SUPPORTING INFORMATION

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EXPERIMENTAL SECTION

Reagents. The random ssDNA library (Lib17), Mipomersen strand, FAM-labeled forward primer and phosphorylated reverse primer were synthesized and purified by Integrated DNA Technologies (Coralville, IA). Each single-stranded DNA (ssDNA) library component consists of a central 60-mer region flanked by two 20-mer PCR primer sequences. The central region includes a 17-nt Mipomersen-binding sequence flanked by two randomized sequences (22- and 21-nt). GoTaq Master Mix and MyOne streptavidin C1 Dynabeads were purchased from Life Technologies (Carlsbad, CA). Unmodified PADNA clones, TAMRA-labeled Mipomersen strand, and BHQ2-labeled clones were synthesized and purified by Biosearch Technologies (San Diego, CA). SA was purchased from Roche Technologies (Germany). iQ SYBR Green Supermix was purchased from Bio-Rad Laboratories (Hercules, CA) for qPCR experiments. λ-exonuclease for single strand generation was purchased from New England BioLabs.

Library Preparation and Immobilization. Lib17 (Table S1) was reconstituted as a 500 μM solution in 1X TE, with concentration confirmed via UV-vis measurement. We mixed 100 pmol of Lib17, or 6.02×10^{13} unique sequences, with 150 pmol of biotinylated Mipomersen and hybridized in 200 μL of pH 7.5 1X PBSMT by heating at 95 °C for 5 min, and then slowly cooling down to room temperature (RT) over 20 min. This mixture was then incubated for 20 minutes at RT with 100 μL of SA-coated magnetic beads in pH 7.5 1X PBSMT. Nonspecifically bound DNA molecules were removed by washing library-bead assemblies with PBST buffer in which the ionic strength was gradually decreased (R1: no wash; R2: x2 in 1X; R3: x1 in 1X, x1 in 1/2 X, x1 in 1/4 X; R4: do R3 wash sequence, then x1 in 1/8 X, x1 in 1/16 X, x1 in 1/32 X; R5-R12: same as R4). The beads were then resuspended in pH 7.5 1X PBSMT overnight. Afterwards, the supernatant was discarded to remove nonspecifically releasing sequences prior to selection.

***In vitro* PADNA Selection.** In R1, the library-bead assemblies were challenged with 200 μL pH 5.2 1X PBSMT for 30 minutes at RT. PADNA molecules that underwent acid-induced structure-switching were separated from Mipomersen-coated beads using a magnetic particle concentrator (Life Technologies). The resulting supernatant was PCR amplified in 100 μL PCR reactions containing 50 μL GoTaq Master Mix, 0.50 μL 100 μM FAM-labeled forward primer and phosphorylated reverse primer, 10 μL PADNA molecules, and 39 μL nuclease-free water. GoTaq polymerase was activated prior to PCR by heating reactions to 95 °C for 15 min, followed by 25 cycles of a three-step PCR reaction (30-s denaturation at 95 °C, 30-s annealing at 56 °C, 30-s extension at 72 °C). 8 μL of PCR mixture was collected and resolved on a 10% PAGE-TBE gel to determine the optimal PCR amplification cycle number with minimal byproducts. Collected PADNA pools from each round were PCR amplified at the optimized cycle number.

After full PCR amplification, phosphorylated double-stranded DNA (dsDNA) was purified using the MinElute PCR Purification Kit (Qiagen). Purified dsDNA was enzymatically digested with 36 μL λ-exonuclease in 21.8 μL 10X lambda exonuclease buffer for 90 minutes at 37C. The resulting ssDNA product was purified using phenol-chloroform extraction/ethanol precipitation overnight at -80 °C. The resulting ssDNA pool was quantified via UV-vis at 260 nm and then prepared for the next round of selection.

Over 12 rounds of selection, we systematically decreased the amount (in pmol) of ssDNA library to apply higher selection stringency (R1-R6: 100, R7: 10, R8: 2, R9-R10: 1, R11-12: 0.1). From R3 onward, we also performed negative selections using 1 μM SA. The negative selections were performed in 200 uL of pH 7.5 1X PBSMT for 30 min at RT.

High-Throughput Sequencing of Isolated PADNAs. After 12 rounds of selection, the enriched pool was PCR amplified with unmodified forward and reverse primers under the reaction conditions described above at an optimized PCR cycle number determined by pilot PCR. These PCR products were then purified by the MinElute PCR Purification Kit (Qiagen) and sequenced with Illumina MiSeq Next Generation Sequencing at the GENEWIZ San Diego Laboratory using a 30% PhiX control.

PADNA Characterization via qPCR. 100 pmol of each individual PADNA ssDNA sequence was mixed with 150 pmol of biotinylated Mipomersen and hybridized in 200 μL of pH 7.5 1X PBSMT by heating at 95 °C for 5 min, and then slowly cooling down to room temperature (RT) over 20 min. This mixture was then incubated for 20 minutes at RT with 100 μL of SA-coated magnetic beads in pH 7.5 1X PBSMT. Our qPCR measurement confirmed that 60% of our PADNAs was immobilized after the preparation step. The PADNA/bead assemblies were challenged with 1X PBSMT (pH 4.5 – pH 9.6) for 30 min at RT and the eluents collected. The quantities of PADNAs released as a result of pH-induced structure-switching were subsequently determined by qPCR. Each PCR reaction contained 10 μL iQ SYBR Green Supermix, 8.8 μL PCR water, 0.1 μL each of 0.1 mM forward and reverse primers and 1 μL DNA template. Fluorescence signals were monitored using the iQ 5 multicolor qPCR Detection System (Bio-Rad) and a ΔCt value was determined for each pH shift. All samples were tested in triplicate. Release ratio was calculated from the following formula: $2^{\Delta Ct}$.

PADNA Characterization via Real-Time Fluorescence Measurements. We mixed 0.5 μM BHQ2-modified PADNA-1 or control sequence with 0.5 uM TAMRA-labeled Mipomersen and hybridized in 200 μL of pH 7.5 1X PBSMT by heating at 95 °C for 5 min, and then slowly cooling down to room temperature (RT) over 20 min. The mixtures were transferred to a black 96-well microplate (Microfluor 2, Thermo Scientific; Waltham MA) and equilibrated for 30 minutes at RT. The pH was cycled between 5.2 and 7.5 using small volumes of 1 M HCl or NaOH and the fluorescence emission was measured at 579 nm

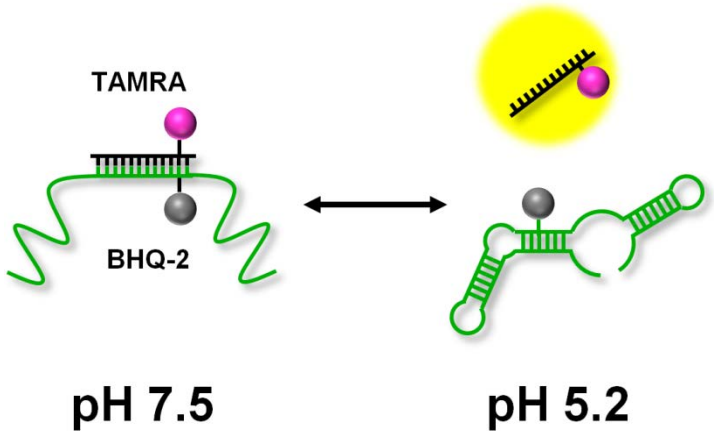
using a TECAN microplate reader (San Jose, CA) with the following settings: excitation wavelength = 525 nm, excitation bandwidth = 5 nm, and emission bandwidth = 5 nm.

Table S1. Oligos used for selection and characterization. All sequences written 5' to 3'.

Forward primer	FAM-AAGTGCCATCGTGCGTATCC
Reverse primer	Phos-TCGTCGTGTCTCCAGTCTAC
Bio-Mipomersen	Biotin-T_{20}-GCCTCAGTCTGCTTCGCACC
Lib17	AAGTGCCATCGTGCGTATCC-N_{22}-GCGAAGCAGACTGAGGC-N_{21}-GTAGACTGGAGACACGACGA
PolyT control	AAGTGCCATCGTGCGTATCC-T_{22}-GCGAAGCAGACTGAGGC-T_{21}-GTAGACTGGAGACACGACGA
TAMRA-Mipomersen	GCC(T-TAMRA)CAGTCTGCTTCGCACC
BHQ2-PADNA-1	AAGTGCCATCGTGCGTATCCTTCACTCCTTGCTCGACAAGAAGCGAAGCAGAC(T-BHQ2)GAGGCGTCCGATGGTCTAATTCTTCAGTAGACTGGAGACACGACGA
BHQ2-polyT control	AAGTGCCATCGTGCGTATCC-T_{22}-GCGAAGCAGAC(T-BHQ2)GAGGC-T_{21}-GTAGACTGGAGACACGACGA
GC-Q	AAGTGCCATCGTGCGTATCCTTC(G)CTCCTTGCTCGACAAG(G)AGCGAAGCAGAC(T-BHQ2)GAGGCGTCCGATGGTCTAATTCTTCAGTAGACTGGAGACACGACGA
AT-Q	AAGTGCCATCGTGCGTATCCTTCACT(T)CTTGCTCGACAAGAAG(T)GAAGCAGAC(T-BHQ2)GAGGCGTCCGATGGTCTAATTCTTCAGTAGACTGGAGACACGACGA
MUT-1	AAGTGCCATCGTGCGTATCCTTC(T)CTCCTTGCTCGACAAGAAGCGAAGCAGACTGAGGCGTCCGATGGTCTAATTCTTCAGTAGACTGGAGACACGACGA
MUT-2	AAGTGCCATCGTGCGTATCCTTC(T)CTCCTTGCTCGACAAG(T)AGCGAAGCAGACTGAGGCGTCCGATGGTCTAATTCTTCAGTAGACTGGAGACACGACGA
MUT-CC	AAGTGCCATCGTGCGTATCCTTC(C)CTCCTTGCTCGACAAG(C)AGCGAAGCAGACTGAGGCGTCCGATGGTCTAATTCTTCAGTAGACTGGAGACACGACGA

Table S2. Release ratios of PADNAs identified by HTS and negative controls.

Sequence ID	Copy number	Sequences without primer sites (5' --> 3')	Release ratio
PADNA-1	91	TTCACTCCTTGCTCGACAAGAAGCGAAGCAGACTGAGGCG TCCGATGGTCTAATTCTTCA	145
PADNA-2	86	TTCACGTTTGTTCTACAAAAGCGAAGCAGACTGAGGCGCGT GCCCTGTCGTGCACCACGG	714
PADNA-3	83	TGAACATTGACGTGTAGTCAATGCGAAGCAGACTGAGGCG CCTCTCTAGTTTGACCTTGG	108
PADNA-4	80	CGCTCCGCTAATATCTATGTTAGCGAAGCAGACTGAGGCG CGATGATGTCTACACTGACG	93
PADNA-6	71	TCCGCTAACTCCGAAAGAGGTAGCGAAGCAGACTGAGGCG CGCCTCCGGCTCGTGTATCG	180
PADNA-7	69	TTCGCAAATTTGGTTACAAATCGCGAAGCAGACTGAGGCGC GCCTTTATGTCTATTCGTT	121
PADNA-9	65	AACTTCACGTCTTCTTGAAAACGCGAAGCAGACTGAGGCG CGTCGAATGGCCTAAACACA	231
PADNA-13	57	TAGCTTCACGATATTTAATACCGCGAAGCAGACTGAGGCGC GTTACCTCCACAGCTTGCT	161
PADNA-18	55	GCTTCACGTTAATTTATTACACGCGAAGCAGACTGAGGCAT GCCTCCTGACGTGATCCAC	205
PADNA-20	54	TTCATGCGGAACTTTTTCCACACGAAGCAGACTGAGGCGC GCCTTTGAGTCGATTTGTAG	201
Lib17	-	N_{22}-GCGAAGCAGACTGAGGC-N_{21}	<1
PolyT	-	T_{22}-GCGAAGCAGACTGAGGC-T_{21}	1



Scheme S1. Real time fluorescence monitoring assay. At pH 7.5, TAMRA-labeled Mipomersen hybridizes with a BHQ2-labeled PADNA molecule, which quenches its fluorescence. When the pH is shifted to 5.2, Mipomersen is released and TAMRA fluorescence is restored.

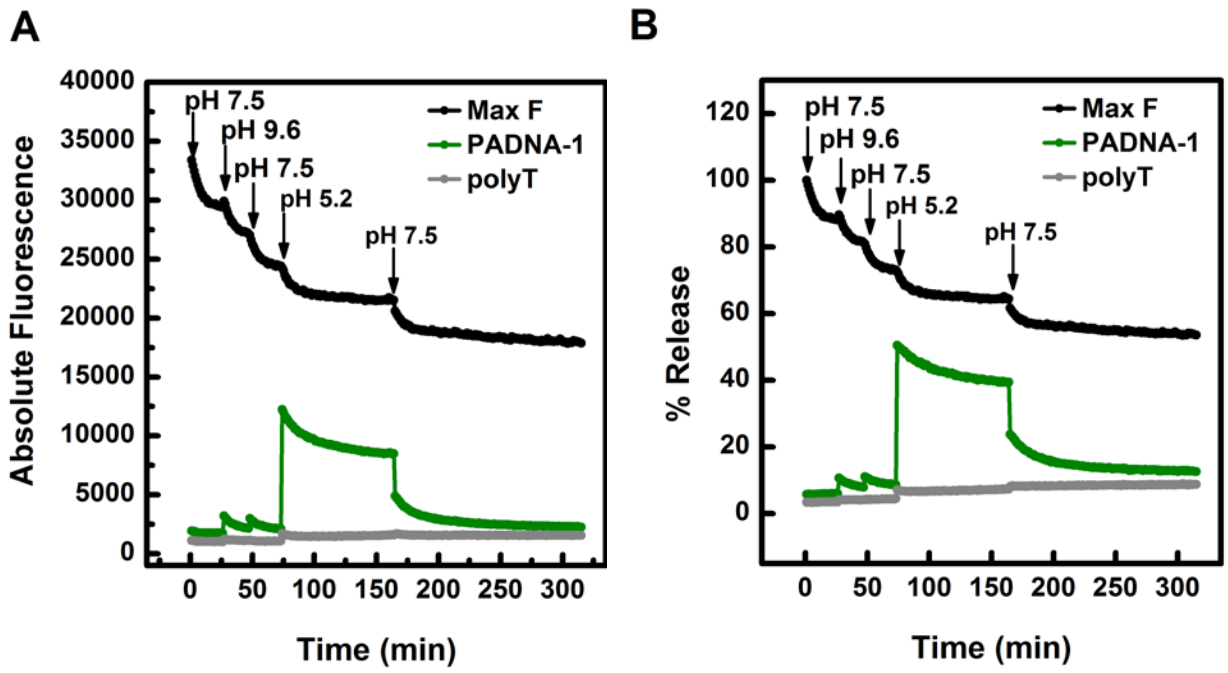


Figure S1. (A) Raw fluorescence data used to produce Figure 3A. Max F represents the signal corresponding to TAMRA-labeled Mipomersen in the absence of BHQ2-labeled PADNA-1. Max F decreases throughout the experiment due to photobleaching of TAMRA. (B) In order to normalize our results relative to TAMRA photobleaching, we calculated the % Release using Equation 1 (below). However, the rate of photobleaching decay for the TAMRA-Mipomersen sample differed slightly from the TAMRA-Mipomersen + BHQ2-PADNA-1 sample and as such, a small photobleaching artifact is visible.

From the absolute fluorescence values, we calculated "% Release" as follows:

$$\% \text{ Release} = \frac{\text{Absolute Fluorescence of Sample}}{\text{Maximum Fluorescence}} \times 100\% \qquad (1)$$

However, as we noted in the manuscript, the maximum % release at pH 5.2 was capped at 50.5%, perhaps due to the influence of TAMRA and BHQ2 modifications. Therefore, we calculated "Normalized % Release", which results in the plot Figure 3A.

$$\text{Normalized \% Release} = \frac{\% \text{ Release}}{50.5\%} \times 100\% \qquad (2)$$

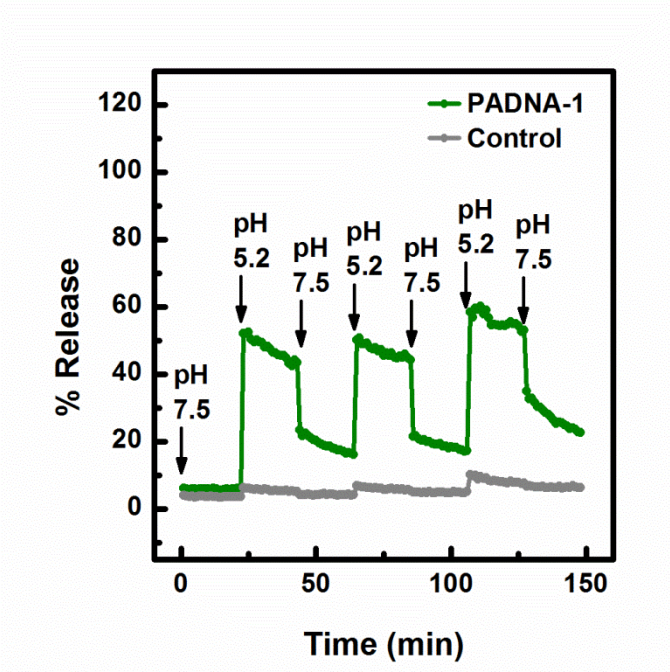


Figure S2. PADNA-1/Mipomersen loading and release is reversible.

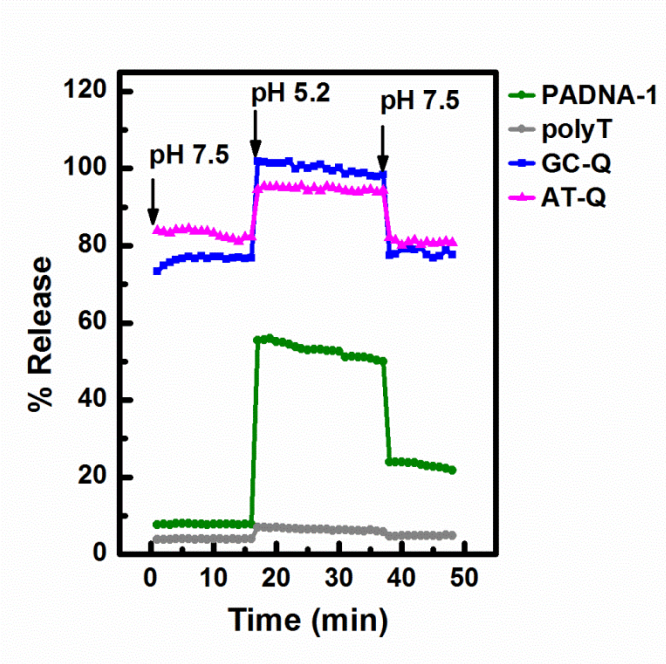


Figure S3. Real-time measurement of fluorophore-labeled Mipomersen release from quencher-labeled mutants of PADNA-1 where C-A mismatches at sites (1) and (2) have been replaced G-C or A-T Watson Crick basepairs. We found that at pH 7.5, the GC mutant (GC-Q) released 76.8% of the loaded Mipomersen. Similarly, the AT mutant (AT-Q) released 82.2% of Mipomersen. This is consistent with our hypothesis for the structure-switching mechanism because G-C or A-T basepairs would stabilize intramolecular folding of PADNA-1 and promote strong release of Mipomersen at pH 7.5. Interestingly, when the GC and AT mutants are challenged with pH 5.2, we observe further Mipomersen release reaching 100%. We believe this effect is likely due to the formation of additional favorable tertiary contacts at low pH.