On analyses of hysteric thermal curves obtained from DNA hybridization

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Nucleic acid chemistry research based on melting and energetics of the DNA double helix has been one of the major topics of molecular biophysics over the past decades. Various modified synthetic oligonucleotides (LNA, PNA etc.) are also an integral part of the said research due to their potential applications as nano-medicines, bio-sensors etc. During the synthesis and characterization of such oligomers, their structural rigidity, stability, functionality along with their stabilization mechanism are of major concern. The phenomenon of hysteresis is commonly observed in several UV thermal experiments (hybridization) and sometimes it is very prominent in modified DNAs. In presence of hysteresis, thermal curves are irreversible and demand a significant effort with multiple steps to produce the reaction specific kinetic and thermodynamic parameters. Here, we describe a unified statistical procedure to analyse such thermal curves with hysteresis. Our method applies to experiments with intra-molecular as well as intermolecular reactions. More specifically; the proposed method allows one to handle the thermal curves for the formation of duplexes, triplexes and various quadruplexes in exactly the same way. The proposed method uses a local polynomial regression (LPR) technique to find the smoothed thermal curves and calculate their slopes. This method is more flexible and easy to implement than the least squares polynomial smoothing which is currently almost universally used for such purposes. Full analyses of the curves including computation of kinetic and thermodynamic parameters can be done using freely available statistical software. At the end, we illustrate our method by analysing irreversible curves encountered in the formations of a G-quadruplex and a LNA (locked nucleic acid) modified parallel duplex.

Keywords: DNA, Thermal melting curves, Hysteresis, Statistical software.

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