

SUPPLEMENTARY MATERIAL

Methods

Enzymatic activity. For catalytic parameters at different concentrations of Na-citrate (Supplementary Figure 1A), caspase-8 (20 nM) was diluted in low-salt assay buffer containing Na-citrate in the concentration range of 0-1.1 M and incubated for 4 h at 37°C prior to addition of the substrate (1-300 μ M).

For activity in the presence of chaotropic salts, a mixture of monomer and dimer caspase-8 (100 nM) was incubated for 15 min at 37°C in low salt assay buffer containing NaCl (control), NaClO₄, MgCl₂, CaCl₂ and NaNO₃ at final concentrations between 0-1.5 M and the activity was measured by adding 100 μ M Ac-IETD-AFC.

Caspase-3 and caspase-7 at 1 nM were incubated in low-salt assay buffer containing Na-citrate between 0-1.4 M and incubated at 37°C for 15 minutes. The activity was determined by addition of 100 μ M Ac-DEVD-AFC. For the kinetics experiments, the caspases were incubated in low-salt and high salt assay buffer and at the specified times (Supplemental Figure 2B), the activity was determined as above.

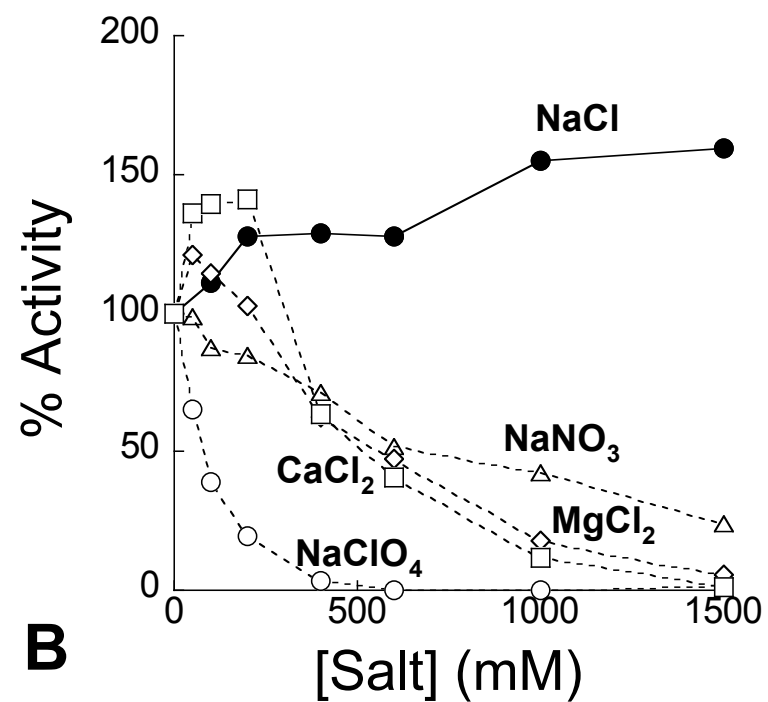
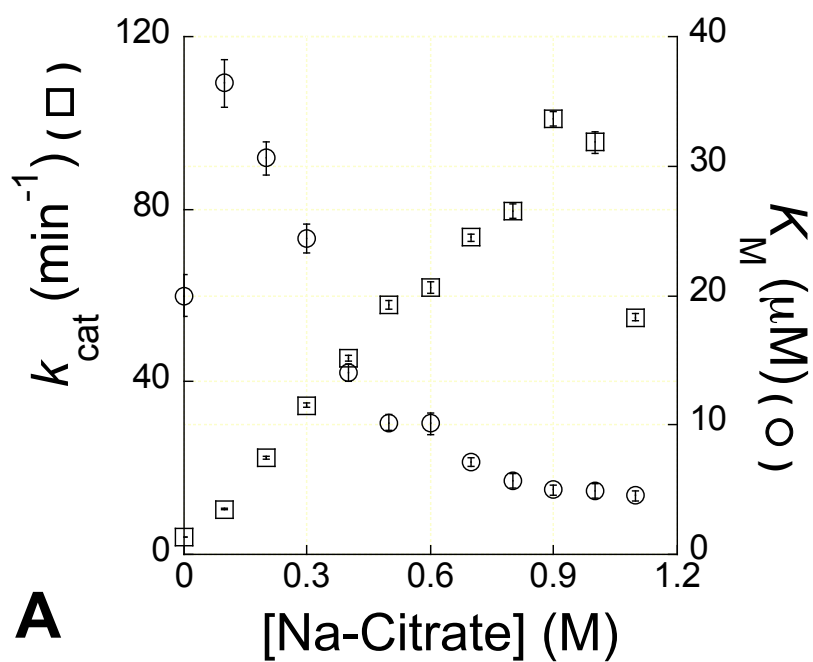
Supplementary Figure Legends

Supplementary Figure 1. A. Value change of the catalytic constant, k_{cat} (\square), and the Michaelis-Menten constant, K_M (\circ), for the activity of wild-type caspase-8 with the increase in concentration of the Na-citrate in assay buffer. B. Inactivation of caspase-8 by chaotropes. Caspase-8 was incubated in assay buffer containing the specified concentrations of chaotropic salts and the percentage of remaining activity is displayed. The control NaCl is a “neutral” salt for the effect upon the protein structure reorganization.

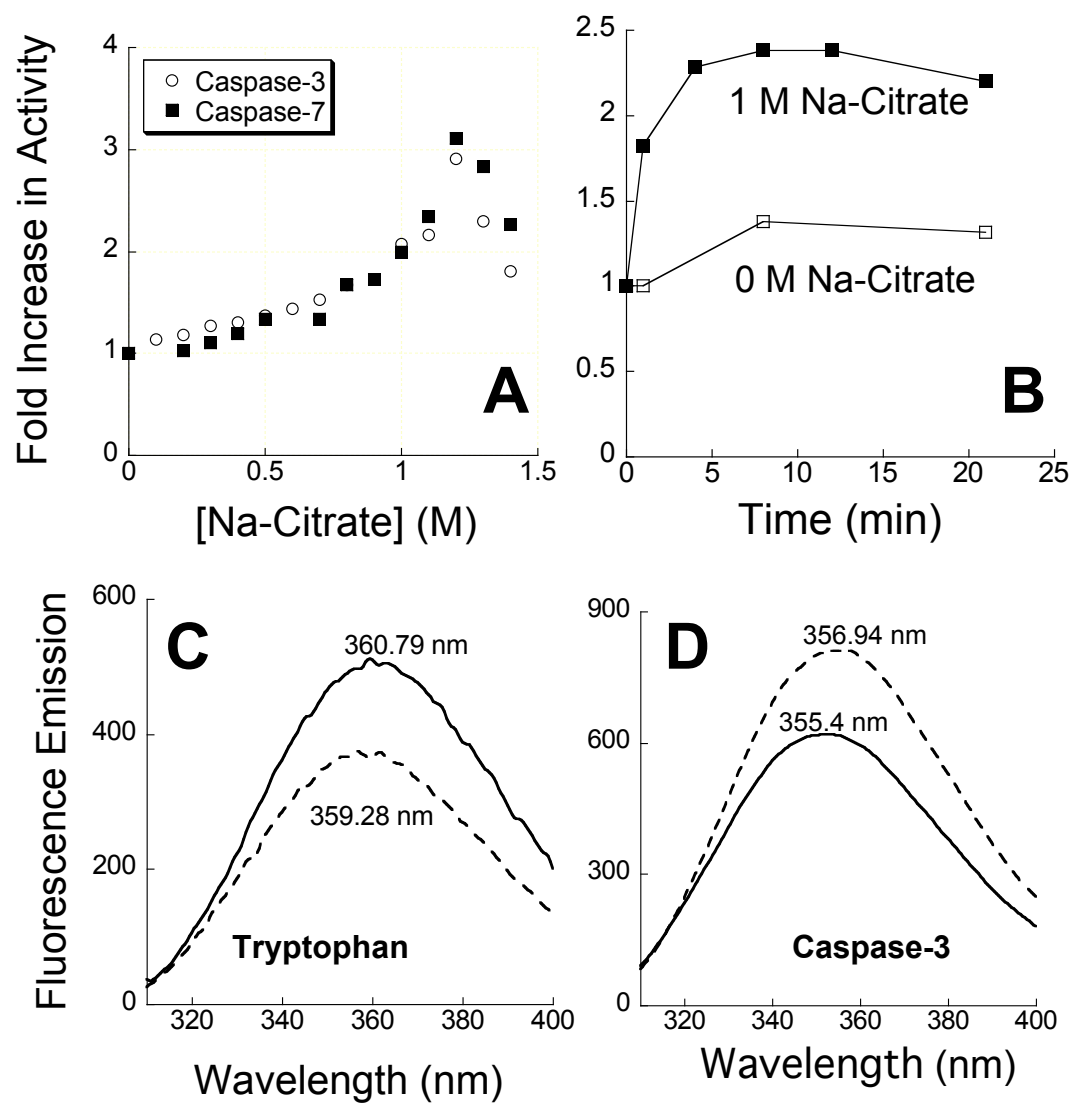
Supplementary Figure 2. A. Fold increase in the activity of caspase-3(\circ) and caspase-7(\blacksquare) after incubation in Na-citrate at the specified concentrations. B. Kinetics of caspase-3 (1 nM) activation in 1 M Na-citrate (\blacksquare) is a fast process. The control for caspase-3 activity in low-salt assay buffer (\square) is shown. C, D. Emission fluorescence spectra of tryptophan (0.5 μM) and caspase-3 (0.5 μM) diluted in phosphate buffer (interrupted lane) or 1 M Na-citrate (solid lane) after excitation at 295 nm.

Supplementary Figure 3. Casp8-D₂A (1 μM) kinetics of activation and average emission change, $\langle\lambda\rangle$ (A) or maximum fluorescence change (B) in 1 M Na-citrate. C. Casp8-WT (1 μM) kinetics of activation and average emission change in 0.7 M Na-citrate. D. Average emission wavelength in the presence of Na-citrate (\blacksquare) versus NaCl (\square) for Casp8-D₂A (1 μM). Interrupted line represents the caspase activity at the specified concentrations of Na-citrate.

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3

