Time-resolved Serial Crystallography: A New Frontier in Drug Discovery

José M. Martín García
jmmartin@iqf.csic.es

Institute of Physical Chemistry Blas Cabrera
CSIC, Madrid

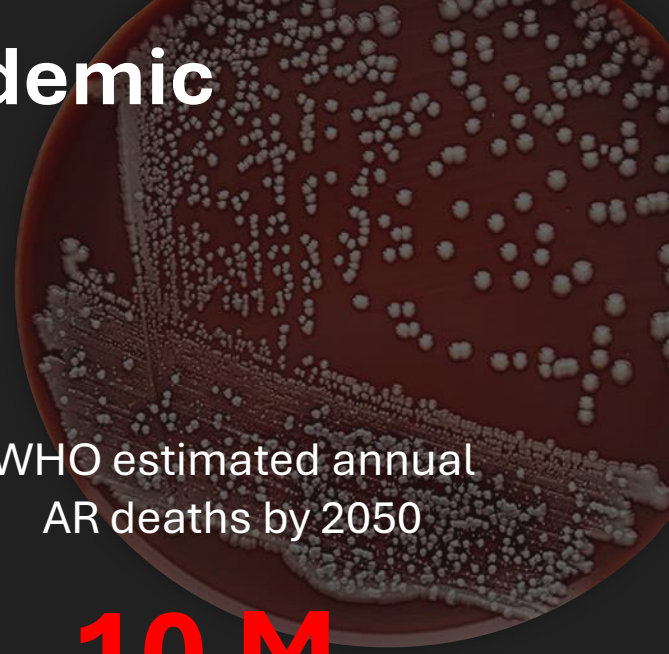
XI AUSE Conference and VI ALBA Users Meeting
Oviedo, September 2nd, 2024

INSTITUTO QUÍMICA FÍSICA BLAS CABRERA

CSIC

Spain XFEL HUB

Antibiotic Resistance: The silent pandemic



People killed annually by AR infections in the EU

35000

People killed annually by AR infections globally

1.27 M

Fourth leading cause of human death today

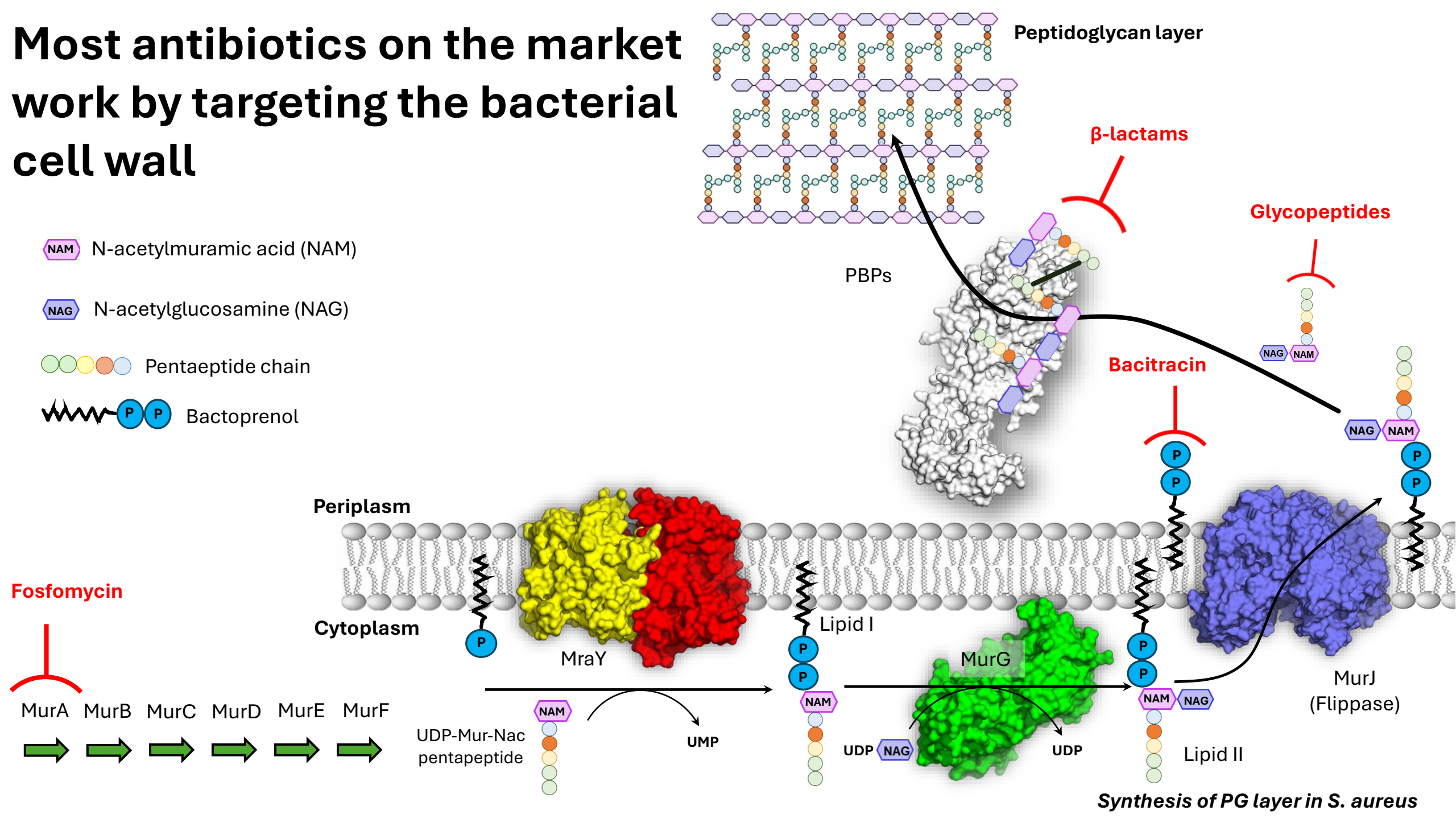
WHO estimated annual AR deaths by 2050

10 M

Cancer causes over 8 M deaths annually

There is an urgent need to advance the development of new and more effective antibiotics.

Most antibiotics on the market work by targeting the bacterial cell wall

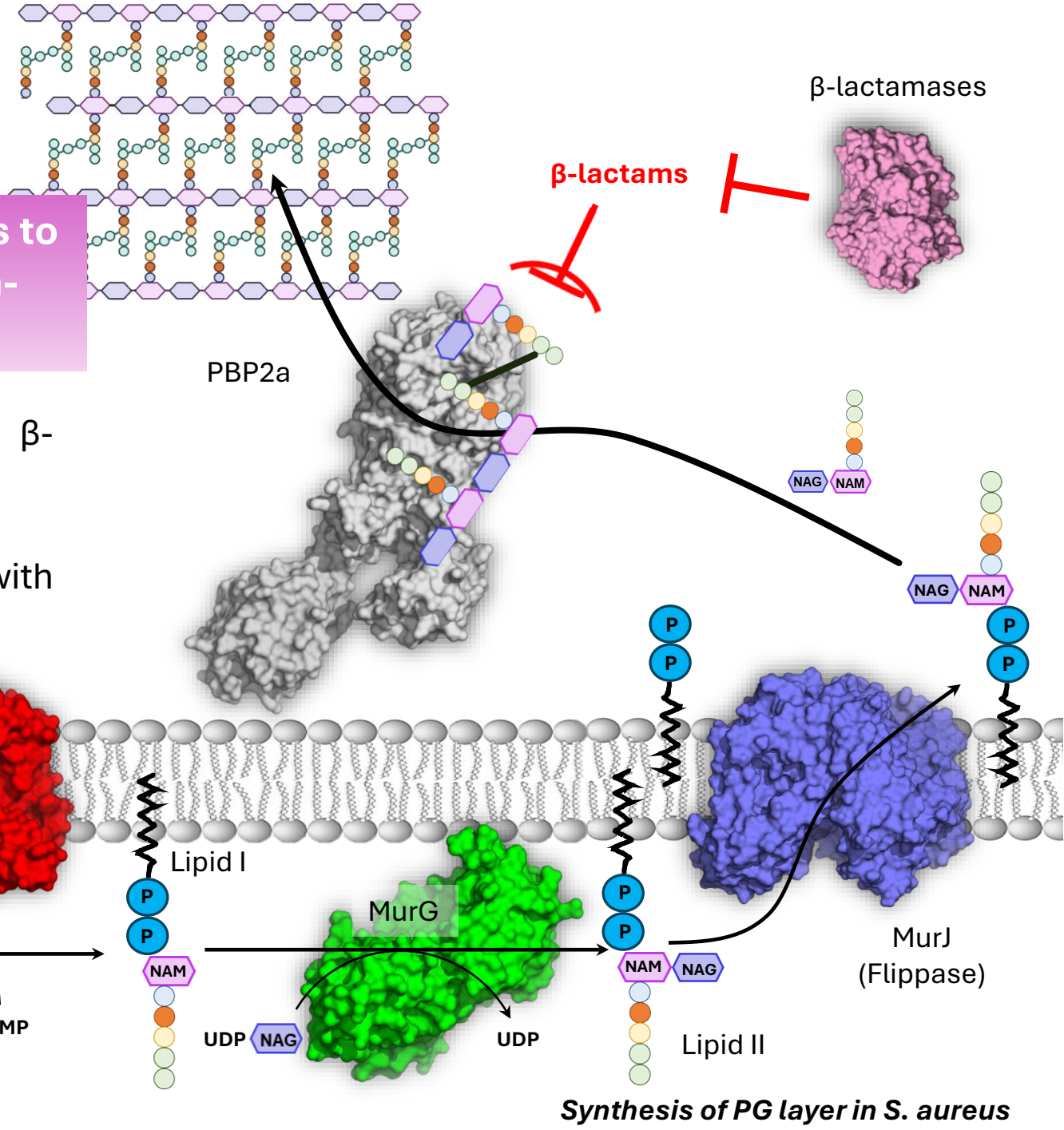


Resistance mechanisms against β-lactam antibiotics

There's an urgent need to develop new inhibitors to block beta-lactamases or to create better non-beta-lactam drugs that can still target PBPs

1) Production of **β-lactamases** to break down the β-lactams antibiotics.

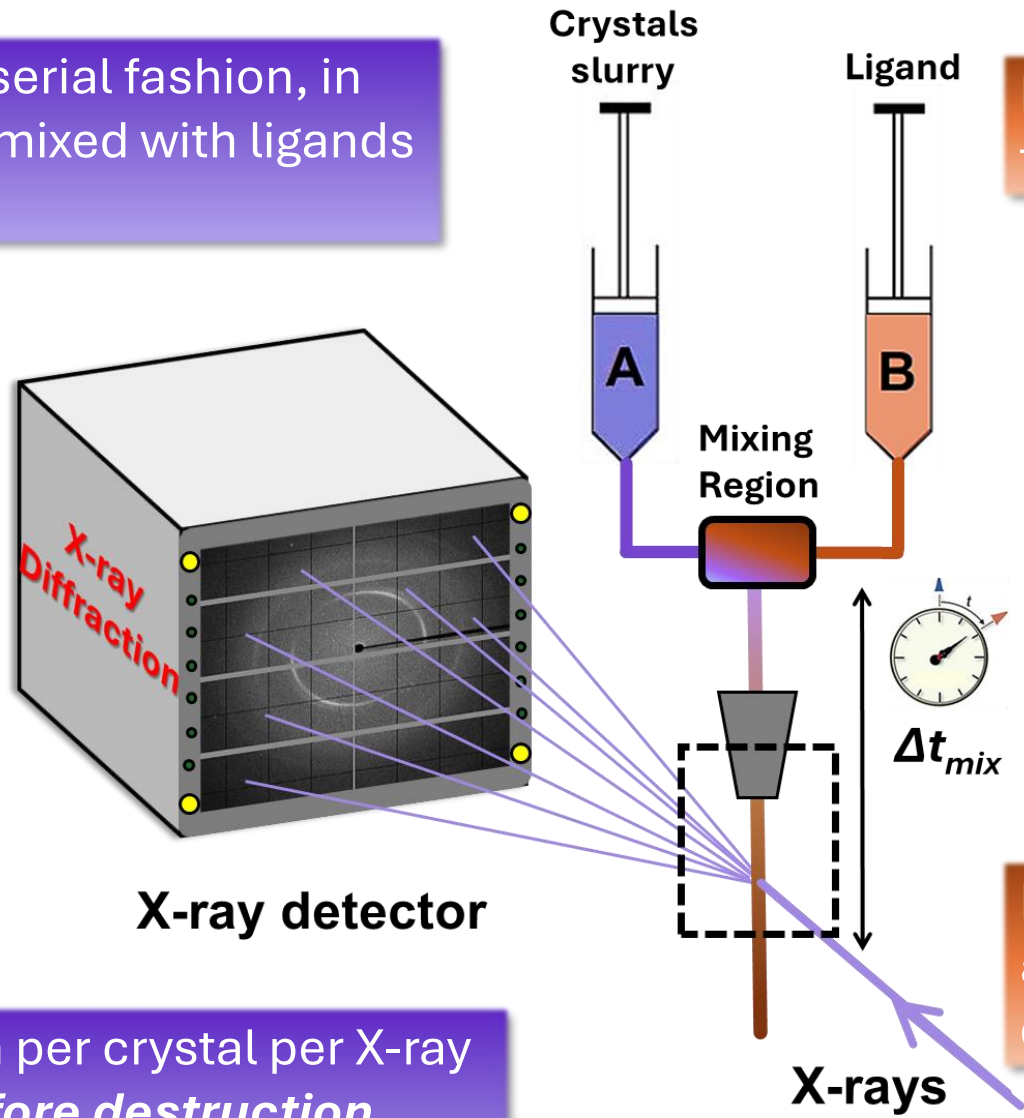
2) Modification of **PBPs** (*e.g.*, PBP2a in *S. aureus*) with reduced binding affinity to β-lactams.



Synthesis of PG layer in S. aureus

Mix-and-inject Time-resolved Serial Femtosecond Crystallography

Microcrystals injected in serial fashion, in random orientations and mixed with ligands using mixing devices

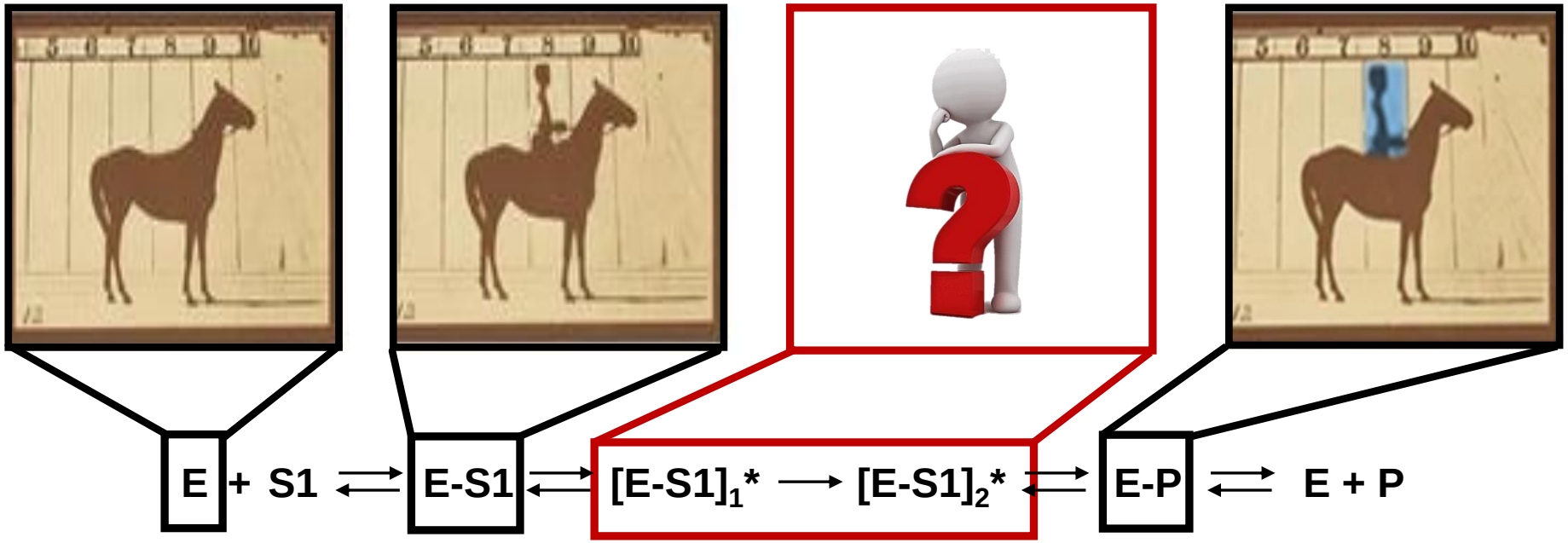


Data collection is performed at room temperature

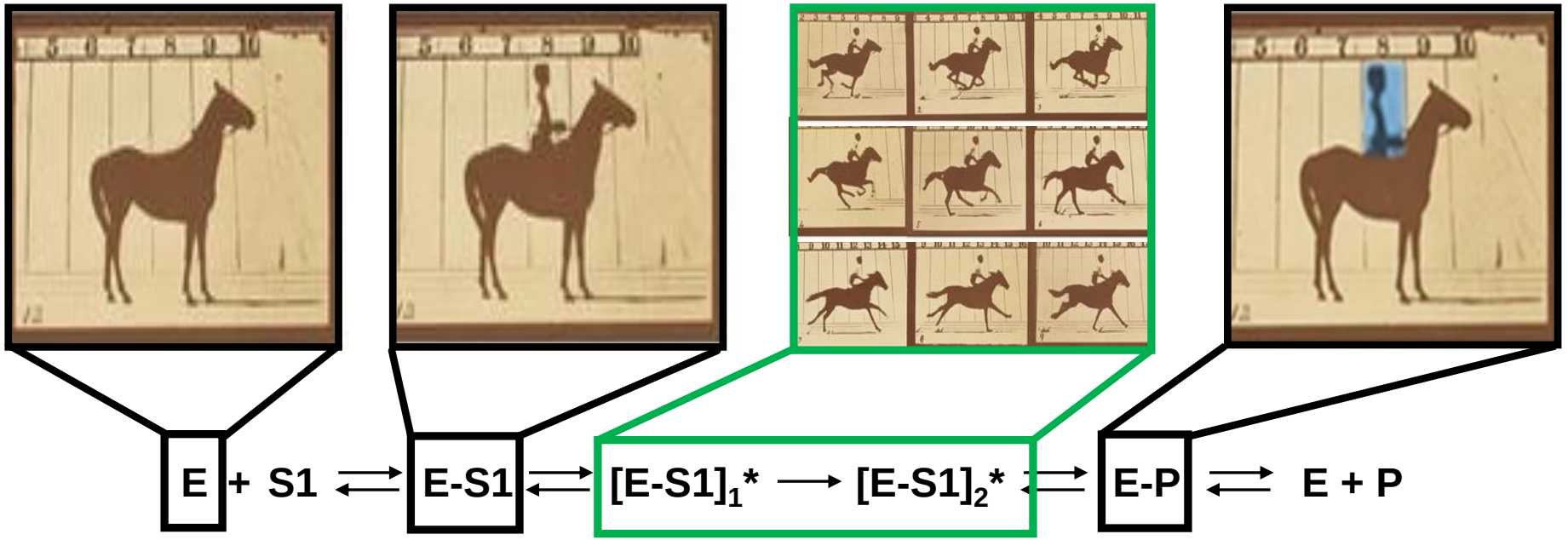
Hundreds of thousands of images for a complete data set are collected at different time points

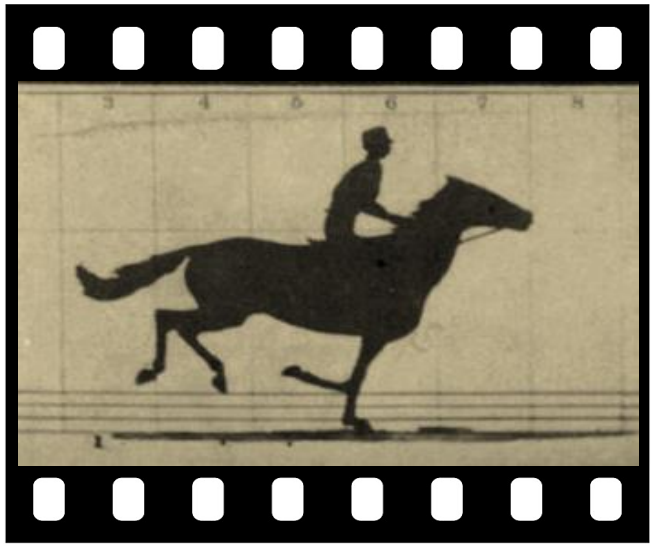
One diffraction pattern per crystal per X-ray pulse…***Diffraction before destruction*** principle

Traditional MX @ synchrotrons limits protein dynamics studies



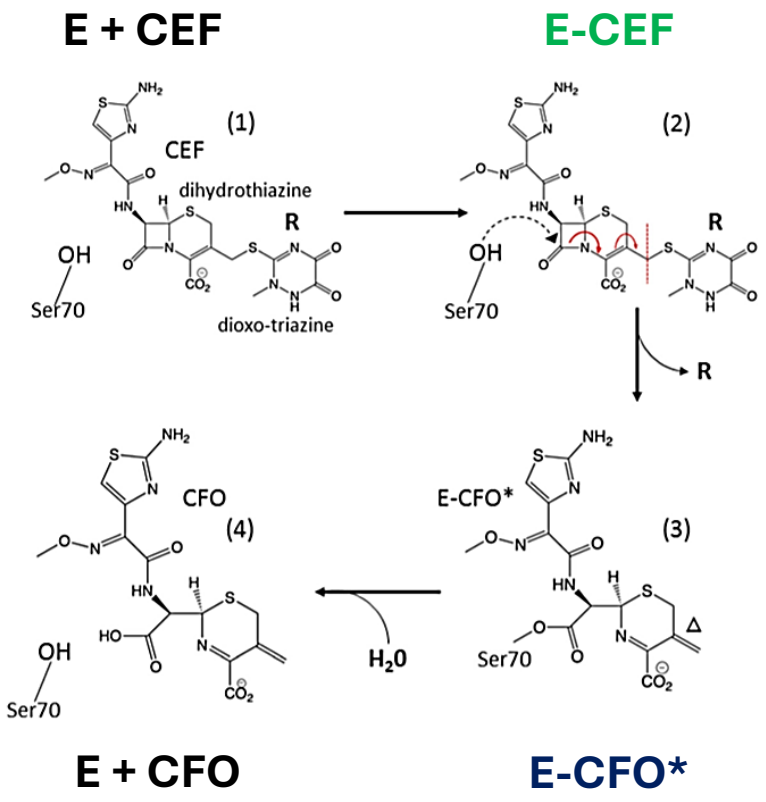
Time-resolved SFX enables function and dynamics

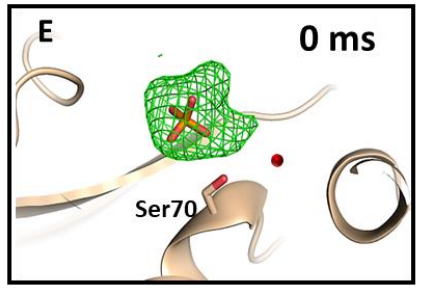


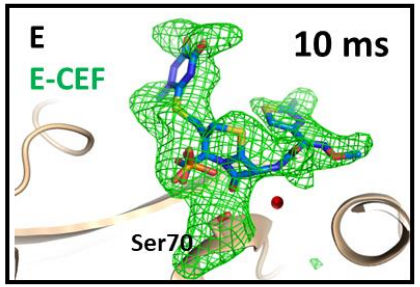


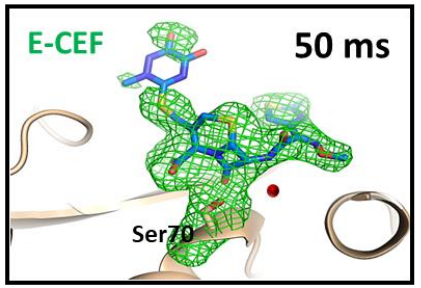
TR-SFX to watch the inactivation of an antibiotic *in real time*

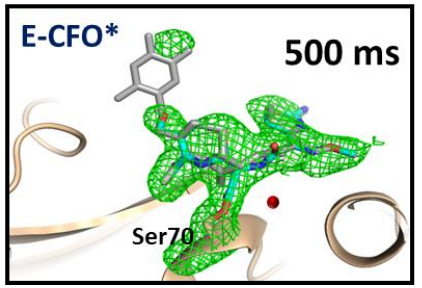
Ceftriaxone is a broad-spectrum cephalosporin antibiotic widely used to treat a variety of bacterial infections

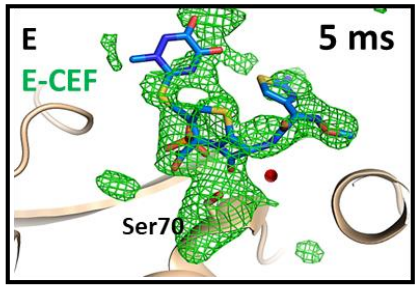


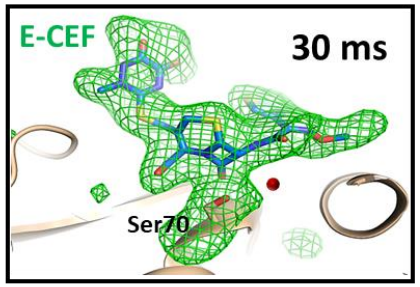


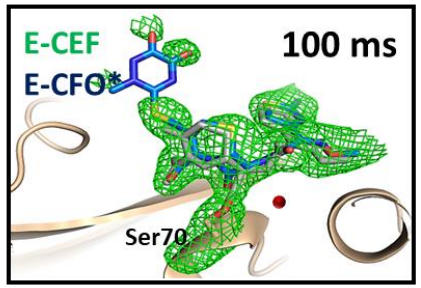




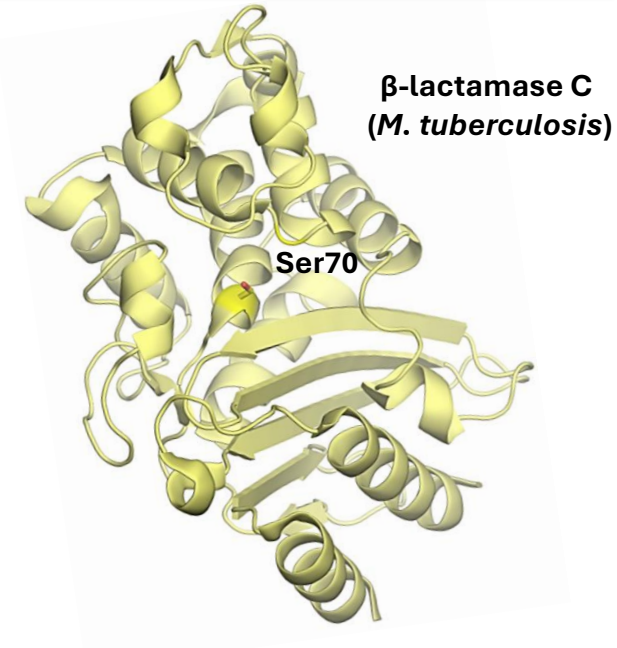


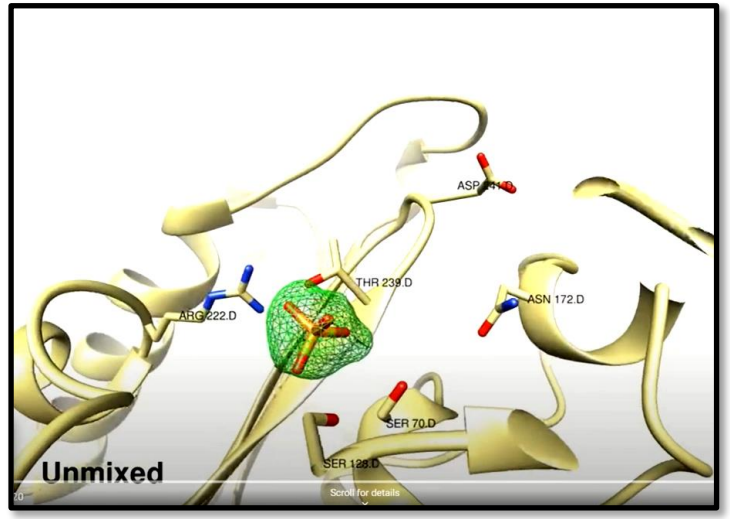






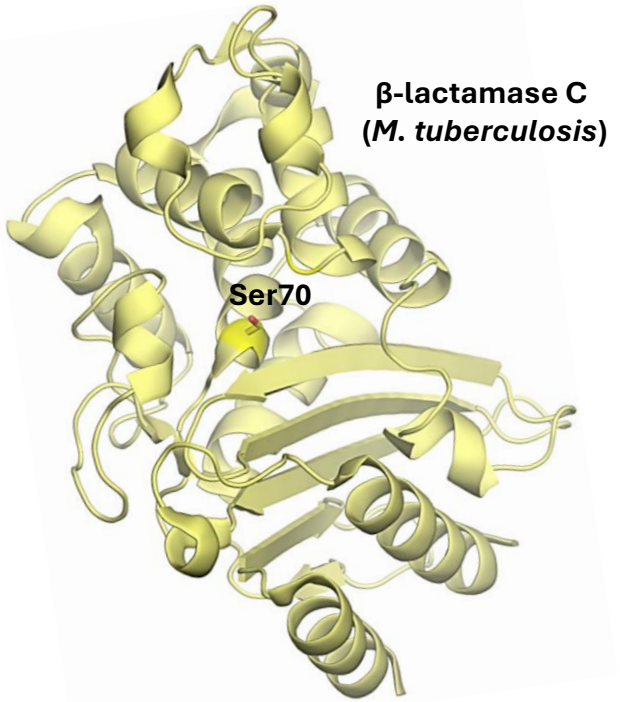
Inactivation of the antibiotic occurs between **100 and 500 ms**



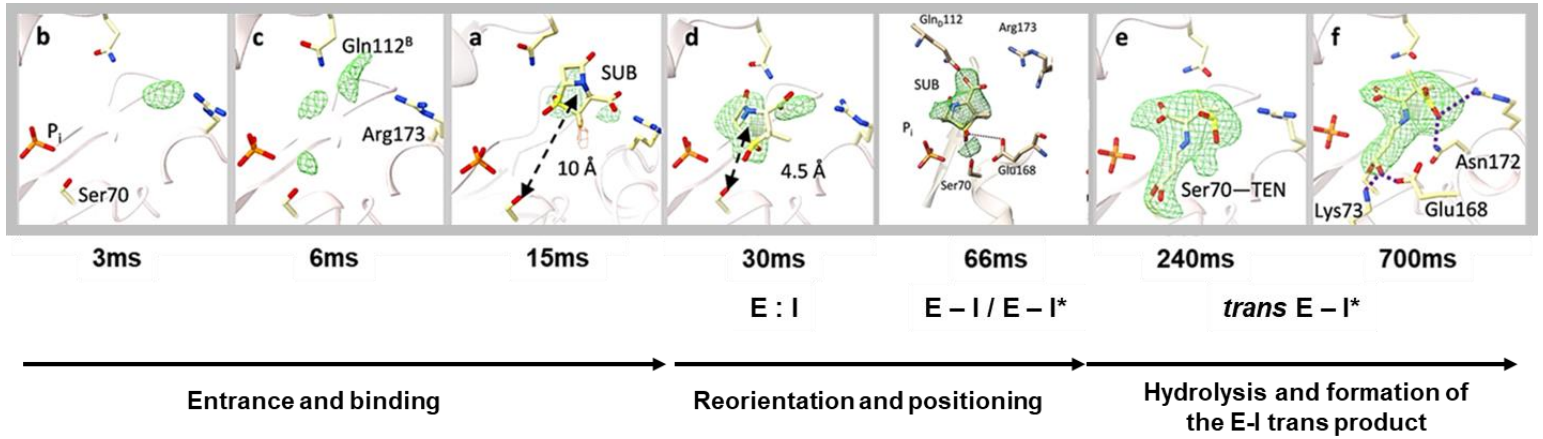


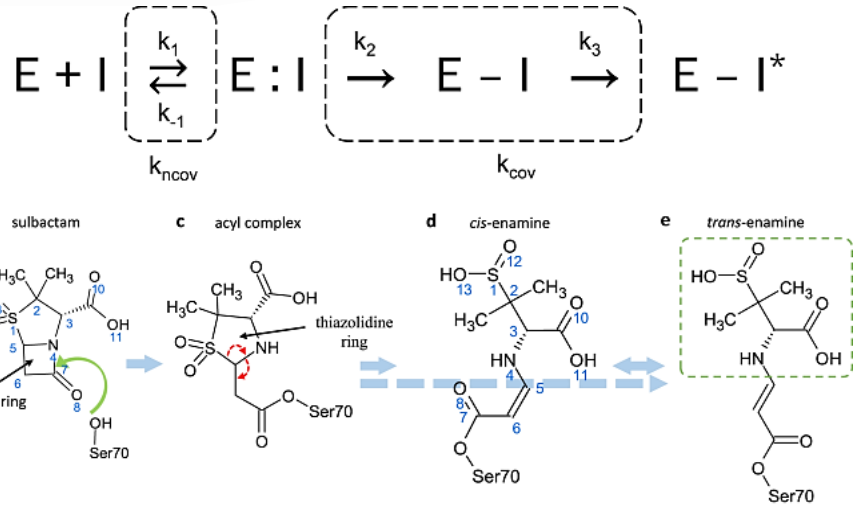
Kupitz C, et al. Structural Dynamics 2016
Olmos JL, et al. BMC Biology 2018
Suraj P, et al. IUCrJ 2021

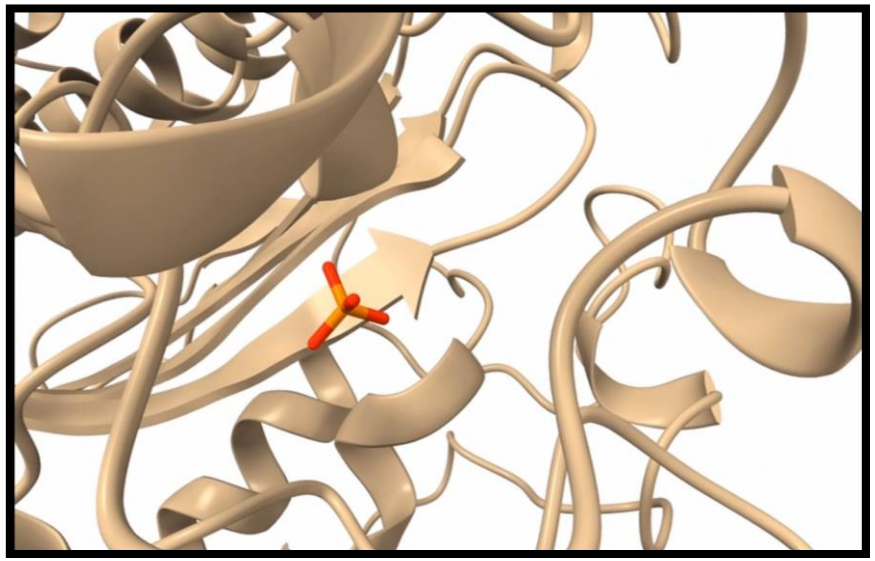
TR-SFX to watch the inactivation of an enzyme *in real time*



Sulbactam is a β-lactamase inhibitor commonly used in combination with β-lactam antibiotics to enhance their effectiveness against bacteria.



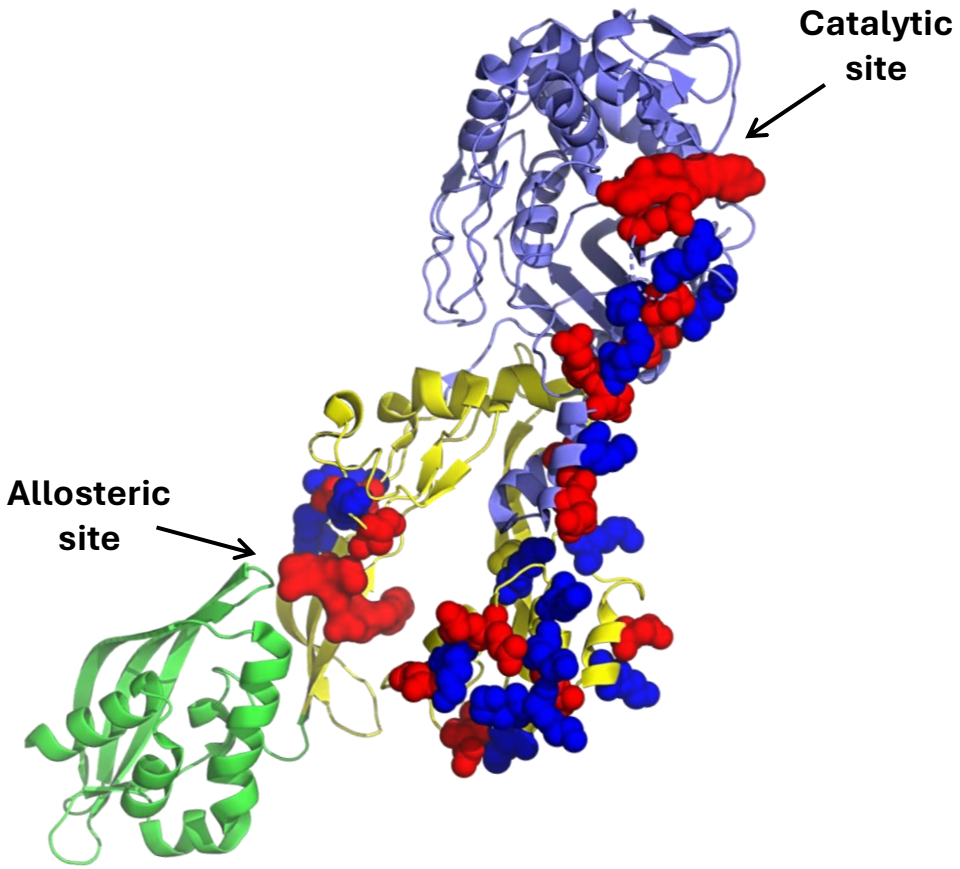




Inactivation of BlaC by SUB occurs **90 ms** upon reaction initiation

Pandey S, et al. IUCrJ 2021
Malla T.N, et al. Nat. Comm. 2023

The PBP2a of *Methicillin-resistance Staphylococcus aureus*



PBP2a + Ceftaroline (PDB 3ZFZ)

What differentiates PBP2a from other PBPs that makes it **resistant to beta-lactams**?

X-ray crystallography revealed the presence of an **allosteric site 60 Å** from the catalytic site

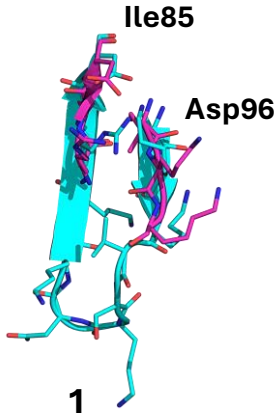
The **allosteric site modulates enzyme activity** through a complex network of salt bridges

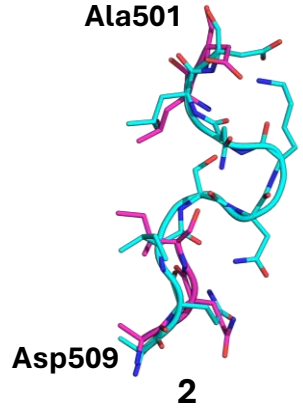
Otero et al. PNAS, 2013

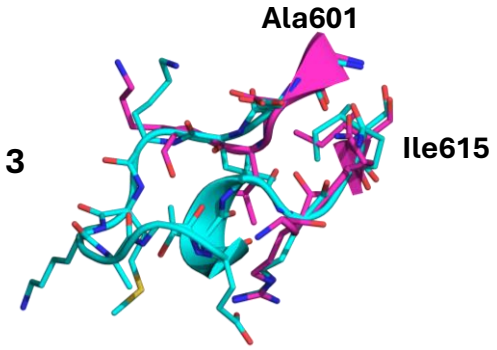
Structure of the apo PBP2a solved at room temperature

Alice Grieco

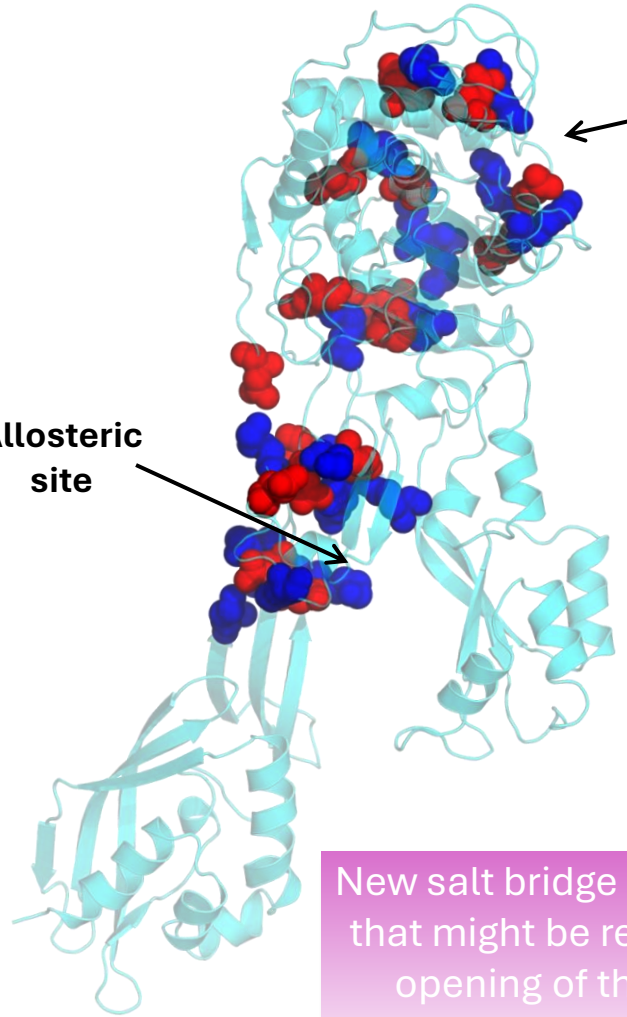
First room temperature PBP2a structure solved to 2.8 Å at XFELs







Three regions never seen before in the apo PBP2a have been modeled



New salt bridge network identified that might be responsible for the opening of the catalytic site

TR-SFX to understand the molecular basis of the allosteric regulatory mechanism of PBP2a to advance the development of allosteric antibiotics to combat MRSA infections.

Grieco A, et al. *Manuscript in preparation*

Acknowledgements

IQF-CSIC

Alice Grieco, PhD

Isabel Quereda-Moraleda, PhD

Celia Lozano Vallhonrat, Msc

Audrey Bivens, Internship

Arizona State University

Sabine Botha

UWM

Marius Schmidt

Suraj Pandey

Tek Malla

European XFEL

Richard Bean

Joachim Schulz

Katerina Dörner

LCLS (SLAC)

Leland Gee

Ray Sierra

Sebastien Boutet

Spain XFEL HUB

talento cm Programa de atracción de talento investigador Comunidad de Madrid

2019-T1/BMD-15552

2023-5A/BMD-28921

MINISTERIO DE CIENCIA E INNOVACIÓN

Financiado por la Unión Europea NextGenerationEU

Plan de Recuperación, Transformación y Resiliencia

AGENCIA ESTATAL DE INVESTIGACIÓN

CNS2022-135713