# Biophysical Constraints Arising from Compositional Context in Synthetic Gene Networks

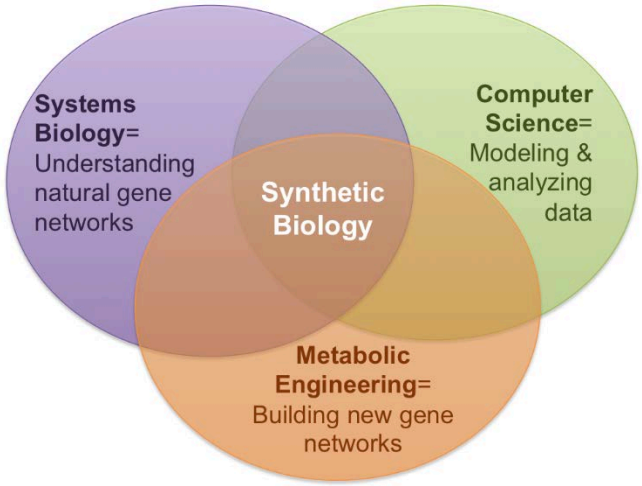
**Enoch Yeung, Aaron J. Dy, Kyle B. Martin, Andrew H. Ng, Domitilla Del Vecchio, James L. Beck, James J. Collins, and Richard M. Murray**

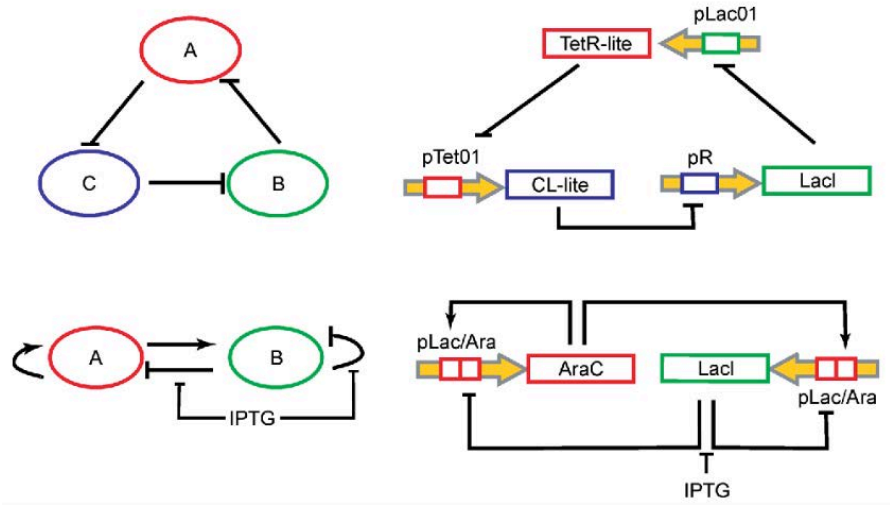
*Cell Systems*
Issue 5, 26 July 2017, Pages 11-24

Yun DENG

7th May, 2020

# Synthetic gene networks



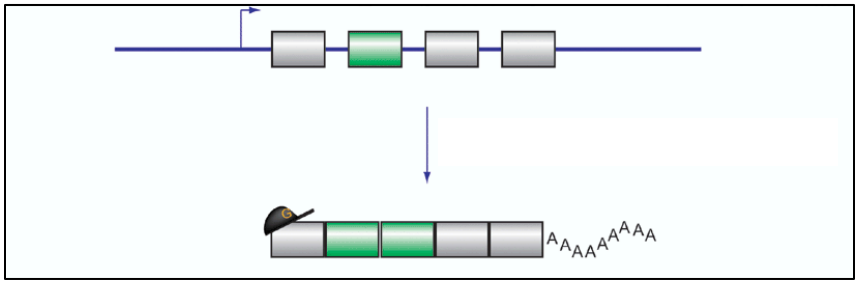

**Synthetic biology** is the application/mixture of system biology, metabolic engineering and computer science based on the deep understanding of gene networks.

**Gene networks** are networks of genes, proteins, small molecules and their regulatory interactions which are involved in the biological process.

*Menn, Su & Wang, Quan Bio, 2017*

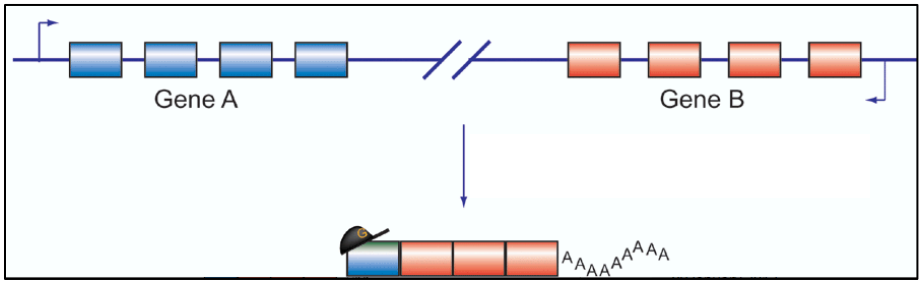
# "Compositional context" of genes

## Intragenic compositional context



- Promoter structure
- Spacing regions between promoter and coding sequences
- Ribosome binding sites etc.
- Be used for circuits design

## Intergenic compositional context




- Spatial arrangement
- Orientation of entire genes on DNA
- Circuits design and performance?

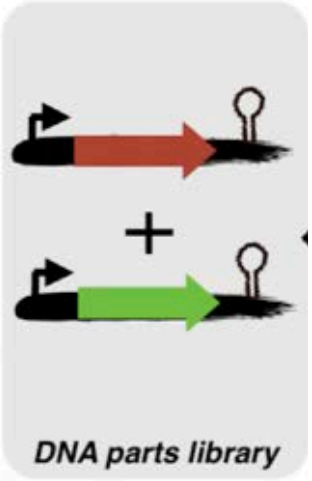
↓

"**Torsional stress**": introduced by unwinding DNA during transcription introduces supercoiling (mechanism for global gene regulation in natural biological system)

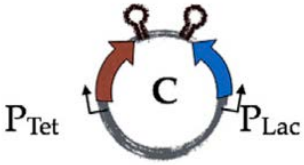
*Horiuchi & Aigaki, 2012 Biol. Cell.*

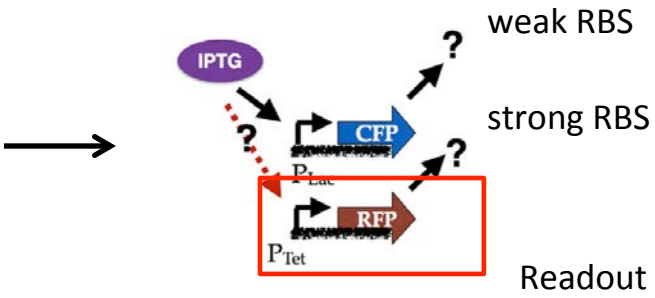
# Outline of the paper

***To systematically examine the effects of intergenic compositional context***

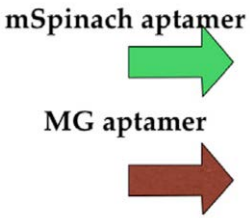



Example plasmid:

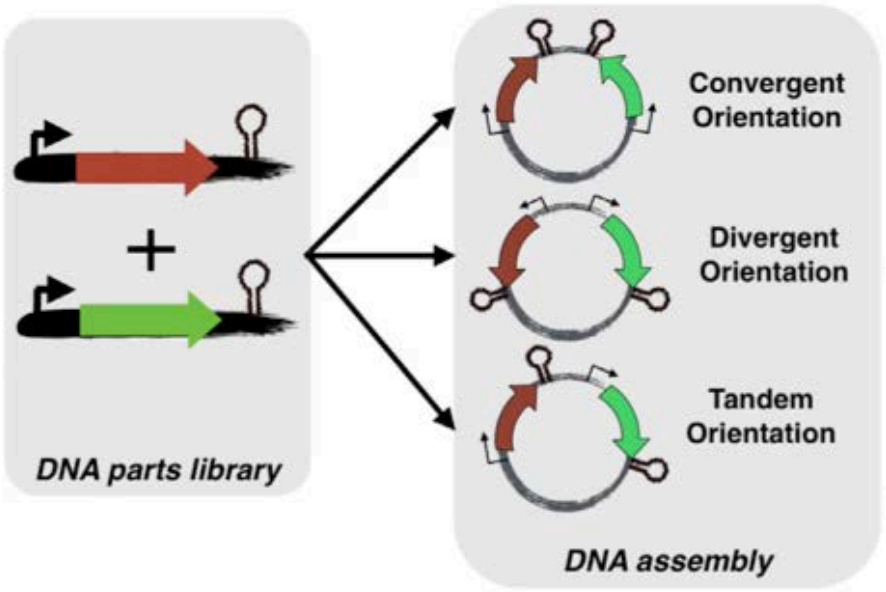



- Inducible promoter: Lac (IPTG) or Tet (ATC) promoter
- A corresponding fluorescent reporter: CFP or RFP

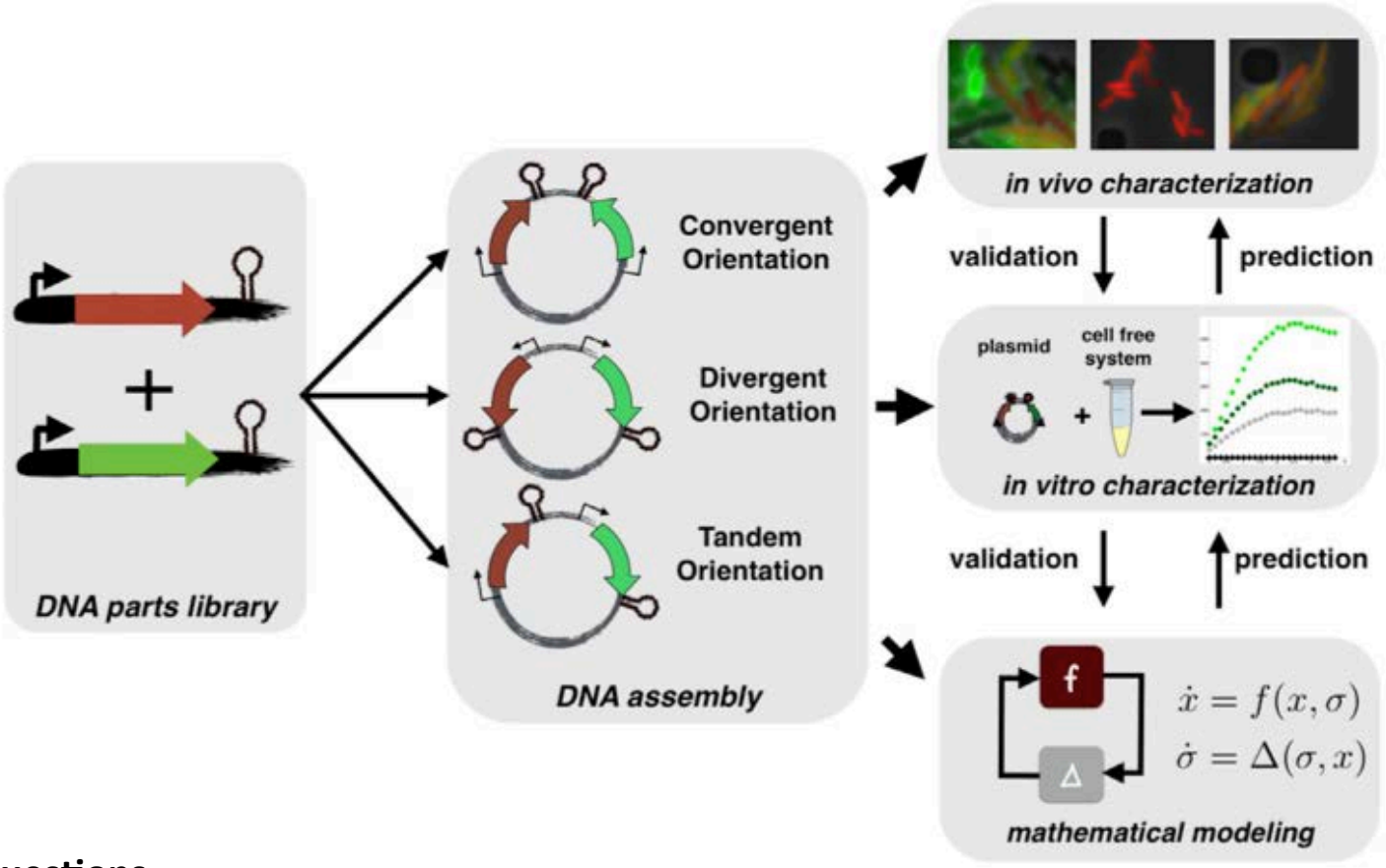


# Outline of the paper




Comparison of gene expression arranged in ***convergent***, ***divergent*** *or* ***tandem orientations***.

# Outline of the paper

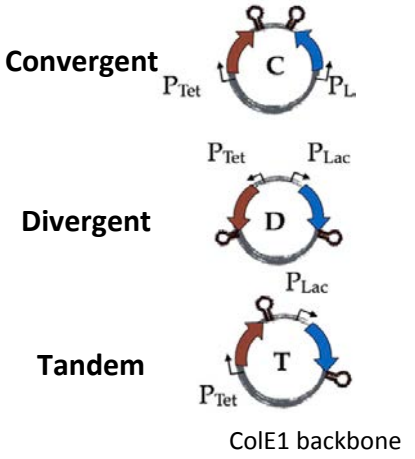



**Research Questions**

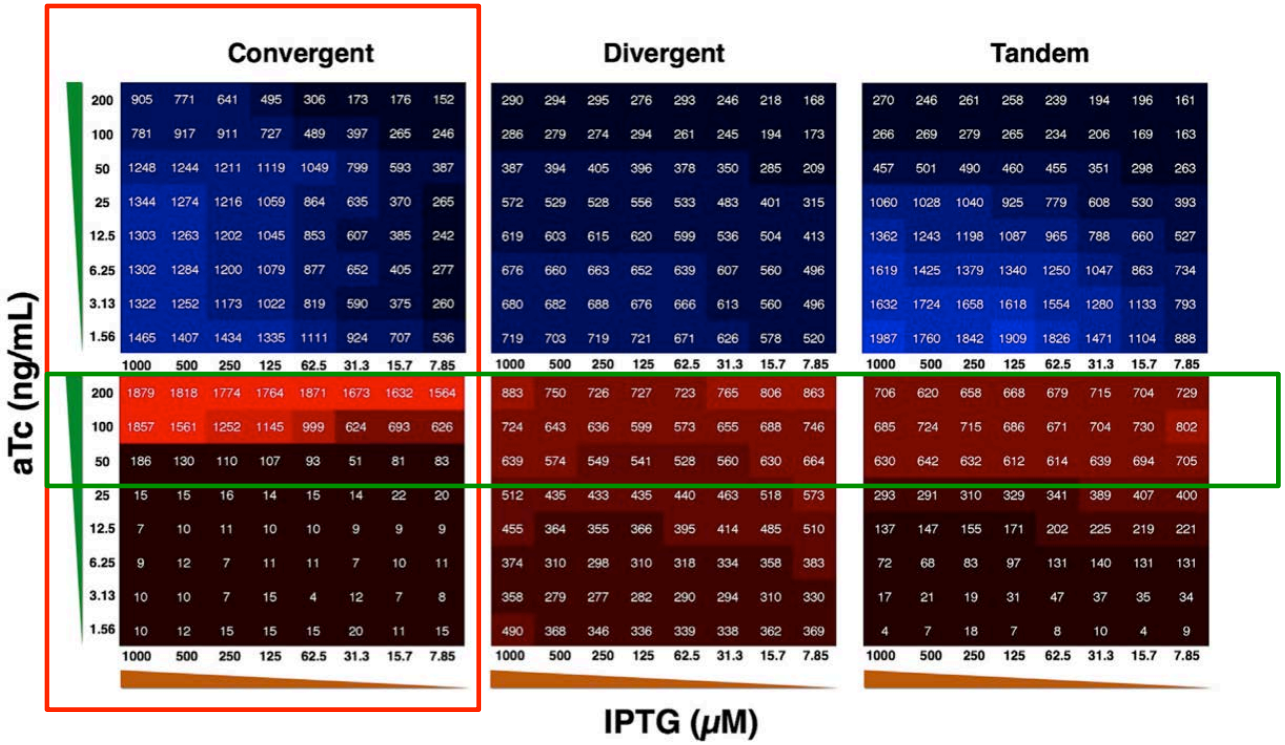
1. How compositional context is going to affect synthetic gene expression?
2. Whether supercoiling can influence the compositional context effect?
3. How to use the compositional context information to better design the synthetic gene networks?

1. To investigate how the compositional context affects the synthetic gene expression?

A. Induction Response




ColE1 backbone



1. To investigate how the compositional context affects the synthetic gene expression?

A. Induction Response

**Hill function**

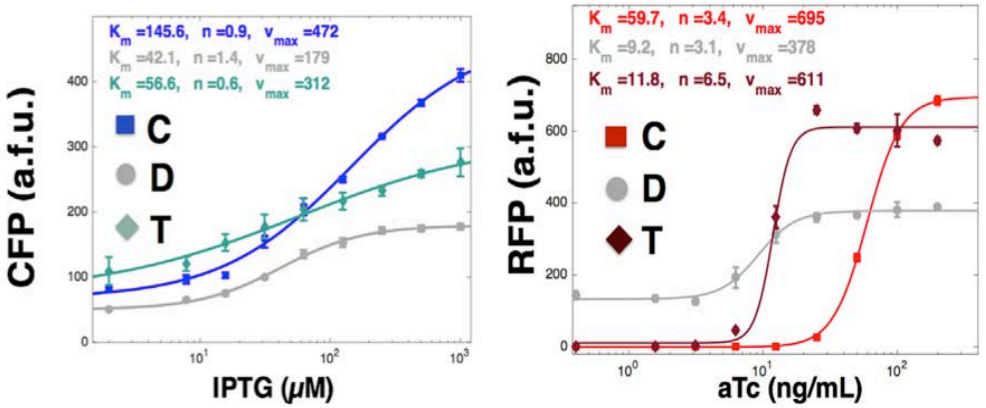
Inducer concentration

Amplitude of expression due to inducer

$$f([I]) = l + \alpha \frac{[I]^n}{K_m^n + [I]^n},$$

Leaky expression

Con. at which induction is half maximal

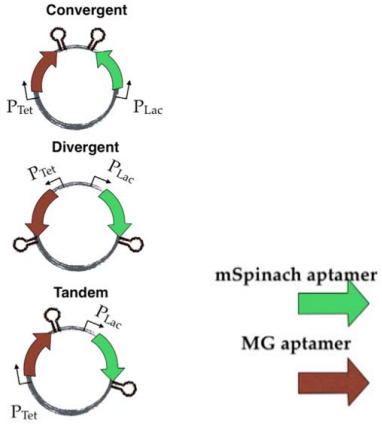


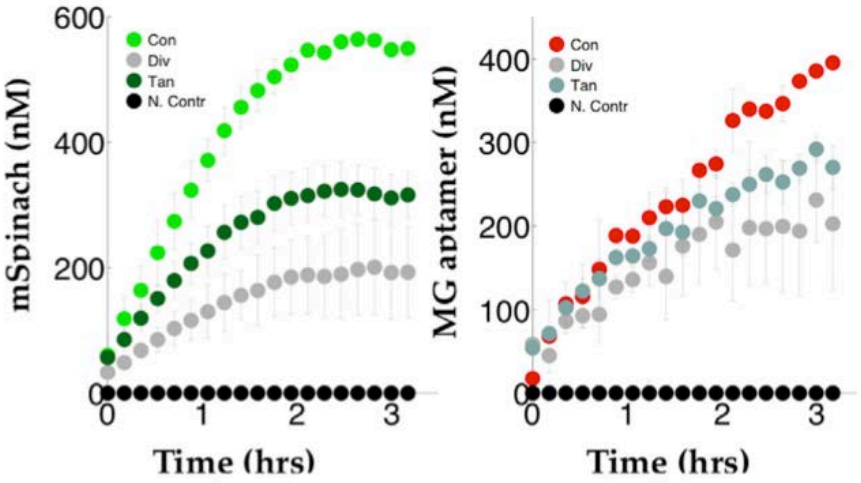

$$V_{max} = l + \alpha$$

Intergenic compositional context alters key features of gene induction, such as dynamic range, maximum expression, and the activation threshold.

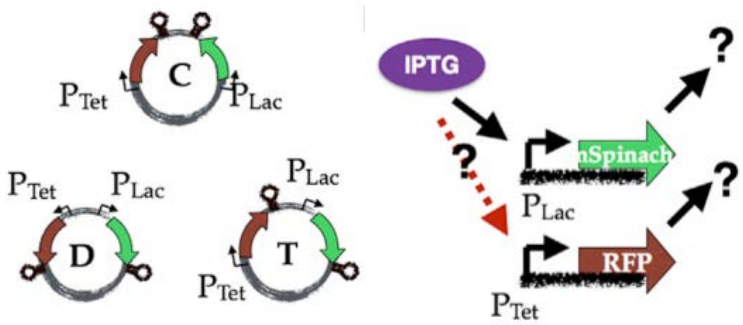
1. To investigate how the compositional context affects the synthetic gene expression?

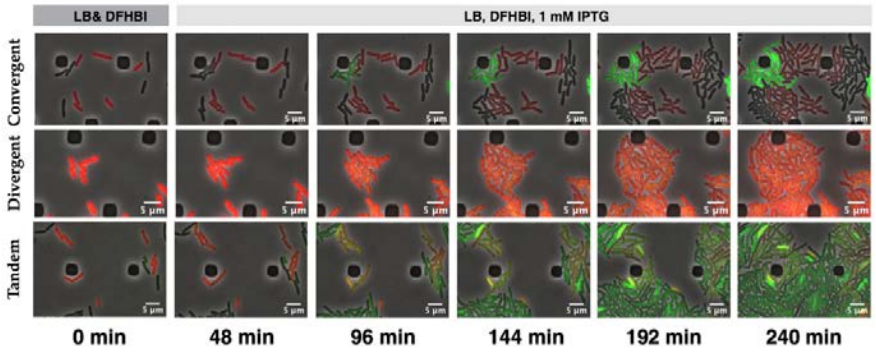
B. Gene transcription




C. Regulatory effects



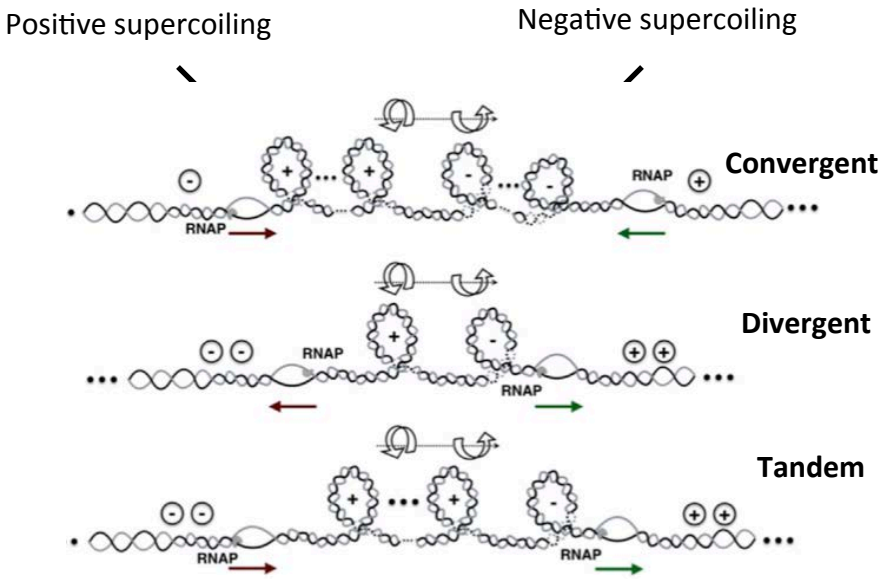




Intergenic compositional context alters gene induction in multiple levels.

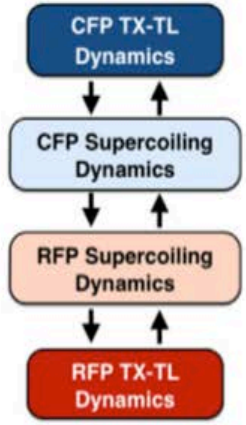
2. To investigate whether supercoiling can influence the compositional context effect?

**Positive supercoiling**

- Torsional stress: naturally oppose the left-handed twist of DNA
- Can be exacerbated by the presence of topological barrier, e.g: DNA binding proteins etc.
- Downstream of transcription bubble-> further impose the resistance against unwinding of DNA
- Reduces initial rate of gene transcription



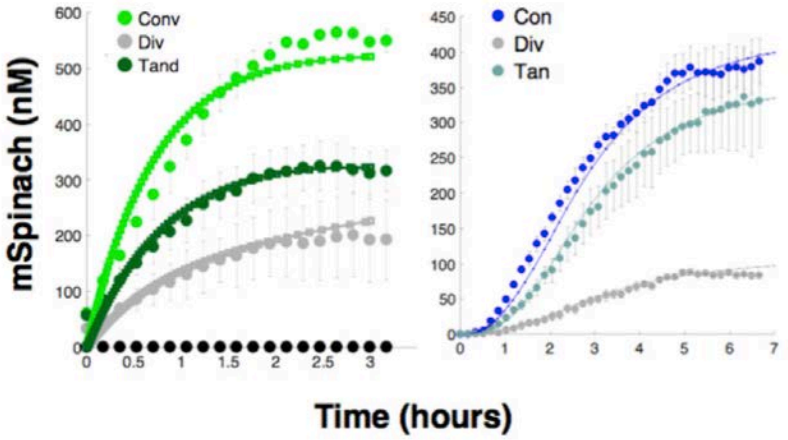
2. To investigate whether supercoiling can influence the compositional context effect?

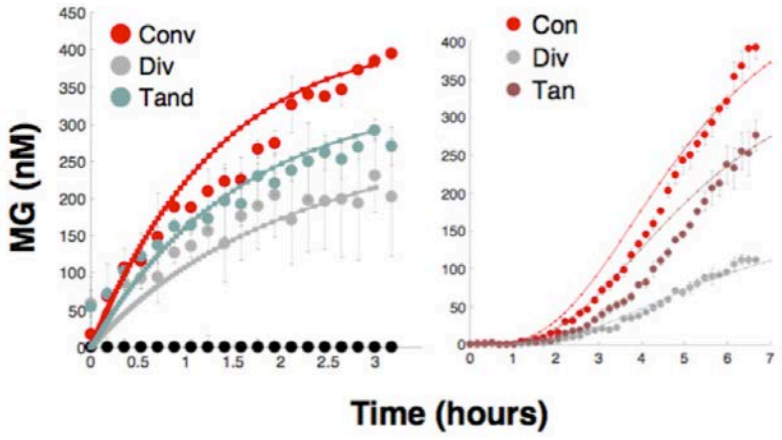



$$\varnothing \xrightarrow{\rho_L} LacI$$
$$LacI \xrightarrow{\delta_p} \varnothing$$
$$LacI + IPTG \underset{k_{ua,\,L}}{\overset{k_{a,\,L}}{\rightleftharpoons}} aLacI$$
$$p_{Lac} + LacI \underset{k_{u,\,L}}{\overset{k_{seq,\,L}}{\rightleftharpoons}} pLacC$$
$$pLacC + IPTG \xrightarrow{k_{a,L}} pLac + aLacI$$
$$R + p_{Lac} \underset{k_r}{\overset{k_f(\sigma_{p,\,S})}{\rightleftharpoons}} CCs$$
$$CC_S \xrightarrow{k_{open}} EC_S$$
$$EC_S \xrightarrow{k_{cat}(\sigma_{t,s})} mS + R + p_{Lac}$$
$$mS \xrightarrow{\delta_m} \varnothing$$

**Factors being considered (ODE model):**

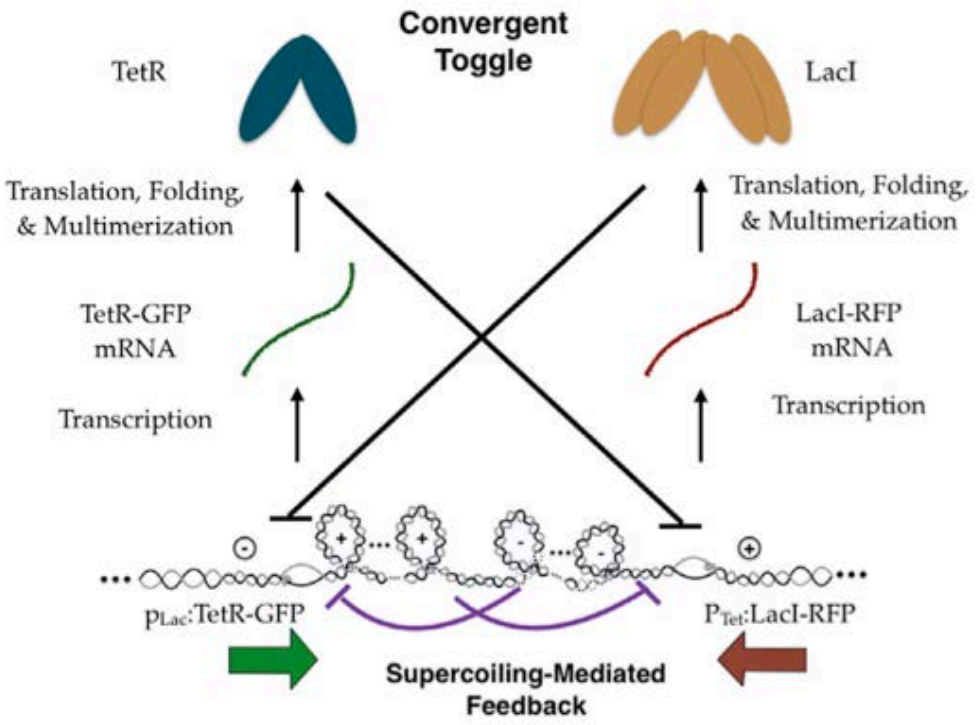
- Local supercoiling density ($\sigma_x$)
- Elongation complex formed (ECx)
- RNA polymerase (R)
- Length of the Plac and PTet promoter (PLx)




Convergent orientation is able to achieve higher levels of expression than its divergent and tandem counterparts

3. How to use compositional context to improve the synthetic gene network design – Toggle switch



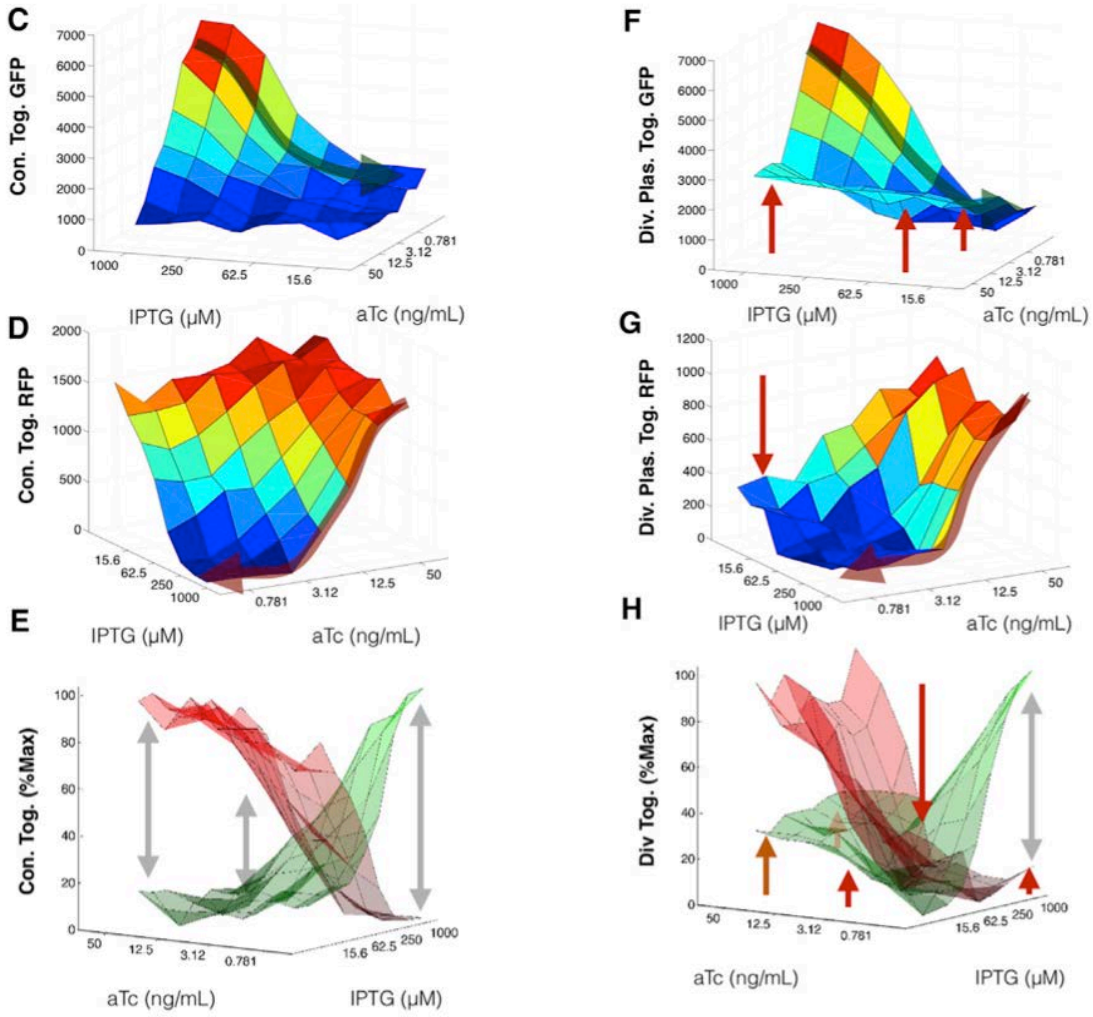

**Advantages:**

- Competing dynamics of positive and negative supercoils between the two genes -> mutual negative feedback
- The co-expression of two genes in convergent orientation were strongly anti-correlated (shown in the previous experiments)

3. How to use compositional context to improve the synthetic gene network design – Toggle switch

Convergent toggle

Divergent toggle




Convergent toggle shows clear separation between high-GFP-low-RFP states and low-GFP-high-RFP states in both of these parameter regimes.

# Take home messages:

- They showed that **intergenic context** creates significant differences in expression consistently across all these design variables.

  - Orientation affects gene expression here with convergent orientation gives highest expression, ultrasensitivity and dynamic range.

- **Supercoiling** (transcriptional interference) is responsible for the observed intergenic context effects and recapitulate the phenomenon in a mathematical model of gene expression incorporating supercoiling.

- Intergenic compositional context effects can be leveraged to design and build a new improved version of the **toggle switch** synthetic gene circuit.

**Thanks for your attention!**
**Questions?**