

An isothermal calorimetry assay for determining steady state kinetic and Ensitrelvir inhibition parameters for SARS-CoV-2 3CL-protease

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SUPPLEMENTARY INFORMATION

Figure 1-SI. Superimposition of the Michaelis-Menten profiles determined for 150 nM 3CL^{pro} by using the *inverse single-injection* method with 0.35 mM (black curve) and 0.15 mM (red curve) substrate.

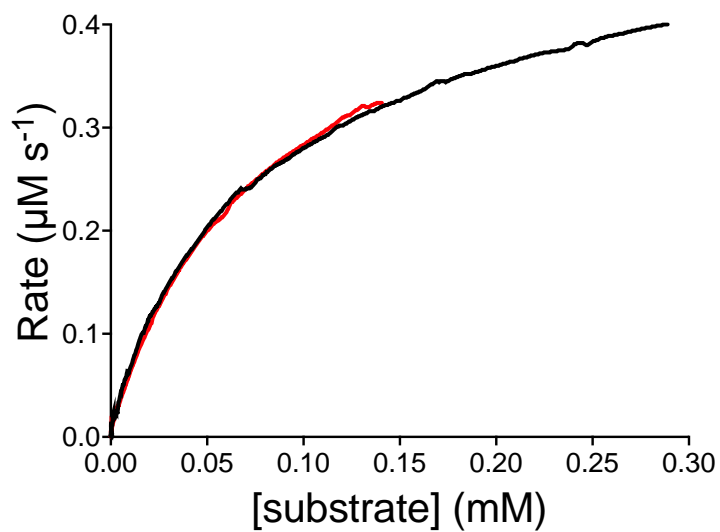


Figure 2-SI. Dependence of 3CL^{pro} V_{max} as a function of monomer concentration at pH 7.5.

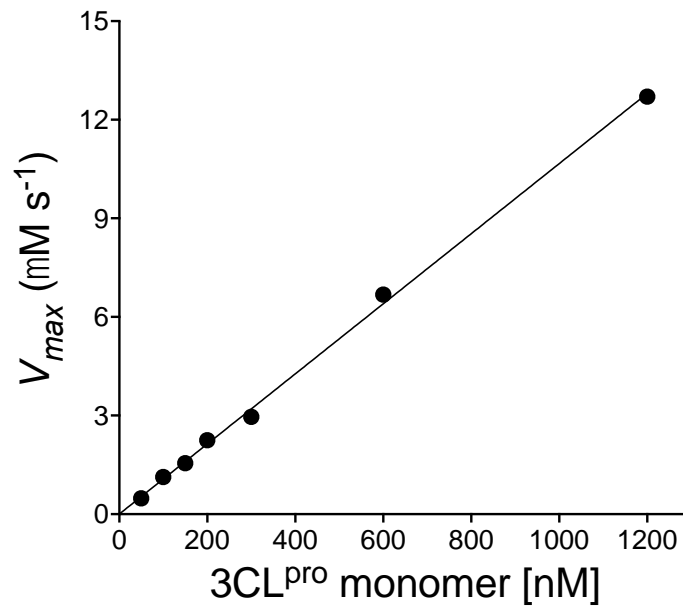


Figure 3-SI. Superimposition of the thermal power recorded over time corresponding to the enzyme activity of 150 nM (black) wild type 3CL^{pro} and its 36 μ M E290A/R298A double mutant (red).

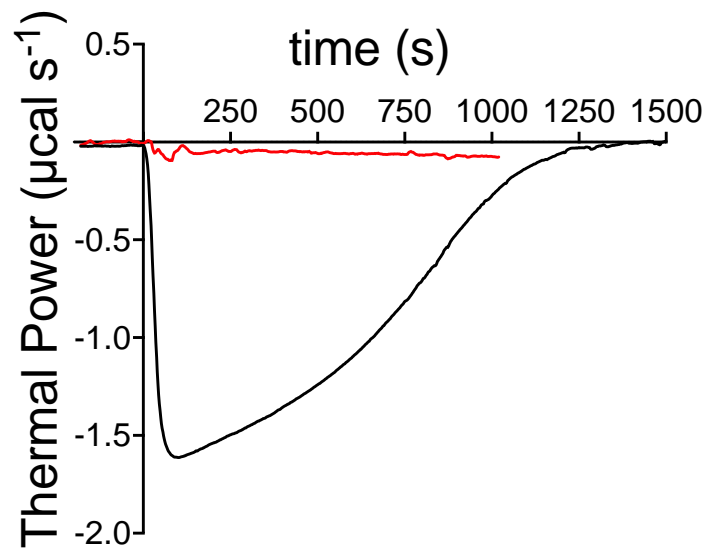
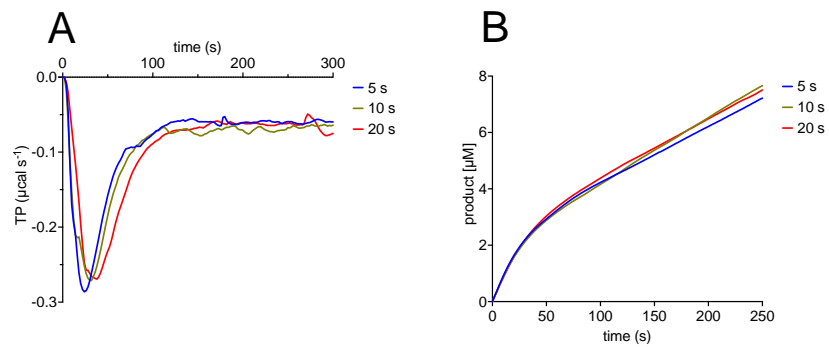


Figure 4-SI. Thermal power over time upon injection the enzyme solution (final concentration of 0.050 μM) to the sample cell containing a fixed concentrations of Ensitrelvir (0.10 μM) and substrate (0.60 mM) at the indicated injection rates (2.34 $\mu\text{L s}^{-1}$, 1.17 $\mu\text{L s}^{-1}$, and 0.59 $\mu\text{L s}^{-1}$, corresponding to 5 s, 10 s, and 20 s of injection time). A table reporting the derived values of v_i and v_s in all three cases is also shown.



	5 s	10 s	20 s
v_i	0.2735	0.2615	0.2565
v_s	0.06181	0.06454	0.0651