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Bacterial Enzymes and Antibiotic Resistance

Lauren Maltz

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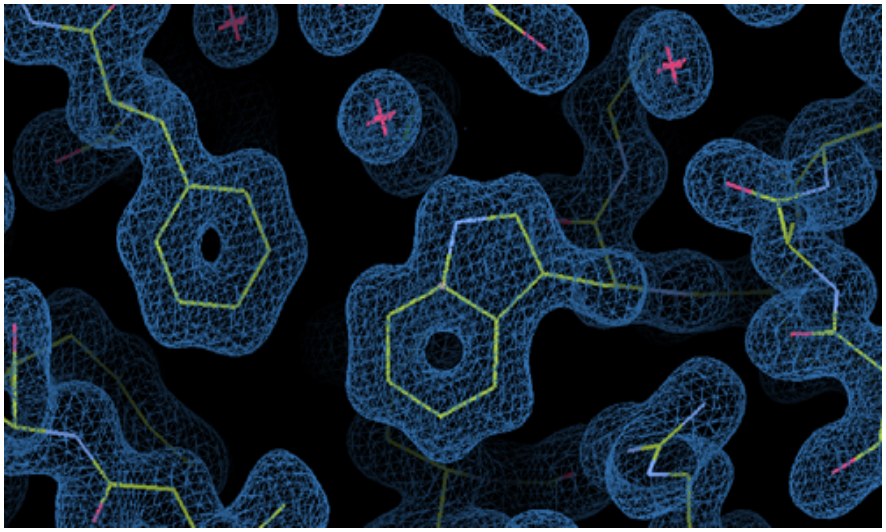
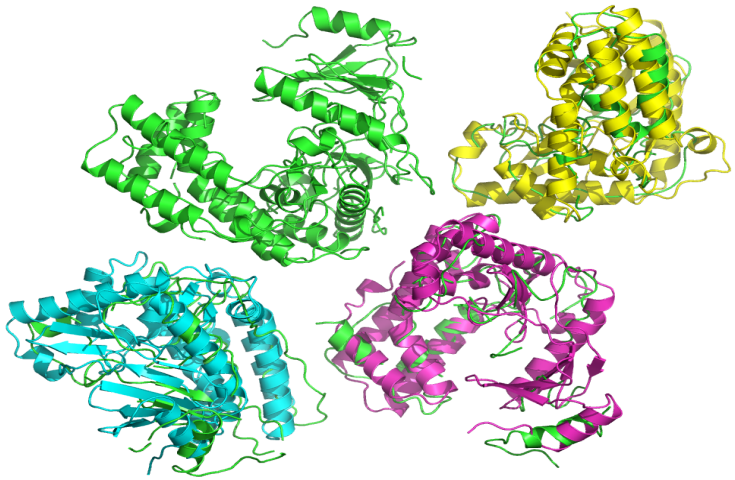


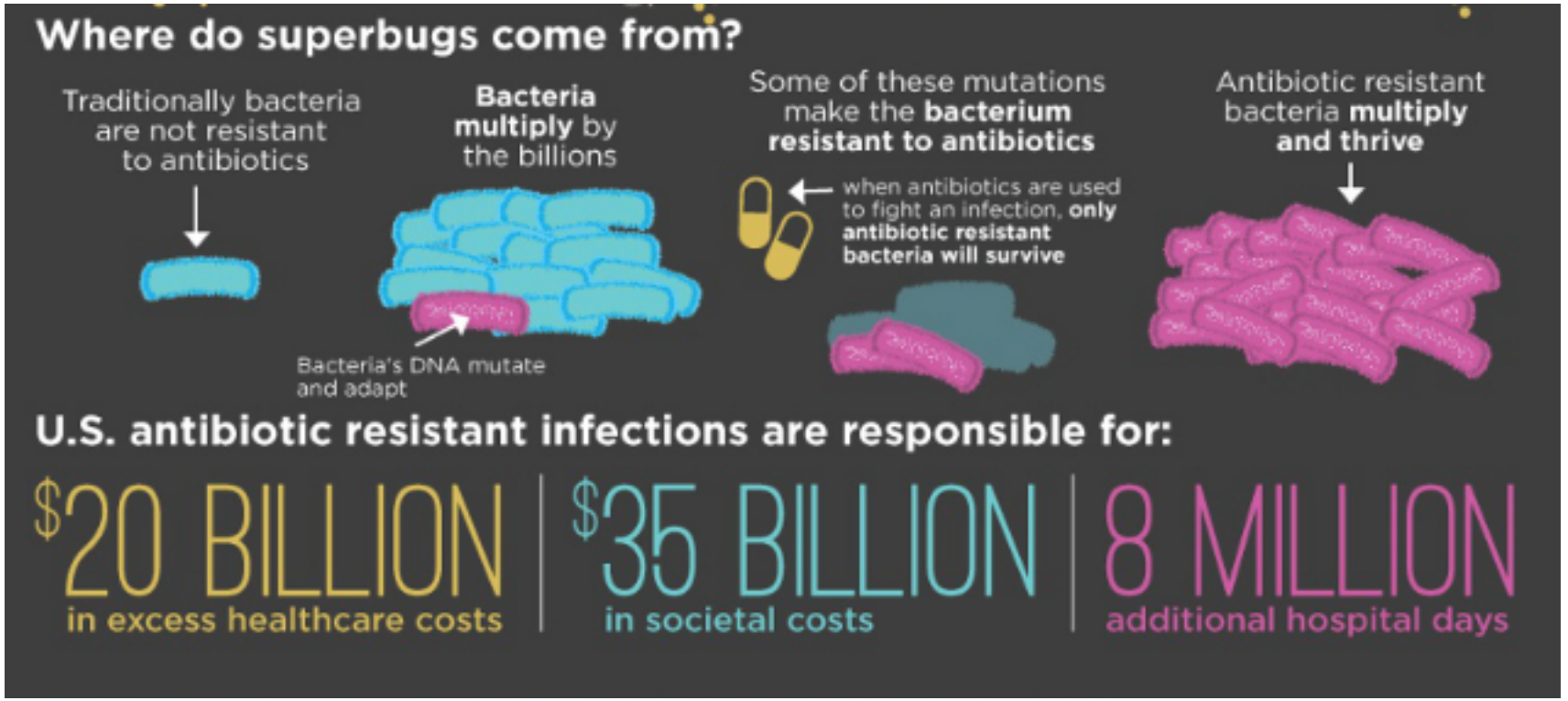


Scientific Abstract

By using protein crystallography and X-ray diffraction, structures of bacterial enzymes were solved to gain a better understanding of how enzymatic modification acts as an antibacterial resistance mechanism. Aminoglycoside phosphotransferases (APHs) are one of three aminoglycoside modifying enzymes that confer resistance to the aminoglycoside antibiotics via enzymatic modification, rendering many drugs obsolete. Specifically, the APH(2”) family vary in their substrate specificities and also in their preference for the phosphate donor (ADP versus GDP). By solving the structures of members of the APH(2”) family of enzymes, we can see how domain movements are important to their substrate specificity. Our structure of the ternary complex of APH(2”)-IIIa with GDP and kanamycin, when compared to the known structures of APH(2”)-IVa, reveals that there are real physical differences between these two enzymes, a structural finding that explains why the two enzymes differ in their preferences for certain aminoglycosides. Another important group of bacterial resistance enzymes are the Class D β-lactamases. Oxacillinase carbapenemases (OXAs) are part of this enzyme class and have begun to confer resistance to ‘last resort’ drugs, most notably carbapenems. Our structure of OXA-143 shows that the conformational flexibility of a conserved hydrophobic residue in the active site (Val130) serves to control the entry of a transient water molecule responsible for a key step in the enzyme’s mechanism. Our results provide insight into the structural mechanisms of these two different enzymes.

Bacterial Superbugs

MRSA



Bacteria

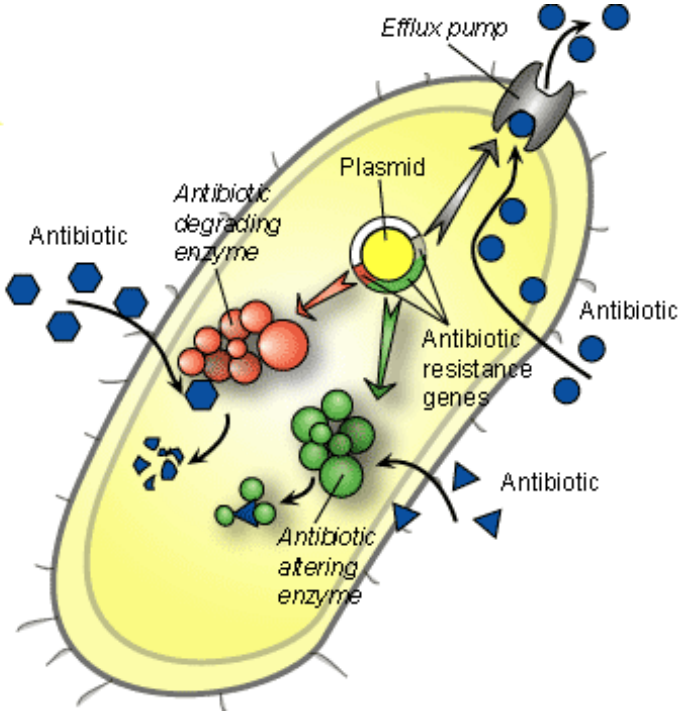
Practices that foster resistance

taking antibiotics for non-bacterial illness
not taking all of antibiotic
non-human use of antibiotics

Antibiotic Resistance Mechanisms

- Diminished cell entry
- Active efflux
- Target Alteration
- Enzymatic modification of drug

β-lactams and aminoglycosides

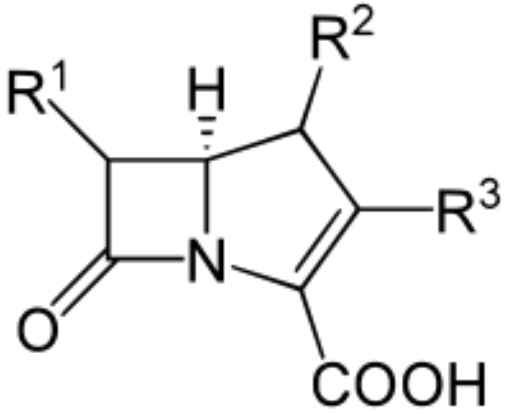


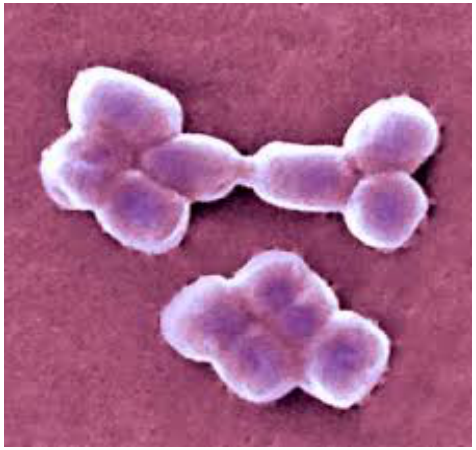
β-lactamases

4 Classes: A, B,C, and D

Class D β-lactamase enzymes → oxacillinases (OXA)

- Deactivate a wide range of antibiotics
  - Carbapenems—"last resort" drugs





Common host species: *Acinetobacter baumannii*

Microscope (left), petri dish (right)

Carbapenem resistance has increased due to the spread of OXA-type β-lactamase

Aminoglycoside Modifying Enzymes (AMEs)

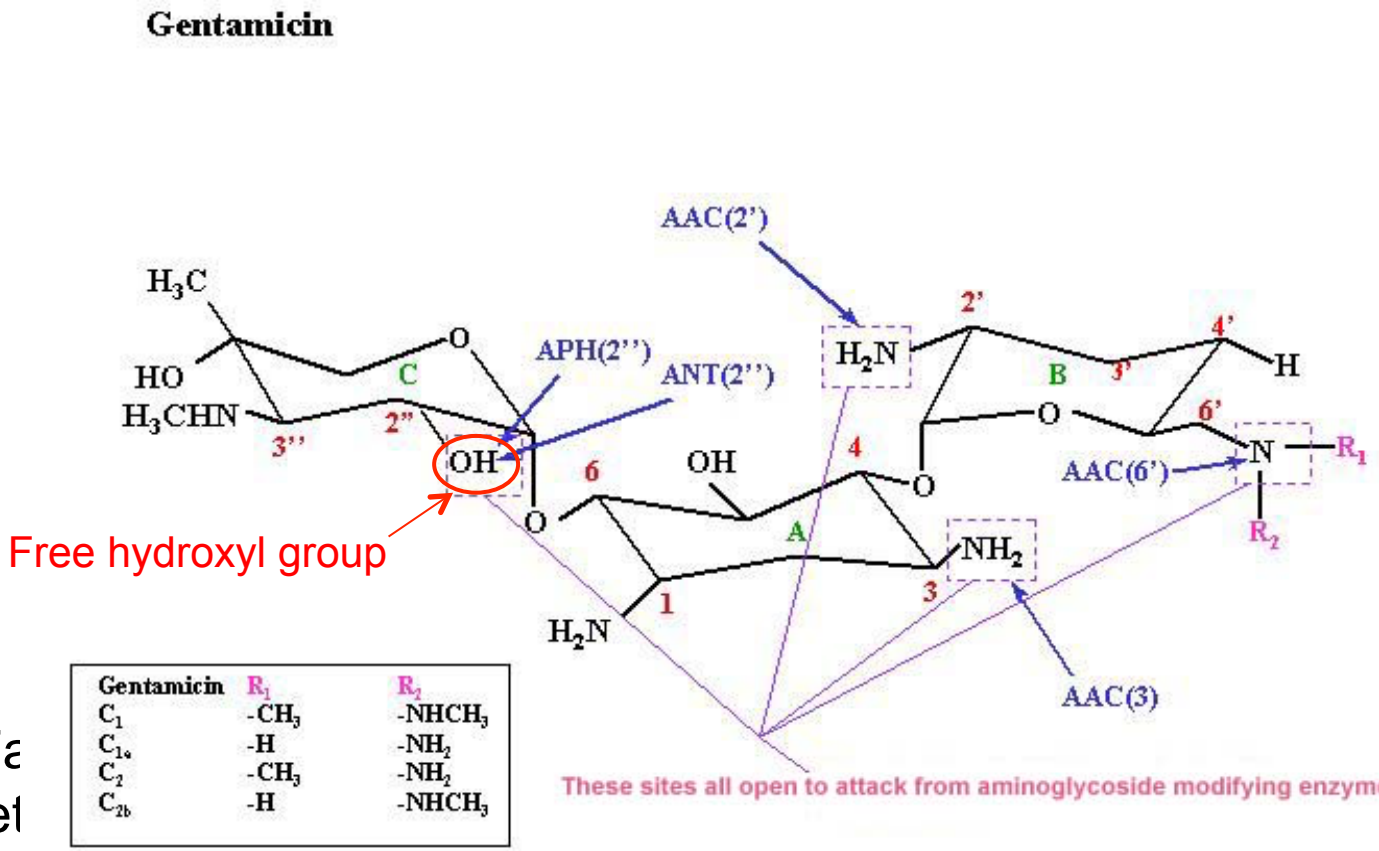
AMEs cause high levels of resistance to aminoglycoside antibiotics

- Promiscuous substrate profiles
- Can be used to treat endocarditis & genetic disorders

3 Families of AMEs:
acetyltransferases, nucleotidyltransferases, phosphotransferases (APHs)

Aminoglycoside Modifying Enzymes (AMEs)

AME

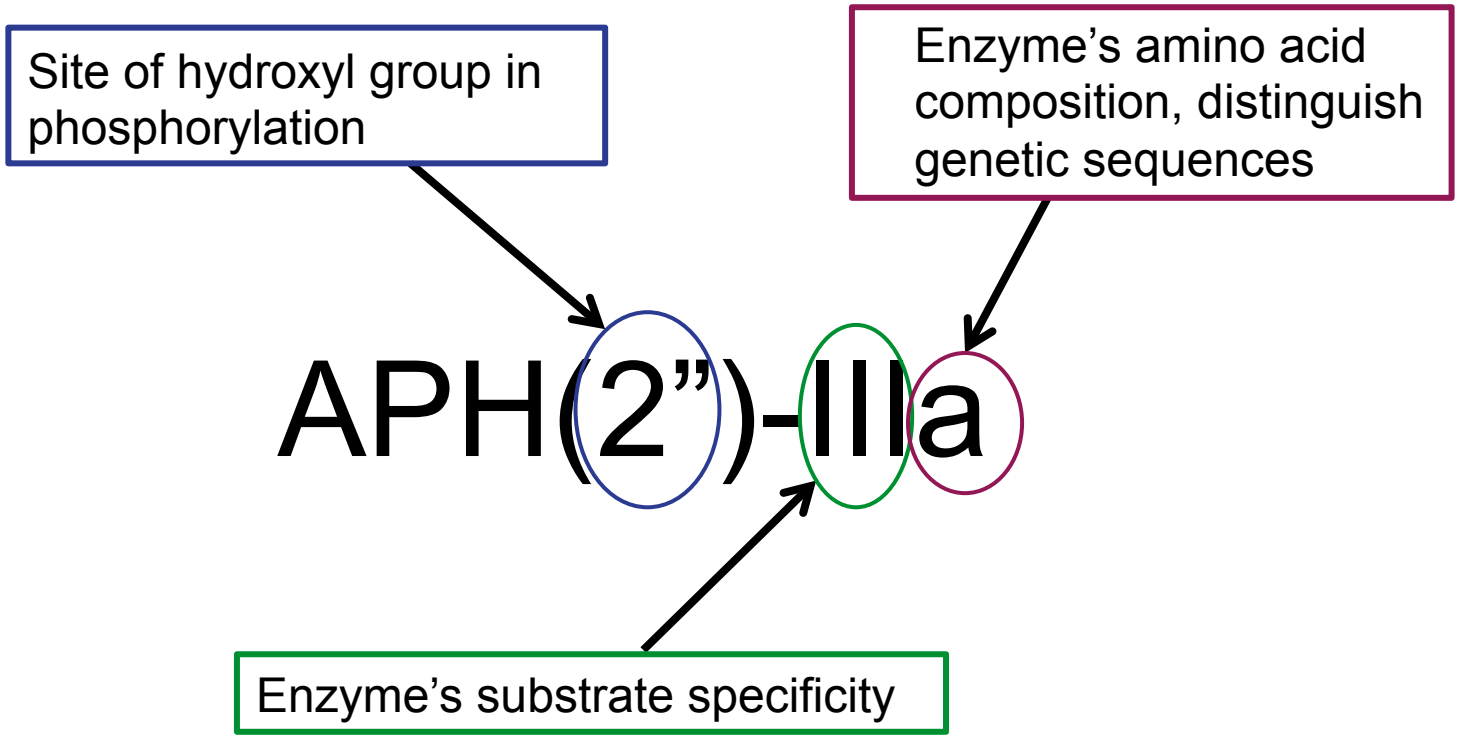


Gentamicin	R_1	R_2
C_1	$-CH_3$	$-NHCH_3$
C_{1a}	$-H$	$-NH_2$
C_2	$-CH_3$	$-NH_2$
C_{2b}	$-H$	$-NHCH_3$

3 Fa
acet
PHs)

Modified from Antimicrob Agents Chemother 1999;43:727-37

Nomenclature of APHs



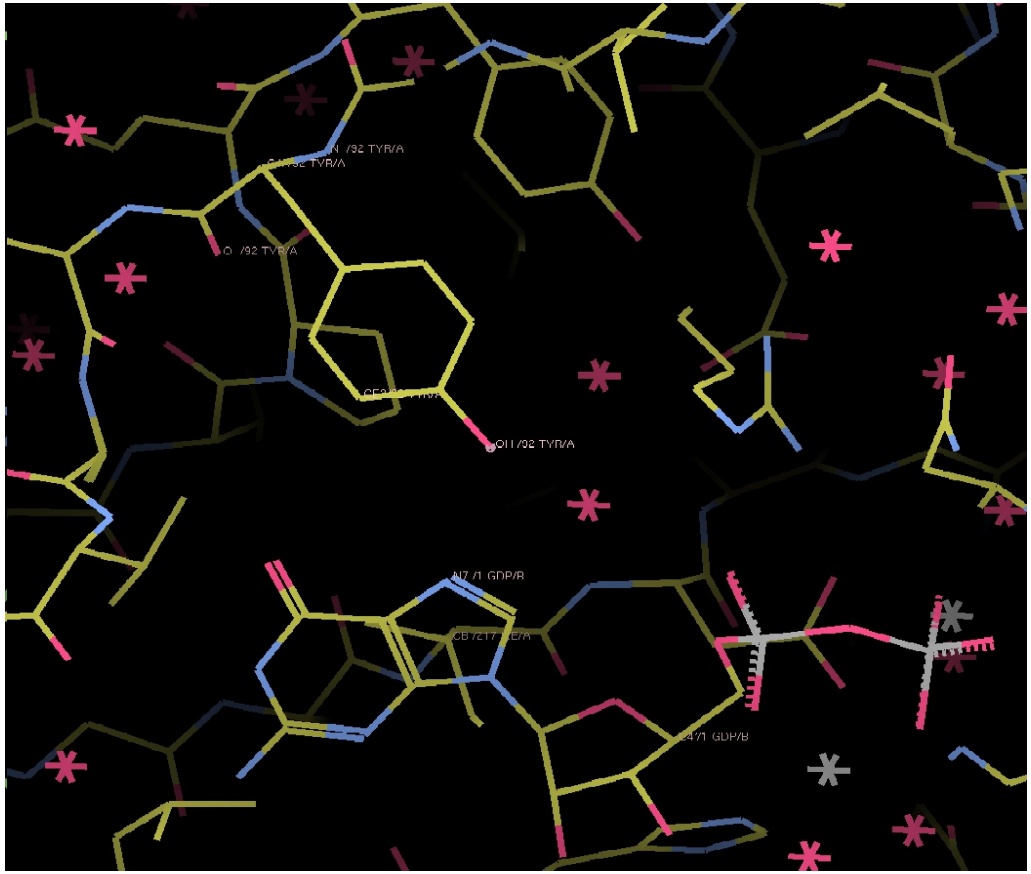
2" Family: Ia, IIa, IIIa, IVa

APH(2”) Family

- Previously considered to solely use ATP for antibiotic modification/phosphate source
- APH(2”)-Ia and APH(2”)-IIIa: GTP preference
- APH(2”)-IIa and APH(2”)-IVa: equal GTP and ATP preference

Why do these enzymes have these specific preferences?

Must look at structure of enzymes
Gatekeeper residues



APH(2”)-IIIa TYR92 on IIa, ADP
APH(2”)-IIIa TYR92, GDP

My Project: APH(2”)-IIIa and OXA-143

Steps of a Protein Crystallography Experiment

1. Purify
2. Crystallize
3. Measure diffraction data
4. Process data, XDS consistently gave the best results for each structure and its data set
5. Molecular replacement: method used to solve structure by used a similar structure as a model, use program MOLREP
   For OXA-143: OXA-24 (87.6% similarity)
   For APH(2”)-IIIa: used wild type with kanamycin and GDP
6. Analyze structures

Enzyme Mechanism

Reaction is occurring characteristic to all beta lactamases:

(1) Acylation Step: Acyl-enzyme intermediate

$$E + S' \rightleftharpoons E\text{-}S$$

(2) Deacylation Step: Breakdown of acyl-enzyme intermediate

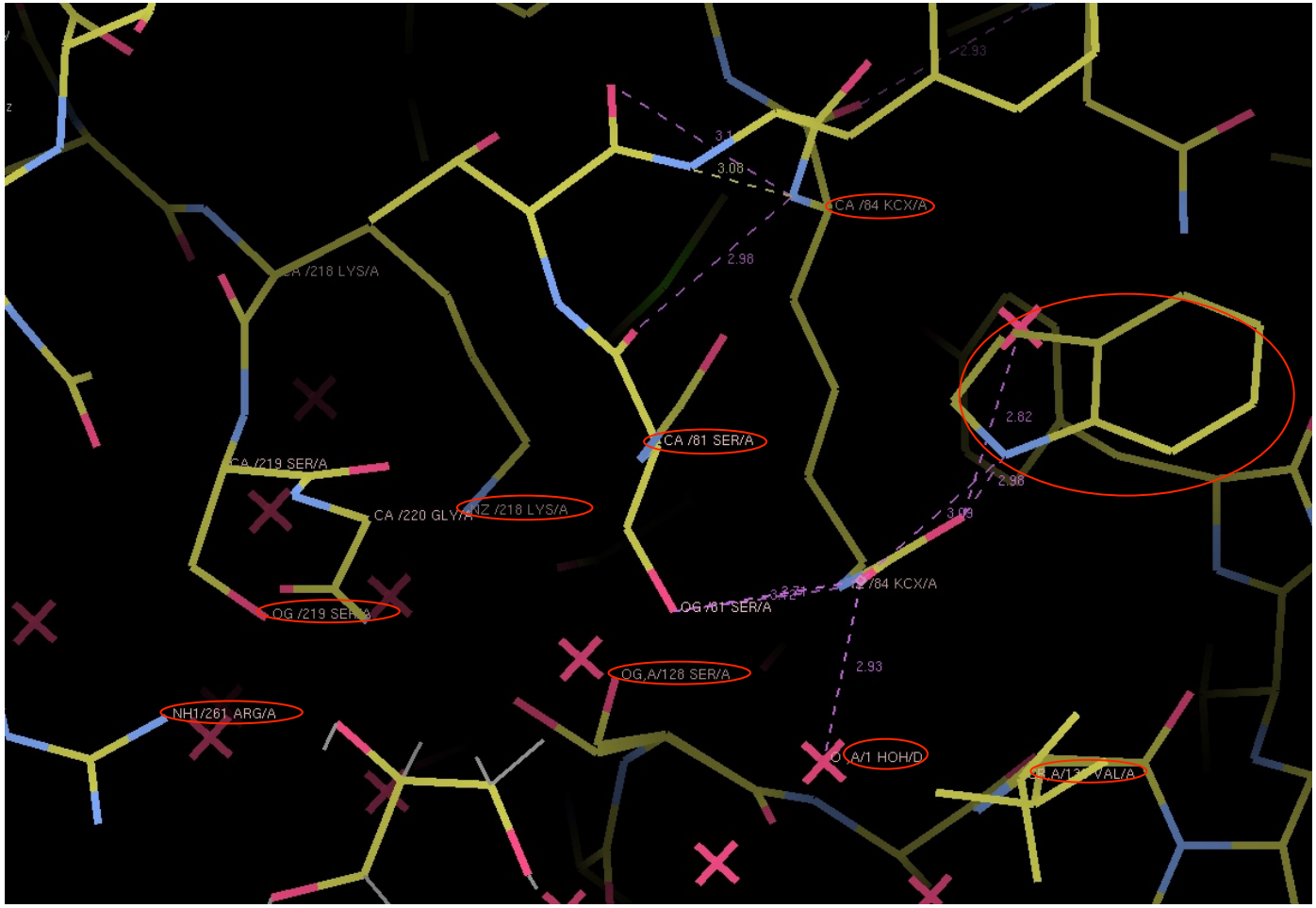
$$E\text{-}S \overset{H_2O}{\rightleftharpoons} E + S^*$$

S' = active substrate
S* = deactivated substrate

OXA-143 structure found to have similar structure to other known OXA enzymes

Results: OXA-143

Important and Active Site Residues:

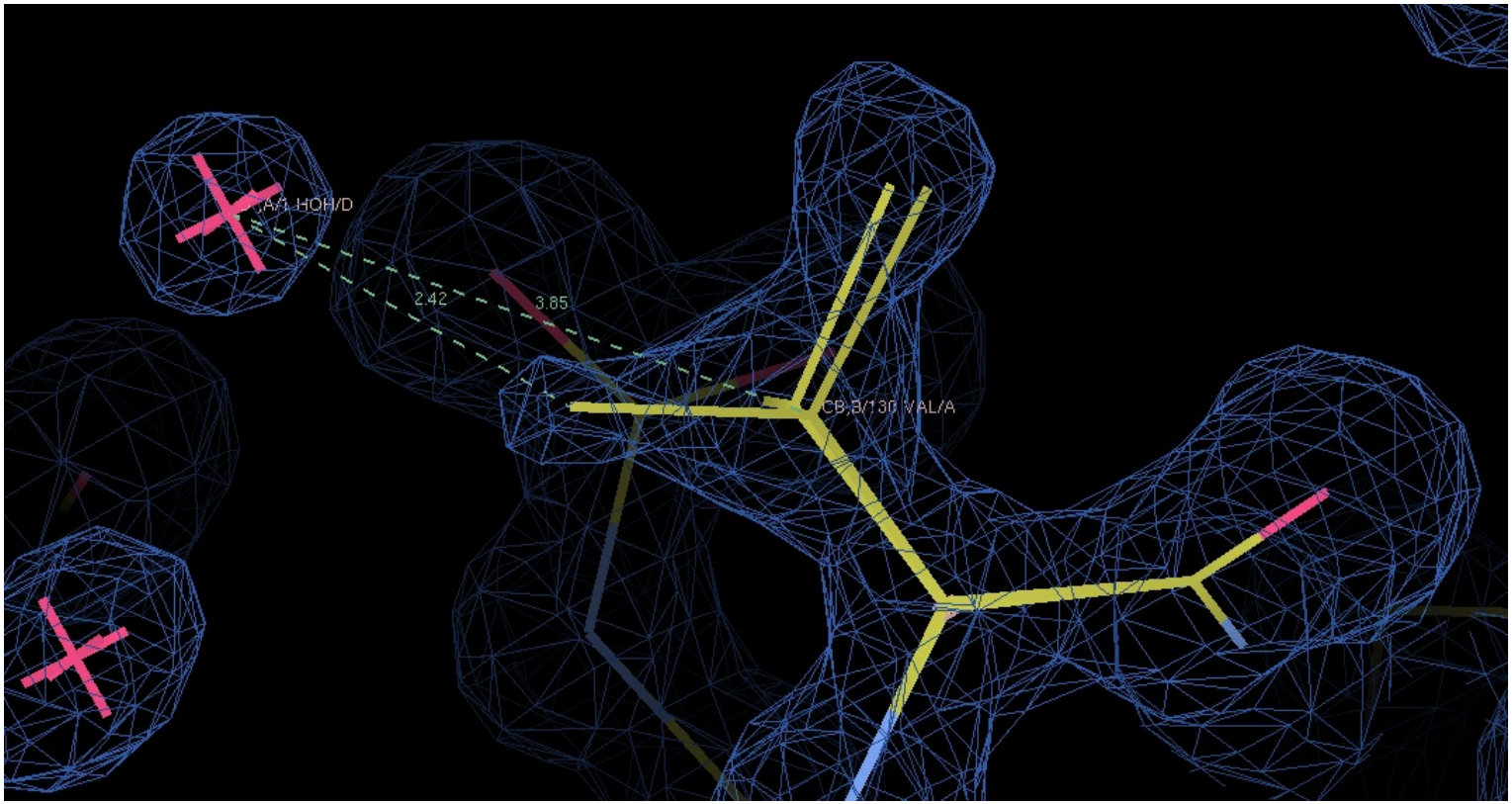


- A1: deacylating water
- Ser81: (1), initial covalent bond
- Lys84: activates A1
- Ser128,Lys218: H bond connecting domains, at base of active site
- Ser219, Arg261: in conjunction, substrate locked in
- Trp167: stabilizes Lys84
- Val130: A1 occupancy control

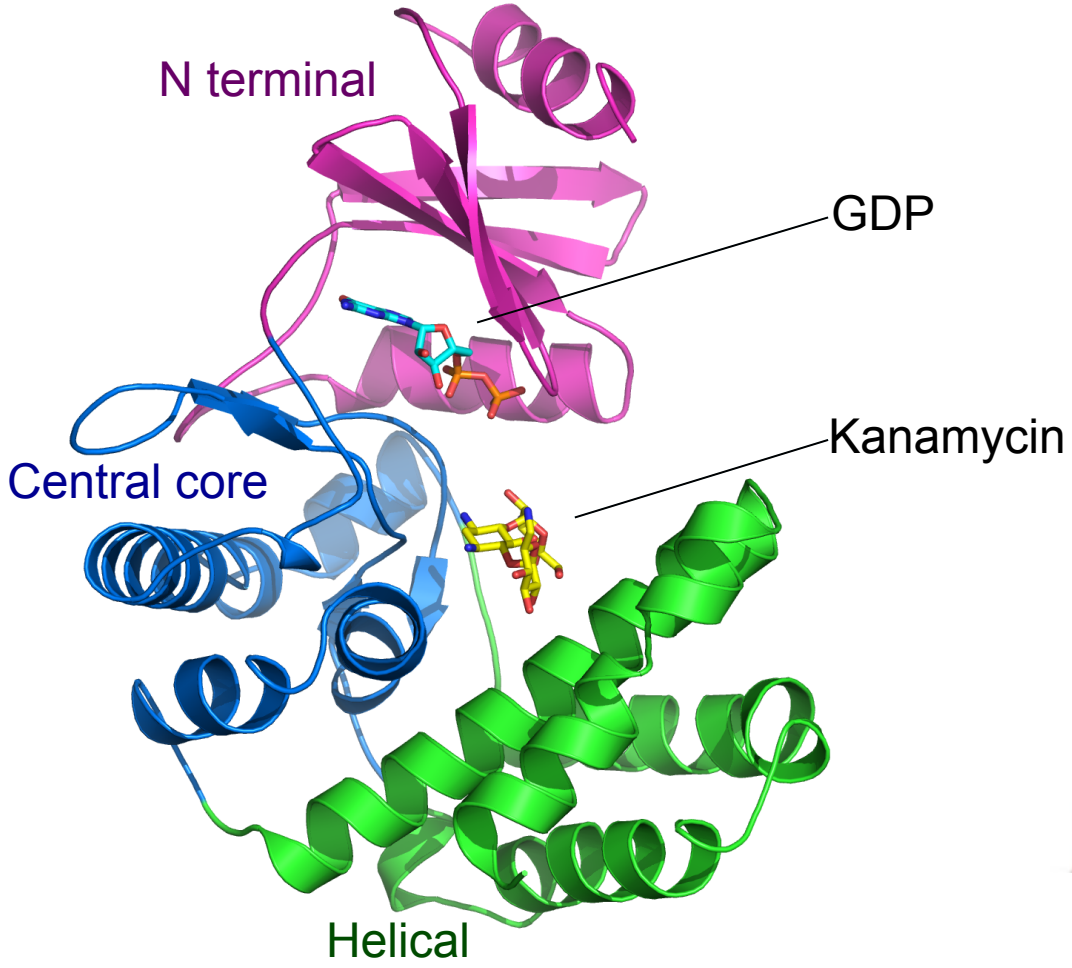
Results: OXA-143

Deacylating Water

- In active site near Valine 130
- Val130 exists in 2 conformations
- Water transiently present depending on the conformation of Val130

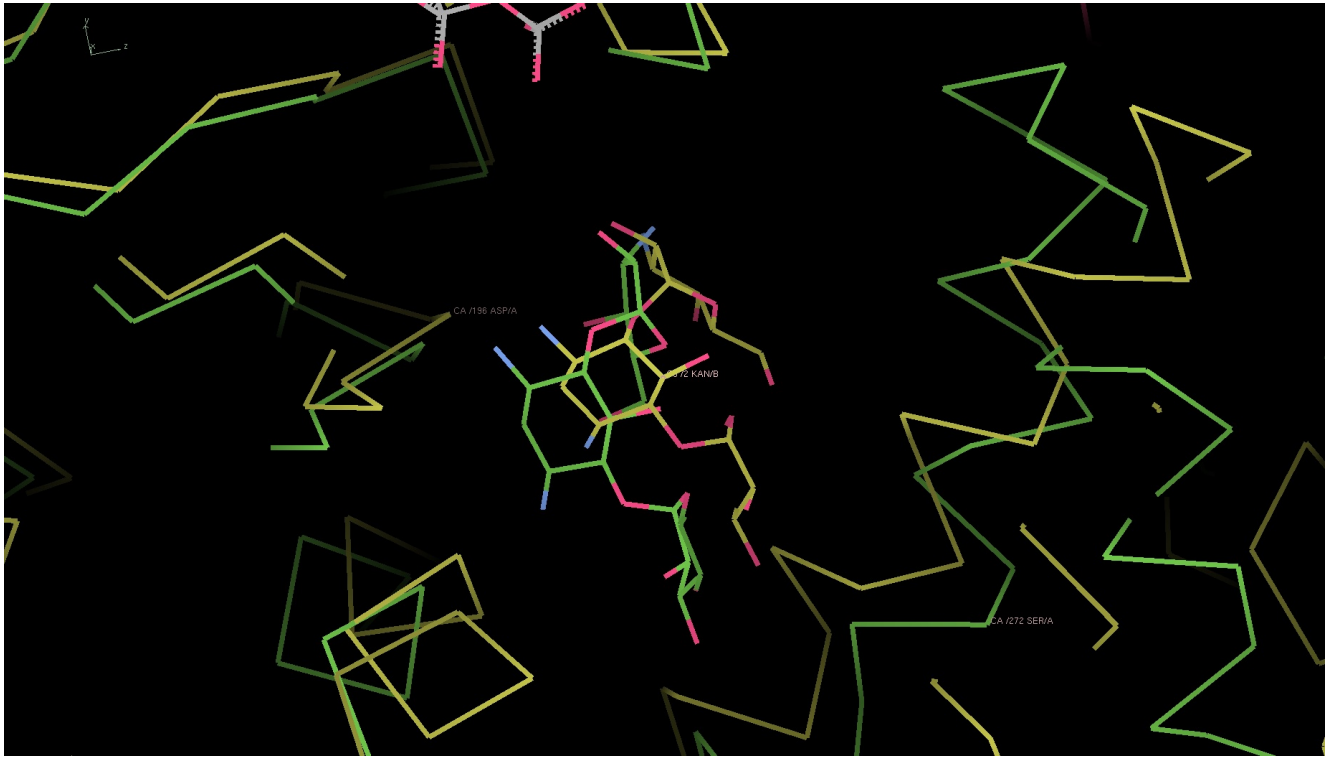


APH(2”)-IIIa Structure



Results: APH(2”)-IIIa

- With and without kanamycin→ no helical movement, same known about IVa from previous work
- Real position differences between IIIa (yellow) and APH(2”)-IVa (green)
  - Kanamycin moved toward active site/helical domain away from the Aspartate in APH(2”)-IIIa
    - Supports known differences in substrate profiles/specificity



APH(2”)-IIIa v. APH(2”)-IVa

Summary

OXA-143

- Sidechain conformation of Val130 is controlling:
  - Access of water
  - Reaction rate of enzyme through deacylation process step 2
- Different control mechanism than other OXAs
  - Drugs have to account for such differences among the OXAs→ pose more difficulty in creating unique inhibitors

APH(2”)-IIIa

- Each APH(2”) is named differently because of their different substrate profiles
  - Supported by real structural differences observed

Acknowledgements

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