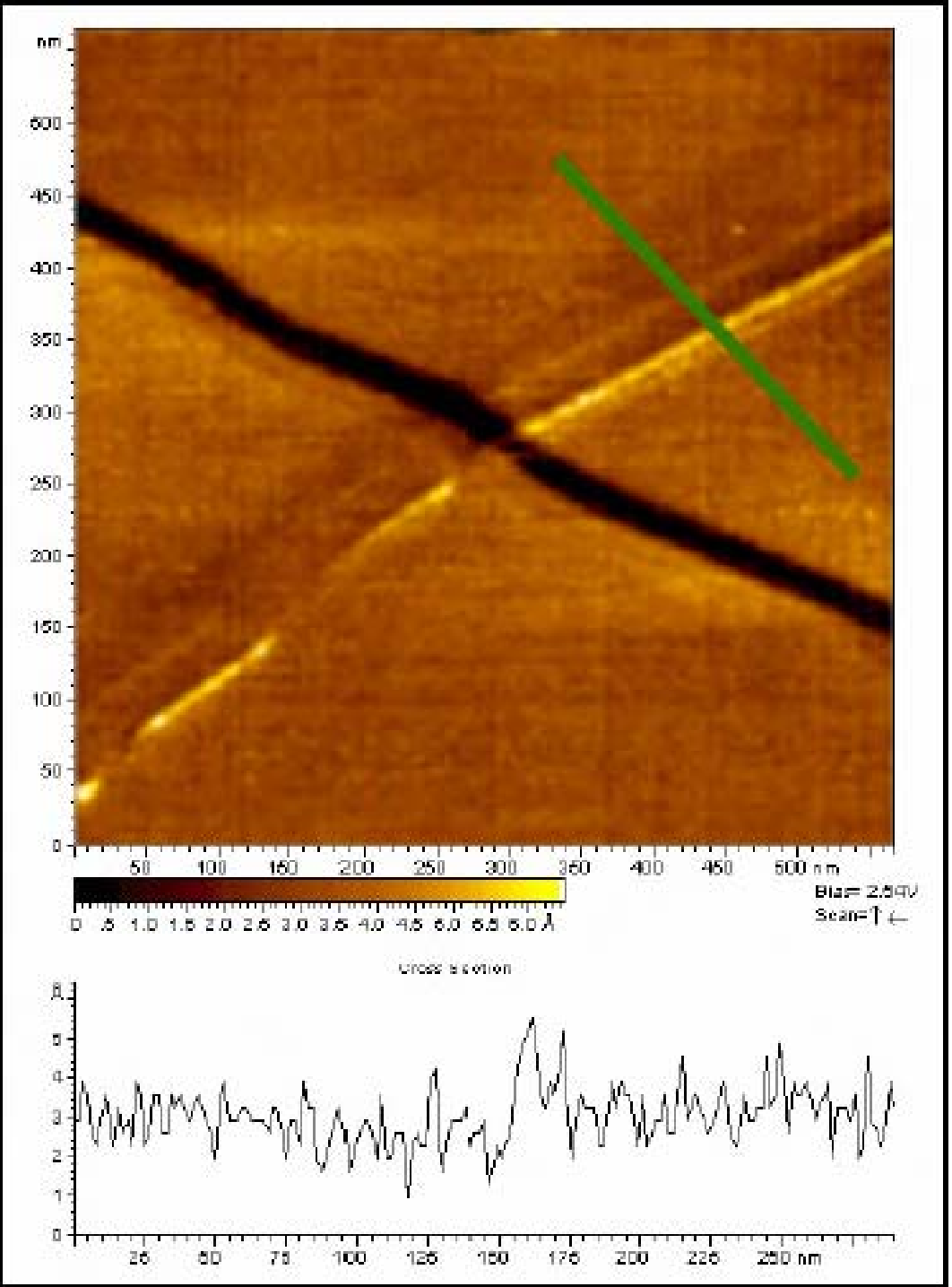
nanowires

PNNL
ANL
KIST

South Africa Limestone Mine

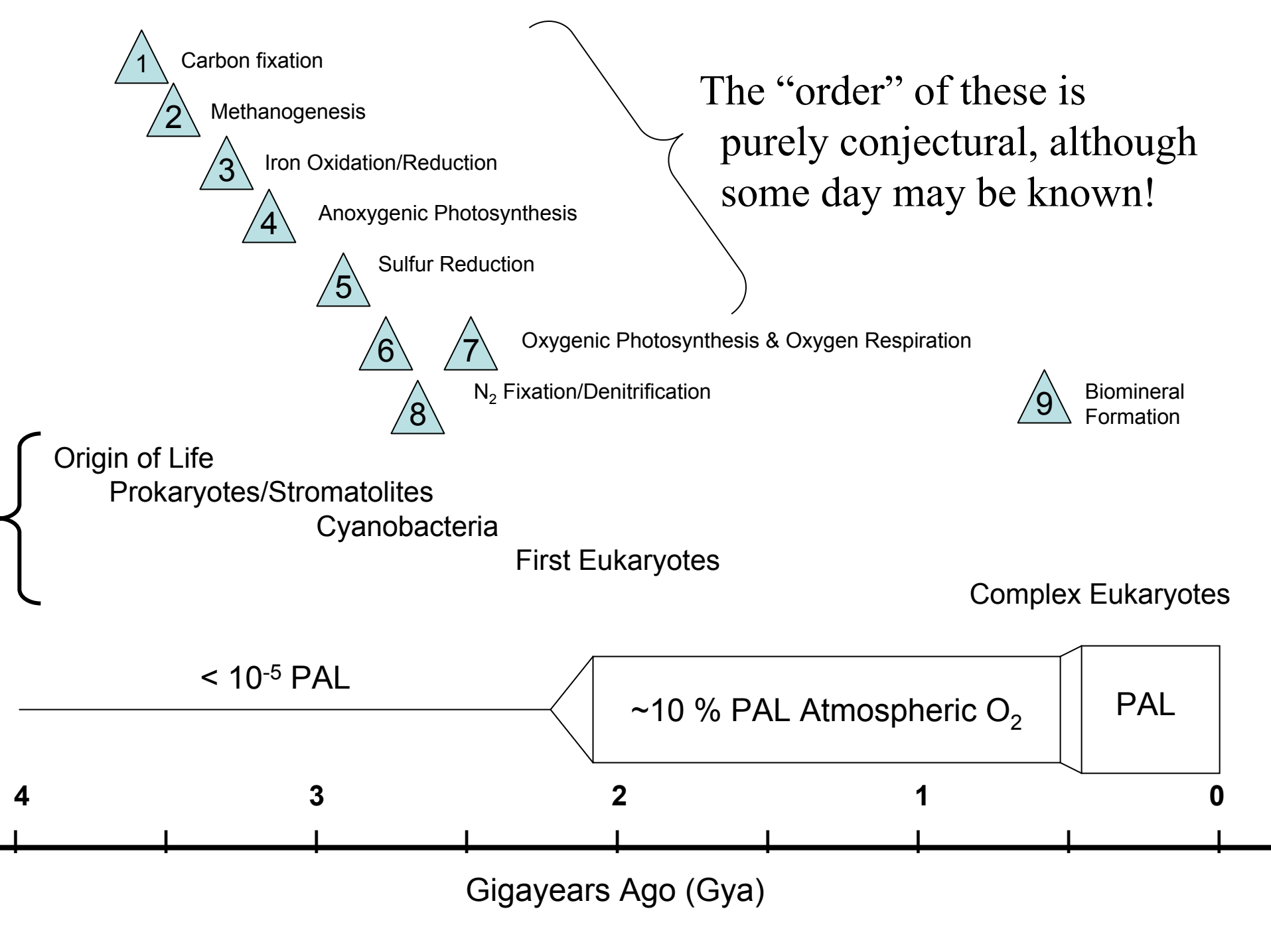


Carbonate-precipitating Eukaryotes



STM scan of pilus from MtrC mutant of MR-1

Here we see that while the pilus appears normal, the conductivity is very low.


1
Carbon fixation
2
Methanogenesis
3
Iron Oxidation/Reduction
4
Anoxygenic Photosynthesis
5
Sulfur Reduction
6
7
Oxygenic Photosynthesis & Oxygen Respiration
8
N_2 Fixation/Denitrification
9
Biomineral Formation
The "order" of these is purely conjectural, although some day may be known!
Origin of Life
Prokaryotes/Stromatolites
Cyanobacteria
First Eukaryotes
Complex Eukaryotes
< 10^{-5} PAL
~10 % PAL Atmospheric O_2
PAL
4
3
2
1
0
Gigayears Ago (Gya)