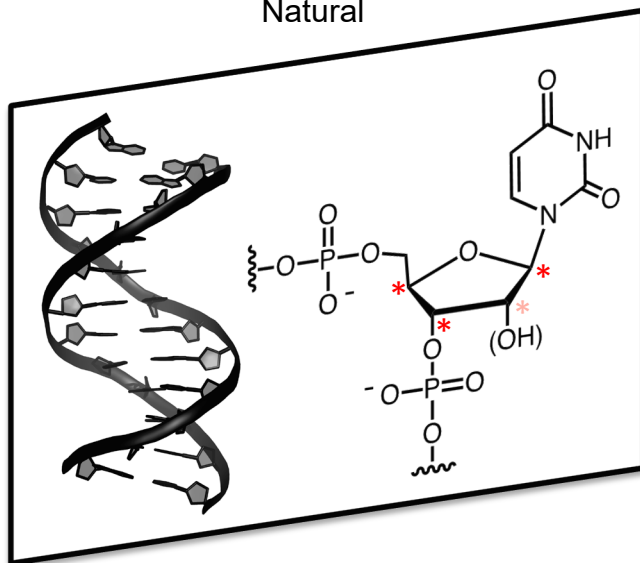




# L-Oligonucleotides

## D-Oligonucleotides

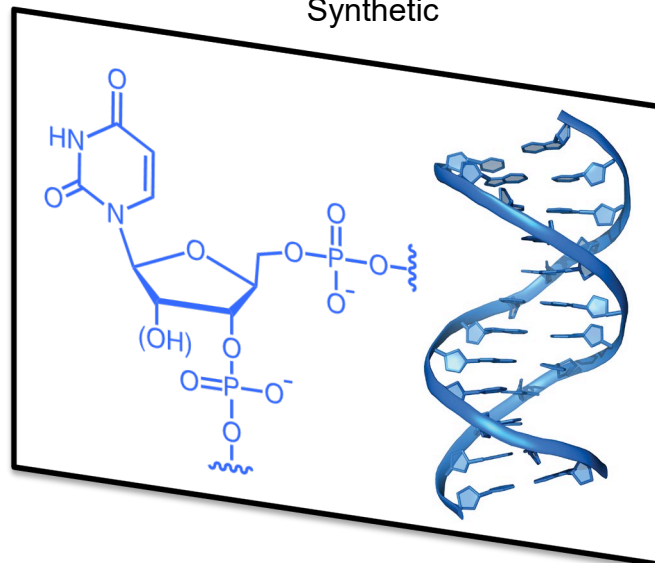
Natural



Mirror

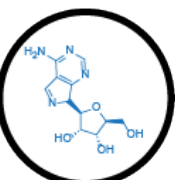
## L-Oligonucleotides

Synthetic



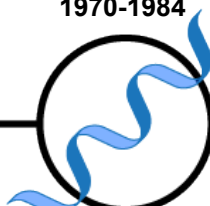
1934

1964



First L-nucleoside synthesized

1970-1984



Solution-phase synthesis and characterization of L-oligonucleotides

1990



Solid-phase synthesis of L-oligonucleotides

1993

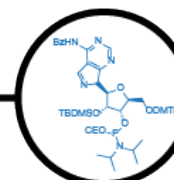
Hybridization between D- and L-oligonucleotides debunked

1996



First L-aptamers (Spiegelmers) reported

2001



L-Nucleoside phosphoramidites become commercially available

2016

Enzymatic synthesis of L-DNA and L-RNA using mirror polymerases

2017

Mirror-image polymerase chain reaction (PCR)

2022

Transcription of mirror-image ribosomal RNAs from enzymatically assembled long mirror-image genes



# L-Oligonucleotides

- L-Oligonucleotides are considered to be orthogonal to the stereospecific environment of natural biology.

- ✓ Resistant towards biological degradation
- ✓ Limited off-target interactions\*
- ✓ Non-immunogenic\*

\*More experimental data needed

**The influence of chirality on the behavior of oligonucleotides inside cells: revealing the potent cytotoxicity of G-rich L-RNA†**

Chen-Hsu Yu and Jonathan T. Szcepanski \*

Yu et al. *Chem. Sci.* 14, 1145-1154 (2023)

**Interrogation of mirror-image L-RNA–protein interactions reveals key mechanisms of single-stranded G-rich L-RNA cytotoxicity and a potential mitigation strategy†**

Chen-Hsu Yu,<sup>a</sup> Xiaomei He, <sup>bd</sup> Rosemarie Elloisa P. Acero,<sup>‡a</sup> Xuan Han,<sup>‡a</sup> Yinsheng Wang<sup>b</sup> and Jonathan T. Szcepanski \*<sup>ac</sup>

Yu et al. *Chem. Sci.* 16, 7560-7572 (2025)

- L-Oligonucleotides have the same physical properties in terms of solubility, hybridization kinetics, and duplex thermal stability as their native D counterparts. This is a major advantage from a design perspective.



- L-Oligonucleotides (<100-nucleotides) can be easily prepared via solid-phase synthesis using readily available reagents.
- Oligonucleotides of opposite stereochemistry (D versus L) do not form contiguous base pairs with each other, which can be both an advantage and a disadvantage.

These properties provide L-oligonucleotides a broad range of opportunities in research and medicine



# L-Oligonucleotides



## Applications

Piwko et al. *ChemBioChem* 23, e202200520 (2022)  
 Shearer et al. *Chem. Sci.* 15, 18239-18258 (2024)

Zhong et al. *ACS Sens.* 4, 566-570 (2019)  
 Zhong et al. *ACS Synth. Biol.* 10, 209-212 (2021)  
 Zhong et al. *J. Am. Chem. Soc.* 145, 17066-17074 (2023)  
 Acero et al. *ACS Sensors* (2025) (In Press)

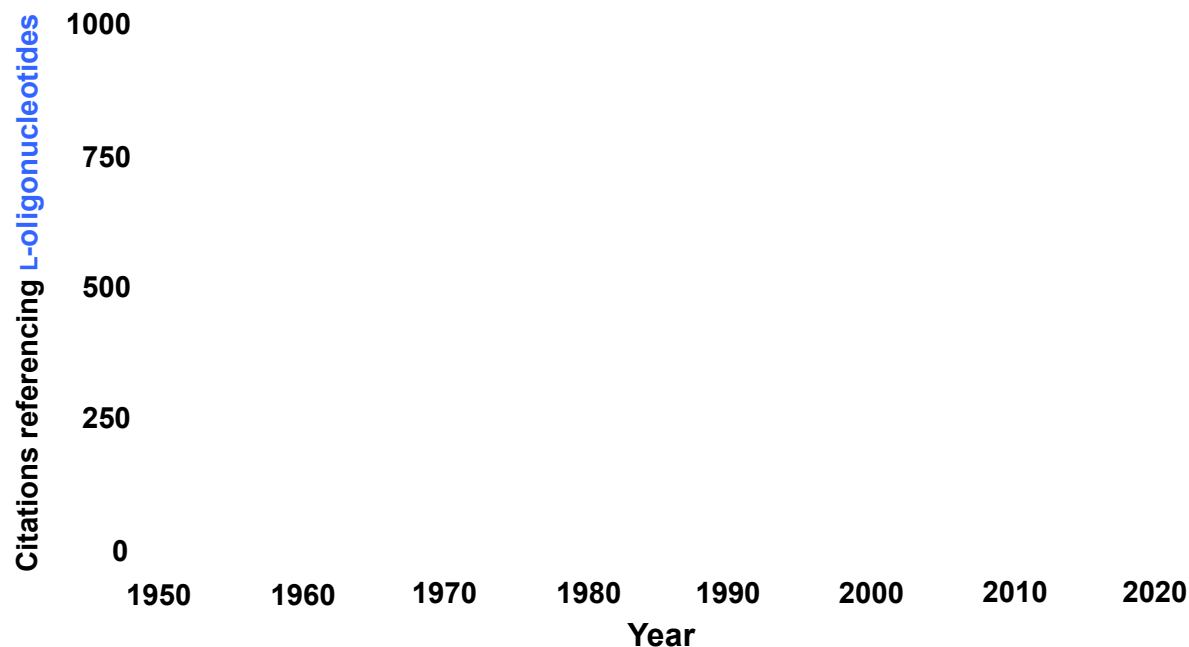
Kabza et al. *J. Am. Chem. Soc.* 139, 1824-1827 (2017)  
 Young et al. *ACS Synth. Biol.* 8, 2756-2759 (2019)

### L-Aptamers in the clinic

 <a href="https://www.tmepharma.com/">https://www.tmepharma.com/</a>	Therapy & Indication	Preclinical	Phase 1/2	Phase 2	Phase 3	Next Inflection Point	Partner/ Collaborator
	NOX-A12 + Radiotherapy ± anti-VEGF Brain cancer / Glioblastoma <small>Orphan Drug Designation Granted in US &amp; EU</small>			Phase 2 protocol approved; Fast Track awarded by FDA		Financing and initiation of randomized Ph 2	
	NOX-A12 + Immunotherapy Pancreatic Cancer			Phase 2 protocol approved		Financing and initiation of randomized Ph 2	Scientific collaboration for Ph1/2 & Ph2 



# L-Oligonucleotides



The impact of L-oligonucleotide research continues to grow

Data obtained from Web of Science

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Welch  
Foundation