Supporting Information for

Evolution of a Histone H4-K16 Acetyl-Specific DNA Aptamer

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Materials and Methods

Oligonucleotide Sequences
DNA Library (Keck Facility at Yale University)
5'-GGCGGCGATGAGGATGAC-N_{48}-ACCACTGCGTGACTGCCC-3
N=1:1:1:1 mixture of A:T:G:C

Forward PCR Primer (IDT, Coralville, IA)
5'-FAM-GGCGGCGATGAGGATGAC-3'
FAM= 6-carboxyfluorescein

Reverse PCR Primer (IDT, Coralville, IA)
5'-Bio-GGGCAGTCACGCAGTGGT-3'
Bio= Biotin

Peptide Sequences
Histone H4 peptide (ASU Protein Facility)
GGKGLGKGGAKRHRK-Bio
Bio= Biotin

Histone H4-K16Ac peptide (Protein Core Laboratory, Baylor College of Medicine)
GGKGLGKGGAK(Ac)RHRK
Ac= acetyl group

Histone H4-K8Ac peptide (Protein Core Laboratory, Baylor College of Medicine)
GGK(Ac)GLGKGGAKRHRK
Ac= acetyl group

Reagents

The DNA library (5'-GGCGGCGATGAGGATGAC-(N48)-ACCACTGCGTGACTGCCC-3) was purchased from the Keck Facility (Yale University). The random region was synthesized using a biased ratio of nucleotide phosphoramidites to give a 1:1:1:1 mixture of A: T: G: C in the oligonucleotide product. The PCR primers were purchased from Integrated DNA Technologies (Coralville, IA). The forward and reverse primers were synthesized with a 6-carboxyfluorescein (6-FAM) and biotin modifications on their 5'-ends, respectively. The H4 peptide tail sequences were synthesized at the ASU Protein Facility, and the acetylated H4 peptide at lysine position 16 (H4-K16Ac) and acetylated H4 peptide at lysine position 8 (H4-K8Ac) were made at the Protein Chemistry Core Laboratory at Baylor College of Medicine. The monoclonal anti-acetyl-Histone H4 (K16) antibody was purchased from Upstate Biotechnology Inc (cat. Number: 07-329).

***In Vitro* Selection**

For each round of selection, the DNA pool was made single-stranded by immobilizing the dsDNA onto streptavidin-coated agarose beads (Millipore) and denaturing the strands with 0.15 M NaOH. The sense strand was collected in the flow-through, neutralized, and folded by heat denaturing the DNA at 95 ºC for 5 min. and cooling on ice in the presence of selection buffer (3 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 10 mM NaCl, and 5 mM $MgCl_2$, pH 7.4). The DNA pool (0.5 μM) was incubated in selection buffer (200 μL) with the H4 peptide (0.5 μM) containing a C-terminal biotin residue for 1 hour at 24 °C, and passed over streptavidin-coated agarose beads to remove molecules that bind to the non-acetylated target. The unbound DNA was collected, purified by ethanol precipitation and used as input for the positive selection step. DNA molecules that remained in the pool were refolded and the DNA pool (0.1 μM) was incubated with histone H4-K16Ac peptide (0.1 nM) in selection buffer for 1 hour at 24 °C. The peptide-bound DNA fraction was separated using capillary electrophoresis (see below), recovered in a vial, and amplified by PCR. After four rounds of selection, the DNA was cloned into a Topo TA plasmid (Invitrogen, CA), transformed into *Escherichia coli* Top10 cells and sequenced at the ASU Sequencing Facility (Table 1).

Capillary Electrophoresis Selection

All capillary electrophoresis (CE) separations were performed on a P/ACE 2100 Capillary Electrophoresis system (Beckman Coulter, Inc., Fullerton, CA). Prior to use, the separation capillary (polyacrylamide coated, 50 µm I.D., 360 µm O.D., total length = 57 cm, length to detector = 50 cm) was rinsed with ultrapure water for 10 min and equilibrated with selection buffer. All experiments were conducted at 20 ºC. A portion of the ssDNA-peptide mixture (~10 nL) was injected onto the separation capillary using pressure injections (1 s, 0.1 psi) and 30 kV was applied across the capillary for 2 min. Laser-induced fluorescence (LIF) was used to monitor the separation of the 6-FAM labeled DNA. Excitation was generated using the 488 nm line of an Ar+ laser (Beckman Coulter) and emission was collected at 520 nm. Two major peaks were observed corresponding to bound and unbound fractions of the DNA (S1). The bound fraction was collected and amplified using PCR. Five CE separations were performed for every round of selection to increase the number of sequence copies in the assay.

Dissociation Constants (K_D) of the Aptamers

Affinity capillary electrophoresis (ACE) was used to obtain K_D values for the different aptamers as previously described (1). Folded clones 4.3, 4.9, and 4.20 were incubated in selection buffer with increasing concentrations of peptide and injected onto the CE instrument. Relative LIF of bound and unbound fractions were recorded for each sample and the peak height of the free DNA was used to generate K_D values by plotting the concentration versus peak height and fit to a 1:1 binding model using the program Origin (Table 2).

Surface Plasmon Resonance

Affinity constants for clone 4.20 aptamer were determined using surface plasmon resonance (SPR) (T100 instrument from Biacore). Streptavidin was immobilized on a CM5 chip using standard NHS/EDC immobilization chemistry. Optimal streptavidin (0.2 µg/ml) immobilization occurred in10mM NaOAc buffer, pH 5.0, resulting in 2500 response units. 5' biotinylated clone 20 was annealed in the presence of 1x TAE Mg^{2+} buffer (20 mM Tris, 20 mM Acetic acid, 2mM EDTA, and 12.5 mM Magnesium acetate, pH 8.0). The annealed aptamer was immobilized on the streptavidin-coated chip yielding over 1,000 response units. The anti-acetyl-Histone H4 antibody was immobilized through standard NHS/EDC immobilization chemistry. PBS tween (10 mM NaH_2PO_4, 138 mM NaCl, 2.7 mM KCl, 0.05% Tween, pH7.4) buffer was used as the running and dilution buffer in the binding assays at a flow rate of 30 µL/min. Each assay consisted of a 60 second contact period and a 260 second dissociation time, allowing the response units to return to baseline. Any nonspecific binding to the reference cell was subtracted from the sample flow cell response. All sensograms were double referenced using buffer injections. A concentration series of H4-K16Ac, H4-K8Ac and H4 peptide ligands were assayed, between 1-500 nM and 0.5-100 µM, respectively. K_D values were determined from equilibrium binding responses using Biacore evaluation software to fit the curves using 1:1 binding (S3).

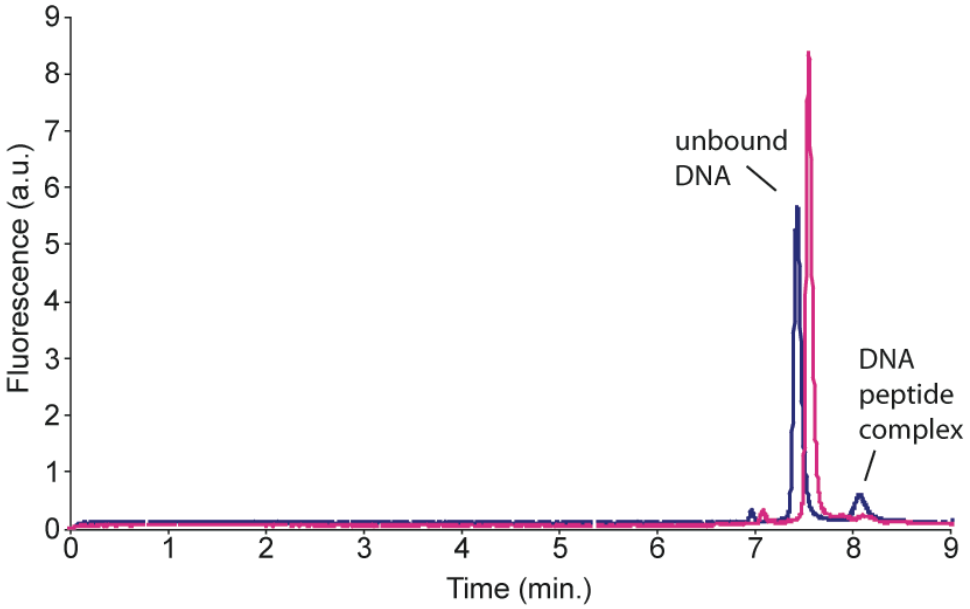

Figure S1. Capillary Electrophoresis Separation of Histone Aptamers. The DNA pool (0.1 μM) from round 1 (pink trace) and round 4 (blue trace) was incubated with histone H4-K16Ac peptide (0.1 nM) and separated by capillary electrophoresis. The unbound DNA migrates as a single peak around 7.5 minutes and the DNA bound to the Histone H4-K16Ac peptide migrates at 8 minutes.

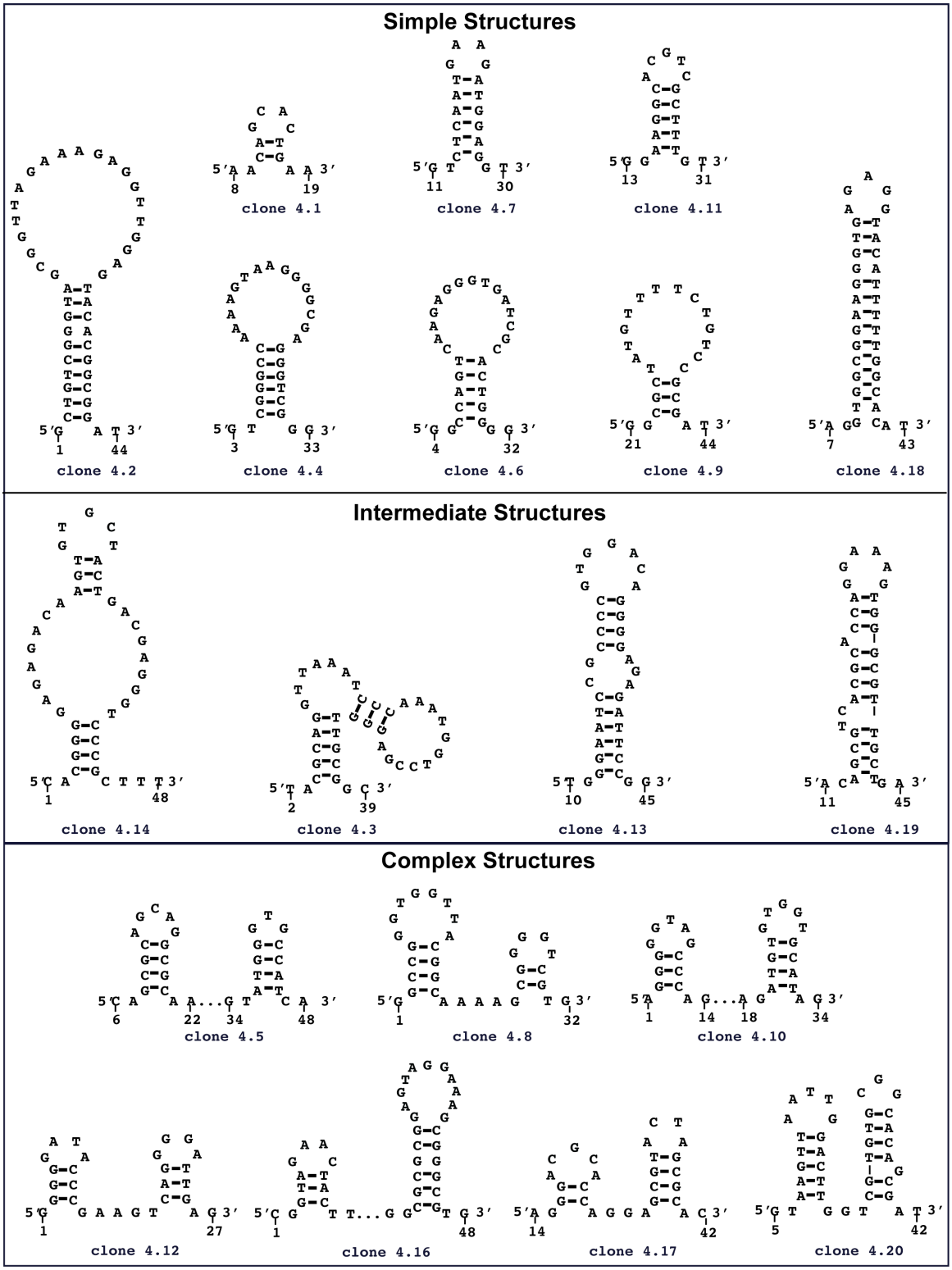

Figure S2. Secondary Structure of Histone H4-K16Ac Aptamers using the DNA version of mFold (2). Clones were categorized into simple, intermediate, and complex structures based on their stem loop, stem loop with bulges, and multiple stem loop structures, respectively.

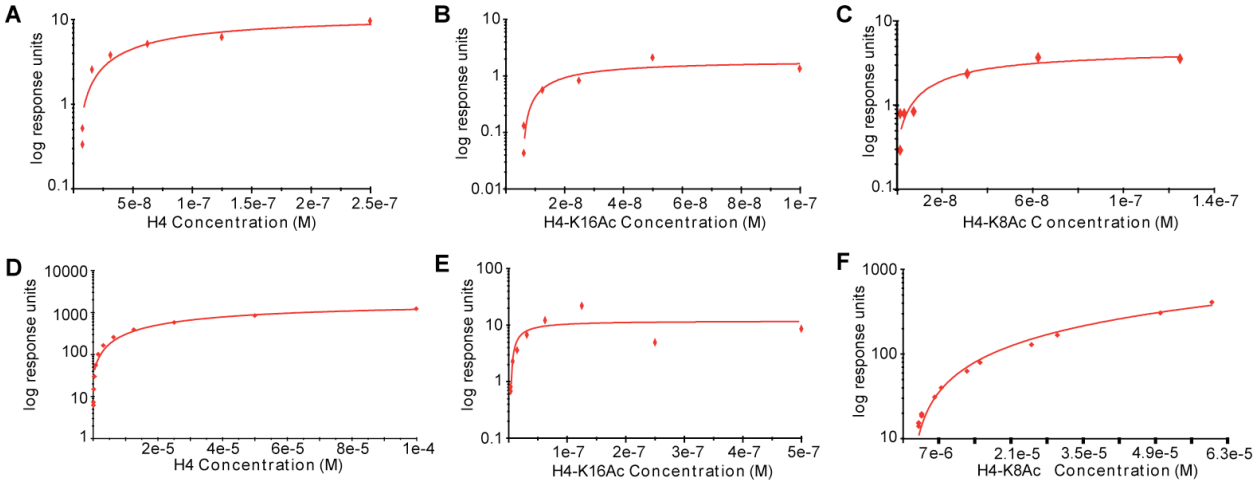

Figure S3. Affinity plots for Anti-H4-K16Ac Antibody and Clone 4.20 Aptamer. (A-C) SPR affinity fits for the monoclonal anti-H4-K16Ac antibody against (A) H4, (B) H4-K16Ac, and (C) H4-K8Ac. (D-F) SPR affinity fits for clone 4.20 against (D) H4, (E) H4-K16Ac, and (F) H4-K8Ac.

Table 1. Round 4 DNA Aptamer Sequences.

Clone	DNA Sequence (5'-3')
4.1	AGGAGGGAACAGCACTGAAGCACATAGGGACCAGAACTAGGCCATTTG
4.2	GCTGTCGGGTAGCGGTTAGAAAGAGGTTGGAGTACACGGCGGATAGAT
4.3	GTACGCAGGTTAAATCCCAAATGGTCCGAGGGTTGCGGCATCAAGGTT
4.4	TGGTCGGGCCAAAAGTAAGGGGCGAGGGTCGGGATGGGCGGGAGAAGC
4.5	CGAGGCAGCGCAGCAGGCGCAAGATGAGGGGGGGTATGGGTGCCATCA
4.6	GGAGGCCAGTCAAGAGGGTGATCGCACTGGGGGTTGGATGGTAGCGT
4.7	TCGCTGTGCAGTCTCAATGAAGATGGAGGTTAATAGGGTGAGGACGGG
4.8	GGCCGGGTGGTTACGGCAAAAGCGGGGTCGTGTCGGAAATGGTGGTAG
4.9	GTCTAAGTAAACTGTGGGAAGGCGCTATGTTTTCTGTCCGCGATACAT
4.10	AGGGGGTAGCCCAGGTCAGATGTGTGGTGCATAGTGCGGGATTGAGGG
4.11	GGGGGGAGGGAGGGAAGGCACGTCGCTTTGTACTGTCGAGGATAGGTG
4.12	GGGGGATACCCGAGTCAGGGGATTGAGTGGTATTAGAGAGAAGGGGTG
4.13	GAAGAGTGGTGGGAATCCGCCCCGTGGACAGGGGAGAGATTCCGGACG
4.14	CACGGGGAGAGACAAGTGTGCTACTGACGAGGGTCCCGCTTT
4.15	CGAGTACGGCATGAGGCCATTGGGCGGCAAGTAACCCCGAGGTTGTAG
4.16	CGGTAGAACTACTTAACCCAGGGCGCGCGGAGTAGGAAAGCGGGCGTG
4.17	TCAATTGGAGGGGAGGGACGCACCAGGAGCGTACTAGCGCACAAGGGG
4.18	GAGGGGAGGTGGCGGAAGGGTGAGAGGTACATTTTTGGCACATAAAG
4.19	AACTGGCAGCCAAGCGTCACGCACCAGGAAAGTGGGCGTTGCTGATCA
4.20	AGACGTAAGTTAATTGGACTTGGTCGTGTGCGGCACAGCGATTGAAAT
	1 10 20 30 40

References

1. Mendosa, S.D.; Bowser, M.T. *J. Am. Chem. Soc.* **2005,** 127, 9382-9383.
2. Zuker, M. *Nucleic Acids Res* **2003**, *31***,** 3406-3415.