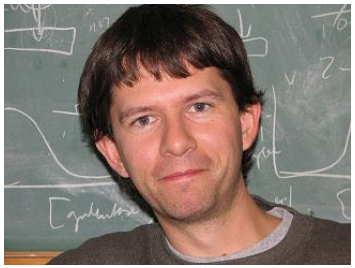
Combining theory and experiments to understand sugar regulation in bacteria

Sandeep Krishna

National Centre for Biological Sciences

Regulation of galactose transport and metabolism

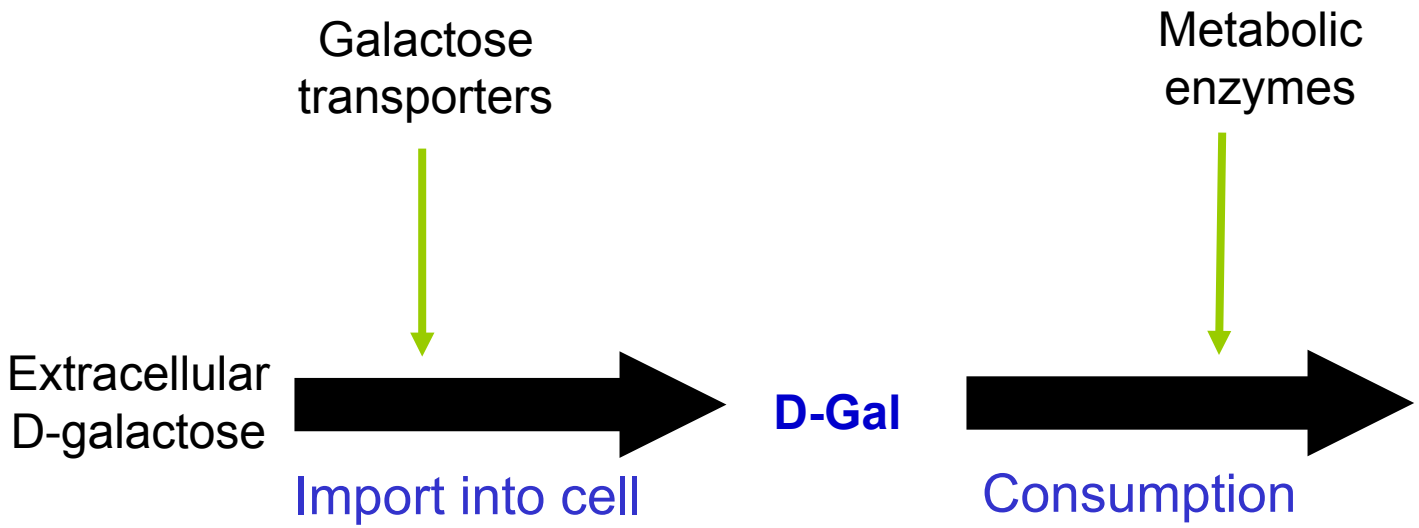
Feed*back* not feedforward!



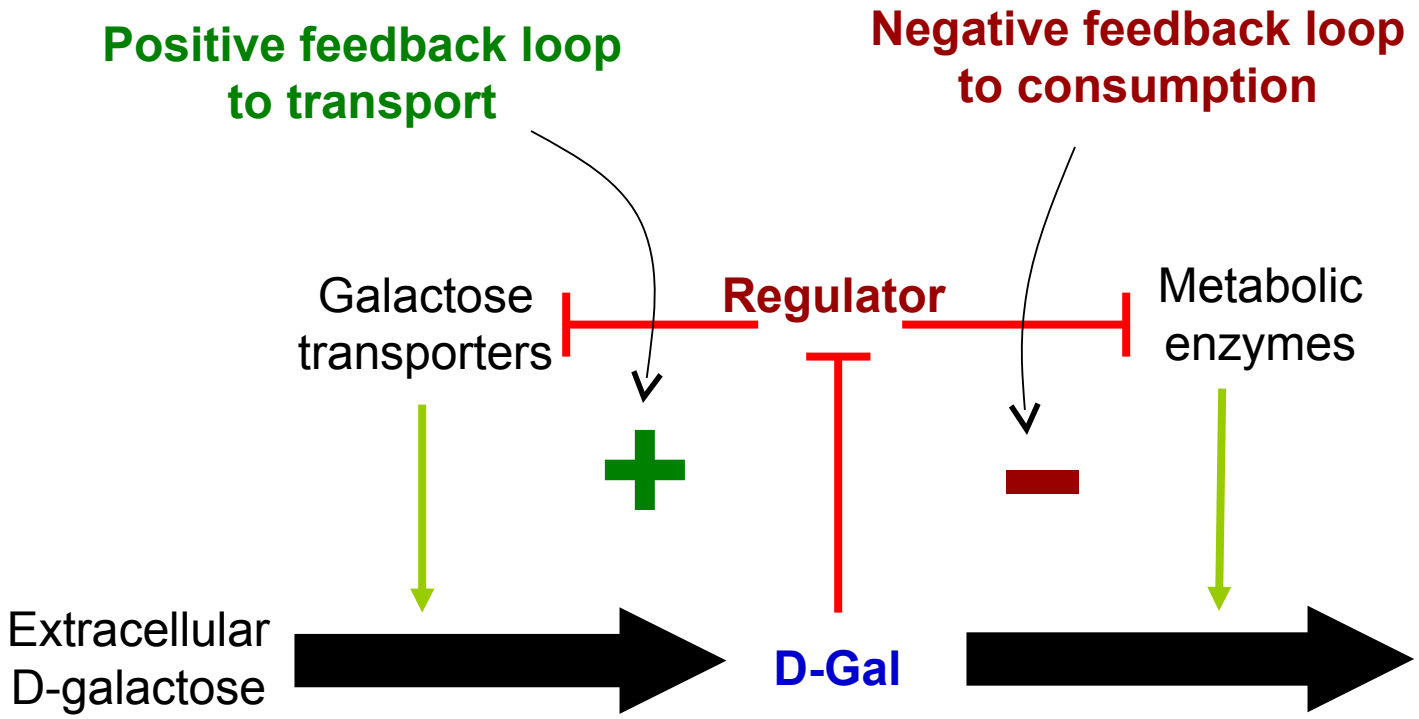
Szabolcs Semsey
(NIH & Copenhagen)

- Feedback loops are 5 times more common than feedforwards
- Feedback loops go through small molecules
- The network is a mess!

Galactose network



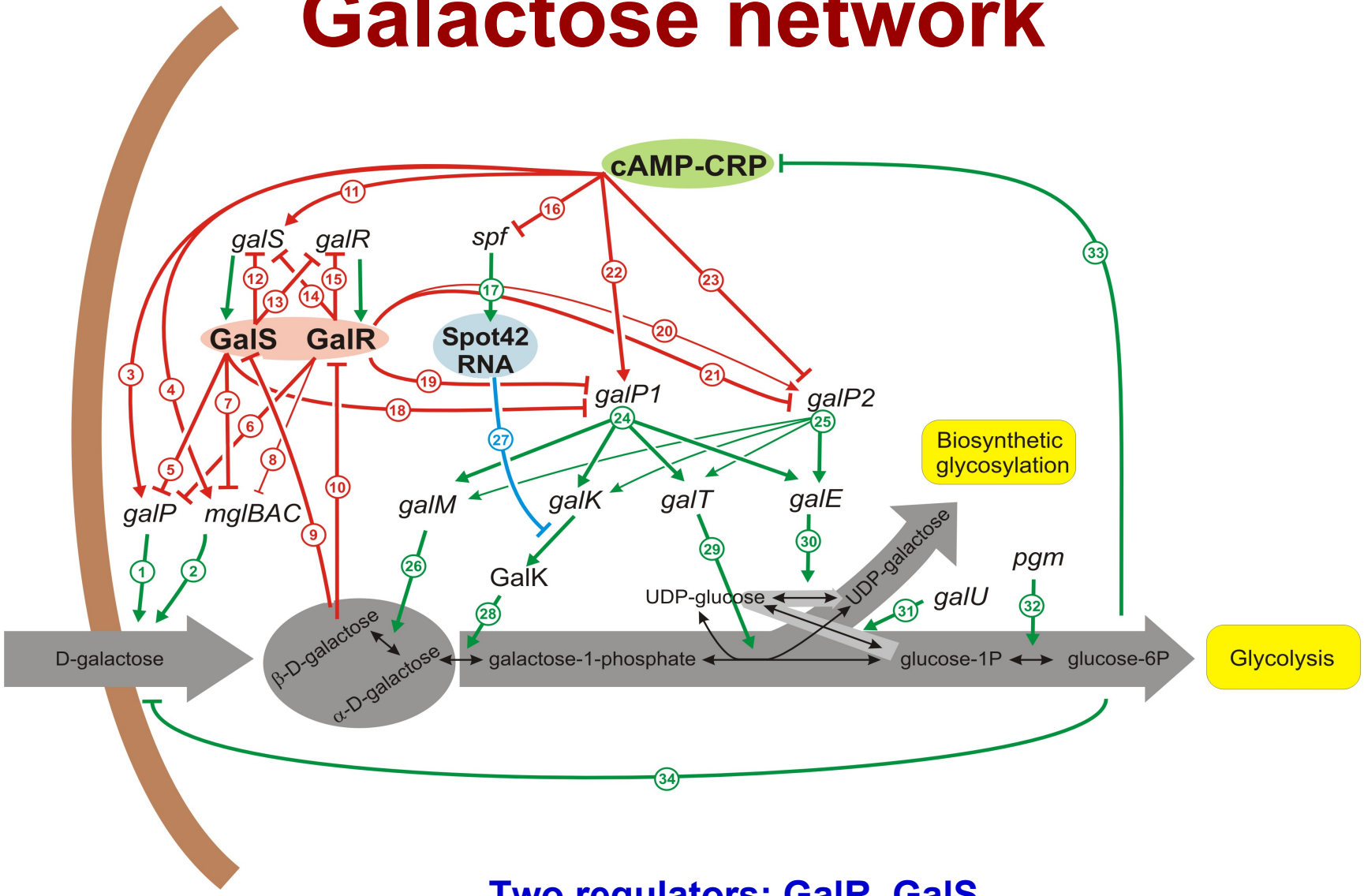
Galactose network



When D-gal is detected, both transport and consumption are increased

This (+ -) two-loop feedback motif maximizes flow through the system

Galactose network



Two regulators: GalR, GalS

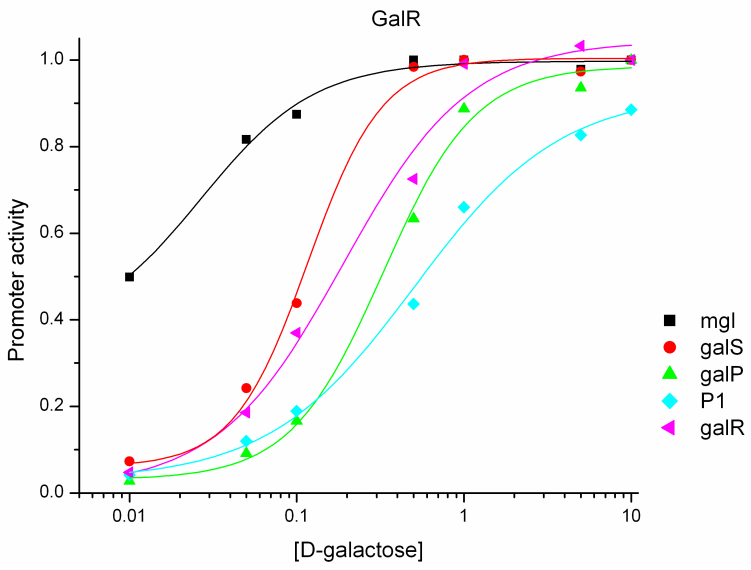
Two input signals: galactose, cyclic AMP

Multiple promoters from which transporters & enzymes are expressed

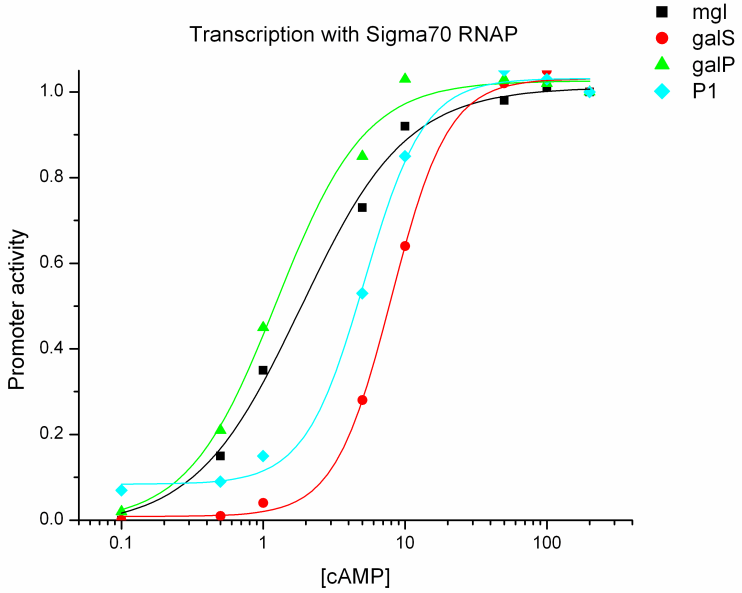
In vitro steady state experiments

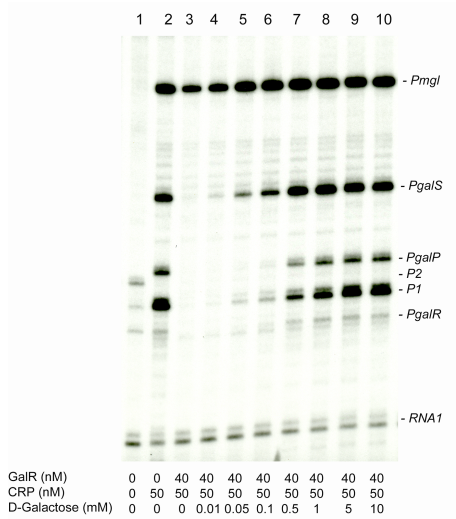
Szabolcs Semsey and Sankar Adhya

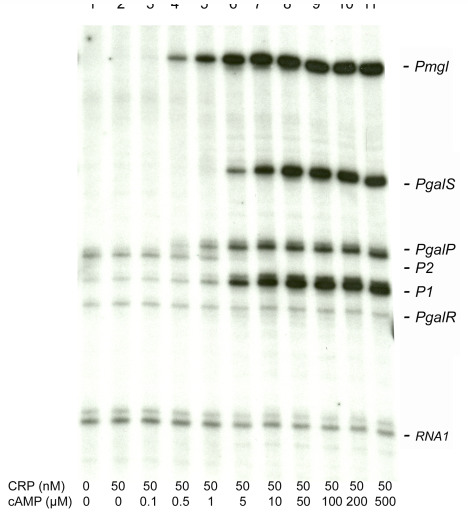
Effect of D-galactose

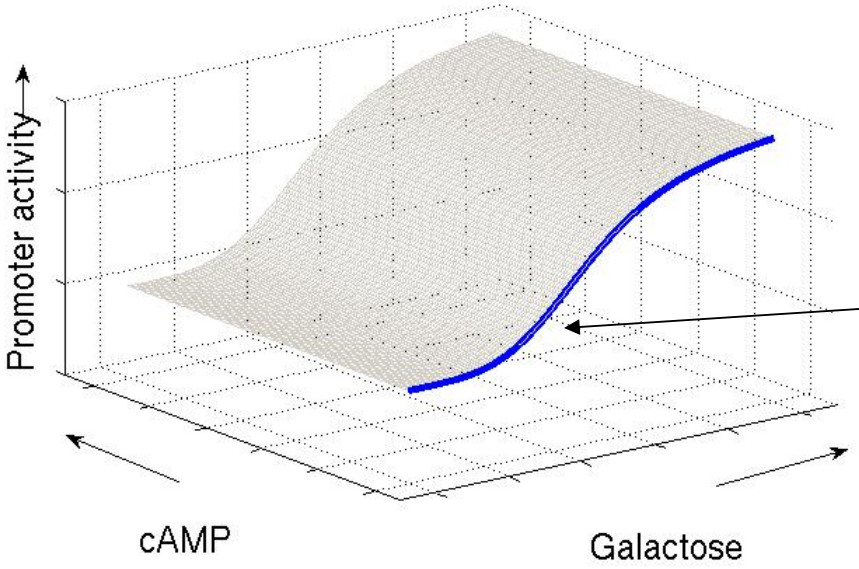


Effect of cAMP

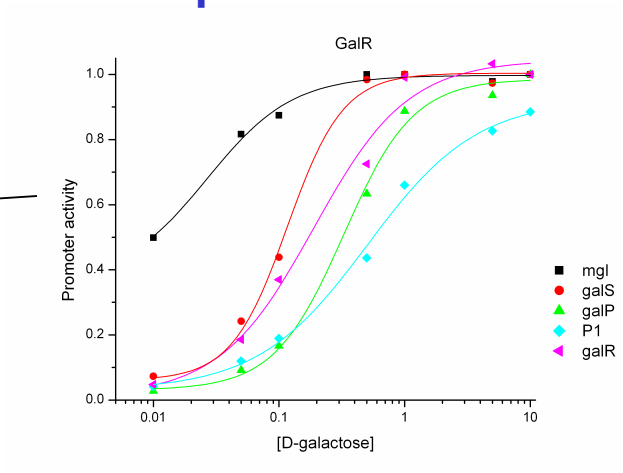




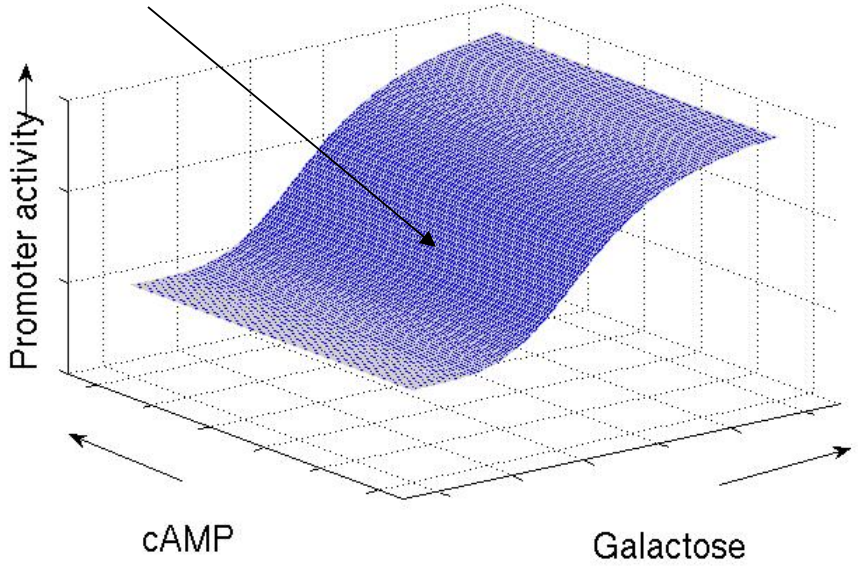




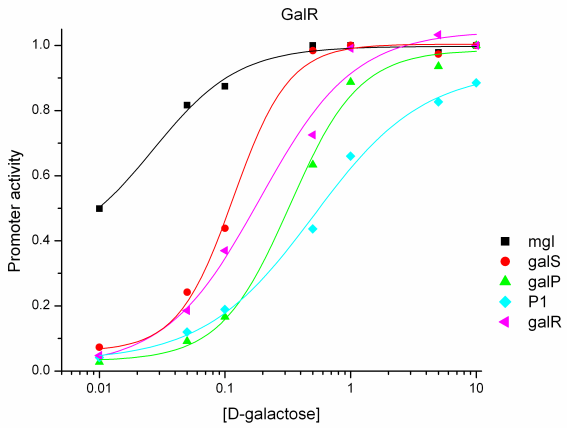
Experimental data



Model reconstructs entire surface from experimental data



Experimental data



Models:

$$R_{active} = \frac{R_{total}}{1 + g / K_g}$$

$$Activity = \frac{1}{1 + (R_{active} / K)^h}$$

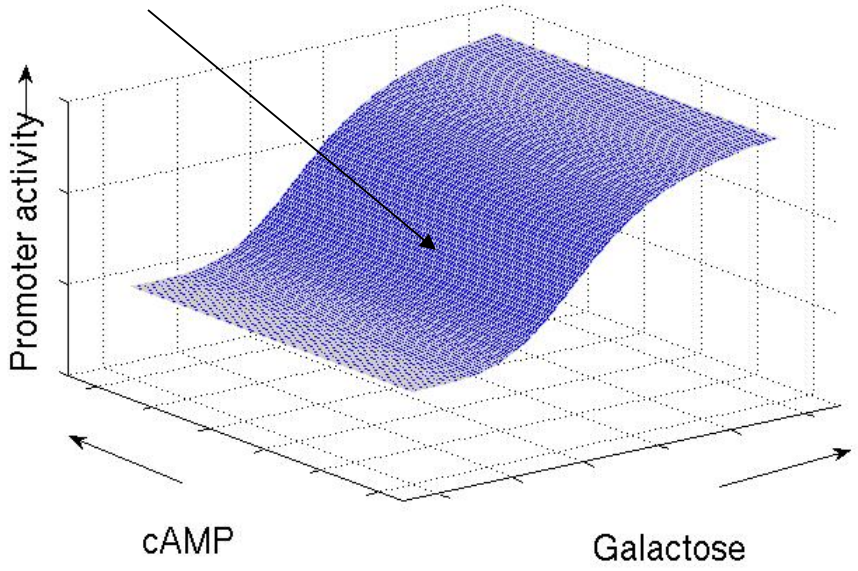
Inactivation of GalR by Galactose

D-Gal ⊣ GalR

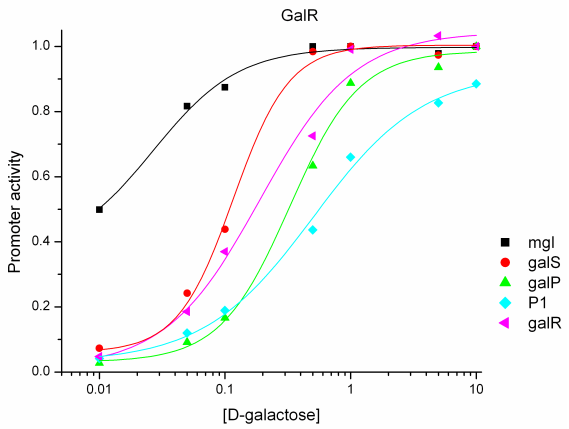
Repression of promoter by active GalR

GalR ⊣ Metabolic enzymes

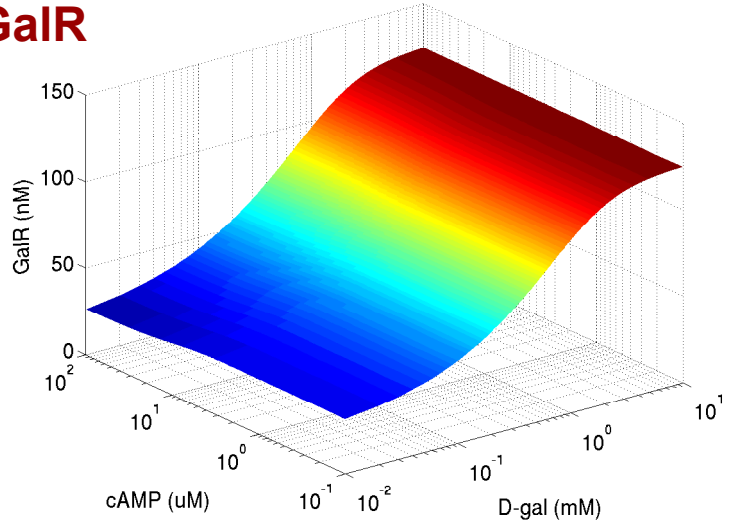
Model reconstructs entire surface from experimental data



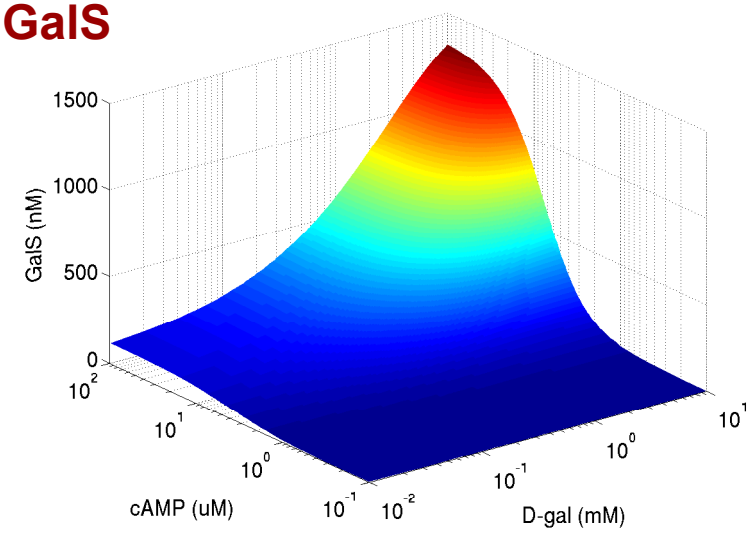
Experimental data

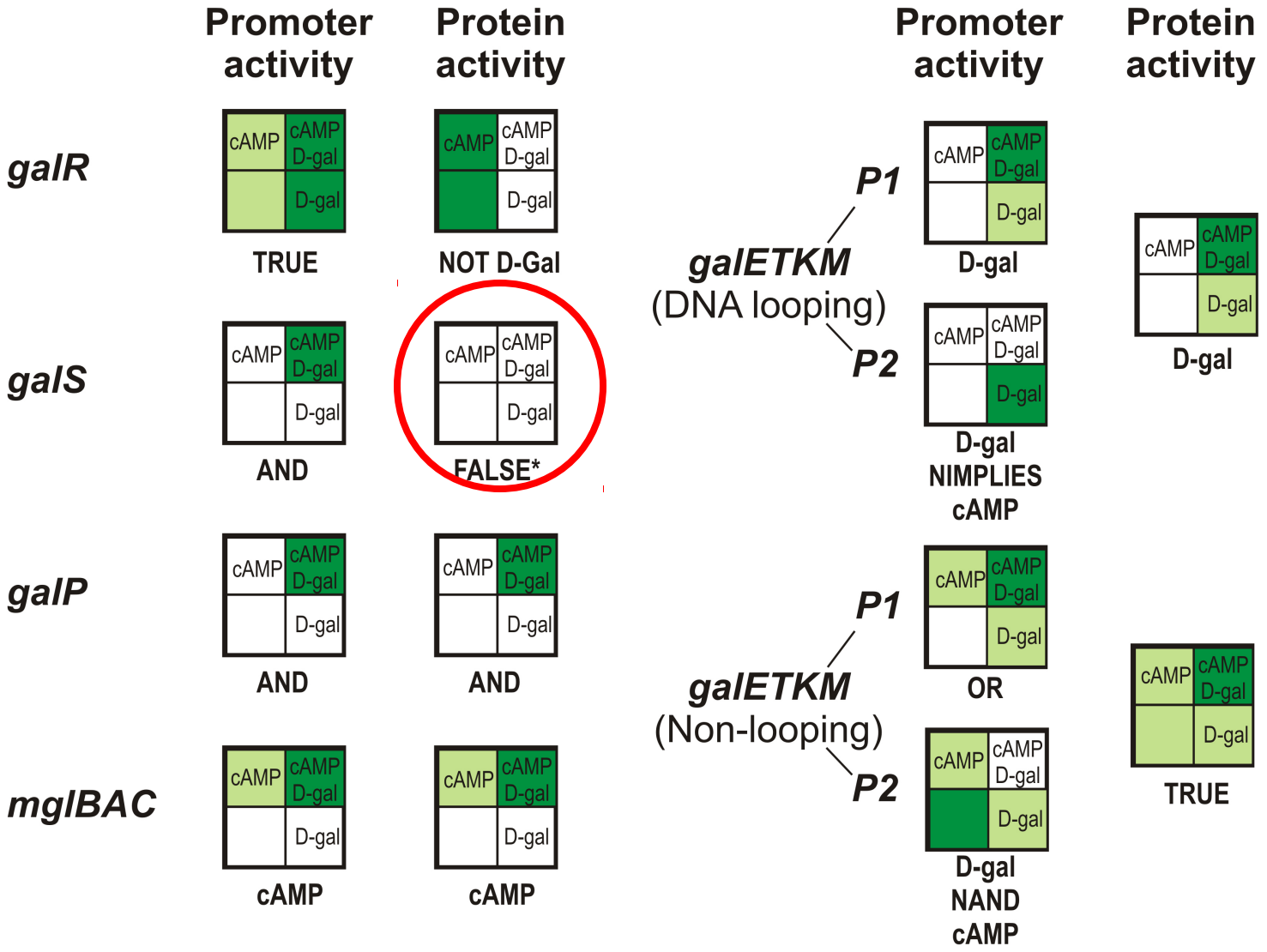


GalR

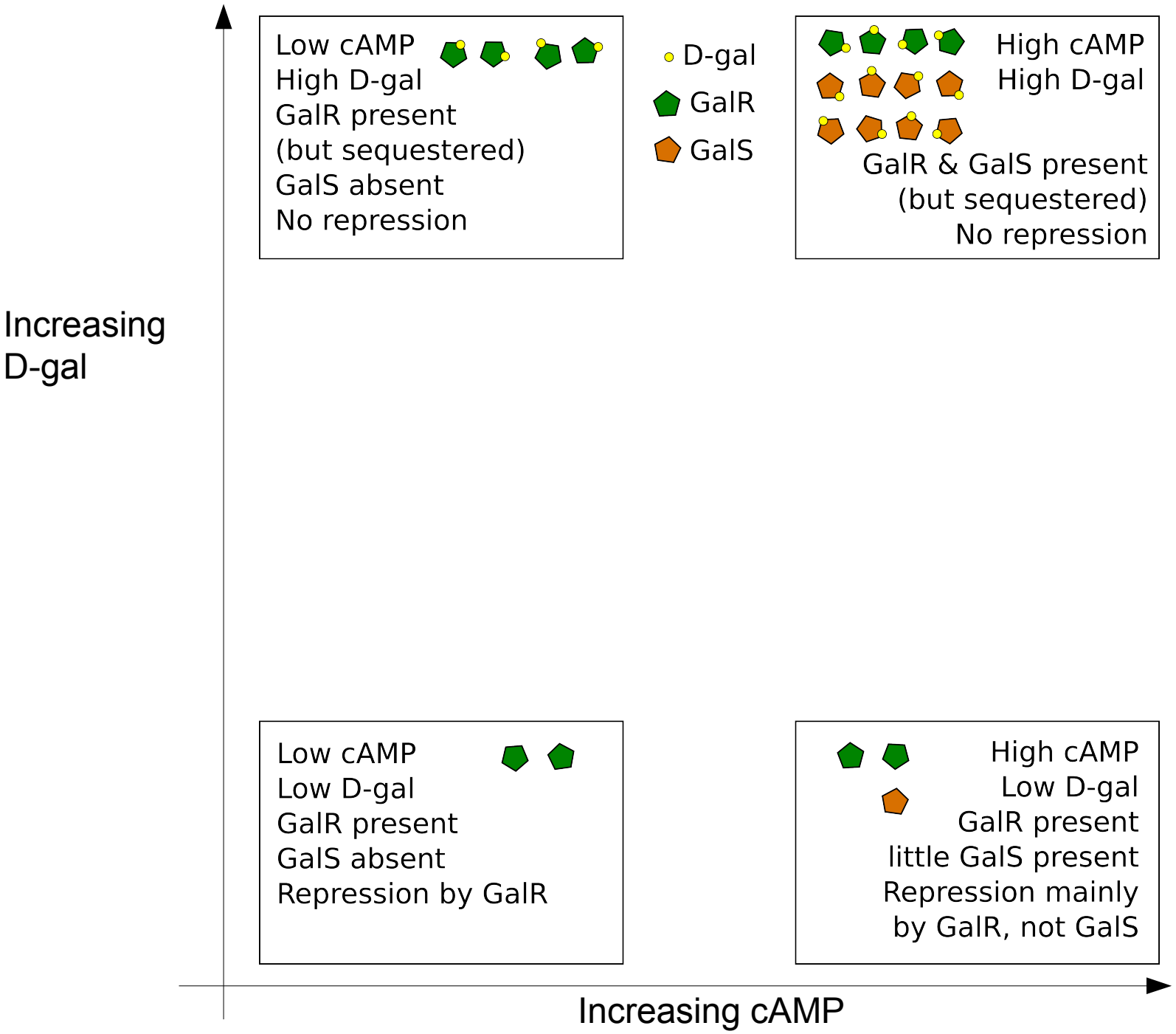


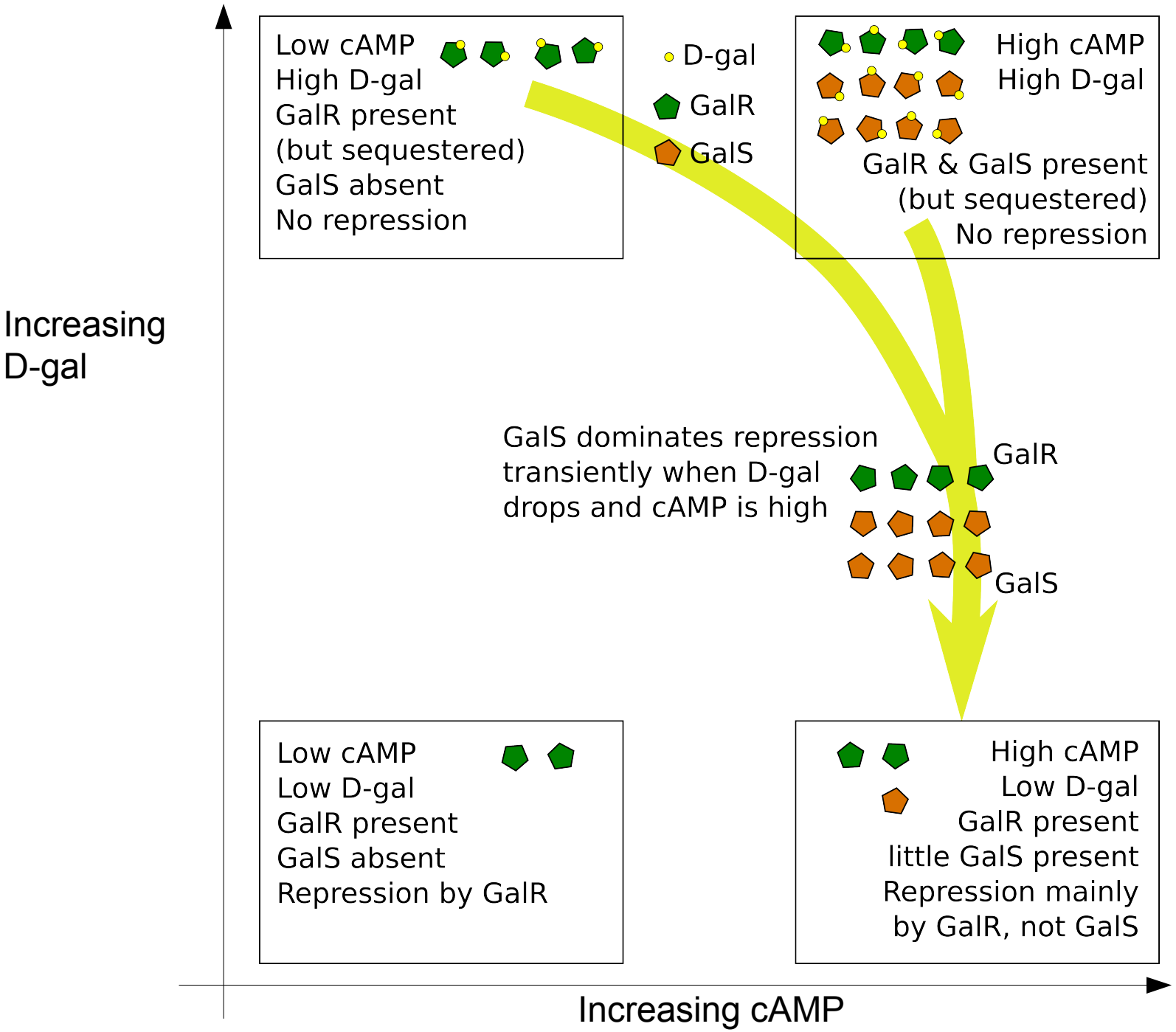
GalS

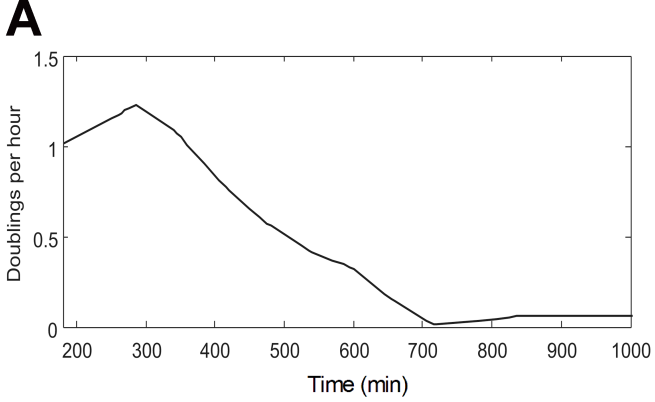


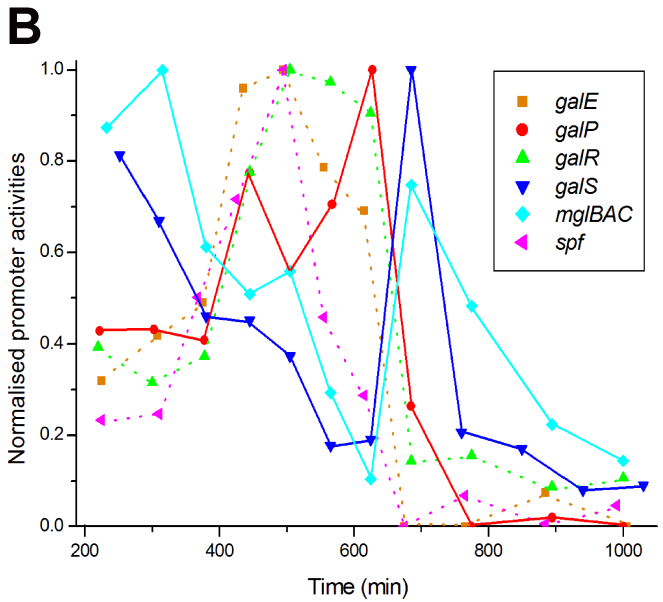


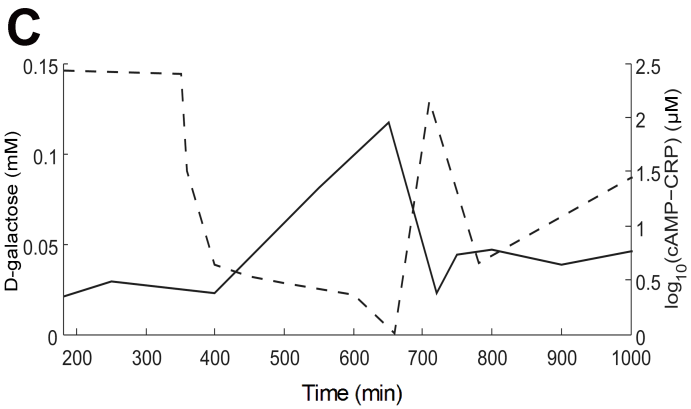
GalS produced only when GalR is inactive → only when Galactose is high
But when Galactose is high, GalS is also inactive


Low cAMP
High D-gal
GalR present
(but sequestered)
GalS absent
No repression
D-gal
GalR
GalS
High cAMP
High D-gal
GalR & GalS present
(but sequestered)
No repression
Increasing
D-gal
Low cAMP
Low D-gal
GalR present
GalS absent
Repression by GalR
High cAMP
Low D-gal
GalR present
little GalS present
Repression mainly
by GalR, not GalS
Increasing cAMP


Low cAMP
High D-gal
GalR present
(but sequestered)
GalS absent
No repression
D-gal
GalR
GalS
High cAMP
High D-gal
GalR & GalS present
(but sequestered)
No repression
GalS dominates repression
transiently when D-gal
drops and cAMP is high
GalR
GalS
Low cAMP
Low D-gal
GalR present
GalS absent
Repression by GalR
High cAMP
Low D-gal
GalR present
little GalS present
Repression mainly
by GalR, not GalS
Increasing
D-gal
Increasing cAMP

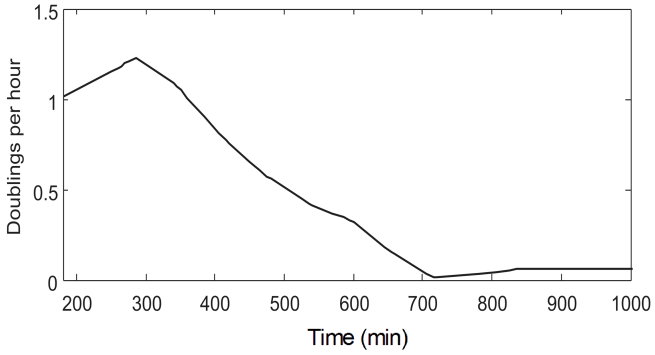






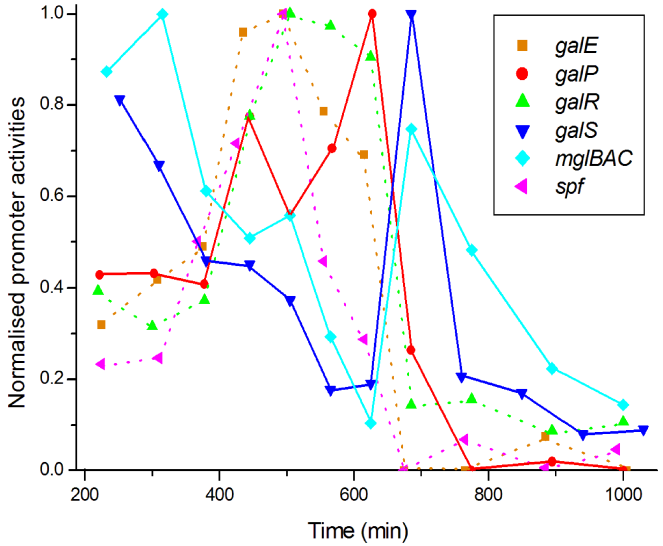
Experiment where a colony of E. coli grows on and depletes a finite amount of galactose

A



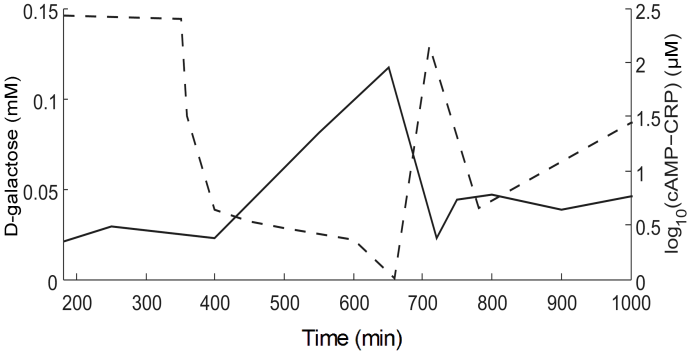
Growth rate vs. time

B



Promoter activities vs. time

C



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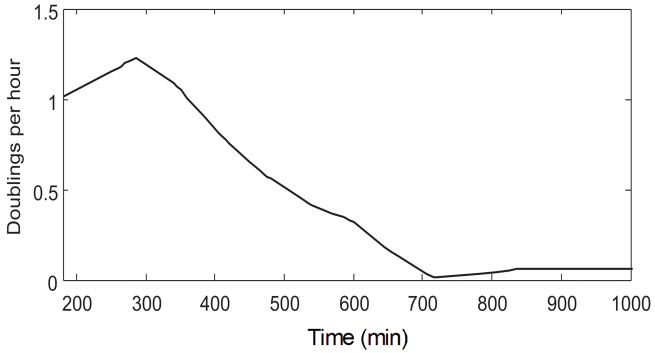

Growth rate vs. time

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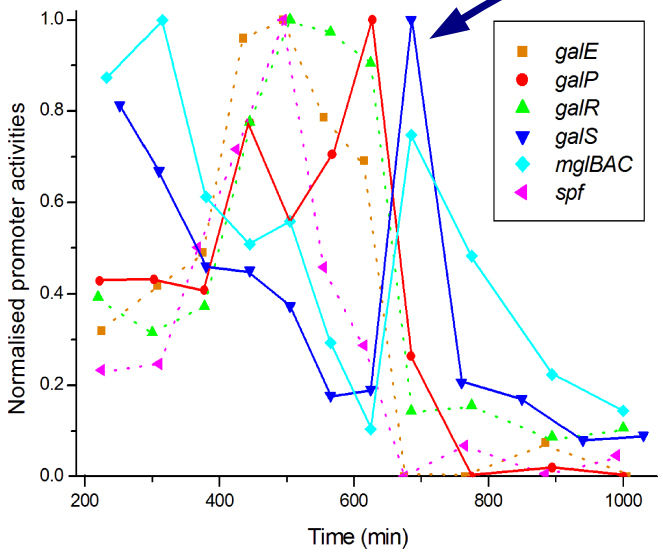
GalS



Promoter activities vs. time

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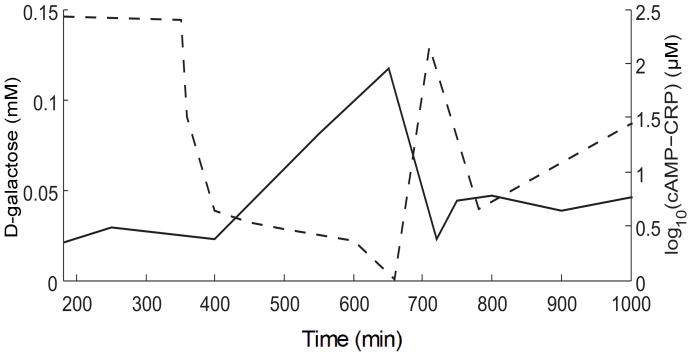

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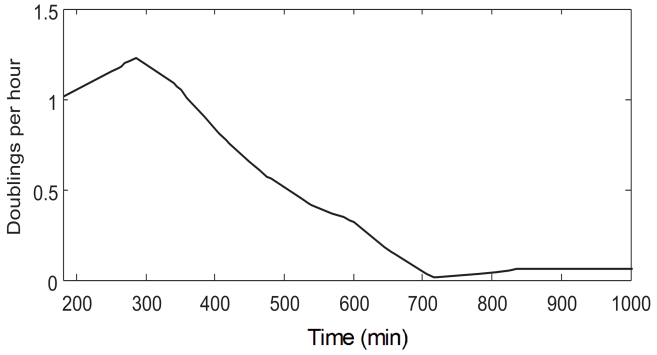

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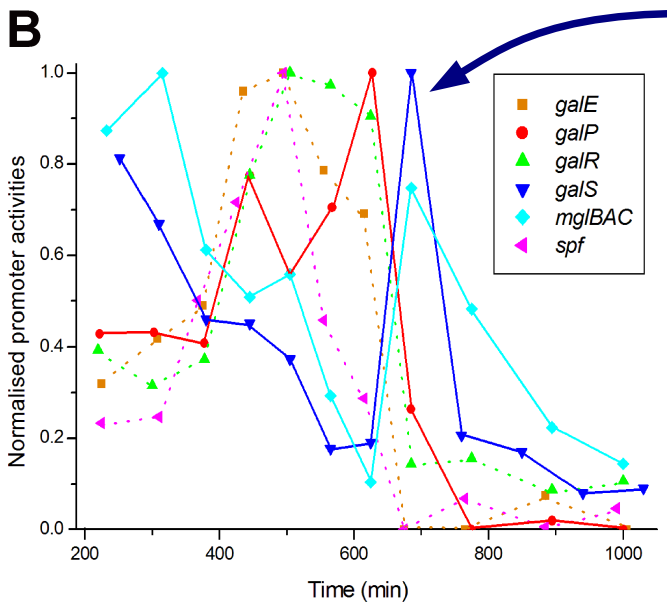

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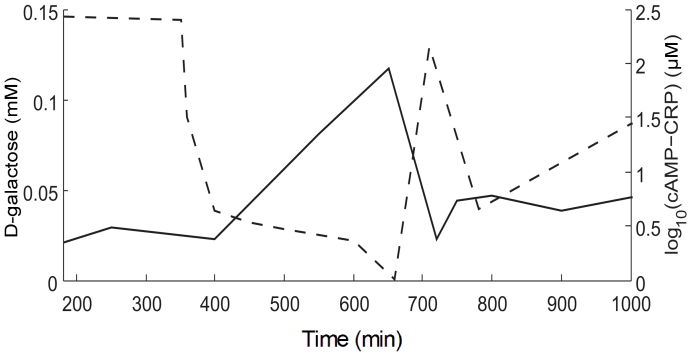
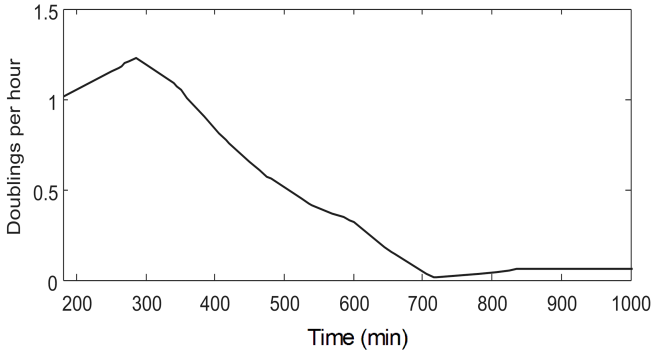


Mathematical model used to back-calculate internal galactose and cAMP levels

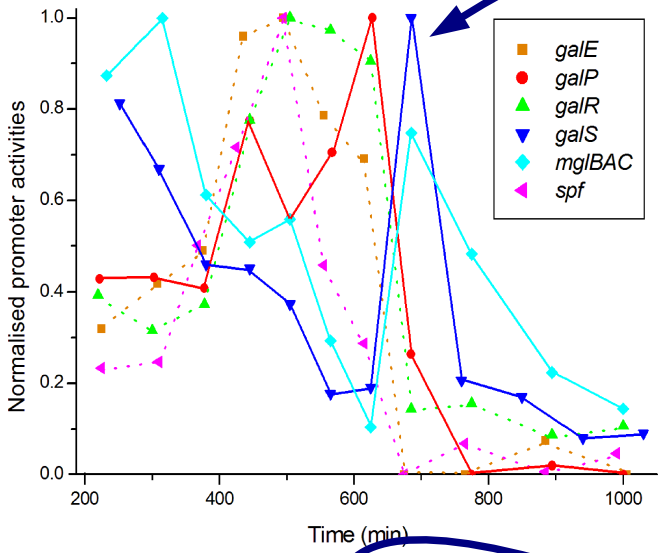
A



Growth rate vs. time

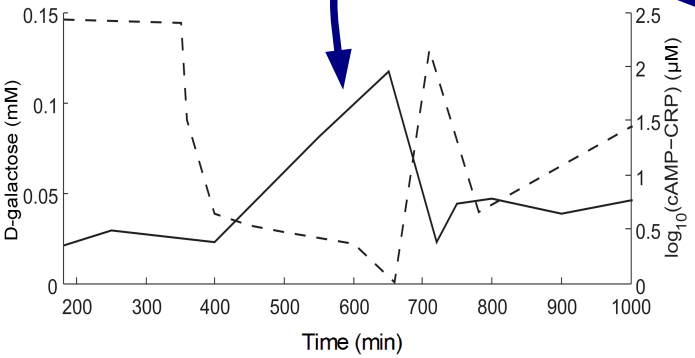
B

GalS

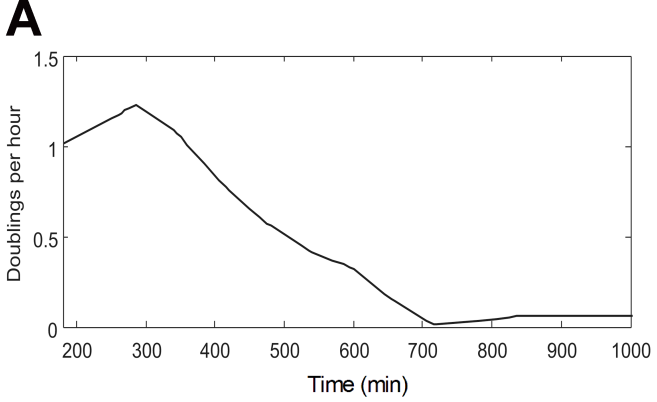


Promoter activities vs. time

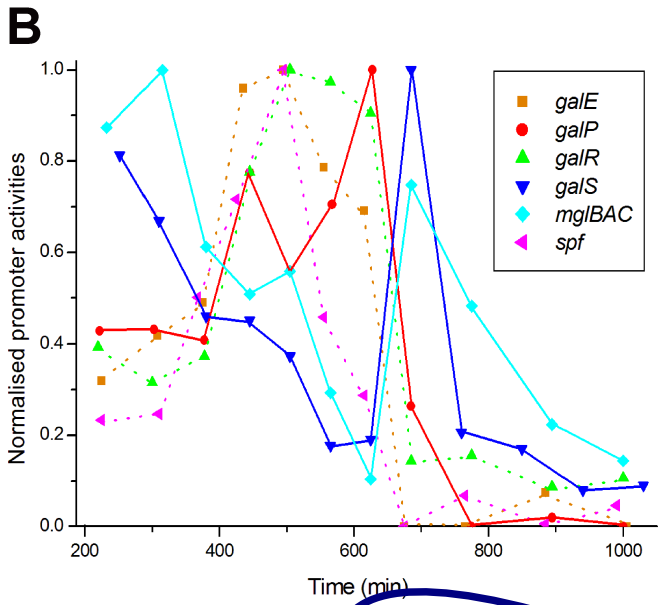
C

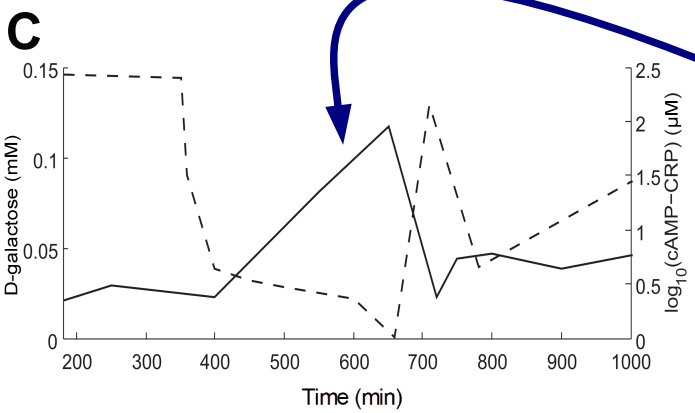


Internal galactose level keeps rising even when external level Is dropping rapidly



Task: precisely control timing of gene expression
Input: continuous monitoring of environment
Decisions: start or stop production of a protein





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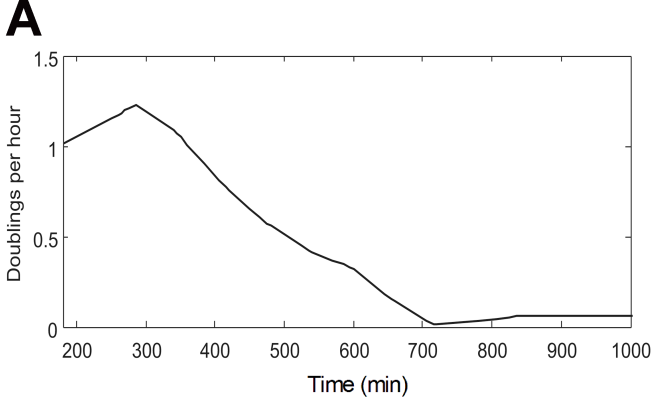

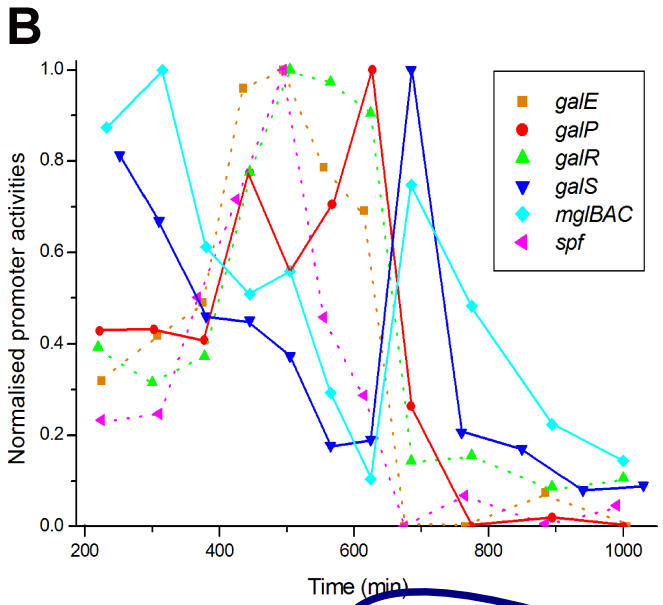

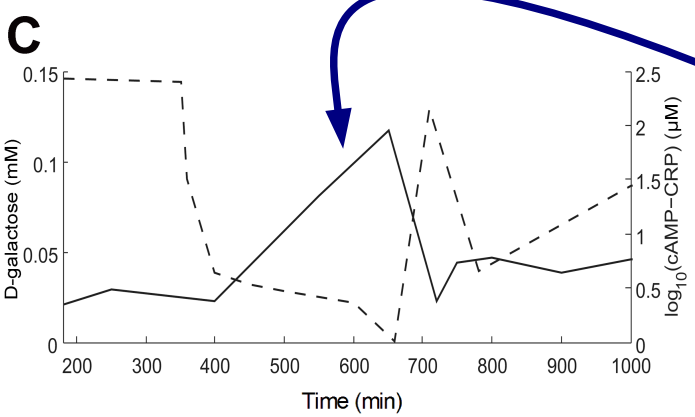

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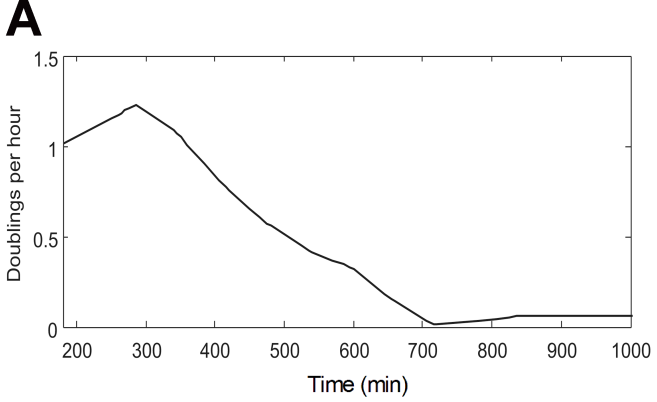
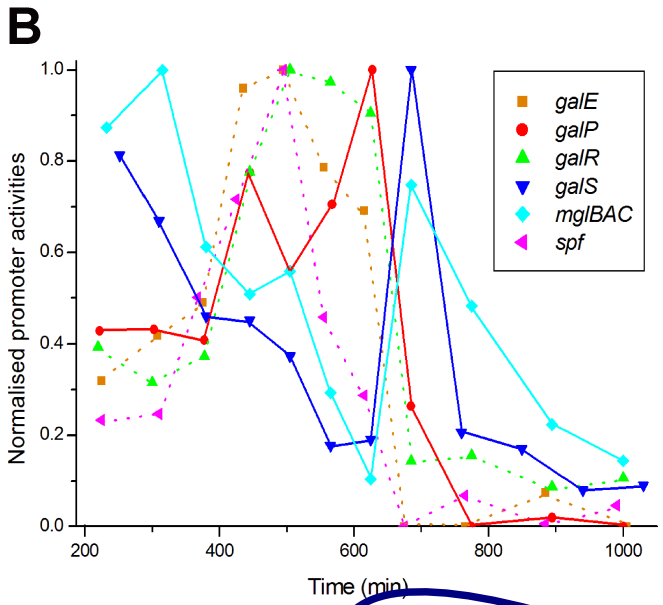
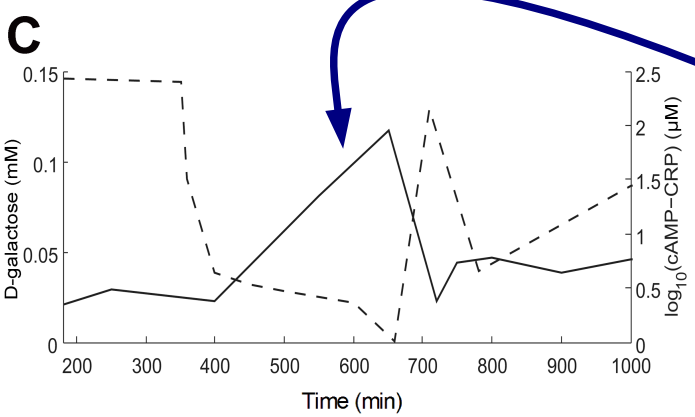
But transporters and enzymes are long-lived so decisions have an effect 2-3 cell generations later

Internal galactose level keeps rising even when external level Is dropping rapidly







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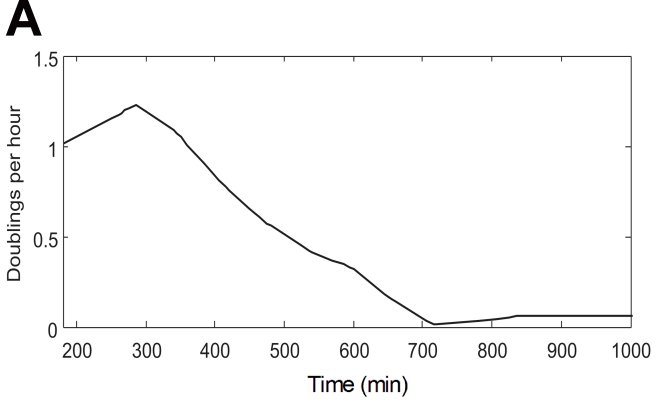
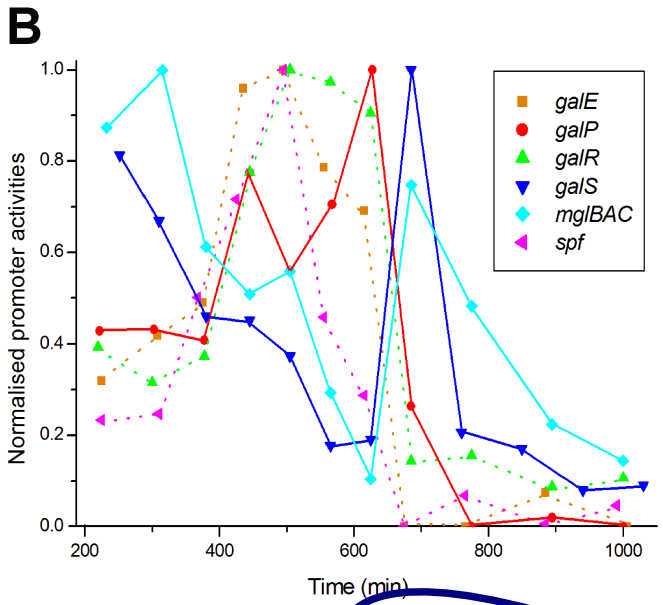
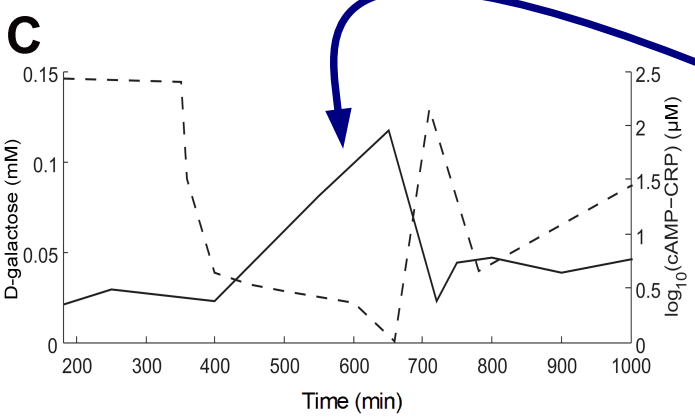
What kind of fluctuating food availability do you want to optimize for?

Internal galactose level keeps rising even when external level Is dropping rapidly







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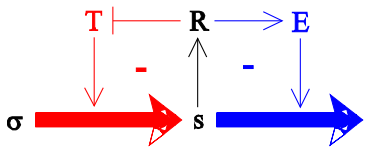
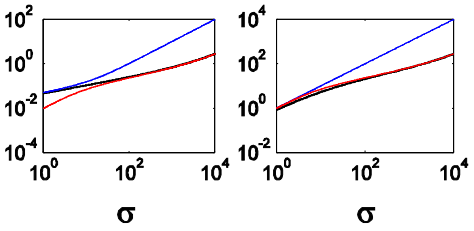
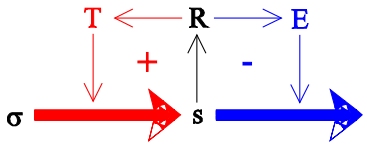
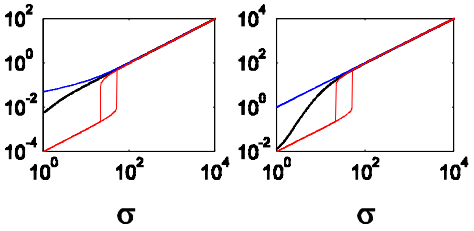
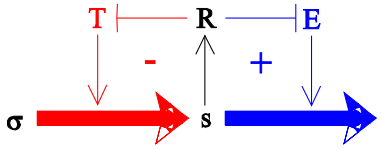
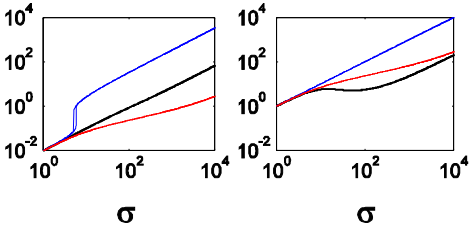
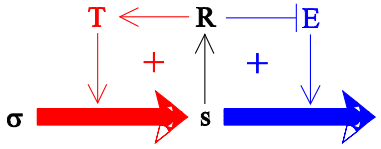
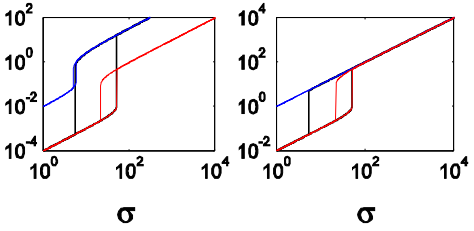
But transporters and enzymes are long-lived so decisions have an effect 2-3 cell generations later

What kind of fluctuating food availability do you want to optimize for?

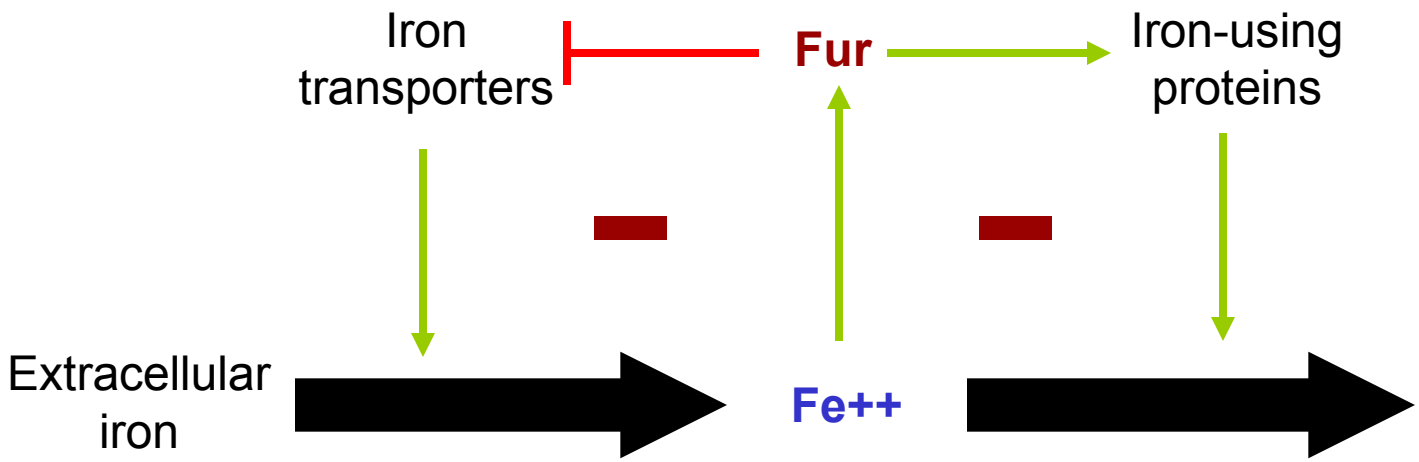
- Continuous, steady level of galactose
- Periodic feast, famine cycles
- Intermittent pulses of galactose

Internal galactose level keeps rising even when external level Is dropping rapidly

Motif	Function	s influx
 **Socialist**	Keeps small molecule concentration relatively constant Found in Fe regulation in mammals **Good for homeostasis**	
 **Consumer**	Maximizes flow Turns on influx quickly Found in sugar regulation Lac, Gal, Ara etc. **Good for food molecules**	
 **Fashion**	When s is small demand is high When s is large demand is low Flow remains relatively constant **Good for flow homeostasis**	
 **Collector**	Bistable Can build up large concentration **Good for accumulation**	

SK, Szabolcs Semsey, Kim Sneppen (2007) *Combinatorics of feedback in cellular uptake and metabolism of small molecules*, **PNAS**.

Iron network



Double negative feedback

- **Helps maintain homeostasis of Fe++**
- **Concentration of intracellular Fe is relatively insensitive to changes in extracellular Fe**