Table S1: Data collection and refinement statistics for selenomethionine derivatized CitA in complex with pyruvate and CitA in presence of NADH. (Statistics for the highest-resolution shell are shown in parentheses)

	CitA-SeMet PDB ID: 8GLB	**CitA-NADH PDB ID: 8GM9**	**CitA-PYR PDB ID: 8GMK**
Wavelength	0.9786	0.9786	0.9787
Resolution range	49.4 - 2.105 (2.18 - 2.105)	37.54 - 2.4 (2.486 - 2.4)	41.34 - 1.81 (1.875 - 1.81)
Space group	P 1 21 1	P 1 21 1	P 31 2 1
Unit cell	75.856 130.127 92.484 90 107.086 90	75.33 121.033 99.584 90 94.731 90	99.524 99.524 141.345 90 90 120
Total reflections	548564 (54078)	233263 (26131)	718577 (63950)
Unique reflections	97981 (9346)	65711 (6925)	74189 (7262)
Multiplicity	5.6 (5.8)	3.5 (3.8)	9.7 (8.8)
Completeness (%)	98.79 (95.02)	94.26 (99.77)	99.87 (99.21)
Mean I/sigma(I)	7.70 (1.00)	6.93 (1.01)	13.12 (0.99)
Wilson B-factor	33.4	48.05	32.41
R-merge	0.2288 (1.852)	0.1686 (1.057)	0.1472 (2.453)
R-meas	0.254 (2.04)	0.2 (1.233)	0.1557 (2.603)
R-pim	0.1084 (0.8468)	0.1063 (0.6311)	0.04995 (0.863)
CC1/2	0.973 (0.366)	0.982 (0.681)	0.996 (0.415)
CC*	0.993 (0.732)	0.995 (0.9)	0.999 (0.766)
Reflections used in refinement	97937 (9345)	65569 (6909)	74164 (7257)
Reflections used for R-free	1999 (190)	1521 (181)	2002 (199)
R-work	0.1879 (0.3115)	0.2198 (0.3355)	0.2099 (0.3927)
R-free	0.2408 (0.3696)	0.2734 (0.3728)	0.2598 (0.4418)
CC(work)	0.963 (0.665)	0.957 (0.808)	0.965 (0.653)
CC(free)	0.932 (0.577)	0.933 (0.768)	0.926 (0.609)
Number of non-hydrogen atoms	12241	11239	6124
macromolecules	11193	10932	5533
ligands	76	89	125
solvent	972	218	466
Protein residues	1473	1441	729
RMS(bonds)	0.004	0.002	0.009
RMS(angles)	0.67	0.48	0.95
Ramachandran favored (%)	97.33	97.27	97.52
Ramachandran allowed (%)	2.67	2.59	2.48
Ramachandran outliers (%)	0	0.14	0
Rotamer outliers (%)	0.8	0.73	0.72
Clashscore	3.25	6.79	7.44
Average B-factor	42.09	59.66	40.83
macromolecules	41.86	59.76	40.49
ligands	49.37	61.76	50.78
solvent	44.15	53.74	42.27

Table S2: Data collection and refinement statistics for CitA variants (Statistics for the highest-resolution shell are shown in parentheses)

	CitA-C143A PDB ID : 8GI7	**CitA-C143K PDB ID : 8GIW**	**CitA-C143S PDB ID : 8S9D**	**CitA-C143W PDB ID : 8S97**
Wavelength	0.9787	0.9787	0.9786	0.9786
Resolution range	57.39 - 3.3 (3.418 - 3.3)	57.76 - 3.15 (3.263 - 3.15)	50.85 - 2.57 (2.662 - 2.57)	50.5 - 2.7 (2.797 - 2.7)
Space group	C 2 2 21	C 2 2 21	I 41 2 2	I 41 2 2
Unit cell	125.001 254.919 192.809 90 90 90	128.597 263.021 196.881 90 90 90	150.253 150.253 231.657 90 90 90	148.472 148.472 230.402 90 90 90
Total reflections	351977 (35165)	437471 (43843)	634581 (63188)	533102 (52883)
Unique reflections	46623 (4587)	57957 (5721)	42440 (4186)	35636 (3488)
Multiplicity	7.5 (7.7)	7.5 (7.7)	15.0 (15.1)	15.0 (15.2)
Completeness (%)	99.90 (100.00)	99.92 (99.95)	99.89 (99.62)	99.81 (98.48)
Mean I/sigma(I)	8.42 (0.92)	12.70 (1.21)	9.63 (0.61)	5.78 (0.52)
Wilson B-factor	87.24	82.67	63.61	66.99
R-merge	0.1373 (0.4316)	0.1102 (0.3562)	0.1836 (2.962)	0.2238 (1.774)
R-meas	0.1477 (0.4633)	0.1184 (0.3822)	0.1902 (3.065)	0.2317 (1.836)
R-pim	0.05395 (0.1675)	0.043 (0.138)	0.04913 (0.787)	0.05985 (0.4694)
CC1/2	0.994 (0.923)	0.996 (0.952)	0.991 (0.63)	0.993 (0.639)
CC*	0.999 (0.98)	0.999 (0.988)	0.998 (0.879)	0.998 (0.883)
Reflections used in refinement	46602 (4587)	57928 (5718)	42403 (4170)	35577 (3435)
Reflections used for R-free	1999 (197)	1997 (197)	1856 (192)	1599 (155)
R-work	0.1722 (0.2976)	0.1847 (0.3103)	0.3133 (0.4322)	0.2423 (0.3818)
R-free	0.1996 (0.3064)	0.2124 (0.3391)	0.3290 (0.4468)	0.2698 (0.4069)
CC(work)	0.971 (0.834)	0.967 (0.835)	0.833 (0.463)	0.909 (0.555)
CC(free)	0.951 (0.766)	0.965 (0.735)	0.830 (0.388)	0.904 (0.577)
Number of non-hydrogen atoms	11295	11426	2848	2831
macromolecules	11219	11245	2788	2796
ligands	72	153	33	16
solvent	4	28	27	19
Protein residues	1478	1482	368	368
RMS(bonds)	0.002	0.002	0.004	0.002
RMS(angles)	0.49	0.53	0.6	0.51
Ramachandran favored (%)	96.73	96.4	96.17	95.08
Ramachandran allowed (%)	3.27	3.6	3.83	4.92
Ramachandran outliers (%)	0	0	0	0
Rotamer outliers (%)	0.09	0	3.57	3.57
Clashscore	4.67	3.04	4.1	5.18
Average B-factor	77.31	72.24	71.14	72.29
macromolecules	77.26	72.04	71.82	72.33
ligands	85.61	90.44	19.02	80.11
solvent	54.25	56.01	64.09	59.74

Table S3: Data collection and refinement statistics for Ebselen modified CitA (Statistics for the highest-resolution shell are shown in parentheses)

	CitA-R149E PDB ID: 8GLL	**CitA-R153M PDB ID: 8GMF**	**CitA-EBS PDB ID: 8GMI**
Wavelength	0.9786	0.9787	0.9787
Resolution range	47.53 - 2.65 (2.745 - 2.65)	91.86 - 2.96 (3.066 - 2.96)	63.06 - 2.7 (2.797 - 2.7)
Space group	P 21 21 21	P 43	P 21 21 21
Unit cell	99.933 129.679 270.153 90 90 90	129.906 129.906 256.698 90 90 90	122.017 167.486 220.997 90 90 90
Total reflections	1055763 (79257)	689427 (68901)	945668 (94264)
Unique reflections	100007 (7923)	88181 (8796)	124596 (12322)
Multiplicity	10.6 (10.0)	7.8 (7.8)	7.6 (7.7)
Completeness (%)	97.38 (78.41)	99.77 (98.33)	99.91 (99.49)
Mean I/sigma(I)	12.47 (2.95)	7.27 (0.60)	6.79 (0.47)
Wilson B-factor	46.41	75.64	66.34
R-merge	0.2488 (1.388)	0.2313 (1.602)	0.224 (2.59)
R-meas	0.2634 (1.477)	0.2479 (1.717)	0.2406 (2.779)
R-pim	0.08301 (0.4833)	0.08896 (0.6152)	0.08717 (1.002)
CC1/2	0.975 (0.347)	0.986 (0.606)	0.993 (0.476)
CC*	0.994 (0.717)	0.997 (0.869)	0.998 (0.803)
Reflections used in refinement	99933 (7913)	87981 (8649)	124504 (12259)
Reflections used for R-free	1999 (159)	1699 (165)	1828 (180)
R-work	0.1892 (0.2458)	0.2100 (0.3310)	0.2142 (0.3613)
R-free	0.2416 (0.3345)	0.2568 (0.3884)	0.2532 (0.4026)
CC(work)	0.957 (0.741)	0.950 (0.658)	0.955 (0.641)
CC(free)	0.938 (0.583)	0.921 (0.467)	0.907 (0.540)
Number of non-hydrogen atoms	23209	22313	22574
macromolecules	22176	22313	22215
ligands	452	0	241
solvent	581	0	118
Protein residues	2940	2948	2938
RMS(bonds)	0.002	0.002	0.002
RMS(angles)	0.46	0.49	0.47
Ramachandran favored (%)	97.04	95.63	97.58
Ramachandran allowed (%)	2.85	4.27	2.42
Ramachandran outliers (%)	0.1	0.1	0
Rotamer outliers (%)	0.54	0.89	1.12
Clashscore	5.19	5.45	4.49
Average B-factor	46.61	70.35	70.3
macromolecules	46.49	70.35	70.33
ligands	59.12	0	76.68
solvent	41.35	0	51.07

Figures

Figure S1: Chromatogram obtained from size exclusion chromatography using Superdex 200 10/300 and SDS-PAGE gel.

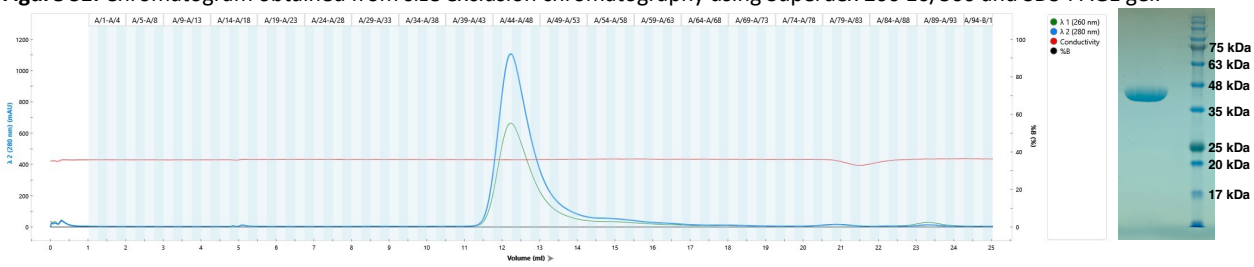

Figure S2: Comparison of *T. thermophilus* citrate synthase structure (*a*) and *M. tb* CitA structure (*b*). CoA and citrate are represented as sticks in the *T. thermophilus* citrate synthase active site. The black boxes indicate the 4 helices at the interface between two *T. thermophilus* citrate synthase monomers (*a*) or the 3 helices at the interface between two *M. tb* CitA monomers (*b*).

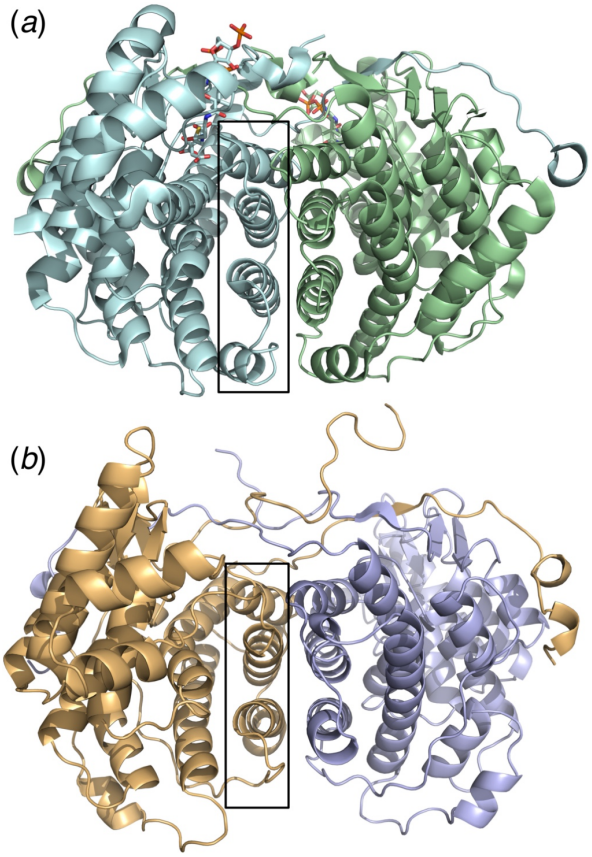

Figure S3: Overall structure of selenomethionine derivatized CitA. The asymmetric unit contains four molecules. The positions of the N-terminus and C-terminus are indicated, as well as the location of the active site. The stars in bold highlight the position of an unknown electron density that will be discussed further in this section.

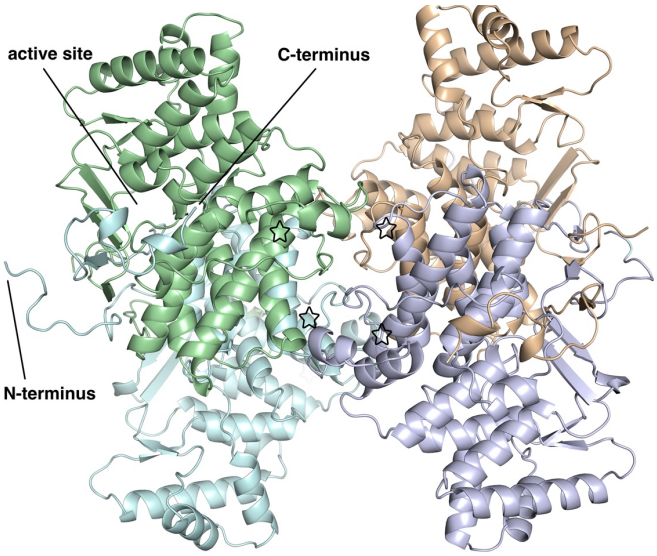

Figure S4: Superimposition of *T. thermophilus* citrate synthase monomer (green) and *M. tb* CitA monomer (blue). The black circle highlights the major difference in secondary structures between both structures (*a*). A close-up view of this region is displayed in (*b*) (Residues N60-V80 in *M. tb* CitA; residues R54-H90 in *T. thermophilus* citrate synthase).

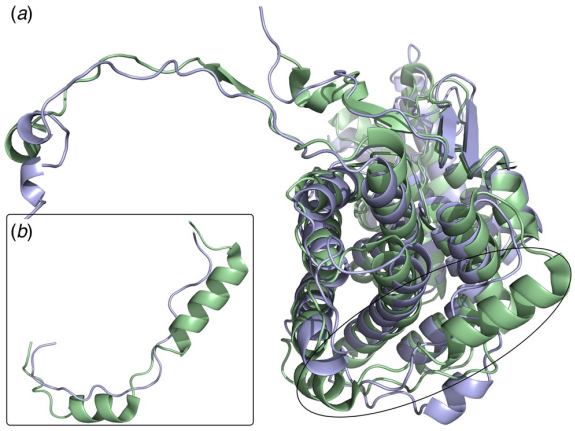

Figure S5: (*a*) Sequence alignment of *M. tb* CitA (top) with *E. coli* citrate synthase. The * represents identical residues. Residues in red form the NADH binding pocket in the *E. coli* citrate synthase.

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sp|P9WPD3|CISY2_MYCTU      ------------------------------MTVVPEN----FVPGLDGV 15
sp|P0ABH7|CISY_ECOLI       MADTKAKLTLNGDTAVELDVLKGTLGQDVIDIRTLGSKGVFTFDPGFTST 50
                                                        : .: .:    * **: ..

sp|P9WPD3|CISY2_MYCTU      VAFTTEIAEPDKDGGALRYRGVDIEDLVSQRVTFGDVWALLVDG------ 59
sp|P0ABH7|CISY_ECOLI       ASCESKITFIDGDEGILLHRGFPIDQLATDSNYLEVCYILLNGEKPTQEQ 100
                           .:  ::*:  * * * * :**. *::*.::   :   : ** .

sp|P9WPD3|CISY2_MYCTU      --NFGSGLPPAEPFPLPIHSGDVRVDVQAG-LAMLAPIWG-----YAPLL 101
sp|P0ABH7|CISY_ECOLI       YDEFKTTVTRHTMIHEQITRLFHAFRRDSHPMAVMCGITGALAAFYHDSL 150
                             :* : :.    :   *       .  ::  :*::. * *     *   *

sp|P9WPD3|CISY2_MYCTU      DIDDATARQQLARASVMALSYVAQSAR--GIYQPAVPQR--IIDECSTVT 147
sp|P0ABH7|CISY_ECOLI       DVNNPRHREIAAFRLLSKMPTMAAMCYKYSIGQPFVYPRNDLSYAGNFLN 200
                           *:::.  *:  *   :  :. :*  .   .* ** *  *  :    . :.

sp|P9WPD3|CISY2_MYCTU      ARFMTRWQ-GEPDPRHIEAIDAYWVSAAEHGMNASTFTARVIASTGADVA 196
sp|P0ABH7|CISY_ECOLI       MMFSTPCEPYEVNPILERAMDRILILHADHEQNASTSTVRTAGSSGANPF 250
                             * *  :  * :*   .*:*   :  *:*  **** *.*. .*:**:

sp|P9WPD3|CISY2_MYCTU      AALSGAIGAMSGPLHGGAPARVLPMLDEVERAGDARSVVKGILDRGE--K 244
sp|P0ABH7|CISY_ECOLI       ACIAAGIASLWGPAHGGANEAALKMLEEISSVKHIPEFVRRAKDKNDSFR 300
                           *.::..*.:: ** ****   .* **:*:. . .  ..*:   *:.:  :

sp|P9WPD3|CISY2_MYCTU      LMGFGHRVYRAEDPRARVLRAAAER----LGAPRYEVAVAVEQAALSELR 290
sp|P0ABH7|CISY_ECOLI       LMGFGHRVYKNYDPRATVMRETCHEVLKELGTKDDLLEVAMELENIALND 350
                           *********:  **** *:* :...    **:    : **:*   ::

sp|P9WPD3|CISY2_MYCTU      ERRPDRAIETNVEFWAAVVLDFARVPANMMPAMFTCGRTAGWCAHILEQK 340
sp|P0ABH7|CISY_ECOLI       PYFIEKKLYPNVDFYSGIILKAMGIPSSMFTVIFAMARTVGWIAHWSEMH 400
                             :: : .**:*::.::*.   :*:.*:..:*: .**.** **  * :

sp|P9WPD3|CISY2_MYCTU      RLG-KLVRPSAIYVGPGPRSPESVDGWERVLTTA 373
sp|P0ABH7|CISY_ECOLI       SDGMKIARPRQLYTGYEKRDFKSDIKR------- 427
                             * *:.**  :*.*   *. :*
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Figure S6: Protein thermal shift assay for CitA wild-type with and without NADH showing no change in the melting temperature.

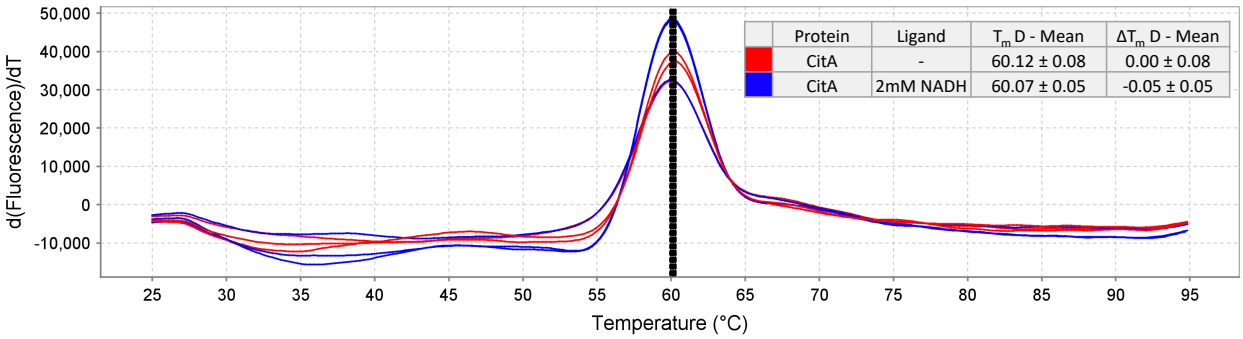


Figure S7: Protein thermal shift assay for CitA variants R149E and R153M against the CitA wild-type

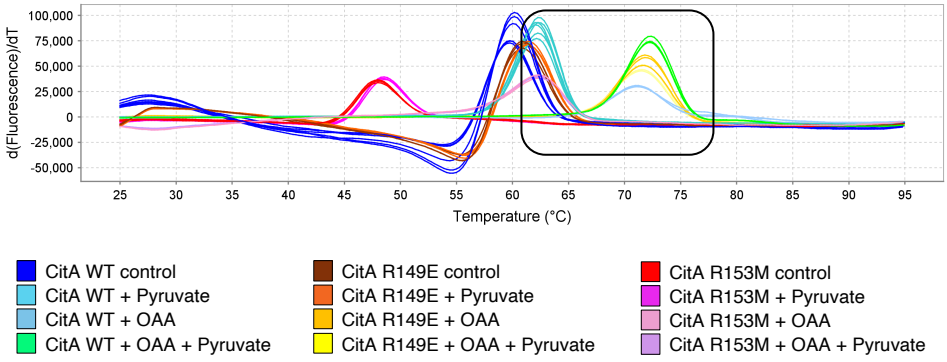
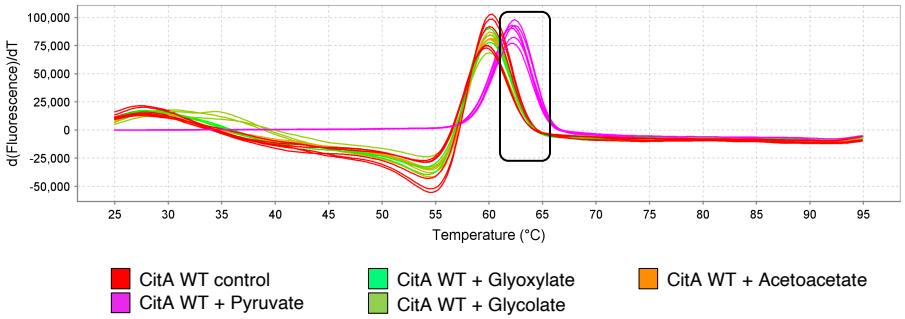


Figure S8: Protein thermal shift assays for CitA wild-type with pyruvate compared against other small metabolites structurally similar to pyruvate



CitA WT control
CitA WT + Pyruvate
CitA WT + Glyoxylate
CitA WT + Glycolate
CitA WT + Acetoacetate

Figure S9: Comparison of the CitA with C143 variants (a) The structural alignment of Pyruvate bound CitA structure with the C143 variants (b) The activity of the C143 variants versus CitA wild-type.

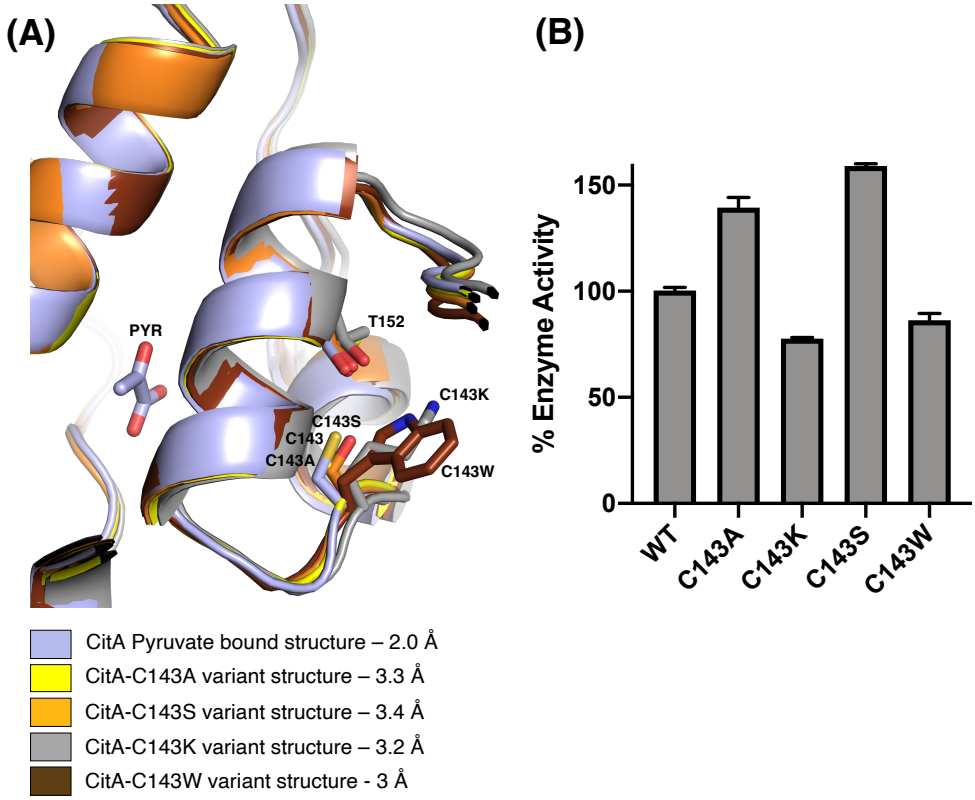


Figure S10: Control experiment of C143S variant with Ebselen