sRNA triggered translational OFF switch

Sven Findeiß

Bioinformatics Group and Interdisciplinary Center for Bioinformatics,
Department of Computer Science,
University Leipzig

34th TBI Winterseminar

February, 2019
Design Idea

sRNA \( (y) \)
- leading A for efficient transcription
- apply terminator design principles\(^1\)

Interaction
- optimize for stable interaction
  without disrupting other constraints

5’ UTR \( (x) \)
- result from another design attempt
- high reporter gene expression

\[
f(xy) = 0.01 \times (G(y|\Phi_{\text{terminator}}) - G(y|\Phi))^2 \\
+ (G(xy|\phi_{\text{unbound}}) + G(xy|\phi_{\text{bound}}) - 2 \cdot G(xy|\Phi))
\]


Design Idea

sRNA \( (y) \)
- leading A for efficient transcription
- apply terminator design principles\(^{[1]}\)

Interaction
- optimize for stable interaction without disrupting other constraints

5’ UTR \( (x) \)
- result from another design attempt
- high reporter gene expression

\[
f(xy) = 0.01 \times (G(y|\Phi_{\text{terminator}}) - G(y|\Phi))^2 + (G(xy|\phi_{\text{unbound}}) + G(xy|\phi_{\text{bound}}) - 2 \cdot G(xy|\Phi))
\]


**Design Idea**

sRNA ($y$)
- leading A for efficient transcription
- apply terminator design principles\[^1\]

Interaction
- optimize for stable interaction
  without disrupting other constraints

5’ UTR ($x$)
- result from another design attempt
- high reporter gene expression

$$f(xy) = 0.01 \times \left( G(y|\Phi_{\text{terminator}}) - G(y|\Phi) \right)^2 + \left( G(xy|\phi_{\text{unbound}}) + G(xy|\phi_{\text{bound}}) - 2 \cdot G(xy|\Phi) \right)$$


Design Idea

sRNA \((y)\)
- leading A for efficient transcription
- apply terminator design principles\[^1\]

Interaction
- optimize for stable interaction without disrupting other constraints

5’ UTR \((x)\)
- result from another design attempt
- high reporter gene expression

\[
f(xy) = 0.01 \times \left( G(y|\Phi_{\text{terminator}}) - G(y|\Phi) \right)^2 + \left( G(xy|\phi_{\text{unbound}}) + G(xy|\phi_{\text{bound}}) - 2 \cdot G(xy|\Phi) \right)
\]


Design Idea

sRNA \((y)\)
- leading A for efficient transcription
- apply terminator design principles\(^1\)

Interaction
- optimize for stable interaction without disrupting other constraints

5’ UTR \((x)\)
- result from another design attempt
- high reporter gene expression

\[
f(xy) = 0.01 \times \left( G(y|\Phi_{\text{terminator}}) - G(y|\Phi) \right)^2 \\
+ \left( G(xy|\phi_{\text{unbound}}) + G(xy|\phi_{\text{bound}}) - 2 \cdot G(xy|\Phi) \right)
\]


sRNA ($y$)

- leading A for efficient transcription
- apply terminator design principles\(^1\)

Interaction

- optimize for stable interaction without disrupting other constraints

5’ UTR ($x$)

- result from another design attempt
- high reporter gene expression

\[
f(xy) = 0.01 \times (G(y|\Phi_{\text{terminator}}) - G(y|\Phi))^2 \\
+ (G(xy|\phi_{\text{unbound}}) + G(xy|\phi_{\text{bound}}) - 2 \cdot G(xy|\Phi))
\]


Design Idea

**sRNA (y)**
- leading A for efficient transcription
- apply terminator design principles\(^1\)

**Interaction**
- optimize for stable interaction
- without disrupting other constraints

**5' UTR (x)**
- result from another design attempt
- high reporter gene expression

\[
f(xy) = 0.01 \times (G(y|\Phi_{\text{terminator}}) - G(y|\Phi))^2 \\
+ (G(xy|\phi_{\text{unbound}}) + G(xy|\phi_{\text{bound}}) - 2 \cdot G(xy|\Phi))
\]

---


Experimental Results I

\[
\text{Effect} = \frac{\langle \text{OFF} \rangle}{\langle \text{ON} \rangle} = p_u \cdot \langle H \rangle + p_b \cdot \langle L \rangle
\]

If \( \langle H \rangle = 800, \langle L \rangle = 50 \) and Effect = 67% \( \Rightarrow \) 35% of all targets are bound.

\[
f(xy) = 0.01 \times \left( G(y|\Phi_{\text{terminator}}) - G(y|\Phi) \right)^2
+ \left( G(xy|\phi_{unbound}) + G(xy|\phi_{bound}) - 2 \cdot G(xy|\Phi) \right)
\]
Experimental Results I

\[
\text{Effect} = \frac{\langle OFF \rangle}{\langle ON \rangle} = p_u \cdot \langle H \rangle + p_b \cdot \langle L \rangle
\]

If \( \langle H \rangle = 800, \langle L \rangle = 50 \text{ and Effect} = 67\% \Rightarrow 35\% \text{ of all targets are bound} \]

\[
f(xy) = 0.01 \times (G(y|\Phi_{\text{terminator}}) - G(y|\Phi))^2 + (G(xy|\phi_{\text{unbound}}) + G(xy|\phi_{\text{bound}}) - 2 \cdot G(xy|\Phi))
\]
Experimental Results I

\[ \text{Effect} = \frac{\langle \text{OFF} \rangle}{\langle \text{ON} \rangle} = p_u \cdot \langle H \rangle + p_b \cdot \langle L \rangle \]

If \( \langle H \rangle = 800, \langle L \rangle = 50 \) and
\[ \text{Effect} = 67\% \]
\[ \Rightarrow \text{35\% of all targets are bound} \]

\[ f(xy) = 0.01 \times (G(y|\Phi_{\text{terminator}}) - G(y|\Phi))^2 + (G(xy|\phi_{\text{unbound}}) + G(xy|\phi_{\text{bound}}) - 2 \cdot G(xy|\Phi)) \]
Experimental Results I

Effect [%] = \frac{\langle OFF \rangle}{\langle ON \rangle} = p_u \cdot \langle H \rangle + p_b \cdot \langle L \rangle \over \langle H \rangle

If \langle H \rangle = 800, \langle L \rangle = 50 and Effect = 67% ⇒ 35% of all targets are bound

f(xy) = 0.01 \times (G(y|\Phi_{\text{terminator}}) - G(y|\Phi))^2
+ (G(xy|\phi_{\text{unbound}}) + G(xy|\phi_{\text{bound}}) - 2 \cdot G(xy|\Phi))
Design Idea

sRNA \((y)\)
- leading A for efficient transcription
- apply terminator design principles\(^{[1]}\)

Interaction
- optimize for stable interaction without disrupting other constraints

5’ UTR \((x)\)
- result from another design attempt
- high reporter gene expression

\[
\begin{align*}
 f(xy) &= 0.01 \times (G(y|\Phi_{\text{terminator}}) - G(y|\Phi))^2 \\
 &\quad + (G(xy|\Phi_{\text{unbound}}) + G(xy|\Phi_{\text{bound}}) - 2 \cdot G(xy|\Phi))
\end{align*}
\]


Design Idea

sRNA \( (y) \)
- leading A for efficient transcription
- apply terminator design principles\(^{[1]}\)

Interaction
- optimize for stable interaction
- without disrupting other constraints

5' UTR \( (x) \)
- result from another design attempt
- high reporter gene expression

\[
f(xy) = 0.01 \times (G(y|\Phi_{\text{terminator}}) - G(y|\Phi))^2 \\
+ (G(xy|\phi_{\text{unbound}}) + G(xy|\phi_{\text{bound}}) - 2 \cdot G(xy|\Phi))
\]

Design Idea

\[ f(xy) = (1 - P(y | \Phi_{unpaired})) + \left(1 - \frac{[xy_{bound}]}{[x]_0}\right) \]

sRNA \((y)\)
- leading A for efficient transcription
- apply terminator design principles\(^\text{[1]}\)

Interaction
- optimize for stable interaction
  without disrupting other constraints

5' UTR \((x)\)
- result from another design attempt
- high reporter gene expression

---


If $\langle H \rangle = 800$, $\langle L \rangle = 50$ and Effect = 23% 
\Rightarrow 82\%$ of all targets are bound

Extend the binding region \rightarrow confusing results

\[ f(D50.1.6) = (1 - P(y|\Phi_{unpaired})) + \left(1 - \frac{xy_{bound}}{[x]_0}\right) \]

\[ f(D50.1.K) = (1 - P(y|\Phi_{unpaired})) + \left(1 - \frac{[x]}{[x]_0}\right) \]
If $\langle H \rangle = 800, \langle L \rangle = 50$ and Effect $= 23\%$

$\Rightarrow 82\%$ of all targets are bound

Extend the binding region
$\Rightarrow$ confusing results

$$f(D50.1.6) = (1 - P(y|\Phi_{unpaired})) + \left(1 - \frac{[xy_{\text{bound}}]}{[x]}_0\right)$$

$$f(D50.1.K) = (1 - P(y|\Phi_{unpaired})) + \left(1 - \frac{[x]}{[x]}_0\right)$$
If $\langle H \rangle = 800$, $\langle L \rangle = 50$ and Effect = 23% 
$\Rightarrow$ 82% of all targets are bound

Extend the binding region → confusing results

$$f(D50.1.6) = (1 - P(y|\Phi_{unpaired})) + \left(1 - \frac{[xy_{bound}]}{[x]_0} \right)$$

$$f(D50.1.K) = (1 - P(y|\Phi_{unpaired})) + \left(1 - \frac{[x]}{[x]_0} \right)$$
If $\langle H \rangle = 800$, $\langle L \rangle = 50$ and Effect = 23%  
$\Rightarrow$ 82% of all targets are bound

Extend the binding region  
$\rightarrow$ confusing results

$$f(D50.1.6) = (1 - P(y|\Phi_{unpaired})) + \left(1 - \frac{[xy_{bound}]}{[x]_0}\right)$$
$$f(D50.1.K) = (1 - P(y|\Phi_{unpaired})) + \left(1 - \frac{[x]}{[x]_0}\right)$$
If $\langle H \rangle = 800$, $\langle L \rangle = 50$ and Effect = 23% \Rightarrow 82\%$ of all targets are bound

Extend the binding region \rightarrow confusing results

\[
f(D50.1.6) = (1 - P(y|\Phi_{unpaired})) + \left(1 - \frac{[xy_{bound}]}{[x]_0}\right)
\]
\[
f(D50.1.K) = (1 - P(y|\Phi_{unpaired})) + \left(1 - \frac{[x]}{[x]_0}\right)
\]
If $\langle H \rangle = 800$, $\langle L \rangle = 50$ and Effect $= 23\%$

$\Rightarrow$ 82% of all targets are bound

Extend the binding region $\rightarrow$ confusing results

$$f(D50.1.6) = (1 - P(y|\Phi_{unpaired})) + \left(1 - \frac{[xy_{bound}]}{[x]_0}\right)$$

$$f(D50.1.K) = (1 - P(y|\Phi_{unpaired})) + \left(1 - \frac{[x]}{[x]_0}\right)$$
If $\langle H \rangle = 800$, $\langle L \rangle = 50$ and Effect = 23% \[ \Rightarrow 82\% \text{ of all targets are bound} \]

Extend the binding region \[ \rightarrow \text{confusing results} \]

\[
f(D50.1.6) = (1 - P(y|\Phi_{unpaired})) + \left(1 - \frac{[xy_{bound}]}{[x]_0}\right)
\]
\[
f(D50.1.K) = (1 - P(y|\Phi_{unpaired})) + \left(1 - \frac{[x]}{[x]_0}\right)
\]
Open Questions

1. Does the synthetic terminator really work?
2. How to verify D50.1.6 regulation?
   - How to verify the interaction?
   - Is it possible to measure duplex concentration?
3. What is wrong with the assumption: “The longer the interaction the more stable it is?”
Open Questions

1. Does the synthetic terminator really work?

2. How to verify D50.1.6 regulation?
   - How to verify the interaction?
   - Is it possible to measure duplex concentration?

3. What is wrong with the assumption:
   “The longer the interaction the more stable it is?”
Termination

PAA-Gel
Blot, Sonde D50.1+SynT (486/1002)

EVC  D50.1  D50.1-K

200 nt
100 nt

Full-length (+T7-Terminator)

SynT-terminiert (ohne T7-Terminator)

PAA-Gel
Blot, Sonde D50.1-K + SynT (998/1002)

D50.1-K  D50.1  EVC

Full-length (+T7-Terminator)

SynT-terminiert (ohne T7-Terminator)
Open Questions

1. Does the synthetic terminator really work?
2. How to verify D50.1.6 regulation?
   - How to verify the interaction?
   - Is it possible to measure duplex concentration?
3. What is wrong with the assumption: “The longer the interaction the more stable it is?”
Open Questions

1. Does the synthetic terminator really work?

2. How to verify D50.1.6 regulation?
   - How to verify the interaction?
   - Is it possible to measure duplex concentration?

3. What is wrong with the assumption:
   “The longer the interaction the more stable it is?”
Open Questions

1. Does the synthetic terminator really work?
2. How to verify D50.1.6 regulation?
   - How to verify the interaction?
   - Is it possible to measure duplex concentration?
3. What is wrong with the assumption: “The longer the interaction the more stable it is?”
Thanks to...

lab members:
- Stefan Hammer
- Felix Kühnl
- Peter F. Stadler
- Manuela Geiß
- Petra Pregel
- Jens Steuck

collaborators:
- Anna Ender, Leipzig
- Chris Günzel, Leipzig
- Mario Mörl, Leipzig
- Christina Weinberg, Leipzig
- Ilka Axmann, Düsseldorf
- Alice Pavlovski, Düsseldorf
- Sebastian Will, Vienna
- Christoph Flamm, Vienna
- Ivo L. Hofacker, Vienna
- Yann Ponty, Palaiseau
- Michael Ryckelynck, Strasbourg
Terminator Efficiency Estimation

SynT efficiency = 88%

\[ \rho_t = \frac{M_{S1} - M_{S3}}{M_{S1} - M_{S3}} \]