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Looking for novel p24 multimerization inhibitors of FIV.

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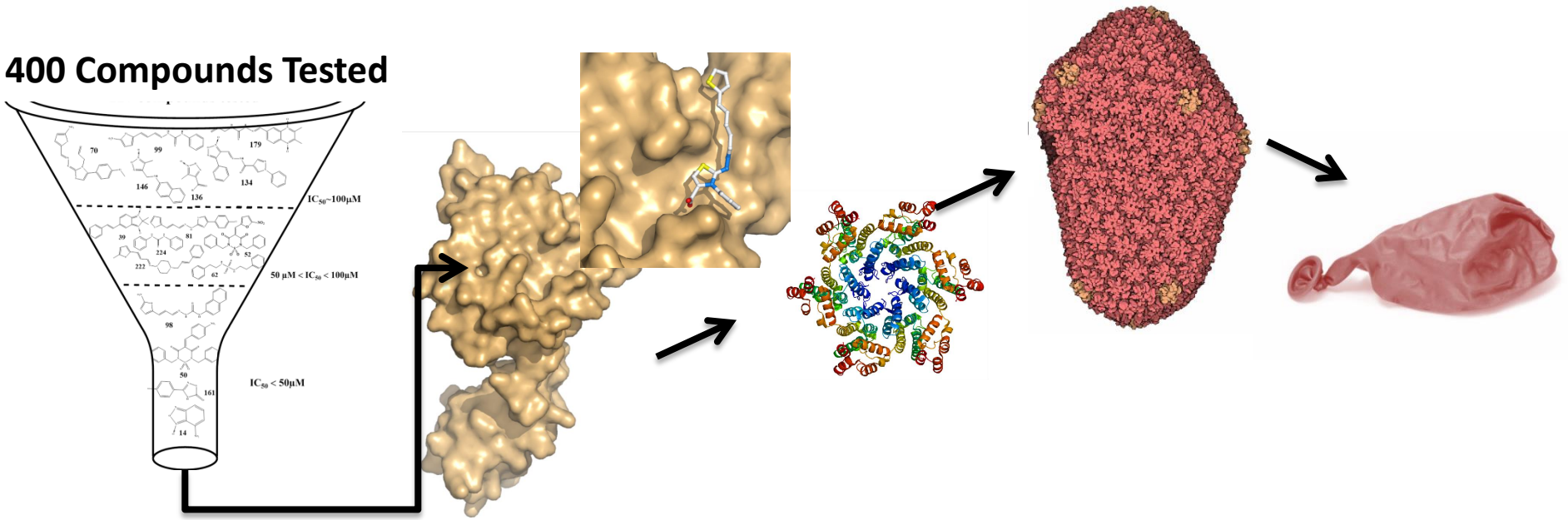
Looking for novel p24 multimerization inhibitors of FIV.

Graphical Abstract

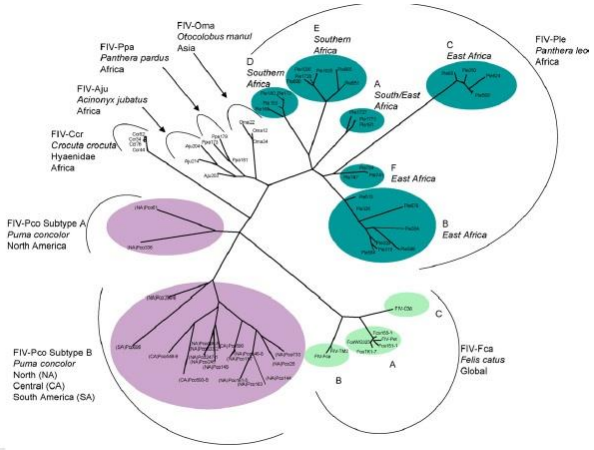


Abstract: Feline immunodeficiency virus (FIV) is a member of the retroviridae family of viruses. It causes an acquired immunodeficiency syndrome (AIDS) in domestic and non-domestic cats worldwide, representing an important veterinary issue. Genome organization of FIV and clinical characteristics of the disease caused by the virus are similar to those of human immunodeficiency virus (HIV). Both viruses infect T lymphocytes, monocytes and macrophages, and their replication cycle in infected cells is analogous. Thus, infection of cats with FIV is also a useful tool to study and develop novel drugs and vaccines against HIV. Anti-retroviral drugs studied extensively in HIV infection have targeted different steps of the virus replication cycle, like the inhibition of virion assembly and maturation. Despite much success of anti-retroviral therapy in slowing HIV progression in humans, similar therapy has not been thoroughly investigated for FIV infection in cats. FIV capsid protein (CA) drives the assembly of the viral particle, which is a critical step in the viral replication cycle. During this step, the CA protein oligomerizes to form a protective coat that surrounds the viral genome. In this article we perform a large screening of four hundred molecules from our in-house library. We used an *in vitro* assembly assay of p24, combined with microscale termophoresis to estimate binding affinity. This screening led to the discovery of around 5 novel hits to drug development and drug design for new antiviral drugs.

Keywords: Assembly inhibitors, immunodeficiency virus, microscale termophoresis

Introduction

- Feline Immunodeficiency Virus
  - Related to HIV
  - AIDS-like disease in felines (domestic cats, wild felines).

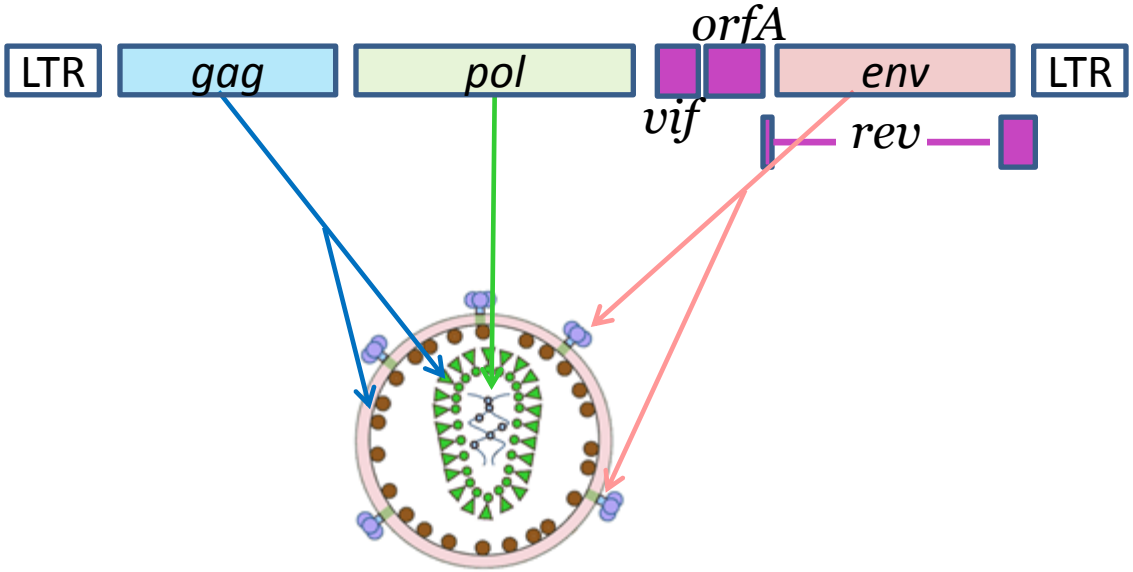


- Veterinary issue:
  - >50 million domestic cats infected worldwide.
  - 2.5% of domestic cats are seropositive for FIV in the United States.

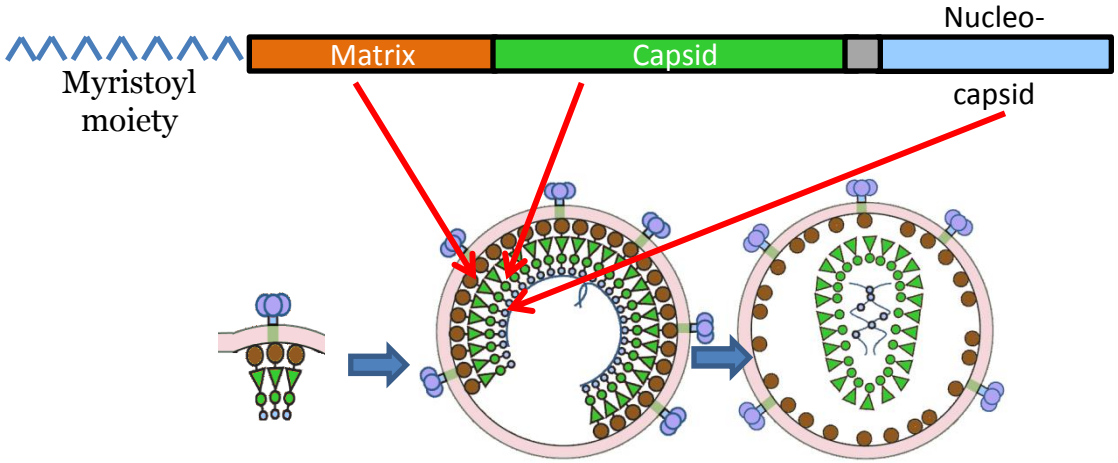
=> Need for the rational development of therapeutic drugs against FIV

Genomic and viral organization of FIV:

- Enveloped virus (~100nm).
- Canonical retroviral genes (gag, pol, env) => structural proteins
- Regulatory/accessory genes.



Gag, motor of the viral particle assembly:

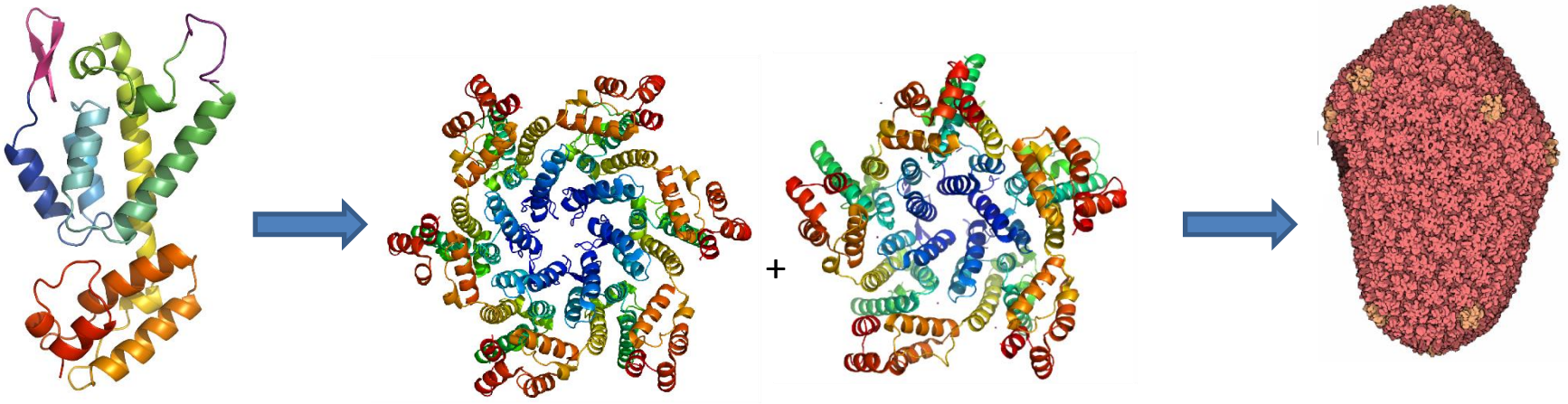


N-terminal myristoylated polyprotein (53kDa) :

- Gag is targetted to the viral membrane through its N-myristyoyl moiety.
- The oligomerization of the polyprotein leads to assembly, budding and maturation of viral particles.
- Proteolysis of the polyprotein by the viral protease leads to a reorganization of its subunits, creating the mature conical viral core which is necessary for infectivity.

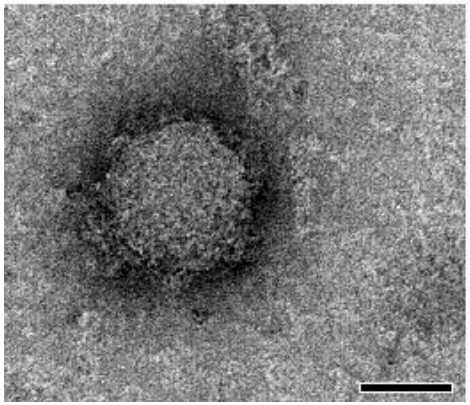
The capsid sub-unit of Gag, the architect of the viral core:

- α-helical protein with two domains separated by a flexible linker
- Hexameric or pentameric in the mature viral particle.



The capsid sub-unit of Gag, the architect of the viral core:

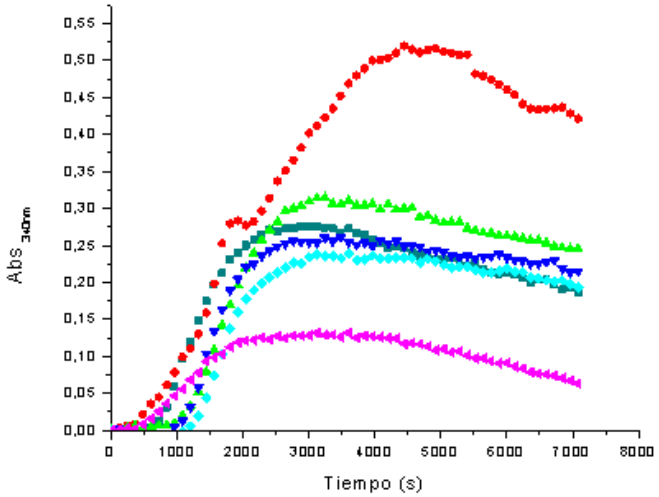
- The recombinant capsid protein can spontaneously assemble *in vitro* in presence of salt:
  - Protein:protein interactions close to those observed in the viral particle.



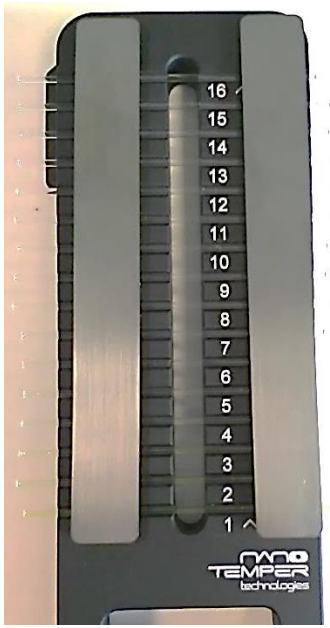
Results and discussion

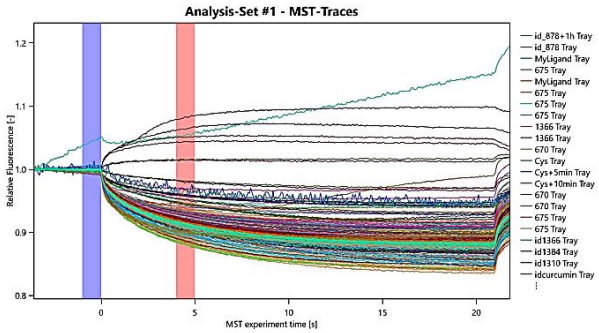
Screening in the polymerization assay of p24.

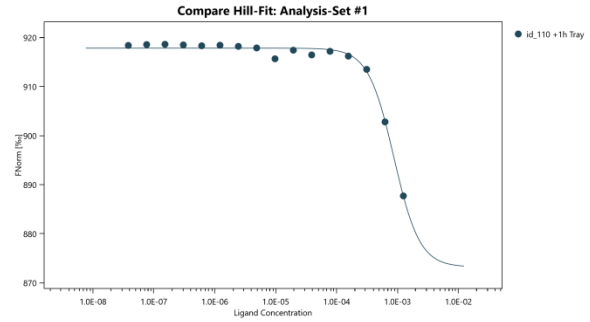
In a multiwall plate reader, using 384 well plates, at 39ºC for 40 min.



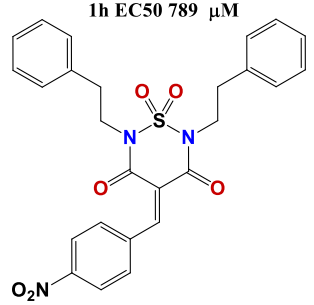
Checking the interaction by thermophoresis.

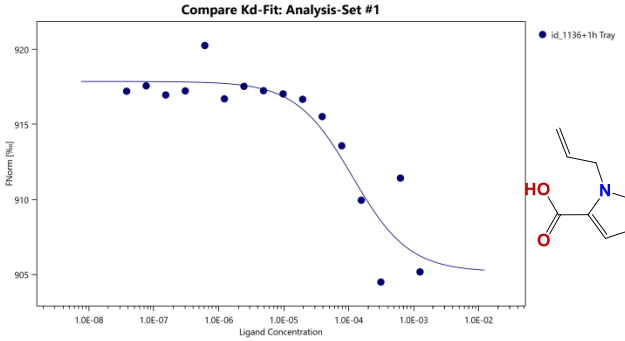




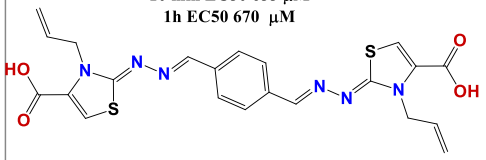


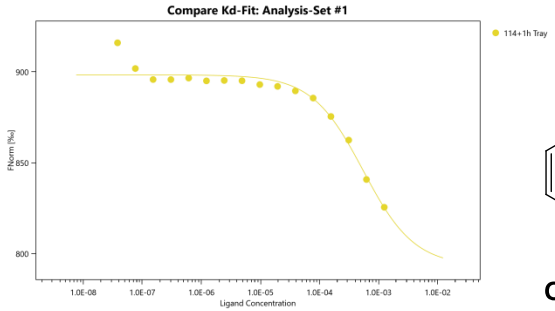
110
binding ⊕
10 min EC50 1.1 mM
1h EC50 789 µM



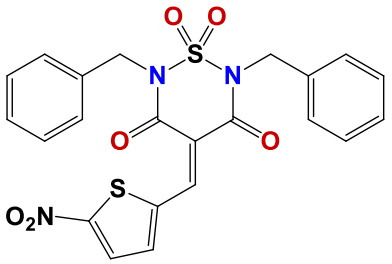


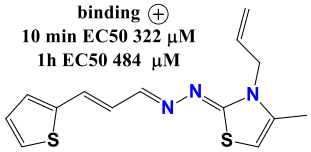
1310
binding ⊕
10 min EC50 655 µM
1h EC50 670 µM



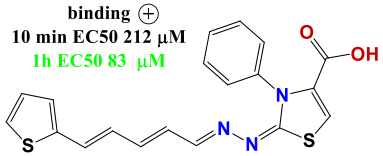


114
binding ⊕

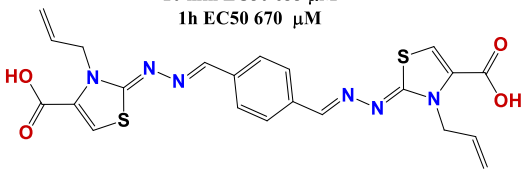




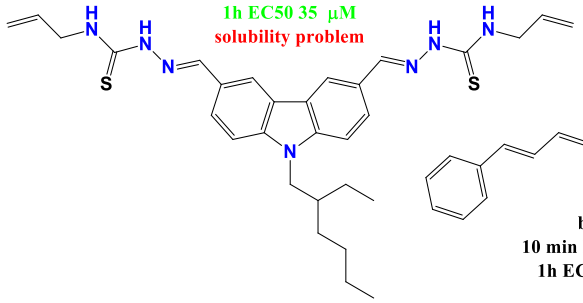
1128
binding ⊕
10 min EC50 322 µM
1h EC50 484 µM



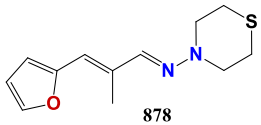
1136
binding ⊕
10 min EC50 212 µM
1h EC50 83 µM



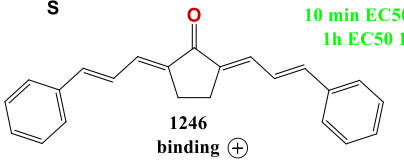
1310
binding ⊕
10 min EC50 655 µM
1h EC50 670 µM



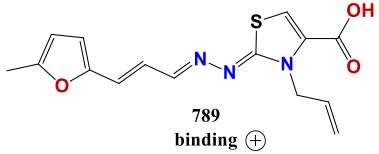
1368
binding ⊕
10 min EC50 no bind
1h EC50 35 µM
solubility problem



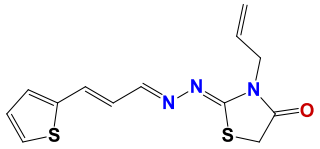
878
binding ⊕
10 min EC50 119 µM
1h EC50 166 nM



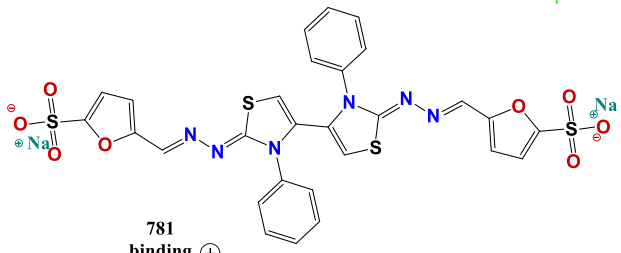
1246
binding ⊕
10 min EC50 390 µM
1h EC50 239 µM



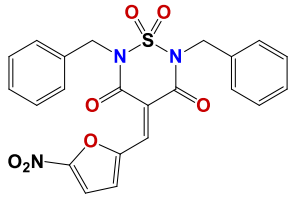
789
binding ⊕
10 min EC50 640 µM
1h EC50 655 µM



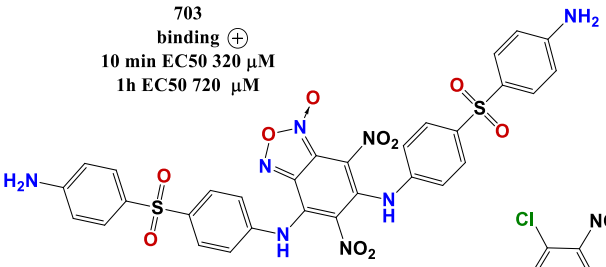
1138
binding ⊕
10 min EC50 242bµM
1h EC50 100 µM



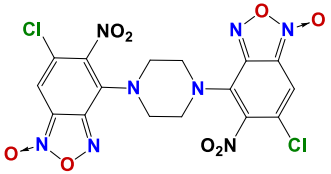
781
binding ⊕



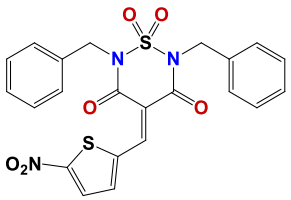
116
binding ⊕



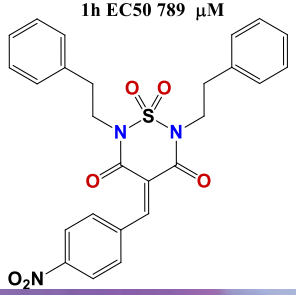
703
binding ⊕
10 min EC50 320 µM
1h EC50 720 µM



704
binding ⊕

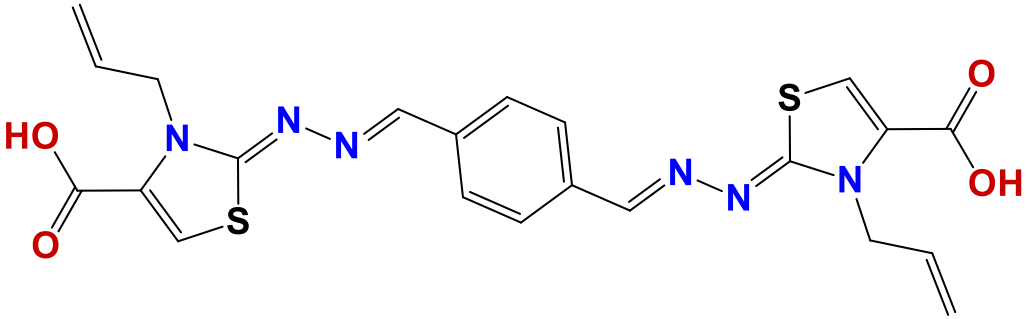


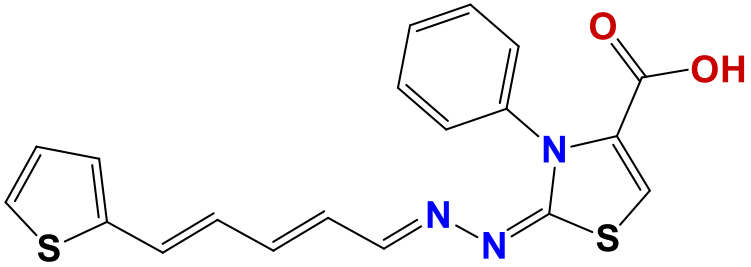
114
binding ⊕

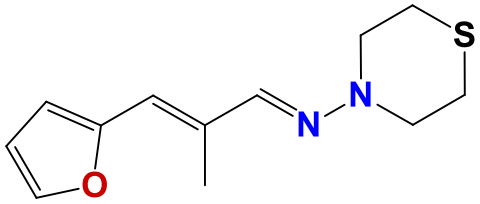


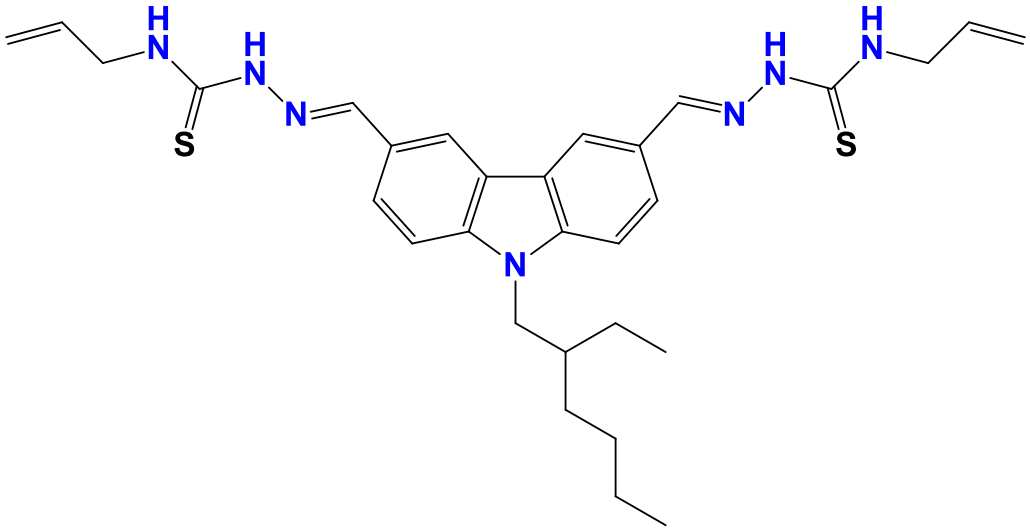
110
binding ⊕
10 min EC50 1.1 mM
1h EC50 789 µM

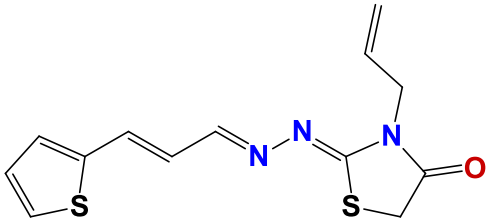
New HITs



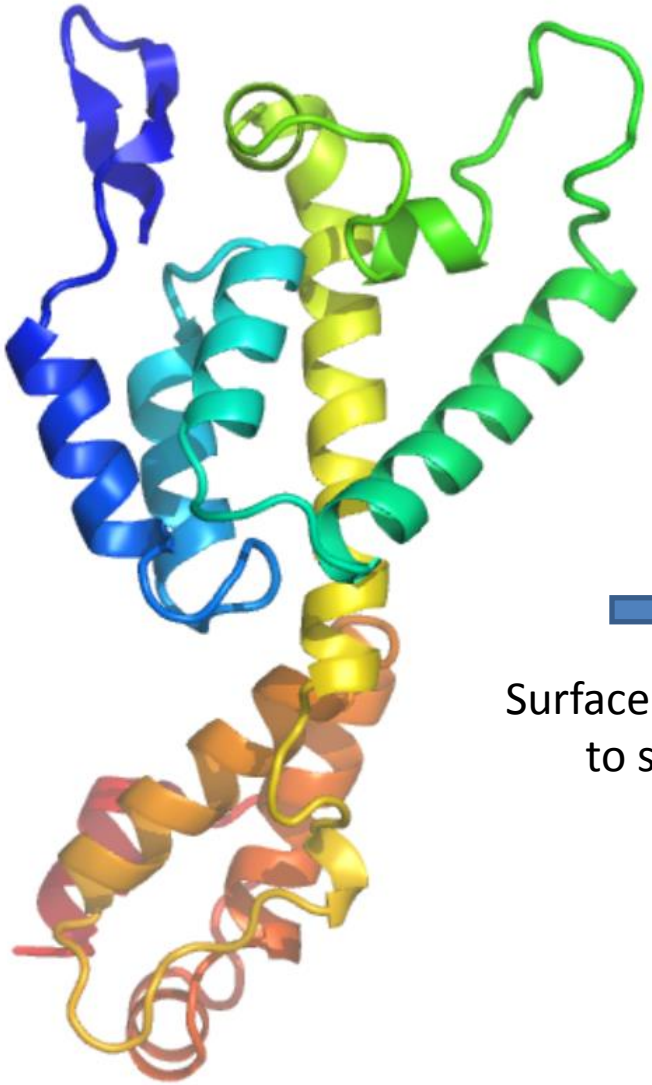




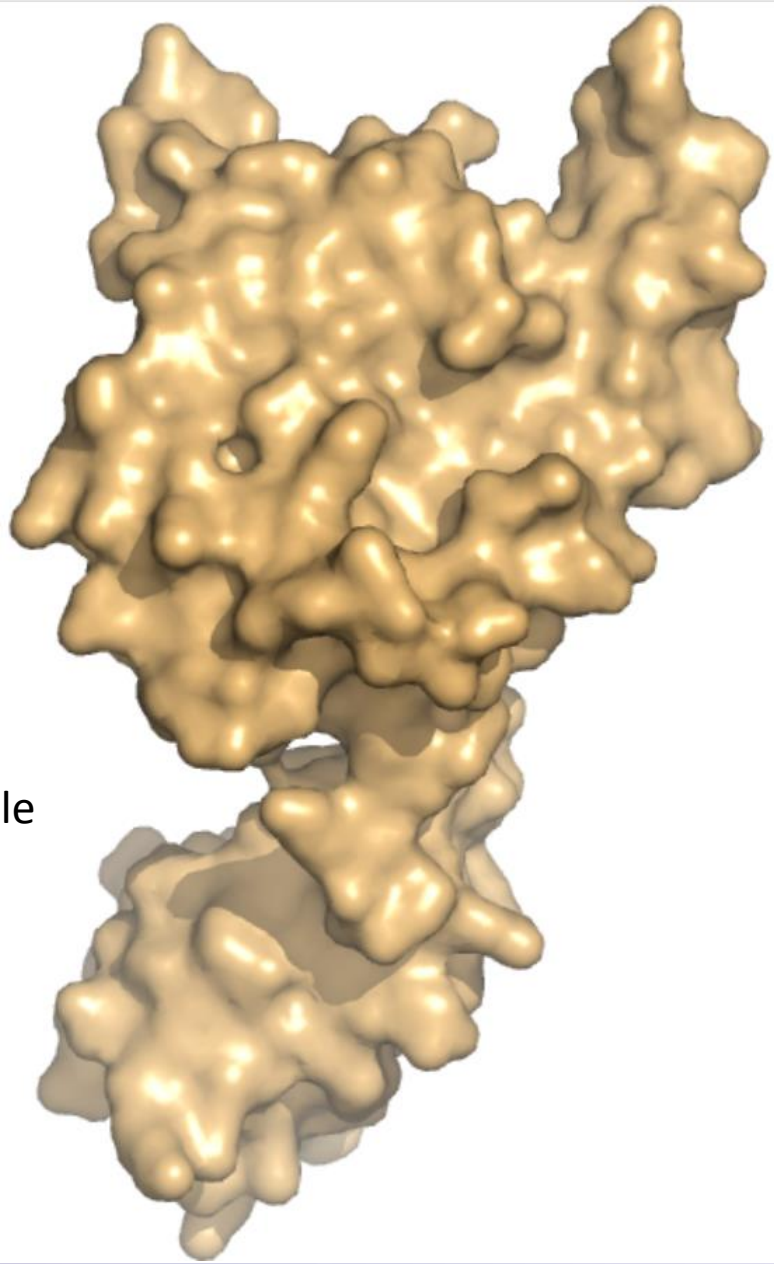


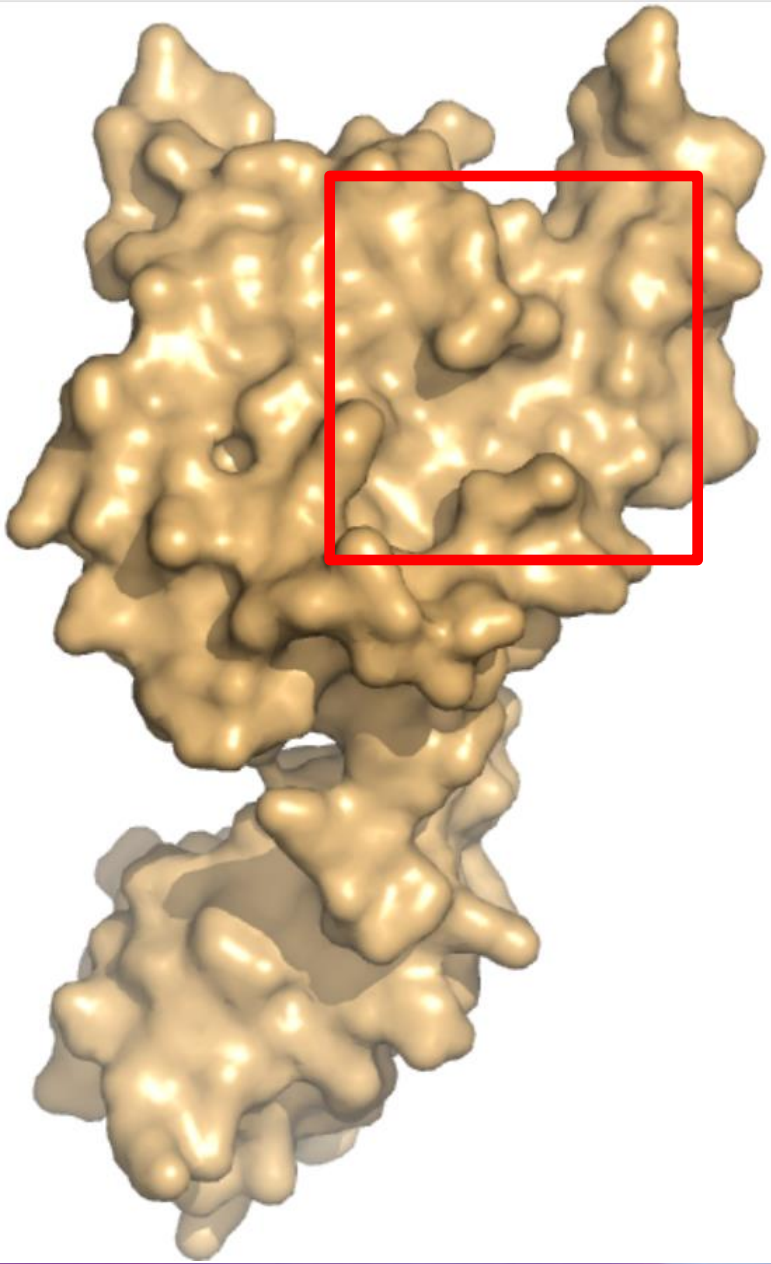


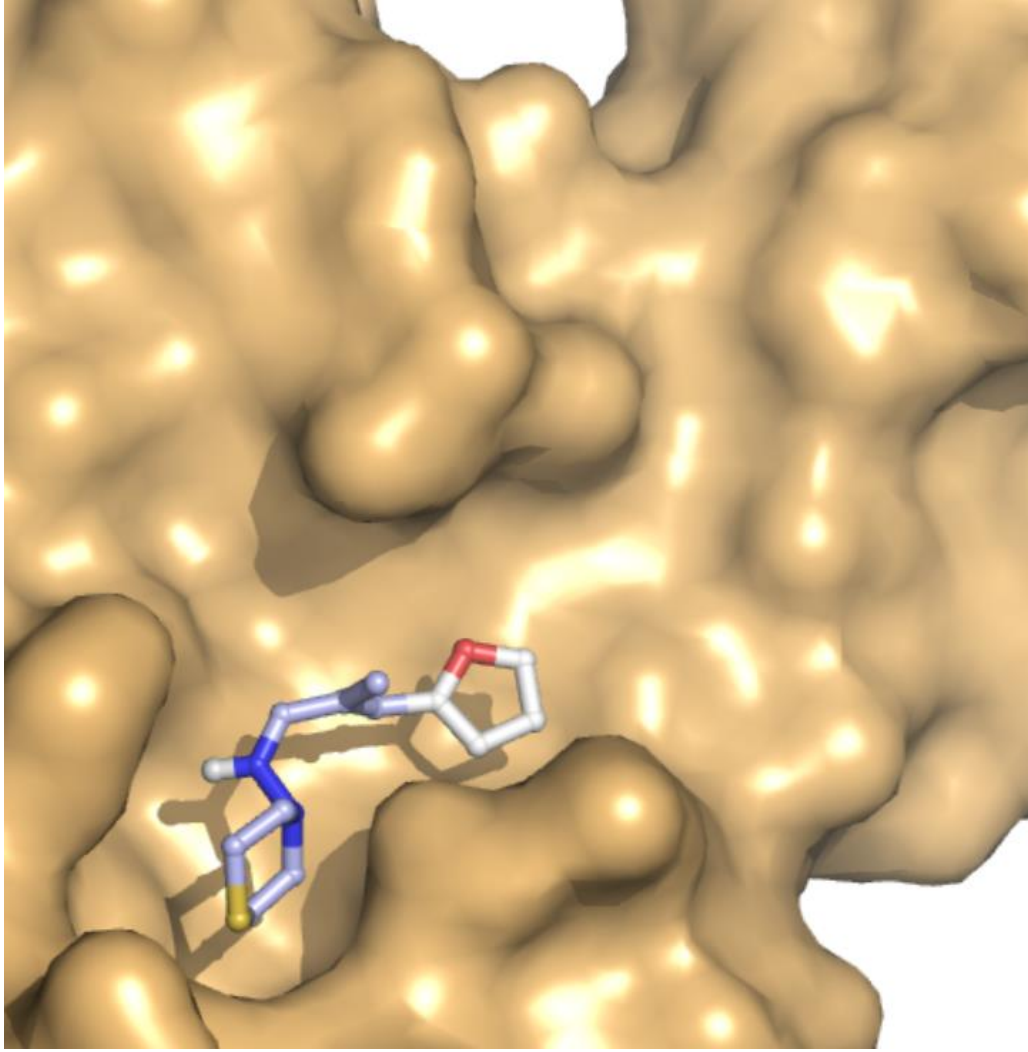
Docking analysis of the HIT-P24 interaction.



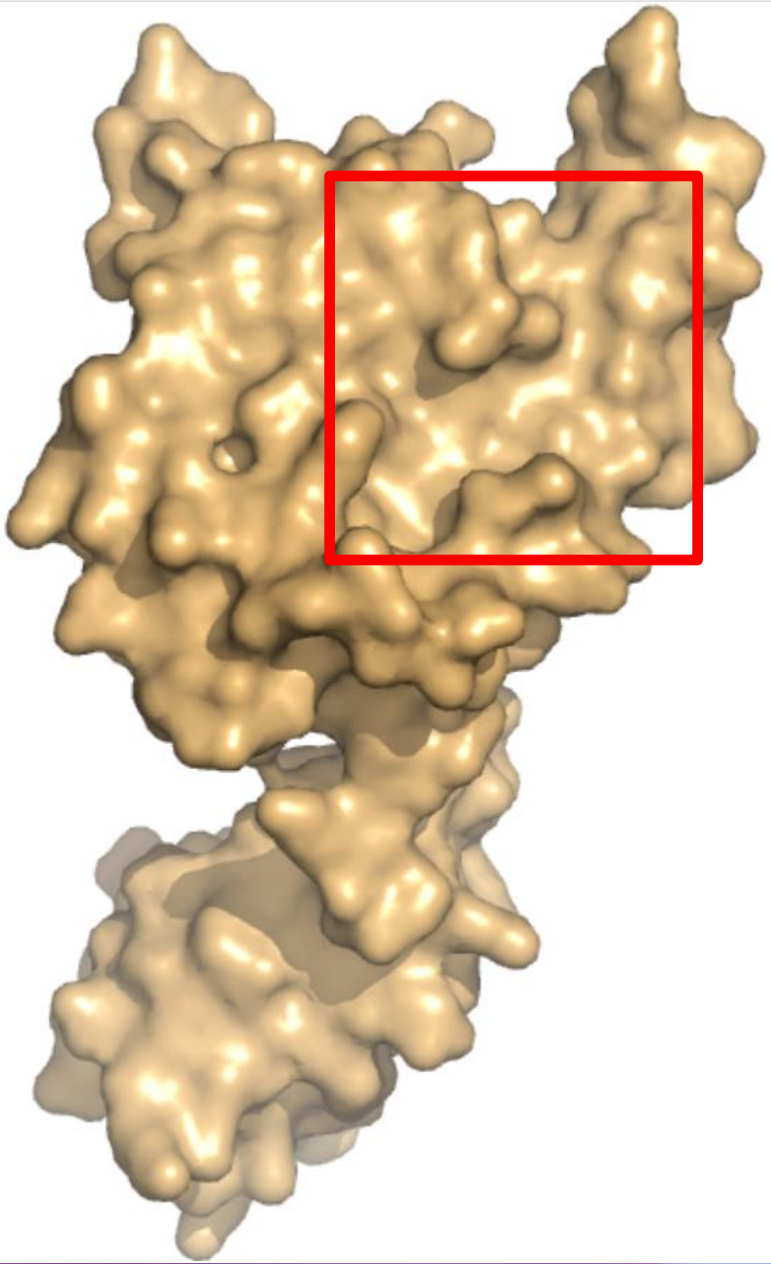
Surface accessible
to solvant

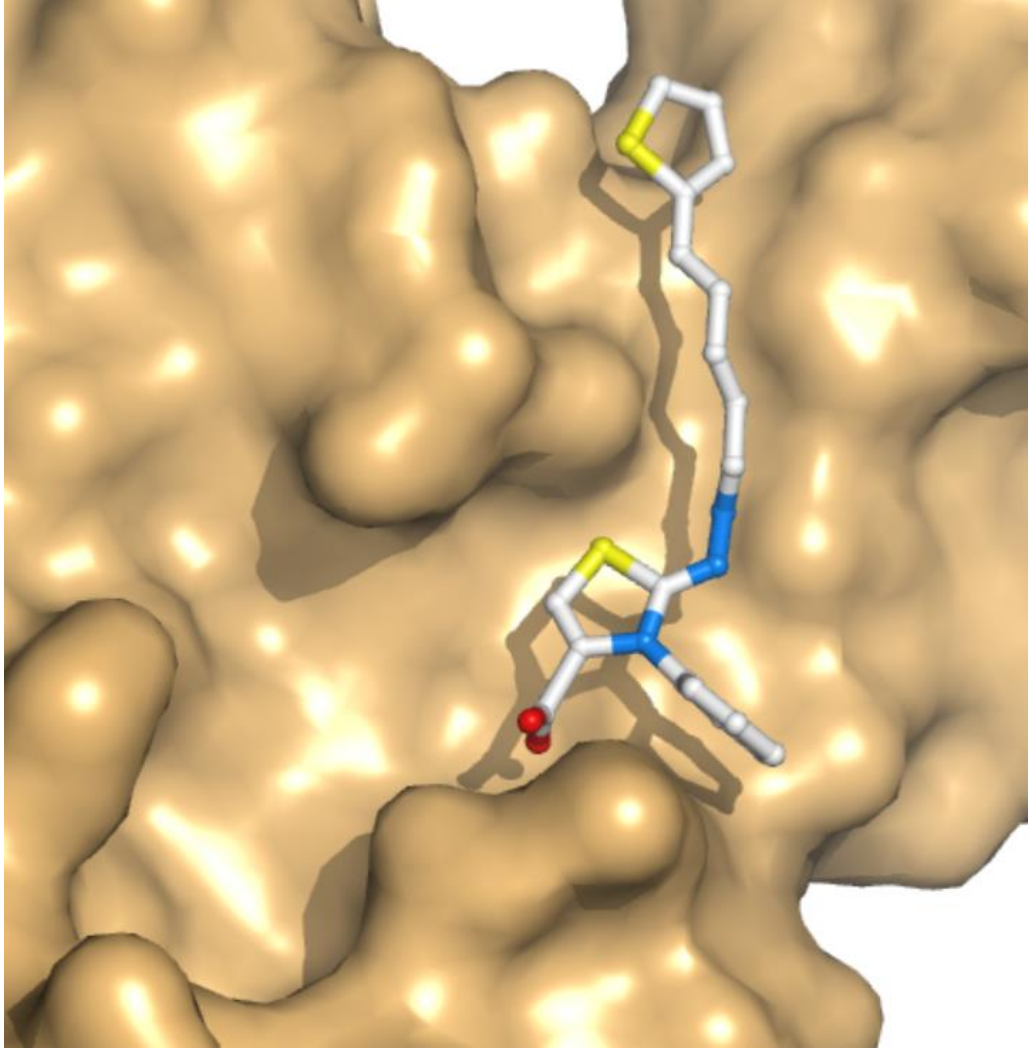






878





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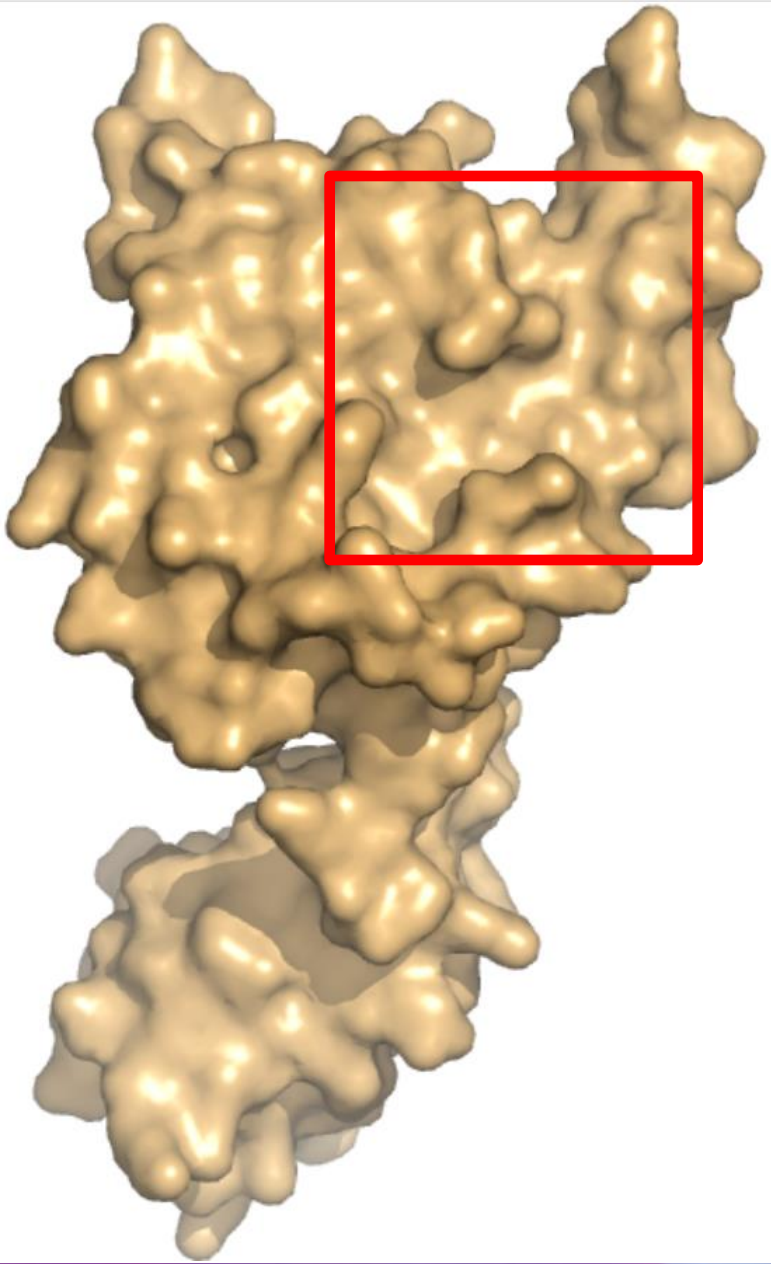

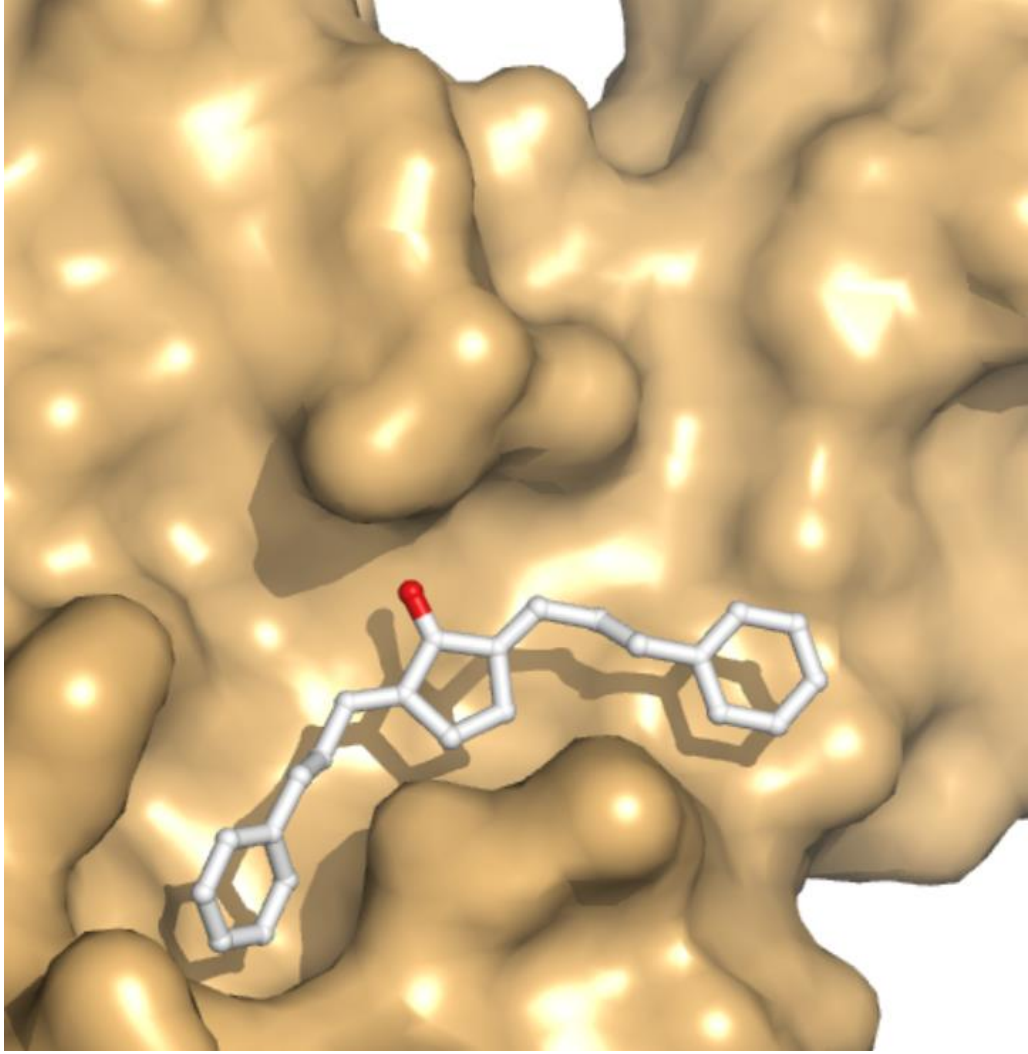

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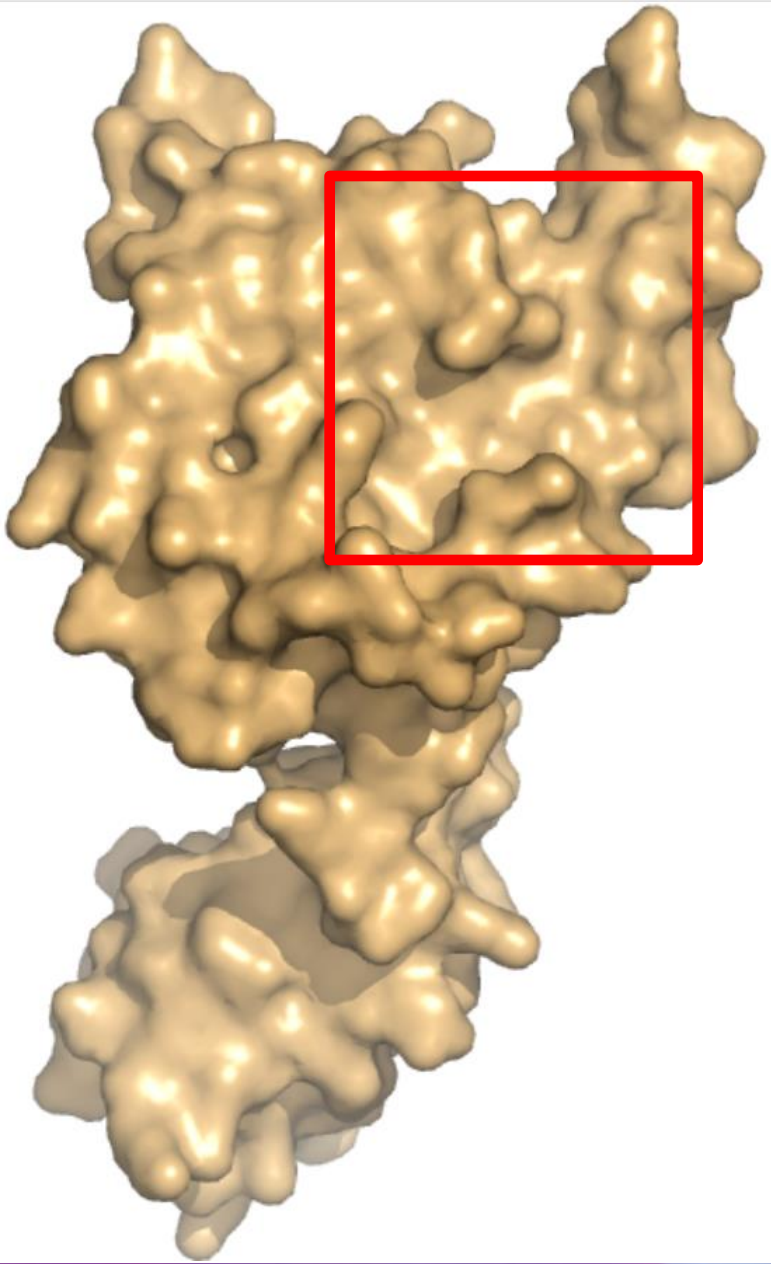

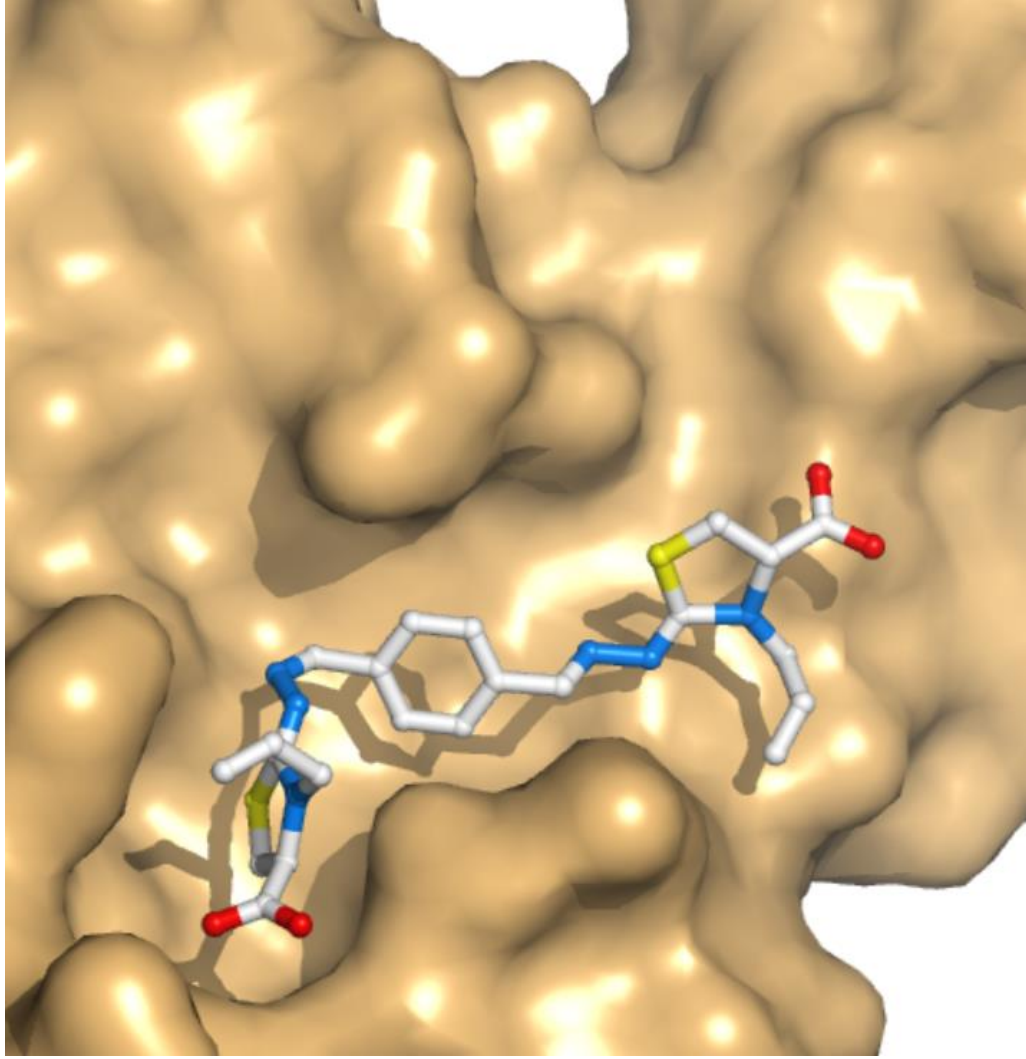

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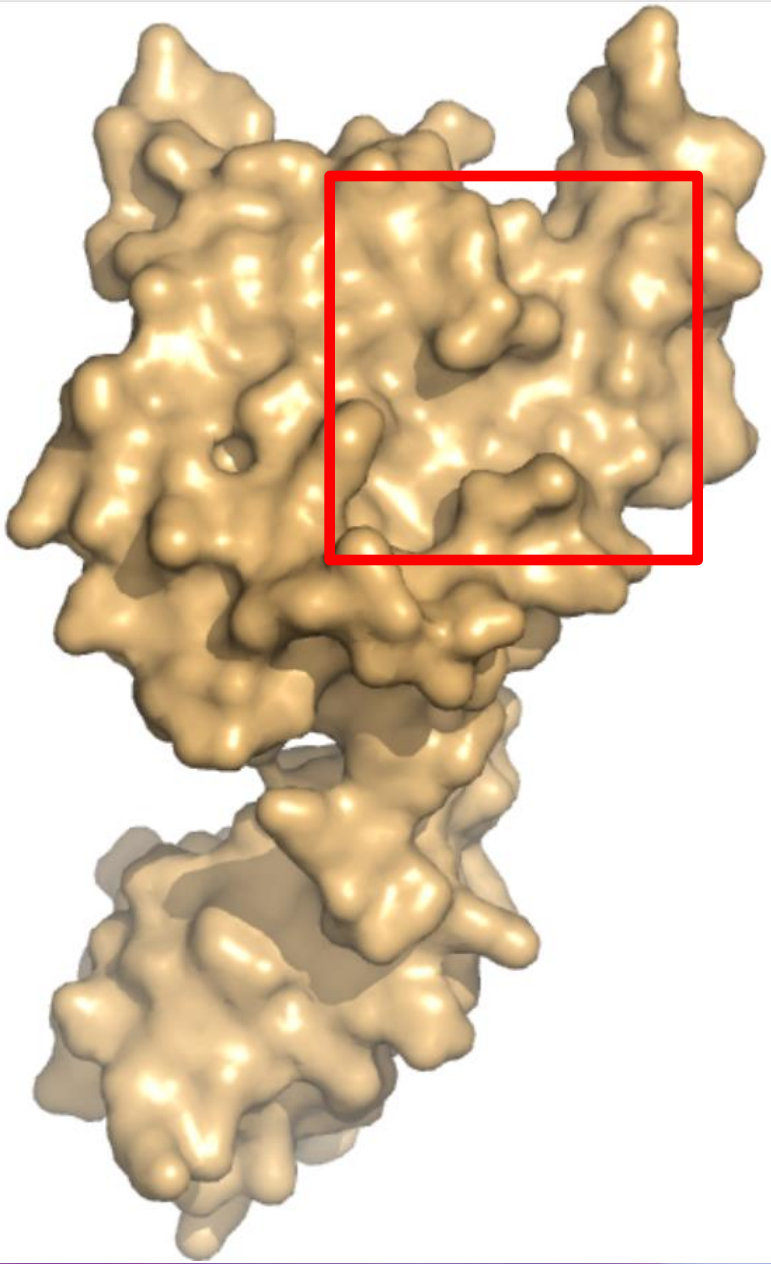
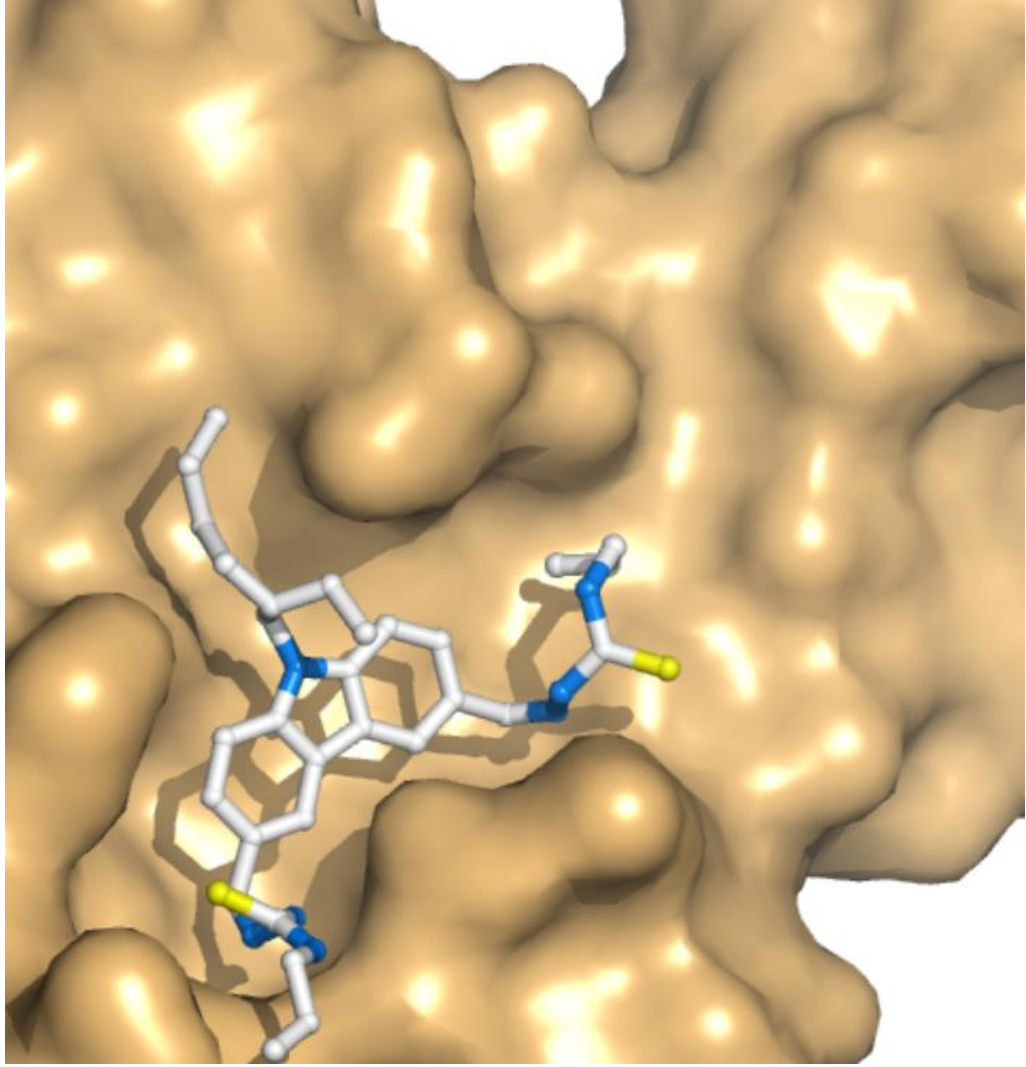
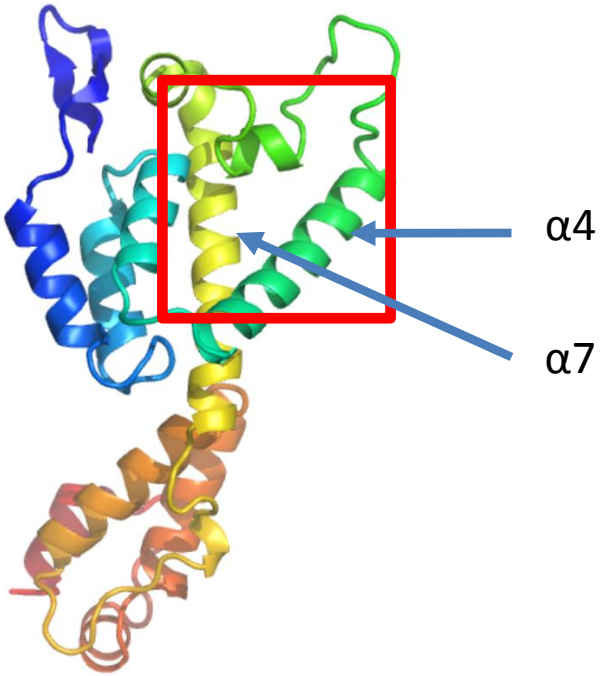


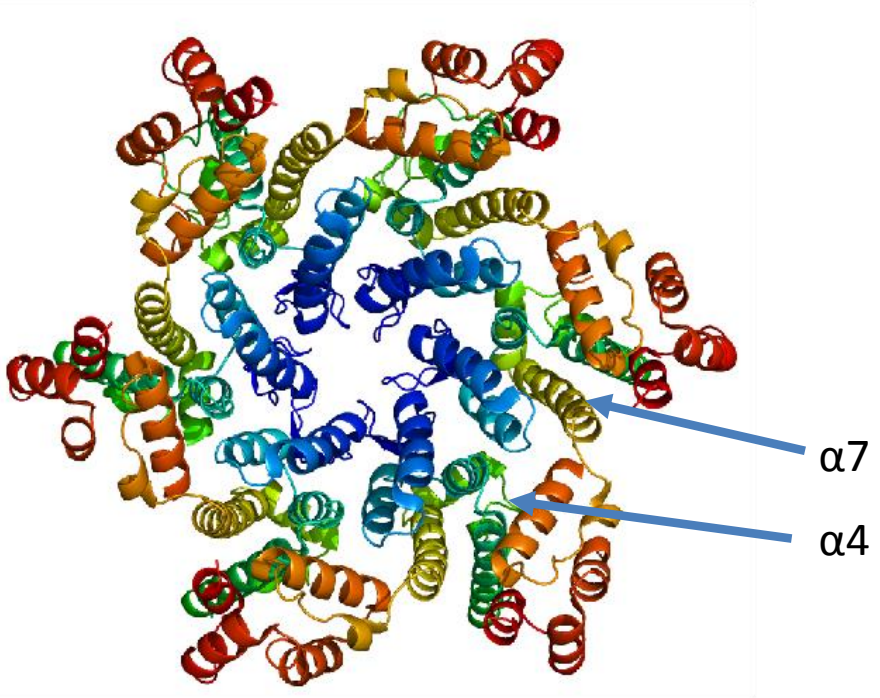


1368

Discussion

Docking experiments reveal a preferential position in the pocket between helices α3, α4 and α7 of FIV p24. In HIV-1, helices α4 and α7 are involved in the protein:protein interactions with neighbouring monomers, leading to the formation of p24 hexamers during capsid formation.





Conclusions

We tested in the assembly assay for p24 of FIV around 400 compounds from our in-house library. We found and checked by thermophoresis and docking 5 new hits for antiviral drug development as p24 assembly interrupters.

Acknowledgments

ESRF ID 23-2 ID 29

PEDECIBA

BioStruct-X

CSIC

Ecos-Sud program

mrmsb Dr. Xavier Robert