

MS04.04.07 SOME FUNCTIONAL INSIGHTS FROM RNA CRYSTAL STRUCTURES. Martin Egli, Dept. of Mol. Pharm. & Biol. Chem., Northwestern University Medical School, 303 East Chicago Ave., Chicago, IL 60611-3008, USA

The conformational features of an RNA A-form duplex with single adenosine bulges in two crystal structures will be discussed. Bulged nucleotides are frequent secondary structure motifs in RNA molecules and are often involved in RNA-RNA and RNA-protein interactions. RNA can be efficiently and selectively cleaved at bulge sites in the presence of divalent metal cations. The bulged As are looped-out, kink the duplex into the minor groove, and cause a marked opening of the normally cavernous RNA major groove. The distinct geometries around the A-bulges in the two structures indicate that bulges can confer considerable local plasticity on the usually rigid RNA double helix. The enhanced backbone flexibility can provide for a linear reorientation of the attacking 2'-oxygen of the bulge nucleotide and the P-O5' bond of the adjacent phosphate, consistent with RNA self-cleavage at bulged sites.

The crystal structure of the RNA duplex [r(CCCCGGGG)]₂ was refined to 1.45 Å resolution using room temperature synchrotron diffraction data. This represents the highest resolution reported to date for an all-RNA oligonucleotide. Analysis of the ordered hydration around the octamer duplex reveals conserved regular arrangements of water molecules in both grooves. Evidence will be provided for an important role of the 2'-hydroxyl groups in the thermodynamic stabilization of RNA relative to DNA beyond their known functions of locking the sugar pucker and mediating 3'→5' intrastrand O2'...O4' hydrogen bonds.

MS04.04.08 THE THREE-DIMENSIONAL STRUCTURE OF AN ALL-RNA HAMMERHEAD RIBOZYME. William G. Scott, John T. Finch and Aaron Klug, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England

We have solved the crystal structure of an all-RNA hammerhead ribozyme by isomorphous replacement. We describe the hammerhead RNA structure from a point of view which shows how the structural elements are disposed to bring the active site nucleotide into the catalytic pocket of the ribozyme. Five potential Mg(II) binding sites can be identified in the hammerhead RNA electron density maps. Of these, one is a newly identified metal site positioned near the ribozyme catalytic pocket, and another site corresponds to a Mn(II) site identified in the previous hammerhead RNA structure. We propose a mechanism for RNA catalytic cleavage on the basis of this new metal-binding site, as well as upon comparisons between the catalytic pocket of the hammerhead RNA and the metal-binding sites in the structurally homologous uridine turn of the anticodon loop in tRNAPhe.

PS04.04.09 CONFORMATIONAL CHARACTERISTICS OF DINUCLEOTIDE STEPS VIA SINGLE-CRYSTAL STRUCTURES OF DNA OLIGOMERS. M. A. El Hassan & C. R. Calladine Department of Engineering, University of Cambridge, Cambridge, CB2 1PZ, UK.

In this paper, we investigate the geometry of DNA dinucleotide steps as revealed by single crystal structures of DNA oligomers. We set up a database of 60 *naked* (= not bound to protein or drug) DNA oligomers, including A-form and B-form oligomers alike. The database contains a total of 400 dinucleotide steps. The geometry of a dinucleotide step is described by the *six step parameters* (Twist, Roll, Tilt, Rise, Slide and Shift) and the *six base-pair parameters* (Propeller, Buckle, Opening, Stretch, Shear and Stagger) as given by the Cambridge Accord¹. The step and base-pair parameters are extracted from the atomic co-ordinates

data of the various oligomers by means of the CEHS scheme that we have recently developed.²

In terms of the leading step parameters: Twist, Roll and Slide, we find that some dinucleotide steps are *Rigid* (= AA/TT, AT, GA/TC), i.e. they adopt more-or-less a single conformation; some are *Bistable* (= GG/CC, GC, CG) i.e. they adopt one of two distinct conformations; and some are *Continuously Flexible* (= CA/TG, TA) i.e. they can lie anywhere in a continuous range of conformations. AC/GT shows characteristics intermediate to those of Rigid and Bistable steps, while AG/CT is not included in our classification due to lack of data. We also find that continuously flexible steps exercise their flexibility along a well-defined single-degree-of-freedom path in the Roll/Slide/Twist conformational space.³

We also present a classification of dinucleotide steps with respect to Propeller. We uncover a striking correlation between the conformational flexibility of a dinucleotide step and the level of Propeller in the constituent base-pairs.⁴ We conclude that the level of Propeller in the base-pairs constituting a dinucleotide step results in a stereo-chemical locking effect, the extent of which determines the conformational mobility or otherwise of a dinucleotide step.

¹Dickerson, R. E. *et al.* (1988). *EMBO. J.* **8**: 1-4

²El Hassan, M. A. & Calladine, C.R. (1995). *J. Mol. Biol.* **251**: 648-664

³El Hassan, M. A. & Calladine, C. R. (1996(a)). Conformational Characteristics of DNA; Empirical Classification and a Hypothesis for the conformational Behaviour of Dinucleotide Steps. *Phil. Trans. Roy. Soc. (A)*. **Accepted for Publication.**

⁴El Hassan, M. A. & Calladine, C. R. (1996(b)). Propeller-Twisting and the flexibility of dinucleotide steps. **Submitted for publication.**

PS04.04.10 X-RAY CRYSTAL ANALYSIS OF THE HUMAN PAPILLOMA VIRUS TYPE-11 E2-BS. James Finley, Ming Luo, University of Alabama-Birmingham, Center for Macromolecular Crystallography, Birmingham, AL USA

The human papilloma viruses, the viruses responsible for common warts, have also been implicated in cancers such as cervical carcinoma. E2, a viral transcription factor, is responsible for regulating both replication of the viral genome and transcription of viral genes including the oncogenic E6 protein. This regulation is accomplished by E2 associating with a specific DNA binding site in the viral genome called the E2 binding site (E2-BS). The sequence E2 binds is ACCN₆GGT where N is any nucleotide. By studying the interaction of the E2 protein with the E2-BS, a deeper understanding of how the virus regulates transcription and replication may be achieved. To Characterize the Human Papilloma Virus Type-II E2 protein's structure and function by X-ray crystallography, three crystallization trials are underway. These include the 207 amino acid N-terminal domain of the E2 protein, the 80 amino acid C-terminal domain of the E2 protein complexed with the E2-BS DNA, and the E2-BS DNA alone. Presently, the DNA sequence dGACCGCGGTC which contains the conserved ACC portion of the E2-BS has been crystallized and x-ray diffraction data has been collected. The crystals diffract to 1.5 Å and appear to be primitive hexagonal.