Biophysical Journal, Volume 98

Supporting Material

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Mutation of Glu-166 blocks the substrate-induced dimerization of SARS coronavirus main protease

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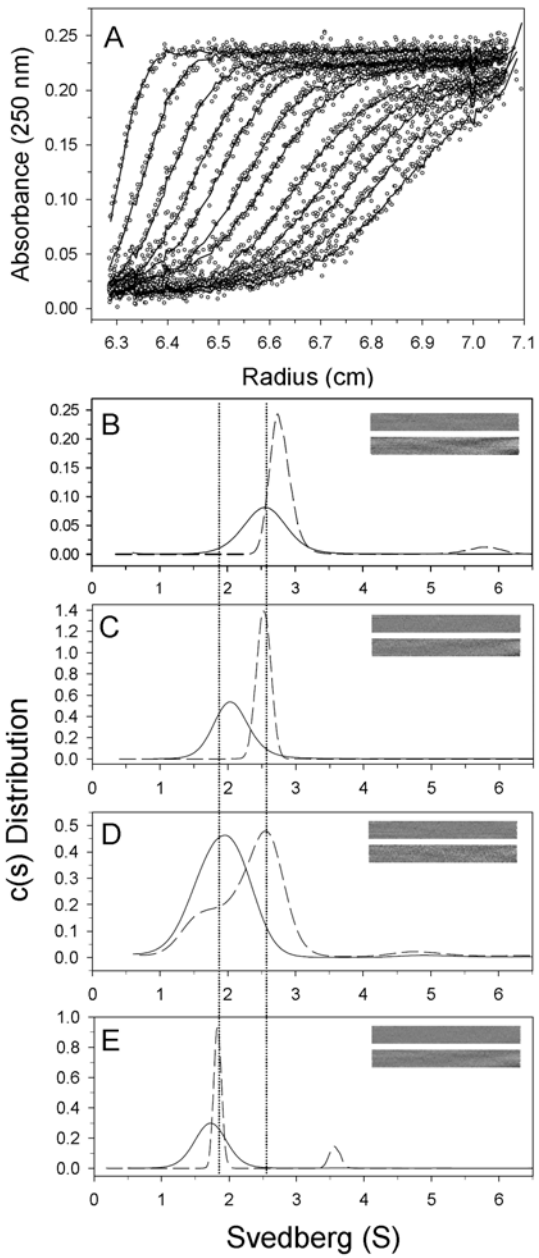
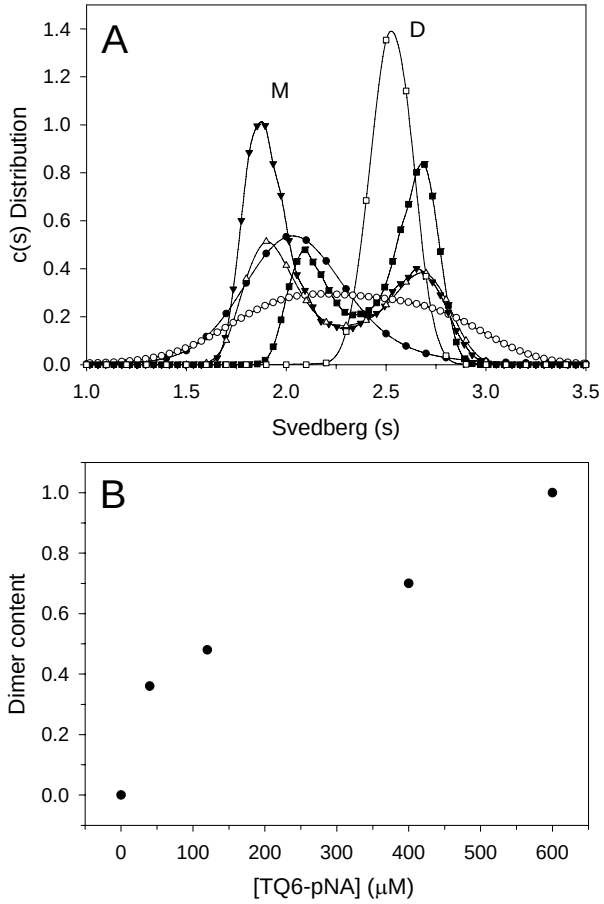
Running title: Substrate-induced dimerization of SARS-CoV main protease

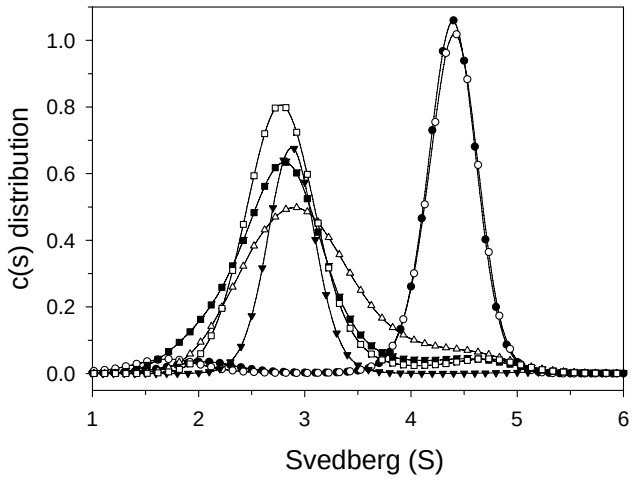
Containing 4 supplemental figures



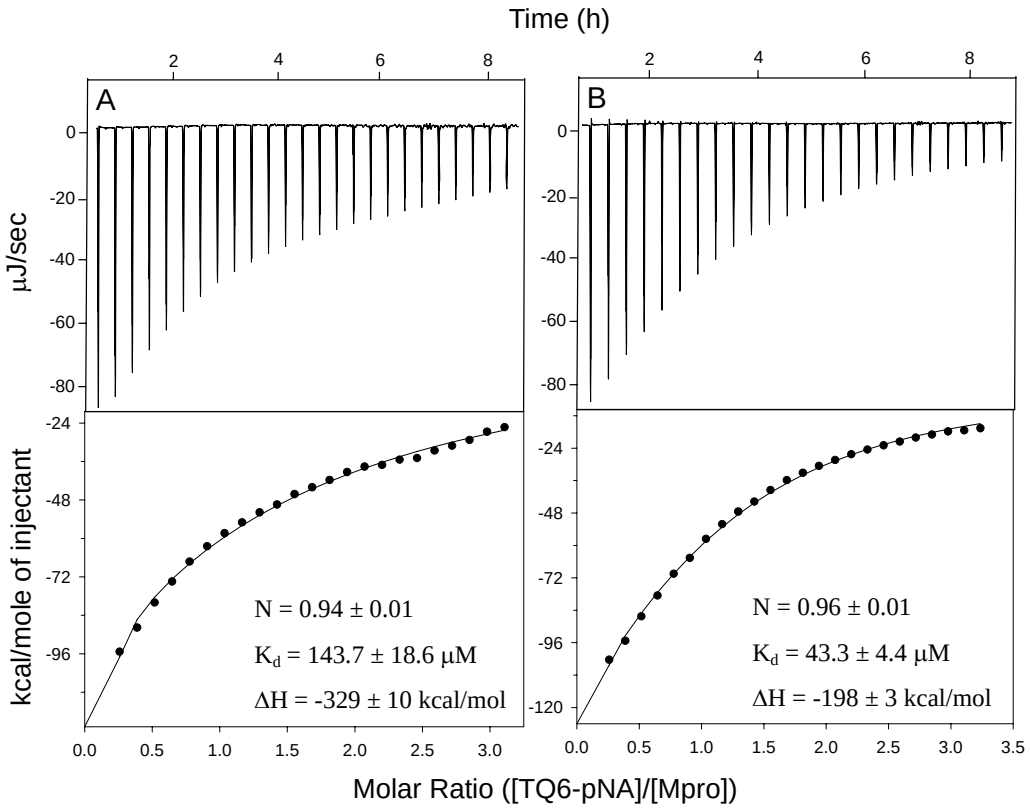
Supplement Fig. 1 Sedimentation velocity experiments of the SARS-CoV Mpro in D_2O. Panel A shows a typical trace of absorbance at 250 nm of the enzyme during the sedimentation velocity experiment. The symbols are experimental data, and the lines are the results fitted to the Lamm equation with SEDFIT program. Panel B-E show the continuous sedimentation coefficient distribution of wild type, R298A, R298L, and R298A/Q299A mutants at 0.2-1.0 mg/ml, respectively. The residual bitmaps of various proteases are shown in the insets. The distributions in D_2O are shown by solid line and those in D_2O with 600 μM TQ6-pNA substrate are by dashed line. The left dotted line indicates the monomer, and the right one is the dimer position.



Supplement Fig. 2 Effect of substrate concentration on the quaternary structure of SARS-CoV Mpro R298A mutants in D_2O. Panel A showed the continuous c(s) distribution of the main protease R298A mutant under TQ6-pNA concentration of 0 (filled circle), 20 μM (open circle), 40 μM (filled triangle), 120 μM (open triangle), 400 μM (filled square), and 600 μM (open square), respectively. The monomer and dimer species were labeled as M and D. Panel B showed the dimer content of R298A in different TQ6-pNA concentrations.



Supplement Fig. 3 Size distribution of SARS-CoV Mpro by AUC. The enzyme without or with substrate was injected into the HiTrap desalting column to remove substrates. The eluted protein was then analyzed by AUC and fitted to the continuous c(s) distribution model. The Mpro and its mutants without or with preincubation of 600 μM substrate were shown as follows: closed circles, wild type; open circles, wild type with substrates; closed triangles, R298A; open triangles, R298A with substrates; closed squares, R298A/Q299A; open squares, R298A/Q299A with substrates.



Supplement Fig. 4 Isothermal calorimetric titration for the substrate TQ6-pNA binding to Mpro E166A (A) and E166A/R298A (B) mutants. The protein concentration was 28.6 μM. The TQ6-pNA (1 mM) of 10 μl was titrated into 2.7 ml of protein solution using 25 injections. Values for the N, K_d and ΔH were determined by the ligand binding analysis with the Digitam program (TA instruments, New Castle, DE). The closed circles show the observed values and the lines represent fitted results.