CALCULATION OF DNA MELTING TEMPERATURES UNDER VARYING SOLVENT CONDITIONS

Martins, H. F. P.[1]*, Essex, J. W.*[1]*, Neylon, C.*[1] *and Weber, G.*[2]
[1]School of Chemistry, University of Southampton, UK, and [2]Departament of Physics, Federal University of Ouro Preto, Ouro Preto, Brazil

The detailed knowledge of DNA melting temperatures is important for several applications in bioinformatics. For instance, for micro-array probe design an accurate calculation of melting temperatures is essential to insure the absence of false positives as well as for the specificity of the probe-target interaction. Recently, we developed a new method which simplifies the calculation of melting temperatures in statistical physics models (G. Weber *et al*, v. 2, p. 55, Nature Physics, 2006). Unlike nearest-neighbour Gibbs free energy fittings, these models consider some important details of the intra-molecular interaction explicitly. Here, we present calculations which consider the solvent interaction in short DNA strands containing a central mismatch, together with experimental results on biotinylated probes and Cy5 labelled targets. We use a multi-dimensional downhill simplex minimization method to fit the experimental data to the calculated melting temperatures which enables us to obtain the solvent parameters of our statistical physics model. Probes and targets are of 20 nucleotides in length and were designed to contain all four possible perfectly matched combinations as well as the twelve possible mismatches. The melting temperatures of the duplexes were measured under different solvent conditions (5xSSPE, 0.1% SDS and 50 mM, 1 M and 2 M urea) and results showed that the matched sequences exhibited higher T_m than their respective mismatches in all solvents studied. Also, all T_ms decreased steadily, as expected, with increasing solvent stringency but remained above room temperature. Support: Research Councils UK and Fapemig.