From Sequence to Success: Streamline your oligonucleotide design and testing

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Introduction

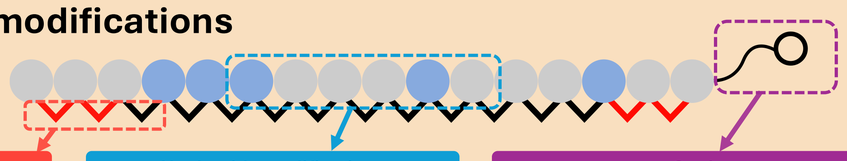
The development timeline for oligonucleotide therapeutics, such as siRNAs and antisense oligonucleotides (ASOs), is significantly accelerated compared to traditional small molecule drug programs. This speed is largely driven by the modular nature of oligo design and the ability to rapidly iterate through candidate sequences. In the early discovery phase, identifying a viable target sequence demands a high-throughput, scalable approach capable of generating and testing plates containing tens to hundreds of oligo variants. These initial screens are critical for pinpointing sequences with optimal binding, efficacy, and minimal off-target activity.

Subsequent cycles of the design-make-test-analyse (DMTA) workflow focus on refining chemical modifications and conjugation strategies to enhance cellular uptake, and tissue-specific delivery, while mitigating off-target and toxicity effects. Each iteration builds on prior data, enabling rational design improvements and accelerating lead optimization.

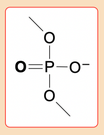
In this poster, we present a breakdown of the key drivers for speed and precision at each stage of oligonucleotide drug discovery. At CatSci we employ computational tools, automation, and data-driven feedback loops to transform early-stage development, enabling biotech startups and research teams to rapidly progress from target validation to lead selection.

Designing Complex Synthetics

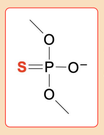
1- Choosing modifications



Phosphate modifications



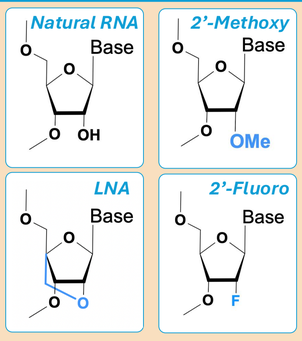
Natural phosphate bond



Phosphorothioate bond

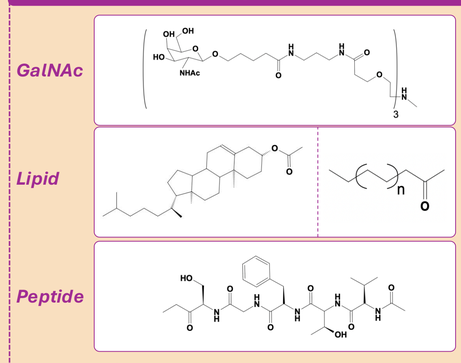
Purpose: Improve *stability, binding affinity, and reduce immune activation*

Nucleotide modifications



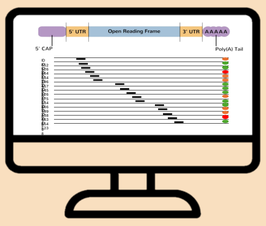
Purpose: Enhance *stability, cellular uptake, and protein binding*

Conjugates

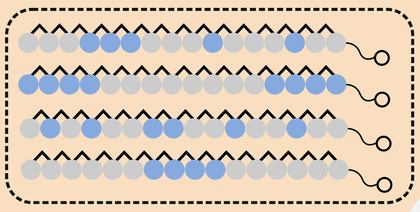


Purpose: Improve *cell-specific delivery, uptake efficiency, and intracellular bioavailability*

2- *In silico* drug design



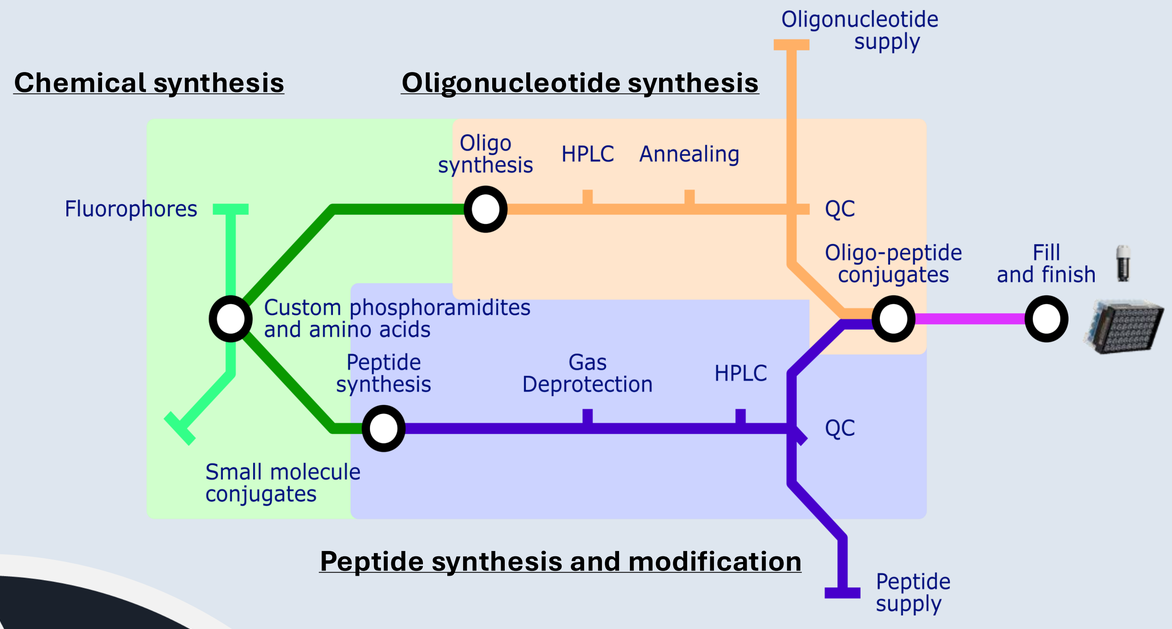
3- *In silico* generated hits



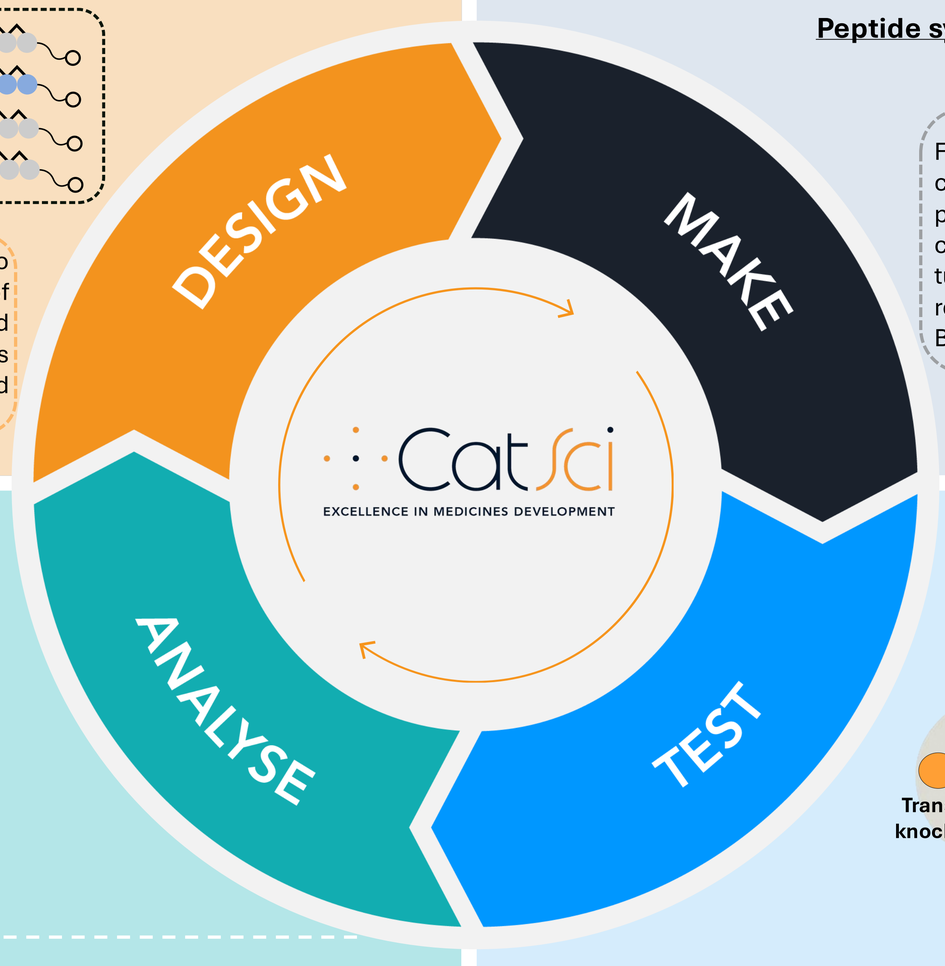
The design integrates chemical and computational engineering to enhance oligonucleotide performance. Targeted modifications of the sugar, backbone or conjugate, improve stability, delivery, and translation, while *in silico* modelling optimises sequence features to yield candidates with predicted efficacy, safety, and manufacturability.

Synthesis of Tides

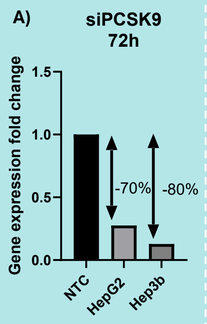
- **CatSci has expertise in oligonucleotide and peptide synthesis**
- **Our strength is the ability to unify complex projects under one roof**



Following the design phase of the oligonucleotide backbone and its conjugate, each component is synthesised within CatSci laboratories prior to the final conjugation step. Every part of the molecule is carefully engineered to optimise efficacy and delivery, while also fine-tuning critical parameters such as stability and inflammatory response. Once the molecule is complete, it is transferred to our Bioscience department for comprehensive testing.



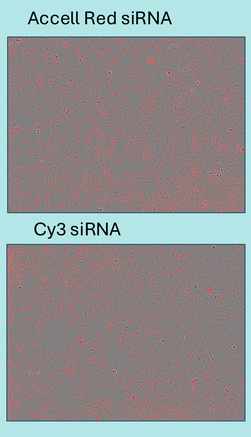
Efficacy

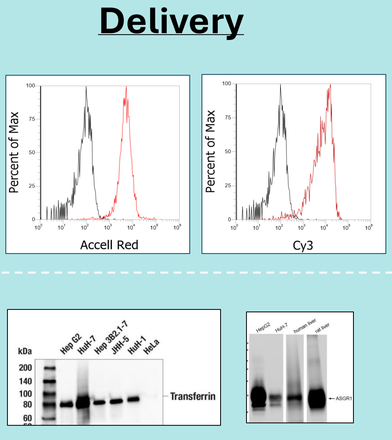


A) Gene expression was assessed using RT-qPCR. The cells were treated with siRNA against PCSK9 for 72h.

B) Protein depletion induced by siRNA. Treatment was assessed using JESS™ Protein Simple automated western blot.

Delivery





A) Uptake of fluorescently tagged cholesterol-conjugated Accell siRNA and transfected non-conjugated siRNA using Lipofectamine monitored on Incucyte SX5.

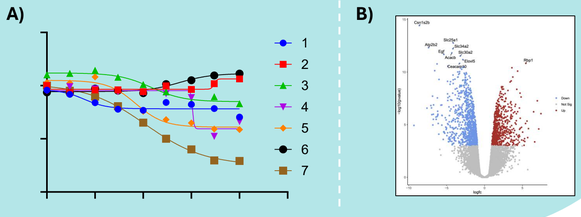
B) Uptake of fluorescently tagged siRNA compounds is quantified by flow cytometry.

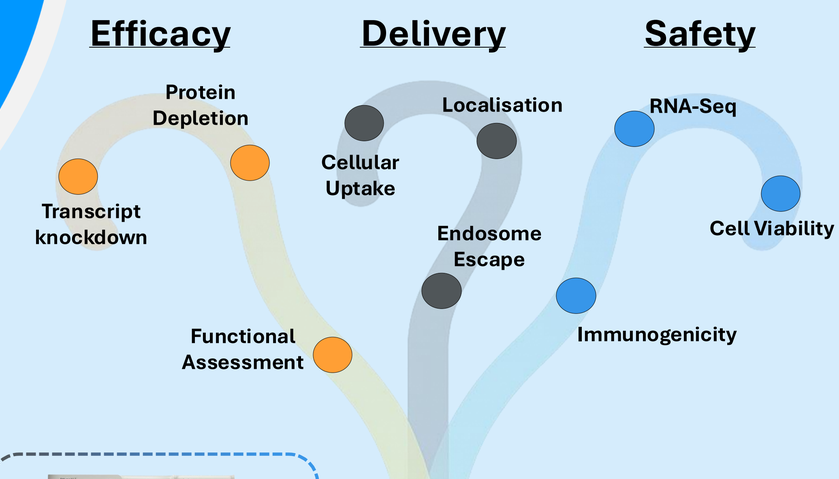
C) Expression of transferrin receptor 1 (TfR-1) and asialoglycoprotein receptor 1 (ASGR-1) enables delivery to the brain and liver, respectively. Taken from: www.cellsignal.com and www.ptglab.com

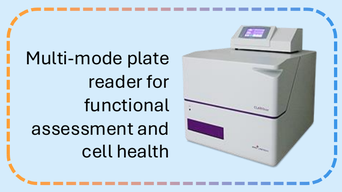
Safety

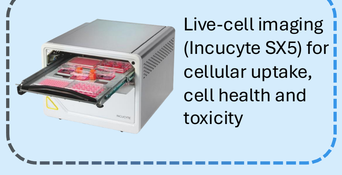
A) Cell viability can be assessed using assays like CellTtiter-Glo®, flow cytometry or live-cell imaging.

B) Example of RNA-Seq volcano plot. RNA-Seq is used to assess on-target and off-target effects of oligonucleotides. Taken from: galaxyproject.github.io









Analysis

Initial assessment of the oligonucleotide molecule begins with a readout of efficacy, usually RT-qPCR, followed by immunoblotting if protein depletion is the desired effect. Once modification or delivery mechanisms are incorporated, efficacy readouts are still used alongside readouts of delivery and cytotoxicity. As the molecules become more complex, so do the assays required to test e.g. functional assays.

Conclusion

From **sequence to success**, CatSci's integrated Design-Make-Test-Analyse (DMTA) workflow accelerates the development of oligonucleotide therapeutics by enabling iterative optimisation informed by high-quality, translatable data. This process is driven by close collaboration between oligonucleotide synthesis and biology teams, ensuring rapid, flexible responses to experimental outcomes and breaking down traditional silos. Our approach is aligned with evolving industry demands in targeted oligonucleotide delivery, supporting the generation of robust data packages for therapeutic advancement.