Figure 1. A) Simulated OMIT MAP at 1.2 σ contours (blue), generated by shaking the coordinates using PDBSET from the CCP4 program suite [1], removing cacodylate molecule from the model, and refining 5 cycles with REFMAC5 [2]. Fo-Fc map at 3.5 σ contours is represented in green. B) The same maps showing the position of several residues in the proximity of the blob corresponding to the cacodylate molecule. Both figures were generated with CCP4MG program [3]. C) Deposited structure (PDB ID. 35NF) showing roughly the same orientation as in A) and B). 2Fo-Fc and Fo-Fc maps are at 1.4 σ and 3.5 σ contours, respectively)

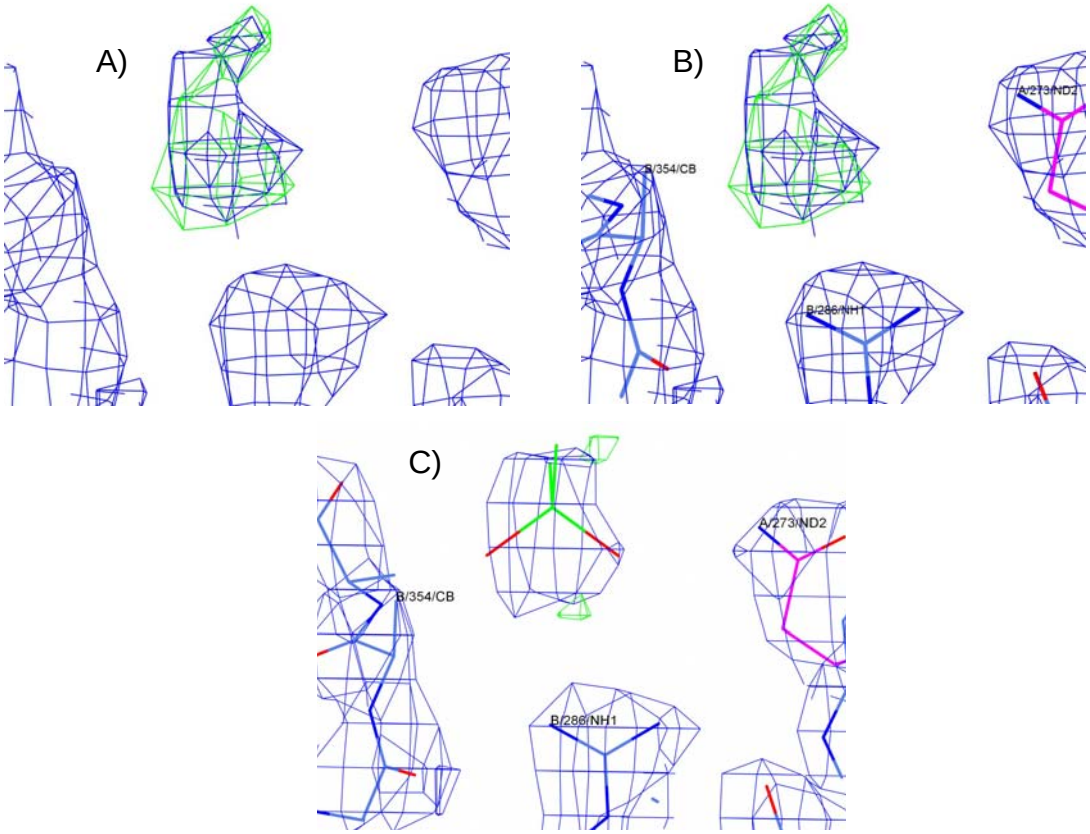


Figure 2. Amino acid sequence alignment of L-carbamoylases. A BLAST search was conducted with BsLcar sequence, using the UNIREF100 sequence cluster [4] to remove sequence redundancy and to reduce the number of sequences. L-carbamoylases with proven activity were included for comparison. AMAB2_GEOSE (GenBank ID Q53389 [5]), AMAB1_GEOSE (GenBank ID P37113 [5]), GeokaLcar (GenBank ID Q8GQG5 [6]), AaurhyuC (GenBank ID Q9F464 [7]), HYUC_PseN (GenBank ID Q01264 [8]) and SmeLcar (GenBank ID Q92MZ4 [9,10]). ClustalW XXL was used for sequence alignment. ESPript [11] was used to generate the images and to show the secondary structure of BsLcar. The consensus sequence appears below. The residues mutated in this work are highlighted in a box; residues forming the conserved bimetallic center appear as asterisks and substrate binding and hydrolysis residues as crosses.

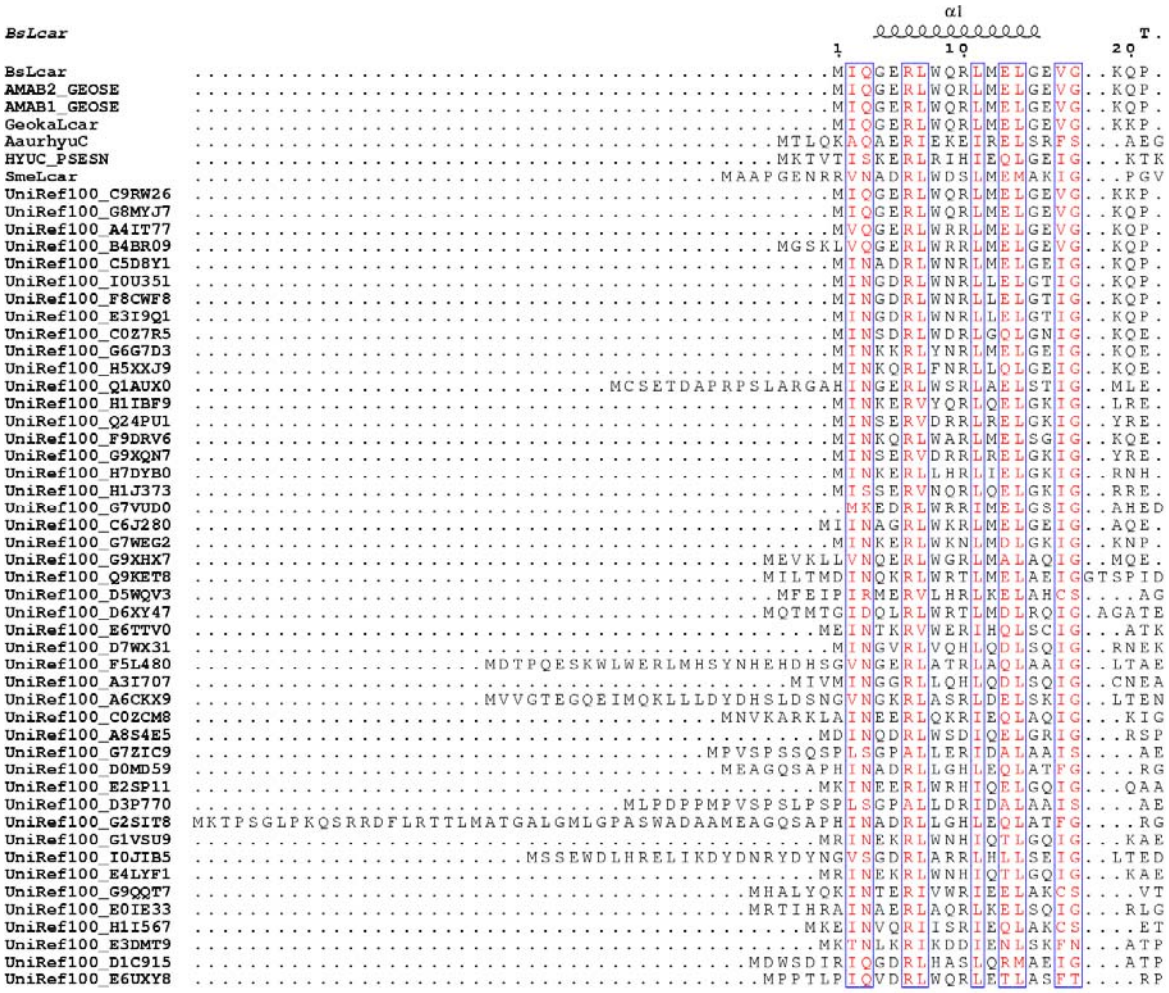


Figure 2. Sequence alignment of L-carbamoylases. (Cont.)

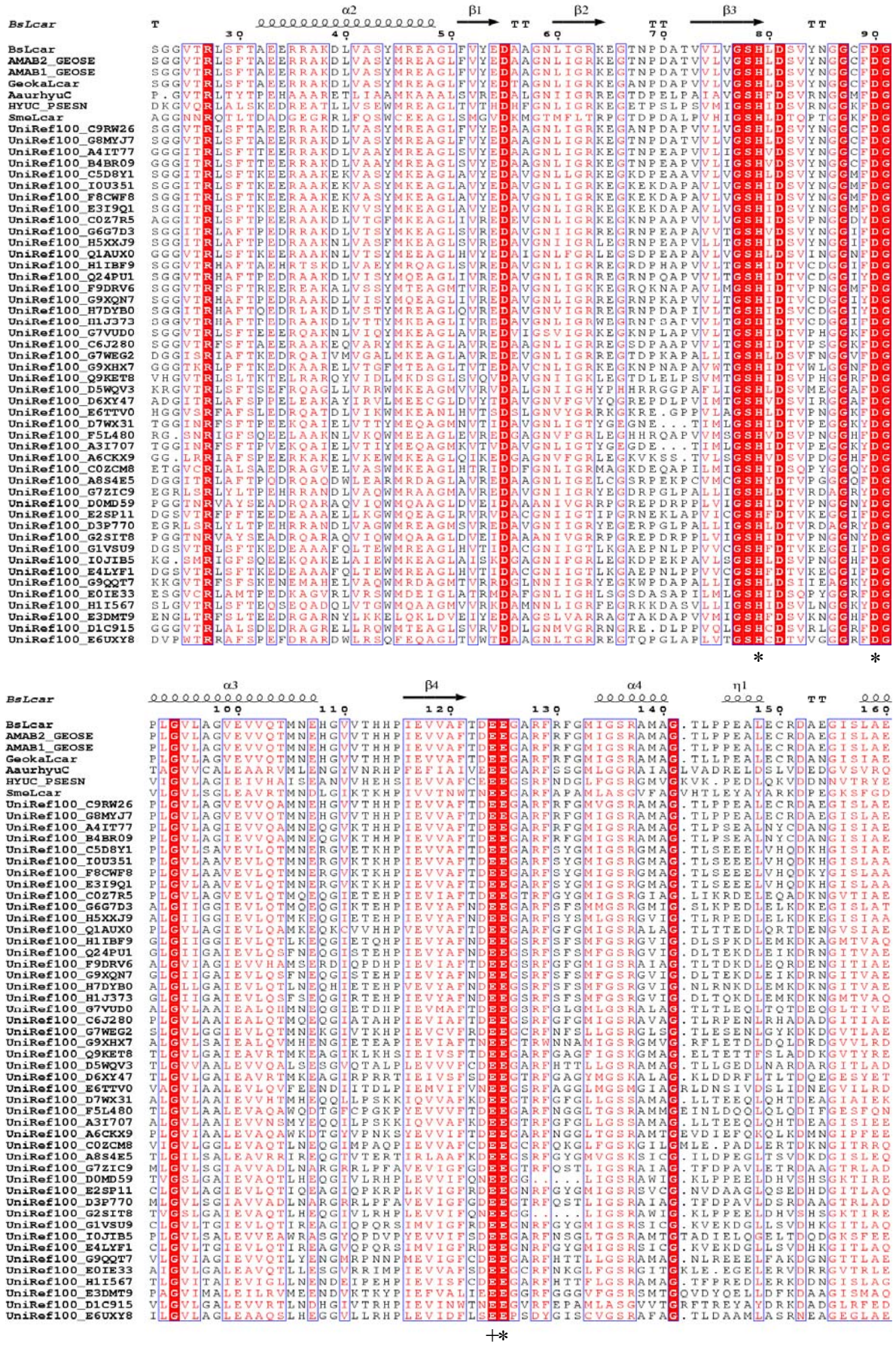


Figure 2. Sequence alignment of L-carbamoylases. (Cont.)

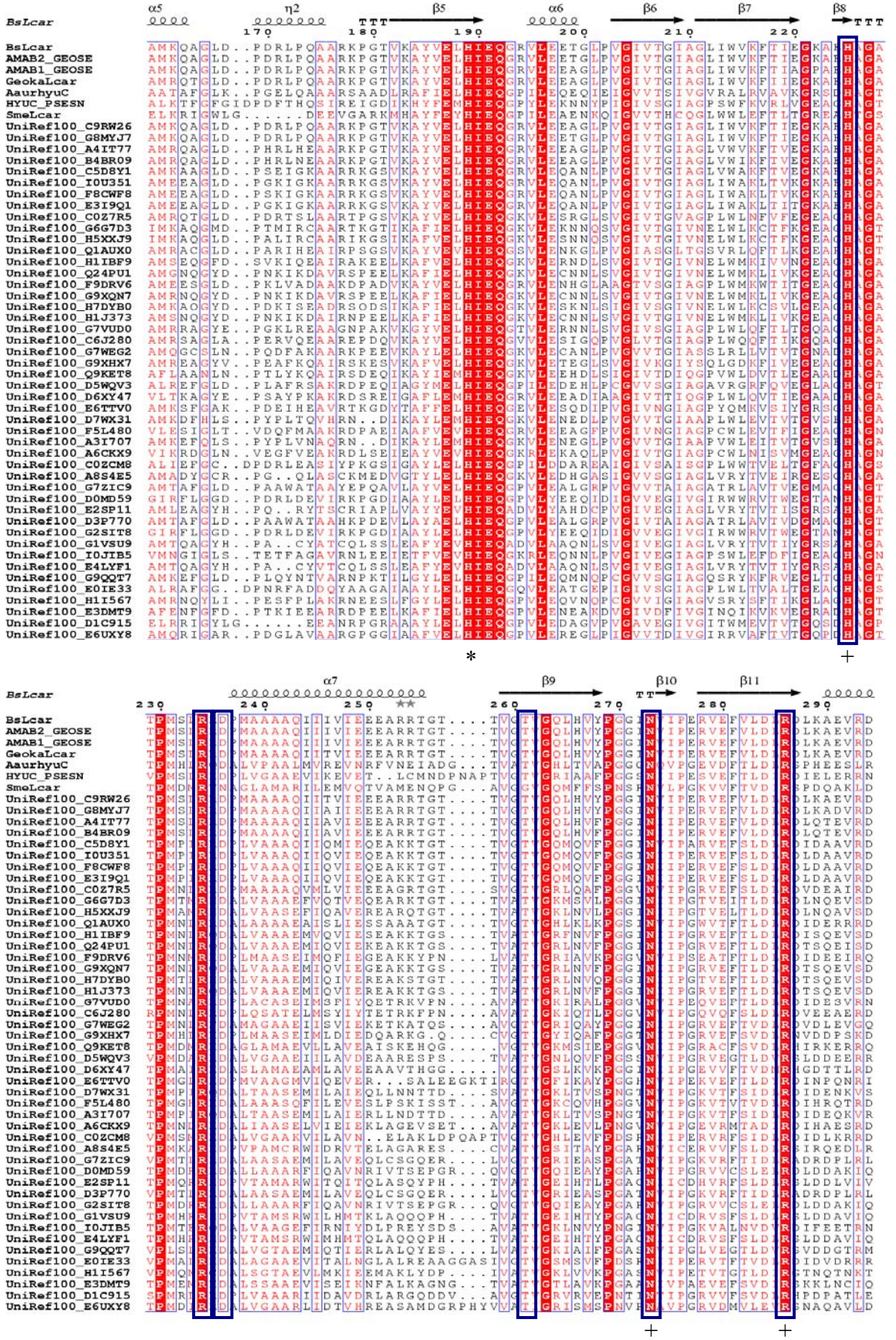


Figure 2. Sequence alignment of L-carbamoylases. (Cont.)

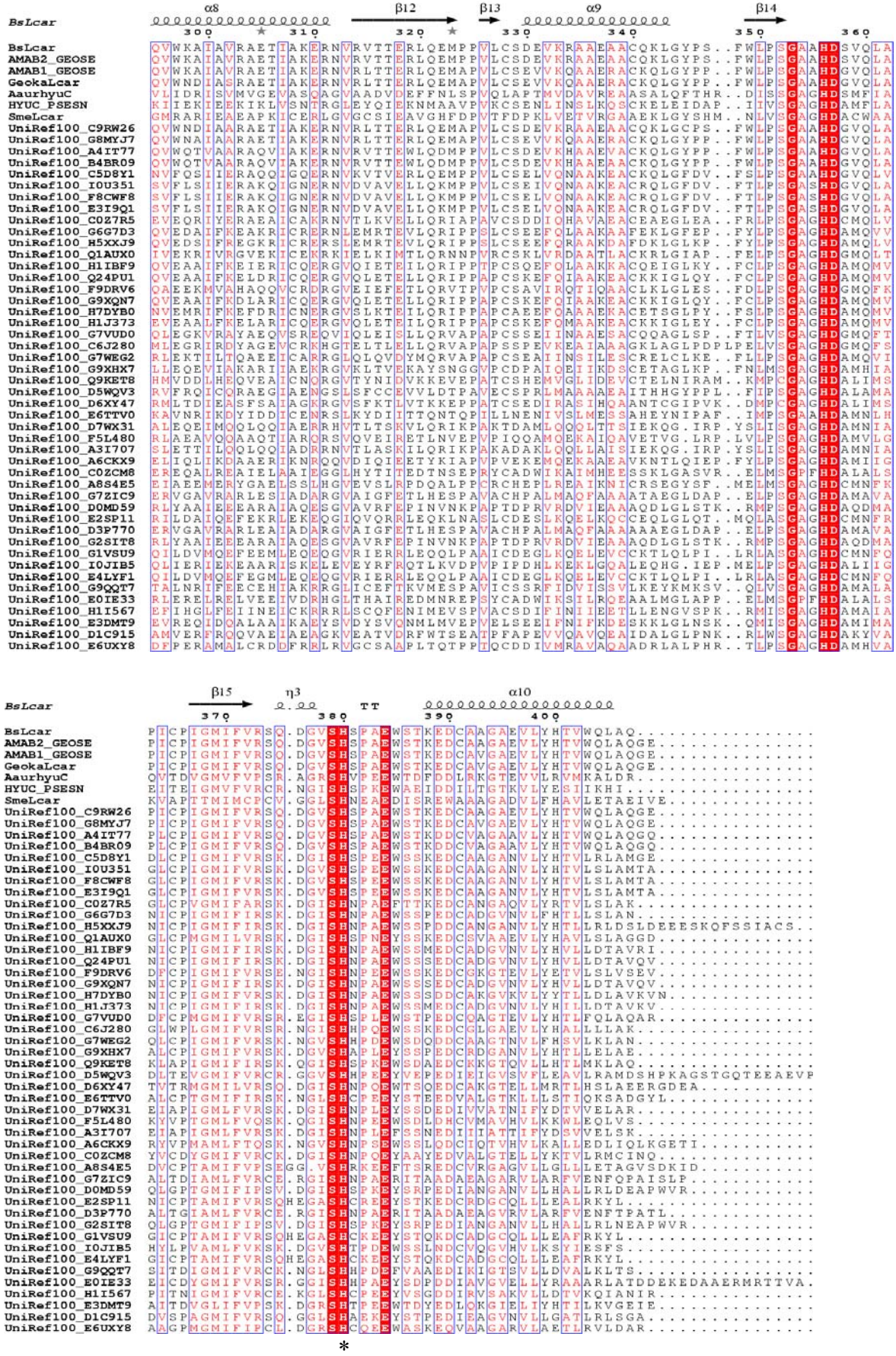


Figure 3. Superposed CD spectra of wild-type and mutated BsLcar species (5µM) in sodium phosphate buffer 100 mM pH 7.5.

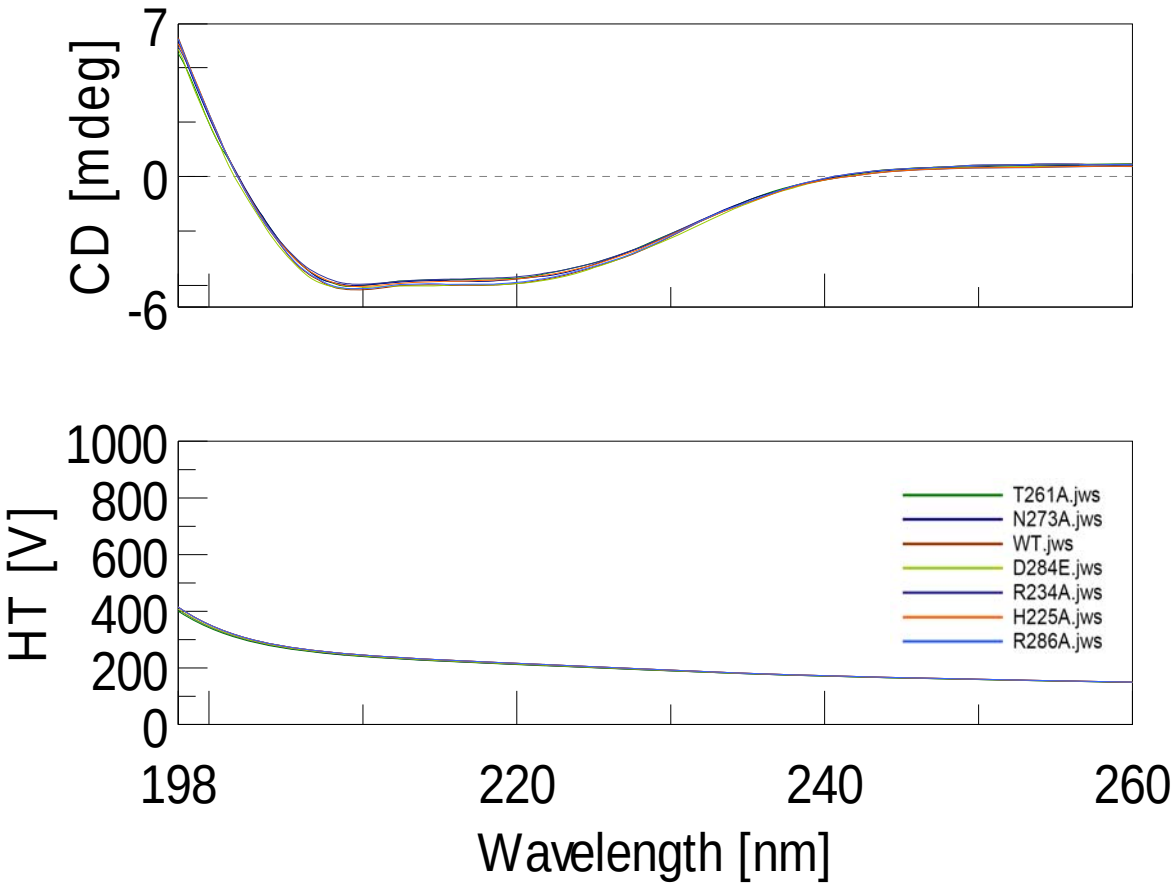
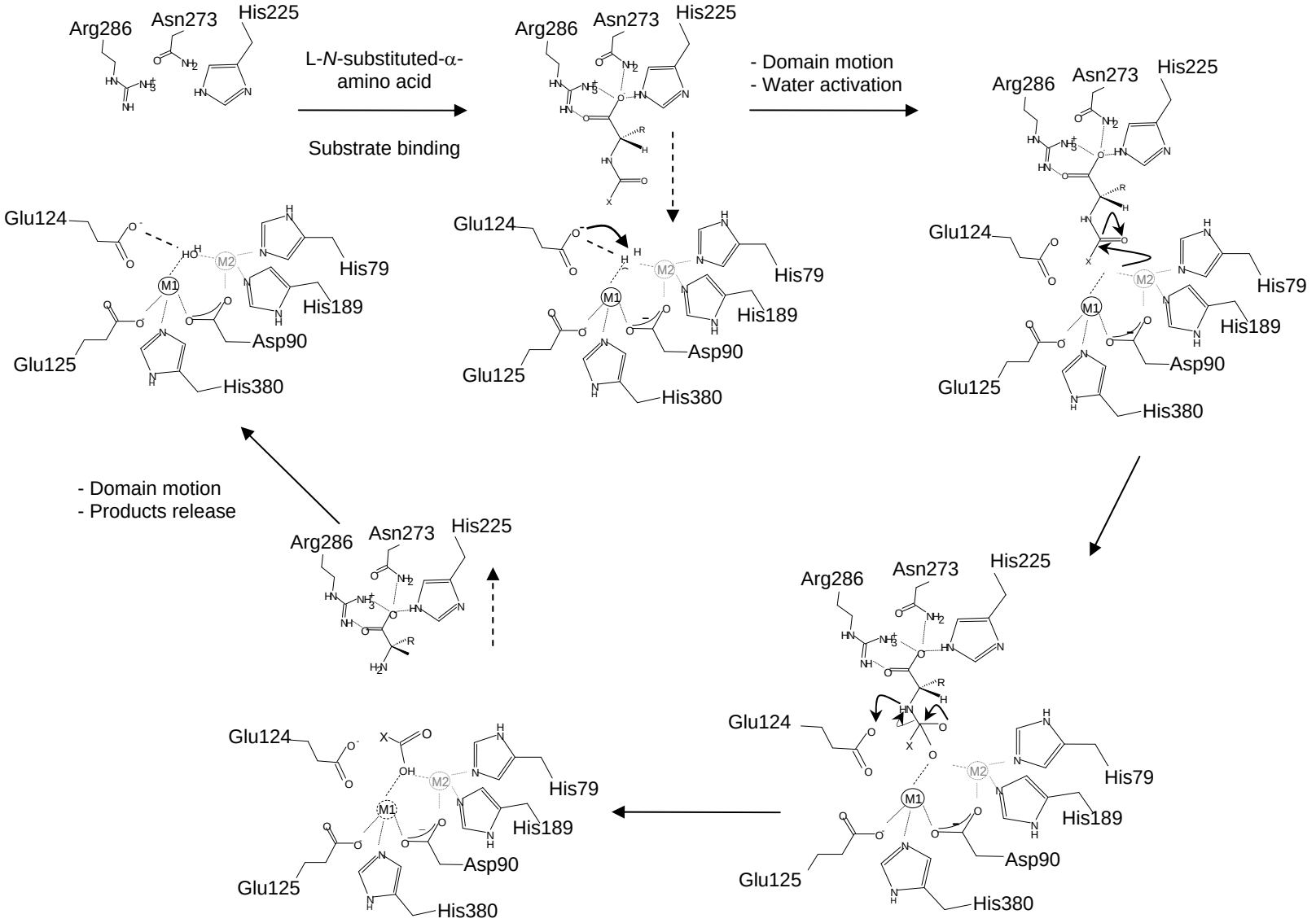


Figure 4. Schematic reaction mechanism of BsLcar toward different L-*N*-substituted-α-amino acids, based on previous proposed mechanisms [12,13]. The second metallic atom (M2) can be present or not, according to the results shown for BsLcar (see Results and discussionsection). X= NH_2, L-*N*-carbamoyl-α-amino acid; X= CH_3, L-*N*-acetyl-α-amino acid; X= H, L-*N*-formyl-α-amino acid; In the product release, (NH_3 +CO_2), acetate or formiate are produced depending on the substrate used (*N*-carbamoyl-, *N*-acetyl- or *N*-formyl-derivative).



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