Synthetic RNA switches working in bacteria

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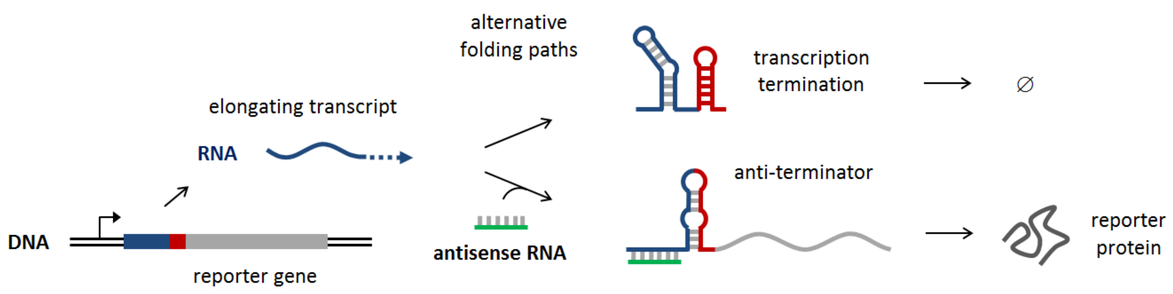
Abstract

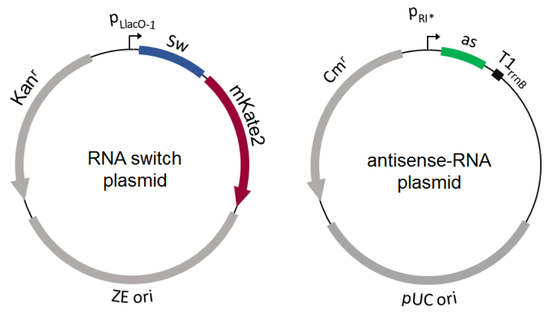
- We use a **learning-by-designing** approach to understand the **link sequence-*folding*-function** in the context of **RNA co-transcriptional folding**.
- We use **antisense RNA–mediated RNA switches** as a convenient model system for addressing the **dual and dynamical inverse-folding problem**.
- Here, we developed a platform to test and validate designed RNA switches *in vivo*, in *Escherichia coli*.

Antisense RNA–mediated RNA switch mechanism



Co-transcriptional termination/anti-termination RNA switch

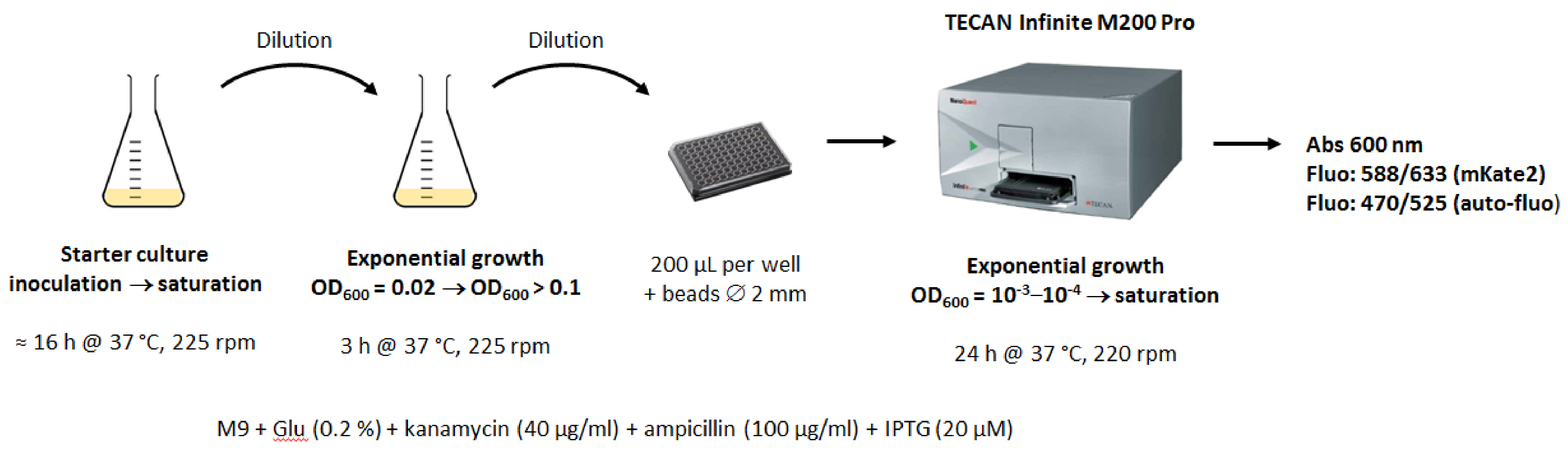
Test platform and designed RNA switches

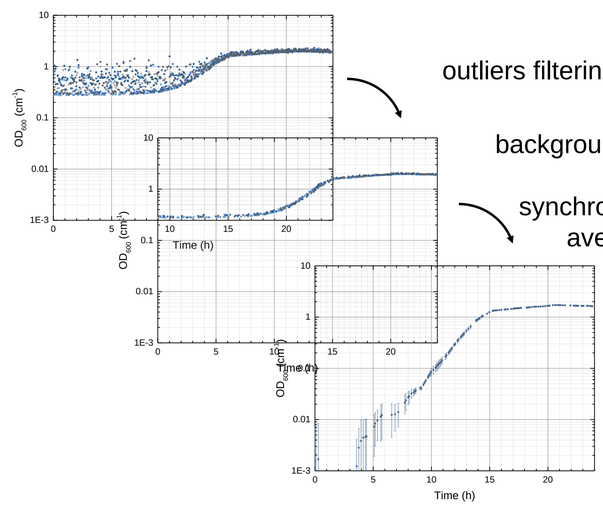


RNA switches:
- Sw-r1v2 (repressible)
- Sw-i2 (inducible)

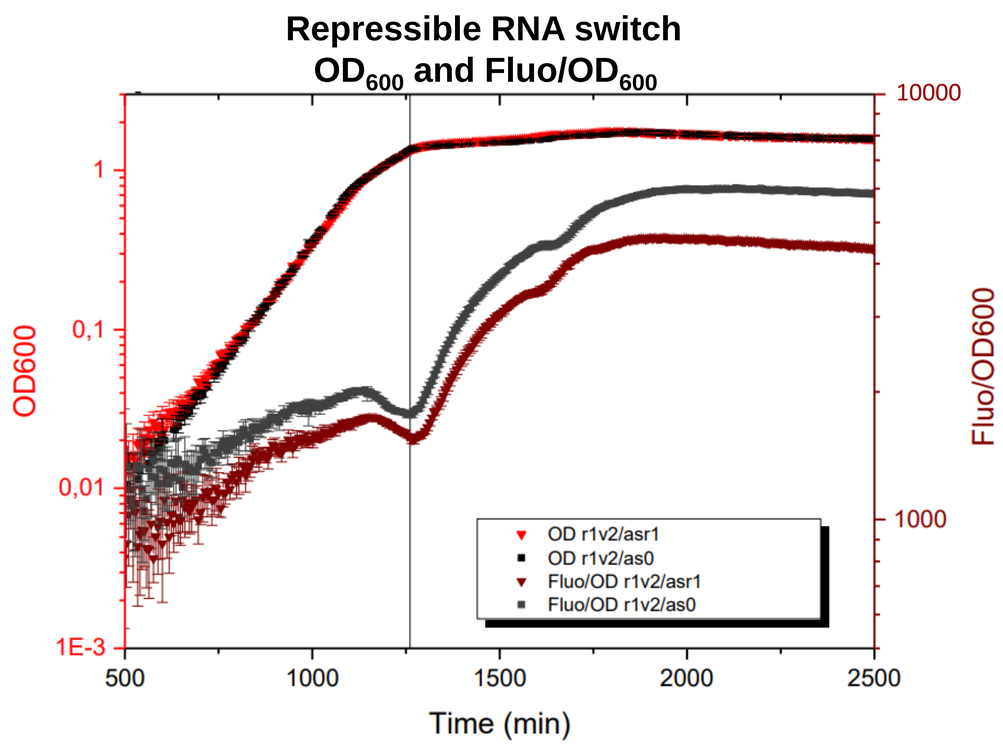
Antisense RNA:
- as-r1
- as-i2
- as-0 (empty)

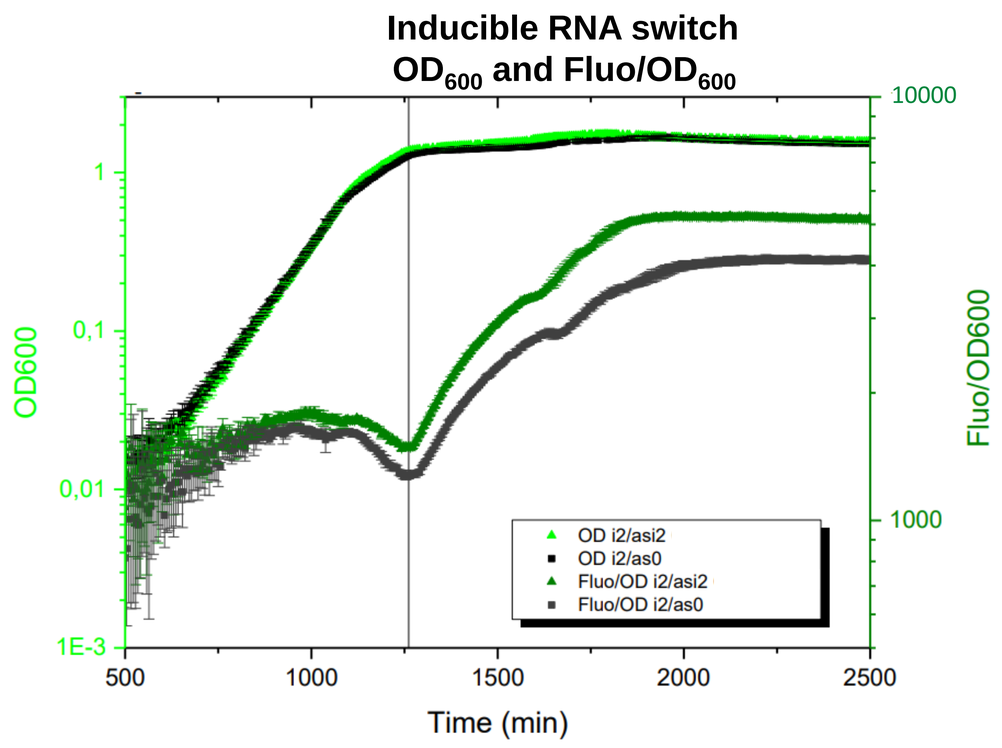
Experimental procedure and data treatment





Data analysis and functional validation *in vivo*





Regulation range observed for the repressible switch in steady-state:

Repression of 16,3 ± 1 %

Conclusion

Results

- Synthetic RNA switches regulating gene expression *in vivo*
- Regulation obtained in a steady state bacterial culture
- Moderate (~30 %) regulation compared to ~90–100 % *in vitro*

Outlook

- Improve dynamic range of regulation
- Monitor RNA expression directly
- Explore new designs
- Develop aptamer-based switches

Regulation range observed for the inducible switch in steady state:

Induction of 8,2 ± 0,5 %