Plant Communications, Volume *4*

Supplemental information

MEP pathway products allosterically promote monomerization of deoxy-D-xylulose-5-phosphate synthase to feedback-regulate their supply

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Supplemental Tables

Supplemental Table S1: Strains used in this study

Name	Description	Antibiotic	Reference
TOP10	F- *mcrA* Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lacZ*ΔM15 Δ *lac*X74 *rec*A1 *ara*D139 Δ(*araleu*)7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G	-	Invitrogen
BL21(DE3)pLysS	F^-*omp*T *hsd*SB (r_B^-, m_B^-) *gal dcm rne*131 (DE3) pLysS (Cm^R)	Cm	Invitrogen
K12 MG1655	F- lambda- ilvG - rfb -50 rph -1	-	(1)
EcAB4-10	K12 MG1655 Δdxr::CAT pBAD-yPMD-hPMK-yMVK-EcIDI	Km/Cm	(2)
EcAM5-1	BL21(DE3) pBAD-yPMD-hPMK-yMVK-EcIDI	Km	(3)

Cm: Chloramphenicol, **Km**: Kanamycin

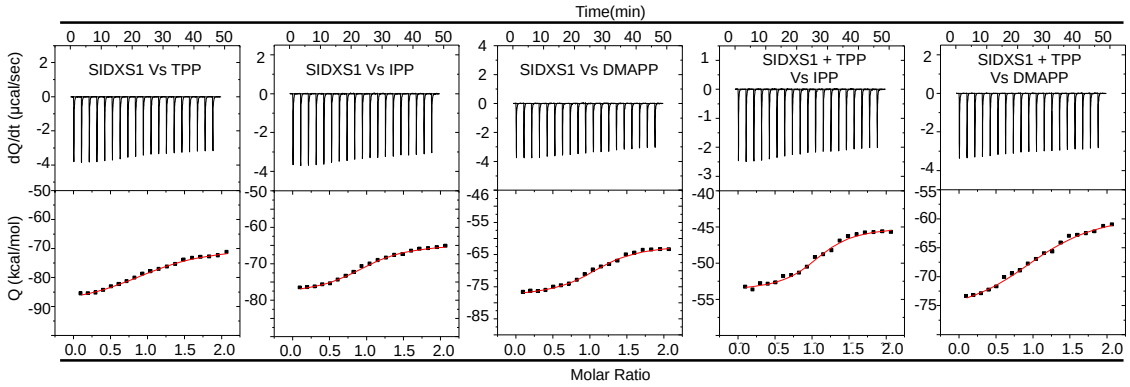
1. **Blattner et al.,** (1997). The complete genome sequence of *Escherichia coli* K-12. Science 277: 1453-1462.
2. **Sauret-Güeto et al.,** (2006). A mutant pyruvate dehydrogenase E1 subunit allows survival of *Escherichia coli* strains defective in 1-deoxy-D-xylulose 5-phosphate synthase. FEBS Lett. 580: 736-740.
3. **Rodriguez-Villalon et al.,** (2008). Carotenoid accumulation in bacteria with enhanced supply of isoprenoid precursors by upregulation of exogenous or endogenous pathways. J. Biotechnol. 135: 78-84.

Supplemental Table S2: Plasmids and primers used in this study.

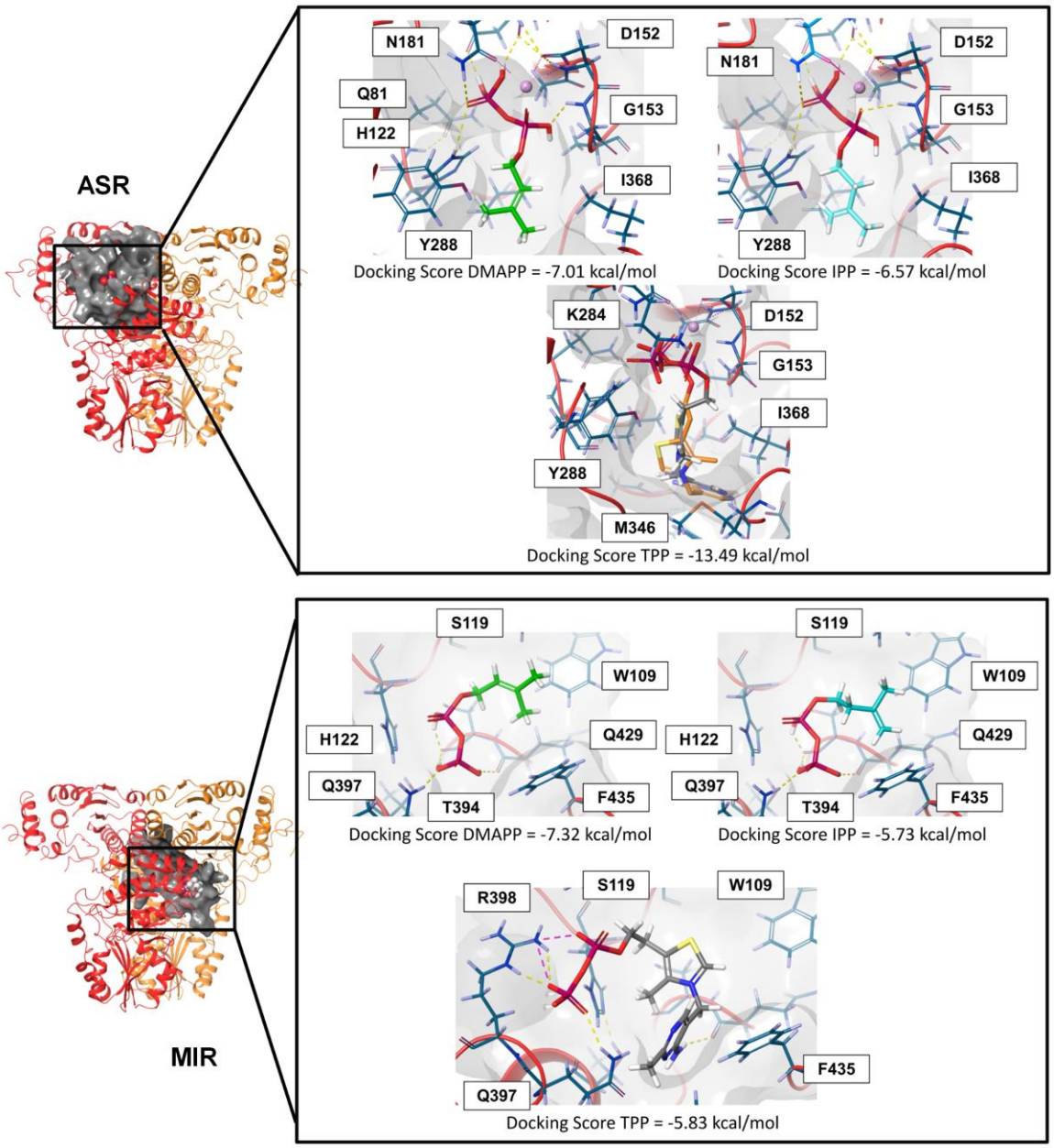
Type				
Plasmids	**Backbone**	**Source**	**Cloning method**	**Tag**
pET23-EcDXS	pET23	Novagen	Ligation	6xHis (C-terminal)
pET23-SlDXS1	pET23	Novagen	Ligation	6xHis (C-terminal)
pGWB420-SlDXS1	pGWB420	(1)	Gateway	myc (C-terminal)
pGWB405-SlDXS1	pGWB405	(1)	Gateway	GFP (C-terminal)
Primers	**Use**	**5' - 3' Sequence**		
EcDXS_NdeI-F	Ligation	CGGCATATGAGTTTTGATATTGC		
EcDXS_XhoI-R	Ligation	ATTCTCGAGTGCCAGCCAGGCCTTG		
NheI_myc_SlDXS1-F	Ligation	ATGGCTAGCGAACAAAAACTCATCTCAGAAGAGGATCTGGCTTCCTTATCAGAATCTGG		
SlDXS1_XhoI-R	Ligation	CTTACTCGAGTGTCATGACCTCTAGAGCCTCTC		
GW_SlDXS1-F	Gateway	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGATGGCTTTGTGTGCTTATGCAT		
GW_SlDXS1-R	Gateway	GGGGACCACTTTGTACAAGAAAGCTGGGTCTGTCATGACCTCTAGAGC		

1. **Nakagawa et al.,** (2007). Improved Gateway binary vectors: high-performance vectors for creation of fusion constructs in transgenic analysis of plants. Biosci. Biotechnol. Biochem. 71**:** 2095-2100.

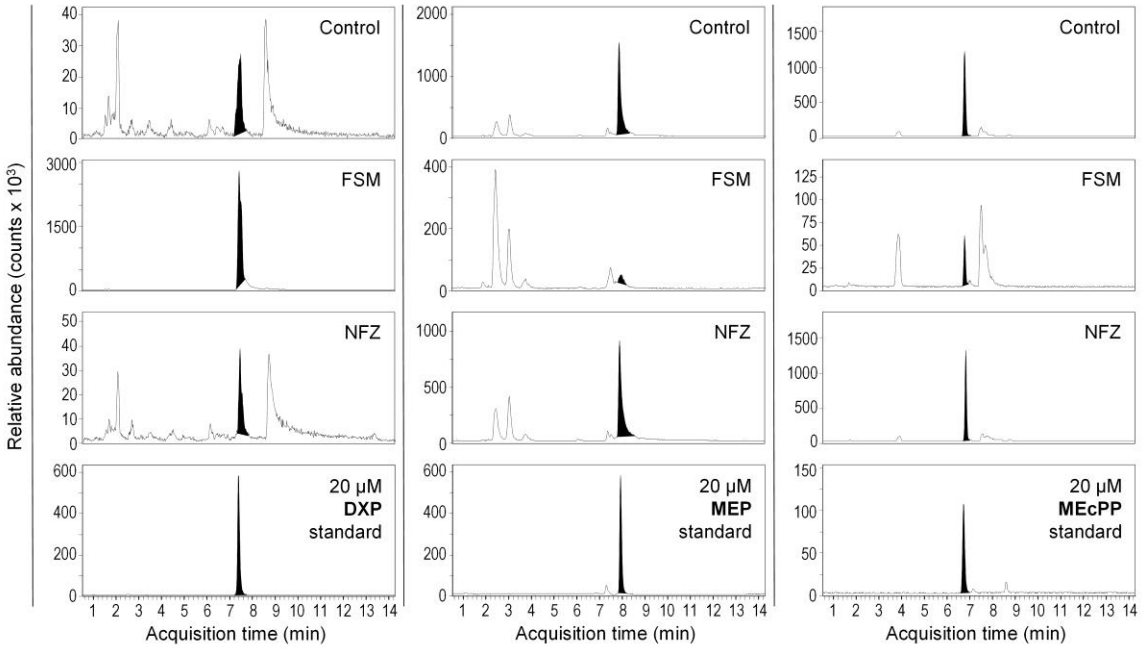
Supplemental Figures



Supplemental Fig S1. Analysis of SlDXS1 interaction with metabolite ligands. ITC plots were obtained from the titration of 20 µM SlDXS1 (either alone or with 100 µM TPP) with 200 µM TPP, IPP or DMAPP as indicated. Assays were performed at 25 ºC in 50 mM HEPES, 150 mM NaCl buffer. The plots in the upper panel show the thermogram (raw thermal power as a function of time), and the plots in the lower panel show the binding isotherm (heat released per injection normalized per mole of ligand injected as a function of the molar ratio, [ligand]/[protein], in the calorimetric cell). The solid lines represent the best fits of the experimental data after non-linear least-squares analysis using a single-site binding model.



Supplemental Fig. S2. EcDXS ligand binding site prediction. Binding sites for ligands are shown in grey: active site region (ASR) and monomer interacting region (MIR). DMAPP, IPP, and TPP carbon atoms are shown in green, cyan, and gray, respectively. In the ASR, the reported binding mode of TPP in the X-ray structure is shown using orange carbon atoms. Residues interacting with ligands are shown, and important residues identified by docking calculations are labeled. Ligand-protein interaction energies are provided in kcal/mol. Hydrogen, oxygen, nitrogen, sulfur, and phosphorous atoms are coloured in white, red, blue, yellow, and purple, respectively.



Supplemental Fig S3. LC-MS chromatograms of plant samples. MEP pathway metabolites were identified in extracts of *N. benthamiana* leaves treated with the indicated inhibitors or a mock solution (control) using mass-to-charge (*m/z*) ratios of 214.024 (DXP), 216.04 (MEP), and 277.996 (MEcPP). Retention times of commercial standards is shown in the bottom plots.