Nucleic Acids Chemistry beyond the Watson-Crick Double Helix (72): Prediction of RNA/DNA hybrid stability under a physiological condition and verification of advantage in CRISPR-Cas9

(*[1]FIBER, Konan University, [2]Slovenian NMR Center, National Institute of Chemistry, [3]EN → FIST Center of Excellence, [4]Faculty of Chemistry and Chemical Technology, University of Ljubljana, [5]FIRST, Konan University*) ○ Dipanwita Banerjee[1], Hisae Tateishi-Karimata[1], Tatsuya Ohyama[1], Marko Trajkovski[2], Maria Toplishek[2], Janez Plavec[2,3,4], and Naoki Sugimoto[1,5]

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Stability prediction for RNA/DNA hybrid duplexes in cellular conditions is essential for understanding of important biological reactions such as replication and transcription and also for development of gene modifying techniques like ASO (Antisense Oligonucleotide) and CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-Cas9. Previously, nearest-neighbor parameters were developed for hybrid duplex in 1 M NaCl buffer[1] which cannot predict hybrid stabilities in a cellular environment as monovalent salt concentration of intracellular and extracellular solutions are much lower than 1 M. Although cellular environments contain a mixture of monovalent and divalent cations of different concentrations, the stability of hybrid duplex under a solution of a typical cellular cation mixture showed more proximity with the stability in the buffer containing 100 mM NaCl compared to that containing 1 M NaCl. Figure 1 exhibits such comparison of stabilities for a hybrid duplex (rGGCUCAAUUGAC/dGTCAATTGAGCC) in buffers containing cellular cation mixture (140 mM K^+, 10 mM Na^+, 1 mM Mg^{2+}, 0.2 μM Ca^{2+}), 1 M NaCl, and 100 mM NaCl indicating that hybrid stability in 100 mM NaCl solution is analogous to that obtained in cellular cation mixture. Therefore, we improved parameters in 100 mM NaCl buffer that can predict stabilities in a physiological salt condition with significant accuracy.[2] Moreover, we demonstrated how improved parameters can help to design efficient short guide RNA in CRISPR technique.[2] Further, we extended our study for structure and stability of hybrid duplexes under molecular crowding conditions as cellular environments are crowded (~400 g L^{-1}) with biomolecules which can largely affect duplex stability.

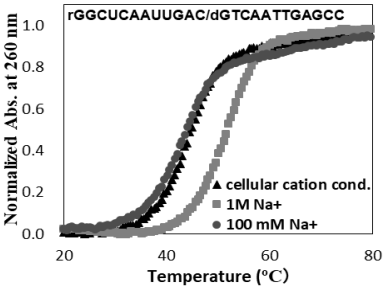

Figure 1. Comparison of melting curves for a representative RNA/DNA hybrid duplex under a solution of typical cellular cation mixture with that in a buffer containing 1 M or 100 mM NaCl concentration.

1) N. Sugimoto, S. Nakano, M. Katoh, A. Matsumura, H. Nakamuta, T. Ohmichi, M. Yoneyama, M. Sasaki, *Biochemistry*, **1995**, *34*, 11211. 2) D. Banerjee, H. Tateishi-Karimata, T. Ohyama, S. Ghosh, T. Endoh, S. Takahashi, N. Sugimoto, *Nucleic Acids Res.*, **2020**, *48*, 12042.