Table 1. Approximated parameters of individual chemical reactions within the riboregulated RNA toggle switch. Values represent rate constants for the reactions and are averaged in units of molecules/s for purposes of Gillespie stochastic modeling. Constants are calculated based on constants for *Escherichia coli* bacteria.

|  |  |  |
| --- | --- | --- |
| Reaction Constant | Chemical Equation | Parameter Value (molecules/s) |
| katc | ADNA 🡪 ARNA | 0.6897 |
| kbtc | BDNA 🡪 BRNA | 0.6897 |
| ka,deg | ARNA 🡪 Ø | 0.0033 |
| kb,deg | BRNA 🡪 Ø | 0.0033 |
| ka,on | ARNA + BRNA 🡪 ARNA:BRNA | 101.4 |
| kb,on | BRNA + ARNA 🡪 ARNA:BRNA | 101.4 |
| ka,off | ARNA:BRNA 🡪 ARNA | 0.5 |
| kb,off | ARNA:BRNA 🡪 BRNA | 0.5 |
| kab,deg | ARNA:BRNA 🡪 Ø | .0033 |

Place this table close to the scheme possibly underneath it

Table 2. Approximated parameters of individual chemical reactions within the riboregulated RNA repressilator. Values represent rate constants for the reactions and are averaged in units of molecules/s for purposes of Gillespie stochastic modeling. Constants are calculated based on constants for *Escherichia coli* bacteria.

|  |  |  |
| --- | --- | --- |
| Reaction Constant | Chemical Equation | Parameter Value (molecules/s) |
| katc | ADNA 🡪 ARNA | 0.6897 |
| kbtc | BDNA 🡪 BRNA | 0.6897 |
| kctc | CDNA 🡪 CRNA | 0.6897 |
| ka,deg | ARNA 🡪 Ø | 0.0033 |
| kb,deg | BRNA 🡪 Ø | 0.0033 |
| kc,deg | CRNA 🡪 Ø | 0.0033 |
| kab,on | ARNA + BRNA 🡪 ARNA:BRNA | 101.4 |
| kbc,on | BRNA + CRNA 🡪 BRNA:CRNA | 101.4 |
| kca,on | CRNA + ARNA 🡪 CRNA:ARNA | 101.4 |
| kab,off | ARNA:BRNA 🡪 ADNA + BRNA | 0.05 |
| kbc,off | BRNA:CRNA 🡪 BDNA + CRNA | 0.05 |
| kca,off | CRNA:ARNA 🡪 CDNA + ARNA | 0.05 |
| kcleave,a | ARNA:BRNA 🡪 ARNA | 0.5 |
| kcleave,b | BRNA:CRNA 🡪 BRNA | 0.5 |
| kcleave,c | CRNA:ARNA 🡪 CRNA | 0.5 |

Fig 1. Representative chemical scheme of toggle switch based on two trans-cleaving hammerhead ribozymes. Ribozyme A cleaves ribozyme B and vice versa after reversibly binding to one another. Translation is not depicted and is assumed to have no effect on transcription nor riboregulation in the circuit.

Fig 2. Timecourse of RNA-based toggle switch with two ribozymes from Gillespie algorithm. Given an initial concentration of ARNA, concentration does not reach some steady state value and eventually drops to zero molecules after 10 hours. BRNA remains low and ABRNA seems to stay constant throughout the reaction despite high initial concentratinos of ARNA, implying high amounts of degradation of ARNA at early times. G is concentration of reporter protein GFP which is encoded on a separate mRNA strand that is cleaved by ribozyme A. When A is not present, GFPRNA is free to be translated and thus fluoresce.

Fig 3. Representative chemical scheme of repressilator based on three trans-cleaving hammerhead ribozymes in a rock-paper-scissor interaction. Ribozyme A binds to and cleaves ribozyme B, ribozyme B binds to and cleaves ribozyme C, and ribozyme C binds to and cleaves ribozyme A. Translation is not depicted and is assumed to have no effect on transcription nor riboregulation in the circuit.

Fig 4. Average trace over 100 seconds of RNA-based repressilator with three ribozymes from Gillespie algorithm. Graph is based on an average sample of 500 repetitions of timecourses from stochastic model. Time and molecules are both kept at low levels in order to clearly observe the oscillations in concentrations. While oscillations are observed, they are irregular and unstable, presumably due to high rates of transcription and degradation of mRNA.