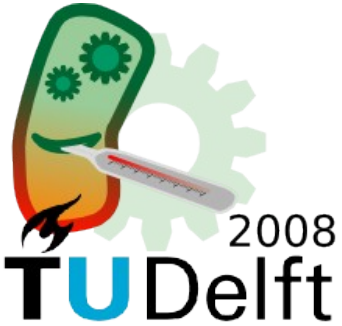

Towards engineering a Biothermometer

Delft University of Technology

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Team and Instructors

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Modeling:
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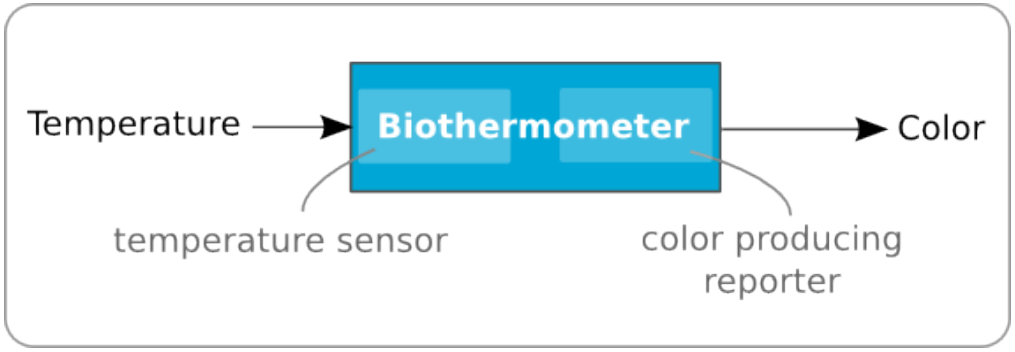
Lab work:
Oscar Stassen
Ruud Jorna

Ethics:
Steven Flipse

Presentation outline

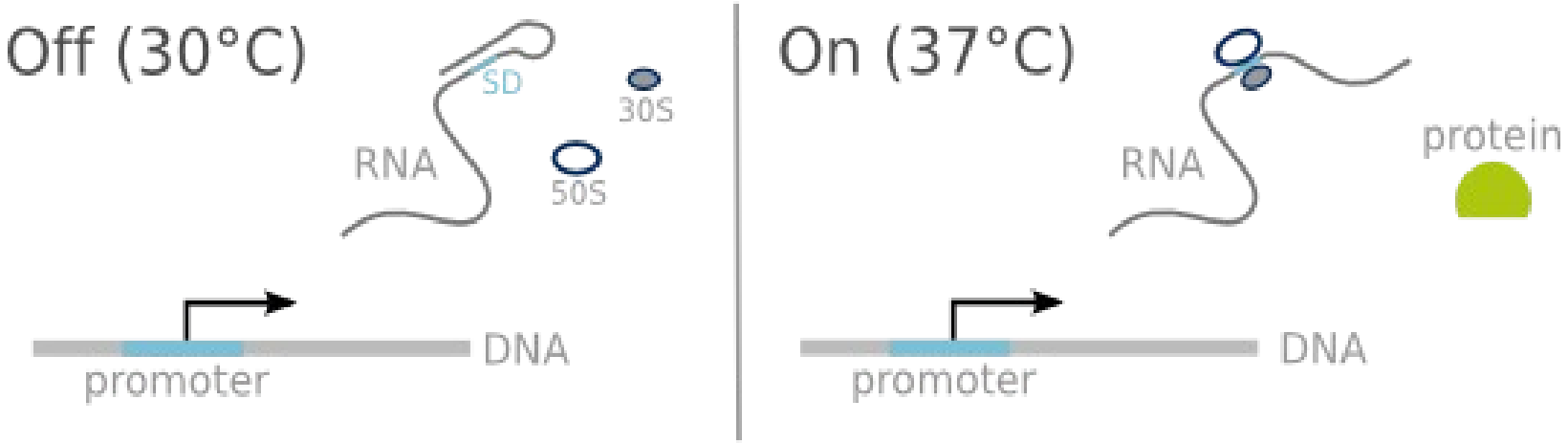
- Background & Basics
- Design
- Laboratory work
- Modeling
- Ethics
- Summary

The biothermometer



- The project is approached as two seperate modules
  - Sensor: RNA thermometer
  - Reporter: isoprenoid biosynthesis pathway
- Potential applications
  - Monitoring temperature during fermentation
  - Temperature inducible protein expression

RNA thermometers



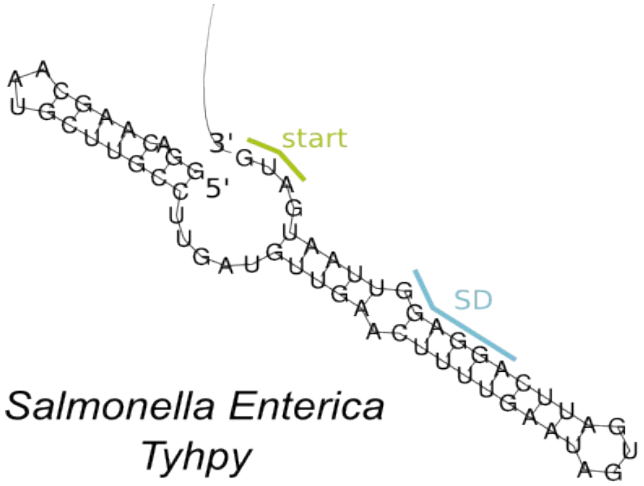
- Functionality
  - Post-transcriptional regulation
  - The hairpin masks ribosome binding site[1]

1. F. Narberhaus, *et al*. 2006.

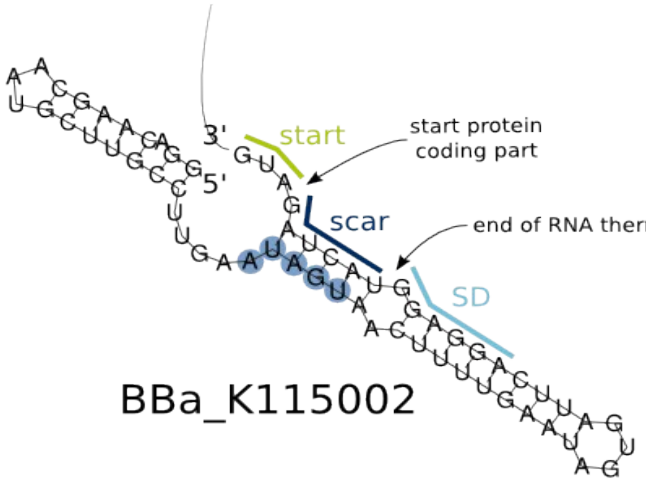
Design

Turning RNA thermometers into BioBricks

- 3 known thermometers[2] turned into BioBricks
- Secondary structure important for functionality
- Adjustment for scar



Salmonella Enterica Tyhpy



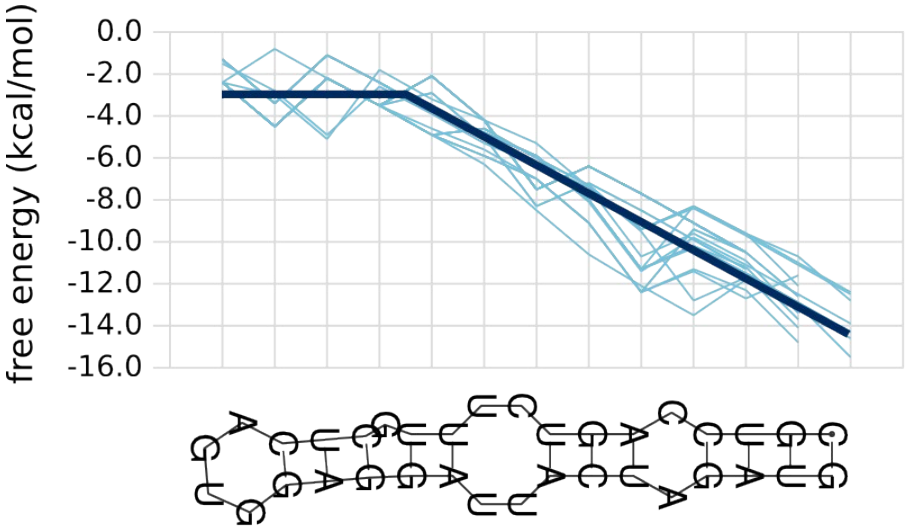
BBa_K115002

2. T. Waldminghaus, *et al.* 2007.

Design

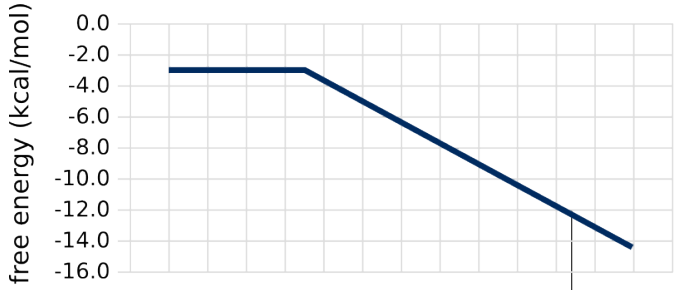
Changing the switching temperature

- Stability profile for 37°C (*RNAfold*)

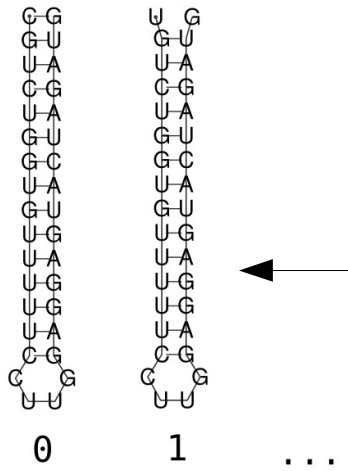


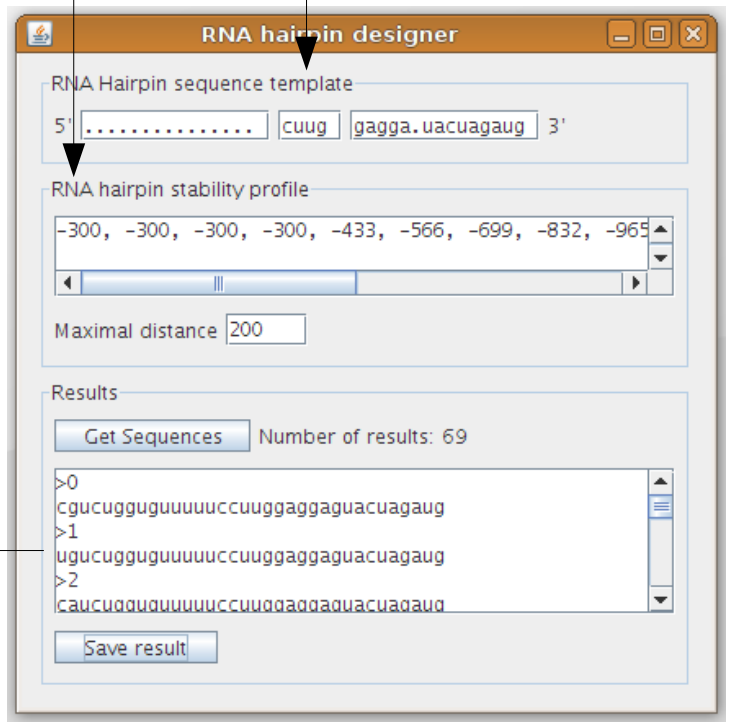
- Similar profile used to design 32°C and 27°C RNA thermometers

Software



- Software tool written
- Produces RNA sequences of hairpin structures with a given stability profile
- Can be used to design RNA thermometers



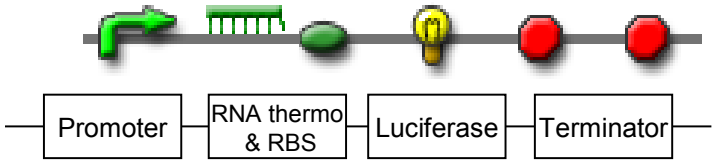


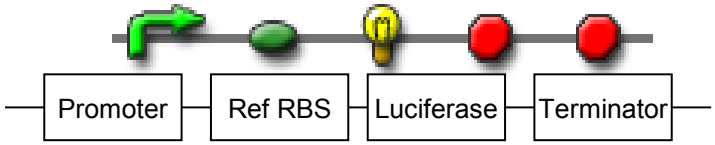
Lab work on RNA thermometers

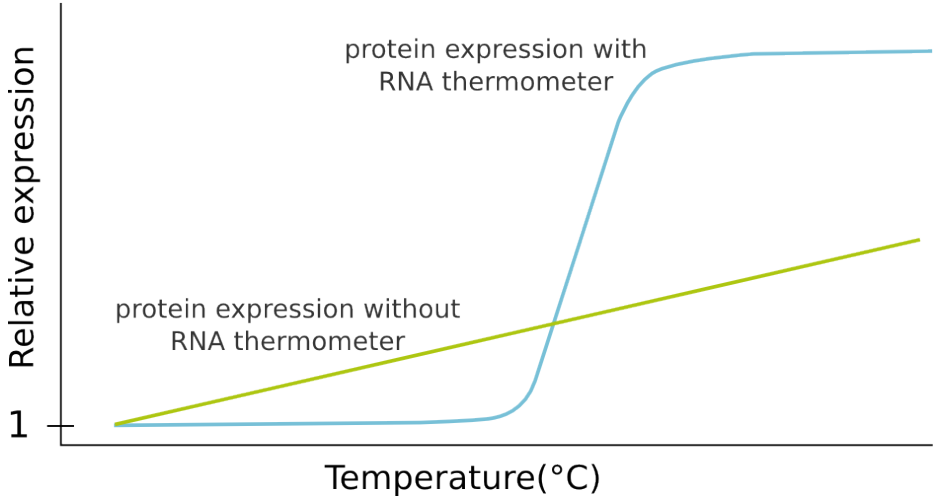
- Assemble BioBricks to make working constructs
- Testing sensitivity
  - Replacing the isoprenoid biosynthesis pathway with luciferase
  - Generate a protocol to measure luciferase (luminescence)
    - Correct luciferase measurements for total protein content

Experimental approach

- Temperature sensitive device (BBa_K115035)
- Reference device (BBa_K115012)







Experimental procedure

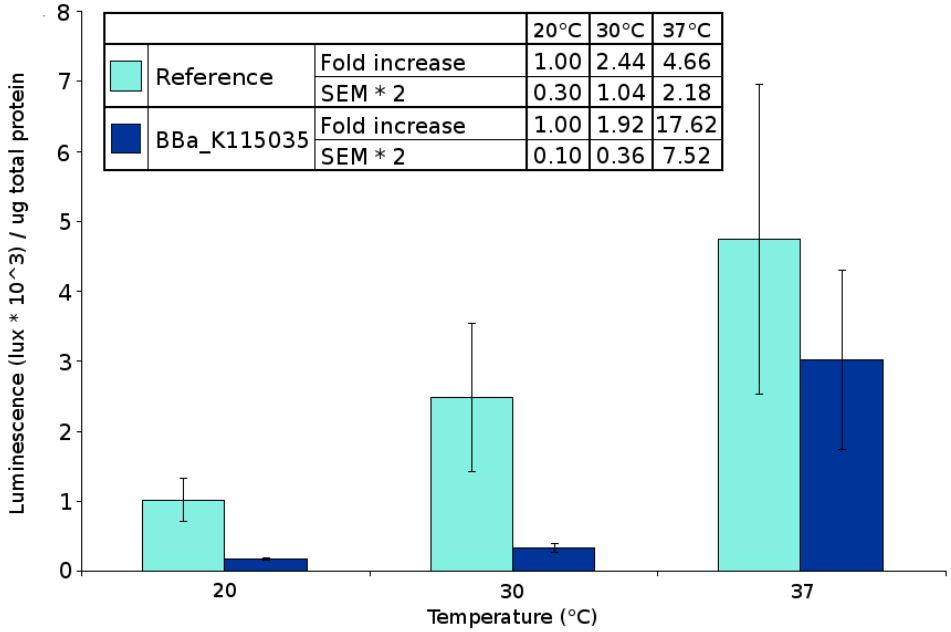
- Grow cultures at different temperatures
- Lyse cells
- Measure
  - total protein content
  - luciferase activity

- Problems in cell lysis
  - Lysis buffer – interferes with protein content
  - Protein precipitation – not reproducible
  - Fastprep – denatures luciferase
  - Beadbeater – not reproducible
  - Sonication – denatures some luciferase but reproducible

Results

- BBa_K115035 is a working device (32°C switch)

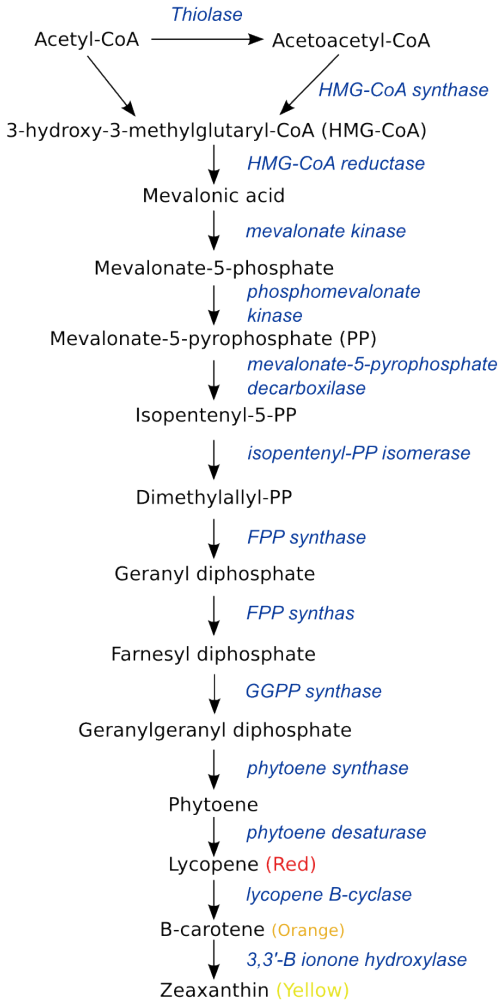
		20°C	30°C	37°C
Reference	Fold increase	1.00	2.44	4.66
	SEM * 2	0.30	1.04	2.18
BBa_K115035	Fold increase	1.00	1.92	17.62
	SEM * 2	0.10	0.36	7.52



- At 30°C a **1.92** fold increase (Ref: 2.44)
- At 37°C a **17.62** fold increase (Ref: 4.66)

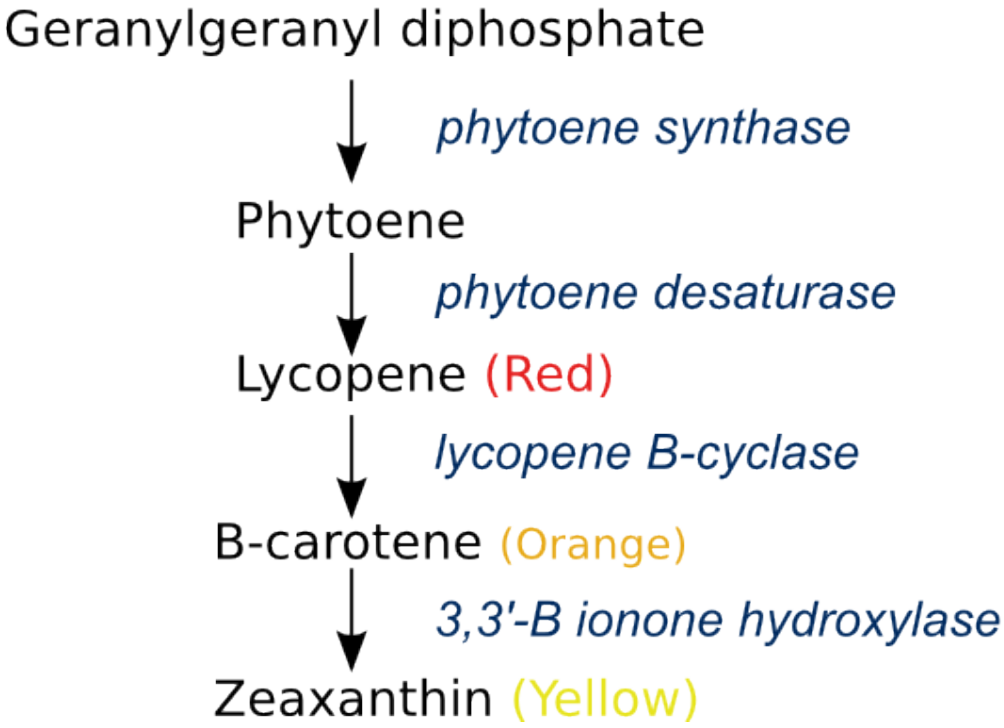
Color Pathway

- 14 enzymes involved
- *Escherichia coli* and *Saccharomyces cerevisiae* genes for FPP production
- Three enzymes obtained in the laboratory:
  atoB — thiolase
  idi — isopentenyl-PP isomerase
  ispA — FPP synthase
- FPP to colorant(s) → Biobricks by Edinburgh 2007



3. V. Martin, *et al*. 2003.

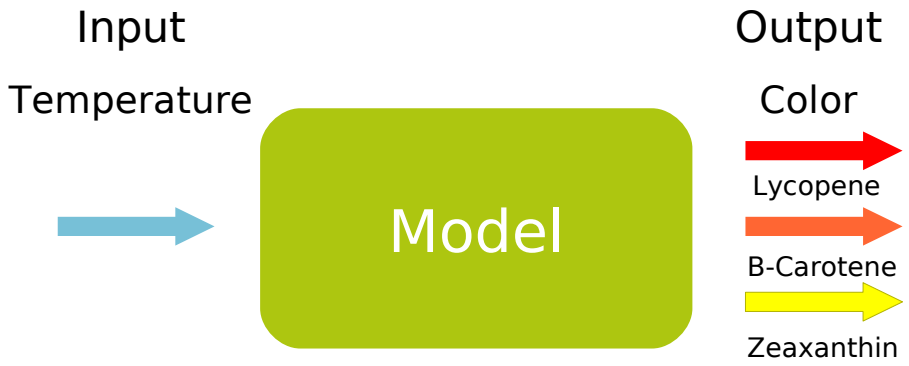
Color Pathway



- FPP to colorant(s) → Biobricks by Edinburgh 2007

Modeling

- Input:
  - Temperature
  - Challenge: Find appropriate model describing temperature sensitivity
- Output:
  - Color
  - Challenge: Define parameter spaces yielding stable dynamic system

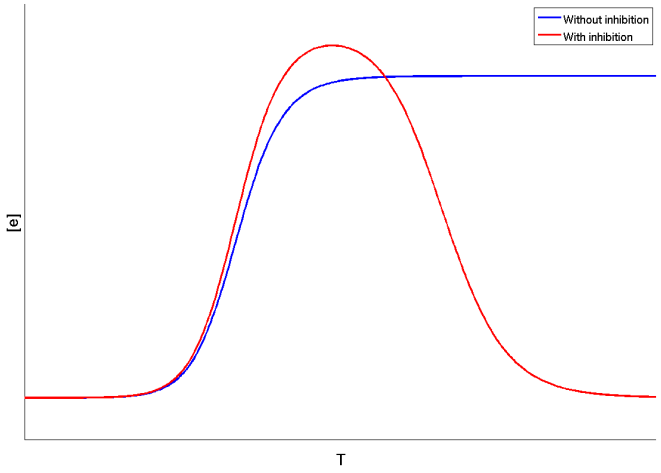


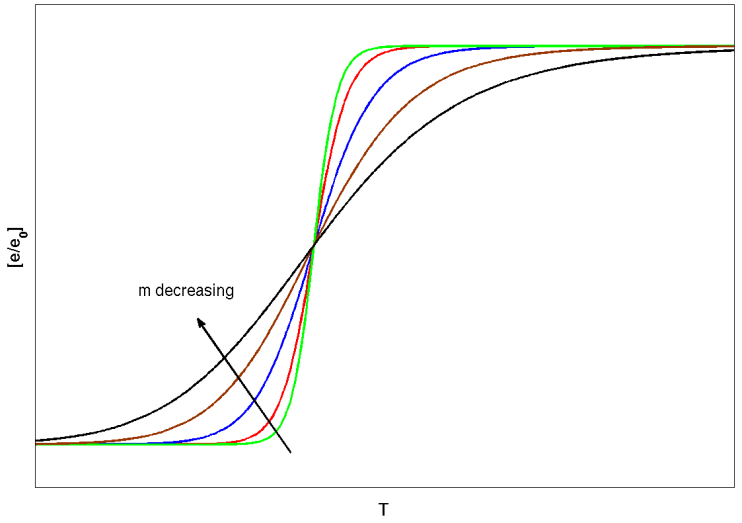
Modeling

- Enzyme production: Hill type
  - m, α, and K are estimated by using luciferase assay results

Without inhibition $$[e] = \alpha \frac{T^m}{T^m + K^m} + 1$$

With inhibition $$[e] = \alpha \frac{T^m}{T^m + K^m} \frac{1}{T^p + K_i^p} + 1$$



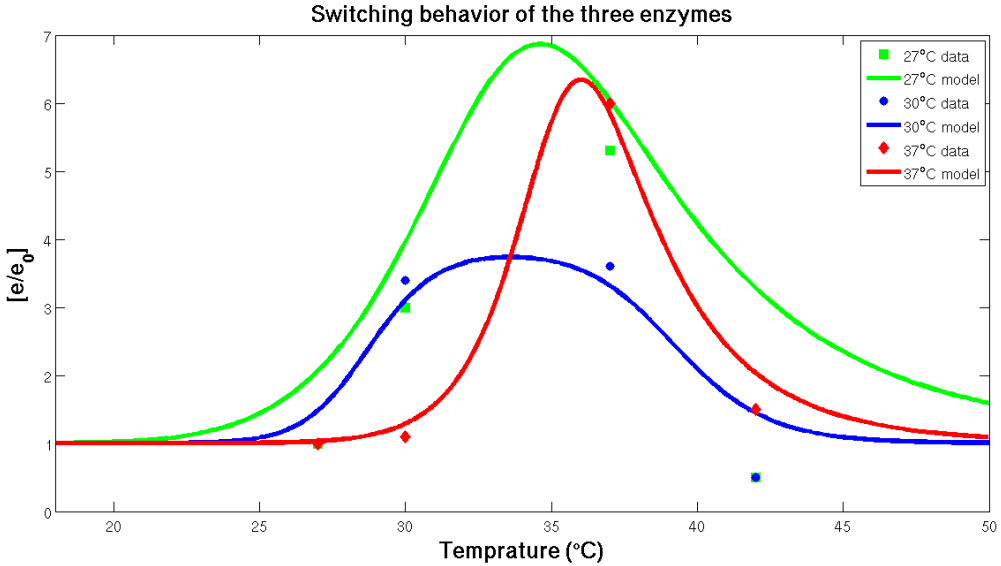


Modeling

- The results show a switch behavior for the model
  - Parameters estimated by Genetic Algorithm

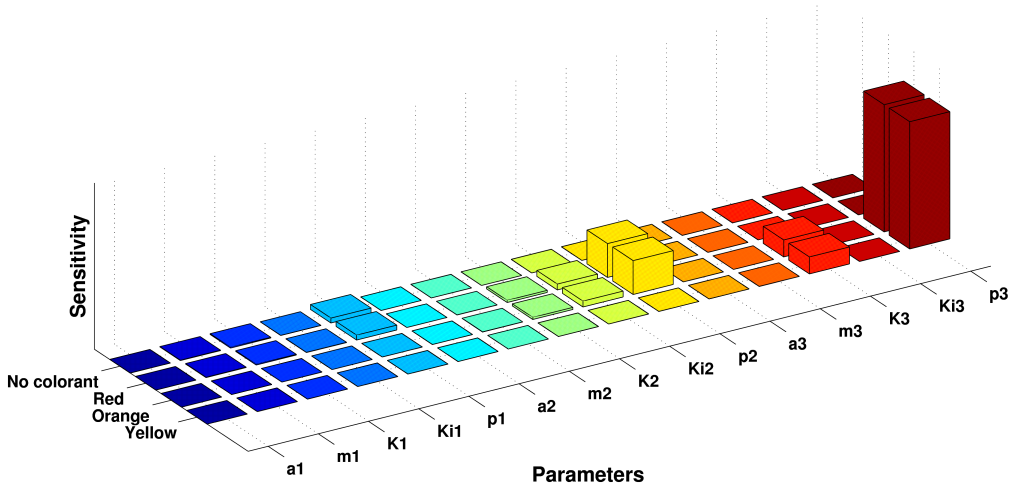
The results for the switch at 30°C is:

$$[e_2 / e_0] = 2 \times 10^{40} \frac{T^{25}}{T^{25} + 28.78^{25}} \frac{1}{T^{25} + 39.25^{25}} + 1$$



Modeling

- Sensitivity analysis
  - The sensitivity analysis on the last four products show the effect of the p parameter on the model



- Bifurcation analysis
  - The bifurcation analysis shows the stability for all estimated parameters

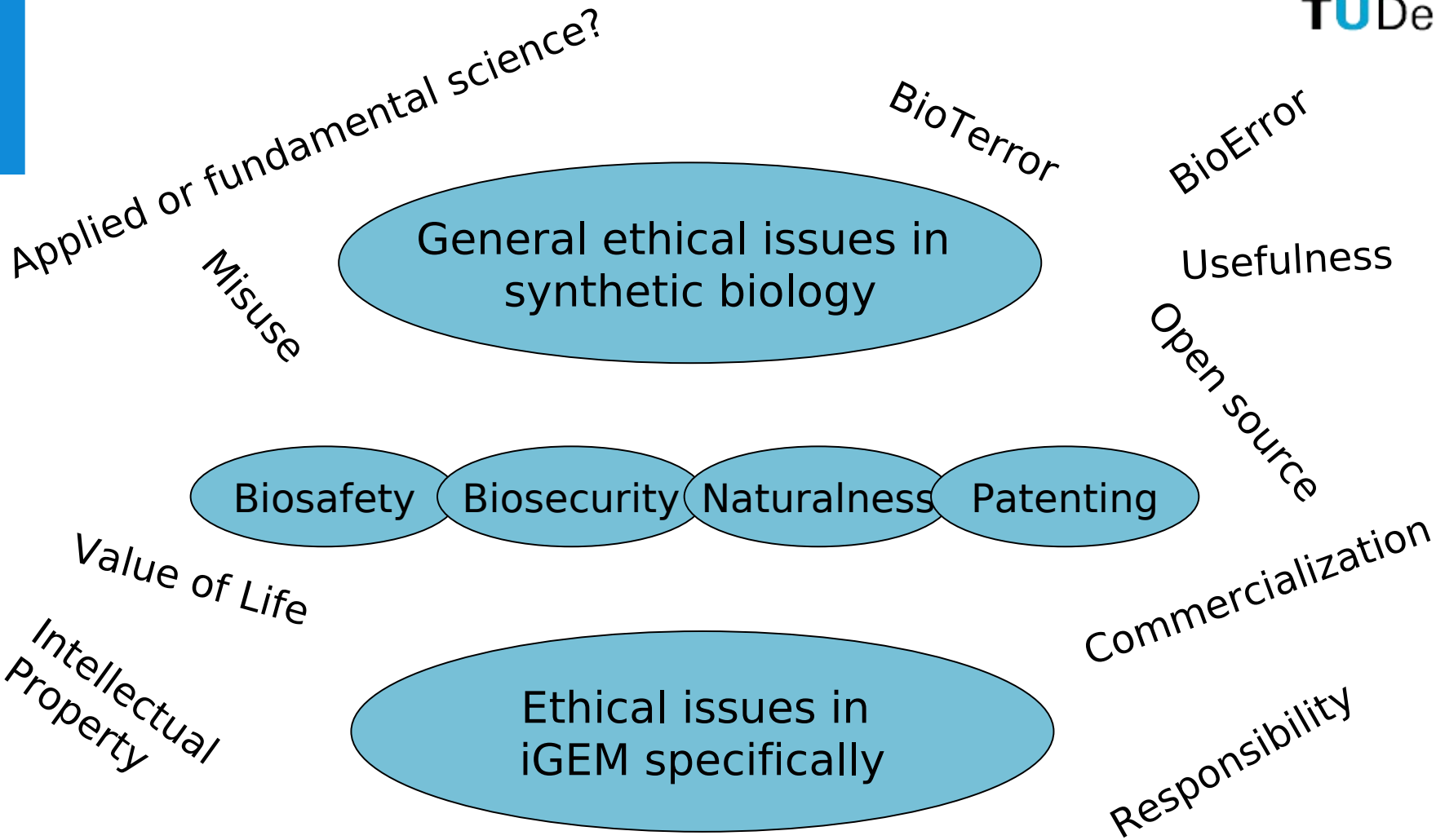
Ethics

- Goal
  - Understanding ethical issues in Synthetic Biology and within iGEM
- Why?
  - Ethical issues important to consider, also during design
- Report
  - “LIFE to LEGO – SynthEthics in the TU Delft iGEM project”
    - Ethical reflections for participants in the open source synthetic biology based iGEM competition

Ethical issues integrated

- Value sensitive design
  - Consider ethical issues in biological design, from the beginning of the project
- Literature survey – Macro ethics
  - Learn which ethical issues play in Sythetic Biology
- Questionnaire – Micro ethics
  - Learn participants' opinions
  - Generate awareness, reflect on ethical issues
- Question of the week
  - Stimulate discussion on ethical issues relevant to the project

The issues identified



Summary

- Software to design RNA thermometers developed
- RNA thermometer (BBa_K115035) confirmed
- Three genes of color pathway cloned
- Mathematical models describing the biothermometer generated
- More awareness on ethical issues by:
  - Implementing value sensitive design
  - Interviews
  - Discussing ethical questions

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2008
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Questions?

The last four products