

# Theophyllin aptamer switch example

## Introduction

Artificial 5'UTR regions were designed which change their structure upon theophyllin binding to the integrated aptamer structure. This triggers the opening of a terminator structure allowing to fully transcribe the mRNA.

## Sequences

The original sequence can be found in `RS3.fa`, a mutated version in `RS3mut.fa`.

## RNAfold with soft-constraints

```
RNAfold < RS3mut.fa
```

include soft constraints which model theophyllin binding

```
RNAfold --motif="GAUACCAG&CCCUUGGCAGC,...((((&)...)))...",-9.22" < RS3mut.fa
```

## Predict ensemble

no switching can be seen in the MFE structure... aha, so it does not work?  
look at ensemble of structures and dot-plot:

```
RNAfold -p --motif="GAUACCAG&CCCUUGGCAGC,...((((&)...)))...",-9.22" < RS3mut.fa
```

aha, high percentage of structures exhibit the aptamer structure bound to the ligand. But what's the percentage?

## Calculate probability of aptamer

use hard constraints to calculate the accessibility of RBS with and without sRNA  
file `RS3_constraint.fa` contains the structure of the theophyllin aptamer, which can be used as hard constraint with `-C`

### **without ligand**

```
RNAfold -p -C <( cat RS3mut.fa RS3_constraint.fa )
```

$\text{prob}(\text{aptamer}) = \exp((\text{pf} - \text{constraint\_energy}) / \text{KT})$   $\text{KT} = ((\text{temperature} + 273.15) * 1.98717) / 1000.0$

calculation should be:  $e^{((-26,04+17,05) \div (((37+273,15) \times 1,98717) \div 1000))} = 0,000000463 = 0,00\%$  of states exhibit have the aptamer structure without ligand

### **with ligand**

```
RNAfold -p -C --motif="GAUACCAG&CCCUUGGCAGC,...((((&)...)))...",-9.22"
<( cat RS3mut.fa RS3_constraint.fa )
```

$\text{prob}(\text{aptamer}) = \exp((\text{pf} - \text{constraint\_energy}) / \text{KT})$   $\text{KT} = ((\text{temperature} + 273.15) * 1.98717) / 1000.0$

calculation should be:  $e^{((-26,55+26,16) \div (((37+273,15) \times 1,98717) \div 1000))} = 0,53110934 = 53,11\%$  of states exhibit have the aptamer structure with ligand

**References** Wachsmuth, Manja, Sven Findeiß, Nadine Weissheimer, Peter F. Stadler, and Mario Mörl. 2013. "De Novo Design of a Synthetic Riboswitch That Regulates Transcription Termination." *Nucleic Acids Research* 41 (4): 2541-51. doi:10.1093/nar/gks1330.