

# **BETA-LACTAMASES: POLYMORPHISM, NEW INHIBITORS AND DNA BIOCHIPS**

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# Microbial resistance against $\beta$ -lactams

- Problem:

Reduction of clinical efficiency of penicillins and cephalosporins



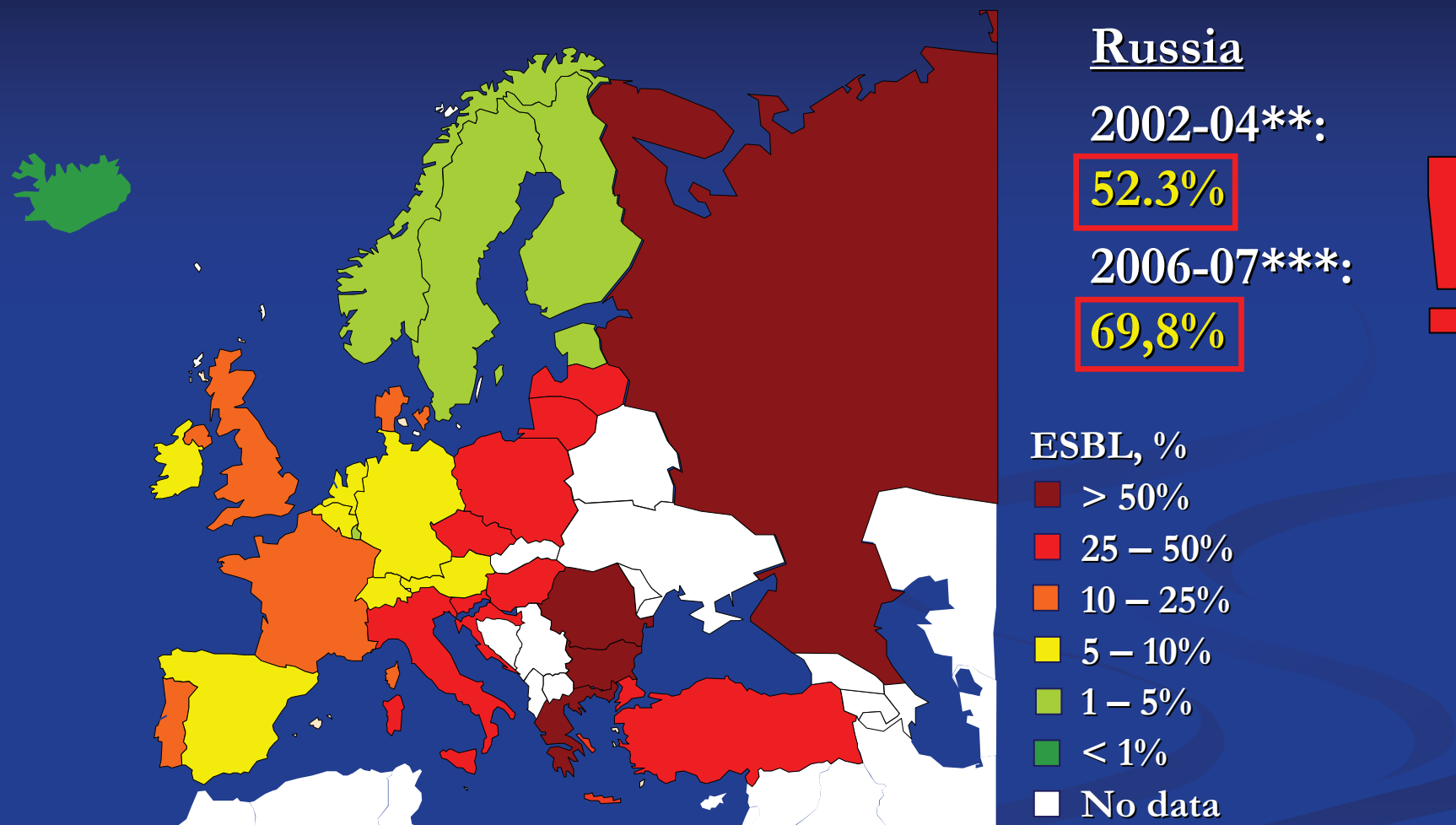
- Reason:

Ability of different species of bacteria to resist  $\beta$ -lactam antibiotics action

# $\beta$ -Lactamase families

- A** plasmid ESBL (TEM, SHV, CTX-M), chromosomal  $\beta$ -lactamases of *Proteus*, *Klebsiella* & *Bacteroides*
- B** Zinc types – metallo  $\beta$ -lactamases
- C** Chromosomal AmpC  $\beta$ -lactamases of most enterobacteria
- D** OXA-class plasmid  $\beta$ -lactamases

# Prevalence of ESBLs among *Enterobacteriaceae* in Europe



\* *EARSS Study, 2007*

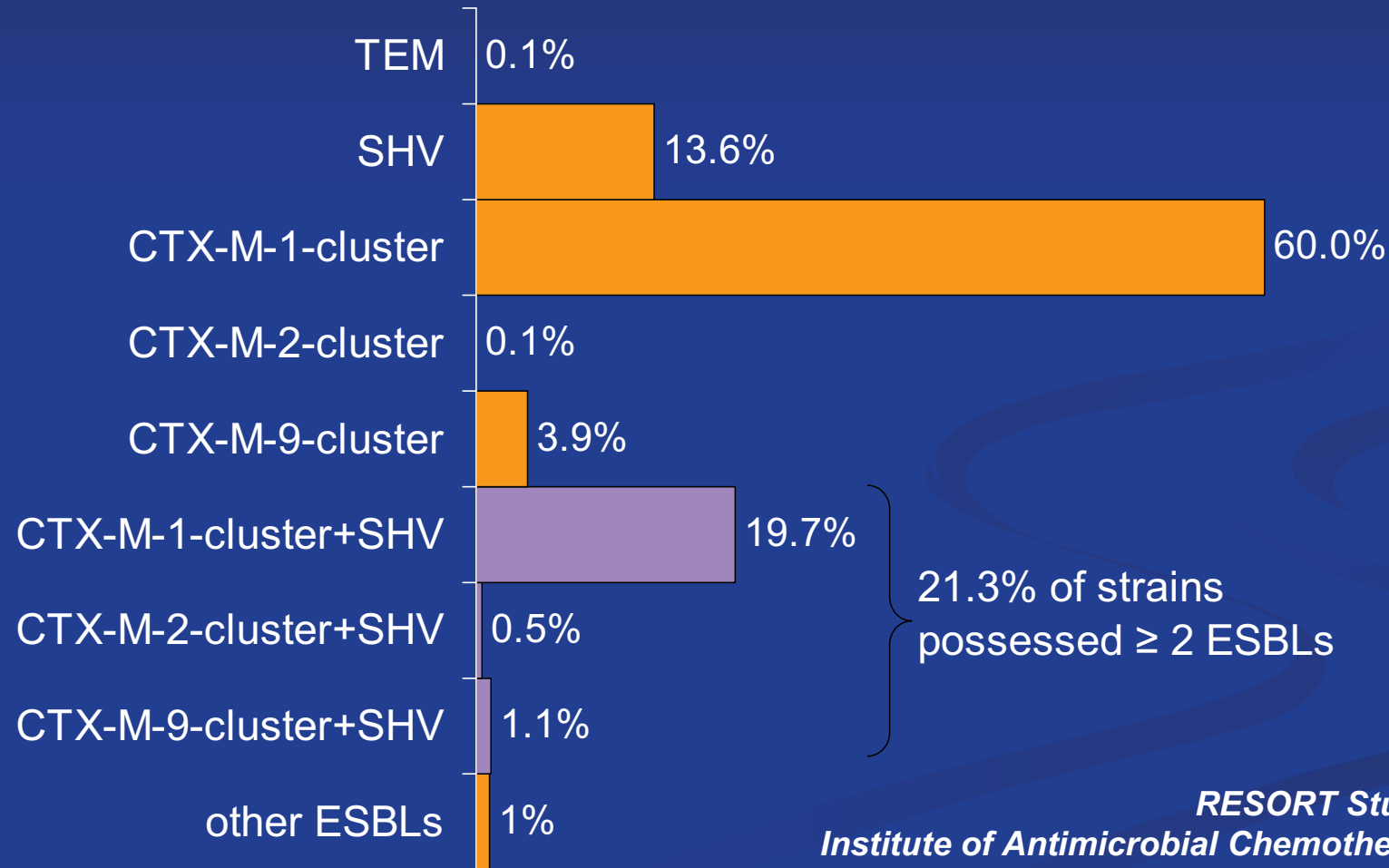
\*\* *Research RESORT, 2002-2004*

\*\*\* *Research РЕВАНШ, 2006-2007*

} *(Inst. of Antimicrobial Chemotherapy, Smolensk)*

# Relative prevalence of different ESBL types and their combination in Russia

n = 718 strains expressing ESBL phenotype



RESORT Study (2002-2004),  
Institute of Antimicrobial Chemotherapy, Smolensk  
(M.Edelstein, et al. 17th ECCMID, 2007, O.1732\_130)

# Extended-spectrum beta-lactamases (ESBLs)

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- Serine  $\beta$ -lactamases
- Typically plasmid-encoded, often associated with mobile elements
- Active against all  $\beta$ -lactams (penicillins, I-IV generations of cephalosporins, aztreonam) except cephamycins and carbapenems
- Inhibited by active-site directed inhibitors (clavulanic acid, sulbactam, tazobactam)
- Main producers are *E.coli*, *K.pneumoniae* and other *Enterobacteriaceae* spp.
- Multiple genetic types: CTX-M, SHV, TEM.

# Why identification of ESBLs is so important?

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- **Confer resistance to the most commonly used antibiotics** which constitute the first-line therapy for nosocomial infections
- **Complex epidemiology: may rapidly spread by clonal transmission or plasmid transfer**
- **Frequently associated with resistance determinants to non- $\beta$ -lactam agents (fluoroquinolones, aminoglycosides, tetracyclines, sulfonamides, etc). ESBL producers are often multiply resistant**
- **Difficult to detect ESBL producers by routine susceptibility tests - common cause of treatment failures with modern cephalosporins**

# Nucleotide polymorphism described for important ESBL types

## ■ TEM (about 160 subtypes)

### TEM-1

4-6  
16  
21  
25-26  
28  
34-35  
38-39  
42  
49  
51  
53  
55  
64  
69  
80  
84  
92  
100  
102  
104  
114-115  
118  
124  
127  
130  
145  
153  
157-158  
163  
164  
165  
173  
175  
179  
182  
184  
204  
215  
218  
221  
224  
226  
234  
237  
238  
240  
244  
248  
251  
262  
275-276  
280  
284

## SHV (about 100 subtypes)

### SHV-1

7-8  
10  
14  
18-19  
22  
25  
35  
43  
44  
54  
61  
64  
69  
75  
79-80  
89  
96  
97  
101  
112-114  
119  
122  
124  
126  
129-130  
134  
139-149  
156  
158  
169  
172-173  
175  
179  
187  
192-193  
195  
202-203  
205-206  
213-214  
226  
234-235  
238  
240  
243  
251  
254-255  
257  
260  
267  
269  
272  
278

## ■ CTX-M (about 80 subtypes)

### CTX-M-1

12  
23  
27  
35  
38  
75  
77  
89  
106  
114  
119  
140  
167  
240  
263  
277  
278  
288

### CTX-M-2

26  
48  
61  
98  
99  
121  
125  
159  
171  
225  
228  
230  
240  
253  
274  
278

### CTX-M-9

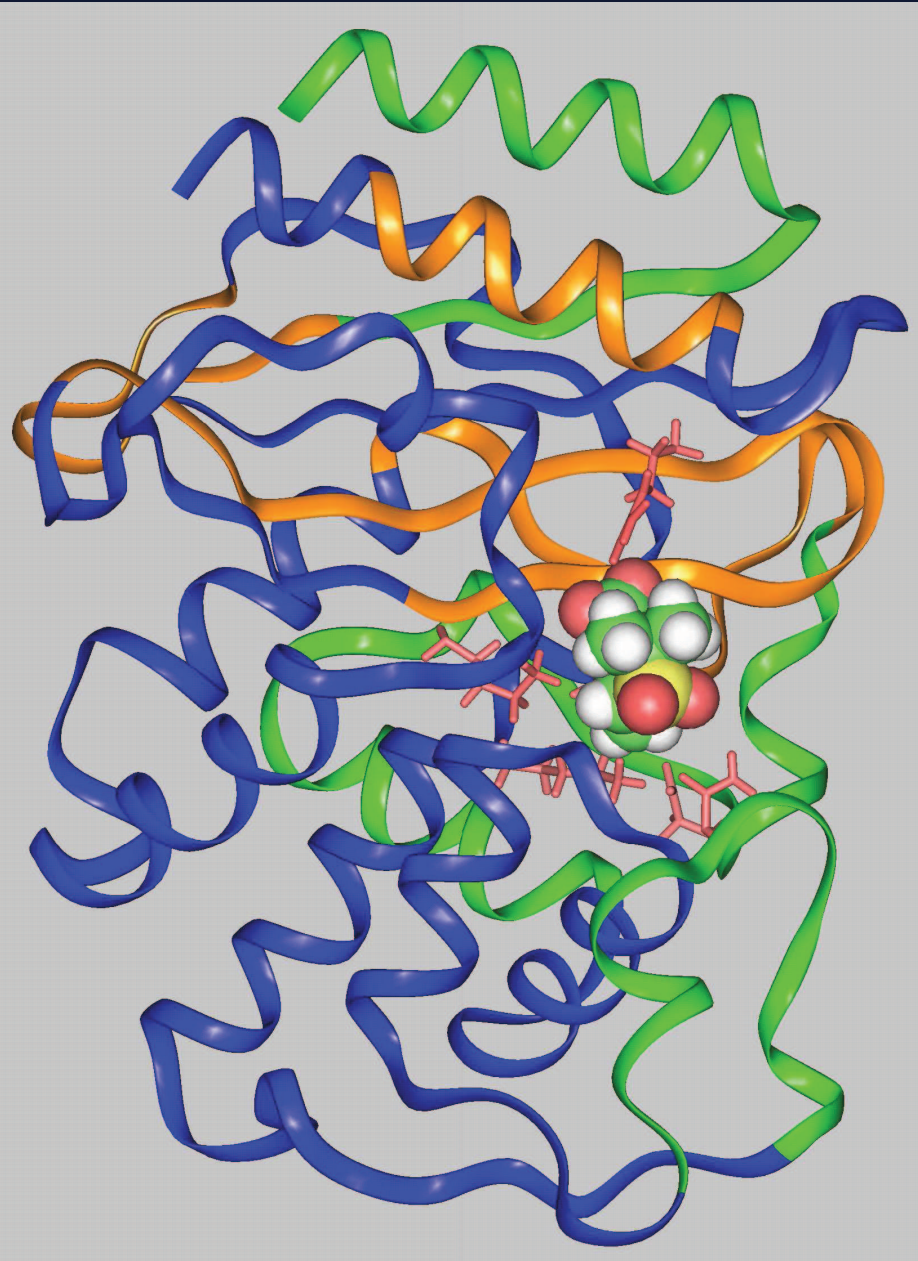
2  
7  
9  
10  
20  
21  
27  
29  
42  
47  
52  
77  
121  
154  
167  
183  
186  
188-  
200  
220  
231  
240  
274  
288

### CTX-M-8

2  
4  
6  
13  
18  
20  
23  
50  
55  
77  
89  
99  
109  
119  
120  
123  
154  
158  
189  
192  
197  
201  
209  
222  
240  
260  
268  
270  
274  
284



## Mutational variability of TEM family of $\beta$ -lactamases

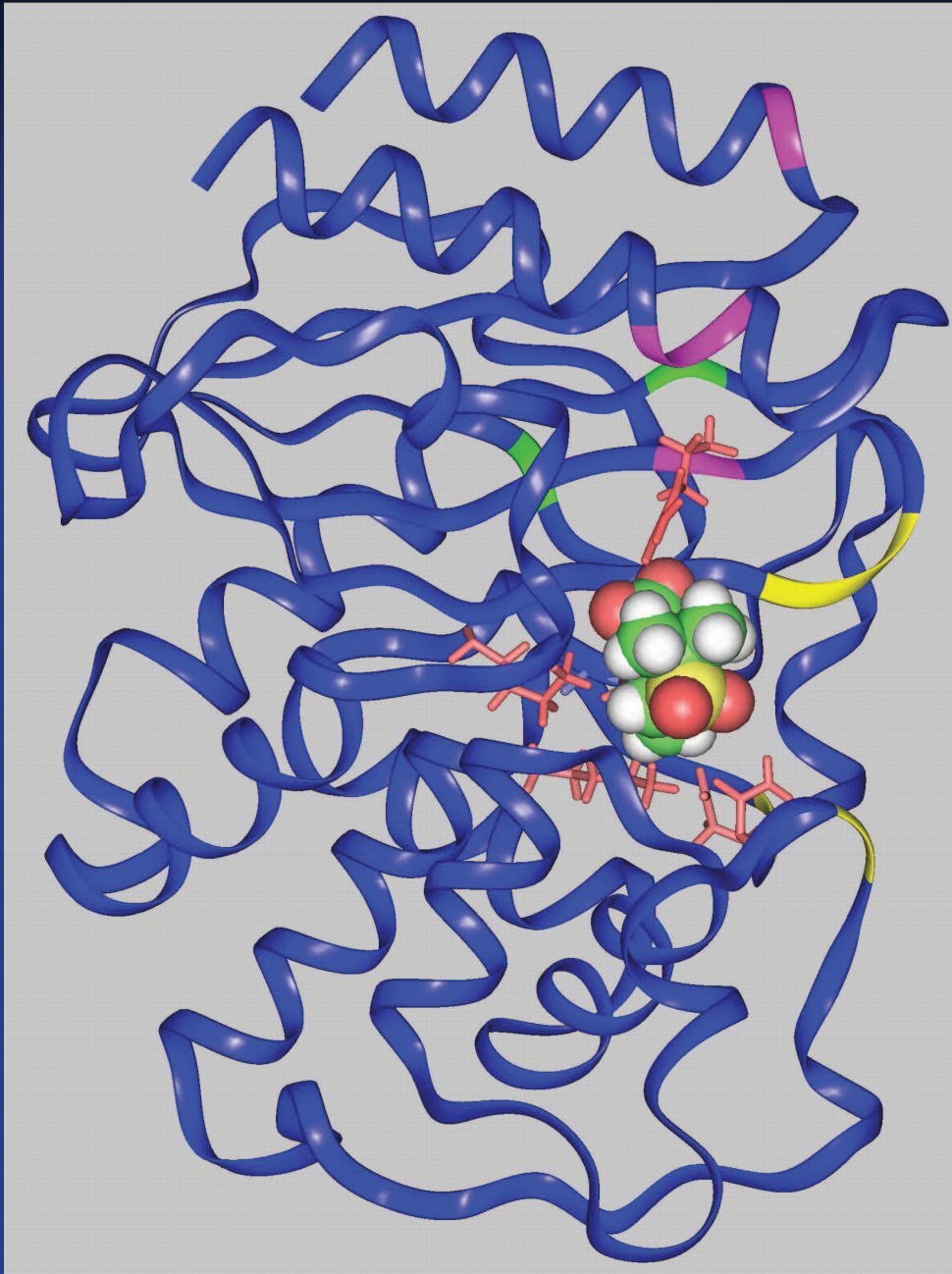


**Orange** color are regions with very high mutation rate.

**Green** – average rate of mutations.

**Blue** –marginal rate.

## Structure of TEM-1 $\beta$ -lactamase with sulbactam



$\beta$ -lactamase TEM-1 is presented as ribbon (blue), showing folding and elements of protein secondary structure.

Active site residues:

Ser 70

Ser 130

Glu 166

Gln 132

Lys 234

Arg 244

Amino acids which mutations cause inhibitory resistance:

Gln39

Met69

Arg244

Arg275

Asn276

Residues causing ESBL phenotype:

Glu 104

Arg 164

Gly 238

Glu 240

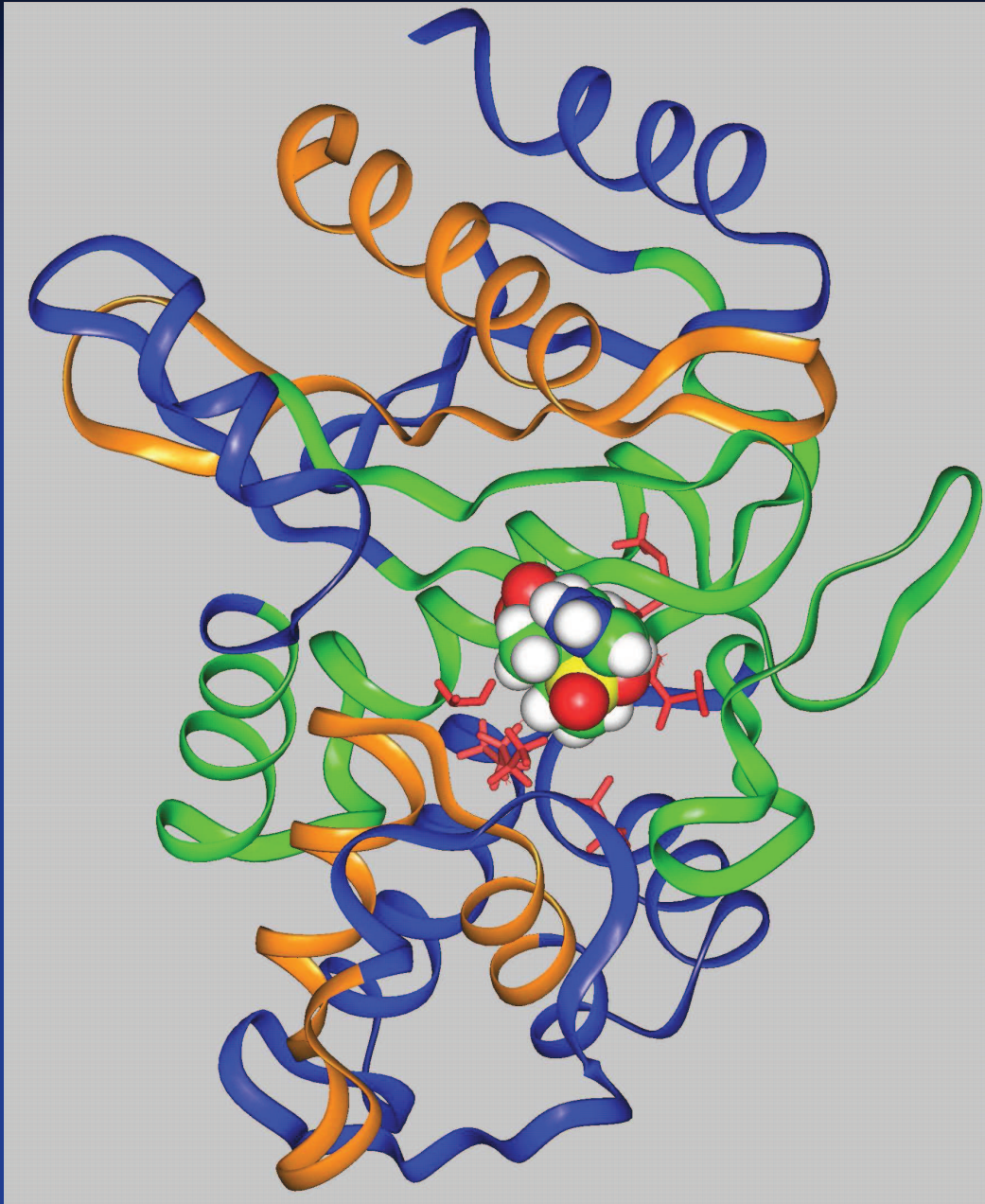
Residues demonstrated high mutation rate in TEM family:

Met 182

Thr 265



## Mutational variability of SHV family of $\beta$ -lactamases

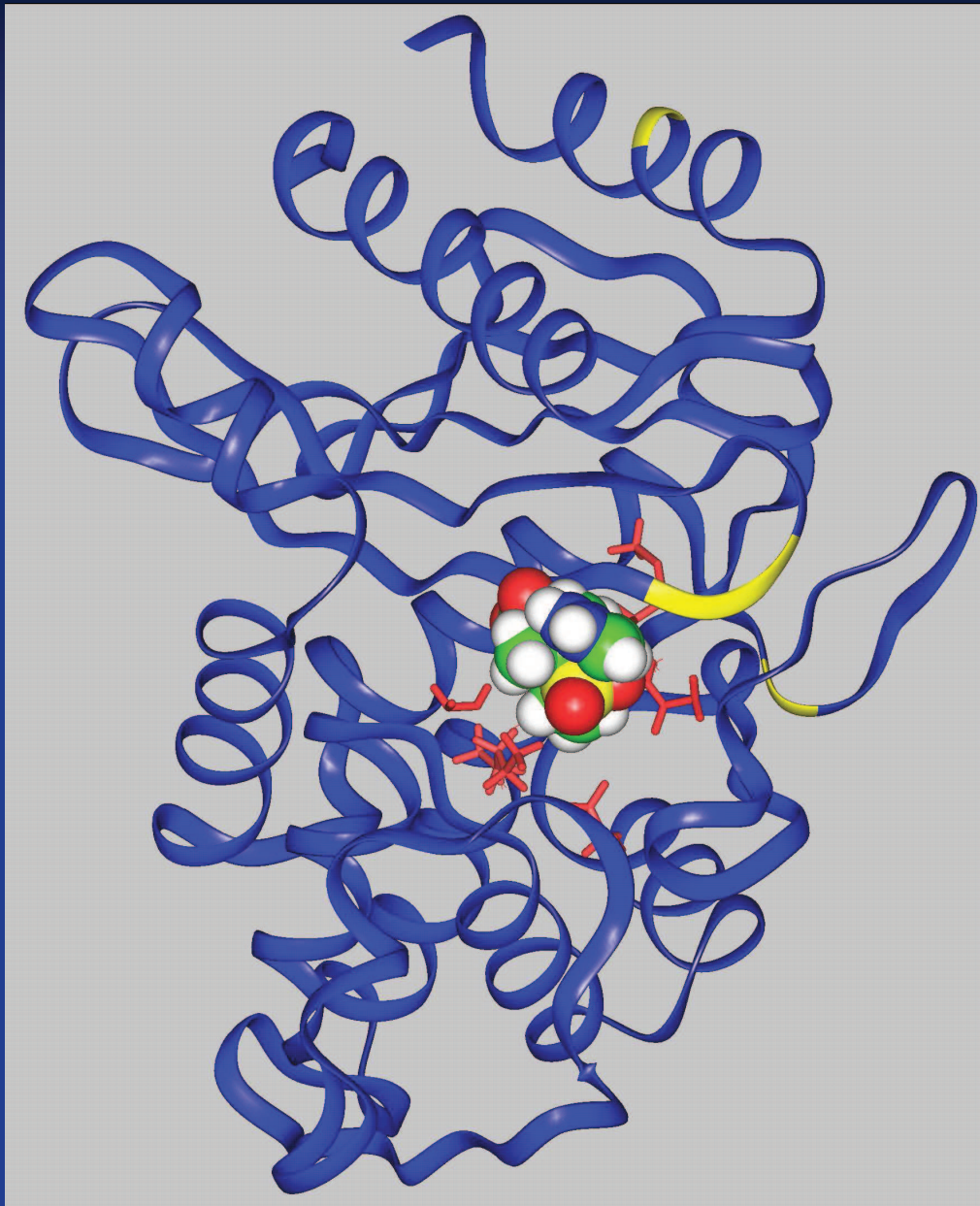


Brown color are regions with very high mutation rate.

Green – average rate of mutations.

Blue –marginal rate.

## Structure of complex between SHV-2 b-lactamase with tazobactam



SHV-2 b-lactamase presented as ribbon (blue). Active site residues are:

Ser 70

Ser 130

Glu 166

Gln 132

Lys 73

Met 69

Amino acids which mutations cause ESBL phenotypes are shown in yellow

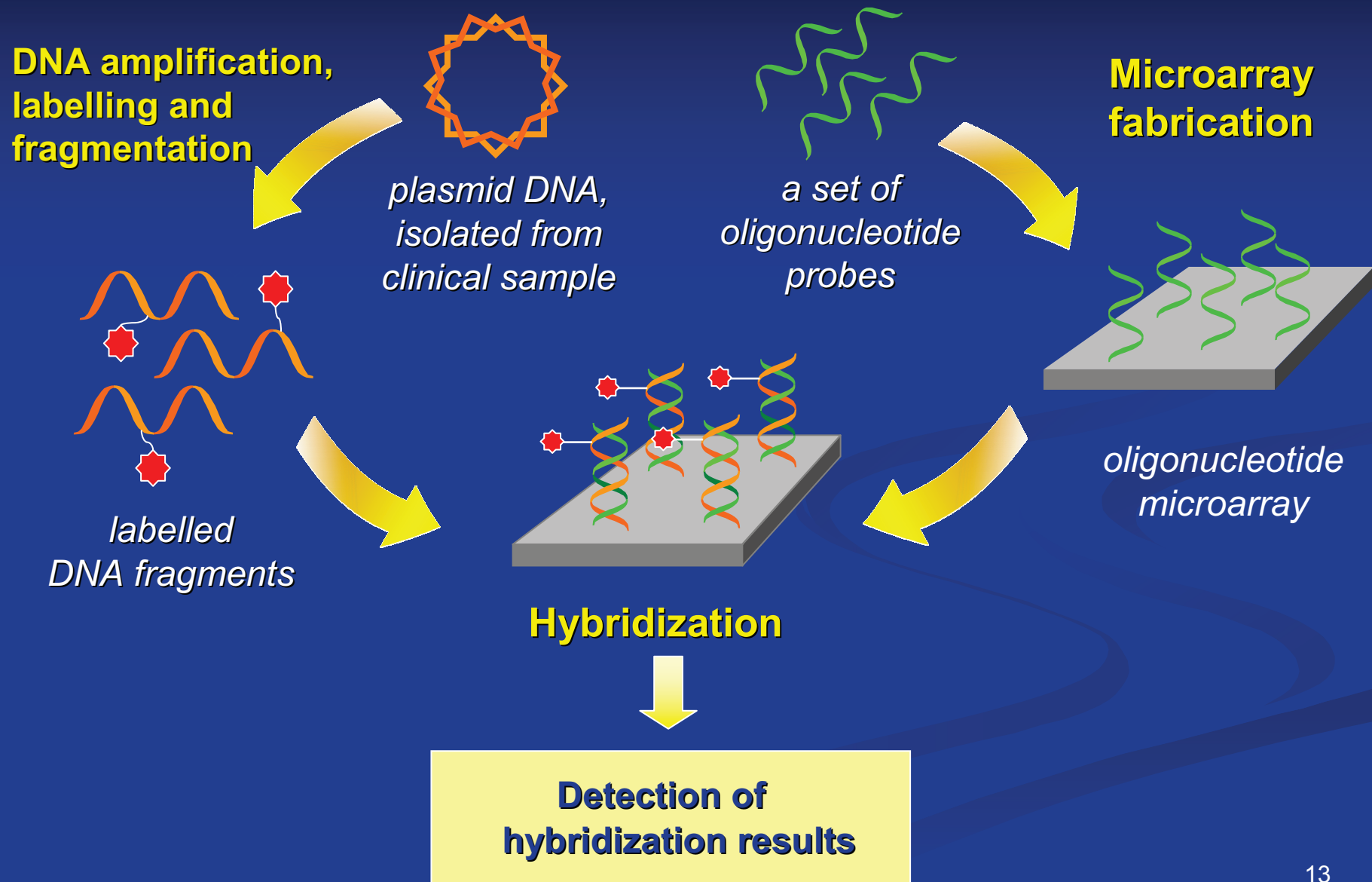
Leu 35

Asp179

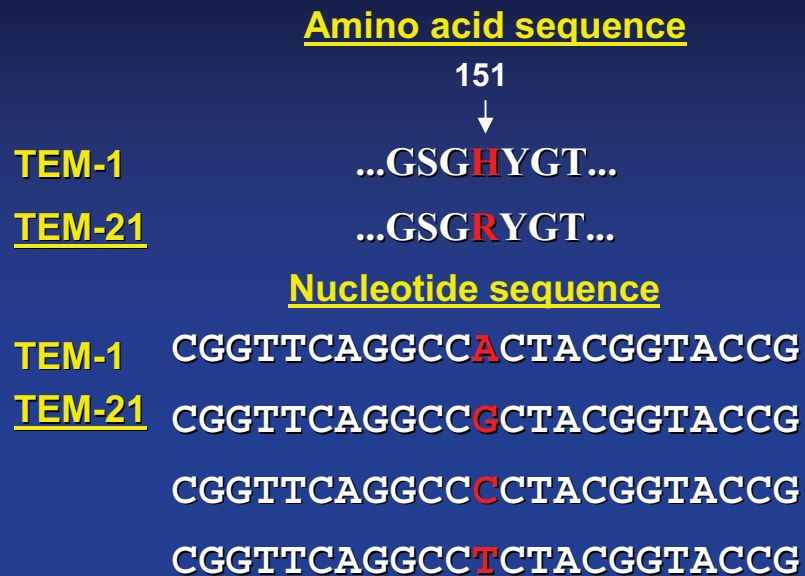
Gly238

Glu240

# DNA microarray technology

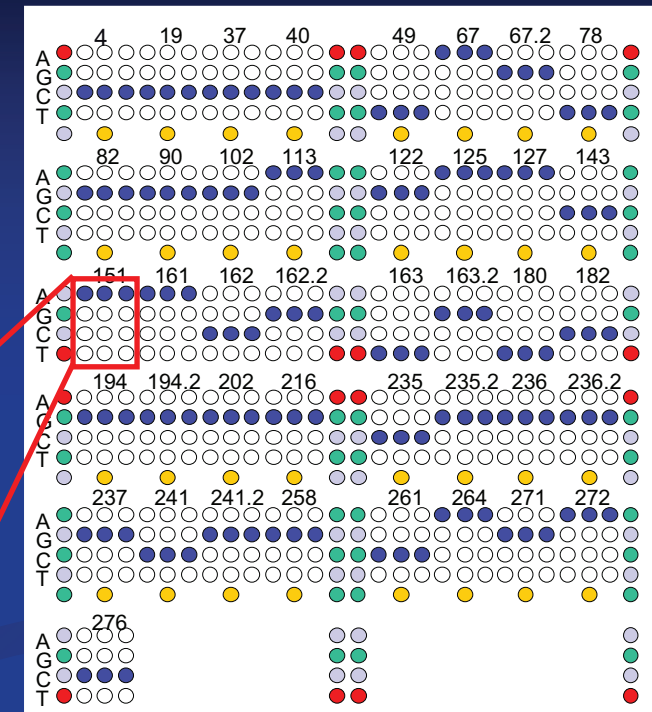


# Strategy of probe design for SNP detection



## Requirements:

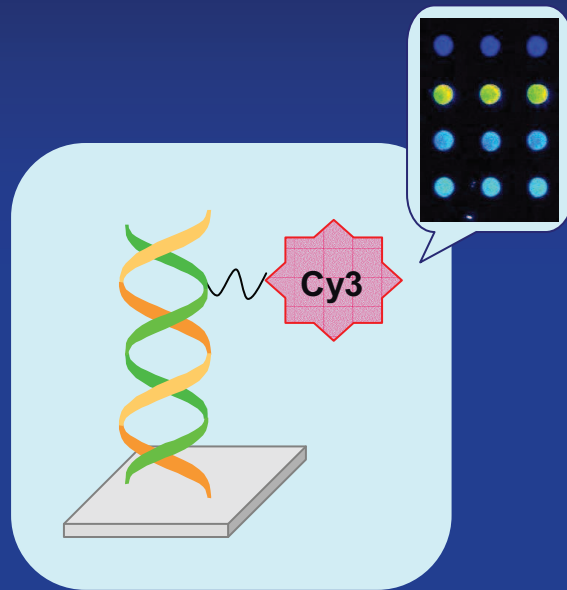
- Mutation at or near the central position within the probe sequence
- Length of probe - 17-24 nucleotides
- Disparity in  $T_m$  for different oligonucleotide groups – no more than 5–10 °C
- (C+G) ~ 35 –70 %
- Minimal probability of dimer and cyclic structures formation for the probe



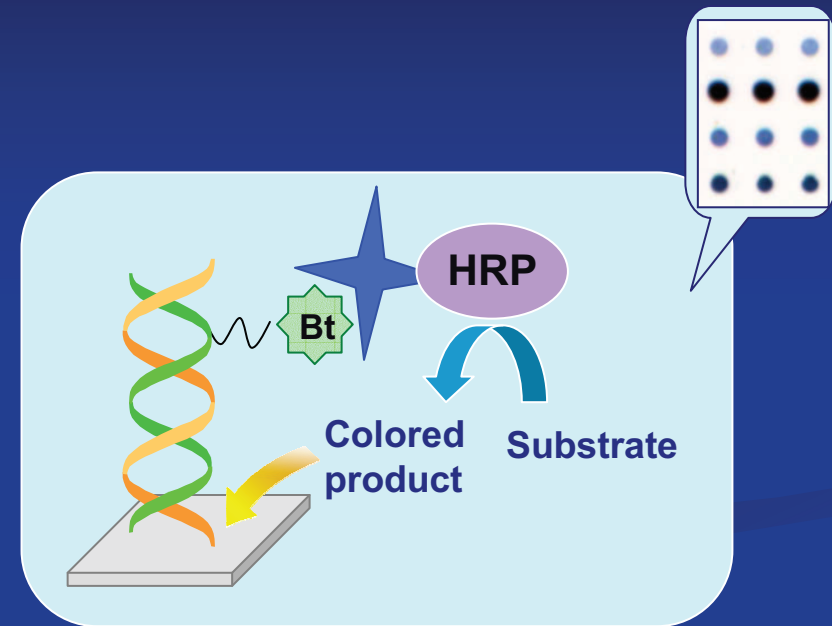
- Spotting control
- Neg. hybridization control
- Pos. hybridization control
- Process control
- Perfect match for TEM-1
- Mismatch for TEM-1



# Different detection systems for DNA microarray technology



DNA-microarray with  
fluorescent detection

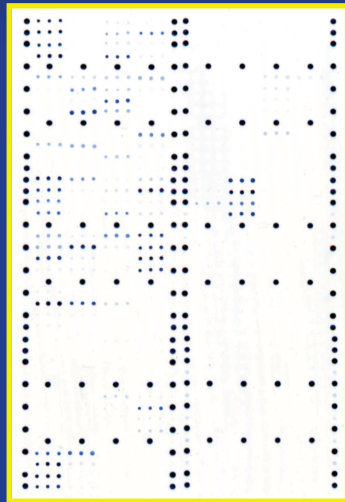


DNA-microarray with  
colorimetric detection

# DNA microarrays developed:

DNA microarrays for ESBL genotyping  
(determination of all known mutations)

Genotyping chip



**Support:** epoxy-coated glass slides

**Size of the array:** 8 mm x 12 mm

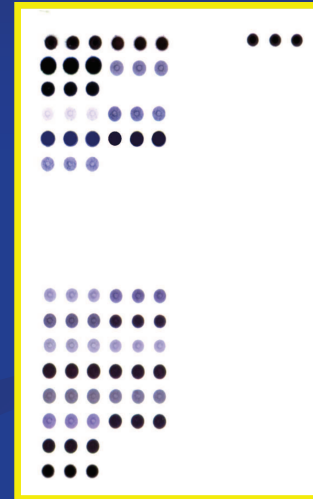
**Spot diameter** ~ 100  $\mu\text{m}$

**Spots per one array :** 1020

**Number of determined mutations:** 67

DNA microarray for identification  
of ESBL type and important  
mutations in encoding genes

Identification chip



**Support:** nitrocellulose membrane

**Size of the array:** 8 mm x 12 mm

**Size of the spot:** ~ 250  $\mu\text{m}$

**Number of spots per array:** 195

**Number of determined mutations:** 19



# Determination of subcluster CTX-M-1 $\beta$ -lactamases with Genotyping chip

CTX-M-3

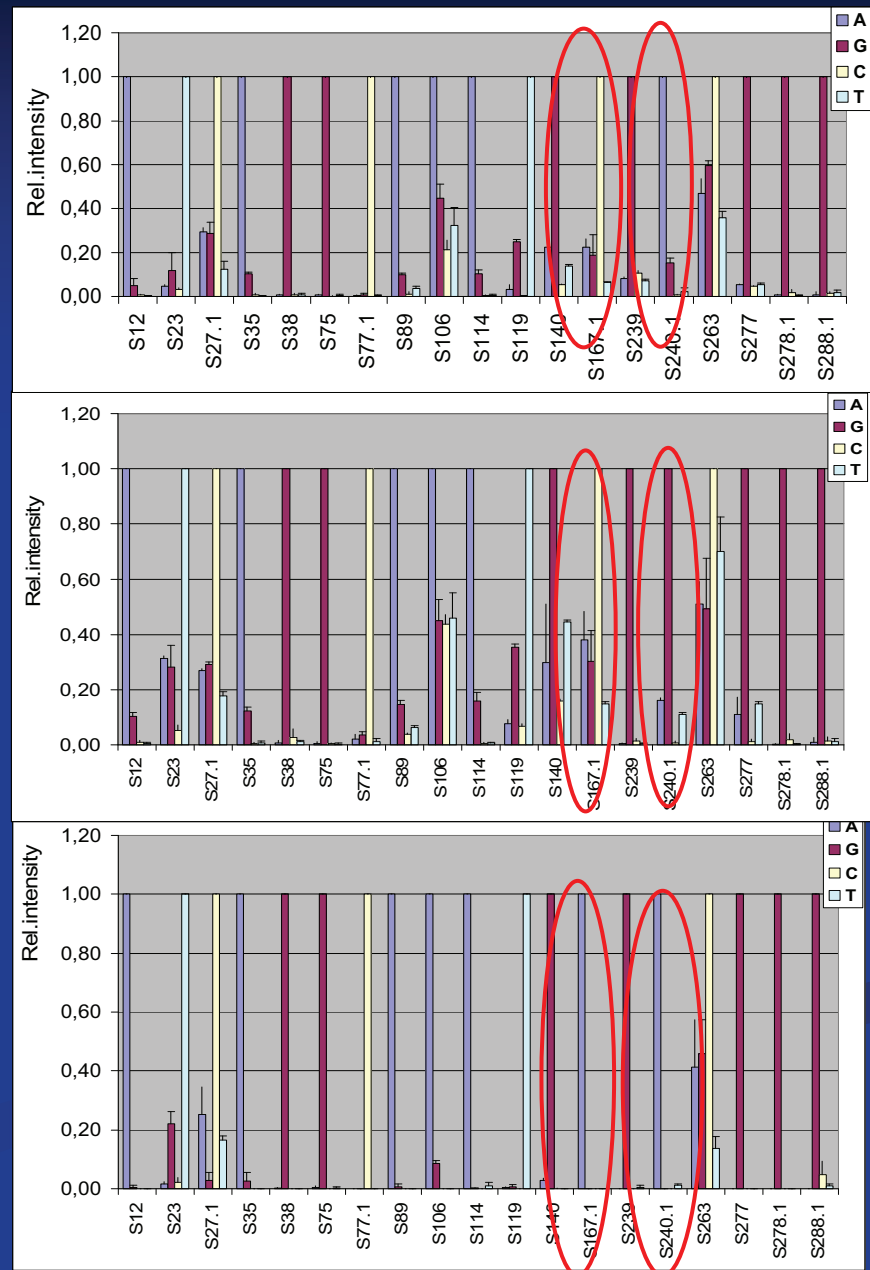
167	240
P( <u>c</u> cg)	D (g <u>a</u> c)

CTX-M-15

167	240
P( <u>c</u> cg)	G (g <u>g</u> c)

CTX-M-42

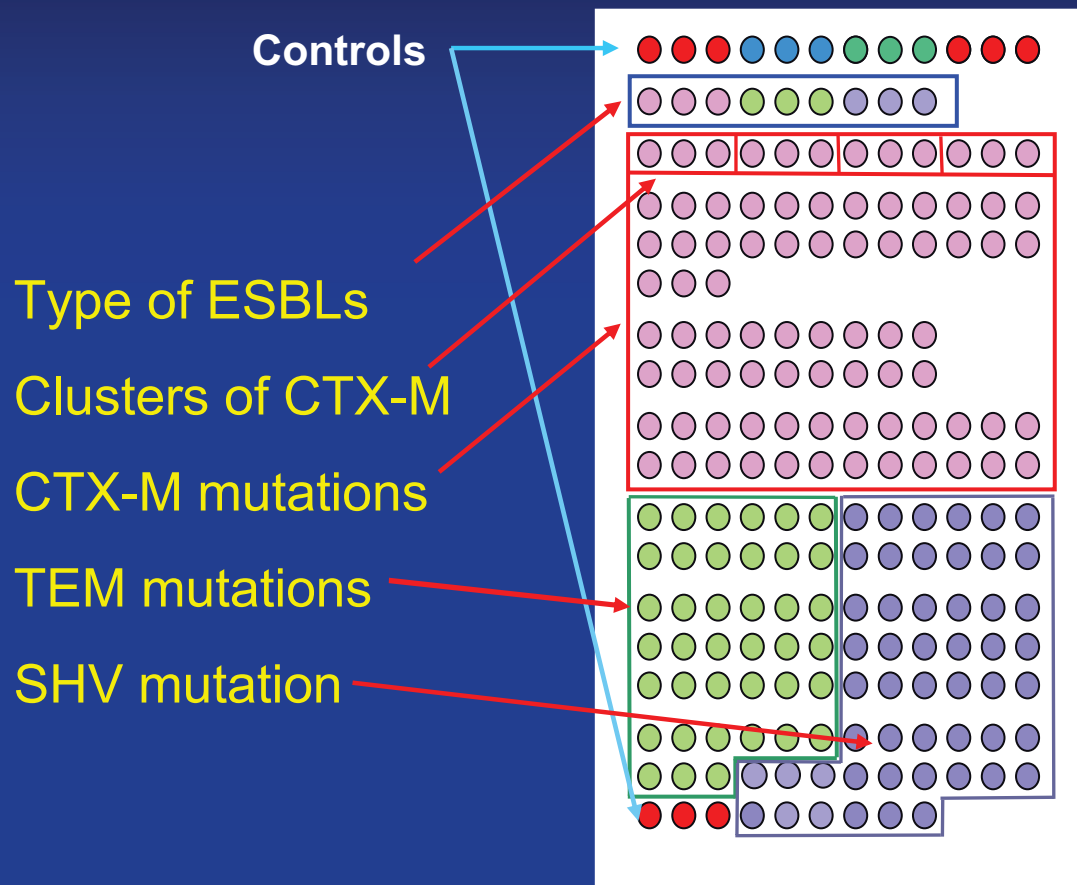
167	240
T ( <u>a</u> cg)	D (g <u>a</u> c)



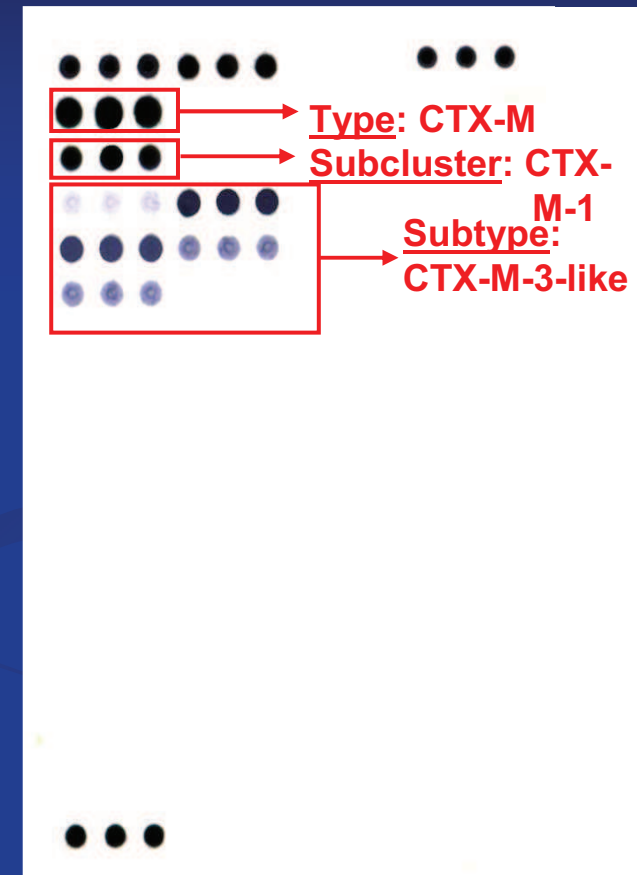
# Validation of Genotyping CTX-M chip

Species	Total no of isolates (n=83)	Genotype by DNA-microarray			
		CTX-M-3 (n=33) 40%	CTX-M-15 (n=39) 47%	CTX-M-5 (n=4) 5%	CTX-M-14 (n=7) 8%
<b>Citrobacter freundii</b>	(n=4)	2	2		
<b>Enterobacter aerogenes</b>	(n=5)	3	2		
<b>Enterobacter cloacae</b>	(n=2)		2		
<b>Escherichia coli</b>	(n=27)	7	14		6
<b>Klebsiella pneumoniae</b>	(n=17)	10	5	1	1
<b>Morganella morganii</b>	(n=1)		1		
<b>Proteus mirabilis</b>	(n=13)	4	7	2	
<b>Proteus penneri</b>	(n=1)	1			
<b>Proteus vulgaris</b>	(n=1)	1			
<b>Providencia rettgeri</b>	(n=3)	1	2		
<b>Providencia stuartii</b>	(n=1)		1		
<b>Salmonella infantis</b>	(n=1)		1		
<b>Salmonella typhimurium</b>	(n=1)			1	
<b>Serratia marcescens</b>	(n=6)	4	2		

# Determination of ESBL types and important SNP with Identification chip

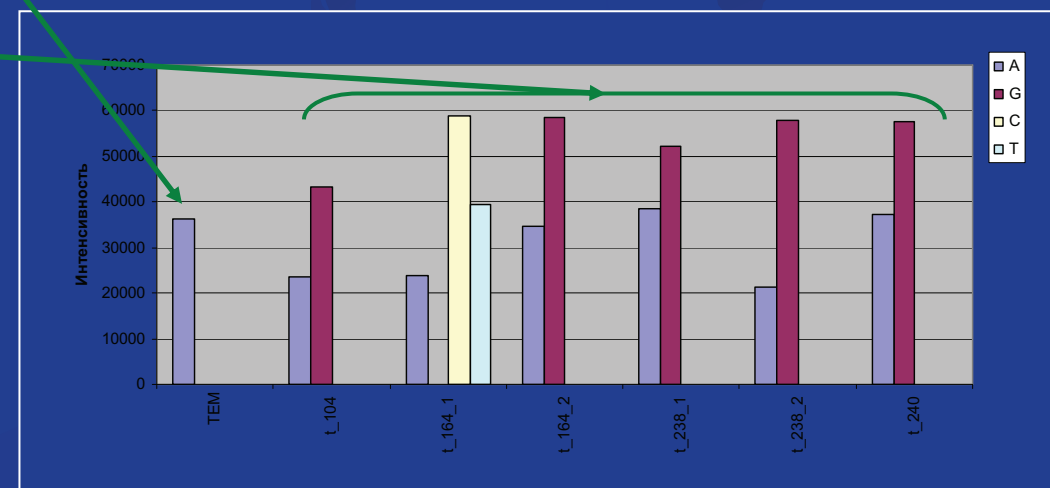
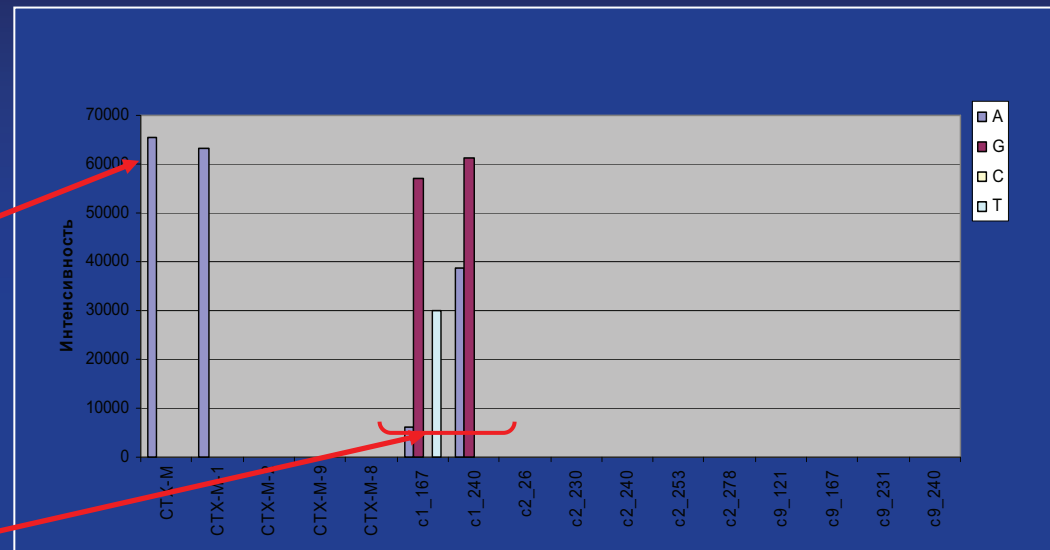
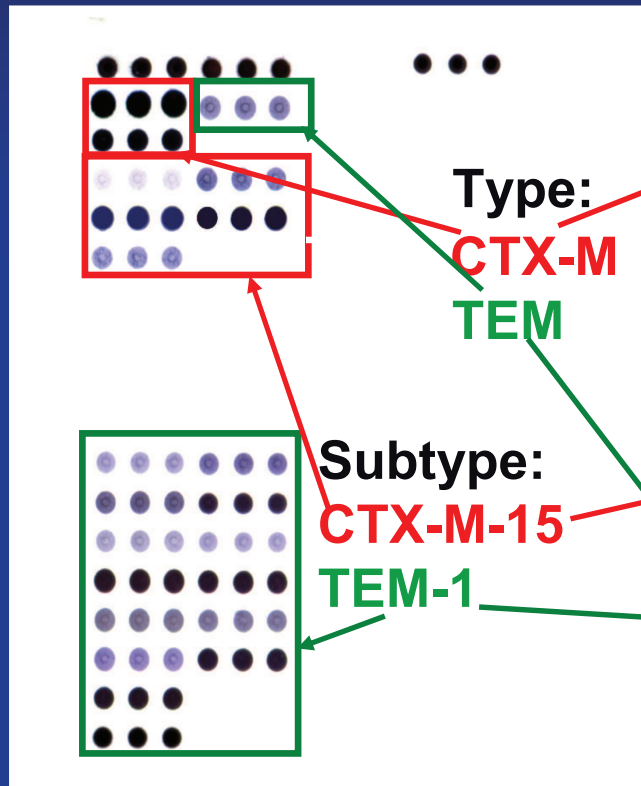


**Support:** nitrocellulose membrane  
**Size of the array:** 8 mm x 12 mm  
**Size of the spot:** ~ 250 µm  
**Number of spots per array:** 195

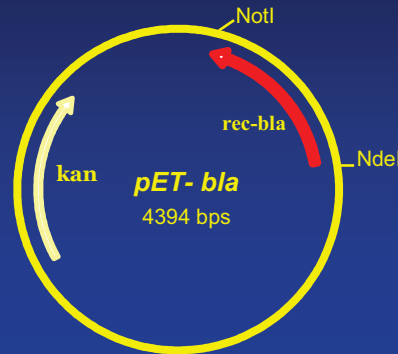


**Result of testing control strain producing CTX-M-3 beta-lactamase**

# Determination of a mixture of TEM-1 и CTX-M-15 with Identification chip



# CREATING *E.coli* STRAIN PRODUCING TEM-1 *b*-LACTAMASE



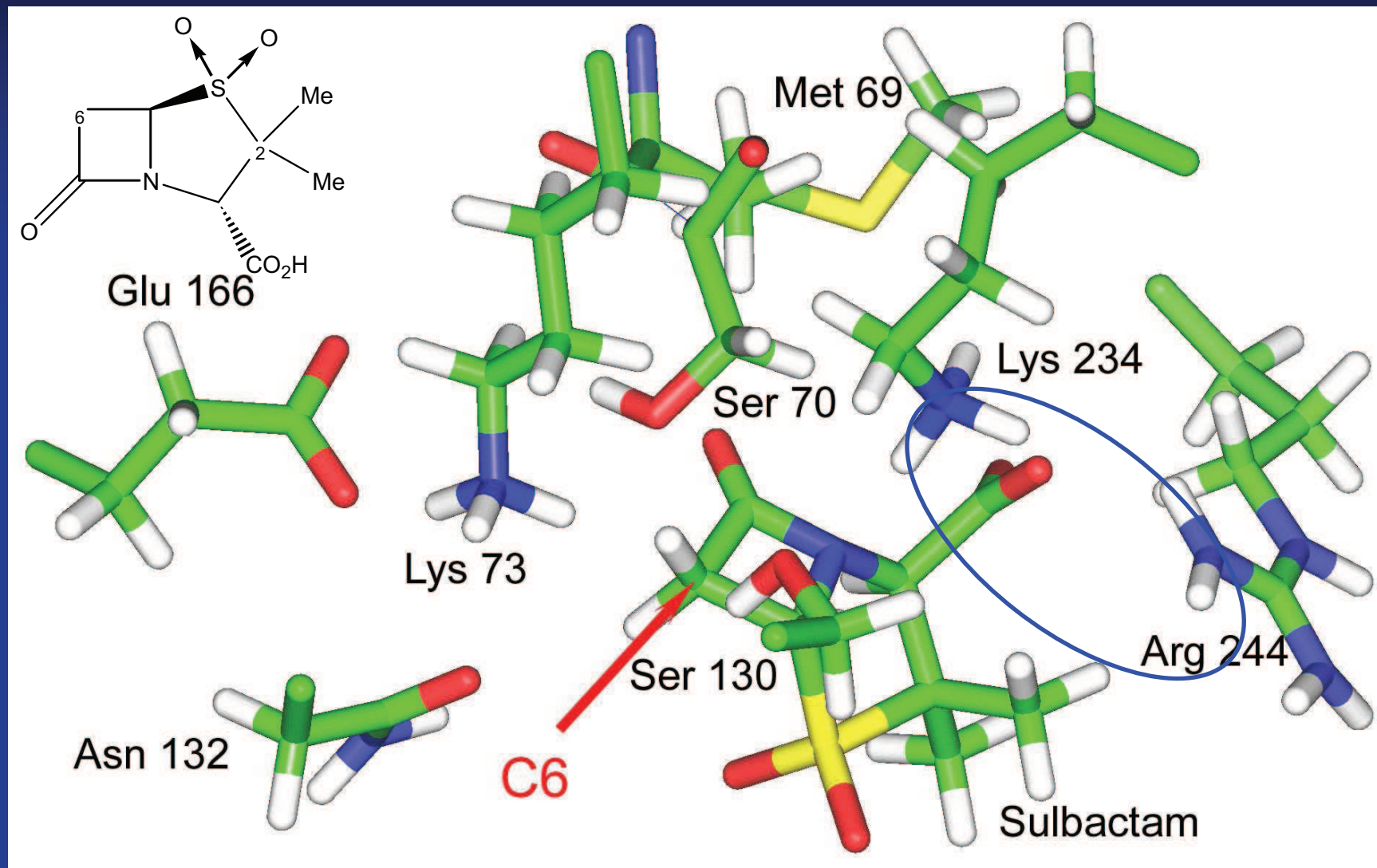
Expression system for *b*-lactamase TEM-1 (class A) production in *E.coli* cells permits to obtain homogeneous preparation of recombinant enzyme.

Kinetic parameters obtained with chromogenic substrate CENTA as follows:

$K_M \text{ eff} = 22 \text{ mM}$ ,  $V_m = 0,39 \text{ mM/s}$ ,  $k_{cat} = 31,2 \text{ s}^{-1}$ ,  $k_{cat}/K_M = 1,4 \text{ mM}^{-1}\text{s}^{-1}$ .

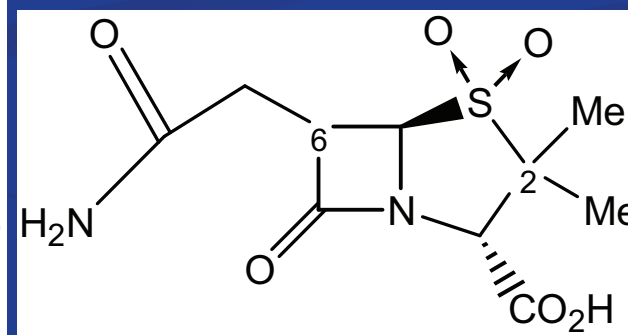
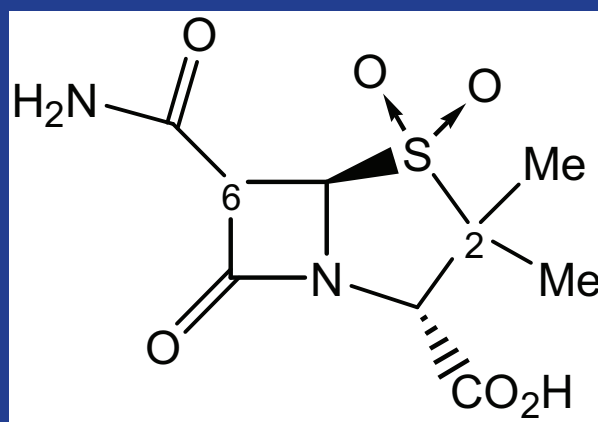
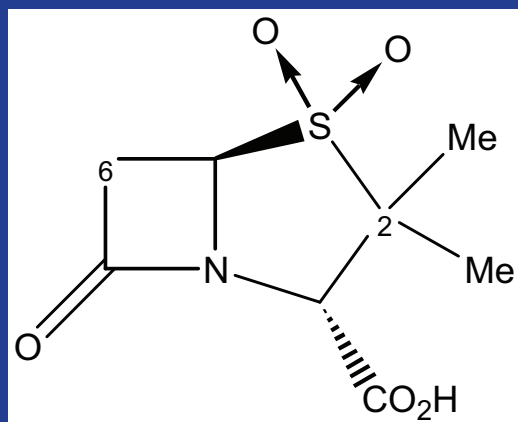
The inhibition constants for recombinant *b*-lactamase TEM-1:  
 $K_i(\text{sulbactam}) = 0,43 \text{ mM}$ ,  $K_i(\text{tazobactam}) = 0,041 \text{ mM}$ ,  $K_i(\text{clavulanic acid}) = 0,046 \text{ mM}$

## Structure of complex of TEM-1 $\beta$ -lactamase with sulbactam



## Suggested sulbactam modification of C6 carbon substitutes

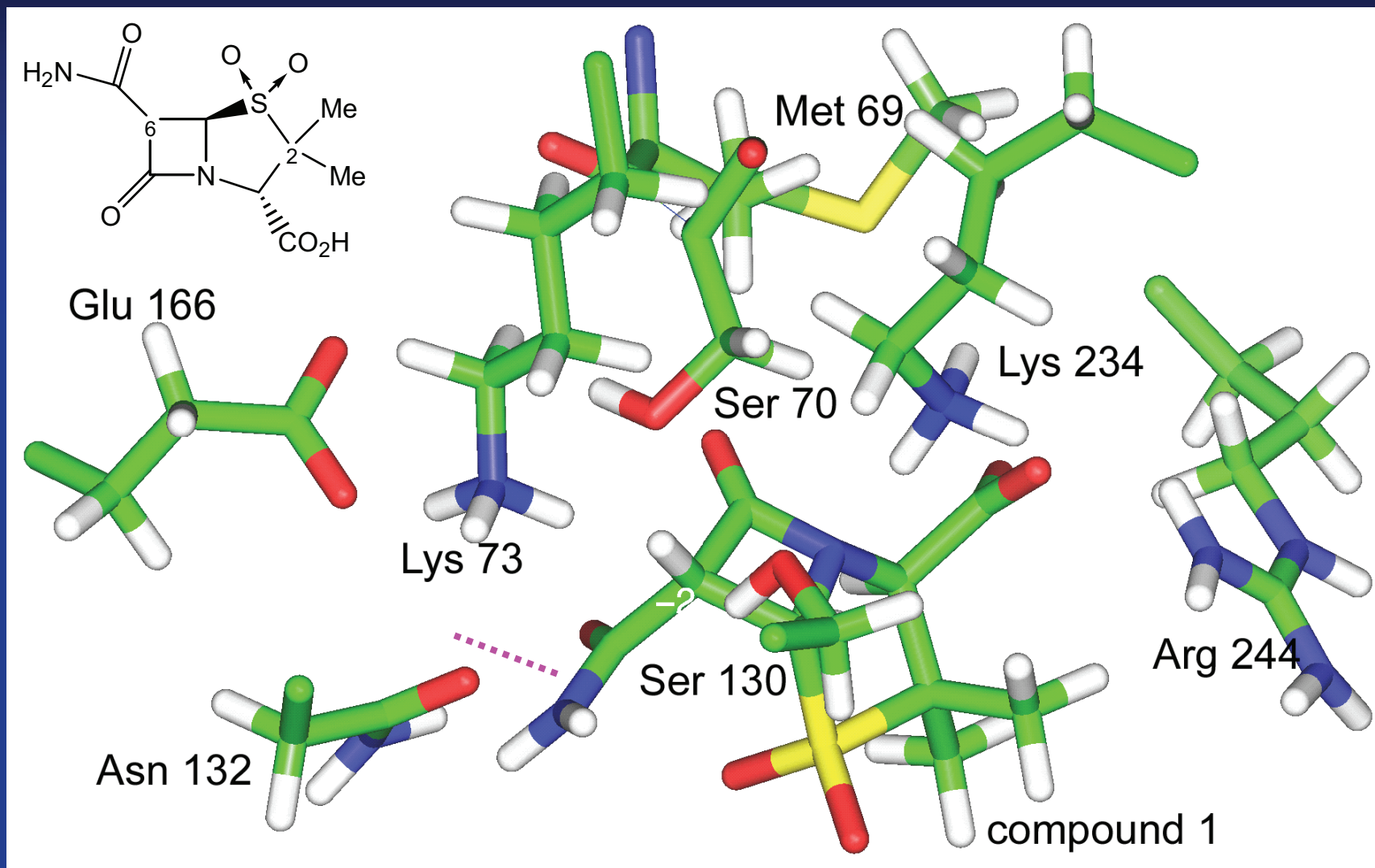
Asn 132 is conservative residues for TEM subclass A of  $\beta$ -lactamases which is located in enzyme active site facing possible C6 substitutes of sulbactam derivatives. Binding could be more strong if it used an ability of Asn132 terminal amide group to establish hydrogen bonds with substrate. Obvious choice would be to use amide group as terminal one for C6 substitute. Such selection would increase chances of establishing the hydrogen bonds between Asn132 and substrate.



**Because of sterical limitations compound with such substitute does not fit in enzyme active site**



## Model complex of prospective inhibitor with TEM b-lactamase active site



$$\Delta E_{\text{bound}} = -2.5 \text{ Kcal/mol}$$



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