

Cryo-EM data collection, refinement and validation statistics

	ACLY–OAA–acetyl-CoA (EMD-23413, PDB 7LLA)	ACLY–OAA–acetyl-CoA (local refinement on ASH domain) (EMD-23389, PDB 7LJ9)	ACLY-E599Q–ATP–citrate–CoA (local refinement on ASH domain) (EMD-23387, PDB 7LIW)
Data collection and processing			
Magnification	45,000	45,000	45,000
Voltage (kV)	200	200	200
Electron exposure (e ⁻ /Å ²)	37	37	40
Defocus range (μm)	0.9-2.8	0.9-2.8	1.0-2.0
Pixel size (Å)	0.87	0.87	0.87
Symmetry imposed	D2	C1	C1
Initial particle images (no.)	719,613	434,952	1,303,057
Final particle images (no.)	108,738	205,702	1,124,928
Map resolution (Å)	3.0	3.0	2.9
FSC threshold	0.143	0.143	0.143
Map resolution range (Å)	2.8-4.0	2.8-4.0	2.7-3.6
Refinement			
Initial model used (PDB code)	6UI9	6UI9	6UUW
Model resolution (Å)	3.3	3.4	3.0
FSC threshold	0.5	0.5	0.5
Model resolution range (Å)	-	-	-
Map sharpening <i>B</i> factor (Å ²)	-123.2	-93.4	-119.5
Model composition			
Non-hydrogen atoms	31,925	14,520	14,638
Protein residues	4,083	1,865	1,871
Ligands	12	6	3
R.m.s. deviations			
Bond lengths (Å)	0.002	0.004	0.003
Bond angles (°)	0.535	0.668	0.543
Validation			
MolProbity score	1.65	1.95	1.53
Clashscore	8.82	10.63	8.72
Poor rotamers (%)	0	0.07	0.26
Ramachandran plot			
Favored (%)	96.97	93.96	97.74
Allowed (%)	3.30	6.04	2.26
Disallowed (%)	0	0	0

SUPPLEMENTARY NOTE

To improve the local resolution of the ASH domains and their bound ligands to generate revised ACLY–OAA–acetyl-CoA (PDB-7LJ9/EMBD-23389) and ACLY-E599Q–ATP–citrate–CoA (PDB-7LIW/EMBD-23387), we followed a previously reported strategy to perform particle symmetry expansion to put all four ASH domains in the same position⁴. Three redundant copies of ASH domains were removed by particle subtraction. Ab-initio models were generated based on partial particles composed of a CSH-module (tetramer) + ASH domain monomer. A good 3D class was selected after heterogenous refinement,

then autorefinement was performed on the good class. ACLY–OAA–acetyl-CoA (PDB-7LLA/EMBD-23413) was generated as described above but without local refinement.