The French Froggies Project!
Our project
Our project

Welcome to Synthetic Physiology!

Saturday, October 6, 12
Welcome to Synthetic Physiology!
Welcome to Synthetic Physiology!

- Compartmentalized system
- Differentiated cells
- Closer to humans
- Longer time scale
- Behaviour as new variable
Why Xenopus?

Xenopus

Family: Pipidae species
Origin: Africa
Stars: Laevis and Tropicalis

Function: Model organism for in vivo studies of molecular, cellular and developmental biology of vertebrates.
Why *Xenopus*?

- Genome is sequenced
- Simple injection into zygote after IVF
- Most major organs developed after five days

*Xenopus*

**Family:** Pipidae species  
**Origin:** Africa  
**Stars:** Laevis and Tropicalis  
**Function:** Model organism for *in vivo* studies of molecular, cellular and developmental biology of vertebrates
Outline

✓ Design & Modeling

✓ Construction

✓ Characterisation
Outline

✓ Design & Modeling
✓ Construction
✓ Characterisation

Now: Our Human Practice!
Human practice
Human Practice

GEM = Genetically Engineered Machines
GEM = Genetically Engineered Machines

Are tadpoles machines?
GEM = Genetically Engineered Machines

Are tadpoles machines?

Are frogs chassis?
GEM = Genetically Engineered Machines

Are tadpoles machines?

Are frogs chassis?

Are you a chassis?
Human Practice
Human Practice
« The price of metaphor is eternal vigilance »

Arturo ROSENBLUETH

Norbert WIENER
In order to tackle these questions we:
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✓ Made two surveys in the team
In order to tackle these questions we:

✓ Made two surveys in the team

✓ Organized debates with the DIYbio community and citizens
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Are you a chassis?

Hey! Call me chassis!
Design
Synthetic endocrinology
Synthetic endocrinology

- Vertebrate organs communicate through hormones
- An orthogonal system could be made taking hormones from plants
- Implementation a synthetic hormonal system using auxin
Synthetic endocrinology

- Vertebrate organs communicate through hormones
- An orthogonal system could be made taking hormones from plants
- Implementation a synthetic hormonal system using auxin

**Auxin emitter**
K812021 & K812020
or K812014

**Auxin receiver**
K812010 + K812012
or K812013
Auxin emitter

- Two enzymes make auxin from tryptophan
- Adapted for eukaryotes from K515000 (Imperial 2011)
- Three alternate devices
Auxin receiver

Auxin arrives in system

TirI binds auxin

Auxin arrives in system

TirI binds auxin

AID binds TirI-auxin

Auxin arrives in system

TirI binds auxin

AID binds TirI-auxin

TirI acts as E3 ligase

Auxin arrives in system

TirI binds auxin

AID binds TirI-auxin

TirI acts as E3 ligase

AID is polyubiquitinated

Auxin arrives in system

TirI binds auxin

AID binds TirI-auxin

TirI acts as E3 ligase

AID is polyubiquitinated

The whole protein is degraded

New two components system:

- Auxin production device
  - A co-injected device
  - A eukaryotic polycistron
  - A multi-chassis system

- Auxin receptor device
  - A co-injected device
  - A eukaryotic polycistron
Modeling

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PDE model

Good representation of diffusion
But parameters very difficult to measure
Emitor
auxin arrival
$C_E$

$P \times S(C_E - C_B)$

Blood
transport
$C_B$

$P' \times S'(C_B - C_R)$

Receptor
auxin to be detected
$C_R$
Emitor
auxin arrival
$C_E$

$P \times S(C_E - C_B)$

Blood
transport
$C_B$

Receptor
auxin to be detected
$C_R$

[auxin in emitter]

[auxin in blood]

[auxin in receptor]

Quantities (mmol)

Time ($\times 10^4$ s)
Auxin production model

Auxin detection model
Auxin production model

Auxin detection model

![Diagram of Auxin Production and Detection Models](image-url)
Auxin production model

Auxin detection model

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**Graphs**

- **IAA** concentration over time (μM)
  - [IAA]
  - [IAM]
  - [dIAA]

- **GFP degradation** over time (μM)
  - [GFP degraded]
  - [IAA-TirI]
  - [TirI-GFP-AID]

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**Legend**

- **IAA**: Indole-3-acetic acid
- **IAM**: Indole-3-acetamide
- **dIAA**: Degraded IAA
- **GFP**: Green fluorescent protein
- **IAA-TirI**: IAA-TirI complex
- **TirI-GFP-AID**: TirI-GFP-AID complex

---

**Auxin transport in Xenopus**

- Realistic geom.
- Simplified geom.

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**Plasmid distribution**

- 5 mm
- 1 mm
- 10 μm
- 100 nm
✓ Multi-scale approach
✓ Modular modeling
Construction
GoldenBricks: Accelerating iGEM cloning

BioBricks
Standardized assembly

Golden Gate
Multiple fragments assembly
GoldenBricks: Accelerating iGEM cloning

BioBricks
Standardized assembly

Golden Gate
Multiple fragments assembly

GoldenBricks
Standardized multiple fragments assembly
GoldenBricks: Accelerating iGEM cloning

GoldenBrick is:

✓ One shot full cassette assembly
✓ BioBrick RFC10 assembly compatible
✓ Golden Gate assembly compatible
GoldenBricks: Accelerating iGEM cloning

GoldenBrick is:
- ✓ One shot full cassette assembly
- ✓ BioBrick RFC10 assembly compatible
- ✓ Golden Gate assembly compatible

New features:
- ✓ DNA shuffling
- ✓ Easy and generic screening
- ✓ Many more...
GoldenBricks: Accelerating iGEM cloning

- H2O
- iGEM plate
- T4 buffer
- T4 ligase
- Bsal
GoldenBricks: Accelerating iGEM cloning

- H2O
- iGEM plate
- T4 buffer
- T4 ligase
- Bsai
GoldenBricks: Accelerating iGEM cloning
GoldenBricks: Accelerating iGEM cloning

37°C

16°C

x 20

Brick 1
AGCG
TCGG
CTCTGG

Brick 2
AGCG
TCGG
GGTCCTC
CCAGAG
GoldenBricks: Accelerating iGEM cloning

- 37°C
- 16°C
- x 20
GoldenBricks: Accelerating iGEM cloning

Transformation

Brick 1: AGCGC
Brick 2: TCCGC
GoldenBricks: Accelerating iGEM cloning

E: EcoRI  B: BsaI  S: SpeI  P: PstI  X: XbaI  Cm: Chloramphenicol  Kan: Kanamycin

Neg. ctrl  T: Terminator

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GoldenBricks: Accelerating iGEM cloning

[Diagram of GoldenBricks with restriction enzyme sites labeled: EcoRI (E), XbaI (X), PstI (P), SpeI (S), BsaI (B).]

Neg. ctrl

Kan

E: EcoRI  B: BsaI
P: PstI  X: XbaI  S: SpeI
GoldenBricks: Accelerating iGEM cloning

EcoRI  XbaI  PstI  SpeI  BsaI

GoldenBricks: Accelerating iGEM cloning

Neg. ctrl  S  5

Kan

E: EcoRI  P: PstI
B: BsaI  X: XbaI  S: SpeI

Saturday, October 6, 12
GoldenBricks: Accelerating iGEM cloning
GoldenBricks: Accelerating iGEM cloning

E: EcoRI  B: BsaI  X: XbaI  P: PstI  S: SpeI

Kan
GoldenBricks: Accelerating iGEM cloning

E: EcoRI  P: PstI
B: BsaI  X: XbaI  S: SpeI
GoldenBricks: Accelerating iGEM cloning

---

**E**: EcoRI  **P**: PstI  
**B**: BsaI  **X**: XbaI  
**S**: SpeI

---

Saturday, October 6, 12
GoldenBricks: Accelerating iGEM cloning
Results:
Results:
Results:

The protocol works, but needs further optimization!
Three new vertebrate backbones

- We biobricked pCS2+ plasmid
- Simple cloning and test procedure
Three new vertebrate backbones

- We biobricked pCS2+ plasmid
- Simple cloning and test procedure
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- We biobricked pCS2+ plasmid
- Simple cloning and test procedure
• We biobricked pCS2+ plasmid
• Simple cloning and test procedure
• Can be used for other vertebrates
• No plasmid replication in *Xenopus*
We biobricked pCS2+ plasmid

Simple cloning and test procedure

Can be used for other vertebrates

No plasmid replication in *Xenopus*

⇒ Transient genetic construction
• We biobricked pCS2+ plasmid

• Simple cloning and test procedure

• Can be used for other vertebrates

• No plasmid replication in *Xenopus*
  
  ➡ Transient genetic construction
  
  ➡ Possibly integrated afterwards
Three new vertebrate backbones

K812000 - Constitutive promoter
CMV prom, fully characterised

CMV promoter
(ubiquitous)
Three new vertebrate backbones

K812000 - Constitutive promoter
CMV prom, fully characterised

J4450 red neg. ctrl.

HindIII

CMV promoter (ubiquitous)

Sall
Three new vertebrate backbones

K812000 - Constitutive promoter
CMV prom, fully characterised
K812000 - Constitutive promoter 
CMV prom, fully characterised
Three new vertebrate backbones

K812000 - Constitutive promoter
CMV prom, fully characterised

K812200 - Pancreas specific
Elastase, result to confirm

K812300 - Heat-shock inducible
HSP70, not characterised yet
Characterization
Plasmid characterization
Plasmid characterization

Plasmid K812000 with the reporter K812031 (sfGFP) injected

Even if ubiquitous promoter (CMV), expression is localized
Plasmid K812000 with the reporter K812010 (GFP-AID) injected

Even if ubiquitous promoter (CMV), expression is localized
Plasmid characterization

Expression of the reporter mCFP (K812032)

Expression of the reporter mCitrine (K812030)

Even if ubiquitous promoter (CMV), expression is localized
Plasmid K812200 (elastase promoter) with the reporter K812031 (sfGFP) injected
Plasmid characterization
Toxicity:
Toxicity:

No effect up to 500 μM of auxin in the water
Absorption:

<table>
<thead>
<tr>
<th></th>
<th>IAA</th>
<th>NAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std.</td>
<td><img src="image1" alt="Graph" /></td>
<td><img src="image2" alt="Graph" /></td>
</tr>
<tr>
<td>0 μM</td>
<td><img src="image3" alt="Graph" /></td>
<td><img src="image4" alt="Graph" /></td>
</tr>
<tr>
<td>250 μM</td>
<td><img src="image5" alt="Graph" /></td>
<td><img src="image6" alt="Graph" /></td>
</tr>
<tr>
<td>500 μM</td>
<td><img src="image7" alt="Graph" /></td>
<td><img src="image8" alt="Graph" /></td>
</tr>
</tbody>
</table>
We experimented chassis to chassis communication

- *E. coli* chassis containing RFP generator or auxin generator
Synthetic ecosystem

• We experimented chassis to chassis communication

• *E. coli* chassis containing RFP generator or auxin generator

Tadpoles fed with bacteria  Control
Conclusions

✓ **Xenopus**: the first vertebrate chassis in iGEM

✓ **New tools** (18 biobricks + 3 plasmids) for SynBio in Xenopus

✓ We designed a **synthetic hormonal system**

✓ We proposed a **new standard** for the Parts Registry

✓ We developed a **modular** and **multi-scale** models for tadpoles

✓ We studied the implications of the introduction of **vertebrates** in iGEM
✓ The path towards synthetic physiology is now open
✓ The path towards synthetic physiology is now open

✓ Future iGEM teams can work with vertebrate systems
Perspectives

✓ The path towards synthetic physiology is now open

✓ Future iGEM teams can work with vertebrate systems

✓ Our hormonal system still needs further characterisation
✓ The path towards synthetic physiology is now open

✓ Future iGEM teams can work with vertebrate systems

✓ Our hormonal system still needs further characterisation

✓ We are going to submit a new RFC for GoldenBricks
A great thanks to our sponsors!