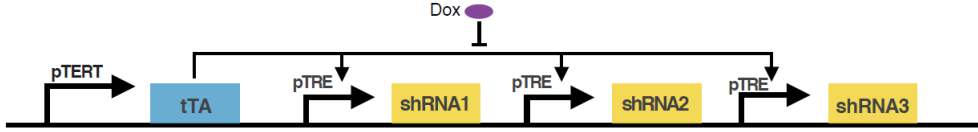# *A simple thermal model to compare the efficiency of two designs of genetic circuits*

I.the genetic circuits with three promoters

└虚数单位

i.General Methods

└一般系统信息

The first step is to write down the total partition function of the system we aim to analyze.Note that the partition function is obtained by summing all of the eventualities associated with the activators, repressors and polymerase molecules being distributed on the DNA.AS shown in the table II, only 9 outcomes are available.This can be represented mathematically as

$$Z_{tot} = \underbrace{Z(P,A,R)}_{empty\ promoter} + \underbrace{e^{-\beta\epsilon_{ad}^s}Z(P,A-1,R)}_{activator} + \underbrace{e^{-\beta\epsilon_{ad}^s}Z(P,A-1,R-1)}_{activator\ and\ repressor} + \underbrace{3\,e^{-\beta\epsilon_{ad}^s}Z(P-1,A,R)}_{one\ RNAP} + \underbrace{3\,e^{-2\,\beta\epsilon_{ad}^s}Z(P-2,A,R)}_{two\ RNAP} +$$

$$\underbrace{e^{-3\beta\epsilon_{pd}^s}Z(P-3,A,R)}_{three\ RNAP} + \underbrace{3\,e^{-\beta\left(\epsilon_{pd}^s+\epsilon_{ad}^s+\epsilon_{ap}\right)}Z(P-1,A-1,R)}_{activator\ and\ one\ RNAP} + \underbrace{3\,e^{-\beta\left(2\,\epsilon_{pd}^s+\epsilon_{ad}^s+\epsilon_{ap}\right)}Z(P-2,A-1,R)}_{activator\ and\ two\ RNAP} + \underbrace{e^{-\beta\left(3\,\epsilon_{pd}^s+\epsilon_{ad}^s+\epsilon_{ap}\right)}Z(P-3,A-1,R)}_{activator\ and\ three\ RNAP}$$

Hence, The partition function of the state that at least one shRNA can be produced can be written as

$$Z_{bound} = 3\,e^{-\beta\epsilon_{ad}^s}Z(P-1,A,R) + 3\,e^{-2\,\beta\epsilon_{ad}^s}Z(P-2,A,R) + e^{-3\,\beta\epsilon_{ad}^s}Z(P-3,A,R) +$$

$$3\,e^{-\beta\left(\epsilon_{pd}^s+\epsilon_{ad}^s+\epsilon_{ap}\right)}Z(P-1,A-1,R) + 3\,e^{-\beta\left(2\,\epsilon_{pd}^s+\epsilon_{ad}^s+\epsilon_{ap}\right)}Z(P-2,A-1,R) + e^{-\beta\left(3\,\epsilon_{pd}^s+\epsilon_{ad}^s+\epsilon_{ap}\right)}Z(P-3,A-1,R)$$

The meaning of $Z(P, A, R)$ is that it's just the partition function of P polymerase molecules and A activators to be bounded on the N_{ns} nonspecific sites as well as R repressors to be bounded on the A activators which can be given by

$$Z(P, A, R) = \underbrace{\frac{N_{ns}!}{P!\,A!\,(N_{ns}-P-A)!}}_{number\ of\ arrangements\ of\ activators\ and\ polymerase\ molecules}\underbrace{\frac{A!}{R!\,(A-R)!}}_{number\ of\ arrangements\ of\ repressors}\underbrace{e^{-\beta P\epsilon_{pd}^{ns}}\,e^{-\beta A\epsilon_{ad}^{ns}}\,e^{-\beta R\epsilon_{rd}^s}}_{the\ Boltamann\ factor\ of\ each\ state}$$

Notation	Meaning
ϵ_{pd}^s	the binding energy of RNA polymerase with its specific DNA target (promoter)
ϵ_{pd}^{ns}	the binding energy of RNA polymerase with its nonspecific DNA target
ϵ_{ad}^s	the binding energy of activator with its specific DNA target (TetO)
ϵ_{ad}^{ns}	the binding energy of activator with its nonspecific DNA target
ϵ_{rd}^s	the binding energy of repressor with its specific DNA target (tTA)
ϵ_{ap}	the "glue" interaction energy between the activator and RNA polymerase
β	$\equiv \dfrac{1}{k_B\,T}$, where k_B is the Boltzmann constant

Table I . Notations and meanings

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Similarly, we may confirm that

$$Z(P-1, A, R)\,e^{-\beta\epsilon_{pd}^s} =$$

$$\frac{N_{ns}!}{(P-1)!\,A!\,[N_{ns}-(P-1)-A]!}\,\frac{A!}{R!\,(A-R)!}\,e^{-\beta(P-1)\epsilon_{pd}^{ns}}\,e^{-\beta A\epsilon_{ad}^{ns}}\,e^{-\beta R\epsilon_{rd}^s}\,e^{-\beta\epsilon_{pd}^s} = \frac{P}{N_{ns}-P-A}\,Z(P, A, R)\,e^{-\beta\epsilon_{pd}^s}$$

We invoke a simplify strategy which depends upon the facts that $N_{ns} \gg$
   $A + P$ and hence there will be almost zero chance of RNA polymerase
      and activator finding each other on the same nonspecific site on the DNA.
         Then, we may get

$$Z(P-1, A, R)\, e^{-\beta \epsilon_{pd}^{s}} \approx \frac{P}{N_{ns}}\, e^{-\beta \epsilon_{pd}^{s}}\, Z(P, A, R)$$

Let us define two important parameters

$$\triangle \epsilon_{pd} \equiv \epsilon_{pd}^{s} - \epsilon_{pd}^{ns}$$

$$\triangle \epsilon_{ad} \equiv \epsilon_{ad}^{s} - \epsilon_{ad}^{ns}$$

which can be used to represent the differences of the specific binding energy and the nonspecific binding energy.
   Given that what we want to calculate is just the probability of promoter occupancy,
we can set the renormalized weight of $Z(P, A, R)$ as 1. As a result,
we can represent other partition functions in the form of the renormalized weight which is showed in Table II.

└表格

Partition Function	State	Renormalized Weight
$Z(P, A, R)$	empty promoter	1
$Z(P-1, A, R)\, e^{-\beta \epsilon_{pd}^{s}}$	one RNAP	$\frac{P}{N_{ns}}\, e^{-\beta \triangle \epsilon_{pd}}$
$Z(P-2, A, R)\, e^{-2\beta \epsilon_{pd}^{s}}$	two RNAP	$\frac{P}{N_{ns}}\, \frac{P-1}{N_{ns}}\, e^{-2\beta \triangle \epsilon_{pd}}$
$Z(P-3, A, R)\, e^{-3\beta \epsilon_{pd}^{s}}$	three RNAP	$\frac{P}{N_{ns}}\, \frac{P-1}{N_{ns}}\, \frac{P-2}{N_{ns}}\, e^{-3\beta \triangle \epsilon_{pd}}$
$Z(P, A-1, R)\, e^{-\beta \epsilon_{ad}^{s}}$	activator	$\frac{A}{N_{ns}}\, \frac{A-R}{A}\, e^{-\beta \triangle \epsilon_{ad}}$
$Z(P, A-1, R-1)\, e^{-\beta \epsilon_{ad}^{s}}$	activator and repressor	$\frac{A}{N_{ns}}\, \frac{R}{A-R-1}\, \frac{A-R}{A}\, e^{-\beta \triangle \epsilon_{ad}}$
$Z(P-1, A-1, R)\, e^{-\beta \left(\epsilon_{pd}^{s}+\epsilon_{ad}^{s}+\epsilon_{ap}\right)}$	activator and one RNAP	$\frac{P}{N_{ns}}\, \frac{A}{N_{ns}}\, \frac{A-R}{A}\, e^{-\beta \left(\triangle \epsilon_{ad}+\triangle \epsilon_{pd}+\epsilon_{ap}\right)}$
$Z(P-2, A-1, R)\, e^{-\beta \left(2\,\epsilon_{pd}^{s}+\epsilon_{ad}^{s}+\epsilon_{ap}\right)}$	activator and two RNAP	$\frac{P}{N_{ns}}\, \frac{P-1}{N_{ns}}\, \frac{A}{N_{ns}}\, \frac{A-R}{A}\, e^{-\beta \left(\triangle \epsilon_{ad}+2\triangle \epsilon_{pd}+\epsilon_{ap}\right)}$
$Z(P-3, A-1, R)\, e^{-\beta \left(3\,\epsilon_{pd}^{s}+\epsilon_{ad}^{s}+\epsilon_{ap}\right)}$	activator and three RNAP	$\frac{P}{N_{ns}}\, \frac{P-1}{N_{ns}}\, \frac{P-2}{N_{ns}}\, \frac{A}{N_{ns}}\, \frac{A-R}{A}\, e^{-\beta \left(\triangle \epsilon_{ad}+3\triangle \epsilon_{pd}+\epsilon_{ap}\right)}$

Table II . Partition functions,

└表格

states and renormalized weight

$$P_{bound} = \frac{Z_{bound}}{Z_{tot}} = \frac{1}{1 + \dfrac{1}{e^{-\beta \triangle \epsilon_{pd}} \left(\frac{3P}{N_{ns}} + \frac{3P(P-1)}{N_{ns}\,N_{ns}} + \frac{P(P-1)(P-2)}{N_{ns}\,N_{ns}\,N_{ns}} \right)} F_{reg}^{-1}(A, R)}$$

where we introduce the regulation factor, $F_{reg}(A, R)$ which is given by

$$F_{reg}(A, R) = \frac{1 + \frac{A}{N_{ns}} \frac{A-R}{A} e^{-\beta(\triangle\epsilon_{ad}+\epsilon_{ap})}}{1 + \frac{A}{N_{ns}} \frac{A-R}{A} e^{-\beta\triangle\epsilon_{ad}} + \frac{A}{N_{ns}} \frac{R}{A-R-1} \frac{A-R}{A} e^{-\beta\triangle\epsilon_{ad}}}$$

As a result of the presence of activators and repressors,

it is as though the number of RNA polymerase molecules has been changed from P to F_{reg} P.

 If we want to compute the probability of the state that all the three promoters are occupied, then it should be
⌊如果

$$P_{bound} = \frac{e^{-\beta(3\,\epsilon_{pd}^s + \epsilon_{ad}^s + \epsilon_{ap})} Z(P-3, A, R) + e^{-\beta(3\,\epsilon_{pd}^s + \epsilon_{ad}^s + \epsilon_{ap})} Z(P-3, A-1, R)}{Z_{tot}}$$

In order to calculate the regulator factor for the regulatory scenario under consideration,
⌊输入行

we need to make estimates for the energy associated with the binding protein to the DNA both

 specifically and non nonspecifically. Binding energies are determined indirectly in experiments which

 measure the equilibrium constant for binding protein to the DNA. If we consider a particular reaction
                ⌊如果

Protein + Dna ⇌ PD

with an equilibrium binding constant

$$K_{bind} = \frac{[PD]}{[P][D]}$$

It is not difficult to get such a relation to relate the microscopic and macroscopic views of binding

$$K_{bind} = V_{cell}\, e^{-\beta\epsilon}$$

where we introduce the parameter ϵ to represent the change of free energy when a single protein binds to a DNA.

 Hence,

$$\frac{K_{pd}^s}{K_{pd}^{ns}} = e^{-\beta\triangle\epsilon_{pd}}$$

$$\frac{K_{ad}^s}{K_{ad}^{ns}} = e^{-\beta\triangle\epsilon_{ad}}$$

With the data showed in Table III, we may obtain
⌊With循环   ⌊表格

$\triangle\epsilon_{pd} \approx -10.6\ k_B\,T$

$\triangle\epsilon_{ad} \approx -11.6\ k_B\,T$

$\epsilon_{ap} \approx -3.9\ k_B\,T$ [6]

Equilibrium Constant	Value (M^{-1})
K_{pd}^s [4]	2×10^{11}
K_{pd}^{ns} [4]	5×10^6
K_{ad}^s [5]	5.59×10^9
K_{ad}^{ns} [5]	5×10^4

Table III. The values of equilibrium constant
⌊表格

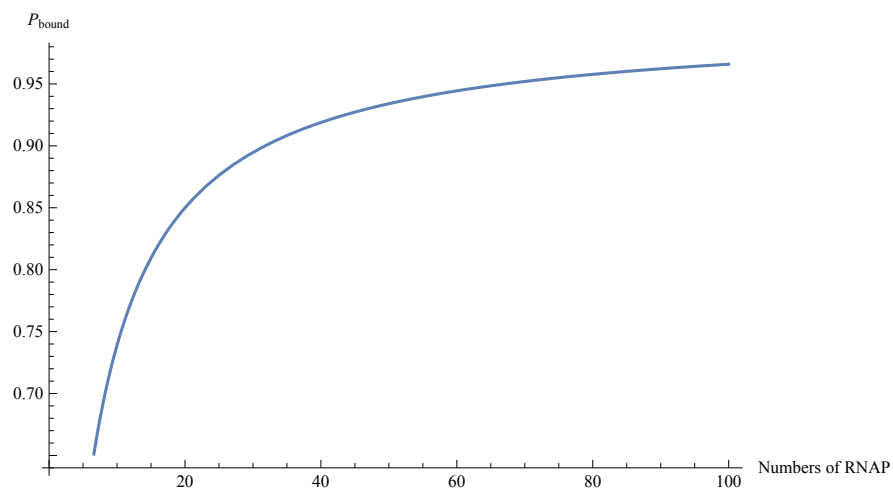## ii. Results

P_{bound}

Figure 1.1 Probability of at least one promoter occupancy as a function of the number of RNA polymerase molecules
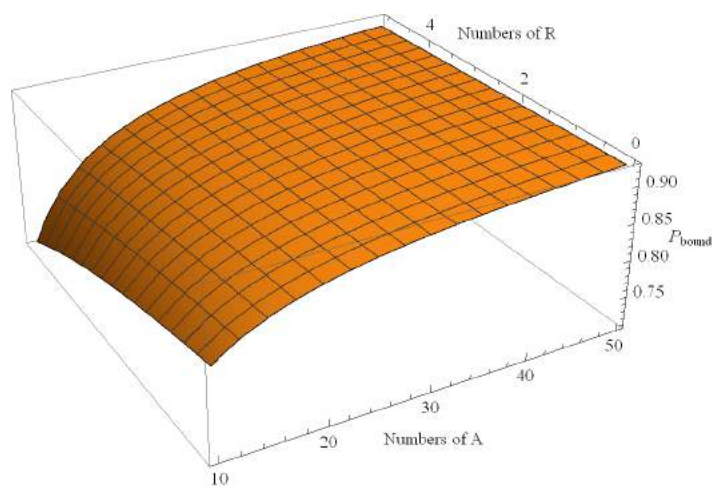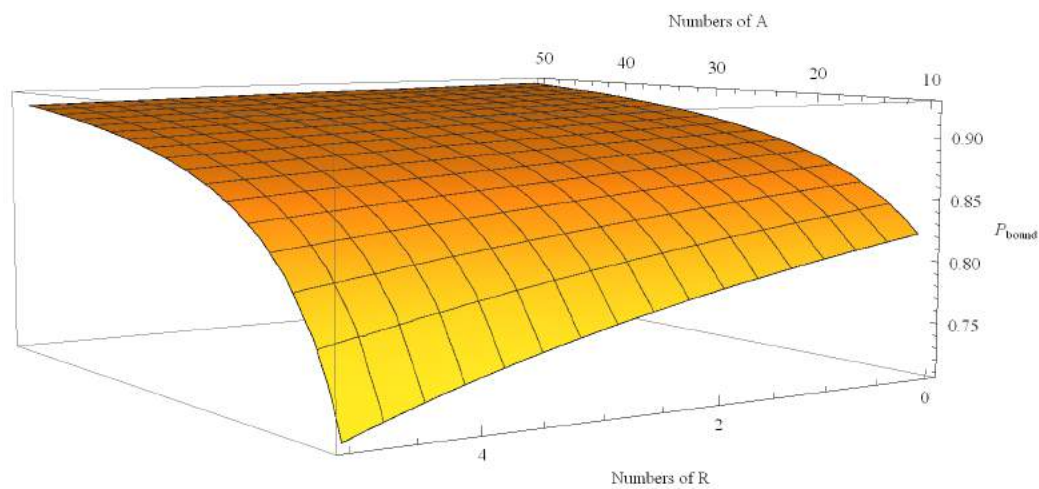
概率

Figure 1.2 Probability of at least one promoter occupancy as a function of the number of activators and repressors
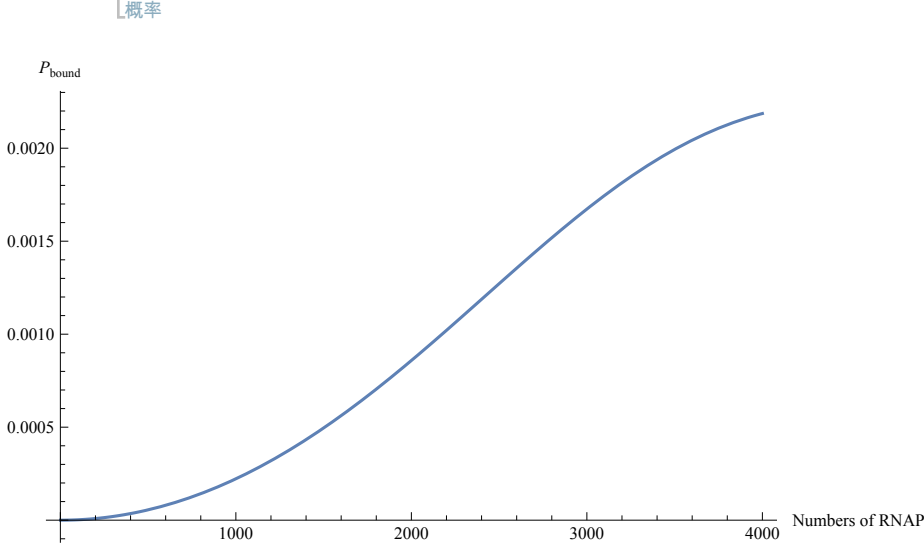└概率

Figure 1.3 Probability of all three promoters occupancy as a function of the number of RNA polymerase molecules
└概率

II.the genetic circuits with one promoter

i.General Methods
└一般系统信息

If the system we consider only contain one promotor,
└如果

the total partition function can be represented mathematically as

$$Z_{tot} = \underbrace{Z(P,A,R)}_{empty\ promoter} + e^{-\beta\epsilon_{ad}^s}\underbrace{Z(P,A-1,R)}_{activator} + e^{-\beta\epsilon_{ad}^s}\underbrace{Z(P,A-1,R-1)}_{activator\ and\ repressor} + e^{-\beta\epsilon_{ad}^s}\underbrace{Z(P-1,A,R)}_{RNAP} + e^{-\beta\left(\epsilon_{pd}^s+\epsilon_{ad}^s+\epsilon_{ap}\right)}\underbrace{Z(P-1,A-1,R)}_{activator\ and\ RNAP}$$

\

Similarily,

$$Z_{bound} = e^{-\beta\epsilon_{ad}^s}Z(P-1,\ A,\ R) + e^{-\beta\left(\epsilon_{pd}^s+\epsilon_{ad}^s+\epsilon_{ap}\right)}Z(P-1,\ A-1,\ R)$$

$$P_{bound} = \frac{Z_{bound}}{Z_{tot}} = \frac{1}{1+e^{\beta\triangle\epsilon_{pd}}\frac{N_{ns}}{P}F_{reg}^{-1}(A,\ R)}$$

where the regulation factor, $F_{reg}(A,\ R)$ is given by

$$F_{reg}(A,\ R) = \frac{1+\frac{A}{N_{ns}}\frac{A-R}{A}e^{-\beta(\triangle\epsilon_{ad}+\epsilon_{ap})}}{1+\frac{A}{N_{ns}}\frac{A-R}{A}e^{-\beta\triangle\epsilon_{ad}}+\frac{A}{N_{ns}}\frac{R}{A-R-1}\frac{A-R}{A}e^{-\beta\triangle\epsilon_{ad}}}$$
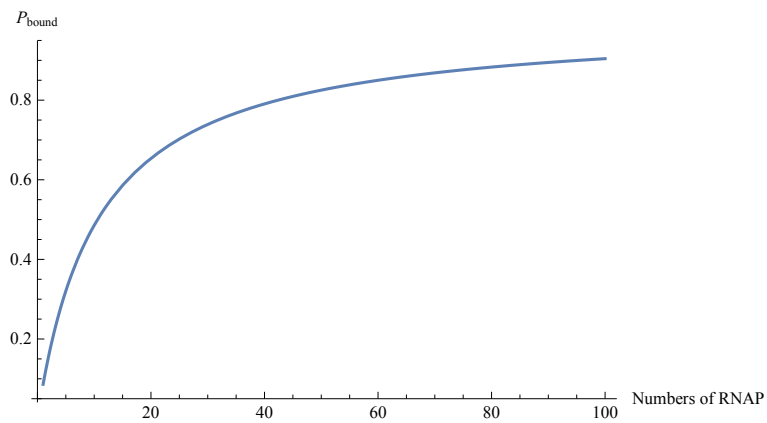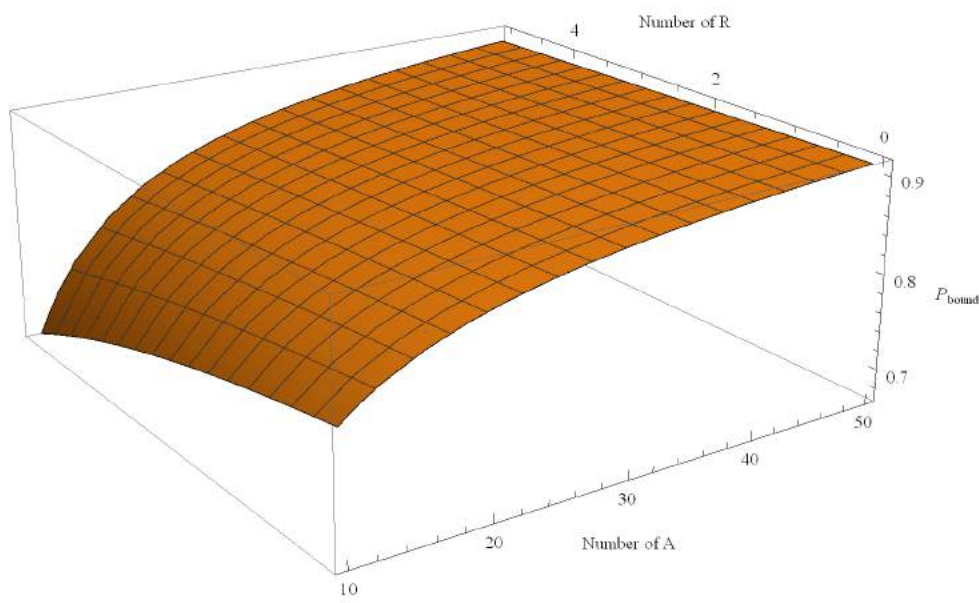
ii.Results

Figure 2.1 Probability of one promoter occupancy as a function of the number of RNA polymerase molecules
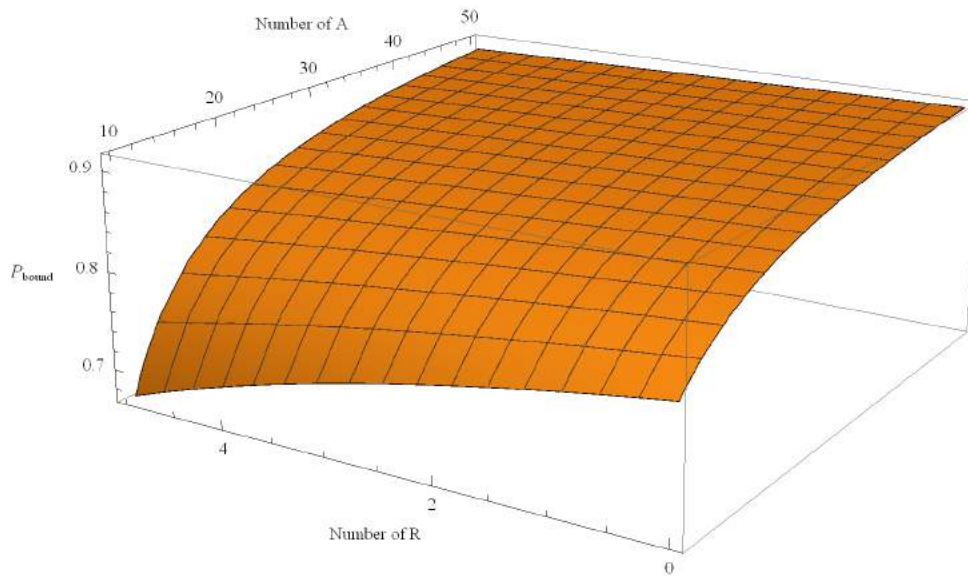概率

Figure 2.2 Probability of one promoter occupancy as a function of the number of activators and repressors
└概率

Compared with the results showed in Figure 1.1 ~ 1.3, it is not difficult to know that the

  second design which only contain one promoter can increase the production of shRNAs sharply.

III.Reference

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