

A Synthetic Biology Approach to Ribosome Collisions and Stalling

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Short Abstract — Translation is well understood, but few predictions at the whole cell level have been experimentally tested regarding ribosome collisions and stalling. In this work, we modify a TASEP model to account for the influence of rare codons on collective ribosomal dynamics. We test this model using a synthetic biology approach, where we are able to obtain quantitative data concerning the influence of ribosome collisions and stalling on translational output.

Keywords — Ribosome Collisions, Ribosome Stalling, TASEP, Systems and Synthetic Biology, Monte-Carlo

I. ABSTRACT

THE fundamentals of translation are well established, but the cellular response to ribosomal stalling and collisions is poorly understood. Previous detailed models for translation, one of which is Totally Asymmetric Simple Exclusion Process (TASEP), predict that ribosomes naturally and frequently interact with each other [1-6]. However, TASEP and similar models typically do not consider the fact that each ribosome's footprint spans many codons, that each ribosome moves along mRNA with a non-uniform rate, or that ribosomes can prematurely dissociate from mRNA.

To this end, we construct a detailed stochastic model for translation that extends earlier efforts [6]. This new model allows for ribosome collisions when their respective footprints overlap. These collisions can then accelerate ribosome rescue, whereby ribosomes insert a fast degradation tag on partially completed peptides and then detach from mRNA. The model also allows for ribosome rescue in the case of stalling, where a ribosome simply pauses at a rare codon. Using this model, we explore the influence of ribosome collisions and stalling on translational output. In the near future, this model will also include the influence of collisions and stalling on mRNA degradation.

We then experimentally test our model predictions using a synthetic biology approach. This involves batch results (96-well microplate experiments) and single cell results (microfluidics and microscopy) for a synthetic circuit in *E. coli*. We designed our synthetic circuit so that two adjacent coding regions for fluorescent proteins are under control of a

common inducible promoter. In between the coding region for these proteins is a region we call the "gate," a potential bottleneck (or stalling site) of elongation. The gate consists of codons that are normally rare in *E. coli*. We then synthetically control the cellular level of tRNA specific to these gate codons, and thus, we can vary these codons from rare to abundant.

Measurement of the two fluorescent proteins provides insight into the influence of ribosome collisions and stalling on translational output. By controlling the tRNA concentration corresponding to our gate, we can continuously transition the gate from an open to a closed state. If the gate is open, then there is a plentiful amount of the tRNA for the gate codons, and translation will continue at a fast pace for all fluorescent proteins. If the gate is closed or infrequently open due to minimal tRNA concentration, then translation of the downstream protein is either stalled or aborted.

The advantage of using our synthetic approach is it allows for quantitative data for (1) rareness of codons related to tRNA levels, and (2) an *in vivo* analysis of ribosomal collisions and stalling. By combining experimental findings with TASEP simulation, it allows us to create a more accurate model of ribosomal interactions than previously possible.

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