Site-specific Mutagenesis and Protein Purification of HIV-1 Protease for DEER Conformational Study

Carlos E. Billini[1,2], Ramadan Ajredini[1], Zhanglong Liu[1], Tang Tran[1], Gail E. Fanucci[1]

[1]Department of Chemistry, University of Florida PO Box 117200, Gainesville, FL 32611

[2]Department of Natural Science, University of Puerto Rico, Rio Piedras, PR 00969

Introduction

The human immunodeficiency virus type 1 (HIV-1) is a lentivirus from the Retroviridae family that starts as an RNA virus and later replicates through the DNA of the infected cells (CD4 cells). The reverse transcriptase (RT) of HIV commits significantly more errors than the human polymerase (HP), i.e. 1 error in 70 nucleotides for the RT versus 1 in 10 billion for the HP. [1] This highly mutative rhythm caused by the RT can lead to evolutionary selection against the attacks made with the inhibitor drugs. Studies suggest that the high mutation rate of HIV-1 virus greatly inhibits researchers from creating a specific drug to control the growth rate of the virus. [2] Among the current HIV-1 drugs, protease inhibitors (PIs) are the most potent to the virus, but the virus continuously develops drug resistant mutations.

To combat this problem, understanding the drug resistance mechanism, especially the structure and dynamics of HIV-1 protease, is crucial to designing the next generation PIs. L90M was identified in 1995 as drug resistant mutant for nelfinavir and saquinavir. Cross drug resistance to nelfinavir can also develop in the presence of other two mutations (D30N and N88D). In this study, we will generate drug resistant mutants by using site-specific mutagenesis. Purified HIV-1 protease will be spin labeled for double electron-electron resonance (DEER) conformational study.

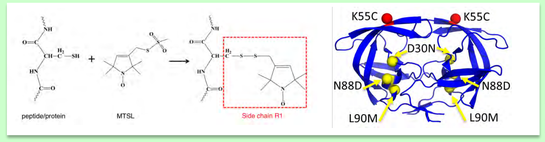


Figure 1. Spin label reaction scheme and drug resistant mutations studied in this study. pdb: 2pk5

Table 1. Inhibition constant (Ki) of the mutants (Coman et al. 2008)

SUBTYPE-MUTANT	RTV	NFV	SQV
B	0.07 (0.01)	1.7 (0.4)	2.2 (0.3)
B-L90M	0.42 (0.07)	5.4 (0.5)	7.5 (0.9)
B-D30N/L90M	0.32 (0.05)	88 (10)	15 (2)
B-N88D/L90M	0.71 (0.13)	12 (1)	49 (4)
B-30/88/90	1.0 (0.2)	88 (7)	47 (6)

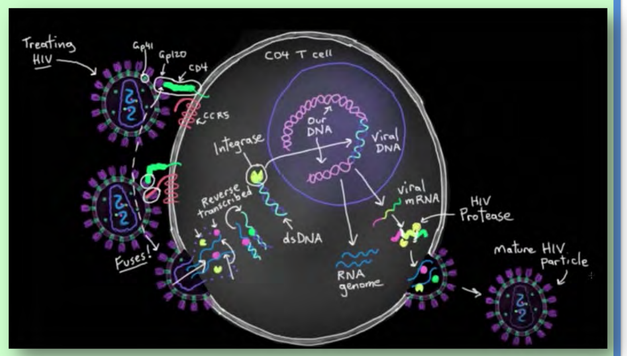


Figure 2: Reproductive cycle of HIV (khan academy, 2016)

Site-specific Mutagenesis

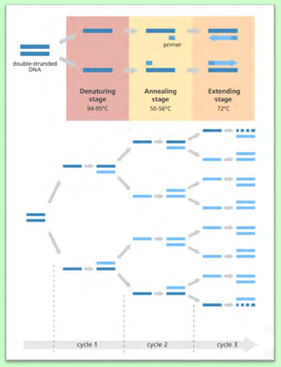
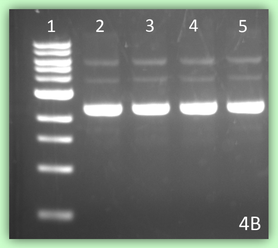


Figure 3. The scheme of PCR reaction for site-specific mutagenesis

D30N	Forward: 5′ GCTGAATACCGGTGCAGATAATACCGTTATCGAGGAAATG 3′ Reverse: 5′ CATTTCCTCGATAACGGTATTATCTGCACCGGTATTCAGC 3′
N88D	Forward: 5′ CGGTTAACATCATCGGCCGCGATCTGCTGACTCAGATTGGCGC 3′ Reverse: 5′ GCGCCAATCTGAGTCAGCAGATCGCGGCCGATGATGTTAACCG 3′
L90M	Forward: 5′ CATCGGCCGCAACCTGATGACTCAGATTGGCG 3′ Reverse: 5′ CGCCAATCTGAGTCATCAGGTTGCGGCCGATG 3′

4A



1: Ladder
2: Reference
3: L90M
4: D30N
5: N88D

Figure 4. Primer designed for D30N, N88D and L90M in 4A. DNA electrophoresis gel indicate that product vectors in 4B

Table 2: Amino acid sequence of B_{si} protease. Yellow residues correspond to the mutation L90M and blue for K55C.

	10 20 30 40 50 60 70 80 90 99
B (reference)	PQITLWKRPL VTIKIGGQLK EALLNTGADD TVIEEMSLPG RWKPKMIGGI GGFICVRQYD QIIIEIAGHK AIGTVLVGPT PVNIIGRNLL TQIGATLNF
B-L90M-1	PQITLWKRPL VTIKIGGQLK EALLNTGADD TVIEEMSLPG RWKPKMIGGI GGFICVRQYD QIIIEIAGHK AIGTVLVGPT PVNIIGRNLL TQIGATLNF
B-L90M-2	PQITLWKRPL VTIKIGGQLK EALLNTGADD TVIEEMSLPG RWKPKMIGGI GGFICVRQYD QIIIEIAGHK AIGTVLVGPT PVNIIGRNLM TQIGATLNF
B-L90M-3	PQITLWKRPL VTIKIGGQLK EALLNTGADD TVIEEMSLPG RWKPKMIGGI GGFICVRQYD QIIIEIAGHK AIGTVLVGPT PVNIIGRNLM TQIGATLNF

Table 3: DNA sequence align with the protein sequence of the L90M mutant.

10 20 30 40
CCA CAA ATC ACT CTG TGG AAA CGT CCG CTG GTC ACC ATT AAA ATT GGC GGT CAA CTG AAA GAA GCG CTG CTG AAT ACC GGT GCA GAT AAT ACC GTT ATC GAG GAA ATG AGC CTG CCG GGT
P Q I T L W K R P L V T I K I G G Q L K E A L L N T G A D N T V I E E M S L P G
50 60 70 80
CGT TGG AAA CCT AAA ATG ATT GGC GGT ATT GGT GGT TTC ATT TGT GTG CGC CAG TAC GAC CAG ATC ATT ATC GAA ATC GCC GGC CAC AAG GCA ATT GGT ACC GTG CTG GTT GGC CCG ACC
R W K P K M I G G I G G F I C V R Q Y D Q I I I E I A G H K A I G T V L V G P T
90 99
CCG GTT AAC ATC ATC GGC CGC AAC CTG ATG ACT CAG ATT GGC GCC ACG CTG AAC TTC
P V N I I G R N L M T Q I G A T L N F

Protein Expression

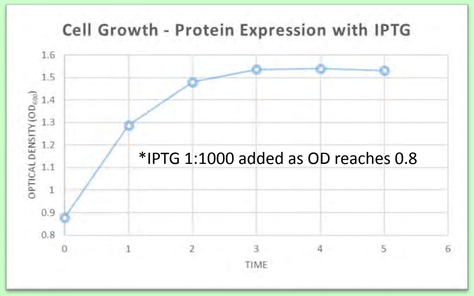
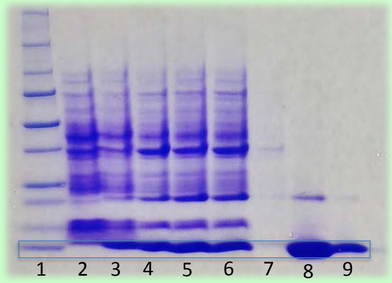


Figure 5. cell growth of L90M while the IPTG was induced

Protein Purification



1: Ladder	6: After IPTG (Overnight)
2: Before IPTG (0 hrs)	7: Supernatant
3: After IPTG (1 hrs)	8: Q column
4: After IPTG (2 hrs)	9: Protein purification
5: After IPTG (3 hrs)	

Figure 6. SDS-PAGE indicating the protein expression with time and the purified L90M mutant

Mass Spectrometry

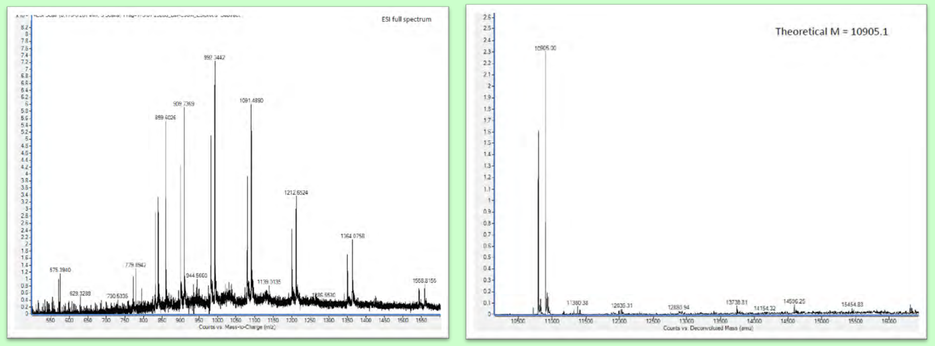


Figure 7. The mass spectrometry indicates that purified spin-labeled L90M was achieved at MW of 10905, while MW of 10797 denotes the L90M mutant without the spin label.

Future Work & Conclusion

There are three mayor mutations for the resistance of Nelfinavir (NFV) which are L90M, D30N and N88D. Site-directed-mutations, side directed spin label (SDSL), and double electron-electron resonance (DEER), can be used to identify the conformation changes of the HIV-1PR drug resistance. By analyzing these conformations, it can help us determine the reason for the lower potency of the inhibitory drugs.

Acknowledgements & Reference

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