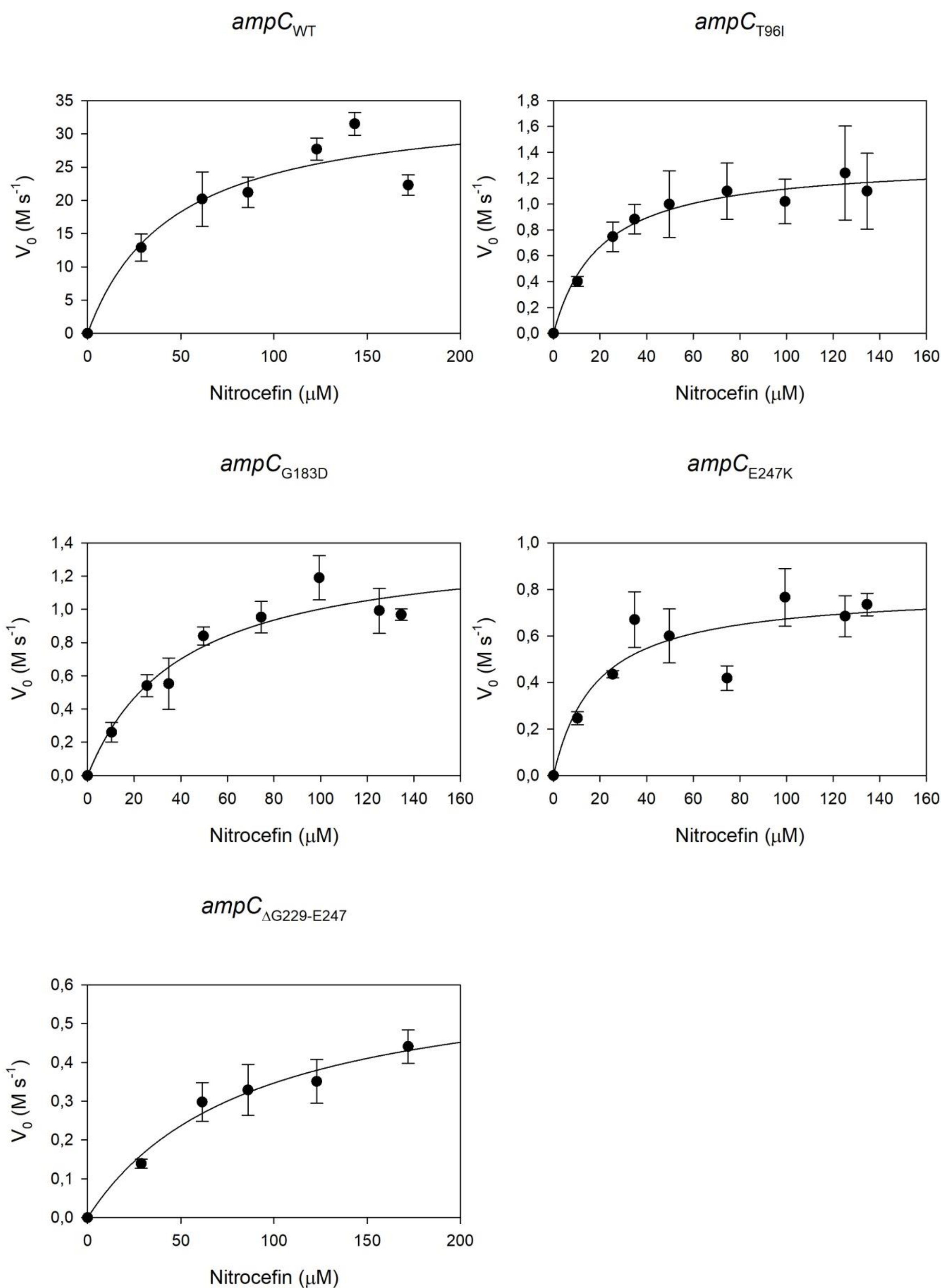



**Figure S1.** Michaelis–Menten plots of wild-type (WT) and mutant AmpC enzymes towards nitrocefin, used as enzymatic activity control. Data points represent averages of three technical replicates and error bars represent the standard deviation.

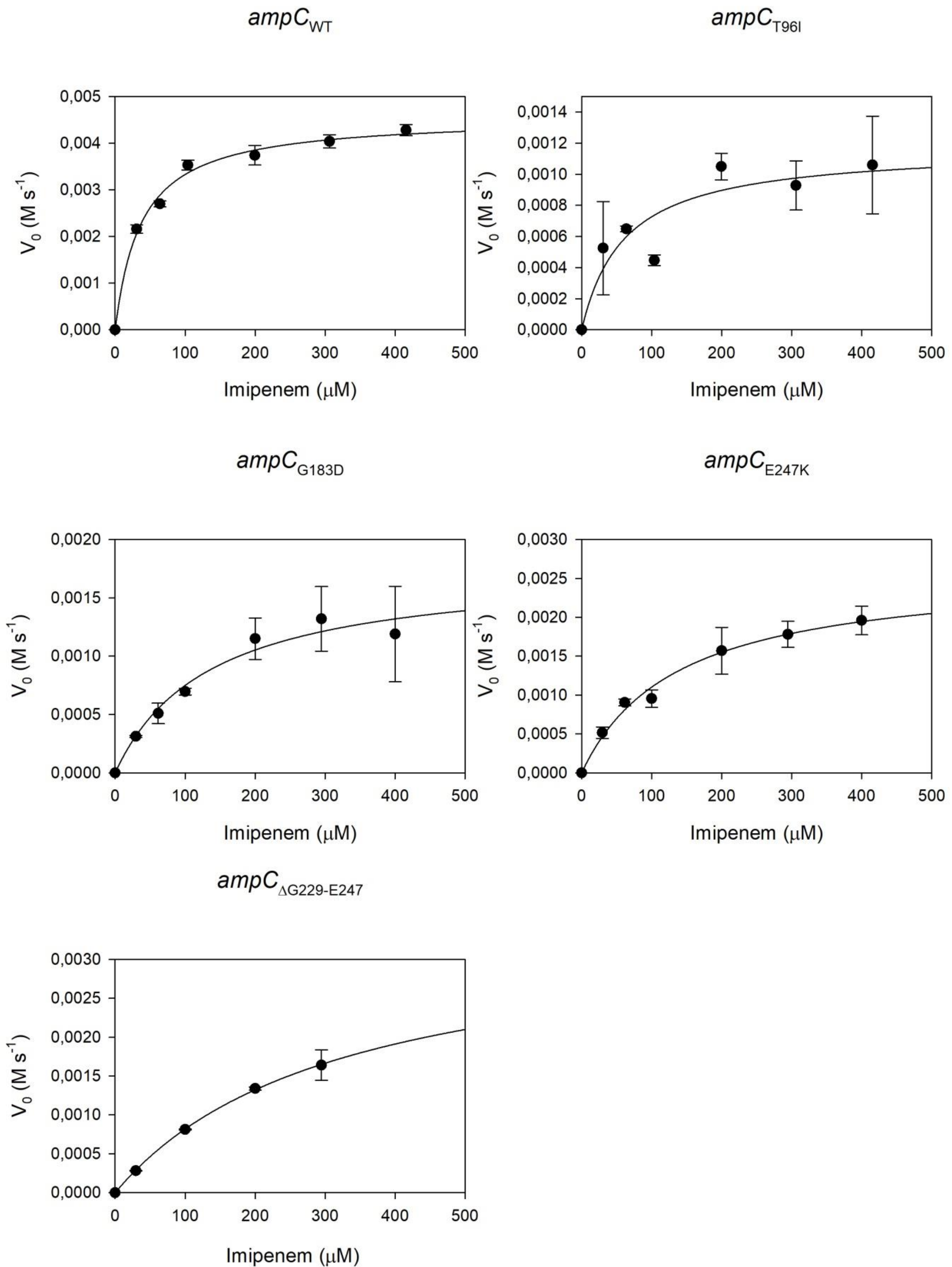



**Figure S2.** Michaelis–Menten plots of wild-type (WT) and mutant AmpC enzymes towards imipenem. Data points represent averages of three technical replicates and error bars represent the standard deviation.

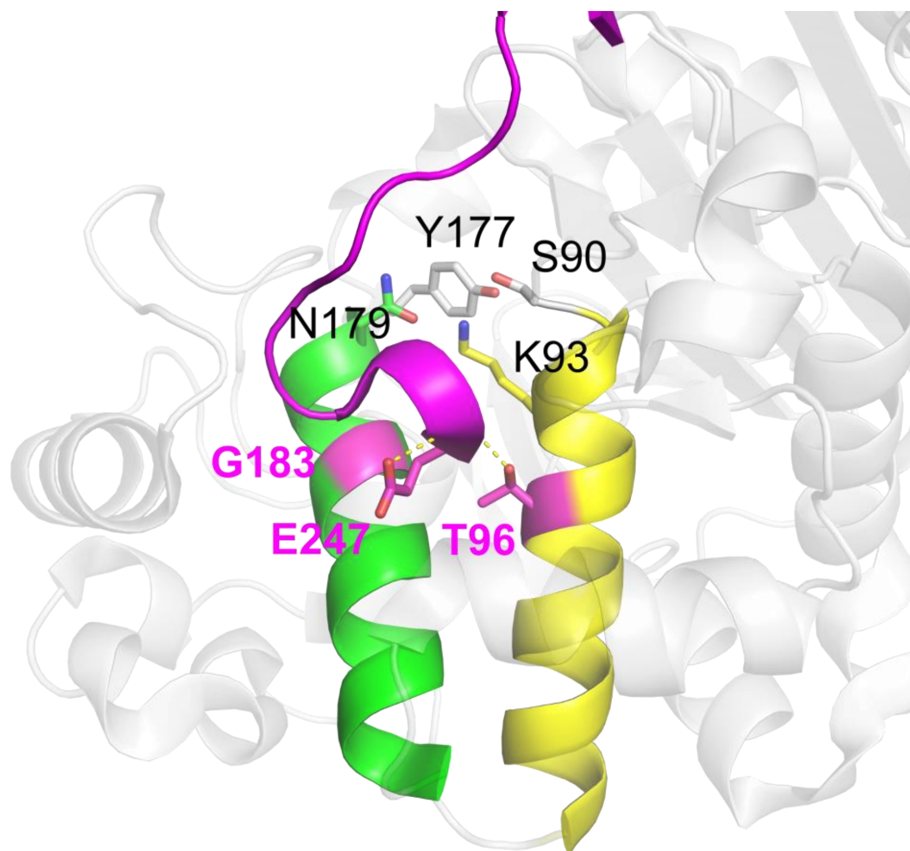
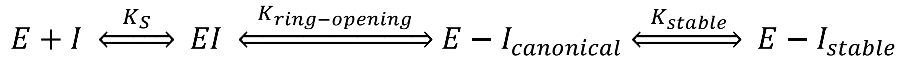
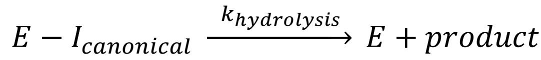



**Figure S3.** *Pseudomonas aeruginosa* PAO1 AmpC structure (PDB 4GZB). α-Helix (91-106) is colored yellow, α-Helix (154-165) is colored green, and Ω-loop (229-247) is colored magenta. Active site amino acids are at the end of the helices. The locations of mutations are labeled in magenta. Hydrogen bonds involving E247, T96 sidechains are indicated by yellow dotted line.

**Derivation of Equation-1.** If we accept Scheme-2 as the mechanism for imipenem degradation:




$$K_S = \frac{[E][I]}{[EI]} \qquad K_{ring-opening} = \frac{[EI]}{[E - I_{canonical}]} \qquad K_{stable} = \frac{[E - I_{stable}]}{[E - I_{canonical}]}$$

Therefore, if hydrolysis is the rate-determining step:

$$\frac{d[product]}{dt} = \frac{\frac{k_{hydrolysis}}{1+K_{ring-opening}+K_{stable}}[E]_{tot}[I]}{\frac{K_S K_{ring-opening}}{1+K_{ring-opening}+K_{stable}}+[I]}$$

Where $[E]_{tot}$ is total enzyme concentration.

And the experimentally determined $k_{cat}$ is equal to $\frac{k_{hydrolysis}}{1+K_{ring-opening}+K_{stable}}$.

And the experimentally determined $K_M$ is equal to $\frac{K_S K_{ring-opening}}{1+K_{ring-opening}+K_{stable}}$.