Supplementary Materials: Mechanism-Guided Discovery of an Esterase Scaffold with Promiscuous Amidase Activity

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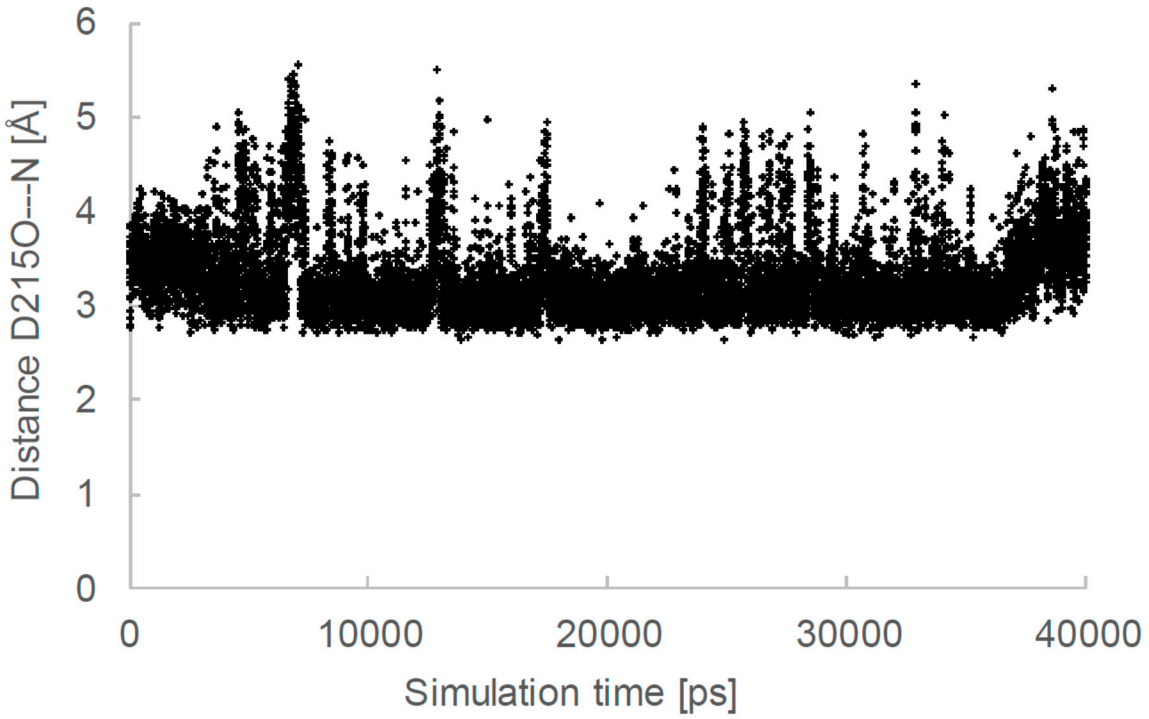

Figure S1. A neutral reacting nitrogen atom leads to unproductive conformations and to spatial arrangements corresponding to the first tetrahedral intermediate that do not capture the essential features of the rate-limiting transition state of nitrogen inversion (not shown). Analyzed heavy-atom distances between the oxygen of the protonated catalytic acid (D215) and the neutral reacting nitrogen atom of the substrate during a 40 ns MD-simulation of patatin are in accordance with a high-abundance of non-productive conformations for which the nitrogen and the catalytic acid are remote.

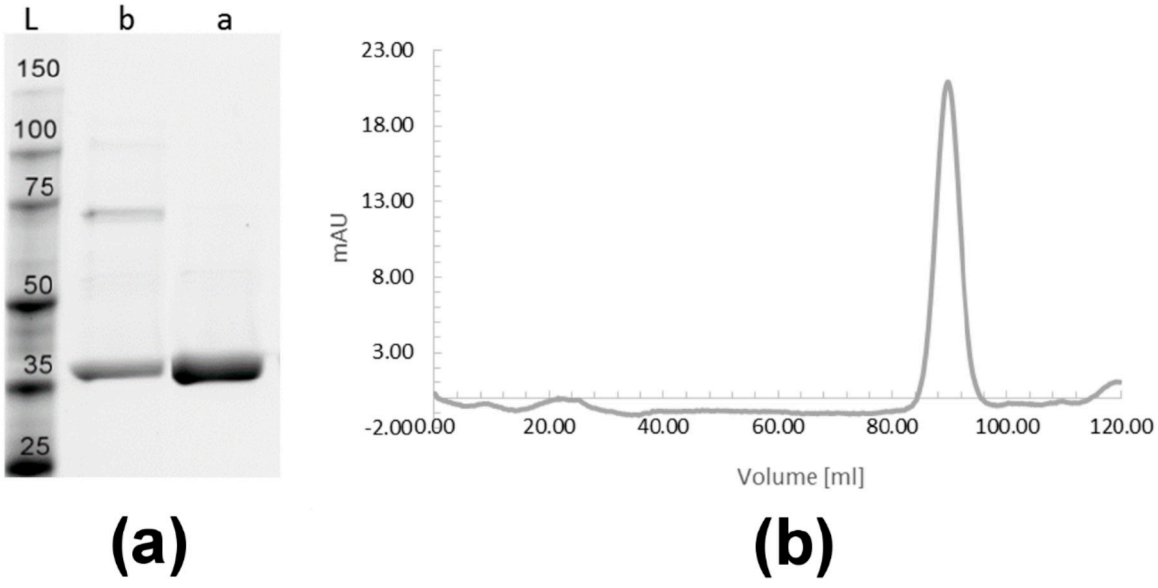

Figure S2. SDS-Page and Size Exclusion Chromatography of patatin. (**a**) SDS-Page with Ladder-L,

purified patatin (Ni-NTA affinity chromatography) before SEC-b, and patatin monomer after SEC-a. (**b**) SEC-chromatogram (Sephacryl S-400) with one distinct signal at 90 ml that corresponds to the monomer band shown in the gel in (**a**).

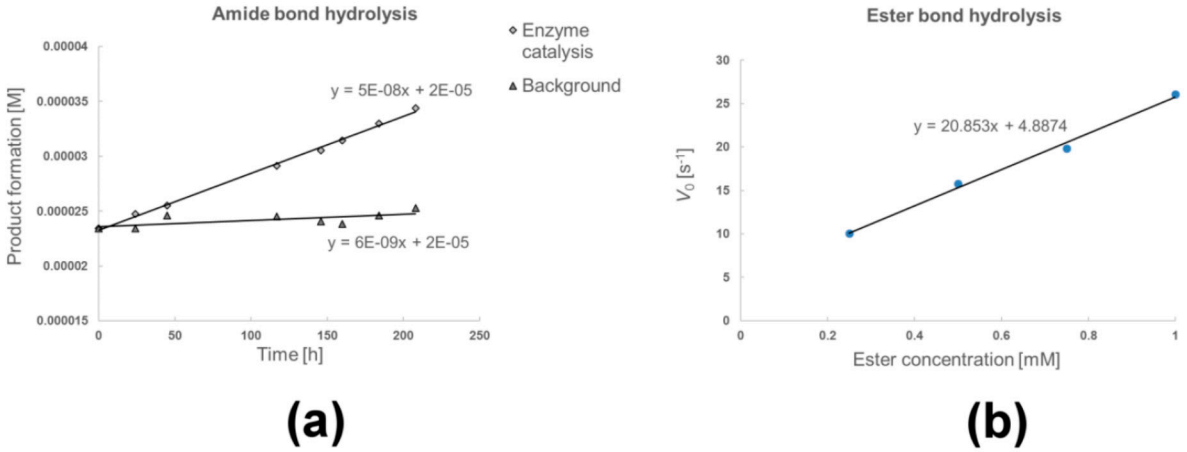

Figure S3. Experimental determination of enzyme kinetics. (**a**) Representative determination of initial velocity (V_0). The shown graph refers to amide bond hydrolysis showing both the enzyme catalyzed reaction (diamonds) and background (triangle) for an initial substrate concentration of *p*-nitrobutyranilide of 1.0 mM. The R_2 value for the fitted line to the experimental data using non-linear regression in Microsoft Excel is 0.99. (**b**) Determination of apparent k_{cat}/K_M for ester bond hydrolysis based on initial velocity (V_0) measurements. For the amide substrate, the substrate concentration was not elevated above 1.0 mM due to solubility issues and lower concentrations could not be followed due to low activity. The data shown has been corrected for active site titration (see Figure S5).

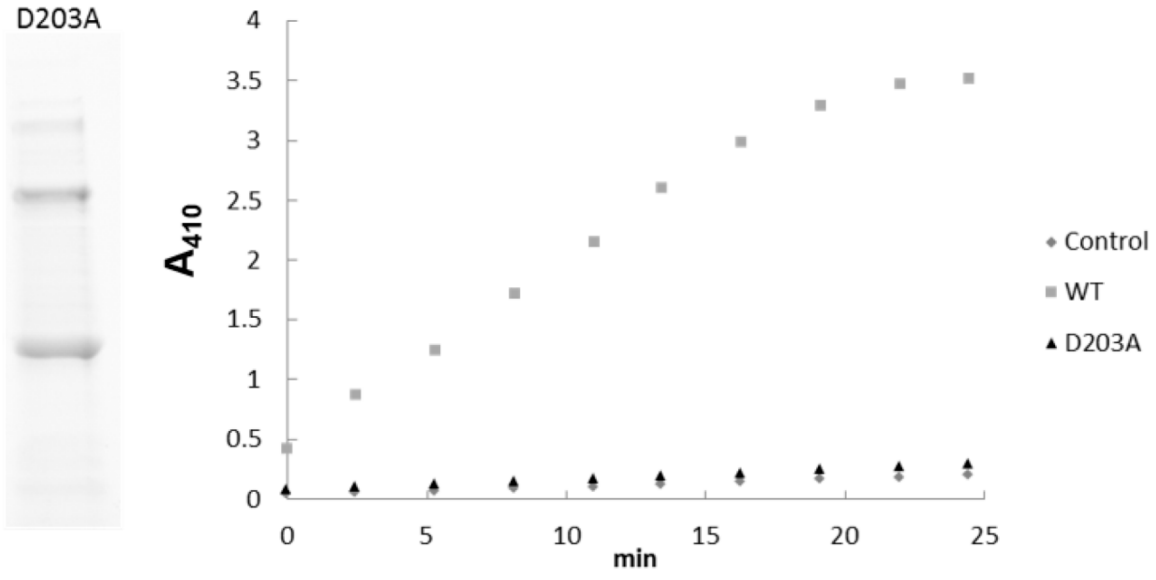

Figure S4. SDS-Page (left) and ester activity measurement (right) of the patatin knock-out D203A mutant (sequence numbering in our synthetic and truncated construct which thus corresponds to D215A in the structure PDB 1OWX). Buffer mixed with substrate was used as control.

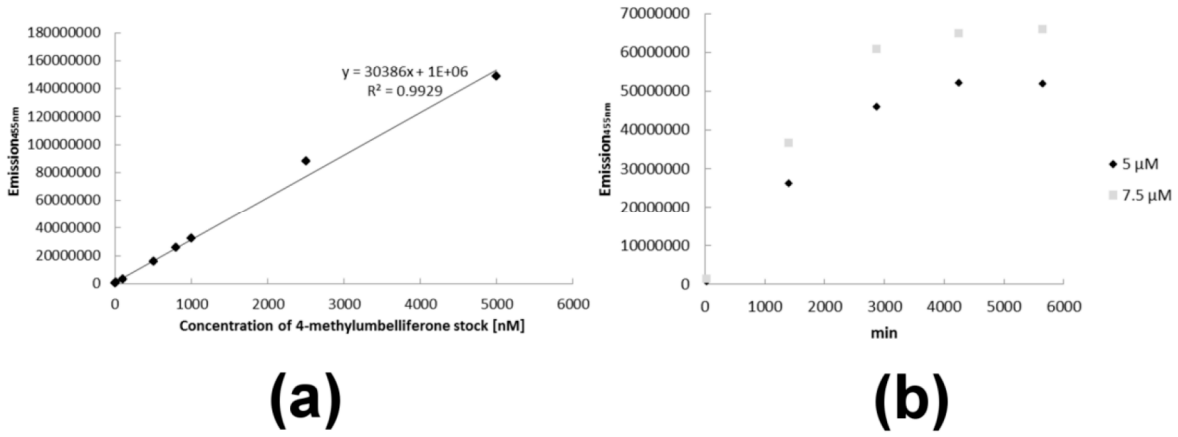

Figure S5. Active site titration of patatin. (a) Standard curve using different concentrations of 4-methylumbelliferone. (b) The fluorescence emission at 455 nm using two different amounts of enzyme (5 µM-diamonds and 7.5 µM-squares) incubated with the inhibitor 4-methylumbelliferyl hexylphosphonate is plotted against the reaction time. The background reaction (buffer + inhibitor) was subtracted from all samples. Saturation is achieved in both samples after approximately 4000 min.