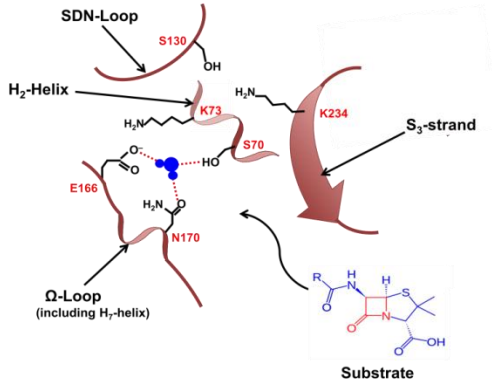
# Biocatalysis is the main mechanism of bacterial resistance to antibiotics

ActaNaturae

*Egorov A.M. et al. Acta Naturae, 2018, 10, 4, 33-48*

## Bacterial Enzymes and Antibiotic Resistance




A. M. Egorov, M. M. Ulyashova, M. Yu. Rubtsova

The resistance of microorganisms to antibiotics has been developing for more than 2 billion years and is widely distributed among various representatives of the microbiological world. The main mechanisms of resistance development are associated with the evolution of superfamilies of bacterial enzymes due to the variability of the genes encoding them. The collection of all antibiotic resistance genes is known as the resistome. Tens of thousands of enzymes and their mutants that implement various mechanisms of resistance form a new community that is called "the enzystome." Analysis of the structure and functional characteristics of enzymes, which are the targets for different classes of antibiotics, will allow us to develop new strategies for overcoming the resistance.

**Bacterial resistance to antibiotics** – a planetary global biological process on the Earth

**Multi and pan-resistant bacteria** - a new challenge for infectious medicine

**Environment** – large reservoir of microbial resistance genes and antimicrobial compounds

Lomonosov Moscow State University
Laboratory of enzyme engineering

# Beta-lactamases – drivers
## of bacterial resistance to beta-lactam antibiotics

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***12th INTERNATIONAL CONFERENCE***
***"BIOCATALYSIS. FUNDAMENTALS & APPLICATIONS"***
**June 24-28 2019, Leonid Sobolev board**

# History of beta-lactam antibiotics

Beta-lactamase inhibitors

**More than 2.5 billions years ago**

Archean era

**1929**

Discovery of penicillin by Alexander Fleming

**1940**

Industrial production of penicillin

**1964**

I generation cephalosporins

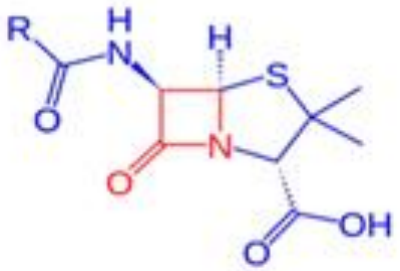
**1982**

III generation cephalosporins

**1985 - 2000**

Carbapenems

# Penicillin -



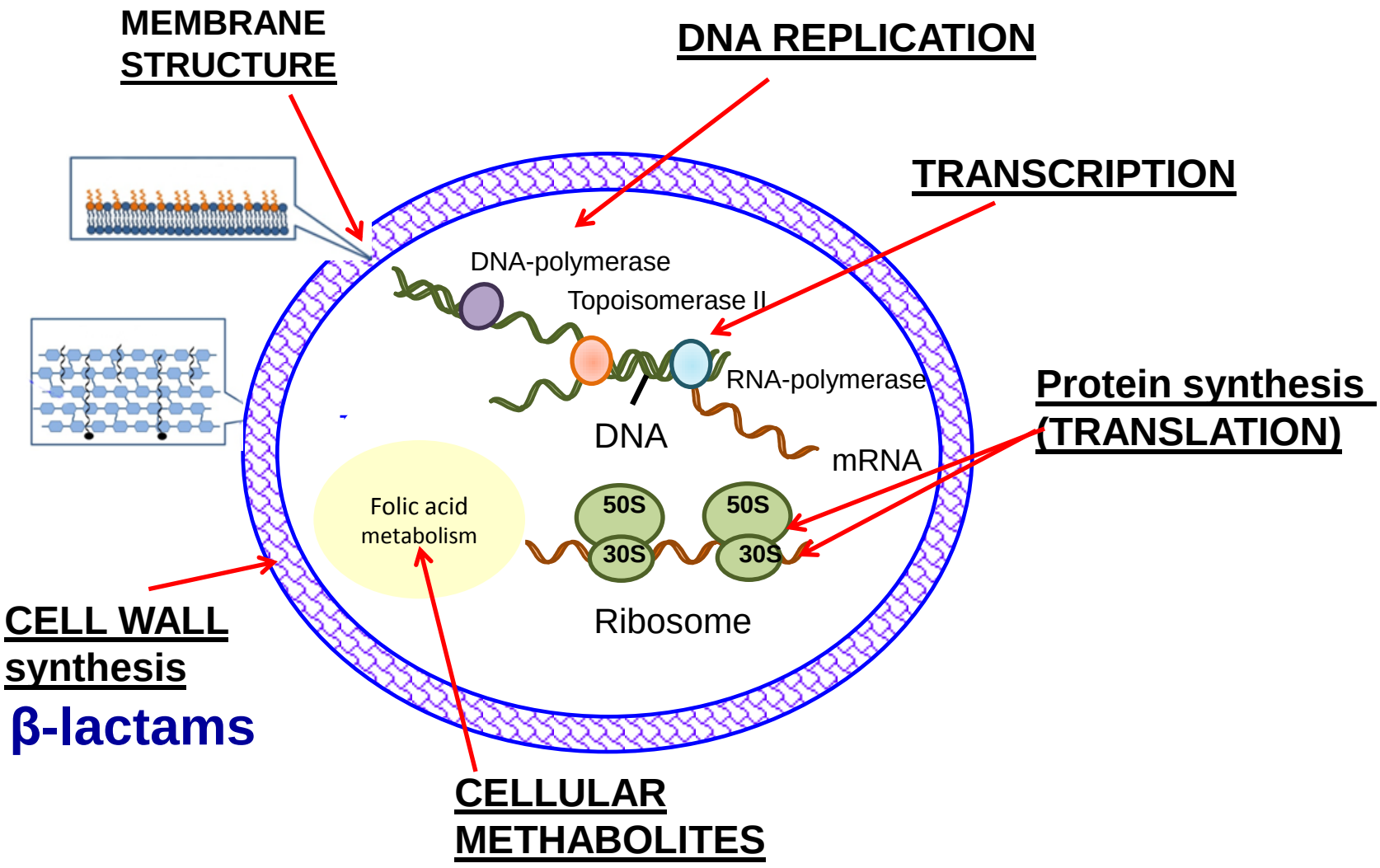

the first natural antibiotic that has been studied and used in clinical medicine

**1928/29 г** – **Alexander Fleming** discovered interspecies struggle of fungus *Penicillium notatum* with *Staphylococcus* cells and called the active compound **“penicillin”**

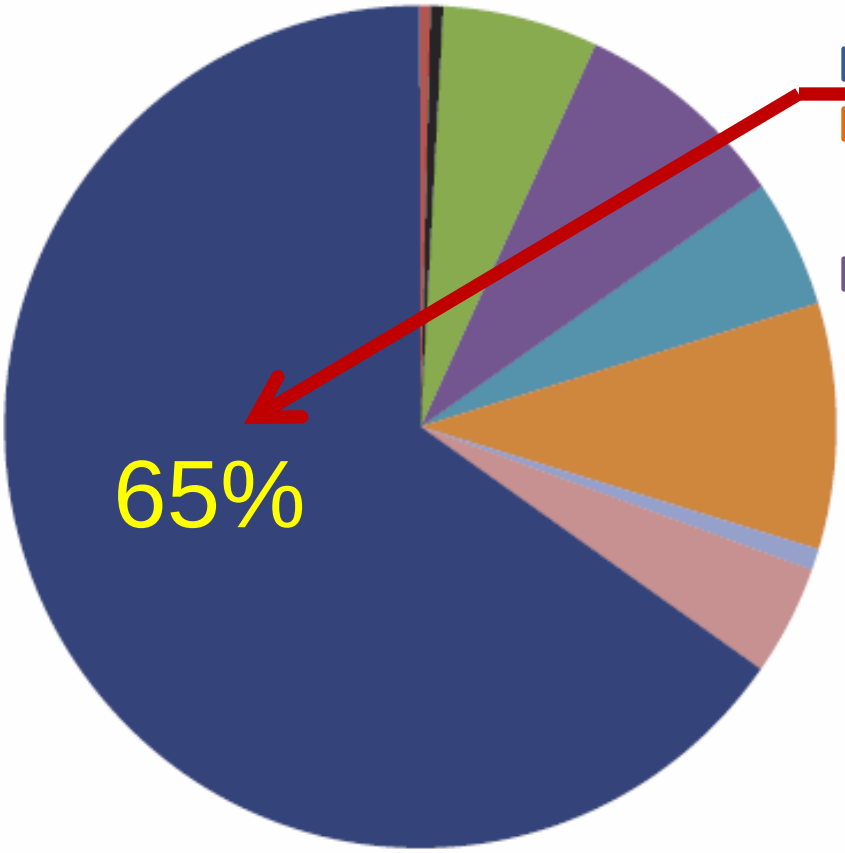
**1939 г. – Howard Florey** and **Ernst Chain** synthesized penicillin and organized its industrial production


Nobel Prize Winners for 1945
in the field of physiology and medicine

# Targets in bacterial cell for antibiotics



# Production of different classes of antibiotics




**Cell wall synthesis**

- beta-lactams
- glycopeptides

**DNA replication**

- fluoroquinolones

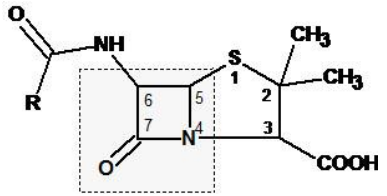
**Protein synthesis**

- macrolides
- aminoglycosides

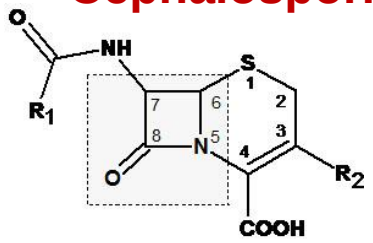
**Membrane structure**

- polymyxins

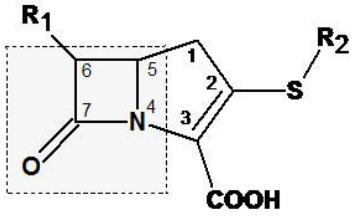
**Penicillins**



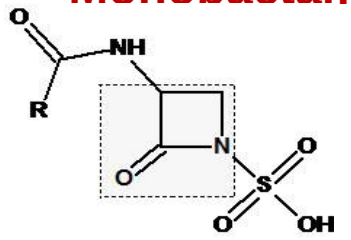
**Cephalosporins**



**Carbapenems**

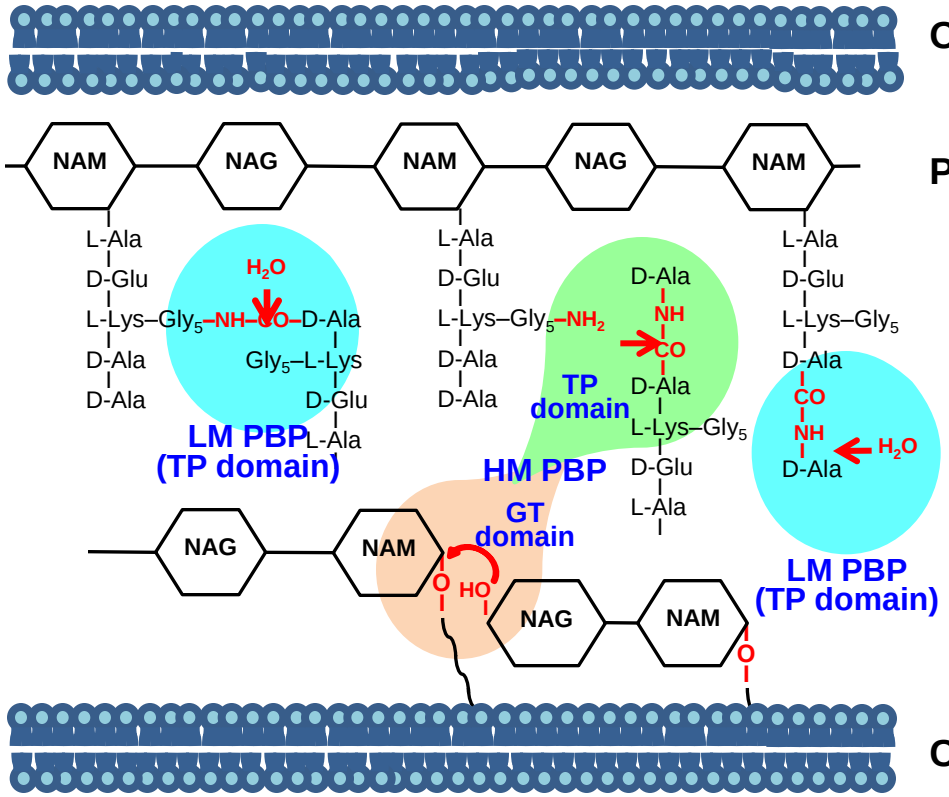


**Monobactams**



*The total production of antibiotics is about 1,000,000 tons, and beta-lactam production is about 600,000 tons (BusinesStat, 2014)*

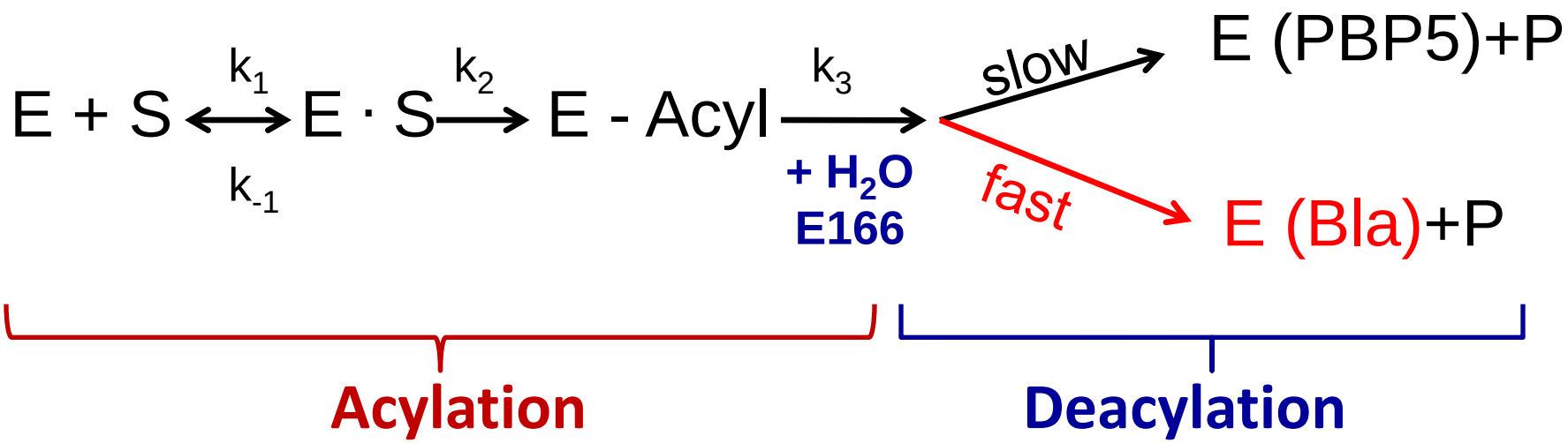
# Main targets for beta-lactam antibiotics – penicillin binding proteins (PBPs)



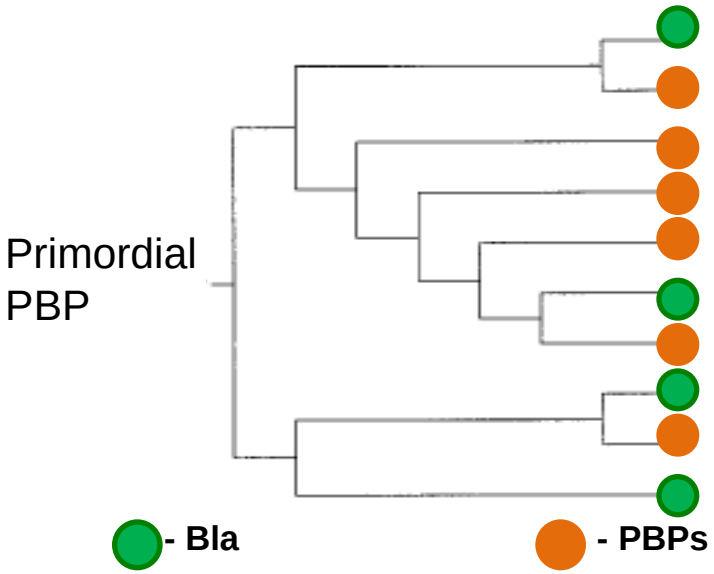

***LM***- *low mol weight*
***HM***- *high mol weight*

- **Beta-lactams are broad-spectrum antibiotics**
- **Low toxicity**
- **No target for beta-lactams in humans**

# Interaction of beta-lactams with enzymes




Co-evolution of PBPs and beta-lactamases (Bla)




| PBP type | Molecular class of BLa |
|---|---|
| LM class C | A (serine) |
| LM class B | B (metallo) |
| LM class B | C (serine) |
| HM class C | D (serine) |

# Beta-lactamases - super-family of enzymes

## Most clinically relevant beta-lactamases

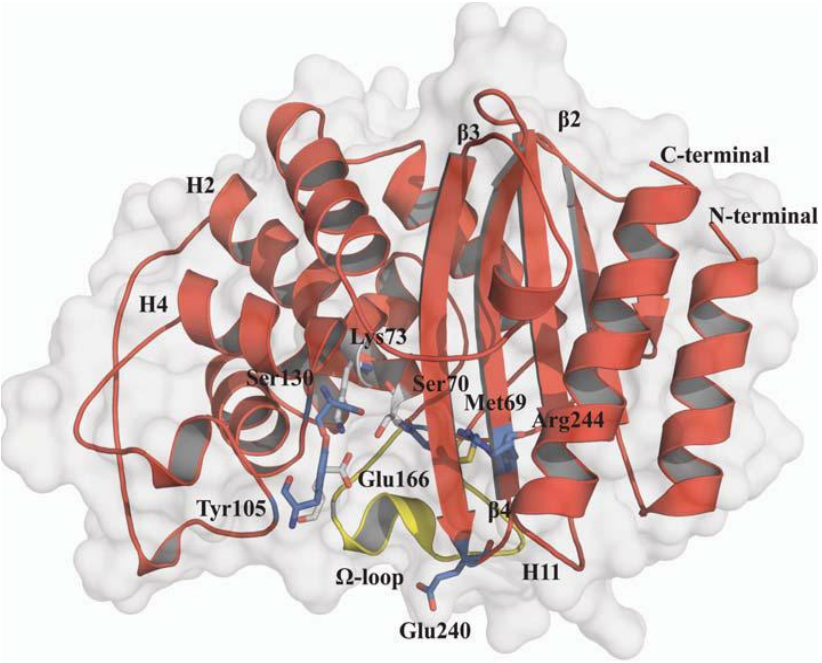
| Class A (serine) | Class B (metallo) | Class C (serine) | Class D (serine) |
|---|---|---|---|
| **TEM** (200 subtypes) | **VIM** (43 subtypes) | **Amp C** (1000 subtypes) | **OXA** (700 subtypes) |
| **SHV** (170 subtypes) | **IMP** (48 subtypes) | | |
| **CTX-M** (160 subtypes) | **NDM** (12 subtypes) | | |
| **KPC** (22 subtypes) | | | |

**4,400** beta-lactamases were isolated or synthesized (2019)

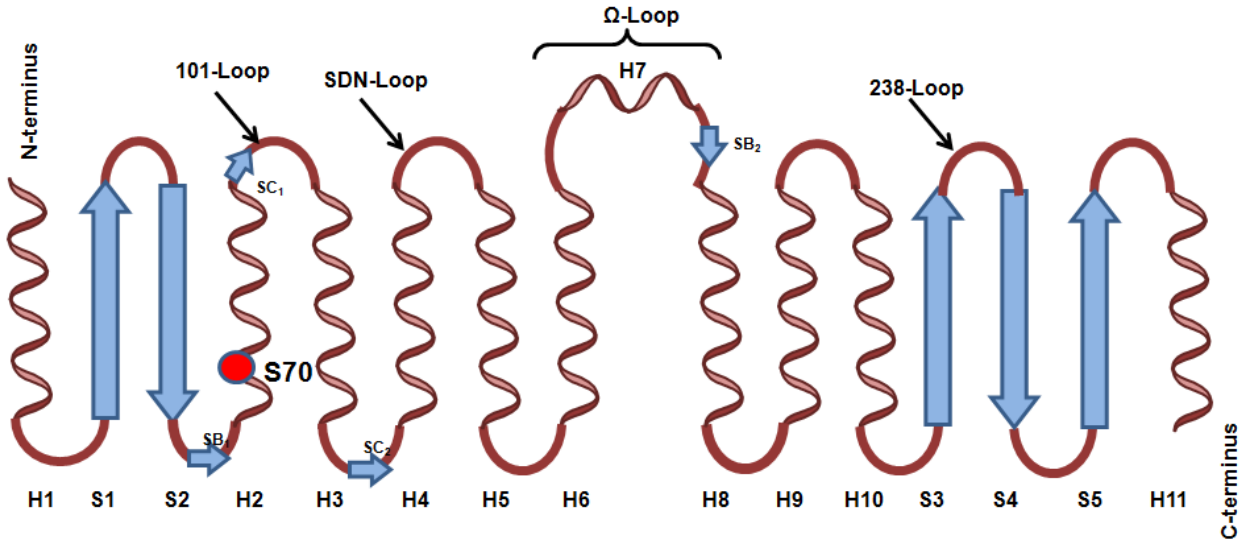
**2,700** enzymes were isolated from clinical bacterial strains (2018)

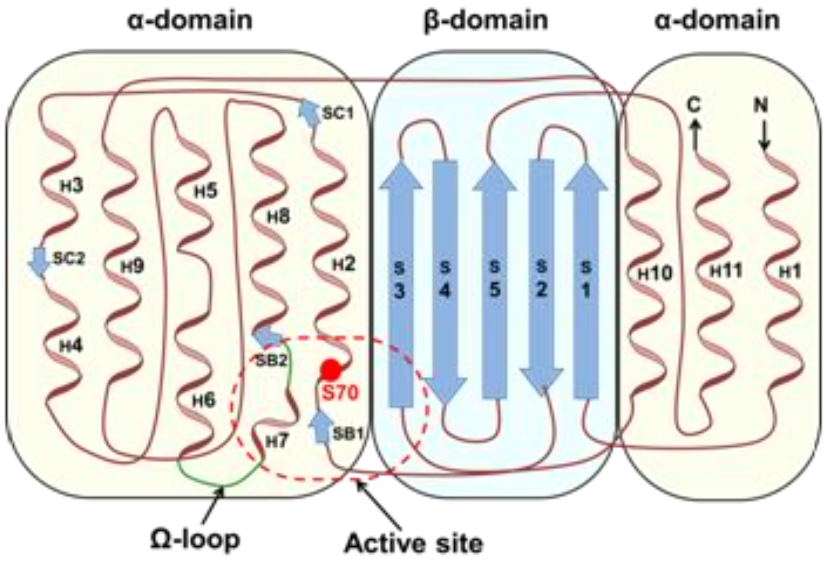
*Beta-Lactamase DataBase (http://bldb.eu)*

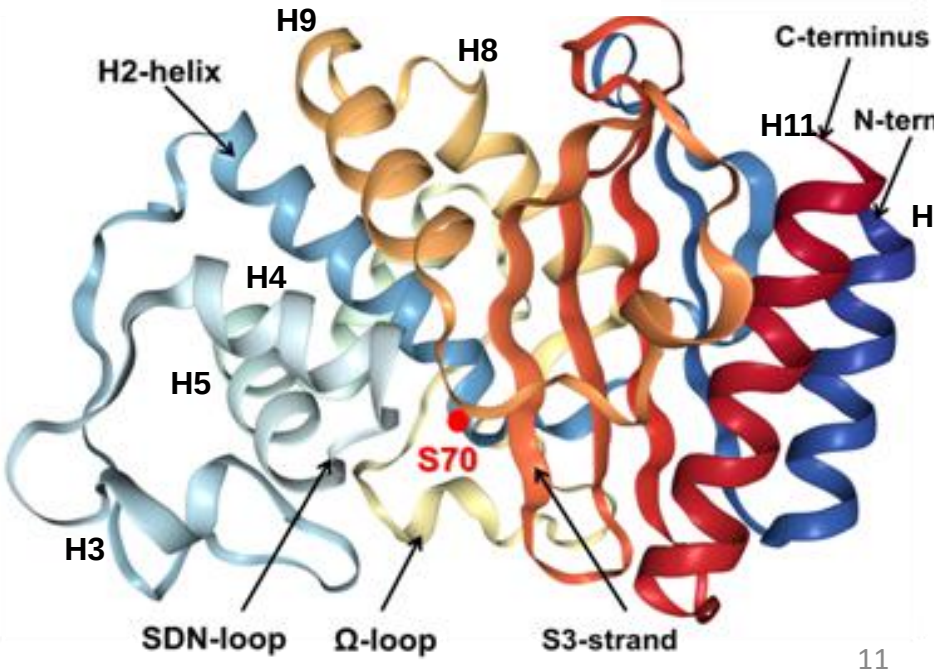
*How do beta-lactamases provide such broad specificity causing the resistance?*



# Structural fold of TEM type beta-lactamases

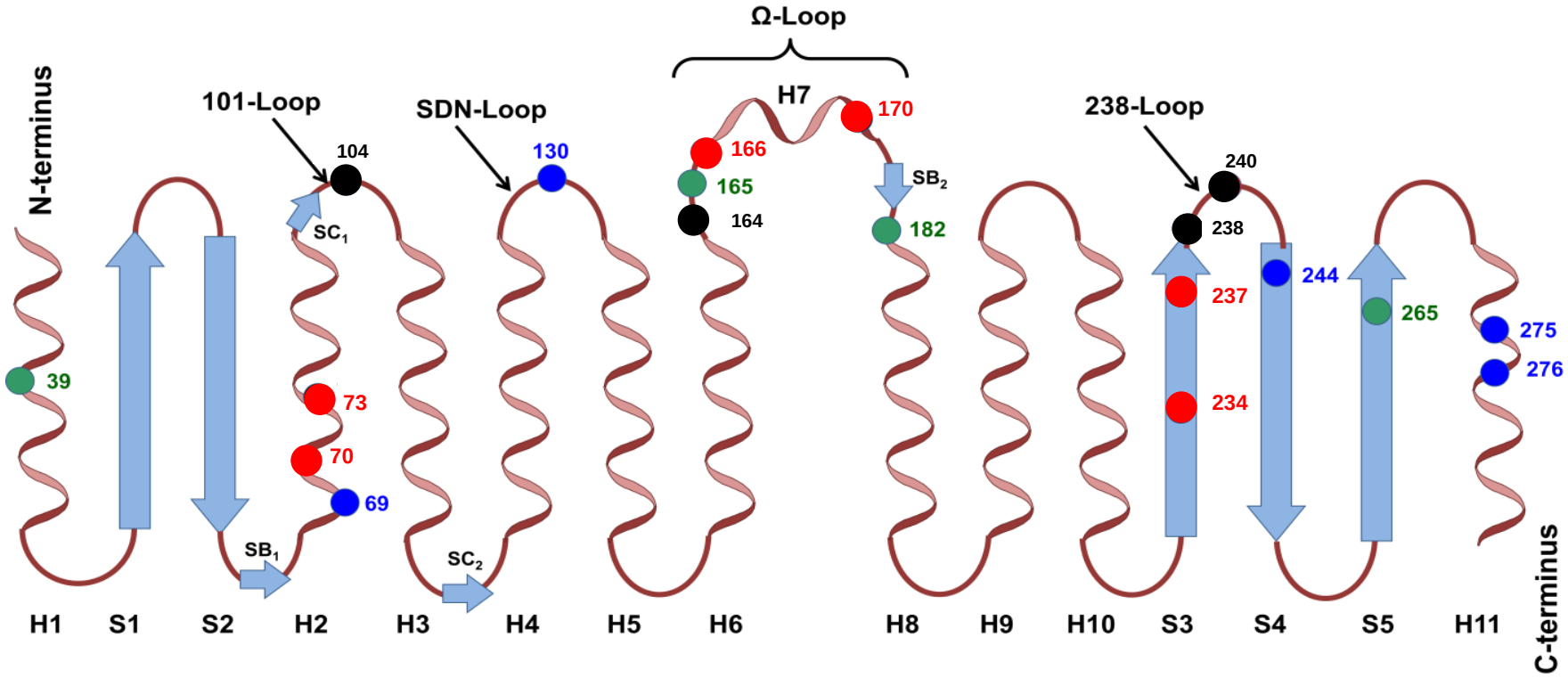


# The variety of beta-lactamases is caused by single mutations and their combinations

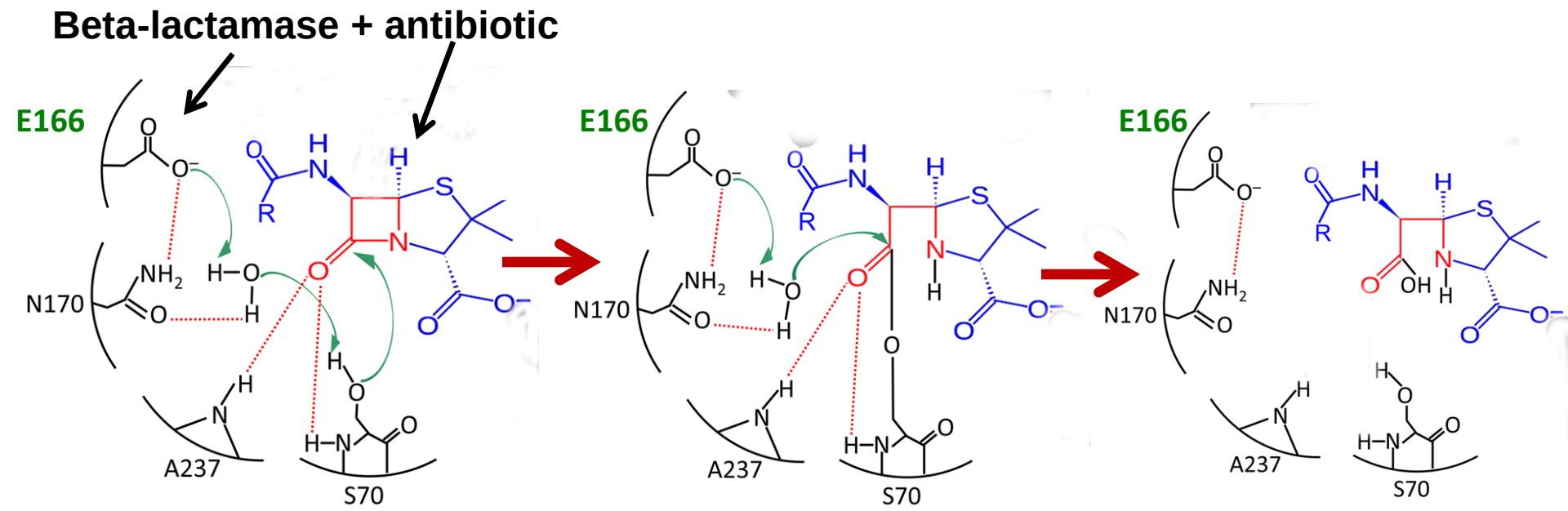
30 % of resides are mutated in TEM type beta-lactamases, most of them are located in the loops




**Red (S70, K73, E166, N170, K234, A237):** catalytic residues
**Black (104, 164, 238, 240):** key mutations responsible for variety of resistance
**Blue (69, 244, 275, 276):** mutations responsible for resistance to inhibitors
**Green (39, 182, 265):** the most common secondary mutations responsible for stability and adaptation of protein structure

# Beta-lactam hydrolysis by TEM beta-lactamases

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E:S \xrightarrow{k_2} E\text{–}S \xrightarrow[H_2O]{k_3} E + P$$



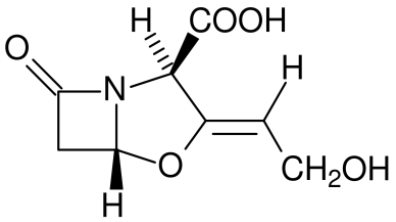

Beta-lactams: $kcat/K_M \sim 10^2$

Competitive inhibitors: $kcat/K_M \sim 0.001$

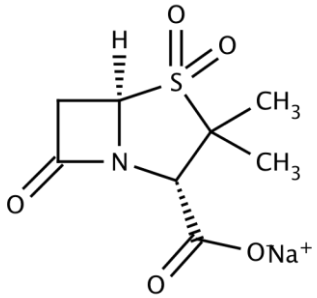
# Inhibitors interacting at the active site of beta-lactamases

## Beta-lactam inhibitors

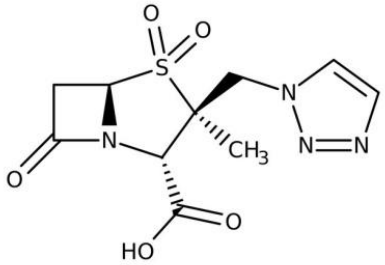
Clavulanic acid



Sulbactam



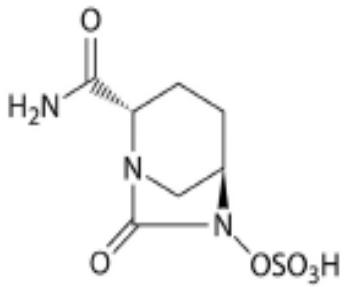
Tazobactam



## Non beta-lactam inhibitors

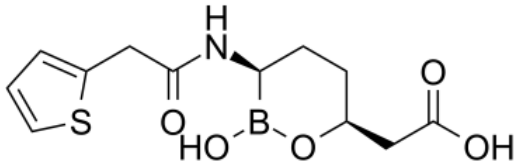
*Diazabicyclooctanes*

Avibactam



*Derivatives of boronic acid*

Vaborbactam



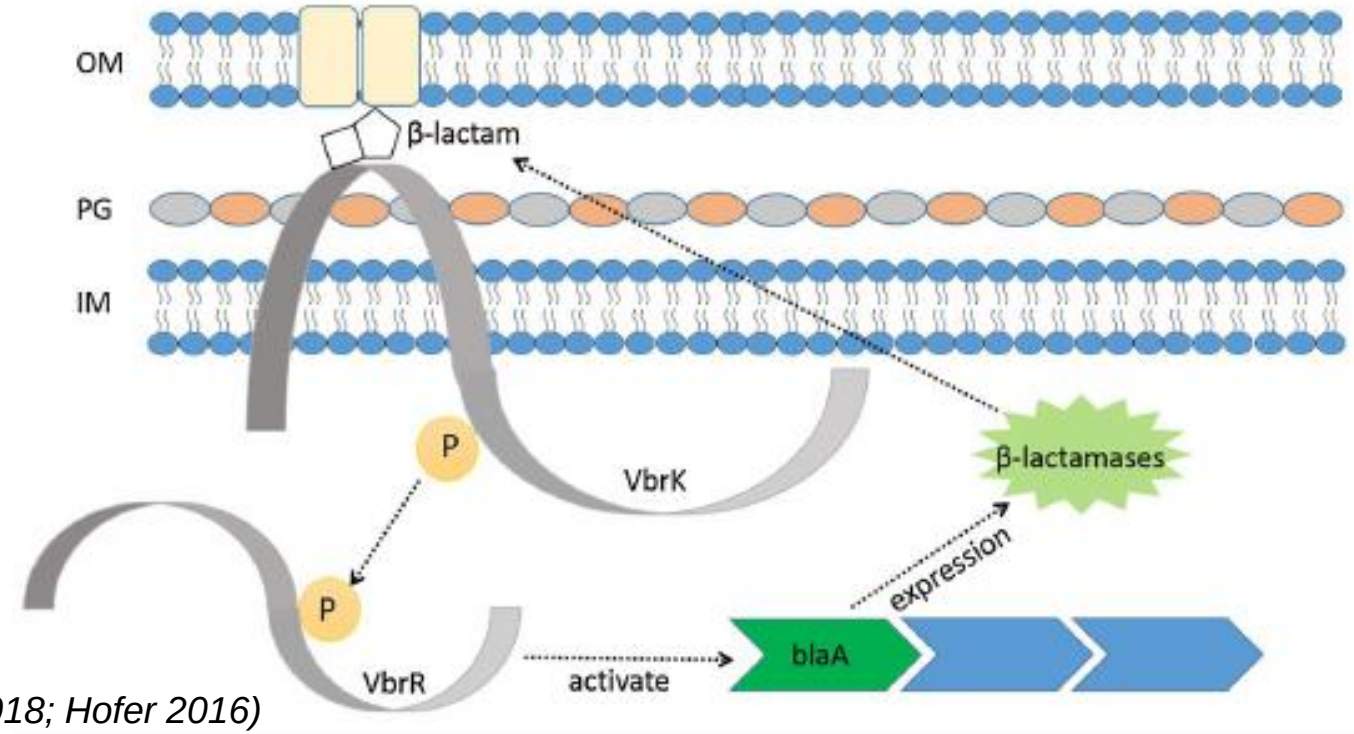
*Inhibitors to the active site **do not protect** against the resistance*

# Beta-lactams stimulate synthesis of beta-lactamases through regulatory systems of bacterial cell

## Bacterial response to beta-lactam antibiotics

1. VbrK histidine kinase is specifically activated by antibiotic via direct binding and then is autophosphorylated.

2. Phosphoryl group is transferred from VbrK to its response regulator VbrR, leading to the beta-lactamase gene expression.




*(Lingzhi 2018; Hofer 2016)*

**Antibiotics –** inductors of beta-lactamases

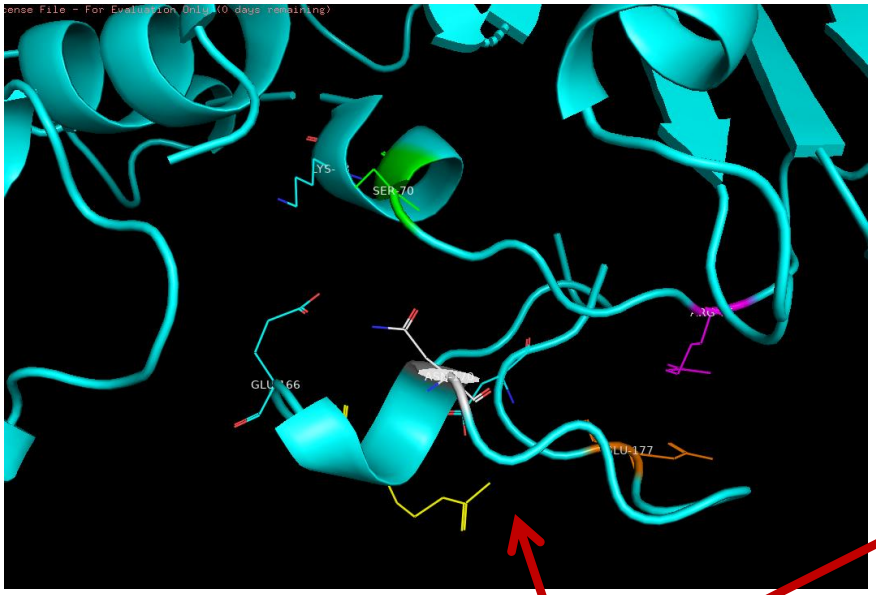
**Beta-lactamases –** drivers of the resistance

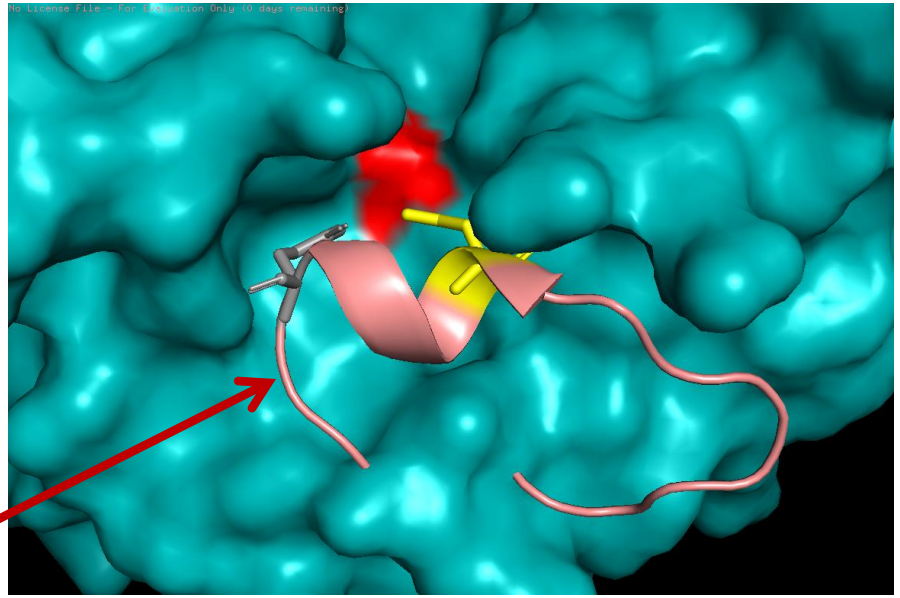
# The complexity of the resistance to beta-lactams

- Many beta-lactamase genes
- Multi- and pan- resistance (combination of several resistance genes)
- Combination with other types of resistance involving bacterial enzymes
- Combination of different resistance mechanisms
- Localization of resistance genes on mobile genetic elements
- Transfer of plasmids to other bacteria
- Growth in the number of new bacterial infections
- Restrictions on the industrial production of antibiotics
- The need to use reserve antibiotics
- Using phages?

# New direction in combating the resistance - microtargeting and allosteric inhibition

## Omega-loop - allosteric target of beta-lactamases



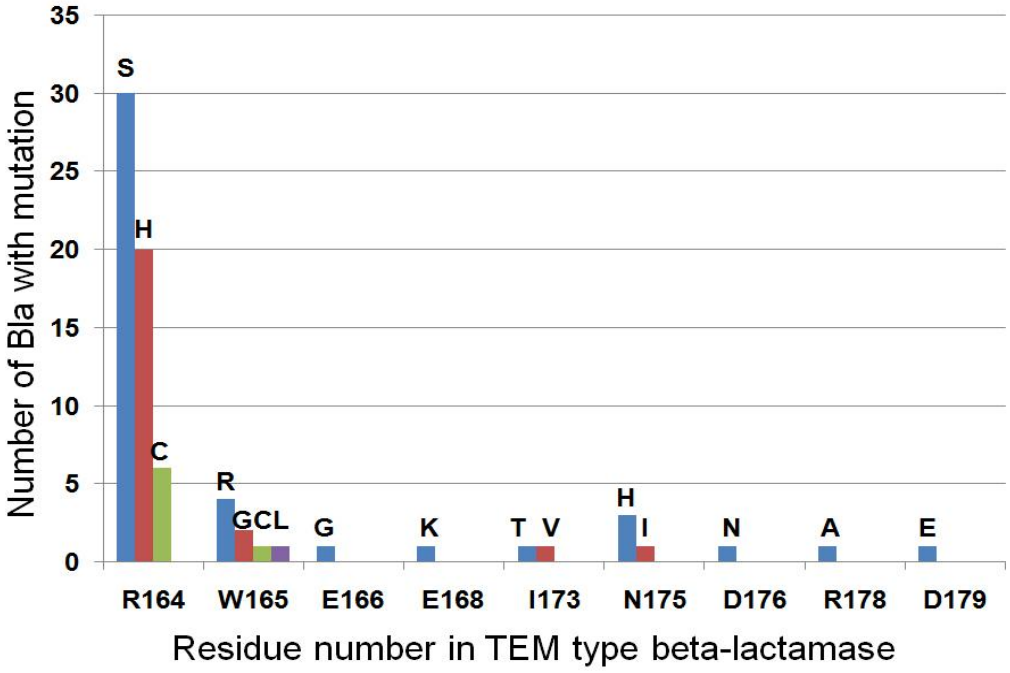



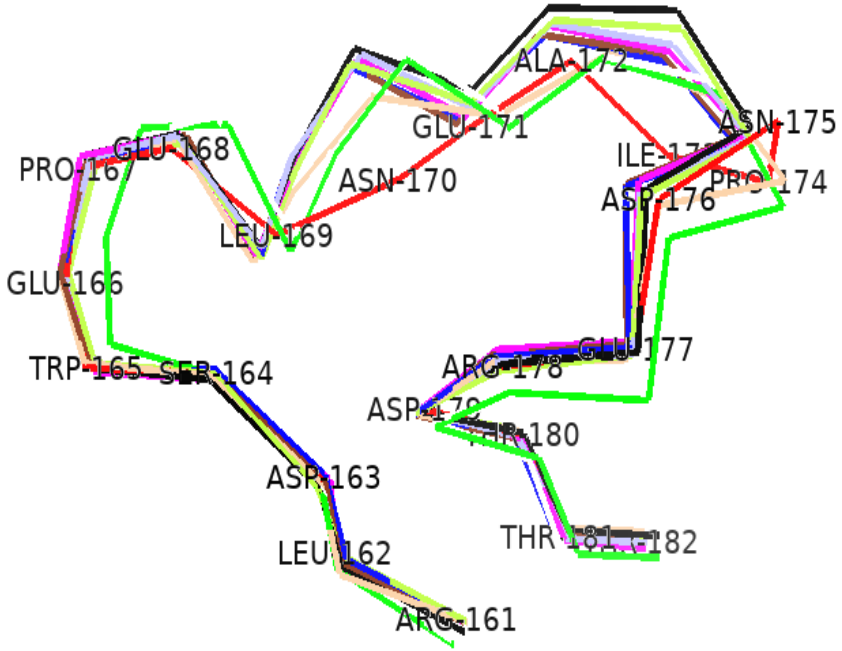
**Omega-loop** in beta-lactamase
(residues 164-179)

# Omega-loop - structural element of all class A beta-lactamases

Omega-loop structural features:

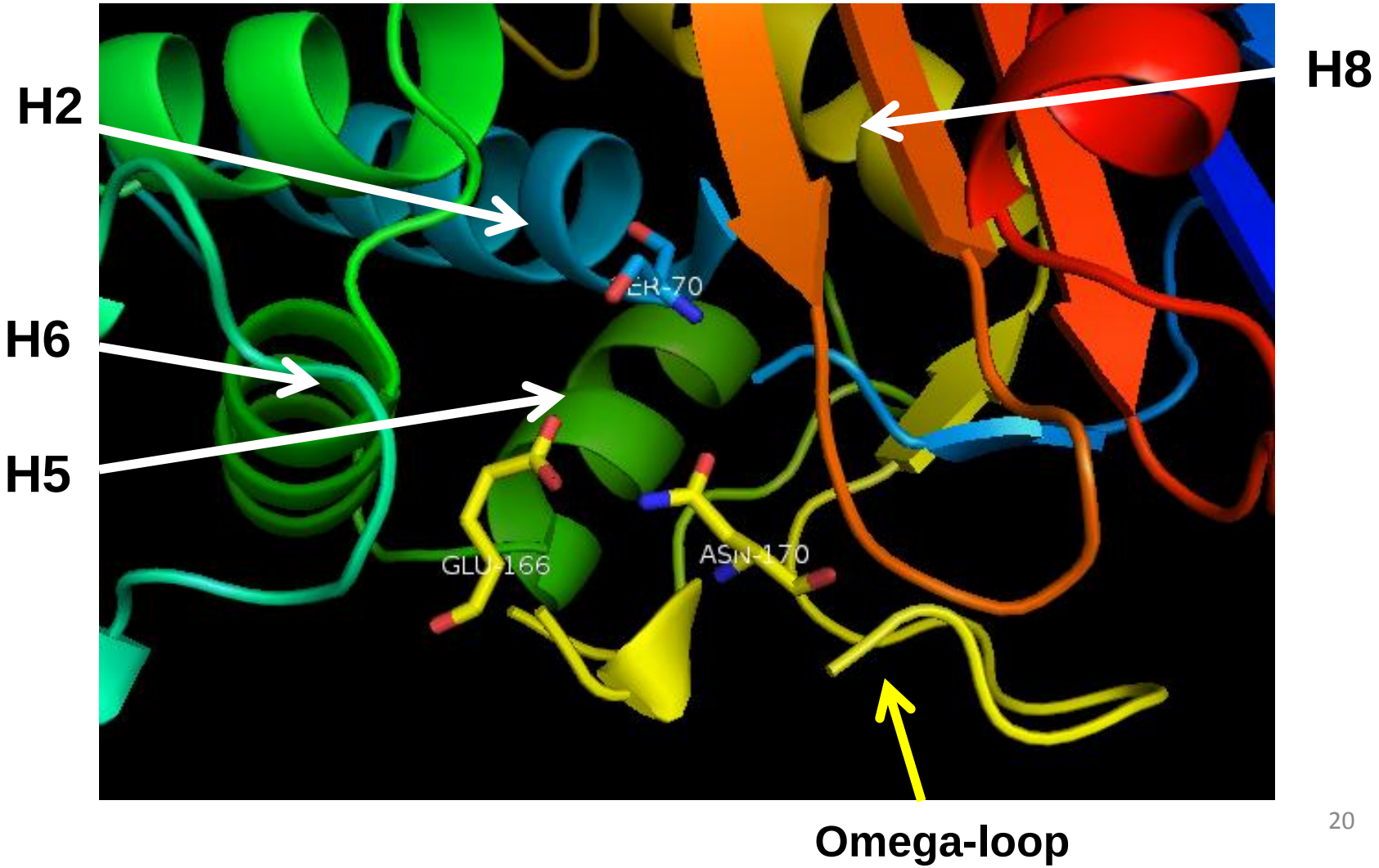
- Located at the entrance to the active site
- Key role of E166 in catalytic cycle (acylation and deaylation)
- Loop residues are linked by ionic and hydrogen bonds
- Conservative structure in class A beta-lactamases
- Solvent availability
- Flexibility and mobility of the loop




Conformation of the omega-loop in class A beta-lactamases

# Omega-loop position in beta-lactamases relative to other structural elements



# Omega-loop - a new target for beta-lactamase inhibitors

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# Resistance grows . . .

**Thanks for your attention!**