The Effects of Viscosity on Nucleic Acid Assembly: A Possible Solution to the Strand Inhibition Problem

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Increased control of molecular self-assembly is important for the further development of DNA-based technologies. While DNA technology research has been carried out almost exclusively in aqueous buffer, alternative solvents could increase our ability to direct DNA assembly. Deep eutectic solvents (DES), closely related to ionic liquids, are characterized by significantly lower melting points than their separate components. Recently, DES have been shown to preserve nucleic acid base pairing and duplex formation, offering a relatively unexplored medium for directing DNA assembly.

In this work, we show that DES provide a highly effective means for tuning DNA thermodynamics and kinetics. We measured DNA duplex thermal stability in a wide range of DES and their aqueous mixtures. We have found that the melting temperature (T_m) of a 32 base pair duplex remains within 10°C of its T_m in aqueous buffer. However, DNA diffusion studies in DES show dramatic molecular sieving effects, with the diffusion of long DNA strands (e.g., 3 kilobases) found to be orders of magnitude slower compared to short oligonucleotides. Reduced diffusion rates in DES allow the formation of intramolecular structures to be kinetically favored over intermolecular duplex formation. Thus, DES can be employed to kinetically trap metastable states that are not accessible in water.

The power of alternative solvents to alter a DNA assembly pathway is illustrated by our ability to circumvent the “strand inhibition problem” by thermal cycling DNA in a DES. Briefly, the strand inhibition problem characterizes the inability to conduct non-enzymatic, template-directed DNA synthesis starting with duplex DNA. The propensity for duplex formation in aqueous solution is so great that, without enzymes, it is not possible to separate duplex strands under conditions that will also allow them to serve as templates for binding of monomers or short oligomers. In our approach, heat is used to separate the strands of a gene-length duplex in DES. When the solution is cooled quickly, the high viscosity of the system results in the kinetic trapping of intramolecular hairpins within the single stranded templates. While the long, single stranded templates are relatively immobile, short complementary oligonucleotides are far less inhibited by viscosity, and can find and bind to their complementary single stranded targets along the long single stranded DNA. Ligation of the ordered oligonucleotides completes circumvention of the strand inhibition problem. This process for template-directed synthesis using a duplex nucleic acid provides a possible solution to a long-standing problem in the origins of life field. Furthermore, our ability to circumvent the strand inhibition problem illustrates the level of control that DES can provide over the kinetic trapping of structures, which could find applications in the creation of DNA-based structures that adopt time-dependent, solvent-directed states.