Electronic Supplementary Information

1. Strains and materials

Reagents for polymerase chain reaction (PCR) -*Ex-Taq* DNA polymerase and T4 DNA ligase - were purchased from Takara (Takara Corp., Japan). pGEM-T easy was purchased from Promega (Madison, USA). A total RNA extraction kit and a nickel-nitrilotriacetic acid (Ni-NTA) superflow column for purification were purchased from Qiagen (Hilden, Germany). Restriction enzymes were obtained from New England Biolabs (MA, USA). pET28a expression vector was purchased from Novagen (Madison, WI). A plasmid isolation kit and oligonucleotide primers were obtained from Bioneer (Daejeon, South Korea). Electrophoresis reagents were from Bio-Rad, and all chemicals for assay were from Sigma-Aldrich (St. Louis, Mo, USA). *Botrytis fuckeliana* was obtained from the Korean Culture Center of Microorganisms (KCCM), Korea. Coenzyme A (CoA), malonyl-CoA, and CoA esters of C_2–C_{18} fatty acids were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2. Cloning, expression, and purification of BPKS

BPKS-encoding open reading frame XM_001555277.1 was amplified from *B. fuckeliana* cDNA with the forward and reverse primers, BCF: G*CATATG*TCAAACAGCACAGAAGGG and BCR: CC*AAGCTT*TCAGTCCAGCTCAACGCCAT (the restriction sites were italicized), and initially cloned with an amino terminal hexa-histidine affinity tag. The purified recombinant protein was judged to be heterogeneous by SDS-PAGE analysis, due to degradation of the carboxy-terminus. Consequently, the Tr-BPKS was amplified with the same forward primer and the reverse primer, BC-TrR: CC*AAGCTT* CAAATGTCCCCACCTACCCC (without 80 extra amino acids at the carboxy terminal), and cloned with an amino-terminal hexa-histidine tag in pET28a. For the production of His_6-tagged BPKS, *E. coli* BL21-CodonPlus (DE3)-RIL harboring pET28a-BPKS was grown at 37 °C in Luria–Bertani broth containing 50 μg/mL kanamycin and 30 μg/mL chloramphenicol. Once the OD_{600} reached 0.4 – 0.6, 100 μM isopropyl β-D-1-thiogalacto-pyranoside (IPTG) was added to induce the overexpression of BPKS. The induced culture was maintained at 30 °C for an additional 6 h. Cells were harvested by centrifugation, resuspended in lysis buffer composed of 20mM Tris–HCl (pH 8.0) and 0.5M NaCl, and disrupted by sonication. A crude cell-lysate was prepared by removing cell debris by centrifugation at 20,000 rpm at 4 °C for 30 min. BPKS was purified by using nickel–nitrilotriacetic acid (Ni–NTA) agarose (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The purified His_6-tagged protein was concentrated and desalted against buffer A [50 mM Tris–HCl (pH 8.0)] using a centrifugal filter (Millipore, Billerica, MA, USA).

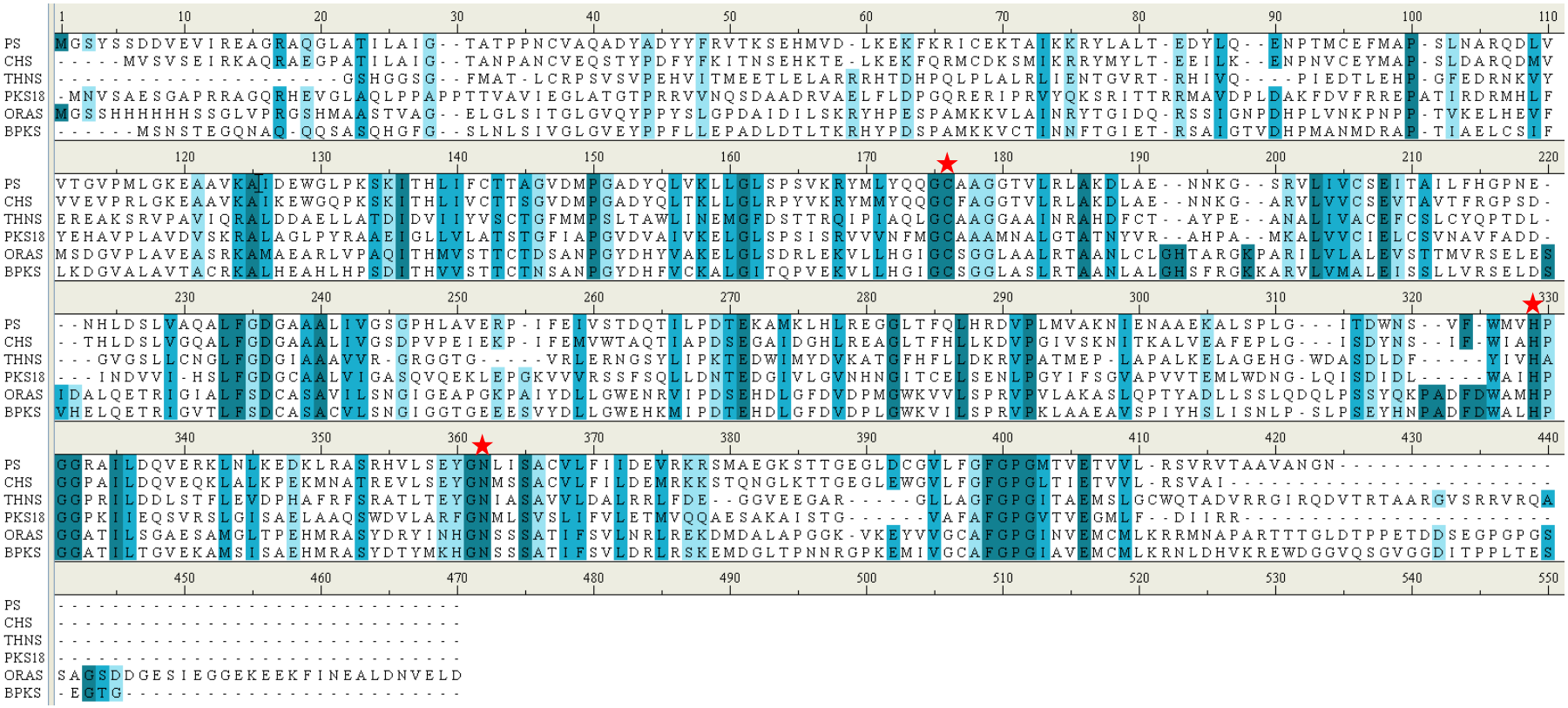

Figure S1 Multiple sequence alignment of various type III polyketide synthases. CHS- chalcone synthase from M*edicago sativa*; PS- 2-pyrone synthase; THNS- 1,3,6,8-tetrahydroxynaphthalene synthase from *Streptomyces coelicolor*A3(2); ORAS- type III polyketide synthase from *Neurospora crassa*; PKS18- type III polyketide synthase from *Mycobacterium tuberculosis.*

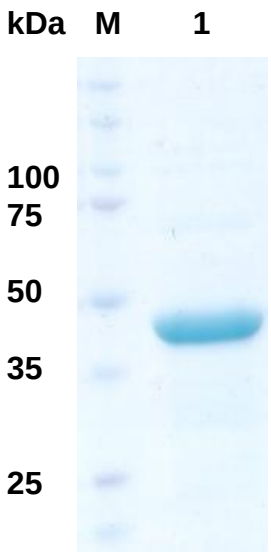

Figure S2 SDS-PAGE analysis of purified BPKS. Lane M- Precision Plus protein standard (Bio-Rad, Hercules, CA); Lane 1- Purified BPKS protein.

3. Polyketide synthase assay and characterization of products

The standard reaction mixture contained 200 μM starter-CoA, 200 μM malonyl CoA, 50 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 100 μg BPKS in a total volume of 500 μL. After the mixture had been preincubated at 30 °C for 3 min, the reaction was initiated by adding malonyl CoA and was allowed to continue for 1 h. The reaction was stopped by adding 20 μL 20% HCl. Products were extracted twice with 500 μL ethyl acetate. The organic layer was dried by N_2 flushing, and the residual material was dissolved in 80 μL CH_3OH for HPLC analysis. An Agilent 1200 Series HPLC and ZORBAX SB-C18 reverse-phase column (3.0×150 mm, 3.5 μm) (Agilent Technologies, Palo Alto, CA) were used by monitoring at 280 nm and at a flow rate of 0.5 mL/min at 25°C. Gradient elution was carried out with H_2O (solvent A) and acetonitrile (solvent B), both containing 0.1% TFA. The mobile phase linear gradient was set for reactions A, and B (Fig. 1): 20% B to 80% B in 30 min; for reaction C: 70% B to 100% B in 30 min. The polyketide products were characterized by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry (LC-APCIMS) analysis on a LCQ DECA XP ion trap (Thermo Finnigan, Waltham, MA). Reactions for LC-APCIMS were scaled up 10-fold compared with those prepared for UV detection.

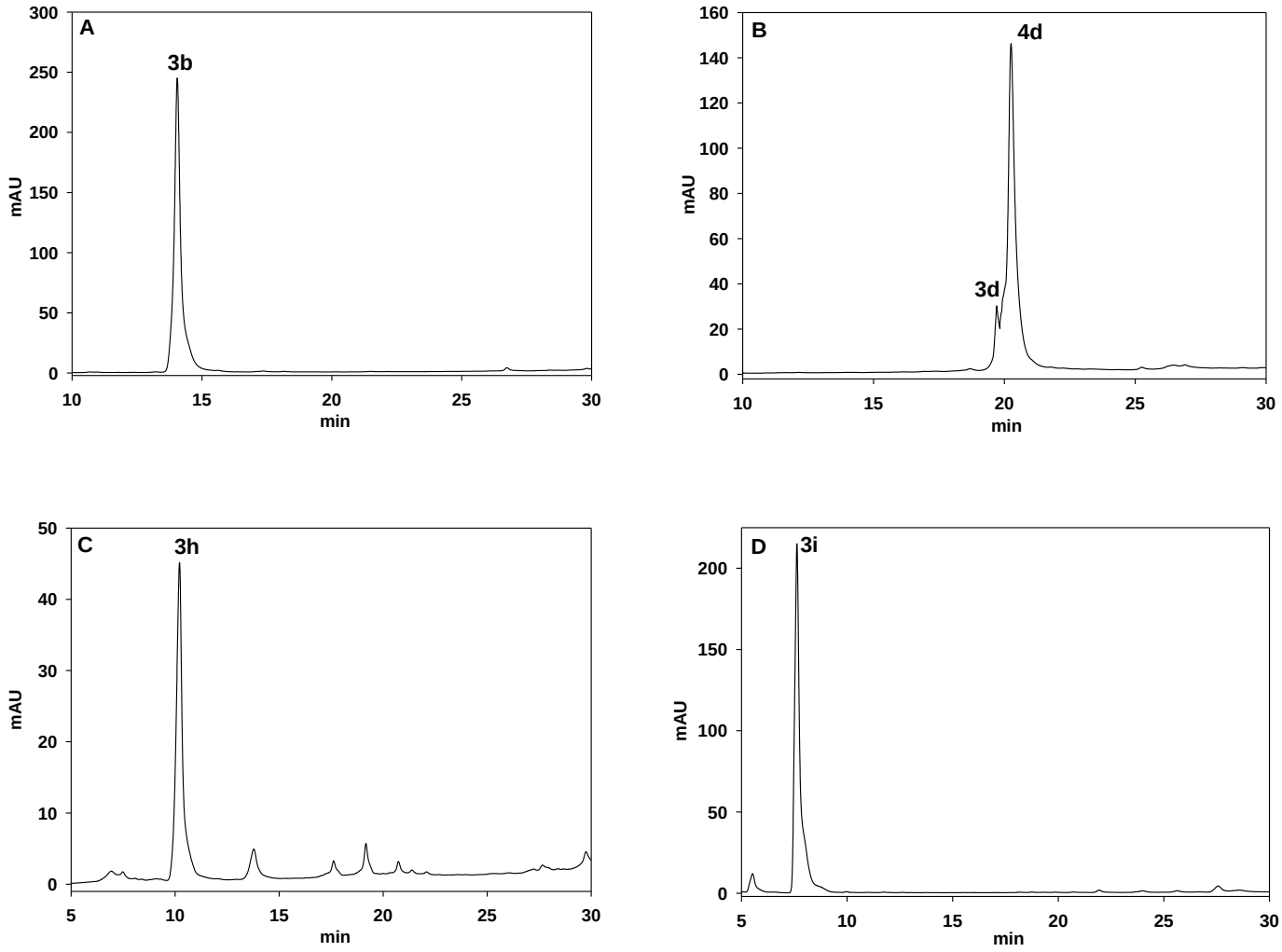

Figure S3 HPLC analysis at $UV_{280}$of the products synthesized by BPKS from various acyl-CoA starter substrates. The reactions contained 200 µM of malonyl-CoA and 200 µM acyl-CoA starters. A, hexanoyl-CoA (**2b**); B, lauroyl-CoA (**2d**); C, benzoyl-CoA (**2g**) and acetoacetyl-CoA (**2h**).

Products from hexanoyl-CoA [**2b**]. 4-hydroxy-6-pentyl-2-pyrone [**3b**]: retention time was 14 min on LC; UV, λ_{max} 286 nm; LC/APCIMS (negative mode), MS: 181[M-H]$^-$; MS/MS (precursor ion at *m/z* 181): 137[M - CO_2-H]$^-$. HRMS (FAB, negative) calcd. for [$C_{10}H_{14}O_3$]$^-$: 181.0871, found: 181.0865.

Products from lauroyl-CoA [**2d**]. 4-hydroxy-6-undecyl-2-pyrone [**3d**]: retention time was 20.4 min on LC; UV, λ_{max} 286 nm; LC/APCIMS (negative mode), MS: 265.2[M-H]$^-$; MS/MS (precursor ion at *m/z* 265.2): 221.2[M - CO_2-H]$^-$. HRMS (FAB, negative) calcd. for [$C_{16}H_{26}O_3$]$^-$: 265.2123, found: 265.2129. 4-hydroxy-6-(2'oxotridecyl)-2-pyrone [**4d**]: retention time was 19.8 min on LC; UV, λ_{max} 286 nm; LC/APCIMS (negative mode), MS: 307.19[M-H]$^-$; MS/MS (precursor ion at *m/z* 307.2): 263.2[M - CO_2-H]$^-$, 125.01[$C_6H_5O_3$]$^-$. HRMS (FAB, negative) calcd. for [$C_{18}H_{28}O_4$] $^-$: 307.2012, found: 307.2023.

Products from stearoyl-CoA [**2g**]. 6-heptadecyl-4-hydroxy-2-pyrone [**3g**]: retention time was 27.4 min on LC; UV, λ_{max} 286 nm; LC/APCIMS (negative mode), MS: 349.2[M-H]$^-$; MS/MS (precursor ion at *m/z* 349.2): 305.2[M - CO_2-H]$^-$. HRMS (FAB, negative) calcd. for [$C_{22}H_{38}O_3$]$^-$: 349.2276, found: 349.2256. 4-hydrocy-6-(2'-oxononadecyl)-2-pyrone [**4g**]: retention time was 22.4 min on LC; UV, λ_{max} 286 nm; LC/APCIMS (negative mode), MS: 391.2[M-H]$^-$; MS/MS (precursor ion at *m/z* 349.2): 347.2[M - CO_2-H]$^-$, 125[$C_6H_5O_3$]$^-$. HRMS (FAB, negative) calcd. for [$C_{24}H_{40}O_4$]$^-$: 391.2315, found: 391.2304.5-(2'-oxononadecyl)-resorcinol [**7g**]: retention time was 22.6 min on LC; UV, λ_{max} 286 nm; LC/APCIMS (negative), MS: 389.3[M-H]$^-$; MS/MS (precursor ion at *m/z* 389.3), 347.2[M - CO_2-H]$^-$, 123[$C_7H_7O_2$]$^-$. HRMS (FAB, negative) calcd. for [$C_{25}H_{42}O_3$]$^-$: 389.3124, found: 389.3167. 2,4-dihydroxy-6-(2',4'-dioxohenicosyl)-benzoic acid [**8g**] retention time was 27.4 min on LC; UV, λ_{max} 286 nm; LC/APCIMS (negative mode), MS: 475.3[M-H]$^-$; MS/MS (precursor ion at *m/z* 475.3), 123[$C_7H_7O_2$]$^-$. HRMS (FAB, negative) calcd. for [$C_{28}H_{44}O_6$]$^-$: 475.3218, found: 475.3221.

Product from benzoyl-CoA [**2h**]. 4-hydroxy-6-phenyl-pyran-2-one [**3h**]: retention time was 10.2 min on LC. UV, λ_{max}325 nm; LC/APCIMS (negative mode), MS: 187 [M-H]$^-$, MS/MS (precursor ion at m/z 187): 143 [M-H-CO_2]$^-$. HRMS (FAB, negative) calcd. for [$C_{11}H_8O_3$]$^-$: 187.1234; found: 187.1238.

Product from acetoacetyl-CoA [**2i**]. 4-hydroxy-6-methyl-2-pyrone (triacetic acid lactone) [**3i**]: retention time was 8.0 min on LC; UV, λ_{max}286 nm; LC/APCIMS (negative mode), MS: 125[M - H]$^-$; MS/MS (precursor ion at *m/z* 125): 81[M - CO_2-H]$^-$. HRMS (FAB, negative) calcd. for [$C_6H_6O_3$]$^-$: 125.0413; found: 125.0418.

4. Determination of kinetic parameters

Standard reactions contained 50 mM Tris-HCl (pH 8.0), malonyl-CoA (100 µM), and 20 µg of BPKS in a total volume of 500 µL. The concentration of priming acyl-CoA varied from 5 to 100 µM for various acyl CoAs. Reaction mixtures were preincubated at 30 °C for four minutes before the addition of malonyl-CoA to start the reaction. Reactions were analyzed using an Agilent

1200 Series HPLC and Prevail C18 reverse-phase column (3.0 ×150 mm, 3.5 µm) (Agilent Technologies, Palo Alto, CA) monitored at 258 nm and under the following conditions: 0.5 mL/min; 25 °C; solvent A: 15 mM ammonium formate, solvent B: 90% methanol, 10% 10 mM ammonium acetate, pH 7.3, adjusted using acetic acid as described by Rubin-Pitel *et al.*[1]. Peaks at 6 min and 8 min corresponded to authentic malonyl-CoA and CoASH, respectively. The experiments were carried out in triplicate, and the initial rates of CoASH production determined from these reactions were averaged. Kinetic parameters (**Table S1**) were determined for the priming substrates from initial velocity measurements of the formation of free CoASH. The enzyme showed maximum CoASH release at pH 8.0 and 30 °C.

Table S1 Specific activity of BPKS protein with different starter units. Results are means (n = 3) with S.E. values less than 15%.

Starter CoA	Specific activity (µmol/min/mg-protein)	Relative activity (%)
Stearoyl CoA	0.24 ± 0.05	88 ± 7
Palmitoyl CoA	0.27± 0.06	100± 9
Myristoyl CoA	0.11 ± 0.02	39 ± 3
Lauroyl CoA	0.06 ± 0.02	23 ± 3
Decanoyl CoA	0.06 ± 0.01	22 ± 2
Octanoyl CoA	0.05 ± 0.02	20 ± 4
Hexanoyl CoA	0.05 ± 0.02	19 ± 4
Butyryl CoA	0.03 ± 0.01	12 ± 1
Acetoacetyl CoA	0.02 ± 0.01	9.1 ± 0.7
Benzoyl CoA	0.03 ± 0.01	11 ± 2

Specific activity, the initial rate of CoASH production (µmol/min) normalized by protein amount (mg) is shown. All the reactions were performed at 30 ^{o}C for 5 min using 100 µM of malonyl CoA and 50 µM of starter CoAs.

Table S2 Comparison of kinetic constants of various starter CoAs determined for type III PKSs.

Starter-CoAs	K_m(μM)	k_{cat} (s^{-1})	k_{cat}/K_m (s^{-1} M^{-1})	Reference
Hexanoyl-CoA				
CysA*	867	0.25	291	2
ORAS	11.3	1.0×10^{-4}	8.8	[This study]
PKS18	19.2	5.5×10^{-3}	286	3
BPKS	1.6	0.05	3.1×10^{4}	[This study]
Palmitoyl-CoA				
PKS18	5.4	0.26	4.8×10^{4}	3
BPKS	3.3	0.92	2.8×10^{5}	[This study]
Stearoyl-CoA				
ORAS	3.5	6.3×10^{-4}	1.8×10^{2}	1
BPKS	3.7	7.5×10^{-1}	2.0×10^{5}	[This study]
Benzoyl-CoA				
AnPKS	18.1	13.5	7.4×10^{5}	4
BPKS	19.8	9.0×10^{-2}	4.5×10^{3}	[This study]

*Kinetic parameters determined for hexanoyl-SNAC

5. Homology modeling and substrate docking

The three dimensional homology model of *B. fuckeliana* was generated using the Build Homology Models (MODELER) module in Discovery Studio 3.0 (DS 3.0, Accelrys Software Inc., San Diego, CA), with the crystal structure of ORAS (3E1H) used as a template. Comparative modeling generated the most probable structure of the query protein by alignment with template sequences, while satisfying spatial restraints and local molecular geometry. Sequence identity between target and template was found to be 65% according to BLAST parameters. The fitness of the models in their current 3D environment was evaluated by Profiles-3D Score/Verify Protein (MODELER) as implemented in DS 3.0. Discrete optimized protein energy scores in MODELER were also calculated to determine the quality of the protein structures. Profile-3D score was 178.23 against a maximum expected score of 197.15. The root mean square deviation (RMSD) between the models and template was calculated by superimposing the model on the template crystal structure to assess the reliability of the models. The RMSD was 0.2 Å based on C-alpha atoms. The generated structure was improved by subsequent refinement of the loop conformations by assessing the compatibility of an amino acid sequence to known PDB structures using the Protein Health module in DS 3.0. The geometry of the loop regions was corrected using Refine Loop/MODELER.

The highest-quality resulting model was used for further docking study. Hydrogen atoms were added to the protein model and were minimized to have stable energy conformations and also to relax the conformation of close contacts. Stearoyl CoA was docked into the CoA binding tunnel of the BPKS model using C-DOCKER in DS 3.0. Random substrate conformations were generated using high-temperature molecular dynamics simulation. Candidate poses were then created using random rigid-body rotations followed by simulated annealing. The protein structure was minimized for energy using a CHARMm forcefield as implemented in DS 3.0. A full potential final minimization was then used to refine the substrate poses. Based on C-DOCKER energy, the docked conformation of the substrate was retrieved for post-docking analysis. The substrate orientation which gave the lowest interaction energy was chosen for subsequent rounds of docking.[5]

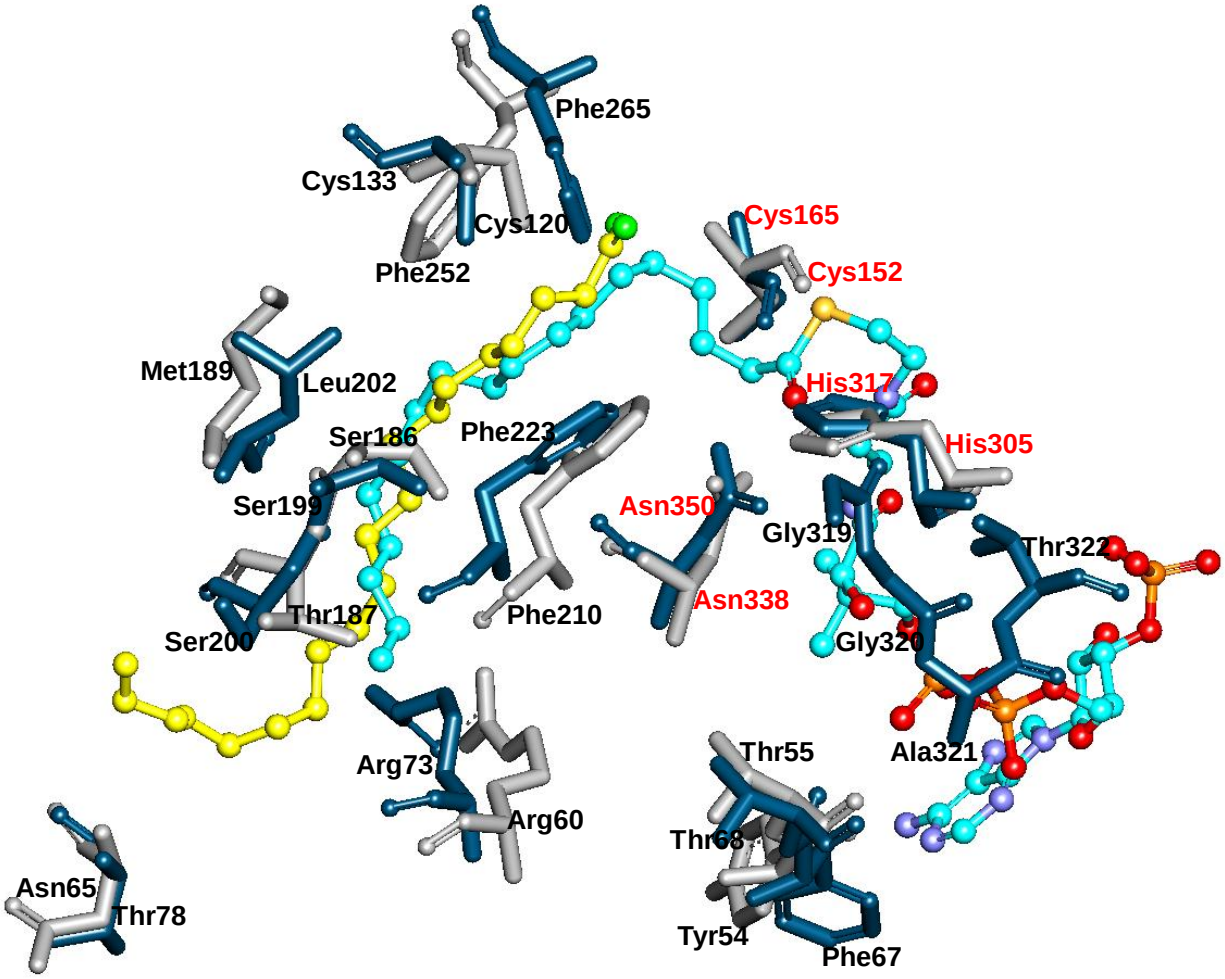

Figure S4 Superposition of the modeled BPKS structure with bound stearoyl-CoA and the ORAS structure co-crystallized with eicosanoic acid (C_{20}). Amino acid residues are shown in stick model colored with white (ORAS) and blue (BPKS). The catalytic amino acid residues and the residues for acyl-CoA binding tunnels are labelled in red and black color, respectively. Ligands are represented in ball and stick model with blue color carbon for stearoyl-CoA and green color for eicosanoic acid.

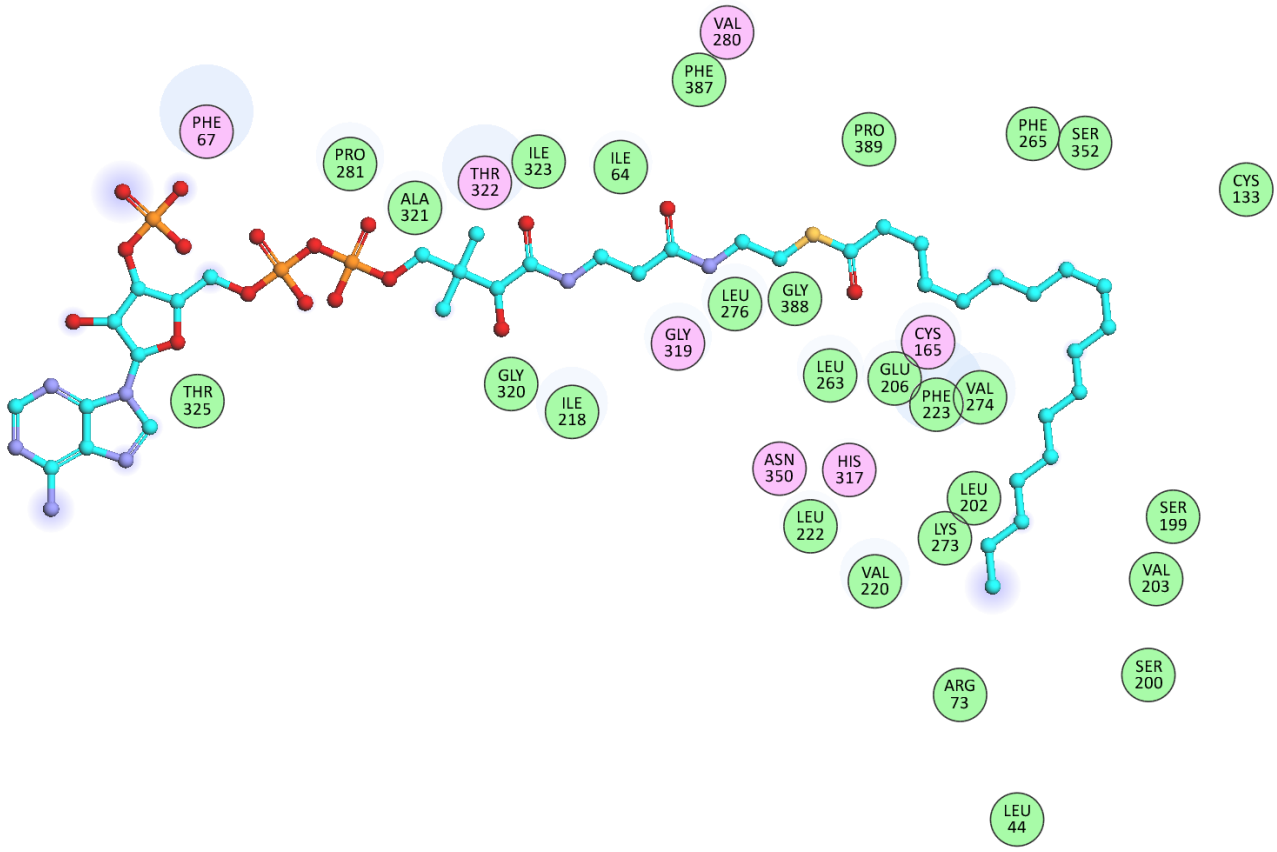

Figure S5 2D representation of the active site and acyl-CoA binding tunnel of BPKS with bound stearoyl-CoA. The residues involved in various events are represented as pink circles (electrostatic interaction) and green circles (van der Waals interaction).

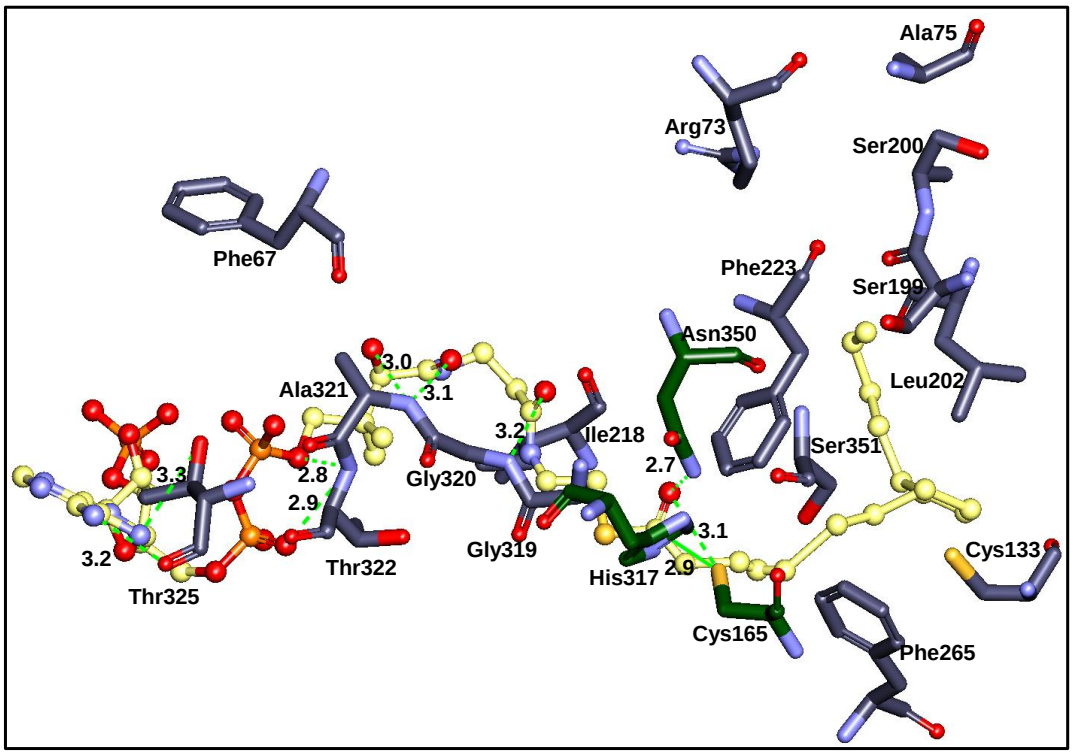

Figure S6 Palmitoyl-CoA docked in the substrate binding tunnel of BPKS. Hydrogen bonds between palmitoyl-CoA and amino acid residues are shown in green dotted lines. Palmitoyl-CoA interaction with the catalytic residue Cys165 is shown in solid line. Amino acid residues are shown in stick model colored with carbon in grey color while catalytic amino acid residues are shown with carbon in green color. Palmitoyl-CoA represented in ball and stick with carbon in yellow color.

References

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