Acta Cryst. (2002). A**58** (Supplement), C56

DAUNOMYCIN/d(TGGGGT): THE FIRST CRYSTAL STRUCTURE OF A DRUG BOUND TO A G4-QUADRUPLEX DNA TELOMERE

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Telomeres occur as multiple repeats of guanine-rich segments at the 3' ends of chromosomes. Most of the telomeric region is double-helical, but a single-stranded overhang protrudes at the extreme 3' terminus, and the guanines in this region can associate strongly to form stacked G4 quadruplexes. There is intense current interest in designing intercalating drug molecules to act as inhibitors of the enzyme telomerase which protects tumor cells against telomere loss during replication. Despite this interest, little is presently known about quadruplex assemblies in vivo, and the only crystal structures which have been reported are for the uncomplexed G4-quadruplexes d(GGGGTTTTGGGG) and d(TGGGGT). We report the first structure determination of a crystal containing a drug molecule bound to a G4 quadruplex - the anti-cancer agent Daunomycin complexed with the telomeric sequence d(TGGGGT). The crystal is monoclinic, space group *C*2, with a = 53.078 Å, b = 47.239 Å, c = 31.914 Å, β = 119.80°. Intensity data were collected at SSRL to 1.17 Å resolution. The asymmetric unit contains four d(TGGGGT) strands in a G4 quadruplex stack, three daunomycin molecules, and three Na cations. The daunomycins are mutually coplanar, but they do not intercalate into the guanine core of the quadruplex. Instead, they make weak π - π stacking interactions with guanines at one end of the quadruplex and stronger π - π interactions with symmetry-related daunomycins from neighbouring asymmetric units. The aliphatic moieties of the daunomycins project into three of the four grooves spiraling around the G4 stack. The overall crystal architecture is highly asymmetric.

Keywords: TELOMERE DAUNOMYCIN DNA CRYSTAL STRUCTURE

Acta Cryst. (2002). A**58** (Supplement), C56

X-RAY CRYSTAL STRUCTURES OF ANTIBIOTIC-RNA COMPLEXES

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Many different antibiotic families are known to target the ribosome and interfere with the protein synthesis. Among them, aminoglycoside antibiotics are bactericidal compounds that interact with the aminoacyl site (A site) on the ribosomal 16S RNA and induce miscoding during translation. X-ray crystallography enabled us to solve at 2.5-2.7Å the structures of complexes between 44-nucleotide long RNA fragments containing the same A-site sequence and three structurally related aminoglycosides: paromomycin, tobramycin and geneticin.
In each structure, the antibiotic interacts in a pocket formed by 4 base pairs, 1 unpaired and 2-bulged adenines. The equivalent number of direct and water-mediated hydrogen bonds explains the micromolar affinity exhibited by these antibiotics towards the A site. Furthermore, the analysis of the contacts provides an explanation for resistance mechanisms that have spread among bacteria to make aminoglycosides inefficient molecules. Inactivating enzymes chemically modify functional groups of the antibiotic or of the A-site nucleotides that are involved in direct hydrogen bonds.

Keywords: RNA CRYSTAL STRUCTURE ANTIBIOTIC RESISTANCE

Acta Cryst. (2002). A**58** (Supplement), C56

AUTOMATED CRYSTAL MOUNTING AND ALIGNMENT SYSTEM AT THE ADVANCED LIGHT SOURCE

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In an effort to realize high-throughput data collection and x-ray structure determination, we have developed and installed an automated cryogenic sample alignment and mounting system on beamline 5.0.3 at the advanced light source. This system has been tested and refined since march, 2001, with reliable and flexible operation demonstrated. In february 2002 another system was been installed in beamline 5.0.2 that is currently in use as well. Rapid mounting and unmounting of the samples increases the efficiency of the crystal screening and data collection process, where many crystals have to be tested for the quality of diffraction as rapidly as possible. The robot has random access to 64 samples (112 in the near future), stored in liquid nitrogen. Mounting of a crystal takes approximately 10 seconds, during which the crystal temperature is maintained below 110 K. Centering of a crystal can be done by the user through the remote controlled xyz goniometer head or automatically by a centering algorithm. To further increase throughput, we have also developed a sample transport/storage system based on 'puck-shaped' cassettes, which can hold 16 samples each. Seven cassettes fit into a standard dry shipping dewar. The development of software tools for controlling automated data collection has been developed and we are currently building into the software the capability to screen and optimize data collection. This system is coupled to a database for tracking of samples, data collected, and project status. Funding by NIH/NIGMS.

Keywords: AUTOMATION BEAMLINE INSTRUMENTATION ROBOTICS

Acta Cryst. (2002). A**58** (Supplement), C56

THE ARP/wARP SUITE FOR THE AUTOMATION OF CRYSTAL STRUCTURE DETERMINATION

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Once the crystallographic phase problem has been solved, a macromolecular crystallographer is faced with the problem of interpreting a map that is not self-explanatory. A reduction of this 3D continuous electron density distribution to a desired set of atoms with type assignments and bonds that constitute a model is a cumbersome task requiring much expertise and time.
The first papers on automatic map interpretation and pattern recognition in a crystallographic context appeared in the early 1970's. Greer's skeletonisation approach was highly successful and is still the most common aid for tracing the main chain in a given electron density map. Recent developments have been mainly related to the core-tracing algorithm and the extensive use of databases also in combination with topological approaches and artificial intelligence techniques. A general framework for knowledge-based structure solution and map interpretation has been formulated based on a Bayesian molecular replacement approach. A similar scheme has been implemented by a template-convolution approach. Progress has been made recently with the development of the data-base assisted template matching, the neural network approach to pattern recognition, automated fragment searching and conformation matching. Still, the current state of automation is still rather modest, relying on the user to actually make all the relevant decisions. ARP/wARP offers capabilities that come close to full automation that allow an essentially complete macromolecular atomic model to be constructed. Newest version of the ARP/wARP suite will be presented and an analysis given of the required quality of initial phases and the resolution of the X-ray data.

Keywords: PROTEIN CRYSTALLOGRAPHY, DENSITY INTERPRETATION, STRUCTURE REFINEMENT