## STRUCTURAL AND DYNAMIC STUDIES OF THE 12 KDA FORM OF PROTEASE FROM MASON-PFIZER MONKEY VIRUS

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Mason-Pfizer monkey virus encodes an aspartic protease (M-PMV PR), which is essential for the correct assembly and maturation of the virion particles. The protease processes viral protein precursors yielding fully functional structural proteins and enzymes. It was demonstrated that the enzyme exists in three active forms with molecular mass of 17, 13, and 12 kDa per monomer, which makes M-PMV PR quite unique among other retroviral proteases [1].

We will report a complete three-dimensional structure of the shortest form of the protease (12 kDa) where both cysteine residues (Cys7 and Cys106) were mutated for alanines to prevent their oxidation [2] and the activity of the protease was suppressed by an exchange of the catalytic aspartate for asparigine in the position 26. Doubly labeled ($^{13}C/^{15}N$) sample was prepared and the resonance assignment was based on triple resonance multidimensional NMR experiments [3]. Based on the calculation of chemical shift index (CSI) approximate positions of secondary structure elements were located. The refinement of the structure was carried out by ARIA software package [4] based on NOE contacts, dihedral angle restraints and hydrogen bonds. To support the structural results we also measured $^{15}N$ relaxation properties of M-PMV PR to obtain a picture of dynamic behavior of the protein.

It turned out that the lower activity of the shortest form of the protease, as compared with the fully active 17 kDa form, is caused by the prevailing monomer in solution. This result was supported by ultracentrifugation experiments. We have proved that the monomeric form of the 12 kDa M-PMV PR is folded similarly as the other retroviral proteases with several distinct features, which will be discussed.

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## NMR RELAXATION STUDIES OF FAST INTERNAL MOTIONS IN NUCLEIC ACIDS

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Investigations of intramolecular dynamics on the nanosecond to picosecond time scale by solution NMR spectroscopy are based on the spin relaxation properties of nuclei such as $^{15}N$ or $^{13}C$. The most commonly measured quantities are the longitudinal relaxation rate constant, $R_1$, the transverse relaxation rate constant, $R_2$, and the steady state nuclear Overhauser effect (NOE). The values of these experimentally obtained parameters can be expressed as linear combinations of spectral density functions. The spectral density function characterizes the overall rotational diffusion of the molecule as well as its intramolecular motions. Two approaches exist for the interpretation of the experimental data. In spectral density mapping, the values of the spectral density function J( ) at characteristic frequencies are determined from the relaxation data [1]. The "model-free" formalism assumes a particular form of the spectral density function, whose parameters then characterize amplitudes and time scales of the molecular motions [2].

The methods for measuring $^{15}N$ relaxation parameters have been well established thanks to a large body of literature on protein dynamics studied through amide nitrogen relaxation. Because of the similarity of the spin environments, the procedures developed for protein amide nitrogen are directly applicable to imino nitrogen of guanine and uracil [3]. However, $^{15}N$ relaxation study of nucleic acids can yield the dynamic properties of guanine and uracil bases only. For a more complete picture, the use of $^{13}C$ relaxation data is highly desirable. The most suitable candidates for relaxation studies in nucleic acids are C8 carbons of purines, C6 carbons of pyrimidines and C1' of the sugar.

In order to bring insight into the internal dynamics of RNA tetraloops we have performed a $^{13}C$ NMR relaxation and molecular dynamics study of 14-nt RNA hairpins GGCACUUCGGUGCC and GGCACGCAAGUGCC (the underlined nucleotides form the loops). The UNCG and GNRA families of stable RNA hairpins (where N is any nucleotide and R is purine) have very similar overall folds. However, the biological roles of these two sequences appear different. The differences have been attributed to distinct dynamical properties of the two sequences [4].

We have measured $R_1$ and $R_{1\rho}$ relaxation rates for C8 of purines, C2 of adenines, C6 and C5 of pyrimidines as well as for C1' of the ribose sugars at several magnetic field strengths. The data have been interpreted in the framework of modelfree analysis characterizing the internal dynamics of the molecules by order parameters and correlation times for fast motions on the picosecond to nanosecond time scale and by contributions of chemical exchange.

While both tetraloops exhibit increased mobility on the fast time scales, with the GCAA loop we have detected a significant contribution of conformational dynamics on the millisecond to microsecond time scale. This is consistent with the observations that the GNRA family appears more

flexible and tolerant of the conformational changes important for molecular recognition.

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## CONFORMATIONAL VARIABILITY OF RNA BACKBONE

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As shown by ribozyme and especially ribosome structures solved in last few years, molecules of RNA form complicated 3D folds which have no match among known DNA structures but their complexity is quite comparable to that of protein folds. Complicated RNA folds are enabled by a high flexibility of the nucleotide backbone but little is known about its conformational behavior. A well refined structure of the large ribosome subunit 50S at 2.4A, NDB structure RR0033 (PDB ID 1JJ2), Ban *et al.* Science **289**, 905 (2000), provides a database of over 2700 nucleotides. This work analyzes conformations of these nucleotides by a combination of Fourier averaging and clustering techniques.

Majority of all nucleotides of RR0033, about 70%, are in the A-type conformation, this main conformational type can be further classified into three subclasses. The remaining 30% of nucleotides with other than A-type conformations were analyzed in a greater detail. The backbone torsion angles for each nucleotide were grouped into eight sets of three angles with the main emphasis on the torsions around the two phosphodiester bonds, O3*-P (torsion zeta) and P-O5* (alpha). Each set of three torsions results in a 3D distribution of points in a parametric torsional space and this distributions was Fourier transformed into densities of nucleotide conformations. Peak positions (maxima) of these maps confine the most probable (di)nucleotide conformations.

Nucleotides belonging to the same peaks in several torsional 3D maps have similar geometry. Such nucleotides were grouped and compared in Cartesian (real) 3D space. In such a way, twelwe types of highly untypical (non-A) nucleotide conformations were identified and their Cartesian coordinates determined. These untypical nucleotide conformations can be useful in e.g.refinement process and are available upon request.

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## STRUCTURE AND DYNAMICS OF RIBOSOMAL 5S RNA AND ITS COMPLEX WITH RIBOSOMAL PROTEIN L25

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Ribosomal 5S RNA (5S rRNA) is an integral component of the large ribosomal subunit in all known organisms with the exception of the small ribosomes of fungal and animal mitochondria. The 5S rRNA of *Escherichia coli* (*E. coli*) interacts with ribosomal proteins L5, L18 and L25 and enhances protein synthesis by stabilization of the ribosome structure but its exact role in protein synthesis is still not known. 5S rRNA contains internal loop - **Loop E**. The Loop E is a salient example of a uniquely structured non-Watson-Crick motif, as it contains seven consecutive non-Watson-Crick base pairs, including wobble G.U base pair and substantial cross-strand purine stacking. This unique duplex architecture together with adjacent sequence helix IV form binding site for ribosomal protein L25.

To understand the structure and function of internal Loop E and interaction between 5S rRNA Loop E and ribosomal protein L25, we have carried out set of molecular dynamics simulations.

Initial structures were directly taken from x-ray crystallography - crystal structure of 5S rRNA Loop E (*E. coli*) [1] and crystal structure of ribosomal protein L25 complexed with the 5S rRNA fragment [2]. Another studied structure was chloroplast Loop E for which there is no atomic resolution structure yet available and which is sufficiently different from bacterial Loop E motifs in sequence, but evolutionarily related to it. Model of chloroplast Loop E was proposed based on homology modeling [3], initial structure for this model was bacterial Loop E, mutation of three base pairs was performed based on the isosteric mutation.

Main focus of our investigation was to study of the structure, dynamics, hydration and cation binding of non-Watson-Crick base pairs and interaction between ribosomal protein L25 and 5S rRNA Loop E. Another aim of this study was to test the usefulness of the MD technique in