

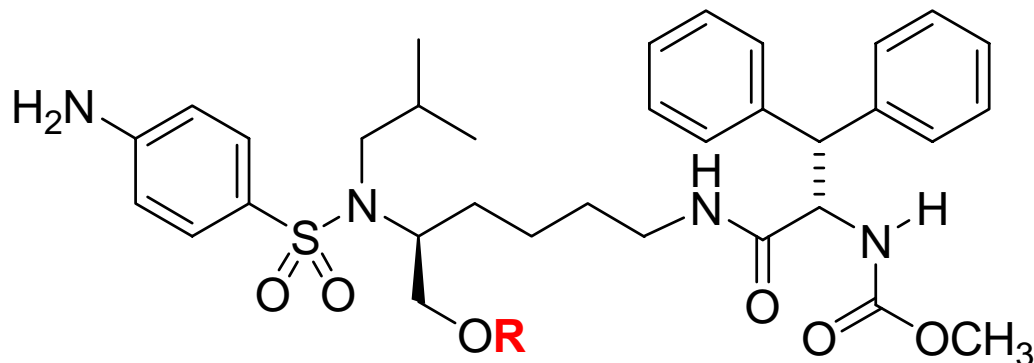
A Structural Basis for Resistance to PL-100

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PL-100 Introduction



R = PO₃Na₂: PPL-100
R = H: PL-100

- A novel lysine-based scaffold for HIV protease inhibition discovered by Ambrilia Biopharma and licensed to Merck
- Reversible inhibitor of HIV protease $K_i = 36 \text{ pM}$
- Protein adjusted EC₉₀ for WT protease = 90 nM
- For resistant virus = 140 nM

Cross Resistance

Fold-Change (FC) of compounds against 63 multi-PI-resistant strains

	Atazanavir	Lopinavir	PL-100
Median FC	15.6	17	3.8
Mean FC	25.7	31.3	8.7
%FC<2.5	16	10	37
%FC<10	38	37	78
%FC>50	22	19	3

- PL-100 displays favorable cross-resistance profile in comparison to current marketed PIs
- PL-100 has the potential for significant clinical efficacy in treatment-experienced patients or patients newly infected with drug-resistant strains

Resistance Selection experiments

Week	PL-100 (Conc μ M)	New mutations
8	(0.1)	T80I
22	(1.0)	T80I
25	(2.5)	K45R/M46I/T80I/P81S
48	(5.0)	K45R/M46I/T80I/P81S

It takes time to raise significant resistance to PL-100

Site directed cross resistance and *in vitro* cross resistance – FC in EC₅₀

Virus	Rep Cap ¹	PL-100	ATV	LPV
WT	++++	1	1	1
K45R	ND	1.51±0.39	0.91	1.62
M46I	ND	0.81±0.18	0.43	0.86
T80I	++++	2.25±0.52	0.48	0.39
P81S	-	NA	NA	NA
K45R/M46I	+++	0.98±0.32	NA	NA
K45R/P81S	-	NA	NA	NA
M46I/P81S	-	NA	NA	NA
T80I/P81S	-	NA	NA	NA
K45R/M46I/P81S	++	NA	NA	NA
K45R/T80I/P81S	-	NA	NA	NA
M46I/T80I/P81S	+	NA	NA	NA
K45R/M46I/T80I/P81S	+++	10.82±2.25	2.70	0.63

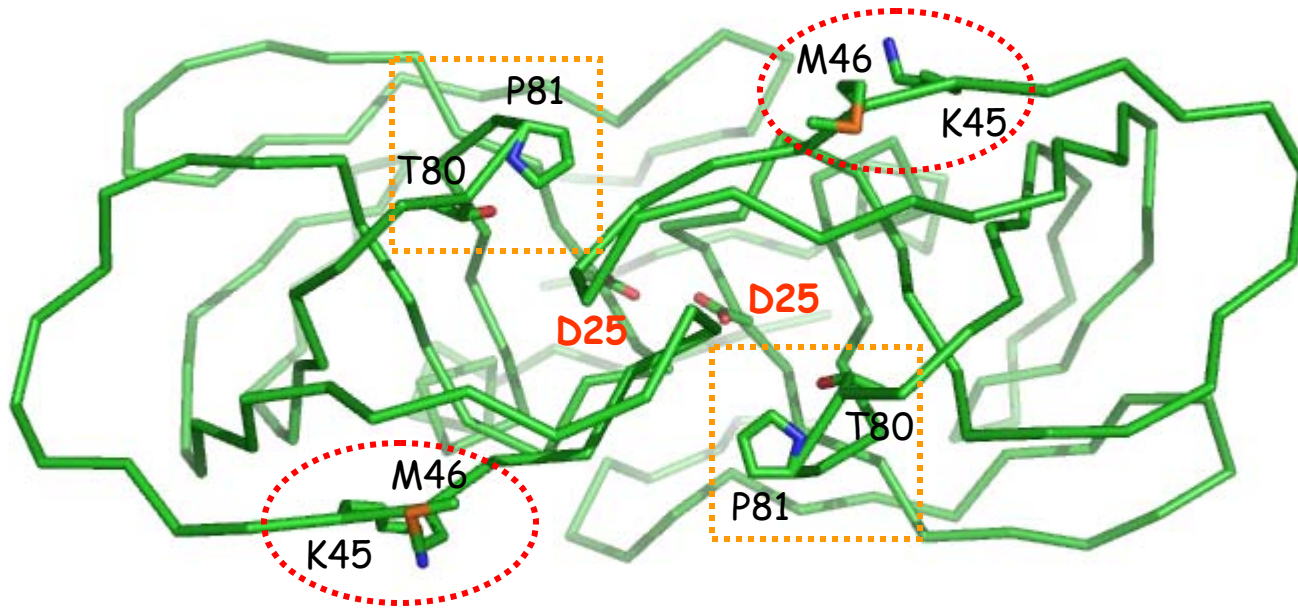
¹As determined by standard replication kinetics in MT4 for 7 days

²As determined by MTT assay in MT4, 6 days post infection

NA; Not available, since viral production did not allow for further testing

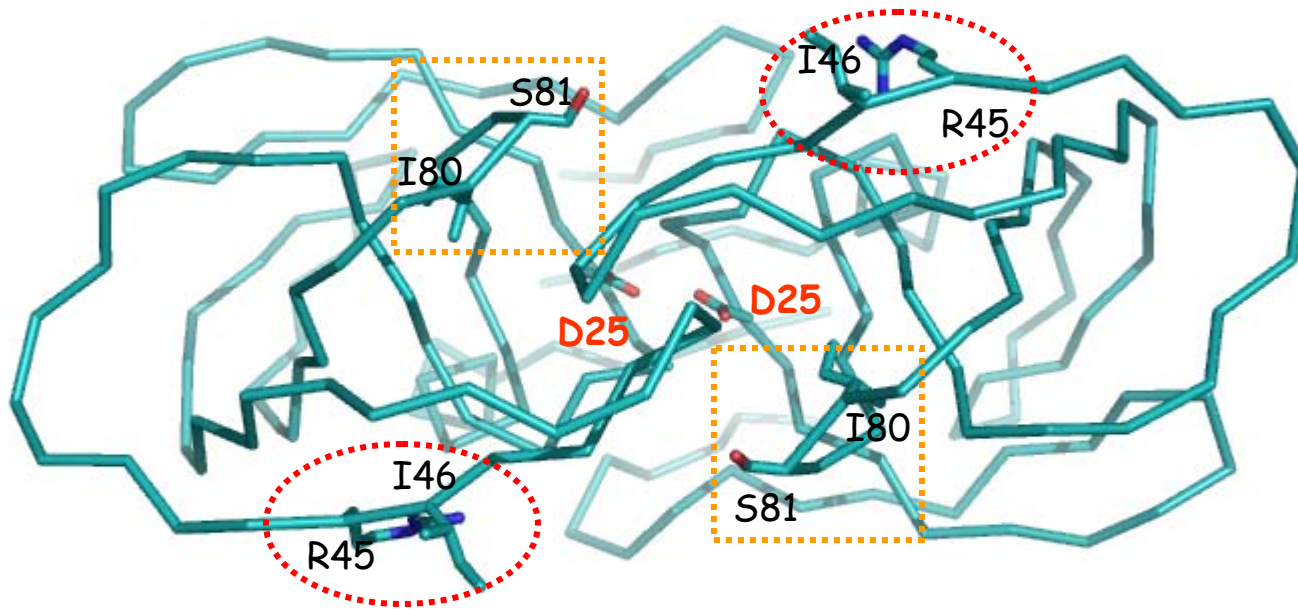
- Single mutations confer a low level of resistance (~2.5FC)
- a moderate resistance (10FC) is conferred in the presence of four mutations

PL-100 Resistant Mutations



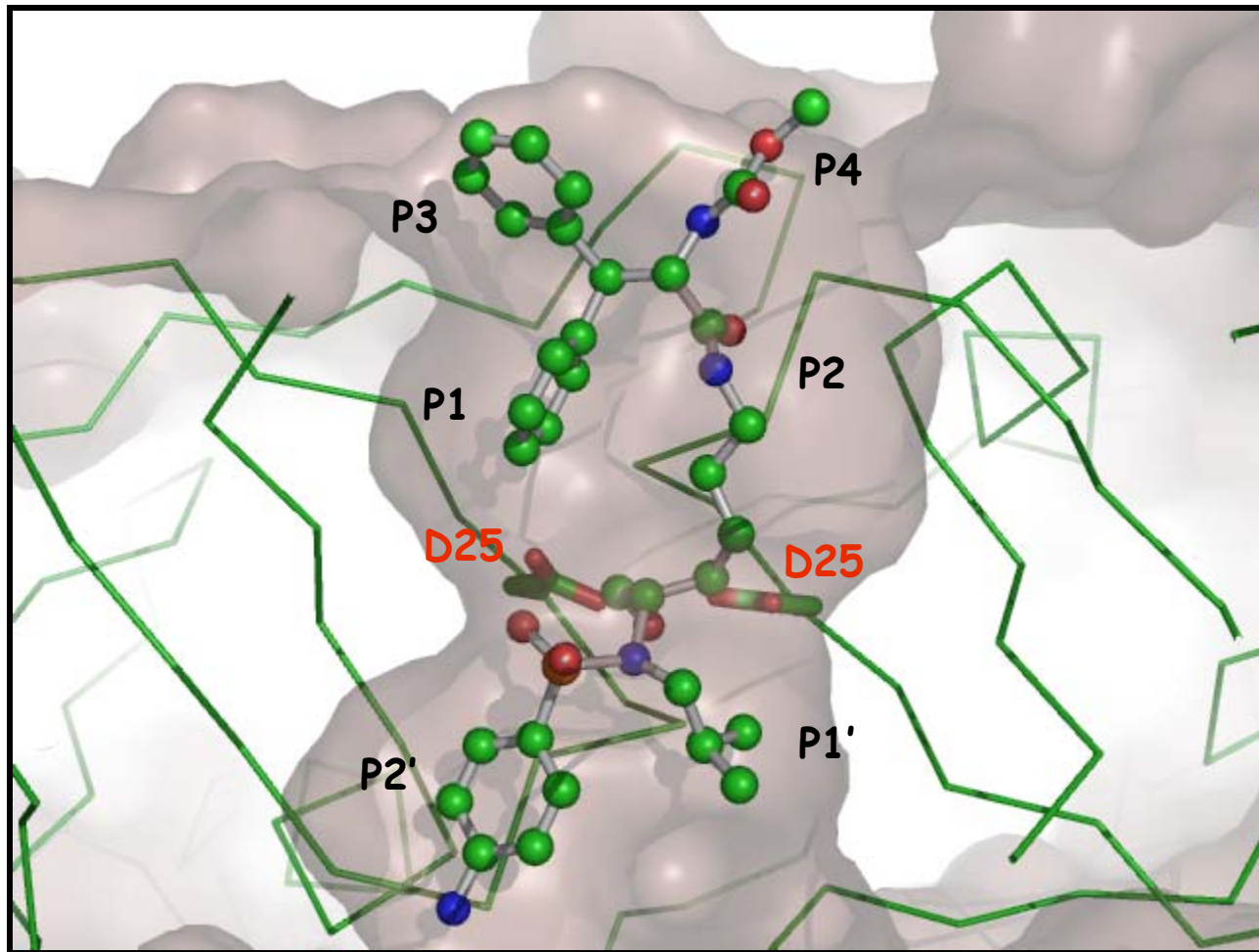
Wild-type HIV-1 protease

PL-100 resistant mutations

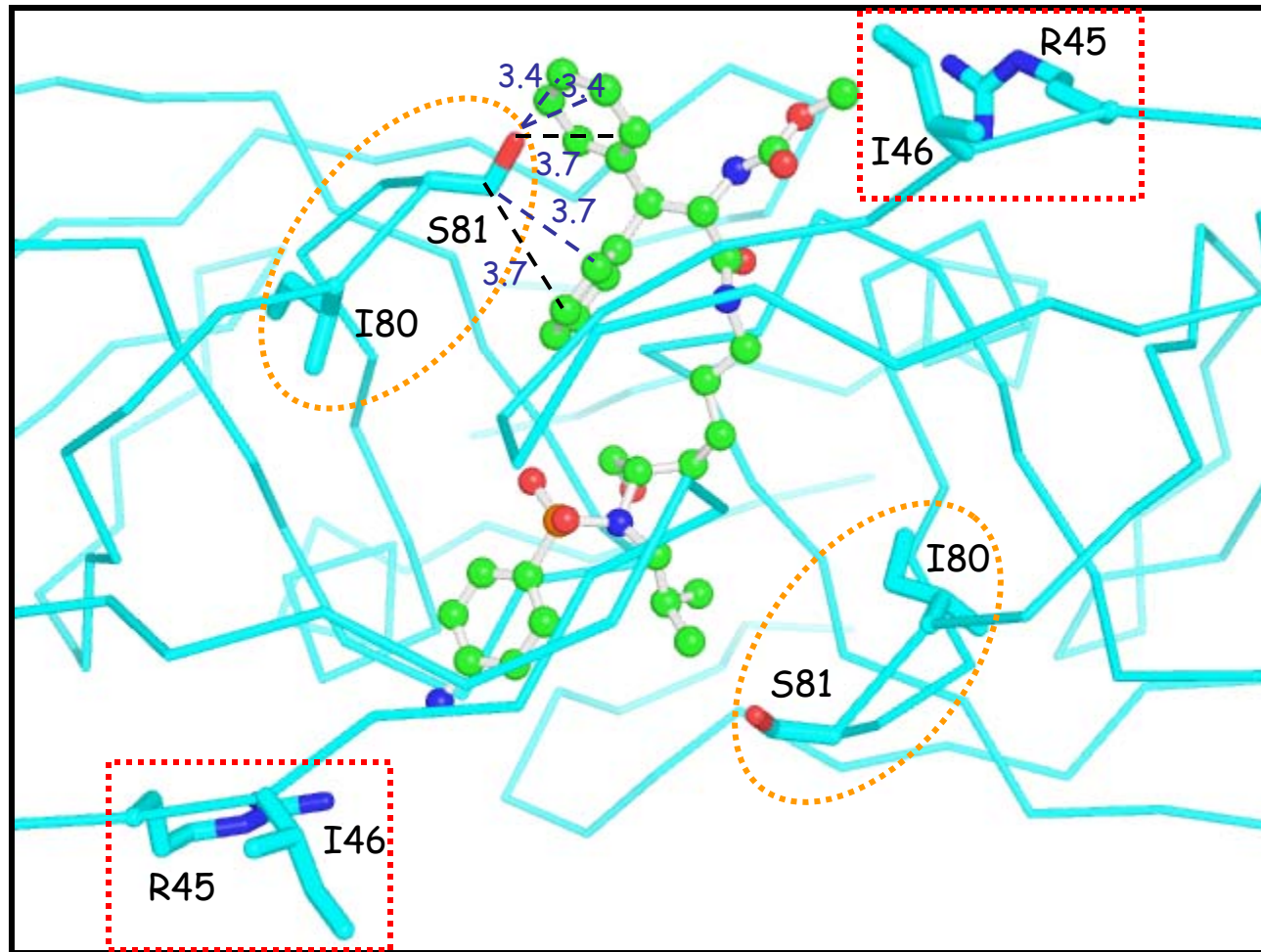


PL-100 Resistant HIV-1 protease

Model of PL-100 in WT protease

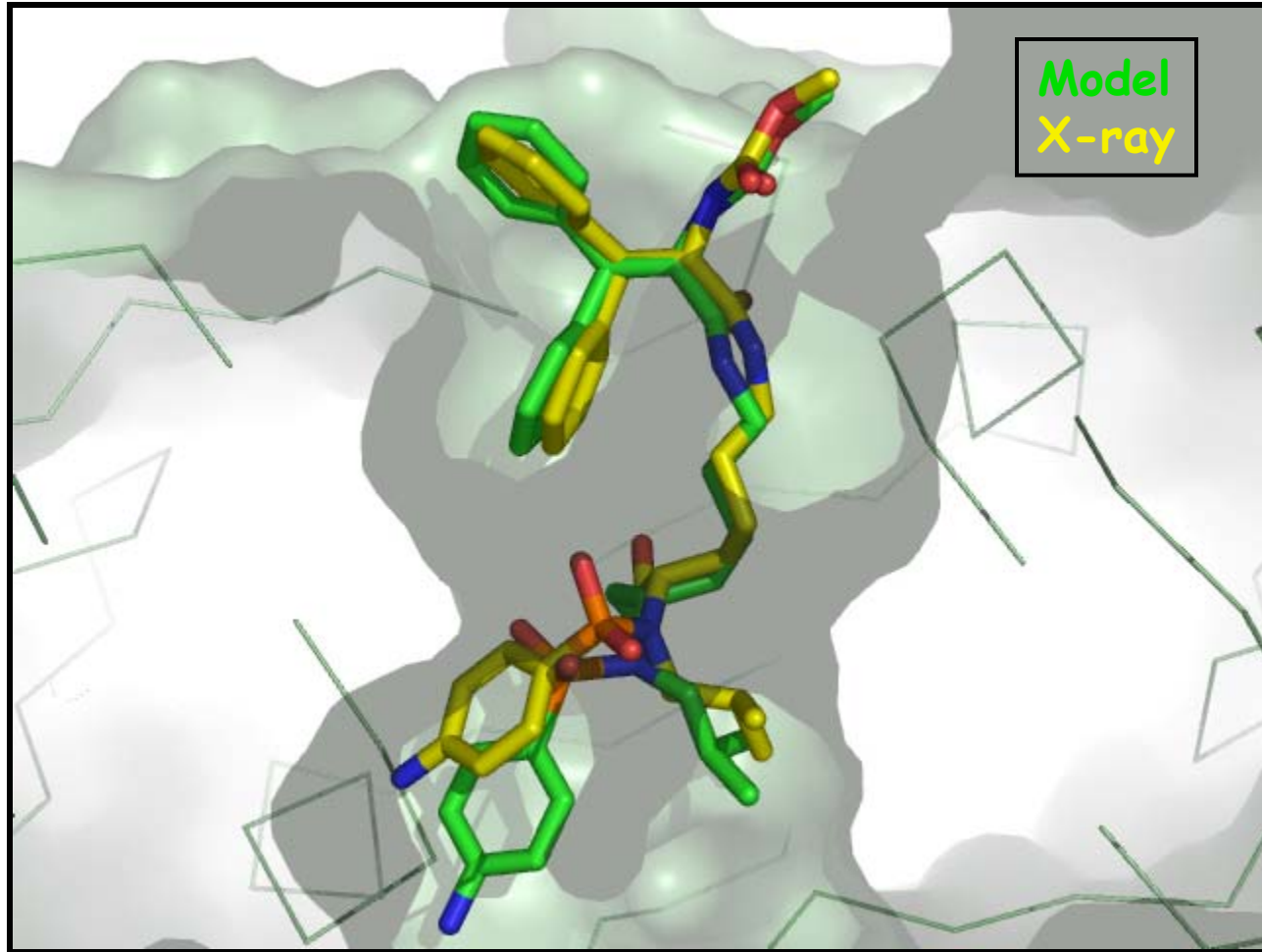


Model of PL-100 in mutant protease

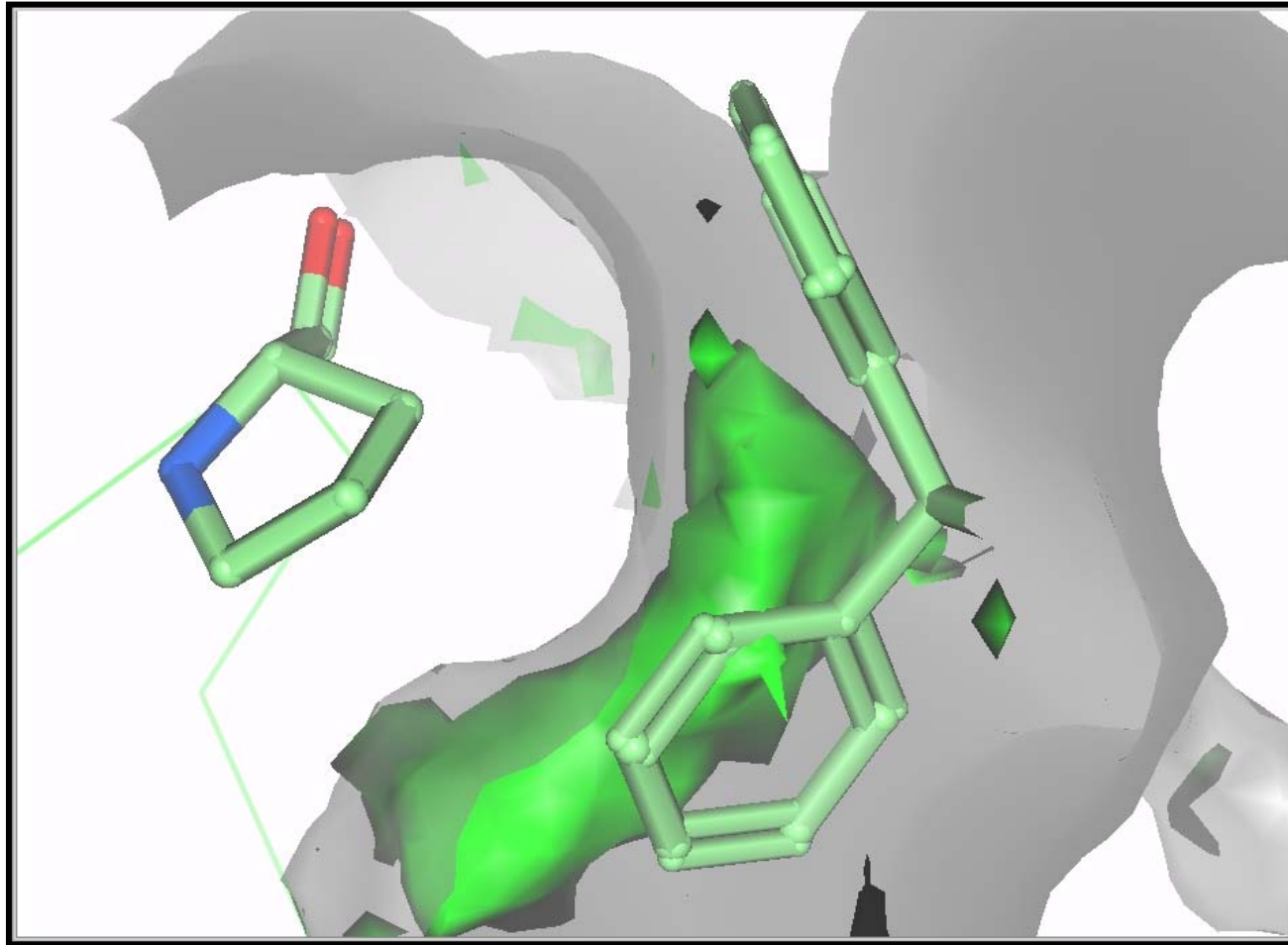


Only S81 is < 4 angstroms from molecule

Comparison of model to X-ray

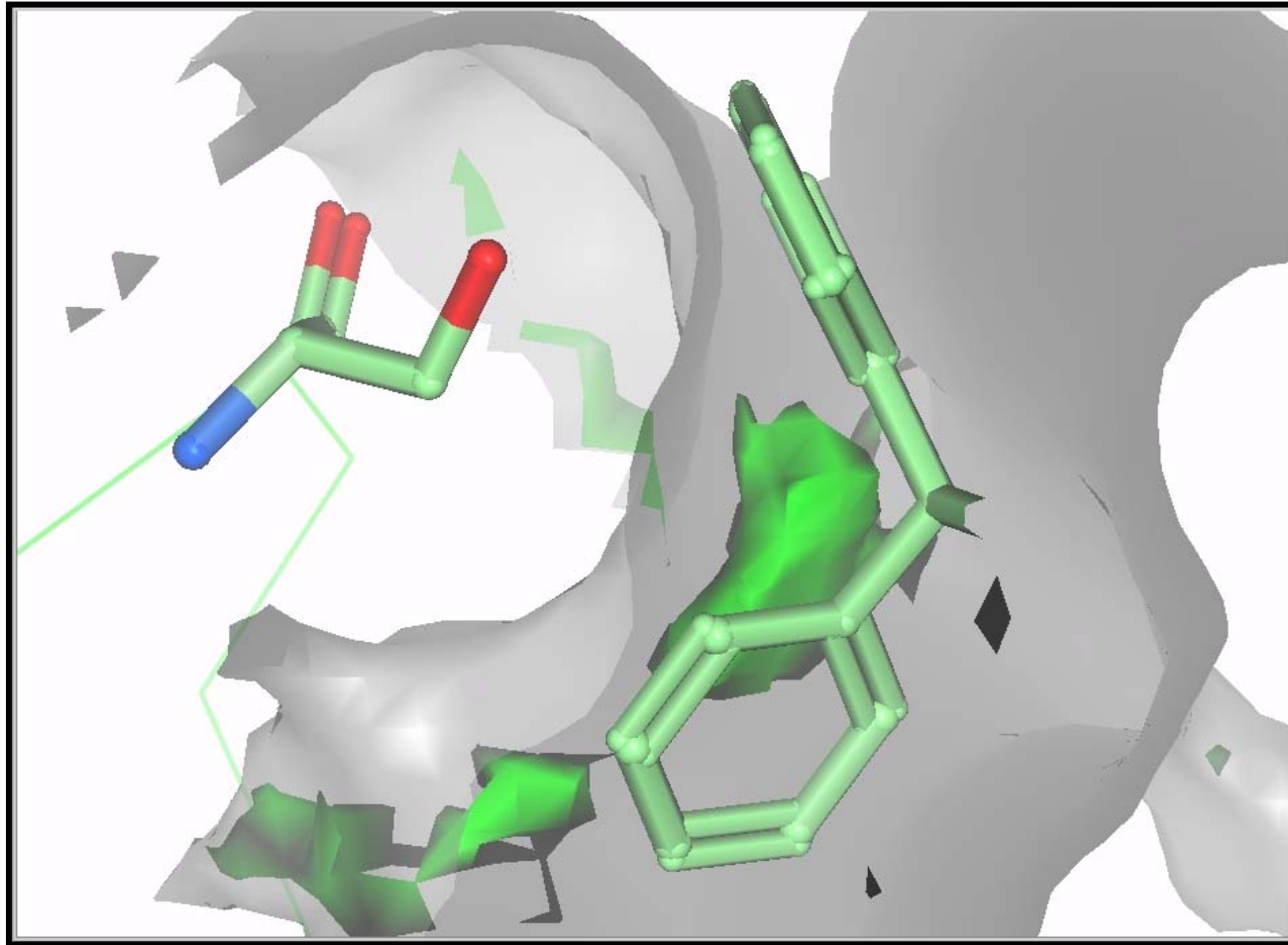


P81S mutation affects binding of di-Phe



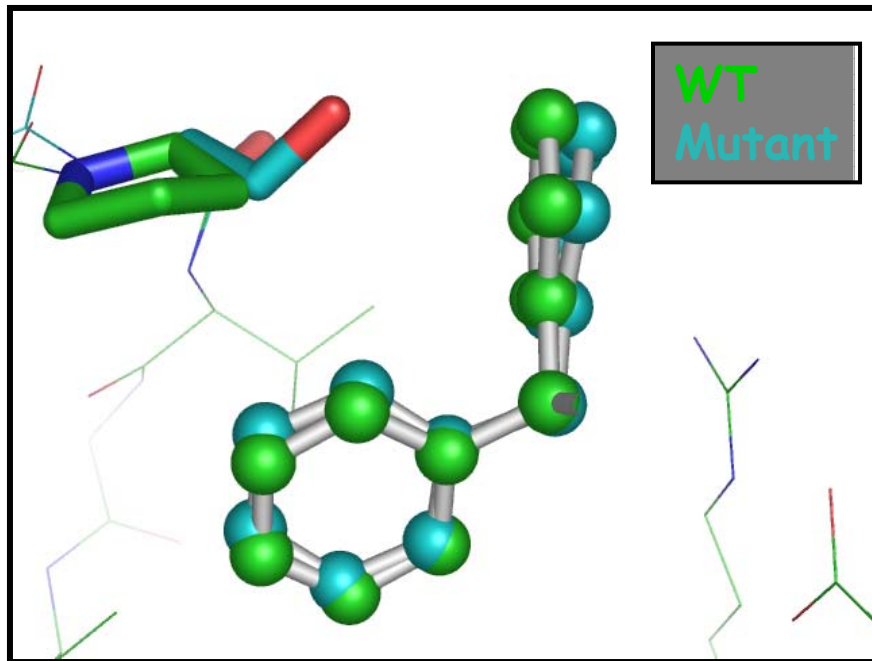
Di-Phenyl groups interact with the hydrophobic space generated by proline

P81S mutation affects binding of di-Phe



P81S takes away available hydrophobic space and leaves a hydrophilic interaction

Calculating the interaction energy of PL-100 in models of the WT and mutant enzymes



	WT	Mutant	Δ
Energy	-53.9	-52.5	1.4
XScore	7.66	7.54	1.12

- The energy difference suggests ~10 fold difference in activity
- The cell based fold-change for the quadruple mutant is ~10 fold

Conclusions

- PL-100 is a potent compound with a low propensity for resistance
- It takes six months of passaging experiments to raise a moderate level of resistance
- A model in the WT and mutant enzyme suggests that the diphenyl alanine is primarily responsible for the lower activity due to the P81S mutation
- An X-ray structure of PL-100 with WT protease confirms the modeling experiment
- This information should aid us in designing better inhibitors and in understanding the resistance of this class