

**Title:** Identification of the active site residues in ATP-citrate lyase's carboxy-terminal portion

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**Supplemental Information**

**Supplementary Table 1. Oligonucleotides with mismatches underlined.**

Name	Sequence	Purpose
KH1.1	CCGGTGCCGGGTATTGGT <u>GCT</u> CGTGTTAAATCTGTC	Forward primer for H491A
KH1.2	GACAGATTTAACACGAG <u>C</u> ACCAATACCCGGCACCGG	Reverse primer for H491A
NS2.1	CTGATTCTGAATGTTG <u>CC</u> GGCACCATCGGTTG	Forward primer for D543A
NS2.2	CTGATTCTGAATGTTG <u>CC</u> GGCACCATCGGTTG	Reverse primer for D543A
VN2.1	GCCGCGTGTGCAGGAAGTTATGT <u>ATTTT</u> CAAGGTGAA GTTATTGTCG	Forward primer to insert TEV protease cleavage site
VN2.2	CGACAATAACTTCACCTTG <u>AAA</u> ATACATAACTTCCTG CACACGCGGC	Reverse primer to insert TEV protease cleavage site

**Supplementary Table 2. Statistics for X-ray diffraction data and model.**

<b>Diffraction data</b>		
X-ray source	CMCF-ID	
Wavelength (Å)	0.97949	
Space group	P3 <sub>2</sub>	
Cell dimensions	$a = b = 82.18 \text{ \AA}, c = 151.20 \text{ \AA}, \alpha = \beta = 90^\circ, \gamma = 120^\circ$	
Resolution range (high resolution) (Å)	75.59 – 1.89 (1.92 – 1.89)	
R <sub>merge</sub> , I/σ, CC <sub>1/2</sub>	0.06 (0.58), 13.5 (1.8), 0.999 (0.545)	
Number of unique reflections	91455 (4474)	
Multiplicity (high resolution)	4.9 (3.6)	
Completeness (high resolution) (%)	99.97 (98.33)	
Wilson B-factor (Å <sup>2</sup> )	39.4	
<b>Model</b>		
Resolution range (high resolution) (Å)	71.17 – 1.90 (1.92 – 1.90)	
Number of reflections in working set	80395 (2215)	
Number of reflections in test set	4287 (117)	
R <sub>free</sub> , R <sub>work</sub>	0.1896 (0.3568), 0.1666 (0.3222)	
Coordinate error (Å)	0.19	
Number of non-hydrogen atoms and average B-factors (Å <sup>2</sup> )		
protein (TLS refinement)	8074	51
water, other	308, 182	45, 78
rmsd from ideal values		
bonds (Å) angles (°)	0.006, 0.667	
Ramachandran plot		
favored, allowed regions (%)	98.54, 1.46	

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CIACLY 347 - - - - - G 347
Nter CS 1 A S S T N L K D V L A S L I P K E Q A R I K T F R Q Q H G N T A V G Q I T V D M S Y G G M R G M K - 49
Cter CS 196 - - - - - 195

CIACLY 348 E V I V E P - - - - - L I R T T I S D D R G - E E P R Y A G Y A A S E L C S K G Y G - - - 383
Nter CS 50 - - - - - G L I Y E T S V L D P D E G I R F R G F S I P E C Q K L L P K A G G 83
Cter CS 196 - - - - - A G S S I G A I D S - - - - - 205

CIACLY 384 - - - - - I E D V I G L L W N K K L P T R E E S E I I K R I V M I S A D H - G P A V S G A F G S I L 427
Nter CS 84 G E E P L P E G L F W L L V T G Q I P T P E Q V S W V S K E W A K R - - - A A L P S H V V T M L D 129
Cter CS 206 - - - - - K L D W S H N F T N M L G Y T D P Q F T E L M R L Y L T I H S D H E G G N V S A H T S H L 250

CIACLY 428 A A C A G I D M P Q A V S A G M T M I - - - - - G P R F G G A V T N A 457
Nter CS 130 N F P T N L H P M S Q L S A A I T A L N S E S N F A R A Y A E G I N R T K Y W - - - - - 168
Cter CS 251 V G S A L S D P Y L S F A A A M N G L A - - - - - G P L H G L A N Q E V 281

CIACLY 458 G K Y F K M A V E D Y P N D - I P G F L S W M K K - - - N V G - P V P G I G H R V K S V K N P D Q R 502
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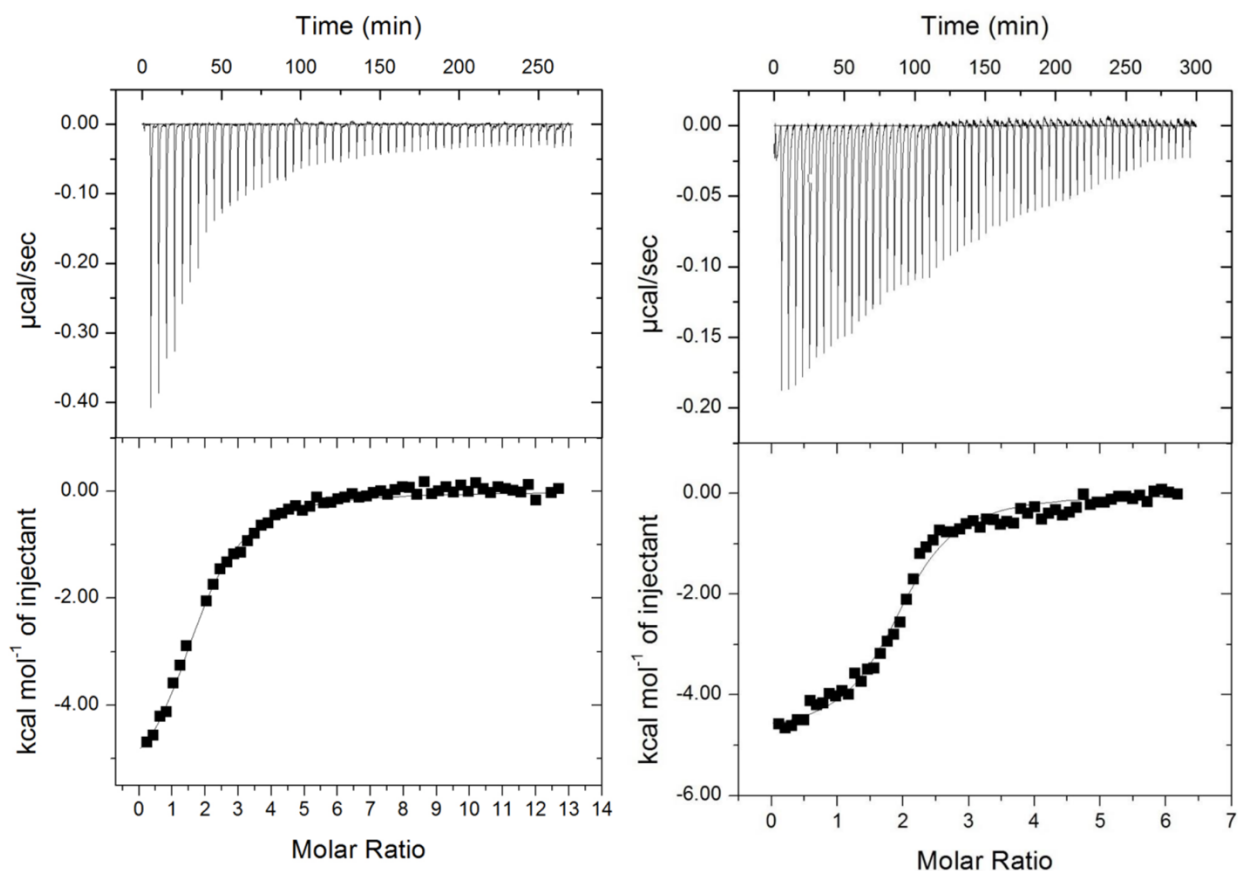
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Cter CS 330 Y T C Q R E F A L K H L - P S D P M F K L V A Q L Y K I V P N V L L E Q G K A K N P W P N V D A H S 378

CIACLY 547 G C I L M D L D F - P V H S L N G F F V L A R T I G M I G H W I D Q N N Q N S R L I R L Y D Y L I N 595
Nter CS 169 - - - - - - - - - - - E F V Y E D A M D L I A K L P C V A A K I Y R N L Y R - - - - - - - - - - - - - - - 195
Cter CS 379 G V L L Q Y Y G M T E M N Y Y T V L F G V S R A L G V L A Q L I W S R A L G F P L E R P K S - - - - - 424

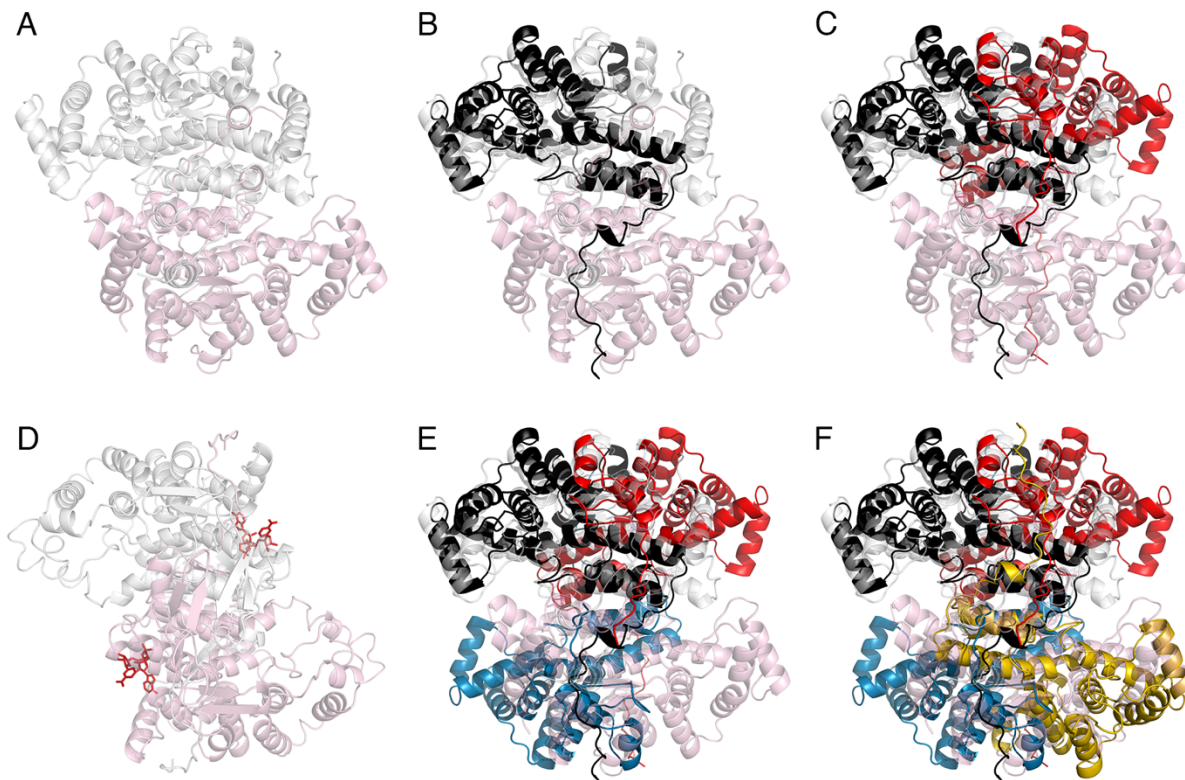
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Cter CS 425 - - - - - - - - - - - M S T A G L E K L S A G G 437

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**Supplementary Figure S1. Structural alignment of the sequence of the C-terminal portion of *CIACLY* with the N- and C-terminal portions of chicken citrate synthase.** The sequences were aligned by identifying which residues had similar positions in the  $\alpha$ -helices. The  $\alpha$ -helices of the C-terminal portion of *CIACLY* are highlighted by red coils above the sequences. When the polypeptide in one structure took a different path from the others, the residues were aligned with gaps in the other sequences. Residues are colored according to the default of Clustal X<sup>1</sup>. The residues mutated in this work are highlighted by rectangles. This figure was drawn using Chimera<sup>2</sup>.



**Supplementary Figure S2. Binding of CoA to *CIACLY* and to the H491A&D543A double mutant.** The top panels depict raw data from isothermal titration calorimetry, i.e. heat generated as a function of time. The bottom panels show the integrated area of each peak plotted against the molar ratio, as well as the curve fitted to the data by nonlinear regression. A. 0.7 mM CoA was titrated into 5  $\mu\text{M}$  *CIACLY*. B. 0.3 mM CoA was titrated into 15  $\mu\text{M}$  H491A&D543A double mutant.



**Supplementary Figure S3. Superposition of the C-terminal portion of *ClACLY* on CS and**

**the CS allosteric site.** A. Chicken CS (PDB ID: 6CSC<sup>3</sup>) is represented by the semitransparent gray and pink ribbon diagram. B. One protomer of the C-terminal portion of *ClACLY* (black) is superposed on the C-terminal portion of a protomer of chicken CS. C. Two protomers of the C-terminal portion of *ClACLY* (black and red) are superposed on a protomer of chicken CS. E and F. Three and four protomers of the C-terminal portion of *ClACLY* (yellow, black, red and blue) are superposed. D. NADH is shown as a red stick model bound to the allosteric site of *E. coli* CS (PDB ID: 1NXG<sup>4</sup>). The allosteric site is located where the second P450 domain is missing from CS. This figure was drawn using PYMOL<sup>5</sup>.

## References

1. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, et al. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* 23:2947–2948.
2. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE (2004) UCSF Chimera - A visualization system for exploratory research and analysis. *J. Comput. Chem.* 25:1605–1612.
3. Usher K, Remington S (1997) PDB ID: 6CSC Chicken citrate synthase complex with trifluoroacetyl-CoA and citrate.
4. Maurus R, Nguyen NT, Stokell DJ, Ayed A, Hultin PG, Duckworth HW, Brayer GD (2003) Insights into the evolution of allosteric properties. The NADH binding site of hexameric type II citrate synthases. *Biochemistry* 42:5555–5565.
5. DeLano WL (2002) The PyMOL Molecular Graphics System.