**HIV Protease Drug Resistance Analysis: PRS17 Multi-Mutant Study**

*A Comprehensive Tutorial for Understanding Molecular Mechanisms of Drug Resistance*

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**1. Introduction and Learning Objectives**

**🎯 What You Will Learn**

By completing this tutorial, you will understand:

* **How HIV protease functions** as a critical drug target
* **How mutations lead to drug resistance** at the molecular level
* **How to analyze protein structures** using ChimeraX software
* **How to interpret structural changes** that affect drug binding
* **The clinical implications** of drug resistance mechanisms

**📋 Prerequisites**

* Basic understanding of proteins (amino acids, 3D structure)
* ChimeraX software installed on your computer
* Internet connection to download PDB files
* No prior structural biology experience required!

**🔬 What We're Analyzing**

**Wild-type HIV Protease (PDB: 1T3R)**

* The normal, unmutated enzyme bound to darunavir
* Resolution: 1.8 Å (very good quality)
* Our reference structure for comparison

**PRS17 Mutant (PDB: 5T2Z)**

* A highly resistant variant with 17 mutations
* Resolution: 1.5 Å (excellent quality)
* Represents extreme drug resistance

**2. Background: Understanding HIV Protease**

**The Biological Context**

HIV protease is essential for viral replication. Think of it as **molecular scissors** that cut long protein chains into smaller, functional pieces. Without these precise cuts, HIV cannot produce infectious virus particles. This makes HIV protease an excellent drug target—if we can block these scissors, we stop HIV from spreading.

**The Clinical Challenge: Drug Resistance**

When patients take protease inhibitors like darunavir, the virus can evolve mutations that allow the protease to continue functioning while reducing drug binding. This is like changing a lock slightly so the original key doesn't fit well, but the door mechanism still works perfectly.

**Understanding Protein Structure Basics**

**Key Terms for Beginners:**

* **Proteins:** Long chains of amino acids that fold into specific 3D shapes
* **Active site:** The "business end" where chemical reactions occur
* **Ligand:** A small molecule (like a drug) that binds to a protein
* **PDB:** Protein Data Bank, a database of 3D protein structures
* **Mutations:** Changes in amino acids (e.g., V82S = Valine at position 82 changed to Serine)

**HIV Protease Architecture**

HIV protease is a **homodimer**—two identical protein chains (A and B) working together like two hands clapping. The key regions include:

**1. Flaps (residues 45-55)**

* Mobile "lids" that open and close
* Allow substrates to enter and trap them for processing
* Move 7-10 Å between open and closed states

**2. Active Site (around residue 25)**

* Contains the catalytic aspartates (Asp25)
* Where the actual cutting reaction happens
* Must be preserved for enzyme function

**3. 80's Loop (residues 80-84)**

* Forms the S1/S1' substrate-binding pockets
* Creates a groove where drugs fit
* Key for drug specificity

**4. Hinge Region (residues 35-37)**

* Controls flap movement dynamics
* Acts like a door hinge mechanism
* Primary target for resistance mutations

**The 17 Mutations in PRS17**

**Flap Region Mutations:**

* **M46L:** Methionine → Leucine (reduced flexibility)
* **G48V:** Glycine → Valine (adds bulk at hinge point)
* **I54V:** Isoleucine → Valine (subtle size change)

**Active Site Mutation:**

* **V82S:** Valine → Serine (only direct active site change)

**Hinge Region Mutations:**

* **E35D:** Glutamate → Aspartate (shorter sidechain)
* **M36I:** Methionine → Isoleucine (hydrophobic core change)
* **S37D:** Serine → Aspartate (adds negative charge)

**Distal Mutation:**

* **K20R:** Lysine → Arginine (forms new salt bridge with E35D)

**Additional Mutations:**

Nine other mutations distributed throughout to maintain viral fitness

**3. Part 1: Loading and Preparing Structures**

**Why we need two structures:**

We compare the normal (wild-type) and mutant proteases to understand what changed—like comparing "before and after" photos.

**Step 1.1: Loading the Protein Structures**

**ChimeraX Commands**

*# Load the wild-type HIV protease with darunavir* **open** 1T3R *# This becomes model #1 in ChimeraX* *# Resolution: 1.8 Å (very good - lower numbers = better quality!)* *# Load the PRS17 mutant with darunavir* **open** 5T2Z *# This becomes model #2 in ChimeraX* *# Resolution: 1.5 Å (excellent quality)*

**💡 Tip:** ChimeraX automatically downloads these structures from the Protein Data Bank when you run these commands.

**Step 1.2: Identifying the Drug Molecule**

**Purpose:** We need to visualize where darunavir binds to understand how mutations affect this binding.

**ChimeraX Commands**

*# Select darunavir in wild-type (drug code: 017)*

**select** #1::name="017"

**color** sel pink *# Select darunavir in mutant* **select**

#2::name="017"

**color** sel pink *# Verify selection - lists all atoms in darunavir*

**info** sel

**What you're seeing:** Darunavir appears as a pink molecule nestled in the center of the protease, like a key in a lock.

**Understanding ChimeraX Selection Syntax**

For beginners, here's how to read ChimeraX commands:

* **#1** = model 1 (wild-type structure)
* **#2** = model 2 (mutant structure)
* **/A or /B** = chain A or chain B
* **:45-55** = residues 45 through 55
* **@CA** = alpha carbon atoms (backbone)
* **::name="017"** = all residues named "017" (our drug)

**4. Part 2: Color-Coding Functional Regions**

**Why Use Color Coding?**

Color-coding helps us:

1. Visually distinguish different functional regions
2. Track changes between wild-type and mutant
3. Create clear, understandable figures

**Our Color Scheme**

* **Red:** Flap regions (mobile parts)
* **Yellow:** Active site and 80's loop (drug binding)
* **Green:** Catalytic aspartates (cutting residues)
* **Orange:** Hinge region (movement control)

**Step 2.1: Coloring the Wild-Type Structure**

**Flap Regions (45-55) - RED**

**ChimeraX Commands**

*# Color the flexible flap regions red*

**select** #1/A:45-55

*# Chain A flap*

**color** sel red

**select** #1/B:45-55

*# Chain B flap*

**color** sel red

**Why these residues?** The flaps are like a Venus flytrap—they open to let substrates in, then close to trap them. They're the most mobile part of the protease.

**Active Site and 80's Loop - YELLOW**

**ChimeraX Commands**

*# Highlight the functional core*

**select** #1/A:25,80-84

**select add** #1/B:25,80-84

**color** sel yellow

**Purpose:** Yellow draws attention to where the drug binds and where catalysis occurs.

**Step 2.2: Labeling Key Residues**

**ChimeraX Commands**

*# Label important residues in wild-type*

**label** #1/A:82@CG1 text "VAL 82" *# Will mutate to Ser*

**label** #1/A:84@CG1 text "ILE 84" *# Key binding residue*

**label** #1/A:25@CG text "ASP 25 (A)" *# Catalytic residue*

**label** #1/B:25@CG text "ASP 25' (B)" *# Partner catalytic residue*

**color** #1/A:25@CG green **color** #1/B:25@CG green

**Step 2.3: Coloring the Mutant Structure**

Apply the same color scheme to the mutant for easy comparison:

**ChimeraX Commands**

*# Mutant flaps - RED*

**select** #2/A:45-55

**color** sel red

**select** #2/B:45-55

**color** sel red *# Mutant active site - YELLOW*

**select** #2/A:25,80-84

**select add** #2/B:25,80-84

**color** sel yellow *# Label mutant residues (note the changes!)*

**label** #2/A:82@CG1 text "m-SER 82" *# Changed from Val!*

**label** #2/A:84@CG1 text "m-ILE 84"

**label** #2/A:25@CG text "m-ASP 25 (A)"

**label** #2/B:25@CG text "m-ASP 25' (B)"

**color** #2/A:25@CG green

**color** #2/B:25@CG green

**🔍 Key Observation: The catalytic Asp25 residues CANNOT mutate—if they did, the enzyme wouldn't work and the virus couldn't replicate.**

**5. Part 3: Measuring Molecular Distances**

**Why Measure Distances?**

Drug binding depends on precise molecular interactions. Think of it like a hand fitting into a glove:

* **Too far apart (>4 Å)** = no interaction
* **Just right (3-4 Å)** = good contact
* **Very close (<3 Å)** = strong interaction or clash

**Understanding the Three Critical Regions**

**Region 1: The Flap Region (45-55) - The Gatekeeper**

**Function:** Two flexible loops that act as a lid over the active site

**Key States:**

* **OPEN:** Flaps separate, substrate enters
* **CLOSED:** Flaps together, trap substrate/drug
* **SEMI-OPEN:** Partially open (common in resistant mutants)

**Important Residues:**

* **Ile50:** Flap tip—first to contact drug
* **Gly48:** Hinge point—provides flexibility
* **Phe53:** Aromatic stacking with drug rings

**Region 2: The S1/S1' Pockets (80-84) - The Binding Groove**

**Function:** Hydrophobic pockets that hold drug P1/P1' groups

**Key Players:**

* **Val82:** Lines pocket wall (becomes Ser in mutant)
* **Ile84:** Forms pocket floor

**Region 3: The Hinge Region (35-37) - The Control Center ⚠️ MOST IMPORTANT**

**Function:** Controls flap movement dynamics

**Critical Interactions:**

* **Glu35:** Forms salt bridges stabilizing flap positions
* **Met36:** Hydrophobic core positioning
* **Ser37:** Hydrogen bonding network

**Step 3.1: Measuring Wild-Type Interactions**

**Flap-Drug Distances**

**ChimeraX Commands**

*# Met46 - Part of flap elbow*

**distance** #1/A:46@CG #1/A:1200@C36

**distance** #1/A:46@CG #1/A:1200@C37 *# Expected: 3.5-4.0 Å (good hydrophobic contact)* *# Gly48 - Flexible hinge point*

**distance** #1/A:48@CG1 #1/A:1200@C36

**distance** #1/A:48@CG1 #1/A:1200@C37 *# Glycine is the smallest amino acid - maximum flexibility* *# Ile50 - FLAP TIP (most important!)*

**distance** #1/A:50@CG1 #1/A:1200@C36

**distance** #1/A:50@CG1 #1/A:1200@C37 *# Expected: 3.0-3.5 Å (tight contact)*

**S1 Pocket Interactions**

**ChimeraX Commands**

*# Val82 - Key pocket residue*

**distance** #1/A:82@CG1 #1/A:1200@C24

**distance** #1/A:82@CG1 #1/A:1200@C25 *# Valine is hydrophobic and bulky - perfect for binding* *# Ile84 - Major pocket residue*

**distance** #1/A:84@CG1 #1/A:1200@C24

**distance** #1/A:84@CG1 #1/A:1200@C25

**Step 3.2: Measuring Mutant Interactions**

**Changed Flap Interactions**

**ChimeraX Commands**

*# Leu46 (was Met46) - MUTATION: M46L*

**distance** #2/A:46@CG #2/B:201@C36

**distance** #2/A:46@CG #2/B:201@C37 *# Leucine is smaller - weaker contacts* *# Val48 (was Gly48) - MUTATION: G48V \*\*\* Major change!*

**distance** #2/A:48@CG1 #2/B:201@C36

**distance** #2/A:48@CG1 #2/B:201@C37 *# Adding bulk where flexibility was needed* *# Like putting a doorstop in a hinge!*

**Altered Pocket Interactions**

**ChimeraX Commands**

*# Ser82 (was Val82) - MUTATION: V82S*

**distance** #2/A:82@CG1 #2/B:201@C24

**distance** #2/A:82@CG1 #2/B:201@C25 *# CRITICAL: Hydrophobic → Polar* *# Like replacing a greasy surface with a sticky one*

**6. Part 4: RMSD Analysis - Quantifying Structural Changes**

**What is RMSD?**

**RMSD = Root Mean Square Deviation**

Think of RMSD like measuring how much a building shifted after an earthquake. It tells us the average distance atoms moved between two structures.

**RMSD Interpretation Scale**

| **RMSD Value** | **Interpretation** | **Visual Analogy** |
| --- | --- | --- |
| 0 Å | Identical structures | Perfect copy |
| 0.1-0.5 Å | Very similar | Normal breathing |
| 0.5-1.0 Å | Noticeable differences | Slight warping |
| 1.0-2.0 Å | Significant changes | Bent but recognizable |
| >2.0 Å | Major reorganization | Substantially deformed |

**Step 4.1: Comparing Different Regions**

**Flap Region RMSD**

**ChimeraX Commands**

**rmsd** #2:45-55@CA to #1:45-55@CA *# Expected: ~1.0 Å (moderate change)* *# Interpretation: Flaps shifted but maintain basic structure* *# Like a door that's slightly warped but still functions*

**Active Site RMSD**

**ChimeraX Commands**

**rmsd** #2:24-28@CA to #1:24-28@CA *# Expected: ~0.2 Å (highly conserved)* *# Interpretation: Catalytic machinery preserved* *# THIS IS CRITICAL - enzyme must remain functional!*

**Hinge Region RMSD - THE SMOKING GUN**

**ChimeraX Commands**

**rmsd** #2:36-37@CA to #1:36-37@CA *# Expected: >2.0 Å (MAJOR reorganization)* *# Interpretation: Primary resistance mechanism revealed!* *# The control center for flap movement is completely rewired*

**Step 4.2: Individual Residue Analysis**

**ChimeraX Commands**

*# Which residue moved most?*

**rmsd** #2:35@CA to #1:35@CA *# Expected: >4.0 Å - LARGEST movement* *# E35D forms new salt bridge with K20R*

**rmsd** #1/A:36@CA to #2/A:36@CA *# Expected: ~2.0 Å* *# M36I reorganizes hydrophobic core*

**rmsd** #1/A:37@CA to #2/A:37@CA *# Expected: ~1.5 Å* *# S37D introduces charge*

**💡 Key Insight: The hinge region changes are like replacing three gears in a watch—the entire mechanism works differently!**

**7. Part 5: Advanced Structural Analysis**

**Step 5.1: Structure Superposition**

**ChimeraX Commands**

*# Align structures on conserved active site* **matchmaker** #2:24-28@CA to #1:24-28@CA

**What this does:**

1. Finds best 3D alignment of active sites
2. Rotates/translates model #2 to overlay model #1
3. Makes differences elsewhere visually apparent

**What you'll see:**

* Active sites overlap perfectly (green)
* Flaps slightly displaced (red)
* Hinge region shows major shift (orange)
* Drug molecules don't overlap perfectly

**Step 5.2: Hydrogen Bond Analysis**

**ChimeraX Commands**

*# Show all hydrogen bonds involving the drug* **hbonds** ligand

**What to look for:**

* Blue lines = hydrogen bonds
* Typical distance: 2.5-3.5 Å
* Darunavir forms 4-6 H-bonds normally
* Some bonds lost in mutant

**Step 5.3: B-Factor Coloring**

**ChimeraX Commands**

*# Color by flexibility/mobility* **color** byattribute bfactor

**Color interpretation:**

* **Blue:** Low B-factor (<20) = rigid
* **Green:** Medium (20-40) = some flexibility
* **Yellow/Orange:** High (40-60) = flexible
* **Red:** Very high (>60) = highly mobile

**Step 5.4: Highlighting Critical Regions**

**ChimeraX Commands**

*# Make hinge region stand out* **select** #1/A:36-37 **color** sel orange **select** #2/A:36-37 **color** sel orange

**Step 5.5: Measuring Catalytic Interactions**

**ChimeraX Commands**

*# Drug OH to catalytic Asp25 (wild-type)*

**distance** #1/A:25@OD1 #1/A:1200@O18 *# Expected: ~2.8 Å (perfect H-bond)* *# Same interaction in mutant*

**distance** #2/A:25@OD1 #2/A:201@O18 *# Should be preserved (~2.8 Å)* *# Compare catalytic geometry*

**distance** #1/A:25@OD1 #1/B:25@OD1 *# WT*

**distance** #2/A:25@OD1 #2/B:25@OD1 *# Mutant* *# Both ~2.8-3.0 Å - catalysis preserved!*

**8. Part 6: Understanding the Resistance Mechanism**

**The Complete Picture: How 17 Mutations Work Together**

**🔴 Primary Resistance Mechanism: Hinge Reorganization**

**Key Players:** E35D + M36I + S37D + K20R

**The Drawbridge Analogy:**

**Normal (Wild-type):**

* Drawbridge opens → ships (substrates) enter
* Closes tightly → traps them for processing
* Drug gets trapped when bridge closes

**Mutant (PRS17):**

* Control center rewired (hinge mutations)
* Drawbridge still opens (substrates enter)
* But doesn't close tightly or long enough
* Drug escapes before being trapped

**Molecular Details:**

* K20R forms NEW salt bridge with E35D
* Pulls hinge into new conformation
* Changes "spring constant" of flap movement
* Result: 100-1000× reduction in drug binding!

**🟡 Secondary Mechanism: Flap Distortion**

**Mutations:** M46L + G48V + I54V

**Synergistic Effects:**

* **G48V:** Adds bulk where flexibility needed (like rock in hinge)
* **M46L:** Reduces hydrophobic contacts
* **I54V:** Fine-tunes flap curl

**Result:** "Twisted" flaps that don't form optimal drug contacts

**🟢 Tertiary Mechanism: Pocket Modification**

**Mutation:** V82S (only active site change)

**Effects:**

* Reduces hydrophobic volume (smaller residue)
* Introduces polarity (OH group)
* Drug doesn't fit as snugly
* But pocket still functional for natural substrates

**Why This Strategy Is Brilliant (From the Virus's Perspective)**

The virus evolved a sophisticated resistance strategy:

1. **Maintains enzyme function** (can still replicate)
2. **Reduces drug binding** (escapes inhibition)
3. **Uses multiple mechanisms** (harder to overcome)
4. **Mutations support each other** (epistasis)

**Clinical Implications**

This explains why:

* Single mutations rarely cause high resistance
* Resistance develops gradually (accumulating mutations)
* Some mutations appear first (primary), others follow (compensatory)
* Next-generation drugs must overcome multiple mechanisms

**9. Part 7: Creating Publication-Quality Visualizations**

**For Scientific Presentations**

**Overview Slide:**

* Show both structures side-by-side
* Use consistent coloring scheme
* Label key regions clearly

**Mechanism Slide:**

* Focus on hinge region (orange)
* Show E35D-K20R salt bridge
* Use arrows for movement

**Drug Binding Slide:**

* Zoom to active site
* Show lost interactions with dotted lines
* Compare wild-type vs mutant

**For Teaching**

1. **Start simple:** Overall protein shape
2. **Add complexity:** Introduce functional regions
3. **Show dynamics:** Use morph animations
4. **Emphasize key points:** Labels and arrows

**For Publications**

* **High resolution:** Use supersample for crisp images
* **Multiple views:** Show different angles
* **Stereo pairs:** For 3D perception in print
* **Consistent style:** Same orientation, lighting, colors

**Saving Your Work**

**ChimeraX Commands**

*# Save ChimeraX session* **save** ~/Desktop/PRS17\_analysis.cxs *# Export high-quality image* **save** ~/Desktop/PRS17\_overview.png width 2400 height 1800 supersample 3 *# Create morph animation* **morph** #1,2 frames 60 *# Save specific view* **view** name myview *# Save current orientation* **view** myview *# Return to saved view*

**10. Summary and Key Takeaways**

**🎓 What You've Learned**

**1. Protein Structure Analysis:**

* Loading and visualizing PDB structures
* Identifying functional regions
* Measuring molecular interactions
* Quantifying structural changes with RMSD

**2. Drug Resistance Mechanisms:**

* Mutations modify but don't break the enzyme
* Multiple mutations work together (epistasis)
* Resistance involves dynamics, not just structure
* Primary mechanism: altered flap dynamics via hinge mutations

**3. The PRS17 Story:**

* **Primary:** Hinge reorganization (E35D, M36I, S37D, K20R)
* **Secondary:** Flap mutations (M46L, G48V, I54V)
* **Tertiary:** Active site change (V82S)
* **Result:** 1000-fold reduction in drug susceptibility!

**🔬 The Bigger Picture**

This analysis demonstrates why HIV treatment requires:

* **Combination therapy:** Multiple drugs targeting different sites
* **Resistance monitoring:** Regular viral sequencing
* **New drug development:** Staying ahead of evolution

**📚 Further Learning Suggestions**

1. **Analyze other mutations:** Compare single vs multiple mutations
2. **Examine other drugs:** How do different inhibitors bind?
3. **Study dynamics:** Molecular dynamics simulations
4. **Read literature:** Search "HIV protease resistance mechanisms"

**💡 Final Thought**

**Drug resistance is an evolutionary arms race. By understanding molecular mechanisms, we can design better drugs and treatment strategies. This approach applies to many diseases, from bacterial infections to cancer.**

**The virus may be clever, but with structural analysis tools, we can be cleverer!**

**11. Appendix: Troubleshooting Guide**

**Common Issues and Solutions**

**Problem: Structures won't load**

* Check internet connection (PDB files download from web)
* Try: open 1T3R from pdb (explicitly specify source)

**Problem: Colors look different**

* Previous commands may affect coloring
* Reset: color #1 tan (default color)
* Reapply desired colors

**Problem: Can't see labels**

* Labels may be hidden behind structure
* Try: label height 0.7 (makes labels bigger)
* Rotate structure to better angle

**Problem: Distances aren't showing**

* Check atom names are correct
* Use select first to verify atoms exist
* Some PDB files use different atom names

**Understanding Atom Naming**

| **Notation** | **Meaning** | **Example** |
| --- | --- | --- |
| CA | Alpha carbon (backbone) | All amino acids have one |
| CB | Beta carbon (first sidechain) | All except glycine |
| CG, CD, CE, CZ | Gamma, delta, epsilon, zeta carbons | Longer sidechains |
| OD1, OD2 | Delta oxygen 1 and 2 | In aspartate |
| CG1, CG2 | When multiple equivalent atoms | In valine |

**Useful ChimeraX Commands**

**ChimeraX Commands**

**cartoon** *# Show as cartoon representation* **surface** *# Show molecular surface* **hydrophobicity** *# Color by hydrophobicity* **sequence** *# Show sequence alignment* **interfaces** *# Analyze chain interfaces* **clashes** *# Find steric clashes* **contacts** *# Find close contacts* **hbonds reveal true** *# Show all H-bonds* **measure rotation #1 #2** *# Measure rotation between structures*

**Resources for Continued Learning**

1. **ChimeraX Documentation:** https://www.rbvi.ucsf.edu/chimerax/docs/
2. **PDB Learning Portal:** https://pdb101.rcsb.org/
3. **HIV Drug Resistance Database:** https://hivdb.stanford.edu/
4. **PyMOL Alternative:** Similar analysis can be done in PyMOL
5. **Research Papers:** Search "PRS17 HIV protease" in PubMed

**Contact and Support**

For questions about:

* **ChimeraX software:** Visit UCSF ChimeraX forums
* **HIV protease biology:** Consult review articles in Nature Reviews Drug Discovery
* **Drug resistance:** See Stanford HIV Drug Resistance Database

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