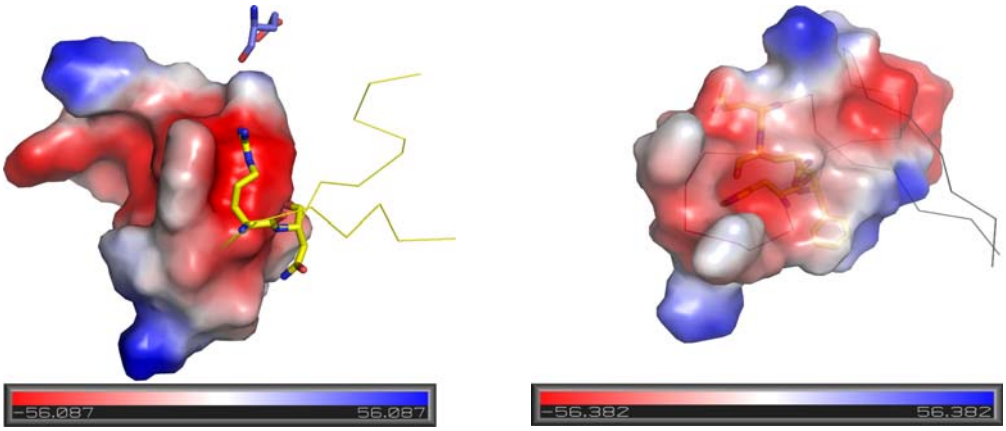
Supplementary material S1.



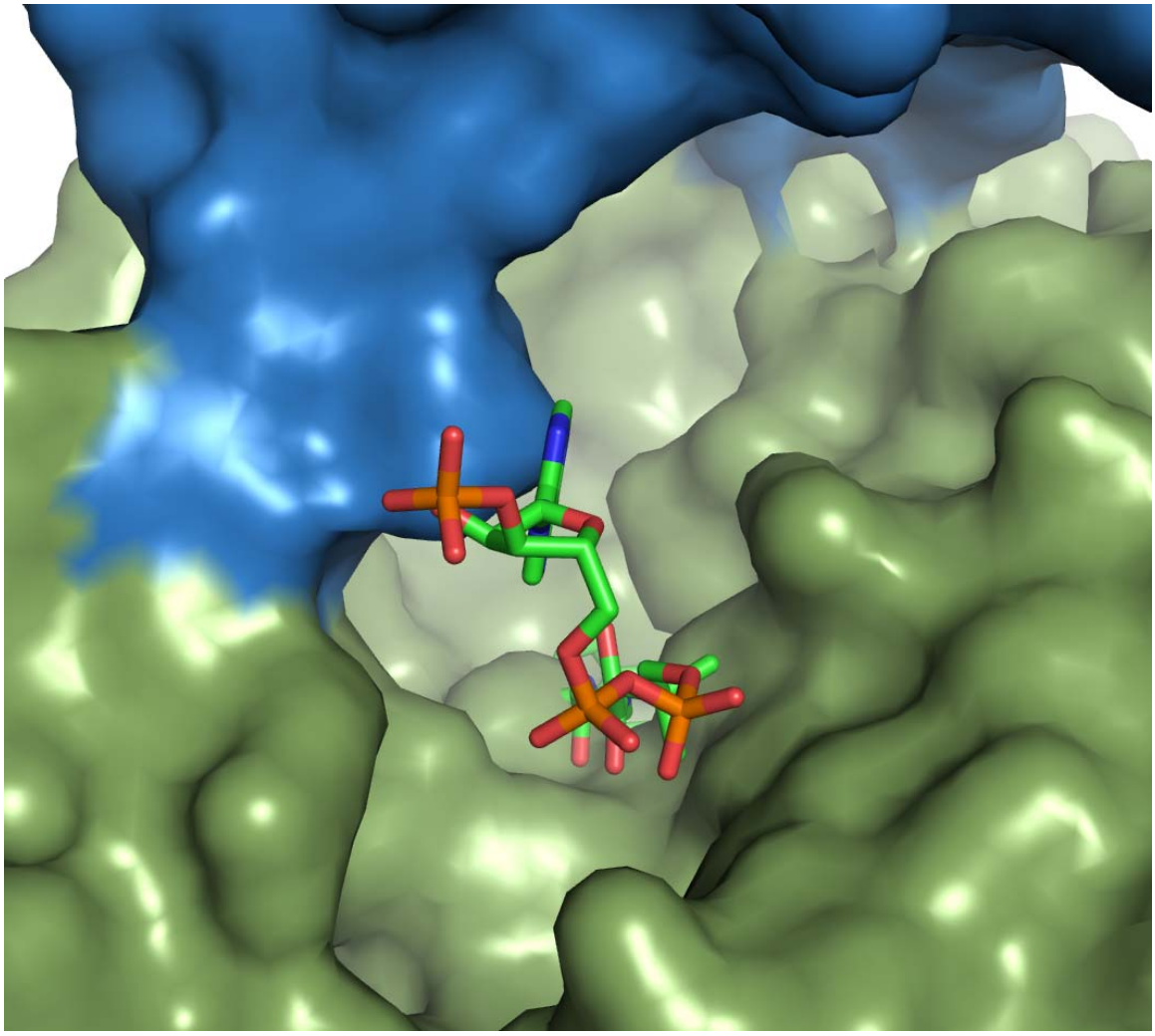
a) Electrostatic surface of *Ec*FAAL insertion motif. Hinge region is shown in yellow. Arg469 binds to the negatively charged pocket. b) Electrostatic surface of the *Lp*FAAL insertion motif.

Supplementary material S2.

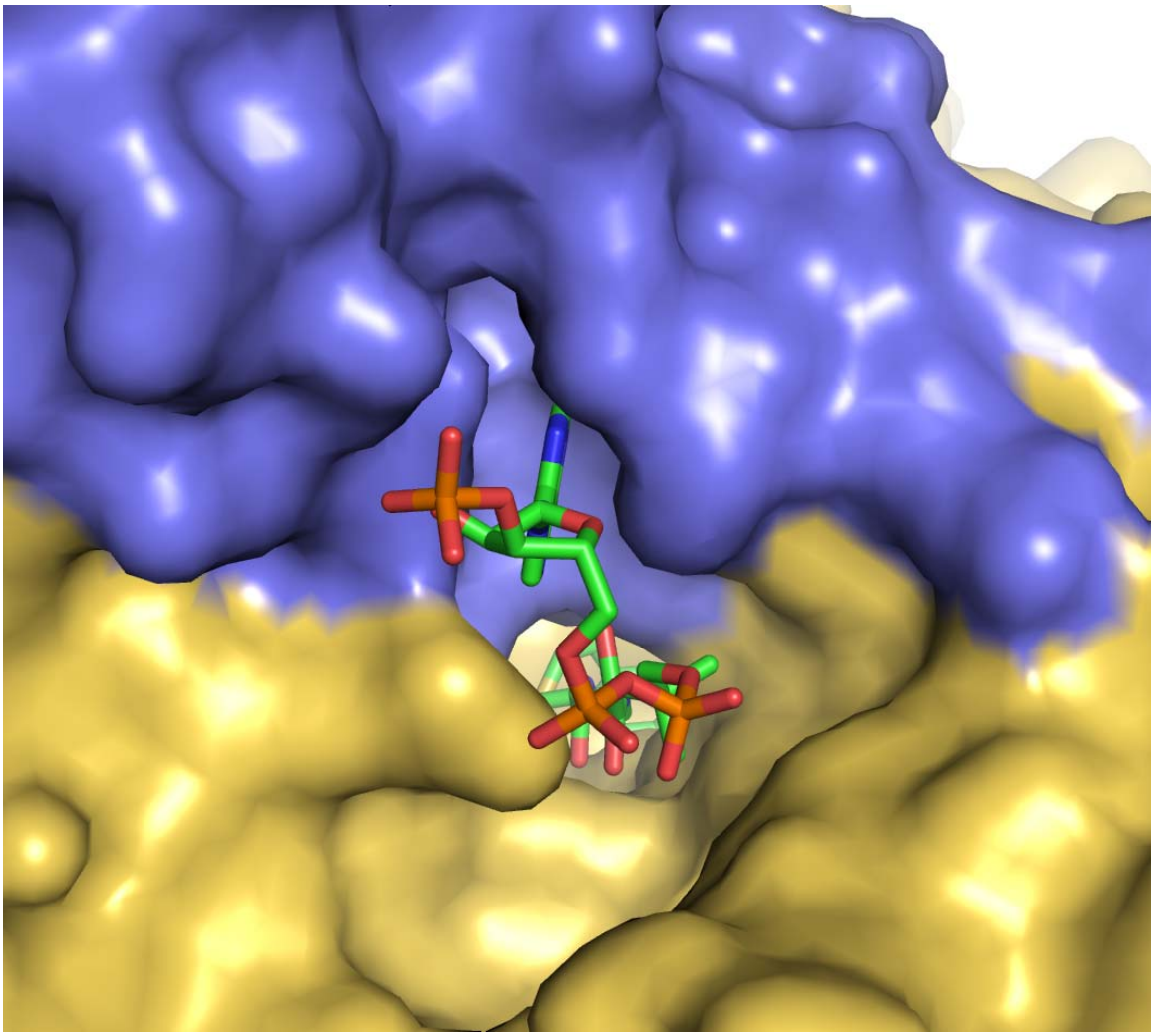
List of residues at the interface of acyl adenylate and *Ec*FAAL or *Lp*FAAL. Residues not conserved are shown in italics. Residues from gate motif highlighted in yellow.

EcFAAL	LpFAAL
N-terminal domain residues	
Thr184	Thr173
Ser185	*Gly175*
Ile208	Ile197
Ile213	*Phe201*
Leu215	*Mse203*
Cys221	*Ile209*
His229	His217
Asp230	Asp218
Mse231	Mse219
Gly235	Gly223
Phe236	Cys224
Thr239	-
Val273	-
Val275	*Ile261*
-	*Phe293*
Gly307	Gly295
Ala308	Ala296
Glu309	Glu297
Pro310	Pro298
Ile311	*Val299*
Cys336	Cys324
Tyr337	Tyr325
Gly338	Gly326
Leu339	Leu327
Ala340	Ala328
Glu341	Glu329
Leu344	Leu332
Ala345	*Leu333*
Phe348	-
Cys387	*Ser374*
Hinge region residues	
Thr443	-
Asp445	Asp440
Val456	Val451
Gly458	-
Arg459	Arg454
C-terminal domain residues	
Ser549	-
Lys551	-

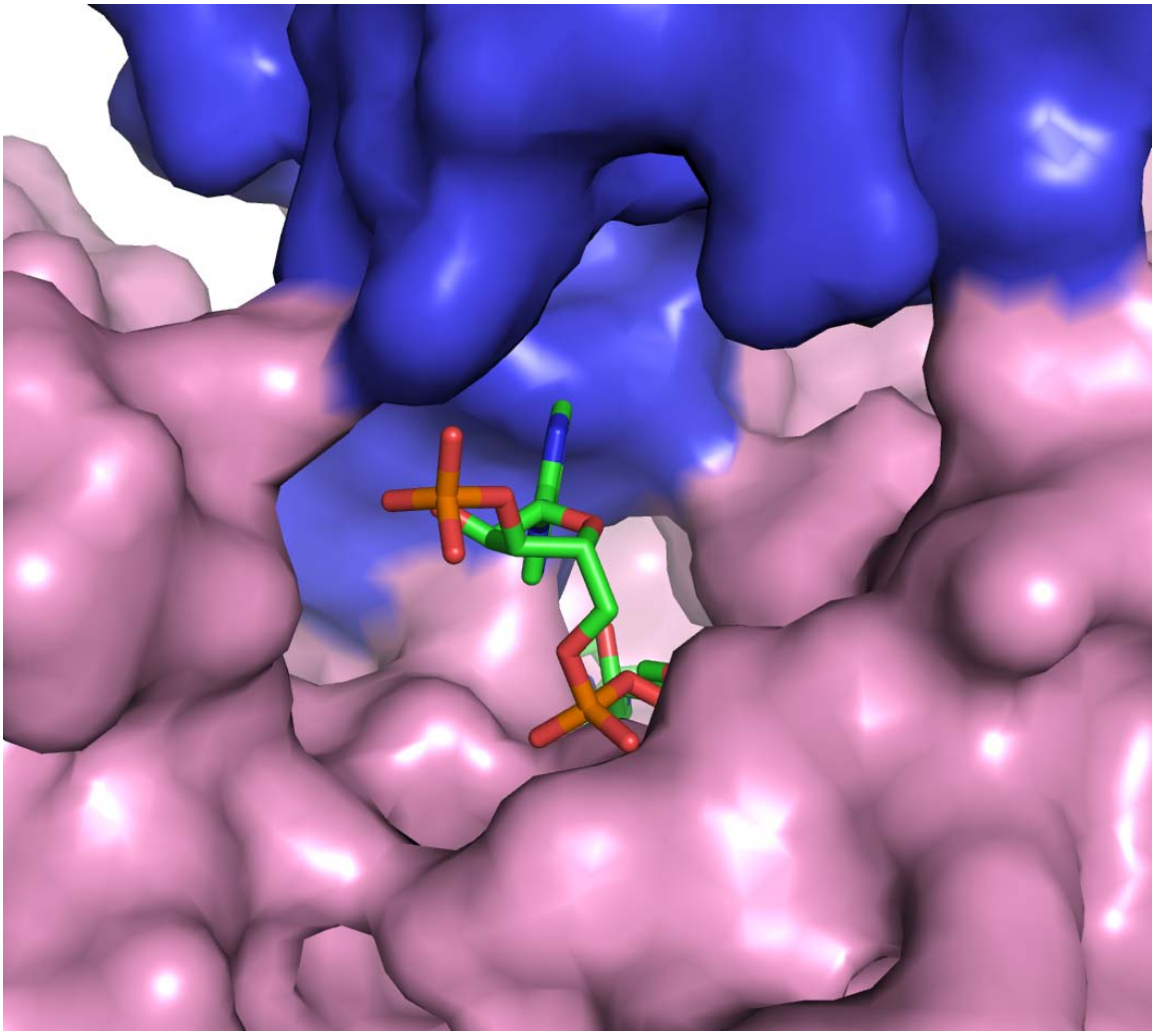
Supplementary material S3-S6:



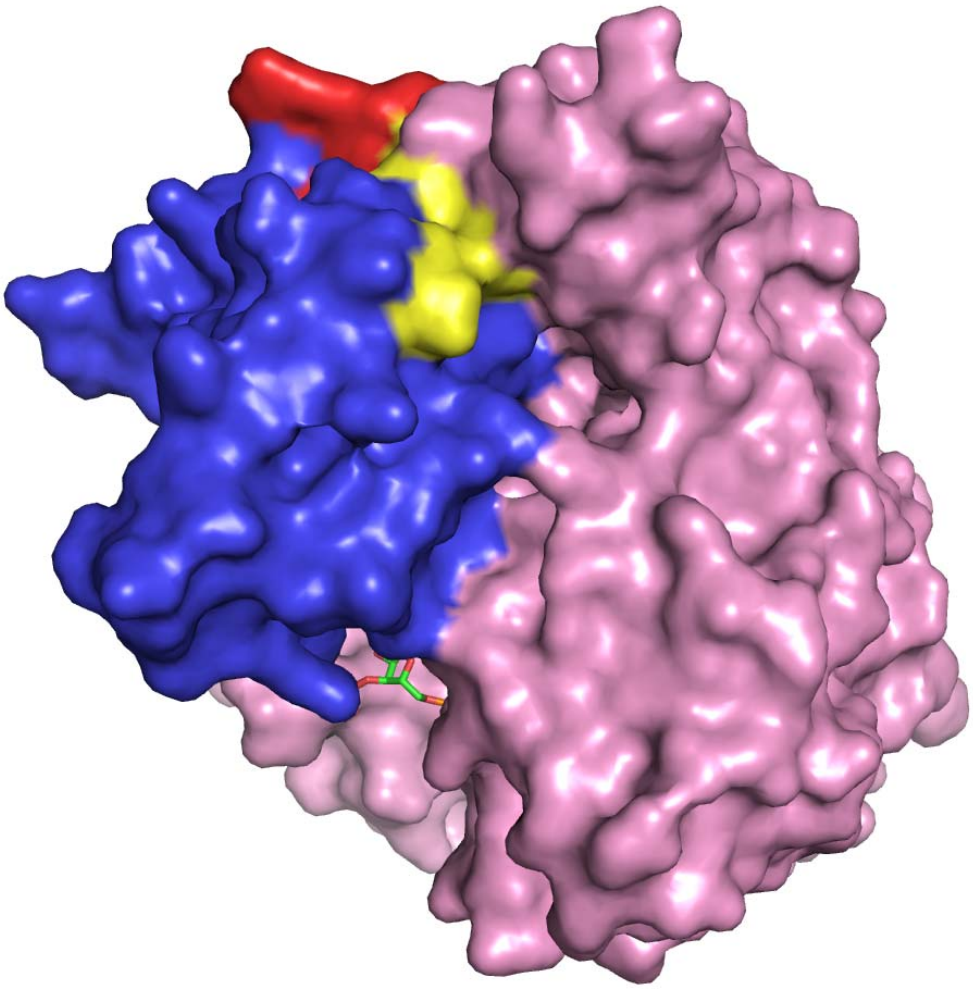
S3. Putative CoA binding cavity in AsFaal. The N and C-terminal domains are shown in green and blue. CoA from the bound structure (3CW9) is overlaid on the unbound structure (3CW8). The cavity is big enough to accommodate CoA. However, critical interactions with CoA are missing.



S4. CoA bound AsFAAL (3CW9). The N and C terminal domains are shown in yellow and blue. It is clear that the C-terminal domain has reoriented to make contacts with CoA.

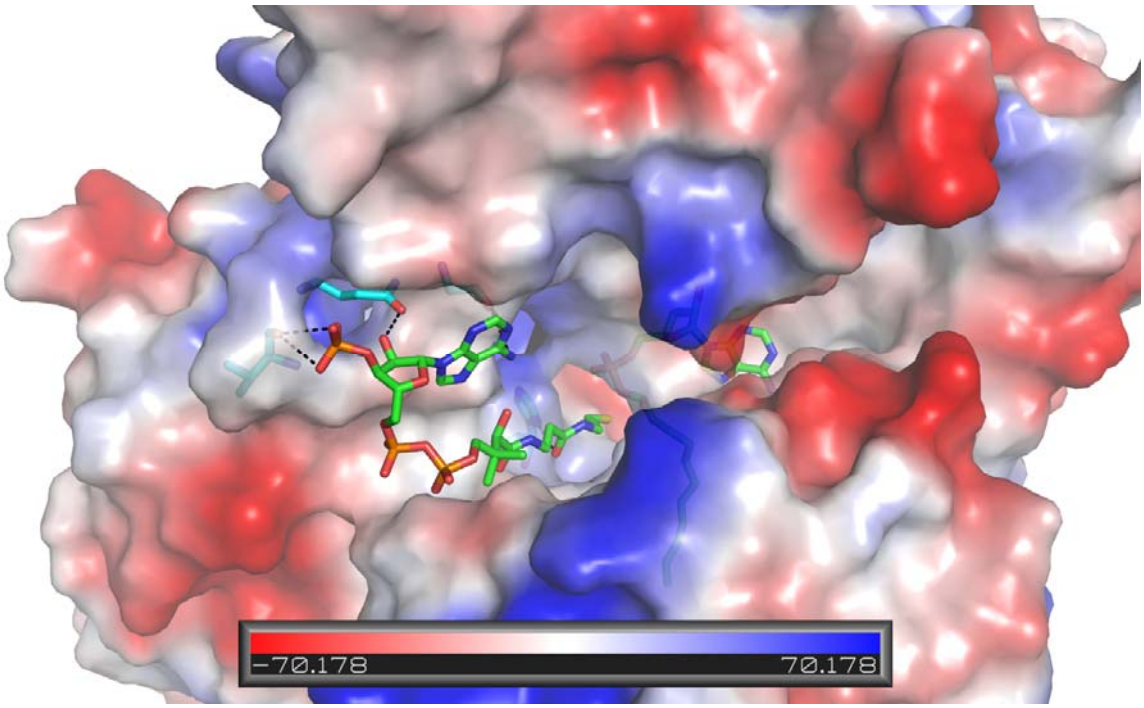


S5. CoA from AsFaal overlayed on EcFaal after least square fit of the two models (3CW8 and 3GQW). The N and C terminal domains are shown in pink and blue. The cavity is big enough to accommodate CoA but the C domain has to move to make critical contacts which is prevented by the strong interaction between the insertion motif and the rest of the protein.



S6. The interaction region between the insertion motif and the C-domain is shown in yellow. These strong interactions restrain C terminal domain movement.

Supplementary material S7



S7. *Ec*FAAL and *Lp*FAAL were modeled to thioester binding conformation by superimposting FAAL C-terminal domain to the *As*FACL (3CW9) C-domain which removes the constraints due to insertion motif interactions. The electrostatic surface analysis shows there are similar non-polar binding pocket for CoA adenylate binding. There are narrow tunnel point to the acyl-adenylate binding site, suggesting the mercaptoethylamine and pantothenic acid group binding. CoA was then model into the binding site.