Engineering Ligand Control of RNA Interference

Thesis by

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Dedicated in loving memory to my mother

Joanne Hendler Heltzer

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ABSTRACT

RNA is a rich and versatile substrate for the construction of information processing devices. These devices detect the levels of specified intracellular biomolecules and control cellular behavior accordingly. With few superficial constraints on the identity of the recognized biomolecule or the targeted gene, RNA-based information processing devices can be rapidly implemented toward various applications in medicine and biotechnology. To advance the design and implementation of RNA-based information processing devices, we delineated general design principles and applied these principles to the construction of devices that operate through RNA interference (RNAi).

RNAi represents an endogenous enzymatic pathway present in humans and other eukaryotes that mediates targeted gene silencing. The pathway has garnered recent interest as a revolutionary biological research tool and as a targeted therapeutic strategy. While RNAi has left an indelible mark on the scientific community, exerting greater control would advance the applicability and safety of this already impressive gene silencing mechanism. Toward this goal, we engineered ligand control of three types of RNAi effectors in mammalian cells: small interfering (si)RNAs, small hairpin (sh)RNAs, and microRNAs (miRNAs). Engineering frameworks enabled facile replacement of the biomolecule sensory and gene targeting domains, thus lending to rapid implementation as biosensors or autonomous control devices. Experimental and computational characterization studies provided a comprehensive understanding of device behavior, thereby facilitating forward design.

Naturally-occurring analogs of RNA-based information processing devices are riboswitches. Riboswitches predominantly mediate dynamic feedback in metabolism and share many traits with current examples of engineered information processing devices. Various experimental characterization studies of riboswitches showed that kinetics underlying events such as conformational switching and ligand binding have a substantial impact on device performance, although these factors remain to be comprehensively evaluated or considered when formulating design principles for synthetic riboswitch construction. We explored the contribution of kinetic factors to riboswitch performance in silico, where model predictions matched experimental observations, including results from our ligand-responsive RNAi effectors. From our modeling results, we developed a general set of design principles that guide riboswitch assembly and performance tuning.

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