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SUPPLEMENTARY INFORMATION

Chemically "barbed" aptamers selected from a base-modified RNA library

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Table S1. Oligonucleotides used in this study and clone sequences from round 16 of selection.

Random library	5'CCGGGCTTTGTGTCACTTNNNNNNNNNNNNNNNNNNNNNNNNNNGCTCGTTCT CCTTCCCTCTCCTATAGTGAGTCGTATTACAGTTG (N:15%/28%/29%/28%:A/G/C/T)
Biotinylated T7 forward primer	5'Biotin-CAACTGTAATACGACTCACTATAGGAGA
IsodC reverse primer	5'isodC-CCGGGCTTTGTGTCACTT
Capture strand (rigidifier binding site underlined), capture sequence in bold	5'**GCTCGTTCTCCTTCCCTCTCC**TTTTTTTTTTCAACACCACAGACCAGTATACCCAGAAATG ACGCAAGCATAGACAAACGATTTAGACATGAGTGCCCCACACAACGAACAAGCTTTTTTTTTA -hexaethyleneglycol spacer-CAACTGTAATACGACTCACTATAGGAGA
Capture strand rigidifier	5'GCTTGTTCGTTGTGTGGGGCACTCATGTCTAAATCGTTTGTCTATGCTTGCGTCATTTCTG GGTATACTGGTCTGTGGTGAA
Unmodified T7 forward primer	5'CAACTGTAATACGACTCACTATAGGAGA
Unmodified Reverse primer	5'CCGGGCTTTGTGTCACTT
Clone 1	CAACTGTAATACGACTCACTATAGGAGATGGAAGGAGAATGAGA**TAGCACCTAACATTTCTTG AAATGA**AAGTGACACAAAGCCCGG
Clone 2	CAACTGTAATACGACTCACTATAGGAGAGGGAAGGAGAACGAGC**AGGCACGTATAACTGACAA ACGCTT**AAGTGACACAAAGCCCGG
Clone 3	CAACTGTAATACGACTCACTATAGGAGAGGGAAGGAGAACGTGC**AGGGTAAAATTTAACTGAC AAGCT**AAGTGACACAAAGCCCGG
Clone 4	CAACTGTAATACGACTCACTATAGGAGAGGGAAGGAGAACGAGC**GGGACAGCTTAACTGACGA AAACTT**AAGTGACACAAAGCCCGG
Clone 5	CAACTGTAATACGACTCACTATAGGAGAGGGAAGGAGAACGAGC**GGGACAGCTTAACTGACGA AAACTT**AAGTGACACAAAGCCCGG
Clone 6	CAACTGTAATACGACTCACTATAGGAGAGGGAAGGAGAATAATA**CCGGGTAGCCTTAACTGAC GCGCTT**AAGTGACACAAAGCCCGG
Clone 7	CAACTGTAATACGACTCACTATAGGAGAGGGAAGGAGAACAAGC**AGGGCAAAATTTAACTGAC AAGCTT**AAGTGACACAAAGCCCGG
Clone 8	CAACTGTAATACGACTCACTATAGGAGAGTGAAGGAGAACGACC**TGGAAACGCATTAACTAAC AAGCTT**AAGTGACAAAAGCCCGG
Clone 9	CAACTGTAATACGACTCACTATAGGAGAGGGAAAGAGAACAAGC**ATAACGGCATTGAGCTTAA CTGTAA**AAGTGACACAAAGCCCGG
Clone 10	CAACTGTAATACGACTCACTATAGGAGAGGGAAGGAGAATAATA**CCGGGTAGCCTTAACTGAC GCGCTT**AAGTGACACAAAGCCCGG

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Table S2. Conditions and PCR recovery efficiency for the selection experiment.

Selection round	Conditions	PCR Cycle # for recovery
1	0.2mg beads, 1hr	14
2	0.2mg beads, 1hr	14
3	0.2mg beads, 1hr	14
4	0.2mg beads, 1hr	14
5	0.2mg beads, 1hr	14
6	0.2mg beads, 1hr	14
7	0.2mg beads, 1hr	10
8	0.2mg beads, 1hr	10
9	0.1mg beads, 30min, 1µM BSA (denatured)	12
10	0.1mg beads, 30min, 1uM BSA (denatured)	12
11	0.1mg beads, 30min, 5uM BSA (denatured)	12
12	0.1mg beads, 30min, 5uM BSA (denatured)	12
13	0.1mg beads, 30min, 5uM BSA (native)	13
14	0.1mg beads, 30min, 5uM BSA (native)	13
15	0.1mg beads, 30min, 15uM BSA (native)	14
16	0.02mg beads, 5min, 15uM BSA (native)	16

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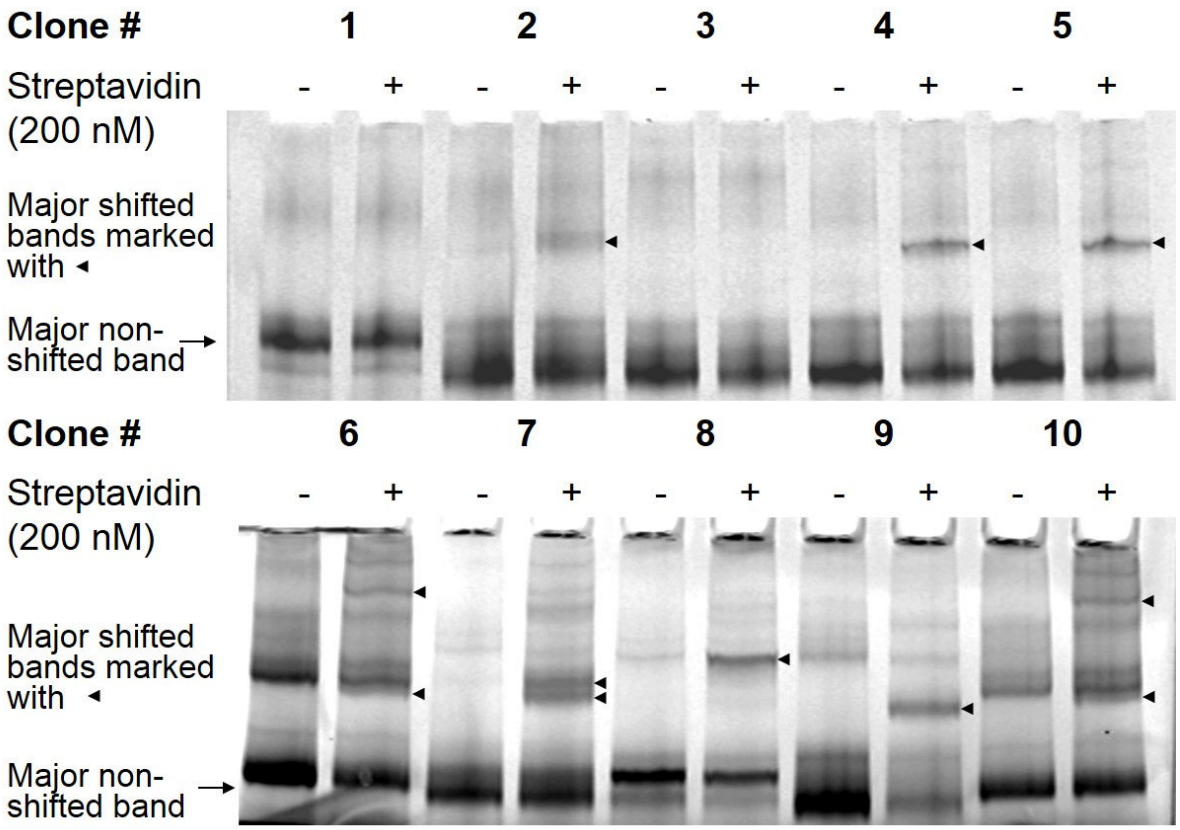

Figure S1. Assay of individual clones for covalent binding activity. RNA was obtained by transcription with ethynyluridine triphosphate in place of uridine triphosphate. RNA samples were labeled with sulfo-HSAB by CuAAC and incubated with or without 200nM streptavidin for 2hr. Samples were denatured in SDS buffer, annealed to a visualization oligo and electrophoresed on 10% (w/v) polyacrylamide SDS gels.

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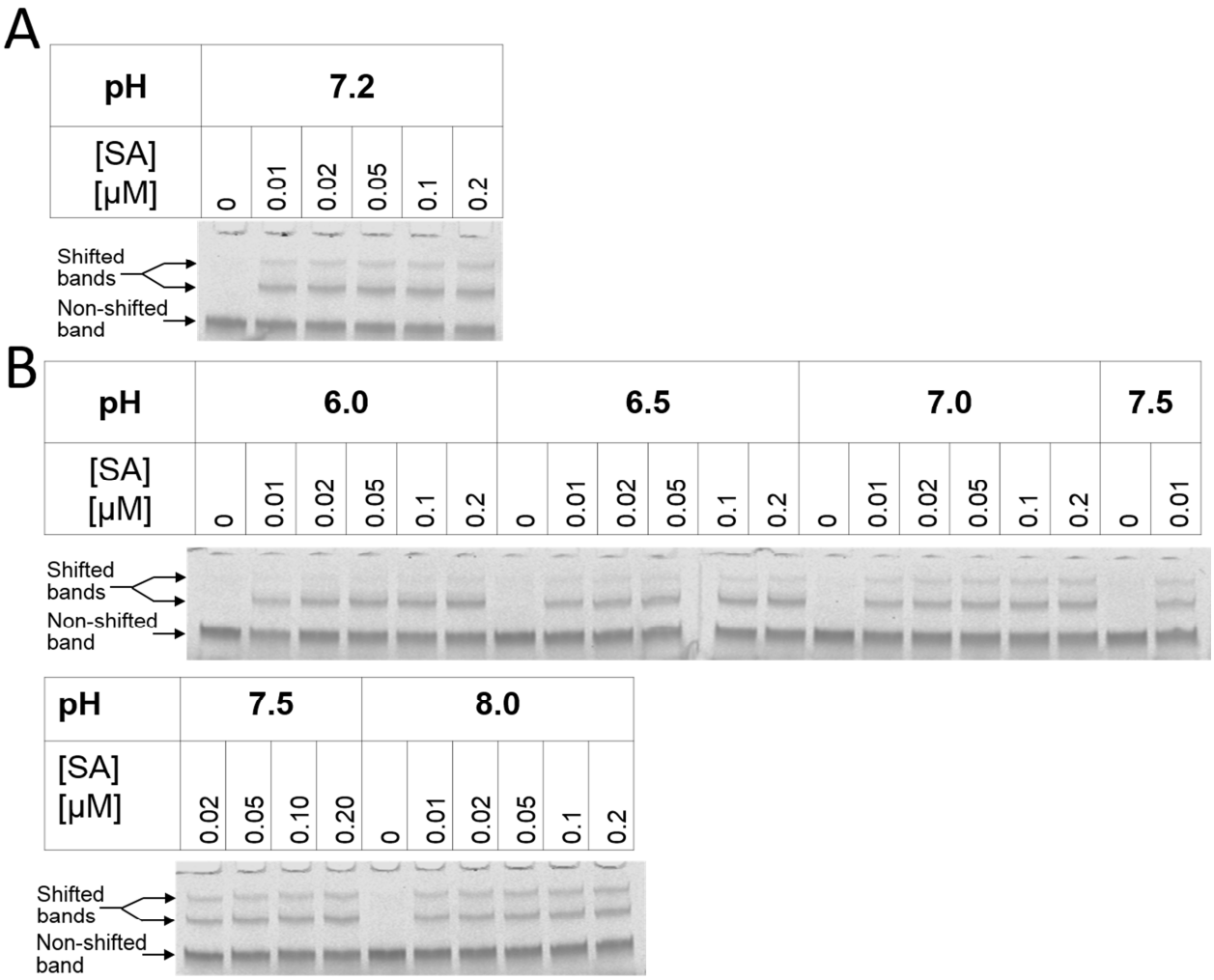

Figure S2. A. Binding analysis of clone 4 at the selection pH (7.2). Clone 4 RNA containing 5-EU was transcribed and purified as described in the materials and methods section. RNA was labeled with sulfo-HSAB by CuAAC and exposed to varying amounts of streptavidin for 2hr. Samples were denatured in SDS buffer, annealed to a visualization oligo and electrophoresed on 10% (w/v) polyacrylamide SDS gels. **B.** Binding analysis of clone 4 at varied pH. Reactions performed as in (A) but at the specified pH.

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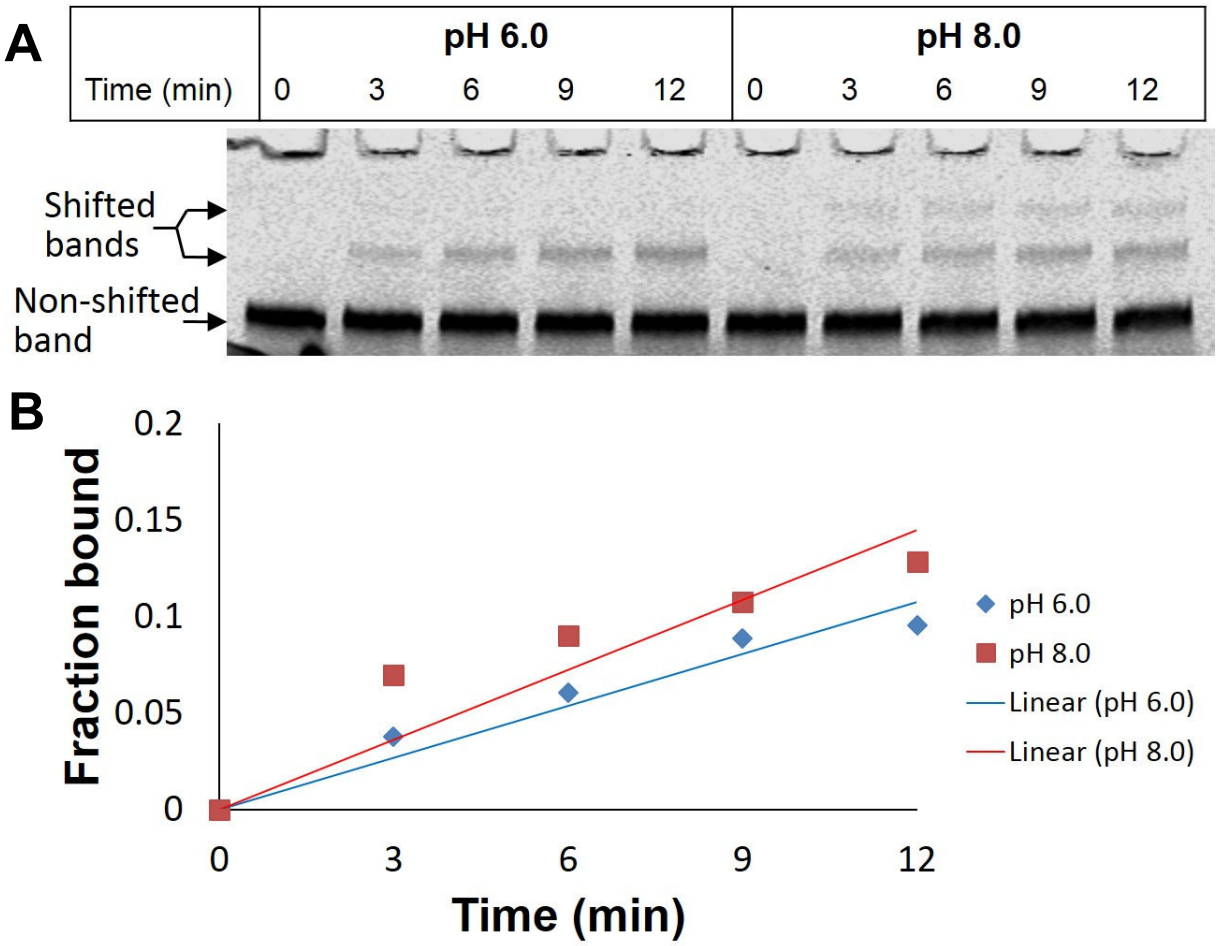

Figure S3. Time-point analysis of reaction rates at varying pH. Clone 4 was labeled as in figure S2 and S3 and reacted with 10nM streptavidin with varying time. **A.** Reactions were stopped with addition of 1% (w/v) SDS and immediate heat-denaturation, and visualized as in Figures S1-S3. **B.** Graphical representation of the initial binding corresponding to (A).

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Figure S4. Native gel shift assay on various formulations of clone 4. Clone 4 RNA was tested in three different formats for the ability to cause a gel shift upon incubation with various streptavidin concentrations. Whereas clone 4 containing both ethynyluridine (lanes 1-5) and the NHS ester hydrolysis product (lanes 6-10) did not cause an observable gel shift in up to 10µM streptavidin, the shifted band for RNA/streptavidin is apparent in 10-200nM streptavidin with the intact NHS ester modification (lanes 11-16). Binding reactions were performed in PBS, pH 7.2. To generate triazolyl RNA, HSAB-modified RNA was heated at 98 °C for 3min and snap cooled on ice. All RNAs were pre-annealed to imaging capture sequence (IRDye700-conjugated) before incubation with streptavidin. Reactions were incubated for 2hr after which native loading dye was added (final glycerol concentration of 2.5% (v/v), trace xylene cyanol loading dye in water). RNAs were electrophoresed for 10min at 250V on a 6% (w/v) polyacrylamide (19:1 acrylamide:bisacrylamide), 0.5x TBE gel.