Analysis of tissue-specificity in unintended off-target occurrences for ASO therapeutics

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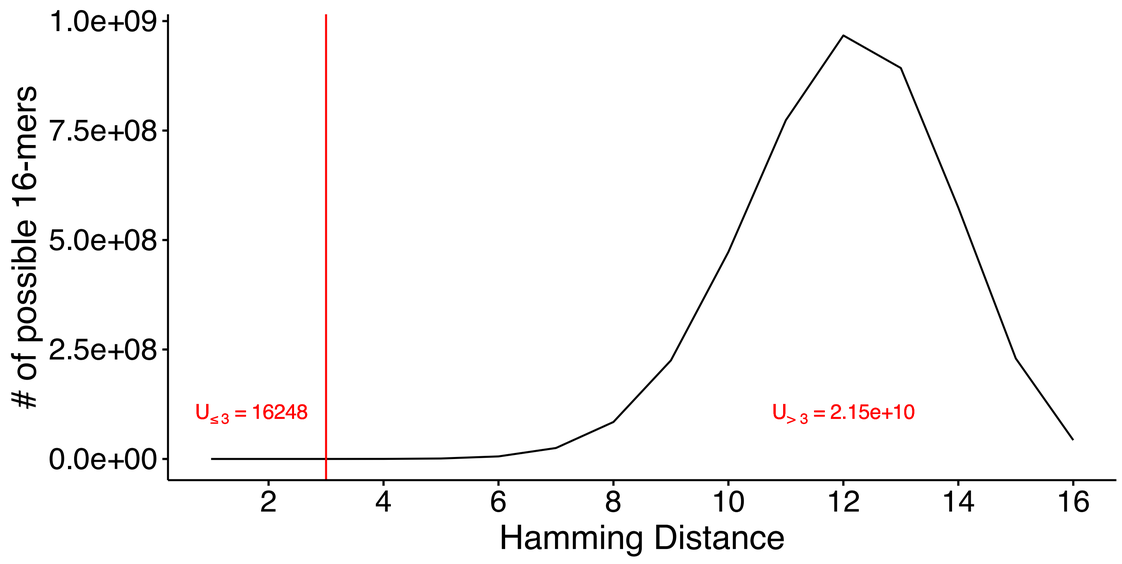
Background

Antisense oligos (ASO) are used in oligo therapeutics to treat rare, chronic, genetic diseases. The most common mechanism is RNase H1 mediated knockdown of mRNA targets [1]. The current challenge in the field is the understanding of factors for efficacy of ASOs. Research has shown that ASO activity can be improved by administering a non-targeting ASO which suggests the existence a nonproductive bulk uptake pathway in the cell [2].

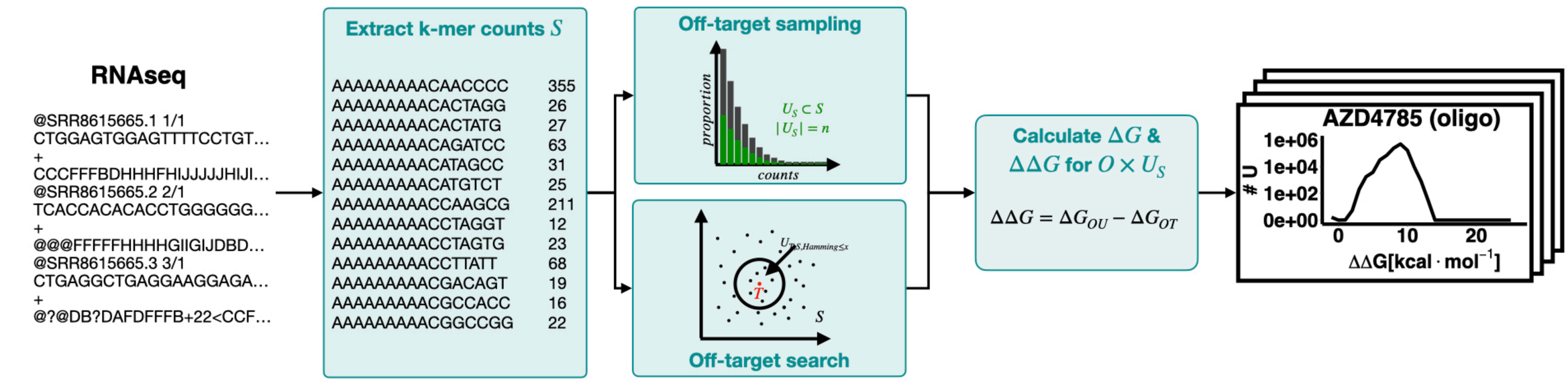
We look at *unintended off-targets*, sequences to which the ASO can bind with low specificity and lower affinity, in the mRNA environment in the cell effecting the target knockdown.

Theoretical Analysis



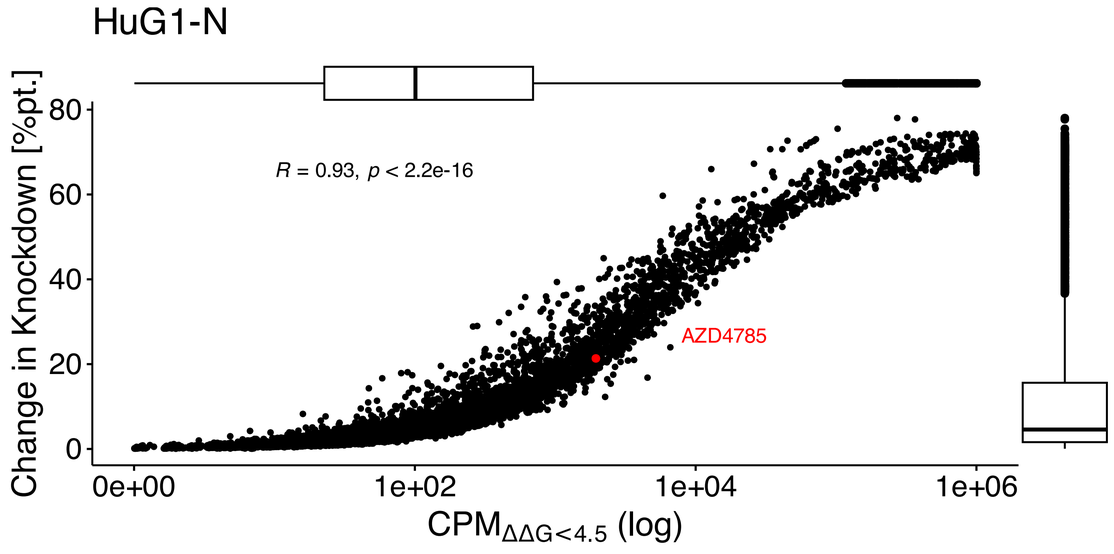
Number of possible 16-mers over the number of mismatches. The distribution shows that a search for unintended off-targets based on Hamming distance to the target site is a non-trivial task and computationally infeasible.

Method

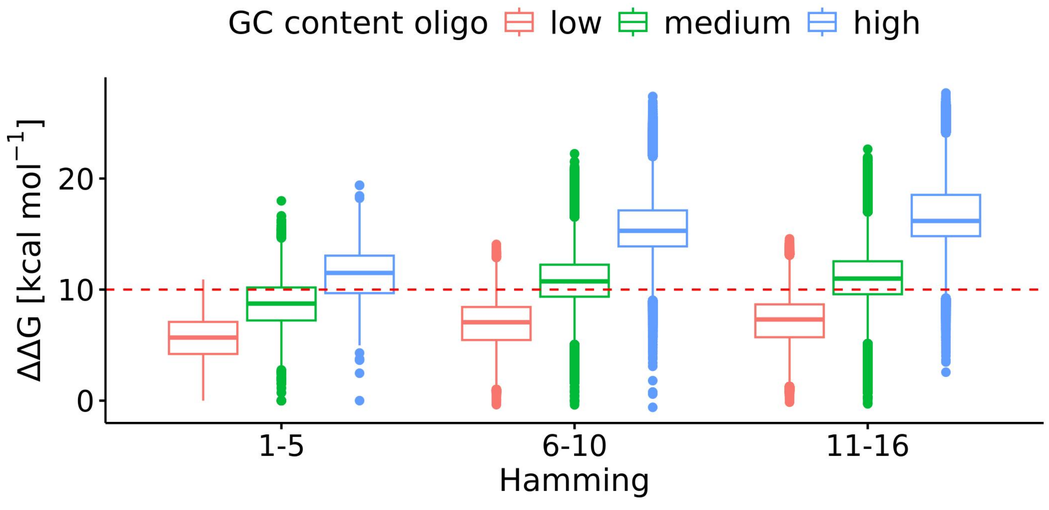


The hybrid approach to extract off-target profiles includes a search for off-target sites with few mismatches to the target sites and a sampling step based on k-mer frequencies. For all sampled and searched unspecific off-targets in U_S the binding energies, ΔG, and differences in binding energies, $\Delta\Delta G$, of the oligo-off-targte complexes OU to the Watson-crick complement of the target site. This pipeline is optimised for extracting off-target profiles for a set of candidate oligos.

Results

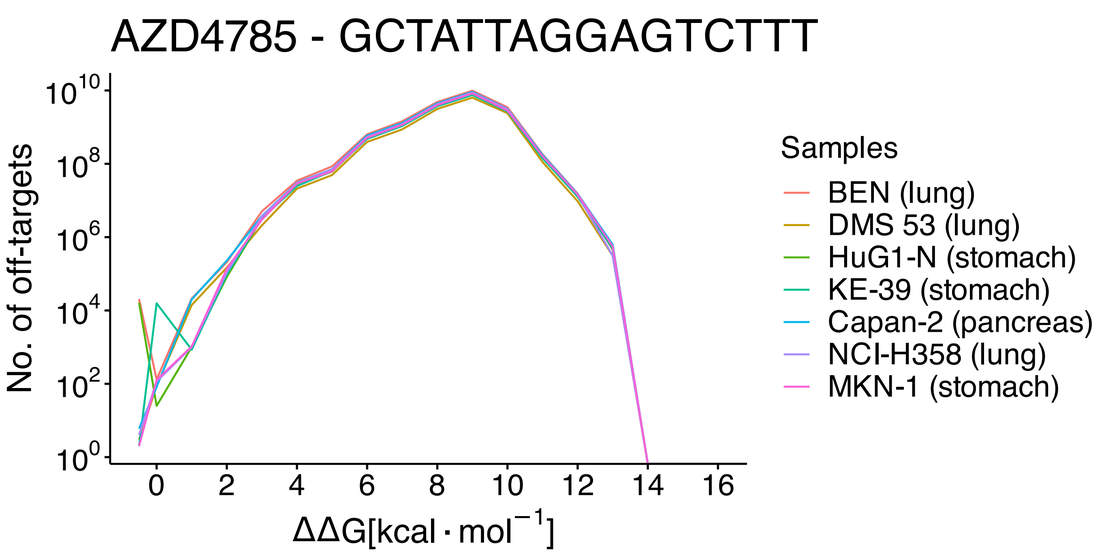


The off-target abundance < 4.5 kcal mol^{-1} correlate significantly with the expected change in knockdown resulting from kinetic simulations.

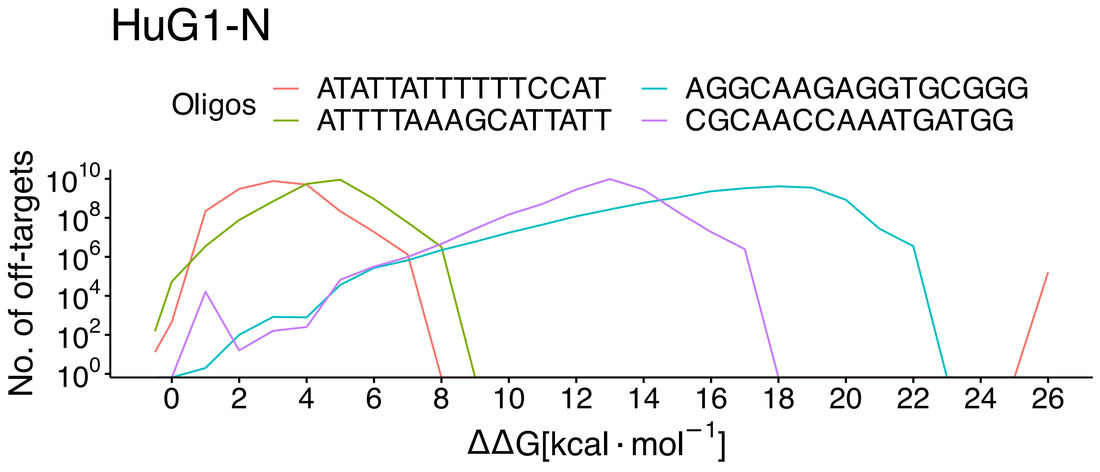


The $\Delta\Delta G$ values are connected to the GC content of the oligo and the Hamming distance between target site and off-target site.

Examples



Off-target profiles for ASO AZD4785 researched for KRAS treatment for different cancer cell lines taken from Cancer Cell Encyclopedia (E-MTAB-2770). A strong tissue-dependency cannot be confirmed.



Off-target profiles can vary significantly across antisense oligonucleotides, hinting to a dependency on GC content and T runs in candidate oligos.

References:
[1] Stanley T. Crooke et al., “Antisense Technology: An Overview and Prospectus,” *Nature Reviews. Drug Discovery* 20, no. 6 (June 2021): 427–53
[2] Annette Buntz et al., “Quantitative Fluorescence Imaging Determines the Absolute Number of Locked Nucleic Acid Oligonucleotides Needed for Suppression of Target Gene Expression,” Nucleic Acids Research 47, no. 2 (January

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