A novel theophylline triggered RNaseP riboswitch
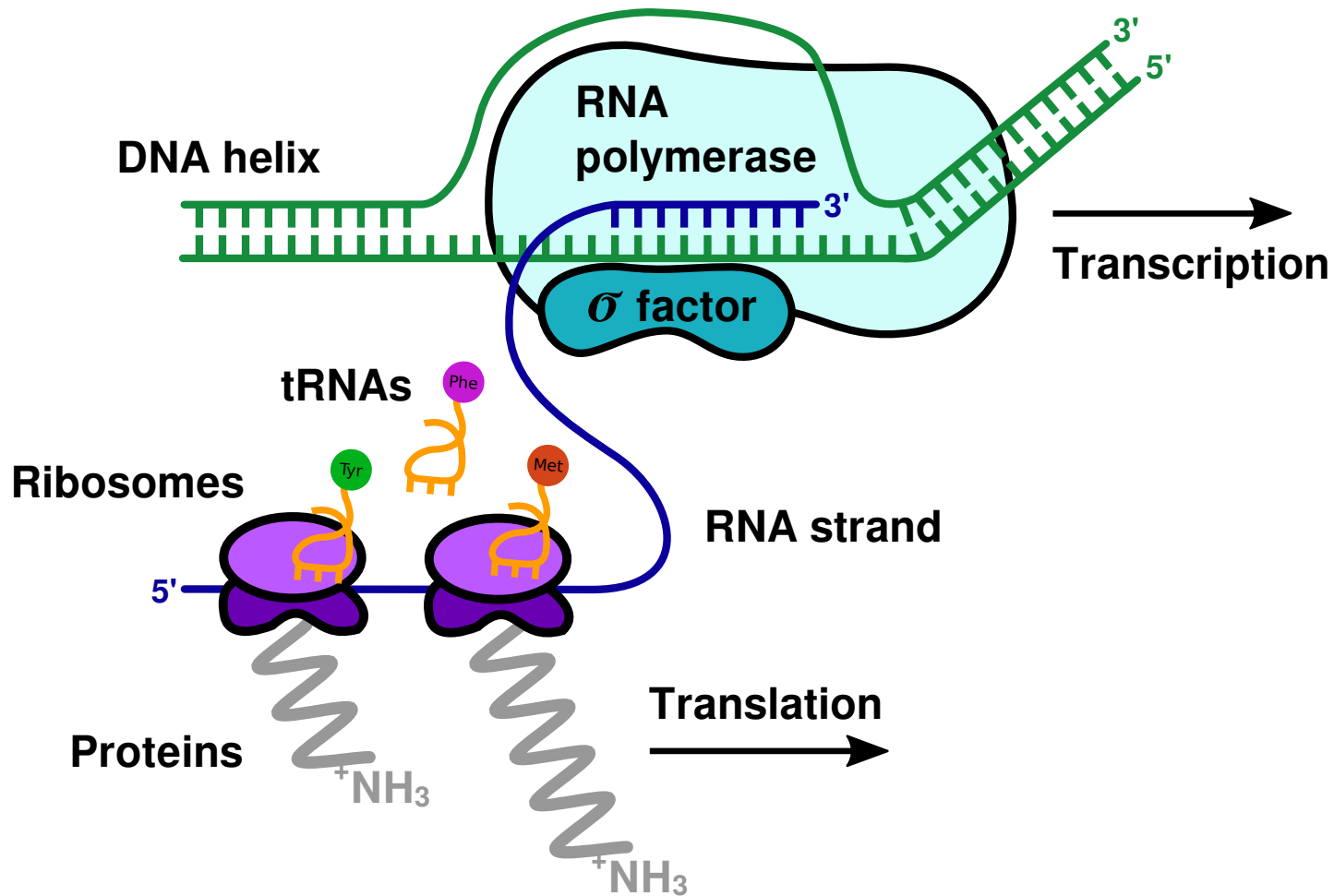
Stefan Hammer
Bled 2018
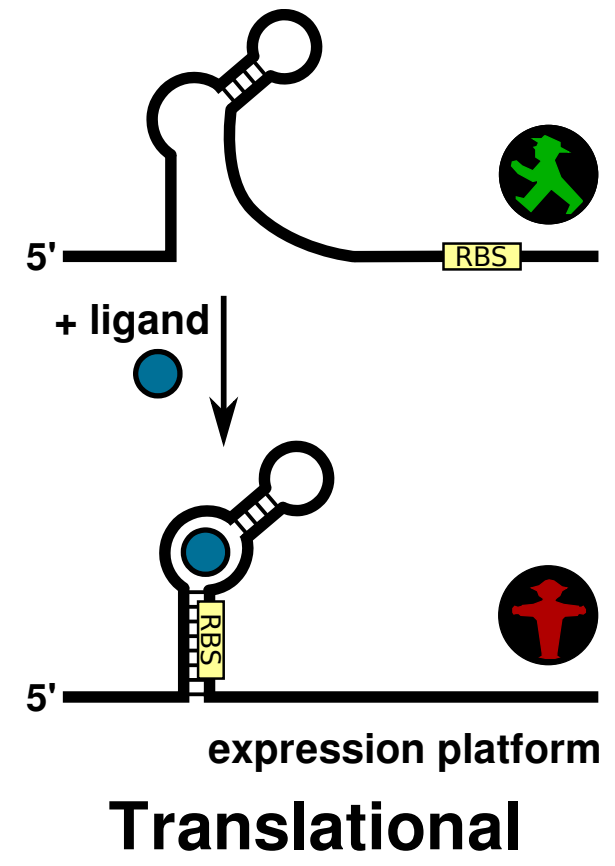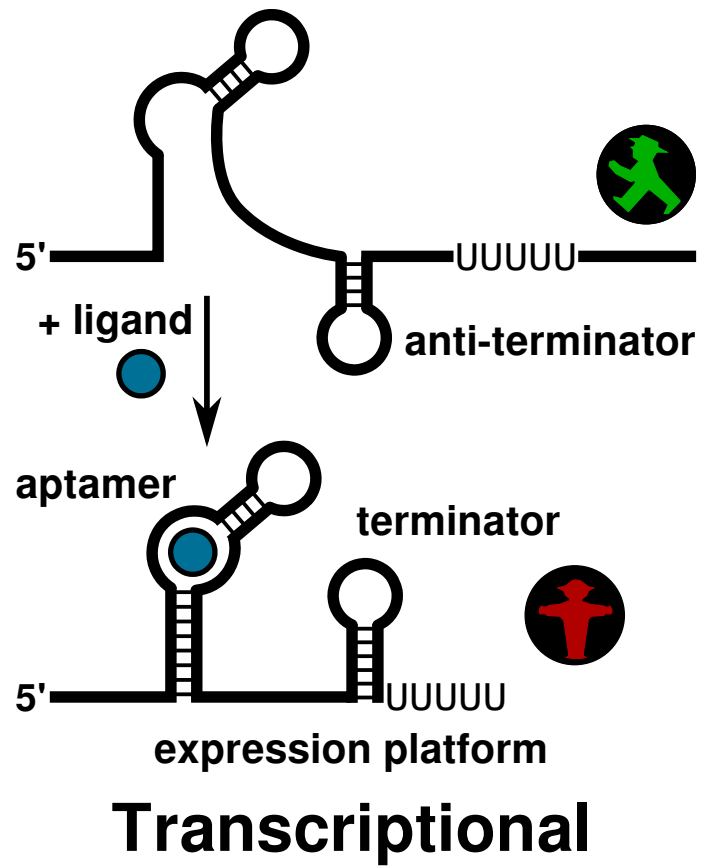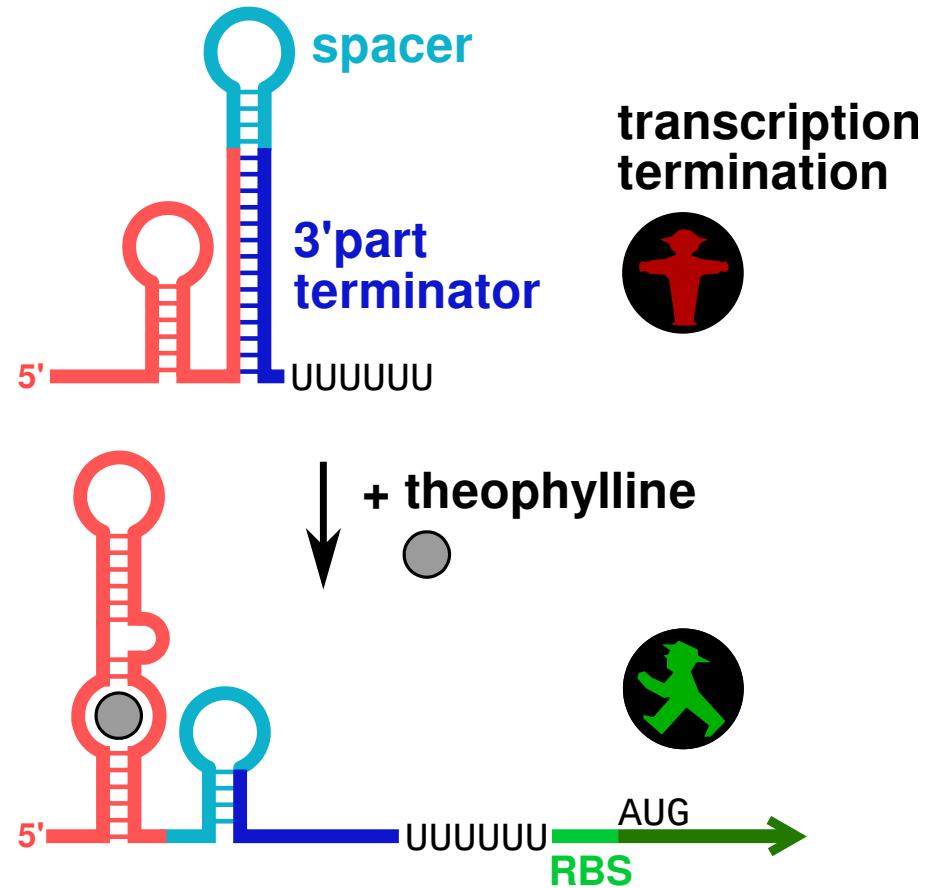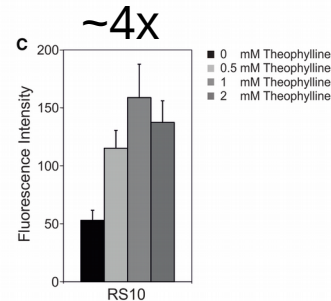
Transcription/Translation

Riboswitches

Artificial Riboswitch

– Theophylline triggered

– Transcriptional

– Reporter Gene

RNaseP riboswitch idea

tRNA processing mechanism
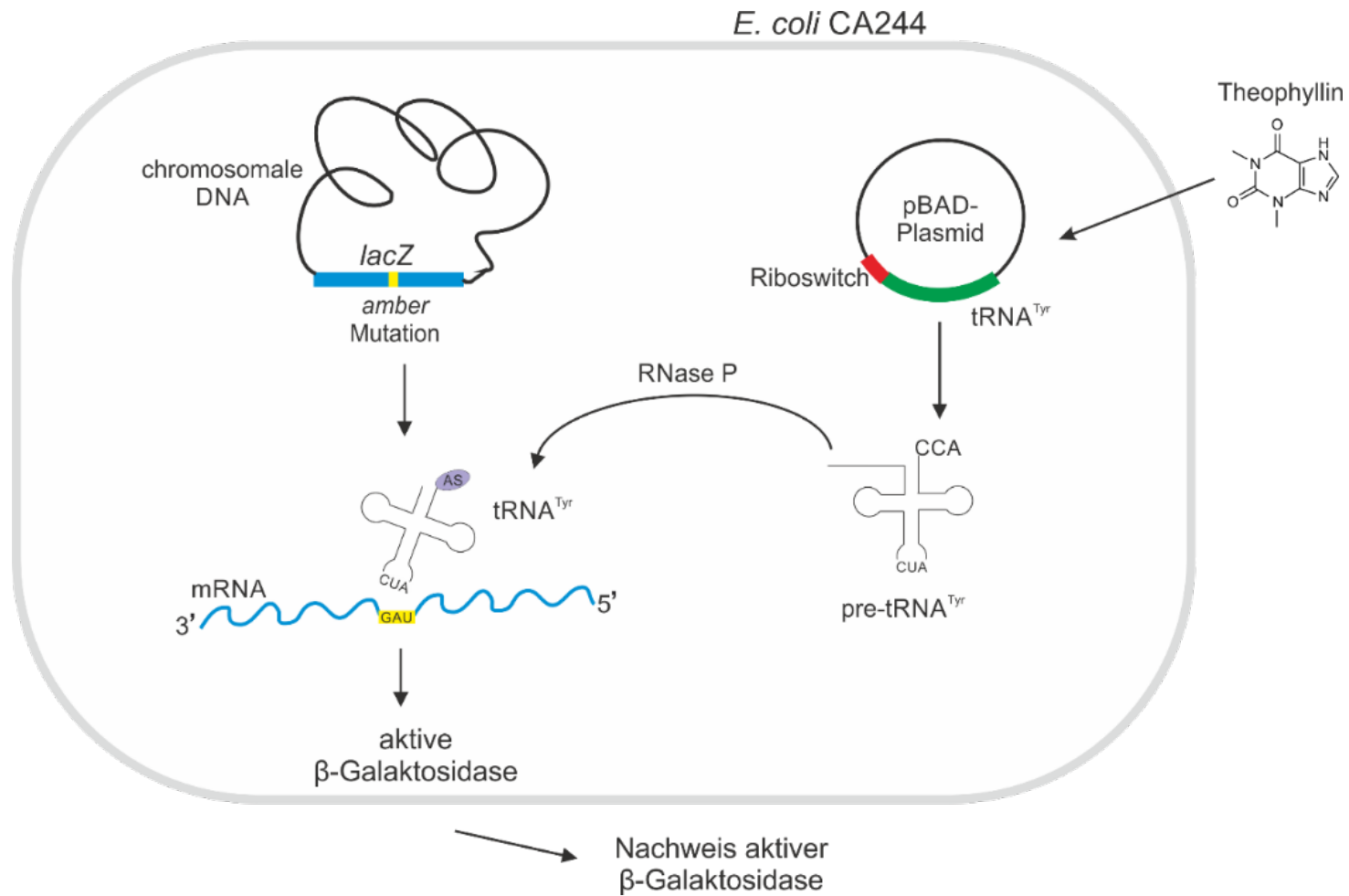
Copy&Paste Design

construct I (RNAseP RS, R273)

> Theophylline RS w/o poly U fused to su3+

AAGUGAUACCAGCAUCGUCUUGAUGCCCUUGGCAGCACUUCAGAAAUCUCUGAAGUGCUGCCAAGGUGGGGUUCC
CGAGCGGCCAAAGGGAGCAGACUCUAAAUCUGCCGUCAUCGACUUCGAAGGUUCGAAUCCUUCCCCCACCACCA

tRNA spacer aptamer

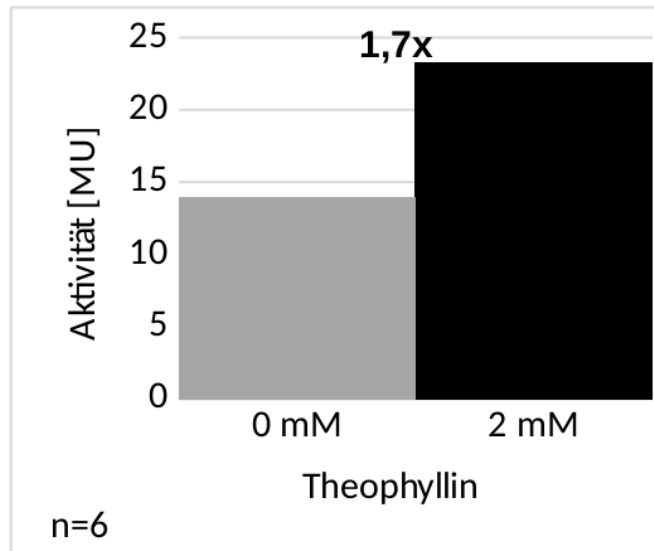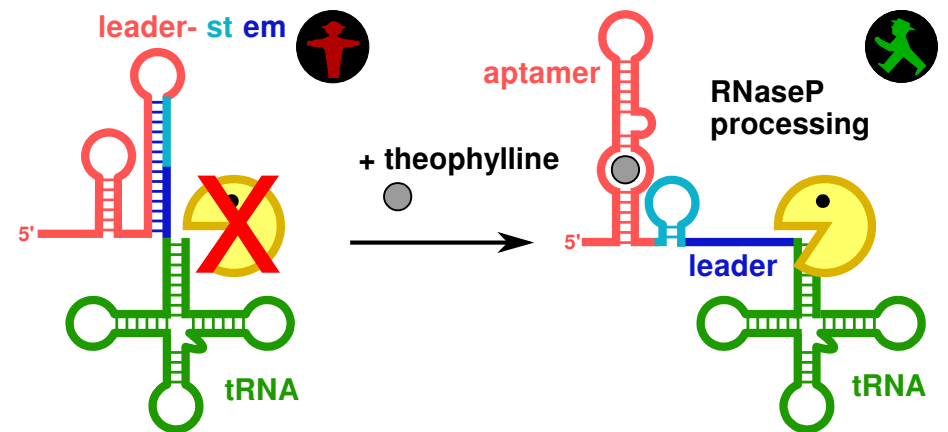-   This construct is without the original 5'-leader sequence of the tRNA

cleavage

Figure 1: ONPG- assay with construcht I (RNase R RS).

Timing is important!

Design Pipeline

Findeiß, Sven, Stefan Hammer, Michael T. Wolfinger, Felix Kühnl, Christoph Flamm, and Ivo L. Hofacker. **2018**. "In Silico Design of Ligand Triggered RNA Switches."
bioRxiv, January, 245464. https://doi.org/10.1101/245464.
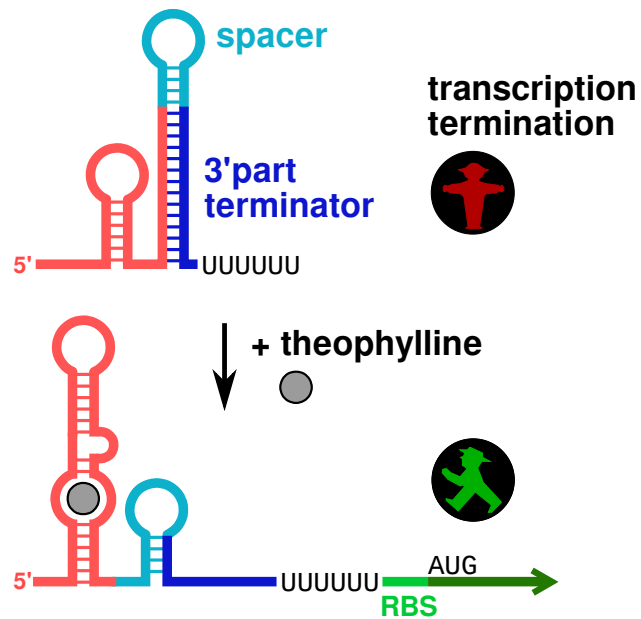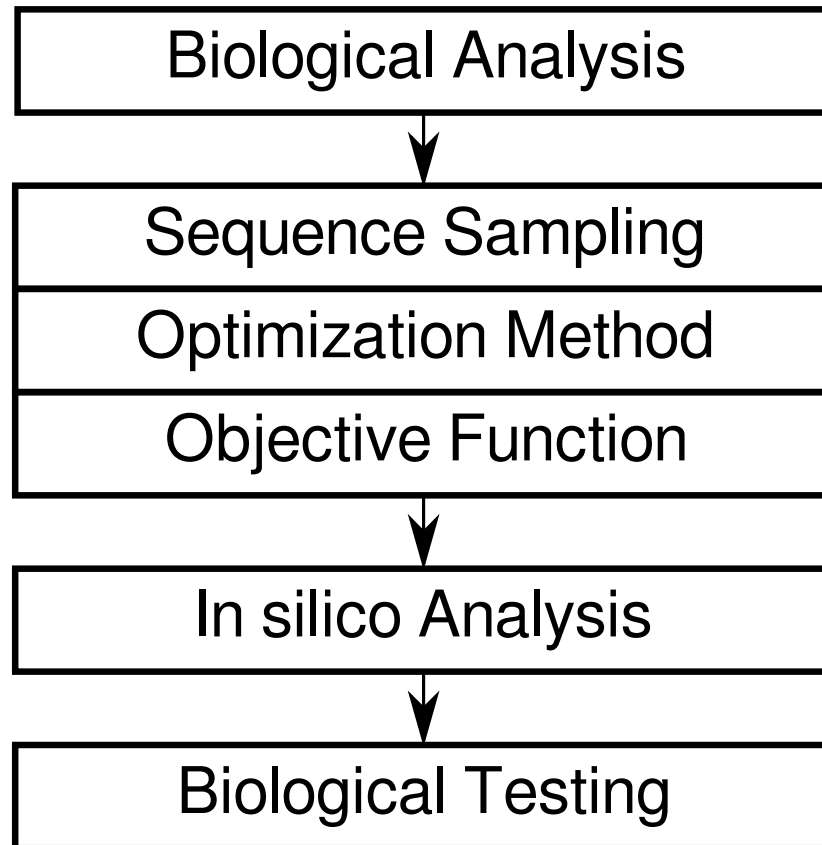
Constrained Sampling
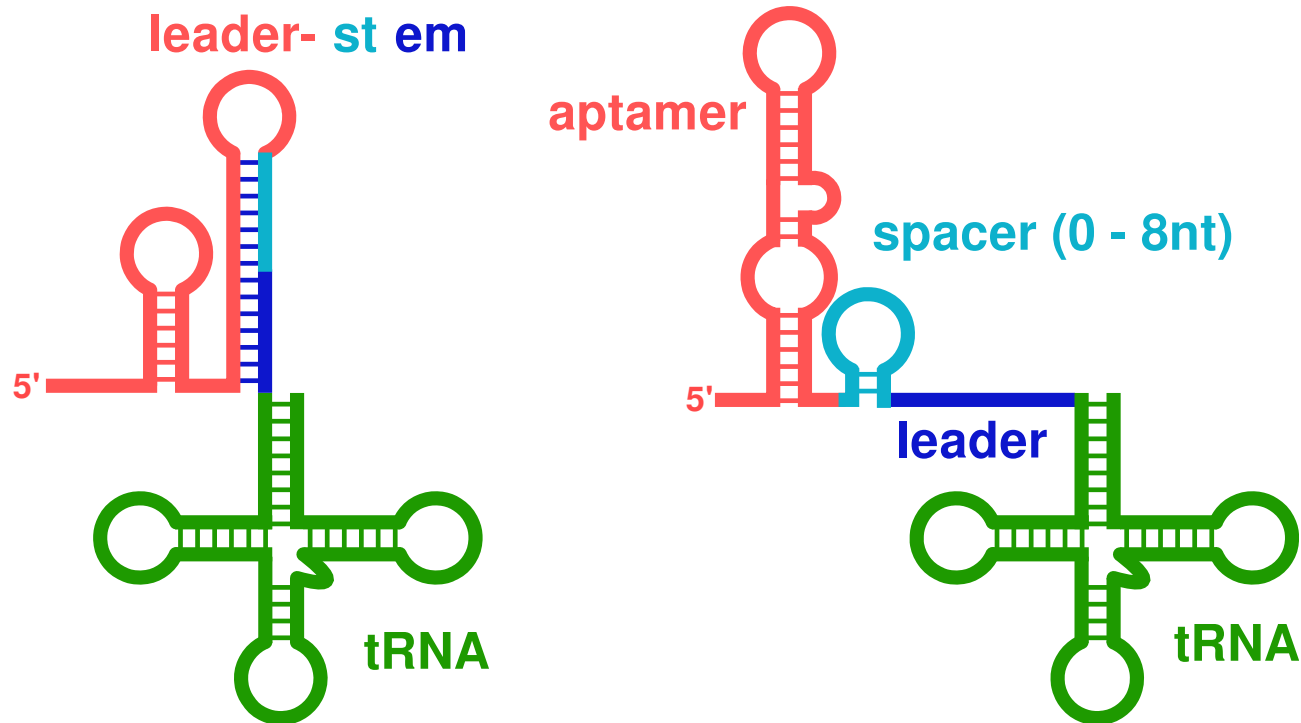
Sequence + Structure constraints →

RNAblueprint → Sequences

Hammer, Stefan, Birgit Tschiatschek, Christoph Flamm, Ivo L. Hofacker, and Sven Findeiß. **2017**. "RNAblueprint: Flexible Multiple Target Nucleic Acid Sequence Design." **Bioinformatics** 33 (18): 2850–58. https://doi.org/10.1093/bioinformatics/btx263.

Objective Functions

- ## Accessibility score [0,1]:

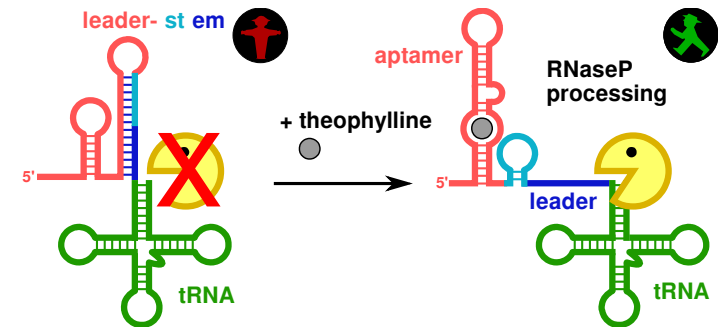$$f(x) = P_{x,C_{theo}}(\Theta_{leader}) \cdot (1 - P_x(\Theta_{leader}))$$

- ## State score [0,1]:

$$f(x) = \underbrace{P_x(\Theta_{leader-stem})}_{\text{leder-stem}\to 1} \cdot \underbrace{\left(\frac{1 - P_x(\Theta_{aptamer}) + P_x(\Theta_{leader-stem})}{2} \right)^2}_{\text{difference to aptamer}\to 1}$$

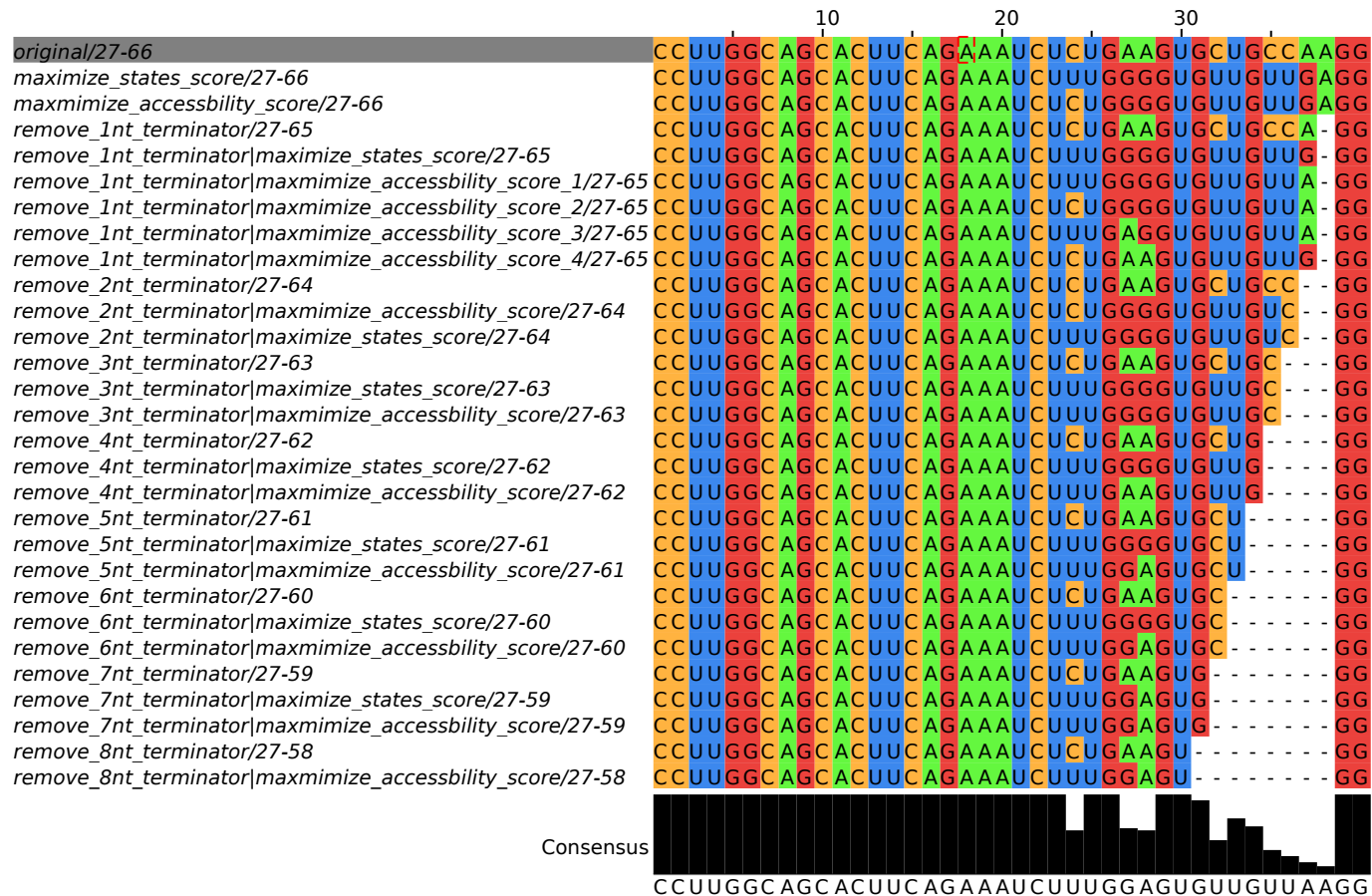$$\cdot \underbrace{P_{x,C_{theo}}(\Theta_{aptamer})}_{\text{aptamer}\to 1} \cdot \underbrace{\left(\frac{1 - P_{x,C_{theo}}(\Theta_{leader-stem}) + P_{x,C_{theo}}(\Theta_{aptamer})}{2} \right)^2}_{\text{difference to leader-stem}\to 1}$$

$x \ldots$ Sequence

$\Theta \ldots$ Structure

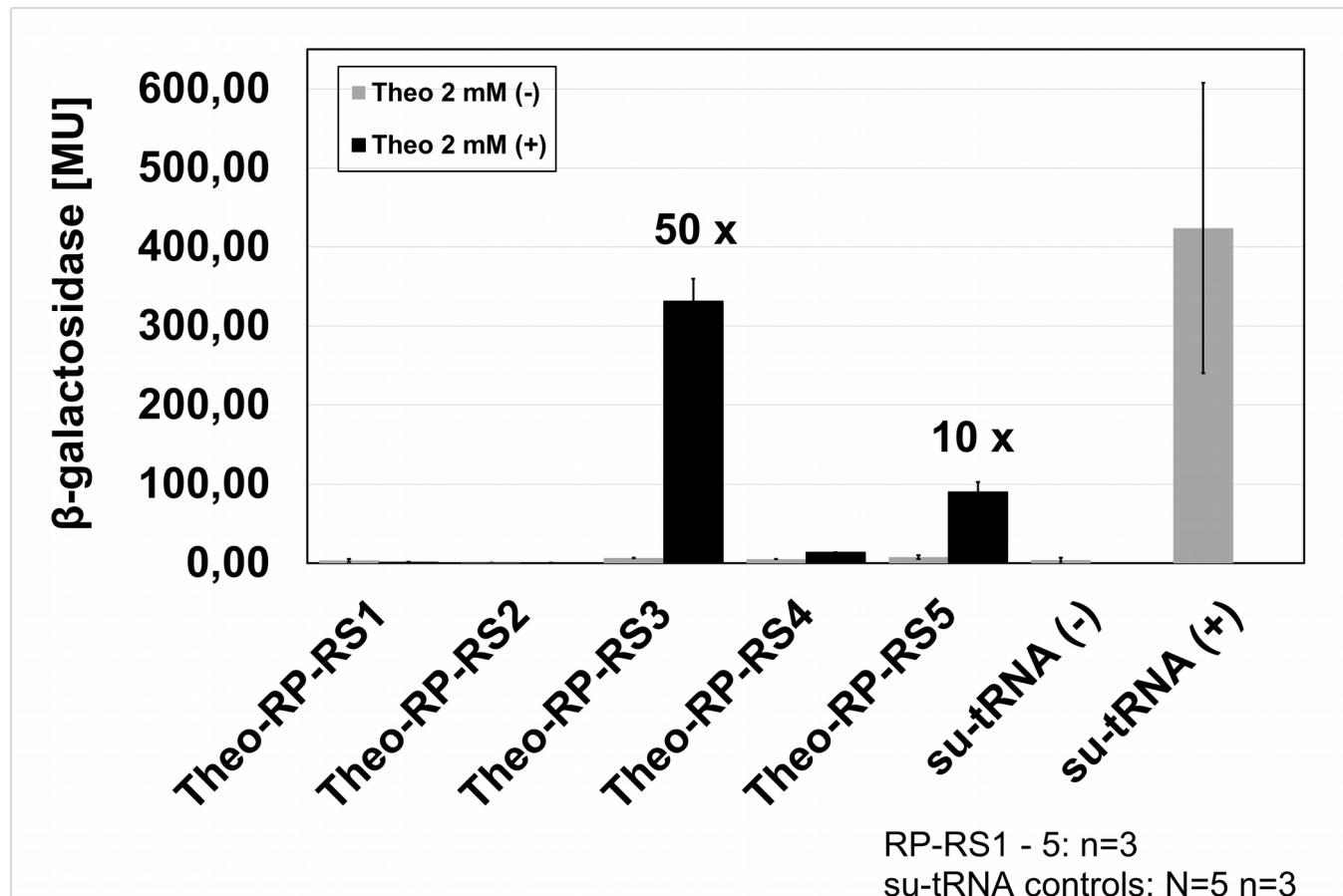$C_{theo} \ldots$ Soft-Constraint for Theophylline

Design Candidates

Analysis and Filtering

- tRNA structure must be maintained
- Verify strong switching behavior
- Avoid undesired structures in ensemble
- Test variation of theophylline binding energies
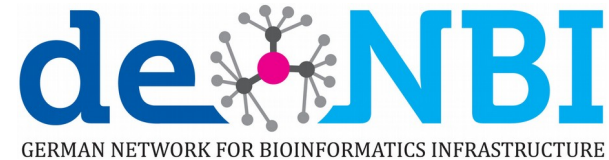- Co-transcriptional analysis without ligand

Laboratory Analysis

Thanks to

- Sven Findeiß
- Felix Kühnl
- Anna Ender
- Maja Etzel

- Mario Mörl
- Peter Stadler

Cotranscriptional View