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RNA therapeutics in the treatment of retinal disease: delivering the potential

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Disclosure: SF is a named inventor on intellectual property licensed to PYC Therapeutics.

A major challenge to nucleic acid therapeutics is achieving efficient and targeted delivery

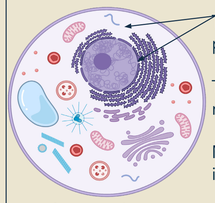


Image from Biorender.com

The opportunity:
The highest value drug targets (DNA, RNA, protein) exist **inside** cells.

The challenge:
The cell membrane has evolved over hundreds of millions of years to keep foreign substances **out.**

The Implication:
Many breakthrough therapeutics fail due to an inability to reach the target.

Nucleic acid drugs
are showing promise in a range of diseases but are hampered by inefficient delivery to target cells.

Antisense oligomer (AO) modulated gene expression

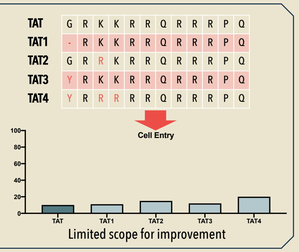
Knockdown gene expression: transcript degradation -RNA silencing, RNaseH induced (DNA analgoues), disrupt the open reading frame (exon skipping)
Alter isoforms: AO mediated exon/splice site selection (RNA analouges; 2'-*O*-modified phosphorothioate 'ASO', phosporodiamidate morpholino oligomers 'PMO')
Block translation, enhance translation, alter mRNA stability: RNA analogues.

Approaches to targeted delivery of AOs

Modality	Class	Scalability	Targeting capacity	In vivo stability	Immunogenicity
* CPP	Synthetic molecule	Excellent	Excellent	Excellent	Low
Antibody	Biological molecule	Moderate	Excellent	Moderate	High
Liposome	Synthetic particle	Moderate	Poor	Poor	Moderate
Dendrimer	Synthetic particle	Excellent	Poor	Excellent	Moderate
Nanoparticle	Synthetic particle	Excellent	Moderate	Excellent	Moderate
Exosome	Biological particle	Poor	Moderate	Poor	Moderate
Viral vector	Biological particle	Poor	Poor	Moderate	High

* CPP-Cell penetrating peptide

Conventional approach to CPP development is to take previously poor-performing CPPs and attempt to increase potency or reduce cytotoxicity through incremental changes that limit diversity.



High-performing cell penetrating peptides selected from our diverse peptide library deliver antisense oligomers (PMO) to the retina

We have developed proprietary screening methods and advanced filtering to identify CPPs with excellent efficacy and tolerability.

What are the libraries? Our libraries contain ~500 million unique peptides from 82 genomes and 118 synthetic viral genes
Diverse : Derived from the genomes of bacteria, archaea and viruses, the peptide libraries are extremely diverse
Targeted: The libraries have been enriched with viral genomes that have evolved to penetrate cells.

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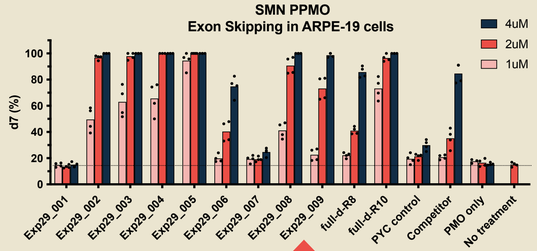
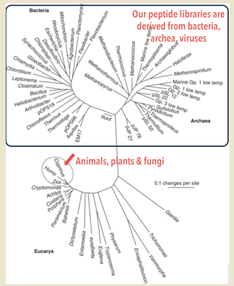
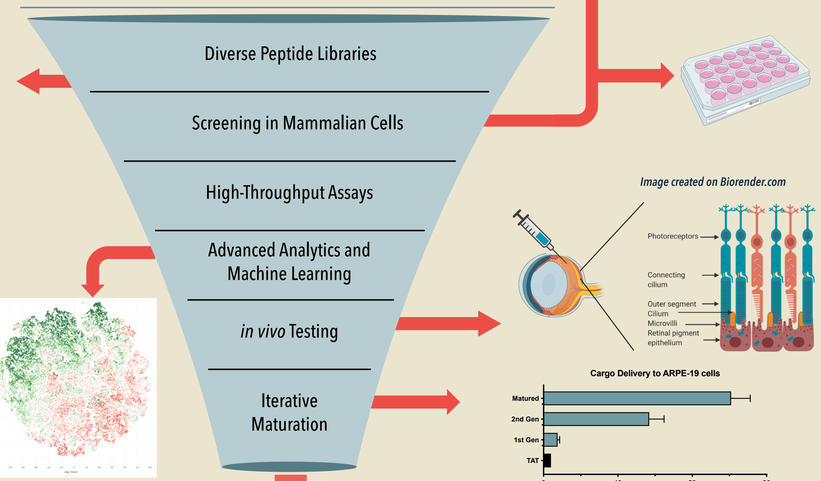
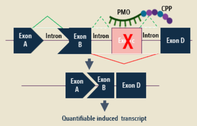




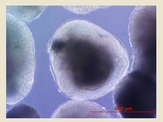


The libraries and machine learning models provide the flexibility to choose appropriate CPPs from our existing diverse panel and to further optimise for various cell and tissue types.

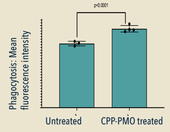
Image created on Biorender.com



Antisense oligomer mediated changes to gene expression

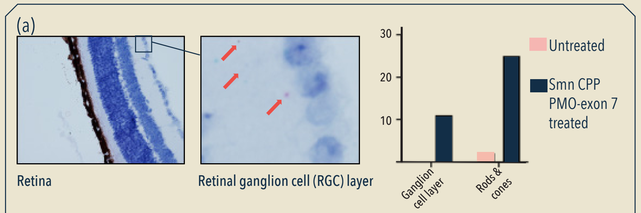


CPP-PMO evaluation in patient induced pluripotent stem cell-derived retinal organoids

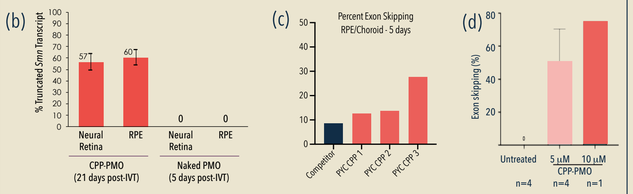


Functional analysis

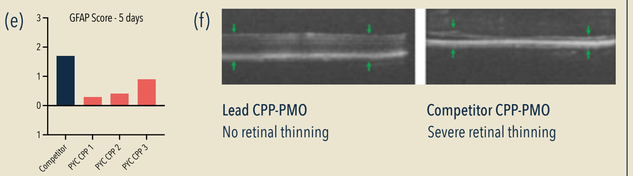
CPP-PMO delivery to retinal cells *in vivo* and to patient-derived cell models



a) Efficient, functional PMO delivery to mouse retina after intravitreal (IVT) injection (3.2 µg reporter Smn CPP-PMO that induces exon 7 skipping) demonstrated in RGC using BaseScope™.



b) Efficient delivery of Smn CPP-PMO to retinal cells *in vivo* by IVT injection of 1.6 µg Smn CPP-PMO and evidence of antisense action in the target tissue in mice (penetration across multiple layers in the intraocular space), with excellent tolerability compared to the competitor PPMO (c). (d) CPP-PMO induced exon skipping in patient iPSC derived retinal organoids.



e) GFAP (marker of injury) expression in retina 5 days after IVT injection of competitor and PYC CPP-PMOs. (f) OCT imaging 21 days after Smn CPP-PMO IVT injection in mice.

CPP-PMO delivery holds promise for the treatment of retinal disease

Our discovery cell penetrating peptides are derived from nature, lack chemical modifications, and yield optimal amino acid sequences with enhanced efficacy and tolerability performance. Our lead CPP traffics the PMO through the vitreous, into the neural retina and retinal pigment epithelium, resulting in enhanced reporter exon skipping, with no evidence of retinal damage.