## Supporting information for

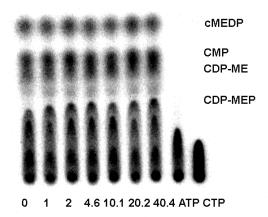
## Absence of Substrate Channeling between Active Sites in the $Agrobacterium \ tume faciens$ IspDF and IspE Enzymes of the Methyl Erythritol Phosphate Pathway<sup>†</sup>

Christian Lherbet $^{\dagger}$ , Florence Pojer $^{\$}$ , Stéphane B. Richard $^{\$}$ , Joseph P. Noel $^{\$}$  and C. D. Poulter $^{\dagger}*$ 

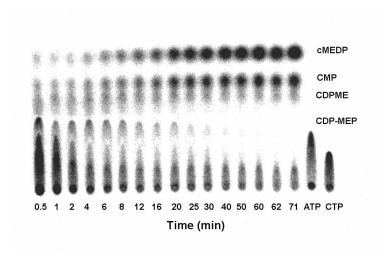
<sup>†</sup>Department of Chemistry, University of Utah, Salt Lake City, Utah 84112

§Jack Skirball Chemical Biology and Proteomics Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037

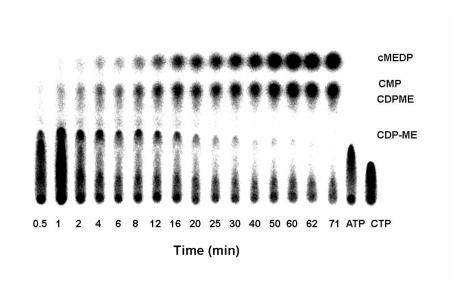
poulter@chem.utah.edu



**Figure SI1**: TLC plate showing the formation of CDP-ME, CDP-MEP, CMP, cMEDP catalyzed by IspDF (0.24  $\mu$ M), IspE (0.31  $\mu$ M) in presence of different concentrations of IspE(D152A) after 15 min reaction at 37°C. ATP and CTP lanes represent the chemical reaction without enzyme.

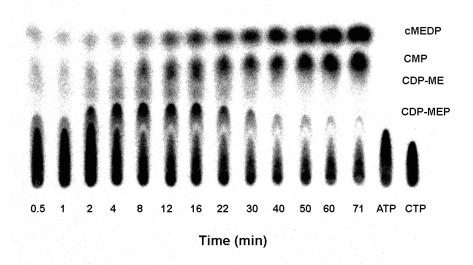


**Figure SI2:** TLC plate showing the time course for the formation of CDP-ME, CDP-MEP, CMP, cMEDP catalyzed by IspDF (0.24  $\mu$ M), IspE (3.12  $\mu$ M). At 61 min an additional 0.5  $\mu$ g portion of each enzyme were added. ATP and CTP lanes represent the chemical reaction without enzyme.

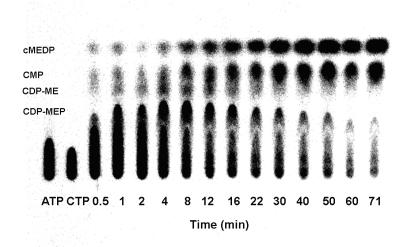


**Figure SI3**: TLC plate showing the time course for the formation of CDP-ME, CDP-MEP, CMP, cMEDP catalyzed by IspDF (0.24  $\mu$ M), IspE (3.12  $\mu$ M) and IspE(D152A)

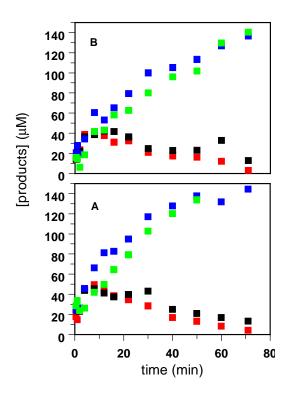
 $(31.2~\mu M)$ . At 61 min an additional 0.5  $\mu g$  portion of each enzyme were added. ATP and CTP lanes represent the chemical reaction without enzyme.



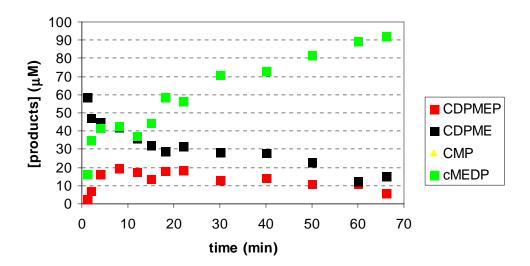
**Figure SI4**: TLC plate showing the time course for the formation of CDP-ME, CDP-MEP, CMP, cMEDP catalyzed by IspDF (0.24  $\mu$ M), IspE (3.12  $\mu$ M) in presence of 30% glycerol. At 61 min an additional 0.5  $\mu$ g portion of each enzyme were added. ATP and CTP lanes represent the chemical reaction without enzyme.



**Figure SI5 :** TLC plate showing the time course for the formation of CDP-ME, CDP-MEP, CMP, cMEDP catalyzed by IspDF (0.24  $\mu$ M), IspE (3.12  $\mu$ M) and IspE(D152A) (31.2  $\mu$ M) in presence of 30% glycerol. At 61 min an additional 0.5  $\mu$ g portion of each enzyme were added. ATP and CTP lanes represent the chemical reaction without enzyme.



**Figure SI6 :** Time courses for the formation of CDP-ME( $\blacksquare$ ), CDP-MEP ( $\blacksquare$ ), CMP ( $\blacksquare$ ), cMEDP ( $\blacksquare$ ) in presence of 30% glycerol. Panel A : IspDF (0.24  $\mu$ M) and IspE (3.12  $\mu$ M); Panel B : IspDF (0.24  $\mu$ M), IspE (3.12  $\mu$ M), IspE(D152A) (31.2  $\mu$ M).



**Figure SI6 :** Time courses for the formation of CDP-ME, CDP-MEP, CMP, cMEDP catalyzed by IspDF (0.24  $\mu$ M), IspE (3.12  $\mu$ M) and IspE(D152A) (30.3  $\mu$ M) in presence of 30% (v/v) glycerol.