High-Throughput Synchrotron Radiation Circular Dichroism (HT-SRCD) for the screening of G-quadruplex ligands

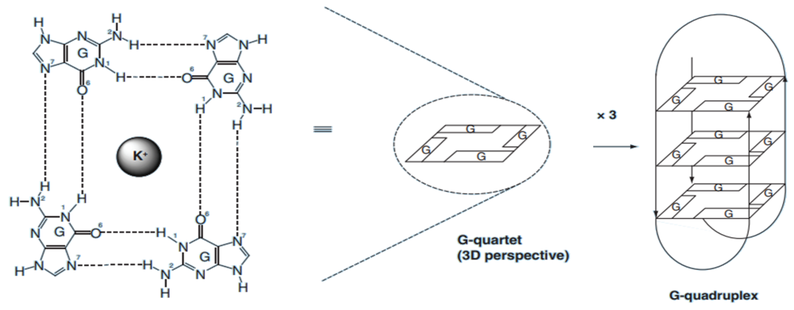
Martina Rotondo[1], Claudia Honisch[2], Barbara Biondi[2], Pietro Spanu[3], Fausta Ulgheri[3], Giovanni Loriga[3], Andrea Beccu[3], Rohanah Hussain[4], Giuliano Siligardi[4], Paolo Ruzza[2]

[1]*Università Degli Studi di Napoli Federico II, Napoli, Italy,*
[2]*Institute of Biomolecular Chemistry of CNR, Padova Unit, Padova, Italy,*
[3]*Institute of Biomolecular Chemistry of CNR, Sassari Unit, Sassari, Italy,*
[4]*Diamond Light Source, Harwell Science and Innovation Campus, Didcot, United Kingdom*

https://doi.org/10.17952/37EPS.2024.P1293

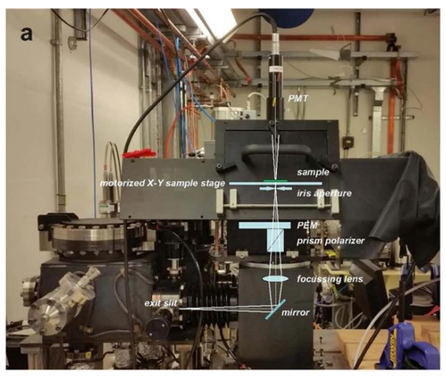
G-quadruplexes (G4) are stable DNA/RNA structures formed by stacking guanine tetrads, supported by Hoogsteen hydrogen bonds and metal cations like K^+ and Na^+. Found in proto-oncogenes, telomeres, and various organisms, G4s are crucial in gene expression. Research aims at finding specific ligands, including peptides, for therapeutic use.

CD spectroscopy and HT-SRCD at Diamond Light Source was used to study G4 topologies and ligand binding. The stability and binding of three G4-forming strands were examined in **10 mM TRIS HCl buffer, pH 7.4, in the presence of either sodium or potassium ions**.

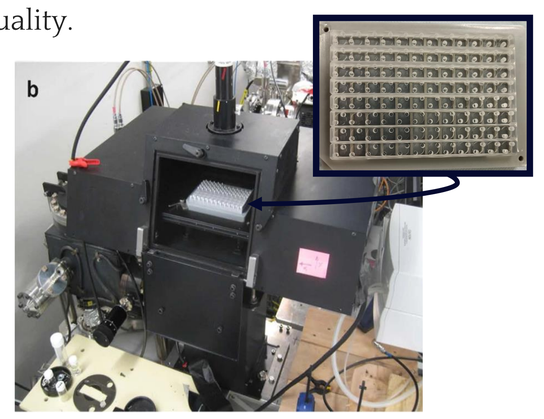


SYNCHROTRON RADIATION

The use of **synchrotron radiation** (SR) of third-generation synchrotrons as a light source has overcome the limitation of the lower photon flux of benchtop CD instruments extending the range of analysis in the vacuum-UV region. The highly collimated SR micro-beam allows measurement of small volume cells of appropriate pathlength whereas the high photon flux improves signal-to-noise ratio, allowing to record just one or few repeated scan per sample with optimal signal quality.



The flexible sample compartment enables various experimental setups, such as a vertical sample compartment designed for multi-well plates used in **high-throughput analysis**. These plates are placed on a motorized X-Y stage for precise and automated movement. Monochromatic light is directed upwards by a plane mirror inside the chamber, which then reflects the light to a photo elastic modulator that creates circularly polarized light before it passes through the sample. **Using multi-well plates allows simultaneous analysis of many samples, significantly accelerating data collection**.

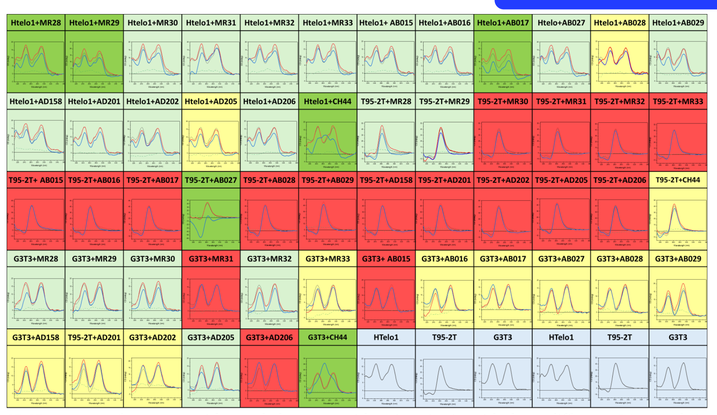


HT-SRCD ANALYSIS

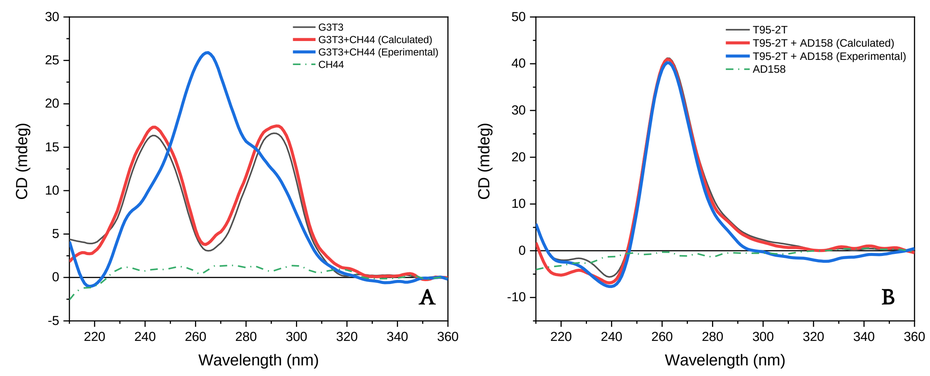
Htelo1
5'-TAGGGTTAGGGTTAGGGTTAGGG-3'
T95-2T
5'-TTGGGTGGGTGGGTGGGT-3'
G3T3
5'-GGGTTTGGGTTTGGGTTTGGG-3'

The binding properties **of seven peptides** and **eleven peptidomimetics** to three selected G4 strands were screened in **two different buffers**, testing 18 different ligands for each G4 strand. **This approach allowed the collection of 360 spectra in just two days.**

EXAMPLE 60-WELL PLATES



SRCD spectra of seven peptides and eleven peptide mimetics in with three different G4 strands in TRIS buffer, pH 7.4 in presence on Na^+ ion (70mM) 60-well plate measured with the vertical chamber for HT-SRCD. The green boxes highlights the spectra in which there is a greater signal variation, the yellow boxes highlights the spectra in which there is a smaller conformational variation, the red boxes highlights the spectra in which there isn't any modification, and the blue boxes highlights the spectra of the G4 alone.

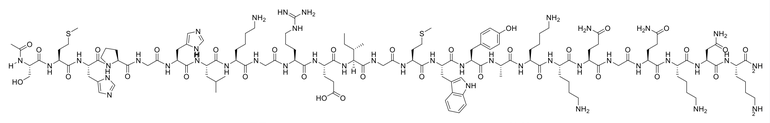


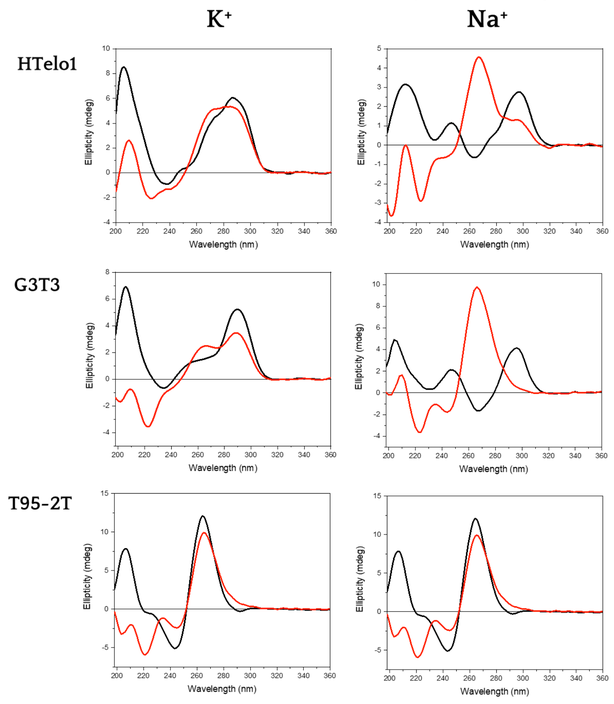
*The spectra show the spectrum of the G4 alone (in black), and an experimental spectrum (in blue) and a calculated spectrum (in red) that are **A)** significantly different and **B)** almost completely overlapping. The dashed line represents the spectrum of the ligand alone*

FURTHMORE ANALYSIS WITH CH44 LIGAND

CIRCULAR DICHROISM

The ligand CH44 is the peptide RAU25, a fragment of the N-terminal domain of the RHAU protein, a RNA helicase.



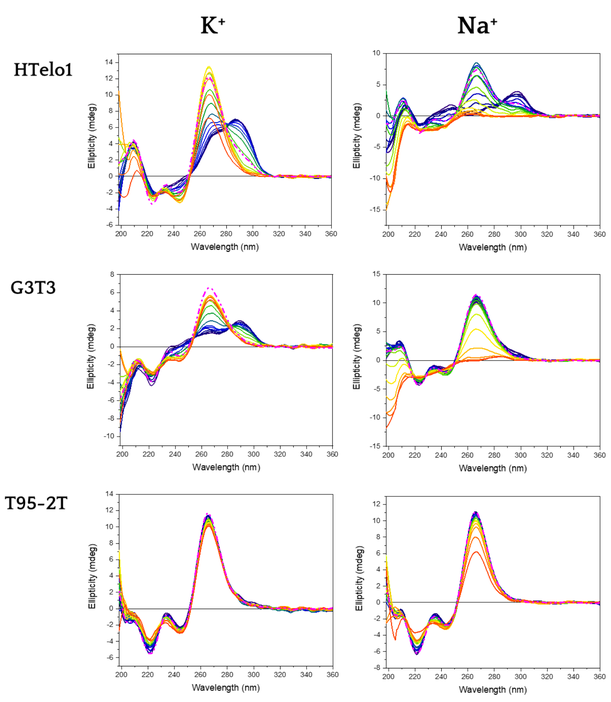


CD spectra of the three different G4 strands (13,5μM) alone (black lines) and in the presence of the RAU-25 peptide (2eq) (red lines) inTRIS buffer, pH 7.4 in presence of K^+ ion (left) or Na^+ ion (right) (70mM)

MELTING ANALYSIS

Sample	Melting Temperature T_m (°C) KCl	NaCl
G3T3 alone	69 ± 0.3	51 ± 0.4
G3T3 + peptide	n.d. *	69 ± 0.3
Htelo1 alone	66 ± 0.3	53 ± 0.3
Htelo1 + peptide	n.d. *	59 ± 0.4 *,#
T95-2T alone	>90	61 ± 0.2
T95-2T + peptide	>90	>90

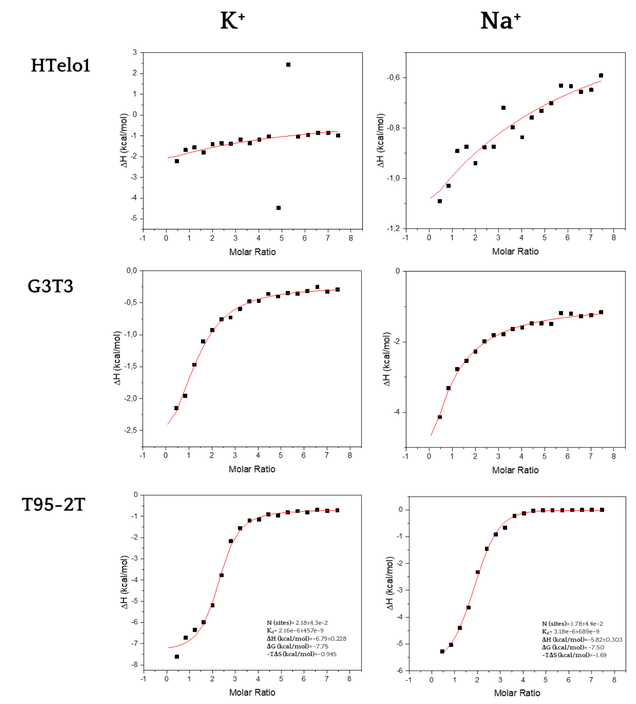
n.d. not determinable because of high stability of the structure. * observed conformational conversion. # determined after 5 h equilibration time.



CD Spectra of G4 forming sequences (13.5 μM) in 10 mM Tris-HCl buffer, pH 7.4, containing 70 mM potassium ions (left) or sodium ions (right) during the annealing process 10°C to 90°C every 5°C. The dashed line represents the oligonucleotide cooled to 20 C after heating at 90°C.

CALORIMETRY

The binding constant and thermodynamic values for the T95-2T-RAU25 complex were calculated. Other ligand-G4 couples with significant changes in CD spectra produce ITC data under the same conditions with large errors, making the measurements unreliable.



ITC curves of G4 forming sequences (25 μM) in 10 mM Tris-HCl buffer, pH 7.4, containing 70 mM potassium ions (left) or sodium ions (right) in presence of increasing amount of RAU-25 peptide (25mM).

CONCLUSION

In this study, we used HTCD by means of synchrotron light as a rapid system for investigating the binding ability of a wide panel of ligands with three different G4s in two different environments (presence of Na+ or K+), allowing us to quickly assess the potential presence of interactions. Subsequent studies with the chosen ligand allowed us to confirm the interactions identified by HTCD. Obviously, these interaction studies will be continued for the other promising ligands found during the screening analyses.

REFERENCES

[1] Del Villar-Guerra R, Trent JO, Chaires JB. *Angew Chem Int Edit.* **2018**;57(24):7171-5.
[2] Ji D, Juhas M, Tsang CM, Kwok CK, Li Y, Zhang Y. *Brief Bioinform.* **2021**;22(2):1150-60.
[3] Honisch C, Ragazzi E, Hussain R, Brazier J, Siligardi G, Ruzza P. *Pharmaceutics.* **2021**; 13(8):1104.

AKNOLEDGEMENT

This work was carried out with the support of Diamond Light Source, Beamline B23 (proposal SM34241-1) , EU funding within the MUR PNRR "National Center for Gene Therapy and Drugs based on RNA Technology" (Project no. CN00000041 CN3 RNA) and MUR PRIN 2020 progetto AEX4TA Natural Products-Assisted Organic Synthesis.