# QM/MM Molecular Dynamics of Polycaprolactone enzymatic synthesis

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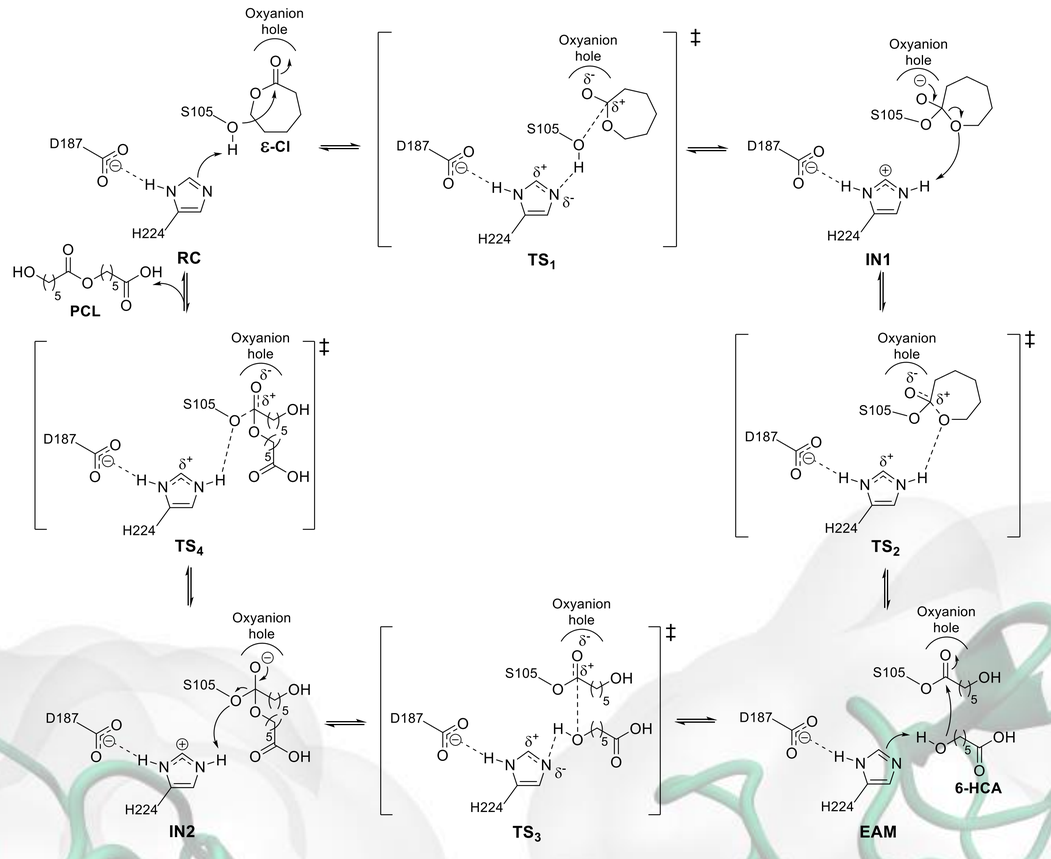
## Abstract

Bioresorbable polymers have gained much attention in the biomedical field, since these materials are non-toxic, biodegradable and biocompatible.[1] Poly(ε-caprolactone) is one of those polymers that, its slow degradation rate has been exploited for tissue engineering, drug-delivery systems and scaffold technologies.[2] The enzyme *Candida antarctica* lipase B (CalB) efficiently catalyzes the ring-opening polymerization (ROP) of ε-caprolactone to products suitable for biomedical applications.[3] Here, we investigated the catalytic cycle for ROP of ε-caprolactone using Quantum Mechanics/Molecular Mechanics Molecular Dynamics (QM/MM MD) calculations.[4]

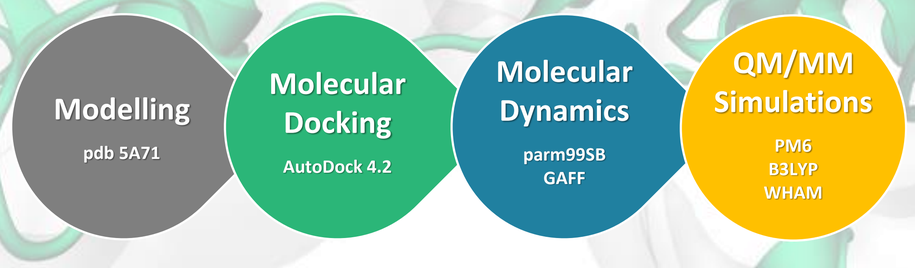
## Catalytic Cycle




**Scheme 1.** Enzymatic mechanism for the biosynthesis of **PCL** model compound by CalB.

CalB, which contains the well described catalytic triad S105, H224 and D187[5] is able to catalyze the synthesis of poly(ε-caprolactone) (**PCL**).[3] The backbone amides of residues T40 and Q106 and the side-chain hydroxyl of T40 stabilize the negative charge developed in the tetrahedral intermediates (**INT-1** and **INT-2**), which are formed by nucleophilic attack by S105 in ε-caprolactone (**ε-Cl**) and a molecule of 6-hydroxycaproic acid (**6-HCA)** to the **EAM**. After product release, the enzyme is ready for another turnover to increase **PCL** polymer chain.

## Workflow




## Discussion

The reaction starts with a nucleophilic attack by the S105 side-chain to the carbonyl group of **ε-Cl** forming **INT-1** which, after ring-opening assisted by H224 (Fig 1), generates the **EAM** structure (Fig 2). The $\mathbf{TS_1}$ is 6.0 ± 0.1 kcal/mol above the reactant complex and the $\mathbf{TS_2}$ overall $\Delta G^{\ddagger}$ is 9.5 kcal/mol (with the PMF corrected with B3LYP).

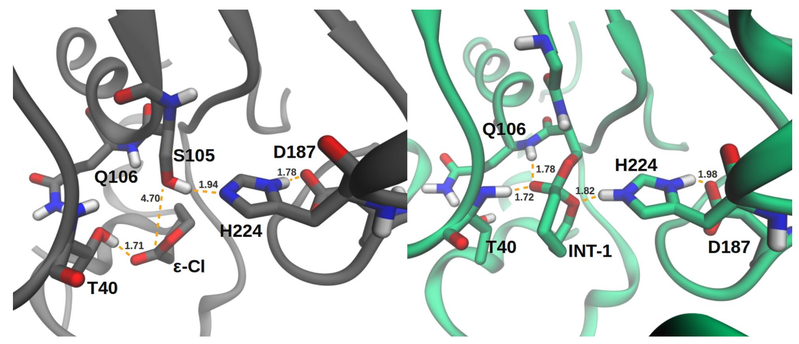



**Fig 1.** Representation of the enzyme active site **RC** with **ε-Cl** (grey) and the **INT-1** complex (dark green).

The alcohol moiety of 6-hydroxycaproic acid **6-HCA** performs a nucleophilic attack to the **EAM** structure (Fig 2). The $\mathbf{TS_3}$ has a free energy barrier of 7.7 ± 0.2 kcal/mol, leading to the formation of the second intermediate structure (**INT-2**, Fig 2).

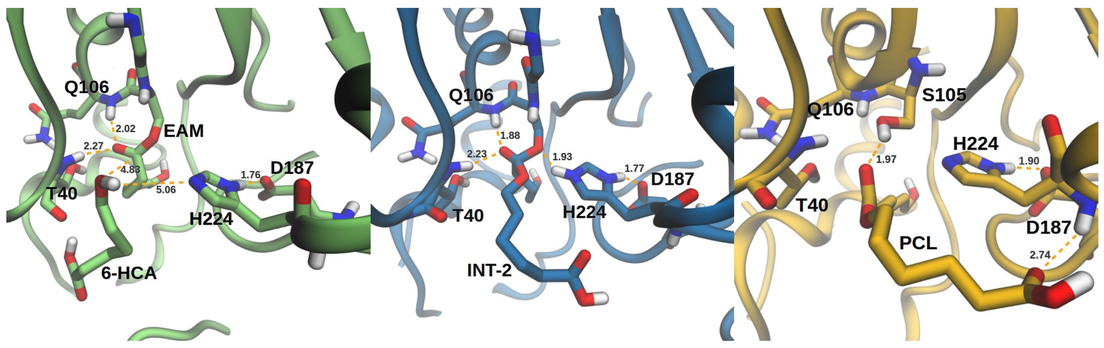



**Fig 2.** Representation of the **EAM** complex with **6-HCA** (light green), **INT-2** complex (blue) and **RC** with **PCL** model compound (dark yellow).

The **INT-2** complex evolves to the release of a **PCL** model compound molecule ($\Delta G^{\ddagger}$ of 4.1 kcal/mol, Fig 2), regenerating the free enzyme **RC** (Fig 1).

We were able to fully characterise the catalytic mechanism for this enzyme and for a **PCL** model compound synthesis.[6] Our results show that the rate-determining step point out to the acylation step and corresponds to the formation of the **EAM** structure, which is in good agreement with the experimental data.[5]

## Final remarks

Poly(ε-caprolactone) has collected much attention among other biodegradable polymers to be applied in tissue engineering, drug-delivery systems and scaffold technologies due to its biodegradability, biocompatibility and tailorability. Our QM/MM MD calculations for the production of **PCL** with this enzyme suggest that the rate-determining step is the formation of the **EAM** structure with an overall free energy of 9.5 kcal/mol. We have compared this mechanism with the one for the thermophilic carboxylesterase AfEST and found that the orientation of the Histidine/Aspartate residues in the **INT-1** and connected transition states ($\mathbf{TS_1}$ and $\mathbf{TS_2}$) differs between both enzymes, providing an explanation for the different catalytic efficiency for this reaction.[6,7]

## References

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## Acknowledgements

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