A Telomeric RNA Quadruplex Crystal Structure

Application Note

X-ray Crystallography

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Background

The Biomolecular Structure Group at the School of Pharmacy (SOP) has relied heavily upon structural biology and crystallographic techniques to lead its drug discovery programme. Important aspects of this programme include the validation of macromolecular targets and the study of quadruplex-ligand interactions. We have now expanded our crystallographic facilities at the SOP by purchasing a Xcalibur Nova system with a Titan detector. The move from a rotating anode/image plate system to a high intensity micro-focus source, coupled to a CCD detector, has enhanced the quality and resolution of data collected in-house.

Here we present the preliminary results of a collection on an unusual macromolecular RNA G-quadruplex structure. The single crystal data was collected to 2.2Å resolution, providing us with a detailed view of the RNA's sugar pucker and hydration structure. The efficient data collection geometry and associated high redundancy of data has enabled us to also visualize the anomalous scattering from the ions in the central channel, and confirm the location of the bromine atoms included to assist in phasing.

Experimental

Guanine-rich RNA quadruplex crystals were grown by the hanging drop vapour diffusion method, using a ribonucleotide sequence containing a bromo-uracil at the 5′ end of the oligonucleotide (Fig. 1).

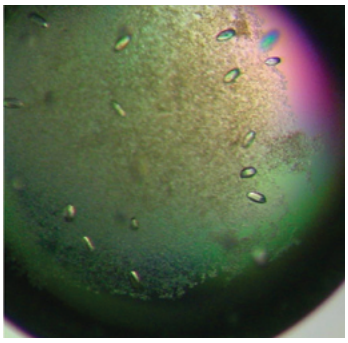

Figure 1. Guanine-rich RNA quadruplex crystals

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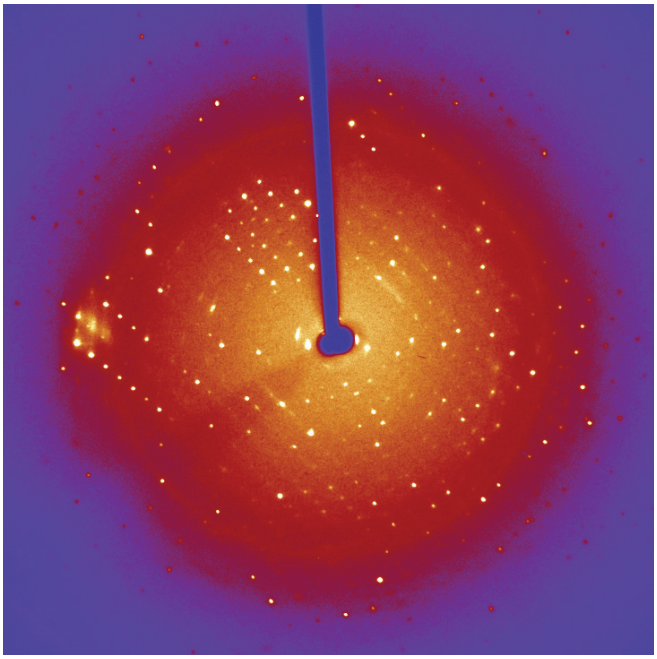

Figure 2. Diffraction image of RNA G-quadruplex crystal

The dataset was collected on a single crystal using an Agilent Technologies (formerly Oxford Diffraction) Nova X-ray source (Cu micro-focus), with a Titan CCD detector. 110 frames of data were collected in 1.0° slices, between omega angles -75.00° and 35.00°. The maximum exposure time was 5 minutes per frame (Fig. 2), with a total collection time of 22 hours. The kappa, phi and detector angles remained constant during data collection (κ = -77.00°, ϕ = -30.00°, d = 3.462°). Agilent's CrysalisPro software was used for data processing. Summary statistics are shown in Table 1. The structure was solved by simple molecular replacement using a DNA model (CCP4[1], refmac5[2]). Coot[3] and refmac5 were used for map fitting and refinement (Fig. 3).

Data collection	
Space group	$P3_121$
Unit cell dimensions:	
a, b, c (Å)	57.583, 57.583, 38.377
α, β, γ (o°)	90.00, 90.00, 120.00
Resolution (Å)	12 - 2.2
R int (%) overall	5 (24)
I / σ	43.38 (4.54) 2.2 Å
Completeness (%)	99.6 (97.3)
Redundancy	6.3 (6.0)
Total reflections	24,789 (2366)
Unique reflections	3952 (397)
*Values in brackets refer to highest resolution shell, 2.28-2.20 Å	

Table 1. Data collection statistics

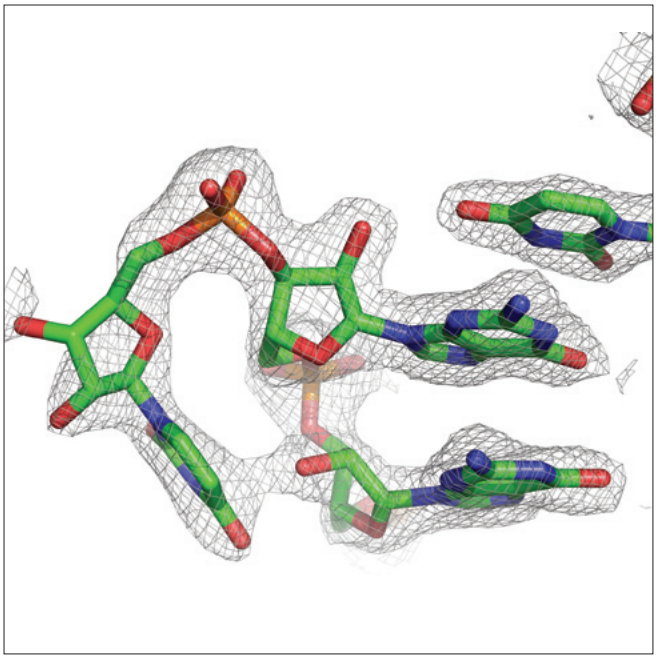

Figure 3. C2′ Hydroxyl group of residue G5 fitted into 2Fo-Fc map (sigma level, 1.0)

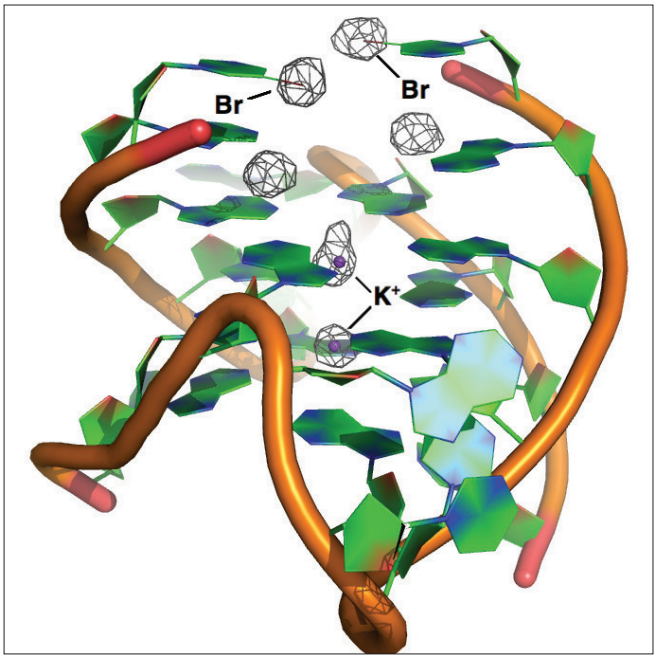

Figure 4. Anomalous scattering from Bromine atoms and Potassium ions. Bromine atoms are attached to residues U1 and U13. Phased anomalous difference map is contoured to 5.0 σ.

Results

In order to further develop DNA quadruplex-targeted ligands, it is important to identify and understand the differences between RNA and DNA quadruplexes. In a step towards this, the structure of a bimolecular RNA quadruplex was solved, using an in-house Agilent diffractometer. The model obtained provides important structural information such as hydroxyl group positioning, allowing these features to be exploited (or avoided) for improved quadruplex-targeted ligand design.

To determine the possibility of observing anomalous scattering from the bromines and potassium ions, the data was remerged, separating the Friedel pairs. The reprocessed data has an overall redundancy of 3.4 (3.2) with 99.5 (95.2) % completeness and an R_{merge} of 3.8 (21.3) % (values in brackets are for the highest resolution shell). Using phasing from the refined model, the anomalous difference Fourier map clearly confirms scattering from the potassium ions (1.07 e) and bromines (1.29 e) (Fig. 4).

References

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