Supporting Information for:

In Vitro Selection of Histone H4 Aptamers for Recognition Imaging Microscopy

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Materials & Methods

Reagents

The DNA library (5'-GGCGGCGATGAGGATGAC-48N-ACCACTGCGTGACTGCCC-3) was purchased from the Keck Facility at 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 moieties on their 5'-ends, respectively. The H4 (GGKGLGKGGAKRHRK), H3 (ARTKQTARKSTGGKA), H2B (SAPAPKKGSKKA VTK), and H2A (GKQGGKTRAKAKTRS) peptide tail sequences were synthesized at the ASU Protein Facility. Recombinant H4, H3, H2A, and H2B proteins were purchased from Millipore.

Capillary Electrophoresis Selection.

All CE separations were performed on a P/ACE 2100 Capillary Electrophoresis system (Beckman Coulter, Inc., Fullerton, CA). Each day 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. For each separation, the capillary was flushed with ultrapure H_2O for 1 min followed by a 3 min rinse with selection buffer (3 mM KCL, 10 mM NaCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, and 5 mM $MgCl_2$). For each selection, the H4 peptide (10 nM) was incubated with 10 µM of the DNA library in selection buffer (final volume, 30 µL) for 30 minutes at room temperature. A portion of this mixture (~10 nL) was injected onto the separation capillary using pressure injection (1 s, 0.1 psi) and 30 kV was applied across the capillary for 2 min. The capillary was equilibrated at 20 ºC for all separations. Laser-induced fluorescence (LIF) was used to monitor the separation. Excitation was generated using the 488 nm line of an Ar+ laser (Beckman Coulter) and emission was collected at 520 nm. Five CE separations were performed for every round of selection to increase the number of sequences in the assay.

PCR Amplification

A PCR master mix consisting of 0.4 mM of each deoxyribonucleotide triphosphate (Sigma, St. Louis, MO), 1 µM forward primer (5'-FAM/GGCGGCGATGAGGATGAC-3'), 1 µM reverse primer (5'-Biotin-GGGCAGTCACGCAGTGGT-3'), and 1x thermpol buffer was prepared. The PCR master mix solution (94 94 µL), CE fraction containing the bound DNA (6 µL), and Taq DNA polymerase (1 µL, 2 units) were added to a thin-walled PCR tube. Functional DNA aptamers were amplified using the polymerase chain reaction (PCR). The double-stranded DNA product was made single-stranded by incubating the mixture with streptavidin-agarose beads and denaturing the duplex with 0.15 M NaOH. The sense strand was collected in the flow-through, neutralized, and used as input for the next round of selection. After 4 rounds of selection, the PCR product was cloned into a Topo TA plasmid (Invitrogen, CA), transformed into *Escherichia coli* Top10 cells and sequenced at the ASU Sequencing Facility.

Dissociation Constants (K_d) of the Aptamers

Affinity capillary electrophoresis (ACE) was used to obtain binding affinities (K_d) for the selected aptamers. As described previously,[S1] each aptamer was mixed with increasing concentrations of peptide in selection buffer and incubated at room temperature for up to 2 hours. Samples were injected onto the column and the peak height of the free DNA was used to estimate the K_d using the following equation:

$$\frac{I_0 - I}{I_0} = \frac{cons\tan t}{K_d + [t\arg et]}$$

where I_o is the height of the free DNA in the absence of target and I is the height at a given concentration of target. K_d values were calculated using a Mechalis-Menton fit in Kaleidagraph.

Atomic Force Microscopy.

The AFM probe was functionalized with a MAL-dPEG™12 NHS linker (Quanta Biodesign, Ltd, Powell, OH), containing a maleimide at the PEG terminus to react with the thiol modified aptamer.[S2] This procedure is described in detail in reference S3. Briefly, UV-cleaned MAC-mode cantilever tips are incubated in a desiccator for up to 2 hours in a solution containing 30 µL of aminopropyltriethoxysilane (APTES) (99%; Sigma–Aldrich) and 10 µL of *N*, *N*-diisopropylethylamine (99%, distilled; Sigma–Aldrich). Next, the amine modified tips are incubated for a second 2 hour period with the PEG crosslinker in a $CHCl_3$ solution (1 mL) containing of 5 µL of triethylamine. The PEG-modified tips are then attached to the thiol-modified aptamer by incubating the aptamer (50 µL) for 1 h with 25 µL of NH_2OH reagent (500 mM $NH_2OH{\cdot}HCl$, 25 mM EDTA, pH 7.5), and 50 µL of buffer (150 mM NaCl, 5 mM Na_2HPO_4, 10 mM EDTA, pH 7.5). Tips were washed three times with buffer and stored in PBS buffer at 4° C. Separately, the histone protein was deposited on the glutaraldehyde-aminopropyltriethoxysilane (GD-APTES) modified mica, as described in (S4). The histone protein (70 µL of a 1 µM solution in PBS) was left on the treated mica surface for 4 hours. The mica was scanned immediately in PBS (pH 7.5) with 1 M NaCl after rinsing several times. AFM MAC mode was used with PicoTREC for recognition image (PicoPlus with picoTREC from MI, Tempe, AZ). Images were recorded at a speed of ~1.6 microns per second with oscillation amplitude of ~6nm.

Table S1. DNA aptamer sequences selected to bind histone H4.[a]

clone	Sequences (5'-3')
1	TCGGGGGGTCTTTTATAAGGGGTGATAAATGCGGGTCCGAAGCCCTAT
2	CGAGCCAGGCGGATAGCAGTTGAATAAGCGTGGGGGCGAACGAGAAC
3	GGACGGTAGAACTATTGTATCACAAAGAAAGGCCGCTAGGAGAGTCTG
4	GCATTAAGGTGTGGCCACTATGGAGATTATTGTGCATGGACTTCTAAG
5	GGCTCCTTAGGCTATGTGTGGTAGTTCTGTGGCGGTGCAAAAGGGAAT
6	CACCAGGGATATGCGGAGCCTGGGGGGGGCTTGGGTATGGGGCCTTAT
7	TGGCTGGGGATGGCCTAAGAGGTCGTGAGAGTGCTCTATGGGCGCAGG
8	TTCGTCTAGGGGTGTTAAAGAGTTAGCGGGAATACGGGTTCGTCACTG
9	TTCACTGAATAGCGGTGGGAGAGTTAATGTAGACGCTGGTTTACGCAG
10	GGAGGGGTCCACCGGGGTGGGGACCTTGAAGGGCAGCGGAGACTGTGG
11	AATTACTTGGGACTAAGCTGAGTGATTTACTTAATGGACCCGTTTTCC
12	GAGGATCCCAGTTGGGATTGATAGCAGATTTATGTTATTTATGGGCTA
13	TGGCCCTTGGCGGGGGAAGGTAAGACATAATCCATCCAACAGAACGTG
14	ATANAATGAGAAGGGGGATTTGCTGTTAATTTTTCCGCCAGATATGGT
15	GGCTCGGGGTNGAGCAGGCTACTAATGGGTGATCCTTTTTCGGCTCC
16	AAGGCGGGTCACGTGCAGCGACTGGTATACCGTTTTTGGGGCAAGCCT
17	TATAGCAAGACGGGCTCGGGATCTCTGATCGCAGGAGCGTGAACGTCG
18	GGGAAGCTAGGATGGACATAATCAGTGTAATGGTCGCTTGTGTTCAAG

[a] Sequences 13 and 15 were randomly chosen for the AFM experiments.

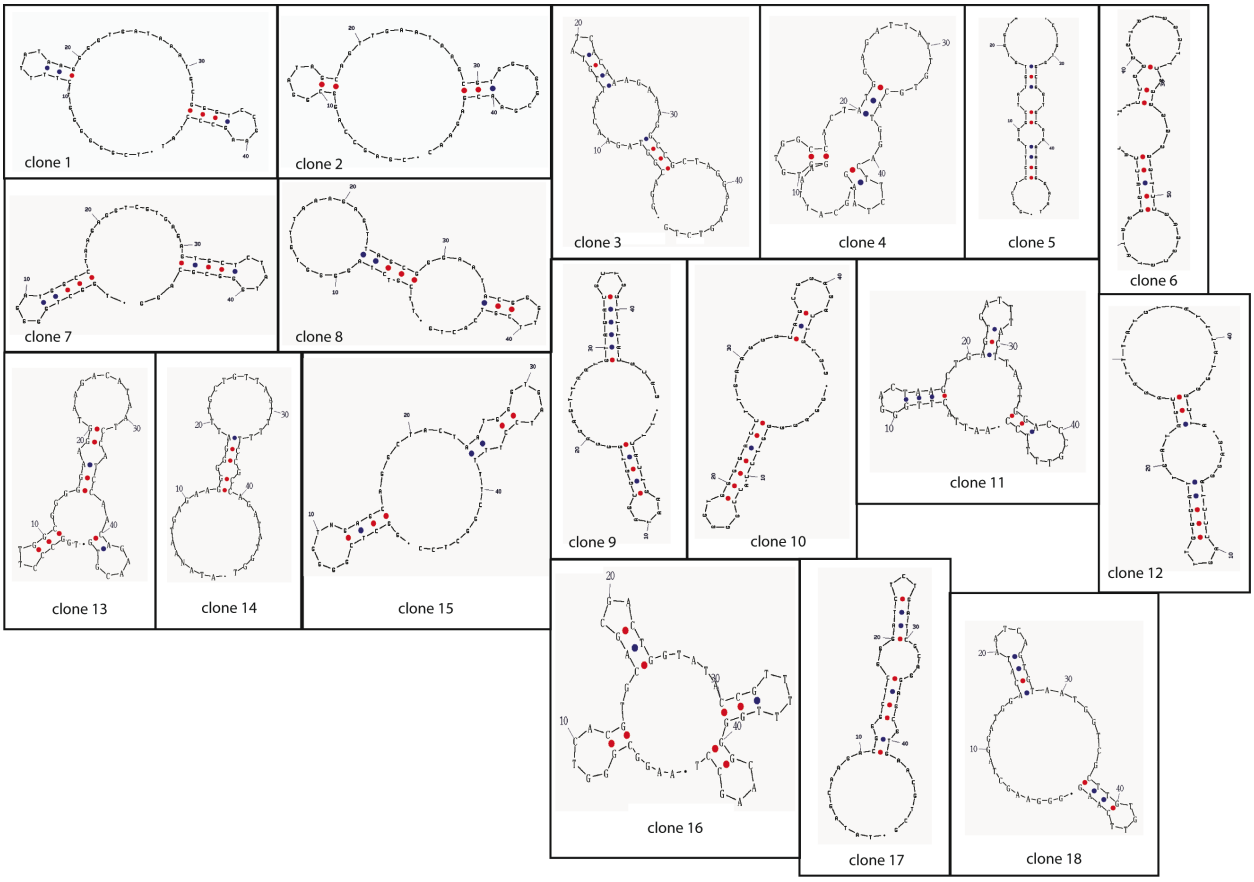

Figure S1. Histone H4 aptamer secondary structures. The secondary structures used in this analysis were computationally generated using the program mFold. All of the structures contain one or more large unpaired regions connected by short stem-loop structures.

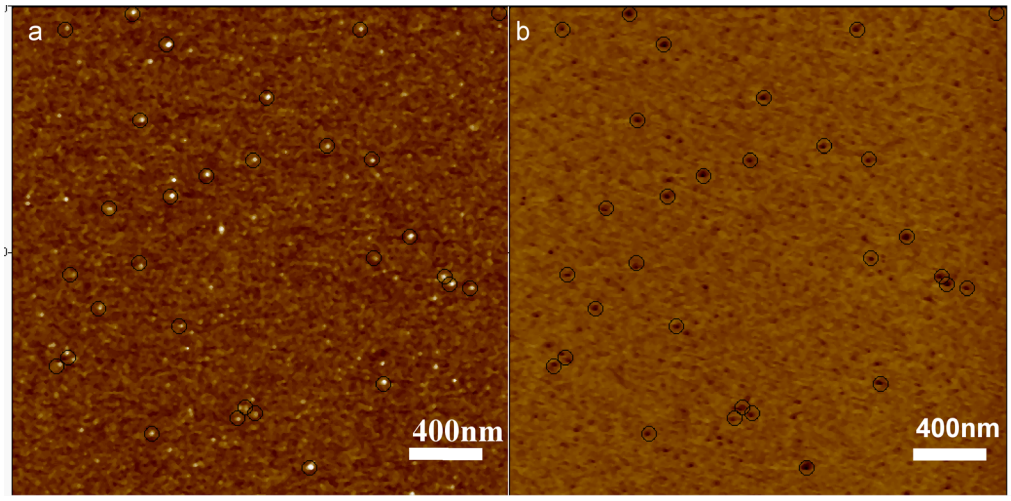

Figure S2. Recognition Imaging of Histone H3 by the anti-H4 aptamer 4.13. Topographic and recognition images of histone proteins imaged with the aptamer modified AFM tip. On the left (a) is the topographic image and on the right (b) is the corresponding recognition image. They are superimposed electronically (marked as black circle). In this image pair, 30 histone H3 proteins are recognized out of 58.

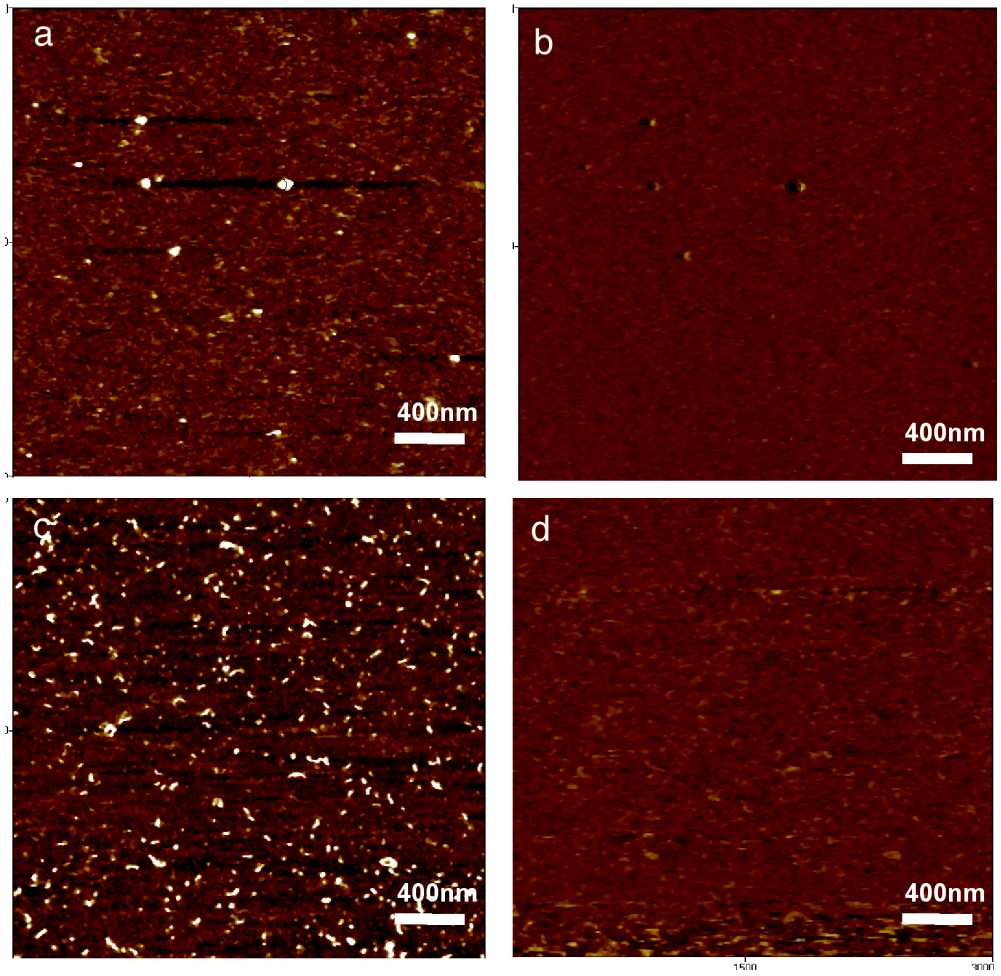

Figure S3. Recognition Imaging of Histone H2A and H2B by the anti-H4 aptamer 4.13. Topographic and recognition images of histone proteins imaged with the aptamer modified AFM tip. On the left (a, c) are topographic images and on the right (b, d) are the corresponding simultaneously acquired recognition images. In the recognition pair seen in panels a and b, only one histone H2B molecule is detected by the aptamer. In the recognition pair seen in panels c and d, no recognition of histone H2A occurs.

References:

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