Modelling the regulatory network of AcrAB-TolC

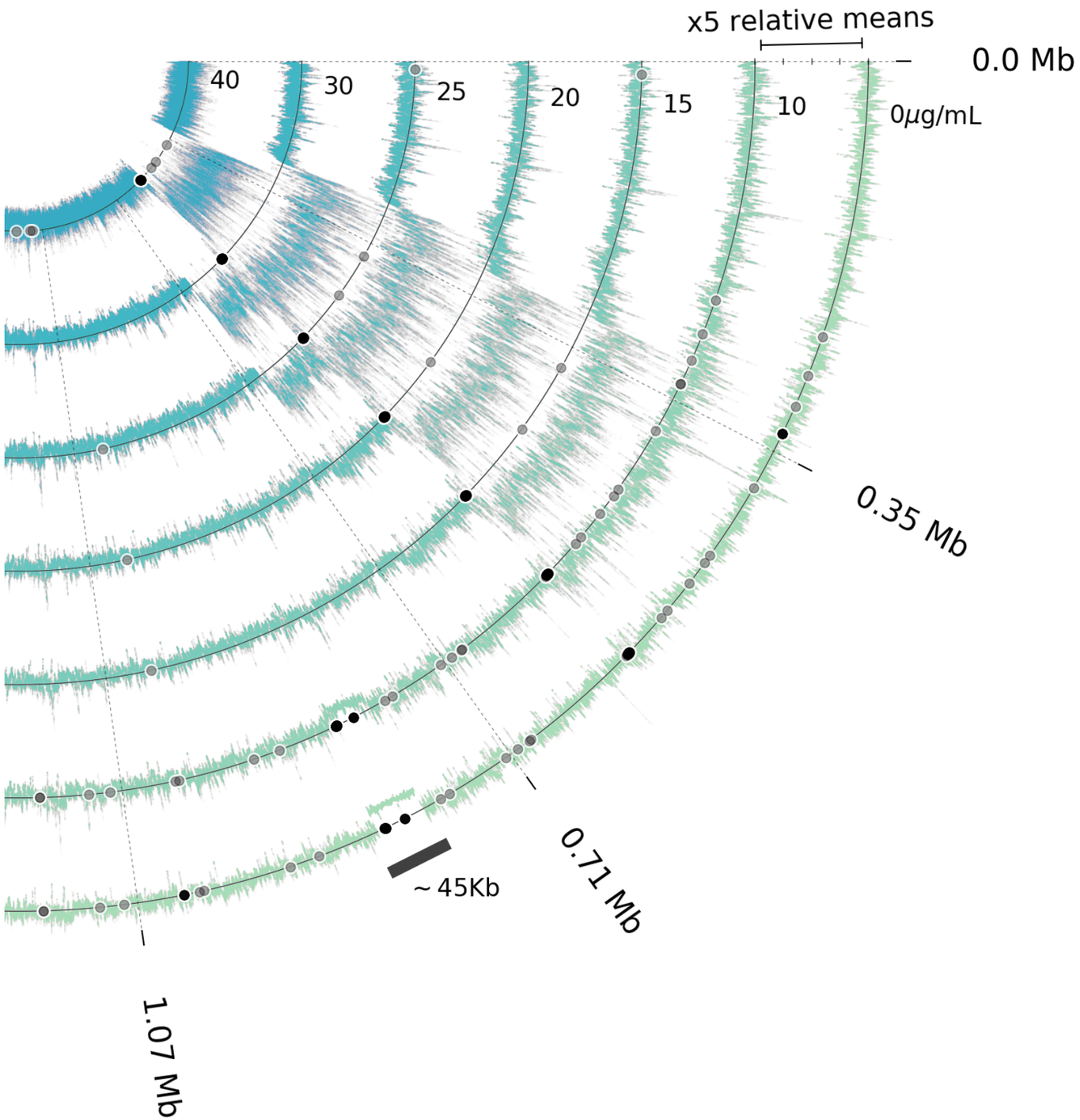
Carlos Reding & Rob Beardmore

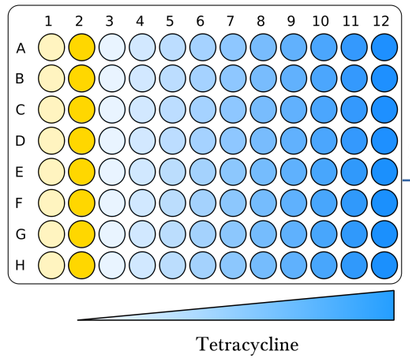
Engineering and Physical Sciences Research Council

European Research Council
Established by the European Commission

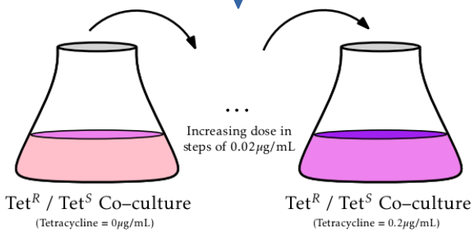
UNIVERSITY OF EXETER

Genome amplification protocol.

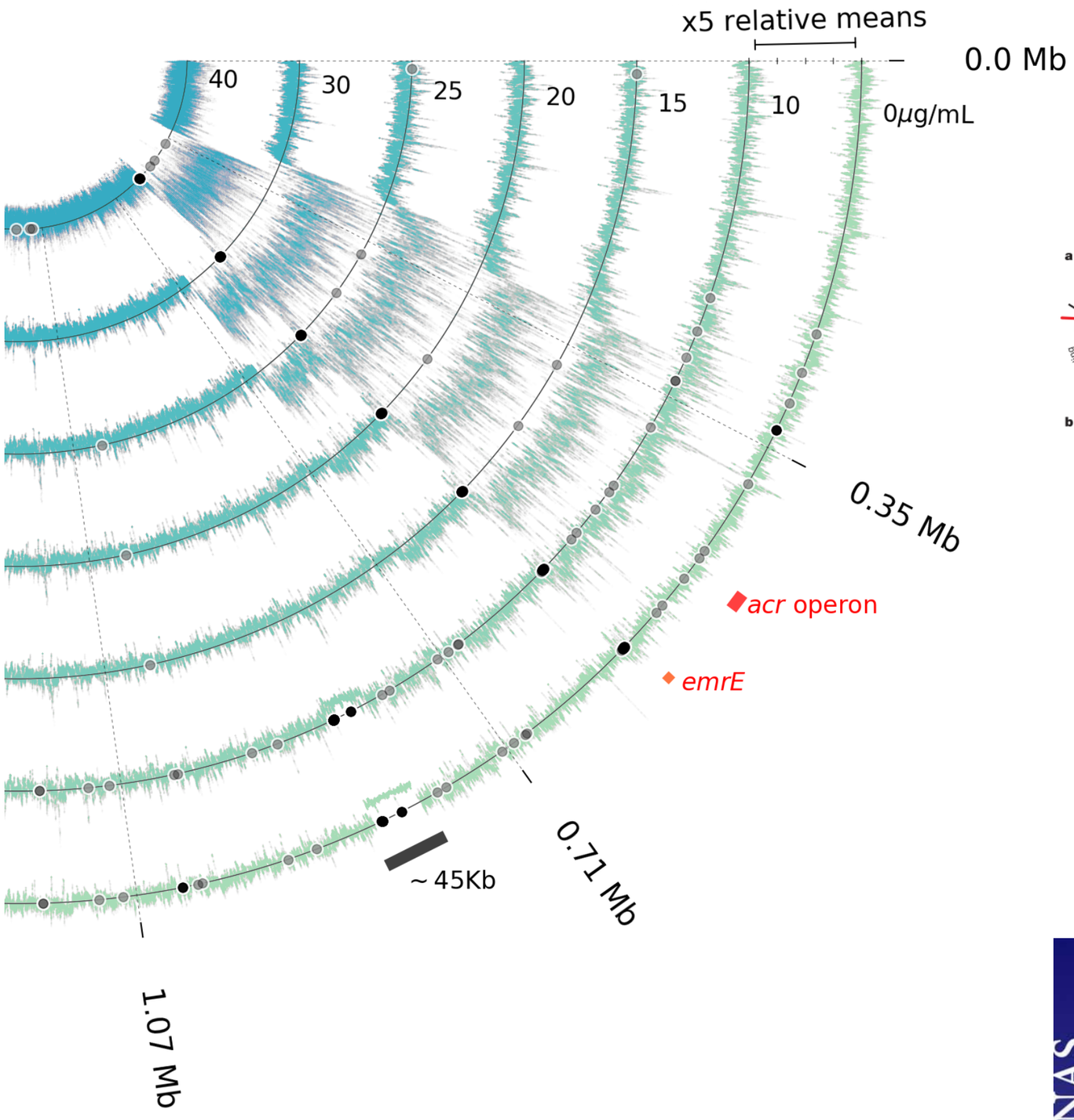


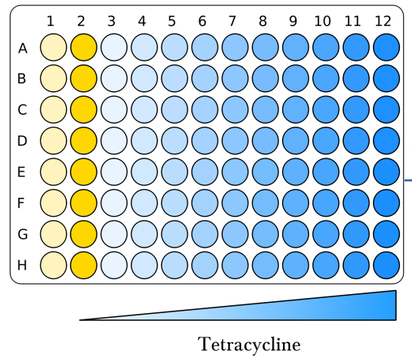


Propagate culture in
same conditions every 24h

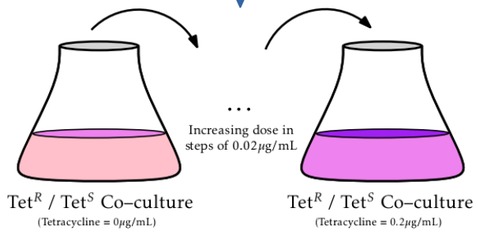


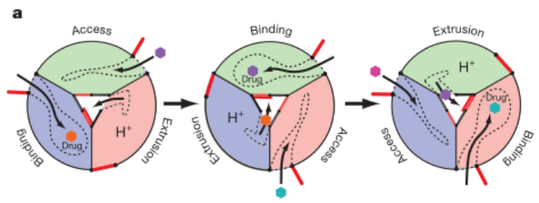
Genome amplification protocol.

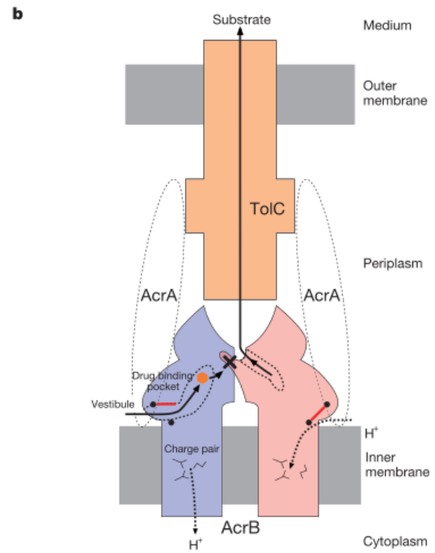




Propagate culture in same conditions every 24h







Nikaido *et al.* Nature (2006)

Highly unspecific.

Observed in the clinic.

PNAS

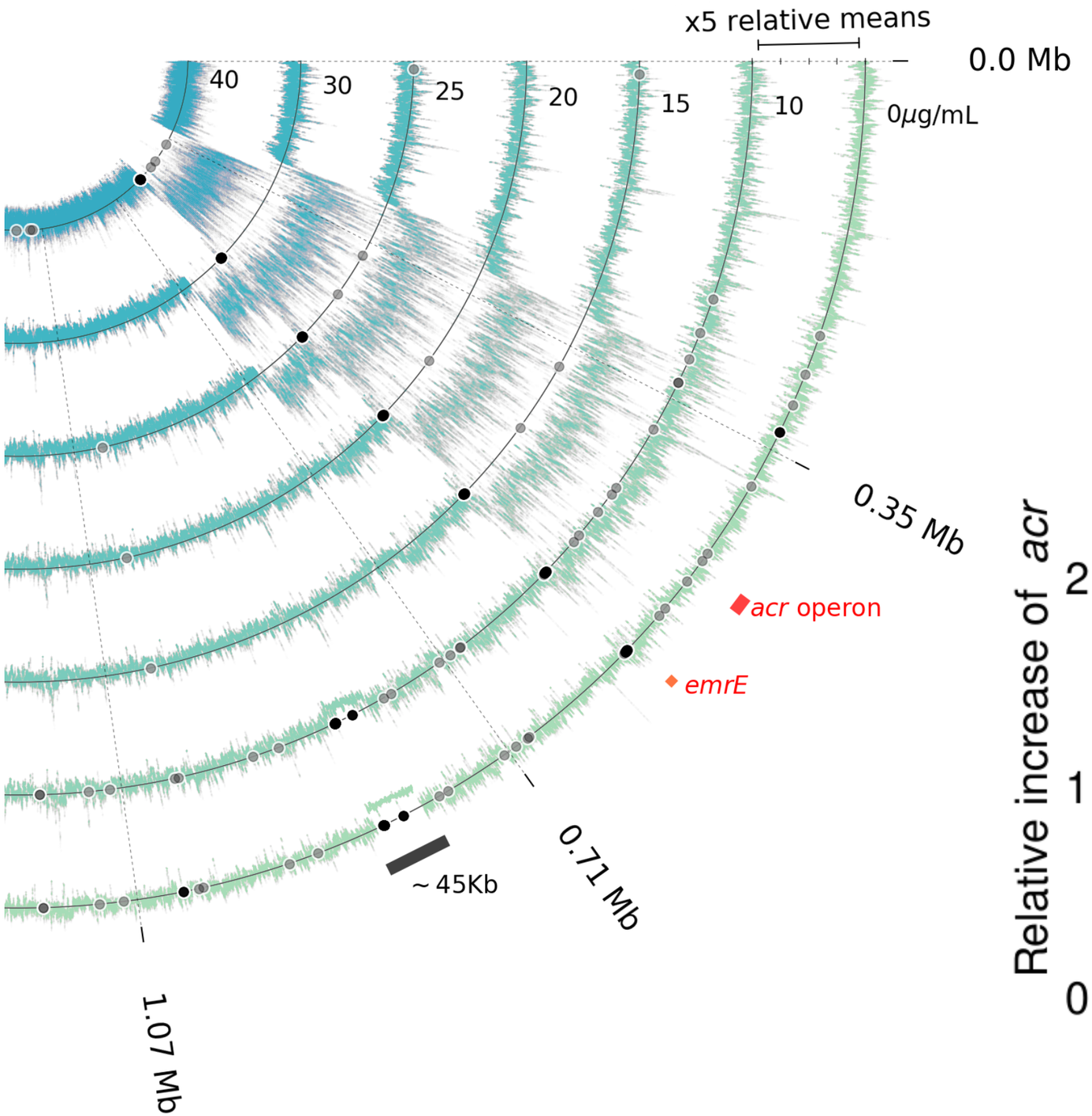
AcrB drug-binding pocket substitution confers clinically relevant resistance and altered substrate specificity

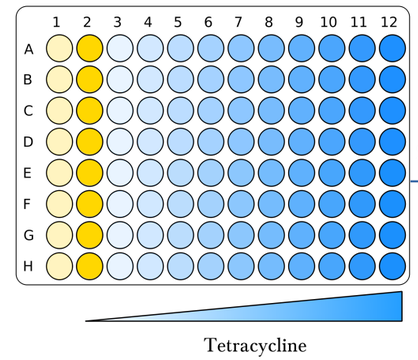
Jessica M. A. Blair[a], Vassiliy N. Bavro[a], Vito Ricci[a], Niraj Modi[b], Pierpaolo Cacciotto[c], Ulrich Kleinekathöfer[b], Paolo Ruggerone[c], Attilio V. Vargiu[c], Alison J. Baylay[a], Helen E. Smith[a], Yvonne Brandon[a], David Galloway[a], and Laura J. V. Piddock[a,1]

[a]Antimicrobials Research Group, School of Immunity and Infection, College of Medical and Dental Sciences, Institute of Microbiology and Infection, The University of Birmingham, Birmingham B15 2TT, United Kingdom; [b]School of Engineering and Science, Jacobs University Bremen, 28759 Bremen, Germany; and [c]Department of Physics, University of Cagliari, 09042 Monserrato, Italy

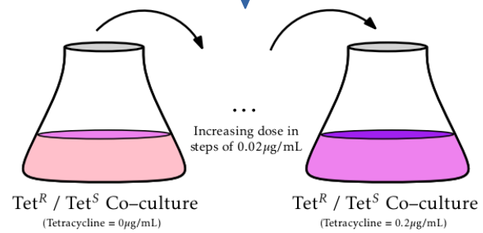
Blair *et al.* Nature (2015)

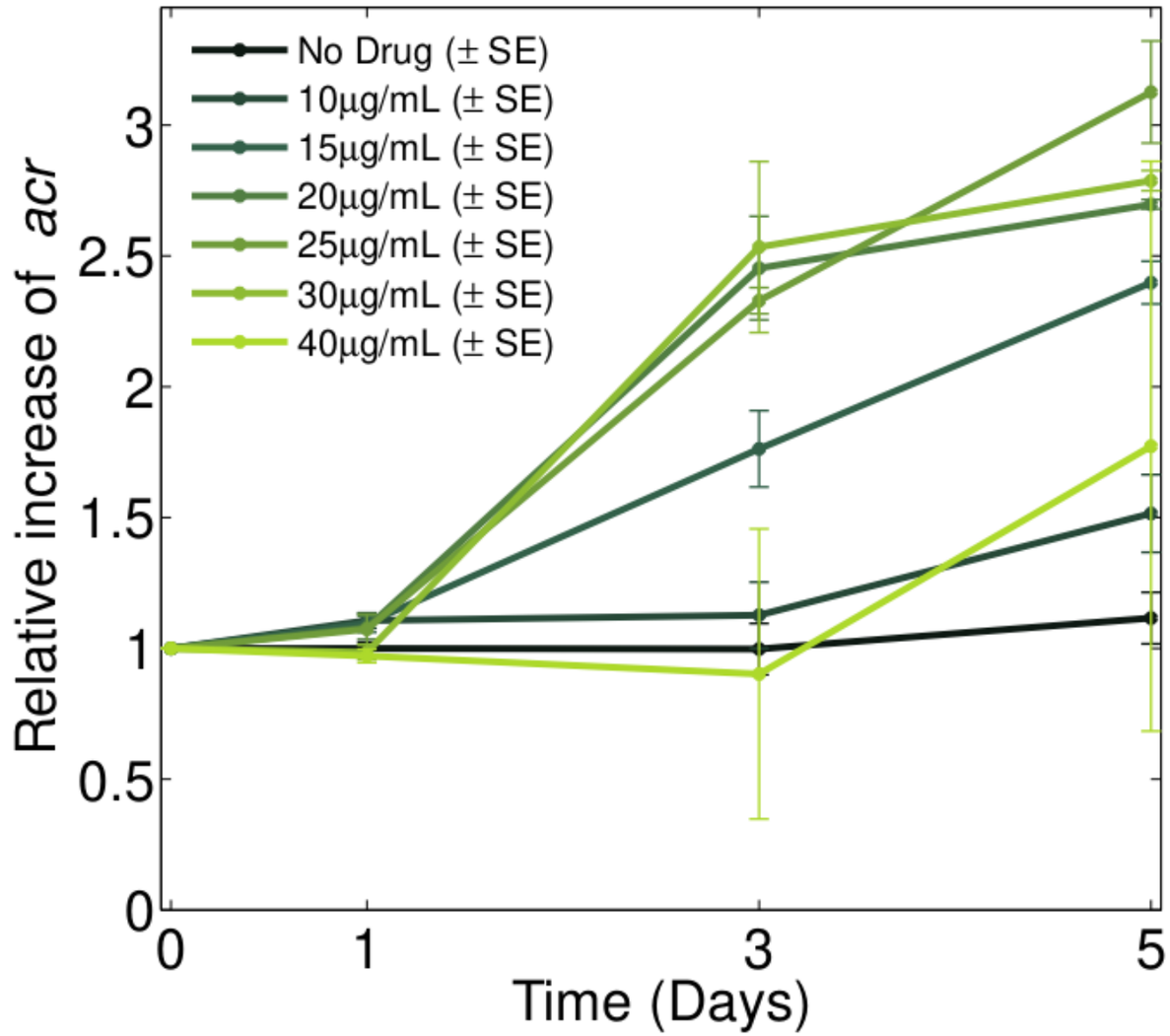
Genome amplification protocol.



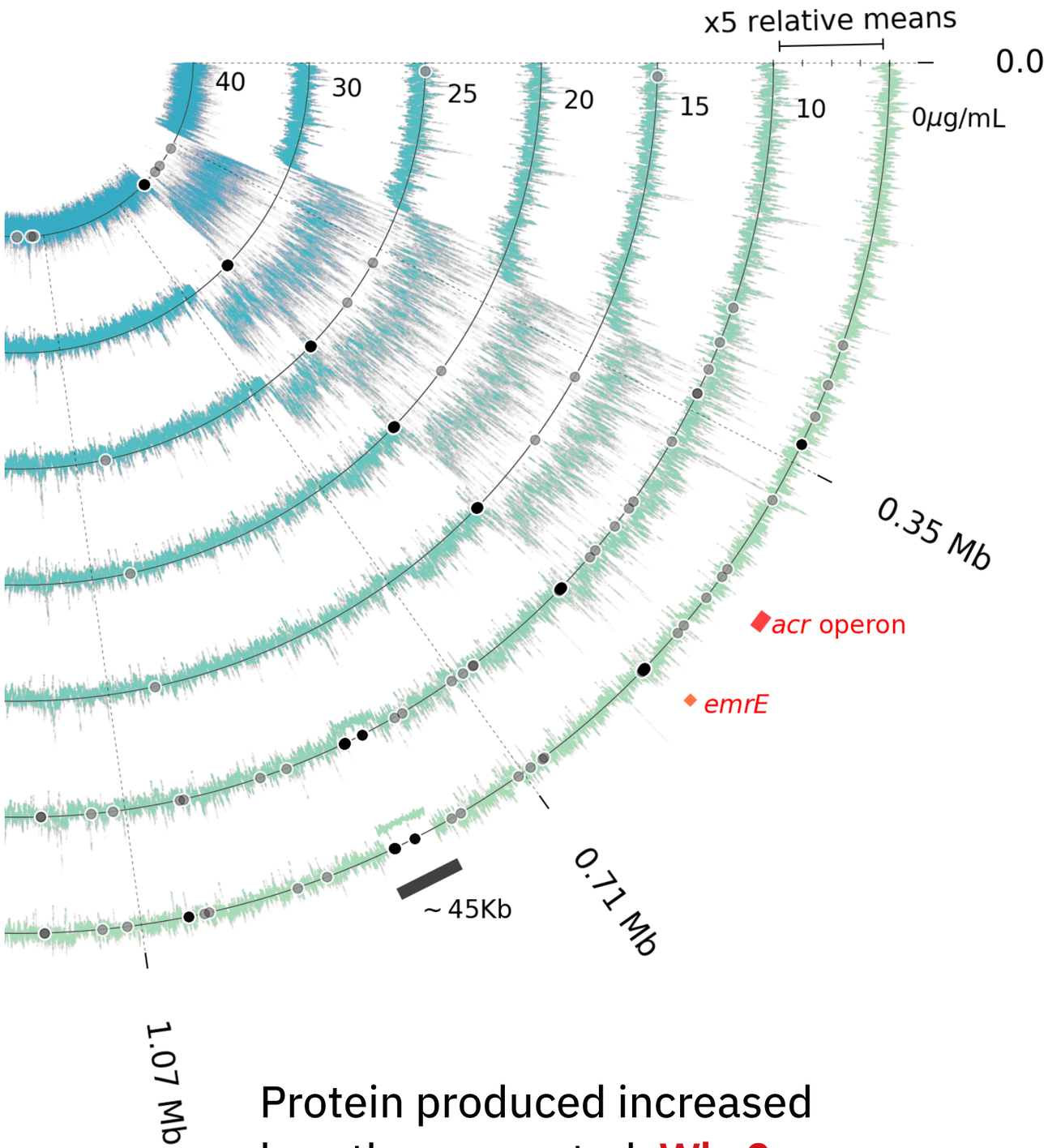


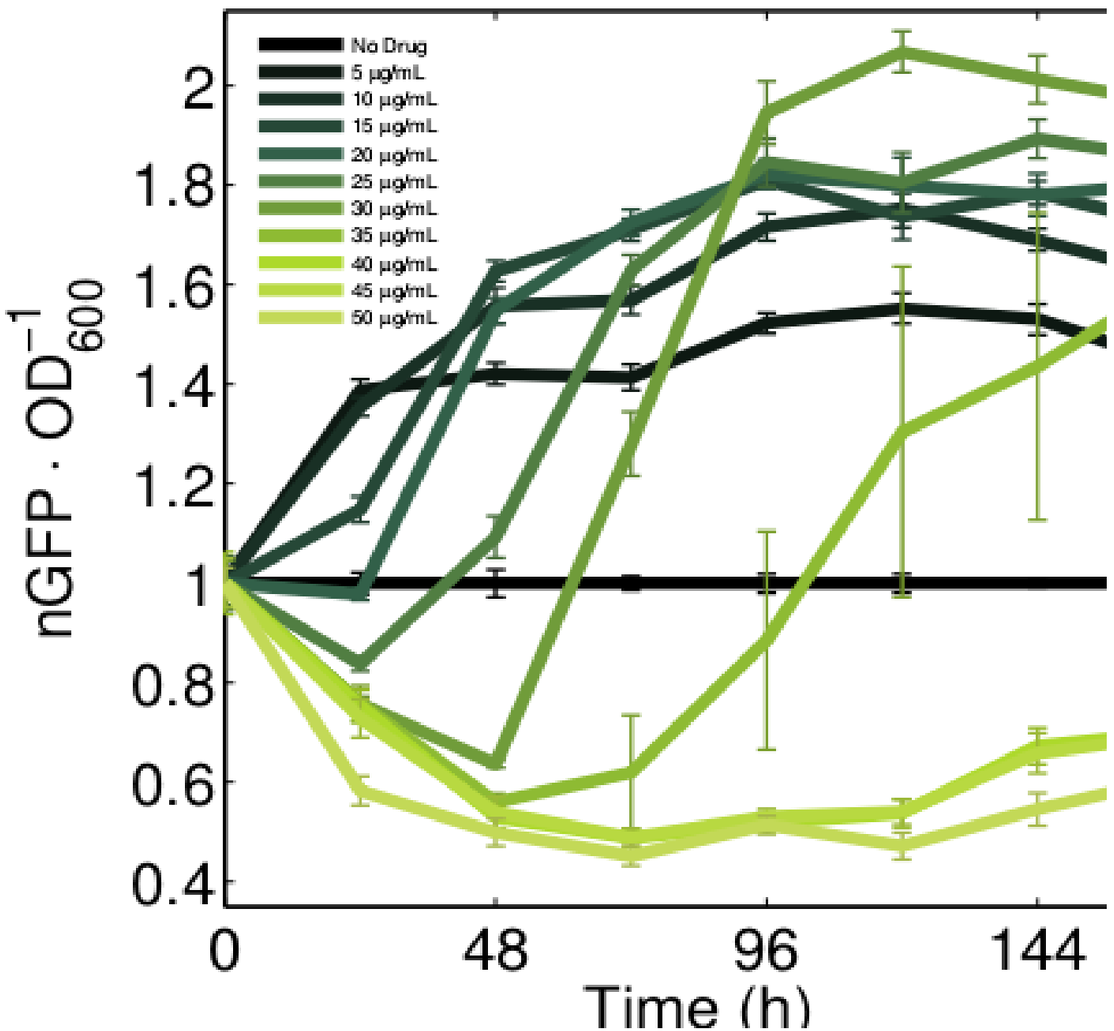
Propagate culture in
same conditions every 24h





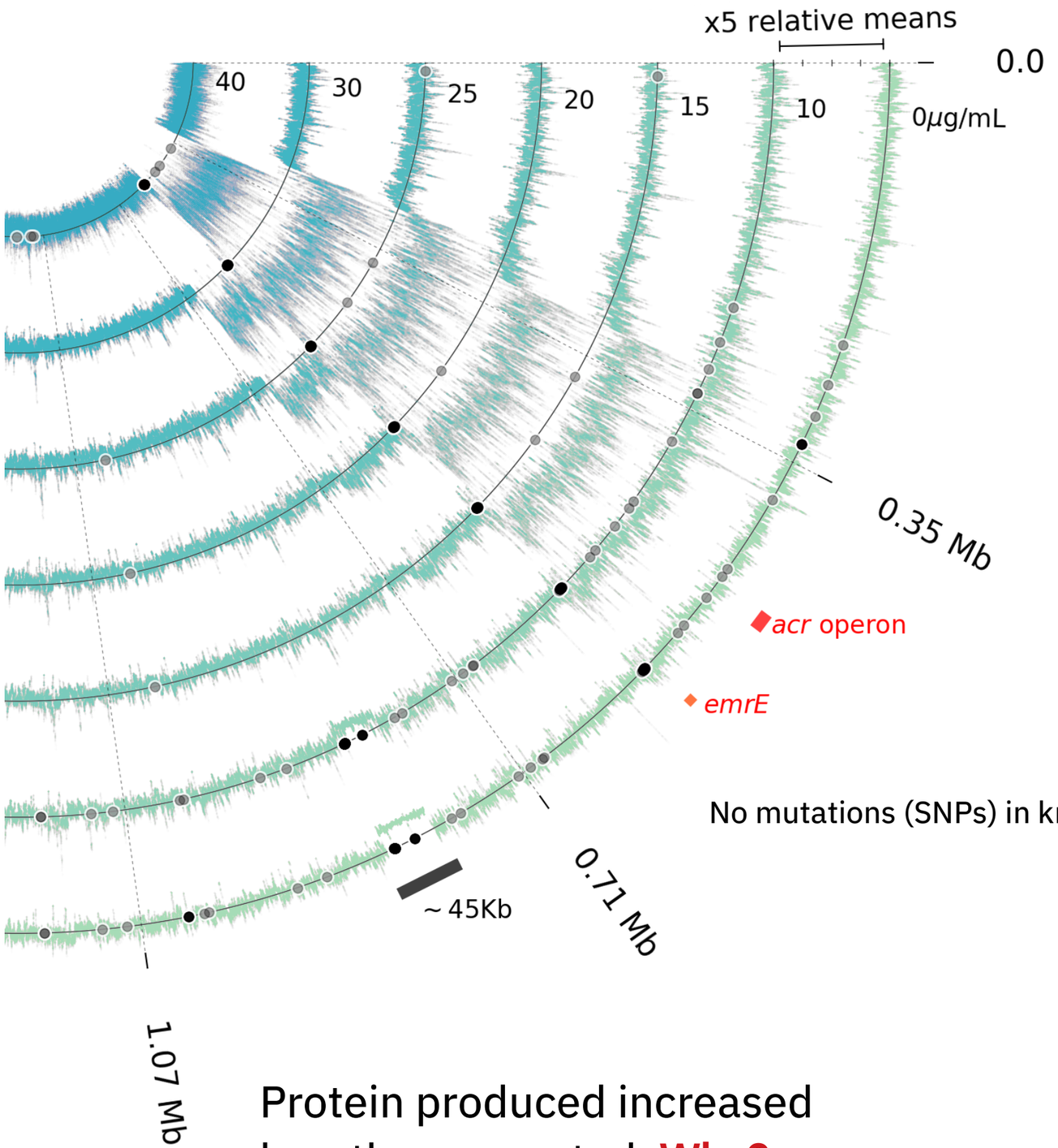
Genome amplification protocol.

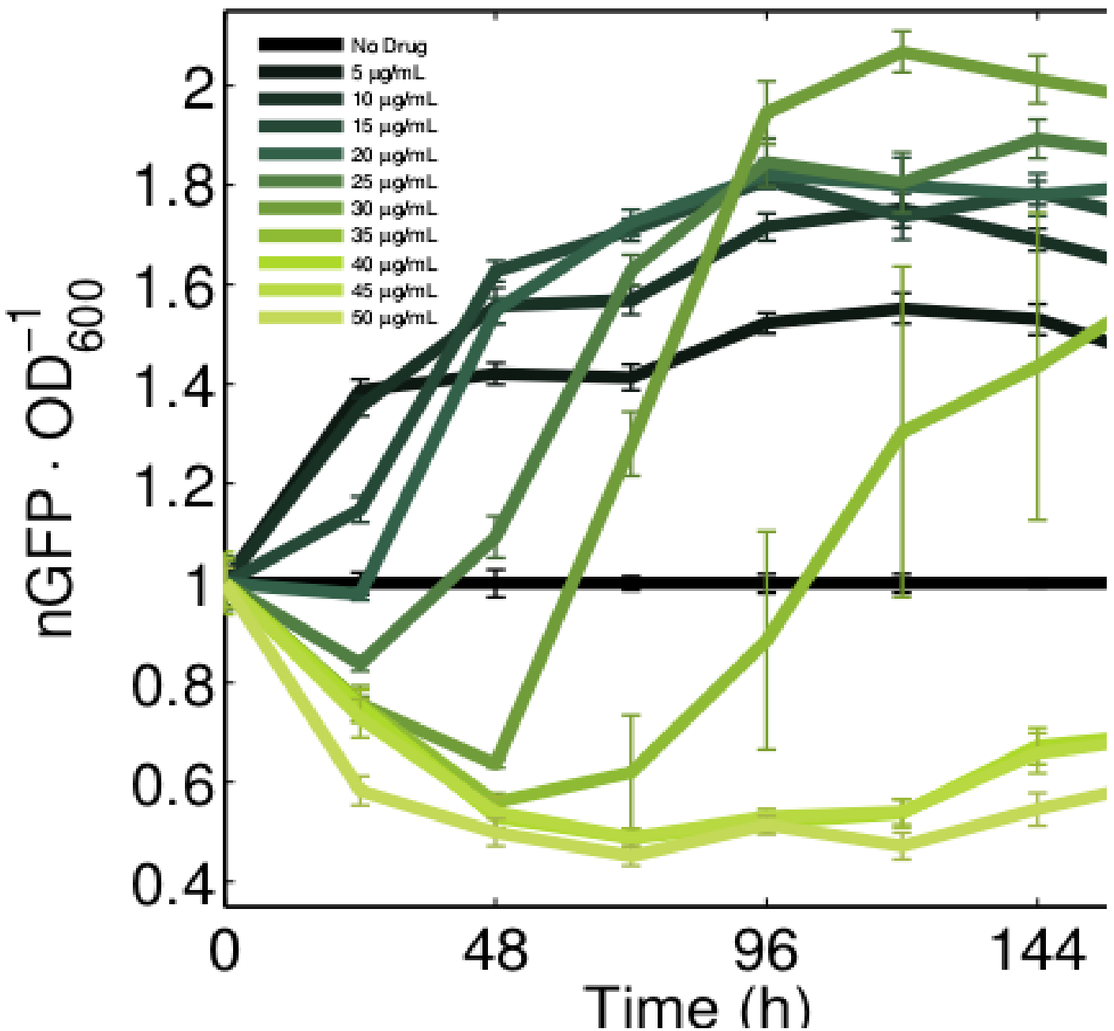




Protein produced increased less than expected. **Why?**

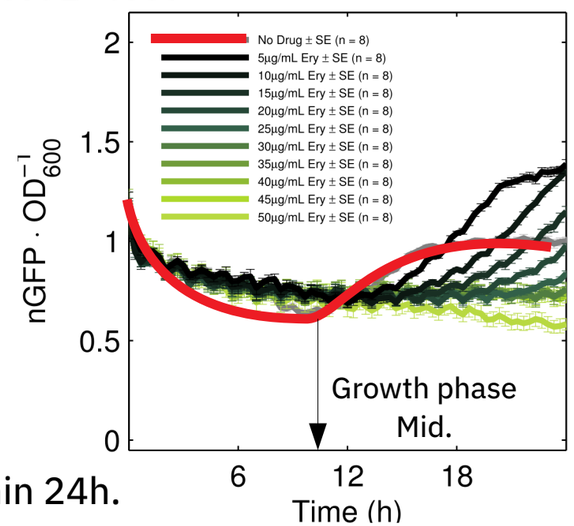
Genome amplification protocol.





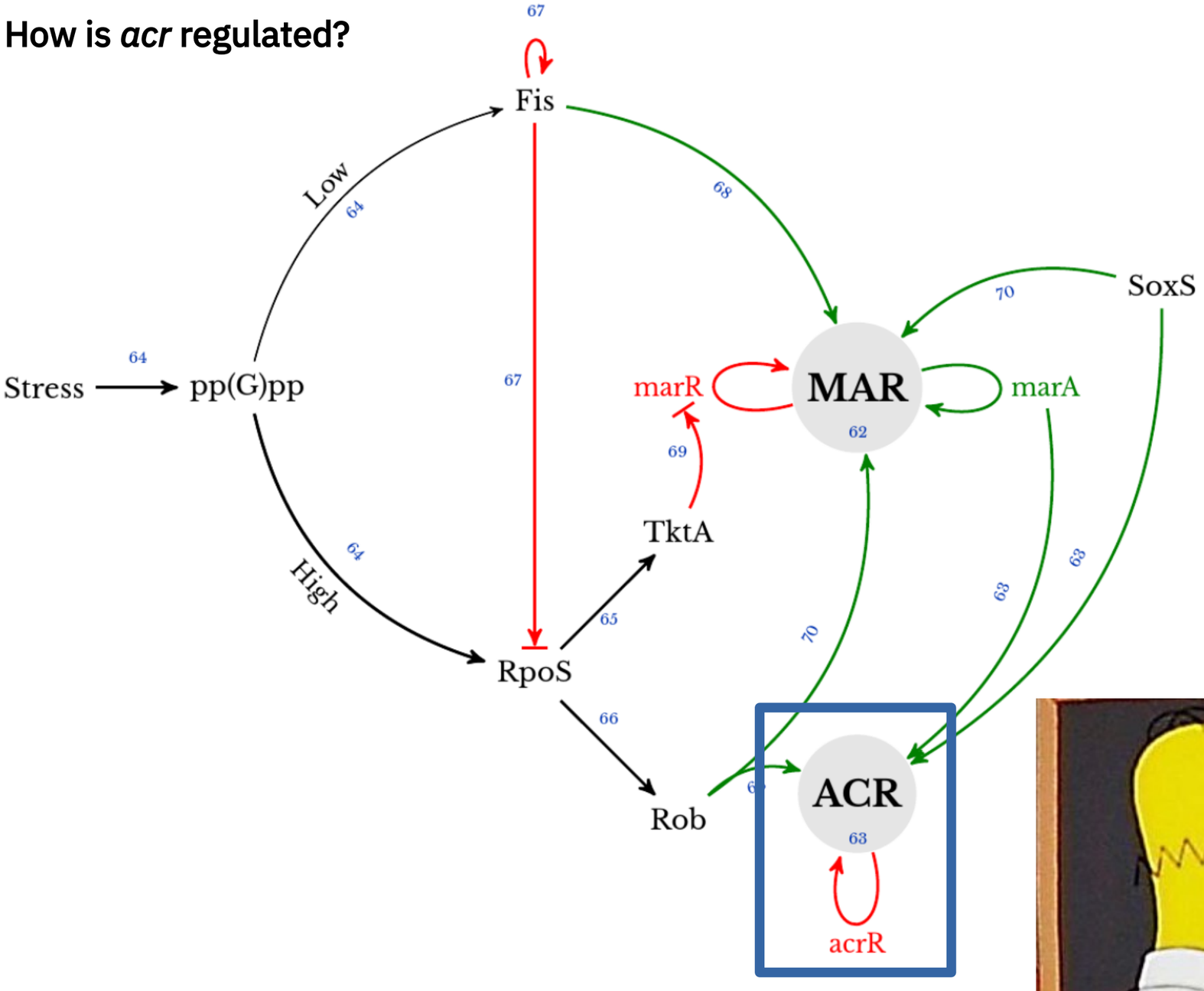
Protein produced increased less than expected. **Why?**

No mutations (SNPs) in known regulators.

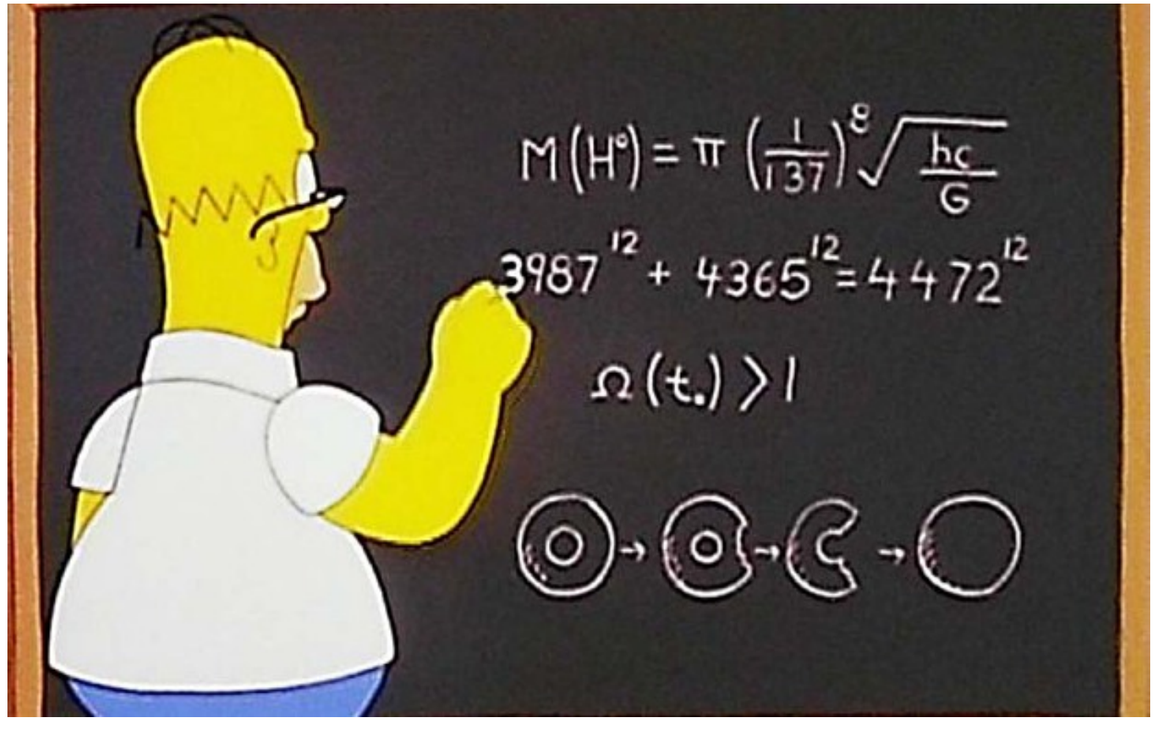


Expression of *acr* plateaus within 24h.

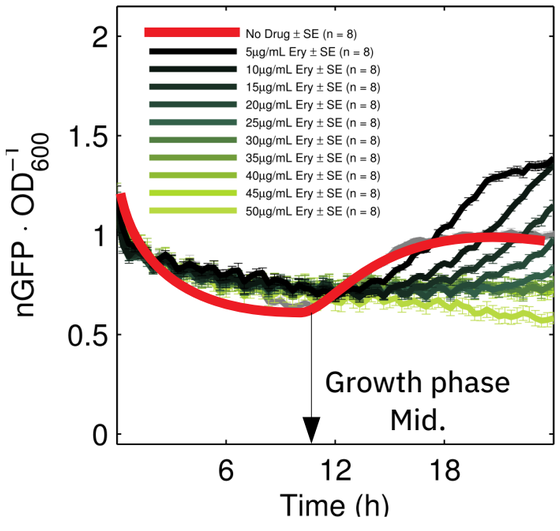
How is *acr* regulated?

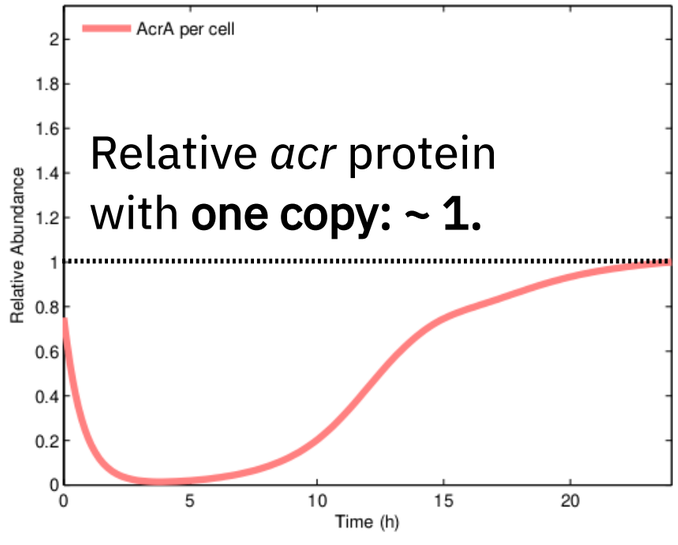


Abundance of this node changes.

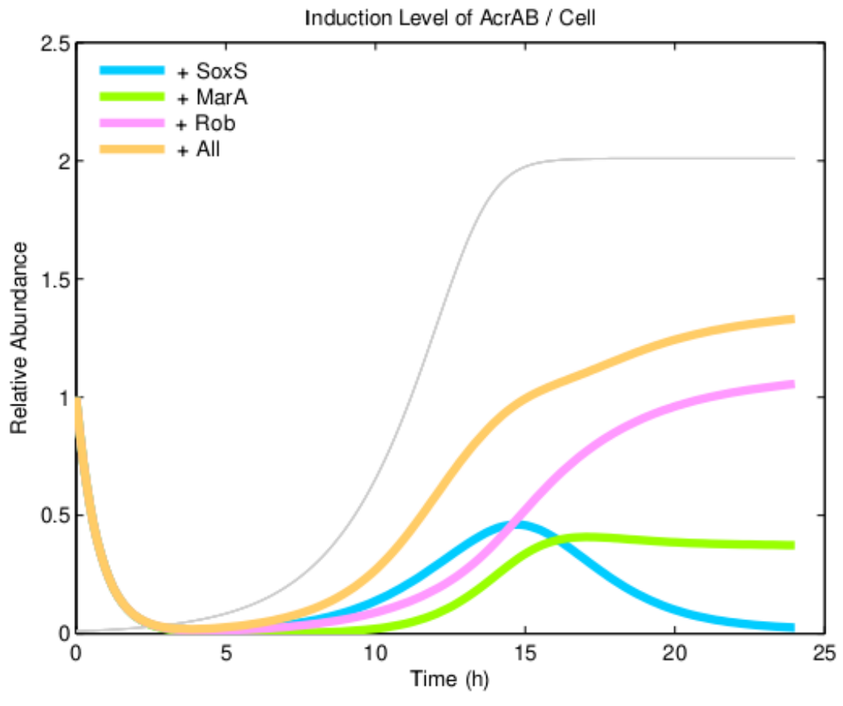


Model of the *acr* operon.

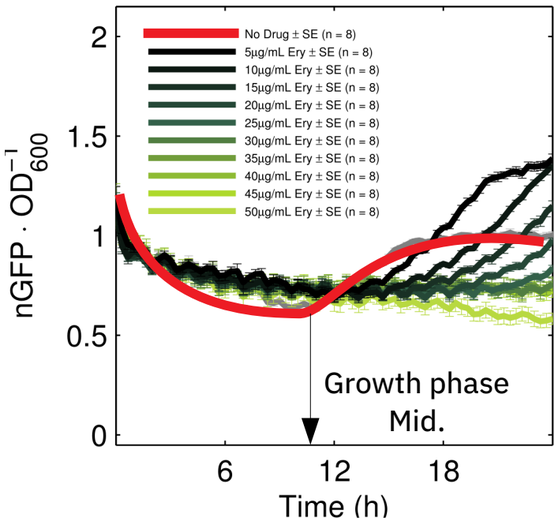




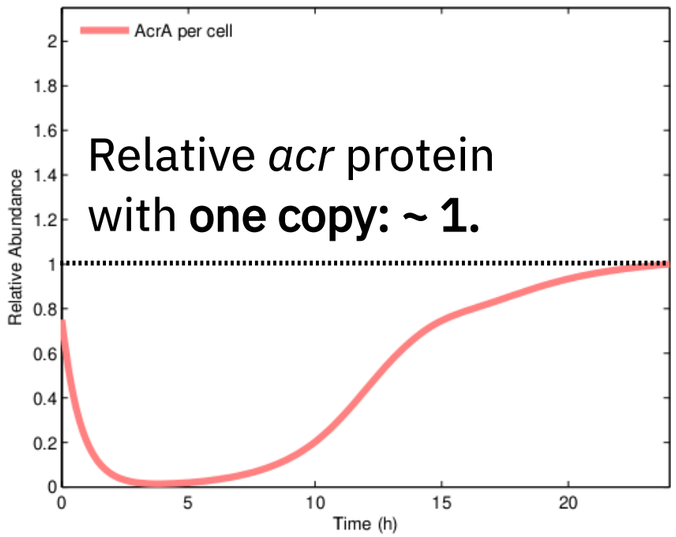
Not perfect, but it's ok...
Captures *acr* dynamics.

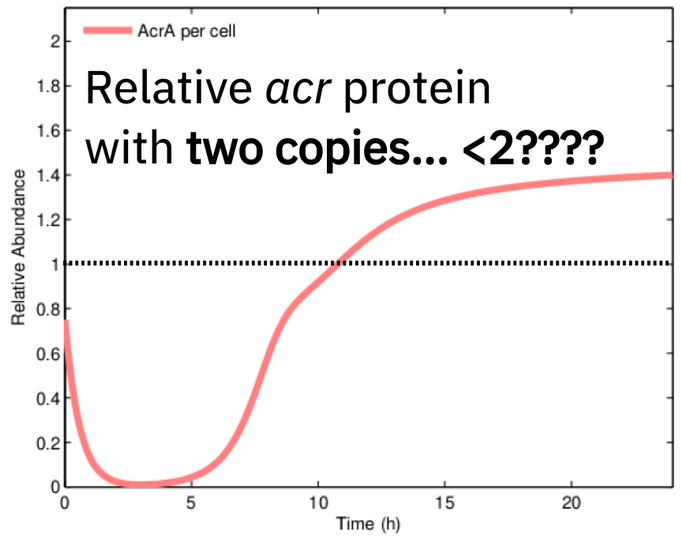


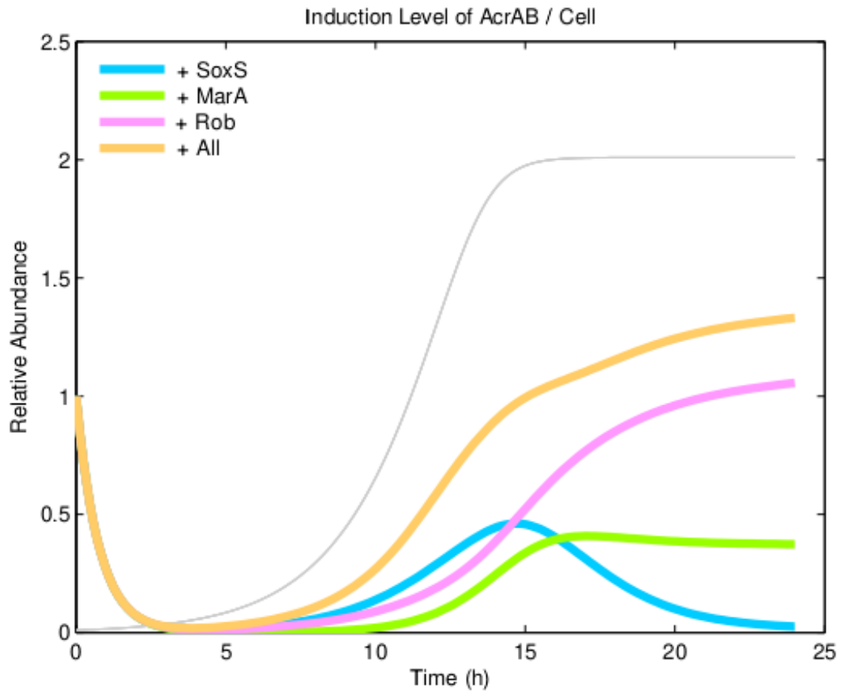
Model of the *acr* operon.

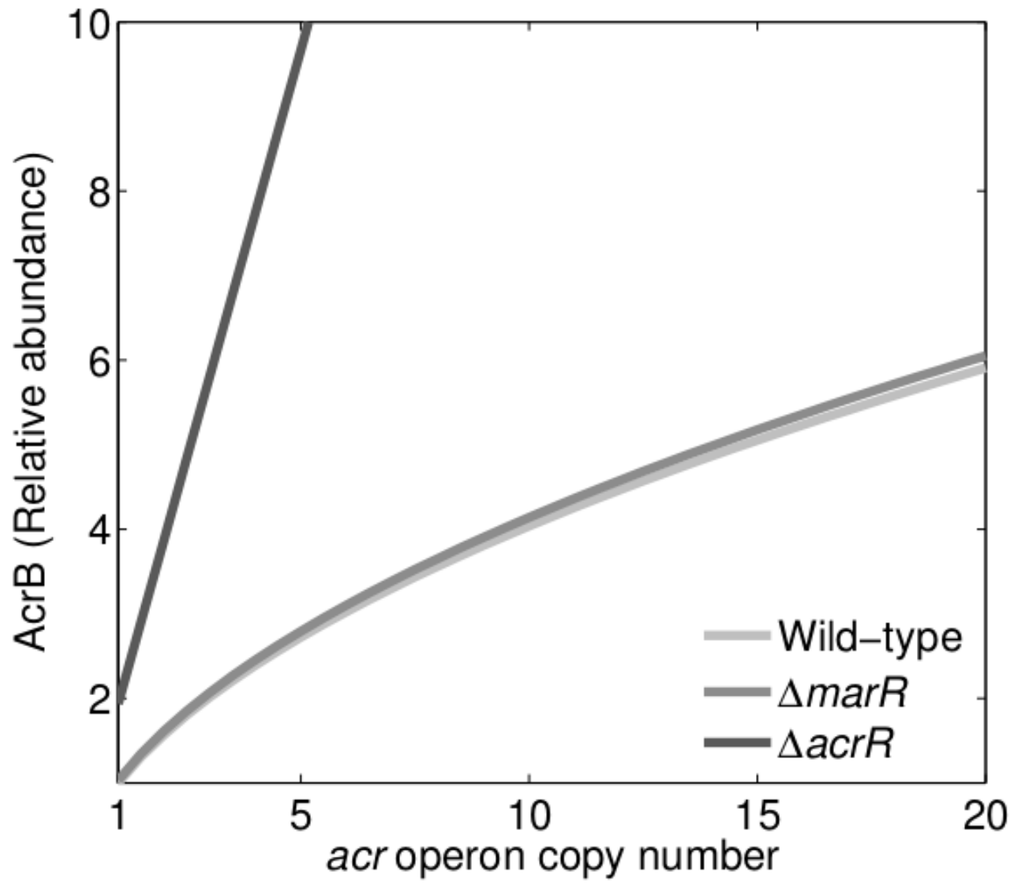


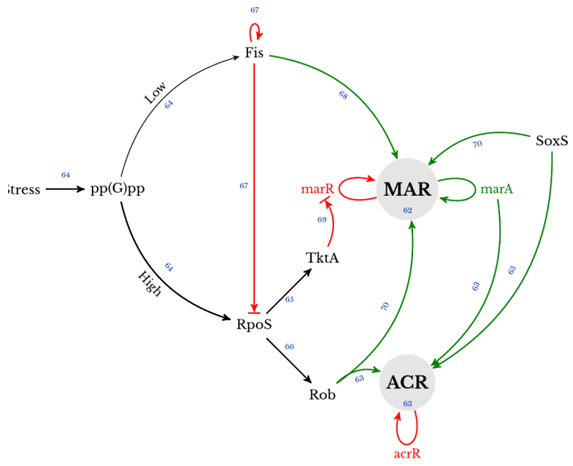
Not perfect, but it's ok...
Captures *acr* dynamics.



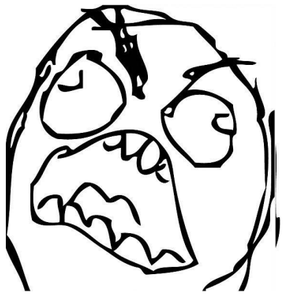




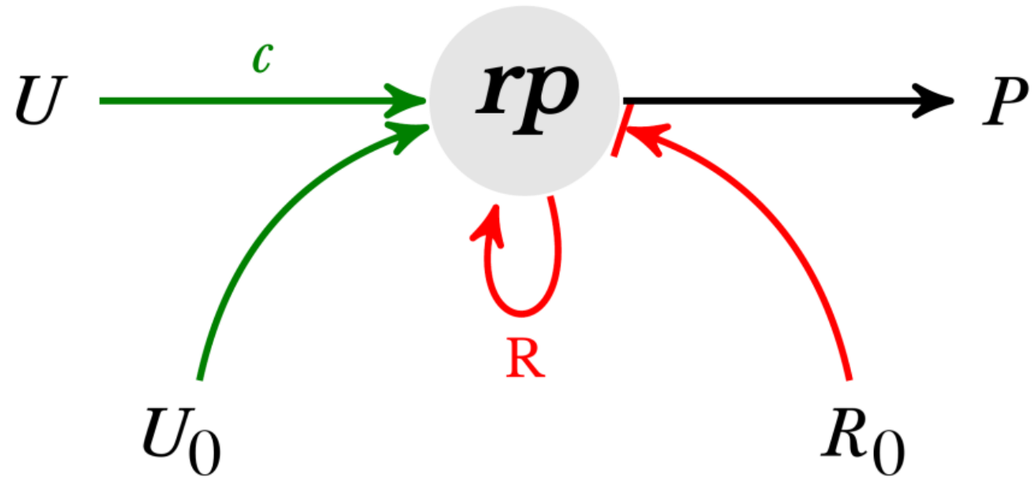




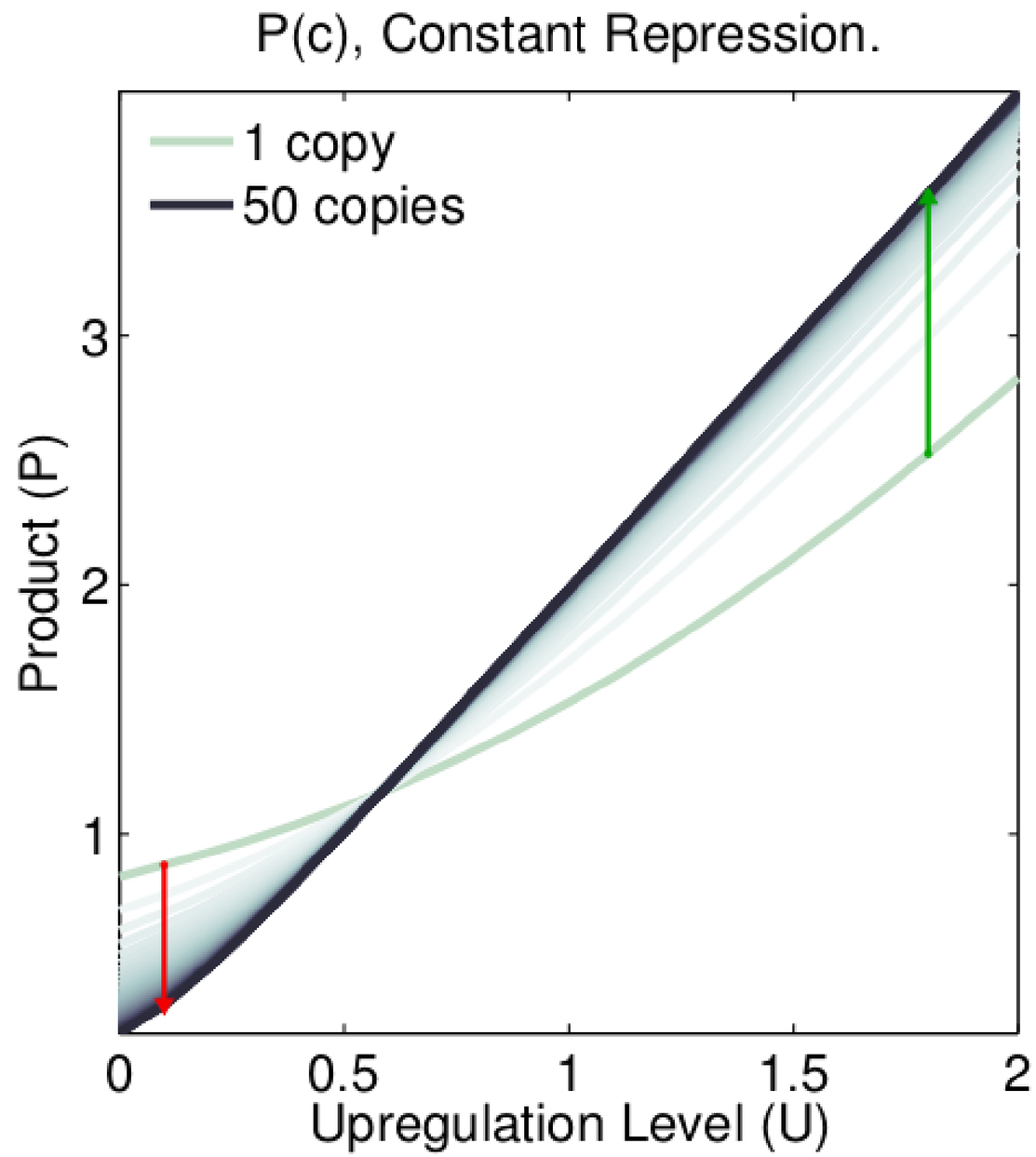
This thing is too complicated!



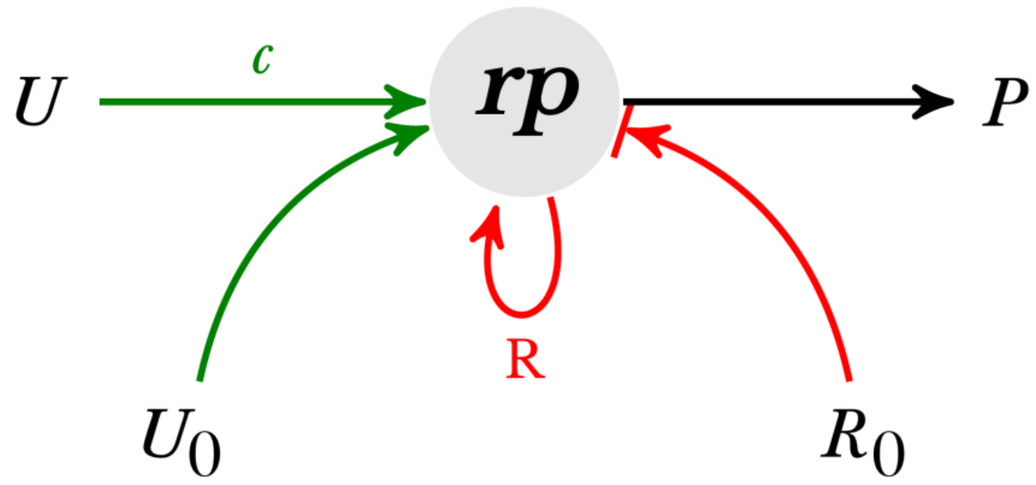
Simpler model: *rp* imaginary operon.



R = repressor, U = up-regulator, c = operon copy number,
R_0 = basal repression, U_0 = basal up-regulation, P = protein

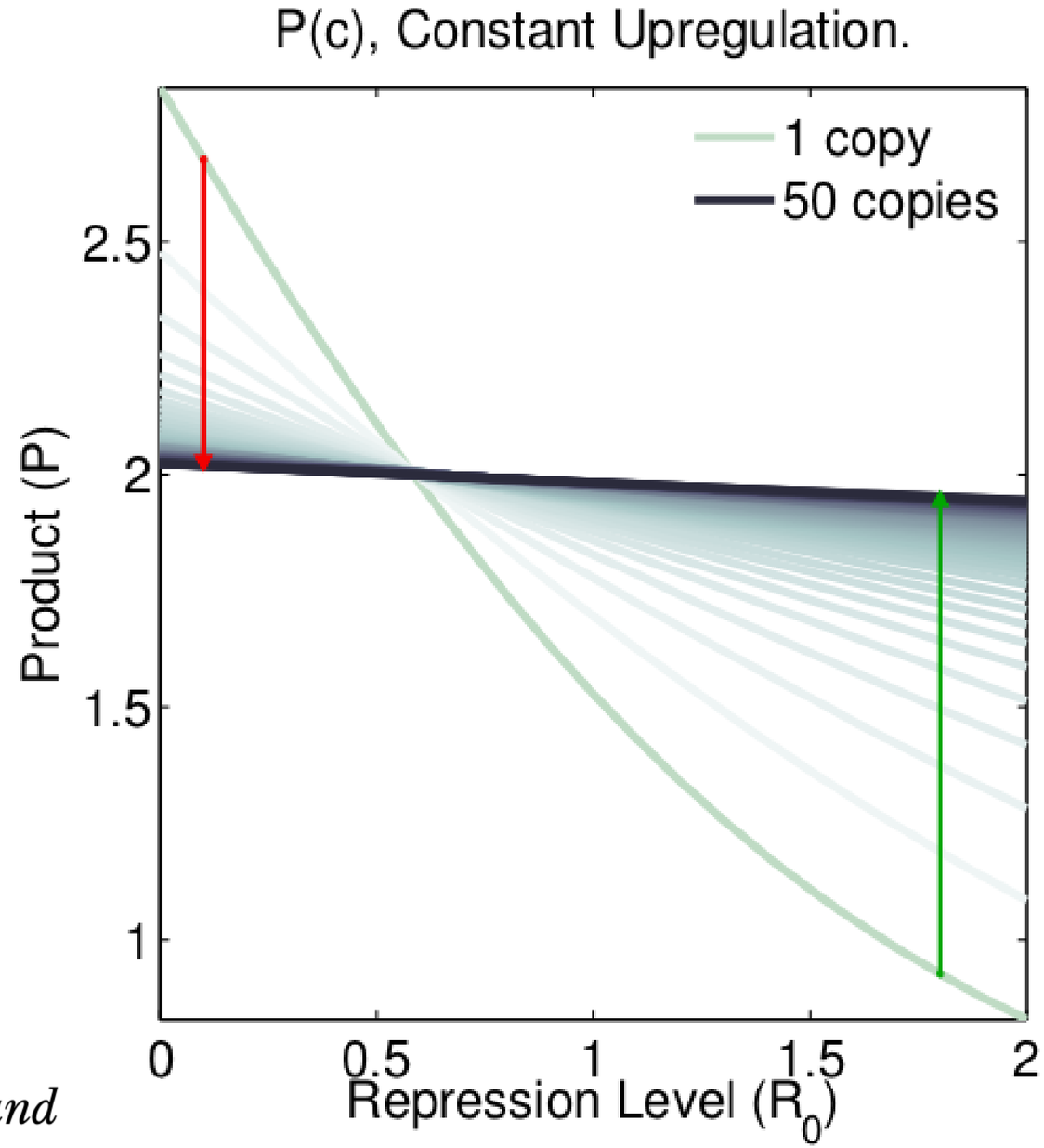


Simpler model: *rp* imaginary operon.



R = repressor, U = up-regulator, c = operon copy number,
R_0 = basal repression, U_0 = basal up-regulation, P = protein

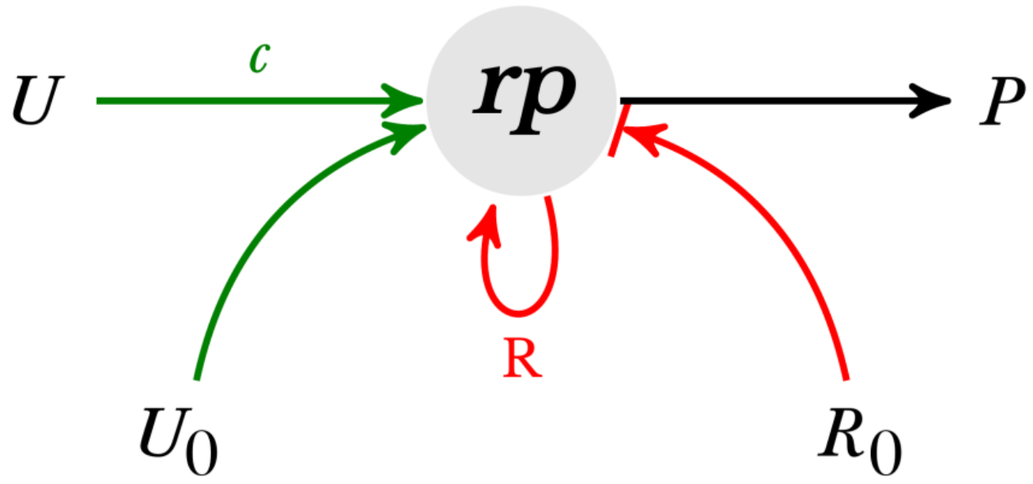
Going from gene to protein doesn't seem to be that straightforward...



"The drivers underlying copy number alterations (CRAs) and transcriptional subtypes are largely unknown, and an integrative analysis (...) may provide a more comprehensive understanding on the information flow from DNA to protein to phenotype."

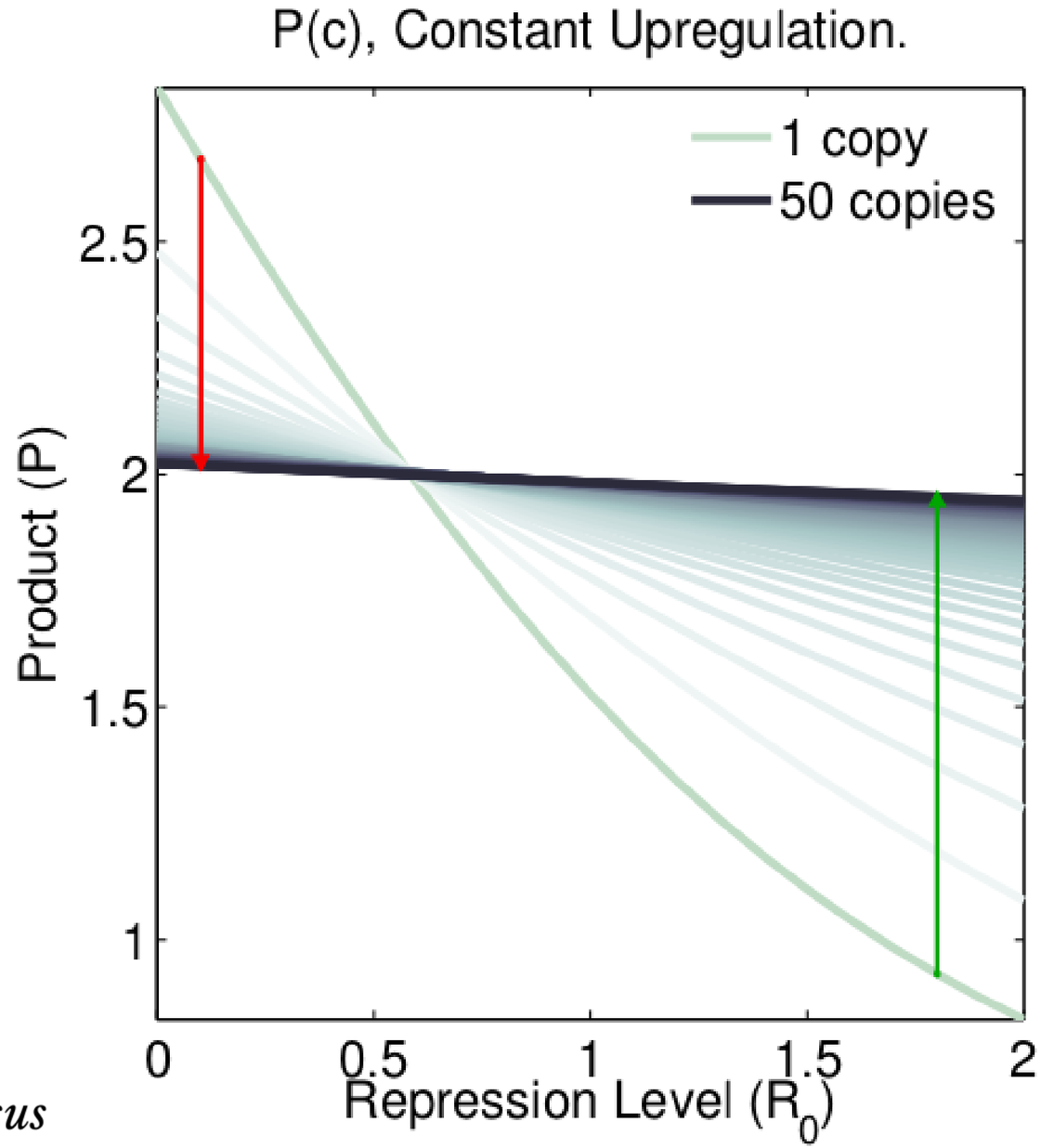
B. Zhang *et al. Nature* **513**, 382 (2014).

Simpler model: *rp* imaginary operon.



R = repressor, U = up-regulator, c = operon copy number,
R_0 = basal repression, U_0 = basal up-regulation, P = protein

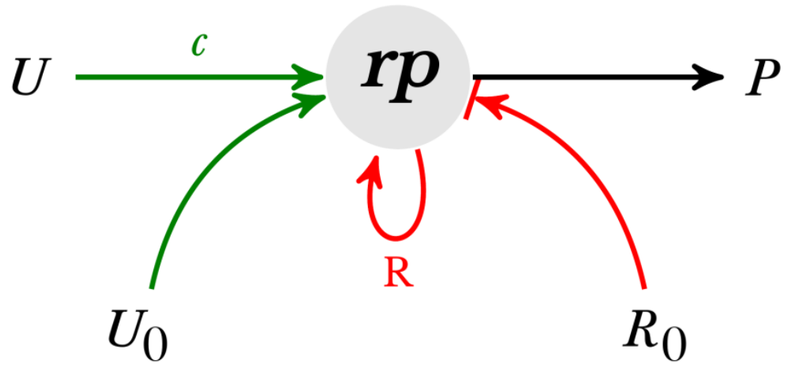
Going from gene to protein doesn't seem to be that straightforward...



"Understanding the contributions of transcriptional versus posttranscriptional control is not simply a matter of academic interest (...): variation of protein expression is poorly correlated with mRNA abundance."

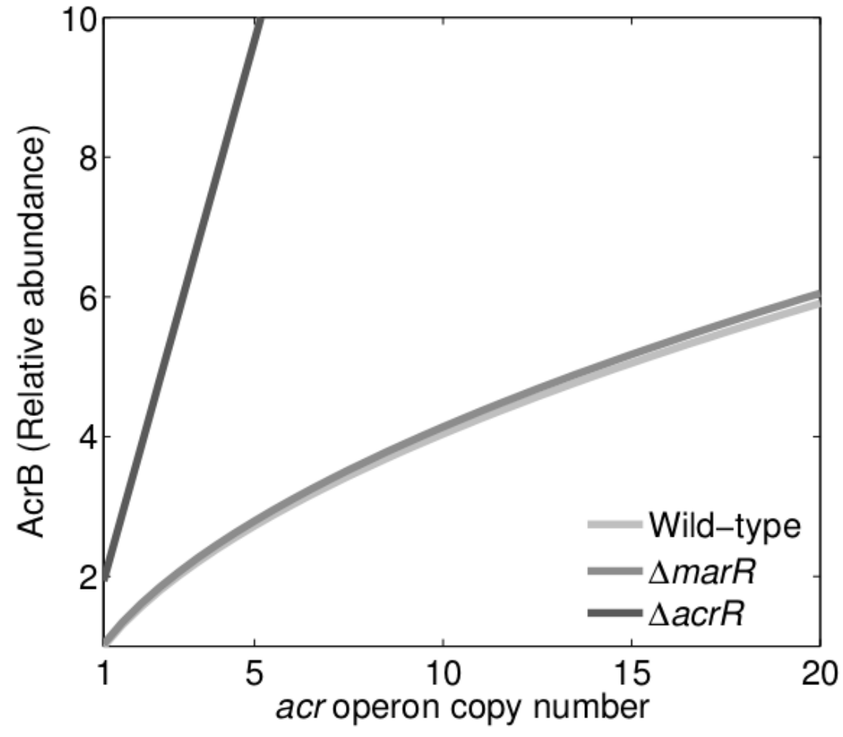
J.J. Li, and M.D. Biggin. *Science* **347**, 6226 (2015).

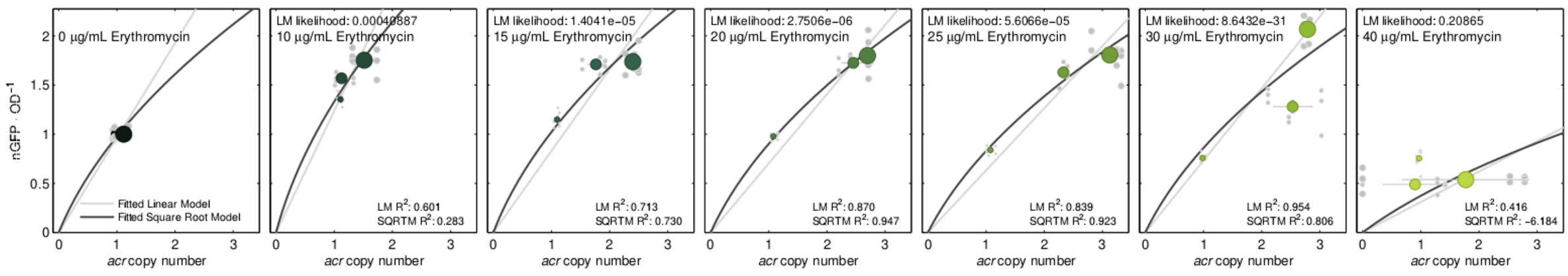
Simpler model: *rp* imaginary operon.



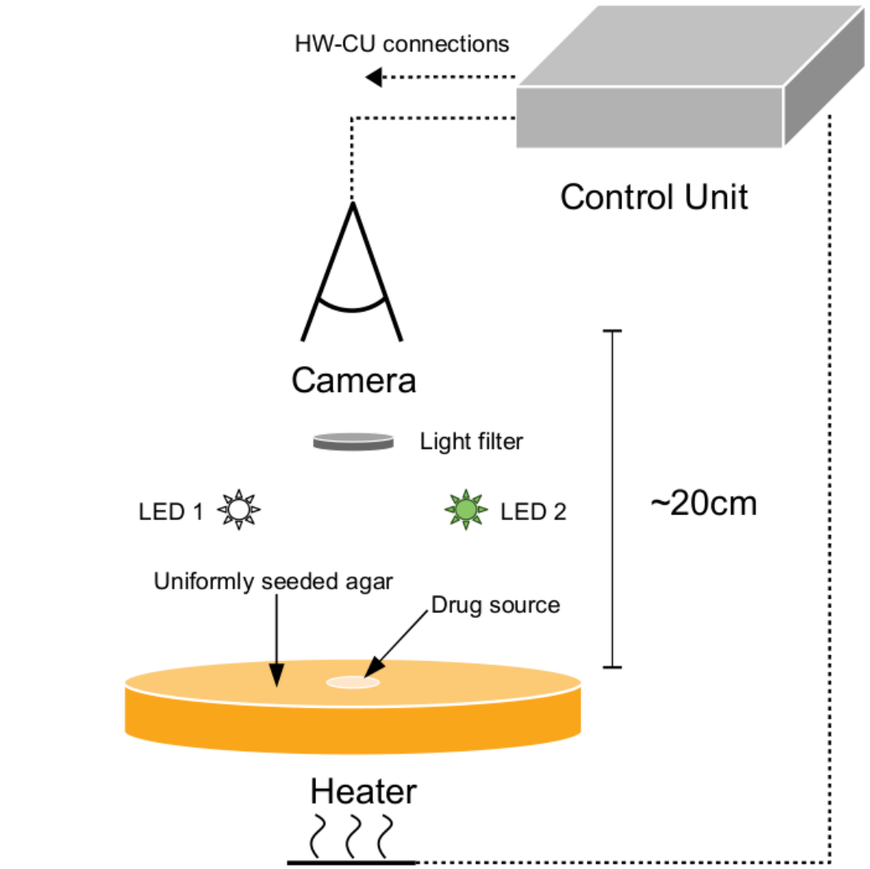
$$P(c) = \alpha + \sqrt{1 + \beta \cdot c}$$

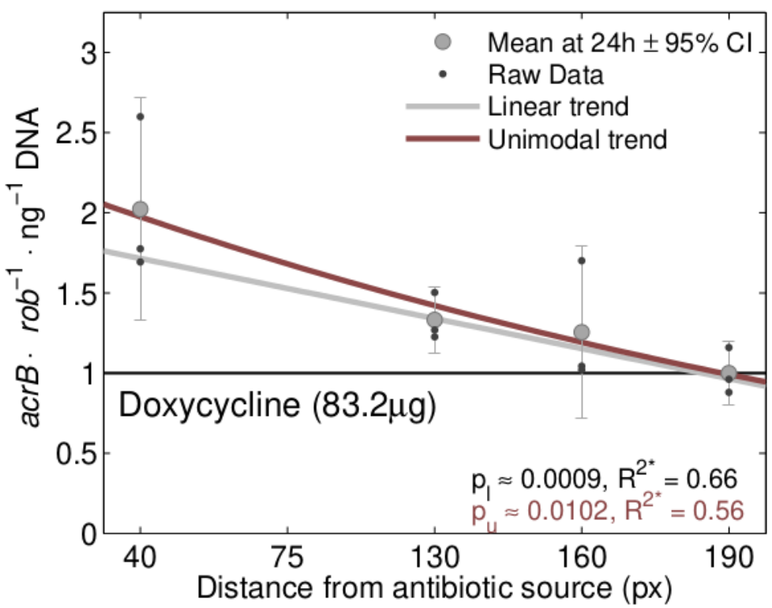
R = repressor, U = up-regulator, c = operon copy number,
R_0 = basal repression, U_0 = basal up-regulation, P = protein

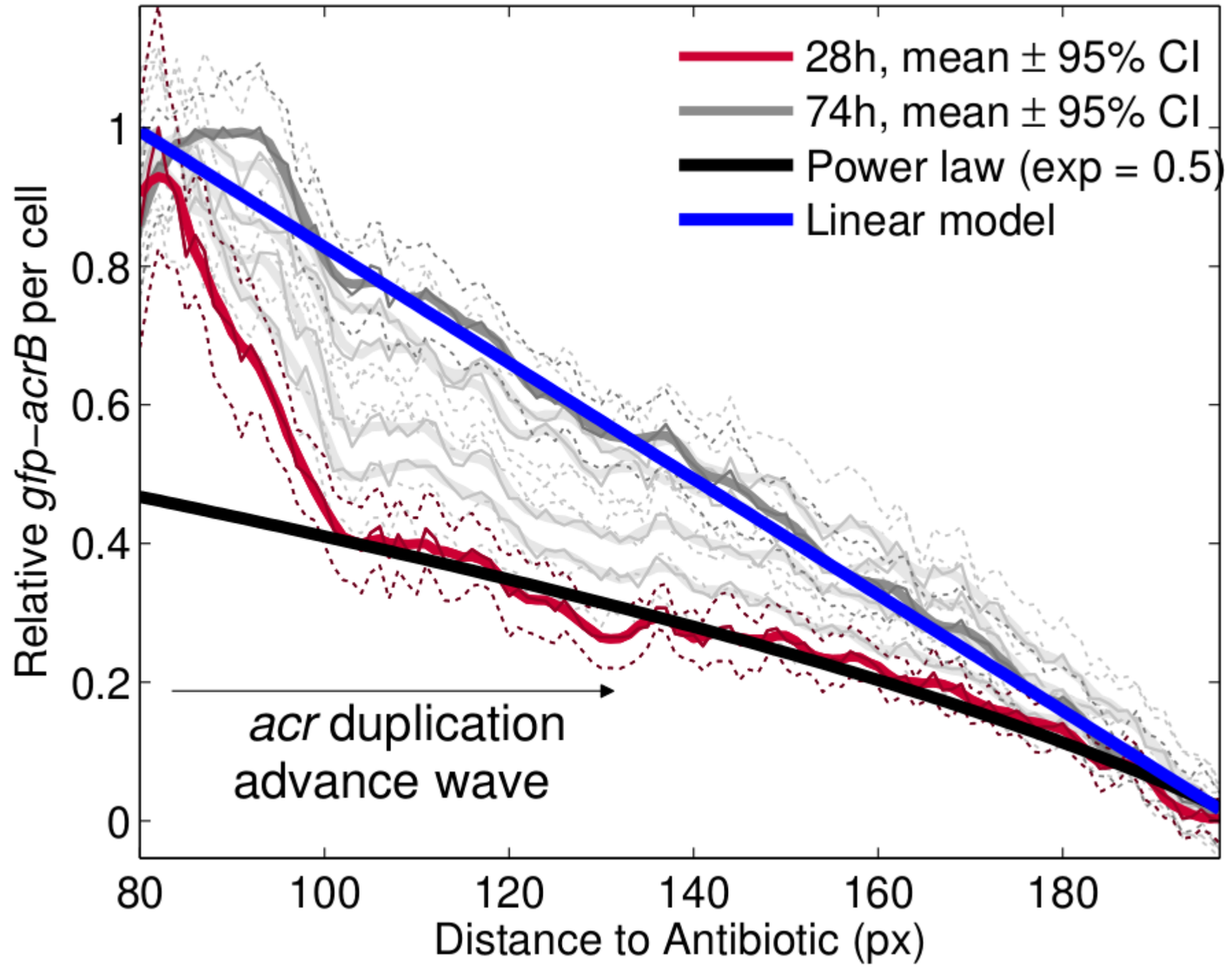




Simpler model: *rp* imaginary operon.







Square root law also applies to antibiograms…
… but it’s broken over time.

Summary

One of the genes encoded by the operon *acr* is a self-repressor.

When *acr* undergoes genomic amplification... so does the repressor.

Because of the above, increasing *acr* copy number does not result in
The proportional increase of *acr* protein (mainly AcrA and AcrB).

A square root law (diminishing returns) is able to explain and
predict the expected protein abundance....

But drugs make *E. coli* break the law: the effect of antibiotics, and the resulting selection of mutants (more than just *acr*) breaks the square root law.

Acknowledgements

People working on lab 309 and 322.

Profs. Robert Beardmore & Ivana Gudelj

EPSRC
Engineering and Physical Sciences Research Council

European Research Council
Established by the European Commission

UNIVERSITY OF EXETER