Supporting Information

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Enzyme	Organism	Natural product	Type	PDB ID	% Identity	RMSD (A)	Ligand(s)	FAD
BexE	*Amycolatopsis orientalis*	BE-7585A	type II PKS	4X4J	100	0.00	FAD	in
PgaE	*Streptomyces PGA64*	gaudimycin	type II PKS	2AQ1	74	0.70	FAD	in
PgaE	*Streptomyces PGA64*	gaudimycin	type II PKS	4ICY	74	0.60	FAD	in
CabE	*Streptomyces H201*	unknown	type II PKS	2QA2	46	0.53	FAD	in
MtmOIV	*Streptomyces argillaceus*	mithramycin	type II PKS	4K5S	48	1.04	FAD, premithramycin B	in
MtmOIV	*Streptomyces argillaceus*	mithramycin	type II PKS	3FMW	48	1.02	FAD	in
OxyS	*Streptomyces rimosus*	oxytetracycline	type II PKS	4K2X	45	0.82	FAD	in
RdmE	*Streptomyces purpurascens*	rhodomycin	type II PKS	3IHG	31	3.10	FAD, aklavinone	out
RebC	*Lechevalieria aerocolonigenes*	rebbecamycin	alkaloid	2R0C	28	4.35	FAD	out
RebC	*Lechevalieria aerocolonigenes*	rebbecamycin	alkaloid	4EIP	28	4.04	FAD, K252c	in
RebC	*Lechevalieria aerocolonigenes*	rebbecamycin	alkaloid	4EIQ	28	3.95	KCT	N/A
RebC	*Lechevalieria aerocolonigenes*	rebbecamycin	alkaloid	2R0G	28	5.59	FAD, 7-carboxy-K252c	in
RebC	*Lechevalieria aerocolonigenes*	rebbecamycin	alkaloid	2R0P	28	3.01	FAD, K252c soaked	out
RebC	*Lechevalieria aerocolonigenes*	rebbecamycin	alkaloid	3EPT	28	4.37	FAD (reduced)	in

Table S1. A table listing the crystal structures of biosynthetic oxygenases that are related to BexE. The % identity and RMSD are relative to the BexE structure. The last column, "FAD" corresponds to the conformation of FAD.

	BexE (PDB ID: 4X4J)
Crystallization	0.1 M MES pH 7.0, 0.2 M ammonium sulfate, 30% PEG 3350
Crystallographic Data	
Wavelength (Å)	1.0000
Space Group	C2
Cell Dimensions (a, b, c) (Å)	150.672, 80.379, 105.079
	α=γ=90° β=126.191°
Resolution (Å)	50.00 - 2.65
No. of observations	218698
No. of unique observations	29488
Completeness %	100.00 (99.8)
I/σ(I)	21.6 (3.9)
R_{merge} %	8.4 (46.9)
Redundancy	7.4
Refinement	
Resolution (Å)	50.00 - 2.65 (2.74 - 2.65)
No. of protein atoms	7162
No. of ligand atoms	121
No. of water atoms	58
R_{free} %	24.56 (35.29)
R_{crys} %	19.62 (29.89)
Geometry	
RMS bonds (Å)	0.005
RMS angles (°)	0.960
Ramachandran Favored (%)	96
Ramachandran Allowed (%)	3.58
Ramachandran Dissallowed (%)	0.42
Average B-factors (Å^2)	
Protein	58.20
Water	55.80
Ligands	48.10

Table S2. BexE crystallographic data collection and refinement statistics. *Numbers in parentheses denote the highest resolution shell.

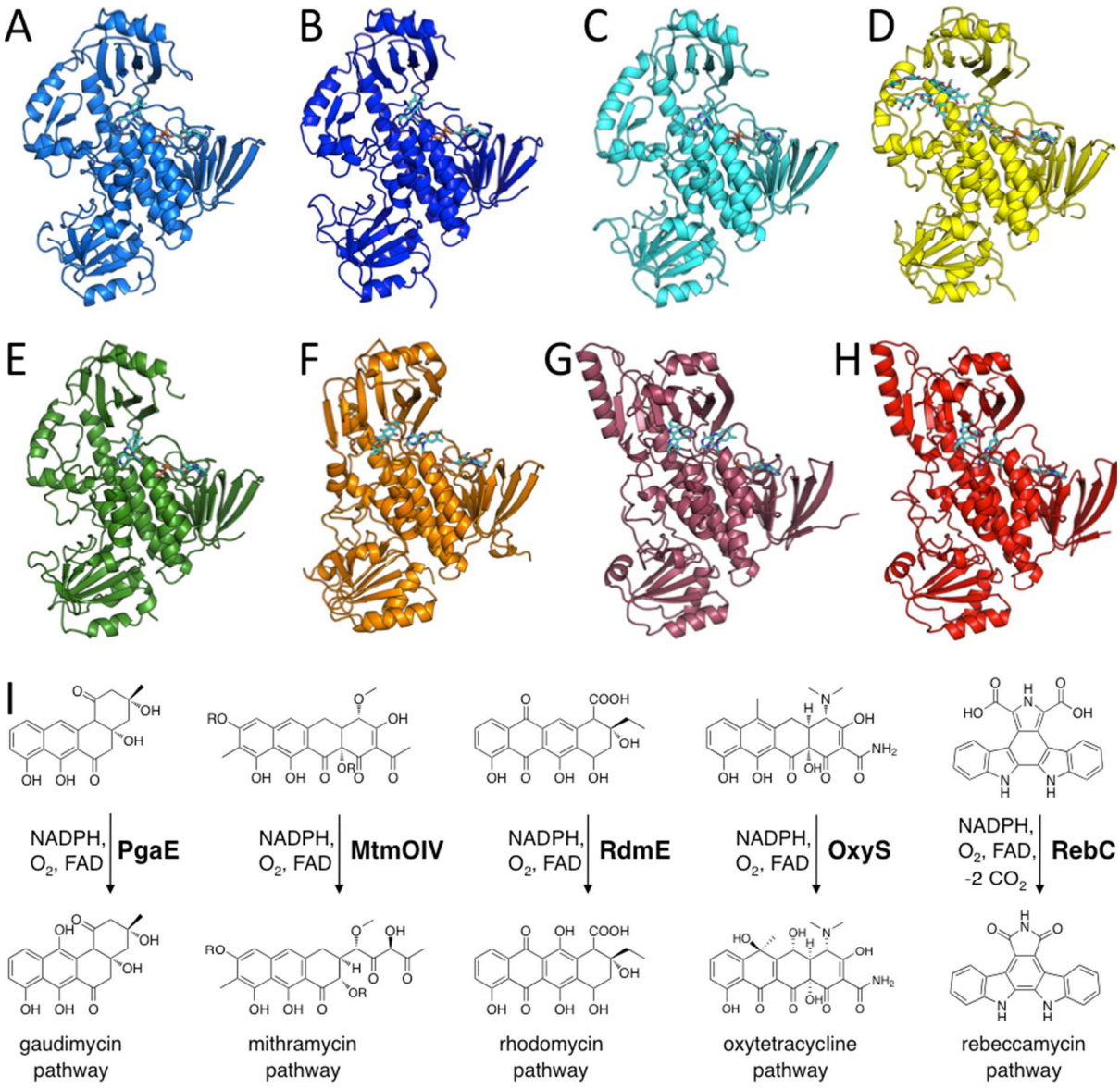

Figure S1. The crystal structures of oxygenases from polyketide and alkaloid biosynthetic pathways. The middle domain is located at the top of each structure, the FAD binding domain in the middle, and the C-terminal domain is at the bottom. (A) BexE (polyketide oxygenase) bound to FAD. (B) PgaE (polyketide oxygenase) bound to FAD. (C) CabE (polyketide oxygenase) bound to FAD. (D) MtmOIV (polyketide oxygenase) bound to FAD and premithramycin B. (E) OxyS (polyketide oxygenase) bound to FAD. (F) RdmE (polyketide oxygenase) bound to FAD and aklavinone. (G) RebC (alkaloid oxygenase) bound to FAD and K252c. (H) RebC (alkaloid oxygenase) bound to FAD and K252c. (I) Reactions catalyzed by oxygenases that are related structurally related to BexE.

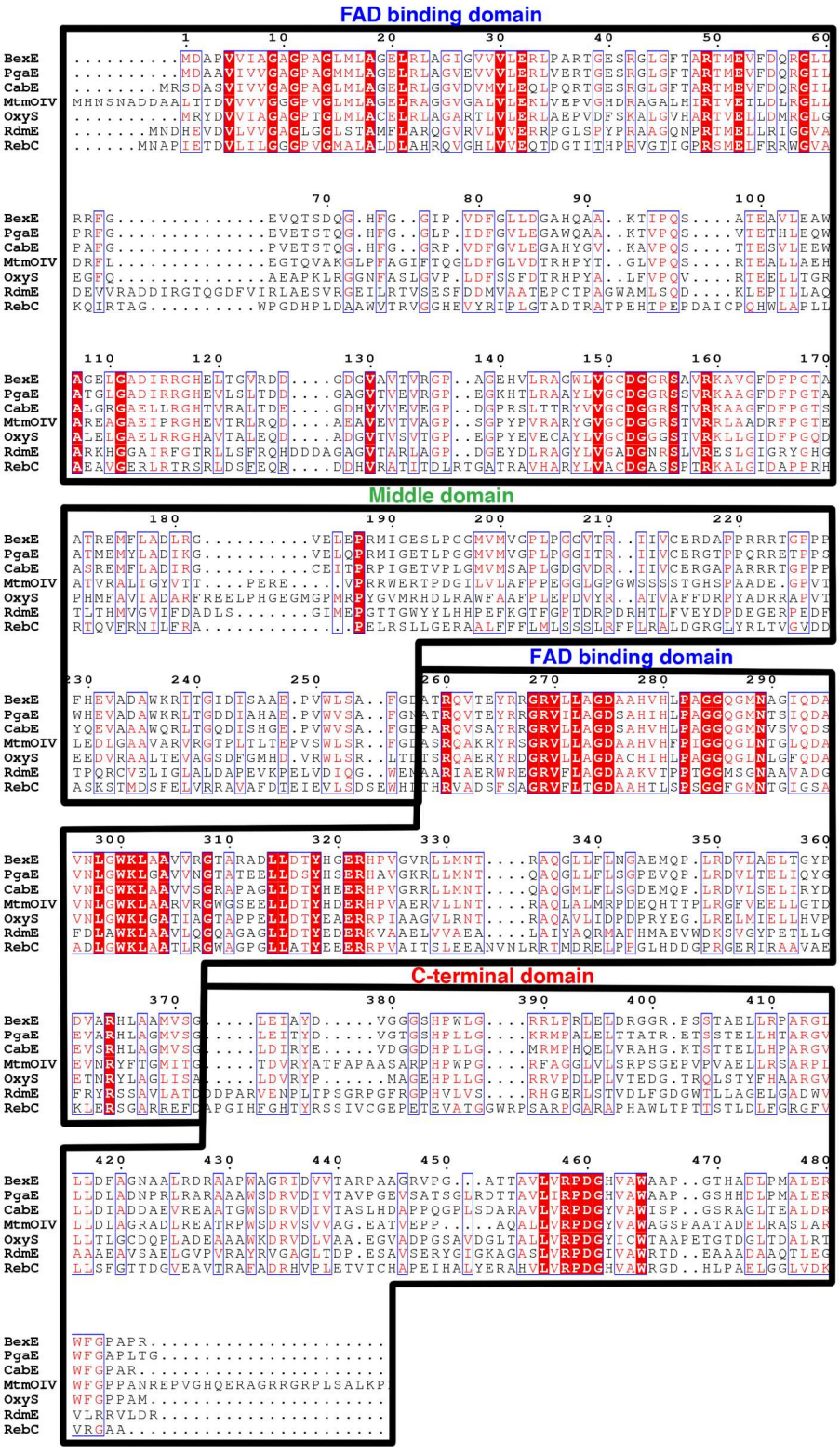

Figure S2. Sequence alignment of BexE with other oxygenases from biosynthetic pathways.

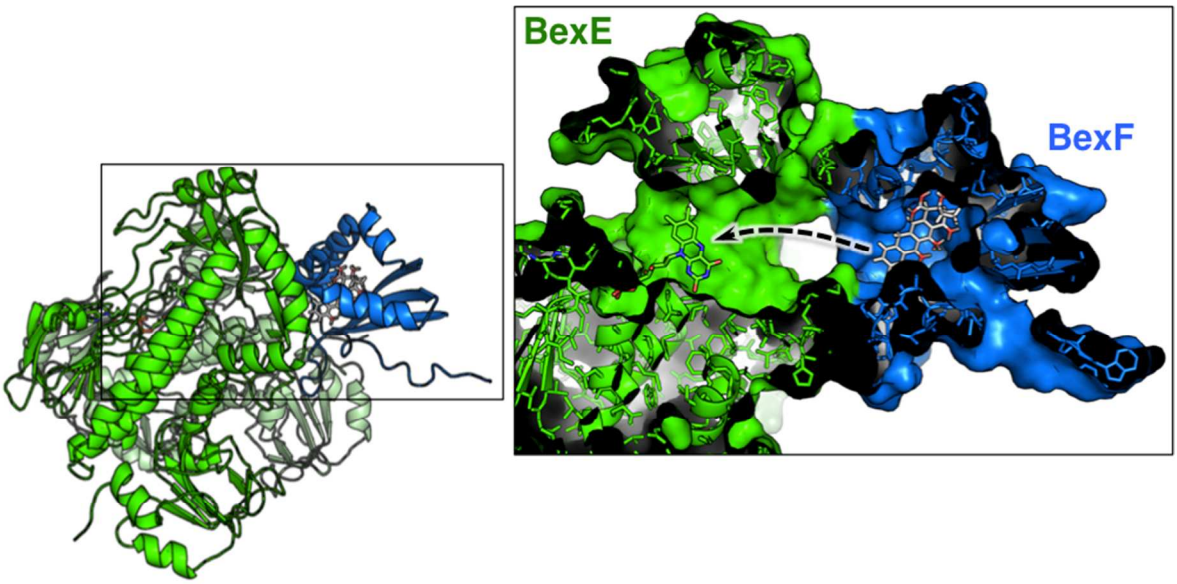

Figure S3. A cartoon representation of the BexF homology model bound to a tetracyclic substrate and docked to the surface of BexE. The BexF model contains a large pocket and active site entrance. When BexF is docked with BexE, a clear interface between the two enzyme active site entrances forms to reveal a tunnel, which we hypothesize is responsible for transferring a reactive substrate from the BexF active site to the BexE active site.