Structural Perspectives on Drug Resistance

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What have we learned from 20 years and hundreds of crystal structures of HIV protease?

A. Insights into Molecular Mechanisms of Resistance
   High to atomic resolution crystal structures of mutants reveal structural alterations that correlate with altered protease activity, stability and inhibition.

   with John Louis; Jozsef Tozser

B. Strategy to Combat HIV Drug Resistance
   Antiviral inhibitors designed to target resistant protease by introducing new polar interactions with conserved structural elements.

   with Arun Ghosh; Hiroaki Mitsuya
Database of ~60 HIV Protease Structures


<table>
<thead>
<tr>
<th>Structures</th>
<th>Resolution (Å)</th>
<th>R-factor %</th>
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<tr>
<td>20 Complexes with substrate analogs</td>
<td>1.1-2.2</td>
<td>10-22</td>
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<tr>
<td>1 Unliganded structure</td>
<td>1.35</td>
<td>16</td>
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<td>2 Complexes with reaction intermediates</td>
<td>1.5</td>
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<td>Complexes with Antiviral Inhibitors</td>
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<tr>
<td>12 with Darunavir</td>
<td>0.84-1.5</td>
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<td>6 with Saquinavir</td>
<td>0.97-1.25</td>
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<td>6 with Indinavir</td>
<td>1.1-1.4</td>
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<td>5 with GRL-98065</td>
<td>1.1-1.6</td>
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<tr>
<td>3 with GRL-06579A, -0105A, -0255A</td>
<td>1.0-1.35</td>
<td>15</td>
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<td>3 HIV-2 PR Complexes with Darunavir, GRL-06579A, GRL-98065</td>
<td>1.2</td>
<td>14-16</td>
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</table>
Advantage of High Resolution X-ray Crystallography

Highly accurate atomic positions at ~1 Å resolution

Increasing significance of changes in distances

> 1 Å  > 0.3 Å  > 0.1 Å

2Fo-Fc  2.2 Å    1.5 Å    0.84 Å

improving resolution

Arg57  Val56  Tyr59  Gln58  Asp60  Arg57
Multiple Conformations in Atomic Resolution Structures

Catalytic Aspartates in V82A/Saquinavir at 0.97 Å Resolution

V82A/Darunavir at 1.1 Å Resolution
Occupancy: 0.6/0.4

Asp25
Asp25’

Catalytic Aspartates in V82A/Saquinavir at 0.97 Å Resolution

Asp30
DRV
Strategy for Drug Design Derived From Conserved Hydrogen Bond Interactions

Important main chain-main chain interactions connect inhibitor with protease flaps and residues 25-29

PR-p2/NC at 1.4Å
Tie et al., FEBS J. 2005
Verification of Darunavir Design

Saquinavir designed as substrate mimic to target wild type PR

PR/SQV structure
Saquinavir forms 3 H-bonds with the PR main chain atoms - nM affinity.
(Tie et al., Proteins, 2007.)

Darunavir designed with more H-bonds with main chain PR atoms to target resistant PR mutants

PR/DRV structure
Darunavir forms 6 H-bonds with the PR main chain atoms - pM affinity

Collaboration with Arun Ghosh
Designs for Antiviral Inhibitors based on Darunavir backbone

Darunavir Ki 16pM, antiviral IC50 4nM

GRL-0255K Ki 6 nM, IC50>1000nM

GRL-0255A Ki 26pM, IC50 4.6 nM

GRL-98065 IC50 0.3nM

GRL-06579A Ki 4.5pM, IC50 1.8nM

Ghosh et al., JMC 2006; 2008; Amano et al., Antimicrob. Agents Chemother 2007; Wang et al., JMC 2007)
New Antiviral Inhibitors

GRL-0255A
\[ Ki = 26\text{pM}, \text{IC}_{50} = 5\text{nM} \]

PR/GRL0255A crystal structure solved at 1.0 Å resolution

New interactions

(Ghosh et al., J. Med. Chem, 2008)
Sites of Drug Resistant Mutations

Resistance mutations from hivdb.stanford.edu, 2008

L23I
L24I
D30N
V32I
V33F
M46IL
I47VA
G48VM
I50VL
F53L
I54VTALM
G73ST
L76V
V82ATFSL
I84VAC
N88DS
L90M
Darunavir Inhibition of HIV-1 PR Mutants

D30N, V32I, M46L and I50V predicted to be resistant to darunavir

Val50 mutant has lost hydrogen bonds with Darunavir. Relative $K_i = 9$

Ala82 has moved to reform favorable interactions with Darunavir. Relative $K_i = 3$

Val84 has lost van der Waals interactions with Darunavir. Relative $K_i = 5$
Mutants Vary in Activity on Substrates and Stability

Catalytic activity varies (kcat/Km)

Stability in urea varies (UC50 is Urea Concentration at 1/2 Vmax)

Kd <5 5 19 22 <5 nM

Reduced dimer stability and loss of intersubunit interactions L24I, I50V, F53L

(Liu et al., J Mol Biol 2005; 2006)
F53L had more open flaps than unliganded WT and has lost intersubunit interactions of side chains of F53 and I50.

F53L has 15% of WT kcat/Km, reduced stability: 60% of WT stability in urea, Kd~20nM, and 10-fold worse Ki for indinavir
Mutant I54V has lost flap interactions with reaction intermediate

I54V showed 40% WT kcat/Km, ~10-20-fold reduced inhibition by DRV or SQV, and no significant change in stability

Structures at 1.5\AA{} resolution, R factor of 0.15
[Kovalevsky et al., Biochem. 2007]
Hydroxyls of Darunavir and Reaction Intermediate TI Superimpose

Fo-Fc map at 3.6 $\sigma$ level shows the TI hydroxyl oxygens.

WT/TI, WT/DRV and unliganded F53L. Arrows show shifts relative to the unliganded structure. Catalytic Asps change orientation with ligands.

OH of darunavir in similar position to OH of TI
Darunavir binds at active site and flap site

Crystal contains two molecular species

60% species: PR_{V32I}/(DRV)\textsubscript{2}

40% species: PR_{V32I}/DRV

Kovalevsky et al. JMB 2006, 363, 161-173
Darunavir Interactions with Flap Residues

The flap site provides a novel target for drug designs. But, no resistant mutations found here after >2 years of darunavir therapy.
Diverse Mechanisms of Drug Resistance

Structural Differences in PR Mutants Correlate with Variation in Catalytic Activity, Inhibition and Stability

1. Reduced interactions with inhibitor and lower inhibition: D30N, V32I, M46L, I50V, I54M, I84V

2. Shift of main chain to accommodate inhibitor: V82A

3. Reduced interactions with reaction intermediate: I54V

4. Reduced intersubunit contacts and stability: L24I, F53L, I50V

Guide designs of next generation of inhibitors for resistant PR
Structural Perspectives on Drug Resistance

Crystal structures refined at 0.84–1.5 Å resolution form a unique resource for structural variation due to mutations or ligands.

Mechanisms of Drug Resistance
Diverse effects of mutations: altered interactions with inhibitor or substrate; reduced protease stability.

Structure-guided Drug Designs
Darunavir and new antiviral inhibitors to combat resistance.
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