An E.coli Cell-Free Expression Toolbox: Application to Synthetic Gene Circuits and Artificial Cells

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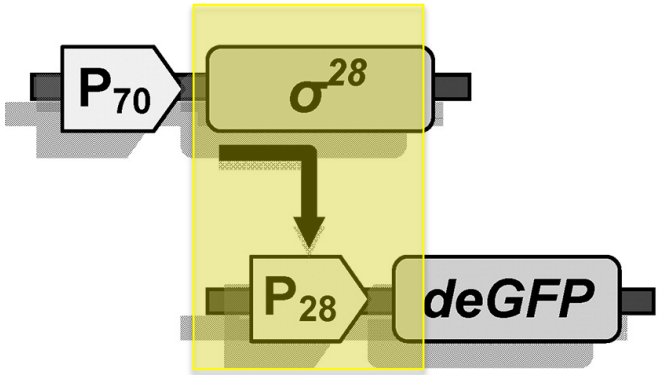
Background

- Transcriptional activation unit
  - Transcriptional activation protein + specific promoter



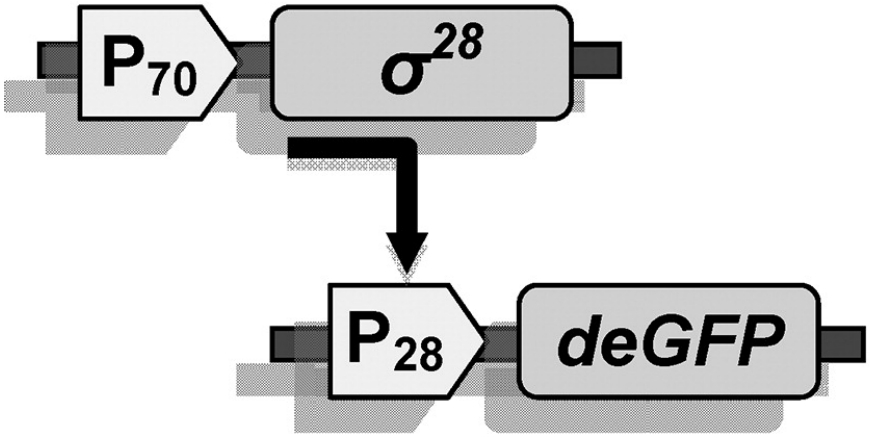
- deGFP: destabilized GFP
- Batch reaction: carried out in a vesicle with all necessary components present

Overview

- Purpose: Create genetic circuits, including cascades, AND gates, and negative feedback loops in a cell-free environment

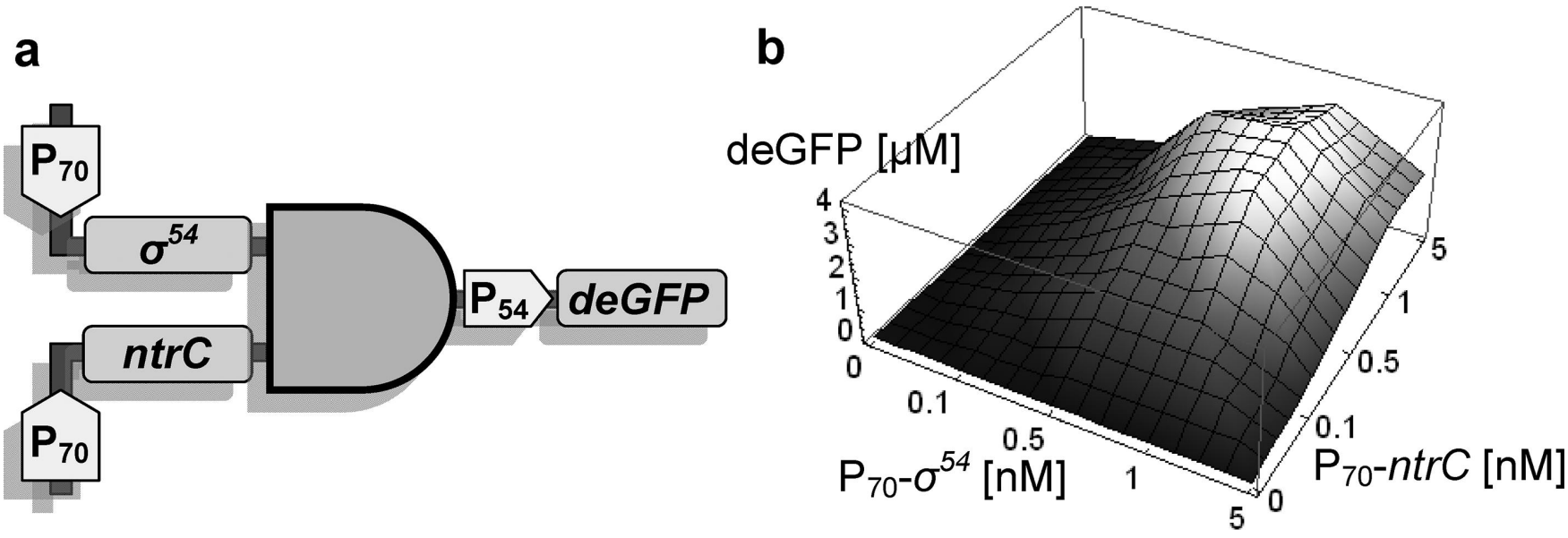
I. Setup of system
II. Transcriptional activation units
    a) Transcription Co-activation: AND gate system
    b) Competition induced transcription regulation
    c) Transcriptional activation cascade: series circuit
    d) Parallel circuits
III. Transcriptional repression units
IV. Long-lived cell-free expression

Setup of System

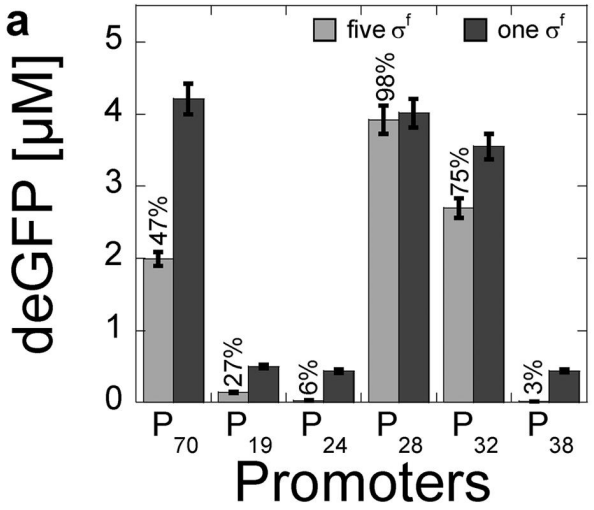


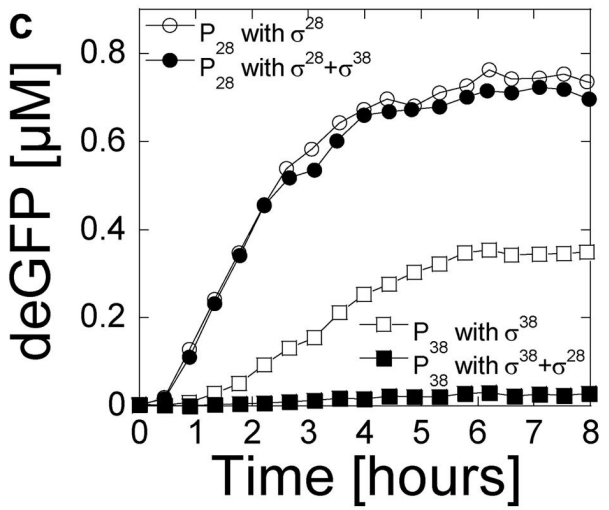
deGFP [μM]		Transcription factor expressed							
		(σ^{70})*	σ^{19}	σ^{24}	σ^{28}	σ^{32}	σ^{38}	T3RNAP	T7RNAP
Promoters	P_{70}	6.40	5.89	5.45	3.96	4.74	6.01	6.06	5.76
	P_{19}	< 0.01	0.89	0.01	0.02	< 0.01	0.01	< 0.01	< 0.01
	P_{24}	0.01	0.03	0.74	< 0.01	0.01	0.03	0.01	0.01
	P_{28}	< 0.01	0.02	< 0.01	8.06	< 0.01	< 0.01	< 0.01	< 0.01
	P_{32}	0.20	0.21	0.20	0.12	5.44	0.20	0.20	0.22
	P_{38}	< 0.01	0.13	0.03	0.01	< 0.01	0.83	0.01	< 0.01
	P_{T3}	0.02	0.02	0.03	< 0.01	0.02	0.03	13.44	0.02
	P_{T7}	0.02	0.03	0.02	< 0.01	< 0.01	0.02	0.02	17.91

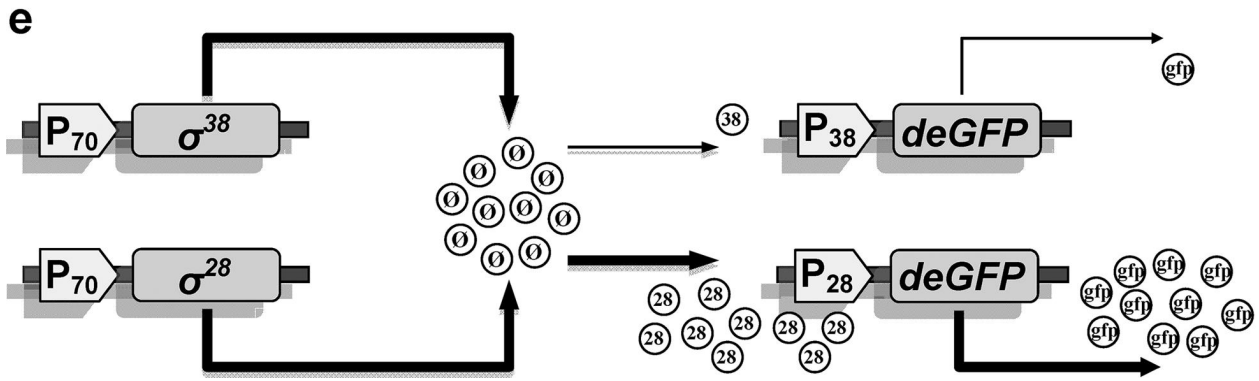
Transcriptional Activation Units
Transcription Co-activation: AND Gate



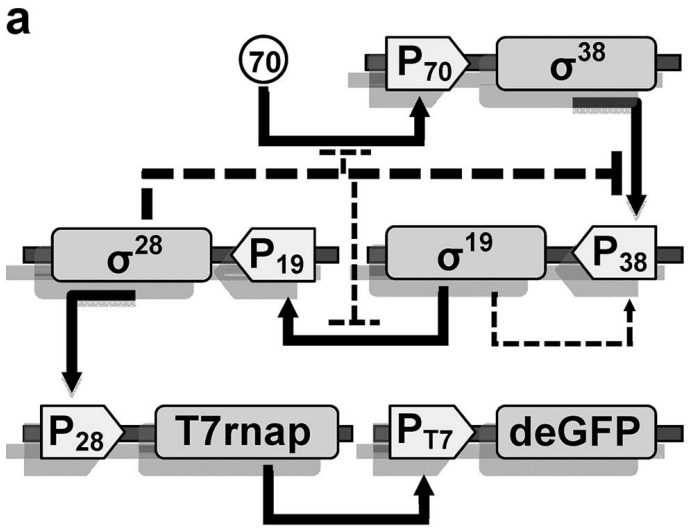
Transcriptional Activation Units
Competition-Induced Transcription Regulation

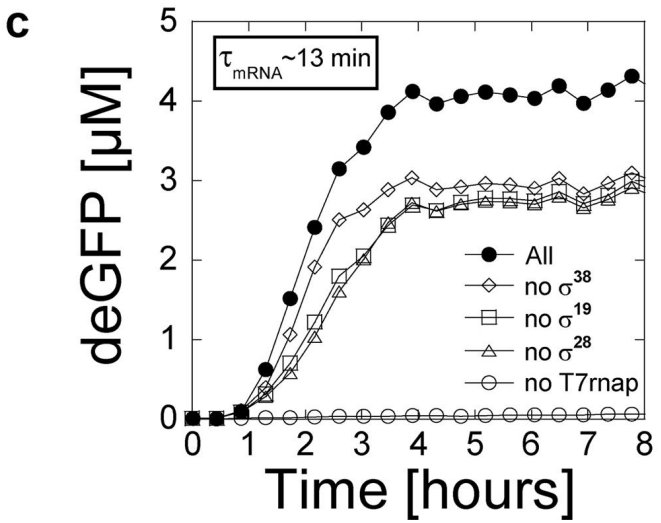


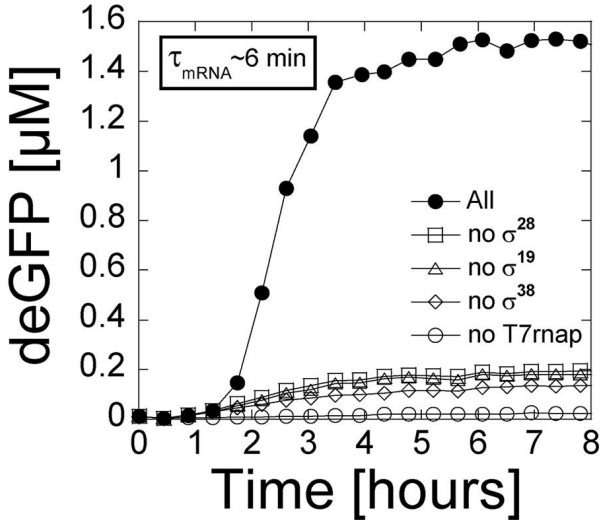




Transcriptional Activation Cascade: Series Circuit

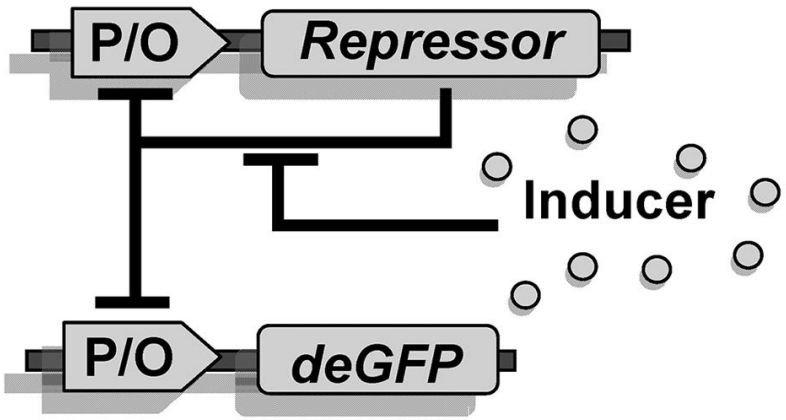






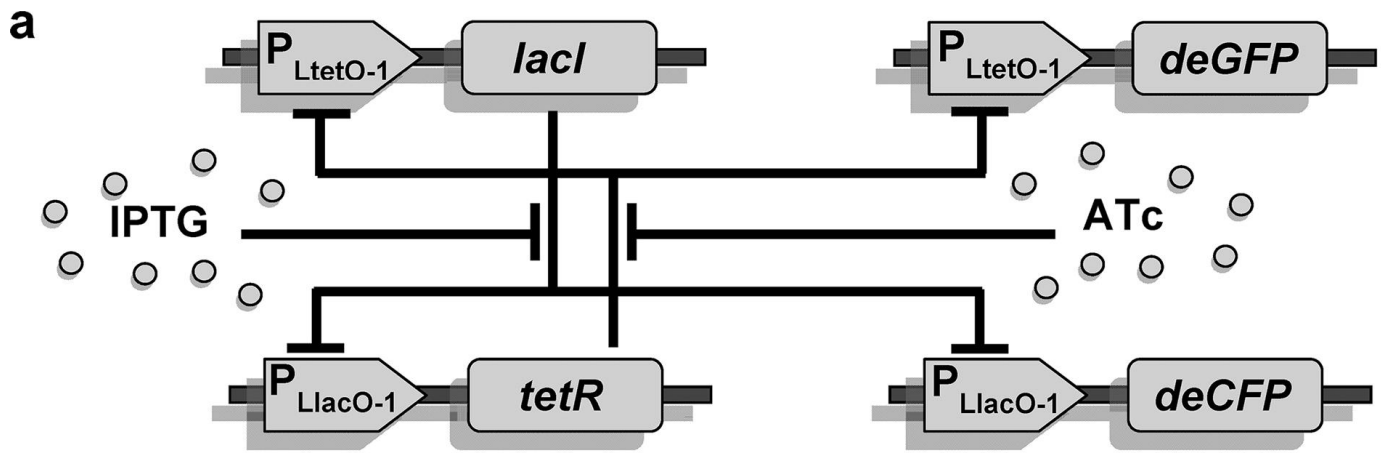
Transcriptional Repression Units

General Repression Unit

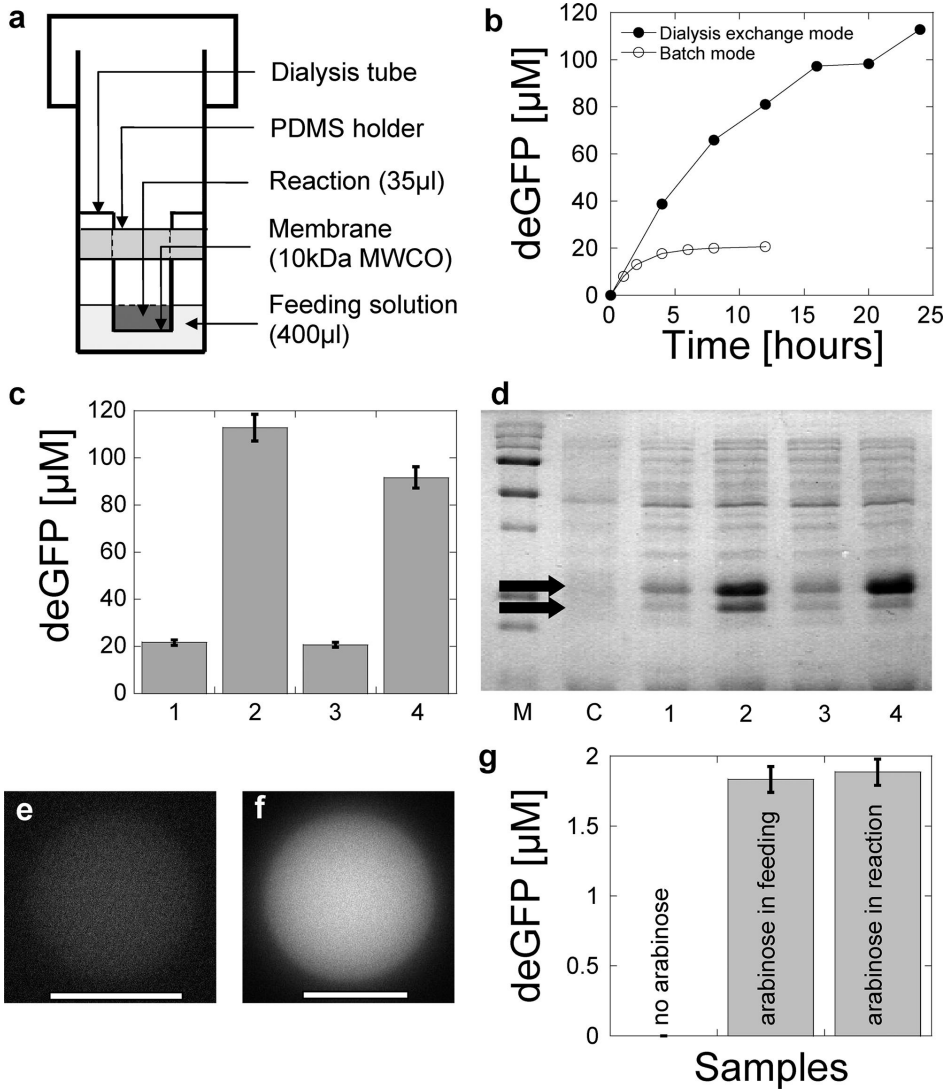


Toggle Switch

Inducer Present	Reporter Present
IPTG	deCFP
ATc	deGFP
IPTG + ATc	deCFP + deGFP
None	None



Long-lived Cell-Free Expression



Conclusions

- Cell-free toolkit can be made using multiple sigma and transcription factors, rather than more simple previous designs
- Genetic circuits, including AND gates, repression units, and series and parallel circuits can be made from this cell-free toolkit
- Cell-free expression time can be greatly increased through dialysis reaction
- Cell-free expression can occur in phospholipid vesicles

Significance

- Provides cell-free systems with greater diversity in expression
  - Lead to more complicated and fine-tuned circuits
- Allows for more stable reactions to occur over longer periods of time
  - Potential for steady state systems, obtain more of desired product
- Creation of artificial cells

Concerns

- Didn’t show results of phospholipid vesicle reaction over time
- Didn’t incorporate AND gate elements into other parts of the genetic circuit

Questions?