

RNA G-quadruplexes in pre-miRNAs: A new way in the target of nucleolin

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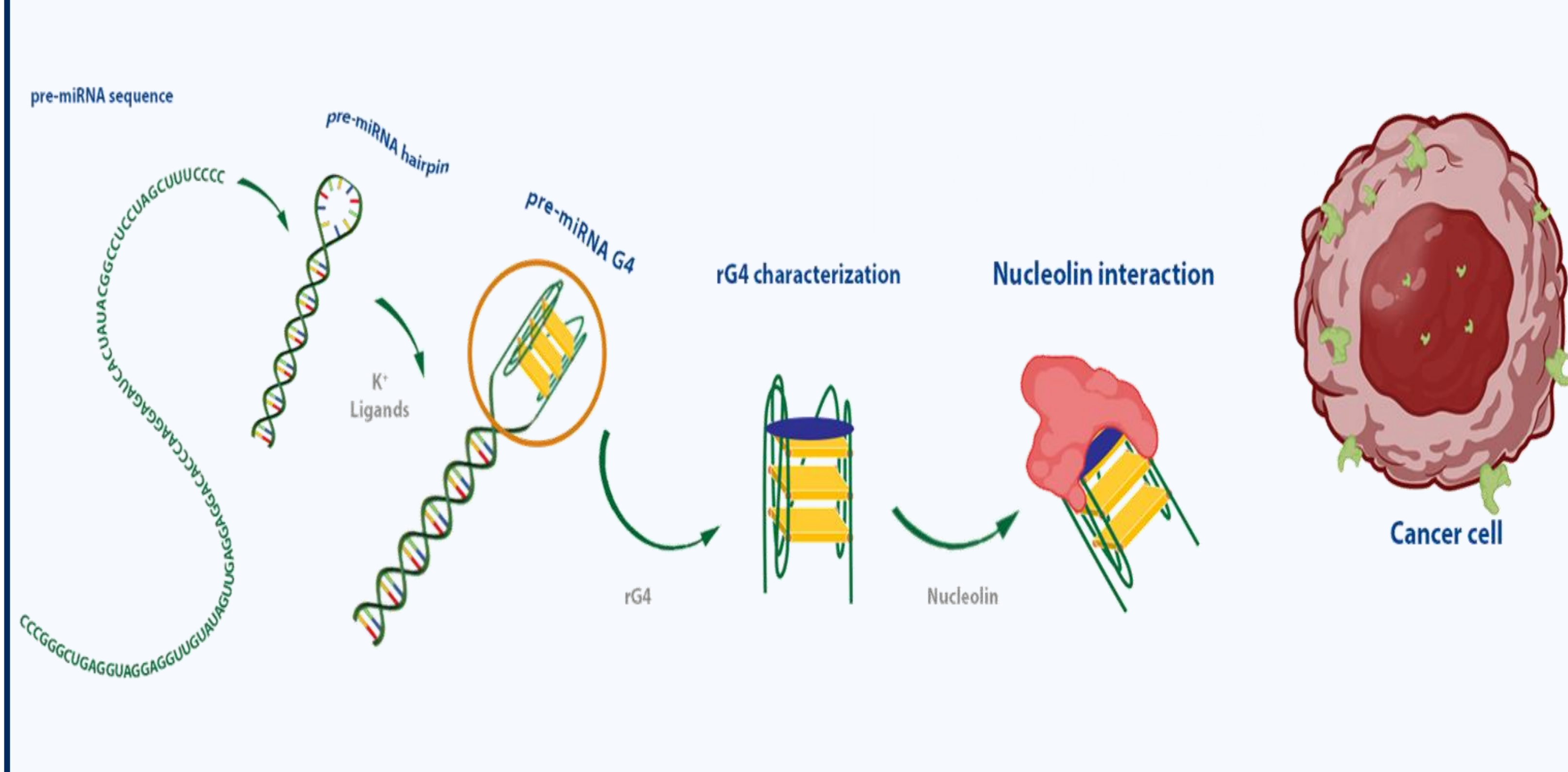
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BACKGROUND

RNA G-quadruplexes (rG4) have been shown to play an important role in some biological processes, including the regulation of microRNA (miRNA) biogenesis and function. Also, it was recently shown that rG4 oligonucleotides found in pre-miRNA-149, pre-miRNA-92b and pre-let-7e bind to ligands and proteins. The protein nucleolin (NCL) was shown to bind rG4s and to modulate their folding and/or stability. Among several pre-miRNA with potential ability to form G4s, those have shown to be deregulated in several cancer types. Therefore, it is important to unveil and characterize its capability to form a G4 structure, and determine the role that some ligands may have in its folding and binding capacity to NCL. Those RNA G4 sequences could be used as supramolecular carriers with affinity for nucleolin.

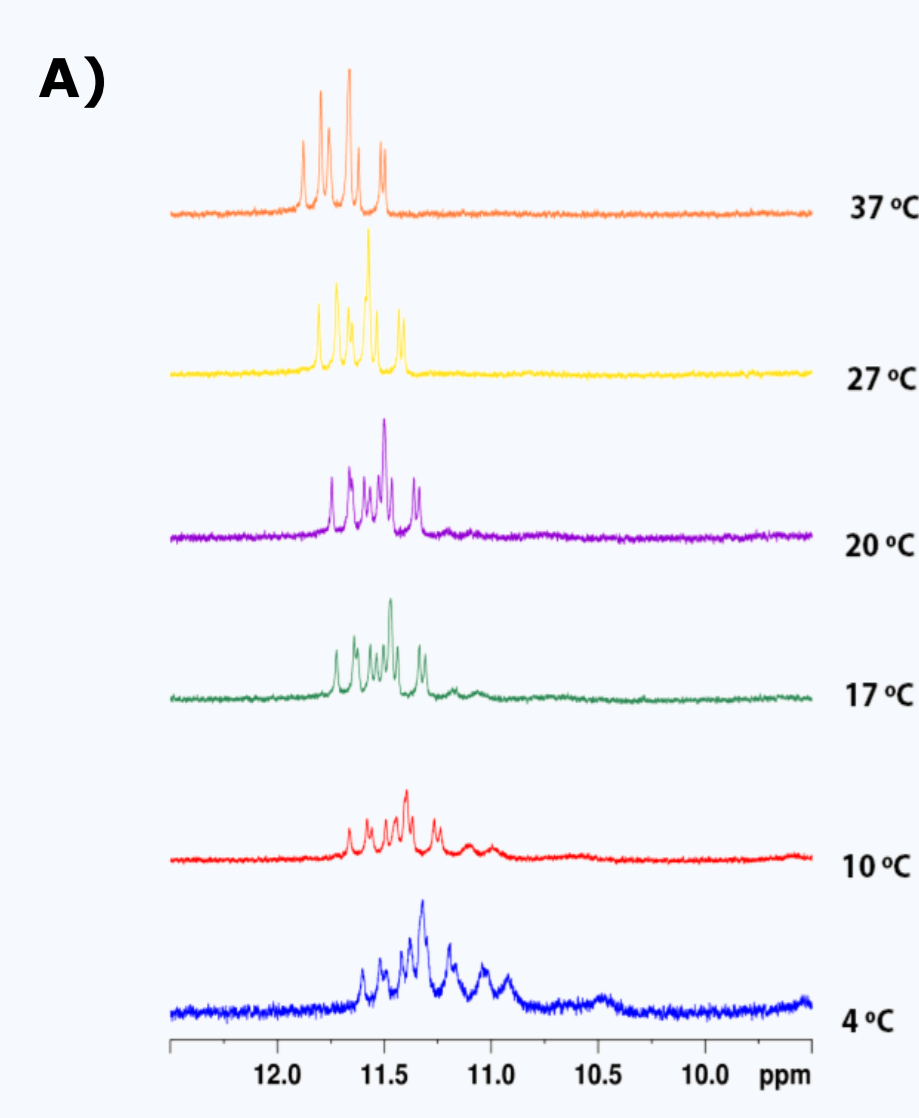
GLOBAL OVERVIEW



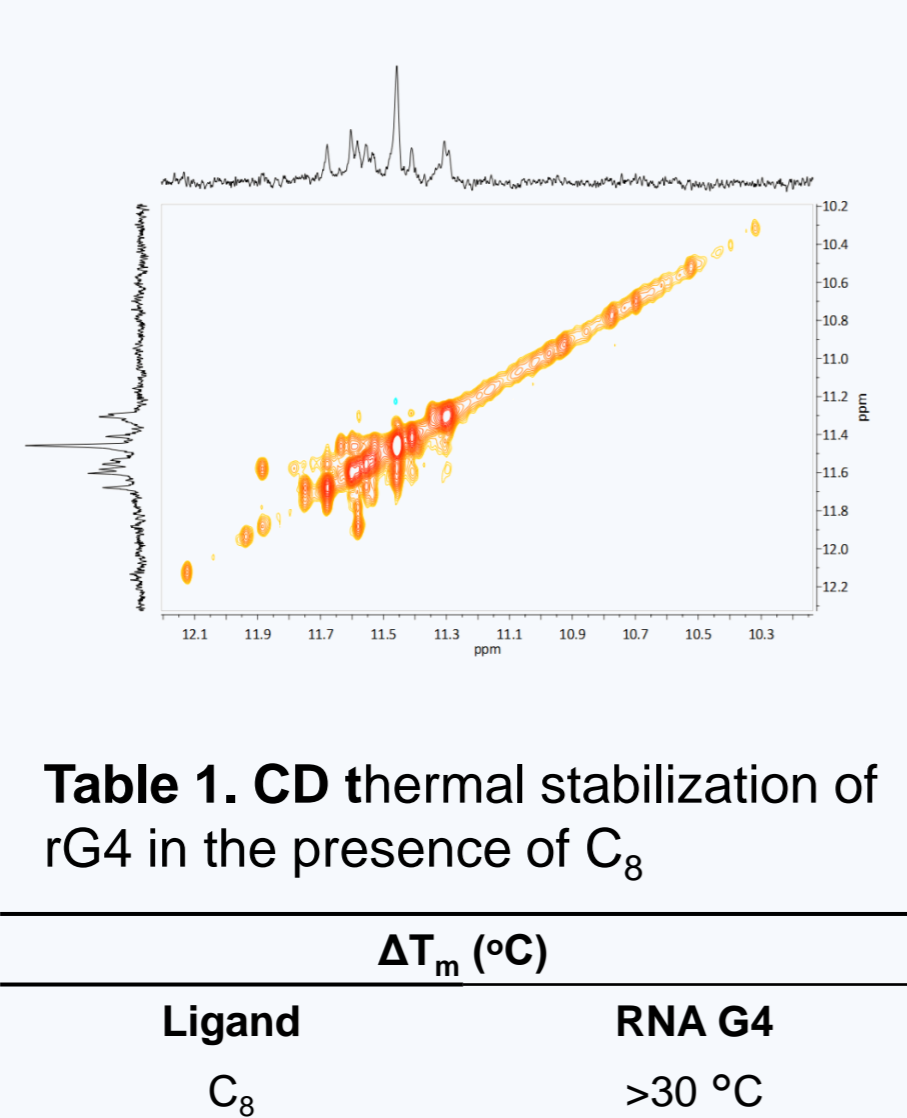
RESULTS AND DISCUSSION

Formation and stabilization of rG4 in pre-miR-149

NMR and CD spectroscopy



B)



In silico analysis

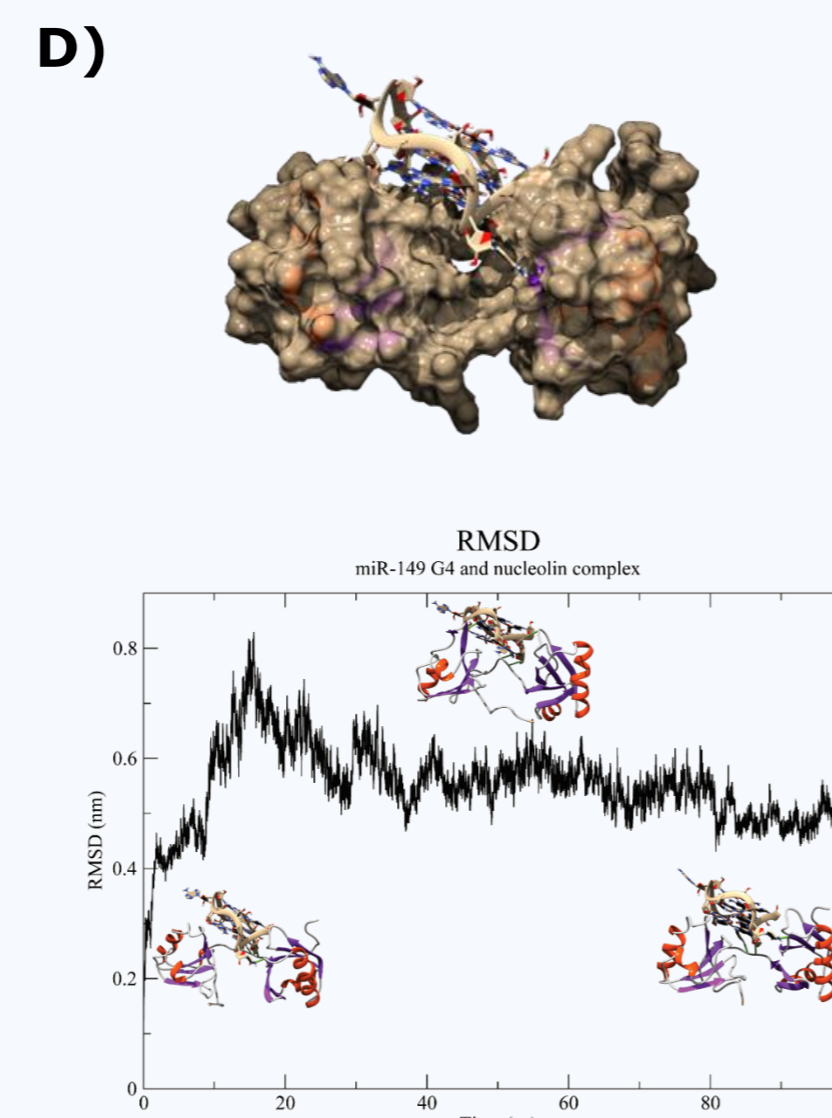


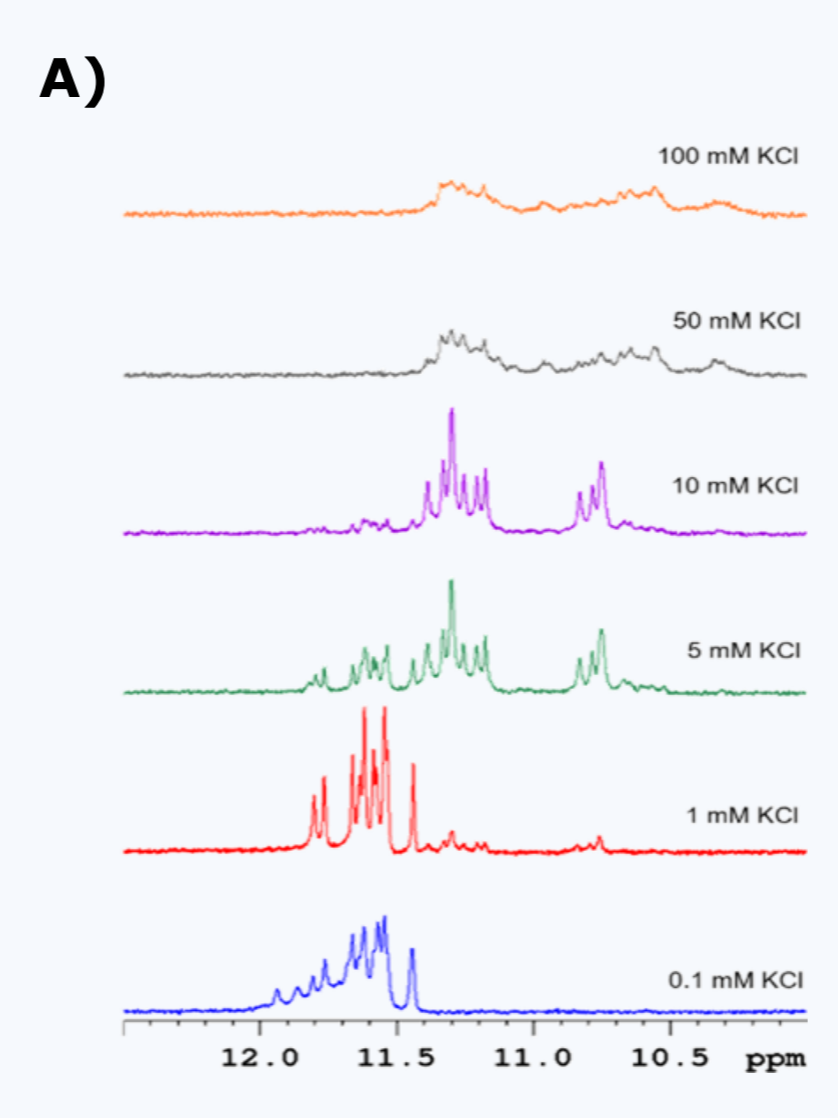
Table 1. CD thermal stabilization of rG4 in the presence of C₈

Ligand	RNA G4
C ₈	>30 °C

Figure 1. A) NMR spectroscopy of rG4 at different temperatures; B) 2D NMR spectrum showing imino region; C) Stabilization of the rG4 in the presence of C₈ and D) Prediction of the interaction of the rG4 with NCL RBD1,2.

Formation and stabilization of rG4 in pre-miR-92b

NMR spectroscopy



B)

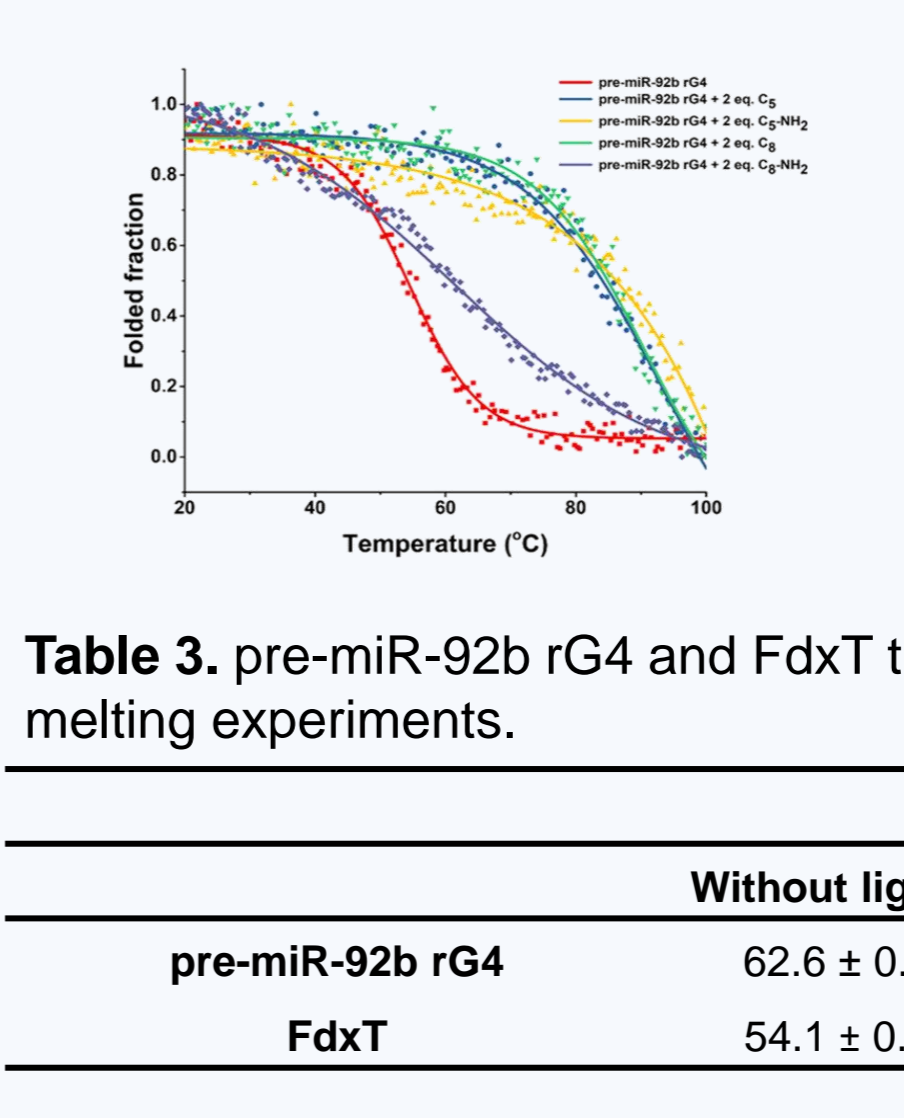


Table 2. CD thermal stabilization of rG4 in the presence of C₈

Ligand	RNA G4
C ₈	>30 °C

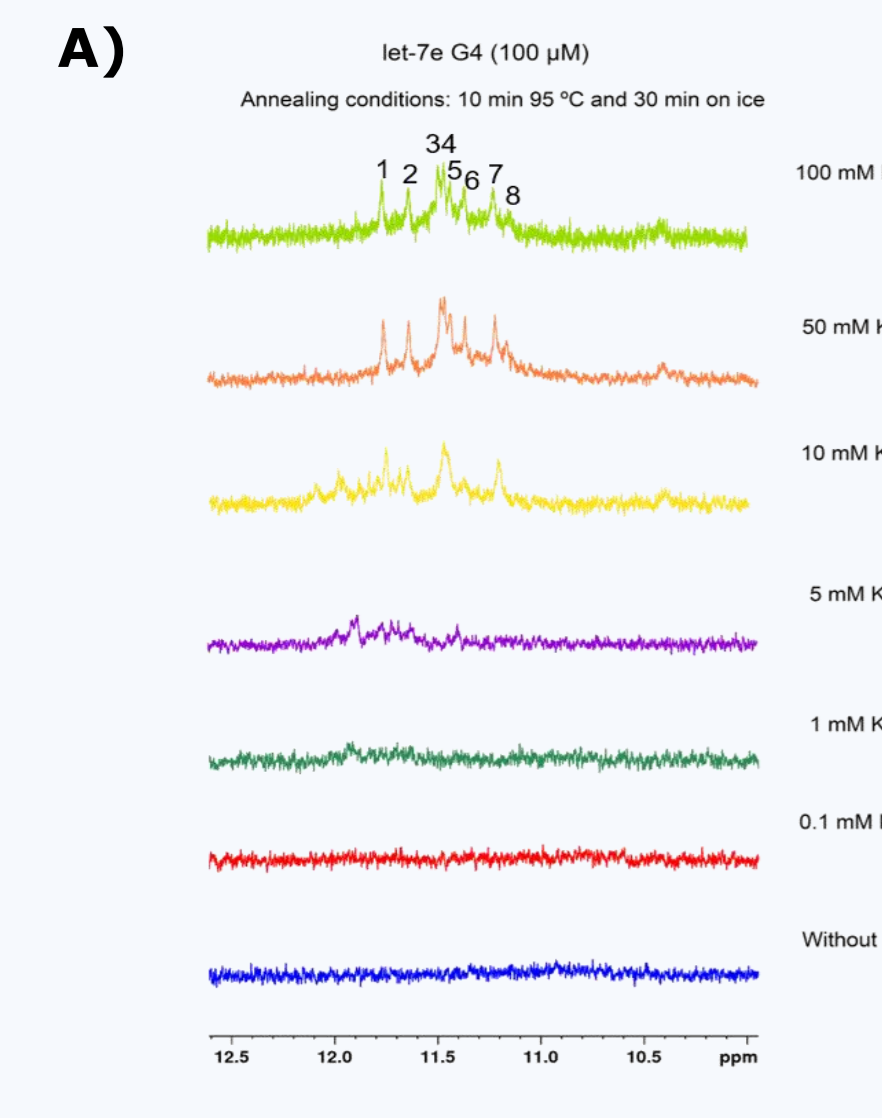
Table 3. pre-miR-92b rG4 and FdxT thermal stabilization (T_m) measured by FRET melting experiments.

	T _m (°C)			
	Without ligand	C ₈	NCL RBD1,2	C ₈ /NCL RBD1,2
pre-miR-92b rG4	62.6 ± 0.2	68.9 ± 0.2	71.0 ± 0.3	84.6 ± 0.5
FdxT	54.1 ± 0.2	54.1 ± 0.1	61.5 ± 0.3	60.2 ± 0.5

Figure 2. A) NMR spectroscopy of rG4 with increasing concentration of KCl; B) CD melting experiments with acridine derivatives; C) Stabilization of the rG4 in the presence of C₈ and NCL.

Formation and stabilization of rG4 in pre-let-7e

NMR spectroscopy



B)

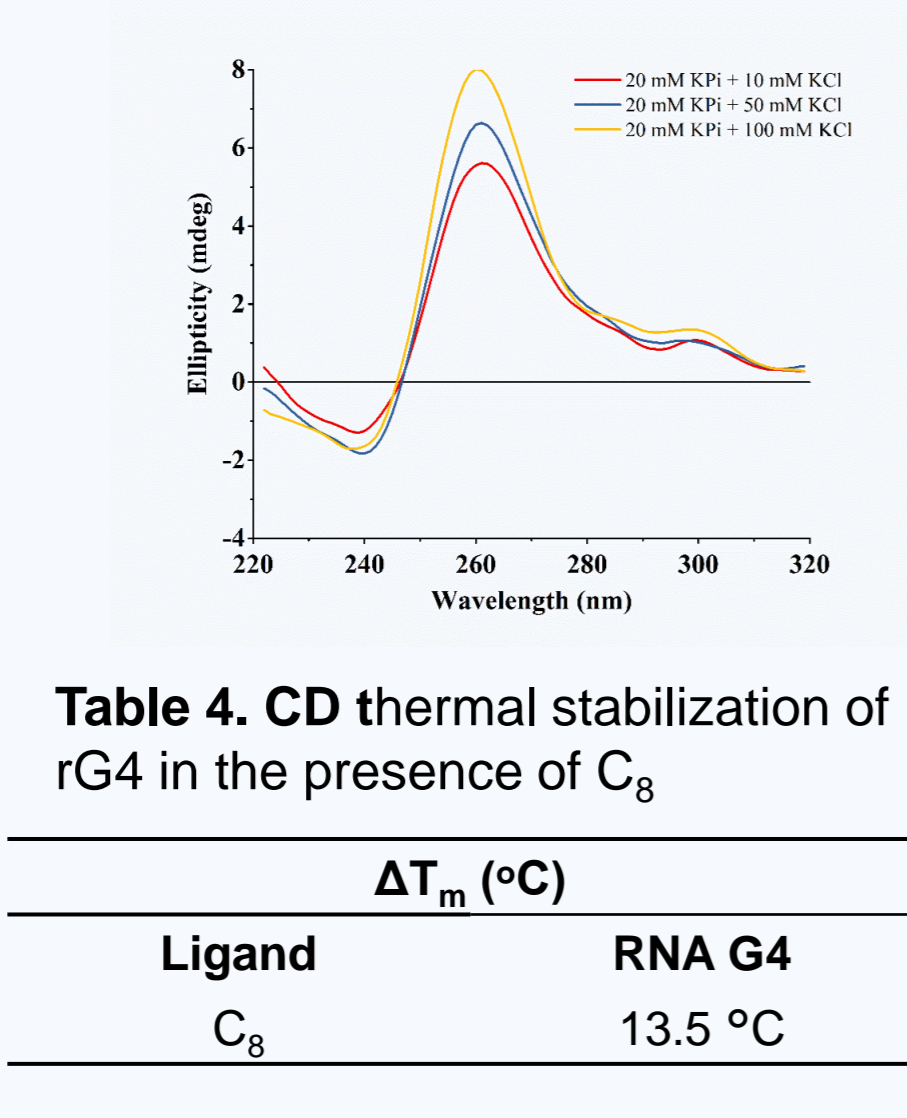


Table 4. CD thermal stabilization of rG4 in the presence of C₈

Ligand	RNA G4
C ₈	13.5 °C

PAGE

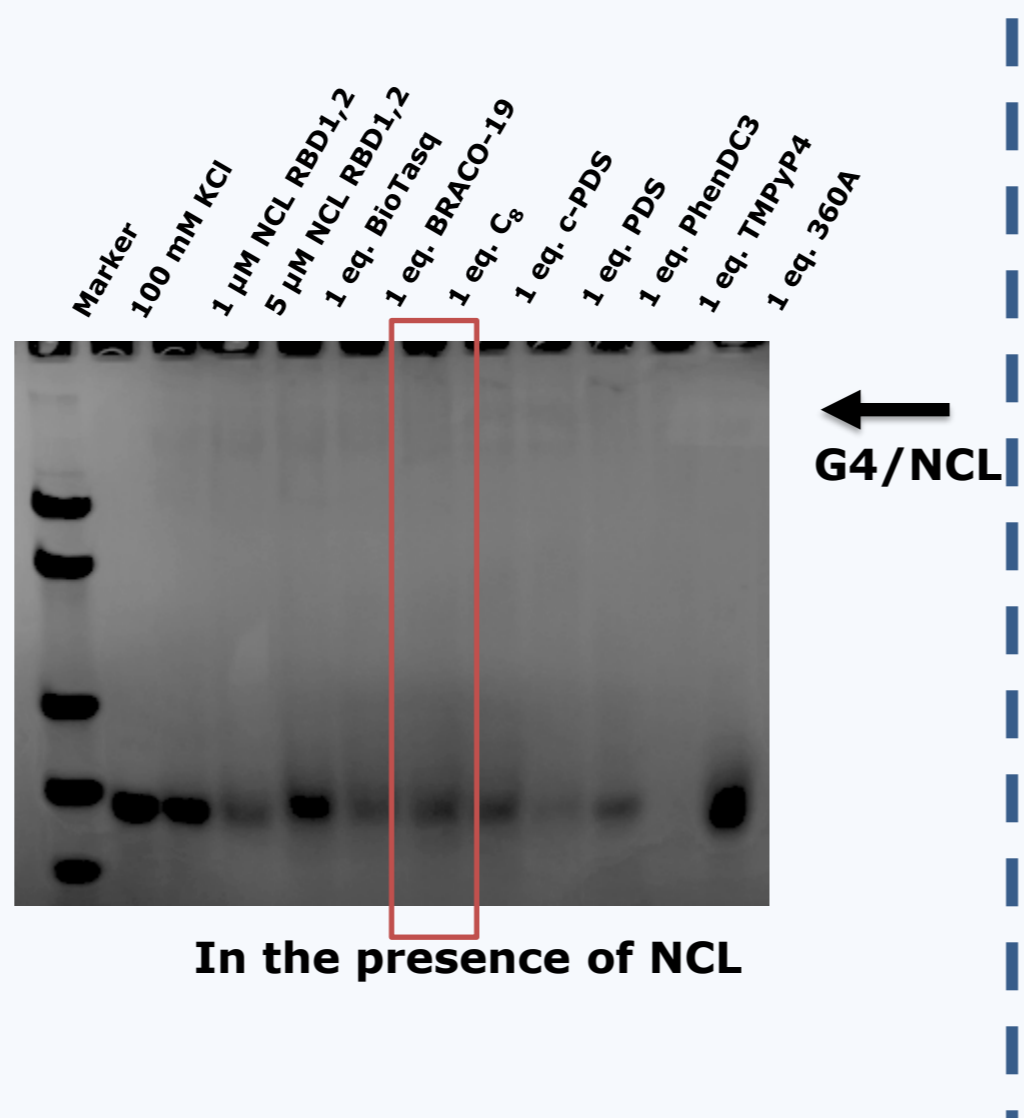


Figure 3. A) NMR spectroscopy of rG4 with increasing concentration of KCl; B) CD spectroscopy of rG4 in presence of KCl and C) PAGE experiments

RNA G-quadruplex in pre-miR-149 as supramolecular carrier for cancer-selective delivery

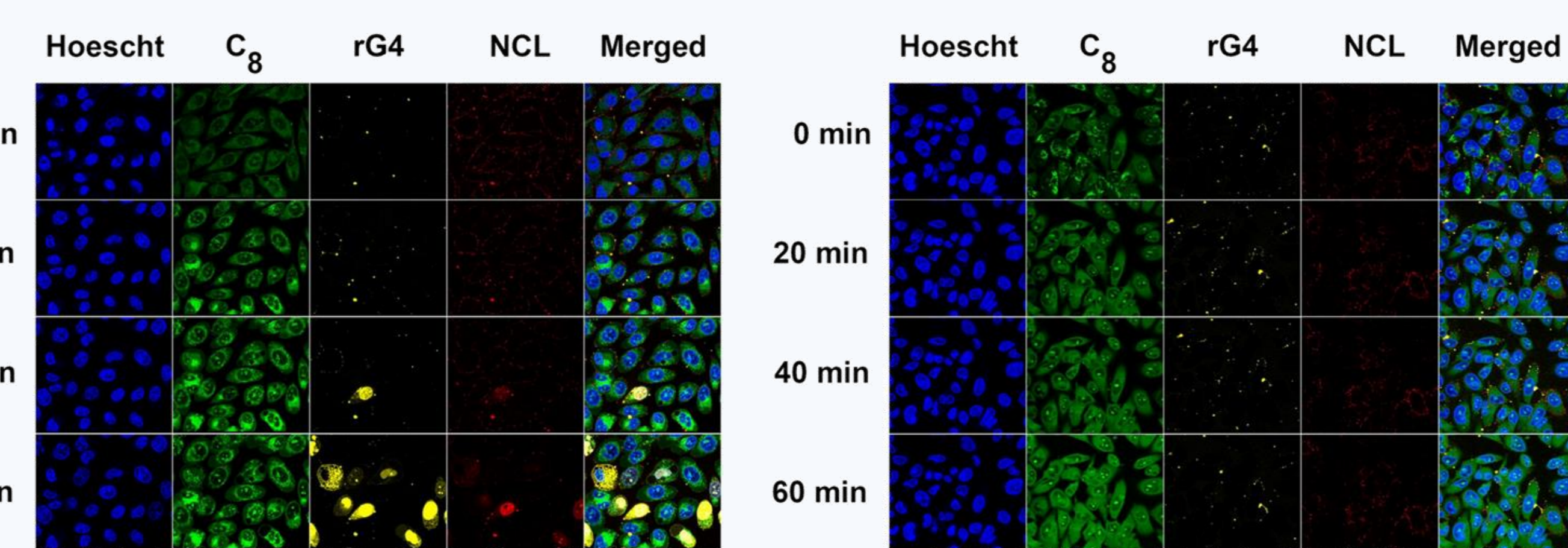


Figure 4. Confocal microscopy showing internalization of rG4/C₈ complex in A) prostate cancer cells and B) healthy cells.

CONCLUSIONS

- ✓ The formation and stabilization of the G4 in pre-miR-149, -92b and -let-7e.
- ✓ The strong ability of C₈ to stabilize G4 in pre-miR-149 and -92b.
- ✓ We proved the internalization of the supramolecular complex rG4/C₈ by prostate cancer cells but not by the healthy cells. We also proved the binding of the complex to NCL in the surface of prostate cancer cells.

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