Highly Selective Cyclic Peptide Ligands for NeutrAvidin and Avidin Identified by Phage Display

Supplementary Material

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Contents:

Figure S1: Sequence of the peptide library.

Di-cysteine constrained cyclic peptide library:

```
gtg  aaaaaattattattcgcaattccttta
 V(M) K  K  L  L  F  A  I  P  L
gttgttcctttctatgcggcccagccggccatgggttgcnnsnnsnnsnnsnnsnnstgc
 V  V  P  F  Y  A  A  Q  P  A  M  G  C  X  X  X  X  X  X  C
ggtggaggcggtgctgcagcgcttactgatactcttcaagctgaaactgatcaacttgaa
 G  G  G  G  A  A  A  L  T  D  T  L  Q  A  E  T  D  Q  L  E
gatgaaaaaagtgctcttcaaactgaaattgctaatcttcttaaagaaaaagaaaaactt
 D  E  K  S  A  L  Q  T  E  I  A  N  L  L  K  E  K  E  K  L
gaatttattcttgctggtgcggccgcaggtgcgccggtgccgtatccggatccgctggaa
 E  F  I  L  A  G  A  A  A  G  A  P  V  P  Y  P  D  P  L  E
ccgcgtgccgcatagactgttgaaagttgtttagcaaaacctcatacagaaaattcattt
 P  R  A  A  -  T  V  E  S  C  L  A  K  P  H  T  E  N  S  F
```

Gene III signaling sequence
Library
Peptide linker
E-TAG
Gene III protein

Figure S2: HABA saturation of NeutrAvidin.

Binding of HABA to NeutrAvidin was determined similarly to the method used by Green (1). Increasing concentrations of HABA were titrated into a fixed concentration of NeutrAvidin (45μM) in phosphate-buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH = 7.4), and the absorbance of each sample was taken at 500 nm on a Beckman DU 520 UV/Vis spectrophotometer. Readings at the appropriate concentrations of HABA were used as background and were subtracted from the raw HABA/NeutrAvidin values. Absorbance caused by non-specific interactions of HABA with NeutrAvidin were measured by addition of an excess (1 mM) of biotin. These values were indistinguishable from the background HABA readings.

As expected, we found that NeutrAvidin binds HABA with a corresponding increase in absorbance at 500 nm (Figure s1). The binding isotherms from the HABA saturation studies were fit as a rectangular hyperbola using,

$$[LR] = \frac{[R_t][L]}{K_d + [L]} \qquad \text{(s1)}$$

where [LR] is the concentration of the HABA-biotin binding protein complex, $[R_t]$ is the total concentration of receptor sites and [L] is free ligand concentration. Non-linear regression analysis produced a dissociation constant of 15 μM for the HABA/NeutrAvidin complex in PBS.

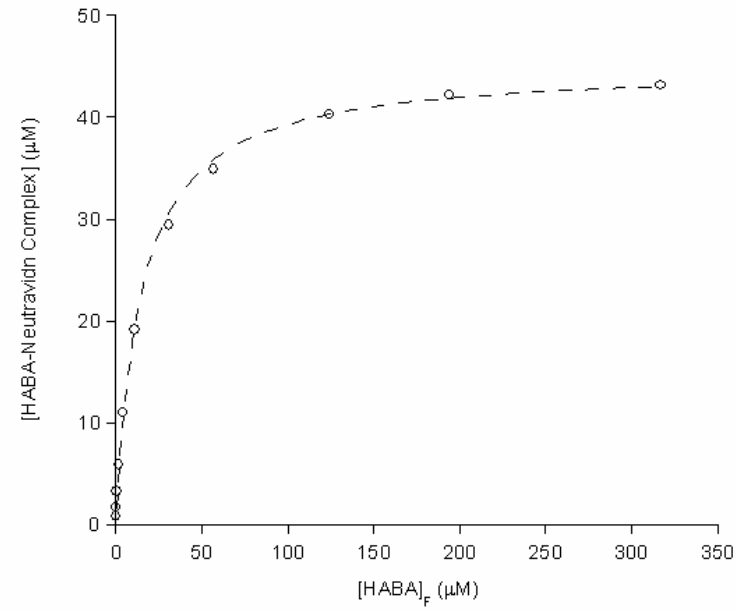

Figure S2: Binding isotherm of HABA to NeutrAvidin.
The dotted line is the best fit to equation s1.

Table S1: Sequences from the cyclic peptide phage display against NeutrAvidin.

Round 3 Sequences																
BSR31:	Met	G	C	D	R	A	T	P	Y	C	G	G	G	G	A	A	A
BSR32:	Met	G	C	D	R	A	S	P	Y	C	G	G	G	G	A	A	A
BSR33:	Met	G	C	D	L	A	S	P	W	C	G	G	G	G	A	A	A
BSR34:	Met	G	C	D	L	A	S	P	W	C	G	G	G	G	A	A	A
BSR35:	Met	G	C	D	R	A	S	P	W	C	G	G	G	G	A	A	A
BSR36:	Met	G	C	D	L	A	S	P	W	C	G	G	G	G	A	A	A
BSR38:	Met	G	C	D	R	S	T	P	Y	C	G	G	G	G	A	A	A
BSR39:	Met	G	C	D	L	A	S	P	W	C	G	G	G	G	A	A	A
BSR310:	Met	G	C	D	R	A	S	P	W	C	G	G	G	G	A	A	A
BSR311:	Met	G	C	D	R	A	T	P	Y	C	G	G	G	G	A	A	A
BSR312:	Met	G	C	D	R	A	S	P	W	C	G	G	G	G	A	A	A
BSR313:	Met	G	C	D	R	A	S	P	Y	C	G	G	G	G	A	A	A
BSR314:	Met	G	C	D	R	A	S	P	Y	C	G	G	G	G	A	A	A
BSR315:	Met	G	C	D	R	A	S	P	Y	C	G	G	G	G	A	A	A
BSR316:	Met	G	C	D	R	A	T	P	Y	C	G	G	G	G	A	A	A
Round 4 Sequences																
BSR41:	Met	G	C	D	R	A	S	P	Y	C	G	G	G	G	A	A	A
BSR42:	Met	G	C	D	R	A	S	P	Y	C	G	G	G	G	A	A	A
BSR43:	Met	G	C	D	R	A	S	P	Y	C	G	G	G	G	A	A	A
BSR44:	Met	G	C	D	R	A	T	P	Y	C	G	G	G	G	A	A	A
BSR45:	Met	G	C	D	R	A	S	P	W	C	G	G	G	G	A	A	A
BSR46:	Met	G	C	D	R	A	S	P	Y	C	G	G	G	G	A	A	A
BSR47:	Met	G	C	D	L	S	T	P	Y	C	G	G	G	G	A	A	A
BSR48:	Met	G	C	D	L	S	T	P	Y	C	G	G	G	G	A	A	A
BSR49:	Met	G	C	D	L	A	S	P	W	C	G	G	G	G	A	A	A
BSR410:	Met	G	C	D	R	A	T	P	Y	C	G	G	G	G	A	A	A
BSR411:	Met	G	C	D	R	A	S	P	Y	C	G	G	G	G	A	A	A
BSR412:	Met	G	C	D	R	A	S	P	Y	C	G	G	G	G	A	A	A
BSR413:	Met	G	C	D	L	A	S	P	W	C	G	G	G	G	A	A	A
BSR414:	Met	G	C	D	R	A	T	P	Y	C	G	G	G	G	A	A	A
BSR415:	Met	G	C	D	R	A	T	P	Y	C	G	G	G	G	A	A	A
BSR417:	Met	G	C	D	R	A	T	P	Y	C	G	G	G	G	A	A	A
BSR418:	Met	G	C	D	R	A	S	P	W	C	G	G	G	G	A	A	A
BSR419:	Met	G	C	D	R	A	S	P	Y	C	G	G	G	G	A	A	A
BSR420:	Met	G	C	D	L	A	S	P	W	C	G	G	G	G	A	A	A
BSR421:	Met	G	C	D	R	A	T	P	Y	C	G	G	G	G	A	A	A
BSR422:	Met	G	C	D	R	A	S	P	Y	C	G	G	G	G	A	A	A
BSR423:	Met	G	C	D	R	A	S	P	Y	C	G	G	G	G	A	A	A
BSR424:	Met	G	C	D	R	A	T	P	Y	C	G	G	G	G	A	A	A
BSR425:	Met	G	C	D	R	A	S	P	Y	C	G	G	G	G	A	A	A
BSR426:	Met	G	C	D	R	A	S	P	Y	C	G	G	G	G	A	A	A
BSR427:	Met	G	C	D	R	A	S	P	Y	C	G	G	G	G	A	A	A
Round 5 Sequences																
BSR51:	Met	G	C	D	L	A	S	P	W	C	G	G	G	G	A	A	A
BSR52:	Met	G	C	D	R	A	S	P	Y	C	G	G	G	G	A	A	A
BSR53:	Met	G	C	D	R	A	S	P	Y	C	G	G	G	G	A	A	A
BSR54:	Met	G	C	D	R	A	S	P	Y	C	G	G	G	G	A	A	A
BSR55:	Met	G	C	D	R	A	S	P	Y	C	G	G	G	G	A	A	A

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BSR57:    Met G C D L A S P W C G G G G A A A
BSR58:    Met G C D R A S P Y C G G G G A A A
BSR59:    Met G C D R A S P Y C G G G G A A A
BSR510:   Met G C D R A S P Y C G G G G A A A
BSR511:   Met G C D R A S P Y C G G G G A A A
BSR512:   Met G C D L A S P W C G G G G A A A
BSR513:   Met G C D R A S P Y C G G G G A A A
BSR514:   Met G C D P A S P Y C G G G G A A A
BSR516:   Met G C D R A A P W C G G G G A A A
BSR516b:  Met G C D R A T P Y C G G G G A A A
BSR517:   Met G C D R A S P Y C G G G G A A A
BSR518:   Met G C D R A S P Y C G G G G A A A
BSR520:   Met G C D R A S P W C G G G G A A A
BSR521:   Met G C D R A S P Y C G G G G A A A
BSR522:   Met G C D L A S P W C G G G G A A A
BSR523:   Met G C D R A S P W C G G G G A A A
BSR524:   Met G C D W S A P Y C G G G G A A A
BSR525:   Met G C D L A S P W C G G G G A A A
BSR526:   Met G C D R A S P Y C G G G G A A A
BSR528:   Met G C D L A S P W C G G G G A A A
BSR529:   Met G C D R A T P Y C G G G G A A A
BSR530:   Met G C D R A S P Y C G G G G A A A
BSR531:   Met G C D R A T P Y C G G G G A A A
BSR532:   Met G C D R A S P Y C G G G G A A A
BSR533:   Met G C D R A T P Y C G G G G A A A
BSR534:   Met G C D R A S P Y C G G G G A A A
BSR535:   Met G C D F A S P W C G G G G A A A
BSR536:   Met G C D F A S P W C G G G G A A A
BSR537:   Met G C D R A S P Y C G G G G A A A
BSR538:   Met G C D R A S P Y C G G G G A A A
BSR541:   Met G C D R A S P Y C G G G G A A A
BSR542:   Met G C D L A S P W C G G G G A A A
BSR543:   Met G C D L S T P Y C G G G G A A A
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1. Green, N.M. (1965) A spectrophotometric assay for avidin and biotin based on binding of dyes by avidin. Biochem J 94: 23C-24C.