

Supporting information

**Refining the model to design  $\alpha$ -chymotrypsin superactivators: the role of the binding mode of quaternary ammonium salts**

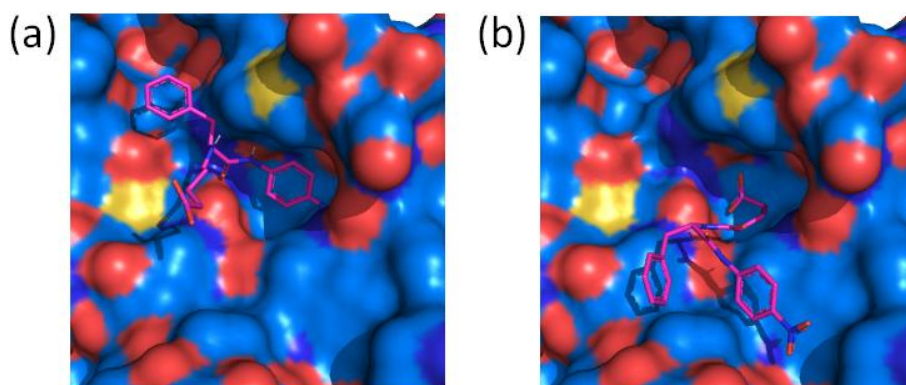
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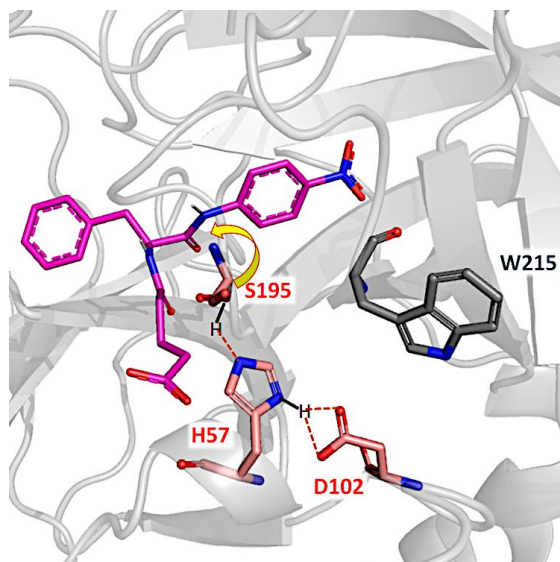
I-67100 Coppito, L'Aquila, Italy.

<sup>b</sup>Department of Chemistry, Biology and Biotechnology, University of Perugia, via Elce di Sotto, 8

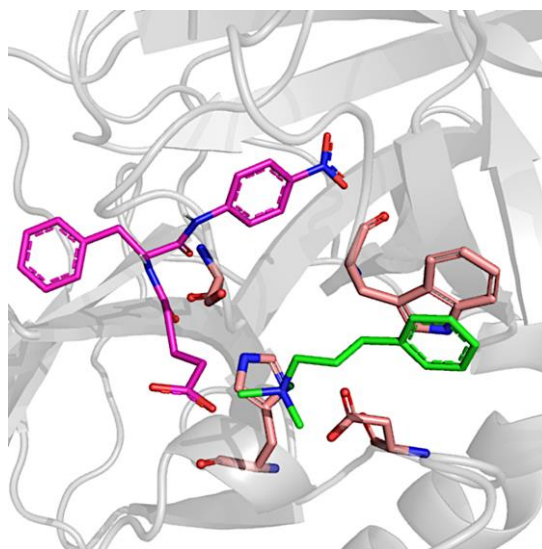
I-06123, Perugia, Italy.



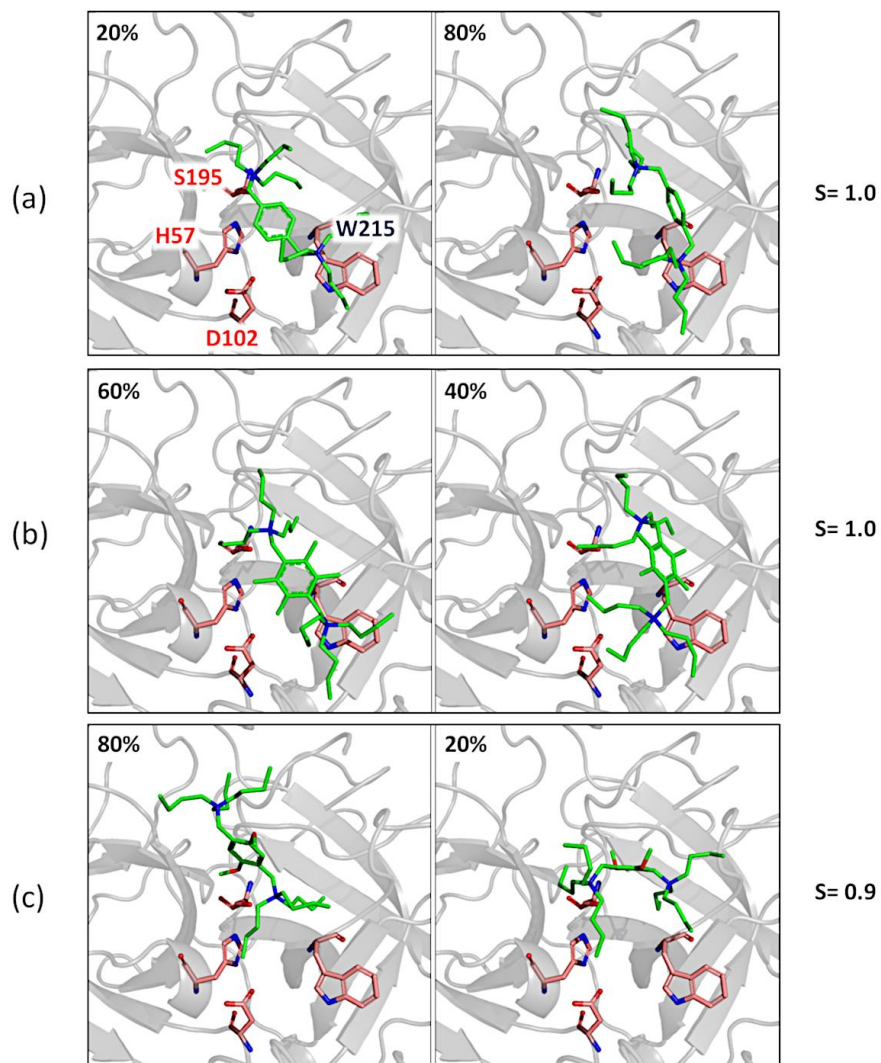
**Figure S1:** Most probable binding poses for GPNA according to FLAP predictions.



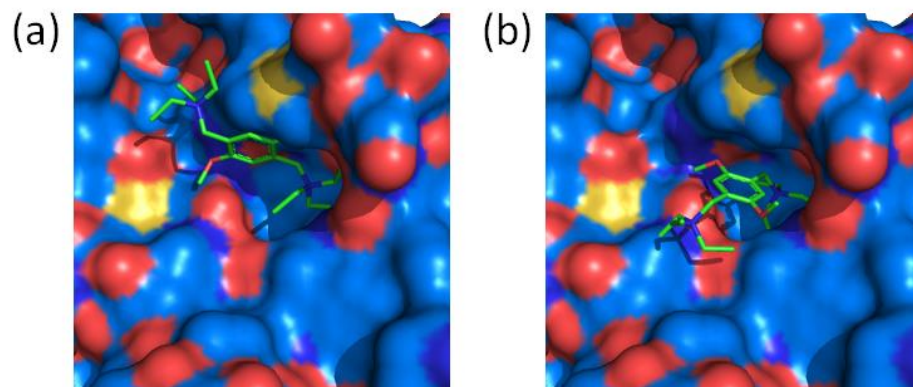
**Figure S2:** Depiction of the mechanism of hydrolysis of GPNA, according to the GPNA reactive pose (as in Figure S1-a).



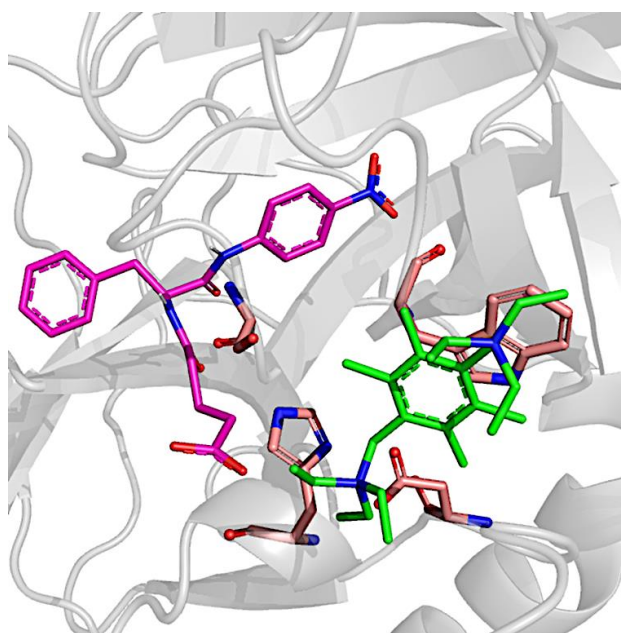
**Figure S3:** Simultaneous visualization of GPNA and PhPrTMABr docked into the  $\alpha$ -CT cavity, with the protein in cartoon mode to highlight the proximity between the ammonium moiety of the additive and the carboxylate moiety of the substrate.



**Figure S4:** Most probable binding poses for bisBAB (a), bisEDuBAB (b) and bisEOMeBAB (c). For each additive, the ten top-ranked binding poses were analyzed and clustered in the two most different poses, associated to a percentage of occurrence. The similarity score  $S$  calculated according to the Glob-Prod descriptor of FLAP is provided (S195:serine 195; H57: histidine 57; W215: tryptophan 215; D102: aspartate 102).



**Figure S5:** Most probable binding poses for bisEOMeEAB according to FLAP predictions.



**Figure S6:** Simultaneous visualization of GPNA and bisEDuEAB docked into the  $\alpha$ -CT cavity.