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Electronic Supplementary Information (ESI)

DNAzyme-Mediated Logic Gate for Programming Molecular Capture and Release on DNA Origami

Feiran Li,[a] Haorong Chen,[a] Jing Pan,[a] Tae-Gon Cha,[a] Igor L. Medintz[b] and Jong Hyun Choi[a*]

[a]School of Mechanical Engineering, Purdue University, West Lafayette, Indiana 47907, United States
[b]Centre for Bio/Molecular Science and Engineering, Code 6900, U.S. Naval Research Laboratory, Washington DC 20375, United States
*Corresponding author. Email: jchoi@purdue.edu

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Materials

All DNA strands were purchased from Integrated DNA Technologies (IDT). Gold nanoparticles (AuNPs) of 10 nm in diameter were acquired from BBI Solutions. Thrombin was obtained from Haematologic Technologies Inc. PD 10 desalting columns were acquired from GE Healthcare Life Sciences. Electrophoresis buffer and reagents as well as nucleic acid standards were obtained from Bio-Rad. All other chemicals were purchased from Sigma-Aldrich.

Experimental Methods

Ru complex conjugation with DNA

The synthesis of ruthenium bipyridine phenanthroline isothiocyanates (Ru-bpy-phen-itc) was reported elsewhere.[1] To conjugate the Ru complex with amino-modified capture strand,[2] Ru-bpy-phen-itc was first dissolved dimethyl sulfoxide (DMSO) to prepare a 20 mM solution. Amino-modified DNA was dissolved in deionized H_2O at 1 mM. Approximately 10 μL Ru-bpy-phen-itc solution was mixed with 10 μL DNA solution. Sodium carbonate buffer (0.1 M, pH 8.5) was added to the mixture until 100 μL to trigger the reaction. The mixture was then incubated at room temperature for 6 hours, with gent tapping of the vial every 2 hours to make sure that the solution is well mixed. After incubation, a PD 10 desalting column was used to purify labelled DNA-Ru sample. 100 μM triethylamine acetate was used as elution buffer. Approximately, 750 μL buffer was used in each elution. Ru-complex-labelled DNA were washed after 3rd or 4th time elution. Purified strands were collected and the concentration was determined by absorbance measurement.

DNA origami synthesis

M13mp18 strands were purchased from Bayou Biolabs. The scaffold strands were mixed with 180 32-nt staple strands in 1x Tris-acetate-ethylenediaminetetraacetic acid (TAE) buffer containing 12.5 mM Mg^{2+} (termed TAEM). The final concentrations of the scaffolds and staple strands were 10 and 100 nM, respectively. The mixture was incubated in a thermal cycler (Bio-Rad S1000). The temperature was elevated to 75 °C and then decreased to 4 °C by 0.1 °C every 6 seconds.[3, 4] After the thermal annealing, the strands self-assemble to form rectangular origami tiles of 100 nm x 70 nm.

Phosphine-capping of AuNPs

AuNPs were incubated overnight with 2.5 mM bis(para-sulfonatophenyl) phenylphosphine dihydrate dipotassium salt (BSPP). The solution was then centrifuged at 13,000 rpm for 15 minutes. The supernatant was discarded and the precipitate was re-dispersed in 2.5 mM BSPP solution. The concentration of AuNPs was determined by measuring the absorption at 520 nm using a NanoDrop 1000 spectrophotometer.

AuNP functionalization

Thiol-modified DNA strands were conjugated with AuNPs (sequence shown below).The DNA strands were mixed with AuNPs at an approximately 200:1 concentration ratio for 12 hours in 0.5x Tris-borate-EDTA (TBE) containing 0.1 M NaCl. After 12 hours, NaCl

concentration was slowly raised to 0.3 M in the next 12 hours at room temperature. The solution was then centrifuged at 13,000 rpm for 15 minutes. The supernatant was discarded and the precipitate was re-dissolved in 1x TBE buffer.[5]

AuNP-origami conjugation

AuNP-modified DNA strands were mixed with DNA origami solution at a concentration ratio of approximately 4:1. The mixture was incubated at room temperature over night for conjugation. The products were then purified by 1.5% agarose gel electrophoresis at 60 V, 4 °C. A desired band was cut and extracted with Freeze 'N Squeeze column (Bio-Rad) which was frozen at -20 °C for 5 minutes and then centrifuged at 13,000 g for 3 minutes. The extracted sample was the purified AuNP-origami.

Thrombin-origami conjugation

Thrombin-binding aptamer (TBA) sequence (5' – GGTTGGTGTGGTTGG - 3') was used for capturing thrombin.[6] To examine the binding specificity, equi-molar mixture of thrombin and glutathione s-transferase (GST) molecules was first mixed with TBA-expressed DNA origami solution at a concentration ratio of 100:1 and incubated at room temperature for 2 hours for conjugation. After conjugation, the mixture was then centrifuged 2 times in a 100 kD centrifugal filter (EMD Millipore) at 5,000 g for 3 minutes. Unfiltered sample was collected and mixed with DNA logic gate strands to release the attached thrombin molecules. The collected thrombin molecules were incubated at 95 °C for 4 minutes to be denatured, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed at 120 V for around 1 hour. The gel was stained by Oriole Fluorescent Gel Stain (Bio-Rad) and visualized under UV irradiation.

Release of analytes (Ru-dye/thrombin/AuNP) from origami tiles

The logic gate complex (LGC) and both initiators were added to analyte-conjugated origami solution at a concentration ratio of logic gate strands: analyte=10: 1. The mixture was then incubated at room temperature for 2 hours.

UV-triggered logic gate experiment

The photocleavable strand S_1^* was mixed with S_2 and S_3, instead of S_1, to prepare a LGC by annealing from 75 to 4 °C. The LGC was then centrifuged at 5,000 rpm for 3 minutes in a 30 kD centrifugal filter (EMD Millipore). The centrifugation was performed 6 times, and the purified LGC was collected and ready to use. UV irradiation was performed using a UV lamp (UVP, model# UVGL-25, λ~254 nm) for about 15 minutes, which was placed approximately 5 cm away from the origami sample.

Agarose gel electrophoresis

Approximately 600 mg agarose was added to 40 mL 0.5x TBE buffer to prepare a 1.5% agarose solution. The solution was then heated to boil to dissolve all agarose. After the solution cooled down, while it did not polymerize, 2 μL 10 mg/mL ethidium bromide was added to the solution and mixed well. The solution was then left in room temperature for polymerization.

PAGE experiment

To prepare 15% native PAGE experiment, approximately 1.875 mL 40% acrylamide, 0.5 mL 10x TBE, and 2.625 mL DI H_2O were mixed well first. Then, 20 µL 25% (w/v) ammonium persulfate (APS) and 10 µL tetramethylethylenediamine (TEMED) were added into the solution. The gel was cast by a gel casting system (Bio-Rad) immediately and ready to use.

SDS-PAGE experiment

SDS-PAGE used in this work was 10% separating gel and 10% stacking gel. To prepare the separating gel, 1.52 mL H_2O, 1.02 mL 40% acrylamide, 1.04 mL 1.5 M Tris (pH 8.8), 40 µL 10% (w/v) SDS were mixed well first. Then, 40 µL 10% (w/v) APS and 4 µL TEMED were added into the solution. Then a separating layer was cast by a gel casting system.

To prepare the stacking gel, 0.98 mL H_2O, 0.5 mL 40% acrylamide, 0.5 mL 0.5 M Tris-HCl (pH 6.8), 20 µL 10% (w/v) SDS were mixed well. Then, 20 µL 10% (w/v) APS and 2 µL TEMED were added into the solution. On top of a freshly prepared separating layer, a stacking layer was cast, and the gel was ready to use.

AFM imaging

All AFM imaging was performed in ambient, unless otherwise stated. Origami solution was first diluted to 1 nM by TAEM buffer. Approximately 10 µL of the diluted solution was then deposited on a freshly cleaved mica. After incubation for 5 – 10 minutes, the solution was blown away with compressed air. The mica surface was further rinsed with about 50 µL DI water and blown dry with compressed air. The AFM imaging was performed with a SCANASYST-AIR probe.

The thrombin origami was imaged in TAEM buffer. To prepare the sample, 10 µL of 1 nM origami sample in TAEM was first deposited on a freshly cleaved mica. After incubation for 5 – 10 minutes, another 30 µL TAEM buffer was added onto the mica surface. The sample was then scanned with a SCANASYST-FLUID+ probe.

Supporting Figures

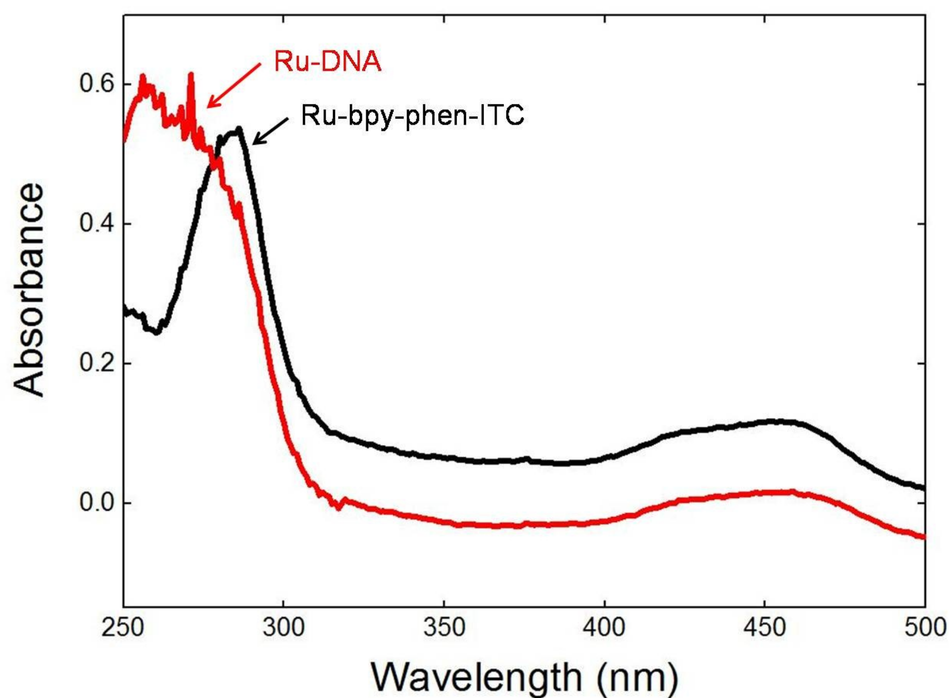

Figure S1. Absorbance of Ru-bpy-phen-ITC and DNA-conjugated Ru-dye

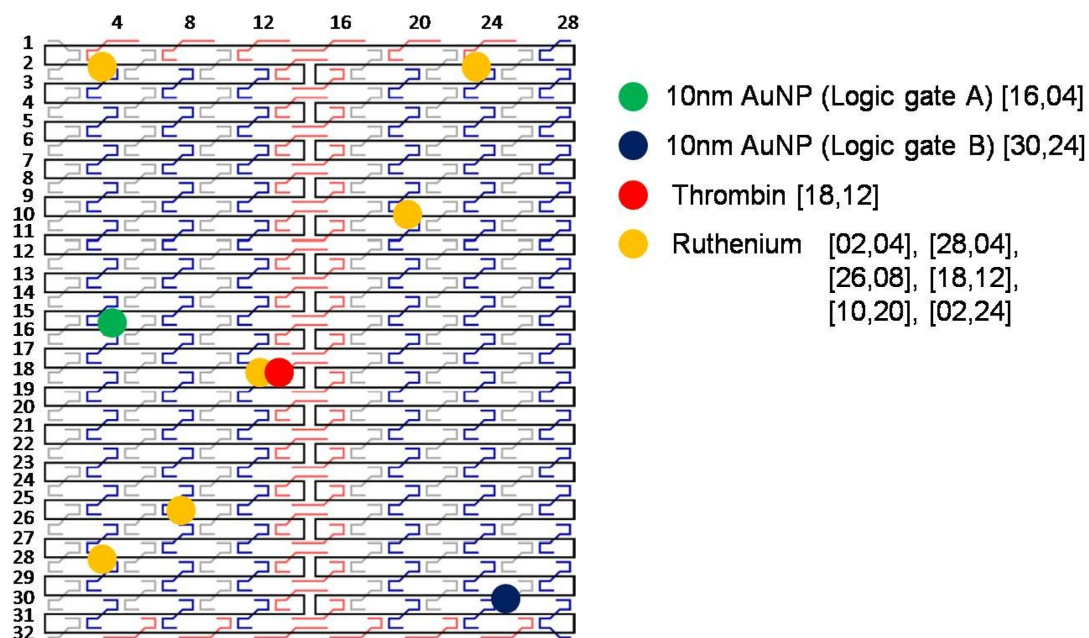

Figure S2. Schematic of the folded pattern of a scaffold (black) along with staples (grey, blue, and red). The staple positions for Ru-dyes, thrombin molecules, and AuNPs are denoted in orange, red, and green/navy, respectively.

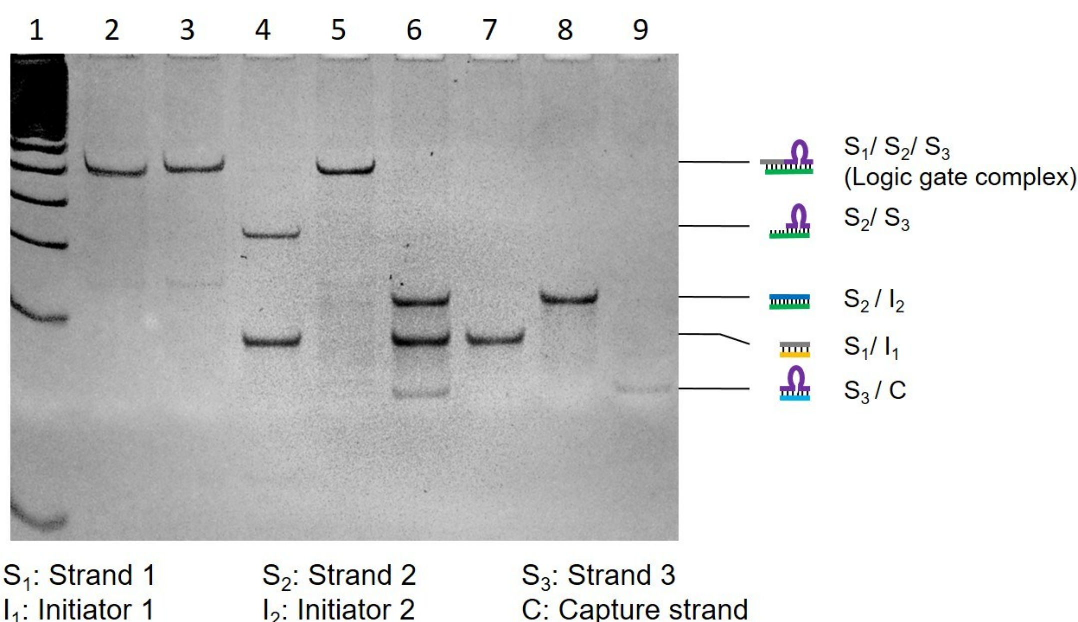

Figure S3. 15% PAGE analysis of the logic gate set B. Lane 1: 20 bp ladder. Lane 2: LGC ($S_1/S_2/S_3$). Lane 3: LGC + C. Lane 4: LGC + I_1 + C. Lane 5: LGC + I_2 + C. Lane 6: LGC + I_1 + I_2 + C. Lane 7: S_1 + I_1. Lane 8: S_2 + I_2. Lane 9: S_3 + C. LGC, capture strand (C), and both initiators are mixed to a final concentration at 0.5 µM. In lines 7-9, S_1/I_1, S_2/I_2, and S_3/C are examined as controls. Only when both initiator strands are present, the logic gate process generates the final product S_3, which subsequently hybridizes with a capture strand, shown as S_3/C band in line 6.

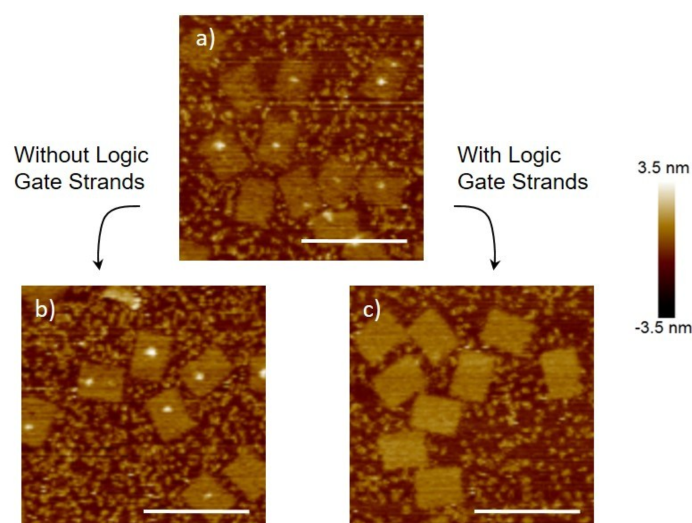

Figure S4. AFM images demonstrating DNAzyme-mediated thrombin release from DNA origami. (a) Thrombin (shown as white dots) captured on DNA origami. (b) In the absence of the logic gate strands, the thrombin molecules are retained on the origami tiles. (c) In the presence of the logic gate strands (LGC, I_1, and I_2) at an equimolar ratio of DNAzyme and capture strands (i.e. S_3: C = 1:1), the analyte molecules are released from DNA origami. Scale bar: 200 nm.

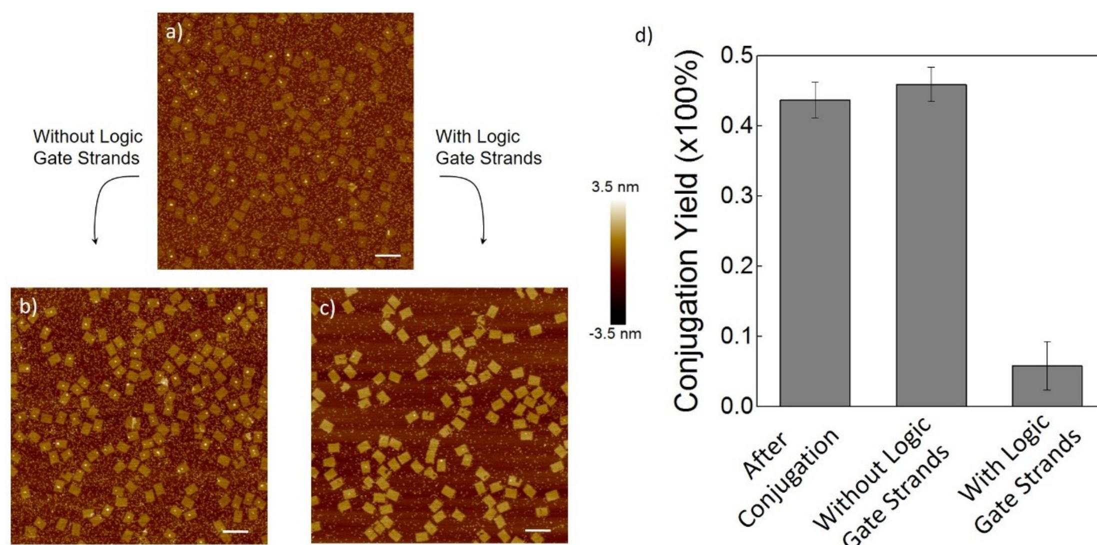

Figure S5. (a)-(c) Representative large-area view of AFM images for statistical analysis of thrombin release from DNA origami tiles. (a) Thrombin molecules (shown as white dots) captured on DNA origami tiles. (b) In the absence of logic gate strands (LGC, I_1, and I_2), the thrombin molecules are retained on the origami tiles. (c) Nearly all thrombin molecules are released from the tiles by the logic gate process. Scale bar: 200 nm. (d) Statistical analysis of thrombin molecules conjugated on DNA origami tiles based on the examination of more than 500 tiles.

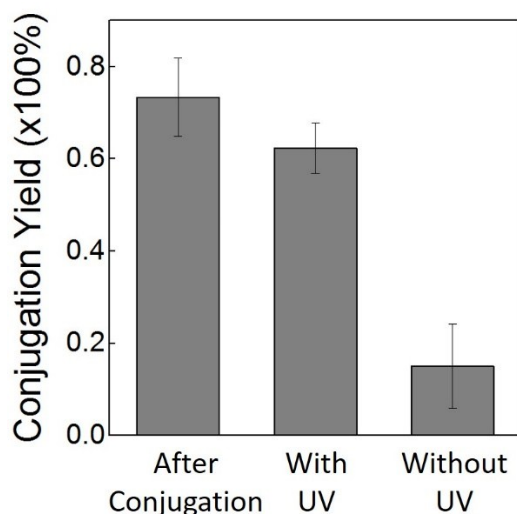

Figure S6. Conjugation yield of AuNPs on DNA origami tiles, in the absence and presence of the logic gate strands. The statistical analysis, corresponding to Fig. 4, is performed based on more than 80 origami tiles.

Sequence Information

All DNA logic gate strands are designed by NUPACK.[7]

Logic gate strand sequence (left to right: 5' – 3')

Logic gate A

Strand 1: ATC TAA CAA CCA CCA CCA AAC CAT CCC ACA CAC CAC

Strand 2: ATG GTT TGG TGG TGG TTG TTA GAT GTC ACT CTG TCC GAA TCA GCA CT

Strand 3: AGT GCT GAT TCG GAC AGG CTA GCT ACA ACG AGA GTG AC

Initiator 1: GTG GTG TGT GGG ATG GTT TGG TGG TGG TTG TTA GAT

Initiator 2: AGT GCT GAT TCG GAC AGA GTG ACA TCT AAC AAC CA

Capture strand: GTC ACT CrArU GTC CGA ATC AGC ACT

Logic gate B

Strand 1: ACC GCG TCT CAT ACA TCA TCT GGC ACA TCA TCT CAC

Strand 2: GCC AGA TGA TGT ATG AGA CGC GGT GTG CAG GTG GAG AGC ATA AAG TC

Strand 3: GAC TTT ATG CTC TCC AGG CTA GCT ACA ACG ACC TGC AC

Initiator 1: GTG AGA TGA TGT GCC AGA TGA TGT ATG AGA CGC GGT

Initiator 2: GAC TTT ATG CTC TCC ACC TGC ACA CCG CGT CTC AT

Capture strand: GTG CAG GrArU GGA GAG CAT AAA GTC

Photo-cleavable logic gate strand

Logic gate A

Strand 1': ATC TAA CAA CCA CCA CCA AAC CAT /iSpPC/CC CAC ACA CCA C

Capture strand for Ru-dye

/5AmMC6/GTG CAG GrArU GGA GAG CAT AAA GTC TTT TTT TTT TTT TTT

Capture strand for AuNP

[16,04]:TTT TTT TTT TTT TTT TTT TTT TTT GTC ACT CrArU GTC CGA ATC AGC ACT /3ThioMC3-D/

[30,24]:TAC GAG TTG AGA ATC CTG AAT TTT GTG CAG GrArU GGA GAG CAT AAA GTC /3ThioMC3-D/

Capture strand for thrombin

TTT TTT TTT TTT TTT GTC ACT CrArU GTC CGA ATC AGC ACT CAT CTC GGT TGG TGT GGT TGG

Staple extension for capture strands

[16,04]: AAA AAA AAA AAA AAA AAA AAA AAA AAA GCC TGA AAG TAA TTC TGT CCA GCA GAA CG

[30,24]: AAA ATT CAG GAT TCT CAA CTC GTA TTT CAA CAT GTA CCG TAA CAC TGA TCA GAA CC

[18,12]: AAA AAA AAA AAA AAA GCA TGT AGC ATT CCA AGA ACG GGT TTT TGA AG

[02,04]: GAA CGG TAC AGA ACA ATA TTA CCG AAT ACC TAA AAA AAA AAA AAA AA

[28,04]: TCA GAC TGC CAC CAG AAC CAC CAC GGC AGG TCA AAA AAA AAA AAA AA

[26,08]: GAA GGT AAA CCA TTA GCA AGG CCG GCA TTT TCA AAA AAA AAA AAA AA

[18,12]: GCA TGT AGC ATT CCA AGA ACG GGT TTT TGA AGA AAA AAA AAA AAA AA

[10,20]: GTC TGG CCA CGT TAA TAT TTT GTT GGT CAT TGA AAA AAA AAA AAA AA

[02,24]: GTC AAA GGA CGC TGG TTT GCC CCA TTT TTC TTA AAA AAA AAA AAA AA

Unmodified staples

Name	Sequence
sTop[03, 05]	GTAATATCCGCCAGAATCCTGAGAGTATAACG
sTop[03, 09]	GAGTAGAAGTGAGGCCACCGAGTAGAGCGGGC
sTop[03, 17]	AAAATCCCTGAGTGTTGTTCCAGTCGATTTAG
sTop[03, 21]	AAATCCTGCTATTAAAGAACGTGGAAGCACTA
sTop[03, 25]	AGCGGTCCGCGAAAAACCGTCTATCAAATCAA
sTop[05, 05]	AGTCTTTACGCTCAATCGTCTGAACCTTGCTG
sTop[05, 09]	CGTGGCACGGCAGATTCACCAGTCACTTGCCT
sTop[05, 17]	TCCAGTCGCGGCCAACGCGCGGGGAAATCGGC
sTop[05, 21]	CACATTAATTGGGCGCCAGGGTGGCAGGCGA
sTop[05, 25]	AAAGCCTGTGAGACGGGCAACAGCTTGCAGCA
sTop[07, 05]	AAAGGAATTAAAACAGAGGTGAGGTGGCTATT
sTop[07, 09]	CAATATCTCGCCTGCAACAGTGCCTAAGAATA
sTop[07, 17]	AAAACGACACTCTAGAGGATCCCCGCCCGCTT
sTop[07, 21]	TAACGCCATTCGTAATCATGGTCAAGCTAACT
sTop[07, 25]	AGGGGGATGAAATTGTTATCCGCTTAAAGTGT
sTop[09, 05]	TCATATTCATAATACATTTGAGGAAACAGTTG
sTop[09, 09]	CAAAGAAATTTACAAACAATTCGACCCTCAAT
sTop[09, 17]	CGACGACAGCTTTCCGGCACCGCTGACGTTGT
sTop[09, 21]	ATCGTAACAGGCAAAGCGCCATTCAAGTTGGG
sTop[09, 25]	ATGGGATAAACTGTTGGGAAGGGCCTGGCGAA
sTop[11, 05]	TGAATACCATGGAAGGGTTAGAACAATTATCA
sTop[11, 09]	GGAGAAACATTTGCACGTAAAACATTGCGGAA
sTop[11, 17]	CATTAAATGGAACGCCATCAAAAATGAGGGGA
sTop[11, 21]	AATTGTAATTCCTGTAGCCAGCTTATGGGCGC
sTop[11, 25]	AAAAACAGTGAGCGAGTAACAACCTGACCGTA
sTop[13, 05]	AAAACATAAACAAACATCAAGAAAGATTGCTT

sTop[13, 09]	CGCTATTATTAACAATTTCATTTGTTACATCG
sTop[13, 17]	TGATAAATTCTACAAAGGCTATCAAAAATTCG
sTop[13, 21]	TCACCATCTCTGGAGCAAACAAGAAATATTTA
sTop[13, 25]	CAAAAGGGATCGTAAAACTAGCATAAAGCCCC
sTop[15, 05]	ATACCGACGAGACTACCTTTTTAAAATCCTTG
sTop[15, 09]	TTCATCTTGGTTATATAACTATATTAAATCGT
sTop[15, 17]	AATAAAGCAAACATTATGACCCTGGTTCTAGC
sTop[15, 21]	ACAGGCAAGAAGCCTTTATTTCAACAGTCAAA
sTop[15, 25]	AATAGTAGTTTAGAACCCTCATATTAAAGATT
sTop[17, 05]	CAAAAGGTTTTAGTATCATATGCGGGTTTGAA
sTop[17, 09]	CGAGCCAGCAGTATAAAGCCAACGTAGTTAAT
sTop[17, 17]	TACGGTGTCCAATTCTGCGAACGAAATTAAGC
sTop[17, 21]	TAGCTCAACATTAGATACATTTCGTAAATCAT
sTop[17, 25]	GGCTTAGATGTTTAGCTATATTTTATTCTACT
sTop[19, 05]	TTTTTATTTATCAACAATAGATAAAGTACCGA
sTop[19, 09]	AAGTACCGATAATATCCCATCCTAGGCATTTT
sTop[19, 17]	GCGGATTGTTCAAATATCGCGTTTAACTAAAG
sTop[19, 21]	TCTTTACCGCGAACCAGACCGGAATAATGCTG
sTop[19, 25]	AAAACGAGCAGGATTAGAGAGTACTTGCGGAT
sTop[21, 05]	AGTTACAAATTCTAAGAACGCGAGGCAAGCCG
sTop[21, 09]	CTAACGAGCCCGACTTGCGGGAGGATTAAACC
sTop[21, 17]	TTTACCAGTTGCAAAAGAAGTTTTGAAGCAAA
sTop[21, 21]	GCATAGTAGTAAAATGTTTAGACTAAATCAGG
sTop[21, 25]	TGCAGATATGCGGAATCGTCATAACAGTTCAG
sTop[23, 05]	AGAGCAAGTGAAAATAGCAGCCTTAATTTGCC
sTop[23, 09]	AACCCACATAAAAACAGGGAAGCGTACCAACG
sTop[23, 17]	ATCATTGTATTATACCAGTCAGGAAACCCTCG
sTop[23, 21]	ATTGGGCTCTACGTTAATAAAACGATTACGAG
sTop[23, 25]	GCCCTGACTTATTACAGGTAGAAATCAACTAA
sTop[25, 05]	CCACGGAAAACAAAGTTACCAGAACAATAATA
sTop[25, 09]	AGGTGGCACAATAATAACGGAATAAGAGAGAT
sTop[25, 17]	TAAGGGAAAACGGTGTACAGACCACAACTTTA
sTop[25, 21]	CTTAGCCGGACCTTCATCAAGAGTAGTAGTAA
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sTop[27, 25]	AGCATCGGGATTATACCAAGCGCGCTGATAAA
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sTop[29, 09]	CTCAGAACCCCCCTTATTAGCGTTCACCAGTA

sTop[29, 17]	GCTTGCTTATAGTTGCGCCGACAACATGAGGA
sTop[29, 21]	CAAAAGGACCACGCATAACCGATAGCTACAGA
sTop[29, 25]	TTTCACGTCTTGCAGGGAGTTAAACGAAAGAC
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sTop[31, 09]	AATAAGTTTTAAAGCCAGAATGGACCGCCACC
sTop[31, 17]	ACAGACAGGTCGTCTTTCCAGACGGTTTATCA
sTop[31, 21]	ACCAGTACCTGTATGGGATTTTGCAAAGGCTC
sTop[31, 25]	GGAACCCAGTTTCAGCGGAGTGAGAATAATTT
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sBot[02, 08]	TATAATCAGAACTCAAACTATCGGATGGATTA
sBot[02, 12]	TGTCCATCGATTAGTAATAACATCACACGACC
sBot[02, 20]	AGAGTCCATTTGATGGTGGTTCCGAGAGGCGG
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sBot[04, 20]	TTTGCGTATTGCGTTGCGCTCACTGGGTACCG
sBot[04, 24]	TTCACCAGGGGTGCCTAATGAGTGTAGCTGTT
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sBot[16, 04]	AAAGCCTGAAAGTAATTCTGTCCAGCAGAACG
sBot[16, 08]	ATTCTTACTAATAAGAGAATATAAGTCCTGAA
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sBot[28, 04]	TCAGACTGCCACCAGAACCACCACGGCAGGTC
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sBot[28, 12]	TTTCATAAACCGCCTCCCTCAGAGAAGCGCAG
sBot[28, 20]	ACCATCGCGCCTTTAATTGTATCGTTAGTAAA
sBot[28, 24]	CGCTGAGGTGAAAATCTCCAAAAATAAACAAC
sBot[30, 04]	AGACGATTCGTATAAACAGTTAATAAACATGA
sBot[30, 08]	AATCCTCATTAACGGGGTCAGTGCCAAGAGAA
sBot[30, 12]	TCTCTGAATTGATGATACAGGAGTTCAGTACC
sBot[30, 20]	TGAATTTTAAACTACAACGCCTGTCACCGTAC

sBot[30, 24]	TTTCAACATGTACCGTAACACTGATCAGAACC
seam[02, 16]	GATAGGGTTTATAAATCAAAAGAAGTAGCAAT
seam[03, 13]	ACTTCTTTACGCAAATTAACCGTTTAGCCCGA
seam[04, 16]	TAATGAATGGAAACCTGTCGTGCCAACAGAGA
seam[05, 13]	TAGAACCCAAGGGACATTCTGGCCAGCTGCAT
seam[06, 16]	GCAGGTCGGGCCAGTGCCAAGCTTAAGCATCA
seam[07, 13]	CCTTGCTGGCAAATGAAAAATCTAGCATGCCT
seam[08, 16]	TCCAGCCAGTATCGGCCTCAGGAATTAATTTT
seam[09, 13]	AAAAGTTTCTTTGCCCGAACGTTAGATCGCAC
seam[10, 16]	AACCAATATTTTGTTAAATCAGCTAACGTCAG
seam[11, 13]	ATGAATATGTAGATTTTCAGGTTTCATTTTTT
seam[12, 16]	TTGAGAGATAATGCCGGAGAGGGTTCAATATA
seam[13, 13]	TGTGAGTGGGAAACAGTACATAAAAGCTATTT
seam[14, 16]	TGTACCAACTCAGAGCATAAAGCTAAGAACGC
seam[15, 13]	GAGAAAACATCCAATCGCAAGACAAAATCGGT
seam[16, 16]	GTTGATTCCTGGAAGTTTCATTCCATTTAACA
seam[17, 13]	ACGCCAACAATTGAGAATCGCCATATATAACA
seam[18, 16]	CGAAAGACCATCAAAAAGATTAAGGGCTGTCT
seam[19, 13]	TTCCTTATAAACCAATCAATAATCAGGAAGCC
seam[20, 16]	AGAGGCTTACGACGATAAAAACCATTTGCACC
seam[21, 13]	CAGCTACACAAGATTAGTTGCTATAAATAGCG
seam[22, 16]	ACTGGCTCGAATTACCTTATGCGAACAAAGTC
seam[23, 13]	AGAGGGTATAACTGAACACCCTGATTTTAAGA
seam[24, 16]	GACAGATGCCGAACTGACCAACTTTTACGCAG
seam[25, 13]	TATGTTAGTGATTAAGACTCCTTATGAAAGAG
seam[26, 16]	CACTACGATTAAACGGGTAAAATATTGAGCCA
seam[27, 13]	TTTGGGAATATCACCGTCACCGACCGTAATGC
seam[28, 16]	TGATACCGTCGAGGTGAATTTCTTCAGAGCCA
seam[29, 13]	CCACCGGATCAAAATCACCGGAACAAACAGCT
seam[30, 16]	AAAGTTTTCCCTCATAGTTAGCGTGCGTCATA
seam[31, 13]	CATGGCTTTTTACCGTTCCAGTAAAACGATCT

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