SUPPORTING INFORMATION

Introduction of a Glycine Linker connecting the Heavy and Light Chains in Synthetic Cardosin B-derived rennet Changes the Specificity of Subpocket S3'

Pedro R. Figueiredo, Sónia F. G. Santos, Beatriz C. Almeida, Isaura Simões,* Alexandra T. P. Carvalho*

CNC – Center for Neuroscience and Cell Biology, Institute for Interdisciplinary Research (IIIUC), University of Coimbra, 3004-504 Coimbra, Portugal

*isimoes@biocant.pt, atpcarvalho@uc.pt

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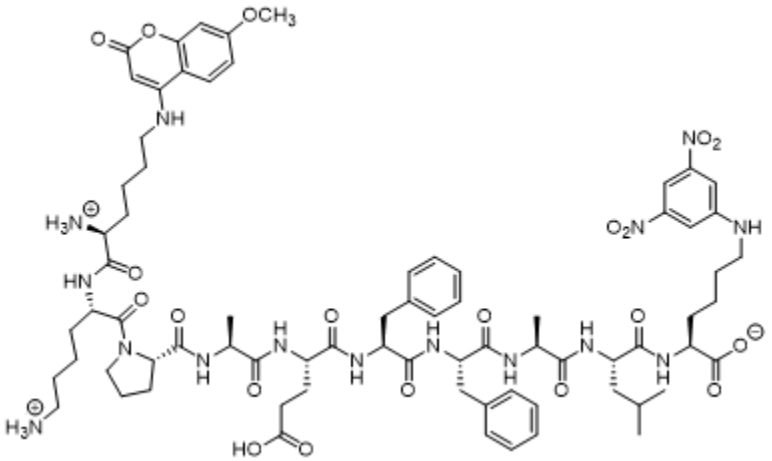

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213  204  208 1
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  1 PEP    1 TEMP     0 **** ****  0 ROOT
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Figure S1. **top**) Fluorogenic substrate structure. **bottom**) fluorogenic substrate mol2 file.

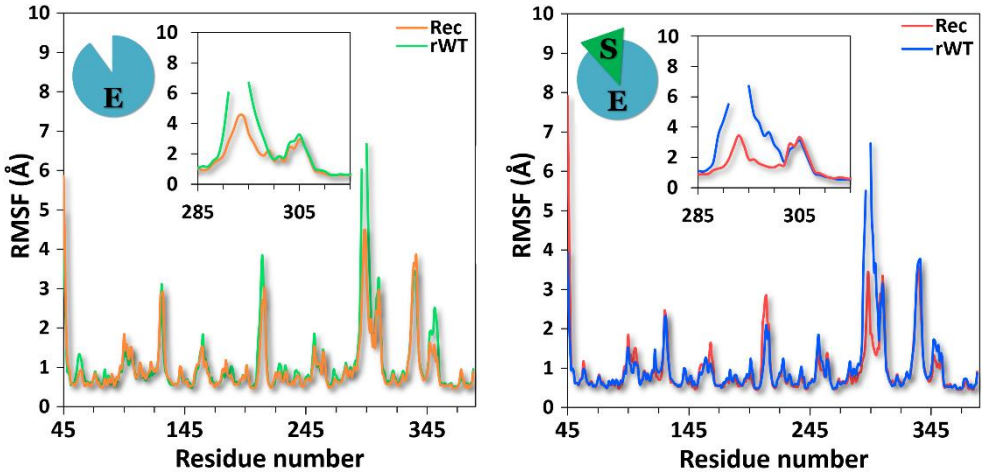

Figure S2. Cα RMSFs for the Rec and rWT enzymes (E) without substrate (S) (**left**) and with substrate (**right**) of cardosin B over the simulated 100 ns. These RMFSs concerned the MD simulations when the catalytic D267 is protonated.

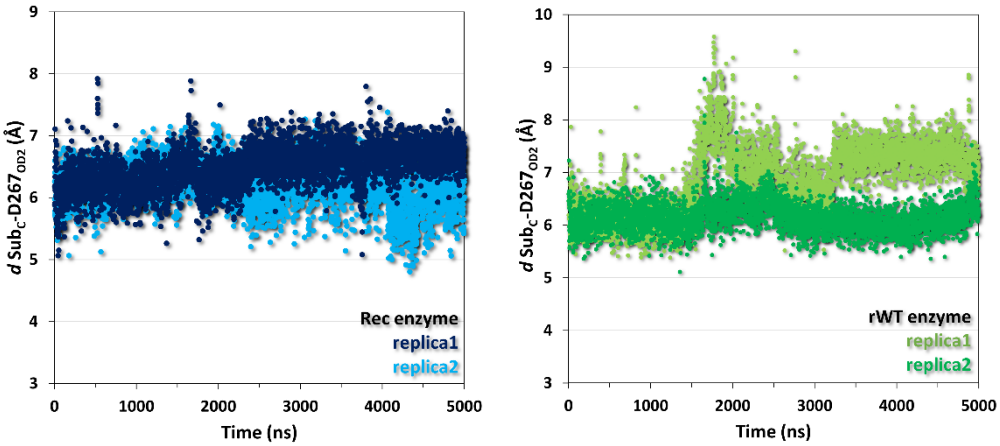

Figure S3. MD replicas plots of the Rec (**left**) and rWT (**right**) for the distance between the phenylalanine carbonyl carbon atom of the fluorogenic peptide and the OD2 atom of the catalytic D267. These plots concerned the MD simulations when the catalytic D267 is protonated.

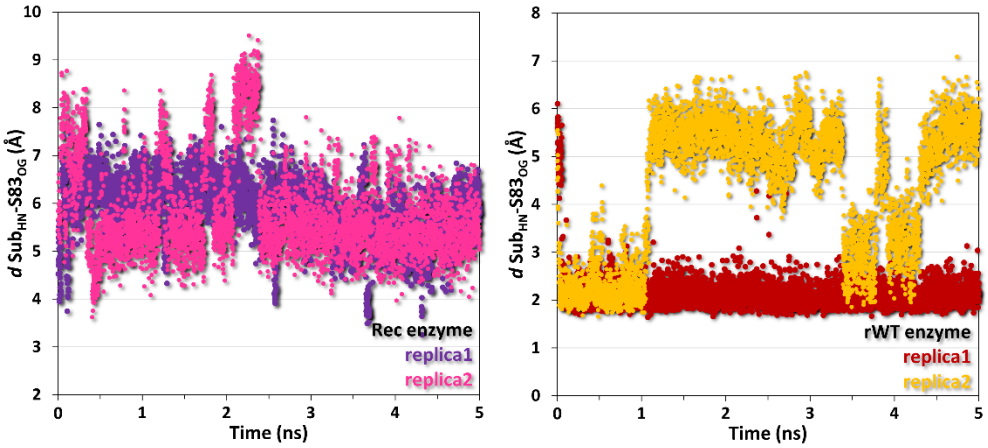

Figure S4. MD replicas plots of the Rec (**left**) and rWT (**right**) for the distance between the phenylalanine amide hydrogen atom of the fluorogenic peptide and the OG atom of the S83 residue. These plots concerned the MD simulations when the catalytic D80 is protonated.

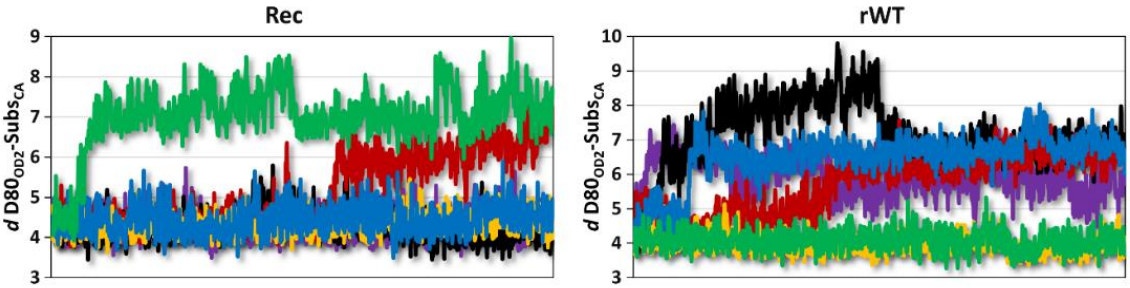

Figure S5. 10 ns MD replicas (6 per plot) for the distance between the phenylalanine carbonyl carbon of the fluorogenic peptide and the OD2 atom of the catalytic D80 of the Rec (**left**) and rWT (**right**) enzymes.

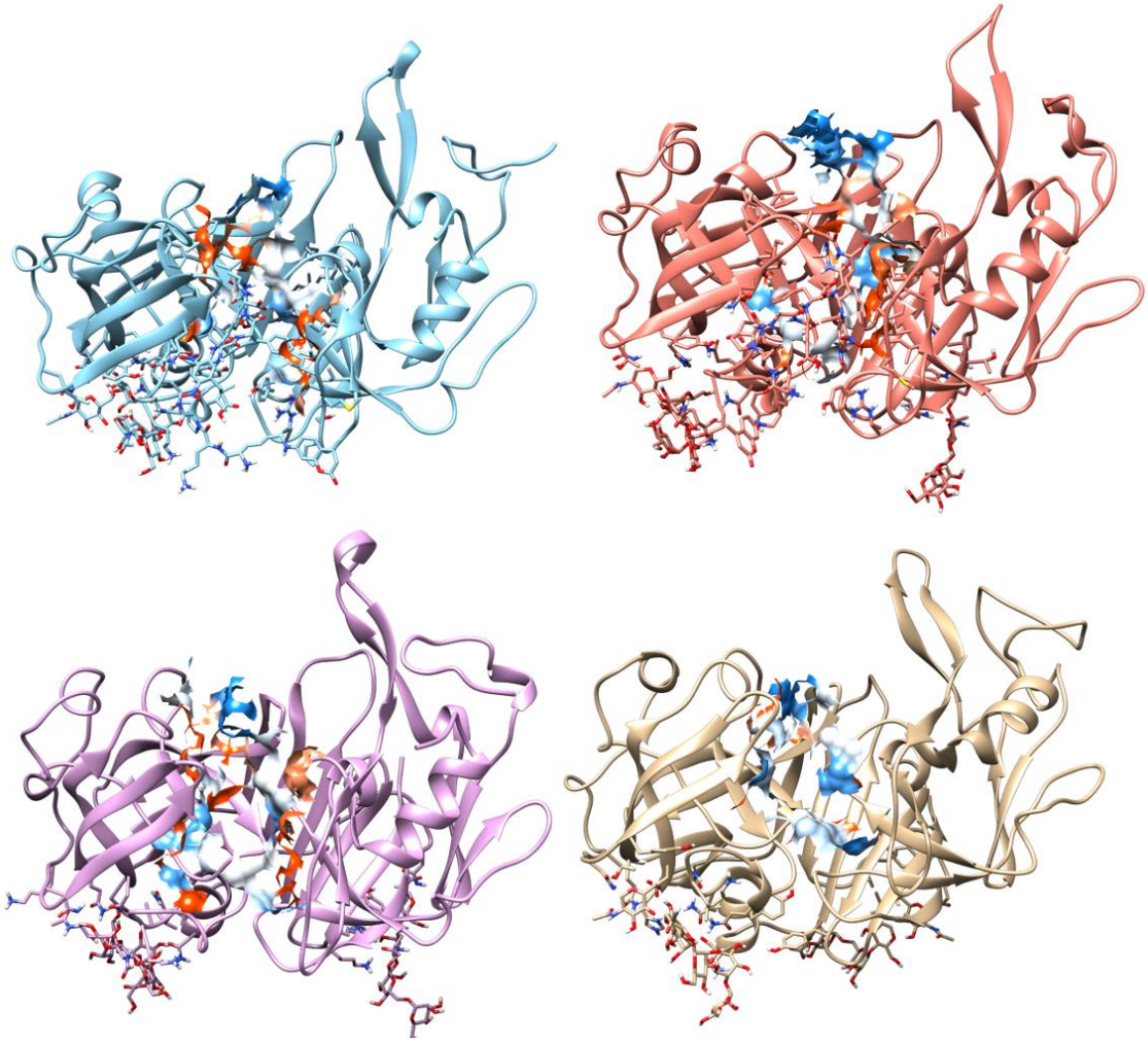

Figure S6. Representation of the main pocket surface for the enzymes with the D80 protonated: **blue**) rWT cluster structure, **pink**) Rec cluster structure. Free enzymes: **purple**) rWT cluster structure, **light brown**) Rec cluster structure. All structures were obtained from the MD simulations. The glycans and substrate (whenever present) are represented in licorice.