










An *In silico* Analysis of Enzyme-Substrate Interaction in α -L-Fucosidases Belonging to the GH29 Family

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Abstract: Human milk is the ideal food for newborns. In addition, thanks to the fucooligosaccharides it contains, it provides protection against gastrointestinal diseases. These oligosaccharides can be synthesized enzymatically using α -L-fucosidases. The aim of this work was to compare the active site of three fucosidases of the GH29 family with *in silico* methods: *Thermotoga maritima*, *Lactobacillus casei*, and *Bifidobacterium longum* subsp. *infantis* to understand the interaction occurring in the enzyme's active site. In addition, the interaction with the substrate was also studied through molecular docking with *p*NP-fucose, a substrate used as a fucosyl donor to synthesize fucooligosaccharides. These *in silico* analyzes showed that the differences in the sequence between fucosidases of the GH29 family are associated with structural characteristics that intervene in the interaction of the substrate in the catalytic site, finding that the affinity of the *T. maritima* fucosidase and the *p*NP-fucose is the largest among the enzymes evaluated in this work, coinciding with that reported by *in vitro* methods.

Keywords: human milk oligosaccharides; fucosidase; glycosyl hydrolase; GH29; molecular docking.

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1. Introduction

Human milk (HM) is the best food for newborns. In addition to its nutritional contribution, it has other benefits, such as protection against gastrointestinal diseases and reducing the risk of obesity, diabetes, and atopic dermatitis, among others [1, 2]. Some of these benefits are provided by human milk oligosaccharides (HMO), which are formed by structures with a lactose nucleus, to which molecules of fucose, glucose, galactose, N-acetylglucosamine, and sialic acid are attached, forming structures of up to 25 units, of which approximately 200 different units have been structurally characterized [3, 4]. Several methods of HMO synthesis have been reported, such as synthetic chemical, enzymatic and recombinant microorganisms, with the purpose of including them in infant formulas made from animal milk because they do not contain oligosaccharides with N-acetylglucosamine, fucose, and many with sialic acid [5]. Two types of enzymes are used in enzymatic synthesis, glycosyltransferases, which catalyze the transfer of a glycosyl group to an acceptor substrate, and glycosyl hydrolases, which hydrolyze oligosaccharides, although they can synthesize oligosaccharides by controlling the

reaction conditions. Fucosylated HMOs are among the most abundant, so enzymes with activity on fucose are among the most sought-after to synthesize them [6], such as fucosyltransferases, which have high specificity and require nucleic acid-activated fucosyl donors that generally have a high cost, while fucosyl hydrolases require less expensive donors, with the disadvantage that the reaction products are also substrates for the hydrolysis reaction. The glycosyl hydrolases with α -L-fucosidase activity belong to the GH29, GH95, GH141, and GH151 families, of which those of the GH29 family have been used for the synthesis of fucooligosaccharides (FUCOS) since they can carry out the transfer of the fucosyl group preserving the position of the α -1 bond of fucose, while with those of other families the same capacity to carry out transfucosylation has not been reported [7].

The reaction mechanism of the GH29 family fucosidases can be seen in Figure 1. The reaction consists of type 1 nucleophilic substitution, carried out by a nucleophilic residue, which is an aspartic acid (ASP), and a general acid-base, which is generally a glutamic acid (GLU), although another ASP can also perform this function. In the first stage, there is an exchange of electrons between the catalytic residues and the fucosyl donor, through which the aspartate achieves the appropriate charge to carry out a nucleophilic attack on the carbon 1 (C1) of the fucose, hydrolyzing the glycosidic bond, releasing the R group of the fucosyl donor and forming the enzyme-substrate complex between ASP and fucose. Later, in the second stage, the fucosyl acceptor is deprotonated by the general acid-base, activating it to carry out the nucleophilic attack on the substrate enzyme complex [ES], transferring the fucose to the acceptor substrate and restoring the charge of the residue that acts as a nucleophile. When a water molecule performs the nucleophilic attack on the enzyme-substrate complex, a hydrolysis reaction is carried out, obtaining a free fucose molecule. Whereas when another molecule, such as lactose carry out this nucleophilic attack, a synthesis reaction is carried out in which a new oligosaccharide is produced, which is also prone to be hydrolyzed by the enzyme [8].

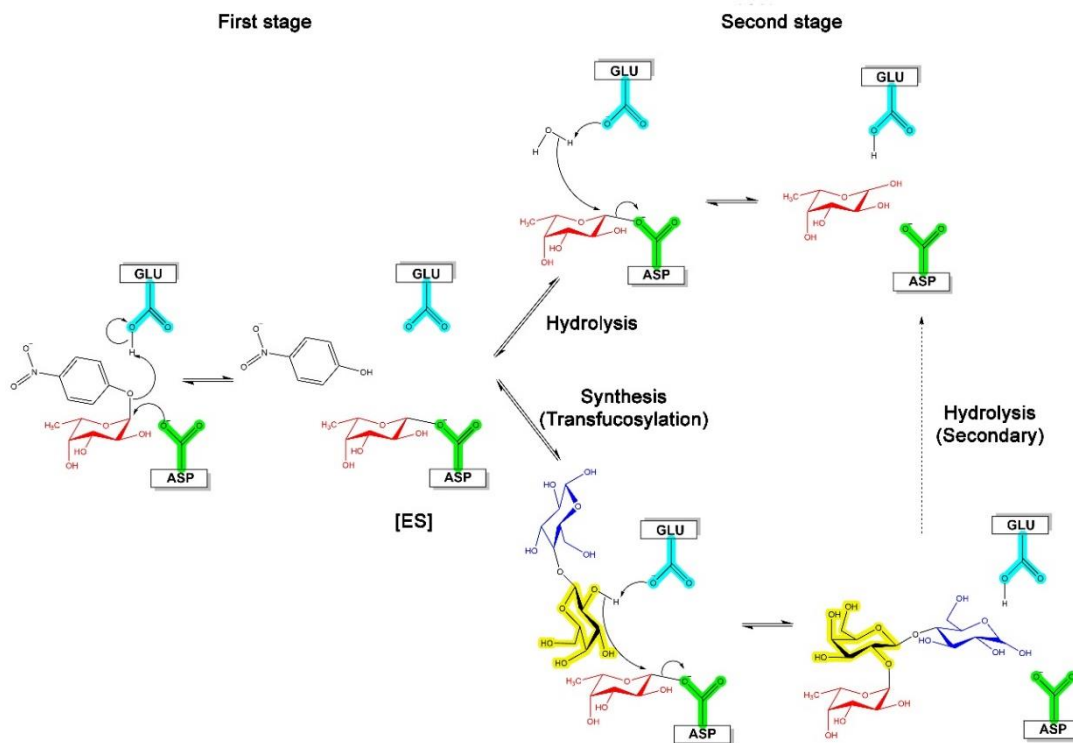


Figure 1. Reaction mechanism of the GH29 family α -L-fucosidase. Fucose is represented in red, glucose in blue, and galactose in yellow.

Among the fucosidases of the GH29 family that has been used for the synthesis of FUCOS, there are *TmFuc* from *Thermotoga maritima* [9], which is found in a hexamer assembly and *AlfC* from *Lactobacillus casei* [10], which is presented in a tetramer assembly, both belonging to the GH29A subfamily, as well as *BiAfcB* from *Bifidobacterium longum* subsp. *infantis* [11] with a dimer assembly belonging to the GH29B subfamily. In order to learn more about the differences between the fucosidases of the GH29 family, in this article, a multi-alignment was performed between the fucosidases *TmFuc* and *AlfC* of the GH29A subfamily and *BiAfcB* of the GH29B subfamily. In addition, the interaction of pNP-Fuc in the three enzymes and its correlation with enzymatic activity was evaluated by means of molecular docking.

2. Materials and Methods

2.1. Crystallographic structures and ligands.

The crystallographic structures of the α -L-fucosidases shown in Table 1 were recovered from the Protein Data Bank database (PDB) [12]. Apoenzyme structures were selected and in complex with fucose or a fucose-like molecule. All models were prepared with *UCSF Chimera* (Version 1.15) [13], conserving only the protein chain A (if there was more than one), and solvent molecules were removed. The missing hydrogens were added to the crystallographic structures according to the pKa of the amino acids at pH 7 in the *PlayMolecule* server using the *ProteinPrepare* tool [14].

The pNP-Fuc (CID: 82473) molecule was obtained from the PubChem database, which was optimized with the *Avogadro* software (Version 1.2.0) [15] with an MMFF94 force field, 1000 steps of the *steepest descent* algorithm, with a convergence of $10e-7$.

Table 1. Crystallographic structures used.

Source organism	Enzyme	Subfamily	PDB ID	Substrate*	Resolution	References
<i>Thermotoga maritima</i>	<i>TmFuc</i>	GH29A	2ZWY	w/o	2.75 Å	[16]
<i>Thermotoga maritima</i>	<i>TmFuc</i>	GH29A	1ODU	Fucose	2.80 Å	[17]
<i>Lactobacillus casei</i>	<i>AlfC</i>	GH29A	6O18	w/o	2.55 Å	[18]
<i>Lactobacillus casei</i>	<i>AlfC</i>	GH29A	6O1A	Fucose	2.60 Å	[18]
<i>Bifidobacterium longum</i> subsp. <i>infantis</i>	<i>BiAfcB</i>	GH29B	3MO4	w/o	1.90 Å	[19]
<i>Bifidobacterium longum</i> subsp. <i>infantis</i>	<i>BiAfcB</i>	GH29B	3UES	Deoxyfucojirimycin	1.60 Å	[20]

* Substrate crystallized with the enzyme; PDB ID: identifier in Protein Data Bank; w/o: no substrate.

2.2. Comparison of fucosidases of the GH29A and GH29B subfamilies.

The crystallographic structures of enzymes without substrate *TmFuc* (2ZWY), *AlfC* (6O18), and *BiAfcB* (3MO4) were superimposed using the MatchMaker tool of the UCSF Chimera software, taking the 2ZWY crystal as a reference. The best alignment was determined based on the standard deviation from the alpha carbons for each residue between the reference crystallographic structure and the other structures (RMSD-ca), using the Needleman-Wunsch algorithm and the BLOSUM-62 matrix, with a multi-alignment between the sequences based on the structure after the overlap. Additionally, a search for other homologous α -L-fucosidases

was carried out, with a percentage of identity with TmFuc greater than 20%, with the local basic alignment search tool for proteins (BLAST®P) [21] of the National Center for Biotechnology Information (NCBI). Images of crystallographic structures were generated with Pymol (The PyMOL Molecular Graphics System, Version 2.5.0, Schrödinger, LLC).

2.3. Molecular docking of TmFuc, AlfC, and BiafcB fucosidases with the fucosyl donor.

The crystallographic structures of the fucosidases without substrate: 2ZWY, 6O18, and 3MO4 were used, and pNP-Fuc was used as the ligand. With AutoDock Tools (version 1.5.6) the search space was delimited to a box containing the active site with the following dimensions: 17.25 x 27.75 x 22.5 Å for TmFuc, 18 x 27 x 28.5 Å for AlfC and 18 x 27.75 x 18.75 Å for BiAfcB. Docking was performed with AutoDock 4.2, using as scoring parameters the Ki value, which was called the binding constant (Kb), and the binding free energy (ΔG_b), which were calculated with the Lamarckian genetic algorithm. 250 runs of the algorithm were performed with an initial population of 150 genes with a maximum of 2×10^3 generations and 25×10^6 energy evaluations.

The complexes obtained by molecular docking of the three α -L-fucosidases were compared with structures obtained from the PDB database in a complex with fucose or a similar molecule, which were considered reference enzyme-substrate complexes. The structures used were the following: 1ODU for TmFuc, 6O1A for AlfC, and 3UES for BiAfcB (in this case, it is an enzyme inhibitor). Images of the different molecular dockings were generated with Discovery Studio Visualizer (BIOVIA, Dassault Systèmes, Discovery Studio Visualizer, v21.1.0.20298, San Diego: Dassault Systèmes, 2020).

To validate the procedure, a redocking was performed with the 1ODU, 6O1A, and 3UES structures with the co-crystallized ligands using the same parameters mentioned above, and the RMSD between the co-crystallized ligand and the ligand obtained from the redocking was calculated with UCSF Chimera.

3. Results and Discussion

3.1. Comparison of fucosidases of the GH29A and GH29B subfamilies.

According to the CAZy classification [22], TmFuc belongs to the GH29 family and is one of the most studied, so it was the first GH29 fucosidase in which the catalytic acid-base residue was identified [23] and from which a three-dimensional structure was obtained [17]. Additionally, TmFuc has been studied in synthesizing FUCOS due to its ability to catalyze transfucosylation [7]. It also has a high similarity to the human fucosidase FUCA1, with which it shares 38% identity, which is of medical importance [24]. A search was done for other α -L-fucosidases similar to TmFuc using the BLAST®P service, finding the following: *Fusarium graminearum* of fungal origin (27% identity), of bacterial origins such as *Bacteroides thetaiotaomicron* (26% identity) and *Paenibacillus thiaminolyticus* (33% identity), and has 35% identity with *Bos taurus* fucosidase [25] of animal origin.

The fucosidases of the GH29 family are subdivided into the GH29A and GH29B subfamilies, which have differences in their sequences that make GH29B more selective towards substrates with α 1-3 and α 1-4 bonds, while GH29A has a lower selectivity hydrolyzing the α 1-2, α 1-3, α 1-4 and α 1-6 bonds [26]. To observe these differences between the subfamilies, TmFuc, which belongs to the GH29A subfamily, was taken as a reference to perform a comparison with the AlfC fucosidase of the GH29A subfamily and one of the

GH29B subfamily, BiAfcB, which have been used in the synthesis of FUCOS [10,11] and come from microorganisms generally recognized as safe (GRAS).

As can be seen in Figure 2A, these enzymes have the conserved domain Alpha_L_fucos (purple region) from the Simple Modular Architecture Research Tool database (SMART accession number: SM00812) [27], which includes them in this family of enzymes.

The sequences that these enzymes have in common are mainly found in their catalytic domains, which can be seen in Figures 2B, 2C, and 2D. The catalytic domain of TmFuc comprises from residue 1 to 357, for BiAfcB from 21 to 359, and in the case of AlfC, the catalytic domain comprises its entire sequence; Unlike TmFuc and BiAfcB, which also have a C-terminal domain composed of folded β s.

When superimposing the crystallographic structures, it was found that TmFuc has a percentage of identity of 26.67% with AlfC and an RMSD of 0.874. As can be seen in Figures 2B and 2C, the conserved sequences between AlfC and TmFuc are located mainly in the α helices corresponding to TmFuc A1, A2, A3, A8, and in the β sheets B2, B3, B7, and B8, which are located around the active site. When performing the overlap between TmFuc and BiAfcB, a 25.27% identity and an RMSD of 1.044 were obtained, coinciding with the 26% identity obtained by Sela *et al.* [20] between the fucosidase gene Blon_2336 from *B. longum* subsp. *infantis* and *T. maritima* fucosidase gene TM0306. In addition, the conserved sequences in the secondary structures corresponding to TmFuc A2, A3, A8, B3, B4, B7, and B8 are observed (Figures 2B and 2D).

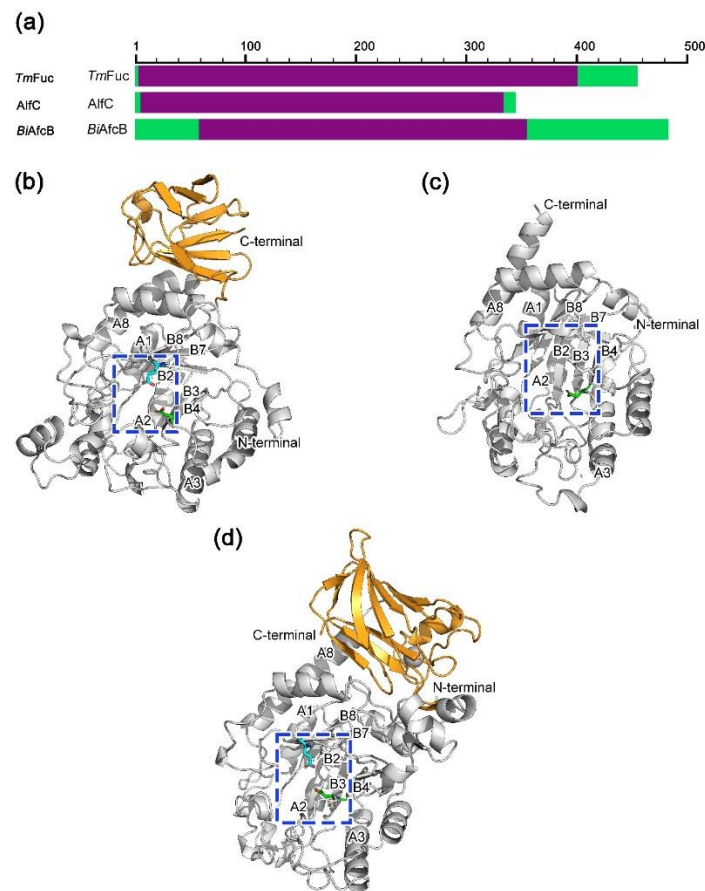


Figure 2. (a) Alignment of the sequence of TmFuc, AlfC, and BiAfcB with the conserved domain Alpha_L_fucos, the general sequence of the enzyme is indicated in green color, the conserved domain Alpha_L_fucos is indicated in purple color. Structure of the three fucosidases; (b) TmFuc; (c) AlfC; (d) BiAfcB. The gray color corresponds to the catalytic domain; the active site is located inside the dotted box, and the orange color corresponds to the C-terminal domain.

In addition to the differences in the percentage of identity and the structural similarity, the GH29 fucosidases analyzed in this work have differences in the active site, which will be reviewed in more detail below. As can be seen in Figure 3A, the enzymes of the GH29A subfamily have an arginine (ARG) that is conserved in this subfamily of fucosidases, like ARG254 in TmFuc and ARG229 in AlfC. This residue has the function of helping to position the substrate by forming a hydrogen bond with the endocyclic oxygen of fucose. On the other hand, in BiAfcB, which belongs to the GH29B subfamily, this ARG is not conserved, but residues TRP213, GLU237, and ASP283 can be found (Figure 3B), which are associated with a galactose binding site and are conserved in other fucosidases of the GH29B subfamily [19]. Regarding the catalytic amino acids, the nucleophile ASP is conserved in the three fucosidases studied, as can be seen in Figures 3A, 3B, and 3C, as ASP224 in TmFuc, ASP200 in AlfC and ASP172 in BiAfcB. While the other catalytic residue that acts as a general acid-base, GLU, is conserved in TmFuc as GLU266 and as GLU217 in BiAfcB (Figure 3A, 3B and 3C), while in AlfC as reported by Klontz *et al.* [18] there are three residues that could perform this function: GLU 39, ASP242 and GLU274, of which ASP242 can be seen in 3C as it is located near the catalytic residue ASP200.

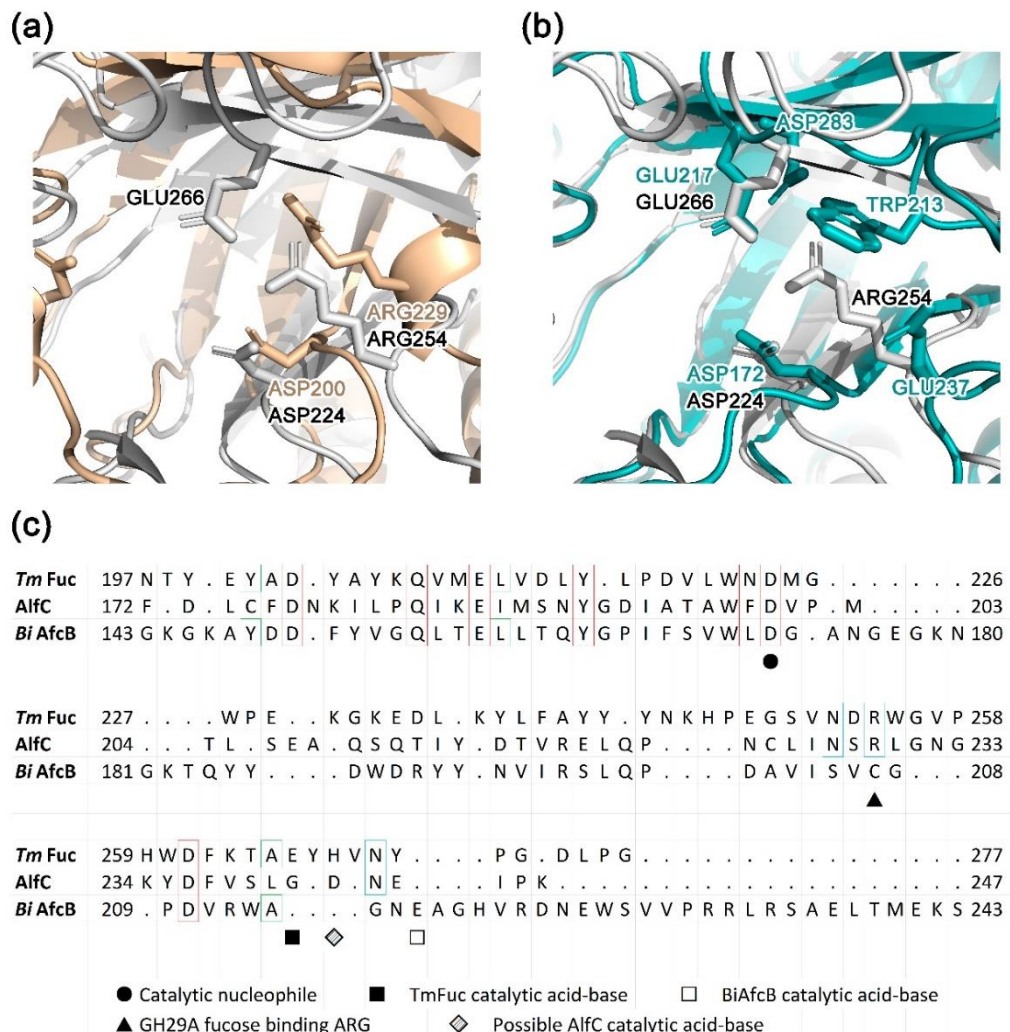


Figure 3. Active site alignment of fucosidases from the GH29A and GH29B subfamilies. (a) TmFuc (gray) and AlfC (brown), two fucosidases from GH29A subfamily. (b) TmFuc (gray) from the GH29A subfamily and BiAfcB (blue) from GH29B subfamily. (c) Multialignment of the TmFuc, AlfC, and BiAfcB sequences showing conserved sequences between TmFuc and AlfC in the blue box, conserved sequences between TmFuc and BiAfcB in the green box, conserved sequences between the 3 in the red box.

By comparing the enzymes of both subfamilies, it was confirmed that there are differences in the sequence between the fucosidases that give rise to their division into the GH29A and GH29B subfamilies, which are related to structural changes that can influence both the affinity and the specificity towards the substrate.

3.2 Molecular docking of fucosidases from the GH29 subfamilies with the fucosyl donor pNP-Fuc.

PNP-Fuc is an activated molecule that is commonly used as a chromogenic substrate to determine the hydrolytic capacity of fucosidases [28], in addition to being used as a fucosyl donor for the synthesis of FUCOS [29, 30]. For this reason, the interaction between this substrate and the enzymes TmFuc and AlfC from the GH29A subfamily, and BiAfcB from the GH29B subfamily, was studied to obtain additional information to that reported in the literature on the affinity of these fucosidases with this substrate [10, 20, 31].

To validate the docking procedure presented below, a redocking was performed with the 1ODU, 6O1A, and 3UES structures using the co-crystallized ligand, obtaining an RMSD of 1.050 Å for 1ODU, 1.937 Å for 6O1A and 0.435 Å for 3UES (supplementary information), so it is considered that the method used to perform the docking is reliable by obtaining an RMSD of less than 2 Å between the co-crystallized ligand and the superposition of the ligand pose obtained from the docking [32, 33].

3.2.1. Molecular docking with TmFuc and the fucosyl donor.

As can be seen in Figure 4A, when docking between TmFuc and pNP-Fuc was performed, the ligand was placed between the catalytic residues, with the fucose portion of the molecule located in the subsite -1 inside the active site cavity (Figures 4A and 4B) and the p-nitrophenol, corresponding to the aglycon located at the +1 subsite, outside the catalytic pocket.

Additionally, as can be seen in Figure 4A, the aromatic ring of the ligand formed pi-alkyl interactions with LEU50 and pi-sigma with MET225, stabilizing the aglycon. In contrast, the C6 of fucose formed pi-alkyl interactions with the aromatic residues PHE290, PHE32, TRP222, and HID34, showing the hydrophobicity of the catalytic pocket. The catalytic residue ASP224, which acts as a nucleophile for the formation of the enzyme-substrate complex, formed a hydrogen bond with the C4 of the fucose, staying at 4 Å from the C1 of the fucose (Figure 4B). The GLU266, which acts as a catalytic residue, is 4.1 Å from the C1 of fucose with a carbon-hydrogen interaction. Meanwhile, ARG254 formed a hydrogen bond with the endocyclic oxygen of fucose. The TmFuc-pNP-Fuc complex presented a K_b of 167.15 μ M and a ΔG_b of -5.6 kcal/mol, a value close to the -5.9 kcal/mol obtained by Pérez-Escalante *et al.* [34].

As a validation method for docking, it was compared with the 1ODU reference crystal [17], which is complex with fucose. As can be seen in Figure 4B, the position of the fucose of pNP-Fuc obtained from the docking was located in a pose very close to that of the reference crystal in subsite -1, with similar distances between the C1 of the fucose and the carboxylic carbon (CC) of the catalytic residues.

3.2.2. Molecular docking with AlfC and the fucosyl donor.

As can be seen in Figure 4A, when docking between AlfC and pNP-Fuc, the ligand was placed close to the ASP200, with the fucose portion of the molecule located at the -1 subsite and the aglycon at the +1 subsite (Figure 4D).

Figure 4C shows the interactions between the ligand and the enzyme, where the aromatic ring of the nitrophenol of pNP-Fuc formed a pi-stacked bond with the TRP40 and the nitro group a hydrogen bond with the ALA154. A pair of pi interactions were also established between TRP198 and TRP283, and C6 of fucose. The HIS18 and HIS87 form hydrogen bonds with the hydroxyls of C4 and C5, while the ARG229 form a hydrogen bond with the endocyclic oxygen of fucose. The catalytic residue ASP200 did not register interaction with the ligand. However, the CC of this catalytic residue is 5.3 Å from the C1 of the fucose (Figure 4D). There is no interaction with the residue that acts as a general acid-base. It is not observed in the vicinity of the active site, coinciding with Klontz *et al.* [18], who reported that this enzyme acts with a clamp mechanism, so this catalytic residue is found at a greater distance than in other fucosidases such as TmFuc, that has the catalytic residues at a closer distance by having a pocket in the active site [17] that allows it to house the -1 subsite inside. In this enzyme-ligand complex, a Kb of 261.42 µM and a ΔGb of -4.89 kcal/mol were obtained.

To validate the docking result, it was compared with the 6O1A reference crystal [18] in complex with fucose. As can be seen in Figure 4D, the fucose portion of the pNP-Fuc molecule was located in a very close position to that of the reference crystal in subsite -1, at a distance of 5.6 Å between the CC of the ASP200 and the C1 of fucose, while, in the reference crystal, this distance is 5.3 Å.

3.2.3. Molecular docking with BiAfcB and the fucosyl donor.

As shown in Figures 4E and 4F, in the molecular docking between BiAfcB and pNP-Fuc, the ligand was also placed between the catalytic residues, with the fucose portion of the molecule located at subsite -1 within the active site cavity, which, unlike TmFuc and AlfC, has an elongated groove shape with two carbohydrate binding sites, one for fucose and a contiguous one for galactose binding [19], on which the aglycon was located, corresponding to subsite +1, without fully inserting itself into said cavity.

As it is shown in Figure 4E, the pNP-Fuc aromatic ring formed pi-stacked interactions with TRP213, pi-alkyl with ALA174, and Pi-anion with ASP172. The C6 of fucose formed Pi-stacked interactions with TRP290 and Pi-alkyl with TRP170 and PHE34 and hydrogen bonds with HIS36, TRP47, and HIE85. The catalytic residue ASP172 does not register interaction with the ligand. Still, it is at 3.7 Å from C1 of fucose, and GLU217, which acts as a general acid-base, is at 4.1 Å from C1 (Figure 4F), with interaction through van der Waals forces. Together these interactions had a Kb of 1420 µM and a ΔGb of -3.4 kcal/mol.

To validate the docking result, it was compared with the 3UES reference crystal [19], which is in complex with the competitive inhibitor deoxyfucojirimycin (DFJ). In Figure 4F, it can be seen that the fucose of pNP-Fuc was located in a position very close to that of the DFJ of the reference crystal at subsite -1, with similar distances between the C1 of the fucose and the CC of the catalytic amino acids to those found in the reference crystal.

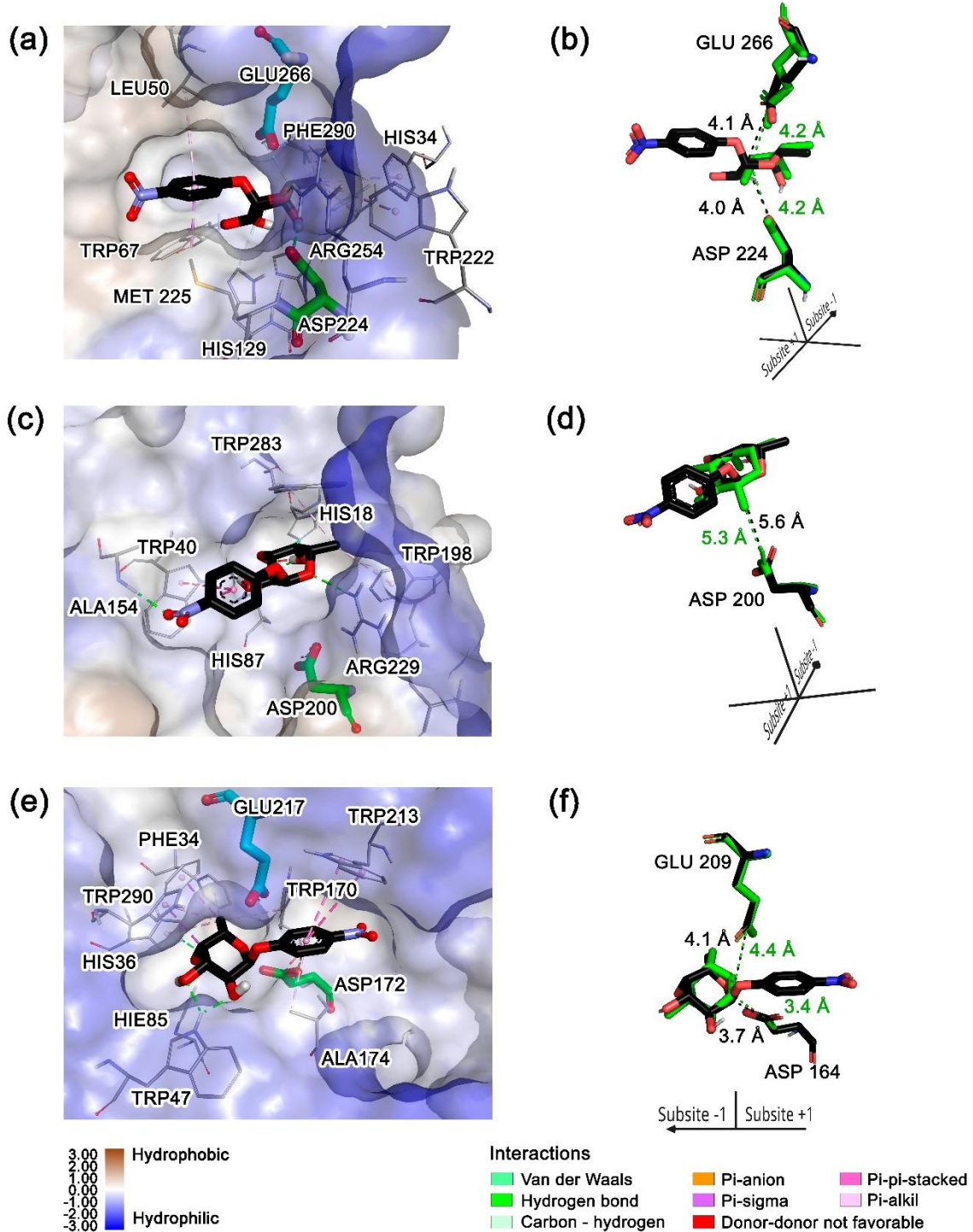


Figure 4. Molecular docking obtained with pNP-Fuc and (a) TmFuc; (c) AlfC; (e) BiAfcB. Comparison of the complexes obtained and the reference crystals (in green color) (b) TmFuc and 1ODU; (d) AlfC and 6O1A; (f) BiAfcB and 3UES.

As can be seen in Table 2, the studied fucosidases from the GH29A subfamily obtained a lower ΔG_b than the α -L-fucosidase from the GH29B subfamily. Therefore, the former seem to form more stable energy complexes with pNP-Fuc. Making an analogy with Gibbs free energy, it is evident that using GH29A; the reaction will be carried out more easily than with GH29B, supporting the data reported in the literature that experimentally demonstrates that this fucosyl donor exhibits a better interaction with TmFuc [19].

Table 2. Free binding energy and K_b obtained from docking between the three fucosidases and pNP-Fuc.

Enzyme	Microorganism	Family	K_b (μM)	ΔG_b (kcal/mol)	K_M pNP-Fuc (μM)
TmFuc	<i>T. maritima</i>	GH29A	167.15	-5.6	200 \pm 3 [31]
AlfC	<i>L. casei</i>	GH29A	261.42	-4.89	700 \pm 30 [18]
BiAfcB	<i>B. longum</i> subsp. <i>infantis</i>	GH29B	1420	-3.88	-

K_b : binding constant; ΔG_b free binding energy.

The differences in ΔG_b obtained may be related to the interactions found in the enzyme-ligand complex. For the case of the GH29A α -L-fucosidases, it was observed that the shape of the active site of TmFuc allowed to bind better the aromatic ring of the ligand by interacting on both sides of the plane of the ring with the hydrophobic pocket while in AlfC, this interaction occurs only between one of the faces of the ligand and a less hydrophobic region of the enzyme. The interaction found with TmFuc coincides with reports of better interaction between the enzyme and molecules that incorporate elongated hydrophobic residues in the aglycone [35].

On the other hand, when comparing the interaction of the GH29A α -L-fucosidases with the GH29B, the interaction of the characteristic ARG residue of GH29A and the endocyclic oxygen of fucose also intervenes, which can perform the function of stabilizing the ligand in the active site [19] in molecular dockings with TmFuc and AlfC; while in molecular docking with BiAfcB, the complex could be less stable due to the characteristics of the active site. BiAfcB has an adjacent cavity reported as a galactose binding site [19], on which the aglycone was placed without completely inserting the p-nitrophenol group of the donor substrate, causing the interactions not to be as favorable.

As can be seen in Table 2, the K_b obtained in the molecular docking for TmFuc was 1.6 times lower than that of AlfC. Since K_b represents the binding and dissociation of the enzyme (α -L-fucosidase) and the ligand (pNP-Fuc), this can be related to the K_M constant, where the binding and dissociation of the enzyme-substrate complex of an enzymatic reaction is related. Therefore, it can be assumed that both TmFuc and AlfC have a higher affinity for pNP-Fuc, which coincides with the K_M reported by Osanjo *et al.* [31] for TmFuc and by Klontz *et al.* [18] for AlfC, while for BiAfcB, a K_b greater than that of the other two fucosidases was obtained by molecular docking, being 8.5 times greater than that of TmFuc, which indicates a lower affinity towards the substrate. As reported by Sakurama *et al.* [19], BiAfcB has low activity on pNP-Fuc, and Sela *et al.* [20], reported low activity of this enzyme on cNP-fucose (chloro-nitrophenyl-fucopyranoside). However, in both investigations, good activity on 3-fucosyl lactose (3'-FL) was reported so that the active site of BiAfcB could better interact with substrates with bonds other than those of pNP-Fuc.

As can be seen from the results obtained, the interactions established by the fucose donor with the enzyme are important and could be related to active site affinity. These differences in interaction may be due to the presence of particular residues of each subfamily of GH29 α -L-fucosidase.

4. Conclusions

This work demonstrated, by *in silico* techniques, that the differences in the amino acid sequences between the fucosidases of the GH29A and GH29B subfamilies exert structural

differences that can influence the interaction they have towards the fucosyl donor substrate so that GH29A fucosidases show a higher affinity for pNP-Fuc than GH29B fucosidases. This complements the existing information in the literature obtained by experimental methods.

In silico methods allow the study of the structural characteristics of different enzymes and their interaction with different substrates that complement the knowledge obtained through experimental methods.

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Conflicts of Interest

The authors declare no conflict of interest.

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Supplementary materials

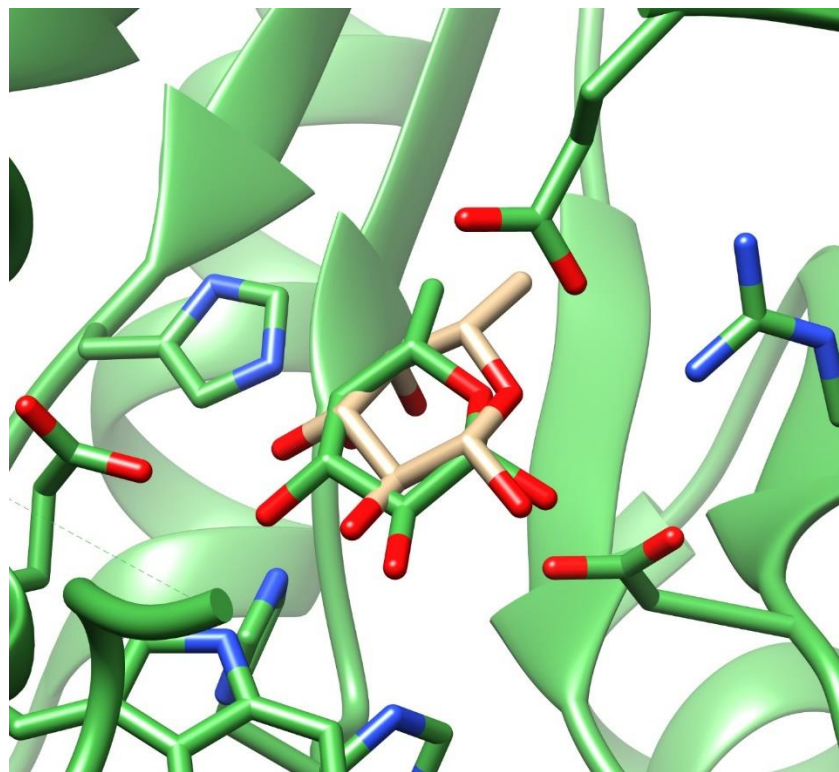


Figure S1. Redocked fucose from 1ODU structure, RMSD of 1.050 Å. Green: 1ODU structure, Brown: redocked fucose.

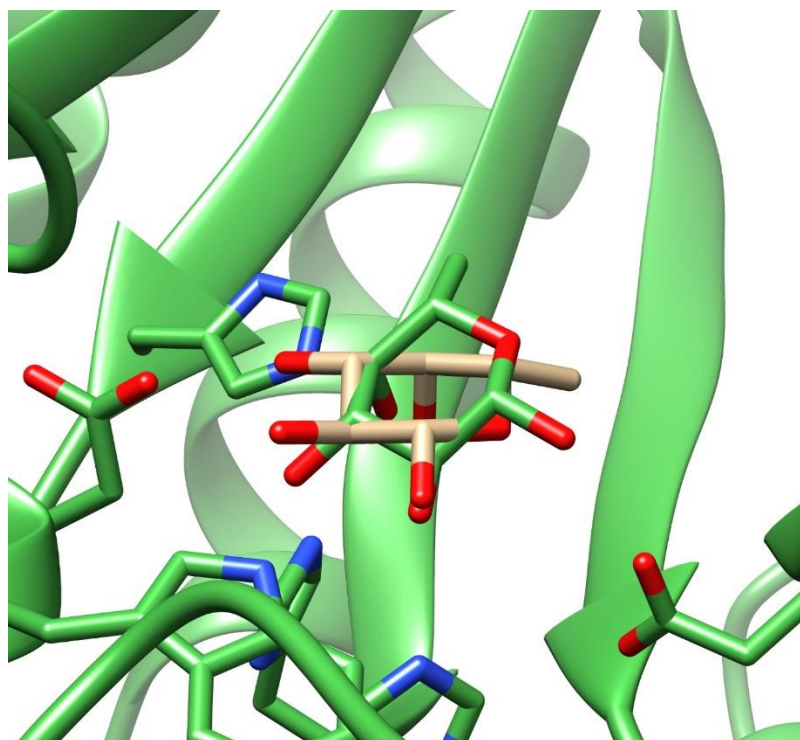


Figure S2. Redocked fucose from 6O1A structure, RMSD of 1.937 Å. Green: 6O1A structure, Brown: redocked fucose.

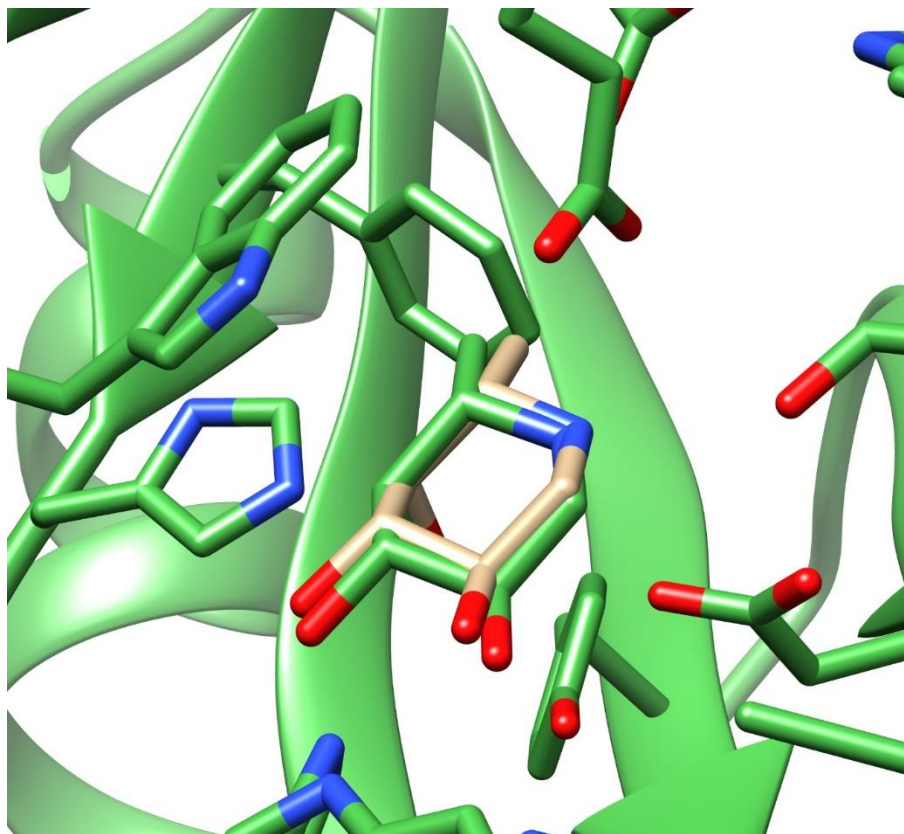


Figure S3. Redocked deoxyfuconojirimycin from 3UES structure, RMSD of 1.435 Å. Green: 3UES structure, Brown: redocked deoxyfuconojirimycin.