Couple applications of the positive feedback motif

Biosensing and protein production

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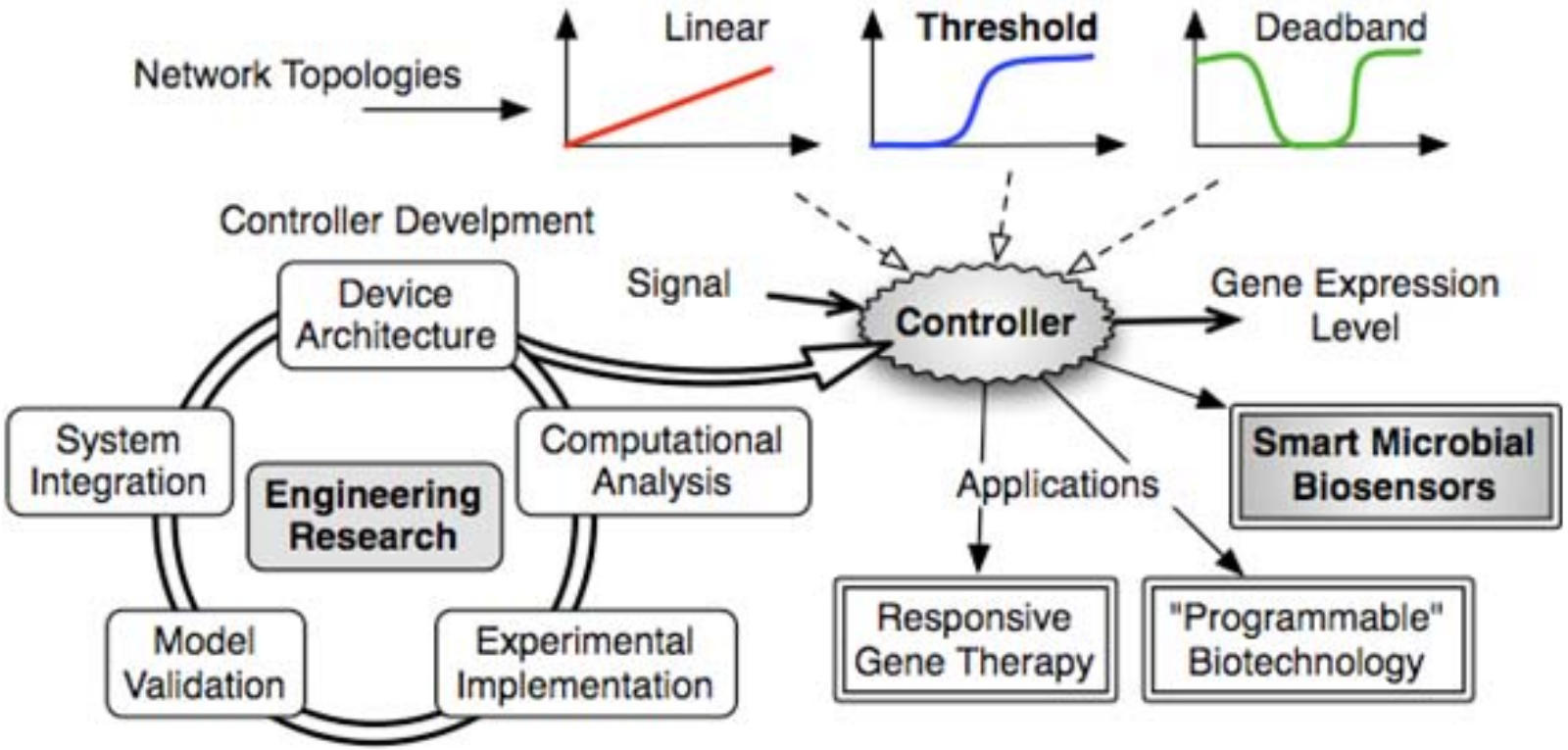
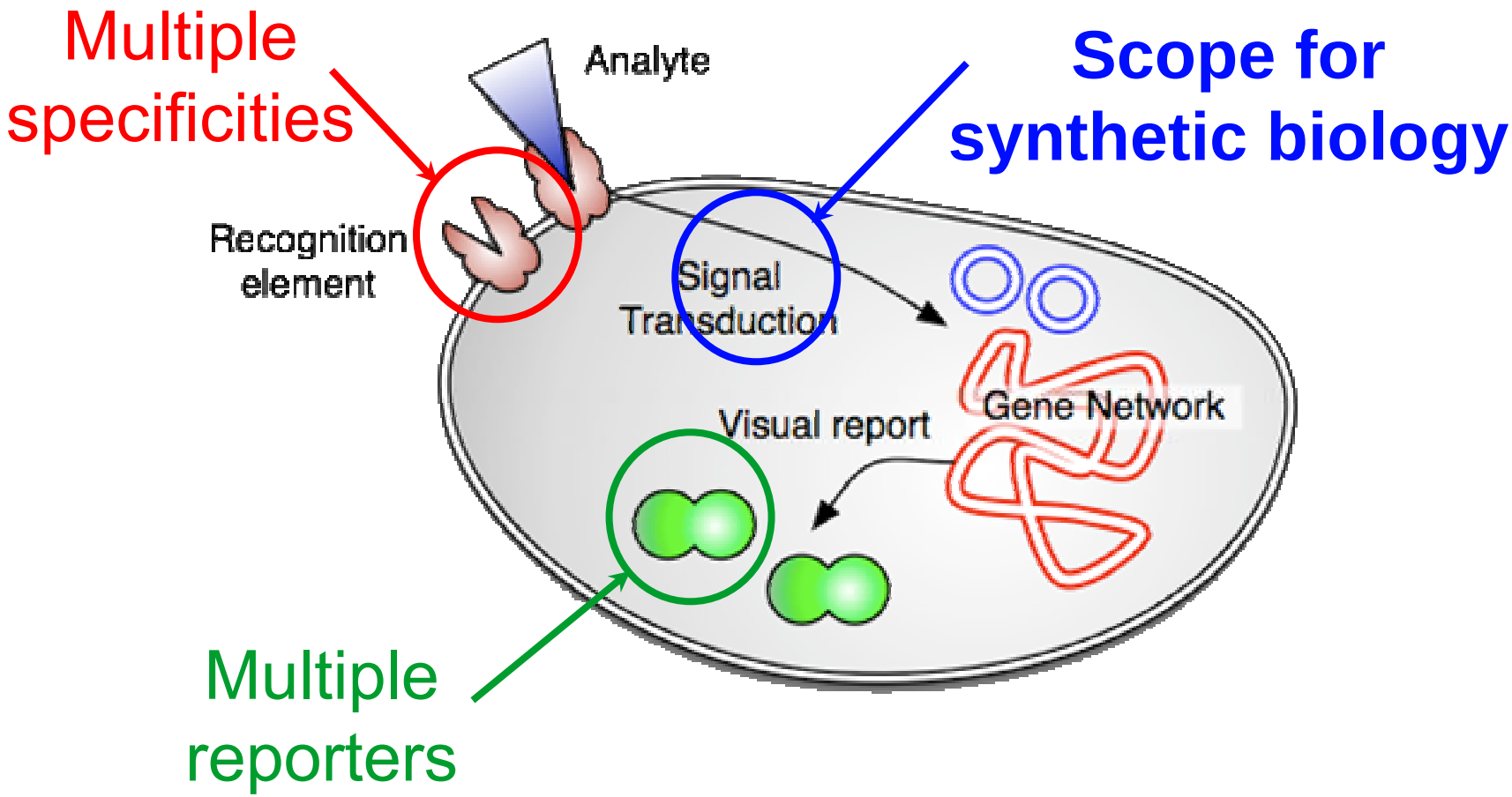
Outline of discussion

- Brief overview of the positive feedback motif
- Applications:
  - Intracellular amplifiers for whole cell biosensors
  - Increasing protein production for pharmacological or research needs
- Programmable biotechnology?

The concept of feedback networks is applicable in any area where gene regulation control is critical

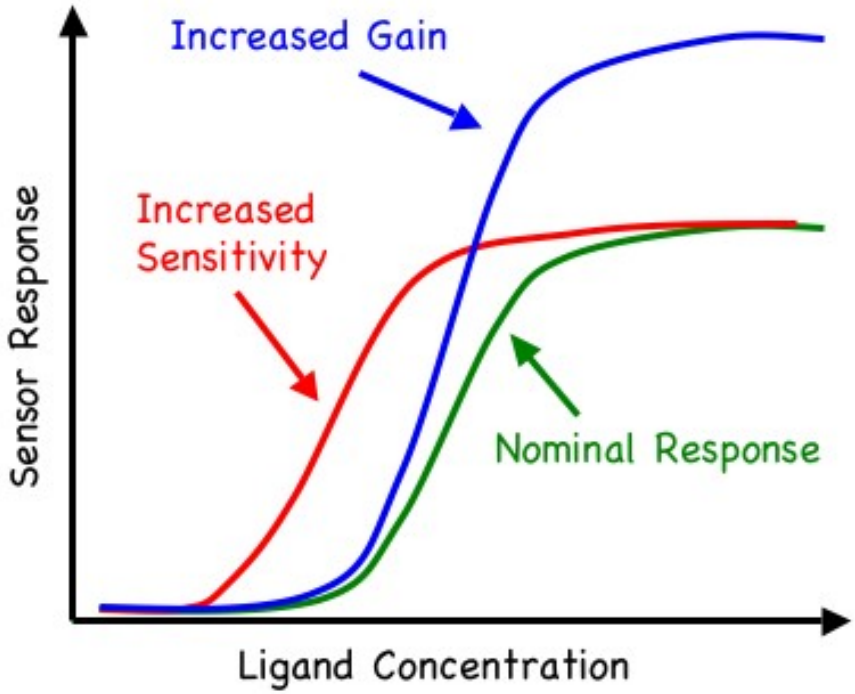


Whole Cell Biosensors (WCBs): Cells engineered to detect and report environmental conditions of interest



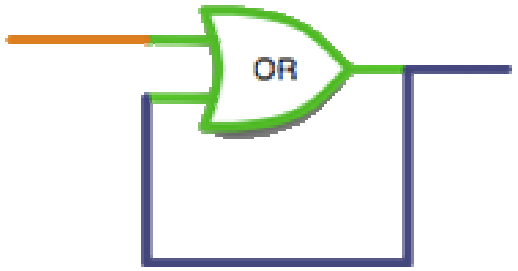
Synthetic gene networks integrated with WCBs provide a novel way of enhancing WCB performance

- By incorporating combinations of positive and negative feedback loops in the gene expression networks it is possible to modulate the device behavior
- Positive feedback leeds to improved sensitivity and “memory-like” behavior
- Negative feedback can change the response threshold

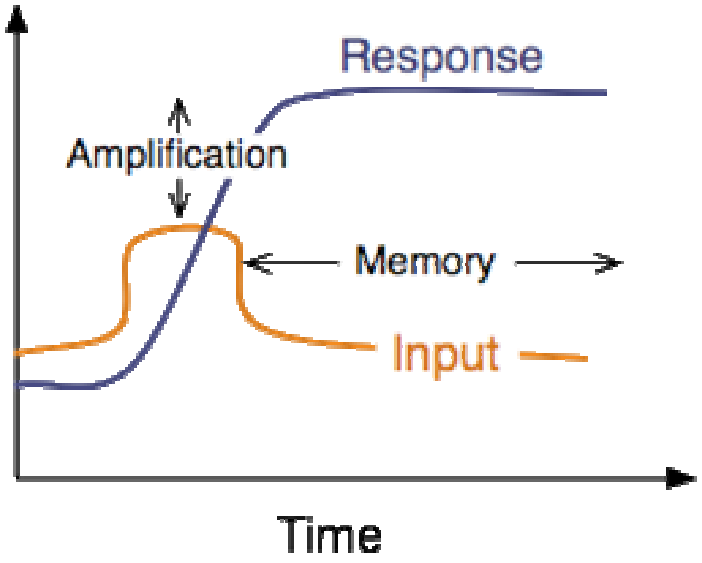


The positive feedback motif leads to a “one-shot” switching behavior

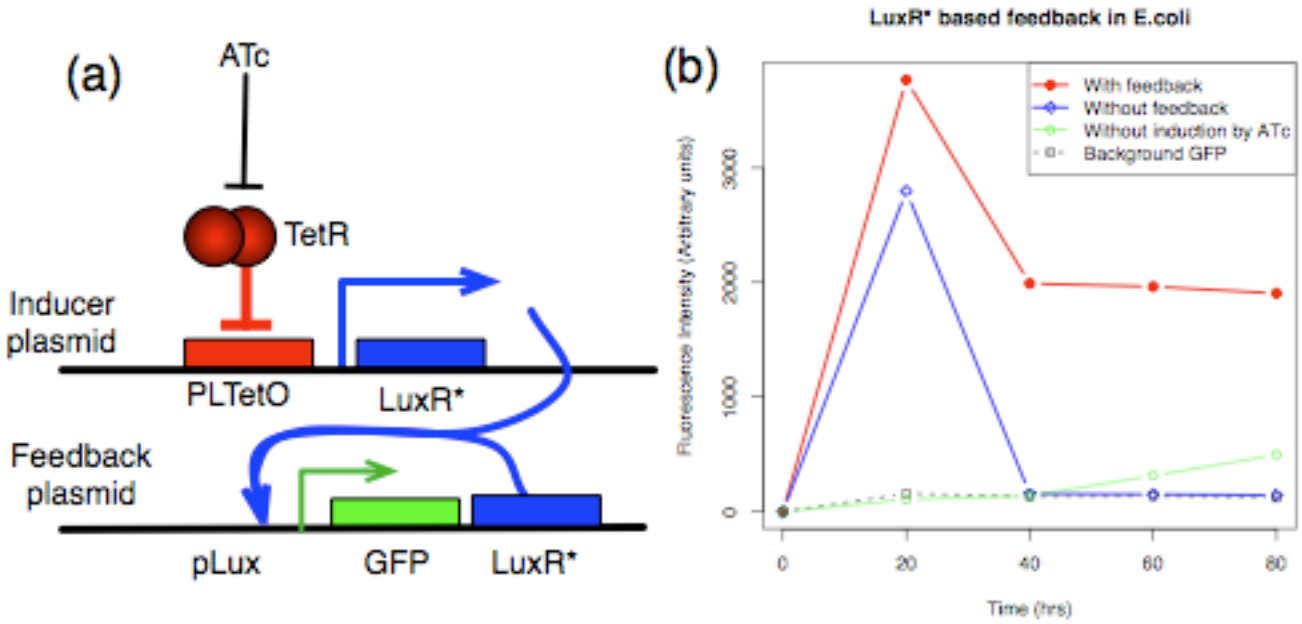
- A transient derepression of a promoter leads to a sustained and stable expression of a gene under its control
- Two consequences of the switching behavior:
  - Increased levels of expression of the gene (**amplification**)
  - A sustained level of expression long after the stimulus (transient derepression) is gone (**memory**)



Positive feedback

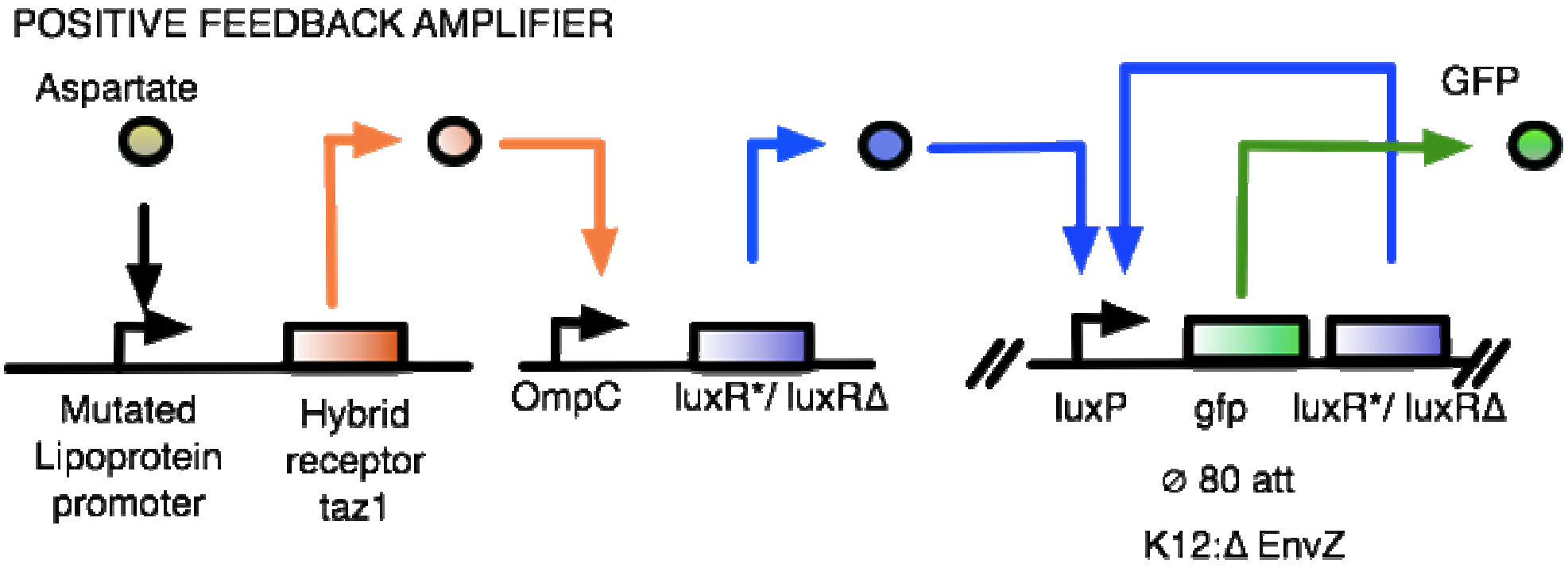


Initial experiments have conclusively demonstrated the memory like effect of positive feedback

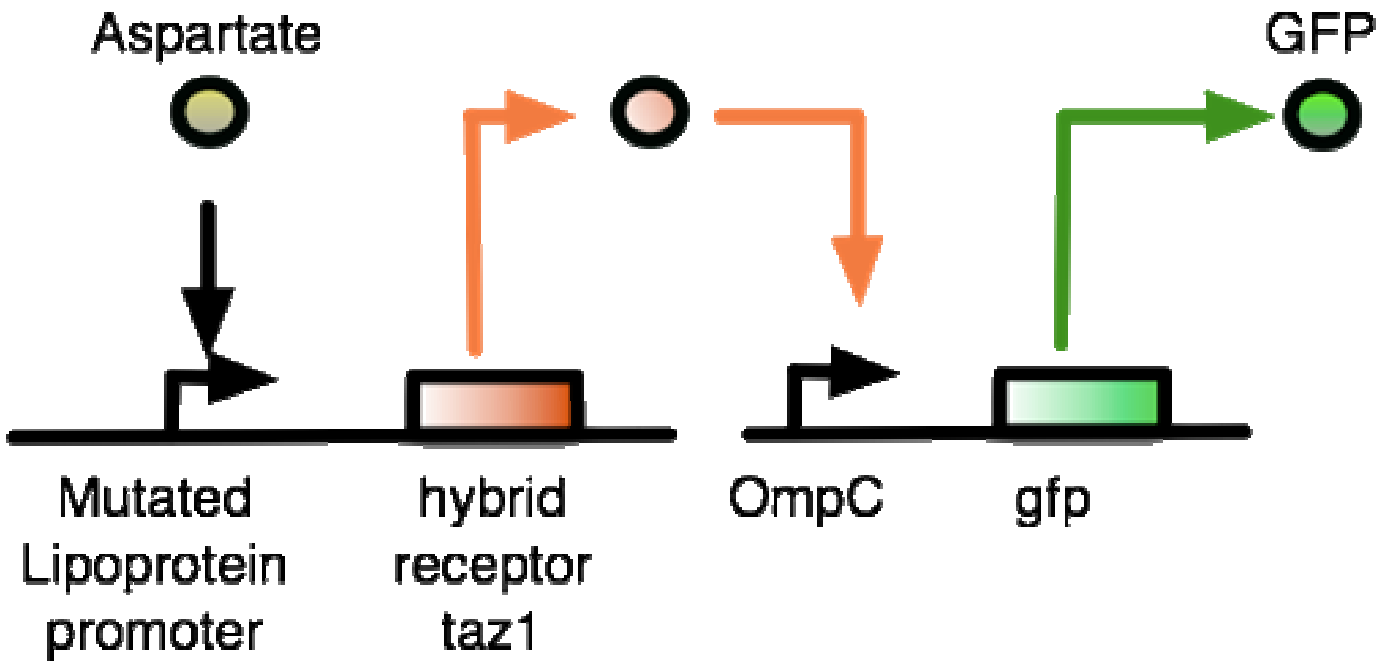


- The “feedback plasmid” sets up a positive feedback the allows a persistent signal (red line) hours after the initial detection of the analyte

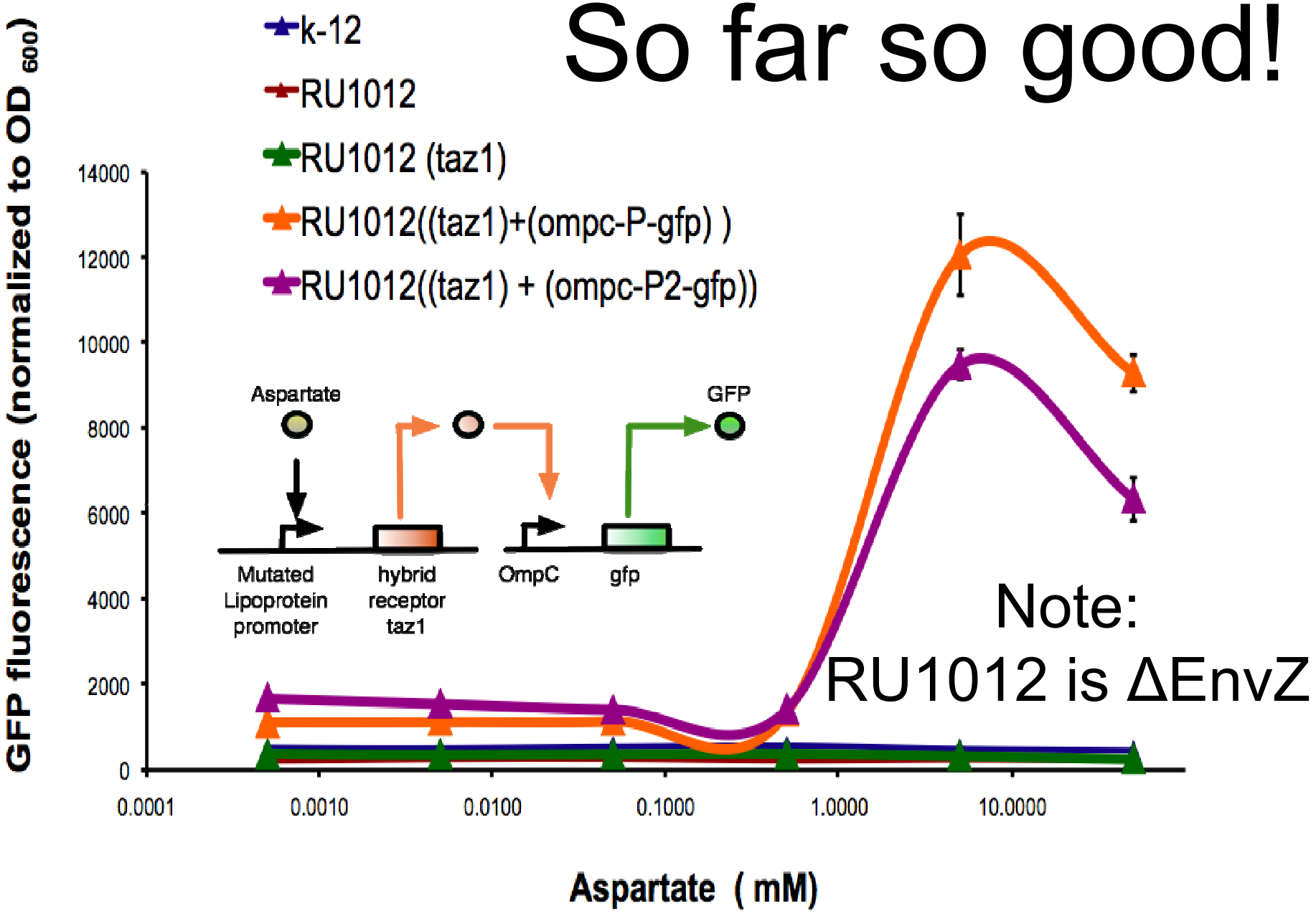
An amplified aspartate biosensor



Testing aspartate recognition subsystem

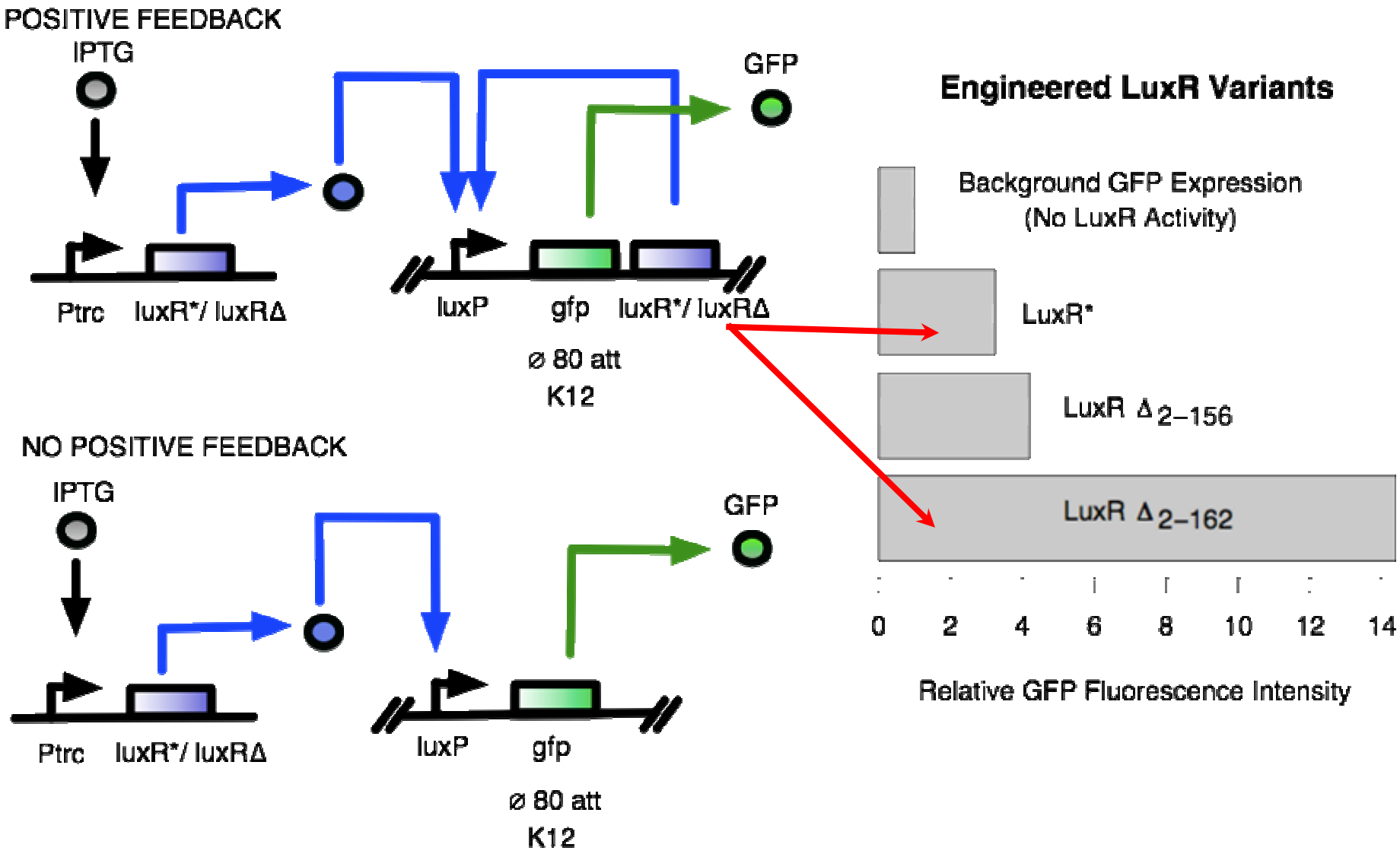


So far so good!

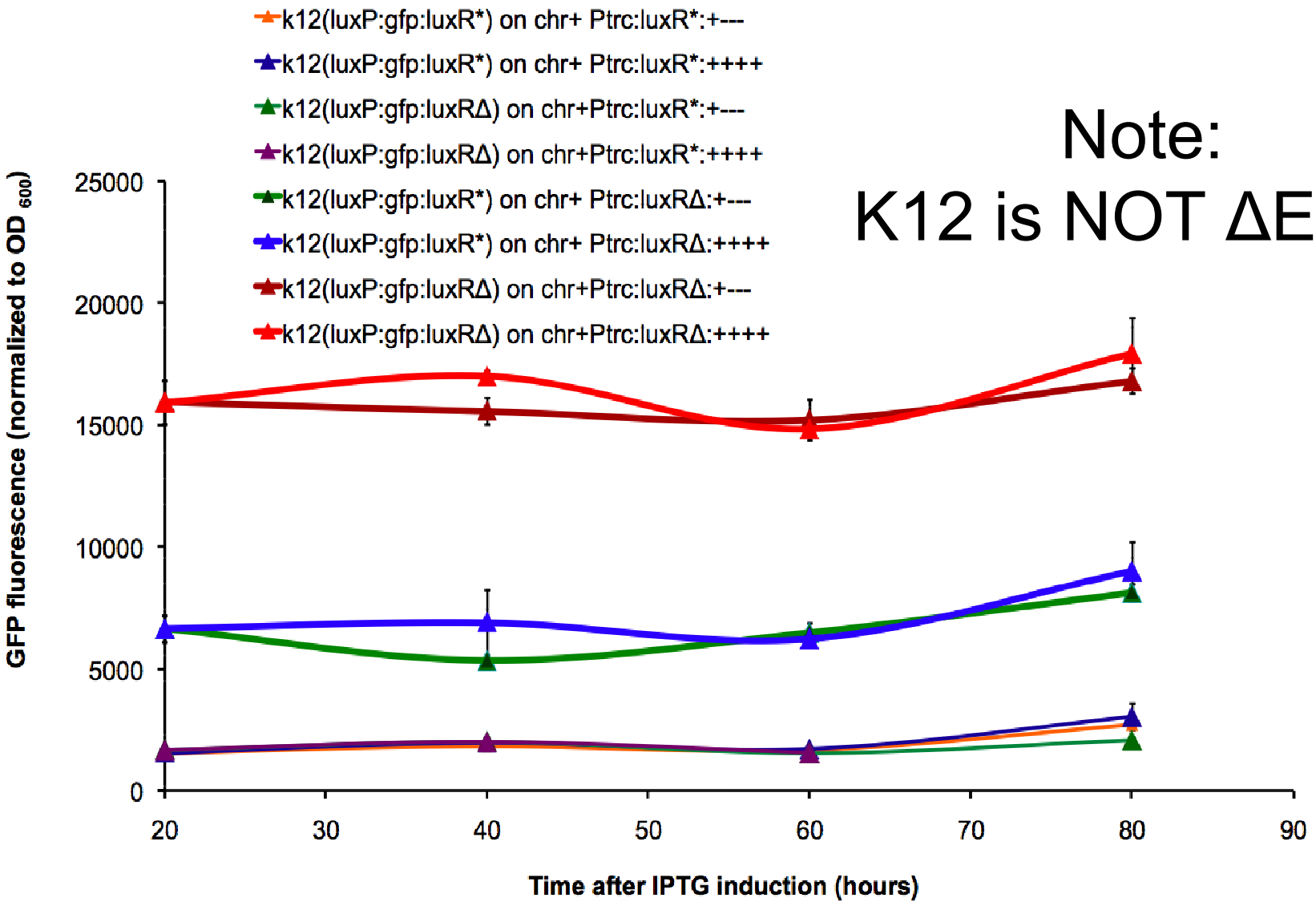


Note:
RU1012 is ΔEnvZ

Testing feedback on chromosome



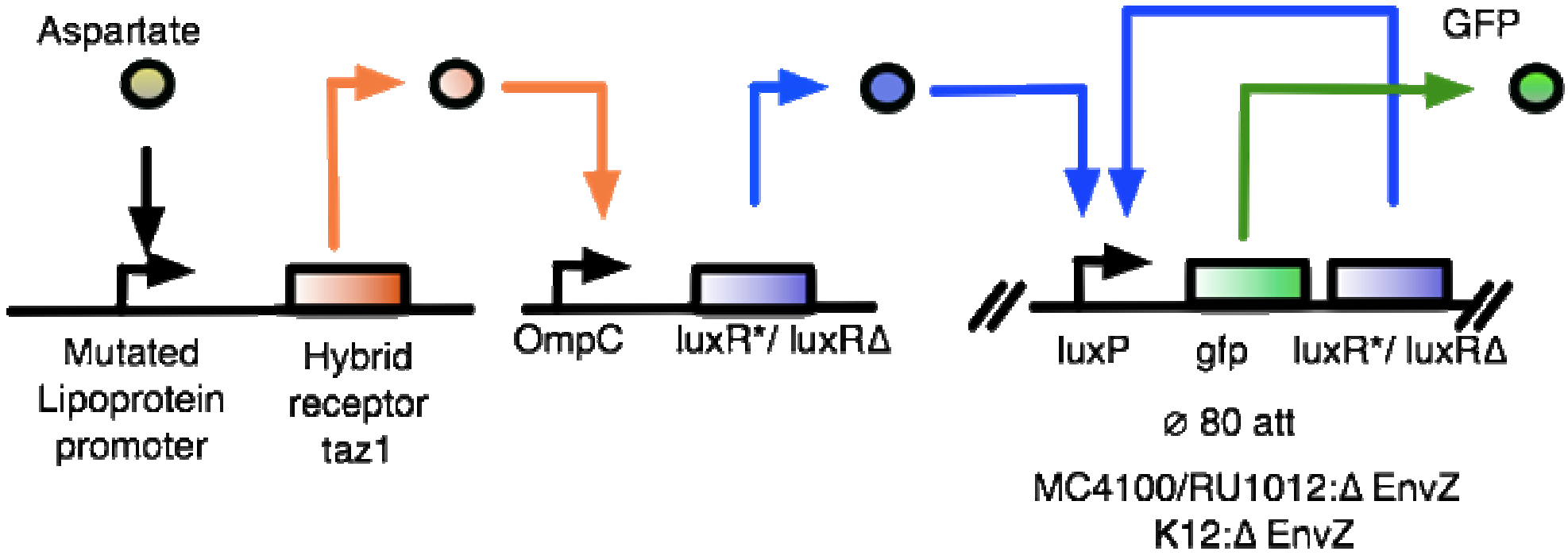
Still looking good



Note:
K12 is NOT ΔEnvZ

Trouble putting 2 & 2 together

POSITIVE FEEDBACK



Multiple workarounds

- Knock out EnvZ from the working K12 integrated system
- Retry MC4100 / RU1012 integrations
- Purchase another K12 derivative with EnvZ knocked out
- **We'll be pipetting until something works!**

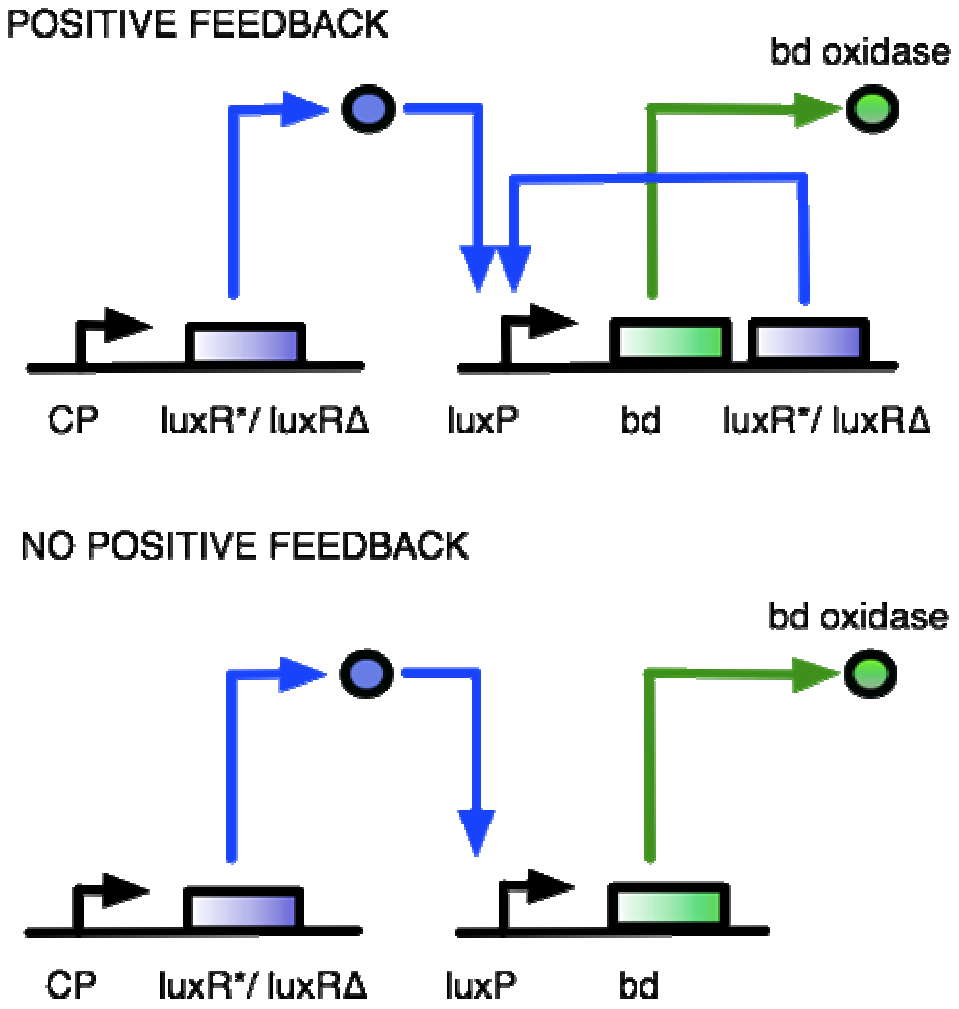
Increased protein production using feedback

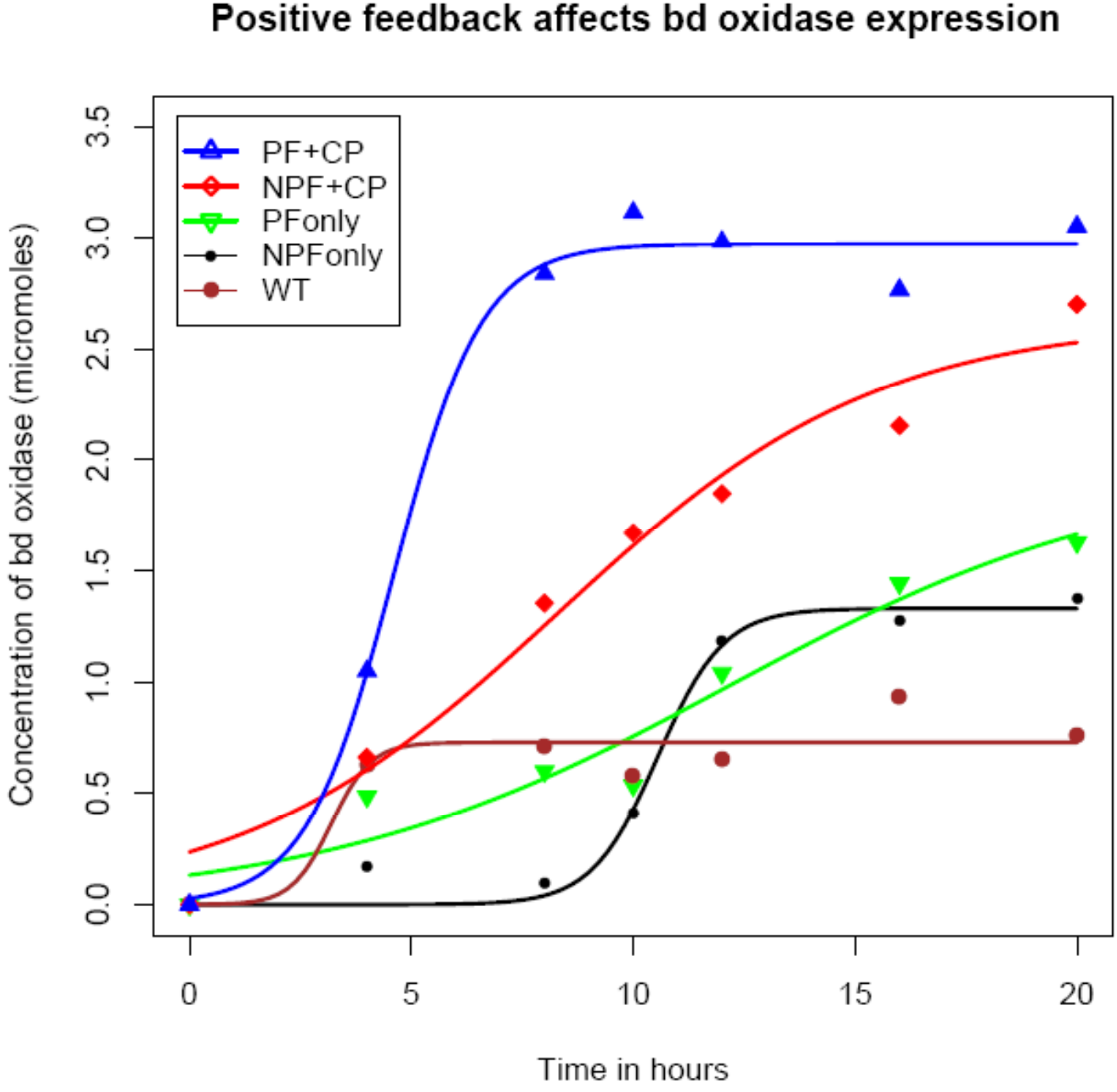
- Single subunit Membrane protein on *E. coli*
- Contains a Cu heme group.
- Terminal oxidase: Interesting from an energetics perspective
- Upregulated during anaerobic conditions
- Producing large quantities for crystallography is expensive (4L cultures for microliters of impure membrane protein)
- Extraction and measurement of bd oxidase is painful

Can we increase protein production efficiency

- Produce protein quicker (Increased rates of production)
- Produce more protein in the same time (Increased final level)
- Produce more protein in the same culture volume. (Smaller / fewer shaker tables)

A simple circuit in a bd-knockout strain




Positive feedback affects bd oxidase expression
PF+CP
NPF+CP
PFonly
NPFonly
WT
Concentration of bd oxidase (micromoles)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
0
5
10
15
20
Time in hours

Teamwork rocks!

- Prof. Chris Rao (Chemical and Biomolecular Engineering)
- Prof. Robert Gennis (Chemistry)
- Kang Wu (Rao group)
- Dr. Jim Hemp and Ke Yang (Gennis group)
- Goutam Nistala and Karan Bansal (Bhalerao group)

Thanks!
Questions and
Feedback?