

Ligand-regulated shRNA switches as programmable network components

Chase L Beisel^{1*}, Travis S Bayer², Kevin G Hoff¹, and Christina D Smolke¹

1. Division of Chemistry and Chemical Engineering

2. Division of Biology

California Institute of Technology, Pasadena, CA 91125, USA

*Email: cbeisel@caltech.edu

Recent progress in the construction of biological networks has opened new avenues of research for understanding and manipulating biological systems¹⁻⁴. Further developments necessitate the design of more advanced components that can be predictably modified to yield optimal system performance. RNA has provided a unique substrate for the design of programmable gene regulatory systems⁵⁻⁷, although current systems lack a framework for translating sequence information into *in vivo* behavior.

Towards these aims, we have engineered an RNA-based platform, which we call an shRNA switch, that provides for ligand control of RNA interference (RNAi) in mammalian cells. shRNA switches are comprised of three modular domains: an shRNA stem, an aptamer, and a competing strand. The shRNA stem encodes the guide strand that activates RNAi-mediated gene silencing following processing of the shRNA switch by the RNAi machinery. The aptamer binds the specified ligand and the competing strand translates this binding interaction into a conformational change from an active to inactive conformation. The presence of ligand coincides with an increase in target gene expression levels by inhibiting processing of the shRNA switch. Plasmid-based expression of an shRNA switch that incorporates the theophylline aptamer⁸ and an EGFP-targeting guide strand displays theophylline-dependent regulation of EGFP in cell culture.

To explore the underlying mechanism of shRNA switch activity and the potential for tuning the component transfer function, we derived a simple mathematical model from our proposed mechanism that predicts the transfer function linking ligand concentration (L) and relative target gene expression levels (f):

$$f = 1 - e \cdot f_{\text{shRNA}} \left[1 + K_{\text{Comp}} \left(1 + K_{\text{Apt}} \cdot L \right) \right]^{-h}, \quad (1)$$

where f_{shRNA} is the knockdown achieved by the original shRNA, K_{Comp} is the partition coefficient between active and inactive conformations, K_{Apt} is the association constant between ligand and formed aptamer, e is the processing efficiency of the shRNA switch by the RNAi machinery, and h is a hill coefficient to account for nonlinearity between siRNA concentration and target expression levels.

Our model predicts that varying K_{Comp} , K_{Apt} , and e uniquely tunes the transfer function. We identified five tuning strategies that specify changes in shRNA switch sequence, mapped to the associated tuning parameters, without disrupting the shRNA stem sequence or secondary structure. For example, modifications targeted to different regions of the competing strand produced similar effects on the transfer function that match the trend for K_{Comp} predicted from our model. Thus, the transfer function can be predictably tuned by modifying the shRNA switch sequence.

Combinatorial implementation of these tuning strategies resulted in strategies for fine tuning the component transfer function. In addition, the modularity inherent in our switch design was used to implement aptamer reuse strategies that resulted in multi-input control over gene expression. An extended model that links secondary structure prediction algorithms to our mechanistic functional model was developed and applied to the *in silico* design of shRNA switches with desired behavior. Therefore, this work describes a molecular platform for the

construction of integrated RNA-based gene regulatory systems and an *in silico* design framework that will greatly aid in the reliable and efficient incorporation of these regulatory components into large-scale biological systems.

References

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