Fragment-based NMR screening for inhibitors of bacterial enzymes

Vladimir Polshakov, Sofia Mariasina, Alexander Rudenko, Alexey Mantsyzov, Roman Romanov, Vladimir Tishkov

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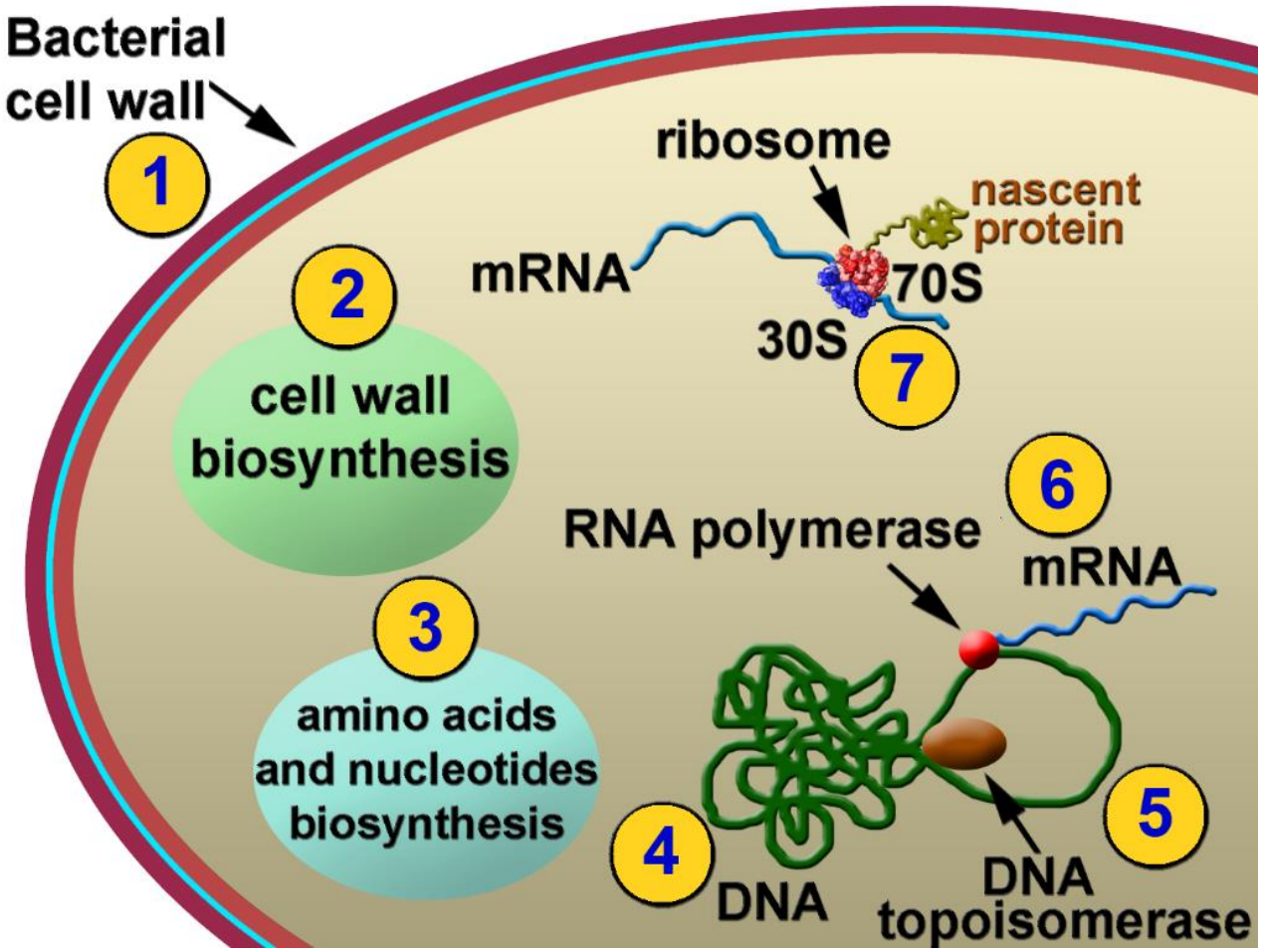
2025

Key targets of antibiotic action

1. Disruption of the bacterial cell wall's integrity;
2. DD-transpeptidase, arabinosyltransferase, enoyl-acyl carrier protein reductase;
3. Dihydrofolate reductase, dihydropteroate synthase, bacterial urease;
4. DNA bases modifications;
5. DNA gyrase;
6. RNA polymerase;
7. Bacterial ribosome.

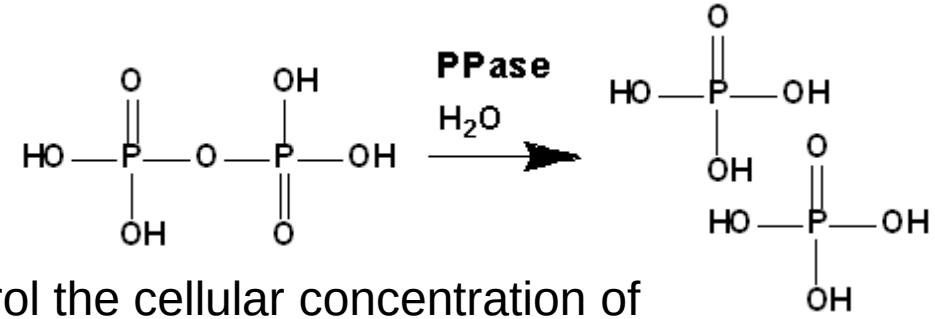
Properties of novel potential bacterial targets:

- **Vital necessity for bacterial cell;**
- **Selectivity** (differences from homologous human systems);
- **Druggability.**



Inorganic pyrophosphatase

Soluble inorganic pyrophosphatases (PPases) catalyze the hydrolysis of pyrophosphate to inorganic phosphate. This is a highly exergonic reaction.



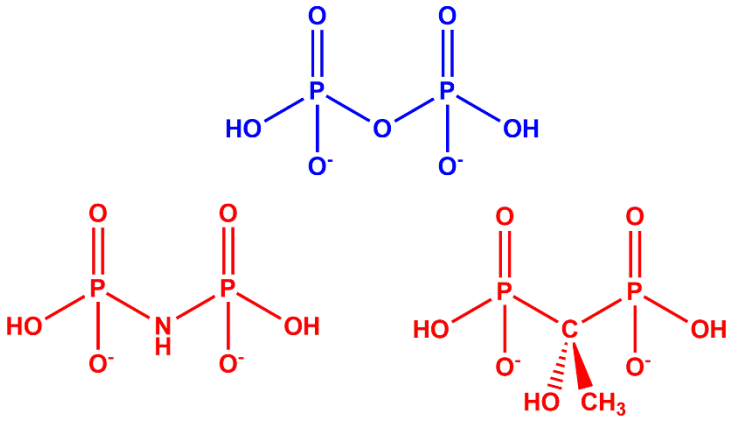
PPases are **essential enzymes** that control the cellular concentration of inorganic phosphates. They are involved in the biosynthesis of nucleic acids, proteins and phospholipids.

There are four classes of PPases: membrane-integral enzyme and the soluble families I, II and III PPases.

The structure of enzymes from different organisms differs significantly, and enzymes from prokaryotic and eukaryotic organisms differ markedly. **This makes selective inhibition of the pathogenic enzyme possible**.

Druggability: several inhibitors (structural analogues of the substrate) are known.

Most of known inhibitors – bioisosteric substrate analogues have low selectivity.



K_i = 2-15 μM 7-3000 μM

PPase from *Mycobacterium tuberculosis*

Class I PPase

Highly cooperative mechanism of catalysis

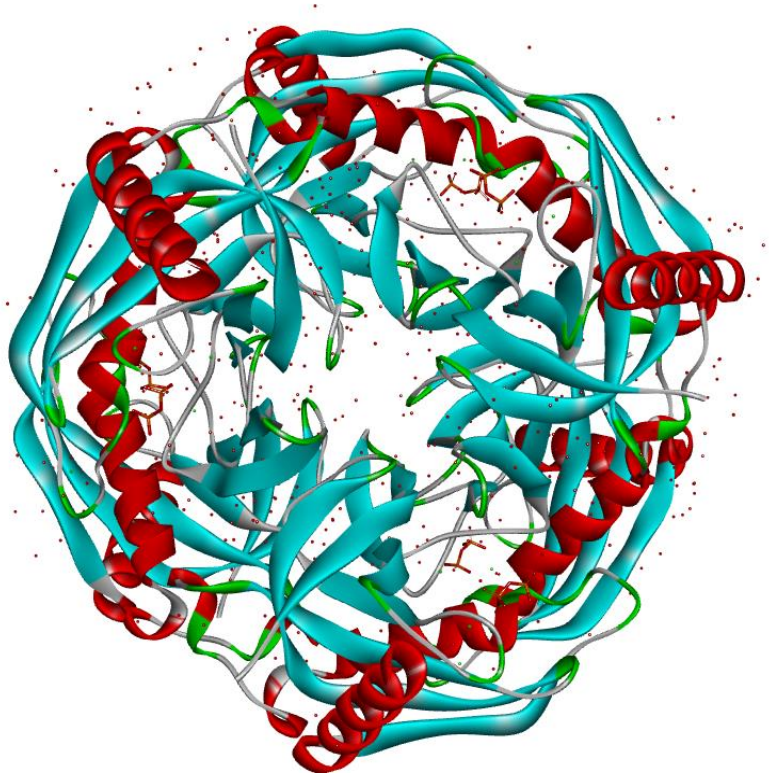
PDB id 4Z74

HTS and X-ray screening for MtPPase inhibitors

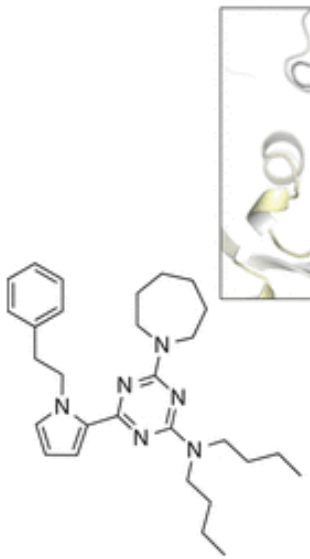
Discovery of Allosteric and Selective Inhibitors of Inorganic Pyrophosphatase from *Mycobacterium tuberculosis*

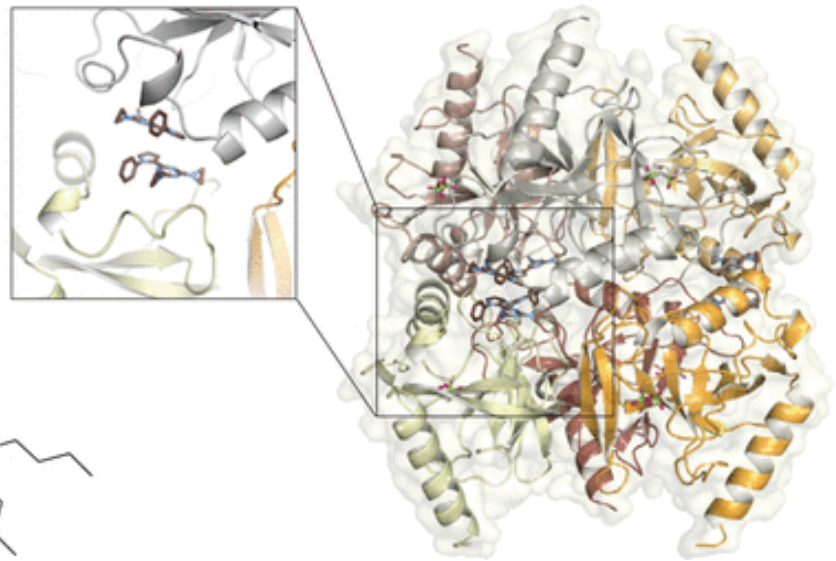
Allan H. Pang[†§], Atefeh Garzan[†§], Martha J. Larsen[‡], Thomas J. McQuade[‡], Sylvie Garneau-Tsodikova[*†], and Oleg V. Tsodikov[*†]

ACS Chem. Biol. 2016, 11, 11, 3084–3092



Hexamer 110 kDa
(6x18.3 kDa, 162 a.o.)





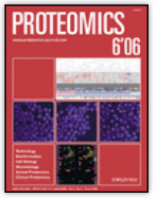
K_i = ~11 μM

Formate dehydrogenasee from *Staphylococcus aureus*

Formate dehydrogenase (FDH) catalyzes the formate ion oxidation to CO_2 coupled with the reduction of NAD^+ to NADH (or $NADP^+$ to NADPH):

$$HCOO^- + NAD^+ \xrightarrow{\textbf{FDH}} CO_2 + NADH$$

FDH plays an extremely important physiological role in the vital activity of several pathogenic bacteria:



Comparative proteome analysis of *Staphylococcus aureus* biofilm and planktonic cells and correlation with transcriptome profiling

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Pages 1867-1877

Alexandra Resch, Stefan Leicht, Marc Saric, Linda Pásztor, Andreas Jakob, Friedrich Götz Prof., Alfred Nordheim

https://doi.org/10.1002/pmic.200500531

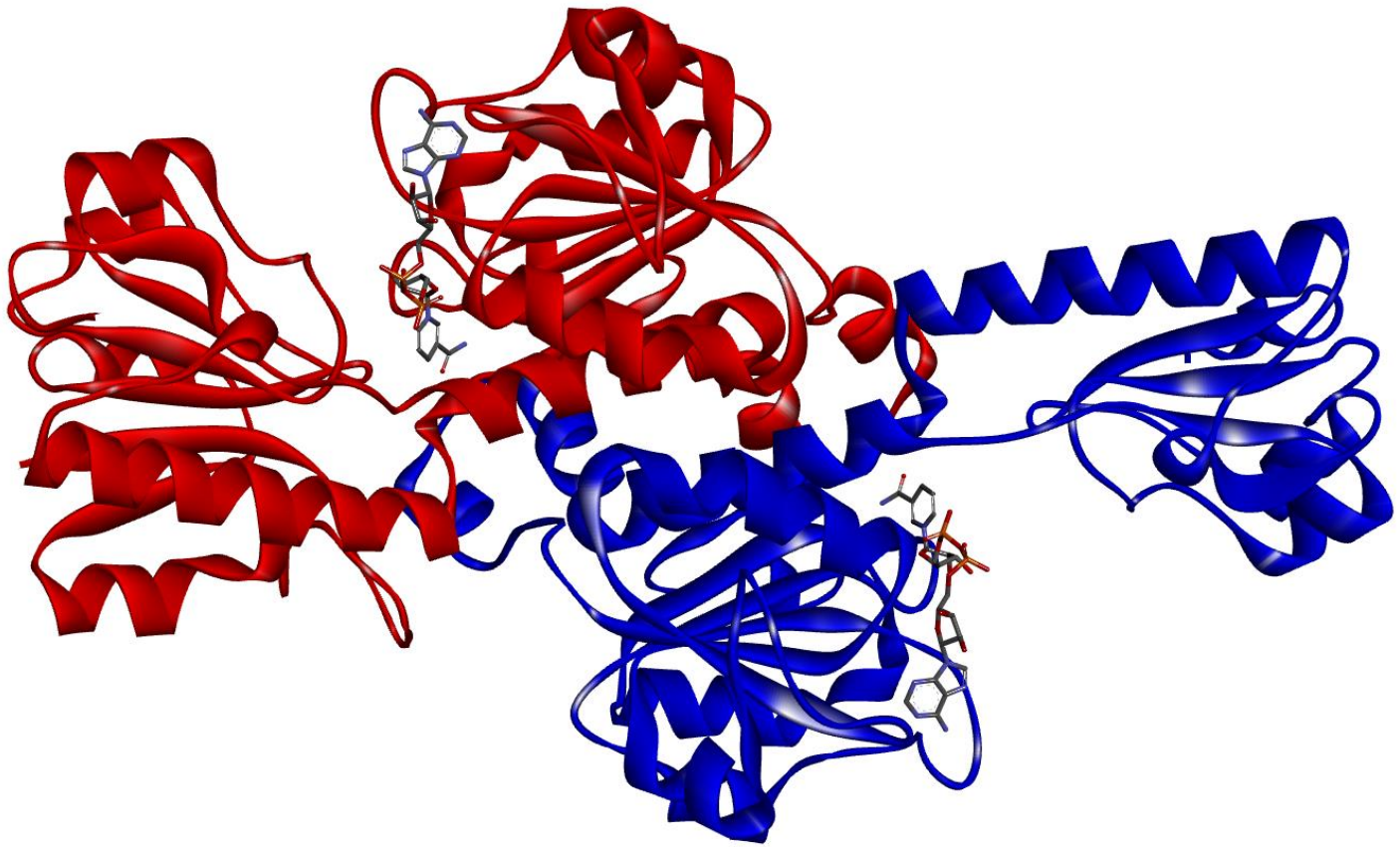
The amount of FDH mRNA in biofilms of *Staphylococcus aureus* increase under the stress **up to 17 times** compared with the planktonic cells.

FDH has been found in bacteria, yeasts, fungi and plants. It has no direct analogue in humans, which ensures the **selectivity of the action** of potential inhibitors.

Druggability: the best known FDH inhibitor is the azide ion, which mimics the transition state structure of the substrate.

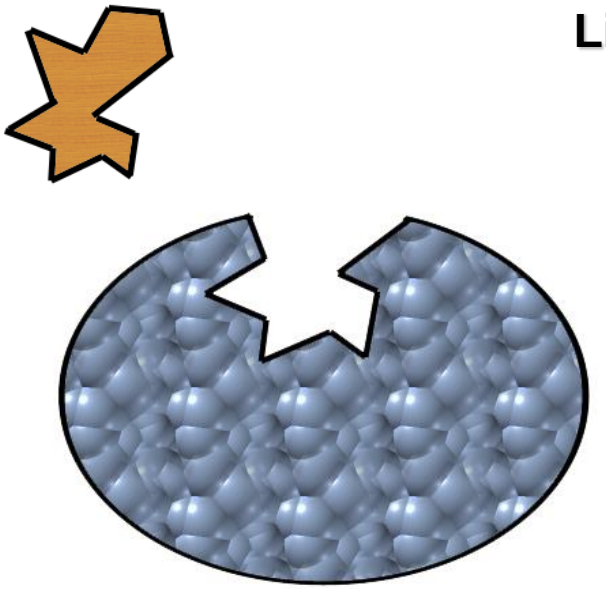
Formate dehydrogenasee from *Staphylococcus aureus*

X-ray structure of SaFDH in complex with NAD^+ (not yet published)



SaFDH is a dimer of two subunits of 368 a.a. each with
a molecular weight of 84 kDa

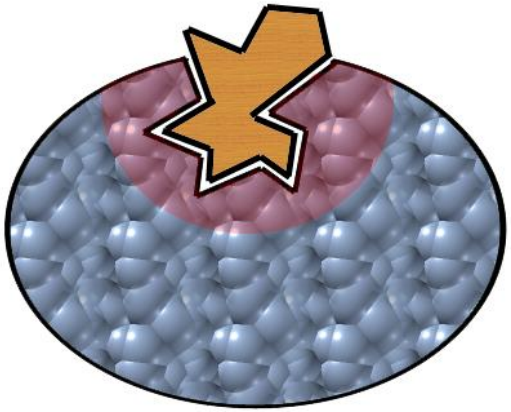
NMR screening of potential inhibitors of MtPPase and SaFDH



Ligand changes:

- Chemical shifts
- Relaxation parameters
- NOEs
- Magnetization transfer

NMR experiments:
STD
WaterLOGSY
NOESY



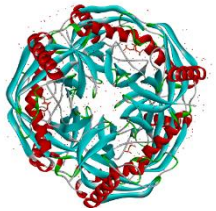
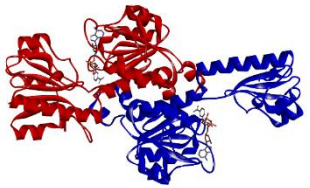
Protein changes:

- Chemical shifts
- Relaxation parameters
- NOEs

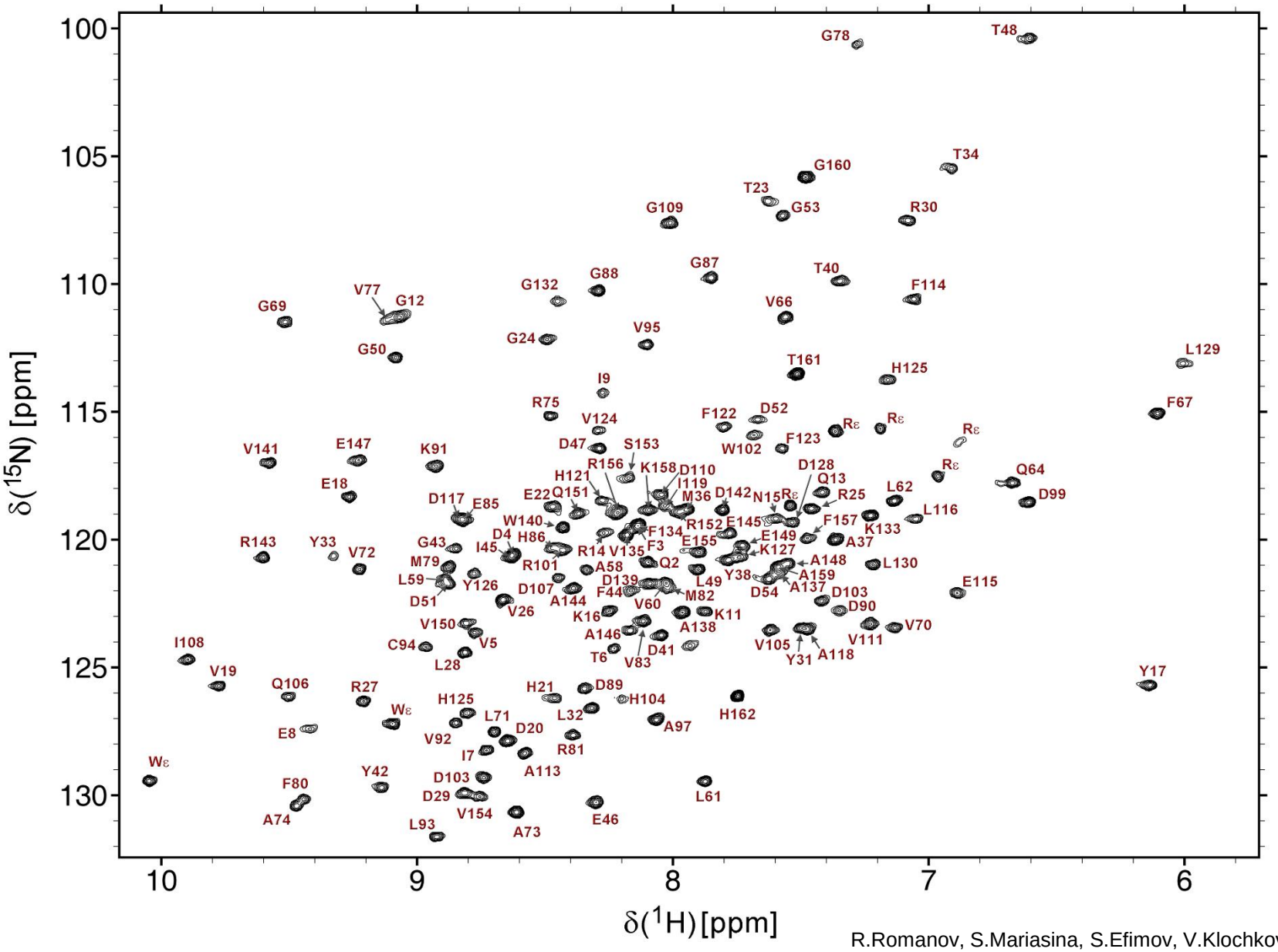
NMR experiments:
TROSY

Monitoring protein-ligand interaction with either ligand or protein changes

Protein samples and NMR experiments

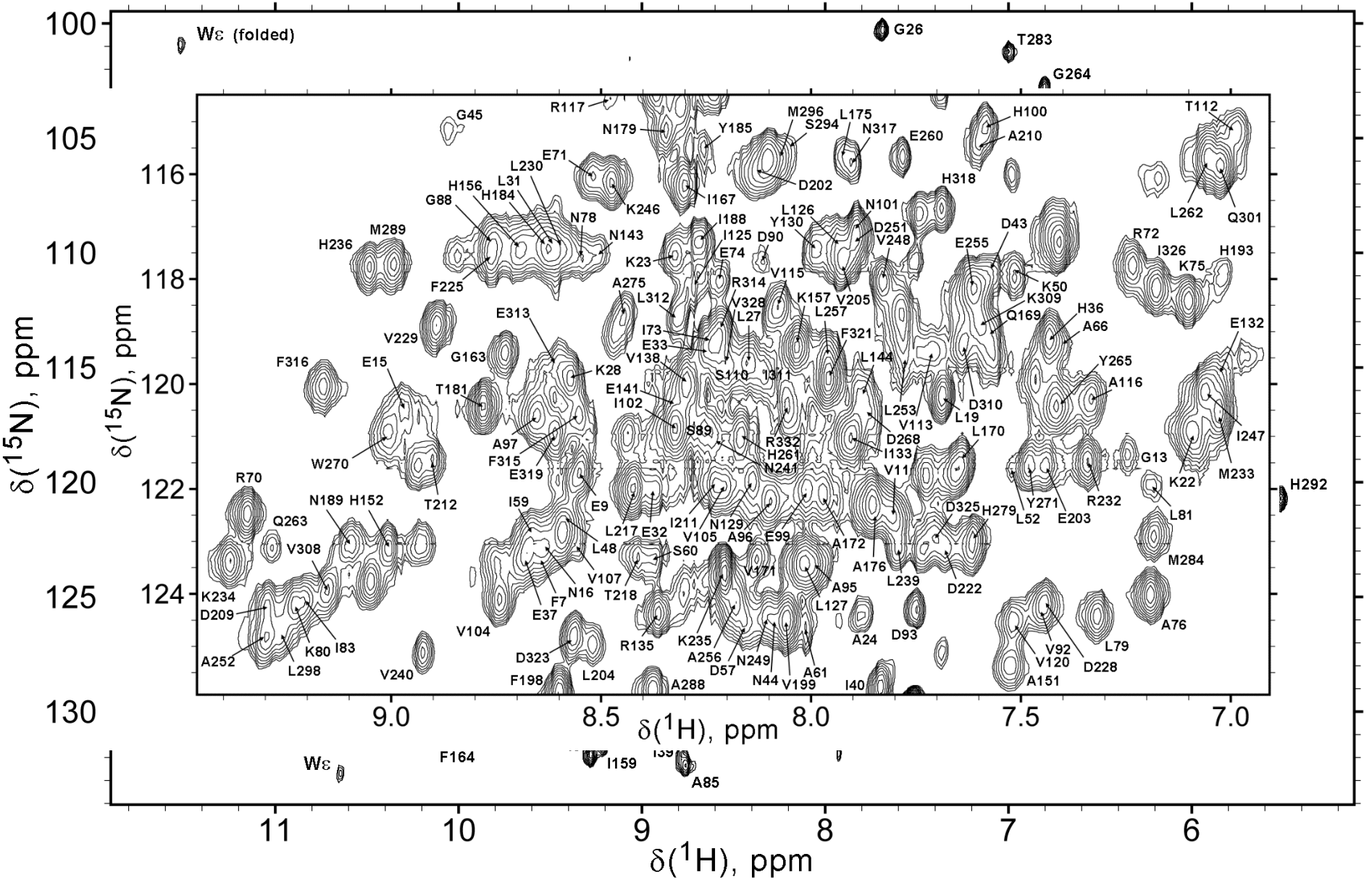
Protein	**MtPPase** 	**SaFDH** 
M.W.	**6 x 18.3 = 110 kDa**	**2 x 42 = 84 kDa**
Expression	***E.coli* culture,** **M9 (^{15}N, ^{13}C, ^{2}D) in D_2O**	***E.coli* culture,** **M9 (^{15}N, ^{13}C, ^{2}D) in D_2O** ↓ **ISOGRO-^{13}C,^{15}N,^{2}D in** **50% D_2O / 50% H_2O**
D/H exchange	**10 s heating in H_2O** **at ~100°C**	**dissolution in H_2O** **(50% slowly exchanged NH)**
NMR samples	**700 MHz, 45°C, H_2O, PBS pH 6.8, 50 mM Arg / 50 mM Glu**	**700 MHz, 45°C, H_2O, PBS pH 6.5, 25 mM Arg / 25 mM Glu**
NMR assignments	**TROSY, 3D tr-HNCA, 3D tr-HN(CO)CA, 3D tr-HNCO, 3D tr-HN(CA)CO, 3D tr-HNCACB, 3D tr-CBCA(CO)NH**	

^{15}N-^{1}H TROSY spectrum of MtPPase after sample/conditions optimization



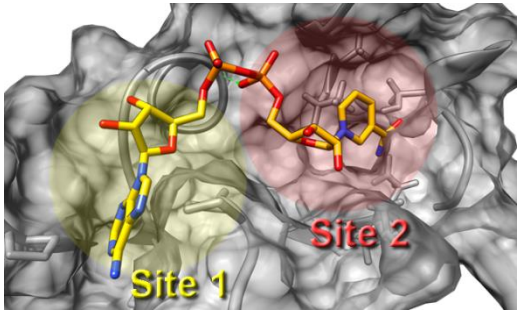
R.Romanov, S.Mariasina, S.Efimov, V.Klochkov, E.Rodina, V. Polshakov (2020) *Biomol. NMR Assign.*, **14**(2): 281-287.

^{15}N-^{1}H TROSY spectrum of the apo-form of SaFDH



The strategy used to search for potential inhibitors of MtPPase and SaFDH

Selection of ligand binding sites



Selection of small fragments using virtual screening

Libraries: InterBioScreen, ChemDiv

~ 1 million per target

Software: DOCK 6.9

Ligand-based NMR screening of hits

STD and WaterLOGSY

155 fragments studied

Two experiments - with and without of target

Positioning of the bound fragments Ligand-based exps

INPHARMA/SAR-by-ILOE

20 fragments studied

Positioning of the bound fragments Protein-based exps

TROSY

12 fragments studied

Synthesis of new compounds

Medicinal chemistry

37 new compounds synthesized

Hit selection

STD, WaterLOGSY, TROSY, *in vitro* enzymatic assays

17 hits selected

Lead selection

Medicinal chemistry, enzymatic assays

2 leads found

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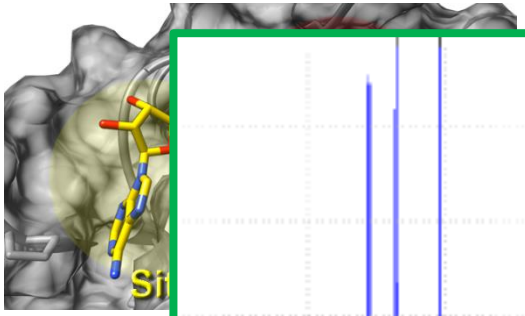

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TRC
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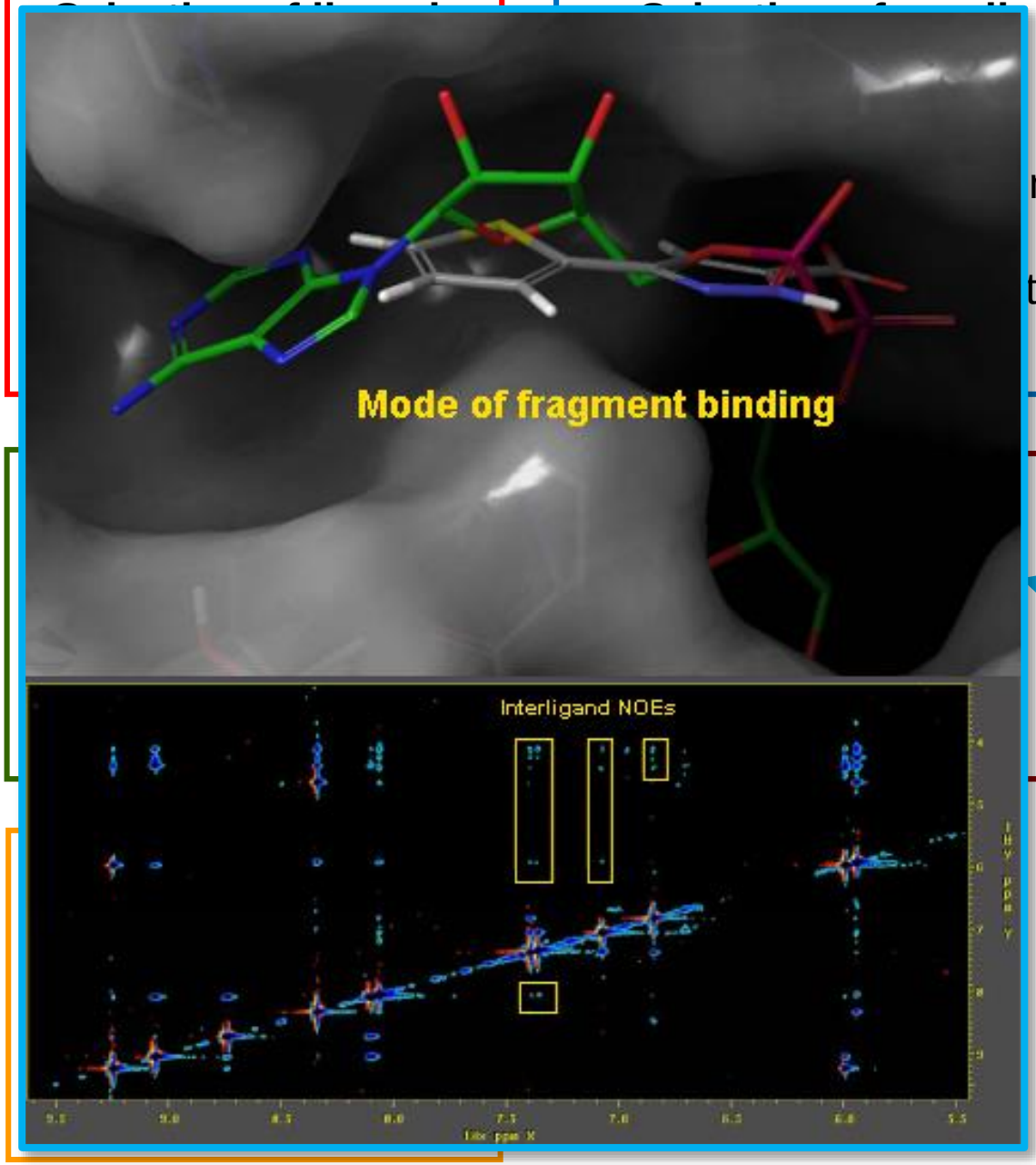

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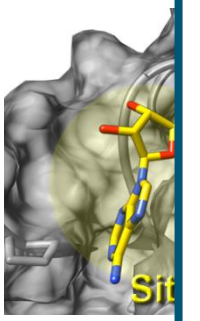
Select
bin



Hit

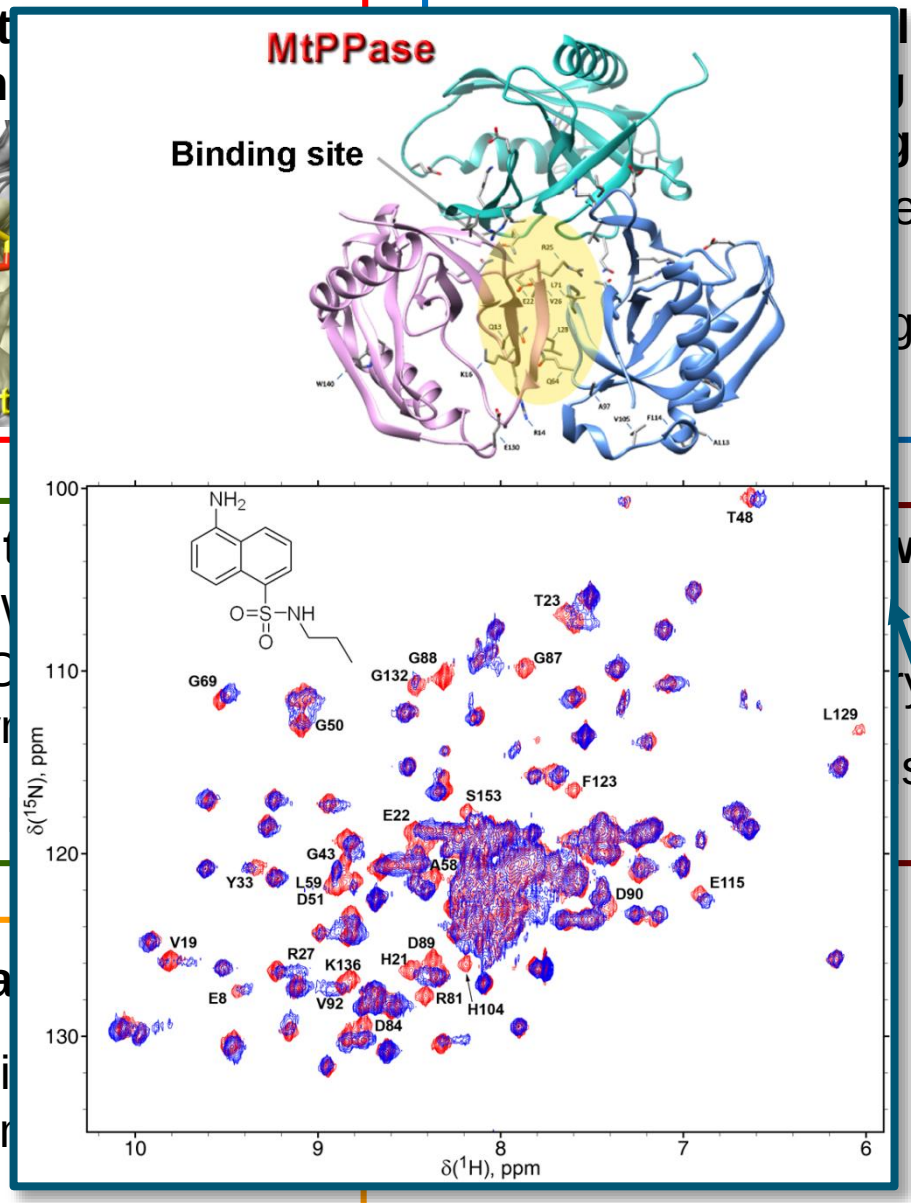
STD, V
TRC
enzyr

17

Lea

Medici
enzyr

2 leads found



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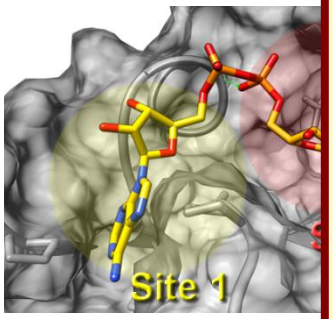


Selection of small

Ligand-based NMR hits

LOGSY

studied

ts - with

f target

Hit selec

STD, WaterL

TROSY, *in*

enzymatic a

17 hits sel

of the nents exps

-by-ILOE

studied

Lead selec

Medicinal che

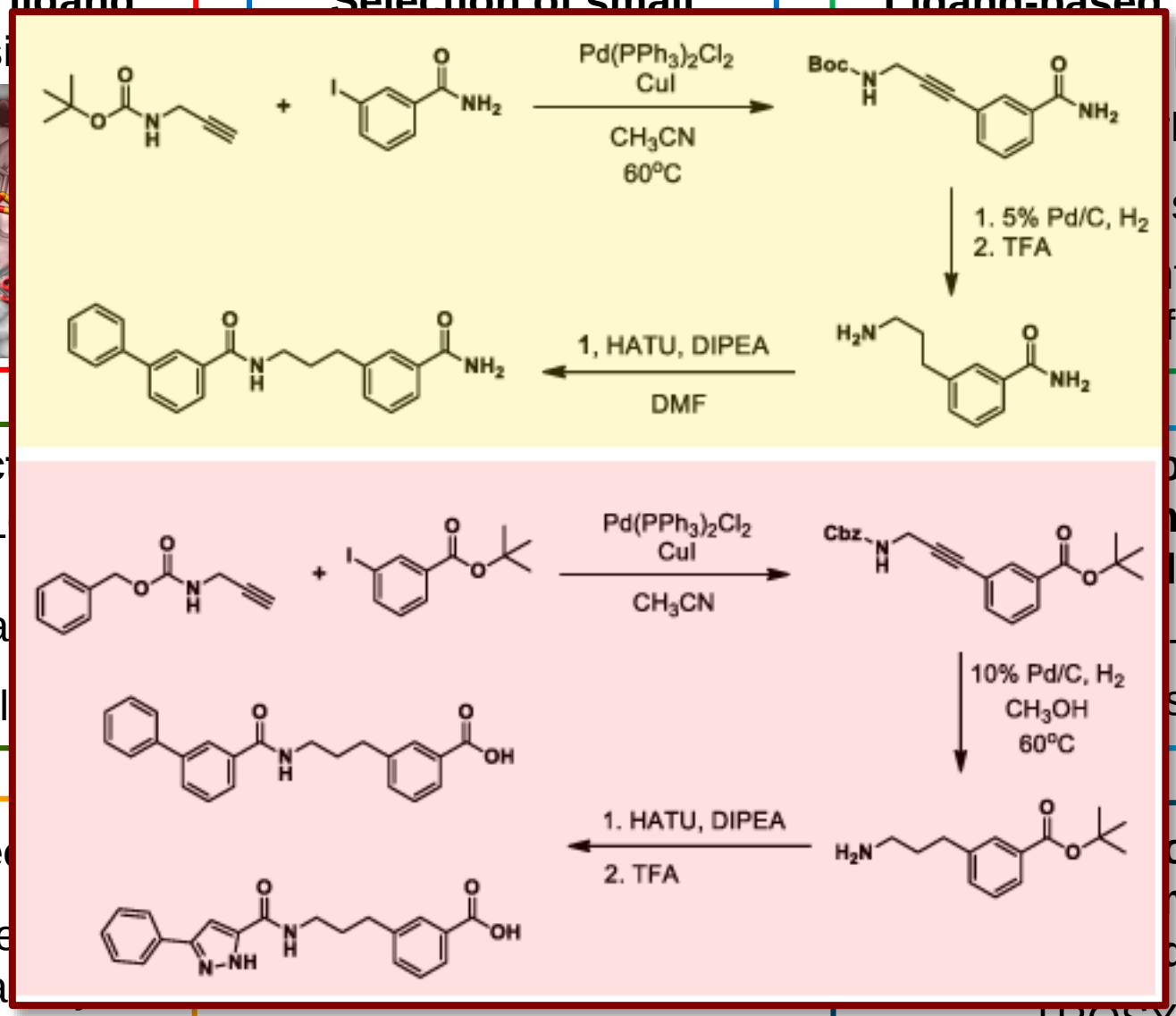
enzymatic a

2 leads found

of the nents exps

TROSY

12 fragments studied



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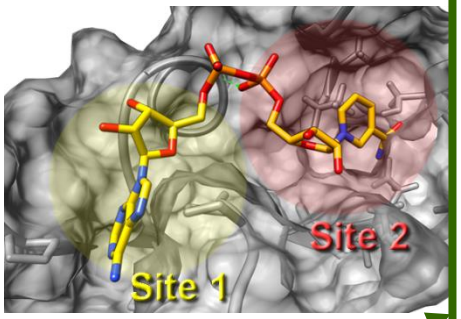
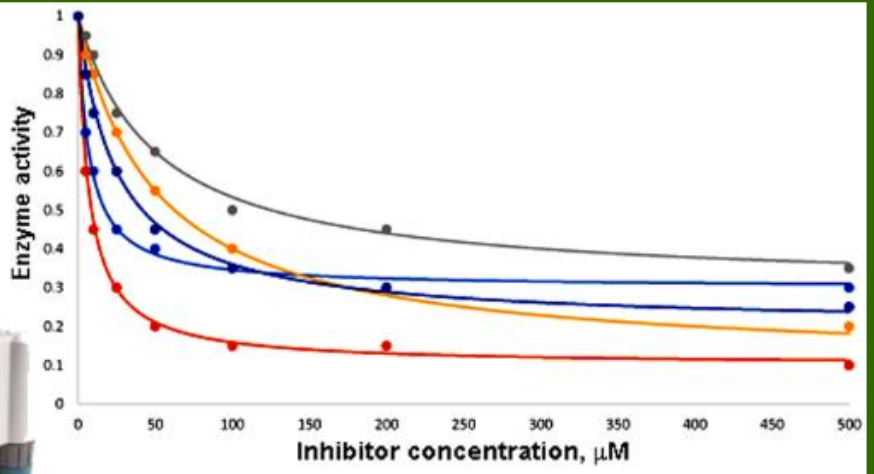


Selection of small fragments using

Ligand-based NMR screening of hits



Hit selection

STD, WaterLOGSY
TROSY, *in vitro* enzymatic assays

17 hits selected

Lead selection

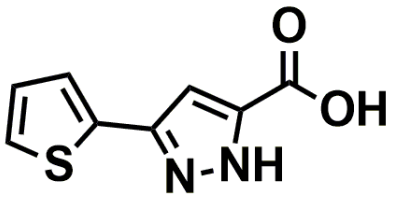
Medicinal chemistry
enzymatic assays

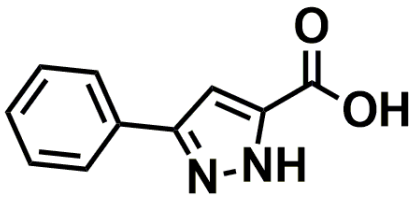
2 leads found

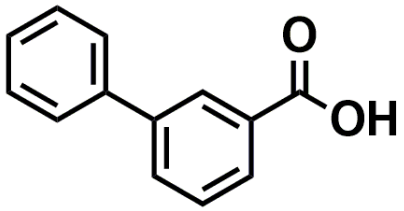
TROSY

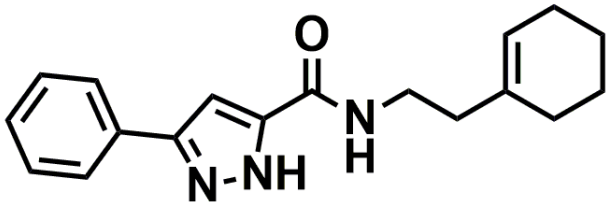
12 fragments studied

The most effective SaFDH inhibitors found

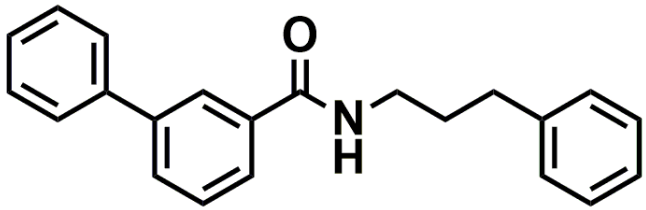




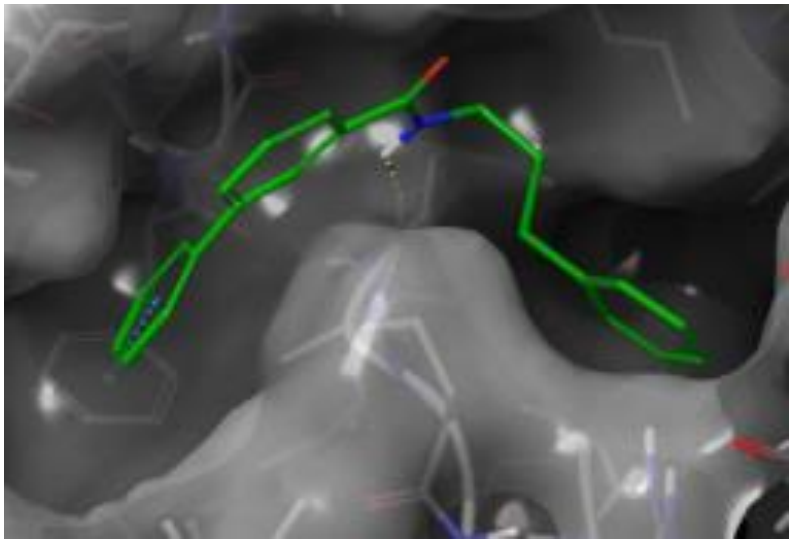


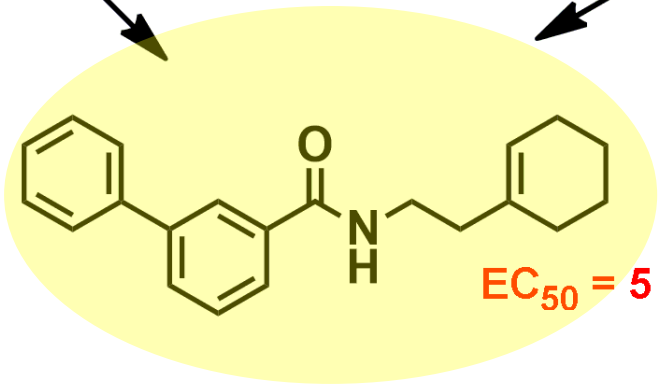


EC_{50} = 20 ± 2 μM



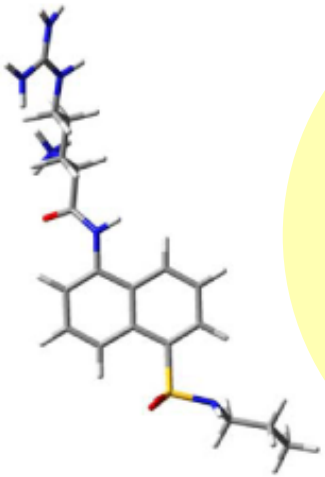
EC_{50} = 7 ± 0.6 μM

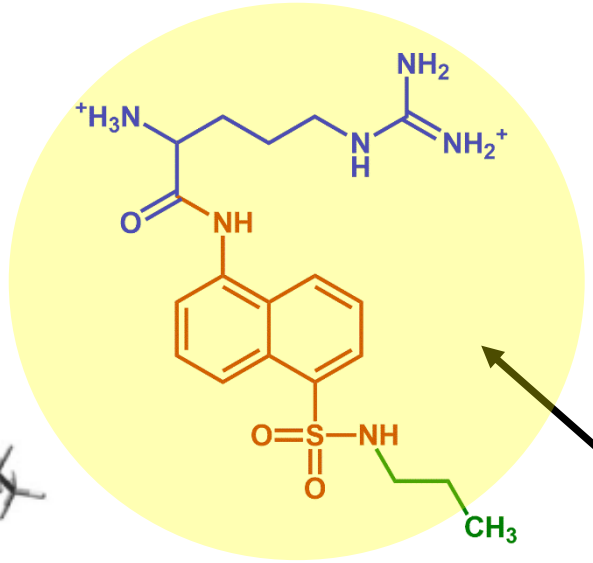




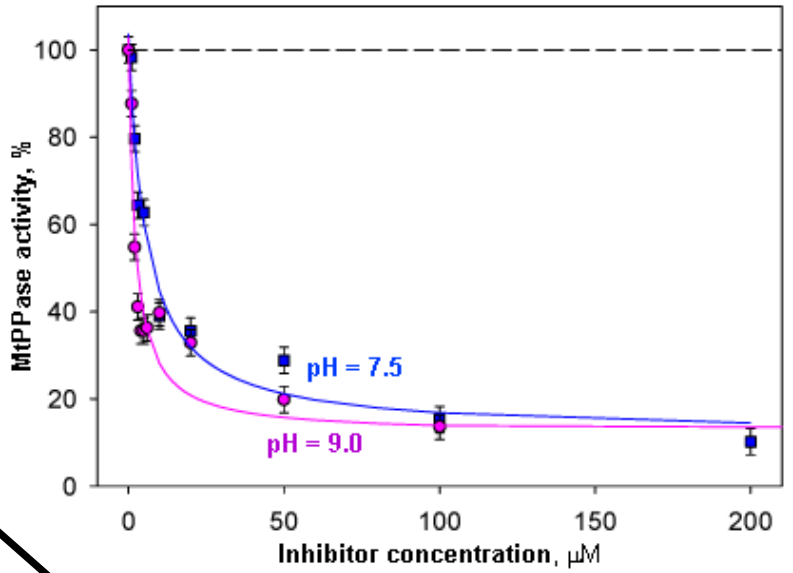
EC_{50} = 5 ± 0.5 μM

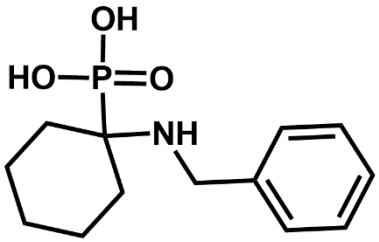
The most effective MtPPase inhibitors found



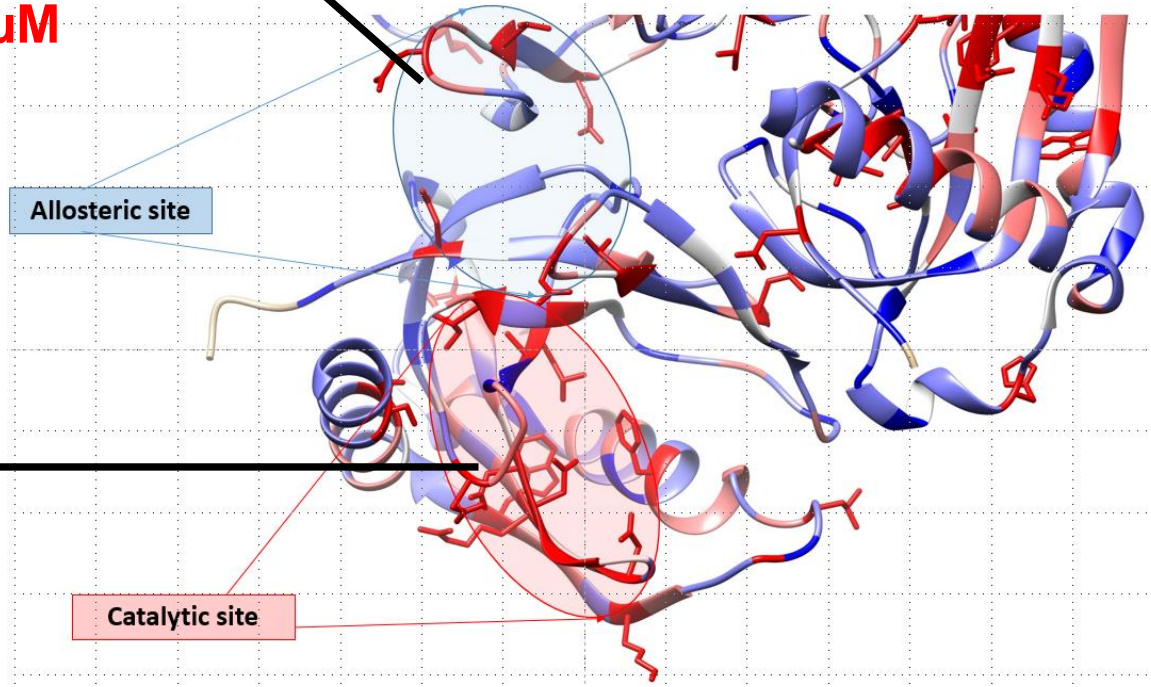


IC_{50} = 7 ± 3 μM





IC50 = 170 ± 10 μM



Conclusions

•Drug design for two potential antibiotic targets from two pathogenic bacteria, *Mycobacterium tuberculosis* and *Staphylococcus aureus*, has been initiated.

•Methods for obtaining samples of ^{15}N- and ^{13}C-labeled proteins have been developed. Protein backbone assignments have been obtained and backbone dynamics have been examined.

•Inhibitors have been designed using fragment-based NMR screening methods. Compounds with an inhibitory concentration in the micromolar range were identified.

•In the case of *Mycobacterium*, allosteric binding inhibitors were discovered. For *Staphylococcus*, NAD^+ competitive inhibitors were designed.

Acknowledgents

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RUDN University, Moscow

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