Molecular Dynamics Alterations in the TEM β-Lactamase Family Induced by Nanobody Binding

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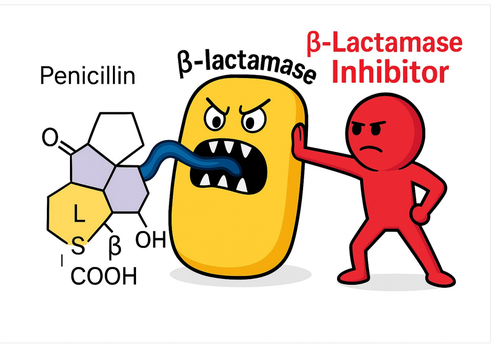
1. Introduction

Bacteria exposed to penicillin antibiotic may produce **penicillinase** (TEM family enzymes) that catalyses the irreversible hydrolysis of penicillin, providing bacterial resistance. The most frequently produced penicillinase is **TEM-1 enzyme** [1]. Camelidae **antibody (nanobody)** has been shown to inhibit TEM-1 enzyme [2].
As consequence of the large clinical usage of penicillin, bacteria have responded by developing improved resistance mechanism. In particular, a mutant of TEM-1 enzyme, TEM-121 enzyme has been identified in pathogen [3]. TEM-121 enzyme differs from TEM-1 enzyme by six amino acid substitutions that are not directly in the active site: Gln39Lys, Glu104Lys, Arg164Ser, Ala237Thr, Glu240Lys, and Arg244Ser.
Both TEM-1 and TEM-121 bind the same Camillidae antibody **cAb_{TEM-1}** and lead to inhibition but with different kinetics profiles.

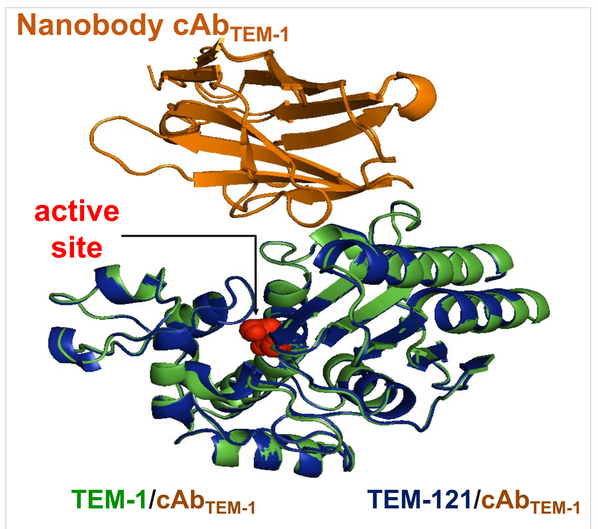


Penicillinase enzyme: bacterial weapons against **penicillin**

This work contributes to understand inhibition of TEM enzymes by the antibody cAb_{TEM-1}.
For this purpose, several research approaches were used:

- **Enzyme kinetics**
- **Crystallography**
- **AI protein's 3D structure prediction (AlphaFold 2)**
- **NMR relaxation**

2. Crystallography

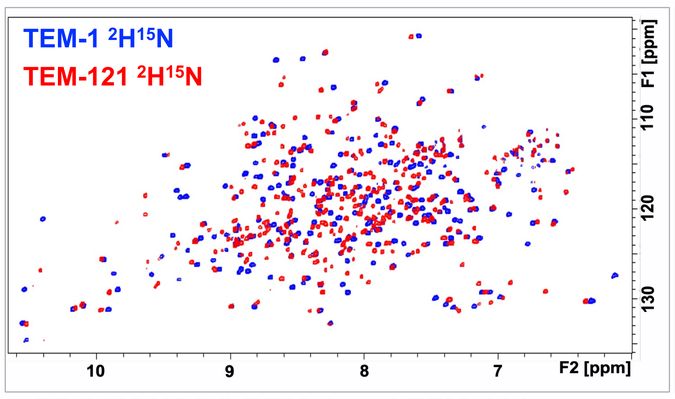


$TEM-1/cAb_{TEM-1}$ and **$TEM-121/cAb_{TEM-1}$** x-ray structures were determined by **Fréderic KERFF (CIP) and Fréderic CAWEZ (CIP)**

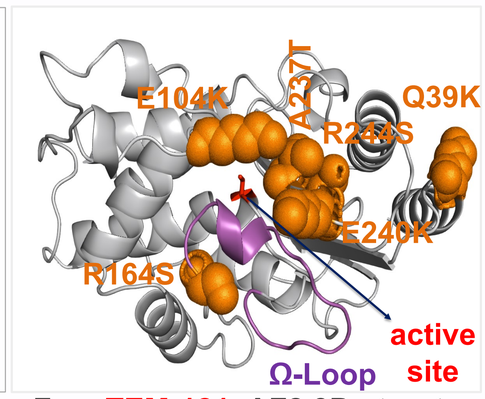
- **cAb_{TEM-1}** inhibits **TEM-1** and **TEM-121**
- **TEM-121** is a **TEM-1 mutant** :
  - **Gln39Lys**
  - **Glu104Lys**
  - **Arg164Ser**
  - **Ala237Thr**
  - **Glu240Lys**
  - **Arg244Ser**
- Both **$TEM-1/cAb_{TEM-1}$** and **$TEM-121/cAb_{TEM-1}$** 3D structures are very similar
- Antibody **does not bind** to **enzymes active sites**, but to the hinge region (residues 213-218) of enzymes **TEM-1** and **TEM-121**

How does inhibition occur ?

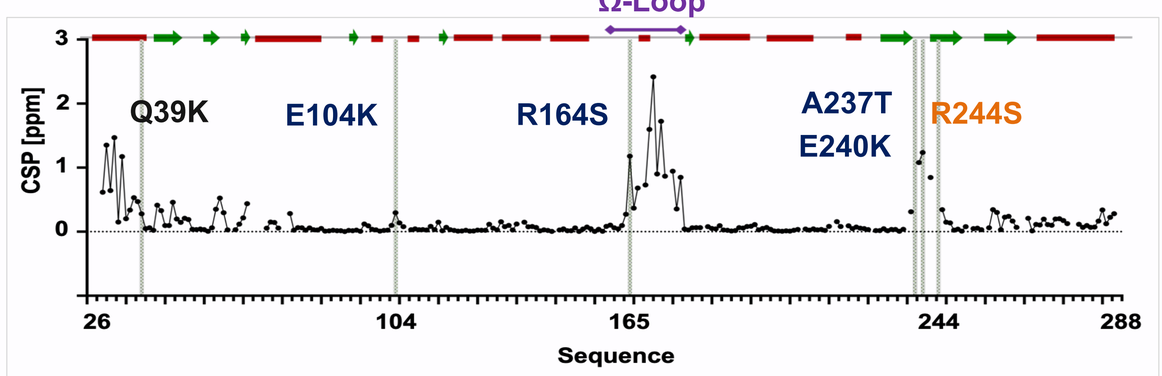
3. NMR Chemical Shifts Perturbations (CSP)



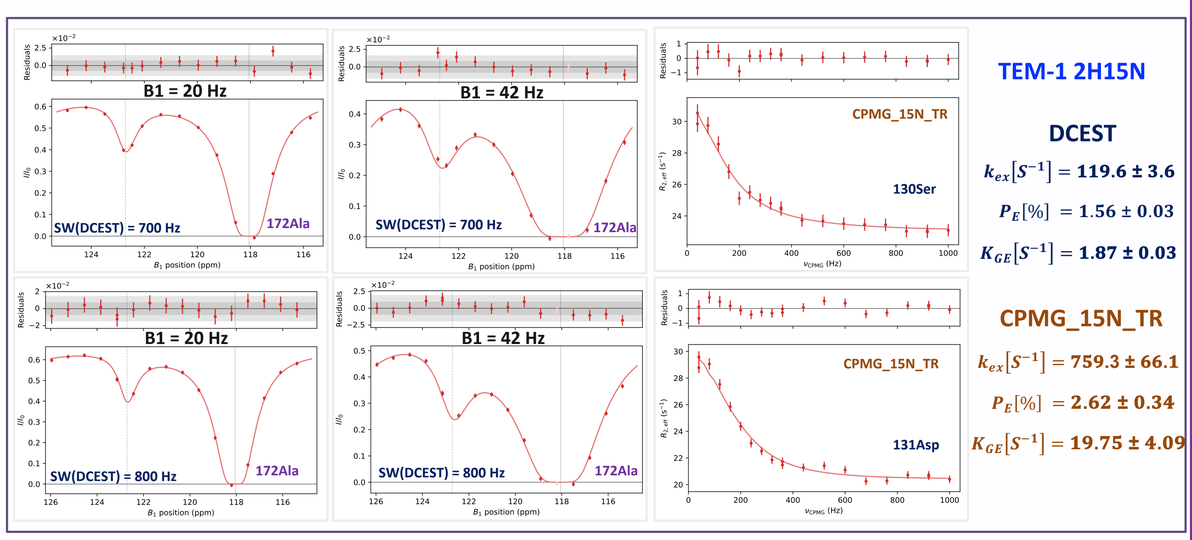
TROSY-HSQC TEM-1 and TEM-121 superposition



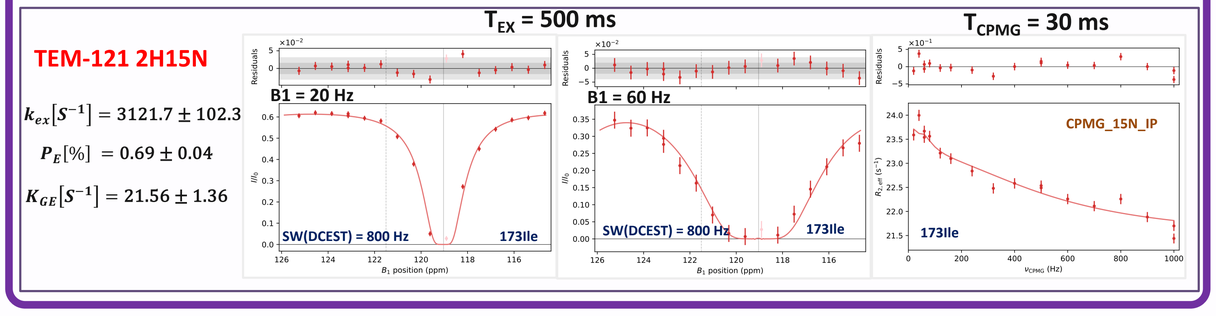
Free **TEM-121**. AF2 3D structure



4. NMR Relaxation at $us - ms$ time scale (1)

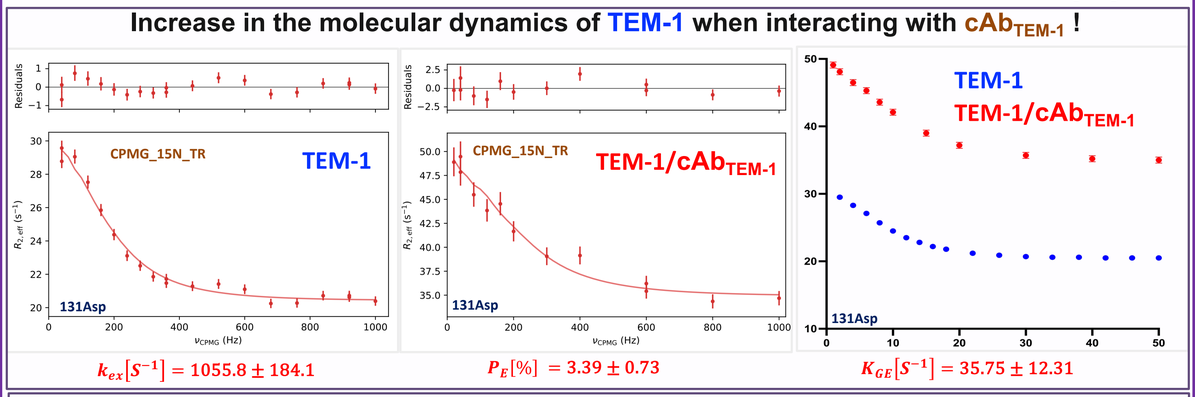


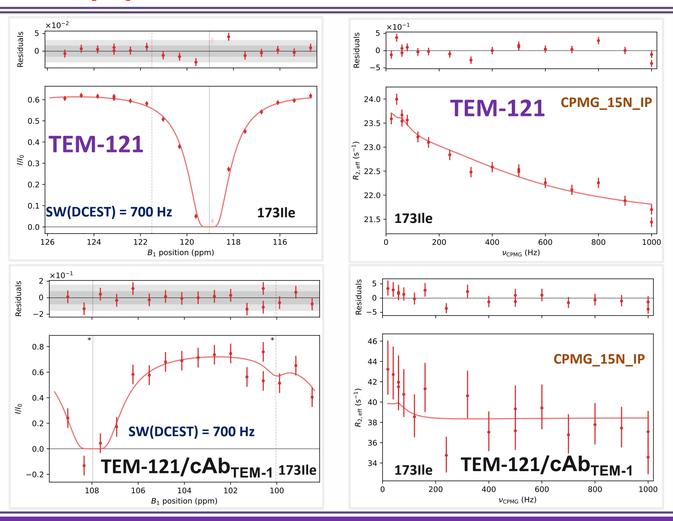
TEM-121 is more mobile than TEM-1 !



5. NMR Relaxation at $us - ms$ time scale (2)

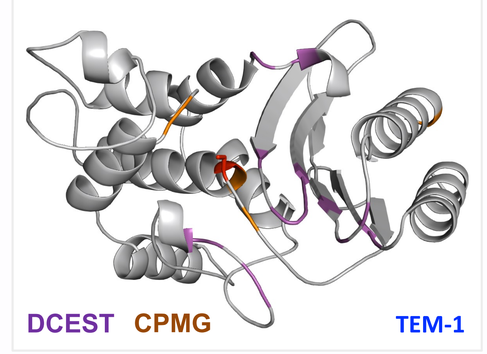
Increase in the molecular dynamics of TEM-1 when interacting with cAb_{TEM-1} !





TEM-121 undergoes global conformational stabilization upon binding to cAb_{TEM-1} !

6. Conclusions



- **TEM-121 is more mobile than TEM-1 !**
  - **This could explain the difference in kinetic profiles**
- **The binding of the nanobody (cAb_{TEM-1}) results in an increase in the molecular dynamics of TEM-1 !**
  - **This could explain the inhibition of TEM-1 by cAb_{TEM-1}.**
- **The binding of the nanobody (cAb_{TEM-1}) results in a decrease in the molecular dynamics of TEM-121 !**
  - **This could explain the inhibition of TEM-121 by cAb_{TEM-1}.**

Following these results, we propose that molecular dynamics perturbations of TEM-1 and TEM-121 enzymes upon nanobody binding are the underlying cause of their inhibition.

7. References

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