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Evaluation of thermodynamic stabilities of in silico designed nucleic acid 3WJ motifs

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Abstract

Nucleic Acid (NA) nanotechnology is a rapidly emerging field demonstrating application of polynucleotides as a versatile biopolymer to fabricate nanostructures of various dimensions and shapes in a programmable and highly predictable way. The folding of DNA or RNA strands into a stable double helix configuration mainly relies on the Watson-Crick (Canonical) base pair composition (G=C and A-T or A-U in the case of RNA), base stacking, and metal ion concentrations. The thermodynamic parameters of DNA B-form helix formation and A-form helix of RNA can be computed using empirically defined sets of nearest neighboring parameters encompassed within the 2D structure predicting programs for example mfold, NUPAC. However, these programs are lacking parameters for a hybrid DNA/RNA base pairing and non-canonical base interactions. In this report, we focused our study to evaluate thermodynamic parameters of several in silico designed three-way junction (3WJ) DNA and hybrid DNA-RNA structural elements. The designed 3WJ motifs contain three helical stems linked with 4,3,2,1, and 0 single stranded Thymidine (T) or Uridine (U) nucleotides. We will report assembly efficiency of the 3WJs investigated by gel shift assay and thermodynamic parameters measured by UV-melting technique. Our experiments reveal that the amount of Ts and Us linkages in the three-way junction dictate the stability of the overall 3WJ conformations. This study is important as we expect it will contribute to the existing set of parameters used for NA structure prediction algorithms as well as provide a guidance for rational design of NA nanostructures.

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