SUPPLEMENTARY DATA

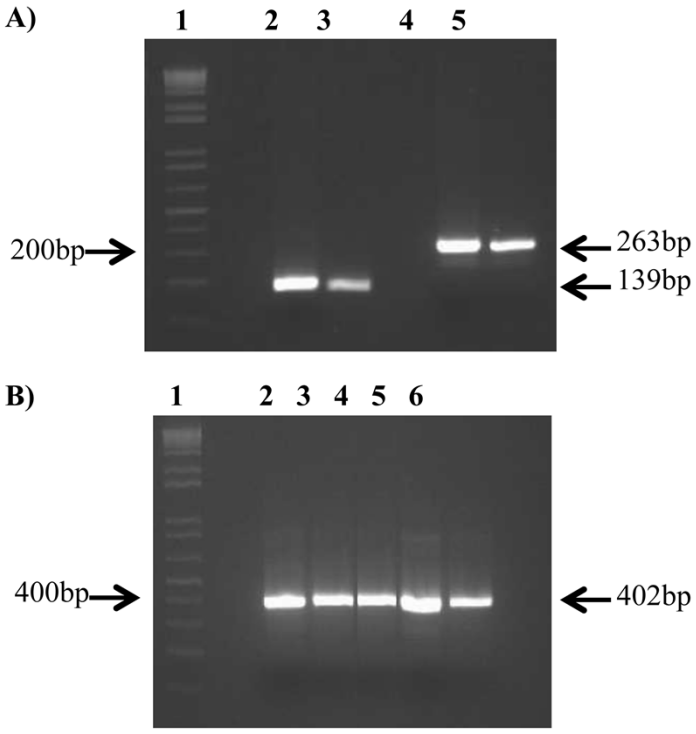

Figure S1. Analysis of mutagenesis PCR products on a 1% agarose gel stained with GelRed™ stain. (A) Two simultaneous PCR reactions were performed to amplify each DNA fragment during the first step PCR. Line 1, 1 kb plus DNA Ladder; Lanes 2-3, PCR product (139bp) obtained with the forward flanking primer (A) and a reverse mutagenic primer (B) for two substitutions (E47D and E47L respectively); Lanes 4–5, PCR product (263bp) obtained with a reverse flanking primer (D) and a forward mutagenic primer (C) for two substitutions (E47D and E47L respectively).(B) Synthesis of the full-length mutated PCR product using the gel purified DNA fragment from the first step PCR and the flanking primers. Lanes 1, 1 kb plus DNA Ladder; Lanes 2-6, full-length mutated of E47A, E47D, E47H, E47L and E47Q DNA fragments respectively.

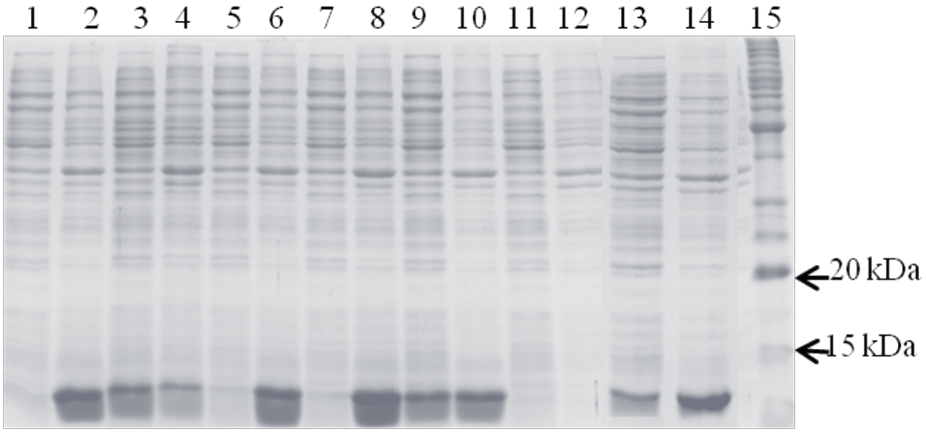

Figure S2. 15% SDS-PAGE analysis of expression of mutant CDA proteins. SF is for soluble fraction and IF for insoluble fraction. Expression of mutants in LB medium (E47d and E47Q) and in TB medium (E47L) plus 6% DMSO after 6h cell growth at 37 °C. Lane 1, E47A (SF); Lane 2, E47A (IF); Lane 3, E47D (SF); Lane 4, E47D (IF); Lane 5, E47H (SF); Lane 6, E47H (IF); Lane 7, E47L (SF); Lane 8, E47L (IF); Lane 9, E47Q (SF); Lane 10, E47Q (IF); Lane 11, pET-23a (+) (SF); Lane 12, pET-23a (+) (IF); Lane 13, E47L (SF); Lane 14, E47L (IF); Lane 15, MW Bench marker (Invitrogen).

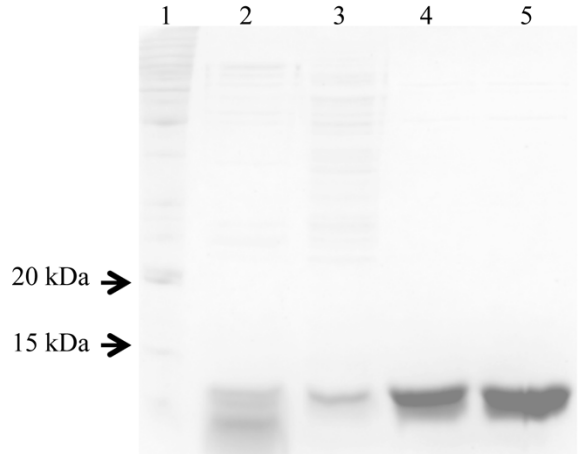

Figure S3. 15% SDS-PAGE of three recombinant proteins purified by liquid chromatography. Lane 1, MW Bench marker (Invitrogen); Lane: 2, E47D (13.91 kDa); Lane 3, E47L (13.92 kDa); Lane 3, E47Q (13.92 kDa); Lane 4, wild-type *Mt*CDA (13.93 kDa).

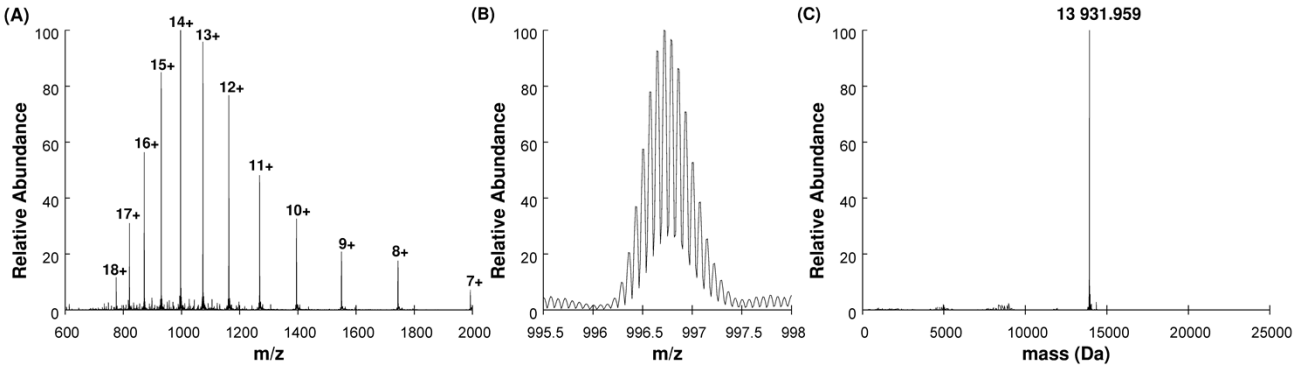

Figure S4. Determination of wtCDA monoisotopic molecular mass by mass spectrometry analysis. (A) ESI-FTMS spectra of wild-type *Mt*CDA spanning from charge state 7+ to 18+. (B) Isotopic envelope of charge state 14+. (C) Deconvoluted spectra of wild-type *Mt*CDA resulted in a peak corresponding to a monoisotopic molecular mass of 13 931.9586 Da. This value matches the theoretical monoisotopic mass for wild-type *Mt*CDA (13 931.9575 Da) with sub parts-per-million mass accuracy (0.0789 ppm).

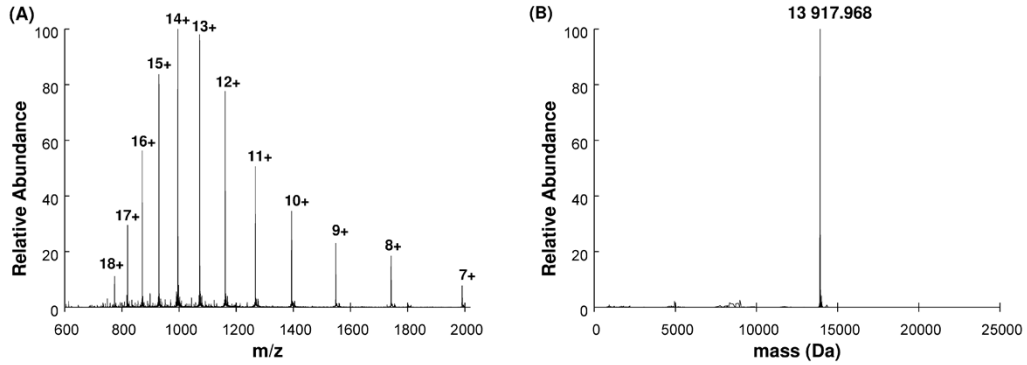

Figure S5. Determination of E47D mutant *Mt*CDA monoisotopic molecular mass. (A) ESI-FTMS spectra with isotopic resolution spanning from charge state 7+ to 18+. (B) Deconvoluted spectra resulted in a peak corresponding to a monoisotopic molecular mass of 13 917.968 Da (theoretical monoisotopic molecular mass of 13 917.942 Da).

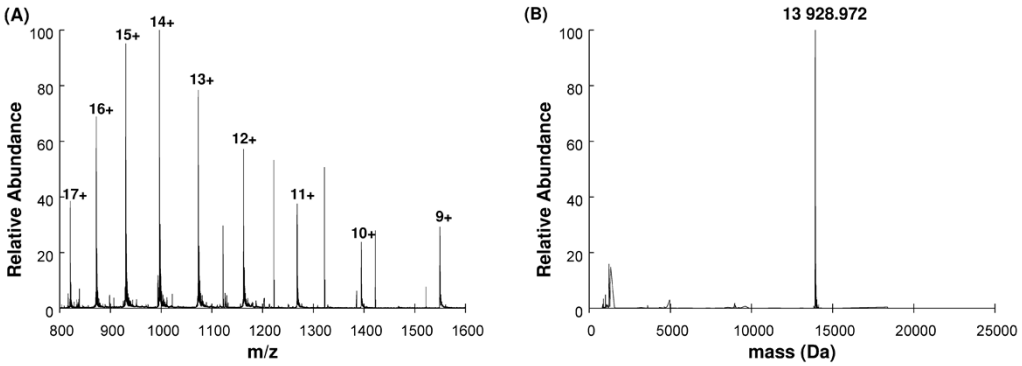

Figure S6. Determination of E47Q mutant *Mt*CDA monoisotopic molecular mass. (A) ESI-FTMS spectra with isotopic resolution spanning from charge state 9+ to 17+. (B) Deconvoluted spectra resulted in a peak corresponding to a monoisotopic molecular mass of 13 928.972 Da (theoretical monoisotopic molecular mass of 13 928.958 Da for E47Q containing one disulfide bond).

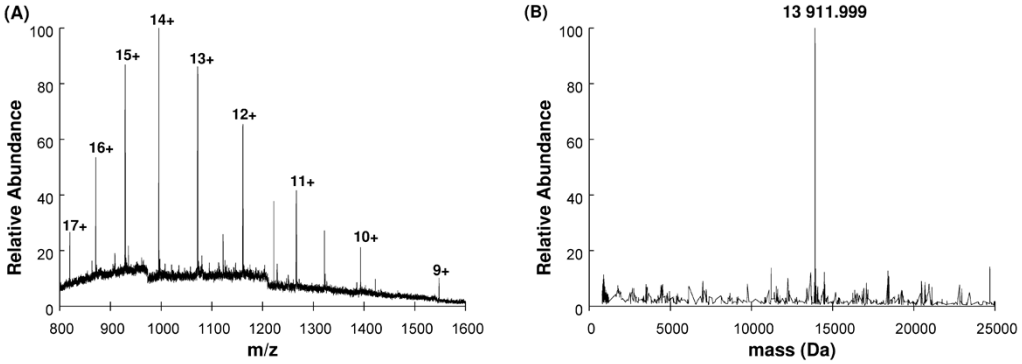

Figure S7. Determination of E47L mutant *Mt*CDA monoisotopic molecular mass. (A) ESI-FTMS spectra with isotopic resolution spanning from charge state 7+ to 18+. (B) Deconvoluted spectra resulted in a peak corresponding to a monoisotopic molecular mass of 13 911.999 Da (theoretical monoisotopic molecular mass of 13 911.968 Da for E47Q mutant containing two disulfide bonds).

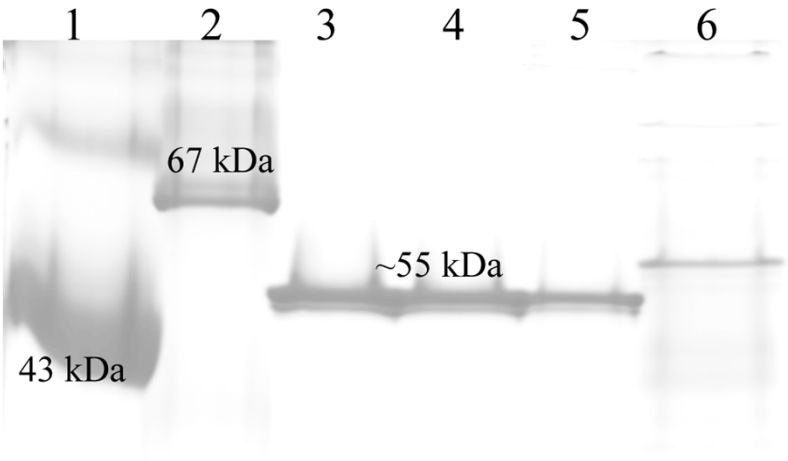

Figure S8: Analysis of oligomeric state of wild type and mutant proteins. Lane 1, Native standard protein Ovalbumin (pI 4.5-4.9); Lane 2, Native standard protein BSA (pI 4.8); Lane 3, wild-type *Mt*CDA (pI 5.72); Lane 4, E47D mutant; Lane 5, E47L mutant; Lane 6, E47Q mutant.

Parameter/ crystal structure	E47D	E47Q
PDB entry	4WIG	4WIF
Crystal parameters		
Spatial group	$C222_1$	$C222_1$
Cell constants (Å) / ° (two molecules / AU[a])	66.00/76.98/112.84	66.59/77.84/111.62
Data collection		
Beamline	XRD2 LNLS	XRD2 LNLS
Wavelength (Å)	1.459	1.459
Resolution range (Å)	112.8 – 1.76	18.67 – 1.69
No. observations	1,153,670	280,176
No. unique reflections[b]	28,875	32,090
Completeness (%)	99.6 (95.4)	97.2 (92.3)
$R_{merge\ (\%)}$[c]	10.8 (52.8)	6.4 (41.1)
I/σ (I)[b]	4.98 (1.4)	6.9 (1.9)
Refinement		
Resolution range (Å)	22.02-1.76 (1.80-1.76)	18,65-1.80 (1.85-1.80)
No. refl. working set	27,379 (1,892)	24,787 (1,688)
No. refl. test set	1,465 (111)	1,321 (83)
Number of atoms (all)	2,145	2,081
Number of atoms (solvent)	303	292
R-factor (%)[d]	21.1 (32.6)	21.4 (23.0)
R-free (%)[e]	22.8 (36.0)	24.9 (26.1)
RMS bond (Å) / (°)[f]	0.007 (1.19)	0.007 (1.22)
Average B-factor (Å^2)		
all	14.5	16.3
Zn[g]	9.4/9.7	12.1/12.0
Residue 47[g]	9.5/10.7	11.8/11.2
Ramachandran (%)[h]	96.3/3.7/0.0	97.3/2.7/0.0

a Asymmetric unit.
b Friedel pairs were treated as identical reflections.
c Rmerge(I) = ΣhklΣj | [I(hkl)j - <I(hkl)>] |/Σhkl Ihkl , where I(hkl)j is the jth measurement of the intensity of reflection hkl and <I(hkl)> is the average intensity.
d R-factor = Σhkl |(|Fo| - |Fc|)|/ Σhkl|Fo|, for the working set, corresponding to 95% of the data.
e R-free = Σhkl |(|Fo| - |Fc|)|/ Σhkl|Fo|, for the test set, corresponding to 5% of the data.
f Deviation from ideal bond lengths/ angles.
g chain A/ chain B
h Number of residues in favored region / allowed region / outlier region

Table S1. Data collection and refinement statistics. Values in parentheses refer to the highest resolution shell.

Atom	B-factor (Å^2)	
	Rotamer 1	Rotamer 2
Nε	14.08	12.97
Oε	13.44	15.65

Table S2. Refined B-factors values for the Oε and Nε atoms in Q47 side chain rotamers 1 and 2 of the E47Q *Mt*CDA mutant.