Comparative biochemistry and physiology of metal-reducing organisms from acidic and neutral pH environments

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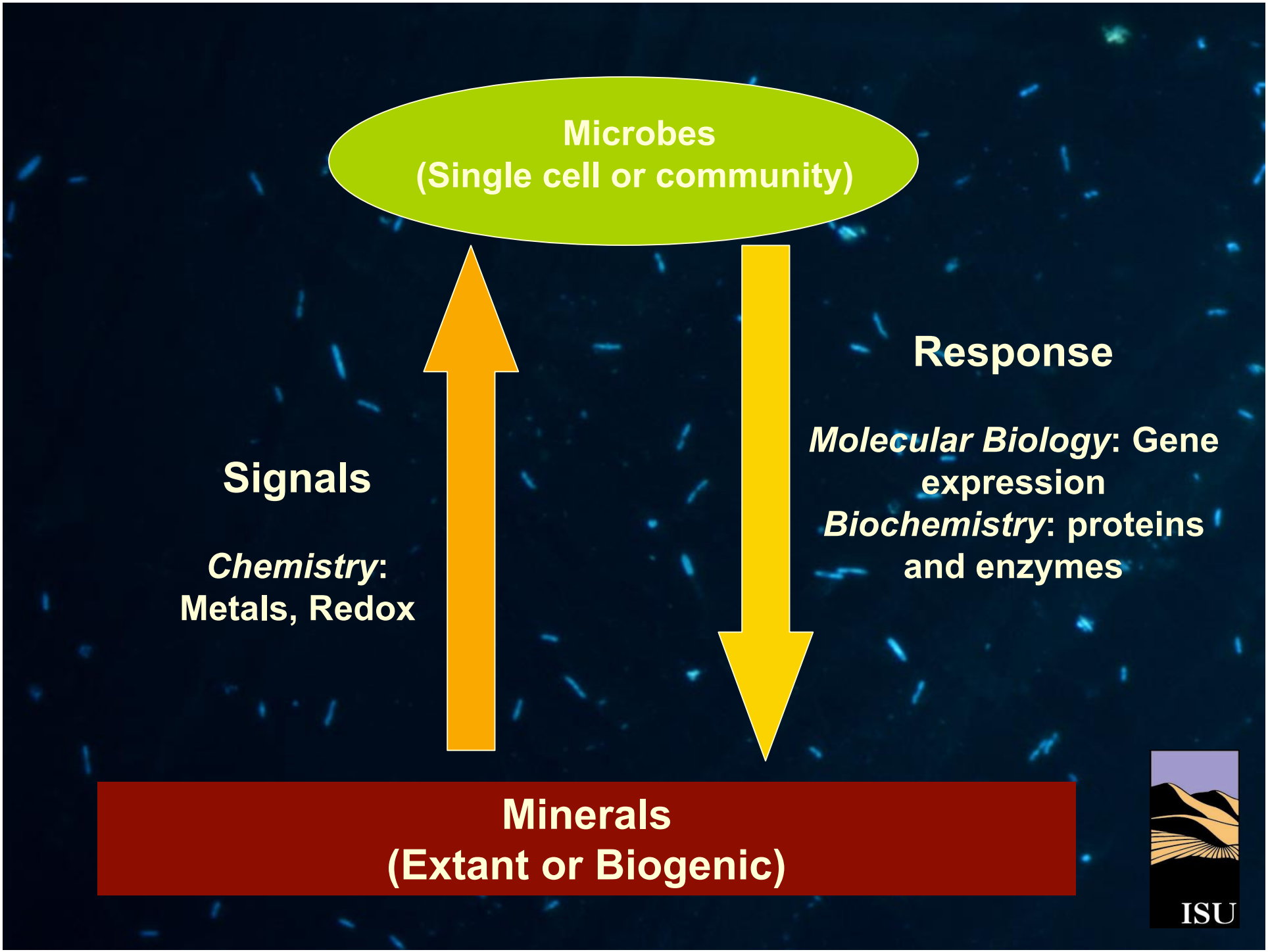
Microbes
(Single cell or community)
Signals
Chemistry:
Metals, Redox
Response
Molecular Biology: Gene expression
Biochemistry: proteins and enzymes
Minerals
(Extant or Biogenic)
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Relevance

- Metal contamination+acid conditions at the FRC
- Soluble vs. insoluble Fe, and the mechanisms for respiration of each

Questions

- What similarities and differences between metal respiration systems of neutrophiles and extremophiles?
- What complement of redox proteins exist in the two physiotypes of bacteria?
- How exactly do redox proteins interact with minerals?
- Do they work independently, or in multi-protein complexes?

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Acidiphilium cryptum JF-5

- True acidophile
- Alphaproteobacteria
- Isolated by K. Kusel from coal mine acid lake, Germany
- Genome by DOE-JGI 2005-2006
- Biochemistry being investigated

Geobacter spp.

- neutrophile
- Deltaproteobacteria
- Isolated from variety of habitats
- Genomes available
- Biochemistry better understood

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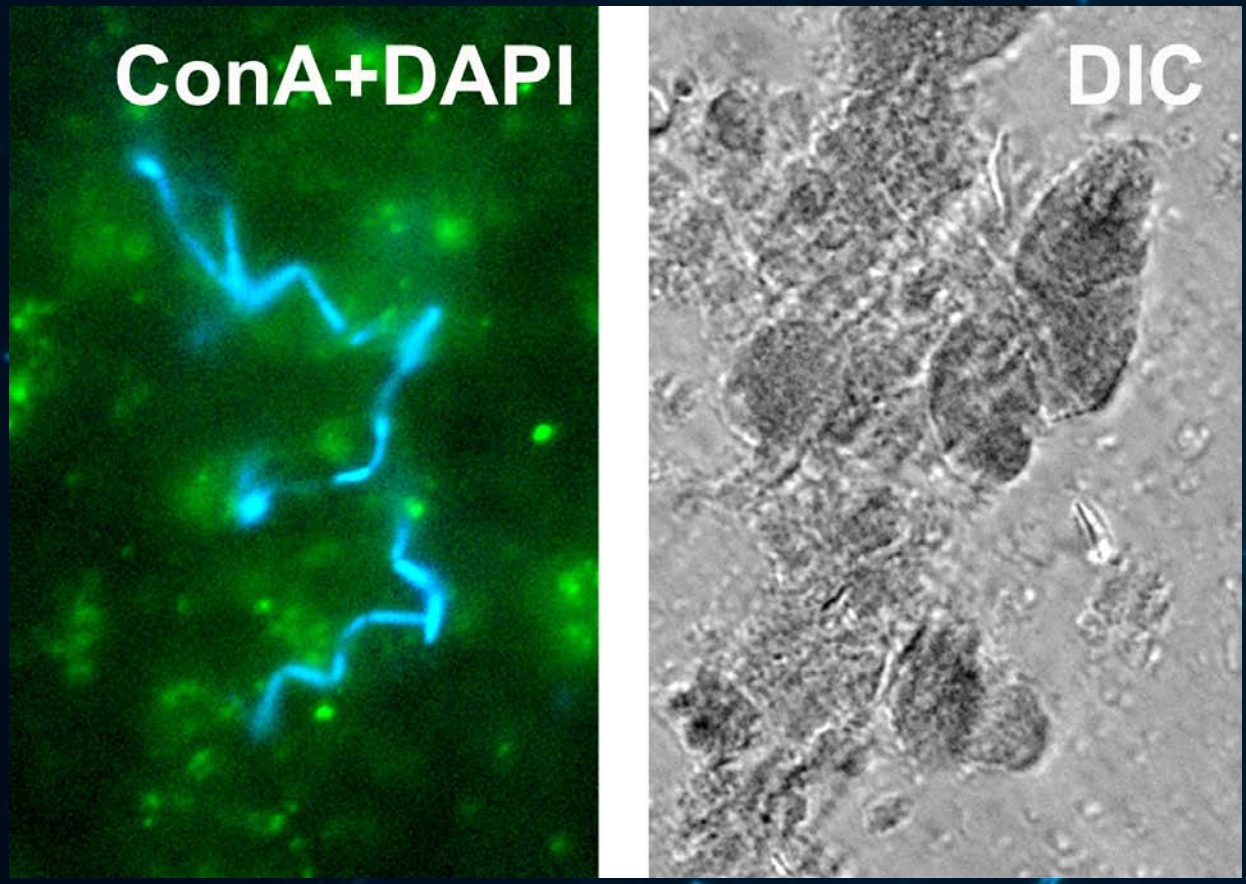
Attachment studies

- Lectin probes
- *Geobacter* on Fe-oxides
- Shows biofilm mineral particle aggregation



Magnuson, T.S., A.L. Neal, and G.G. Geesey. 2004. Microb. Ecol. 48: 578-588

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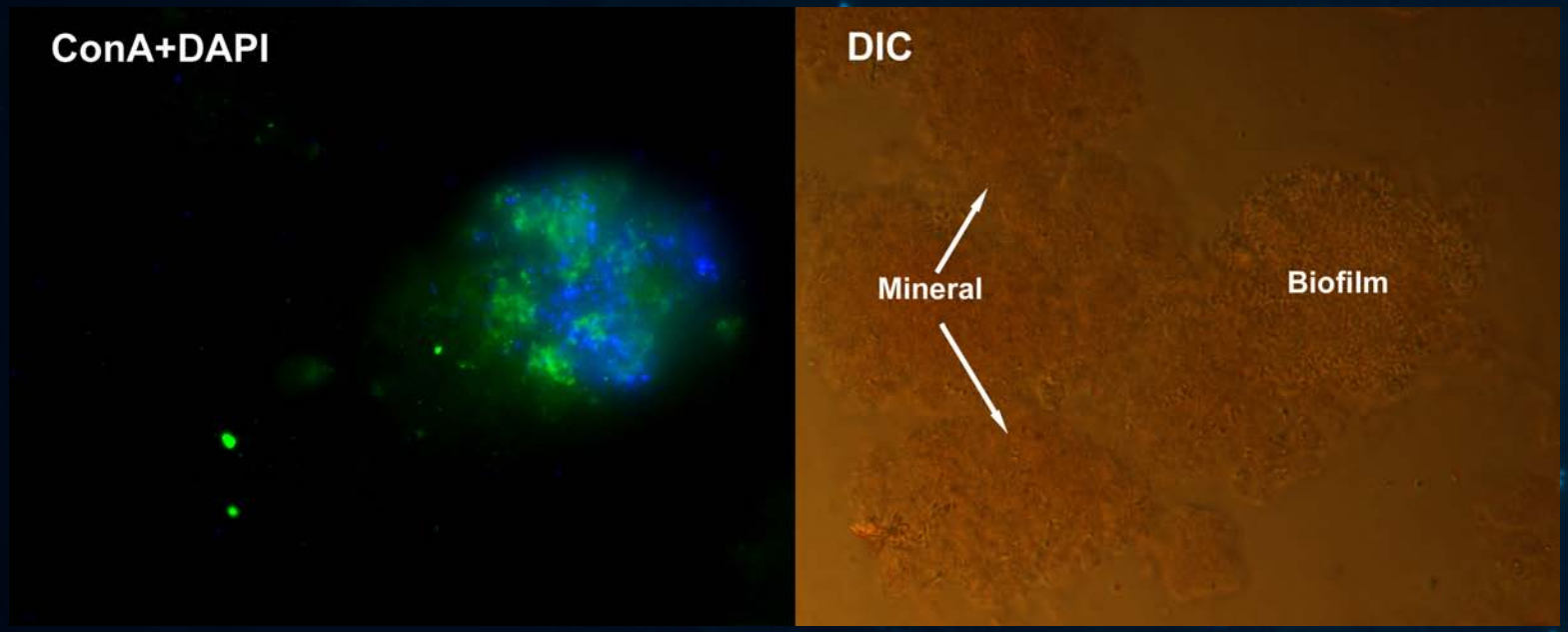
Attachment studies



- Lectin probes
- *Acidiphilium* loosely attached to mineral surface
- Aggregate mineral particles

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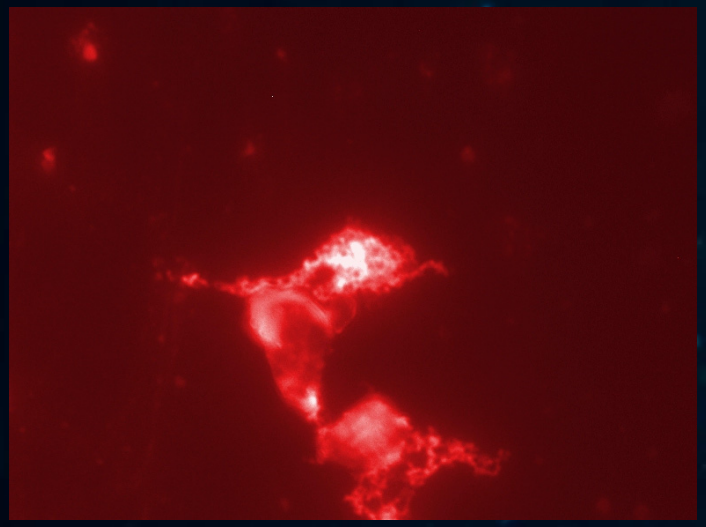
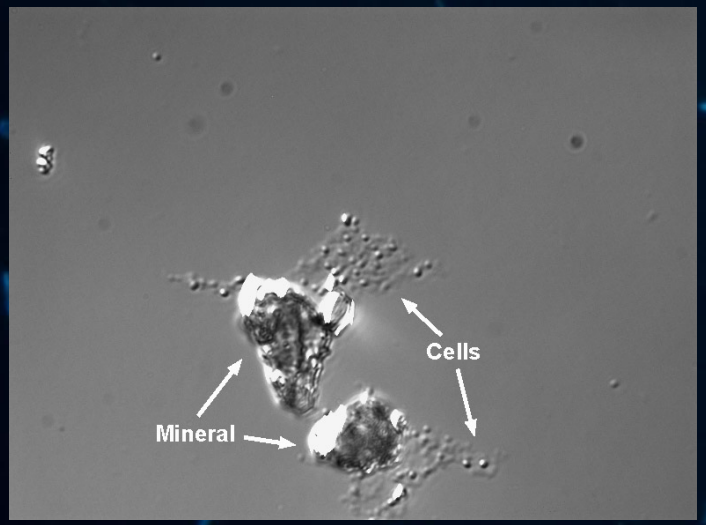
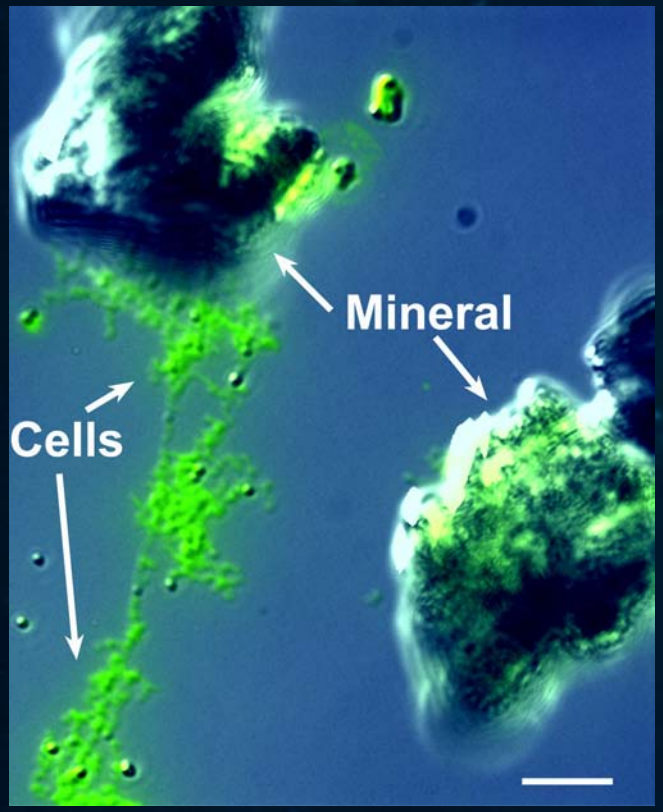
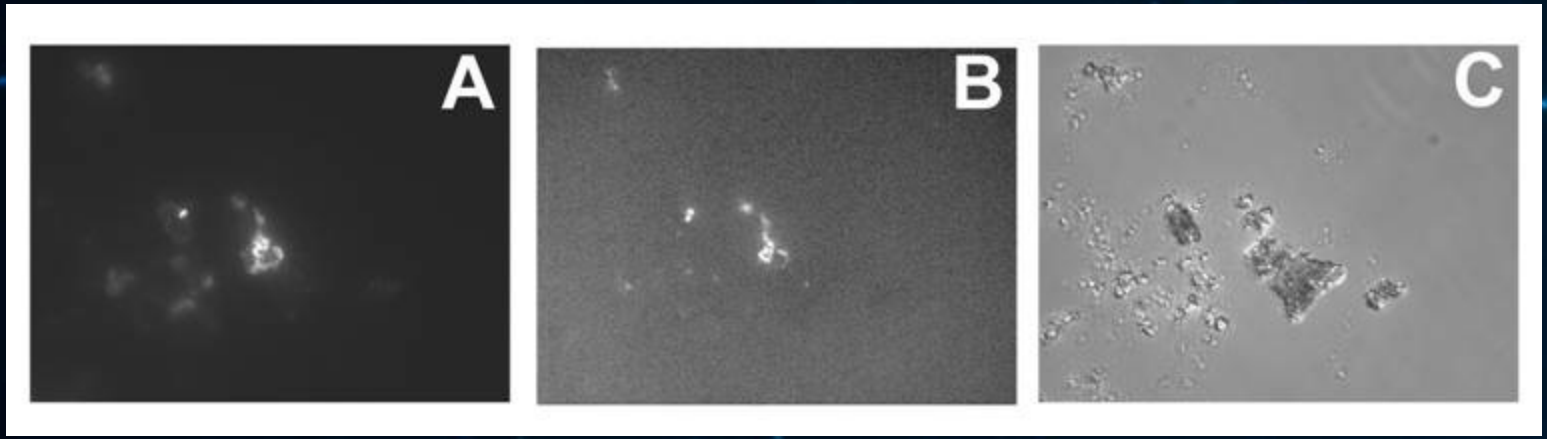
IS-RT-PCR analysis of *Geobacter*





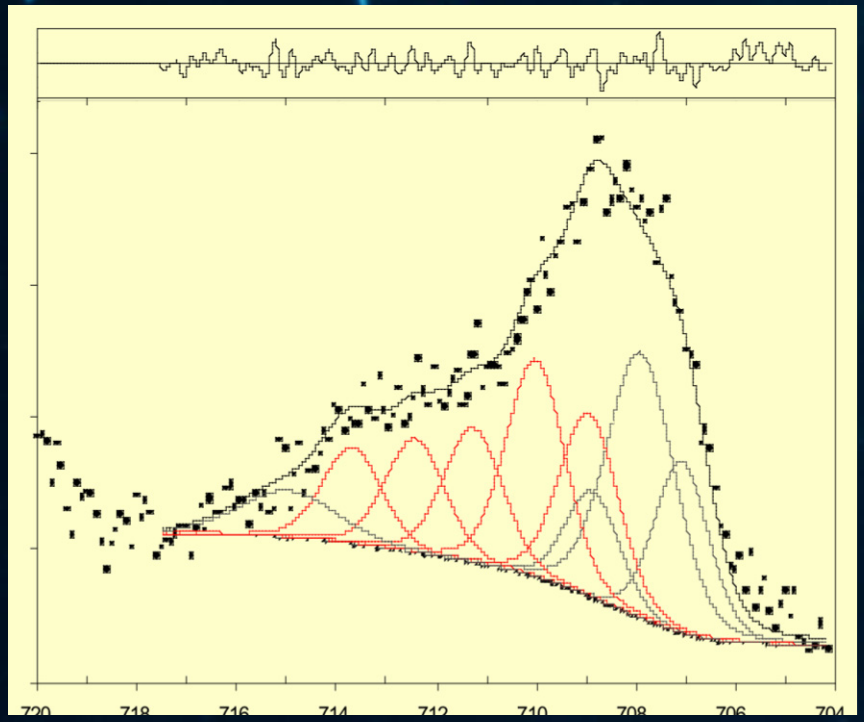


Geobacter-Colonized Ferrihydrite: *in situ* images…



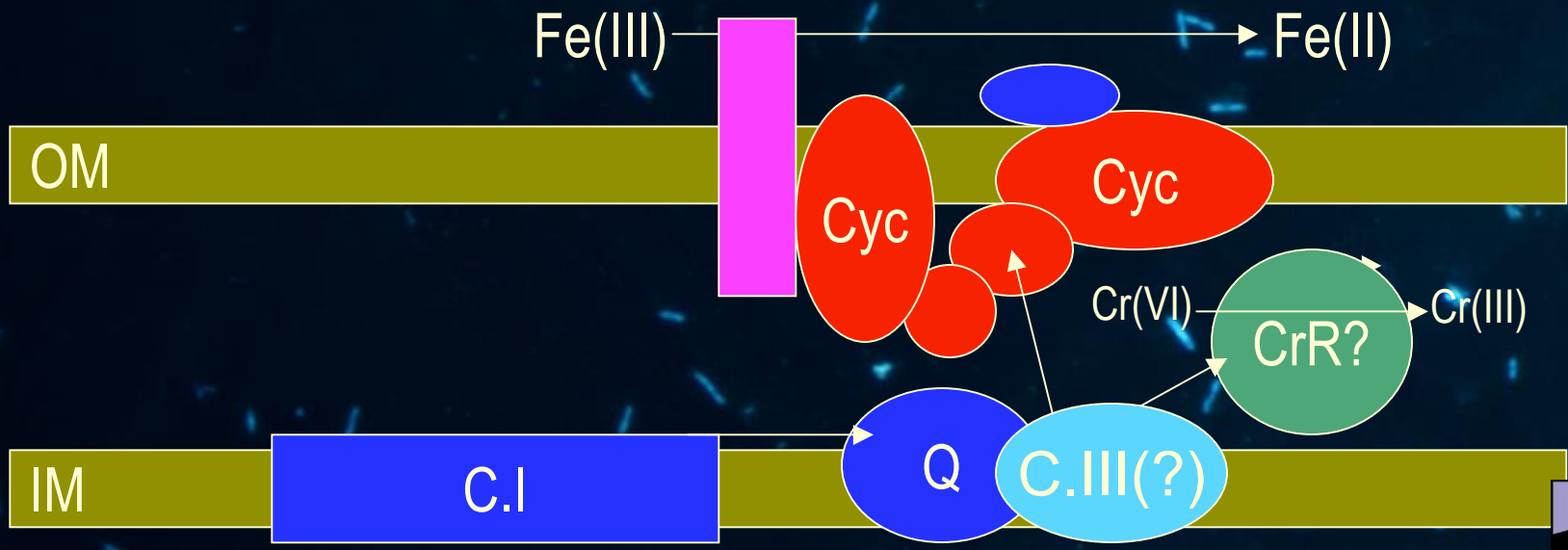
XPS surface analysis of sample from same culture

- Gene expression detected on ferrihydrite substrate
- Strong evidence for biologic mineral transformation- 45% Fe(II)



The paradigm of metal reduction

- In neutrophiles– mediated by cytochromes c (electron carriers)
- Gene redundancy and very high levels of cytochromes c
- Acidophilic Fe-oxidizing bacteria—possess membrane associated large mass cytochromes c
- Hypothesis—Acidophilic Fe-reducing bacteria use similar cytochromes, which have thermodynamic properties suitable for metal reduction
- Molecular circuits (parallel or series) allow electron transfer to an insoluble substrate



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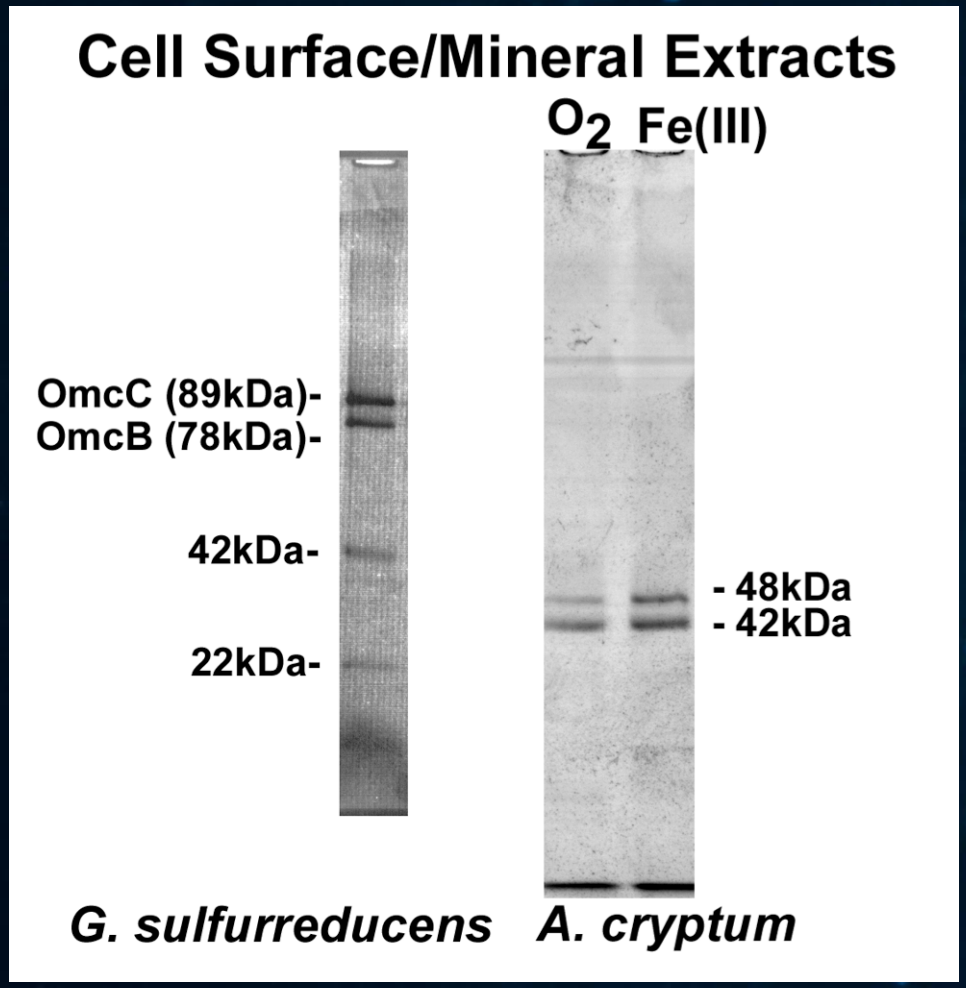
SDS-PAGE of *Acidiphilium* and *Geobacter* cytochromes

- Treat cell/mineral suspension with EDTA/Zwittergent
- Remove cells/mineral
- Purification and analysis



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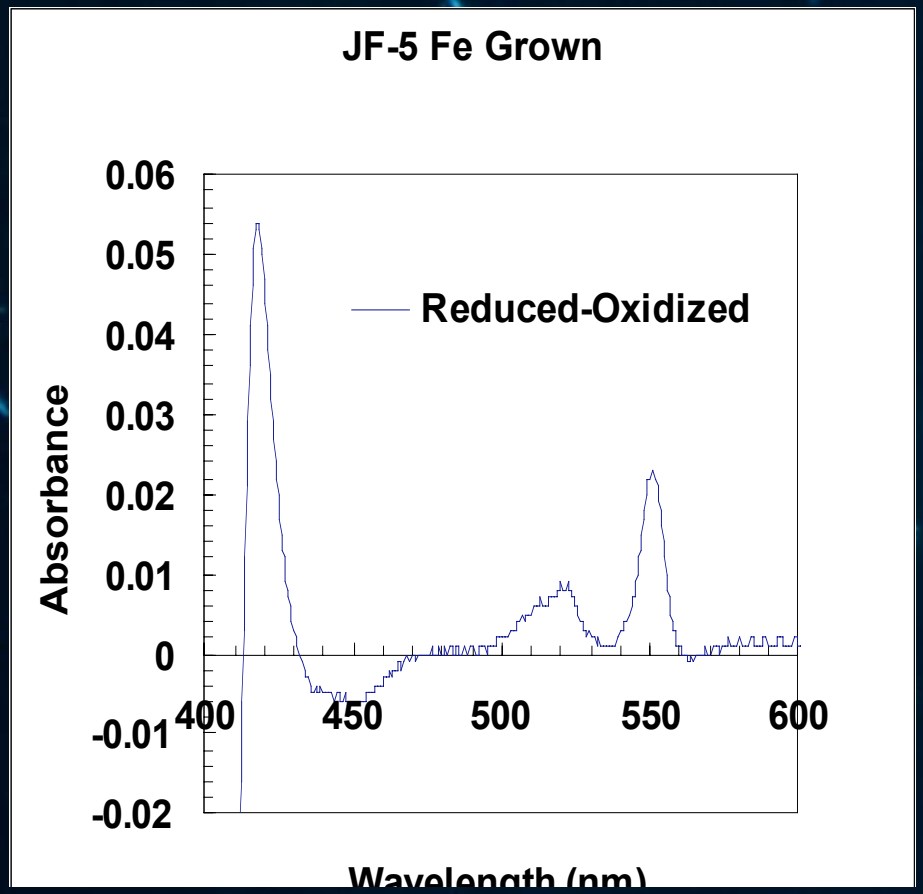
Cytochromes c from *Acidiphilium*

- Cell surface extract
- Amount significantly less than mesophilic DIRB
- Classic heme c spectrum



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Protease digestion of cell surface proteins

A. cryptum

- Cell suspensions treated with protease
- Cell surface proteins extracted using standard protocol
- Extract resolved on SDS-PAGE and stained for heme c
- Heme staining fragments result from partial digestion

Discrete digestion products
Limited exposure to outside?

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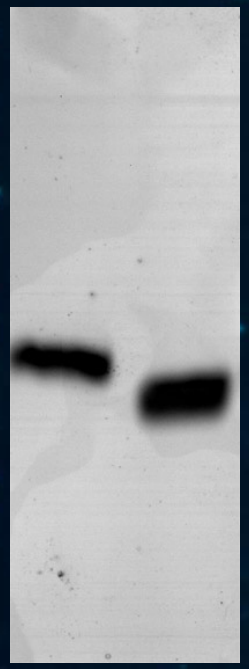
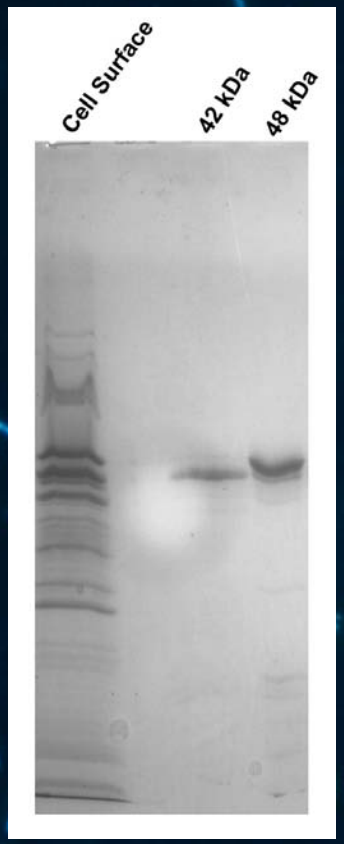
Purification of cytochromes c from *Acidiphilium*

- Cell surface extract (EDTA, Zwittergent)
- Ion-exchange, GF chromatography
- 4 cytochromes now purified (2 membrane, 2 periplasmic)



Periplasmic



OM

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Properties of purified cytochromes c from *Acidiphilium*

Mass (kDa)	Heme c content	Heme coordination	Fe reduction	Cr Reduction
10.1	1	His-Met	+	+
11.2	1	His-Met	+	+
42	3	??	??	+
48	3	??	??	??

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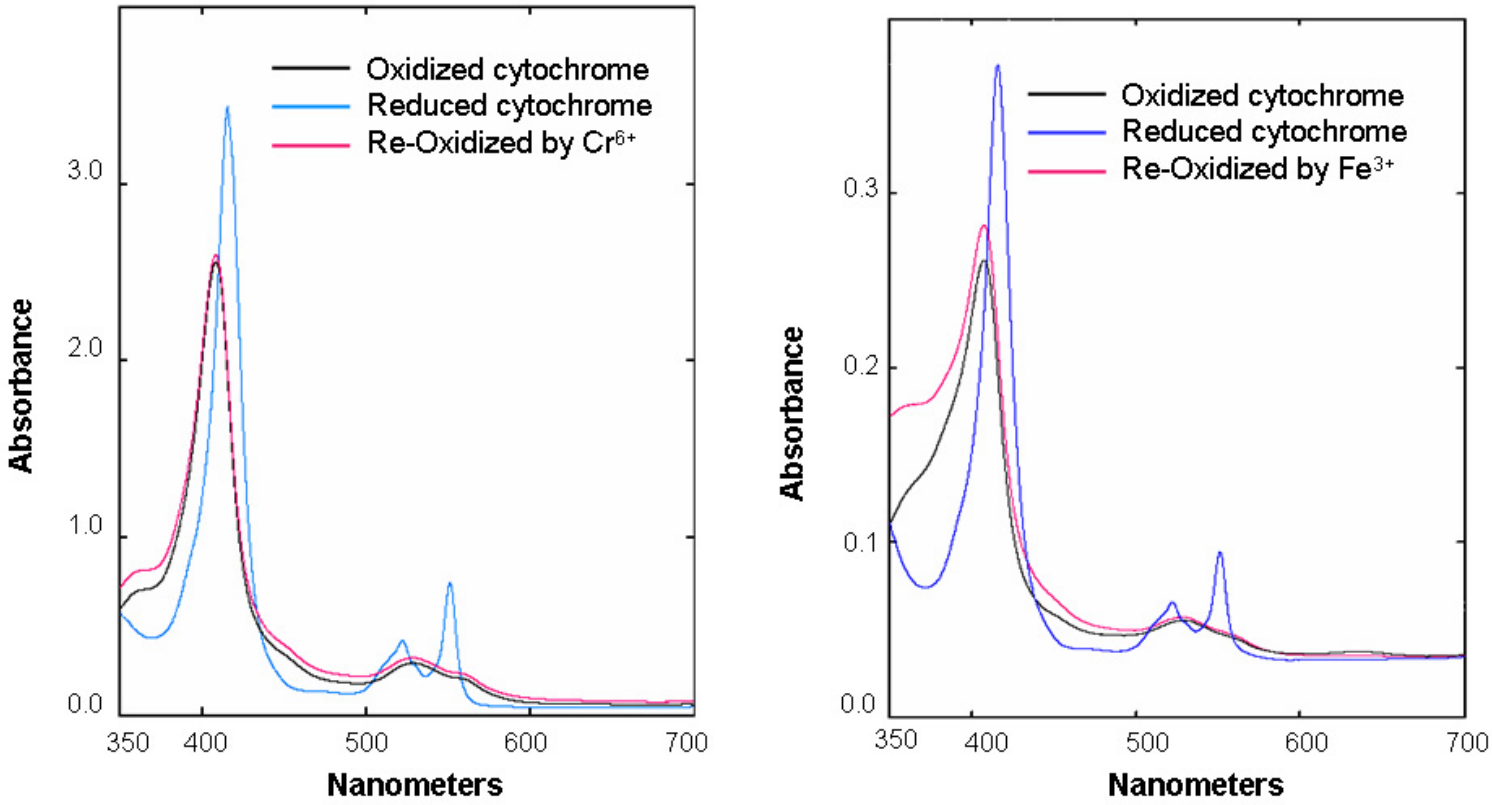
Metal reduction by *Acidiphilium* cytochromes c



Metal Reduction by the 10.1 kDa CytC of *A. cryptum*

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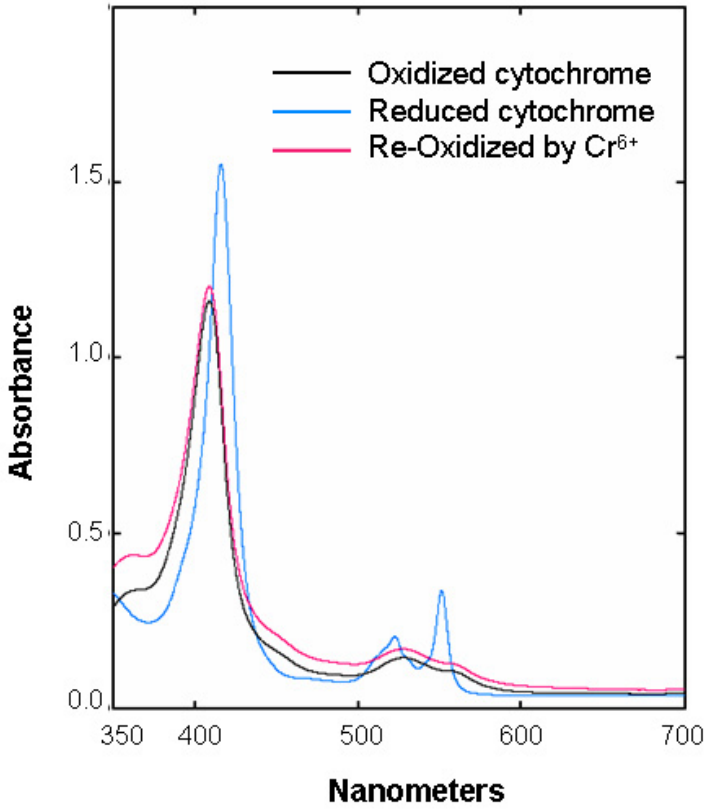
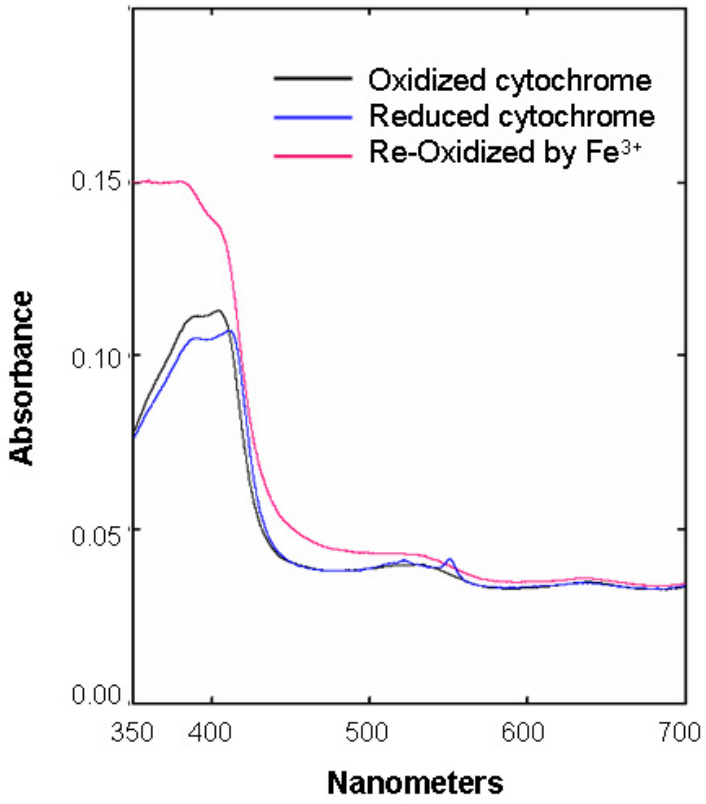
Metal reduction by *Acidiphilium* cytochromes c





Metal reduction by the 11.2 kDa CytC of *A. cryptum*

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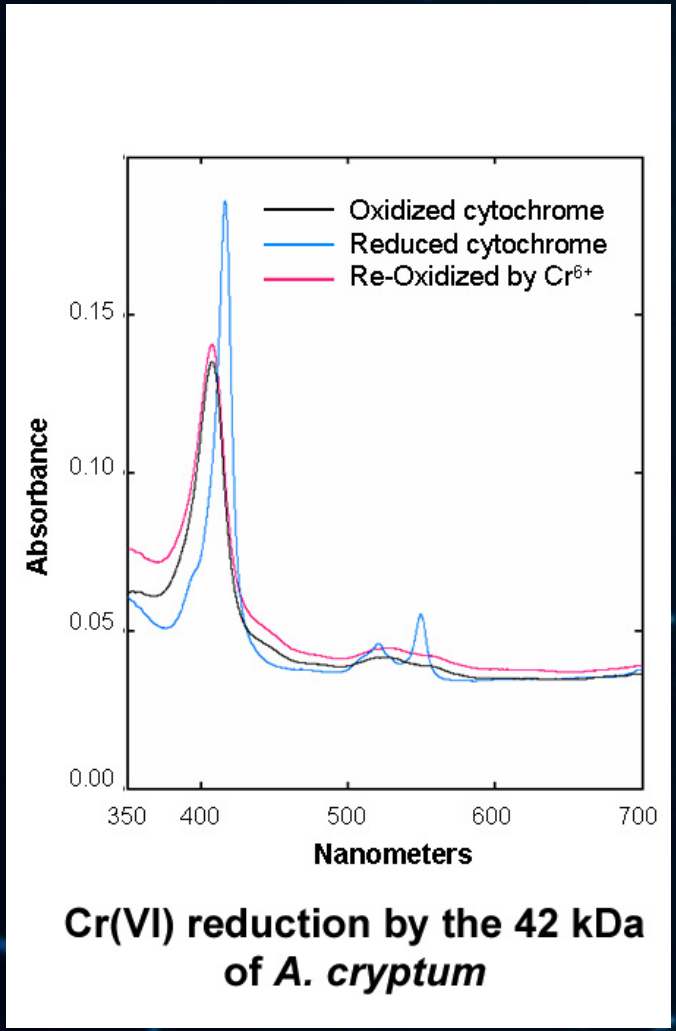
Metal reduction by *Acidiphilium* cytochromes c



Cr(VI) reduction by the 42 kDa of *A. cryptum*

Data for the 48 kDa cytochrome forthcoming

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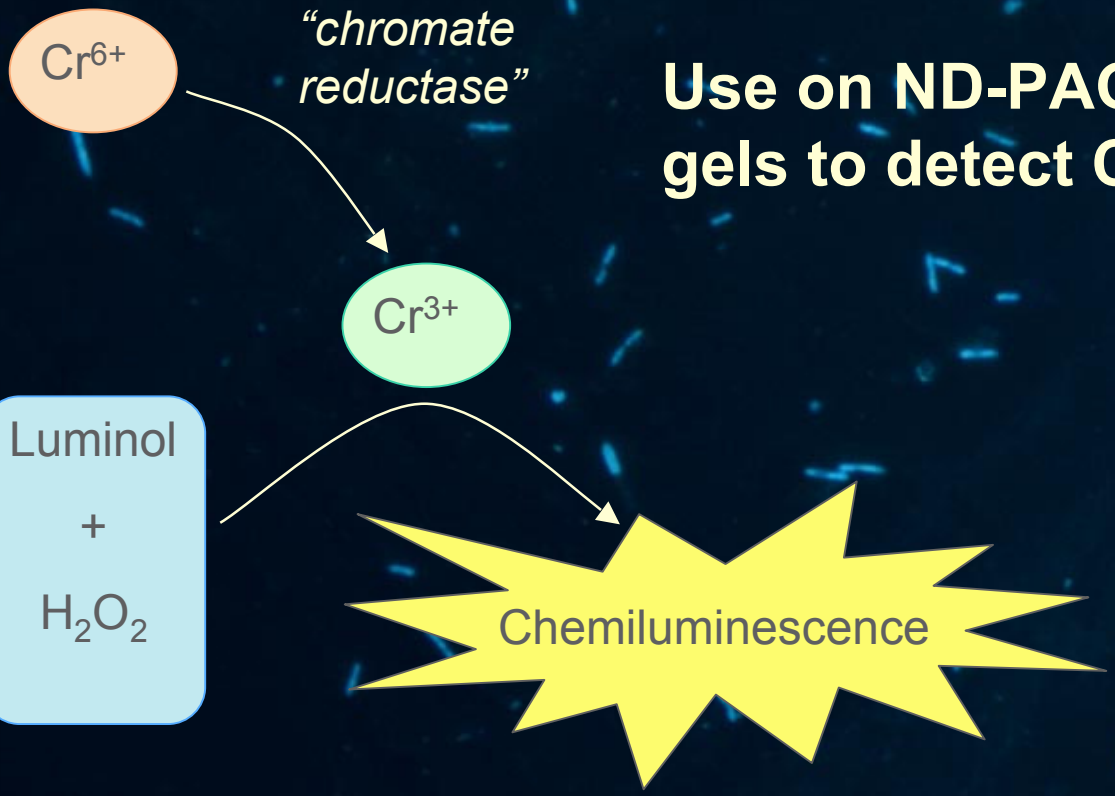
Detection of chromate reductase activity



Use on ND-PAGE gels to detect CrR

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Conclusions

- Cytochromes c present in *Acidiphilium* (OM and Periplasmic)
- Lower abundance than neutrophiles-reflective of utilization of soluble vs. solid-phase Fe??
- Attachment and biofilm formation evident
- Preliminary redox studies suggest that cytochromes have ability to reduce Fe and/or Cr

Future directions

- Protein-mineral interactions and conformational change during mineral reduction
- U(VI) reduction
- Multiprotein complexes
- Genome-enabled studies parallel to those with *Shewanella*, *Geobacter*

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Funding, Research Team, and Collaborators

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- M. Swenson, T. Tyler, K. Kusel, A.L. Neal, G.G. Geesey

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