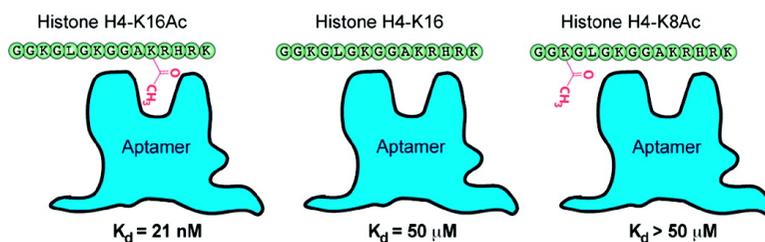


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Evolution of a Histone H4-K16 Acetyl-Specific DNA Aptamer

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The development of high quality affinity reagents to human proteins represents a major challenge in basic and applied biomedicine. Many large-scale biological assays rely on the use of antibodies to interrogate the nature and function of the human proteome.¹ Unfortunately, only a small portion of human proteins have antibodies that are available for use in routine molecular and cellular biology assays.² Even less common are antibodies with high affinity and specificity to specific post-translational modifications (PTMs), and caution is often urged when using antibodies to detect modified proteins in biological samples.^{2,3} This shortfall has created a tremendous need for new molecular tools that maintain many of the recognition properties of antibodies but overcome or avoid some of their limitations.⁴

Aptamers, pieces of single-stranded DNA or RNA that fold into three-dimensional structures with binding sites that are complementary in shape and charge to target antigens, have received much attention as possible alternatives to traditional antibodies.⁵ Because these molecules can be produced *in vitro* by test tube evolution methods, their recognition and binding properties can be tailored to specific target antigens. Indeed, aptamers have now been created to bind virtually any target including ions, small molecules, drugs, peptides, proteins, and even whole cells.⁶ Despite these advances, very few aptamers have been identified that bind specific protein PTMs. In fact, only one literature-reported aptamer exists that binds a PTM, and this aptamer shows only a 10-fold preference against the unmodified target.^{7,8}

In this report, we address the question of whether aptamers can be created that bind subtle PTMs and distinguish their site of occurrence in a protein sequence. We chose histone H4 acetylated at lysine 16 (H4-K16Ac) as our target due to the importance of this modification in regulating gene activation and silencing.⁹ Because the K4-H16Ac modification is located on the N-terminal tail, which is a region of the protein that remains unfolded and accessible to chromatin modifying enzymes when assembled into nucleosomes, we used a 15-mer peptide containing residues Gly6 to Lys20 to represent this portion of the protein.¹⁰ The use of synthetic peptides as target molecules helped to simplify the selection, as pure histone H4-K16Ac protein is not readily available.

The initial library contained $\sim 10^{14}$ distinct single-stranded DNA molecules with 48 random nucleotide positions flanked on both sides with constant primer-binding sites for PCR. The selection strategy (Figure 1) involved a negative selection step to remove molecules that bind to the unmodified histone H4 (H4-K16) tail sequence, followed by a positive selection step to enrich for molecules that bind to the desired H4-K16Ac target. For each round of selection, the single-stranded DNA pool was passed through an affinity matrix displaying the H4 peptide sequence to selectively

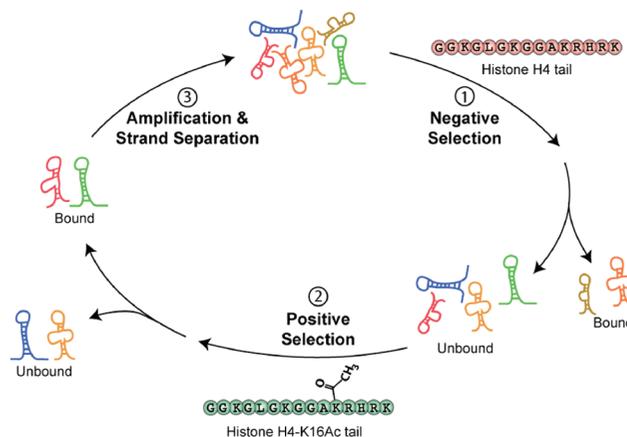


Figure 1. Selection strategy used to identify H4-K16Ac-specific aptamers. The selection included a negative followed by a positive selection step to ensure high specificity binding.

remove molecules that bind to the nonacetylated H4 tail. Those molecules that remained in the pool were incubated with the H4-K16Ac peptide, and functional aptamers were separated from the DNA pool by injecting the mixture onto a neutral coated capillary. Five injections were made per round of selection, and $\sim 10^{11}$ unique DNA sequences were sampled in the starting pool.

We chose the capillary electrophoresis (CE) protocol for the positive selection step because this technique enables solution-based separation of bound aptamers from the unbound pool.¹¹ We felt that this approach would help reduce the occurrence of nonspecific binders, which is sometimes a problem with traditional bead-based selections. CE-based selections have the added benefit of ultrahigh partitioning, which enables the discovery of high affinity aptamers in a minimum number of selection rounds.¹¹ Electrophoresis was performed using an electric field of 30 kV in a 57 cm long capillary with an inner diameter of 50 μm . Under these conditions, the unbound DNA migrated faster than the DNA-peptide complex, which enabled us to collect the bound DNA in a separate vial by applying pressure to the column after the unbound DNA passed into a waste vial. The DNA from each round of selection was amplified by PCR and made single-stranded by denaturing the DNA product on streptavidin-coated agarose beads. After four rounds of *in vitro* selection and amplification, a second peak became visible in the CE chromatogram (Supporting Information, SI), indicating that the pool had become enriched in molecules with high affinity to the H4-K16Ac histone tail.

DNA molecules obtained from round 4 of the selection were cloned and sequenced. Analysis of these sequences (SI) revealed no one dominant sequence or class of related sequences, but rather a large number of unrelated sequences. We and others have reported similar results,¹¹ which suggests that the high partition coefficient and minimum number of selection rounds associated with CE-

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SELEX help facilitate the discovery of many high affinity aptamers. To categorize the different aptamers, all of the sequences were organized into different groups (SI) based on the complexity of their predicted secondary structure. mFold was used to identify the lowest energy motif for each aptamer. Group I contained aptamers whose structures were dominated by a single stem-loop. Group II contained aptamers with an internal bulge-loop. Group III consisted of aptamers with multiple stem-loop structures. From this set, we chose clones 4.9, 4.3, and 4.20 to represent aptamers with simple, intermediate, and complex secondary structures (Figure 2), respectively.

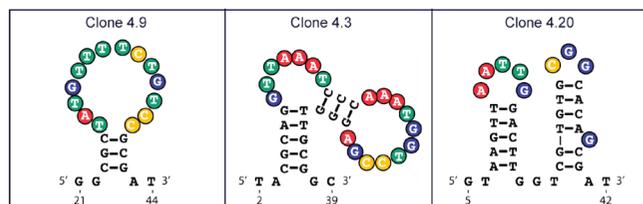


Figure 2. Predicted secondary structure of H4-K16Ac binding aptamers. Clone 4.9, 4.3, and 4.20 represent aptamers with simple, intermediate, and complex secondary structures, respectively.

We screened these three aptamers for binding using affinity capillary electrophoresis (ACE) to measure the dissociation constant (K_d) of each aptamer to the H4-K16Ac target. Clone 4.20 had the highest binding affinity with a K_d of 47 ± 24 nM, while clones 4.9 and 4.3 bind the target with K_d 's of 83 ± 75 and 140 ± 30 nM, respectively (SI). As a validation method, we examined the affinity and specificity of clone 4.20 using a more sensitive Biacore T-100 surface plasmon resonance (SPR) instrument. A biotinylated version of clone 4.20 was immobilized onto a streptavidin-coated biosensor chip, and the binding response to each target was measured at different concentrations. The maximum binding response taken from each sensogram was plotted versus peptide concentration, and the data were fit to a standard hyperbolic curve (SI).

Based on the notion that aptamers with more elaborate secondary structures contain more information content than aptamers with simpler structural motifs,¹² we chose clone 4.20 to examine the specificity for the H4-K16Ac tail sequence. The binding affinity constant of clone 4.20 to H4-K16Ac was compared with the affinity of this aptamer to the unmodified variant H4-K16. Analysis of the affinity plots revealed that clone 4.20 binds the H4-K16Ac target with a K_d of 21 nM and discriminates against the unmodified H4-K16 tail by a dramatic 2400-fold (Table 1), corresponding to a $\Delta\Delta G$ of binding of 4.6 kcal/mol. To examine whether clone 4.20 is specific to the acetyl modification at position 16 in the tail, a second binding assay was performed using a peptide with an acetyl group at lysine position 8 (H4-K8Ac). SPR analysis showed that clone 4.20 is >2400-fold more selective against an acetylated lysine residue at position 8 in the H4 tail sequence, thereby demonstrating that this aptamer is both modification and location specific.

For comparison purposes, the same binding assays were performed using a standard chip quality antibody raised to bind the

Table 1. Dissociation Constants and Specificity Values of Aptamer 4.20 and H4-K16Ac Antibody by SPR

peptide	clone 4.20		H4-K16Ac antibody	
	K_d (nM)	specificity ^a	K_d (nM)	specificity ^a
H4-K16Ac	21 ± 11	-	5.9 ± 3.5	-
H4	$50\,000^b$	2400	87 ± 25	15
H4-K8Ac	$>50\,000^b$	>2400	94 ± 32	16

^a Specificity is defined as K_d (off-target)/ K_d (on-target). ^b Experimental error $\sim 10\%$.

H4-K16Ac target. This antibody, which is advertised as a highly specific affinity reagent, binds to the desired H4-K16Ac target with a K_d of 6 nM but shows only 15- and 16-fold specificity against the H4-K16 and H4-K8Ac off-target sequences (Table 1). This result highlights a common problem among many commercial antibodies, which is their limited ability to distinguish close PTMs in biological assays.¹³

In summary, we describe the creation of a highly specific DNA aptamer to an important histone PTM. The generality of this approach coupled with the low number of selection steps provides a facile method for generating high quality protein affinity reagents. We suggest that molecules with similar properties could be made that bind a wide range of PTMs with high affinity and specificity.

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Supporting Information Available: Experimental information. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) (a) Hober, S.; Uhlen, M. *Curr. Opin. Biotechnol.* **2008**, *19*, 30–35. (b) Uhlen, M. *Mol. Cell. Proteomics* **2007**, *6*, 1455–1456.
- (2) Blow, N. *Nature (London)* **2007**, *447*, 741–744.
- (3) Kazanekci, C. C.; Kowalski, A. J.; Ding, T.; Rittling, S. R.; Denhardt, D. T. *J. Cell. Biochem.* **2007**, *102*, 925–935.
- (4) Taussig, M. J. *Nat. Methods* **2007**, *4*, 13–17.
- (5) (a) Ellington, A. D.; Szostak, J. W. *Nature (London)* **1990**, *346*, 818–822. (b) Tuerk, C.; Gold, L. *Science* **1990**, *249*, 505–510.
- (6) (a) Wilson, D. S.; Szostak, J. W. *Annu. Rev. Biochem.* **1999**, *68*, 611–647. (b) Nimjee, S. M.; Rusconi, C. P.; Sullenger, B. A. *Ann. Rev. Med.* **2005**, *56*, 555–583. (c) Bunka, D. H. J.; Stockley, P. G. *Nat. Reviews* **2006**, *4*, 588–596.
- (7) Gilbert, B. A.; Sha, M.; Wathen, S. T.; Rando, R. R. *Bioorg. Med. Chem.* **1997**, *5*, 1115–1122.
- (8) Aptamers have been selected that block sites where PTMs occur. For example: Vaish, N. K. *Nat. Biotechnol.* **2002**, *20*, 810–815.
- (9) Shogren-Knaak, M. A.; Ishii, H.; Sun, J.; Pazin, M. J.; Davie, J. R.; Peterson, C. L. *Science* **2006**, *311*, 844–847.
- (10) Luger, K.; Mäder, A. W.; Richmond, R. K.; Sargent, D. F.; Richmond, T. J. *Nature (London)* **1997**, *389*, 251–260.
- (11) (a) Mendonsa, S. D.; Bowser, M. T. *J. Am. Chem. Soc.* **2004**, *126*, 20–21. (b) Mosing, R. K.; Mendonsa, S. D.; Bowser, M. T. *Anal. Chem.* **2005**, *77*, 6107–6112. (c) Mendonsa, S. D.; Bowser, M. T. *J. Am. Chem. Soc.* **2005**, *127*, 9382–9282. (d) Lin, L.; Hom, D.; Lindsay, S. M.; Chaput, J. C. *J. Am. Chem. Soc.* **2007**, *129*, 14568–14569.
- (12) Carothers, J. M.; Oestreich, S. C.; Szostak, J. W. *J. Am. Chem. Soc.* **2006**, *128*, 7929–7937.
- (13) Zichi, D.; Eaton, B.; Singer, B.; Gold, L. *Curr. Opin. Chem. Biol.* **2008**, *12*, 78–85.

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