# Selection and characterization of aptamers to inhibit *in vitro* activity of histone acetyl transferase 1 (HAT1)

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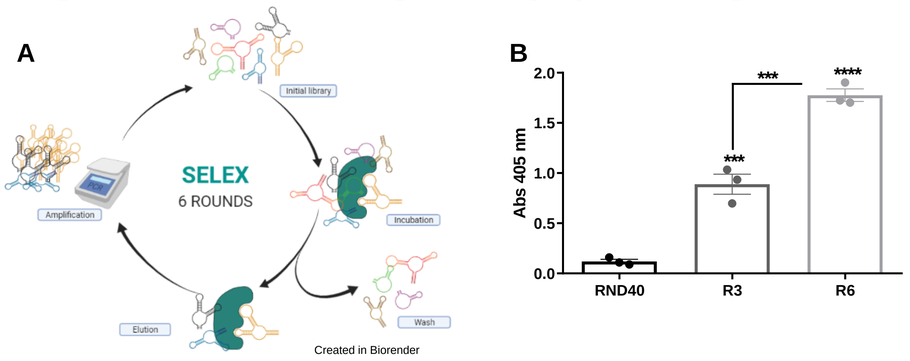
## INTRODUCTION

Histone Acetyltransferase 1 (HAT1) belongs to the HATs family of enzymes. In fact, it was the first to be described within this group, although it is the least known. However, in recent years it has aroused growing interest due to its involvement in multiple pathologies. The overexpression of this enzyme is related to a poor prognosis and survival in cancer patients as well as in viral infections and other pathologies in which inflammation is involved. Thus, many authors propose HAT1 as a potential therapeutic target.

Aptamers are RNA or ssDNA oligonucleotides able to recognize a target molecule with high affinity and specificity because of their tertiary structure. Using the systematic evolution of ligands by exponential enrichment technology (SELEX), DNA aptamers against HAT1 were isolated from a library of ssDNA after 6 rounds of selection. Enrichment was monitored using the enzyme-linked oligonucleotide assay (ELONA). The selected aptamer populations were analysed to obtain specific aptamers in two different ways: Sanger and Next-Generation (NGS) sequencing. Two aptamers named apHAT63 and apHAT610 were optimized by truncating part of their sequences and checking their affinity towards HAT1. The analysis of their secondary structures predicted using mFold and QGRS Mapper software indicated the presence of G-quadruplex structures. Original aptamers and apHAT63T (truncated) were characterized, analysing their inhibitory activity in *in vitro* assays, showing IC50s in the nanomolar range for apHAT63T and apHAT610.

## RESULTS

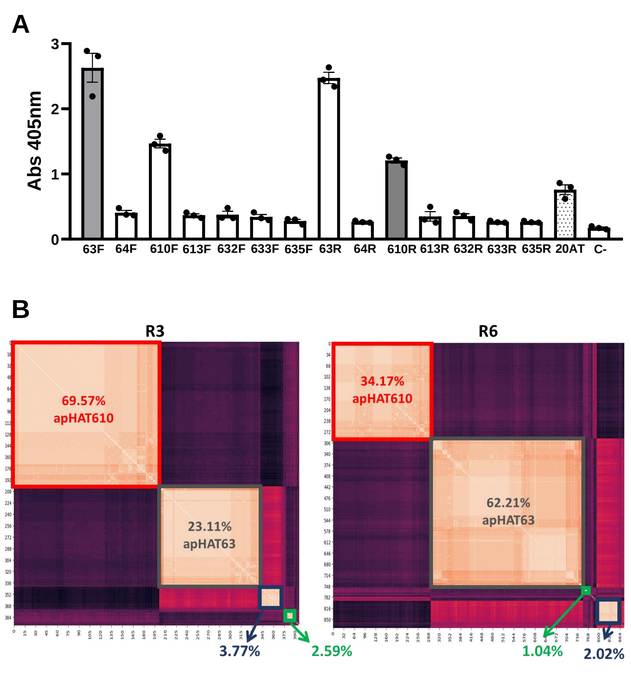
### Figure 1. Selection of high-affinity aptamers against HAT1




(**A**) SELEX-method scheme used in the selection of DNA aptamers against HAT1. We carried out 6 rounds of selection using a NI-NTA resin that binds the purified HAT1 protein fused to the 6xHIS tail in which the protein was incubated with the initial library in physiological conditions.

(**B**) Rounds 3 and 6 (R3, R6) were analyzed by ELONA to check the evolution of the selection. In both rounds analyzed, there are aptamers that recognized HAT1 with high affinity, being statistically significant when compared to the initial population (RND40).

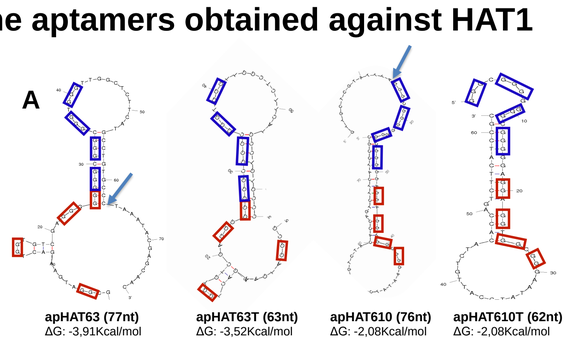
### Figure 2. Analysis of specific aptamers by SANGER and NGS methods




(A) Aptamers isolated from R3 and R6 after cloning, were analyzed by ELONA to study their affinity to HAT1. Aptamers 63 and 610 reached values at least twice the value of the negative control. Both strands of each clone (F and R chains) recognized the target, but we chose 63 F and 610 R chains which showed G-quadruplex (data not shown). 20AT represents an unspecific aptamer.

(B) We performed a Next-Generation Sequencing (NGS) of R3 and R6. The sequences obtained from each round (1658 in R3 and 5775 in R6) were grouped by similarity, resulting in 4 differentiated clusters in both rounds. Two of these groups correspond to the aptamers apHAT63 and apHAT610 obtained by Sanger (A). In the heatmaps, it can be seen the evolution of the populations throughout the SELEX.

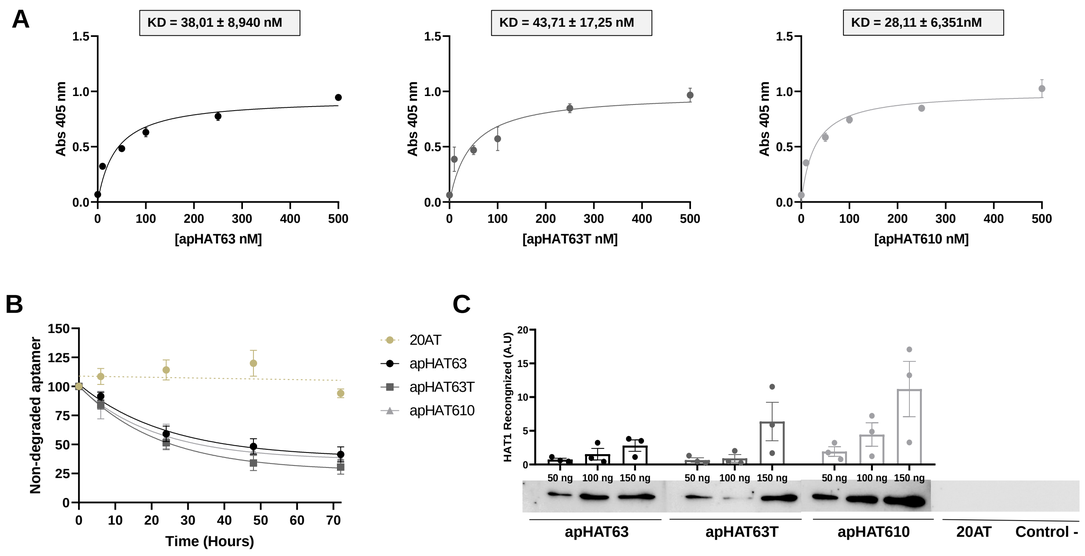
### Figure 3. Optimization of the aptamers obtained against HAT1




(**A**) Secondary structures and G-quadruplex were obtained by mFold and QGRS Mapper programs. Both aptamers and their truncated forms have two G-quadruplex structures. Considering the lower free energy (ΔG), apHAT63 has the more stable structure.

(**B**) The four aptamers were synthesized and tested by ELONA. The two original aptamers and apHAT63T specifically recognize HAT1, and there is statistical significance of this recognition against a cell lysate. ApHAT610T was discarded from the study.

### Figure 4. Characterization of the aptamers obtained against HAT1

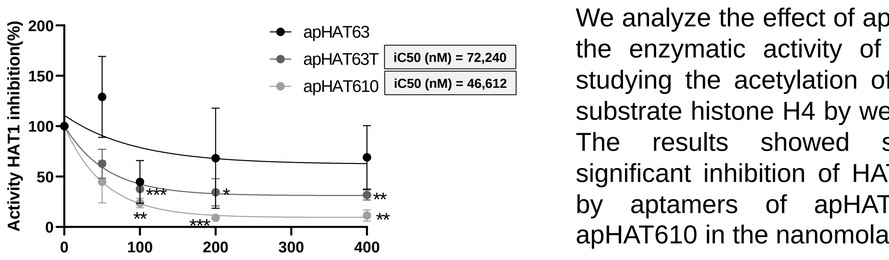



(**A**) ELONA assays were performed in order to obtain the dissociation constants (KD) of the aptamers.

(**B**) Serum resistance of HAT1-aptamers was analyzed by electrophoresis. Results showed that aptamers are highly stable even after 24 hours of treatment.

(**C**) Apta Western-Blot of the aptamers to determine their affinity for HAT1. The most representative image and the graph show that the 3 aptamers have affinity towards the target, and that the affinity of apHAT610 is higher, with concentration-dependent recognition.

### Figure 5. Aptamers inhibit *in vitro* HAT1 activity




We analyze the effect of aptamers on the enzymatic activity of HAT1 by studying the acetylation of its direct substrate histone H4 by western blot. The results showed statistically significant inhibition of HAT1 activity by aptamers of apHAT63T and apHAT610 in the nanomolar range.

## CONCLUSIONS

1. Aptamers apHAT63 and apHAT610 have been the most represented sequences in both Sanger and NGS sequencing. Furthermore, the sequences analysis by NGS shows an evolution of the aptamer population towards 4 unique and differentiated groups that share a high similarity within each cluster.
2. We have selected, optimized, and characterized four DNA aptamers (apHAT63, apHAT610, and their truncated sequences), and three of them recognize HAT1 with high affinity and specificity.
3. Two of these aptamers have shown inhibitory effects in the enzymatic assay of HAT1 activity in the nanomolar range. Therefore, we can conclude that we have developed inhibitors against the potential therapeutic target HAT1.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