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Structural insight into acyl-ACP thioesterase towards substrate specificity design

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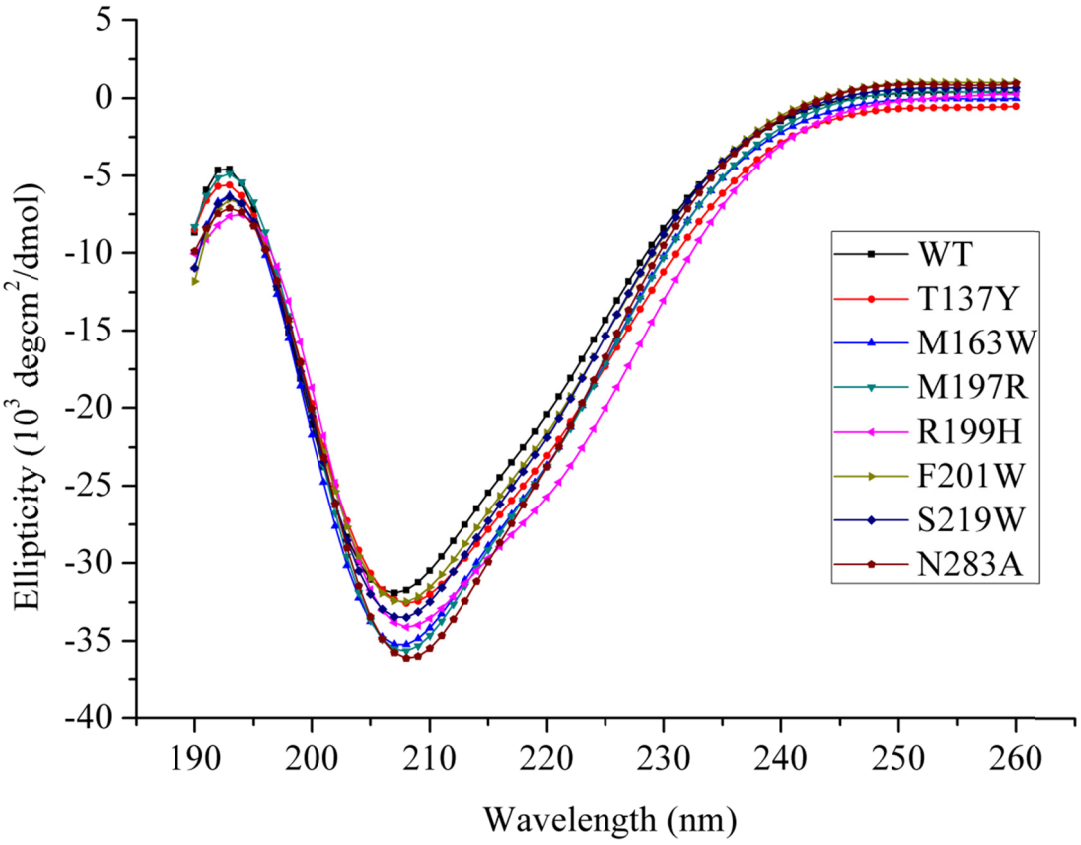

Figure S1 Circular dichroism spectroscopy of UcFatB and its mutants

Structural and enzymatic assay proved that T137, M163, M197, R199, F201, S219 and N283 residues consisted of the UcFatB substrate binding pocket. Circular dichroism spectroscopy of UcFatB showed that the secondary structure of T137Y, M163W, M197R, R199H, F201W, S219W and N283A mutations were well-retained comparing with the wild type of the enzyme.

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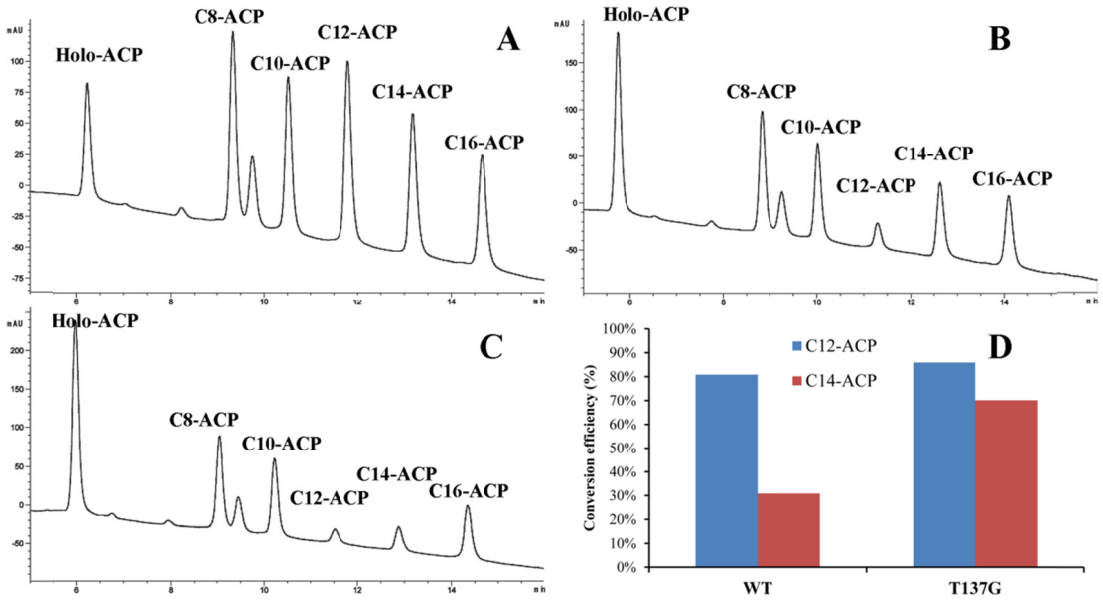

Figure S2 Relative selectivity assay of UcFatB

(A) Preparation of synthetic (C8-C16)-ACP pool *in vitro*, the synthetic acyl-ACPs from C8-C16 were quantified by retention time and peak area on a C8-ODS column. The reaction mixture without enzyme was set as control, the Y-axis represents the absorbance at 210 nm (B) The relative selectivity of UcFatB was assayed by the acyl-ACP decrement comparing with the control, UcFatB preferred C12-ACP to C14-ACP obviously. (C) The relative selectivity of UcFatB_T137G was assayed by the acyl-ACP decrement comparing with the control, the T137G mutant hydrolyzed C12-ACP and C14-ACP with approximate selectivity. (D) The relative substrate conversion efficiency of UcFatB WT and T137G to C12 and C14 substrates. UcFatB preferred C12-ACP to C14-ACP by 2.6 times while UcFatB_T137G hydrolyzed C12-ACP and C14-ACP in an approximate velocity, with a C14/12 relative activity of 0.81.

Table S1 Data-collection and refinement statistics for UcFatB

Data Set	UcFatB
Data collection	
Space group	$P2_12_12_1$
Unit-cell parameters(Å,°)	a=62.60, b=69.87, c=148.93
	α=90, β=90, γ=90
Molecules in asymmetric unit	2
Wavelength (Å)	1.0001
Total reflections	301915 (30574)
Unique reflections	25401 (2457)
Resolution(Å)	46.62-2.42
Rsym† (%)	12.44 (47.41)
Mean I/σ(I)	12.60 (3.40)
Completeness (%)	99.78 (98.28)
Multiplicity	11.9 (12.4)
Wilson B-factor	45.80
Refinement resolution (Å)	46.62-2.51
Rwork ‡ (%)	20.08 (27.13)
Rfree § (%)	26.00 (38.54)
No of atoms	4370
Protein	4253
Ligand/ion	-
Water	117
R.m.s.d	
Bond lengths(Å)	0.009
Bond angles(°)	1.22
Ramachandran (%)	
Allowed	99.8
Outliers	0.19
Clashscore	11.15
Average B-factor (Å^2)	51.30

*Values in parentheses are for the highest resolution shell.

†Rsym = Σ|I-<I>|/Σ<I>, where I is the observed intensity, and <I> is the average intensity of multiple observations of symmetry related reflections.

‡ Rwork= Σhkl||Fobs|-|Fcalc||/Σhkl|Fobs|

§ Rfree is calculated from 5% of the reflections excluded from refinement.

Methods

Circular dichroism spectroscopy

Circular dichorism spectroscopy was acquired on a Biologic M450 spectropolarimeter (BioLogic Science Inc) to monitor the secondary structure changes of the enzyme. Spectra of each protein were measured in 25 μM PBS buffer at 25°C. The light source was a xenon lamp, and the slit was 1.0 nm wide. Quartz cuvettes (1 mm path) were used for measurements, and the protein concentrations were 0.67 mg/mL. Data was collected between 190 and 260 nm at a scan rate of 1 nm/s.

Preparation of synthetic (C8-C16)-ACP pool in *vitro*

The acyl-ACP was separately prepared by acyl-ACP synthetase (AaaS) using fatty acid (8:0, 10:0, 12:0, 14:0, 16:0) as acyl donor as described in the text. Each 50 μL reaction assay contained 25 mM Tris-HCl, 2 mM DTT, 5 mM $MgCl_2$, 5 mM ATP, 50 μM holo-acyl-carrier-protein, 200 μM fatty acid and 0.1 μg purified AaaS protein. And the holo-ACP was completely converted to acyl-ACP at 30 °C for 30 min. The acyl-ACPs were purified by an additional gel chromatography and adjusted to the same concentration before mix.

Relative selectivity assay of UcFatB

The relative selectivity of UcFatB was assayed in a reaction mixture (final volume of 50 μL) containing 47 μL lysis buffer，1 μL UcFatB (0.1 mg/mL for WT and 0.56 mg/L for T137G mutant), 1 μL synthetic (C8-C16) acyl-ACP mix (1 mM). Reactions were carried out at 27 °C for 2 min and terminated by addition of 2 μL 10% TFA. Then 10 μL samples were loaded onto the HPLC system for determination of the reduction efficiency of the substrates. Each assay was measured with three separate replicates.