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ASOkai: A Modular Open-Source Framework for Systematic ASO Design and Evaluation

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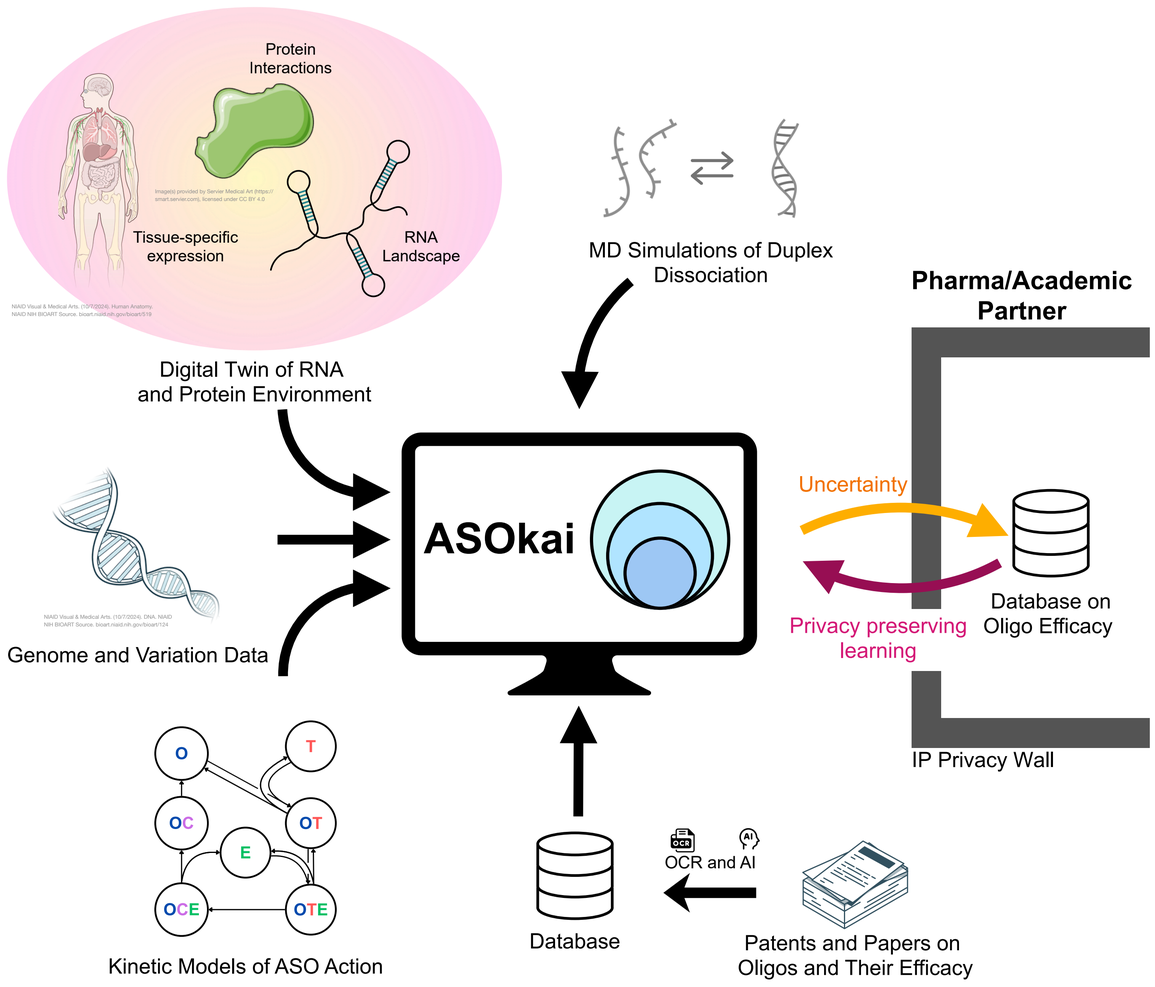
Introduction

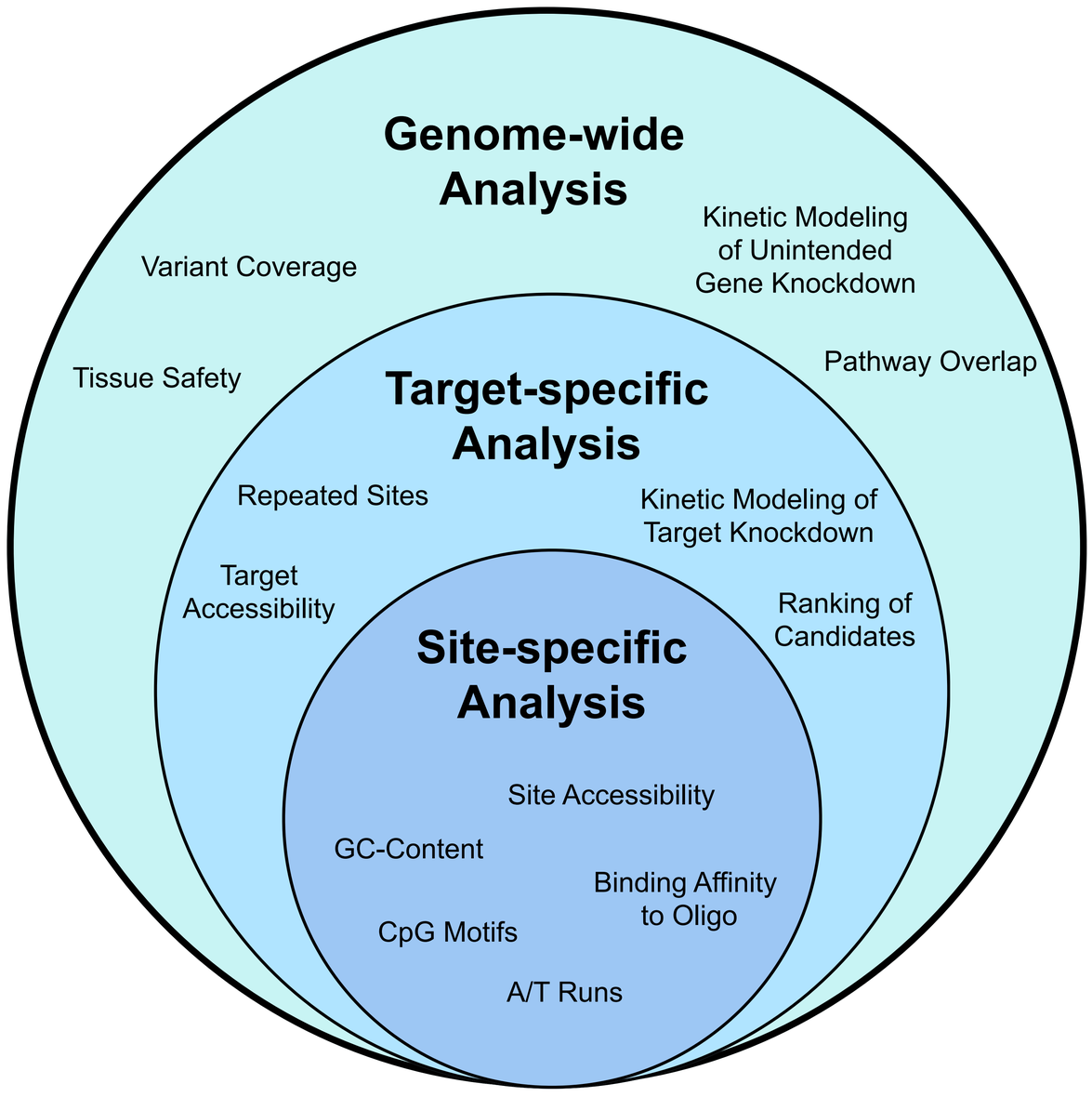
The challenge in designing effective antisense oligonucleotide (ASO) therapeutics lies in the rapidly expanding combinatorial space of candidate binding sites, chemical modifications, and backbone configurations, making exhaustive experimental screening impractical. Prioritizing candidates demands evaluating them against complex sequence, thermodynamic, and specificity constraints.

To address this, we present ASOkai, an open-source Python framework for systematic ASO design and evaluation. ASOkai integrates a growing suite of complementary analysis modules, covering sequence features, off-target risk, target accessibility, and beyond, into a unified, reproducible, and automation-ready workflow.

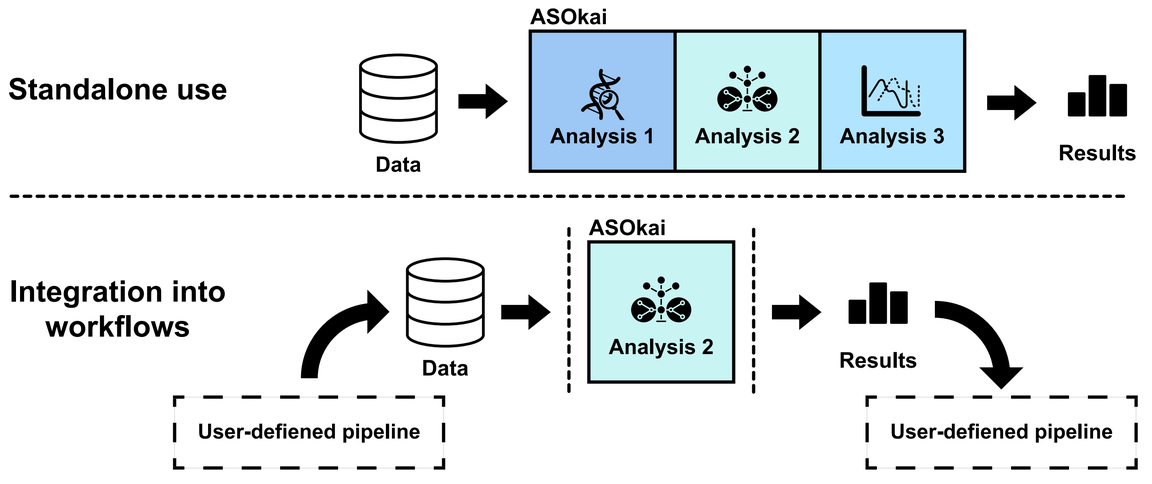


ASOkai Workflow

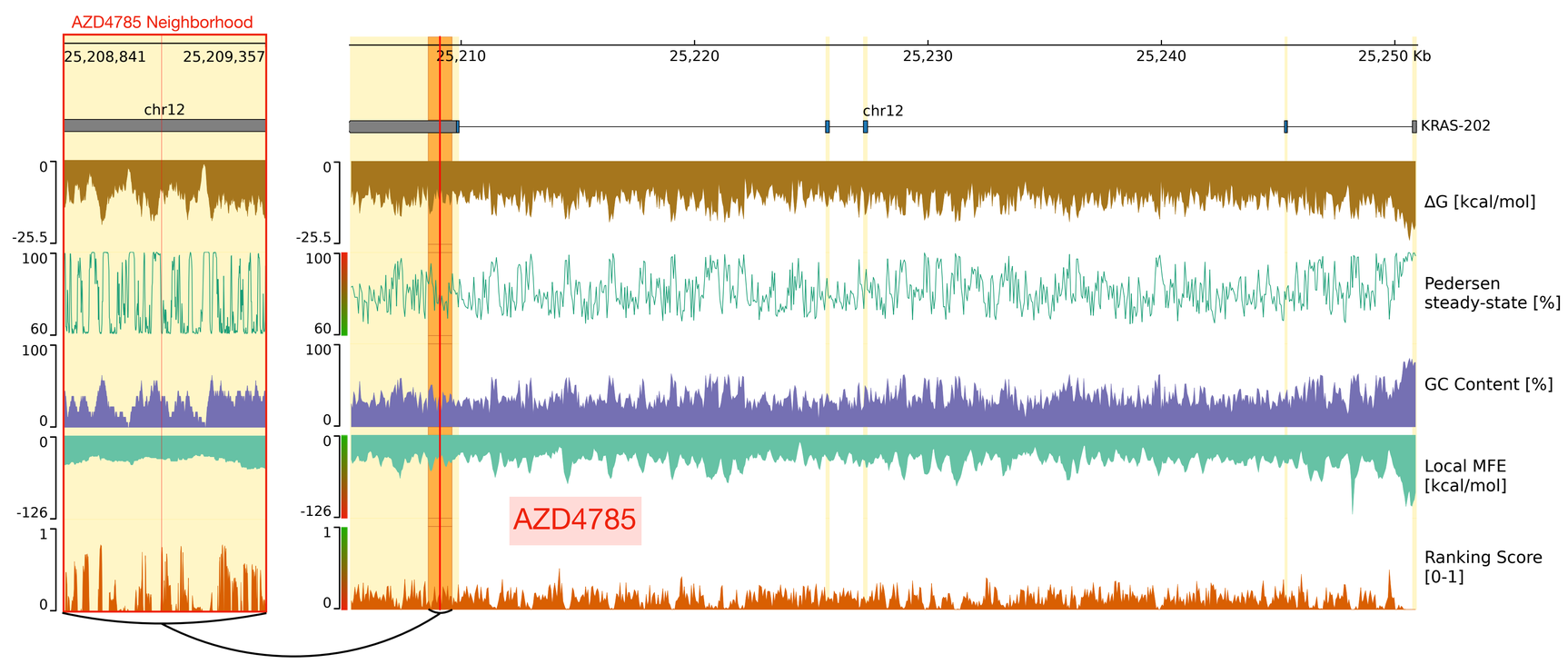
The platform organizes candidate analysis into three nested levels of increasing scope — site-specific, target-specific, and genome-wide — progressing from extraction and filtering of binding sites based on sequence features and genome-wide uniqueness, through assessment of target accessibility and repeated sites [3], to kinetic modeling of target knockdown and unintended gene suppression arising from off-target interactions [1][4]. A machine learning model trained on empirical antisense data then integrates these multidimensional evaluations to identify optimal binding sites and their corresponding oligonucleotides.



ASOkai supports both standalone use and integration into existing computational biology workflows through a portable, interoperable workflow interface and is built to support end-to-end ASO evaluation while remaining flexible to new methods and use cases. A privacy-preserving learning framework further enables collaboration with external data sources without compromising data confidentiality [2].



Example: Targeting KRAS



Tag:	**AZD4785 [5]**
Sequence:	GCTATTAGGAGTCTTT
GC Content:	37.5 %
ΔG Binding site:	-12.70 kcal/mol
Homodimer ΔG	-3.0 kcal/mol
Approximate knockdown:	43.81 %
Repeated sites:	0
# spec. Off-targets:	189
Length T run:	3
Length AT run:	5
Rank:	9,499/45,676 sites
percentile:	20.8 %

References

[1] L. Pedersen, P. H. Hagedorn, M. W. Lindholm, and M. Lindow, “A Kinetic Model Explains Why Shorter and Less Affine Enzyme-recruiting Oligonucleotides Can Be More Potent,” Molecular Therapy - Nucleic Acids, vol. 3, p. e149, Jan. 2014, doi: 10.1038/mtna.2013.72.
[2] S. Tavara, A. Schliep, and D. Basu, “Federated Learning of Oligonucleotide Drug Molecule Thermodynamics with Differentially Private ADMM-Based SVM,” in Machine Learning and Principles and Practice of Knowledge Discovery in Databases, M. Kamp, I. Koprinska, A. Bibal, T. Bouadi, B. Frénay, L. Galárraga, J. Oramas, L. Adilova, G. Graça, et al., Eds., Cham: Springer International Publishing, 2021, pp. 459–467. doi: 10.1007/978-3-030-93733-1_34.
[3] L. Pedersen et al., “Targeting Repeated Regions Unique to a Gene Is an Effective Strategy for Discovering Potent and Efficacious Antisense Oligonucleotides,” Molecular Therapy Nucleic Acids, vol. 19, pp. 124–131, Mar. 2020, doi: 10.1016/j.omtn.2019.10.040.
[4] N. Gocht, A. Khatova, F. Karlsson, and A. Schliep, “Implications of unintended off-target binding on efficacy of Antisense Oligonucleotides,” Jul. 2024.
[5] S. J. Ross et al., “Targeting KRAS-dependent tumors with AZD4785, a high-affinity therapeutic antisense oligonucleotide inhibitor of KRAS,” Sci Transl Med, vol. 9, no. 394, p. eaal5253, Jun. 2017, doi: 10.1126/scitranslmed.aal5253.