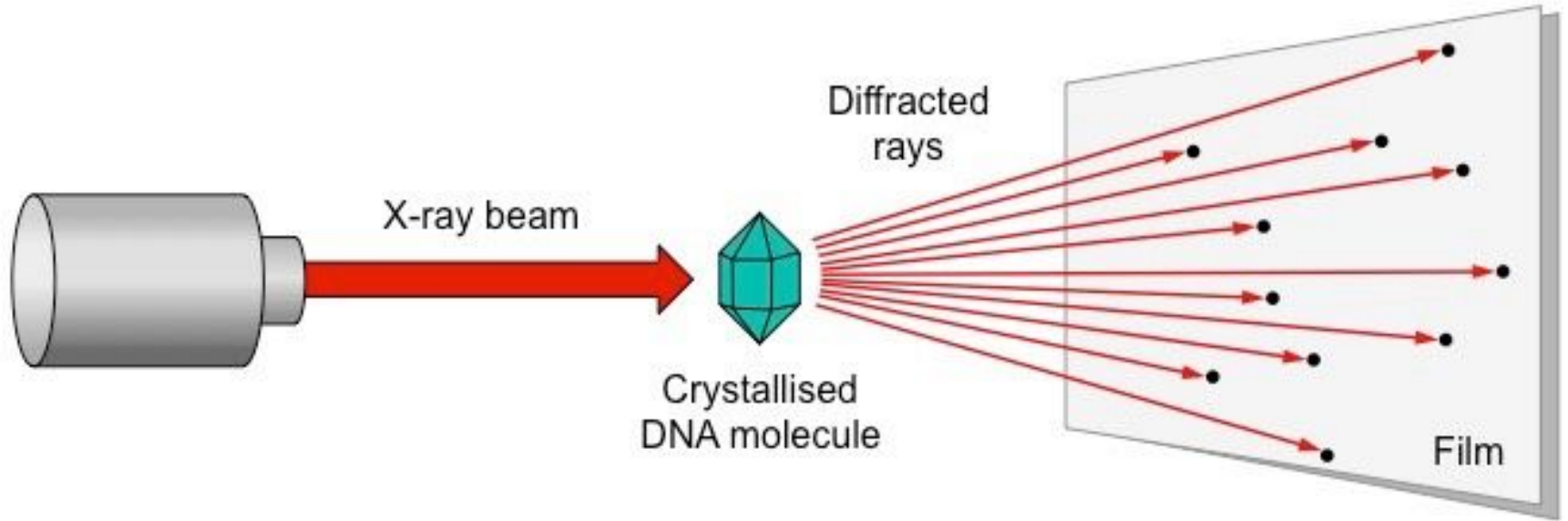


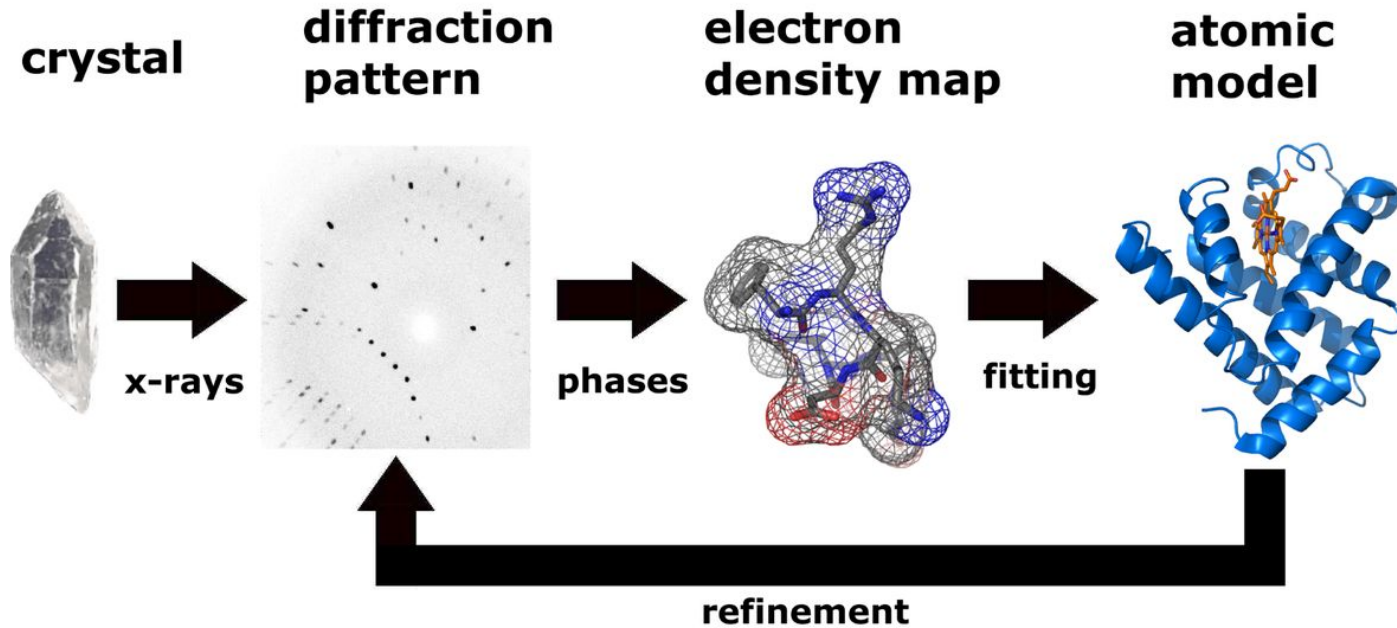
# Structural Determination & Analysis of Protein 6J17

Malvika Agarwal, Nikhila Butani, Bushra Haque, Harleen Sangha

# A brief look at X-ray Crystallography



# X-ray Crystallography Steps



# X-ray Crystallography Steps

GROUP PROJECT

2. Crystallization

4. Phase Determination

6. Model Building

8. Model Validation

3. Data Collection

5. Phase Improvement

7. Model Refinement

9. Model Analysis

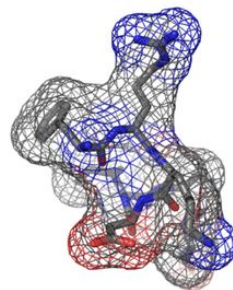
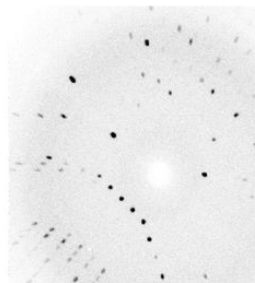
**crystal**

**diffraction  
pattern**

**electron  
density map**

**atomic  
model**

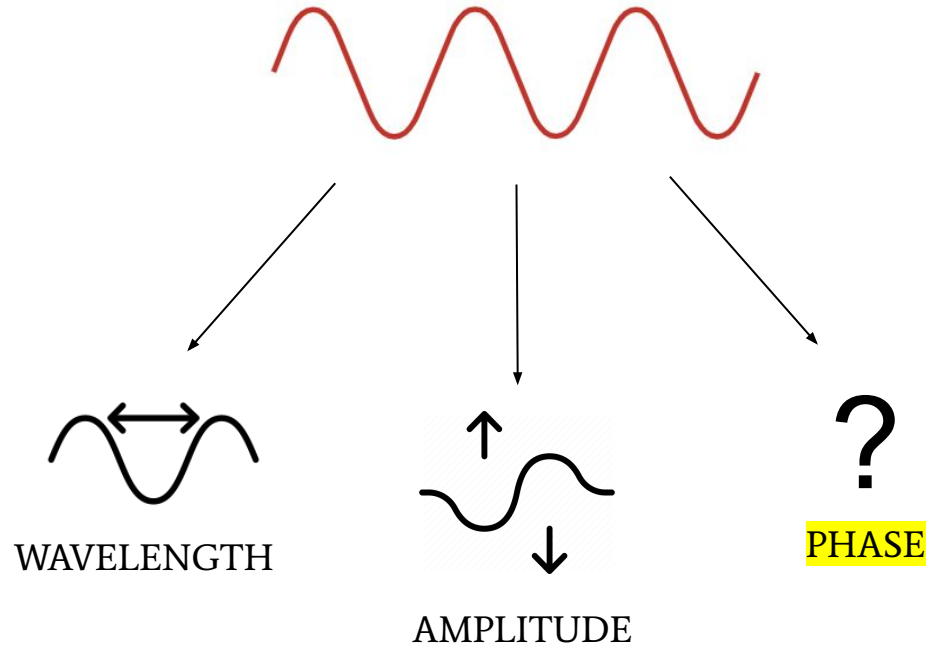
1. Pure Protein



10. Follow-Up Studies



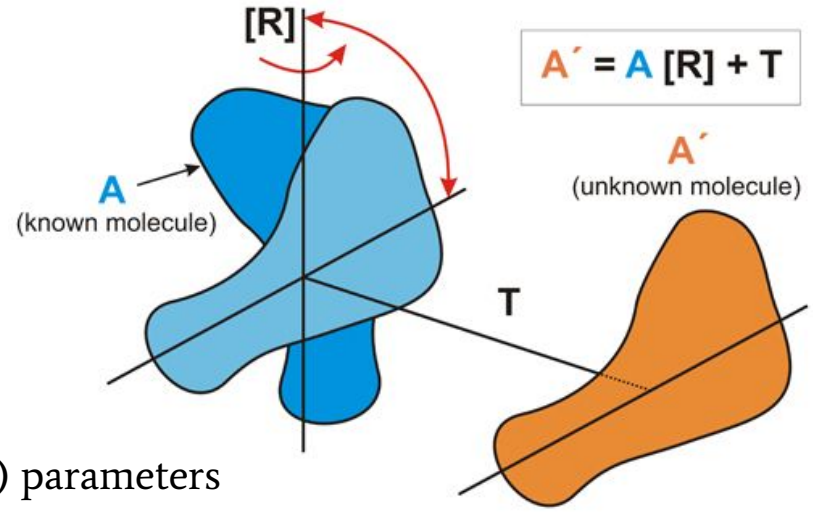
# Solving the Phase Problem



**Solution: Molecular Replacement**

# Molecular Replacement

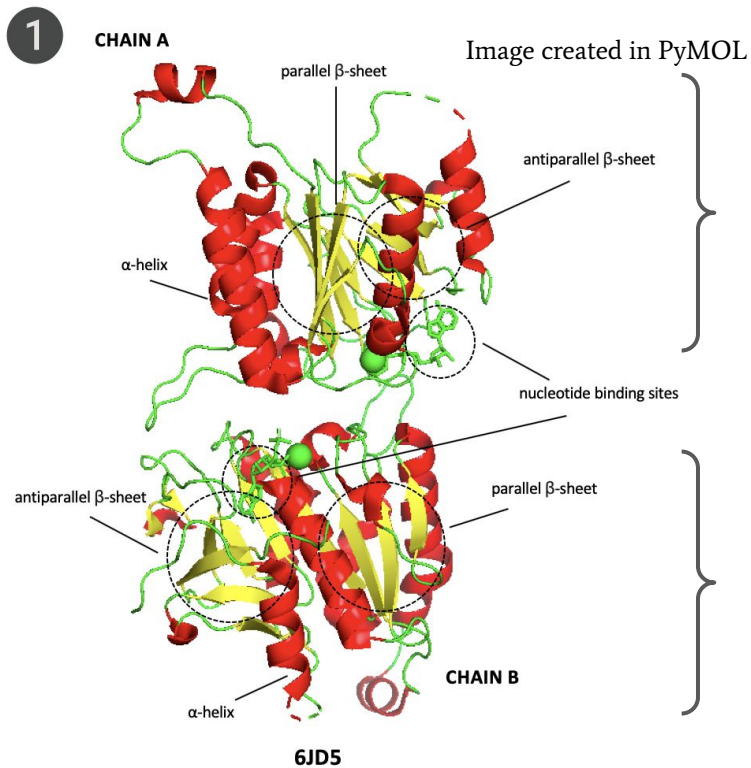
- Borrowing phase from a known, homologous protein model
- Homologous model is positioned within the unit cell of unknown target crystal



- Use of: (1) Rotational [R] (2) Translational (T) parameters
- Solve the electron density equation



# Challenges with Molecular Replacement



Protein Model

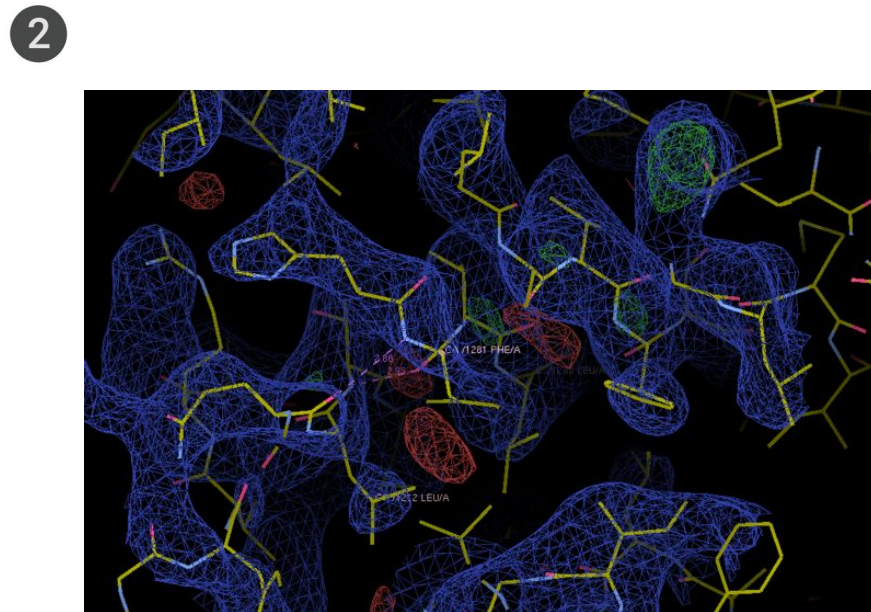


Image created in Coot

Model Bias

# Output from Phaser

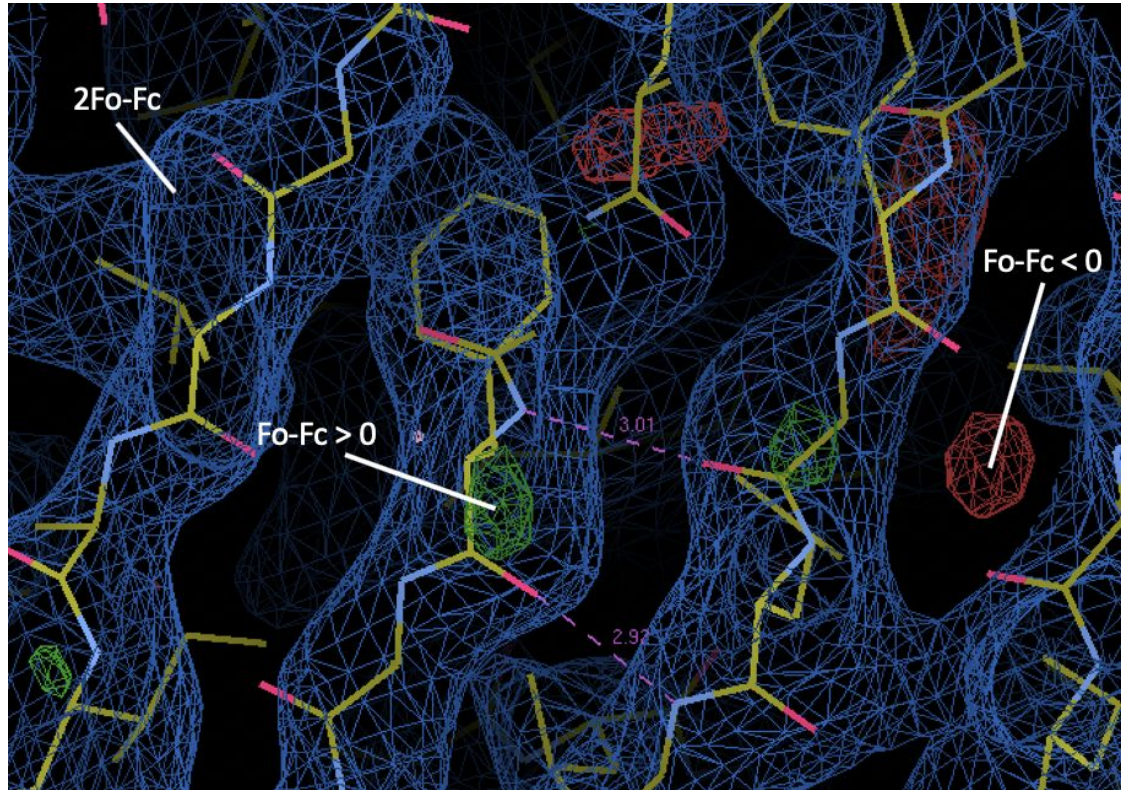
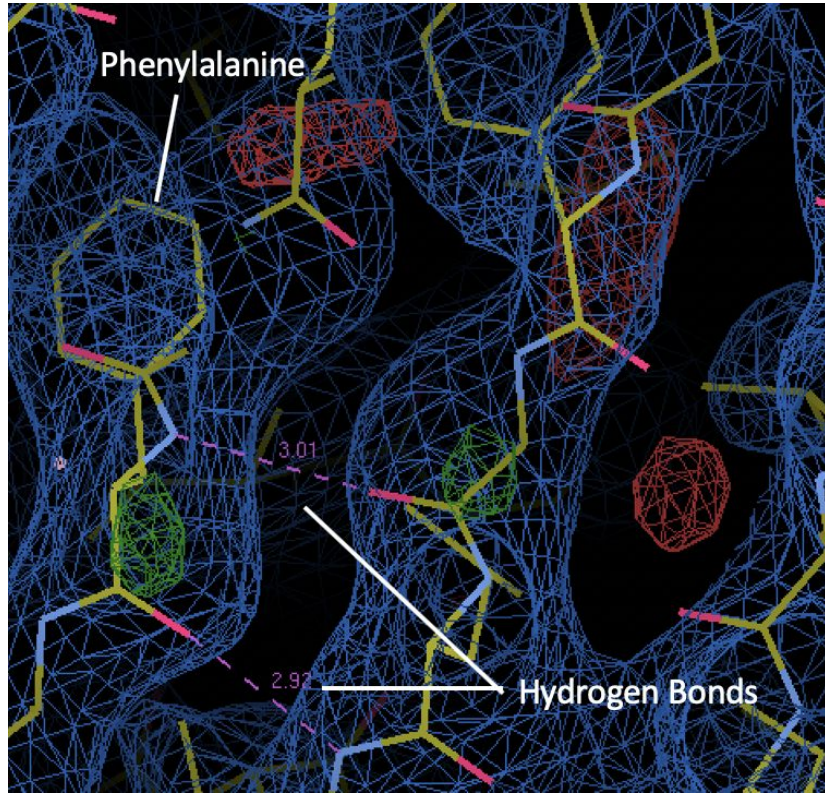


Image created in Coot

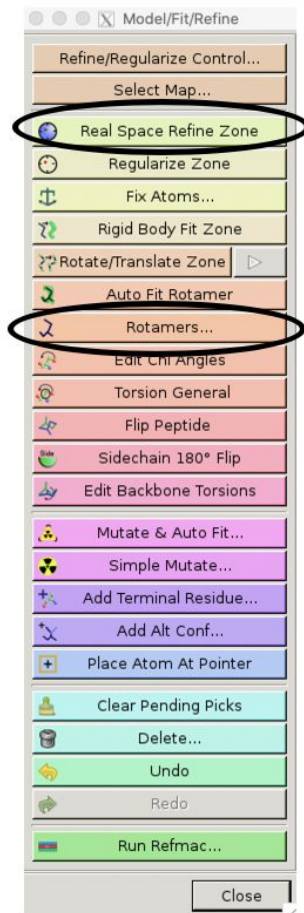




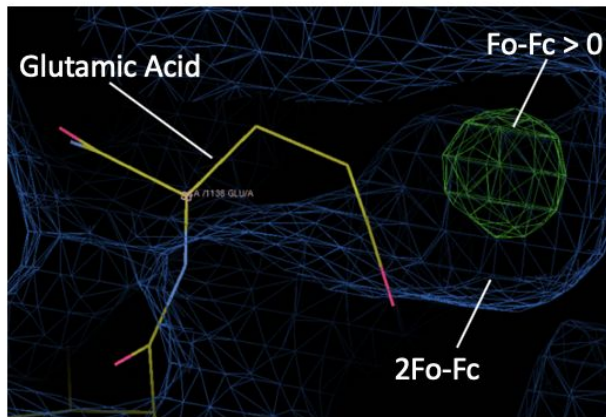
## Analyzing the Electron Density Map

Image created in Coot

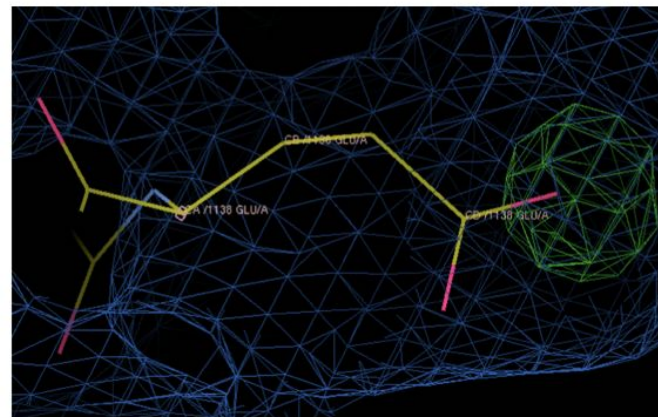
# Manual Refinement in Coot



Before Refinement



After Refinement



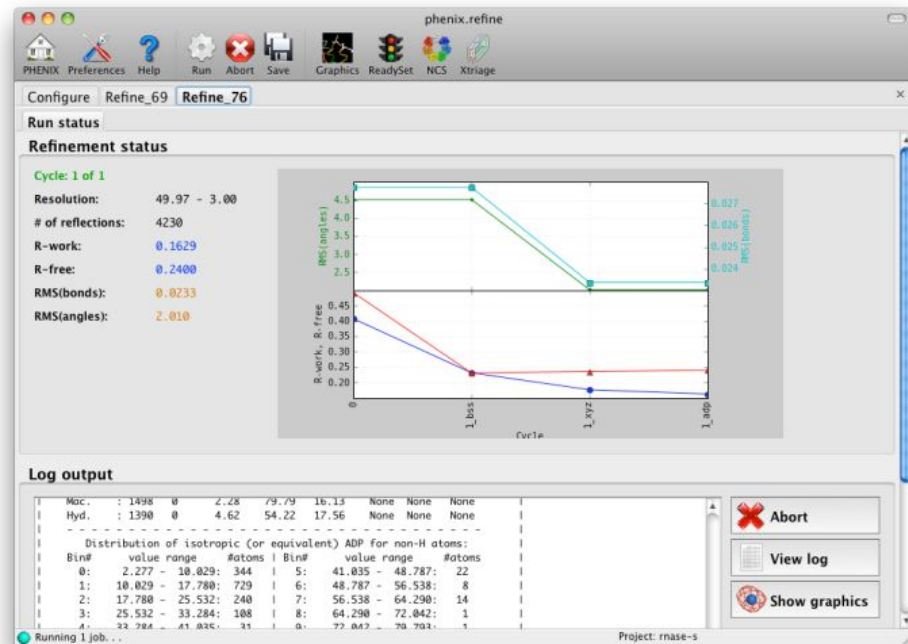
# Computational Refinement using Phenix

## Coordinate Refinement

Atomic positions of proteins  
in the electron density mesh

## ADP Refinement

How much atomic motion is  
at a position in the structure



# Model Validation

FINISHED

Resolution: 46.35 – 1.98

# of reflections: 19173

R-work: 0.2308

R-free: 0.2623

RMS(bonds): 0.0165

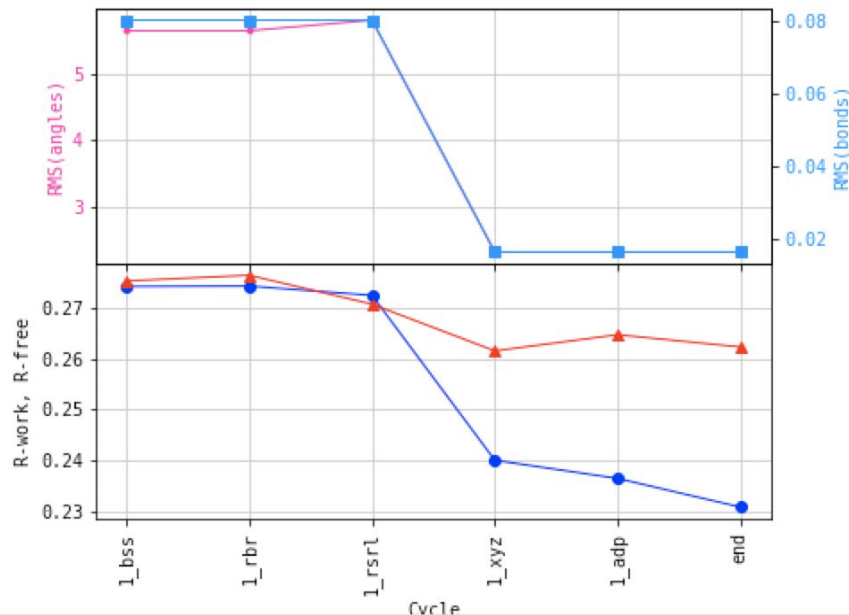
RMS(angles): 2.319

Clashscore: 15.0

Ramachandran favored: 89.88

Ramachandran outliers: 3.24

Rotamer outliers: 0.00



1

R-WORK & R-FREE

2

BASIC GEOMETRY

3

CLASHES

# Model Validation

- Blue dots = angles avoiding steric collisions
- Red dots = angles causing clashes between atoms

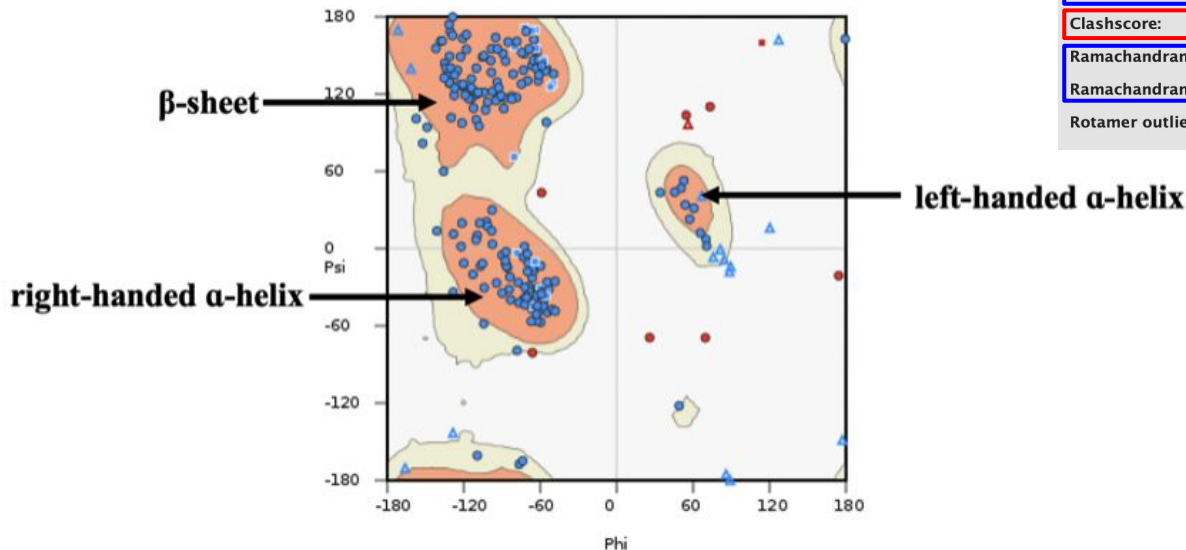


Image obtained from Coot

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1

R-WORK & R-FREE

2

BASIC GEOMETRY

3

CLASHES



# Crystallography Table

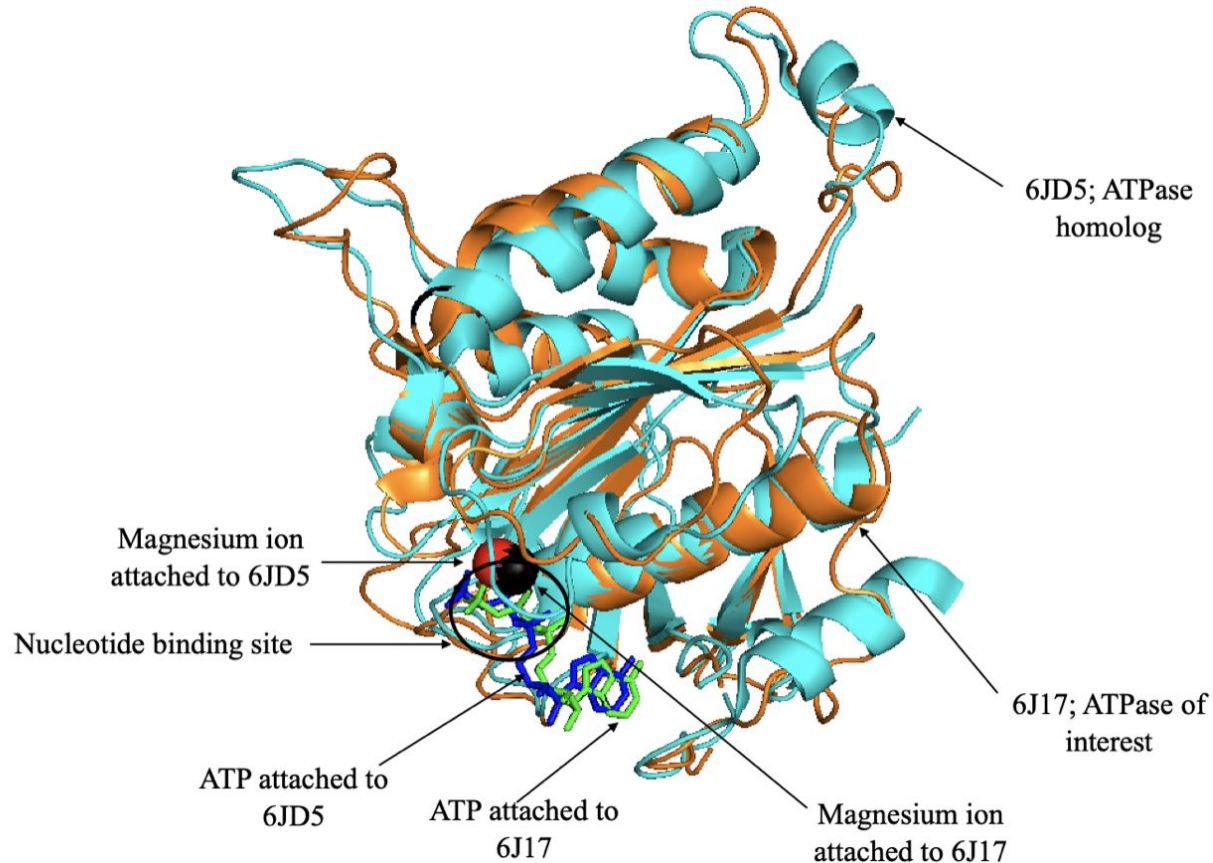
Crystal Statistics (PDB: 6J17)	
Data Collection	
Resolution range (Å)	46.35 - 1.98 (2.051 - 1.98)
Space group	P 21 21 21
Unit cell dimensions	53.363, 56.628, 93.532, 90, 90, 90
Unique reflections	19109 (1552)
Completeness (%)	93.70 (76.82)
Wilson B-factor	30.38

Crystal Statistics (PDB: 6J17)	
Refinement	
Reflections used in refinement	19079 (1534)
Reflections used for R-free	1915 (156)
R-work	0.2621 (0.3029)
R-free	0.2631 (0.3002)
Number of non-hydrogen atoms	2135
macromolecules	1955
ligands	32
solvent	148
Protein residues	254
RMS(bonds)	0.08
RMS(angles)	5.71
Ramachandran favored (%)	90.16
Ramachandran allowed (%)	4.92
Ramachandran outliers (%)	4.92
Rotamer outliers (%)	2.4
Clashscore	53.33
Average <i>B</i> factor	44.38
macromolecules	44.68
ligands	25.94
solvent	44.38

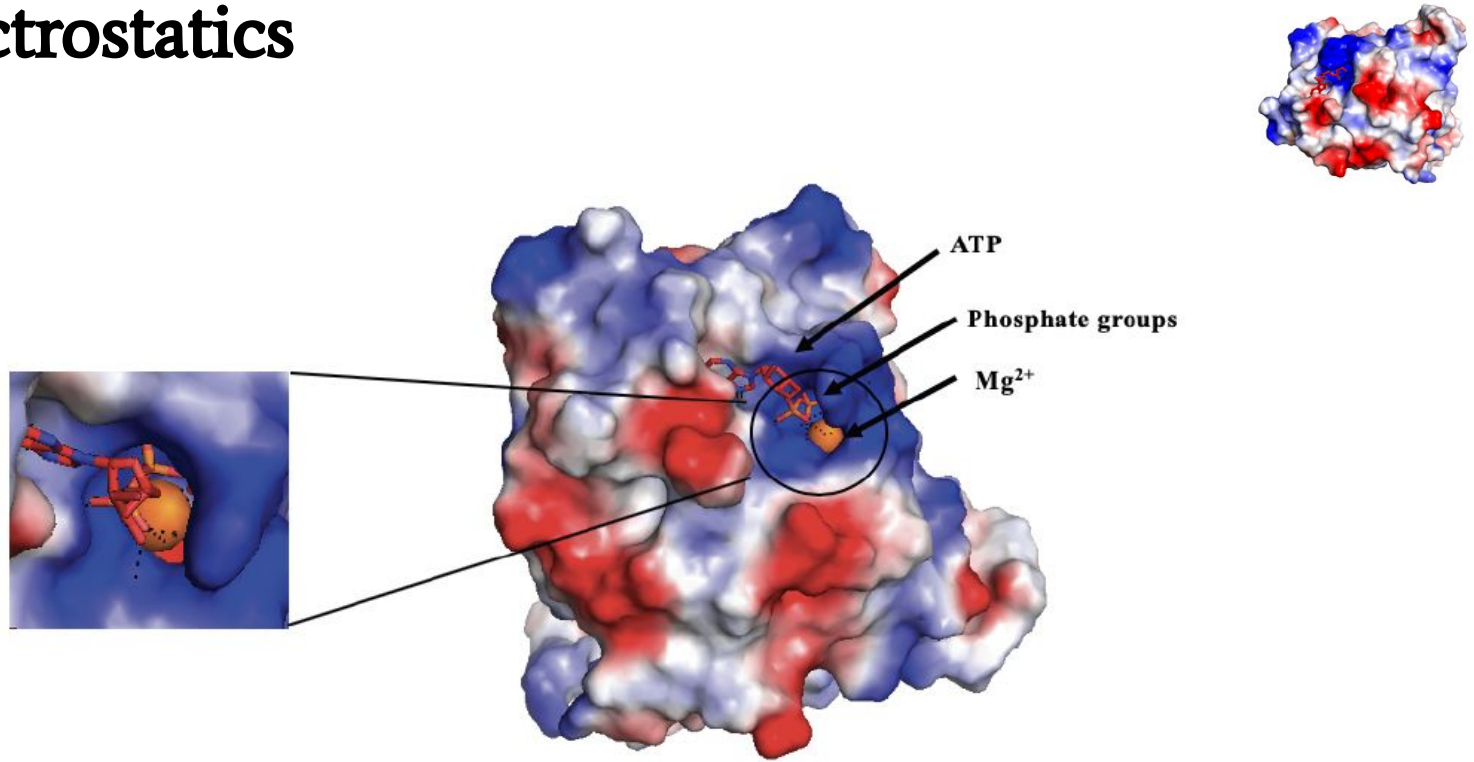
# Sequence Homology

Score	Expect	Method	Identities	Positives	Gaps
60.1 bits(144)	1e-14	Compositional matrix adjust	75/266(28%)	116/266(43%)	27/266(10%)
Query 34		GPGSPGMAAPPVRLLPNLAPHAVGE---LYRGPDL---VIGQREEDLAPVILDLAAN	86		
		GPGS ++ LP + A+ E +++G D L IG+R E L PV + L +			
Sbjct 1		GPGSHAS----LQRLPQRVELSAIVEHEAVHQGGDDLSIAFAIGERHE-LGPVPIKLRES	55		
Query 87		PLLMVFGDARSGKTTLLRHIIRTVREHSTADRVAFTVLDLR-----LHLVDEPLFPDNEY	141		
		P LM+ G GKTT L I V + + T++D + L + P + Y			
Sbjct 56		PGLMILGRQCGKTTALVAIGEAVMNRFSPPQAQLTLIDPKTAPHGLRDLHAPGY-VRAY	114		
Query 142		TANIDRIIPAMLGLA-NLIEARRPPAGMSAAELSRWT-FAGHTHYLIIDDV-DQVPDSPA	198		
		+ D I + LA ++ R PP G+S EL + G H+++IDDV D P			
Sbjct 115		AYDQDEIDEVITELAQQILLPRLPPKGLSQEELRALKPWEGPRHFVLIDDVQDLRPAQSY	174		
Query 199		MTGPYIGQRPWTPLIGLLAQAGDLGLRVIVTGRATGSAHLLMTSPLLRRFNDLQATTML	258		
		P +G W L+ +A +GL V T + A + M P ++ + L +			
Sbjct 175		PQKPPVGAALWK----LMERARQVGLHVFSTRNSANWATMPM-DPWVKSQTSAKVAQLYM	229		
Query 259		AGNPADSGKIRGERFARLPAGRAILL	284		
		+P + R R LP GR +L+			
Sbjct 230		DNDPQNRIN-RSVRAQTLPPGRGLLV	254		

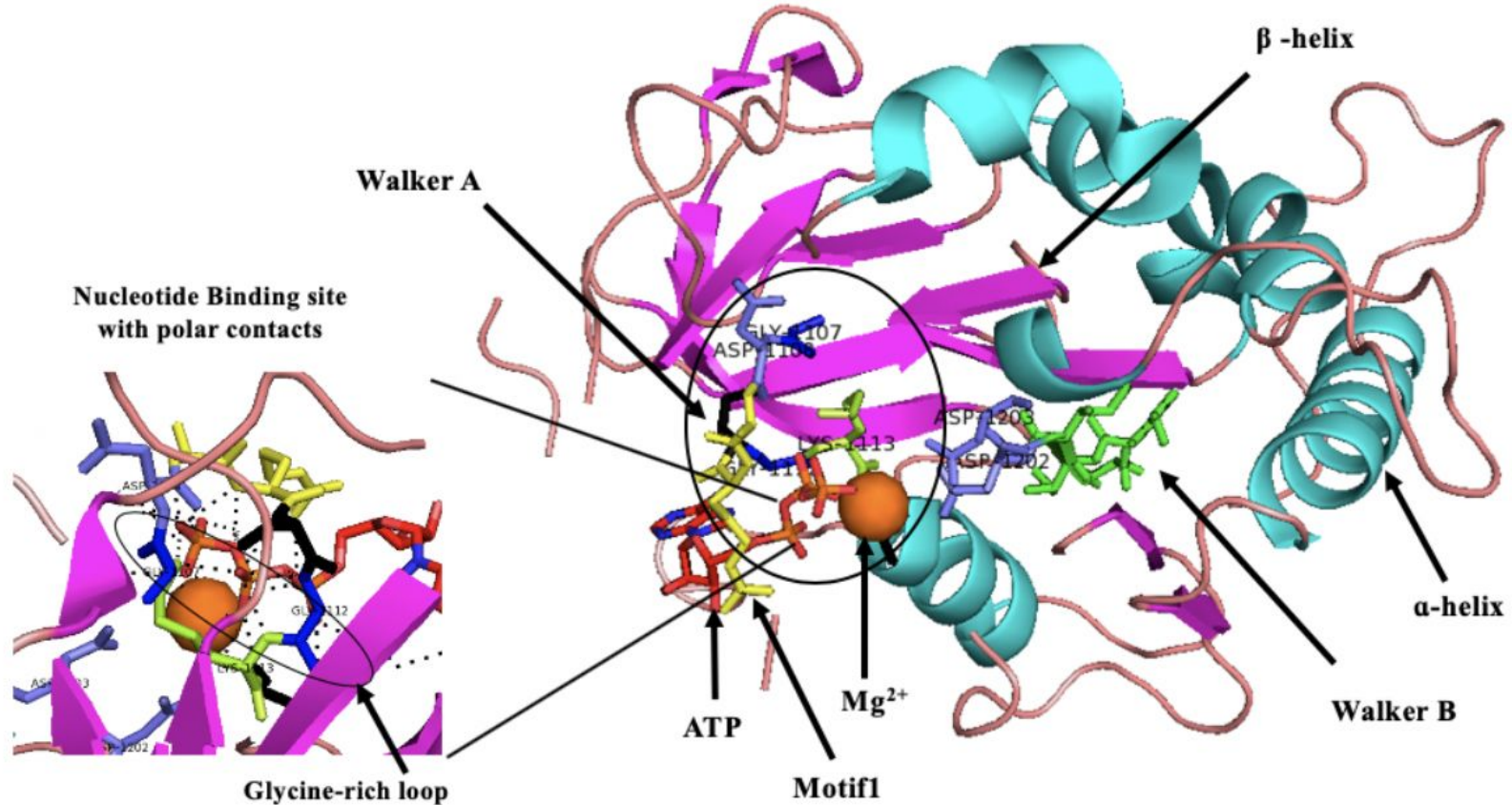
# Homologous Overlap using Pymol



# Electrostatics



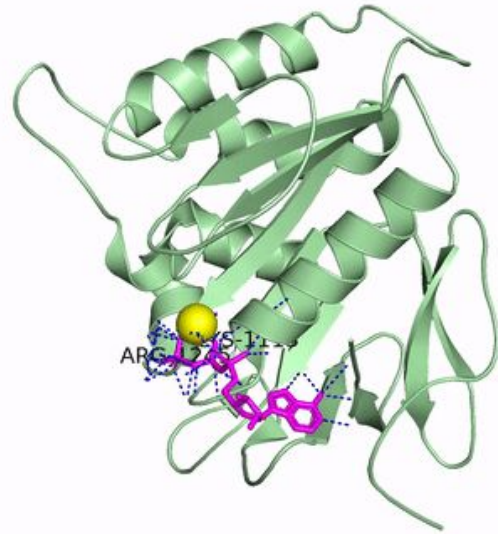
# Amino acids responsible for ATPase function





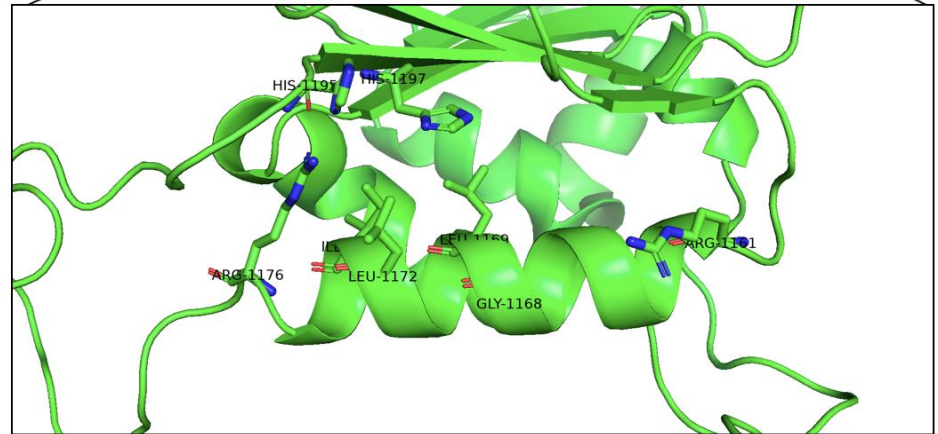
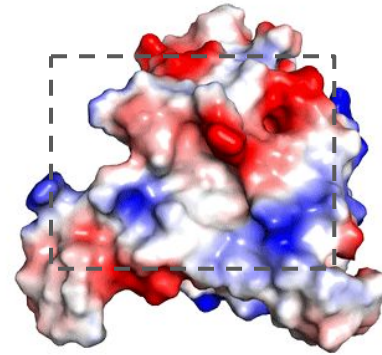
# Residues Important in Function

- Lys1113 is essential for ATP hydrolysis
- Arg1245 is required for the binding of ATP



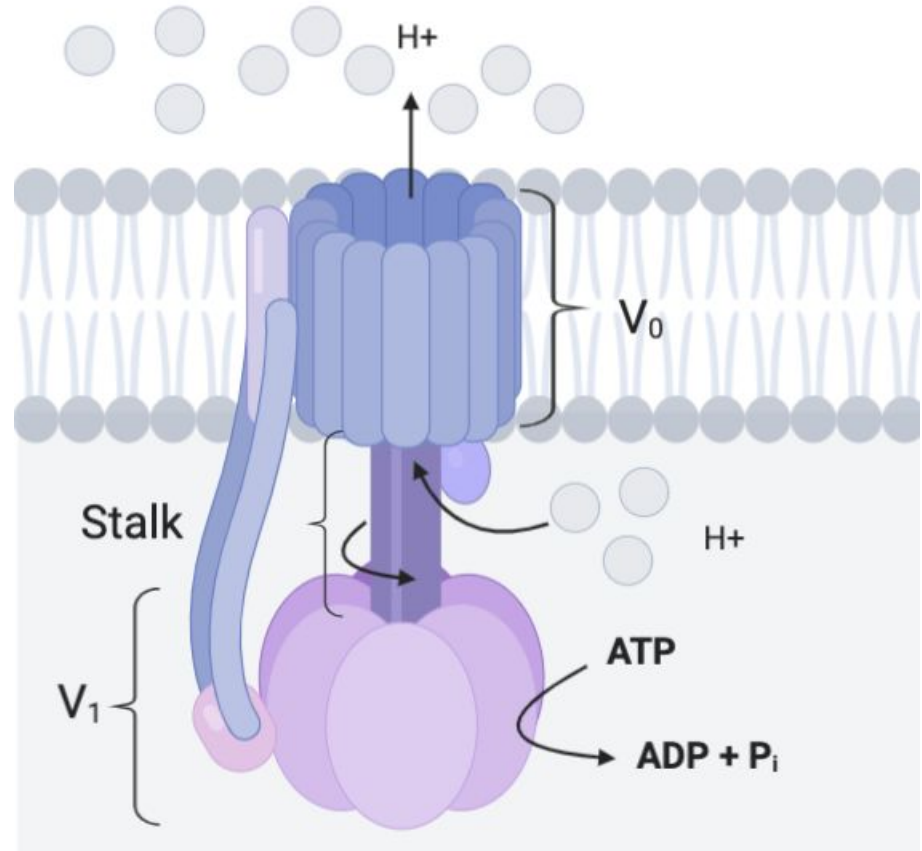
# Protein-Protein Interactions

- Protein binding pocket determined using electrostatics
- Interaction with other residues
  - E.g. Virulence factors
- Influence on ATPase activity
- Not highly conserved



# ATPases

- Enzymes which catalyze ATP hydrolysis reactions
- Energy is released and used to drive other processes
- 3 components:  $V_0$ , Stalk, and  $V_1$
- E.g.  $H^+$ -ATPase



# ATPases as Drug Targets

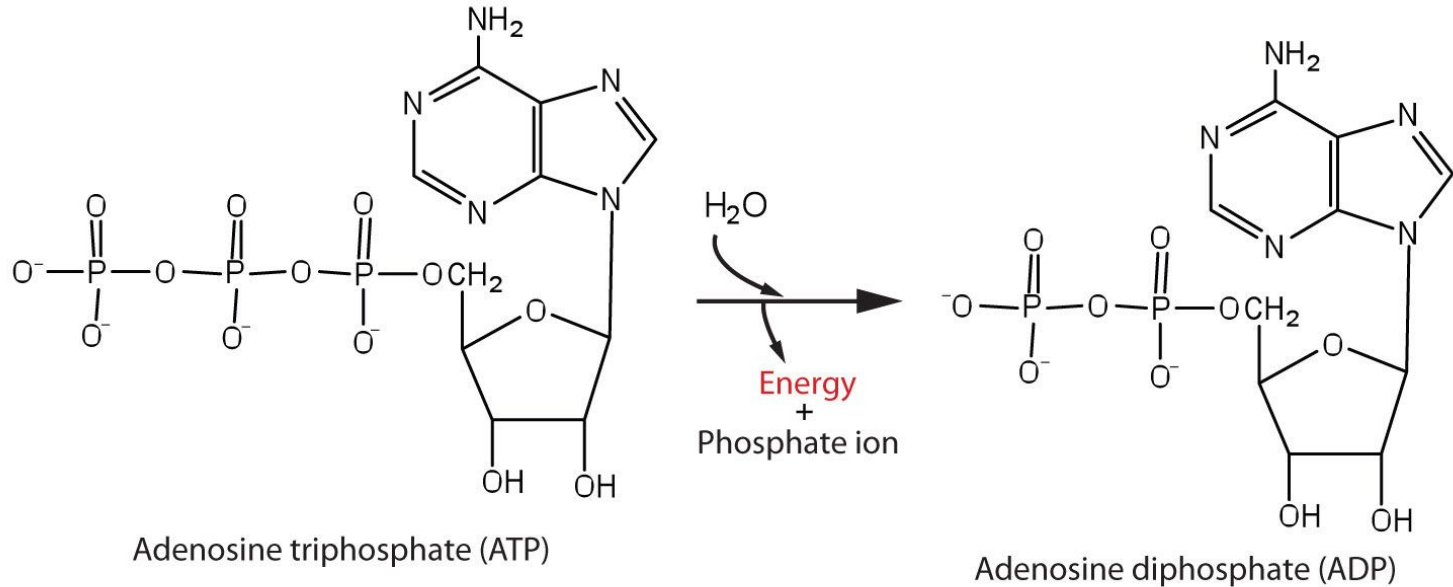
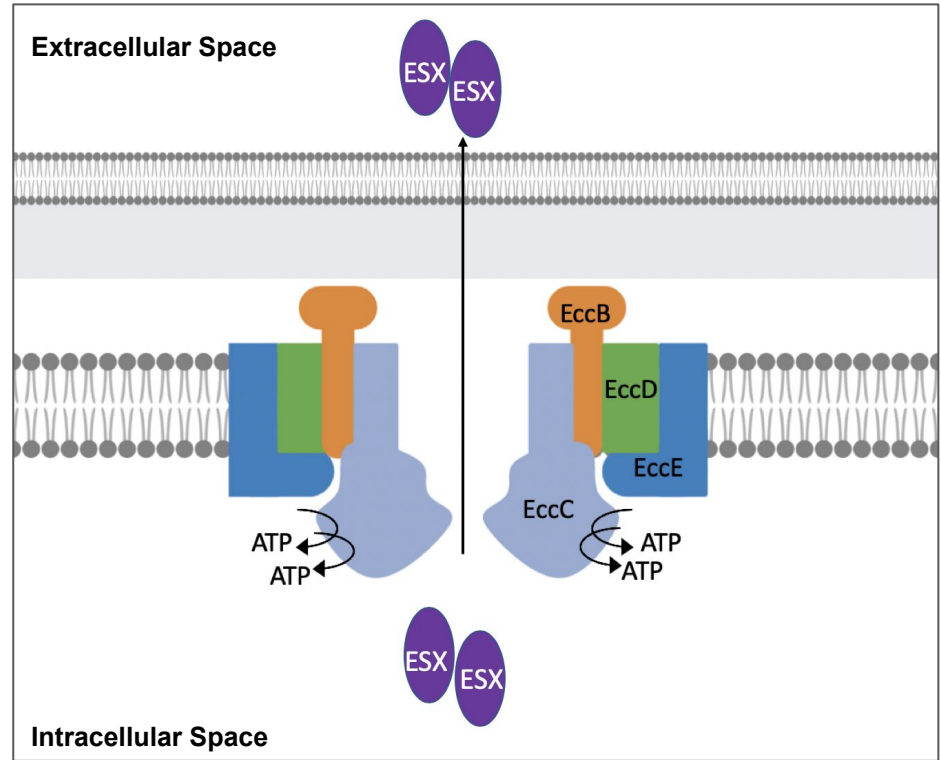


Image from: (ATP—the Universal Energy Currency, n.d.)

# EccC-ATPase<sub>3</sub> Function

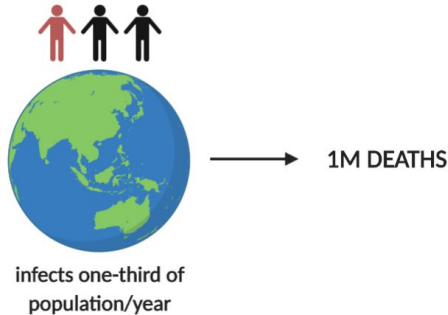
- EccC-ATPase<sub>3</sub> found in *M. tuberculosis*
- Active transport to diffuse molecules across the bacterial cell envelope using ATP hydrolysis generated energy



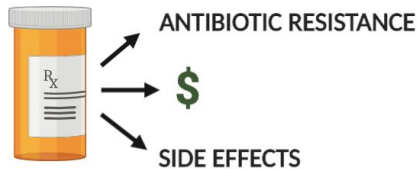


# Novel Drug Target

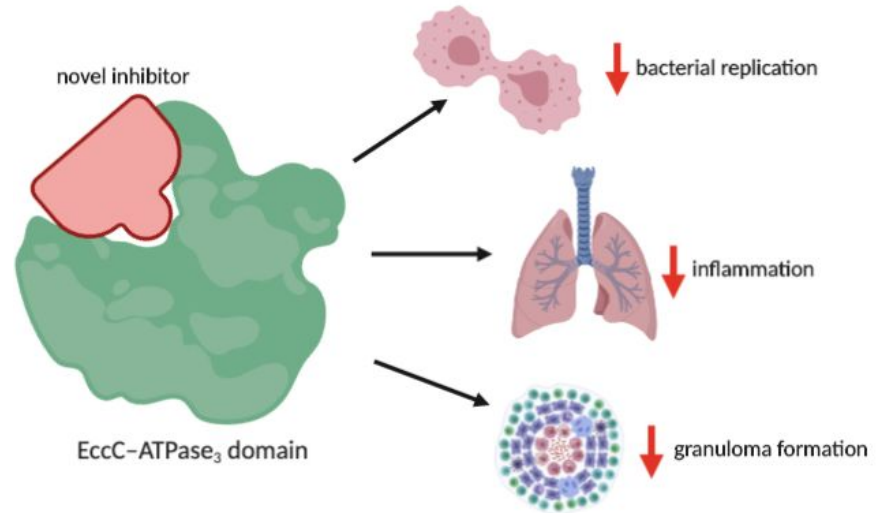
- *M. tuberculosis* is the causative agent of tuberculosis



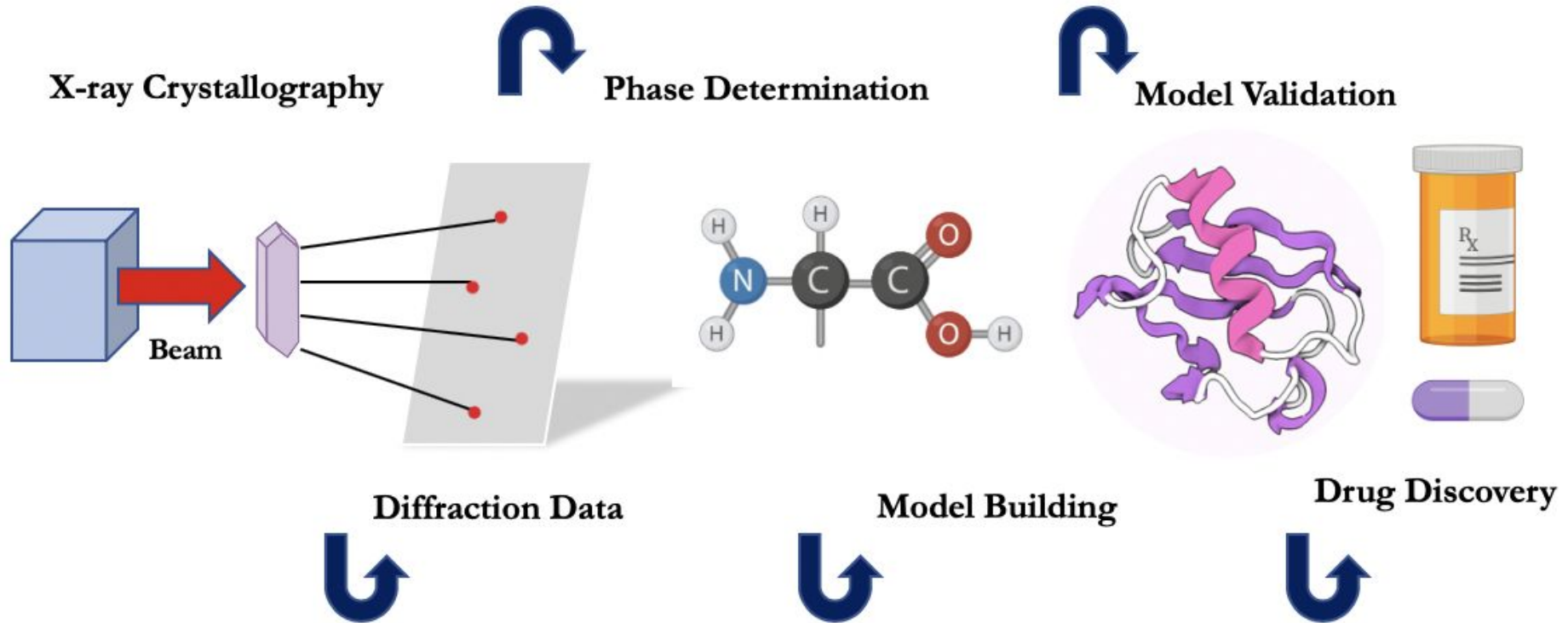
- Decreased efficacy of current drugs



- ATPase<sub>3</sub> domain can serve as a novel drug target



# PATHWAY TO DRUG DISCOVERY



# Acknowledgments

Dr. Sara Andres, Assistant Professor

Lucas Koechlin, Teaching Assistant

Tim Klein, Teaching Assistant

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