FEEDBACK ARCHITECTURES TO REGULATE FLUX OF COMPONENTS IN ARTIFICIAL GENE NETWORKS

Giulia Giordano
Elisa Franco
Richard M. Murray
We can make **nanoscale** shapes and patterns.

Can we produce and **control** the shape of nanostructures with artificial gene networks?

Nucleic acids (DNA/RNA) self-assemble.
Research motivation

We can build a **complex** system in a biomolecular environment...

**Industrial** assembly

- Arduino microcontroller

**Molecular** assembly

- RNA cube (Afonin et al., Nature Nanotech 2010)
We can build a complex system in a biomolecular environment...

**Industrial** assembly

*Arduino microcontroller*

...and, to **optimize production** process, we want as many components as we need → **scalable flux control architectures** tailored to synthetic gene networks

**Molecular** assembly

*RNA cube (Afonin et al., Nature Nanotech 2010)*
General problem statement
Outline

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- Different output interconnection schemes
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- Positive vs negative feedback schemes: a comparison
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- Hints for a viable DNA strand implementation
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- Different output interconnection schemes
- Positive vs negative feedback schemes: a comparison
- Hints for a viable DNA strand implementation
- Conclusions
Flux regulation: the basic idea

\[ T_i \beta_i R_i + T_i, \quad R_1 + R_2 \rightarrow P \]

Surplus / shortage of one reagent \(\rightarrow\) mismatch
Flux regulation: the basic idea

$$T_i \beta_i R_i + T_i, \quad R_1 + R_2 \rightarrow P$$

Surplus / shortage of one reagent → mismatch

Two strategies can **balance** reagents’ production
Flux regulation: the basic idea

- **negative feedback**
  \[ T_i \xrightarrow{\beta_i} R_i + T_i, \quad R_1 + R_2 \xrightarrow{k} P \]

Surplus / shortage of one reagent → mismatch

Two strategies can balance reagents’ production

\[ T_i^* \xrightarrow{\alpha_i} T_i, \quad [T_i^{\text{tot}}] = [T_i] + [T_i^*] \]

\[ R_i + T_i \xrightarrow{\delta_i} T_i^* \]

\[ [R_i^{\text{tot}}] = [R_i] + [T_i^*] + [P] \]
Flux regulation: the basic idea

- **negative feedback**
  \[ T_i \beta_i R_i + T_i, \quad R_1 + R_2 \xrightarrow{k} P \]

- **positive feedback**
  \[ T_i \alpha_i T_i, \quad [T_i^{\text{tot}}] = [T_i] + [T_i^*] \]
  \[ R_i + T_i \delta_i T_i^* \]
  \[ [R_i^{\text{tot}}] = [R_i] + [T_i^*] + [P] \]

Surplus / shortage of one reagent → mismatch

Two strategies can balance reagents’ production

- **self-repression**
- **cross-activation**
Different output interconnection schemes with $n$ genelets

**single product** connection scheme (always 1 product) →

**neighbor** connection scheme ($n$ products)

**handshake** connection scheme ($\frac{n(n-1)}{2}$ products) →
**Negative feedback - handshake/neighbor**

**Activation:** \( T_i^* \xrightarrow{\alpha_i} T_i \), \( [T_i^{\text{tot}}] = [T_i] + [T_i^*] \)

**Reagents production:** \( T_i \xrightarrow{\beta_i} R_i + T_i \)

**Self–repression:** \( R_i + T_i \xrightarrow{\delta_i} T_i^* \)

**Products formation:** \( R_i + R_j \xrightarrow{k_{ij}} P_{ij} \)

\( [R_i^{\text{tot}}] = [R_i] + [T_i^*] + \sum_j [P_{ij}] \)
Activation: $T_i^* \xrightarrow{\alpha_i} T_i, \quad [T_i^{\text{tot}}] = [T_i] + [T_i^*]$

Reagents production: $T_i \xrightarrow{\beta_i} R_i + T_i$

Self–repression: $R_i + T_i \xrightarrow{\delta_i} T_i^*$

Products formation: $R_i + R_j \xrightarrow{k_{ij}} P_{ij}$

$[R_i^{\text{tot}}] = [R_i] + [T_i^*] + \sum_j [P_{ij}]$

$[T_i]' = \alpha_i ([T_i^{\text{tot}}] - [T_i]) - \delta_i [R_i][T_i]$,  

$[R_i]' = \beta_i [T_i] - \delta_i [R_i][T_i] - \sum_j k_{ij} [R_i][R_j]$,  

$[P_{ij}]' = k_{ij} [R_i][R_j]$
Activation: $T_i^* \alpha_i T_i$, \quad $[T_i^{\text{tot}}] = [T_i] + [T_i^*]$

Reagents production: $T_i \beta_i R_i + T_i$

Self-repression: $R_i + T_i \delta_i T_i^*$

Product formation: $\sum_{i=1}^{n} R_i k P$

$[R_i^{\text{tot}}] = [R_i] + [T_i^*] + [P]$
Activation: \( T^*_i \alpha_i \rightarrow T_i \), \( [T^{\text{tot}}_i] = [T_i] + [T^*_i] \)

Reagents production: \( T_i \beta_i \rightarrow R_i + T_i \)

**Self–repression:** \( R_i + T_i \delta_i \rightarrow T^*_i \)

Product formation: \( \sum_{i=1}^{n} R_i k \rightarrow P \)
\( [R^{\text{tot}}_i] = [R_i] + [T^*_i] + [P] \)

\[
[T_i]' = \alpha_i ([T^{\text{tot}}_i] - [T_i]) - \delta_i [R_i][T_i],
\]

\[
[R_i]' = \beta_i [T_i] - \delta_i [R_i][T_i] - k \prod_{i=1}^{n}[R_i],
\]

\[
[P]' = k \prod_{i=1}^{n}[R_i]
\]
Positive feedback - handshake/neighbor

Inactivation: \( T_i \xrightarrow{\alpha_i} T_i^* \), \( [T_i^{\text{tot}}] = [T_i] + [T_i^*] \)

Reagents production: \( T_i \xrightarrow{\beta_i} R_i + T_i \)

**Cross–activation:** \( R_i + T_j^* \xrightarrow{\delta_{ij}} T_j \)

Products formation: \( R_i + R_j \xrightarrow{k_{ij}} P_{ij} \)

\( [R_i^{\text{tot}}] = [R_i] + \sum_j [T_j] + \sum_j [P_{ij}] \)
Positive feedback - handshake/neighbor

Inactivation: \[ T_i^* = T_i^{\alpha_i} \]  
\[ [T_i^{\text{tot}}] = [T_i] + [T_i^*] \]

Reagents production: \[ T_i \beta_i \rightarrow R_i + T_i \]

Cross–activation: \[ R_i + T_j^* \delta_{ij} \rightarrow T_j \]

Products formation: \[ R_i + R_j^k_{ij} \rightarrow P_{ij} \]  
\[ [R_i^{\text{tot}}] = [R_i] + \sum_j [T_j] + \sum_j [P_{ij}] \]

\[ [T_i]' = -\alpha_i [T_i] + \sum_j \delta_{ij} [R_j]([T_i^{\text{tot}}] - [T_i]) \]

\[ [R_i]' = \beta_i [T_i] - \sum_j k_{ij} [R_i][R_j] - \sum_j \delta_{ji} [R_i]([T_j^{\text{tot}}] - [T_j]) \]

\[ [P_{ij}]' = k_{ij} [R_i][R_j] \]
Inactivation: $T_i \xrightarrow{\alpha_i} T_i^*,$ \quad $[T_i^{\text{tot}}] = [T_i] + [T_i^*]$

Reagents production: $T_i \xrightarrow{\beta_i} R_i + T_i$

$\delta_i$ \quad \text{global cross–activation strength on genelet i due to all the others}

Product formation: $\sum_{i=1}^{n} R_i \xrightarrow{k_i} P$

$[R_i^{\text{tot}}] = [R_i] + \sum_{j \neq i} [T_j] + [P]$
Inactivation: $T_i^\alpha_i T_i^* \rightarrow T_i^\alpha_i T_i^*$, $[T_i^{\text{tot}}] = [T_i] + [T_i^*]$

Reagents production: $T_i^\beta_i R_i + T_i$

$\delta_i$ global cross–activation strength on genelet $i$ due to all the others

Product formation: $\sum_{i=1}^{n} R_i^k \rightarrow P$

$[R_i^{\text{tot}}] = [R_i] + \sum_{j \neq i} [T_j] + [P]$

$[T_i]' = -\alpha_i [T_i] + \delta_i ([T_i^{\text{tot}}] - [T_i]) \prod_{j \neq i} [R_j]$

$[R_i]' = \beta_i [T_i] - k \prod_{i=1}^{n} [R_i] - \delta_i [R_i] \prod_{j \neq i} ([T_j^{\text{tot}}] - [T_j])$

$[P]' = k \prod_{i=1}^{n} [R_i]$
Parameter variation sensitivity

### Negative feedback

![Negative feedback graph](image)

### Positive feedback

![Positive feedback graph](image)

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Feedback flux regulation in gene networks
## Negative vs positive feedback: a comparison

<table>
<thead>
<tr>
<th>Negative feedback schemes</th>
<th>Positive feedback schemes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Negative</strong> feedback</td>
<td><strong>Positive</strong> feedback</td>
</tr>
<tr>
<td>schemes</td>
<td>schemes</td>
</tr>
<tr>
<td>fast</td>
<td>fast</td>
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<tr>
<td>time response</td>
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<tr>
<td>easy</td>
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<tr>
<td>to scale up (each gene</td>
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<td>controls its own production rate)</td>
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<td>loosely speaking, &quot;</td>
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<td>stabilize</td>
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<tr>
<td>the system</td>
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<tr>
<td>not enhance output</td>
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<tr>
<td>production rate</td>
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<tr>
<td>⇒ better to avoid</td>
<td>⇒ better for genes in</td>
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<tr>
<td>accumulation of unused reagents</td>
<td>high demand</td>
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<td>slower</td>
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<tr>
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<tr>
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<tr>
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Negative vs positive feedback: a comparison

Negative feedback schemes
- fast time response

Positive feedback schemes
### Negative vs positive feedback: a comparison

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Negative feedback schemes

- **fast** time response
- **easy** to scale up (each gene controls its own production rate)
- loosely speaking, “stabilize” the system

Positive feedback schemes
Negative feedback schemes

- **fast** time response
- **easy** to scale up (each gene controls its own production rate)
- loosely speaking, “**stabilize**” the system
- **not enhance output** production rate

Positive feedback schemes

- **slow** time response
- **difficult** to scale up (each gene is controlled by others, growing # interactions)
- small feedback constant to avoid unbounded increase
- **maximize output** production rate

⇒ better for genes in high demand
Negative feedback schemes

- **fast** time response
- **easy** to scale up (each gene controls its own production rate)
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- **slower** time response

Negative vs positive feedback: a comparison
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- **slower** time response
- **difficult** to scale up (each gene is controlled by others, growing # interactions)
# Negative vs Positive Feedback: A Comparison

- **Negative feedback schemes**
  - *fast* time response
  - *easy* to scale up (each gene controls its own production rate)
  - loosely speaking, “stabilize” the system
  - *not enhance output* production rate

- **Positive feedback schemes**
  - *slower* time response
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### Negative feedback schemes
- **Fast** time response
- **Easy** to scale up (each gene controls its own production rate)
- Loosely speaking, “**stabilize**” the system
- **Not enhance output** production rate

### Positive feedback schemes
- **Slower** time response
- **Difficult** to scale up (each gene is controlled by others, growing # interactions)
- Small feedback constant to avoid **unbounded increase**
- **Maximize output** production rate
**Negative feedback schemes**
- **fast** time response
- **easy** to scale up (each gene controls its own production rate)
- loosely speaking, “stabilize” the system
- **not enhance output** production rate
  ⇒ better to **avoid accumulation** of unused reagents

**Positive feedback schemes**
- **slower** time response
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- **easy** to scale up (each gene controls its own production rate)
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**Positive feedback schemes**
- **slower** time response
- **difficult** to scale up (each gene is controlled by others, growing # interactions)
- small feedback constant to avoid **unbounded increase**
- **maximize output production rate**

⇒ better for genes in **high demand**
DNA strand implementation - DNA domains

(a) 3 and (b) 4 genelets handshake negative feedback scheme implemented with artificial gene networks (transcriptional circuits)
A new, more detailed model can be built including all the reactions occurring.

\[ n = 3 \]

\[ n = 4 \]
A new, more detailed model can be built including all the reactions occurring

\[ n = 3 \]

Rate–regulation effective
⇒ circuits can be experimentally implemented and tested
Conclusions

- Synthetic $n$–gene systems: regulate production of RNA species interacting to produce complexes
Conclusions

- **Synthetic** $n$–gene systems: *regulate production* of RNA species interacting to produce complexes.

- **Numerical analysis** of negative / positive feedback architectures.
Conclusions

- Synthetic \( n \)-gene systems: regulate production of RNA species interacting to produce complexes
- Numerical analysis of negative / positive feedback architectures
- Negative autoregulation: better scalability and faster response
Conclusions

- Synthetic $n$–gene systems: *regulate production* of RNA species interacting to produce complexes

- **Numerical analysis** of negative / positive feedback architectures

- **Negative** autoregulation: better *scalability* and *faster* response

- **Viable DNA strand implementation** of negative–autoregulated 3 and 4 gene systems: *good performance*
Conclusions

- Synthetic \( n \)-gene systems: regulate production of RNA species interacting to produce complexes

- **Numerical analysis** of negative / positive feedback architectures

- **Negative** autoregulation: better **scalability** and faster response

- **Viable DNA strand implementation** of negative–autoregulated 3 and 4 gene systems: good performance

- Predictions useful for future experiments
Synthetic $n$–gene systems: *regulate production* of RNA species interacting to produce complexes

Numerical analysis of negative / positive feedback architectures

Negative autoregulation: better *scalability* and faster response

Viable DNA strand implementation of negative–autoregulated 3 and 4 gene systems: *good performance*

Predictions *useful for future experiments*

Thank you!