SUPPLEMENTAL INFORMATION

CRYSTAL STRUCTURE OF HETERODIMERIC HEXAPRENYL DIPHOSPHATE SYNTHASE FROM *MICROCOCCUS LUTEUS* B-P 26 REVEALS THAT THE SMALL SUBUNIT IS DIRECTLY INVOLVED IN THE PRODUCT CHAIN LENGTH REGULATION

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Supplemental Figure Captions

Supplemental Figure 1. Characterized homo- and heterooligomeric *trans*-prenyltransferases with their sources and PDB codes if available. These include heterotetrameric GPPs from *Mentha piperita* (1-3), heterodimeric HexPPs from *Micrococcus luteus* B-P 26 (4-9), heterodimeric HepPPs from *Bacillus subtilis* (4,10-14) and *Bacillus stearothermophilus* (15,16), heterotetrameric SPPs from *Mus musculus* (17) and *Homo sapience* (17), and heterotetrameric DPPs from *Schizosaccharomyces pombe* (18) and *Homo sapience* (17), homodimeric GPPs from *Abies grandis* (19), homodimeric FPPs from *E. coli* (20), *Gallus gallus* (21,22), *Homo sapience* (23,24) and *Trypanosoma cruzi* (25), homodimeric GGPPs from *Saccharomyces cerevisiae* (26), *Sinapis alba* (27), *Pantoea ananatis* (28), *Pyrococcus horikoshii* Ot3 and *Thermus thermophilus* (29), homohexameric GGPPs from *Homo sapience* (30,31), homodimeric HexPPs from *Sulfolobus solfataricus* (32,33), homodimeric OPPs from *Thermotoga maritima* (34), homodimeric SPPs from *Micrococcus luteus* (35) and *Arabidopsis thaliana* (36), and homodimeric DPPs from *Agrobacterium tumefaciens* (37).

Supplemental Figure 2. Arrangement of the two heterodimers ((HexA-HexB) and (HexA'-HexB')) in an asymmetric unit. (A) Front view of the heterodimers (*cylindrical helices*). Two HexB molecules, the large subunits of the two heterodimers, are shown by *blue* (chain B) and *green* (chain D) *helices* (A-Q, A'-Q'). The other small subunits, two HexA molecules, are shown by *red* (chain A) and *yellow* (chain C) *helices* (R-X, R'-X'). Magnesium ions are shown by *pink spheres*. The *black line* represents the non-crystallographic 2-fold axis at the center of the two heterodimers. (B) Top view of the *Ml*-HexPPs heterodimers and the topology of the subunit assembly. The topology diagram is also drawn from the top side (aspartate-rich motif side) of the heterodimer. The color codes are the same as in (A). The first and the second aspartate-rich motifs in HexB molecules, FARM (DD$XX_{2\text{-}4}$D) and SARM (DD*XX*D) are shown by *stick* models. Inf-A (~1800 Å^2) and Inf-B (~750 Å^2) are the intra- and inter-heterodimeric interfaces, respectively. The *closed black circle* indicates the 2-fold axis. (C) Top view of the *Mp*-GPPs heterotetramer (*cylindrical helices*) and the topology of the subunit assembly. Two LSU molecules, the large subunits of the heterotetramer, are shown by *yellow* (chain D) and *lightgreen* (chain A) *helices*. The first and the second aspartate-rich motifs in LSU molecules, FARM (DD$XX_{2\text{-}4}$D) and SARM (DD*XX*D) are shown by *stick* models. The other small subunits, two SSU molecules, are shown by *rightbrown* (chain C) and *magenta* (chain B) *helices*. Inf-A (~1900 Å^2) and Inf-B (~800 Å^2) are the intra- and inter-heterodimeric interfaces, respectively. The *closed black circle* indicates the 2-fold axis.

Supplemental Figure 3. Product analyses of the recombinant *Ml*-HexPPs using the TLC autoradiogram. Position of authentic standard (C_{30}) (lane 1). The reaction product catalyzed by *Ml*-HexPPs sample before (lane 2) and after (lane 3) tag-removal and by HexPPs from *S. solfataricus* (33) (lane 4). Ori., origin; S.F., solvent front.

Supplemental Figure 4. Amino-acid sequence alignment of the small (A) and the large (B) components of heterodimeric *trans*-prenyltransferases. The sequences of the small components of

Ml-HexPPs, HepPPs from *B. subtilis* and *B. stearothermophilus* are displayed as *Ml*-HexA, *Bsu*-HepI and *Bst*-HepI', respectively, and those for the large components of *Ml*-HexPPs, HepPPs from *B. subtilis* and *B. stearothermophilus* are displayed as *Ml*-HexB, *Bsu*-HepII and *Bst*-HepII', respectively. Identical and similar amino-acid residues are highlighted by *black* and *white boxes,* respectively. Each secondary structure of HexA or HexB is shown above each sequence. Residues forming the hydrophobic cleft wall in HexA and HexB (as illustrated in Figures 4B and 4C) are highlighted in *red* and *blue* with *arrows,* respectively. The figures were prepared with the program ESPript (38).

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