

Unlocking RNA as a Drug Target: Novel Hit Discovery Strategies for Small Molecule Binders

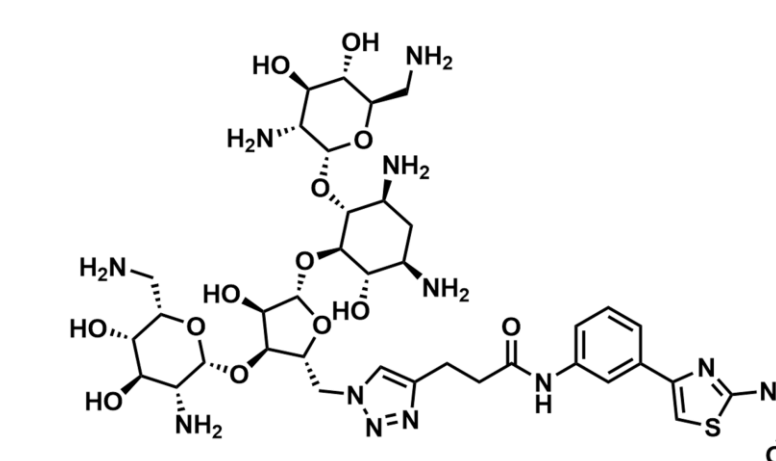
Rose Bigley¹, James Craswell¹, Michael Carter², Philip Fallon² & Nicholas Bland¹

¹Domainex Ltd, Sigma Building, 40 South Street, Unity Campus, Pampisford, CB22 3FW, UK, ²Domainex Ltd, Chesterford Research Park, Saffron Walden, CB10 1XL, UK;

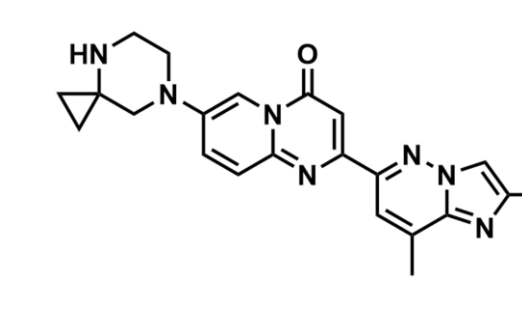
RNA as a Drug Target

- Drug discovery is evolving beyond the traditional paradigm of binary interactions between small molecules and protein targets. Among the most challenging targets are those considered "undruggable" at the protein level
- An attractive approach to targeting such proteins, is to hit them at the RNA stage. Targeting RNA, before it is translated into deleterious proteins, represents a promising new frontier in therapeutic development
- Domainex is pioneering a platform for RNA-targeted drug discovery, aiming to intercept disease at its earliest molecular stages

- Traditionally RNA-directed therapeutics were typically anti-sense oligonucleotides or siRNAs
- Small molecules have become of increasing interest in the field of RNA drug discovery
- One of the most famous small molecules that targets RNA is Evrysdi (ridisplam) a treatment for 5q Spinal Muscular Atrophy (SMA)
- As the field progresses new molecules are constantly being identified, such as neomycin-S which can bind human pre-miRNA



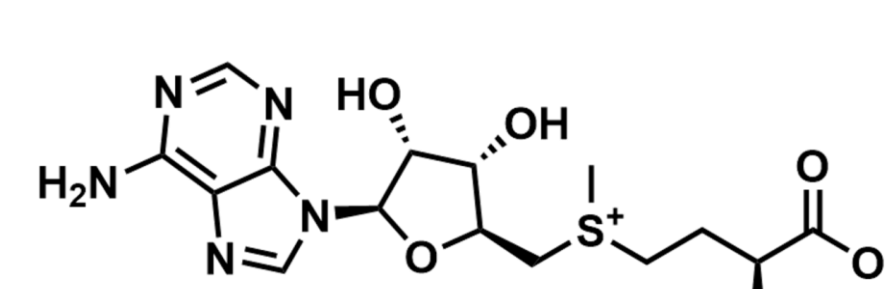
Neomycin-S
Inhibitor
pre-miRNA 372/373



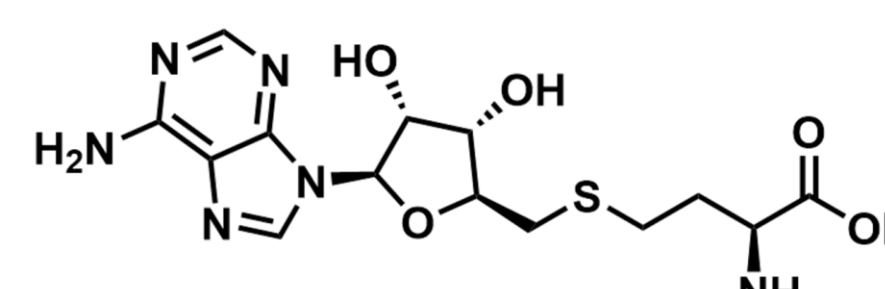
Evrysdi (ridisplam)
FDA approved drug
Treatment for SMA

Biophysical Assays for Small Molecule RNA Binders

- Riboswitches are structured RNA molecules that are essential for regulating bacterial metabolism, making them attractive targets for novel antibiotics
- Domainex utilised its suite of cutting-edge biophysical technologies to measure binding of small molecules to the riboswitch SAM-VI
 - SAM-VI's endogenous ligands are S-Adenosylmethionine (SAM) and S-Adenosylhomocysteine (SAH)
- Assays were developed using the NanoTemper Dianthus and Grating-Coupled Interferometry (GCI)
- Both methods showed excellent recapitulation of published values providing an excellent platform for any RNA-targeted drug discovery programme

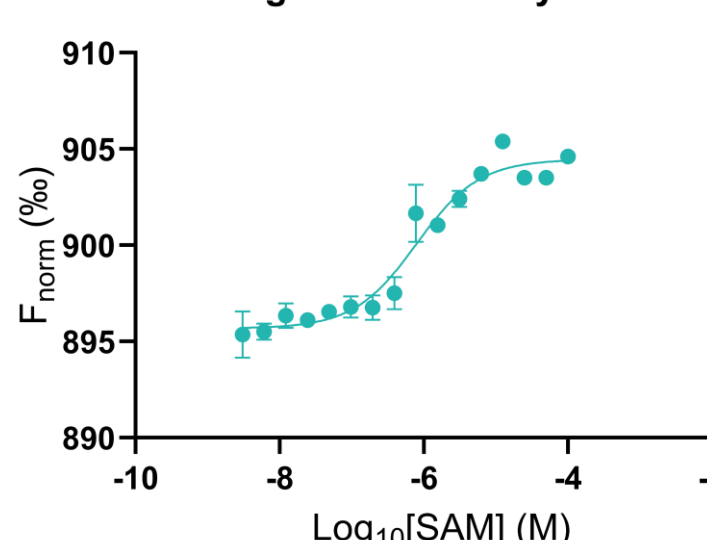


S-Adenosylmethionine (SAM)

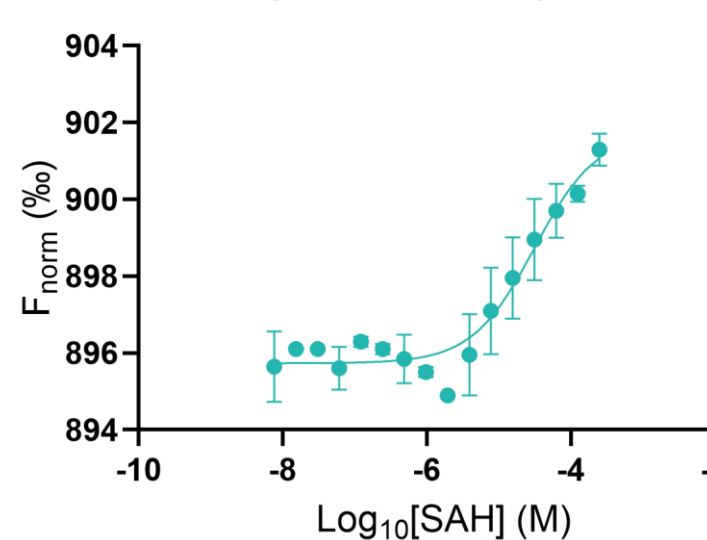


S-Adenosylhomocysteine (SAH)

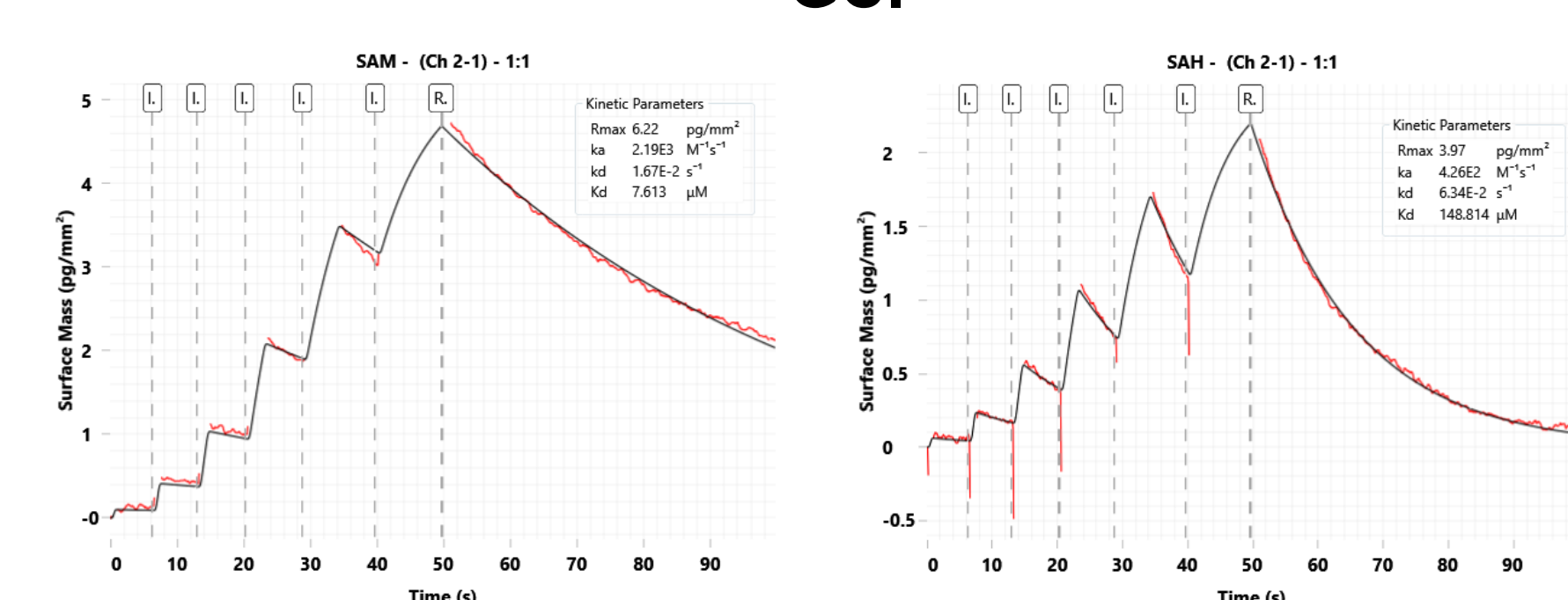
SAM Binding Determined by the Dianthus



SAH Binding Determined by the Dianthus



GCI



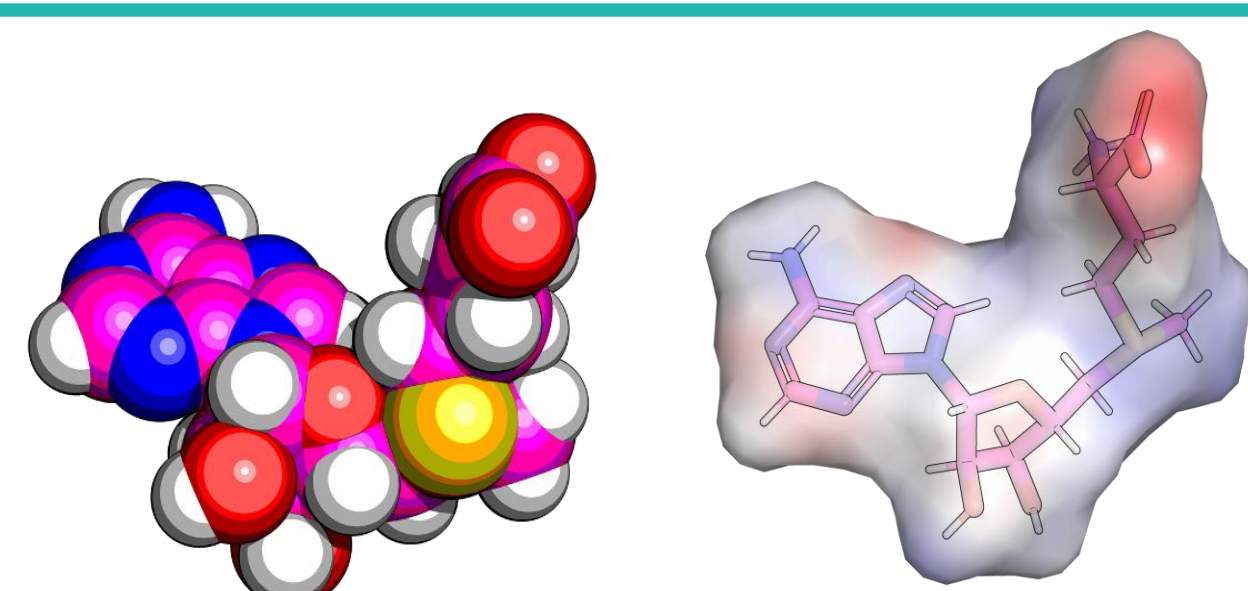
SAM	ka (M ⁻¹ s ⁻¹)	kd (s ⁻¹)	K _D (µM)
Literature*	2.98E+03	9.8E-03	3.72
Domainex	2.19E+03	1.67E-02	7.61

SAH	ka (M ⁻¹ s ⁻¹)	kd (s ⁻¹)	K _D (µM)
Literature*	3.88E+02	5.4E-02	150
Domainex	4.26E+02	6.34E-02	149

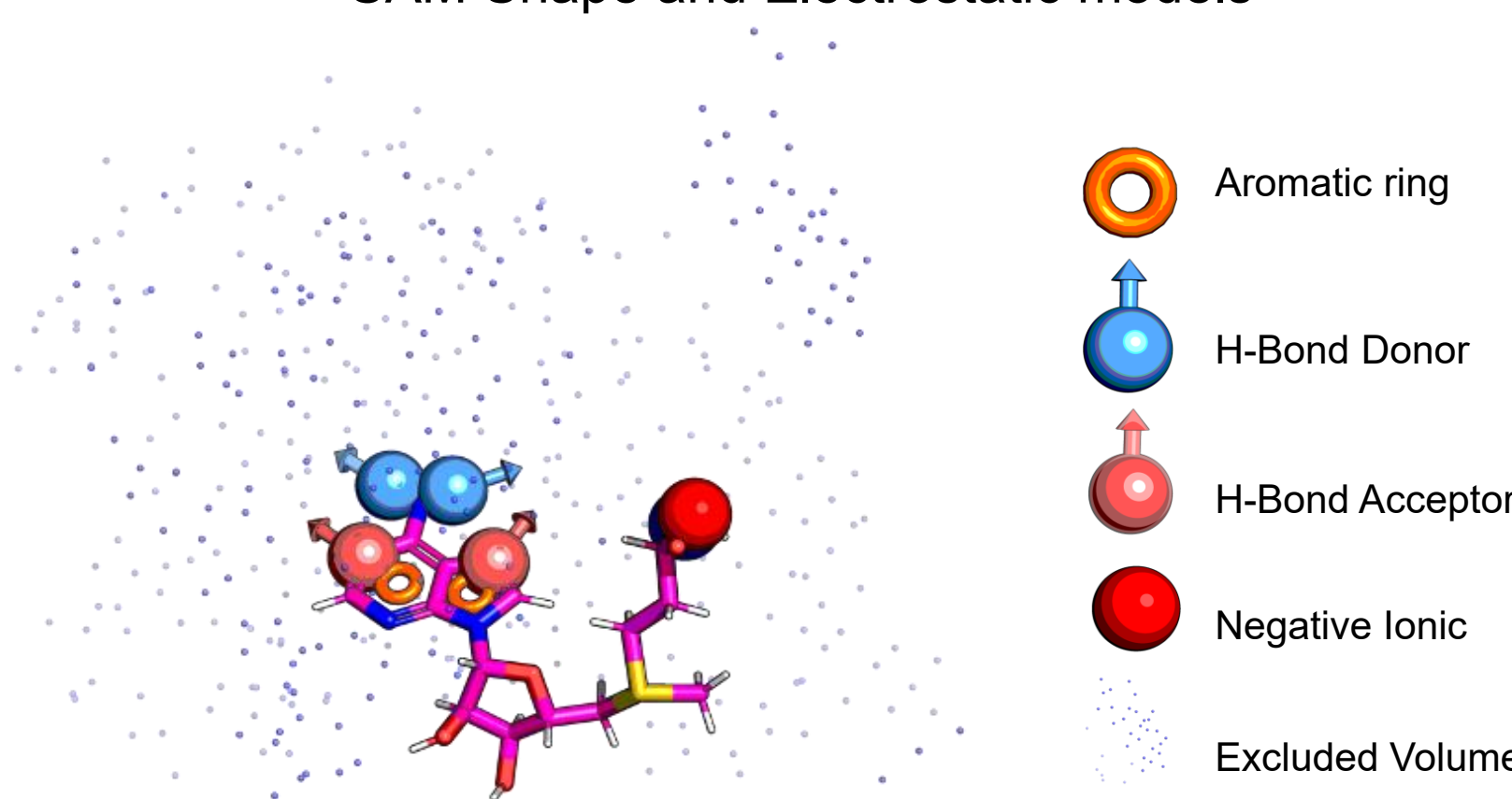
*Almena Rodriguez et al., J. Med. Chem. 2025, 68, 8, 8659-8678

Computational Approaches to Identify RNA-binding Fragments

- Virtual Screening of Domainex's proprietary fragment library
 - 1200 fragments
- Developed Ligand-Based Drug Design (LBDD) models to identify small molecules targeting SAM-VI RNA
- Constructed Shape and Electrostatic similarity models using Phase (Schrödinger) to capture key physicochemical complementarity to known SAM-VI RNA binders
- Built Pharmacophore models using Phase (Schrödinger) to define essential interaction features and guide Virtual Screening
- LBDD models enabled rapid scoring of Domainex's fragment library using prior structural and ligand information

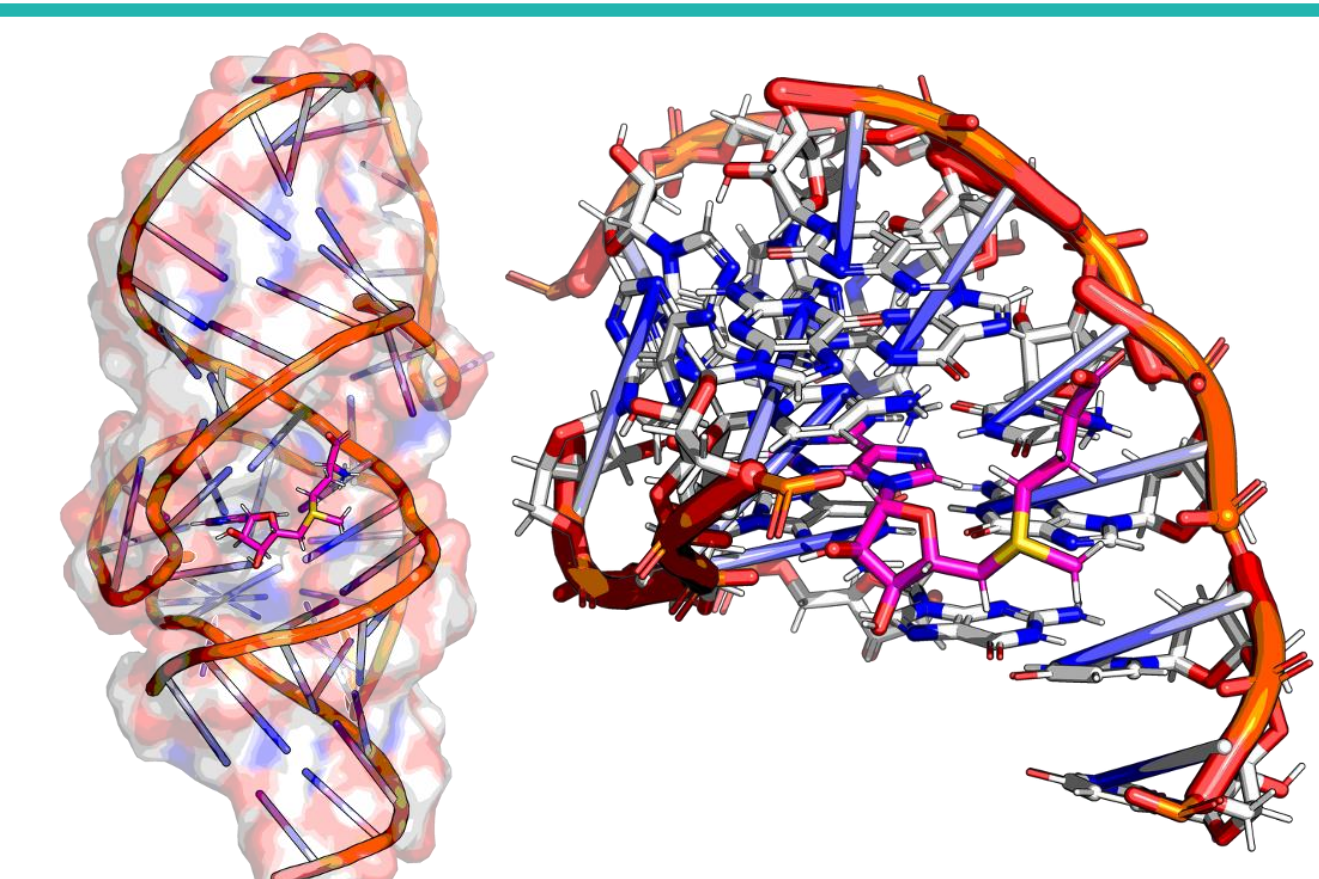


SAM Shape and Electrostatic models

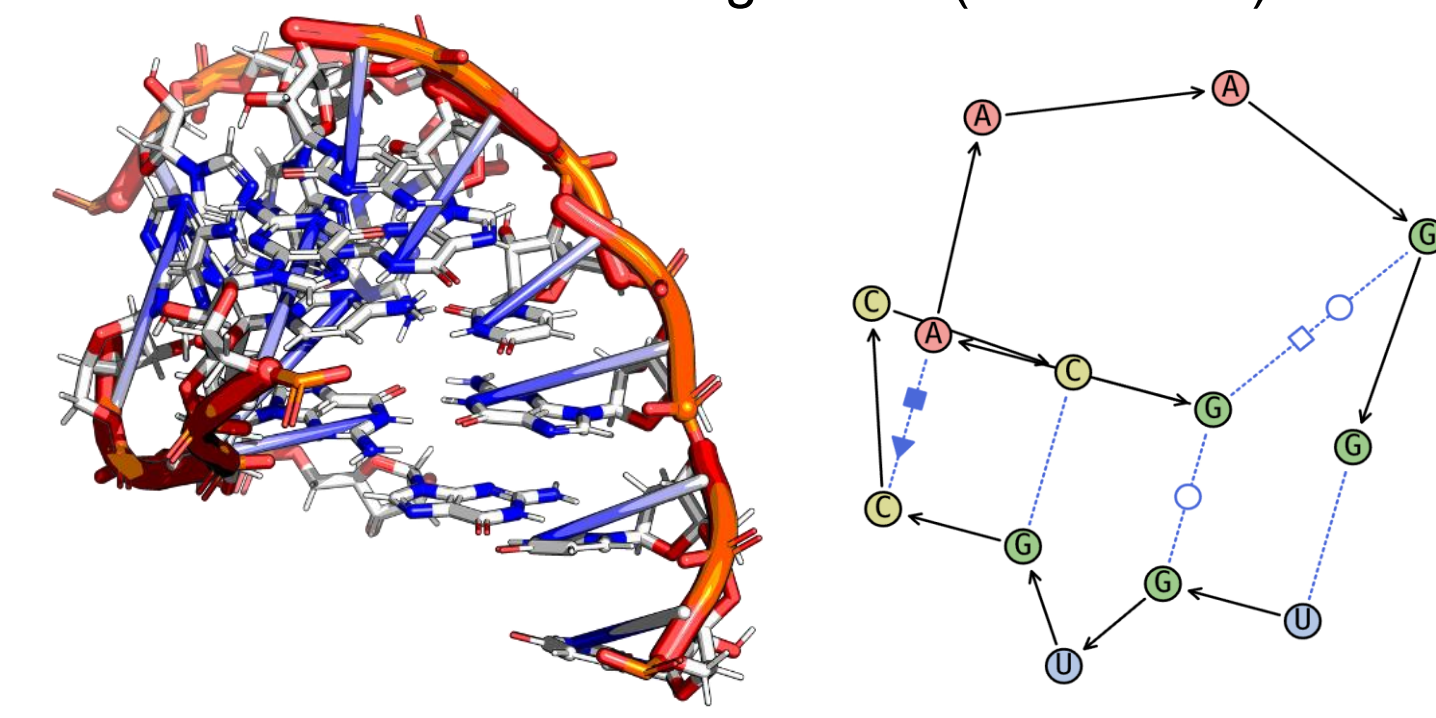


SAM Pharmacophore model

- Implemented Structure-Based Drug Design (SBDD) workflows leveraging Glide (Schrödinger) for high-precision SAM-VI RNA-ligand docking
- Integrated RNAmigos2, a deep graph learning algorithm trained to predict RNA-ligand binding probabilities based on 3D RNA binding sites leveraging 2.5D graph-based binding site features
- Combined physics-based docking scores with AI/ML-derived interaction likelihoods to enhance confidence in hit prioritisation
- Established a hybrid LBDD/SBDD virtual screening workflow that leveraged both physics-based and AI/ML scoring functions to score and rank Domainex's fragment library
- Utilised Pareto ranking to identify fragments on the Pareto frontier across all Virtual Screening models, selecting 100 fragments for experimental screening



SAM-VI Docking Model (PDB:6LAS)

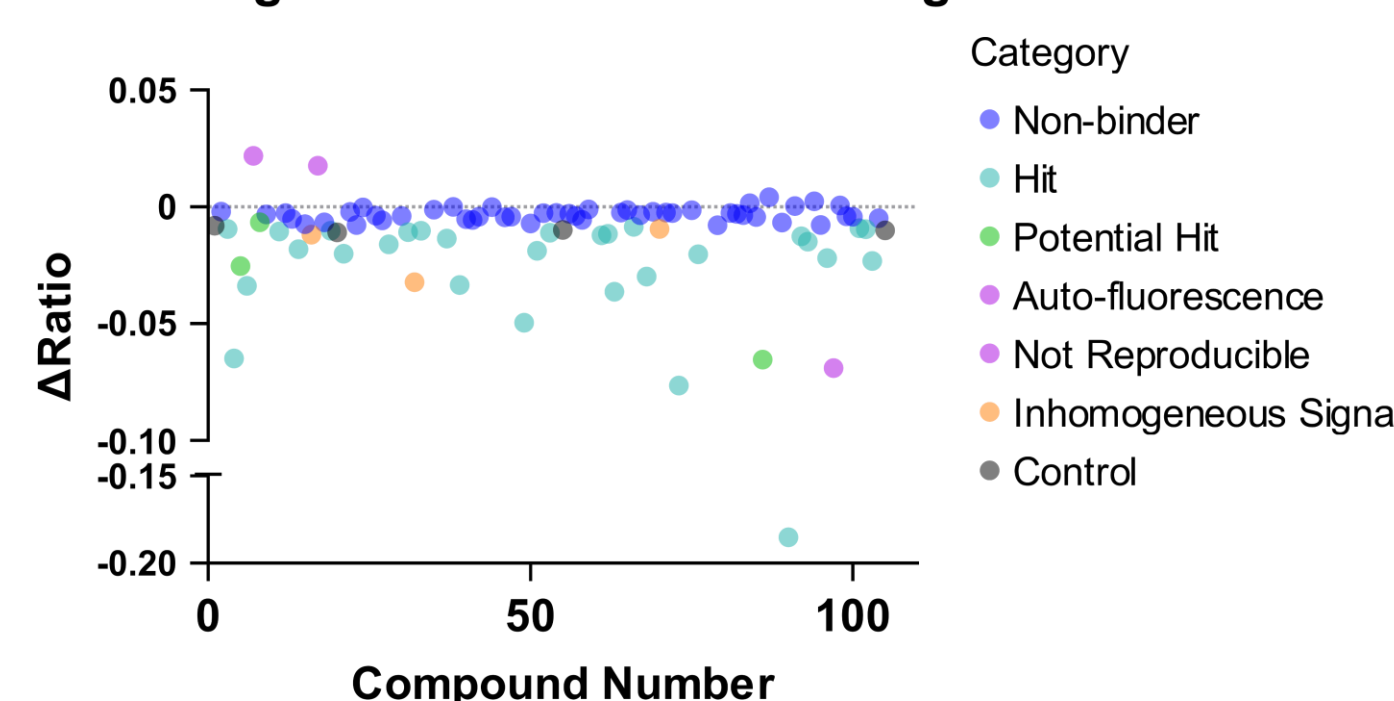


SAM-VI 3D Binding Site - RNAmigos2 2.5D Graph

Screening of Computationally Selected Fragments

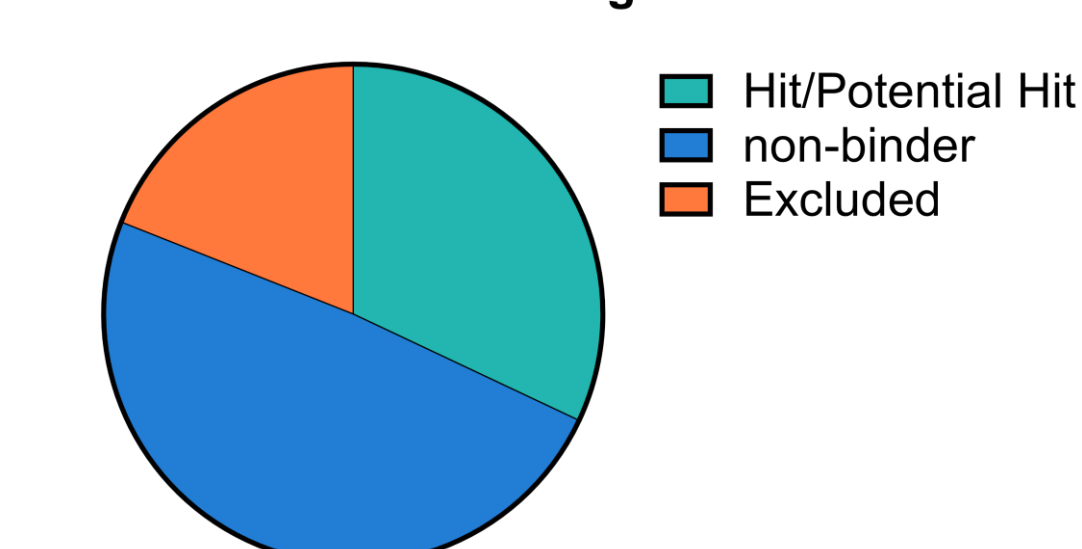
- Spectral shift is an attractive technology for screening RNA
 - RNA requires a simple modification - addition of Cy5
 - The 384 well based format allows for rapid turnaround of screening data
- 100 fragments were screened at 500µM with both SAM and SAH controls
- Compounds were automatically classified into 6 categories allowing for easier classification and interpretation
- Only a small number of compounds cause interference by autofluorescence - an advantage of using a far-red dye

Single Concentration Screening



- A third of compounds screened were confirmed as binders of the SAM-VI riboswitch
- Approximately 20% of compounds were excluded due to assay interference
- Almost half of compounds did not return a significant Δratio and were classified as non-binders

Breakdown of Screening Results



Summary

- Small molecule binding to RNA is an exciting new modality in drug discovery
- Domainex's suite of computational and biophysical techniques provides a powerful platform to explore the stimulating new frontier of RNA-directed small molecule therapies

Services/Contact

If you would like to learn more about applying our drug discovery platforms, please contact: enquiries@domainex.co.uk

