LINKING VISCOSITY TO HIGHER-ORDER INTERACTIONS IN CHEMICALLY MODIFIED OLIGONUCLEOTIDE SOLUTIONS

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Background and aim

Pharmaceutical industries have seen substantial growth in the number of approved **oligonucleotide therapeutics** over the past decade. [1] However, their development is often hindered by their **unpredictable high viscosity in solution**, which complicates formulation and manufacturing processes. [2]

In this study, we explore the **viscosity** behaviour of equimolar mixtures of RNA-based 16-mers **Poly(A)** and **Poly(U)** sequences bearing common **chemical modifications**, aiming to relate **viscosity changes** induced by these modifications to changes in **molecular interactions.** Better understanding of these effects is fundamental to **optimising** the future **design** and **development** of **oligonucleotide formulations**.

Results: Viscosity

Viscosity of **modified oligonucleotides is** measured using a **microfluidic viscometer** to determine which **chemical modifications** induce **viscosity changes** compared with an **unmodified** sequence. (**Figure 1**)

The **GalNAc conj. sample** exhibited **higher viscosity** than the **unmodified sample**, indicating an influence of the **GalNAc moiety** on viscosity behaviour.
The **2'-MOE sample** exhibited **higher viscosity** than the **2'-OMe sample**, indicating an **effect** of the **substituent** in the **2'-position** on **viscosity.**

The **LNA-modified sample** and the **siRNA-mimicking sequences** showed **no significant viscosity differences** compared to the **unmodified** sequence, indicating minimal impact on viscosity.

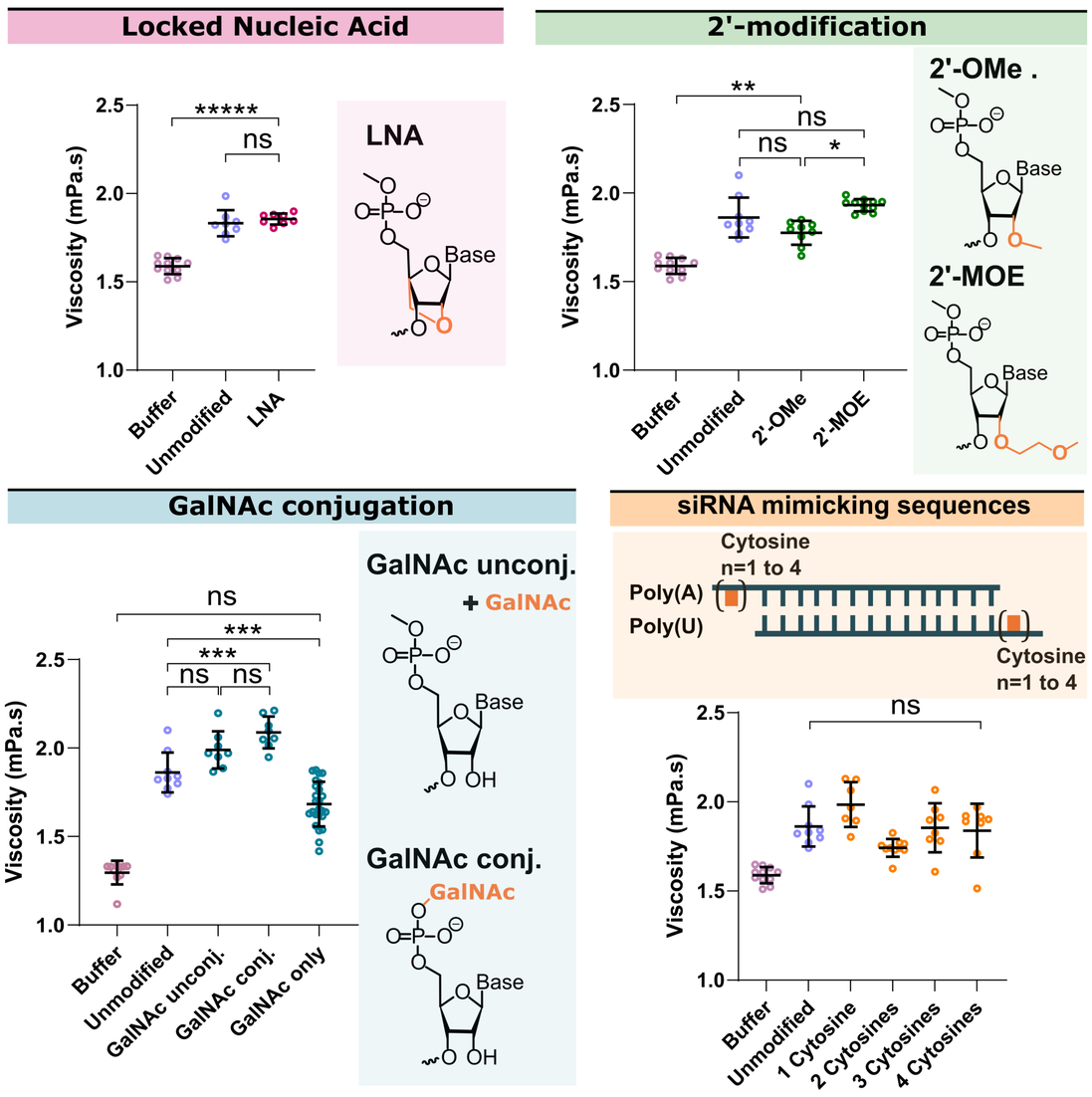

Fig. 1. *Viscosity of modified Poly(A)/Poly(U) duplexes in 10 mM phosphate buffer in D_2O at 10° C. The total oligonucleotide concentration is 2 mmol/L, with Poly(A) and Poly(U) equimolar. (n=10) Statistical significance was assessed using pairwise t-tests with Tukey adjustment for comparisons between the unmodified and the modified samples. ns, p> 0.05; *, p< 0.05; **, p< 0.01; ***, p <0.001 and *****, p< 0.00001.*

Results: Structural characterisation

To **better understand** the **viscosity differences** observed between samples, **structural characterisations** were performed on **systems displaying distinct** viscosity behaviours. (**Fig. 2**)

Birefringence observed by **microscopy** indicates **self-organisation** of the chains in **concentrated** regimes through **end-to-end π–π stacking** between oligonucleotide strands. (**Fig. 2A**) This type of organisation is referred to as **liquid crystals.** [3]

^{1}H NMR signal at **11 ppm** demonstrates **Watson-Crick base pairing** between the poly(A) and the poly(U) strands in **diluted** regimes. (**Fig. 2B**) [4]

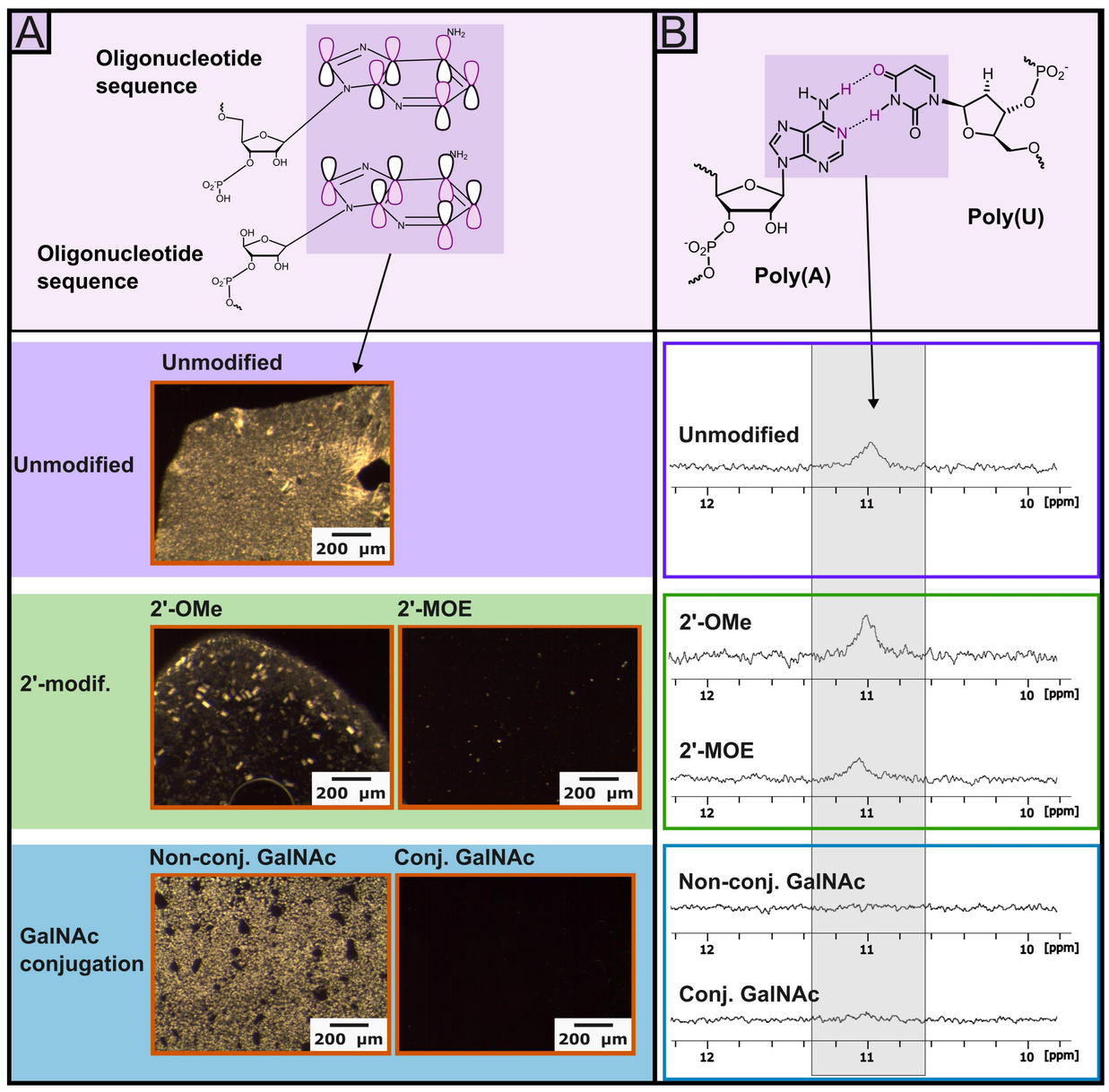

Fig. 2. (A) *Polarised light microscopy images of modified oligonucleotide solutions at 20 mmol/L in a 10 mM phosphate buffer in D_2O. The presence of birefringence indicates the formation of liquid crystal structures. (**B**) Imino proton region (10-15 ppm) of the ^{1}H NMR spectra of the chemically modified oligonucleotide solutions at 0.1 mmol/L in H_2O/D_2O (90:90 v/v). Spectra were acquired with WATERGATE suppression on a 500 MHz NMR.*

Despite **base pairing** between strands (**Fig. 2B**), the **size of the 2'-substituent** affects **chain self-organisation**, as shown by the more **scattered liquid crystal** textures observed for the **2'-OMe** and **2'-MOE** samples. (**Fig. 2A**) **Increased steric bulk in the 2'-MOE modification** likely contributes to **higher viscosity** compared with the **2'-OMe analogue**: the larger substituent **sterically** excludes **close interstrand** approach, increasing effective excluded volume and solution viscosity.

The **GalNAc conjugation** likely **increases viscosity** through **increased chain length**, while **preventing** both **liquid crystal** formation (**Fig. 2A**) and **base pairing** (**Fig. 2B**)

Conclusions and Future work

1. Among the chemical modifications studied, **GalNAc conjugation** and **bulkier 2'-substituents** increase **viscosity.**

2. Structural characterisation indicates that **increased chain length**, and **larger 2'-substituents** are responsible for **these viscosity increases.**

3. Viscosity and structural characterisations will be performed at **higher concentrations** as **interactions** vary with **concentration regime.**

Acknowledgements

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[1] V. Vinjamuri; J.Pan ; P.Peng (2024) J. Pharm. Sci. 113 (7), 1749-1768 [2] J. Muslehiddinoglu et al. (2020) Nucleic Acid Ther. 30 (4),189–197 [3] T. [3] Bellini, G.Zanchetta, R. Cerbino et al. (2012) Proc Natl Acad Sci, 109 (4) 1110-1115. [4] F. Barnwal, V. Yang, G. Varani (2017) Arch Biochem Biophys., 628, 42-56