



A Scalable Cellular Logic Technology Using Zinc-Finger Proteins

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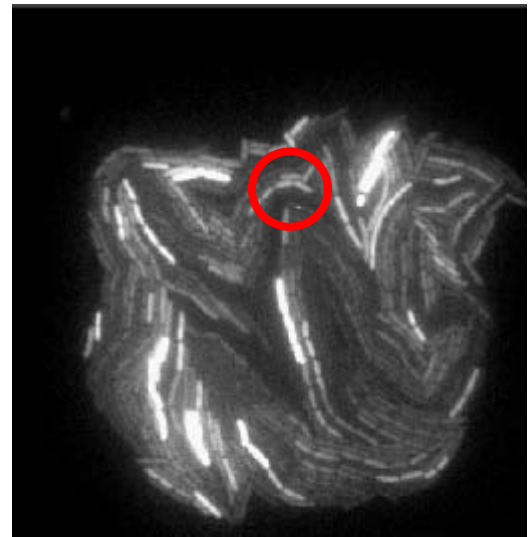
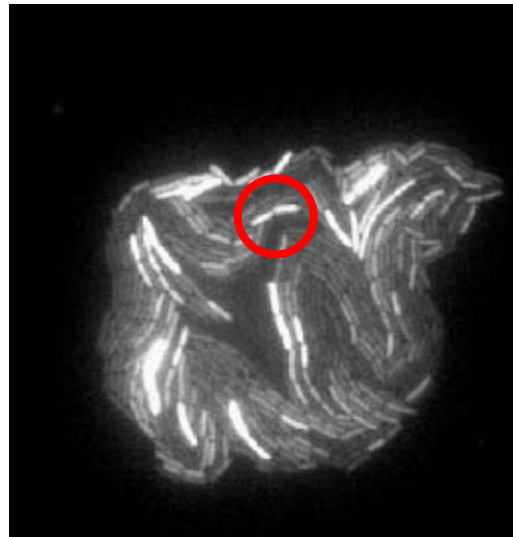
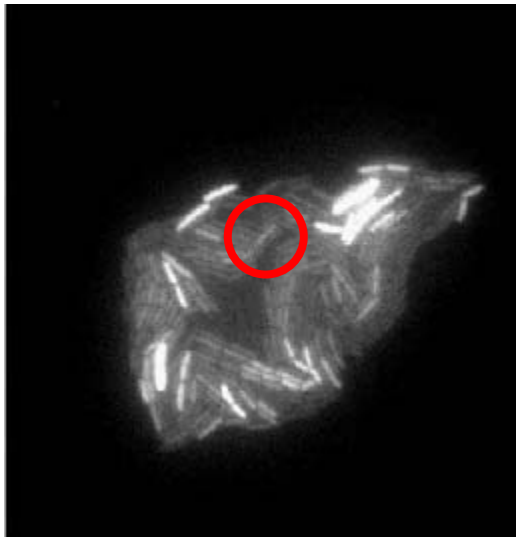
Synthetic Biology

- Synthetic biology hopes to bring engineering practices common in other engineering disciplines to the field of molecular genetics and thus create a novel nanoscale computational substrate
- Advantages
 - Tightly integrated biological inputs and outputs
 - Easily grow thousands of computational engines
 - Natural use of directed evolution
- Disadvantages
 - Speed is on the order of millihertz (tens of seconds)
 - Modest computational capability of each engine

Synthetic biology is not an attempt to replace silicon computing!

Synthetic Biology Applications

- Autonomous biochemical sensors
- Biomaterial manufacturing
- Programmed therapeutics
- Smart agriculture
- Engineered experimental systems for biologists



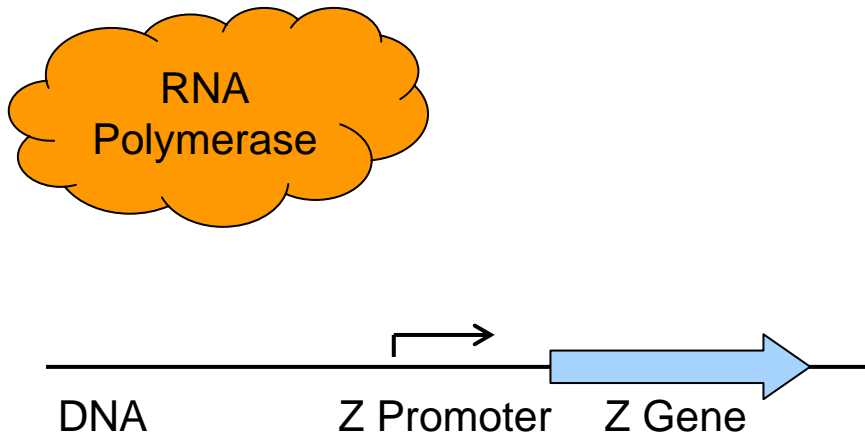
M. Elowitz and S. Leibler
A synthetic oscillatory network of transcriptional regulators
Nature, January 2000

Outline

- Background
 - Protein expression basics
 - Transcription-based cellular logic
 - Zinc-Finger Proteins (ZFPs)
- Proposed ZFP Logic Technology
- Evaluation
 - Analytical model
 - Simulation results
- Future Work and Conclusions

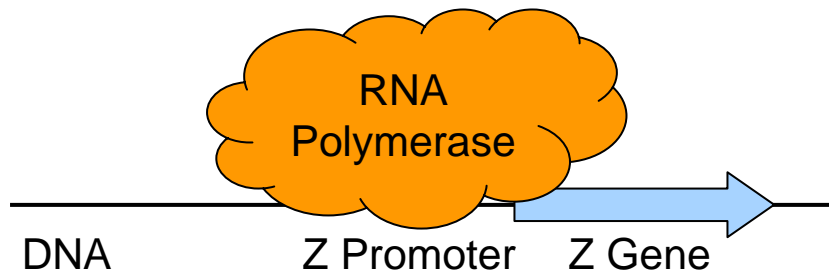
Protein Expression Basics

- RNA polymerase binds to promoter
- RNAP transcribes gene into messenger RNA
- Ribosome translates messenger RNA into protein



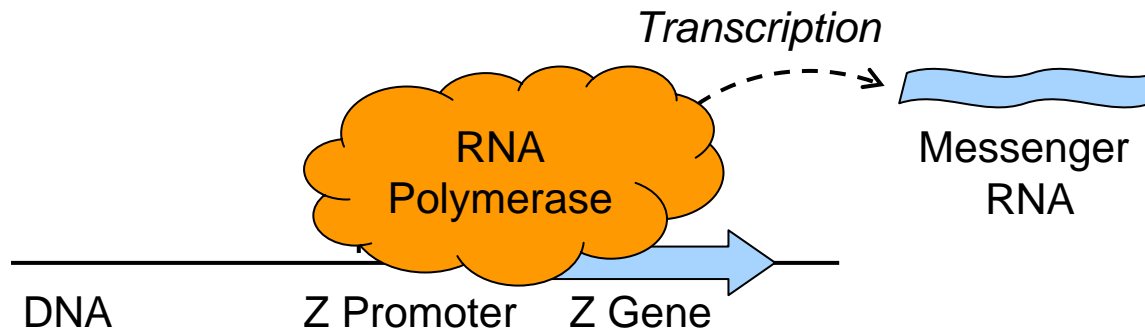
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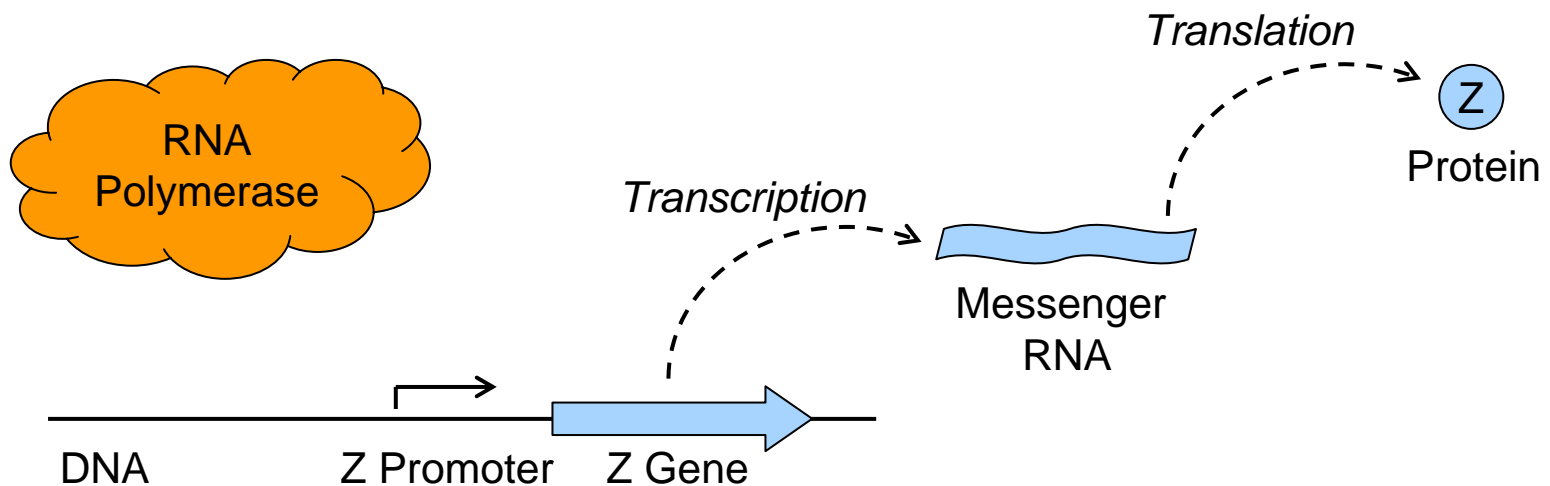
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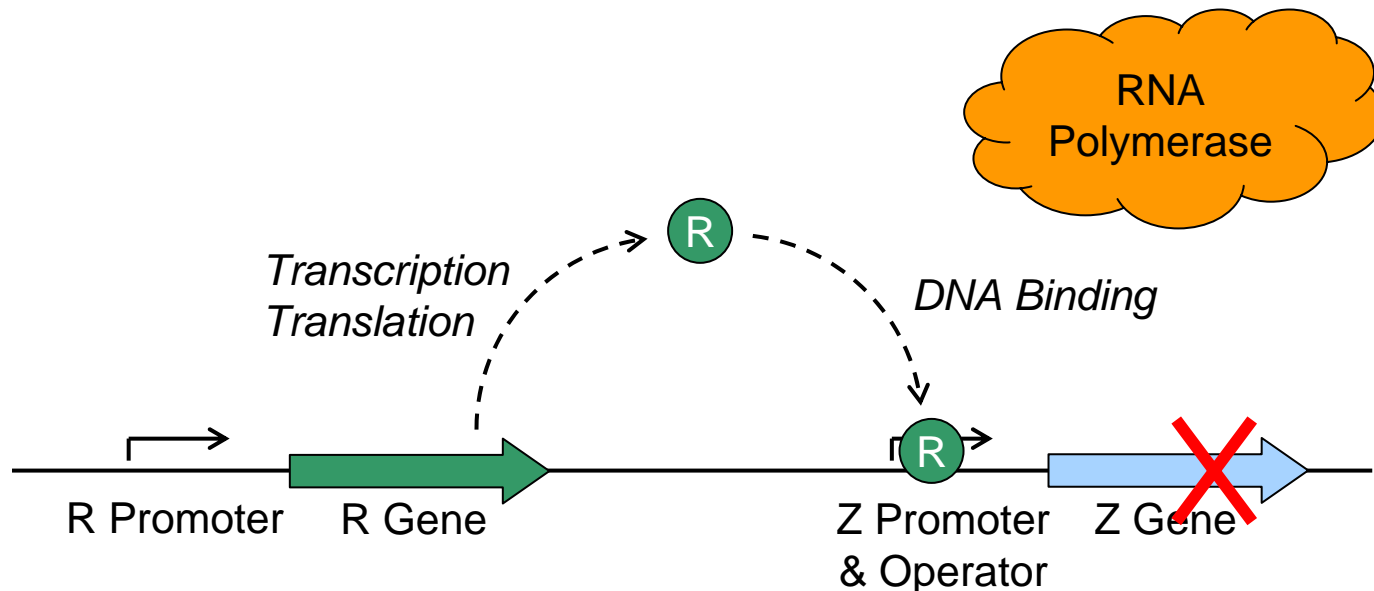
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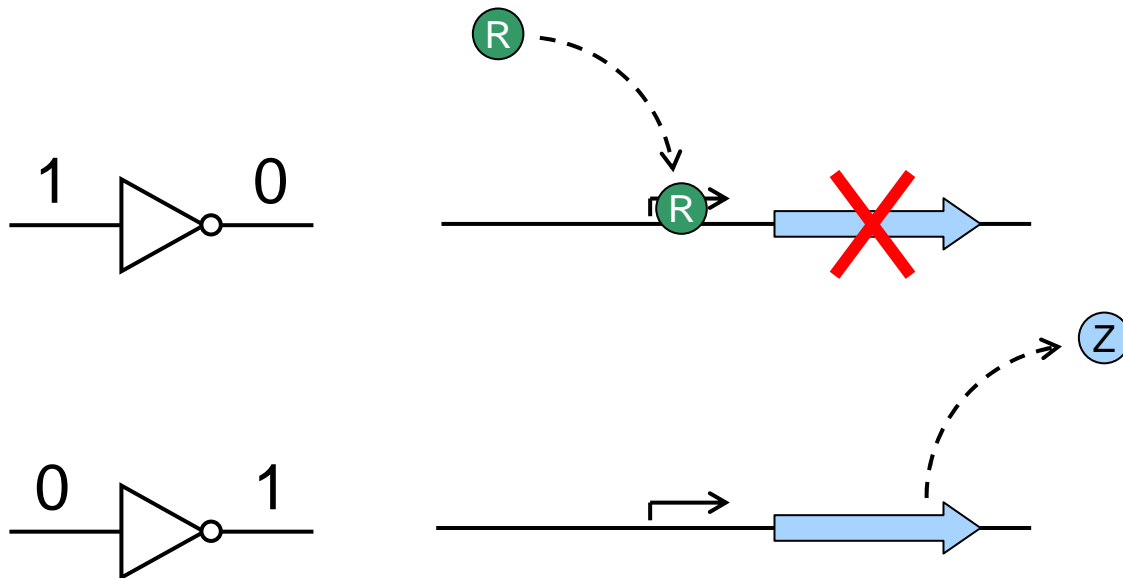
Regulation Through Repression

- Repressor proteins can bind to the promoter and block the RNA polymerase from performing transcription
- The DNA site near the promoter recognized by the repressor is called an **operator**
- The target gene can code for another repression protein enabling regulatory cascades



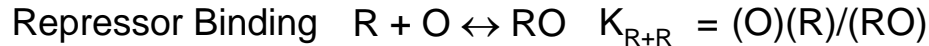
Transcription-Based Inverter

- Protein concentrations are analogous to electrical wires
- Proteins are not physically isolated, so unique wires require unique proteins



Simple Inverter Model

Chemical Equations



Total Concentration Equations

Total Operator $(O_T) = (O) + (RO)$

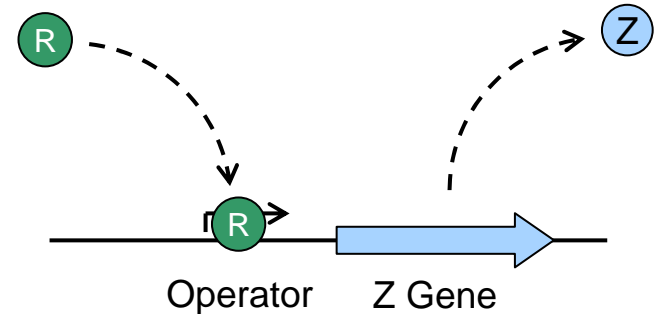
Total Repressor $(R_T) = (R) + (RO) \approx (R)$ if $(R_T) \gg (O)$

Transfer Function Derivation

$$\frac{(O)}{(O_T)} = \frac{(O)}{(O) + (RO)} = \frac{1}{1 + (RO)/(O)} = \frac{1}{1 + (R)/K_{R+R}}$$

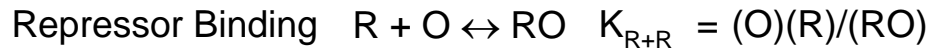
$$\frac{d(Z)}{dt} = k_x \cdot (O) - k_{deg} \cdot (Z) = 0 \text{ at equilibrium}$$

$$(Z) = \frac{k_x}{k_{deg}} (O) = \frac{k_x}{k_{deg}} \cdot \frac{(O_T)}{1 + (R)/K_{R+R}}$$



Simple Inverter Model

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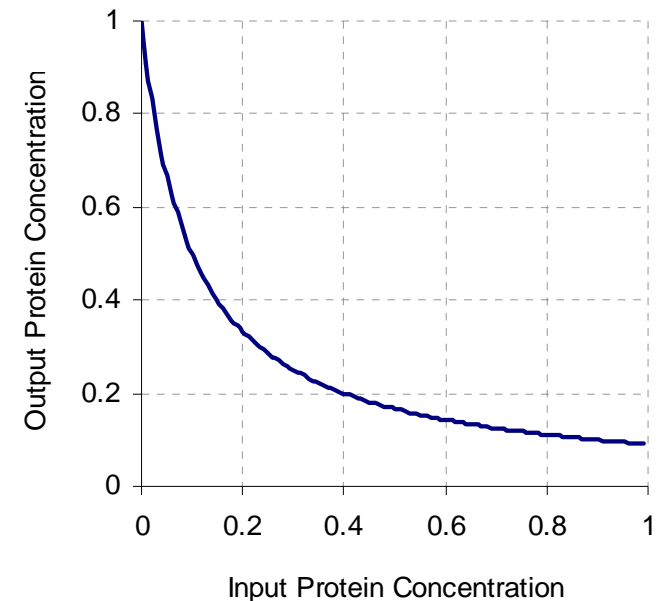
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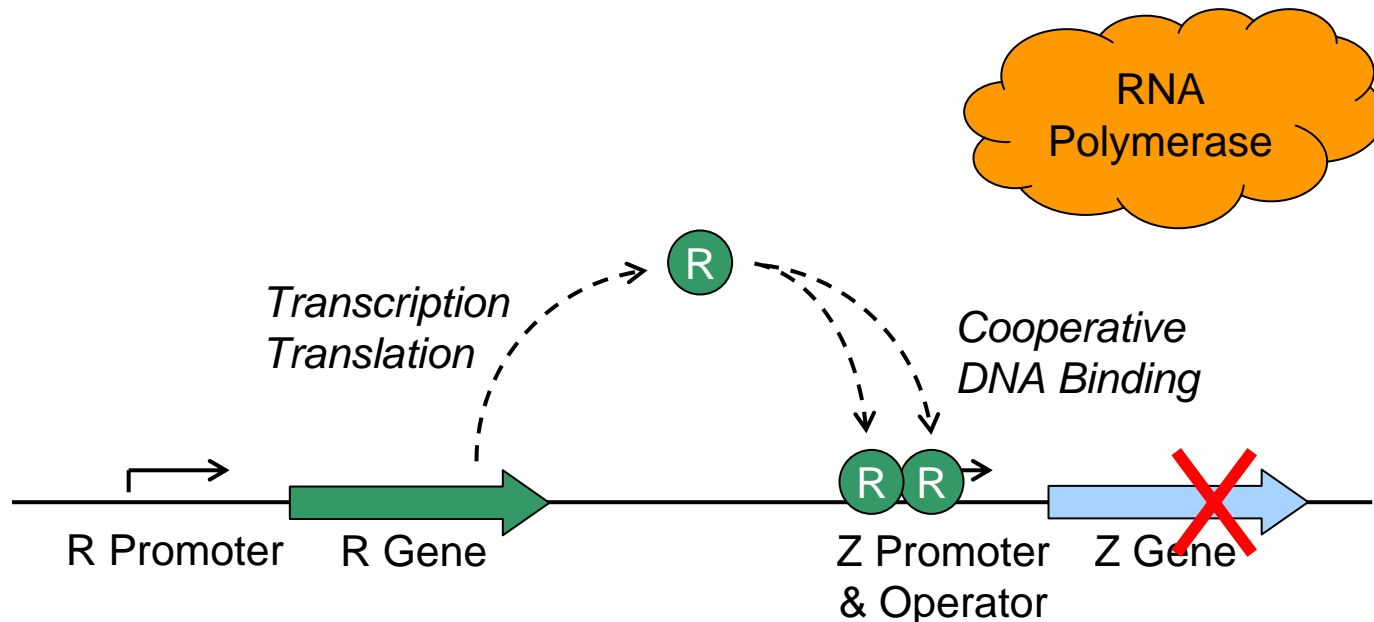
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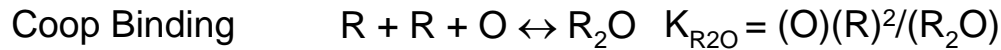
Cooperativity

- Cooperative DNA binding is where the binding of one protein increases the likelihood of a second protein binding
- Cooperativity adds more non-linearity to the system
 - Increases switching sensitivity
 - Improves robustness to noise



Cooperative Inverter Model

Chemical Equations



Total Concentration Equations

Total Operator $(O_T) = (O) + (R_2O)$

Total Repressor $(R_T) = (R) + 2 \cdot (R_2O) \approx (R)$ if $(R_T) \gg (O)$

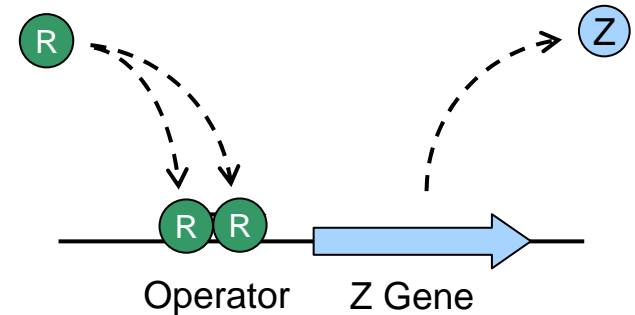
Transfer Function Derivation

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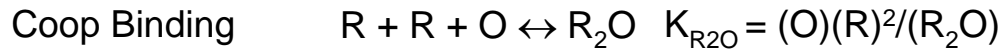
$$(Z) = \frac{k_x}{k_{deg}} (O) = \frac{k_x}{k_{deg}} \cdot \frac{(O_T)}{1 + (R)^2/K_{R_2O}}$$

Cooperative Non-Linearity



Cooperative Inverter Model

Chemical Equations



Total Concentration Equations

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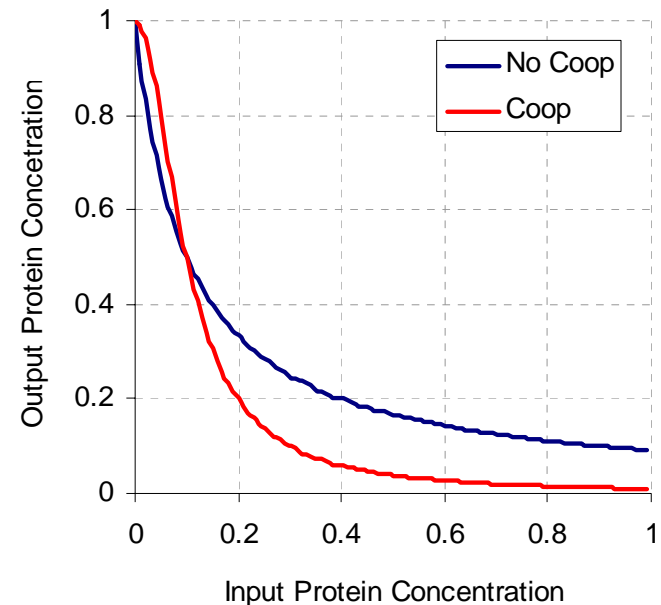
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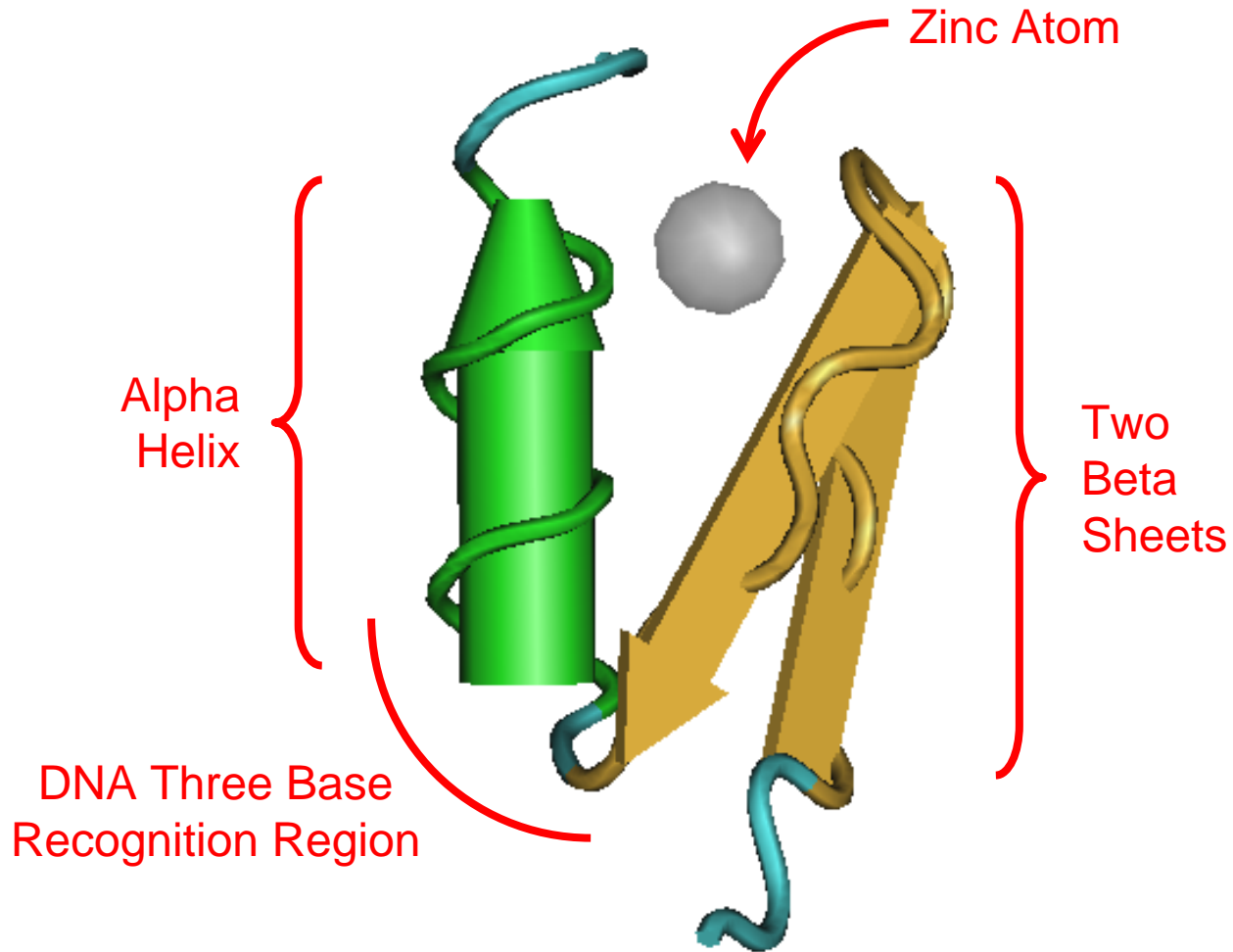


Cooperative Non-Linearity

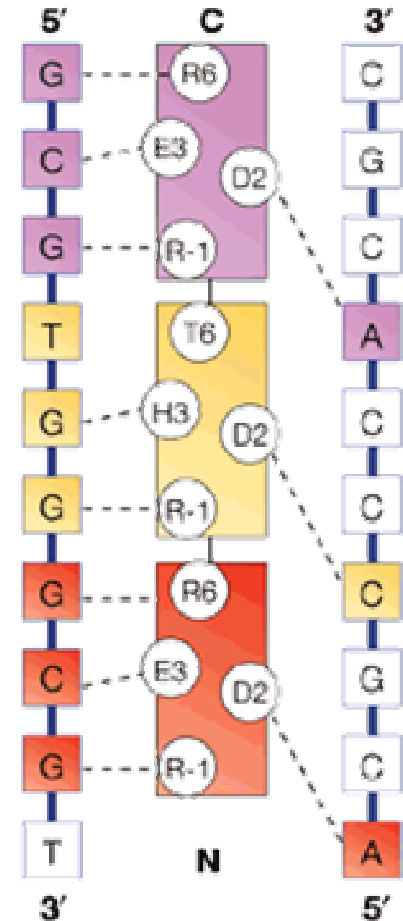
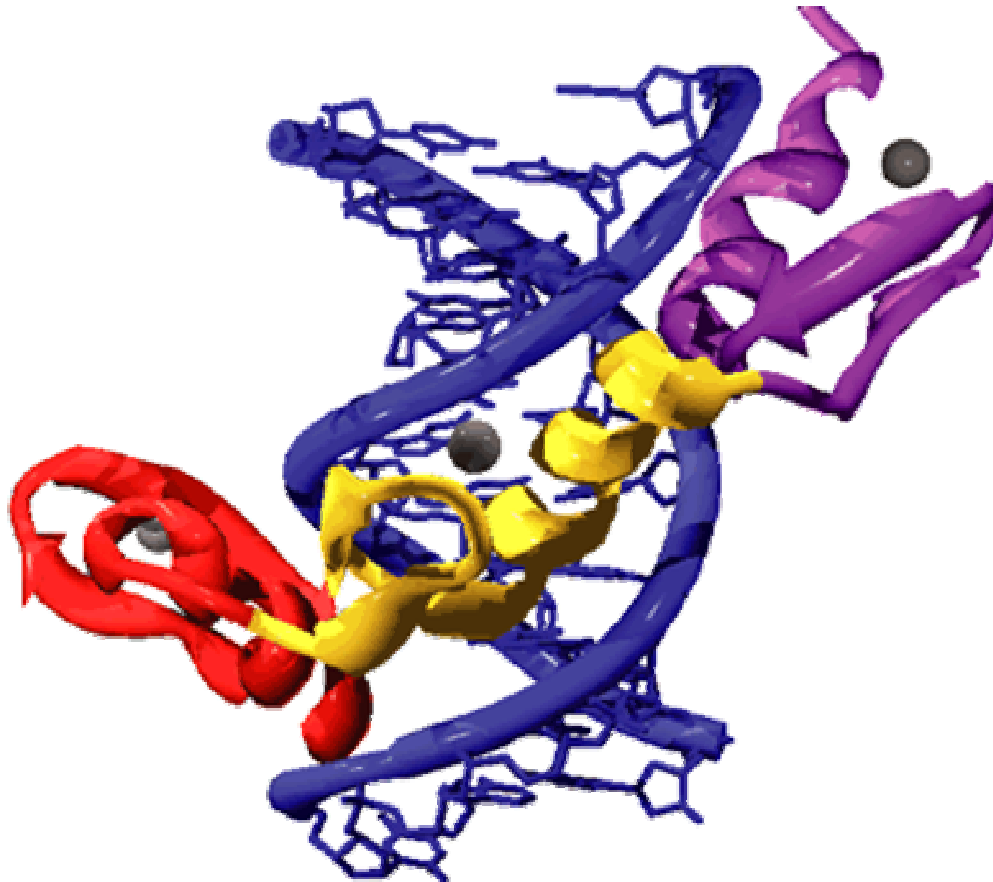
Cellular Logic Summary

- Current systems are limited to less than a dozen gates
 - Three inverter ring oscillator [Elowitz00]
 - RS latch [Gardner00]
 - Inter-cell communication [Weiss01]
- A natural repressor-based logic technology presents serious scalability issues
 - Scavenging natural repressor proteins is time consuming
 - Matching natural repressor proteins to work together is difficult
- Sophisticated synthetic biological systems require a scalable cellular logic technology with good cooperativity
 - Zinc-finger proteins can be engineered to create many unique proteins relatively easily
 - Zinc-finger proteins can be fused with dimerization domains to increase cooperativity
 - A cellular logic technology of only zinc-finger proteins should hopefully be easier to characterize

Single Zinc-Finger Structure



Poly-Finger ZFPs



A.C. Jamieson, J.C. Miller, and C.O. Pabo.
Drug discovery with engineered zinc-finger proteins.
Nature Reviews Drug Discovery, May 2003

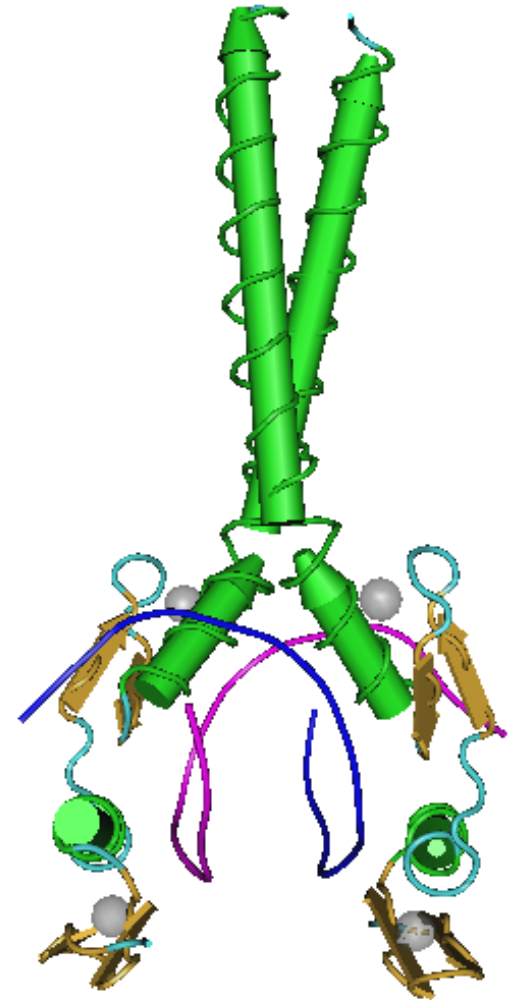
Engineering ZFPs

- Early hopes for a code to simply map amino-acid residues to DNA bases have not materialized [Choo94]
- Some success has been had engineering ZFP fingers to recognize GNG sequences [Dreier00, Segal99]
- These GNG fingers can then be easily composed into poly-finger ZFPs
- Recent work has broadened these techniques to include ANNA fingers [Dreier01]

We are nearing the point where an appropriate poly-finger ZFP can be easily composed from a library of fingers to recognize almost any DNA sequence

Engineering ZFP Dimers

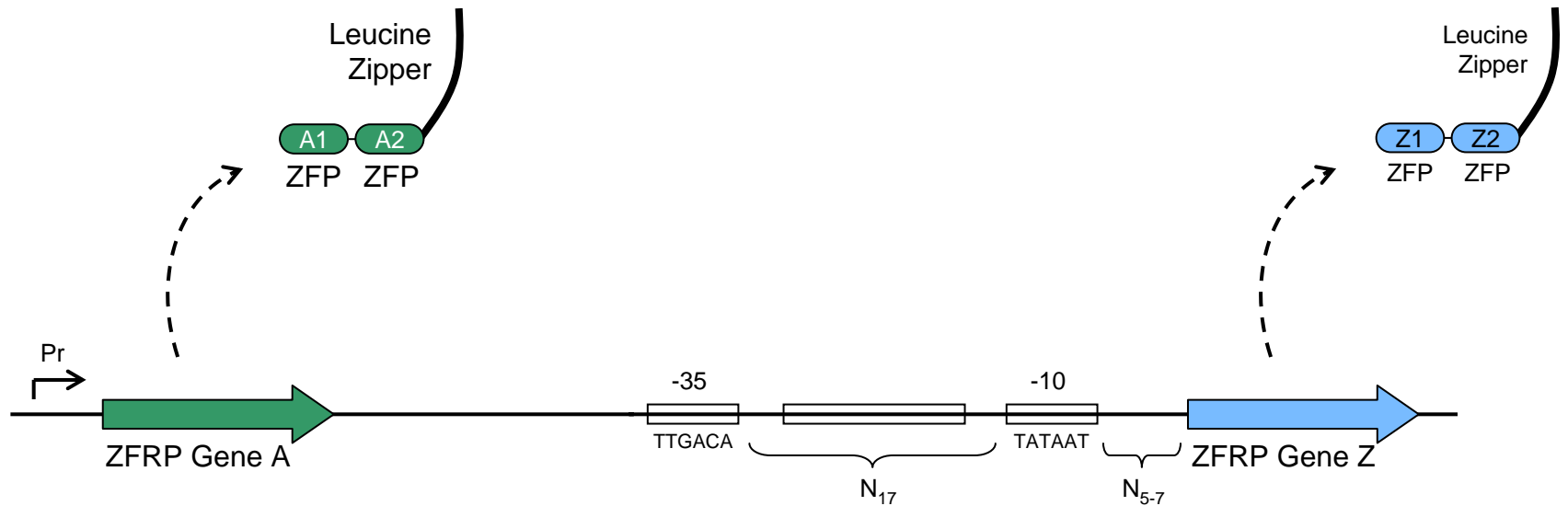
- Dimerization is the natural phenomenon where two proteins bind together
- Dimerization is a form of cooperative DNA binding and increases cooperativity
- Two-finger ZFPs have been fused to GCN4 leucine zipper dimerization domains to create cooperative ZFP DNA binding proteins [Wolfe00]



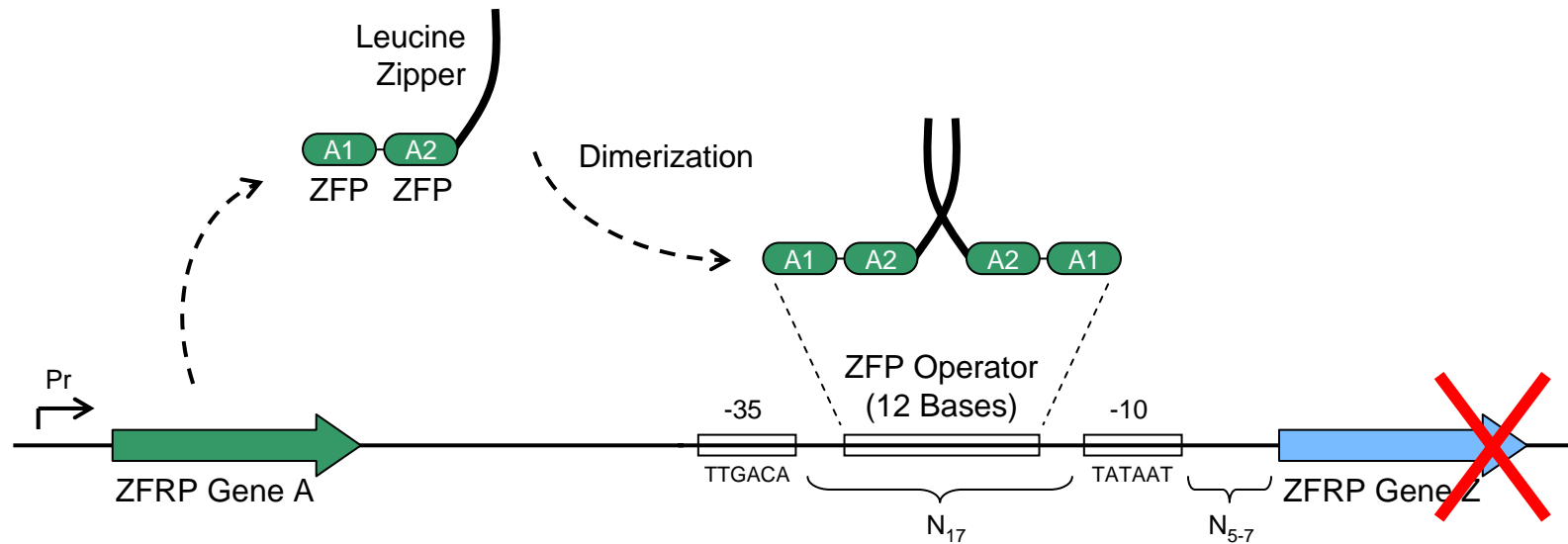
Proposed ZFP Logic Technology

- Use two-finger ZFPs fused to a GCN4 leucine zipper as basic repressor monomer
- Each gate/wire has a unique engineered ZFP
- Why two-finger monomers?
 - Recognizes 6 base pairs permitting an encoding space suitable for hundreds of gates
 - Specificity suitable for *E. coli* genome
 - Affinity suitable for biologic circuit dynamics
- Since all gates have identical leucine zipper dimerization domains, monomers from different gates could dimerize causing **inter-gate interference**

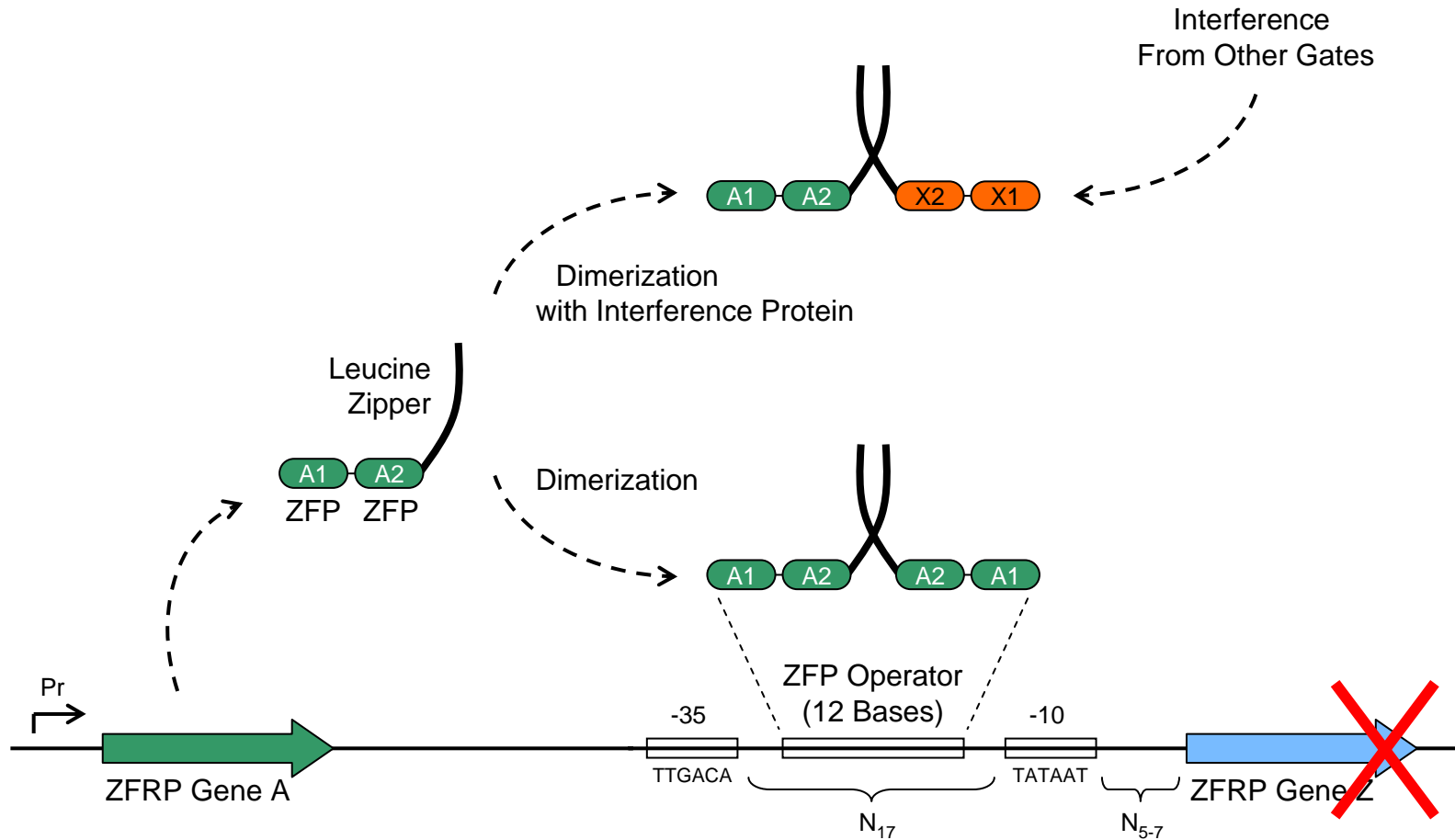
Proposed ZFP Logic Technology



Proposed ZFP Logic Technology



Proposed ZFP Logic Technology



Analytical Model

Dimerization	$R + R \leftrightarrow R_2$	$K_{R+R} = \frac{(R)^2}{(R_2)} = e^{E_{dim}/RT}$
Dimer Binding	$O + R_2 \leftrightarrow R_2O$	$K_{R_2+O} = \frac{(O)(R_2)}{(R_2O)} = e^{2E_{op}/RT}$
Monomer Binding	$O + R \leftrightarrow OR$	$K_{R+R} = \frac{(O)(R)}{(OR)} = e^{E_{op}/RT}$
Monomer Binding	$R + O \leftrightarrow RO$	$K_{R+R} = \frac{(O)(R)}{(RO)} = e^{E_{op}/RT}$
Cooperative Binding	$OR + R \leftrightarrow R_2O$	$K_{OR+R} = \frac{(OR)(R)}{(R_2O)} = e^{(E_{op}+E_{dim})/RT}$
Cooperative Binding	$RO + R \leftrightarrow R_2O$	$K_{RO+R} = \frac{(RO)(R)}{(R_2O)} = e^{(E_{op}+E_{dim})/RT}$
Protein Synthesis	$O \rightarrow O + Z$	k_x
Protein Decay	$Z \rightarrow$	k_{deg}
Dimerization	$X + X \leftrightarrow X_2$	$K_{X+X} = \frac{(X)^2}{(X_2)} = e^{E_{dim}/RT}$
Inter-Gate Interference	$X + R \leftrightarrow XR$	$K_{X+R} = \frac{(X)(R)}{(XR)} = e^{E_{dim}/RT}$

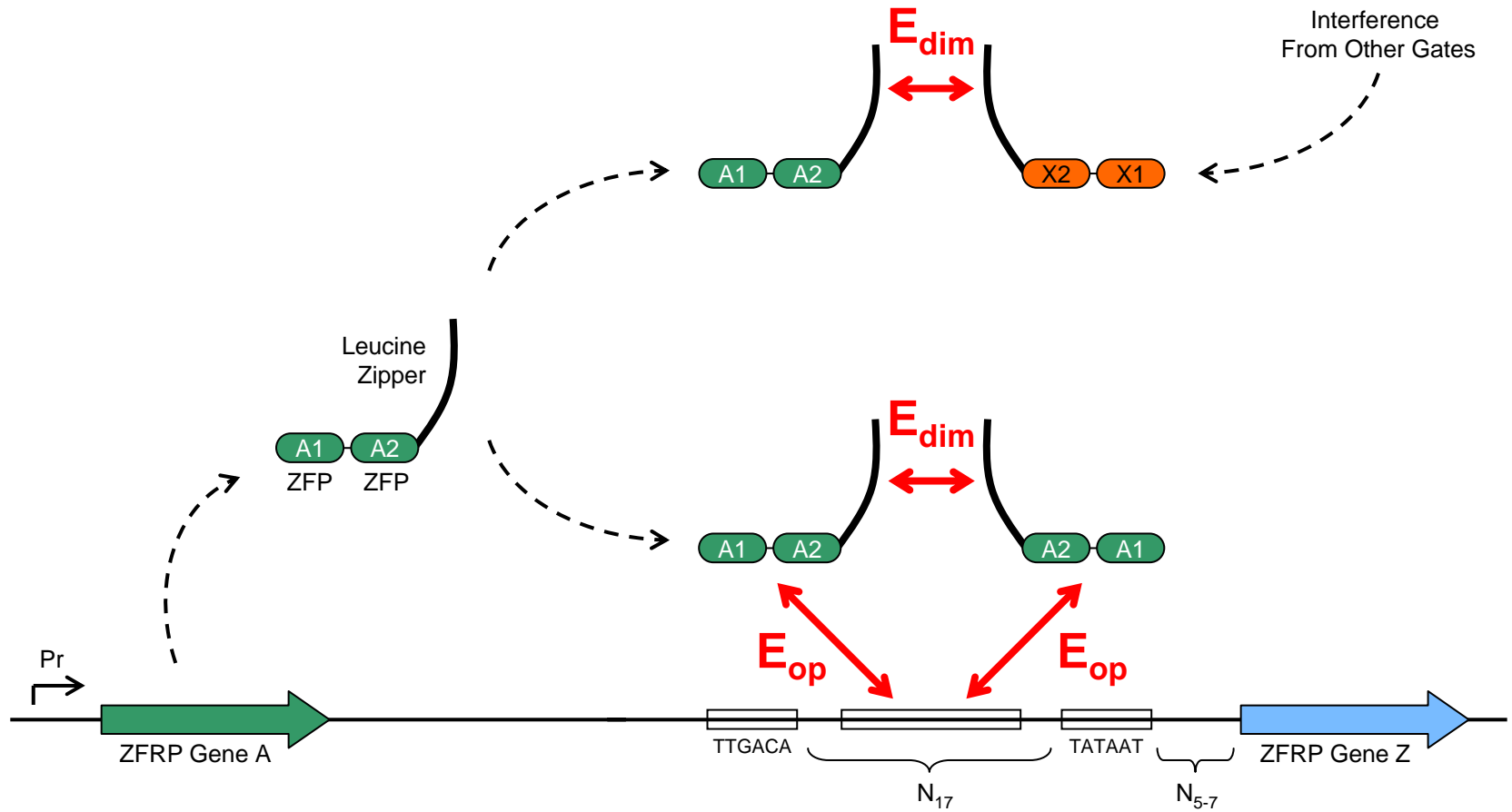
K : Equilibrium dissociation constant

k : Dynamic rate constant

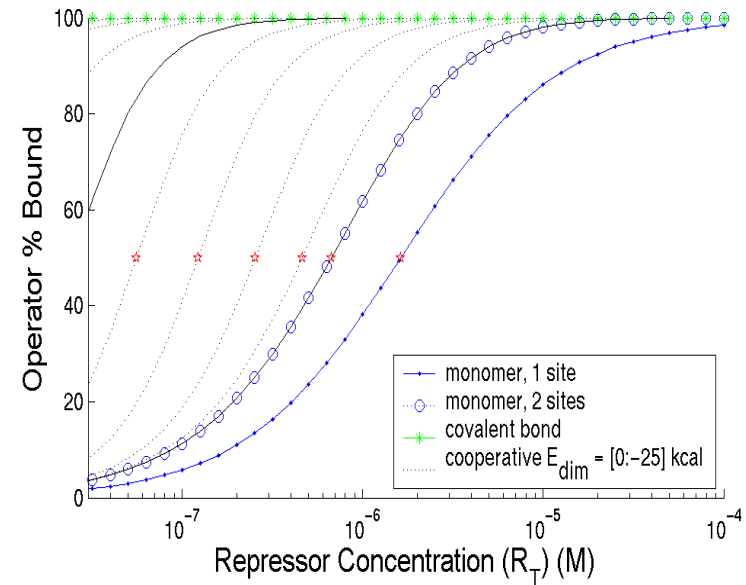
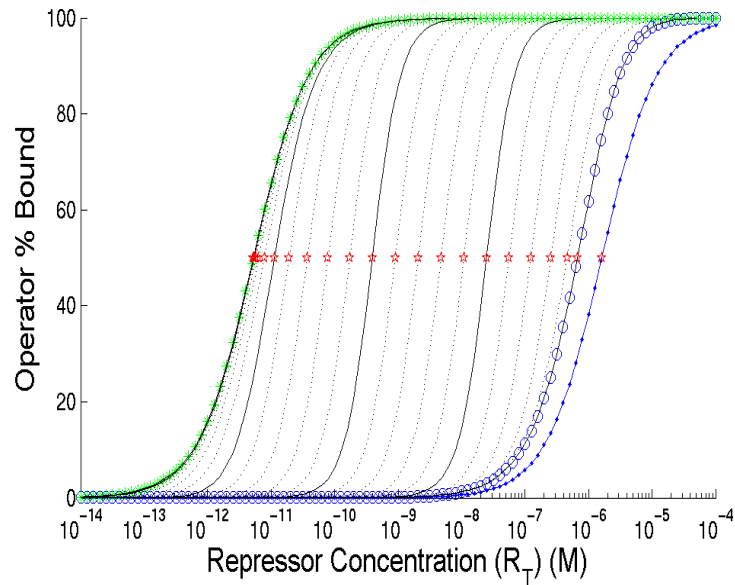
E : Binding energy or change in potential energy caused by the reaction

More negative E means the reaction is more likely to occur

Dimerization and Operator Energy

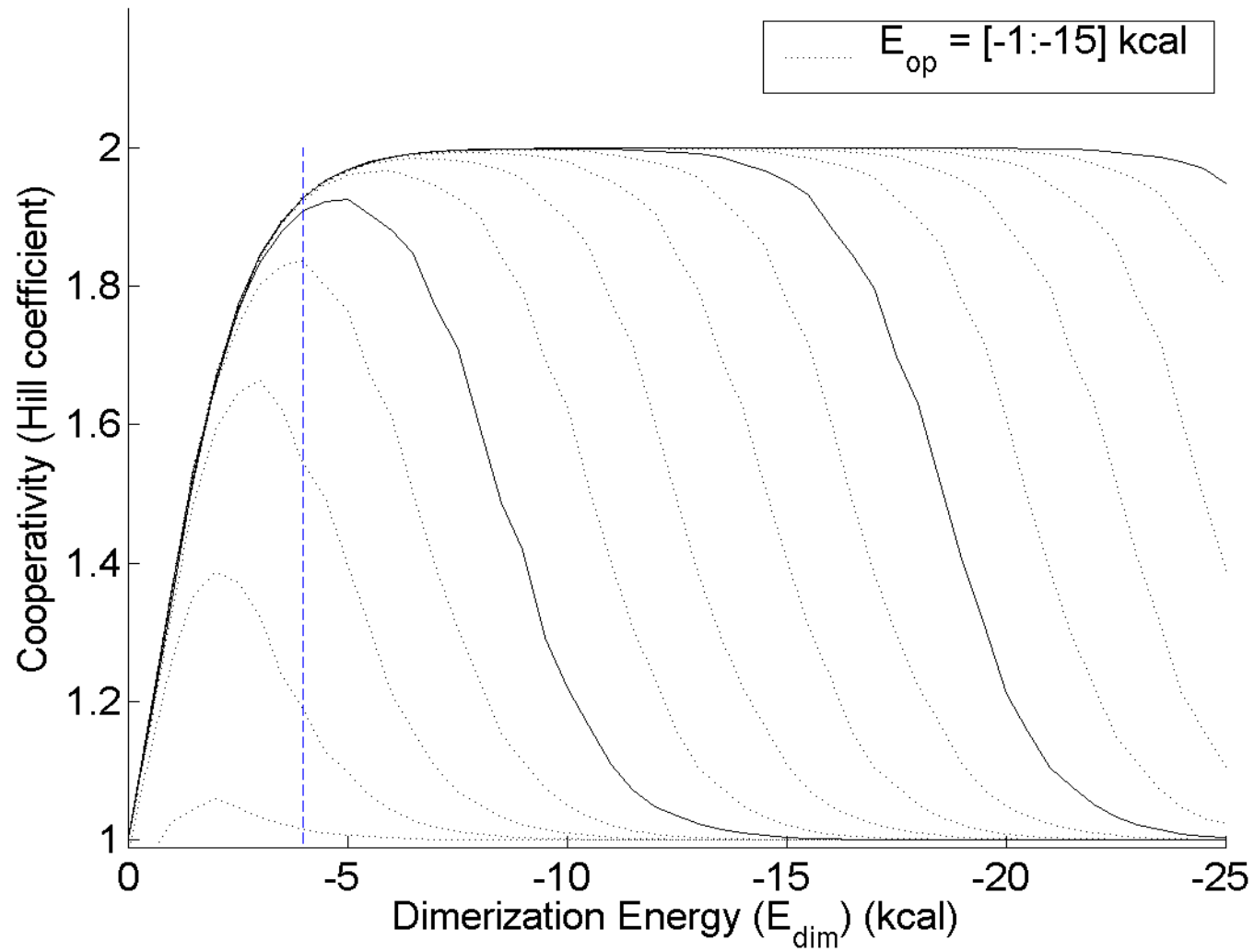


Percent Operator Bound

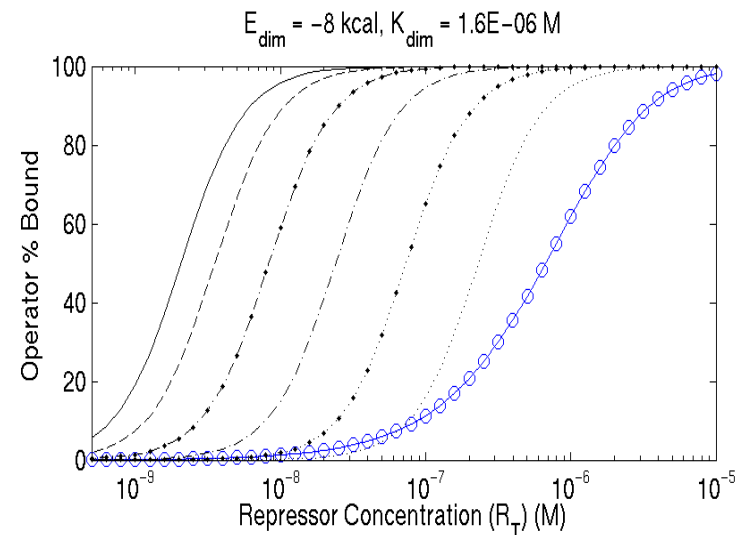
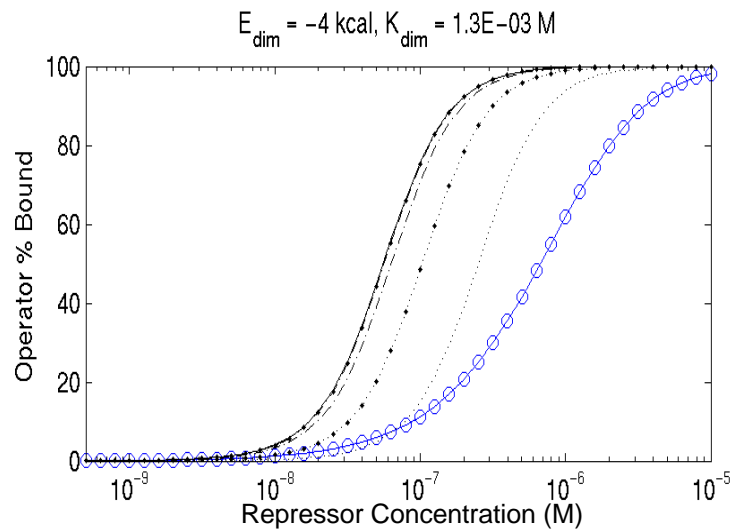
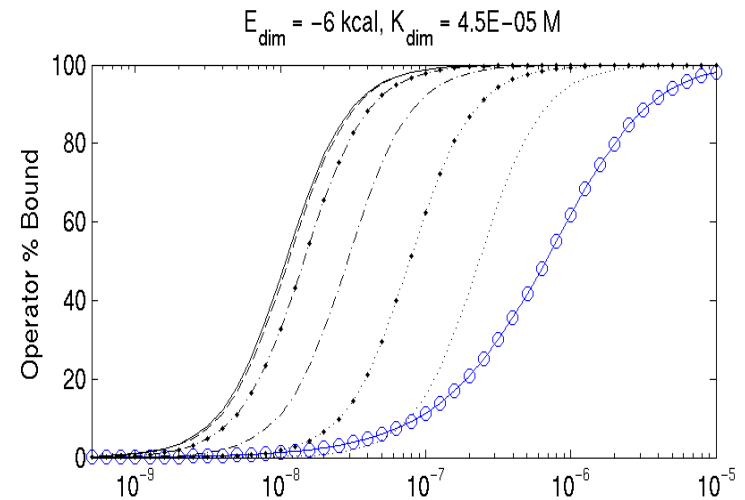
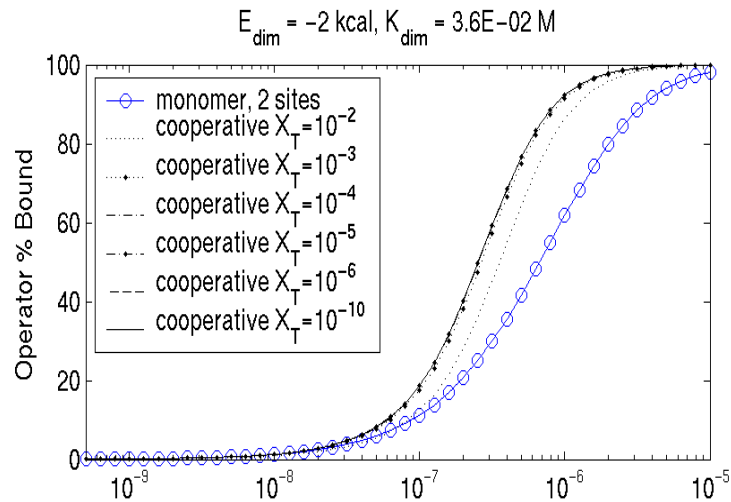


- For **very low dimerization energies**, system approaches uncooperative repressor monomer system
- For **very high dimerization energies**, system approaches uncooperative covalently bonded repressor system
- For **moderate dimerization energies**, the system is cooperative ie. the slope of the curve is steeper than for the uncooperative systems

Cooperativity



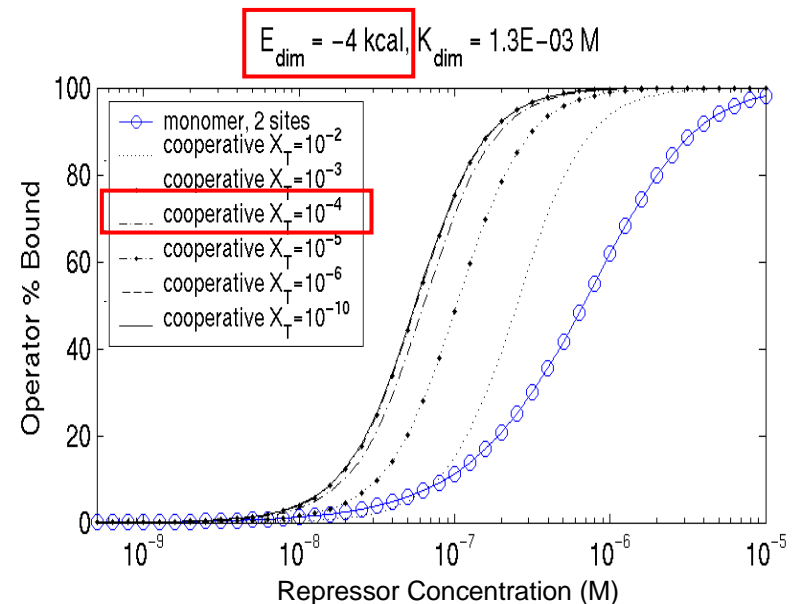
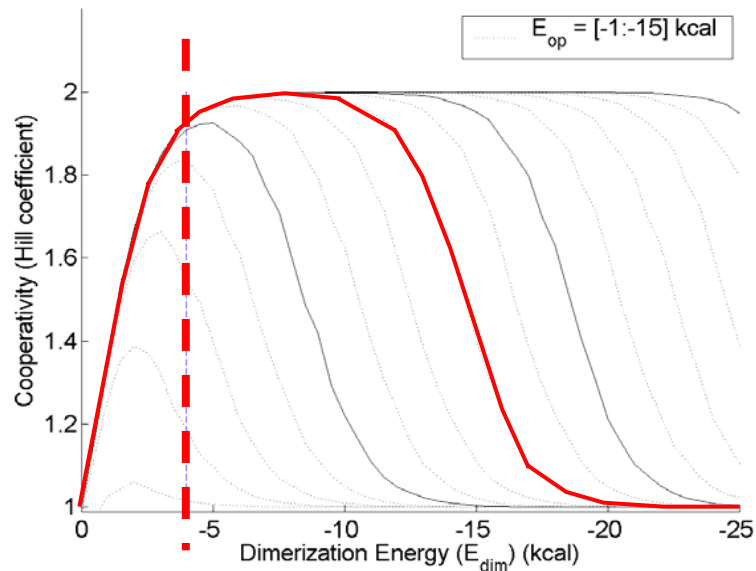
Inter-Gate Interference



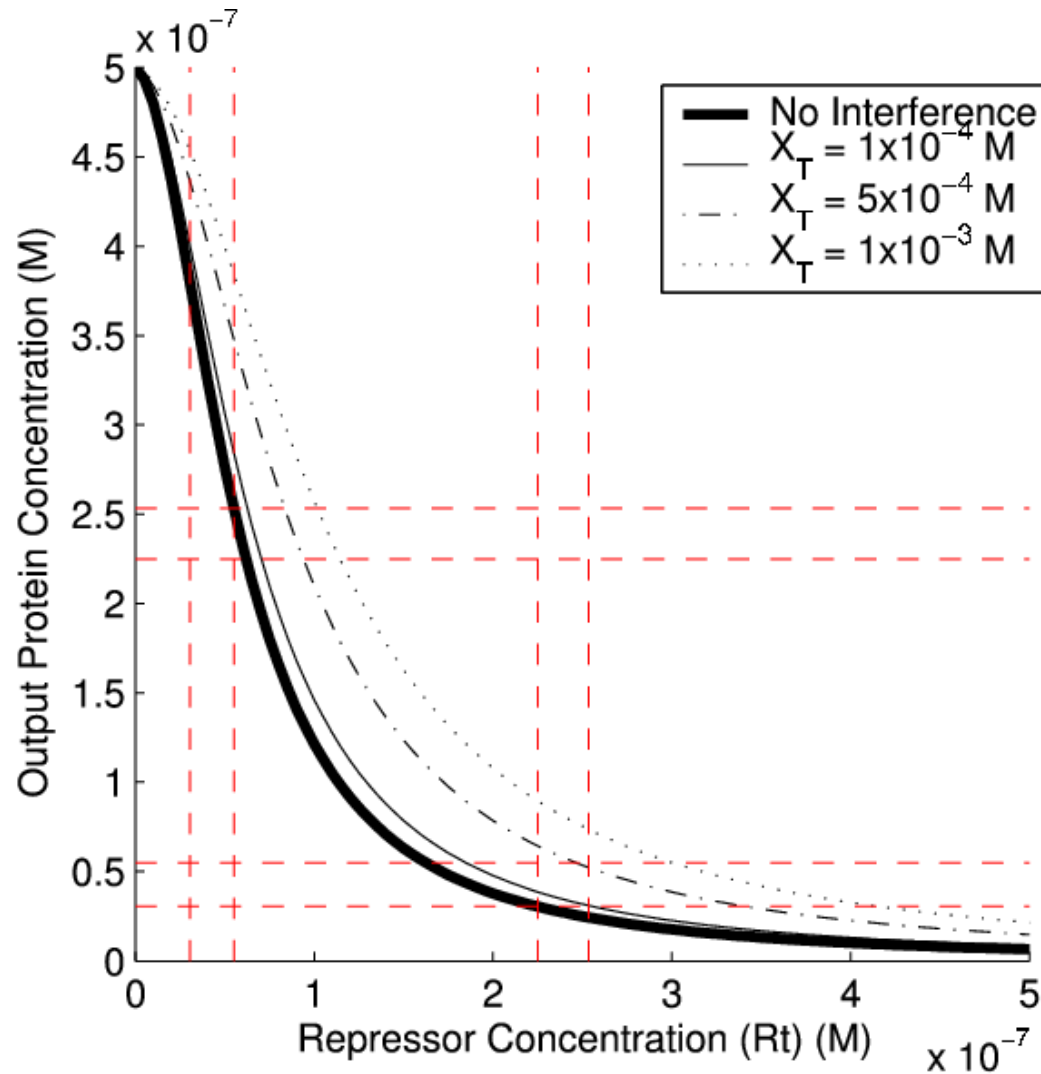
Desired Dimerization Energy

- Tradeoffs in setting the dimerization energy
 - Stronger dimerization energy increases cooperativity
 - Stronger dimerization energy increases inter-gate interference

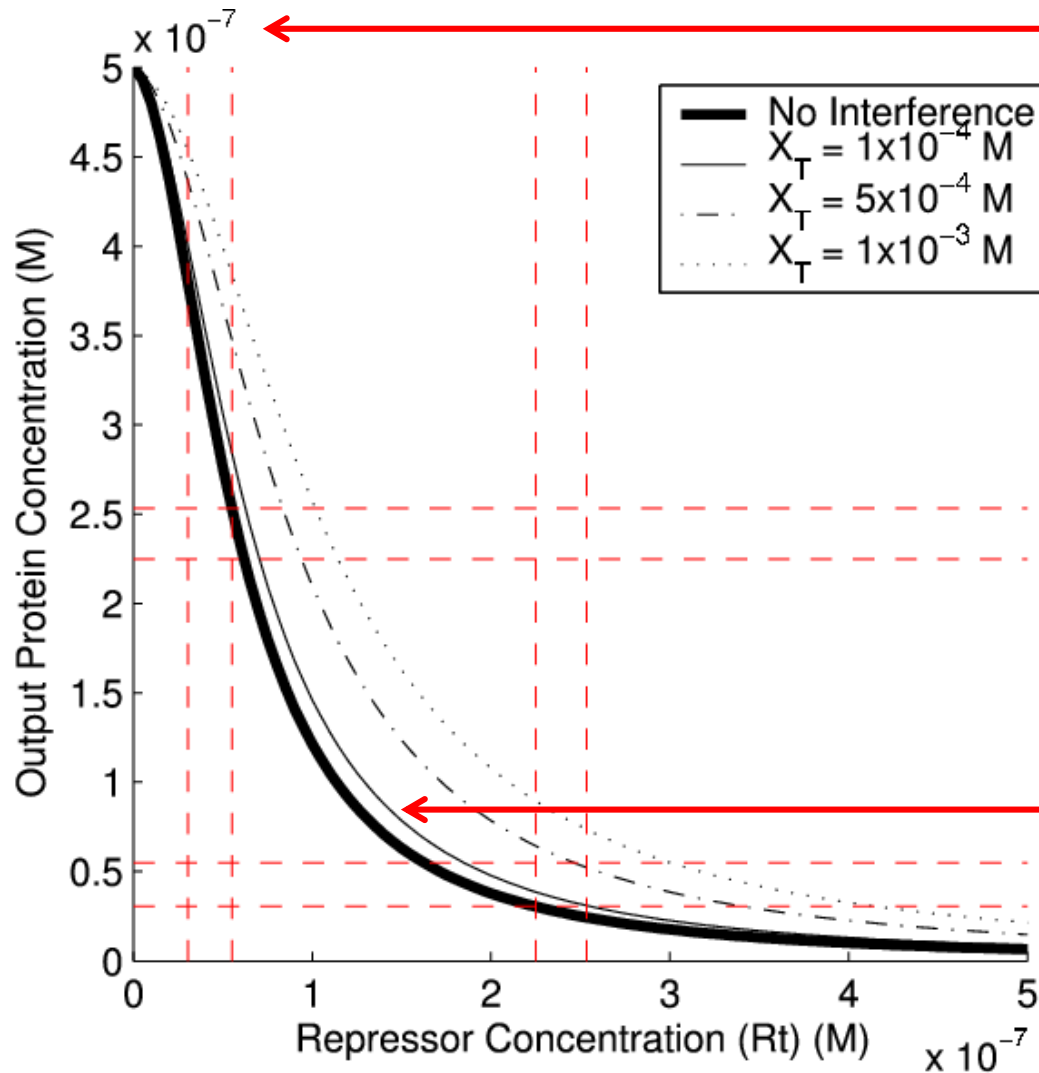
We desire the weakest dimerization energy which still achieves the maximum cooperativity



Transfer Curve and Interference



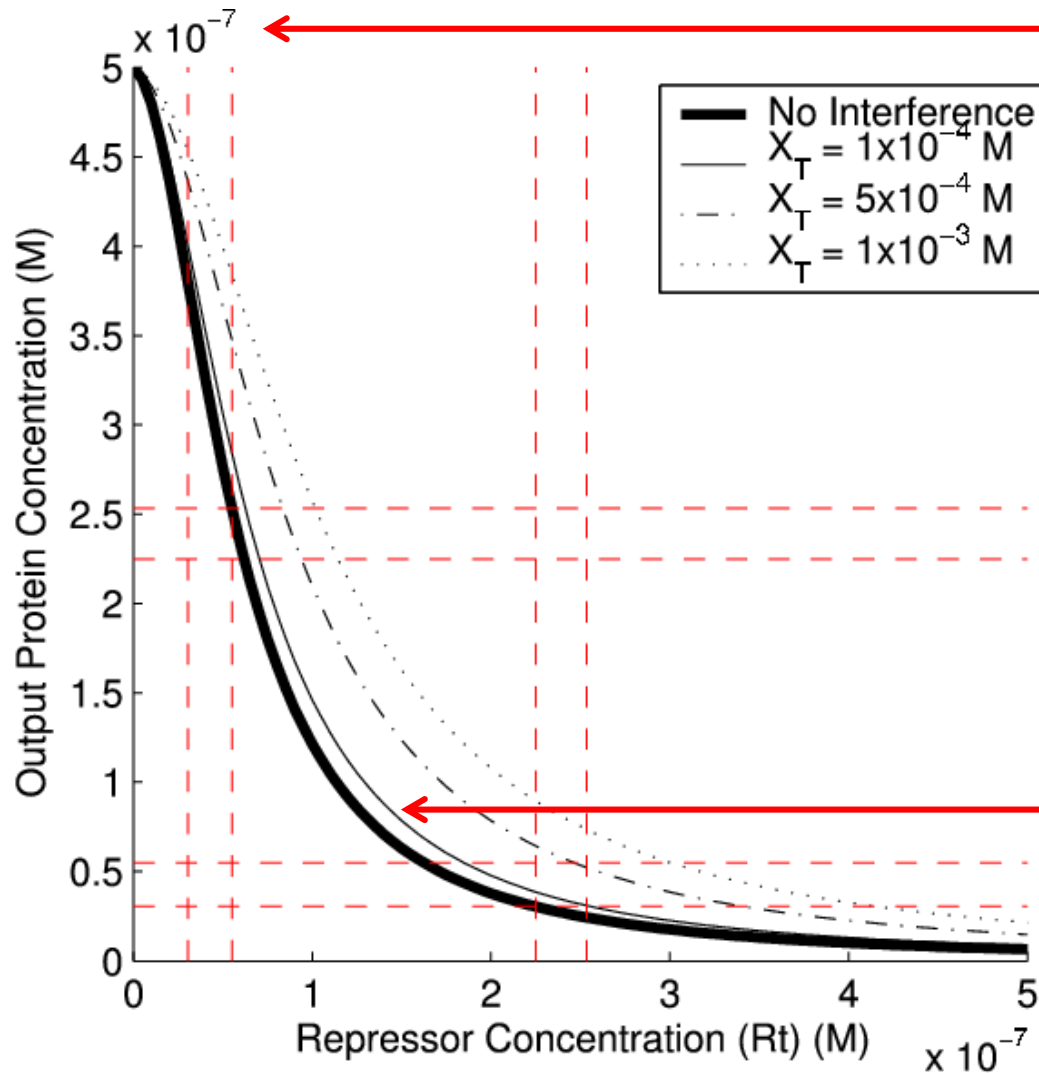
Transfer Curve and Interference



Max output protein concentration per gate is 5×10^{-7} M

Inter-gate interference must be below 10^{-4} M

Transfer Curve and Interference

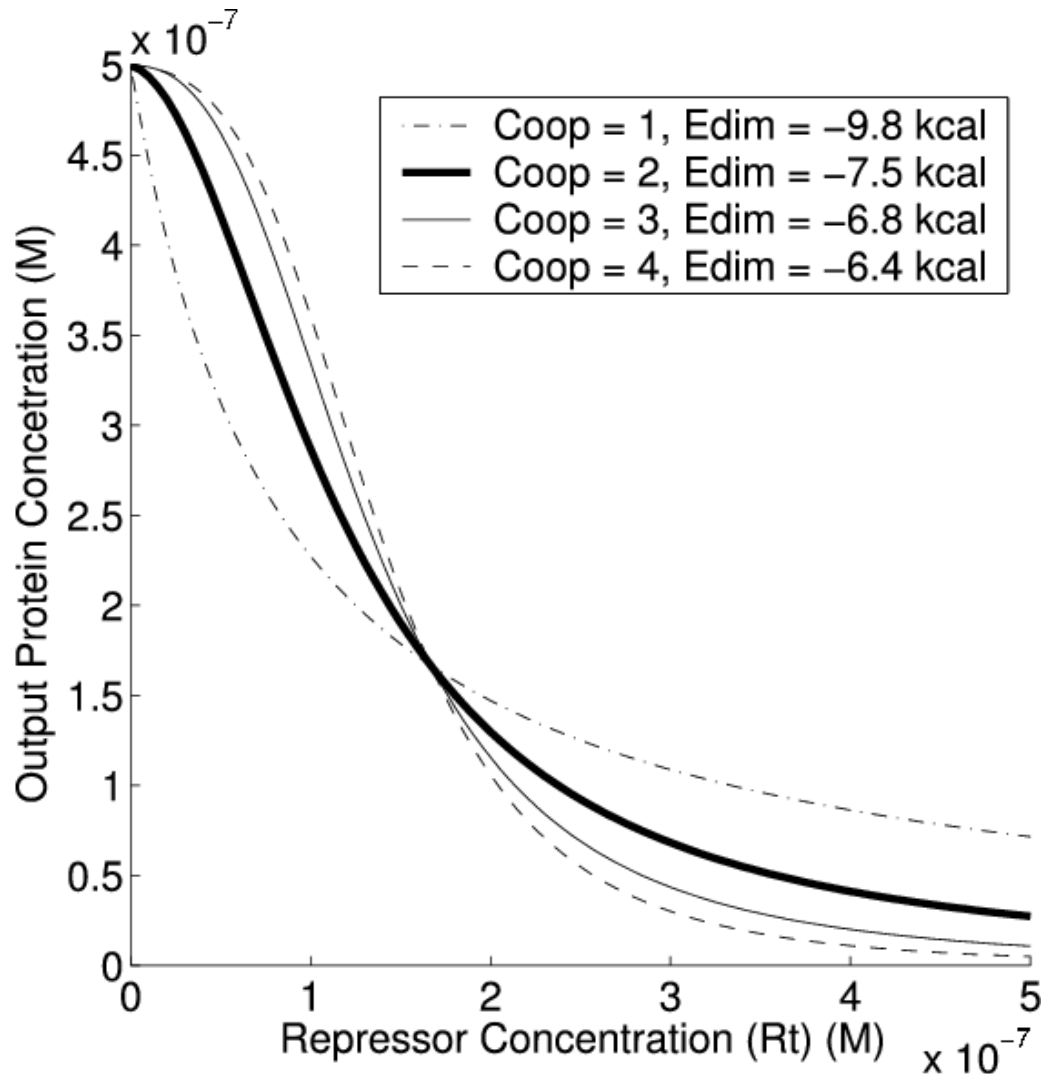


Max output protein concentration per gate is 5×10^{-7} M

To first order, could have $10^{-4} / 5 \times 10^{-7} \approx 200$ gates

Inter-gate interference must be below 10^{-4} M

Transfer Curve and Cooperativity



Future Work

- Model and Design Improvements
 - Model system transient response
 - Model stochastic effects
 - Design a system with increased cooperativity
- Implementation
 - Simple test circuits to investigate use of two finger ZFP dimer as a cooperative repressor in *E. coli*
 - Engineered zinc-finger system with heterodimers to implement more complex logic gates

Conclusions

- Current natural repressor-based biological circuits are limited to less than a dozen gates
- A cellular logic technology based on zinc-finger proteins should enable hundreds of gates
- Careful engineering of the dimerization energy can help mitigate inter-gate interference without sacrificing cooperativity