Electronic Supplementary Material (ESI) for Chemical Communications

DNA nanostructure serum stability: greater than the sum of its parts

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Supplementary Information

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S-I. General

StainsAll®, acetic acid, tris(hydroxymethyl)-aminomethane (Tris), formamide and urea were used as purchased from Aldrich. Acetic acid and boric acid were purchased from Fisher Scientific and used without further purification. Nucleoside (dA, dC, dG and T) derivatized and universal 1000Å LCAA-CPG supports with loading densities between 25-40 µmol/g and reagents used for automated DNA synthesis were purchased through Bioautomation Corporated. Size-exclusion columns (sephadex G-25, DNA grade) were purchased from Glen Research. Fetal bovine serum (FBS) and phosphate buffered saline (PBS) were purchased from Wisent Corporated. 1xTBE buffer is composed of 0.09M Tris and Boric acid (TB) and 2 mM EDTA with a pH ~8.3. 1xTAMg buffer is composed of 45 mM Tris and 12.6 mM $Mg(OAc)_2 \cdot 6H_2O$. The pH of the 1xTAMg buffer was adjusted to 8 using glacial acetic acid. Dulbecco's Modified Eagle Medium (DMEM) was purchased from Invitrogen. The T4 DNA Ligase (400,000 units/ml) was purchased from New England BioLabs.

S-II. Instrumentation

Standard automated oligonucleotide solid-phase synthesis was performed on a Mermade MM6 synthesizer from Bioautomation. Gel electrophoresis experiments were carried out on an

acrylamide 20 X 20 cm vertical Hoefer 600 electrophoresis unit. Annealing of all structures was conducted using an Eppendorf Mastercycler Pro. DNA quantification was performed using a BioTek Synergy HT microplate reader.

S-III. Solid phase synthesis of 3D DNA 'clip' strands

General Procedure for Solid-Phase DNA Synthesis:

DNA synthesis was performed on a 1 μmole scale, starting from the required nucleotide modified or universal 1000 Å LCAA-CPG solid-supports. Coupling efficiency was monitored after removal of the dimethoxytrityl (DMT) 5′-OH protecting groups. The hexaethylene glycol (**HEG**) and hexane diol (**C6**) phosphoramidites were purchased from ChemGenes Inc. Phosphoramidites **HEG** and **C6** were initially diluted with dichloromethane (DCM) to a concentration of 0.1 M in a glove box. For DNA couplings, approximately 10-fold excess of each phosphoramidite was used in comparison to DNA. For off-column couplings, an equal volume of ethylthiotetrazole (0.1M in acetonitrile, Glen Research) was combined with each phosphoramidite and manually coupled on the DNA solid support with an extended reaction time of 10 minutes. After coupling, supports were removed from the glove box and returned to the DNA synthesizer for oxidation, capping and deblock steps. The chemical phosphorylation reagent (**P**, Glen Research, cat # 10-1900) was coupled in a similar fashion, however this compound was initially dissolved in acetonitrile. All sequences, modified and unmodified, were fully deprotected in concentrated ammonium hydroxide (25 ^{o}C/1.5 hours followed by 60 ^{o}C/2 hour).

Supplementary Table 1| Oligonucleotides prepared via solid-phase synthesis. The **TTTT** represents a short non-base pairing spacer that is inserted within each strand and ends up near the junction points (or hybridization regions) of assembled 3D structures. Non-nucleoside phosphoramidites hexaethylene glycol (**HEG**), 1,6-hexanediol (**C6**) and chemical phosphorylation reagent (**P**) have been inserted selectively into individual sequences as indicated.

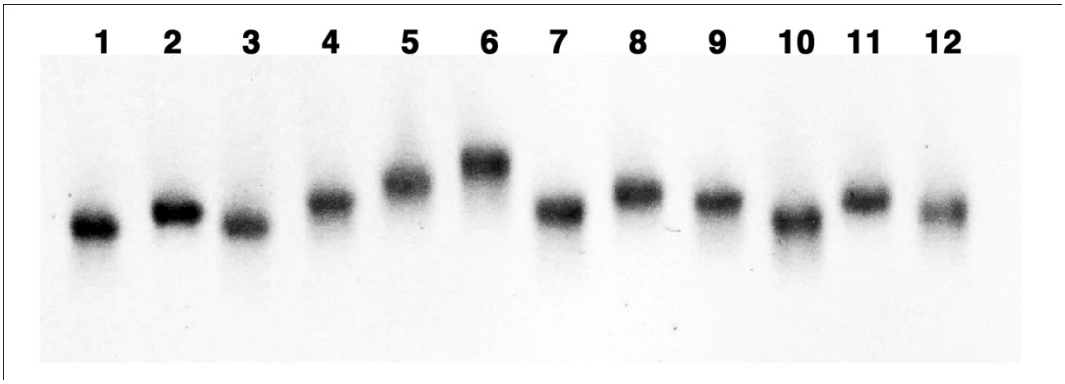
Name	Sequence (5′ → 3′)
1	TCGCTGAGTA**TTTT**GCCTGGCCTTGGTCCATTTG**TTTT**GCAAGTGTGGGCA CGCACAC**TTTT**CGCACCGCGACTGCGAGGAC**TTTT**CACAAATCTG
2	CACTGGTCAG**TTTT**CCACCAGCTAGATGTTGAAG**TTTT**TACTCAGCGACAG ATTTGTG**TTTT**CGCTCTTCTATACTGGCGGA**TTTT**GGTTTGCTGA
3	CCACACTTGC**TTTT**GTCGACACAGTAGCAGTGTG**TTTT**CTGACCAGTGTCA GCAAACC**TTTT**CCATGACGATGCACTACATG**TTTT**GTGTGCGTGC

1_H	**(HEG)**TCGCTGAGTA**TTTT**GCCTGGCCTTGGTCCATTTG**TTTT**GCAAGTGTG GGCACGCACAC**TTTT**CGCACCGCGACTGCGAGGAC**TTTT**CACAAATCTG**(H EG)**
2_H	**(HEG)**CACTGGTCAG**TTTT**CCACCAGCTAGATGTTGAAG**TTTT**TACTCAGCG ACAGATTTGTG**TTTT**CGCTCTTCTATACTGGCGGA**TTTT**GGTTTGCTGA**(HE G)**
3_H	**(HEG)**CCACACTTGC**TTTT**GTCGACACAGTAGCAGTGTG**TTTT**CTGACCAGT GTCAGCAAACC**TTTT**CCATGACGATGCACTACATG**TTTT**GTGTGCGTGC**(H EG)**
1_6	**(C6)**TCGCTGAGTA**TTTT**GCCTGGCCTTGGTCCATTTG**TTTT**GCAAGTGTGGG CACGCACAC**TTTT**CGCACCGCGACTGCGAGGAC**TTTT**CACAAATCTG**(C6)**
2_6	**(C6)**CACTGGTCAG**TTTT**CCACCAGCTAGATGTTGAAG**TTTT**TACTCAGCGA CAGATTTGTG**TTTT**CGCTCTTCTATACTGGCGGA**TTTT**GGTTTGCTGA**(C6)**
3_6	**(C6)**CCACACTTGC**TTTT**GTCGACACAGTAGCAGTGTG**TTTT**CTGACCAGTG TCAGCAAACC**TTTT**CCATGACGATGCACTACATG**TTTT**GTGTGCGTGC**(C6)**
1_P	**P**TCGCTGAGTA**TTTT**GCCTGGCCTTGGTCCATTTG**TTTT**GCAAGTGTGGGCA CGCACAC**TTTT**CGCACCGCGACTGCGAGGAC**TTTT**CACAAATCTG
2_P	**P**CACTGGTCAG**TTTT**CCACCAGCTAGATGTTGAAG**TTTT**TACTCAGCGACA GATTTGTG**TTTT**CGCTCTTCTATACTGGCGGA**TTTT**GGTTTGCTGA
3_P	**P**CCACACTTGC**TTTT**GTCGACACAGTAGCAGTGTG**TTTT**CTGACCAGTGTC AGCAAACC**TTTT**CCATGACGATGCACTACATG**TTTT**GTGTGCGTGC
1-TOP	CAAATGGACCAAGGCCAGGC
1-BOT	GTCCTCGCAGTCGCGGTGCG
2-TOP	CTTCAACATCTAGCTGGTGG
2-BOT	TCCGCCAGTATAGAAGAGCG
3-TOP	CACACTGCTACTGTGTCGAC
3-BOT	CATGTAGTGCATCGTCATGG

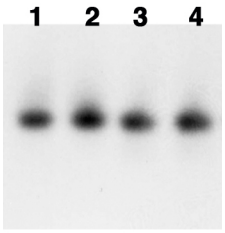
C1	TCGCTGAGTA**TTTT**TCAACTGCTC**TTTT**GCAAGTGTGGGCACGCACAC**TTT T**TCAACTGCTC**TTTT**CACAAATCTG
C2	CTATCGGTAG**TTTT**TCAACTGCTC**TTTT**TACTCAGCGACAGATTTGTG**TTTT** TCAACTGCTC**TTTT**CAACTAGCGG
C3	CACTGGTCAG**TTTT**TCAACTGCTC**TTTT**CTACCGATAGCCGCTAGTTG**TTTT** TCAACTGCTC**TTTT**GGTTTGCTGA
C4	CCACACTTGC**TTTT**TCAACTGCTC**TTTT**CTGACCAGTGTCAGCAAACC**TTTT** TCAACTGCTC**TTTT**GTGTGCGTGC
B	CAGATTTGTGTACTCAGCGA

Purification:

Each of the 96mer crude products was purified on an 8% polyacrylamide/8M urea polyacrylamide gel (PAGE; up to 20 OD_{260} of crude DNA per gel) at constant current of 30 mA for 2 hours (30 min at 250V followed by 1.5 hr at 500V), using the 1x TBE buffer. Following electrophoresis, the plates were wrapped in plastic and placed on a fluorescent TLC plate and illuminated with a UV lamp (254nm). The bands were quickly excised, and the gel pieces were crushed and incubated in 12 mL of sterile water at 60 ^{o}C for 12-16 hours. Samples were then dried to ca. 1 mL, desalted using size exclusion chromatography (Sephadex G-25 columns, Glen Research), and carefully quantified (OD_{260}) using UV-Vis spectroscopy. Smaller strands (**1-TOP, 1-BOT, 2-TOP, 2-BOT, 3-TOP and 3-BOT**) were purified using a 15% PAGE mixture and running conditions of 30 min at 250V followed by 45 min at 500V.



Supplementary Figure S1| Denaturing PAGE Analysis of synthesized oligonucleotides. Denaturing PAGE (8%, 1xTBE) gel ran for 30 minutes at 250V and then 1 hr at 500 V; Lane 1-**1**, Lane 2- **2**, Lane 3- **3**, Lane 4- $\mathbf{1}_{H}$, Lane 5- $\mathbf{2}_{H}$, Lane 6- $\mathbf{3}_{H}$, Lane 7- $\mathbf{1}_{6}$, Lane 8 – $\mathbf{2}_{6}$, Lane 9- $\mathbf{3}_{6}$, Lane 10- $\mathbf{1}_{P}$, Lane 11- $\mathbf{2}_{P}$ and Lane 12- $\mathbf{3}_{P}$.



Supplementary Figure S2| Denaturing PAGE Analysis of synthesized oligonucleotides for open linear trimer analogues. Denaturing PAGE (8%, 1xTBE) gel ran for 30 minutes at 250V and then 1 hr at 500 V; Lane 1-**C1**, Lane 2- **C2**, Lane 3- **C3**, Lane 4- **C4**,

S-IV. Assembly and characterization of 3D DNA structure TP

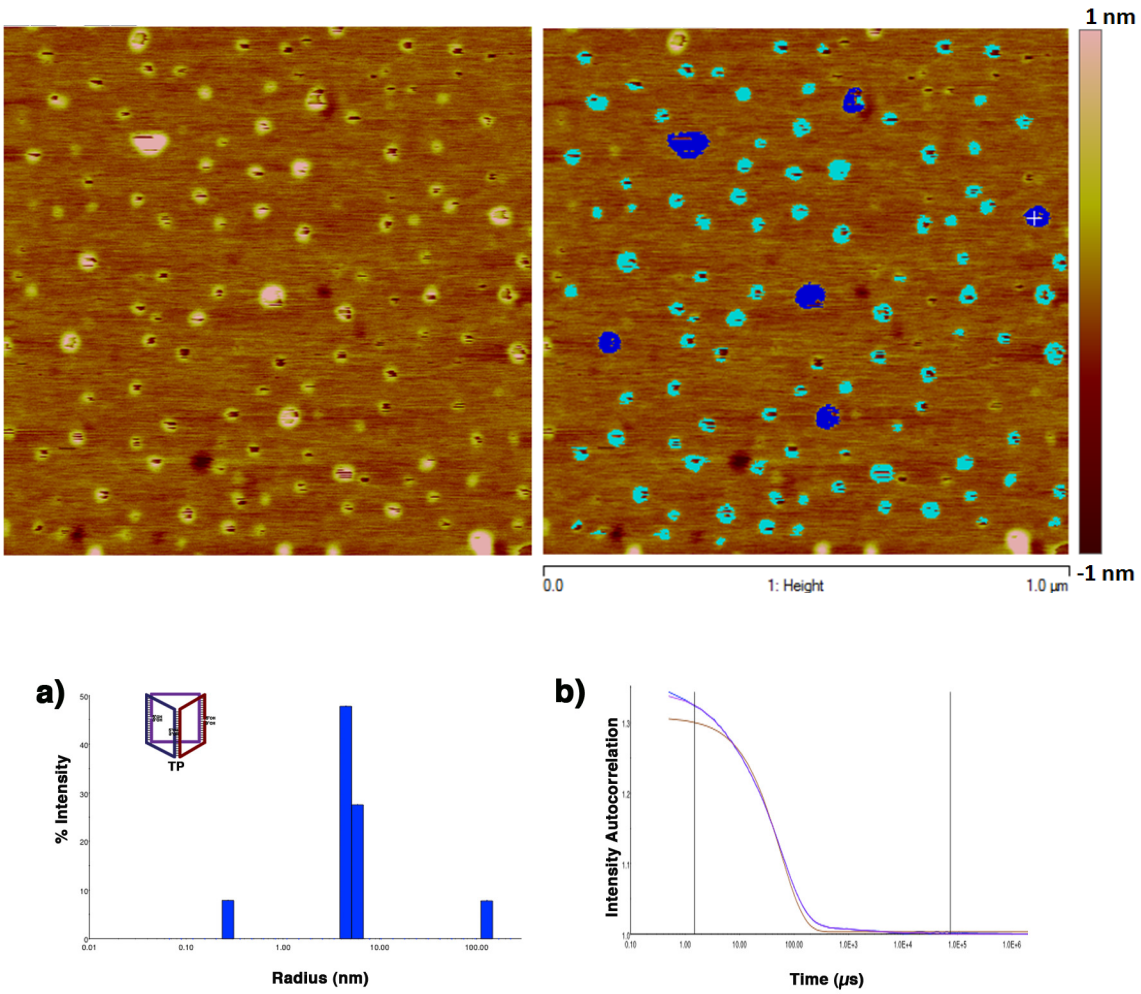
In general, equimolar amounts of each of the three strands (**1** – **3**) were combined in 1xTAMg buffer at a final 3D concentration of 1.25 μM. Samples were then subjected to a simple annealing protocol whereby strands were brought to 95 ºC for 5 minutes and cooled back to 4 ºC over 4 minutes. Table S2 shows each of the individual strand combinations used to assemble triangular prisms **TP**, $\mathbf{TP_H}$, $\mathbf{TP_6}$ and $\mathbf{TP_P}$, which were analyzed in manuscript Figure 2b. To limit the amounts of magnesium used in the degradation studies, DNA structures **TP**, $\mathbf{TP_H}$, $\mathbf{TP_6}$ and $\mathbf{TP_P}$ were additionally concentrated using microcon centrifugal filtration devices (30K MWCO). Before concentration, filter devices were washed with autoclaved Milli-Q water (2 x 450 μL) by centrifugation at 13,400 rpm and 4 °C for 8 minutes. Each sample was then centrifuged (13,400 rpm, 10 minutes, 4 °C). The final volumes for each sample were adjusted with 1xTAMg buffer to give overall 3D concentrations of 25 - 30 μM.

Supplementary Table 2| Strands combinations used to prepare each 3D triangular prismatic structure.

Structure	*Component strands*	*[3D] for annealing (μM)*
TP	1, 2, 3	1.25
$\mathbf{TP_{HEG}}$	1_{HEG}, 2_{HEG}, 3_{HEG}	1.25
$\mathbf{TP_{C6}}$	1_{C6}, 2_{C6}, 3_{C6}	1.25
$\mathbf{TP_P}$	1_P, 2_P, 3_P	1.25
M	C1, B	1.25
D	C1, C4, B	1.25
T	C1, C4, C3, B	1.25

To help determine the physical properties of each 3D DNA structure in solution, DLS experiments were performed. Each structure (14 μL, 1.25 μM) was analyzed with DLS measurements being taken on a DynaPro99 (Protein Solution/Wyatt) instrument operating at 24 °C and using a laser wavelength of 824 nm.

Supplementary Figure S3| AFM (Top) and DLS (Bottom) analysis of TP. DLS regularization distribution histogram (a) and associated correlation function (b) for TP. % PD observed was 14 %. Small peaks likely due to buffer components and maybe a small amount of intermolecular dimer.



DLS and AFM were performed on preformed TPs in 1xTAM buffer. DLS measurements of the unmodified TP structure were performed on a DynaPro99 (Protein Solution/Wyatt) instrument operating at 24 °C and using a laser wavelength of 824 nm. Samples were diluted to a concentration of 5 μM uM and 14 μL was used for measurements. Measurements indicate TP has a hydrodynamic radius of 5.8 ± 0.5 nm. AFM samples were measured using a Multimode

microscope equipped with the Nanoscope IIIa controller (Digital Instruments, Santa Barbara, CA). AFM silicon probes (model AC160TS from Asylum Research) with resonance frequency ~200-400 kHz and spring constant ~12-103 N/m or RTSEP NanoProbe tips (Veeco, Santa Barbara, CA) with resonance frequency 200-400 kHz, spring constant ~20-80 N/m (tip radius < 10 nm) were used. Images were analyzed using NanoScope® (DI) and worked up using a 3rd order plane fit which corrects for sample tilt. DNA samples were diluted to 25 nM with Millipore water, and 3 uL of this solution was applied to freshly cleaved mica. After 60s the mica surface was washed with 50-60 uL of water which was then wicked away using filter paper. AFM samples were dried under air for 30 min and then put under vaccum for 12 hours. AFM images shows a homodisperse population with average height values of 0.7 ± 0.4 nm.

TPs are deposited on mica and air-dried overnight, therefore 3D structures are expected to be collapsed and distorted, leading to the ring-like structures. Particles included in the height analysis can be selected with a height filter using the Nanoscope software (light blue selection S3 top right). Structures that are clearly unrelated (larger aggregates) can be excluded from the selection (dark blue selection S3 top right). The Nanoscope software then generates the particle analysis table included below based on this selection.

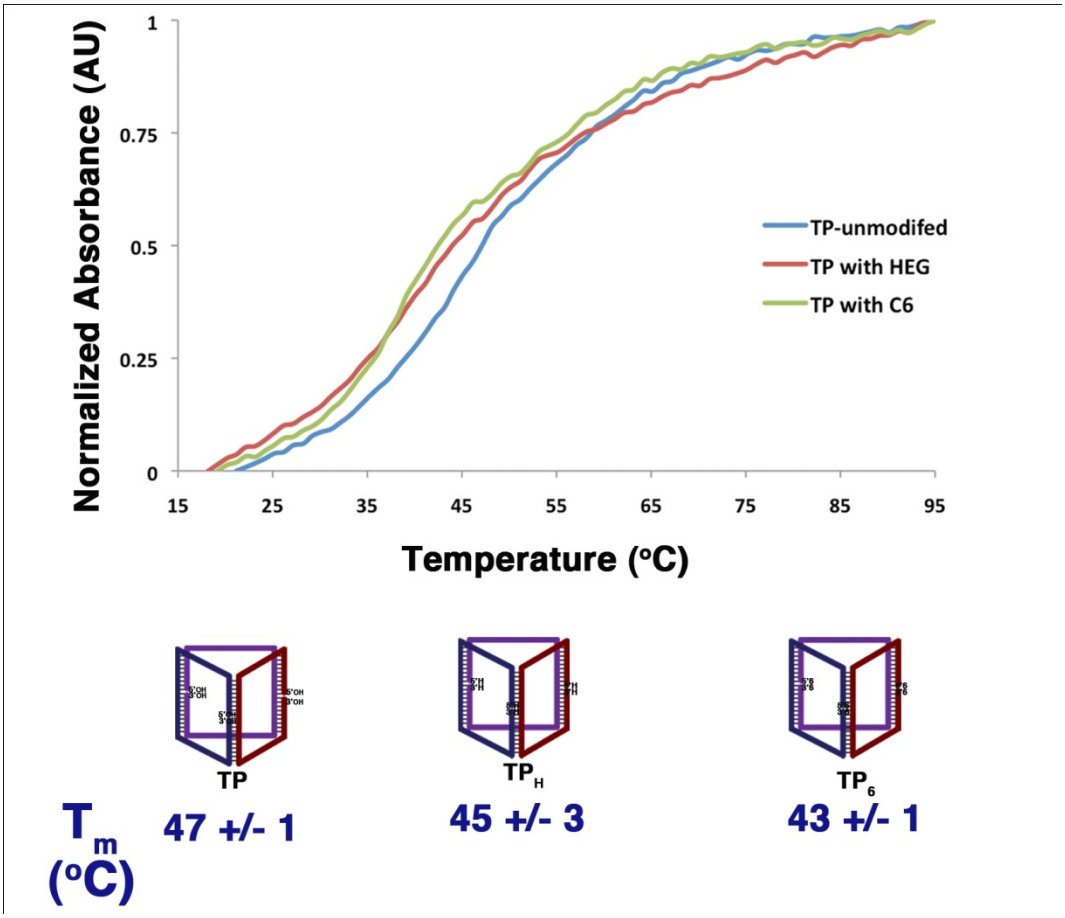
Supplementary Table 3| Summary of AFM particle analysis for height and diameter of TP.

Parameter	**Mean**	**Minimum**	**Maximum**	**Sigma**
Total Count	120.000	120.000	120.000	0.000
Density (/nm^2)	120.000	120.000	120.000	0.000
Height (pm)	709.757	229.516	2672.106	397.138
Area (nm^2)	522.423	95.367	2998.352	415.919
Diameter (nm)	24.219	11.019	61.787	8.865

A number of previous studies have shown that the height of a DNA double helix, measured by AFM is consistently lower than the expected 2 nm. In many experiments, the largest observed heights are in fact ~0.5 nm. Moreno-Herrero et al (Ultramicroscopy 96, (2003) 167) showed that this was due to a ~0.8nm thick salt layer on mica, in which DNA strands are embedded. Chen et al have recently suggested that these discrepancies are also due to tip-induced deformation of the soft DNA molecules under AFM imaging conditions, and to inconsistent-imaging dynamics, in which the cantilever oscillates in the attractive regime on substrate background but in the repulsive regime on the sample (Ultramicroscopy 107 (2007) 275). Thus, previous AFM studies of double-stranded DNA show significantly lower heights than 2 nm.

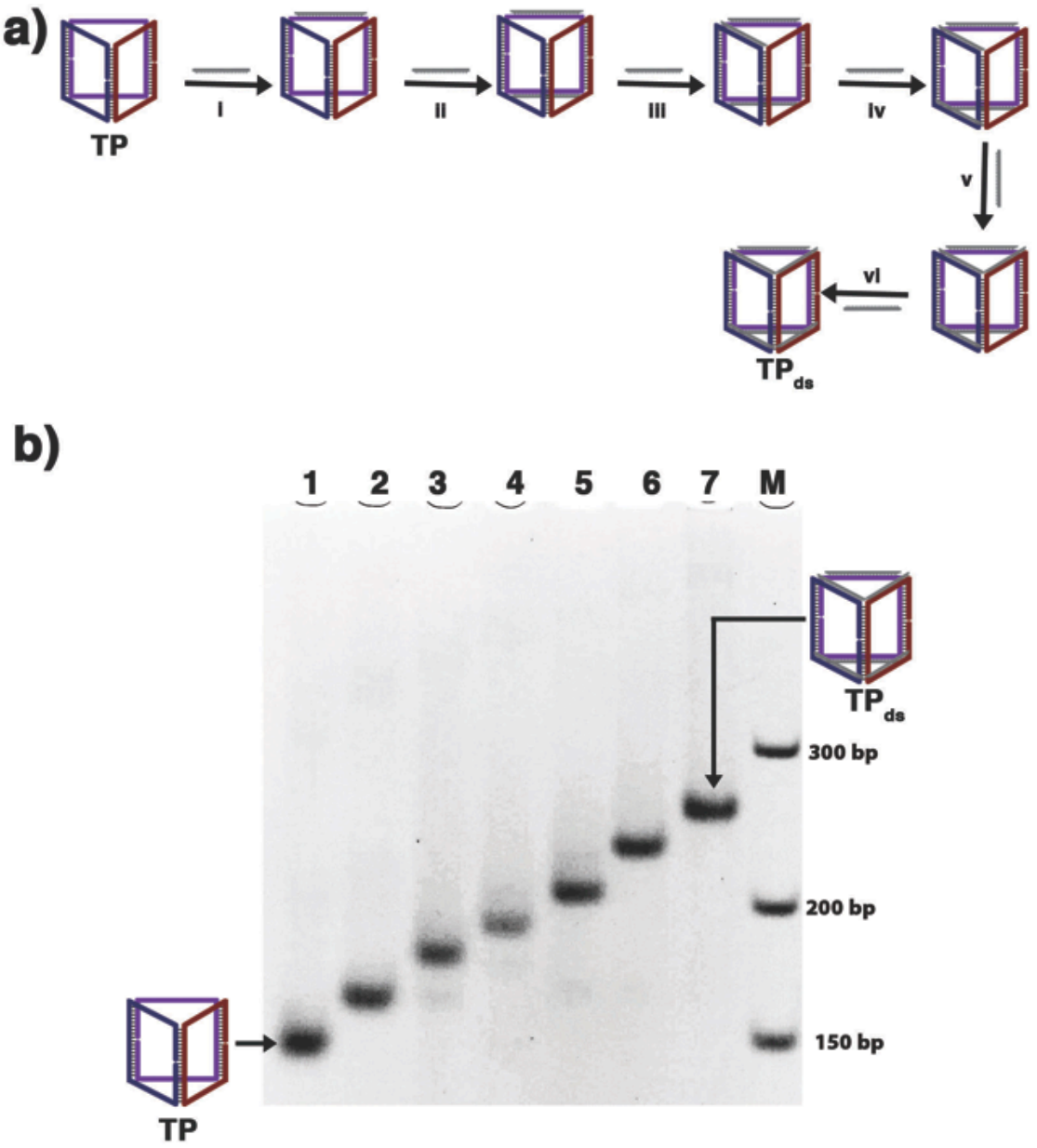
Keeping these effects in mind, as well as the previously described distortion of these structures on the mica surface upon drying, the heights observed here are reasonable, and consistent with previous measurements (see J. Am. Chem. Soc. 2012, 134, 11998).

UV absorption spectra (normalized A260 vs. temperature) were recorded with a Varian CaryBio 300 UV/Vis spectrometer equipped with a temperature controller. Samples were initially prepared as described in section S-VIa and concentrated to 25-35 μM. Structures **TP**, $\mathbf{TP_H}$ and $\mathbf{TP_6}$ (375 pmol/structure) were then each diluted with 1xTAMg buffer up to a total volume of 100 μL. To each of these samples was added 1.4 mL PBS buffer (Wisent), bringing the total volume to 1.5 mL and [3D] = 250 nM, which provide enough material to perform denaturation studies in triplicate (0.5 mL/run). The temperature of the denaturation experiments ranged from 10 - 95 ºC and was performed with a temperature ramp of 1.0 ºC/min. Thermal denaturation temperatures (T_m) were determined after buffer blank subtraction from the first derivatives maximum of the normalized absorbance vs. temperature curve (Figure S6). The curves obtained for **TP**, $\mathbf{TP_{HEG}}$ and $\mathbf{TP_{C6}}$ are all quite broad, due likely in part to the sequence asymmetric make-up of each duplex region within the structure. Both chemical modifications at the 3'/5' terminal positions do not drastically alter the melting profiles obtained. The values reported beneath Figure S3 are an average of three measurements with less than 5% error.



Supplementary Figure S4| Thermal denaturation studies on TP, TP_{HEG} and TP_{C6}, (triplicate measurements).

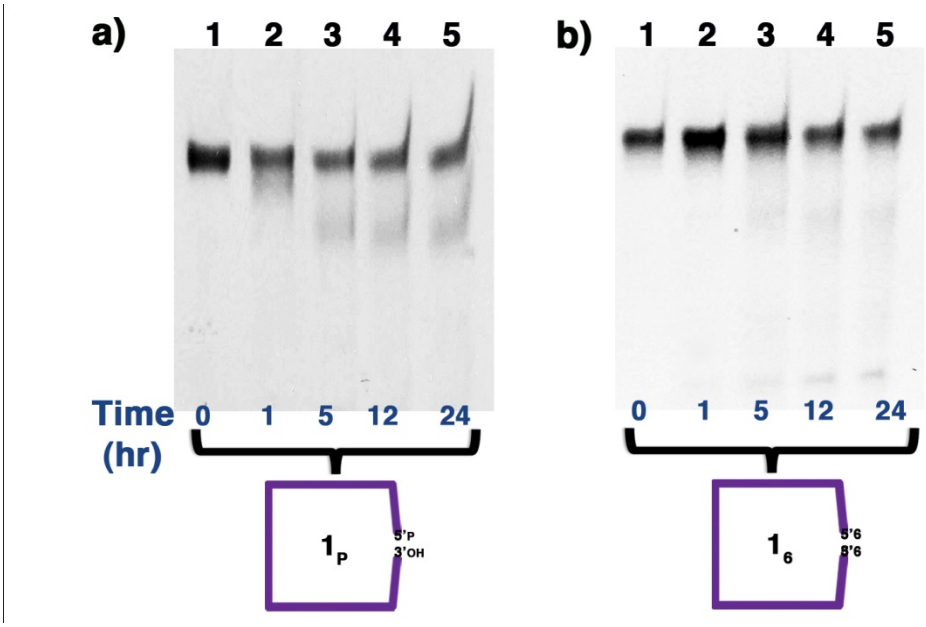
The ssDNA regions within **TP** were designed to be sequence asymmetric such that each region could be individually addressed by addition of the a corresponding 20mer complement strand (see Table S2 above for sequences) to yield fully double-stranded structure $\mathbf{TP_{ds}}$ (Figure S3a). As shown in Figure S3b, **TP** could be addressed in a sequential manner with increasing numbers of 20mer complement strands to ultimately yield $\mathbf{TP_{ds}}$. It should be noted that this asymmetric sequence arrangement within the 3D DNA structure allows for a multitude of potential strand orientations with functional or chemically modified oligonucleotides.



Supplementary Figure S5| Availability of single-stranded regions in TP to hybridization. a) Schematic representation for the step-wise addition of 20mer complement strands (i – vi) to each single-stranded region of **TP** to generate fully double-stranded structure $\mathbf{TP_{ds}}$. **b)** Native PAGE (6%, 1 x TAMg, 250V, 60 mA, 5 hr, RT) analysis of $\mathbf{TP_{ds}}$ formation; Lane 1-**TP**, Lane 2- **TP/1-TOP**, Lane 3- **TP/1-TOP/1-BOT**, Lane 4- **TP/1-TOP/1-BOT/2-TOP**, Lane 5- **TP/1-TOP/1-BOT/2-TOP/2-BOT**, Lane 6- **TP/1-TOP/1-BOT/2-TOP/2-BOT/3-TOP**, Lane 7- **TP/1-TOP/1-BOT/2-TOP/2-BOT/3-TOP/3-BOT ($\mathbf{TP_{ds}}$)** and Lane M - base-pair marker.

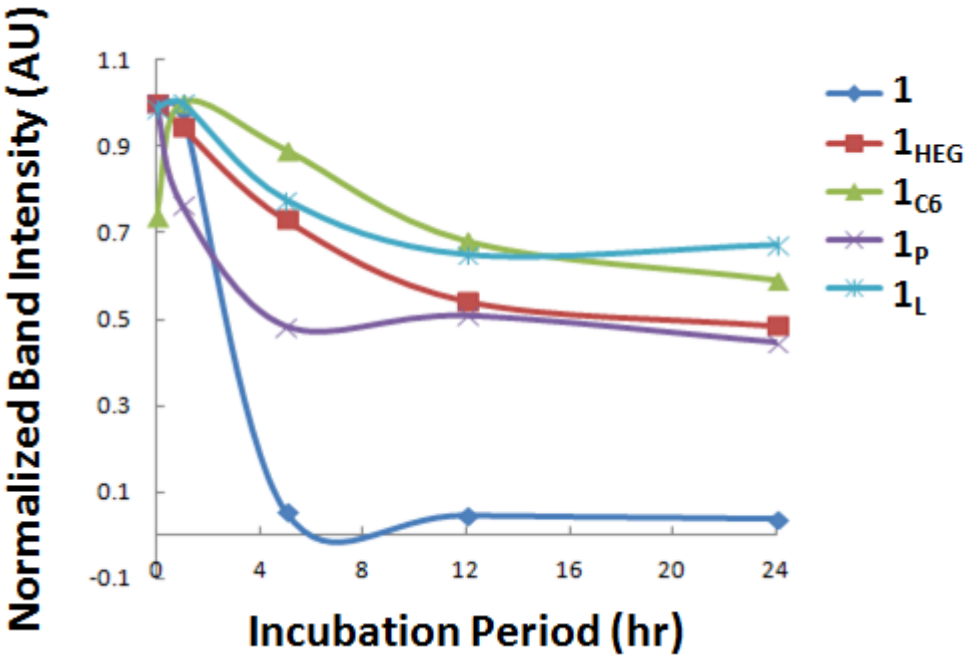
V. Stability studies in fetal bovine serum (FBS)

For initial degradation studies, DNA strands were concentrated to a stock solution of 40 μM in 1xTAMg buffer. As an example, strand **1** (40 μM, 2 μL) was first diluted with DMEM media (88 μL). To this mixture was added a fresh sample of undiluted FBS (10 μL) with slight mixing to make the overall % of FBS 10% (v/v). An aliquot was immediately taken out (10 μL), formamide (5 μL) added and then stored at -4 °C as the t =0 hr time point. The remaining sample was then incubated at 37 °C and similar aliquots were removed and treated as described above at time points of 1, 5, 12 and 24 hr. Digested products were analyzed by denaturing PAGE (8%, 15 mA, 250V → 30 min followed by 500V → 1 hr). In addition to the results found in manuscript Figure 1b, degradation assays were also performed on modified strands $\mathbf{1_P}$ and $\mathbf{1_{C6}}$ (Figure S3).



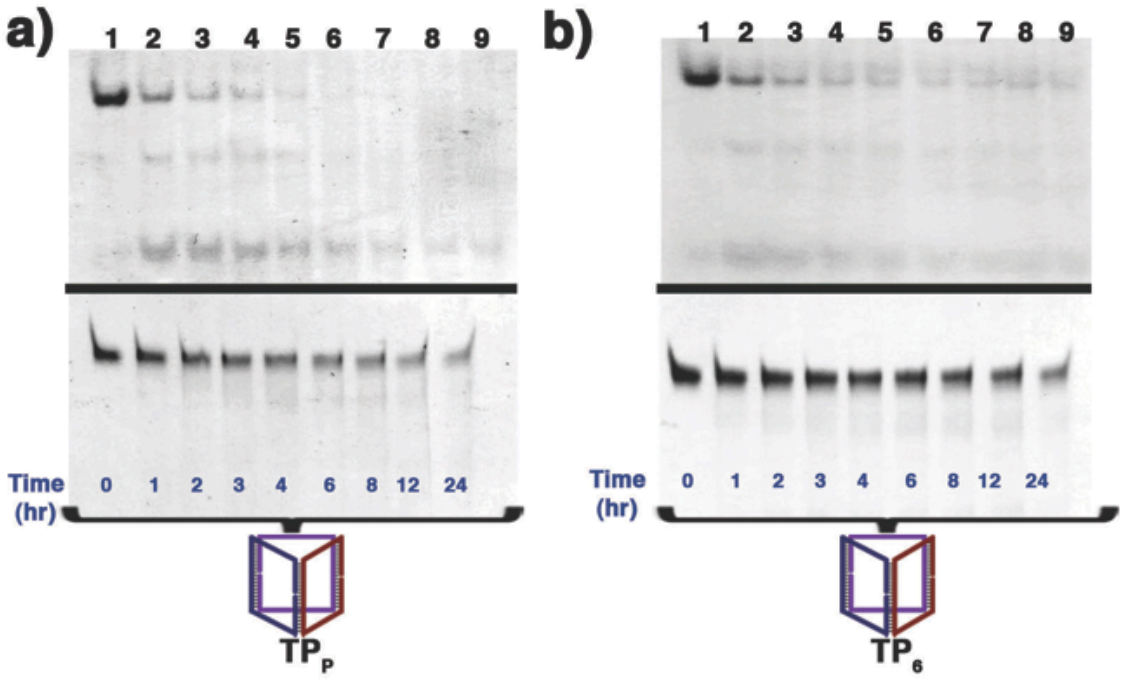
Supplementary Figure S6| Degradation assay results for modified oligonucleotides $\mathbf{1_P}$ and $\mathbf{1_6}$. Denaturing PAGE (8%, 1 x TBE, 250V → 30 min, 500 V → 1 hr, 15 mA, RT) analyses for a) $\mathbf{1_P}$ and b) $\mathbf{1_{C6}}$. Time points where samples were removed from the FBS mixture are indicated on each gel.

The intensity of the PAGE bands corresponding to each time point were then analyzed with ImageJ to obtain area values that could be plotted and used to graphically demonstrate the longevity of each strand under these serum conditions. As shown in Figure S4, unmodified strand **1** undergoes quick degradation of the full 96mer strand within a 2 hr incubation window. Each of the modified strands, $\mathbf{1_{HEG}}$, $\mathbf{1_{C6}}$, $\mathbf{1_P}$, and $\mathbf{1_L}$, show marked improvements to stability in serum nucleases.



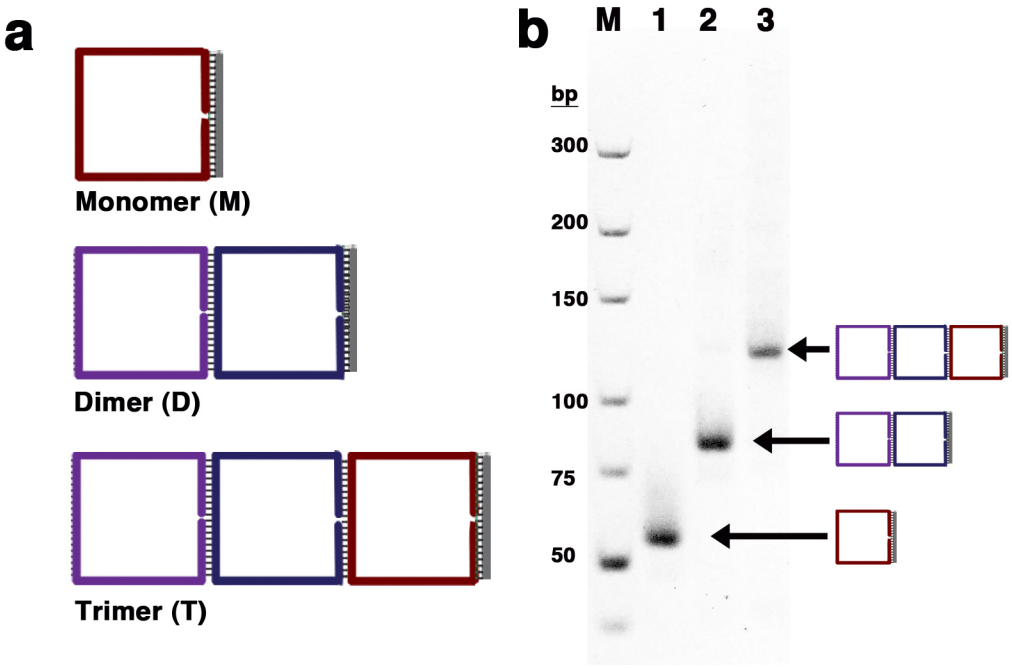
Supplementary Figure S7| Combined degradation assay results single-stranded oligonucleotides. A cumulative analysis of the normalized PAGE intensity demonstrates the stability of modified strands to exonuclease attack. Trend lines added to guide reader (not fitted data).

For degradation studies on each 3D structure, stock solutions between 25 - 35 μM in 1xTAMg buffer were prepared (see section S-VIa). As an example, **TP** ([3D] = 32.3 uM, 1.24 μL) was first diluted with 1xTAMg (0.76 uL) and DMEM media (88 μL). The additional 1xTAMg maintains the concentration of magnesium used within each degradation assay. To this mixture was then added undiluted FBS (10 μL) with slight mixing to make the overall % of FBS 10% (v/v). An aliquot was immediately taken out (10 μL) and either glycerin:EDTA(50 mM) (4 μL:1μL) or formamide (5 μL) added and stored at -4 °C as the t =0 hr time points. The remaining sample was then incubated at 37 °C and similar aliquots were removed and treated as described above at time points of 1, 2, 3, 4, 6, 8, 12 and 24 hr. Digested products were then analyzed by either native or denaturing PAGE. In addition to the profiles indicated in manuscript Figure 3a (**TP** and $\mathbf{TP_{HEG}}$), degradation assays were also performed for structures $\mathbf{TP_{C6}}$ and $\mathbf{TP_P}$ (Figure S6).



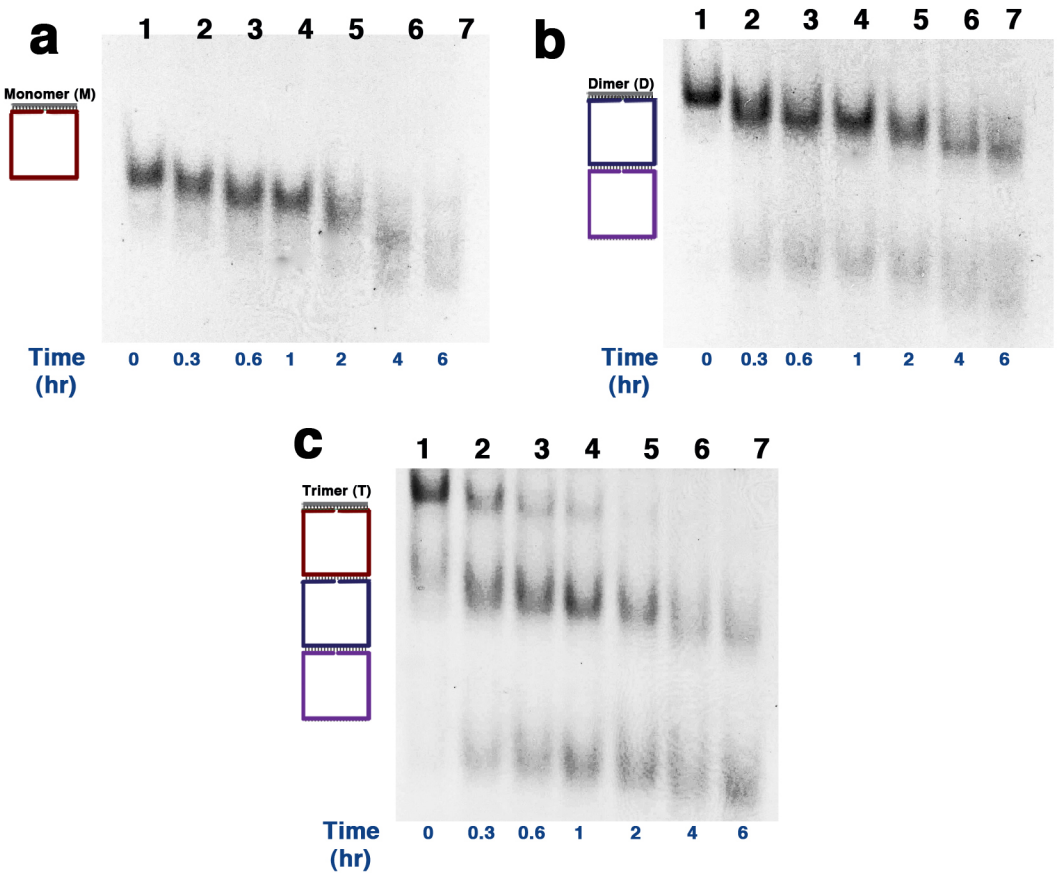
Supplementary Figure S8| Degradation assay results for modified TP_P and TP_{C6}. Native PAGE (6%, 1x TAMg, 250V 4 hr) top and denaturing PAGE (8%, 1 x TBE, 250V → 30 min, 500 V → 1 hr, 15 mA, RT) bottom, for a) **TP_P** and b) TP_{C6}. Time points where samples were removed from the FBS mixture are indicated on each gel.

As outlined in the manuscript, a number of linear structures were prepared so as to compare degradation profiles with those obtained for the 3D DNA objects (Figure S10a). Each linear structure is closed via hybridization to 20mer strand **b** (see Table S2 for annealing conditions). Unlike **TP**, the three-component trimer **T** is unable to close back onto the unhybridized end of the structure. As shown in Figure S10b, linear structures **M**, **D** and **T** are formed in excellent yields.



Supplementary Figure S9| Desgn and analysis of linear structures. a, Schematic representaion of linear structures monomer (**M**), dimer (**D**) and trimer (**T**). **b,** Native PAGE (6%, 1 x TAMg, 250V, 60 mA, 3 hr, RT); Lane M – marker, Lane 1-**M**, Lane 2- **D** and Lane 3- **T**.

All of the degradation assay results obtained for structures **M**, **D** and **T** (Figure S11) reveal quick digestion of the closed structures within only one hour. It would appear that these variants contain nicked junctions that are far more accessible to nuclease digestion. Especially in the case of direct linear analog **T** (Figure S11c), rapid destruction of the structure occurs, which is in contrast to the longevity observed for closed 3D structure **TP**. It is still unclear as to what factors contribute to the inherent stability observed for some of the 3D structures, especially **TP** and **RP**. Further investigations are underway to better elucidate the structural basis of this nuclease stability.



Supplementary Figure S10| Typical degradation assay results for linear DNA structures M, D and T. Native PAGE (6%, 1 x TAMg, 250V, 60 mA, 3 hr, RT) analyses were performed for each structure. Time points where samples were removed from the FBS mixture are indicated on each gel. **a** (**M**), **b** (**D**) and **c** (**T**).

FBS samples are collected over 24 hrs and analyzed using PAGE; normalized band intensities are then plotted with respect to time. The data is well described by exponential decay (Equation 1). Integrating and rearranging Equation 1 generates Equation 2, which is used to fit our data and extract a decay constant (λ). The decay constant is inversely related to the lifetime (τ) (Equation 3), and is reported for FBS degradation comparisons. The half-life $t_{1/2}$ = lifetime (τ) x ln2. In general, data interpretation using exponential decay analysis was used for comparison with other groups (Bermudez, Chem. Commun., 2009, 7036–7038); the mean lifetime values derived from this analysis are directly related to changes in band intensity. It is therefore the comparative trends in band intensity disappearance that are most significant. The lifetime values derived from exponential decay interpretation do contain intrinsic fitting errors reflected by the R^2 value, and should therefore be taken as a semi-quantitative assessment of serum stability.

Equation 1: $$\frac{dN}{dt} = -\lambda N$$

Equation 2: $$N(t) = N_o\, e^{-\lambda t}$$

Equation 3: $$\tau = \frac{1}{\lambda}$$

N: band intensity

N_o: initial band intensity

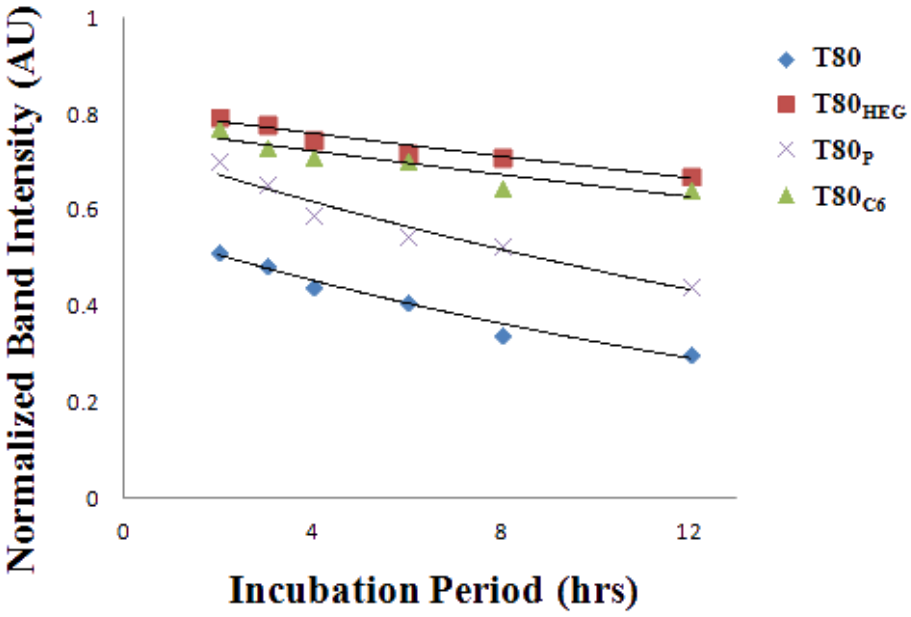
λ: decay constant (hrs^{-1})

τ: lifetime (hrs)

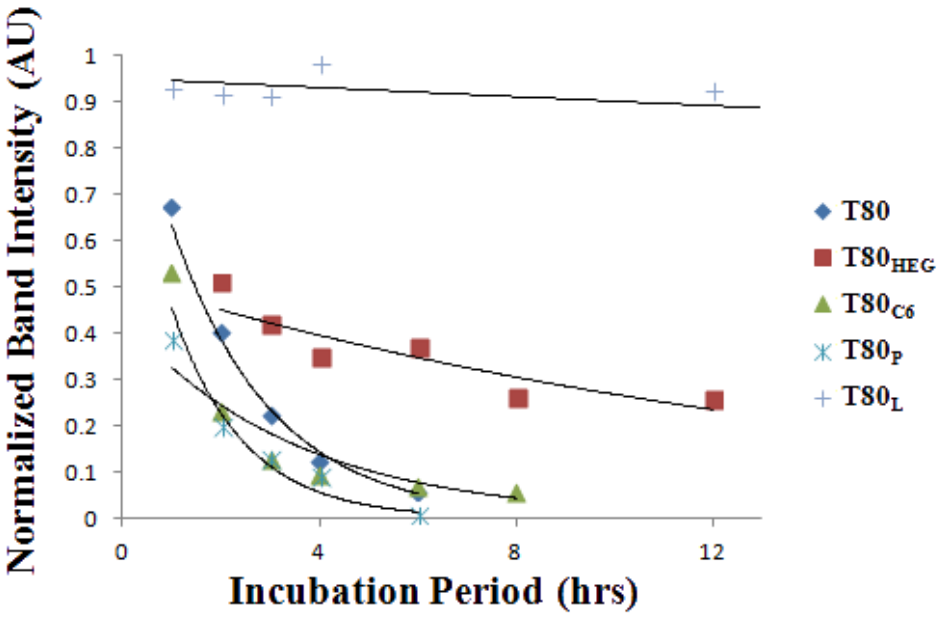
t: time (hrs)

Supplementary Table 4| Summary of exponential decay analysis for all single clips and assembled structures.

	ss Clips			TP Denaturing			TP Native		
Sample	**λ (hrs^{-1})**	**R^2**	**τ (hrs^{-1})**	**λ (hrs^{-1})**	**R^2**	**τ (hrs^{-1})**	**λ (hrs^{-1})**	**R^2**	**τ (hrs^{-1})**
Unmod.	0.275	0.756	3.63	0.055	0.973	18.18	0.494	0.987	2.02
HEG	0.051	0.989	19.61	0.016	0.945	62.50	0.065	0.818	15.38
C6	0.075	0.988	13.33	0.018	0.872	55.56	0.287	0.835	3.48
PO_4	0.136	0.966	7.35	0.044	0.961	22.73	0.696	0.946	1.44
LIG	0.037	0.954	27.03	na	na	na	0.005	0.637	200.00

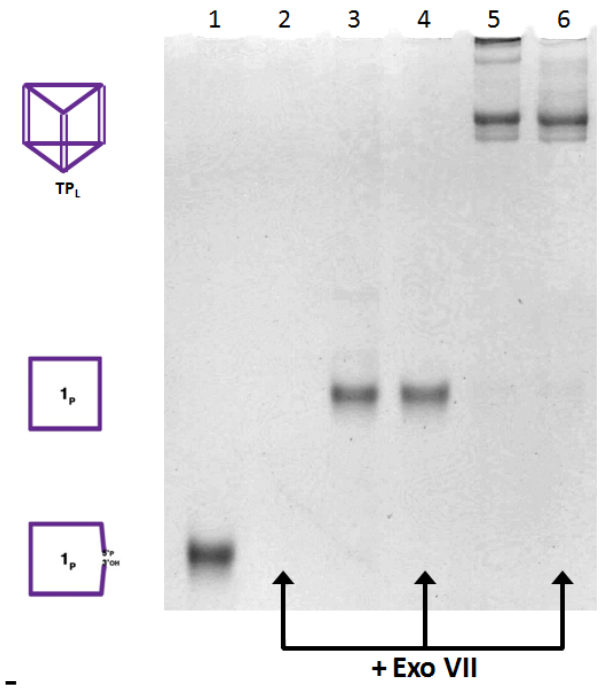


Supplementary Figure S11| Overlay of degradation assays for TP structures using denaturing PAGE, band intensity trends are fit using exponential first order decay analysis.



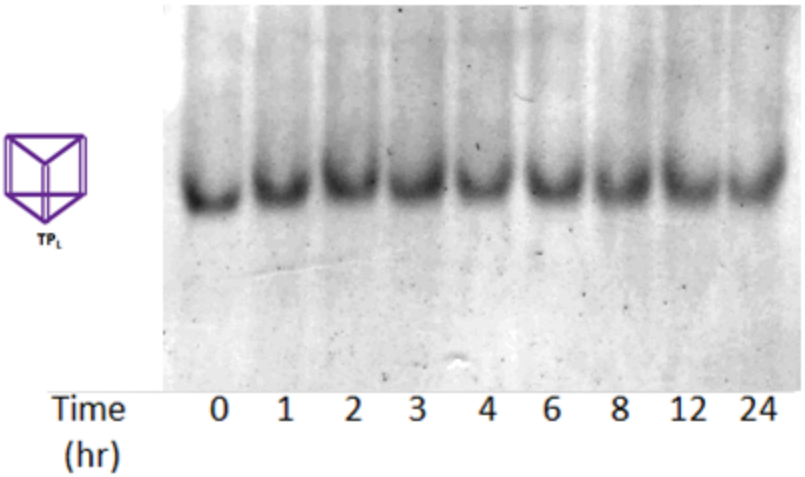
Supplementary Figure S12| Overlay of degradation assays for TP structures using native PAGE, band intensity trends are fit using exponential first order decay analysis.

VI. Assembly and FBS studies for ligated (T4 Ligase) structure 1_L and TP_L



Supplementary Figure S13| Preparation and digestion controls for ligated samples. Denaturing (8%, 1 x TBE, , 250V → 30 min, 500V → 1hr, 15 mA, RT, RT)

All samples were ligated following supplier experimental conditions. All nicked regions targeted for ligation were first prepared with the complement strands or assembled into the target 3D triangular prism for a final concentration of 5 uM (single clip or assembled TP); T4 Ligase was then added to the solution and samples were incubated 8-12 hours. Samples were heated to 90°C for 10 min., quickly cooled to RT and incubated with Exo VII as a clean-up step (4 hrs, 37 °C). Ligated products were isolated using denaturing PAGE gel excision, extraction and desalting. Successful ligation of intended products is confirmed using Exo VII digestion (4 hrs, 37 °C) and 8% denaturing PAGE.



Supplementary Figure S14| FBS degradation assay of ligated TP_L. Native PAGE (6%, 1 x TAMg, 250V → 4hr, 15 mA, RT). Time points where samples were removed from the FBS mixture are indicated on each gel.