New Data Science in Nucleic Acids Chemistry (10): Quantitative analysis for factors affecting i-motif formation in living cells estimated by the pseudo-cellular system

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The structural versatility of DNA is profoundly influenced by its surrounding environments, with the canonical duplex structure being just one facet of its dynamic conformational landscape. Beyond the conventional duplex, DNA exhibits the ability to adopt alternative structures such as triplexes, G-quadruplexes and i-motifs. The intricacies of DNA folding are further modulated by environmental factors, including the presence of cosolutes such as polyethylene glycol (PEG) and Ficoll, as well as cations like K^+ and Mg^{2+}.[1] These factors have been demonstrated to either stabilize noncanonical DNA structures or induce destabilization of short duplexes, thus adding an additional layer of complexity to the regulation of DNA stability.[2] However, molecular environment within living cells influencing i-motif structures are unknown. Intracellular environments are densely populated with an array of macromolecules, creating a highly crowding conditions and ionic strength setting for DNA structures like i-motif (Figure 1).

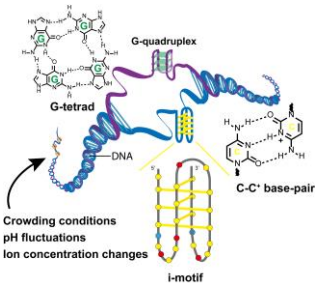

Figure 1. Scheme of intracellular environments providing conditions for i-motif formation.

The *CDH1* gene encodes E-cadherin, which is a protein responsible for cell adhesion. *CDH1* is a tumor suppressor gene, which contains many C-rich sequences that can form i-motif. In this study, as a typical example of an oncogene, we selected some C-rich sequences from *CDH1* to conduct a systematic study of pH dependence examining the relationship between intracellular conditions and i-motif dynamics in the context of cancer. Firstly, we employed biophysical techniques such as CD and UV spectroscopy to examine the stability of *CDH1*-derived C-rich sequences under varying pH and ion concentrations with cosolute mimicking cancer cell environments. Typically, we use PEG as a common cosolute. For example, i-motifs with PEG8000 is stable than in dilute solution. Our results demonstrated that i-motif stability was markedly altered in cosolute conditions, suggesting crowding conditions influences largely i-motif formation. In this presentation, we will show quantitative analysis of the determinants impacting i- motif formation within living cells, utilizing a novel pseudo-cellular system.

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