ASOkai - An open-source framework for ASO design using kinetics and AI

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schlieplab.org, SchliepLAB github.com/Schlieplab/ASOkai

Background

In light of the enormous combinatorial search space for drug design for Antisense Oligonucleotide (ASOs), *in silico* drug design becomes increasingly important as demonstrated by publication of tools like PFRED [1] for siRNA and ASO design, and machine learning (ML) based approaches like eSkip-Finder [2] for Exon-Skipping ASOs and ASOptimizer [3] for targeting IDO1 with optimised modifications.

Commonly, sequence features as well as chemical and thermodynamic features are used to forecast the potential efficacy of drug candidates.

ASO Features

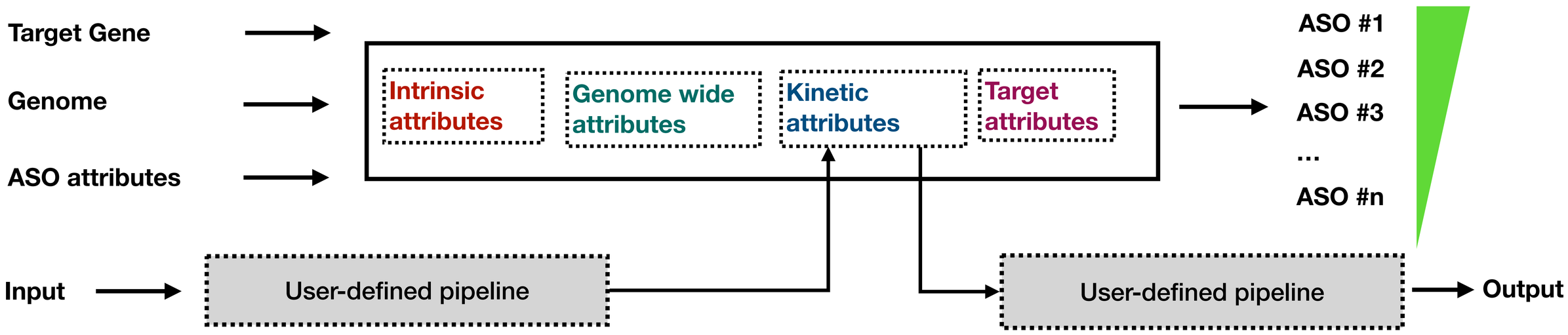
Intrinsic Attributes:
- GC content
- Longest T-run
- Longest AT-run

Genome-wide attributes:
- Specific off-targets
- Unspecific off-targets
- Location

Kinetic Attribute:
- Target-level after ASO administration
- Kinetic models excluding/including off-target presence

Target attributes:
- Secondary target sites
- Target accessibility

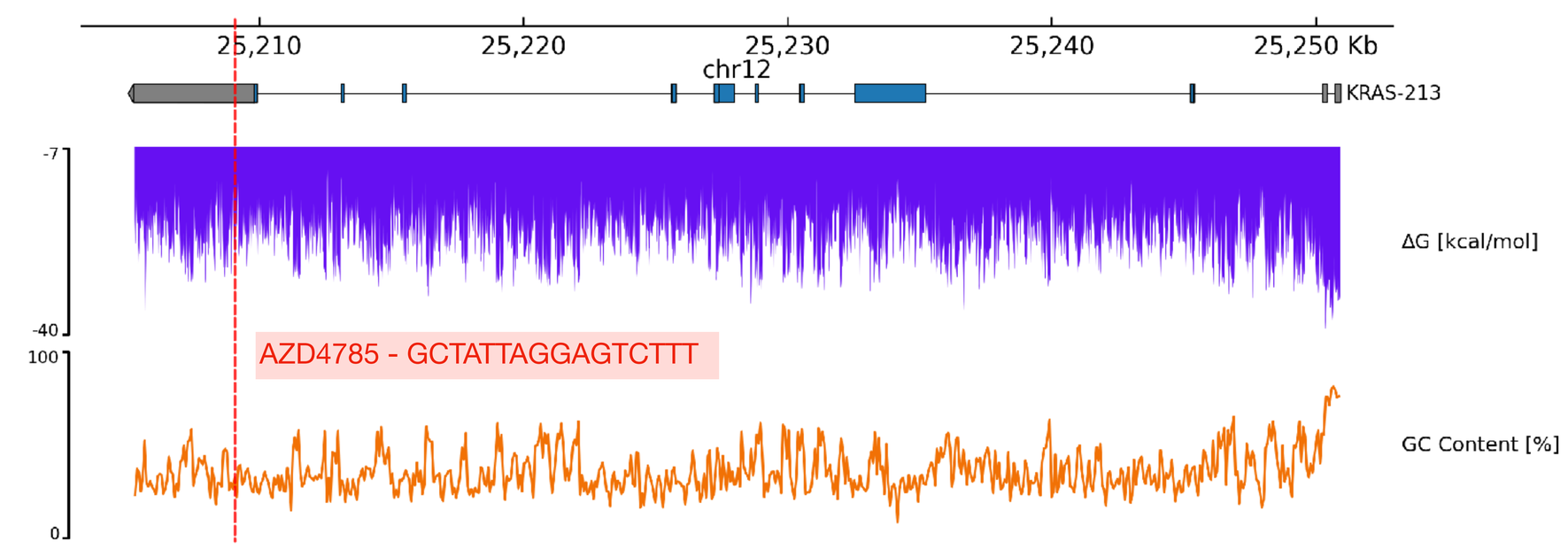
A Modular System



Our modular, open-source pipeline includes analytical features from different aspects around ASO drug design, commonly used attributes like GC content as well as attributes usually requiring human experts. In particular, our focus is on extensive analysis of specific and unspecific off-targets and their impact on potential target knockdown evaluated through kinetic simulations.

While the pipeline can be utilised as a ready-to-use workflow, all individual analytical steps are available to be integrated in already existing user-defined drug design workflows.

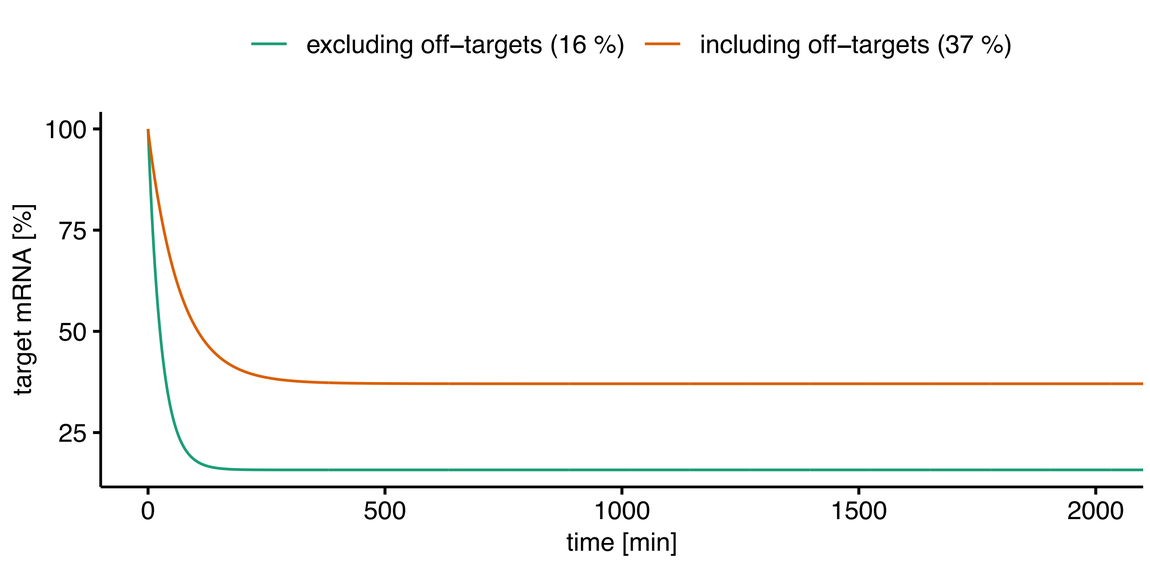
Targeting KRAS



The tumor suppressor gene KRAS becomes an oncogene when mutated and is among the most frequently mutated genes across all cancer types with mutations occurring in 61% of gastrointestinal tract, 16% of lung, and up to 90% of pancreatic cancers. [5,6]

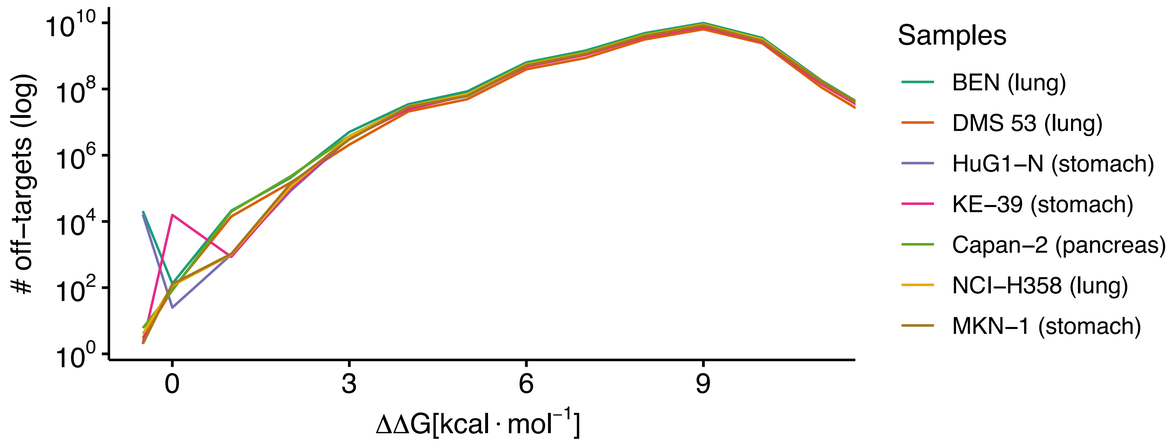
Tag:	**AZD4785**
Sequence:	GCTATTAGGAGTCTTT
GC Content:	37.5 %
ΔG Binding site:	-23.9 kcal/mol
Approx. Target Percentage:	15.77 %
Secondary target sites:	0
# spec. Off-targets:	189
Length T run:	3
Length AT run:	5

Target knockdown trajectories



Two target knockdown trajectories are shown for ASO AZD4785 excluding off-targets and including off-targets for the cell line HuG1-N. Considering unspecific off-targets for this cell line shows a decreased target knockdown by ~21 %pt. Stochastic simulations using a kinetic model introduced by Pedersen [4] and extended to include off-target profiles generated from RNAseq samples evaluate the potential target knockdown including and excluding off-targets respectively. By utilising AI models predicting target knockdown based on kinetic simulations, potential drug candidates can already be evaluated in the initial screening phase, while more detailed simulations are made available through interfaces to standard stochastic simulation packages.

Off-target Profiles for AZD4785



Here, off-target profiles are generated from seven cancer cell lines commonly associated with increased KRAS expression. The difference in binding energy between Oligo-target complexes and Oligo-Off-target complexes correlates significantly with the impact of target knockdown. AI models predicting off-target profiles on sample or genome level give resource friendly alternatives for pre-screening of oligo candidates.

References

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