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Capturing hammerhead ribozyme structures in action by modulating the rate of general base catalysis

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We have obtained pre-catalytic (enzyme-substrate complex) and post-catalytic (enzyme-product complex) crystal structures of an active full-length hammerhead ribozyme that cleaves in the crystal. Using the natural satellite tobacco ringspot virus (sTRSV) hammerhead RNA sequence, the self-cleavage reaction was modulated by substituting the general base of the ribozyme, G12, with A12, a purine variant with a much lower pKa that does not significantly perturb the ribozyme's atomic structure. The active but slowly cleaving ribozyme thus permits isolation of enzyme-substrate and enzyme-product complexes without modifying the nucleophile or leaving group of the cleavage reaction, nor any other aspect of the substrate. The pre-dissociation enzyme-product complex structure reveals RNA and metal ion interactions potentially relevant to transition-state stabilization that are absent in pre-catalytic structures.

Keywords: hammerhead ribozyme, general base catalysis, active conformation

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Ligand binding and structural rearrangements of quadruplexes containing human telomeric sequences

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Telomere maintenance is integral to the progression of human cancer and its disruption through the stabilization of novel G-quadruplex DNA structures by small molecule ligands is an attractive strategy for anti-cancer therapies. Knowledge of the biologically relevant folded topology adopted by these single-stranded telomeric sequences is critical for the design of drugs that selectively target and stabilize these structures. In an attempt to validate and design selective ligands we have used crystallographic techniques to provide a detailed understanding of the mode of ligand binding to human telomeric DNA. We will report on two classes of ligands that have co-crystallized in complex with both an intramolecular and a bimolecular quadruplex DNA of human telomeric sequence. A tetra-substituted naphthalene diimidine and the experimental anticancer drug BRACO-19, a 3,6,9-trisubstituted acridine. Both ligands have been shown to bind tightly to telomeric DNA, inhibit telomerase enzymatic activity resulting in telomere shortening. These crystal structures reveal that the quadruplex topology in both sequences is unchanged by the addition of the ligands, with the ligands binding to the external 5' and 3' planar G-tetrad surfaces. There is however, some remodelling to the previously observed TTA loop structures to provide additional sites for interaction. These new DNA/ligand structures have enabled us to identify the modes of ligand binding and apply a rational, structure based approach to design for these classes of ligands. Structural aspects of ligand interaction and design will be discussed along with future implications for selective quadruplex-binding ligands.

Keywords: anticancer drug structural study, DNA-ligand interactions, drug discovery and design

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Crystal structures of DNA-bound Co(III)·bleomycins

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Bleomycins constitute a widely studied class of complex DNA cleaving natural products that are used to treat squamous cell carcinomas, lymphomas, and testicular carcinomas. The bleomycins consist of a disaccharide-modified metal-binding domain connected to a bithiazole/C-terminal tail via a methylvalerate-Thr linker. Their mechanism of action is to induce DNA damage after oxygen activation through site-selective cleavage of duplex DNA at 5'GT/C sites. Using a host-guest crystallographic approach, we have determined the structures of two isoforms of Co(III)·bleomycin (A2 and B2) bound to 5'GT containing oligonucleotides. The host in this case is the N-terminal fragment of Moloney murine leukemia virus reverse transcriptase and the guest, a hexadecanucleotide including preferred 5'GT bleomycin binding sites. Both A2 and B2 isoforms of Co(III)·bleomycin were soaked into preformed host-guest crystals and their structures determined at 3.0 and 2.8 Å, respectively. Distinct modes of intercalation of the bithiazole/C-terminal tail domains of each isoform correlate with different orientations for the methylvalerate-Thr linker while retaining similar hydrogen bonding interactions between the linker and the DNA. Minor groove binding and base-specific hydrogen bonding of the metal binding and disaccharide domains is also retained in the two isoforms. Modeling of a hydroperoxide ligand coordinated to Co(III) suggests that the drug molecule is ideally positioned for C4'H abstraction. Our studies reveal that intercalation of the bithiazole/C-terminal domain is independent of ordered minor groove binding, that linker flexibility is necessary for the molecule to bind to the DNA, and that the disaccharide may play a more important role in DNA binding than was previously suggested.

Keywords: DNA-drug complexes, DNA-drug interactions, biological crystallography

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Molecular recognition and the DNA Holliday junction

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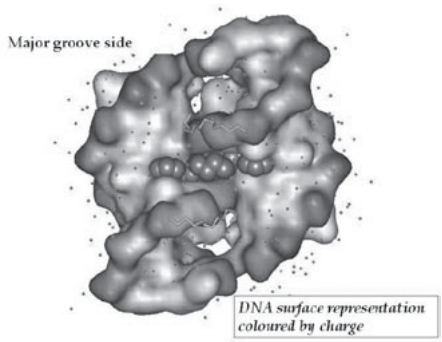
The Cardin group has recently been successful[1] in determining the modes of binding to the DNA Holliday junction of a bisintercalator. The acridine chromophores of the bisintercalator replaced two adenine bases at the junction, see Figure. The mode of binding of these so-called bisintercalators is not intercalative, but displacement of the adenosines at position 6 of the crossover strands, and their replacement with acridine groups in the AT basepair. The 4-carboxamide sidechains thread through to the minor groove side of the junction but make no specific hydrogen bond. The acridine chromophore also makes no specific interaction with the unpaired thymine. Rather, the binding is stabilised by stacking interactions, good steric fit and charge neutralisation. The Cardin group characterised the crosslinking of duplex DNA by the same compound[2]. This talk will include recent unpublished results demonstrating that recognition of the junction can take place in solution.



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Keywords: DNA, Holliday, intercalator

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X-ray Raman scattering: A probe of soft X-ray absorption edges using hard X-rays

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Non-resonant x-ray Raman scattering has emerged as a valuable, complementary, and in some cases unique tool to study truly bulk sensitive soft x-ray absorption edges with high energy x-rays, in particular if electrons or soft x-rays are difficult to use as a probe. Nowadays, dedicated experimental endstations are accessible at third generation synchrotron radiation sources which is reflected in a strong increase of x-ray Raman scattering studies during the past decade. This development is accompanied by a considerable progress in understanding non-resonant x-ray Raman spectra theoretically. For low momentum transfers in the so-called dipole limit the measured x-ray Raman spectra can be compared with results of soft x-ray absorption studies. If the momentum transfer is increased non-dipole transitions contribute significantly to the spectra so that the unoccupied density of states can be studied symmetry selectively which has been widely used in, e.g., exciton spectroscopy. Although the main focus of such experiments was set on the study of the very near edge regions it can be used also to access the extended x-ray absorption fine structure. Specifically the bulk sensitivity of this technique makes experiments feasible in which complicated sample environments are needed. Hence manifold studies on liquids and solids under high pressure conditions have been accomplished. This presentation gives a short introduction to non-resonant x-ray Raman spectroscopy. The special properties of this technique will be emphasized and exemplified by discussing recent studies of liquids, complex materials and samples under extreme conditions.

Keywords: X-ray scattering, absorption spectroscopy, complex materials

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Pressure-induced structural transition in oxides at high pressure: Inelastic X-ray scattering study

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The structures of crystalline and amorphous oxides at high pressure are essential to understand their thermodynamic and electronic properties. Experimental studies of pressure-induced structural changes in the archetypal low-z oxide glasses and crystals (i.e. borates and silicates), however, limited due to the lack of suitable experimental probes. The inherent difficulties of current technologies pose major challenges for probing structural changes of low-z glasses over a wide pressure ranges. Recent progress in in-situ high pressure inelastic x-ray scattering (IXS) with advanced x-ray optics and diamond anvil cell technology, has enabled us to reveal pressure-induced structural changes in archetypal low z- amorphous and crystalline oxides. Here, we report our recent progress about IXS studies of borates and silicates at high pressure. Pressure-induced structural changes in Na-borate glasses are characterized by a single densification pathway in stark contrast to the multiple pathways shown in Li- and pure borate glasses. Oxygen, boron, and lithium K-edge spectroscopy using IXS reveals the nature of electronic bonding changes in diverse amorphous and crystalline silicates at high pressure up to 40 GPa. The result unveils the important role cation field strength plays in pressure-induced structural changes in oxide glasses. We also account for these differences with a conceptual model that utilizes pressure rigidity (the resistance to structural changes with increased pressurization) defined by the variance of the ratio of energy difference between high and low pressure states to its pressure gradient. The results and methods here give improved prospects for atomistic origins of a gradual -to an abrupt coordination transformation in amorphous and crystalline oxides.

Keywords: inelastic x-ray scattering, amorphous oxides, high-pressure

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New applications of q-dependent XRS across the periodic table

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