located at the interface between A-band and M-line. It has been shown by Centner et al. [2] that MURF-1, a member of the RING finger proteins, binds to the two Ig-domains A168 and A169 in proximity to the kinase. Thus, its binding might be involved in the regulation of titin kinase. The structure of this tandem Ig domain has been solved. Ig domains, also in titin, are involved in many protein-protein interactions and this interconnects titin with other muscle proteins and pathways. Here, we will present new structures near titin kinase and from a downstream signaling pathway of titin kinase (Gautel et al., unpublished data).

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Keywords: muscle proteins, immunoglobulin structure, protein interaction

P.04.04.3

Acta Cryst. (2005). A**61**, C218

Carving a Beanstalk: the Structure of ΔS2 from Human Myosin II

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S2 is the flexible coiled coil that connects light meromyosin to the N-terminal motor domain of myosin II. S2 interacts with other proteins of thick filament and can lead to fatal familial hypertrophy (FFH) when mutated. We have determined the structure of a 126-residue N-terminal fragment of S2 in two different crystal forms. The WT protein diffracted to 2.7 Å resolution in a $C222_1$ cell of a=40, b=46, c=373 Å. Cryo-protection was difficult and data could only be reduced in XDS. Phases were derived from 2-λ MAD data collected from a mercury derivative. Only SHELXD with SHARP generated interpretable electron density maps. The protein is a parallel dimeric coiled coil of 187 Å lying stretched out along the c-axis.

The FFH-associated E924K-mutant crystallised in P1 with a=40, b=42, c=98 Å; α=91, β=93, c=107°. Molecular replacement was not successful and crystals were highly radiation sensitive, giving non-traceable electron density maps when anomalous phasing from SeMet-labelled or heavy-atom-soaked crystals was employed. It was, however, possible to locate 4 mercury atoms from anomalous data. These co-ordinates together with the position of cysteine residues in the WT structure were used in a semi-brute-force approach to derive the relative orientation of two coiled coils in the asymmetric unit. The model was refined to 2.5 Å with R=27.3 and R_{free}=34.9 %. The two extended coiled coils run anti-parallel with neighbouring molecules lying head-to-tail such that they form quasi-endless filaments.

Keywords: coiled coil proteins, MAD, brute force

P.04.05.1

Acta Cryst. (2005). A**61**, C218

Preliminary X-ray Analysis of RNA Oligomers Containing CUG Repeats

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Human genome contains so many different types of repetitive sequences. Some of them are tandem repeats of trinucleotides, and their unusual expansions cause genetic diseases including type 1 myotonic dystrophy (DM1) and Huntington's disease (HD). The unit sequence for DM1 is CTG in the 3'-untranslated region of the myotonic dystrophy protein kinase (DMPK) gene, and that for HD is CAG in the ORF of exon-1 of the HD gene.

The two complementary sequences may induce increase or decrease of the repeats during DNA replication or repair of DNA. The direct origin of DM1 is, however, the transcribed RNA fragments with CUG repeats, which forms a specific structure and inhibits other protein syntheses. In the present study, structural versatility of such DNA and RNA fragments has been examined.

In the case of $(CUG)_n$, native PAGEs show that the even repeat (n=even number) is more stable than the odd repeat. This may be ascribed to the structural difference at the hairpin head. The PAGEs also suggest that duplex formation is dependent on coexisting cationic species and their concentration. Crystal data of $(CUG)_6$ are *a*=*b*=39.6 and *c*=141.0Å, space group *R*32 and one oligomer in the asymmetric unit. An approximate crystal structure has been solved by molecular replacement techniques at 1.9 Å resolution and shows that the fragment forms a duplex similar to an A-form RNA.

Keywords: RNA structure, X-ray analysis, genetic disease

P.04.05.2

Acta Cryst. (2005). A**61**, C218

Crystal Structures of Two Minor Groove Binders Complexed with d(CGCGAATTCGCG)$_2$

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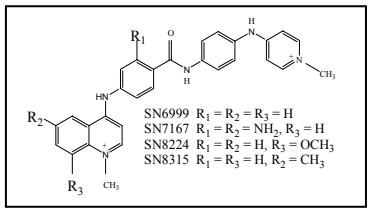
Quinolinium quaternary salts (QQS) are anticancer drugs [1] and bind reversibly in the minor groove of AT rich sites in DNA. The crystal structures of SN6999 [2] and SN7167 [3] complexed with CGC[e^{6}G]AATTCGCG and CGCGAATTCGCG respectively have been solved previously at a resolution of about 2.5 Å. We have recently solved the structures of two new QQS compounds, SN8224 and SN8315, complexed with the dodecamer CGCGAATTCGCG. These two structures are at higher resolution (1.6 and 1.8 Å) and crystallise in similar conditions. We are able to compare the four complex structures and reach conclusions about minor groove requirements for QQS compounds bound to unmodified DNA.



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Keywords: DNA, minor groove binders, anticancer drugs

P.04.05.3

Acta Cryst. (2005). A**61**, C218-C219

A new Highly Symmetric DNA G-4 Quadruplex/ Drug Structure

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Guanine-rich DNA telomeres occur at the 3' ends of chromosomes. They can associate into four-stranded assemblies known as stacked G-4 quadruplexes. It has been found that the enzyme telomerase protects tumour cells but not normal cells from telomere loss during replication. Telomerase has therefore become an exciting new target for anti-cancer drug design. Small molecules which can stabilise the formation of G-4 quadruplexes may inhibit telomerase activity.

We recently determined the crystal structure of the daunomycin complex with the telomeric sequence d(TGGGGT) [1]. We now report the 1.08 Å structure of daunomycin complexed to d(GGGG). The crystals are tetragonal, space group I4, a = b = 40.21, c = 49.83 Å. The final R is 16.1%. The asymmetric unit contains 2 independent strands of d(GGGG), 4 drug molecules, and eight Na and 2 Mg cations. The crystallographic 4-fold axis generates the biological unit which consists of 2 high-symmetry G-4 quadruplexes between which are 4 layers of daunomycin molecules with 4 daunomycins per layer.

[1] Clark G.R., Pytel P.D., Squire C.J, Neidle S., *J. Amer. Chem. Soc.*, 2003, **125(14)**, 4066-4067.

Keywords: G4-quadruplexes, DNA-drug interactions, telomeres

P.04.05.4

Acta Cryst. (2005). A**61**, C219

Structural Studies on Acridine Derivatives Binding to Telomeric DNA

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Acridine derivatives are known to inhibit a variety of nuclear enzymes, such as topoisomerases and telomerases, by binding or intercalating to DNA. This class of compounds is of great interest in the development of novel anticancer agents, some of which are currently under clinical trial [1, 2].

Despite the obvious pharmaceutical interest and recent successes in determining the crystal structure of some of the compounds complexed with DNA [1,2,3], a lot is still unknown about the mechanisms of action, binding preferences and biological targets.

In this study a variety of techniques is employed to investigate the binding behaviour of a selection of drugs to DNA. Fiber diffraction is used to obtain information about sequence preferences and to analyze structural changes in the DNA upon drug binding using a continuous polymer. Data is usually obtained at lower resolution and complements crystal diffraction studies. Crystal diffraction is then used to analyze DNA-drug complexes in oligonucleotides at high resolution. With the information gained, neutron diffraction studies are planned to analyze the hydrogen bonding patterns of the DNA-drug complexes.

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Keywords: DNA-drug complexes, X-ray fiber diffraction, X-ray crystallography

P.04.05.5

Acta Cryst. (2005). A**61**, C219

Crystallographic Studies of *Homo sapiens* A-sites Complexed with Aminoglycosides

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Toxicity resulting from the clinical use of aminoglycoside antibiotic drugs is known to originate from the binding of these drugs to the *Homo sapiens* A-sites. In order to design antibiotics with higher selectivity for bacterial ribosomes and less toxicity to eukaryotes, further structural investigations have been carried out with a number of A-site complexes. In all cases, the structure solution by molecular replacement was not straightforward and required simultaneous applications of several programs and various approaches.

In the case of the cytoplasmic A-site with paromomycin, a $P2_12_12$ crystal with one RNA duplex in the asymmetric unit was obtained. The same solution was found with *AMoRe* using the bulk-solvent correction technique and with *PHASER*. After applying normal-mode refinement for only the central stem region in the 10-5.0 Å resolution range, R_{free} and CC_{free} values are 30.5% and 32.3%, respectively.

The crystal of the mitochondrial A-site with tobramycin (space group *P1*) contains two RNA duplexes in the asymmetric unit. Orientation of the duplexes has been found by combination of information from *PHASER*, the self-rotation function from *GLRF* and other sources. Position of the duplexes was obtained essentially from the packing analysis.

The solution of other similar complexes (1 or 2 strands in the A.U., trigonal unit cells) also required specific approaches.

Keywords: RNA structure, antibiotic binding, crystal structures

P.04.05.6

Acta Cryst. (2005). A**61**, C219

X-ray Analyses of DNA Dodecamers Containing 2'-Deoxy-5-formyluridine

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It is known that formylation of thymine base induces purine transition in DNA replication. In order to establish the structural basis for such mutagenesis, crystal structures of two kinds of DNA dodecamers d(CGCGRATf^{5}UCGCG) with f^{5}U=2'-deoxy-5-formyluridine and R=A or G have been determined. The f^{5}U residues form a Watson-Crick-type pair with A[1,2] and two types of pairs (wobble and reversed wobble) with G[3] (*see* figure), the latter being the first example. Structural modeling suggests that the DNA polymerase can accept the reversed wobble pair with G, as well as the Watson-Crick pair with A.

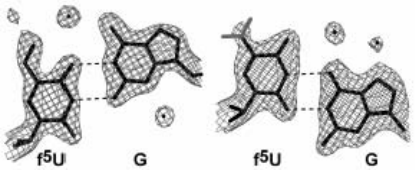

Figure 2|*Fo*|-|*Fc*| maps around the f^{5}U residues found in crystals of f^{5}U:G.

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Keywords: DNA crystallography, mutagenesis, novel structures

P.04.05.7

Acta Cryst. (2005). A**61**, C219

A 1:1 Binding Mode for Netropsin in the Minor Groove of d(GGCCAATTGG)

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The naturally occurring antitumor drug netropsin from *Streptomyces netropsis* binds preferentially to the minor groove of AATT-rich B-DNA. The decamer d(GGCCAATTGG) forms an octamer B-DNA double helix with 2 overhanging G-bases, able to form triple helices. This crystal engineering technique allows enhancing the resolution of minor groove binders such as DAPI [1], distamycin [2] and netropsin with approximately 0.5 Å.

A 98.5% complete dataset was collected at EMBL beamline BW7B (DESY in Hamburg). The structure was solved by molecular replacement using the decamer-DAPI structure [1] as a starting model and further refined to completion using Refmac5.1.24, R factor of 20.0% (including 68 water molecules). The enhanced resolution to 1.75 Å resulted in an unambiguous determination of the drug conformation and orientation.

Bifurcated hydrogen bonds are formed between the amide N-atoms of the drug and the N3(A) and (O2)T base atoms, cataloging the structure to Class I. As the bulky NH_2-group on G is believed to prevent binding of the drug, the detailed nature of several of the amidinium and guanidinium end contacts were further investigated by *ab initio* quantum chemical methods.

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Keywords: nucleic acids, netropsin, ab-initio calculations