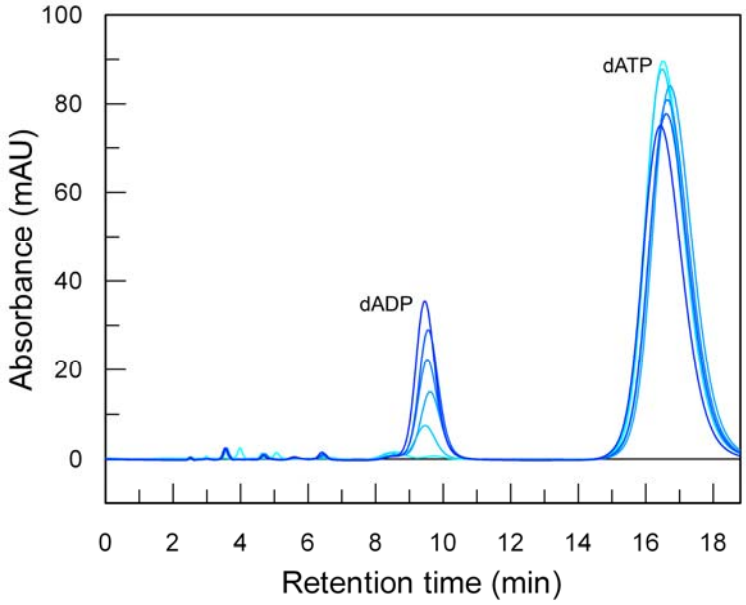

Figure S1. dADP increase as a result of hydrolytic activity of Apaf-1. Samples of a reaction mixture containing 10 µM Apaf-1 and 1 mM dATP were analyzed by HPLC at t = 0, 1, 2, 3, 4, and 5 h. Darker hues correspond to later time points.

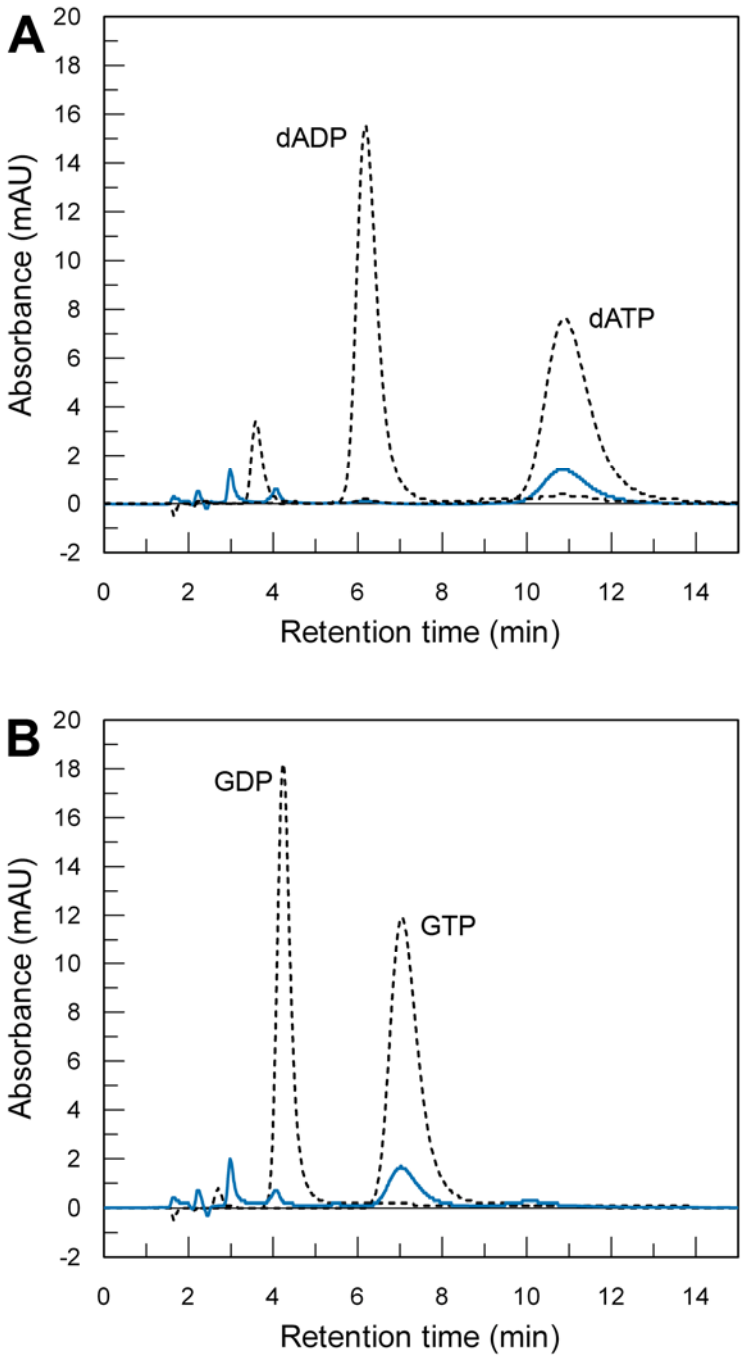

Figure S2. Hydrolytic activity of apoptosomes. Apoptosomes assembled with dATP (A) or GTP (B), respectively, were isolated via gel filtration and analyzed by HPLC. The blue curves correspond to the nucleotide extracted from the respective apoptosomes, the black dotted curves correspond to the respective di- and triphosphates measured as references.

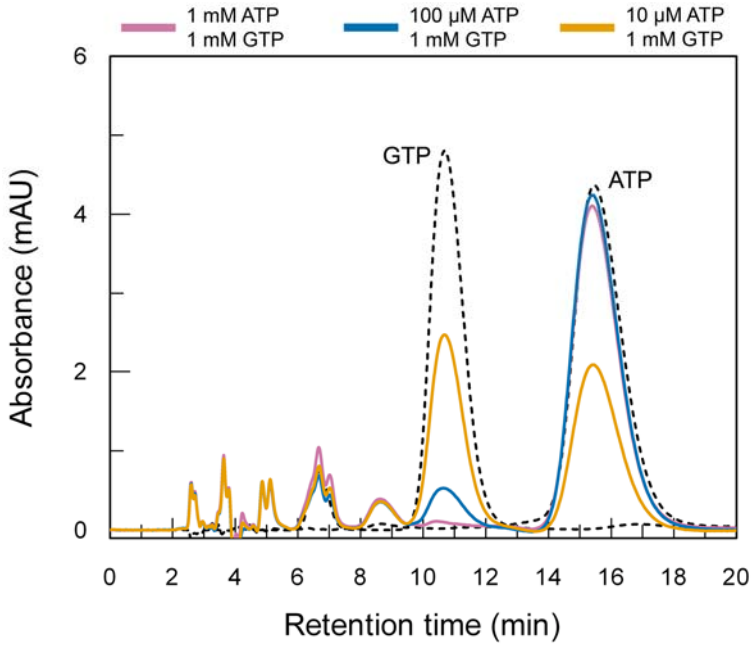

Figure S3. Nucleotide content of apoptosomes assembled with different ratios of ATP and GTP. Apoptosomes assembled with different ratios of ATP and GTP were isolated via gel filtration and analyzed by HPLC (flow rate 1.0 ml/min).

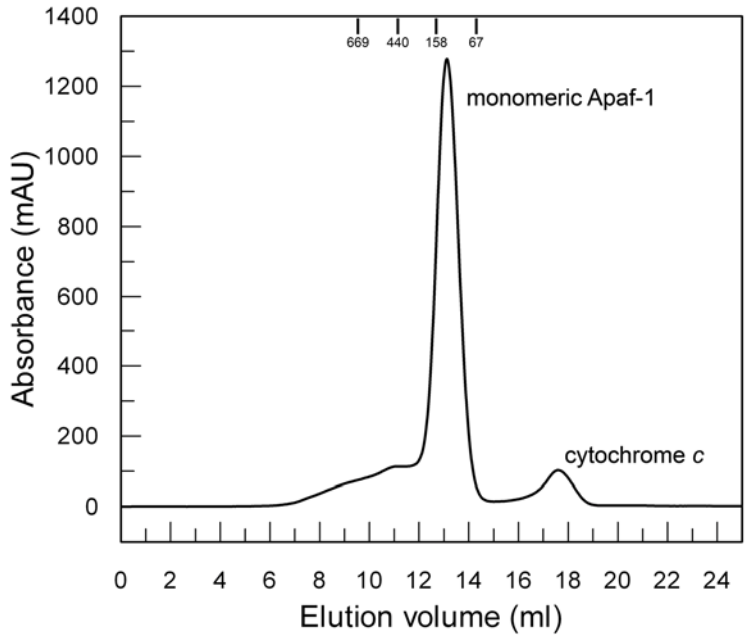

Figure S4. Gel filtration elution profile of apoptosomes run in high salt buffer. Apoptosomes were applied to a Superdex S200 gel filtration column equilibrated with buffer containing 1 M NaCl. The eluate contains monomeric Apaf-1 and cytochrome *c*. The elution volumes of globular marker proteins are indicated.

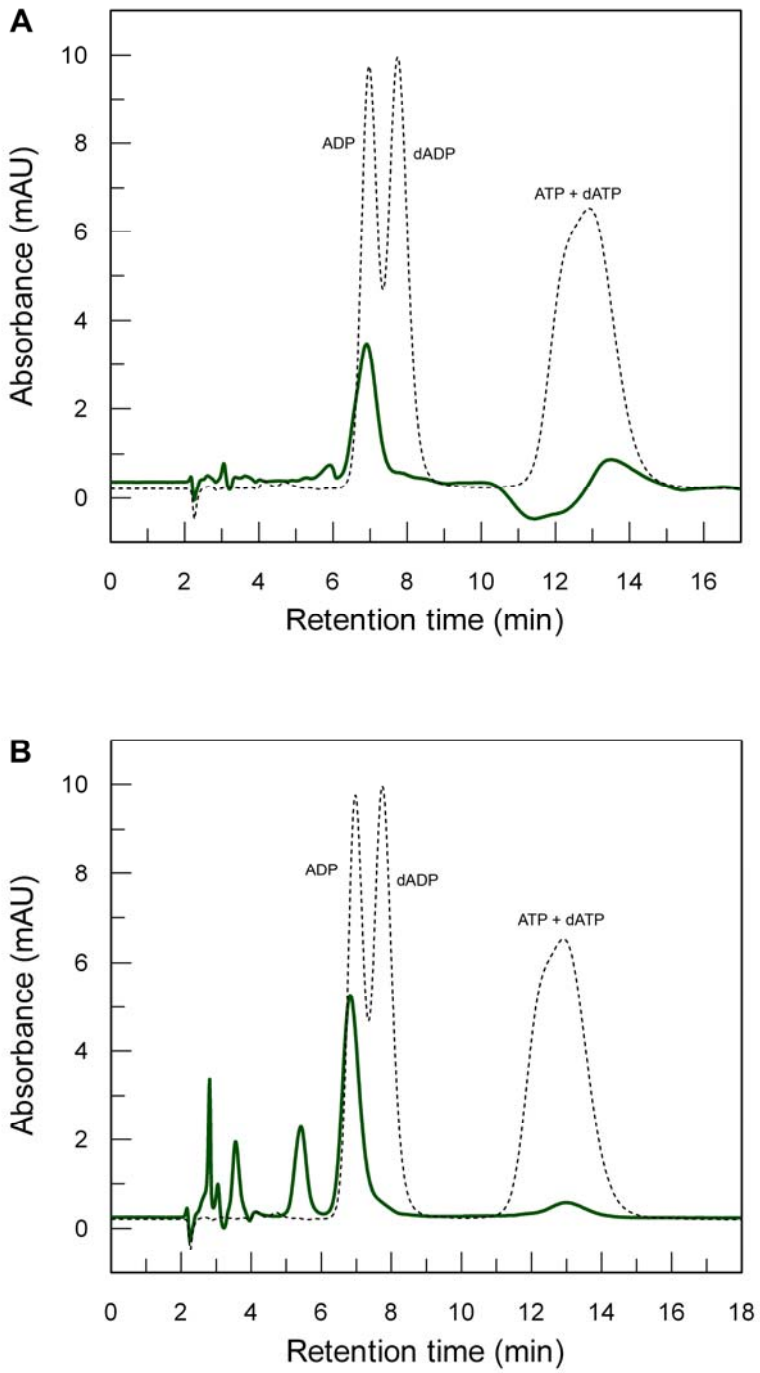

Figure S5. Nucleotide extracts from purified Apaf-1 using alternative denaturation methods. Apaf-1 was dialyzed against buffer containing 50 mM HEPES (pH 7.5) and 50 mM NaCl to remove potential contaminating free nucleotide. (A) The protein was then treated with phenol/chloroform/isoamyl alcohol and the aqueous phase was subjected to HPLC (as described in *Experimental procedures*). (B) The dialyzed protein was directly subjected to HPLC, i.e. the protein was denatured on the pre-column.