A Pseudoknot in the BVDV 5'UTR Internal Ribosome Entry Site

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Insights into the secondary and tertiary structure of the Bovine Viral Diarrhea Virus Internal Ribosome Entry Site

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ABSTRACT
The internal ribosome entry site (IRES) RNA of bovine viral diarrhoea virus (BVDV), an economically significant *Pestivirus*, is required for the cap-independent translation of viral genomic RNA. Thus, it is essential for viral replication and pathogenesis. We applied a combination of high-throughput biochemical RNA structure probing (SHAPE-MaP) and *in silico* modelling approaches to gain insight into the secondary and tertiary structures of BVDV IRES RNA. Our study demonstrated that BVDV IRES RNA in solution forms a modular architecture composed of three distinct structural domains (I–III). Two regions within domain III are represented in tertiary interactions to form an H-type pseudoknot. Computational modelling of the pseudoknot motif provided a fine-grained picture of the tertiary structure and local arrangement of helices in the BVDV IRES. Furthermore, comparative genomics and consensus structure predictions revealed that the pseudoknot is evolutionarily conserved among many *Pestivirus* species. These studies provide detailed insight into the structural arrangement of BVDV IRES RNA H-type pseudoknot and encompassing motifs that likely contribute to the optimal functionality of viral cap-independent translation element.

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Internal ribosome entry site (IRES); bovine viral diarrhoea virus (BVDV); selective 2′-hydroxyl acylation analysed by primer extension and mutational profiling (SHAPE-MaP); H-type pseudoknot; covariance analysis

Introduction

Bovine viral diarrhoea virus (BVDV) is a member of the genus *Pestivirus*, family Flaviviridae, that includes the causative agents of economically significant diseases of cattle, pigs, and sheep. The BVDV infections in cattle lead to decreased fertility and milk production, slow foetal growth, diarrhoea, respiratory symptoms, reproductive and immunological dysfunction [1]. According to the ICTV virus taxonomy profile [2], two main genotypes of BVDV, namely type 1 (*Pestivirus* A) of low-virulence and type 2 (*Pestivirus* B) of high-virulence, have been recognized and are estimated to cause considerable economic loss [3]. Besides the two BVDV genotypes, several genetic isolates have been distinguished within BVDV type 1 [4,5].

The BVDV genome is a positive-sense single-stranded RNA (12.5 kb) that codes for one open reading frame (ORF). The coding region is preceded by the distinctly structured 5′ untranslated region (5′ UTR), which folds into the Internal Ribosomal Entry Site (IRES) [6]. By definition, IRES is the RNA domain that recruits ribosomes to the internal region of mRNAs to initiate translation through a cap-independent pathway. IRES domains can be found in genomic RNAs of many pathogenic viruses. In Hepatitis C virus (HCV) genomic RNA, it mediates the initiation of translation by recruiting the subset of canonical translation initiation factors (eIF2, eIF3), methionine tRNA (Met-tRNAi), and by orienting the 40S ribosomal subunit at the translational initiation codon [7,8]. Cricket paralysis virus (CrPV) IRES RNA directs ribosomes without the requirement of additional translational factors [9], while IRES RNAs of poliovirus (PV) [10] and foot and mouth disease virus (FMDV) [11] rely on binding of eIFs, Met-tRNAi, and additional proteins referred to as the IRES trans-acting factors (ITAFs) [5,12–14].

According to previous structural studies, the viral IRES RNAs fold into intricate secondary and tertiary arrangements that provide a structural scaffold for cellular initiation factors and trigger conformational changes in the 40S ribosomal that drive translation initiation by an active mechanism [15–18]. For example, the HCV IRES consists of four structurally defined domains (I–IV), with domain II inducing the open conformation of the 40S subunit and III and IV forming a functionally essential pseudoknot [19]. Pseudoknots are formed upon base-pairing of a single-stranded region of RNA in the loop of a hairpin or a bulge to a stretch of complementary nucleotides elsewhere in the RNA chain. The flexibility and transient formation of pseudoknots might

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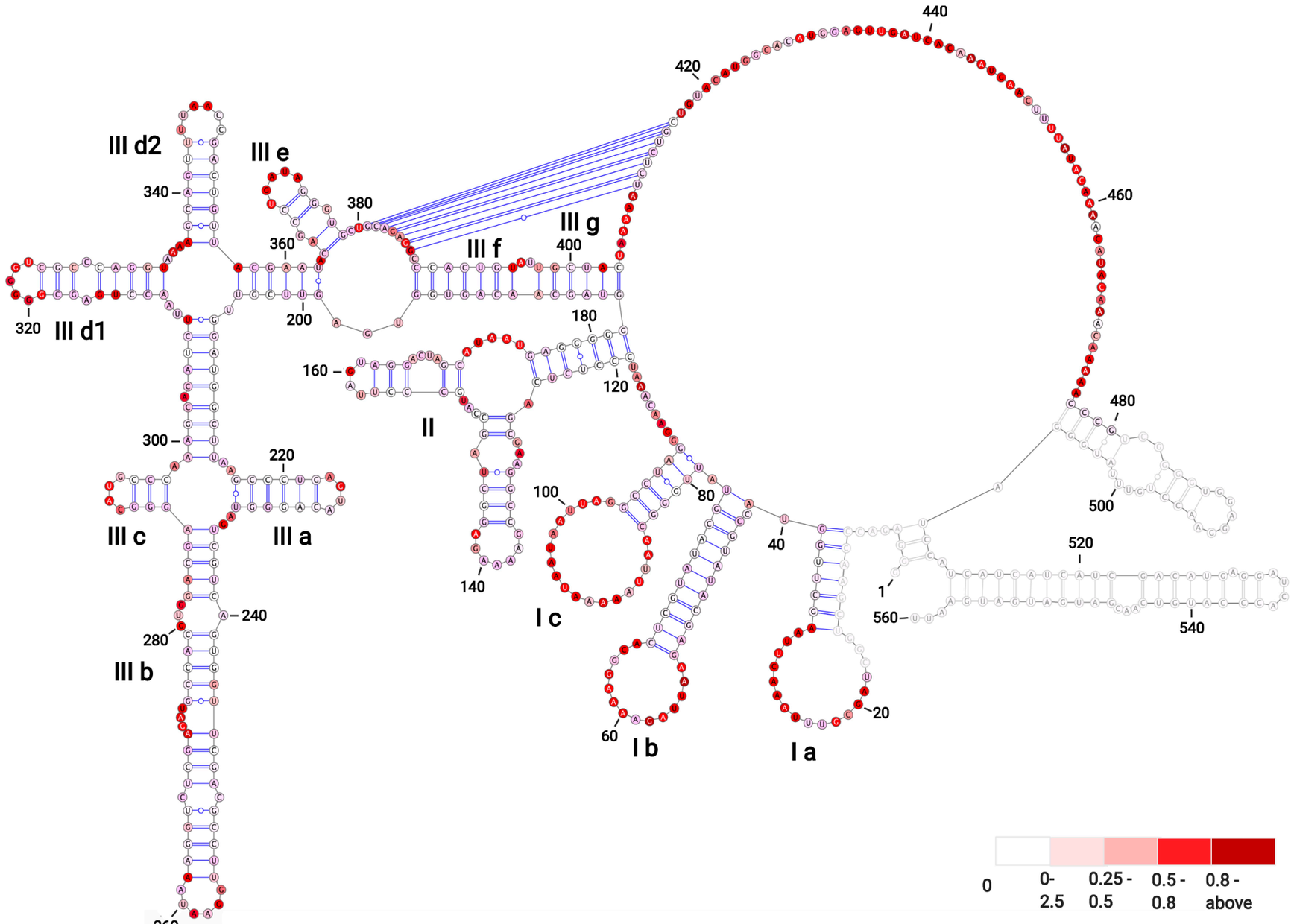

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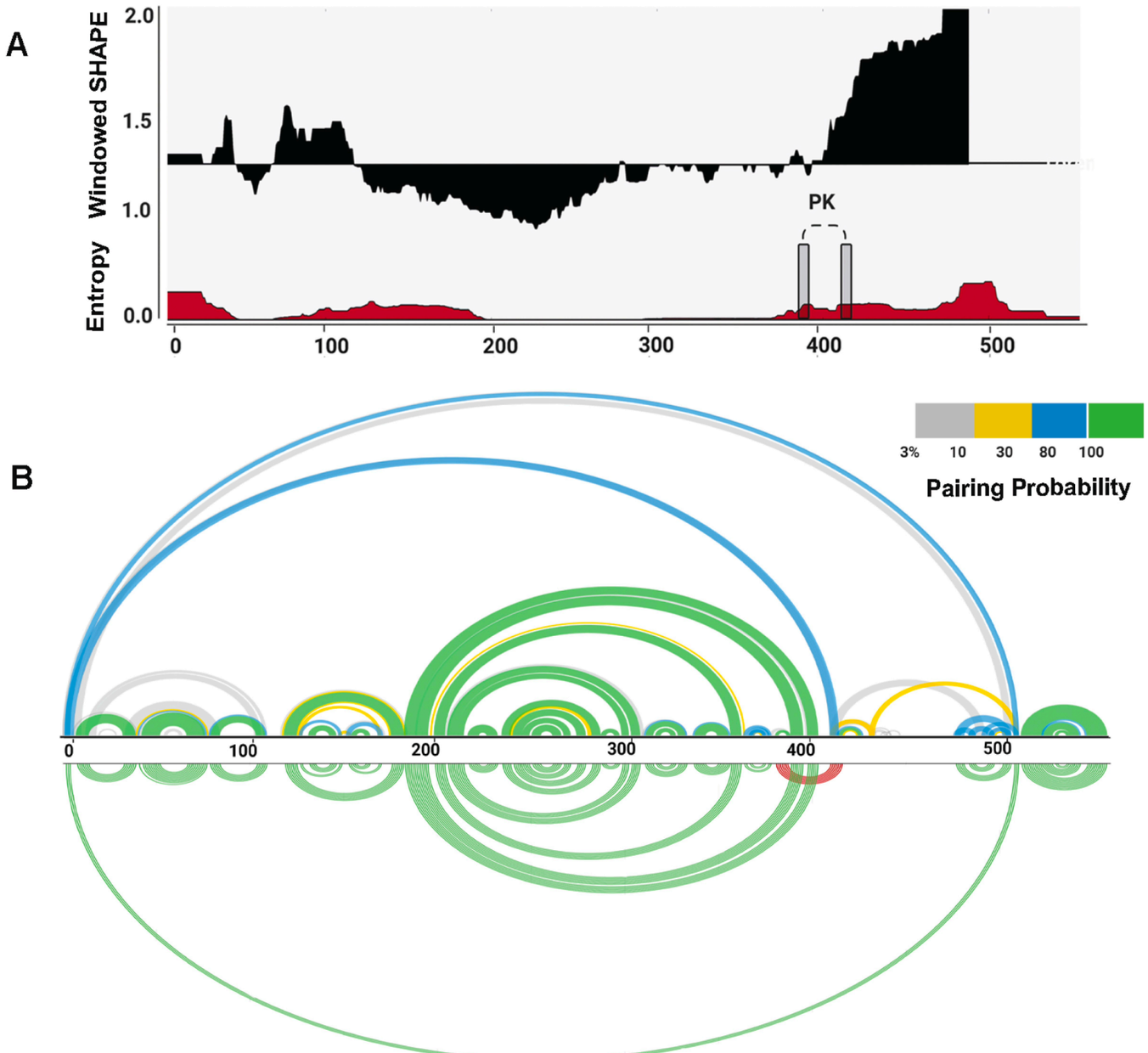

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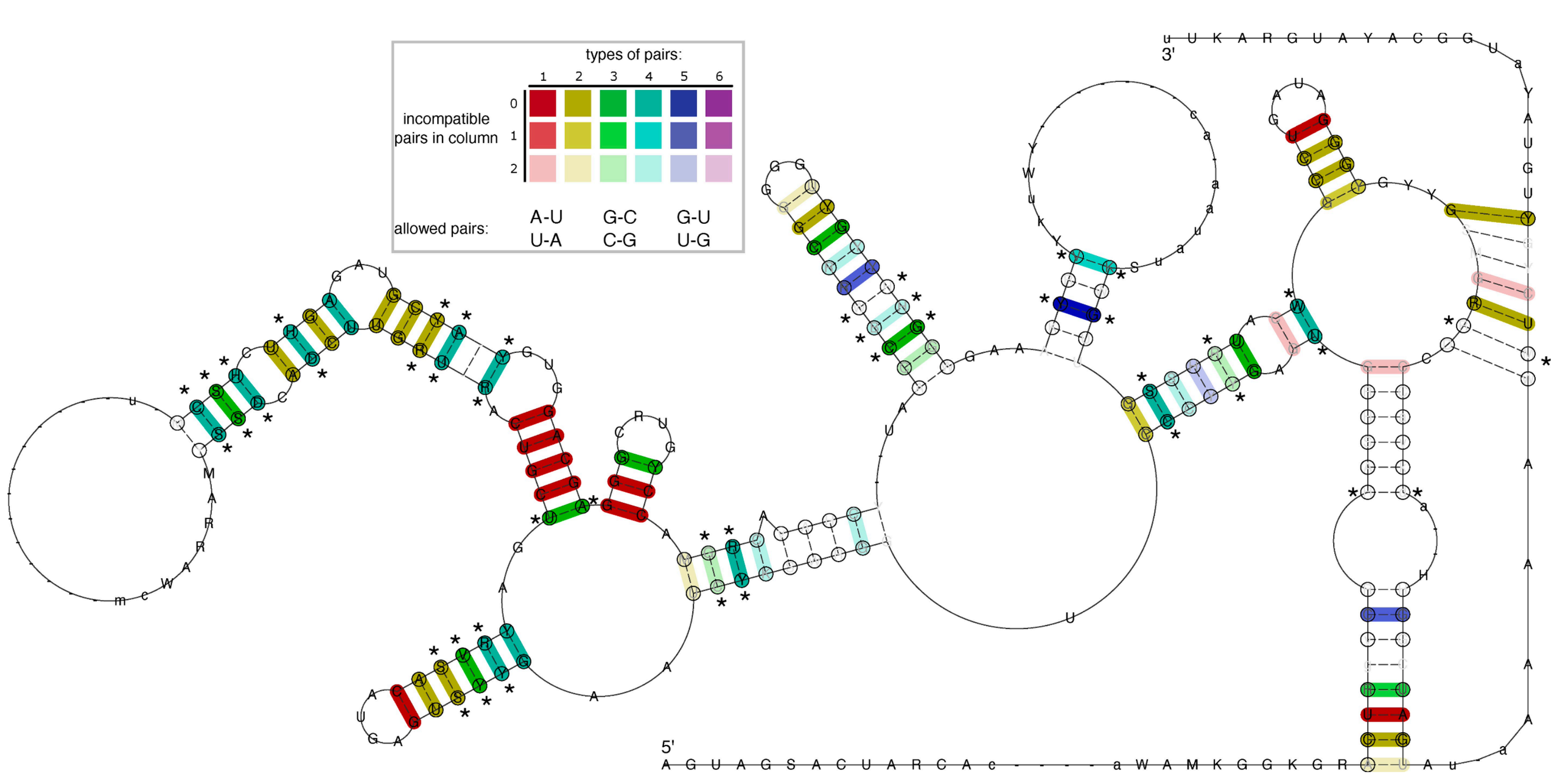

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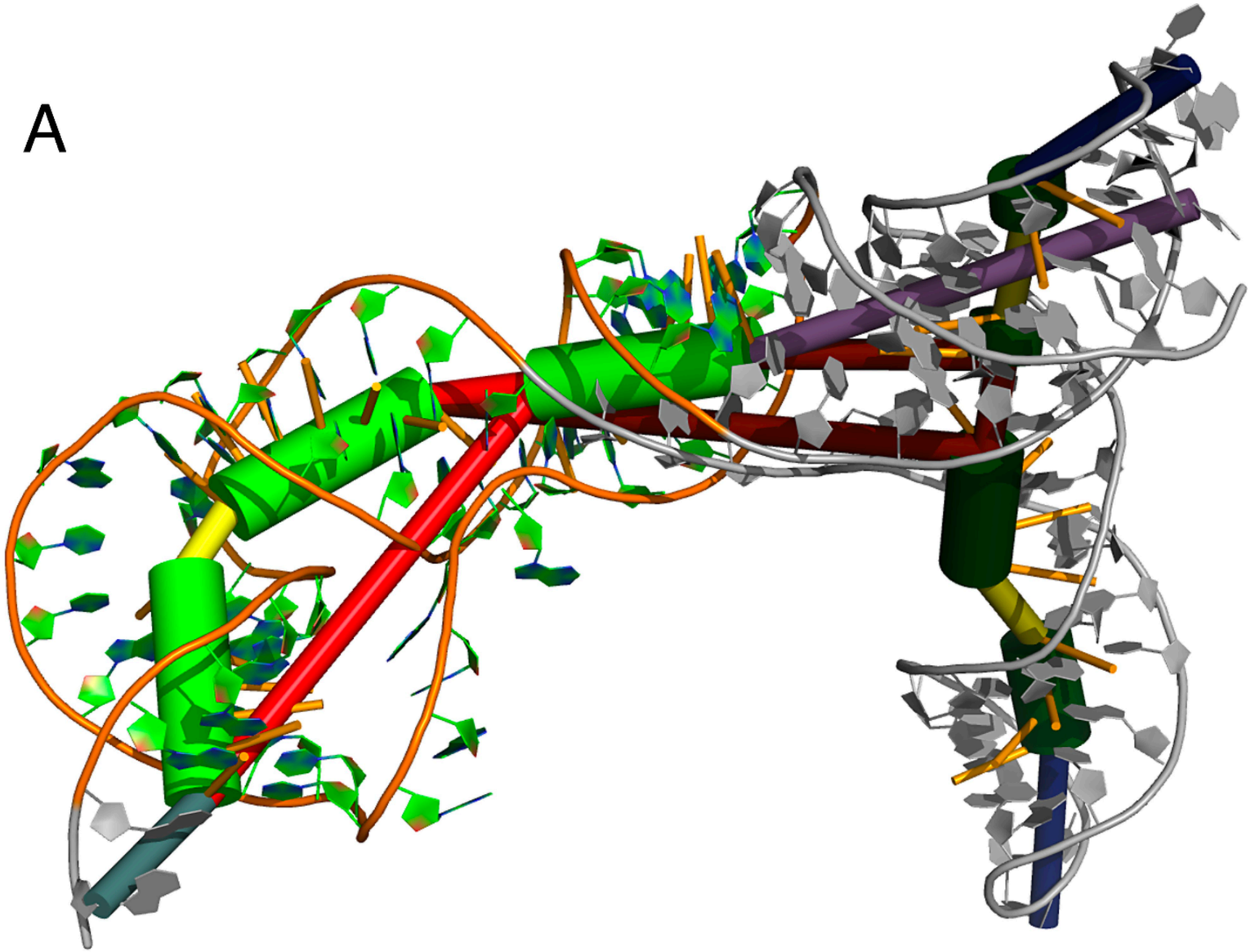

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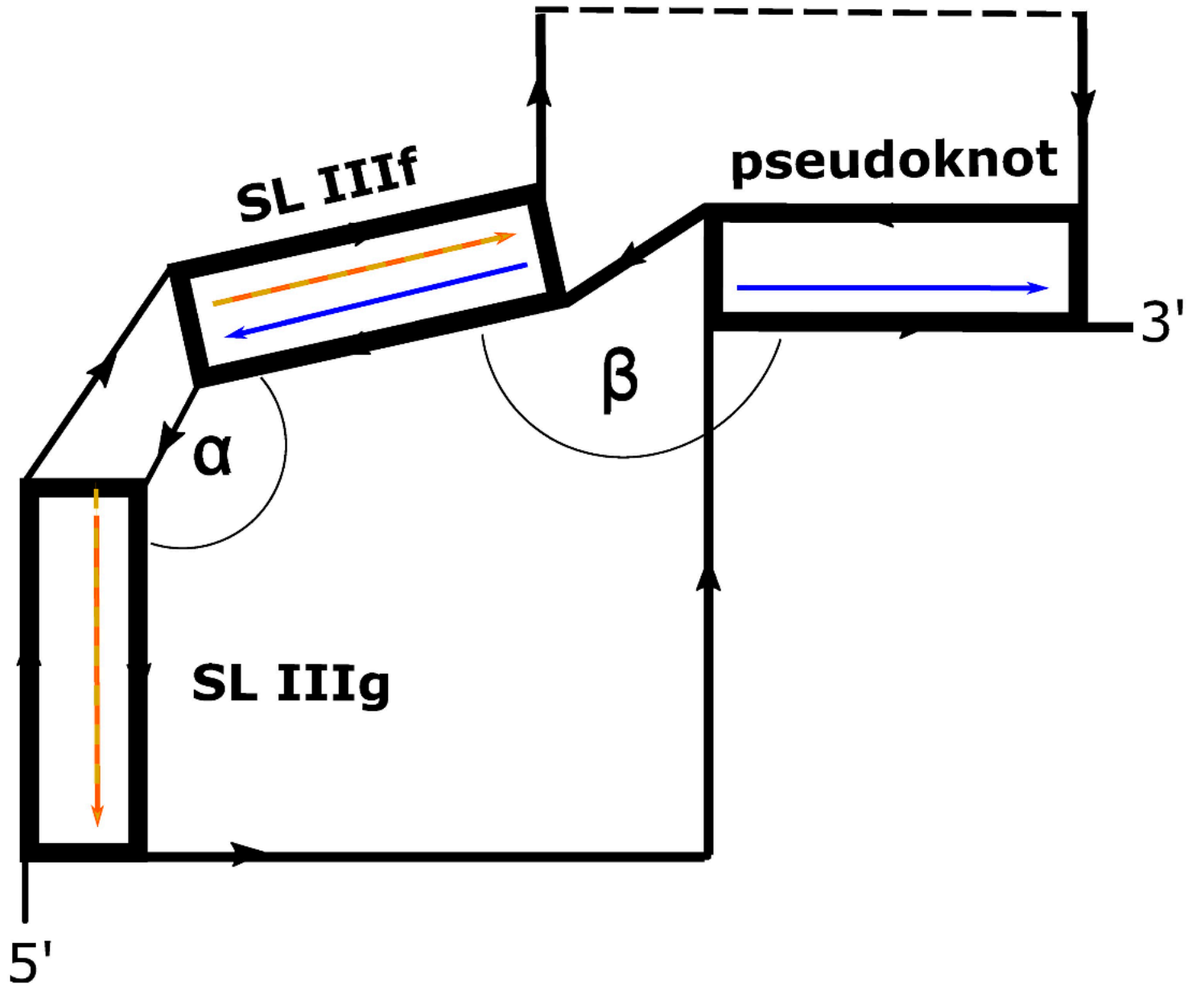

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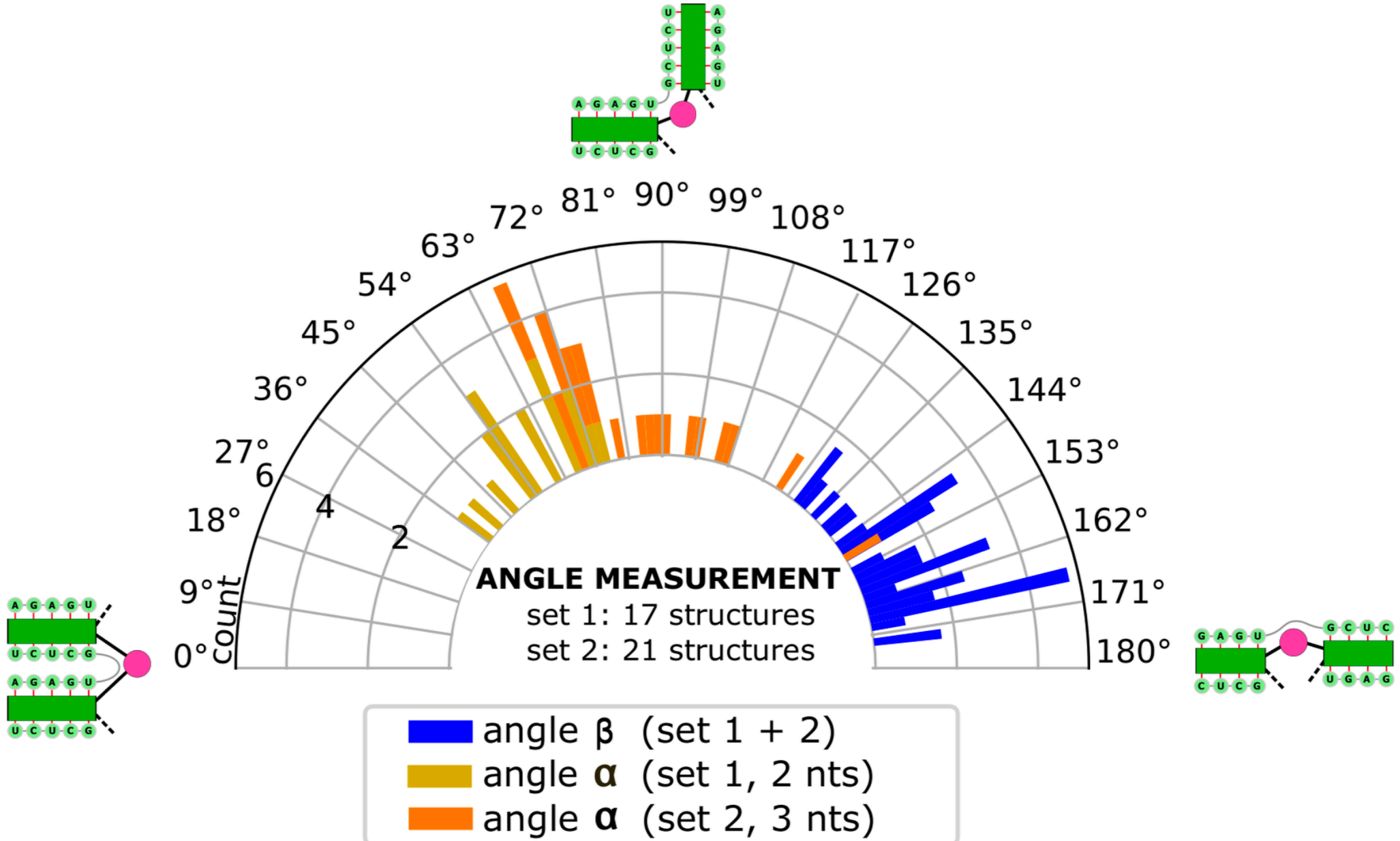

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