Discovery of HIV capsid inhibitors

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Introduction

- ✓ Human inmunodefficiency Virus (HIV) affects around 38 million people in the world[1].
- ✓ Current treatment is a combination of 4 drugs against different viral proteins[2,3].
- ✓ Therapeutic failure due to resistance to multiple drugs has been reported[4,5].
- ✓ HIV capsid protein (CA) correct assembly is crucial for virus replication and infectivity[6].
- ✓ A virtual screening (Atomwise) identified 84 molecules that interact with a conserved region in the interfase between CA monomers.
- ✓ No commercial drugs are available yet against CANC as a therapeutic target[7].

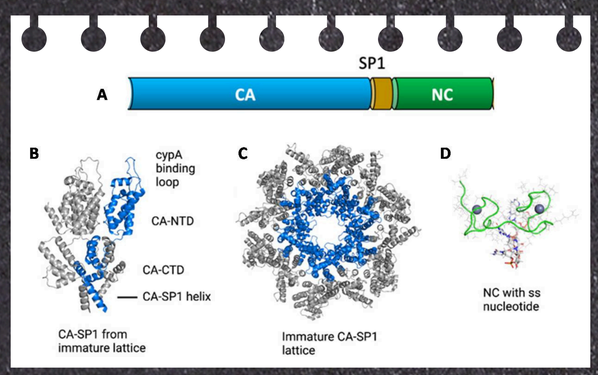


FIGURE 1 (A) CANC fusion protein scheme showing domains in different colors. (B) CASP1 structure side view with CA-NTD and CTD colored in blue (C) Immature CA-SP1 lattice , top view, with single hexamer colored blue (D) NC structure with single-stranded oligonucleotide and zinc ions shown as spheres. Modified from[8].

Objectives

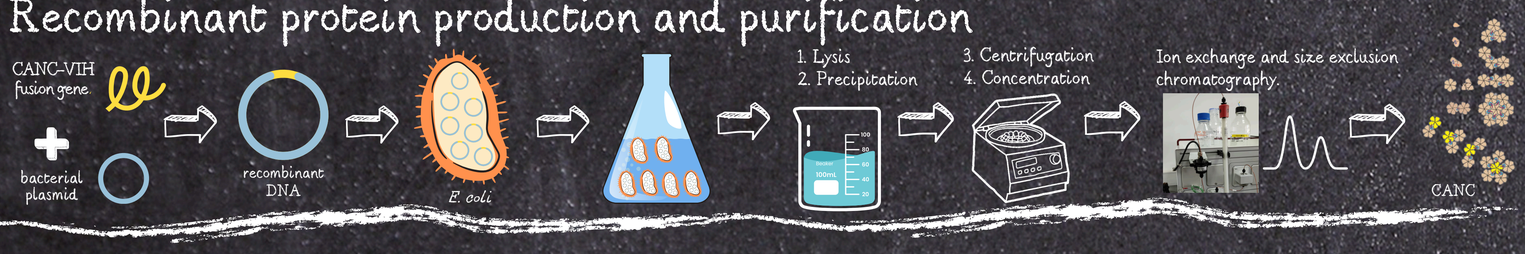
General aim: Identify molecules that affect HIV CANC multimerization process *in vitro*

Specific aims:

1. Transform *E. coli* BL21(DE3) with a plasmid containing CANC codifying sequence and express and purify the recombinant CANC fusion protein.
2. Optimize the *in vitro* multimerization assay.
3. Evaluate the effect of different molecules on CANC multimerization.
4. Evaluate the compounds that affect capsid multimerization in a cellular infection model

Results and Discussion

1 Recombinant protein production and purification



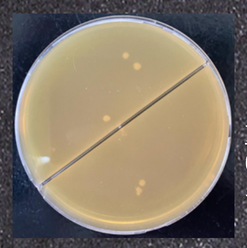


FIGURE 2 *E. coli* BL21(DE3) colonies transformed with pET11a plasmid containing the sequence codifying HIV CANC fusion protein.

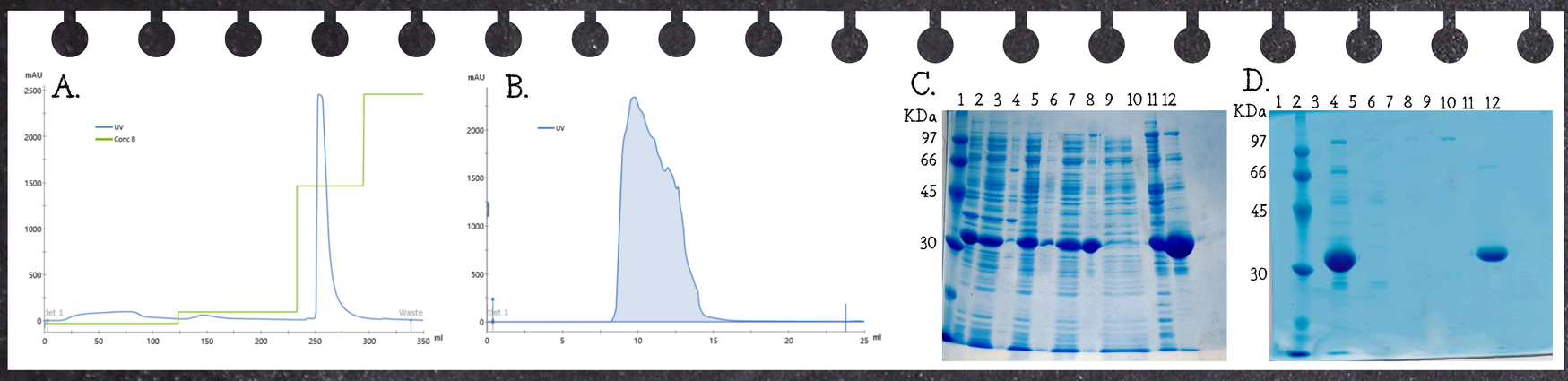
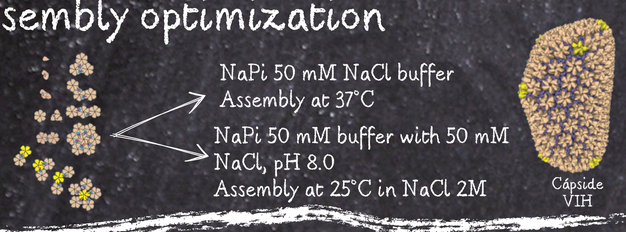
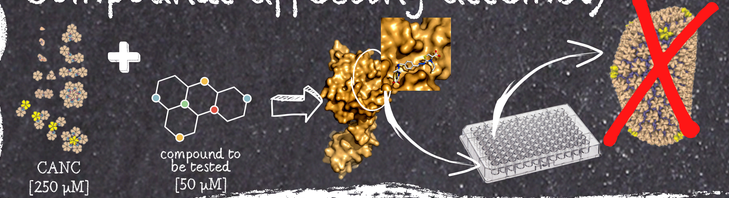


FIGURE 3. Recombinant CANC purification. (A) Ion exchange chromatograhpy. CM FF 16/10 (GE) column was equilibrated with 25 mM potassium phosphate buffer pH 6.0 with 50 mM NaCl and 5 mM βME. Elution was achived by increasing NaCl concentration in 3 steps of 5, 60 and 100% buffer B (25 mM potassium phosphate, pH 6.0 with 1 M NaCl and 5 mM βME). (B) Size exclusion chromatgraphy in a Superdex 75 10/300 (GE) column equilibrated with a 20 mM Tris buffer pH 7.5 with 5 mM $MgCl_2$, 140 mM KCl, 10 mM NaCl and 5 mM βME. Fractions collected from (C) lysis and precipitation steps, and from (D) ion exchange and size exclusion chromatography were analyzed in 12% SDS-PAGE in reducing conditions.

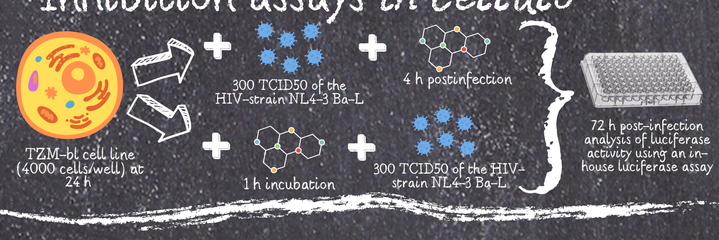
2 Assembly optimization



3 Compounds affecting assembly



4 Inhibition assays *in cellulo*



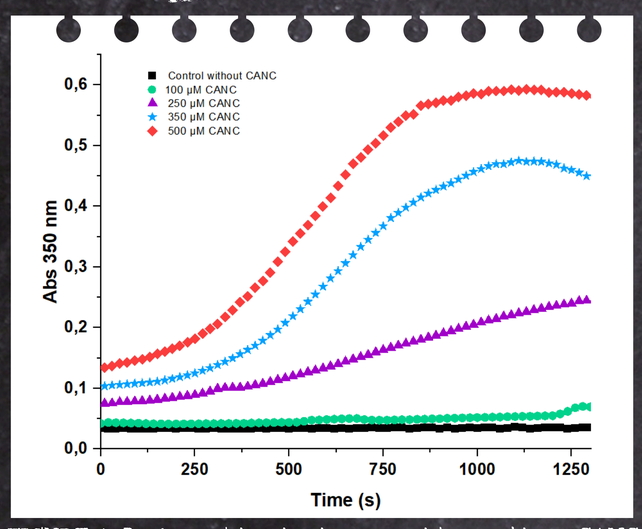


FIGURE 4. *In vitro* multimerization assay with recombinant CANC. The experiment was performed using 100, 250, 350 or 500 µM CANC in NaPi 50 mM buffer pH 8.0 with 50 mM NaCl, using temperature as a trigger. These experiments were performed in a 96-well plate using a final volumen of 100 µL and absorbance at 350 nm was measured in a VarioskanTM Flash Multimode Reader.

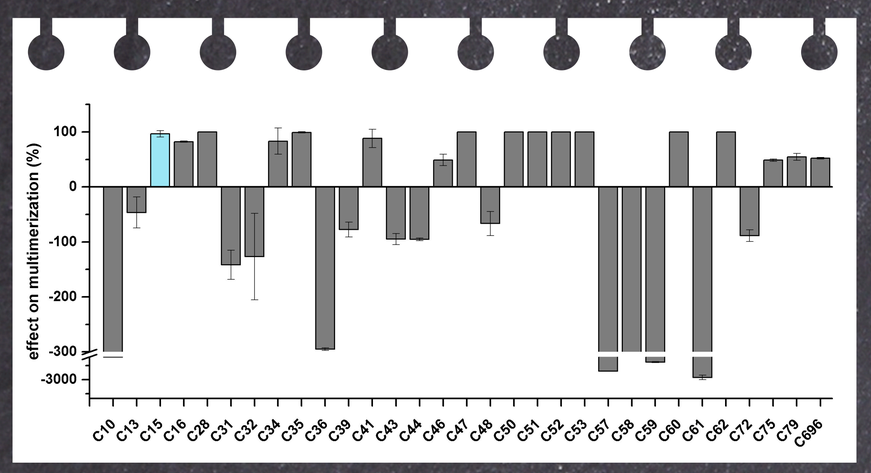
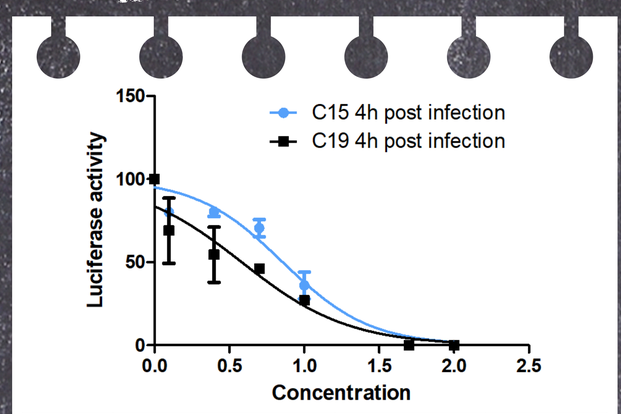


FIGURE 5. Evaluation of CANC assembly rates in the presence of selected compounds at 50 µM. We identified 31 compounds that reduce or accelerate the assembly process in more than 50%, when compared to a control assay. 17 compounds inhibited the assembly process (C15, C16, C28, C34, C35, C41, C46, C47, C50, C51, C52, C53, C60, C62, C75, C79, C696) while 14 compounds accelerated it (C10, C13, C31, C32, C36, C39, C43, C44, C48, C57, C58, C59, C61, C72). C15 colored in blue is the only compound that was active in both assays, inhibiting CANC assembly both *in vitro* and *in cellulo*.



Compound	*in vitro*	EC_{50} *in cellulo*	
		4 h postinfection	1 h incubation
C19	inihbitor (Internal control) EC50 = 7.2 µM	2.6 ± 0.18 µM	7.6 ± 0.16 µM
C15	inhibitor 100% at 50 µM	8.6 ± 0.15 µM	13 ± 0.2 µM

FIGURE 6. An initial screening in a cellular model of HIV infection was performed with all 84 molecules at 5 µM final concentration. For the compounds with a significant inhibition, the IC_{50} was determined. In the assayed conditions, C15 inhibited the infection *in cellulo* in a dose-dependent manner.

Conclusions

Pure recombinant CANC was obtained and the purification conditions were optimized.
The *in vitro* assembly assay was optimized using temperature as a trigger for multimerization.
84 compounds were evaluated and 31 were found to disrupt CANC assembly *in vitro*, either slowing down or accelerating the process.
One compound decreased viral load in a cellular model of HIV infection.

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Aknowledgements

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