Development of a ribonuclease containing a G4-specific binding motif for programmable RNA cleavage

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We developed a ribonuclease for site-specific targeting and cleavage of single-stranded RNA. The engineered RNase protein was constructed by incorporating two independent functional domains, an RNase HI domain that could cleave the RNA strand in a DNA-RNA hybrid, and a domain of the RHAU protein that could selectively recognize a parallel DNA G-quadruplex (G4). The newly designed RNase first recruits a DNA guide oligonucleotide containing both a parallel G4 motif and a template sequence complementary to the target RNA. This RNase:DNA complex targets and efficiently cleaves the single-stranded RNA in a site-specific manner. A major cleavage site occurs at the RNA region that is complementary to the DNA template sequence. The newly designed RNase can serve as a simple tool for RNA manipulation and probing RNA structure.

RNA plays a central role in various biological processes including gene expression, gene regulation and catalysis1–4. Many RNA transcripts are of several thousand nucleotides long, and understanding the structure and function of full-length RNA is not always possible. Thus, fragmentation of the RNA molecules is an attractive approach for RNA research5. RNA-based enzymes (ribozymes), which can catalyse RNA cleavage in a sequence-specific manner, include artificially selected ribozymes and natural ribozymes, such as those involved in RNA splicing and RNA processing6,7. In addition, development of DNAzymes for RNA cleavage has also been reported8. However, the use of protein enzymes (ribonucleases or RNases) for fragmentation of the RNA molecules is a widely used method5. Unlike Type II restriction enzymes that cleave DNA in a site-specific fashion9, most of RNases exhibit non-specific cleavage activity5,10.

Development of RNase systems for site-specific RNA cleavage would greatly help the studies of RNA structure and function. An artificial site-specific RNA endonuclease was generated by incorporating an RNA cleavage domain with a series of Pumilio/fem-3-binding factor domains that could specifically recognize an 8-nt RNA sequence and cleave near the binding site11. RNase H enzymes, consisting of a RNA-DNA hybrid binding domain and a catalytic domain, can cleave the RNA strand in a RNA-DNA hybrid12. Fusing the catalytic domain RNase HI with a sequence-specific zinc-finger protein resulted in an enzyme that cleaved the RNA strand in a RNA-DNA hybrid at 5 nt from the recognition sequence5. However, this technique will require the specific nucleotide sequence to be present at the desired location of the RNA molecule. DNA oligonucleotide-targeted RNA cleavage provides an alternative approach for fragmentation of RNA molecules13–19. Hybridization of a short DNA oligonucleotide was early proposed to direct the RNase H-mediated cleavage at a specific site of a RNA molecule13. Later, RNase H was used for site-specific RNA cleavage using the complementary chimeric 2′-O-methyl15,20 DNA or 2′-O-methyl RNA-flanked DNA21. To enhance the RNA site-specific cleavage efficiency, a short antisense DNA oligonucleotide was covalently conjugated to RNase H to generate a hybrid ribonuclease8,19. Especially, the RNase H-antisense oligonucleotide conjugate was shown to efficiently cleave target RNA transcripts and inhibit gene expression in vitro and in living cells18,19. In this approach, the RNase H-antisense oligonucleotide chemical conjugation is the limiting step for varying target sites1.

It has been established that RNA can act as a template for a ribonucleoprotein to selectively recognize and knock out complementary messenger RNA (mRNA) including the miRNA and siRNA machineries22,23. Recently, powerful CRISPR-Cas systems have been applied for RNA recognition and cleavage. The crisprRNA-Cas protein

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complex can directly recognize and cleave target RNAs. The type VI effector Cas13 acts as a crRNA-guided ribonuclease function which can degrade RNAs. The type III-associated RNase (Csm3 and Csm6) targets and cleaves the viral DNA and its transcriptional RNAs. Programmable RNA recognition and site-specific cleavage by Cas9 has been reported. By using specifically designed DNA PAMmers, the Cas9 system can selectively bind and cleave RNA targets in a site-specific manner in vitro. Without addition of DNA PAMers, recognition of a natural PAMer of RNA target by NmeCas9 also allows site-specific cleavage of the RNA target. Despite these advances, it is not simple to manipulate these CRISPR-Cas systems for programmable RNA recognition and cleavage due to the protein complexity and a need for guide RNA, additive DNA-PAMer or rPAM presence. This study aimed to establish a simple ribonuclease approach for programmable site-specific RNA cleavage, based on a DNA guide and protein-DNA structure-specific recognition.

G-quadruplexes (G4) are four-stranded structures formed by stacking of multiple G-tetrads. G4 structures are highly polymorphic: four strands of the G-tetrad core can be parallel (oriented in the same direction) or non-parallel. In cell, the formation of G4 structures has been implicated in many biological processes such as telomere maintenance, replication, transcription and translation. G4 structure provides a potential target site for recognition by small molecules and proteins. The specific interaction between the N-terminal domain of the RH1 protein and a G4 has been characterized. Incorporating fluorescent protein to this RH1 peptide motif provided a useful probe for discrimination between different G4 topologies.

In this study we generate a novel RNase, when formed a complex with a guide DNA strand containing both a parallel G4 motif and a template sequence, is capable of site-specific cleavage of a single-stranded RNA at a predetermined position, providing a powerful tool for RNA manipulation.

Results

It was shown that while a short 16-aa RH1 recognition motif (aa 53–68) is sufficient for specific recognition of a parallel G4, longer RH1 peptides exhibit higher binding affinity with RH140 (aa 53–192) displaying a dissociation constant (Kd) in the nanomolar range. Possibly due to the packing of several helices formed at the N-terminal of RH140. We designed a ribonuclease RH140-RNase HI by incorporating a catalytic domain of RNase HI from B. halodurans to the RH140 peptide. The newly designed RNase is expected to be capable of recruiting a DNA strand containing both a parallel G4 motif and an antisense DNA template sequence. The RNase-DNA complex can selectively target and cleave a single RNA strand at the predetermined position that is complimentary to the template DNA (Fig. 1).

Two RNases proteins, the hybrid RH140-RNase HI and the control wild-type RNase HI catalytic domain (Supplementary Table S1), were cloned, expressed and purified. RNases with good purity was obtained as monitored by SDS-PAGE (Supplementary Fig. S2).

To determine whether the fusion protein RH140-RNase HI and the DNA strand bearing a parallel G4 motif formed a complex, a binding assay was performed by gel electrophoresis mobility shift assay (Supplementary Fig. S3). RH140-RNase HI was observed to bind the DNA strand B1 containing a parallel G4 motif (Table 1) causing a change in the B1 mobility in the gel. In a negative control, upon addition of the same amount of RH140-RNase HI to B0 (a DNA strand without a G4 motif) (Table 1), no change in the mobility of B0 was observed. The wild-type RNase HI did not show any binding to B1 and B0 (Supplementary Fig. S3). Note that the RNase HI construct used contains only the catalytic domain and lacks the nucleic acid binding N-terminal domain. The selective recognition of a parallel G4 by RH140 allowed RH140-RNase HI to form a complex with DNA bearing a parallel G4 motif, bringing the RNase HI domain close to the RNA substrate for enhancement of catalytic activity.

The RNA cleavage activity of RNase-DNA complexes was tested. The RNA substrate R1 was labelled with Fluorescein (FAM) at the 5' end (Table 1). The complexes of the RNase and DNA oligonucleotides were well mixed at the 1:1 ratio before adding to the reaction. Incubation of the RH140-RNase HI:B1 complex with R1 showed that the cleavage product of R1 was increased in a time-dependent manner (Supplementary Fig. S4). It took around 45 minutes for RH140-RNase HI:B1 (0.3 μM:0.3 μM) to fully cleave the substrate R1 (10 μM) (Fig. 2a). A specific cleavage site occurred inside of the complementary region between RNA and DNA (see below). Control experiments using the same amount of mixture of RH140-RNase HI:B0 (Fig. 2b) or RNase
HI:B1 (Fig. 2c) showed only minor cleavage (at the same cleavage site) after the same incubation time with R1. These results revealed that RHAU140-RNase HI:B1 can specifically target and efficiently cleave R1. The complex of RHAU140-RNase HI:DNA consisting of a G4 motif would provide a powerful tool for direct cleavage of RNA at a predetermined position.

Table 1. Oligonucleotide sequences used in this study. R1 is RNA while other sequences are DNA. The G4-forming motif is underlined. T₈ and T₄ linkers between the G4 motif and the template sequence are shown in boldface.

| Name | Sequence |
|------|----------|
| R1   | 5′-FAM-CCAUGAUAUGCACGUGUUGAAGUCUU-3′ |
| B1   | 5′-TTGGGTGGGTGGGTGGGTTCGACGACGGCATCATC-3′ |
| B0   | 5′-TTGACTAAGTGCCTTAGTTCAACGACGACGGCATCATC-3′ |
| C1   | 5′-TTGGGTGGGTGGGTGGGTCTTTCGGGTGGGTGGTTCGACGACGACGGCATCATC-3′ |
| D1   | 5′-TTGGGTGGGTGGGTGGGTCTTTCGGGTGGGTGGTTCGACGACGACGGCATCATC-3′ |
| E1   | 5′-TTGGGTGGGTGGGTGGGTCTTTCGGGTGGGTGGTTCGACGACGACGGCATCATC-3′ |
| F1   | 5′-TTGGGTGGGTGGGTGGGTCTTTCGGGTGGGTGGTTCGACGACGACGGCATCATC-3′ |

Figure 2. Cleavage of the RNA strand R1 by using the complexes of (a) RHAU140-RNase HI:B1, (b) RHAU140-RNase HI:B0 and (c) RNase HI:B1. The RNA strand R1 was labelled with FAM at the 5′-end. Reactions were carried out by incubation of RHAU140-RNase HI:B1 (0.3 µM:0.3 µM), RHAU140-RNase HI:B0 (0.3 µM:0.3 µM) and RNase HI:B1 (0.3 µM:0.3 µM) with R1 (10 µM) in reaction buffer at 37 °C. The samples which were collected after 5, 15, 30 and 45 minutes were analysed by using a denaturing gel with 7 M urea.

Discussion

Development of a novel ribonuclease by fusing separate functional protein domains is a useful approach for programmable site-specific RNA recognition and cleavage. We generated a recombinant ribonuclease, RHAU140-RNase HI by using the catalytic domain of RNase HI and the G4-recognition domain RHAU140. The engineered RHAU140-RNase HI protein selectively binds to a short antisense guide DNA bearing a parallel G4 motif (G4-DNA), leading to the formation of a complex of RHAU140-RNase HI:G4-DNA. This complex can directly bind and cleave an RNA target at a predetermined position.

In fact, RNase H family itself is able to target and cleave the RNA strand of a DNA-RNA hybrid12. However, the antisense DNA oligonucleotide and RNase components need to co-localize at the desired cleavage site of the RNA molecule for the reaction to happen, hence, limiting the efficiency of site-specific RNA cleavage. Pre-loading of the DNA template on the enzyme, such as in a DNA oligonucleotide-conjugated RNase H, was shown to
enhance the direct binding and site-specific cleavage of the RNA target. However, this approach requires chemical conjugation between the DNA template and the RNase H enzyme. On the other hand, the systems of a zinc-finger protein conjugated RNase and an artificial RNA endonuclease, while having the advantage of being protein-only sequence-specific RNases, would require particular RNA sequences at the desired locations of the RNA substrate. Sequence-specific ribonuclease systems using non-covalently bound RNA templates including miRNA, siRNA and crRNA are powerful and have been exploited both by the nature and in biotechnology.

Our approach is similar regarding the use of a nucleic acid template. However, while the specificity and efficiency might not meet that of the natural systems, its simplicity and the use of a more robust and inexpensive DNA template could make our approach attractive.

The complex of RHAU140-RNase H:G4-DNA acts as a nucleoprotein enzyme to catalyze the site-specific RNA cleavage. Major cleavage sites occur inside the RNA region that is complementary to the DNA template and depend on nucleotide sequence of the DNA-RNA hybrid. All of major cleavage sites in our assays were at a 5′-guanine, consistent with a previous report on multiple nucleotide preferences of the cleavage sites of RNase H. The linker between RHAU140 and the RNase H domain may also affect the position of the cleavage site(s). However, the effect of the linker between the G4 motif and the template region was not observed. The site-specific cleavage position can be further restrained and optimized by using a DNA guide strand containing modified nucleotides.

Generally, RNase H can cleave non-specifically any RNA sequence in a DNA-RNA hybrid. Thus, in principle any RNA segment that is unique in the RNA molecule can be chosen as the cleavage target in our approach. On the other hand, the cleavage efficiency and specific cleavage site(s) depend on the sequence of the chosen target segment. Based on previous studies, a RNA target sequence with highly-efficient cleavage site(s) can be chosen for programmable RNA cleavage. The cleavage site can be further restricted with a chimeric design of the DNA template sequence. In our approach, the DNA guide can be easily constructed by adding a DNA template sequence, complementary to the RNA target segment, to a stable parallel G4 motif, e.g. TT(G3T)4, not complementary to any part of the RNA molecule. This G4-DNA guide, forming a complex with RHAU140-RNase H, can direct an efficient cleavage of the single-stranded RNA target in a site-specific manner. The cleavage of the RNA strand can be readily monitored and demonstrated by gel electrophoresis.

This study takes advantage of a G4-recognition motif of RHAU to construct an RNase that can recognize a G4-containing DNA template for programmable site-specific RNA cleavage. We have demonstrated the concept using a G4-binding motif of RHAU with a 140-aa length and a nanomolar-range affinity (10 nM < Kd < 100 nM) to parallel G4s. In principle, a full-length catalytic dead mutant of RHAU with much higher affinity to G4s can also be used. However, the large size of the full-length RHAU (> 1000 aa) might limit its applications. On the other hand, a short G4-recognition motif of RHAU can be further engineered to have a higher affinity to G4s. The newly developed RNase can also be used to probe the formation of G4 structures in RNA, when using DNA templates not containing a G4.
This approach can be extended to other G4-binding proteins\cite{Saida2005} or proteins recognizing other structural motifs such as Z-DNA\cite{Choudhury2019} and hairpin loop\cite{Saida2005}, as well as small chemical motifs such as biotin\cite{Morris2019} and digoxigenin\cite{Saida2005}.

**Conclusion**

A novel RNase was developed to site-specifically target and cleave single-strand RNA, providing a powerful tool for RNA structure and function studies. The RNase was generated by incorporating the catalytic domain of RNase H1 to the N-terminal domain of RHAU which can form a complex with a DNA guide strand bearing a parallel G4 motif and a template segment. The RNase:DNA complex can selectively target and efficiently cleave single-stranded RNA at the DNA-RNA hybrid position.

**Methods**

**Construction, expression and purification of RHAU140-RNase HI and RNase HI.** The Ribonuclease protein RHAU140-RNase HI was generated by incorporating the RHAU140 peptide to the RNase HI catalytic domain from *B. halodurans*. DNA encoding for RHAU140-RNase HI was synthesized by IDT Singapore Pte Ltd. It was amplified by PCR using a pair of primer ON1 (5′-gcgggtcagccgctcgctcggcagtcaaggcag-3′) and ON2 (5′-accactcagttcttgctcggctcttcgcaatc-3′). The PCR product was then cloned into treated pET-Duet1 (Merck) at BamHI and XhoI, resulting in plasmid RHAU140-RNase HI. The wild-type RNase HI was generated by PCR using a pair of primer ON3 (5′-ccggtcagccgctcgctcggcagtcaaggcag-3′) and ON2 (5′-accaactcagttcttgctcggctcttcgcaatc-3′). This PCR product was then cloned into treated vector pET-Duet1 (Merck) at BamHI and XhoI, resulting in plasmid RNase HI (Supplementary Fig. S1).

The plasmids of RHAU140-RNase HI and RNase HI were transformed into *E. coli* strain BL21 (DE3) for protein expression. The bacteria were cultured in LB medium containing 100 µg/ml of ampicillin and the cells were grown at 37 °C, shaking at 220 rpm to an OD$_{600}$ of 0.6–0.8. Then, IPTG was added to a final concentration of 0.5 mM. The cells were incubated for 10 hours at 16 °C, shaking at 180 rpm before being harvested. The pellet was re-suspended into the BugBuster protein extraction reagent containing 500 mM imidazole. The insoluble material was removed by centrifugation at 20,000 rpm, for 1 hour at 4 °C. The soluble fraction was applied to a column filled with Ni-agarose beads. The column was washed with 30 volumes of buffer (10 mM potassium phosphate, 100 mM potassium chloride, 10 mM imidazole, pH 7). The proteins were then eluted by buffer (10 mM potassium phosphate, 100 mM potassium chloride, 200 mM imidazole, pH 7) and the high concentration of imidazole was removed by dialysis buffer (10 mM potassium phosphate, 100 mM potassium chloride, pH 7). The purity of desired proteins was analysed by SDS-PAGE (Supplementary Fig. S2).

**Gel mobility shift assay.** The gel mobility shift assay was performed using native PAGE of 10% polyacrylamide in 1X TBE (Tris-borate-EDTA), 20 mM potassium phosphate, 100 mM potassium chloride, pH 7.0. The DNA oligonucleotides B1 containing a G4 motif (10 µM) and B0 without a G4 motif (10 µM) were incubated with 50 µM RHAU140-RNase HI or 50 µM RNase HI. The mixtures were subjected to the native PAGE and all the images were taken by using the Gel Doc UV shadowing technique (Alpha Innotech).

**RNA cleavage activity assay.** The RNA target (R1), derived from the EGFP gene and consisting of 30 nt, was labelled with FAM at the 5′ end. DNA oligonucleotides B0, B1, C1, D1, E1 and F1 having 20 nt that are complementary to different parts of the RNA sequence. A G4-forming motif TTTG(T)$_n$, which is not complementary to the RNA target, was introduced to B1, C1, D1, E1 and F1 (Table 1). All RNA and DNA oligonucleotides were purchased from IDT Singapore Pte Ltd. The complex of proteins and DNA oligonucleotides were well mixed at the 1:1 ratio before adding to the reaction. RNA cleavage activity assays were performed with 10 µM R1 in the 1X reaction buffer (50 mM Tris–HCl, 100 mM KCl, 3 mM MgCl$_2$, 10 mM DTT, pH 8.3). A specific cleavage site occurred inside of the complementary region between RNA and DNA. Reaction was stopped by adding equal volume of formamide. For cleavage site mapping, T1 RNase and alkaline hydrolysis (Fermentas) cleavage was performed as RNA ladders. The samples were resolved on a 15% polyacrylamide–7 M urea gel and imaged by the Gel Doc (Alpha Innotech).

**References**

1. Doudna, J. A. & Cech, T. R. The chemical repertoire of natural ribozymes. *Nature* **418**, 222–228 (2002).
2. Cech, T. R. & Steitz, J. A. The noncoding RNA revolution-trashing old rules to forge new ones. *Cell* **157**, 77–94 (2014).
3. Morris, K. V. & Mattick, J. S. The rise of regulatory RNA. *Nat Rev Genet* **15**, 423–437 (2014).
4. Mortimer, S. A., Kidwell, M. A. & Doudna, J. A. Insights into RNA structure and function from genome-wide studies. *Nat Rev Genet* **15**, 469–479 (2014).
5. Sulej, A. A., Tuszyńska, L., Skowronek, K. J., Nowotny, M. & Bujnicki, J. M. Sequence-specific cleavage of the RNA strand in DNA-RNA hybrids by the fusion of ribonuclease H with a zinc finger. *Nucleic Acids Res* **40**, 11563–11570 (2012).
6. Puerta-Fernandez, E., Romero-Lopez, C., Barroso-delJesus, A. & Berzal-Herranz, A. Ribozymes: recent advances in the development of RNA tools. *FEBS Microbiol Lett* **27**, 75–97 (2003).
7. Doudna, J. A. & Lorsch, J. R. Ribozyme catalysis: not different, just worse. *Nat Struct Mol Biol* **12**, 395–402 (2005).
8. Santoro, S. W. & Joyce, G. F. A general purpose RNA-cleaving DNA enzyme. *Proc Natl Acad Sci USA* **94**, 4262–4266 (1997).
9. Pingoud, A., Fuxreiter, M., Pingoud, V. & Wende, W. Type II restriction endonucleases: structure and mechanism. *Cell Mol Life Sci* **62**, 685–707 (2005).
10. Saida, F. & Odaert, B. RNA recognition and cleavage by sequence-specific endoribonucleases. *Protein Peptide Lett* **14**, 103–111 (2007).
11. Choudhury, R., Tsai, Y. S., Dominguez, D., Wang, Y. & Wang, Z. Engineering RNA endonucleases with customized sequence specificities. *Nat Commun* **3**, 1147 (2012).
12. Nowotny, M., Gadamakov, S. A., Crouch, R. J. & Yang, W. Crystal structures of RNase H bound to an RNA/DNA hybrid: substrate specificity and metal-dependent catalysis. *Cell* **121**, 1055–1066 (2005).
13. Donis-Keller, H. Site specific enzymatic cleavage of RNA. *Nucleic Acids Res* **7**, 179–192 (1979).
14. Morgan, R. D., Dalton, M. & Stote, R. A Unique Type-ii Restriction Endonuclease from Acinetobacter-Lwoffi N. *Nucleic Acids Res* **15**, 7201–7201 (1987).
15. Hayase, Y., Inoue, H. & Ohtsuka, E. Secondary structure in formylmethionine RNA influences the site-directed cleavage of ribonuclease H using chimeric 2′-O-methyl oligodeoxynucleotides. Biochemistry 29, 8793–8797 (1990).
16. Kanaya, S. et al. A hybrid ribonuclease H. A novel RNA cleaving enzyme with sequence-specific recognition. J Biol Chem 267, 8492–8498 (1992).
17. Ma, W. P., Hamilton, S. E., Stowell, J. G., Byrn, S. R. & Davisson, V. J. Sequence specific cleavage of messenger RNA by a modified ribonuclease H. Bioorg Med Chem 2, 169–179 (1994).
18. Walton, C. M., Wu, C. H. & Wu, G. Y. A ribonuclease H-oligo DNA conjugate that specifically cleaves hepatitis B viral messenger RNA. Bioconjugate Chem 12, 770–775 (2001).
19. Fukuma, T., Walton, C. M., Wu, C. H. & Wu, G. Y. Conjugation of an antisense oligodeoxynucleotide to ribonuclease H results in sequence-specific cleavage and intracellular inhibition of HCV gene expression. Bioconjugate Chem 14, 295–301 (2003).
20. Inoue, H., Hayase, Y., Iwai, S. & Ohtsuka, E. Sequence-Dependent Hydrolysis of RNA Using Modified Oligonucleotide Splints and RNase H. FEBS Lett 215, 327–330 (1987).
21. Duss, O., Maris, C., von Schroetter, C. & Allain, F. H. A fast, efficient and sequence-independent method for flexible multiple segmental isotope labeling of RNA using ribozyme and RNase H cleavage. Nucleic Acids Res 38, e188 (2010).
22. Czech, B. & Hannoun, G. J. Small RNA sorting: matchmaking for Argonautes. Nat Rev Genet 12, 19–31 (2011).
23. Wilson, R. C. & Doudna, J. A. Molecular mechanisms of RNA interference. Annu Rev Biophys 42, 217–239 (2013).
24. Hale, C. R. et al. RNA-Guided RNA Cleavage by a CRISPR RNA-Cas Protein Complex. Cell 139, 945–956 (2009).
25. Abudayyeh, O. O. et al. RNA targeting with CRISPR-Cas13. Nature 550, 280–284 (2017).
26. Jiang, W. Y., Samai, P. & Marraffini, L. A. Degradation of Phage Transcripts by CRISPR-Associated RNases Enables Type III CRISPR-Cas Immunity. Cell 164, 710–721 (2016).
27. O’Connell, M. R. et al. Programmable RNA recognition and cleavage by CRISPR/Cas9. Nature 516, 263–266 (2014).
28. Rousseau, B. A., Hou, Z., Gramelspacher, M. J. & Zhang, Y. Programmable RNA Cleavage and Recognition by a Natural CRISPR-Cas9 System from Neisseria meningitidis. Mol Cell 69, 906–914 (2018).
29. Gellert, M., Lipsett, M. N. & Davies, D. R. Helix Formation by Guanylic Acid. Proc Natl Acad Sci USA 48, 2013–2018 (1962).
30. Sen, D. & Gilbert, W. Formation of Parallel 4-Stranded Complexes by Guanine-Rich Motifs in DNA and Its Implications for Meiosis. Nature 334, 364–366 (1988).
31. Patel, D. J., Pian, A. T. & Kuryavyi, V. Human telomere, oncogenic promoter and 5′-UTR G-quadruplexes: Diverse higher order DNA and RNA targets for cancer therapeutics. Nucleic Acids Res 35, 7429–7455 (2007).
32. Burge, S., Parkinson, G. N., Hazel, P., Todd, A. K. & Neidle, S. Quadruplex DNA: sequence, topology and structure. Nucleic Acids Res 34, 5402–5415 (2006).
33. Maitels, N. & Gray, L. T. The G4 genome. PLoS Genet 9, e1003468 (2013).
34. Rhodes, D. & Lipps, H. J. G-quadruplexes and their regulatory roles in biology, Nucleic Acids Res 43, 8627–8637 (2015).
35. Mergny, J. L. & Helene, C. G-quadruplex DNA: A target for drug design. Nat Med 4, 1366–1367 (1998).
36. Balasubramanian, S., Hurley, L. L. & Neidle, S. Targeting G-quadruplexes in gene promoters: a novel anticancer strategy? Nat Rev Drug Discov 10, 261–275 (2011).
37. Heddi, B., Cheong, V. V., Martadinata, H. & Phan, A. T. Insights into G-quadruplex specific recognition by the DEAH-box helicase RHAU: Solution structure of a peptide-quadruplex complex. Proc Natl Acad Sci USA 112, 9608–9613 (2015).
38. Lattmann, S. et al. The DEAHbox RNA helicase RHAU binds an intramolecular RNA G-quadruplex in TERC and associates with telomerase holoenzyme. Nucleic Acids Res 39, 9390–9404 (2011).
39. Dang, D. T. & Phan, A. T. Development of Fluorescent Protein Probes Specific for Parallel DNA and RNA G-Quadruplexes. Chembiochem 17, 42–45 (2016).
40. Chen, M. C. et al. Structural basis of G-quadruplex unfolding by the DEAH/RHAU helicase DHX36. Nature 558, 465–469 (2018).
41. Gorski, S. A., Vogel, J. & Doudna, J. A. RNA-based recognition and targeting: sowing the seeds of specificity. Nat Rev Mol Cell Biol 18, 215–228 (2017).
42. Schultz, S. J., Zhang, M. & Champoux, J. J. Multiple nucleotide preferences determine cleavage-site recognition by the HIV-1 and M-MuLV RNases H. J Mol Biol 397, 161–178 (2010).
43. Kielbinski, L. J., Hagedorn, P. H., Lindow, M. & Vinther, J. RNase H sequence preferences influence antisense oligonucleotide efficiency. Nucleic Acids Res 45, 12932–12944 (2017).
44. Schwartz, T.,roud, M. A., Lowenhaupt, K., Herbert, A. & Rich, A. Crystal structure of the Zalpha domain of the human editing enzyme ADAR1 bound to left-handed Z-DNA. Science 284, 1841–1845 (1999).
45. Oubridge, C., Io, N., Evans, P. R., Teo, C. H. & Nagai, K. Crystal structure at 1.92Å resolution of the RNA-binding domain of the U1A spliceosomal protein complexed with an RNA hairpin. Nature 372, 432–438 (1994).
46. Lim, K. H., Huang, H., Pralle, A. & Park, S. Stable, high-affinity streptavidin monomer for protein labeling and monovalent biotin detection. Biotechnol Bioeng 110, 57–67 (2013).
47. Tinberg, C. E. et al. Computational design of ligand-binding proteins with high affinity and selectivity. Nature 501, 212–216 (2013).

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Author Contributions
A.T.P and D.T.D. conceived the idea. D.T.D. performed experiments under the supervision of A.T.P. Both authors designed experiments, analysed data, and co-wrote the paper.

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