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Understanding the covalent inhibition of Clavulanate against β-lactamases (TEM-1 and KPC-2) with QM/MM screening methods.

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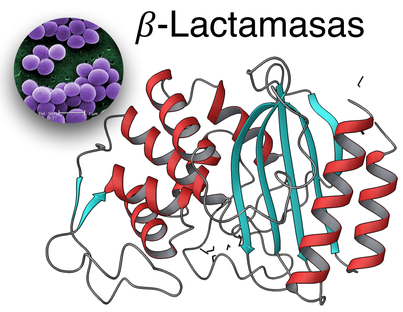
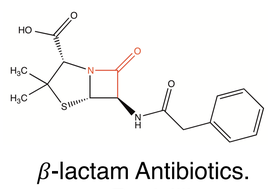
Abstract

β-lactamases are a primary cause of bacterial resistance to β-lactam antibiotics for many important human pathogens (particularly Gram-negative bacteria) [1]. Inhibitors of β-lactamase have been implemented as a dual therapy with antibiotics, but there are only four inhibitors clinically approved and resistance to these compounds is also rising [2]. For β-lactam inhibitors, after acylation, the opening of five-membered ring leads to the formation of a transient imine intermediate then it rearranges several times to form a trans or cis final enamine inhibition-products. Slow hydrolysis of this product by the enzyme leads to an inhibited β-lactamase [2]. A computational study of reaction mechanism for the first step on the deacylation of the inhibitor clavulanate with TEM-1 (inhibited) and KPC-2 (hydrolyzed) enzymes using QM/MM Umbrella Sampling with DFTB method is presented [3]. 2D free energies surfaces for the reactions were calculated using the weighted histogram analysis method (WHAM) and the minimum energy path (MEP) was identified; where the highest point along the MEP is taken as the transition state giving the activation free energy "ΔG_{calc}".Our results show that TEM-1 and KPC-2 have an approximate 5 kcal/mol difference in ΔG_{calc}. Such results are in good agreement with inhibition experimental data for two enzymes in which KPC-2 is less inhibited by clavulanate than TEM-1. We hope our methodology can assist the design and development of covalent inhibitors through a computational screening of inhibitory activity of other molecules.

Introduction





β-lactam Antibiotics. Penicillin.

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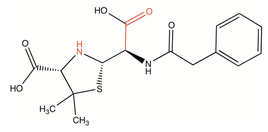
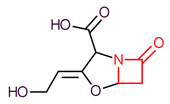


Inactivated Penicillin

Antibiotic Resistance



β-lactamico Inhibitor. Clavulanate (CLA).

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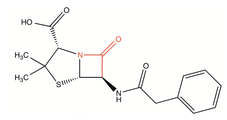
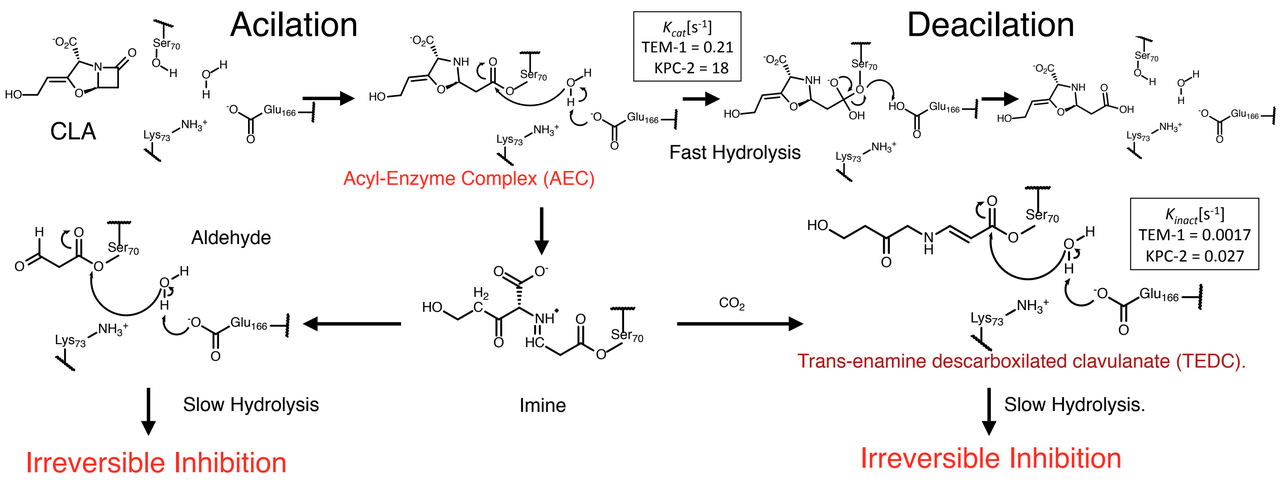
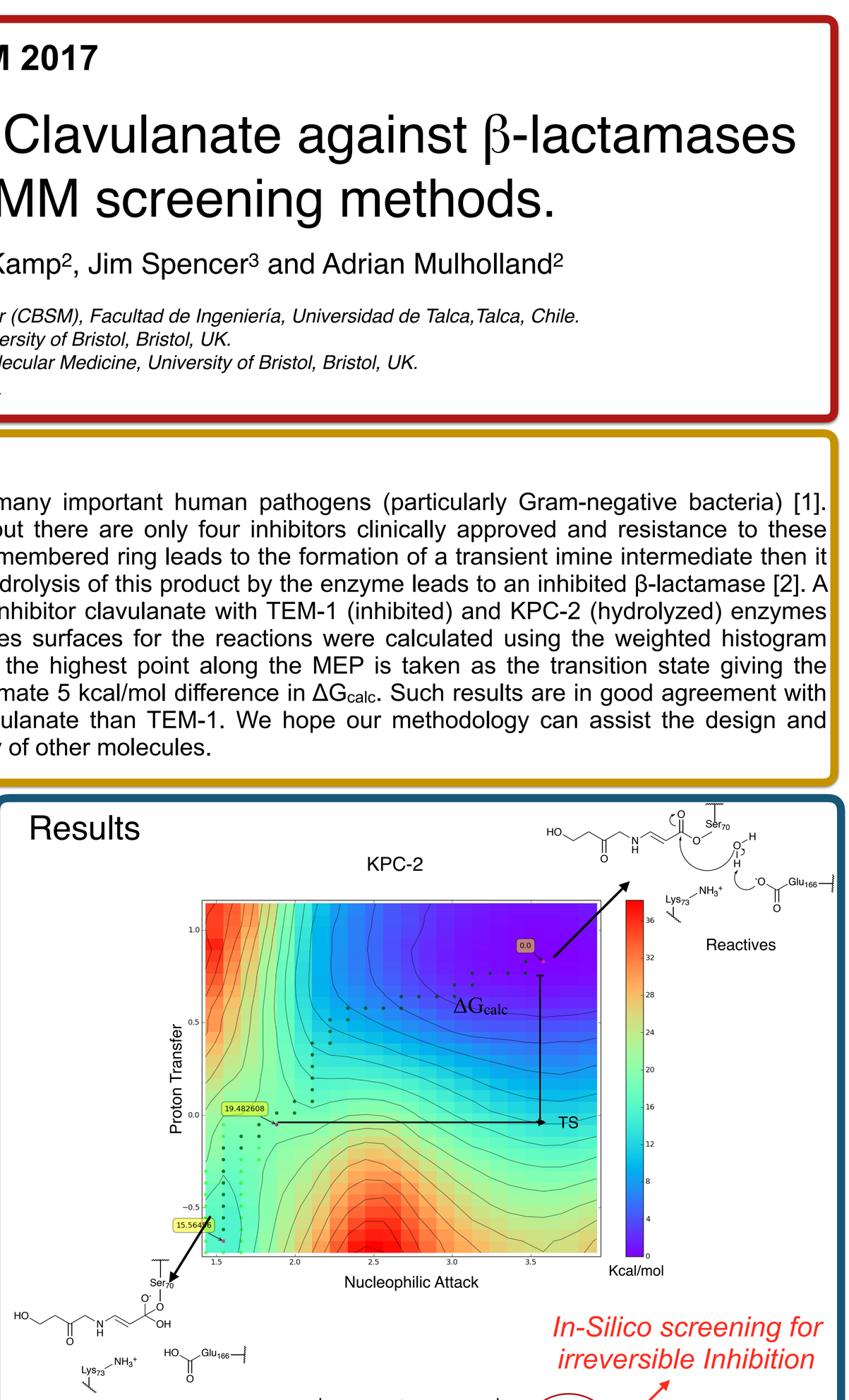


Penicillin



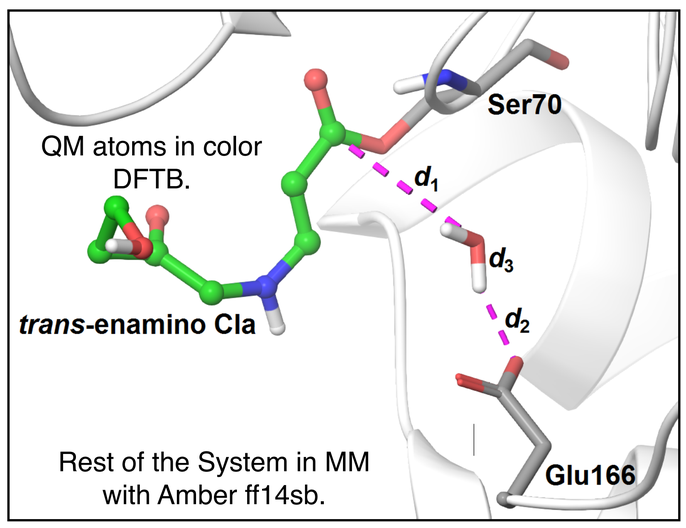
Results



In-Silico screening for irreversible Inhibition

β-lactamase	K_{inact}(s^{-1})	ΔG_{exp} (kcal/mol)	ΔG_{calc} (kcal/mol)
KPC-2	0.027	19.72	18.76 ± 0.6
TEM-1	0.0017	21.36	23.85 ± 1.25
ΔΔG		-1.65	-5.08

Methodology



QM/MM MD Umbrella Sampling.

1) Complexes model creation (KPC-2 y TEM-1).
2) Adduct parametrization.
3) Molecular Dynamics (MD).
4) QM/MM MD, 6 replicas of 50 ps.
5) *US* of reaction coordinates.
   5.1) Distance.
      d_1 = CC(Ser70) - O(DW).
      d_2 = O(Glu166)–H(DW) .
      d_3 = H(DW)– O(DW).
   5.2) Reaction coordinates definition.
      R_x = (d_2 - d_3). Proton Transfer.
      R_y = d_1. Nucleophilic Attack.
   5.3) Initial coordinates for Minimun Energy Path from (MEP) of Meropenem.
   5.4) 2ps QM/MM MD simulation per windows, 0.1 Å .
   5.5) Scan of the surface:
      R_y from 1.4 to4.0 Å.
      R_x from -0.8 to 1.1 Å.
   5.6) 3 Umbrella Sampling QM/MM MD replicas.
6) Free Energy Estimation.
   1) Potential of Mean Force (PMF) with WHAM.
   2) Estimation of Transition State (TS) and "ΔG_{calc}".

Conclusiones.

- The activation free energies calculated for TEDC with TEM-1 and KPC-2 are in the same magnitud order as the experimental values for the inactivation constant.
- These computational methodology aloud us to discriminate the inhibitory activity of CLA against KPC-2 and TEM-1 β-lactamase.

Perspectives.

- Calculate others covalent complexes during CLA inhibition.
- These protocol could potentially be use as a *In-silico* screening for inhibitory activity of covalent inhibitors.
- Expansion to others protein-ligands systems of medical interest.

Acknowledgments.

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