# Swiss++ ETH iGEM team

# Teaching cells how to add numbers

ETH Zürich

iGEM 2006

# Who are we and where are we from?


Image © 2006 NASA
Image © 2006 TerraMetrics

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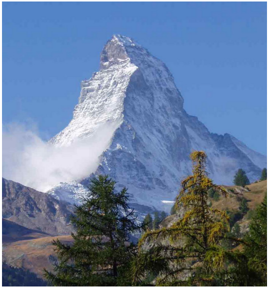
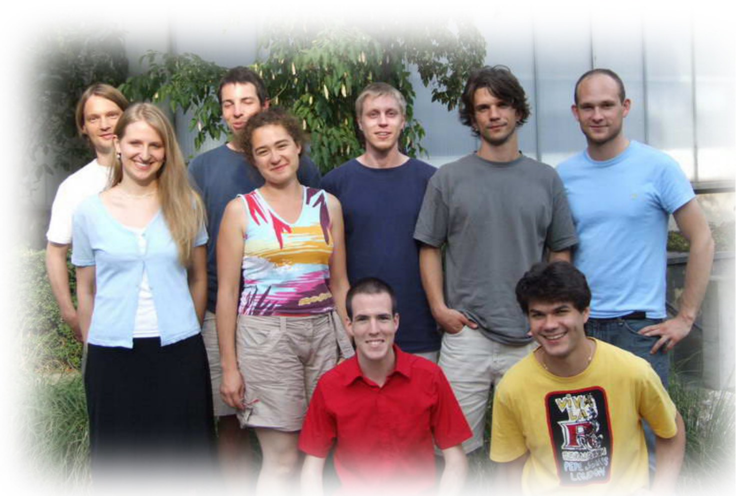


# The ETH Zurich iGEM team 2006



# Contents

1. Applications of our System
2. Model based design – the XOR gate
3. Biological Implementation of the gates
4. Experiments and Results

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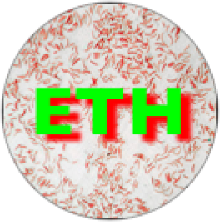
# Direct application of our system

perfect match

less congruent



artistic

# Adding numbers

$$4 + 18 = ?$$

# Adding numbers

$$4 + 18 = ?$$

# One decimal position after the other

|  |  |  |
|---|---|---|
|  | 0 | 4 |
| + | 1 | 8 |
| Carry |  |  |
| Sum |  |  |

# One decimal position after the other

| | | |
|---|---|---|
| | 0 | 4 |
| + | 1 | 8 |
| Carry | | |
| Sum | | |

# One decimal position after the other

| | | |
|---|---|---|
| | 0 | 4 |
| + | 1 | 8 |
| Carry | 1 | |
| Sum | | 2 |

# One decimal position after the other

$$
\begin{array}{rcc}
 & 0 & 4 \\
+ & 1 & 8 \\
\hline
\text{Carry} & 1 & \\
\text{Sum} & & 2 \\
\hline\hline
\end{array}
$$

# One decimal position after the other

| | | |
|---|---|---|
| | 0 | 4 |
| + | 1 | 8 |
| Carry | 1 | |
| Sum | 2 | 2 |

# One decimal position after the other

$$
\begin{array}{rcc}
 & 0 & 4 \\
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\text{Carry} & 1 & \\
\text{Sum} & 2 & 2 \\
\hline\hline
\end{array}
$$

# Same principle for binary addition

$$\begin{array}{rcc} & 0 & 1 \\ + & 1 & 1 \\ \hline \text{Carry} & & \\ \text{Sum} & & \\ \hline\hline \end{array}$$

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|---|---|---|
| | 0 | 1 |
| + | 1 | 1 |
| Carry | 1 | |
| Sum | | 0 |

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\text{Carry} & 1 & \\
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\hline\hline
\end{array}$$

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# Same principle for binary addition

$$\begin{array}{rccc}
 & & 0 & 1 \\
+ & & 1 & 1 \\
\hline
\text{Carry} & \color{blue}{1} & 1 & \\
\text{Sum} & & 0 & 0 \\
\hline\hline
\end{array}$$

# Same principle for binary addition

$$\begin{array}{rccc} & & 0 & 1 \\ + & & 1 & 1 \\ \hline \text{Carry} & 1 & 1 & \\ \text{Sum} & 1 & 0 & 0 \\ \hline\hline \end{array}$$

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# Logic used for calculations in binary

| A | B | Carry | Sum |
|---|---|---|---|
| 0 | 0 | 0 | 0 |

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|---|---|---|---|
| 0 | 0 | 0 | 0 |
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| 1 | 0 | 0 | 1 |
| 1 | 1 | 1 | 0 |

| A | B | XOR |
|---|---|---|
| 0 | 0 | 0 |
| 0 | 1 | 1 |
| 1 | 0 | 1 |
| 1 | 1 | 0 |

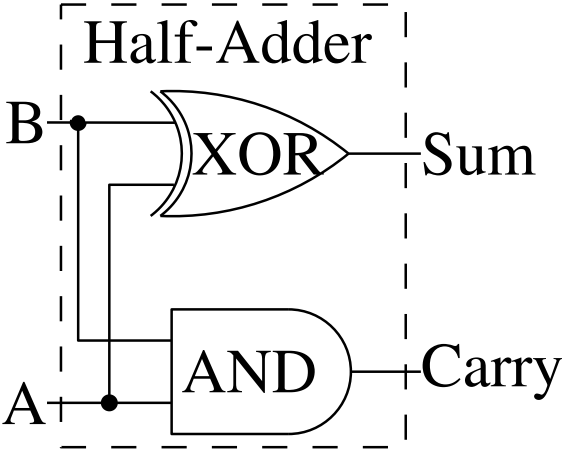
# Logic used for calculations in binary

| A | B | Carry | Sum |
|---|---|---|---|
| 0 | 0 | 0 | 0 |
| 0 | 1 | 0 | 1 |
| 1 | 0 | 0 | 1 |
| 1 | 1 | 1 | 0 |

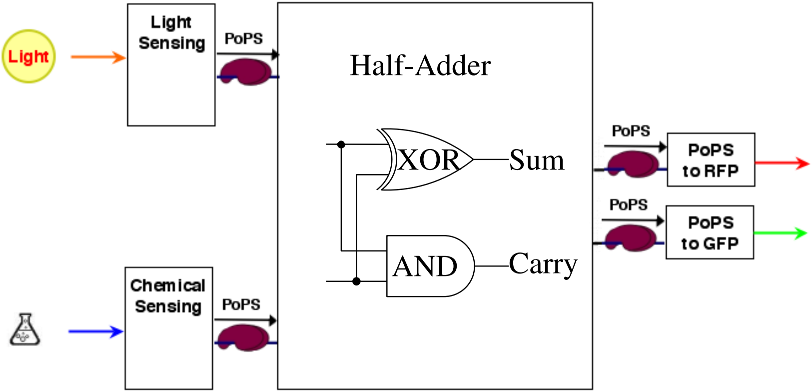
| A | B | AND |
|---|---|---|
| 0 | 0 | 0 |
| 0 | 1 | 0 |
| 1 | 0 | 0 |
| 1 | 1 | 1 |

| A | B | XOR |
|---|---|---|
| 0 | 0 | 0 |
| 0 | 1 | 1 |
| 1 | 0 | 1 |
| 1 | 1 | 0 |

# Logic used for calculations in binary

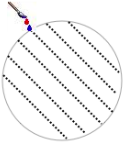


# Building a system around the half-adder



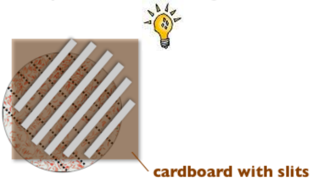
# Building a system around the half-adder

1. put chemical to plate

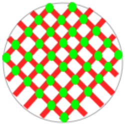


2. let bacteria grow uniformly

3. expose them to light



4. expected result



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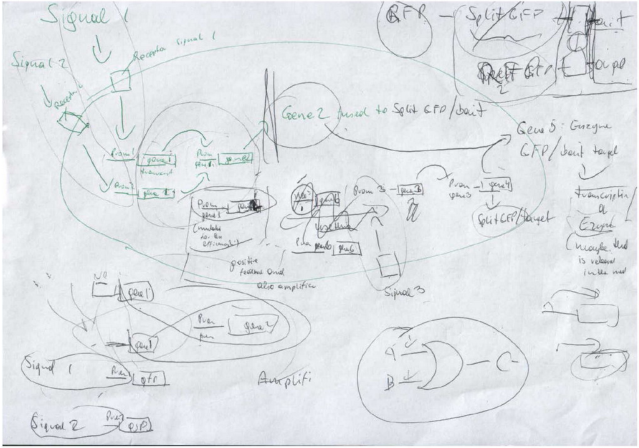
# XOR concepts



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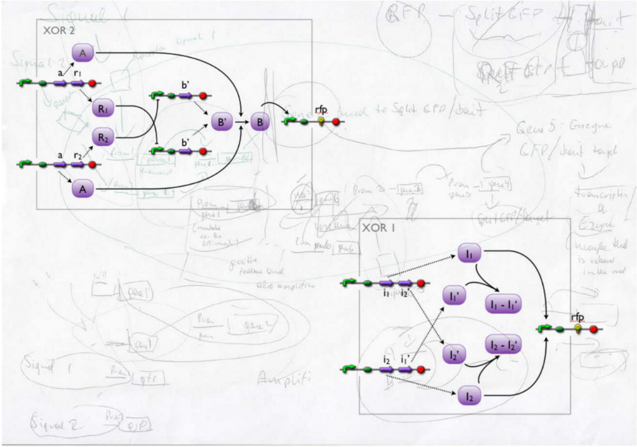

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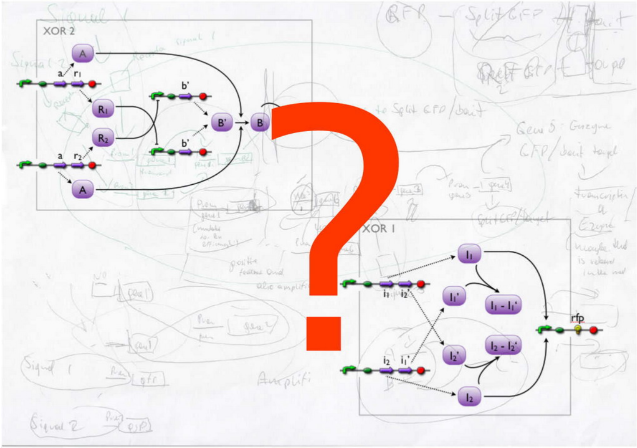

# Requirements

1. Biologically feasible
2. Appropriate steady-state behavior
3. Robust to uncertainty
4. Appealing

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→ estimated using a model

# Dynamic system model

$$\frac{\partial \vec{c}(t)}{\partial t} = \vec{f}(\cdot)$$

$\vec{c}(t)$: Concentrations

# Dynamic system model

$$\frac{\partial \vec{c}(t)}{\partial t} = N \cdot \vec{r}(\vec{c}(t), \vec{u}(t), \vec{p}, t)$$

| | |
|---|---|
| $\vec{c}(t)$: | Concentrations |
| $N$: | Stoichiometric matrix |
| $\vec{r}(\cdot)$: | Reaction rates (kinetic rate law) |
| $\vec{u}(t)$: | Inputs / external influences |
| $\vec{p}$: | Parameters (kinetic constants, ...) |

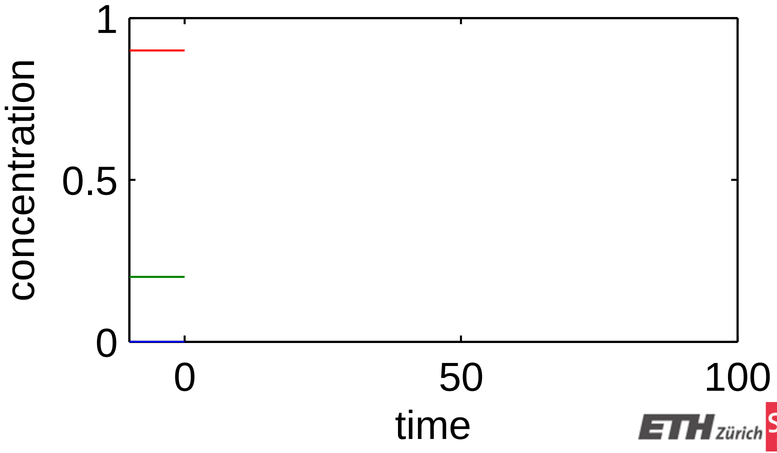
# Dynamic system simulation

No closed-form solutions

# Dynamic system simulation

No closed-form solutions
→ numeric integration

# Steady-state



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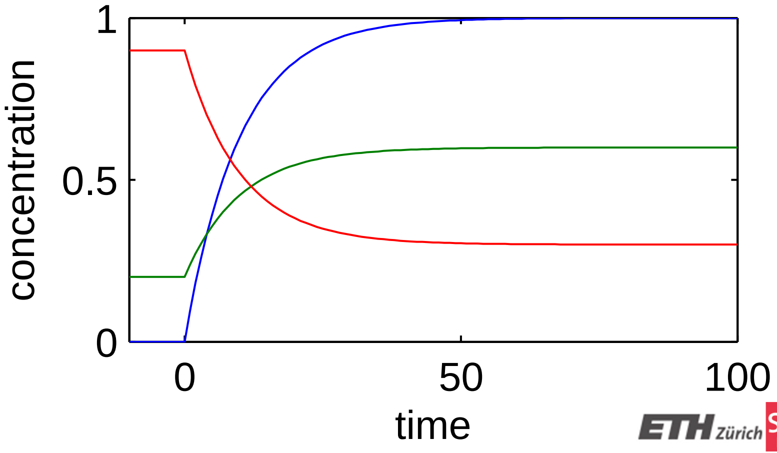

# Steady-state

Using steady-state as output because:

- **convenient** to measure: system remains there
- **robust** to disturbances
- **exists** in most practical systems

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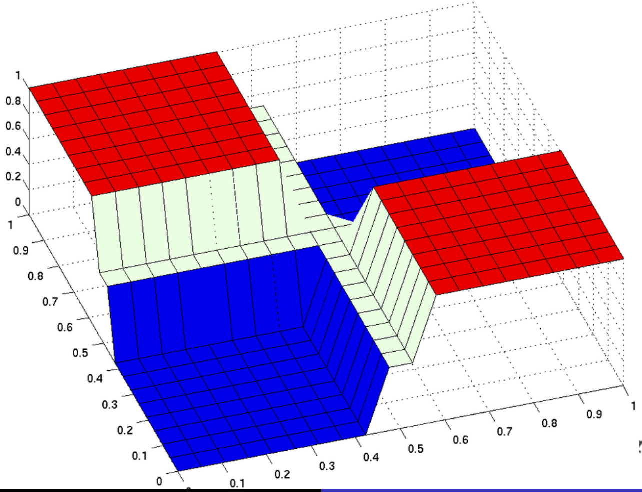
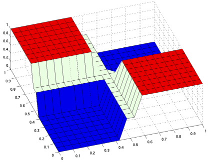
# Steady-state behavior of a good XOR



# Simulated steady-state behavior of the concepts



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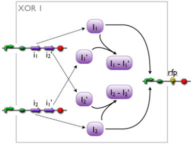

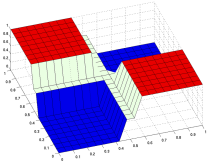

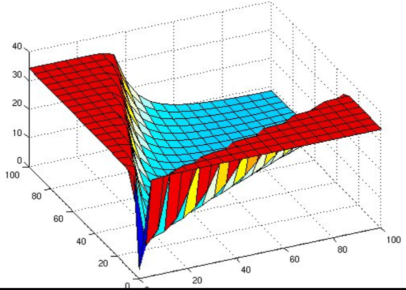

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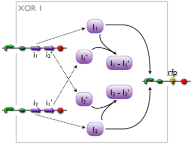

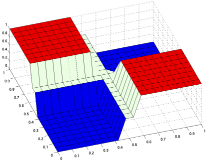

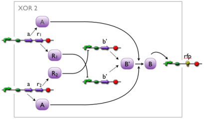

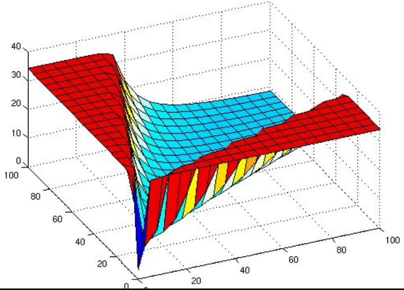

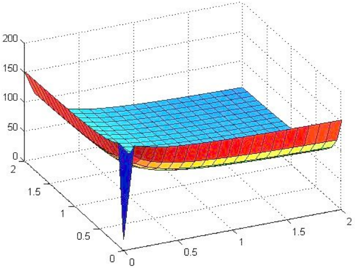

# Steady-state sensitivity

## Steady-state sensitivity

- measures influence of parameter on steady-state → robustness
- algebraically derivable from model

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# Steady-state sensitivity

Sensitivity = 2

# Steady-state sensitivity

Sensitivity = 2

Parameter change by 1%

# Steady-state sensitivity

Sensitivity = 2

Parameter change by 1%
↓
steady-state changes by 2%

# Steady-state sensitivity

relative parameter change

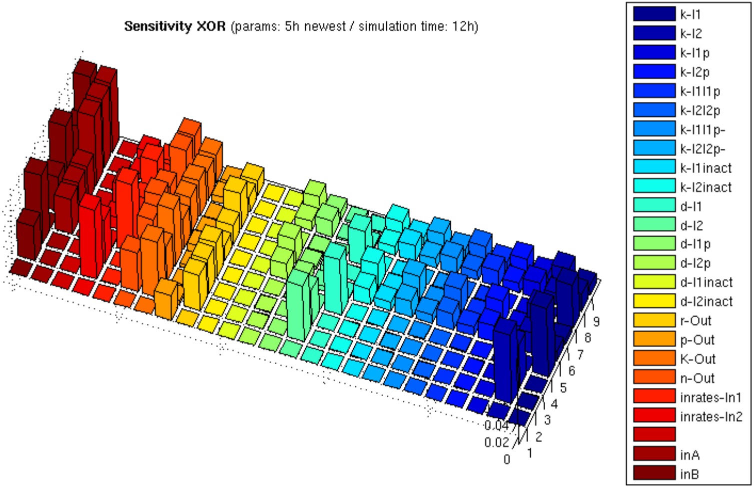
×

sensitivity

=

steady-state change

# Steady-state sensitivity: XOR output



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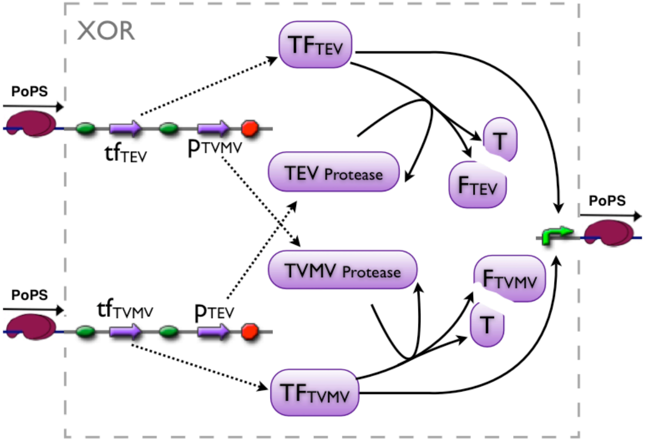
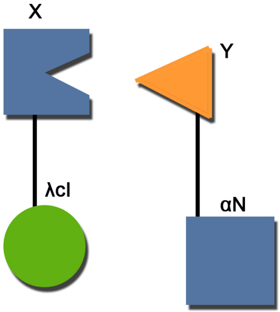
# XOR overall system

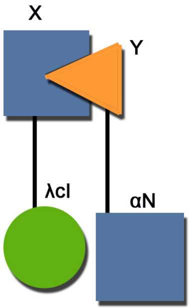


# XOR inspiration



Reference: Dove and Hochschild, 1998

# XOR inspiration



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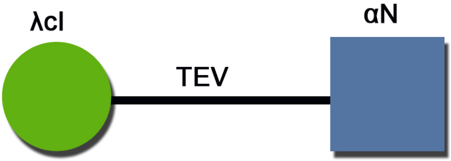
# XOR main elements



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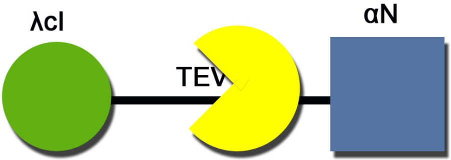

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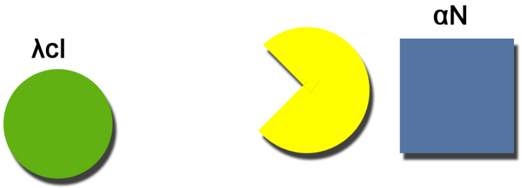

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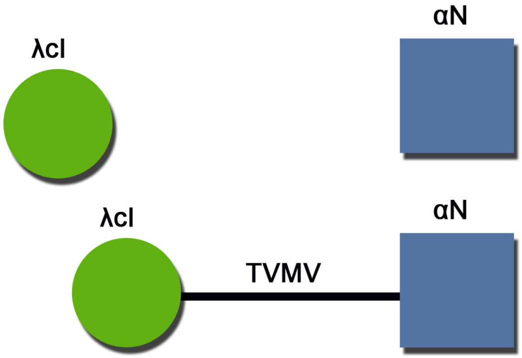

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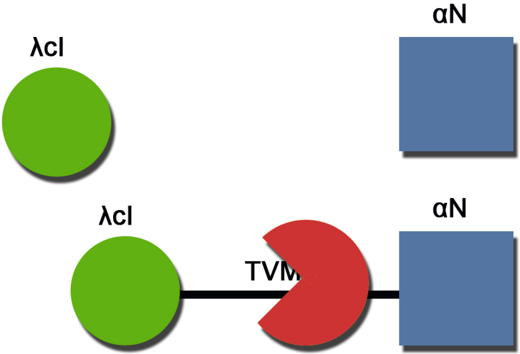

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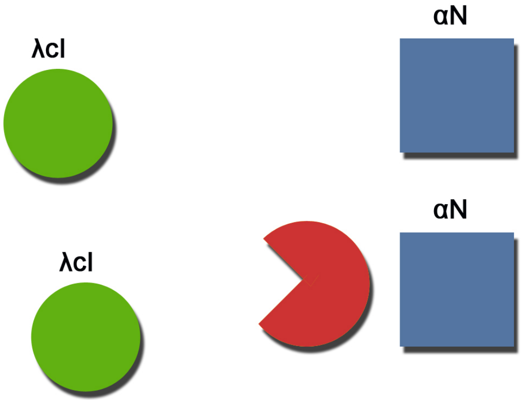

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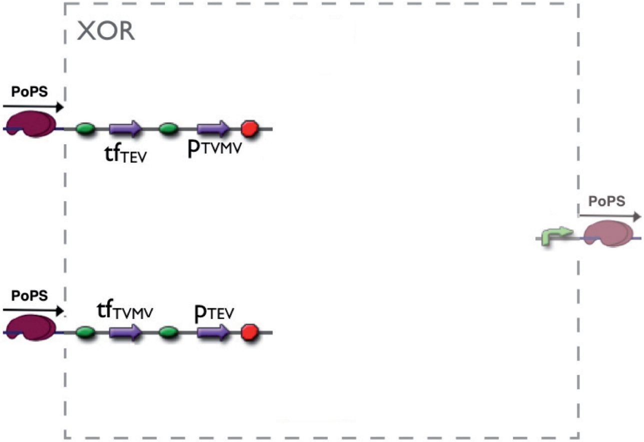

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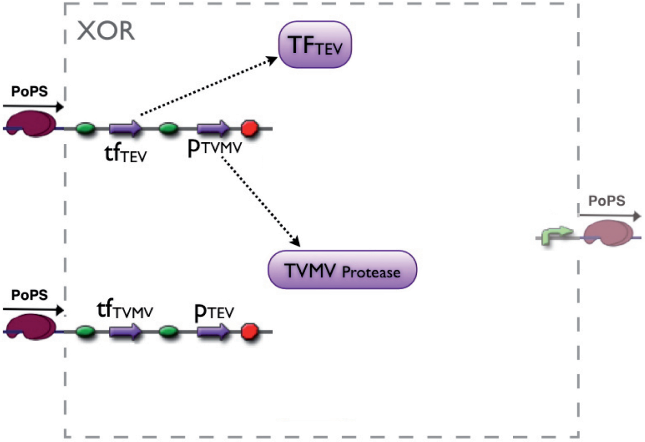

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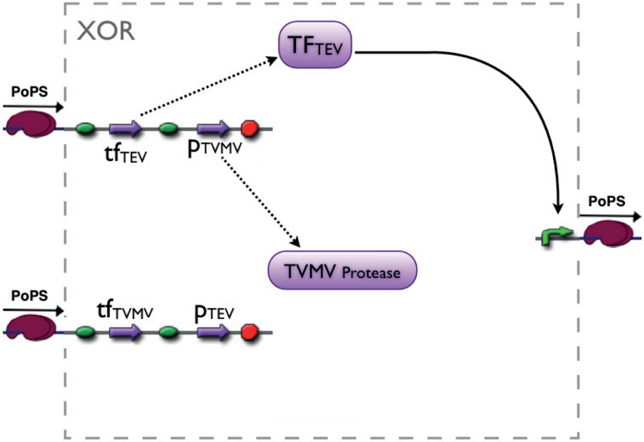

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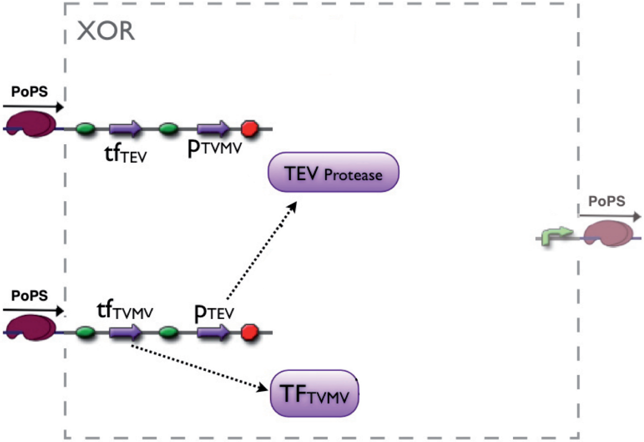

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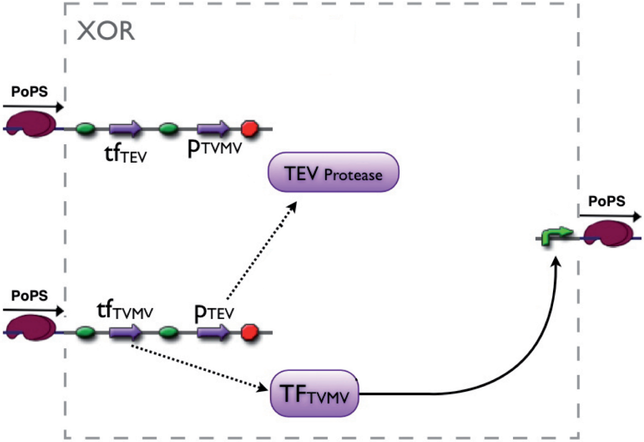

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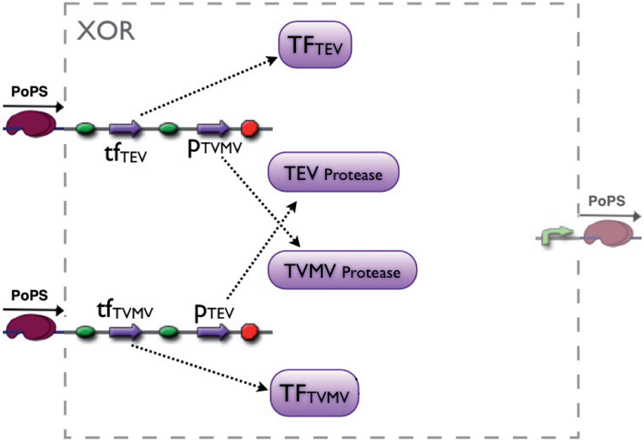

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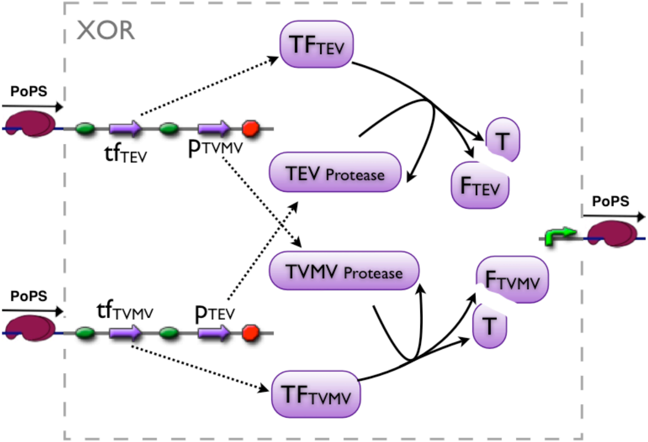

# Design Advantages of the XOR

## The XOR gate

- Very specific proteases, almost no off-target effects
- Active Proteases can be expressed in vivo
- In a functional *TF*, *AD* and *DBD* can be separated by various linkers

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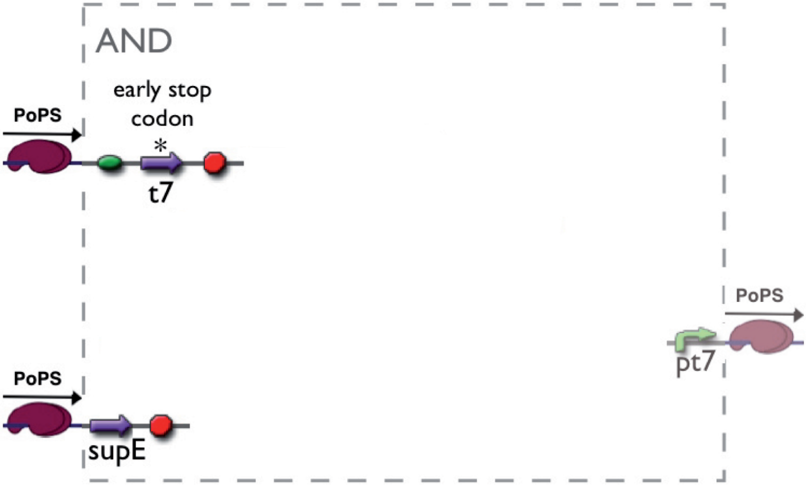

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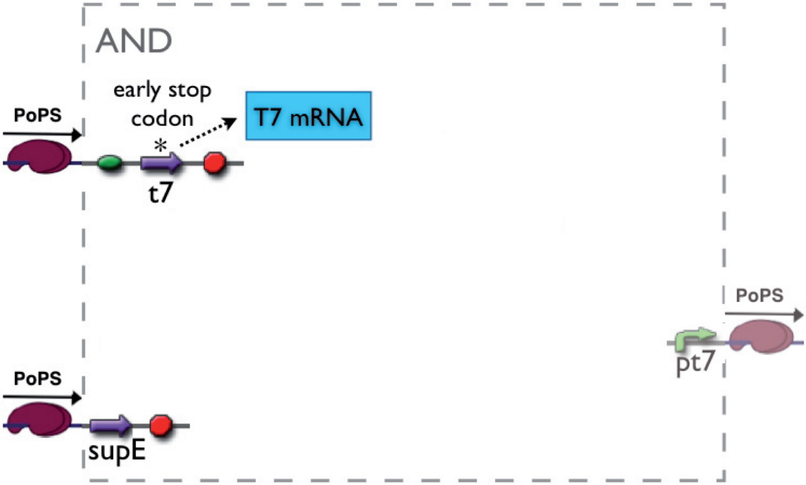

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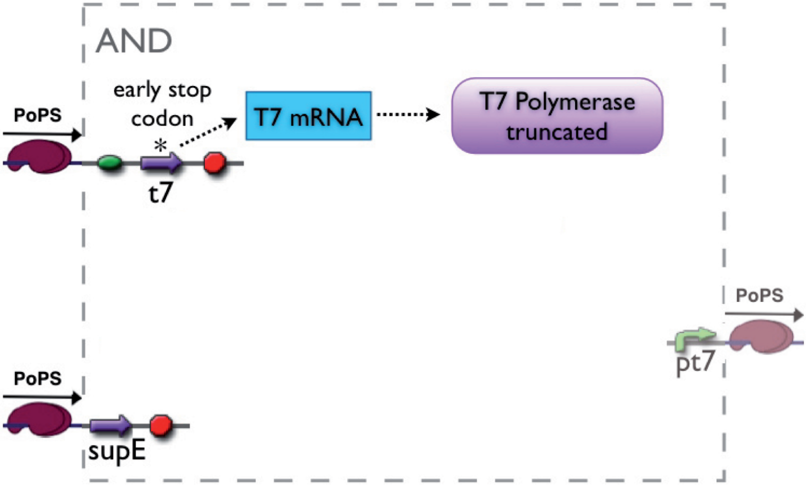

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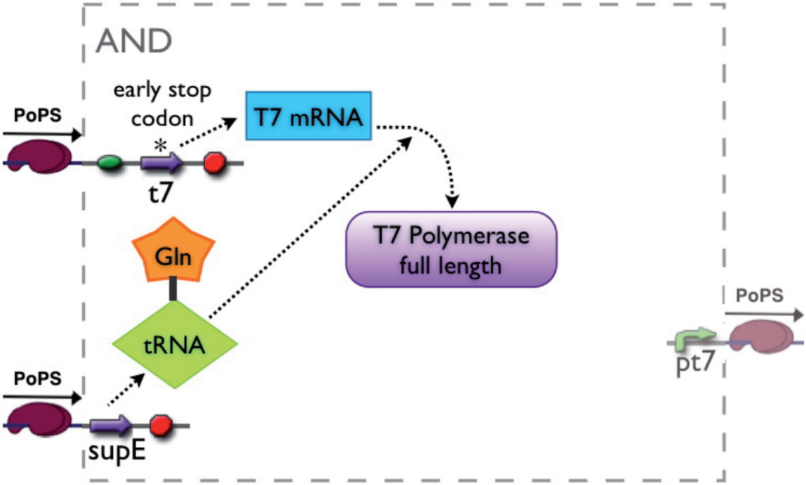

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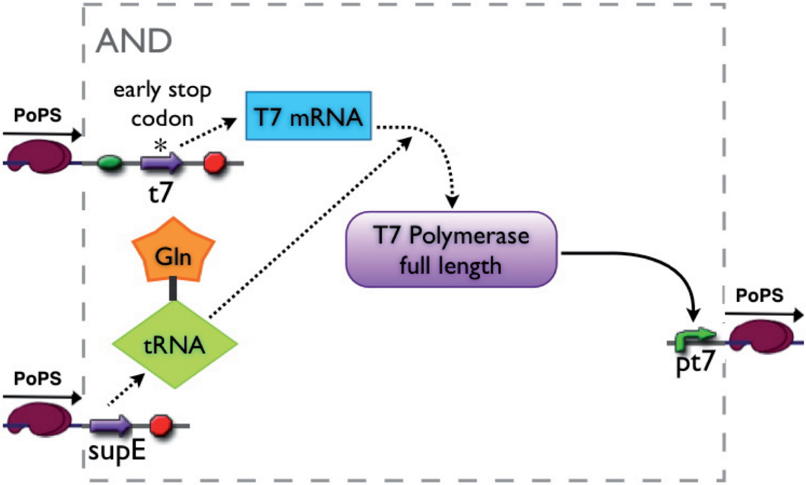

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   - System modeling
   - Steady-state behavior simulation
   - Steady-state sensitivity analysis
3. Biological Implementation of the gates
   - The XOR-gate
   - The AND-gate
4. Experiments and Results
   - Experiments
   - Summary

# Input testing: chemical and light sensing

- Chemical sensing
- Light Sensing - from UCSF group

Light

No light

# Gates Testing

- Test the gates operation via 2 inducible promoters
- Characterize behavior by varying strength and duration of inputs

# Expected results

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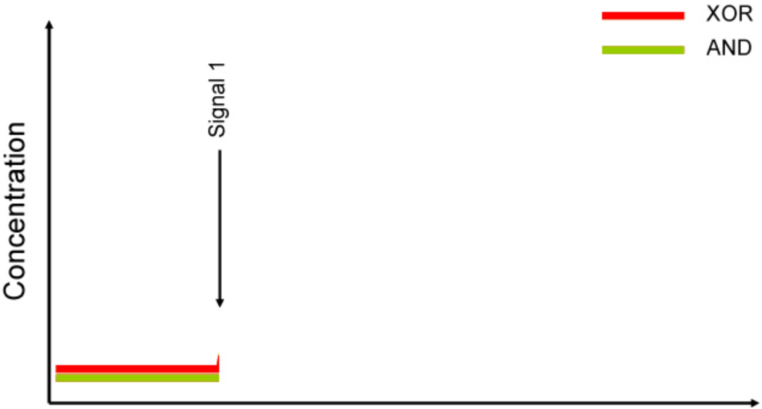
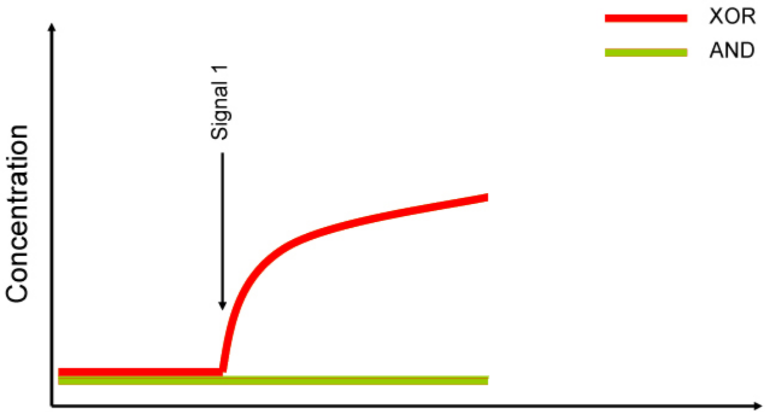
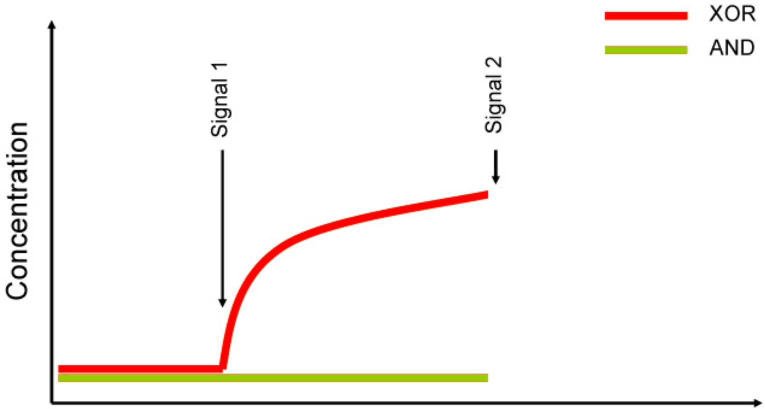


# Expected results



# Expected results



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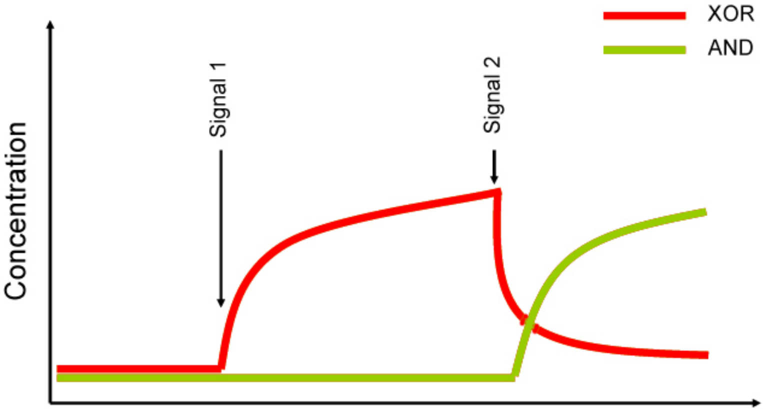

# System status

- Design and order DNA for XOR, AND gates
- Test chemical and light sensing systems
- Clone reporter genes into XOR, AND plasmids
- Combine gate segments: XOR (3), AND (2)
- Clone gates into plasmids and test separately
- Combine sensing and gate parts and test entire system

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# Summary

- Design Half Adder
  - Perform addition in bacteria
  - Pattern recognition
- Logic gates
  - XOR two similar transcription factors with specific proteases
  - AND suppressor tRNA
- Learned a lot and enjoyed the process

# Acknowledgments

**Many thanks to the iGEM organisation team**
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- Lab-people: Giorgia, Alex and Eric
- Ambassadors: Jonas, Tamara and Robin
- Anselm Levskaya and UCSF team

# . . . and thank you

. . . and of course thank YOU for your attention.
Are there any questions?