Screening novel HIV-1 inhibitors targeting cyclophilin A by structure-based and ligand-based in silico screening

Norihito Kawashita[1,2], Yu-Shi Tian[1], Chris Verathamjamras[3], Kousuke Okamoto[1], Teruo Yasunaga[2], Kazuyoshi Ikuta[2], Masanori Kameoka[2,3], Tatsuya Takagi[1,2]

[1] Graduate School of Pharmaceutical Sciences, Osaka University
[2] Research Institute for Microbial Diseases, Osaka University
[3] Thailand-Japan Research Collaboration Center on Emerging and Re-emerging Infections

Joint International Tropical Medicine Meeting 2013

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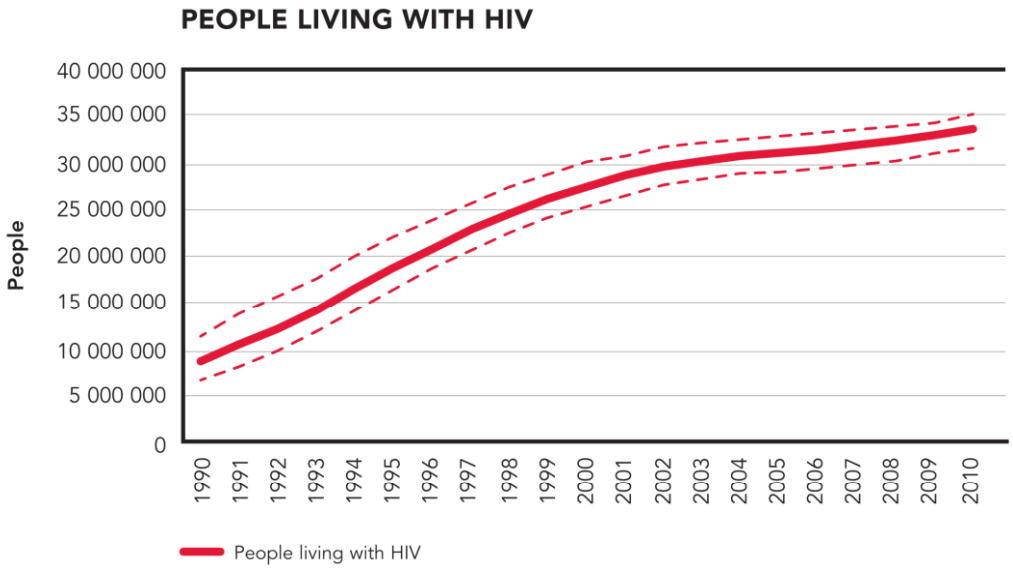
Introduction

About 35 million people were infected HIV until 2012.[1)]

PEOPLE LIVING WITH HIV



The number of newly-infected patients has been decreased since 1997 and AIDS-related deaths has been decreased since 2005.[2)]

1) 2013 UNAIDS GLOBAL REPORT 2013
2) UNAIDS REPORT ON THE GLOBAL AIDS EPIDEMIC 2010

Antiretroviral therapy (ART)

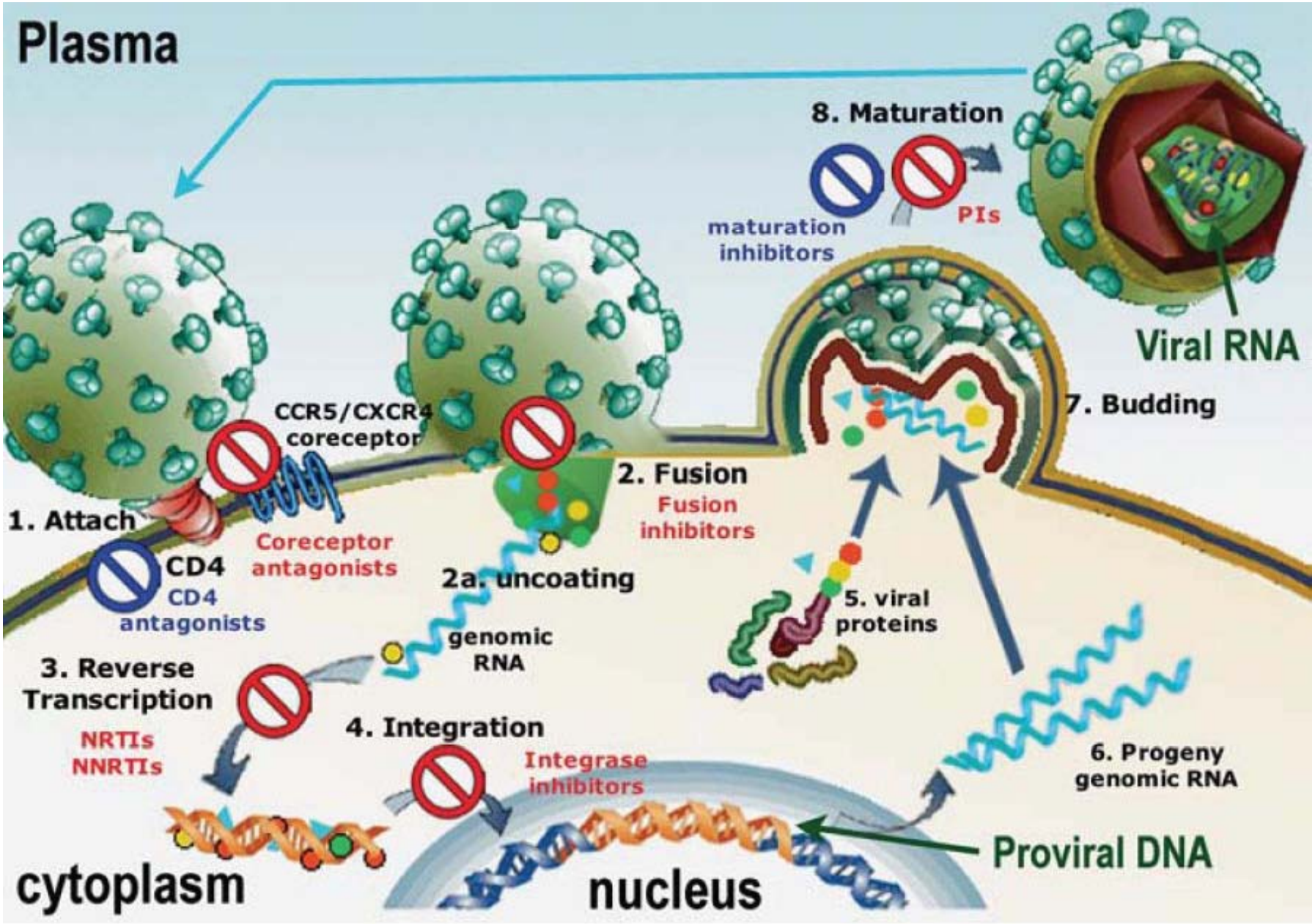
Highly active antiretroviral therapy (HAART) was started in 1996, the mortality and severe syndrome by AIDS were then dramatically improved.

An example of treatment guideline
（First line treatment in Japan in 2013）

- 2 NRTIs and 1 NNRTI
- 2 NRTIs and 1 PI
- 2 NRTIs and 1 INSTI

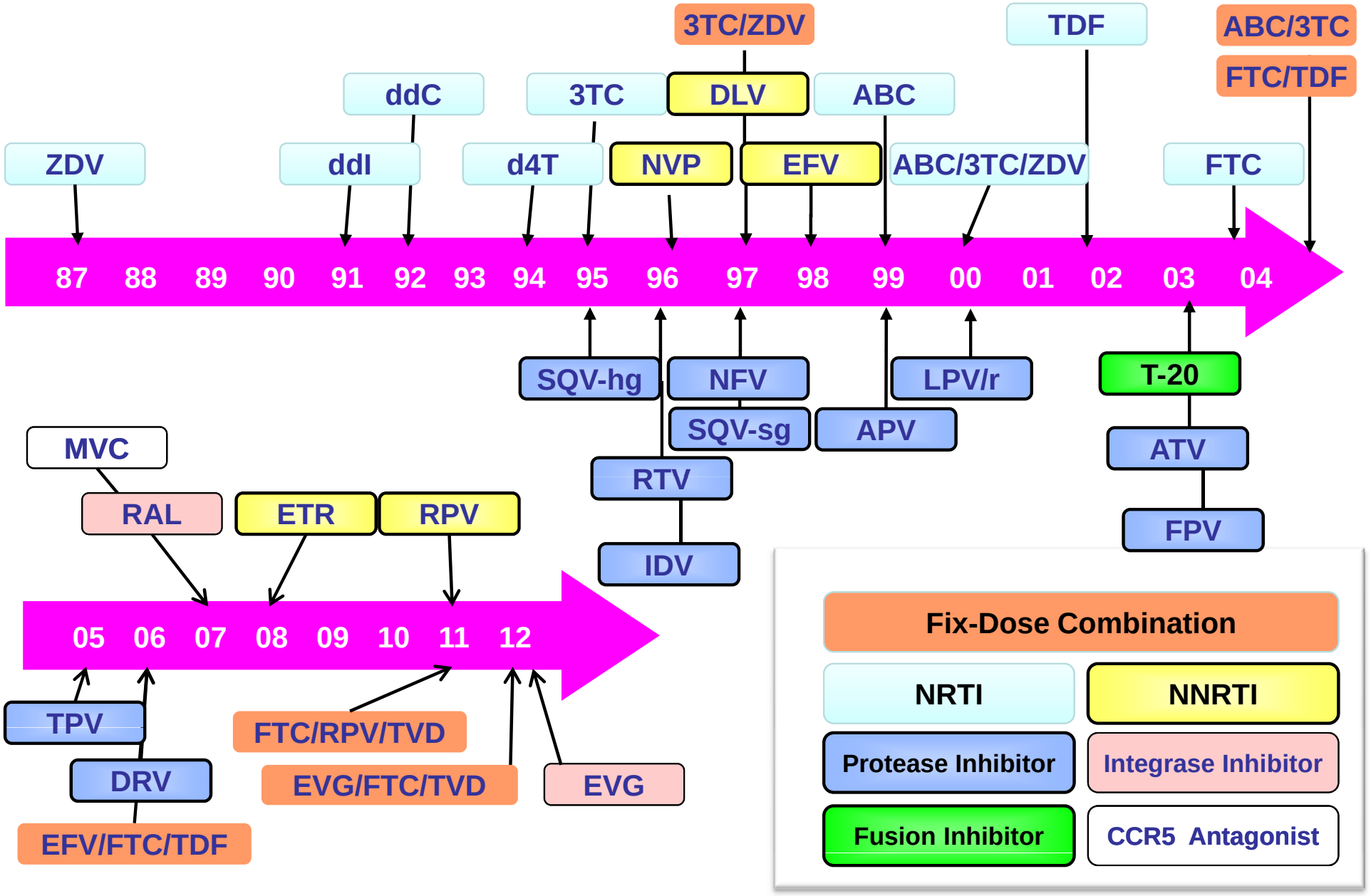
- NRTI: Nucleoside type reverse transcriptase inhibitor
- NNRTI: Non-nucleoside type reverse transcriptase inhibitor
- PI: Protease Inhibitor
- INSTI: Integrase inhibitor

Target of anti-HIV drugs



Sierra-Aragón S, Walter H. *Intervirology*, **55**, 84-97, (2012).

Anti-HIV drugs



Shirasaka, T. *Rinsho & Biseibutu*, **40**, 21-28, (2013).

Problems of ART

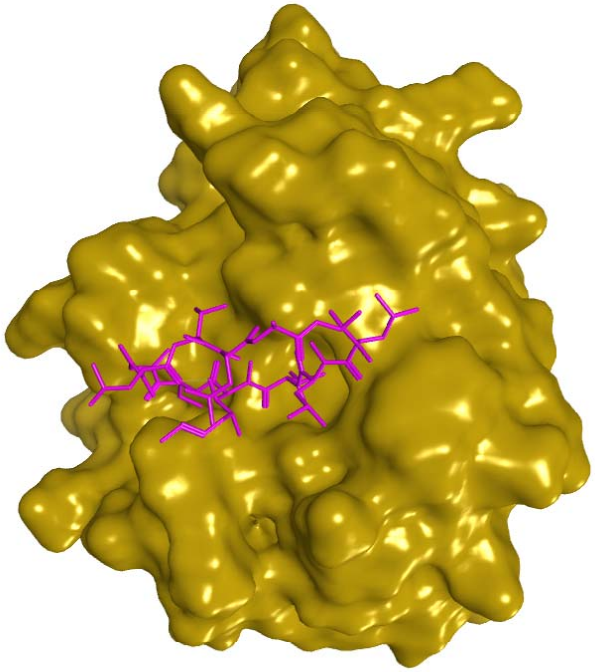
- Long-term toxicity
- Drug-drug interaction
- Difficulty of adherence
- Not permanent cure
- Higher cost
- Drug resistance

Drug target of our study

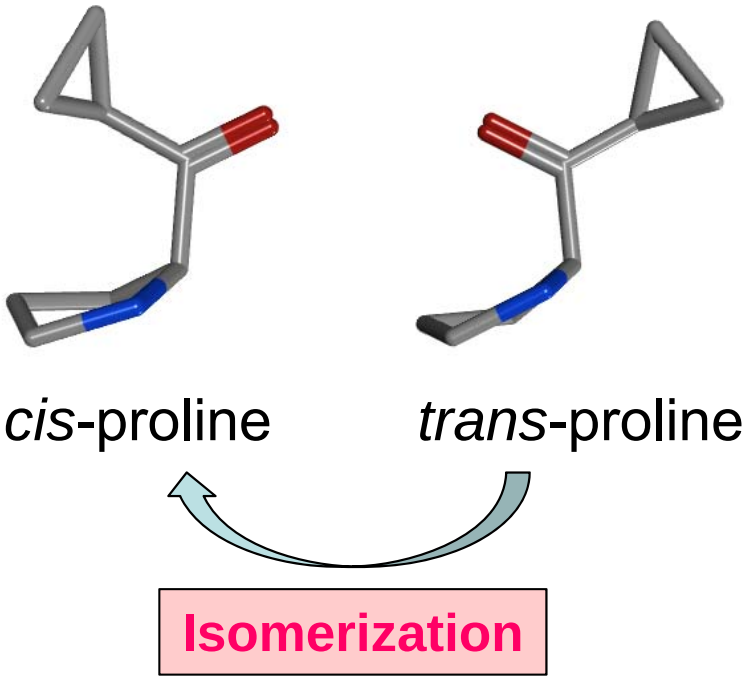
- Various anti-HIV agents have been developed, drug resistance is still one of severe problems to anti-HIV treatment yet. It is important to deal with it by increasing the number of drug.
- For a number of drugs were developed on known HIV proteins, drug development to novel target was required.
- We focused cyclophilin A (CypA) for a novel drug target of anti-HIV agent.

Cyclophilin A (CypA)

- **Cyclophilin A** (**CypA**) was first discovered as the receptor of immunosuppressive drug Cyclosporin A (CsA).
- One of the main function of CypA is **peptidyl prolyl isomerase** (**PPIase**) activity, which is converted from *trans*- form to *cis*- form at proline residues.

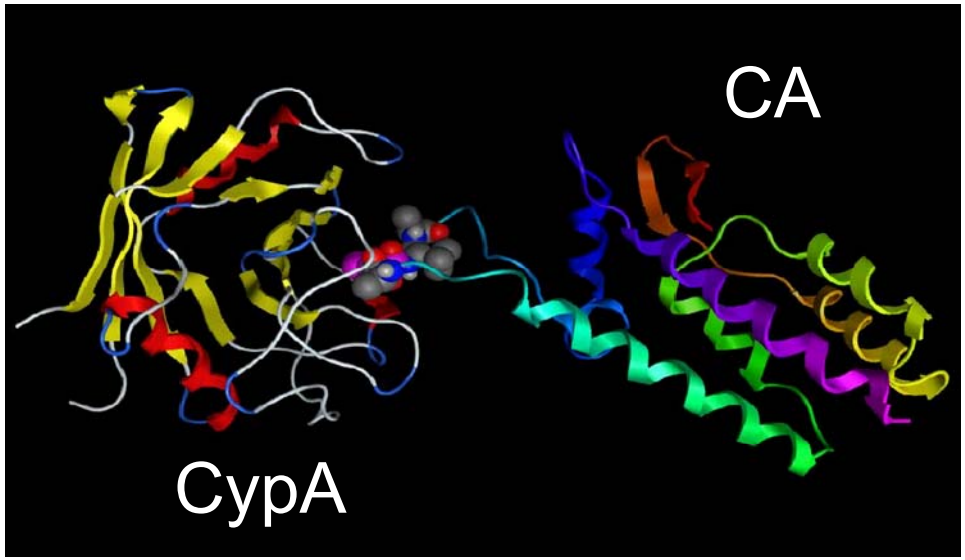


Structure of CypA and CsA
(PDB ID: 1MF8)

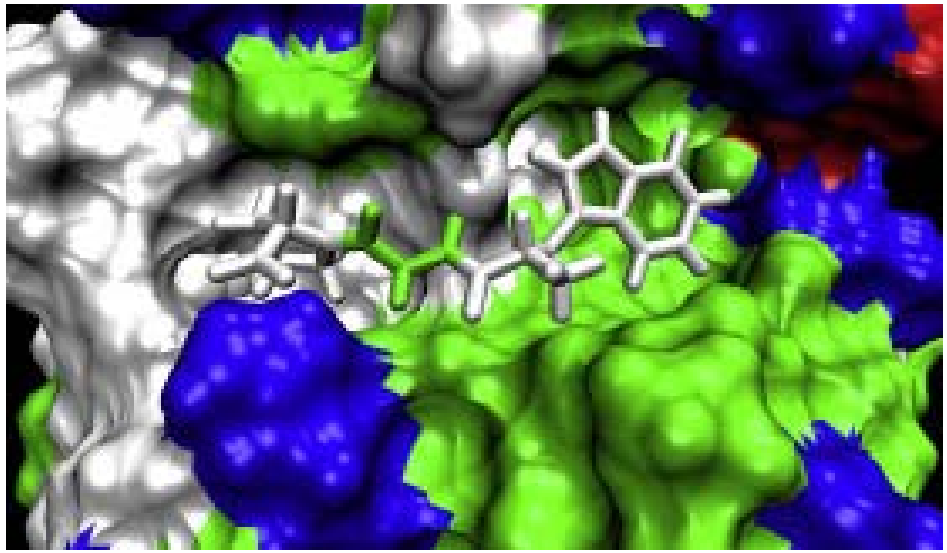


Role of CypA

- It was reported that HIV capsid protein (CA) binds to the PPIase active site of CypA, and the interaction is essential to enhance viral infectivity.[1)]
- The molecule which inhibit the interaction may have a potential to be developed as an anti-HIV drug.[2)]



Interaction between CypA and HIV capsid protein (CA).

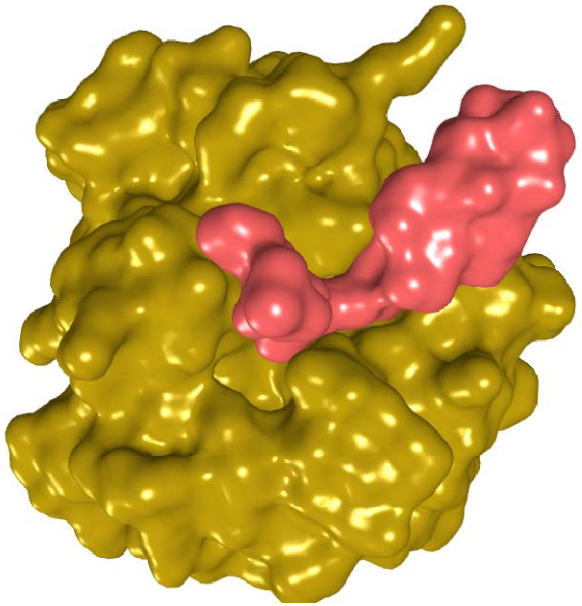


Interaction between CypA and Pro-Gly-Trp tripeptide.

1) Thali M, *et. al. Nature*, **372**, 363-365, (1994). 2) Pang X. *et. al. Eur. J. Med. Chem.* **46**, 1701-1705, (2011).

Antiviral Target of CypA

- CypA is an important host factor for a number of viruses (HCV, HBV, Influenza, SARS, Rotavirus, *etc.*) .[1)]
- However, no CypA inhibitor has been approved in clinical use for antiviral agent.
- In this study, we explored the compounds which binds to CypA using structure-based *in silico* screening.

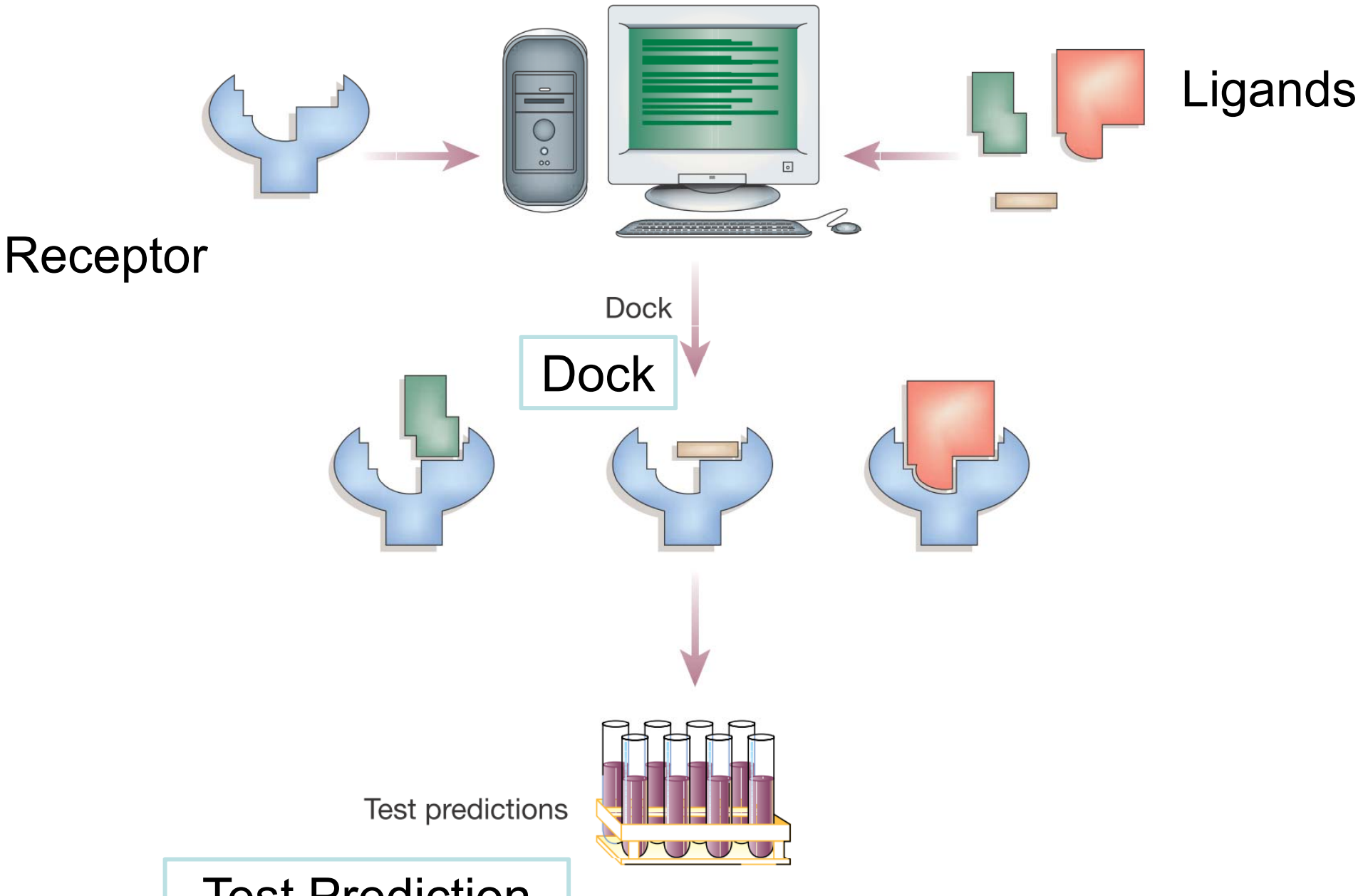


Interaction between CypA and HIV gag (partial sequence).

PDB ID: 1FGL

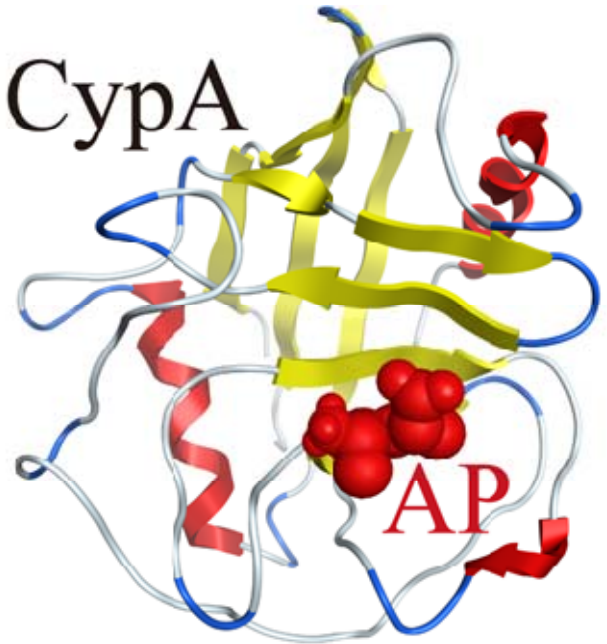
1) Liu X., *et. al. Viruses*, **5**, 182-191, (2013).

Summary of Screening



Shoichet, B. K. *Nature*, **432**, 862-865, (2004).

■Used target structure

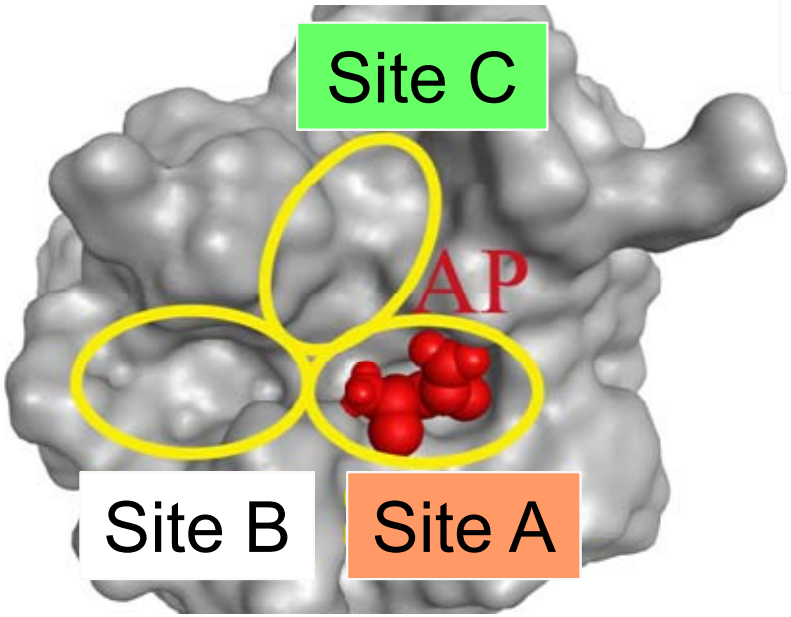


Co-crystal structure of CypA and Alanine-Proline dipeptide (AP)
(PDB entry: 2CYH, Resolution: 1.64 Å)

Preparation of protein

（Add hydrogen atom, delete the other ligand, structure optimization of the side chain atoms）

Receptor structure

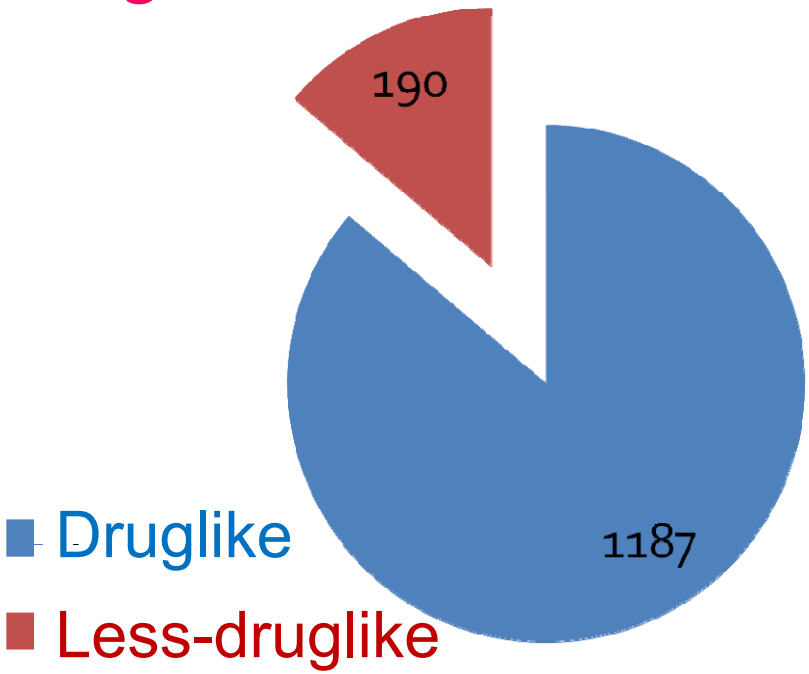


■Search for the active site

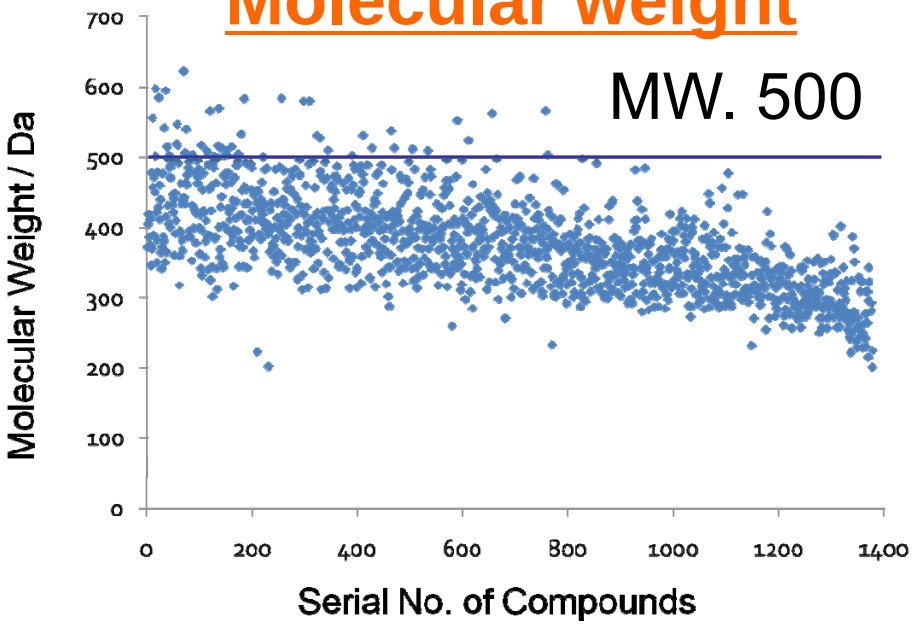
Site A, B, and C were found by MOE alpha site finder module.

Because each site is too small, all sites were set to the docking site.

Druglikeness



Molecular weight



Compound database

All-chemy compound database (1,377 molecules) obtained from ChemGenesis Co. Ltd. (http://www.chemgenesis.com/)

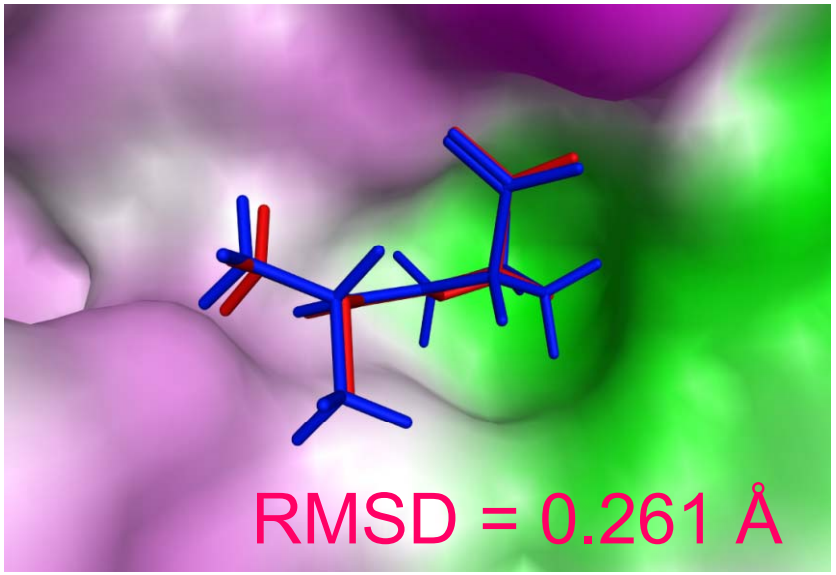
1) Convert to MOE Database format
2) Delete ions
3) Structure optimization
(Forcefield: MMFF94x)

Ligand database

- Value of Druglikeness（Lipinski's rule of 5）was calculated.

  Molecular Weight (MW) ≤ 500,
  logP ≤ 5, hydrogen donor ≤ 5,
  hydrogen acceptor ≤ 10

  are better for oral agent.



- RMSD (root mean squared deviation) represents the sample standard deviation of the differences between predicted values and observed values.

$$\mathrm{RMSD} = \sqrt{\frac{1}{N}\sum_{i=1}^{i=N}\delta_i^2}$$

Docking Scheme

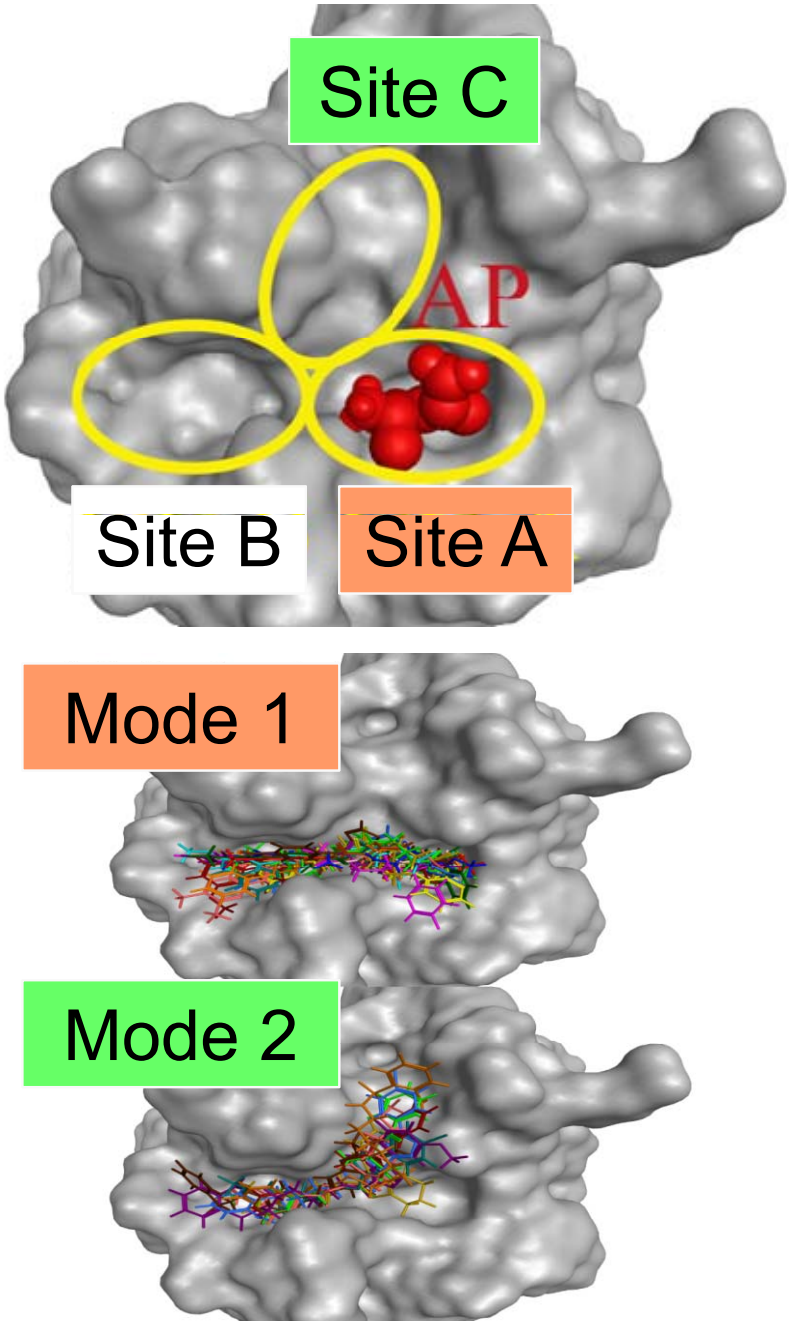
1) Generated conformations
2) Placement (Triangle Matcher)
3) Scoring (London dG)
4) Structure optimization
   (Forcefield: MMFF94x)
5) Re-scoring (London dG)

Evaluation of Docking

Ala-Pro (AP) dipeptide was re-docked into CypA to confirm whether the docking argorism is suitable for this system.

Compound database

All-Chemy database (1,377 compounds)



■Compound selection for biological assay

- The docking score was from −10.75 to −7.14, and compounds which showed less than −10 were 31. (about 2 % of 1,377)
- 29 of 31 compounds were available, which used for candidate to biological assay.

Two docking modes (**Mode 1** and **2**) were obtained from 29 compounds.

Mode 1: 20 compounds（Site A + Site B）
Mode 2: 9 compounds
（Site B and/or Site B + Site C）

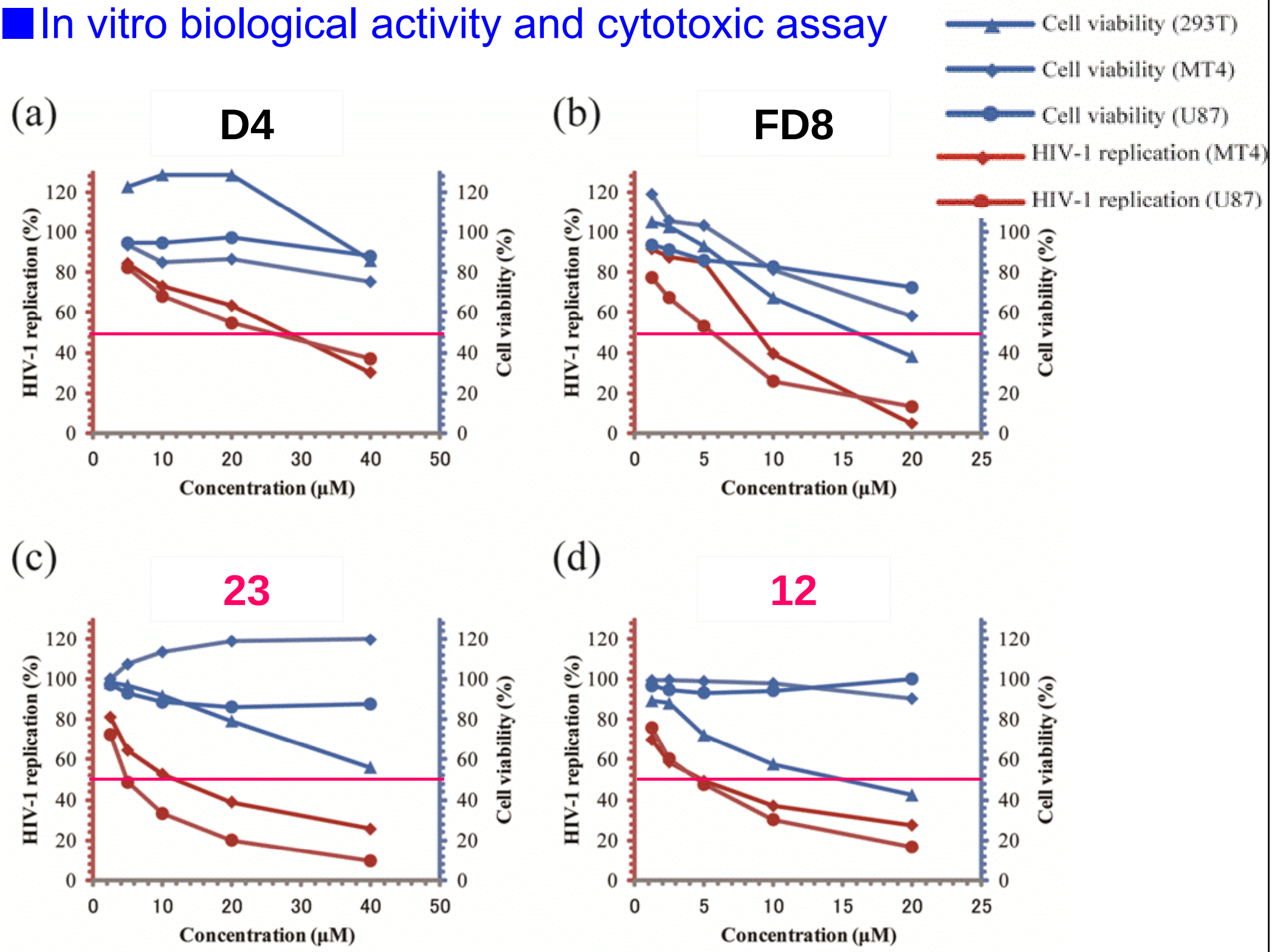
In vitro assay for anti-HIV activity and toxicity

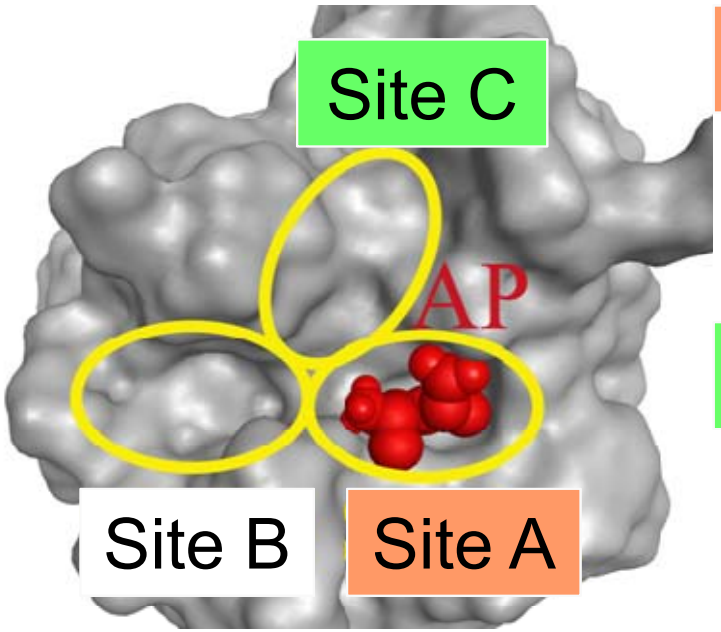
- We used 29 candidate compounds and 2 positive controls (D4 and FD8) were used for this assay.
- Biological assays were performed to determine whether the 29 compounds and the 2 positive controls exerted inhibitory effects on viral replication in the single replication cycle of HIV-1 and induced cellular toxicity.

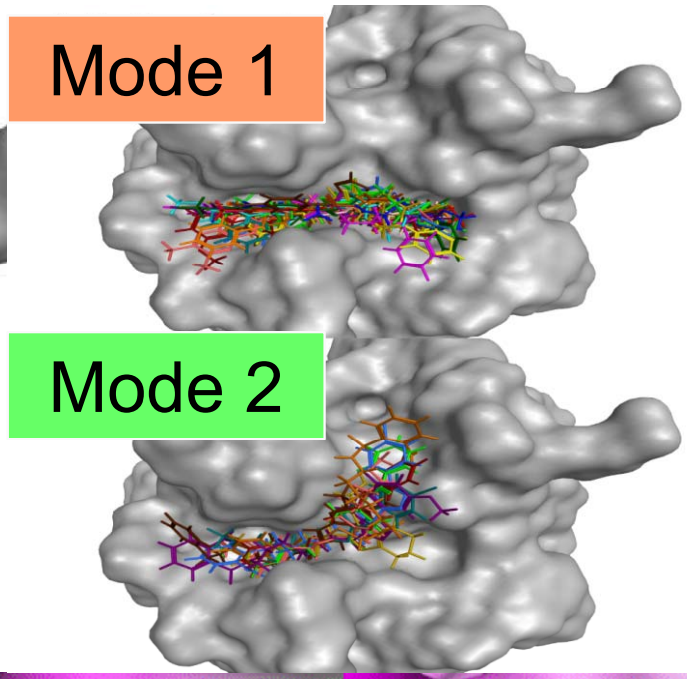
  293T cells (virus-producing cells), MT-4 (T cell line), U87.CD4.CXCR4(HIV-1 reporter cell line) were used as viral target cells.
- Cytotoxicity was evaluated by WST-1 cell growth system.

These experiments were performed by Mr.Chris and Assosiate Prof. Kameoka.

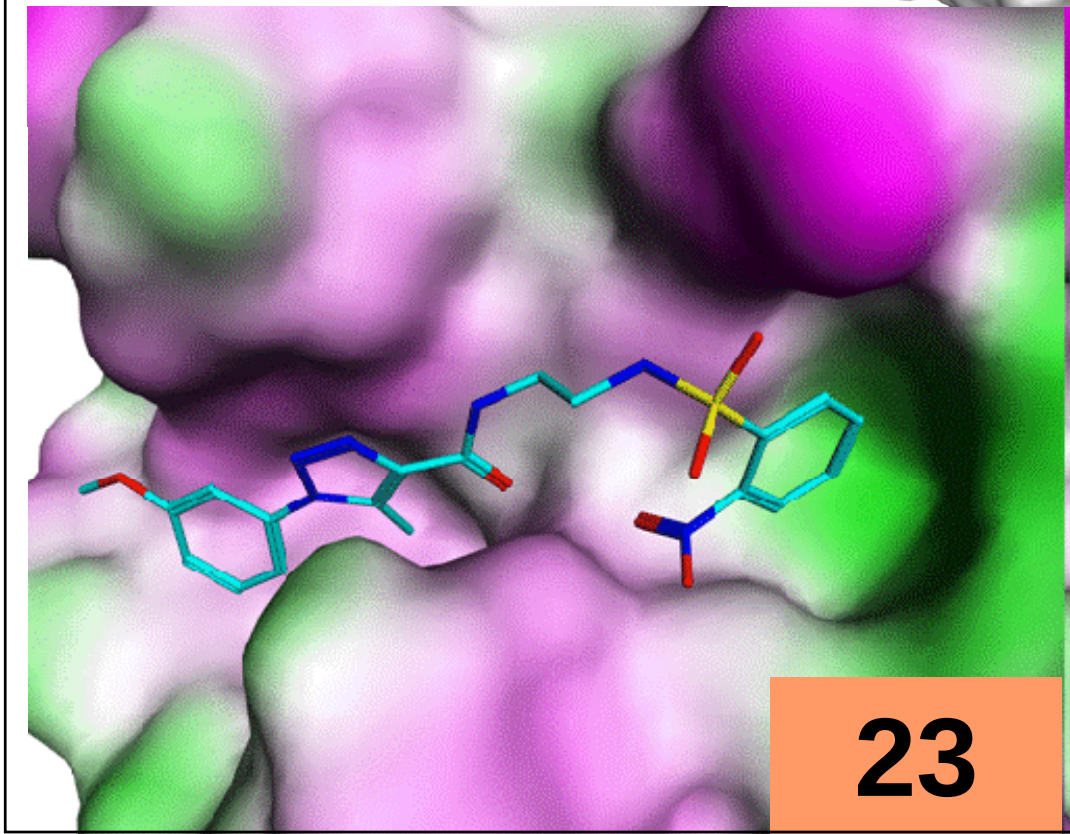
In vitro biological activity and cytotoxic assay

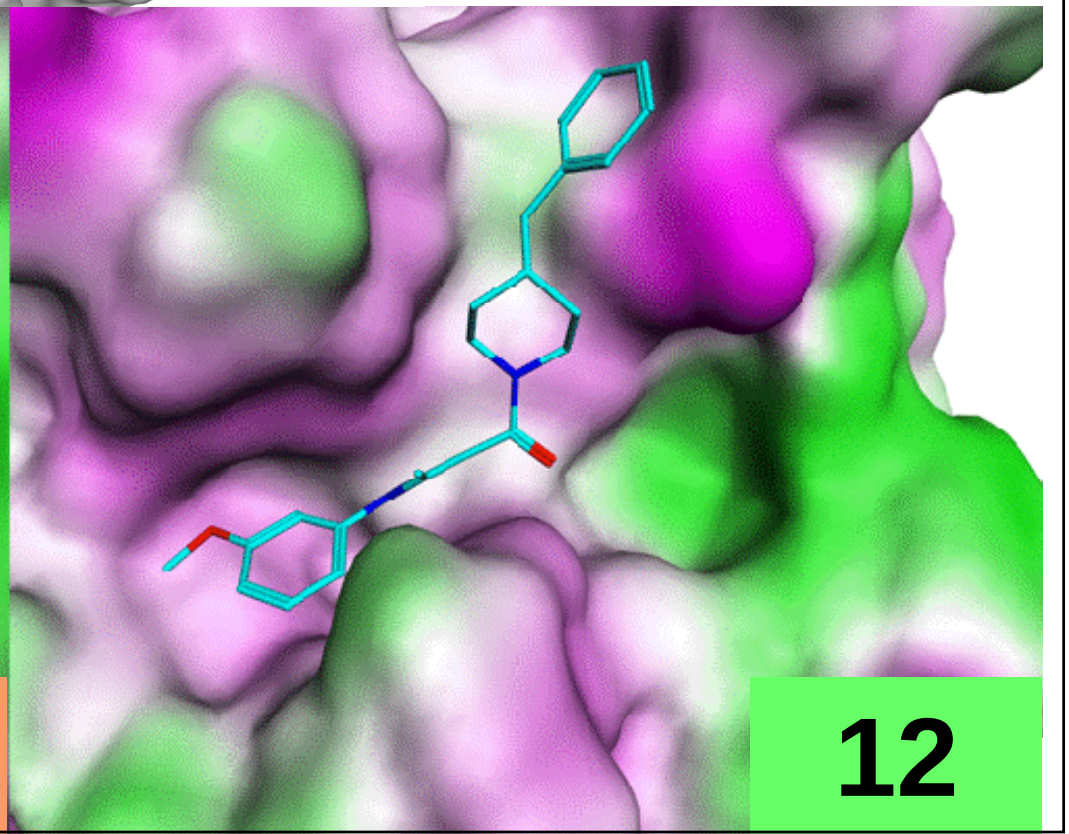




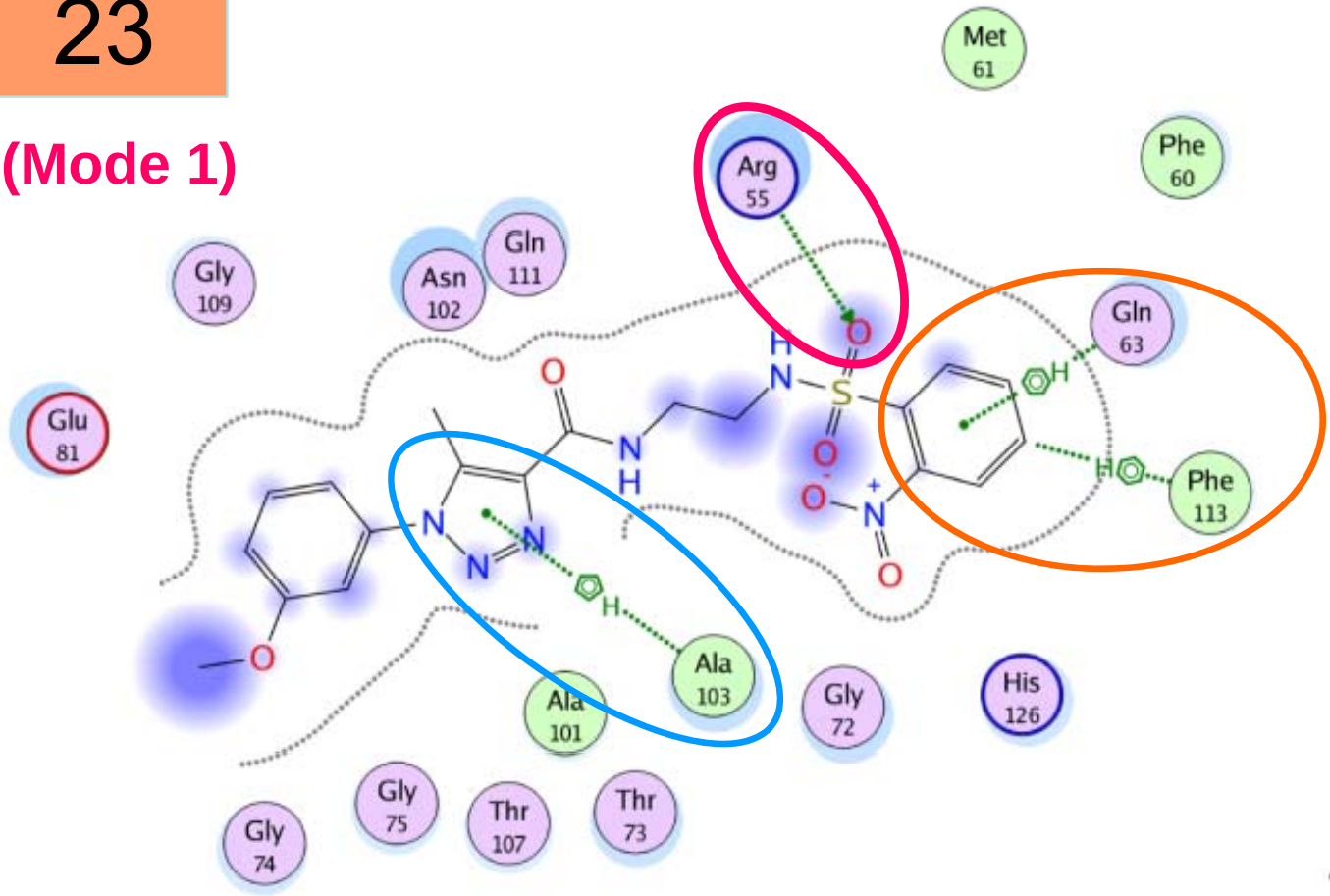


23 is the most stable pose of **Mode 1** which covered site A and B, while **12** is the most of **Mode 2** which covered site B and C.

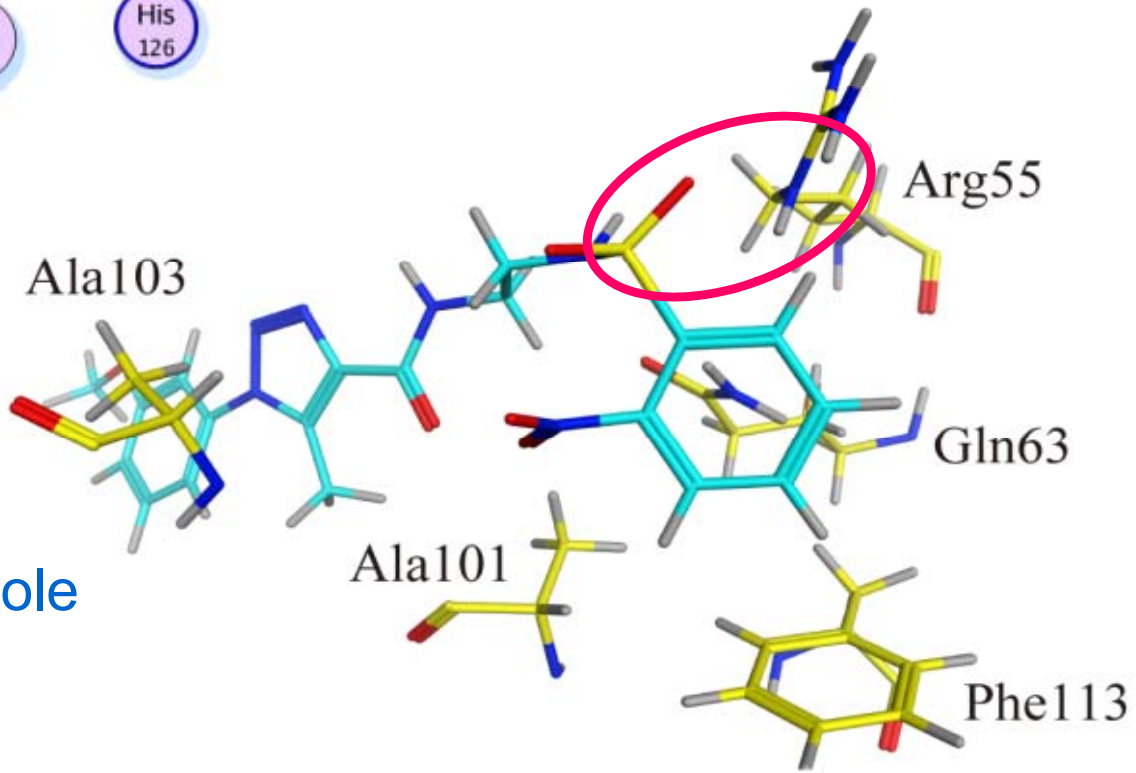




(Mode 1)



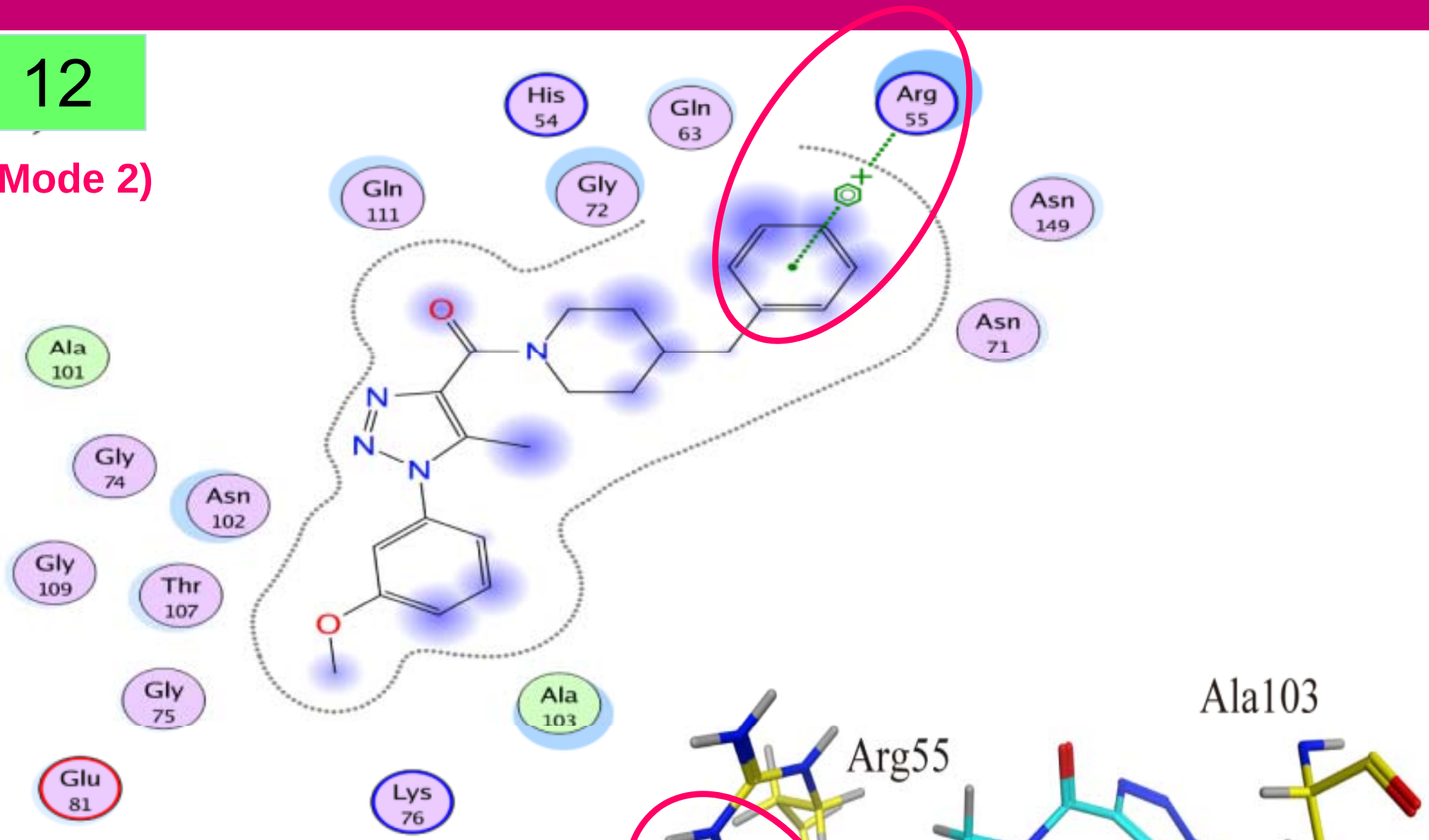
- Hydrogen bond formation between sulfonyl group and **Arg55**.

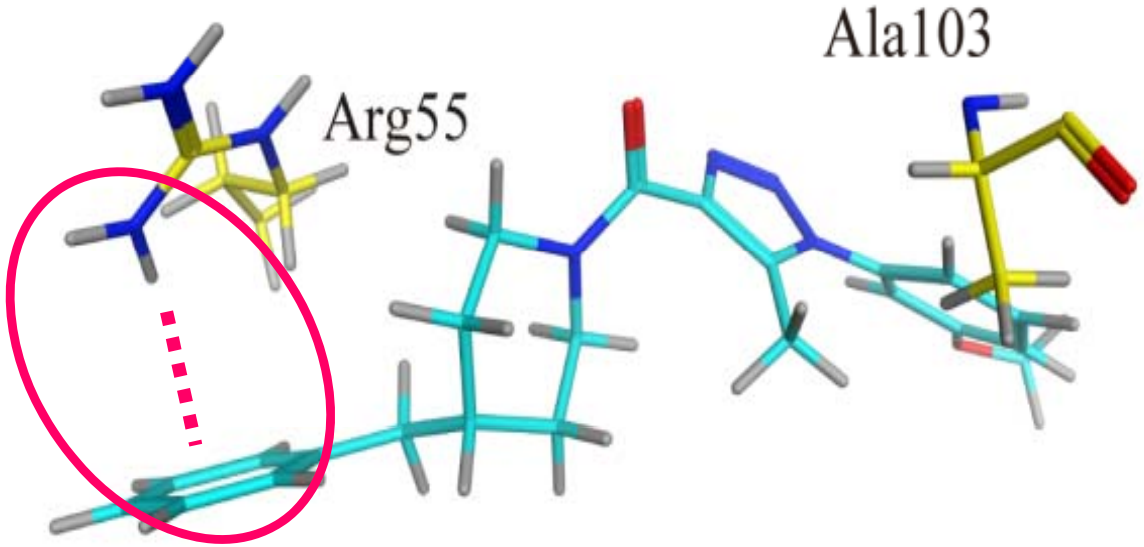


- CH-π interaction between benzene ring and **Gln63** or **Phe113**.
- CH-π interaction between triazole ring and **Ala103**.

12

(Mode 2)



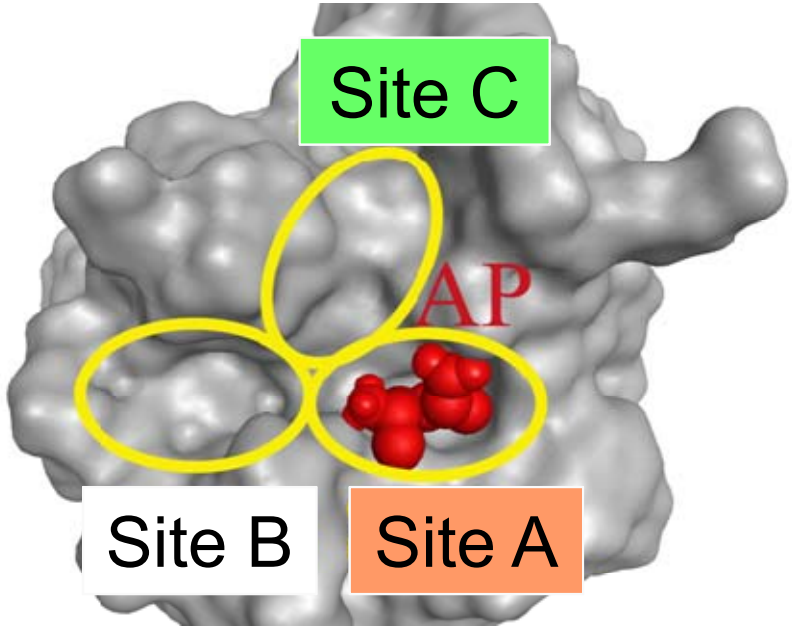


Cation-π interaction

between benzene ring of **12** and Arg55.

Future Plans

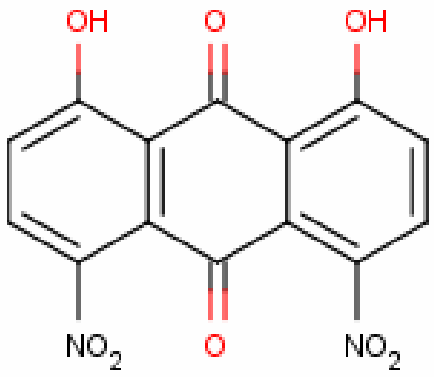
- We could not find the compound which bind to all sites. However, a novel compound may be designed by obtaining important information of the interaction from the active compounds.



- One of the screening method is ligand-based virtual screening by fingerprint and pharmacophore from the active compounds.

Molecular Fingerprint

It represents the presence or absence of a partial structure, and this is often used for similarity search of compounds.



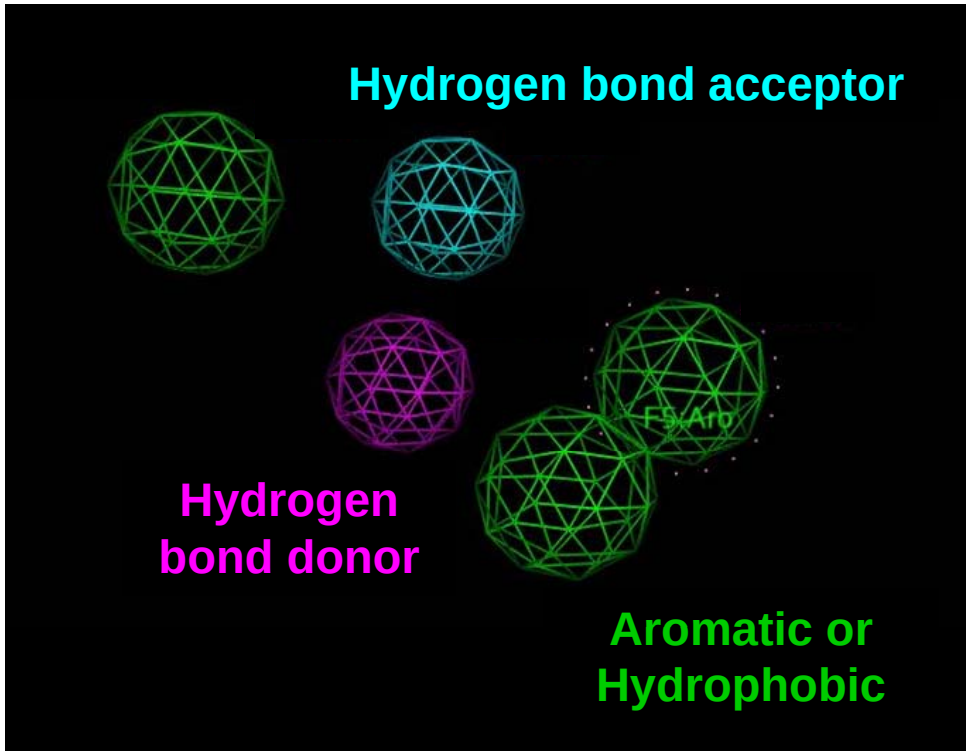
For example, carbonyl (=O), hydroxyl (-OH), and benzene ring are present, while chlorine (-Cl), amino (-NH_2), and aliphatic group are absent in this molecule.

Fingerprint of the molecule is shown below.

=O	-OH	-NO_2	Aromatic	-Cl	-NH_2	-CH_3	-CH_2-
1	1	1	1	0	0	0	0

Pharmacophore

Pharmacophore is an ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger (or block) its biological response“, and it is often used for ligand-based screening.



Conclusion

- The present work involves the discovery of HIV-1 inhibitors targeting CypA via in silico and biological screening methods.
- 29 compounds selected from a database, together with CsA, were examined for antiviral activities. Two of the compounds **12** and **23** exhibited anti-HIV-1 activities with relatively low cytotoxicity at the effective concentration inhibiting viral growth by 50%.
- Therefore, our compounds may be used as lead compounds for elucidating CypA inhibitors for clinical applications.

Thank you for your attention!