

Engineering Yeast for Heavy Metal Waste Remediation

Supplemental Materials

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SUPPLEMENTAL – Stats



Who should care?



Who should care?



The yeast market is one of the most mature industries in the world

Brew



Package



Disseminate



Store



Weight removed per yeast dry weight

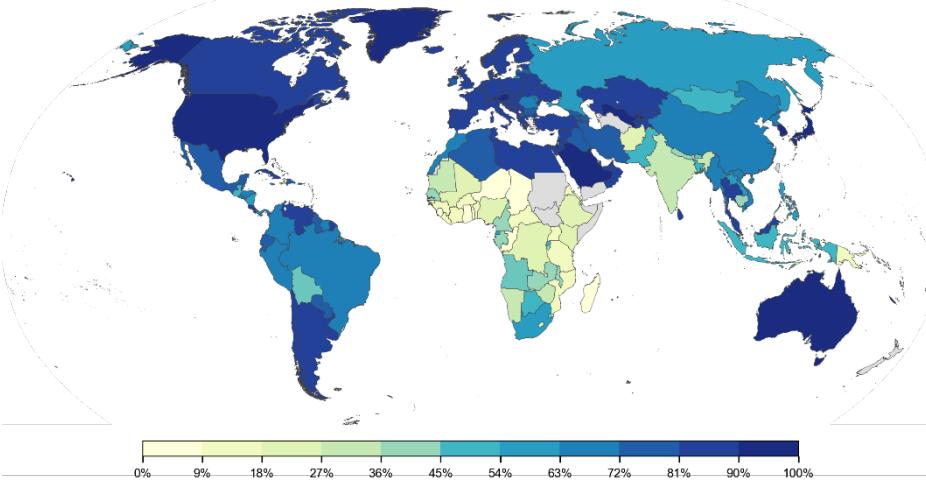
	Cr	Mn	Fe	Cu	Zn	As	Cd	Hg	Pb
MW	51.9	54.9	55.8	58.9	63.38	74.9	112.41	200.5	207.2
low	0	10	10	50	10	0	60	40	60
high	25	500	30	100	50	25	100	60	80
hyper threshold	0.10%	1.00%	0.10%	0.10%	1.00%	0.01%	0.01%	0.10%	1.00%
percent capture (low)	0.00%	0.11%	0.11%	0.59%	0.13%	0.00%	1.35%	1.60%	2.49%
percent capture (high)	0.26%	5.49%	0.56%	1.18%	0.63%	0.32%	2.25%	2.41%	3.32%
mutants	Sull, Sul2	S*BC, S*BCT	FTR1, FTR4, S+C1	CTR1, CTR3, S+C1	n/a	Pho84, Pho87, Pho89	S*BCT, mCd, ΔM17	ΔM17, ΔH2, ΔC4, ΔHM	ΔM17, ΔH2, ΔC4, ΔHM

Weight removed per protein dry weight

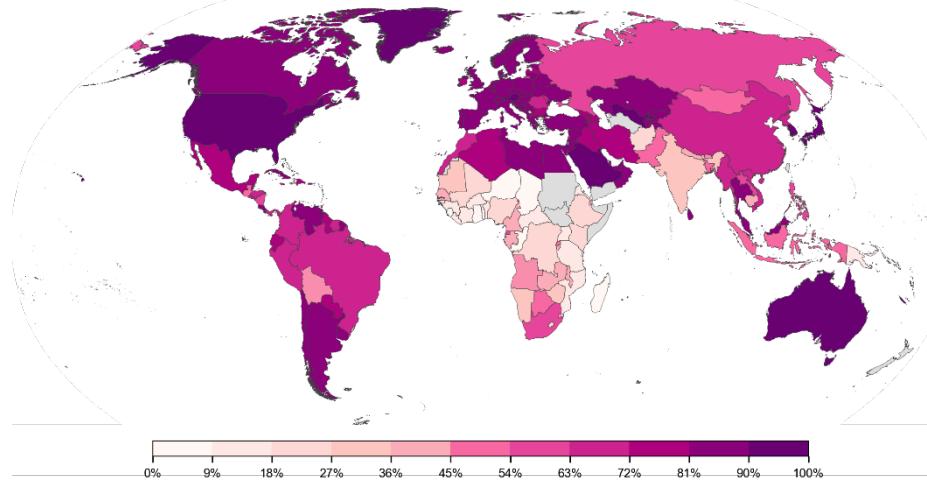
	Mn	Co	Ni	Cu	Zn	Cd	Hg	Pb	Ca
MW	54.94	58.93	58.69	63.546	65.38	112.411	200.592	207.2	40.078
pyrG (62.5 kDa)	92.7 0.08%	45 0.04%	204.7 0.19%	462.7 0.47%	413.1 0.43%	259.7 0.47%	669.7 2.15%	387 1.28%	- -
glnA (54 kDa)	341.3 0.35%	130 0.14%	186.3 0.20%	228.3 0.27%	699.7 0.85%	372.7 0.78%	166 0.62%	433.3 1.66%	- -
+CaM (70.3 kDa)	-	-	-	-	-	-	-	-	226.3 0.13%
+MT1A (58.9 kDa)	172.3 0.16%	108 0.11%	137.3 0.14%	634.3 0.68%	813.7 0.90%	541.7 1.03%	313.3 1.07%	482 1.70%	- -

Global water accessibility and burden

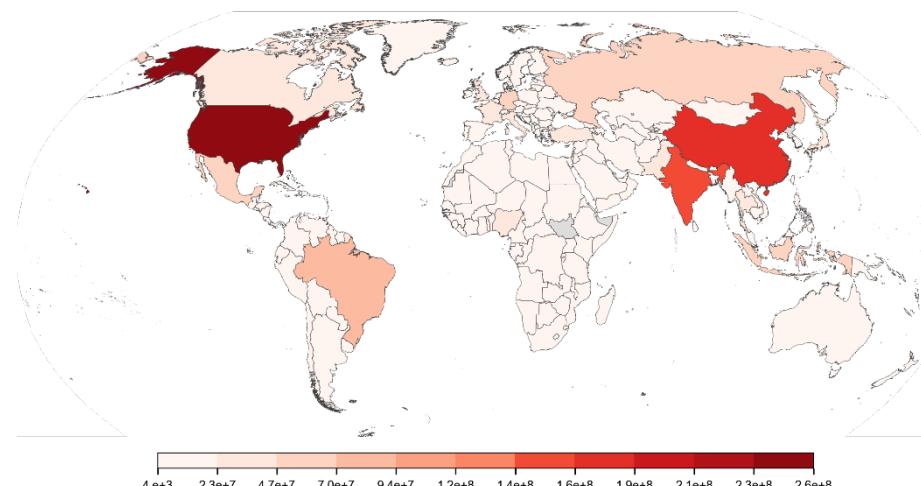
Access to clean water



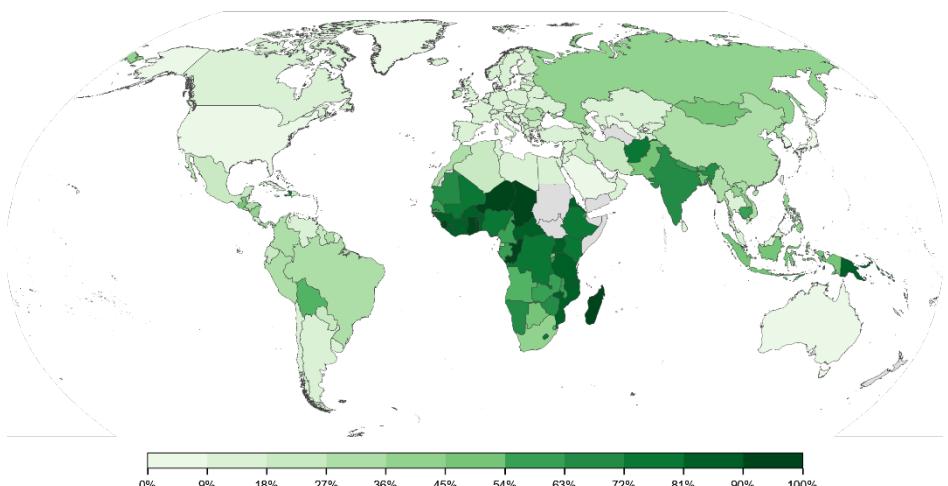
Water usage



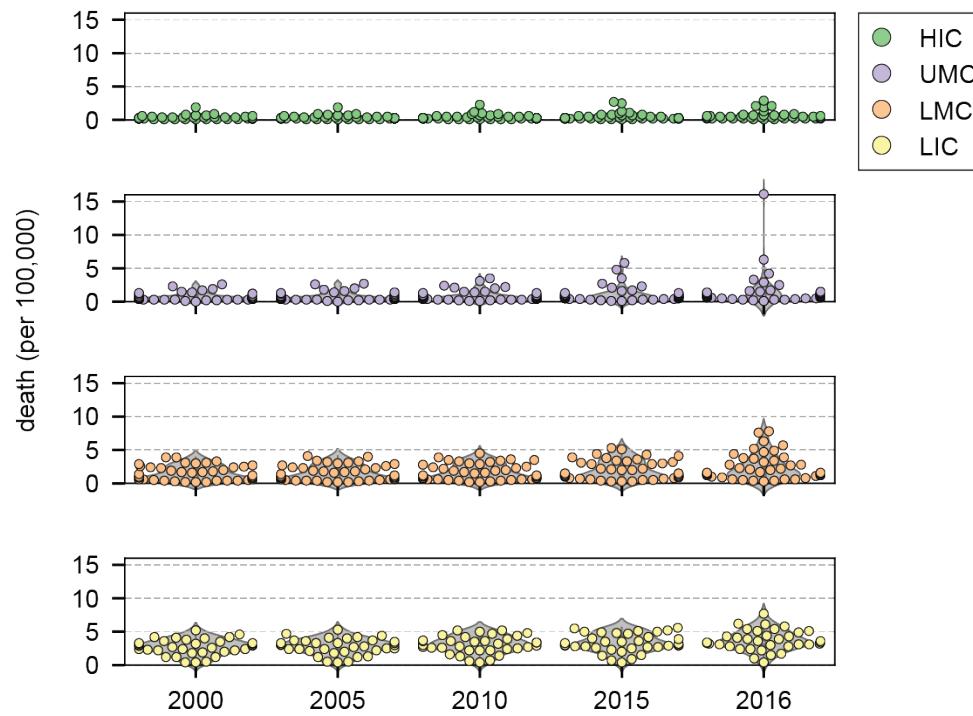
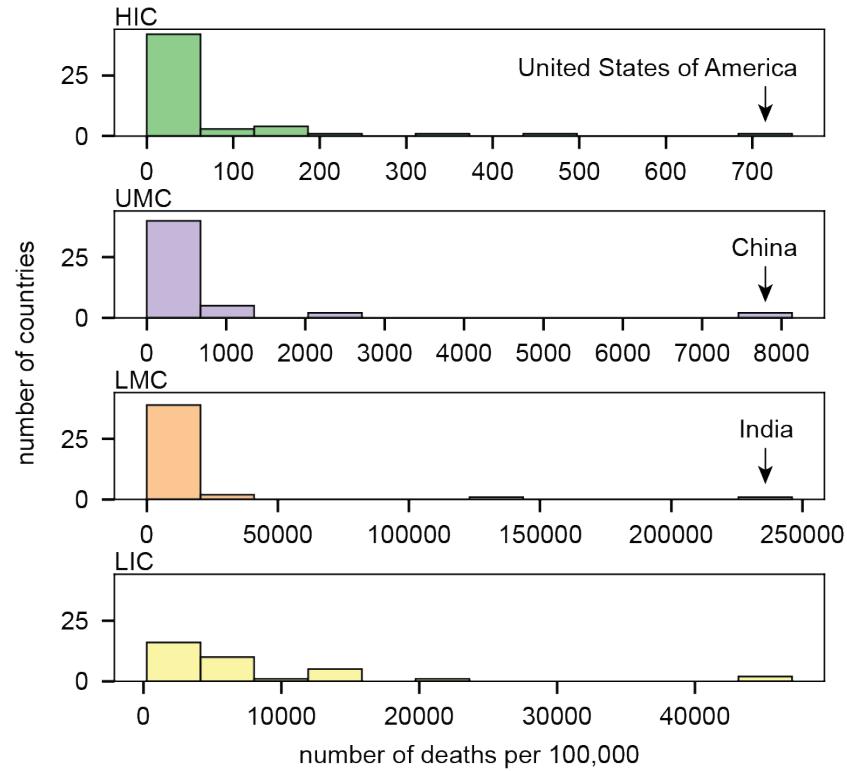
Waste production



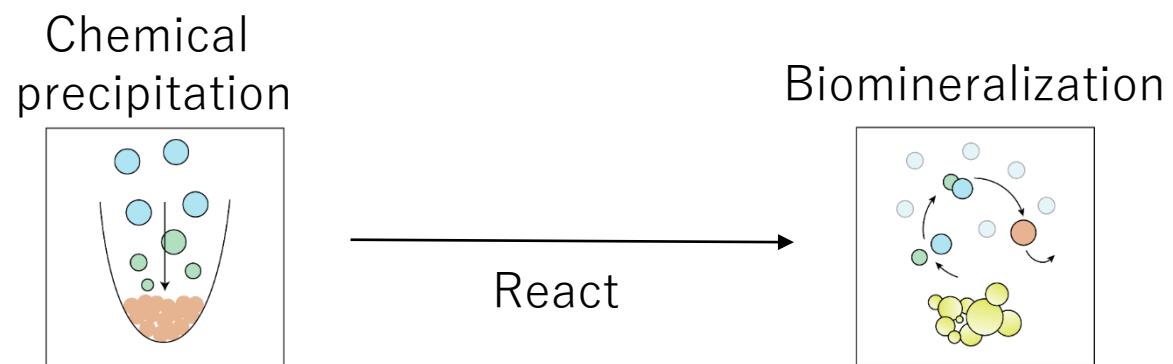
Death due to contaminated water



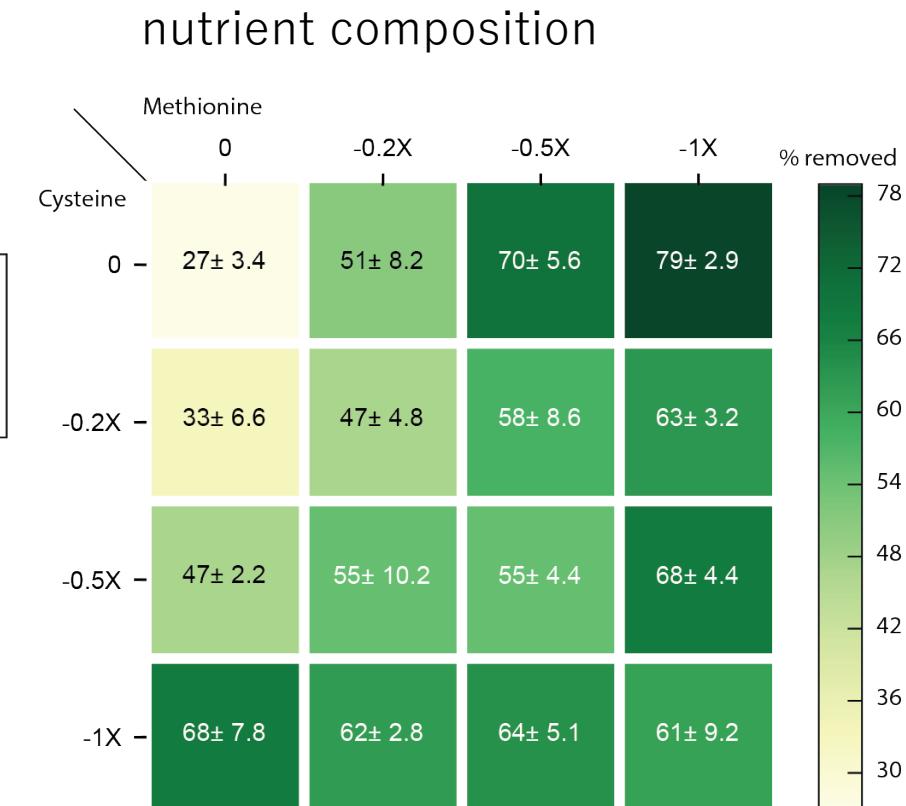
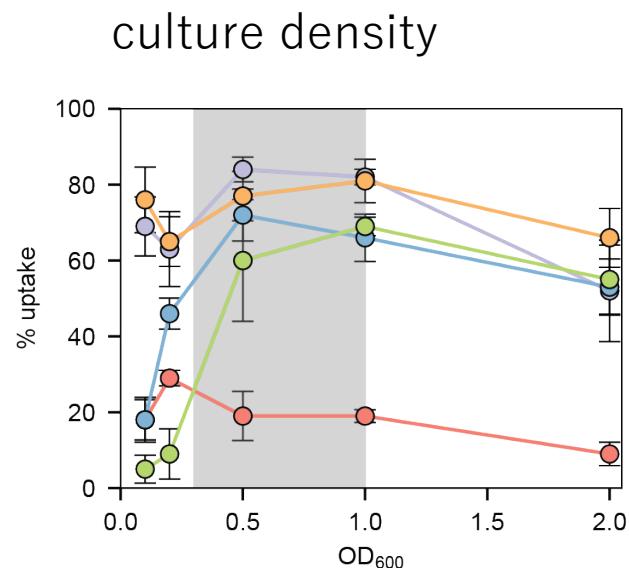
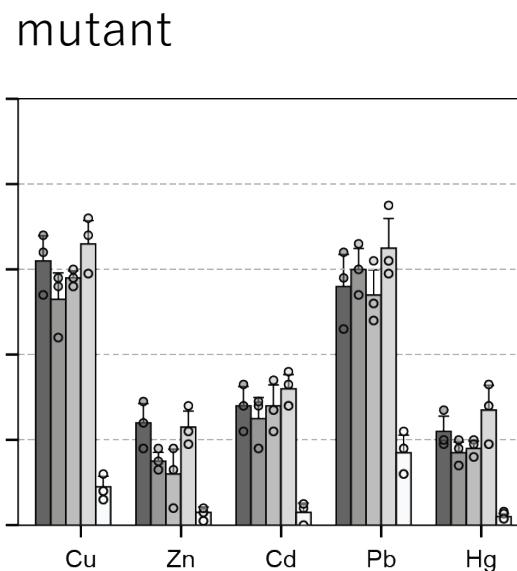
Global water accessibility and burden



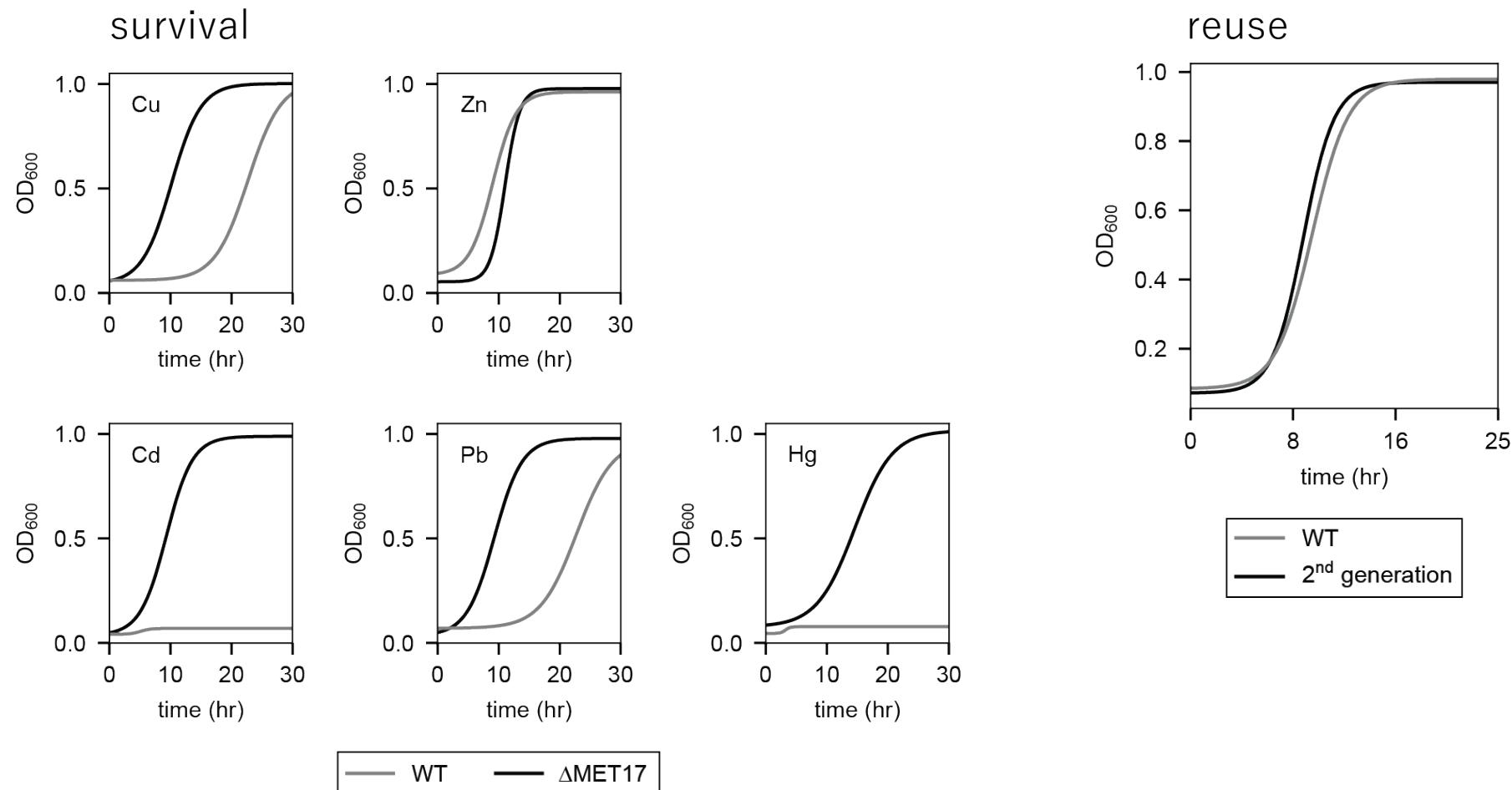
SUPPLEMENTAL – Chemical Precipitation



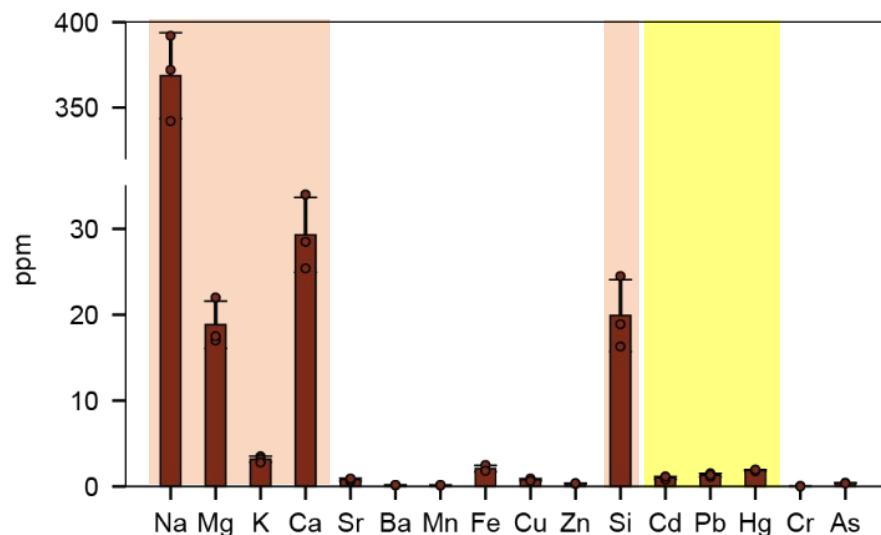
Optimizing culture and nutrient composition for metal sulfide precipitation



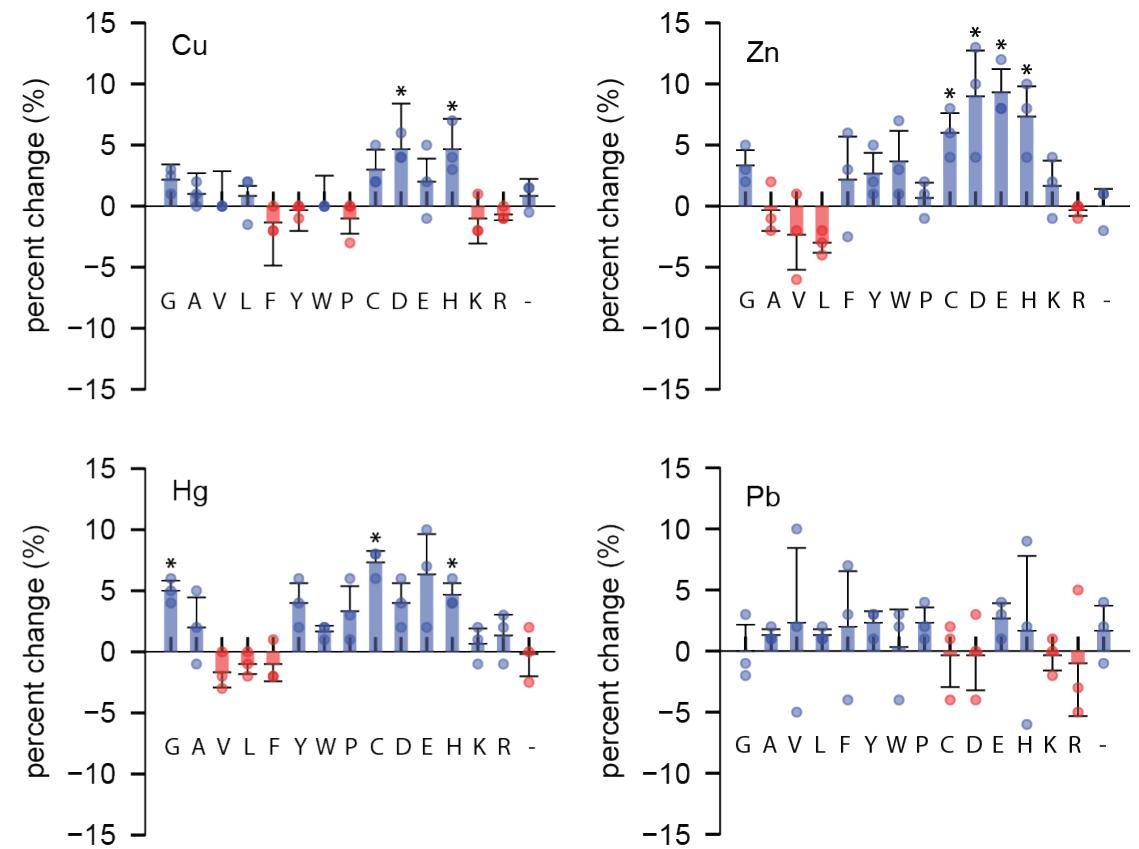
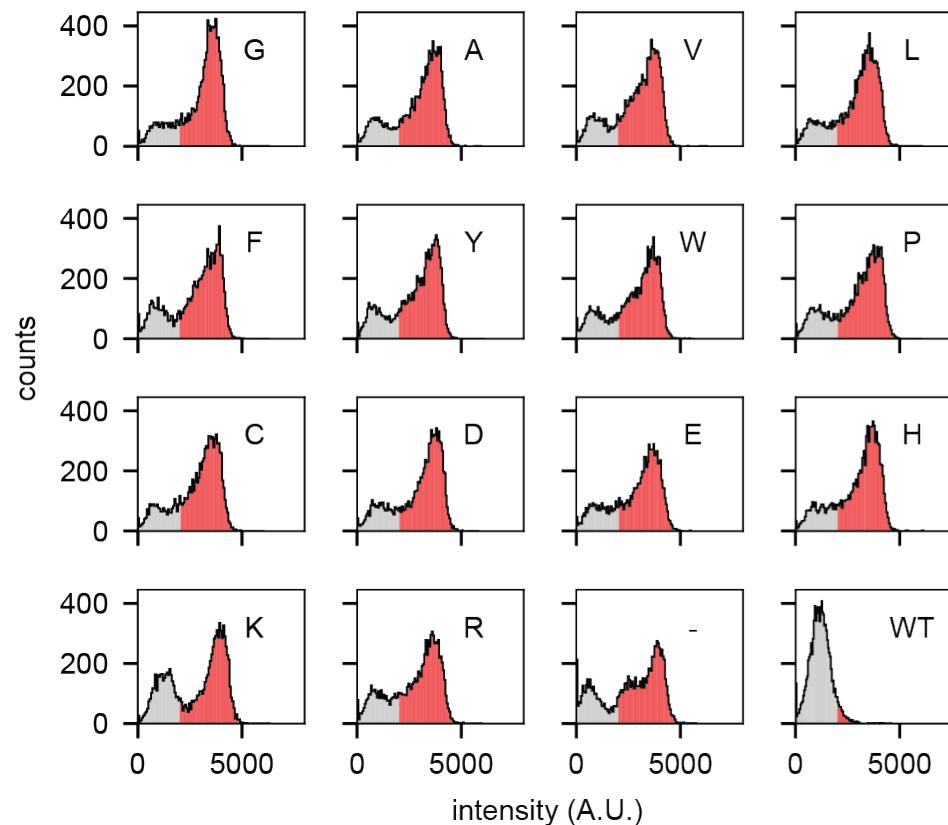
H_2S production increases heavy metal tolerance



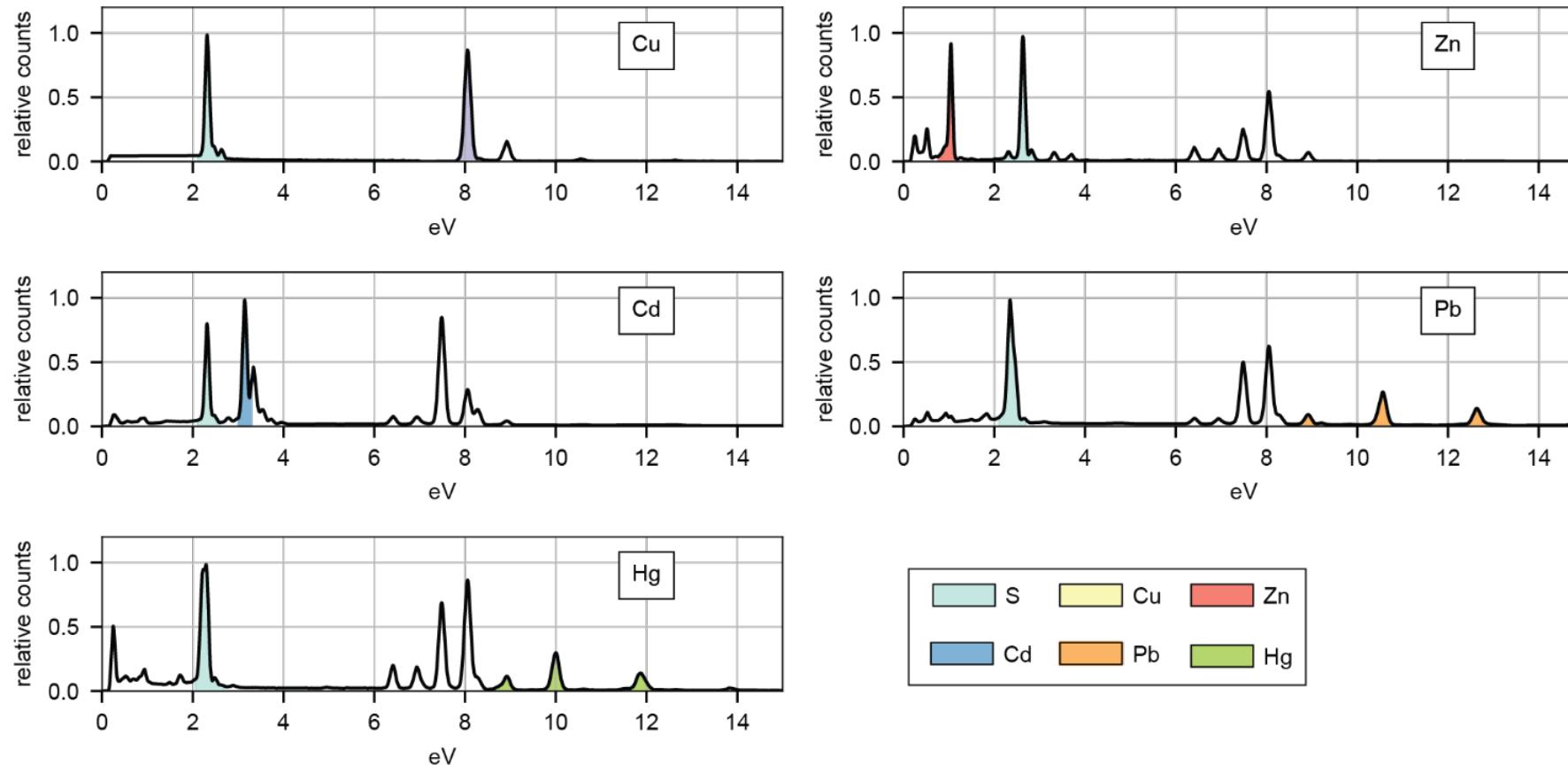
Sample from the Athabasca Oil Sands has a high content of clay and heavy metals.



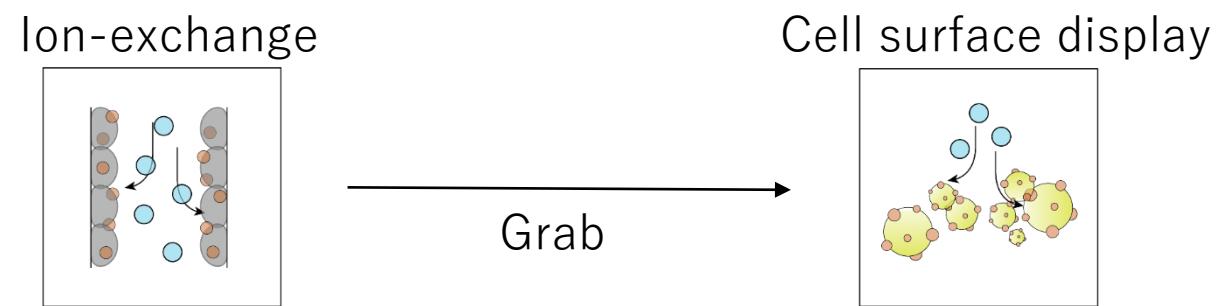
Yeast display of hexa-peptides tunes metal sulfide precipitation efficiencies



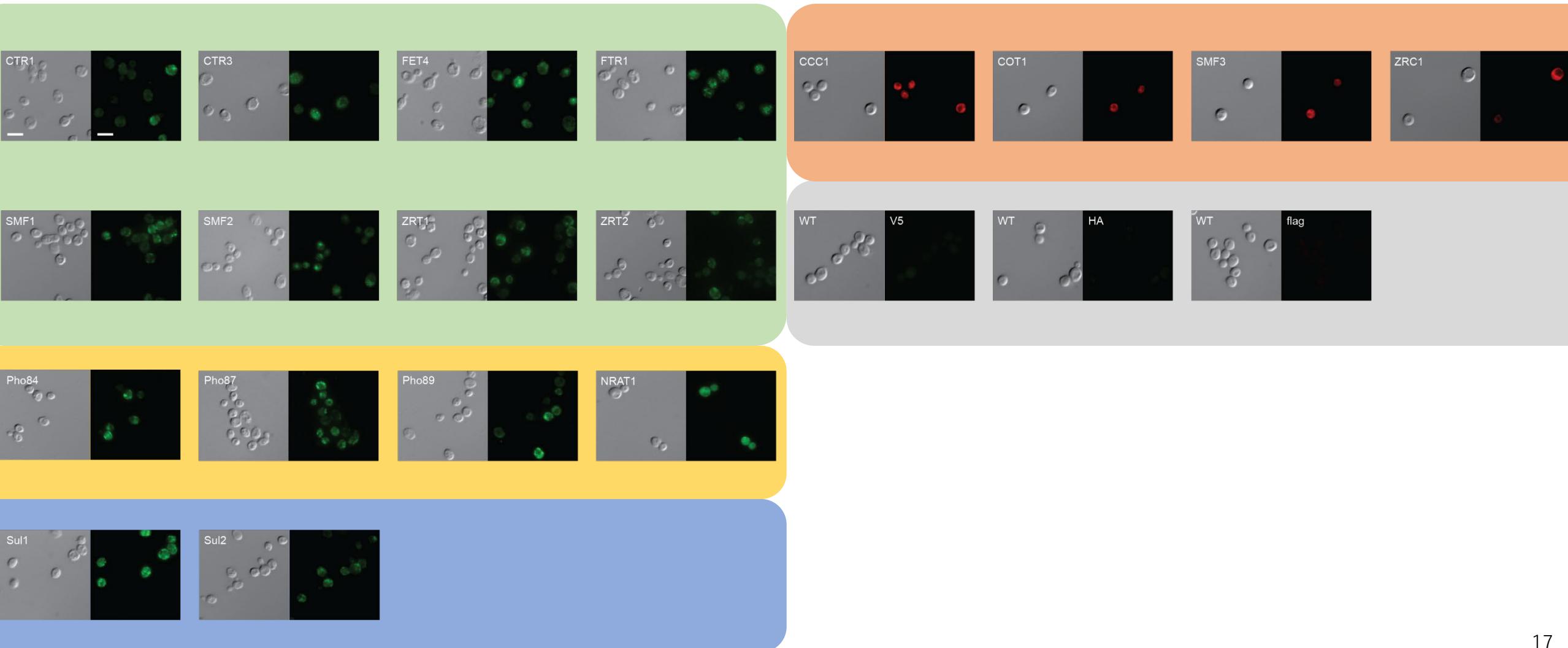
Metal sulfide precipitation produces 1:1 ratio of metal to sulfur, as analyzed by EDX



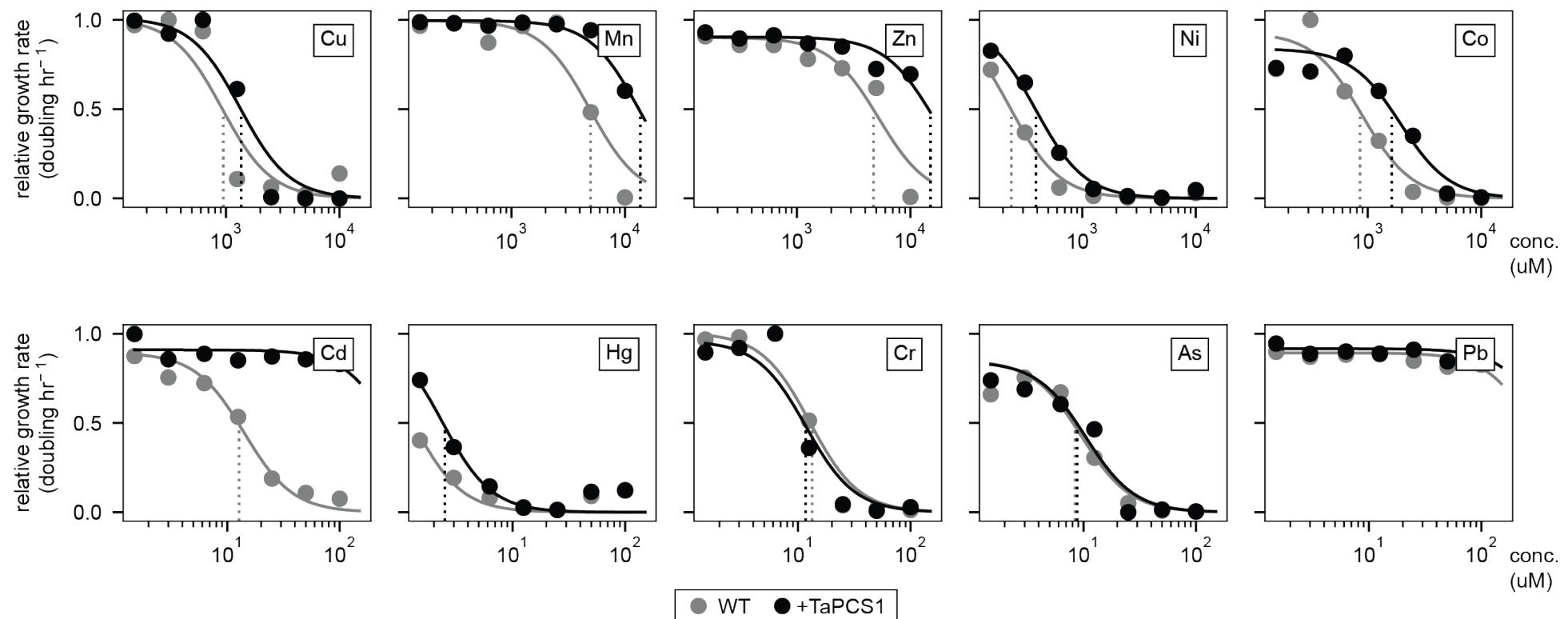
SUPPLEMENTAL – Absorption



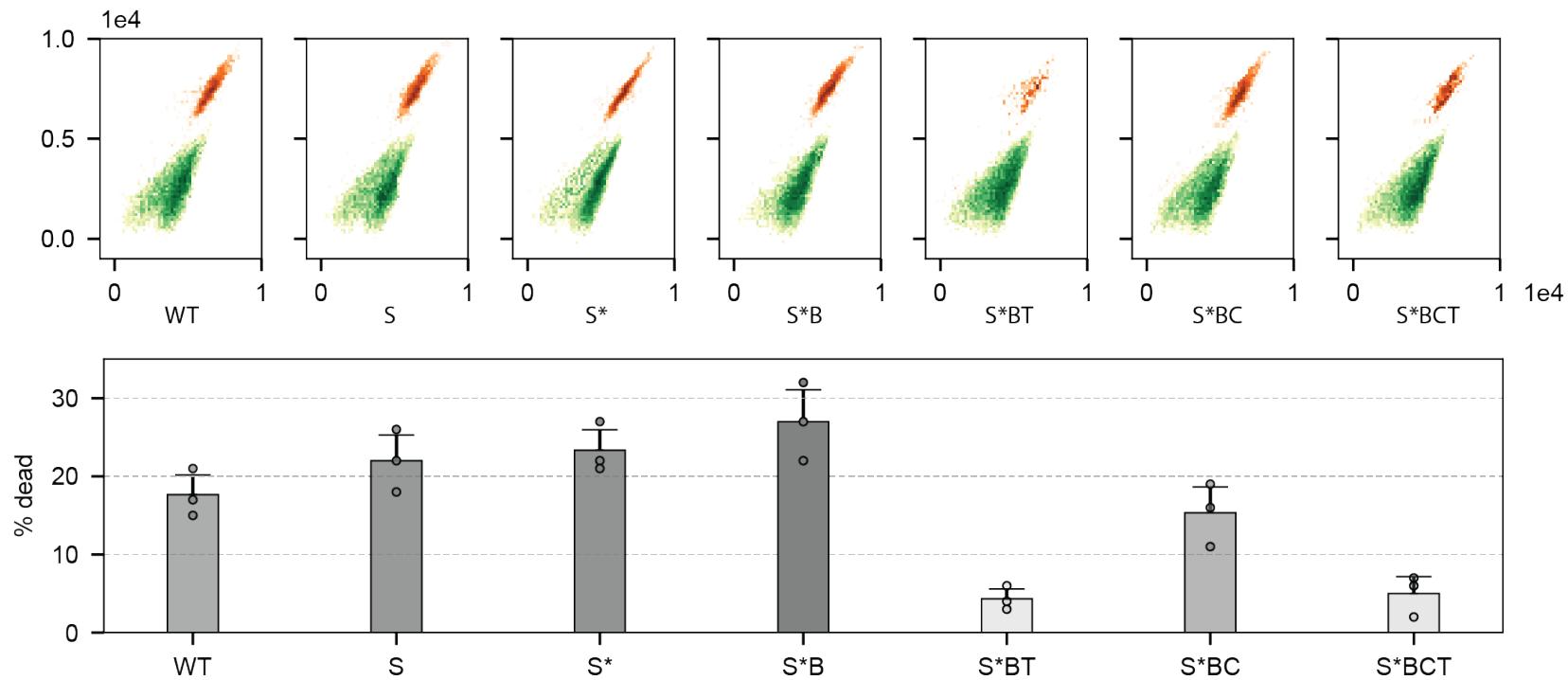
Examining transporter expression & localization using fluorescence microscopy



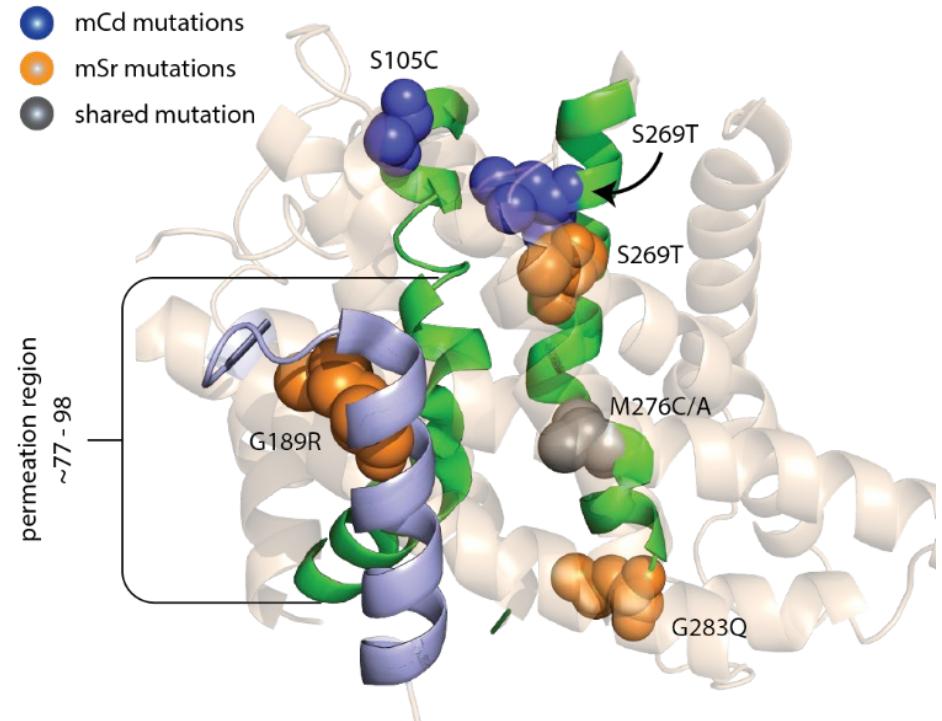
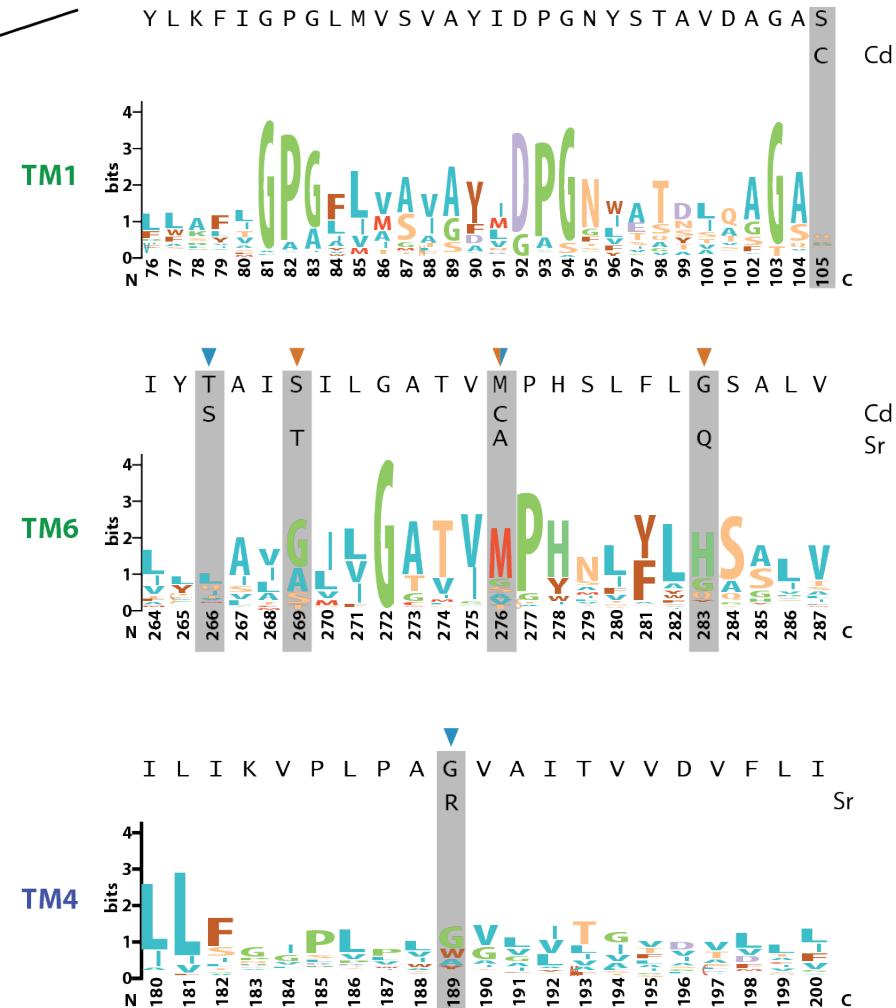
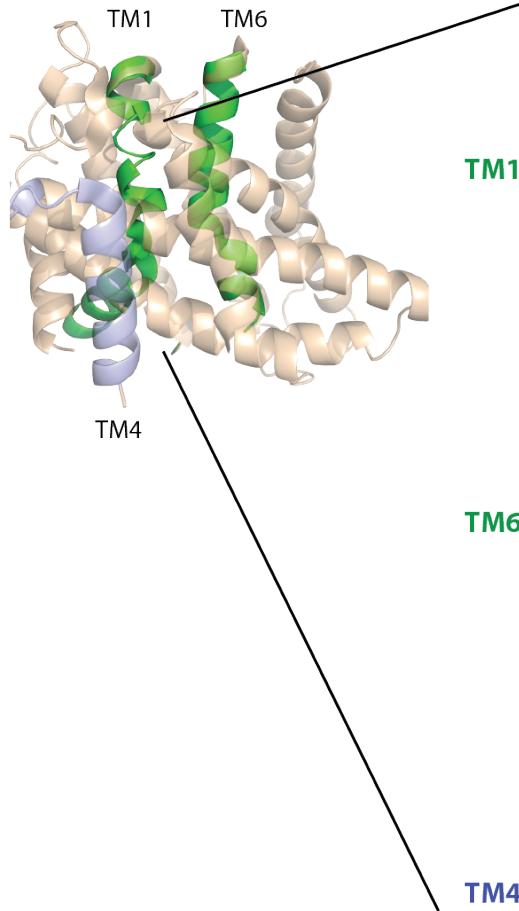
TaPCS1 increases heavy metal tolerance particularly against Cd



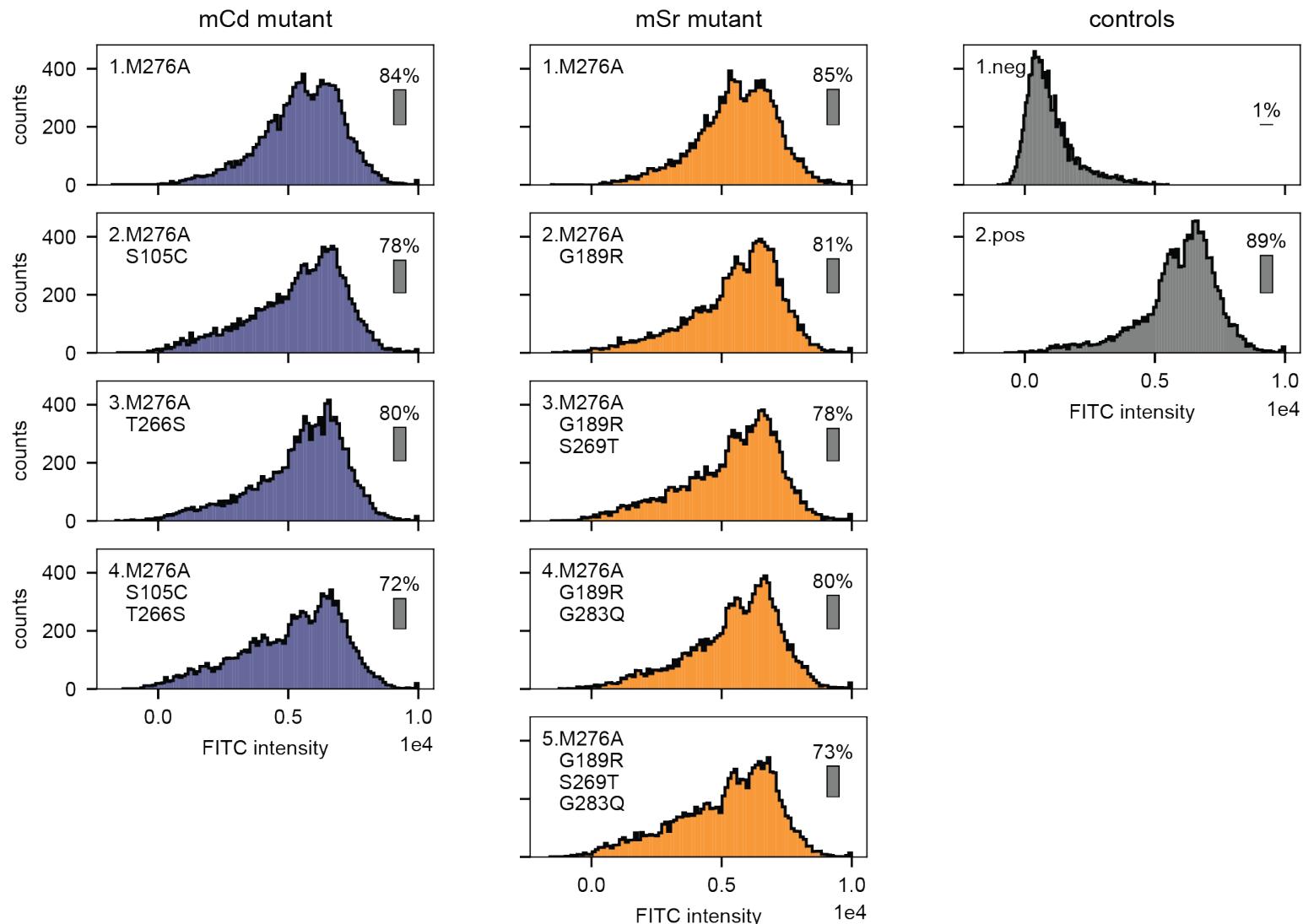
TaPCS1 and CCC1 help to improve metal tolerance



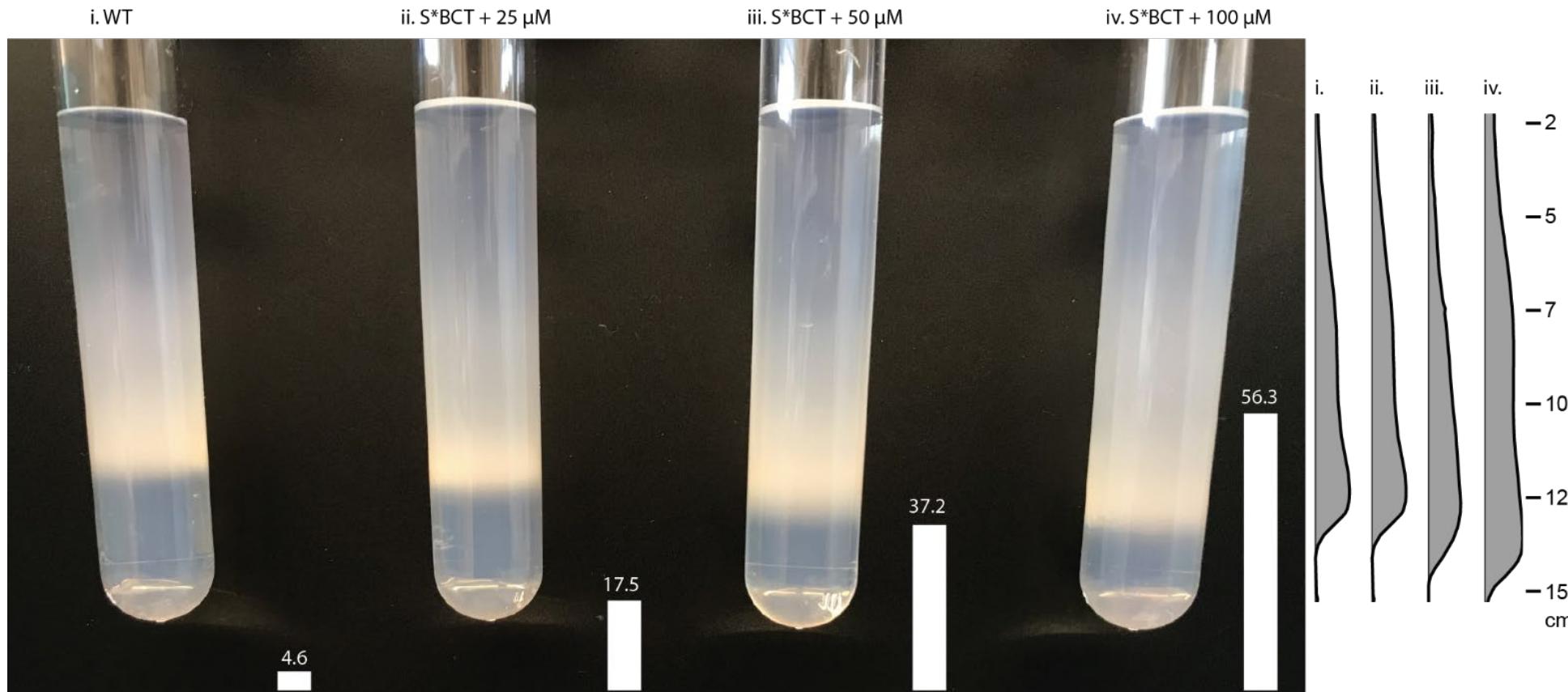
Mutation regions on SMF1



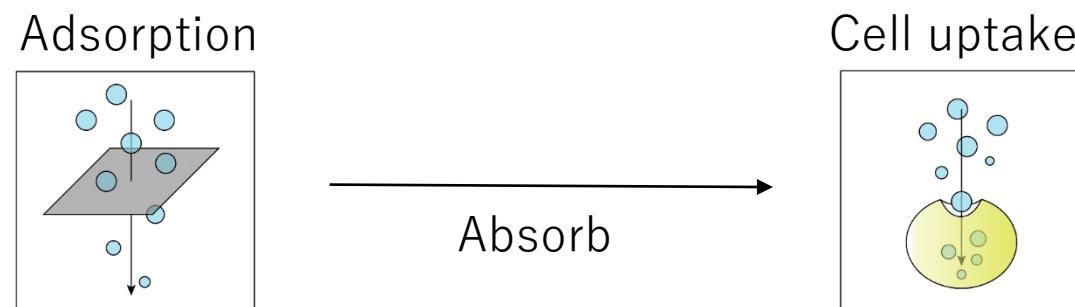
SMF1 expression is moderately impacted by mutations in creating mCd and mSr



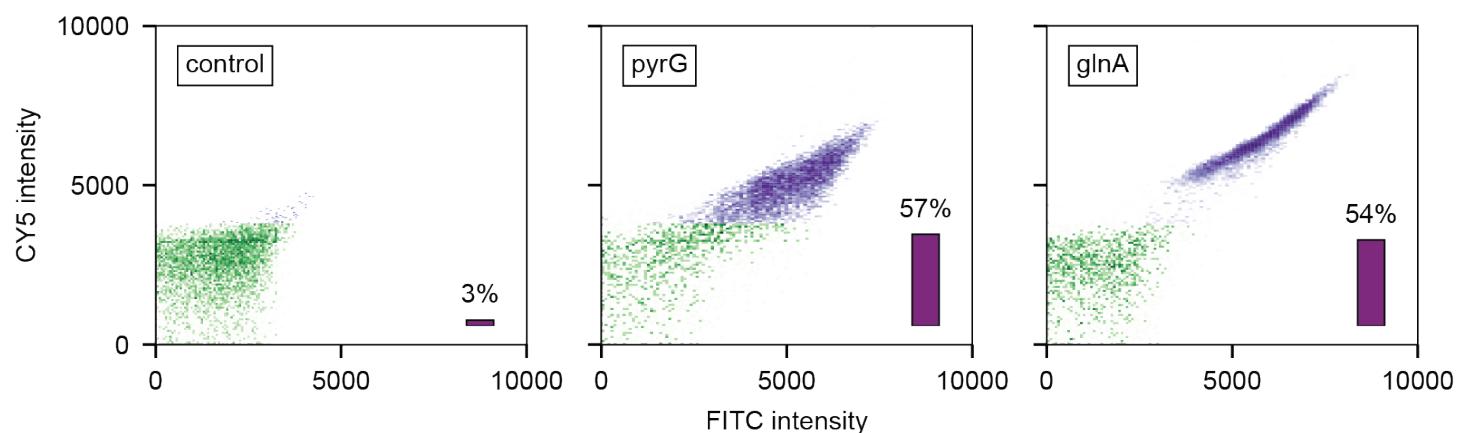
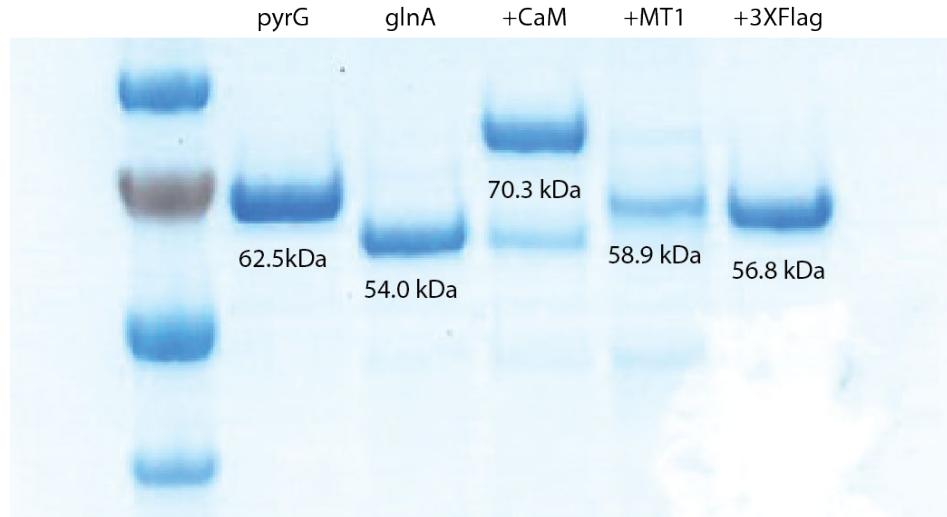
Positive control of density gradient centrifugation screening for transporter mutants



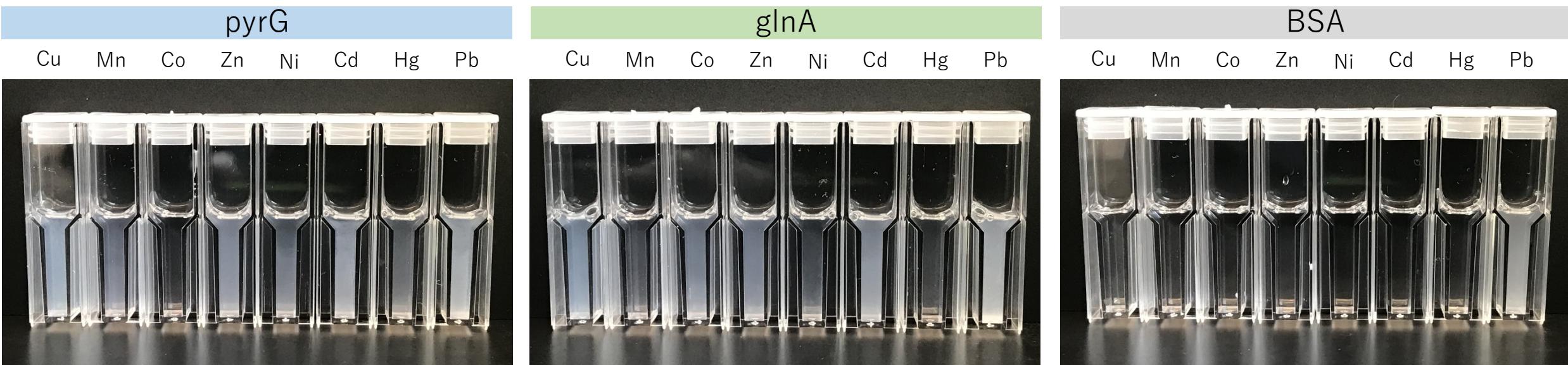
SUPPLEMENTAL – Ion-exchange



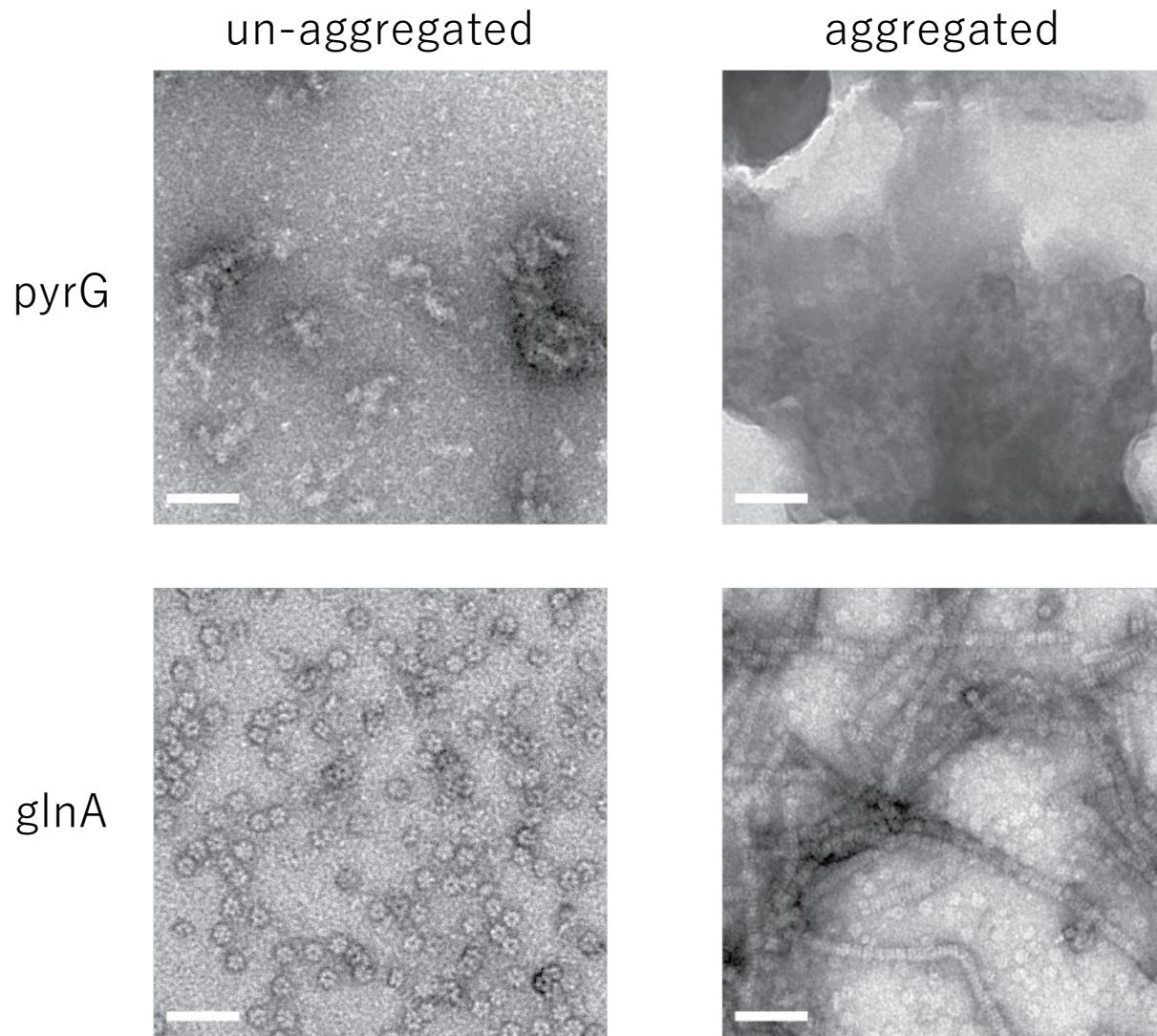
Confirming protein expression



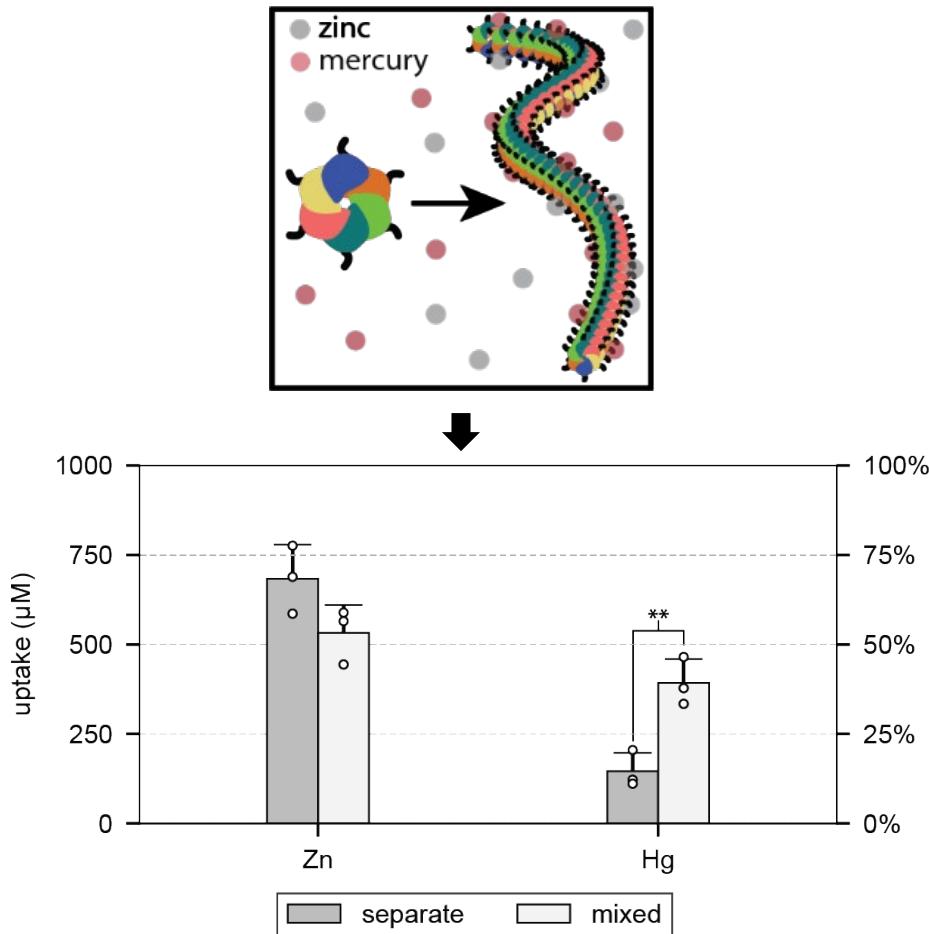
Aggregation of *pyrG* and *glnA* in response to metals, compared to BSA control



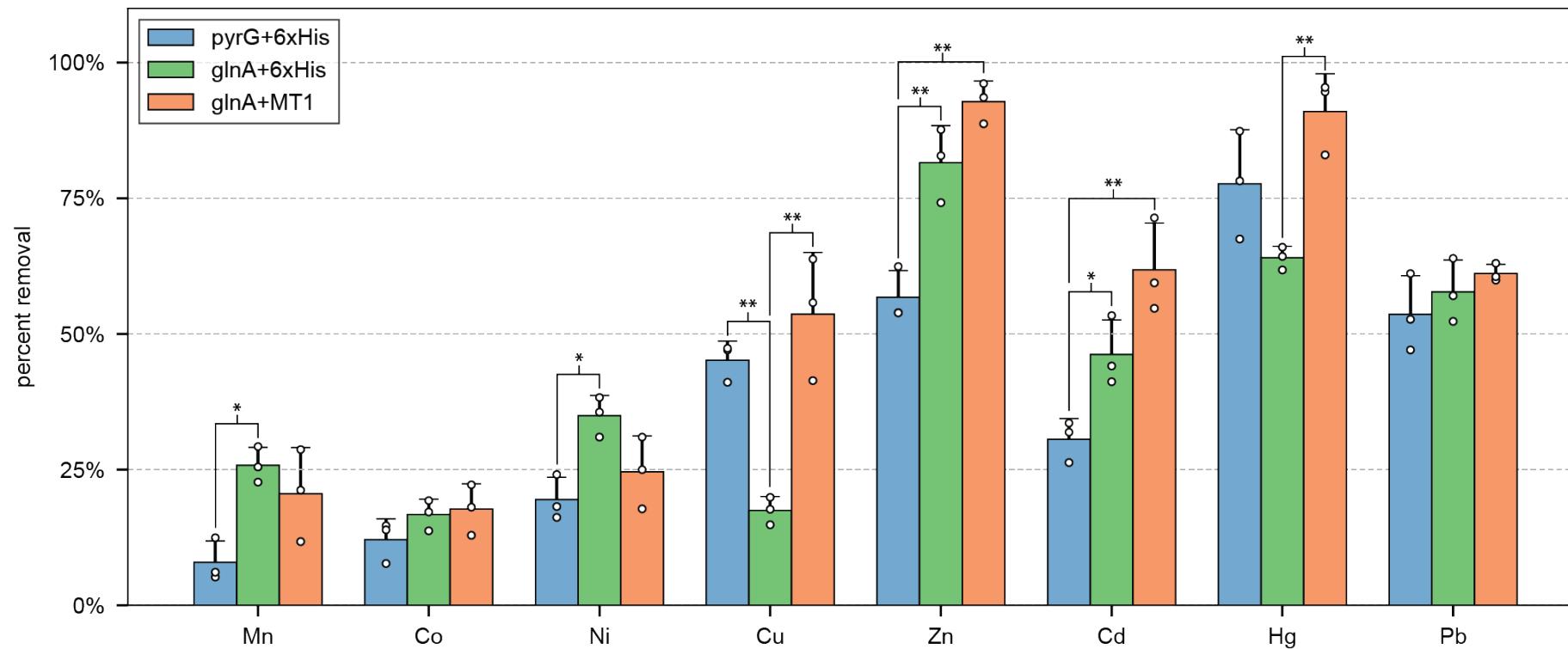
High resolution sample images of pyrG and glnA in the monomer and aggregated forms



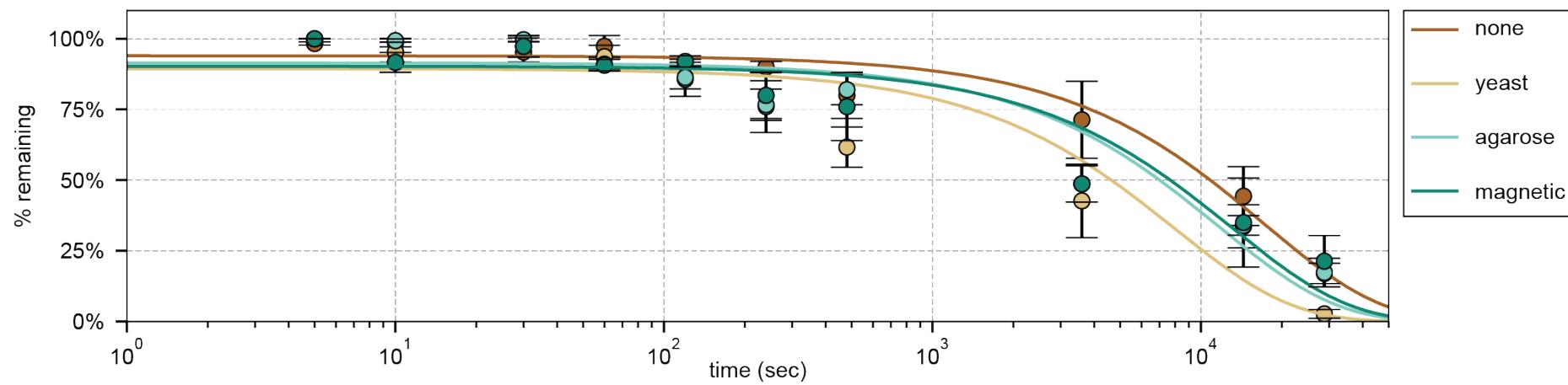
Mixing metals with one that induces high aggregation can increase removal of the other



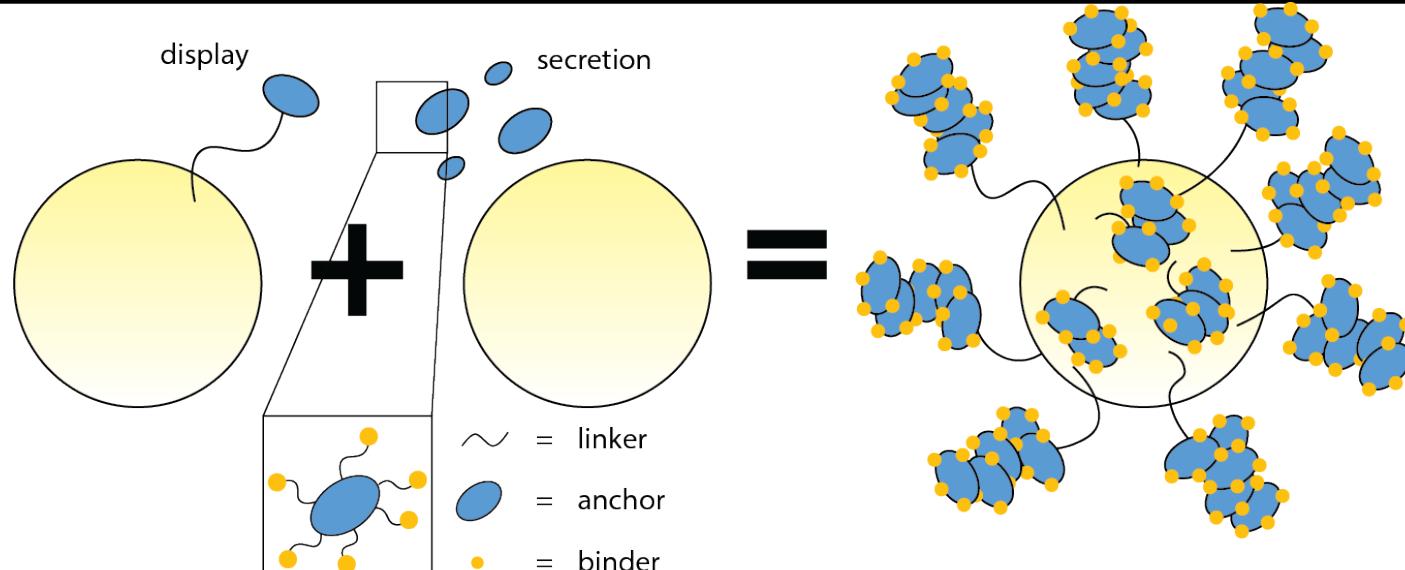
Comparison of the metal uptake profiles of pyrG+6xHis, glnA+6xHis, and glnA+MT1A



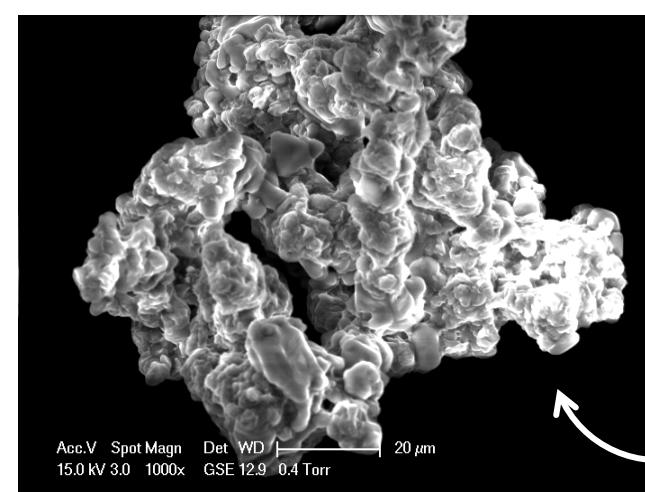
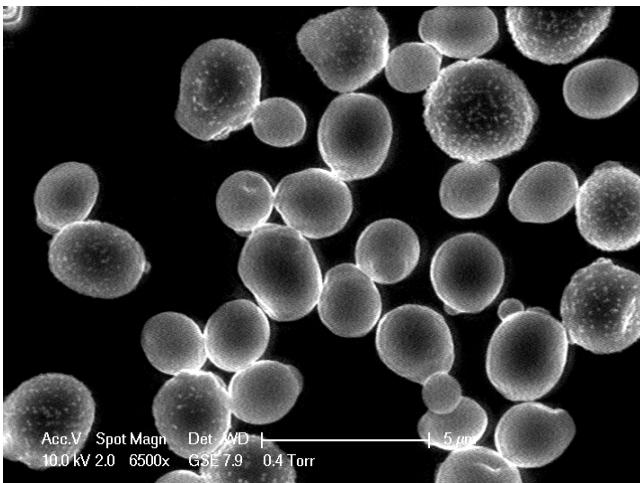
Sedimentation controls of agarose/magnetic beads, or yeast display with non-bound glnA



An aside: a fully biological platform



traditional
yeast display



multiplier
protein display

poor protein secretion
& expression