## Parameters verification

$P$

P means the effective number of ribosome binding to mRNA.According to hypothesis(1),the mRNA strand is abundant with ribosomes except stem loop.Due to the absence of stem loop in mRNA2, the calculation formula should be considered respectively:

As for mRNA and mRNA1,

P=(length of mRNA or mRNA1 sequence - length of stem loop sequence)/3

As for mRNA2,

P=length of mRNA2 sequence/3

From the formulas above,we get the following table:

| $P$ /bp | Reaction 2 | Reaction 4 | Reaction 5 |
|---|---|---|---|
| Circuit 2 | 5488/3 | 1061/3 | 4415/3 |
| Circuit 5 | 5488/3 | 1061/3 | 4415/3 |
| Circuit 9 | 5488/3 | 1061/3 | 4415/3 |
| Circuit 11 | 5488/3 | 1061/3 | 4415/3 |

$N_{NS}$

$N_{NS}$ means the number of non-specific sites in mRNA.Honestly,we cannot know the number exactly,but we can estimate it.Obviously the promoter doesn't belong to non-specific sites.And secondly because ribosomes are unable to bind to the stem loop,stem loop doesn't belong to them as well. Besides, mRNA2 doesn't contain the stem loop, thereby the calculation fomula should be considered respectively too:

As for mRNA and mRNA1,

$N_{NS}$ = length of mRNA or mRNA1 sequence - length of RBS sequence - length of stem loop

As for mRNA2,

$N_{NS}$ =length of mRNA2 sequence - length of RBS sequence

We can get the following table from the formulas above:

| $N_{NS}$ /bp | Reaction 2 | Reaction 4 | Reaction 5 |
|---|---|---|---|
| Circuit 2 | 5476 | 1049 | 4403 |
| Circuit 5 | 5476 | 1049 | 4403 |
| Circuit 9 | 5476 | 1049 | 4403 |
| Circuit 11 | 5476 | 1049 | 4403 |

Annotation:

1.Both the length of RBS sequence in circuit 1-5 are 12bp

2.The length of stem loop sequence in circuit 2 is 37bp;circuit 5 is 63bp; circuit 9 is 42bp; circuit 11 is 66bp.

$K_{pd}^{S}$、$K_{pd}^{NS}$

$K_{pd}^{S}$、$K_{pd}^{NS}$ means dissociation constants for specific binding and dissociation constants for non-specific binding. In terms of literature[5], $K_{pd}^{S}$ =0.06 $\pm$ 0.005.Because the probability of ribosome binding to mRNA's non-specific sites is very small, $K_{pd}^{NS}$ must be a large number.Therefore,we can choose one as an estimated value. In our model,we define it as 100.

[mRNA](0)

Because the initial concentration of mRNA cannot be measured, here we replace it approximately with the concentration of mRNA in 90min. In terms of the experimental statistics provided by wet lab, we obtained the concentration of mRNA in 90min in different circuits(unit: mol/L):

| Circuit | 2 | 5 | 9 | 11 |
|---|---|---|---|---|
| [mRNA](90) / (mol/L) | 1.465e-7 | 1.242e-7 | 4.093e-8 | 2.153e-8 |

As for mRNA1 and mRNA2, we also use the concentration in 90min instead of the initial concentration. The concentration can be calculated by the concentration of mRNA in 90min and reaction(3):

According to the empirical formula, we have calculated the $K_{d1}$ in different circuits

So the reaction rate is $v = K_{d1} \times [mRNA_1](90)$ . The concentration of mRNA1 and mRNA2 in 90min are $[mRNA_1](90) = [mRNA_2](90) = v \times 90$

Reaction rate constant

Substituting these data above into formula(3.1), we could obtain the relevant reaction rate constant.

| Circuit | 2 | 5 | 9 | 11 |
|---|---|---|---|---|
| $K_{p1}/\mathrm{hr}^{-1}$ | 18.86 | 22.26 | 67.51 | 128.36 |
| $K_{p11}/\mathrm{hr}^{-1}$ | 0.676 | 0.551 | 4.005 | 2.184 |
| $K_{p12}/\mathrm{hr}^{-1}$ | 0.676 | 0.551 | 4.005 | 2.184 |