

**P014** Engineered riboswitches to control gene expression  
by small molecules

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Riboswitches are newly discovered regulatory elements. They consist solely of RNA, sense their ligand in a preformed binding pocket and perform a conformational switch in response to ligand binding resulting in altered gene expression. We have utilized rational design and *in vitro* evolution to tap the enormous potential of RNA for molecular sensing and conformational changes and developed novel conditional gene expression systems based on small molecule-binding engineered riboswitches.

Tetracycline-dependent regulation can be imposed on an mRNA in yeast by inserting an aptamer in its 5' UTR. Translation of a reporter gene was suppressed up to 30-fold upon addition of tetracycline. Biochemical and genetic analyses determined that binding of the ligand connects the aptamer intramolecularly thereby inhibiting initial steps of translation. In addition, we created a translational control element by combining the theophylline aptamer with a communication module for which a one-nucleotide slipping mechanism had been proposed. This structural element was inserted close to the bacterial ribosome binding site at a position just interfering with translation in the non ligand-bound form. Addition of the ligand then shifts the inhibitory element to a distance which permits efficient translation.

We present here two novel regulatory mechanisms for engineered riboswitches. Their use of helix slippage or intramolecular linkage makes them different from natural riboswitches.