Major geographic areas where Mercury rejections exceed 0.5 tonnes per year.
The Device

Mercuro-Coli

Importance to quantify mercury contained in water

Elemental Mercury Vapor → Dry Deposit → HgO, Hg^{2+} → Wet Deposit

Mercury Permeation → Bioaccumulation MetHg

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Meeting with a Chemical Industry

Industrial Needs:
- Precise quantification (ECD: 50µg/L)
- Reliable technics
The Device

Mercuro-Coli

State of the art
atomic absorption spectrometry

Mercuro-coli

Detection + Quantification
The Device

Mercuro-Coli

Mercuro-coli

Detection + Quantification

Easy to handle

Cheap

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The Device

Mercuro-Coli

• Specifications:

- Single bacterial strain
- Comparative measurement
- Visual result

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• Single Bacterial Strain
• Comparative Measurement
  – The reference molecule IPTG

![Diagram of IPTG Concentration and Position](image-url)
• Comparative Measurement
  – Emergence of 2 bacterial behaviors:
• Comparative Measurement
  – Emergence of 2 bacterial behaviors:
The Device

Mercuro-Coli

• Visual Result
  – At the boundary both senders and receivers interact:
The Genetic Network

Mercurio-Coli

2-way Switch  Coloration  Power Button
The Genetic Network

Mercuro-Coli

- 3-level Genetic Network

Power Button

2-way Switch

Coloration

Post-Transcriptional Regulation

Toggle Switch

Quorum Sensing and Coloration

AHL/CinR Complex

Lycopene
The Genetic Network

Mercuro-Coli

• 2-way Switch ➡ Switch between 2 pathways
- 2-way Switch coupled with Quorum Sensing

1 Pathway  \( \equiv \) 1 Behavior
The Genetic Network

Mercuro-Coli

- 2-way Switch coupled with Quorum Sensing
The Genetic Network

Mercuro-Coli

• 2-way Switch coupled with Quorum Sensing
Modeling and Experiments
Mercuro-Coli

2-way Switch  Coloration  Power Button
Modeling AND Experiments

Mercuro-Coli

Hg²⁺  2-way Switch  IPTG

Sender  Receiver

Coloration
Modeling AND Experiments

Mercuro-Coli

- 2-way Switch
  
  aTc
  
  IPTG
  
  2-way Switch
• 2-way Switch ODEs

\[
\frac{d[TetR]}{dt} = \frac{k_{pLac} [pLac]_{tot}}{\beta} - \delta_{TetR} [TetR] \\
1 + \left( \frac{[lacI_{total}]}{K_{pLac} + \frac{K_{pLac} [IPTG]}{K_{lacI-IPTG}}} \right)
\]
Evolution of TetR concentration (1mM IPTG)

\[ aTc = 50 \text{ ng/mL} \]
\[ aTc = 150 \text{ ng/mL} \]
• Biological Results
• Evolution of GFP fluorescence (1mM IPTG)
- Biological bistability

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• Biological bistability
• Biological bistability
• Stochastic study of bistability
• Quorum Sensing
\[
\frac{d[QS_i]}{dt} = \eta([QS_e] - [QS_i]) - \delta_{QS_i}[QS_i] + k'_\text{QS-production}[\text{CinI}]
\]

\[
\frac{d[QS_e]}{dt} = \rho v_c \eta([QS_i] - [QS_e]) - \delta_{QS_e}[QS_e] + D_{\text{diff}} \frac{\partial^2[QS_e]}{\partial x^2}
\]

\[
\frac{d[\text{CinR}^*]}{dt} = k_{\text{comp}}[\text{CinR}][QS_i]
\]

\[
\frac{d[\text{Lyco}]}{dt} = k_{p\text{Cin}}[p\text{Cin}][\text{CinR}^*]
\]
Modeling and Experiments

Mercuro-Coli

• Result on the plate

![Image](image.png)

\[ aTc = 50 \text{ ng/mL} \]

\[ aTc = 150 \text{ ng/mL} \]
• Result on the plate
• Power Button
Post-transcriptional network
• Post-transcriptional network
• GFP fluorescence obtained with fha

GFP Fluorescence
• The power switch using RsmA & rsmY

![GFP fluorescence graph]

- Autofluorescence
- Fha
- Fha + RsmA
- Fha + RsmA + rsmY

GFP fluorescence
• Channels on the device
• Channels on the device
• Device specifications

- **Response time**: $\approx 1$ hour
- **Sensitivity**: $= 10^{-7} \text{ M (20µg/L)}$
- **10% precision**: $10^4$ bacteria per channel
Human Practice

Mercuro-Coli

Interdisciplinarity

Promotion
Human Practice

Mercuro-Coli

• Synthetic Biology

An interdisciplinary approach of living systems!
Human Practice

Mercuro-Coli

• How to improve the interaction between Biologists & Modelers?
Human Practice
Mercuro-Coli

Synthetic Biology is essentially an interdisciplinary approach of living systems. It is not only limited to the construction of the networks, where genes and proteins are in interactions, but attempts to understand how a dynamic behaviour emerges.

The assistance in understanding these interactions is at the very heart of Modeling.

Therefore, Biologists and Modelers must work together.

1. What is a model?
A model is the mathematical translation of a biological or physical phenomenon. In this case, hypotheses are formulated in order to specify the equation of system, which is quite often very complex in the early stages of modeling.

2. What is the purpose of a model?
The model predicts the evolution of the system. However, it has to be experimentally validated in order to fit the biological process as much as possible.

3. How to proceed?

- **Know what we want to model!**
- **How to proceed?**

EvoI. Follow the evolution of the GFP protein concentration within the cell.

Let's take a simple case: 

- *| Synthesis of GFP protein
- **| Destruction of GFP protein

GFP concentration, [GFP], depends on several parameters. The most important are: the Synthesis and the Degradation.

\[
\frac{d[GFP]}{dt} = \text{Synthesis} - \text{Degradation}
\]

**Expression of the Synthesis**

- * | Synthesis of GFP protein
- ** | Degradation of GFP protein

**Expression of the Degradation**

- * | Synthesis of GFP protein
- ** | Degradation of GFP protein

In a more complex case, presence of a repressor.

**The Biologist's intervention**

If we have a repressor R, which inhibits the expression of GFP, we have the following equation:

\[
\frac{d[R]}{dt} = \text{Synthesis} - \text{Degradation}
\]

The Biologist can impact the repressor R to control the concentration of GFP.

**The regulation of the differential equations provides the solution [GFP]**

Confrontation of the predictions to the experimental data

Data and hypotheses underlying the model

The model

Experiments plan

Experiments can lead to predictions and approximations

Once validated, the model is used to load experiments, which, in turn, will provide relevant informations that will enrich the model gradually.

2011 World Championship Jamboree
Synthetic Biology is essentially an interdisciplinary approach to living systems. It is not only limited to the construction of the networks, where genes and proteins are in interactions but attempts to understand how a dynamic behavior emerges.

The essence in understanding these interactions is at the very heart of modeling.

Therefore, Biologists and Modelers must work together.

1. Construction of a genetic network

   - **Promoter**: A site on the DNA where RNA polymerase initiates transcription.
   - **Transcription**: The process by which RNA is synthesized from DNA templates.
   - **Translation**: The process by which RNA is translated into protein.
   - **Protein**: A macromolecule that has a specific three-dimensional structure and functions in the cell.

2. How to regulate our circuit?

   - Some molecules can inhibit or facilitate the binding of the RNA polymerase on the promoter. This then initiates transcription and translation, respectively.
   - Some protein represses the promoter and might itself be repressed by other molecules.

3. What a modeler should also know...

   - **Degradation**
     - Molecules and proteins have a certain lifetime. Those that are not translated will be degrading over time.
   - **Synthesis rate**
     - Each promoter is characterized by its synthesis rate, which is the number of promoter sites.

4. The modeler intervention

   - The study of the system's behavior needs to be completed by modeling, which allows the description of the prediction of the system's behavior.
   - **Driving biological experiments**

---

2011 World Championship Jamboree
Human Practice

Mercuro-Coli

• Test organization


Quiz

Group A (With Flyers)

Group B (Without Flyers)
Test results

- Group A (With Flyers) average score: 8
- Group B (Without Flyers) average score: 4
Human Practice

Mercuro-Coli

• Test results

Did the flyers help you to better understand the questions?

- 84% Positive
- 16% Negative
Human Practice

• Test results

Did the flyers help you to better interact with your partner?

- 62% Positive
- 38% Negative
• Promoting Synthetic Biology
  – Conferences
  – Media Attention (GreNews/A savoir/Radio)
• Major achievements

- Innovative mercury biosensor ➔ Industrial interest!
- Proof of concept of 3 modules:
  - 2 Way-Switch
  - Quorum sensing
  - Power button
- 19 biobricks added.
- Models giving all necessary specifications of the device.
- New iGEM flyers for biologists & modelers.
- iGEM Grenoble 2012 is launched!
Acknowledgments

Mercuro-Coli

• Instructors
  - Hans Geiselmann
  - Franz Bruckert
  - Marianne Weidenhaupt
  - Hidde De Jong
  - Delphine Ropers
  - Robert Baptist

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  - Stéphane Pinhal
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Mercuro-Coli

• Sponsors

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• Sponsors

• « CEA- Direction des Sciences du vivant »
• « CEA- Direction des Sciences de la Matière »
• « CEA-Direction de la Recherche Technologique »
• Programme Transversal Nanotoxicologie
• Programme Transversal Technologies pour la Santé
• Programme transversal Nanosciences
Thank you for your attention

Questions?
Translational regulation

Mercuro-Coli

Influence of a translational regulation system on the switch

given IPTG gradient with an aTc concentration of 10^{-6} M

Low TetR concentration: Switch at lower concentration of IPTG (red curve).
Translational regulation

Mercuro-Coli
Influence of RsmA production on the growth of E. coli DH5α?

- pVLT31-rsmA or empty
- No growth difference
Translational regulation

Mercuro-Coli

mag operon leader sequence

Fhal gene leader sequence

rsmY sRNA sequence

Constructs used to characterise RsmA system
Translational regulation

Mercuro-Coli

Flow cytometer: dot plots of the 40000 bacteria analysed
Translational regulation

Mercuro-Coli

Flow cytometer: first test of the leader sequences
Translational regulation

Mercuro-Coli

Flow cytometer: compilation of controls and the two leader sequence signal
Translational regulation

Mercuro-Coli

Relative RBS strength to the reference RBS. SD of four independent measurements.
Translational regulation

Mercuro-Coli

Effect of RsmA and RsmA + rsmY on fha-GFP signal

Single, double, and triple transformations. Using RsmA, fha-GFP signal is much decreased. Upon transcription of rsmY, fha-GFP signal rises.
Translational regulation

Mercuro-Coli

Effect of RsmA and RsmA + rsmY on fha-GFP signal

Using values from the whole population, RsmA appears more important, rsmY effect is shunted by 30%. Clone variability?
Translational regulation
Mercuro-Coli

Effect of RsmA and RsmA + rsmY on mag-GFP signal

Single, double, and triple transformations. mag-GFP sigla is very low, and totally shut off when bacteria contain rsmA.
Translational regulation

Cellular context of the RsmA system

Environmental signals
- LadS
- RetS
- GacS/GacA

RsmY/RsmZ

Indirect regulation
- via direct regulation of regulatory factors

Direct regulation
- via mRNA binding and block of translation

Genes downregulated in the rsmA mutant
- T3S genes
- Type IV pili genes
- Iron homeostasis genes

Genes upregulated in the rsmA mutant
- hcnABC operon
- PA0277 gene
- PA2541 operon
- PA3732 operon
- PA4492 operon
- T6S genes

Brenic et Lory, 2009
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Translational regulation

An other translational regulation system: dsrA / rpoS

Bacterial Small RNA Regulators. Nadim Majdalani et al, 2005