Directed self-assembly of genomic sequences into monomeric and polymeric branched DNA structures

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Supplementary Information

Designing of Oligonucleotides

Oligonucleotides have been designed from the forward and reverse primers of different genes that include β-actin, Cu-Zn superoxide dismutase (SOD1), Mn-superoxide dismutase (SOD2), catalase (CAT), and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) of *Rattus norvegicus* (*primers of these genes were used for gene expression study in our previous reports; Chattopadhyay et al., Comp. Biochem. Physiol, 2007 and Subudhi U. and Chainy G.B.N. Mol. Biol. Rep. 2012*). Since the forward and reverse primers of a gene are not complementary and do not have any secondary structures, they are explored for bDNA preparation. For a single monomer unit four oligonucleotide strands are required. And each strand has two parts, the internal region and the external region. Forward and reverse primers of a particular gene are used to generate a 30 nt long internal region. Similarly external parts of a strand are derived from forward and reverse primers of a different gene. The thymine containing loop (either 3T or 5T) which joins both the internal and external region, provides the rigidity or flexibility to the bDNA structure. Hence, for a single monomer unit, primers of four different genes are being used. Both desalted and PAGE purified oligonucleotides were purchased from Integrated DNA Technologies, Inc.

In the present study, eighteen different strands (A, B, C, D, I, and J with 3T loop, E, F, G, H, K, and L with 5T loop and M, N, O, P, Q, and R without any loop) have been designed to assemble into monomeric and polymeric structures. Each strand is 66 nt long with 3T loop or 70 nt long with 5T loop. In each strand, the internal 30 nt (designed from

one gene) are flanked by external oligonucleotides containing 15 nt each (designed from a different gene). The thymine containing loop, which determines the flexibility or rigidity of the bDNA structure is present at the junction of internal and external oligonucleotides (Fig. S1a). The complementary nature of the oligos is as follows. Internal oligos of strand A or E hybridize with the internal oligos of strand B or F. Similarly, internal oligos of strand C or G bind with the internal oligos of strand D or H. Nevertheless, external oligos of strands B and F bind to the external oligos of strands C and G, respectively (Scheme 1and 2). Therefore, different combination of strands results into diverse structures. For self-assembly among the monomer units, strands A (or E) and D (or H) are designed from β-actin gene with complementary overhangs (Fig. S1b). The generality of this new approach was also verified by replacing the overhangs of the monomer unit with other strands I (or K) and J (or L) which are derived from G3PDH. However, only monomeric structures were produced using non-complementary overhangs (Fig. S1c).

Nucleic acid sequences of different strands

Different strands are mentioned below with different colour representing external oligos (blue or green), 3T or 5T loop (black), and internal oligos (pink or red). Except black, same colour oligos are complementary in nature and hence follow Watson-Crick base pairing. Strands A, B, C, and D having 3T in the loop and strands E, F, G, and H having 5T in the loop are derived from exon regions of β-actin, CAT, SOD1, and SOD2 as depicted in Figure 1. Another set of strands are also derived where β-actin is replaced with G3PDH. The external oligos of strands A, D, E, and H are replaced with nucleotide sequences derived from G3PDH without changing other regions. Hence strands I, J, K, and L are derived from the exon regions of G3PDH, SOD1, and SOD2. The sequences of M, N, O, P, Q and R is similar to A, B, C, D, I and J except the absence of thymine loop.

A: 5’ CTG ACC GAG CGT GGC TTT CTG AGG AGA GCA GCG CTT GGC CAG CGC CTC TTT CCT GCT TGC TGA TCG 3’

B: 5’ CTG AGT GAC GTT GTG TTT GAG GCG CTG GCC AAG CGC TGC TCT CCT CAG TTT CGA ATG GAG AGG CAG 3’

C: 5’ CTG CCT CTC CAT TCG TTT TGA GCA GAA GGC AAG TAG CAG GAC AGC AGA TTT CAC AAC GTC ACT CAG 3’

D: 5’ GCC ACG CTC GGT CAG TTT TCT GCT GTC CTG CTA CTT GCC TTC TGC TCA TTT CGA TCA GCA AGC AGG 3’

E: 5’ CTG ACC GAG CGT GGC TTTTT CTG AGG AGA GCA GCG CTT GGC CAG CGC CTC TTTTT CCT GCT TGC TGA TCG 3’

F: 5’ CTG AGT GAC GTT GTG TTTTT GAG GCG CTG GCC AAG CGC TGC TCT CCT CAG TTTTT CGA ATG GAG AGG CAG 3’

G: 5’ CTG CCT CTC CAT TCG TTTTT TGA GCA GAA GGC AAG TAG CAG GAC AGC AGA TTTTT CAC AAC GTC ACT CAG 3’

H: 5’ GCC ACG CTC GGT CAG TTTTT TCT GCT GTC CTG CTA CTT GCC TTC TGC TCA TTTTT CGA TCA GCA AGC AGG 3’

I: 5’ CTC AAG ATT GTC AGC TTT CTG AGG AGA GCA GCG CTT GGC CAG CGC CTC TTT AGA TCC ACA ACG GAT 3’

J: 5’ GCT GAC AAT CTT GAG TTT TCT GCT GTC CTG CTA CTT GCC TTC TGC TCA TTT ATC CGT TGT GGA TCT 3’

K: 5’ CTC AAG ATT GTC AGC TTTTT CTG AGG AGA GCA GCG CTT GGC CAG CGC CTC TTTTT AGA TCC ACA ACG GAT 3’

L: 5’ GCT GAC AAT CTT GAG TTTTT TCT GCT GTC CTG CTA CTT GCC TTC TGC TCA TTTTT ATC CGT TGT GGA TCT 3’

M: 5’ CTG ACC GAG CGT GGC CTG AGG AGA GCA GCG CTT GGC CAG CGC CTC CCT GCT TGC TGA TCG 3’

N: 5’ CTG AGT GAC GTT GTG GAG GCG CTG GCC AAG CGC TGC TCT CCT CAG CGA ATG GAG AGG CAG 3’

O: 5’ CTG CCT CTC CAT TCG TGA GCA GAA GGC AAG TAG CAG GAC AGC AGA CAC AAC GTC ACT CAG 3’

P: 5’ GCC ACG CTC GGT CAG TCT GCT GTC CTG CTA CTT GCC TTC TGC TCA CGA TCA GCA AGC AGG 3’

Q: 5’ CTC AAG ATT GTC AGC CTG AGG AGA GCA GCG CTT GGC CAG CGC CTC AGA TCC ACA ACG GAT 3’

R: 5’ GCT GAC AAT CTT GAG TCT GCT GTC CTG CTA CTT GCC TTC TGC TCA ATC CGT TGT GGA TCT 3’

Self-assembly study

Self assembly among oligos was carried out in TAE/Mg_2^+ buffer. The TAE/Mg_2^+ buffer consisted of Tris base (40 mM, pH 8.0), acetic acid (20 mM), EDTA (2 mM) and $Mg(Ac)_2$ (12.5 mM). In 25 µl reaction each oligo were combined in equimolar (25 pmol each) ratio, denatured at 95°C for 9 min and then cooled to 4°C with ramp rate 0.3°C/sec using a thermal cycler (S1000, Bio-Rad). All the self-assembly reactions were performed 5 times in the same experimental condition. bDNA samples were then directly used for characterization, without further fractionation or purification.

Native Polyacrylamide gel electrophoresis

Gels contained 10% polyacrylamide (29:1 acrylamide/bisacrylamide) and were run on a SE260 electrophoresis unit (Hoffer) at 4°C (150V, constant voltage) with TAE as running buffer. After electrophoresis, the gels were stained with ethidium bromide and documented in FluroChemE system (Cell Biosciences).

Circular dichroism (CD) analysis

The circular dichroism (CD) spectra of self-assembled bDNAs were measured at 25°C using a Chirascan spectrophotometer (Applied Photophysics) operated with a scan speed of 60 nm/min, a bandwidth of 1 nm and a time per point of 0.5 sec. Spectra were recorded between 320 and 200 nm with a cell of path length of 10 mm. All the spectra were corrected for hybridizing buffer background. Three spectra were averaged for getting the final spectra.

DNA melting curve analysis

The DNA melting curves of self-assembled bDNAs was assessed using a Realplex master cycler (Eppendorf) using SYBR Green I. Self assembly among oligos was carried out in TAE/Mg_2^+ buffer. In 10 µl reaction each oligo were combined in equimolar (25 pmol each) ratio in presence of 1x concentration of SYBR Green I as mentioned by the manufacturer. The mixture was denatured at 95°C for 9 min and then cooled to 4°C with ramp rate 0.3°C/sec using Realplex, followed by thermal melting at 4.5°C/min till 95°C. All the reactions were performed 3 times in the same experimental condition and the mean value of fluorescence were plotted against temperature.

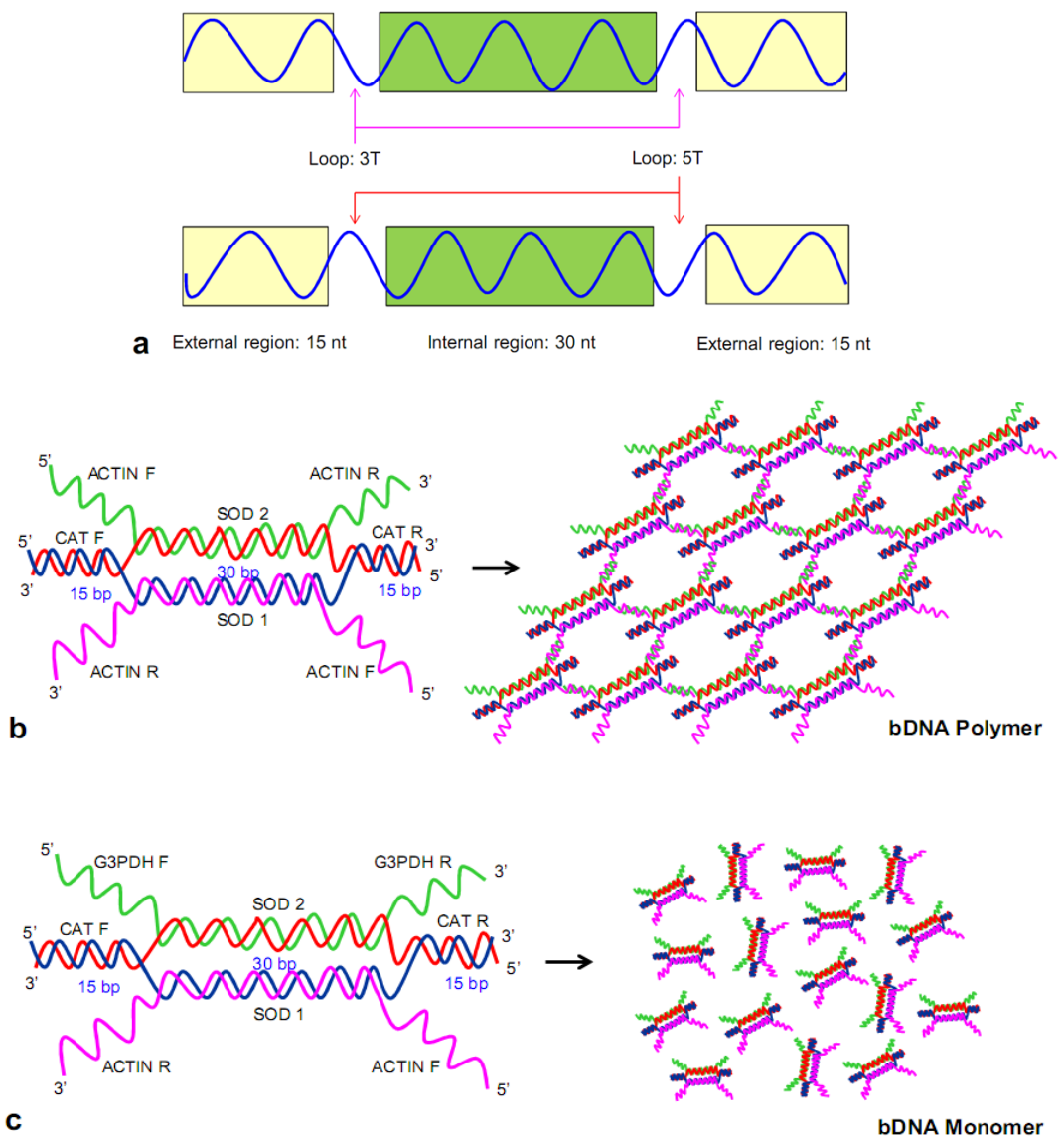

Figure S1. Basic designing of individual oligos. (a) Individual oligos have three parts, the internal 30 nt is flanked by two external ssDNA containing 15 nt each. At the junction of internal and external oligos a loop is present with three or five numbers of thymine, which determine the flexibility or rigidity of the structure. Therefore two sets of oligos were designed one with 3T and the other set with 5T at the loop. (b) Monomer-I with complementary overhangs further self-assemble into polymeric bDNA structure which contains large number of single-stranded arms and double-stranded ends. (c) The two external oligos are devoid of complementary overhangs hence results into only monomeric structure (IBCD or KFGH) after self-assembly. The external oligos of one strand is derived from G3PDH, where the other one is designed from β-actin.

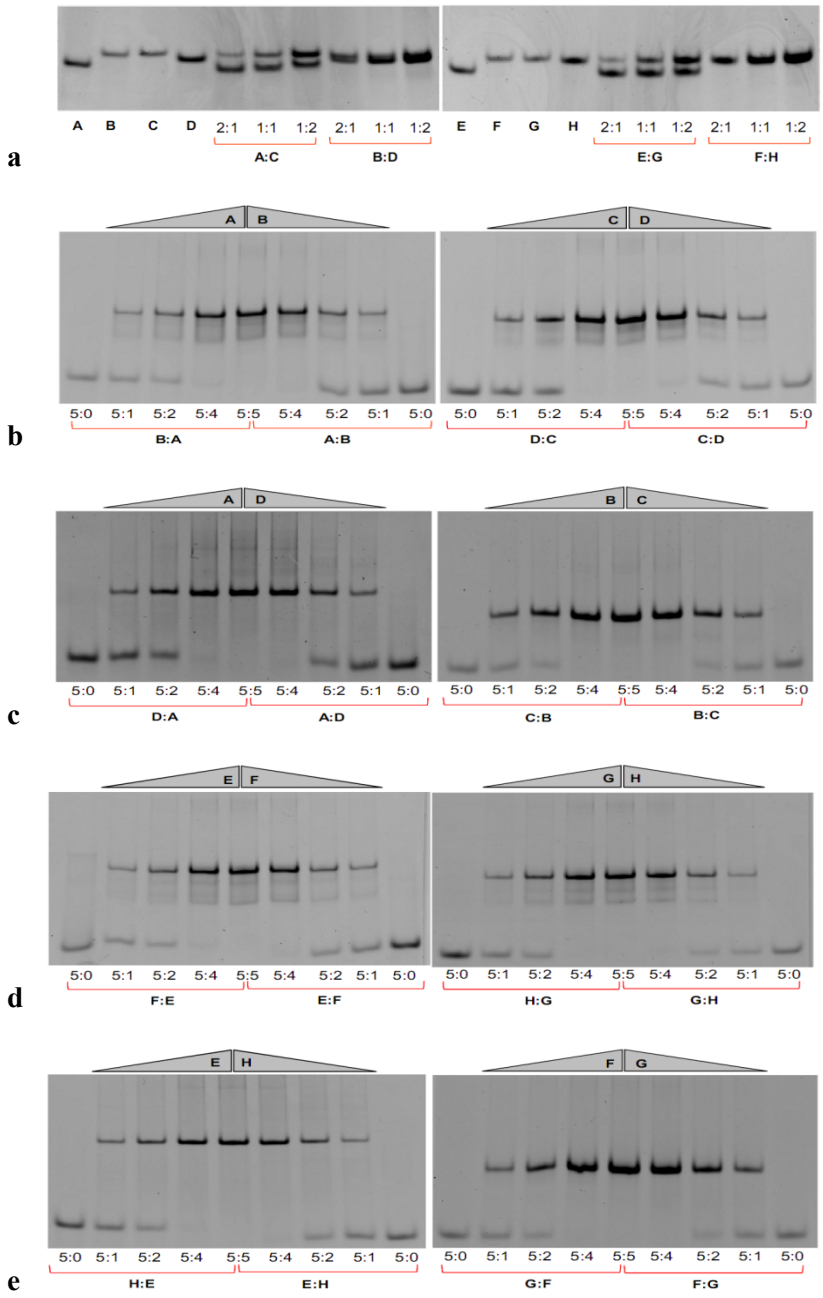

Figure S2. Characterization of the self-assembled di-oligo complexes with different molar concentrations (0.4, 0.8, 1.6 and 2 µM) in nPAGE (10%). The sample composition and ratio between two oligos is mentioned at the bottom of each gel (1:1 or 5:5 ratio is equivalent to 2 µM oligos each). (a) Gels show the individual oligos and non-interacting oligos. (b-e) Gels show two different oligos combine to form di-oligo complexes.

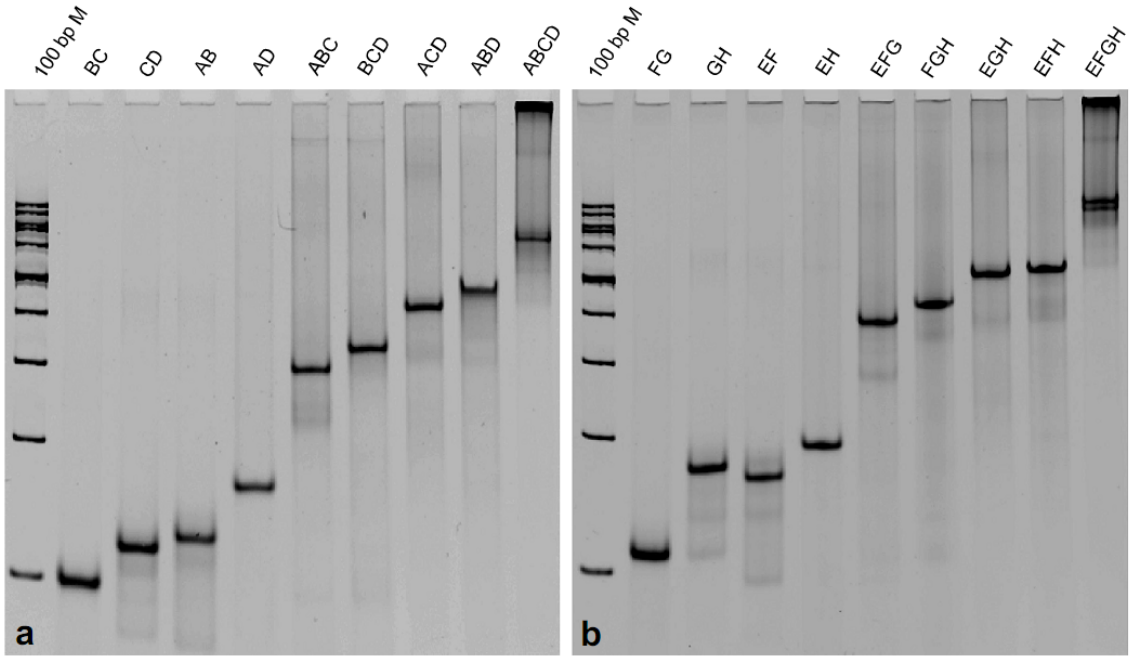

Figure S3: Characterization of the self-assembled bDNA structures in nPAGE (10%). The sample composition is labeled on the top of each lane. (a) Self-assembly of desalted-oligos A, B, C, and D in different combination. (b) Self-assembly of desalted oligos E, F, G, and H in different combination. Gels showing the differential migration pattern of di, tri, and tetra-oligo complexes and polymeric structure against 100 bp DNA ladder. The four different strands are derived from the exon regions of β-actin, SOD1, SOD2, and CAT.

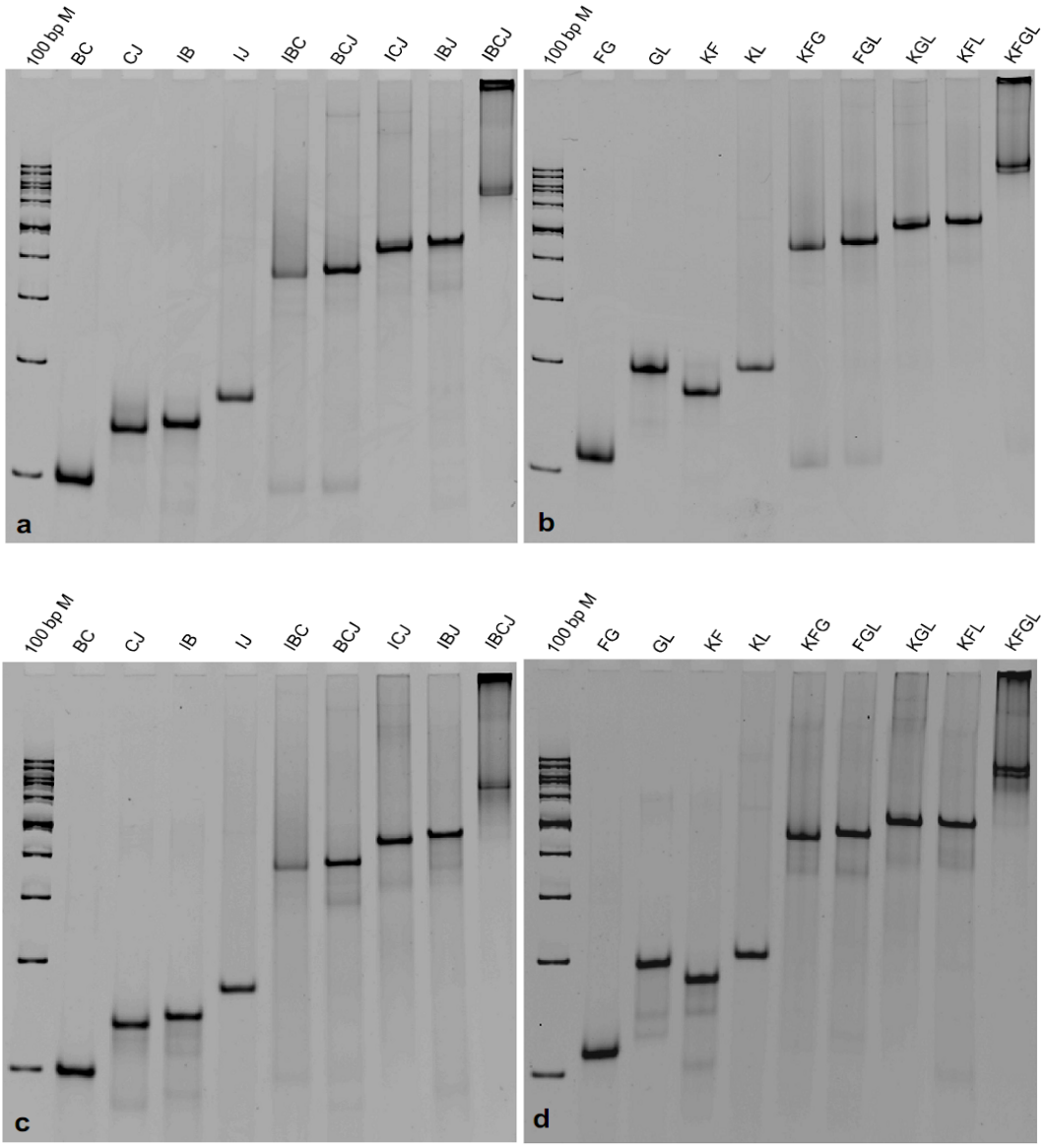

Figure S4. Characterization of the self-assembled bDNA structures in nPAGE (10%). The sample composition is labeled on the top of each lane. Gels showing the differential migration pattern of di, tri, and tetra-oligo complexes and polymeric structure against 100 bp DNA ladder. The four different strands are derived from the exon regions of G3PDH, SOD1, SOD2, and CAT. (a) Self-assembly of PAGE-purified oligos I, B, C, and J in different combination. (b) Self-assembly of PAGE-purified oligos K, F, G, and L in different combination. (c) Self-assembly of desalted oligos I, B, C, and J in different combination. (d) Self-assembly of desalted oligos K, F, G, and L in different combination.

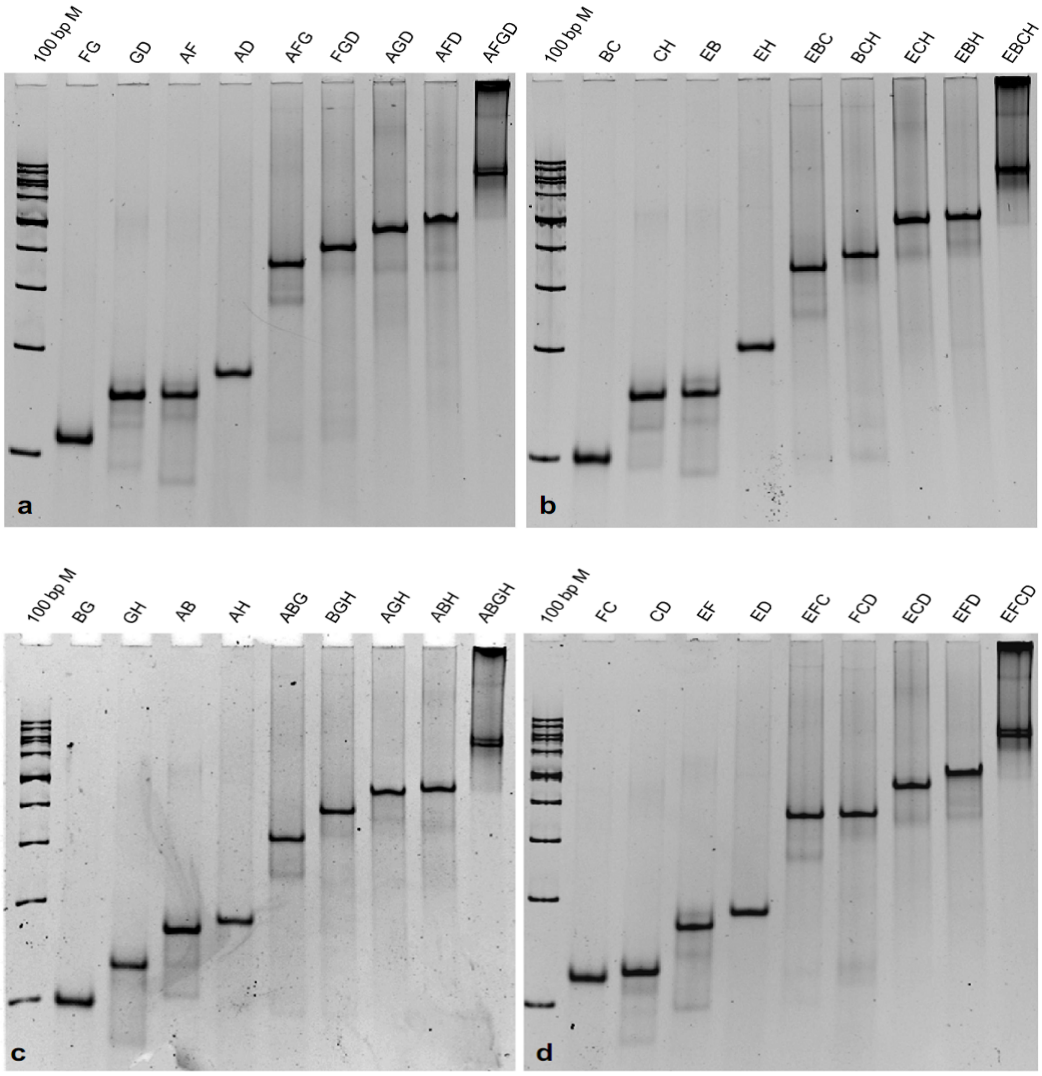

Figure S5. Effect of loop length on self-assembly and characterization of the self-assembled bDNA structures in nPAGE (10%). The sample composition is labeled on the top of each lane. Gels showing the differential migration pattern of di, tri, and tetra-oligo complexes and polymeric structure against 100 bp DNA ladder. (a) Self-assembly of strands A, F, G, and D in different combination, where external strands contain 3T and internal strands contain 5T in the loop. (b) Self-assembly of strands E, B, C, and H in different combination, where internal strands with 3T and external strands with 5T. (c) Self-assembly of strands A, B, G, and H in different combination, where strands A and B contain 3T and strands G and H contain 5T. (d) Self-assembly of strands E, F, C, and D in different combination, where strands E and F contain 5T and strands C and D contain 3T.

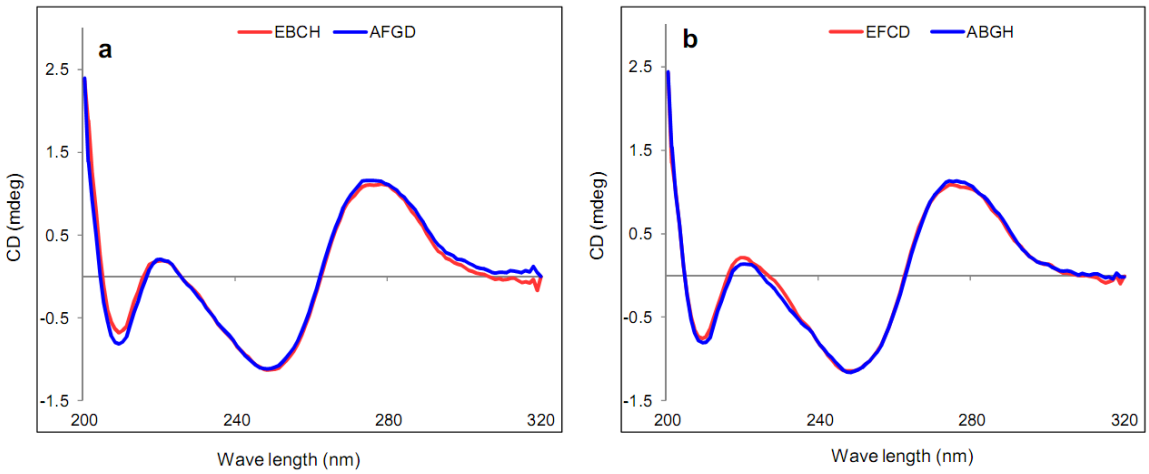

Figure S6. Circular dichroism (CD) characterization of self-assembled bDNAs. All bDNA structures (EBCH, AFGD, EFCD and ABGH) exhibit typical B-DNA conformation.