Towards engineering a BioThermometer

I. Emrah Nikerel

Department of Biotechnology, Delft University of Technology

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Outline

- Overview of team and work
- Principle of the RNA thermometer
- Designing thermo sensitive parts
- Making colored colonies
- Modeling an RNA thermometer
- Lab results
- To do
- Ethics

A bit of History

spring, summer 2007 Domenico came up with the idea

fall 2007 W(H)e managed to convince others (everybody, including the rector)

winter, spring 2008 Team set up, brainstorming, actual work

The project

After many brainstorming sessions, we decided to construct temperature-sensing bacteria *Escherichia coli* that changes color at different temperatures. Possible uses:

- temperature reporter system in large-scale fermentations
- temperature-inducible protein production system.

The ethical issues in design and possible implementation of a commercial product are also addressed.

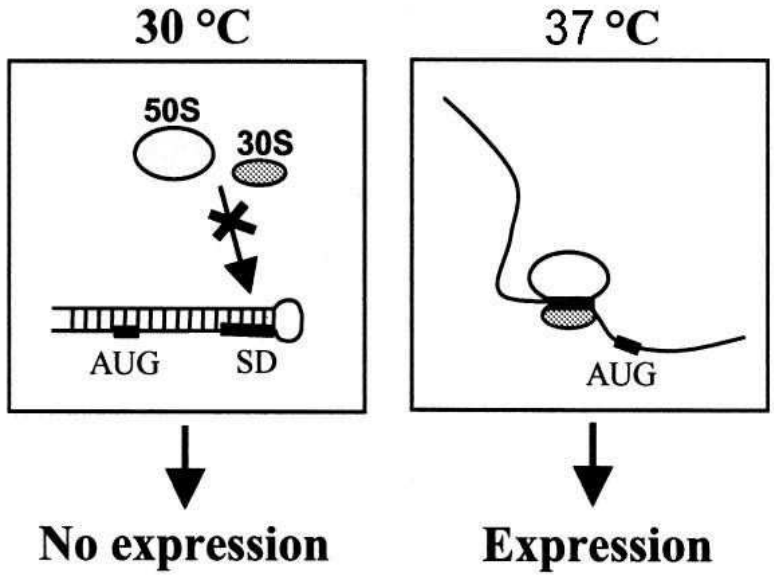
Team, workload

- Design and WIKI $\longrightarrow$ Bas
- Modeling $\longrightarrow$ Rad, Farzad
- Ethics $\longrightarrow$ Steven
- Wet lab work $\longrightarrow$ Oscar, Ruud

The RNA thermometer concept

The functionality of this thermometer relies on the post-transcriptional regulation of a temperature-sensitive RNA structure:

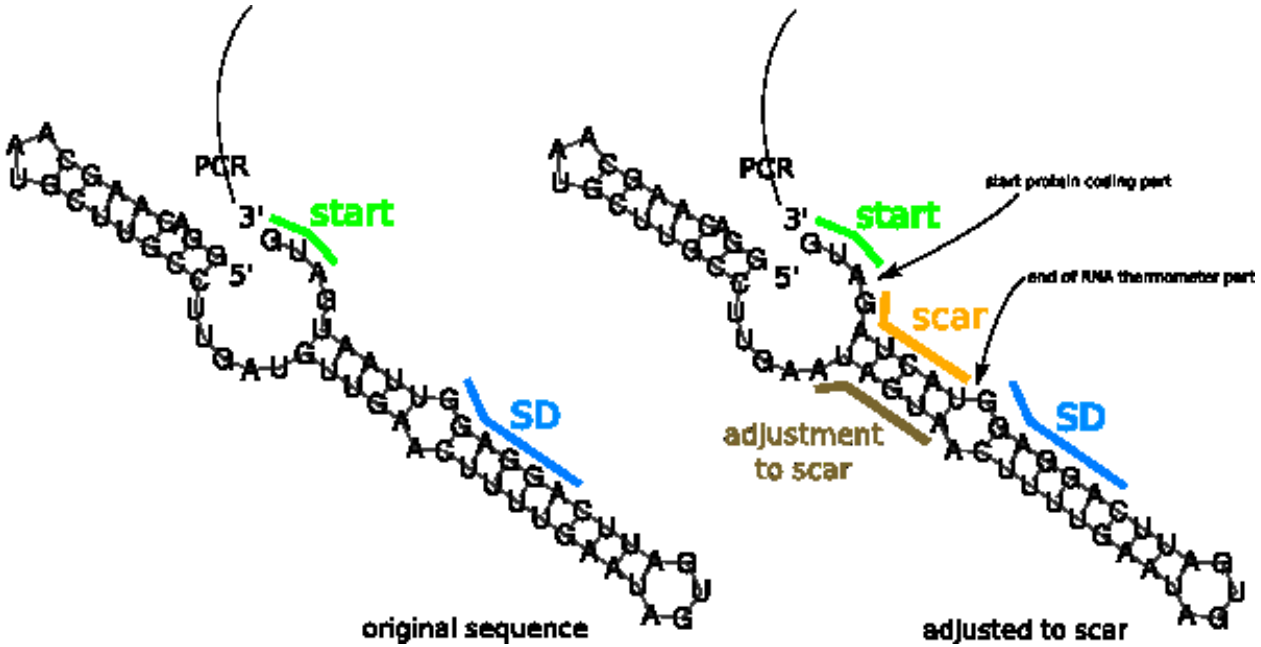
it opens and enables the ribosome to bind, only when the temperature exceeds a certain threshold.



Design of an RNA thermometer

Sequence adjustments

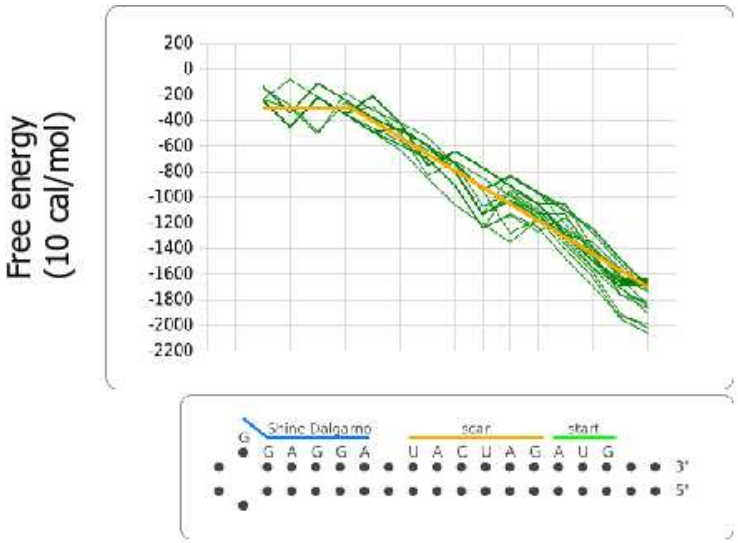
Three thermometers, found in the literature and converted into Standard Biological Part (part of IGEM idea/requirements)



Design of an RNA thermometer

Actual design for temperature sensitivity

- Switching point of the RNA thermometer depends on the stability of the temperature sensitive hairpin.
- Data analysis showed that the temperature sensitive hairpin of 32 ROSE RNA thermometers show a similar stability



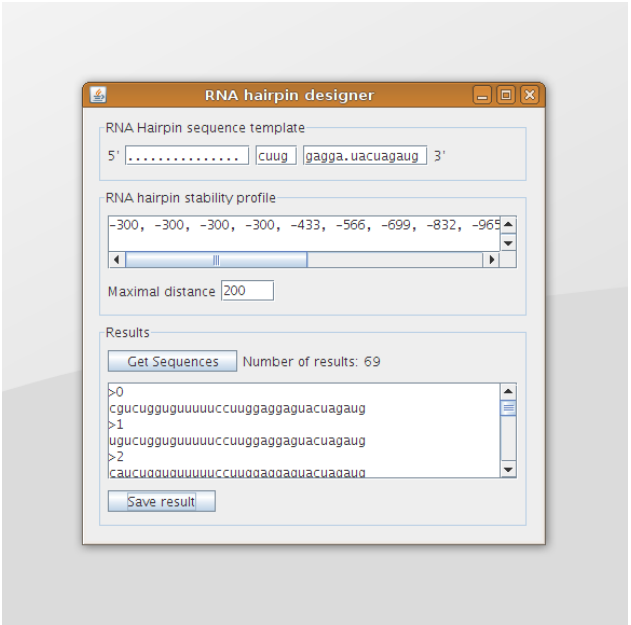
Design of an RNA thermometer

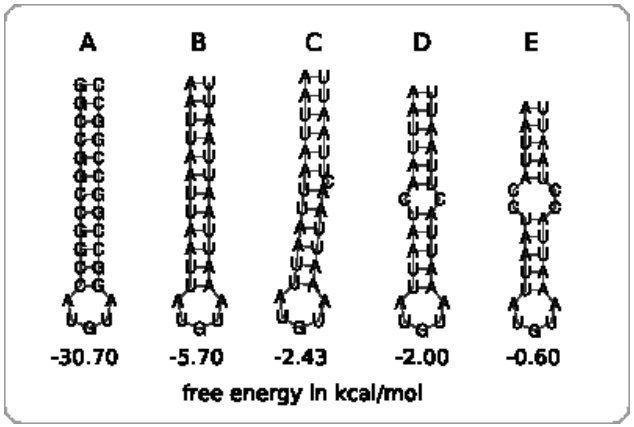
Actual design for temperature sensitivity

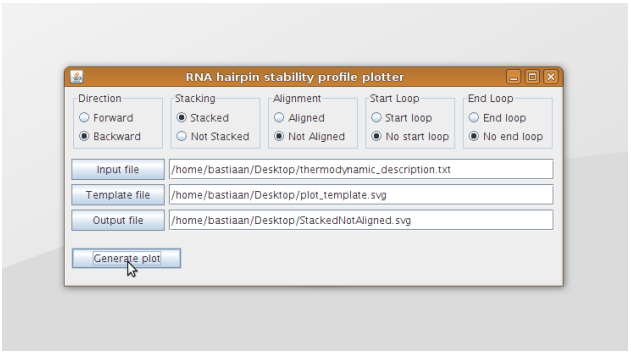
- Two RNA thermometers with a lower switching temperature are designed based on the found stability for the ROSE RNA thermometers
- The designed hairpins have the same stability at different temperatures, which should result in a different switching point

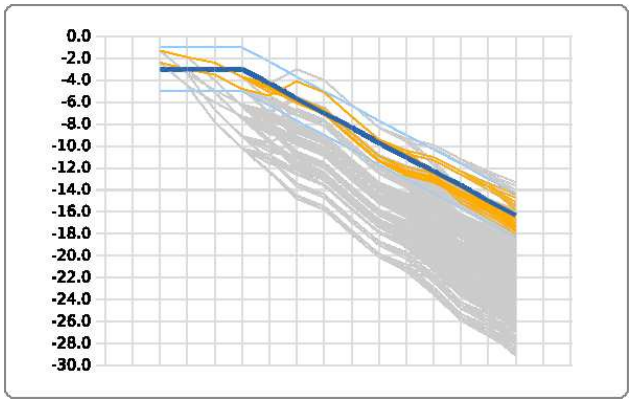
A software is written

A software (with *some* GUI) is written to generate the sequence for the RNA structure that would "melt" at the desired temperature.



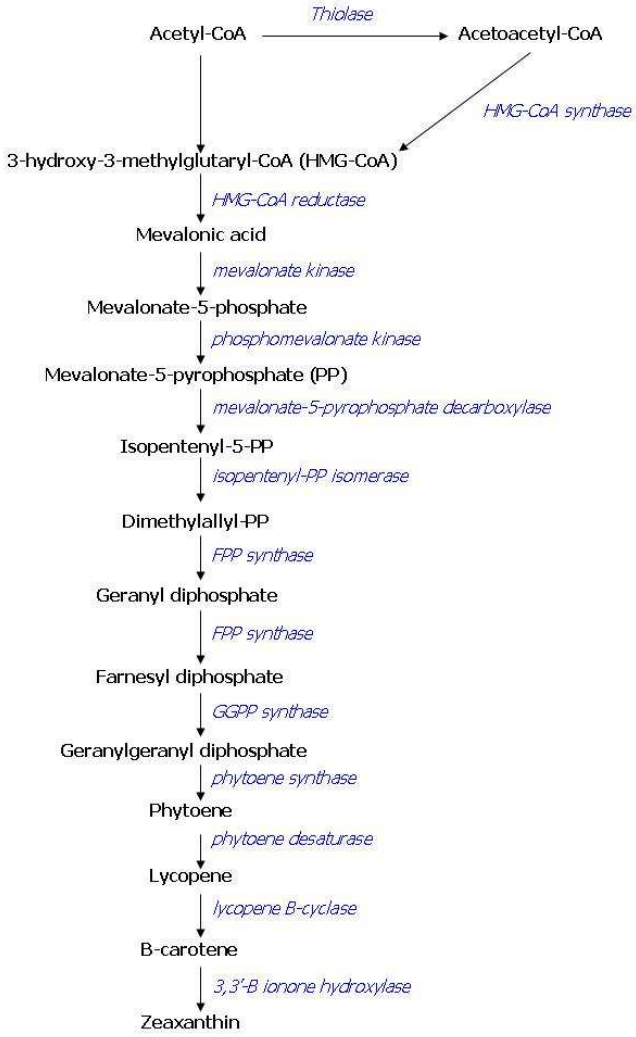






Designing colored *E. coli* cells

- Total of 14 enzymes to produce yellow color
- E. coli and S. cerevisiae genes to produce FPP
- Edinburgh BioBrick can be used



Abstraction of the system

Modeling approaches and tasks

Input: Temperature sensitivity

The enzyme synthesis controlled by the temperature

Challenge: Finding a suitable model for the temperature dependence of the synthesis for the color producing enzymes.

Output: Color production part

Challenge: Solving the Michaelis-Menten kinetic equations, obtain the dynamics of pathway intermediates.

modeling the dynamics of the pathway

$$\longrightarrow X_{i-1} \xrightarrow{e_i} X_i \xrightarrow{e_{i+1}} X_{i+1}$$

$$\frac{dX_i}{dt} = v_i - v_{i+1} \qquad \frac{de_i}{dt} = v_{prod} - v_{deg}$$

- For the enzyme production, we coined Hill-type like effect of the Temperature

  $$v_{prod} = \frac{v^+ T^m}{T^m + K^m}$$

- For the degradation, we used fist order degradation

  $$v_{deg} = k \cdot e_i$$

For the color production, we used simple michaelis menten kinetics.

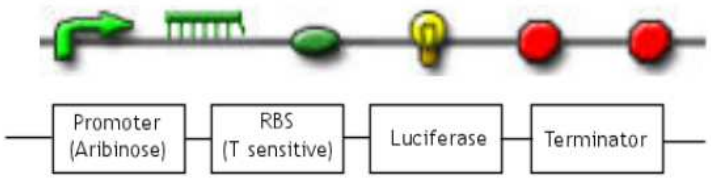
$$v_i = \frac{k_{cat} \cdot X_{i-1}}{X_{i-1} + K_M}$$

Modeling perspectives

- Infer on the dynamics (determine the time frame)
- Design experiments (dynamic/steady state)
- Estimate the parameters (v^+, m, K, k_{cat}, K_M)
- Perform sensitivity analysis for the undeterminable parameters

Wet-Lab work

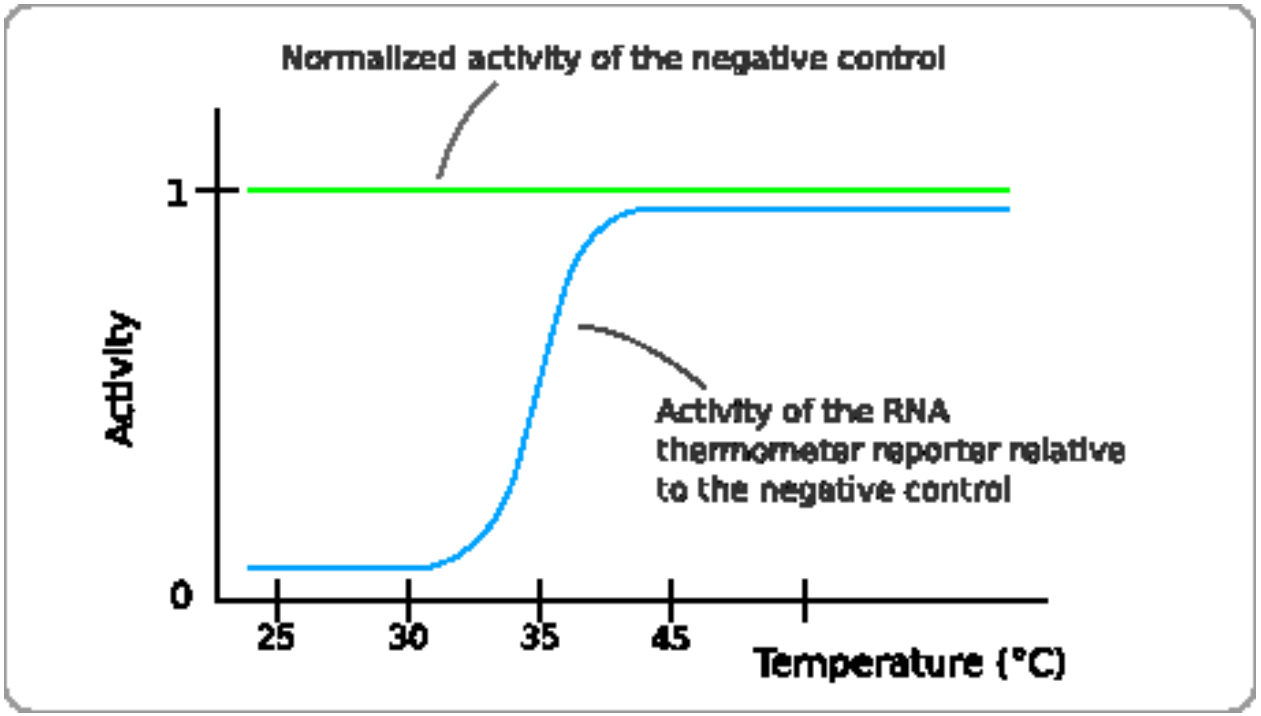
- Order the temperature sensitive constructs
- Testing temperature sensitive parts with luciferase assays (to make sure that $e = e(T)$ works)
- Cloning genes for color pathway



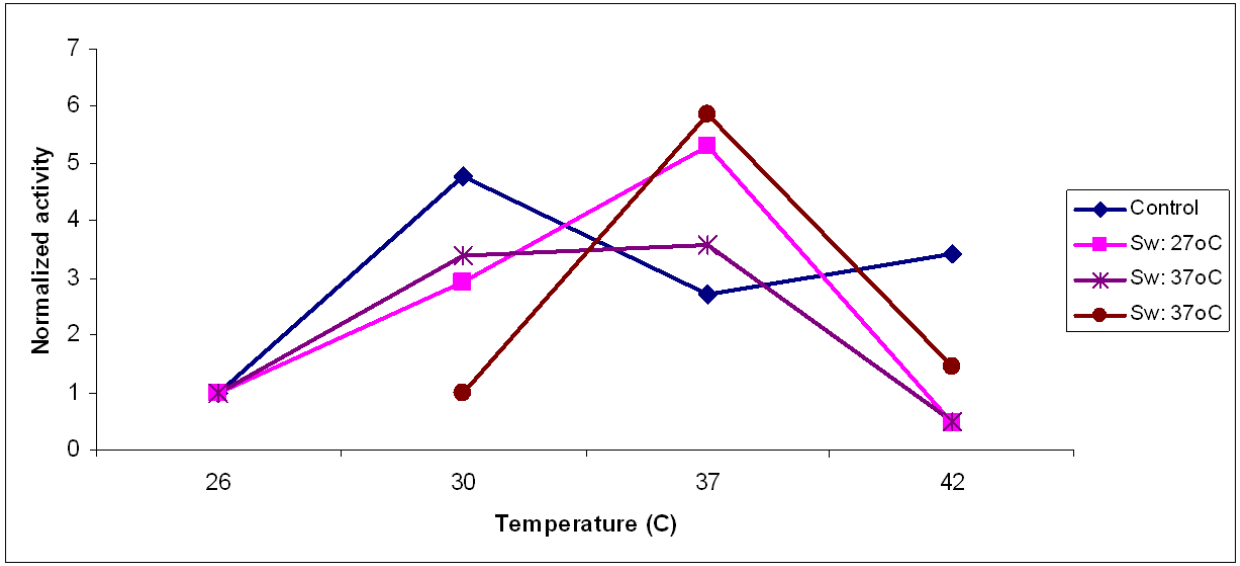
Results (Lab work)

- Temperature sensitive parts
  - done (Most) constructs are ready
  - ongoing Luciferase could be measured for five constructs (including control)
  - ongoing Problem with protein measurements / lysis buffer
- Color Pathway part
  - done Three genes generated as BioBrick: atoB, idi, ispA
  - ongoing Performing PCRs on other genes

Results, expectations



Actual results



:) Not all the constructs behave the same way, the temperature sensitivity works (in some way..)

:S No hard switch, rather a transition period

:(Background still under determined

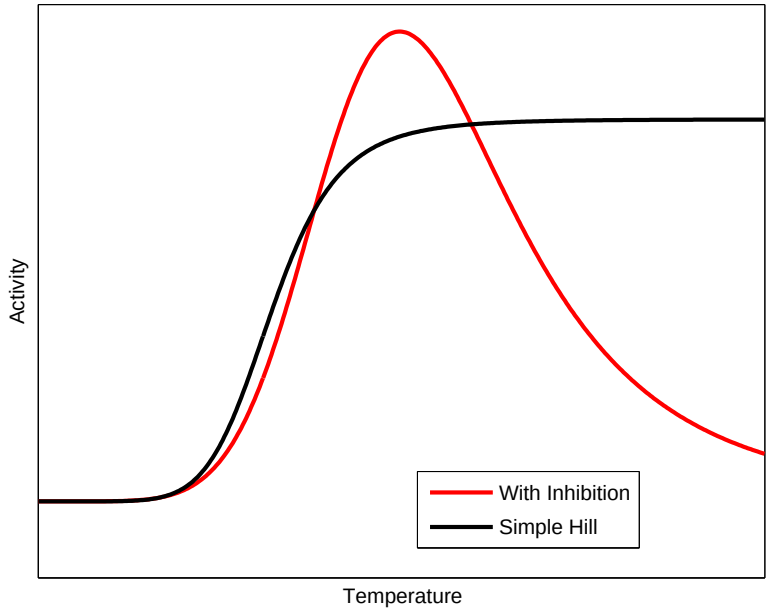
To do in the lab

- Improve protein measurements
- Perform missing luciferase measurements (provide errorbars)
- Perform color pathway PCRs
- Coordinate with the modeling crew for additional experiments

Modeling revisited

The production term is revisited to include the boiling of the metabolism:

$$v_{prod} = \frac{v^{+}T^{m}}{T^{m} + K^{m}} \cdot \frac{1}{K_I + T^{n}}$$



Ethics (Life to Lego)

Ethical reflections for participants in the Open Source Synthetic Biology based International Genetically Engineered Machine (IGEM) competition
SynthEthics in the TU Delft iGEM project
Understanding ethical considerations in Synthetic Biology and within iGEM on:

- Macro level
- Micro level

Zoom in to Macro Level

- **A definition of SB** discourse in semantics
  Gradations of naturalness, (de)constructing
- **Risks in SB** (T)Errorism
  Novel technology or novel technological approach
  Open source
- **SB terminology** Contradictio in Terminis
  How synthetic can biology be?
  The banner implications
- **PR stunt or Public backlash** SB in the media
  Hype or promise
- **Intellectual Property** usefulness or commercialization
  Nave? Capitalism, cash cows, academic/industrial interest, terrorism

A glimpse of the questionnaire torture

Question: Do you see the usefulness of the final product important?

Participant A

A. Do you mean of the final product or of the knowledge we gain?
Q. The end product.
A. The usefulness of the product, I don't think that is very important.

Q. Should it have been more important?
A. No, we are currently in too early a stage, we are learning rather than producing.
Q. So that means generating knowledge?

A. Yes, we do this purely to gain knowledge, that's my opinion

Participant B

A. Right now, because little work has been done, usefulness has been down rated. Usefulness should have more importance in design. Try to figure out in which areas the temperature sensor on nano scale can be useful.

Participant C

A. I think we should think about this more. For example, which applications can be thought of? It's nice to make a thermometer, but what can we really do with it? Which realistic things can be achieved? Currently, it's not very clear what we can do with it. That is important for the iGEM contest. But...

Q. At this moment there is too little a focus on the applications?
A. Yes, I think that we don't think about that enough.

A glimpse of the questionnaire torture

Question 1: Should the research be open source?
Question 2: What happens if it leads to know-how to turn lead into gold?

Participant A

A. If this does not give you neither better or fundamental understanding of biology, neither does it give any useful application, I don't really think it is a useful application that you mention. Besides being able to make money out of it.
Q. Enormous amounts of money…
A. One of the things of iGEM is this idea of progressing of science, of international effort. If you just submit your BioBrick it would be ok to participate.
Q. Do you think the TU Delft board would be happy with us participating in iGEM, with an application that could generate enormous amounts of money for the TU?
A. I don't think they would be very happy, I expect. Now I understand what you mean. You have a nice application and in a way you have the choice to submit it to iGEM and let it free or you can patent it and get money out of it…
Q. So where would the border be for you then?
A. In my case, I would just go for the open source. With these things it would just be something that would be patented; I think I would lose my main drive and just drop it.
Q. So the fundamental science and the usefulness are really a drive for you?
A. In our application, it is not a problem that we are doing only fundamental science, but the whole idea is doing it in a transparent way, in an open source framework. You saw in the beginning, when brainstorming, […] said that if the application relates to biofuels, he could not help, the same said […]. They have that constraint, I would not like to have it for myself.

Participant B

A. The point with this example is that it has great influence on the world. I don't think… Eh, within iGEM, you successively develop something. At first, it is quite idealistic, and at a certain moment you find out that perhaps you can do something with it. What you would do with it then, submit it or keep it for yourself, I can't really say at the moment.

Participant C

A. To be honest, I would tend to *not* submit it to iGEM. That wouldn't be very smart. I think I would be crazy to submit a great finding that I did, as open source in iGEM.

Results on ethics discussion

- Scientists do not form a "homogenic" community
  Conflicting arguments among participants
- A fundamental conflict
  Application oriented vs. Science oriented
- Preventing misuse, responsibilities?
- Value sensitive design.. Failed? Or too early in design?

Question of the Week!

How would you like to see ethics incorporated in your daily work and how do you see ethicists and scientists work together?

Who should be responsible for safety or security, and why, and who should be liable in case of "emergency"?

Summary

Still work to do in a number of items, but:

- Algorithm of temperature sensitivity was made
- BioBricks were made
- Luciferase could be measured
- Parameters for modeling can be estimated
- Ethics are covered
- Hotels/flight booked for the Jamboree (November 7-9)

The "thanks to" slide

- Bas, Farzad, Oscar, Ruud, Rad, Steven
- Domenico, Emrah, Janine, Marco
- Jack Pronk, Loesje, Ton van Maris, Esengül, Fred Hagen, Ibo van der Poel, Marcel Reinders