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

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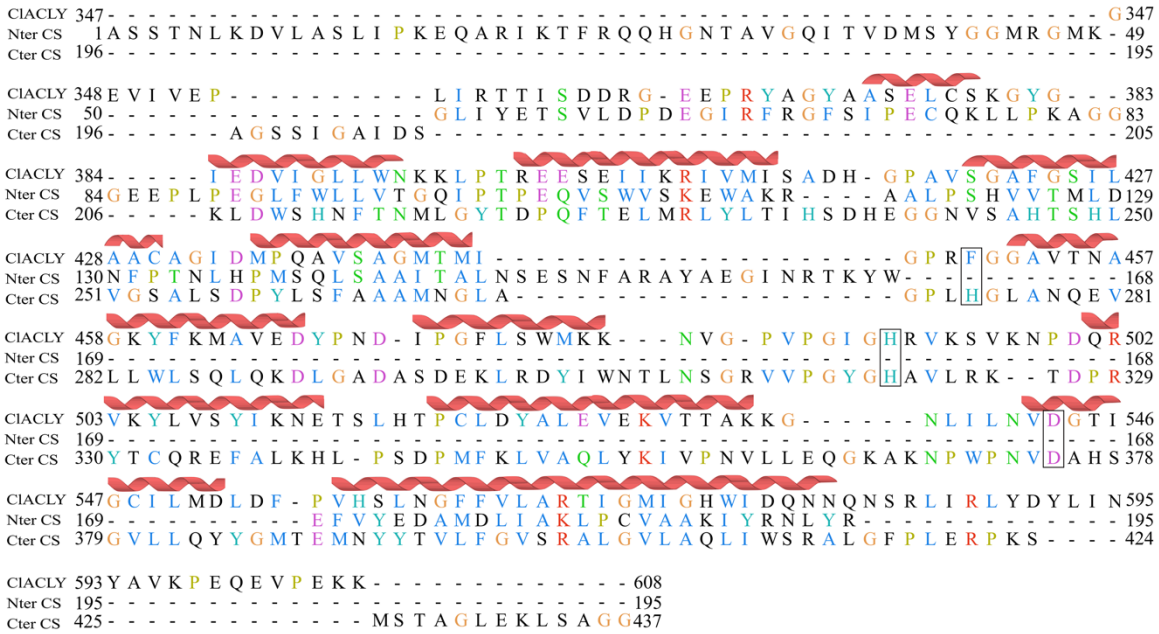
Supplemental Information

Supplementary Table 1. Oligonucleotides with mismatches underlined.

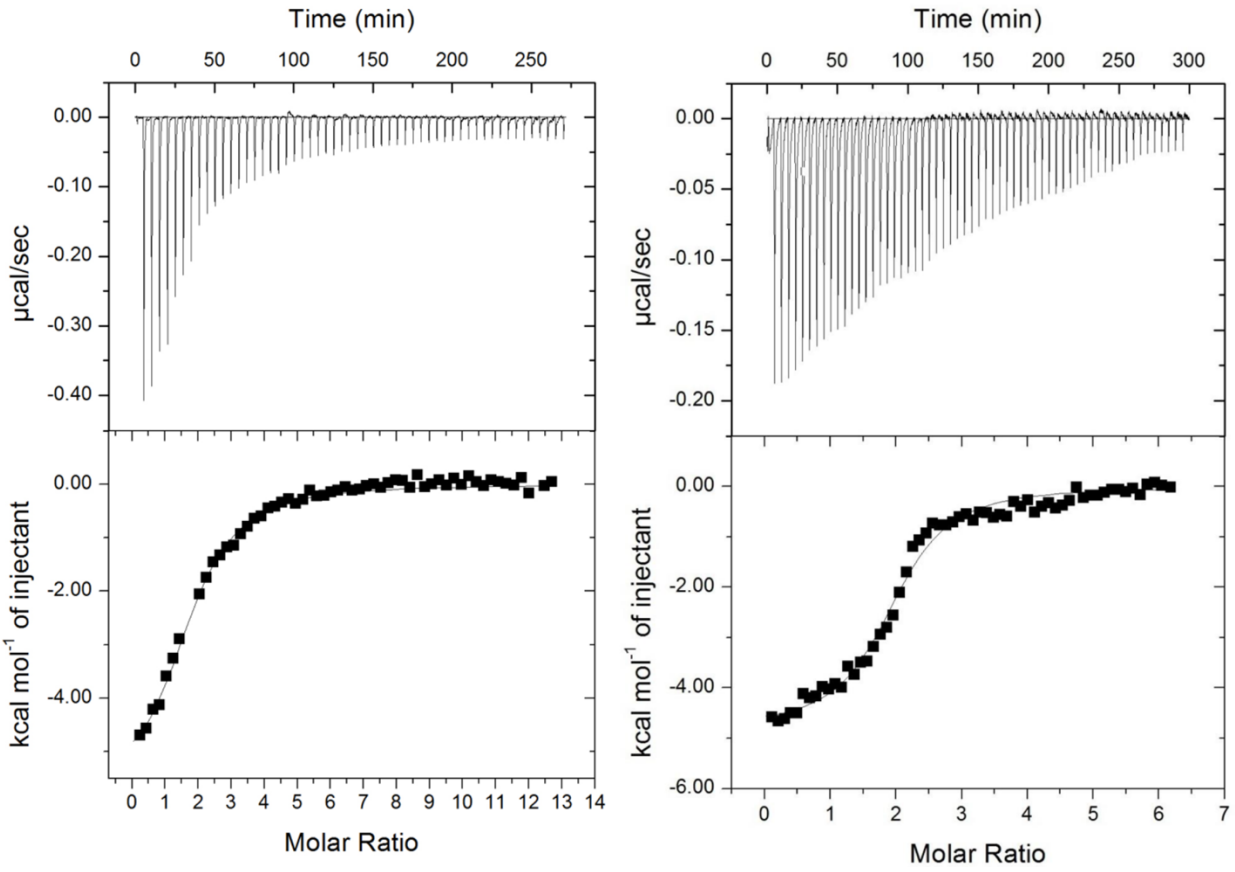
Name	Sequence	Purpose
KH1.1	CCGGTGCCGGGTATTGGTGCTCGTGTTAAATCTGTC	Forward primer for H491A
KH1.2	GACAGATTTAACACGAGCACCAATACCCGGCACCGG	Reverse primer for H491A
NS2.1	CTGATTCTGAATGTTGCCGGCACCATCGGTTG	Forward primer for D543A
NS2.2	CTGATTCTGAATGTTGCCGGCACCATCGGTTG	Reverse primer for D543A
VN2.1	GCCGCGTGTGCAGGAAGTTATGTATTTTCAAGGTGAA GTTATTGTCG	Forward primer to insert TEV protease cleavage site
VN2.2	CGACAATAACTTCACCTTGAAAATACATAACTTCCTG CACACGCGGC	Reverse primer to insert TEV protease cleavage site

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

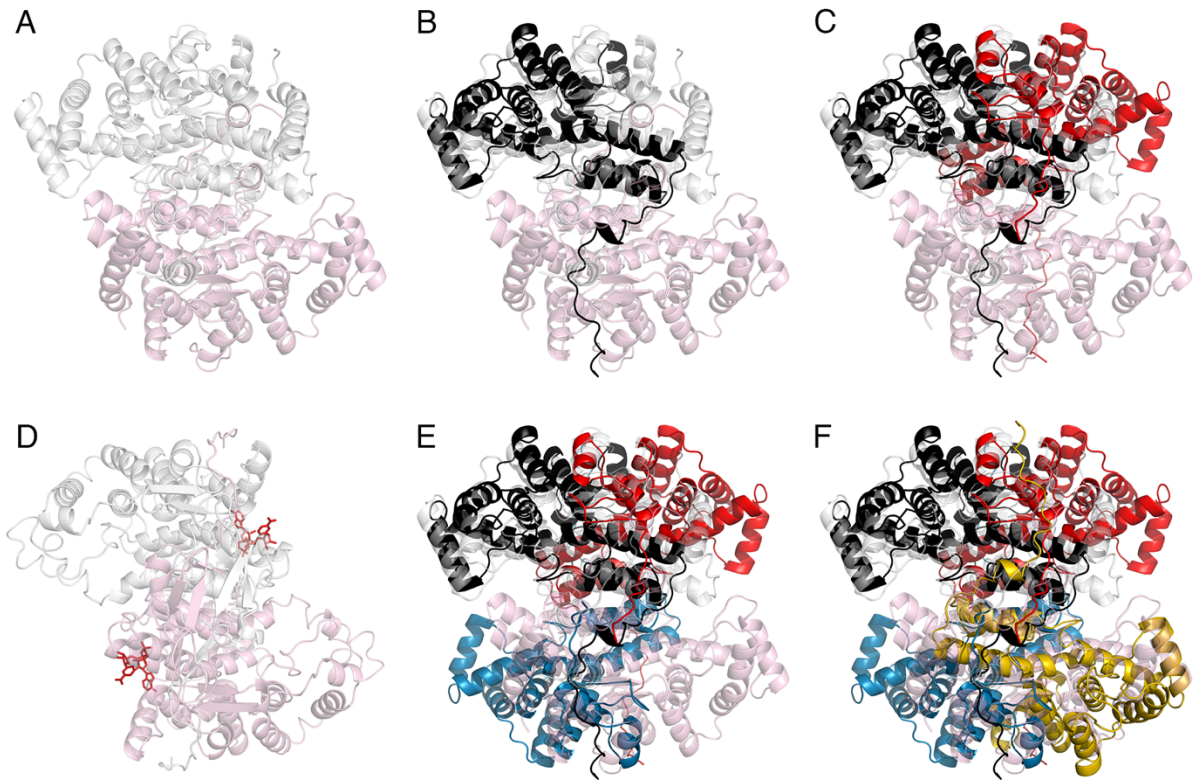
Diffraction data		
X-ray source	CMCF-ID	
Wavelength (Å)	0.97949	
Space group	$P3_2$	
Cell dimensions	$a = b = 82.18$ Å, $c = 151.20$ Å, $\alpha = \beta = 90^o$, $\gamma = 120^o$	
Resolution range (high resolution) (Å)	75.59 – 1.89 (1.92 – 1.89)	
R_{merge}, I/σ, $CC_{1/2}$	0.06 (0.58), 13.5 (1.8), 0.999 (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^2)	39.4	
Model		
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_{free}, R_{work}	0.1896 (0.3568), 0.1666 (0.3222)	
Coordinate error (Å)	0.19	
Number of non-hydrogen atoms and average B-factors (Å^2)		
protein (TLS refinement)	8074	51
water, other	308, 182	45, 78
rmsd from ideal values		
bonds (Å) angles (^{o})	0.006, 0.667	
Ramachandran plot		
favored, allowed regions (%)	98.54, 1.46	



Supplementary Figure S1. Structural alignment of the sequence of the C-terminal portion of *Cl*ACLY with the N- and C-terminal portions of chicken citrate synthase. The sequences were aligned by identifying which residues had similar positions in the α-helices. The α-helices of the C-terminal portion of *Cl*ACLY 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[1]. The residues mutated in this work are highlighted by rectangles. This figure was drawn using Chimera[2].



Supplementary Figure S2. Binding of CoA to *Cl*ACLY 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 µM *Cl*ACLY. B. 0.3 mM CoA was titrated into 15 µM H491A&D543A double mutant.



Supplementary Figure S3. Superposition of the C-terminal portion of *Cl*ACLY on CS and the CS allosteric site. A. Chicken CS (PDB ID: 6CSC[3]) is represented by the semitransparent gray and pink ribbon diagram. B. One protomer of the C-terminal portion of *Cl*ACLY (black) is superposed on the C-terminal portion of a protomer of chicken CS. C. Two protomers of the C-terminal portion of *Cl*ACLY (black and red) are superposed on a protomer of chicken CS. E and F. Three and four protomers of the C-terminal portion of *Cl*ACLY (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[4]). The allosteric site is located where the second P450 domain is missing from CS. This figure was drawn using PYMOL[5].

References

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