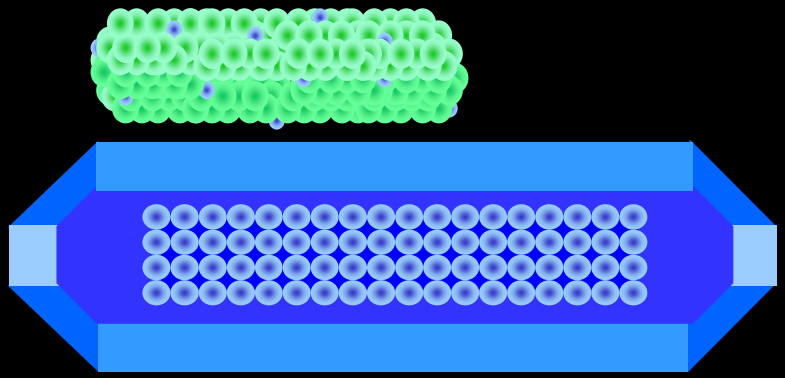
MINERAL SPECIFIC PROTEINS SYNTHESIZED BY BACTERIA

Steven Lower
Ruchi Yongsunthon
Brian Lower
Michael Hochella, Jr.

Ohio State University
Virginia Tech



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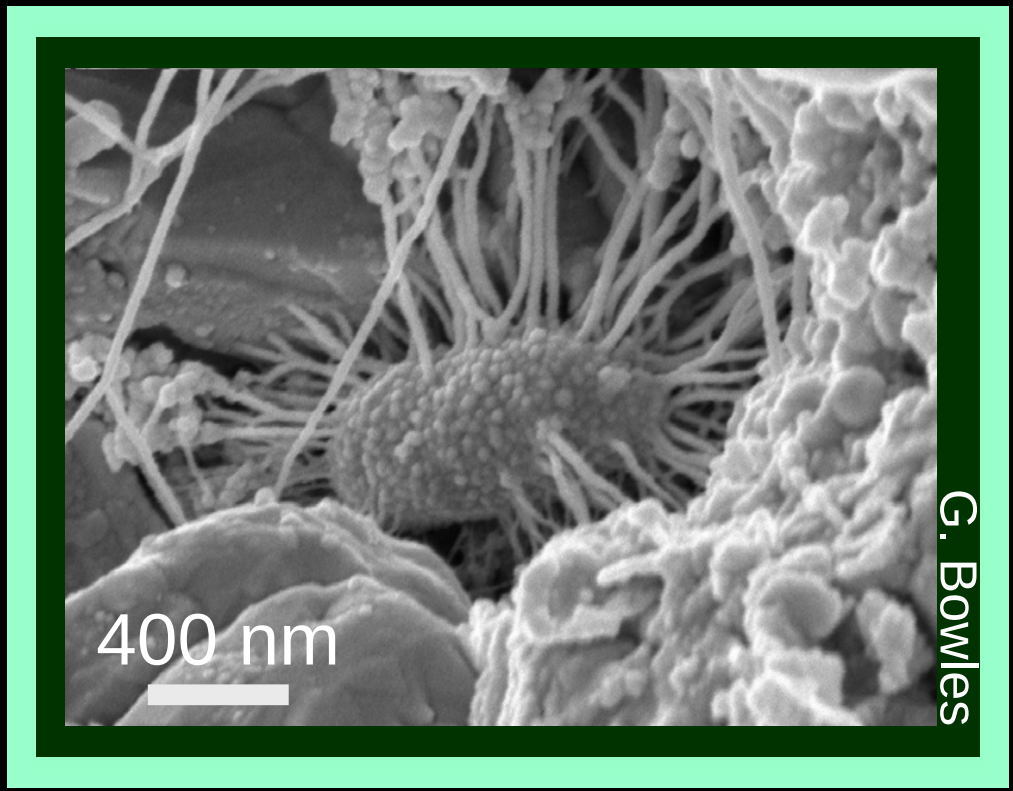
bacterium-mineral interface

interfacial forces and proteins

**forces control, and are themselves modulated by, the expression of biopolymers on a bacterium's surface*



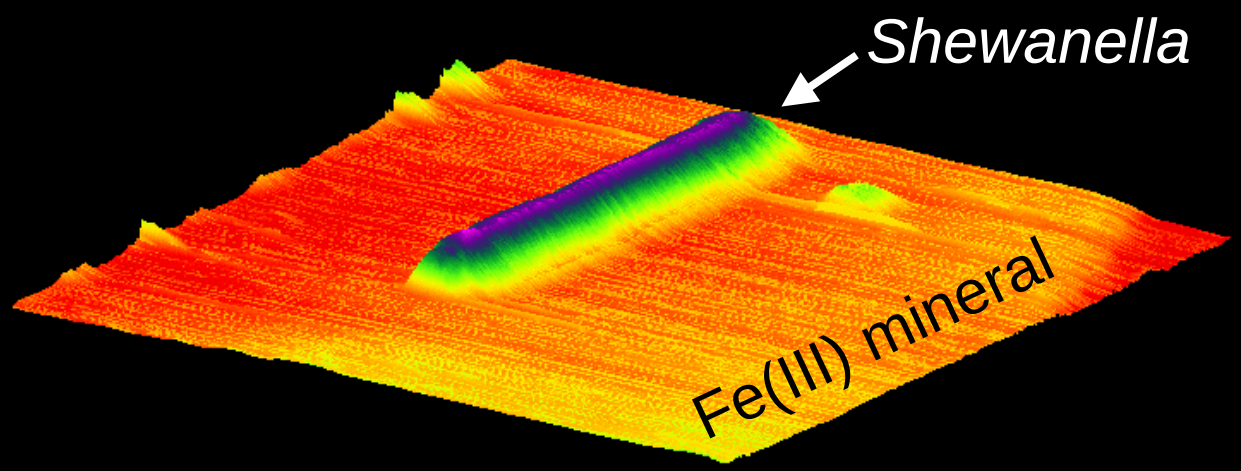
A comparison of two organisms that work in the realm of the nanometer.

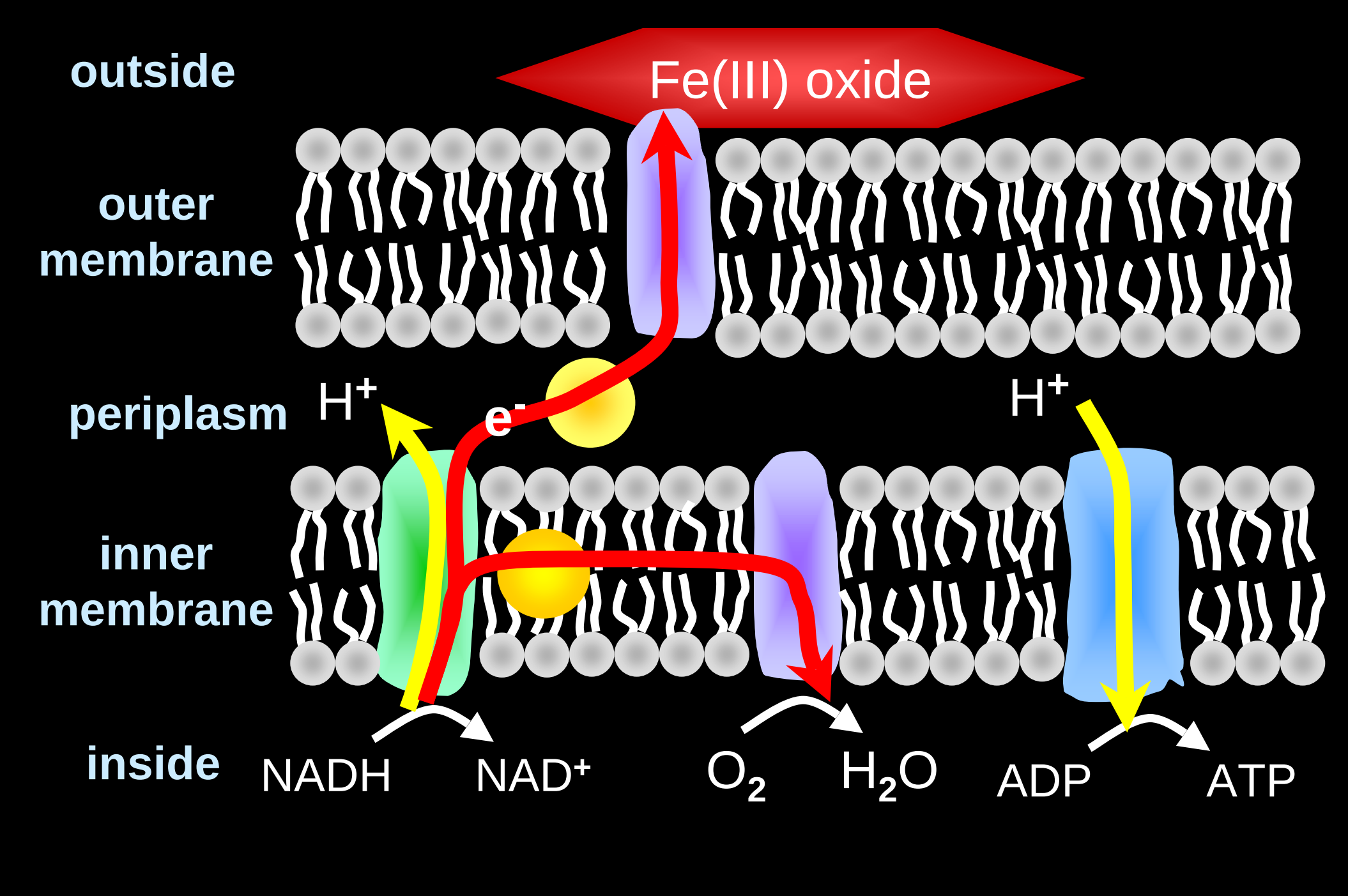
	Microorganisms	Humans
# of cells	10^{30}	10^{9}
# of species	10^{6}	10^{0}
# years	10^{9}	10^{6}

Shewanella interactions with Fe oxyhydroxides

metal reducing bacteria & oxides

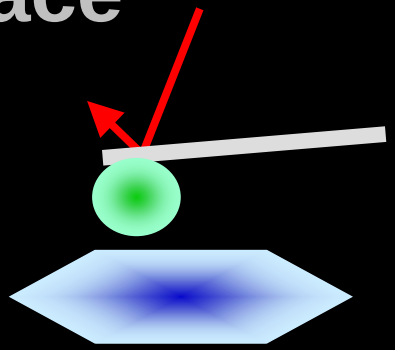
- primitive form of respiration using Fe(III) solids
- remediation of organic and inorganic pollutants in surface and subsurface environments




outside
Fe(III) oxide
outer membrane
periplasm
H+
e-
H+
inner membrane
inside
NADH
NAD+
O2
H2O
ADP
ATP

Cell-material interface

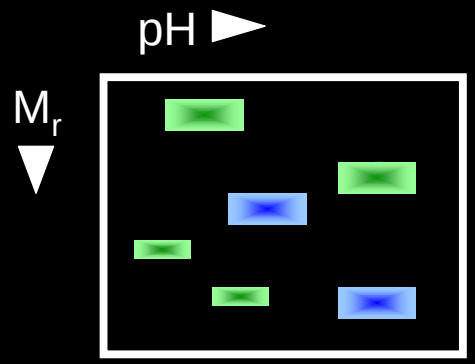
discover forces and proteins



force microscopy

$F \sim e^{-D}$

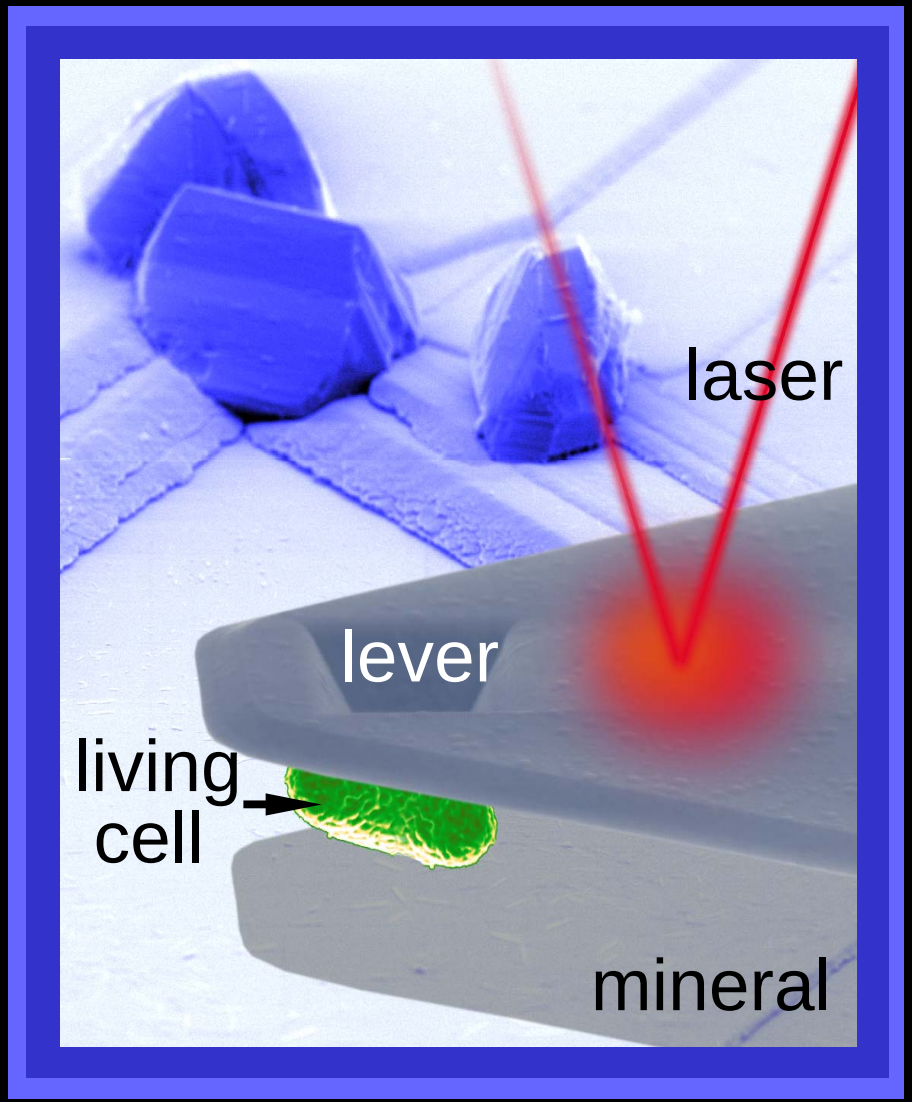
theory



2D gel electrophoresis

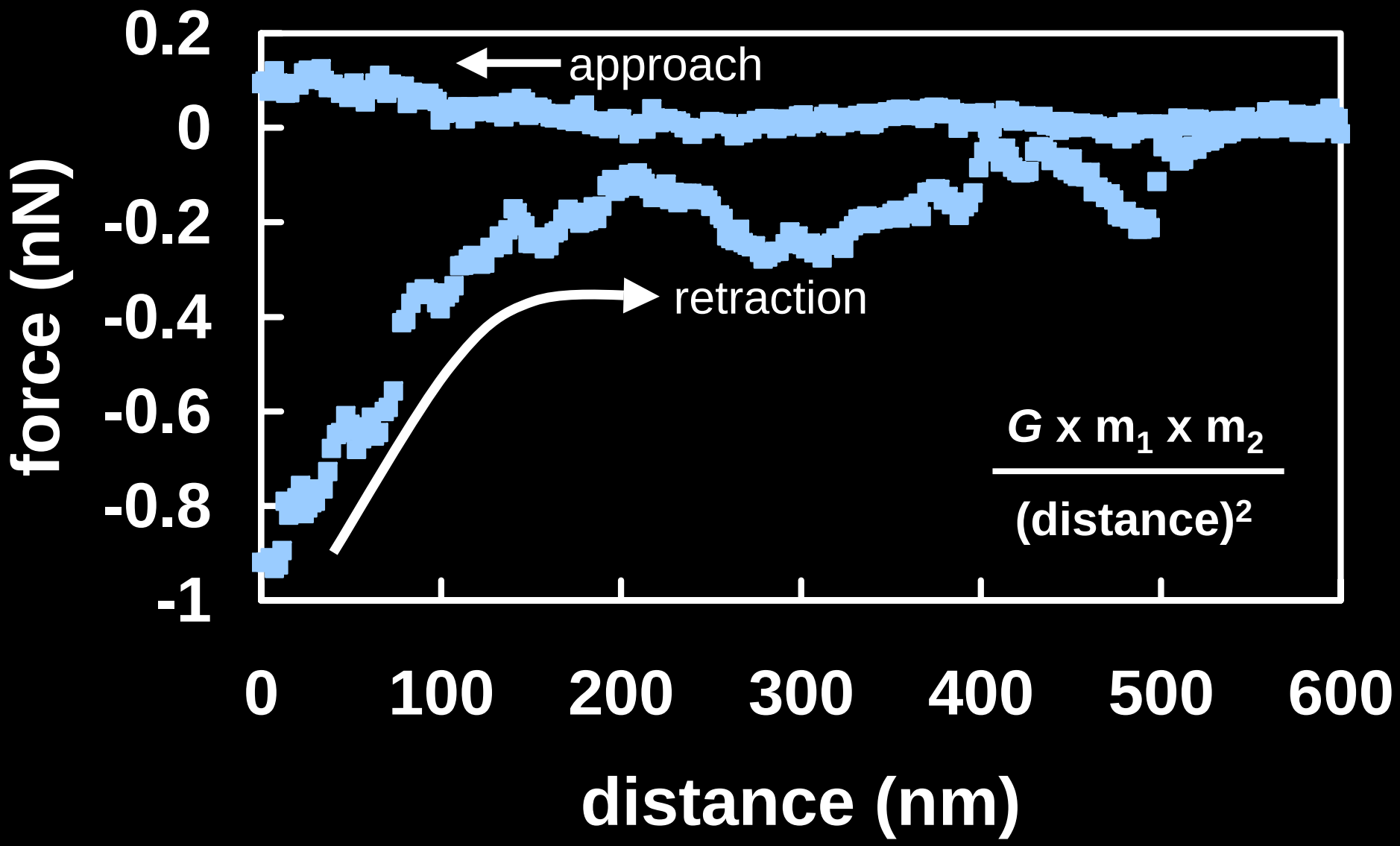
mimic and utilize information

biological force microscopy



Force-distance curves

using different minerals, bacteria, and solutions



electrostatic and van der Waals forces

DLVO Theory

$$F(d) = \frac{4\,\pi\,\sigma_1\,\sigma_2\,R}{\varepsilon\,\varepsilon_o\,\kappa}\,e^{-\kappa d} - \frac{H\,R}{6\,d^2}$$

σ = surface charge

R = radius

ε = dielectric constant

ε_o= vacuum permittivity

d = distance

κ = 1 / Debye length

~(salt concentration) $^{-1/2}$

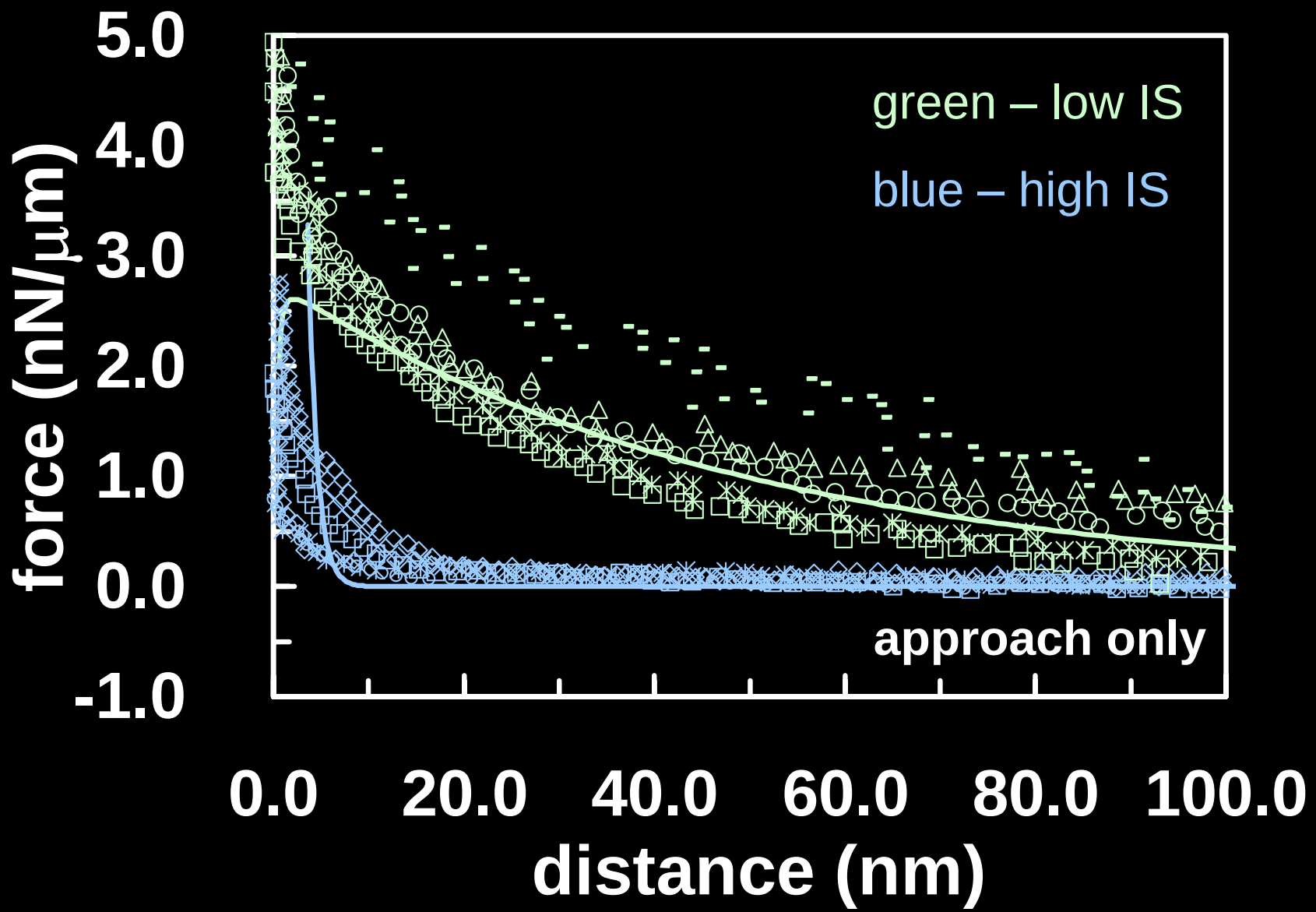
H = Hamaker constant

R = radius

d = distance

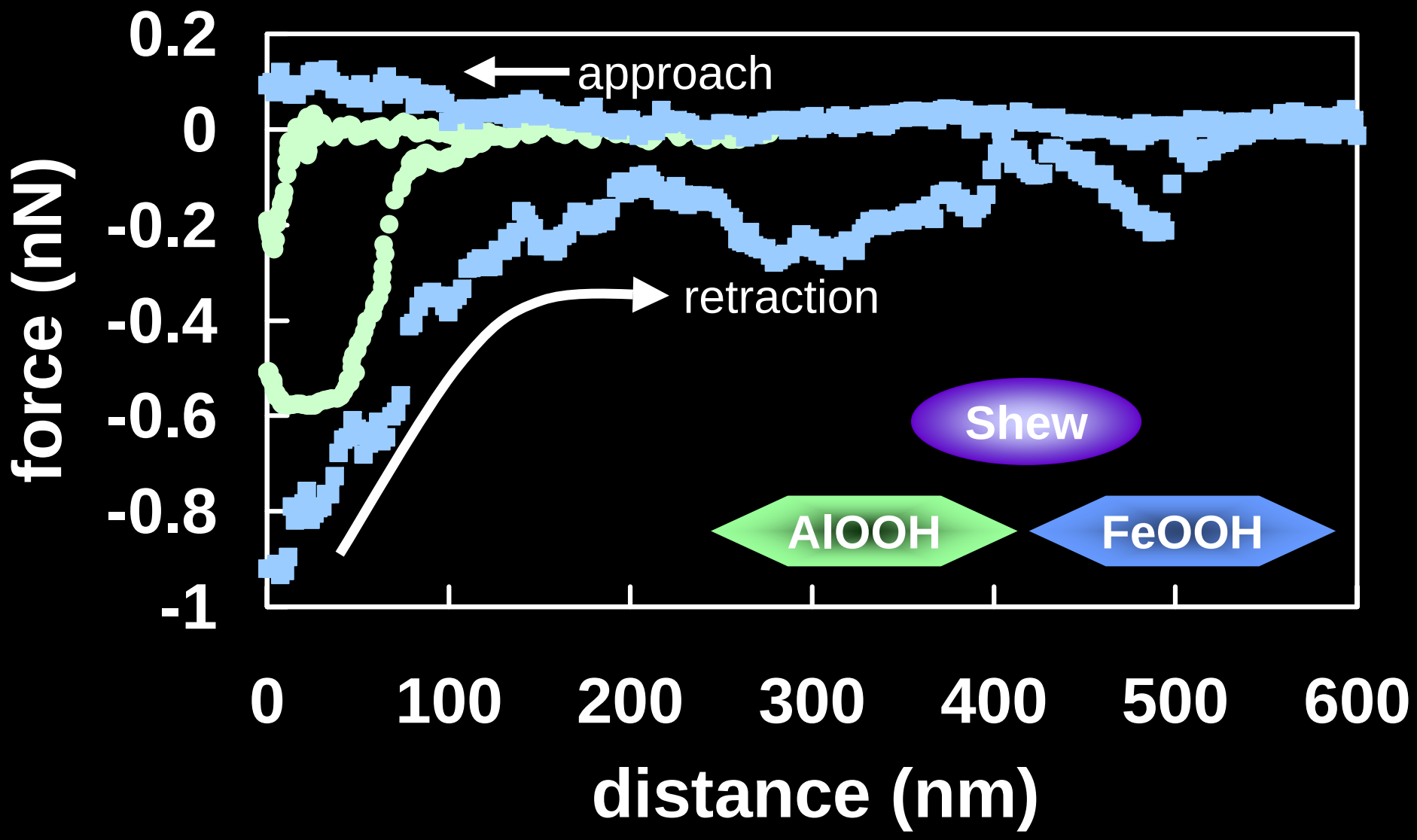
subsurface transport in the environment

(approach measurements between G- bacterium and a silicate)



Force-distance curves

(retraction forces between *Shewanella and AlOOH vs FeOOH*)



energy values between *Shewanella* and mineral

(as function of oxygen concentration)

attoJoules (10^{-18} J)

	diaspore (AlOOH)	goethite (FeOOH)
aerobic	39 ± 7	26 ± 6
anaerobic	41 ± 4	137 ± 20

Control experiment with nonviable cells ~6 aJ (did not change with mineral, oxygen concentration, or contact time)

outside

Fe(III) oxide

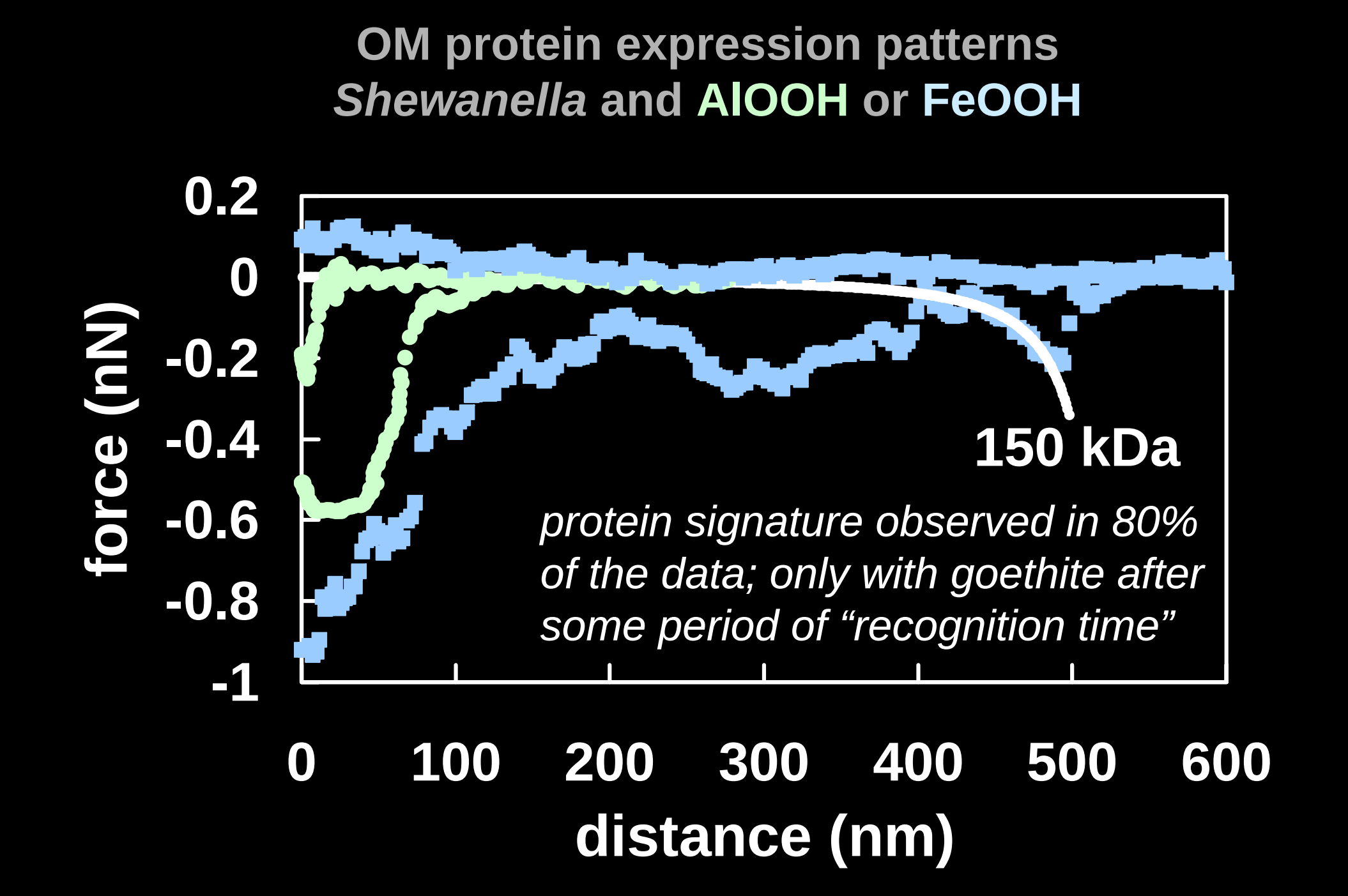
outer membrane

Protein folding/unfolding

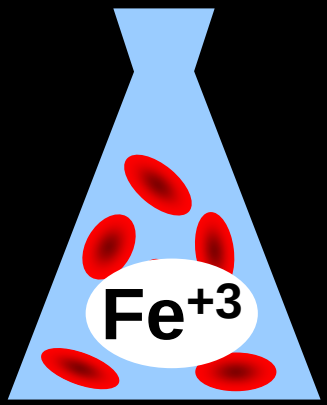
Worm-like Chain Model

$$F(d) = (k\,T / b)\,[0.25\,(1 - d / L)^{-2} - 0.25 + d / L]$$

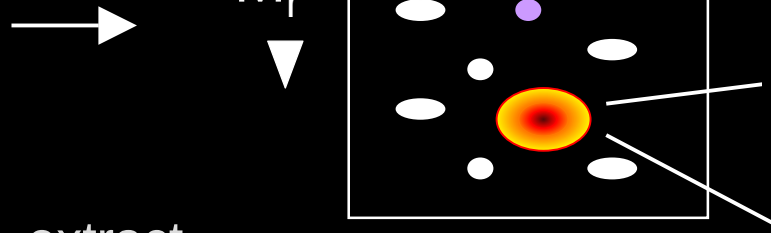
d = distance or extension
k = Boltzmann's constant
T = temperature
b = persistence length (0.38nm C_α-C_α in protein)
L = contour length (length of stretched protein chain)


OM protein expression patterns
Shewanella and AlOOH or FeOOH
force (nN)
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
150 kDa
protein signature observed in 80% of the data; only with goethite after some period of "recognition time"
0
100
200
300
400
500
600
distance (nm)

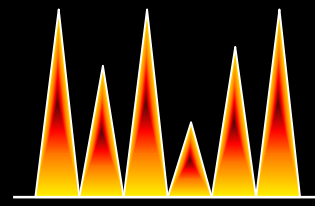
2D Gel Electrophoresis of OM Proteins



extract membrane proteins

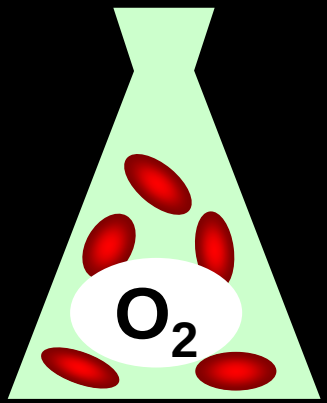


excise proteins & fragment into peptides

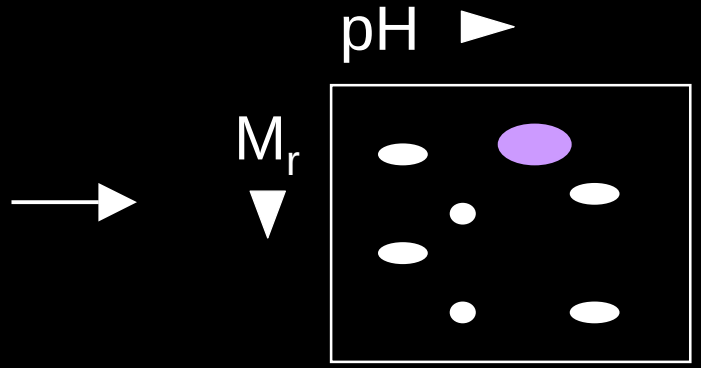


mass spec & database search

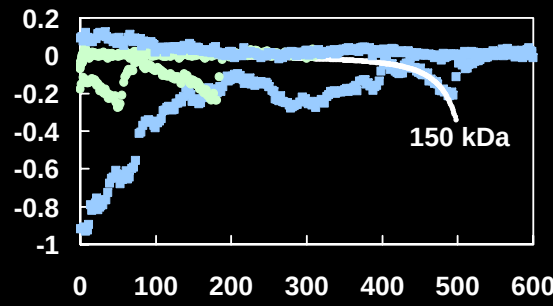
compare with force signature



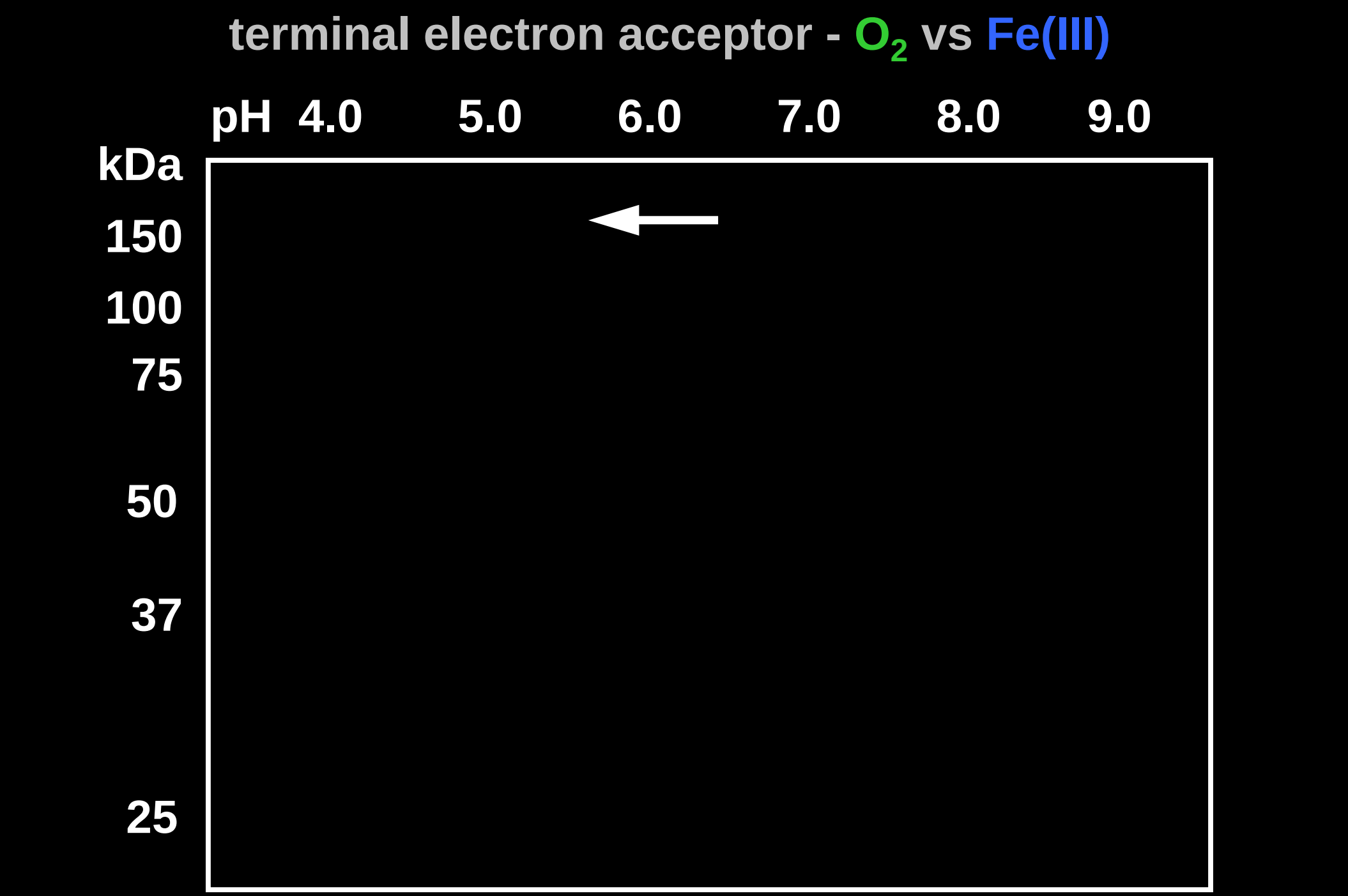
culture cells



separate proteins



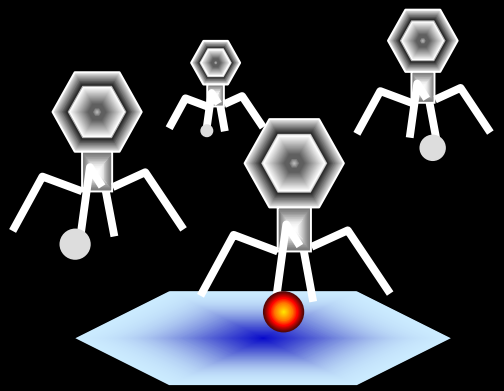
force microscopy


terminal electron acceptor - O2 vs Fe(III)
pH 4.0 5.0 6.0 7.0 8.0 9.0
kDa
150
100
75
50
37
25

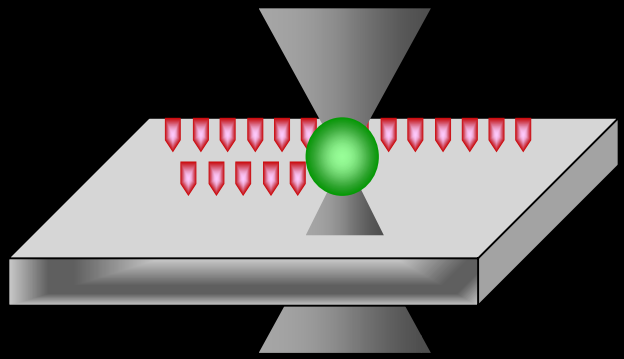
Cell-material interface

discover forces and proteins

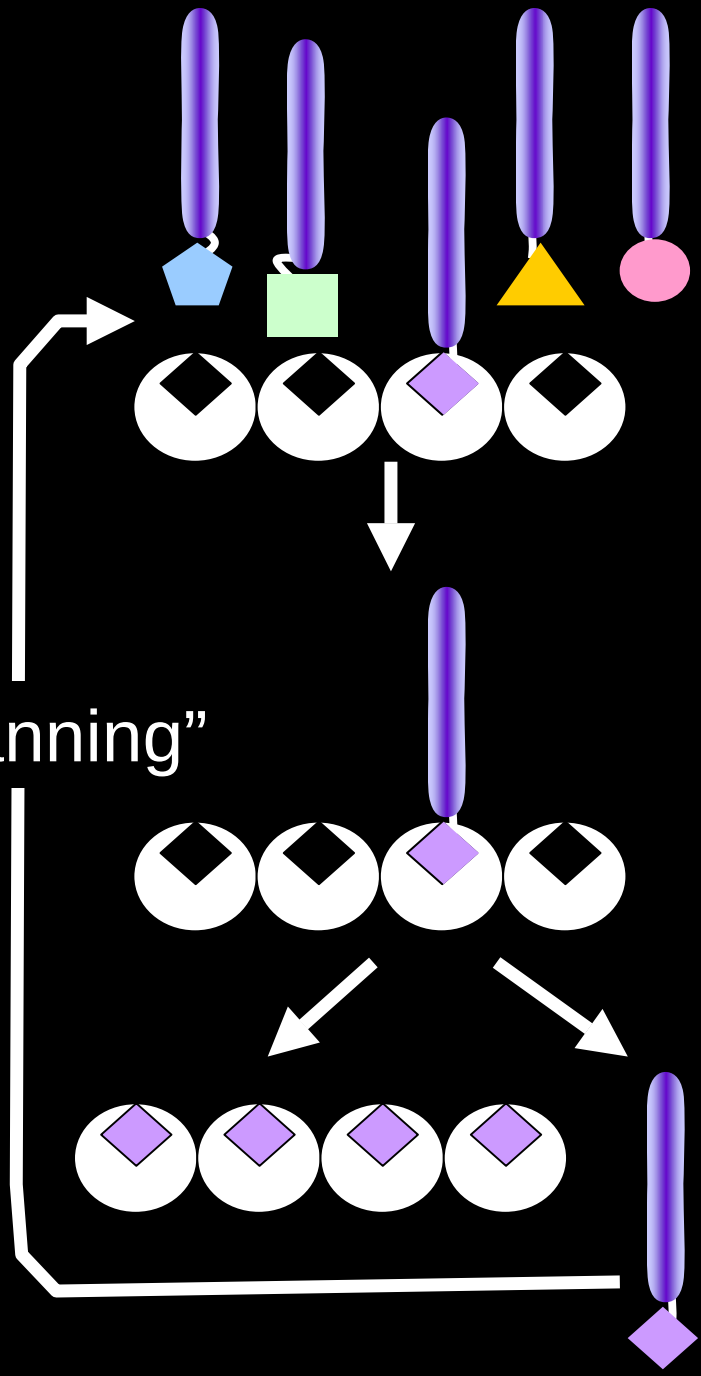
mimic and utilize information



phage display library



cell lithography



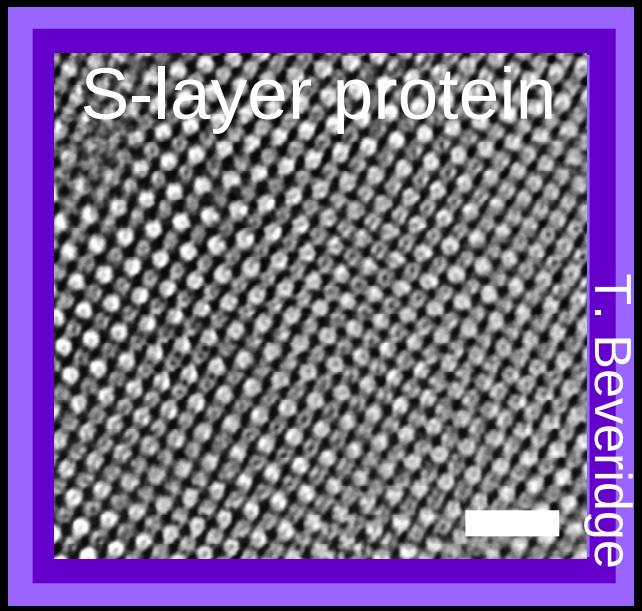
“bio-panning”

peptide phage library expose
to target mineral

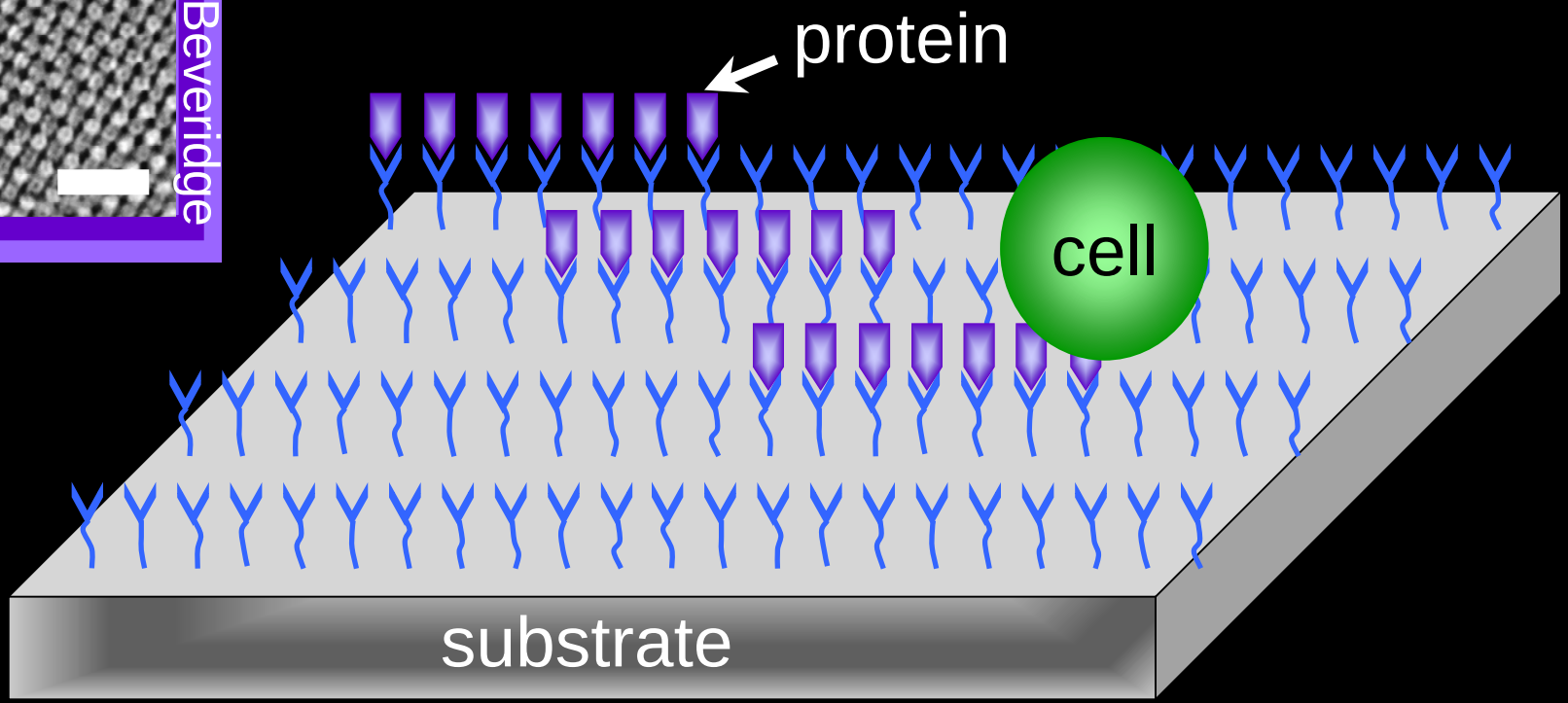
wash away unbound phage
elute bound phage

isolate clones, sequence DNA
to find mineral-binding motif,

biological cell lithography



bacterium as living “pen” that produces and secretes genetically engineered proteins



bacterium-mineral interface

nanoscale forces and proteins

- quantify natural forces of affinity between inorganic crystalline phases and proteins synthesized by bacteria
- use theoretical models and protein expression patterns to identify putative mineral specific proteins
- mimic natural specificity by attempting to fabricate peptides with unique mineral-binding motifs
- use living microbial cells as a lithographic tool

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Steven Lower – Ohio State University – Lower.9@osu.edu