Mathematical Modeling and Kinetic Analysis of a Type II Fatty Acid Synthase with Applications in Oleochemical Production

by

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Thesis directed by Assistant Professor Jerome Fox

Abstract

The design and manipulation of biological systems is complicated by the complexity of the interdependent chemical and physical processes on which they rely. A striking variety of biological processes result from the interdependence of competing—and sometimes overlapping—catalytic steps. The full spectrum of pathway behaviors cannot be understood without quantitative models of these processes. This project aims to address this gap in understanding by developing a mechanistic kinetic model of the fatty acid synthase (FAS) of *Escherichia coli*. Fatty acids are precursors to many valuable oleochemicals (alcohols, ketones and alkanes for example), and the use of FASs to overproduce fatty acids is an important goal of applied biocatalysis. Unfortunately, to date, simultaneous control over both total production and chain length remains challenging. This project comprises three studies. In the first, I develop a detailed kinetic model of saturated fatty acid biosynthesis and use it to examine previously unexplained FAS behaviors, and to explore new strategies for controlling FAS outputs. The results of this work indicate that promiscuous thioesterases can, when coupled with changes in the concentrations of other FAS components, permit precise control over both the average length of fatty acids and their total production; narrow product profiles, however, require either a specialized thioesterase or mutants of key FAS components. In the second study, I expand the kinetic model to include the production of
unsaturated fatty acids, and I use it to explore the advantages conferred by functional redundancy (i.e., the inclusion of two enzymes with overlapping catalytic activities) in fatty acid pathways. The results of this analysis indicate that functional redundancy can help decouple competing biochemical objectives (e.g., the total production, average length, and unsaturated fraction of fatty acids). In the third study, I expand the model further to include oleochemical production. This revised model effectively captures trends in oleochemical production exhibited by engineered strains of *E. coli*. Broadly, the results of the three studies help explain the structure and activity of FAS systems and provide new strategies for engineering these systems for the microbial production of oleochemicals.
Dedicated to my parents, Angelica and David, and to my sister, Monika
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CHAPTER I: INTRODUCTION

This project uses kinetic modeling to improve our understanding of metabolic pathway design strategy in oleochemical production by microbial hosts. Oleochemicals (aliphatic compounds produced by biological sources) are an exciting renewable source of chemical products normally derived from crude oil. To generate oleochemicals from microbial sources, the biocatalytic machinery of the host is redirected towards excess production of a desired oleochemical target. This process of cellular re-design (referred to as metabolic engineering) is challenging due to the complexity of cellular processes and regulatory behaviors. Furthermore, often only a qualitative understanding of the relevant biocatalytic pathways and regulatory mechanisms is available. A metabolic engineer must therefore intuit strategies based on literature experience and select the most viable for experimental exploration. This process is highly limited by the time and expense of experiments, and considerable effort is often required to achieve optimal production. Additionally, surprising or unintuitive results in the optimization process may be difficult to explain without quantitative insight into pathway behavior. Quantitative models may also provide a means of understanding the unique advantages of differing pathway designs. The development of detailed quantitative models of biocatalytic networks can provide valuable information on engineering strategy and understanding of biological rationale in pathway design.

This work focuses on the development of a detailed kinetic model of the *E.coli* fatty acid synthase (FAS) and oleochemicals derived from fatty acids. This choice was made for the following reasons: i) *E.coli* is well studied, there are detailed kinetic studies on FAS enzymes ii) *E.coli* FAS is a common metabolic engineering target, there are numerous studies producing fatty acids and derived products iii) metabolic engineering of FAS is challenging due to the nonlinear
response of its iterative design, and so is likely to benefit from quantitative modeling iv) There are
detailed *in vitro* kinetic studies of a pathway reconstitution of FAS. Kinetic studies of the pathway
*in vitro* are of particular significance as numerous pathway behaviors observed in the *in vitro*
reconstitution translate to *in vivo* behavior. This suggests that FAS pathway outputs may be
predicted *in vivo* using a kinetic model that neglects upstream processes or other cellular
interactions and regulatory behavior. Furthermore, any discrepancies in behavior between the
model and *in vivo* behavior that are validated *in vitro* are shown to derive from effects not related
to pathway kinetics.

The model of *E.coli* FAS for this project was constructed in three phases: i) an initial model
consisting of saturated fatty acid synthesis ii) an expansion of the model to include unsaturated
fatty acid synthesis as well as lipid A and the initial phospholipid reactions iii) and a final
expansion of the model to include downstream oleochemical products.
CHAPTER II: ANALYSIS OF INTERDEPENDENT KINETIC CONTROLS OF FATTY ACID SYNTHASES

Alex Ruppe and Jerome Fox

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2.1 | ABSTRACT

Biocatalytic systems (e.g., multi-enzyme pathways) enable the conversion of simple sugars into complex products under ambient conditions and, thus, represent promising platforms for the synthesis of renewable fuels and chemicals. Unfortunately, to date, many of these systems have proven difficult to engineer without a detailed understanding of the kinetic relationships that regulate the concerted action of their constituent enzymes. This study develops a mechanistic kinetic model of the fatty acid synthase (FAS) of Escherichia coli, and uses that model to determine how different FAS components work together to control the production of free fatty acids—precursors to a wide range of oleochemicals. Perturbational analyses indicate that the modification or overexpression of a single FAS component can depress fatty acid production (a commonly observed phenomenon) by sequestering the proteins with which it interacts and/or by depleting common substrate pools. Multi-parameter studies, in turn, suggest that simple changes in the ratios of FAS components can alter the average length of fatty acids, but show that specialized enzymes (i.e., highly specific ketoacyl synthases or thioesterases) are required for narrow product profiles. Intriguingly, a sensitivity analysis indicates that two components primarily influence—and, thus, enable fine control over—total production, while the enzymes that regulate product profile are more broadly influential. Findings reveal the general importance of kinetic considerations in efforts to engineer fatty acid biosynthesis and provide strategies—and a kinetic model—for incorporating those considerations into FAS designs.
2.2 | INTRODUCTION

The biocatalysts that enable fatty acid synthesis in microbial cells offer a promising means of producing fuels and chemicals from renewable feedstocks. Fatty acid synthases (FASs)—multi-domain complexes (type I) or multi-enzyme mixtures (type II) that convert glucose-derived metabolites into structurally varied fatty acids—can supply precursors to a wide range of enzymatically synthesizable oleochemicals (e.g., alcohols, alkyl esters, methyl ketones, and alkanes).\(^1\) To date, most efforts to engineer the product profiles of FASs have relied on changes in the identity or expression level of individual catalytic components;\(^2,3\) these approaches have enabled the synthesis of novel varieties of fatty acids but by neglecting the kinetic relationships that allow different components to work together, they have struggled to achieve tight, yield-independent control over product distributions.\(^4,5\) The development of general strategies to tune the product profiles of FASs thus remains a longstanding challenge of applied biocatalysis.\(^6\)

The fatty acid pathway of *Escherichia coli* (*E. coli*) is a well-studied type II FAS that demonstrates the difficulty of controlling fatty acid production in microbial cells.\(^7,8\) It builds fatty acids in three main steps (Figure 2.1): initiation, elongation, and termination. Initiation begins when FabD (a transacylase) transfers the malonyl group of malonyl-CoA to an acyl carrier protein (ACP), where FabH (a \(\beta\)-ketoacyl-ACP synthase) condenses it with acetyl-CoA to form acetoacyl-ACP. Elongation, two carbons at a time, occurs through repeated (i) reduction of acetoacyl-ACP to acyl-ACP by three enzymes—FabG (a \(\beta\)-ketoacyl-ACP reductase), FabZ or FabA (\(\beta\)-hydroxyacyl-ACP dehydratases), and FabI (an enoyl-acyl-ACP reductase)—and (ii) condensation of acyl-ACPs with malonyl-ACP by FabB or FabF (\(\beta\)-ketoacyl-ACP synthases). Termination—the release of free fatty acids—results from thioesterase-catalyzed hydrolysis of acyl-ACPs. The
FAS of *E. coli* does not contain a thioesterase, per se, but it is often supplemented with one in engineered strains.⁹,¹⁰

**Figure 2.1.** The fatty acid synthase (FAS) of *E. coli*. This type II FAS formally includes FabD, FabH, FabG, FabZ, FabA, FabI, FabB, FabF, and holo-ACP; many studies, however, supplement it with a periplasmic variant of TesA.⁹,¹⁹,²³ Our model captures the activities of the seven enzymes (orange) necessary for *de novo* biosynthesis of fatty acids from malonyl-CoA and acetyl-CoA.

ACP s are often overlooked as simple substrate carriers, but recent evidence suggests that they may play important regulatory roles.¹¹ The archetypal type II ACP consists of an α-helical bundle with a central hydrophobic cleft.⁸ This cleft sequesters acyl chains in solution and, upon enzyme-ACP association, undergoes a conformational change that releases those chains into neighboring active sites.¹² Differences in the strength—and conformational repercussions—
various ACP-substrate and ACP-enzyme interactions could, thus, modulate the flux of intermediates through the fatty acid pathway.

Most efforts to control fatty acid production in *E. coli* have incorporated enzymes with non-native activities.\textsuperscript{13,14} Examples include ketoacyl synthases with enhanced activities on branched acyl-CoAs,\textsuperscript{15} ketoacyl-ACP reductases that alter the location or stereochemistry of double bonds,\textsuperscript{16,17} and thioesterases that target short acyl-ACPs.\textsuperscript{4,18,19} When overexpressed in *E. coli*, these enzymes have enabled useful changes in product profiles (e.g., the enhanced synthesis of short or branched fatty acids); they have not, however, yielded precise control over product distributions (they have tended to generate broad distributions with numerous side products\textsuperscript{13,14,19}) or afforded mechanistic insights that explain that lack of control.

Detailed studies of fatty acid synthesis in *E. coli* indicate that complex kinetic relationships between enzymes regulate FAS activity in a nonintuitive manner.\textsuperscript{20–22} We will briefly describe two: (i) In an investigation of the FAS reconstituted in vitro, Khosla and colleagues observed that high concentrations of FabH, FabF, thioesterase, or holo-ACP reduced rates of fatty acid synthesis\textsuperscript{23}; they attributed this inhibitory effect to the sequestration of essential proteins (i.e., the removal of holo-ACP by excess enzymes that bind to this protein, or vice versa). (ii) In an analysis of *E. coli* engineered to express plant-derived thioesterases, Silver and colleagues showed that cerulenin, an inhibitor of FabF and FabB, could increase yields of fatty acids\textsuperscript{5}; they hypothesized that this improvement resulted from both (i) the enhanced availability of medium-chain acyl-ACPs targeted by thioesterases and (ii) the reduced accumulation of long-chain acyl-ACPs that inhibit FabH.\textsuperscript{24} The results of these two studies show how the kinetics of interdependent catalytic steps control fatty acid production in a nonlinear manner that complicates the rational rewiring of FAS activity.
In this study, we developed a mechanistic kinetic model of the FAS of *E. coli* and used it to determine how different enzymes work together to control rates of fatty acid synthesis and overall product profiles. This analysis departs from previous quantitative investigations of fatty acid production (e.g., flux balance analyses, which incorporate cell-wide reaction stoichiometries but neglect reaction kinetics) in its focus on the mechanistic and kinetic details of a small number of highly influential steps: the enzymatic reactions responsible for the synthesis of saturated fatty acids. We used our model to accomplish three tasks: (i) to test previously posited hypotheses concerning the mechanistic origin of unexpected experimental results (e.g., the inhibitory effect of high concentrations of FAS components), (ii) to carry out multi-parameter studies of interdependent catalytic steps (i.e., studies that are experimentally intractable), and (iii) to develop new strategies to tune the product profiles of FASs. With these analyses, we sought to develop a general kinetic framework for engineering fatty acid synthesis in microbial systems.

2.3 | RESULTS AND DISCUSSION

2.3.1 | Development of a Mechanistic Kinetic Model. We constructed a detailed kinetic model of the FAS of *E. coli* by incorporating the activities of seven enzymes necessary to convert malonyl-CoA and acetyl-CoA to saturated fatty acids (Figure 2.1 and Table 2.1): FabD, FabH, FabG, FabZ, FabI, FabF, and TesA. We included TesA, a periplasmic thioesterase from *E. coli*, because it generates large amounts of free fatty acids and is commonly included in engineered systems.9,10 (These systems include a cytosolic variant of TesA; we refer only to this variant in our analysis). We excluded FabA and FabB, two jointly regulated enzymes required for the construction of unsaturated fatty acids,26 for two reasons: (i) They have overlapping activities with FabZ and FabF and, thus, complicate the systematic analysis of unique catalytic steps,27 and (ii)
previous studies suggest that they exert a negligible influence on overall rates of synthesis.\textsuperscript{11,23,27}

We based all rate equations on kinetic mechanisms reported in detailed experimental analyses of individual enzymes, and we avoided \textit{a priori} equilibrium assumptions by including independent association and disassociation steps for each heteromeric complex (Tables 2.1 and A.1). Our final model contained 154 equations and 83 unique kinetic constants (Appendix A).

We note: Five of the modeled enzymes (FabH, FabG, FabZ, FabI, and FabF) form homooligomeric complexes that may give rise to cooperative interactions between complexed proteins;\textsuperscript{8} previous studies have observed such interactions in dimers of FabH and tetramers of FabG.\textsuperscript{28,29} By modeling all enzymes as monomers, we assumed that cooperative changes in binding constants were small, relative to both (i) differences in binding constants between enzymes and (ii) the precision afforded by model fits.
Table 2.1. Kinetic Mechanisms and Parameters

†FabD*, FabH*, and FabF* represent refer to acyl-enzyme intermediates.
‡For the sensitivity analysis of the expanded model: b_{11} scales k for 1 and 2; b_{12}, k for 3; b_{23}, k for 10; b_{31}, K_{eq} for 2; b_{32}, K_{eq} for 3; b_{33}, K_{eq} for 10; c_{21}, k_{cat} for 6; c_{22}, k_{cat} for 7; c_{23}, k_{cat} for 9; and c_{23}, k_{cat} for 11.
2.3.2 | Parameterization of the Kinetic Model. We based initial estimates of model parameters on the results of detailed kinetic studies. For most enzymes, we used a combination of (i) measured turnover numbers and equilibrium constants reported in the literature or (ii) fits to published kinetic data (Table A.4). For TesA, we supplemented these fits with new kinetic measurements that allowed us to estimate its affinity for holo-ACP (Methods, Figure A.2E, and Table A.3).

We optimized estimates of kinetic parameters by fitting our model to experimental measurements of FAS activity. In brief, we used twelve scaling parameters to link groups of similar kinetic terms to one another (e.g., $c_2$ scales estimates of $k_{cat}$ for the four enzymes that constitute the elongation cycle; Table 2.1), and we optimized these parameters with a simultaneous fit to three datasets: (i) a time course of total fatty acids produced by a reconstituted FAS,\textsuperscript{23} (ii) the product distribution generated by a strain of *E. coli* overexpressing TesA,\textsuperscript{19} and (iii) initial rates of fatty acid synthesis exhibited by reconstituted FASs with varying concentrations of FabH (Figure 2.2).\textsuperscript{23} This diverse set of data helped to prevent overfitting and, thus, to ensure that the model could accurately predict fatty acid production under a broad range of FAS compositions. We note: The reference study for the third dataset did not report the time used to measure initial rates,\textsuperscript{23} so we assumed a measurement time of 2.5 minutes (i.e., the time taken for ~ 10 turnovers) and optimized our model against normalized initial rates (e.g., Figure 2.2C).
Figure 2.2. Optimization of the kinetic model. We optimized our model with simultaneous fits to three datasets: (A) a time course of total fatty acids generated by a reconstituted FAS (1 μM of each Fab, 10 μM holo-ACP, 10 μM TesA, 1 mM NADPH, 1mM NADH, 0.5 mM malonyl-CoA, and 0.5 mM acetyl-CoA), (B) the product distribution generated by a strain of E. coli overexpressing TesA, and (C) initial rates of fatty acid synthesis (calculated over 2.5 min) exhibited by reconstituted FASs with varying concentrations of FabH (i.e., mixtures with 0.2 mM acetyl-CoA that are otherwise identical to A). We used the in vivo product distribution (B) as an approximation for the in vitro distribution (not reported) at 12.5 minutes; for C, we optimized our model against normalized initial rates. Panel C compares two modified models: In the first (triangles), FabH cannot bind to ACP or acyl-ACPs. In the second, the first revised model is further modified with two FabDs that generate FabH- and FabF-specific pools of malonyl-ACP. Only the second model is insensitive to high concentrations of FabH, suggesting that inhibition results from competition between FabH and FabF for malonyl-ACP.
2.3.3 | Excess FabH Inhibits Fatty Acid Synthesis by Depleting Malonyl-ACP. We began our analysis of FAS kinetics by using our model to examine the inhibitory effect of high concentrations of FabH. Previous studies have posited that this effect might result from either (i) the sequestration of ACPs (i.e., high concentrations of FabH might bind to holo-ACP and/or acyl-ACPs and, thus, sequester them from the reaction mixture) or (ii) the depletion of malonyl-ACP, a substrate of FabF. To test the first explanation, we eliminated terms describing binding of FabH to holo- and acyl-ACPs (Table A.1); this modification yielded only a slight reduction in inhibition (Figure 2.2C), suggesting that component sequestration was not its primary cause. To test the second explanation, we further modified our model by adding variants of FabD that generate FabH- and FabF-specific pools of malonyl-ACP. This modification had two prominent effects: (i) it reduced initial rates (an expected outcome, given the reduced concentration of malonyl-ACP available for initiation), and (ii) it eliminated the inhibitory effect of high concentrations of FabH (Figure 2.2C). Our analysis, thus, indicates that high concentrations of FabH inhibit FAS activity by competing with FabF for malonyl-ACP.

2.3.4 | Excess holo-ACP, FabF, and TesA Inhibit Fatty Acid Synthesis by Sequestering FAS Components. We tested the ability of our model to capture trends in data to which it was not fit by examining the inhibitory effects of holo-ACP, FabF, and TesA reported by Khosla and colleagues. To our satisfaction, our model predicted these effects (i.e., fractional reductions in initial rates were similar to those observed in in vitro experiments; Figure 2.3A, 2.3C, and 2.3E) and, thus, appeared to accurately capture the mechanisms by which FAS components influence overall activity.
An inventory of bound and free forms of holo-ACP, FabH, and TesA indicate that excess concentrations of these species inhibit fatty acid synthesis through component sequestration (Figures 2.3B, 2.3D, and 2.3F). To examine this effect more directly, we removed terms describing the binding (i) all enzymes to holo-ACP, (ii) FabF to holo-ACP, or (iii) TesA to holo-ACP. Unlike with FabH (Figure 2.2C), these modifications eliminated inhibition (Figures 2.3A, 2.3C, and 2.3E) and, thus, indicated that component sequestration was its primary cause. The results of this analysis are intriguing because they imply that the strength of enzyme-ACP interactions determines the optimal composition of FASs (an implication supported by previous observations that heterologous ACPs can reduce FAS activity30,31). Efforts to exchange or modify ACPs—or the enzymes with which they interact—are, thus, likely to require compositional re-optimization, a step rarely taken in metabolic engineering.
Figure 2.3. The influence of ACP, FabF, and TesA on FAS activity. Initial rates of fatty acid synthesis exhibited by reconstituted FASs (1 μM of each Fab, 10 μM TesA, 10 μM holo-ACP, 1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, and 0.2 mM acetyl-CoA, 2.5 min) with varying concentrations of (A) ACP, (C) FabF, and (E) TesA. The model captures the inhibitory effects of each component and provides a mechanistic explanation: (A) High concentrations of ACP reduce initial rates by (B) sequestering enzymes from the reaction mixture. In A, a modified model that lacks interactions between enzymes and holo-ACP shows no inhibition (dashed blue line). Similarly, high concentrations of (C) FabF and (E) TesA reduce initial rates by (D and F) sequestering ACP. Modified models that lack (D) FabF-ACP or (F) TesA-ACP interactions show no inhibition (dashed blue lines). Note that for TesA both versions of the model show an initial rising trend at low concentrations, and the discrepancy in maximal initial rate location likely stems from inaccuracies in the estimation of TesA-ACP binding strength.
2.3.5 | Excess FabI and FabZ Can Enhance Rates of Fatty Acid Synthesis. Many studies of FASs seek to improve rates of fatty acid synthesis by identifying and removing metabolic bottlenecks.\textsuperscript{32,33} In one such study, Khosla and colleagues observed that high concentrations of FabZ and FabI (relative to a base system) could enhance FAS activity \textit{in vitro} and \textit{in vivo}.\textsuperscript{23} Motivated by this result, we used our model to examine the influence of these two enzymes on rates of fatty acid synthesis. Our results revealed trends similar to those observed \textit{in vitro}: As concentrations of FabI and FabZ increased, initial rates increased in a hyperbolic manner (Figure 2.4). Importantly, model-predicted optima occurred at lower enzyme concentrations than experimental optima, but a reduction in modeled values of $k_{\text{cat}}$ for both enzymes reduced this discrepancy; our model may thus overestimate activities of FabI and FabZ.

Overall, trends in initial rates indicate that both FabZ and FabI can increase FAS activity, but show that FabZ does so over a wider range of concentrations than FabI (i.e., a range that may be more likely to include physiologically relevant conditions). The pronounced influence of FabZ likely results from the combined effects of its slow production of enoyl-ACP and the rapid consumption of enoyl-ACP by FabI; enoyl-ACP thus acts as a rate-limiting intermediate. A formal analysis of reaction intermediates supports this assertion: FabZ enhances rates of fatty acid synthesis until steady-state concentrations of enoyl-acyl-ACP plateau (Figures A.4C and A.4D). The “valve-like” behavior of FabZ, alongside previous reports of the titer-enhancing benefits of FabZ overexpression,\textsuperscript{23,25,34} suggests that this enzyme catalyzes a rate-limiting step in fatty acid synthesis.
Figure 2.4. The influence of FabI and FabZ on FAS activity. Initial rates of fatty acid synthesis exhibited by reconstituted FASs (1 μM of each Fab, 10 μM TesA, 10 μM holo-ACP, 1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, and 0.2 mM acetyl-CoA) with varying concentrations of (A) FabI and (B) FabZ. As enzyme concentrations increase, initial rates increase in a hyperbolic manner. Model-predicted optima occur at lower enzyme concentrations than experimental optima, but when modeled values of $k_{cat}$ for FabI or FabZ are reduced by hundredfold and tenfold, respectively, predicted and experimental trends agree well (dashed blue lines). FabZ improves initial rates over a wider range of enzyme concentrations than FabI and may, thus, limit FAS activity over a wider range of conditions.
2.3.6 | **Carbon Flux Affects Total Production and Chain Length.** Metabolic engineers often seek to improve the yields of biological products by increasing the flux of carbon to the pathways that make them.\textsuperscript{35-37} FASs are unique among metabolic pathways, however, because their cyclic structure enables carbon to enter simultaneously at two steps: initiation and elongation. Changes in flux are, thus, likely to alter total production and product profile in a simultaneous—and largely nonintuitive—manner. We used our model to explore this influence.

In brief, we modeled FAS compositions with different concentrations of malonyl-CoA (a surrogate for flux) and measured total production and average chain length at 12.5 minutes. At this biologically relevant time point (c.a., half the doubling time of *E. coli*\textsuperscript{38}), differences in FAS outputs between compositions result from differences in the steady state kinetics of fatty acid production (Appendix A Note 3). Figure 2.5A shows the results of our analysis. As concentrations of malonyl-CoA increase, both total production and average chain length increase in a hyperbolic manner. Associated changes in initiation and elongation events help to explain these trends (Figure 2.5B): A gradual increase in the number of initiation events (the number of acyl-ACPs that will eventually exit the FAS as fatty acids) enhances total production, while an abrupt increase in the ratio of elongation to initiation events (the relative activities of FabF and FabH on malonyl-ACP) causes abrupt elongation. These finding suggest two regimes: (i) At low carbon fluxes, changes in flux modify chain length by altering the relative kinetics of initiation and elongation reactions. (ii) At medium-to-high fluxes, by contrast, they affect only overall rates of synthesis.
Figure 2.5. The influence of carbon flux on FAS outputs. (A) Total production and average length of fatty acids generated by modeled FASs (1 μM of each Fab, 10 μM TesA, 10 μM holo-ACP, 1 mM NADPH, 1 mM NADH, and 0.5 mM acetyl-CoA, 12.5 min) with varying concentrations of malonyl-CoA. As concentrations of malonyl-CoA increase, production levels increase gradually, while average length increases abruptly over a narrow range of concentrations. (B) Enhanced production levels correlate with an increase in initiation events; average chain length, with an increase in the ratio of elongation to initiation events (i.e., the relative activities of FabF and FabH on malonyl-ACP).
2.3.7 The Relative Concentrations of FabH, FabF, and TesA Enable Independent Tuning of Total Production and Chain Length. The results of our analysis of carbon flux are intriguing because they indicate that changes in the relative rates of interdependent steps can have a pronounced influence on FAS outputs. To explore this effect further, we examined the influence of changes in the ratios of FabH, FabF, and TesA—three enzymes previously targeted in metabolic engineering studies—on total production and chain length. To our surprise, simple adjustments in the relative concentrations of these enzymes yielded both (i) changes in average length that preserved total production (e.g., region 1 in Figure 2.6) and (ii) changes in total production that preserved average length (e.g., region 2 in Figure 2.6). Importantly, the breadth of compositions associated with each adjustment was length-specific, and few compositions could achieve lengths of 12 or below without reducing production levels (a result suggestive of the need for additional modifications to achieve short- or medium-chain products). The findings of our compositional analysis are striking because they indicate that simple changes in the ratios of FAS components (i.e., changes that might be introduced with modifications to ribosome binding sites) may enable fine-tuning of FAS outputs.
**Figure 2.6.** The influence of relative concentrations of FabH, FabF, and TesA on FAS outputs. Ternary diagrams show (A) total production and (B) average length of fatty acids generated by modeled FASs (1 μM of each Fab, 10 μM holo-ACP, 1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, and 0.5 mM acetyl-CoA, 12.5 minutes) in which ratios of FabH, FabF, and TesA vary (i.e., [FabH]+[FabF]+[TesA]=12 μM). Compositional adjustments in region 1 change average length (C_{12}-C_{16}) but leave production levels nearly unaltered (38-43 μM); adjustments in region 2, by contrast, alter production levels (25-44 μM), but not chain length (C_{18}). The reference point (filled circle) denotes the composition examined in Figure 2.2A.
2.3.8 | **Optimal Concentrations of FabF are Thioesterase-Dependent.** The limited natural supply of medium-chain fatty acids (i.e., 4 to 12 carbons) has motivated many efforts to synthesize these molecules in microbial hosts.\(^{18,19,41}\) Heterologous thioesterases specific for medium-chain acyl-ACPs can accomplish this feat, but they tend to reduce yields by causing a suboptimal balance of initiation, elongation, and/or termination rates.\(^{42-44}\) In one intriguing study, Silver and colleagues adjusted this balance—and improved yields of medium-chain fatty acids in *E. coli*—by using an inhibitor of FabF to slow rates of elongation.\(^5\) Motivated by their work—and the desire to identify a “genetically encodable” (i.e., inhibitor-free) solution to the same problem—we used our model to optimize concentrations of FabF around different varieties of thioesterase.

Briefly, we parameterized the substrate specificities of plant-derived thioesterases specific for C\(_4\), C\(_8\), or C\(_{12}\) acyl-ACPs (Figure 2.7A) and examined their ability to generate fatty acids in the presence of varying concentrations of FabF. This analysis afforded two interesting observations: (i) The total production of FASs with specialized thioesterases (e.g., CpFatB1 and UcFatB) showed a pronounced sensitivity to FabF concentration (Figure 2.7B). (ii) Optimal, production-maximizing concentrations of FabF increased with the average chain length of FAS products (Figure 7C). This second observation is consistent with *in vivo* studies, which indicate that optimal FabF activity scales with the length of thioesterase targets.\(^5\)

Optimal FabF concentrations could plausibly reflect (i) a minimization of FabH inhibition (i.e., a reduction in the concentration of long-chain acyl-ACPs that inhibit FabH\(^{24}\)) or (ii) a maximization of substrate availability (i.e., an increase in the concentration of acyl-ACPs targeted by thioesterases). When we ran our model in the absence of FabH inhibition, however, optimal concentrations of FabF remained unchanged (Figure 2.7C). Our results thus indicate that FabF-derived improvements in the total production result from an increase in the concentration of acyl-
ACPs targeted by thioesterases. This finding is consistent with both (i) the heightened sensitivity of highly specific thioesterases to FabF (i.e., suboptimal concentrations of FabF can quickly shift substrate lengths away from the target) and (ii) the high FabF requirements of thioesterases specific for long-chain acyl-ACPs (which require multiple elongation steps).
Figure 2.7. Analysis of the interdependence of thioesterase specificity and FabF concentration. (A) Product distributions for modeled FASs (1 μM of each Fab, 10 μM thioesterase, 10 μM holo-ACP, 1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, and 0.5 mM acetyl-CoA, 12.5 min) containing thioesterases specific for C₄ (BfTES), C₈ (CpFatB1), C₁₂ (UcFatB), and C₁₄ acyl-ACPs (TesA). (B) Total production by FASs from A with varying concentrations of FabF. FASs that contain thioesterases with narrow substrate specificities (e.g., CpFatB1 and UcFatB) show a pronounced sensitivity to FabF concentration. (C) Optimal concentrations of FabF increase with the length of thioesterase targets in both (i) the base model and (ii) a modified model that lacks inhibition of FabH by acyl-ACPs. This result suggests that optimal FabF concentrations do not minimize inhibition, but rather, maximize the availability of acyl-ACPs targeted by thioesterases.
2.3.9 | Narrow Distributions of Fatty Acids Require Specialized Enzymes. Our analysis of FAS compositions highlights two approaches for modifying the length of fatty acids: (i) changes in the concentrations of catalytic components and (ii) changes in the identities of those components. To compare these two approaches to each other, we examined the product distributions afforded by each. We began by optimizing the enzyme concentrations of the FAS to build fatty acids with average lengths of 8, 10, 12, and 14 carbons (Figure 2.8A). As expected, compositions with high concentrations of TesA and high TesA/FabF ratios generated short fatty acids, while compositions with low concentrations of TesA and low TesA/FabF ratios generated long fatty acids. The distributions afforded by these compositions, however, were broad and, thus, suboptimal for the production of fine chemicals. Accordingly, we next evaluated the ability of novel thioesterases to build narrow product profiles by optimizing appropriately modified FASs around narrow distributions centered at C₈ and C₁₂. Interestingly, only FASs with highly specific thioesterases could generate these distributions (Figure 2.8B). Results thus indicate that changes in component ratios are sufficient to control the mean—but not the breadth—of fatty acid profiles.

Specialized variants of FabF provide an alternative—if less commonly explored—strategy for adjusting product distributions. In an early study, Dahesh and colleagues showed that steric obstructions in the binding site of this enzyme could prevent the elongation of long-chain acyl-ACPs. To evaluate the control afforded by these “elongation-restricted” mutants, we optimized the enzyme concentrations of mutant-containing FASs around narrow distributions of fatty acids; to our surprise, mutants of FabF could achieve these distributions (Figure 2.8B). Our analyses thus indicate that both highly specific thioesterases and sterically hindered ketoacyl synthases permit the production of narrow product profiles.
Figure 2.8. Analysis of product distributions. (A-B) Top: Modeled FAS compositions (10 μM holo-ACP, 1 mM NADPH, 1 mM NADH, and 0.5 mM acetyl-CoA, 12.5 min) with concentrations of FabD, FabH, FabG, FabZ, FabI, FabF, and TesA optimized for specified average chain lengths. Differences in colored area reveal differences in total enzyme concentration. Bottom: product distributions associated with each composition. (B) Top: FAS compositions optimized for specified product distributions (orange). Compositions are identical to those in A but, where indicated, include non-native components (i.e., CpFatB1, UcFatB, or versions of FabF that cannot elongate beyond 8 or 12 carbons) in place of native ones. Narrow distributions require specialized thioesterases or elongation-restricted mutants of FabF.
2.3.10 | A Sensitivity Analysis Yields General Rules for Tuning FASs. Having established the ability of our model to recreate—and help explain—trends from \textit{in vitro} and \textit{in vivo} data, we used it to develop general rules for tuning FAS outputs. In brief, we applied the Morris method, a global sensitivity analysis, to identify the kinetic parameters that most strongly influence (i) average chain length, (ii) total production, and (iii) our original model objective (i.e., a measure of the similarity of predicted and measured trends in Figure 2.2A and 2.2B). This method supplies a metric, termed the normalized “elementary effect”, for the sensitivity of a specified output to a model parameter. We began by examining the sensitivity of each output to the 12 fit parameters; several lumped parameters (e.g., $c_2$, which scales $k_{\text{cat}}$ for multiple enzymes), however, were highly influential (Figure 2.9A), so we decomposed them into enzyme-specific terms and re-ran our analysis (Figure 2.9B). Results of this second test indicated that average chain length was most sensitive to the substrate specificity of TesA and to the activities of TesA and FabF; total production, to the activities of FabD and FabZ. The model objective exhibited a sensitivity that simply combined the two sets of effects. The contributions of TesA and FabF to chain length are consistent with their reported ability to alter product profiles;\textsuperscript{5,18,19} the contributions of FabZ and FabD to production, in turn, agree with their reported effects on yield and titer.\textsuperscript{23,25,46}

The results of our sensitivity analysis suggest an important limit on the effects afforded by single-component adjustments to FAS compositions. TesA and FabF influence both chain length and total production, while FabD and FabZ affect only the latter. Changes in the identity and/or expression level of individual components thus enable fine-tuning of total production, but not product profile, which requires coordinated changes in multiple enzymatic steps.
Figure 2.9. Sensitivity analysis. (A) The normalized mean elementary effect for each fit parameter (Table 2.1). Average chain length is most sensitive to $c_2$, $c_3$, $d_1$, and $d_2$; total production, to $b_1$, $b_2$, $b_3$, and $c_2$. (B) The normalized mean elementary effects of an expanded model show enzyme-specific contributions: Average length is most sensitive to the substrate specificity of TesA ($d_1$ and $d_2$) and the activities of FabF and TesA ($c_2$ and $c_3$, respectively), while total production is most sensitive to the activities of FabD ($b_1$, $b_2$, and $b_3$) and FabZ ($c_2$; Table 2.1). The model objective exhibits an additive sensitivity. A comparison of sensitivities indicates that FabD and FabZ influence only one output (i.e., production level), while TesA and FabF contribute to two.
2.4 | CONCLUSIONS

Metabolic pathways use complex systems of interacting catalysts to convert simple inputs (e.g., glucose) into complex, dynamically adjustable outputs (e.g., the plasma membrane). Efforts to engineer these pathways thus require an understanding of the kinetic relationships that govern catalytic collaboration within them. In this study, we developed a mechanistic kinetic model of the *E. coli* FAS and used it to determine how different enzymes work together to control FAS outputs. The model’s ability to predict trends from a range of *in vitro* and *in vivo* datasets indicates that the reactions on which it focuses (i.e., those catalyzed by the seven enzymes necessary to build saturated fatty acids) are largely responsible for those trends.

The central findings help explain perplexing results from experimental studies and provide new strategies for controlling fatty acid production in microbial systems. Perturbational analyses indicate that the modification and/or overexpression of one FAS component can depress fatty acid production (a commonly observed phenomenon) by sequestering the proteins with which it interacts and/or by depleting common substrate pools. Importantly, we show that suboptimal concentrations of FabF can reduce fatty acid synthesis by lowering concentrations of acyl-ACPs targeted by thioesterases—a common target of metabolic engineering. Compositional studies, in turn, suggest that both (i) coordinated changes in the concentrations of catalytic components and (ii) adjustments to the substrate specificities of those components can alter the average length of fatty acids, but indicate that adjustments to the distributions of those lengths require thioesterases and/or ketoacyl synthases with appropriately focused substrate specificities.

The results of this study suggest two general rules for FAS design: (i) They indicate that the exchange of nonnative components with native ones (e.g., alternative versions of ACP, TesA, FabF, or FabH for wild-type variants) will likely require concomitant changes in concentration or
activity of other FAS components. (ii) They suggest that single-component adjustments—at least within the FAS of *E. coli*—enable independent tuning of production levels, but not product profiles. The relevance of the specific controls identified (i.e., FabD, FabZ, TesA, and FabF) to other FASs merits exploration in future work; the general ability of some components to adjust one output, while others influence several, however, is broadly interesting because it is rarely considered in engineering efforts. Broadly, the findings of this study highlight the precision afforded by coordinated changes in the composition of FASs, and provide new strategies for building biocatalytic systems (and microbial hosts) with precisely defined product profiles.

2.5 | MATERIALS AND METHODS

2.5.1 | Assembly and Solution of the Kinetic Model. Our mechanistic kinetic model captures the combined activities of the enzymes depicted in Figure 2.1 with a system of rate equations derived from the mechanisms depicted in Tables 2.1 and A.1 (Appendix A). Our model uses physiologically relevant concentrations of enzymes, substrates, and cofactors as described by Khosla and colleagues.23 We generated numerical solutions to our final system of differential equations (a system that is stiff at early time points) by using the MATLAB solver ode15s with a relative error tolerance of 1e-4 and an absolute error tolerance of 1e-6.

2.5.2 | Parameterization of the Kinetic Model. We based initial estimates of model parameters on the results of detailed experimental studies (Table A.4). We used estimates of $k_{cat}$ as given, and we decomposed estimates of $K_d$ and $K_M$, which rely on equilibrium or steady-state assumptions, by

$$K_d = \frac{k_{off}}{k_{on}} \quad \text{(Eq. 2.1)}$$
\[ K_D = K_M - \frac{k_{cat}}{k_{on}} \quad \text{(Eq. 2.2)} \]

using equations 2.1 and 2.2, alongside literature-based estimates of \( k_{off} \). In our fitting routine, we adjusted values of \( k_{off} \) by using equations 2.1 and 2.2 to recalculate \( k_{on} \) under the assumption that \( K_M \) or \( K_D \) are constant.

2.5.3 | Materials and Resources. We purchased reagents for buffer and media from Thermo Fisher Scientific (Waltham, MA), substrates and cofactors for kinetic experiments from Sigma-Aldrich (St. Louis, MO), enzymes for cloning from New England Biolabs (Ipswich, MA), and kits for DNA extraction and purification from Qiagen (Hilden, Germany). We performed distributed computing with the Google Cloud Compute Engine (https://cloud.google.com/).

2.5.4 | Design of Plasmids. We used three plasmids to express apo-ACP, TesA, and Sfp (a 4´-phospho-pantetheinyl transferase from Bacillus subtilis) in \( E. coli \). For TesA, we isolated genomic DNA from \( E. coli \) by using the DNeasy Blood and Tissue Kit (Qiagen); we amplified the TesA gene by using primers that add 6X polyhistidine tags to the N-terminus (we removed its native N-terminal signal sequence): ATATCCATGGGCAGCAGCCATCATCATCATCATCACATGGCGGACACGGTTATTGATTCTG (forward) and TTTTTGGATCCATTATGAGTCATGATTACTAAAGGCTGCAACTGCTTCGCCAT (reverse); and we cloned the amplified gene into pET16b (Novagen). For Sfp and apo-ACP, we used plasmids with N-terminal polyhistidine-tagged versions of each protein (i.e., pET29b and pET22b, respectively); these plasmids were supplied by the Barkart lab. We verified all sequences with Sanger sequencing (Quintara Bio).
2.5.5 | **Expression and Purification of Proteins.** We expressed TesA, apo-ACP, and Sfp by carrying out the following steps: (i) We transformed each plasmid into *E. coli* BL21(DE3) by using heat shock. (ii) We spread the transformed cells onto antibiotic-containing LB plates (100 µg/mL carbenicillin or 50 µg/mL kanamycin) and incubated them at 37°C for 12 hours. (iii) We used a single colony, thus generated, to inoculate 20 mL LB media (100 µg/mL carbenicillin), and we placed this culture in an incubator shaker (37 ºC, 150 rpm) for 4-5 hours until it turned visibly cloudy. (iv) We used our initial culture to inoculate 1 L of expression media (20g tryptone, 10g yeast extract, 5g NaCl, 4g M9 salts, 4g glucose, 100mg carbenicillin), and we placed this new culture in an incubator shaker (37 ºC, 150 rpm) for 2-3 hours until it reached an OD600 of 0.5-0.8. (v) We induced expression by adding isopropyl β-D-1-thiogalactopyranoside (IPTG) at a concentration of 0.1-0.5 mM, and we returned the culture to an incubator shaker set to 20 ºC. (vi) After 16-20 hours, we pelleted the cells.

We purified all proteins with the following steps: (i) We lysed the cell pellets by incubating them (1 h, rocking platform) with lysis buffer (4 ml/g pellet): 4 ml B-PER [Thermo Fisher], 1 mg MgSO4, 2 mg Naα-p-Tosyl-L-arginine methyl ester hydrochloride, 3.5 mg tris(2-carboxyethyl)phosphine (TCEP), 3.75 µl phenylmethylsulfonyl fluoride, 0.5 mg Lysozyme, and 10 µl DNase). (ii) We clarified the lysate by pelleting the cell debris and adding saturated ammonium sulfate at 20% (v/v), followed by immediate centrifugation. (iii) We exchanged the protein into Tris-HCl buffer (50 mM Tris-HCl, pH 7.5, 0.5 mM TCEP) by using a desalting column (HiPrep 26/10, GE Healthcare). (iv) We flowed the desalted lysate over a nickel column (HisTrap HP) and eluted the purified protein with imidazole (a step gradient of Tris-HCl buffer with 300 mM imidazole). (v) We exchanged the eluent back into Tris-HCl buffer (50 mM Tris-HCl, pH 7.5, 0.5 mM TCEP), flowed the resulting solution over an anion exchange column (HiPrep Q HP 16/10,
GE Healthcare), and eluted the protein with NaCl (a gradient of Tris-HCl buffer with 1M NaCl).

(vi) We stored final desalted proteins in 20% glycerol at -80°C.

2.5.6 | **Synthesis of Holo-ACP.** We synthesized holo-ACP by using Sfp to transfer the 4′-phosphopantetheinyl moiety of coenzyme A to apo-ACP.\(^{50}\) In brief, we incubated apo-ACP, Sfp, and CoA at 37°C for 3 hours (50µM ACP, 2µM Sfp, 200µM CoA, 1 mM MgCl\(_2\), 1 mM TCEP, 50mM Tris HCl, pH 7.5), and we used anion exchange to separate holo-ACP pools and apo-ACP from the final mixture. We stored the purified holo-ACP as described above.

2.5.7 | **Parameterization of TesA Activity.** To parameterize the activity of TesA, we fit values of \(k_{\text{cat}}\) and \(K_M\) to previously collected kinetic data describing the activity of TesA on acyl-CoAs of different lengths (Figure A.2A; Table A.2);\(^{19}\) and we used eqs. 2.1 and 2.2 to convert values of \(K_M\) to estimates of \(K_d\) (for this effort, we estimated \(k_{\text{off}}\) to be 2.0 s\(^{-1}\), an intermediate value; Table A.4). For \(C_4\), \(C_{18}\), and \(C_{20}\) acyl-CoAs, we estimated values of \(k_{\text{cat}}\), \(K_M\), and \(K_d\) by extrapolating trends exhibited by values of \(k_{\text{cat}}\), \(K_M\), and \(K_d\) for \(C_6\)-\(C_{16}\) acyl-CoAs (Figs A.2B-A.2D).

In our optimization routine, we adjusted the activity of TesA with three fit parameters. The first, \(c_3\), scales estimates of \(k_{\text{cat}}\) by retaining their relative values (Figure A.2B); the second two, \(d_1\) and \(d_2\), adjust a subset of \(K_d\)’s with a linear free-energy relationship (Eq. 2.3, Figure A.3D).

\[
LN(K_D) = d_1 Length + d_2 \quad \text{(Eq. 2.3)}
\]

In brief, we retained original estimates of \(K_d\) for short substrates (≤ \(C_{12}\)) and used the linear free-energy relationship for long substrates (i.e., Eq. 2.3 passes through the point \([C_{12}, \ln(K_{d,C_{12}})]\) with \(d_1\) and \(d_2\) as fit parameters). Interestingly, despite their different bases of estimation, final values of \(\ln(K_d)\) for all acyl-ACPs fell onto a single line (Figure A.3D).
2.5.8 | Parameterization of Enzyme-holo-ACP Binding. We measured the affinity of TesA for holo-ACP by examining TesA-catalyzed hydrolysis of p-nitrophenyl-butyrate (pNP4) in the presence of holo-ACP (Figure A.2E). In brief, we prepared a 96-well plate (100µL, 50mM Tris-HCl, pH 7.5, 0.1 mM TCEP, 0.1µM TesA, 5 % DMSO) with varying concentrations of pNP4 (100µM-10mM) and holo-ACP (0, 2, and 10 µM), and we monitored hydrolysis by measuring absorbance at 405 nm (4-nitrophenol) with a SpectraMax M2 plate reader. To our surprise, the kinetic data fit well to an activation model in which the TesA-ACP complex had a K_d of ~9µM (Figure A.2E, Table A.3). The ability of holo-ACP to activate TesA-catalyzed hydrolysis of small substrates is intriguing, but holo-ACP and acyl-ACPs likely share the same binding site. We, thus, used the K_d determined in our kinetic study as an estimate of K_I for competitive inhibition.

Previous studies indicate that holo-ACP binds to FabG, FabZ, FabI, and FabF;\textsuperscript{51-54} data on the affinity of these interactions, however, is scarce. We used our estimate of K_d for the TesA-ACP complex as an initial estimate of the K_d’s for complexes between holo-ACP and FabG, FabZ, FabI, and FabF. By contrast, for FabH, where the inhibitory effect of holo-ACP is ill-defined,\textsuperscript{15,54} we assumed a binding constant that is 100-fold weaker than those of other enzymes.

2.5.9 | Parameterization of FabH Inhibition. Long-chain acyl-ACPs regulate flux through the fatty acid pathway by inhibiting FabH—a form of negative feedback.\textsuperscript{48} We parameterized this inhibition in three steps: (i) We fit a detailed kinetic model of FabH-catalyzed condensation of acetyl-CoA and malonyl-ACP (lines 3 and 4 in Table 2.1) to previously reported initial rates measured at varying concentrations of these two substrates (Figures A.3A and A.3B);\textsuperscript{55} (ii) with this model as a starting point, we fit inhibition constants for palmitoyl-ACP (i.e., K_{I,1} and K_{I,2} as defined in Table A.1) to initial rates measured under varying concentrations of palmitoyl-ACP.
(Figures A.3C and A.3D); and (iii) we estimated length-specific values of inhibition constants by fitting them to data showing the inhibitory effects of acyl-ACP of various lengths (Figure A.3E).

2.5.10 | Parameterization of FabZ. The equilibrium of the dehydration reaction catalyzed by FabZ favors substrates over products by a ratio of ~4:1;\(^{56}\) however, to develop our model, we approximated this reaction as irreversible in the presence of FabI, which rapidly consumes enoyl-acyl-ACP.\(^{22}\) An examination of the concentrations of β-hydroxyacyl-ACP and enoyl-acyl-ACP, which are present at a ratio of ~60:1 in the base model and ~500:1 in the model with a reduced \(k_{cat}\) for FabZ (Figure 2.4B), supports this approximation (Figure A.4A and A.4B).

2.5.11 | Optimization of the Kinetic Model. We optimized estimates of scaling parameters by carrying out the following steps: (i) We used diffusion calculations (Appendix A Note 1) and previously reported kinetic measurements to estimate physically relevant ranges of each kinetic parameter (Table A.5) and, subsequently, to estimate ranges of each scaling parameter (Table A.6). (ii) We constructed 500 initial guesses of 11 scaling parameters (all but “e” as defined in Table 2.1) by randomly sampling the range associated with each (i.e., we sampled a uniform distribution defined by the logarithm of the upper and lower limits of each range). (iii) We used distributed computing to determine if the model, when parameterized with the 500 sets of guesses, could predict the time-course profile and product distribution depicted in Figures 2.2A and 2.2B. (We note: The experimental systems used to generate Figures 2.2A-2.2C, 2.3, 2.4, and A.5 include FabA and FabB, which are neglected by our model; our fits—and, later, our model predictions—assume that these enzymes make a negligible contribution to trends in production rates and length-specific product distributions of the FAS). (iv) We compared predicted and measured trends with
Obj_A = SSE_{set1} * SSE_{set2}; here, SSE_{set1} and SSE_{set2} represent the sums of squared errors for Figures 2.2A and 2.2B, respectively. (v) We used the 20 best-performing sets of guesses (i.e., those with the lowest values of Obj_A) to fit our model to the data in Figures 2.2A and 2.2B. Here, we used MATLAB function “fminsearch” (an implementation of the Nelder-Mead simplex method) with Obj_A. (vi) We used the two best optimized sets of parameters to assess our model’s ability to predict the inhibitory effects of FabH and FabF (i.e., Figures 2.2C and 2.3C); poor agreement between predicted and experimental trends, however, suggested that our model did not accurately capture the strength of interactions between enzymes and holo-ACP. (vii) We introduced a twelfth parameter—\( e \), which scales \( K_D \)’s for the binding of both (a) FabH and FabF to holo-ACP and (b) FabH to acyl-ACPs—and we re-optimized our model to the data in Figures 2.2A-2.2C. Here, we used the two parameter sets from step vi and Obj_B = SSE_{set1} * SSE_{set2} * SSE_{set3}, where SSE_{set3} represents the sum of squared errors for Figure A.2C. (vii) The set of fit parameters with the lowest value of Obj_B served as our starting point for all analyses described in this study (Table A.6).

2.5.12 Parameterization of Plant-Derived Thioesterases. We parameterized the activity and substrate specificity of plant-derived thioesterases by using datasets from two in vivo studies: The first dataset included total fatty acids generated by strains of \textit{E. coli} containing TesA or UcFatB2 (Figure A.9A).\textsuperscript{57} The second dataset included product distributions generated by strains containing BfTES, CpFatB1, or UcFatB2 (Figure A.9B-A.9D).\textsuperscript{5} When combined, these datasets allowed us to parameterize both the activities and substrate specificities of BfTES, CpFatB1, or UcFatB2. For context, BfTES was most active on C\(_4\) acyl-ACP and showed slight activity on C\(_6\) and C\(_8\) acyl-ACPs (Figure A.9B); CpFatB1 was most active on C\(_8\) acyl-ACP and showed minor activity on C\(_6\) and C\(_{10}\) acyl-ACPs (Figure A.9C); and UcFatB2 was most active on C\(_{12}\) acyl-ACP.
and showed some activity on C\textsubscript{10} and C\textsubscript{14} acyl-ACPs (Figure A.9D). We optimized kinetic parameters by fitting FASs with plant-derived thioesterases to the production levels depicted in Figure A.9A. For BfTES, we adjusted absolute values of k\textsubscript{cat} and K\textsubscript{d}, but maintained their relative values between substrates (i.e., for C\textsubscript{4}, C\textsubscript{6}, and C\textsubscript{8} acyl-ACPs, we based initial estimates of k\textsubscript{cat} and K\textsubscript{d} on measurements of k\textsubscript{cat} and K\textsubscript{d} for TesA activity on C\textsubscript{16}, C\textsubscript{18}, and C\textsubscript{20} substrates, respectively; for all other substrates, we set values of k\textsubscript{cat} to zero). For CpFatB1 and UcFatB2, we adjusted values of k\textsubscript{cat} and K\textsubscript{d} for major substrates by simultaneously moving both parameters along the lines depicted in Figures A.2B and A.2D; for minor substrates, we held values of k\textsubscript{cat} and K\textsubscript{d} constant. (We based initial estimates of k\textsubscript{cat} and K\textsubscript{d} for major substrates on measurements of k\textsubscript{cat} and K\textsubscript{d} for TesA activity on C\textsubscript{6} acyl-CoA; for minor substrates, we used TesA activity on C\textsubscript{20} acyl-CoA; for all other substrates, we set values of k\textsubscript{cat} to zero).

2.5.13 | Parameterization of Mutants of FabF. We modeled elongation-restricted mutants of FabF by eliminating their activity on substrates longer than the specified “restriction length”. For example, for the mutant incapable of elongating beyond eight carbons (Figure 2.8B, left), we set values of k\textsubscript{cat} for C\textsubscript{8}-C\textsubscript{20} to zero; for the mutant incapable of elongating beyond twelve carbons (Figure 2.8B, right), in turn, we set values of k\textsubscript{cat} for C\textsubscript{12}-C\textsubscript{20} to zero. This parameterization is consistent with the results of \textit{in vitro} studies, which indicate that elongation-restricted mutants of FabF retain their activities on substrates shorter than the restriction length.\textsuperscript{45}

2.5.14 | Sensitivity Analysis of the Kinetic Model. We performed a sensitivity analysis of scaling parameters (Figure 2.9A) and enzyme-specific decompositions of those parameters (Figure 2.9B) by calculating the mean elementary effects as defined by the Morris Method.\textsuperscript{58} (An
elementary effect is the mean of a set of derivatives calculated at semi-random points within a model’s parameter space).\textsuperscript{59} We restricted the values of parameters explored in the sensitivity analysis to ranges that spanned up to three orders of magnitude (centered at the initial fit parameters). We used the SAFE toolbox\textsuperscript{60} to implement the analysis with the radial method (r = 100 trajectories) as described previously.\textsuperscript{59} Figure A.10, which illustrates the convergence of the mean elementary effects, provides additional details of our analysis.
References

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CHAPTER III: A KINETIC RATIONALE FOR FUNCTIONAL REDUNDANCY IN FATTY ACID BIOSYNTHESIS

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3.1 | ABSTRACT

Cells build fatty acids with biocatalytic assembly lines in which a subset of enzymes often exhibit overlapping activities (e.g., two enzymes catalyze one or more identical reactions). Although the discrete enzymes that make up fatty acid pathways are well characterized, the importance of catalytic overlap between them is poorly understood. This study develops a detailed kinetic model of the fatty acid synthase (FAS) of Escherichia coli and pairs that model with a fully reconstituted in vitro system to examine the capabilities afforded by functional redundancy in fatty acid synthesis. The model captures—and helps explain—the effects of experimental perturbations to FAS systems and provides a powerful tool for guiding experimental investigations of fatty acid assembly. Compositional analyses carried out in silico and in vitro indicate that FASs with multiple partially redundant enzymes enable tighter (i.e., more independent and/or broader-range) control of distinct biochemical objectives—the total production, unsaturated fraction, and average length of fatty acids—than FASs with only a single multi-functional version of each enzyme (i.e., one enzyme with the catalytic capabilities of two partially redundant enzymes). Maximal production of unsaturated fatty acids, for example, requires a second dehydratase that is not essential for their synthesis. This work provides a kinetic, control-theoretic rationale for the inclusion of partially redundant enzymes in fatty acid pathways and supplies a valuable framework for carrying out detailed studies of FAS kinetics.

3.2 | INTRODUCTION

Cells use sophisticated, multi-component biocatalytic systems to build the fatty acids required for phospholipid homeostasis, energy storage, and other metabolic functions.¹ These fatty acid synthases (FASs), as they are commonly referred, consist of mixtures of discrete or physically
linked enzymes.² FASs have remained a longstanding focus of biochemical research for their fundamental importance and for their use as platforms for the production of oleochemicals (e.g., fuels and alcohols);³-⁶ Over the years, many biochemical studies have examined the structures and catalytic activities of individual FAS components;⁷ the outputs and regulatory controls afforded by the concerted activities of multiple components working together within complete FAS systems, however, remain incompletely understood, difficult to predict, and challenging to engineer.⁸-¹⁰

Intriguingly, many FASs include enzymes with overlapping activities. This seemingly inefficient attribute could result from incomplete evolutionary specialization; its widespread occurrence in different systems (Table B.₁¹,¹²), however, suggests a functional role. The type II FAS of Escherichia coli (E. coli) provides an illustrative example. In this system, an acyl carrier protein (ACP) guides growing acyl chains between discrete enzymes, which catalyze successive rounds of reduction and condensation (Figure 3.1A). Two specialized enzymes facilitate the production of unsaturated fatty acids: FabA, which catalyzes the allylic rearrangement of trans-dec-2-enoyl-ACP to cis-dec-3-enoyl-ACP, and FabB, which elongates cis-dec-3-enoyl-ACP by condensing it with malonyl-ACP.¹⁴ In addition to their specialized catalytic roles, FabA and FabB also carry out several reactions primarily catalyzed by FabZ and FabF, a β-hydroxyacyl-ACP dehydratase and a β-ketoacyl-ACP synthase, respectively.¹⁵-¹⁶ The functional advantage of this catalytic overlap—if any—remains poorly understood.
Figure 3.1. Development of a detailed kinetic model. (A) The complete fatty acid synthase (FAS) of *E. coli*. FabA and FabB specialize in (blue) the assembly of unsaturated fatty acids, but also carry out several additional steps also carried out by FabZ and FabF. Our model captures the activities of all nine enzymes necessary to convert acetyl-CoA and malonyl-CoA to free fatty acids. (B-C) We optimized our kinetic model with simultaneous fits to (B) the product distribution of a strain of *E. coli* that overexpresses TesA, (C) initial rates of fatty acid synthesis by three reconstituted FASs (asterisk), and two previously described kinetic datasets (Figure B.2). The model overestimates the initial rate of a FAS depleted in FabI and may, thus, overestimate the activity of this enzyme. General agreement between measured rates and predicted rates (bars without an asterisk), however, suggests that the model captures the kinetic contributions of individual FAS components. In B-C, the FAS compositions include 1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, 0.5 mM acetyl-CoA, 10 μM ACP, 10 μM TesA, and 1 μM of each Fab (except where indicated); data indicate (B) 12-min or (C) 30-sec time points; and error bars show the standard deviation of triplicate measurements or modeled data. Error bars for C were not supplied by the reference study.
The substrate specificities of FabZ and FabF are certainly distinct from those of their more versatile counterparts. FabZ is most active on C₄-C₆ β-hydroxy-ACPs and easily accepts unsaturated substrates, while FabA favors C₈-C₁₂ β-hydroxy-ACPs and is generally more active on saturated acyl chains. FabF and FabB, by contrast, exhibit similar activities on most substrates with the notable exception of C₁₆:1-ACP, which FabF elongates more readily than FabB. Despite their distinct substrate specificities, however, FabZ and FabF are not strictly required to produce saturated or unsaturated fatty acids; their contributions to the versatility of FAS outputs remain a matter of speculation.

We developed a mechanistic kinetic model of the complete FAS of *E. coli* and used it, alongside a reconstituted *in vitro* system, to examine the biochemical repercussions of functional redundancy in fatty acid synthesis. The FAS has numerous biochemically influential outputs; we chose to focus on free fatty acids because they are important signaling molecules, metabolic intermediates, and oleochemical precursors. In our study, we use the term “partially redundant” to refer to enzymes with catalytic activities that overlap (without consideration for differences in substrate specificity) with the catalytic activity of at least one other enzyme. FabA, FabZ, FabF, and FabB are partially redundant enzymes. With these enzymes in mind, we focused on two questions: (i) How do partially redundant enzymes influence the production of unsaturated fatty acids, an output that requires nonredundant steps? (ii) How do partially redundant enzymes help to control different—and, perhaps, competing—biochemical objectives (i.e., the total production, unsaturated fraction, and average length of fatty acids)? These questions, when answered, could help illuminate the importance of functional redundancy in fatty acid biosynthesis, a physiologically important and industrially relevant metabolic process.
3.3 | RESULTS

3.3.1 | Development of a Detailed Kinetic Model. We constructed a detailed kinetic model of the complete FAS of *E. coli* by introducing two essential modifications to our previous model of saturated fatty acid synthesis. We incorporated (i) the activities of FabA and FabB and (ii) the reversibility of the FabA- and FabZ-catalyzed dehydration reactions. As before, we based all rate equations on detailed reaction mechanisms reported in experimental studies of purified enzymes, and we avoided a priori equilibrium assumptions by including independent association and dissociation steps for all heteromeric complexes. Our final model, which contained 313 equations and 135 unique kinetic constants, captured the activities of all nine enzymes necessary to convert malonyl-CoA and acetyl-CoA to saturated and unsaturated free fatty acids. We note: TesA, a cytosolic variant of a periplasmic thioesterase, is not an essential component of the FAS, but it facilitates the production of free fatty acids and is included in many engineered systems; it is a representative termination enzyme.

We parameterized our model by using estimates of kinetic constants reported in the literature (Table B.4). For most enzymes, we used experimentally determined turnover numbers or equilibrium constants, as described previously. For FabA, FabZ, FabB, and FabF, we used fits to published kinetic data that allowed us to resolve differences in their substrate specificities (Figure B.1).

We optimized kinetic parameters by fitting the model to a diverse set of experimental data. In brief, we used 14 scaling parameters to link groups of similar kinetic constants to one another, and we optimized these parameters with a simultaneous fit to three datasets: (i) the product distribution of a strain of *E. coli* that overexpresses TesA (Figure 3.1B), (ii) initial rates of fatty acid synthesis by several FASs reconstituted *in vitro* (Figure 3.1C and Figure B.2C), and (iii)
a time course of total fatty acid production by a reconstituted FAS (Figure B.2A\textsuperscript{28}). This large dataset reduces the influence of measurement error (which was not reported in the reference studies) and ensures the model’s ability to capture different FAS compositions. Our final model recreated important trends in the target datasets but, notably, overestimated the initial rate of a FAS depleted in FabI—an indication that it may overestimate the activity of this enzyme. General agreement between measured and predicted initial rates for several mixtures (i.e., agreement obtained without fitting; Figure 3.1C), however, suggests that the model adequately captures the kinetic contributions of different FAS components.

\section*{3.3.2 Analysis of Unsaturated Fatty Acid Biosynthesis.} To examine the contribution of the partially redundant enzymes to the biosynthesis of unsaturated fatty acids, we modeled their removal from the FAS system (Figure 3.2A). Mixtures lacking either FabA or FabB could not produce unsaturated fatty acids; these enzymes are thought to be required for the introduction of the double bond into growing acyl chains,\textsuperscript{31,32} and we modeled their activities accordingly (we will return to this assumption later). The removal of FabF, by contrast, had a nearly imperceptible effect, while the removal of FabZ lowered the fraction of unsaturated fatty acids; the latter effect, which has been observed in an experimentally reconstituted FAS\textsuperscript{29} and is consistent with the higher unsaturated fractions afforded by FabZ overexpression in cells,\textsuperscript{33} lacks an obvious cause.
Figure 3.2. Analysis of unsaturated fatty acid biosynthesis. (A) The fraction of unsaturated fatty acids (i.e., the unsaturated fraction) generated by a modeled FAS with different enzymes removed. (B) The unsaturated fraction generated by modeled FASs with increasing concentrations of FabZ. Note: FabAZ is a version of FabA with the same activity as FabZ on saturated and unsaturated substrates. (C) An analysis of the influence of FabZ on the unsaturated fraction afforded by FASs with FabA, FabAZ, or versions of FabAZ that retain native (i.e., FabA-like) activity on (i) unsaturated substrates or (ii) different chain lengths. We note: In all FASs, [FabA]+[FabZ] = 2 μM. For systems containing all FAS components (black bars), we optimized the FabA/FabZ ratio to maximize the unsaturated fraction. (D) A depiction of the contributions of FabZ and FabA. Low concentrations of FabZ facilitate the assembly of unsaturated fatty acids by enhancing the production of β-hydroxy-decanoyl-ACP, which FabA rapidly dehydrates and isomerizes to cis-dec-3-enoyl-ACP; high concentrations of FabZ depress production by outcompeting FabA for β-hydroxy-decanoyl-ACP. (E) The unsaturated fraction generated by modeled FASs with increasing concentrations of (inset) FabF and (main) FabB. (F) The unsaturated fraction afforded by (i) wild-type ACP or (ii) ACPD38A in two systems: (left) a strain of E. coli and (right) a modeled FAS. We fit the in vivo data collected for the wild-type ACP by adjusting the concentrations of FabB and FabA. We predicted the changes in fatty acid production caused by the mutant of ACP, in turn, by swapping in the experimentally measured Kd for the FabB-ACPd38a complex. In A-C and E-F, the FASs include 1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, 0.5 mM acetyl-CoA, 10 μM ACP, 10 μM TesA, and 1 μM of each Fab, except where indicated, and data correspond to 12-min time points. The error bars and shaded regions indicate the standard deviation of modeled or measured (F only) data.
We probed the contribution of FabZ further by modeling FAS compositions with different concentrations of this component. As concentrations of FabZ increased, the fraction of unsaturated fatty acids increased to a peak value and decreased thereafter (Figure 3.2B). High concentrations of FabA enhanced unsaturated fatty acid production in the absence of FabZ but could not achieve the same peak fraction afforded by its presence (Figure 3.2B). The nonmonotonic influence of FabZ is explained by trends in steady-state concentrations of reaction intermediates (Figure B.3): As concentrations of FabZ increase from zero, this enzyme enhances the production of β-hydroxy-decanoyl-ACP, which FabA rapidly dehydrates and isomerizes to cis-dec-3-enoyl-ACP—a precursor for all unsaturated fatty acids. As concentrations of FabZ increase further, however, it begins to “outcompete” FabA for β-hydroxy-decanoyl-ACP and, thus, to decrease the production of cis-dec-3-enoyl-ACP. (FabA can isomerize the trans-dec-2-enoyl-ACP generated by FabZ, but the steady-state concentration of this intermediate is far lower than that of β-hydroxy-decanoyl-ACP; Figure B.3B). Interestingly, FabA also exerts a nonmonotonic effect as concentrations rise by directing flux toward cis-dec-3-enoyl-ACP at low concentrations and toward trans-dec-2-enoyl-ACP at high concentrations (Figure B.4).

The results of our kinetic analyses thus indicate that low concentrations of FabZ can enhance the production of unsaturated fatty acids beyond the levels afforded by FabA alone. FabZ has two distinguishing features that could enable this effect: (i) a preference for short-chain substrates (i.e., C₄-C₆) and (ii) a heightened activity on unsaturated β-hydroxyacyl-ACPs. We used our model to evaluate each feature. To begin, we created a “FabZ-like” version of FabA with the same activity on saturated and unsaturated substrates (FabA_z, which retains the native isomerase activity of FabA); in the presence of this variant, FabZ ceased to enhance the production of unsaturated fatty acids—an unsurprising result, given the lack of distinguishing features between
the two enzymes (Figures 3.2B-3.2C). Next, we reverted each attribute of FabA back to its native state. The reversion of FabA’s low activity on unsaturated substrates, but not its preference for intermediate chain lengths, restored the influence of FabZ on unsaturated fatty acid synthesis (Figure 3.2C). Steady-state concentrations of reaction intermediates provide a straightforward explanation (Figure B.5): In the absence of FabZ, the low activity of FabA on unsaturated β-hydroxyacyl-ACP’s causes them to accumulate in the reaction mixture and reduces flux through the unsaturated branch of the fatty acid pathway, yielding a low unsaturated fraction. A low concentration of FabZ can alleviate this effect.

Next, we turned our attention to FabF and FabB. As concentrations of FabF increased, the fraction of unsaturated fatty acids stayed constant (Figure 3.2E). This result is consistent with the established insensitivity of FAS kinetics to acyl-ACP elongation. In brief, FabF does not carry out a rate-limiting step and, thus, has little influence on the concentrations of indirect reaction intermediates (Figure B.6). FabB, by contrast, enhanced the production of unsaturated fatty acids in a hyperbolic manner (Figure 3.2E). Additional FabA amplified this effect; extra FabZ reduced it. The influence of FabB is consistent with experimentally established links between FabB overexpression and the accumulation of unsaturated fatty acids in vivo. The effects of FabA and FabZ, in turn, highlight their respective contributions to the accumulation and depletion of cis-dec-3-enoyl-ACP. Our study of the β-ketoacyl-ACP synthases thus suggests that FabB—but not FabF—can alter carbon partitioning between saturated and unsaturated branches of the fatty acid pathway.

Next, we used the model to investigate an intriguing structural perturbation. A recent study identified a mutant of ACP (D38A) that weakens its affinity for FabB and—likely, as a result—reduces the production of unsaturated fatty acids in vivo. We examined the influence of this
mutation on fatty acid production in two steps: First, we adjusted concentrations of FabA and FabB in our reference system to achieve the unsaturated fractions produced at three growth temperatures examined in the aforementioned study. (We note: *E. coli* responds to drops in temperature by synthesizing more unsaturated fatty acids; Figure 3.2F). Second, we changed the $K_d$ for all FabB-C$_i$ACP complexes to the experimentally determined value for the FabB-C$_8$ACP$_{D38A}$ complex. This single change captured the reduction in unsaturated fatty acids observed *in vivo* (Figure 3.2F). This result is interesting because it suggests that the influence of the D38A mutation results from the weakened affinity of ACP$_{D38A}$ for FabB (i.e., the only effect captured by our model) and not from changes in the stability of other enzyme-ACP complexes. It also demonstrates that our model can capture experimentally observed shifts in FAS activity through the introduction of a single experimentally measured binding parameter.

### 3.3.3 Control of Competing Biochemical Objectives

Total production and average chain length are—in addition to unsaturated fraction—biochemically and industrially important objectives of FAS systems. To determine how functional redundancy contributes to each of these objectives, we used our model to examine tradeoffs between them. In brief, we restricted the total enzyme concentrations of FASs depleted in various components to the total concentration of our reference system and, at different production levels, we optimized enzyme compositions to maximize or minimize either (i) the fraction of unsaturated fatty acids or (ii) the average length of fatty acids (Figures B.7-B.9). This analysis allowed us to construct phase diagrams that show the ranges of unsaturated fractions or chain lengths accessible at each production level (Figure 3.3A and 3.3C). For all FASs, high production levels reduced access to both large unsaturated fractions and short chain lengths; this finding suggests that the different objectives compete with one
another. The absence of FabZ amplified the tradeoff between total production and the unsaturated fraction, while the absence of both FabZ and FabF caused a similar—if less pronounced—effect on competition between total production and chain length.
Figure 3.3. Analysis of competing objectives. (A) The ranges of unsaturated fatty acids afforded by modeled FASs with different components removed (18 μM total enzyme, 1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, 0.5 mM acetyl-CoA, 10 μM ACP, 12 min; all shaded regions extend to 0). The maximal unsaturated fraction decreases as total production increases; this tradeoff is most pronounced in the absence of FabZ. Note: The yellow and purple shaded regions terminate at the maximum production levels of the indicated FASs. Also, some regions correspond to FAS compositions with identical phase diagrams (e.g., the gray region corresponds to both (i) the reference system and (ii) the reference system without FabF). (B) The enzyme compositions of the complete FAS (black) and the FabZ-less FAS (purple) that yield the maximum unsaturated fractions at the highest and lowest production levels plotted in A (open circles). Letters indicate associated Fab enzymes; T corresponds to TesA. Note: FabG and FabD are absent because their concentrations do not change significantly (Appendix B, Fig. B.8). (C) The ranges of average chain
lengths afforded by modeled FASs with different components removed (as in A). The range of accessible chain lengths decreases as total production increases. (D) The compositions of the complete FAS (black) and the FabZ/F-less FAS (yellow) that yield the maximum and minimum average chain lengths at the highest production levels plotted in C (open circles). The error bars and line thicknesses in A-D represent the standard deviation of modeled data. (E) A sensitivity analysis shows enzyme-specific contributions to three objectives. Left: In the complete FAS, discrepancies between the contributions of partially redundant enzymes to total production, unsaturated fraction, and chain length reflect their ability to help balance those objectives. Middle: The removal of FabZ broadens the influence of FabA across the three objectives. Right: The removal of FabF has a similar effect on the contribution of FabB.

The enzyme compositions at modeled optima reveal the origins of observed tradeoffs. Maximal unsaturated fractions require high concentrations of FabA and FabB, while maximal production levels require high concentrations of (i) FabH, which enhances initiation rates, and (ii) FabZ and FabI, which enhance flux through the saturated branch of the fatty acid pathway (Figure 3.3B). The removal of FabZ limits flux through this branch, necessitating compensating changes in FabA and FabB that reduce unsaturated fatty acid synthesis (i.e., more FabA and less FabB push flux to the saturated branch). Maximal production of short-chain fatty acids, in turn, requires additional TesA and less FabF and FabB, while high production levels restrict the depletion of β-ketoacyl-ACP synthases, which are required to sustain high flux (Figure 3.3D). These conflicting requirements explain the positive slope of the lower boundary in Figure 3.3C; the higher concentrations of β-ketoacyl-ACP synthases required by higher production levels restrict the minimum average chain length (i.e., it becomes longer). The removal of FabF reduces the maximal chain length, likely a result of the low activity of FabB on C₁₆ substrates.

We visualized the distinct influences of partially redundant enzymes more directly by using a global sensitivity analysis to examine their contributions to different objectives—total production, unsaturated fraction, and average chain length. That is, we examined the sensitivity of each objective to changes in the concentration of each enzyme in our model. Our results indicate
that FabZ has a strong influence on both total production and unsaturated fraction, while FabA influences only the latter. FabF has a pronounced effect on average chain length, while FabB alters mainly the unsaturated fraction (Figure 3.3E). Discrepancies in the contributions of partially redundant enzymes to competing objectives suggest that these enzymes help balance tradeoffs between them. We resolved this effect further by carrying out sensitivity analyses of FASs depleted in FabZ or FabF (Figure 3.3E); in this analysis, the removal of each enzyme expanded the influence of its counterpart (i.e., FabA in the absence of FabZ, or FabB in the absence of FabF) across multiple objectives. This finding is intriguing because it indicates that partially redundant enzymes enable independent control over distinct biochemical objectives that are more closely linked—that is, more highly interdependent—in less redundant systems.

3.3.4 | Analysis of other Biochemical Influences. The FAS of *E. coli* supplies substrates for lipid biosynthesis, a metabolic process that could alter the behavior of the FAS system by competing for shared intermediates. To evaluate the influence of lipid biosynthesis on our modeling results, we integrated reactions for lipid A and phospholipid assembly into our kinetic model (Figure B.12). We began by adding kinetic equations that describe (i) the production of lipid A and (ii) the initiation of phospholipid biosynthesis (Appendix B Methods). These equations assume Michaelis-Menten kinetics, a simplification that reflects the lack of available kinetic data for the 11 added enzymes (though, we note that the equations for the lipid A pathway came from a previously validated kinetic model).\textsuperscript{44} We parameterized the expanded model with estimates of kinetic constants and substrate concentrations reported in the literature,\textsuperscript{44-48} and we optimized it with fits to experimental measurements of free fatty acid and lipid synthesis by a TesA-expressing strain of *E. coli* (Figure B.13). Intriguingly, when we used the expanded model to repeat both (i)
titration studies and (ii) sensitivity analyses, trends in FAS outputs remained unchanged from the original model. This consistency indicates that these trends are insensitive to lipid biosynthesis.

A complete cell includes a multitude of biochemical reactions that could influence FAS activity. To evaluate the ability of our base model (i.e., the model that lacks lipid biosynthesis) to capture FAS activity in living cells, we recreated perturbations reported in three in vivo studies. We began by reducing the concentration of TesA in the reference composition (all three in vivo studies used native expression levels of this enzyme). With this new composition as our baseline, we simulated the over- or under-expression of various components by adjusting their concentrations by 30-fold. To our satisfaction, simulated shifts in unsaturated fractions and total production levels resembled those observed in vivo. This finding is consistent with (i) the model’s ability to capture ACP-derived shifts in the unsaturated fraction in vivo (Figure 3.2F) and (ii) the insensitivity of trends in FAS kinetics to lipid biosynthesis (i.e., the consistent behavior of the expanded model; Figure B.14). The three findings, taken together, indicate that the observed contributions of component-specific perturbations to FAS activity originate from effects internal to the FAS system (i.e., effects examined in this study).

3.3.5 | Evaluation of Predictions with a FAS Reconstituted in Vitro. We sought to confirm the most interesting predictions of our model by testing them with a reconstituted FAS. To begin, we overexpressed and purified the ten constituent proteins of the E. coli system (Figure B.19). We note: For this effort, we used a fused dimer of FabZ, an enzyme that forms an obligate dimer in vivo; this homooligomeric fusion facilitates overexpression of the functional enzyme. We assayed the purified enzymes by measuring the conversion of NADPH to NADP+, a previously validated assay for FAS activity. Our reconstituted FAS achieved an initial rate of 3.9 μM
palmitic acid equivalents per minute and reached ~ 25 μM palmitic acid equivalents after 7 minutes (Figure 3.4A); this rate and productivity are remarkably consistent with previously reported measurements of the same in vitro system (i.e., 3.7 μM/min and ~25 μM palmitic acid equivalents after 7 minutes29).
Figure 3.4. Experimental analysis of FAS activity. (A) A time course of total fatty acid production by an experimentally reconstituted FAS (1.3 mM NADPH, 0.5 mM malonyl-CoA, 0.1 mM acetyl-CoA, 10 μM ACP, 10 μM TesA, and 1 μM of each Fab). The black line and shaded region show the mean and standard error, respectively, of n = 4 independent reactions. (B) A comparison of
measured (black) and modeled (white) initial rates of different FASs (substrates and cofactors as in A with rates measured at 2.5 min). (C) Initial rates of FASs with different ratios of FabZ and FabA (as in A with [FabZ] + [FabA] = 2 μM). (D) Total fatty acid production for a subset of systems described in C. (E-F) Initial rates of FASs with indicated concentrations of (E) FabA and FabZ or (F) FabB and FabF. The removal of FabZ enhances the influence of FabA on initial rate; the removal of FabF has a similar effect on FabB. (G) The unsaturated fractions afforded by a subset of FASs from E and F measured by GC/MS analysis (2 hours, see Appendix B Methods). Bars (B and D) and solid circles (C, E, F, and G) show the average of n ≥ 3 biological replicates; open circles denote each replicate; error bars denote standard error. Error bars for model results in B were calculated as described in the Appendix B methods.

We tested our base model by comparing predicted and measured initial rates of fatty acid production for several FAS compositions (Figure 3.4B). The model captured trends in initial rates for most of the FASs examined but underpredicted the rate of a system with 30 μM FabH. Previous studies suggest that FabH enhances initial rates at low concentrations and inhibits them at high concentrations (e.g., 30 μM28), likely by outcompeting FabF for malonyl-ACP.24 With this mechanism in mind, we hypothesized that the concentration of FabH at which inhibition emerges might increase with the concentration of ACP. Simulations of FabH titrations in the presence of varying amounts of ACP support this hypothesis, and experimental measurements of initial rates at 100 μM FabH show that this enzyme can, indeed, become inhibitory in our system (Figure B.17). The fraction of active ACP in our in vitro system may, thus, exceed the active fractions in the FASs used to train our model.

Our model also overpredicts—though somewhat mildly—the initial rates of FASs with additional FabF or FabA. In particular, FabF is less inhibitory in the model than in the in vitro system. This effect could reflect an underestimate of (i) the modeled affinity of FabF for ACP and/or (ii) the in vitro concentration of FabF; a stronger binding constant and higher enzyme concentration enhance the inhibitory effect of FabF in our model (Figure B.18A). FabA, by contrast, appears more active in the model than in the in vitro system; a reduction in the modeled
activity of FabA improves agreement between model and experiment (Figure B.18B).

Our modeling analysis of tradeoffs between competing biochemical objectives indicates that maximal production levels require a disproportionately high flux through the saturated branch of the fatty acid pathway. We tested this prediction with in vitro kinetic measurements of FASs with different proportions of FabZ and FabA (i.e., [FabZ] + [FabA] = 2 μM). As expected, initial rates increased as the relative proportion of FabZ grew larger (Figure 3.4C); measurements of total fatty acids generated in vitro showed a similar trend (Figure 3.4D). Small amounts of FabZ also increased the production of unsaturated fatty acids while leaving the level of saturated products unaltered (relative to a FabZ-less system), a result consistent with the ability of FabZ to enhance unsaturated fatty acid synthesis (i.e., as we observe in Figure 3.2B); a high concentration of FabZ, in turn, enhanced both species, a finding consistent with the restricted access to large unsaturated fractions at high production levels.

The results of our sensitivity analysis indicate that the removal of redundant FAS components can expand the influence of remaining components across multiple objectives. The removal of FabZ, for example, enhances the contribution of FabA to total production; the depletion of FabF has a similar effect on FabB. To test these predictions in vitro, we examined fatty acid synthesis by FASs with different concentrations of FabA or FabB. As expected, initial rates were insensitive to both (i) FabA in the presence of FabZ and (ii) FabB in the presence of FabF, but increased with FabA and FabB concentrations when their redundant counterparts were absent (Figures 3.4E and 3.4F). By contrast, both FabA and FabB enabled substantial enhancements to unsaturated fractions (i.e., 40% or more) in the presence and absence of their redundant counterparts (Figure 3.4G). This result supports our finding that partially redundant enzymes
facilitate independent control over total production and unsaturated fraction—two objectives that become more closely linked in less redundant systems.

Intriguingly, FASs lacking FabA or FabB continued to produce unsaturated fatty acids, albeit at lower levels than complete FAS systems (Figure 3.4G). To assess the contribution of enzyme contaminants to this effect, we used quantitative proteomics to measure all proteins in our stocks of FabZ and FabF. The FabZ stock had a FabA impurity of less than 0.5 % (i.e., FabA relative to FabZ); the FabF stock had no detectable FabB. The unsaturated fractions afforded by FabZ and FabF thus appear to result from secondary activities of these enzymes. Close inspection of the literature supports this finding: Although FabA and FabB are required for *E. coli* to grow in the absence of unsaturated fatty acids, studies of cell extracts from strains that lack—or contain defective versions of—FabA or FabB have observed low levels unsaturated fatty acid synthesis.31,32,51 An analysis of reconstituted FASs also shows measurable unsaturated fatty acid production in the absence of FabA.29 FabZ and FabF thus appear to be capable of building unsaturated fatty acids—an often-overlooked capability. These secondary activities do not change the general conclusions of our analysis (as demonstrated by the consistency between model predictions and the experimental results depicted in Figure 3.4); however, they are certainly worth integrating into future versions of the model.

### 3.4 | DISCUSSION AND CONCLUSION

Many biochemical systems include enzymes with partially redundant activities (signaling networks, metabolic pathways, and cellular surfaces, to name a few52-56); the importance of this functional overlap, however, is rarely clear. In this study, we combined a detailed kinetic model with an *in vitro* system to investigate the capabilities afforded by functional redundancy in fatty
acid synthesis. Our model captures the effects of experimental perturbations to FAS systems (i.e., changes in the concentrations and/or activities of specific components in vitro and in vivo) and helps explain the mechanistic origin of those effects.

The modeling analyses indicate that the high activity of FabZ on unsaturated β-hydroxy-acyl-ACP's allows it to enhance the production of unsaturated fatty acids beyond the levels afforded by FabA alone. This effect is consistent with biostructural differences between the two enzymes: The linear binding pocket of FabA, which easily accommodates C_{10} substrates, is well suited—and, perhaps, optimized—for the allylic rearrangement of trans-dec-2-enoyl-ACP to cis-dec-3-enoyl-ACP, but not the dehydration of unsaturated long-chain intermediates, a deficiency remedied by the elongated and kinked binding pocket of FabZ. The contribution of FabZ to unsaturated fatty acid synthesis thus demonstrates an intriguing form of synergy in which two partially redundant enzymes work together to enhance a specialized function.

Compositional analyses carried out in silico and in vitro, in turn, indicate that partially redundant enzymes enable independent control over competing biochemical objectives. Total production and unsaturated fraction, for example, appear to be independently adjustable in the presence of partially redundant enzymes but become tightly coupled in their absence; the additional flexibility afforded by functional redundancy may thus facilitate independent control of growth rate and membrane composition—though, additional research is needed to support this biological effect. The contribution of redundancy to tradeoffs between total production and chain length, by contrast, is less pronounced, a limitation that may reflect the dominant influence of thioesterase specificity on the length of free fatty acids._\textsuperscript{9,57,58}_

Bacteria with less redundant FASs than _E. coli_ certainly exist. Examples include (i) _Helicobacter pylori_ and _Bacillus subtilis_, which couples FabZ and a desaturase (i.e., FabX or Des,
which act on decanoyl-ACP or phospholipids, respectively,\textsuperscript{12,59} (ii) \textit{Streptococcus pneumoniae}, which uses an isomerase in place of FabA (FabM\textsuperscript{60}), and (iii) \textit{Aerococcus viridans}, which exploits a single multifunctional dehydratase/isomerase (FabQ\textsuperscript{61}). At the extreme, \textit{Chlamydia trachomatis} uses a non-redundant FAS that cannot produce unsaturated fatty acids. This organism builds branched fatty acids from branched acyl-CoAs, which help it control its membrane fluidity without adjusting saturation.\textsuperscript{62} Our findings do not imply that alternative FAS systems are incapable of adjusting their outputs; they suggest that some of these systems—those with individual multifunctional enzymes—are more limited in the breadth of accessible adjustments (as in Figure 3.3A), and indicate that others—those with specialized enzymes—may simply reflect alternative solutions to biological control.

This work highlights the power of combining kinetic models with \textit{in vitro} systems to study metabolic pathways. The fate of fatty acids \textit{in vivo}—their transport into and out of the lipid membranes, their degradation and functionalization, and their interaction with proteins in crowded environments—is incompletely understood;\textsuperscript{8,63,64} the mechanisms by which pathway modifications influence final product profiles can, thus, be extremely difficult—if not impossible—to examine with cell-based studies. By removing the fatty acid pathway from the cellular milieu, the kinetic model and \textit{in vitro} system described in this study supply powerful tools for carrying out detailed mechanistic studies of fatty acid biosynthesis. Consistency between \textit{in silico}, \textit{in vitro}, and \textit{in vivo} studies, in turn, demonstrate that these tools can supply important, biologically relevant insights into the structure and function of cellular systems.
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CHAPTER IV: A DETAILED KINETIC MODEL FOR OLEOCHEMICAL BIOSYNTHESIS IN *E. coli*

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Manuscript in preparation
4.1 | INTRODUCTION

Concerns over the long-term sustainability of petrochemical reserves have stimulated considerable interest in oleochemicals as an effective replacement.\textsuperscript{1,2} Production of oleochemicals currently derive largely from less sustainable plant and animal sources.\textsuperscript{3} Microbes have considerable potential advantages in oleochemical production in terms of efficiency of feedstock conversion and capability for production of diverse functional groups.\textsuperscript{4} The ability to tailor chain lengths to medium and short chain oleochemicals is of particular interest due to the higher value of these products. However, making use of this potential of microbial systems involves considerable challenges in optimizing and redesigning relevant metabolic pathways. The production of fatty acids by fatty acid synthases (FAS) are of considerable importance as this pathway generates the precursor aliphatic chains for oleochemicals. The extensively studied \textit{E.coli} FAS is a common target for metabolic engineering, generally seeking to maximize yields and/or control chain length specificity of fatty acids. The dependence of chain length distributions on relative pathway kinetics results in the need for new strategies and optimization for differing distributions.\textsuperscript{5} The use of modeling to capture pathway kinetics is therefore needed to accelerate the optimization and strategy design process.

Models used in biology span a large range of spatial and temporal resolutions. Several key assumptions or simplifications are usually required to specify the governing equations and structure of models. As a result, model development requires careful consideration of the scope of investigation or engineering. Classical metabolic engineering seeks to increase the amount of feedstock converted to a final product and so typically examines the metabolic pathways at steady state using stoichiometric constraints. This flux balance method requires accurate information on the mapping of biochemical reactions but has the significant advantage of not requiring further
kinetic information. Because of its low computational cost, genome wide models are possible, and optimization can often involve tuning transcriptional levels of all biocatalytic steps from feedstock to final product while still ensuring basic cell growth needs are met. Several models of fatty acid metabolism utilizing this method have been developed, suggesting strategies for increasing carbon flux into the fatty acid pathway.\(^5\)\(^6\) However, as these models neglect enzyme kinetics, they cannot optimize pathway behavior sensitive to kinetics such as chain length distribution.

Models of pathway kinetics typically involve representing the concentration of all components and intermediates of the relevant enzymatic reactions with ordinary differential equations (ODE) or partial differential equations if spatial heterogeneity is relevant. As the FAS consists of freely diffusing constituent enzymes located in the cytoplasm we assume that spatial effects can be neglected. We further neglect any stochastic effects (reasonable given that we examine only fast reactions and do not consider transcription). Michaelis-Menten rate equations are often used as rate equations based on a rapid equilibrium assumption (this is often reasonable and significantly reduces the number of parameters needed). We did not include any simplifying assumptions for enzyme kinetics as the wealth of kinetic information for \textit{E.coli} FAS allowed for effective parameter estimation. Our model thus consists of continuous deterministic ODEs based on rate equations that capture substrate binding (\(k_{\text{on}}/k_{\text{off}}\)), the reaction step (\(k_i/k_r\) if reversible or \(k_{\text{cat}}\) if irreversible) and product unbinding for reversible reactions (\(k_{\text{on}}/k_{\text{off}}\)). We also capture pathway inhibitory mechanisms (feedback inhibition of long chain acyl-ACPs on FabH, and competitive inhibition of FAS enzymes with ACP).

Our initial model version included the enzymes required for saturated fatty acid synthesis from malonyl-CoA and acetyl-CoA (FabD, FabH, FabG, FabZ, FabI, FabF, and TesA). We parameterized this model by incorporating kinetic measurements from \textit{in vitro} enzymatic studies.
and parameter fits to diverse pathway datasets (time course of production, chain length distributions, and initial rates of enzyme titrations). We validated our model by capturing initial rate trends of \textit{in vitro} data from the Khosla group not used in fitting. We performed a range of \textit{in silico} experiments that demonstrated chain length distributions could be shifted using compositional optimization of pathway components while controlling the shape of distributions (for example tight distributions) required modifications to either the termination enzyme (TesA) or the final elongation enzyme (FabF). Our subsequent model introduced unsaturation via the addition of partially redundant enzymes FabA and FabB (isozymes of FabZ and FabF respectively that perform the same reactions in addition to incorporating unsaturation). We found that unsaturated fraction showed greater uncoupling from production and chain length distributions in systems with partial redundancy.

In this study we examined the influence of fatty acid production on downstream oleochemical production by expanding the model to include pathways converting fatty acids or FAS intermediates to ketones, alcohols, esters and alkanes (Figure 4.1). We validated the model by comparison to metabolic engineering efforts using the added pathways to generate oleochemicals. We then explored various unintuitive or unexpected results observed in metabolic engineering. Finally, we contrasted different design strategies targeting the production of alcohols and ketones to determine if there were any advantages relating to production or distribution of oleochemicals for differing pathway designs.
Figure 4.1. Oleochemical biosynthesis in *E. coli*. (A) The fatty acid synthase (FAS) of *E. coli* (orange) can supply precursors for a broad set of enzymatically synthesizable oleochemicals. Many engineered pathways exploit pools of free fatty acids, where length is controlled by the substrate specificities of thioesterases, or acyl-ACPs. Colors highlights important products and the distinct enzymatic steps required for their synthesis.

4.2 | MATERIALS AND METHODS

4.2.1 | Assembly and solution of a kinetic model of FAS activity. In prior work, we developed a detailed kinetic model of the FAS of *E. coli* by modeling the nine enzymes necessary to convert malonyl-CoA and acetyl-CoA to saturated and unsaturated fatty acids (Figure 4.1). The modeled FAS includes a cytosolic variant of the periplasmic thioesterase TesA, which is commonly used for the overproduction of free fatty acids in engineered strains. Our model, in brief, uses rate equations based on detailed reaction mechanisms and includes separate association
and dissociation steps for all heteromeric complexes. In this study, we refined the model by incorporating commonly ignored secondary activities of FabZ and FabF. Both enzymes can produce unsaturated fatty acids in *vitro* and *in vivo*—although, less effectively than FabA and FabB. We added rate equations describing (i) the allylic rearrangement of trans-dec-2-enoyl-ACP to cis-dec-3-enoyl ACP by FabZ and (ii) the condensation of cis-dec-3-enoyl-ACP with malonyl-ACP by FabF. The new equations are analogous to those used for FabA and FabB (Table C.1); we used the same binding parameters \( k_{on} \) and \( k_{off} \) but adjusted values of \( k_{cat} \) for all four enzymes by fitting to reported measurements of the unsaturated fractions produced by reconstituted FASs lacking FabA, FabZ, FabB, or FabF. Our model captured the relative differences in unsaturated fractions between FAS mixtures (the fit objective) but predicted lower total concentrations of unsaturated products (Tables C.1 and C.2). This finding is consistent with our observation that *in vitro* systems tend to produce larger unsaturated fractions than *in vivo* systems and, relatedly, with our use of an *in vivo* product profile to optimize the original model. We re-optimized our adjusted model by fitting its 14 scaling parameters to the broad set of experimental data used for our initial optimization process; most parameters changed marginally from their original value (Table C.3).

Our model consists of a system of rate equations and mass balances. We solved it by using the MATLAB solver ode15s with relative and absolute error tolerances of \( 10^{-6} \); a sparsity matrix and vectorization step reduced solve time.

### 4.2.2 | Methyl ketone biosynthesis.

We expanded our model by incorporating two pathways for methyl ketone biosynthesis. The first exploits β-oxidation of free fatty acids; the second relies on thiolase-mediated elongation of acyl-CoAs (Figure 4.1). Both pathways generate β-ketoacyl-
CoAs, which can be hydrolyzed by a β-ketoacyl-CoA thioesterase (FadM) to generate β-keto fatty acids; these unstable molecules spontaneously decarboxylate to form methyl ketones.

We began with β-oxidation. We incorporated the activities of FadD, FadE, FadB, and FadM with single- or bi-substrate Michaelis-Menten equations (Table C.4), and we estimated kinetic parameters from measurements reported in the literature (Table C.5). To facilitate fitting, we carried out a sensitivity analysis of newly added $k_{cat}$ values by using the Morris Method to measure their influence on total production at 12 minutes (Figure C.1). In prior work, this time point has recapitulated trends in FAS activity in vitro and in silico.7–10 Our analysis indicated that the activities of (i) FadD, FadE, and FadM on their primary substrates and (ii) TesA on acyl-CoAs were highly influential. Accordingly, we optimized corresponding values of $k_{cat}$ by fitting to several datasets that describe methyl ketone production by CpFatB and BTE through the β-oxidation pathway.11 Where appropriate, we modeled acyl-CoA oxidase, a more active alternative to FadE, by increasing the FadE concentration by tenfold. Our initial fits underpredicted methyl ketone production by BTE, so we re-fit relevant datasets by including its $k_{cat}$ on acyl-ACPs as an additional fit parameter. Our final model captured trends in methyl ketone production between different strains but underpredicted both (i) the production of 2-nonanone by BTE and (ii) the production of palmitic acid by TesA (Figure 4.2). The underprediction of 2-nonanone likely results from the narrow in vivo product profile used to parameterize BTE in our model; this profile may change slightly between chains. For the second discrepancy, we speculate that the large experimental concentrations of palmitic acid may result from the contamination of membrane lipids, which contain large amounts of this fatty acid (we have observed such contamination in our studies).
We validated our model by assessing its ability to predict the influence of (i) reduced growth temperatures and (ii) thioesterase exchange on the production of methyl ketones (Figure 4.2). For the first study, we modeled low growth temperatures by reducing concentrations of FabA and FabB, as described previously. Trends in ratios of unsaturated methyl ketones were similar between model and experiment. For the second study, we exchanged TesA for BTE. Trends in ratios of methyl ketones between model and experiment were, once again, similar, with the notable exception of C15/C13 for BTE—a ratio modeled to be zero. This discrepancy is, once again, attributable to the narrow profile used to parameterize BTE.9,13

Figure 4.2. Biosynthesis of methyl ketones. (A)-(B) The model concentrations of methyl ketones are fit to five experimental conditions11 (1-4 includes CpFatB while the system in 5 utilizes BTE) while simultaneously fitting to the ratios of fatty acid to methyl ketone measured in two conditions from a separate study (left is without FadM overexpression and right is with FadM overexpression).12 (C) The model trends for ratios of ketones at two different temperatures are compared to measured values showing similar relative behavior.12 (D) The model trends for two thioesterases TesA and BTE are compared to experiment.12 Here, while the trends have similar relative shifts for C11/C13 the modeled BTE has higher specificity.
We incorporated a thiolase pathway by starting with our model of β-oxidation. Briefly, we added a thiolase enzyme using a bi-substrate Michaelis-Menten equation, and we turned “off” FadE and FadB by setting enzyme concentrations to zero. We used values of $k_{\text{cat}}$ and $K_m$ measured for *E. coli* FadA as initial estimates for kinetic parameters assuming no chain length substrate specificity.\(^{14}\) An *in vivo* thiolase pathway expressing CpFatB as the thioesterase compared a variety of thiolases including ecFadA and found a thiolase from *R. eutropha* BktB had the highest activity.\(^{11}\) We used the ratio of ketone production of ReBktB to EcFadA to estimate the ratio of $k_{\text{cat}}$ between the two enzymes. We then validated our thiolase pathway by comparing our distribution of ketones to the measured distribution for ReBktB.\(^{11}\)

### 4.2.3 Alcohol biosynthesis

Several pathways enable the biosynthesis of alcohols in *E. coli*. We incorporated a two-step pathway consisting of (i) a carboxylic acid reductase (CAR) and (ii) a native aldehyde reductase (Ahr), which we modeled with Michaelis-Menten equations for enzyme kinetics. Briefly, we used measured values of $k_{\text{cat}}$ and $K_m$ for each chain length to parameterize CAR and for Ahr we estimated $k_{\text{cat}}$ by using the measured value of $k_{\text{cat}}$ for C\(_4\) and scaling it for other chain lengths with ratios of initial rates (assuming that $K_m$ did not change with chain length).\(^{15,16}\) For chain lengths that were not measured we assumed values of the nearest measured chain length. For activity on unsaturated chain lengths that were not measured we assumed the same parameters as on saturated chain lengths. We validated our parameterization by comparing to an *in vivo* distribution of fatty alcohols utilizing TesA as the thioesterase.\(^{15}\) We found good agreement between the modeled and measured distribution although our model produces more C\(_{14}\) vs C\(_{12}\) fatty alcohols (Figure 4.3A).
Figure 4.3. Oleochemical biosynthesis. (A)-(B) The distribution of fatty alcohols and FAME in model and experiment are compared for validation.\textsuperscript{15} (C) The fraction of FAEE distribution in a TesA expressing strain is fit by tuning elongation rate (FabF and FabB concentrations are increased to 10.4µM from 1µM).\textsuperscript{22} (D)-(E) The fraction of FAEE for (D) chFatB2 and (E) atFatA3 expressing strains are examined for validation.\textsuperscript{22} (F) The fraction of alkanes is fit with $k_{cat}$ on C17:1; interestingly the model cannot fully capture the amount of C17:1 produced in experiment.\textsuperscript{19} (G)-(H) The trends of alkane fraction at different temperatures are compared between model and experiment.\textsuperscript{19} The model and experiment trends agree well except for 18ºC, which showed lower production in the experiment relative to the higher temperatures, possibly explaining the break with the trend.
4.2.4 | **Alkane biosynthesis.** We incorporated alkane biosynthesis with a two-step pathway consisting of (i) an acyl-acyl carrier protein reductase (AAR) and (ii) an aldehyde deformylating oxygenase (ADO). We used Michaelis-Menten kinetics for rate equations. AAR was parameterized by estimating $k_{\text{cat}}$ from a *Cryptosporidium parvum* AAR $k_{\text{cat}}$ and scaling the $k_{\text{cat}}$ for varying chain lengths using measured *in vivo* alkane distributions (assuming same $K_m$ for all chain lengths).\(^{17}\) ADO was parameterized by using the measured $k_{\text{cat}}$ value for C\(_{16}\) for all chain lengths except for C\(_{18:1}\) which we fit to the *in vivo* production profile (due to an abundance of C\(_{17:1}\) alkanes produced, indicating unusually high activity; Figure 4.3F).\(^{18}\) We validated our model of alkane biosynthesis by comparison to experimental trends in fraction of alkanes with varying temperature (Figure 4.3G-H).\(^{19}\) We represent the effect of temperature on distributions by increases in FabA/FabB concentrations resulting in increases in unsaturated fatty acid production.

4.2.5 | **FAME and FAEE biosynthesis.** We incorporated fatty acid methyl ester (FAME) production with a single enzymatic modification step utilizing DmJHAMT to convert fatty acid to its methyl ester. We used Michaelis-Menten kinetics and parameterized $k_{\text{cat}}$ and $K_m$ by using the measured values for C\(_{12}\) fatty acid, assuming no specificity for $K_m$ and scaling $k_{\text{cat}}$ with reaction rate as a function of chain length.\(^{20}\) Validation of the model distribution of FAME with measured *in vivo* distributions shows good agreement (Figure 4.3B).

Fatty acid ethyl esters (FAEE) were added to the model with a single enzymatic step utilizing atfA (a wax ester synthase). We used Michaelis-Menten kinetics and estimated $k_{\text{cat}}$ and $K_m$ values with no chain length specificity as indicated by activity measurements as a function of chain length.\(^{21}\) The measured distributions of FAEE in a strain expressing TesA and atfA were unusually biased towards long chains so we fit the distribution by increasing FabF/FabB
concentrations to 10.4µM from 1µM (Figure 4.3C). We validated our parameterization and elongation rate adjustment by comparison to distributions expressing atfA and alternative thioesterases chFatB2 and atFatA3 in place of TesA (Figure 4.3D-E).22

4.2.6 | Sensitivity analysis. We used the Morris Method sensitivity analyses of important variables and kinetic parameters (e.g., enzyme concentrations or kinetic constants). This method is an efficient means of estimating relative global sensitivity of parameters. The method as defined by Morris23 calculates a set of derivatives with respect to the changed parameter by sampling randomly in parameter space. The mean elementary effect is the average derivative with respect to a given parameter for a variety of parameter conditions and is the estimate of sensitivity. We implemented this method using the SAFE toolbox24 with a radial method and Latin hypercube sampling (r = 1,000 trajectories) and we further verified the convergence of the mean elementary effects (Figure C.1).

4.3 | CONCLUSION

Our expansion of the model to include oleochemical production relies on high quality kinetic data. We focused on studies that carried out detailed kinetic measurements of purified enzymes or that used previously characterized enzymes. For ketone production, we had access only to detailed measurements of substrate specificity for FadD, but we found that fitting the overall k_{cat} of FadD and several other enzymes was sufficient to capture observed trends in product distributions and production levels. For studies that measured substrate specificity, such as several that focused on the production fatty alcohols and FAME, we found that our model could recapitulate experimentally observed product profiles without fitting. In cases where
measurements of substrate specificity were sparse, such as in studies of alkanes and FAEE, we had to fit either elongation rates (for FAEE) or substrate specificities (for alkanes) to capture observed distributions. These fits were sufficient to recapitulate observed trends in oleochemical production for cases that were not fit. Together, our analysis of various oleochemical-producing strains highlights the versatility of our kinetic model as a tool for metabolic engineering.

In future work, we will use our model to compare and contrast alternative pathways for a subset of important oleochemicals. We will focus on methyl ketones and alcohols, which have several metabolic routes that have been described in recent studies. In brief, we will examine the access of these routes to different combinations of production level, chain lengths, and unsaturated fractions—attributes that affect the economics of microbial oleochemical production. We expect some pathways to be better suited for specific product profiles (e.g., narrow distributions of methyl ketones), and we expect their suitability to be a function of the currently known repertoire of FAS enzymes, such as thioesterases with different substrate specificities.

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CHAPTER V: OUTLOOK

5.1 Expansion and refinement of a detailed kinetic model of fatty acid biosynthesis

As a famous aphorism suggests, all models are wrong, but some are useful. This thesis describes the systematic development of a detailed kinetic model of fatty acid biosynthesis in *E. coli* and the use of that model to examine experimental data. The final model can recreate—and help explain—a diverse set of unexpected trends from *in vitro* and *in vivo* experiments, and supply new strategies for controlling fatty acid synthesis in engineered strains (e.g., the total production, unsaturated fraction, and average chain length of fatty acids). Our model provides a powerful tool for guiding experimental studies of FAS systems.

Future versions of the model should be guided by trends in experimental data that it cannot capture (e.g., trends where mechanistic explanations are desired but presently elusive); quantitative agreement between the model and experiment is also important, though only insomuch as it is required for the design and optimization of FAS systems. We anticipate refining future versions of the model by exploring new approaches for assessing error and quality of fit. This work could facilitate the expansion of the model to include new FAS-relevant reactions (e.g., by enabling the design of a minimal set of experiments) or the development of alternative, system-specific model versions (e.g., the design of a model for the type I FAS of *Saccharomyces cerevisiae*).

Metabolic pathways—in contrast with many of the more common (or classical) reaction systems found in chemical engineering—have a large number of complex catalysts and physically distinct reaction intermediates that are difficult to probe in reacting systems (e.g., living cells).\(^1\) Experimental data is, as a result, limited in its availability and variable in its quality.\(^2\) The loose constraints on model parameters that result imply that many possible fits may produce similar quantitative results in fitting and validation.\(^3,4\) The development of a systematic approach for
evaluating different types of fits could prove informative.\textsuperscript{5} To start, our model uses 12-14 scaling parameters to link groups of similar kinetic terms to one another. We explored several alternative sets of scaling parameters, but the number of parameter combinations is enormous; superior versions of our model certainly exist, and a systematic approach to find them could be informative. As we state above, however, this effort should be accompanied by a specific set of questions or engineering challenges that justify the added effort, as the current version of the model is sufficient to analyze and explain a diverse set of kinetic phenomena. An exemplary engineering challenge might be the development of a kinetic model of fatty acid synthesis in a less well-studied biological system; an automated approach for fitting a model from a limited set of data could be advantageous.

Methods for identifying important features of data will be important for both the expansion of our kinetic model and its generalization to other organisms. For example, when we examined changes in FAS kinetics associated with increasing concentrations of TesA, we saw a rise and fall in initial rate.\textsuperscript{6} This trend could be characterized with two important features: (i) a peak with a negative second derivative and a zero first derivative and (ii) a peak location. The first feature, which captures the important qualitative influence of TesA (i.e., its importance at low concentrations and its inhibitory effect at high concentration) is much more important than the second. This feature deserves a greater weight.

The incorporation of error in measured parameter values could improve our estimates of model error. We note, however, that most kinetic parameters have few reported measurements, and those measurements are often reported for different (model) substrates. Accordingly, the value of more advanced error estimates would have to be weighed against the relevance of the measurements on which those estimates are based. The results of our sensitivity analyses and
existing error estimates (which assume a normal distribution of enzyme and substrate concentrations) suggest that error in estimates of model parameters, which could be computationally costly to implement for all parameters, are unlikely to alter overall trends in modeled FAS outputs.⁶,⁷

5.2 In vivo validation

One of the most exciting capabilities of our model is its ability to suggest new—and largely nonintuitive—strategies for the design of FAS systems. In future work, we plan to test some of our designs by using the Marionette strain of *E. coli*. In brief, this strain, which was developed by the Voigt Group at Massachusetts Institute of Technology,⁸ uses small-molecule biosensors to control the transcription of up to 12 genes. The authors optimized it by using directed evolution to minimize both background expression (i.e., leakiness) and inter-sensor crosstalk; the resulting strain enables 100-fold shifts in gene expression with commercially available inducers.⁸ Katie Mains (Fox Group) has begun using this strain to test our model-informed approach for using changes in the ratio of FabF, FabH, and TesA (e.g., “ratiometric tuning”) to control the average length and total production of fatty acids in *E. coli*. Her work will provide a proof-of-concept study that will guide the assembly of more robust industrial strains. Notably, the cost of inducers limits the use of the Marionette strain in industrial processes; the optimal ratios suggested by this strain, however, could be integrated into industrial *E. coli* strains through the use of ribosome binding sites (e.g., RBSs) and promoters of different strengths.⁹,¹⁰ Additional regulatory controls (e.g., sensors that regulate the transcription of FAS components in response to the concentrations of FAS intermediates¹¹) could also improve the performance of inducer-free strains; our model could help guide their design.
5.3 A sustainable future

*E. coli* is one of the most important industrial microorganisms; it is used for the production of polyhydroxybutyrate,¹² 1,3-propanediol,¹³,¹⁴ amino acids,¹⁵ and therapeutic proteins.¹⁶ *E. coli* has several advantages for oleochemical production: (i) It is a model organism, so its metabolic pathways and regulatory networks are well resolved (at least, in comparison to less well studied systems).¹⁷ (ii) It can produce a broad range of chemicals—including oleochemicals—at high titers.¹⁷ (iii) It can grow under both aerobic and anaerobic conditions (an attribute that can help with biological control). (iv) It can use both pentose and hexose sugars as well as sugar alcohols and organic acids as substrates.¹⁸ In light of these advantages, we (and others) believe that *E. coli* could be used as platform for the production of a diverse set of oleochemicals in an industrial setting.

Several organisms offer important advantages over *E. coli*. Many fungal systems (e.g., *Neurospora crassa*),¹⁹ for example, can secrete cellulases that allow them to grow on cellulosic biomass, a cheaper substrate than sugar. Photosynthetic microbes such as cyanobacteria, in turn, can fix CO₂,²⁰ though the need for light penetration limits the size of their cultures.²¹ Alternatively, some yeast systems can produce enormous quantities of fatty acids (e.g., *Yarrowia lipolytica*)²² or survive under conditions that minimize the risk of contamination (e.g., low pH²³ and/or high temperatures²⁴). The soil bacterium *Corynebacterium glutamicum* is a well-known industrial organism due to its effectiveness in large scale production of amino acids,²⁵ and there is considerable interest in expanding its product profile.²⁶ To date, the FASs of these microbial systems have proven more difficult to tune than the FAS of *E. coli*, but research on their use for producing oleochemicals is ongoing. In the end, we believe that a detailed understanding of FAS
kinetics in *E. coli* will inform efforts to engineer fatty acid synthesis in other systems, which may prove advantageous for the industrial production of varieties of products.

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APPENDIX A: SUPPORTING INFORMATION FOR CHAPTER II

Note 1. Estimation of Diffusion Limits. We estimated the upper limits of rate constants for intermolecular association ($k_{on}$) by treating this process as a diffusion-limited reaction between two uniformly reactive spheres. In brief, we used the Stokes-Einstein equation (Eq. A.1) to estimate diffusion coefficients ($D$) and the Smoluchowski expression (Eq. A.2) to estimate rate constants based on those coefficients. In Eqs. A.1-A.2, $k_B$ is the Boltzmann constant, $T$ is temperature (300 K), $\eta$ is the dynamic viscosity of water, $r$ is the radius of a protein or ligand (e.g., small-molecule substrate), and subscripts A and B denote distinct binding partners. We estimated the radii of proteins and ligands from their volumes (i.e., we assumed a spherical volume of $4/3*\pi r^3$), and we determined those volumes with the following steps: For proteins, we

$$D = \frac{k_B T}{6 \pi \eta r} \quad \text{(Eq. A.1)}$$

$$k_{on} = 4\pi(D_A + D_B)(r_A + r_B) \quad \text{(Eq. A.2)}$$

used Eq. A.3, where $MW$ is the molecular weight of the protein (Da), and $V$ is its volume (nm$^3$); this empirical relationship assumes a partial molar volume of 0.73 cm$^3$/g, a reasonable estimate for a wide range of proteins. For ligands, we used the PhysChem module of the ACD/Labs property prediction service (www.chemspider.com). Estimates of $k_{on}$, thus determined, were similar between different varieties of protein-protein or protein-ligand interactions (i.e., within each class of interaction, the standard deviation of $k_{on}$ was ~ 2%), so we used average values of $6.29 \times 10^2$ μM$^{-1}$ s$^{-1}$ for all protein-protein interactions and $1.65 \times 10^3$ μM$^{-1}$ s$^{-1}$ for all protein-ligand interactions.
Note 2. Titration Experiments with FabD and FabG. We validated our kinetic model by comparing predicted trends in initial rates of fatty acid synthesis to previously reported measurements of those rates. Specifically, we examined titration data for ACP, FabH, FabZ, FabI, FabF, and TesA (Figures 2.2-2.4), six components previously observed to enhance or inhibit fatty acid synthesis. We did not examine similar data for FabD and FabG, however, because the kinetic contributions of these enzymes are controversial and, thus, represent poor observables for model validation. (For example, the results of an in vitro analysis suggest that FAS activity is insensitive to changes in the concentrations of FabD and FabG, but findings from an in vivo study show that overexpression of FabD can increase fatty acid production in E. coli.) Despite the absence of an expected set of trends, we completed our titration studies by examining the influence of FabD and FabG on FAS activity (Figure A.5). Intriguingly, initial rates were insensitive to changes in the concentrations of FabG (above very low concentrations) but showed a pronounced sensitivity to FabD—a result consistent with our sensitivity analysis (Figure 2.9) and with the influence of carbon flux (which is “gated” by FabD) on total production (Figure 2.5).

Note 3. The Influence of Measurement Time and Substrate Concentration. In many of the studies reported in the main text, we report total production and product distribution at 12.5 minutes. To determine how this choice of time point affected observed trends in FAS outputs, we repeated the analyses reported in Figures 2.6 and 2.7B at 2.5 and 25 minutes. Interestingly, total fatty acid production differed by ~fivefold between these time points, but general trends in production and chain length remained similar between them (Figures A.6, and A.8). The consistent trends exhibited at 2.5, 12.5, and 25 minutes suggest that differences in outputs of various FAS compositions—at least within the 2.5- to 25-minute span of times—result from differences in the
steady-state kinetics of fatty acid production, not from differences in production at early or late time points (i.e., discrepancies that would grow or diminish with sample time).

Substrate concentrations represent another possible source of bias in our kinetic analysis. For much of our study, we used substrate concentrations (0.5 mM for malonyl-CoA and 0.5 mM for acetyl-CoA) based on physiologically relevant FAS compositions examined by Khosla and colleagues. To determine how the choice of concentrations affected observed trends in FAS outputs, we repeated the analysis of Figure 2.6 at different concentrations of malonyl-CoA and acetyl-CoA (Figure A.7). To our satisfaction, trends in production and chain length remained similar across different substrate concentrations (within a reasonable range of concentrations).
A.1 Supporting Figures and Tables

*Michaelis-Menten Kinetics:*

\[
[\mathcal{E}] + [S] \xrightarrow{k_{+1}} [\mathcal{E}S] \xrightarrow{k_{cat}} [\mathcal{E}] + [P]
\]

\[
K_M = \frac{k_{-1} + k_{cat}}{k_{-1}}
\]

*Competitive Inhibition:*

\[
[E] + [I] \xleftrightarrow{K_I} [EI]
\]

*Mixed Inhibition:*

\[
[E] + [I] \xleftrightarrow{K_{I,2}} [E*I]
\]

*Activation:*

\[
[E] + [S] \xleftrightarrow{K_A} [ES] \xrightarrow{k_{cat}} [E] + [P]
\]

\[
[AES] \xrightarrow{\beta k_{cat}} [AE] + [P]
\]

\[
[E] + [S] \xleftrightarrow{K_A} [ES]
\]

\[
[E] + [A] \xleftrightarrow{K_X} [AE]
\]

\[
[AE] + [S] \xleftrightarrow{\alpha K_A} [AES]
\]

\[
[ES] + [A] \xleftrightarrow{\alpha K_X} [AES]
\]

Figure A.1. Kinetic models. We reference these models in the main text and supporting information.
Figure A.2. Analysis of TesA kinetics. (A) We estimated values of $k_{\text{cat}}$ and $K_M$ for TesA-catalyzed hydrolysis of acyl-CoAs by fitting a Michaelis-Menten model (solid lines) to previously reported kinetic data (filled circles; Table A.2). (B) Values of $k_{\text{cat}}$ determined from fits described in A (filled circles) or an extrapolation of those fits (open circles). We estimated values of $k_{\text{cat}}$ for C$_{18}$ and C$_{20}$ acyl-CoAs by extrapolating the linear trend exhibited by values of $k_{\text{cat}}$ for C$_{10}$-C$_{16}$ acyl-CoAs ($r^2 = 0.943$); we estimated a $k_{\text{cat}}$ for C$_4$ acyl-CoA, in turn, by averaging the $k_{\text{cat}}$’s of C$_6$-C$_{10}$ acyl-CoAs. (C) Values of $K_M$ determined from fits described in A (filled circles) or an extrapolation of those fits (open circles). Values of $K_M$ for C$_6$-C$_{16}$ acyl-CoAs fit well to a second-order polynomial ($r^2 = 0.97$), so we used this polynomial to estimate values of $K_M$ for C$_4$, C$_{18}$, and C$_{20}$ acyl-CoAs. (D) Values of ln($K_d$) determined from $K_M$’s described in C (filled circles) or an optimization of our kinetic model (open circles). We note: For C$_4$-C$_{20}$ acyl-CoAs, experimentally derived and model-based estimates of ln($K_d$) overlap with one another. (E) Initial rates of TesA-catalyzed hydrolysis of p-nitrophenyl-butyrater (pNP4) in the presence of increasing concentrations
of ACP. Lines represent fits to an activation model (Table A.3), which allowed us to estimate a $K_d$ for the ACP-TesA complex (i.e., $K_\alpha$ in Figure A.1).
Figure A.3. Analysis of FabH inhibition. We parameterized the inhibition of FabH by acyl-ACPs by using kinetic measurements supplied by a detailed study of this phenomenon.\(^8\) (A-B) To begin, we fit a mechanistic kinetic model of FabH-catalyzed condensation of acetyl-CoA and malonyl-CoA (lines 3 and 4 in Table 2.1, open circles) to initial rates of condensation (filled circles) determined at different concentrations of (A) acetyl-CoA and (B) malonyl-CoA. (C-D) We fit a model for competitive inhibition to initial rates determined in the presence of varying concentrations of \(\text{C}_{16}\)-ACP and (C) acetyl-CoA or (D) malonyl-CoA; that is, we fit only \(K_{I,1}\) and \(K_{I,2}\) as defined in Table A.1 and retained the kinetic parameters determined in A-B. (E) We estimated length-specific values of \(K_{I,1}\) and \(K_{I,2}\), in turn, by fitting them to measurements of initial rates made in the presence of acyl-ACPs of different lengths (“NA” indicates an initial rate
determined in the absence of acyl-ACPs). Table A.4 shows estimates of $k_{\text{off}}$ and $k_{\text{on}}$ based on our initial fit to this data, and Table A.7 shows the final inhibition parameters of our optimized model.
Figure A.4. Analysis of FabZ kinetics. (A-B) The ratio of β-hydroxyacyl-ACP to enoyl-acyl-ACP generated by modeled FASs (1 μM of each Fab, 10 μM TesA, 10 μM holo-ACP, 1 mM NADPH, 1 mM NADH, and 0.5 mM acetyl-CoA, 12.5 min) with varying concentrations of FabZ. In both (A) the base model and (B) the model in which we reduced the $k_{cat}$ of FabZ by tenfold, this ratio is high, relative to the ratio expected at equilibrium (~4:1). (C) FabZ enhances rates of fatty acid synthesis until (D) concentrations of enoyl-acyl-ACP cease to increase.
Figure A.5. Titration of FabD and FabG. (A-B) Initial rates of fatty acid synthesis exhibited by reconstituted FASs (1 μM of each Fab, 10 μM TesA, 10 μM holo-ACP, 1 mM NADPH, 1mM NADH, 0.5 mM malonyl-CoA, and 0.2 mM acetyl-CoA, 2.5 min) with varying concentrations of (A) FabD and (B) FabG. Rates are highly sensitive to FabD, but not FabG, a result consistent with our sensitivity analysis (Figure 2.9).
Figure A.6. The influence of measurement time on compositional effects. (A-B) Ternary diagrams show total production (left) and average length (right) of fatty acids generated by modeled FASs (1 μM of each Fab, 10 μM holo-ACP, 1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, 0.5 mM acetyl-CoA) in which ratios of FabH, FabF, and TesA vary (i.e., [FabH]+[FabF]+[TesA] = 12 μM). (A) 2.5 minutes and (B) 25 minutes. Compositions with low concentrations of FabF show disproportionately low fatty acid production at 2.5 minutes (i.e., in the diagram on the upper left, the lower right is blue); general trends across the diagrams, however, remain similar between measurement times and, thus, appear to reflect differences in the steady-state kinetics of fatty acid production between compositions.
Figure A.7. The influence of substrate concentration on compositional effects. (A-B) Ternary diagrams show the total production (left) and average length (right) of fatty acids generated by modeled FASs (1 μM of each Fab, 10 μM holo-ACP, 1 mM NADPH, and 1 mM NADH) in which ratios of FabH, FabF, and TesA vary (i.e., [FabH]+[FabF]+[TesA] = 12 μM) at 12.5 minutes. Patterns are similar for (A) 500μM of malonyl CoA and 500μM acetyl-CoA and (B) 2500μM malonyl CoA and 2500μM acetyl-CoA; they, thus, appear to be independent of substrate concentration (within a reasonable range of concentrations).
Figure A.8. The influence measurement time of the observed contributions of FabF. (A-C) Total fatty acid production generated by modeled FASs (1 µM of each Fab, 10 µM TesA, 10 µM holo-ACP, 1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, 0.5 mM acetyl-CoA) with varying concentrations of FabF at (A) 2.5, (B) 12.5, and (C) 25 minutes. The consistency of trends across time points suggests that these trends result from differences in the steady-state kinetics of fatty acid production, not from differences in production at early or late time points (i.e., discrepancies that should change with sample time).
Figure A.9. Analysis of plant-derived thioesterases. (A) Relative titers (i.e., palmitic acid equivalents) of fatty acids generated by strains of *E. coli* containing different thioesterases,\textsuperscript{10,11} we normalized each titer by that of the TesA-containing strain. (B-D) Approximate product distributions generated by strains containing (B) BfTES, (C) CpFatB1, and (D) UcFatB; we normalized each plot by total production.\textsuperscript{11} We note: The reported product profiles of all strains\textsuperscript{11} showed low concentrations of off-target fatty acids (e.g., C\textsubscript{10}, C\textsubscript{12}, C\textsubscript{14}, C\textsubscript{16}, and C\textsubscript{18} for BfTES);
both the low concentrations of these products and their consistency across different thioesterase-containing strains, however, suggest that they arise from nonspecific background activities. Accordingly, panels B-D show only products close in length to the thioesterase-specific products (i.e., those most likely to be “true” off-target products of the thioesterases under study). For A-D, we prepared the model profiles by optimizing thioesterase-specific compositions to A (see main text); Table A.8 shows the final optimized kinetic parameters for plant-derived thioesterases.
Figure A.10. Analysis of the convergence of elementary effects (EE). (A-F) We ensured convergence of elementary effects by examining estimates determined from different numbers of model evaluations. In brief, (i) we determined the total number of evaluations (N) necessary to carry out sensitivity analyses of the kinetic model (A, C, and E) and the expanded version of that model (B, D, and F) by multiplying the number of trajectories (r, the number of initial points used to calculate elementary effect) by the number of model variables (M) plus one (i.e., N = r*[M+1] = 100*[12+1] or 100*[19+1]). (ii) We estimated the mean elementary effect for each trajectory (i.e., collection of 100 points). (iii) We averaged the mean elementary effects estimated from different subsets of trajectories chosen at random among all subsets (e.g., 20, 40, 60, 80, 100 trajectories for each model). The figures show the results of our sensitivity analyses of three objectives: (A-B) average length, (C-D) total production, and (E-F) a model objective sensitive to
both length and total production (i.e., $\text{Obj}_A$, the product of the sums of squared errors between predicted and measured trends in Figures 2A and 2B; see Materials and Methods).
Table A.1. Mechanisms of Inhibition.

\[
\text{FabH} + \text{ACP} \underset{k_{\text{off-FabH-Inh-1}}}{\overset{k_{\text{on-FabH-Inh-1}}}{\rightleftharpoons}} \text{FabH} \cdot \text{ACP} \tag{S1}
\]

\[
\text{FabH} + \text{ACP} \underset{k_{\text{off-FabH-Inh-2}}}{\overset{k_{\text{on-FabH-Inh-2}}}{\rightleftharpoons}} \text{FabH} \cdot \text{ACP} \tag{S2}
\]

\[
\text{FabH}^* + \text{ACP} \underset{k_{\text{off-FabH-Inh-3}}}{\overset{k_{\text{on-FabH-Inh-3}}}{\rightleftharpoons}} \text{FabH}^* \cdot \text{ACP} \tag{S3}
\]

\[
\text{FabG} + \text{ACP} \underset{k_{\text{off-FabG-Inh}}}{\overset{k_{\text{on-FabG-Inh}}}{\rightleftharpoons}} \text{FabG} \cdot \text{ACP} \tag{S4}
\]

\[
\text{FabZ} + \text{ACP} \underset{k_{\text{off-FabZ-Inh}}}{\overset{k_{\text{on-FabZ-Inh}}}{\rightleftharpoons}} \text{FabZ} \cdot \text{ACP} \tag{S5}
\]

\[
\text{FabI} + \text{ACP} \underset{k_{\text{off-FabI-Inh}}}{\overset{k_{\text{on-FabI-Inh}}}{\rightleftharpoons}} \text{FabI} \cdot \text{ACP} \tag{S6}
\]

\[
\text{FabF} + \text{ACP} \underset{k_{\text{off-FabF-Inh}}}{\overset{k_{\text{on-FabF-Inh}}}{\rightleftharpoons}} \text{FabF} \cdot \text{ACP} \tag{S7}
\]

\[
\text{TesA} + \text{ACP} \underset{k_{\text{off-TesA-Inh}}}{\overset{k_{\text{on-TesA-Inh}}}{\rightleftharpoons}} \text{TesA} \cdot \text{ACP} \tag{S8}
\]

*ACP refers to holo-ACP.
**FabD*, *FabH*, and *FabF* represent refer to acyl-enzyme intermediates.
***In our analysis of FabH inhibition (Fig. A.3), we defined values of $K_{I,1}$ and $K_{I,2}$ (Fig. A.1) as follows: $K_{I,1} = \frac{k_{\text{off-FabH-Inh-2}}}{k_{\text{on-FabH-Inh-2}}}$ and $K_{I,2} = \frac{k_{\text{off-FabH-Inh-3}}}{k_{\text{on-FabH-Inh-3}}}$.
### Table A.2. Kinetic Parameters for TesA-Catalyzed Hydrolysis of Acyl-CoAs.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>$k_{cat}$ (1/s)</th>
<th>$K_M$ (μM)</th>
<th>$K_a$ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TesA</td>
<td>C$_6$-CoA</td>
<td>5.50</td>
<td>1090</td>
<td>294</td>
</tr>
<tr>
<td>TesA</td>
<td>C$_8$-CoA</td>
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<tr>
<td>TesA</td>
<td>C$_{10}$-CoA</td>
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<td>27.3</td>
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<td>C$_{12}$-CoA</td>
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<tr>
<td>TesA</td>
<td>C$_{14}$-CoA</td>
<td>49.5</td>
<td>265</td>
<td>4.0</td>
</tr>
<tr>
<td>TesA</td>
<td>C$_{16}$-CoA</td>
<td>108</td>
<td>642</td>
<td>2.25</td>
</tr>
</tbody>
</table>

*C$_i$-CoA refers to acyl-CoA with $i$ carbons in its acyl chain.

*Parameters determined from the fit described in Fig. A.2A.

### Table A.3. Kinetic Parameters for ACP-Mediated Inhibition of FabH.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
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</thead>
<tbody>
<tr>
<td>$K_A$</td>
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</tr>
<tr>
<td>$K_X$</td>
<td>8.96</td>
<td>μM</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>0.862</td>
<td>unitless</td>
</tr>
<tr>
<td>$\beta$</td>
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<td>unitless</td>
</tr>
<tr>
<td>$k_{cat}$</td>
<td>3.97</td>
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</tbody>
</table>

*Parameters determined from the fit described in Figure A.2E.
### Table A.4. Estimates of Kinetic Parameters.

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<th>Enzyme</th>
<th>Parameter</th>
<th>Model Label</th>
<th>Value</th>
<th>Units</th>
<th>Source</th>
</tr>
</thead>
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<tr>
<td>FabD</td>
<td>KM-mCoA</td>
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<td>6.0 E1</td>
<td>µM</td>
<td>Estimate from K_m*</td>
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<tr>
<td>FabD</td>
<td>k_on-mCoA</td>
<td>k2_1f</td>
<td>1.33 E-3</td>
<td>µM⁻¹ s⁻¹</td>
<td>Estimate from FabD</td>
</tr>
<tr>
<td>FabD</td>
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<td>k2_1r</td>
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<td>s⁻¹</td>
<td></td>
</tr>
<tr>
<td>FabD</td>
<td>kcatFabD*</td>
<td>k2_2f</td>
<td>1.58 E3</td>
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</tr>
<tr>
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<tr>
<td>FabD</td>
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<td>Estimate from K_m*</td>
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<tr>
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<td>s⁻¹</td>
<td></td>
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<tr>
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<td>kcatFabH*</td>
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<td>1.58 E3</td>
<td>s⁻¹</td>
<td>Used k_catFabD* from FabD</td>
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<tr>
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<td>k3_2r</td>
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<td>µM⁻¹ s⁻¹</td>
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</tr>
<tr>
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<td>µM</td>
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<tr>
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<td>µM⁻¹ s⁻¹</td>
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<td>FabH</td>
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<tr>
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<td>2.99E2</td>
<td>s⁻¹</td>
<td>8, this study</td>
</tr>
<tr>
<td>FabH</td>
<td>k_off-C18ACP</td>
<td>koff-1-2, C18</td>
<td>7.77E1</td>
<td>s⁻¹</td>
<td>8, this study</td>
</tr>
<tr>
<td>FabH</td>
<td>k_off-C20ACP</td>
<td>koff-1-2, C20</td>
<td>3.98E1</td>
<td>s⁻¹</td>
<td>8, this study</td>
</tr>
<tr>
<td>FabH</td>
<td>k_off-C(4-20)ACP-FabH*</td>
<td>koff-1-3, C(4-20)</td>
<td>1.55E0</td>
<td>µM⁻¹ s⁻¹</td>
<td>8, this study</td>
</tr>
<tr>
<td>FabH</td>
<td>k_off-C(4-12)ACP-FabH*</td>
<td>koff-1-3, C(4-12)</td>
<td>3.67E1</td>
<td>s⁻¹</td>
<td>8, this study</td>
</tr>
<tr>
<td>FabH</td>
<td>k_off-C14ACP-FabH*</td>
<td>koff-1-3, C14</td>
<td>4.67E1</td>
<td>s⁻¹</td>
<td>8, this study</td>
</tr>
<tr>
<td>FabH</td>
<td>k_off-C16ACP-FabH*</td>
<td>koff-1-3, C16</td>
<td>1.17E1</td>
<td>s⁻¹</td>
<td>8, this study</td>
</tr>
<tr>
<td>FabH</td>
<td>k_off-C18ACP-FabH*</td>
<td>koff-1-3, C18</td>
<td>1.32E1</td>
<td>s⁻¹</td>
<td>8, this study</td>
</tr>
<tr>
<td>FabH</td>
<td>k_off-C20ACP-FabH*</td>
<td>koff-1-3, C20</td>
<td>3.89E0</td>
<td>s⁻¹</td>
<td>8, this study</td>
</tr>
<tr>
<td>FabH</td>
<td>k_cat</td>
<td>kcat3</td>
<td>3.13 E0</td>
<td>s⁻¹</td>
<td></td>
</tr>
<tr>
<td>FabG</td>
<td>KM-NADPH</td>
<td>N/A</td>
<td>1.0 E-2</td>
<td>mM</td>
<td></td>
</tr>
</tbody>
</table>
Table A.4 (cont.). Estimates of Kinetic Parameters.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Parameter</th>
<th>Model Label</th>
<th>Value</th>
<th>Units</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>FabG</td>
<td>k_{on-NADPH}</td>
<td>k4_1f</td>
<td>1.54 E-3</td>
<td>μM^{-1} s^{-1}</td>
<td>Used k_{on-NADPH} from FabI</td>
</tr>
<tr>
<td>FabG</td>
<td>k_{off-NADPH}</td>
<td>k4_1r</td>
<td>7.93 E-2</td>
<td>s^{-1}</td>
<td>Used k_{off-NADPH} from FabI</td>
</tr>
<tr>
<td>FabG</td>
<td>KM-fkaACP</td>
<td>N/A</td>
<td>1.70 E-2</td>
<td>mM</td>
<td>19</td>
</tr>
<tr>
<td>FabG</td>
<td>k_{on-fkaACP}</td>
<td>k4_2f</td>
<td>1.28 E-3</td>
<td>μM^{-1} s^{-1}</td>
<td>Estimate from K_{m}*</td>
</tr>
<tr>
<td>FabG</td>
<td>k_{off-fkaACP}</td>
<td>k4_2r</td>
<td>2.17 E-2</td>
<td>s^{-1}</td>
<td>Used k_{off-ACP} from FabD</td>
</tr>
<tr>
<td>FabG</td>
<td>k_{cat}</td>
<td>kcat4</td>
<td>5.90 E-1</td>
<td>s^{-1}</td>
<td>18</td>
</tr>
</tbody>
</table>

**For these estimates, we used a measured value of k_{off} for the complex between C_{4} acyl-CoA and FabI.**

***To clarify, we used k_{cat} as an order-of-magnitude estimate of the forward acyl-transfer constant.***

****For this estimate, we assumed a rate constant approximately tenfold higher than k_{on-mCoA}.
For this estimate, we assumed a rate constant approximately tenfold higher than $k_{\text{on}}$.

To clarify, we used $k_{\text{on}}$.

For these estimates, we used a measured value of $k_{\text{off}}$.

*For these estimates, we used Eqs. 1 and 2 from the main text.

**For these estimates, we used a measured value of $k_{\text{off}}$ for the complex between C$_4$ acyl-CoA and FabI.

***To clarify, we used $k_{\text{cat}}$ as an order-of-magnitude estimate of the forward acyl-transfer constant.

****For this estimate, we assumed a rate constant approximately tenfold higher than $k_{\text{off}, \text{mCoA}}$.

Table A.5. Ranges of Kinetic Parameters.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Source (low)</th>
<th>Source (high)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FabD</td>
<td>$K_m$-$\text{mCoA}$</td>
<td>6.0E1 – 2.50E2</td>
<td>μM</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>FabD</td>
<td>$k_{\text{on}}$-$\text{mCoA}$</td>
<td>1.28E-3 – 1.85E1</td>
<td>μM$^{-1}$ s$^{-1}$</td>
<td>13</td>
<td>26, this study*</td>
</tr>
<tr>
<td>FabD</td>
<td>$k_{\text{off}}$-$\text{mCoA}$</td>
<td>8.0E-2 – 1.11E3</td>
<td>s$^{-1}$</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>FabD</td>
<td>$k_{\text{rFabD}}$</td>
<td>9.34E-4 – 5.20E3</td>
<td>s$^{-1}$</td>
<td>14,26,27</td>
<td>12,26, this study*</td>
</tr>
<tr>
<td>FabD</td>
<td>$k_{\text{rFabD}}$</td>
<td>1.28E-3 – 1.85E1</td>
<td>μM$^{-1}$ s$^{-1}$</td>
<td>13</td>
<td>26, this study*</td>
</tr>
<tr>
<td>FabD</td>
<td>$K_m$-$\text{ACP}$</td>
<td>4.0E1 – 3.51E2</td>
<td>μM</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>FabD</td>
<td>$k_{\text{on}}$-$\text{ACP}$</td>
<td>5.02E-2 – 3.70E0</td>
<td>μM$^{-1}$ s$^{-1}$</td>
<td>15</td>
<td>1, this study*</td>
</tr>
<tr>
<td>FabD</td>
<td>$k_{\text{off}}$-$\text{ACP}$</td>
<td>2.17E-2 – 1.30E3</td>
<td>s$^{-1}$</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>FabD</td>
<td>$k_{\text{rFabD}}$-$\text{ACP}$</td>
<td>9.34E-4 – 5.20E3</td>
<td>s$^{-1}$</td>
<td>14,26,27</td>
<td>12,26, this study*</td>
</tr>
<tr>
<td>FabD</td>
<td>$k_{\text{rFabD}}$-$\text{ACP}$</td>
<td>1.28E-3 – 3.70E0</td>
<td>μM$^{-1}$ s$^{-1}$</td>
<td>13</td>
<td>1, this study*</td>
</tr>
<tr>
<td>FabD</td>
<td>$k_{\text{cat}}$</td>
<td>4.67E-4 – 2.60E3</td>
<td>s$^{-1}$</td>
<td>14,27</td>
<td>12</td>
</tr>
<tr>
<td>FabH</td>
<td>$K_m$-$\text{aCoA}$</td>
<td>4.0E1 – 6.0E1</td>
<td>μM</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>FabH</td>
<td>$k_{\text{on}}$-$\text{aCoA}$</td>
<td>1.28E-3 – 1.87E1</td>
<td>μM$^{-1}$ s$^{-1}$</td>
<td>13</td>
<td>26, this study*</td>
</tr>
<tr>
<td>FabH</td>
<td>$k_{\text{off}}$-$\text{aCoA}$</td>
<td>8.0E-2 – 1.12E3</td>
<td>s$^{-1}$</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>FabH</td>
<td>$k_{\text{rFabH}}$</td>
<td>9.34E-4 – 5.20E3</td>
<td>s$^{-1}$</td>
<td>14,26,27</td>
<td>12,26, this study*</td>
</tr>
<tr>
<td>FabH</td>
<td>$k_{\text{rFabH}}$</td>
<td>1.28E-3 – 3.70E0</td>
<td>μM$^{-1}$ s$^{-1}$</td>
<td>13</td>
<td>1, this study*</td>
</tr>
<tr>
<td>FabH</td>
<td>$K_m$-$\text{mACP}$</td>
<td>5.0E0 – 2.0E1</td>
<td>μM</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>FabH</td>
<td>$k_{\text{on}}$-$\text{mACP}$</td>
<td>5.02E-2 – 3.70E0</td>
<td>μM$^{-1}$ s$^{-1}$</td>
<td>15</td>
<td>1, this study*</td>
</tr>
<tr>
<td>FabH</td>
<td>$k_{\text{off}}$-$\text{mACP}$</td>
<td>2.17E-2 – 7.40E1</td>
<td>s$^{-1}$</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>
We estimated these values by using the diffusion calculations described in Note 1.

Table A.5 (cont.). Ranges of Kinetic Parameters.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Source (low)</th>
<th>Source (high)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FabH</td>
<td>$k_{cat}$</td>
<td>2.7E-1 – 4.71E1</td>
<td>s$^{-1}$</td>
<td>16</td>
<td>12,17</td>
</tr>
<tr>
<td>FabG</td>
<td>$K_M$-NADPH</td>
<td>1.0E1</td>
<td>μM</td>
<td>18</td>
<td>N/A</td>
</tr>
<tr>
<td>FabG</td>
<td>$k_{on}$-NADPH</td>
<td>1.54E-3 – 2.16E1</td>
<td>μM$^{-1}$s$^{-1}$</td>
<td>13</td>
<td>26, this study*</td>
</tr>
<tr>
<td>FabG</td>
<td>$k_{off}$-NADPH</td>
<td>7.90E-2 – 2.16E2</td>
<td>s$^{-1}$</td>
<td>13</td>
<td>Calculated from maximum $K_m$ and $k_{on}$</td>
</tr>
<tr>
<td>FabG</td>
<td>$k_{cat}$</td>
<td>1.40E-2 – 2.65E2</td>
<td>s$^{-1}$</td>
<td>18</td>
<td>28</td>
</tr>
<tr>
<td>FabZ</td>
<td>$k_{on}$-βhaACP</td>
<td>5.02E-2 – 3.70E0</td>
<td>μM$^{-1}$s$^{-1}$</td>
<td>15</td>
<td>1, this study*</td>
</tr>
<tr>
<td>FabZ</td>
<td>$k_{off}$-βhaACP</td>
<td>2.17E-2 – 2.07E2</td>
<td>s$^{-1}$</td>
<td>15</td>
<td>Calculated from maximum $K_m$ and $k_{on}$</td>
</tr>
<tr>
<td>FabZ</td>
<td>$k_{cat}$</td>
<td>7.80E-2 – 7.20E1</td>
<td>s$^{-1}$</td>
<td>20</td>
<td>9.29</td>
</tr>
</tbody>
</table>

*We estimated these values by using the diffusion calculations described in Note 1.
Table A.6. Optimized Scaling Parameters for the Kinetic Model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description*</th>
<th>Estimated Range**</th>
<th>Optimized Value***</th>
</tr>
</thead>
<tbody>
<tr>
<td>a₁</td>
<td>Scales $k_{\text{off}}$ for 1-4</td>
<td>1E0 to 1.4E4</td>
<td>4.498 E3</td>
</tr>
<tr>
<td>a₂</td>
<td>Scales $k_{\text{off}}$ for acyl-ACPs for 5-11</td>
<td>1E0 to 1.28E4</td>
<td>1.586 E1</td>
</tr>
<tr>
<td>a₃</td>
<td>Scales $k_{\text{off}}$ for 12</td>
<td>1E0 to 1.86E5</td>
<td>4.319 E3</td>
</tr>
<tr>
<td>b₁</td>
<td>Scales $k_r$ for 1-3 and 10</td>
<td>0.128 to 3.7E2</td>
<td>2.474 E1</td>
</tr>
<tr>
<td>b₂</td>
<td>Scales $K_{\text{eq}}$ for acyl transfer in 1</td>
<td>2.46E-7 to 1.98E3</td>
<td>5.277 E-2</td>
</tr>
<tr>
<td>b₃</td>
<td>Scales $K_{\text{eq}}$ for acyl transfer in 2, 3, 10</td>
<td>2.46E-7 to 3.96E2</td>
<td>8.541 E-2</td>
</tr>
<tr>
<td>c₁</td>
<td>Scales $k_{\text{cat}}$ for 4</td>
<td>1E-3 to 1.5E1</td>
<td>1.651 E0</td>
</tr>
<tr>
<td>c₂</td>
<td>Scales $k_{\text{cat}}$ for 6, 7, 9, 11</td>
<td>1E-2 to 2.4E2</td>
<td>8.794 E1</td>
</tr>
<tr>
<td>c₃</td>
<td>Scales $k_{\text{cat}}$ for 12</td>
<td>7.45E-5 to 1E0</td>
<td>1.902 E-2</td>
</tr>
<tr>
<td>d₁</td>
<td>Substrate specificity of TesA; see Eq. 3 in main text.</td>
<td>-5E-1 to 5E-1</td>
<td>-2.897 E-1</td>
</tr>
<tr>
<td>d₂</td>
<td>Substrate specificity of TesA; see Eq. 3 of main text.</td>
<td>0 to 1E1</td>
<td>5.443 E0</td>
</tr>
<tr>
<td>e</td>
<td>Scales inhibition of (i) FabH/ F by holo-ACPS and (ii) FabH by acyl-ACPs.</td>
<td>1E0 to 9E2</td>
<td>2.872 E1</td>
</tr>
</tbody>
</table>

*The numbers in these descriptions correspond to lines (i.e., reactions) of Table 2.1.
**We determined these ranges from the ranges of associated scaled parameters described in Table A.5.
***We determined these parameters by optimizing our kinetic model.
### Table A.7. Optimized Binding Parameters for ACPs and Acyl-ACPs.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Kd (μM)</th>
<th>kon (μM⁻¹ s⁻¹)</th>
<th>koff (s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FabH</td>
<td>ACP</td>
<td>3.14E1</td>
<td>6.92E-4</td>
<td>2.17E-2</td>
</tr>
<tr>
<td>FabH</td>
<td>C₄/ C₆/ C₈/C₁₀-ACP</td>
<td>1.51E2</td>
<td>3.09E-1</td>
<td>4.66E1</td>
</tr>
<tr>
<td>FabH</td>
<td>C₁₂-ACP</td>
<td>1.51E2</td>
<td>3.09E-1</td>
<td>4.66E1</td>
</tr>
<tr>
<td>FabH</td>
<td>C₁₄-ACP</td>
<td>2.87E1</td>
<td>3.09E-1</td>
<td>8.87E0</td>
</tr>
<tr>
<td>FabH</td>
<td>C₁₆-ACP</td>
<td>3.37E1</td>
<td>3.09E-1</td>
<td>1.04E1</td>
</tr>
<tr>
<td>FabH</td>
<td>C₁₈-ACP</td>
<td>8.76E0</td>
<td>3.09E-1</td>
<td>2.70E0</td>
</tr>
<tr>
<td>FabH</td>
<td>C₂₀-ACP</td>
<td>4.48E0</td>
<td>3.09E-1</td>
<td>1.38E0</td>
</tr>
<tr>
<td>FabH*</td>
<td>C₄/ C₆/ C₈/C₁₀-ACP</td>
<td>8.24E-1</td>
<td>1.55E0</td>
<td>1.28E0</td>
</tr>
<tr>
<td>FabH*</td>
<td>C₁₂-ACP</td>
<td>8.24E-1</td>
<td>1.55E0</td>
<td>1.28E0</td>
</tr>
<tr>
<td>FabH*</td>
<td>C₁₄-ACP</td>
<td>1.05E0</td>
<td>1.55E0</td>
<td>1.63E0</td>
</tr>
<tr>
<td>FabH*</td>
<td>C₁₆-ACP</td>
<td>2.63E-1</td>
<td>1.55E0</td>
<td>4.08E-1</td>
</tr>
<tr>
<td>FabH*</td>
<td>C₁₈-ACP</td>
<td>2.95E-1</td>
<td>1.55E0</td>
<td>4.58E-1</td>
</tr>
<tr>
<td>FabH*</td>
<td>C₂₀-ACP</td>
<td>8.74E-1</td>
<td>1.55E0</td>
<td>1.36E-1</td>
</tr>
<tr>
<td>TesA</td>
<td>holo-ACP</td>
<td>9.00E0</td>
<td>2.41E-3</td>
<td>2.17E-2</td>
</tr>
<tr>
<td>FabG</td>
<td>holo-ACP</td>
<td>9.00E0</td>
<td>2.41E-3</td>
<td>2.17E-2</td>
</tr>
<tr>
<td>FabZ</td>
<td>holo-ACP</td>
<td>9.00E0</td>
<td>2.41E-3</td>
<td>2.17E-2</td>
</tr>
<tr>
<td>FabI</td>
<td>holo-ACP</td>
<td>9.00E0</td>
<td>2.41E-3</td>
<td>2.17E-2</td>
</tr>
<tr>
<td>FabF</td>
<td>holo-ACP</td>
<td>3.14E-1</td>
<td>6.92E-2</td>
<td>2.17E-2</td>
</tr>
</tbody>
</table>

*ACP refers to holo-ACP; Cᵢ-ACP refers to an acyl-ACP with i carbons in its acyl chain.

**We determined these parameters by optimizing our kinetic model.

### Table A.8. Optimized Kinetic Parameters for Various Thioesterases

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>kcat (1/s)</th>
<th>Kd (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BfTES</td>
<td>C₄-ACP</td>
<td>2.35</td>
<td>1.45</td>
</tr>
<tr>
<td>BfTES</td>
<td>C₆-ACP</td>
<td>1.70</td>
<td>2.62</td>
</tr>
<tr>
<td>BfTES</td>
<td>C₈-ACP</td>
<td>1.05</td>
<td>4.73</td>
</tr>
<tr>
<td>BfTES</td>
<td>C₁₀-ACP</td>
<td>0.40</td>
<td>8.53</td>
</tr>
<tr>
<td>CpFatB1</td>
<td>C₆-ACP</td>
<td>0.105</td>
<td>274</td>
</tr>
<tr>
<td>CpFatB1</td>
<td>C₈-ACP</td>
<td>2.77</td>
<td>0.989</td>
</tr>
<tr>
<td>CpFatB1</td>
<td>C₁₀-ACP</td>
<td>0.033</td>
<td>13.8</td>
</tr>
<tr>
<td>UcFatB</td>
<td>C₁₀-ACP</td>
<td>0.105</td>
<td>274</td>
</tr>
<tr>
<td>UcFatB</td>
<td>C₁₂-ACP</td>
<td>1.37</td>
<td>3.52</td>
</tr>
<tr>
<td>UcFatB</td>
<td>C₁₄-ACP</td>
<td>0.033</td>
<td>13.8</td>
</tr>
</tbody>
</table>

*Cᵢ-ACP refers to an acyl-ACP with i carbons in its acyl chain.

**We determined these parameters by optimizing our kinetic model.
Table A.9. Analysis of the Sensitivity of Average Length to Scaling Parameters.

<table>
<thead>
<tr>
<th>Objective</th>
<th>Parameter</th>
<th>Min</th>
<th>Max</th>
<th>EE Mean</th>
<th>EE SD</th>
<th>Mean/SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avg. Length</td>
<td>d₂</td>
<td>0</td>
<td>10</td>
<td>11.34</td>
<td>3.40</td>
<td>0.30</td>
</tr>
<tr>
<td>Avg. Length</td>
<td>d₁</td>
<td>-0.5</td>
<td>0</td>
<td>7.08</td>
<td>2.64</td>
<td>0.37</td>
</tr>
<tr>
<td>Avg. Length</td>
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*This table shows the mean elementary effects used to generate Figure 2.9A.

**We used Objₐ, the product of the sums of squared errors of predicted and experimental results in Figures 2.2A and 2.2B (see Materials and Methods).
A.2 Model Equations

\[
[FabD] = [FabD]_{\text{tot}} - [FabD \cdot \text{Malonyl-CoA}] - [FabD^*] - [FabD^* \cdot \text{ACP}] \quad \text{Eq. A.4}
\]

\[
[FabH] = [FabH]_{\text{tot}} - [FabH \cdot \text{Acetyl-CoA}] - [FabH^*] - [FabH^* \cdot \text{Malonyl-ACP}] \\
- \sum_{n=2}^{10} [FabH \cdot C_{2n}\text{Acyl-ACP}] - \sum_{n=2}^{10} [FabH^* \cdot C_{2n}\text{Acyl-ACP}] - [FabH \cdot \text{ACP}] \quad \text{Eq. A.5}
\]

\[
[FabG] = [FabG]_{\text{tot}} - [FabG \cdot \text{NADPH}] - \sum_{n=2}^{10} [FabG \cdot \text{NADPH} \cdot C_{2n}\beta\text{-ketoacyl-ACP}] \\
- [FabG \cdot \text{ACP}] \quad \text{Eq. A.6}
\]

\[
[FabZ] = [FabZ]_{\text{tot}} - \sum_{n=2}^{10} [FabZ \cdot C_{2n}\beta\text{-hydroxyacyl-ACP}] - [FabZ \cdot \text{ACP}] \quad \text{Eq. A.7}
\]

\[
[FabI] = [FabI]_{\text{tot}} - [FabI \cdot \text{NADH}] - \sum_{n=2}^{10} [FabI \cdot \text{NADH} \cdot C_{2n}\text{Enoylacyl-ACP}] - [FabI \cdot \text{ACP}] \quad \text{Eq. A.8}
\]

\[
[TesA] = [TesA]_{\text{tot}} - \sum_{n=2}^{10} [TesA \cdot C_{2n}\text{acyl-ACP}] - [TesA \cdot \text{ACP}] \quad \text{Eq. A.9}
\]

\[
[FabF] = [FabF]_{\text{tot}} - \sum_{n=2}^{10} [FabF \cdot C_{2n}\text{acyl-ACP}] - \sum_{n=2}^{10} [C_{2n}\text{FabF}^*] \\
- \sum_{n=2}^{10} [C_{2n}\text{FabF}^* \cdot \text{Malonyl-ACP}] - [FabF \cdot \text{ACP}] \quad \text{Eq. A.10}
\]

\[
\frac{d[\text{Acetyl-CoA}]}{dt} = k_{\text{off-acyl-CoA}}[FabH \cdot \text{Acetyl-CoA}] - k_{\text{on-acyl-CoA}}[FabH][\text{Acetyl-CoA}] \quad \text{Eq. A.11}
\]

\[
\frac{d[\text{ACP}]}{dt} = k_{\text{off-ACP}}[FabD^* \cdot \text{ACP}] - k_{\text{on-ACP}}[FabD^*][\text{ACP}] \\
+ \sum_{n=2}^{10} k_{\text{catTesA}}, C_{2n}[TesA \cdot C_{2n}\text{Acyl-ACP}] + \sum_{n=2}^{10} k_{f,FabF}[FabF][C_{2n}\text{Acyl-ACP}] \\
- \sum_{n=2}^{10} k_{r,FabF}[C_{2n}\text{FabF}^*][\text{ACP}] + k_{\text{off-FabH-Inh-1}}[FabH \cdot \text{ACP}] \\
- k_{\text{on-FabH-Inh-1}}[FabH][\text{ACP}] + k_{\text{off-FabG-Inh}}[FabG \cdot \text{ACP}] \\
- k_{\text{on-FabG-Inh}}[FabG][\text{ACP}] + k_{\text{off-FabZ-Inh}}[FabZ \cdot \text{ACP}] - k_{\text{on-FabZ-Inh}}[FabZ][\text{ACP}] \\
+ k_{\text{off-FabZ-Inh}}[FabZ \cdot \text{ACP}] - k_{\text{on-TesA-Inh}}[TesA][\text{ACP}] + k_{\text{off-TesA-Inh}}[TesA][\text{ACP}] \\
- k_{\text{on-TesA-Inh}}[TesA][\text{ACP}] + k_{\text{off-FabF-Inh}}[FabF \cdot \text{ACP}] - k_{\text{on-FabF-Inh}}[FabF][\text{ACP}] \quad \text{Eq. A.12}
\]

\[
\frac{d[\text{NADPH}]}{dt} = k_{\text{off-NADPH}}[FabG \cdot \text{NADPH}] - k_{\text{on-NADPH}}[FabG][\text{NADPH}] \quad \text{Eq. A.13}
\]
\[
\frac{d\text{[NADH]}}{dt} = k_{\text{off,NADH}} \cdot \text{[FabI} \cdot \text{NADH]} - k_{\text{on,NADH}} \cdot \text{[FabI]} \cdot \text{[NADH]}
\]

Eq. A.14

\[
\frac{d\text{[Malonyl-CoA]}}{dt} = k_{\text{off-mCoA}} \cdot \text{[FabD} \cdot \text{Malonyl-CoA]} - k_{\text{on-mCoA}} \cdot \text{[FabD]} \cdot \text{[Malonyl-CoA]}
\]

Eq. A.15

\[
\frac{d\text{[CoA]}}{dt} = k_{\text{f,FabD}} \cdot \text{[FabD} \cdot \text{Malonyl-CoA]} - k_{\text{r,FabD}} \cdot \text{[FabD}*[CoA]}
\]

\[
- k_{\text{f,FabH}} \cdot \text{[FabH]} \cdot \text{[Acetyl-CoA]} - k_{\text{r,FabH}} \cdot \text{[FabD]} \cdot \text{[Acetyl-CoA]}
\]

Eq. A.16

\[
\frac{d\text{[Malonyl-ACP]}}{dt} = k_{\text{f,FabD}*ACP} \cdot \text{[FabD}* \cdot \text{Malonyl-ACP]} - k_{\text{r,FabD}*} \cdot \text{[FabD]} \cdot \text{[ACP]}
\]

\[
- \sum_{n=2}^{9} k_{\text{off-F}_{mACP}} \cdot \text{[C}_{2n} \text{FabF}* \cdot \text{Malonyl-ACP]}
\]

\[
+ \sum_{n=2}^{9} k_{\text{on-F}_{mACP}} \cdot \text{[C}_{2n} \text{FabF}*][\text{Malonyl-ACP]}
\]

Eq. A.17

\[
\frac{d\text{[CO}_2\text{]}}{dt} = k_{\text{catFabH}} \cdot \text{[FabH}* \cdot \text{Malonyl-ACP]} + \sum_{n=2}^{10} k_{\text{catFabF}} \cdot \text{[C}_{2n} \text{FabF}* \cdot \text{Malonyl-ACP]}
\]

Eq. A.18

\[
\text{d[C}_{4\beta}\text{-ketoacyl-ACP]} \text{dt} = k_{\text{catFabH}} \cdot \text{[FabH}* \cdot \text{Malonyl-ACP]}
\]

\[
+ k_{\text{offβkaACP}} \cdot \text{[FabG} \cdot \text{NADPH} \cdot \text{C}_{4\beta}\text{-ketoacyl-ACP]}
\]

\[
- k_{\text{onβkaACP}} \cdot \text{[FabG} \cdot \text{NADPH} \cdot [\text{C}_{4\beta}\text{-ketoacyl-ACP]}
\]

Eq. A.19

\[
\sum_{n=3}^{10} \frac{d\text{[C}_{2n\beta}\text{-ketoacyl-ACP]} \text{dt}}{} = \sum_{n=3}^{10} k_{\text{offβkaACP}} \cdot \text{[FabG} \cdot \text{NADPH} \cdot \text{C}_{2n\beta}\text{-ketoacyl-ACP]}
\]

\[
- \sum_{n=3}^{10} k_{\text{onβkaACP}} \cdot \text{[FabG} \cdot \text{NADPH} \cdot \text{[C}_{2n\beta}\text{-ketoacyl-ACP]}
\]

\[
+ \sum_{n=3}^{10} k_{\text{catFabF}} \cdot \text{[C}_{2n} \text{FabF}* \cdot \text{Malonyl-ACP]}
\]

Eq. A.20

\[
\sum_{n=2}^{10} \frac{d\text{[C}_{2n\beta}\text{-hydroxyacyl-ACP]} \text{dt}}{} = \sum_{n=2}^{10} k_{\text{offβhaACP}} \cdot \text{[FabZ} \cdot \text{C}_{2n\beta}\text{-hydroxyacyl-ACP]}
\]

\[
- \sum_{n=2}^{10} k_{\text{onβhaACP}} \cdot \text{[FabZ]} \cdot \text{[C}_{2n\beta}\text{-hydroxyacyl-ACP]}
\]

\[
+ \sum_{n=2}^{10} k_{\text{catFabG}} \cdot \text{[FabG} \cdot \text{NADPH} \cdot \text{C}_{2n\beta}\text{-ketoacyl-ACP]}
\]

Eq. A.21
\[
\sum_{n=2}^{10} \frac{d[C_{2n}\text{Enoylacyl-ACP}]}{dt} = \sum_{n=2}^{10} k_{\text{off-eacACP}} [\text{FabI} \cdot \text{NADH} \cdot C_{2n}\text{Enoylacyl-ACP}]
- \sum_{n=2}^{10} k_{\text{on-eacACP}} [\text{FabI} \cdot \text{NADH}][C_{2n}\text{Enoylacyl-ACP}]
+ \sum_{n=2}^{10} k_{\text{catFabZ}} [\text{FabZ} \cdot C_{2n}\beta\text{-hydroxyacyl-ACP}]
\]

\[
\sum_{n=2}^{10} \frac{d[C_{2n}\text{acyl-ACP}]}{dt} = \sum_{n=2}^{10} k_{\text{catFabI}} [\text{FabI} \cdot \text{NADH} \cdot C_{2n}\text{Enoylacyl-ACP}]
+ \sum_{n=2}^{9} k_{\text{off-FacACP}} [\text{FabF} \cdot C_{2n}\text{Acyl-ACP}]
- \sum_{n=2}^{9} k_{\text{on-FacACP}} [\text{FabF}][C_{2n}\text{Acyl-ACP}]
+ \sum_{n=2}^{9} k_{\text{off-FabH-Inh-2}} [\text{FabH} \cdot C_{2n}\text{Acyl-ACP}]
- \sum_{n=2}^{9} k_{\text{on-FabH-Inh-2,C2n}} [\text{FabH}][C_{2n}\text{Acyl-ACP}]
+ \sum_{n=2}^{9} k_{\text{off-FabH-Inh-3}} [\text{FabH}^* \cdot C_{2n}\text{Acyl-ACP}]
- \sum_{n=2}^{9} k_{\text{on-FabH-Inh-3,C2n}} [\text{FabH}^*][C_{2n}\text{Acyl-ACP}]
\]

\[
\frac{d[FabD \cdot \text{Malonyl-CoA}]}{dt} = k_{\text{on-mCoA}}[\text{FabD}][\text{Malonyl-CoA}] - k_{\text{off-mCoA}}[\text{FabD} \cdot \text{Malonyl-CoA}]
+ k_{F-FabD}[\text{FabD}^*][\text{CoA}] - k_{F-FabD^*}[\text{FabD} \cdot \text{Malonyl-CoA}]
\]

\[
\frac{d[\text{FabD}^*]}{dt} = k_{F-FabD^*}[\text{FabD} \cdot \text{Malonyl-CoA}] - k_{F-FabD^*}[\text{FabD}^*][\text{CoA}] + k_{\text{off-ACP}}[\text{FabD}^* \cdot \text{ACP}]
- k_{\text{on-ACP}}[\text{FabD}^*][\text{ACP}]
\]

\[
\frac{d[\text{FabD}^* \cdot \text{ACP}]}{dt} = k_{\text{on-ACP}}[\text{FabD}^*][\text{ACP}] - k_{\text{off-ACP}}[\text{FabD}^* \cdot \text{ACP}] + k_{F-FabD^*ACP}[\text{FabD}][\text{Malonyl-ACP}]
- k_{F-FabD^*ACP}[\text{FabD}^* \cdot \text{ACP}]
\]
\[
\frac{d[\text{FabH} \cdot \text{Acetyl-CoA}]}{dt} = k_{\text{on-acCoA}}[\text{FabH}][\text{Acetyl-CoA}] - k_{\text{off-acCoA}}[\text{FabH} \cdot \text{Acetyl-CoA}]
\]
\[+ k_{r-\text{FabH}^\bullet}[\text{FabH}^\bullet][\text{CoA}] - k_{f-\text{FabH}^\bullet}[\text{FabH} \cdot \text{Acetyl-CoA}]\]

Eq. A.27

\[
\frac{d[\text{FabH}^\bullet]}{dt} = k_{f-\text{FabH}^\bullet}[\text{FabH} \cdot \text{Acetyl-CoA}] - k_{r-\text{FabH}^\bullet}[\text{FabH}^\bullet][\text{CoA}]
\]
\[- k_{\text{off-mACP}}[\text{FabH}^\bullet][\text{Malonyl - ACP}]
\[+ \sum_{n=2}^{10} k_{\text{off-FabH-Inh-3,2n}}[\text{FabH}^\bullet \cdot \text{C}_{2n} \text{Acyl-ACP}]
\[- \sum_{n=2}^{10} k_{\text{on-FabH-Inh-3,2n}}[\text{FabH}^\bullet][\text{C}_{2n} \text{Acyl-ACP}]\]

Eq. A.28

\[
\frac{d[\text{FabH}^\bullet \cdot \text{Malonyl - ACP}]}{dt} = k_{\text{on-mACP}}[\text{FabH}^\bullet][\text{Malonyl - ACP}]
\[- k_{\text{off-mACP}}[\text{FabH}^\bullet \cdot \text{Malonyl - ACP}] - k_{\text{catFabH}}[\text{FabH}^\bullet \cdot \text{Malonyl - ACP}]\]

Eq. A.29

\[
\frac{d[\text{FabG} \cdot \text{NADPH}]}{dt} = k_{\text{on-NADPH}}[\text{FabG}][\text{NADPH}]
\[- k_{\text{off-NADPH}}[\text{FabG} \cdot \text{NADPH}]
\[+ \sum_{n=2}^{10} k_{\text{off\text{aACP}}} [\text{FabG} \cdot \text{NADPH} \cdot \text{C}_{2n} \beta\text{-ketoacyl-ACP}]
\[- \sum_{n=2}^{10} k_{\text{on\text{aACP}}} [\text{FabG} \cdot \text{NADPH} \cdot \text{C}_{2n} \beta\text{-ketoacyl-ACP}]\]

Eq. A.30

\[
\sum_{n=2}^{10} \frac{d[\text{FabG} \cdot \text{NADPH} \cdot \text{C}_{2n} \beta\text{-ketoacyl-ACP}]}{dt}
\]
\[= \sum_{n=2}^{10} k_{\text{on\text{aACP}}} [\text{FabG} \cdot \text{NADPH} \cdot \text{C}_{2n} \beta\text{-ketoacyl-ACP}]
\[- \sum_{n=2}^{10} k_{\text{off\text{aACP}}} [\text{FabG} \cdot \text{NADPH} \cdot \text{C}_{2n} \beta\text{-ketoacyl-ACP}]
\[- \sum_{n=2}^{10} k_{\text{catFabG}} [\text{FabG} \cdot \text{NADPH} \cdot \text{C}_{2n} \beta\text{-ketoacyl-ACP}]\]

Eq. A.31

\[
\sum_{n=2}^{10} \frac{d[\text{FabZ} \cdot \text{C}_{2n} \beta\text{-hydroxyacyl-ACP}]}{dt}
\]
\[= \sum_{n=2}^{10} k_{\text{on\text{aACP}}} [\text{FabZ}][\text{C}_{2n} \beta\text{-hydroxyacyl-ACP}]
\[- \sum_{n=2}^{10} k_{\text{off\text{aACP}}} [\text{FabZ} \cdot \text{C}_{2n} \beta\text{-hydroxyacyl-ACP}]
\[- \sum_{n=2}^{10} k_{\text{catFabZ}} [\text{FabZ} \cdot \text{C}_{2n} \beta\text{-hydroxyacyl-ACP}]\]

Eq. A.32
\[
\frac{d[\text{FabI} \cdot \text{NADH}]}{dt} = k_{\text{on-NADH}}[\text{FabI}][\text{NADH}] - k_{\text{off-NADPH}}[\text{FabI} \cdot \text{NADH}] + \sum_{n=2}^{10} k_{\text{off-eacACP}}[\text{FabI} \cdot \text{NADH} \cdot \text{C}_{2n}\text{Enoylacyl-ACP}] - \sum_{n=2}^{10} k_{\text{on-eacACP}}[\text{FabI} \cdot \text{NADH}[\text{C}_{2n}\text{Enoylacyl-ACP}]
\]

\[
\sum_{n=2}^{10} \frac{d[\text{TesA} \cdot \text{C}_{2n}\text{acyl-ACP}]}{dt} = \sum_{n=2}^{10} k_{\text{on-acACP}}[\text{TesA}][\text{C}_{2n}\text{Acyl-ACP}] - \sum_{n=2}^{10} k_{\text{off-acACP}}[\text{TesA} \cdot \text{C}_{2n}\text{Acyl-ACP}] - \sum_{n=2}^{10} k_{\text{catTesA}}[\text{TesA} \cdot \text{C}_{2n}\text{Acyl-ACP}]
\]

\[
\sum_{n=2}^{9} \frac{d[\text{FabF} \cdot \text{C}_{2n}\text{acyl-ACP}]}{dt} = \sum_{n=2}^{9} k_{\text{on-F,acACP}}[\text{FabF}][\text{C}_{2n}\text{Acyl-ACP}] - \sum_{n=2}^{9} k_{\text{off-F,acACP}}[\text{FabF} \cdot \text{C}_{2n}\text{Acyl-ACP}] + \sum_{n=2}^{9} k_{r-\text{FabF}}[\text{C}_{2n}\text{FabF*}][\text{ACP}] - \sum_{n=2}^{9} k_{r-\text{FabF}}[\text{FabF} \cdot \text{C}_{2n}\text{acyl-ACP}]
\]

\[
\sum_{n=2}^{9} \frac{d[\text{C}_{2n}\text{FabF*}]}{dt} = \sum_{n=2}^{9} k_{r-\text{FabF}}[\text{FabF} \cdot \text{C}_{2n}\text{acyl-ACP}] - \sum_{n=2}^{9} k_{r-\text{FabF}}[\text{C}_{2n}\text{FabF*}][\text{ACP}] + \sum_{n=2}^{9} k_{\text{off-FmACP}}[\text{C}_{2n}\text{FabF*} \cdot \text{Malonyl-ACP}] - \sum_{n=2}^{9} k_{\text{on-FmACP}}[\text{C}_{2n}\text{FabF*}][\text{Malonyl-ACP}]
\]

\[
\sum_{n=2}^{9} \frac{d[\text{C}_{2n}\text{FabF*} \cdot \text{Malonyl-ACP}]}{dt} = \sum_{n=2}^{9} k_{\text{on-FmACP}}[\text{C}_{2n}\text{FabF*}][\text{Malonyl-ACP}] - \sum_{n=2}^{9} k_{\text{off-FmACP}}[\text{C}_{2n}\text{FabF*} \cdot \text{Malonyl-ACP}] - \sum_{n=2}^{10} k_{\text{catFabf}}[\text{C}_{2n}\text{FabF*} \cdot \text{Malonyl-ACP}]
\]
\[
\sum_{n=2}^{10} \frac{d[FabH \cdot C_{2n}\text{Acyl-ACP}]}{dt} = \sum_{n=2}^{10} \left( k_{\text{on-FabH-Inh-2}} [FabH][C_{2n}\text{Acyl-ACP}] \right) - \sum_{n=2}^{10} k_{\text{off-FabH-Inh-2,C2n}} [FabH \cdot C_{2n}\text{Acyl-ACP}]
\]

\[
\sum_{n=2}^{10} \frac{d[FabH^* \cdot C_{2n}\text{Acyl-ACP}]}{dt} = \sum_{n=2}^{10} \left( k_{\text{on-FabH-Inh-3}} [FabH^*][C_{2n}\text{Acyl-ACP}] \right) - \sum_{n=2}^{10} k_{\text{off-FabH-Inh-3,C2n}} [FabH^* \cdot C_{2n}\text{Acyl-ACP}]
\]

\[
\frac{d[TesA \cdot ACP]}{dt} = k_{\text{on-TesA-Inh}} [TesA][ACP] - k_{\text{off-TesA-Inh}} [TesA \cdot ACP]
\]

\[
\frac{d[FabH \cdot ACP]}{dt} = k_{\text{on-FabH-Inh-1}} [FabH][ACP] - k_{\text{off-FabH-Inh-1}} [FabH \cdot ACP]
\]

\[
\frac{d[FabG \cdot ACP]}{dt} = k_{\text{on-FabG-Inh}} [FabG][ACP] - k_{\text{off-FabG-Inh}} [FabG \cdot ACP]
\]

\[
\frac{d[FabZ \cdot ACP]}{dt} = k_{\text{on-FabZ-Inh}} [FabZ][ACP] - k_{\text{off-FabZ-Inh}} [FabZ \cdot ACP]
\]

\[
\frac{d[FabI \cdot ACP]}{dt} = k_{\text{on-FabI-Inh}} [FabI][ACP] - k_{\text{off-FabI-Inh}} [FabI \cdot ACP]
\]

\[
\frac{d[FabF \cdot ACP]}{dt} = k_{\text{on-FabF-Inh}} [FabF][ACP] - k_{\text{off-FabF-Inh}} [FabF \cdot ACP]
\]
A.3 MATLAB Code and Associated Files

Code associated with this work is available online at: https://pubs.acs.org/doi/10.1021/acscatal.8b03171

1. Combined_Pathway_Solver. This program initiates and solves the kinetic model with the specified input parameters and options of files 2-8.

2. Combined_Pathway_Model. This program contains material balances and differential equations used to model FAS activity.

3. LeastSquaresCalc. This program calculates sums of squared errors between predicted and experimental trends (Experimental_Dataset.csv).

4. param_func. This file parameterizes the model with specified input parameters.

5. kcat.csv. This file contains the estimates of $k_{cat}$ used by param_func.

6. km_est.csv. This file contains the estimates of $K_m$ used by param_func.

7. est_param.csv. This file contains estimates of all ratios of $k_{on}$ to $k_{off}$ used by param_func.

8. Experimental_Dataset.csv: contains the digitized values of previously reported experimental data.⁴
Appendix A References

APPENDIX B: SUPPORTING INFORMATION FOR CHAPTER III

B.1 METHODS

B.1.1 | Assembly and Solution of the Kinetic Model. In prior work, we developed a kinetic model that captures the activities of seven enzymes necessary for the de novo biosynthesis of saturated fatty acids from malonyl-CoA and acetyl-CoA in Escherichia coli.\(^1\) In this study, we modified our prior model by (i) incorporating the activities of FabA and FabB and (ii) introducing reactions that capture the reversibility of the FabA- and FabZ-catalyzed dehydration reactions. The final model consists of a system of rate equations (Figure B.1) derived from the mechanisms depicted in Table B.2. We solved this model by using the MATLAB solver ode15s with relative and absolute error tolerances of \(10^{-6}\). In order to reduce solve time—an essential step for the analyses discussed in this study—we provided a sparsity matrix to the solver (i.e., a matrix that specifies the non-zero values of the Jacobian matrix) and vectorized the model.

B.1.2 | Parameterization of the Kinetic Model. Our initial estimates of model parameters reflect the results of detailed experimental studies performed over the last several decades.\(^2\)\(^-\)\(^4\) In brief, we used measured values of \(k_{\text{cat}}\) and \(K_d\), and when direct measurements of intermolecular association were not available, we converted values of \(K_M\) to estimates of \(K_d\) (a reasonable approximation when \(k_{\text{cat}}\) is small, relative to \(k_{\text{off}}\); Eq. B.1). To avoid \textit{a priori} equilibrium

\[
K_M = \frac{k_{\text{off}}+k_{\text{cat}}}{k_{\text{on}}} \quad \text{Eq. B.1}
\]

\[
K_M \approx K_d = \frac{k_{\text{off}}}{k_{\text{on}}} \quad \text{Eq. B.2}
\]
assumptions, we decomposed each $K_d$ into kinetic constants by using Eq. B.2 and literature-based estimates of $k_{off}$ (Table B.4). Finally, we equated kinetic parameters for saturated and unsaturated fatty acids (i.e., saturated parameters = unsaturated parameters) for all enzymes, except FabA, FabZ, FabB, and FabF—enzymes for which substrate-specific measurements are available.

**B.1.3 Parameterization of FabZ, FabA, FabF, and FabB.** We parameterized the substrate specificities of FabZ, FabA, FabF, and FabB by using datasets from two *in vitro* studies (Figure B1). The first dataset describes the specific activity of FabZ and FabA on β-hydroxyacyl-ACPs of varying lengths. For substrates on which each enzyme exhibited the highest activity (i.e., “reference” substrates), we used estimates of $k_{cat}$ and $K_a$ (i.e., $1/K_d$) described in Table B.4; for all other substrates, we used the same $k_{cat}$, and we adjusted $K_a$ to match specific activity (Table B.3). Kinetic studies of FabA, where gating residues reduce its affinity for short-chain fatty acids, suggest that the length of substrates affects $K_M$ more strongly than $k_{cat}$. The second dataset describes the specific activity of FabF and FabB on acyl-ACPs. Here, we used estimates of $k_{cat}$ and $K_a$ for a reference substrate (Table B.4) and assumed a fixed ratio between specific activity and $k_{cat}$ for all other substrates (Table B.3). We parameterized activities on substrates that lacked kinetic data, in turn, by using values of $k_{cat}$ and $K_a$ for substrates similar in length. Finally, we modeled the activity of FabA on unsaturated substrates to be 3% of its activity on saturated substrates; this preference is supported by a comparison of the activities of FabA and FabZ on C_{14:1} acyl-ACP. For all other enzymes, we incorporated enzyme activities on unsaturated substrates by using kinetic parameters for saturated substrates of the same lengths.

We added two additional scaling parameters to our revised model: $f$, which scales $k_{fwd}$ for FabA-catalyzed isomerization of *trans*-dec-2-enoyl-ACP to *cis*-dec-3-enoyl-ACP, and $c_4$, which
scales $k_{\text{cat fwd}}$ and $k_{\text{cat rvs}}$ (such that $K_{eq}$ is constant at 0.25) for FabZ. These parameters allowed our model to capture the trends in the datasets used for optimization.

**B.1.4 | FAS Compositions.** Our model uses physiologically relevant concentrations of enzymes, substrates, and cofactors described by Khosla and colleagues. Our reference condition includes 1 µM of each Fab enzyme, 10 µM ACP, 10 µM TesA, 500 µM malonyl-CoA, 500 µM acetyl-CoA, 1 mM NADPH, and 1 mM NADH. Other FAS systems include adjustments to this composition as described in the figures.

**B.1.5 | Optimization of the Kinetic Model.** We optimized estimates of scaling parameters by carrying out the following steps: (i) We estimated physically relevant ranges of each scaling parameter as described previously (Table B.6). (ii) We constructed 500 sets of initial guesses of the value of each scaling parameter by randomly sampling uniform distributions defined by the logarithm of the upper and lower limits of their physically relevant ranges (1). (iii) We evaluated each set of guesses by assessing its ability to capture—without fitting—the time-course profile, product distribution, and initial rates depicted in Fig. B.2. For this analysis, we used $Obj_1$ (Eq. B.3), where

$$Obj_1 = SSE_1 \cdot SSE_2 \cdot SSE_3$$  \hspace{1cm} \text{Eq. B.3}$$

$SSE_1$, $SSE_2$, and $SSE_3$ are the sums of squared errors for the time course (Fig. B.2A), product distribution (Fig. B.2B), and set of initial rates (Fig. B.2C), respectively. (iv) We used the best-performing set of initial guesses and $Obj_2$ (Eq. B.4) to fit our model to the time-course profile and
\[ Obj_2 = SSE_1 \cdot SSE_2 \] \hspace{1cm} \text{Eq. B.4}

product distribution depicted in Fig. B.2. (v) We used the optimized set of scaling parameters to fit the model to the full dataset depicted in Fig. B.2 (Obj_1). (vi) We observed that Obj_1 overweights initial rate data, so we refined our final fit by using Obj_3 and Obj_4 in series (Eq. B.5

\[ Obj_3 = SSE_1^2 \cdot SSE_2 \cdot SSE_3 \] \hspace{1cm} \text{Eq. B.5}

\[ Obj_4 = SSE_1 \cdot SSE_2 \cdot (1 + SSE_{3A}) \cdot (1 + SSE_{3B}) \] \hspace{1cm} \text{Eq. B.6}

and B.6), where SSE_{3A} and SSE_{3B} are the sums of squared errors for the first (SSE_{3A}) and latter (SSE_{3B}) three initial rates used in our initial fit (Fig. B.2C). We concluded the optimization when additional fits ceased to change the value of Obj_4 by more than 1%. Table B.6 reports the final scaling parameters used for all analyses described in this study.

**B.1.6 | Analysis of Error in Model Predictions.** We estimated error in modeling results by sampling normal distributions of protein and substrate concentrations. In brief, at each indicated composition, we selected 300 concentrations of each system constituent (i.e., a protein or substrate) from normal distributions centered at the stated concentrations of those constituents (with standard deviations of 5% of the mean); we input these randomly selected concentrations into our model; and we used the standard deviation of the model output as an estimate of error. Accordingly, each plotted FAS output (e.g., each specific estimate of total production, unsaturated fraction, or chain length) shows the mean and standard deviation of 300 model runs.
B.1.7 | Thermal Regulation and FabB-ACP Binding. *E. coli* responds to decreasing temperatures by increasing the production of unsaturated fatty acids.\(^9\text{-}^{11}\) Accordingly, to model the effect of low growth temperatures (Figure 3.2F), we increased the concentrations of FabA and FabB to match the unsaturated fractions generated by *E. coli* at those temperatures. To model the influence of an ACP mutant at the same temperatures, we left the temperature-specific concentrations of FabA and FabB unchanged and equated the K\(_d\) for all FabB-C\(_i\)ACP\(_{D38A}\) complexes to the experimentally measured K\(_d\) for the FabB-C\(_8\)ACP\(_{D38A}\) complex (Figure 3.2F).

B.1.8 | Sensitivity Analysis of Enzyme Concentrations. We examined the sensitivity of different objectives (i.e., total production, average chain length, and unsaturated fraction at 12 min) to the concentrations of various FAS components by calculating mean elementary effects (Figure 3.3E). A mean elementary effect, as defined by the Morris Method,\(^{12,13}\) is the mean of a set of derivatives (i.e., the change in a specific objective with respect to the change in the concentration of a specific enzyme) calculated at different FAS compositions. Here, we defined the sample space of concentrations as a uniform distribution of (i) 0.1-10 μM for essential enzymes (i.e., those required for the production of fatty acids) and (ii) 0-10 μM for partially redundant enzymes (i.e., FabZ/A and FabF/B). Broader ranges of concentrations (i.e., 0.1-100 μM and 0-100 μM) yielded similar elementary effects (Figure B.15). We implemented our analysis with the SAFE toolbox (14) using a radial method with Latin hypercube sampling (r = 1,000 trajectories for 10,000 points sampled), and we verified that the mean elementary effects converged (Fig. B.10).
Assembly and Parameterization of an Expanded Kinetic Model. We evaluated the influence of lipid biosynthesis on our modeling results by expanding our kinetic model (Fig. B.12). We began by incorporating the entire lipid A pathway. We used MATLAB to replicate a previously validated kinetic model for lipid A biosynthesis in *E.coli*, and we ensured that it produced matching rates of lipid A production with the original model (the original model was based in COPASI which is incompatible with MATLAB). Next, we added the kinetic equations (all of which assumed Michaelis-Menten kinetics, Table B.8A) and parameter values of this model to our base model with three adjustments: (i) We expanded two equations—those of LpxL and LpxM, which assumed an excess of lauroyl and myristoyl-ACP, respectively—from one-substrate to two-substrate Michaelis-Menten equations (Table B.8B). (ii) We replaced constant terms for FAS intermediates (β-hydroxymyristoyl-ACP, ACP, lauroyl-ACP, and myristoyl-ACP) with terms describing their concentrations in our original model. (iii) We reduced the substrate concentration for UDP-GlcNac (a substrate in the first step of lipid A biosynthesis) from 5mM (an excess value used in the original paper) to 430 µM, which was measured *in vivo*.

We approximated the contribution of phospholipid biosynthesis by incorporating the first two steps of this pathway. These steps, which are catalyzed by P1sB and P1sC, rely on FAS intermediates (palmitoyl-ACP, palmitoleoyl-ACP, oleoyl-ACP, and *cis*-vaccenoyl-ACP) and thus compete with FAS enzymes for substrates. For both reactions, we used a two-substrate Michaelis-Menten equation (Table B.8C), which we parameterized with measured values of $K_M$ from *in vitro* kinetic measurements of P1sB. (We note: We assumed identical kinetic parameters for P1sC, see Table 3.9B). Finally, we set the concentration of G3P (*sn*-glycerol 3-phosphate) to 0.18 mM, which was measured *in vivo*.
B.1.10 | Optimization of the Expanded Model. We optimized our expanded kinetic model with fits to experimental measurements of free fatty acid and lipid production by a TesA-overexpressing strain of *E. coli*\(^{18}\) (Fig. B.13). In brief, we used our reference FAS composition for stationary growth (1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, 0.5 mM acetyl-CoA, 10 μM ACP, 10 μM TesA, and 1 μM of each Fab) and assumed a ratio of lipid A to phospholipids of 0.12 (per molecule basis\(^{19}\)) for both stationary and logarithmic phases. We retained all FAS kinetic parameters and fit (i) the concentration of TesA in the log phase of growth, (ii) \(k_{\text{cat}}\) for LpxA, (iii) and \(k_{\text{cat}}\) for both PIsB and PIsC. Note: This fitting procedure assumes a higher concentration of TesA, which is induced in the log phase, in the stationary phase (the phase in which the majority of free fatty acids is produced). Final kinetic constants appear in Table B.10.

B.1.11 | Materials. We used *E. coli* BL21(DE3) (New England Biolabs) for cloning and protein expression. We purchased decanoic acid, myristic acid, and pentadecanoic acid standards from Acros Organics; dodecanoic acid from Alfa Aesar; malonyl CoA (malonyl coenzyme A lithium salt), acetyl CoA (acetyl coenzyme A sodium salt), and all other fatty acids (including methyl esters) from Millipore Sigma; and NADPH (Nicotinamide adenine dinucleotide phosphate) from Cayman Chemical.

B.1.12 | Cloning and Molecular Biology. We used pET vectors to overexpress FAS proteins in *E. coli*. Briefly, we extracted genomic DNA from *E. coli* BL21(DE3) with the DNeasy Blood and Tissue kit (Qiagen), and we amplified genes for FabA-FabZ and the leaderless segment of TesA with the primers described in Table B.11. We cloned each gene into pET15b, pET16b, or pET28a
vectors (Novagen) with restriction digest and ligation. For FabD, FabE, FabF, FabH, FabI, FabZ, and ‘TesA, we used primers to add N-terminal polyhistidine tags to inserted genes; for FabA, FabB, and FabG, by contrast, we used the N-terminal tag present in the expression plasmid. A pET22b and pET29b vector encoding ACP and Sfp, respectively, were kind gifts from the Burkart Group of the University of California, San Diego.

FabZ is an obligate dimer that can be difficult to overexpress in its monomeric form, so we generated a fused dimer using an established method. In brief, we designed primers to split the pET28a-FabZ vector in half immediately before and after the FabZ gene, such that each amplified fragment included the gene for FabZ. We purchased a DNA fragment encoding a linker segment from Integrated DNA Technologies, and we amplified this segment to include overlaps that would enable plasmid assembly. We digested the three fragments for two hours with DpnI (New England Biolabs) and assembled them into the final plasmid-encoded dimer with Gibson assembly. We verified all plasmids with Sanger sequencing (Quintara Biosciences).

B.1.13 | Protein Expression and Purification. We expressed and purified FabD, FabH, FabG, FabA, FabI, FabF, FabB, FabZ (a fused dimer), ACP, and TesA by following standard procedures. We carried out expression at 22 °C for 14-18 hours in rich induction media (20 g/L tryptone, 10 g/L yeast extract, and 5 g/L NaCl, 4 g/L M9 salts, 0.4% (w/v) glucose). We lysed pelleted cells by rocking them for 1 hour at room temperature with lysis buffer (per g of cell pellet): 4mL B-PER (Thermo), 2 mg TAME, 2 mg magnesium sulfate heptahydrate, 3.5 mg TCEP, and 3.75 µl PMSF, 0.5 mg lysozyme, and 200 units DNase I (New England Biolabs). We pelleted the cell lysate and purified protein from the supernatant with a nickel-affinity column followed by desalting and anion exchange columns, with the exception of FabZ, which was only carried through the first desalting
step due to high purity after the nickel-affinity column (here, we used a HisTrap HP column with 50 mM Tris-HCl, pH 7.5, 0.5 mM TCEP, 300 mM NaCl and 0-500 mM imidazole; a HiPrep 26/10 desalting column with 50 mM Tris-HCl, pH 7.5 or 8.5, 0.5 mM TCEP; and a HiPrep Q HP 16/10 column with 50 mM Tris-HCl, pH 7.5 or 8.5, 0.5 mM TCEP and 0-1 M NaCl). To increase the percentage of holo-ACP in our final protein solution, we utilized Sfp to transfer the 4-phosphopantetheinyl moiety of coenzyme A to apo-ACP as described previously\(^1\) and ran the reaction mixture over the anion exchange column a second time. We confirmed the purity of final protein using SDS-PAGE (Fig. B.19) and stored it at -80°C in Tris buffer (7-850 µM protein, 50 mM Tris-HCl, pH 7.5, 0.5 mM TCEP, 20% glycerol). We purchased all chromatography columns from GE healthcare.

**B.1.14 |** Kinetic Analysis of Fatty Acid Synthases. We reconstituted FASs in 100-µL or 1-ml reactions (96-well plates and 2-ml glass vials, respectively). All reactions contained 0-10 µM of each FAS enzyme in a standard background (10 µM holo-ACP, 10 µM TesA, 10 mM HEPES, pH 7.4, 150 mM NaCl, 0.5 mM TCEP). Each 100-µL reaction, in turn, contained 1.3 mM NADPH, 500 µM malonyl-CoA, 100 µM acetyl-CoA,\(^8\) while each 1-ml reaction, which generated high levels of fatty acids sufficient for GC/MS analysis, contained 2.6 mM NADPH, 1.5 mM malonyl-CoA, and 300 µM acetyl-CoA. For all experiments, our negative control contained 10 µM of TesA, 1 µM of FabA-Z, and a background that lacked malonyl-CoA, acetyl-CoA, and holo-ACP.

We determined initial rates of fatty acid synthesis by using the UV spectrophotometric assay developed by Xiao et al.\(^8\) In short, we followed the oxidation of NADPH to NADP+ by monitoring absorbance at 340 nm in a SpectraMax M2 plate reader, and we converted NADPH concentrations to palmitic acid equivalents by assuming a stoichiometry of 12 µM NADPH:1 µM
palmitic acid. For all reactions, we subtracted the signal produced by our negative control from the signals generated by reconstituted FASs, and we calculated initial rates over 2.5 minutes.

B.1.15 | Extraction of Fatty Acids. We examined the product profiles of FASs by carrying out the following steps (a modification of the procedure described by Sarria et al.\textsuperscript{21}): (i) We quenched each reaction by adding 125 μL acetic acid. (ii) We prepared each quenched reaction for extraction by adding 125 μL of 10% NaCl (w/v), 50 μL 90.5 mg/L pentadecanoic acid (an internal standard), and 500 μL ethyl acetate. (iii) We vortexed this mixture for 10 s and centrifuged it for 20 minutes at ~2,500 g. (iv) We transferred 250 μL of the ethyl acetate layer to a clean glass vial and evaporated the solvent to concentrate the solution. (v) We resuspended the dried fatty acids in 1 mL of 30:1 methanol and 37% (v/v) HCl, and we allowed esterification to proceed at 50°C for one hour before returning the reactions to room temperature. (vi) We added 500 μL water and 500 μL hexane, vortexed for 10 seconds, and used 100 μL of the hexane layer for GC/MS analysis. We generated standard curves for all reported chain lengths, relating sample concentration to integrated peak area, by performing the above protocol with known amounts of authentic fatty acid standards (Fig. B.20).

B.1.16 | Quantification of Fatty Acids by GC/MS. We quantified fatty acids with a ThermoFisher Trace 1310/ ISQ7000 MS equipped with a TG-5SILMS column (ThermoFisher Scientific, 30 m x 0.25 mm x 0.25 μm). For all runs, we used the following method: (i) Inject at 1 ml/min with a split ratio of 10:1 and an inlet temperature of 280°C, (ii) hold at 80°C (5 min), (iii) increase to 230°C (20°C/min), (iv) increase to 260°C (10°C/min), and (v) hold at 260°C (1 min).
To identify fatty acids, we used select ion mode (SIM) to scan for the molecular ion (i.e., m/z =74 and 87). We subtracted peak areas present in control samples from sample peak areas for each analyte to account for any contamination caused by use of plastics during protein purification. To quantify the fatty acids present in unknown samples, we used standard curves of fatty acids for all chain lengths (C₈-C₁₈, including C₁₂₋₁, C₁₄₋₁, C₁₅, C₁₆₋₁, and C₁₈₋₁; Fig. B.20), described above.

B.1.17 | Quantification Proteomics. We quantified the protein impurities in select enzyme stocks (i.e., FabZ and FabF) by using quantitative proteomics. In brief, we digested FabZ and FabF samples overnight with trypsin, analyzed the digested samples on an Orbitrap LC/MS/MS, and quantified their protein constituents using the iBAQ method.²²,²³ We quantified impurities by normalizing iBAQ values to the most abundant protein (i.e., FabZ or FabF).

B.2 Notes

Note 1. Estimation of Dissociation Constants. We used experimentally derived estimates of equilibrium constants to constrain values of kon and koff for all heteromeric complexes (i.e., Kd = koff/kon; Materials and Methods). To summarize, a measurement of Kd for the FabB-C₈ACP complex served as the Kd for all FabB-CiACP complexes, and we determined substrate-specific values of Kd for FabA and FabZ from fits to kinetic data (Figure B.1). We estimated values of Kd for all other enzymes, in turn, from measurements of KM (i.e., Kd ∼ KM; Table B.4). Our final model captured the influence of a mutant of ACP on unsaturated fatty acid production with the introduction of a single experimentally measured Kd (Figure 3.2F). This result suggests that the model adequately captures the relative stabilities of different enzyme-ACP complexes, and it demonstrates, more broadly, the model’s value as a tool for interpreting experimental data.
B.3 Figures and Tables

Figure B.1. Analysis of the substrate specificities of FabZ, FabA, FabF and FabB. (A) The specific activities of FabZ and FabA on β-hydroxyacyl-ACPs of varying lengths. (B) Estimates of $K_a$ (i.e., $1/K_d$) for FabZ and FabA. We used estimates of $k_{cat}$ and $K_a$ described in Table B.4 for the substrate on which each enzyme exhibited the highest activity ($C_6$ for FabZ and $C_{10}$ for FabA) and assumed a linear relationship between $K_a$ and specific activity for all other substrates (i.e., we held $k_{cat}$ constant). (C) The specific activities of FabF and FabB on acyl-ACPs of varying lengths. (D) Estimates of $k_{cat}$ for FabF and FabB. Here, we used values of $k_{cat}$ and $K_a$ for the preferred substrates ($C_{10}$ for FabF and $C_{12}$ for FabB, Table B.4) and assumed a linear relationship between specific activity and $k_{cat}$ for all other substrates (i.e., we held $K_a$ constant). In both B and D, we estimated $k_{cat}$ and $K_a$ for substrates that lacked kinetic data by equating them to the experimentally rooted values of $k_{cat}$ and $K_a$ for substrates closest in length (e.g., in B, estimates of $K_a$ for $C_{16}$-$C_{20}$ are the same).
Figure B.2. Optimization of the kinetic model. We optimized our kinetic model with simultaneous fits to three datasets: (A) A time course of total fatty acids generated by a reconstituted FAS (1 μM of each Fab, 10 μM holo-ACP, 10 μM TesA, 1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, and 0.5 mM acetyl-CoA), (B) the product distribution of a strain of E. coli that overexpresses TesA, and (C) the relative initial rates of fatty acid production by four reconstituted FASs (1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, and 0.5 mM acetyl-CoA). We used the product distribution from B as an approximation for the distribution in A (not reported) at 12 min. For C, we assumed a measurement time of 2.5 minutes for the first composition and 0.5 minutes for all others; these times are consistent with the time course profiles of the reported studies. In C, we also normalized all initial rates by the rates of reference systems in which the
highlighted enzymes had different concentrations (for the first composition, $[\text{FabH}] = 1 \, \mu M$; for the latter three, $[\text{FabA}] = 1 \, \mu M$, $[\text{FabZ}] = 10 \, \mu M$, $[\text{FabB}] = 1 \, \mu M$, and $[\text{FabI}] = 10 \, \mu M$).
Figure B.3. Titration of FabZ. (A-C) Steady-state concentrations of (A) β-hydroxy-decanoyl-ACP, (B) trans-dec-2-enoyl-ACP, and (C) cis-dec-3-enoyl-ACP generated by a modeled FAS (1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, 0.5 mM acetyl-CoA, 10 μM ACP, 10 μM TesA, and 1 μM of each Fab, 12 min) with varying concentrations of FabZ. (D-E) Increasing concentrations of FabZ cause (D) a steady decline in the ratio of β-hydroxy-decanoyl-ACP to trans-dec-2-enoyl-ACP and (E) a small depression followed by a gradual increase in the ratio of trans-dec-2-enoyl-ACP to cis-dec-3-enoyl-ACP. In brief, low concentrations of FabZ increase steady-state concentrations of β-hydroxy-decanoyl-ACP, while higher concentrations of FabZ “outcompete” FabA for β-hydroxy-decanoyl-ACP and, thus, decrease the production of cis-dec-3-enoyl-ACP.
Figure B.4. Titration of FabA. (A-C) Steady-state concentrations of (A) β-hydroxy-decanoyl-ACP, (B) trans-dec-2-enoyl-ACP, and (C) cis-dec-3-enoyl-ACP generated by a modeled FAS (1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, 0.5 mM acetyl-CoA, 10 μM ACP, 10 μM TesA, and 1 μM of each Fab, 12 min) with varying concentrations of FabA. (D-E) Increasing concentrations of FabA cause (D) a sharp depression followed by a gradual increase in the ratio of trans-dec-2-enoyl-ACP to cis-dec-3-enoyl-ACP and (E) a sharp increase followed by a gradual decrease in the fraction of unsaturated fatty acids. Lower concentrations of FabZ (i.e., < 1 μM, the concentration in the reference system) increase the peak unsaturated fraction.
Figure B.5. Analysis of the contribution of FabZ to unsaturated fatty acid biosynthesis. (A) Steady-state concentrations of unsaturated β-hydroxy-acyl-ACPs and (B) the final unsaturated fractions generated by a modeled FAS (1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, 0.5 mM acetyl-CoA, 10 μM ACP, 10 μM TesA, and 1 μM of each Fab, 12 min) with varying concentrations of FabA and FabZ. In the absence of FabZ, the low activity of FabA on unsaturated β-hydroxyacyl-ACPs causes them to accumulate, slowing the production of unsaturated fatty acids. Small amounts of FabZ alleviate this effect.
Figure B.6. Titration of FabF. Fatty acid production by a modeled FAS with (1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, 0.5 mM acetyl-CoA, 10 μM ACP, 10 μM TesA, and 1 μM of each Fab) with varying concentrations of FabF. Changes in the concentration of FabF, which does not catalyze a rate-limiting step in fatty acid synthesis, have little influence on (A) total production, (B) the fraction of unsaturated fatty acids, or the concentrations of (C) β-keto-decanoyl-ACP, (D) β-hydroxy-decanoyl-ACP, and (E) cis-dec-3-enoyl-ACP at 12 minutes.
Figure B.7 Enzyme compositions that achieve the maximum fraction of unsaturated fatty acids. The fraction of total protein in (A) a complete FAS and (B-D) FASs lacking (B) FabZ, (C) FabF, and (D) both FabZ and FabF (18 μM total enzyme, 1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, 0.5 mM acetyl-CoA, 10 μM ACP, 12 min). These compositions achieve the maximum unsaturated fractions at the indicated production levels; the compositions that achieve the minimum unsaturated fractions are not shown as in all cases, they merely lack FabA or FabB.
Figure B.8. Enzyme compositions that achieve the maximum average chain length. The fraction of total protein in (A) a complete FAS and (B-F) FASs lacking (B) FabZ, (C) FabA, (D) FabB, (E) FabF, and (F) both FabZ and FabF (18 μM total enzyme, 1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, 0.5 mM acetyl-CoA, 10 μM ACP, 12 min). These compositions achieve the maximum average chain length at the indicated production levels.
Figure B.9. Enzyme compositions that achieve the minimum average chain length. The fraction of total protein in (A) a complete FAS and (B-F) FASs lacking (B) FabZ, (C) FabA, (D) FabB, (E) FabF, and (F) both FabZ and FabF (18 μM total enzyme, 1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, 0.5 mM acetyl-CoA, 10 μM ACP, 12 min). These compositions achieve the minimum average chain length at the indicated production levels.
Figure B.10. Analysis of the convergence of mean elementary effects (EE). We ensured the convergence of mean elementary effects by carrying out the following steps: (i) We determined the total number of model evaluations ($N$) necessary to carry out the sensitivity analysis by using the equation $N = r \times [M+1] = 1000 \times [9+1]$, where $M$ is the number of modeled enzymes and $r$ is the number of initial points used to calculate each elementary effect. (ii) We estimated the mean elementary effect for each trajectory (i.e., collection of 10 points). (iii) We averaged the mean elementary effects estimated from different sets of trajectories chosen at random among all subsets (e.g., 200, 400, 600, 800, 1000 trajectories). The figures show the convergence of sensitivity analyses carried out with maximum enzyme concentrations of 10 $\mu$M (A,C,E) or 100 $\mu$M (B,D,F) for three objectives: (A-B) total production, (C-D) average chain length, and (E-F) unsaturated fraction. In all cases the minimum enzyme concentrations were 0 for partially redundant enzymes (FabZ, FabA, FabF, and FabB) and 0.1 $\mu$M for all other enzymes.
Figure B.11. Sensitivity analyses of fatty acid biosynthesis. (A-B) A comparison of elementary effects for FASs in which the maximum concentration of each enzyme is (A) 10 µM or (B) 100 µM. (In both cases, minimum concentrations are limited to 0 or 0.1 µM; Materials and Methods). The sensitivities of various enzymes are similar for the two ranges.
Figure B.12. Biosynthesis of lipid A and phospholipids. (A) A depiction of the complete fatty acid synthase (FAS) of *E. coli* with interfacing steps (i.e., steps that use FAS intermediates) from lipid A (blue) and phospholipid (green) pathways. (B) The complete lipid A pathway. (C) The first two steps of the phospholipid pathway. Our expanded kinetic model of fatty acid synthesis includes the 11 enzymatic steps depicted in B and C. (D) Structures of the substrates, intermediates, and products of the lipid A and phospholipid pathways.
Figure B.13. Optimization of kinetic parameters for lipid biosynthesis. We optimized our expanded kinetic model with fits to experimental measurements of (A) free fatty acid and lipid synthesis by a TesA-overexpressing strain of *E. coli* and (B) the ratio of lipid A to phospholipids (0.12 on per molecule basis). For this fit, we adjusted three parameters—(i) the concentration of TesA in log phase of growth, (ii) $k_{\text{cat}}$ for LpxA, (iii) and $k_{\text{cat}}$ for both PlsB and PlsC—and retained all other kinetic parameters and enzyme concentrations used in our reference system (1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, 0.5 mM acetyl-CoA, 10 μM ACP, 10 μM TesA, and 1 μM of each Fab, 12 min). Table B.10 lists final parameter values.
Figure B.14. The influence of lipid biosynthesis on trends in FAS kinetics. We evaluated the influence of lipid A and phospholipid biosynthesis on our modeling results by repeating the analysis depicted in (A) Fig. 3.2B, (C) Fig. 3.2C, and (D) Fig. 3.2E with our expanded kinetic model. (C-F) Trends in FAS outputs remain unchanged. The error bars and shaded regions indicate the standard deviation of modeled data.
Figure B.15. The influence of lipid biosynthesis on FAS sensitivity. We evaluated the influence of lipid A and phospholipid biosynthesis on our sensitivity analysis by repeating the global analysis depicted in (A) Fig. 3.3E. (B) The sensitivities of different objectives to changes in the concentrations of each enzyme remain unchanged with one exception: the contributions of TesA, FabF, and FabB to total production increase in the expanded model. This enhanced influence reflects the competition between these enzymes and LpxL, LpxM, PlsB, and PlsC for acyl-ACPs. Importantly, the central finding of our analysis—the ability of partially redundant enzymes to permit independent control over competing biochemical objectives that become more closely coupled in their absence—remains unchanged.
Figure B.16. Analysis of perturbations to FAS systems. We evaluated our model’s ability to capture FAS activity in living cells by recreating several perturbations from published studies of the *E. coli* FAS. Perturbations included the overexpression (+) of (A) FabA, FabB, or both FabA and FabB,
(B) FabZ,
and (C) FabF. We recreated these adjustments in our base model (which neglects lipid biosynthesis) by increasing the concentrations of the indicated components by 30-fold (1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, 0.5 mM acetyl-CoA, 10 μM ACP, 1 μM of each Fab, and 0.33 μM TesA, which was not overexpressed in these studies; 12 min). We note: For B, which showed an unusually low unsaturated fraction in the native system (relative to both A and previous studies of the *E. coli* FAS), we used the “FabZ-overexpressed” case as our reference system and evaluated the influence of a 30-fold reduction in FabZ. In all cases trends in the unsaturated fraction or normalized production (i.e., a metric that allows us to compare changes in production between strains) were similar between model and experiment. This consistency suggests that the influence of enzymes on FAS activity originate from effects internal to the FAS system (i.e., effects captured by our kinetic model).
Figure B.17. Analysis of FabH inhibition. (A) Initial rates of fatty acid production by an experimentally reconstituted FAS (1 μM of each Fab, 10 μM holo-ACP, 10 μM TesA, 1.3 mM NADPH, 0.5 mM malonyl-CoA, and 0.1 mM acetyl-CoA; 2.5min) with varying concentrations of FabH. FabH enhances initial rates at low concentrations and depresses them at high concentrations. (B) A modeled FabH titration with 30 μM ACP (and the remaining FAS as in A) shows a trend similar to that observed in A. (C) In our modeling analysis, the optimal concentration of FabH—and, thus, the concentration at which inhibition emerges—increases with the concentration of ACP. Differences in the fraction of active ACP between reconstituted mixtures may, thus, give rise to differences in the observed inhibitory effect of FabH.
Figure B.18. Analysis of the contributions of FabF and FabA to FAS activity. In Figure 3.4B, our model overpredicts initial rates of FASs with (i) 10 µM FabF and (ii) 10 µM FabA and no FabZ. (A) Initial rates of fatty acid production by a modeled FAS with increasing concentrations of FabF. High concentrations of FabF and/or a strong FabF-ACP binding constant (i.e., a ten-fold reduction in $K_d$, blue line) can enhance the inhibitory effect of this enzyme. (B) A comparison of initial rates of modeled and experimentally reconstituted FASs (as in Fig. 3.4B). To prepare the plot on the right, we fit the initial rate of an FAS with 10 µM FabA and 0 µM FabZ by reducing the activity of FabA (i.e., we reduce $k_{on}$ with ACP by approximately ten-fold) and, using the revised model, we compared modeled and experimental rates for the FAS with 0 µM FabZ. In general, a reduction in FabA activity achieved better agreement between model and experiment. For all plots, we used the modeled FAS composition described in our paper (1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, 0.5 mM acetyl-CoA, 10 µM ACP, 10 µM TesA, and 1 µM of each Fab). Error bars represent standard deviation of modeled and measured (for B) data.
Figure B.19. Sds-page of purified FAS components. A criterion t gx stain-free gel (4-20%, biorad) loaded with 1 µg of each protein and stained with imperial™ protein stain (thermo fisher scientific; secondary staining enabled visualization of fabz). We imaged the gel with a bio rad gel doc ez imager. All enzymes appeared at their expected molecular weights, with the exception of holo-acp which migrates with a higher-than expected molecular weight, as reported in previous studies.\textsuperscript{24,29} Note: as we used a homodimer of fabz, this enzyme appears at twice its monomeric molecular weight.
Figure B.20. Standard curves for GC-MS analyses. We prepared standards of fatty acids at concentrations of 0, 1.2, 2.5, 5, 10, and 20 mg/L in water (from stock solutions in ethyl acetate), and we subjected these standards to our extraction and derivatization procedure (Materials and Methods). The plots show the integrated peak areas of different concentrations. Linear fits from each plot allowed us to determine the concentrations of fatty acids in experimental samples.
Figure B.21. Standard curve for spectrophotometric assay of FAS activity. This plot shows the absorbance of NADPH (340 nm) at various concentrations (10 mM HEPES, 150 mM NaCl, 0.5 mM TCEP, pH 7.4). A linear fit to this plot allowed us to convert absorbance measurements to concentrations of NADPH in aqueous reaction mixtures.
<table>
<thead>
<tr>
<th>Cases</th>
<th>Description</th>
<th>Source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FabA/FabZ</td>
<td>Both FabA and FabZ have dehydratase activity; only FabA has isomerase activity.</td>
<td><em>Escherichia coli</em> General: all α- and γ-proteobacteria.</td>
<td>(3, 30)</td>
</tr>
<tr>
<td>FabB/FabF</td>
<td>Both FabB and FabF can elongate acyl-ACPs by condensing them with malonyl-ACP. Only FabB can elongate cis-dec-3-enoyl-ACP generated by FabA.</td>
<td><em>Escherichia coli</em> General: all α- and γ-proteobacteria.</td>
<td>(3, 30)</td>
</tr>
<tr>
<td>FabI/FabL</td>
<td>FabI/FabV are functionally redundant with respect to Fabl (i.e., they can rescue growth of Fabl-deficient strains) but differ significantly from FabI in sequence Bacillus subtilis (FabL), Pseudomonas aeruginosa (FabV)</td>
<td></td>
<td>(31–34)</td>
</tr>
<tr>
<td>FabI/FabK</td>
<td>FabI and FabK from <em>E. faecalis</em> can rescue growth of a FabI-deficient strain of <em>E. coli</em> Both enzymes thus appear to exhibit reductase activity.</td>
<td><em>Enterococcus faecalis</em></td>
<td>(35)</td>
</tr>
<tr>
<td>ACP1/ACP2</td>
<td>Some type I FASs possess two ACPs, but only one is necessary to produce fatty acids.</td>
<td><em>Rhodosporidium toruloides</em> General: Several fungal FAS</td>
<td>(36)</td>
</tr>
</tbody>
</table>
Table B.2A. Kinetic Mechanisms

(1) \( \text{FabD} + \text{Malonyl-CoA} \overset{k_{\text{on-mCoA}}}{\underset{k_{\text{off-mCoA}}}{\rightleftharpoons}} \text{FabD} \cdot \text{CoA} \overset{k_{\text{FabD}+}}{\rightarrow} \text{FabD}^+ + \text{CoA} \)

(2) \( \text{FabD}^+ + \text{ACP} \overset{k_{\text{on-ACP}}}{\rightarrow} \text{FabD}^+ \cdot \text{ACP} \overset{k_{\text{FabD}^+ \cdot \text{ACP}}}{\rightarrow} \text{ACP} + \text{FabD} + \text{Malonyl-ACP} \)

(3) \( \text{FabH} + \text{Acetyl-CoA} \overset{k_{\text{on-aCoA}}}{\rightarrow} \text{FabH}^+ \cdot \text{CoA} \overset{k_{\text{FabH}^+ \cdot \text{CoA}}}{\rightarrow} \text{FabH}^+ + \text{CoA} \)

(4) \( \text{FabH}^+ + \text{ACP} \overset{k_{\text{on-mACP}}}{\rightarrow} \text{FabH}^+ \cdot \text{ACP} \overset{k_{\text{FabH}^+ \cdot \text{ACP}}}{\rightarrow} \text{ACP} + \text{FabH}^+ + \text{CO}_2 \)

(5) \( \text{FabG} + \text{NADPH} \overset{k_{\text{on-NADPH}}}{\rightarrow} \text{FabG} \cdot \text{NADPH} \overset{k_{\text{FabG} \cdot \text{NADPH}}}{\rightarrow} \text{FabG} \cdot \text{NADPH} \cdot \text{ACP} \overset{k_{\text{FabG} \cdot \text{NADPH} \cdot \text{ACP}}}{\rightarrow} \beta\text{-ketoacyl-ACP} \)

(6) \( \text{FabG} \cdot \text{NADPH} \cdot \text{ACP} \overset{k_{\text{FabG} \cdot \text{NADPH} \cdot \text{ACP}}}{\rightarrow} \beta\text{-ketoacyl-ACP} \overset{k_{\text{FabG} \cdot \text{NADPH} \cdot \text{ACP}}}{\rightarrow} \beta\text{-hydroxyacyl-ACP} + \text{FabG} + \text{NADP}^+ \)

(7) \( \text{FabZ} + \text{ACP} \overset{k_{\text{on-ACP}}}{\rightarrow} \text{FabZ} \cdot \text{ACP} \overset{k_{\text{FabZ} \cdot \text{ACP}}}{\rightarrow} \text{H}_2\text{O} + \text{Enoyl-acyl-ACP} \)

(8) \( \text{FabZ} \cdot \text{ACP} \overset{k_{\text{FabZ} \cdot \text{ACP}}}{\rightarrow} \text{Enoyl-acyl-ACP} \overset{k_{\text{FabZ} \cdot \text{ACP}}}{\rightarrow} \text{Enoyl-acyl-ACP} \)

(9) \( \text{FabZ} + \text{NADH} \overset{k_{\text{on-NADH}}}{\rightarrow} \text{FabZ} \cdot \text{NADH} \overset{k_{\text{FabZ} \cdot \text{NADH}}}{\rightarrow} \text{Enoyl-acyl-ACP} \)

(10) \( \text{FabZ} \cdot \text{NADH} \overset{k_{\text{FabZ} \cdot \text{NADH}}}{\rightarrow} \text{Enoyl-acyl-ACP} \overset{k_{\text{FabZ} \cdot \text{NADH}}}{\rightarrow} \text{FabZ} + \text{NAD}^+ \)

(11) \( \text{FabF} + \text{ACP} \overset{k_{\text{on-ACP}}}{\rightarrow} \text{FabF} \cdot \text{ACP} \overset{k_{\text{FabF} \cdot \text{ACP}}}{\rightarrow} \text{FabF}^+ + \text{ACP} \)

(12) \( \text{FabF}^+ + \text{ACP} \overset{k_{\text{FabF}^+ \cdot \text{ACP}}}{\rightarrow} \beta\text{-ketoacyl-ACP} \overset{k_{\text{FabF}^+ \cdot \text{ACP}}}{\rightarrow} \text{FabF} + \text{CO}_2 \)
Table B.2B. Kinetic Mechanisms.

(13) \[ \text{TesA} + \text{ACP} \xrightleftharpoons{k_{off \rightarrow ACP}} \text{TesA} \cdot \text{ACP} \xrightarrow{k_{cat \rightarrow ACP}} \text{TesA} + \text{ACP}\]

(14) \[ \text{FabA} + \text{ACP} \xrightleftharpoons{k_{off \rightarrow ACP}} \text{FabA} \cdot \text{ACP} \xrightarrow{k_{cat \rightarrow ACP}} \text{FabA} + \text{ACP}\]

(15) \[ \text{FabA} \cdot \text{ACP} \xrightarrow{k_{off \rightarrow ACP}} \text{FabA} + \text{ACP}\]

(16) \[ \text{FabA} + \text{ACP} \xrightleftharpoons{k_{off \rightarrow ACP}} \text{FabA} \cdot \text{ACP} \xrightarrow{k_{cat \rightarrow ACP}} \text{FabA} + \text{ACP}\]

(17) \[ \text{FabA} + \text{ACP} \xrightleftharpoons{k_{off \rightarrow ACP}} \text{FabA} \cdot \text{ACP} \xrightarrow{k_{cat \rightarrow ACP}} \text{FabA} + \text{ACP}\]

(18) \[ \text{FabB} + \text{ACP} \xrightleftharpoons{k_{off \rightarrow ACP}} \text{FabB} \cdot \text{ACP} \xrightarrow{k_{cat \rightarrow ACP}} \text{FabB} + \text{ACP}\]

(19) \[ \text{FabB} + \text{ACP} \xrightleftharpoons{k_{off \rightarrow ACP}} \text{FabB} \cdot \text{ACP} \xrightarrow{k_{cat \rightarrow ACP}} \text{FabB} + \text{ACP}\]

(20) \[ \text{FabB} + \text{ACP} \xrightleftharpoons{k_{off \rightarrow ACP}} \text{FabB} \cdot \text{ACP} \xrightarrow{k_{cat \rightarrow ACP}} \text{FabB} + \text{ACP}\]

(21) \[ \text{FabB} + \text{ACP} \xrightleftharpoons{k_{off \rightarrow ACP}} \text{FabB} \cdot \text{ACP} \xrightarrow{k_{cat \rightarrow ACP}} \text{FabB} + \text{ACP}\]
Table B.3A. Kinetic Parameters for the Specificities of FabZ, FabA, FabF, and FabB.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate*</th>
<th>Specific Activity**</th>
<th>Units</th>
<th>Estimated or Measured</th>
<th>$K_a = 1/K_d$ (1/μM)**</th>
<th>$k_{cat}$ (1/s)**</th>
</tr>
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<tbody>
<tr>
<td>FabZ</td>
<td>C$_7$-β-hydroxyacyl-ACP</td>
<td>1.30E+00</td>
<td>nmole/min/μg</td>
<td>Measured</td>
<td>8.38E-03</td>
<td>2.72 E-01</td>
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<td>FabZ</td>
<td>C$_6$-β-hydroxyacyl-ACP</td>
<td>2.77E+00</td>
<td>nmole/min/μg</td>
<td>Measured</td>
<td>1.79E-02</td>
<td>2.72 E-01</td>
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<tr>
<td>FabZ</td>
<td>C$_6$-β-hydroxyacyl-ACP</td>
<td>8.20E-01</td>
<td>nmole/min/μg</td>
<td>Measured</td>
<td>5.29E-03</td>
<td>2.72 E-01</td>
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<td>FabZ</td>
<td>C$_{10}$-β-hydroxyacyl-ACP</td>
<td>1.03E+00</td>
<td>nmole/min/μg</td>
<td>Measured</td>
<td>6.64E-03</td>
<td>2.72 E-01</td>
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<tr>
<td>FabZ</td>
<td>C$_{12}$-β-hydroxyacyl-ACP</td>
<td>5.54E-01</td>
<td>nmole/min/μg</td>
<td>Measured</td>
<td>3.57E-03</td>
<td>2.72 E-01</td>
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<td>FabZ</td>
<td>C$_{14}$-β-hydroxyacyl-ACP</td>
<td>1.53E-01</td>
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<td>C$_{16}$-β-hydroxyacyl-ACP</td>
<td>2.91E-01</td>
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<td>2.72 E-01</td>
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<tr>
<td>FabZ</td>
<td>C$_{20}$-β-hydroxyacyl-ACP</td>
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<td>Estimated</td>
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<td>FabA</td>
<td>C$_7$-β-hydroxyacyl-ACP</td>
<td>2.18E-01</td>
<td>nmole/min/μg</td>
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<td>C$_9$-β-hydroxyacyl-ACP</td>
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<td>FabA</td>
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<td>2.57E+00</td>
<td>nmole/min/μg</td>
<td>Measured</td>
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<td>FabA</td>
<td>C$_{12}$-β-hydroxyacyl-ACP</td>
<td>1.93E+00</td>
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<td>FabA</td>
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<td>2.18E-01</td>
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<td>Measured</td>
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<tr>
<td>FabA</td>
<td>C$_{16}$-β-hydroxy-cis-9-acyl-ACP</td>
<td>3.43E-03</td>
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<td>Estimated</td>
<td>2.38E-05</td>
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<td>FabA</td>
<td>C$_{18}$-β-hydroxyacyl-ACP</td>
<td>9.59E-02</td>
<td>nmole/min/μg</td>
<td>Measured</td>
<td>6.66E-04</td>
<td>2.72 E-01</td>
</tr>
</tbody>
</table>

*Orange highlights designate estimates of kinetic parameters described in Table B.3. We used these values to anchor our estimates of kinetic parameters for the other substrates (see Fig. B.2).
* C$_i$ refers to $i$ carbons in an acyl chain. Unless otherwise noted, we assumed the kinetic parameters for unsaturated substrates to be the same as those of saturated substrates.
**Measured specific activities of FabZ/A$^5$ and FabF/B$^7$. We estimated the specific activities of substrates that lacked kinetic data by equating them to the specific activities of substrates closest in length.
***Estimated values (see Note 1 and Fig. B.2)
****We estimated these values by assuming a specific activity ratio between unsaturated and saturated substrates of 0.0358 (i.e., the ratio for C$_{14}$ substrates$^5$).
Table B.3B. Estimates of Kinetic Parameters for the Substrate Specificities of FabZ, FabA, FabF, and FabB.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate*</th>
<th>Specific Activity**</th>
<th>Units</th>
<th>Estimated or Measured</th>
<th>$K_d$ =1/$K_d$ (1/μM)**</th>
<th>$k_{cat}$ (1/s)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>FabA</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;-β-hydroxy-cis-11-acyl-ACP</td>
<td>3.43E-03****</td>
<td>nmole/min/μg</td>
<td>Estimated</td>
<td>2.38E-05</td>
<td>2.72 E-01</td>
</tr>
<tr>
<td>FabA</td>
<td>C&lt;sub&gt;20&lt;/sub&gt;-β-hydroxy-cis-13-acyl-ACP</td>
<td>9.59E-02</td>
<td>nmole/min/μg</td>
<td>Measured</td>
<td>6.66E-04</td>
<td>2.72 E-01</td>
</tr>
<tr>
<td>FabA</td>
<td>C&lt;sub&gt;20&lt;/sub&gt;-β-hydroxy-cis-13-acyl-ACP</td>
<td>3.43E-03****</td>
<td>nmole/min/μg</td>
<td>Estimated</td>
<td>2.38E-05</td>
<td>2.72 E-01</td>
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<tr>
<td>FabF</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;-acyl-ACP</td>
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<td>FabF</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;-acyl-ACP</td>
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<td>Estimated</td>
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<tr>
<td>FabF</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;-acyl-ACP</td>
<td>2.33E+01</td>
<td>μU/μg</td>
<td>Estimated</td>
<td>7.14E-02</td>
<td>2.86E+00</td>
</tr>
<tr>
<td>FabF</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;-acyl-ACP</td>
<td>2.55E+01</td>
<td>μU/μg</td>
<td>Measured</td>
<td>7.14E-02</td>
<td>3.13E+00</td>
</tr>
<tr>
<td>FabF</td>
<td>C&lt;sub&gt;12&lt;/sub&gt;-acyl-ACP</td>
<td>2.30E+01</td>
<td>μU/μg</td>
<td>Measured</td>
<td>7.14E-02</td>
<td>2.82E+00</td>
</tr>
<tr>
<td>FabF</td>
<td>C&lt;sub&gt;14&lt;/sub&gt;-acyl-ACP</td>
<td>7.37E+00</td>
<td>μU/μg</td>
<td>Measured</td>
<td>7.14E-02</td>
<td>9.05E-01</td>
</tr>
<tr>
<td>FabF</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;-acyl-ACP</td>
<td>8.67E+00</td>
<td>μU/μg</td>
<td>Measured</td>
<td>7.14E-02</td>
<td>6.95E-02</td>
</tr>
<tr>
<td>FabF</td>
<td>C&lt;sub&gt;20&lt;/sub&gt;-acyl-ACP</td>
<td>8.67E+00</td>
<td>μU/μg</td>
<td>Measured</td>
<td>7.14E-02</td>
<td>1.06E+00</td>
</tr>
<tr>
<td>FabF</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;-acyl-ACP</td>
<td>5.66E-01</td>
<td>μU/μg</td>
<td>Estimated</td>
<td>7.14E-02</td>
<td>6.95E-02</td>
</tr>
<tr>
<td>FabF</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;-acyl-ACP</td>
<td>5.66E-01</td>
<td>μU/μg</td>
<td>Estimated</td>
<td>7.14E-02</td>
<td>1.06E+00</td>
</tr>
<tr>
<td>FabB</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;-acyl-ACP</td>
<td>1.46E+01</td>
<td>μU/μg</td>
<td>Estimated</td>
<td>2.63E-02</td>
<td>2.48E+00</td>
</tr>
<tr>
<td>FabB</td>
<td>C&lt;sub&gt;8&lt;/sub&gt;-acyl-ACP</td>
<td>1.46E+01</td>
<td>μU/μg</td>
<td>Estimated</td>
<td>2.63E-02</td>
<td>2.48E+00</td>
</tr>
<tr>
<td>FabB</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;-acyl-ACP</td>
<td>1.67E+01</td>
<td>μU/μg</td>
<td>Estimated</td>
<td>2.63E-02</td>
<td>2.83E+00</td>
</tr>
<tr>
<td>FabB</td>
<td>C&lt;sub&gt;12&lt;/sub&gt;-acyl-ACP</td>
<td>1.65E+01</td>
<td>μU/μg</td>
<td>Estimated</td>
<td>2.63E-02</td>
<td>2.80E+00</td>
</tr>
<tr>
<td>FabB</td>
<td>C&lt;sub&gt;14&lt;/sub&gt;-acyl-ACP</td>
<td>1.71E+01</td>
<td>μU/μg</td>
<td>Estimated</td>
<td>2.63E-02</td>
<td>2.90E+00</td>
</tr>
<tr>
<td>FabB</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;-acyl-ACP</td>
<td>2.14E+00</td>
<td>μU/μg</td>
<td>Estimated</td>
<td>2.63E-02</td>
<td>3.63E-01</td>
</tr>
<tr>
<td>FabB</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;-acyl-ACP</td>
<td>3.55E+00</td>
<td>μU/μg</td>
<td>Estimated</td>
<td>2.63E-02</td>
<td>6.02E-02</td>
</tr>
<tr>
<td>FabB</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;-cis-9-acyl-ACP</td>
<td>3.92E-01</td>
<td>μU/μg</td>
<td>Estimated</td>
<td>2.63E-02</td>
<td>6.02E-02</td>
</tr>
<tr>
<td>FabB</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;-acyl-ACP</td>
<td>3.92E-01</td>
<td>μU/μg</td>
<td>Estimated</td>
<td>2.63E-02</td>
<td>6.02E-02</td>
</tr>
<tr>
<td>FabB</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;-cis-11-acyl-ACP</td>
<td>3.92E-01</td>
<td>μU/μg</td>
<td>Estimated</td>
<td>2.63E-02</td>
<td>6.02E-02</td>
</tr>
</tbody>
</table>

†Orange highlights designate estimates of kinetic parameters described in Table B.3. We used these values to anchor our estimates of kinetic parameters for the other substrates (see Fig. B.2).

*C<sub>i</sub> refers to *i* carbons in an acyl chain. Unless otherwise noted, we assumed the kinetic parameters for unsaturated substrates to be the same as those of saturated substrates.

**Measured specific activities of FabZ/A<sup>2</sup> and FabF/B<sup>7</sup>. We estimated the specific activities of substrates that lacked kinetic data by equating them to the specific activities of substrates closest in length.

***Estimated values (see Note 1 and Fig. B.2)

****We estimated these values by assuming a specific activity ratio between unsaturated and saturated substrates of 0.0358 (i.e., the ratio for C<sub>14</sub> substrates<sup>3</sup>).
Table B.4A. Estimates of Kinetic Parameters

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Parameter</th>
<th>Label</th>
<th>Value</th>
<th>Units</th>
<th>Source</th>
<th>Detail</th>
</tr>
</thead>
<tbody>
<tr>
<td>FabD</td>
<td>$k_D$ mCoA</td>
<td>N/A</td>
<td>6.0 E1</td>
<td>μM</td>
<td>(37)*</td>
<td>Fixed</td>
</tr>
<tr>
<td>FabD</td>
<td>$k_o$ mCoA</td>
<td>k2_1f</td>
<td>1.3 E-3</td>
<td>μM⁻¹  s⁻¹</td>
<td>Calculated from $k_D$</td>
<td>Initial</td>
</tr>
<tr>
<td>FabD</td>
<td>$k_o$ FabD</td>
<td>k2_1r</td>
<td>8.0 E-2</td>
<td>s⁻¹</td>
<td>(38)**</td>
<td>Initial</td>
</tr>
<tr>
<td>FabD</td>
<td>$k_f$ FabD</td>
<td>k2_2f</td>
<td>1.58 E3</td>
<td>s⁻¹</td>
<td>Estimate from FabD $k_{cat}$</td>
<td>Initial</td>
</tr>
<tr>
<td>FabD</td>
<td>$k_f$ FabD</td>
<td>k2_2r</td>
<td>1.0 E-2</td>
<td>μM⁻¹  s⁻¹</td>
<td>****</td>
<td>Initial</td>
</tr>
<tr>
<td>FabD</td>
<td>$k_D$ ACP</td>
<td>N/A</td>
<td>4.0 E1</td>
<td>μM</td>
<td>(37)*</td>
<td>Fixed</td>
</tr>
<tr>
<td>FabD</td>
<td>$k_o$ ACP</td>
<td>k2_3f</td>
<td>5.43 E-4</td>
<td>μM⁻¹  s⁻¹</td>
<td>Calculated from $k_D$</td>
<td>Initial</td>
</tr>
<tr>
<td>FabD</td>
<td>$k_o$ ACP</td>
<td>k2_3r</td>
<td>2.17 E-2</td>
<td>s⁻¹</td>
<td>(40)</td>
<td>Initial</td>
</tr>
<tr>
<td>FabD</td>
<td>$k_f$ ACP</td>
<td>k2_4f</td>
<td>1.58 E3</td>
<td>s⁻¹</td>
<td>Estimate from FabD $k_{cat}$</td>
<td>Initial</td>
</tr>
<tr>
<td>FabH</td>
<td>$k_D$ aCoA</td>
<td>N/A</td>
<td>4.0 E1</td>
<td>μM</td>
<td>(41)*</td>
<td>Fixed</td>
</tr>
<tr>
<td>FabH</td>
<td>$k_o$ ACoA</td>
<td>k3_1f</td>
<td>2.0 E-3</td>
<td>μM⁻¹  s⁻¹</td>
<td>Calculated from $k_D$</td>
<td>Initial</td>
</tr>
<tr>
<td>FabH</td>
<td>$k_f$ FabH</td>
<td>k3_1r</td>
<td>8.0 E-2</td>
<td>s⁻¹</td>
<td>(38)**</td>
<td>Initial</td>
</tr>
<tr>
<td>FabH</td>
<td>$k_f$ FabH</td>
<td>k3_2f</td>
<td>1.58 E3</td>
<td>s⁻¹</td>
<td>Used $k_f$ FabD from FabD</td>
<td>Initial</td>
</tr>
<tr>
<td>FabH</td>
<td>$k_f$ FabH</td>
<td>k3_2r</td>
<td>1.0 E-2</td>
<td>μM⁻¹  s⁻¹</td>
<td>****</td>
<td>Initial</td>
</tr>
<tr>
<td>FabH</td>
<td>$k_D$ mACP</td>
<td>N/A</td>
<td>5.0 E0</td>
<td>μM</td>
<td>(41)*</td>
<td>Fixed</td>
</tr>
<tr>
<td>FabH</td>
<td>$k_o$ mACP</td>
<td>k3_3f</td>
<td>4.34 E-3</td>
<td>μM⁻¹  s⁻¹</td>
<td>Calculated from $k_D$</td>
<td>Initial</td>
</tr>
<tr>
<td>FabH</td>
<td>$k_o$ mACP</td>
<td>k3_3r</td>
<td>2.17 E-2</td>
<td>s⁻¹</td>
<td>Used $k_o$ ACP from FabD</td>
<td>Initial</td>
</tr>
<tr>
<td>FabH</td>
<td>$k_{cat}$</td>
<td>kcat3</td>
<td>3.13 E0</td>
<td>s⁻¹</td>
<td>(42)</td>
<td>Initial</td>
</tr>
<tr>
<td>FabG</td>
<td>$k_D$ NADPH</td>
<td>N/A</td>
<td>1.0 E-2</td>
<td>mM</td>
<td>(43)*</td>
<td>Fixed</td>
</tr>
<tr>
<td>FabG</td>
<td>$k_o$ NADPH</td>
<td>k4_1f</td>
<td>7.93 E-3</td>
<td>μM⁻¹  s⁻¹</td>
<td>Calculated from $k_D$</td>
<td>Initial</td>
</tr>
<tr>
<td>FabG</td>
<td>$k_o$ NADPH</td>
<td>k4_1r</td>
<td>7.93 E-2</td>
<td>s⁻¹</td>
<td>Used $k_o$ NADPH from FabH</td>
<td>Initial</td>
</tr>
<tr>
<td>FabG</td>
<td>$k_D$ βkaACP</td>
<td>N/A</td>
<td>1.70 E-2</td>
<td>mM</td>
<td>(44)*</td>
<td>Fixed</td>
</tr>
<tr>
<td>FabG</td>
<td>$k_o$ βkaACP</td>
<td>k4_2f</td>
<td>1.28 E-3</td>
<td>μM⁻¹  s⁻¹</td>
<td>Calculated from $k_D$</td>
<td>Initial</td>
</tr>
<tr>
<td>FabG</td>
<td>$k_o$ βkaACP</td>
<td>k4_2r</td>
<td>2.17 E-2</td>
<td>s⁻¹</td>
<td>Used $k_o$ ACP from FabD</td>
<td>Initial</td>
</tr>
<tr>
<td>FabG</td>
<td>$k_{cat}$</td>
<td>kcat4</td>
<td>5.90 E-1</td>
<td>s⁻¹</td>
<td>(43)</td>
<td>Initial</td>
</tr>
<tr>
<td>FabZ</td>
<td>$k_D$ βkaACP</td>
<td>N/A</td>
<td>5.60 E1</td>
<td>μM</td>
<td>(45)*</td>
<td>Fixed</td>
</tr>
<tr>
<td>FabZ</td>
<td>$k_o$ βkaACP</td>
<td>k5_1f</td>
<td>3.88 E-4</td>
<td>μM⁻¹  s⁻¹</td>
<td>Calculated from $k_D$</td>
<td>Initial</td>
</tr>
<tr>
<td>FabZ</td>
<td>$k_o$ βkaACP</td>
<td>k5_1r</td>
<td>2.17 E-2</td>
<td>s⁻¹</td>
<td>Used $k_o$ ACP from FabD</td>
<td>Initial</td>
</tr>
<tr>
<td>FabZ</td>
<td>$k_{eq}$ Rxn</td>
<td>N/A</td>
<td>2.5 E-1</td>
<td>unitless</td>
<td>(46) (Keq of FabA)</td>
<td>Fixed</td>
</tr>
<tr>
<td>FabZ</td>
<td>$k_{cat fwd}$</td>
<td>kcat5</td>
<td>2.72 E-1</td>
<td>s⁻¹</td>
<td>(5)</td>
<td>Initial</td>
</tr>
<tr>
<td>FabZ</td>
<td>$k_{cat rvs}$</td>
<td>kcat5_rvs</td>
<td>1.09 E0</td>
<td>s⁻¹</td>
<td>Calculated from $k_{eq}$</td>
<td>Initial</td>
</tr>
<tr>
<td>FabZ</td>
<td>$k_{KD eac ACP}$</td>
<td>N/A</td>
<td>5.60 E1</td>
<td>s⁻¹</td>
<td>Used $k_D$ of substrate</td>
<td>Fixed</td>
</tr>
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</table>
For these reversible reactions, we assumed similar binding of substrate and product. We estimated values of $K_d$ as an order-of-magnitude estimate of the forward acyl-transfer constant.

For this estimate, we assumed a rate constant approximately tenfold higher than $k_{on}\cdot m\text{CoA}$.

For these reversible reactions, we assumed similar binding of substrate and product (i.e., values of $K_d$, $k_{on}$, and $k_{off}$ were the same for both product and reactant).

Table B.4B. Estimates of Kinetic Parameters

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Parameter</th>
<th>Label</th>
<th>Value</th>
<th>Units</th>
<th>Source</th>
<th>Detail</th>
</tr>
</thead>
<tbody>
<tr>
<td>FabI</td>
<td>$k_{cat}$</td>
<td>kcat6</td>
<td>4.0E0</td>
<td>s⁻¹</td>
<td>(49)</td>
<td>Initial</td>
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<tr>
<td>TesA</td>
<td>$k_{en}-C_{4a}ACP$</td>
<td>k7_1f-C4</td>
<td>4.59 E-3</td>
<td>μM⁻¹ s⁻¹</td>
<td>(1, 50)</td>
<td>Initial</td>
</tr>
<tr>
<td>TesA</td>
<td>$k_{off}-C_{4a}ACP$</td>
<td>k7_1f-C6</td>
<td>7.38 E-3</td>
<td>μM⁻¹ s⁻¹</td>
<td>(1, 50)</td>
<td>Initial</td>
</tr>
<tr>
<td>TesA</td>
<td>$k_{on}-C_{6a}ACP$</td>
<td>k7_1f-C8</td>
<td>4.10 E-2</td>
<td>μM⁻¹ s⁻¹</td>
<td>(1, 50)</td>
<td>Initial</td>
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<tr>
<td>TesA</td>
<td>$k_{cat}-C_{10a}ACP$</td>
<td>k7_1f-C10</td>
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<td>μM⁻¹ s⁻¹</td>
<td>(1, 50)</td>
<td>Initial</td>
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<tr>
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<td>$k_{on}-C_{12a}ACP$</td>
<td>k7_1f-C12</td>
<td>3.03 E-1</td>
<td>μM⁻¹ s⁻¹</td>
<td>(1, 50)</td>
<td>Initial</td>
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<tr>
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<td>$k_{on}-C_{14a}ACP$</td>
<td>k7_1f-C14</td>
<td>5.42 E-1</td>
<td>μM⁻¹ s⁻¹</td>
<td>(1, 50)</td>
<td>Initial</td>
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<td>k7_1f-C16</td>
<td>0.96669</td>
<td>μM⁻¹ s⁻¹</td>
<td>(1, 50)</td>
<td>Initial</td>
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<td>$k_{on}-C_{18a}ACP$</td>
<td>k7_1f-C18</td>
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<td>μM⁻¹ s⁻¹</td>
<td>(1, 50)</td>
<td>Initial</td>
</tr>
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<td>k7_1f-C20</td>
<td>3.08 E0</td>
<td>μM⁻¹ s⁻¹</td>
<td>(1, 50)</td>
<td>Initial</td>
</tr>
<tr>
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<td>$k_{on}-C_{20a}ACP$</td>
<td>k7_1r</td>
<td>2.17E0</td>
<td>s⁻¹</td>
<td>(1)</td>
<td>Initial</td>
</tr>
<tr>
<td>TesA</td>
<td>$k_{cat}-C_{4}$</td>
<td>kcat7-C4</td>
<td>6.13 E0</td>
<td>s⁻¹</td>
<td>(1, 50)</td>
<td>Initial</td>
</tr>
<tr>
<td>TesA</td>
<td>$k_{cat}-C_{6}$</td>
<td>kcat7-C6</td>
<td>5.50 E0</td>
<td>s⁻¹</td>
<td>(1, 50)</td>
<td>Initial</td>
</tr>
<tr>
<td>TesA</td>
<td>$k_{cat}-C_{8}$</td>
<td>kcat7-C8</td>
<td>1.12 E1</td>
<td>s⁻¹</td>
<td>(1, 50)</td>
<td>Initial</td>
</tr>
<tr>
<td>TesA</td>
<td>$k_{cat}-C_{10}$</td>
<td>kcat7-C10</td>
<td>1.71 E0</td>
<td>s⁻¹</td>
<td>(1, 50)</td>
<td>Initial</td>
</tr>
<tr>
<td>TesA</td>
<td>$k_{cat}-C_{12}$</td>
<td>kcat7-C12</td>
<td>2.73 E1</td>
<td>s⁻¹</td>
<td>(1, 50)</td>
<td>Initial</td>
</tr>
<tr>
<td>TesA</td>
<td>$k_{cat}-C_{14}$</td>
<td>kcat7-C14</td>
<td>4.95 E1</td>
<td>s⁻¹</td>
<td>(1, 50)</td>
<td>Initial</td>
</tr>
<tr>
<td>TesA</td>
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<td>kcat7-C16</td>
<td>1.08 E2</td>
<td>s⁻¹</td>
<td>(1, 50)</td>
<td>Initial</td>
</tr>
<tr>
<td>TesA</td>
<td>$k_{cat}-C_{18}$</td>
<td>kcat7-C18</td>
<td>1.32 E2</td>
<td>s⁻¹</td>
<td>(1, 50)</td>
<td>Initial</td>
</tr>
<tr>
<td>TesA</td>
<td>$k_{cat}-C_{20}$</td>
<td>kcat7-C20</td>
<td>1.66 E2</td>
<td>s⁻¹</td>
<td>(1, 50)</td>
<td>Initial</td>
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<tr>
<td>FabF</td>
<td>$K_{D-F_{m}}ACP$</td>
<td>N/A</td>
<td>1.40 E-2</td>
<td>mM</td>
<td>(51)*</td>
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<tr>
<td>FabF</td>
<td>$k_{on}-F_{m}ACP$</td>
<td>k8_1f</td>
<td>1.55 E-3</td>
<td>μM⁻¹ s⁻¹</td>
<td>Calculated from $K_d$</td>
<td>Initial</td>
</tr>
<tr>
<td>FabF</td>
<td>$k_{off}-F_{m}ACP$</td>
<td>k8_1r</td>
<td>2.17 E-2</td>
<td>s⁻¹</td>
<td>Used $k_{off}-ACP$ from FabD</td>
<td>Initial</td>
</tr>
<tr>
<td>FabF</td>
<td>$k_{off}-F_{m}ACP$</td>
<td>k8_2f</td>
<td>1.58 E3</td>
<td>s⁻¹</td>
<td>Used $k_{off}$ from FabD**</td>
<td>Initial</td>
</tr>
<tr>
<td>FabF</td>
<td>$k_{on}-F_{m}ACP$</td>
<td>k8_2r</td>
<td>1.0 E-2</td>
<td>μM⁻¹ s⁻¹</td>
<td>***</td>
<td>Initial</td>
</tr>
<tr>
<td>FabF</td>
<td>$K_{D-F_{m}}ACP$</td>
<td>N/A</td>
<td>8.20 E-3</td>
<td>mM</td>
<td>(52)*</td>
<td>Fixed</td>
</tr>
<tr>
<td>FabF</td>
<td>$k_{\text{on-F}_m\text{ACP}}$</td>
<td>$k_{8_3f}$</td>
<td>$2.65 \times 10^{-3}$</td>
<td>$\mu M^{-1} s^{-1}$</td>
<td>Calculated from $K_D$</td>
<td>Initial</td>
</tr>
<tr>
<td>FabF</td>
<td>$k_{\text{off-F}_m\text{ACP}}$</td>
<td>$k_{8_3r}$</td>
<td>$2.17 \times 10^{-2}$</td>
<td>$s^{-1}$</td>
<td>Used $k_{\text{FabD}}$ from FabD**</td>
<td>Initial</td>
</tr>
<tr>
<td>FabF</td>
<td>$k_{\text{cat}}$</td>
<td>$k_{\text{cat}8}$</td>
<td>$3.13 \times 10^{-2}$</td>
<td>$s^{-1}$</td>
<td>(52)</td>
<td>Initial</td>
</tr>
<tr>
<td>FabB</td>
<td>$K_{D-B_{\text{acACP}}}$</td>
<td>N/A</td>
<td>$3.80 \times 10^{-1}$</td>
<td>$\mu M$</td>
<td>(53)*</td>
<td>Fixed</td>
</tr>
<tr>
<td>FabB</td>
<td>$k_{\text{on-B}_{\text{acACP}}}$</td>
<td>$k_{10_1f}$</td>
<td>$1.55 \times 10^{-3}$</td>
<td>$\mu M^{-1} s^{-1}$</td>
<td>Calculated from $K_D$</td>
<td>Initial</td>
</tr>
<tr>
<td>FabB</td>
<td>$k_{\text{off-B}_{\text{acACP}}}$</td>
<td>$k_{10_1r}$</td>
<td>$2.17 \times 10^{-2}$</td>
<td>$s^{-1}$</td>
<td>Used $k_{\text{off-ACP}}$ from FabD</td>
<td>Initial</td>
</tr>
<tr>
<td>FabB</td>
<td>$K_{D-B_{\text{cisACP}}}$</td>
<td>N/A</td>
<td>$3.80 \times 10^{-1}$</td>
<td>$\mu M$</td>
<td>Est. based on sat. rxn.</td>
<td>Fixed</td>
</tr>
<tr>
<td>FabB</td>
<td>$k_{\text{on-B}_{\text{cisACP}}}$</td>
<td>$k_{10_4f}$</td>
<td>$1.55 \times 10^{-3}$</td>
<td>$\mu M^{-1} s^{-1}$</td>
<td>Est. based on sat. rxn.</td>
<td>Initial</td>
</tr>
<tr>
<td>FabB</td>
<td>$k_{\text{off-B}_{\text{cisACP}}}$</td>
<td>$k_{10_4r}$</td>
<td>$2.17 \times 10^{-2}$</td>
<td>$s^{-1}$</td>
<td>Est. based on sat. rxn.</td>
<td>Initial</td>
</tr>
<tr>
<td>FabB</td>
<td>$k_{\text{rwdfabB}^*}$</td>
<td>$k_{10_2f}$</td>
<td>$1.58 \times 10^{3}$</td>
<td>$s^{-1}$</td>
<td>Used $k_{\text{FabD}}$ from FabD**</td>
<td>Initial</td>
</tr>
<tr>
<td>FabB</td>
<td>$k_{\text{revfabB}^*}$</td>
<td>$k_{10_2r}$</td>
<td>$1.0 \times 10^{-2}$</td>
<td>$\mu M^{-1} s^{-1}$</td>
<td>***</td>
<td>Initial</td>
</tr>
<tr>
<td>FabB</td>
<td>$K_{D-B_{\text{mACP}}}$</td>
<td>N/A</td>
<td>$8.20 \times 10^{-3}$</td>
<td>$mM$</td>
<td>(52)*</td>
<td>Fixed</td>
</tr>
<tr>
<td>FabB</td>
<td>$k_{\text{on-B}_{\text{mACP}}}$</td>
<td>$k_{10_3f}$</td>
<td>$2.65 \times 10^{-3}$</td>
<td>$\mu M^{-1} s^{-1}$</td>
<td>Calculated from $K_D$</td>
<td>Initial</td>
</tr>
<tr>
<td>FabB</td>
<td>$k_{\text{off-B}_{\text{mACP}}}$</td>
<td>$k_{10_3r}$</td>
<td>$2.17 \times 10^{-2}$</td>
<td>$s^{-1}$</td>
<td>Used $k_{\text{FabD}}$ from FabD**</td>
<td>Initial</td>
</tr>
<tr>
<td>FabB</td>
<td>$k_{\text{cat}}$</td>
<td>$k_{\text{cat}10}$</td>
<td>$2.90 \times 10^{-2}$</td>
<td>$s^{-1}$</td>
<td>(52)</td>
<td>Initial</td>
</tr>
</tbody>
</table>

†Orange highlights designate estimates of kinetic constants for preferred substrates; we used these values to anchor the estimates describe in Table B.3. For FabB and FabF, we used measurements of $k_{\text{cat}}$ on lauroyl-ACP.

*We estimated values of $K_D$ from measurements of $K_M$ (i.e., $K_D \approx K_M$)

**To clarify, we used $k_{\text{cat}}$ as an order-of-magnitude estimate of the forward acyl-transfer constant.

***For this estimate, we assumed a rate constant approximately tenfold higher than $k_{\text{on-mCoA}}$. 

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Table B.4C. Estimates of Kinetic Parameters

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Parameter</th>
<th>Label</th>
<th>Value</th>
<th>Units</th>
<th>Source</th>
<th>Detail</th>
</tr>
</thead>
<tbody>
<tr>
<td>FabA</td>
<td>$K_D$-lhaACP</td>
<td>N/A</td>
<td>5.60 E1</td>
<td>μM</td>
<td>(45)*</td>
<td>Fixed</td>
</tr>
<tr>
<td>FabA</td>
<td>$k_{on}$-lhaACP</td>
<td>$k_{9_1f}$</td>
<td>3.88 E-4</td>
<td>μM$^{-1}$ s$^{-1}$</td>
<td>Calculated from $K_D$</td>
<td>Initial</td>
</tr>
<tr>
<td>FabA</td>
<td>$k_{off}$-lhaACP</td>
<td>$k_{9_1r}$</td>
<td>2.17 E-2</td>
<td>s$^{-1}$</td>
<td>Used $k_{off}$-ACP from FabD</td>
<td>Initial</td>
</tr>
<tr>
<td>FabA</td>
<td>$k_{cat}$</td>
<td>$k_{cat9}$</td>
<td>2.72 E-1</td>
<td>s$^{-1}$</td>
<td>(5)</td>
<td>Initial</td>
</tr>
<tr>
<td>FabA</td>
<td>$k_{cat_rvs}$</td>
<td>$k_{cat9_rvs}$</td>
<td>1.09 E0</td>
<td>s$^{-1}$</td>
<td>(5)</td>
<td>Initial</td>
</tr>
<tr>
<td>FabA</td>
<td>$k_{cat_unsat}$</td>
<td>$k_{cat9_un}$</td>
<td>2.72 E-1</td>
<td>s$^{-1}$</td>
<td>Est. based on sat. rxn.</td>
<td>Initial</td>
</tr>
<tr>
<td>FabA</td>
<td>$k_{cat_rvs_unsat}$</td>
<td>$k_{cat9_rvs_un}$</td>
<td>1.09 E0</td>
<td>s$^{-1}$</td>
<td>Est. based on sat. rxn.</td>
<td>Initial</td>
</tr>
<tr>
<td>FabA</td>
<td>$K_D$-eacACP</td>
<td>N/A</td>
<td>5.60 E1</td>
<td>μM</td>
<td>Est. with $K_D$ of substrate</td>
<td>Fixed</td>
</tr>
<tr>
<td>FabA</td>
<td>$k_{off}$-eacACP</td>
<td>$k_{9_3f}$</td>
<td>2.17 E-2</td>
<td>s$^{-1}$</td>
<td>**</td>
<td>Initial</td>
</tr>
<tr>
<td>FabA</td>
<td>$k_{on}$-eacACP</td>
<td>$k_{9_3r}$</td>
<td>3.88 E-4</td>
<td>μM$^{-1}$ s$^{-1}$</td>
<td>**</td>
<td>Initial</td>
</tr>
<tr>
<td>FabA</td>
<td>$K_D$-cisACP</td>
<td>N/A</td>
<td>5.60 E1</td>
<td>μM</td>
<td>Est. with $K_D$ of substrate</td>
<td>Fixed</td>
</tr>
<tr>
<td>FabA</td>
<td>$k_{off}$-cisACP</td>
<td>$k_{9_4f}$</td>
<td>2.17 E-2</td>
<td>s$^{-1}$</td>
<td>**</td>
<td>Initial</td>
</tr>
<tr>
<td>FabA</td>
<td>$k_{on}$-cisACP</td>
<td>$k_{9_4r}$</td>
<td>3.88 E-4</td>
<td>μM$^{-1}$ s$^{-1}$</td>
<td>**</td>
<td>Initial</td>
</tr>
</tbody>
</table>

* Orange highlights designate estimates of kinetic constants for preferred substrates; we used these values to anchor the estimates describe in Table B.3.

** We estimated values of $K_D$ from measurements of $K_M$ (i.e., $K_d \approx K_M$)

** For these reversible reactions, we assumed similar binding of substrate and product (i.e., values of $K_d$, $k_{on}$ and $k_{off}$ were the same for both product and reactant).
# Table B.5. Estimates of Kinetic Parameters for Inhibition

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Parameter</th>
<th>Label</th>
<th>Value</th>
<th>Units</th>
<th>Source</th>
<th>Detail</th>
</tr>
</thead>
<tbody>
<tr>
<td>FabH</td>
<td>$K_D$-ACP</td>
<td>N/A</td>
<td>3.80 E1</td>
<td>µM</td>
<td>(53)*</td>
<td>Fixed</td>
</tr>
<tr>
<td>FabH</td>
<td>$k_{on}$-ACP</td>
<td>$k_{on}$-1</td>
<td>2.41E-05</td>
<td>µM$^{-1}$ s$^{-1}$</td>
<td>(1, 54)</td>
<td>Initial</td>
</tr>
<tr>
<td>FabH</td>
<td>$k_{off}$-ACP</td>
<td>$k_{off}$-1</td>
<td>2.17E-02</td>
<td>s$^{-1}$</td>
<td>(1, 54)</td>
<td>Initial</td>
</tr>
<tr>
<td>FabH</td>
<td>$k_{on}$-(4-20)ACP</td>
<td>$k_{on}$-1, (4-20)</td>
<td>3.09E-1</td>
<td>µM$^{-1}$ s$^{-1}$</td>
<td>(1, 54)</td>
<td>Initial</td>
</tr>
<tr>
<td>FabH</td>
<td>$k_{off}$-(4-12ACP</td>
<td>$k_{off}$-1, (4-12)</td>
<td>1.34E3</td>
<td>s$^{-1}$</td>
<td>(1, 54)</td>
<td>Initial</td>
</tr>
<tr>
<td>FabH</td>
<td>$k_{off}$-C(4,20)ACP</td>
<td>$k_{off}$-1, C(4,20)</td>
<td>1.17E1</td>
<td>s$^{-1}$</td>
<td>(1, 54)</td>
<td>Initial</td>
</tr>
<tr>
<td>FabH</td>
<td>$k_{off}$-(4,12ACP-FabH*</td>
<td>$k_{off}$-1, (4-12)</td>
<td>1.32E1</td>
<td>s$^{-1}$</td>
<td>(1, 54)</td>
<td>Initial</td>
</tr>
<tr>
<td>FabH</td>
<td>$k_{off}$-(C-20ACPFabH*)</td>
<td>$k_{off}$-1, C-20</td>
<td>3.89E0</td>
<td>s$^{-1}$</td>
<td>(1, 54)</td>
<td>Initial</td>
</tr>
<tr>
<td>FabG</td>
<td>$K_D$-ACP</td>
<td>N/A</td>
<td>3.80 E1</td>
<td>µM</td>
<td>(53)*</td>
<td>Fixed</td>
</tr>
<tr>
<td>FabZ</td>
<td>$K_D$-ACP</td>
<td>N/A</td>
<td>3.80 E1</td>
<td>µM</td>
<td>(53)*</td>
<td>Fixed</td>
</tr>
<tr>
<td>FabA</td>
<td>$K_D$-ACP</td>
<td>N/A</td>
<td>3.80 E1</td>
<td>µM</td>
<td>(53)*</td>
<td>Fixed</td>
</tr>
<tr>
<td>FabF</td>
<td>$K_D$-ACP</td>
<td>N/A</td>
<td>3.80 E1</td>
<td>µM</td>
<td>(53)*</td>
<td>Fixed</td>
</tr>
<tr>
<td>TesA</td>
<td>$K_D$-ACP</td>
<td>N/A</td>
<td>3.80 E1</td>
<td>µM</td>
<td>(53)*</td>
<td>Fixed</td>
</tr>
</tbody>
</table>

*We used the measured $K_d$ for the FabB-C$_8$ACP complex to estimate the $K_d$ for competitive inhibition by ACP.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description*</th>
<th>Optimized Value**</th>
<th>Minimum Value</th>
<th>Maximum Value***</th>
</tr>
</thead>
<tbody>
<tr>
<td>a1</td>
<td>Scales $k_{\text{off}}$ for 1-4</td>
<td>1.425 E5</td>
<td>1.0 E-1</td>
<td>2.0 E5</td>
</tr>
<tr>
<td>a2</td>
<td>Scales $k_{\text{off}}$ for acyl-ACPs for 5-12 and 14-21</td>
<td>7.598 E3</td>
<td>1.0 E-1</td>
<td>2.0 E5</td>
</tr>
<tr>
<td>a3</td>
<td>Scales $k_{\text{off}}$ for 13</td>
<td>4.277 E1</td>
<td>1.0 E-1</td>
<td>1.0 E5</td>
</tr>
<tr>
<td>b1</td>
<td>Scales $k_1$ for 1-3,11, 18, 20</td>
<td>4.021 E4</td>
<td>1.0 E-1</td>
<td>1.0 E5</td>
</tr>
<tr>
<td>b2</td>
<td>Scales $K_{\text{eq}}$ for acyl transfer in 1</td>
<td>6.678 E-3</td>
<td>1.0 E-5</td>
<td>1.0 E0</td>
</tr>
<tr>
<td>b3</td>
<td>Scales $K_{\text{eq}}$ for acyl transfer in 2, 3, 11,18, 20</td>
<td>2.850 E-1</td>
<td>1.0 E-5</td>
<td>1.0 E0</td>
</tr>
<tr>
<td>c1</td>
<td>Scales $k_{\text{cat}}$ for 4</td>
<td>4.646 E0</td>
<td>1.0 E-3</td>
<td>1.5 E1</td>
</tr>
<tr>
<td>c2</td>
<td>Scales $k_{\text{cat}}$ for 6, 10, 12, 16, 19, 21</td>
<td>8.885 E1</td>
<td>1.0 E-1</td>
<td>2.40 E2</td>
</tr>
<tr>
<td>c3</td>
<td>Scales $k_{\text{cat}}$ for 13</td>
<td>5.388 E-3</td>
<td>1.0 E-4</td>
<td>1.0 E0</td>
</tr>
<tr>
<td>c4</td>
<td>Scales $k_{\text{cat}}$ for 7</td>
<td>2.180 E3</td>
<td>1.0 E-1</td>
<td>2.0 E5</td>
</tr>
<tr>
<td>d1</td>
<td>Scales substrate specificity of TesA</td>
<td>-2.857 E-1</td>
<td>-5.0 E-1</td>
<td>5.0 E-1</td>
</tr>
<tr>
<td>d2</td>
<td>Scales substrate specificity of TesA</td>
<td>3.349 E0</td>
<td>-1.0 E1</td>
<td>1.0 E1</td>
</tr>
<tr>
<td>e</td>
<td>Scales inhibition of (i) FabH and FabF by ACPs and (ii) FabH by acyl-ACPs</td>
<td>2.887 E0</td>
<td>1.0 E-1</td>
<td>1.0 E3</td>
</tr>
<tr>
<td>f</td>
<td>Scales $K_{\text{eq}}$ for isomerization in 16</td>
<td>1.590 E1</td>
<td>1.0 E-1</td>
<td>1.0 E3</td>
</tr>
</tbody>
</table>

*The numbers in these descriptions correspond to lines (i.e., reactions) of Table B.1.

**We determined these values by optimizing our kinetic model (see Fig B.2).

***Estimated upper limits for scaling parameters. The maximum value of each scaling parameter depends on the values of other scaling parameters; we derived these values using our optimized values of scaling parameters. During model fitting, we estimated new maximum values for each scaling parameter as follows: For a, b, d, and e, we chose the maximum value for which the values of $k_{\text{cat}}$ do not exceed their diffusion limits, as described previously. For c, we chose a maximum value that achieved catalytic rate constants equal to their maximum observed literature values. For f, we assumed the isomerization reaction to be reversible with a weak preference for products and thus chose a range that included—but did not require—such a preference.

****Parameters are as described previously, except for the new parameters c4 and f which are defined in the Materials and Methods section (Parameterization of FabZ, FabA, FabF, and FabB).
Table B.7. Analysis of the Sensitivity of Pathway Objectives to Enzyme Concentration*

<table>
<thead>
<tr>
<th>Objective</th>
<th>Enzyme</th>
<th>Min</th>
<th>Max</th>
<th>EE Mean</th>
<th>EE SD</th>
<th>Mean/SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturation</td>
<td>FabD</td>
<td>0.1</td>
<td>10</td>
<td>2.55E-02</td>
<td>1.32E-01</td>
<td>1.94E-01</td>
</tr>
<tr>
<td>Saturation</td>
<td>FabH</td>
<td>0.1</td>
<td>10</td>
<td>1.06E-01</td>
<td>1.19E-01</td>
<td>8.90E-01</td>
</tr>
<tr>
<td>Saturation</td>
<td>FabG</td>
<td>0.1</td>
<td>10</td>
<td>3.26E-03</td>
<td>2.23E-02</td>
<td>1.46E-01</td>
</tr>
<tr>
<td>Saturation</td>
<td>FabZ</td>
<td>0</td>
<td>10</td>
<td>1.15E-01</td>
<td>1.48E-01</td>
<td>7.73E-01</td>
</tr>
<tr>
<td>Saturation</td>
<td>FabA</td>
<td>0</td>
<td>10</td>
<td>1.49E-01</td>
<td>2.96E-01</td>
<td>5.05E-01</td>
</tr>
<tr>
<td>Saturation</td>
<td>FabI</td>
<td>0.1</td>
<td>10</td>
<td>2.94E-01</td>
<td>5.73E-01</td>
<td>5.12E-01</td>
</tr>
<tr>
<td>Saturation</td>
<td>FabB</td>
<td>0</td>
<td>10</td>
<td>2.73E-01</td>
<td>2.59E-01</td>
<td>1.05E+00</td>
</tr>
<tr>
<td>Saturation</td>
<td>TesA</td>
<td>0.1</td>
<td>10</td>
<td>8.52E-03</td>
<td>2.39E-02</td>
<td>3.57E-01</td>
</tr>
<tr>
<td>Avg. Length</td>
<td>FabD</td>
<td>0.1</td>
<td>10</td>
<td>1.86E-01</td>
<td>1.47E+00</td>
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<tr>
<td>Avg. Length</td>
<td>FabH</td>
<td>0.1</td>
<td>10</td>
<td>1.30E+00</td>
<td>1.16E+00</td>
<td>1.12E+00</td>
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<td>Avg. Length</td>
<td>FabG</td>
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<td>10</td>
<td>2.77E-02</td>
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<td>1.06E-01</td>
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<tr>
<td>Avg. Length</td>
<td>FabZ</td>
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<td>10</td>
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<td>FabA</td>
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<td>10</td>
<td>2.22E-01</td>
<td>5.85E-01</td>
<td>3.80E-01</td>
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<tr>
<td>Avg. Length</td>
<td>FabI</td>
<td>0.1</td>
<td>10</td>
<td>3.13E-01</td>
<td>7.55E-01</td>
<td>4.14E-01</td>
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<td>Avg. Length</td>
<td>FabB</td>
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<td>10</td>
<td>9.45E-01</td>
<td>8.37E-01</td>
<td>1.13E+00</td>
</tr>
<tr>
<td>Avg. Length</td>
<td>FabF</td>
<td>0</td>
<td>10</td>
<td>2.39E+00</td>
<td>1.59E+00</td>
<td>1.50E+00</td>
</tr>
<tr>
<td>Avg. Length</td>
<td>TesA</td>
<td>0.1</td>
<td>10</td>
<td>3.19E+00</td>
<td>2.85E+00</td>
<td>1.12E+00</td>
</tr>
<tr>
<td>Production</td>
<td>FabD</td>
<td>0.1</td>
<td>10</td>
<td>1.16E+00</td>
<td>5.21E+00</td>
<td>2.23E-01</td>
</tr>
<tr>
<td>Production</td>
<td>FabH</td>
<td>0.1</td>
<td>10</td>
<td>2.85E+01</td>
<td>2.74E+01</td>
<td>1.04E+00</td>
</tr>
<tr>
<td>Production</td>
<td>FabG</td>
<td>0.1</td>
<td>10</td>
<td>1.08E+00</td>
<td>8.76E+00</td>
<td>1.24E-01</td>
</tr>
<tr>
<td>Production</td>
<td>FabZ</td>
<td>0</td>
<td>10</td>
<td>9.26E+00</td>
<td>4.39E+01</td>
<td>2.11E-01</td>
</tr>
<tr>
<td>Production</td>
<td>FabA</td>
<td>0</td>
<td>10</td>
<td>2.28E+00</td>
<td>7.36E+00</td>
<td>3.11E-01</td>
</tr>
<tr>
<td>Production</td>
<td>FabI</td>
<td>0.1</td>
<td>10</td>
<td>2.52E+00</td>
<td>1.14E+01</td>
<td>2.22E-01</td>
</tr>
<tr>
<td>Production</td>
<td>FabB</td>
<td>0</td>
<td>10</td>
<td>2.09E+00</td>
<td>6.56E+00</td>
<td>3.19E-01</td>
</tr>
<tr>
<td>Production</td>
<td>FabF</td>
<td>0</td>
<td>10</td>
<td>2.75E+00</td>
<td>8.49E+00</td>
<td>3.24E-01</td>
</tr>
<tr>
<td>Production</td>
<td>TesA</td>
<td>0.1</td>
<td>10</td>
<td>2.76E+00</td>
<td>5.86E+00</td>
<td>4.71E-01</td>
</tr>
</tbody>
</table>

*This table shows the mean elementary effects used to generate Fig. 3.3E. Note: A large standard deviation indicates that the influence of a particular parameter is highly dependent on the values of other parameters.*55
Table B.8A. Rate equations for the biosynthesis of lipid A

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Rate Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>LpxC, LpxK, LpxL, LpxM, MsbA</td>
<td>rate = ( \frac{k_{cat}[E][S]}{K_m + [S]} )</td>
</tr>
<tr>
<td>LpxH</td>
<td>rate = ( \frac{k_{cat}[E][S]}{(K_m + [S])(1 + \frac{[P]}{K_i})} )</td>
</tr>
<tr>
<td>LpxA, LpxB, WaaA</td>
<td>rate = ( \frac{k_{cat}[E][S_1][S_2]}{(K_{m1} + [S])(K_{m2} + [S_2])} )</td>
</tr>
<tr>
<td>LpxD</td>
<td>rate = ( \frac{k_{cat}[E][S_1][S_2]}{(K_{m1} + [S])(K_{m2} + [S_2])(1 + \frac{[P]}{K_i})} )</td>
</tr>
</tbody>
</table>

Table B.8B. Rate equations for the biosynthesis of lipid A

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Original Equation</th>
<th>New Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>LpxL, LpxM</td>
<td>rate = ( \frac{k_{cat}[E][S]}{K_m + [S]} )</td>
<td>rate = ( \frac{k_{cat}[E][S_1][S_2]}{(K_{m1} + [S])(K_{m2} + [S_2])} )</td>
</tr>
</tbody>
</table>

Table B.8C. Rate equations for phospholipid synthesis

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Rate Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PlsB, PlsC</td>
<td>rate = ( \frac{k_{cat}[E][S_1][S_2]}{(K_{m1} + [S])(K_{m2} + [S_2])} )</td>
</tr>
</tbody>
</table>
Table B.9A. Kinetic parameters for Lipid A and phospholipid biosynthesis.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDP-GlcNAc</td>
<td>Concentration</td>
<td>430</td>
<td>µM</td>
<td>(16)</td>
</tr>
<tr>
<td>CMP-KDO</td>
<td>Concentration</td>
<td>4.957E3</td>
<td>µM</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxA</td>
<td>Concentration</td>
<td>1.6457</td>
<td>µM</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxA</td>
<td>( K_{m1} )</td>
<td>0.82</td>
<td>mM</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxA</td>
<td>( K_{m2} )</td>
<td>0.0016</td>
<td>mM</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxA</td>
<td>( k_{cat, fwd} )</td>
<td>0.0071</td>
<td>s(^{-1})</td>
<td>fit*</td>
</tr>
<tr>
<td>LpxA</td>
<td>( k_{cat, rvs} )</td>
<td>717</td>
<td>s(^{-1})</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxC</td>
<td>Promoter</td>
<td>0.0025</td>
<td>µM</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxC</td>
<td>( K_m )</td>
<td>0.00019</td>
<td>mM</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxC</td>
<td>( k_{cat} )</td>
<td>3.3</td>
<td>s(^{-1})</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxC</td>
<td>( k_{trans} )</td>
<td>0.148</td>
<td>s(^{-1})</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxC</td>
<td>( k_{degrade} )</td>
<td>9.62E-5</td>
<td>s(^{-1})</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxD</td>
<td>Concentration</td>
<td>1.1227</td>
<td>µM</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxD</td>
<td>( K_{m1} )</td>
<td>0.0025</td>
<td>mM</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxD</td>
<td>( K_{m2} )</td>
<td>0.0032</td>
<td>mM</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxD</td>
<td>( k_{cat} )</td>
<td>23</td>
<td>s(^{-1})</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxD</td>
<td>( K_i )</td>
<td>0.0094</td>
<td>mM</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxH</td>
<td>Concentration</td>
<td>0.4387</td>
<td>µM</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxH</td>
<td>( K_m )</td>
<td>0.0617</td>
<td>mM</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxH</td>
<td>( k_{cat} )</td>
<td>47</td>
<td>s(^{-1})</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxH</td>
<td>( K_i )</td>
<td>0.015</td>
<td>mM</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxB</td>
<td>Concentration</td>
<td>0.9517</td>
<td>µM</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxB</td>
<td>( K_{m1} )</td>
<td>0.287</td>
<td>mM</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxB</td>
<td>( K_{m2} )</td>
<td>0.381</td>
<td>mM</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxB</td>
<td>( k_{cat} )</td>
<td>129</td>
<td>s(^{-1})</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxK</td>
<td>Concentration</td>
<td>1.071</td>
<td>µM</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxK</td>
<td>( K_m )</td>
<td>0.04</td>
<td>mM</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxK</td>
<td>( k_{cat} )</td>
<td>2.1</td>
<td>s(^{-1})</td>
<td>(15)</td>
</tr>
<tr>
<td>WaaA</td>
<td>Promoter</td>
<td>0.0025</td>
<td>µM</td>
<td>(15)</td>
</tr>
<tr>
<td>WaaA</td>
<td>( K_{m1} )</td>
<td>0.088</td>
<td>mM</td>
<td>(15)</td>
</tr>
<tr>
<td>WaaA</td>
<td>( K_{m2} )</td>
<td>0.052</td>
<td>mM</td>
<td>(15)</td>
</tr>
<tr>
<td>WaaA</td>
<td>( k_{cat, 1} )</td>
<td>16.7</td>
<td>s(^{-1})</td>
<td>(15)</td>
</tr>
<tr>
<td>WaaA</td>
<td>( k_{cat, 2} )</td>
<td>1.9</td>
<td>s(^{-1})</td>
<td>(15)</td>
</tr>
<tr>
<td>WaaA</td>
<td>( K_i )</td>
<td>0.0317</td>
<td>mM</td>
<td>(15)</td>
</tr>
</tbody>
</table>

*Parameter value fit in this study, as described in methods.
Table B.9B. Kinetic parameters for Lipid A and phospholipid biosynthesis.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>WaaA</td>
<td>$k_{\text{translate}}$</td>
<td>0.176</td>
<td>s$^{-1}$</td>
<td>(15)</td>
</tr>
<tr>
<td>WaaA</td>
<td>$k_{\text{degrade}}$</td>
<td>1.8E-4</td>
<td>s$^{-1}$</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxL</td>
<td>Concentration</td>
<td>2.30</td>
<td>µM</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxL</td>
<td>$K_m$</td>
<td>0.015</td>
<td>mM</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxL</td>
<td>$k_{\text{cat}}$</td>
<td>131</td>
<td>s$^{-1}$</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxM</td>
<td>Concentration</td>
<td>9.2197</td>
<td>µM</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxM</td>
<td>$K_m$</td>
<td>0.00275</td>
<td>mM</td>
<td>(15)</td>
</tr>
<tr>
<td>LpxM</td>
<td>$k_{\text{cat}}$</td>
<td>0.6</td>
<td>s$^{-1}$</td>
<td>(15)</td>
</tr>
<tr>
<td>MsbA</td>
<td>Concentration</td>
<td>0.5106</td>
<td>µM</td>
<td>(15)</td>
</tr>
<tr>
<td>MsbA</td>
<td>$K_m$</td>
<td>0.021</td>
<td>mM</td>
<td>(15)</td>
</tr>
<tr>
<td>MsbA</td>
<td>$k_{\text{cat}}$</td>
<td>166</td>
<td>s$^{-1}$</td>
<td>(15)</td>
</tr>
<tr>
<td>FtsH</td>
<td>Concentration</td>
<td>1.435</td>
<td>µM</td>
<td>(15)</td>
</tr>
<tr>
<td>FtsH$^*_{\text{LpxC}}$</td>
<td>$k_{\text{FtsH}}$</td>
<td>2.0</td>
<td>mM$^{-1}$ s$^{-1}$</td>
<td>(15)</td>
</tr>
<tr>
<td>FtsH$^*_{\text{LpxC}}$</td>
<td>$k_{\text{active}}$</td>
<td>0.14</td>
<td>mM$^{-1}$ s$^{-1}$</td>
<td>(15)</td>
</tr>
<tr>
<td>FtsH$^*_{\text{LpxC}}$</td>
<td>$k_{\text{inactive}}$</td>
<td>0.1</td>
<td>s$^{-1}$</td>
<td>(15)</td>
</tr>
<tr>
<td>FtsH$^*_{\text{WaaA}}$</td>
<td>$k_{\text{FtsH}}$</td>
<td>6.8</td>
<td>mM$^{-1}$ s$^{-1}$</td>
<td>(15)</td>
</tr>
<tr>
<td>FtsH$^*_{\text{WaaA}}$</td>
<td>$k_{\text{active}}$</td>
<td>32.3</td>
<td>mM$^{-1}$ s$^{-1}$</td>
<td>(15)</td>
</tr>
<tr>
<td>FtsH$^*_{\text{WaaA}}$</td>
<td>$k_{\text{inactive}}$</td>
<td>0.1</td>
<td>s$^{-1}$</td>
<td>(15)</td>
</tr>
<tr>
<td>PlsB</td>
<td>Concentration</td>
<td>0.3569</td>
<td>µM</td>
<td>(56)</td>
</tr>
<tr>
<td>PlsB</td>
<td>$K_m$ (C16:ACP)</td>
<td>15</td>
<td>µM</td>
<td>(57)</td>
</tr>
<tr>
<td>PlsB</td>
<td>$K_m$ (C18:1-ACP)</td>
<td>25</td>
<td>µM</td>
<td>(57)</td>
</tr>
<tr>
<td>PlsB</td>
<td>$K_m$ (G3P)</td>
<td>140</td>
<td>µM</td>
<td>(57)</td>
</tr>
<tr>
<td>PlsB</td>
<td>$k_{\text{cat}}$</td>
<td>3874</td>
<td>s$^{-1}$</td>
<td>fit*</td>
</tr>
<tr>
<td>PlsC</td>
<td>Concentration</td>
<td>0.3569</td>
<td>µM</td>
<td>(56)**</td>
</tr>
<tr>
<td>PlsC</td>
<td>$K_m$ (C16:ACP)</td>
<td>15</td>
<td>µM</td>
<td>(57)**</td>
</tr>
<tr>
<td>PlsC</td>
<td>$K_m$ (C18:1-ACP)</td>
<td>25</td>
<td>µM</td>
<td>(57)**</td>
</tr>
<tr>
<td>PlsC</td>
<td>$K_m$ (LysP)</td>
<td>140</td>
<td>µM</td>
<td>(57)**</td>
</tr>
<tr>
<td>PlsC</td>
<td>$k_{\text{cat}}$</td>
<td>3874</td>
<td>s$^{-1}$</td>
<td>fit*</td>
</tr>
</tbody>
</table>

*Parameter value fit in this study, as described in methods.
**PlsC abundance was not measured, so we assumed the same concentration as for PlsB.
***We used measured PlsB $K_m$ values to estimate PlsC $K_m$ values.
### Table B.10. Optimization of kinetic parameters for lipid and phospholipid biosynthesis.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Parameter</th>
<th>Optimized Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>TesA</td>
<td>Concentration in log phase</td>
<td>0.59</td>
<td>µM</td>
</tr>
<tr>
<td>LpxA</td>
<td>$k_{cat, fwd}$</td>
<td>0.0071</td>
<td>s$^{-1}$</td>
</tr>
<tr>
<td>PIsB/PIsC</td>
<td>$k_{cat}$</td>
<td>3874</td>
<td>s$^{-1}$</td>
</tr>
</tbody>
</table>

### Table B.11. Primers for cloning.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>FabA</td>
<td>atatcatatgcagataacgcaacg</td>
<td>atatgaattcaaggctagtccagagcagcagcagcag</td>
<td>(24)</td>
</tr>
<tr>
<td>FabB</td>
<td>atatcatatgaaacgcatgcaggtcatttacccgg</td>
<td>atatctcgagatggctagtcttttcagcagaagcagcagcag</td>
<td>(24)</td>
</tr>
<tr>
<td>FabF</td>
<td>atatcatatgcaaggcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcag</td>
<td>ttgatcatctattagcatctttttaaagataaatg</td>
<td>This study</td>
</tr>
<tr>
<td>FabD</td>
<td>atatcatatgcaaggcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcag</td>
<td>ttgatcatctattagcatctttttaaagataaatg</td>
<td>This study</td>
</tr>
<tr>
<td>FabG</td>
<td>atatcatatgcatatgcaaggcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcag</td>
<td>atatgaattcatgacaagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcag</td>
<td>This study</td>
</tr>
<tr>
<td>FabH</td>
<td>atatcatatgcaaggcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcag</td>
<td>tggatctactagaaacgaaccagcgcggagccc</td>
<td>This study</td>
</tr>
<tr>
<td>FabI</td>
<td>atatcatatgcaaggcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcag</td>
<td>ttgatcatctattagcatctttttaaagataaatg</td>
<td>This study</td>
</tr>
<tr>
<td>FabZ</td>
<td>atatcatatgcaaggcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcag</td>
<td>atatgaattcatgacaagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcag</td>
<td>This study</td>
</tr>
<tr>
<td>TesA</td>
<td>atatcatatgcaaggcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcag</td>
<td>ttgatcatctattagcatctttttaaagataaatg</td>
<td>This study</td>
</tr>
<tr>
<td>SpFabM</td>
<td>ggcatacatatgcagataacgcatatgcaagcagcagcagcagcagcagcagcagcagcagcagcagcag</td>
<td>ggcatacatatgcagataacgcatatgcaagcagcagcagcagcagcagcagcagcagcagcagcagcag</td>
<td>This study</td>
</tr>
<tr>
<td>FabZ Linker</td>
<td>ggcatacatatgcagataacgcatatgcaagcagcagcagcagcagcagcagcagcagcagcagcagcag</td>
<td>ggcatacatatgcagataacgcatatgcaagcagcagcagcagcagcagcagcagcagcagcagcagcag</td>
<td>This study</td>
</tr>
<tr>
<td>FabZ Dimer 1st half</td>
<td>ggcatacatatgcagataacgcatatgcaagcagcagcagcagcagcagcagcagcagcagcagcagcag</td>
<td>ggcatacatatgcagataacgcatatgcaagcagcagcagcagcagcagcagcagcagcagcagcagcag</td>
<td>This study</td>
</tr>
<tr>
<td>FabZ Dimer 2nd half</td>
<td>ggcatacatatgcagataacgcatatgcaagcagcagcagcagcagcagcagcagcagcagcagcagcag</td>
<td>ggcatacatatgcagataacgcatatgcaagcagcagcagcagcagcagcagcagcagcagcagcagcag</td>
<td>This study</td>
</tr>
</tbody>
</table>

*Restriction sites appear in bold.*
B.4 Model Equations

\[ [\text{FabD}] = [\text{FabD}]_{\text{tot}} - [\text{FabD} \cdot \text{Malonyl-CoA}] - [\text{FabD}^\ast] - [\text{FabD} \cdot \text{ACP}] \quad \text{Eq. B.1} \]

\[ [\text{FabH}] = [\text{FabH}]_{\text{tot}} - [\text{FabH} \cdot \text{Acetyl-CoA}] - [\text{FabH}^\ast] - [\text{FabH} \cdot \text{Malonyl-ACP}] \]
\[ - \sum_{n=2}^{10} [\text{FabH} \cdot \text{C}_{2n} \text{Acyl-ACP}] - \sum_{n=10}^{10} [\text{FabH} \cdot \text{C}_{2n:1} \text{Acyl-ACP}] \]
\[ - \sum_{n=2}^{10} [\text{FabH}^\ast] \cdot \text{C}_{2n} \text{Acyl-ACP} - \sum_{n=6}^{10} [\text{FabH}^\ast] \cdot \text{C}_{2n:1} \text{Acyl-ACP} - [\text{FabH} \cdot \text{ACP}] \]

\[ [\text{FabG}] = [\text{FabG}]_{\text{tot}} - [\text{FabG} \cdot \text{NADPH}] - \sum_{n=2}^{10} [\text{FabG} \cdot \text{NADPH} \cdot \text{C}_{2n} \beta\text{-ketoacyl-ACP}] \]
\[ - \sum_{n=6}^{10} [\text{FabG} \cdot \text{NADPH} \cdot \text{C}_{2n:1} \beta\text{-ketoacyl-ACP}] - [\text{FabG} \cdot \text{ACP}] \]

\[ [\text{FabZ}] = [\text{FabZ}]_{\text{tot}} - \sum_{n=2}^{10} [\text{FabZ} \cdot \text{C}_{2n} \beta\text{-hydroxyacyl-ACP}] - \sum_{n=6}^{10} [\text{FabZ} \cdot \text{C}_{2n:1} \beta\text{-hydroxyacyl-ACP}] \]
\[ - \sum_{n=2}^{10} [\text{FabZ} \cdot \text{C}_{2n} \text{Enoylacyl-ACP}] - \sum_{n=6}^{10} [\text{FabZ} \cdot \text{C}_{2n:1} \text{Enoylacyl-ACP}] \]
\[ - [\text{FabZ} \cdot \text{ACP}] \]

\[ [\text{FabI}] = [\text{FabI}]_{\text{tot}} - [\text{FabI} \cdot \text{NADH}] - \sum_{n=2}^{10} [\text{FabI} \cdot \text{NADH} \cdot \text{C}_{2n} \text{Enoylacyl-ACP}] \]
\[ - \sum_{n=6}^{10} [\text{FabI} \cdot \text{NADH} \cdot \text{C}_{2n:1} \text{Enoylacyl-ACP}] - [\text{FabI} \cdot \text{ACP}] \]

\[ [\text{TesA}] = [\text{TesA}]_{\text{tot}} - \sum_{n=2}^{10} [\text{TesA} \cdot \text{C}_{2n} \text{acyl-ACP}] - \sum_{n=6}^{10} [\text{TesA} \cdot \text{C}_{2n:1} \text{acyl-ACP}] - [\text{TesA} \cdot \text{ACP}] \quad \text{Eq. B.5} \]

\[ [\text{FabA}] = [\text{FabA}]_{\text{tot}} - \sum_{n=2}^{10} [\text{FabA} \cdot \text{C}_{2n} \beta\text{-hydroxyacyl-ACP}] \]
\[ - \sum_{n=6}^{10} [\text{FabA} \cdot \text{C}_{2n:1} \beta\text{-hydroxyacyl-ACP}] - \sum_{n=2}^{10} [\text{FabA} \cdot \text{C}_{2n} \text{Enoylacyl-ACP}] \]
\[ - \sum_{n=6}^{10} [\text{FabA} \cdot \text{C}_{2n:1} \text{Enoylacyl-ACP}] - [\text{FabA} \cdot \text{C}_{10\text{cis}-3}\text{-Enoylacyl-ACP}] \]
\[ - [\text{FabA} \cdot \text{ACP}] \]

\[ [\text{FabF}] = [\text{FabF}]_{\text{tot}} - \sum_{n=2}^{9} [\text{FabF} \cdot \text{C}_{2n} \text{acyl-ACP}] - \sum_{n=6}^{9} [\text{FabF} \cdot \text{C}_{2n:1} \text{acyl-ACP}] \]
\[ - \sum_{n=2}^{9} [\text{C}_{2n} \text{FabF}^\ast] - \sum_{n=6}^{9} [\text{C}_{2n:1} \text{FabF}^\ast] - \sum_{n=2}^{9} [\text{C}_{2n} \text{FabF}^\ast \cdot \text{Malonyl-ACP}] \]
\[ - \sum_{n=6}^{9} [\text{C}_{2n:1} \text{FabF}^\ast \cdot \text{Malonyl-ACP}] - [\text{FabF} \cdot \text{ACP}] \]
\[ [\text{FabB}] = [\text{FabB}]_{\text{tot}} - \sum_{n=2}^{9} [\text{FabB} \cdot C_{2n}\text{-acyl-ACP}] - \sum_{n=6}^{9} [\text{FabB} \cdot C_{2n:1}\text{-acyl-ACP}] \]  
\[ - \sum_{n=2}^{9} [C_{2n}\text{-FabB}] - \sum_{n=6}^{9} [C_{2n:1}\text{-FabB}] - \sum_{n=2}^{9} [\text{FabB}\# \cdot \text{Malonyl-ACP}] \]  
\[ - \sum_{n=6}^{9} [C_{2n:1}\text{-FabB}\# \cdot \text{Malonyl-ACP}] - [\text{FabB} \cdot C_{16}\text{-cis-3-Enoylacyl-ACP}] \]  
\[ - [C_{10}\text{-cis-3-FabB}\#] - [C_{10}\text{-cis-3-FabB}\# \cdot \text{Malonyl-ACP}] - [\text{FabB} \cdot \text{ACP}] \]

\[
\frac{d[\text{Acetyl-CoA}]}{dt} = k_{\text{off-ACoA}}[\text{FabH} \cdot \text{Acetyl-CoA}] - k_{\text{on-ACoA}}[\text{FabH}][\text{Acetyl-CoA}] \]

\[
\frac{d[\text{ACP}]}{dt} = k_{\text{off-ACP}}[\text{FabD}\# \cdot \text{ACP}] - k_{\text{on-ACP}}[\text{FabD}\#][\text{ACP}] 
+ \sum_{n=2}^{10} k_{\text{catTesAC2n}}[\text{TesA} \cdot C_{2n}\text{Acyl-ACP}] 
+ \sum_{n=6}^{10} k_{\text{catTesAC2n}}[\text{TesA} \cdot C_{2n:1}\text{Acyl-ACP}] + \sum_{n=2}^{9} k_{\text{FabF} \#}[\text{FabF}][C_{2n}\text{Acyl-ACP}] 
+ \sum_{n=6}^{9} k_{\text{FabF} \#}[C_{2n:1}\text{Acyl-ACP}] 
+ k_{\text{FabF} \#}[\text{FabB}][C_{10}\text{-cis-3-Enoylacyl-ACP}] - k_{\text{FabF} \#}[C_{10}\text{-cis-3-FabB}\#][\text{ACP}] 
- \sum_{n=2}^{9} k_{\text{FabF} \#}[C_{2n}\text{FabF}\#][\text{ACP}] 
- \sum_{n=2}^{9} k_{\text{FabF} \#}[C_{2n:1}\text{FabF}\#][\text{ACP}] 
- \sum_{n=2}^{9} k_{\text{FabF} \#}[C_{2n}\text{FabB}\#][\text{ACP}] - \sum_{n=6}^{9} k_{\text{FabB} \#}[C_{2n:1}\text{FabB}\#][\text{ACP}] 
+ k_{\text{off-FabH-Lih-1}}[\text{FabH} \cdot \text{ACP}] - k_{\text{on-FabH-Lih-1}}[\text{FabH}][\text{ACP}] 
- k_{\text{off-FabG-Lih}}[\text{FabG} \cdot \text{ACP}] - k_{\text{on-FabG-Lih}}[\text{FabG}][\text{ACP}] 
+ k_{\text{off-FabZ-Lih}}[\text{FabZ} \cdot \text{ACP}] - k_{\text{on-FabZ-Lih}}[\text{FabZ}][\text{ACP}] + k_{\text{off-FabI-Lih}}[\text{FabI} \cdot \text{ACP}] 
- k_{\text{on-FabZ-Lih}}[\text{FabZ}][\text{ACP}] + k_{\text{off-TesA-Lih}}[\text{TesA} \cdot \text{ACP}] - k_{\text{on-TesA-Lih}}[\text{TesA}][\text{ACP}] 
+ k_{\text{off-JacF-Lih}}[\text{FabF} \cdot \text{ACP}] - k_{\text{on-JacF-Lih}}[\text{FabF}][\text{ACP}] 
\]

\[
\frac{d[\text{NADPH}]}{dt} = k_{\text{off-NADPH}}[\text{FabG} \cdot \text{NADPH}] - k_{\text{on-NADPH}}[\text{FabG}][\text{NADPH}] \]

\[
\frac{d[\text{NADH}]}{dt} = k_{\text{off-NADH}}[\text{FabI} \cdot \text{NADH}] - k_{\text{on-NADH}}[\text{FabI}][\text{NADH}] \]

\[
\frac{d[\text{Malonyl-CoA}]}{dt} = k_{\text{off-mCoA}}[\text{FabD} \cdot \text{Malonyl-CoA}] - k_{\text{on-mCoA}}[\text{FabD}][\text{Malonyl-CoA}] \]

\[
\frac{d[\text{CoA}]}{dt} = k_{\text{f-FabD}}[\text{FabD} \cdot \text{Malonyl-CoA}] - k_{\text{r-FabD}}[\text{FabD}][\text{CoA}] 
- k_{\text{f-FabH}}[\text{FabH}][\text{Acetyl-CoA}] - k_{\text{r-FabH}}[\text{FabD}][\text{Acetyl-CoA}] 
\]
\[
\frac{d[\text{Malonyl-ACP}]}{dt} = k_{f,FabD*ACP}[\text{FabD*} \cdot \text{ACP}] - k_{f,FabD*ACP}[\text{FabD}][\text{ACP}]
\]
\[
- k_{\text{on-mACP}}[\text{FabH*}][\text{Malonyl-ACP}] - k_{\text{off-mACP}}[\text{FabH*} \cdot \text{Malonyl-ACP}]
\]
\[
+ \sum_{n=2}^{9} k_{\text{off-F}_{m\text{ACP}}}[C_{2n}\text{FabF*} \cdot \text{Malonyl-ACP}]
\]
\[
- \sum_{n=2}^{9} k_{\text{on-F}_{m\text{ACP}}}[C_{2n}\text{FabF*}][\text{Malonyl-ACP}]
\]
\[
+ \sum_{n=6}^{9} k_{\text{off-F}_{m\text{ACP}}}[C_{2n:1}\text{FabF*} \cdot \text{Malonyl-ACP}]
\]
\[
- \sum_{n=6}^{9} k_{\text{on-F}_{m\text{ACP}}}[C_{2n:1}\text{FabF*}][\text{Malonyl-ACP}]
\]
\[
+ \sum_{n=2}^{9} k_{\text{off-B}_{m\text{ACP}}}[C_{2n}\text{FabB*} \cdot \text{Malonyl-ACP}]
\]
\[
- \sum_{n=2}^{9} k_{\text{on-B}_{m\text{ACP}}}[C_{2n}\text{FabB*}][\text{Malonyl-ACP}]
\]
\[
+ \sum_{n=6}^{9} k_{\text{off-B}_{m\text{ACP}}}[C_{2n:1}\text{FabB*} \cdot \text{Malonyl-ACP}]
\]
\[
- \sum_{n=6}^{9} k_{\text{on-B}_{m\text{ACP}}}[C_{2n:1}\text{FabB*}][\text{Malonyl-ACP}]
\]
\[\text{Eq. B.15}\]

\[
\frac{d[\text{CO}_2]}{dt} = k_{\text{catFabH}}[\text{FabH*} \cdot \text{Malonyl-ACP}] + \sum_{n=2}^{10} k_{\text{catFabF}}[C_{2n}\text{FabF*} \cdot \text{Malonyl-ACP}]
\]
\[
+ \sum_{n=6}^{10} k_{\text{catFabF}}[C_{2n:1}\text{FabF*} \cdot \text{Malonyl-ACP}]
\]
\[
+ \sum_{n=2}^{10} k_{\text{catFabB}}[C_{2n}\text{FabB*} \cdot \text{Malonyl-ACP}]
\]
\[
+ \sum_{n=6}^{10} k_{\text{catFabB}}[C_{2n:1}\text{FabB*} \cdot \text{Malonyl-ACP}]
\]
\[\text{Eq. B.16}\]

\[
\frac{d[C_4\beta-\text{ketoacyl-ACP}]}{dt} = k_{\text{catFabH}}[\text{FabH*} \cdot \text{Malonyl-ACP}]
\]
\[
+ k_{\text{off B}_{\text{ACP}}}[\text{FabG} \cdot \text{NADPH} \cdot C_4\beta-\text{ketoacyl-ACP}]
\]
\[
- k_{\text{on B}_{\text{ACP}}}[\text{FabG} \cdot \text{NADPH}][C_4\beta-\text{ketoacyl-ACP}]
\]
\[\text{Eq. B.17}\]
\[
\sum_{n=3}^{10} \frac{d[C_{2n}\beta\text{-ketoacyl-ACP}]}{dt} = \sum_{n=3}^{10} k_{\text{off}}[\text{FabG} \cdot \text{NADPH} \cdot C_{2n}\beta\text{-ketoacyl-ACP}]
- \sum_{n=3}^{9} k_{\text{on}}[\text{FabG} \cdot \text{NADPH}][C_{2n}\beta\text{-ketoacyl-ACP}]
+ \sum_{n=3}^{9} k_{\text{cat FabF}}[C_{2n}\text{FabF}^* \cdot \text{Malonyl-ACP}]
+ \sum_{n=3}^{9} k_{\text{cat FabB}}[C_{2n}\text{FabB}^* \cdot \text{Malonyl-ACP}]
\]

\[
\sum_{n=6}^{10} \frac{d[C_{2n:1}\beta\text{-ketoacyl-ACP}]}{dt} = \sum_{n=6}^{10} k_{\text{off}}[\text{FabG} \cdot \text{NADPH} \cdot C_{2n:1}\beta\text{-ketoacyl-ACP}]
- \sum_{n=6}^{9} k_{\text{on}}[\text{FabG} \cdot \text{NADPH}][C_{2n:1}\beta\text{-ketoacyl-ACP}]
+ \sum_{n=6}^{9} k_{\text{cat FabF}}[C_{2n:1}\text{FabF}^* \cdot \text{Malonyl-ACP}]
+ \sum_{n=6}^{9} k_{\text{cat FabB}}[C_{2n:1}\text{FabB}^* \cdot \text{Malonyl-ACP}]
\]

\[
\sum_{n=2}^{10} \frac{d[C_{2n}\beta\text{-hydroxyacyl-ACP}]}{dt} = \sum_{n=2}^{10} k_{\text{off}}[\text{FabZ} \cdot C_{2n}\beta\text{-hydroxyacyl-ACP}]
- \sum_{n=2}^{10} k_{\text{on}}[\text{FabZ}][C_{2n}\beta\text{-hydroxyacyl-ACP}]
+ \sum_{n=2}^{10} k_{\text{off A,F fab A}}[\text{FabA} \cdot C_{2n}\beta\text{-hydroxyacyl-ACP}]
- \sum_{n=2}^{10} k_{\text{on A,F fab A}}[\text{FabA}][C_{2n}\beta\text{-hydroxyacyl-ACP}]
+ \sum_{n=2}^{10} k_{\text{cat FabG}}[\text{FabG} \cdot \text{NADPH} \cdot C_{2n}\beta\text{-ketoacyl-ACP}]
\]

Eq. B.18

Eq. B.19

Eq. B.20
\[
\sum_{n=6}^{10} d[C_{2n:1}\beta\text{-hydroxyacyl-ACP}] \\
\frac{dt}{dt} = \sum_{n=6}^{10} k_{\text{off},\text{haACP}} [\text{FabZ} \cdot C_{2n:1}\beta\text{-hydroxyacyl-ACP}] \\
- \sum_{n=6}^{10} k_{\text{on},\text{haACP}} [\text{FabZ}] [C_{2n:1}\beta\text{-hydroxyacyl-ACP}] \\
+ \sum_{n=6}^{10} k_{\text{off},\text{A,haACP}} [\text{FabA} \cdot C_{2n:1}\beta\text{-hydroxyacyl-ACP}] \\
- \sum_{n=6}^{10} k_{\text{on},\text{A,haACP}} [\text{FabA}] [C_{2n:1}\beta\text{-hydroxyacyl-ACP}] \\
+ \sum_{n=6}^{10} k_{\text{cat},\text{FabG}} [\text{FabG} \cdot \text{NADPH} \cdot C_{2n:1}\beta\text{-ketoacyl-ACP}] \\
\]

\[
\sum_{n=2}^{10} d[C_{2n}\text{Enoylacyl-ACP}] \\
\frac{dt}{dt} = \sum_{n=2}^{10} k_{\text{off},\text{eacACP}} [\text{FabI} \cdot \text{NADH} \cdot C_{2n}\text{Enoylacyl-ACP}] \\
- \sum_{n=2}^{10} k_{\text{on},\text{eacACP}} [\text{FabI} \cdot \text{NADH}] [C_{2n}\text{Enoylacyl-ACP}] \\
+ \sum_{n=2}^{10} k_{\text{off},\text{Z,eacACP}} [\text{FabZ}] [C_{2n}\text{Enoylacyl-ACP}] \\
- \sum_{n=2}^{10} k_{\text{on},\text{Z,eacACP}} [\text{FabZ}] [C_{2n}\text{Enoylacyl-ACP}] \\
+ \sum_{n=2}^{10} k_{\text{off},\text{A,eacACP}} [\text{FabA}] [C_{2n}\text{Enoylacyl-ACP}] \\
- \sum_{n=2}^{10} k_{\text{on},\text{A,eacACP}} [\text{FabA}] [C_{2n}\text{Enoylacyl-ACP}] \\
\]

\[
\frac{d[C_{10}\text{cis-3-Enoylacyl-ACP}]}{dt} = k_{\text{off},\text{AcisACP}} [\text{FabA} \cdot C_{10}\text{cis-3-Enoylacyl-ACP}] \\
- k_{\text{on},\text{AcisACP}} [\text{FabA}] [C_{10}\text{cis-3-Enoylacyl-ACP}] \\
+ k_{\text{off},\text{B,AcACP}} [\text{FabB} \cdot C_{10}\text{cis-3-Enoylacyl-ACP}] \\
- k_{\text{on},\text{B,AcACP}} [\text{FabB}] [C_{10}\text{cis-3-Enoylacyl-ACP}] \\
\]
\[
\sum_{n=6}^{10} \frac{d[C_{2n:1} \text{ Enoylacyl-ACP}]}{dt} = \sum_{n=6}^{10} k_{\text{off-eacACP}}[\text{FabI} \cdot \text{NADH} \cdot C_{2n:1} \text{ Enoylacyl-ACP}]
- \sum_{n=6}^{10} k_{\text{on-eacACP}}[\text{FabI} \cdot \text{NADH}][C_{2n:1} \text{ Enoylacyl-ACP}]
+ \sum_{n=6}^{10} k_{\text{off-Z_eacACP}}[\text{FabZ}][C_{2n:1} \text{ Enoylacyl-ACP}]
- \sum_{n=6}^{10} k_{\text{on-Z_eacACP}}[\text{FabZ}][C_{2n:1} \text{ Enoylacyl-ACP}]
+ \sum_{n=6}^{10} k_{\text{off-A_eacACP}}[\text{FabA}][C_{2n:1} \text{ Enoylacyl-ACP}]
- \sum_{n=6}^{10} k_{\text{on-A_eacACP}}[\text{FabA}][C_{2n:1} \text{ Enoylacyl-ACP}]
\]

Eq. B.24

\[
\sum_{n=2}^{10} \frac{d[C_{2n} \text{ acyl-ACP}]}{dt} = \sum_{n=2}^{10} k_{\text{catFabI}}[\text{FabI} \cdot \text{NADH} \cdot C_{2n} \text{ Enoylacyl-ACP}]
+ \sum_{n=2}^{10} k_{\text{off-acACP,C2n}}[\text{TesA} \cdot C_{2n} \text{ Acyl-ACP}]
- \sum_{n=2}^{9} k_{\text{on-acACP,C2n}}[\text{TesA}][C_{2n} \text{ Acyl-ACP}]
+ \sum_{n=2}^{9} k_{\text{off-FacACP}}[\text{FabF} \cdot C_{2n} \text{ Acyl-ACP}] - \sum_{n=2}^{9} k_{\text{on-FacACP}}[\text{FabF} \cdot C_{2n} \text{ Acyl-ACP}]
- \sum_{n=2}^{9} k_{\text{on-bacACP}}[\text{FabB}][C_{2n} \text{ Acyl-ACP}]
+ \sum_{n=2}^{9} k_{\text{off-bacACP}}[\text{FabB}][C_{2n} \text{ Acyl-ACP}]
+ \sum_{n=2}^{10} k_{\text{off-FabH-Inh-2}}[\text{FabH} \cdot C_{2n} \text{ Acyl-ACP}]
- \sum_{n=2}^{10} k_{\text{on-FabH-Inh-2,C2n}}[\text{FabH}][C_{2n} \text{ Acyl-ACP}]
+ \sum_{n=2}^{10} k_{\text{off-FabH-Inh-3}}[\text{FabH*} \cdot C_{2n} \text{ Acyl-ACP}]
- \sum_{n=2}^{10} k_{\text{on-FabH-Inh-3,C2n}}[\text{FabH*}][C_{2n} \text{ Acyl-ACP}]
\]

Eq. B.25
\[\sum_{n=6}^{10} \frac{d[C_{2n:1\text{acyl-ACP}}]}{dt} = \sum_{n=6}^{10} k_{\text{catFabI}}[\text{FabI} \cdot \text{NADH} \cdot C_{2n:1\text{Enoylacyl-ACP}}] + \sum_{n=6}^{10} k_{\text{off-acyACP,C}2n}[\text{TesA} \cdot C_{2n:1\text{Acyl-ACP}}] - \sum_{n=6}^{9} k_{\text{on-acyACP,C}2n}[\text{TesA}] [C_{2n:1\text{Acyl-ACP}}] + \sum_{n=6}^{9} k_{\text{off-FacACP}}[\text{FabF} \cdot C_{2n:1\text{Acyl-ACP}}] - \sum_{n=6}^{9} k_{\text{on-FacACP}}[\text{FabF} \cdot C_{2n:1\text{Acyl-ACP}}] - \sum_{n=6}^{9} k_{\text{on-BacACP}}[\text{FabB}][C_{2n:1\text{Acyl-ACP}}] + \sum_{n=6}^{9} k_{\text{off-BacACP}}[\text{FabB}][C_{2n:1\text{Acyl-ACP}}] + \sum_{n=6}^{9} k_{\text{on-FabH-Inh-2}}[\text{FabH} \cdot C_{2n:1\text{Acyl-ACP}}] - \sum_{n=6}^{9} k_{\text{on-FabH-Inh-2,C}2n}[\text{FabH}][C_{2n:1\text{Acyl-ACP}}] + \sum_{n=6}^{10} k_{\text{off-FabH-Inh-3}}[\text{FabH*} \cdot C_{2n:1\text{Acyl-ACP}}] - \sum_{n=6}^{10} k_{\text{on-FabH-Inh-3,C}2n}[\text{FabH*}][C_{2n:1\text{Acyl-ACP}}]\]

\[\frac{d[\text{FabD} \cdot \text{Malonyl-CoA}]}{dt} = k_{\text{on-mCoA}}[\text{FabD}][\text{Malonyl-CoA}] - k_{\text{off-mCoA}}[\text{FabD} \cdot \text{Malonyl-CoA}] + k_r[\text{FabD*}][\text{CoA}] - k_f[\text{FabD*}][\text{FabD} \cdot \text{Malonyl-CoA}]
\]

\[\frac{d[\text{FabD*}]}{dt} = k_f[\text{FabD} \cdot \text{Malonyl-CoA}] - k_r[\text{FabD*}][\text{CoA}] + k_{\text{off-ACP}}[\text{FabD*} \cdot \text{ACP}] - k_{\text{on-ACP}}[\text{FabD*}][\text{ACP}]
\]

\[\frac{d[\text{FabD*} \cdot \text{ACP}]}{dt} = k_{\text{on-ACP}}[\text{FabD*}][\text{ACP}] - k_{\text{off-ACP}}[\text{FabD*} \cdot \text{ACP}] + k_r[\text{FabD*ACP}][\text{FabD}][\text{Malonyl-ACP}] - k_f[\text{FabD*ACP}][\text{FabD*} \cdot \text{ACP}]
\]
\[
\begin{align*}
\frac{d[FabH \cdot Acetyl-CoA]}{dt} &= k_{on-\text{Acetyl-CoA}}[FabH][Acetyl-CoA] - k_{off-\text{Acetyl-CoA}}[FabH \cdot Acetyl-CoA] \\
&+ k_{r,FabH}[FabH^*][CoA] - k_{f,FabH}[FabH \cdot Acetyl-CoA] \\
\frac{d[FabH^*]}{dt} &= k_{f,FabH^*}[FabH \cdot Acetyl-CoA] - k_{r,FabH^*}[FabH^*][CoA] + k_{off-mACP}[FabH^*] \cdot \text{Malonyl-ACP} \\
&- k_{on-mACP}[FabH^*][\text{Malonyl-ACP}] \\
&+ \sum_{n=2}^{10} k_{off-FabH-Inh-3,C2n}[FabH^* \cdot C_{2n}Acyl-ACP] \\
&- \sum_{n=2}^{10} k_{on-FabH-Inh-3,C2n}[FabH^*][C_{2n}Acyl-ACP] \\
&+ \sum_{n=6}^{10} k_{off-FabH-Inh-3,C2n}[FabH^* \cdot C_{2n:1}Acyl-ACP] \\
&- \sum_{n=6}^{10} k_{on-FabH-Inh-3,C2n}[FabH^*][C_{2n:1}Acyl-ACP] \\
\frac{d[FabH^* \cdot Malonyl-ACP]}{dt} &= k_{on-mACP}[FabH^*][\text{Malonyl-ACP}] \\
&- k_{off-mACP}[FabH^* \cdot \text{Malonyl-ACP}] - k_{catFabH}[FabH^* \cdot \text{Malonyl-ACP}] \\
\frac{d[FabG \cdot NADPH]}{dt} &= k_{on-NADPH}[FabG][NADPH] - k_{off-NADPH}[FabG \cdot NADPH] \\
&+ \sum_{n=2}^{10} k_{off\beta\text{kaACP}}[FabG \cdot NADPH \cdot C_{2n}\beta\text{-ketoacyl-ACP}] \\
& - \sum_{n=2}^{10} k_{on\beta\text{kaACP}}[FabG \cdot NADPH][C_{2n}\beta\text{-ketoacyl-ACP}] \\
&+ \sum_{n=6}^{10} k_{off\beta\text{kaACP}}[FabG \cdot NADPH \cdot C_{2n:1}\beta\text{-ketoacyl-ACP}] \\
& - \sum_{n=6}^{10} k_{on\beta\text{kaACP}}[FabG \cdot NADPH][C_{2n:1}\beta\text{-ketoacyl-ACP}] \\
\sum_{n=2}^{10} \frac{d[FabG \cdot NADPH \cdot C_{2n}\beta\text{-ketoacyl-ACP}]}{dt} &= k_{on\beta\text{kaACP}}[FabG \cdot NADPH][C_{2n}\beta\text{-ketoacyl-ACP}] \\
& - k_{off\beta\text{kaACP}}[FabG \cdot NADPH \cdot C_{2n}\beta\text{-ketoacyl-ACP}] \\
& - k_{catFabG}[FabG \cdot NADPH \cdot C_{2n}\beta\text{-ketoacyl-ACP]}
\end{align*}
\]
\[
\sum_{n=6}^{10} \frac{d[FabG \cdot \text{NADPH} \cdot C_{2n:1}\beta-\text{ketoacyl-ACP}]}{dt} = \sum_{n=6}^{10} k_{on\beta\text{kaACP}}[\text{FabG} \cdot \text{NADPH}][C_{2n:1}\beta-\text{ketoacyl-ACP}]
- \sum_{n=6}^{10} k_{off\beta\text{kaACP}}[\text{FabG} \cdot \text{NADPH} \cdot C_{2n:1}\beta-\text{ketoacyl-ACP}]
- \sum_{n=6}^{10} k_{cat\text{FabG}}[\text{FabG} \cdot \text{NADPH} \cdot C_{2n:1}\beta-\text{ketoacyl-ACP}]
\]

Eq. B.35

\[
\sum_{n=2}^{10} \frac{d[FabZ \cdot C_{2n}\beta-\text{hydroxyacyl-ACP}]}{dt} = \sum_{n=2}^{10} k_{on\beta\text{haACP}}[\text{FabZ}][C_{2n}\beta-\text{hydroxyacyl-ACP}]
- \sum_{n=2}^{10} k_{off\beta\text{haACP}}[\text{FabZ} \cdot C_{2n}\beta-\text{hydroxyacyl-ACP}]
- \sum_{n=2}^{10} k_{cat\text{FabZ-fwd}}[\text{FabZ} \cdot C_{2n}\beta-\text{hydroxyacyl-ACP}]
+ \sum_{n=2}^{10} k_{cat\text{FabZ-rvs}}[\text{FabZ} \cdot C_{2n}\text{Enoylacyl-ACP}]
\]

Eq. B.36

\[
\sum_{n=6}^{10} \frac{d[FabZ \cdot C_{2n:1}\beta-\text{hydroxyacyl-ACP}]}{dt} = \sum_{n=6}^{10} k_{on\beta\text{haACP}}[\text{FabZ}][C_{2n:1}\beta-\text{hydroxyacyl-ACP}]
- \sum_{n=6}^{10} k_{off\beta\text{haACP}}[\text{FabZ} \cdot C_{2n:1}\beta-\text{hydroxyacyl-ACP}]
- \sum_{n=6}^{10} k_{cat\text{FabZ-fwd}}[\text{FabZ} \cdot C_{2n:1}\beta-\text{hydroxyacyl-ACP}]
+ \sum_{n=6}^{10} k_{cat\text{FabZ-rvs}}[\text{FabZ} \cdot C_{2n:1}\text{Enoylacyl-ACP}]
\]

Eq. B.37
\[ \sum_{n=2}^{10} \frac{d[FabZ \cdot C_{2n} \text{Enoylacyl-ACP}]}{dt} = \sum_{n=2}^{10} k_{\text{catFabZ-fwd}}[FabZ \cdot C_{2n} \beta\text{-hydroxyacyl-ACP}] - \sum_{n=2}^{10} k_{\text{catFabZ-rvs}}[FabZ \cdot C_{2n} \text{Enoylacyl-ACP}] + \sum_{n=2}^{10} k_{\text{on-Z_eacACP}}[FabZ][C_{2n} \text{Enoylacyl-ACP}] - \sum_{n=2}^{10} k_{\text{off-Z_eacACP}}[FabZ \cdot C_{2n} \text{Enoylacyl-ACP}] \]  

\[ \sum_{n=6}^{10} \frac{d[FabZ \cdot C_{2n:1} \text{Enoylacyl-ACP}]}{dt} = \sum_{n=6}^{10} k_{\text{catFabZ-fwd}}[FabZ \cdot C_{2n:1} \beta\text{-hydroxyacyl-ACP}] - \sum_{n=6}^{10} k_{\text{catFabZ-rvs}}[FabZ \cdot C_{2n:1} \text{Enoylacyl-ACP}] + \sum_{n=6}^{10} k_{\text{on-Z_eacACP}}[FabZ][C_{2n:1} \text{Enoylacyl-ACP}] - \sum_{n=6}^{10} k_{\text{off-Z_eacACP}}[FabZ \cdot C_{2n:1} \text{Enoylacyl-ACP}] \]  

\[ \sum_{n=2}^{10} \frac{d[FabA \cdot C_{2n} \beta\text{-hydroxyacyl-ACP}]}{dt} = \sum_{n=2}^{10} k_{\text{on\betahaACP}}[FabA][C_{2n} \beta\text{-hydroxyacyl-ACP}] - \sum_{n=2}^{10} k_{\text{off\betahaACP}}[FabA \cdot C_{2n} \beta\text{-hydroxyacyl-ACP}] - \sum_{n=2}^{10} k_{\text{catFabA-fwd}}[FabA \cdot C_{2n} \beta\text{-hydroxyacyl-ACP}] + \sum_{n=2}^{10} k_{\text{catFabA-rvs}}[FabA \cdot C_{2n} \text{Enoylacyl-ACP}] \]
\[ \sum_{n=6}^{10} \frac{d[FabA \cdot C_{2n:1}\beta\text{-hydroxyacyl-ACP}]}{dt} = \sum_{n=6}^{10} k_{\text{on} \beta\text{haACP}} [FabA][C_{2n:1}\beta\text{-hydroxyacyl-ACP}] 
- \sum_{n=6}^{10} k_{\text{off} \beta\text{haACP}} [FabA \cdot C_{2n:1}\beta\text{-hydroxyacyl-ACP}] 
- \sum_{n=6}^{10} k_{\text{cat FabA-fwd}} [FabA \cdot C_{2n:1}\beta\text{-hydroxyacyl-ACP}] 
+ \sum_{n=6}^{10} k_{\text{cat FabA-rvs}} [FabA \cdot C_{2n:1}\text{Enoylacyl-ACP}] \]

\[ \sum_{n=2}^{10} \frac{d[FabA \cdot C_{2n}\text{Enoylacyl-ACP}]}{dt} = \sum_{n=2}^{10} k_{\text{cat FabA-fwd}} [FabA \cdot C_{2n}\beta\text{-hydroxyacyl-ACP}] 
- \sum_{n=2}^{10} k_{\text{cat FabA-rvs}} [FabA \cdot C_{2n}\text{Enoylacyl-ACP}] 
+ \sum_{n=2}^{10} k_{\text{on } A_{\text{eacACP}}} [FabA][C_{2n}\text{Enoylacyl-ACP}] 
- \sum_{n=2}^{10} k_{\text{off } A_{\text{eacACP}}} [FabA \cdot C_{2n}\text{Enoylacyl-ACP}] 
- k_{\text{cat-unsat-fwd}} [FabA \cdot C_{10}\text{Enoylacyl-ACP}] 
+ k_{\text{cat-unsat-rvs}} [FabA \cdot C_{10}\text{cis-3-Enoylacyl-ACP}] \]

\[ \sum_{n=6}^{10} \frac{d[FabA \cdot C_{2n:1}\text{Enoylacyl-ACP}]}{dt} = \sum_{n=6}^{10} k_{\text{cat FabA-fwd}} [FabA \cdot C_{2n:1}\beta\text{-hydroxyacyl-ACP}] 
- \sum_{n=6}^{10} k_{\text{cat FabA-rvs}} [FabA \cdot C_{2n:1}\text{Enoylacyl-ACP}] 
+ \sum_{n=6}^{10} k_{\text{on } A_{\text{eacACP}}} [FabA][C_{2n:1}\text{Enoylacyl-ACP}] 
- \sum_{n=6}^{10} k_{\text{off } A_{\text{eacACP}}} [FabA \cdot C_{2n:1}\text{Enoylacyl-ACP}] \]

\[ \frac{d[FabA \cdot C_{10}\text{cis-3-Enoylacyl-ACP}]}{dt} = k_{\text{cat-unsat-fwd}} [FabA \cdot C_{10}\text{Enoylacyl-ACP}] 
- k_{\text{cat-unsat-rvs}} [FabA \cdot C_{10}\text{cis-3-Enoylacyl-ACP}] 
+ k_{\text{on } A_{\text{cisACP}}} [FabA][C_{10}\text{cis-3-Enoylacyl-ACP}] 
- k_{\text{off } A_{\text{cisACP}}} [FabA \cdot C_{10}\text{cis-3-Enoylacyl-ACP}] \]
\[
\begin{align*}
\frac{d[FabI \cdot NADH]}{dt} &= k_{on-NADH}[FabI][NADH] - k_{off-NADPH}[FabI \cdot NADH] \\
&+ \sum_{n=2}^{10} k_{off-eacACP}[FabI \cdot NADH \cdot C_{2n}\text{Enoylacyl-ACP}] \\
&- \sum_{n=2}^{10} k_{on-eacACP}[FabI \cdot NADH][C_{2n}\text{Enoylacyl-ACP}] \\
&- \sum_{n=6}^{10} k_{on-eacACP}[FabI \cdot NADH][C_{2n:1}\text{Enoylacyl-ACP}] \\
&+ \sum_{n=6}^{10} k_{off-eacACP}[FabI \cdot NADH \cdot C_{2n:1}\text{Enoylacyl-ACP}]
\end{align*}
\]

Eq. B.45

\[
\sum_{n=2}^{10} \frac{d[FabI \cdot NADH \cdot C_{2n}\text{Enoylacyl-ACP}]}{dt} = \sum_{n=2}^{10} k_{on-eacACP}[FabI \cdot NADH][C_{2n}\text{Enoylacyl-ACP}] \\
- \sum_{n=2}^{10} k_{off-eacACP}[FabI \cdot NADH \cdot C_{2n}\text{Enoylacyl-ACP}] \\
- \sum_{n=2}^{10} k_{catFabI}[FabI \cdot NADH \cdot C_{2n}\text{Enoylacyl-ACP}]
\]

Eq. B.46

\[
\sum_{n=6}^{10} \frac{d[FabI \cdot NADH \cdot C_{2n:1}\text{Enoylacyl-ACP}]}{dt} = \sum_{n=6}^{10} k_{on-eacACP}[FabI \cdot NADH][C_{2n:1}\text{Enoylacyl-ACP}] \\
- \sum_{n=6}^{10} k_{off-eacACP}[FabI \cdot NADH \cdot C_{2n:1}\text{Enoylacyl-ACP}] \\
- \sum_{n=6}^{10} k_{catFabI}[FabI \cdot NADH \cdot C_{2n:1}\text{Enoylacyl-ACP}]
\]

Eq. B.47

\[
\sum_{n=2}^{10} \frac{d[TesA \cdot C_{2n}\text{acyl-ACP}]}{dt} = \sum_{n=2}^{10} k_{on-acACP}[TesA][C_{2n}\text{Acyl-ACP}] - \sum_{n=2}^{10} k_{off-acACP}[TesA \cdot C_{2n}\text{Acyl-ACP}] \\
- \sum_{n=2}^{10} k_{catTesA}[TesA \cdot C_{2n}\text{Acyl-ACP}]
\]

Eq. B.48

\[
\sum_{n=6}^{10} \frac{d[TesA \cdot C_{2n:1}\text{acyl-ACP}]}{dt} = \sum_{n=6}^{10} k_{on-acACP}[TesA][C_{2n:1}\text{Acyl-ACP}] - \sum_{n=6}^{10} k_{off-acACP}[TesA \cdot C_{2n:1}\text{Acyl-ACP}] \\
- \sum_{n=6}^{10} k_{catTesA}[TesA \cdot C_{2n:1}\text{Acyl-ACP}]
\]

Eq. B.49
\[
\sum_{n=2}^{9} \frac{d[FabF \cdot C_{2n} \text{acyl-ACP}]}{dt} = \sum_{n=2}^{9} k_{on \cdot F_{acACP}} [FabF][C_{2n} \text{Acyl-ACP}] - \sum_{n=2}^{9} k_{off \cdot F_{acACP}} [FabF \cdot C_{2n} \text{Acyl-ACP}] \\
+ \sum_{n=2}^{9} k_{r \cdot FabF} [C_{2n} \text{FabF}^*][ACP] - \sum_{n=2}^{9} k_{f \cdot FabF} [FabF \cdot C_{2n} \text{acyl-ACP}]
\]

**Eq. B.50**

\[
\sum_{n=2}^{9} \frac{d[C_{2n} \text{FabF}^*]}{dt} = \sum_{n=2}^{9} k_{f \cdot FabF} [FabF \cdot C_{2n} \text{acyl-ACP}] - \sum_{n=2}^{9} k_{r \cdot FabF} [C_{2n} \text{FabF}^*][ACP] \\
+ \sum_{n=2}^{9} k_{off \cdot F_{mACP}} [C_{2n} \text{FabF}^* \cdot \text{Malonyl-ACP}] \\
- \sum_{n=2}^{9} k_{on \cdot F_{mACP}} [C_{2n} \text{FabF}^*][\text{Malonyl-ACP}]
\]

**Eq. B.51**

\[
\sum_{n=2}^{9} \frac{d[C_{2n} \text{FabF}^* \cdot \text{Malonyl-ACP}]}{dt} = \sum_{n=2}^{9} k_{on \cdot F_{mACP}} [C_{2n} \text{FabF}^*][\text{Malonyl-ACP}] \\
- \sum_{n=2}^{9} k_{off \cdot F_{mACP}} [C_{2n} \text{FabF}^* \cdot \text{Malonyl-ACP}] \\
- \sum_{n=2}^{9} k_{cat FabF} [C_{2n} \text{FabF}^* \cdot \text{Malonyl-ACP}]
\]

**Eq. B.52**

\[
\sum_{n=6}^{9} \frac{d[FabF \cdot C_{2n:1} \text{acyl-ACP}]}{dt} = \sum_{n=6}^{9} k_{on \cdot F_{acACP}} [FabF][C_{2n:1} \text{Acyl-ACP}] \\
- \sum_{n=6}^{9} k_{off \cdot F_{acACP}} [FabF \cdot C_{2n:1} \text{Acyl-ACP}] + \sum_{n=6}^{9} k_{r \cdot FabF} [C_{2n:1} \text{FabF}^*][ACP] \\
- \sum_{n=6}^{9} k_{f \cdot FabF} [FabF \cdot C_{2n:1} \text{acyl-ACP}]
\]

**Eq. B.53**

\[
\sum_{n=6}^{9} \frac{d[C_{2n:1} \text{FabF}^*]}{dt} = \sum_{n=6}^{9} k_{f \cdot FabF} [FabF \cdot C_{2n:1} \text{acyl-ACP}] - \sum_{n=6}^{9} k_{r \cdot FabF} [C_{2n:1} \text{FabF}^*][ACP] \\
+ \sum_{n=6}^{9} k_{off \cdot F_{mACP}} [C_{2n:1} \text{FabF}^* \cdot \text{Malonyl-ACP}] \\
- \sum_{n=6}^{9} k_{on \cdot F_{mACP}} [C_{2n:1} \text{FabF}^*][\text{Malonyl-ACP}]
\]

**Eq. B.54**
\[ \sum_{n=6}^{9} \frac{d[C_{2n+1} \text{FabF}^* \cdot \text{Malonyl-ACP}]}{dt} = \sum_{n=6}^{9} k_{\text{on-FmACP}}[C_{2n+1} \text{FabF}^*][\text{Malonyl-ACP}] - \sum_{n=6}^{10} k_{\text{off-FmACP}}[C_{2n+1} \text{FabF}^* \cdot \text{Malonyl-ACP}] - \sum_{n=6}^{9} k_{\text{catFabf}}[C_{2n+1} \text{FabF}^* \cdot \text{Malonyl-ACP}] \]

Eq. B.55

\[ \sum_{n=2}^{9} \frac{d[\text{FabB} \cdot C_{2n} \text{acyl-ACP}]}{dt} = \sum_{n=2}^{9} k_{\text{on-B_{acACP}}}[\text{FabB}][C_{2n} \text{Acyl-ACP}] - \sum_{n=2}^{9} k_{\text{off-B_{acACP}}}[\text{FabB} \cdot C_{2n} \text{Acyl-ACP}] + \sum_{n=2}^{9} k_{r-\text{FabB}}[C_{2n} \text{FabB}^*][\text{ACP}] - \sum_{n=2}^{9} k_{f-\text{FabB}}[\text{FabB} \cdot C_{2n} \text{acyl-ACP}] \]

Eq. B.56

\[ \sum_{n=2}^{9} \frac{d[C_{2n} \text{FabB}^*]}{dt} = \sum_{n=2}^{9} k_{f-\text{FabB}}[\text{FabB} \cdot C_{2n} \text{acyl-ACP}] - \sum_{n=2}^{9} k_{r-\text{FabB}}[C_{2n} \text{FabB}^*][\text{ACP}] + \sum_{n=2}^{9} k_{\text{off-BmACP}}[C_{2n} \text{FabB}^* \cdot \text{Malonyl-ACP}] - \sum_{n=2}^{9} k_{\text{on-BmACP}}[C_{2n} \text{FabB}^*][\text{Malonyl-ACP}] \]

Eq. B.57

\[ \sum_{n=2}^{9} \frac{d[C_{2n} \text{FabB}^* \cdot \text{Malonyl-ACP}]}{dt} = \sum_{n=2}^{9} k_{\text{on-BmACP}}[C_{2n} \text{FabB}^*][\text{Malonyl-ACP}] - \sum_{n=2}^{10} k_{\text{off-BmACP}}[C_{2n} \text{FabB}^* \cdot \text{Malonyl-ACP}] - \sum_{n=2}^{9} k_{\text{catFabb}}[C_{2n} \text{FabB}^* \cdot \text{Malonyl-ACP}] \]

Eq. B.58

\[ \sum_{n=6}^{9} \frac{d[\text{FabB} \cdot C_{2n:1} \text{acyl-ACP}]}{dt} = \sum_{n=6}^{9} k_{\text{on-B_{acACP}}}[\text{FabB}][C_{2n:1} \text{Acyl-ACP}] - \sum_{n=6}^{9} k_{\text{off-B_{acACP}}}[\text{FabB} \cdot C_{2n:1} \text{Acyl-ACP}] + \sum_{n=6}^{9} k_{r-\text{FabB}}[C_{2n:1} \text{FabB}^*][\text{ACP}] + \sum_{n=6}^{9} k_{f-\text{FabB}}[\text{FabB} \cdot C_{2n:1} \text{acyl-ACP}] \]

Eq. B.59
\[ \sum_{n=6}^{9} \frac{d[C_{2n:1}\text{FabB}^*]}{dt} \]

Eq. B.60

\[ = \sum_{n=6}^{9} k_{f-\text{FabB}*}[\text{FabB} \cdot C_{2n:1}\text{acyl-ACP}] - \sum_{n=6}^{9} k_{r-\text{FabB}^*}[C_{2n:1}\text{FabB}^*][\text{ACP}] \\
+ \sum_{n=6}^{9} k_{\text{off-b}_{m\text{ACP}}}[C_{2n:1}\text{FabB}^* \cdot \text{Malonyl-ACP}] \\
- \sum_{n=6}^{9} k_{\text{on-b}_{m\text{ACP}}}[C_{2n:1}\text{FabB}^*][\text{Malonyl-ACP}] \]

\[ \sum_{n=6}^{9} \frac{d[C_{2n:1}\text{FabB}^* \cdot \text{Malonyl-ACP}]}{dt} \]

Eq. B.61

\[ = \sum_{n=6}^{9} k_{\text{on-b}_{m\text{ACP}}}[C_{2n:1}\text{FabB}^*][\text{Malonyl-ACP}] \\
- \sum_{n=6}^{10} k_{\text{off-b}_{m\text{ACP}}}[C_{2n:1}\text{FabB}^* \cdot \text{Malonyl-ACP}] \\
- \sum_{n=6}^{9} k_{\text{catFabB}}[C_{2n:1}\text{FabB}^* \cdot \text{Malonyl-ACP}] \]

\[ \frac{d[\text{FabB} \cdot C_{10}\text{cis-3-Enoylacyl-ACP}]}{dt} \]

Eq. B.62

\[ = k_{\text{on-c}_{a\text{ACP}}}[\text{FabB}][C_{10}\text{cis-3-Enoylacyl-ACP}] \\
- k_{\text{off-c}_{a\text{ACP}}}[\text{FabB} \cdot C_{10}\text{cis-3-Enoylacyl-ACP}] \\
+ k_{f-\text{FabB}^*}[C_{10}\text{cis-3-FabB}^*][\text{ACP}] - k_{r-\text{FabB}^*}[\text{FabB} \cdot C_{10}\text{cis-3-Enoylacyl-ACP}] \]

\[ \frac{d[C_{10}\text{cis-3-FabB}^*]}{dt} \]

Eq. B.63

\[ = k_{f-\text{FabB}^*}[\text{FabB} \cdot C_{10}\text{cis-3-Enoylacyl-ACP}] - k_{r-\text{FabB}^*}[C_{10}\text{cis-3-FabB}^*][\text{ACP}] \\
+ k_{\text{off-b}_{m\text{ACP}}}[C_{10}\text{cis-3-FabB}^* \cdot \text{Malonyl-ACP}] \\
- k_{\text{on-b}_{m\text{ACP}}}[C_{10}\text{cis-3-FabB}^*][\text{Malonyl-ACP}] \]

\[ \frac{d[C_{10}\text{cis-3-FabB}^* \cdot \text{Malonyl-ACP}]}{dt} \]

Eq. B.64

\[ = k_{\text{on-b}_{m\text{ACP}}}[C_{10}\text{cis-3-FabB}^*][\text{Malonyl-ACP}] \\
- k_{\text{off-b}_{m\text{ACP}}}[C_{10}\text{cis-3-FabB}^* \cdot \text{Malonyl-ACP}] \\
- k_{\text{catFabB}}[C_{10}\text{cis-3-FabB}^* \cdot \text{Malonyl-ACP}] \]

\[ \sum_{n=2}^{10} \frac{d[\text{FabH} \cdot C_{2n}\text{Acyl-ACP}]}{dt} \]

Eq. B.65

\[ = \sum_{n=2}^{10} k_{\text{on-FabH-Inh-2}}[\text{FabH}][C_{2n}\text{Acyl-ACP}] \\
- \sum_{n=2}^{10} k_{\text{off-FabH-Inh-2}}[\text{FabH} \cdot C_{2n}\text{Acyl-ACP}] \]
\[
\sum_{n=6}^{10} \frac{d[\text{FabH} \cdot \text{C}_{2n:1}\text{Acyl-ACP}]}{dt} = \sum_{n=6}^{10} k_{\text{on-FabH-Inh-2}}[\text{FabH}][\text{C}_{2n:1}\text{Acyl-ACP}] - \sum_{n=6}^{10} k_{\text{off-FabH-Inh-2,C}_{2n}}[\text{FabH} \cdot \text{C}_{2n:1}\text{Acyl-ACP}]
\]

Eq. B.66

\[
\sum_{n=2}^{10} \frac{d[\text{FabH}^* \cdot \text{C}_{2n}\text{Acyl-ACP}]}{dt} = \sum_{n=2}^{10} k_{\text{on-FabH-Inh-1}}[\text{FabH}^*][\text{C}_{2n}\text{Acyl-ACP}] - \sum_{n=2}^{10} k_{\text{off-FabH-Inh-3,C}_{2n}}[\text{FabH}^* \cdot \text{C}_{2n}\text{Acyl-ACP}]
\]

Eq. B.67

\[
\sum_{n=6}^{10} \frac{d[\text{FabH}^* \cdot \text{C}_{2n:1}\text{Acyl-ACP}]}{dt} = \sum_{n=6}^{10} k_{\text{on-FabH-Inh-1}}[\text{FabH}^*][\text{C}_{2n:1}\text{Acyl-ACP}] - \sum_{n=6}^{10} k_{\text{off-FabH-Inh-3,C}_{2n}}[\text{FabH}^* \cdot \text{C}_{2n:1}\text{Acyl-ACP}]
\]

Eq. B.68

\[
\frac{d[\text{TesA} \cdot \text{ACP}]}{dt} = k_{\text{on-TesA-Inh}}[\text{TesA}][\text{ACP}] - k_{\text{off-TesA-Inh}}[\text{TesA} \cdot \text{ACP}]
\]

Eq. B.69

\[
\frac{d[\text{FabH} \cdot \text{ACP}]}{dt} = k_{\text{on-FabH-Inh-1}}[\text{FabH}][\text{ACP}] - k_{\text{off-FabH-Inh-1}}[\text{FabH} \cdot \text{ACP}]
\]

Eq. B.70

\[
\frac{d[\text{FabG} \cdot \text{ACP}]}{dt} = k_{\text{on-FabG-Inh}}[\text{FabG}][\text{ACP}] - k_{\text{off-FabG-Inh}}[\text{FabG} \cdot \text{ACP}]
\]

Eq. B.71

\[
\frac{d[\text{FabZ} \cdot \text{ACP}]}{dt} = k_{\text{on-FabZ-Inh}}[\text{FabZ}][\text{ACP}] - k_{\text{off-FabZ-Inh}}[\text{FabZ} \cdot \text{ACP}]
\]

Eq. B.72

\[
\frac{d[\text{FabI} \cdot \text{ACP}]}{dt} = k_{\text{on-FabI-Inh}}[\text{FabI}][\text{ACP}] - k_{\text{off-FabI-Inh}}[\text{FabI} \cdot \text{ACP}]
\]

Eq. B.73

\[
\frac{d[\text{FabF} \cdot \text{ACP}]}{dt} = k_{\text{on-FabF-Inh}}[\text{FabF}][\text{ACP}] - k_{\text{off-FabF-Inh}}[\text{FabF} \cdot \text{ACP}]
\]

Eq. B.74
B.5 MATLAB Code and Associated Files

Code associated with this work is available online at: [https://github.com/jmfoxai/functionalredundancy](https://github.com/jmfoxai/functionalredundancy)

1. Combined_Pathway_Solver_script. This script initiates and solves the kinetic model with the specified input parameters and options described in files 4-10.

2. Simple_Pathway_Script. This script has simplified inputs for ease of use, solving the same system as Combined_Pathway_Solver_script but with only initial conditions, enzyme concentration and time range of solution specified in this script. These inputs are then passed to Combined_Pathway_Sover_function.

3. Combined_Pathway_Sover_function. This function is called by Simple_Pathway_Script, and initiates and solves the kinetic model with specified inputs from Simple_Pathway_Script.

4. Combined_Pathway_Model_Unsaturated_Opt_vec. This function contains material balances and differential equations used to model FAS activity.

5. LeastSquaresCalc. This function calculates sums of squared errors between predicted and experimental trends (Experimental_Dataset.csv).

6. param_func. This function parameterizes the model with specified input parameters.

7. km_est.csv. This file contains the estimates of $K_m$ used by param_func.

8. est_param.csv. This file contains estimates of $k_{on}$, $k_{off}$ and $k_{cat}$ used by param_func.

9. JpMat.mat This file contains the sparsity pattern matrix for the model function.

10. Experimental_Dataset.csv: contains the digitized values of previously reported experimental data.\(^{24}\)

11. Simple_Pathway_Script_Lipid. This script is analogous to Simple_Pathway_Script but includes lipid A and phospholipid synthesis.
12. Combined_Pathway_Solver_Lipid. This function is called by Simple_Pathway_Script_Lipid and is analogous to Combined_Pathway_Solver_function.

13. Combined_Pathway_Model_Lipid. This function contains the base FAS model along with the additional lipid A and phospholipid equations and parameters and is analogous to Combined_Pathway_Model_Unsaturated_Opt_vec.
Appendix B References

C.1 Supporting Figures and Tables

Figure C.1. Sensitivity analysis of ketone production. In (A) the normalized elementary effects for varying $k_{\text{cat}}$ from reference values (range of 0.1 to 10 fold) is shown. FadE and FadD are shown to have the largest effect on ketone production, while FadB has negligible influence (likely due to its high base activity). FadD and FadB have two $k_{\text{cat}}$ values in their mechanisms, hence the numbering FadD1 and FadD2. TE CoA represents the catalytic activity of the thioesterase on acyl-CoA. In (B) the elementary effect as a function of the number of model evaluation is shown indicating that the elementary effects are converged for the full sample size (24,000 model evaluations).
Table C.1. Enzyme compositions used to refine the kinetic model of FAS activity

<table>
<thead>
<tr>
<th>Case</th>
<th>FabA</th>
<th>FabZ</th>
<th>FabF</th>
<th>FabB</th>
<th>Experimental Unsaturation</th>
<th>Fit Model Unsaturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0.74</td>
<td>0.1651</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.34</td>
<td>0.0759</td>
</tr>
<tr>
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<td>1</td>
<td>0</td>
<td>1</td>
<td>0.84</td>
<td>0.1616</td>
</tr>
<tr>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.56</td>
<td>0.1077</td>
</tr>
</tbody>
</table>

*All compositions included 1 mM NADPH, 1 mM NADH, 0.5 mM malonyl-CoA, 0.5 mM acetyl-CoA, 10 μM ACP, 10 μM TesA, and 1 μM of each Fab.
*We obtained values of unsaturation from Fig. 4G in reference.

Table C.2. Results of our optimization to FAS compositions from Table C.1.

<table>
<thead>
<tr>
<th>Case</th>
<th>Experimental Unsaturation Ratio</th>
<th>Model Unsaturation Ratio</th>
<th>Relative $k_{cat}$ Ratio</th>
<th>Fit Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>FabZ/FabA</td>
<td>0.459</td>
<td>0.459</td>
<td>0.1795</td>
<td></td>
</tr>
<tr>
<td>FabF/FabB</td>
<td>0.667</td>
<td>0.667</td>
<td>0.1236</td>
<td></td>
</tr>
</tbody>
</table>

Table C.3. Optimized Scaling Factors for Kinetic Model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description*</th>
<th>Optimized Value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>a1</td>
<td>Scales $k_{off}$ for 1-4</td>
<td>1.065 E5</td>
</tr>
<tr>
<td>a2</td>
<td>Scales $k_{off}$ for acyl-ACPs for 5-12 and 14-21</td>
<td>7.726 E3</td>
</tr>
<tr>
<td>a3</td>
<td>Scales $k_{off}$ for 13</td>
<td>4.618 E0</td>
</tr>
<tr>
<td>b1</td>
<td>Scales $k_{r}$ for 1-3,11, 18, 20</td>
<td>6.289 E4</td>
</tr>
<tr>
<td>b2</td>
<td>Scales $K_{eq}$ for acyl transfer in 1</td>
<td>5.309 E-3</td>
</tr>
<tr>
<td>b3</td>
<td>Scales $K_{eq}$ for acyl transfer in 2, 3, 11,18, 20</td>
<td>2.954 E-1</td>
</tr>
<tr>
<td>c1</td>
<td>Scales $k_{cat}$ for 4</td>
<td>4.289 E0</td>
</tr>
<tr>
<td>c2</td>
<td>Scales $k_{cat}$ for 6, 10, 12, 16, 19, 21</td>
<td>8.778 E1</td>
</tr>
<tr>
<td>c3</td>
<td>Scales $k_{cat}$ for 13</td>
<td>5.962 E-3</td>
</tr>
<tr>
<td>c4</td>
<td>Scales $k_{cat}$ for 7</td>
<td>2.164 E3</td>
</tr>
<tr>
<td>d1</td>
<td>Scales substrate specificity of TesA</td>
<td>-2.965 E-1</td>
</tr>
<tr>
<td>d2</td>
<td>Scales substrate specificity of TesA</td>
<td>3.658 E0</td>
</tr>
<tr>
<td>e</td>
<td>Scales inhibition of (i) FabH and FabF by ACPs and (ii) FabH by acyl-ACPs</td>
<td>9.912 E0</td>
</tr>
<tr>
<td>f</td>
<td>Scales $K_{eq}$ for isomerization in 16</td>
<td>1.047 E1</td>
</tr>
</tbody>
</table>

*The numbers in these descriptions correspond to lines (i.e., reactions) of Table B.1 in our previous paper.
**We determined these parameters by optimizing our kinetic model.
Table C.4. Rate equations for oleochemical pathways

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Rate Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>FadD (two reactions), each reaction uses two substrates</td>
<td>[ \text{rate} = \frac{k_{\text{cat}}[E][S_1][S_2]}{(K_{m1} + [S_1])(K_{m2} + [S_2])} ]</td>
</tr>
<tr>
<td>FadE/ACO</td>
<td>[ \text{rate} = \frac{k_{\text{cat}}[E][S_1][S_2]}{(K_{m1} + [S_1])(K_{m2} + [S_2])} ]</td>
</tr>
<tr>
<td>FadB hydratase activity</td>
<td>[ \text{rate} = \frac{k_{\text{cat}}[E][S]}{K_m + [S]} ]</td>
</tr>
<tr>
<td>FadB dehydrogenase activity</td>
<td>[ \text{rate} = \frac{k_{\text{cat}}[E][S_1][S_2]}{(K_{m1} + [S_1])(K_{m2} + [S_2])} ]</td>
</tr>
<tr>
<td>FadM</td>
<td>[ \text{rate} = \frac{k_{\text{cat}}[E][S]}{K_m + [S]} ]</td>
</tr>
<tr>
<td>FadA</td>
<td>[ \text{rate} = \frac{k_{\text{cat}}[E][S_1][S_2]}{(K_{m1} + [S_1])(K_{m2} + [S_2])} ]</td>
</tr>
</tbody>
</table>

Table C.5. Estimates of Kinetic Parameters

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FadD</td>
<td>Km_ATP</td>
<td>36*</td>
<td>µM</td>
<td>2</td>
</tr>
<tr>
<td>FadD</td>
<td>kcatD1</td>
<td>0.029</td>
<td>s⁻¹</td>
<td>3</td>
</tr>
<tr>
<td>FadD</td>
<td>Km_CoA</td>
<td>40.67*</td>
<td>µM</td>
<td>2</td>
</tr>
<tr>
<td>FadE</td>
<td>Km_FA-CoA</td>
<td>0.947*</td>
<td>µM</td>
<td>4</td>
</tr>
<tr>
<td>FadE</td>
<td>Km_FAD</td>
<td>1.9**</td>
<td>µM</td>
<td>4</td>
</tr>
<tr>
<td>FadE</td>
<td>kcatE</td>
<td>19.03**</td>
<td>s⁻¹</td>
<td>5</td>
</tr>
<tr>
<td>FadB</td>
<td>Km_enoyl-acyl-CoA</td>
<td>53</td>
<td>µM</td>
<td>6</td>
</tr>
<tr>
<td>FadB</td>
<td>kcatB1</td>
<td>775</td>
<td>s⁻¹</td>
<td>6</td>
</tr>
<tr>
<td>FadB</td>
<td>Km_beta-hydroxy-acyl-CoA</td>
<td>69</td>
<td>µM</td>
<td>6</td>
</tr>
<tr>
<td>FadB</td>
<td>Km_NAD⁺</td>
<td>2.0</td>
<td>µM</td>
<td>6</td>
</tr>
<tr>
<td>FadB</td>
<td>kcatB2</td>
<td>382</td>
<td>s⁻¹</td>
<td>6</td>
</tr>
<tr>
<td>FadM</td>
<td>Km-FadM_beta-keto-acyl-CoA</td>
<td>8.43</td>
<td>µM</td>
<td>7</td>
</tr>
<tr>
<td>FadM</td>
<td>kcatM</td>
<td>6</td>
<td>s⁻¹</td>
<td>7</td>
</tr>
<tr>
<td>FadA</td>
<td>Km_CoA</td>
<td>102</td>
<td>µM</td>
<td>6</td>
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<tr>
<td>FadA</td>
<td>Km-FadA_beta-keto-acyl-CoA</td>
<td>96</td>
<td>µM</td>
<td>6</td>
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<tr>
<td>FadA</td>
<td>kcatA</td>
<td>117</td>
<td>s⁻¹</td>
<td>6</td>
</tr>
</tbody>
</table>

*We used the average value of the three charge isoforms

*We used the average value for acyl chain lengths 4, 8 and 16.
Appendix C References


6. He, X. Y. & Yang, S. Y. Histidine-450 is the catalytic residue of L-3-hydroxyacyl coenzyme A dehydrogenase associated with the large α-subunit of the multienzyme complex of fatty acid oxidation from Escherichia coli. *Biochemistry* (1996) doi:10.1021/bi960374y.