Supporting Information

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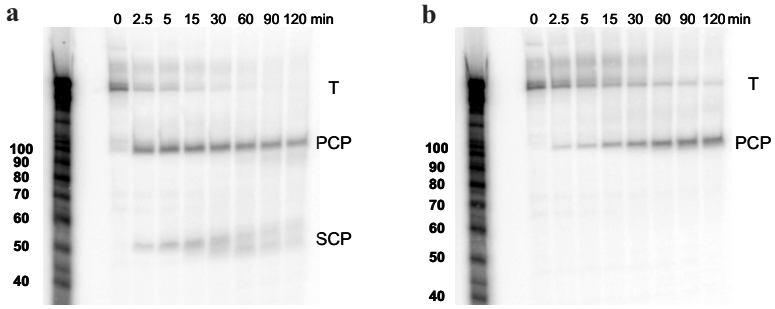

Fig. S1. Comparison of AON and siRNA cleavage reactions when targeting unmodified substrate. Two-hour cleavage reactions in the presence of 2.5 μM AON (*a*) or 2.5 μM siRNA (*b*) are analyzed on 10% sequencing gels. The time above each lane indicates when an aliquot of the reaction was stopped. The 182-nt mRNA target of unknown structure, primary cleavage product, and secondary cleavage products are indicated by T, PCP, and SCP respectively.

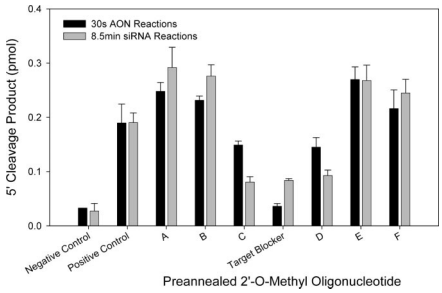

Fig. S2. Amount of product formed in reactions of induced structure over the entire 182-nt target. Cleavage product formation for each reaction was calculated by normalization to the amount of target in the negative control lanes of each gel as shown in Fig. 2, and the mean of at least three independent reactions is plotted (±1 SD).

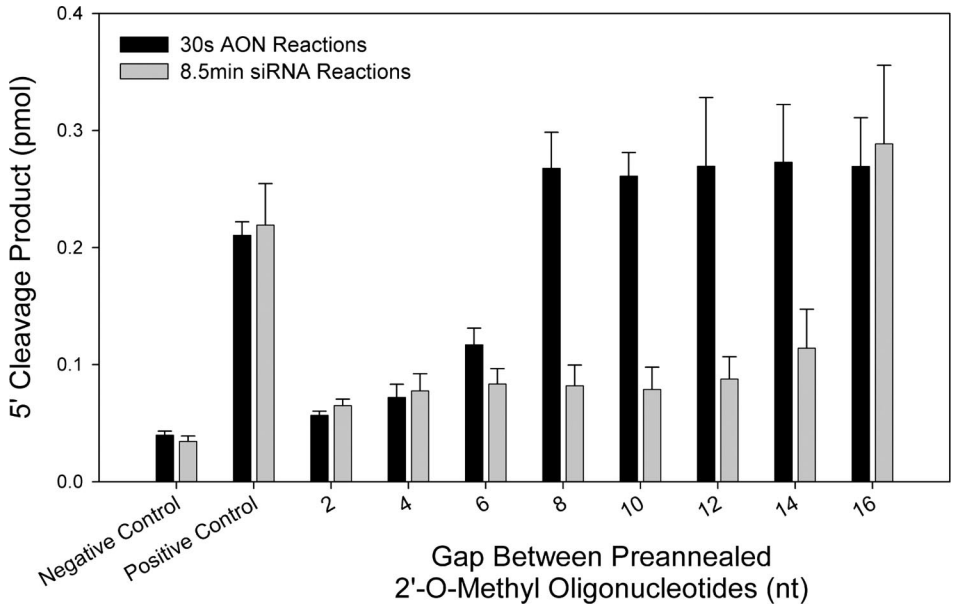

Fig. S3. Product formation as a function of accessible bases in target site. Cleavage activity is reported as a function of the number of unhybridized bases between the two pre-annealed 2′OMe ON. The amount of cleavage product was calculated by normalization to the amount of target in the negative controls of each gel, as shown in Fig. 3, and the mean of at least three independent reactions is plotted (±1 SD).

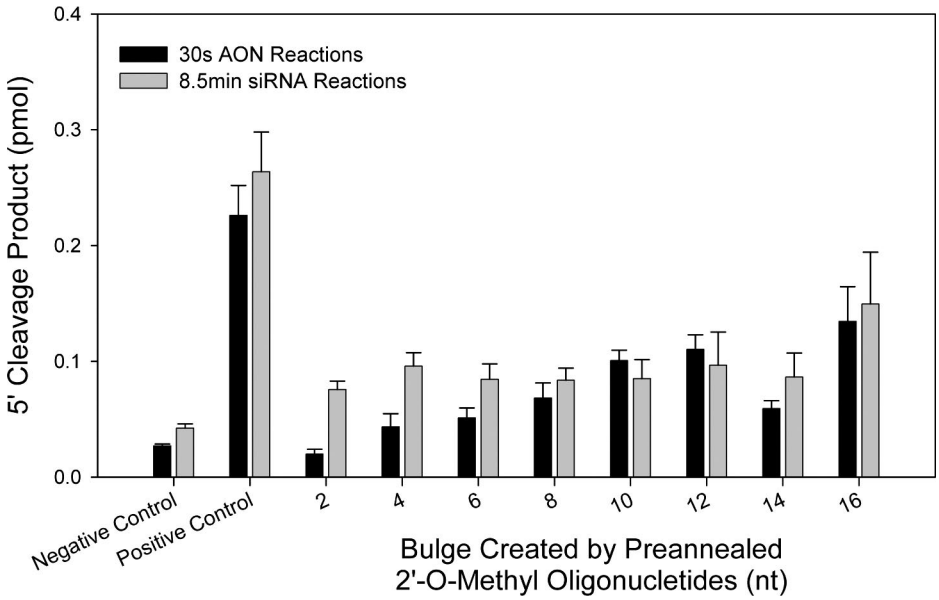

Fig. S4. Product formation as a function of the number of bases in induced bulge. The amount of cleavage product was calculated by normalization to the amount of target in the negative controls of each gel, as shown in Fig. 4, and the mean of at least three independent reactions is plotted (±1 SD).

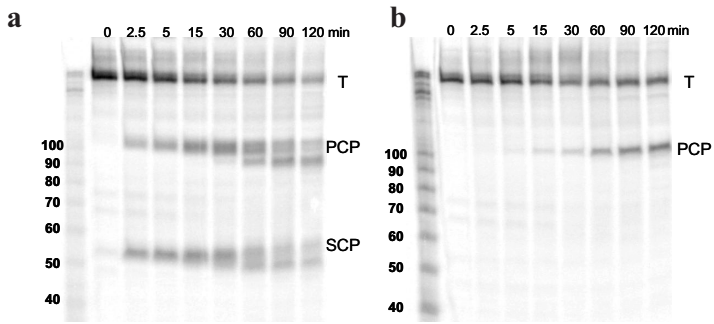

Fig. S5. Extended time course targeting a 6-nt induced bulge. Two-hour cleavage reactions in the presence of 2.5 μM AON (*a*) or 2.5 μM siRNA (*b*) are analyzed on 10% sequencing gels. The double-stranded mRNA target with a 6-nt mismatched bulge, primary cleavage product, and secondary cleavage products are indicated by T, PCP, and SCP, respectively.

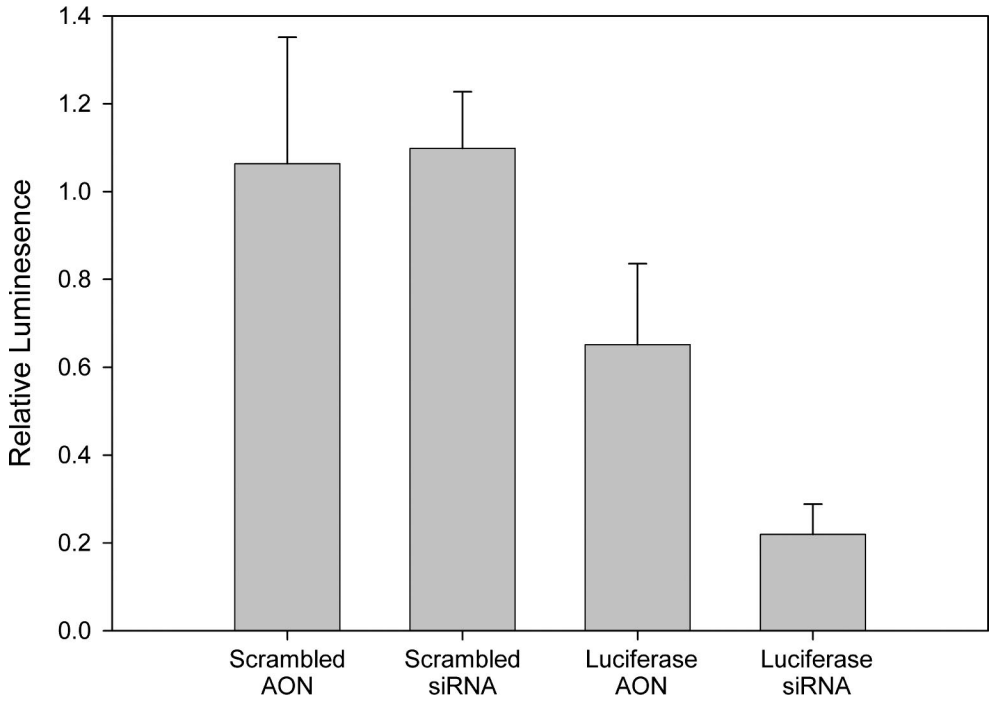

Fig. S6. Dual luciferase assay in K562 cells. Reduction in luminescence was compared for the same AON and siRNA used in *in vitro* experiments. For both the scrambled controls and knockdown experiments, 0.8 nmol of total oligo was nucleofected into K562 cells. Twenty-four hours after nucleofection, cells were lysed, and the ratio of firefly to Renilla luciferase was determined and normalized to that of the samples with luciferase vectors alone. The mean of at least three independent experiments is plotted (±1 SD).

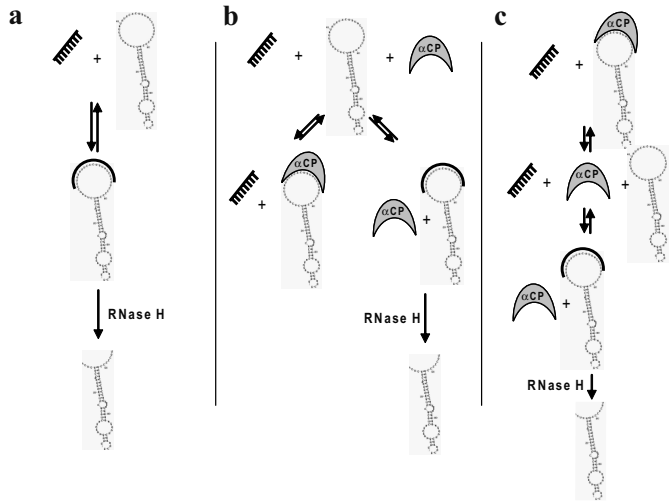

Fig. S7. *In vitro* AON cleavage reaction schemes for R7α1 and αCP. (*a*) Reaction conditions to determine rate of cleavage in the absence of αCP-1. (*b*) Mixing of AON and αCP-1 before introduction of R7α1 substrate allows two competing binding reactions to establish. (*c*) Preincubation of R7α1 and αCP preforms the α-complex before cleavage reaction. αCP dissociation must take place in order for the AON to facilitate cleavage of R7α1.

Table S1. Sequences of 2′-*O*-methyl oligonucleotides used to model intramolecular mRNA structure

Group I 2′-*O*-methyl oligonucleotides		Group II 2′-*O*-methyl oligonucleotides
Name	Sequence (5′ to 3′)	Sequence 5′ to 3′–Gap (nt)–5′ to 3′
A	CCGAACGGACAUUUCGAAG	GCCCAUAUCGUUUCA–2–UCUGUGAUUUGUAUU
B	GUUUCAUAGCUUCUGCCAA	CCCAUAUCGUUUCAU–4–UUCUGUGAUUUGUAU
C	AGCCCAUAUCGUUUCAUAG	CCAUAUCGUUUCAUA–6–AUUCUGUGAUUUGUA
Target Blocker	AUUUGUAUUCAGCCCAUAU	CAUAUCGUUUCAUAG–8–GAUUCUGUGAUUUGU
D	CGAUUCUGUGAUUUGUAUU	AUAUCGUUUCAUAGC–10–CGAUUCUGUGAUUUG
E	UGCAUACGACGAUUCUGUG	UAUCGUUUCAUAGCU–12–ACGAUUCUGUGAUUU
F	AAUUGAAGAGAGUUUUCAC	AUCGUUUCAUAGCUU–14–GACGAUUCUGUGAUU
		UCGUUUCAUAGCUUC–16–CGACGAUUCUGUGAU

Group III 2′-O-methyl oligonucleotides	
Mismatches (nt)	Sequence (5′ to 3′)
2	UCUGUGAUUUGUAUUacGCCCAUAUCGUUUCA
4	UUCUGUGAUUUGUAUcacuCCCAUAUCGUUUCAU
6	AUUCUGUGAUUUGUAgcacuaCCAUAUCGUUUCAUA
8	GAUUCUGUGAUUUGUcgcacuaaCAUAUCGUUUCAUAG
10	CGAUUCUGUGAUUUGgcgcacuaaaAUAUCGUUUCAUAGC
12	ACGAUUCUGUGAUUUagcgcacuaaacUAUCGUUUCAUAGCU
14	GACGAUUCUGUGAUUcagcgcacuaaacgAUCGUUUCAUAGCUU
16	CGACGAUUCUGUGAUccagcgcacuaaacgcUCGUUUCAUAGCUUC

Each group of 2′-*O*-Me oligonucleotides was used in experiments where short regions of double-stranded character were induced over a large area of the 182-nt luciferase target (Group I), in pairs on the target site with a variable number of free target bases (Group II), or used to make the entire target site double-stranded with a variable internal loop (Group III). Between the sequence of each Group II 2′-*O*-Me oligonucleotide, the number of unhybridized target bases is indicated. In Group III 2′-*O*-Me oligonucleotides, the lowercase letters indicate bases that are not complementary with the mRNA target. All sequences (and gaps) have been checked against oligonucleotide synthesis sheets