# Increasing the Thermal Stability of Self-Assembling DNA Nanostructures by Incorporation of isoG/isoC Base Pairs

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## ABSTRACT

Self-assembling DNA nanostructures are currently being developed for a variety of applications in nanofabrication and molecular computing. For many uses, these nanostructures would benefit from increased thermal stability. However, increasing the lengths of sticky-ends that encode interactions between building blocks (DNA cross-tiles) could have an adverse effect on the observed rates of assembly errors and therefore be undesirable. An alternative approach described here, makes use of recently-developed unnatural isomers of guanine and cytosine, isoG and isoC. These isomers form a new base pair that has three hydrogen bonds and interaction strength comparable to the existing G/C pairing. Moreover, the addition of the isoG/isoC pair effectively increases the DNA workspace from four bases to six, facilitating a larger range of unique sequences, and enabling greater flexibility for nanostructure design. In this study, the thermal stabilities of two types of DNA cross-tile lattice (fully-corrugated 4x4 nanogrid) were compared: 1) The original lattice design using natural base pairs, and 2) A modified lattice design in which all A/T basepairs in the tile sticky-ends were substituted with isoG/isoC. We observed an increase in lattice melting temperature of approximately 11°C, from $T_m$ = 42±1°C to $T_m$ = 53±1°C, and at the same time there was no change in the tile melting temperature ($T_m$ = 62 ±1°C). We have successfully demonstrated a marked increase in the thermal stability of a complex DNA nanostructure via the incorporation of isoG/isoC basepairs. Future studies will include incorporation of isoG/isoC within the tile building blocks in order to increase the thermal stability of the tiles.

***Keywords***: DNA nanotechnology, self-assembly, nanostructure, multicode.

## 1 DNA SELF-ASSEMBLIES

The development of programmable self-assembling DNA "tiles" has been a recent trend in nanotechnology[1-6]. Uniquely programmed single-stranded DNA oligonucleotides, when properly annealed, form cross-tile structures through Watson-Crick base pairing [7]. Unpaired bases on the ends of the branches form sticky-ends, which enable the tiles to preferentially bind to other tiles. Tile combination in this way can lead to the formation of massive two-dimensional lattices, called nanogrids, composed of tens of thousands of tiles. Selective binding of streptavidin onto biotinylated tiles has also been demonstrated[7], for both regular and irregular, complex patterns[8]. This proves the feasibility of using nanogrid as a scaffold for the selective binding of a large array of other molecular components [9-16]. The technology thus far holds potential applications for, among others, DNA computing, nanocircuitry, nanoscale "factories" with the use of selectively-bound enzymes, and programmable nanoscale machines.

Work is ongoing in improving upon existing DNA nanostructures in areas such as electrical conductivity [17-18] and thermal stability. Here we document a significant increase in this latter aspect, thermal stability, through the incorporation of isoG/isoC basepairs in the DNA nanostructure.

## 2 EXPANDED BASE PAIR SET

DNA has found its way into nanotechnology because it can form molecular switches, assemble into 3-dimensional memory elements, create high density molecular recognition bio-chips and assemble into defined structures. This is mainly because of the simple rules used to create DNA architectures and the expanded tools used to screen and manipulate DNA nanostructures. Yet the genetic code need not be limited to the two standard nucleotide base pairs known in nature. Rather, non natural nucleobase pairs forming additional base pairs are possible and the chemical syntheses for many new bases are developed [19-23]. We have employed one of the most advanced expanded base pairs made from isoguanosine (isoG) and isocytosine (isoC) into nanotechnology.

We term additional base pair chemistry MultiCode® technology. The additional informational content that MultiCode technology builds into DNA allows for greater diversity. For example, if the four naturally occurring bases are used to make a library of 7-mers, there are only $4^7$ or 16,384 possibilities. In contrast, a six letter system generates 279,936 different 7-mers. This increase in diversity is important as most of those combinations will form mismatched nanostructures even if special conditions are employed to help prevent it. MultiCode technology also allows for construction of shorter sequences with similar diversity. To build specificity, assembly typically takes

place at temperatures near the melting temperatures ($T_m$): the $T_m$ for a 12-mer duplex can be around 36° C while an 8-mer under the same conditions would have a melting temperature of 24° C (~room temperature). In practice, specific assembly could then occur at room temperature conditions. However, what may be the most important aspect of short sequences (less than 9 bases) is that they have no secondary or tertiary structure at room temperature. Since single stranded nucleic acid sequences can fold into structures that can be hard or even impossible to model, assembly to complementary sequences can be problematic. In the simplest of cases, intra-molecular self-assembly forms due to hairpin or stem-loop formation. In these cases, assembly to complementary sequences is blocked. Occasionally, raising assembly temperature can help unblock the effect. Yet because they are intramolecular, the effective concentration is that of water (55 M) and therefore their melting temperatures tend to be high (50° to 70° C). With shorter sequences, intramolecular structure formation is minimized or eliminated (as is the case for sequences shorter than 9 bases in length).

The expansion of the code broadens the scope that DNA will have in the field. Compared to earlier reports, our chemistry advancements make this chemistry available and affordable which should facilitate the expansion of the DNA code for additional nanotech uses.

## 3 METHODS

The design for cross-tile fully-corrugated nanogrid was used as a base template [7]. Sequences were altered by substituting A and T bases in the sticky-ends. A was replaced by isoG and T by isoC. The modified strands were synthesized by EraGen Biosciences, Inc. (www.eragen.com), while all other strands were synthesized by Integrated DNA Technology, Inc. (www.idtdna.com). The oligos were purified by denaturing polyacrylamide (15%) gel electrophoresis. The DNA concentration was determined by ultraviolet absorption at 260nm. For annealing, the individual oligos were mixed together stoichiometrically at 0.25µM in 1X TAE/$Mg^{2+}$ buffer (40mM Tris-HCl (pH 8.0), 20mM acetic acid, 2mM EDTA, 12.5mM magnesium acetate) and slowly cooled from 95°C to 20°C over a period of 2 days. Melting was performed with 120µL of pre-annealed DNA lattice at 0.25µM using a Cary UV-Vis spectrometer. 120µL of 1X TAE/$Mg^{2+}$ buffer was used as a control. The temperature of the lattice was increased from 20°C to 90°C at 0.2°C/min, before being cooled to 20°C again at the same rate. Derivatives of the melting curves were calculated using software provided by Cary.

## 4 RESULTS

2x2 tile arrays and extended lattice were formed for both regular unmodified and isoG/isoC modified experiments. UV-absorption at 260 nm was measured against temperature over the duration of the melt. 2x2 tile arrays were designed to form the fixed size objects shown in Panels c & d. Extended tile lattice was designed to grow without bounds (melt data shown in Panels e & f).

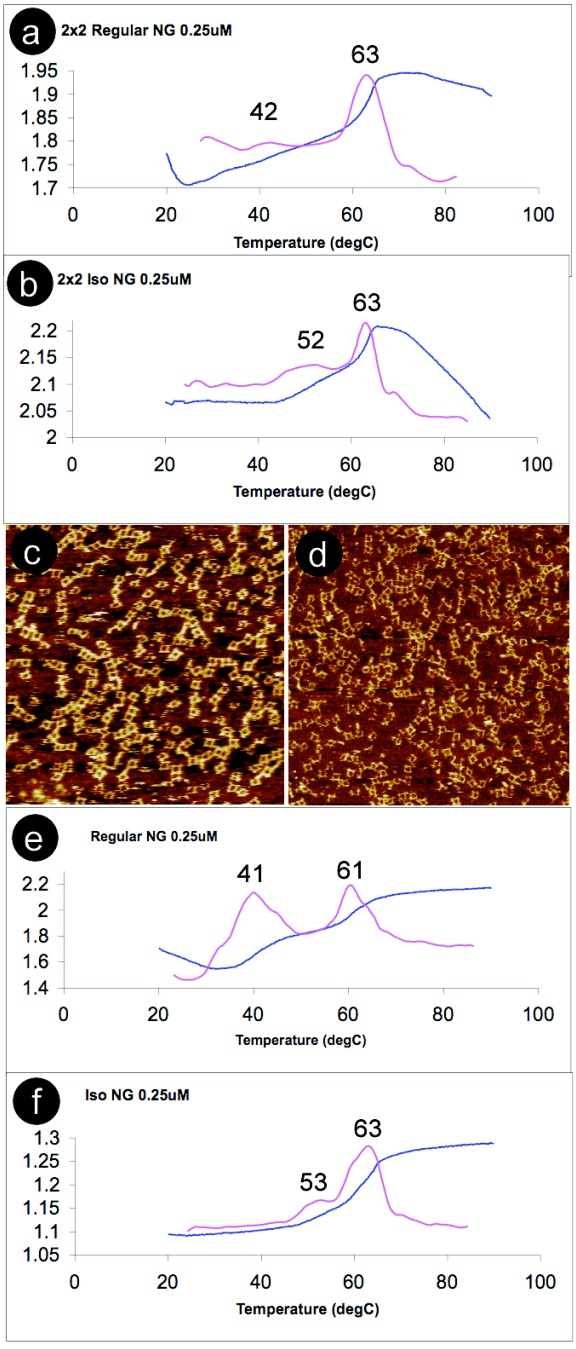


**Figure 1.** UV absorption data for thermal melts of 2x2 cross-tile array with regular sticky-ends (panel a) and isoG/isoC-containing sticky ends (panel b). Atomic force microscope images of 2x2 cross-tile array with regular sticky-ends (panel c) and isoG/isoC-containing sticky ends (panel d). Panels e & f show thermal melt data for regular and isoG/isoC extended nanogrid, respectively.

Fig 1 panels a & b show the increase in lattice melting temperature from 42°C for regular unmodified nanogrid to 52°C for nanogrid containing isoG/isoC in the sticky ends. Tile melting temperature (63°C) remained unchanged since the sticky end modifications only affect inter-, and not intra-tile bonds. Similar results are observed for melting of extended lattice, with isoG/isoC containing lattice breaking down approximately 10 degrees higher than regular lattice and individual tiles melting to single DNA strands at about 63°C, as previously.

## 5 CONCLUSIONS

We have successfully demonstrated the ability to markedly increase the thermal stability of a DNA cross-tile lattice through the replacement of A/T basepairs with isoG/isoC basepairs in the sticky ends. The implications of these results are far-reaching. First, by enabling the nanostructures to remain stable at a higher temperature, more avenues for applications are opened, since thermal environmental constraints become less restrictive. Second, our findings prove the feasibility of isoG/isoC incorporation in DNA nanostructures. This essentially expands the usable genetic alphabet from 4 bases to 6 bases, resulting in much broader diversity in the range of unique complex sequences that can be used. These results represent another large step in the development of self-assembling DNA nanostructure technology.

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