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STRUCTURAL CHARACTERISATION OF DOSRS – A TWO-COMPONENT SIGNAL TRANSDUCTION SYSTEM RESPONSIBLE FOR MYCOBACTERIUM TUBERCULOSIS ENTRY TO DORMANT STATE

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Mycobacterium tuberculosis is a very successful pathogen, to a great extent, due to its ability to persist in a dormant state within the host for many years. Entry into a dormant state has been tightly linked to occurrence of hypoxic conditions. Hypoxia and nitric oxide were both shown to lead to induction of DosRS genes. DosRS proteins form a classical two-component signal transduction system where histidine kinase (DosS) undergoes autophosphorylation in response to changes in the environment (i.e. nutrients, oxygen, light, toxins) and then phosphorylates cognate response regulator (DosR) that elicits response. DosR is a transcription activator that through DosRS system induces a regulon of approximately 50 genes that are associated with dormancy.

We have initiated structural investigation of the N-terminal sensory region of DosS histidine kinase in order to identify molecular nature of the stimuli that initiates responses to hypoxic conditions, and to elucidate molecular mechanism of signal transduction within DosRS.

DosS sensor contains two GAF domains that are ubiquitously found in signalling proteins and are known to bind variety of ligands primarily cyclic nucleotides. We have demonstrated that proximal GAF domain of DosS binds haem and identified histidine 149 as critical for haem binding. This is the first GAF domain identified to bind haem. We are carrying out structural characterisation of these domains using UV/VIS spectroscopy, X-ray crystallography and NMR methods. Based on our findings we were able to propose a model of histidine kinase activation in response to a drop in oxygen pressure that would result in conversion to a dormant state of the mycobacterium.

RIP PHASING AND RADIATION DAMAGE ON BROMOU-RNA

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The intense X-ray fluxes associated with third generation synchrotron sources can result in significant damage to cryo-cooled macromolecular crystals [1-4]. For brominated compounds commonly used with nucleic acids, even a relatively moderate X-ray dose can induce sufficient debromination to prevent structure determination [3]. However, specific X-ray damage can provide additional phase information, ultimately allowing to determine a protein structure using a single crystal from a native macromolecule [2, 4].

In the present study, fluorescence spectra were measured at regular time intervals along data collection on a crystal of a brominated 23-nucleotide RNA fragment. Modification of the fluorescence spectrum correlates well with the cumulated X-ray dose and these measurements help to follow the radiation-induced debromination event. Thus, the decrease of bromine occupancy can be determined along data collection which is of particular interest for the Radiation damage Induced Phasing method.

In addition, we have made striking verifications of the influence of crystal orientation relative to the beam polarization on the height of a 'white line' (i.e. on the value of f''). We will show that, depending on the crystal orientation, the white line can disappear completely.

These results are not limited to bromine and can be extended to any element having an absorption edge in the commonly available wavelength/energy range.

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CRYSTAL STRUCTURE OF THE EXTENDED DUPLEX FORM OF THE HIV-DIS RNA BOUND TO AMINOGLYCOSIDE ANTIBIOTICS

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All retroviral genomes consist in two homologous single stranded RNAs. HIV-1 Dimerization Initiation Site (DIS) is a strongly conserved stem-loop in the 5' non-coding region of the genomic RNA. Alteration of the DIS strongly affects RNA dimerization, packaging and reverse transcription, and dramatically reduces viral infectivity. The DIS loop contains a self-complementary sequence and initiates genome dimerization by forming a loop-loop complex. This initial complex is further stabilized into an extended duplex form upon interaction with the viral NCp7 nucleocapsid protein. We have previously solved crystal structures of the DIS loop-loop complex and extended duplex. These structures revealed an unexpected resemblance with the eubacterial 16S ribosomal aminoacyl-tRNA site (A-site), which is the target of aminoglycoside antibiotics. Similarities exist at the primary and secondary structure level, but also at the tertiary structure level, as revealed by comparison of the respective DIS and A-site crystal structures. Footprinting experiments showed that some aminoglycosides specifically bind to the DIS, with an affinity and geometry similar to that observed for A-site. In agreement with these predictions, we solved crystal structures of the DIS kissing-loop bound to various aminoglycoside antibiotics, showing that two aminoglycosides are bound per loop-loop complex. Using these results, chemical probing was performed on HIV-1 genome in infected cells and on viral RNA extracted from virions, showing that DIS remains accessible to aminoglycosides in vivo. It is therefore a valuable target for a potential new antiviral drug.

We will now present the 1.5 Å resolution crystal structure of the extended duplex form of the DIS bound to aminoglycoside antibiotics, showing that these molecules can also be targeted against the viral RNA after maturation by the nucleocapsid protein. Interestingly, the structure solution was not straightforward and introduction of a new kind of modified nucleoside (2'-methylseleno-uridine) was used for phasing by MAD.

AUTOMATED FITTING OF NUCLEIC ACID FRAGMENTS INTO ELECTRON DENSITY MAPS

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A method for automatic building of nucleic acid structures has been developed. Molecular fragments are located in electron density maps by a phased rotation conformation and translation function, as implemented in the program NUT. The fragments are RNAbone DNAbone (each has about 17 atoms from NA backbone) that represent a mononucleotide of phosphate-sugar-phosphate type. Fragments are flexible and all torsion angles can be varied during the search. For computation reasons the search is restricted by a table of allowed conformations, conformation families. RNAbone and DNAbone are suitable for intermediate crystallographic resolution of about 2.0 to 3.0 Å. Individual fragments are connected into polynucleotide chains by a program HEL. In the case of RNAbone/DNAbone, nitrogenous bases can also be modeled into the density. The result of connecting RNAbone/DNAbone fragments is a PDB file. The procedures were tested on RNA and DNA structures ranging form a small nucleotide (1QYL) to ribosomal structures (1FFK, 1J5E). About 70% to 100% of the structure can be built, depending on resolution, fragment used and phase quality.A rigid double helical fragment NAhelix of 90 atoms is used to locate stretches of regular A-RNA or DNA structures. The position and orientation of the fragment can be refined as rigid body, its conformation is fixed. Using NAhelix fragment, only about 70% of the structure can be located in a typical RNA structure. NAhelix is suitable for lower resolution structures.

SOLVATION OF NUCLEIC ACIDS: MOLECULAR DYNAMICS SIMULATIONS AND DATABASE SCREENING

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Modeling accurately the solvation of nucleic acid systems is an important issue since it has been shown that water, together with the surrounding ionic atmosphere, is an essential component of RNA and DNA structure. I will present some examples related to our own experience of including ionic species (Mg^{2+}, K^{+}, Cl^{-}) in MD simulations of RNA systems. A new web service, called SwS ("Solvation web Service" for nucleic acids), will also be presented. This web service, based on the nucleic acid structures contained in the NDB, is devoted to the statistical analysis of their first solvation shell and has been developed to allow accurate comparisons between theoretical and experimental data.

NMR PARAMETERS FOR NUCLEIC ACIDS: CAN QUANTUM MECHANICS HELP?

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Understanding of chemical processes of all biologically active molecules is related to the knowledge of their molecular structure and dynamical behavior at given conditions. For solid state, the structure of nucleic acids (NAs) is predominantly determined by X-ray crystallography and latest achievements by this experimental technique represent fundamental contribution to the field. Molecular structure of NAs in native environment is frequently studied by the methods of molecular spectroscopy, where the most prominent role is attributed to the nuclear magnetic resonance (NMR).

Structural parameters of NAs can be resolved with the NMR spectroscopy only indirectly and the interpretation of measured NMR spectra clearly suffers from lack of information necessary for the reliable correlation with corresponding geometry descriptors.[1] NMR studies of the NAs mostly rely on empirical interpretation of spectroscopy parameters.[2] Such an approach can hardly cover all effects like dependence on local geometry or specific hydration/solvation. Theoretical modeling of NMR parameters can thus facilitate their structural interpretation and in many cases also validate current interpretation schemes.

The following theoretical studies carried out during last years show the potential of theoretical modeling of NMR parameters for structural studies of NAs. Specific NMR J-coupling constants in guanine base can be used for the discrimination between canonical and mismatched type of base pairing in DNA hairpin molecule [3] and inclusion of water solvent leads to more reliable model and thus better agreement of calculated and experimental J-couplings.[4] Calculated intermolecular NMR J-couplings between ions Mg^{2+}, Zn^{2+} and guanine base, show their capability for detection of the binding motif in hammerhead ribozyme [5]; specific direct and water mediated contacts of metal ion to the guanine base can be distinguished.[6] Theoretical calculation of NMR cross-correlation relaxation rates in DNA nucleosides unveils its significant degree of conformational dependence on sugar to base orientation as well as on sugar pucker what improves currently established schemes for its structural interpretation.[7] Theoretical prediction of specific J-coupling constants between atoms of NA base and backbone phosphate across the P=O•••H-C motif indicate its possible detection for some rare but important backbone topologies, as seen for example in the reverse kink-turn motif. [8] Complete set of the J-coupling constants calculated between atoms of the backbone in NAs can be used for the discrimination among NA backbone structural patterns.[9]

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TRIPLE HELICAL FRAGMENTS AND RESOLUTION ENHANCEMENT BY CRYSTAL ENGINEERING

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Nucleic acid triplexes can be considered as the result from the sequence-specific association between a single stranded oligonucleotide and a double helix. Triple helix formation is believed to play a role in numerous biochemical processes, e.g. regulation of transcription and replication, genetic recombination of homologous sequences, chromosome folding,... One of the most important applications of triple-stranded complexes is their potential role to regulate gene expression in vivo.

As not much structural knowledge is available on triplex DNA, we introduced a novel way of obtaining structural details of DNA triplets by the use of overhanging bases. The ability of overhanging bases to form triplexes opens up possibilities for obtaining ordered crystals of triple helical fragments by extending the length of the overhanging strands and applying crystal-engineering techniques.

Detailed models of a parallel G•GC triplet and of a parallel and antiparallel (G•GC)2 triple helical fragment have been obtained by the carefully chosen nonamer d(GCGAATTCG) and decamer d(GGCCAATTGG) [1,3], leading to the interaction in the major groove of the unpaired guanine residues with the GC Watson-Crick base pairs. The nonamer d(GCGAATTCG) crystallized in the B-DNA conformation with unpaired guanine bases at its ends. Two crystallographic independent parallel Hoogsteen G•GC base triplets are formed by interaction of the guanine bases with the terminal C•G base pairs of neighbouring double helices [1,2]. The decamer d(GGCCAATTGG) forms an octamer B-DNA helix with two overhanging G-bases, which are able to form both parallel Hoogsteen and anti-parallel reverse-Hoogsteen (G•GC)2 triple helical fragments [3,4].

This crystal engineering technique, which mimics triple helical fragments in the crystal lattice of d(GGCCAATTG G) can at the same time be used to improve the resolution of the obtained diffraction data. We have previously reported the 1.9 ÅA resolution structure determination of the minor groove binder DAPI (4',6-diamidino-2-phenylindole) with d(GGCCAATTG G), revealing a novel off-centered binding with a hydrogen bond between the drug and a CG base pair [5]. Structure determinations of the same decamer with distamycin at 2.38 Å and 1.85 Å, revealed two 1:1 binding modes for distamycin in the minor groove [6]. The same crystal engineering technique could be used to improve the resolution of the 1:1 d(GGCCAATTGG)-netropsin complex to 1.75 Å [7].

In order to introduce a third subsequent T•AT triplet, the decamer was further extended with one overhanging thymine residue. These thymines can interact with AT base pairs forming T•AT triplets and hence extend the triple helical fragment to three triplets.

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LOOP REMODELLING IN TELOMERIC DNA AND ITS IMPLICATION FOR LIGAND DESIGN

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The integrity of telomeric DNA and its maintenance is a hallmark of human cancer. Small molecule ligands that target the single stranded 3' ends of telomeric DNA are actively being developed as potential anti-cancer therapeutic agents. Recent structural studies on these guanine rich DNA sequences have revealed a diverse group structures folded into quadruplexes with a range of competing topologies. Structural investigations are currently underway to determine the modes of interaction of these quadruplex binding ligands both to the well defined guanine tetrads but also to the loop regions to improved binding affinity and selectivity. Using our recently acquired structural data on both native human telomeric sequences and in complex with ligands, we will discuss the modes of ligand binding and their effects.

DNA BINDING MODES OF "BISINTERCALATORS" AND OTHER MYSTERIES

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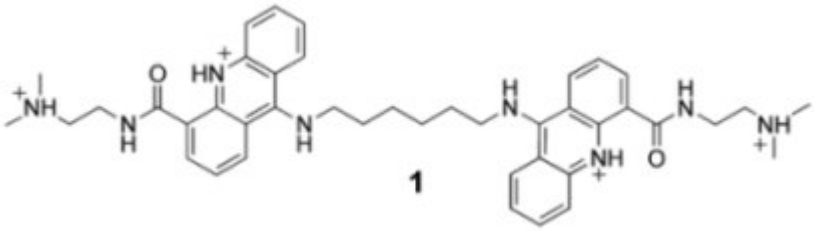
Molecule 1 is one of a family of compounds designed as DNA bisintercalators. They were developed because of the long residence times found for these compounds compared with comparable monointercalators. According to the classical binding model, they should intercalate at sites two steps apart in the DNA ladder, with the linker in one of the grooves. So far, however, we have never observed binding according to this model, by using the method of cocrystallisation of these compounds with DNA oligonucleotides, although the solution evidence had been interpreted as bisintercalation. Recently we have shown that 1 crosslinks the duplex d(CGTACG)2, having crystallised in a novel packing in space group P4322. We solved the structure using SAD data at the Sr edge from beamline BM14 at the ESRF. The linker in this molecule is apparently too short for it to be a bisintercalator.



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