SUPPORTING INFORMATION:

Design and Characterization of Compact, Programmable, Multistranded Non-Immunostimulatory Nucleic Acid Nanoparticles Suitable for Biomedical Applications

*Ross Brumett[1], Leyla Danai[2], Abigail Coffman[1], Yasmine Radwan[2], Megan Teter[1], Hannah Hayth[1], Erwin Doe[1], Katelynn Pranger[1], Sable Thornburgh[1], Allison Dittmer[1], Zhihai Li[1], Tae Jin Kim[3], Kirill A. Afonin[2], and Emil F. Khisamutdinov[1]**

[1] Department of Chemistry, Ball State University, Muncie, IN 47306, USA

[2] Department of Chemistry, University of North Carolina at Charlotte, Charlotte, NC, 28223, USA

[3] Department of Physical Sciences, West Virginia University Institute of Technology, Beckley, WV, 25801, USA

* Corresponding Author:

Department of Chemistry, Ball State University, Muncie, IN 47306. Email: kemil@bsu.edu

Supporting Table S1. DNA triangle sequences utilized in this project.

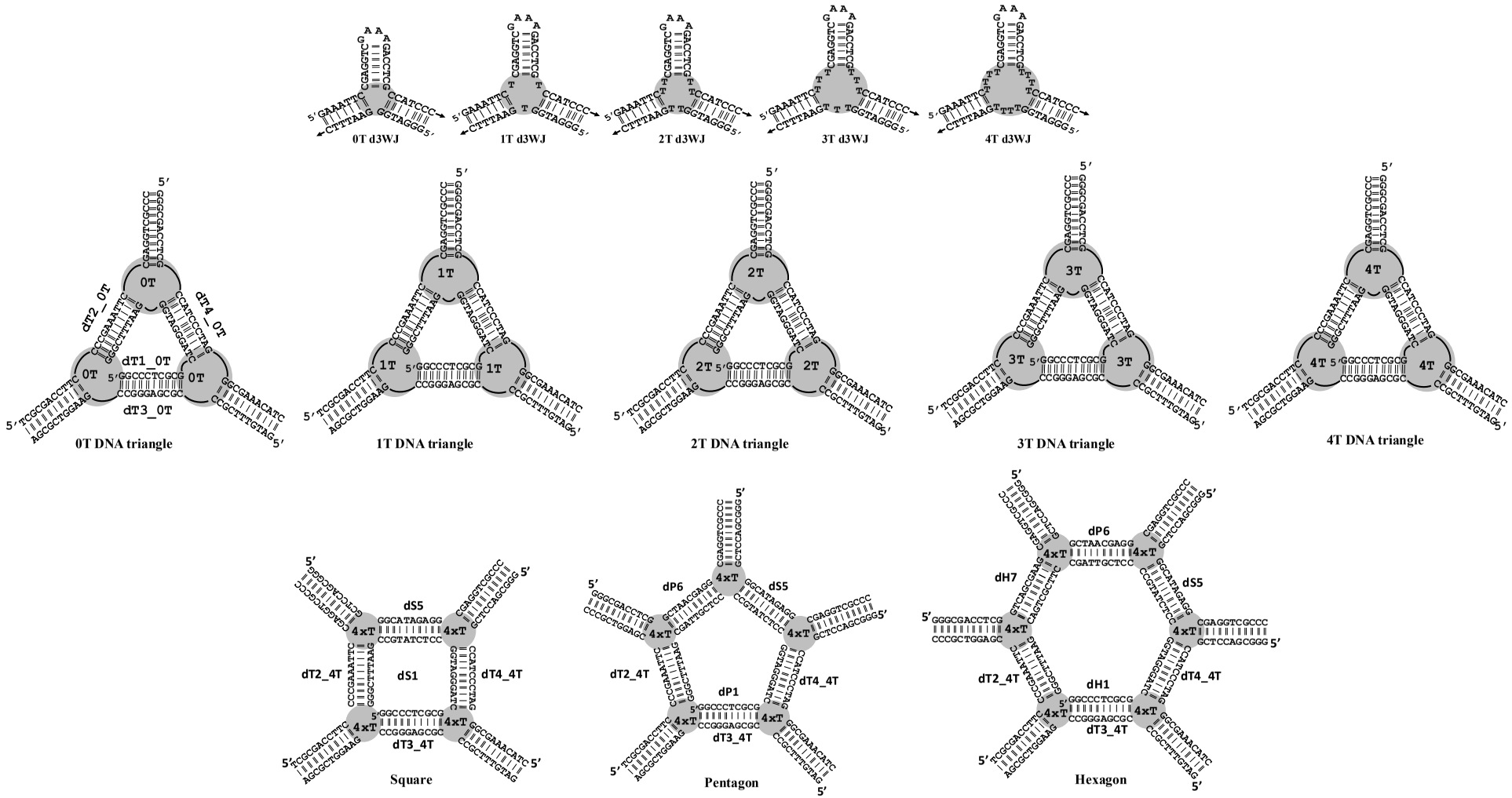
DNA Complex name	Strands name	Sequences
0T d3WJ	ssDNA1 noT	GAA ATT CCG AGG TCG AAA GAC CTC GCC ATC CC
	ssDNA2 noT	GGG ATG GGA ATT TC
1T d3WJ	ssDNA1 1xT	GAA ATT CTC GAG GTC GAA AGA CCT CGT CCA TCC C
	ssDNA2 1xT	GGG ATG GTG AAT TTC
2T d3WJ	ssDNA1 2xT	GAA ATT CTT CGA GGT CGA AAG ACC TCG TTC CAT CCC
	ssDNA2 2xT	GGG ATG GTT GAA TTT C
3T d3WJ	ssDNA1 3xT	GAA ATT CTT TCG AGG TCG AAA GAC CTC GTT TCC ATC CC
	ssDNA2 3xT	GGG ATG GTT TGA ATT TC
4T d3WJ	ssDNA1 4xT	GAA ATT CTT TTC GAG GTC GAA AGA CCT CGT TTT CCA TCC C
	ssDNA2 4xT	GGG ATG GTT TTG AAT TTC
0T DNA triangle	dT1_0T	GGCCCTCGCG CTAGGGATGG GAATTTCGGG
	dT2_0T	TCGCGACCTTC CCCGAAATTC CGAGGTCGCCC
	dT3_0T	GATGTTTCGCC CGCGAGGGCC GAAGGTCGCGA
	dT4_0T	GGGCGACCTCG CCATCCCTAG GGCGAAACATC
1T DNA triangle	dT1_1T	GGCCCTCGCG T CTAGGGATGG T GAATTTCGGG
	dT2_1T	TCGCGACCTTC T CCCGAAATTC T CGAGGTCGCCC
	dT3_1T	GATGTTTCGCC T CGCGAGGGCC T GAAGGTCGCGA
	dT4_1T	GGGCGACCTCG T CCATCCCTAG T GGCGAAACATC
2T DNA triangle	dT1_2T	GGCCCTCGCG TT CTAGGGATGG TT GAATTTCGGG
	dT2_sh2T	TCGCGACCTTC TT CCCGAAATTC TT CGAGGTCGCCC
	dT3_2T	GATGTTTCGCC TT CGCGAGGGCC TT GAAGGTCGCGA
	dT4_2T	GGGCGACCTCG TT CCATCCCTAG TT GGCGAAACATC
3T DNA triangle	dT1_3T	GGCCCTCGCG TTT CTAGGGATGG TTT GAATTTCGGG
	dT2_3T	TCGCGACCTTC TTT CCCGAAATTC TTT CGAGGTCGCCC
	dT3_3T	GATGTTTCGCC TTT CGCGAGGGCC TTT GAAGGTCGCGA
	dT4_3T	GGGCGACCTCG TTT CCATCCCTAG TTT GGCGAAACATC
4T DNA triangle	dT1_4T	GGCCCTCGCG TTTT CTAGGGATGG TTTT GAATTTCGGG
	Alexa 488_4T*	/5Alex488N/TT GGCCCTCGCG TTTT CTAGGGATGG TTTT GAATTTCGGG
	dT2_4T	TCGCGACCTTC TTTT CCCGAAATTC TTTT CGAGGTCGCCC
	dT3_4T	GATGTTTCGCC TTTT CGCGAGGGCC TTTT GAAGGTCGCGA
	dT4_4T	GGGCGACCTCG TTTT CCATCCCTAG TTTT GGCGAAACATC

*This strand used to assemble Alexa-488 fluorogenic DNA nanoparticle.

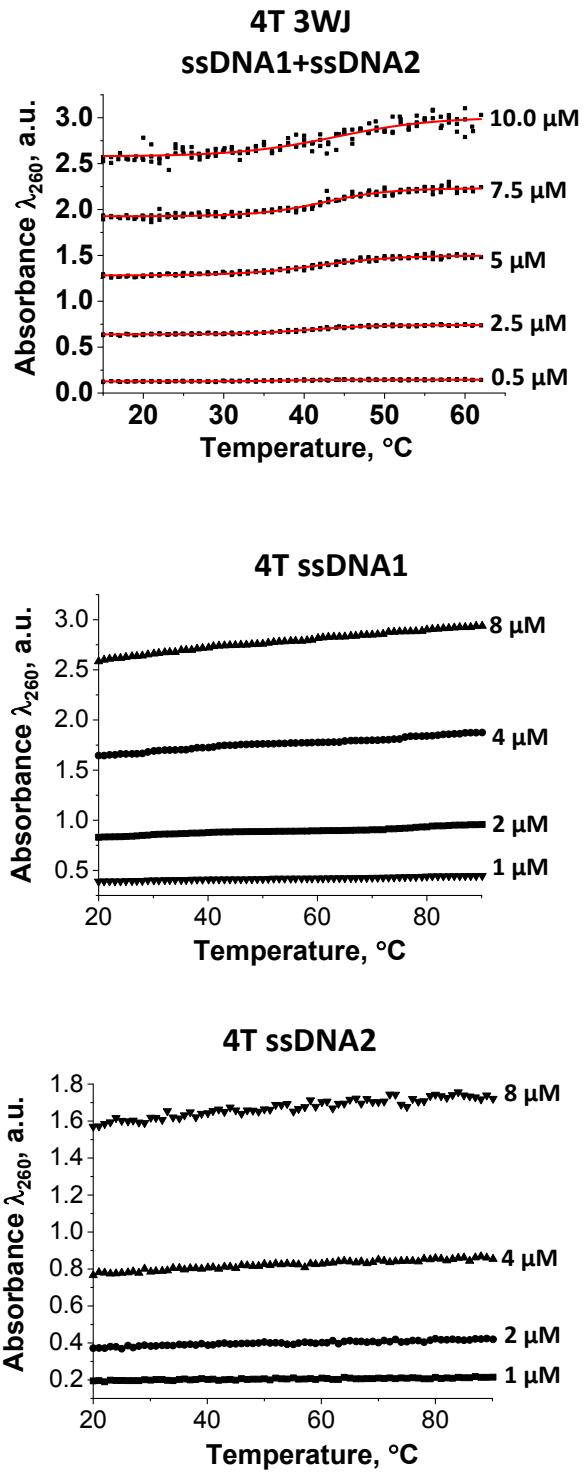
Supporting Table S2. DNA Polygon sequences utilized in this project.

DNA NP name	Strands name	Sequence
SQR	dS1	G GCC CTC GCG TTT TCT AGG GAT GGT TTT CCT CTA TGC CTT TTG AAT TTC GGG
	Alexa 488_dS1*	G GCC CTC GCG TTT TCT AGG GAT GGT TTT CCT CTA TGC CTT TTG AAT TTC GGG
	dT2_4T	TCGCGACCTTCTTTTCCCGAAATTCTTTTCGAGGTCGCCC
	dT3_4T	GATGTTTCGCCTTTTCGCGAGGGCCTTTTGAAGGTCGCGA
	dT4_4T	GGGCGACCTCGTTTTCCATCCCTAGTTTTGGCGAAACATC
	dS5	GGGCGACCTCGTTTTGGCATAGAGGTTTTCGAGGTCGCCC
PENT	dP1	G GCC CTC GCG TTT TCT AGG GAT GGT TTT CCT CTA TGC CTT TTC CTC GTT AGC TTT TGA ATT TCG GG
	Alexa 488_dP1*	/5Alex488N/TT G GCC CTC GCG TTT TCT AGG GAT GGT TTT CCT CTA TGC CTT TTC CTC GTT AGC TTT TGA ATT TCG GG
	dT2_4T	TCGCGACCTTCTTTTCCCGAAATTCTTTTCGAGGTCGCCC
	dT3_4T	GATGTTTCGCCTTTTCGCGAGGGCCTTTTGAAGGTCGCGA
	dT4_4T	GGGCGACCTCGTTTTCCATCCCTAGTTTTGGCGAAACATC
	dS5	GGGCGACCTCGTTTTGGCATAGAGGTTTTCGAGGTCGCCC
	dP6	GGGCGACCTCGTTTTGCTAACGAGGTTTTCGAGGTCGCCC
HEX	dH1	G GCC CTC GCG TTT TCT AGG GAT GGT TTT CCT CTA TGC CTT TTC CTC GTT AGC TTT TCT TCG CTG ACT TTT GAA TTT CGG G
	Alexa 488_dH1*	/5Alex488N/TT G GCC CTC GCG TTT TCT AGG GAT GGT TTT CCT CTA TGC CTT TTC CTC GTT AGC TTT TCT TCG CTG ACT TTT GAA TTT CGG G
	dT2_4T	TCGCGACCTTCTTTTCCCGAAATTCTTTTCGAGGTCGCCC
	dT3_4T	GATGTTTCGCCTTTTCGCGAGGGCCTTTTGAAGGTCGCGA
	dT4_4T	GGGCGACCTCGTTTTCCATCCCTAGTTTTGGCGAAACATC
	dS5	GGGCGACCTCGTTTTGGCATAGAGGTTTTCGAGGTCGCCC
	dP6	GGGCGACCTCGTTTTGCTAACGAGGTTTTCGAGGTCGCCC
	dH7	GGGCGACCTCGTTTTGTCAGCGAAGTTTTCGAGGTCGCCC

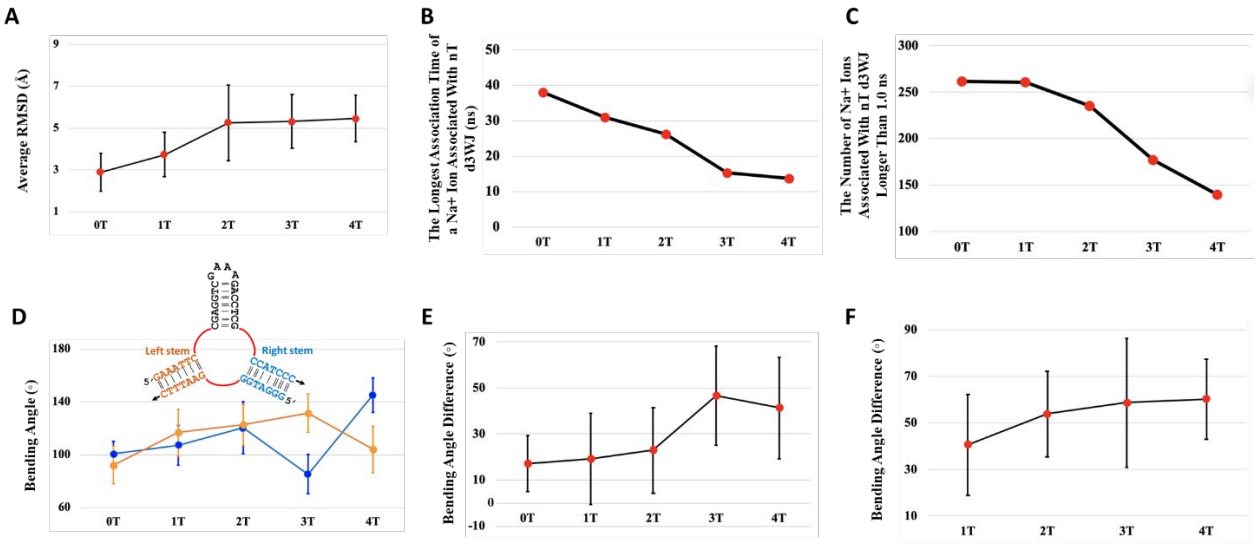
*These strands were used to assemble Alexa-488 fluorogenic DNA nanoparticles.



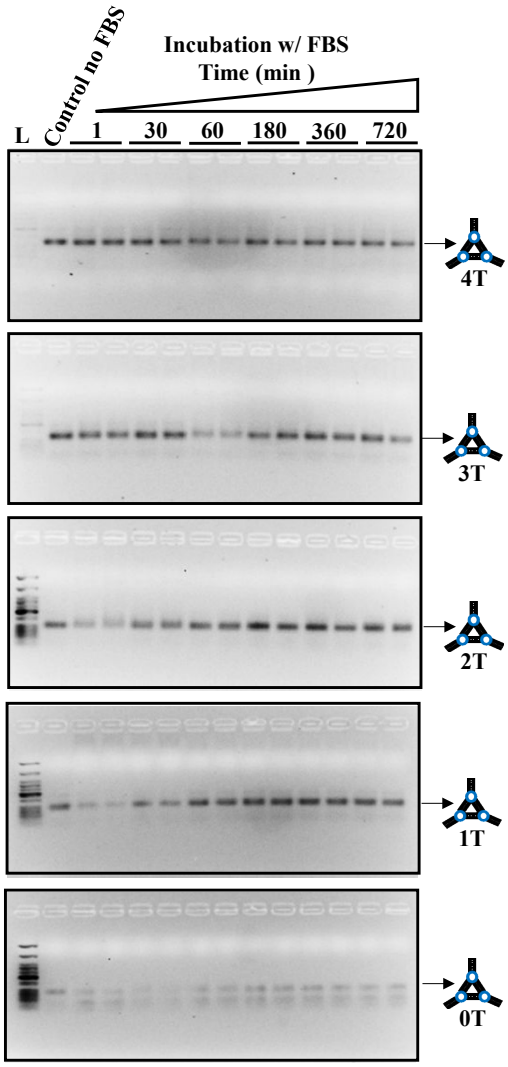
Supporting Figure S1. DNA secondary structures used in this project.



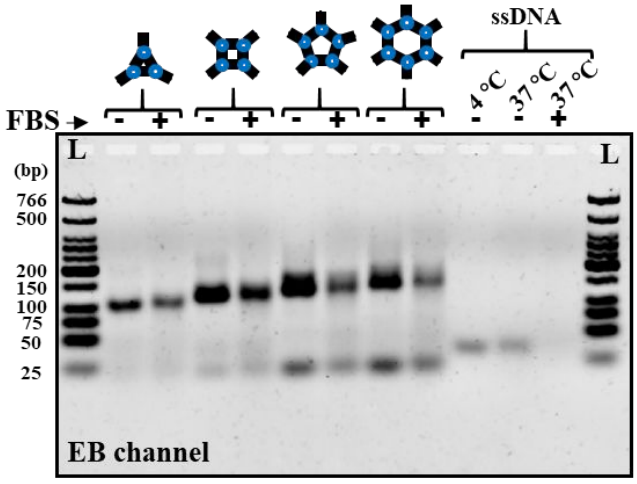
Supporting Figure S2. Examples of UV-melt profiles for 4T ssDNA1 and 4T ssDNA2 in CB1 buffer at various concentrations showing no melting transition as compared to 4T d3WJ.



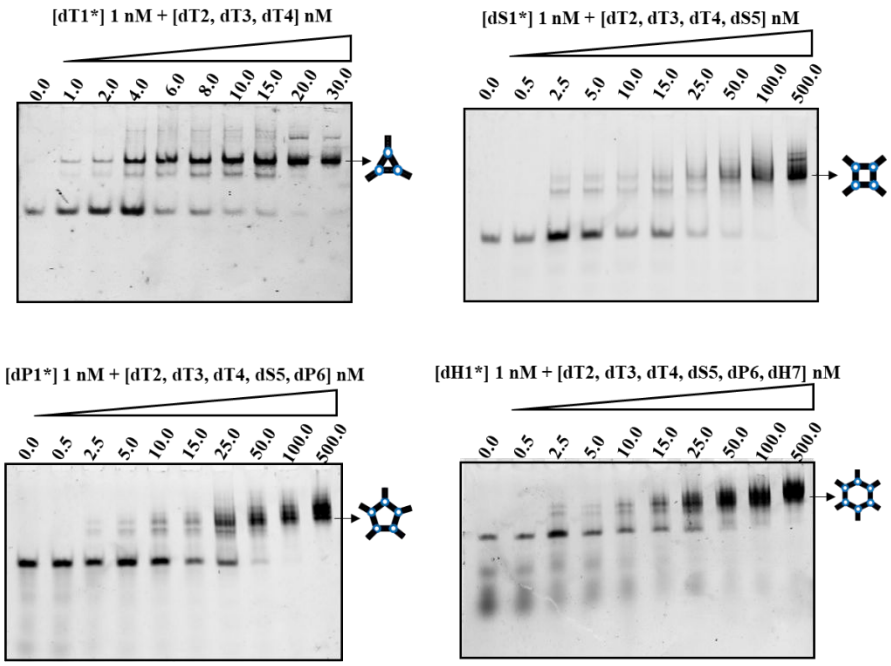
Supporting Figure S3. MD simulation data. A) The average root-mean-square deviation (RMSD) of nT d3WJ (n = 0 − 4). Vertical bars indicate standard deviation values of each RMSD. B) the longest association time of Na^+ ion associate with d3WJs. C) the number of Na^+ ions associated with d3WJs longer than 1.0 ns. D) The average bending angles of the left (orange) and the right (blue) stems. E) The average bending angle difference between the left and the right stems. F) average bending angle difference between nT d3WJ (n = 1 − 4) and 0T d3WJ. Vertical bars indicate standard deviations of bending angle.



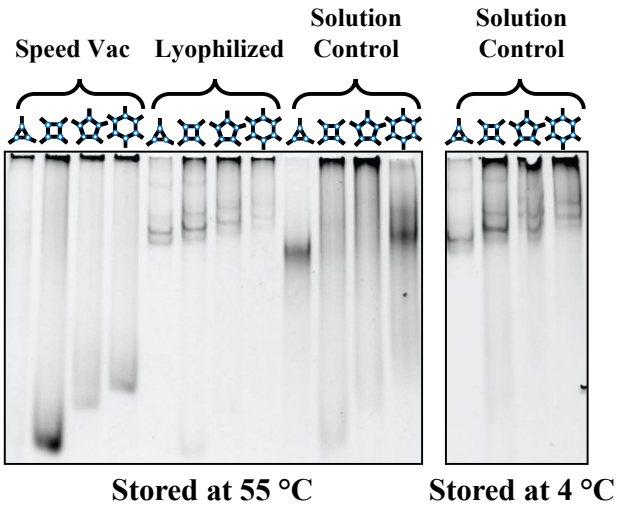
Supporting Figure S4. Stability of triangle DNA nanoparticles in 10% FBS at various time points evaluated by 4% agarose gel electrophoresis. Lane L corresponds to DNA molecular ladder (Low Weight DNA, New England Biolabs).



Supporting Figure S5. DNA Polygon stability assay in 10% FBS solution assayed by 4% agarose gel electrophoresis. The single stranded DNA (ssDNA) 5'- GGCCCTCGCGTTTTCTAG GGATGGTTTTCCTCTATGCCTTTTGAATTTCGGG-3' was used as a positive control. Lane L is Low Weight DNA molecular ladder (NewEngland Biolabs).



Supporting Figure S6. Examples of 7 % native PAGE analysis of DNA polygon formation. The fixed concentration (1 nM) of Alexa488-labeled central DNA strands were titrated with incremental addition of equimolar solution of unlabeled DNA strands covering 0.5 nM- 500 nM range.



Supporting Figure S7. DNA polygon structural integrity assessment by 8% native PAGE after prolonged storage in dehydrated form in harsh conditions