Supporting Information for

Molecular recognition of fluorine impacts substrate selectivity in the fluoroacetyl-CoA thioesterase FlK

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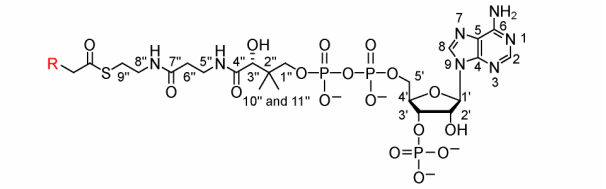
Supplementary Figures

Supplementary Figures

Table S1. Acyl-CoA numbering scheme for 1D NMRs and $^{1}H/^{13}C$ HMBC crosspeaks.



Crosspeak numbering for HMBC spectra							
1	$H_{1''}C_{2''}$	**10**	$H_{10''}C_{11''}$	**19**	$H_{5''}C_{7''}$	**28**	H_2C_4
2	$H_{1''}C_{3''}$	**11**	$H_{10''}C_{2''}$	**20**	$H_{5''}C_{6''}$	**29**	H_2C_6
3	$H_{1''}C_{11''}$	**12**	$H_{10''}C_{1''}$	**21**	$H_{6''}C_{7''}$	**30**	H_8C_4
4	$H_{1''}C_{10''}$	**13**	$H_{10''}C_{3''}$	**22**	$H_{6''}C_{5''}$	**31**	H_8C_5
5	$H_{3''}C_{11''}$	**14**	$H_{11''}C_{10''}$	**23**	$H_{8''}C_{7''}$	**32**	$H_{1'}C_2$
6	$H_{3''}C_{10''}$	**15**	$H_{11''}C_{2''}$	**24**	$H_{8''}C_{9''}$	**33**	$H_{1'}C_4$
7	$H_{3''}C_{2''}$	**16**	$H_{11''}C_{1''}$	**25**	$H_{9''}C_{1RAc}$	**34**	$H_{1'}C_{4'}$
8	$H_{3''}C_{1''}$	**17**	$H_{11''}C_{3''}$	**26**	$H_{9''}C_{8''}$	**35**	$H_{2'}C_{1'}$
9	$H_{3''}C_{4''}$	**18**	$H_{5''}C_{4''}$	**27**	$H_{2RAc}C_{1RAc}$	**36**	$H_{3'}C_{4'}$

Figure S1. $^{1}H/^{13}C$ HMBC spectrum of *(S)*-2-fluoropropionyl-CoA in D_2O.

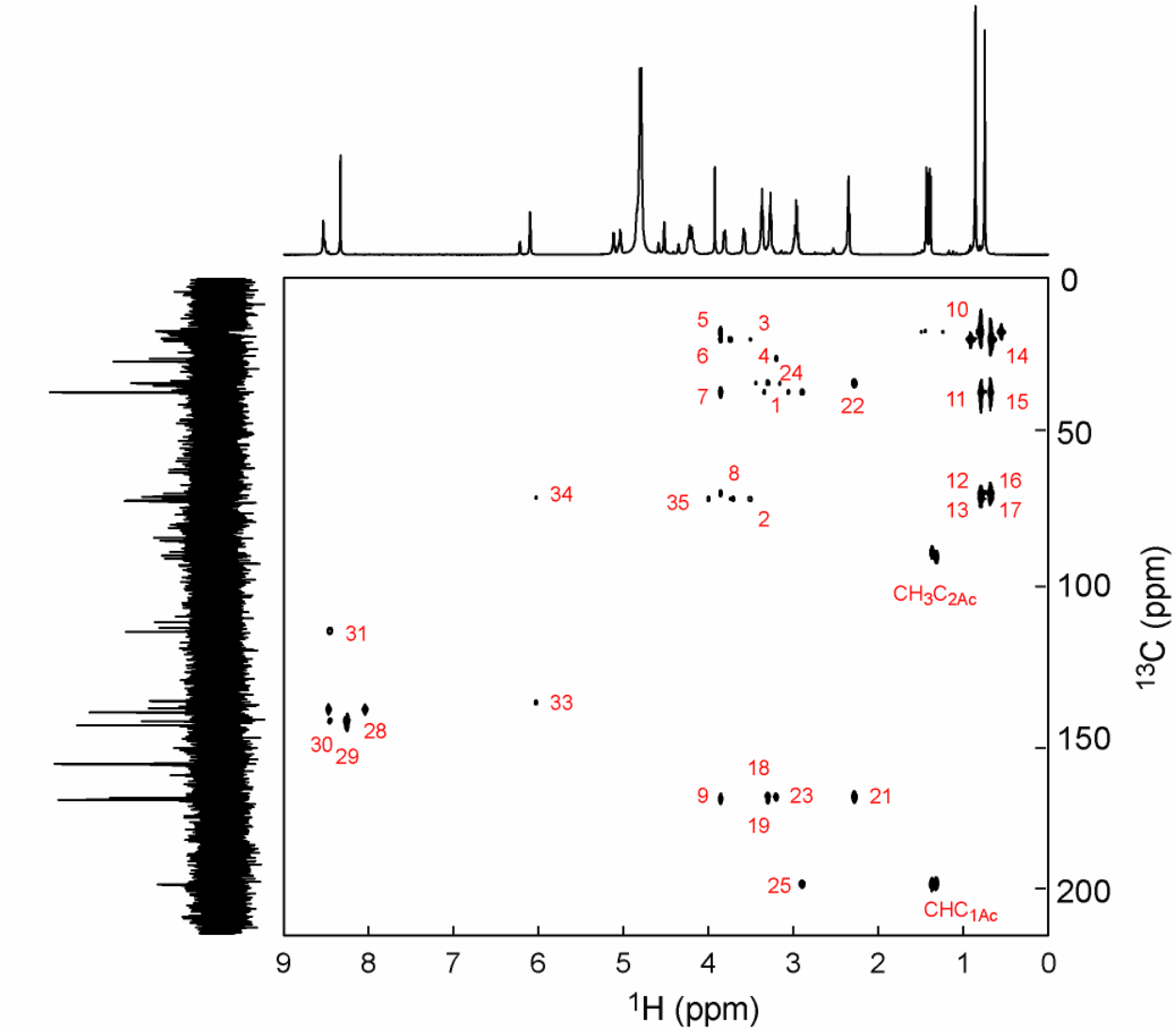


Figure S2. $^{1}H/^{13}C$ HMBC spectrum of *(R)*-2-fluoropropionyl-CoA in D_2O.

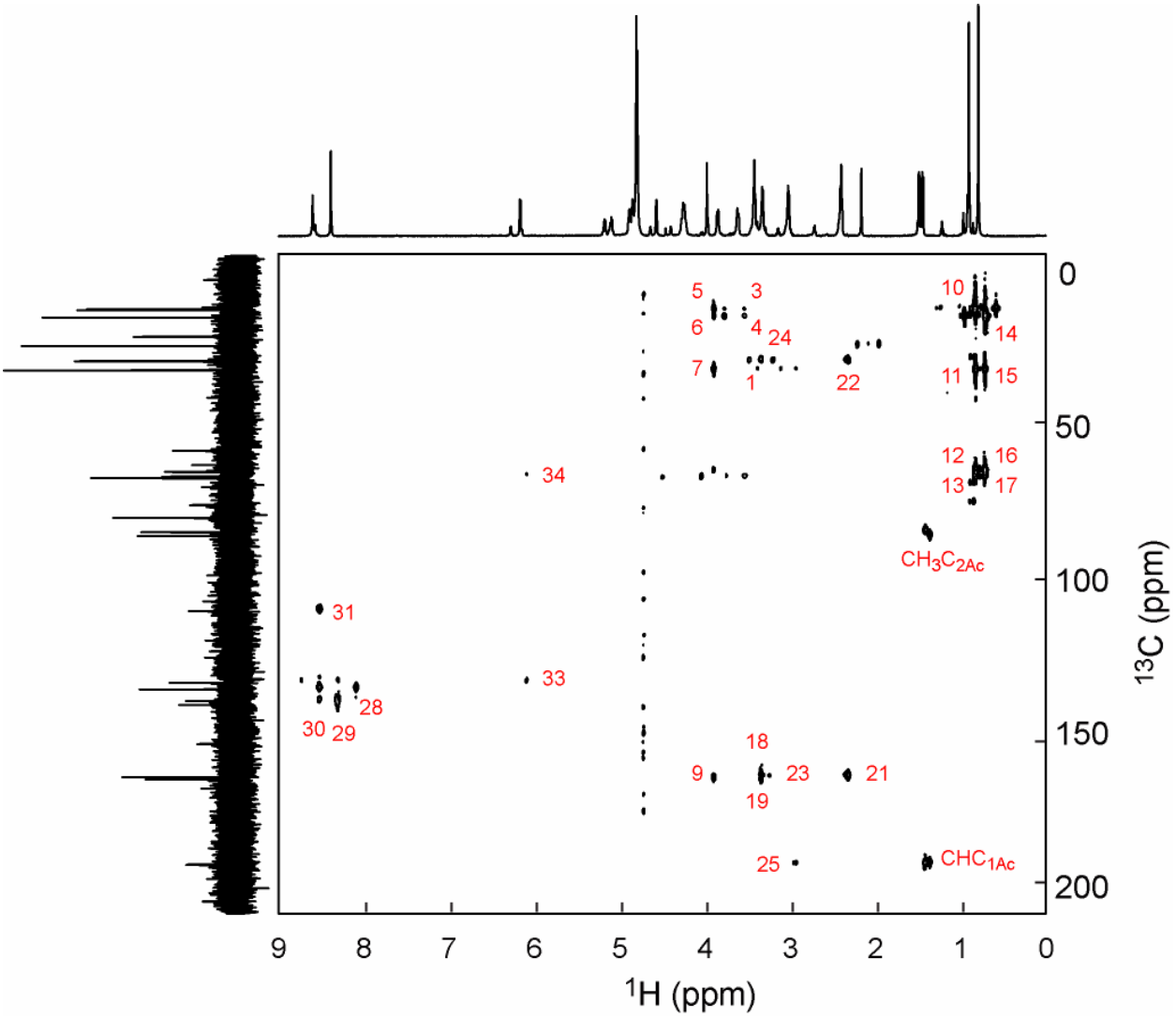


Figure S3. The catalytic triad of FlK-T42S. In the FlK-T42S mutant (PDB ID: 3KVU), both Ser 42 and His 76 populate two different rotatmers, and Glu 50 is rotated relative to its position in the wild-type enzyme (*1*). Chain A, grey; chain B, slate; carbon, grey; nitrogen, blue; oxygen red.

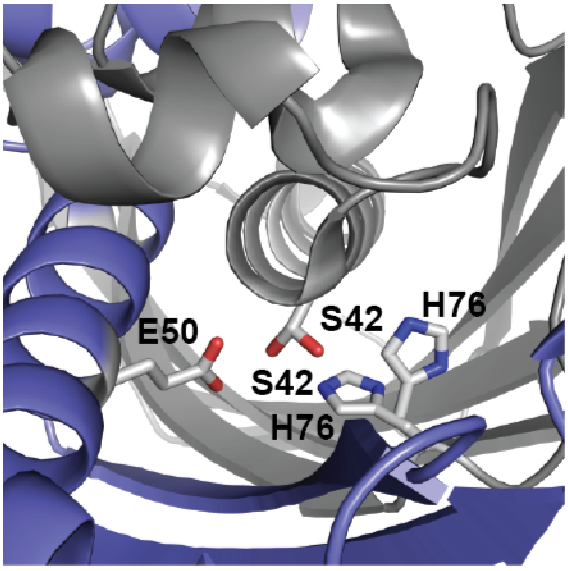


Figure S4. Simulated steady-state kinetic data for FIK-T42S-catalyzed acetyl-CoA hydrolysis. Data were simulated using the same K_D for both wild-type and mutant and using the acylation and deacylation rate constants measured using pre-steady-state kinetic analysis.

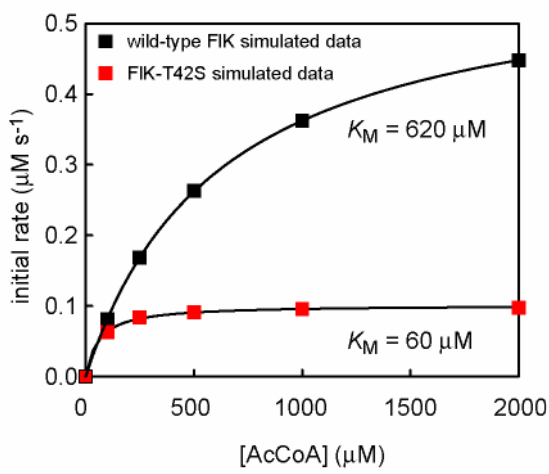


Figure S5. Time courses for hydrolysis of (*RS*)-2-fluoropropionyl-CoA. (A) 50 μM. (B) 100 μM. (C) 200 μM. (D) 250 μM.

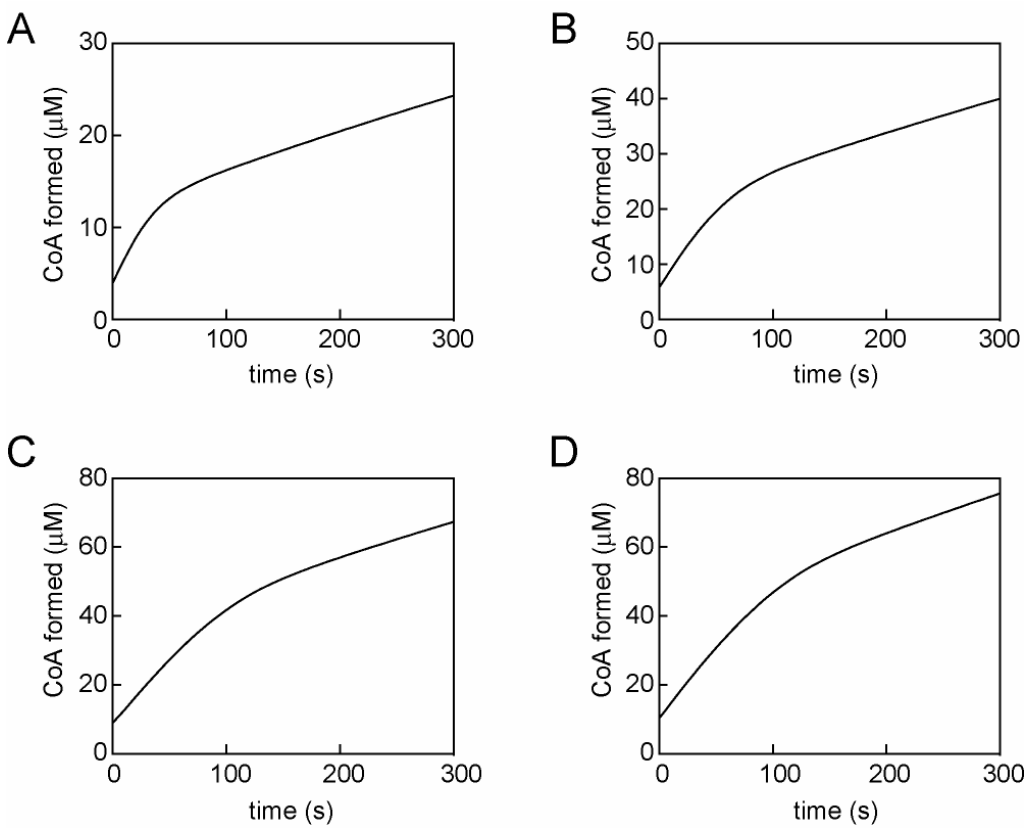


Figure S6. Taft plot for k_{cat} of various acyl-CoA FIK substrates. 2-fluoropropionyl-CoA substrates are shown in red. Data shown in black are from (*2*). σ* values for acyl-CoAs with a single α-substituent are from (*3*). The σ* value for 2-fluoropropionyl-CoA was calculated by adding the values for the F and Me substituents as described in (*4*). Et, butyryl-CoA; Me, propionyl-CoA; H, acetyl-CoA; Br, bromoacetyl-CoA; Cl, chloroacetyl-CoA; F, fluoroacetyl-CoA; (*S*)-F, Me, (*S*)-2-fluoropropionyl-CoA; (*R*)-F, Me, (*R*)-2-fluoropropionyl-CoA; CN, cyanoacetyl-CoA.

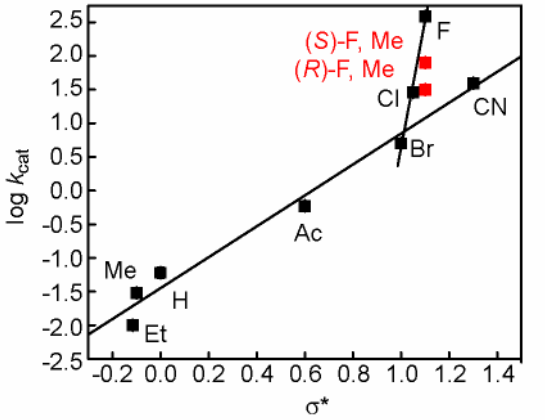
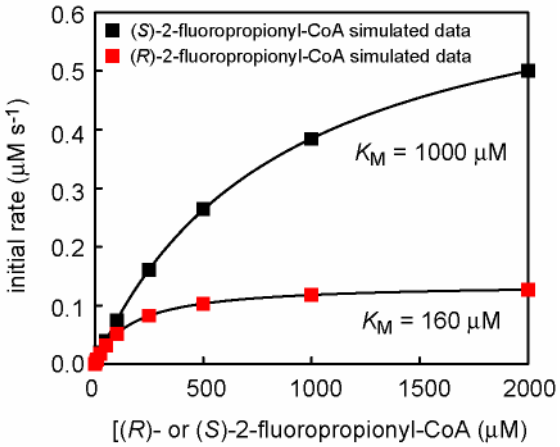


Figure S7. Simulated steady-state kinetic data for FIK-catalyzed hydrolysis of (*S*)- and (*R*)-2-fluoropropionyl-CoA. At constant K_D, the changes in the measured kinetic constants are sufficient to explain the difference in K_M between the two substrates.



Literature cited

1. Dias, M. V., Huang, F., Chirgadze, D. Y., Tosin, M., Spiteller, D., Dry, E. F., Leadlay, P. F., Spencer, J. B., and Blundell, T. L. (2010) Structural basis for the activity and substrate specificity of fluoroacetyl-CoA thioesterase FlK, *J. Biol. Chem.* **285**, 22495-22504.
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