Engineering Yeast for Heavy Metal Waste Remediation

PhD Defense

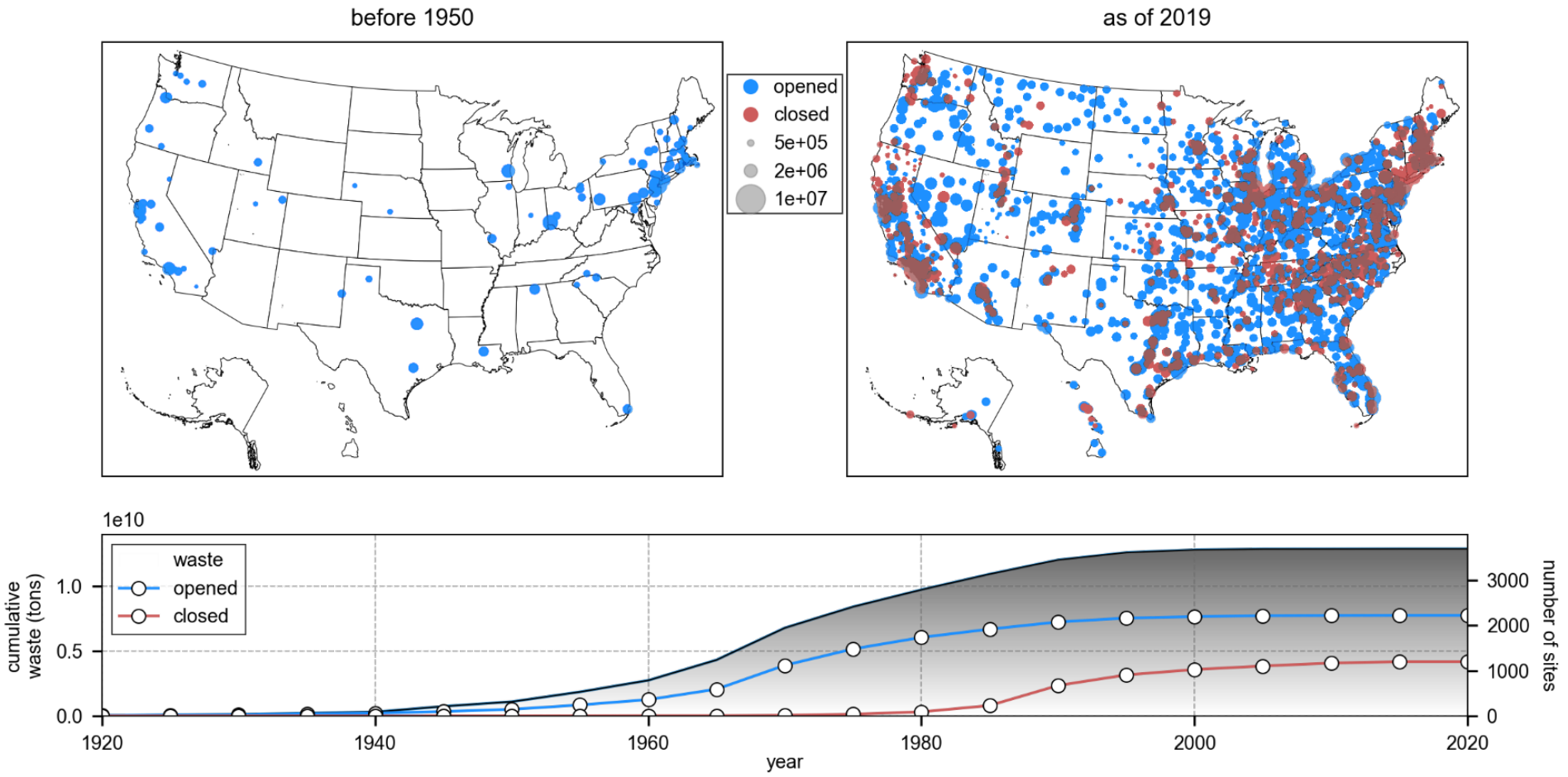
George Sun

June 5th, 2019

Talking about waste

Who cares?

Talking about waste – by numbers



Talking about waste – by numbers

3 billion pounds per year (1.5 million tons) (USA)

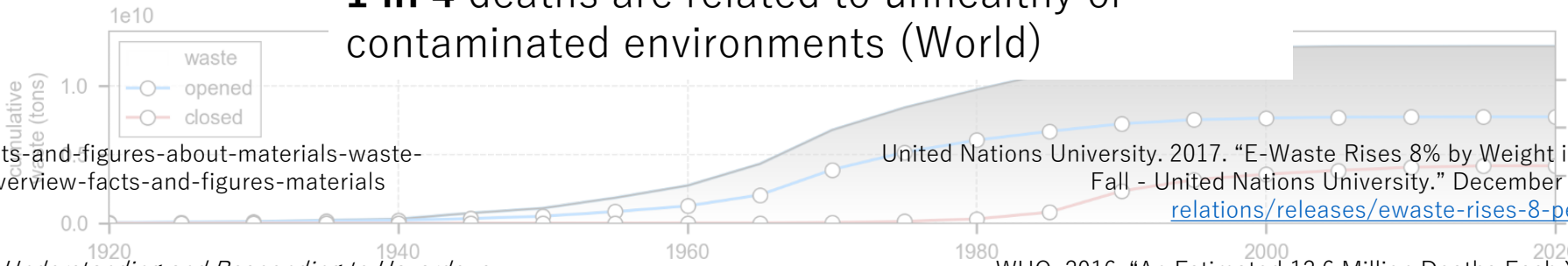
MSW: **4.48 pounds** (2 kg) per person per day (USA)

13,000 reported mining sites

500,000 are abandoned yet still polluting (USA)

Ewaste: **14 pounds** (6.35 kg) per person per year (World)

1 in 4 deaths are related to unhealthy or contaminated environments (World)



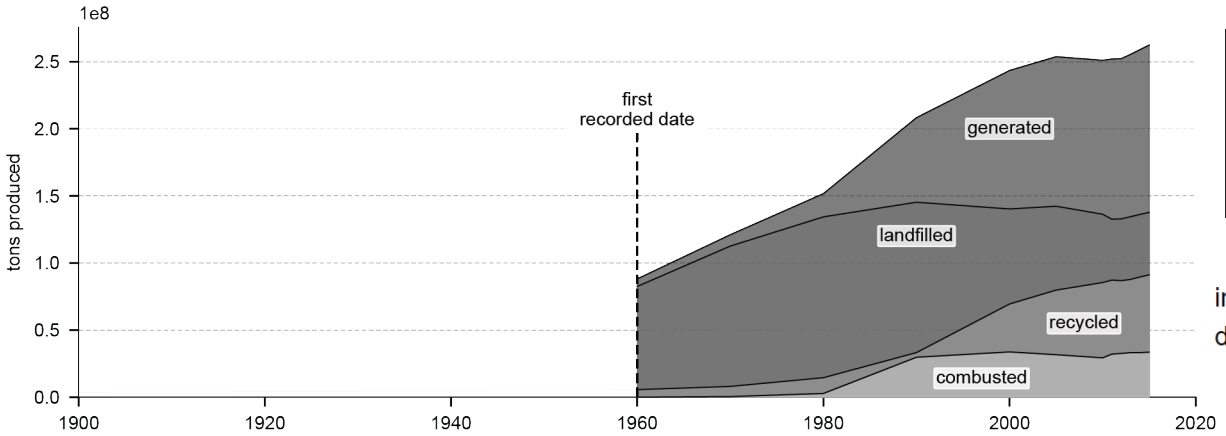
https://www.epa.gov/facts-and-figures-about-materials-waste-and-recycling/national-overview-facts-and-figures-materials

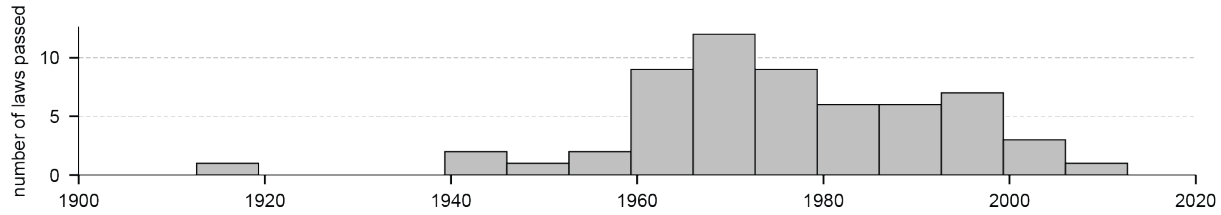
United Nations University. 2017. "E-Waste Rises 8% by Weight in 2 Years as Incomes Rise, Prices Fall - United Nations University." December 14, 2017. https://unu.edu/media-relations/releases/ewaste-rises-8-percent-by-weight-in-2-years.html.

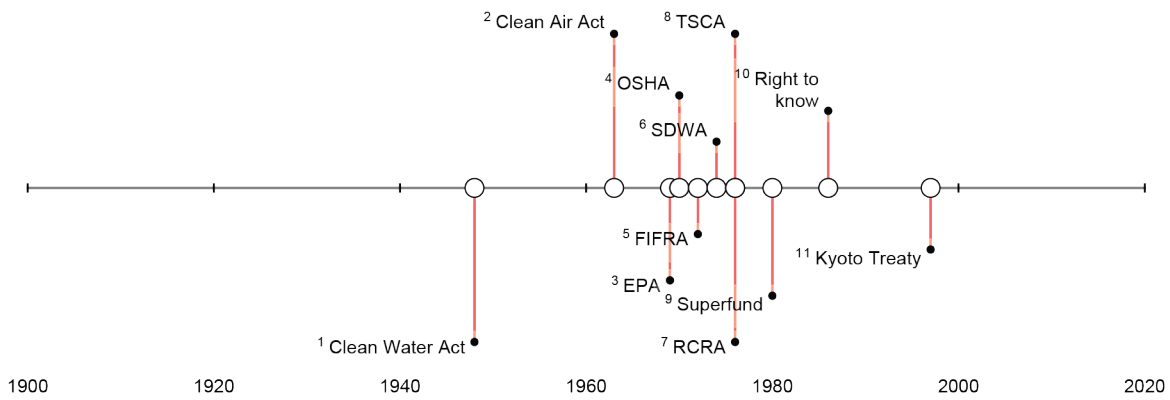
DeGraff, Jerome V. 2007. *Understanding and Responding to Hazardous Substances at Mine Sites in the Western United States*. Geological Society of America.

WHO. 2016. "An Estimated 12.6 Million Deaths Each Year Are Attributable to Unhealthy Environments," March. https://www.who.int/news-room/detail/15-03-2016-an-estimated-12-6-million-deaths-each-year-are-attributable-to-unhealthy-environments.

Talking about waste – by policy







American Environmental Policy since 1964

Richard N. L. Andrews

Subject: 20th Century: Post-1945, Political History, Environmental History Online Publication Date: Feb 2018

DOI: 10.1093/acrefore/9780199329175.013.408

Beginning in the 1980s, the long-standing Progressive ideal of the "public interest" was increasingly supplanted by a narrative of "government overreach," and the 1990s witnessed campaigns to delegitimize the underlying evidence justifying environmental policies by labeling it "junk science" or a "hoax."

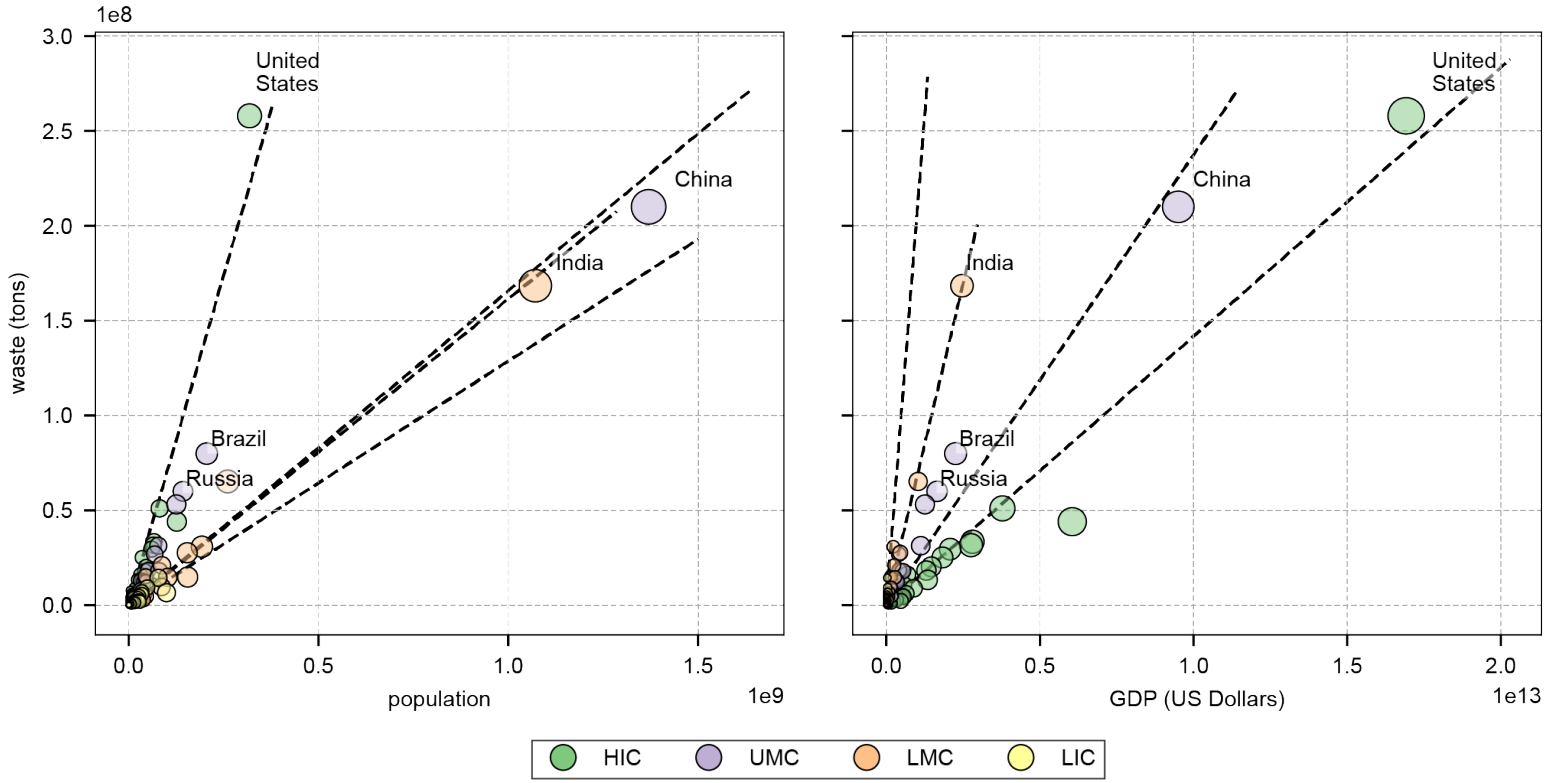
Does It Really Pay to Be Green? An Empirical Study of Firm Environmental and Financial Performance: An Empirical Study of Firm Environmental and Financial Performance

Andrew A. King, Michael J. Lenox

First published: 08 February 2008 | https://doi.org/10.1162/108819801753358526 | Cited by: 28

The relationship between underlying capabilities and environmnetal management is likely to be complex and contingent... It suggests that "When does it pay to be green?" may be a more important question than "Does it pay to be green?"

Talking about waste – using trends



HIC = high income country

(e.g. USA)

UMC = upper middle-income country (e.g. China)

LMC = lower middle-income country (e.g. India)

LIC = lower income country

(e.g. Zambia)

	tons per 1000 people	R^2
HIC	69.4	0.94
UMC	16.6	0.91
LMC	16.1	0.97
LIC	12.8	0.68

	tons per million $	R^2
HIC	14.2	0.96
UMC	23.7	0.96
LMC	67.8	0.99
LIC	206	0.59

http://faculty.ucr.edu/~jorgea/econ181/wdr_2008.pdf

An explanation of waste production under the lens of societal and cultural changes

American Sociological Association

The Historical Nature of Cities: A Study of Urbanization and Hazardous Waste Accumulation

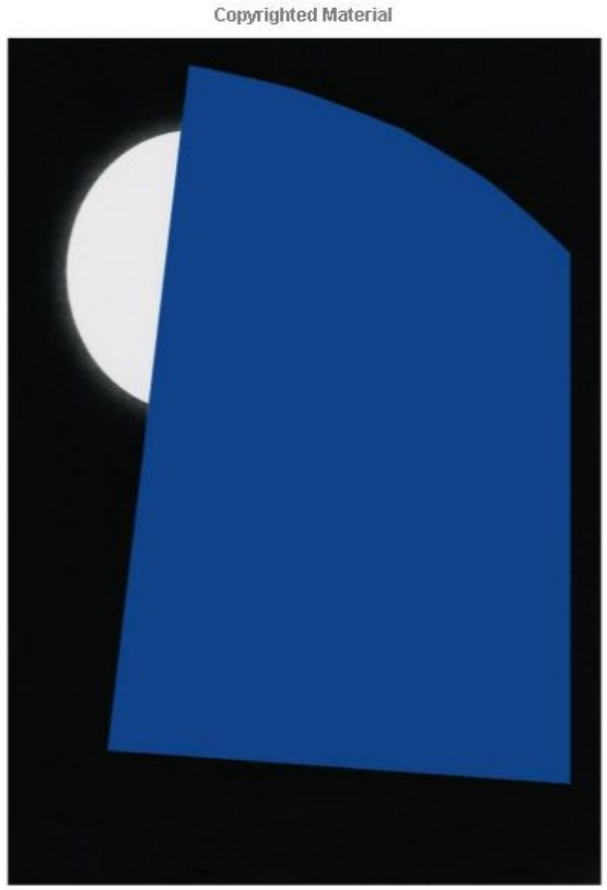
James R. Elliott[a] and Scott Frickel[b]

American Sociological Review
78(4) 521–543

DOI: 10.1177/0003122413493285
http://asr.sagepub.com
SAGE

Major conclusions

- "Out of site, out of mind"
- "Someone else will do it"



"…a society increasingly preoccupied with the future (and also with safety), which generates the notion of risk"

"…risk society; that is, a condition wherein the growth of knowledge has everywhere created 'manufactured uncertainty' (for example the risk of ecological disaster), so that scientific expertise is increasingly called upon to alleviate the effects of earlier applications of science."

We have trash in space

Who cares?

BBC NEWS

https://www.bbc.com/news/av/science-environment-38091707/time-to-take-out-the-space-trash

Who should care?

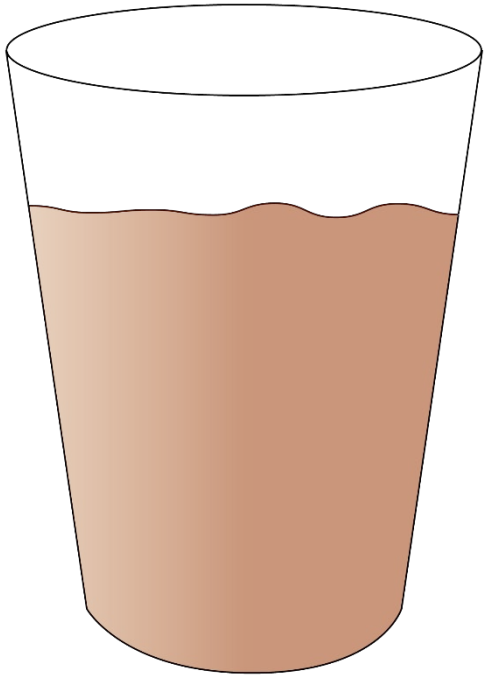
the engineers and scientist

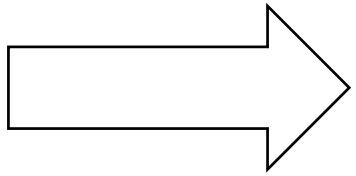
Who should care?

more people should try

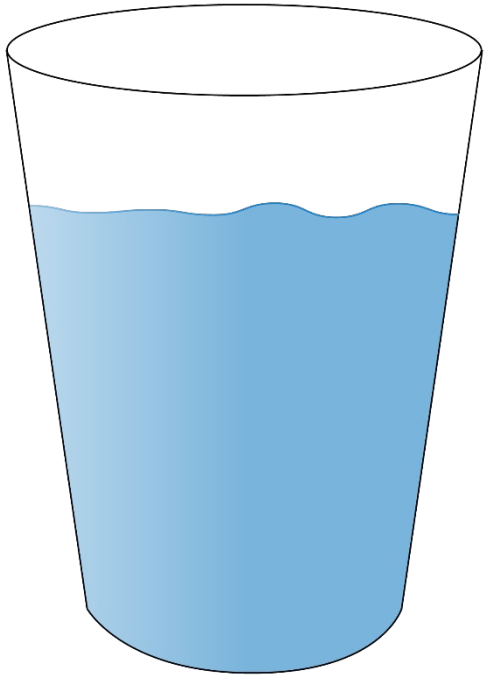
The goal – clean water

Dirty



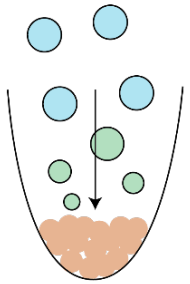


Clean



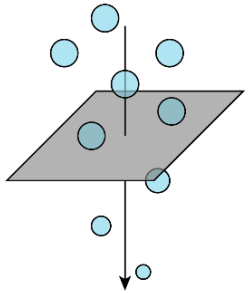
Current remediation strategies rely on physicochemical methods

Chemical Precipitation



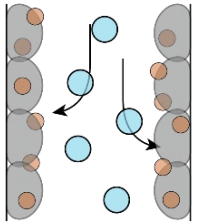
React

Absorption



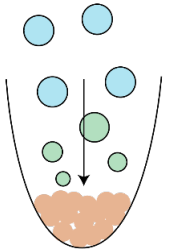
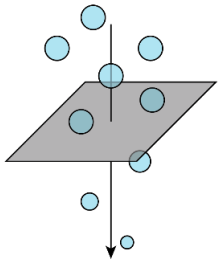
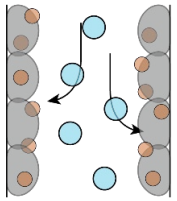
Absorb

Ion-exchange



Bind

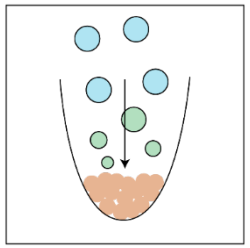
Cost, specificity, and technology accessibility are limitations for adoption

		specificity	re-usability	cost to make	cost to operate
Chemical Precipitation		LOW	NO	LOW	HIGH
Absorption		LOW	NO	VARIES	VARIES
Ion-exchange		VARIES	YES	HIGH	HIGH

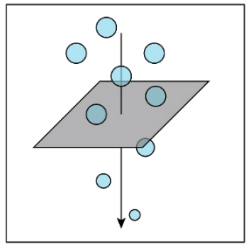
Designing biological analogies to physicochemical processes

Synthetic

Chemical precipitation



Absorption



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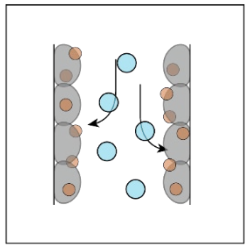


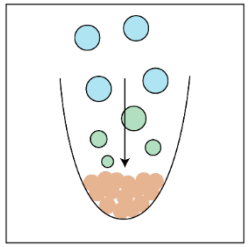
Biological

- Sustainable
- Scalable
- Autonomous
- Genetically encoded

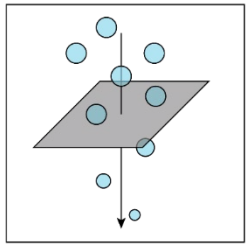
Designing biological analogies to physicochemical processes

Synthetic

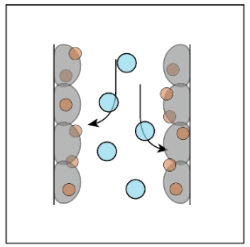
Chemical precipitation



Absorption

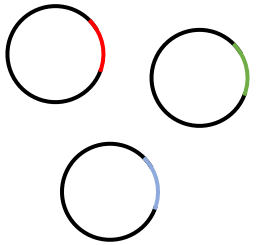


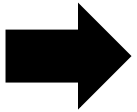
Ion-exchange



Biological

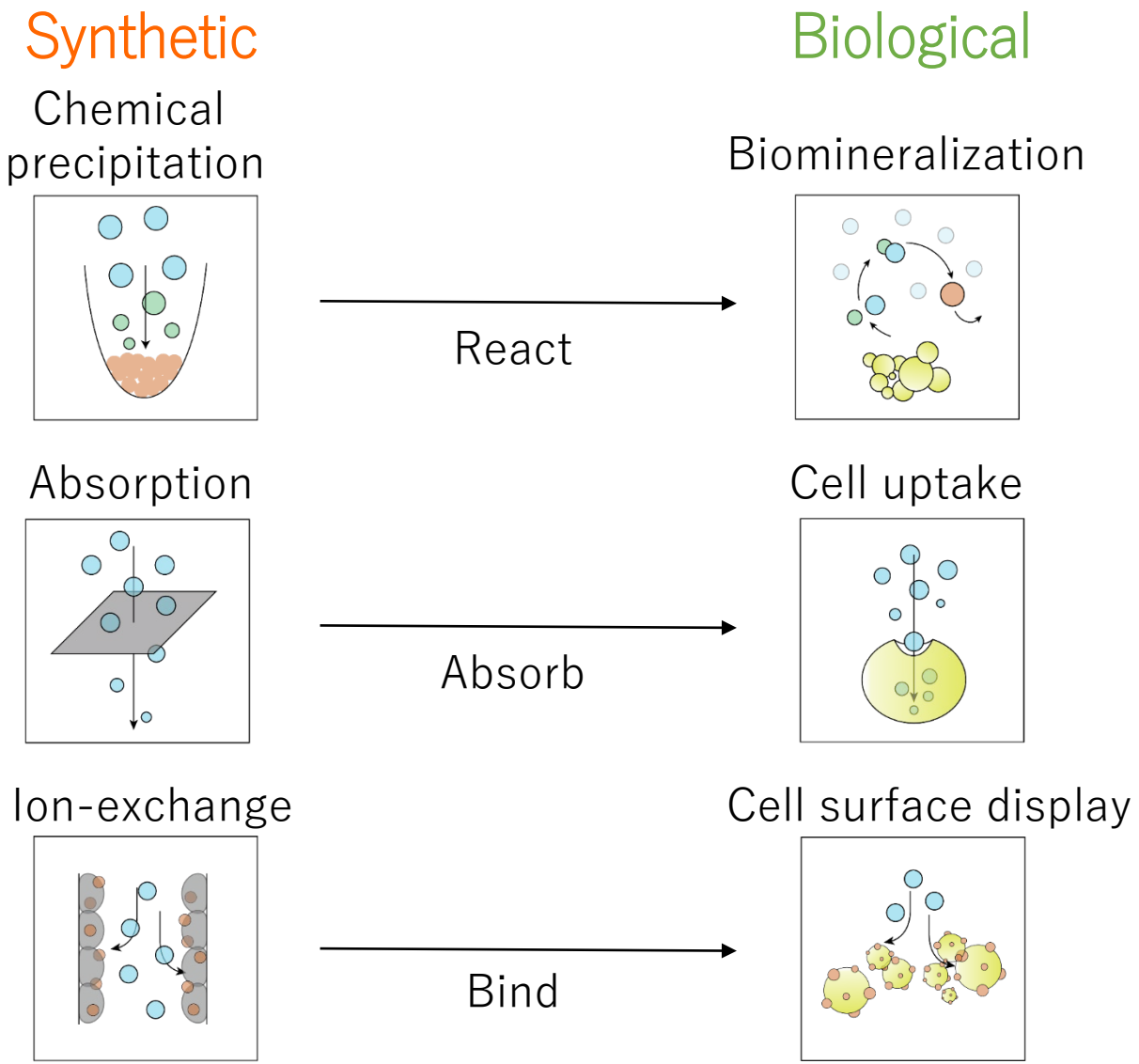
DNA



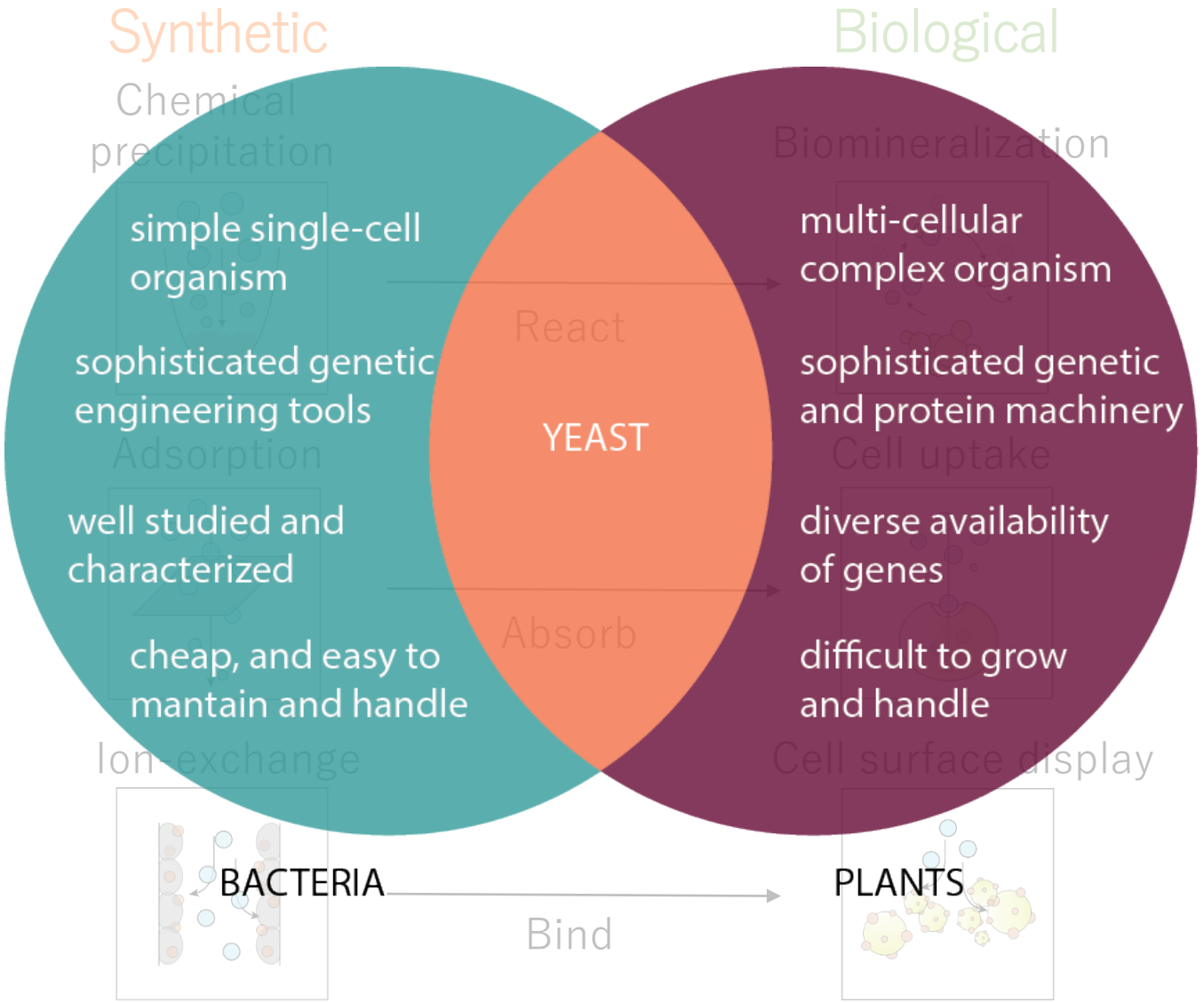


FUNCTION

Designing biological analogies to physicochemical processes

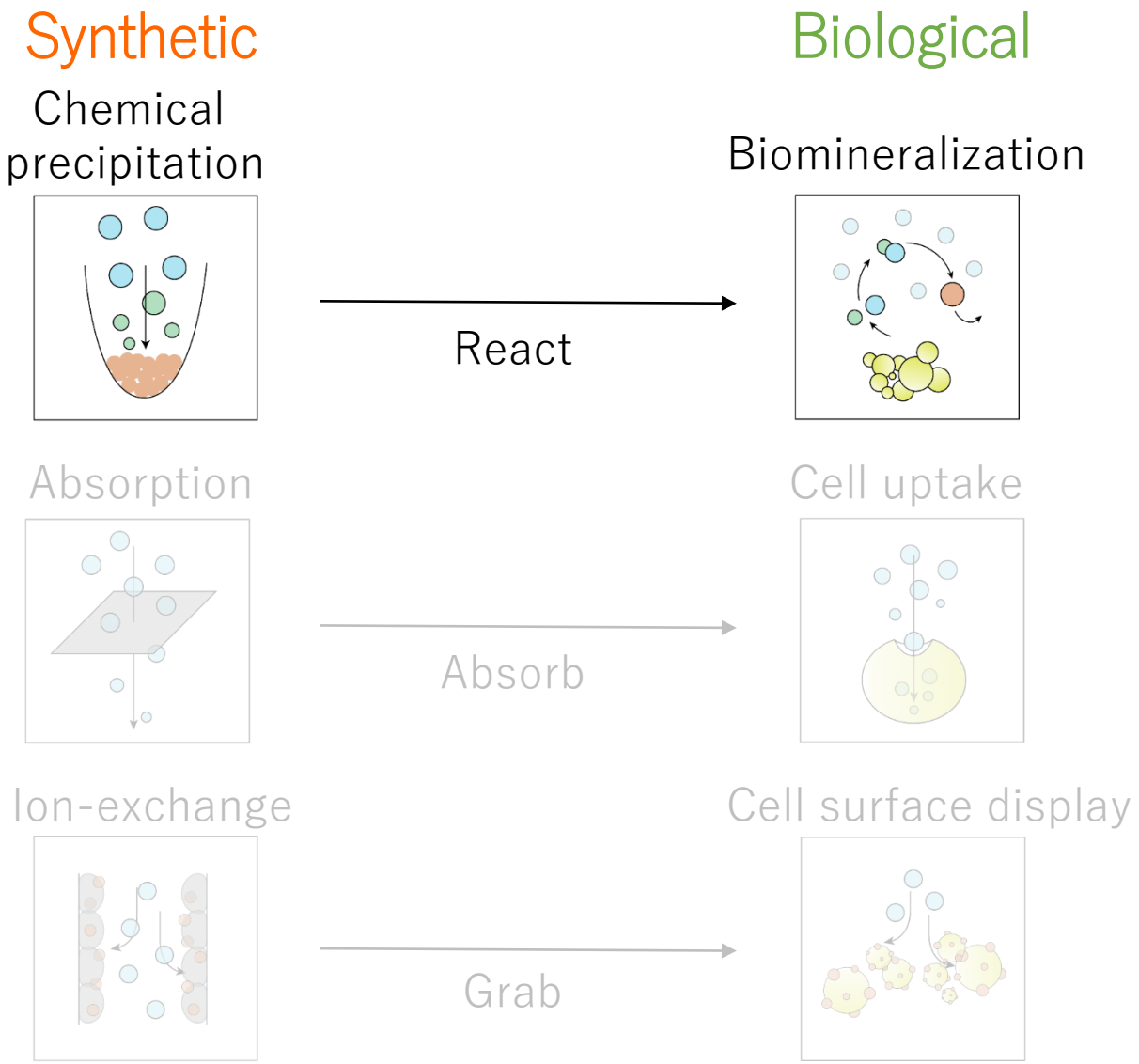


Designing biological analogies to physicochemical processes



Why use yeast?

Biomineralization as an analogy to chemical precipitation



Biomineralization as an analogy to chemical precipitation

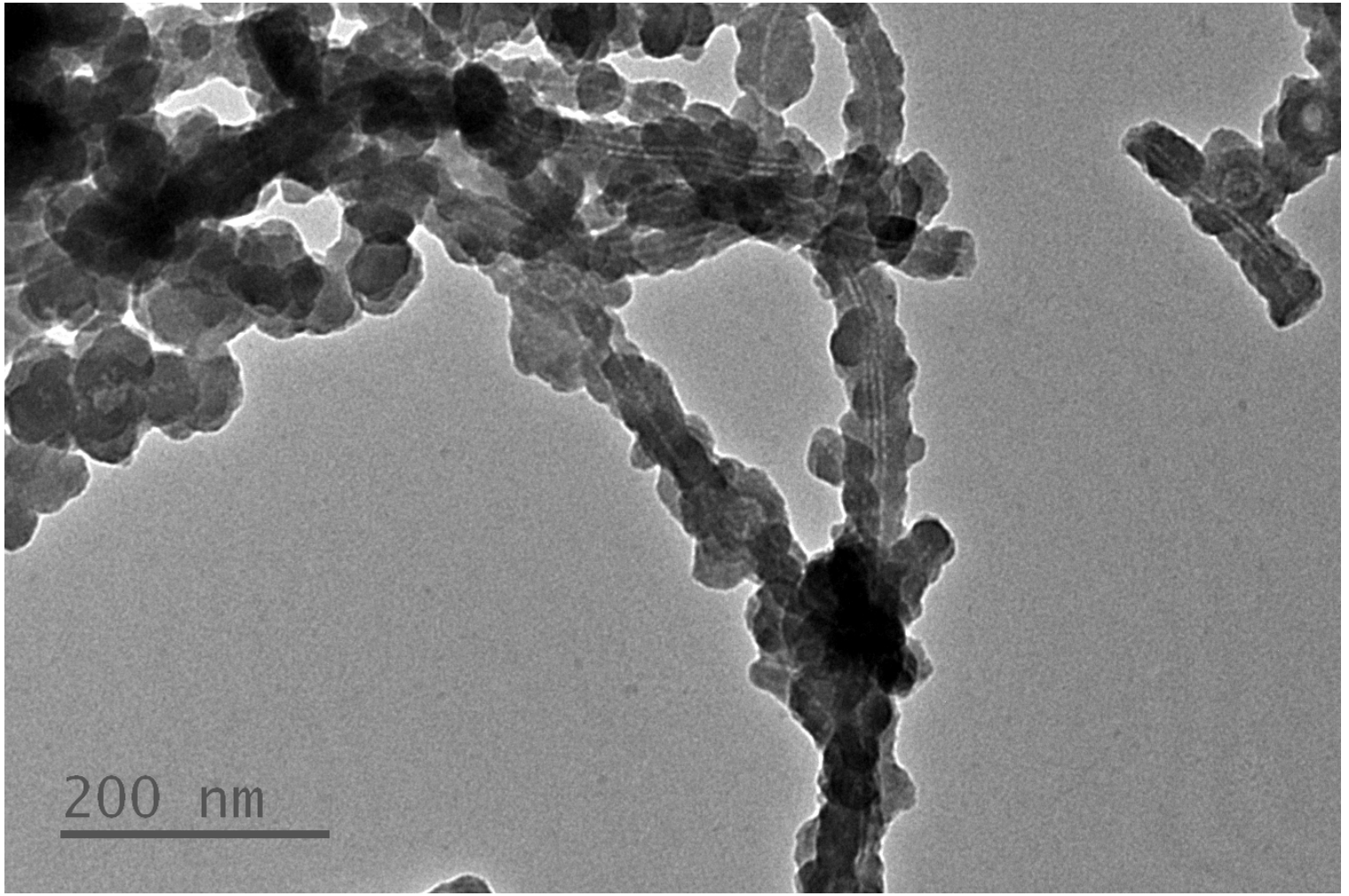
pH > 8

sacrificial chemicals:

- Lime (CaOH)
- Iron (Fe(III))
- Sulfides (H_2S)

production of sludge

Biomineralization as an analogy to chemical precipitation



rarely occurs

highly controlled for specific tissues (bones, shells, etc.)

sacrificial chemicals:

- phosphates (PO_4^{3-})
- carbonates (CO_3^{2-})
- sulfates (SO_4^{2-})

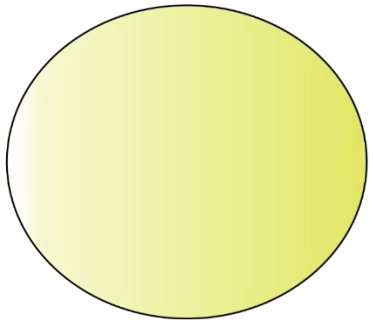
Inspiration for chemical precipitation from the wine industry

poor winemaking generated odorous and distasteful wines

chemical culprit:

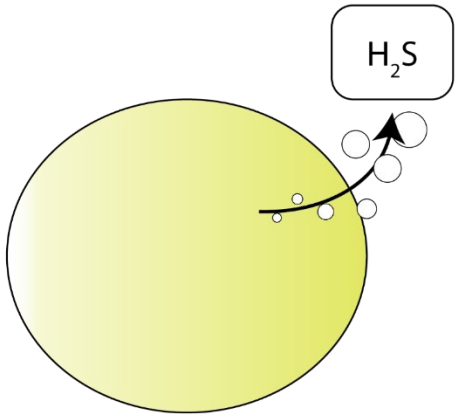
- hydrogen sulfide (H_2S)

Engineering H_2S production in yeast

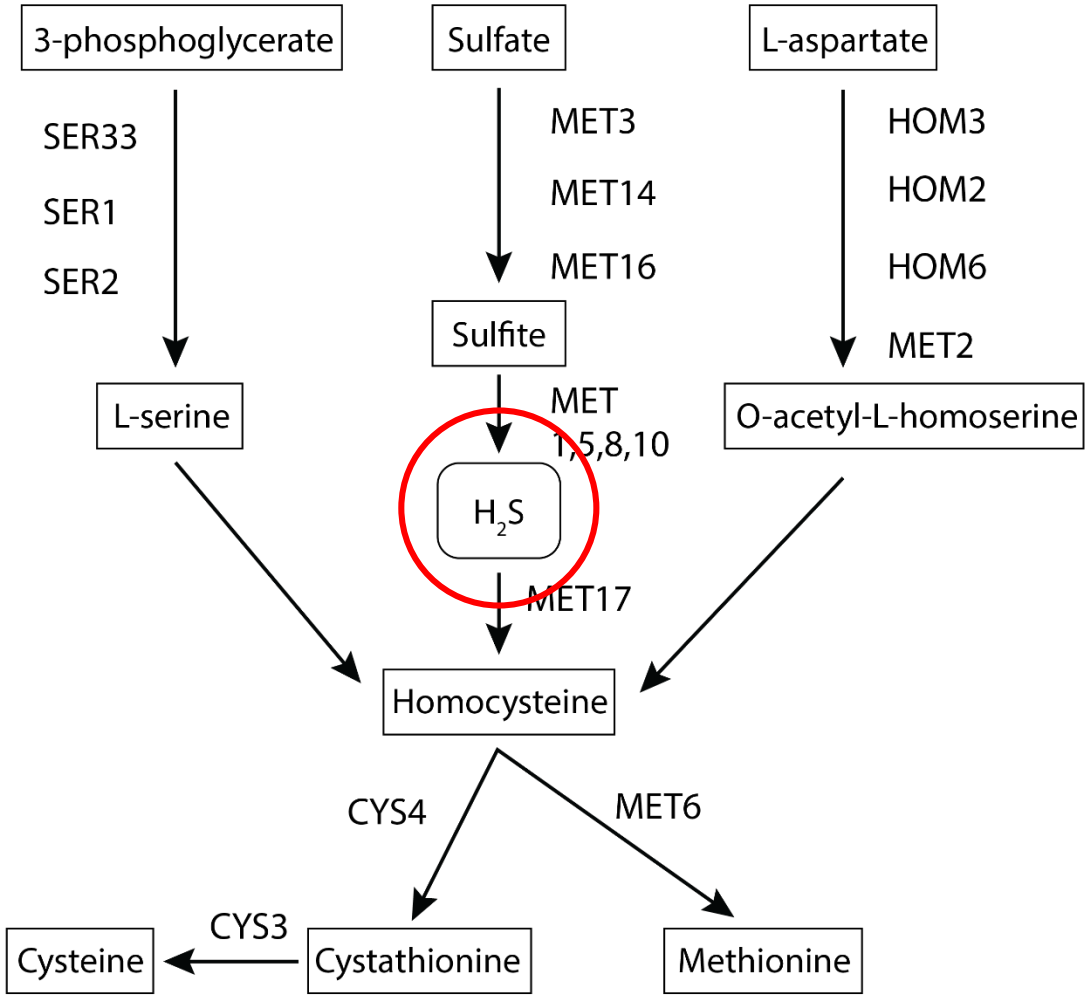


yeast cell

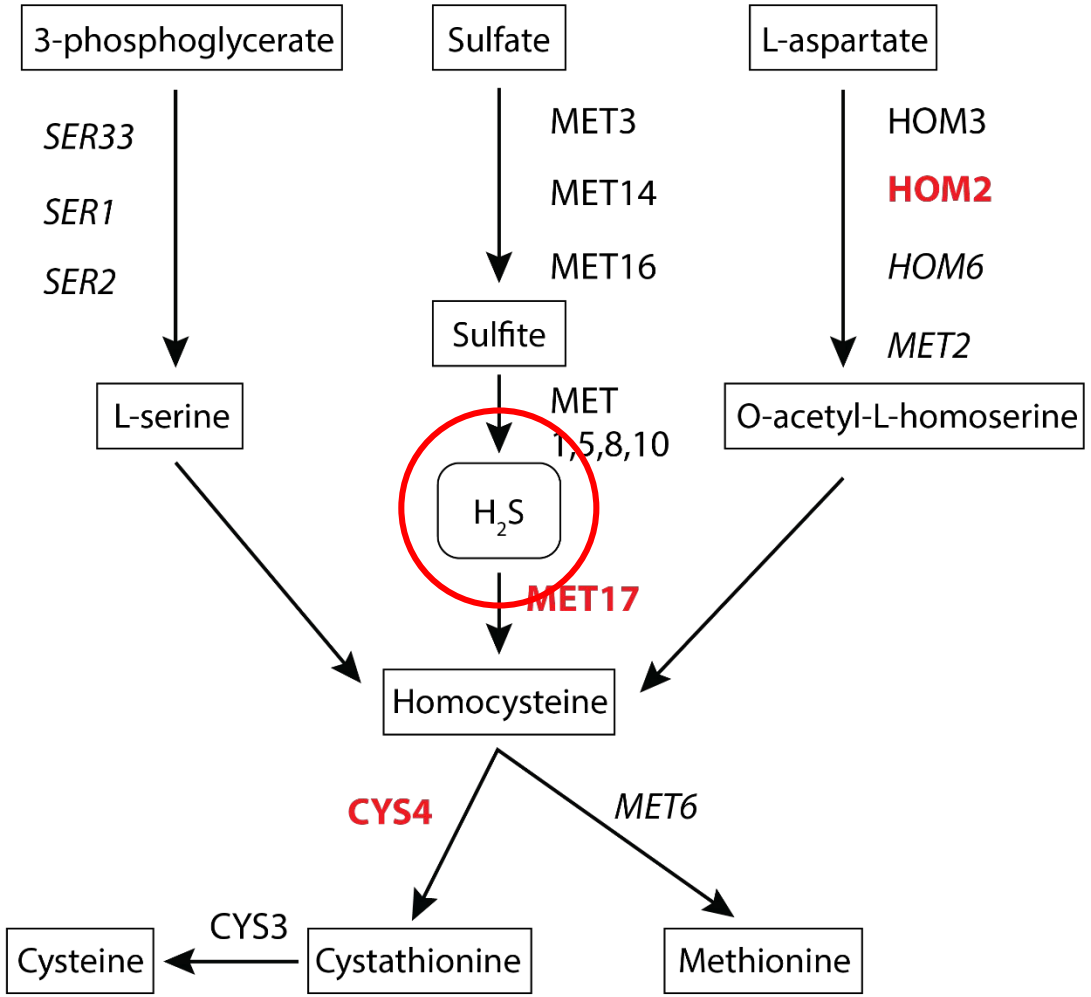
Engineering H_2S production in yeast



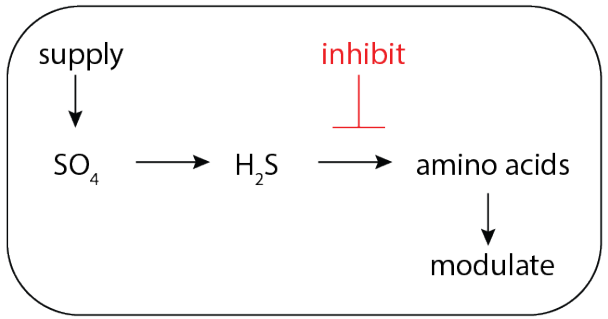
Engineering H_2S production in yeast

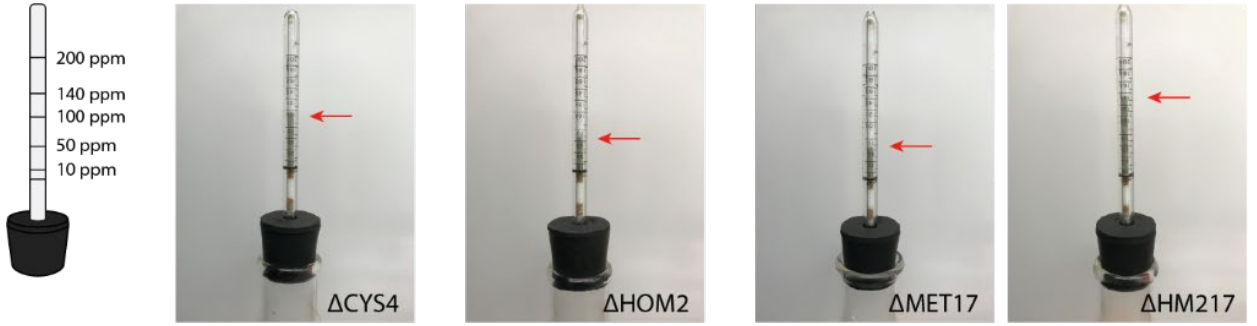


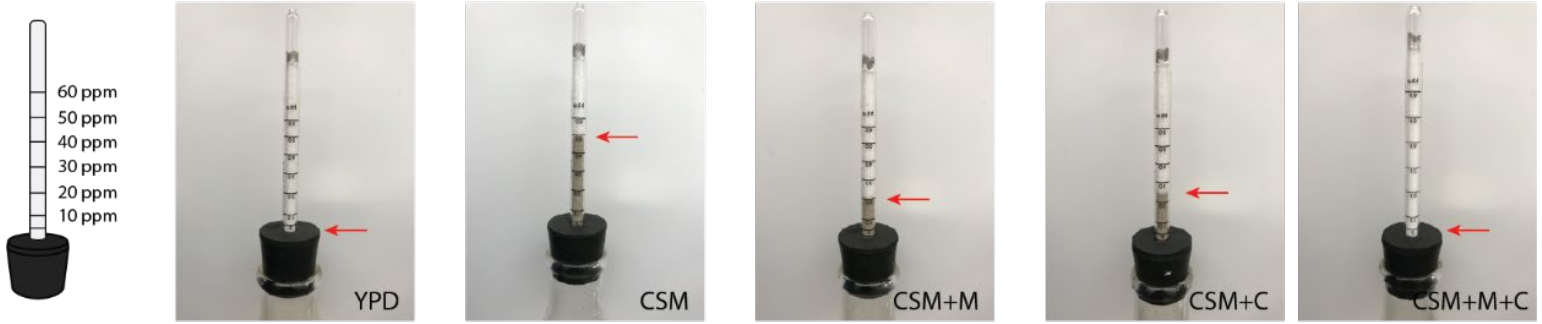
Engineering H_2S production in yeast

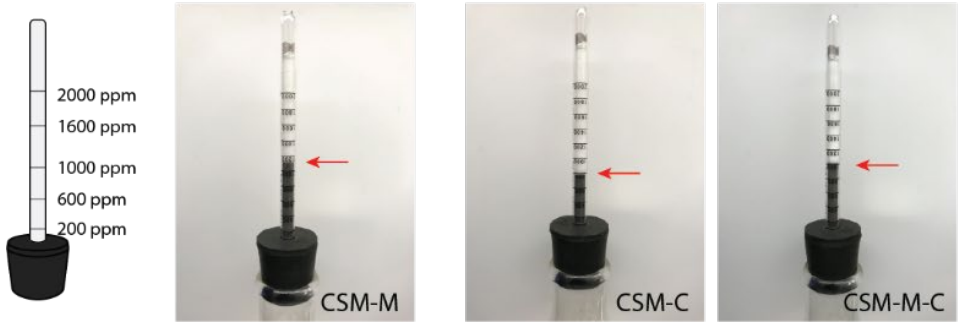


Quantifying and controlling H_2S production using simple culturing methods

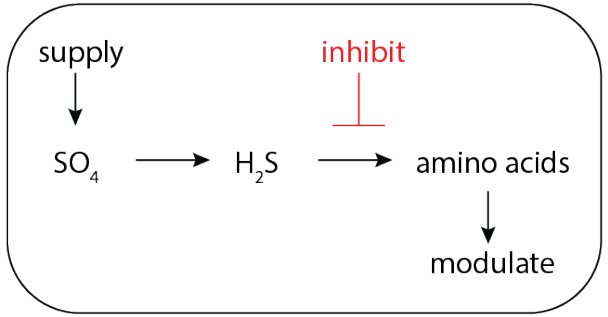


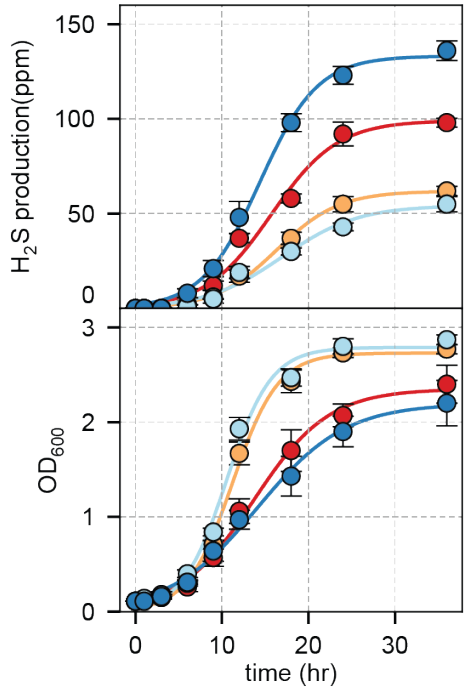






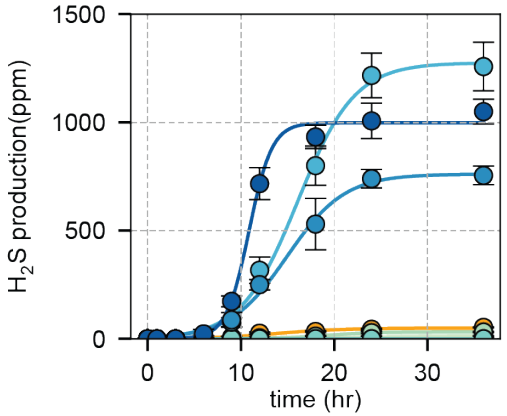
Controlling yeast H_2S production with different knockouts and culture conditions





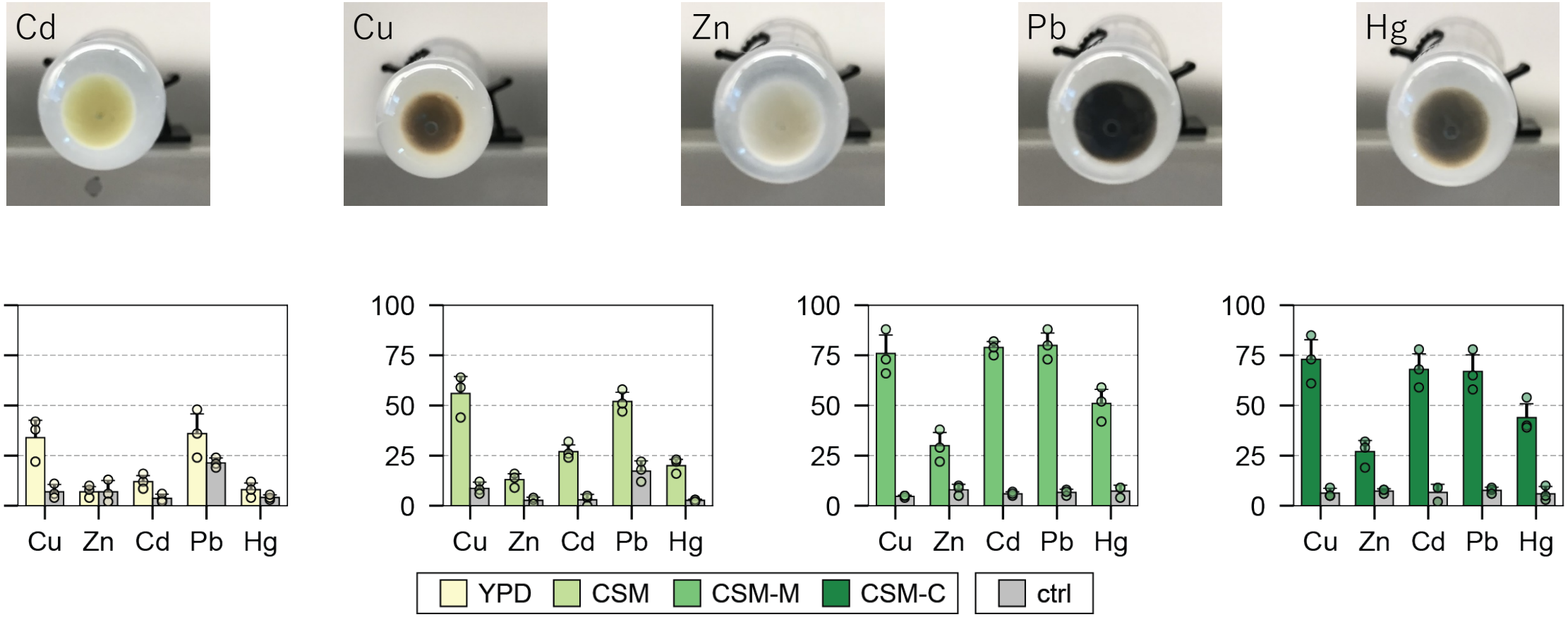
	A (ppm)	$t_{1/2}$ (hr)	r (ppm hr^{-1})
CYS4	99 ± 3.0	16 ± 0.48	2.36 ± 0.22
HOM2	62 ± 2.5	16 ± 0.66	1.57 ± 0.21
MET17	54 ± 4.5	17 ± 0.94	1.12 ± 0.13
HM217	133 ± 2.4	14 ± 0.46	3.62 ± 0.36

	OD_{600}	$t_{1/2}$ (hr)	r (OD hr^{-1})
CYS4	1.67 ± 0.09	14 ± 1.77	0.03 ± 0.01
HOM2	2.73 ± 0.04	11 ± 0.38	0.09 ± 0.01
MET17	2.79 ± 0.81	11 ± 2.40	0.10 ± 0.05
HM217	2.29 ± 0.77	14 ± 4.87	0.05 ± 0.02

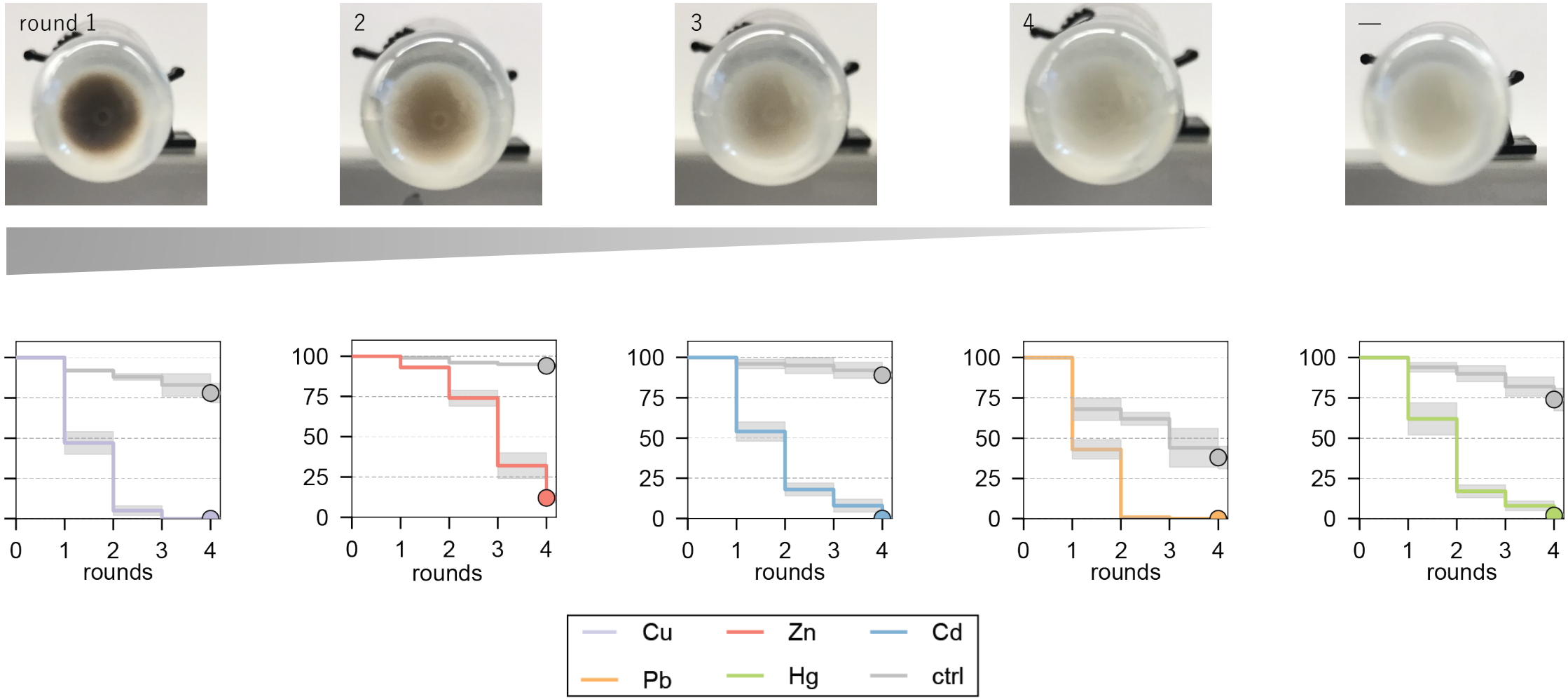


	A (ppm)	$t_{1/2}$ (hr)	r (ppm hr^{-1})
YPD	0 ± 0	0 ± 0	0 ± 0
CSM	50 ± 7.4	14 ± 2.3	1.26 ± 0.54
CSM+M	12 ± 6.4	23 ± 1.0	0.31 ± 0.82
CSM+C	32 ± 4.2	19 ± 1.3	1.07 ± 0.70
CSM+M+C	0 ± 0	0 ± 0	0 ± 0
CSM-M	1275 ± 80	16 ± 2.0	38.2 ± 7.51
CSM-C	761 ± 102	15 ± 1.4	22.4 ± 5.12
CSM-M-C	998 ± 92	11 ± 4.2	74.9 ± 17.7

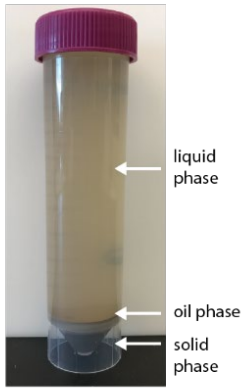
Removing heavy metals using H_2S generated from yeast

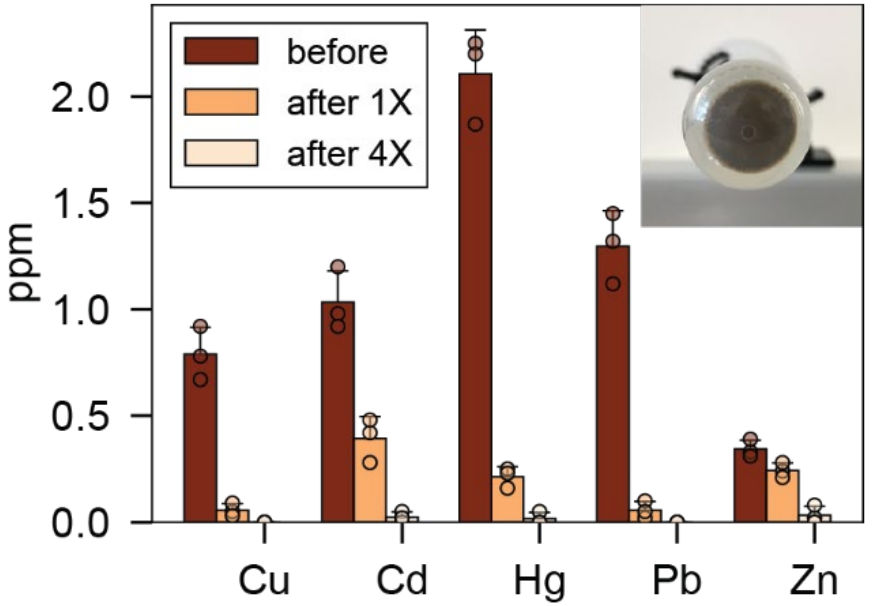


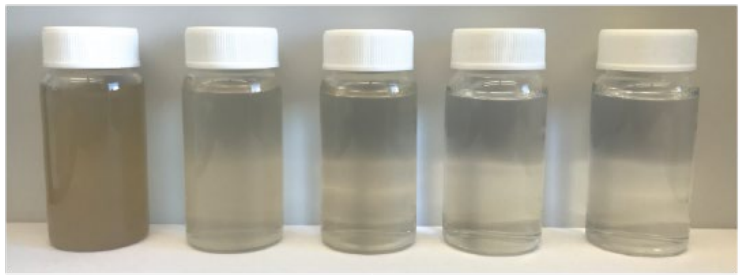
Multiple rounds of yeast mediated H_2S precipitation can completely eliminate metal



Removing heavy metals from the Canadian Athabasca Oil Sands

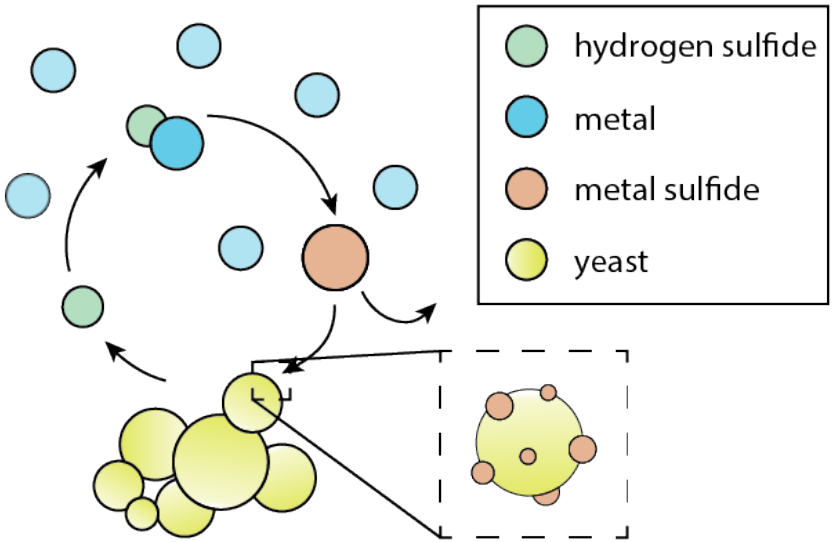


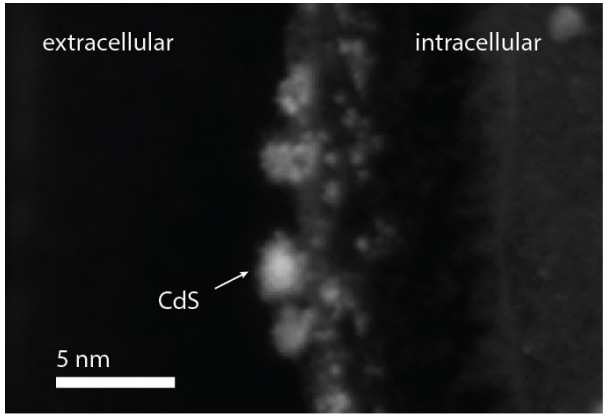


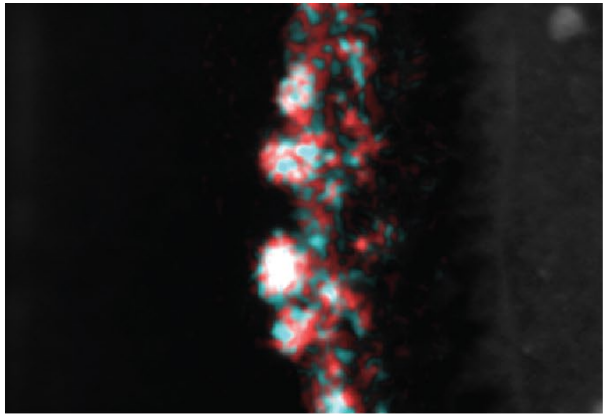


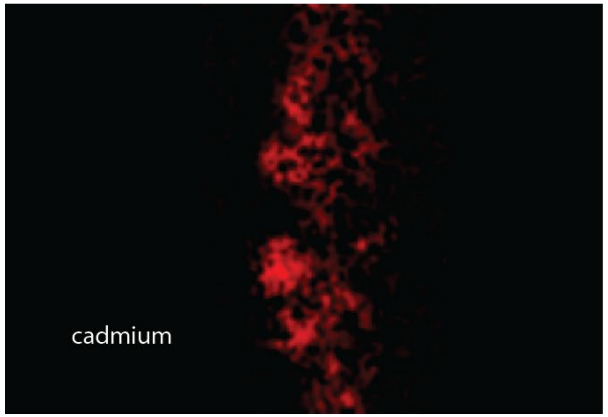
round 1 2 3 4 -

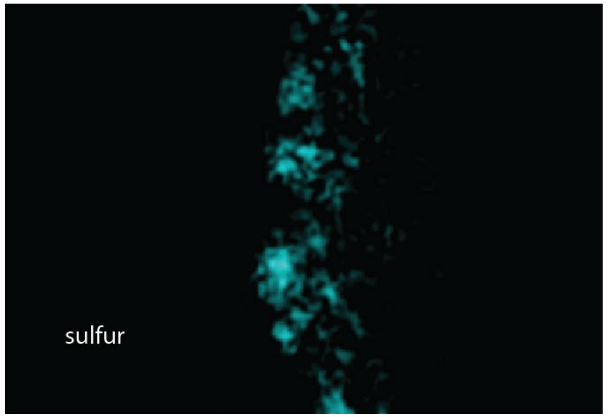
Controlling metal sulfide precipitation based on sulfur production rate and yeast display



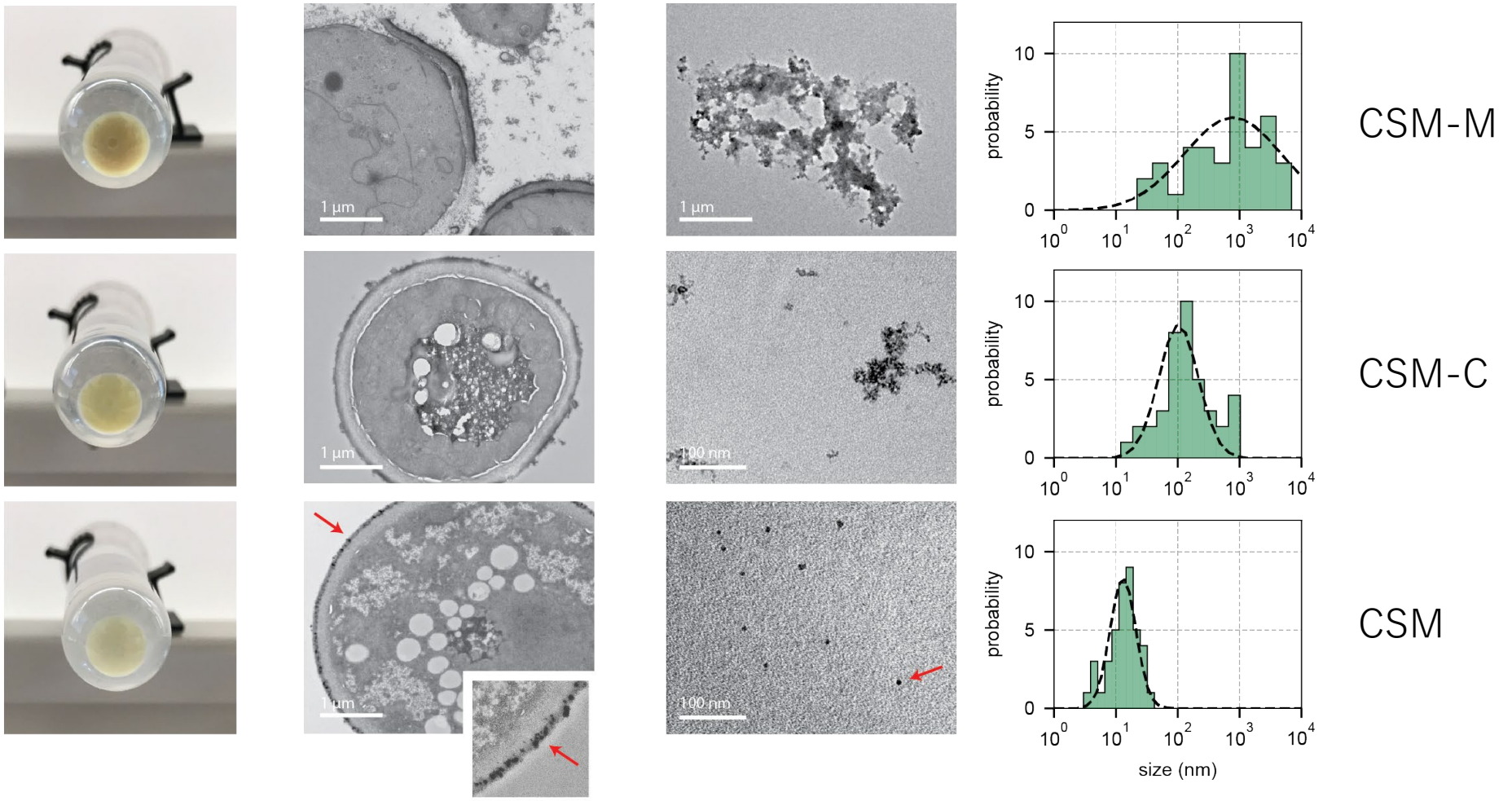




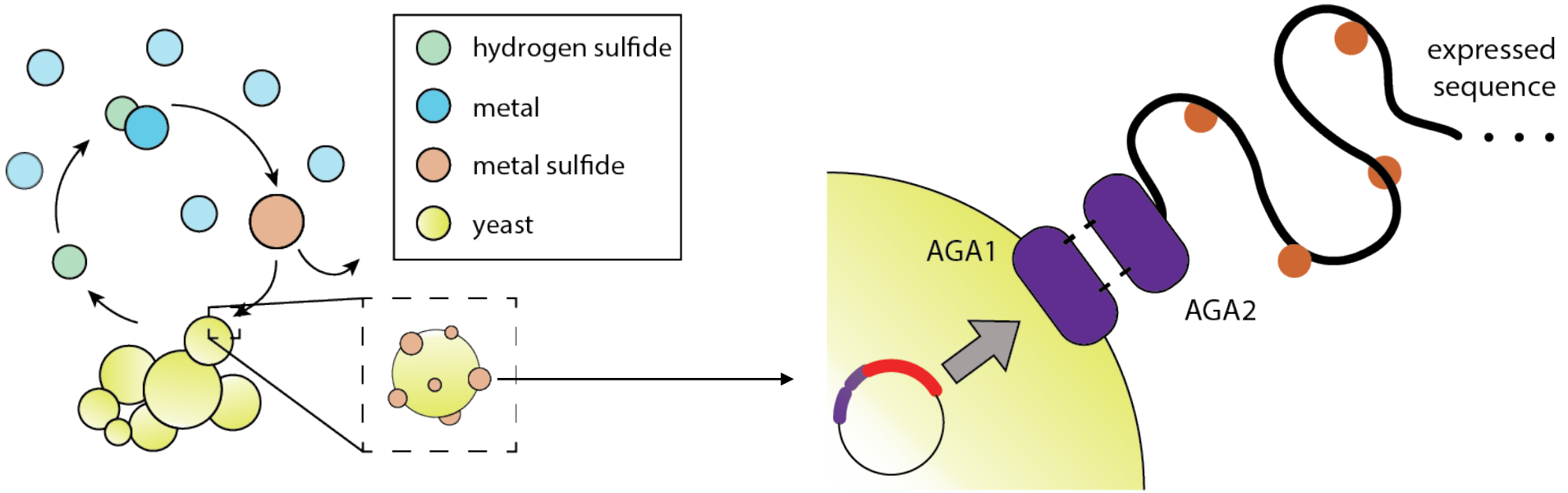




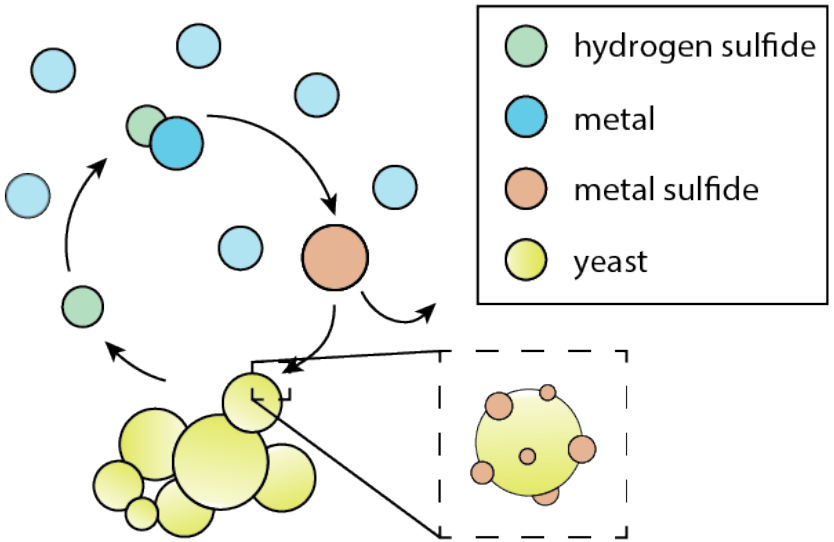
Controlling H_2S production rates improves metal sulfide size distribution formation

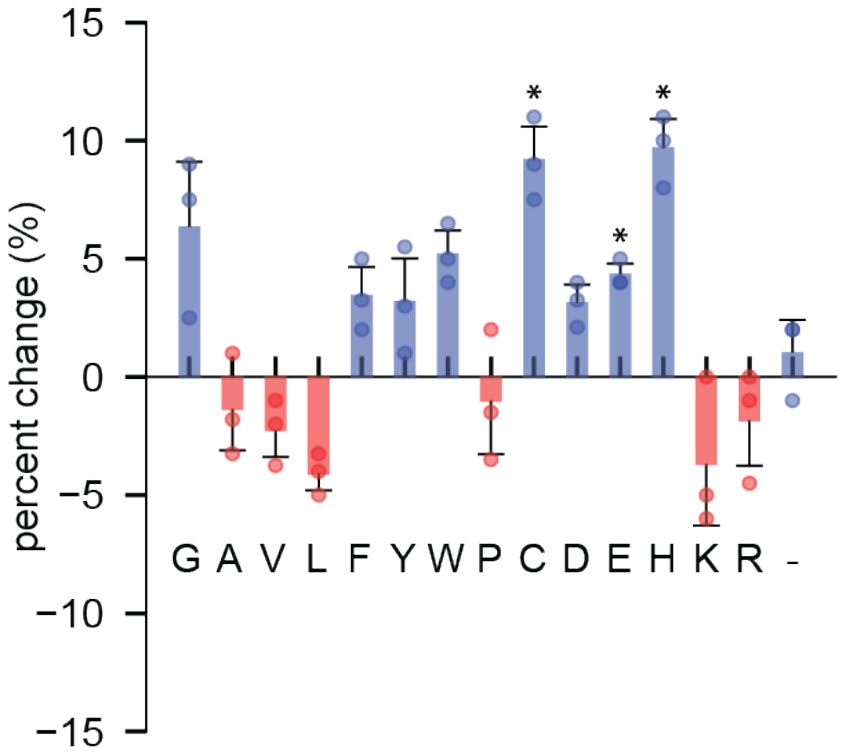


Controlling metal sulfide precipitation based on sulfur production rate and yeast display

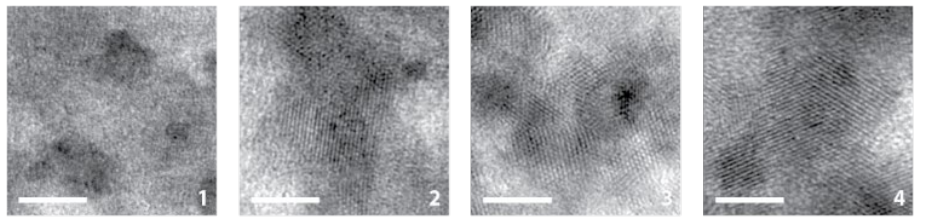


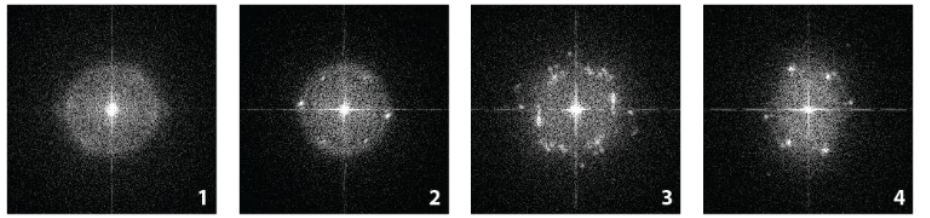
Controlling metal sulfide precipitation based on sulfur production rate and yeast display

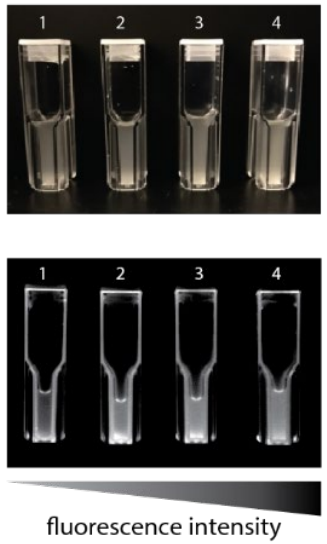


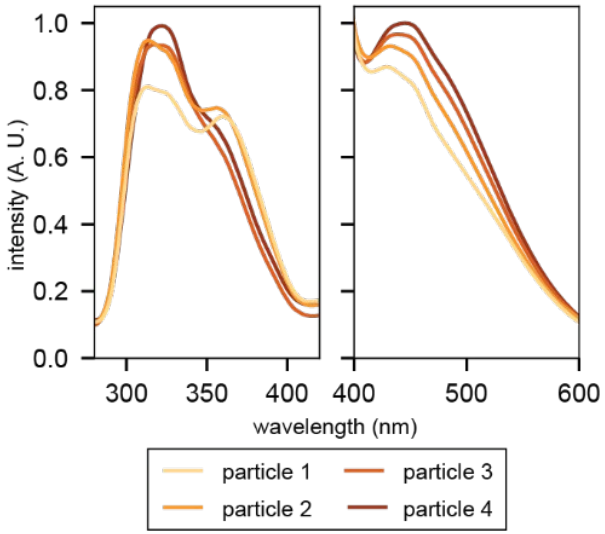


Improving metal sulfide morphology and crystallinity using yeast display







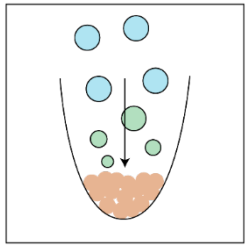


1 = GGGGGG
2 = CCCCCC
3 = GGCGGC
4 = GCCGCC

Takeaway

Synthetic

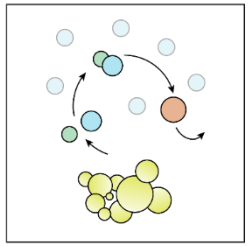
Chemical precipitation



React

Biological

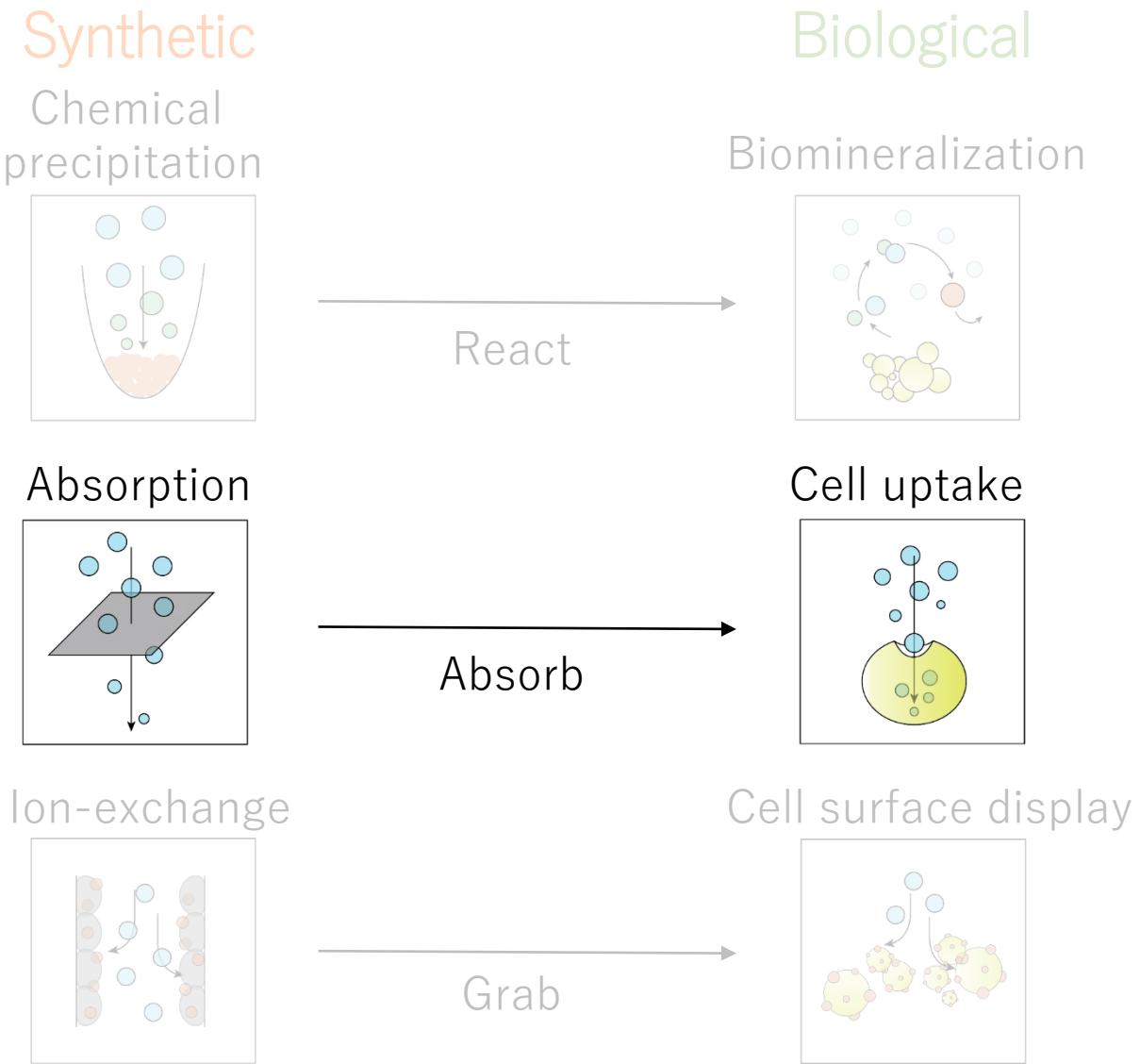
Biomineralization



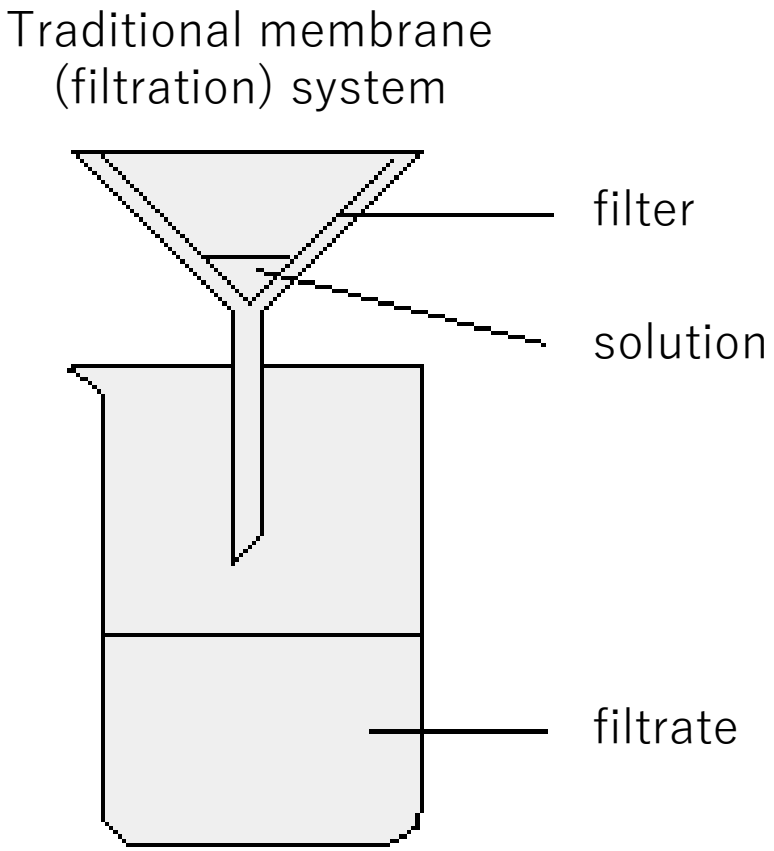
TAKEAWAY

- Engineering S^{2-} production
- Knockouts and culture conditions tune H_2S production
- Metal precipitation of Cu, Zn, Cd, Hg, and Pb > 50% for 1 round
- Controlled metal sulfide particle formation, compared to production of sludge

Cellular metal uptake as an analogy to absorption

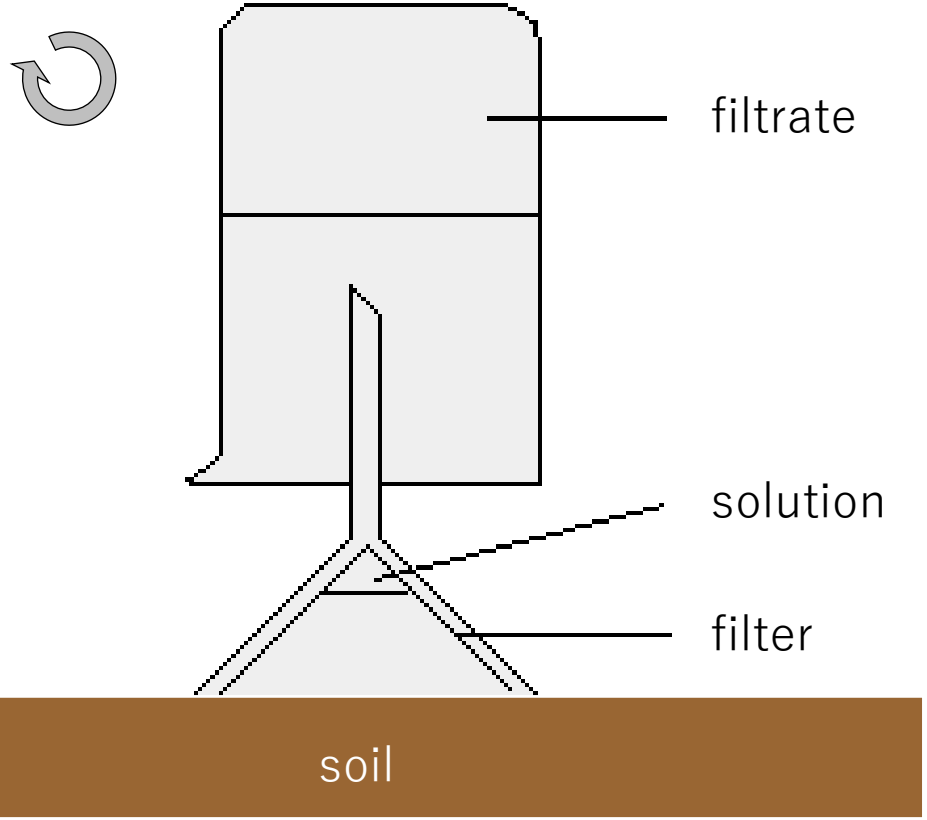


Absorption requires a filter and a sequestration body to retain contaminants



Absorption requires a filter and a sequestration body to retain contaminants

Traditional membrane (filtration) system

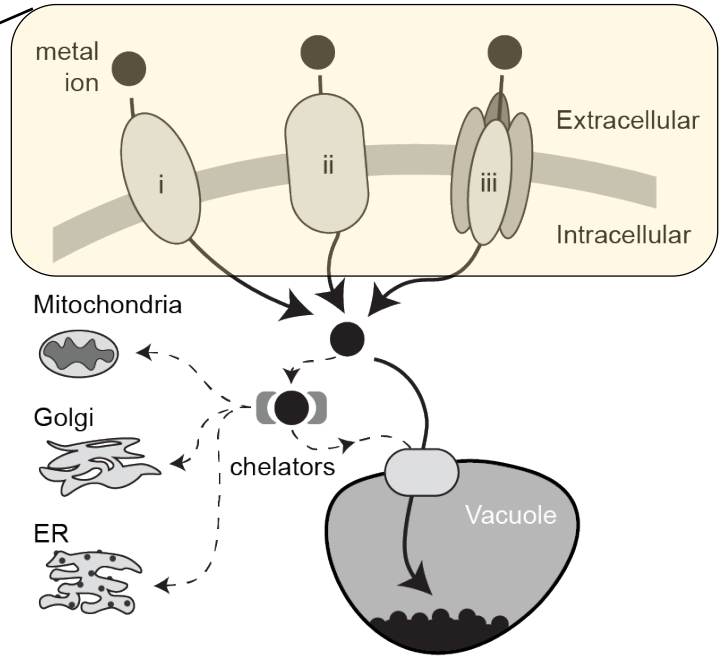


Plant (phyto-) remediation

Hyperactive membrane transporters confer hyperaccumulation in plants, and yeast.

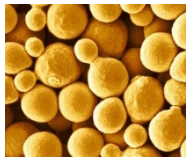
Plants

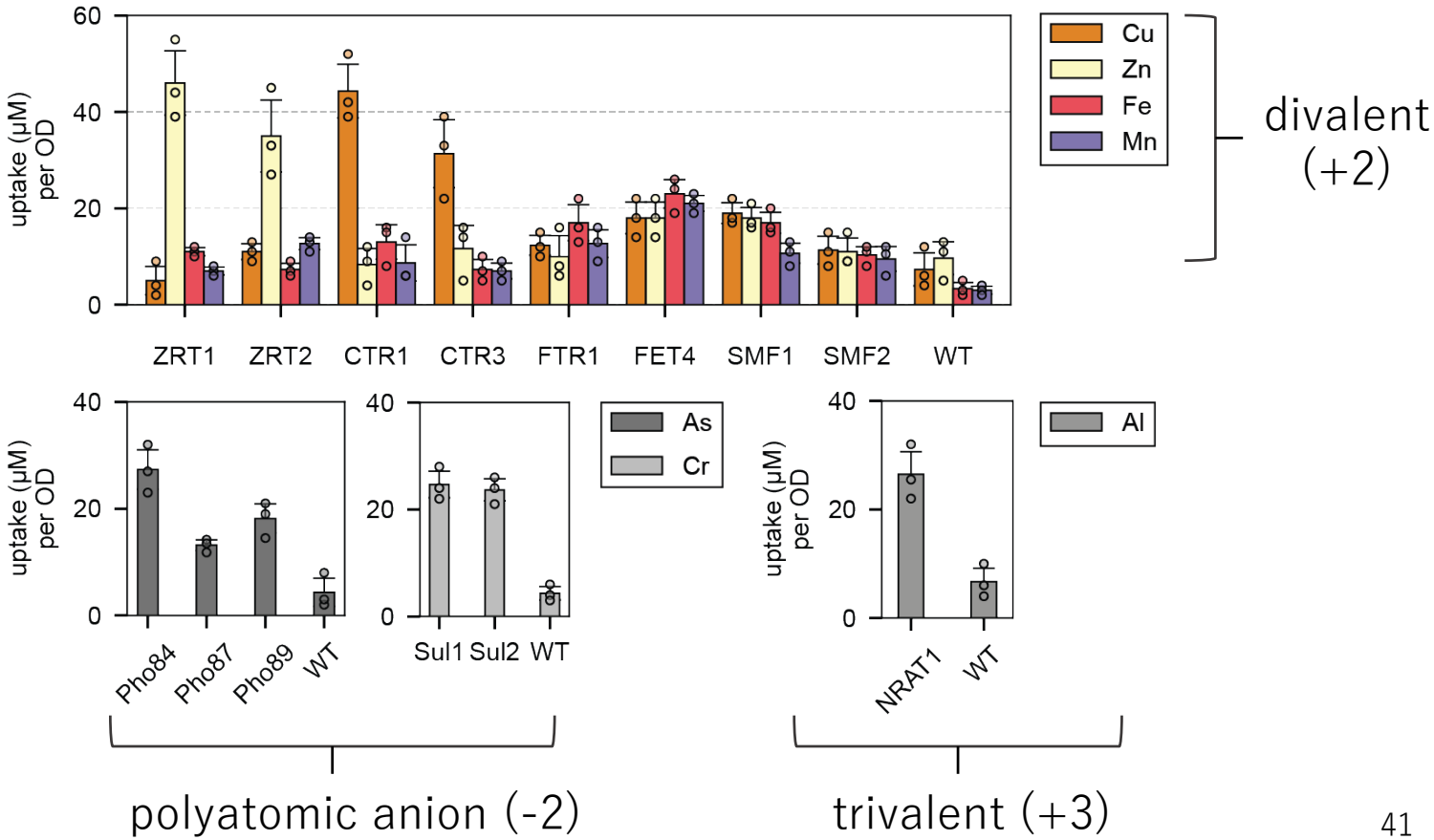
Gatekeepers: metal transporters



i = native transporters
ii = permeases
iii = non-native transporters

Yeast



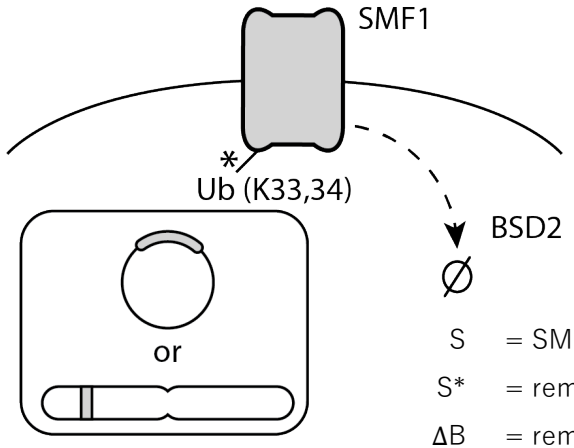


Establishing engineering requirements for enhancing metal uptake

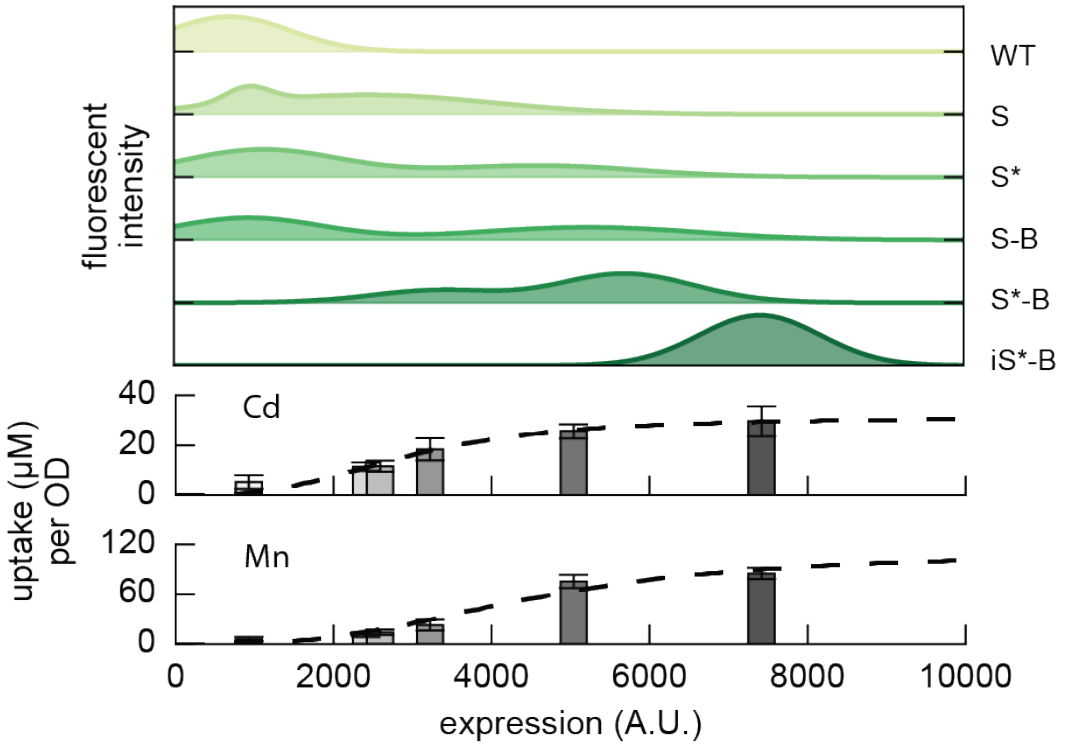
1. Overexpress transporter
2. Increase expression longevity
3. Access other compartments (i.e. vacuole)
4. Increase metal tolerance

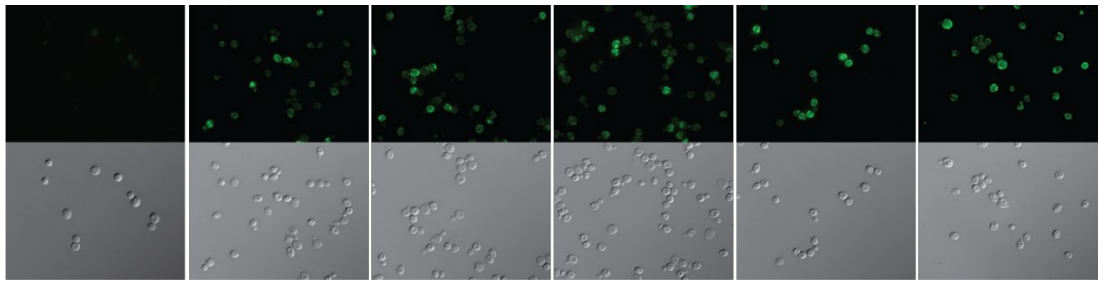
Increasing membrane transporter expression

1. Overexpress transporter
2. Increase expression longevity
3. Access other compartments (i.e. vacuole)
4. Increase metal tolerance



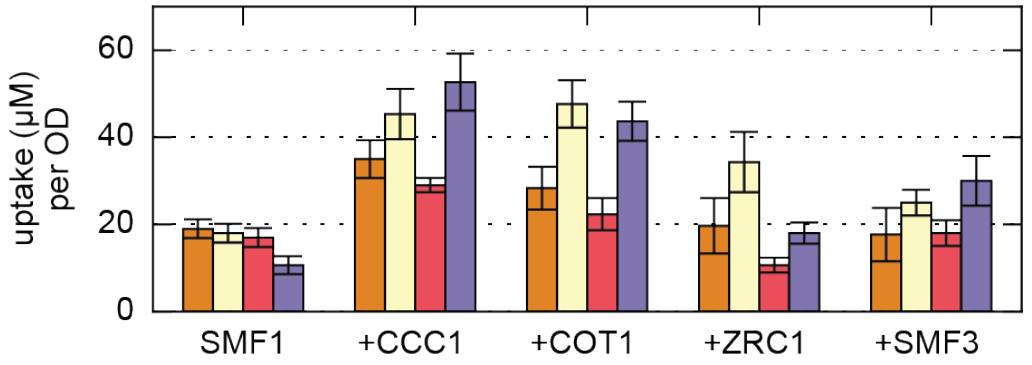
S = SMF1
S* = removed ubiquitination site
ΔB = removed ubiquitination ligase
iS = integrated SMF1



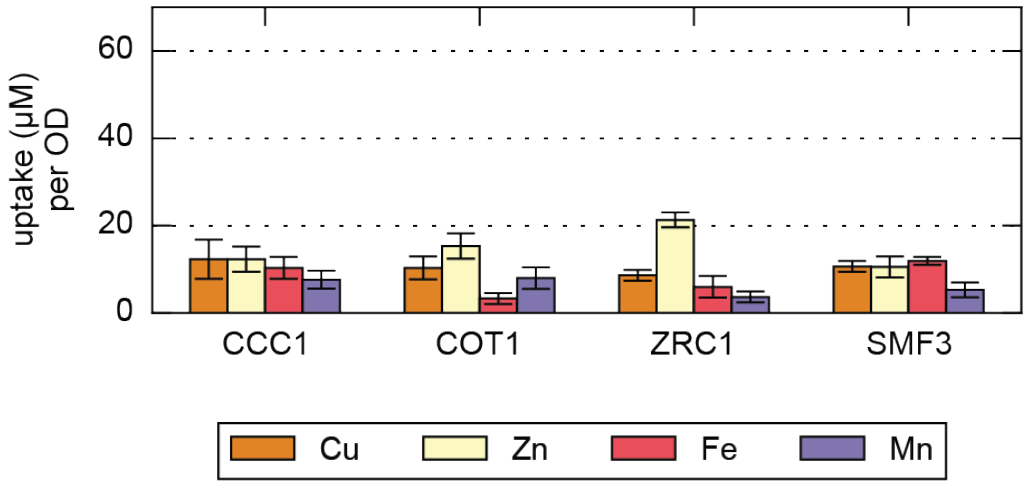


Metals can be further compartmentalized in vacuoles

1. Overexpress transporter
2. Increase expression longevity
3. Access other compartments (i.e. vacuole)
4. Increase metal tolerance



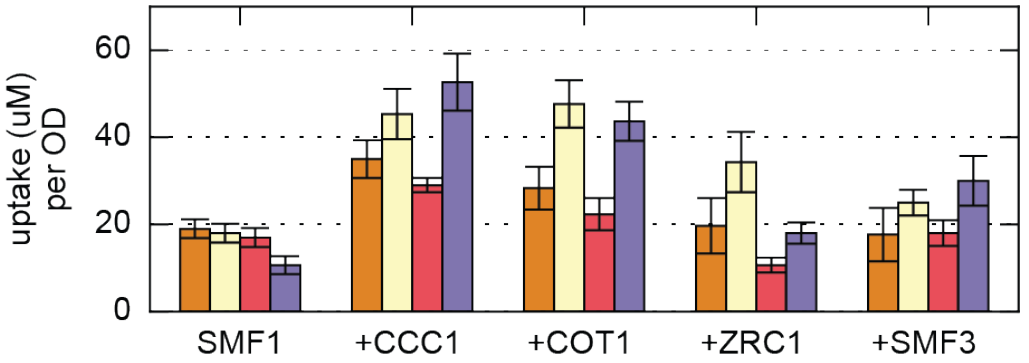
overexpression of vacuole transporter = ↑ uptake capacity



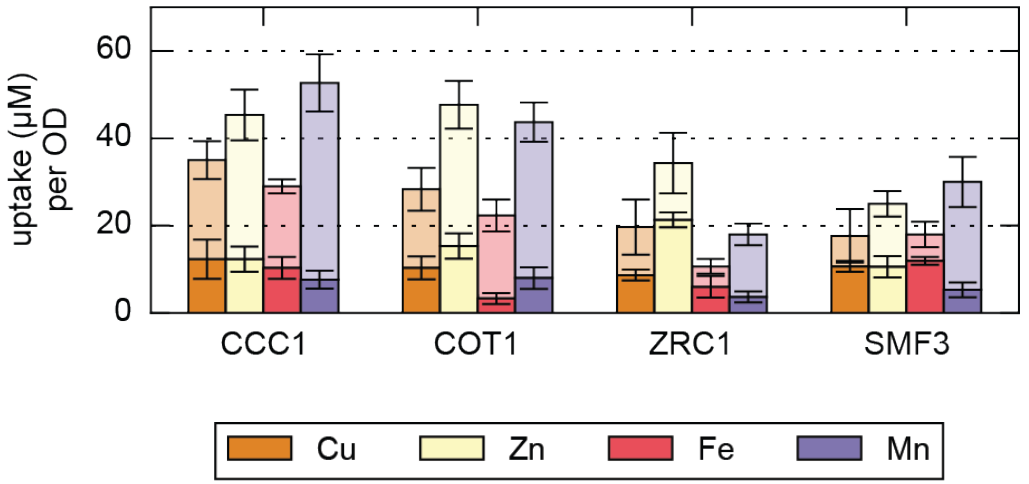
surface transport is the limiting "reaction"

Metals can be further compartmentalized in vacuoles

1. Overexpress transporter
2. Increase expression longevity
3. Access other compartments (i.e. vacuole)
4. Increase metal tolerance



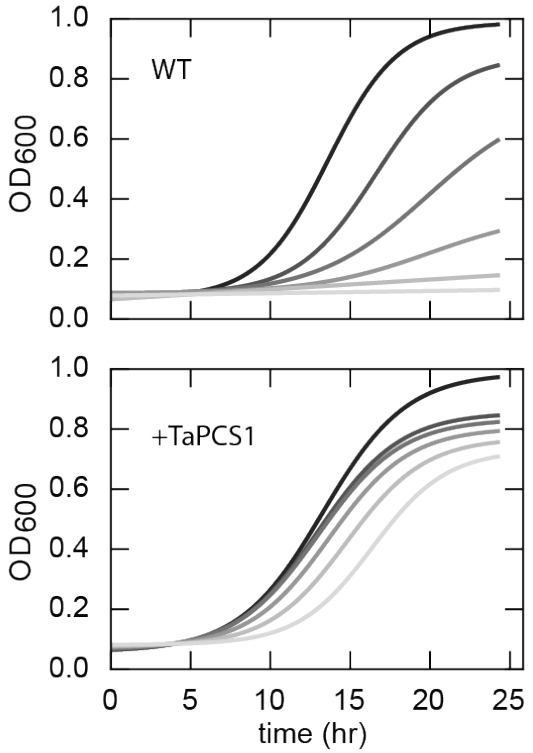
overexpression of vacuole transporter = ↑ uptake capacity

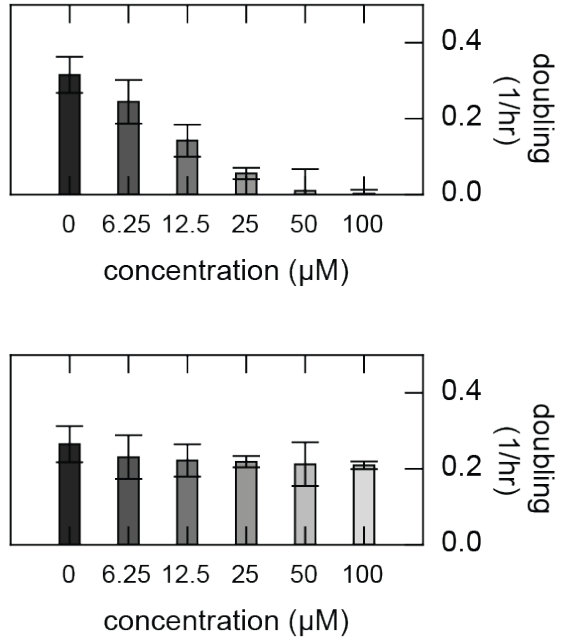


surface transport is the limiting "reaction"

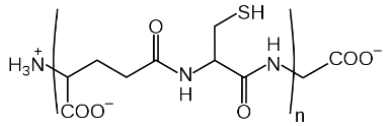
Enhancing cell tolerance to internalized heavy metals

1. Overexpress transporter
2. Increase expression longevity
3. Access other compartments (i.e. vacuole)
4. Increase metal tolerance

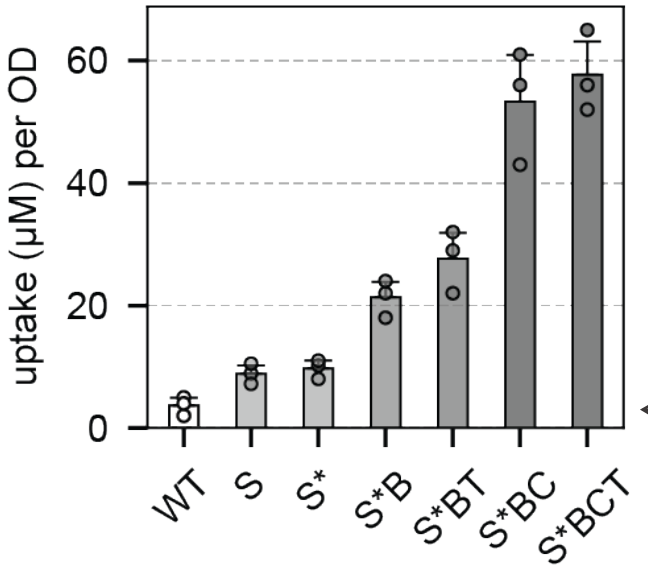


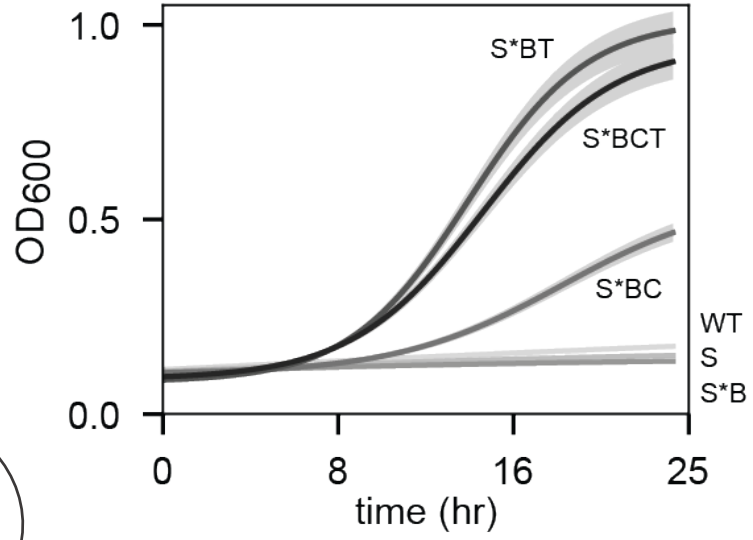


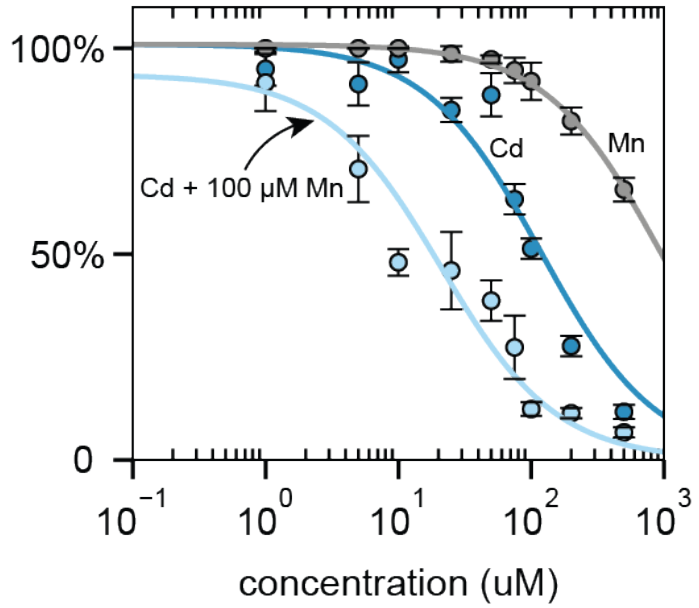
+TaPCS1 (T) Cys→ γGlu-Cys→ $(\gamma Glu-Cys)_n$-Gly



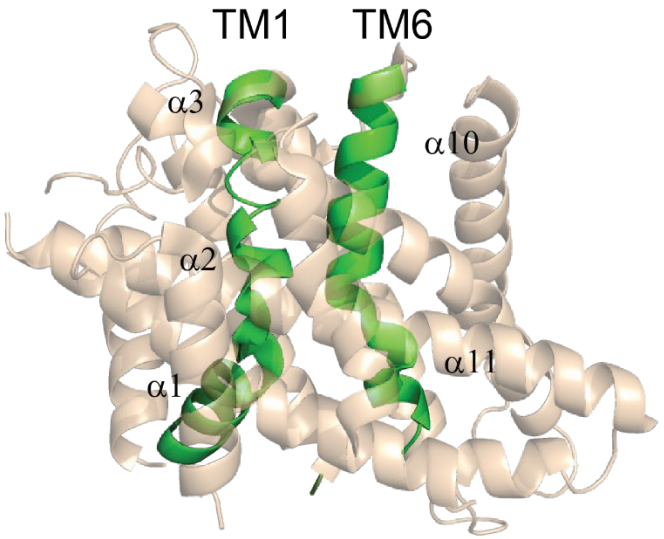
Combining design criteria into a single yeast strain for enhanced metal removal

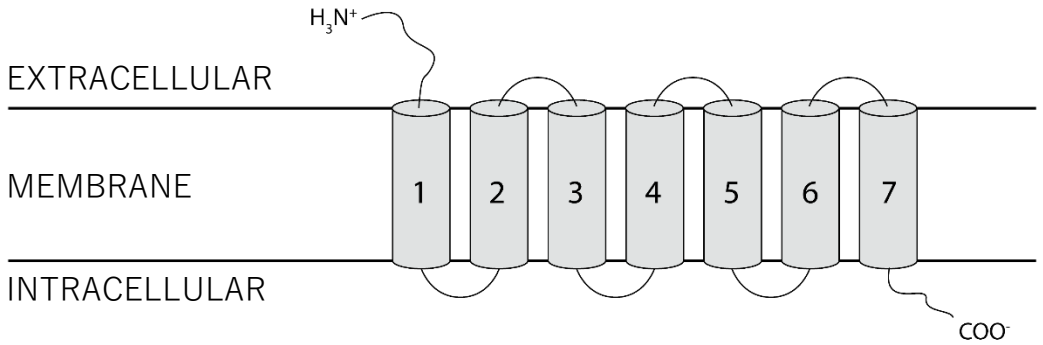




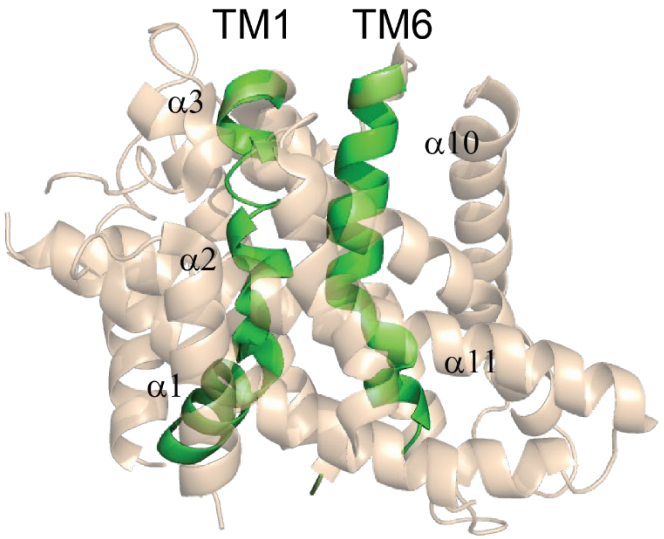


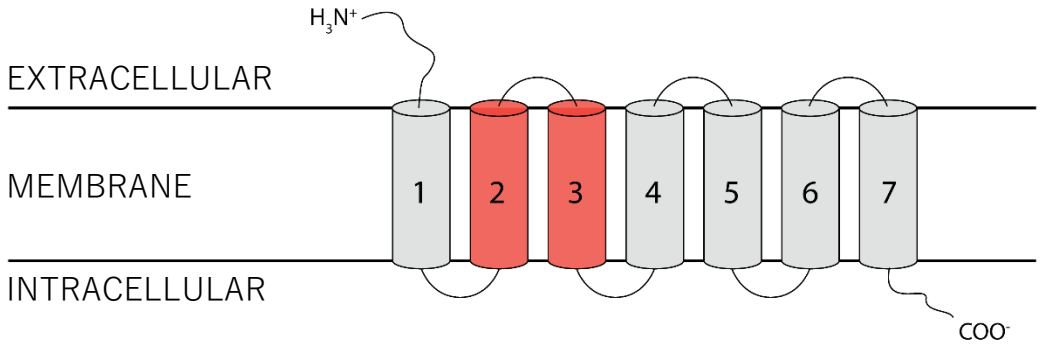
Altering metal transport specificity by engineering membrane transporters



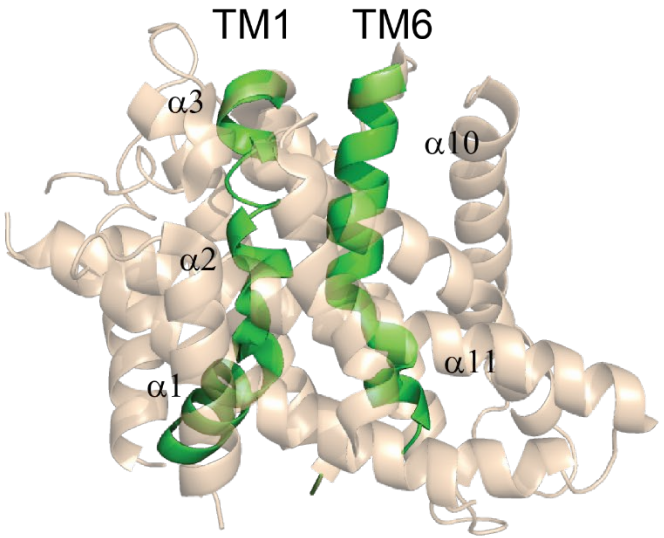


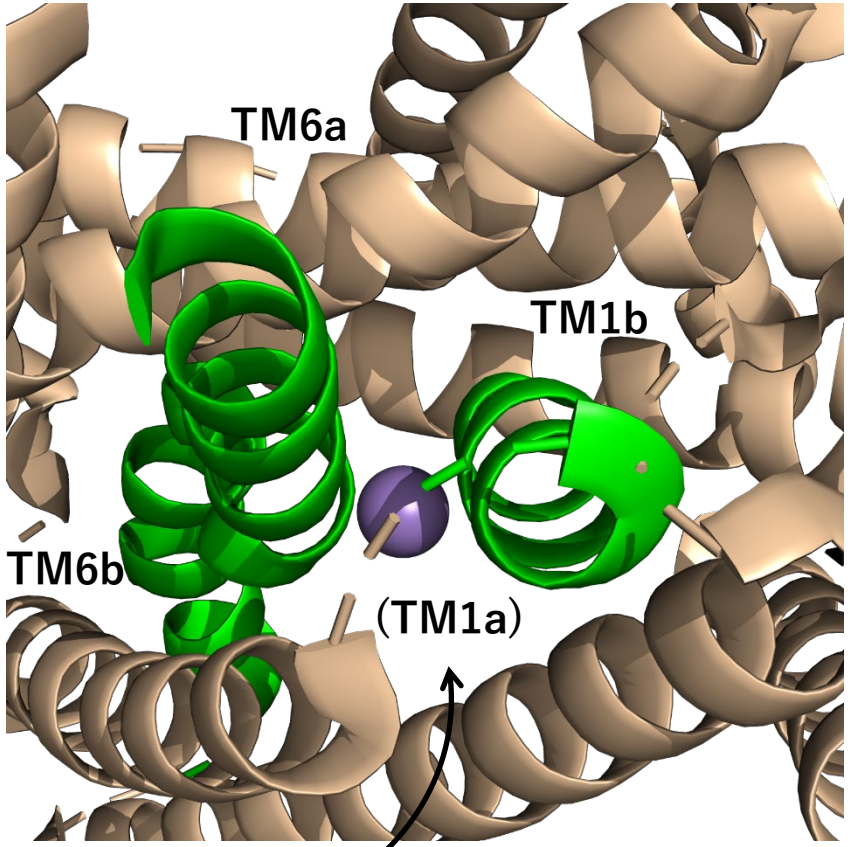
Altering metal transport specificity by engineering membrane transporters





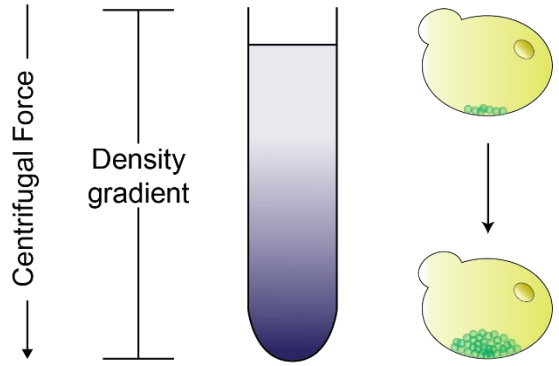
Altering metal transport specificity by engineering membrane transporters

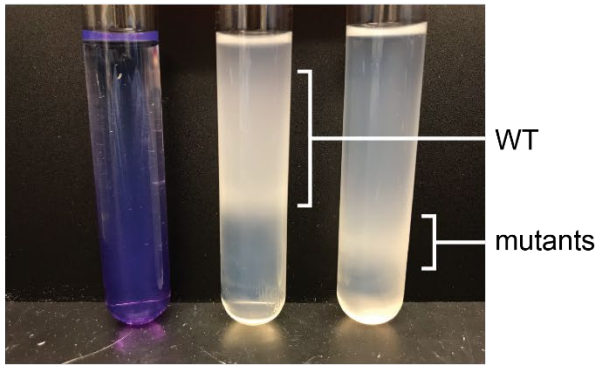


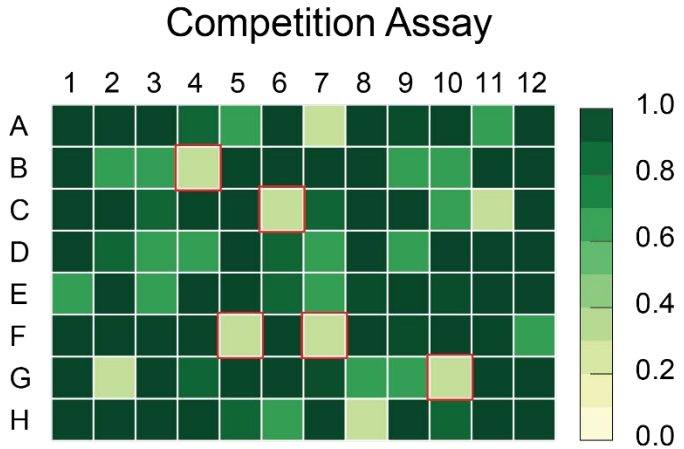


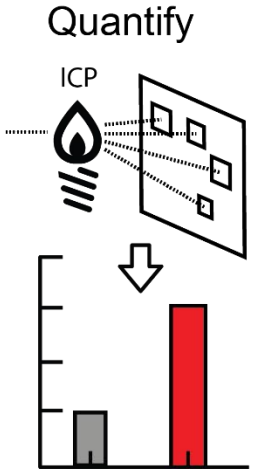
Rationally mutate

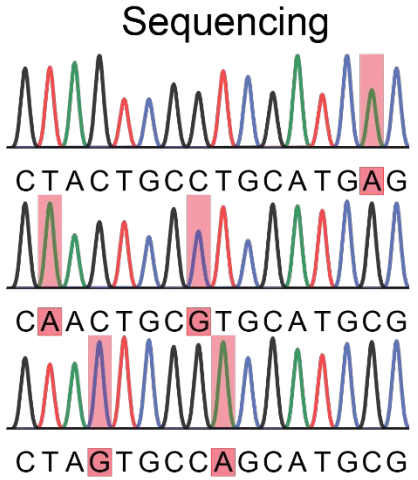
Designing a high throughput method for screening mutant metal transporters



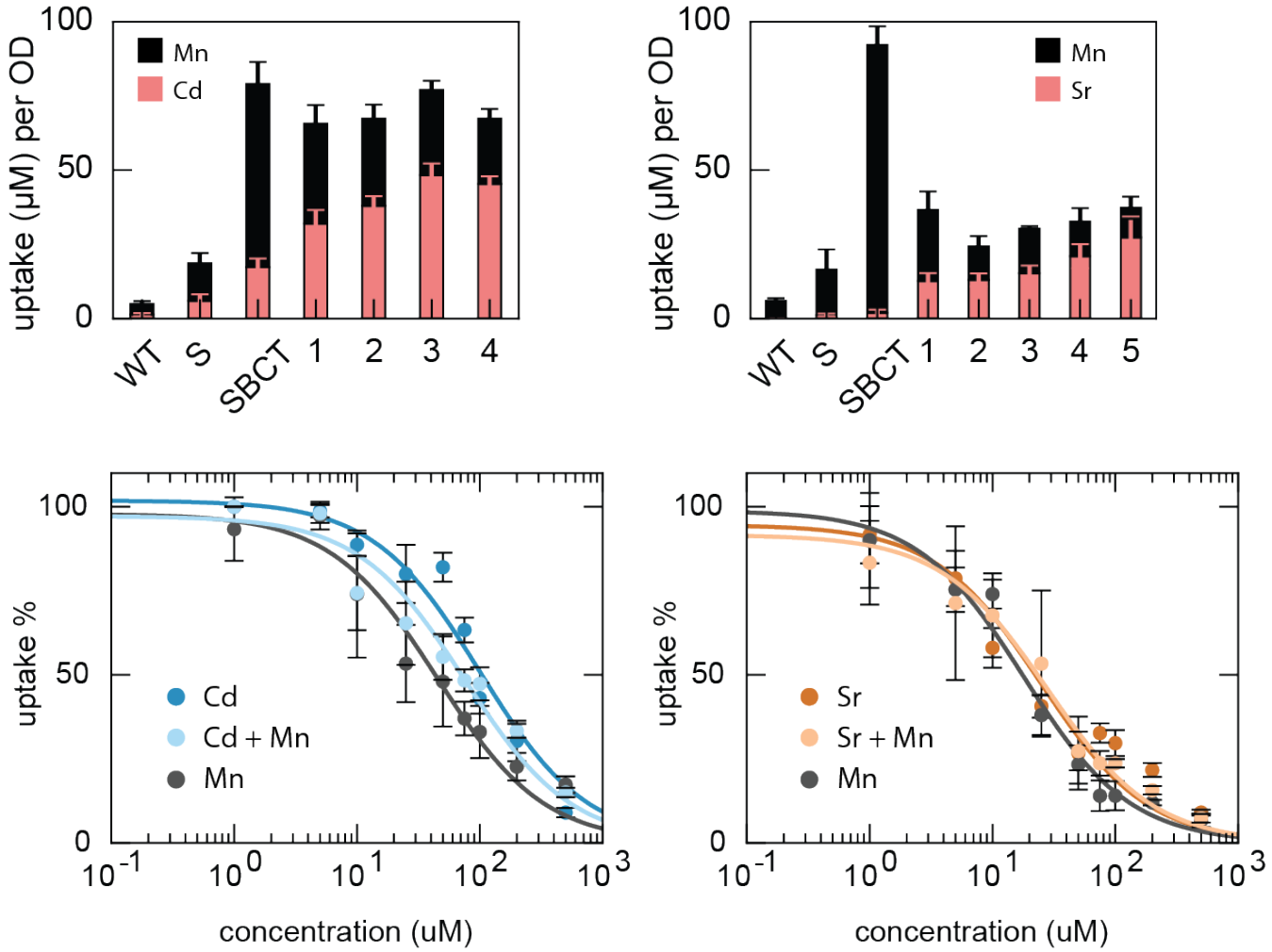








Creating cadmium and strontium transporters with reduced manganese uptake

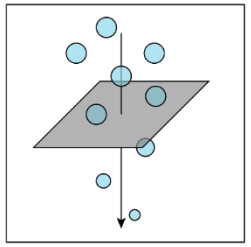


Takeaway

Synthetic

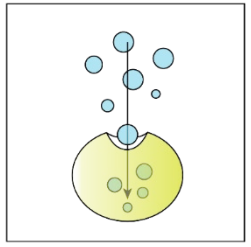
Biological

Absorption



Absorb

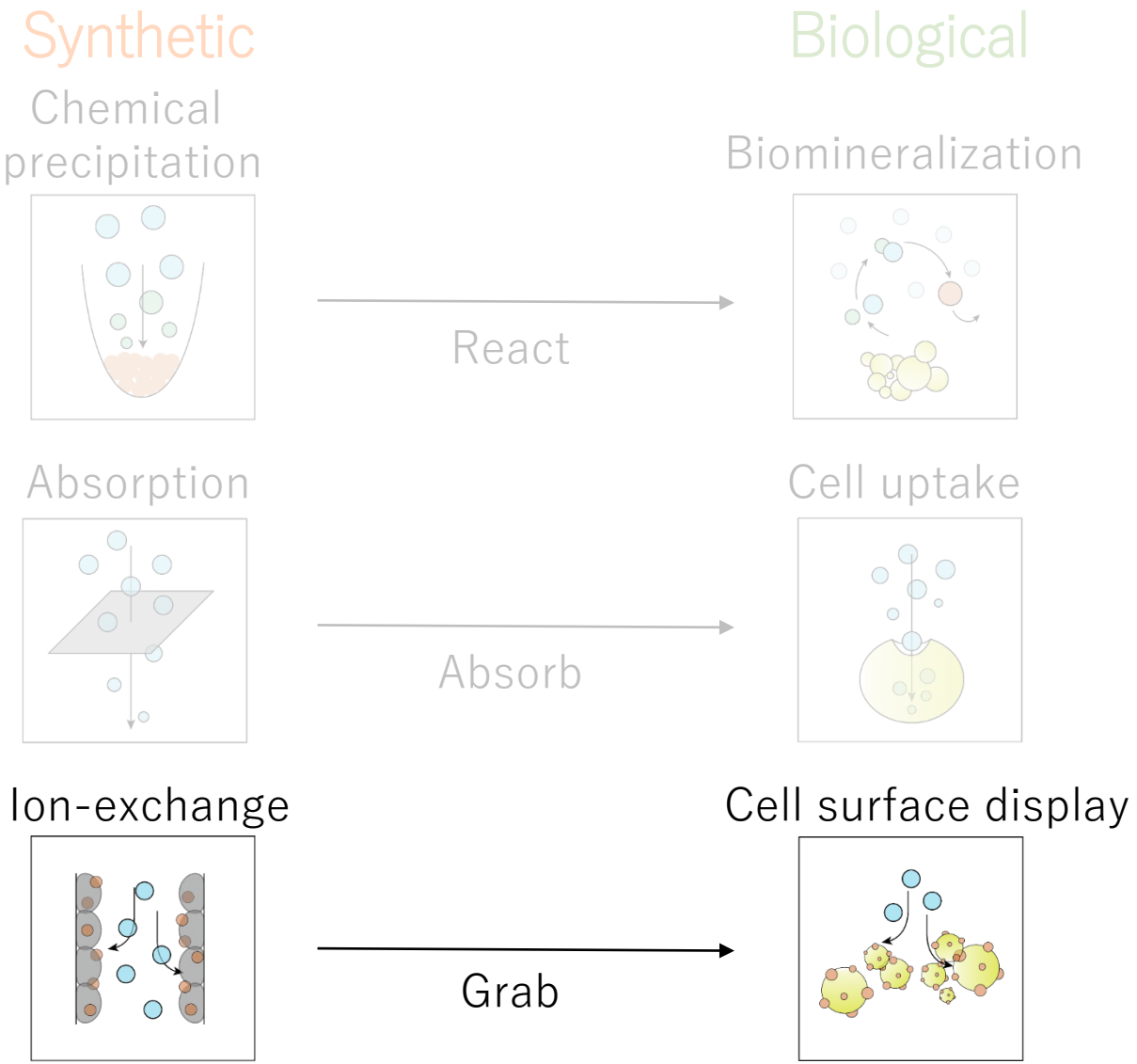
Cell uptake



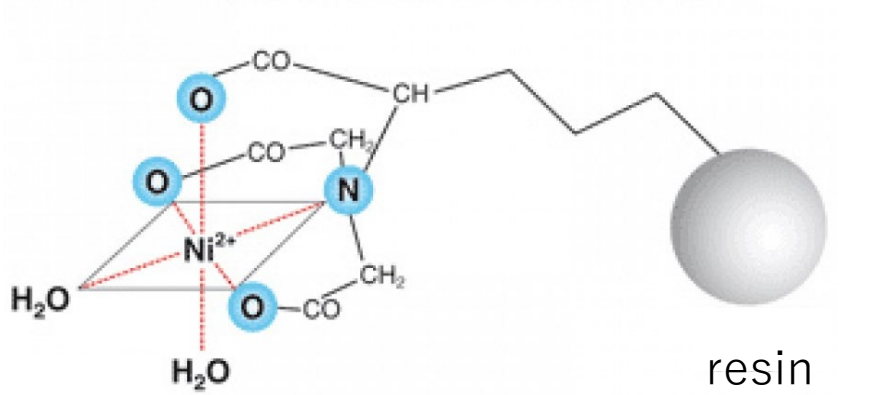
TAKEAWAY

- Membrane transporters confer metal hyperaccumulation
- Increasing transporter expression, longevity, and metal tolerance enhances metal uptake
- Membrane transporters offer high metal specificity, and can be engineered for other metals

Cell and protein chelation as an analogy to ion-exchange



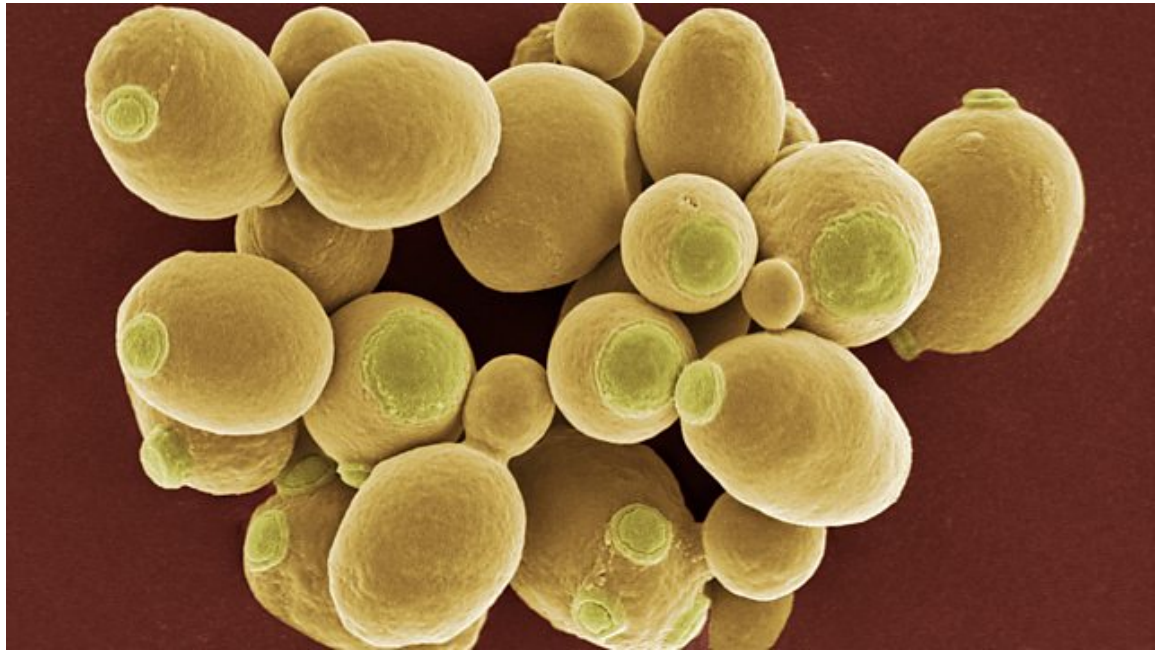
Ion-exchange has two primary components: the resin and the exchanger



exchanger
(-SO_3^-, -$COOH^-$)

resin
(agarose)

Ion-exchange has two primary components: the resin and the exchanger



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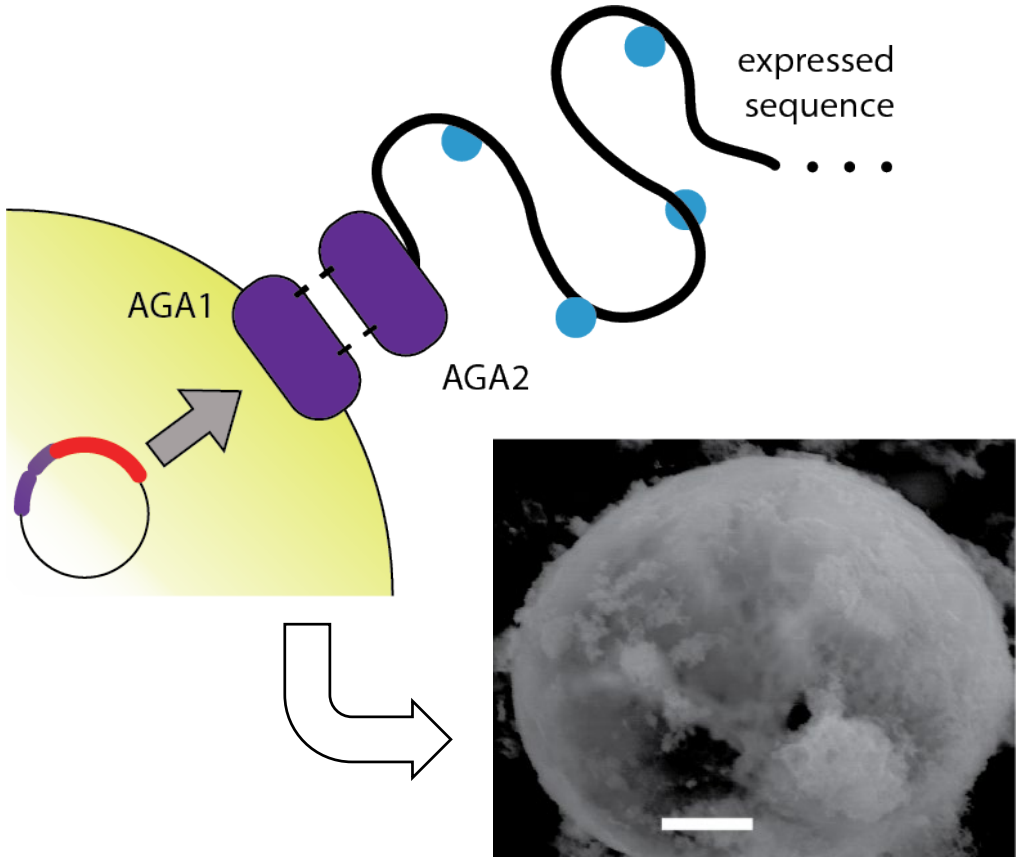
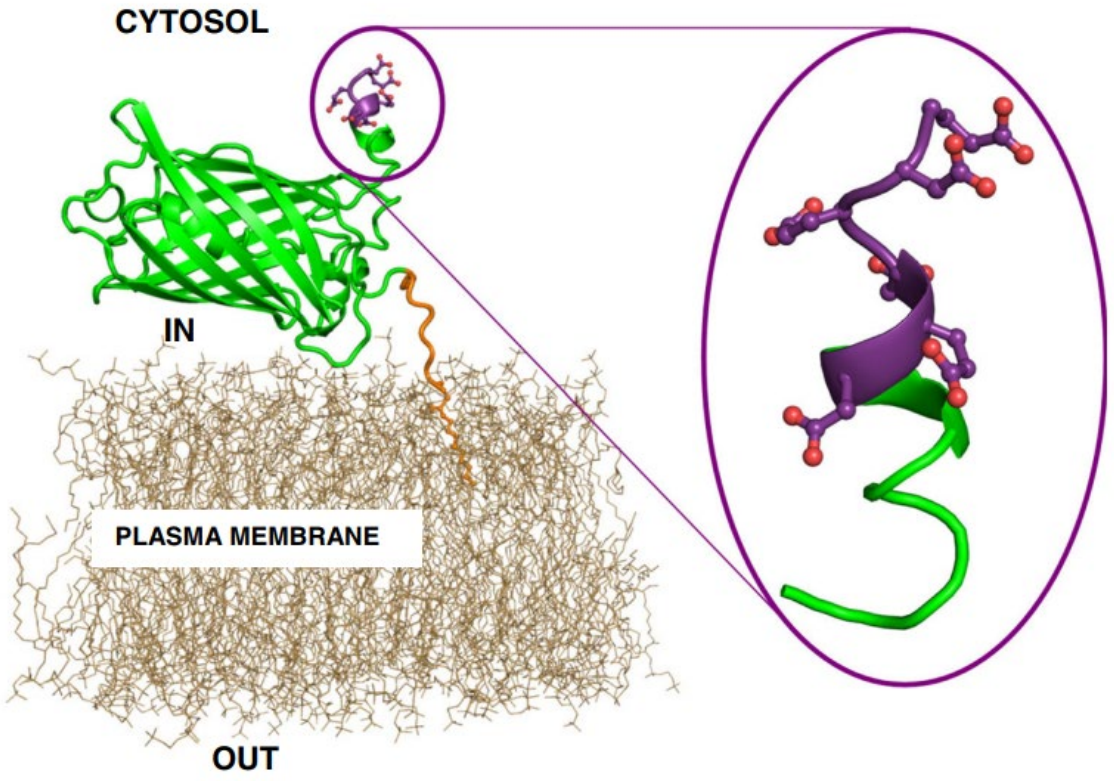
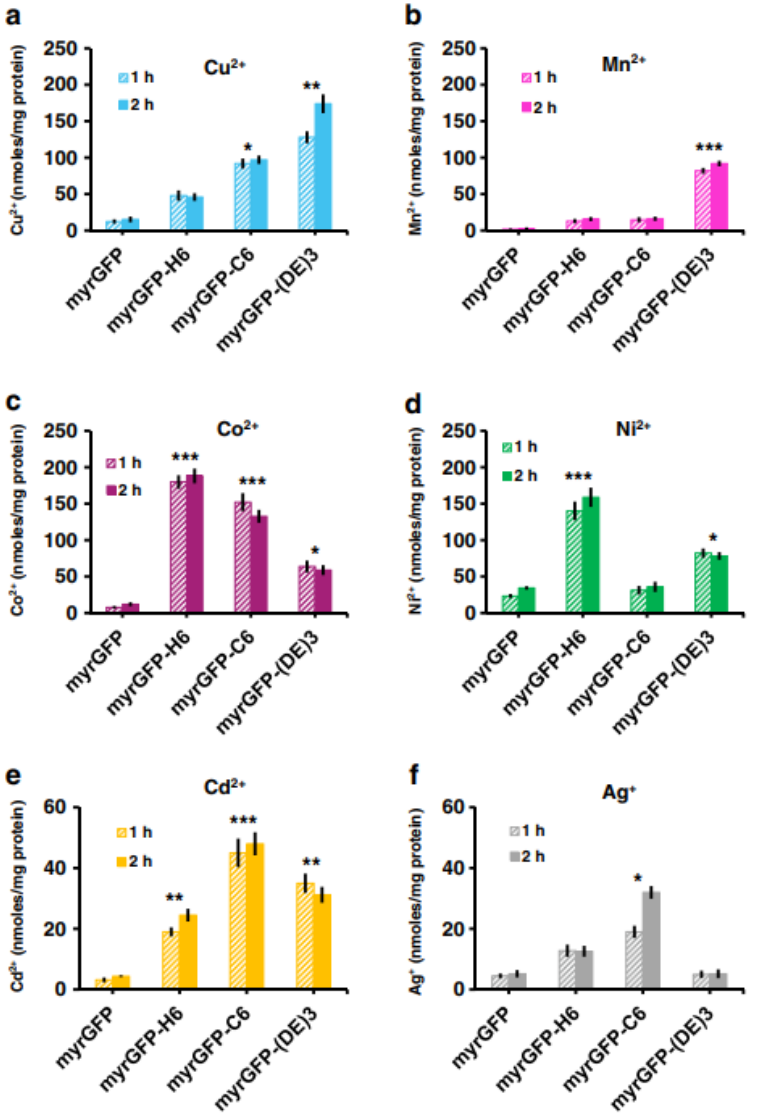


Past studies using display technology showed metal removal, but may not be enough



Ruta, Lavinia Liliana, et al. "Heavy metal accumulation by Saccharomyces cerevisiae cells armed with metal binding hexapeptides targeted to the inner face of the plasma membrane." (2017)



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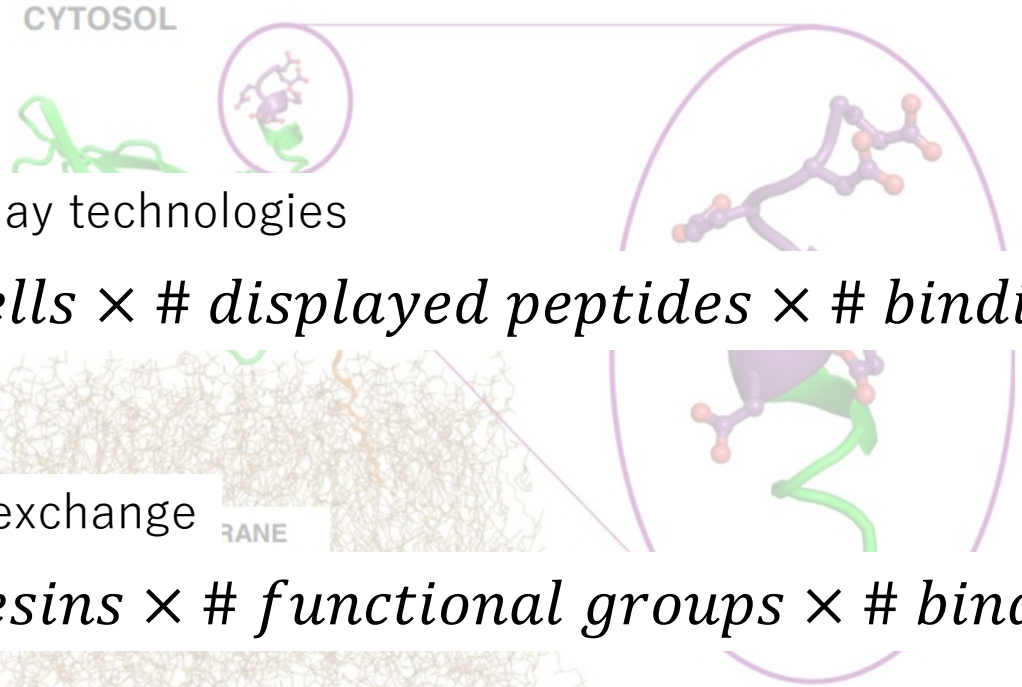
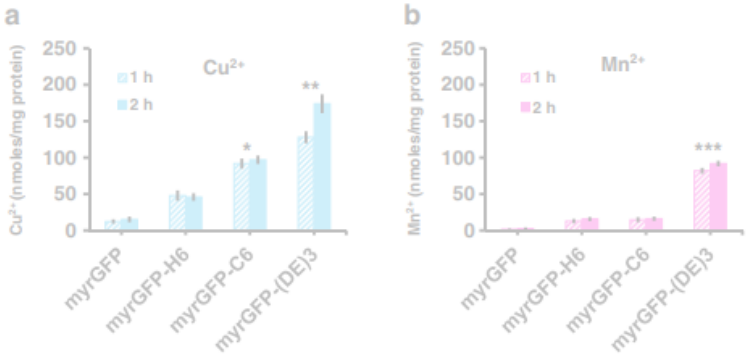
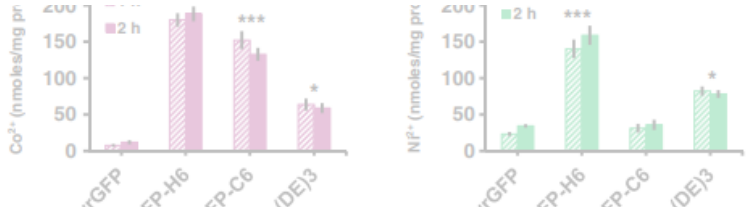


display technologies

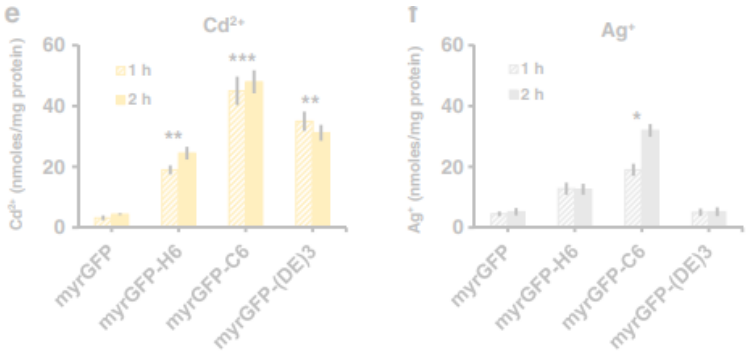
$$\#\ cells \times \#\ displayed\ peptides \times \#\ binding\ sites \approx 2\ nano\ molar\ (\mathbf{10^{-9}})$$





ion-exchange

$$\#\ resins \times \#\ functional\ groups \times \#\ binding\ sites > milli\ molar\ (\mathbf{10^{-3}})$$



Ruta, Lavinia Liliana, et al. "Heavy metal accumulation by Saccharomyces cerevisiae cells armed with metal binding hexapeptides targeted to the inner face of the plasma membrane." (2017)

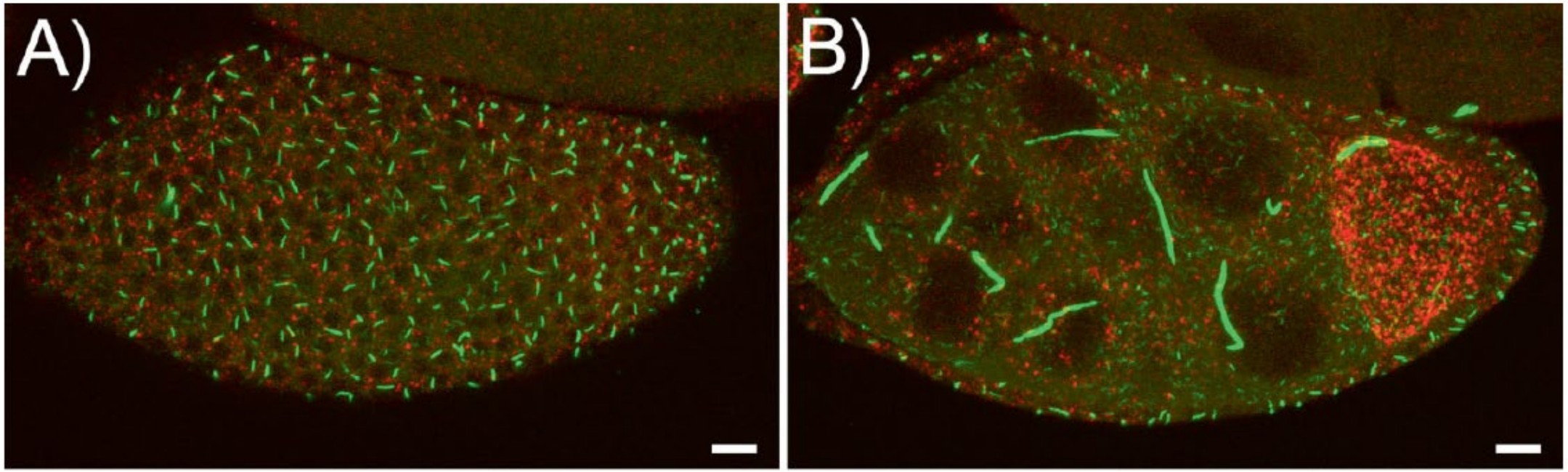
Discovery of aggregating proteins can inspire new methods for metal-exchange removal

BioEssays

Insights & Perspectives | Free Access

The enigmatic cytoophidium: Compartmentation of CTP synthase via filament formation

Ji-Long Liu

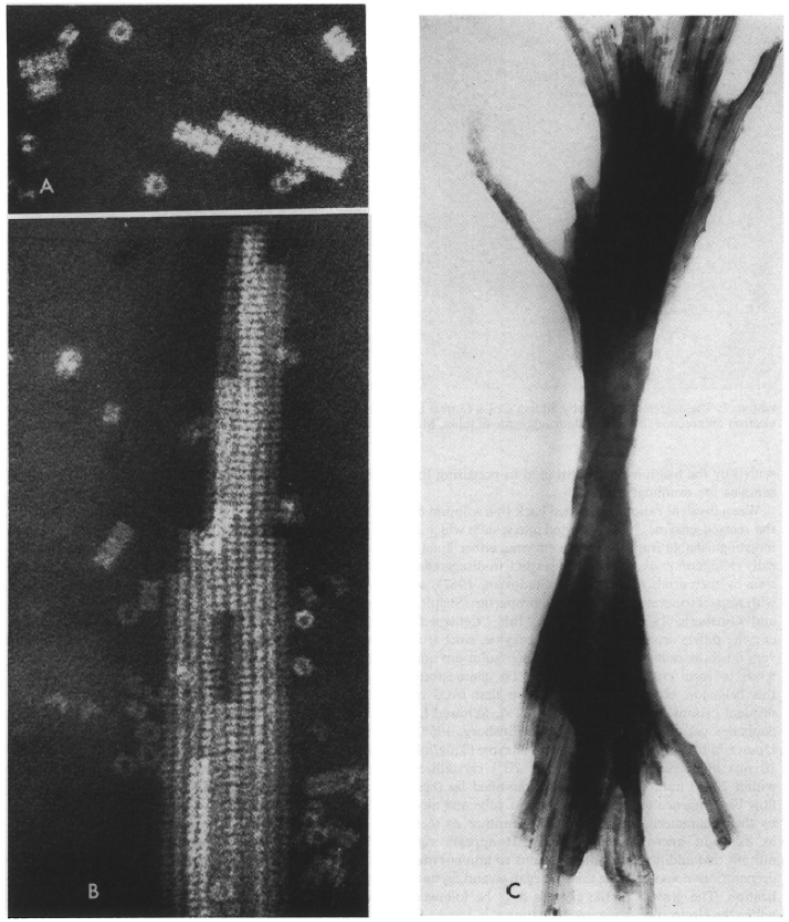


Numerous proteins with known enzymatic function have unique aggregating properties

Table I. **Proteins that assemble into intracellular structures**

Foci-forming protein	Biological process
Prs4p	5-phosphoribose 1-diphosphate biosynthetic process
Acs1p	Acetate fermentation/acetyl-CoA biosynthetic process/histone acetylation
Glt1p	**Ammonia assimilation cycle/glutamate biosynthetic process**
Ssd1p	Cell wall organization/chronological cell aging/replicative cell aging
Ura7p	**CTP synthesis**
Hsp42p	Cytoskeleton organization/response to stress
Rnr4p	Deoxyribonucleotide biosynthetic process
Rnr2p	Deoxyribonucleotide biosynthetic process
Psa1p	**GDP-mannose biosynthetic process/protein amino acid glycosylation**
Gln1p	Glutamine biosynthetic process/nitrogen compound metabolic process
Dug2p	Glutathione catabolic process
Gly1p	Glycine biosynthetic process/threonine catabolic process
Gsy2p	Glycogen biosynthetic process
Gdb1p	Glycogen catabolic process
Gph1p	Glycogen catabolic process
Hem2p	Heme biosynthetic process
His4p	Histidine biosynthetic process
Hek2p	Intracellular mRNA localization/telomere maintenance via telomere
Rim20p	Invasive growth in response to glucose limitation/protein processing/proteolysis
Sam1p	Methionine metabolic process/S-adenosylmethionine biosynthetic process
Sam2p	Methionine metabolic process/S-adenosylmethionine biosynthetic process
Hsp104p	Protein folding
Ssa1p	Protein folding
Sse2p	Protein folding
Ssa2p	Protein folding
Sis1p	Protein folding
Rpn9p	Proteosome assembly/ubiquitin-dependent protein catabolic process
Gcd6p	**Regulation of translation initiation**
Sui2p	**Regulation of translation initiation**
Gcd2p	**Regulation of translation initiation**
Gcd7p	**Regulation of translation initiation**
Gcn3p	**Regulation of translation initiation**
Sgt2p	Response to heat (glutamine-rich cytoplasmic protein of unknown function)
Thr1p	Threonine metabolic process
YAR009Cp	Transposition, RNA mediated
YLR143Wp	Unknown
YMR253Cp	Unknown

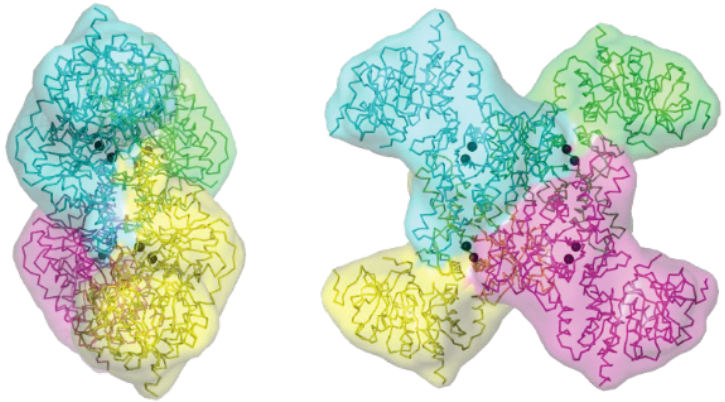
Narayanaswamy, R. *et al.* Widespread reorganization of metabolic enzymes into reversible assemblies upon nutrient starvation. *PNAS* 106, 10147–10152 (2009).

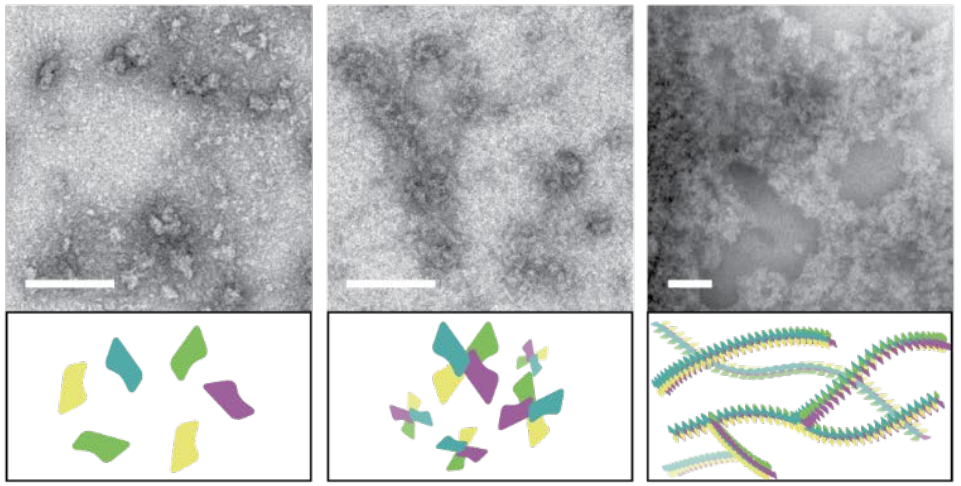


Valentine, R. C., Shapiro, B. M. & Stadtman, E. R. Regulation of glutamine synthetase. XII. Electron microscopy of the enzyme from Escherichia coli. *Biochemistry* 7, 2143–2152 (1968).

pyrG and glnA from *E. coli* have metal-inducible aggregation behavior

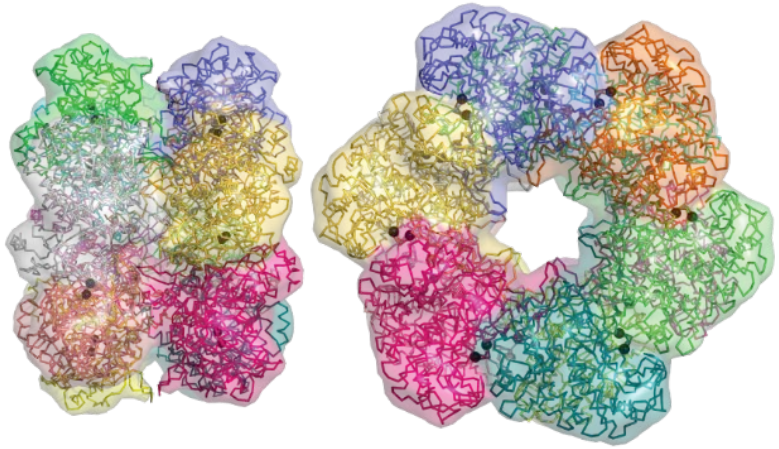
pyrG

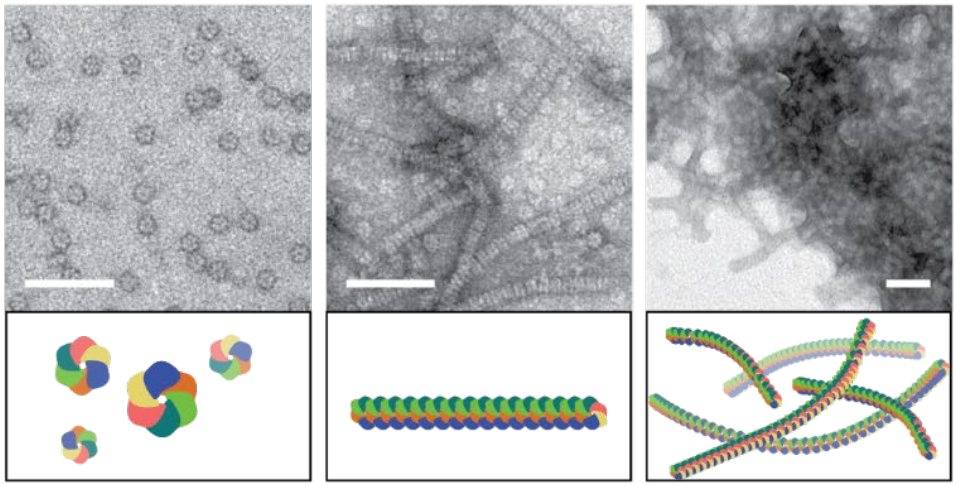




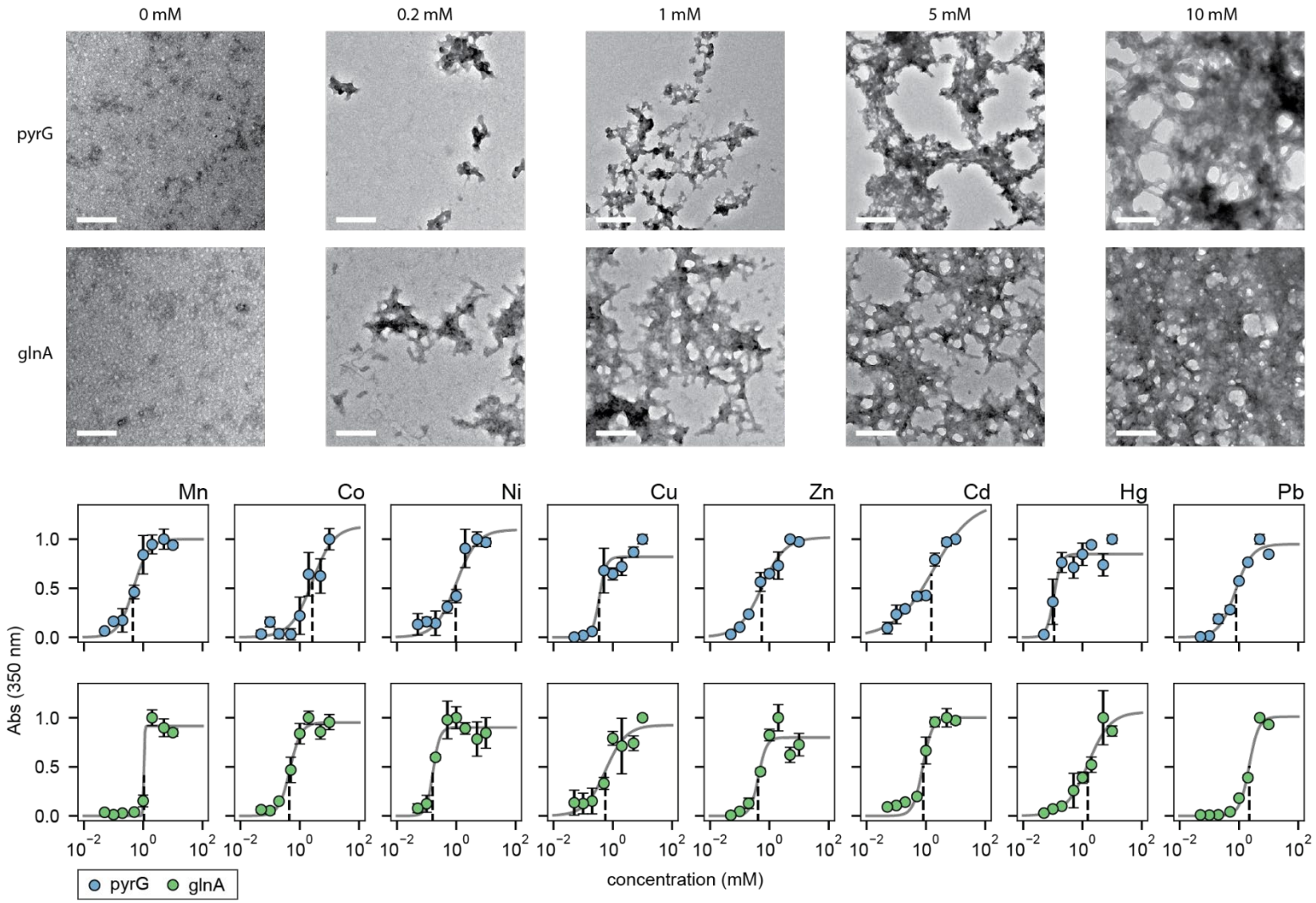
scale bars = 50 nm

glnA



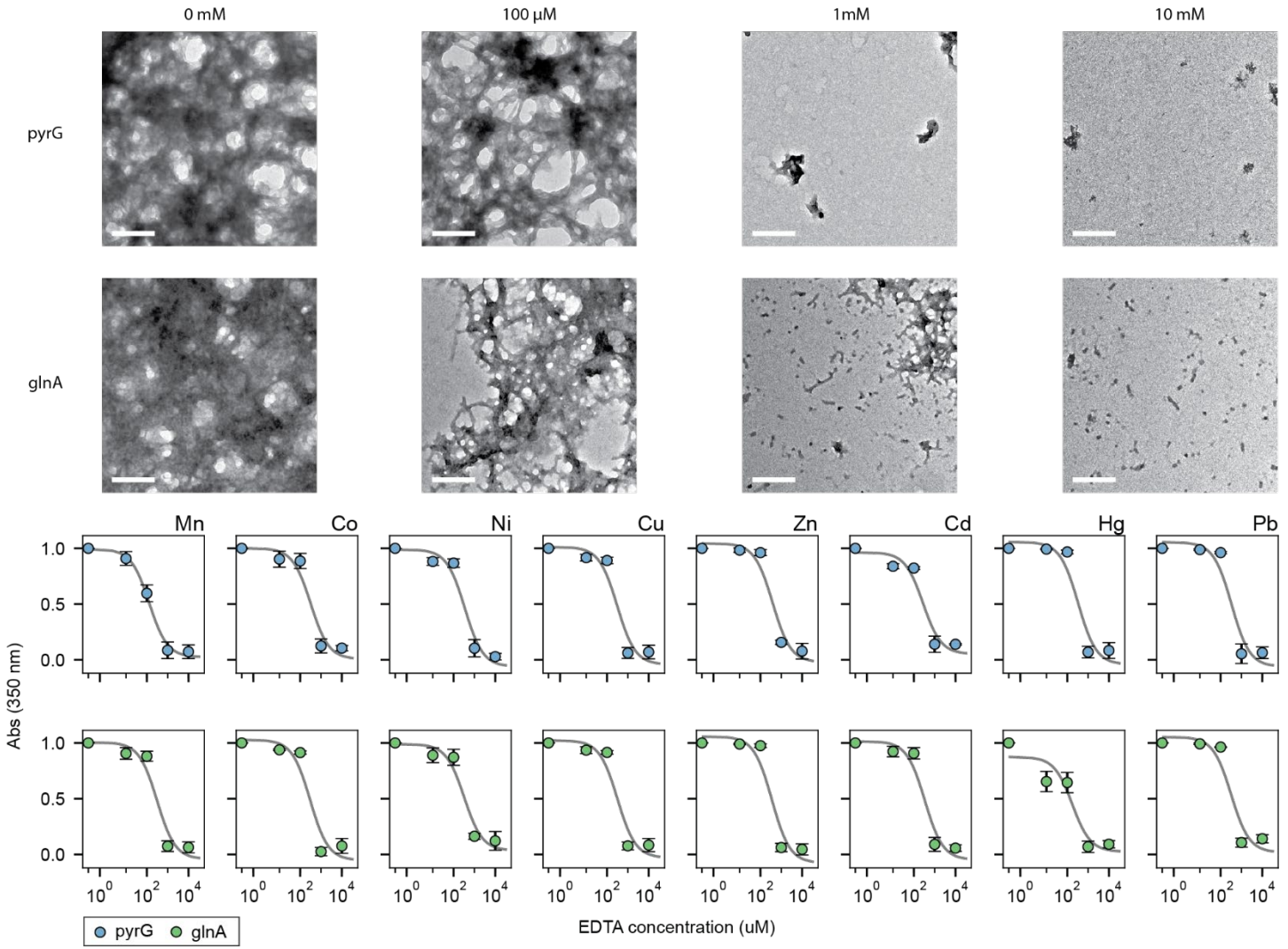


Aggregation intensity is induced by metal concentration



scale bars = 200 nm

Absence or competition of metals reverses aggregation



scale bars = 200 nm

Engineering these proteins to act as both ‘resin’ and ‘exchanger’

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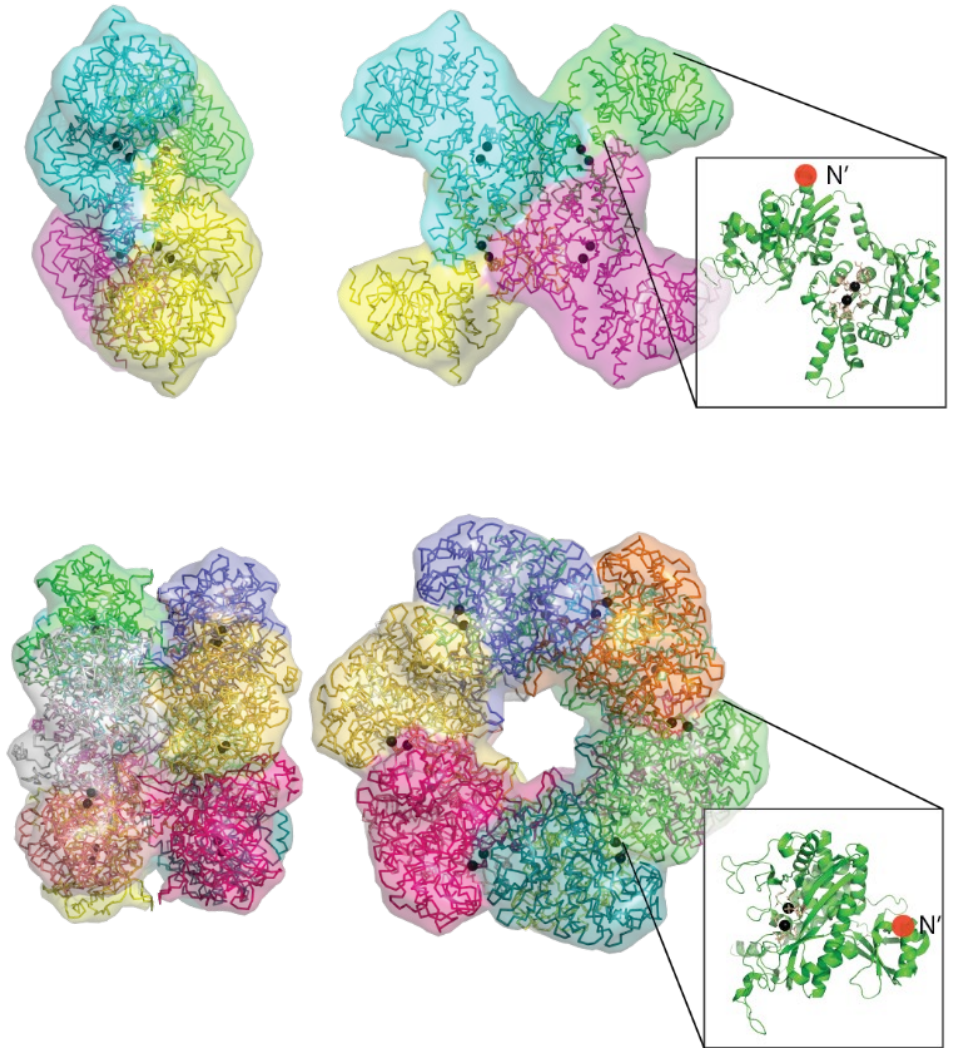
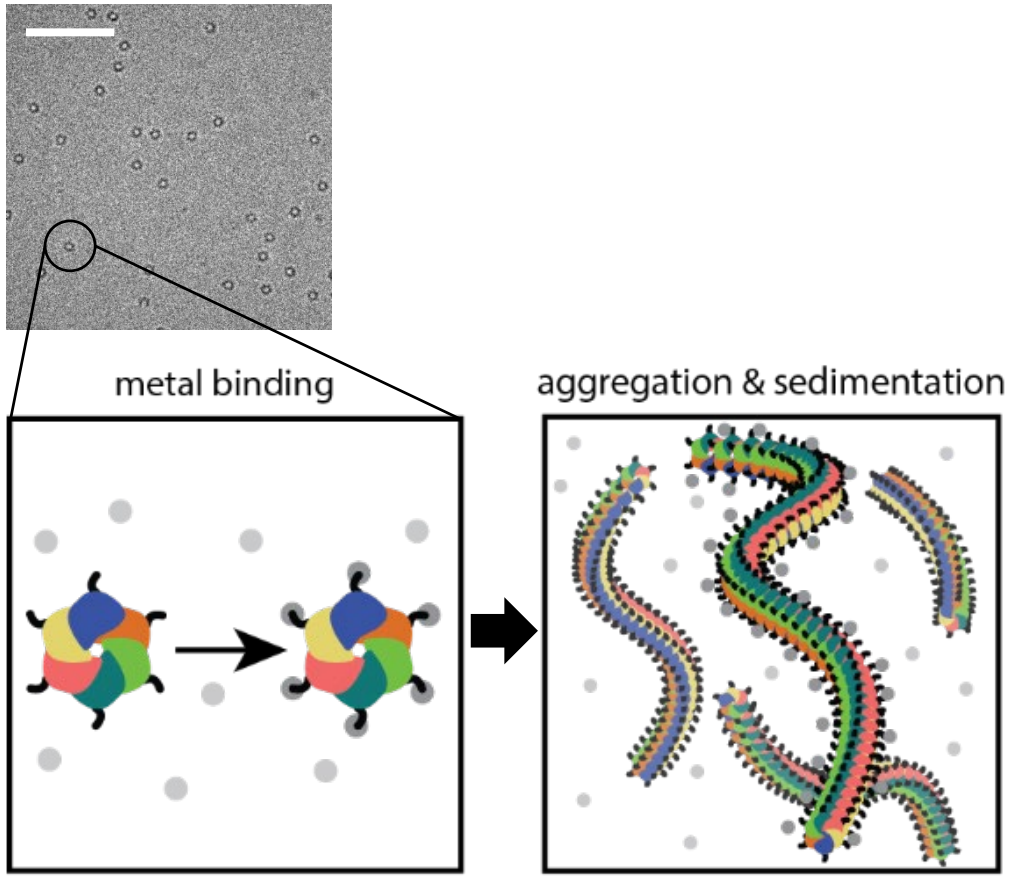
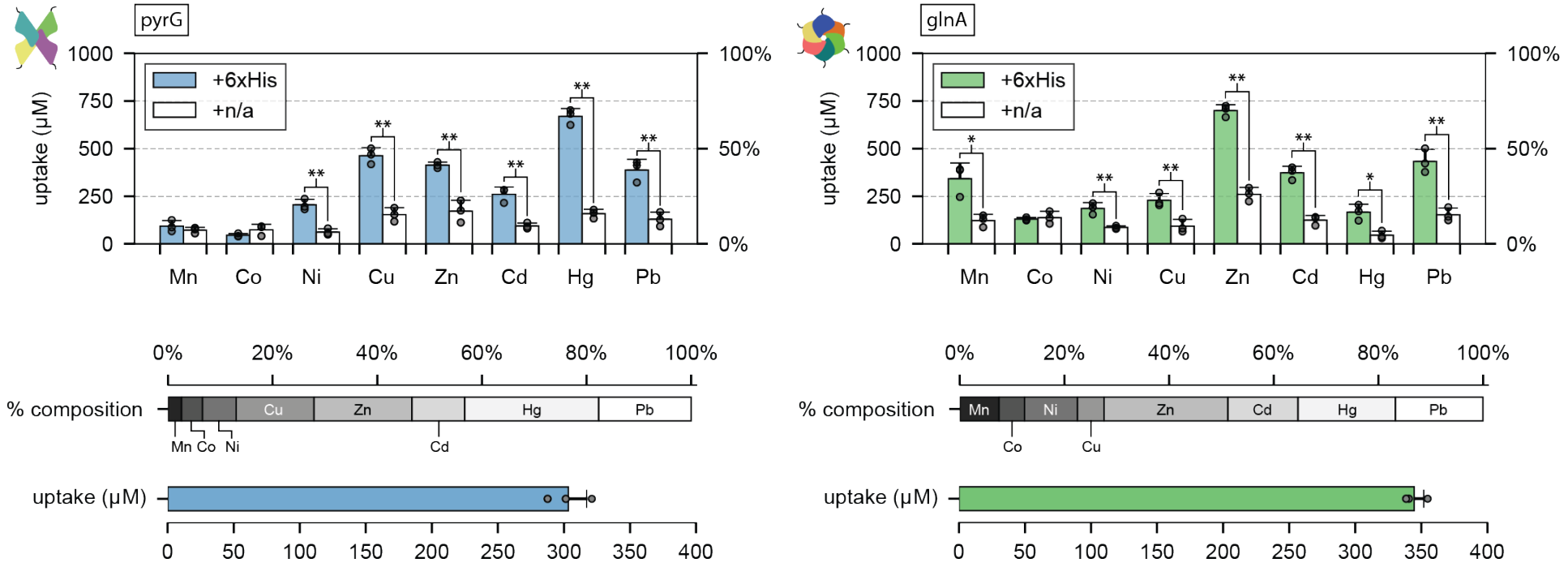


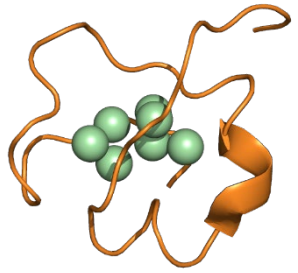


Surface modification with a His-tag helps chelate and sediment bound metals

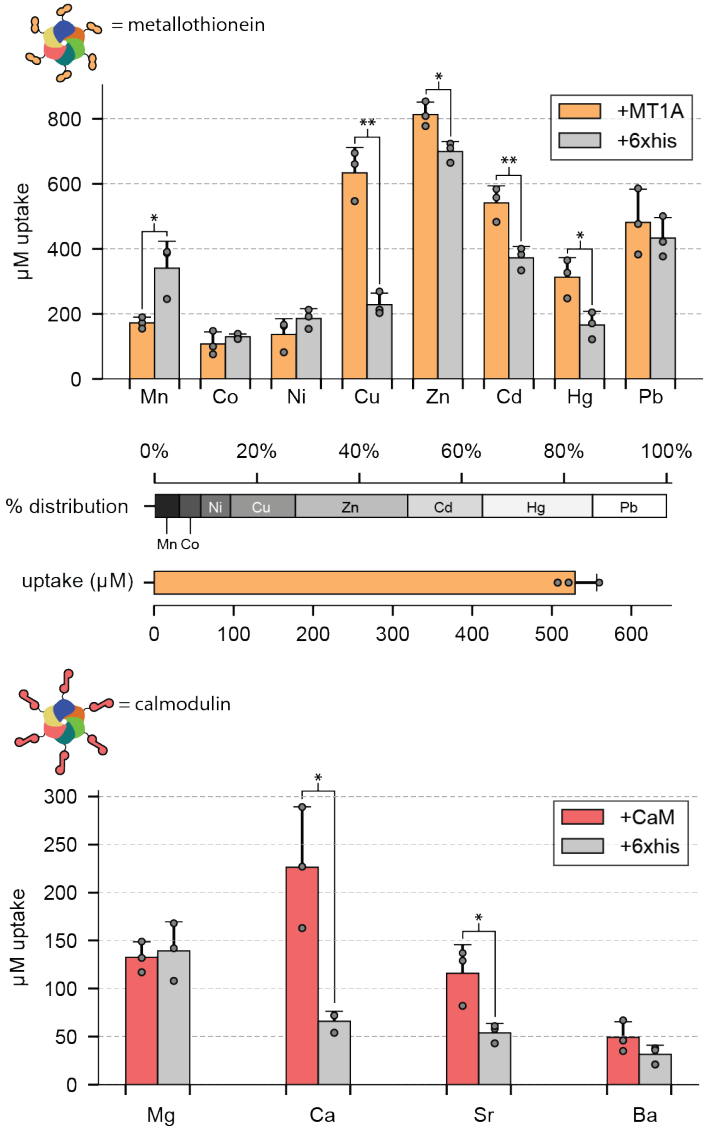
Metal removal profile is determined by aggregation propensity and exchanger



Changing the metal binding domain alters the metal removal profile

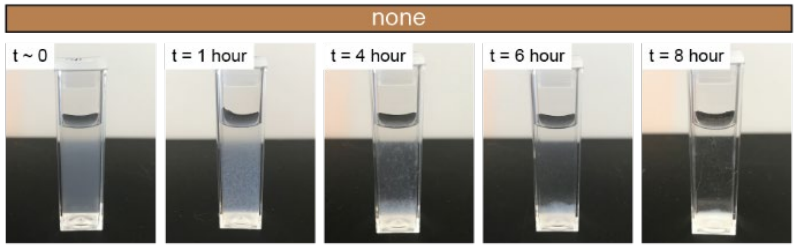


metallothionein
PDB: 1AQR



calmodulin
PDB: 1EXR

Further addition of a binding motif allows for anchorage onto denser substrates



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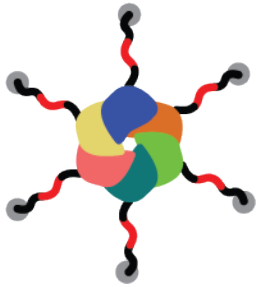

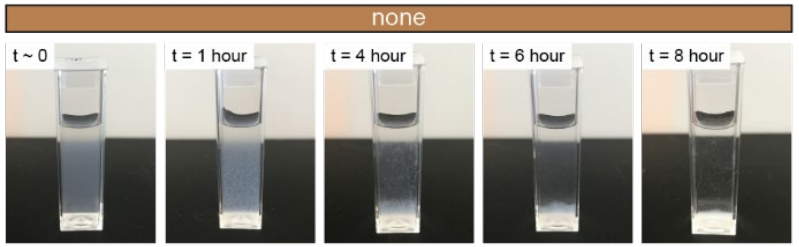

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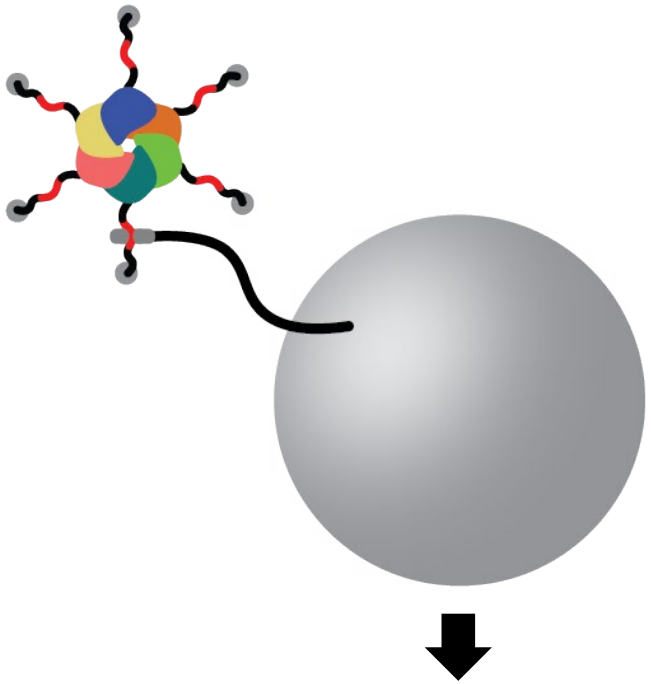
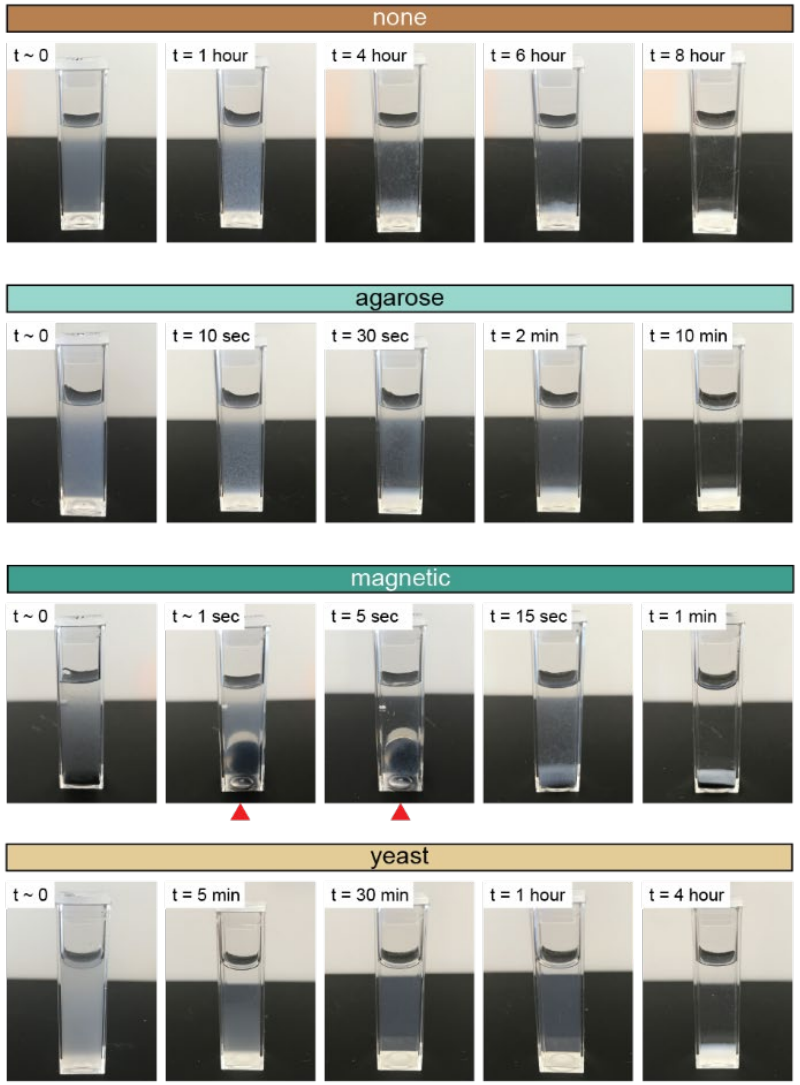
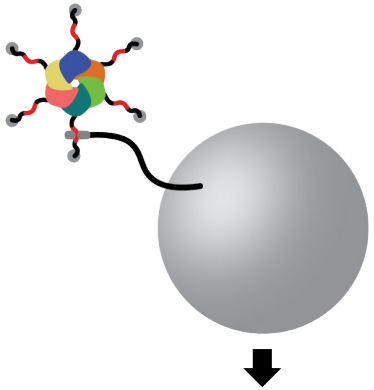


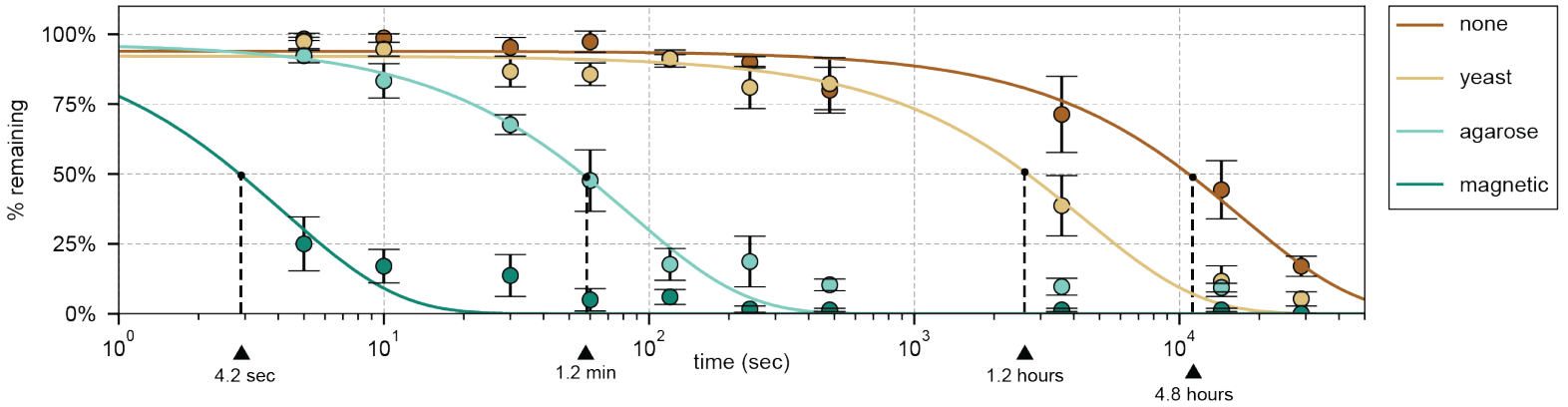
gravity
magnetically
biologically



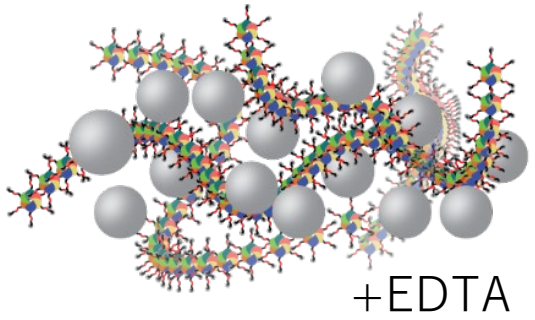
Further addition of a binding motif allows for anchorage onto denser substrates

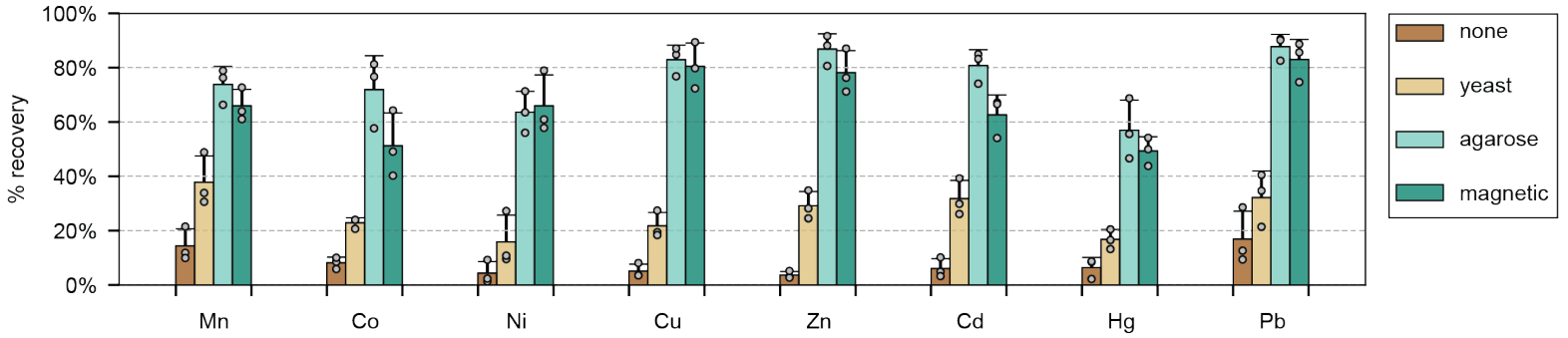
sedimentation





recovery



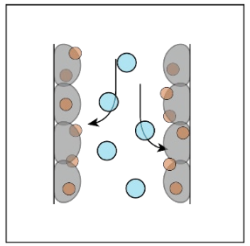


Takeaway

Synthetic

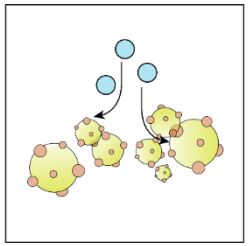
Biological

Ion-exchange



Grab

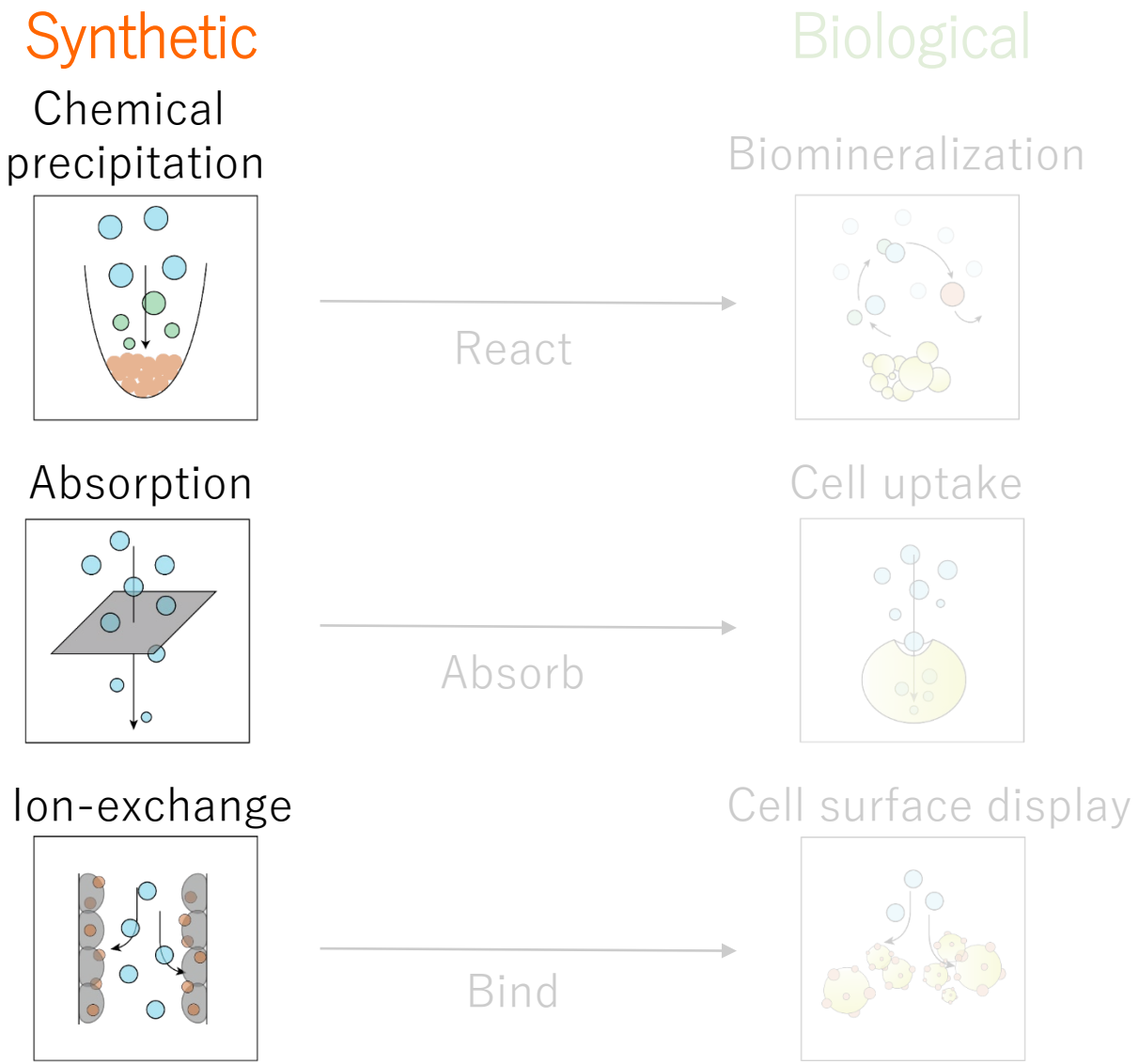
Cell surface display



TAKEAWAY

- Display technology is possible, but does not scale
- Aggregation behavior of recently discovered proteins can be engineered to be ion-exchange like
- Surface modifications offer new ways to remove metals

The bigger picture: insight on industrial logistics, scalability, and accessibility



The infrastructure for physicochemical processes is complex

React

Absorb

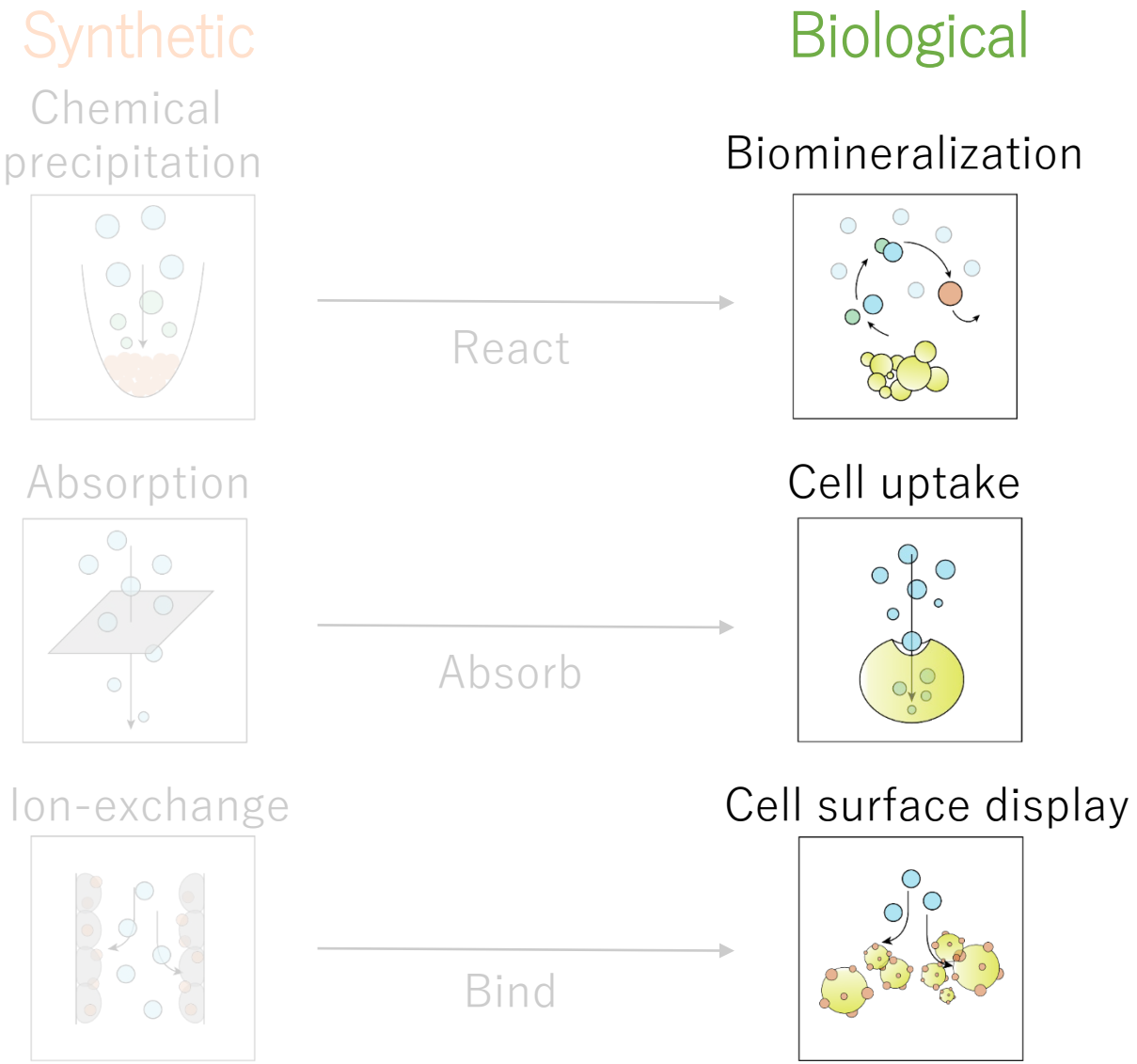
Bind

Infrastructure

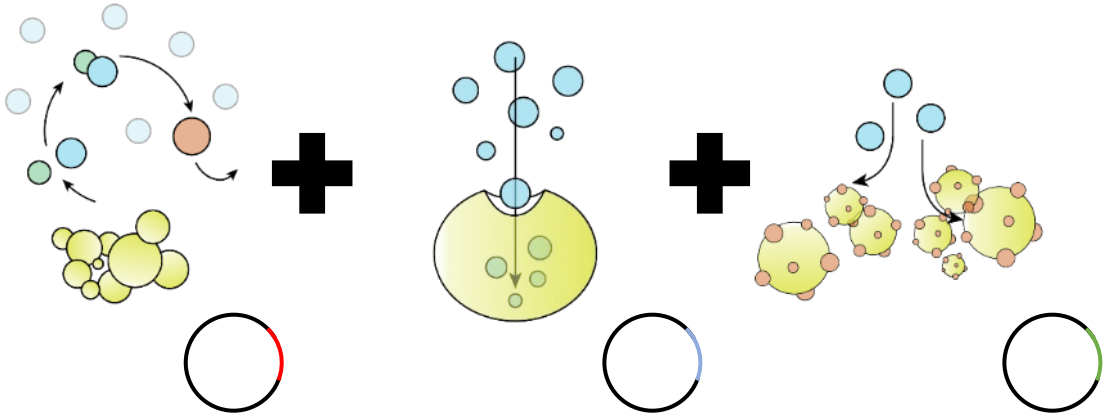
Modularity

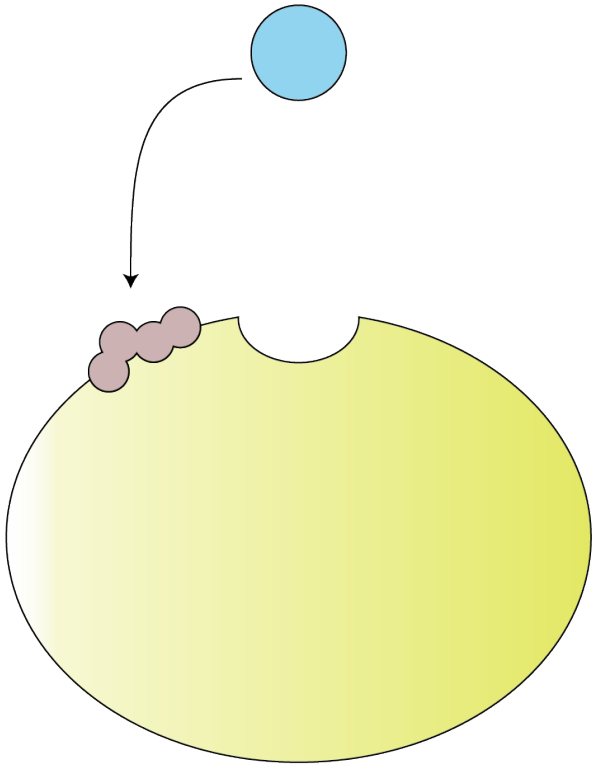
Maintenance

The bigger picture: insight on industrial logistics, scalability, and accessibility



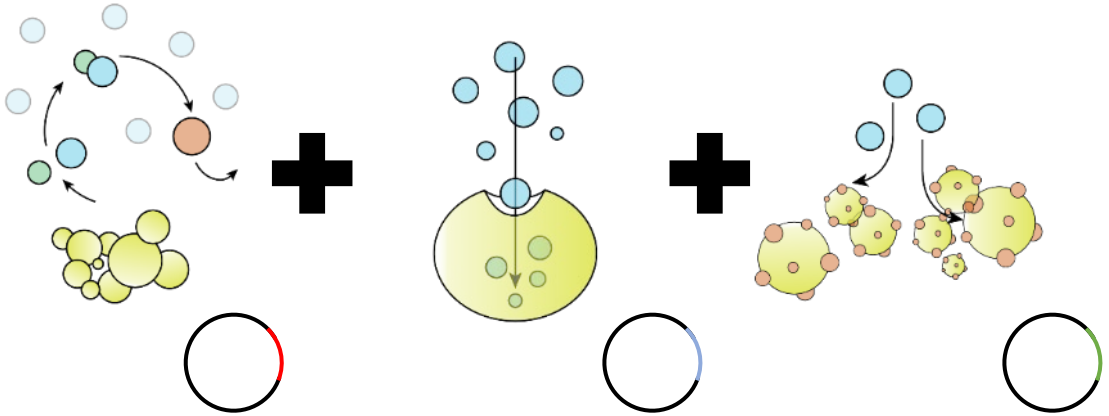
Yeast are both the factories and engineers, they only need instructions

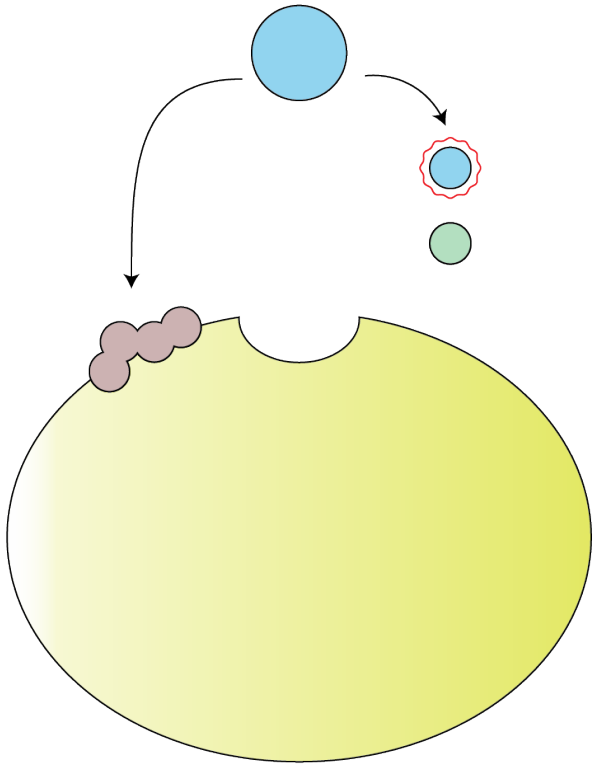




Strategy 1

Yeast are both the factories and engineers, they only need instructions





Strategy 1

Strategy 1+3

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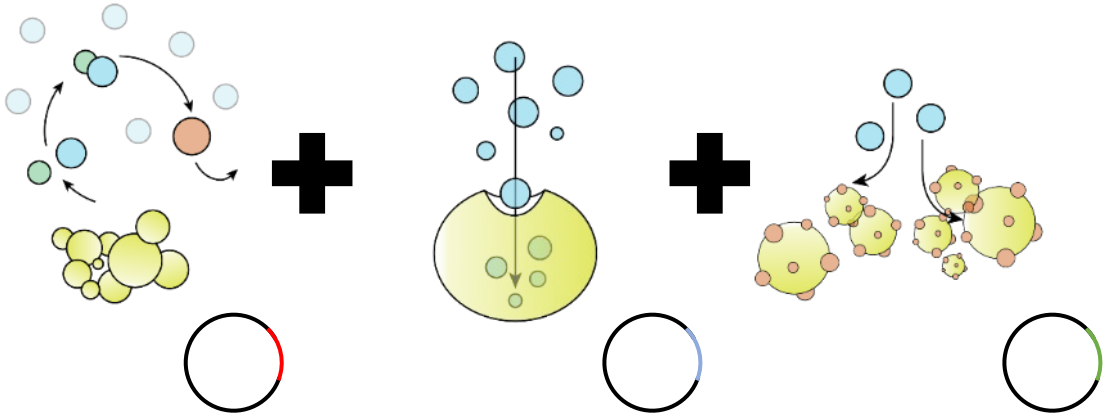

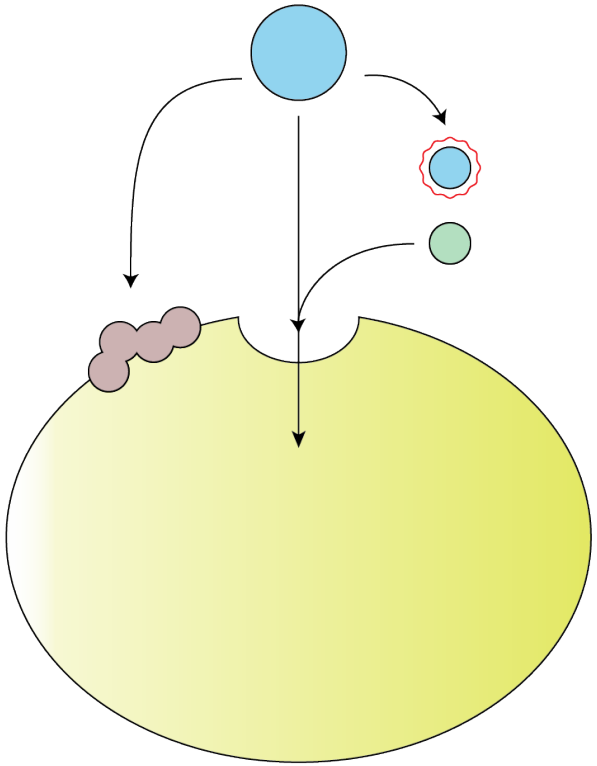

Strategy 1

Strategy 1+3

Strategy 2+3

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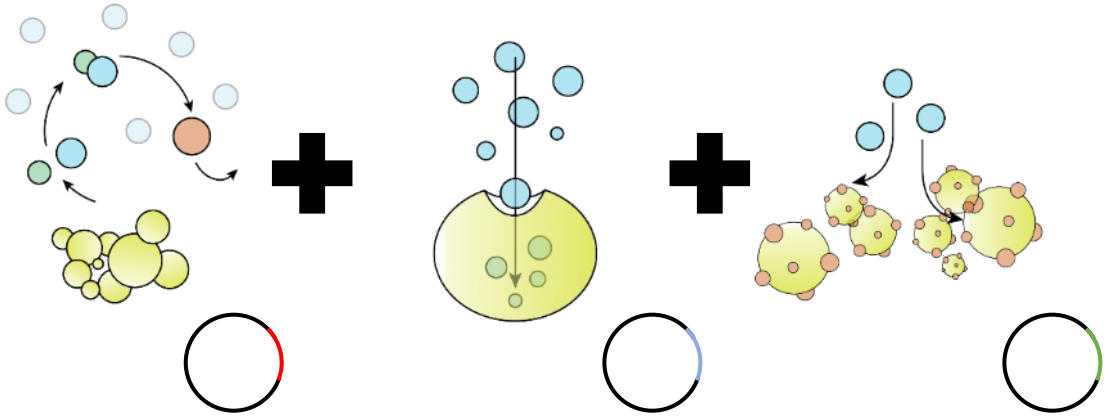

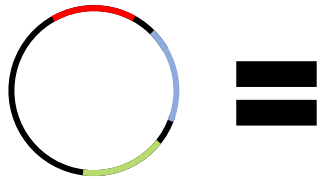

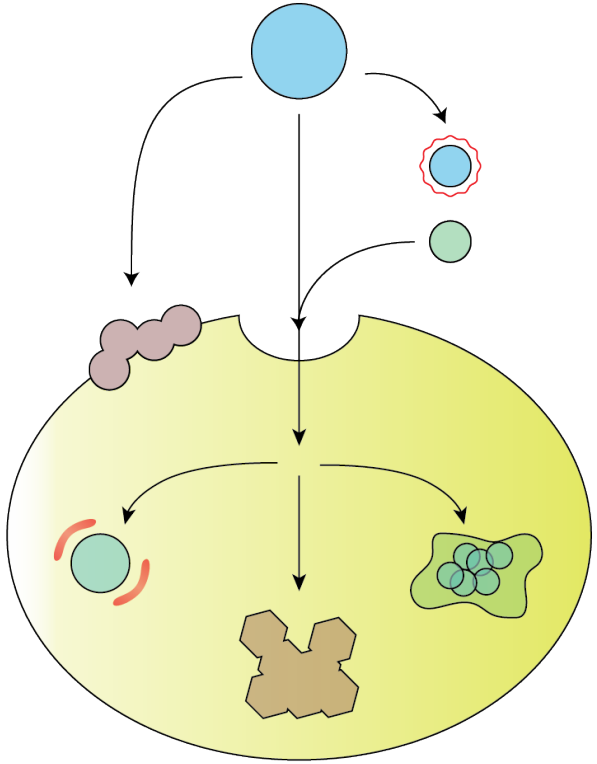

Strategy 1

Strategy 1+3

Strategy 2+3

Strategy 1+2+3

Strategy … 4, 5, 6, etc

Cost of physicochemical processes versus expected cost of yeast

[1]

method	cost (US$)
chemical precipitation	$ 20–500
ab(d)sorption	$ 50–150
ion-exchange	$ 50–200

metal	EPA limit (ppm)	metal mass (g)	yeast mass (g)	culture volume (L)	cost (US$)
Cr	0.1	100	10000	20000	$ 3200
Mn	0.05	50	5000	10000	$ 1600
Fe	0.3	300	30000	60000	$ 9600
Co*	0.1	100	10000	20000	$ 3200
Ni	0.1	100	10000	20000	$ 3200
Cu	1.3	1300	130000	260000	$ 41600
Zn	5	5000	500000	1000000	$ 160000
As	0.01	10	1000	2000	$ 320
Cd	0.005	5	500	1000	$ 160
Hg	0.002	2	200	400	$ 64
Pb	0.015	15	1500	3000	$ 480

10 OD_{600}
1% yeast biomass
[2] 16 cents per L

[1] Gupta, Vinod Kumar, et al. "Chemical treatment technologies for waste-water recycling—an overview." *Rsc Advances* 2.16 (2012): 6380-6388.
[2] Harrison, Roger G., Paul Todd, Paul W. Todd, Scott R. Rudge, and Demetri P. Petrides. 2015. *Bioseparations Science and Engineering*. Oxford University Press.

[*] cobalt toxicity limit was inferred

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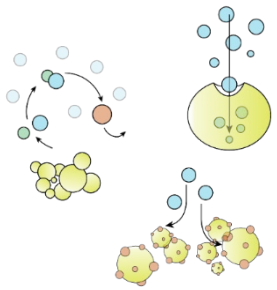
The potential impact of the yeast market on water remediation

[3] 195 billion liters of yeast

1.07 million tons of yeast



1% capture capacity dry weight

10,000 tons of metal removed

9.8 trillion liters of water remediated at 1 ppm (EPA limit)
or
~50X ratio of yeast culture to water remediated

[4] **3.9 million** olympic swimming pools

[5] 309 thousand pools &
10 million residential pools in the US

[3] Barth-Haas Group. 2019. "Worldwide Beer Production, 2017 | Statistic." Statista. May 12, 2019. https://www.statista.com/statistics/270275/worldwide-beer-production/.

[4] https://www.livestrong.com/article/350103-measurements-for-an-olympic-size-swimming-pool/

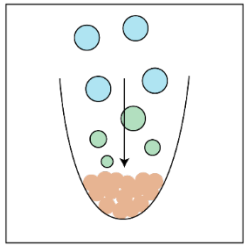
[5] https://www.thespruce.com/facts-about-pools-spas-swimming-safety-27371

Biological platforms provide a natural alternative to physicochemical technologies

Synthetic

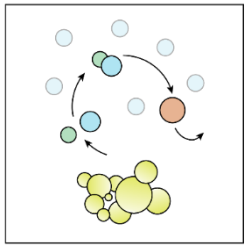
Biological

Chemical precipitation

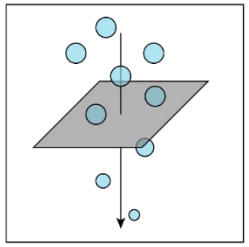


React

Biomineralization

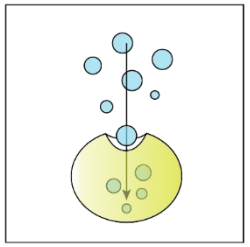


Absorption

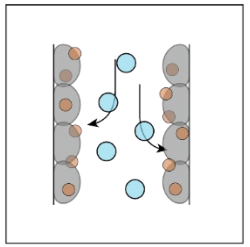


Absorb

Cell uptake

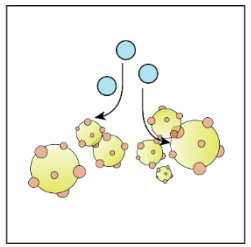


Ion-exchange

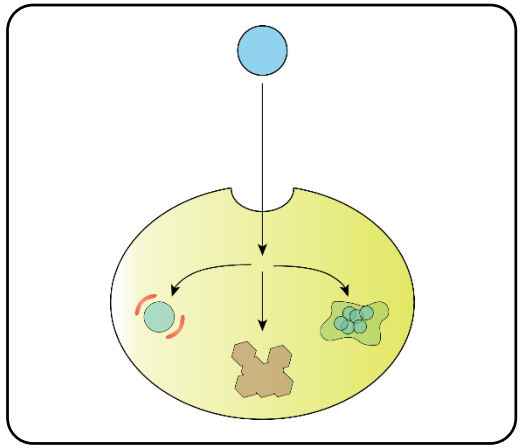


Bind

Cell surface display

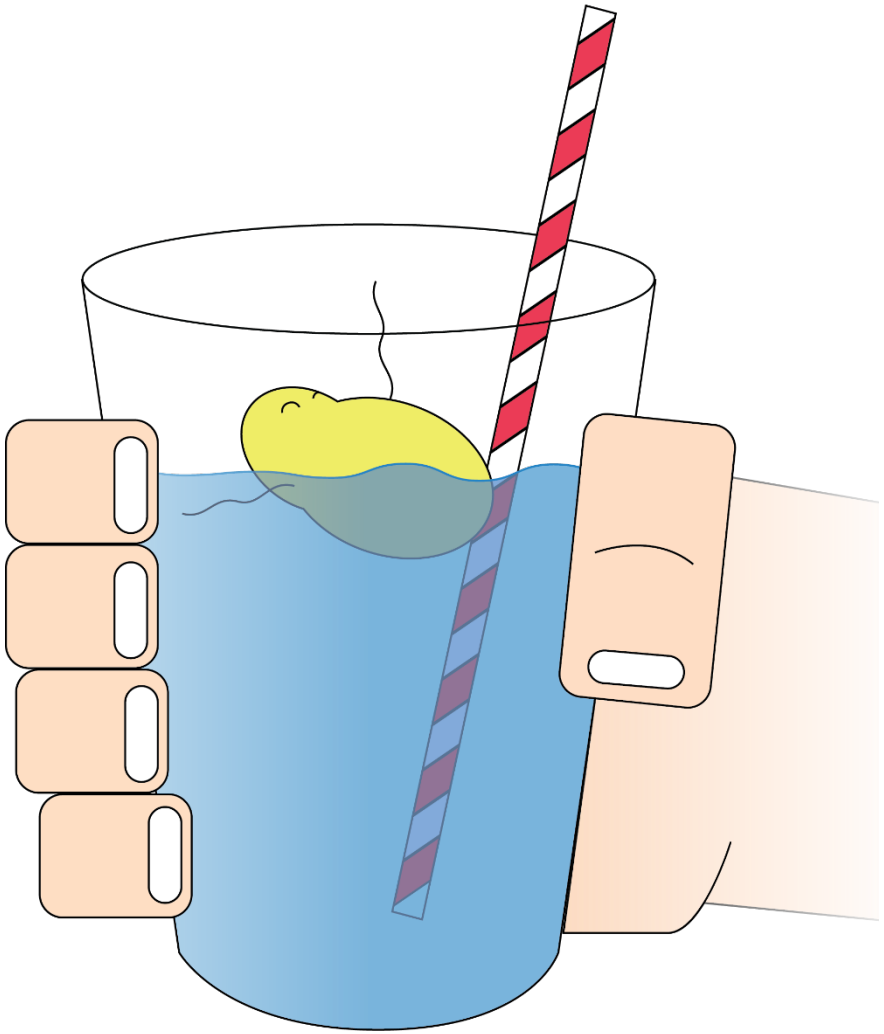


Fully customized yeast strain



Real world waste treatment

Happy Drinking



Thank you

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- Summer Morrill
- Dan Corbi
- Hilla Weidberg

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- Prerna Bhargava
- Diana Chien
- Sean Clarke
- Comm Fellows

Facilities

- CMSE – ICP, TEM, SEM, XRD, Fluor.
- DCIF – NMR
- Koch Flow Core
- Koch Microscopy Core
- Koch High Throughput Core
- Koch Nanotechnology Core
- Whitehead Keck Microscopy

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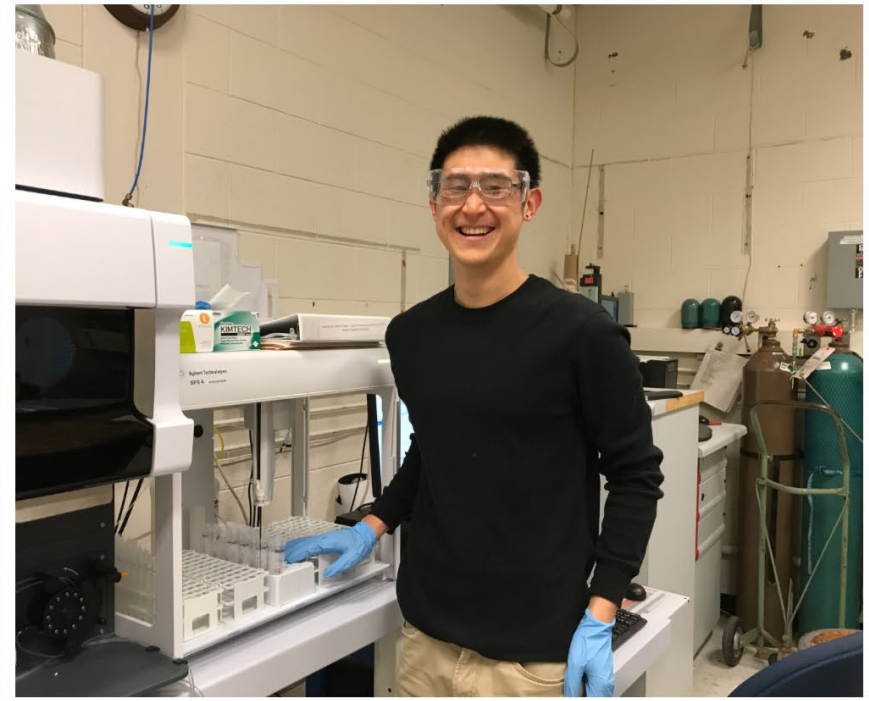
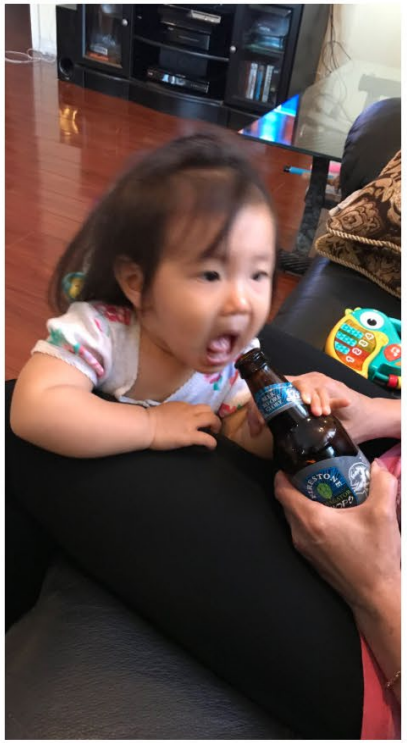
- Bose Grant
- CEHS Pilot Grant
- Shell


The Royal Room






































ROLL ON
YOU
BEARS





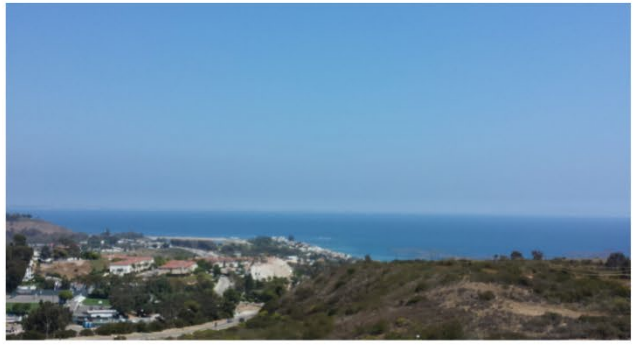




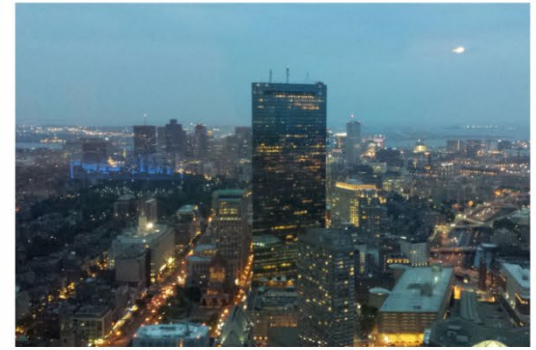




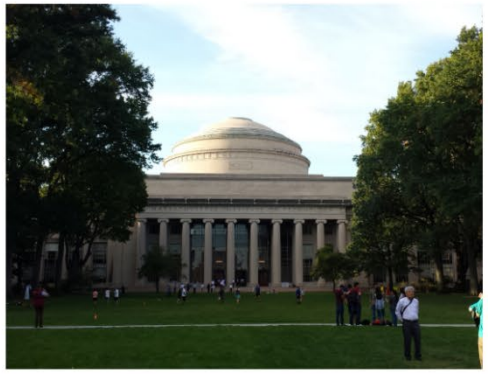
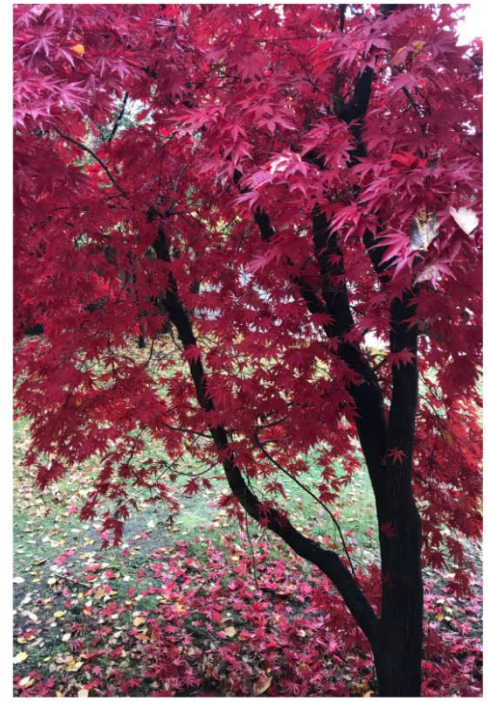


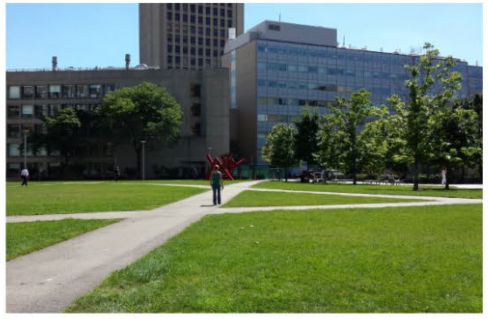


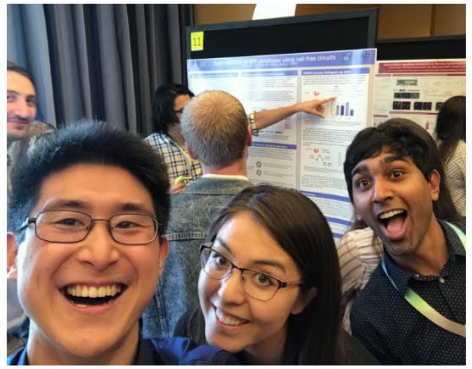




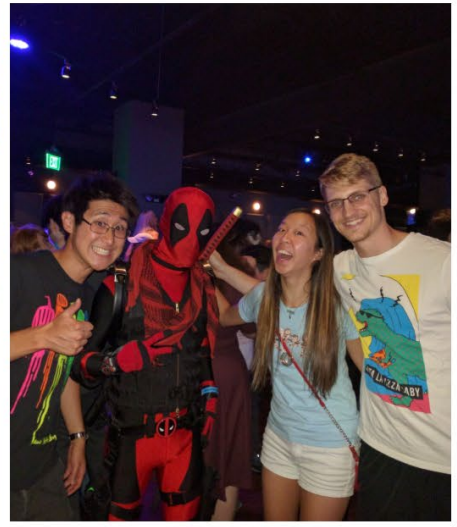


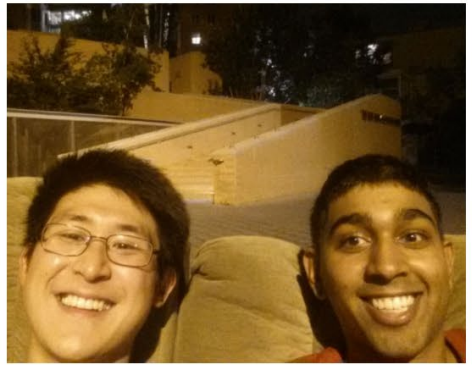






ESCAPE
SHE'S HOT
ZOMBIE SLAYER LEVEL 100
NO Brains FOR you!
WE WON
MY BOSS MADE ME Do This
I'M SMARTER THAN A ZOMBIE






Questions