

Bacillus subtilis

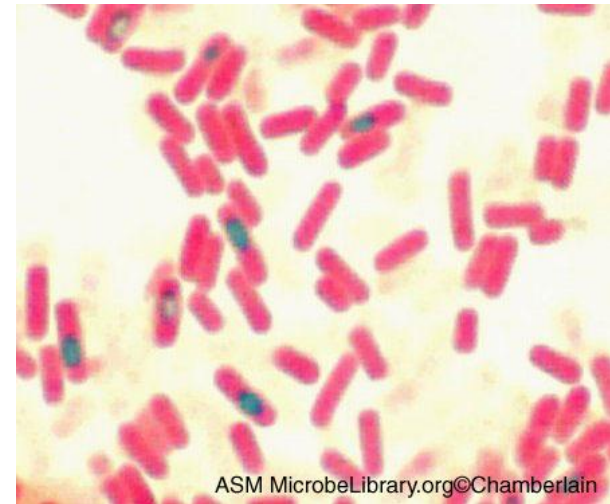
Gram positive rod shaped Bacterium

Widely distributed in the environment

Discovered in 1835 but rediscovered in 1941 by the German Army in N Africa

Produces the protease subtilisin and has immunostimulatory effects

Can form spores and shows competence



ASM MicrobeLibrary.org©Chamberlain

Competence

Competence is the ability of a cell to take up extracellular DNA from the environment.

Competence is usually triggered by nutritional shortages or adverse conditions.

Competence

Under environmental stress, a small fraction of cells transiently differentiate into the state of competence.

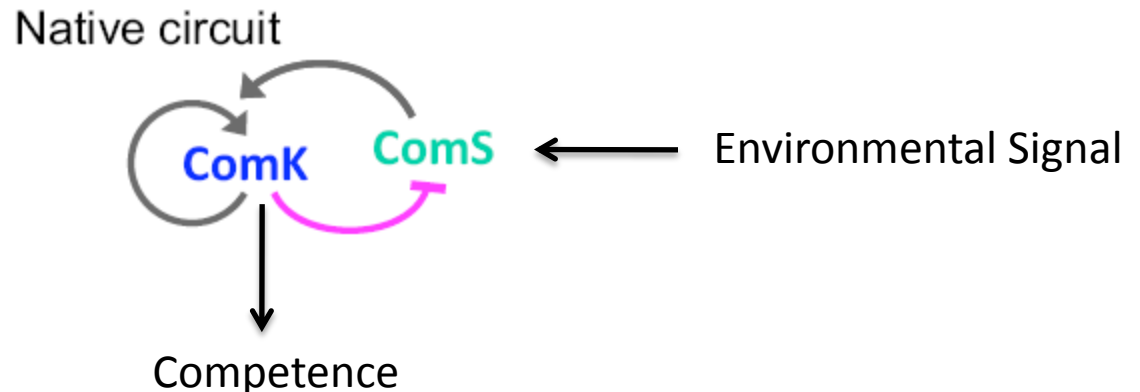
Competence is regulated by a simple circuit comprising of ComK and ComS.

ComK is necessary to trigger competence (controls over 140 other genes)

Competence

ComK activates its own expression (positive feedback).

ComK also regulates a negative feedback loop that is used to turn off the ComK activation.



Testing Two Designs

Architecture-Dependent Noise Discriminates Functionally Analogous Differentiation Circuits. Tolga C agatay, Marc Turcotte, Michael B. Elowitz, Jordi Garcia-Ojalvo,4 and Gurol M. Suel. Cell, (2009), 139, 1-11.

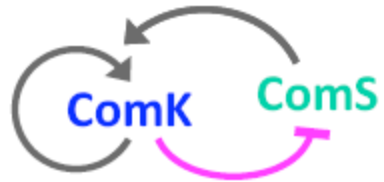
<http://www.elowitz.caltech.edu/publications/CompNoiseArch.pdf>

<http://www.elowitz.caltech.edu/publications/CompNoiseArch-supp.pdf>

Two Circuits

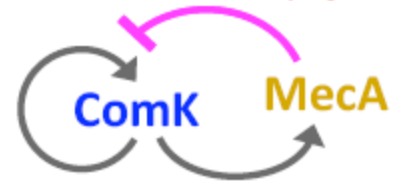
A

Native circuit

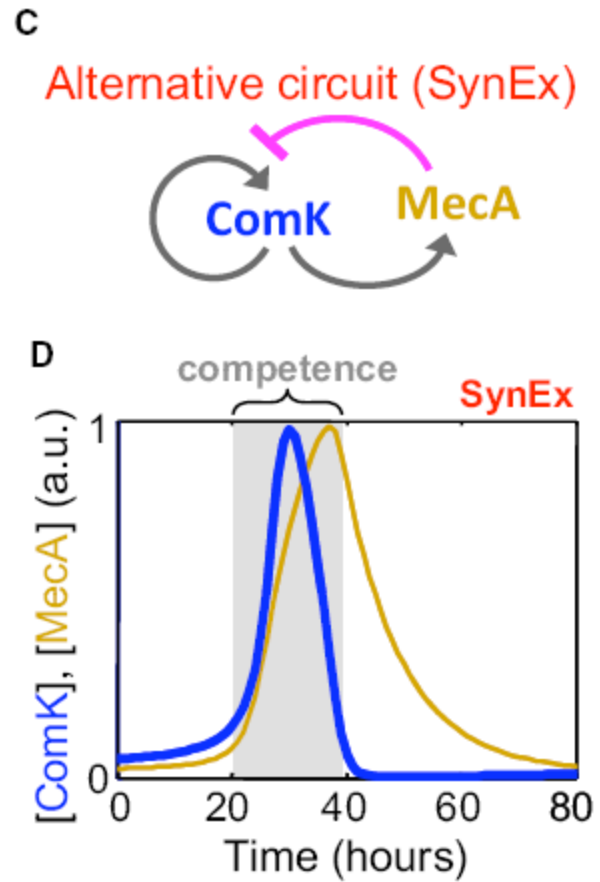
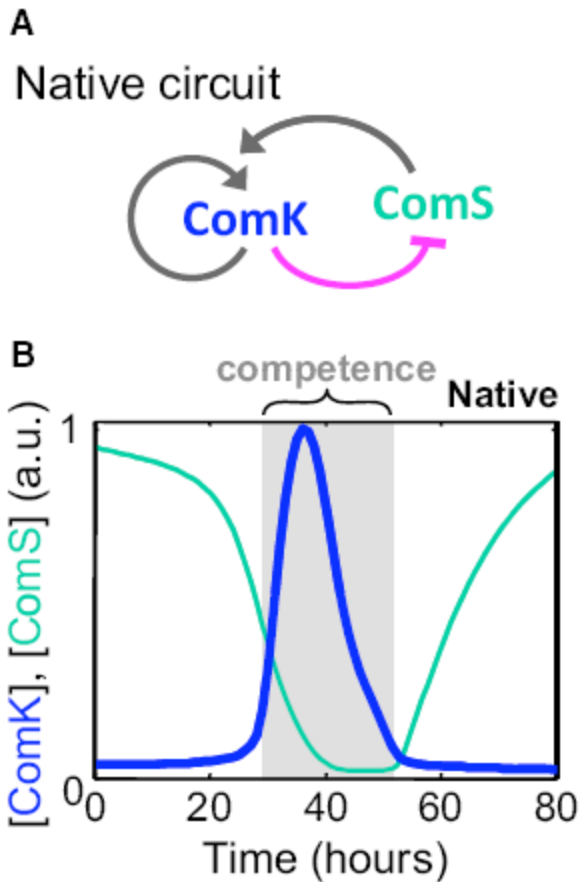


C

Alternative circuit (SynEx)

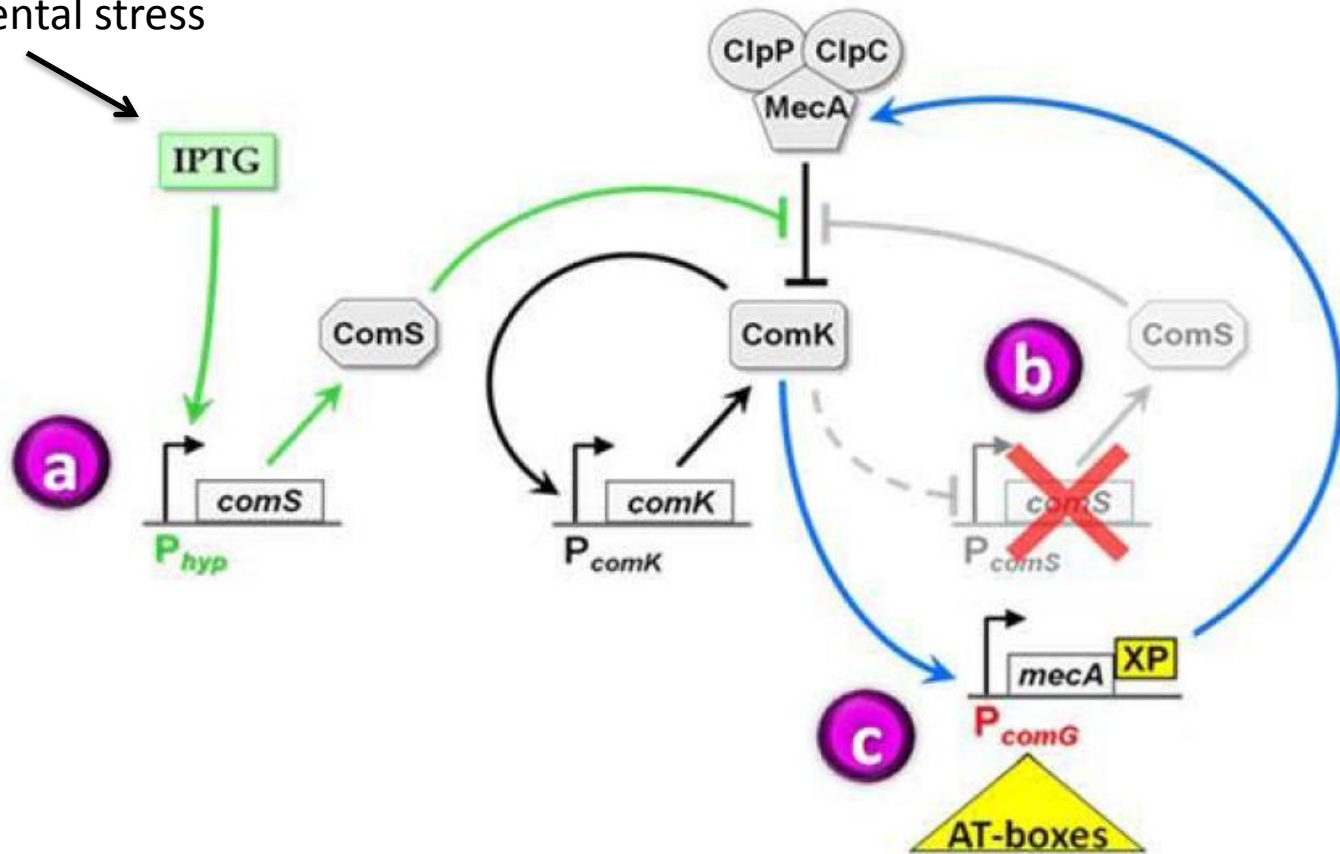


Two Circuits

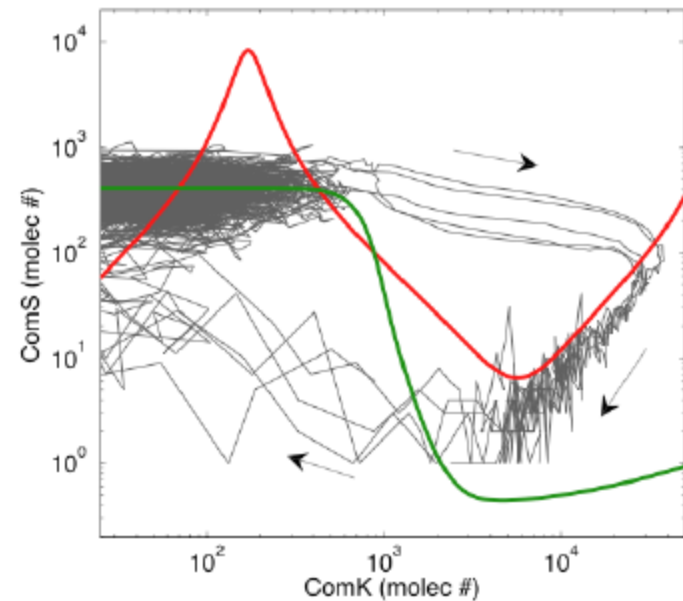
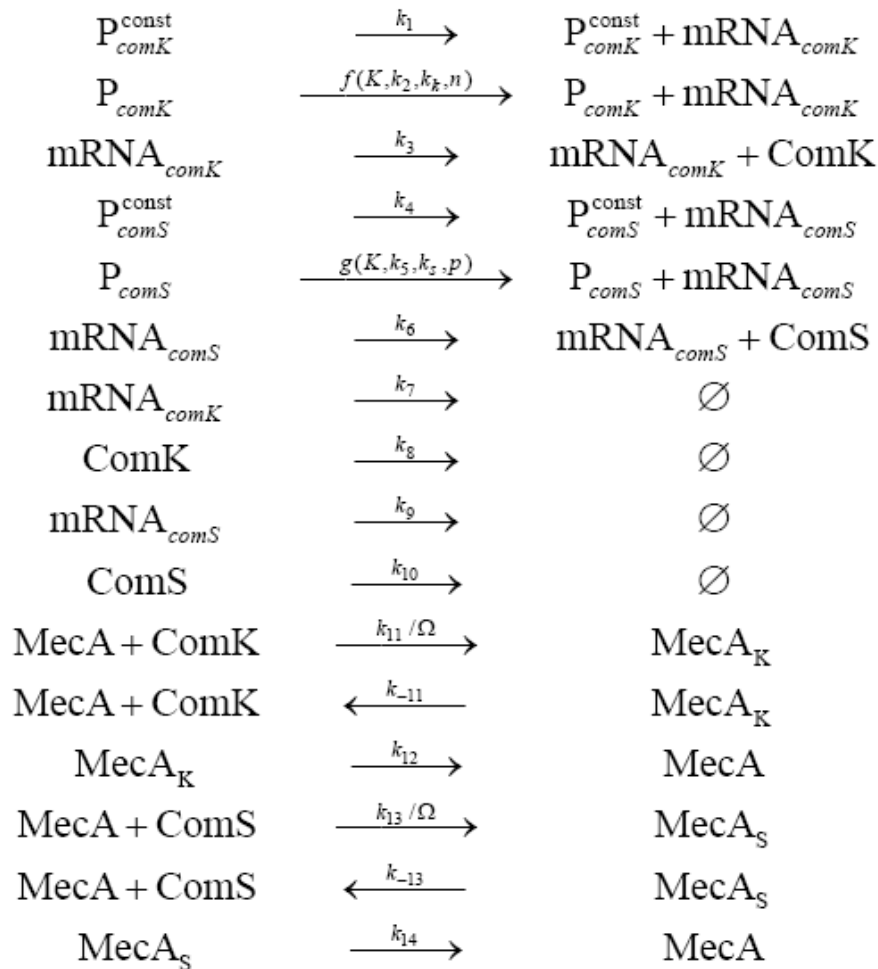


Modified Circuit

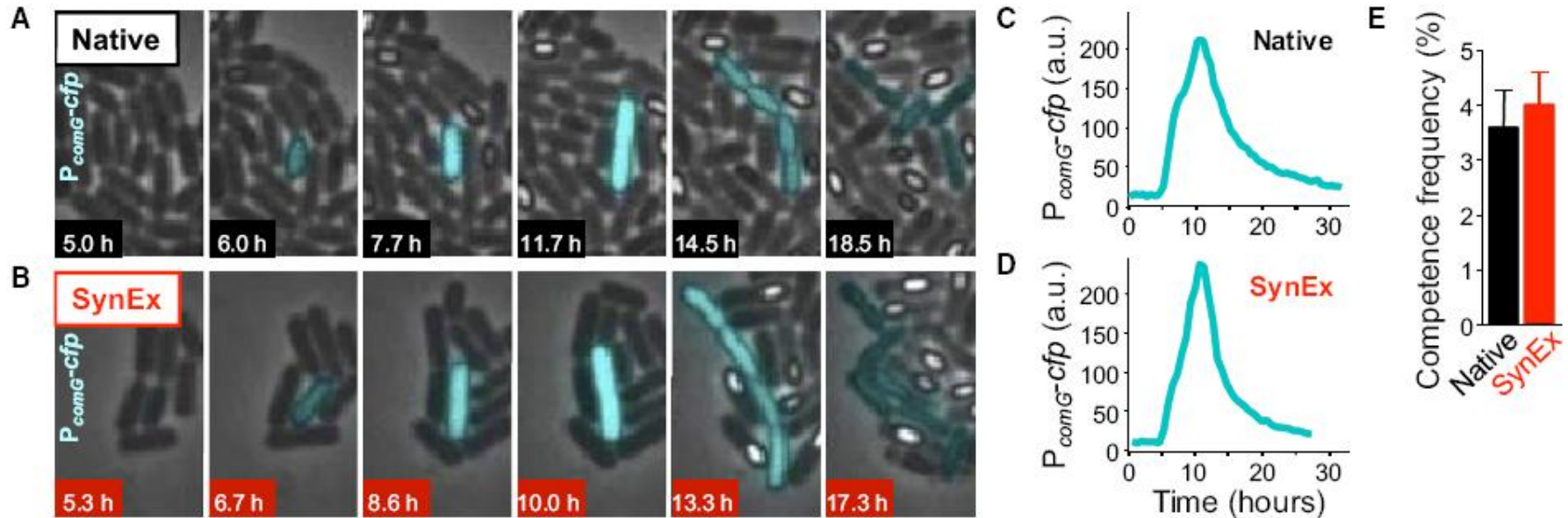
Simulated environmental stress



Computer Model

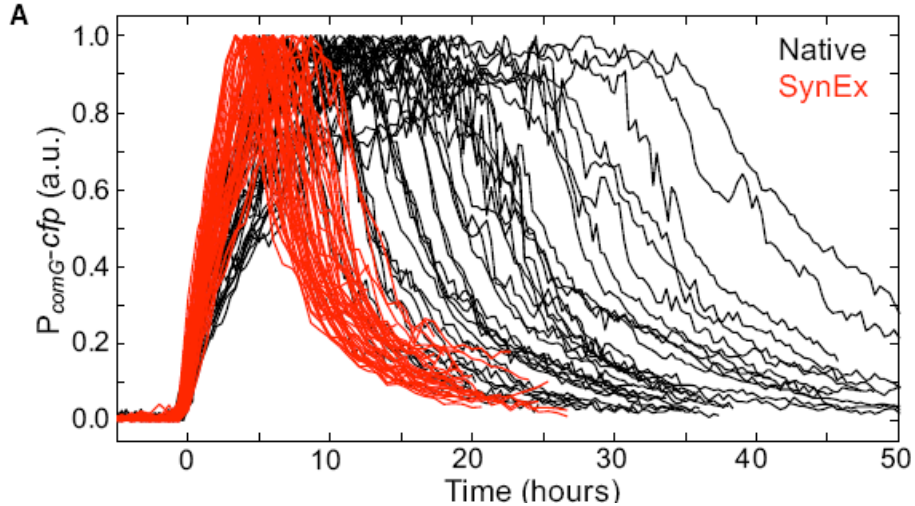


Initial Experiments

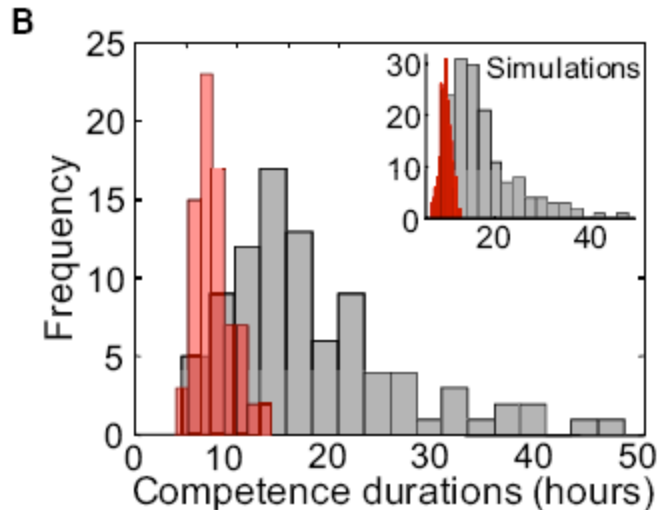


Mathematical modeling was used to guide and tune the SynEx circuit. Simulations showed that high affinity on the ComK to MecA loop (P_{comG}) would prevent excitable dynamics, therefore the promoter was reduced in strength.

Frequency Experiments



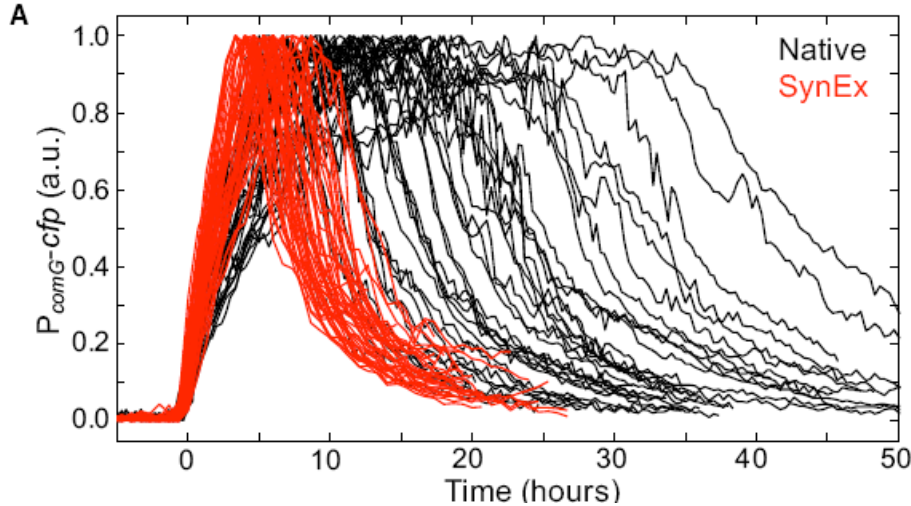
Competency duration times much shorter in the engineered circuit as measured by PcomG-cfp.



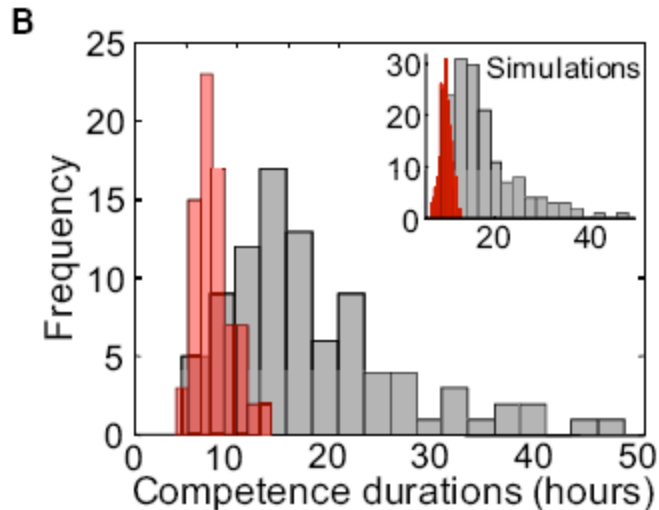
Frequency of competency duration times from experiment and simulation.

How to they explain this? Back to the simulation model

Frequency Experiments



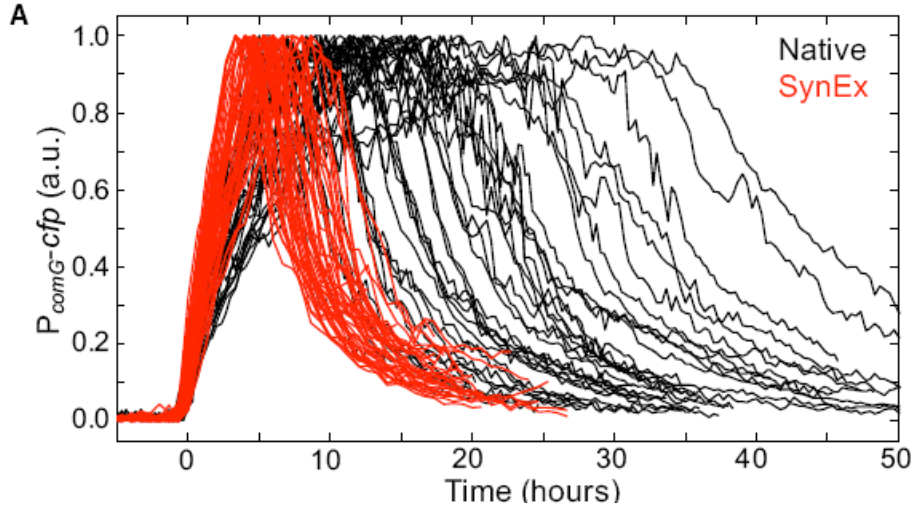
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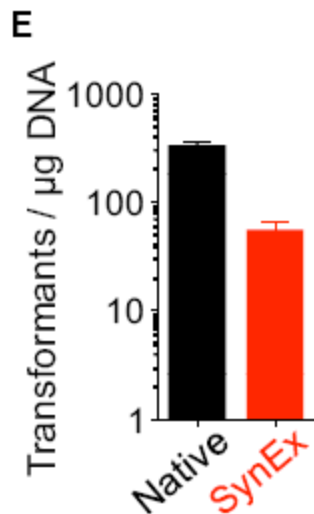
Frequency of competence duration times from experiment and simulation.

Does this really matter?

Frequency Experiments



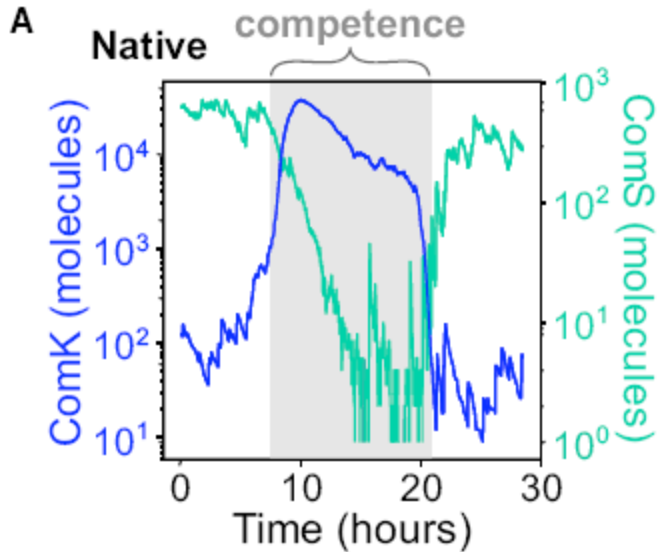
Competency duration times much shorter in the engineered circuit as measured by PcomG-cfp.



Probably yes because it affects how often A cell manages to pick up DNA.

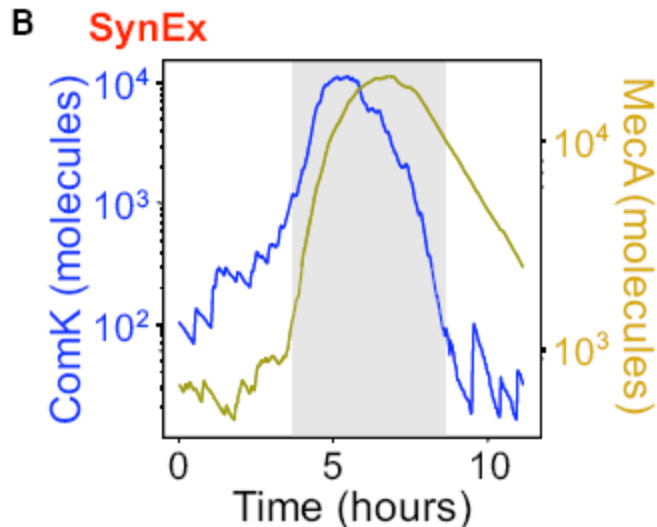
How to they explain this? Back to the simulation model

Simulation Experiments



Competency duration times much shorter in the engineered circuit as measured by PcomG-cfp.

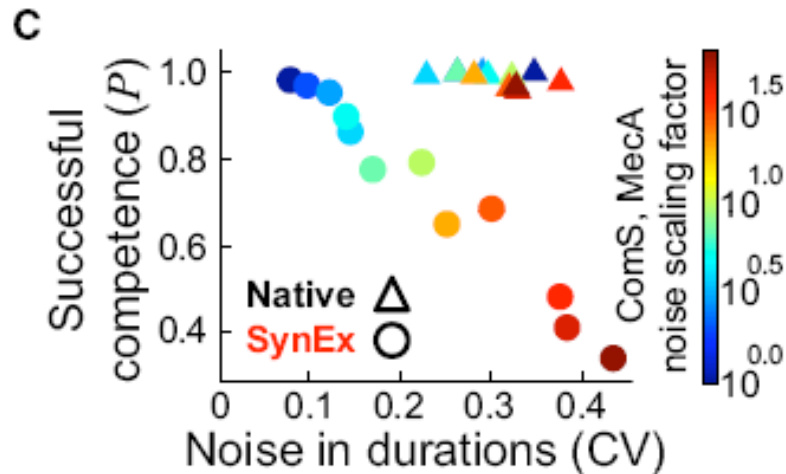
Given the noise in ComS, it takes Longer to switch off ComK



Frequency of competency duration times from experiment and simulation.

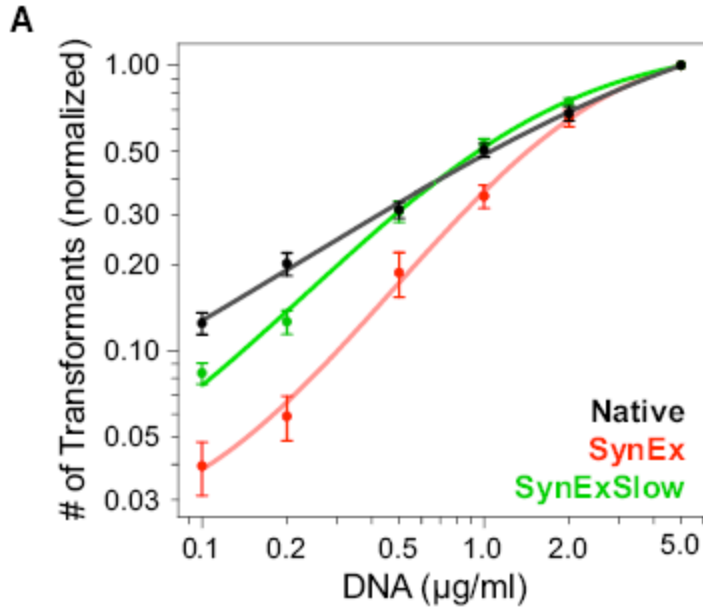
Simulation Experiments

Increase the expression level by changing the promoter strengths and degradation rates so that the mean levels are unchanged but the noise is reduced (averaged out)



As the relative noise is reduced the rate of successful competence goes up. (CV = coefficient of variation, standard deviation / mean)

Selective Advantage



Native circuit is more robust under varying extracellular DNA concentrations.

Eg, at 0.1 µg/ml, the number of transformations is almost 10 times lower in the engineered circuit.