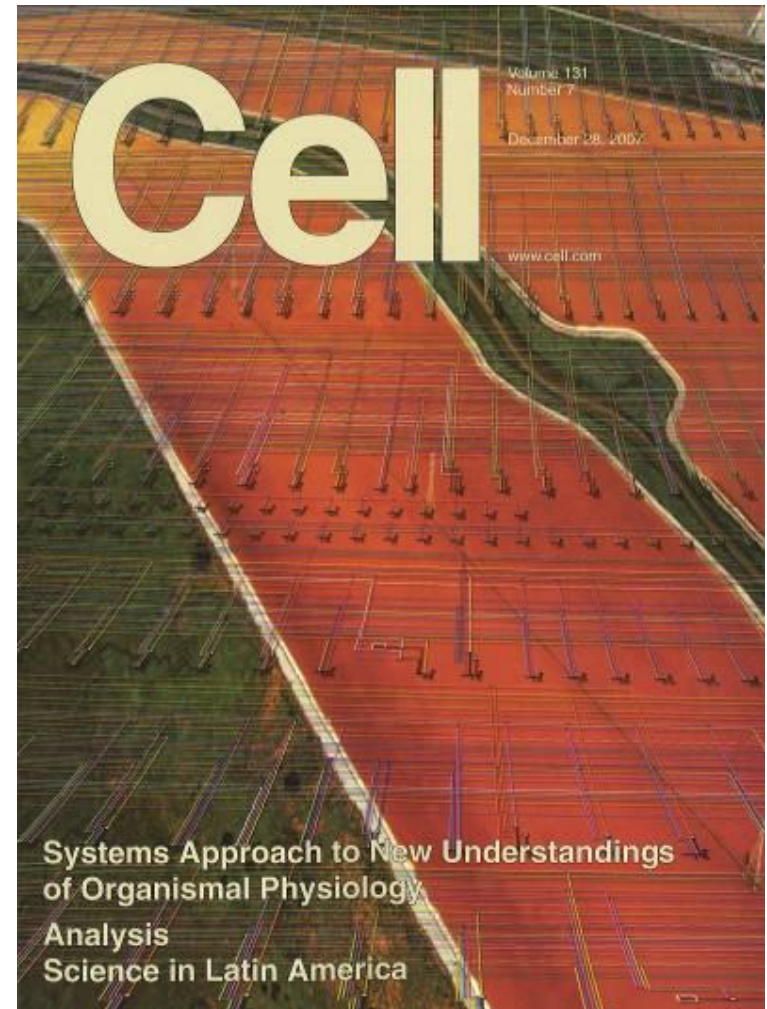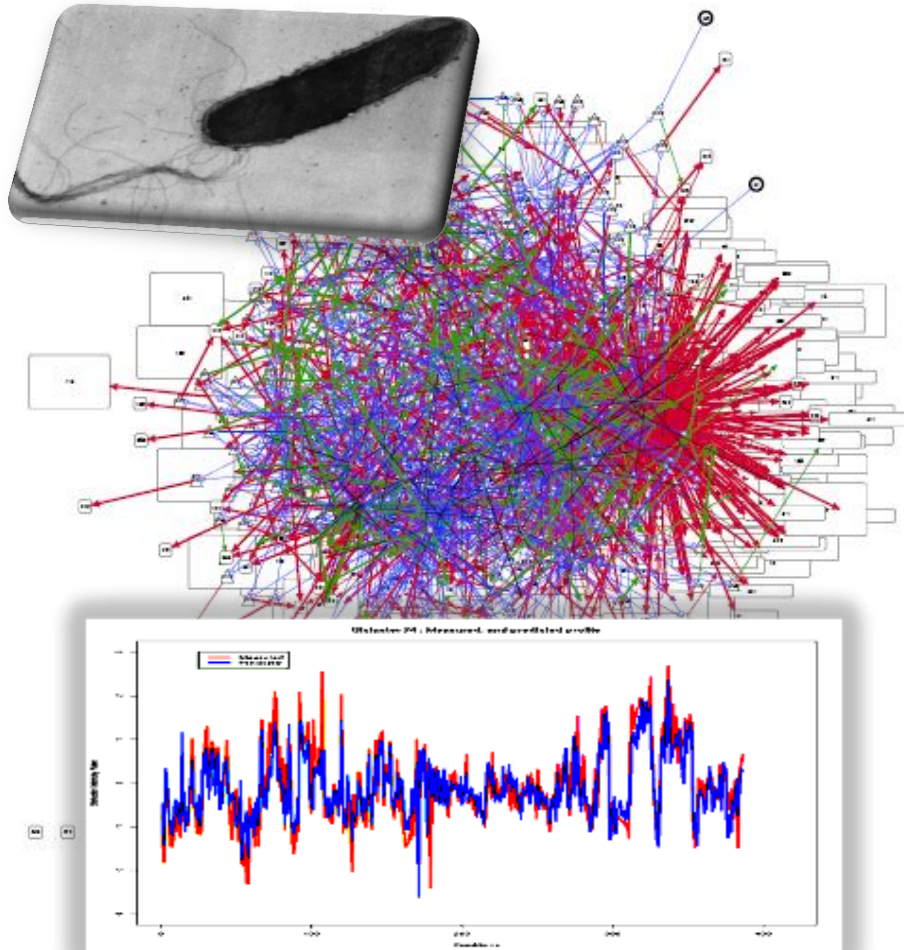# Регуляция гомеостаза ионов меди у *Halobacterium salinarum*: компьютерное моделирование и экспериментальный анализ

Regulation of Cu homeostasis in *H. salinarum*: computational modeling and experimental analysis

Александр Ратушный
*Alexander Ratushny*

Model Organism

Halobacterium salinarum

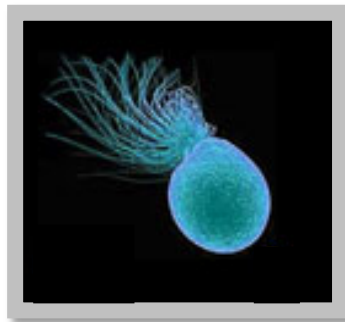Bonneau *et.al.* Cell (2007); Baliga NS Science (2008)

Archaea

Archaea are one of three distinct domains of life on Earth. They are adapted to almost all extreme environments from Methanogens in swamps, to Halophilic Archaea in Salt lakes, and to Thermophilic Archaea in deep ocean thermal vents or hot springs.
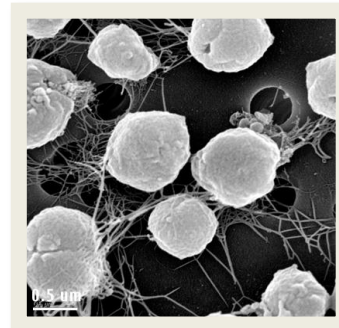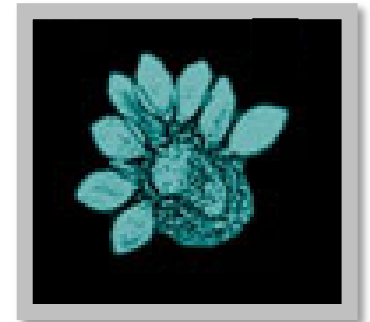
Halobacterium salinarum
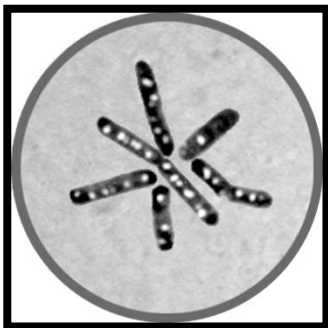
Pyrococcus furiosis
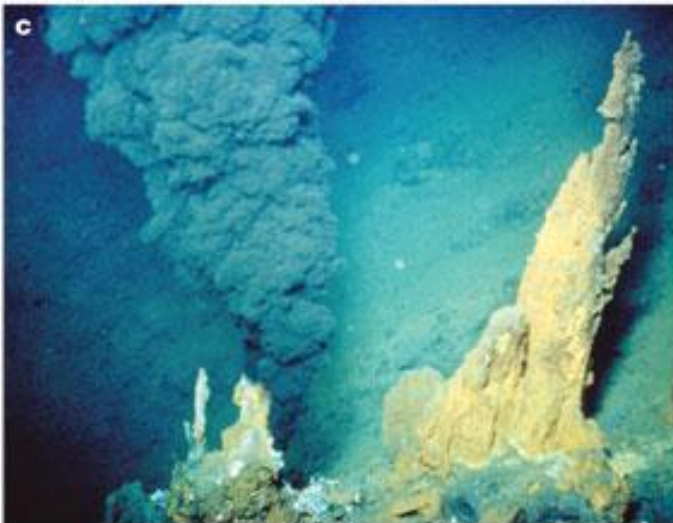

Methanococcus maripaludis

Sulfolobus solfataricus

Archaea

20% of the total biomass

Global Oceans
1.3×10^{28}
Archaea

3.1×10^{28}
Bacteria
(Karner et al. 2001)

Allers & Mevarech, 2005

Nature Reviews | Genetics

Reasons why *H. salinarum NRC-1* is an ideal model organism for systems biology research

a. It is non-pathogenic and easy-to-culture to very high cell densities (>10^8 cells/ml).

b. It has a relatively small genome (2.6Mbp) that is completely sequenced[25]

c. Over 62% of all protein coding genes in *H. salinarum NRC-1* have been characterized through extensive proteomic analysis[26].

d. Key genetic information processing and DNA repair mechanisms are eukaryotic-like [27].

e. It is easily lysed in low salt buffers facilitating high-throughput RNA and protein preparations[20].

f. It is biochemically, genetically and genomically tractable with a range of molecular biology, computational and high-throughput functional genomic tools[19,28-34].

g. A network with an exhaustive number of inferred interactions/associations among the *H. salinarum NRC1* proteins and protein-DNA interactions for general transcription factors has been constructed through evolutionary comparisons and ChIP-chip experiments[5]. More importantly, all of this information has been processed along with gene expression profiling using machine learning algorithms[29,30] to infer a global predictive gene regulatory network[24].

h. Complete genome sequences of several closely related halophiles [25,35-37] will facilitate comparative genomics for extracting detailed information.
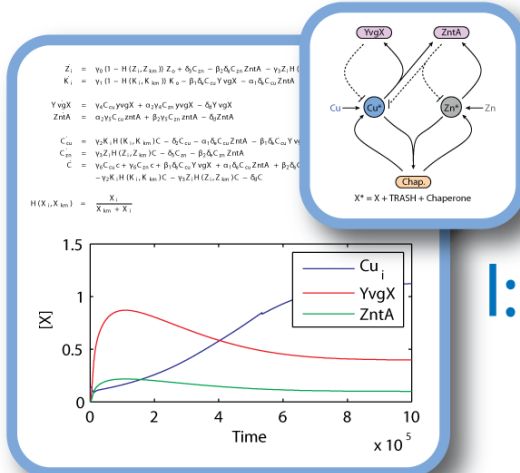
Resources for systems analysis of *H. salinarum NRC-1*

We have crafted tools for comprehensive systems analysis of *Halobacterium salinarum NRC-1* –from high-throughput experimental analysis, to network inference, visualization, and exploration of complex data.

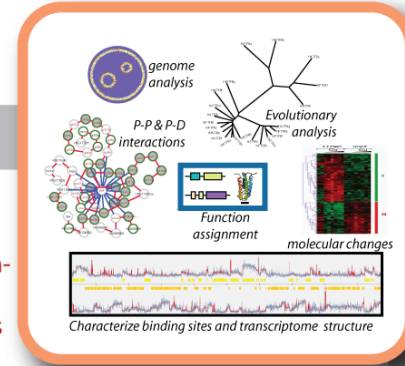Several of these tools have been previously published and include

- classical genetics (gene knockouts[20-23,34], overexpression and epitope tagging[5] etc.), microarrays[19,21,23],

- quantitative proteomics (using ICAT[19] and iTRAQ[20,22]),

- protein surveys[26],

- protein complex immunoprecipitation for determining protein-protein and protein-DNA interactions[5],

- computational algorithms for localizing protein-DNA interactions[38] and regulatory network inference[29,30]

- and software for simultaneous exploration of diverse databases in a framework that enables software interoperability[31,39]
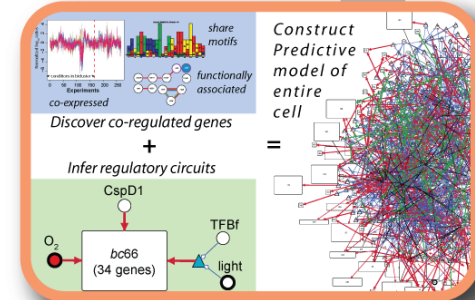
Research Model

YvgX   ZntA

$X^* = X + TRASH + Chaperone$

$H(X_i, X_{km}) = \dfrac{X_i}{X_{km} + X_i}$

[X] — 1.5, 1, 0.5

Cu$_i$
YvgX
ZntA

Time   x 10^5

I: Define and simulate mass-action kinetics

I: Formulate and test new hypotheses via global-systems analysis

genome analysis

P-P & P-D interactions

Evolutionary analysis

Function assignment

molecular changes

Characterize binding sites and transcriptome structure

RE-ENGINEER

Cu efflux

(YvgX)   (ZntA)

Cu   Zn/Cu/Ni/Co

702H/2581H (chaperone)

1179C (TRASH domain)

yvgX

Zn/Cu/Ni/Co efflux

Unknown regulator(s)

zntA

Gene(s)   a. activate   b. repress   Gene Product

x and y

Characterize sub-circuit behavior

II: Develop algorithms to integrate diverse data and construct predictive models

share motifs   *functionally associated*   *co-expressed*

Construct Predictive model of entire cell

Discover co-regulated genes

+

Infer regulatory circuits

CspD1
O$_2$ bc66 (34 genes)   TFBf   light

=

II: Construct modules and experimentally validate dynamics

Media Mixer   Cells In   Waste

Draw Pump   Cell Waste

yvgX mRNA
MK-1 GFP + Cu
Sma3 GFP + Cu
AuraA1179C GFP + Cu

Normalized Expression

Time (minutes)

Mean fluorescence (Arb)

Time (minutes)

III: Develop software to explore models and design experiments to test model predictions

VISUALIZATION
Cytoscape: Interactions
DMV: Matrices
Genome Browser

KEGG (metabolic pathways)

NCBI (gene, protein)
DAVID (Functional annotations)

DATABASES
Microarray
Proteomics
ChIP-chip
Annotations
Protein CoIP

BOSS   **FIREGOOSE**   **EMBL STRING** (Functional Associations)

RMI   RMI   RMI

ANALYSIS
R statistical environment (Bioconductor)
MeV; MatLab

SBEAMS (Halobacterium Functional Annotations)

Gaggle Microformat

Baliga lab

Wackett et. al, *App. Env. Microbiol*, 2004

Article

A systems view of haloarchaeal strategies to withstand stress from transition metals

Amardeep Kaur,[1,3] Min Pan,[1,3] Megan Meislin,[1] Marc T. Facciotti,[1] Raafat El-Gewely,[2] and Nitin S. Baliga[1,4]

[1]Institute for Systems Biology, Seattle, Washington 98103-8904 USA; [2]University of Tromso, 9037 Tromso, Norway

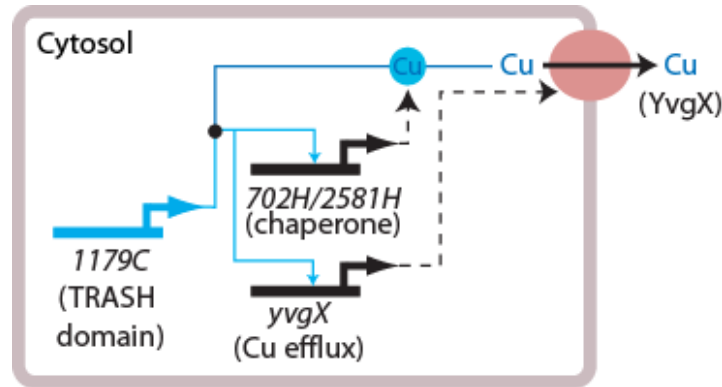# A systems-level model for transition metal stress response of *Halobacterium NRC-1*
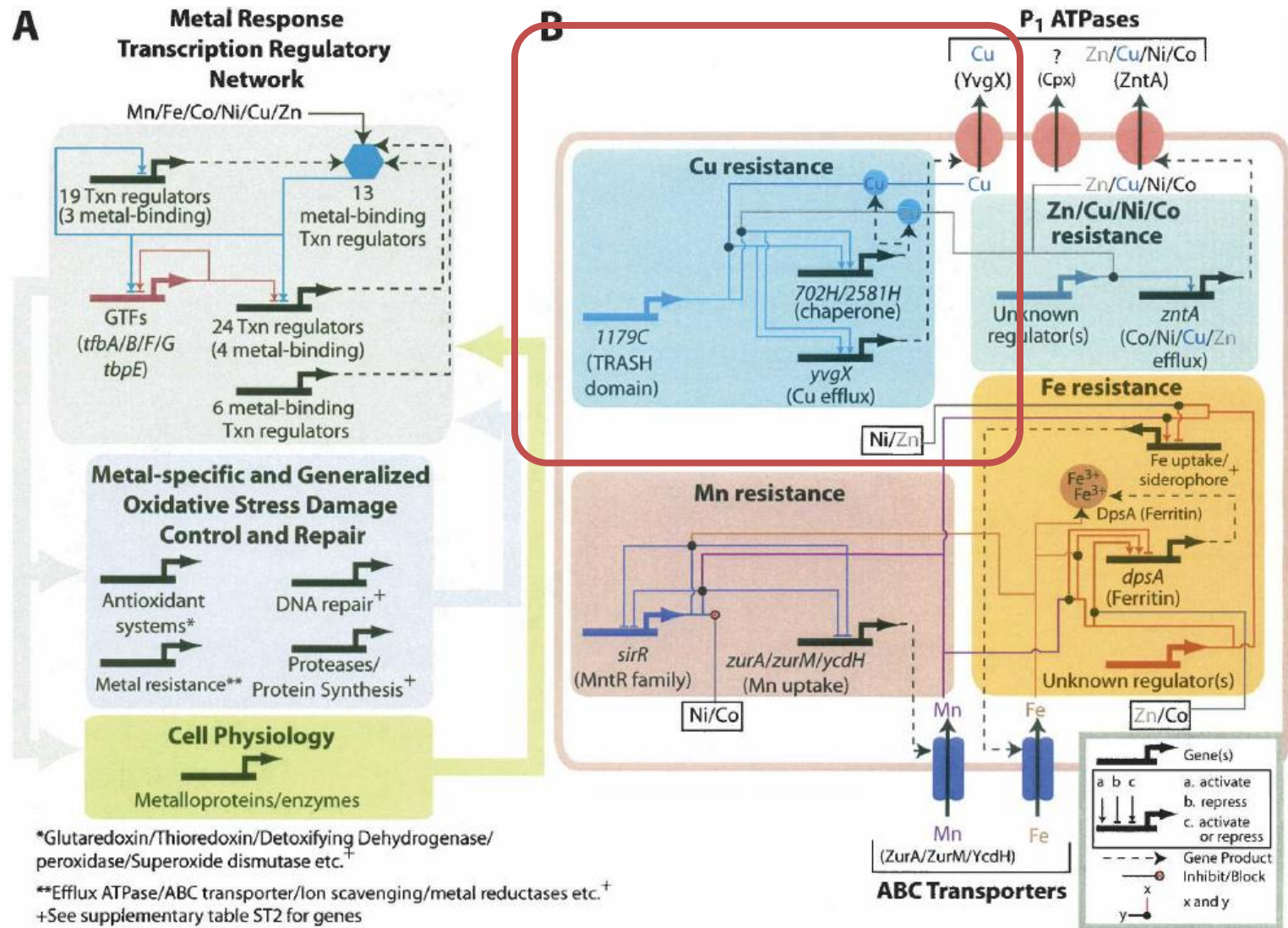
Cu: essential and hazardous

- Crucial micronutrient
  - Co-factor in electron transport
  - Redox detoxification
- Misregulation linked to:
  - Wilson's disease
  - Menke's syndrome } Cu import / efflux disorders
  - Alzheimer's
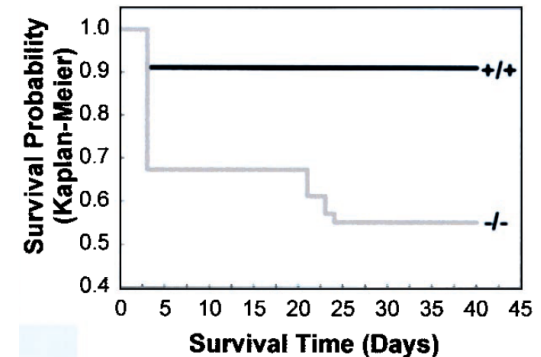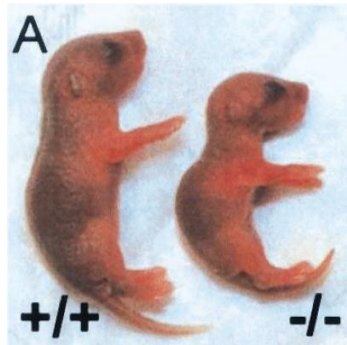
Hamza *et.al*, *PNAS*, 2001

Cu Efflux in *H. salinarum*

0702   2581

BLAST Alignment
Score: 26.6
E-value: 3×10^{-5}

Experimental Methods

- Assay for YvgX expression
  - Transcriptionally fused GFP expression

- Assay for intracellular Cu
  - Inductively coupled plasma mass spectrometry (ICP-MS)

- Compare:
  - Deletion of chaperones
  - Constitutive overexpression of chaperones
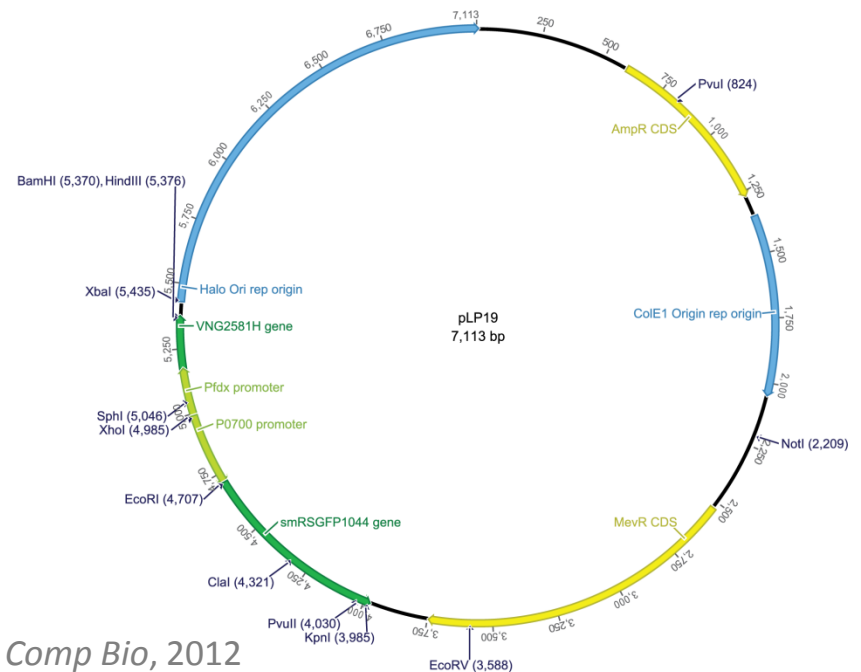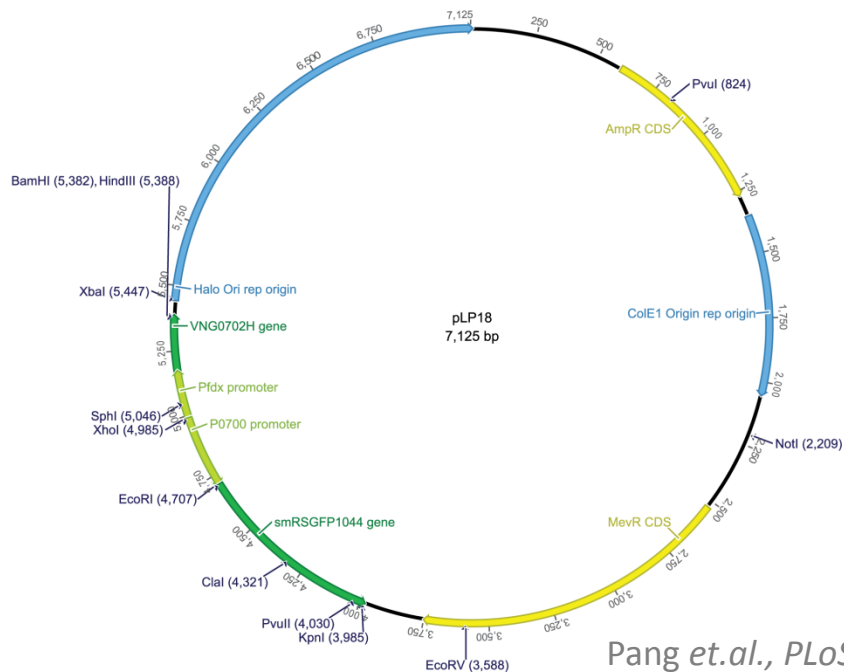
Pang *et.al., PLoS Comp Bio,* 2012

Cu Efflux has pulse dynamics
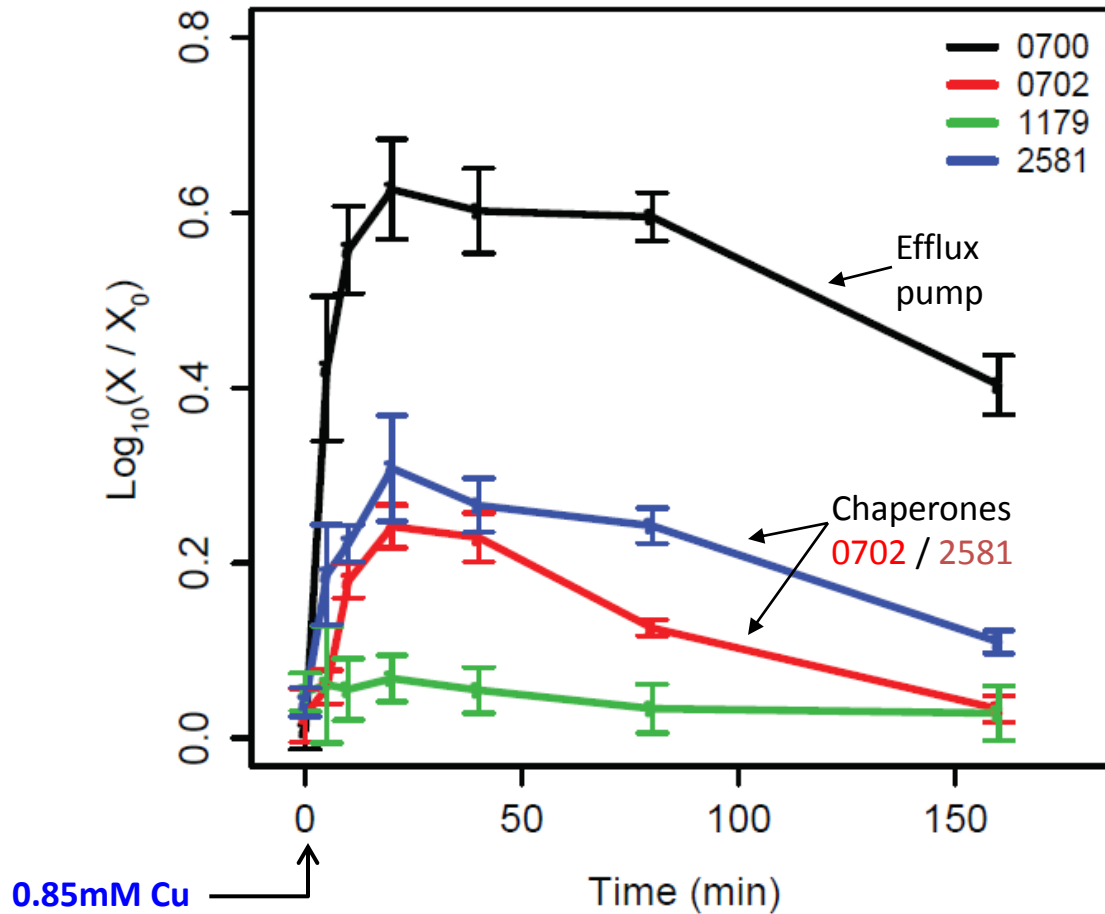
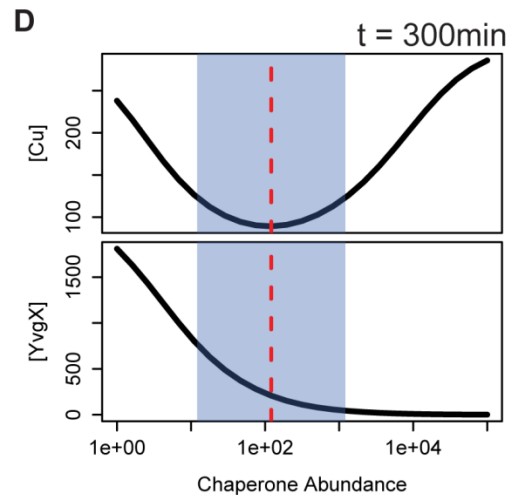mRNA profiles show pulse responses for efflux pump and metallochaperones

Pang *et.al., PLoS Comp Bio,* 2012

Metallochaperone Function

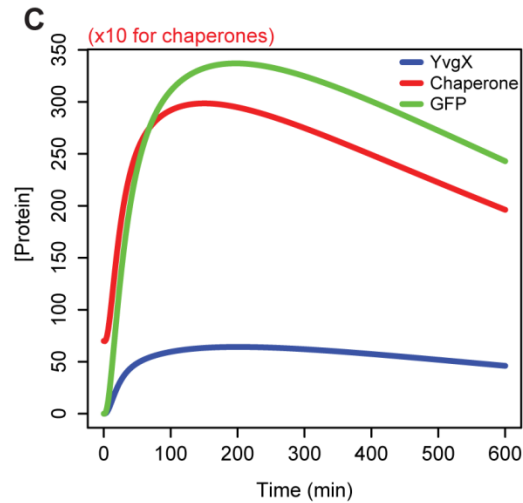**Wild-type**

Cu

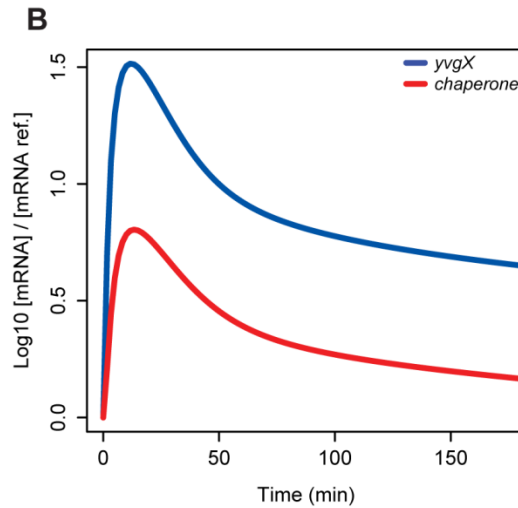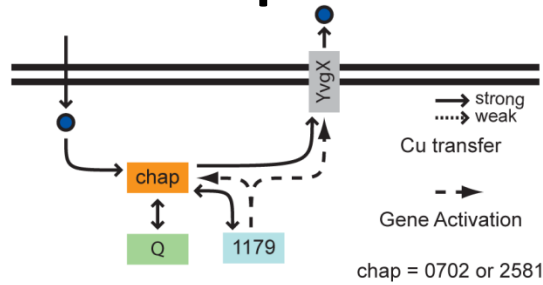1179 ⟷ a / b ⟷ YvgX

a / b = 0702 / 2581
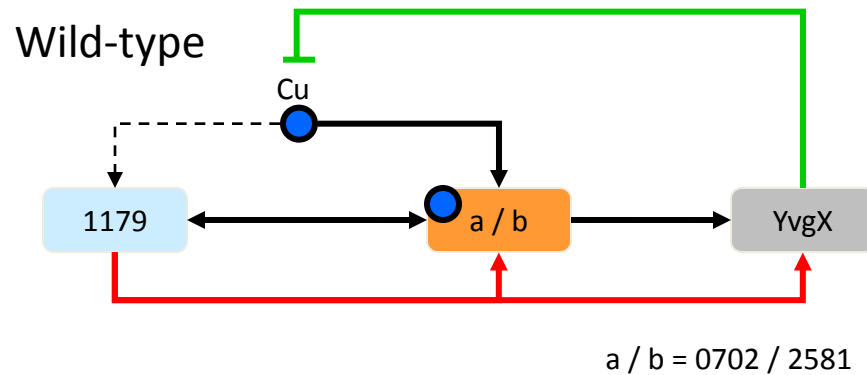
Chaperones are a functionally important element in Cu efflux
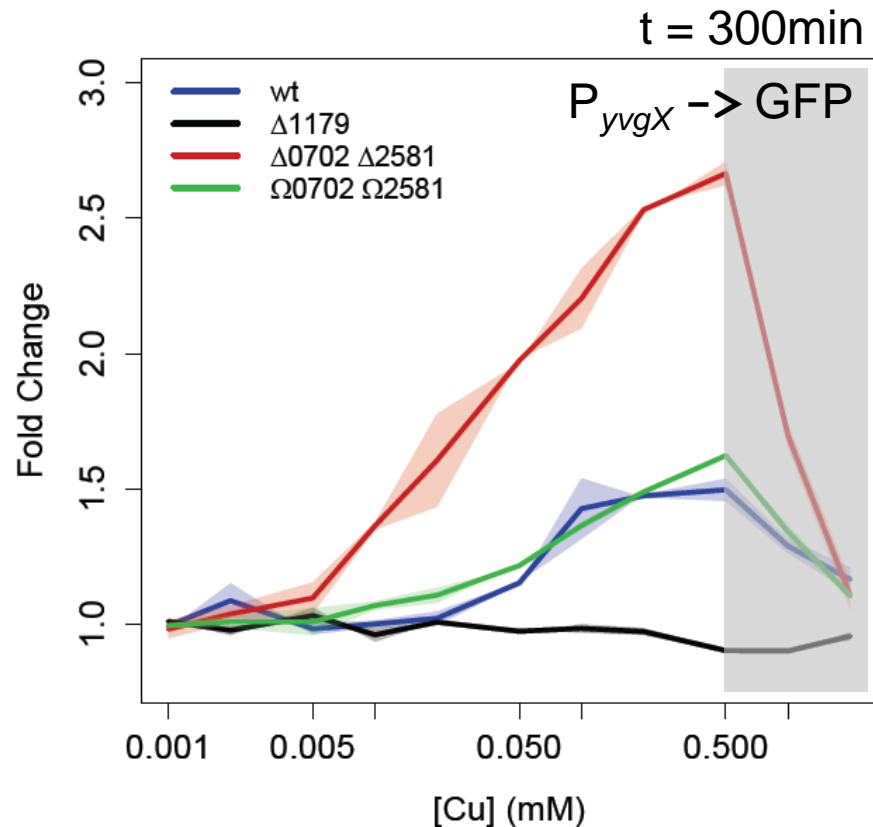
Trafficks Cu between sensory and efflux components
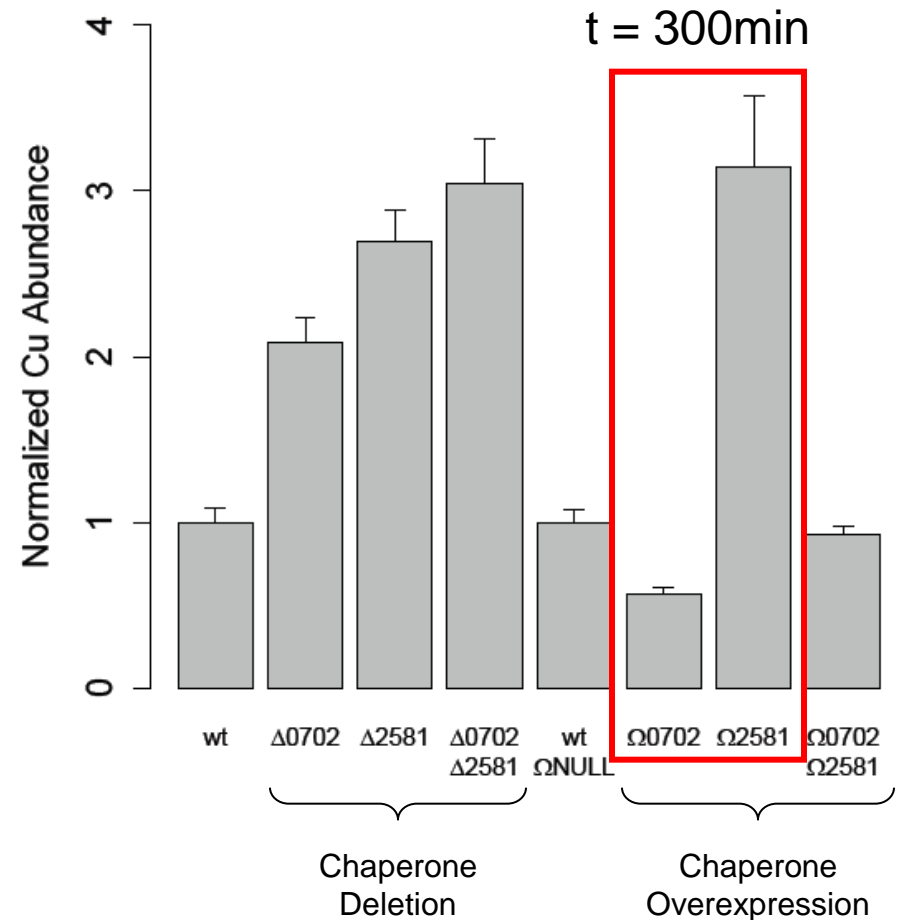
Mediates negative feedback

A simple model for Cu Efflux
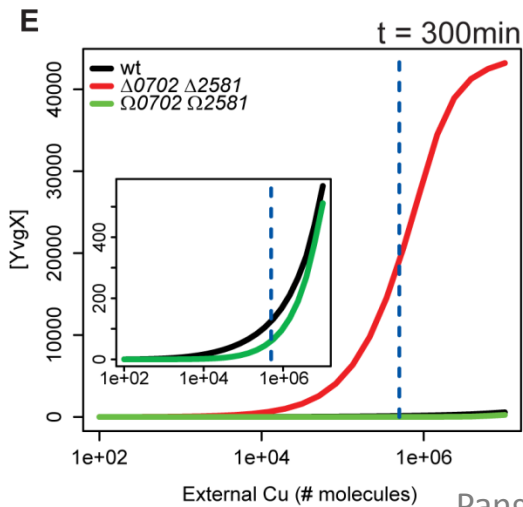
Transcriptional dynamics of YvgX and associated consequences on intracellular Cu levels
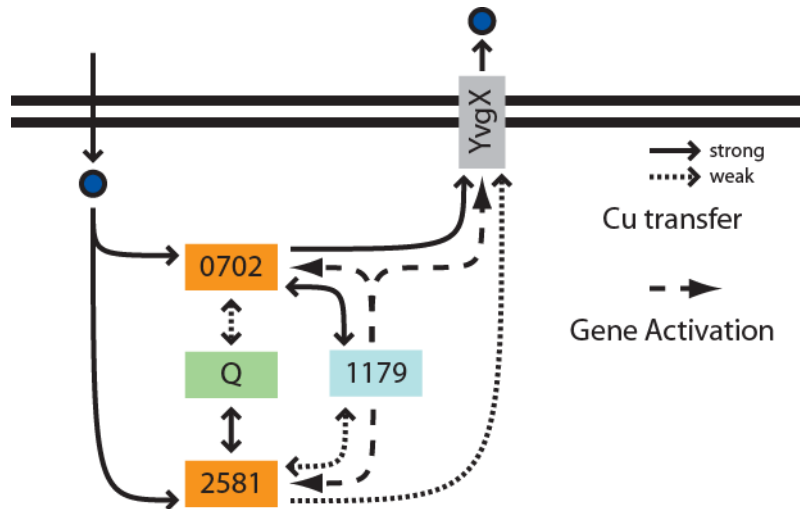

Chaperone **deletion** increases sensitivity to Cu

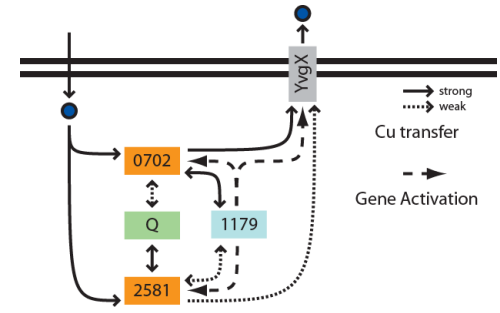Chaperone **overexpression** quantitatively similar to **wt**
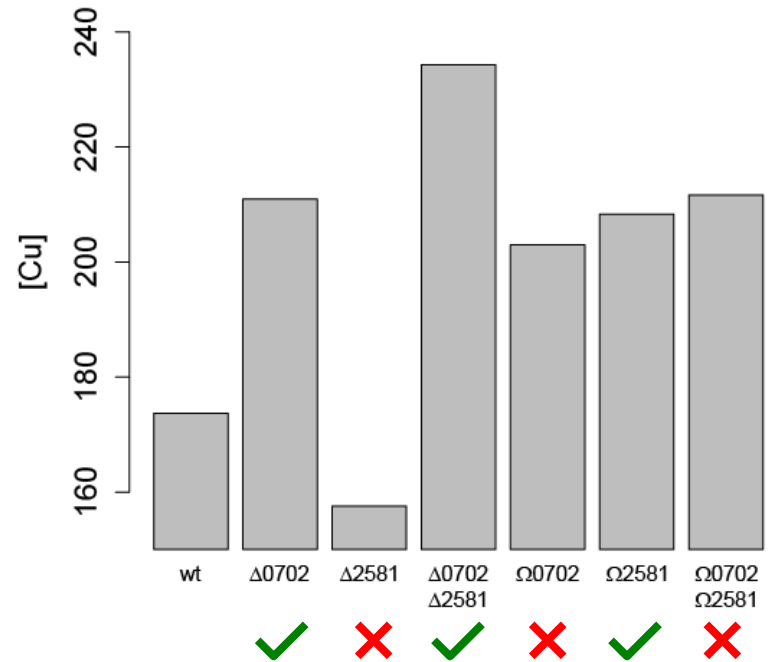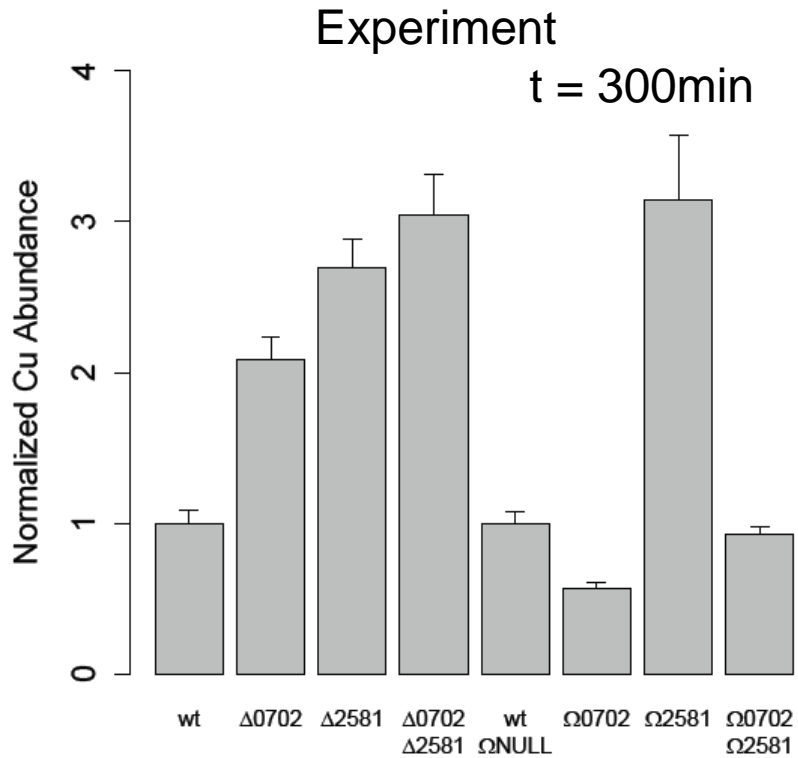
Unique chaperone roles

Two Chaperone Model 1

Parameter	Default	Model 0	Model 1.1
k.D2581.P1179.Cu.binding	0.01	0	
k.D2581.P1179.Cu.dissociation	0.1	0	
k.M2581.degradation	0.003262	0	
k.M2581.transcription	0.424167	0	
k.OE2581.transcription	0.004242	0	
k.P0700.Cu.by.P2581.F1	0.01	0	
k.P0700.Cu.by.P2581.R1	0.1	0	
k.P0700.Cu.by.P2581.F2	1	0	0.1
k.P0702.Cu.bind	0.01		
k.P0702.Cu.by.P1179.F1	0.01		
k.P0702.Cu.by.Q.F1	0.001		
k.P0702.Cu.by.P2581.F1	0.01	0	0
k.P0702.Cu.by.P2581.R1	0.1	0	0
k.P0702.degradation	0		
k.P2581.Cu.bind	0.01	0	
k.P2581.Cu.by.P1179.F1	0.01	0	
k.P2581.Cu.by.P0702.F1	0.01	0	0
k.P2581.Cu.by.P0702.R1	0.1	0	0
k.P2581.Cu.by.Q.F1	0.001	0	
k.P2581.Cu.debind	0.001	0	
k.P2581.Cu.degradation	0	0	
k.P2581.degradation	0	0	
k.P2581.translation	0.030769	0	
k.P1179.Cu.by.P0702.F1	0.01		
k.P1179.Cu.by.P0702.R1	0.1		
k.P1179.Cu.by.P0702.F2	0.1		
k.P1179.Cu.by.P2581.F1	0.01	0	
k.P1179.Cu.by.P2581.R1	0.1	0	
k.P1179.Cu.by.P2581.F2	0.1	0	0.01
k.Q.Cu.by.P0702.F1	0.01		

Pang *et.al., PLoS Comp Bio*, 2012
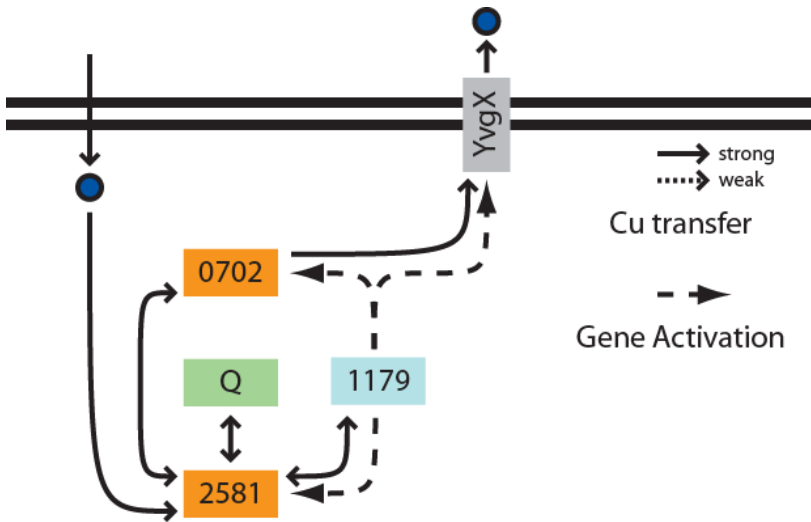
Experiment
t = 300min
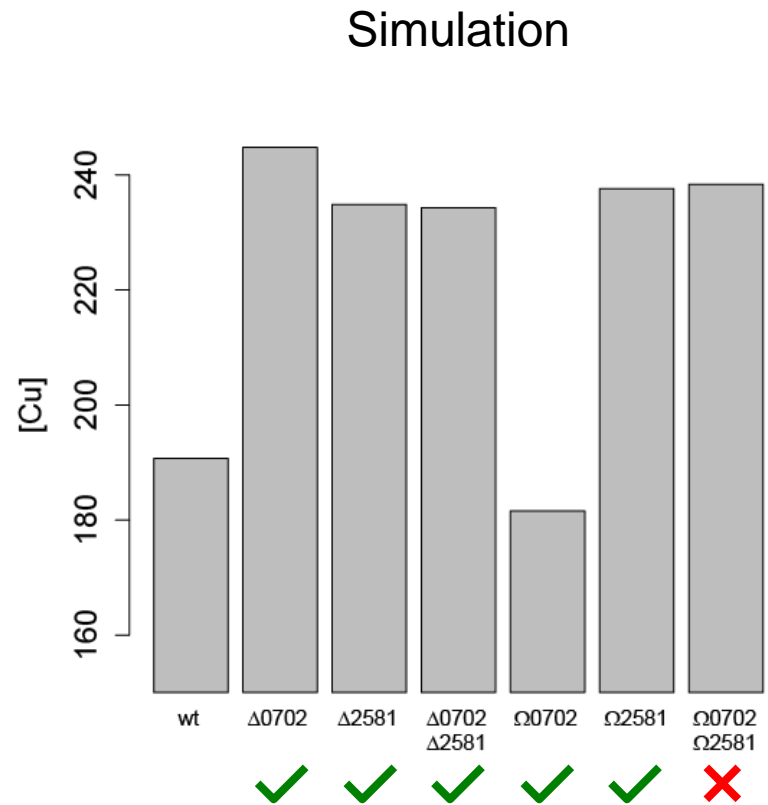
Simulation

Pang *et.al., PLoS Comp Bio*, 2012

Unique chaperone roles

Two Chaperone Model 2

Parameter	Default	Model 0	Model 1.1	Model 1.2
k.D2581.P1179.Cu.binding	0.01	0		
k.D2581.P1179.Cu.dissociation	0.1	0		
k.M2581.degradation	0.003262	0		
k.M2581.transcription	0.424167	0		
k.OE2581.transcription	0.004242	0		
k.P0700.Cu.by.P2581.F1	0.01	0		0
k.P0700.Cu.by.P2581.R1	0.1	0		0
k.P0700.Cu.by.P2581.F2	1	0	0.1	0
k.P0702.Cu.bind	0.01			
k.P0702.Cu.by.P1179.F1	0.01			0
k.P0702.Cu.by.Q.F1	0.001			0
k.P0702.Cu.by.P2581.F1	0.01	0	0	
k.P0702.Cu.by.P2581.R1	0.1	0	0	
k.P0702.degradation	0			
k.P2581.Cu.bind	0.01	0		
k.P2581.Cu.by.P1179.F1	0.01	0		
k.P2581.Cu.by.P0702.F1	0.01	0	0	
k.P2581.Cu.by.P0702.R1	0.1	0	0	
k.P2581.Cu.by.Q.F1	0.001	0		
k.P2581.Cu.debind	0.001	0		
k.P2581.Cu.degradation	0	0		
k.P2581.degradation	0	0		
k.P2581.translation	0.030769	0		
k.P1179.Cu.by.P0702.F1	0.01			0
k.P1179.Cu.by.P0702.R1	0.1			0
k.P1179.Cu.by.P0702.F2	0.1			0
k.P1179.Cu.by.P2581.F1	0.01	0		
k.P1179.Cu.by.P2581.R1	0.1	0		
k.P1179.Cu.by.P2581.F2	0.1	0	0.01	
k.Q.Cu.by.P0702.F1	0.01			0

Pang *et.al., PLoS Comp Bio*, 2012

Experiment
t = 300min
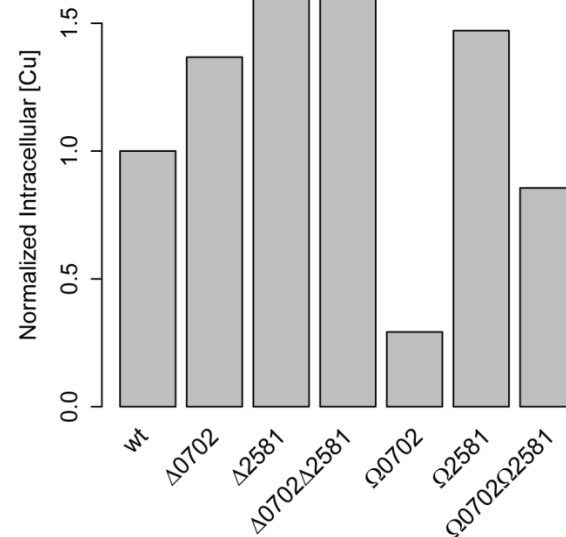
Simulation

Pang *et.al., PLoS Comp Bio,* 2012
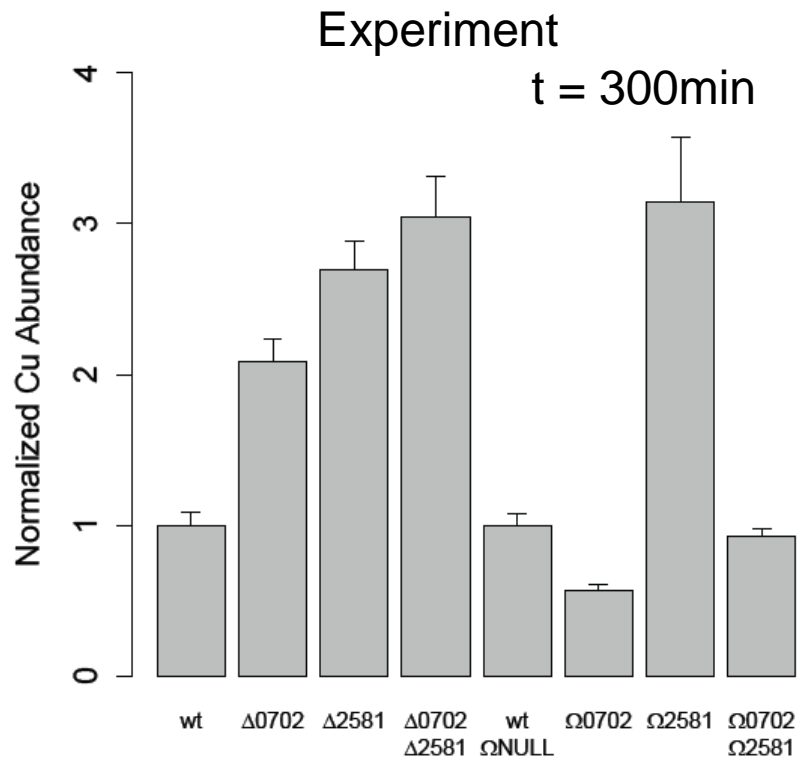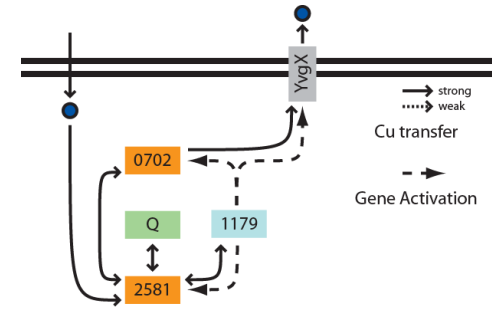
Steady-state model for intracellular *Cu*

$$Cu_{in} = \frac{k_0 + \dfrac{c_2}{k_{21}}}{1 + \dfrac{c_2}{k_{21}} + f(c_2, k_{20}, k_{22})\dfrac{c_1}{k_{11}} + f(c_2, 0, k_{23})}$$

where

$$f(x, k_1, k_2) = \frac{k_1 + \dfrac{x}{k_2}}{1 + \dfrac{x}{k_2}}$$

c_1 and c_2 are concentrations of 0702 and 2581 metallochaperones, respectively
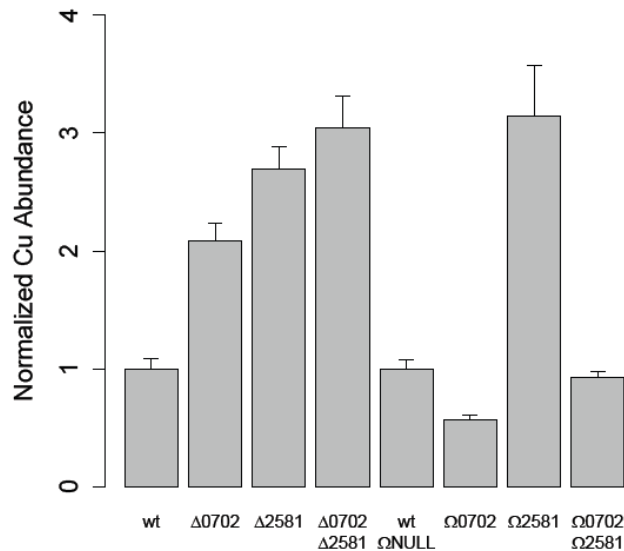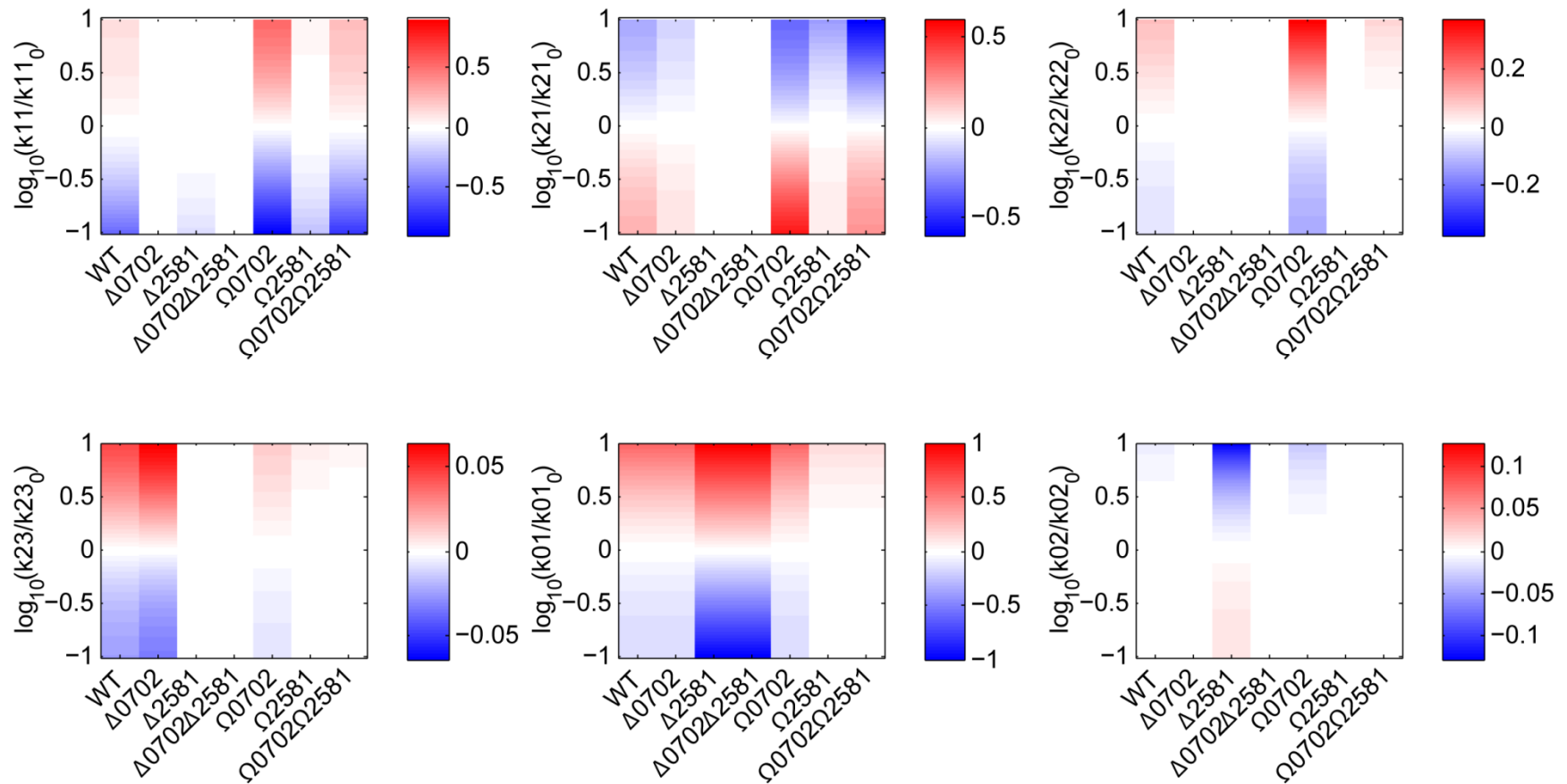

Experiment

In silico simulated levels of intracellular Cu for different genetic backgrounds

Sensitivity analysis of the steady-state GHF model

GHF model ⇒ Improvement of the portrait model

Added

- basal Cu affinity of 0702

- basal Cu trafficking of 2581 to YvgX

- degradation (on the order of cell growth) to the chaperones. Specifically, 2581 (MC2) is degraded slightly faster than 0702 (MC1).
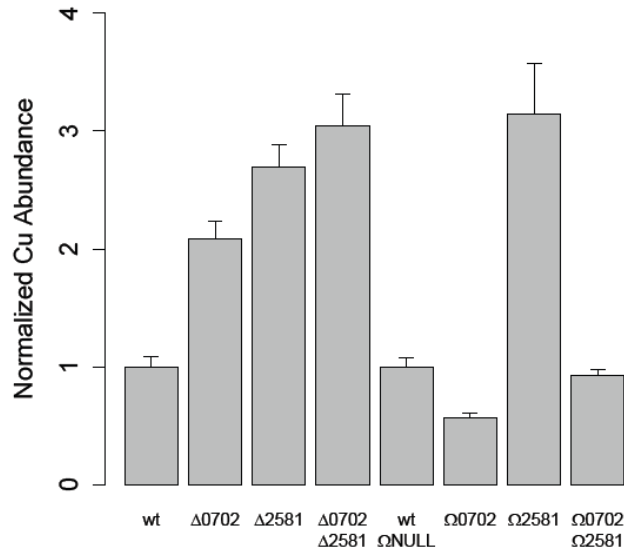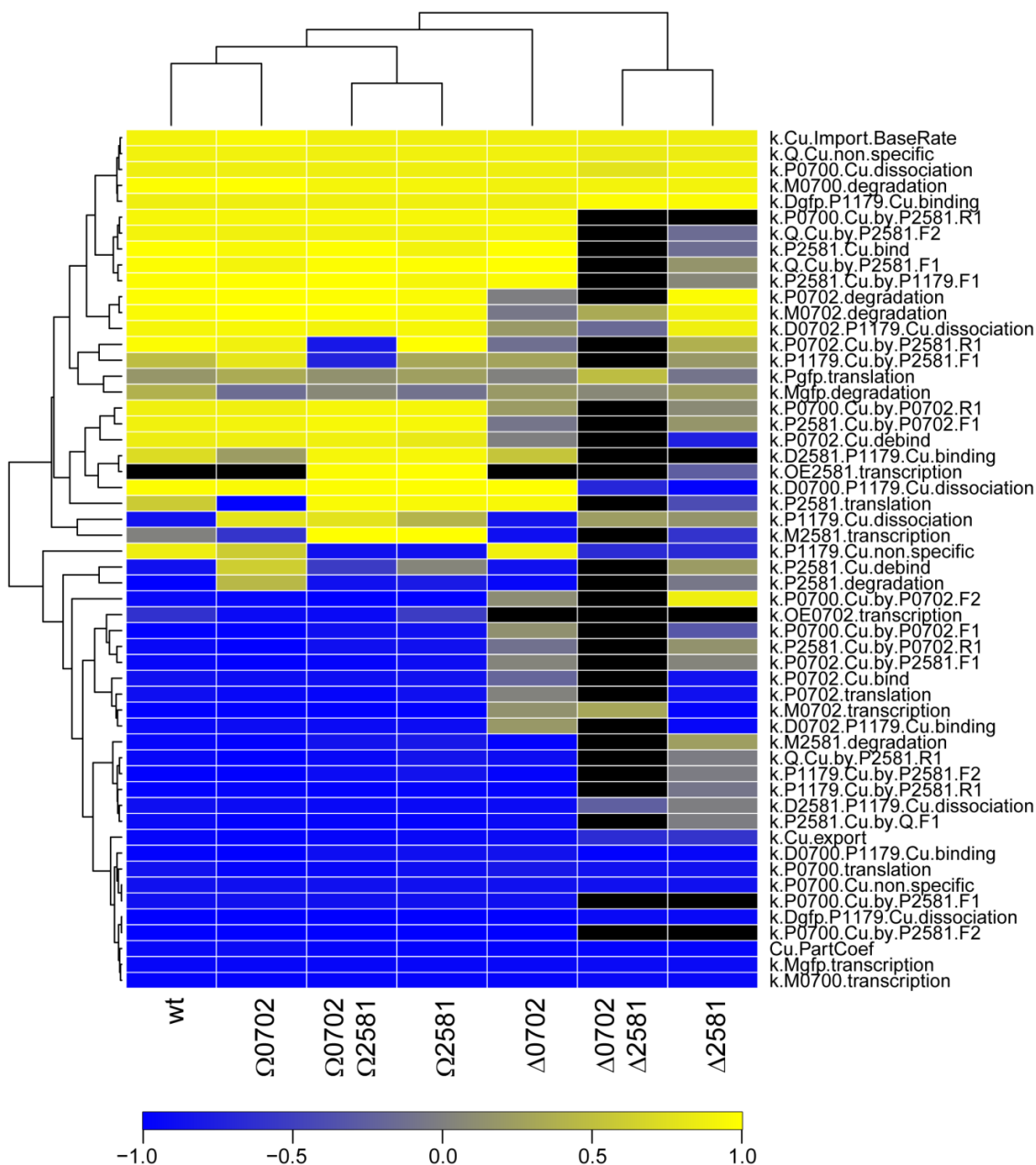
Modified

- the equilibrium for Cu mediated 0702-2581 dimers that are formed during the Cu 'hand-off' - made their prevalence lower.

- the activation rate of 1179 by 2581

Experiment
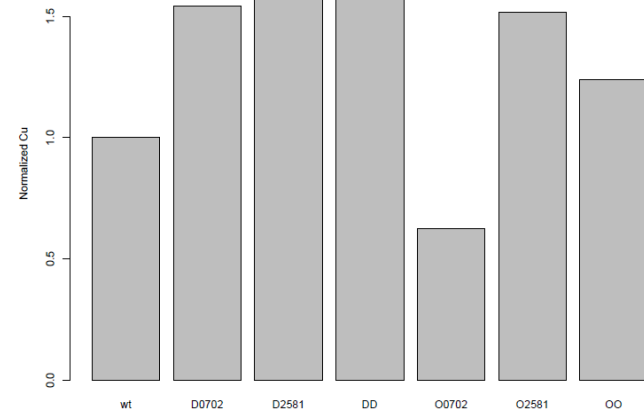
Portrait model simulation results

Sensitivity analysis of the portrait model

Reducing the activity of VNG0702H results in an increase in intracellular Cu. This can occur in several ways:
- the binding affinity of VNG0702H can be reduced (decreasing the equilibrium ratio between apo-/Cu-bound VNG0702H, or apo-VNG0702H / Cu-VNG2581H).
- trafficking efficiency of VNG0702H to VNG0700G can be reduced.
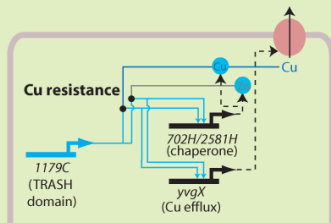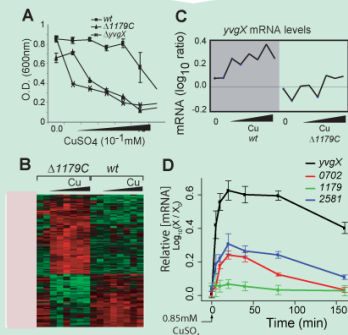- the lifetime of VNG0702H proteins can be reduced via increased specific degradation.
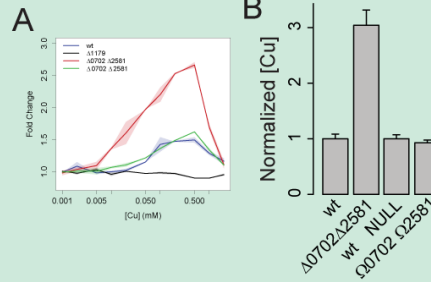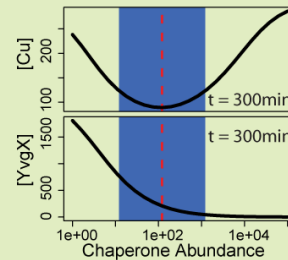
ITERATION 1 — ITERATION 2 — ITERATION 3

MODEL

EGRIN
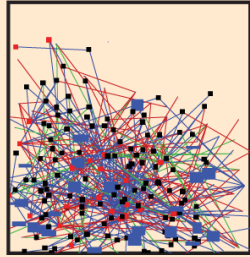
Model 0
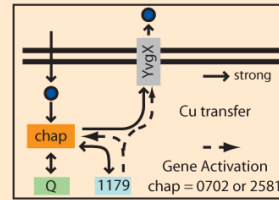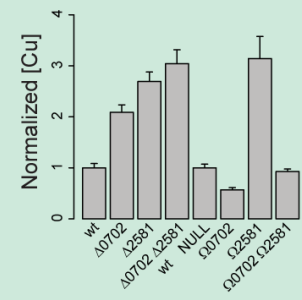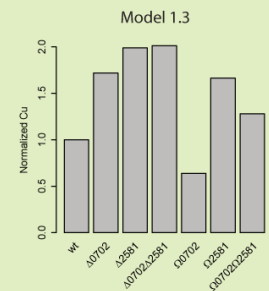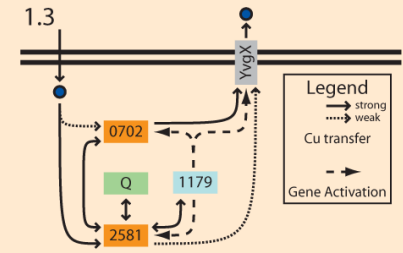
Generalized Hill function method

PREDICTION

EXPERIMENT

Acknowledgements

Baliga and Aitchison Groups, ISB
- **Lee Pang**
- Amardeep Kaur
- Min Pan
- Aaron Brooks
- Danielle (Miller) Durudas
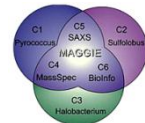- Nitin Baliga
- John Aitchison

Adams Group, UGa
- Aleksandar Cvetkovic
- Sunil Kumar
- Michael W. W. Adams

ENIGMA, LBL:
- Adam Arkin

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  - MAGGIE
  - Enigma

- Center for Systems Biology