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Impact of nitrogen-containing backbone linkages on stereopure antisense oligonucleotides in the CNS

Pachamuthu Kandasamy

Oligonucleotide Therapeutics Society
Sept. 26-29, 2021

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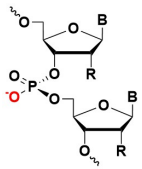
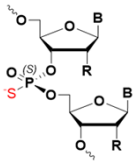
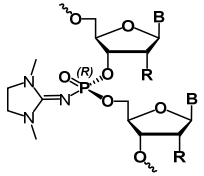
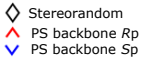
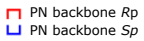
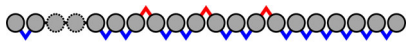
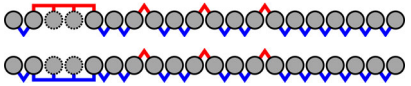
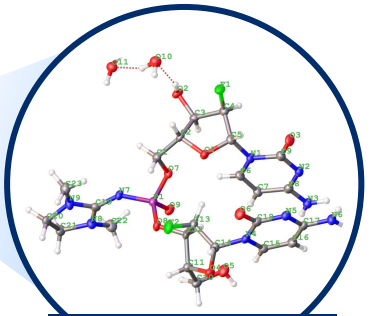
Expanding repertoire of backbone modifications with novel PN backbone chemistry

Backbone linkages

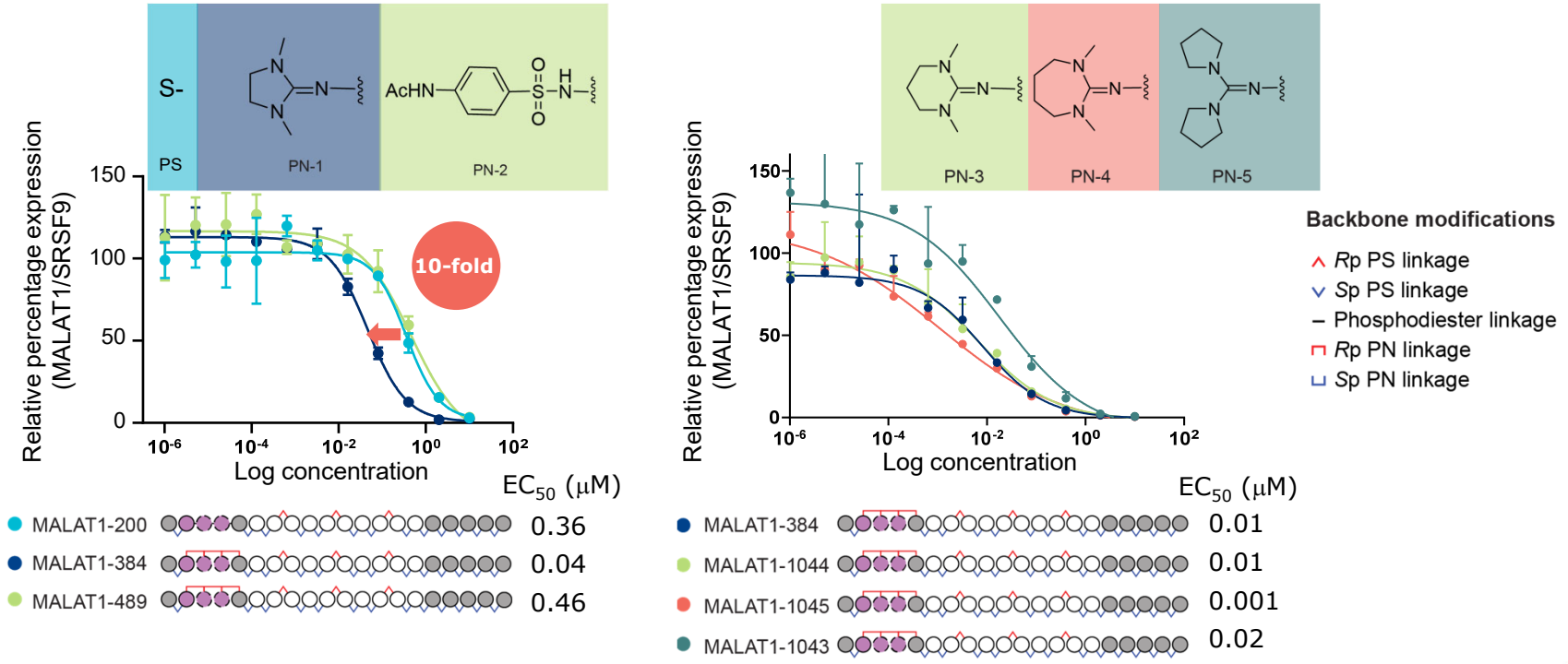
	PO	PS	PN
Backbone modification (X)	Phosphodiester 	Phosphorothioate 	Phosphoramidate diester 
Stereochemistry	Not chiral	Chiral 	Chiral 
Charge	Negative	Negative	Neutral
Depiction			
PRISM backbone modifications	PO/PS PO/PS/PN		



Phosphoryl guanidine x-ray structure

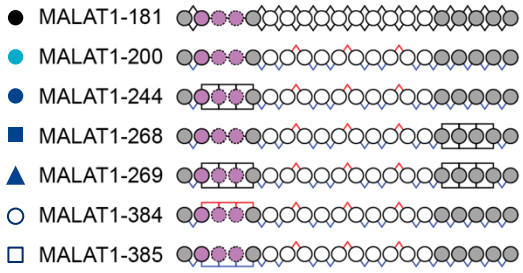
Molecule structure illustrative of backbone modification patterns

PN chemistry increases potency *in vitro* 10-fold



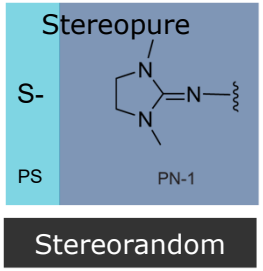
iCell neurons were treated with increasing concentrations of oligonucleotide. MALAT1 RNA was normalized to SRSF9. Mean ± sd are shown, n=3 per concentration. Half maximal effective concentrations (EC_{50}) are calculated with GraphPad Prism software.

PN-1 backbone improves cellular potency without impacting RNase H activity

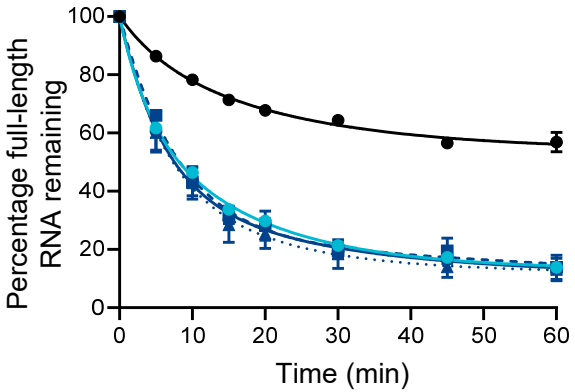


Backbone modifications

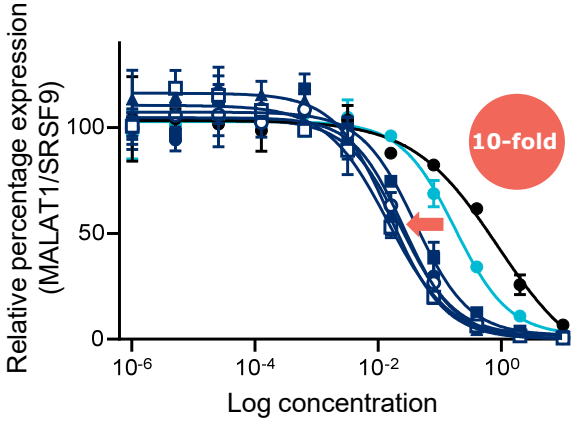
- Stereorandom PS linkage
- *Rp* PS linkage
- *Sp* PS linkage
- Phosphodiester linkage
- Stereorandom PN linkage
- *Rp* PN linkage
- *Sp* PN linkage



RNase H assay



iCell neurons

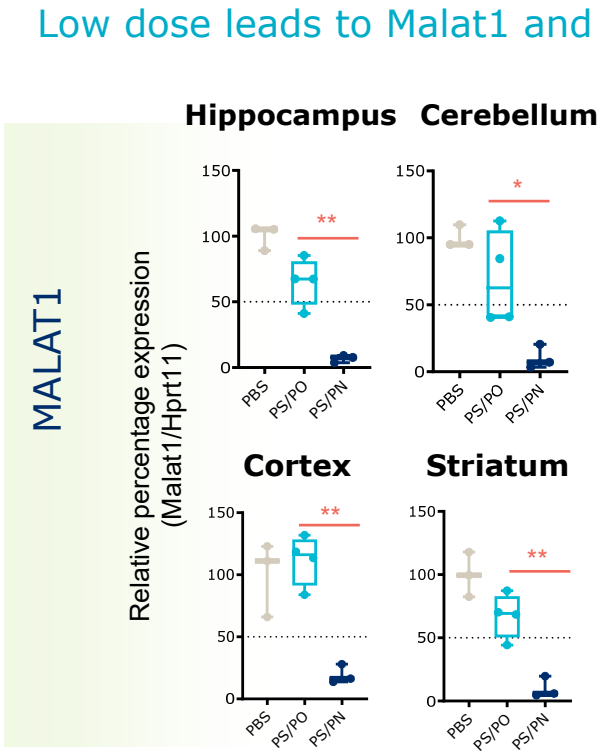


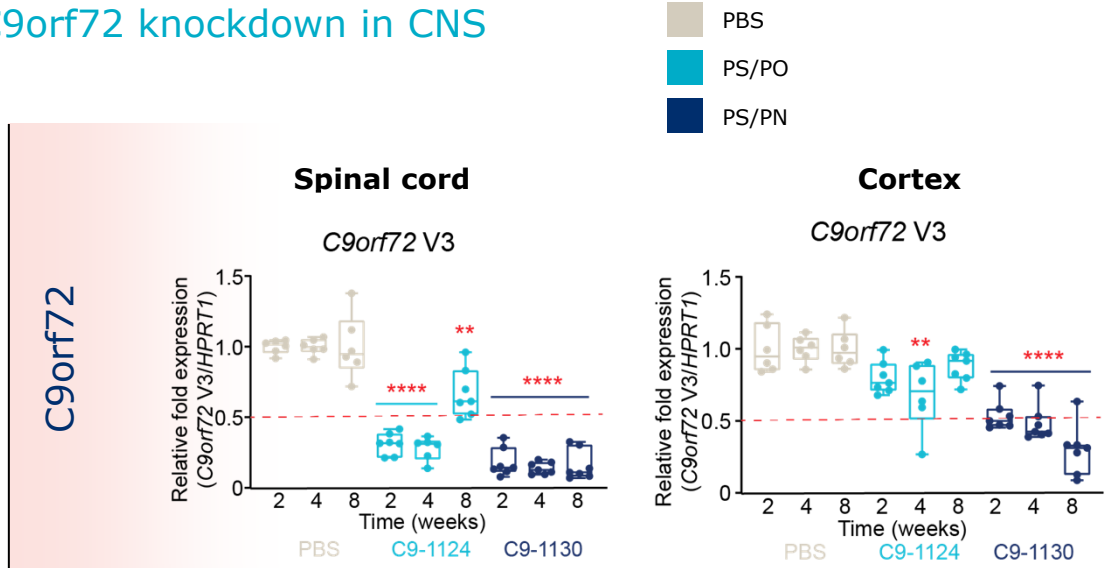
- PN-1 matches stereopure PS in RNase H assay
- PN-1 increases potency 10-fold over stereopure PS in neurons

(Middle) Biochemical RNase H assays performed on MALAT1 RNA-oligonucleotide duplex. Mean ± sem are shown, n=3 per time point. (Right) iCell neurons were treated with increasing concentrations of oligonucleotide. MALAT1 RNA was normalized to SRSF9. Mean ± sd are shown, n=2 per concentration. Half maximal effective concentrations (EC_{50}) are calculated with GraphPad Prism software.

PN-1 chemistry increases potency and durability in mouse CNS for multiple targets

Low dose leads to Malat1 and C9orf72 knockdown in CNS





- PN-1 increases potency and durability throughout CNS
- Application of PN-1 to WVE-004 (C9orf72) extended duration to at least 6 months

(Left) Mice received a single 100 μg ICV injection (n=3 per group). Relative percentage Malat1 expression (normalized to Hprt1) is shown for the indicated tissues 10-weeks post-dose. Stats: 1-way ANOVA. (Right) C9BAC mice received 2 x 50 μg ICV injection (n=7 per group). C9orf72 V3 is normalized to Hprt1. Stats: 2-way ANOVA *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 PBS, phosphate buffered saline