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.

Sequenzes

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 whats 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 )
```

prob(aptamer) = exp((pf - constraint_energy) / KT) KT = ((temperature + 273.15)*1.98717)/1000.0

calculation should be: eˆ((-26,04+17,05)÷(((37+273,15)×1,98717)÷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 )
```

prob(aptamer) = exp((pf - constraint_energy) / KT) KT = ((temperature + 273.15)*1.98717)/1000.0

calculation should be: eˆ((-26,55+26,16)÷(((37+273,15)×1,98717)÷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.