SUPPORTING INFORMATION

Table 1: Primers for mutants of β′ and α

	Sequence of the primers (from 5′-3′)
Y138F-β′ (f)[a]	GAATGTTCACTCAGAGATG**TTC**AGTTTGCTGATAGACAC
Y138-β′ (r)[b]	GTGTCTATCAGCAAACT**GAA**CATCTCTGAGTGAACATTC
C218S-α (f)	CCGCCCACAACTTTCTAGC**TCT**TTTCTTCTGAGTATG
C218S-α (r)	CATACTCAG AAGAAAA**AGA**G CTAGAAAGTT GTGGGCGG
C218A-α (f)	CCGCCCACAACTTTCTAGC**GCT**TTTCTTCTGAGTATG
C218A-α (r)	CATACTCAG AAGAAAA**AGC**GCTAGAAAGTT GTGGGCGG
C429S-α (f)	GCAGCAACCTG**TCC**ACAGAAATAGTGGAGTACACC
C429S-α (r)	GGTGTACTCCACTATTTCTGT**GGA**CAGG TTGCTGC
C429A-α (f)	CCATCAAATGCAGCAACCTG**GCC**ACAGAAATAGTGGAG
C429A-α (r)	CTCCACTATTTCTGT**GGC**CAGGTTGCTGCATTTGATGG
C444S-α (f)	GAGGTTGCTGTT**TCT**AATTTGGCTTCCCTGGCCC
C444S-α (r)	GGGCCAGGGAAGCCAAATT**AGA**AACAGCAACCTC
C444A-α (f)	GAGGTTGCTGTT**GCT**AATTTGGCTTCCCTGGCCC
C444A-α (r)	G GGCCAGGGAAGCCAAATT**AGC**AACAGCAAC CTC
C787S-α (f)	GGAGAATAGAGATGAA**TCT**CTGATGTGTGGATCCTG
C787S-α (r)	CAGGATCC ACACATCAG**AGA**TTCATCTC TATTCTCC
C787A-α (f)	GCTCTTTGGAGAATAGAGATGAA**GCT**CTGATGTGTGGATCCTG
C787A-α (r)	CAGGATCC ACACATCAG**AGC**TTCATCTCTATTCTCCAA AGAG
C790S-α (f)	GGAGAATAGAGATGAATGTCTGATG**TCT**GGATCCTG
C790S-α (r)	CAGGATCC**AGA**CATCAGACATTCATCTCTATTCTCC
C790A-α (f)	GCTCTTTGGAGAATAGAGATGAATGTCTGATG**GCT**GGATCCTG
C790A-α (r)	CAGGATCC**AGC**CATCAGACATTCATCTC TATTCTCCAAAGAGC
E431Q-α (f)	GCAACCTGTGCACA**CAA**ATAGTGGAGTACACC
E431Q-α (r)	GGTGTACTCCACTAT**TTG**TGTGCACAGG TTGC
E431D-α (f)	GCAACCTGTGCACA**GAC**ATAGTGGAGTACACC
E431D-α (r)	GGTGTACT CCACTAT**GTC**TGTGCACAGG TTGC

The mutation sites are highlighted.
[a] forward primer (f).
[b] reverse primer (r).

Figure S1. Analysis by HPLC of products generated during inactivation of $\alpha_n(\beta'2)_m$ by [5-^{3}H]-F_2CDP.The inactivation mixture contained final concentrations of α, β', 1.2 μM; [5-^{3}H]-F_2CDP, 1.2 μM, 3mM ATP, 5mM DTT. Subsequent to removal of the protein from the small molecules by YM3 centricon and removal of the phosphates with alkaline phosphatase, the filtrate was injected into an Altech Adsorbosphere Nucleotide Nucleoside C-18 column at a flow rate of 1mL/min. The elution buffer contained: Buffer A, 10 mM NH_4OAc, pH 6.8; Buffer B: 100% methanol. A 10 min isocratic elution was followed by a linear gradient to 40% B over 30 min. The elution profile monitored by A_{267nm} (−) and scintillation counting (■). Cytosine (0.65 eq.) was released (retention time 10.5 min) and 1.35 eq. of F_2C (retention time 30.5 min) was recovered.

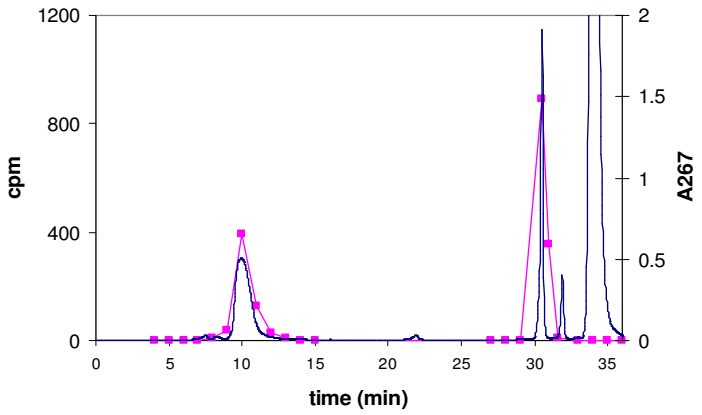

Figure S2. SDS-PAGE analysis of the α/β (6 μM) inactivated by F_2CDP (30 μM) and ATP (3 mM) at 37 °C for 5 min. Each sample was mixed with 2x loading buffer ± β-ME or boiling for 2 min before loading as indicated. A band at 120 kDa is observed in Lanes 2, 3 (inactivation in the presence of DTT, without boiling) and Lanes 5, 6 (inactivation in the absence of DTT, without boiling).

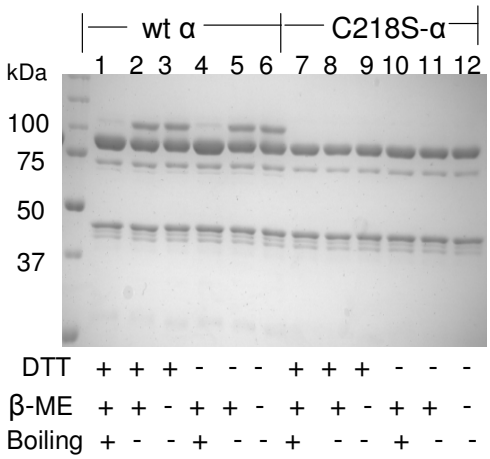

Figure S3. Gel filtration molecular weight standards (GE healthcare). Ovalbumin, 43 kDa; conalbumin, 75 kDa; aldolase, 158 kDa; catalase 232 KDa; ferritin, 440 kDa; and blue dextran, 2000 kDa (♦ from left to right).

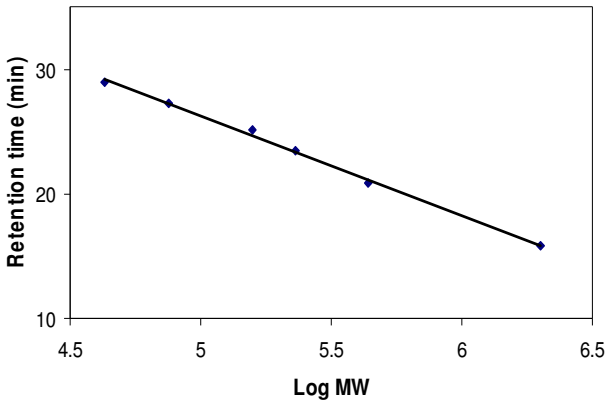

Figure S4. SEC of a control with β′2, α and α2 with TTP. (A) β′2 (81 kDa) migrates at 26 min with an apparent MW 116 kDa. A S200 column (GE healthcare) was used. (B) α (25 mM DTT). The first peak that elutes at 21.7 min is a contaminant that co-purifies with α, the second protein elutes at 25.6 min, with apparent MW 88 kD. (C) α2 (100 μM TTP). The protein peak that elutes at 22 min is the contaminant that co-purifies with α. The second protein elutes at 24 min with an apparent MW 189 kD. Superose 12 column was used in (B) and (C).

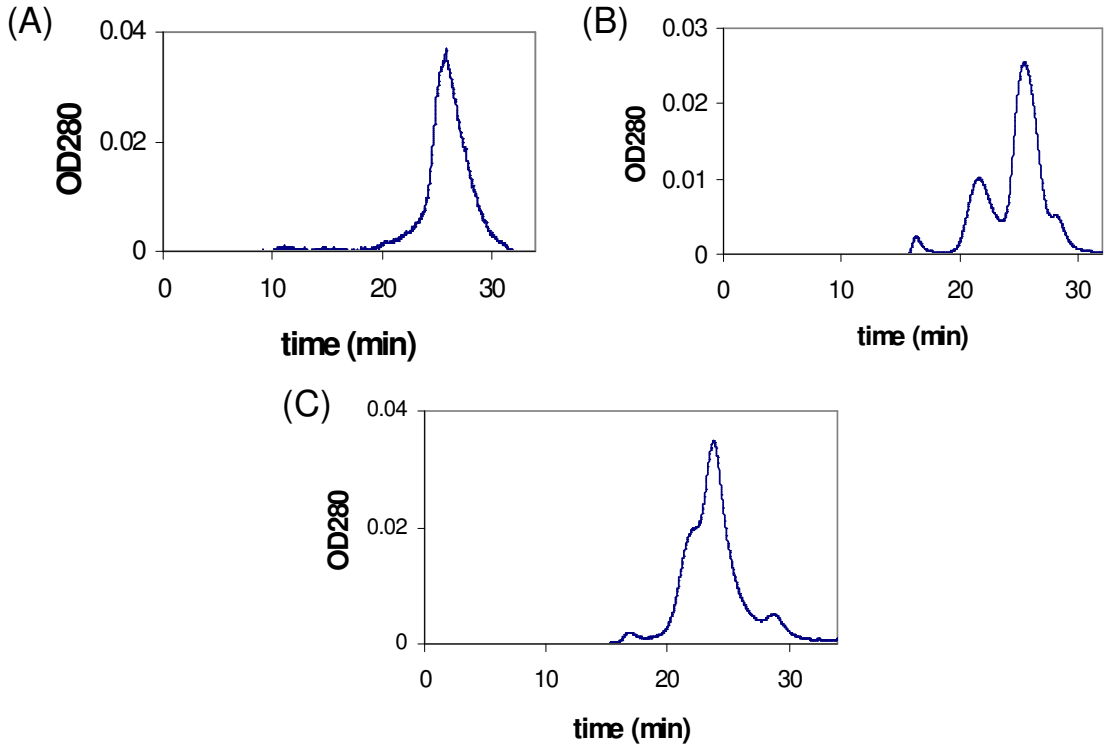

Figure S5. *E. coli* α and β (0.05- 0.4 μM) standards at different concentrations analyzed by SDS-PAGE to determine the ratio of α, β′ in Figure 3B.