Supporting Information For:

Thermodynamic Aspects of DNA Nanoconstruct Stability and Design

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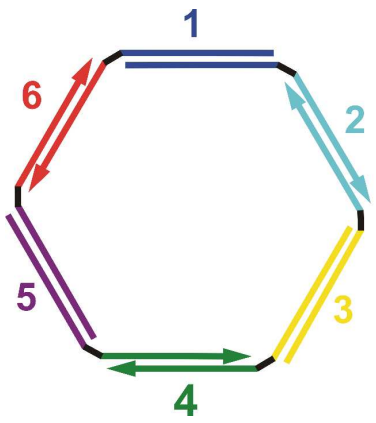
Sequences used:

Oligo	Sequence
UM1	5' C CATA CATA C TT C CACA GCAT C 3'
UM2	5' G TATG TATG G TT C AACC TCCT C 3'
UM3	5' G GCTC TACA G TT G AGGA GGTT G 3'
UM4	5' C TGTA GAGC C TT G TAGT GTGA G 3'
UM5	5' C ACCA ACAA C TT C TCAC ACTA C 3'
UM6	5' G TTGT TGGT G TT G ATGC TGTG G 3'
tC^O11	5' C CATA tC^OATA C TT C CACA GCAT C 3'
tC^O22	5' G TATG TATG G TT C AAtC^OC TCCT C 3'
tC^O33	5' G GCTC TAtC^OA G TT G AGGA GGTT G 3'
tC^O54	5' C ACCA ACAA C TT C TCAtC^O ACTA C 3'
tC^O55	5' C ACCA AtC^OAA C TT C TCAC ACTA C 3'
tC^O16	5' C CATA CATA C TT C CAtC^OA GCAT C 3'



Unmodified sequences are named according to the numbers in the figure. The numbers at each side of the hexagon refer to the sequence which is on the outside in the figure *i.e.* closest to the number. For the tC^O-modified sequences the naming is as follows; the first number refers to the corresponding unmodified strand and the second number refers to which side the tC^O is positioned.

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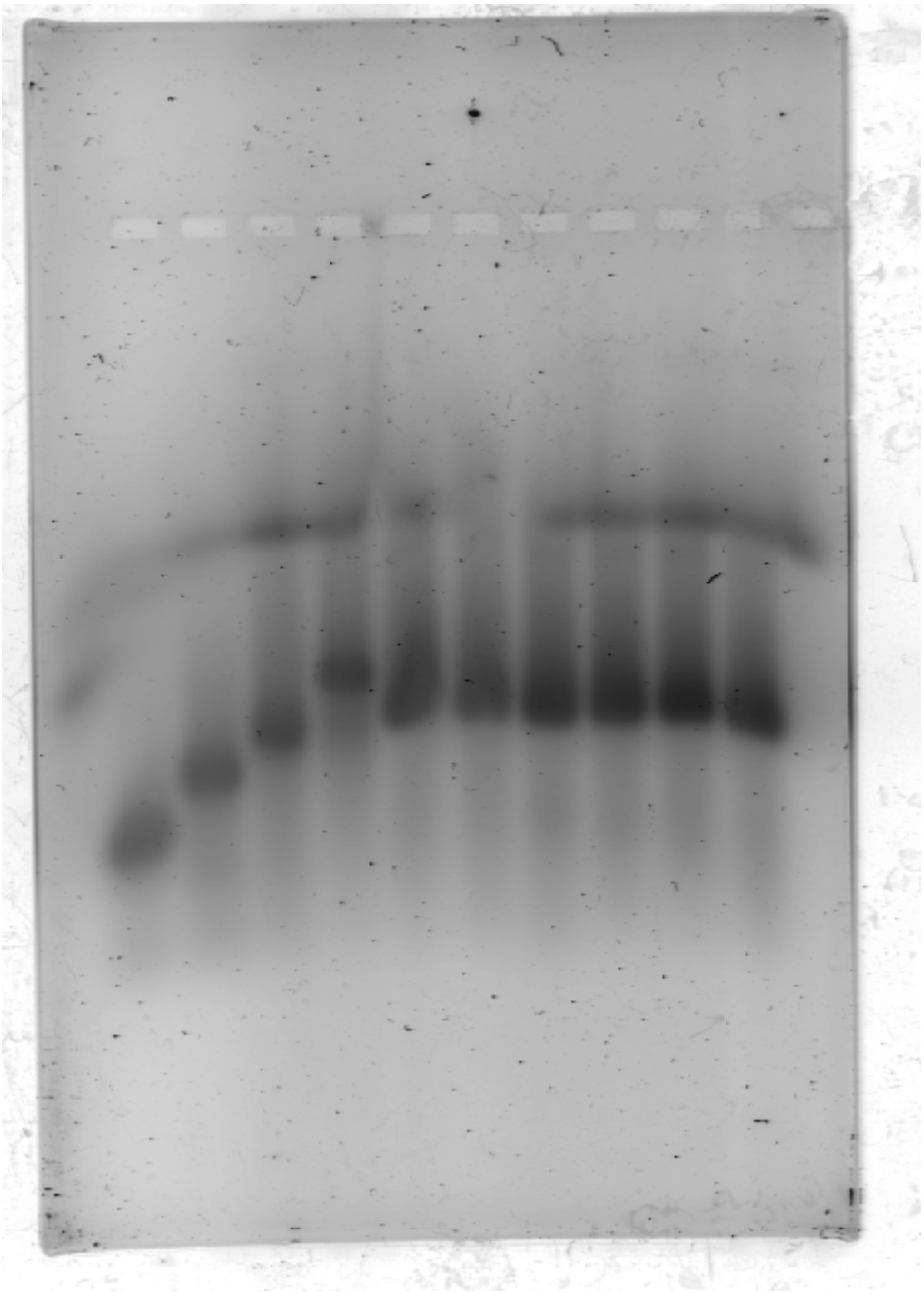
Figure S1



3 4 5 L H H1 H2 H3 H4 H5

Electrophoresis measurements were perfomed to confirm that the structures were indeed unique. In Figure S1: 3, 4, 5, L denote linear structures made from 3, 4, 5 and 6 oligonucleotides respectively and H denotes the unmarked ring-closed structure used in the melting experiments which has the same number of bases as L. H1, H2, H3, H4 and H5, are the same structure as H but with the tC^{O} label at sides 1, 2, 3, 4, 5 respectively, as used in the melting experiments.

Samples were loaded on a 4.5% Metaphor agarose gel in phosphate buffer and run for 3 hours at 4.4 Vcm^{-1} and 3˚C. The gel was then stained with SybrGold (Invitrogen) which stains for both double and single-stranded DNA and scanned on a Fluorimager 595 (Molecular Dynamics) – λ_{ex} = 488 nm, emission obtained with a 530 nm band pass filter.

No single-stranded oligonucleotides were seen in the gel indicating all structures had the correct number of strands (the horizontal band between the wells and the main bands is an artefact from the staining). There is a stepwise retardation on increasing the length of the structure from 3 to 6 strands. The ring-closed structures migrate slightly faster than the linear forms but more slowly than the linear analogue containing the same number of bases. This proves that the ring-closure has in fact taken place. All ring-closed structures are the same irrespective of the position of the tC^{O}.