62% of the people we interviewed are totally ignorant about this danger.
And you?
PCBs
PCBs

PolyChloro Biphenylys

• A family of synthetic compounds
• High chemical and thermal stability
Mostly used as insulating fluids in electrical equipment

But also in coolants, paints and cements, as stabilizing additives in flexible PVC, pesticides...
• Produced and used during the 2\textsuperscript{nd} half of the 20\textsuperscript{th} century

400 million tons produced worldwide
PCB toxicity

Water and soil contamination
Increasing PCB concentration

Most PCBs are:
- Carcinogenic
- Endocrine disruptors

Dangerous for human health and the environment

Water and soil contamination

Ban of PCBs production and use in France in 1987
PCBs in the environment

• Contaminated soils and waters (sediments)

Sites polluted by PCBs in France

28% of French population: Higher exposure than PTMI (Provisional Tolerable Monthly Intake)

Are PCBs biodegradable?

First PCB degrading bacteria discovered here!

Hudson river

- Bacterial communities can degrade PCBs
- Bacteria degrading PCBs have been isolated and studied
- But none of them can totally degrade PCBs
2 step PCB degradation

**ANAEROBIC PATHWAY**
(e.g. *Dehalobium chlorocoercia*)

Highly chlorinated PCBs → Lowly chlorinated PCBs

**Dechlorination**

**AEROBIC PATHWAY**
(e.g. *Burkholderia xenovorans LB 400*)

Lowly chlorinated PCBs → Acetyl coenzyme A

**Oxidative degradation**
PCB degradation pathways chosen

**ANAEROBIC PATHWAY**
(e.g. *Dehalobium chlorocoercia*)

**AEROBIC PATHWAY**
(e.g. *Burkholderia xenovorans LB 400*)
Objectives of the PCB Busters’ project

1. To combine the anaerobic and aerobic degradation pathways in a single bacterium

   Choice of the chassis: *E. coli*
   - Quick growth
   - Dissemination control
   - Aerobic and anaerobic metabolisms

2. To construct a PCB sensor system
Objective: build bacteria strain able to detect PCBs in water
In a *Pseudomonas pseudoalcaligenes* strain:

- **BphR2**: Inactive
- **PCB**: Active

**Genes:**
- **PbphR1** BBa_K1155001
- **PbphA1** Bba_K1155002
PCB sensor system

Constitutive promoter \( \rightarrow \) \( bphR2 \) \( \rightarrow \) \( PbphR1 \) \( PbphA1 \) \( \rightarrow \) \( lacZ \)

**+PCB**
- BphR2 is active
- Blue signal

**-PCB**
- BphR2 is inactive
- No signal
PCB sensor system

Constitutive promoter → \textit{bphR2} → \textit{PbphR1} → \textit{PbphA1} → \textit{lacZ}

<table>
<thead>
<tr>
<th></th>
<th>BphR2 is active</th>
<th>Blue signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>+PCB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-PCB</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BphR2 is active when PCB is present, resulting in a blue signal. BphR2 is inactive when PCB is absent, resulting in no signal.
Objective: build bacteria able to completely degrade PCBs.
PCB degradation system

It involves two pathways:
- Anaerobic: reductive dechlorination
- Aerobic: oxidative degradation
- No bacteria are able to perform both pathways

Objective:
To combine aerobic and anaerobic steps of PCB degradation in *E.coli*
PCB degradation system

In a *E. coli* strain:

- **FNR**
  - Active

- **O$_2$**
  - FNR
    - Inactive

- **PnirB** BBa_K1155004
- **PnarG** BBa_K1155005
- **PnarK** BBa_K1155006
- **Pndh** BBa_K1155000
Expression of all the PCB degradation enzymes in *E. coli*

- **Reductive dechlorination**
  - FNR
  - Constitutive promoter
  - *fnr*
  - PnarK
  - PnarG
  - PnirB

<table>
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<tr>
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<tbody>
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Expression of all the PCB degradation enzymes in *E. coli*

- FNR is active
- Reductive Dechlorination
- Oxidative degradation

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Expression of all the PCB degradation enzymes in *E. coli*.

- **O₂**
  - FNR is active
  - Reductive Dechlorination: No

- **+O₂**
  - FNR is inactive
  - Reductive Dechlorination: No
  - Oxidative degradation
Expression of all the PCB degradation enzymes in *E. coli*

**Diagram:**
- FNR
- Constitutive promoter
- fnr
- PnarK
- PnarG
- PnirB
- Reductive dechlorination
- Pndh*
- Oxidative degradation

**Table:**

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Testing the system

Reporting gene AmiLCP (Uppsala 2011 - BBa_K592009)
Testing the system

Reporting gene AmiICP (Uppsala 2011 - BBa_K592009)
Testing the system

Constitutive promoter

- $\text{O}_2$
  - FNR is active
  - Pndh* repressed
  - No

+ $\text{O}_2$
  - FNR is inactive
  - Pndh* no repressed
  - Violet signal
Pndh* - *amilCP* expression

**AEROBIC CONDITIONS**

**ANAEROBIC CONDITIONS**
Summary

Work done:

• three FNR activated promoters
• one FNR repressed promoter
• Improved a reporter system
• Characterize Pdnh* promoter by using RBS_AmilCP_Term construction

Work in progress:

• Finish the sensor system
• Characterize NarG, NarK and NirB promoters
• Finish the construction of the degradation system
Modeling

The $O_2$ switching system
Patrick Amar and Loïc Paulevé. *HSIM: an hybrid stochastic simulation system for systems biology*, *Electronic Notes in Theoretical Computer Science*
A → B + C [P]
HSIM reactions

A → B + C [P]

A + B → C + D [P]
Random walk simulation scheme
Random walk simulation scheme
Random walk simulation scheme

\[ A + B \rightarrow C + D \]

REACTION PROBABILITY \( P \) ?
Random walk simulation scheme

A + B $\rightarrow$ C + D
Modeling our oxygen sensor

FNR

O₂

Bound FNR

Biological system

Computer model
Modeling our oxygen sensor

FNR + O₂ → FNR

Biological system

Computer model
Modeling our oxygen sensor

**Biological system**

FNR + O₂ → Bound FNR

**Computer model**

FNR + O₂ → FNR

FNR → O₂

FNR + Bound FNR
Modeling our oxygen sensor

Biological system

FNR

$O_2$

Bound FNR

Computer model

FNR + $O_2$ → FNR

FNR + Bound FNR

Bound FNR → FNR + Bound FNR

FNR + Bound FNR

FNR + Bound FNR
Modeling our oxygen sensor

FNR + \( \text{O}_2 \) \( \rightarrow \) Bound FNR

FNR

Bound FNR

Biological system

Computer model

FNR + \( \text{O}_2 \) \( \rightarrow \) FNR

FNR

Bound FNR

Bound FNR

Bound FNR +
Results

- Green reporter (lack of oxygen)
- Red reporter (oxygen present)
- FNR bound to promoter sites
A lighter HSIM simulator, wiki-integrated!

Programmed by Damir Vodenicarevic, based on the work of Patrick Amar and Loïc Paulevé [HSIM: an hybrid stochastic simulation system for systems biology, Electronic Notes in Theoretical Computer Science www.elsevier.nl/locate/entcs]
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Human Practices

Perception of our project by the public

- Safety
- Open Source
Perception of our project by the public

Have you ever heard about PCBs?

- Yes: 38%
- No: 62%

What do you think about our PCBbuster project?

- In favor: 25%
- Against: 26%
- No opinion: 49%
Perception of our project by the public

- Safety questions
- Intellectual Property
PS Bacterial Filter

Gemote system
Sensor detection
Anaerobic and aerobic combination
Open Source Reflection

Meetings:
- Comparison free software/synthetic biology
- Bioterrorism history
- Democratization of science
- Bioeconomy
- Biohacking

Interview: Ellen Jorgensen

« I think that people are curious »
« DNA is everywhere »
« Synthetic biology is very powerful! »
PS PCR : open source thermo cycler
PS-PCR: heating/cooling
PS-PCR: heating/cooling

- Peltier element
  (3.78€)
PS-PCR: heating/cooling

- Peltier element
  (3.78€)

- Heat sink
PS-PCR : sample holder
PS-PCR : thermal block
PS-PCR: control electronics

Power board

Logic board

12V
From PSU

USB
To computer
PS-PCR: computer software

Open source control software, in collaboration with Pierre-Yves
PS-PCR: minimal cost

TOTAL COST ~ 30€
Test

PS-PCR
15µL
Conclusion

$\text{PbphR1}$
$\text{PbphA1}$

$I\text{GEM PARI\text{-}SACLAY}
\text{PCB BUSTERS}
\text{open source}$

Safety First
Thank you!

Super advisors

Sylvie  Jean-Luc  Philippe  Claire  Nguyễn  Solenne