Argonne NATIONAL LABORATORY

The Membrane Proteome of Shewanella oneidensis *MR-1*

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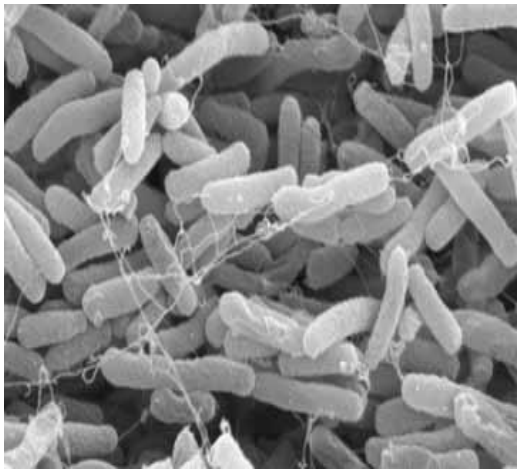


ERSP PI Meeting

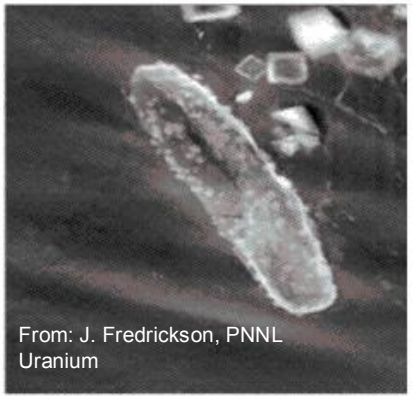
April, 2006

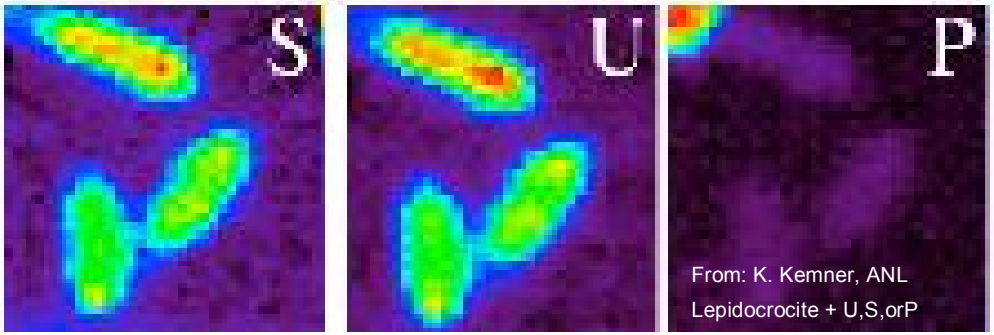
Oak Ridge National Laboratory

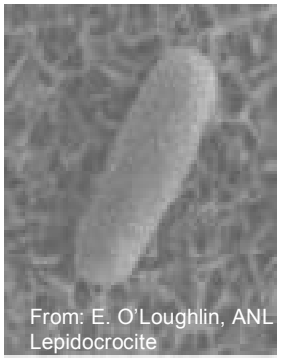
University of Southern California

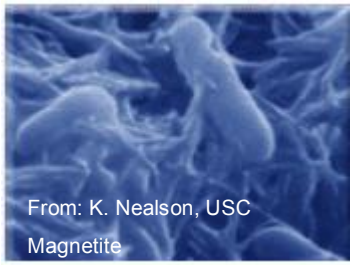
Shewanella MR-1 is in Touch with its Environment

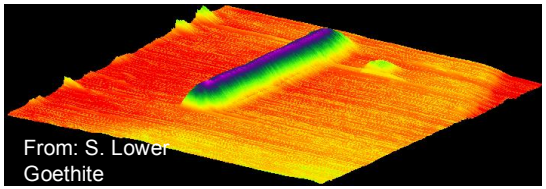
In natural habitats, MR-1 interacts with insoluble metals as terminal electron acceptors – the cell membrane is the point of contact.



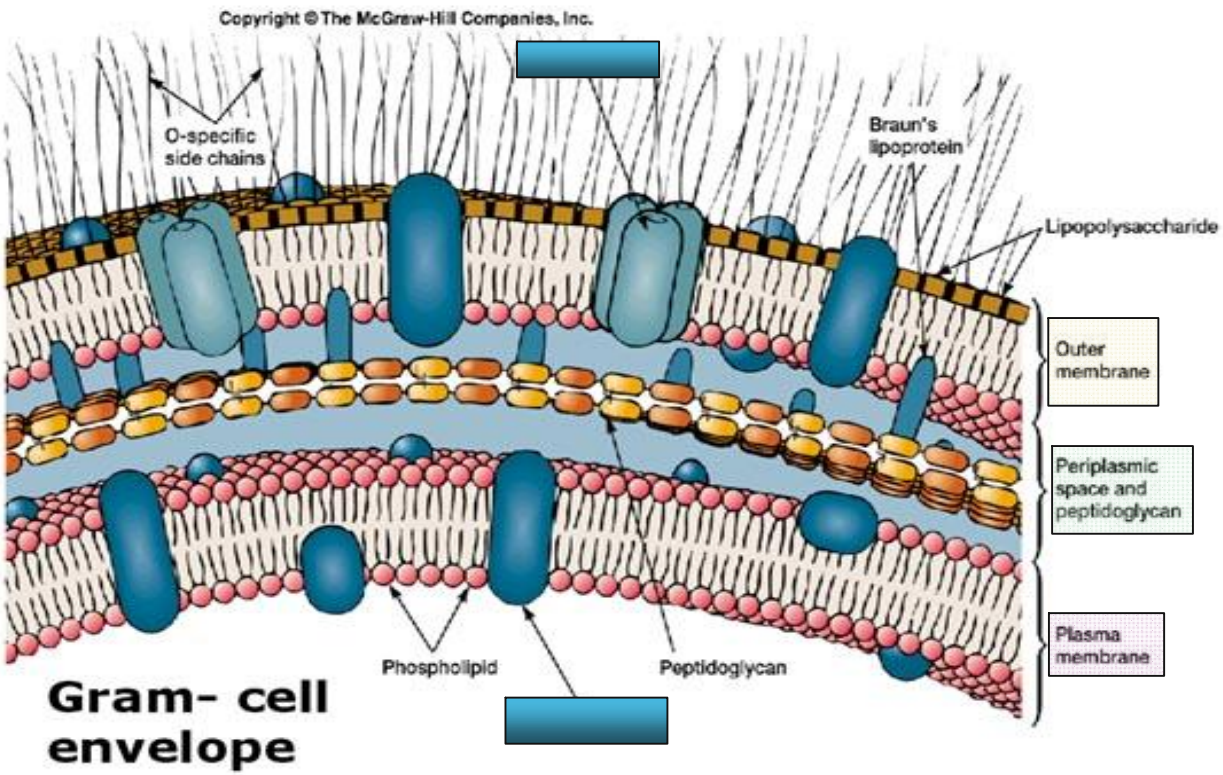








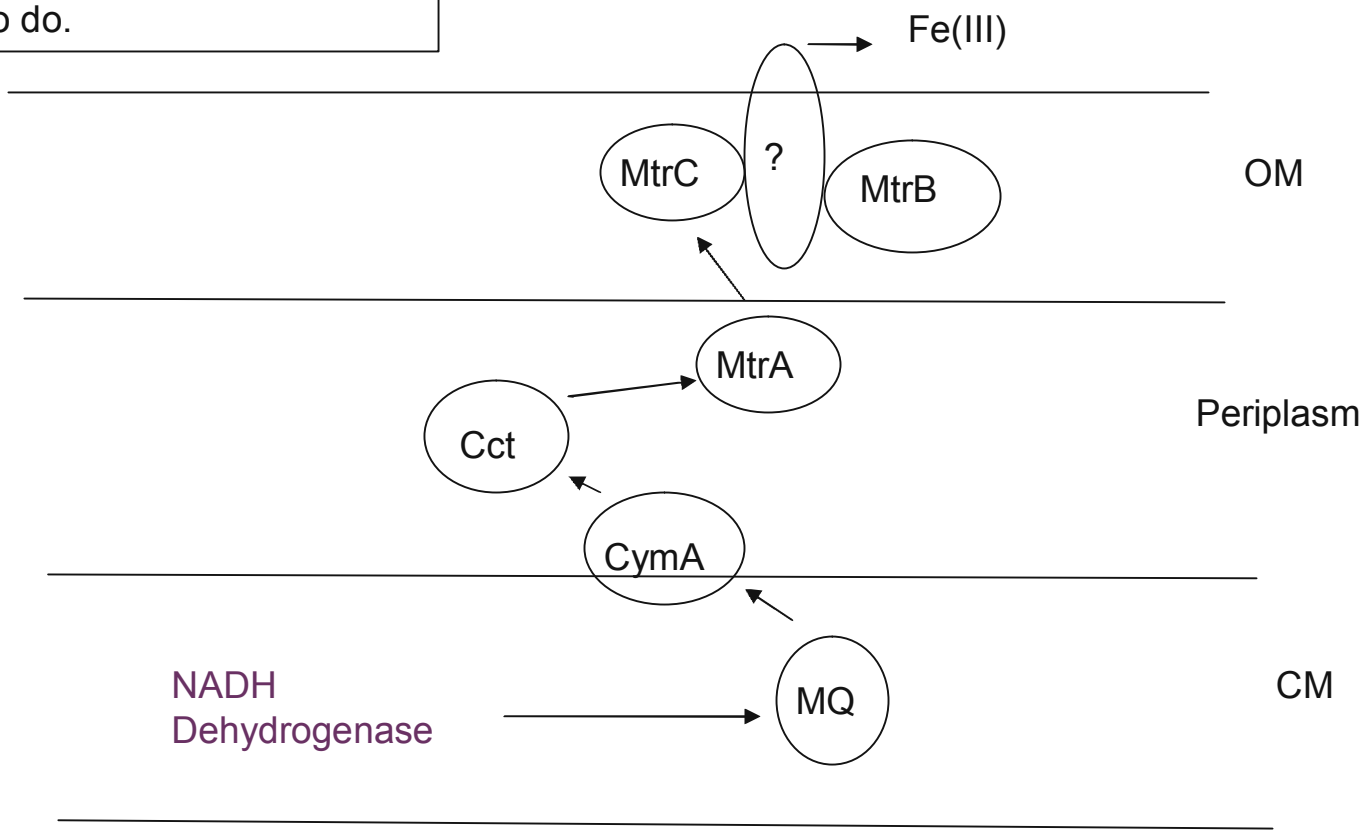
Gram Negative Microbe Membrane



- Membrane has 3 layers
- Proteins in each layer serve unique functions
- Proteins are essential in cell interaction with the environment

What Are the Proteins in These Membrane Compartments?

Mutation work has revealed some of the players, but there is more to do.



(Belieav, Myers and Myers)

Identification of Expressed Membrane Proteins

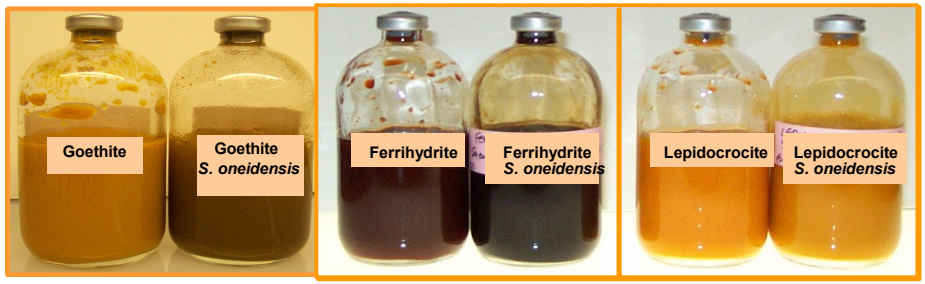
Step #1: Business as usual – identify proteins expressed in response to soluble electron acceptors

➢MR-1 grown aerobically or anaerobically with fumarate

Step #2: Move closer to natural environment – identify proteins expressed in response to insoluble electron acceptors

➢MR-1 grown anaerobically with insoluble iron oxide as electron acceptor

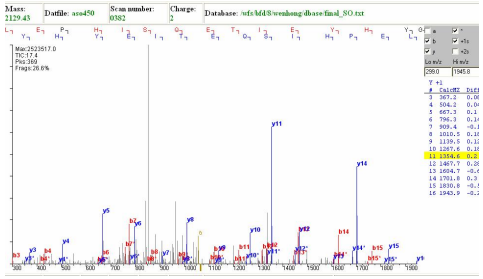
- Goethite
- Lepidocrocite
- Ferrihydrite



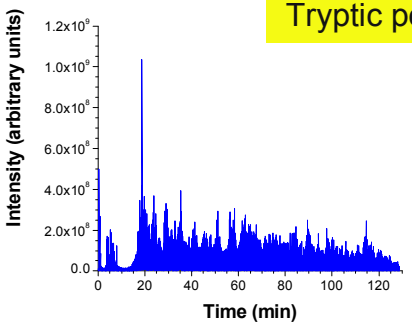
In collaboration with E. O'Loughlin, ANL

Analysis: Two Complementary Proteomics Approaches

Denatured proteins



Tryptic peptides



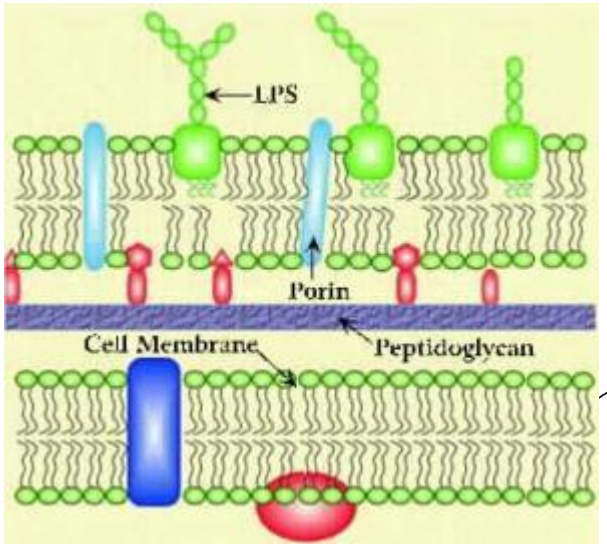
2DE with LC/MS-MS of particular proteins for the assessment of relative abundance and the identification of specific proteins showing differential expression (ANL)

"Shotgun" Proteomics with LC-LC/MS-MS for the identification of ALL proteins present in membrane preparations

(ORNL – N. VerBerkmoes/ R. Hettich)

Enrichment of outer and inner membrane proteins

The challenge is to separate the two membrane components and then released the proteins from those components while minimizing cross contamination.

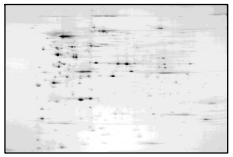


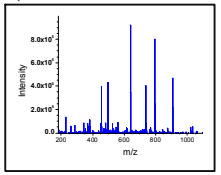
EDTA-lysozyme-Brij lysis protocol (Myers and Myers 1992)

Sucrose Gradient Centrifugation

Denature/solubilize membrane proteins

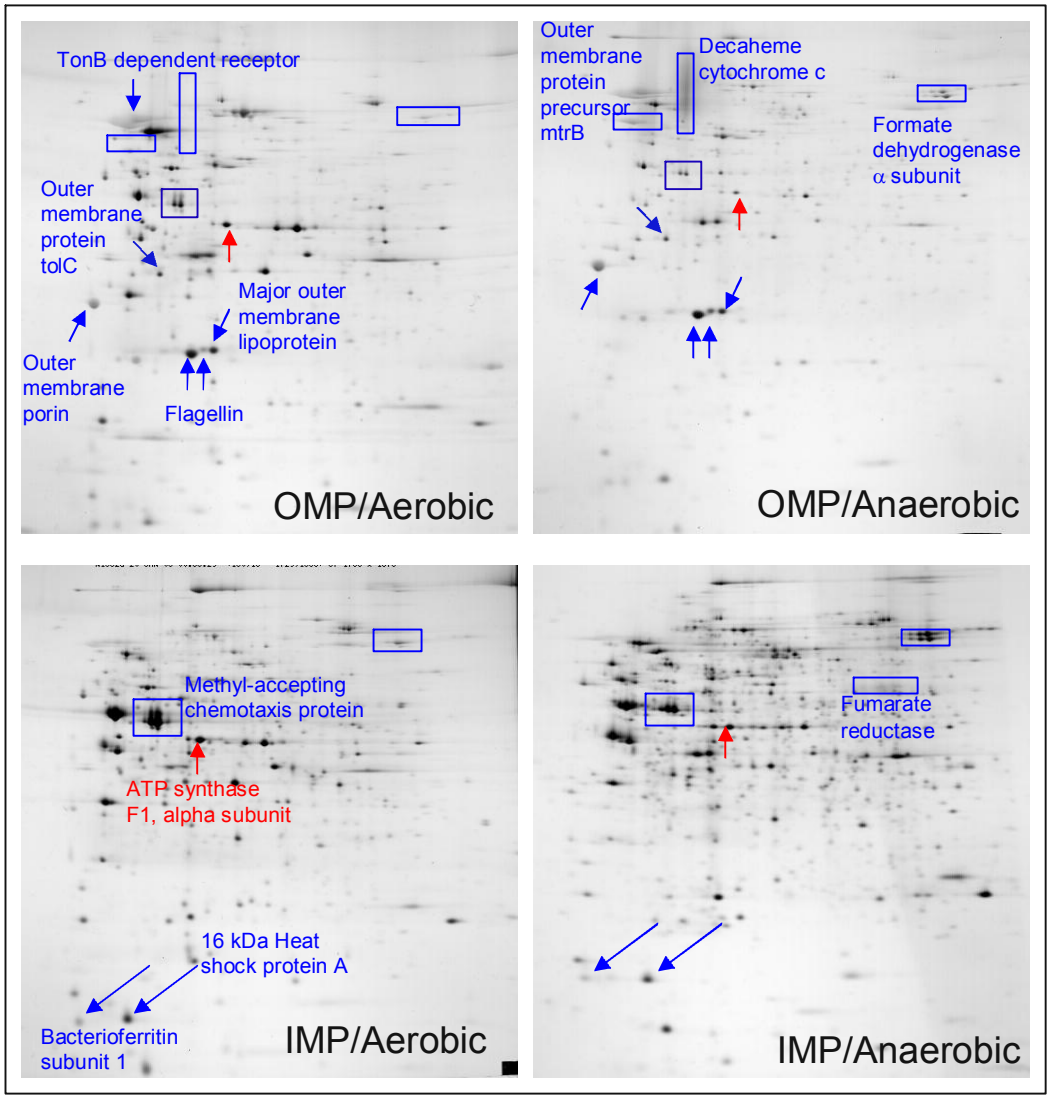
Trypsin digest membrane proteins





Outer and Inner Membrane Proteins: Aerobic vs. Anaerobic With Fumarate

Association of specific proteins with outer or inner membranes observed.



Differential protein expression observe in both membrane compartments.

Outer and Inner Membrane Proteins: LC/LC-MS/MS Analysis

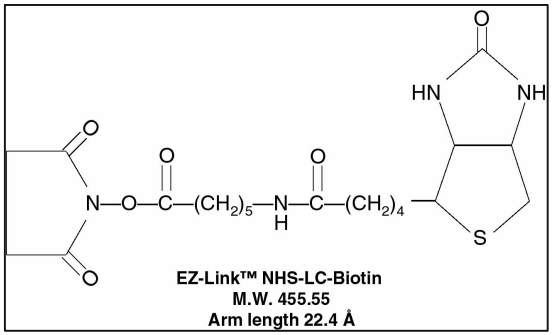
- 2DLC/MS-MS (LTQ) analysis of outer and inner membrane preparations from cells grown aerobically provided additional identifications.
  - Outer membrane prep: 76 (2 or more peptides);
    1307 (1 peptide)
    80% overlap with inner membrane identifications
  - Inner membrane prep: 877 (2 or more peptides);
    1333 (1 peptide)
    58% overlap with outer membrane identifications

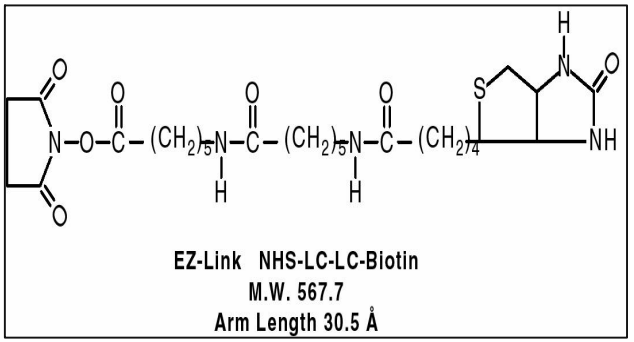
Mixtures are too complex to elucidate the proteins actually in contact with the extracellular environment.

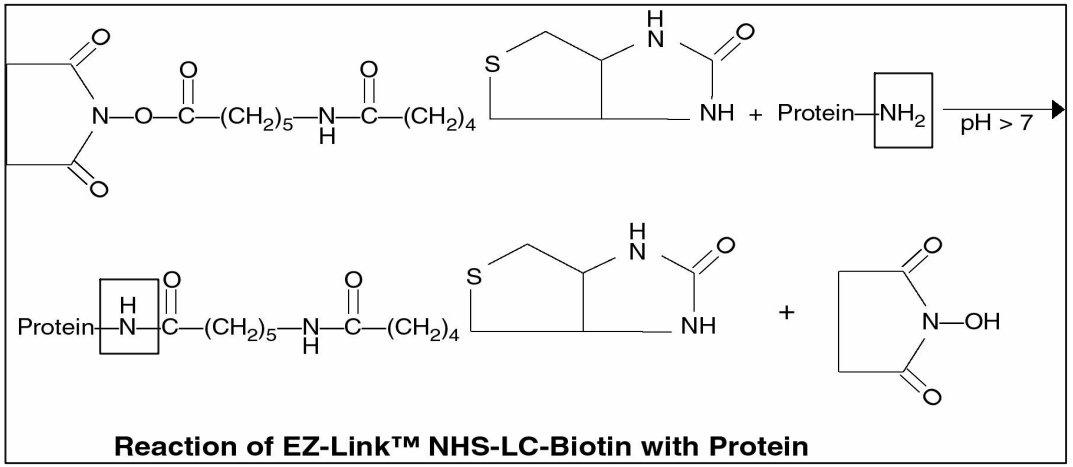
Cell surface protein enrichment is needed.

Approaches to Determining Surface Location of Proteins

- Radiolabeling with I-125 –radioisotope; intracellular labeling
- Proteinase K digestion – antibodies to identify specific proteins lost
- Biotinylation – nonisotopic method of tagging all proteins; less intracellular labeling





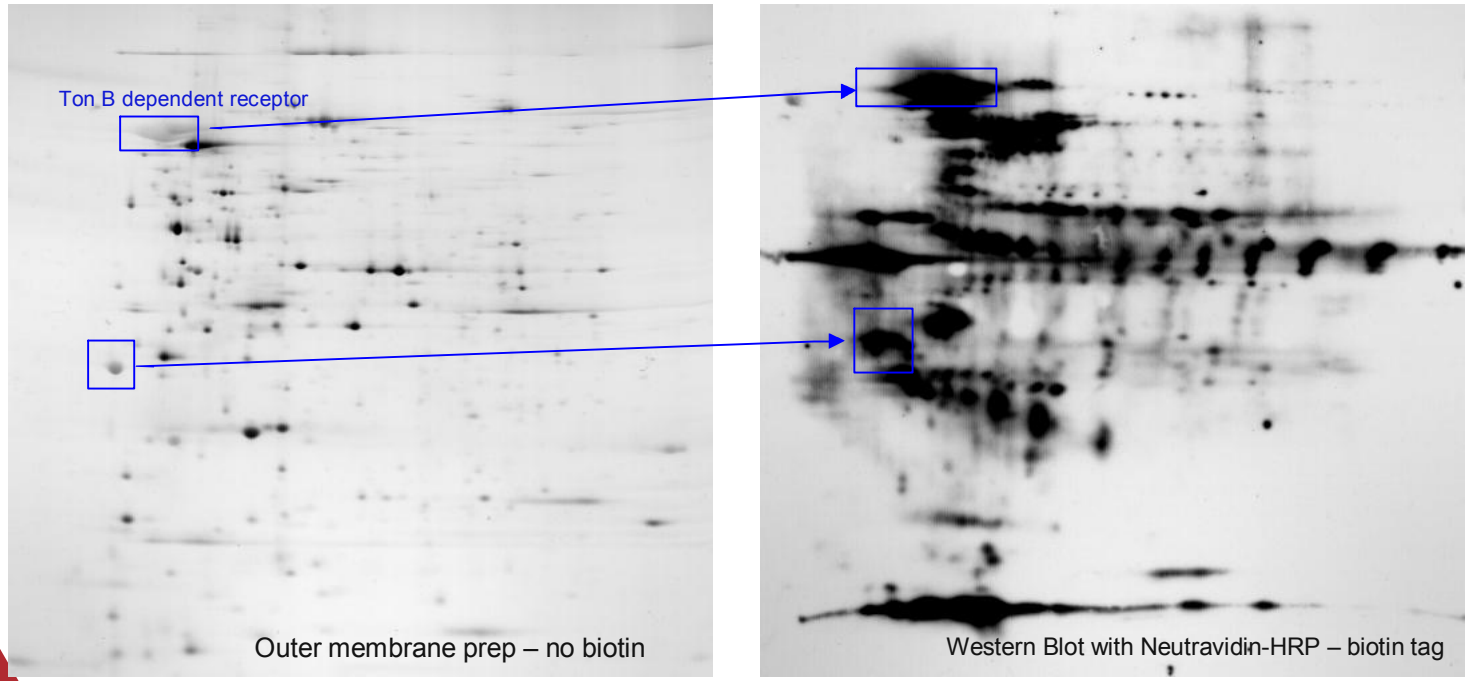


Intact Cells are Biotinylated

- Cells are labeled in culture
- Zwittergent 3-14 is used to release membrane proteins
- Biotinylated proteins are captured by avidin affinity chromatography
- Biotinylated proteins are analyzed by 2DE and 2DLC/MS-MS

Syto9-Green fluorescence/Live cells

Propidium iodide-Red fluorescence/Dead cells

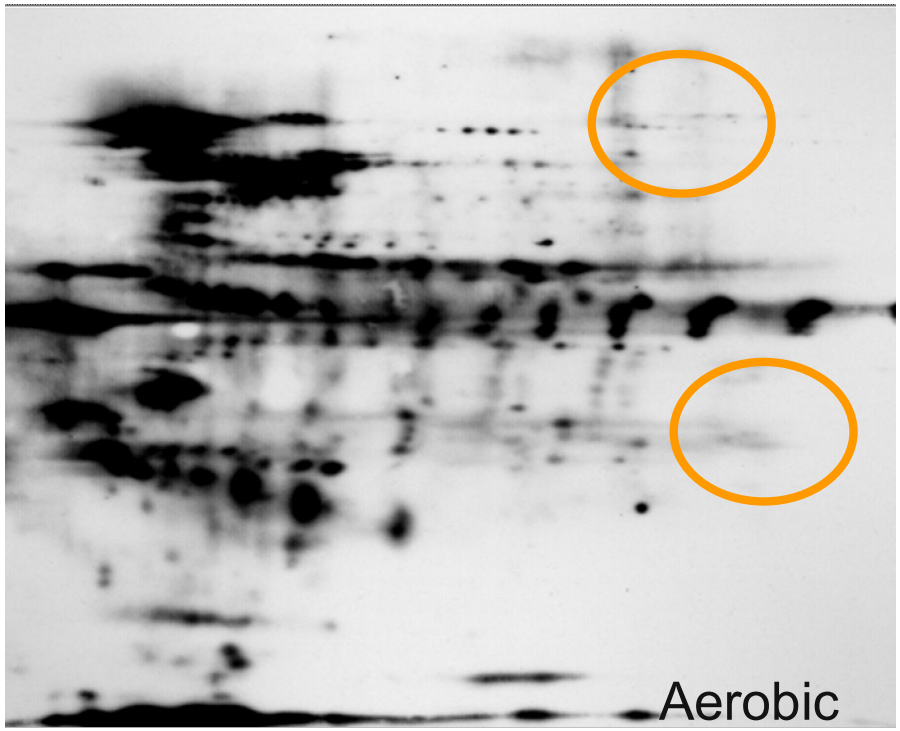


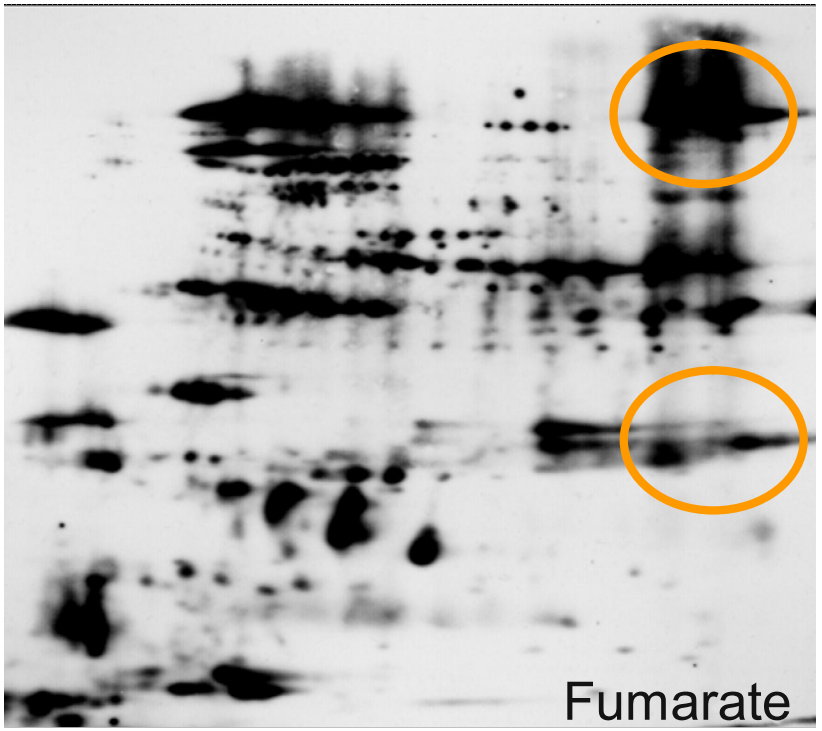
Biotinylation increased sensitivity of detection.

LC/LC-MS/MS Confirms Biotinylation Captures Outer Membrane Proteins

outer membrane porin, putative	decaheme cytochrome c (omcA)	conserved hypothetical protein
outer membrane protein TolC	hypothetical protein	conserved hypothetical protein
outer membrane protein OmpH	cytochrome c	conserved hypothetical protein
TonB-dependent receptor, putative	cytochrome c oxidase, cbb3-type, subunit II	periplasmic glucan biosynthesis protein, putative
TonB-dependent receptor domain protein	MSHA pilin protein MshA (mshA)	survival protein surA (surA)
tolB protein (tolB)	agglutination protein (aggA)	decaheme cytochrome c (omcB)
TPR domain protein	conserved hypothetical protein	polyamine ABC transporter, periplasmic polyamine-binding protein
peptidase, M16 family	peptidoglycan-associated lipoprotein (pal)	flagellar hook-associated protein FliD
peptidase, M13 family	cytochrome c (cytcB)	conserved hypothetical protein
heme transport protein (hugA)	outer membrane protein precursor MtrB	ferric alcaligin siderophore receptor
MotA/TolQ/ExbB proton channel family protein	multidrug resistance protein, AcrA/AcrE family	periplasmic nitrate reductase (napA)
sulfate ABC transporter, periplasmic sulfate-binding protein	lipoprotein, putative	OmpA family protein
major outer membrane lipoprotein, putative	ATP synthase F1, beta subunit	thiol:disulfide interchange protein DsbE
ubiquinol-cytochrome c reductase, iron-sulfur subunit (petA)	formate dehydrogenase, alpha subunit	

Biotinylated Proteins From Aerobic and Anaerobic with Fumarate Growth





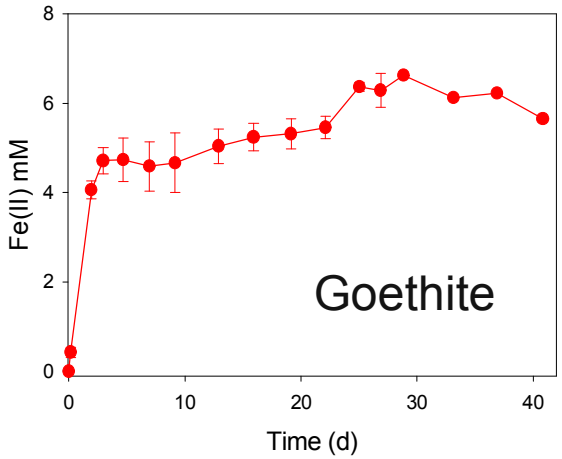
Differentially Expressed Surface Proteins (2DLC/MS-MS

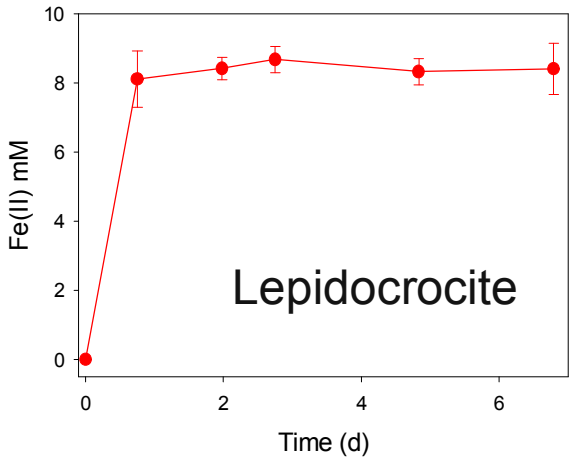
Detected in Anaerobic but not Aerobic Cells
phage shock protein A
decaheme cytochrome c
general secretion pathway protein D
TPR domain protein
transcriptional regulator RpiR family
conserved hypothetical protein (gi7597239)
PqiB family protein
flagellin
conserved hypothetical protein (gi7589906)
outer membrane protein TolC
conserved hypothetical protein (gi7595997)
agglutination protein

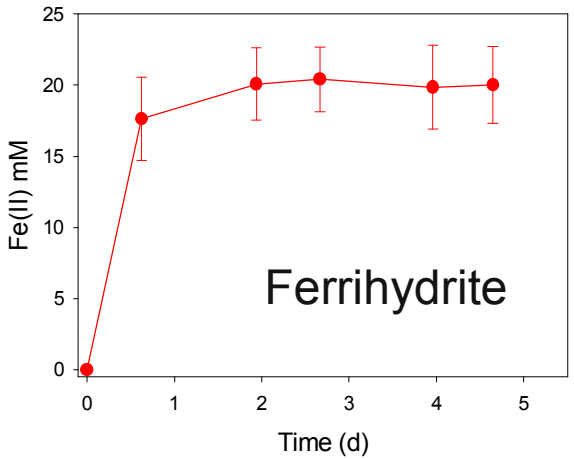
Significantly More Abundant in Anaerobic Cells
hemolysin protein putative
Hypothetical protein (gi7587156)
adhesion protein putative
hypothetical protein (gi7587828)
outer membrane protein
anaerobic dimethyl sulfoxide reductase B subunit
anaerobic dimethyl sulfoxide reductase A subunit
conserved hypothetical protein (gi7589906)
cytochrome c551 peroxidase
16 kDa heat shock protein A
RNA pseudouridylate synthase family protein
universal stress protein family
conserved hypothetical protein (gi7599217)
formate dehydrogenase iron-sulfur subunit
hypothetical protein (gi7597502)
putative lipoprotein, putative

Similar Abundance in Aerobic and Anaerobic Cells
General diffusion Gram-negative porins, putative
ATP synthase F1 epsilon subunit
ATP synthase F1 beta subunit
ATP synthase F1 gamma subunit
ATP synthase F1 alpha subunit
ATP synthase F0 B subunit

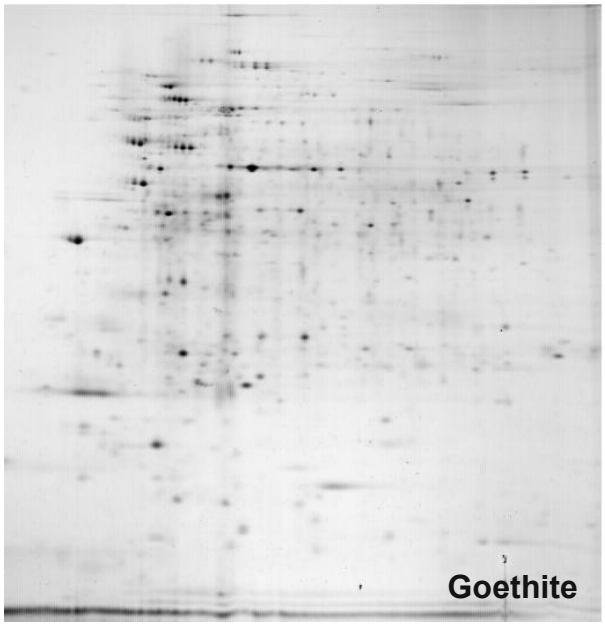
Proteomics of MR-1 Grown with Insoluble Iron Oxides

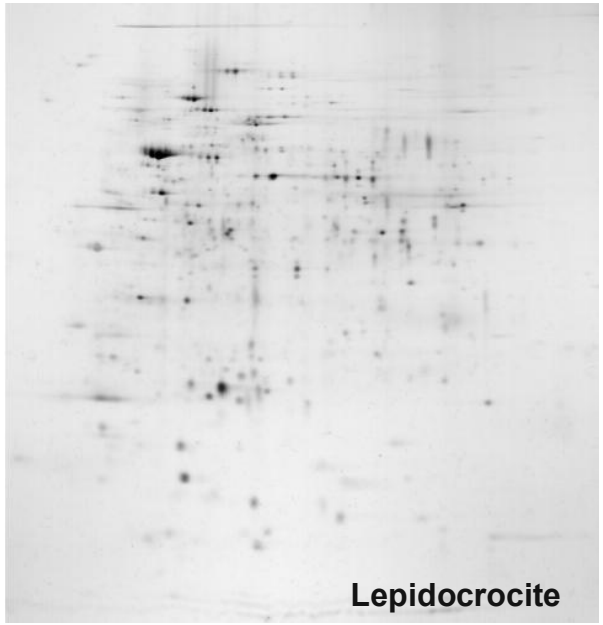


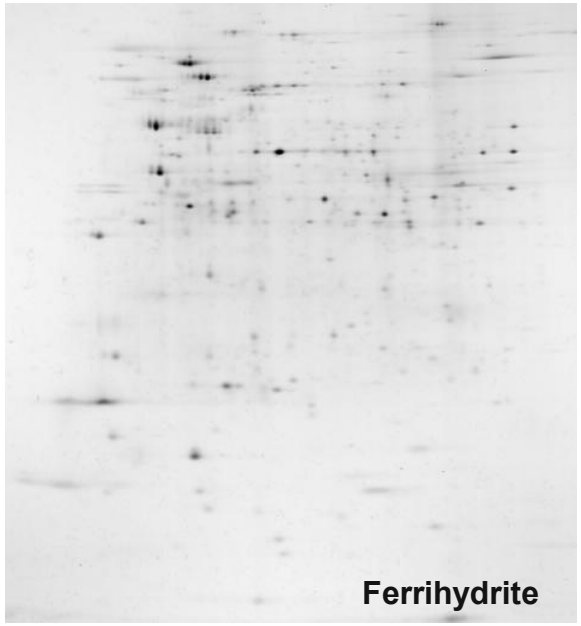




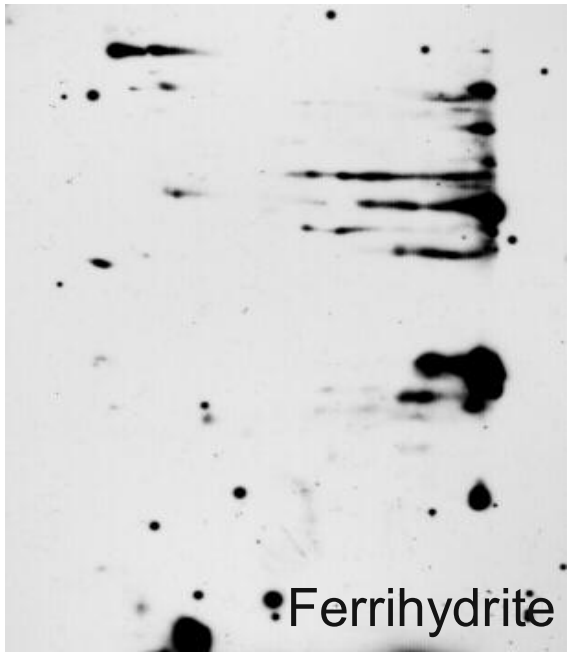
Differential Protein Expression Obvious from Total Lysate 2DE Patterns



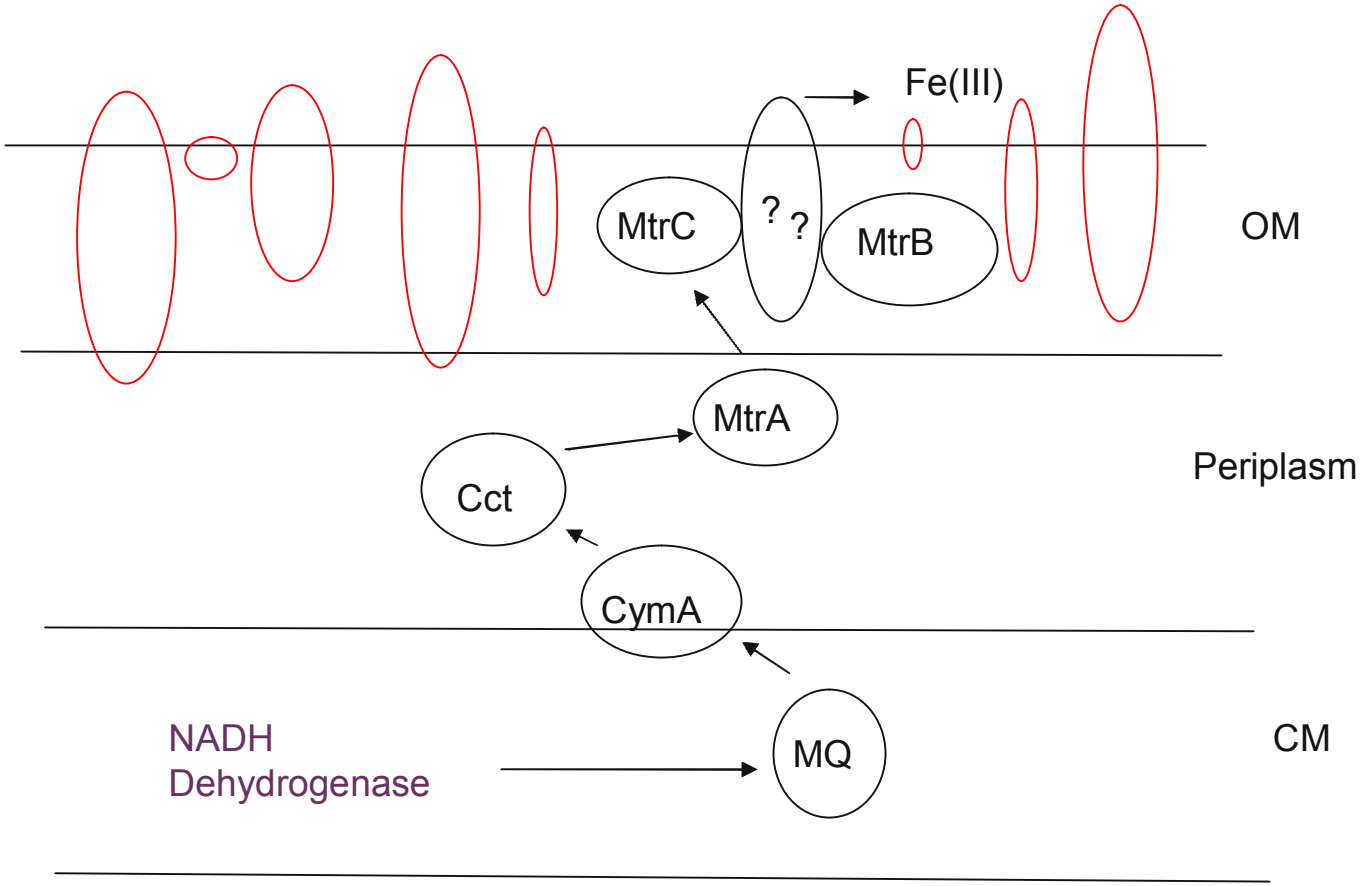




Surface Proteome Also Distinctive



A Model of the Membrane Proteome Required for Fe(III) reduction



Summary

•Biotinylation of intact cells provides a valuable tool for identifying the proteins exposed at the surface of the cell.

•The membrane proteome of *S. oneidensis* MR-1 is dynamic, varying in content in response to terminal electron acceptors.

•Surface membrane proteome of cells grown with insoluble iron oxides is significantly different from that of cells grown with soluble terminal electron acceptors.

•A number of proteins in addition to c-type cytochromes comprise the variable component of the membrane surface.

"The effects of Omp35 on anaerobic electron acceptor use are therefore likely indirect. The results demonstrate the ability of non-electron transport proteins to influence anaerobic respiratory phenotypes." From Maier and Myers BMC Microbiology 2004

•Understanding the membrane protein configuration of microbes in the environment will be an important component of determining the fate and transport of contaminants.

Acknowledgement

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Collaborators: **E.J. O' Loughlin (ANL)**

Robert Hettich (ORNL)

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