Electronic Supplementary Material

Photo-controlled release of paclitaxel and model drugs from RNA pyramids

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MATERIALS AND METHODS

Characterization of the self-assembled RNA pyramid nanoparticle

All constructs were assembled as described above, and 6 × loading dye (40% sucrose, 0.01% bromophenol blue, 0.01% xylene cyanol) was mixed with each RNA sample before loading on the non-denaturing gels. All native gels were run in 1 × TBM (89 mM Tris, 200 mM boric acid, and 2.5 mM $MgCl_2$) buffer at 4 °C, 90 V. Ethidium bromide (EB) solution was used for gel stain. Typhoon FLA 7000 (GE Healthcare) was used to image the stained gels.

Dynamic light scattering (DLS) was utilized to measure the apparent hydrodynamic size and zeta potential of RNA pyramid (100–200 nM in 1 × Tris buffer at 25°C) using Zetasizer nano-ZS (Malvern Instrument, LTD) as described previously.[1] The laser wavelength used in this measurement is 633 nm. Three independent measurements were carried out to obtain the data.

Temperature gradient gel electrophoresis (TGGE) analysis was performed on 4% native PAGE ina buffer containing 50 mM TRIS pH = 8.0, 100 mM NaCl, and 0.2 mM $MgCl_2$ as previously reported.[2] A gradient temperature (30-76°C) was applied perpendicular to the electrical current, and the experiment was run for 1 h at 20 W. S3 was fluorescently labeled and a total RNA concentration of 1.5 μM was used in the TGGE analysis. Quantification of the intact nanoparticles was performed using Image J software. Boxes with equal size were drawn around the lanes corresponding to the pyramid complexes, and each quantified value for intact RNA pyramid nanostructures was divided by the sum of the values calculated in the corresponding lane. OriginPro 8 software was then used to plot the melting temperature curve.

Single particle image processing and 3D reconstruction

The image processing software package EMAN2.1, was used for the micrograph evaluation, particle picking, contrast transfer function correction, 2-D reference-free class averaging, initial model building and 3-D refinement of the cryo-EM data. We boxed a total of 1522 particles to generate the 2D class averages for building the initial models. At last, 1103 particles were used for final refinement, applying the c4 symmetry. The resolution for the final map was estimated by the 0.143 criterion of FSC curve without any mask. A 25 Å Gauss low-pass filter was applied to the final 3D maps displayed in the Chimera software package.

Spinach and malachite green aptamer fluorescence measurements

Native 6% PAGE was used to detect the fluorescence emission of fluorogenic pyramid nanoparticles. The gel was stained simultaneously in a mixture of 5 μM MG and 5 μM DFHBI and scanned for the MG-apt fluorescence λ_{exc} centered at 635 nm and for spinach-APT fluorescence λ_{exc} centered at 473 nm. Fluorescence emission in solution was measured, as previously reported.[1,3] Briefly, assembled square nanoparticles (0.1 μM) harboring MG and spinach aptamers in TMS buffer were mixed with MG (2 μM) and DFHBI (2 μM) (Lucerna, Inc) and incubated at room temperature for 30 min. 3WJ nanoparticles harboring MG and SPIN were used as a positive control. [4]Fluorescence was measured using a fluorospectrometer (Horiba Jobin Yvon), excited at 450 nm (565 - 750 nm scanning for emission) for spinach and 615 nm (625 - 750 nm scanning for emission) for MG dyes.

HBV ribozyme activity assay

The HBV ribozyme activity assay was carried out as previously detailed. [5] Briefly, the HBV RNA substrate was 5'-Cy5 labeled using the Mirus Cy5 labeling kit. The labeled substrate was incubated with RNA pyramid nanoparticle harboring HBV ribozyme for 60 min at 37 °C in buffer containing 20 mM $MgCl_2$, 20 mM NaCl, and 50 mM Tris-HCl (pH 7.5). 3WJ nanoparticle harboring HBV ribozyme served as a positive control, and Multi-Pyramid without a HBV ribozyme was used as a negative control. The cleaved fragments were analyzed on the Typhoon fluorescent imaging system under the Cy5 channel.

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Streptavidin (STV) aptamer binding assay

50μL 400nM multifunctional RNA pyramids harboring STV aptamer were incubated with STV agarose resin (Thermo Scientific) in binding buffer (PBS with 10 mM Mg^{2+}) at room temperature for 1 hr. Then the resin was spun at 500×g for 1 min and the supernatant was collected. 50 μL of binding buffer was added to the resin and the resin washed for three times. The wash fractions were also collected. After washing, 5 mM biotin was added to the resin three times to elute the RNA. All the collected supernatant, washing, and elution fractions were measured by Nanodrop for absorbance at 260 nm. RNA pyramids without STV aptamer were used as negative control.

ATP aptamer binding assay

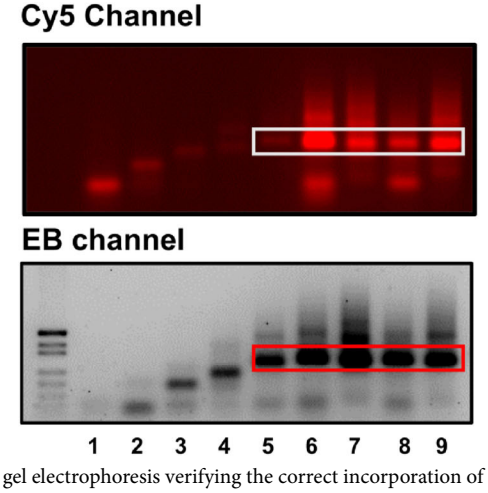
S5-ATP and S1-MG RNA was 5'-end labeled with ^{32}P using γ-^{32}P-ATP (Perkin Elmer, NEG002A250UC) using T4 PNK method. Radiolabeled S1-MG was used for assembly of radioactive multifunctional pyramid (Multi-Pyr*) with or without ATP aptamer. S5-ATP with 5-end labeling of ^{32}P was used as a positive control. After washing with 100 μl of distilled water and then with 100 μl of binding buffer (300 mM NaCl, 20 mM Tris, pH 7.6, 5 mM $MgCl_2$) on adenosine 5'-triphosphate–agarose resin (Sigma-Aldrich, Cat. No. A2767), 1 μg (2.5×10^{-5} μmol) of ^{32}P-labeled RNA in 100 μl binding buffer was applied to the resin. The resin was then washed with 100 μl of binding buffer for four times. Then the ATP-agarose resin was subjected to scintillation counting on 1900 TR Liquid Scintillation Counter (Packard).

Conjugation of fluorophore and paclitaxel on short RNA oligomers

Fluorophore conjugation was completed through NHS ester reaction with primary amine on RNA strands.[6] Cyanine5 NHS ester were purchases from Lumiprobe. Conjugation reactions were carried out by mixing a 1:10 molar ratio of primary amine labeled short RNA oligomers: NHS Ester - Fluorophore in 0.1 M sodium bicarbonate buffer, pH = 8.5. The conjugation reactions were incubated at room temperature for 4 hours while protected from light. Following incubation, the reactions were ethanol precipitated and washed twice with cold 75% ethanol to remove the majority of unreacted fluorophore, facilitating HPLC purification. The paclitaxel conjugation was conducted via CuAAC click reaction. Alkyne-labeled RNA oligomers were mixed with homemade azido-modified paclitaxel (RNA: paclitaxel = 1: 10) with the aid of CuBr stabilized by TBTA. This reaction takes 1 hour at 37 °C in dark. The RNA-paclitaxel conjugates were ethanol precipitated and washed twice before HPLC purification. 16% urea PAGE was used to characterize the conjugation of Cy5 and paclitaxel on short RNA strands.

Serum stability of RNA pyramids harboring PC spacer

Serum stability assay of RNA pyramid was carried out as previously described.[1] RNA nanocages (1 μM) with and without PC spacers were incubated in 10% FBS solution 37 °C for 0-24 hour. After removal from 37 °C incubation after their respective time points, the samples were frozen on dry ice to prevent any further degradation. The samples were then analyzed on 6% native PAGE followed by fluorescent imaging by Typhoon imaging system under ethidium bromide and Cy5 channel. Quantification analysis was performed using ImageJ software and the plot was generated by Numbers software.



	1	2	3	4	5	6	7	8	9
S1						+	+	+	+
S2		+	+	+	+		+	+	+
S3			+	+	+	+		+	+
S4				+	+	+	+		+
S5					+	+	+	+	
S1-Cy5	+	+	+	+	+				
S2-Cy5						+			
S3-Cy5							+		
S4-Cy5								+	
S5-Cy5									+

Figure S1 3% agarose gel electrophoresis verifying the correct incorporation of five ssRNA into pyramid structure using Cy5-labeled strands.

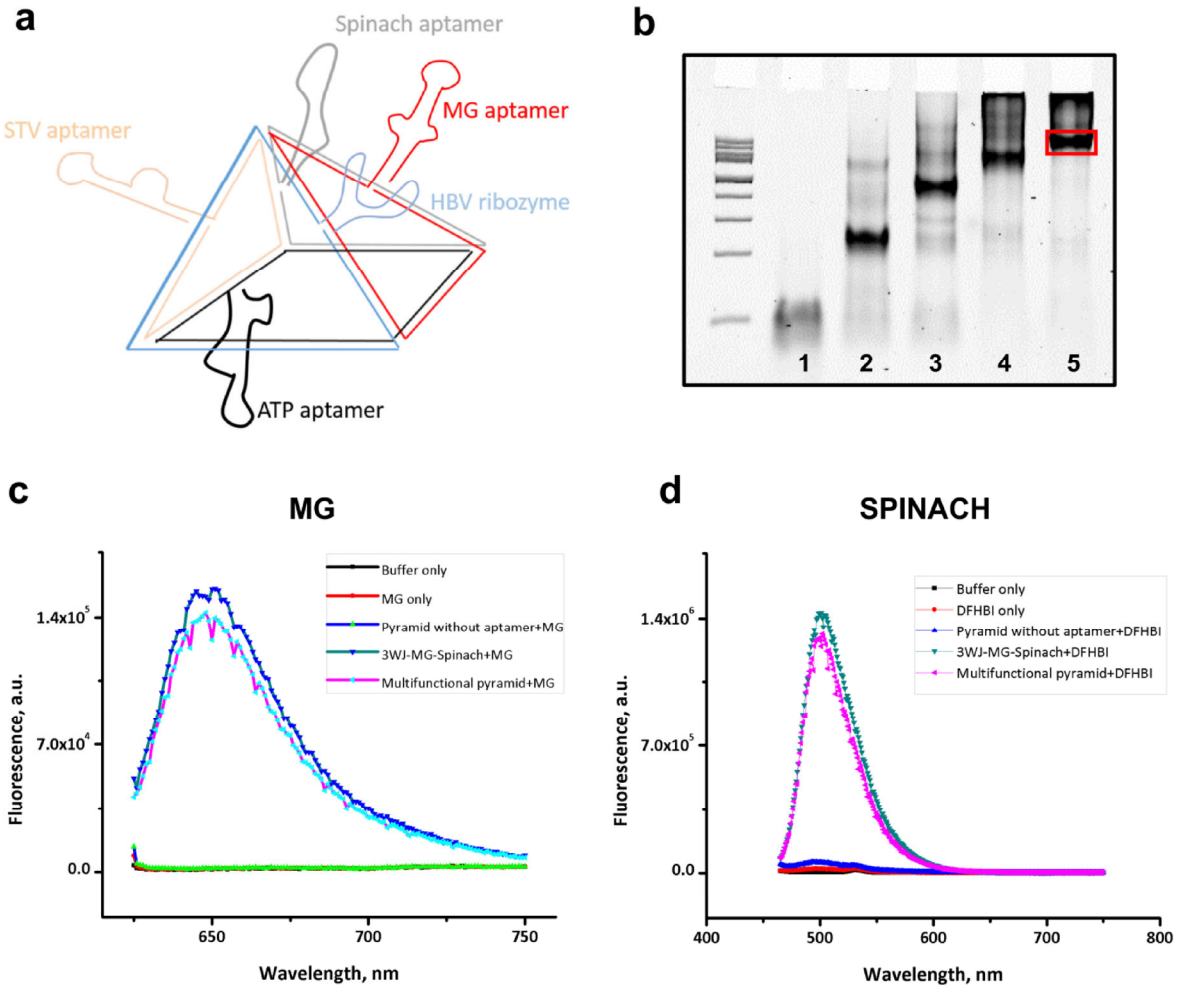


Figure S2 Design and assembly of multifunctional RNA pyramid nanoparticle. (a) Illustration of multifunctional RNA pyramid (Multi-Pyr) with Malachite green (MG) aptamer, spinach (SPIN) aptamer, streptavidin (STV) aptamer, hepatitis B virus (HBV) ribozyme, and ATP aptamer. **(b)** Native PAGE assembly gel showing the step-wise assembly of multifunctional RNA pyramid nanoparticle, lane 5 is assembled Multi-Pyr. **(c)** Malachite green aptamer assay. Only multifunctional RNA pyramid and 3WJ-MG-SPIN showed increased fluorescence upon incubation with malachite green dye. **(d)** Spinach aptamer assay. Only multifunctional RNA pyramid and 3WJ-MG-SPIN showed increased fluorescence upon incubation with DFHBI dye.

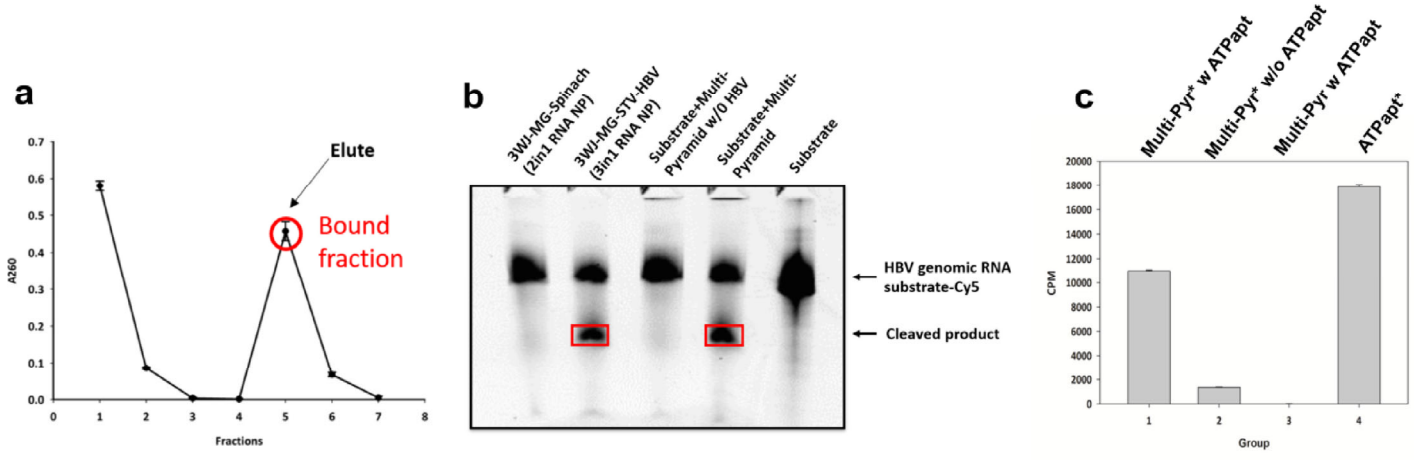


Figure S3 Functional assays of multifunctional RNA pyramid. (a) STV binding assay using STV affinity column showing the function of STV aptamer. **(b)** HBV ribozyme function assay by cleaving Cy5-labeled HBV genomic RNA substrate. Cleaved products are boxed in red. **(c)** ATP-binding assay of multifunctional pyramid using radiolabeled RNA strands. Multi-Pyr* w/o ATPapt and Multi-Pyr w ATPapt were used as negative controls. S5-ATP was used as a positive control. Asterisk denotes the ^{32}P labeling.

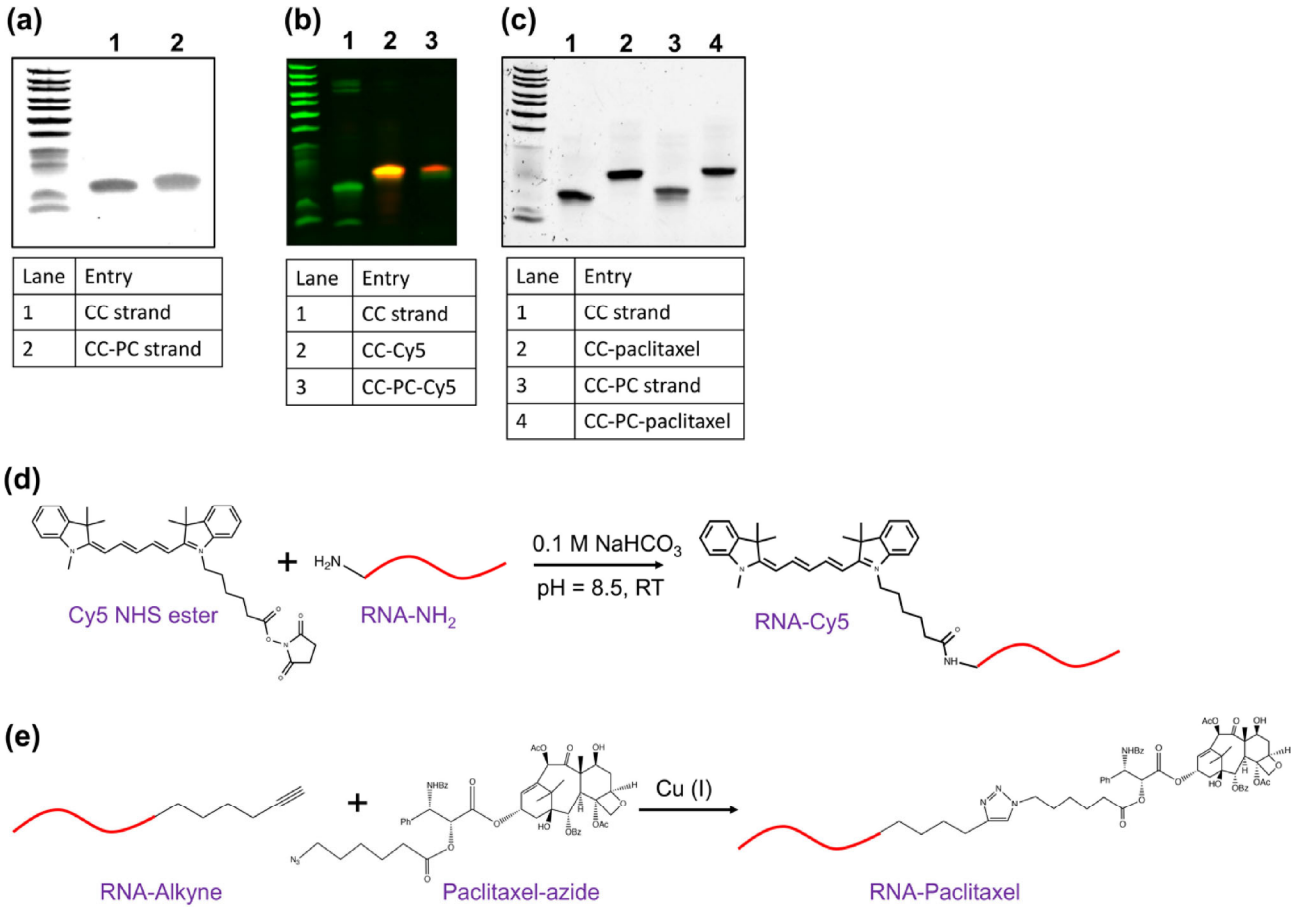


Figure S4 RNA synthesis and conjugation. **(a)** Denaturing urea PAGE showing the synthesis of CC and CC-PC RNA strand. **(b)** Cy5 conjugation on short RNA strands with or without PC spacer. **(c)** Denaturing urea PAGE characterization of paclitaxel conjugation on short RNA strand with or without PC spacer. Ultra Low Ranger ladder (Thermo Fisher) was used in these gels. Schematic of conjugation of **(d)** Cy5 and **(e)** Paclitaxel on RNA strand.

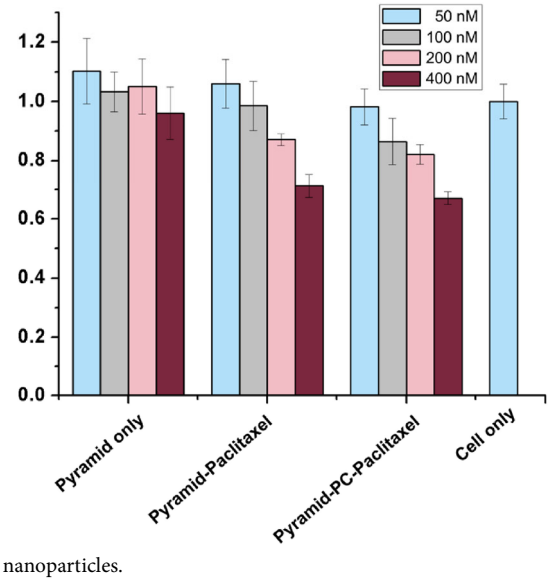


Figure S5 Cell viability assay of various pyramid nanoparticles.

Table S1 RNA sequences of pyramid strand 1-5

Strand	Sequence
1	5'- GGGAGCAGAUC CGU GGUCUCUCAC CUCCCGUACA CAUAUUAC UUU GUU CACGCAACUGCUCCAUCUGUCA AUG U GUA CACUCAUACAC -3'
2	5'- GGGACAGAUA CGGU ACG GAUCUGCUCC CGUGUAUGAG UG UAC UUU GUU UCGCUCAUGUCUCCAGUGCCGU AUG U GUACGCAGAA CACC -3'
3	5'- GGCUGGUUGUC UCA ACC GUAUCUGUCCC GGUGUUCUGCG UAC UUU GUUACCGAGC GUCCAUAGAA CUCUAAUG U GUA UUCUCUCGCAU -3'
4	5'- GGAGGUGAGAG ACC UGAGACAACC AGCCAUGCGA GAGAAUAC UUU GUU CUGGUACAGCUAGCAGAUGCAC AUG U GUA AUAUGUGUACG -3'
5	5'- GGACGCUCGGU AAU CAUACGGCAC UGGAGACAUG AGCGAAAU CAUUGACAGA UGGAGCAGUU GCGUGAAU CAUGUGCAUC UGCUAGCUGU ACCAGAAU CAU UAGAGUUCUAU -3'

Table S2 RNA sequences of multifunctional pyramid

Strand	Sequence
1-MG aptamer	5'- GGAUCCCGACUGGCGAGAGCCAGGUAACGAAUGGAUCC GGGAGCAGAUC CGU GGUCUCUCAC CUCCCGUACA CAUAUUAC UUU GUU CACGCAACUGCUCCAUCUGUCA AUG U GUA CACUCAUACAC -3'
2-HBV ribozyme	5'- GGGACGAAAAAAAACAAAUUCUUUACUGAUGAGUCCGUGAGGAC GAAACGGGUCAAAAAAAACGUCCC GGGACAGAUA CGGU ACG GAUCUGCUCC CGUGUAUGAG UG UAC UUU GUU UCGCUCAUGUCUCCAGUGCCGU AUG U GUACGCAGAA CACC -3'
3-STV aptamer	5'- GGAUGCGGCCGCCGACCAGAAUCAUGCAAGUGCGUAAGAUAGU CGCGGGUCGGCGGCCGCAUCC GGCUGGUUGUC UCA ACC GUAUCUGUCCC GGUGUUCUGCG UAC UUU GUUACCGAGC GUCCAUAGAA CUCUAAUG U GUA UUCUCUCGCAU -3'
4-Spinach aptamer	5'- GGACGCAACUGAAUGAAAUGGUGAAGGACGGGUCCAGGUGUGG CUGCUUCGGCAGUGCAGCUUGUUGAGUAGAGUGUGAGCUCCGU AACUAGUCGCGUCC GGAGGUGAGAG ACC UGAGACAACC AGCCAUGCGA GAGAAUAC UUU GUU CUGGUACAGCUAGCAGAUGCAC AUG U GUA AUAUGUGUACG -3'
5-ATP aptamer	5'- GGGUUGGGAAGAAACUGUGGCACUUCGGUGCCAGCAACCC GGACGCUCGGU AAU CAUACGGCAC UGGAGACAUG AGCGAAAU CAUUGACAGA UGGAGCAGUU GCGUGAAU CAUGUGCAUC UGCUAGCUGU ACCAGAAU CAU UAGAGUUCUAU -3'

Table S3 RNA sequences of extended pyramid and CC strands

Strand	Sequence
S1-Ext	5'- GGGAGCAGAUC CGU GGUCUCUCAC CUCCCGUACA CAUAUUAC UUU GUU CACGCAACUGCUCCAUCUGUCA AUG U GUA CACUCAUACAC UUUCGAAGUCAGCAUACGAA-3'
S3-Ext	5'- GGCUGGUUGUC UCA ACC GUAUCUGUCCC GGUGUUCUGCG UAC UUU GUUACCGAGC GUCCAUAGAA CUCUAAUG U GUA UUCUCUCGCAU UUUCGAAGUCAGCAUACGAA-3'
CC RNA	5'-fUfUfCrGfUrAfUrGfCfUrGrAfCfUfUfCrG-3'
CC-PC	5'-PC-fUfUfCrGfUrAfUrGfCfUrGrAfCfUfUfCrG-3'

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