Degradation-Driven Failure Minimization in Genetic Circuits Through Model Checking

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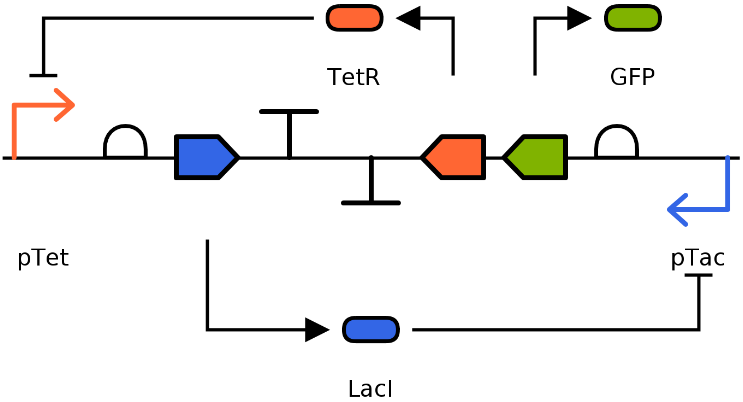
Genetic Toggle Switch



Genetic Toggle Switch

Desired Behavior
The toggle switch should stay in the specified (on/off) state.
Unintended state reversal is a failure (undesired state).

Our Goal

Research Question
Can we modify degradation rates of LacI, TetR, and YFP to reduce failure probability?

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Why degradation?
We can tune performance by utilizing degradation tags.

CTMC Models

- Two models represent the circuit initialized in the "on" (YFP high) and "off" (YFP low) states
- Desired behaviors are mutually exclusive
  - Calls for using two models
  - Creates an optimization problem
- Models are represented as *continuous-time Markov chains* (CTMCs)

CTMC Models

In a CTMC model:

- Each assignment of variable values is a *state*
- Each reaction/update is a *transition*
- Transitions occur at a specified *rate*
- Transition rates correspond to a *probability* of choosing a certain transition

CTMC Models

- CTMC transient analysis gives an *exact* probability
- We use the PRISM probabilistic model checker to do transient analysis
- We specify behavior in *Continuous Stochastic Logic* (CSL):
  YFP Low: $P_{=?}[\ \texttt{true}\ U^{[0,2100]}\ \texttt{YFP} \geqslant 30\ ]$
  YFP High $P_{=?}[\ \texttt{true}\ U^{[0,2100]}\ \texttt{YFP} < 10\ ]$

Rate Variation

- Varied degradation rates (k_d) of YFP, LacI, and TetR across 9 values: $[0.00375, 0.005, 0.00625, 0.0075, 0.00875, 0.01, 0.01125, 0.0125, 0.015]$
- Values are spaced around iBioSim's default k_d of 0.0075
- We explore 3^9 combinations:
  $(0.00375, 0.00375, 0.00375)$
  $(0.00375, 0.00375, 0.005)$
  $(0.00375, 0.005, 0.00375)$
  $(0.005, 0.00375, 0.00375)$
  $(0.00375, 0.00375, 0.00625)$
  $\ldots$

Methodology

For each combination of degradation rates for YFP, LacI, and TetR:

- Python script updates the model's degradation rates
- PRISM CTMC transient analysis finds the probability of an undesired state switch within about one cell cycle

Methodology

After all models are tested:

- Script processes and ranks resulting failure probabilities, considering:
  - Each rate combination affects circuit behavior differently
  - Improving the YFP high model may harm the YFP low model
  - A metric is needed to weigh the probability pairs
- Script recommends optimal combinations of degradation rates

Metrics

Metrics penalize both high probabilities and imbalanced probabilities. They include:

- Simple sum ($p_{\text{high}} + p_{\text{low}}$),
- Average plus difference ($(p_{\text{high}} + p_{\text{low}})/2 + \text{abs}(p_{\text{high}} - p_{\text{low}})$),
- Sum of squares ($p_{\text{high}}^2 + p_{\text{low}}^2$) and cubes ($p_{\text{high}}^3 + p_{\text{low}}^3$),
- and several others

The variety of metrics allows flexibility: engineers can choose their priorities

Metrics

For example:

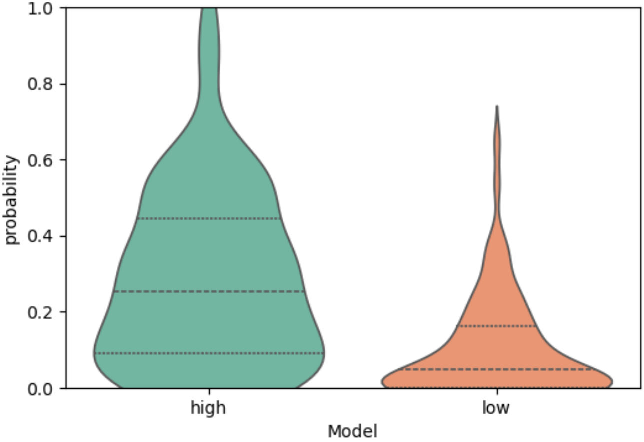
- $p_{\text{high}} = 0.3$ and $p_{\text{low}} = 0.3$ is balanced
- $p_{\text{high}} = 0.6$ and $p_{\text{low}} = 0.0$ has the same average, but is not balanced

Probability Distribution

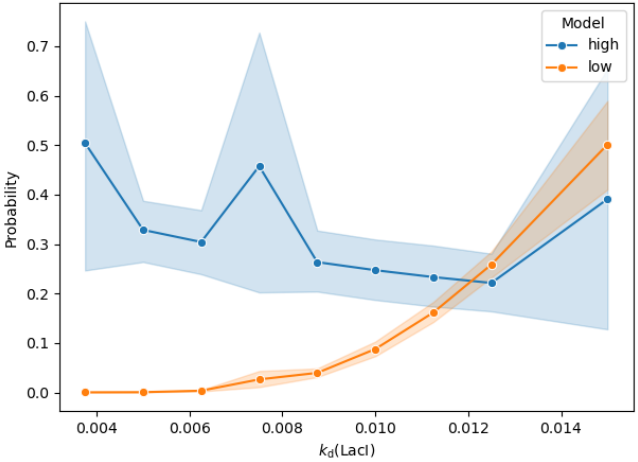
Preliminary analysis shows that:

- Each degradation rate has a unique impact on failure probability
- The YFP high model sees more variation than the YFP low model
- The YFP low model sees a generally-low failure probability

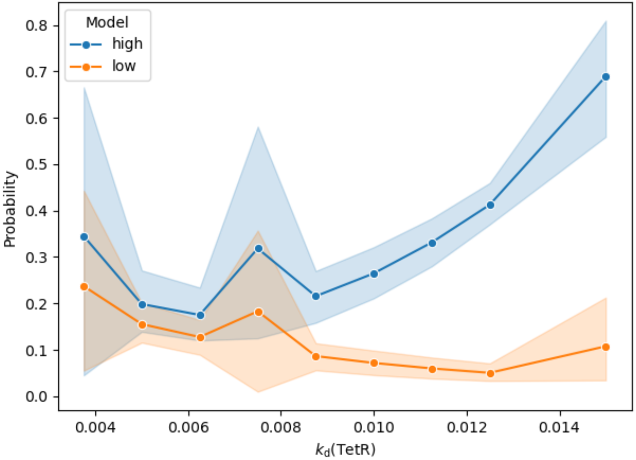
Probability Distribution



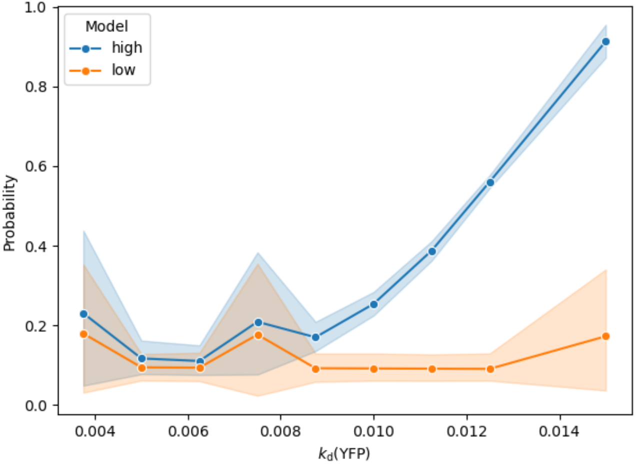
Probability vs LacI



Probability vs TetR



Probability vs YFP



Observations

- Optimizing robustness in one model reduces stability in the other
- This model calls for a balanced profile across YFP, LacI, and TetR
- Each degradation rate has a unique impact
- Modifying degradation rates can greatly impact probability of failure

Best Combinations

For 20 metrics over all parameter combinations, winning combinations for YFP, LacI, TetR are:

- (0.00375, 0.00375, 0.00375) – 5 metrics
- (0.00375, 0.015, 0.00375) – 5 metrics
- (0.015, 0.00375, 0.015) – 5 metrics
- (0.00625, 0.00625, 0.005) – 2 metrics
- (0.015, 0.00375, 0.00375) – 2 metrics
- (0.015, 0.015, 0.00375) – 1 metric

Conclusion

We choose (YFP, LacI, TetR) = (0.00375, 0.00375, 0.00375) because:

- It was selected by five metrics
- Manual validation found it to be best overall
- It provides a reasonable balance between probability of failure for both the high and low models

Discussion

Limitations:

- We rely on a combinatorial analysis of degradation rates
- This approach is minimally scalable

Future Work:

- Validate these results in the lab
- Use parameter synthesis techniques and heuristics
  to scale to more complex genetic circuits

Thank You

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