

## Quadruplex formation between a triazole-acridine conjugate and guanine-containing repeat DNA sequences. Atomic force microscopy and voltammetric characterisation

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The telomeres are responsible for the protection of the chromosomes ends, being involved in more than 80% of all cancers. One of the key steps in human carcinogenesis is the activation of the telomeres maintenance system that allows the continued proliferation of cancer cells. G-quadruplexes (GQs) are four-stranded higher-order structures formed by folding of a single (intra-molecular) or by the intermolecular association of two, three or four separate guanine rich DNA strands, stabilised by the presence of monovalent cations, notably sodium and potassium. The occurrence of GQ sequences in telomeres, promoter regions and other genomic locations was determined by the direct visualisation of GQ formation in cell nuclei, in the cytoplasm and at telomeres, which revealed the crucial role of these structures as targets for anticancer drugs.

A large number of potent GQ-binding ligands which stabilize or promote GQ formation have been described in the literature. The GQ ligands in telomeres prevent GQ from unwinding and opening the telomeric ends to telomerase, thus indirectly targeting the telomerase enzyme complex and inhibiting its catalytic activity. Acridines are heterocyclic compounds some of which have been used as chemotherapeutic agents in human cancer. A number of acridine derivatives have been specifically synthesized with the purpose of increasing binding affinity and selectivity for human telomeric DNA GQs. In particular the GQ-targeting acridine derivatives BRACO-19 and RHPS4 have been important tools for studying the antitumor activity of this general class of agents. However, they are relatively non GQ-selective, having also significant binding affinity for duplex DNA. More recently, a series of triazole-linked acridine ligands, e.g. GL15, with enhanced selectivity for human telomeric GQs binding versus duplex DNA binding have been designed, synthesized and evaluated.

The *Tetrahymena* telomeric repeat sequence d(TG<sub>4</sub>T) forms parallel-stranded tetra-molecular GQs in the presence of Na<sup>+</sup> and K<sup>+</sup> ions [1] and is considered to be a simple model for biologically relevant GQs. It has also provided high resolution structural data on drug-DNA interactions. Synthetic polynucleotides poly(dG) and poly(G) [2] are also widely used as models to determine the interaction of drugs with G-rich segments of DNA. In this context, the interactions of the short-length sequence d(TG<sub>4</sub>T) and long poly(G) sequence with the triazole-acridine conjugate GL15, were investigated at the single-molecule level, using a novel approach, based on the combination of two powerful analytical techniques, atomic force microscopy (AFM) and voltammetry [3].

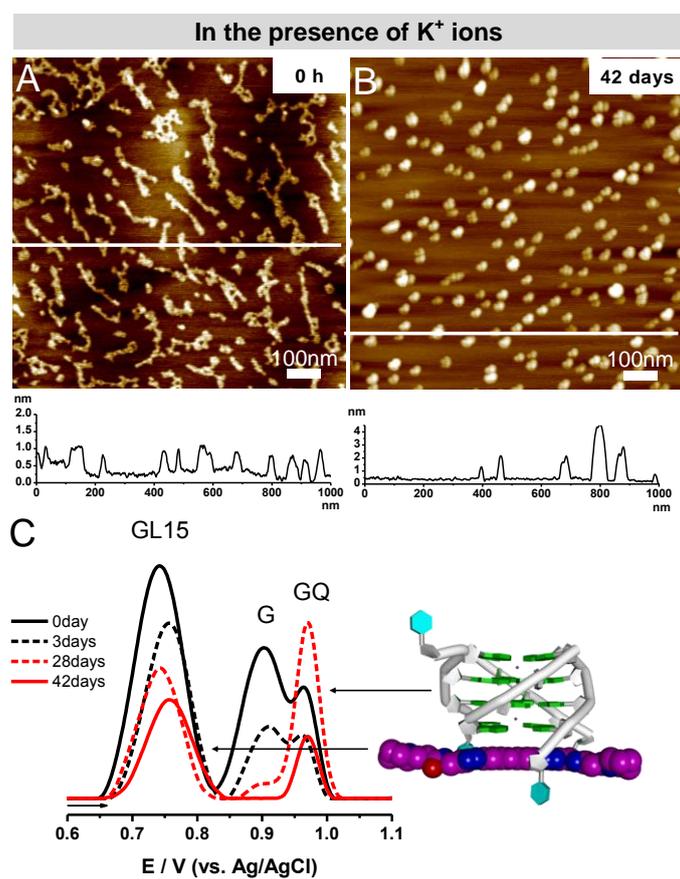
The interaction of GL15 with d(TG<sub>4</sub>T) and poly(G) was evaluated based on changes in structure and redox behaviour, enhanced by the presence of Na<sup>+</sup> or K<sup>+</sup> ions. GL15 interacted with both sequences, in a time dependent manner and GQ formation was detected. AFM showed the adsorption of GQs as small d(TG<sub>4</sub>T) and poly(G) spherical aggregates and large GQ-based poly(G) assemblies, and voltammetry showed the decrease and disappearance of GL15 and guanine oxidation peak currents, and appearance of the G-quadruplex oxidation peak (Fig. 1).

The GL15 molecule strongly stabilized and accelerated GQ formation in both Na<sup>+</sup> and K<sup>+</sup> ion-containing solution, although only K<sup>+</sup> promoted the formation of perfectly aligned tetra-molecular GQs. The small-molecule complex with the d(TG<sub>4</sub>T) GQ is discrete and approximately globular, whereas the GQ complex with poly(G) is formed at a number of points along the length of the polynucleotide, analogous to beads on a string. An excellent correlation was observed between the d(TG<sub>4</sub>T) and poly(G) structural changes and redox behaviour, before and after interaction with GL15, and was directly influenced by the presence of monovalent Na<sup>+</sup> or K<sup>+</sup> ions in solution.

## References

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[3] A. M. Chiorcea Paquim, A. D. R. Pontinha, R. Eritja, G. Lucarelli, S. Sparapani, S. Neidle, A. M. Oliveira Brett, *Anal. Chem.* **87** (2015) 6141.

## Figures



**Figure 1** – GL15–d(TG<sub>4</sub>T) after different incubation times in the presence of  $K^+$  ions: (A, B) AFM images and cross-section profiles through the white dotted lines and (C) differential pulse voltammograms baseline corrected.