Biosensors: Development and Environmental Testing

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Metals of interest

Copper: maximum drinking water level standard 1.3 mg/L

aquatic chronic exposure limit 30.3 µg/L

Human tolerance relatively high
Used for microbial pest control
Some lower organisms acute toxicity (phytoplankton, zooplankton, amphibians)

Cadmium: maximum drinking water standard 0.005 mg/L

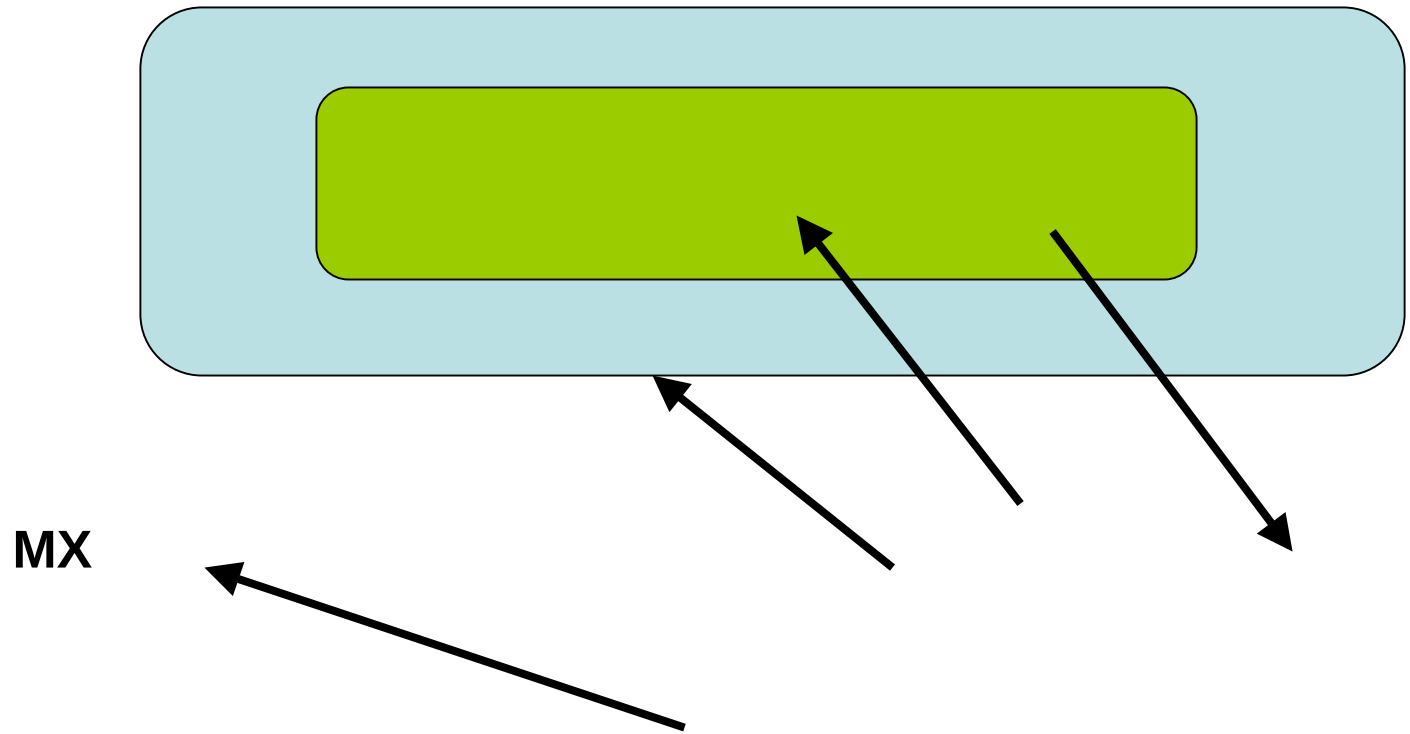
aquatic chronic exposure limit 2.7µg/L

Higher animal toxicity: accumulates in kidneys and liver, produces bone and blood problems
Carcinogenic (Group B1)
Minor use as pesticide

Cu is a trace element: required for certain enzymatic and carrier proteins

Cd no known cellular use—toxic- may disrupt Ca and Zn metabolism

Both cause oxidative stress
Both act on –SH proteins

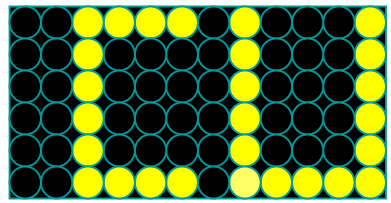


Cell concentrations regulated by chelation, sorption, uptake, and efflux mechanisms

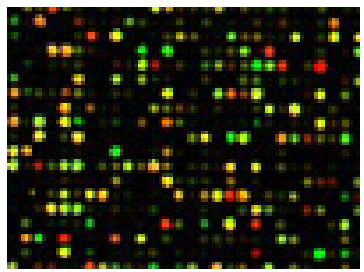
Biosensors

Final goals:

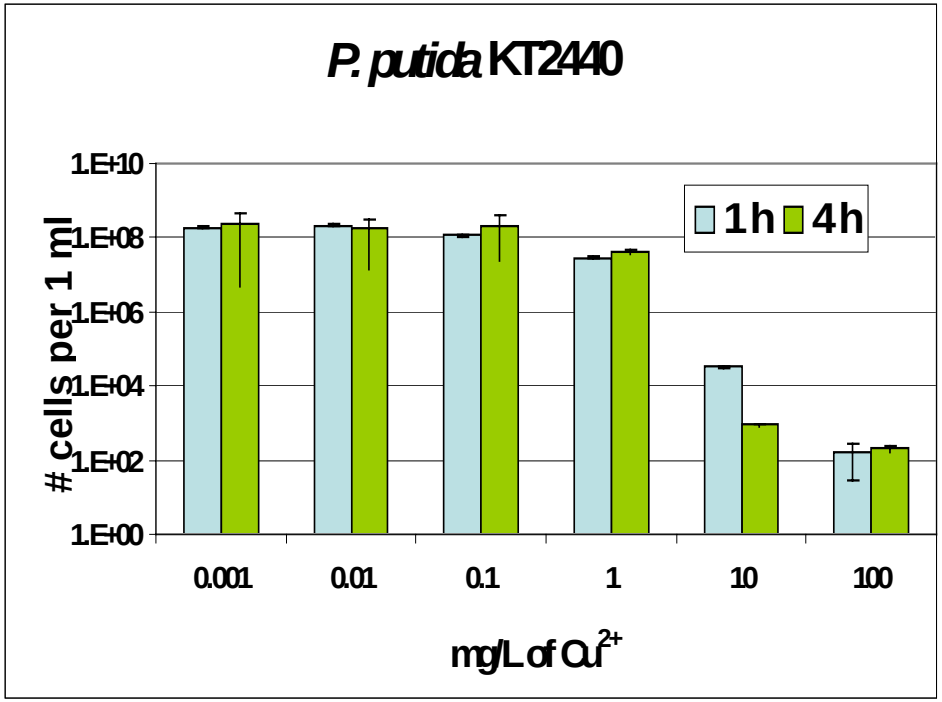
1) An array of promoter fusions that respond differentially and specifically with light output upon exposure to toxic metals

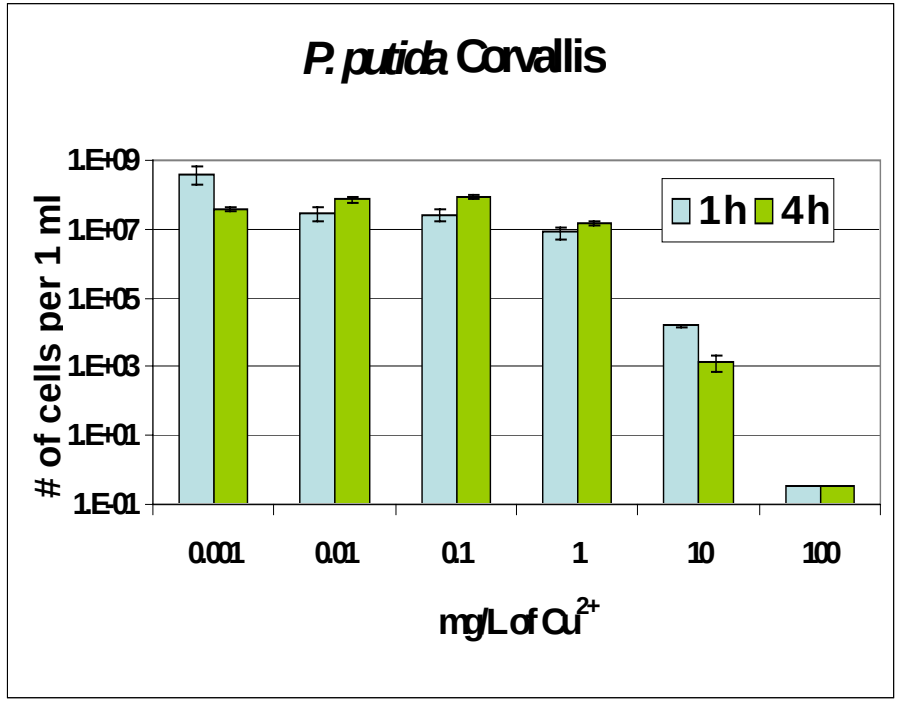


2) A gene chip array to detect transcript abundance from cells responding to toxic metals. Pattern of gene activation would specify the metal.

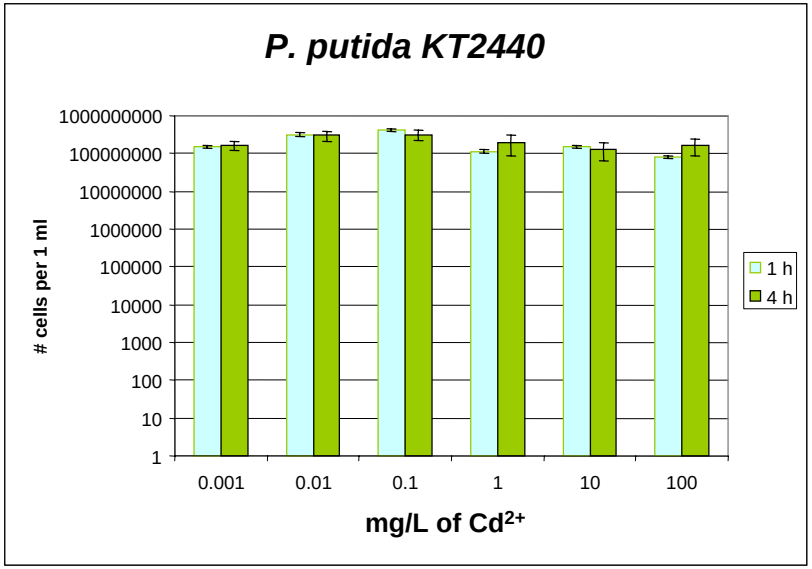


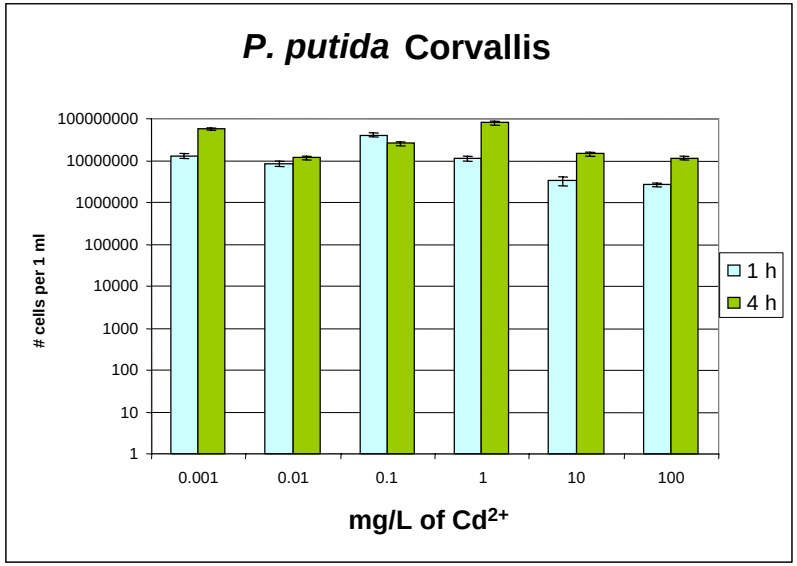
Test organisms: *P. putida* strains





Cells of both pseudomonads are killed by concentrations of Cu starting between 1 and 10 mg/L

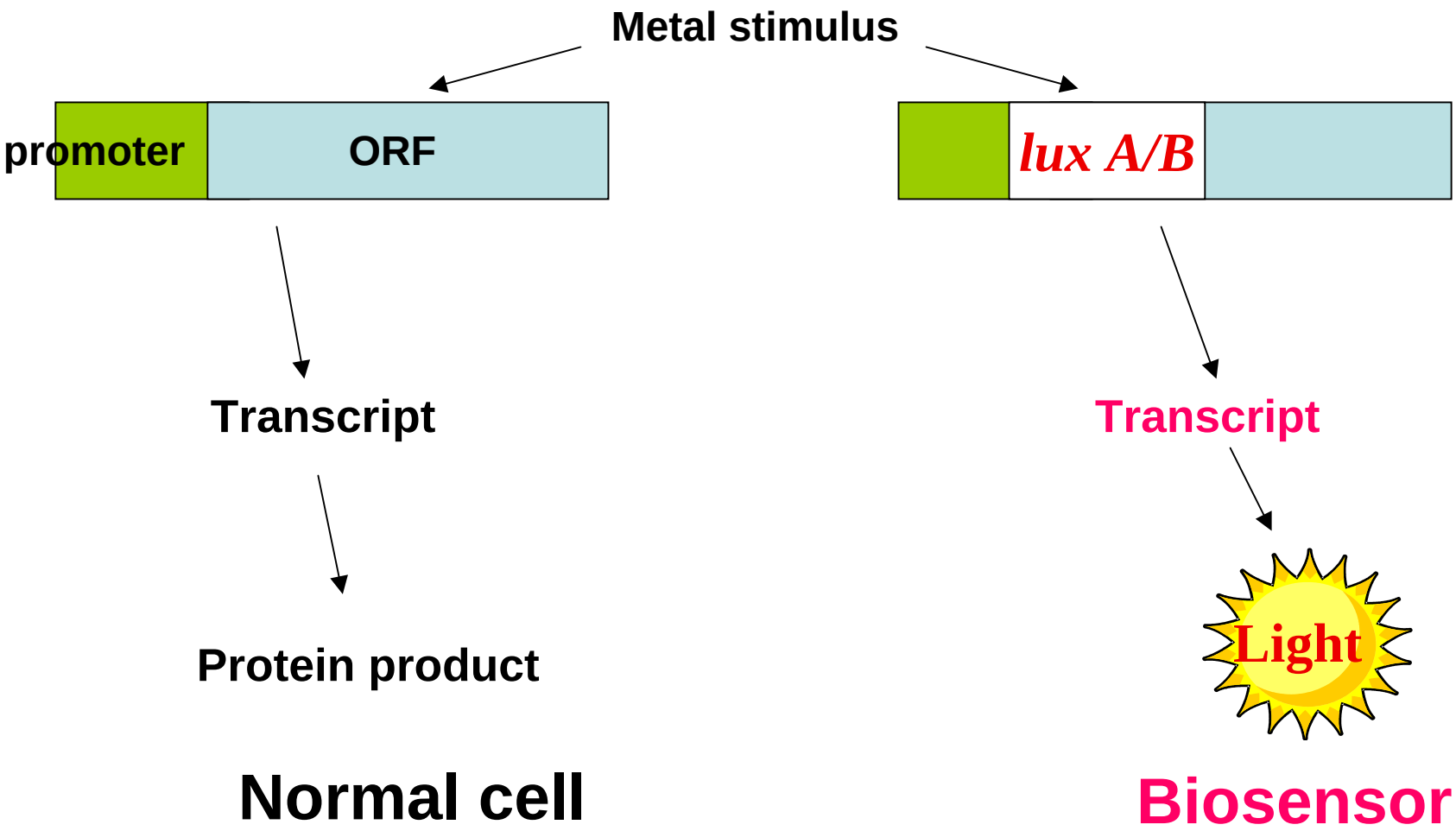


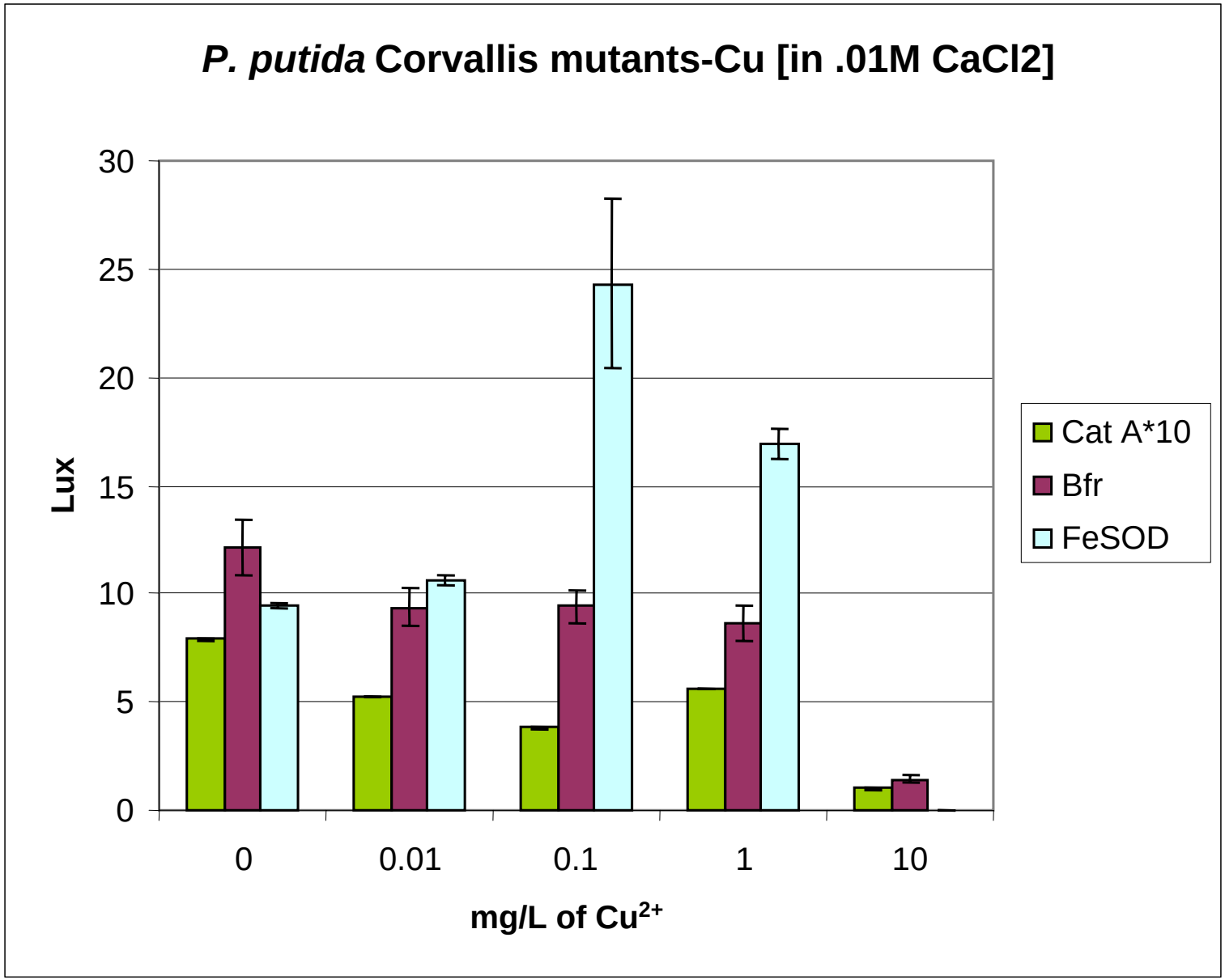


The pseudomonad cells show little change in culturability with Cd even at doses of 100 mg/L.

Both wild type isolates behave similarly showing resistance to Cd

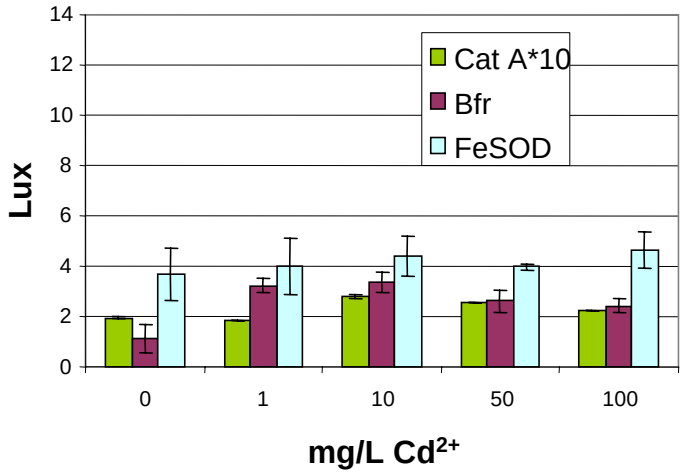
Generation of Luciferase Biosensor



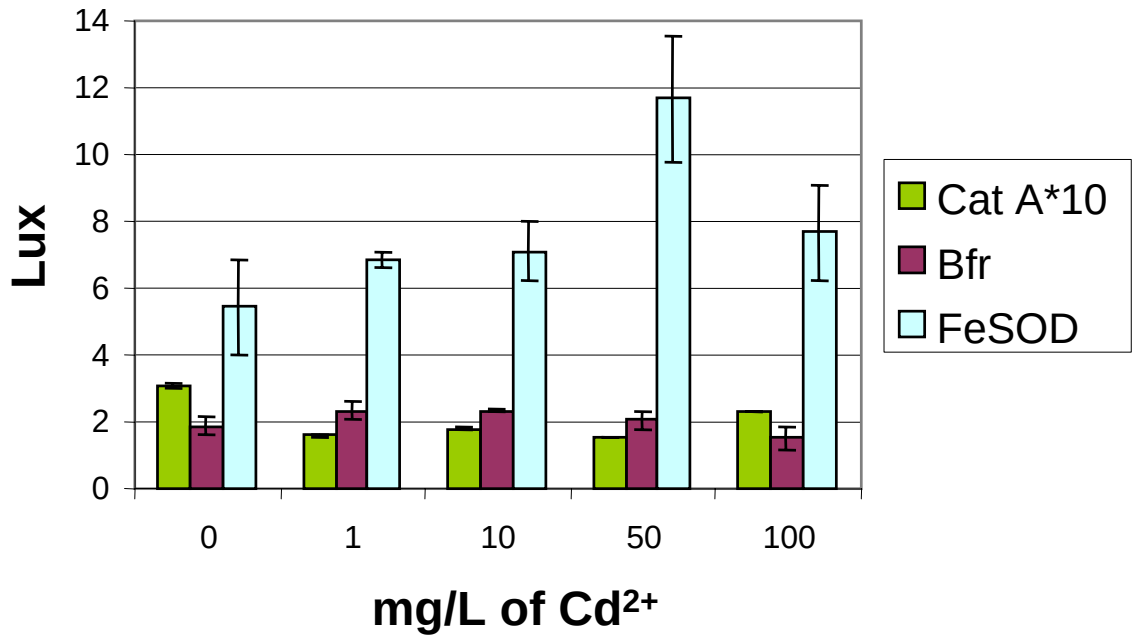

P. putida Corvallis mutants-Cu [in .01M CaCl2]
Lux
30
25
20
15
10
5
0
0
0.01
0.1
1
10
mg/L of Cu2+
Cat A*10
Bfr
FeSOD

Cadmium detection

P. putida Corvallis mutants-Cd [in .01M $Ca(NO_3)_2$]



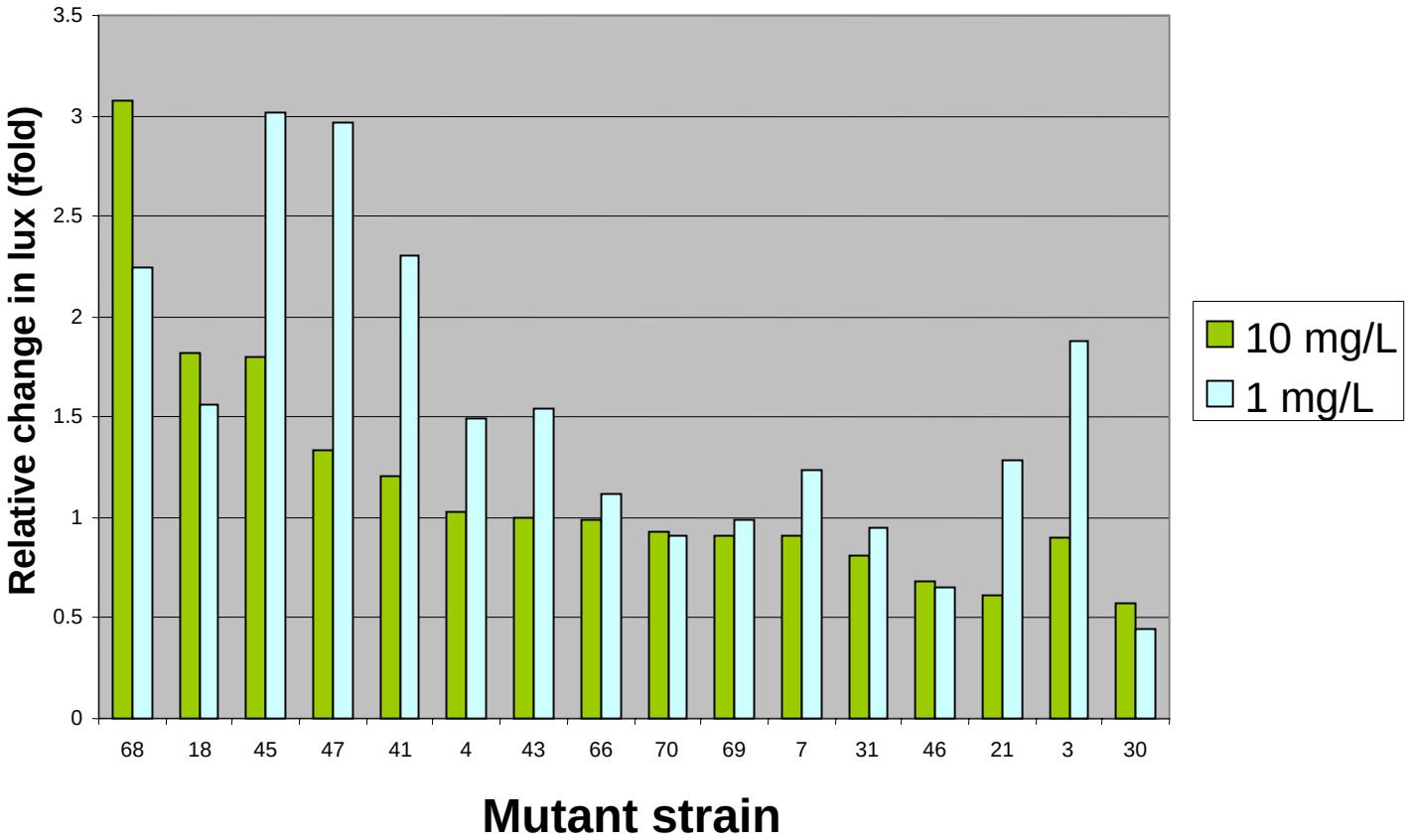
P. putida Corvallis mutants-Cd [in .01M KNO_3]



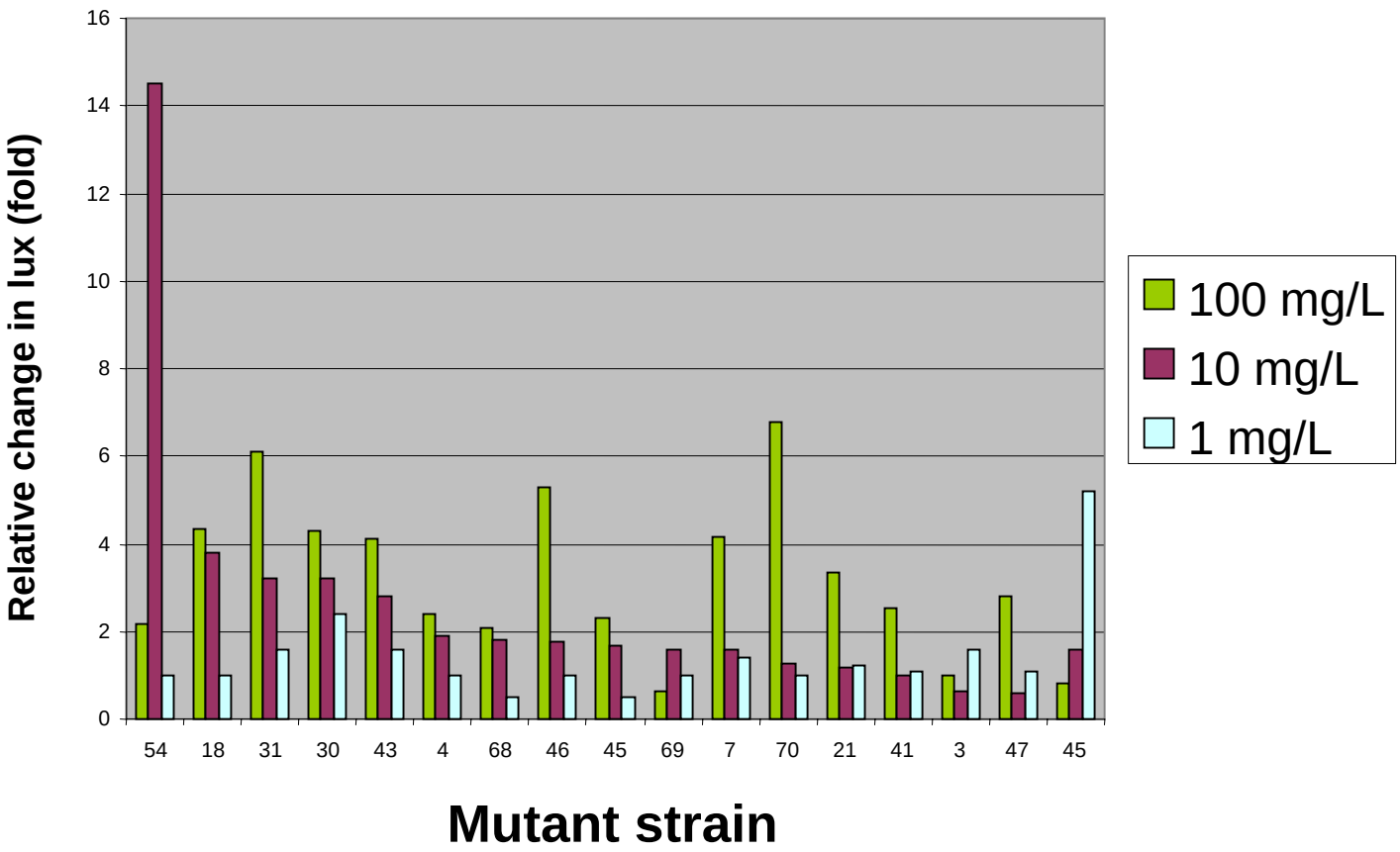
FeSOD mutant is most sensitive

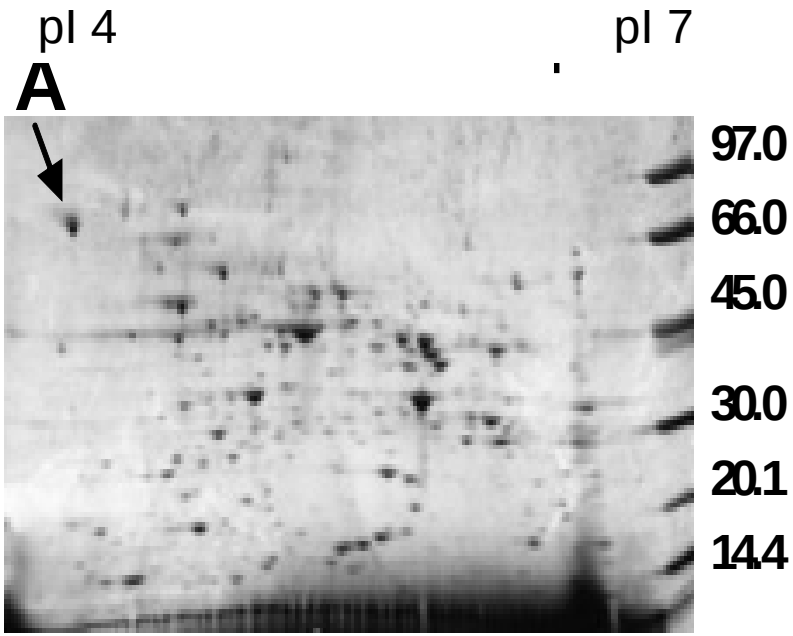
Ca inhibits the Cd response

Response of KT2440 mutants to Cu

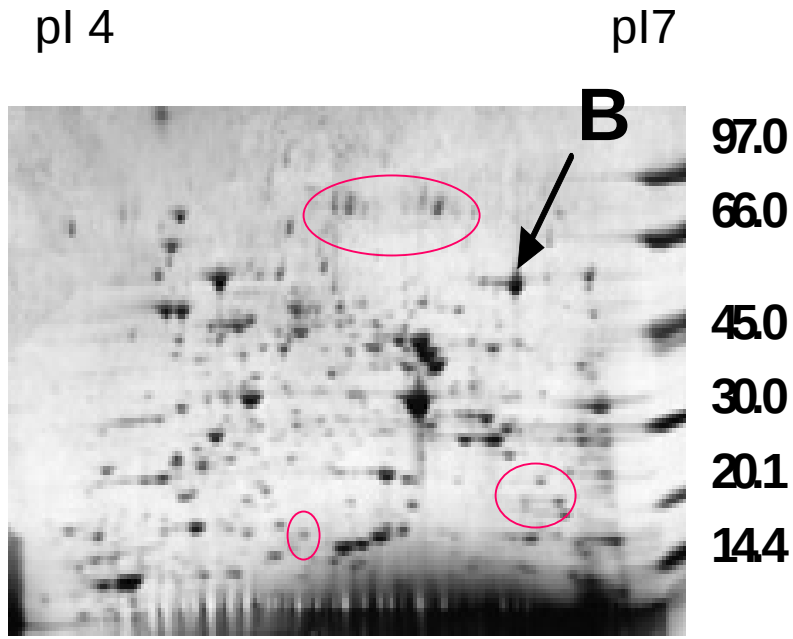


Response of KT2440 mutants to Cd



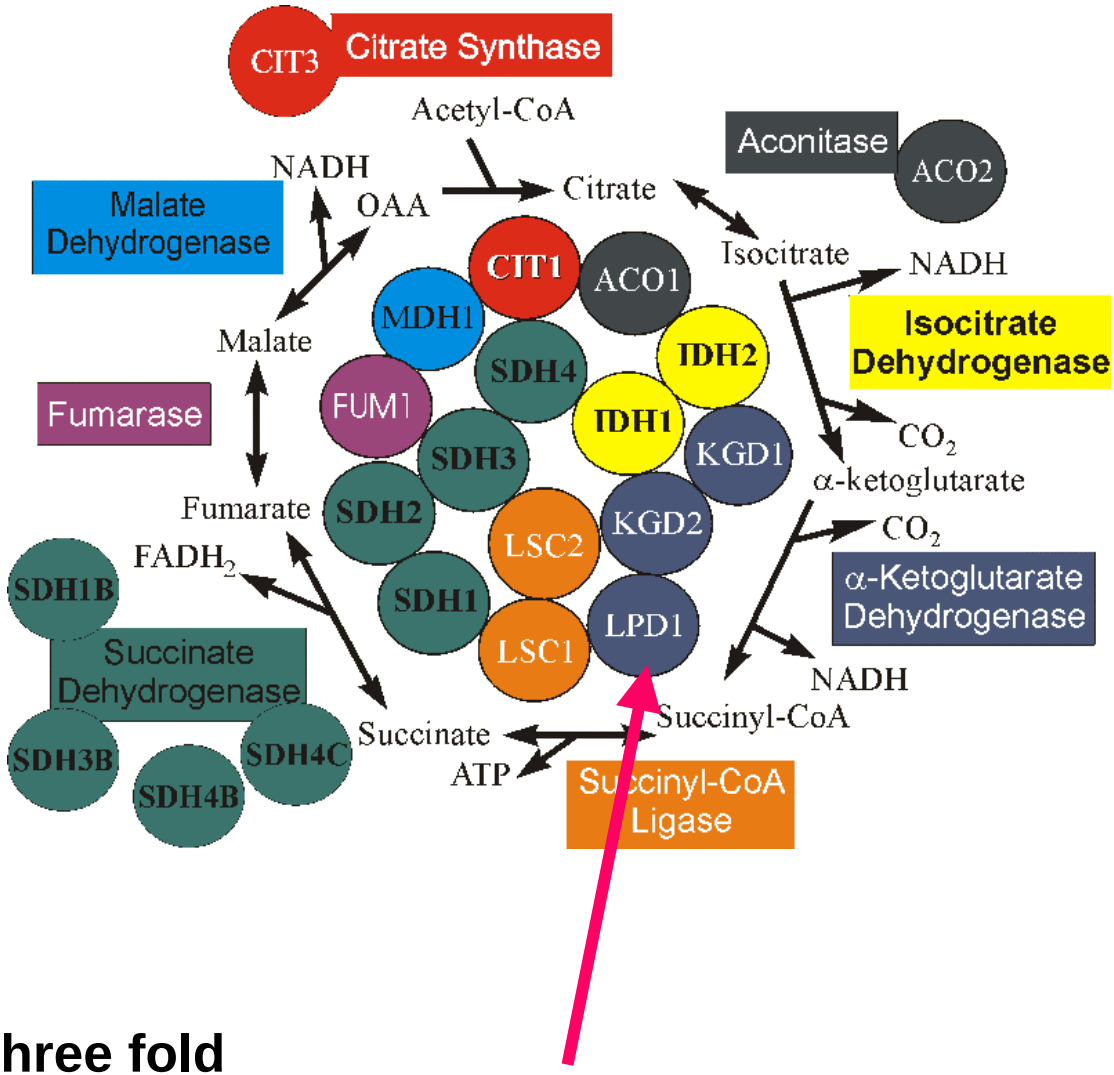


Control



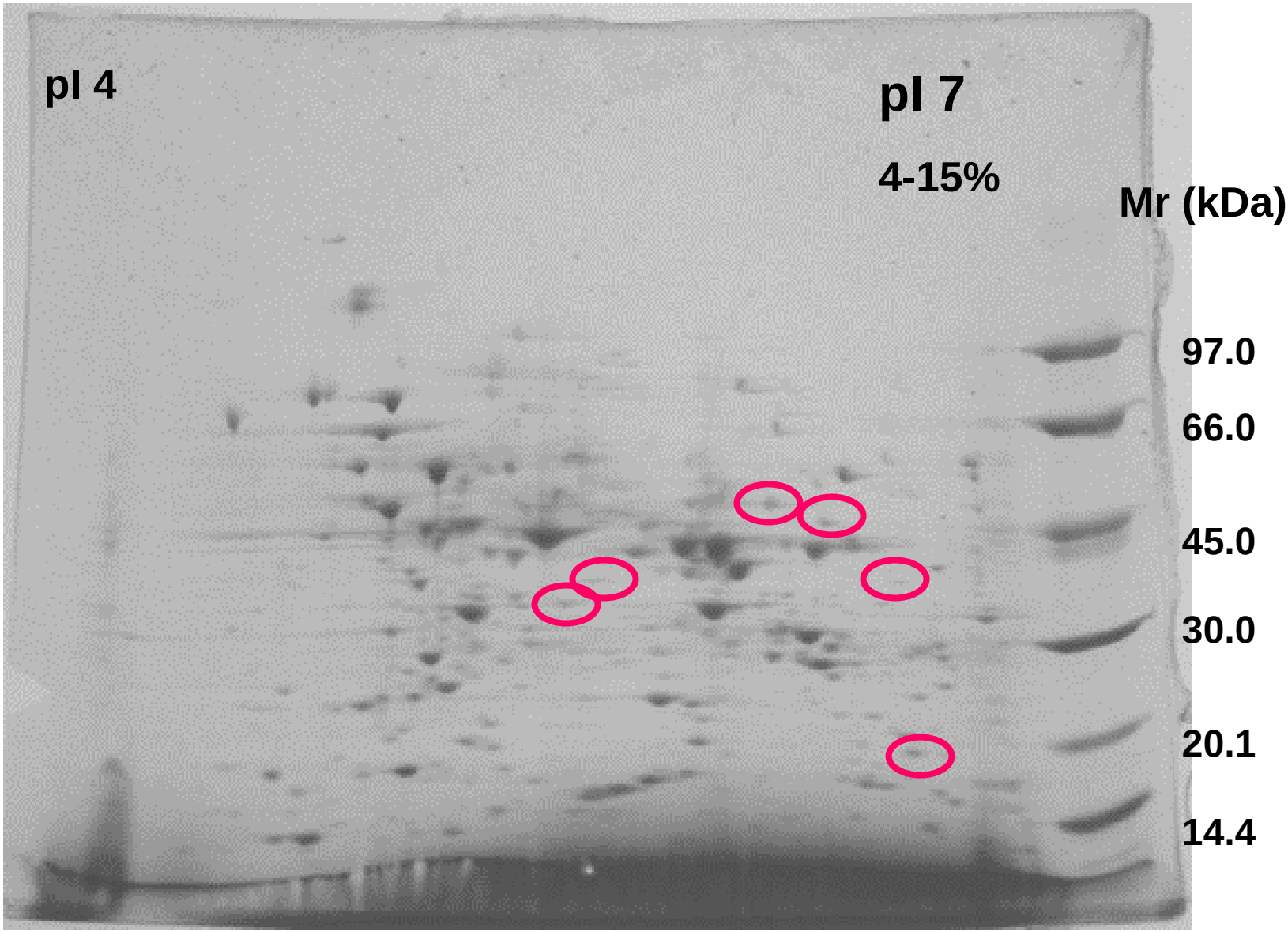
Treatment

(10 mg/l Cu^{++})



Spot B: increased three fold

Corresponded to lipoamide dehydrogenase in TCA cycle



Peptides of increased Intensity.

KT2440 0.01M $Ca(NO_3)_2$ 100 ppm Cd

Conclusions

1) **The two approaches for biosensor development are feasible**

2) ***luxAB*::Insertional mutants that detect Cu and Cd differentially have been identified**
Gene loci await determination

3) **Peptides that increase upon Cu exposure are detected and one has been correlated with a specific function**