Gene expression dynamics limit efficacy of ASOs in kinetic simulations

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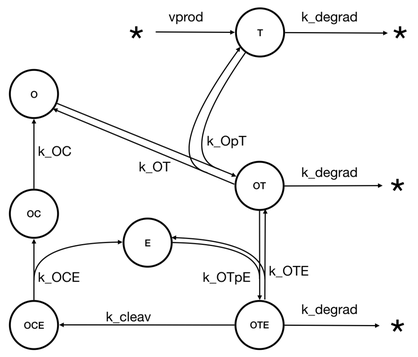
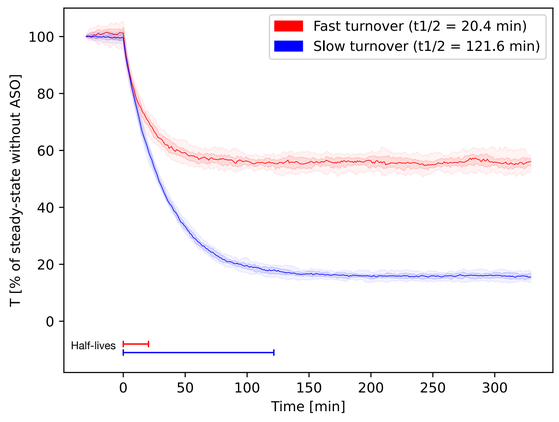
Abstract

Antisense oligonucleotides (ASOs) are usually optimized under the simplifying assumption that their target mRNA is at steady state. However, gene expression is a highly dynamic process, and the abundance of transcripts of most human genes fluctuates widely [1, 2]. In this work, we extend the kinetic model of RNase-H-mediated ASO action of Pedersen et al. [3] to study the performance of ASOs under the temporal regulation of the target mRNA transcription.

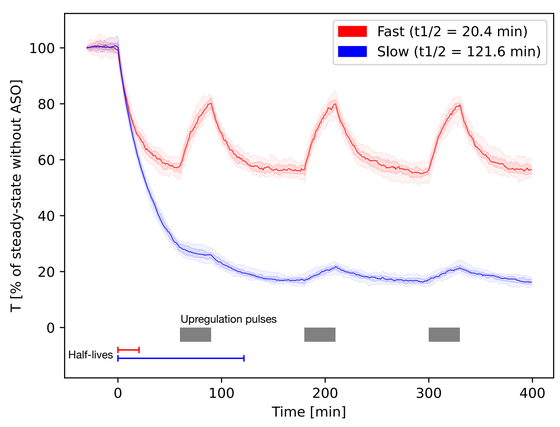
Target expression dynamics affect ASO efficacy

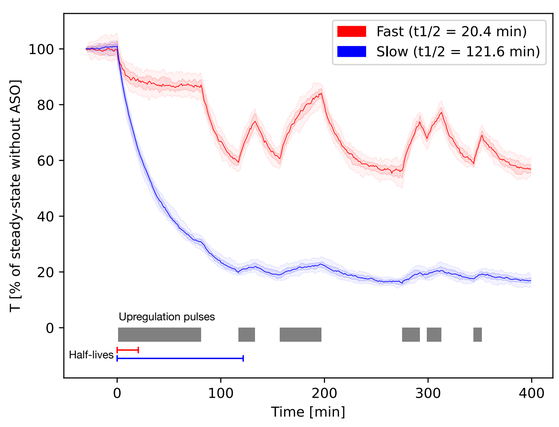


T	Target mRNA	OTE	ASO:target:RNase H complex
O	ASO	OCE	ASO:cleaved target:RNase H complex
OT	ASO:target complex	OC	ASO:cleaved target complex
E	RNase H enzyme		



Previous studies provide strong evidence for the inverse relationship between the turnover rate of the target mRNA and the efficacy of siRNAs [4], which have mechanism of action similar to ASOs. Pedersen et al. [3] used their kinetic model (above, left) to show that, when all other parameters of the system are kept unchanged, increased target degradation rate leads to decreased ASO efficacy and potency.





We extended the model to allow for pulsating upregulation of the target mRNA production. In the presence of the upregulation signal, the production rate of the mRNA was increased 1.5-fold, and the degradation rate stayed the same. This can be interpreted either as a short upregulation of the gene expression (e.g. in response to some external signal) or as gene bursting with rather long time between bursts.
For the first simulation (above, left) we used a fixed pulse pattern. For the second simulation (above, right) a stochastic model of transcription with the same average time interval between the two consecutive pulses as was used in the simulation with the fixed pulse pattern.
In both simulations ASO fails to suppress the upregulation peaks of the target with fast turnover rate.

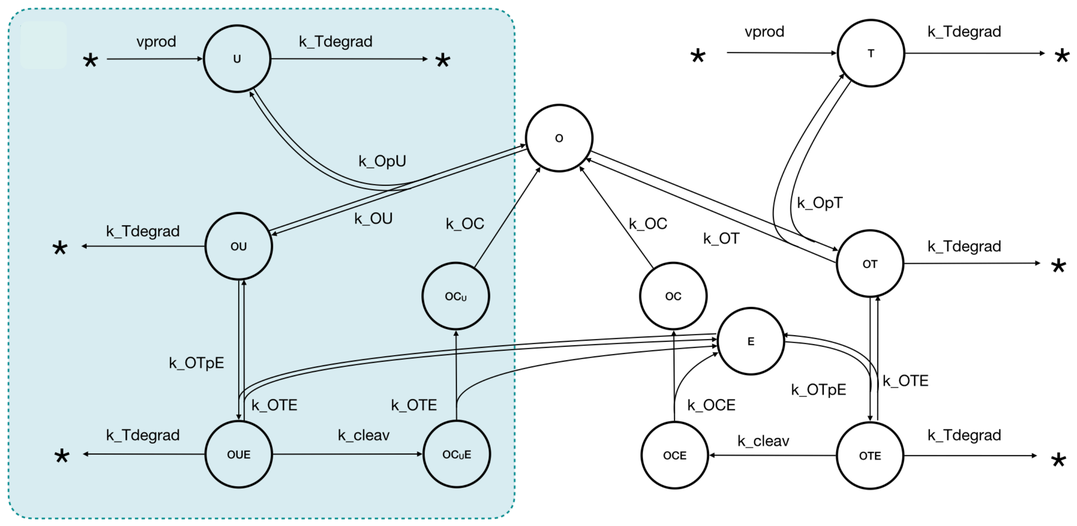
Tissue-level effects

Simulation results show that unaccounted target mRNA dynamics can be one of the reasons of decreased *in vivo* ASO efficacy, as compared to the *in vitro* efficacy. All simulations shown here can be interpreted as simulations of a single cell. However, for the *in vivo* ASO treatment, we should consider the tissue level of cellular organization. Cells in a tissue can either have asynchronous expression of target mRNA, or be synchronized.

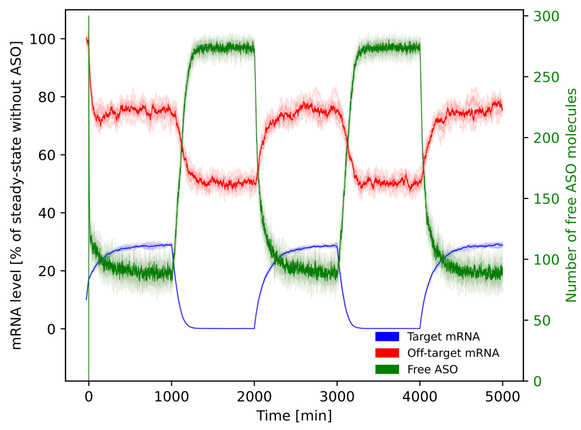
The first scenario applies, e.g., for cell-cycle-regulated genes in mature tissues. Noisy transcriptional bursting due to stochastic transcription also results in asynchrony. In this case, the peaks of upregulation of the transcription will be lost in the noise, and the ASO efficacy should stay unchanged or only slightly decrease.

The second scenario, where all cells in a tissue are synchronized, may occur in response to systemic factors or environmental stress. The upregulation peaks may be noticeable at the tissue level. In this case ASO treatment may be less effective.

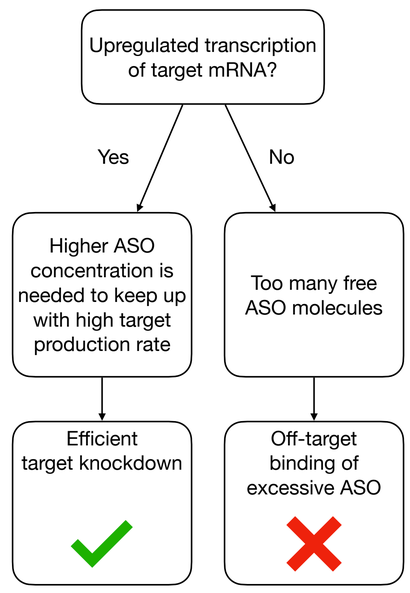
Target expression dynamics impact ASO safety



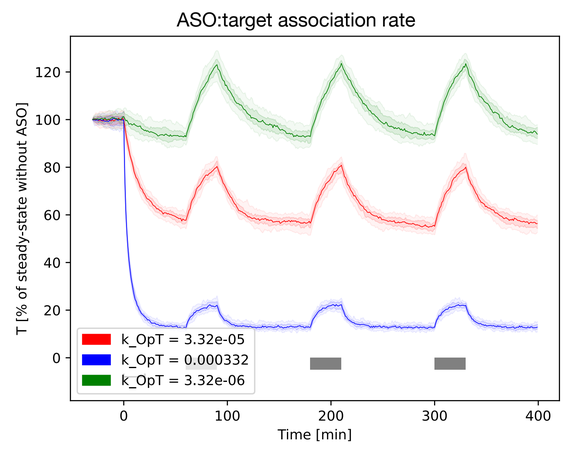
A kinetic model, based on the model developed by Pedersen et al. [3], for the system that includes unspecific RNA off-targets, was used to simulate the situation where ASO was designed to be efficient in knocking down the dynamically regulated target mRNA at the peaks of upregulation. Aiming for silencing during the upregulation peaks can lead to increased toxicity in periods where no upregulation is happening.

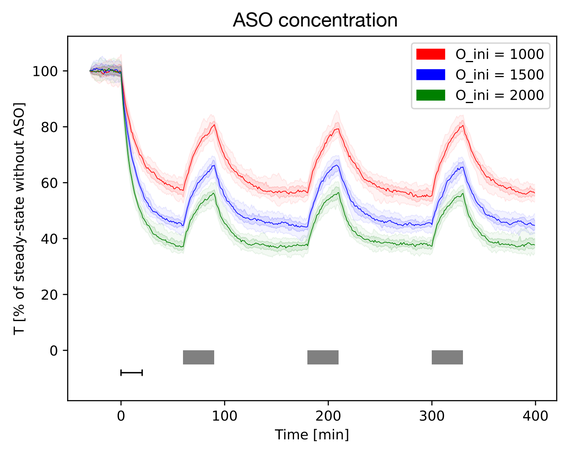


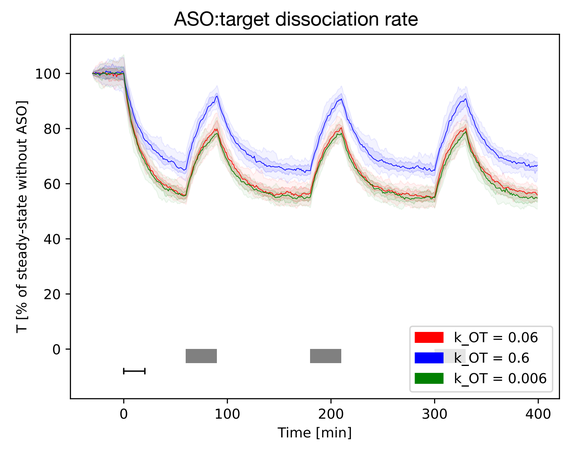
The baseline level of target mRNA is 100 molecules, and during the upregulation peaks its production rate is increased by the factor of 100. The baseline level of off-target mRNA is 1000 molecules, and the affinity of ASO:off-target complex is two times smaller than the affinity of ASO:target complex. Number of ASO molecules added to the cell is 500.



Improving ASO efficacy for dynamically expressed target mRNA







Changing different parameters of the kinetic model demonstrated that the efficacy of ASO in presence of dynamically expressed target can be influenced by changing effective ASO concentration in the cell, as well as by increasing the affinity of the ASO:target complex.

References

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Acknowledgements

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