

Supplementary Material

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Title: Structural Asymmetry of Procaspase-7 Bound to a Specific Inhibitor

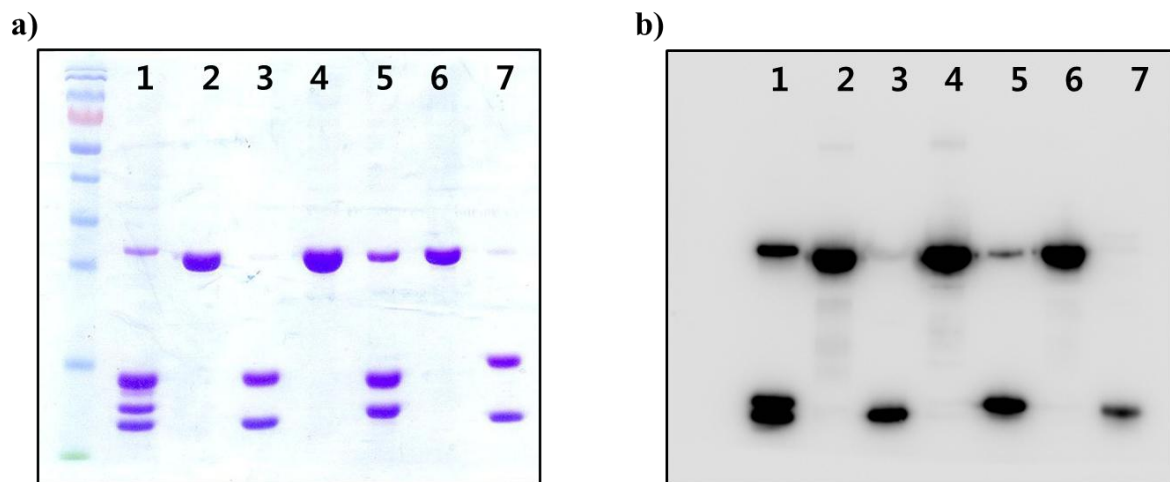


Figure S1. Western blot analysis of procaspase-7 variants. Purified caspase-7 variants (Protein **II**, **III**, and **IV**) were separated by 12% SDS-PAGE and transferred to PVDF membrane, blocked with 5% skim milk in PBST (PBS with 0.05% Tween 20) for 1 h at RT. After washed with PBST, the membrane was incubated with anti-caspase-7 antibody (Abcam, UK) for 1 h at RT, then incubated with horseradish peroxidase-conjugated anti-rabbit antibody (Abcam, UK) for 1 h, and washed with PBST. Blots were developed using enhanced chemiluminescence (ECL) detection system (Pierce, USA). 1, Protein **I** ($\Delta 46$ caspase-7); 2, Protein **II** ($\Delta 46/195\text{TS-6aa}$ procaspase-7); 3, $\Delta 46/195\text{TS-6aa}$ procaspase-7 (**II**) processed with thrombin; 4, Protein **III** ($\Delta 46/195\text{TS}$ procaspase-7); 5, Protein **III** ($\Delta 46/195\text{TS}$ procaspase-7) processed with thrombin; 6, Protein **IV** ($\Delta 46/\text{D198A}/205\text{TI}$ procaspase-7); 7, $\Delta 46/\text{D198A}/205\text{TI}$ procaspase-7 (**IV**) processed with thrombin. a) SDS-PAGE analysis. b) Western blot analysis (probed with anti-caspase-7 antibody).

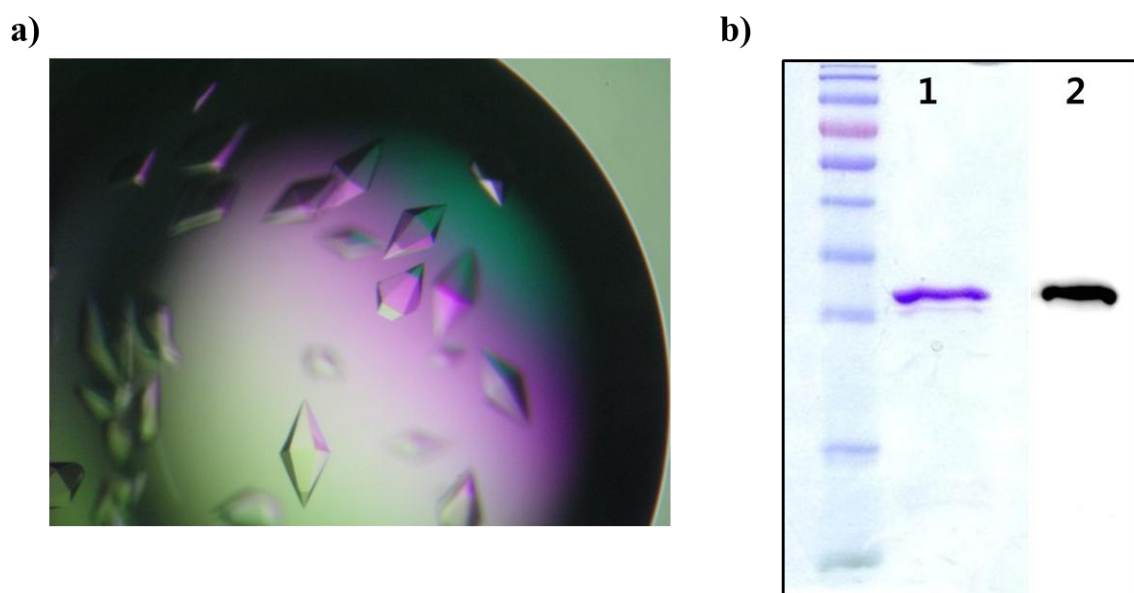


Figure S2. SDS-PAGE and Western blot analysis of inhibitor-bound $\Delta 46/D198A/205TI$ procaspase-7 crystal. a) Crystals of Protein **IV** bound to Ac-DEVD-CHO. b) Western blot analysis (1, SDS-PAGE; 2, Western blot). Crystals were washed with reservoir solution and dissolved in SDS-PAGE sample buffer (60 mM Tris-Cl pH 6.8, 25% glycerol, 2% SDS, 14.4 mM 2-mercaptoethanol, 0.1% bromophenol blue), which was separated by two sets of 12% SDS-PAGE. The protein on one gel was stained with coomassie brilliant blue (a), and the protein on the other gel was transferred to PVDF membrane, which was blocked with 5% skim milk in PBST (PBS with 0.05% Tween 20) for 1 h at RT. The membrane was probed using anti-caspase-7 antibody (Abcam, UK) and secondary antibody and the blot was developed using ECL detection system (Pierce, USA) (b).

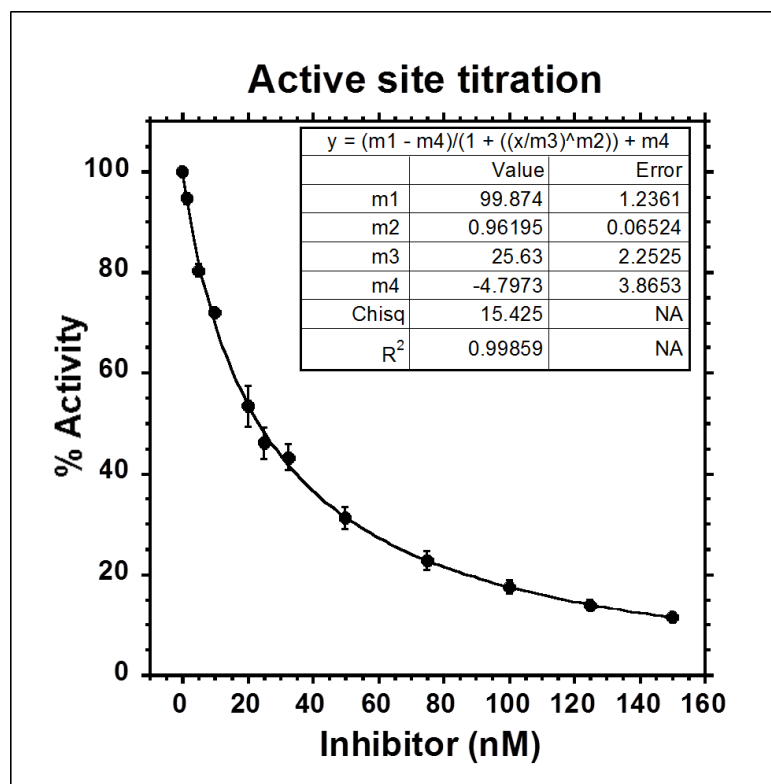


Figure S3. Active site titration of $\Delta 46/D198A/205TI$ procaspase-7 (Protein **IV**) using Ac-DEVD-CHO. Protein **IV** (100 nM) was treated with Ac-DEVD-CHO (0, 1.25, 5, 10, 20, 25, 32.5, 50, 75, 100, 125, 150 nM) for 30 min on ice. The remaining activity was determined using colorimetric caspase substrate, Ac-DEVD-*p*NA, as described in Materials and Method. IC₅₀ was determined by fitting the data in Figure S3 to a four parameter logistic equation ($y = (m1 - m4)/[1 + (x/m3)^{m2}] + m4$; m1: estimated response at zero concentration, m2: slope factor, m3: mid-range concentration, m4: estimated response at infinite concentration, y: % activity in the enzyme reaction rate, x: inhibitor concentration) (Finney 1983) using Kaleida Graph 4.0 (Synergy Software).

Finney, D.J. (1983). *Clin. Chem.* **29**, 1762-1766.