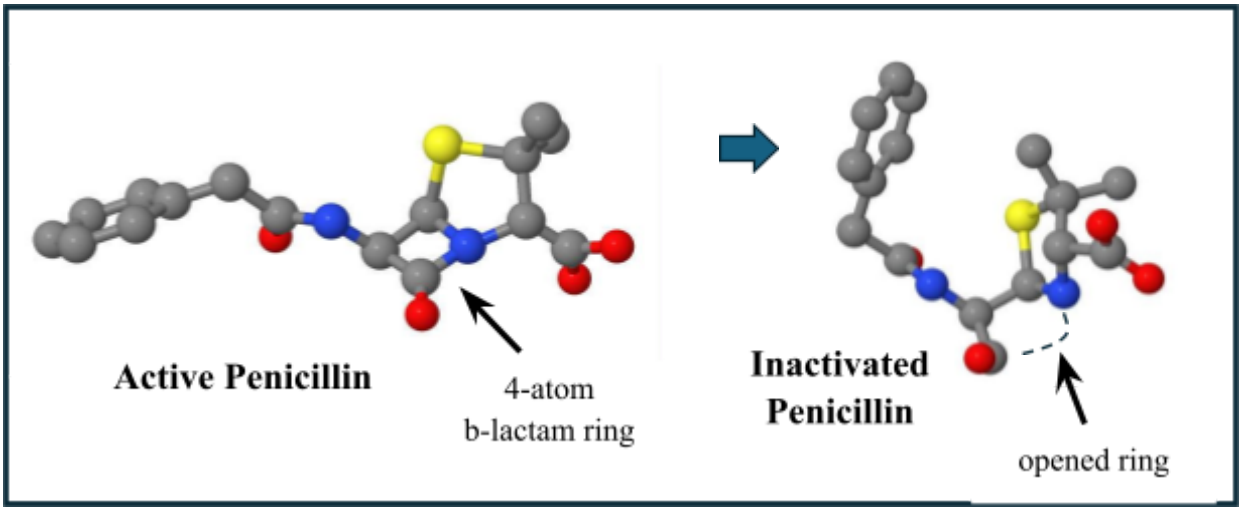
***β*-Lactamase**… A Guided Jmol Exploration

This is a ***Guided Exploration*** of a bacterial enzyme – ***β-lactamase*** – that can bind to and destroy penicillin.

Penicillin is an antibiotic that kills bacteria by inhibiting the enzyme **PBP** (**P**enicillin **B**inding **P**rotein) that crosslinks the bacterial cell wall. Without a strong, crosslinked cell wall, the bacteria explode as a result of the osmotic pressure that exists inside the cell. By deploying ***β-lactamase*** enzymes on its surface, bacteria can intercept and destroy penicillin before it has a chance to inhibit the crosslinking enzyme. This is one way in which bacteria can evolve resistance to an antibiotic.

Penicillin is known as a beta-lactam antibiotic. It contains an unusual 4-atom lactam ring. ***β-lactamase*** is a bacterial enzyme that opens this ring….and in so doing, inactivates the antibiotic.



This *Guided Exploration* is based on the structure of ***β-lactamase*** *a*s described in the PDB file **5KMW.pdb.**

In 2016, Patricia Langan and colleagues solved the 3D structure of the Toho 1 β-lactamase with penicillin bound in its active site. This structure was notable in that it was the first structure to capture the penicillin in the enzyme's active site.

The overarching **QUESTION** that his Jmol Exploration is designed to answer is… ***Why was Langan et.al. successful in capturing the penicillin in the active site while many other groups had not been able to do this?***

This Jmol exploration is based on the following paper:

Primary Citation: *The structure of Toho β-lactamase in complex with penicillin reveals the role of Tyr105 in substrate recognition.* Patrica Langan, Venu Gopal Vandavasi, Kevin Weiss, Jonathan Cooper, Stephan Ginell and Leighton Coates. FEBS Open Bio 6 (2016) 1170-1177

Note to teachers….about this paper: This is a complicated paper. We don't recommend that you give this paper to your students to explore without some help. Here's why.

You will note that the title says something about Tyr150 and its role in substrate recognition. This is NOT why we chose this paper. Instead, we chose this paper because…

(i) it tells a story about how this research group was able to capture the penicillin bound in the active site of β-lactamase, and

(ii) it has a great introduction (not the abstract) that summarizes the background to this field of study. Several pertinent paragraphs from this introduction are presented below. (We do recommend that you give this introduction to your students.)

From the Introduction…..

Penicillin, a β-lactam antibiotic, was first observed by Alexander Fleming in 1928 when he saw that mold growing on an accidentally contaminated Petri dish destroyed the bacteria surrounding it [1]. This discovery changed the course of medical history. Unfortunately, however, resistant bacteria have been evolving alongside antibiotic agents long before their discovery by humans [2]. Bacteria have obtained resistance to β-lactam antibiotics in various ways, but most commonly by their expression of β-lactamases. Despite evolving resistance to β-lactam antibiotics they remain the most widely used class due to their low toxicity. This is due to the fact that they interact with bacterial penicillin binding proteins (PBPs) which are absent in humans. PBPs are enzymes involved in the construction of the bacterial cell wall. β-lactams are able to form stable acyl-enzyme complexes with PBPs, rendering them inactive, unable to maintain and construct the cell wall, ultimately causing cell lysis. As there is no human counterpart of this enzyme, and disruption of its activity is fatal to the bacterial cell, it is important that we continue to develop our understanding of how β-lactam antibiotics are bound and inactivated by β-lactamase enzymes [3,4].

Wild-type β-lactamase rapidly hydrolyze and release b-lactam antibiotics, making it virtually impossible to trap the acyl-enzyme intermediate for study. In class A b-lactamase enzymes Glu166 activates the hydrolytic water required for deacylation of the tetrahedral intermediate. Therefore, mutating Glu166 to an alanine halts the reaction at the acyl-enzyme adduct (Fig. 1, stage 3) enabling this state to be studied.

The **β-Lactamase Guided Jmol Exploration** can be found here: [3D Molecular Designs - Beta Lactamase (centerforbiomolecularmodeling.org)]

The text from that **β-Lactamase Guided Jmol Exploration** is provided here ….

Click Here to look at the structure of B-lactamase

Click and drag the Jmol image to the right to spin it around to examine it's secondary structure.

QUESTION: Do you see a 5-stranded beta-sheet and clusters of alpha helices ... all joined by short loops of protein structure?

Click Here to show the secondary structures color-coded (yellow beta-sheet and red alpha helices)

Now let's find the active site. When penicillin binds to beta-lactamase, it forms a covalent bond to Ser70. **So,... where is Ser70?**

Click Here to show a Jmol image with the sidechain of Ser70 displayed. Notice that the active site appears to be located in a groove between two compact domains of the enzyme.

Now let's display the penicillin bound in the active site of the enzyme.

Click Here to display the penicillin.

Zoom in on Ser70 -- and notice that the penicillin is covalently bound to this amino acid. Notice also that the 4-atom lactam ring of the penicillin has been opened -- inactivating the penicillin.

Now back to our original question -- **Why was Langan et.al. successful in trapping penicillin in the active site of beta-lactamase, while many other research groups had failed to do this?**

The answer to this question is found in amino acid 166. Langan and colleagues knew from other research into the chemistry of this active site that Glu166 played a critical role in destabilizing the covalent bond that connected penicillin to the Ser70 sidechain. Normally this covalent bond only lasts for a very short time before it is cleaved by Glu166, releasing the penicillin.

Find amino acid #166 in the Jmol image to the right. (If you 'hover' your mouse over each amino acid -- its identity will be reported on the screen.) When you find amino acid 166 you will see that it is not Glu (Glutamic acid). Instead, it is Ala (Alanine).

So,... the answer to the above question is that Langan et.al. created a variant form of β-lactamase in which Glu166 was changed to Ala166. As a result, the covalent bond connecting penicillin to the enzyme was not broken....and they observed the antibiotic bound in the active site.

If you zoom in on the penicillin, you will notice that it is now covalently linked to the sidechain of Ser70.

Click Here to see a zoomed in view of the active site -- with the Ala166 sidechain displayed as a green sphere.

Additional note to teachers: ***Never display your art in an art gallery.*** If you do, your art will be displayed along with hundreds of other art pieces…. all competing for the attention of the gallery visitors. We have a similar problem with the 12 different instructional tools that we have created to support our ***Modeling Infection and Immunity*** workshop. In spite of all that competition from the other materials you experienced in that summer course… I hope that some of you will field-test the **β-lactamase physical model** and the **β-lactamase Guided Jmol Exploration** with your students….and provide us with some feedback.

If you do,… here is one more follow-on activity you can have your students explore:

Have your students compare the structure of β-lactamase with the structure of a PBP (Penicillin Binding Protein). The punchline here is that b-lactamase evolved from a PBP. Don't tell your students this…. Instead, see if they can see the similarities between these two structures and then draw their own conclusions about where β-lactamase came from.

To do this, have your students use the Protein Exploratorium (https://learn.3dmoleculardesigns.com/protein-exploratorium) to load the 1PWC.pdb.