Supporting Information

High Affinity Aptamer for the Detection of the Biogenic Amine Histamine

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List of contents

S1 Materials

Histamine and tryptamine were purchased from Acros Organics. Tyramine, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), N-hydroxysuccinimide (NHS), phosphate-buffered saline pH 7.4, Trizma-HCl, goat anti-rabbit-HRP antibody conjugate, skimmed milk powder, TMB substrate for ELISA and 11-mercapto-1-undecanoic acid (MUA) were provided by Sigma Aldrich (Spain). Polyclonal antibody against histamine was purchased from Abcam (Spain). Streptavidin-polyHRP conjugate from SDT, PCR reagents, Dynabeads M-270 Carboxylic Acid and Dynabeads M-270 Amine magnetic beads and maleimide-activated plates were obtained from Fisher Scientific (Spain). The SiMAG-Carboxyl magnetic beads were obtained from Chemicell GmbH (Germany) and the DNA purification kits (Gel DNA recovery Kit and Oligo Clean & Concentrator kit) were from Zymo Research (provided by Ecogen S.R.L., Spain). The DNA library used for the SELEX, the primers and the oligonucleotides were purchased from Biomers (Germany). The Epoxy activated sepharose 6B was purchased from GE Healthcare. Ultra-pure milli-Q water (18.2 MΩ.cm) was used for all experiments.

S2 Histamine immobilization on magnetic beads

Histamine was immobilized on Dynabeads M-270 Carboxylic Acid magnetic beads (2.8 µm diameter) via carbodiimide coupling as described before[1] with few modifications. A 25 mg/ml histamine solution prepared in milli-Q water was used for immobilization and any unreacted carboxylic groups remaining on the magnetic bead surface were blocked using 50 µl of 50 mM Tris pH 7.4 during a 15 minutes incubation at room temperature under tilt rotation. The conjugated magnetic beads were re-suspended in Tris-binding buffer (20 mMTris-HCl, 100 mM NaCl, 2 mM $MgCl_2$, pH 7.4) and the presence of histamine on the surface of the beads was confirmed by a qualitative assay using a polyclonal histamine antibody. Briefly, 3 µl of the conjugated beads (histamine-magnetic beads) were washed three times with 200 µl of PBS (10 mM phosphate, 138 mM NaCl, 2.7 mM KCl, pH 7.4), followed by blocking with 200 µl of 2 % skimmed milk in PBS-Tween (PBS, 0.05 % v/v Tween-20) for 30 minutes at room temperature. After that, the beads were washed four times with PBS-Tween and then 50 µl of anti-histamine antibody (5 µg/ml in PBS) was added to the beads followed by a 30-minute incubation. After another washing step, 50 µl of anti-rabbit-HRP antibody conjugate (1/20,000 dilution in PBS-Tween) was added and allowed to incubate with the beads for 30 minutes. After a final washing step, 50 µl of TMB substrate was added and 5 minutes later the reaction was stopped by the addition of 50 µl of 1 M H_2SO_4. The absorbance was read at 450 nm in a SpectraMax microplate reader (bioNova Scientifics, S.L) and naked beads (carboxylic acid modified magnetic beads without immobilized histamine) were used as control. Similarly, Chemicell SiMAG-Carboxyl magnetic beads (1 µm

diameter) were immobilized with histamine for comparison and the results are shown below in Figure S1. Similar levels of histamine was successfully detected on both types of beads using the histamine polyclonal antibody. Because of the high background signal obtained from the naked Chemicell SiMAG-Carboxyl beads used as negative control, they were excluded.

S3 Histamine immobilization on sepharose resin

Approximately 143 mg of epoxy-activated sepharose 6B resin was added to a microspin chromatography column and was washed three times with water. The column was then centrifuged at 11000 rpm for 1 min to remove any remaining water traces and 500 µl of histamine solution (6 mg/ml in water) were added, followed by overnight incubation at room temperature under mild shaking. The resin was washed again three times with water and centrifuged. Finally, the histamine-sepharose resin was blocked with 50 mM of Tris pH 8 for 2 hours at room temperature under mild shaking. Successful immobilization of histamine on the resin was verified with a qualitative assay using a histamine polyclonal antibody as explained above in Section S2.

S4 Histamine immobilization on maleimide-activated microplate

Initially the plate was washed with 200 µl of PBS, followed by the addition of 50 µl of 100 µM of 11-mercapto-1-undecanoic acid (MUA) in PBS and overnight incubation at 4°C. The plate was washed three times with PBS and the carboxyl groups of the immobilized MUA were activated using an EDC/NHS mixture (10 mg/ml each) in MES buffer for 20 minutes. The plate was washed again and 50 µl of 20 mM histamine in PBS was added and incubated for 1 hour under shaking. Finally, the plate was washed with PBS, blocked with 50 mM Tris pH 7.4 for 15 minutes, washed again with PBS and used directly.

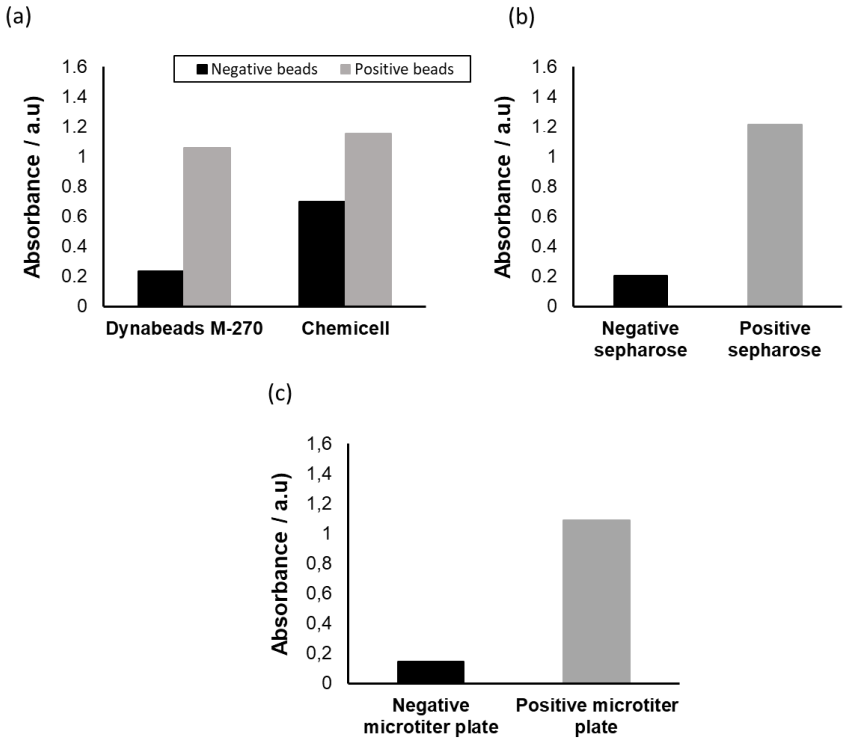


Figure S1 Evaluation of the immobilization level of histamine on different types of matrix: (a) carboxyl-modified magnetic beads; (b) sepharose resin; (c) maleimide microtiter plate.

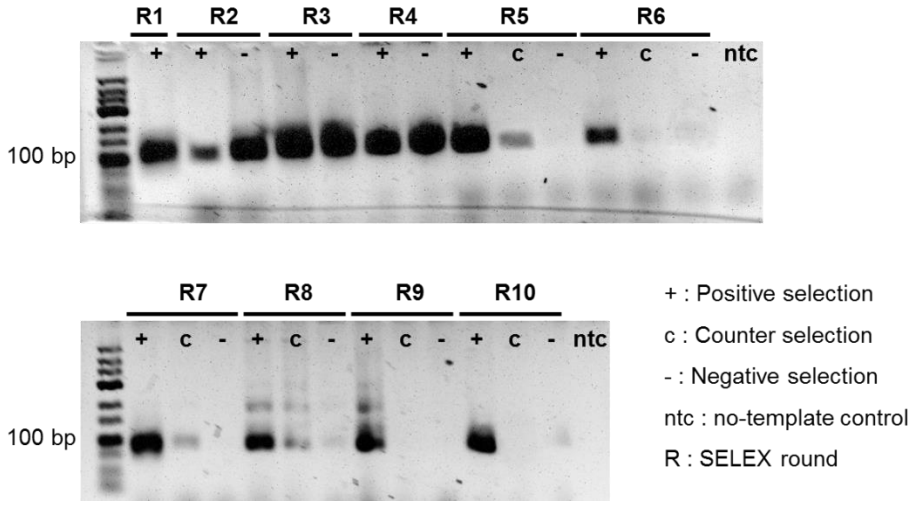


Figure S2 Summary of histamine SELEX. Agarose gels corresponding to the different selection steps performed at each round.

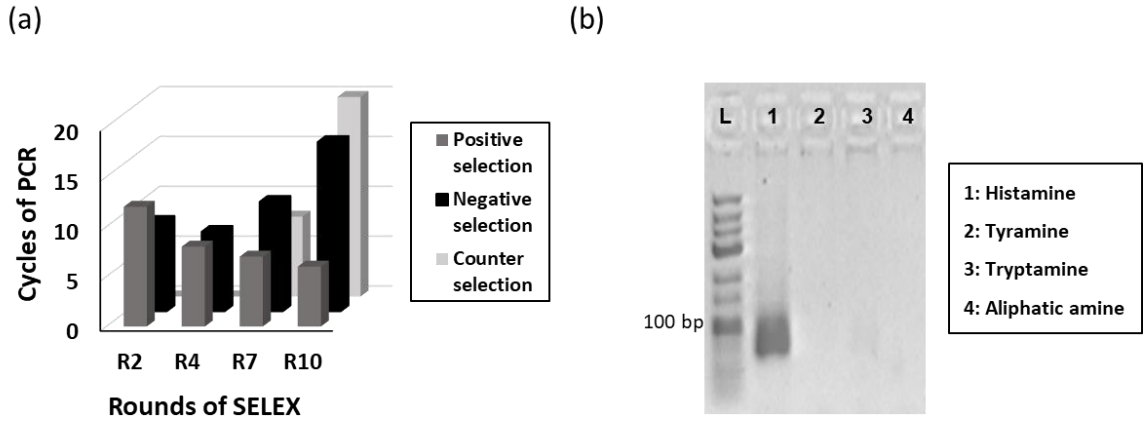
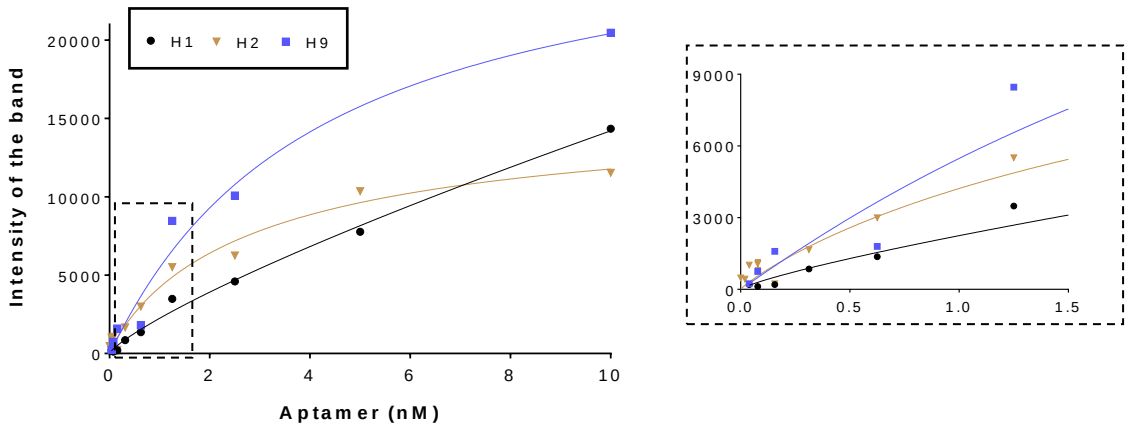


Figure S3 Evolution of the histamine aptamer selection process: (a) PCR cycles required to amplify DNA; (b) Specificity of the last selection round (10th) from histamine SELEX. [R = Round Number of SELEX].



Aptamer	K_D (nM)	R^2
H1	21.51 ± 7.43	0.9911
H2	3.08 ± 1.13	0.9787
H9	4.08 ± 2.21	0.9753

Figure S4 APAA assay for K_D determination of histamine aptamer candidates.

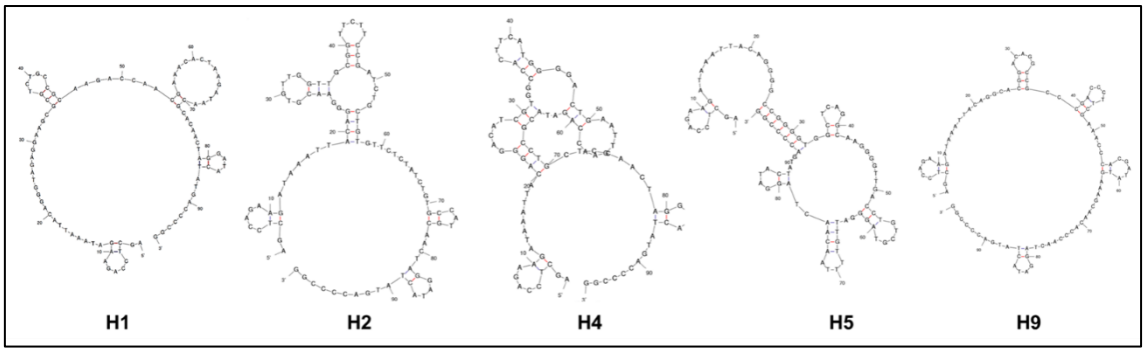
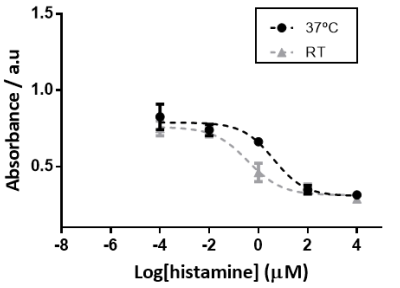


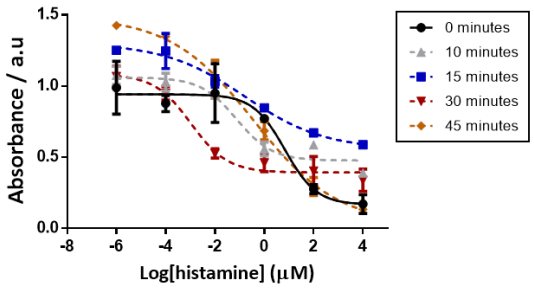
Figure S5 Predicted structures of the aptamer candidates.

(a)



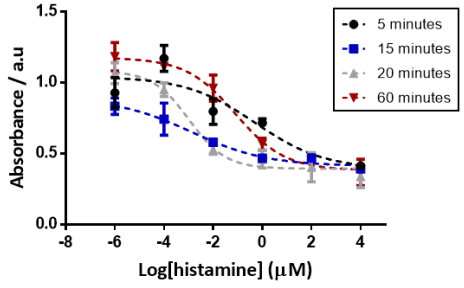
Temperature	LOD (nM)	R square
37°C	435	0.963
22°C	54	0.970

(b)



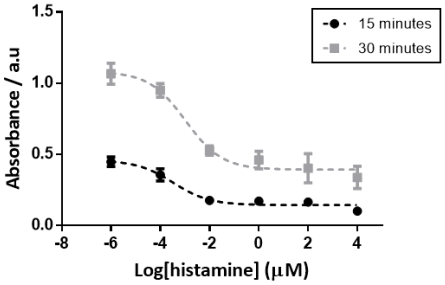
Pre-incubation	LOD (nM)	R square
0 min	740	0.927
10 min	9.422	0.940
15 min	2.091	0.972
30 min	0.193	0.957
45 min	0.255	0.990

(c)



Incubation	LOD (nM)	R square
5 min	1787.369	0.7995
15 min	13.371	0.935
20 min	0.193	0.957
60 min	1.903	0.973

(d)



SA-polyHRP	LOD (nM)	R square
15 min	0.091	0.946
30 min	0.193	0.959

Figure S6 Effect of (a) temperature, (b) pre-incubation time, (c) incubation time and (d) concentration of streptavidin-polyHRP (SA-polyHRP) on the competitive bead assay.

Table S1 Summary of the results obtained from NGS of histamine SELEX.

	Histamine beads	Tryptamine beads
Total sequences	327,645	255,574
Sequences length (bp)	25 - 289	25 – 358
Sequences 85-105 bp (% total)	194,534 (59.4 %)	156,947 (61.4 %)
Top 100 unique sequences (% of 85-105 bp)	0.72 %	0.67 %

Table S2 Histamine aptamer candidates.

Aptamer	Sequence (5' to 3')
H1	AGCTCCAGAAGATAAATTACAGGGTAGAGGAAGCGCGTCTGCCGCAAGAC CAACGAAACACTAAGATAACGCACAACTAGGATACTATGACCCCGG
H2	AGCTCCAGAAGATAAATTACAGGGAACGTGTTGGTTGCGGTTCTTCCGATC TGCTGTGTTCTCTATCTGTGCCATGCAACTAGGATACTATGACCCCGG
H3	AGCTCCAGAAGATAAATTACAGGATACACCAAGGCATACGCAAGCCCCTC CAACGGGCCCATGGCCAGGTCACCAACTAGGATACTATGACCCCGG
H4	AGCTCCAGAAGATAAATTACAGGGACATCGTGGCCACTTCATGGGGACTG AATTGAACCAGATACGCCTGCTCCAACTAGGATACTATGACCCCGG
H5	AGCTCCAGAAGATAAATTACAGGGGCCGGGGTGGCTCAGGCAAGGGGTTG ACCTGTCGTAGGGATTGTTTTAACAACTAGGATACTATGACCCCGG
H9	AGCTCCAGAAGATAAATTACAGGCACGGACAGGCCGCCCGACCCTTCGAA ACCCACGATATGAAAGCAACACCCAACTAGGATACTATGACCCCGG

REFERENCES

(1) Nadal, P.; Pinto, A.; Svobodova, M.; Canela, N.; O'Sullivan, C. K. *PLoS One* **2012**, *7*, e35253.