Supporting Information

DNA-based nanoscaffolds as vehicles for 5-fluoro-2'-deoxyuridine oligomers in colorectal cancer therapy

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Material and methods

Reagents: The standard phosphoramidites and ancillary reagents used on the oligonucleotide synthesis were obtained from Applied Biosystems and Link Technologies Ltd. 5'-Fluorescein CE phosphoramidite (FITC), Cyanine-3-CE phosphoramidite (Cyanine 540), 5'-Cholesterol-CE phosphoramidite and 5-FdU-CE phosphoramidite were acquired from Link Technologies. 5-Fluoro-2'-deoxyuridine (FdU) was purchased from Alfa Aesar. Centrifugation devices Nanosep 10K omega were purchased to Pall Life Science. Matrix for MALDI-TOF experiments was composed by 2',4',6'-trihydroxiacetophenone monohydrate (THAP, Aldrich) and ammonium citrate dibasic (Fluka). Solvents for HPLC analysis were prepared using triethylammonium acetate (TEAA) and acetonitrile (Merck) as mobile phase. The rest of the chemicals are analytical reagent grade from commercial sources as specified. Circular genomic DNA from the virus M13mp18, 10, 50, and 1Kbp DNA Ladders were purchased from New England Biolabs and 100 bp DNA Ladders from Promega. Control drug, 5-Fluorouracil (5-FU) was purchased from Sigma. Ultrapure water (Millipore) was used in all experiments. HTB-38 was purchased from American Type Culture Collection, Manassas, VA and HCC2998 cell line was kindly provided by Dr. Diego Arango (Molecular Oncology Group; CIBBIM-Nanomedicine, Vall d'Hebron Institut of Research (VHIR)). Both cell lines were grown in DMEM supplemented with 10% fetal bovine serum (FBS), 50 i.u ml^{-1} penicillin and 50 µg ml^{-1} streptomycin. Cultures were grown at 37 °C in 5% CO2 humidified atmosphere. Cell membrane dyes WGA-488 and WGA-555 were kindly provided by the confocal microscopy service (ThermoFisher). Annexin V-FITC kit was obtained from ThermoFisher and used following manufacturer's instructions.

Instrumentation: Modified staple-strands for engineer the two DNA nanostructures were synthesized on an ABI 3400 DNA Synthesizer (Applied Biosystems). Analytic RP-HPLC was performed on a Waters chromatography system using XBridge™ OST C_{18} (4.6 × 50 mm, 2.5 µm) column. The HPLC solvent used were; A, 5% ACN in 100 mM triethylammonium acetate (TEAA) (pH = 7) and solvent B, 70% ACN in 100 mM TEAA (pH = 7). Mass spectra were recorded on a MALDI Voyager DE RP time-of-flight (TOF) spectrometer (Applied Biosystems) equipped with nitrogen laser at 337 nm using a 3ns pulse. The matrix used contained 2,4,6-trihydroxyacetophenone (THAP, 10 $mgmL^{-1}$ in ACN/ water 1:1) and ammonium citrate (50

mgmL^{-1} in water). Molecular absorption spectra between 220 and 550 nm were recorded with a Jasco V650 spectrophotometer. The temperature was controlled with an 89090A Agilent Peltier device. Hellma quartz cuvettes were used. The DNA nanostructures were annealed in an Eppendorf thermocycler (Boeker). Gels were imaged with a Gene Genius Bioimaging system (Syngene). AFM images were obtained by liquid tapping on a Nanoscope 3A Multimode 4 AFM, using Bruker SNL-10 ultra-sharp silicon tips. Imaging was then conducted using a TEM microscope, Jeol JEM 1010 100 kv with CCD Megaview 1kx1k (CCIT- University of Barcelona). The MTT and colony assays were measured in an automated spectrophotometric plate reader Glomax multi detection system (Promega). Internalization and apoptosis assays were measured with a Guava easyCyte™ flow cytometer (Millipore) and data was analysis with Guavasoft 3.1.1. The confocal images were acquired by using a TCS SP2 confocal microscope equipped with live cell chamber system (Leica).

Synthesis of modified oligonucleotides: Standard staple-strand oligonucleotides were purchased to Sigma. Modified staple-strands were synthesized on ABI 3400 DNA Synthesizer, using the 200-nmol scale synthesis and the standard protocols. FITC, FdU, cholesterol and Cy3 phosphoramidites were site-specifically inserted into the standard sequence oligonucleotide. FdU_n oligonucleotides at the 3'-end were introduced using controlled pore glass functionalized with a 3'-succinyl-5-FdU solid support. To this end, the FdU was dimethoxytritilated and then was treated with anhydride succinic to obtain the corresponding succinate. Finally, the controlled pore glass solid support with 5-FdU was prepared following standard protocol.[1, 2]

Modified oligonucleotides were deprotected with ammonia solution overnight at room temperature, followed by 1h at 55ºC. The FdU containing oligonucleotides were purified by OPC (oligonucleotide purified cartridge, Glen Research). The Cholesterol-staples were desalted by Sephadex G-25 with 30% acetonitrile (NAP-10, GE Healthcare). All the oligonucleotide staples were analyzed by RP-HPLC (Table S3) using two different HPLC gradients with a flow rate of 1 mlmin^{-1}. For the modified FdU staples the gradient used was: 0–50% acetonitrile in 20 min and for the modified staples with cholesterol, fluorescein and Cy3 moieties the gradient of acetonitrile was increased stepwise, starting with 0–15% in the initial 5 min, 15-40% over 1 min and 40-85% over the last 9 min. The length and homogeneity of the oligonucleotides were checked by MALDI-TOF (Table S3). The modified oligonucleotide concentration was

determined by absorbance measurements (260 nm) and their extinction coefficient calculated. These samples were kept dry at -20 ºC until further use. All the modified oligonucleotides sequences are detailed in **Table S1**. The standard strands for the DNA origami and DNA tetrahedron assemble are described below in **Table S2**.

DNA nanostructures stability in cell medium. DNA tetrahedron (70 nmols) and DNA origami samples (10 nmols) were dissolved in cell medium (DMEM plus 10% Fetal bovine serum) and shacked at 37 °C for different periods of time (0, 15, 30, 60, 120, 240, 480 min and 24 h). At the desired time the samples were removed from the shaker and frozen until further use. Then the samples were verified by gel electrophoresis. 2.5 % agarose in TAE/12.5 mM Mg buffer and run for 50 min at 100 V 1% agarose gels with the same buffer for the DNA origami. In the case of Td gels were run at 4 °C.

Table S1. Modified staple-strands sequences for DNA tetrahedron and DNA origami**.**

DNA tetrahedron	
S1- FdU_{10}	AGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCA TAGTAGACGTATCACC-**$(FdU)_{10}$**
S2a	CTTGCTACACGATTCAGACT
Chol-S2a	**Chol-**CTTGCTACACGATTCAGACT
S2b	TAGGAATGTTCGACATGCGAGGGTCCAATACCGACGATTACAG
Chol-S2b-FdU_{10}	**Chol-**TAGGAATGTTCGACATGCGAGGGTCCAATACCGACGA TTACAG-**$(FdU)_{10}$**
S3a	GGTGATAAAACGTGTAGCAA
Chol-S3a	**Chol-**GGTGATAAAACGTGTAGCAA
S3b	GCTGTAATCGACGGGAAGAGCATGCCCATCCACTACTATGGCG
Chol-S3b	**Chol-**GCTGTAATCGACGGGAAGAGCATGCCCATCCACTACTAT GGCG
S4a –	CCTCGCATGACTCAACTGCC
Chol-S4a	**Chol-**CCTCGCATGACTCAACTGCC
S4b –	TGGTGATACGAGGATGGGCATGCTCTTCCCGACGGTATTGGAC
Chol-S4b	**Chol-**TGGTGATACGAGGATGGGCATGCTCTTCCCGACGGTAT TGGAC
S4c	CCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATGCTCTT CCCGACGGTATTGGAC-TTGATGAATGGTGGGTGAGAGG
Fluorescein-S5	**Fluorescein-**CCTCTCACCCACCATTCATC
Cy3-S5	**Cy3-**CCTCTCACCCACCATTCATC
DNA origami	**Sequence (5' to 3')**
t-5r4e-FdU_{13}	AAAGGCCGCTCCAAAAGGAGCCTTAGCGGAGT-**$(FdU)_{13}$**
t-3r2f-FdU_{14}	TGTAGCATAACTTTCAACAGTTTCTAATTGTA-**$(FdU)_{14}$**
t3r4e-FdU_{14}	GTTTGCCACCTCAGAGCCGCCACCGCCAGAAT-**$(FdU)_{14}$**
t5r2f-FdU_{14}	AATGCCCCATAAATCCTCATTAAAAGAACCAC-**$(FdU)_{14}$**
t-3r8e-FdU_{10}	AGTAATCTTCATAAGGGAACCGAACTAAAACA-**$(FdU)_{10}$**
t3r6f-FdU_{10}	CCGGAAACTAAAGGTGAATTATCATAAAAGAA-**$(FdU)_{14}$**
Chol-t-3r14f	**Chol-**TTT-TCAGAAGCCTCCAACAGGTCAGGATTTAAATA

Chol-t-3r18f	**Chol**-TTT-CAAAATTAGGATAAAAATTTTTAGGATATTCA
Chol-t5r14f	**Chol**-TTT-TCATTACCGAACAAGAAAAATAATAATTCTGT
Chol-t5r18f	**Chol**-TTT-AGGCGTTAGGCTTAGGTTGGGTTAAGCTTAGA
Chol-t-1r22f	**Chol**-TTT-GCTCATTTCGCGTCTGGCCTTCCTGGCCTCAG
Chol-t1r22e	**Chol**-TTT-TTTAACGTTCGGGAGAAACAATAACAGTACAT
Chol-t-1r30f	**Chol**-TTT-GAATAGCCACAAGAGTCCACTATTAAGCCGGC
Chol-t1r30e	**Chol**-TTT-GTTGTAGCCCTGAGTAGAAGAACTACATTCTG

FdU_n n in subscript stands for the number of FdU monomers, Chol for a cholesterol moiety and Cy3 for Cyanine 540.

Table S2. List of the helper strands used to engineer the DNA tetrahedron and DNA origami. All these oligonucleotides were purchased from commercial sources as described in the Materials and Methods section.

20 bp regular DNA Tetrahedron	
S1	AGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGT AGACGTATCACC
S2	CTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAATAC CGACGATTACAG
S3	GGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCATC CACTACTATGGCG
S4	CCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATGCTCTTCCCG ACGGTATTGGAC
DNA origami staples	
t1r0g	AGGGTTGATATAAGTATAGCCCGGAATAGGTG
t1r2e	TAAGCGTCGGTAATAAGTTTTAACCCGTCGAG
t1r2f	AGTGTACTATACATGGCTTTTGATCTTTCCAG
t1r4e	AACCAGAGACCCTCAGAACCGCCACGTTCCAG
t1r4f	GAGCCGCCCCACCACCGGAACCGCTGCGCCGA
t1r6e	GACTTACGTAAAGGTCGCAACATACCGTCACC
t1r6f	AATCACCACCATTTGGGAATTAGACCAACCTA
t1r8e	TTATTACGTAAAGGTCGCAACATACCGTCACC
t1r8f	TACATACACAGTATGTTAGCAAACTGTACAGA
t1r10e	TGAACAAAGATAACCCACAAGAATAAGACTCC
t1r10f	ATCAGAGAGTCAGAGGGTAATTGAACCAGTCA
t1r12e	TATTTTGCACGCTAACGAGCGTCTGAACACCC
t1r12f	TCTTACCAACCCAGCTACAATTTTAAAGAAGT
t1r14e	ATCGGGCTGACCAAGTACCGCACTCTTAGTTGC
t1r14f	GGTATTAATCTTTCCTTATCATTCATATCGCG
t1r16e	CATATTTATTTCGAGCCAGTAATAAATCAATA
t1r16f	AGAGGCATACAACGCCAACATGTATCTGCGAA
t1r18e	ACAAAGAAAATTTCATCTTCTGACAGAATCGC
t1r18f	TTTTAGTTCGCGAGAAAACTTTTTTTATGACC

t1r20e	AAATCAATCGTCGCTATTAATTAAATCGCAAG
t1r20f	CTGTAAATATATGTGAGTGAATAAAAAGGCTA
t1r22e	TTTAACGTTCGGGAGAAACAATAACAGTACAT
t1r22f	CTTTTACACAGATGAATATACAGTGCCATCAA
t1r24e	TTATTAATGAACAAAGAAACCACCTTTTCAGG
t1r24f	ATTTTGCGTTTAAAAGTTTGAGTACCGGCACC
t1r26e	CTAAAGCAAATCAATATCTGGTCACCCGAACG
t1r26f	AAACCCTCTCACCTTGCTGAACCTAGAGGATC
t1r28e	CTAAAAGCAAATCAATATCTGGTCACCCGAACG
t1r28f	GCGTAAGAAGATAGAACCCTTCTGAACGCGCG
t1r30e	GTTGTAGCCCTGAGTAGAAGAACTACTTCTG
t1r30f	ATCACTTGAATACTTCTTTGATTAGTTGTTCC
t1r32h	TACAGGGCGCGTACTATGGTTGCTAATTAACC
t3r0g	TGCTCAGTACCAGGCGGATAAGTGGGGGTCAG
t3r2e	GGAAAGCGGTAACAGTGCCCGTATCGGGGTTT
t3r2f	TGCCTTGACAGTCTCTGAATTTACCCCTCAGA
t3r4e	GTTTGCCACCTCAGAGCCGCCACCGCCAGAAT
t3r4f	GCCACCACTCTTTTCATAATCAAATAGCAAGG
t3r6e	TTATTCATGTCACCAATGAAACCATTATTAGC
t3r6f	CCGGAAACTAAAGGTGAATTATCATAAAAGAA
t3r8e	ATACCCAAACACCACGGAATAAGTGACGGAAA
t3r8f	ACGCAAAGAAGAACTGGCATGATTTGAGTTAA
t3r10e	GCGCATTAATAAGAGCAAGAAACAATAACGGA
t3r10f	GCCCAATAGACGGGAGAATTAACTTTCCAGAG
t3r12e	AGGTTTTGGCCAGTTACAAAATAAACAGGGAA
t3r12f	CCTAATTTAAGCCTTAAATCAAGAATCGAGAA
t3r14e	CTAATTTACCGTTTTTATTTTCATCTTGCGGG
t3r14f	CAAGCAAGCGAGCATGTAGAAACCAGAGAATA
t3r16e	ACGCTCAACGACAAAAGGTAAAGTATCCCATC
t3r16f	TAAAGTACCAGTAGGGCTTAATTGCTAAATTT
t3r18e	TATGTAAAGAAATACCGACCGTGTTAAAGCCA
t3r18f	AATGGTTTTGCTGATGCAAATCCATTTTCCCT

t3r20e	TTGAATTATTGAAAACATAGCGATTATAACTA
t3r20f	TAGAATCCCCTTTTTTAATGGAAACGGATTCG
t3r22e	ACAGAAATCTTTGAATACCAAGTTAATTTCAT
t3r22f	CCTGATTGAAAGAAATTGCGTAGAAGAAGGAG
t3r24e	CGACAACTTCATCATATTCCTGATCACGTAAA
t3r24f	CGGAATTACGTATTAAATCCTTTGGTTGGCAA
t3r26e	GCCACGCTTTGAAAGGAATTGAGGAAACAATT
t3r26f	ATCAACAGGAGAGCCAGCAGCAAAATATTTTT
t3r28e	GTCACACGATTAGTCTTTAATGCGGCAACAGT
t3r28f	GAATGGCTACCAGTAATAAAAGGGCAAACTAT
t3r30e	GTAAAAGACTGGTAATATCCAGAAATTCACCA
t3r30f	CGGCCTTGGTCTGTCCATCACGCATTGACGAG
t3r32h	CACGTATAACGTGCTTTCCTCGTTGCCACCGA
t5r0g	CCTCAAGAGAAGGATTAGGATTAGAAACAGTT
t5r2e	ACAAACAACTGCCTATTTCGGAACCTGAGACT
t5r2f	AATGCCCCATAAATCCTCATTAAAAGAACCAC
t5r4e	TCGGCATTCCGCCGCCAGCATTGATGATATTC
t5r4f	CACCAGAGTTCGGTCATAGCCCCCTCGATAGC
t5r6e	ATTGAGGGAATCAGTAGCGACAGACGTTTTCA
t5r6f	AGCACCGTAGGGAAGGTAAATATTTTATTTTG
t5r8e	GAAGGAAAAATAGAAAATTCATATTTCAACCG
t5r8f	TCACA ATCCC GAGGA AACGC AATAA TGAAATA
t5r10e	CTTTACAGTATCTTACCGAAGCCCAGTTACCA
t5r10f	GCAATAGCAGAGAATAACATAAAAACAGCCAT
t5r12e	GAGGCGTTTCCCAATCCAAATAAGATAGCAGC
t5r12f	ATTATTTATTAGCGAACCTCCCGACGTAGGAA
t5r14e	TAAGTCCTGCGCCCAATAGCAAGCAAGAACGC
t5r14f	TCATTACCGAACAAGAAAAATAATAATTCTGT
t5r16f	CCAGACGACAAATTCTTACCAGTAGATAAATA
t5r18e	TAACCTCCAATAAGAATAAACACCTATCATAT
t5r18f	AGGCGTTAGGCTTAGGTTGGGTTAAGCTTAGA
t5r20e	AAAACAAACTGAGAAGAGTCAATATACCTTTT

t5r20f	TTAAGACGATTAATTACATTTAACACAAAATC
t5r22e	AACCTACCGCGAATTATTCATTTCACATCAAG
t5r22f	GCGCAGAGATATCAAAATTATTTGTATCAGAT
t5r24e	GGATTTAGTTCATCAATATAATCCAGGGTTAG
t5r24f	GATGGCAAAAGTATTAGACTTTACAAGGTTAT
t5r26e	AGGCGGTCTCTTTAGGAGCACTAAACATTTGA
t5r26f	CTAAAATAAGTATTAACACCGCCTCGAACTGA
t5r28e	GAAATGGAAAACATCGCCATTAAACAGAGGTG
t5r28f	TAGCCCTATTATTTACATTGGCAGCAATATTA
t5r30e	AGAAGTGTCATTGCAACAGGAAAAAATCGTCT
t5r30f	CCGCCAGCTTTTATAATCAGTGAGAGAATCAG
t5r32h	AGCGGGAGCTAAACAGGAGGCCGAGAATCCTG
t-1r0g	TATCACCGTACTCAGGAGGTTTAGATAGTTAG
t-1r2e	ACGTTAGTTCTAAAGTTTTGTCGTGATACAGG
t-1r2f	CGTAACGAAAATGAATTTTCTGTAGTGAATTT
t-1r4e	CAATGACAGCTTGATACCGATAGTCTCCCTCA
t-1r4f	CTTAAACAACAACCATCGCCCACGCGGGTAAA
t-1r6e	AAACGAAATGCCACTACGAAGGCAGCCAGCAA
t-1r6f	ATACGTAAGAGGCAAAAGAATACACTGACCAA
t-1r8e	CCAGGCGCGAGGACAGATGAACGGGTAGAAAA
t-1r8f	CTTTGAAAATAGGCTGGCTGACCTACCTTATG
t-1r10e	GGACGTTGAGAACTGGCTCATTATGCGCTAAT
t-1r10f	CGATTTTAGGAAGAAAAATCTACGGATAAAAA
t-1r12e	TTTGCCAGGCGAGAGGCTTTTGCAATCCTGAA
t-1r12f	CCAAAATAAGGGGGTAATAGTAAAAAAAGATT
t-1r14e	TTTTAATTGCCCGAAAGACTTCAACAAGAACG
t-1r14f	AAGAGGAACGAGCTTCAAAGCGAAAGTTTCAT
t-1r16e	CGAGTAGAACAGTTGATTCCCAATATTTAGGC
t-1r16f	TCCATATATTTAGTTTGACCATTAAGCATAAA
t-1r18e	CTGTAATAGGTTGTACCAAAAACACAAATATA
t-1r18f	GCTAAATCCTTTTGCGGGAGAAGCCCGGAGAG
t-1r20e	TCAGGTCATTTTTGAGAGATCTACCCTTGCTT

t-1r20f	GGTAGCTATTGCCTGAGAGTCTGGTTAAATCA
t-1r22e	AAATAATTTTTAACCAATAGGAACAACAGTAC
t-1r22f	GCTCATTTCGCGTCTGGCCTTCCTGGCCTCAG
t-1r24e	GCTTCTGGCACTCCAGCCAGCTTTACATTATC
t-1r24f	GAAGATCGTGCCGGAAACCAGGCAGTGCCAAG
t-1r26e	CCCGGGTACCTGCAGGTCGACTCTCAAATATC
t-1r26f	CTTGCATGCCGAGCTCGAATTCGTCCTGTCGT
t-1r28e	GGGAGAGGCATTAATGAATCGGCCACCTGAAA
t-1r28f	GCCAGCTGCGGTTTGCGTATTGGGAATCAAAA
t-1r30e	AGTTTGGACGAGATAGGGTTGAGTGTAATAAC
t-1r30f	GAATAGCCACAAGAGTCCACTATTAAGCCGGC
t-1r32h	GAACGTGGCGAGAAAGGAAGGGAATGCGCCGC
t-3r0g	CCCTCAGAACCGCCACCCTCAGAAACAACGCC
t-3r2e	TGCTAAACTCCACAGACAGCCCTCTACCGCCA
t-3r2f	TGTAGCATAACTTTCAACAGTTTCTAATTGTA
t-3r4e	ATATATTCTCAGCTTGCTTTCGAGTGGGATTT
t-3r4f	TCGGTTTAGGTCGCTGAGGCTTGCAAAGACTT
t-3r6e	CTCATCTTGGAAGTTTCCATTAAACATAACCG
t-3r6f	TTTCATGATGACCCCCAGCGATTAAGGCGCAG
t-3r8e	AGTAATCTTCATAAGGGAACCGAACTAAAACA
t-3r8f	ACGGTCAATGACAAGAACCGGATATGGTTTAA
t-3r10e	ACGAACTATTAATCATTGTGAATTTCATCAAG
t-3r10f	TTTCAACTACGGAACAACATTATTAACACTAT
t-3r12e	ACTGGATATCGTTTACCAGACGACTTAATAAA
t-3r12f	CATAACCCGCGTCCAATACTGCGGTATTATAG
t-3r14e	GAAGCAAAAAAGCGGATTGCATCAATGTTTAG
t-3r14f	TCAGAAGCCTCCAACAGGTCAGGATTTAAATA
t-3r16f	TGCAACTAGGTCAATAACCTGTTTAGAATTAG
t-3r18e	CAACGCAAAGCAATAAAGCCTCAGGATACATT
t-3r18f	CAAAATTAGGATAAAAATTTTTAGGATATTCA
t-3r20e	AGAGAATCAGCTGATAAATTAATGCTTTATTT
t-3r20f	ACCGTTCTGATGAACGGTAATCGTAATATTTT

t-3r22e	CTTTCATCTCGCATTAAATTTTTGAGCAAACA
t-3r22f	GTTAAAATAACATTAAATGTGAGCATCTGCCA
t-3r24e	TTCGCCATGGACGACGACAGTATCGTAGCCAG
t-3r24f	GTTTGAGGTCAGGCTGCGCAACTGTTCCCAGT
t-3r26e	TCATAGCTTGTAAAACGACGGCCAAAGCGCCA
t-3r26f	CACGACGTGTTTCCTGTGTGAAATTTGCGCTC
t-3r28e	TGGTTTTTCTTTCCAGTCGGGAAAAATCATGG
t-3r28f	ACTGCCCGCTTTTCACCAGTGAGATGGTGGTT
t-3r30e	TGGACTCCGGCAAAATCCCTTATACGCCAGGG
t-3r30f	CCGAAATCAACGTCAAAGGGCGAAAAGGGAGC
t-3r32h	CCCCGATTTAGAGCTTGACGGGGAAAAGAACG
t-5r0g	CTCAGAGCCACCACCCTCATTTTCCGTAACAC
t-5r2e	GAGAATAGGTCACCAGTACAAACTCCGCCACC
t-5r2f	TGAGTTTCAAAGGAACAACTAAAGATCTCCAA
t-5r4e	AAAGGCCGCTCCAAAAGGAGCCTTAGCGGAGT
t-5r4f	AAAAAAGGCTTTTGCGGGATCGTCGGGTAGCA
t-5r6e	GCGAAACAAGAGGCTTTGAGGACTAGGGAGTT
t-5r6f	ACGGCTACAAGTACAACGGAGATTCGCGACCT
t-5r8e	CCAAATCATTACTTAGCCGGAACGTACCAAGC
t-5r8f	GCTCCATGACGTAACAAAGCTGCTACACCAGA
t-5r10e	AAAGATTCTAAATTGGGCTTGAGATTCATTAC
t-5r10f	ACGAGTAGATCAGTTGAGATTTAGCGCCAAAA
t-5r12e	TAAATATTGAGGCATAGTAAGAGCACAGGTAG
t-5r12f	GGAATTACCATTGAATCCCCCTCACCATAAAT
t-5r14e	TACCTTTAAGGTCTTTACCCTGACAATCGTCA
t-5r14f	CAAAAATCATTGCTCCTTTTGATAATTGCTGA
t-5r16e	TTTCATTTCTGTAGCTCAACATGTTTAGAGAG
t-5r16f	ATATAATGGGGGCGCGAGCTGAAATTAACATC
t-5r18e	TATATTTTCATACAGGCAAGGCAAAGCTATAT
t-5r18f	CAATAAATAAATGCAATGCCTGAGAAGGCCGG
t-5r20e	CATGTCAAAAATCACCATCAATATAACCCTCA
t-5r20f	AGACAGTCTCATATGTACCCCGGTTTGTATAA

t-5r22e	ACCCGTCGTTAAATTGTAAACGTTAAAACTAG
t-5r22f	GCAAATATGATTCTCCGTGGGAACCGTTGGTG
t-5r24e	GGCGATCGCGCATCGTAACCGTGCGAGTAACA
t-5r24f	TAGATGGGGTGCGGGCCTCTTCGCGCAAGGCG
t-5r26e	GCTCACAAGGGTAACGCCAGGGTTTTGGGAAG
t-5r26f	ATTAAGTTTTCCACACAACATACGCCTAATGA
t-5r28e	AGCTGATTACTCACATTAATTGCGTGTTATCC
t-5r28f	GTGAGCTAGCCCTTCACCGCCTGGGGTTTGCC
t-5r30e	TATCAGGGCGAAAATCCTGTTTGACGGGCAAC
t-5r30f	CCAGCAGGCGATGGCCCACTACGTGAGGTGCC
t-5r32h	GTAAAGCACTAAATCGGAACCCTAAAACCGTC

Table S3. HPLC analysis and MALDI-TOF characterization of modified staples required for DNA Td and DNA origami assembly. Calculated (calc) and experimental (exp) molecular weights are compiled for comparison.

Sample	rt (min)	MW calc/ exp	Sample	rt (min)	MW calc/ exp
DNA Tetrahedron					
S1-FdU$_{10}$	[a]5.4	22423.1/22464.0	**Chol-S4a**	14.0	6591.8/6593.7
Chol-S2a	13.6	6645.8/6646.9	**Chol-S4b**	13.9	13924.5/13909.6
Chol-S2b-FdU$_{10}$	13.9	16912.1/16978.4	**Fluorescein-S5**	5.3	6855.8/6872.9
Chol-S3a	13.9	6807.9/6810.3	**Cy3-S5**	7.5	6399.4/6406.3
Chol-S3b	13.7	13831.4/13820.7			
DNA origami					
FdU$_{10}$	[a]5.0	3020.0/3018.1			
t-5r4e-FdU$_{13}$	[a]6.1	13897.5/13904.5	**Chol-t-3r18f**	12.6	11378.2/11380.3
t-3r2f-FdU$_{14}$	[a]6.3	14100.6/14119.7	**Chol-t5r14f**	13.0	11308.2/11307.2
t3r4e-FdU$_{14}$	[a]6.1	14028.5/14034.5	**Chol-t5r18f**	12.7	11496.9/11495.2
t5r2f- FdU$_{14}$	[a]6.1	14011.6/14011.7	**Chol-t-1r22f**	13.2	11216.7/11220.9
t-3r8e-FdU$_{10}$	[a]5,5	12923.0/12933.4	**Chol-t1r22e**	12.9	11370.9/11365.3
t3r6f-FdU$_{10}$	[a]5.4	12987.1/12983.7	**Chol-t-1r30f**	13.0	11317.8/11314.0
Chol-t-3r14f	12.7	11326.2/11328.7	**Chol-t1r30e**	12.9	11369.8/11367.5

FdU$_n$ n in subscript stands for the number of FdU monomers, Chol for a cholesterol moiety and Cy3 for Cyanine 540.[a] The HPLC gradient was increased from 0–50% acetonitrile in 20 min; the remaining samples were analyzed following the HPLC gradient increased from; 0–15% over 5 min, followed by 15-40 % over 1 min and 40-85% over 9 min.

Table S4. Averaged hydrodynamic size determined for Td and DNA origami through dynamic light scattering (DLS). The sizes of unmodified Td (Td) and DNA origami (Origami-N) are listed and compared with FdU_n- and cholesterol-modified nanostructures, Td-1F, Td-1F-2C, Td-1F-3C, Td-1F-4C, Td-2F-3C, Origami-4F, Origami-6F, Origami-4F-4C and Origami-6F-8C. Data was measured in triplicate. The polydispersity index (PdI) and respective standard deviation (SD-PdI) are also reported.

Sample	Hydrodynamic size (d nm)	SD	Pdl	SD-Pdl
DNA Tetrahedron				
Td	24.5	±5.8	0.44	±0.01
Td-1F	26.4	±4.4	0.45	±0.07
Td-1F-2C	94.3	±4.8	0.38	±0.02
Td-1F-3C	100.5	±4.8	0.39	±0.19
Td-1F-4C	187.7	±34.7	0.27	±0.11
Td-2F-3C	76.4	±14.8	0.43	±0.17
DNA origami				
Origami-N	124.3	± 3.8	0.26	± 0.09
Origami-4F	133.5	± 6.47	0.39	± 0.06
Origami-6F	117.7	±2.2	0.23	± 0.01
Origami-4F-4C	131.2	± 3.2	0.34	± 0.05
Origami-6F-8C	141.2	± 6.2	0.33	± 0.05

Table S5. This table summarizes the concentration of DNA nanoscaffolds and their respective total number of equivalents of FdU. The control FdU_{10} is also listed.

Sample	Number of FdU units	Molar concentration of DNA nanoscaffolds (μM)	Total number of FdU equivalents (μM)
DNA Tetrahedron			
Control FdU_{10}	10	0.5	5
Td-1F	10	0.5	5
Td-1F-2C	10	0.5	5
Td-1F-3C	10	0.5	5
Td-1F-4C	10	0.5	5
Td-2F-3C	20	0.25	5
DNA origami			
Origami-4F	55	0.002	0.110
Origami-6F	75	0.002	0.150
Origami-4F-4C	55	0.002	0.110
Origami-6F-8C	75	0.002	0.150

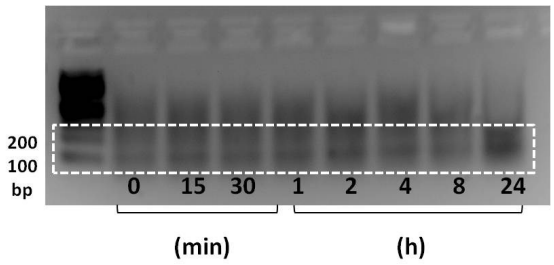

Figure S1. Long-term stability of the DNA Td in cell culture medium measured in 2.5% agarose gel electrophoresis. Each lane represents the migration of standard Td after different times of incubation with DMEM plus 10 % FBS, at 37 °C, (lane 2) t= 0, (lane 3) t= 15 min, (lane 4) t= 30 min, (lane 5) t= 1h, (lane 6) t= 2h, (lane 7) t= 4h, (lane 8) t= 8h and (lane 9) t= 24 h. Lane (1) shows the migration of the DNA ladder with 100 bp.

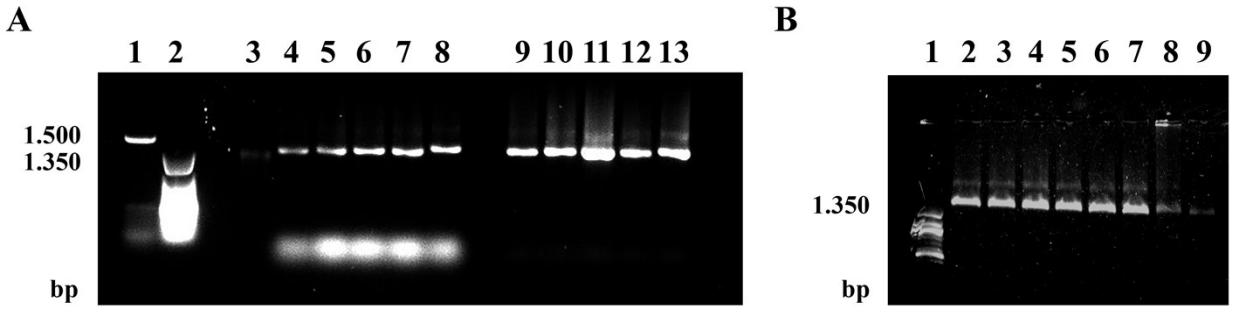

Figure S2. Gel electrophoresis analysis and stability of DNA origami, 1% agarose gel. **A,** Shows the formation and purification of the different DNA origami prepared. (1) 10 bp ladder, (2) 50 bp ladder, (3) M13 scaffold alone, (4-8) DNA origami formation (9-13) DNA origami after purification, (4, 9) DNA origami, (5, 10) DNA origami 4-F, (6, 11) DNA origami-4F-4C, (7, 12) DNA origami-6F, (8, 13) DNA origami-6-F-8C. **B,** Shows the stability of the DNA origami over time in DMEM plus 10 % FBS at 37 °C (1) 50 bp ladder, (2) t= 0, (3) t= 15 min, (4) t= 30 min, (5) t= 60 min, (6) t= 120 min, (7) t= 240 min, (8) t= 480 min and (9) t= 24 h.

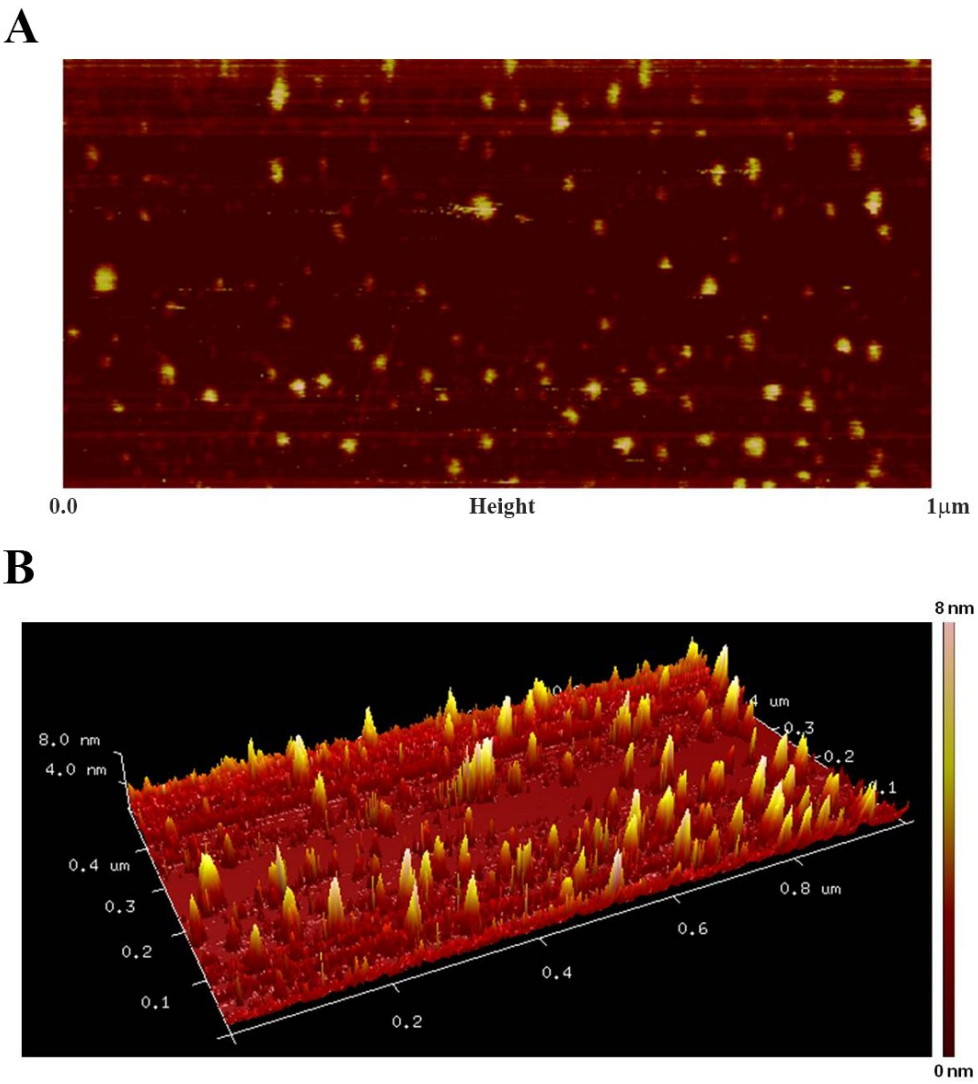

Figure S3: Additional AFM images of DNA Td. **A,** AFM image showing DNA tetrahedron deposited on mica surface. **B,** 3D representations of the DNA Td deposited in mica. Color bar indicates the height of the nanostructures.

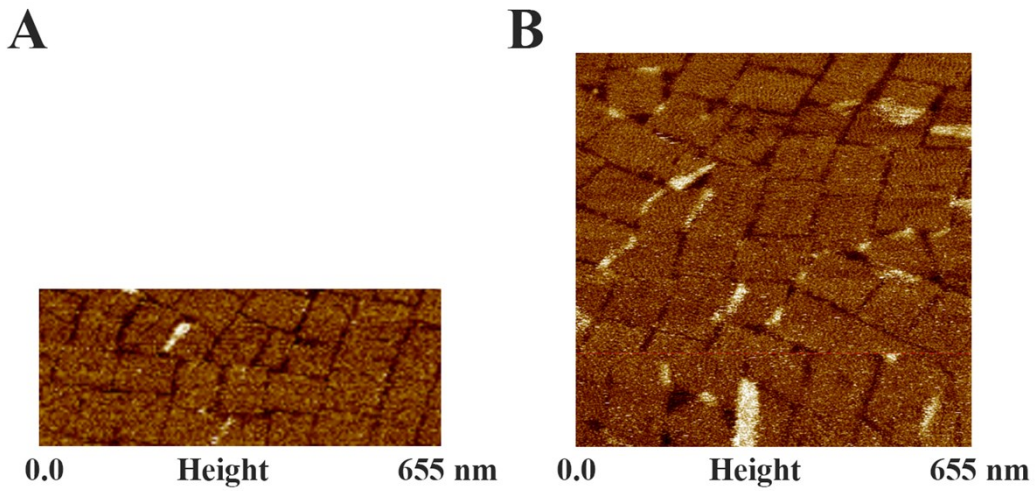

Figure S4. AFM images of the unmodified DNA origami. Image size 328 nm x 656 nm and 656 nm x 656 nm Image height: -2.0 nm to 2.7 nm.

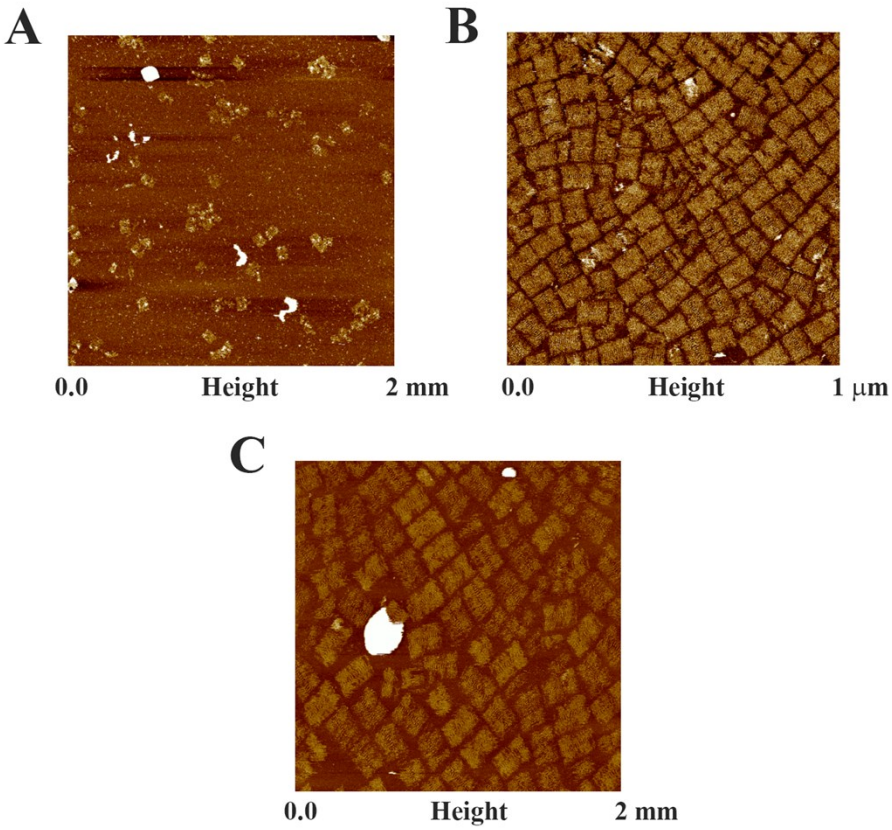

Figure S5. AFM images of the DNA origami containing **A,** 4 FdU staples, **B,** 4 FdU-4C and **C,** 6 FdU-8C staple displaced in different positions. DNA origami were formed correctly, showing that the introduction of the modified tiles did not affect its assembly.

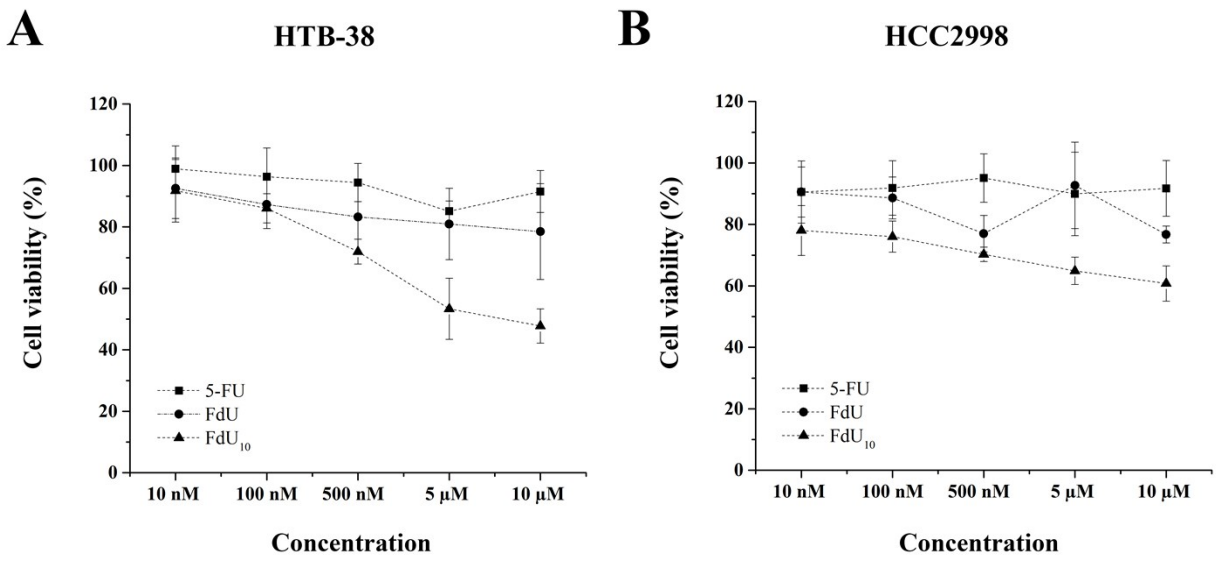

Figure S6. 5-fluoro-uracil (5-FU), 5-fluoro-2'-deoxyuridine (FdU) and polymeric 5-fluoro-2'-deoxyuridine (FdU_{10}) cytotoxic activity measured through MTT assay in, **A,** HTB-38 and **B,** HCC2998 cells. The values obtained for 5-FU, FdU and FdU_{10} are shown as fill squares, circles and triangles, respectively. All the data are normalized to DMSO and PBS both used as control for drugs and polymeric form, respectively. The concentration assayed ranges from 10 nanomolar (nM) to 10 micromolar (µM). Data represent the mean values and standard deviation measured in duplicate for three independent experiments.

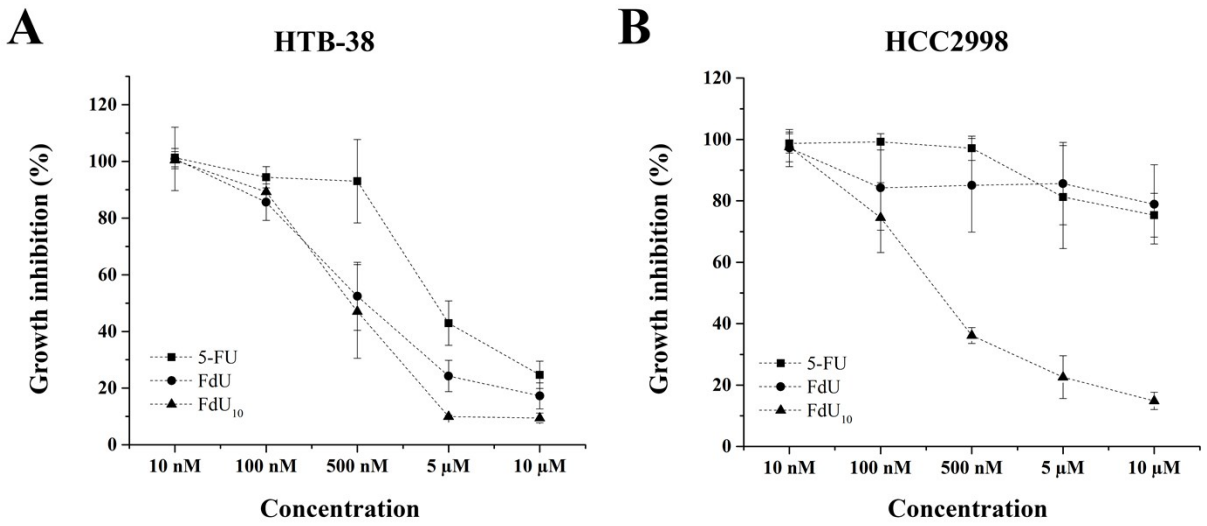

Figure S7. Growth inhibition assays. 5-fluoro-uracil (5-FU), 5-fluoro-2'-deoxyuridine (FdU) and polymeric 5-fluoro-2'-deoxyuridine (FdU_{10}) were assayed in two different types of colorectal cancer cell **A,** HTB-38 and **B,** HCC2998. The values obtained for 5-FU, FdU and FdU_{10} are shown as fill square, circles and triangles, respectively. All the data are normalized to DMSO and PBS both used as control for drugs and polymeric form, respectively. The concentration assayed range from 10 nanomolar (nM) to 10 micromolar (µM). Data represent the mean values and standard deviation measured in duplicate for three independent experiments.

References

1. Gupta KC, Kumar P, Bhatia D and Sharma AK, *Nucleosides Nucleotides* 1995, **14**, 829.
2. M. J. Damha, P. A. Giannaris and S. V. Zabarylo, *Nucleic Acids Res*, 1990, **18**, 3813-3821.