D. vulgaricate

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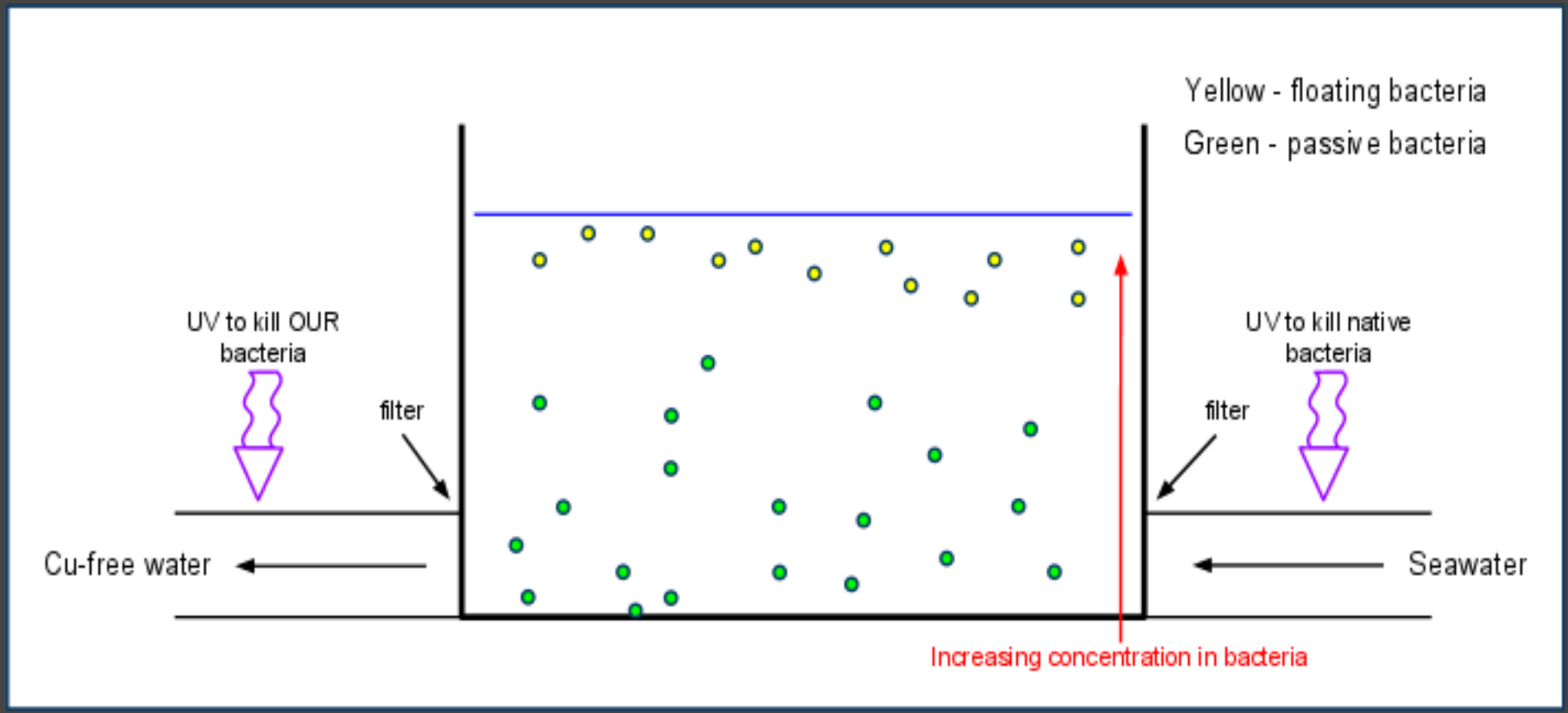
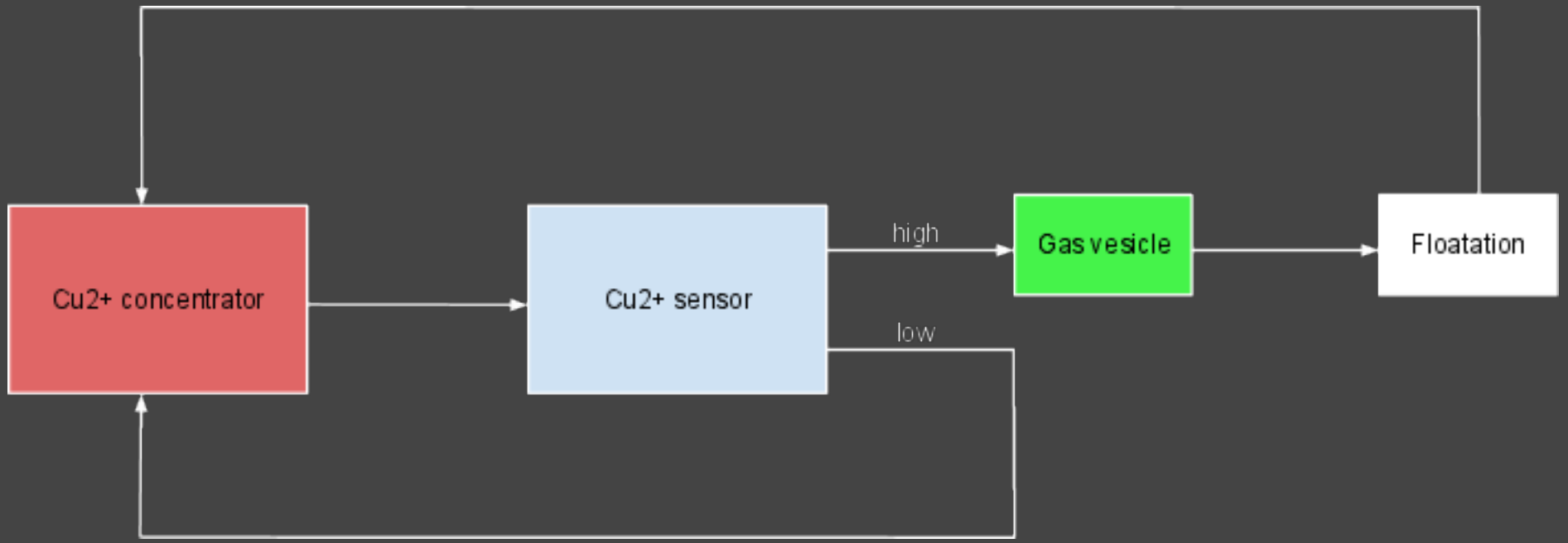
Changes

- Decided to import tunicate abilities to bacteria
- Previous research done on importing to *E. coli* , but this is not a useful chassis (salt water)
- Cu^{2+} as new target ion
- Shift to systems engineering and feasibility
- Goal: design a constant-throughput system for concentrating metals

Design


Yellow - floating bacteria
Green - passive bacteria
UV to kill OUR bacteria
filter
UV to kill native bacteria
filter
Cu-free water
Seawater
Increasing concentration in bacteria

Devices


Cu2+ concentrator
Cu2+ sensor
high
low
Gas vesicle
Floatation

Devices

- **Cu^{2+} concentrator**: entire point of the project - continually binds Cu^{2+} from the seawater and brings it into the cell

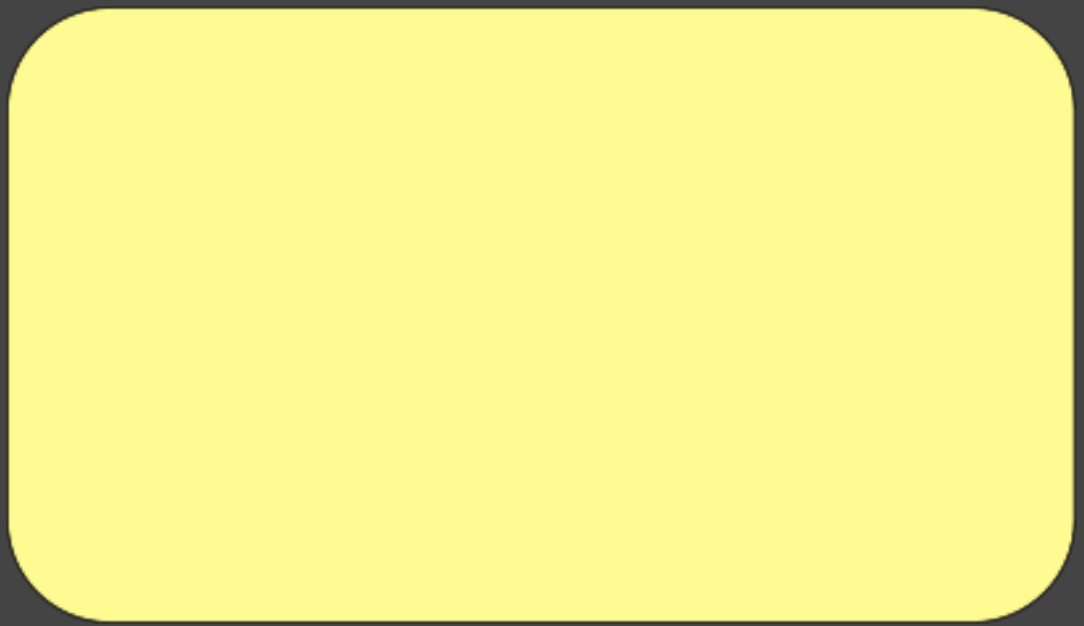
- **Cu^{2+} sensor**: detects levels of Cu^{2+} in the cell, allows properly-timed flotation

- **Flotation device** (gas vesicle generator): triggered by Cu^{2+} sensor - brings Cu-rich bacteria to surface for harvesting

Parts

Chassis - *Desulfovibrio vulgaris*

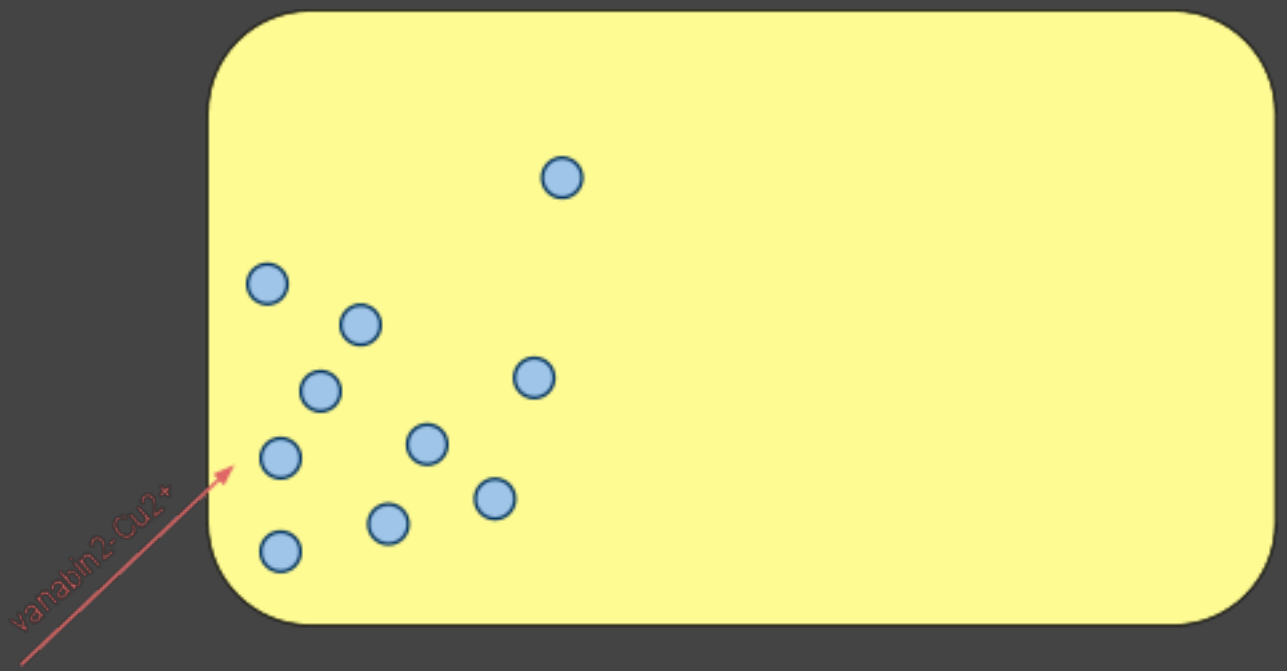
- Salt-tolerant
- Sequenced genome
- Resistant to very high osmotic stress
- Survivable in environments with a variety of heavy metals



Parts

Cu^{2+} binder - MBP-vanabin2

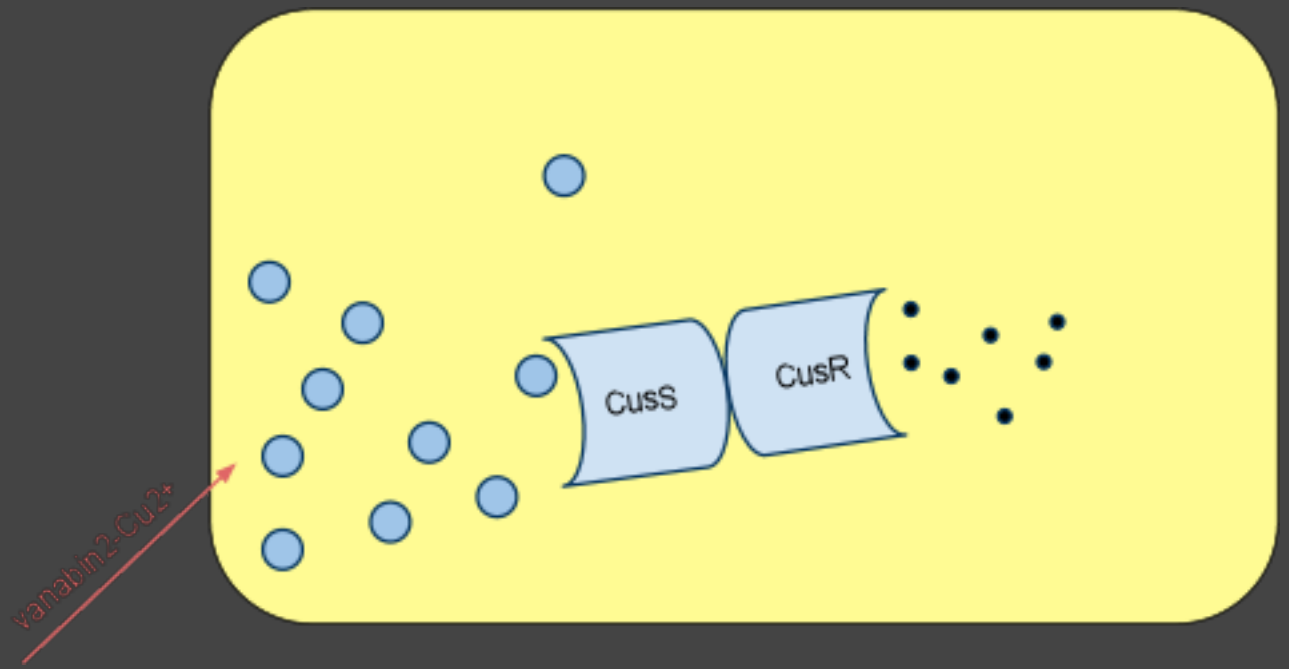
- Vanabin2 protein binds vanadium in tunicate
- Successfully transported to *E. coli* and fused to MBP (maltose binding protein
- In *E. coli* , binds Cu^{2+} to concentration of 882ng/mg



Parts

Cu^{2+} sensor - CusR/CusS two component signal system

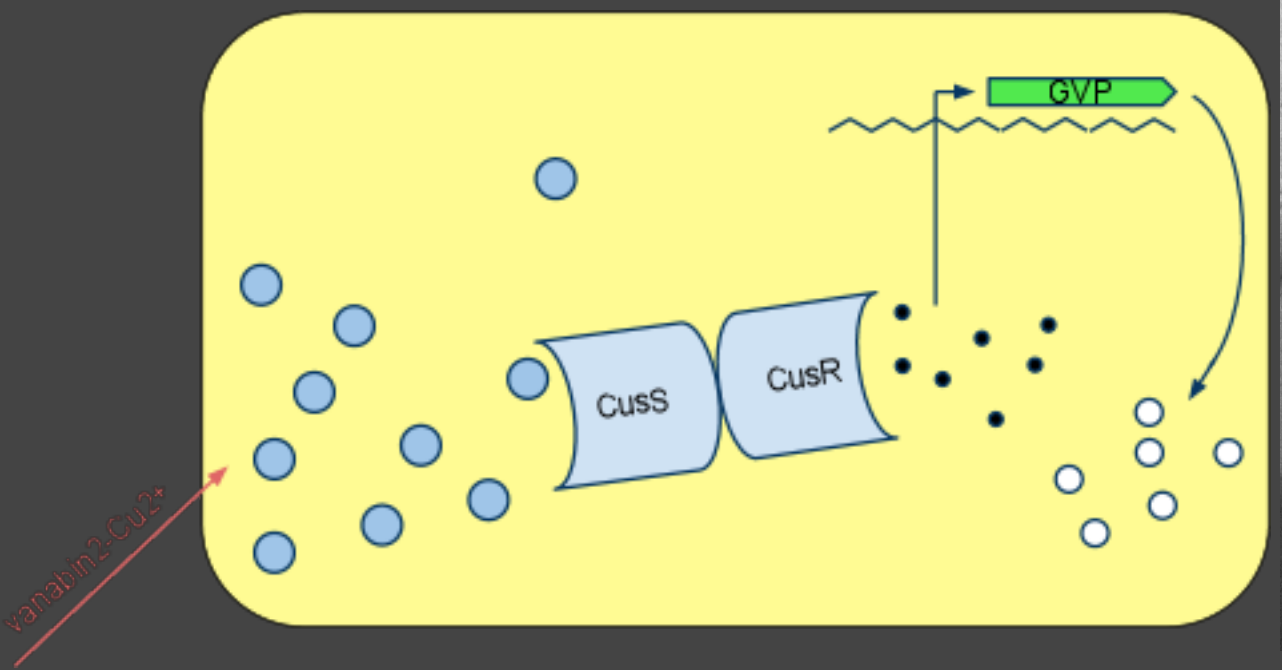
- Originally found in *E. coli* as a transmembrane sensor
- CusS phosphorylates CusR, which then is an activator
- Hope to bring CusS inside cell, fuse to CusR

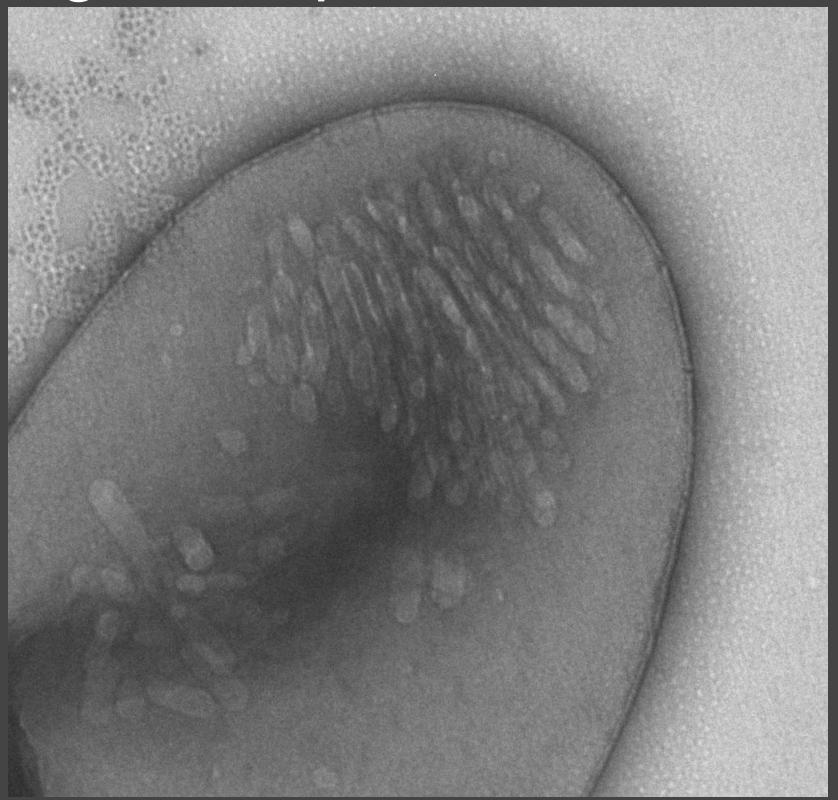


Parts

Flotation - Gas Vesicle polycistonic gene

- Creates gas-filled organelles inside the cell
- Buoyancy of cell increased, resulting in flotation
- Previously activated by an arsenic-regulated promoter

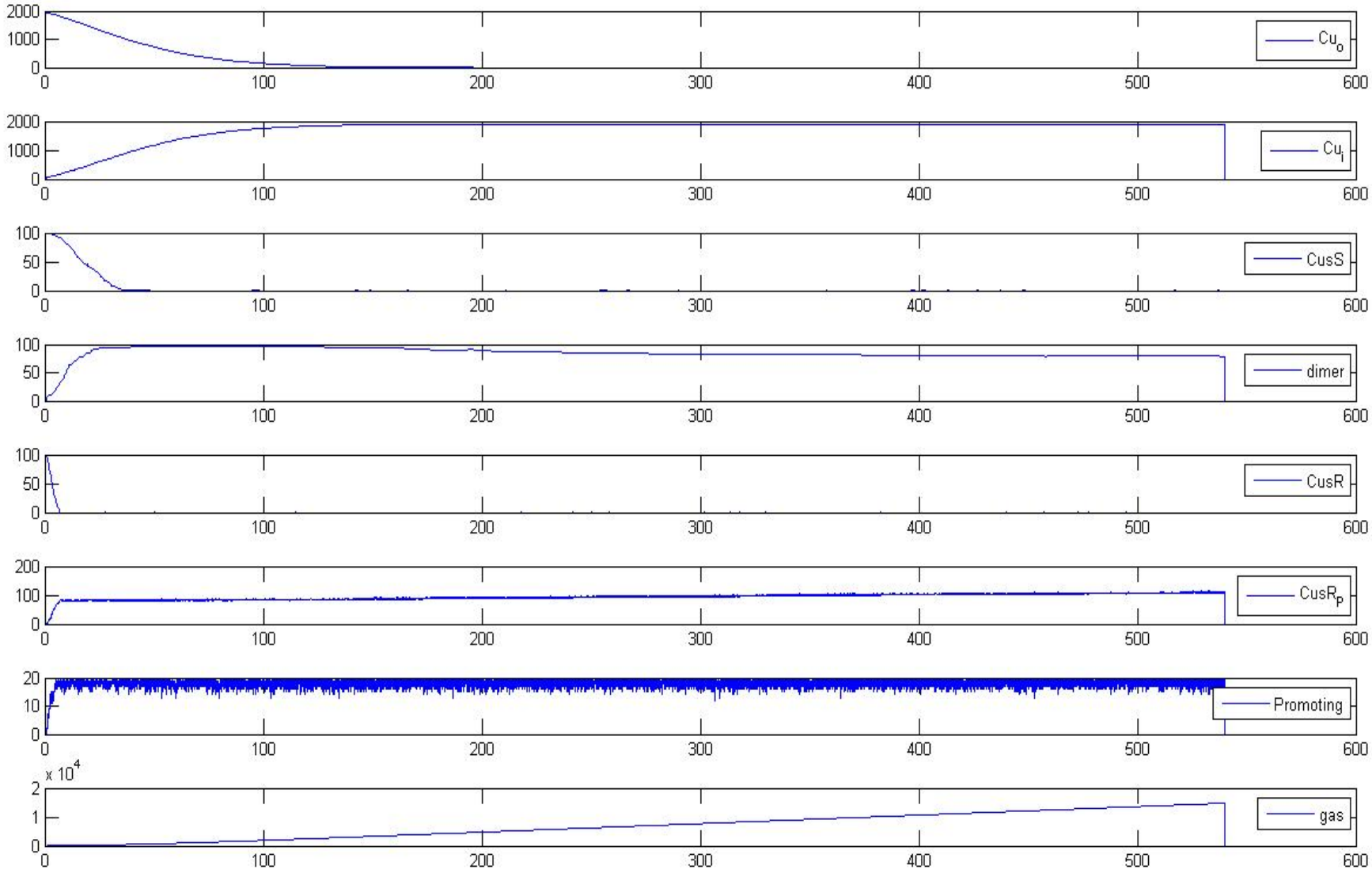




Equations:

1) Cu^{2+} + Vanabin2 = Cu Complex
2) Cu Complex is brought inside cell
3) Cu Complex = Cu^{2+} + Vanabin2
4) Vanabin2 is exported
5) Cu^{2+} + CusS = CusS_Cu
6) CusS_Cu + CusS_Cu = CusS_Cu_2
7) CusS_Cu_2 + CusR = CusR_P + CusS_Cu_2
8) CusR_P binds to GVP Promoter
9) Promoter then increases expression of GVP

Timing Diagram



Error-Checking and Testing

- *Implement one part at a time!*
- test components in *D. vulgaris* individually first.

1. Insert gene(s) for Cu^{2+} concentrator.
    1. If cells die, the Cu overload is probably killing them, therefore new chassis.
    2. if no death occurs, preform assay to determine amount of Cu^{2+} in cells
2. Insert normal genes for signaling pathway, attach promoter to GFP or LacZ
    1. if death occurs, try again
    2. if no death occurs, culture on X-Gal or check for GFP to see if it functions
    3. if it does not function, assay for CusS and CusR to see what is wrong

Error Checking and Testing

3. place GVP genes into chassis with promoter known to work
    1. if cell dies, find new chassis
    2. if cell lives, check if it floats or not
    3. if it doesn't float, assay for proteins to see if GVP is being made
4. Hook up concentrator and normal signaling pathway to check for denaturing of proteins
5. Hook up concentrator, normal pathway and GVP, determine if you have expression
6. Start mutagenizing CusS until you have one that works inside the cell membrane

Impact

- Proof of concept using copper
- Hope to tune the system for other metals
- Not environmentally destructive
- Tremendous resources in ocean
- Mesh with current lithium extraction techniques

Outstanding Issues

Internalizing Cu^{2+} two-component system

- Current state, is transmembrane
- Unknown if removing CusS from membrane, fusing to CusR is feasible
- Possibility of crosstalk between sensor and native features in cell
- Alternative: batch processing of seawater, measure concentration drop outside cell

Outstanding Issues

Implementation

- Vanabins untried in our chassis
- *D. vulgaris* has complex interactions with many ions which give it its durability
- Promoting GVP with CusR
- Sufficiency of GVP to overcome random turbulence

GO