# Automated Design of Synthetic Bacterial Small RNAs

Amin Espah Borujeni[1], Emily Dong[2], and Howard M. Salis[3]

***Short Abstract* — Small RNAs (sRNAs) are short RNAs that regulate translation and mRNA stability in bacteria. We have developed a statistical thermodynamic model that predicts the translation initiation rate of a mRNA when regulated by a sRNA, building on our recent work that quantitatively predicts mRNA translation initiation rates (Salis et. al., Nature Biotechnology, 2009). We combine this biophysical model with optimization to automatically design synthetic sRNAs to control protein expression *in trans*. We utilize the biophysical model to quantitatively predict the regulatory functions of small RNAs in *Escherichia coli*, including their targets and the extent of their activation or repression of translation.**

***Keywords* — synthetic biology, gene expression, small RNA, statistical thermodynamics, optimization**

## I. Purpose

Small RNAs (sRNAs) are short, non-coding RNAs that regulate translation and mRNA stability in bacteria[1]. Escherichia coli employs 80 to 100 sRNAs to control its metabolism, stress response, and biofilm formation, making these regulatory RNAs as ubiquitous as transcription factors. The mechanism in which sRNAs bind to mRNAs and alter their translation initiation rate are qualitatively understood; however, a quantitative biophysical model will be needed to better understand the dynamics of gene regulatory networks and to rationally control protein expression for biotechnology applications.

We have created a statistical thermodynamic model that predicts the translation initiation rate of a natural or synthetic mRNA when regulated by a natural or synthetic sRNA, building on our recent work that quantitatively predicts mRNA translation initiation rates[2]. We employ a free energy model[3] to calculate the Gibbs free energies of the sRNA, sRNA-sRNA, mRNA, sRNA-mRNA, mRNA-ribosome, and sRNA-mRNA-ribosome molecular interactions. These calculations explicitly depend on the sRNA and mRNA nucleotide sequences, including the protein coding sequence. We combine these calculations with the mRNA and sRNA concentrations to predict protein expression levels on a proportional scale from 1 to 100,000 or more. By combining this biophysical model with optimization, we design activating and repressing synthetic sRNAs that increase or decrease protein expression by a selected factor, over a 1000-fold range.

We use mRFP1 and GFPmut3b fluorescent protein reporters in a ColE1-BAC dual plasmid system to experimentally test both activating and repressing synthetic sRNAs, employing 40-hour cultures to obtain steady-state fluorescence data. We also employ synthetic sRNAs to control chromosomal protein expression, knocking-up or knocking-down expression levels without requiring chromosomal modification.

The presented design method enables the automated generation of many synthetic sRNAs, on demand, to regulate the expression of many plasmid-born or chromosomally encoded proteins according to a user's specifications[4-7].

Acknowledgements: This work was funded by a DARPA Young Faculty Award.

[1]Department of Chemical Engineering, Penn State University, University Park, PA

[2]Department of Biological Engineering, Penn State University, University Park, PA

Corresponding e-mail: salis@psu.edu

## References

[1] Waters, L. & Storz, G. Regulatory RNAs in bacteria. *Cell* **136**, 615-628 (2009).

[2] Salis, H.M., Mirsky, E.A. & Voigt, C.A. Automated design of synthetic ribosome binding sites to control protein expression. *Nat Biotechnol* **27**, 946-50 (2009).

[3] Xia, T. et al. Thermodynamic Parameters for an Expanded Nearest-Neighbor Model for Formation of RNA Duplexes with Watson-Crick Base Pairs†. *Biochemistry* **37**, 14719-14735 (1998).

[4] Temme, K. et al. Induction and relaxation dynamics of the regulatory network controlling the type III secretion system encoded within Salmonella pathogenicity island 1. *J Mol Biol* **377**, 47-61 (2008).

[5] Tabor, J.J. et al. A synthetic genetic edge detection program. *Cell* **137**, 1272-81 (2009).

[6] Salis, H., Tamsir, A. & Voigt, C. Engineering bacterial signals and sensors. *Contrib Microbiol* **16**, 194-225 (2009).

[7] Salis, H. & Kaznessis, Y.N. Computer-aided design of modular protein devices: Boolean AND gene activation. *Phys Biol* **3**, 295-310 (2006).