6.14.10
Meeting with Dr. Rao

try metal sensitive promoter to regulate efflux pump
collection system under a stationary phase promoter instead of a low affinity promoter. Takes longer to turn on. Or heat shock, temperature sensitive promoters. Look into those used for control of capsule formation for stationary phase promoters.

No to chemotaxis and thermotaxis as collection system

How can we expect efflux system to affect binding metal to TF—shouldn't be a big deal, reach steady state. Affinity of TF higher than affinity of pump.

Try a bunch of membrane proteins
send Dr. Rao a copy of the paper
use the designs described in the papers for fusing metallothioneine to membrane protein, not RFC25. using pore protein shouldn't matter.

Protein half life—depends on the protein. TF tend to be more like minutes. GFP tends to be about 24 hours.

Agglutination—fimbrae and add powdered yeast and they'll clump together
Flocculation—division mutants.

Site directed mutagenesis with DNA fragment instead of on a plasmid—needs to be on a plasmid.
mega primer PCR might work.

take berkeley's biobricks for membrane proteins.
clone out all the membrane proteins, try them out. Put them in high copy number plasmid.

with initial cloning, put into something without a promoter??

compare cells with MT to cells without.

Assays/diagnostic tools—outer membrane gel. -- boil cells, outer membrane prep, run on gel to visualize protein.

GFP isn't functional in the periplasm. mCherry might be functional in the periplasm, don't know about outside the cell.

How much can we fit on one plasmid—up to maybe 10-12 Kb, not including the backbone. Clone operon out, make into biobrick. ABC transporter will be high affinity, other would be lower affinity.

clone out gold binder, strong promoter (Tac, T7—need special cell for T7 to work)—see if it works. Could possibly secrete it, recover it with a his tag (bind on nickel column).

see if gas vesicles work

Clone out outer membrane proteins,

Find biobrick with GFP without promoter. Send Dr. Rao sequence of the gene it regulates and the first 500 bp upstream of gene it regulates.

Next Steps:
Clone out each component—outer membrane proteins
See if gas vesicles work
Express gold binder on strong promoter to see if it works

Tac—strong promoter

T7—super strong promoter, need special cell

Find sequence for promoter—send Dr. Rao plasmid (used for reporter)< and sequence we want to use for promoter, and 500 bp upstream of e. coli gene regulated by Arsenic
put in front of GFP to see if we can get it to work