# An unusual His/Asp acid/base dyad in glycosidase catalysis. Insight from QM/MM MD simulations.

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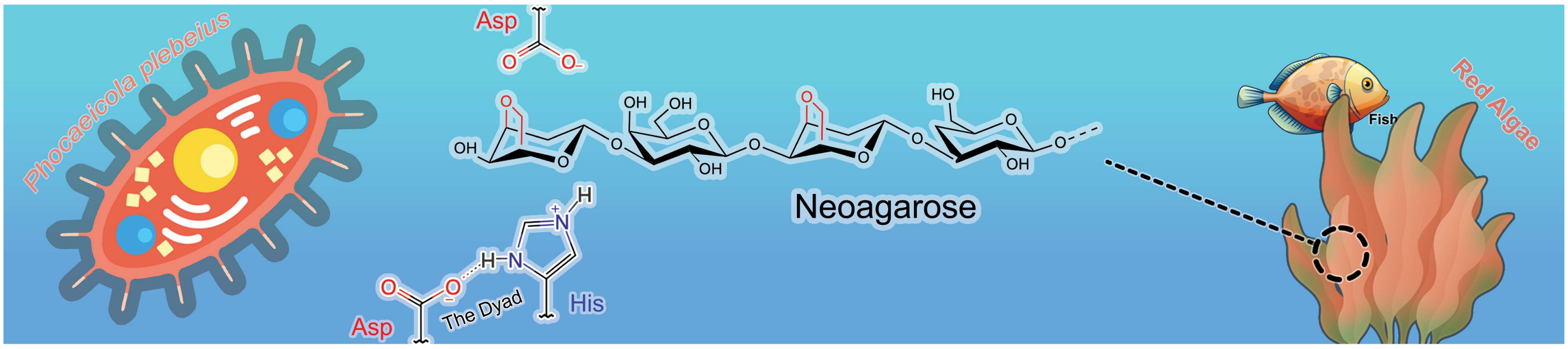
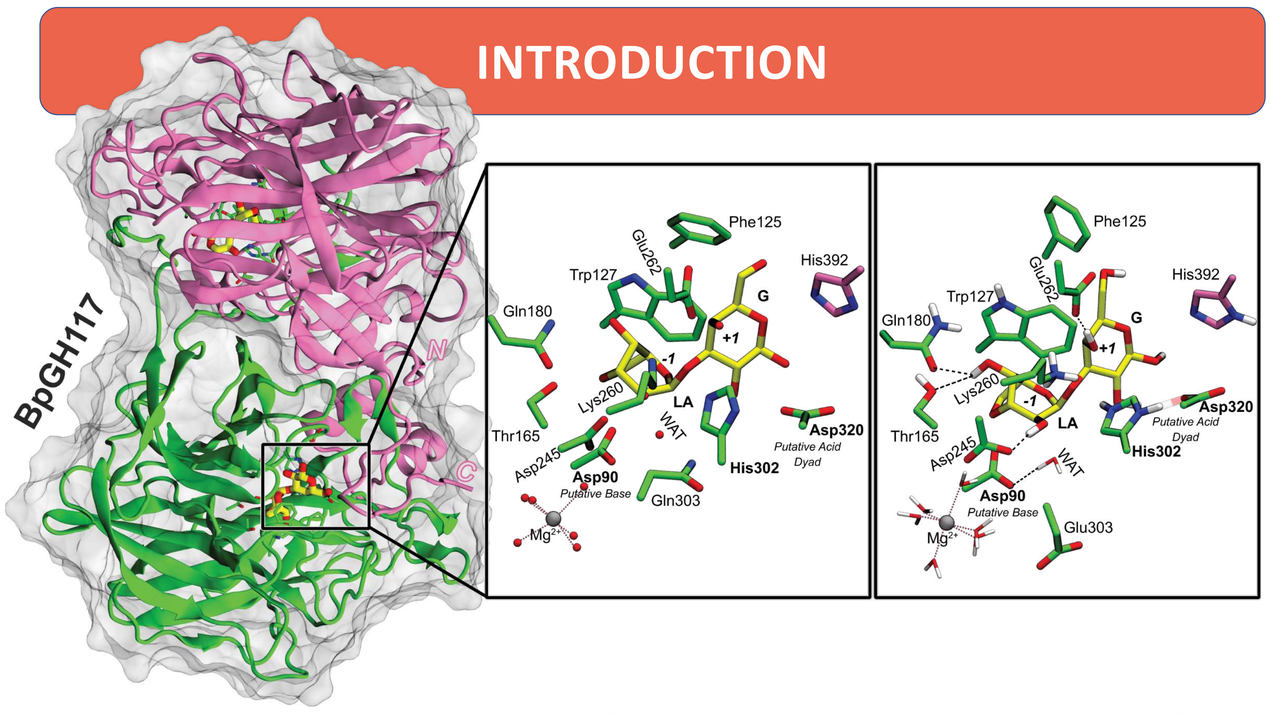



## INTRODUCTION



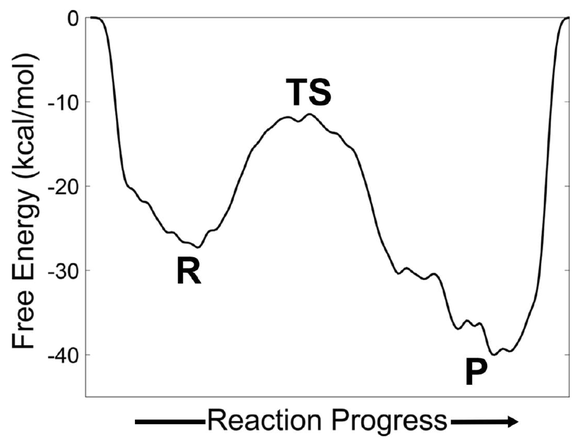

**Agarose** is a linear polysaccharide commonly found in agarophyte red algae. It is composed of longer chains of alternating α-1,3-linked bicyclic 3,6-anhydro-L-galactose (**LA**) and β-1,4-linked D-galactose (**G**) units (also known as **neoagarobiose**). The **bridged structure of LA** residues in agarose allows for the formation of organized helical structures, resulting in the creation of a high-strength gels. These flexible gels are resistant to degradation, making them useful for a variety of applications, including microbiological, molecular biological, and food-related uses.(1) **The enzyme 3,6-anhydro-α-(1,3)-L-galactosidase**,(2) **GH117**(3) an enzyme of *Phocaeicola plebeius*, a bacterium in the human gut microbiota.(4) This exo-acting enzyme removes the **LA** residue from the non-reducing end of neoagaro-oligosaccharides, an important step in the **degradation and utilization of agarose**.(5) Structural analysis suggests that **Asp90 and His302** may function as a **general base and general acid**, respectively, in the catalytic reaction. This is unusual as only two other GHs have been identified to employ a His residue as the general acid.

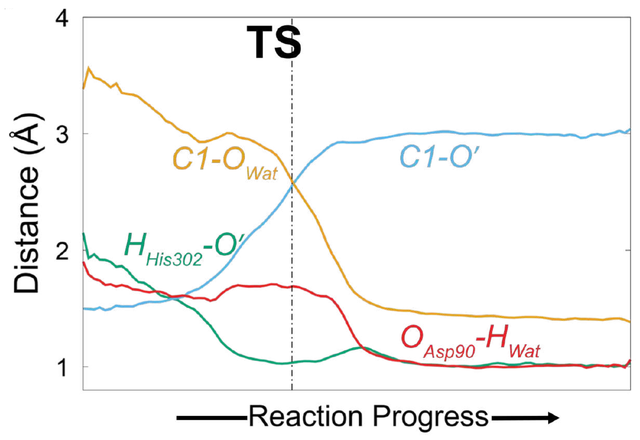
## METHODS

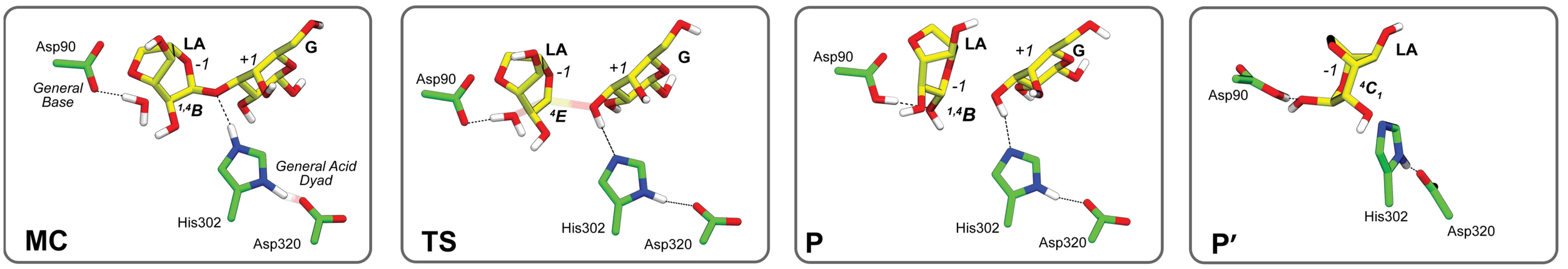
We mainly used **QM**(DFT)/**MM Metadynamics** simulations to investigate the mechanistic details in the active site. The Cremer-Pople **Puckering** collective variables (CV) were used to identify the available conformations of the *-1* sugar (LA). Then, a single CV was set to explore the reaction mechanism, linearly combining all the main bond distances expected to be broken or formed during the reaction.

## RESULTS

We identified two possible conformers in the active site, $^{1,4}B$ **and** $^{4}C_{1}$. Notably, the **boat** conformation exhibits the L-galactoside leaving group in an axial orientation and slightly lower energy minimum. The reaction free energy profile obtained from the simulation displays an exergonic reaction, with an **energy barrier of 15.8 kcal·mol$^{-1}$**. These results are in very good agreement with the energy barrier estimated from experimental kinetic data (15.60 kcal·mol$^{-1}$ and 15.89 kcal·mol$^{-1}$, for two GH117 enzymes from *Cellvibrio sp.*).(24) This indicates that the reaction involving **Asp90 and His302** as **catalytic base and acid**, respectively, is feasible.




## CONCLUSIONS

The mechanism of *Bp*GH117 involves an **aspartate-stabilized histidine residue** as **catalytic acid**, together with **an aspartate** playing the role of **general base**. The reaction follows an $S_N2$ mechanism and involves an oxocarbenium ion-like transition state in which the LA sugar adopts a $^{4}E$ conformation, following a $^{1,4}B \rightarrow [^{4}E]^{\ddagger} \rightarrow [^{1,4}B]$ catalytic itinerary. **His302 works in tandem with Asp320**, relays a proton from one end to the other, as needed during the chemical reaction. Therefore, the results obtained for *Bp*GH117 neoagarobiosidase can probably be extended to other His-Asp dyad GHs that are also exo-acting GHs, although differing in structure and substrate specificity. These previous experimental investigations together with the present computational results highlight distinct strategies used by GHs to catalyse the hydrolysis of the glycosidic bond.

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