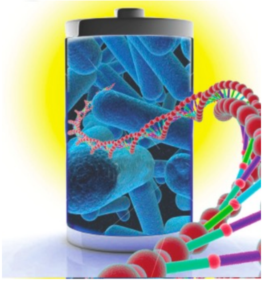
Evolving Organisms to Grow New Nanomaterials for Energy, the Environment, and Medicine



Angela M. Belcher

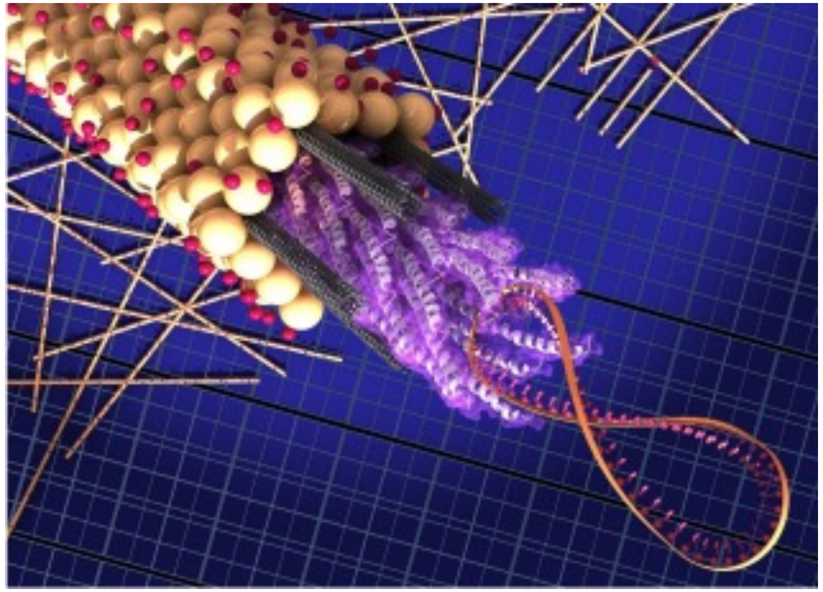
And Her Dream Team

MIT BE BIOLOGICAL ENGINEERING

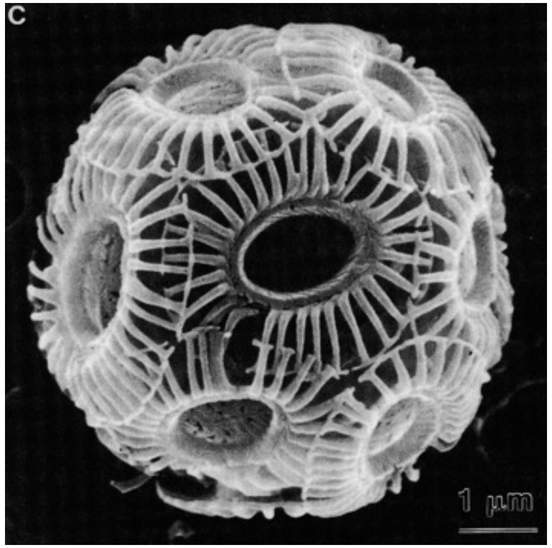
DMSE DEPARTMENT OF MATERIALS SCIENCE & ENGINEERING

KOCH INSTITUTE for Integrative Cancer Research at MIT

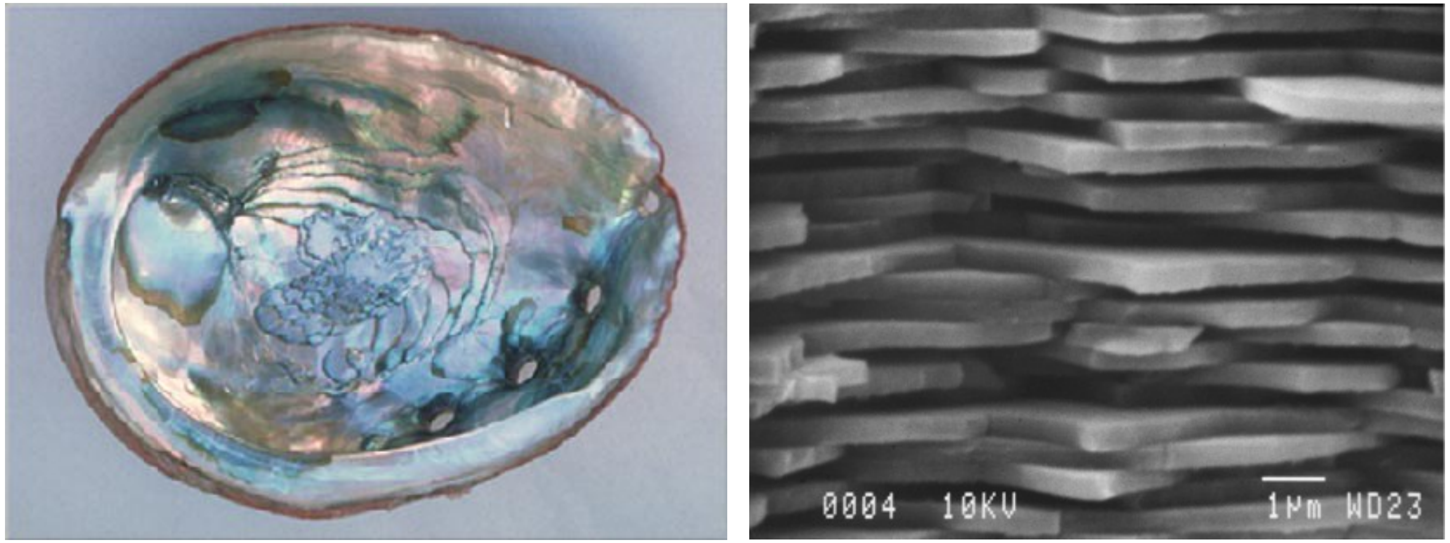
MARBLE CENTER FOR CANCER NANOMEDICINE



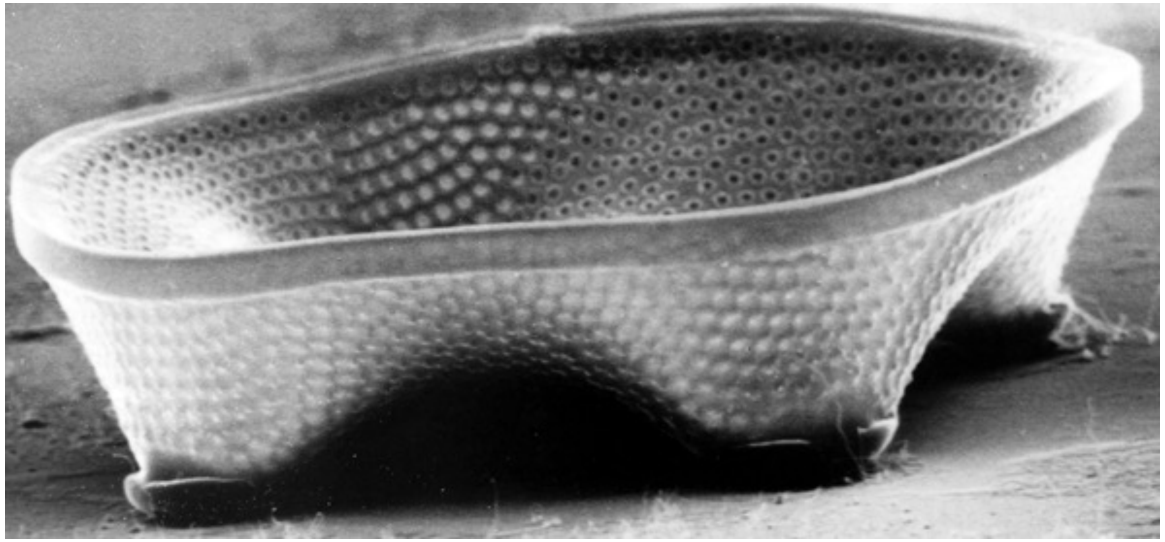
Biology already does this at a nanoscale



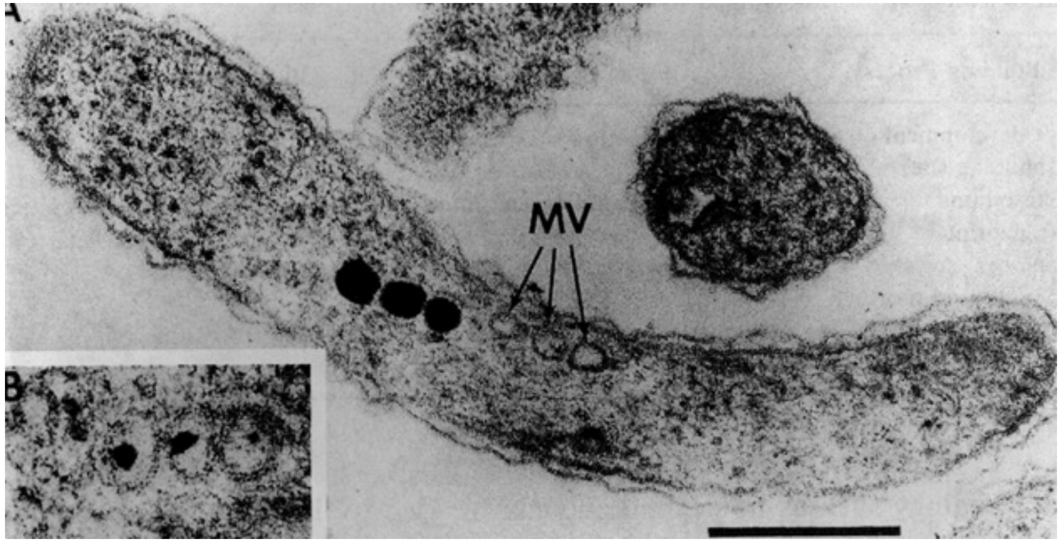
Coccosphere $CaCO_3$



Abalone shell $CaCO_3$

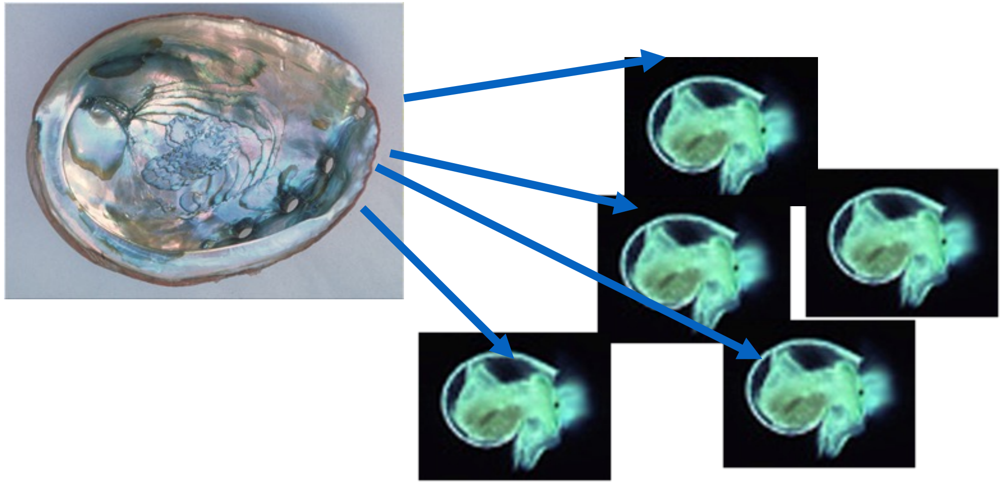


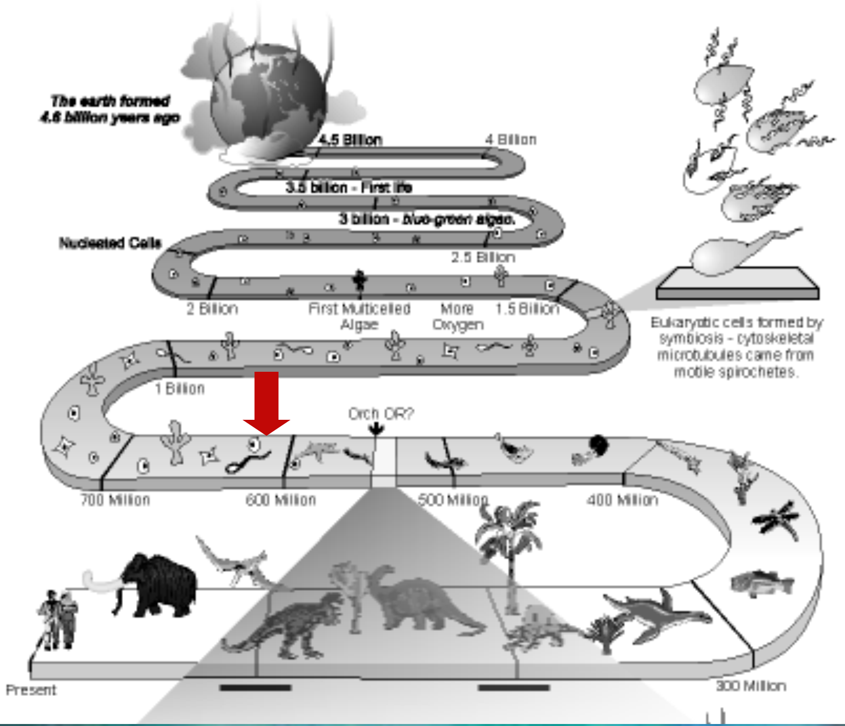
Diatom SiO_2

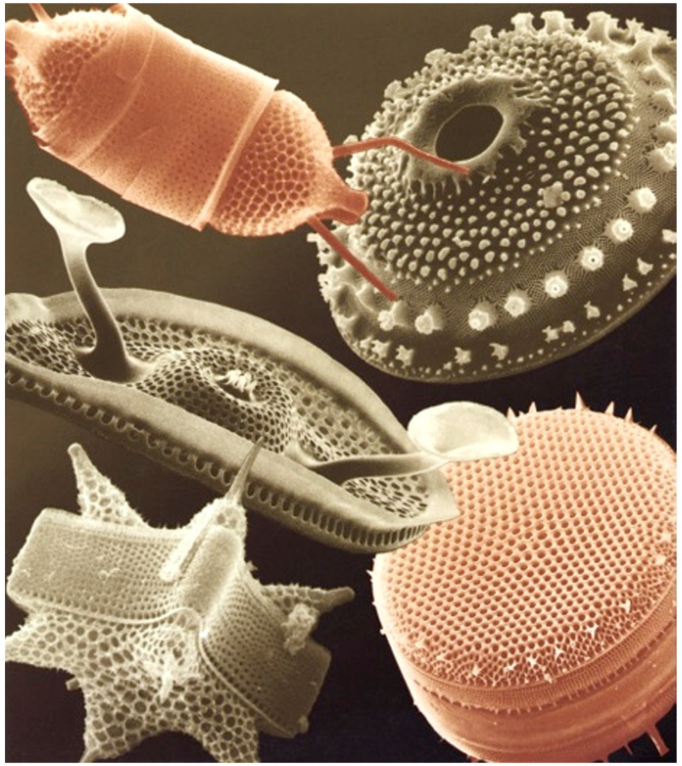


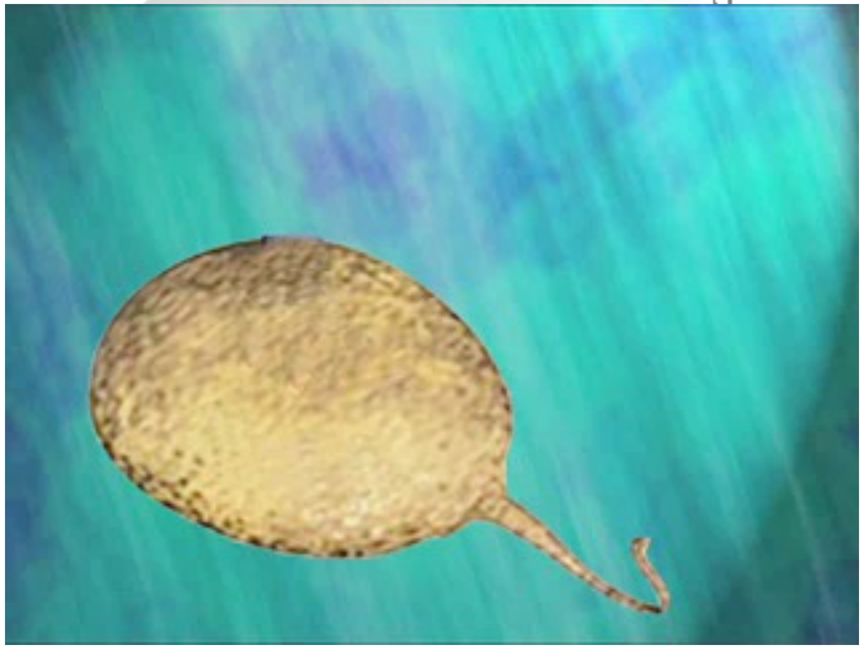
Magnetotatic bacterium Fe_3O_4

Genetic Control of Materials



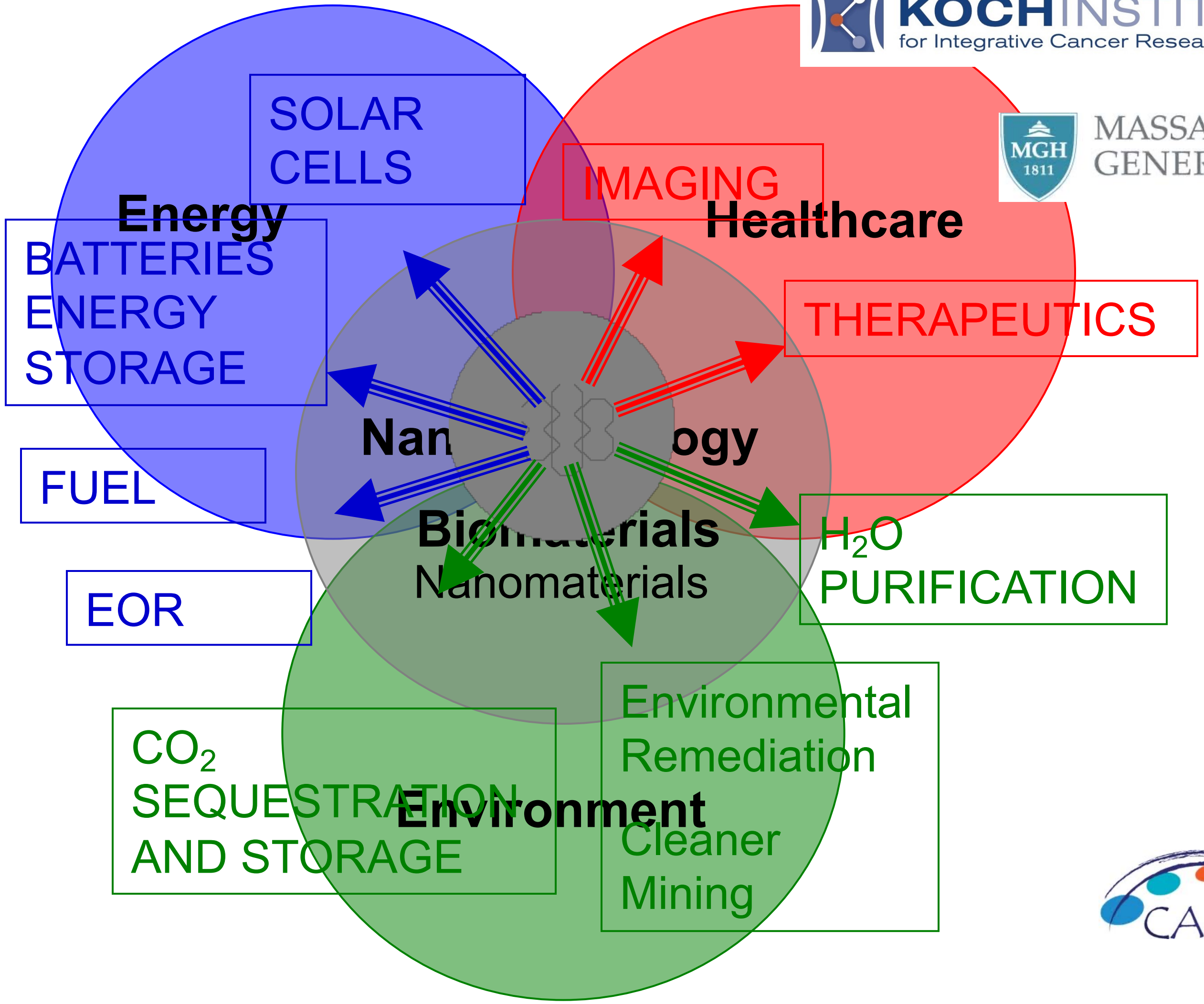






Significant Global Issues- Materials Opportunities

6x6
Biotechnologies



siluria TECHNOLOGIES

KOCH INSTITUTE for Integrative Cancer Research at MIT

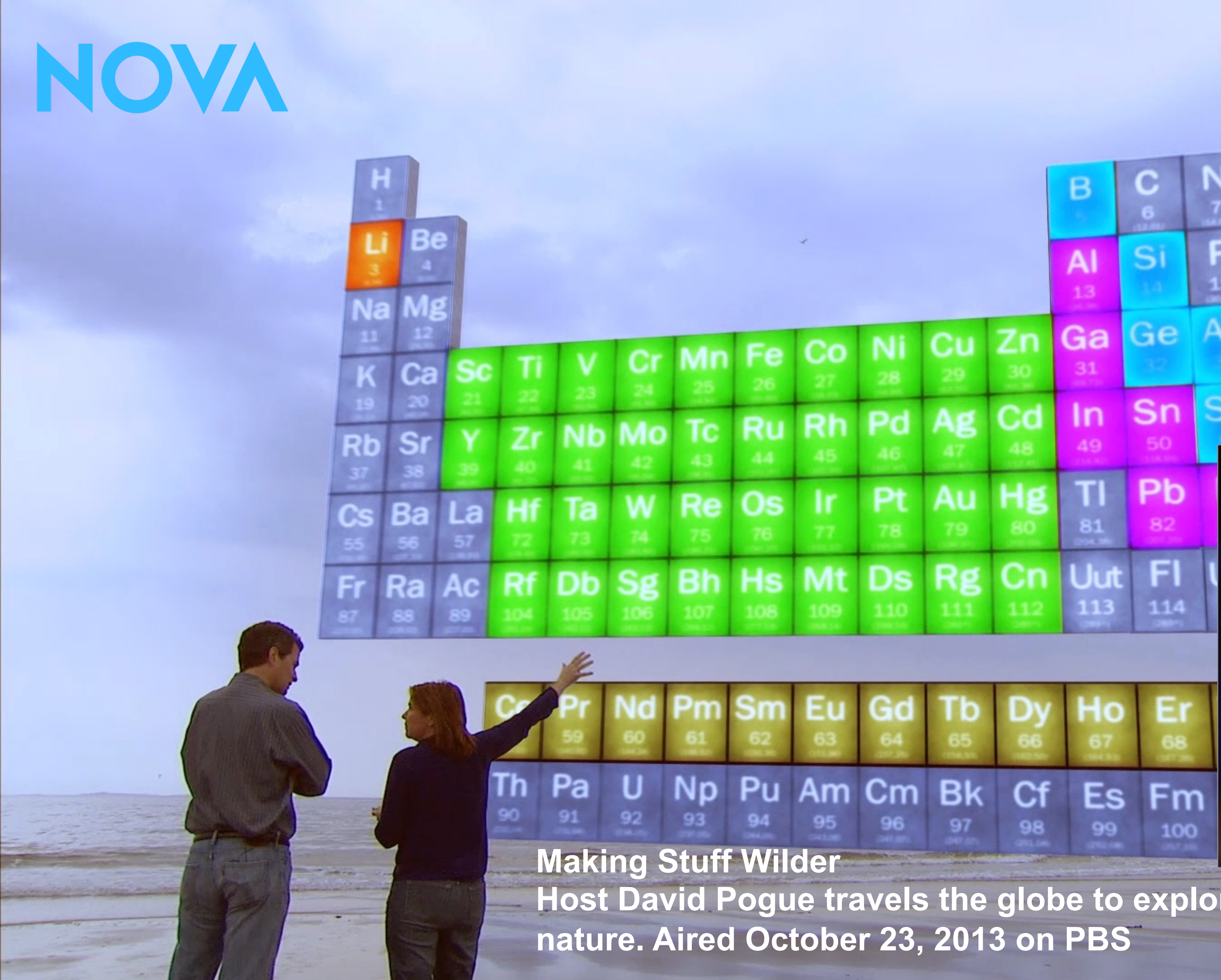
CisionVision

MASSACHUSETTS GENERAL HOSPITAL

gensaic

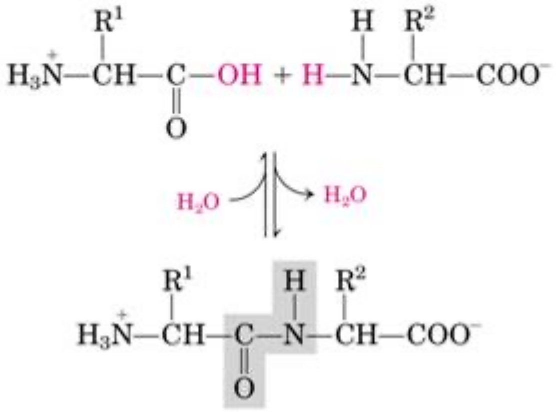
CAMBRIOS

NOVA

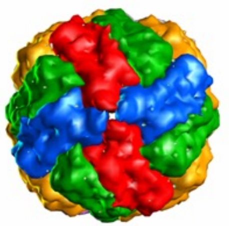


Making Stuff Wilder
Host David Pogue travels the globe to explore new technologies inspired by nature. Aired October 23, 2013 on PBS

Biological Tool Kit

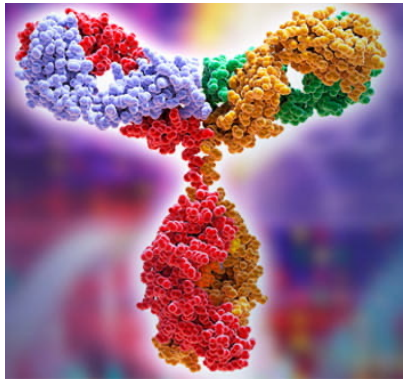


Amino acids, peptide, proteins

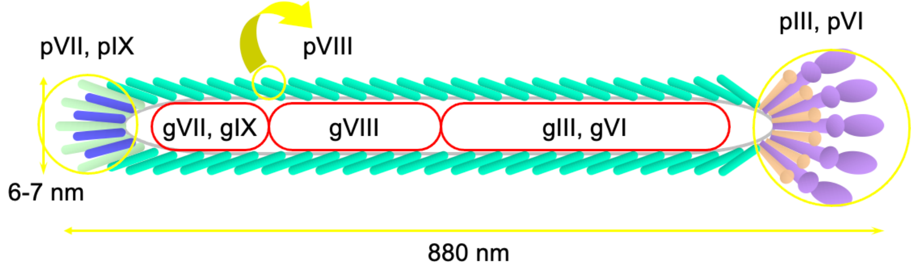


Protein Cages

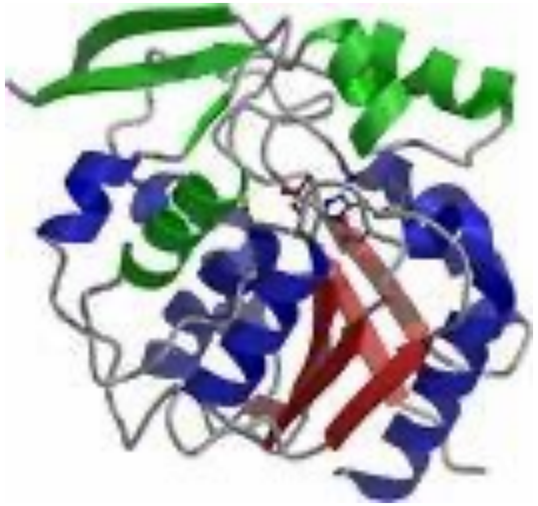
Microbes

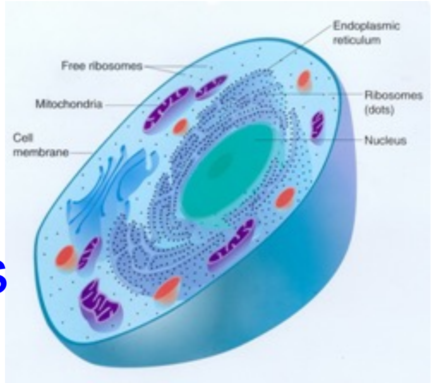


Antibodies



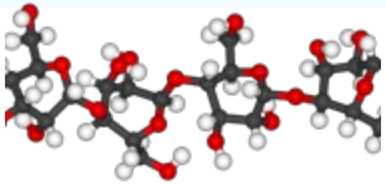
Viruses



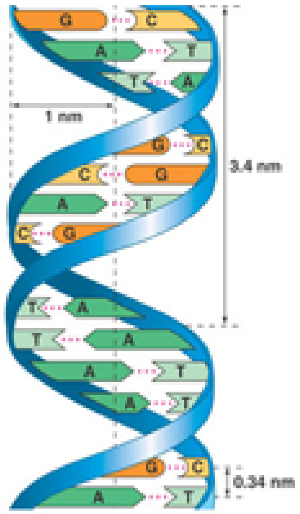


Cells as factories

Polysaccharides



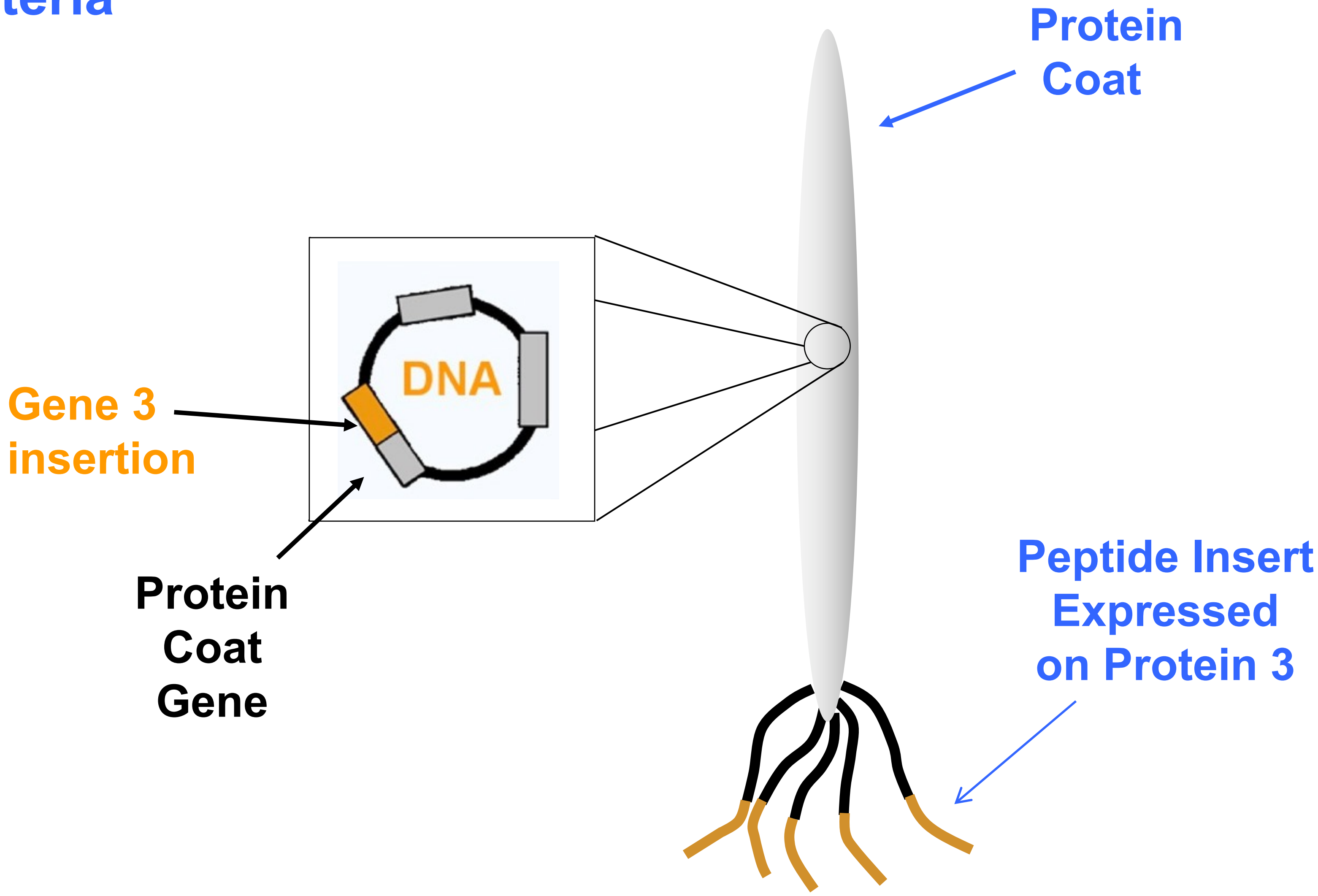
Nucleic Acids



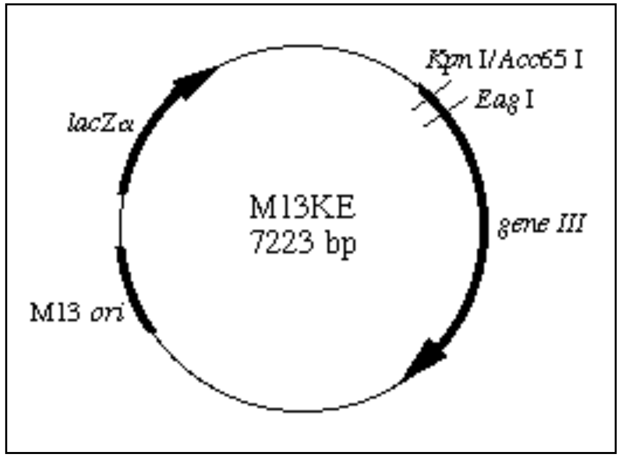
A Combinatorial Approach…..

Using Bacteriophage, or “phage”

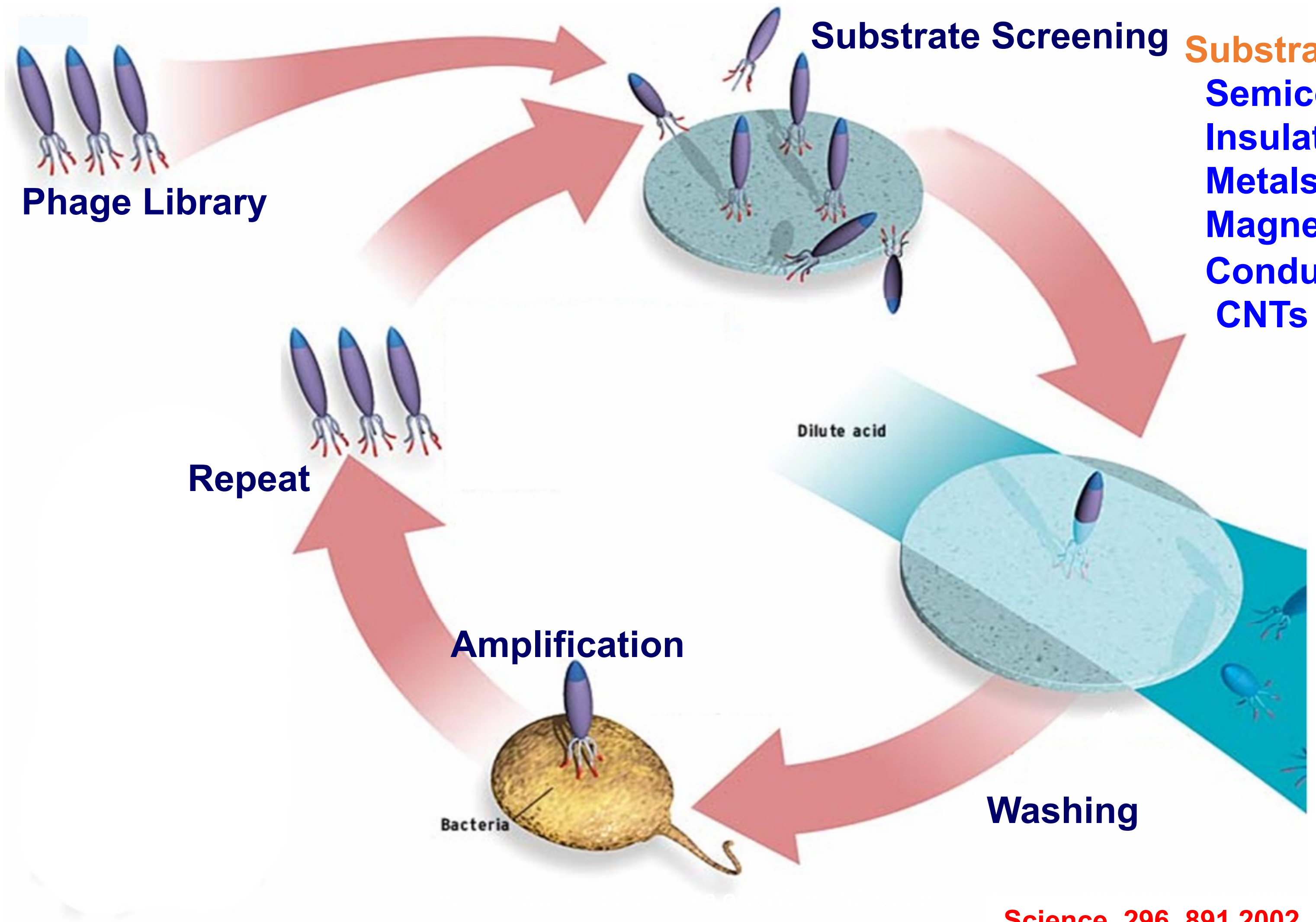
A virus that infects bacteria



(New England BioLabs)



Peptide Libraries & Selection



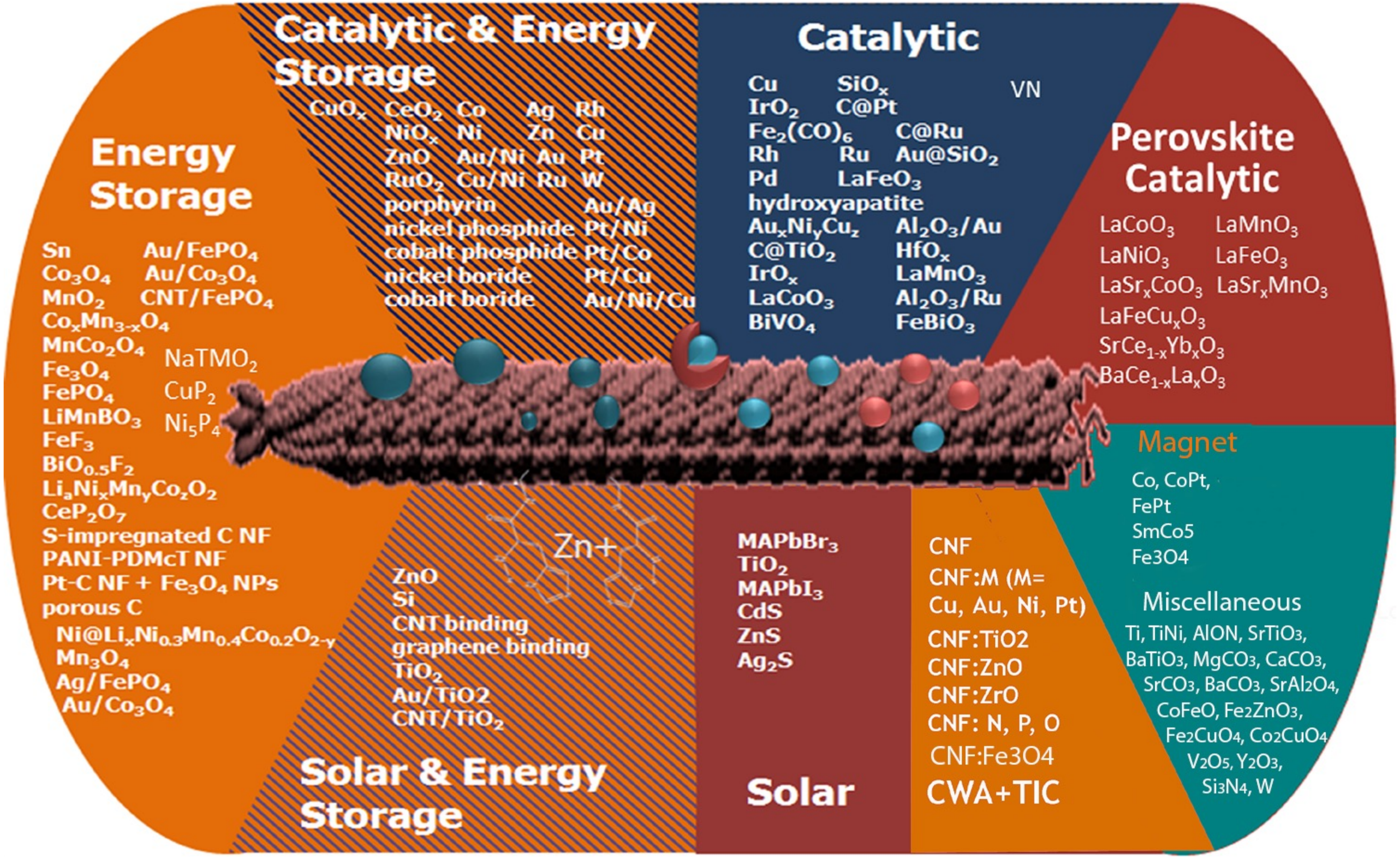
Substrates:

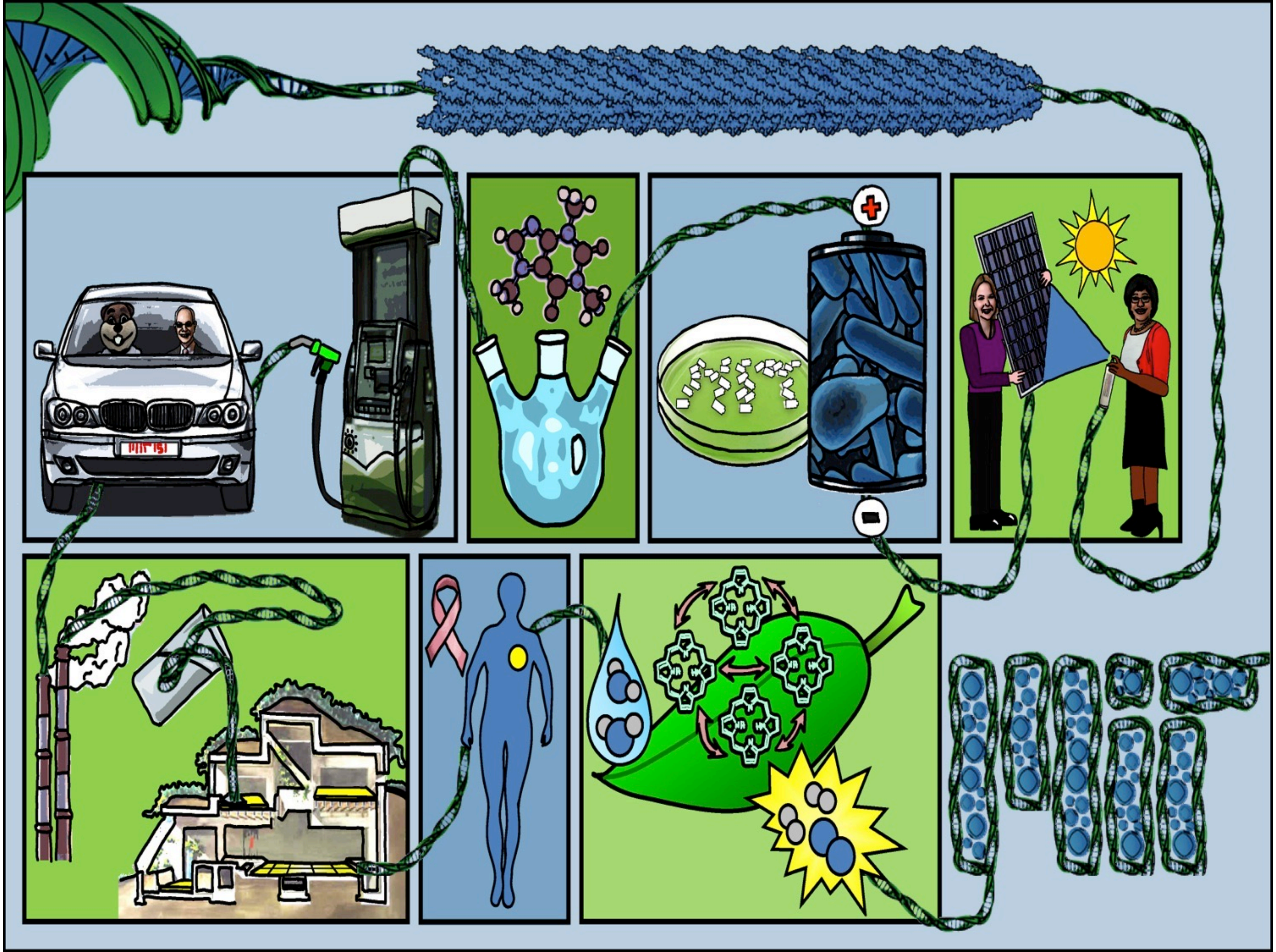
- Semiconductors
- Insulators
- Metals
- Magnetic Materials
- Conducting polymers
- CNTs

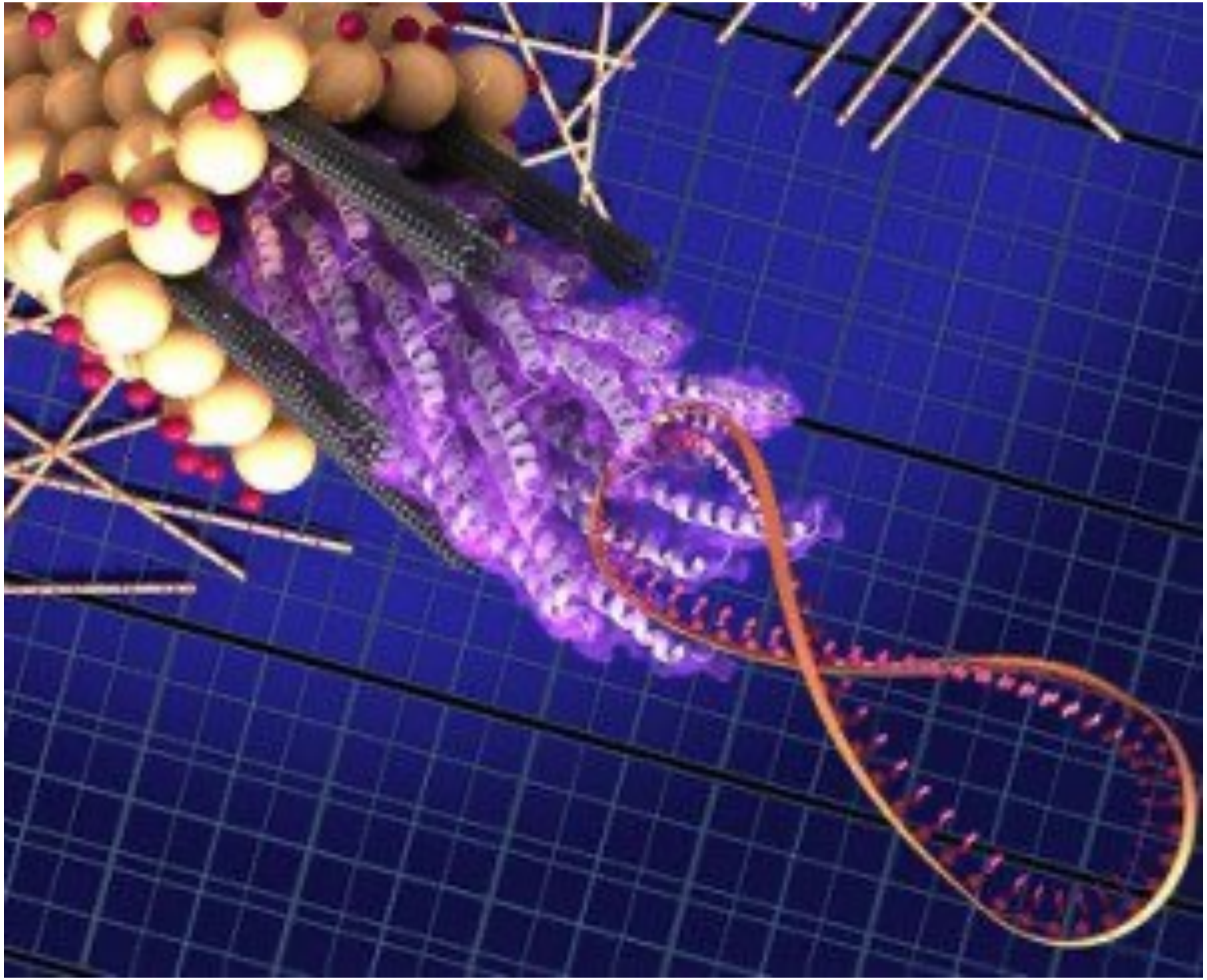
Science, 296, 891,2002
Science, 303, 213, 2004
Science, 312,885, 2006
Science , 324,1051, 2009

Nature,405, 665, 2000
Nature Materials,4,496 2005
Nature Materials, 5,234,2006
Nature Nanotechnology, 2010
Nature Nanotechnology, 2011
Nature Nanotechnology, 2012
Nature Communications, 2013
Nature Communications, 2014
Nature Materials, 2015

Produced 135^+ New Biotemplated Nanomaterials







Energy storage using M13 bacteriophage

Bio-Enabled Energy Storage on a Continuum of Scales

Unmanned Aerial Vehicles

Virus-Templated Metal Nanofoams

Electric Ground Vehicles

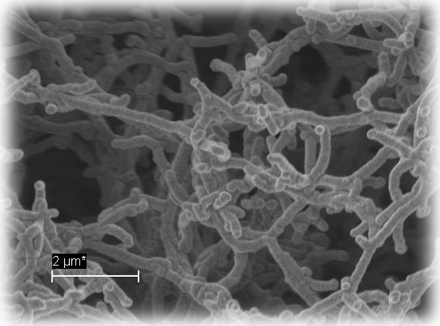
Lithium-Oxygen,
Sodium-Oxygen,
Sulfur-Based Cathodes,
Sodium Intercalation,
Carbon Nanotube/Active Material Bio-enabled Composites

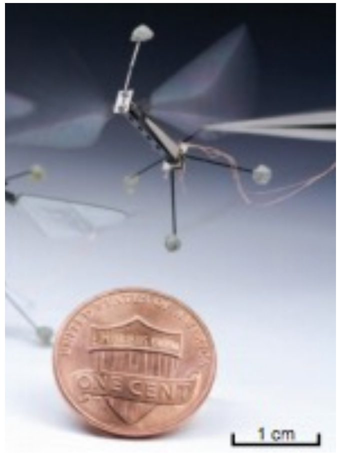
Li-O_2
(12,000 Wh/kg Theor.)

Small Scale ⟷ Large Scale

Above: Raven UAV
Below Left: Virus-templated Nickel current collector
Below Right: "Robobee"
Ma, *Science*, 2013

Power Density Limited





Wearable Electronics

Electrospun Interpenetrating Battery Cloths

Below: Electrospun polymer matte

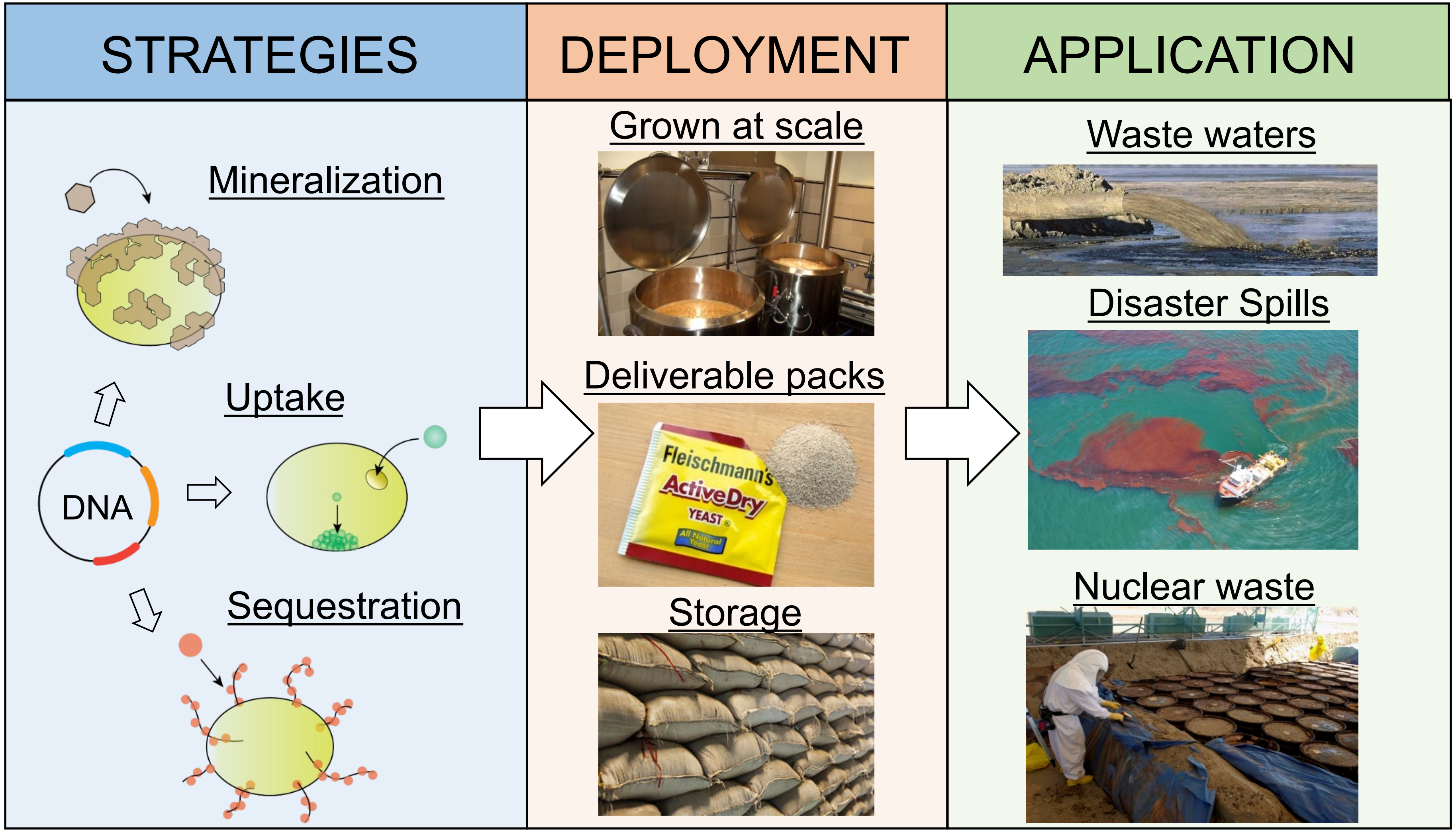
Cost and Portability Limited

Grid and Microgrid

Biotemplated Sodium-Ion Batteries
Lithium and Sodium Sulfur Batteries
Flow Batteries

Yeast for natural waste management

- We are re-purposing yeast to clean up toxic waste.
- Humans have been using yeast for over *5,000* years. The technology to store, dry, and reconstitute yeast have already been optimized.
- We are targeting heavy metals from mining runoff, oil sands, and organic runoff from dyes.



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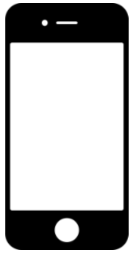
Trivia

What do these two things have in common?



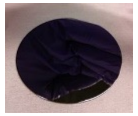
Day-to-day electronics

Copper
Electronic traces and connectors

Cobalt
Strengthening alloy; coloration

Lead
Power electronics; physical & electrical support

Cadmium
Batteries; LEDS; strengthening alloy



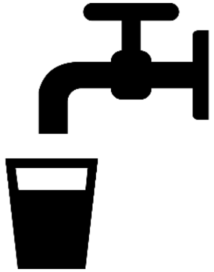
Arsenic
Batteries; LEDS; strengthening alloy

Mercury
Construction of sensors and transducers

Chromium
Plating; protective coating; coloration

Drinking water

Pretty much everywhere

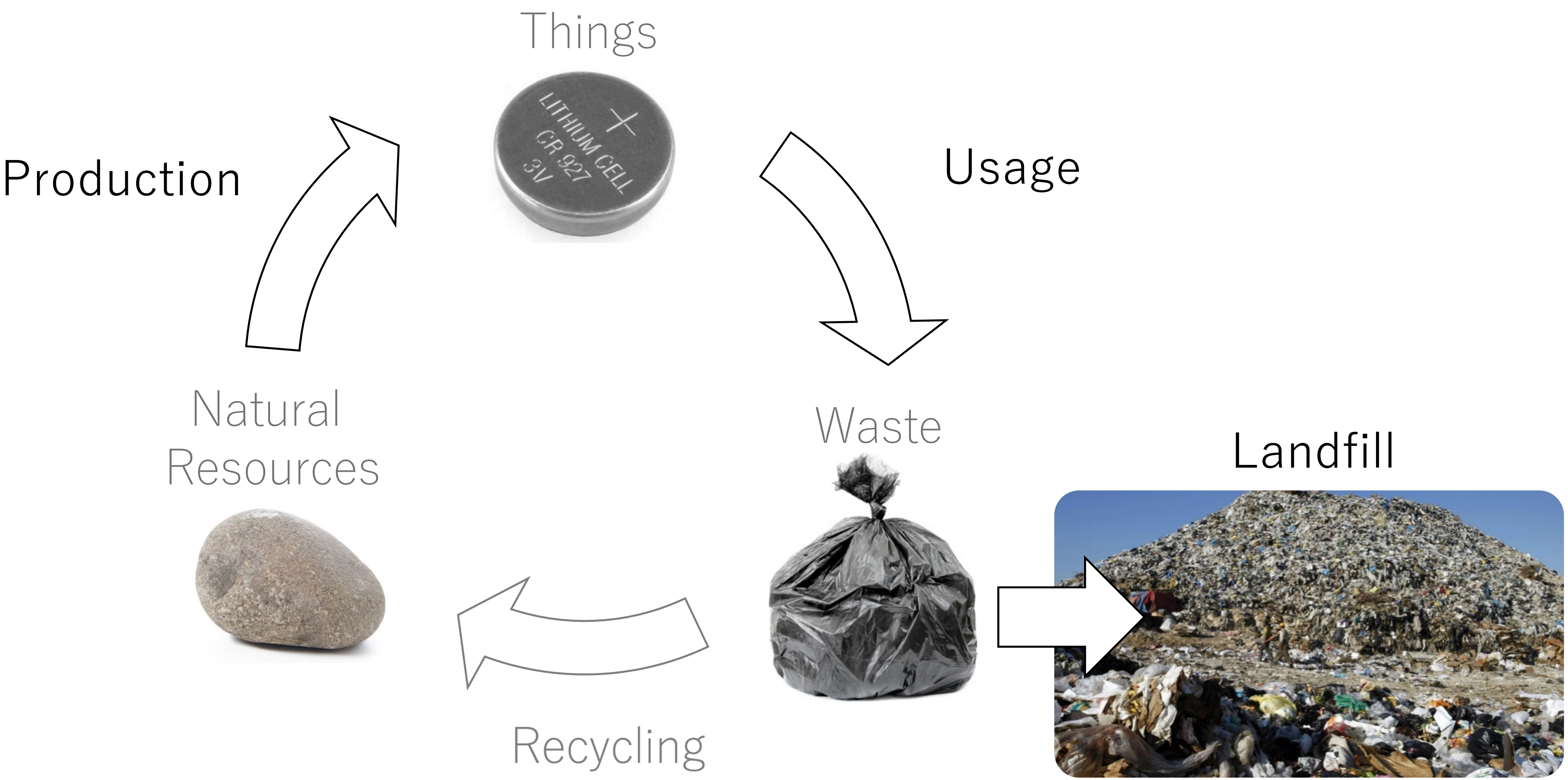


- Liver and kidney damage
- Anemia
  Thyroid and nerve problems (e.g. ear)
- Developmental retardation
  Slew of physiological ailments
- Nervous system damage
  Cancer
- Multisystem disease, acutely toxic
  Cancer
- Multisystem disease, acutely toxic
  Cancer
- Multisystem disease, acutely toxic
  Cancer (e.g. Lung)

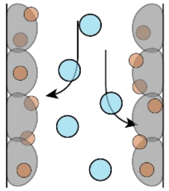
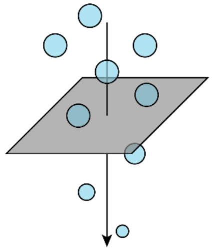
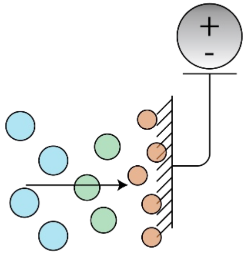
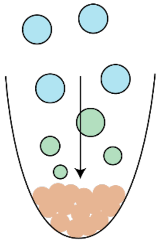
Waste management: an incomplete cycle

Dr. George Sun

Waste management: an incomplete cycle



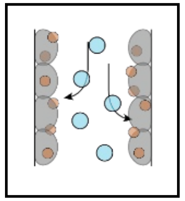
Current physicochemical processes

		specificity	reusability	cost to make	cost to operate
Ion-exchange		VARIES	YES	HIGH	HIGH
Adsorption		LOW	NO	VARIES	~
Electro-chemical		VARIES	VARIES	HIGH	HIGH
Chemical Precipitation		LOW	NO	LOW	HIGH

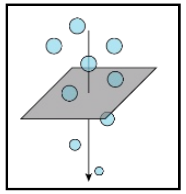
Physicochemical *vs* bioremediation

Synthetic

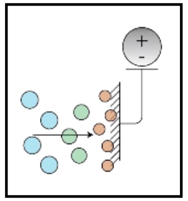
Ion-exchange



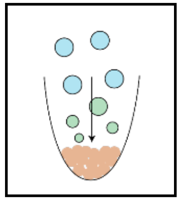
Adsorption



ElectroChem

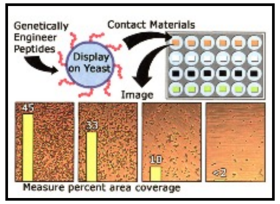


Precipitation

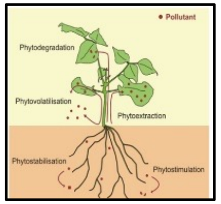


Biological

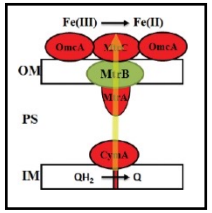
Cell surface display



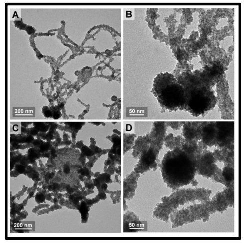
Cell uptake



Oxidases & Reductases



Biomineralization



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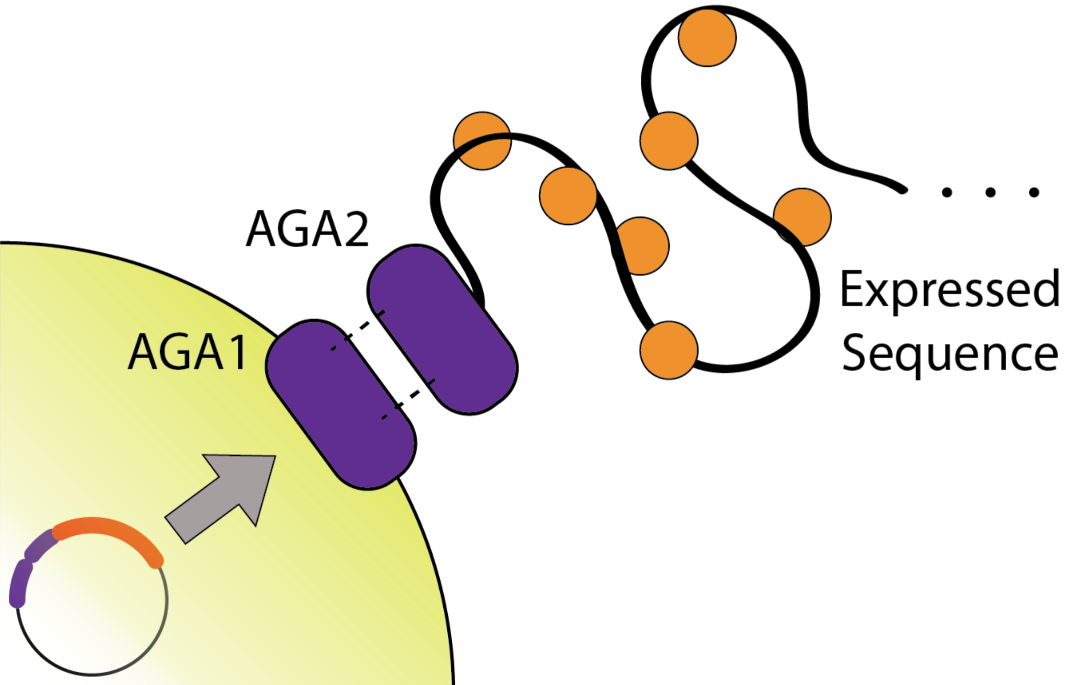
Strategy #1: *a.k.a.* ion-exchange

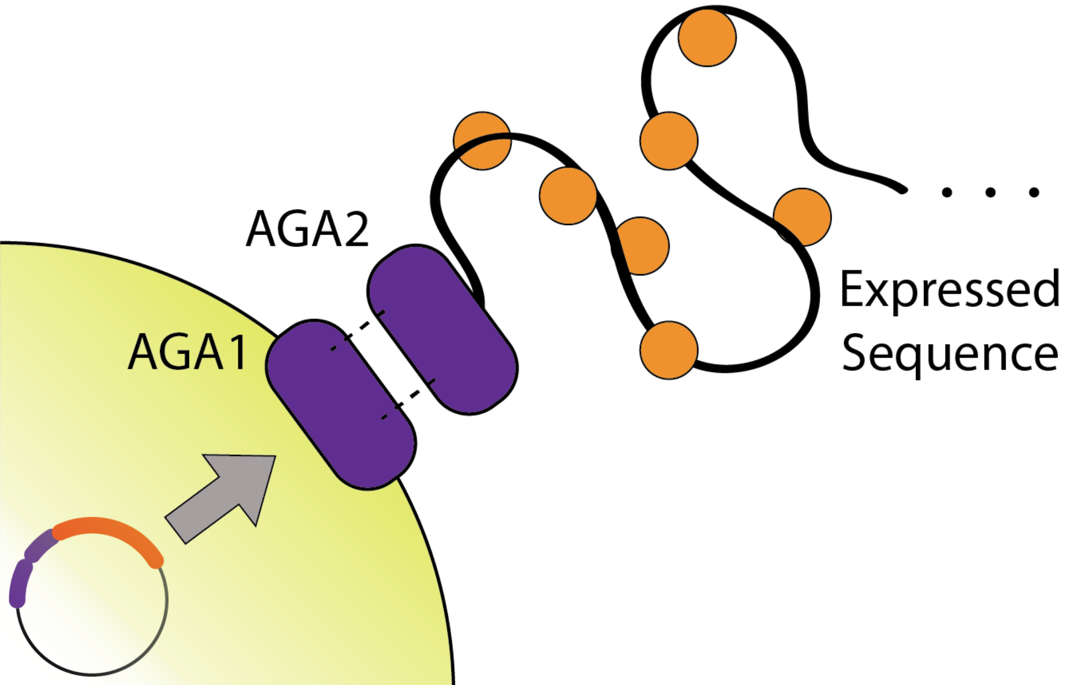


Yeast display for metal uptake

~ probably not good enough ~

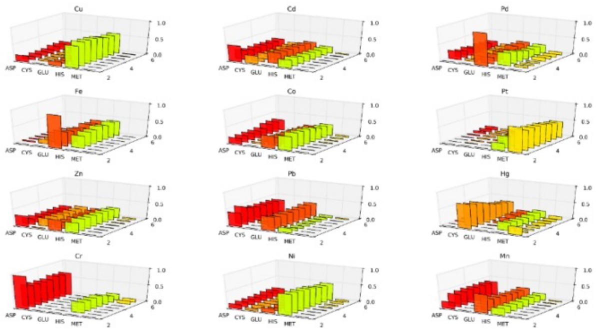
Yeast Display : # cells $\times$ # displayed peptides/proteins $\times$ # binding sites $\approx \mu$M

Ion Exchange : # density of resin $\times$ # functional groups $\times\ 1 \gg$ mM



Methods

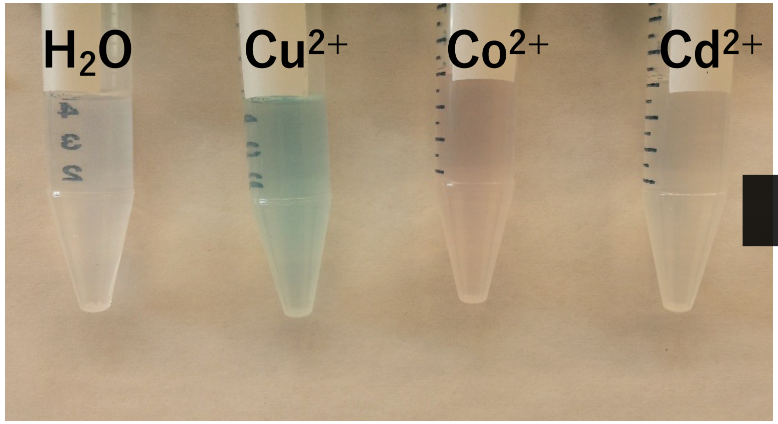
- Computational methods
  - Machine learning
  - + empirical testing and screening



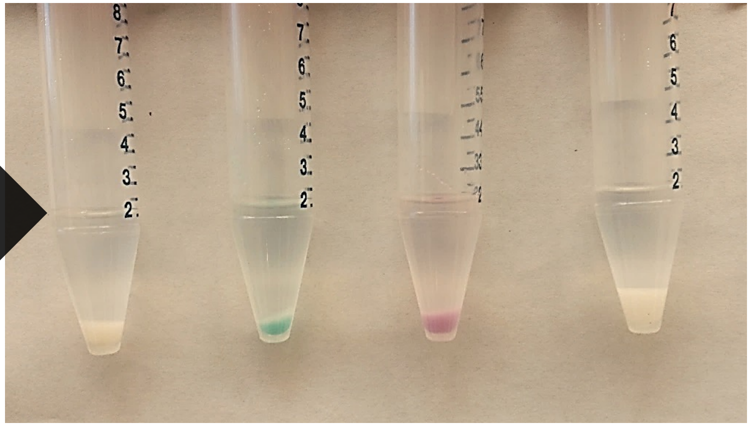
- ··· Use nature

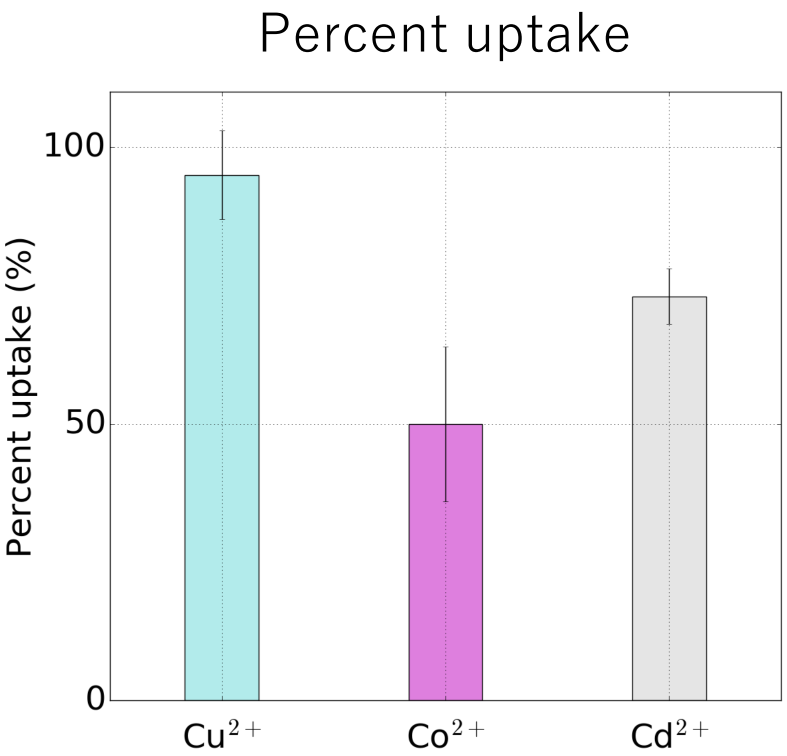
Yeast Display Capture

no cells

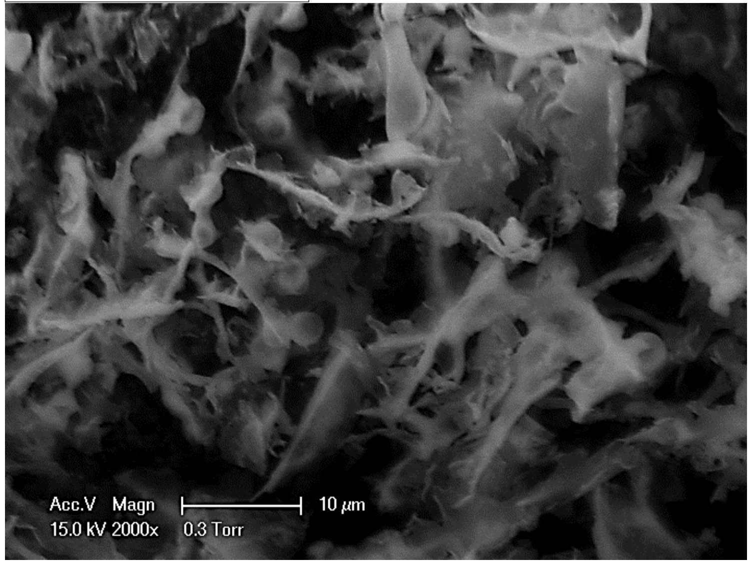


+ cells

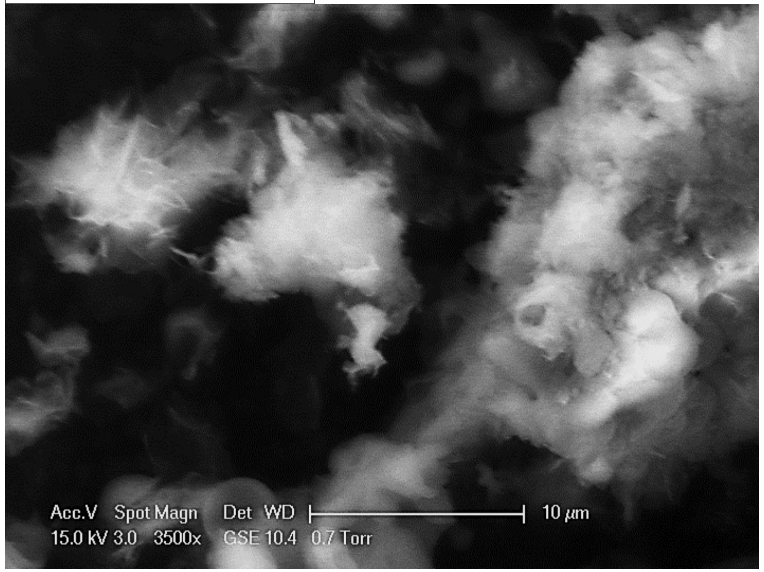




1 mM Cd^{2+}



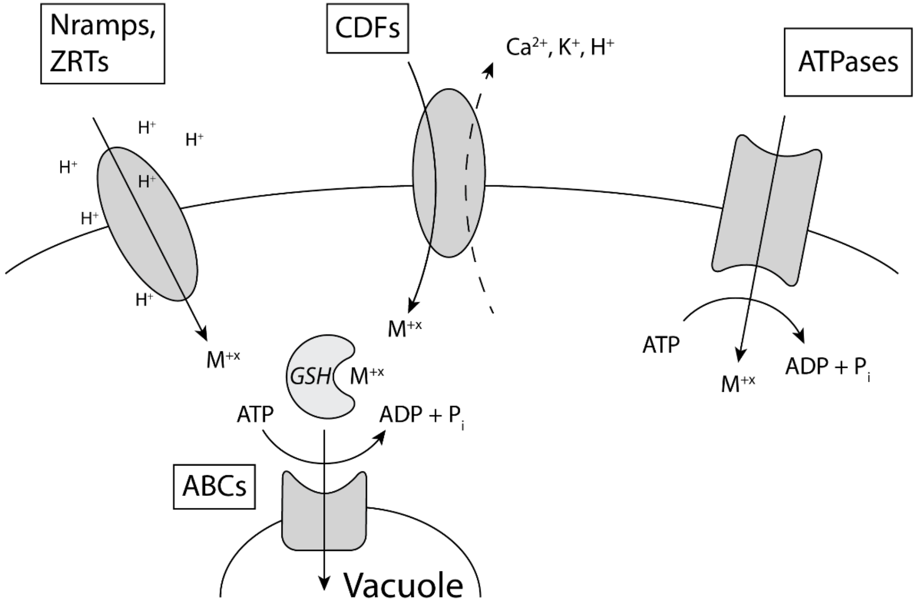
1 mM Zn^{2+}



Observations

- Similar trends for: Ni^{2+}, Zn^{2+}, Sr^{2+}
- Not effected by Ca^{2+}, Mg^{2+}, Na^{+}, Li^{+}
- Engineering aggregation for Pb^{2+}, Hg^{2+}, and lanthanide metals

Strategy #2: *a.k.a.* absorption



Transporter	Metal	Where	Requires
CDF	Mn/Fe/Zn/Co/Cd	varies	antiports counter ions
ZIP	Fe/Zn	cytoplasm	proton gradient
Nramp	Fe/Zn/Mn/Co/Ca/Cu/Ni/Pb	cytoplasm	antiports H^{+}
ATPases	Cu/Ag/Zn/Cd/Pb/Co/Ca/Mg	cytoplasm	ATP
ABCs	phytochelatin+metal complex	vacuole	ATP

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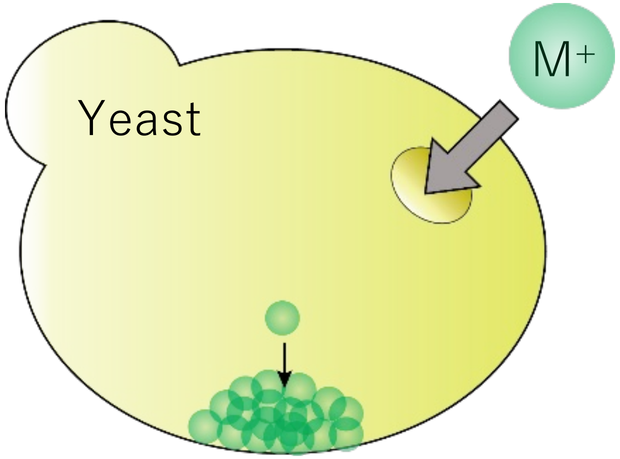
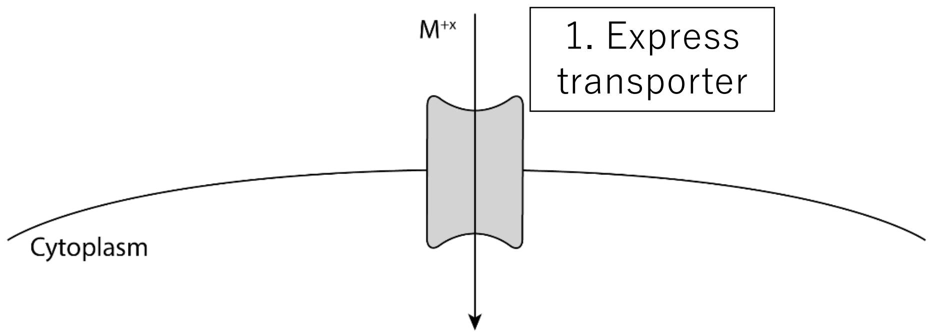
Strategy #2: *a.k.a.* absorption

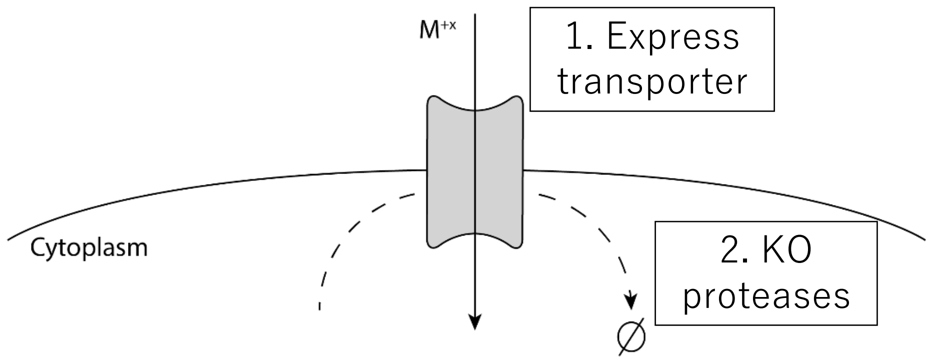


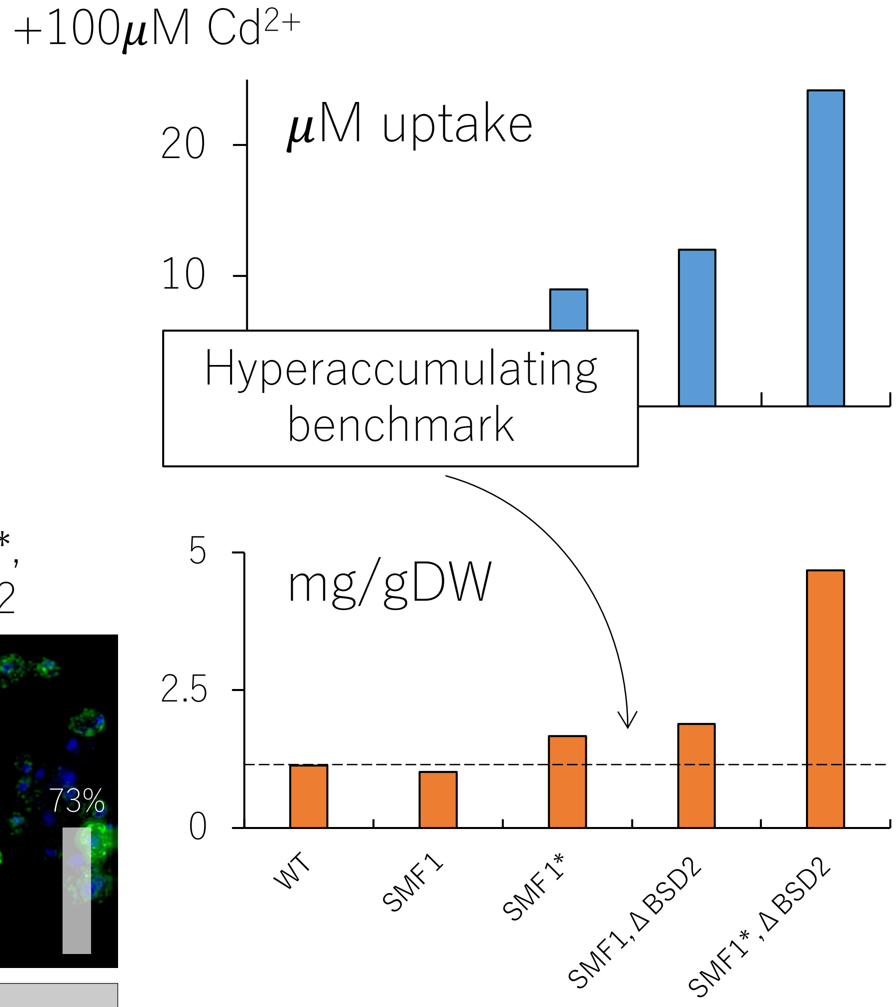
Transporter	Metal	Where	Requires
CDF	Mn/Fe/Zn/Co/Cd	varies	antiports counter ions
ZIP	Fe/Zn	cytoplasm	proton gradient
Nramp	Fe/Zn/Mn/Co/Ca/Cu/Ni/Pb	cytoplasm	antiports H^+
ATPases	Cu/Ag/Zn/Cd/Pb/Co/Ca/Mg	cytoplasm	ATP
ABCs	phytochelatin+metal complex	vacuole	ATP

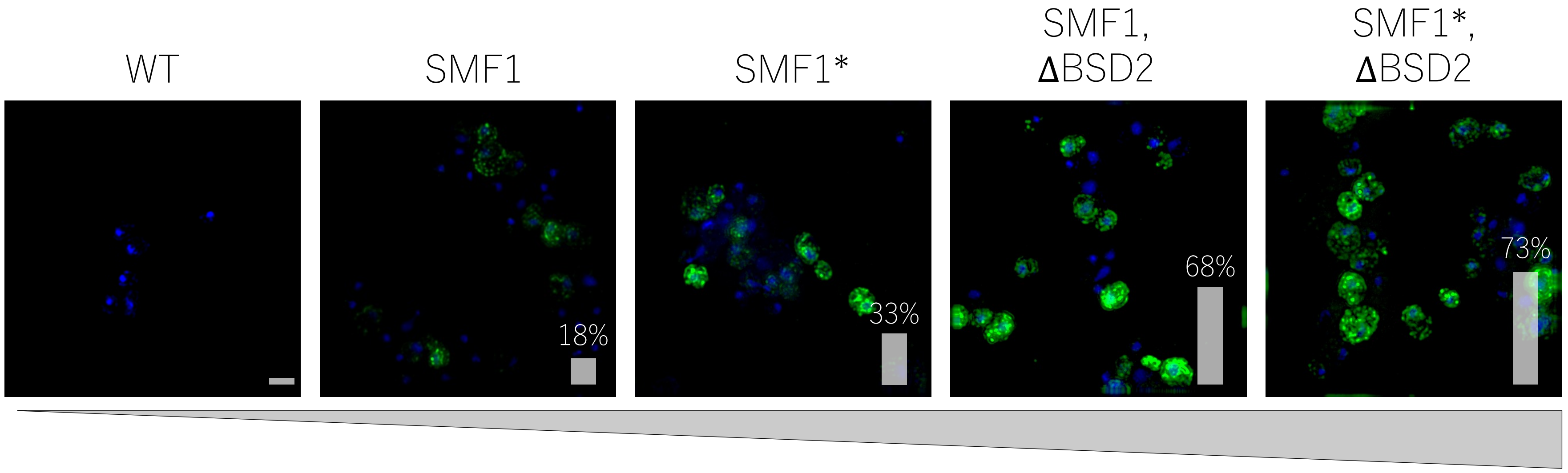
Metal capture using yeast transporters



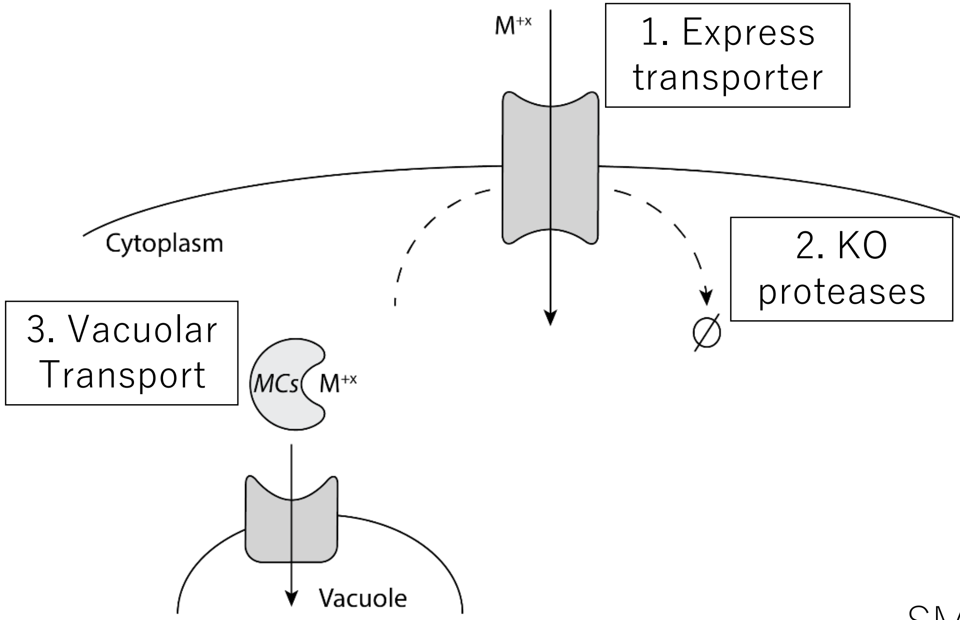
Metal capture using yeast transporters

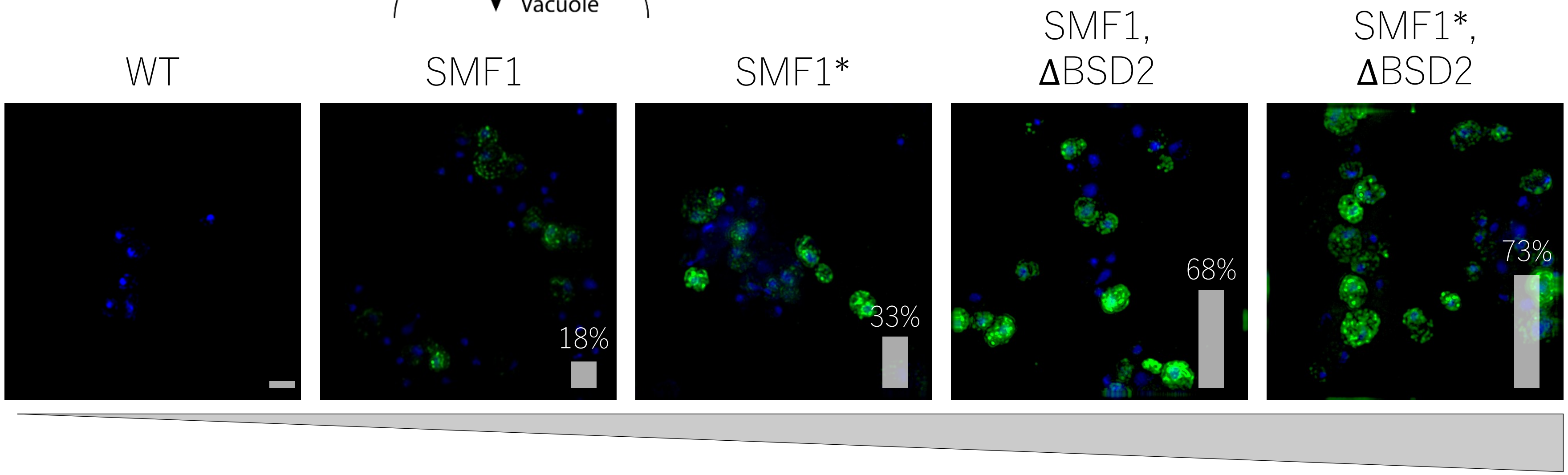


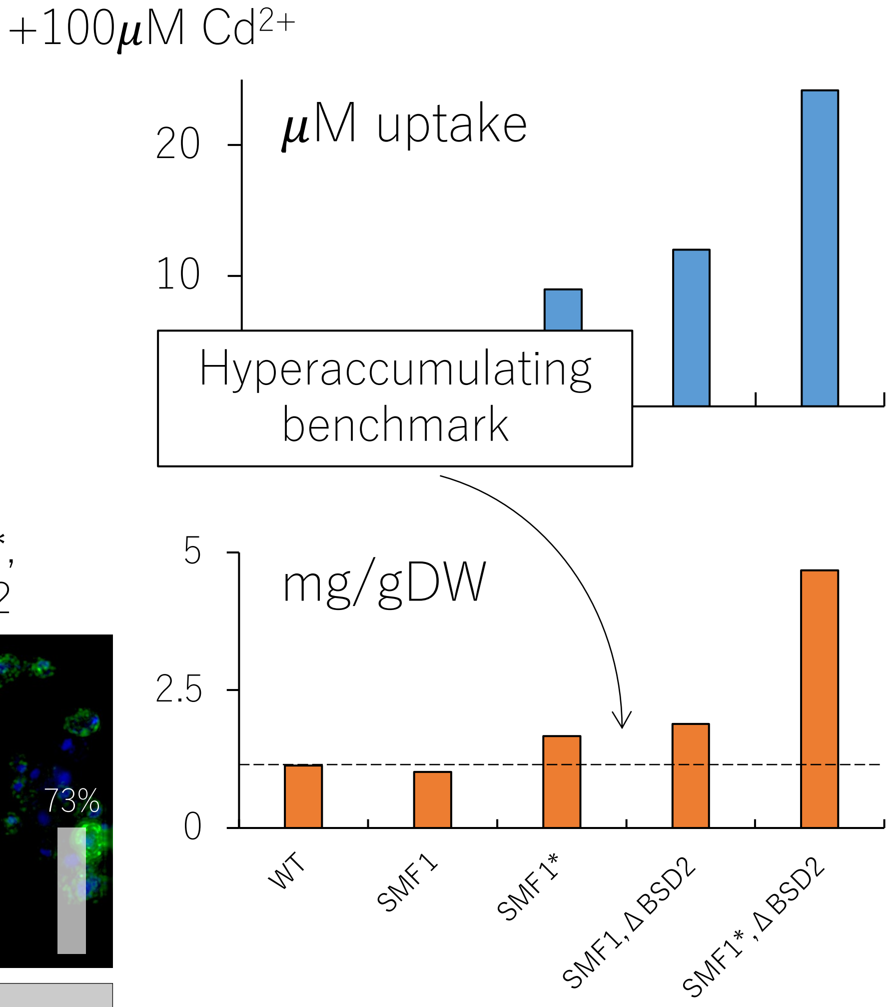




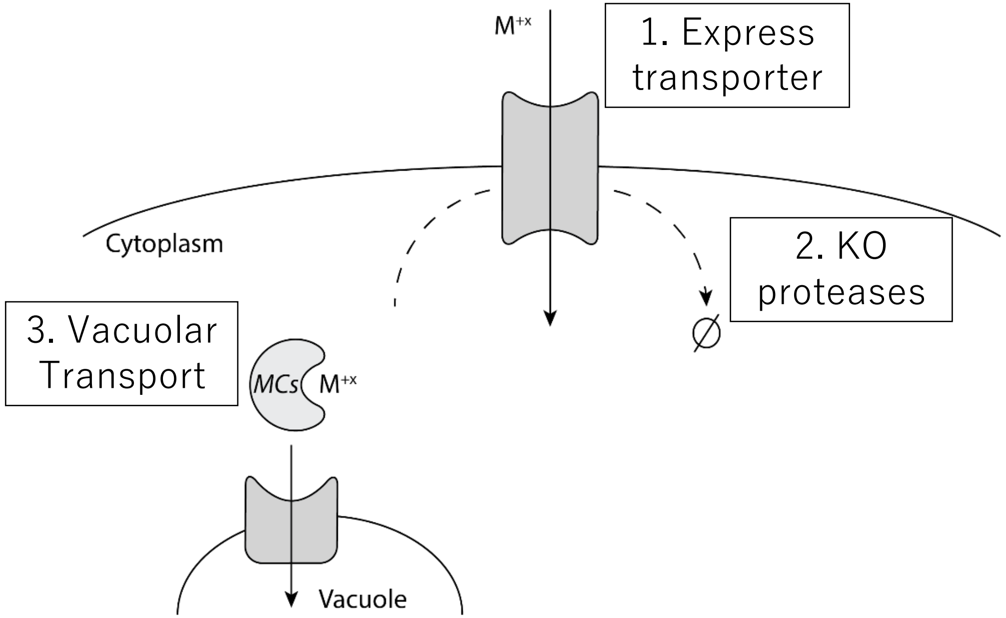
Metal capture using yeast transporters

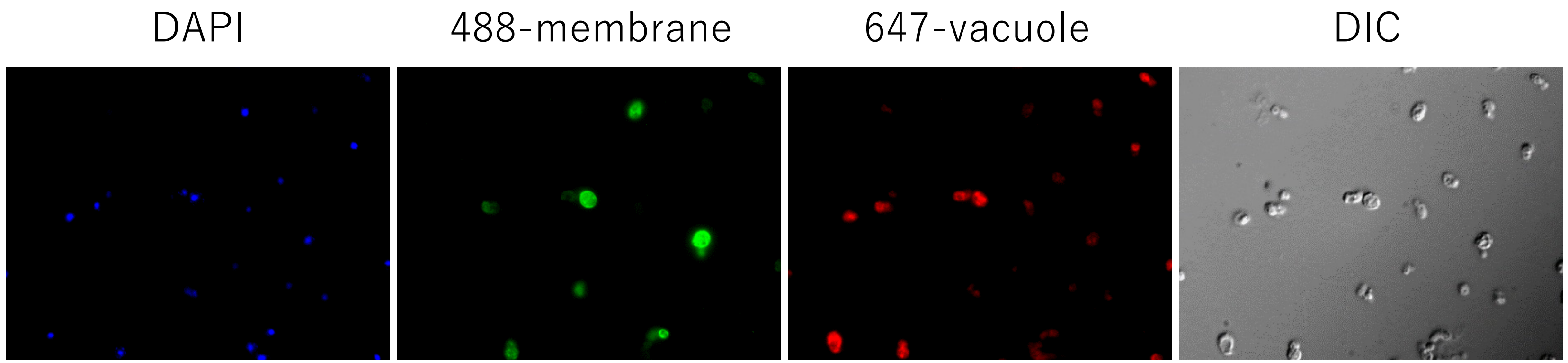


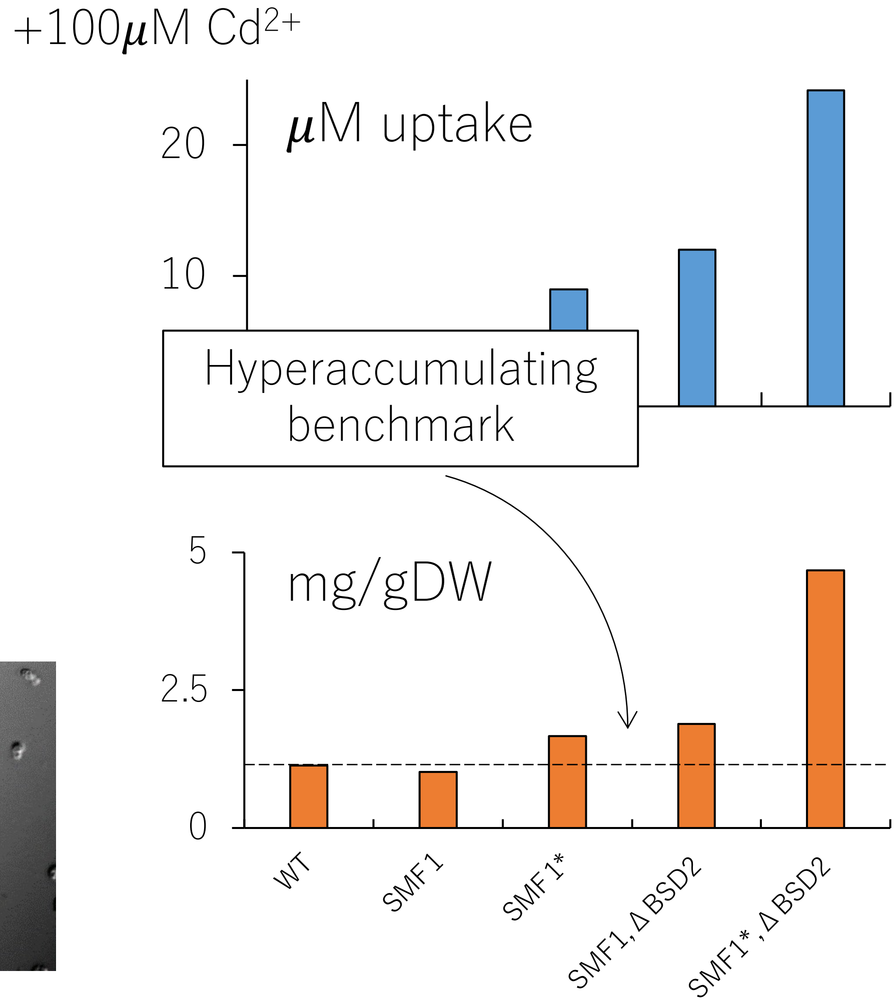




Metal capture using yeast transporters







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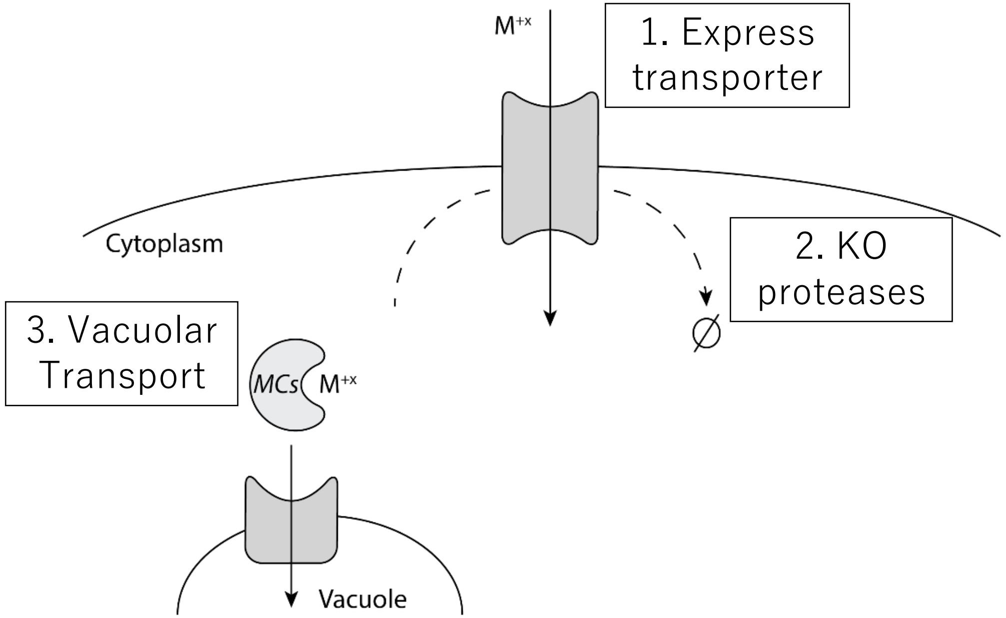

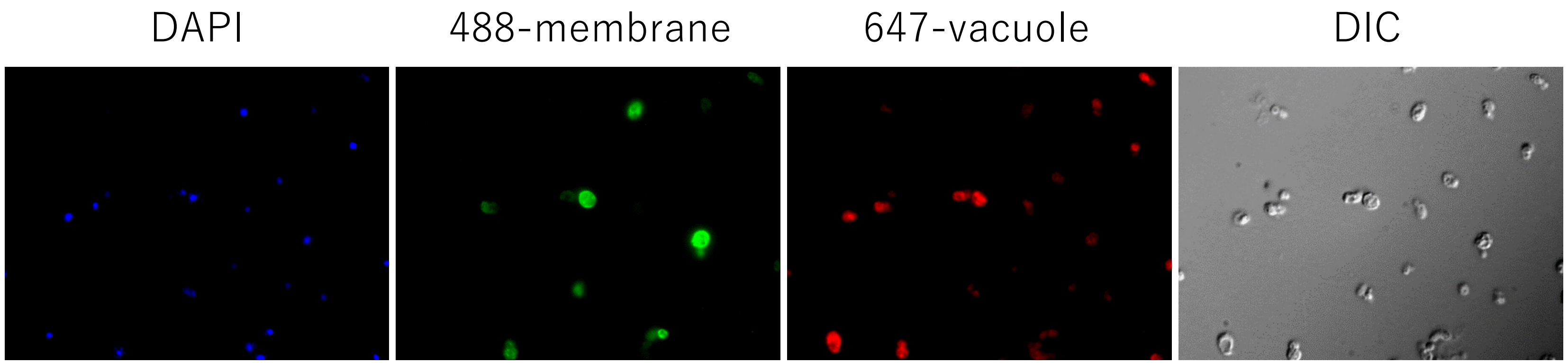

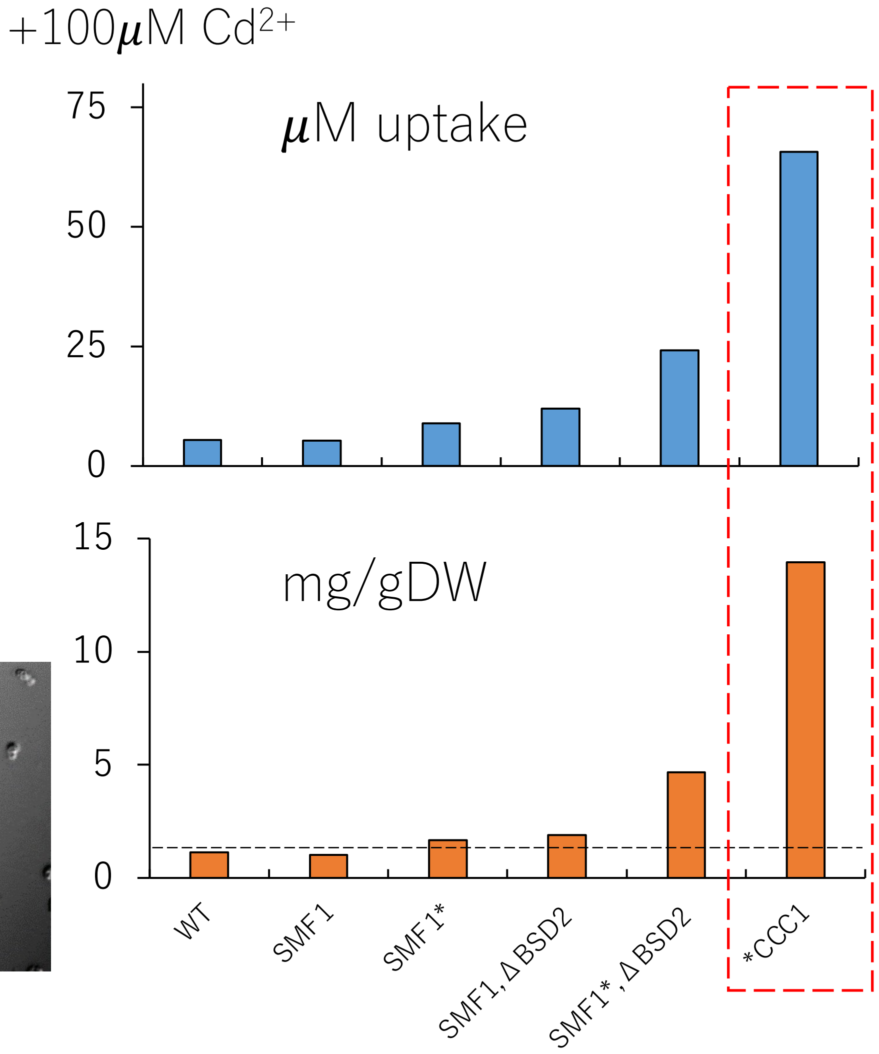

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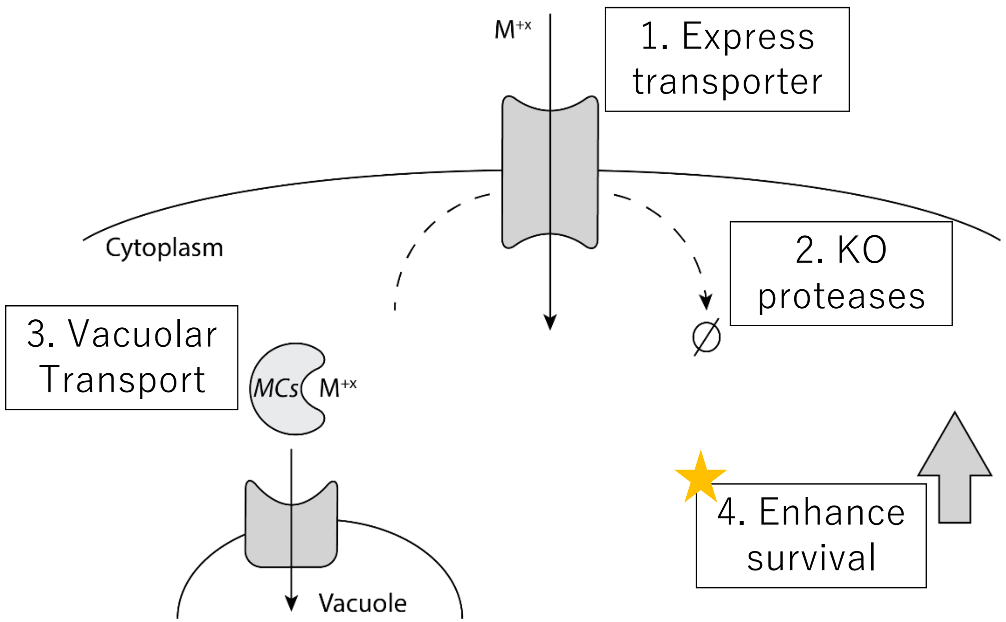
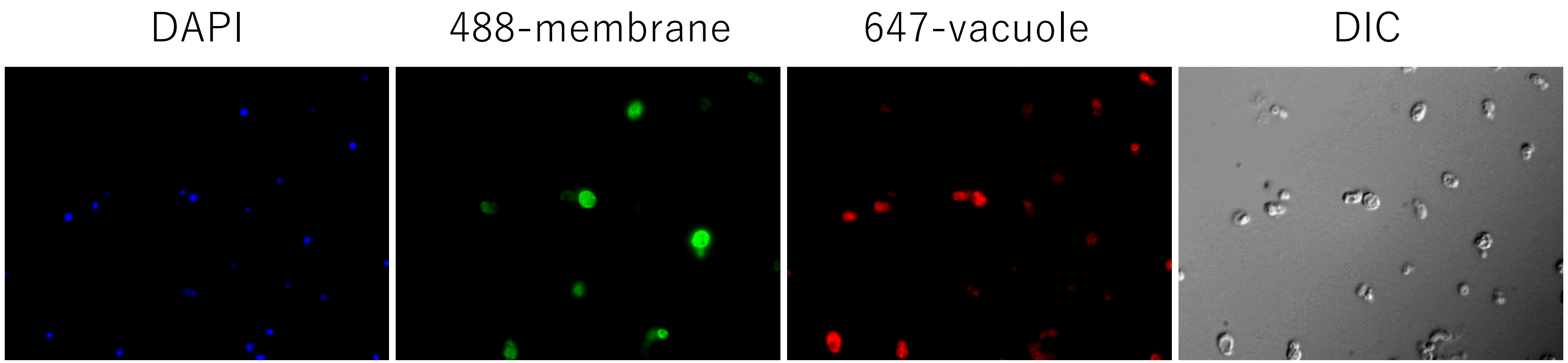
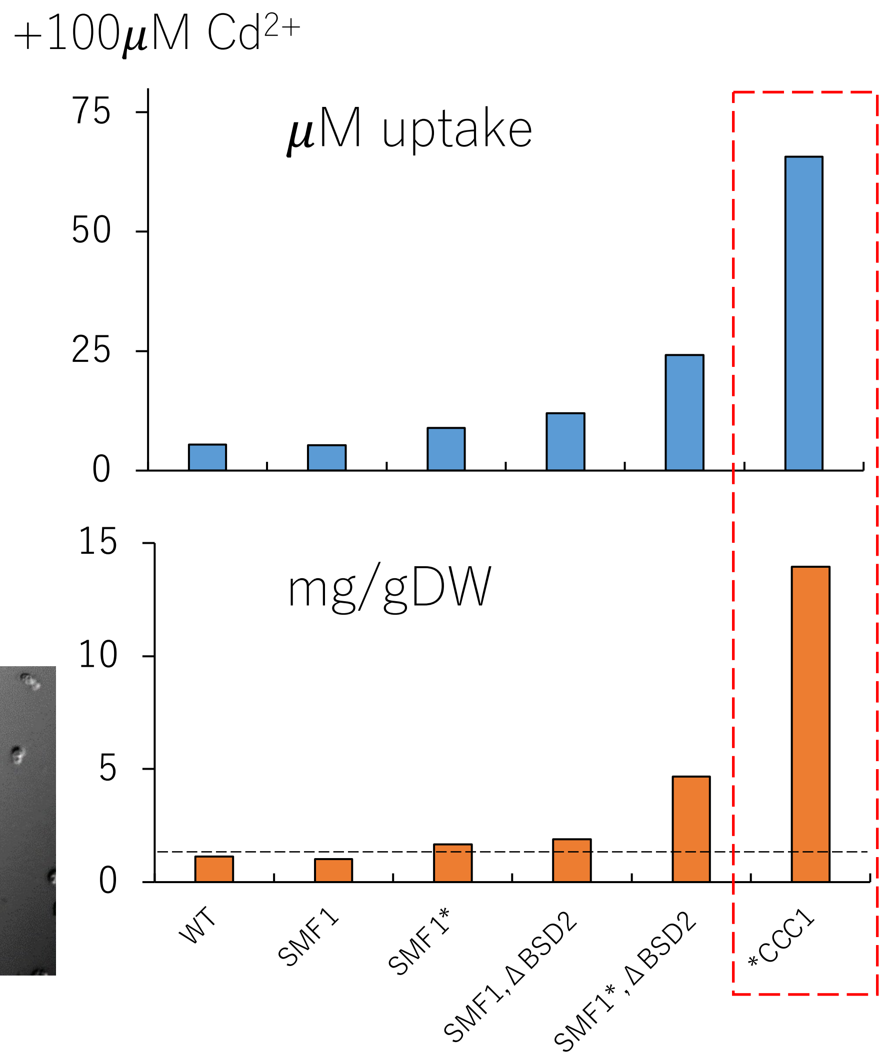
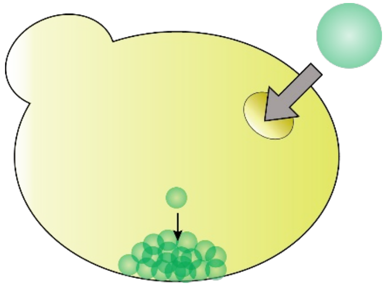






Strategy #2: Summary



Rationally engineer and screen metal transporters

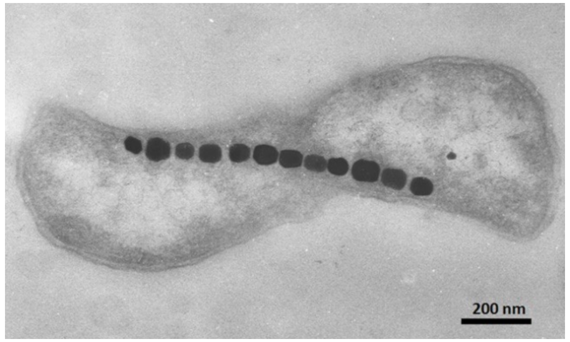
1. Engineer transporters for higher uptake efficiency and kinetics
2. Engineer transporters for greater specificity
3. Pilot studies of density gradient centrifugation to screen for enhanced metal transporters

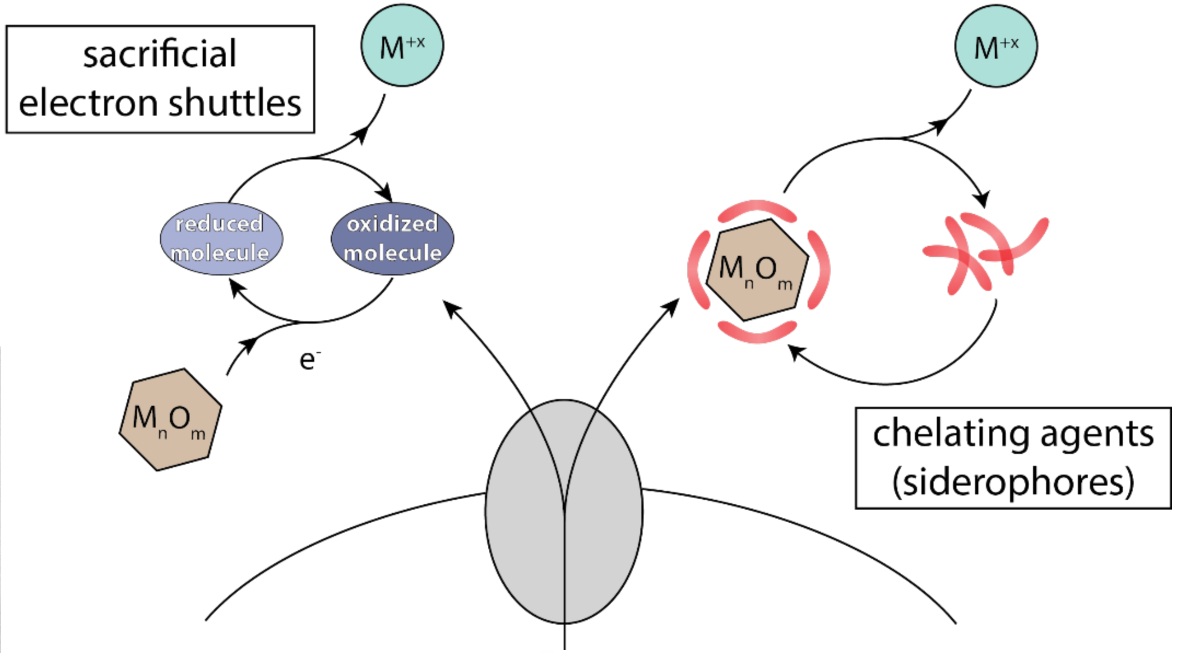
Control metal localization in the cell

1. Hijack glutathione and metallothionein pathway to sequester metals
2. Express metal vacuole transporters
3. Increase cell tolerance to metal uptake

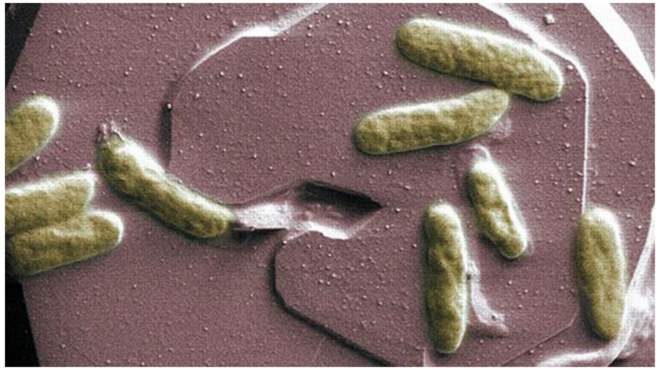
Strategy #3: *a.k.a.* precipitation

Magnetosomes





Metal eating microorganisms



Mineralized materials

Dr. George Sun

Small digression:
Nothing in life is free

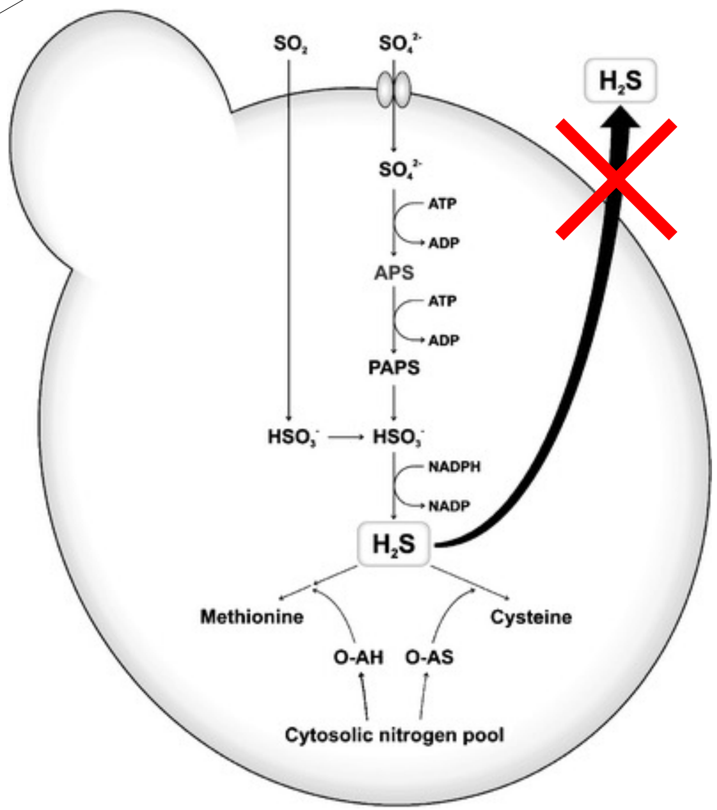
Metal Reduction

- Electrons (e^-) have to come from somewhere
- Donations of e^- have to overcome metals redox potential (ΔG^o ~usually high)

Chemical precipitation

- Metals need to react with something
- Reactive species that produce insoluble compounds (CO_3^{2-}, PO_4^{3-}, SO_4^{2-}) are not common
- Require changes in pH

Wine Industry



- Fermentation caused yeast to produce gaseous odors
- Primary culprit was sulfur compounds

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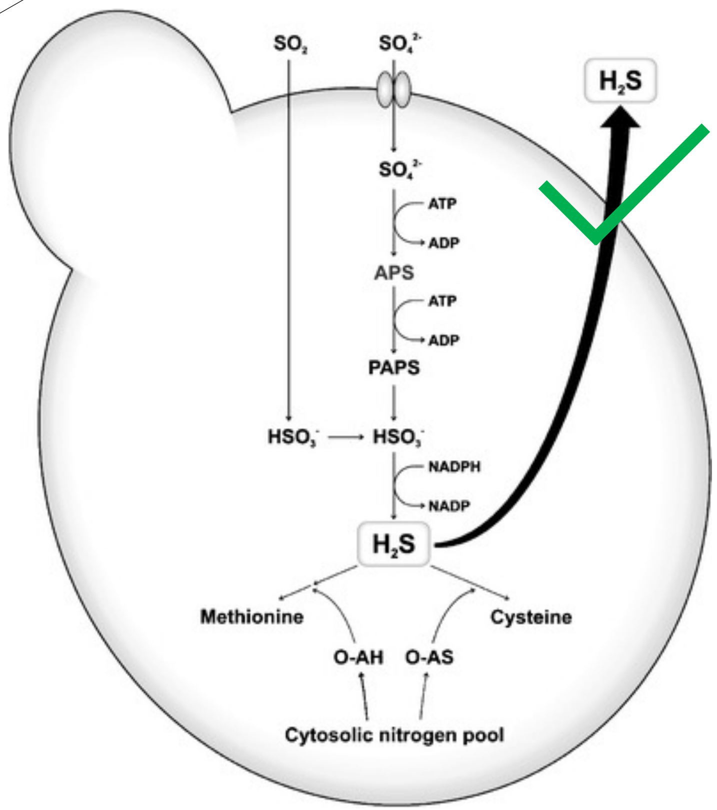
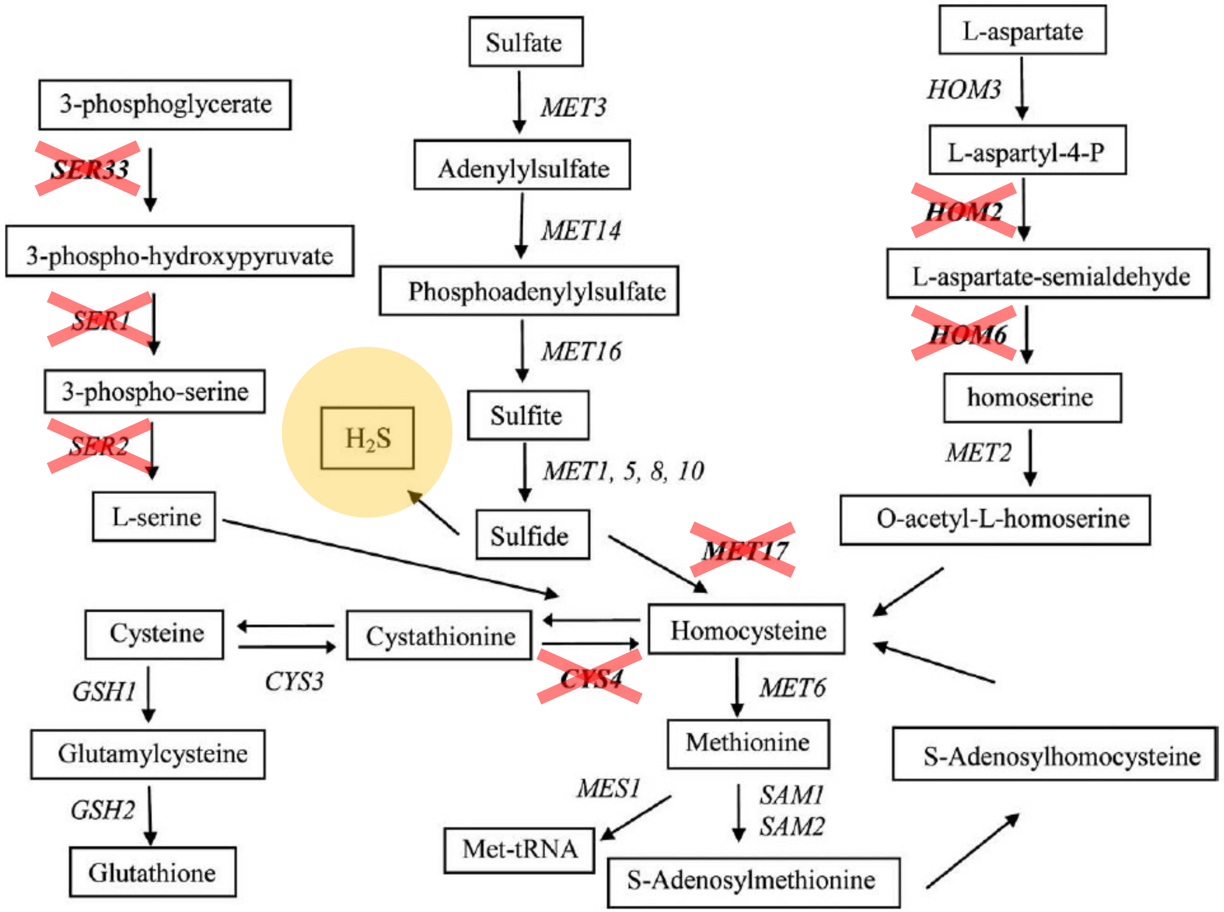


Metabolic engineering for H_2S production

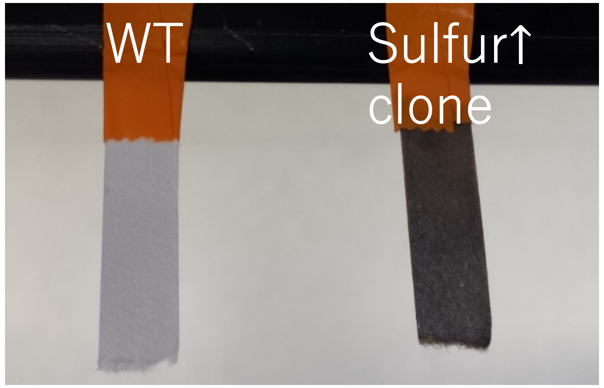
Engineering H_2S strains



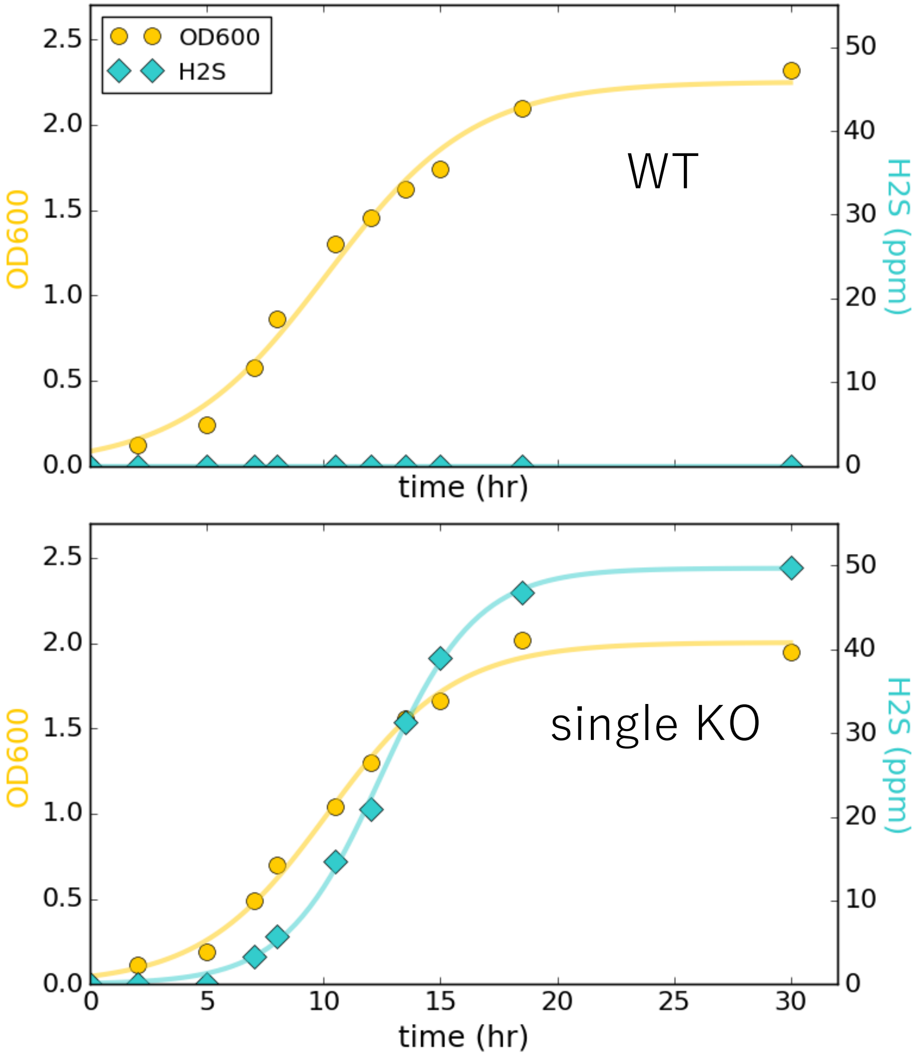
Linderholm, A. L., et. al. Identification of Genes Affecting Hydrogen Sulfide Formation in Saccharomyces cerevisiae. *Appl. Environ. Microbiol.* **74,** 1418–1427 (2008).

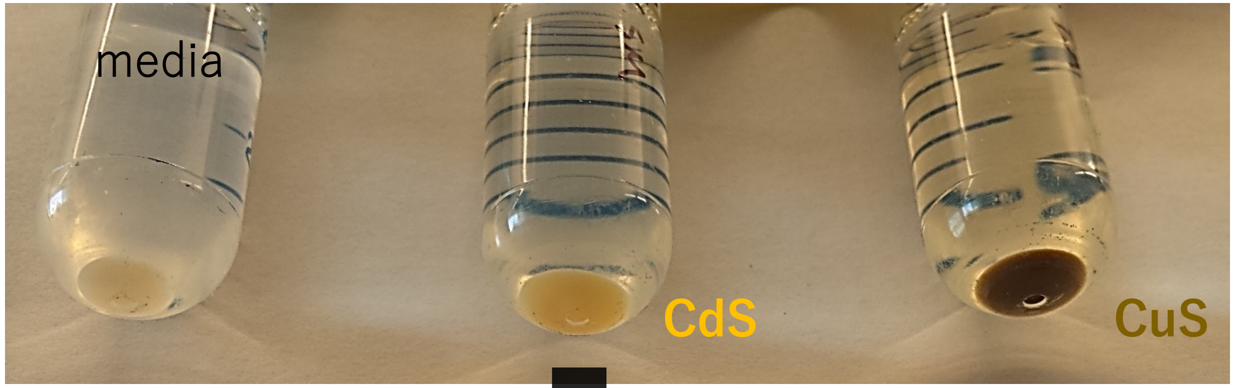
Engineering H_2S strains

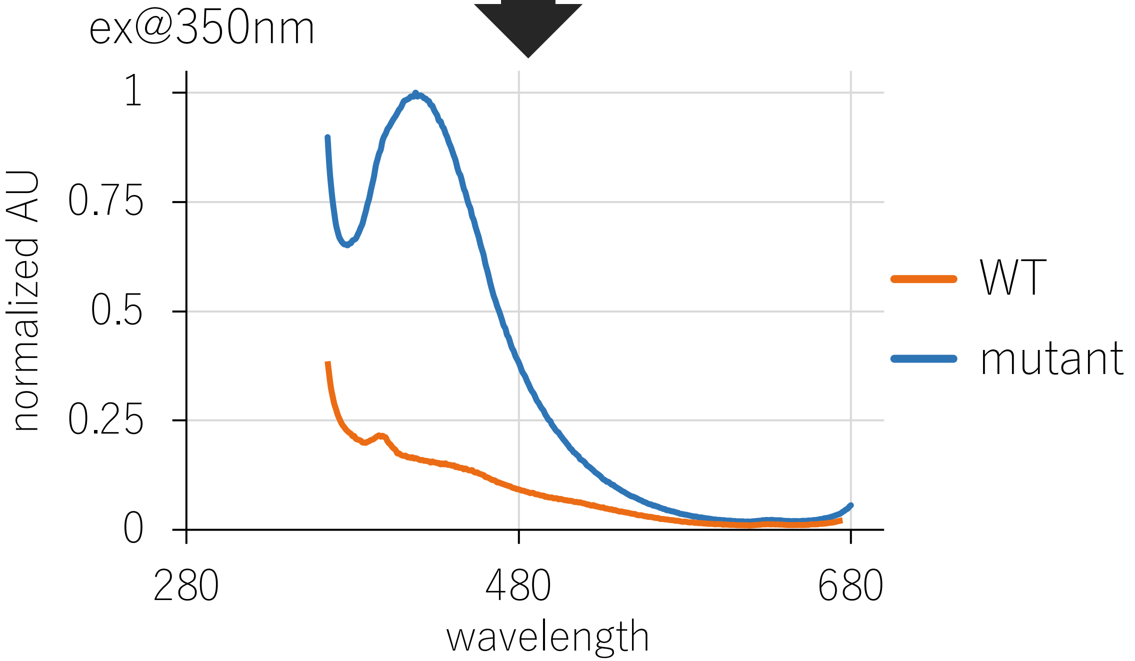
Lead acetate indicator strips



Effective removal of metals through sulfide precipitation

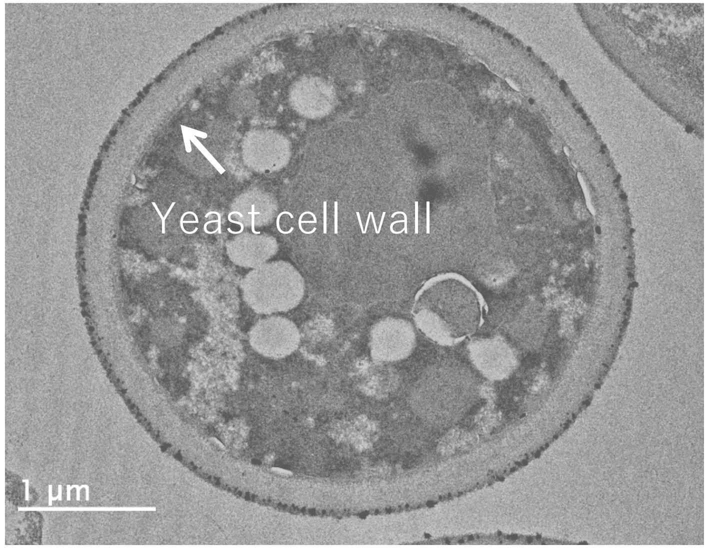


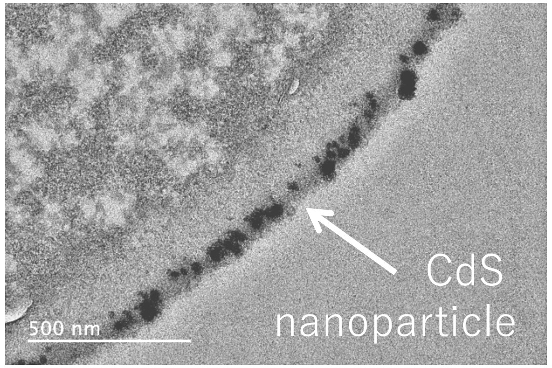


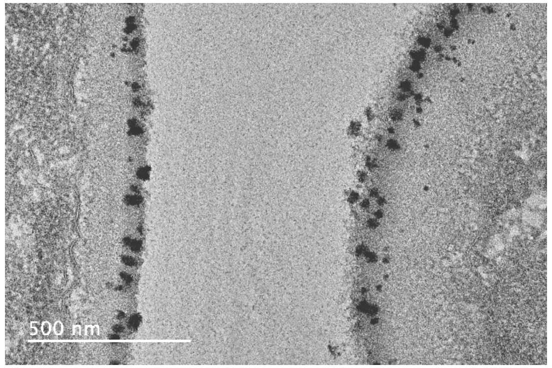


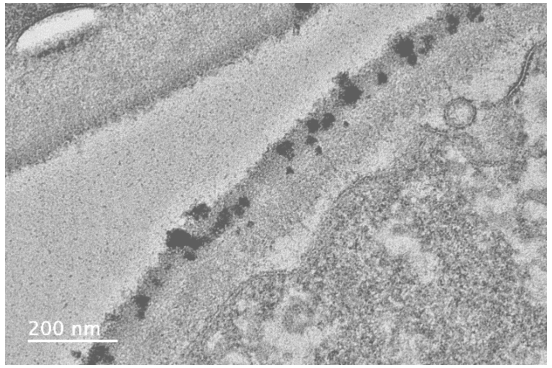
Taking a closer look: quantum dot synthesis

CdS precipitation

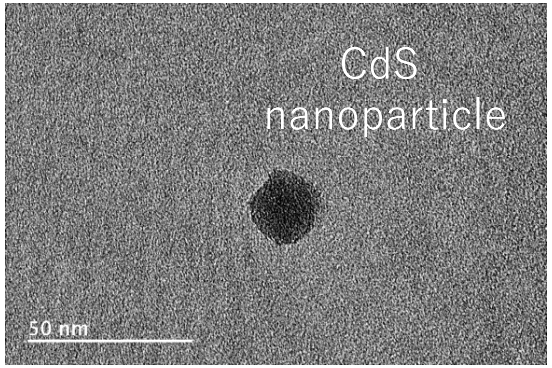


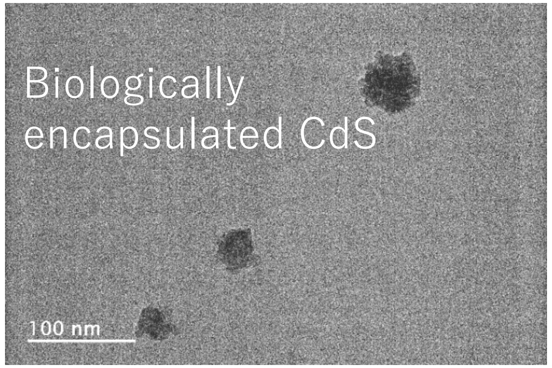






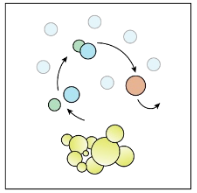
Purified CdS from yeast



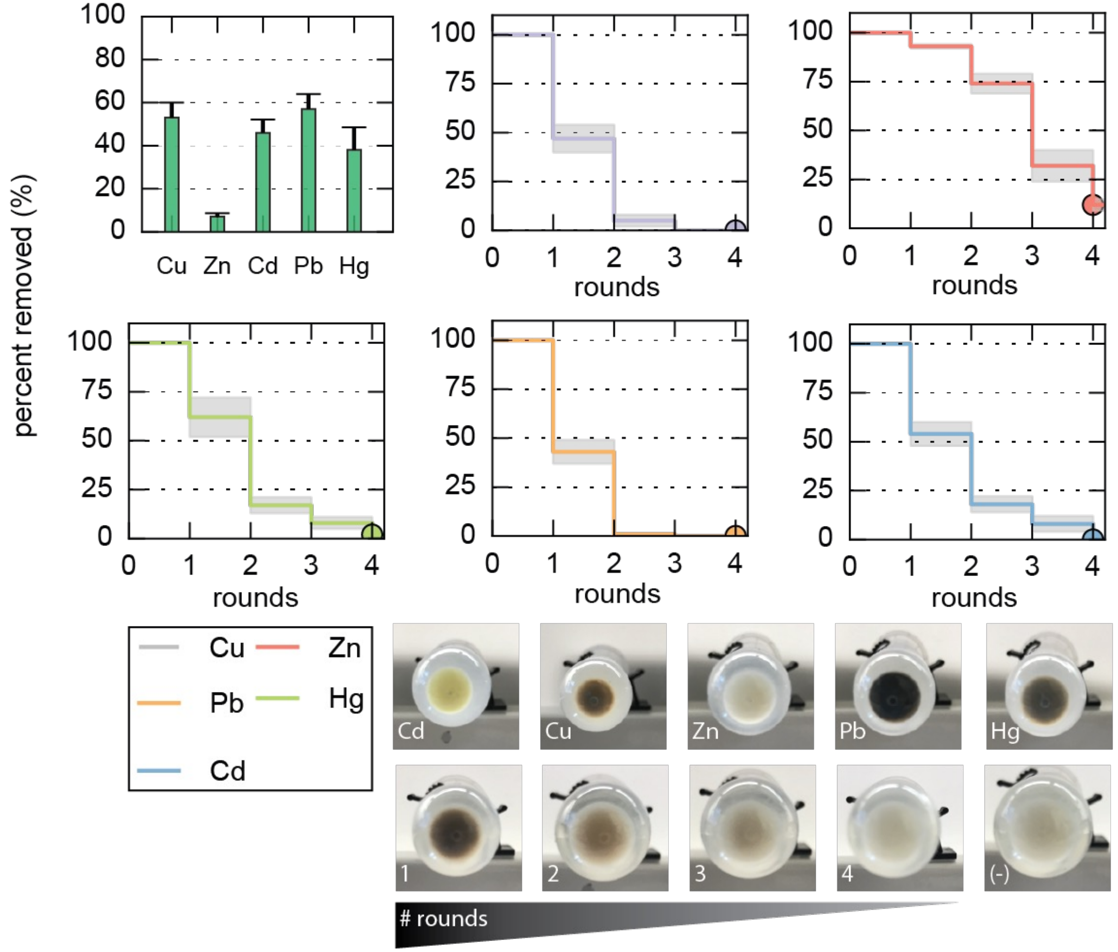


Alberta Canada Oil Sands

Bio-precipitation of heavy metals

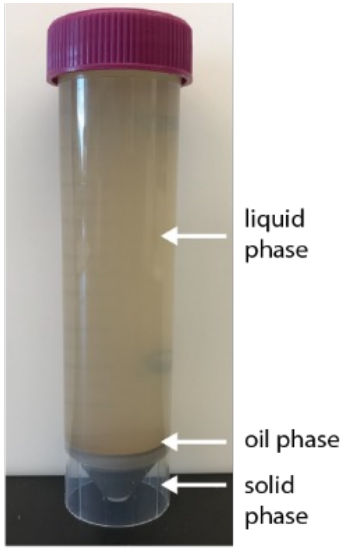


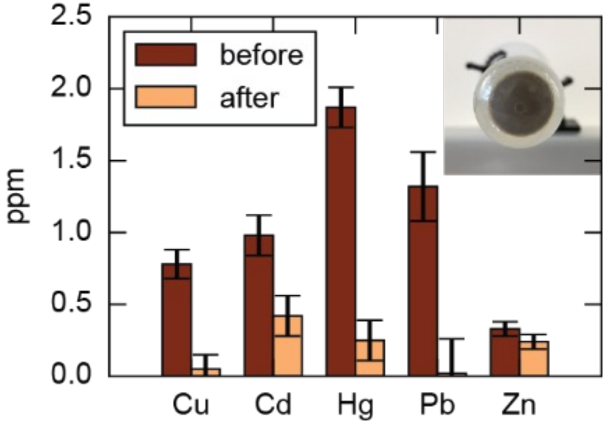
Metabolic engineering for heavy metal precipitation, *we can clean to EPA standards for Cu, Pb, Hg, Cd, Zn*



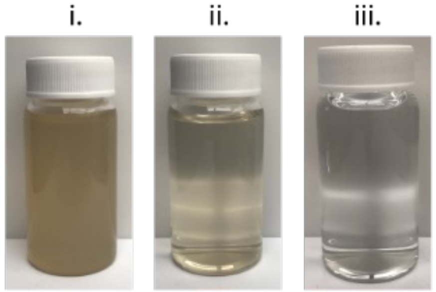
Nat Sustain **3,** 303–311 (2020)

Real world example:
Canadian Athabasca Oil Sands



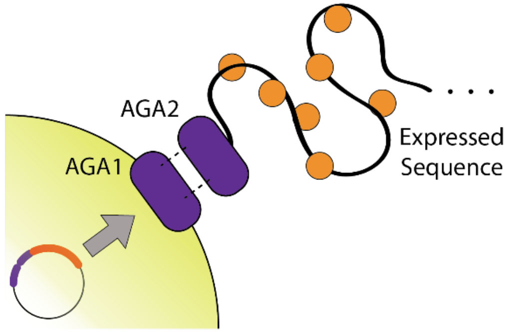


Before---middle--after



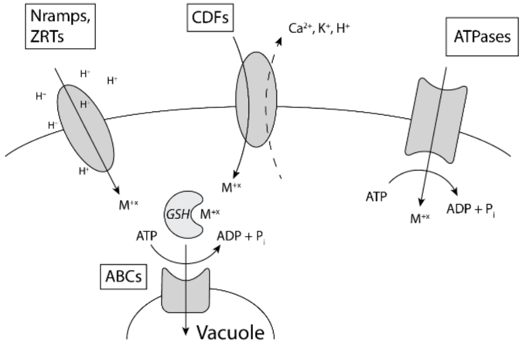
Yeast has proven to be a promising platform for metal remediation

Strategy #1: *a.k.a.* ion-exchange



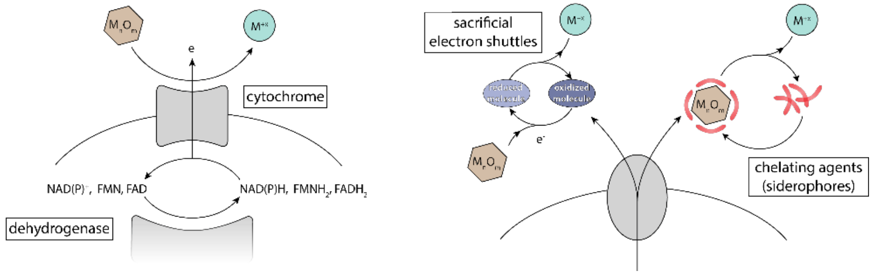
- Use "multiplier" proteins to enhance capture capacity
- Either use computational or experimental methods to create metal-specific capture motifs

Strategy #2: *a.k.a.* absorption



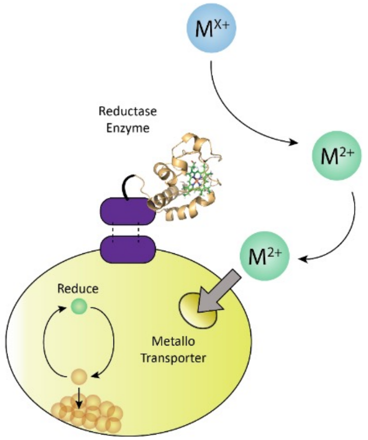
- Metal transporters are an effective method to sequester specific metals
- There exist multiple options to engineer enhanced meta hyperaccumulating in yeast

Strategy #3: *a.k.a.* precipitation



- Use byproducts to react or precipitate metal species
- Reacted compounds can be useful for other applications
- Use biomineralization peptides to facilitate the growth of precipitated metals

Combination Strategy

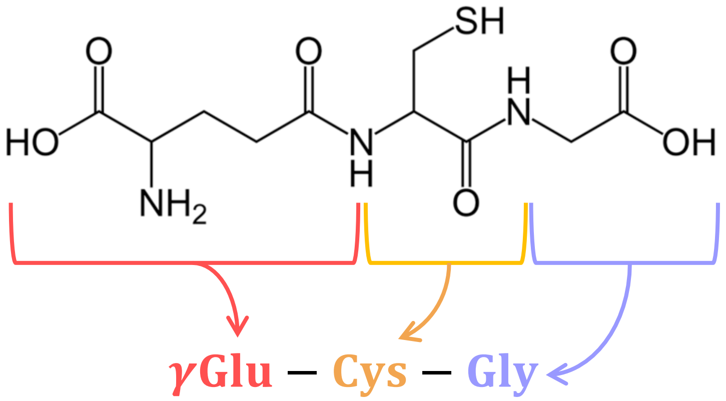


- Intelligently combine strategies 1—3 to create multi-purpose bioremediating yeast.
- Work with beer and wine industries to scale and package yeast for environmental applications

Survival

Using wheat phytochelatin synthases to enhance metal tolerance

Glutathione

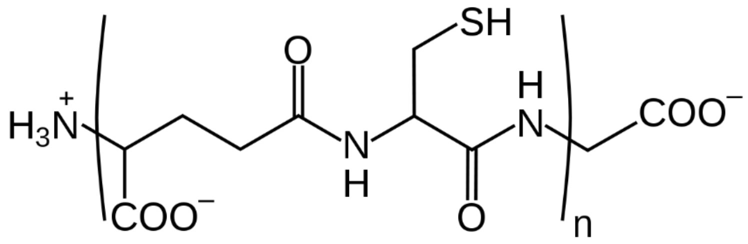


+

Wheat Proteins

=

Phytochelatin



Clemens, S., Kim, E. J., Neumann, D. & Schroeder, J. I. Tolerance to toxic metals by a gene family of phytochelatin synthases from plants and yeast. *The EMBO Journal* **18,** 3325–3333 (1999).

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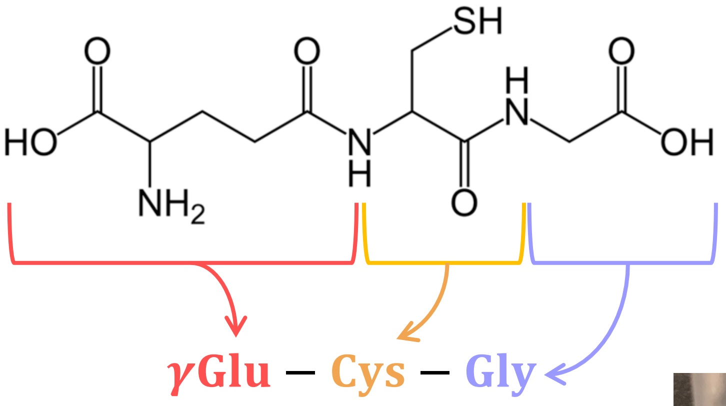
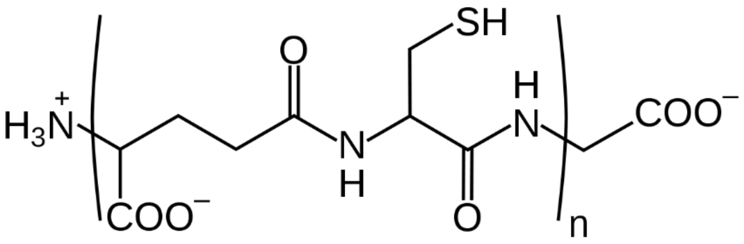
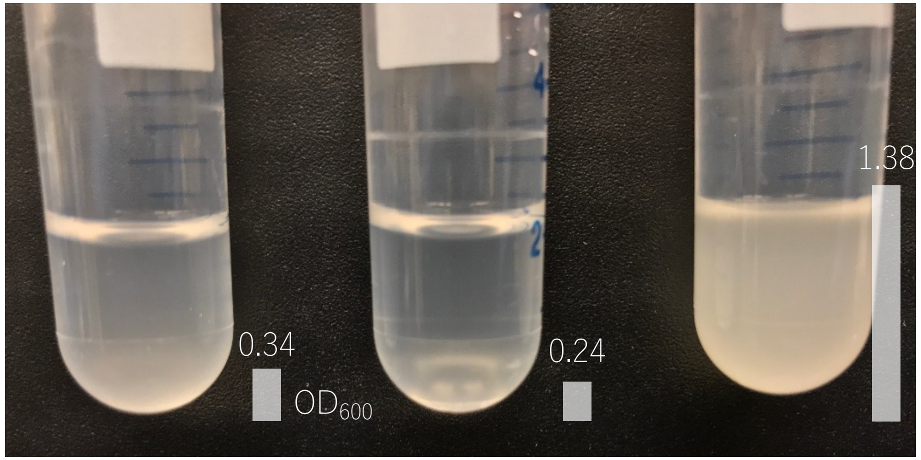


Whole Foods

Phytochelatin



WT un-induced induced



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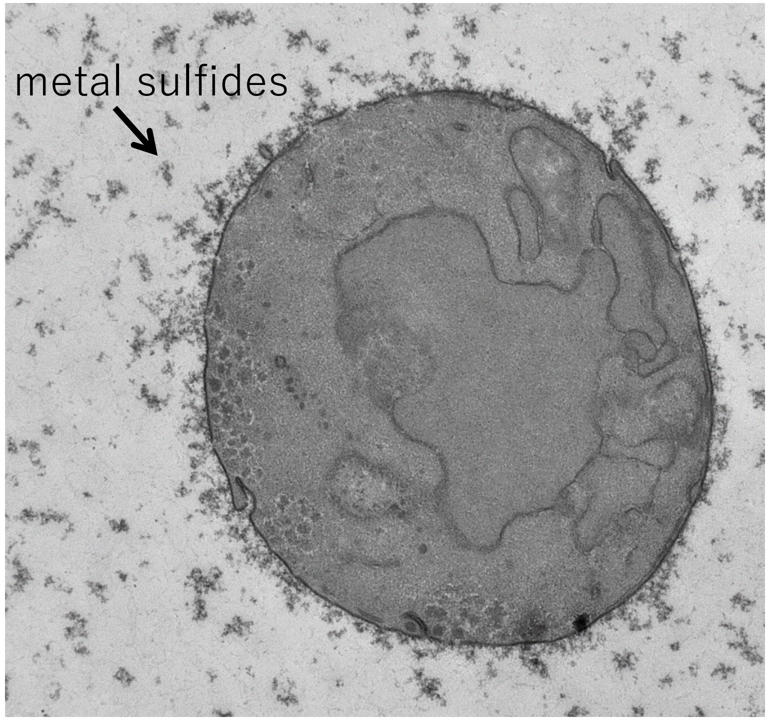
QD formation

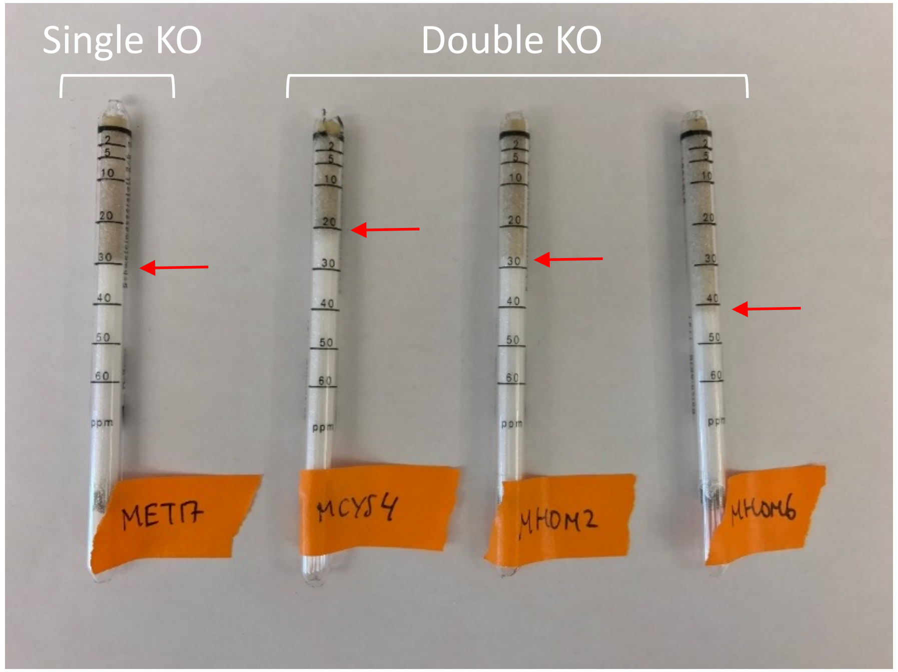
Understanding and controlling kinetics of sulfur production is critical for QD formation



Observations

- Rapid overproduction of quantum dots precipitates in solution
- Depending on the growth conditions, quantum dot formation and degrade the yeast cell wall

Knockout permutations confer a variety of H_2S production capabilities

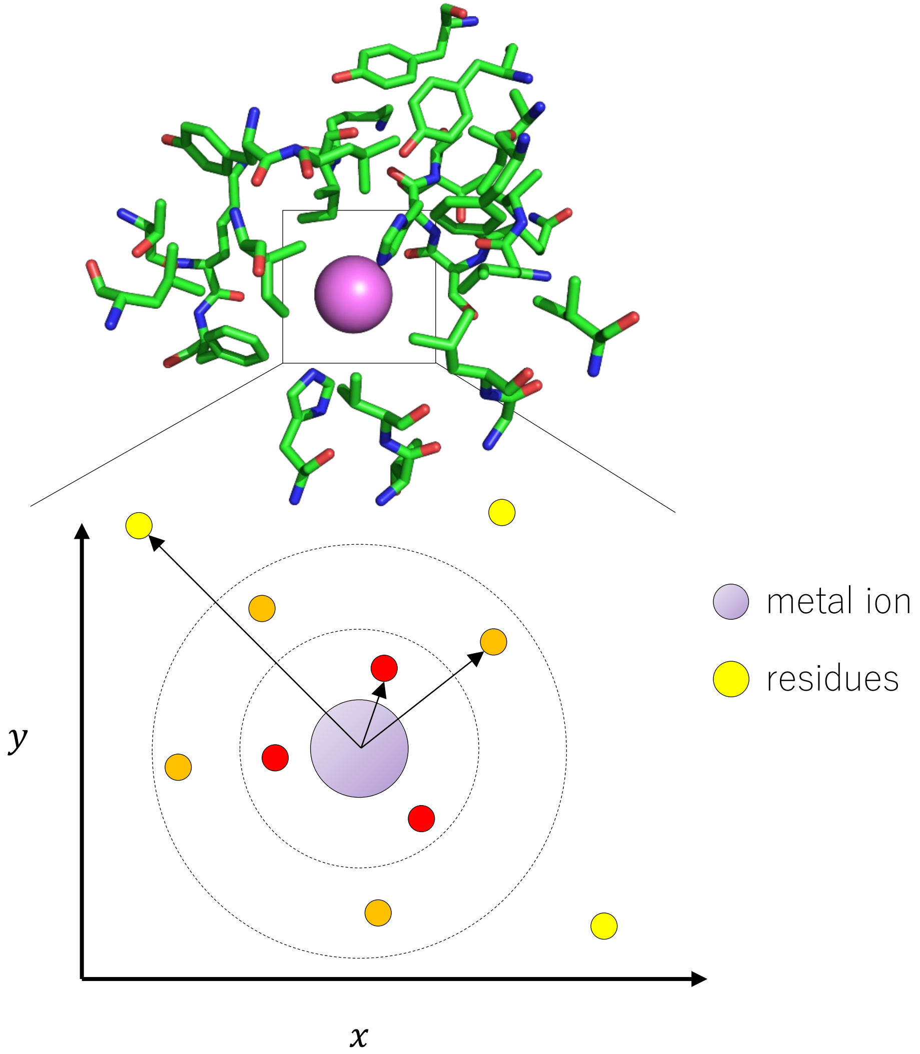


Machine learning is a tractable discovery strategy that can aid in screening experiments

Reasoning

- Relatively small search space. Finite sequences and combinations.
- “Solutions” already exist with experimentally discovered peptides.
- Large structural databases to learn from.
- Information will not be deterministic, but informative.

Structural Modeling



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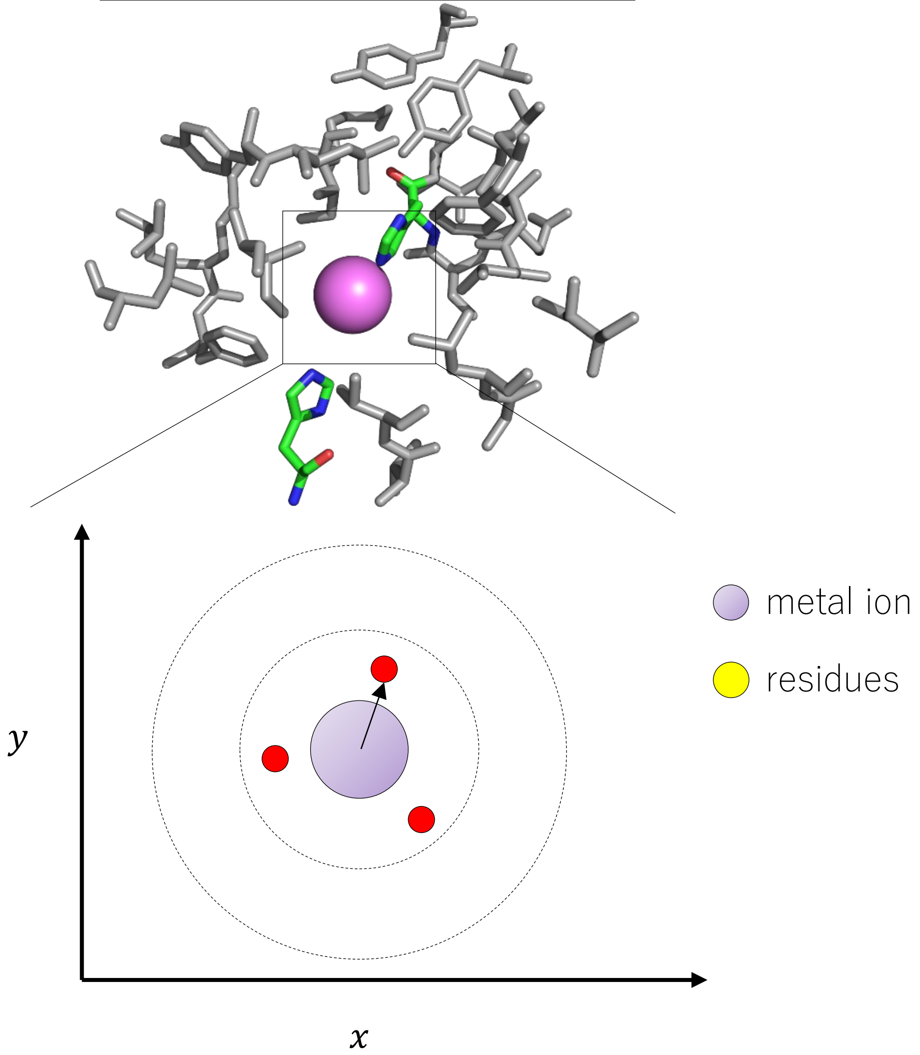
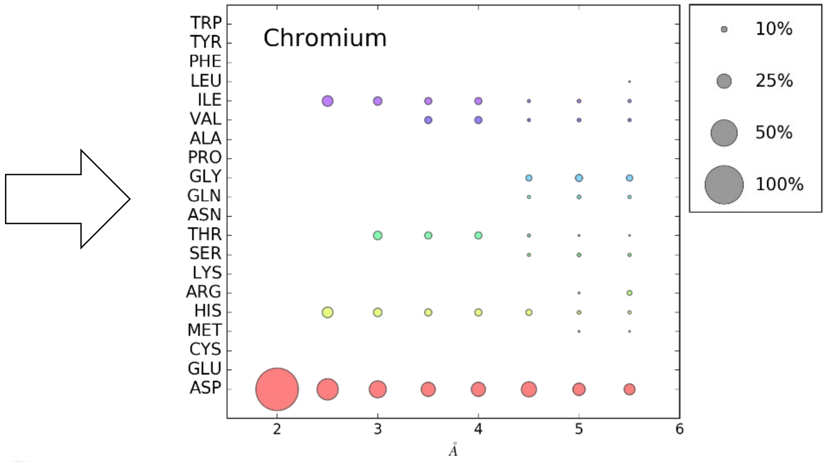


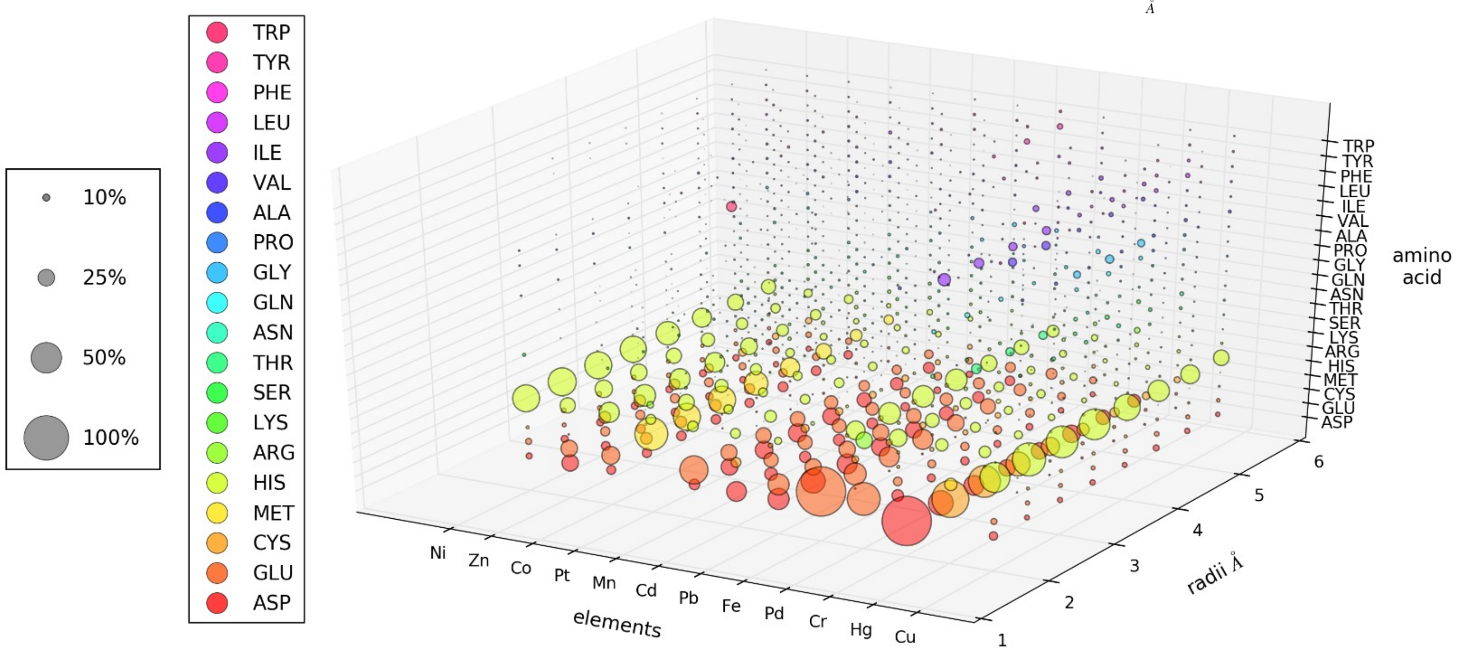
Machine Learning

Nearest neighbor (NN) search is an informative data extraction method

<u>Algorithm</u>

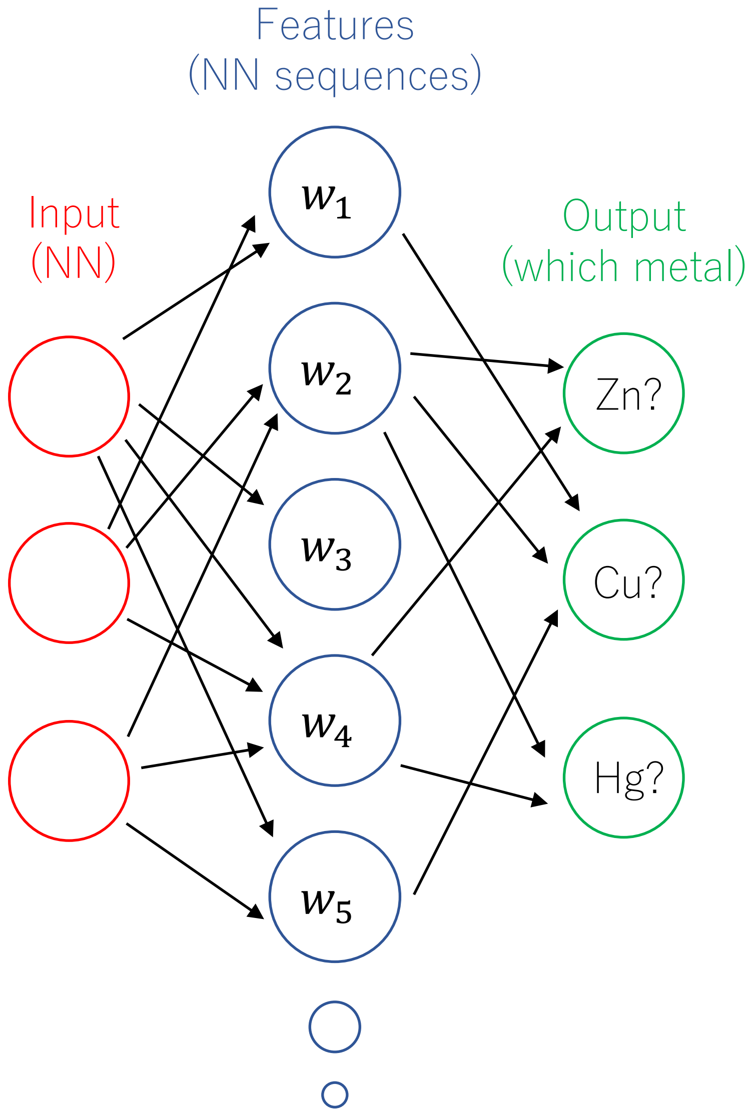
1) Search for metal center
2) Find nearest neighbors at given radii
3) Count residues
4) Repeat

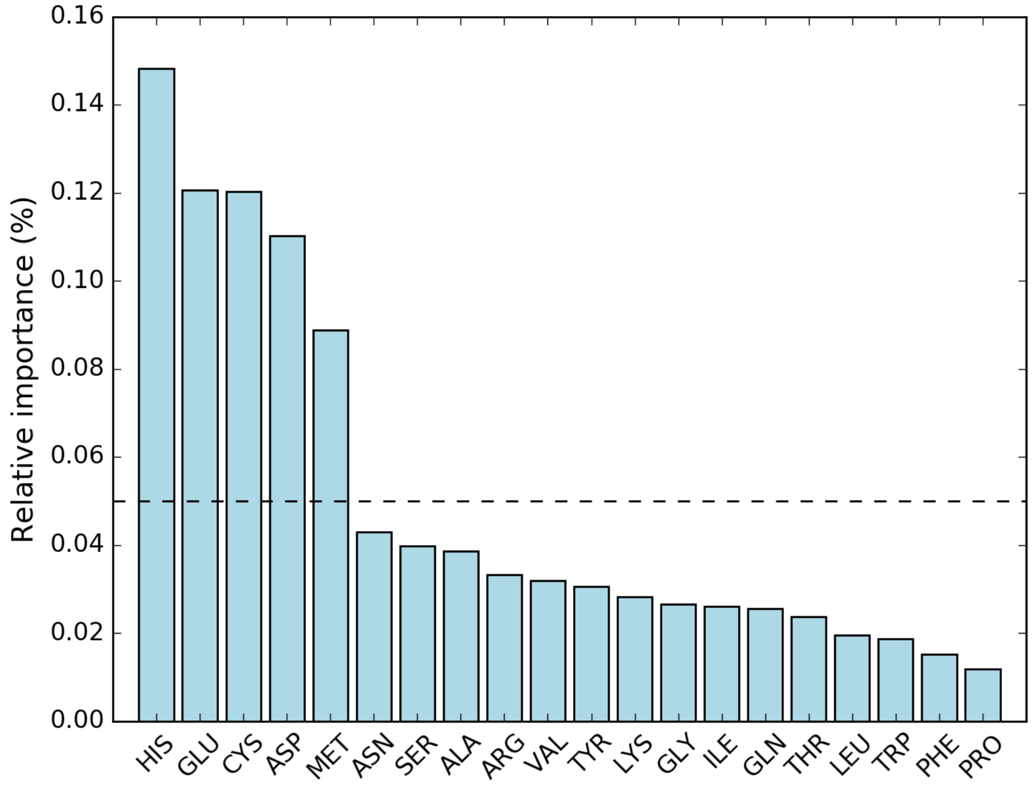




Machine Learning

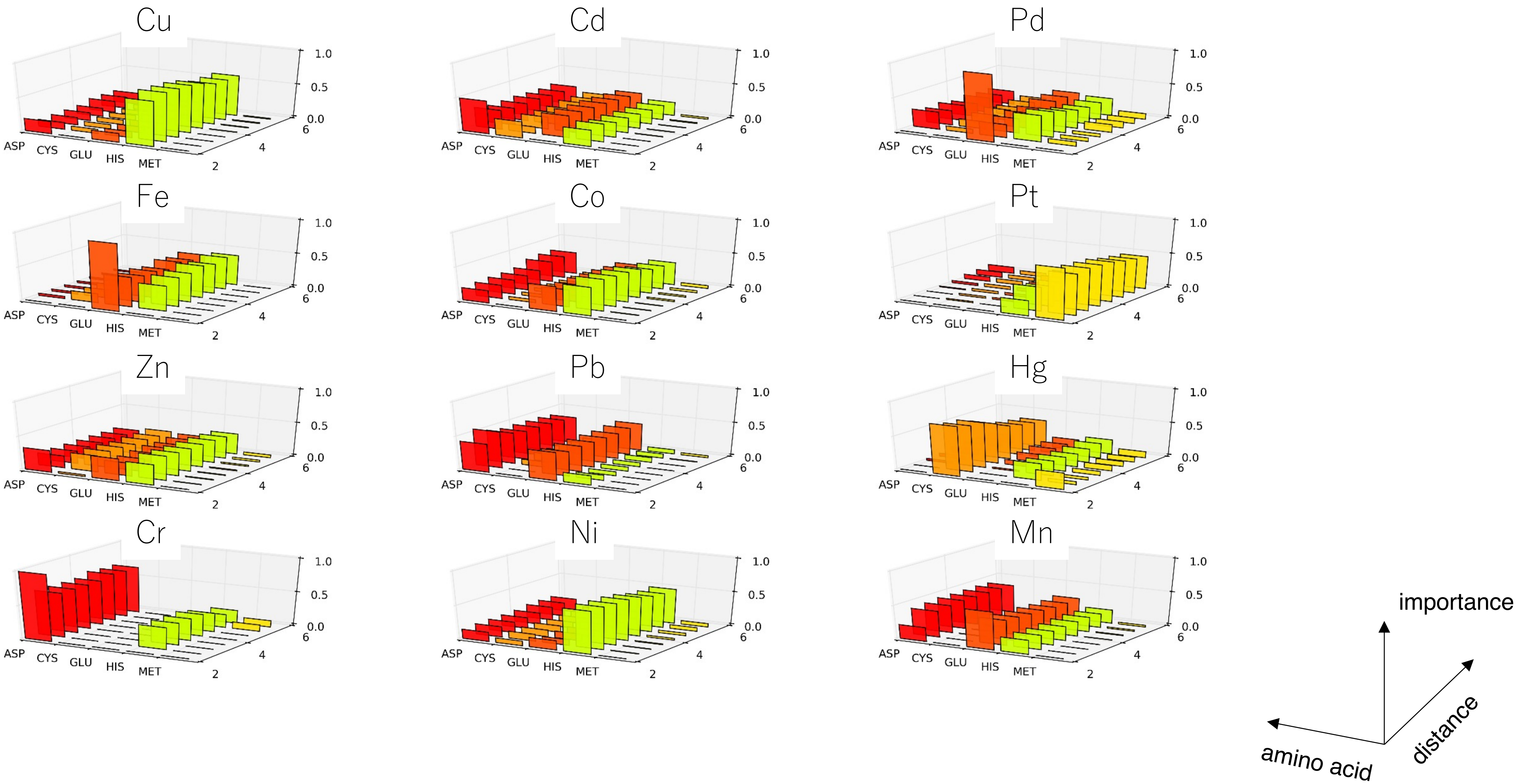
His, Glu, Cys, Asp, Met are important residues that confer metal binding





Machine Learning

Unique profiles per element exist, confirming the possibility of designing selective peptide binders



Machine Learning

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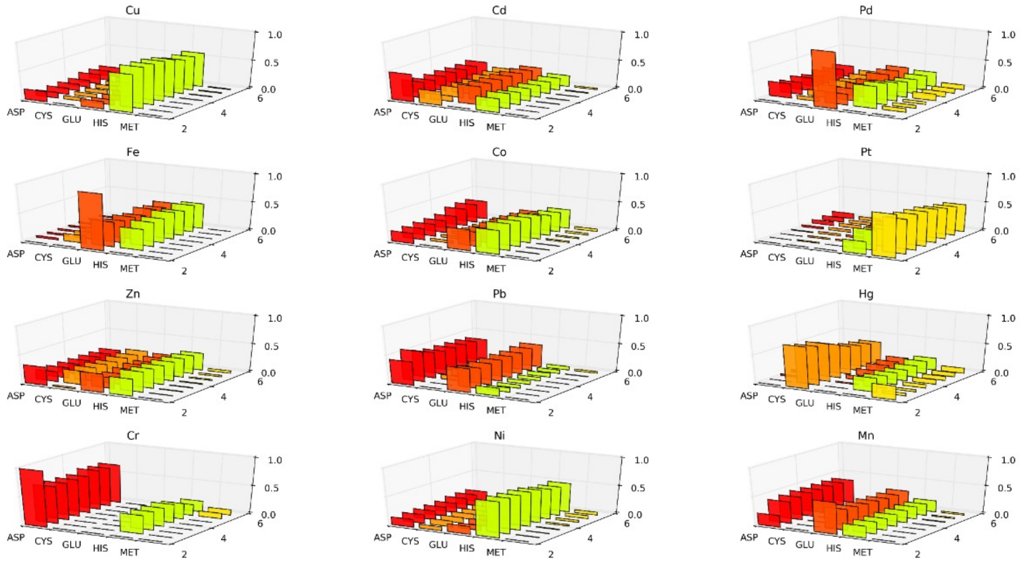
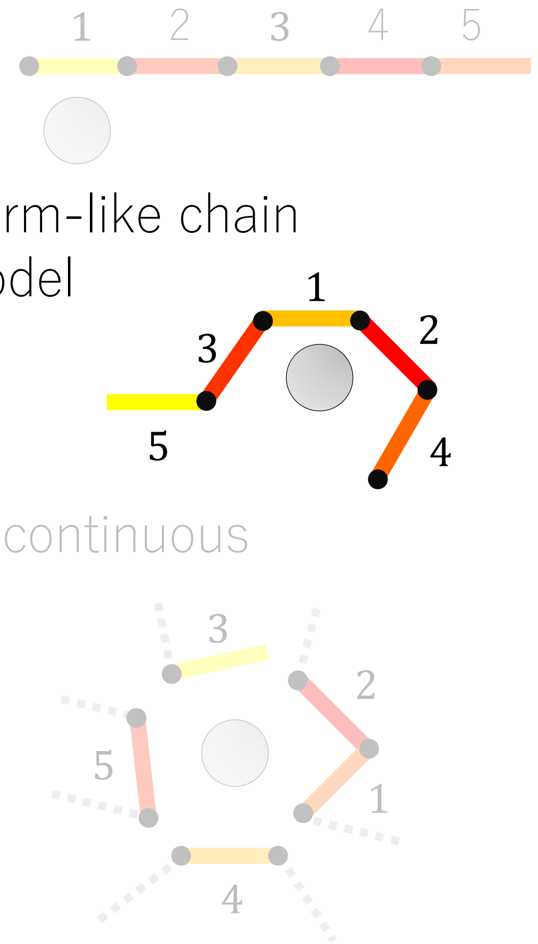
AA versus distance profiles → How to model distance



1) linear

2) worm-like chain model

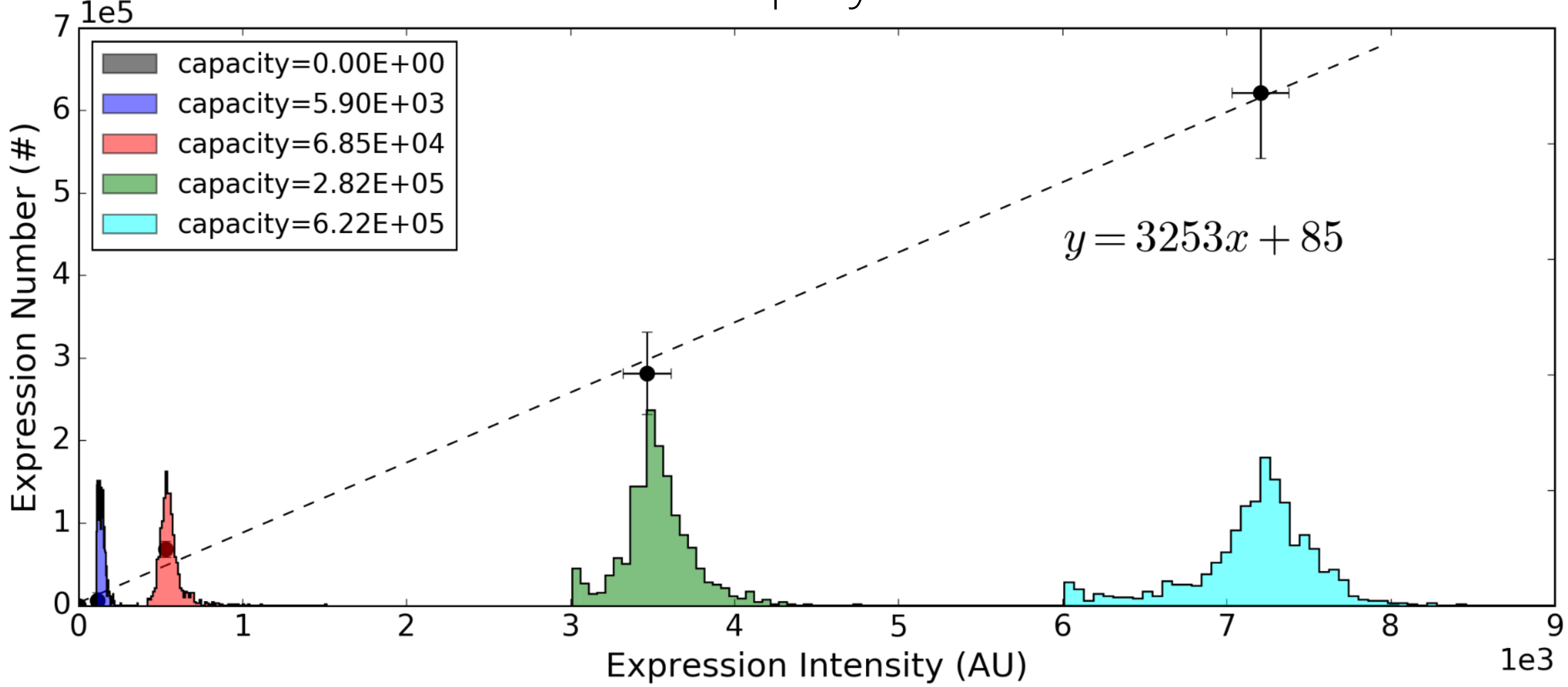
3) discontinuous



Yeast Display

Quantifying expression level using antibody coated calibration beads

Standard Curve
Yeast Display Levels



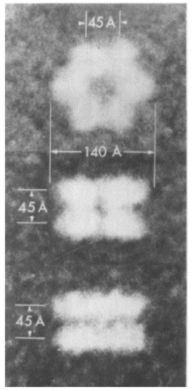
y = expression #
x = FACS intensity

Yeast Display Estimates

- Large proteins (>50 kD) = **10,000-50,000 #/cell**
- Small peptides (10-100 AA) = **50,000-500,000 #/cell**

MultiProt. Engineering “multiplier” proteins

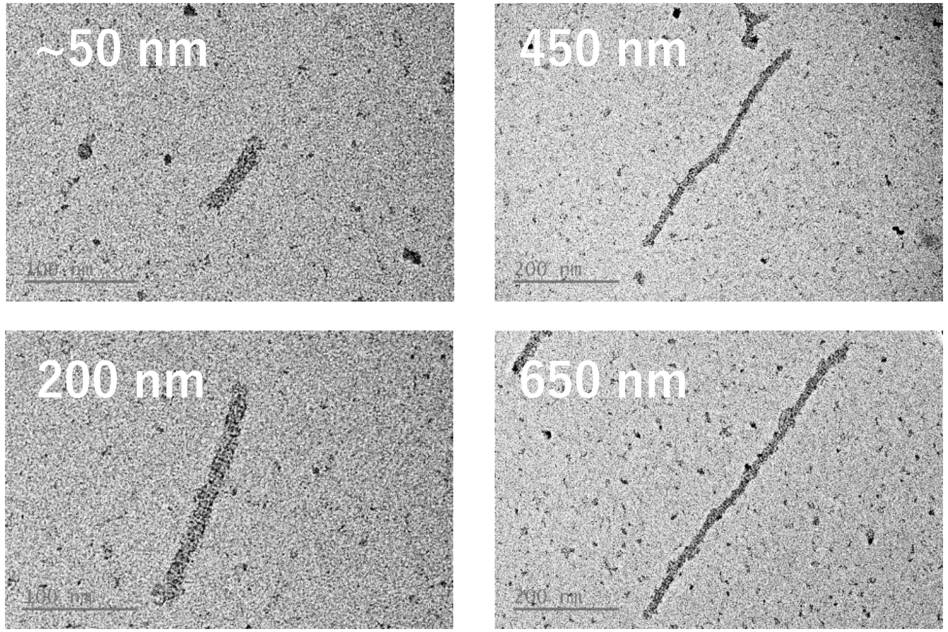
multiplier subunit



Dimension

Radius	~ 14 nm
Thickness	4.5 nm

Controlled oligomerization



- Metal induced aggregation
- Controlled growth using EDTA
- Mechanism not well understood