Electronic Supplementary Material (ESI) for Chemical Science.

Electronic Supplementary Information

IspH-RPS1 and IspH-UbiA: "Rosetta Stone" Proteins

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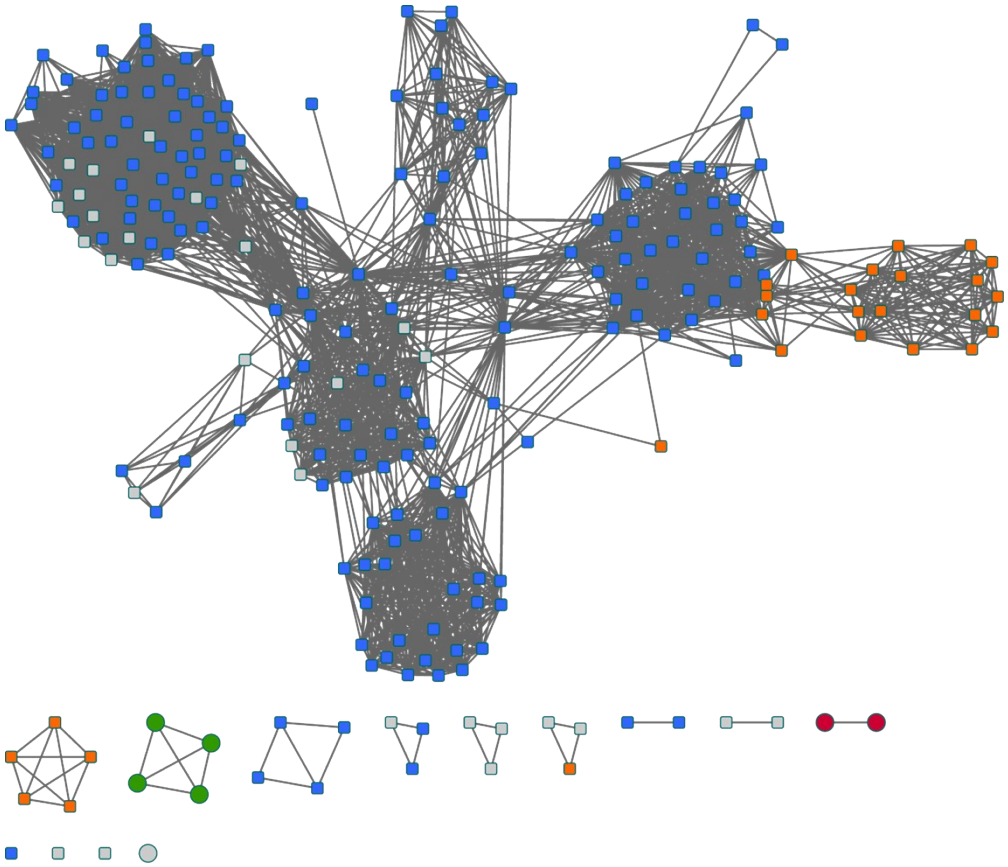

Figure S1. Sequence similarity network of IspH-RPS1 cluster, expectation-value (e-value) of 10^{-160}. Blue: Clostridia. Orange: Negativicutes. Green: Thermotogae. Red: Fusobacteria. Circle: IspH-RPS1 with 6 S1 repeats.

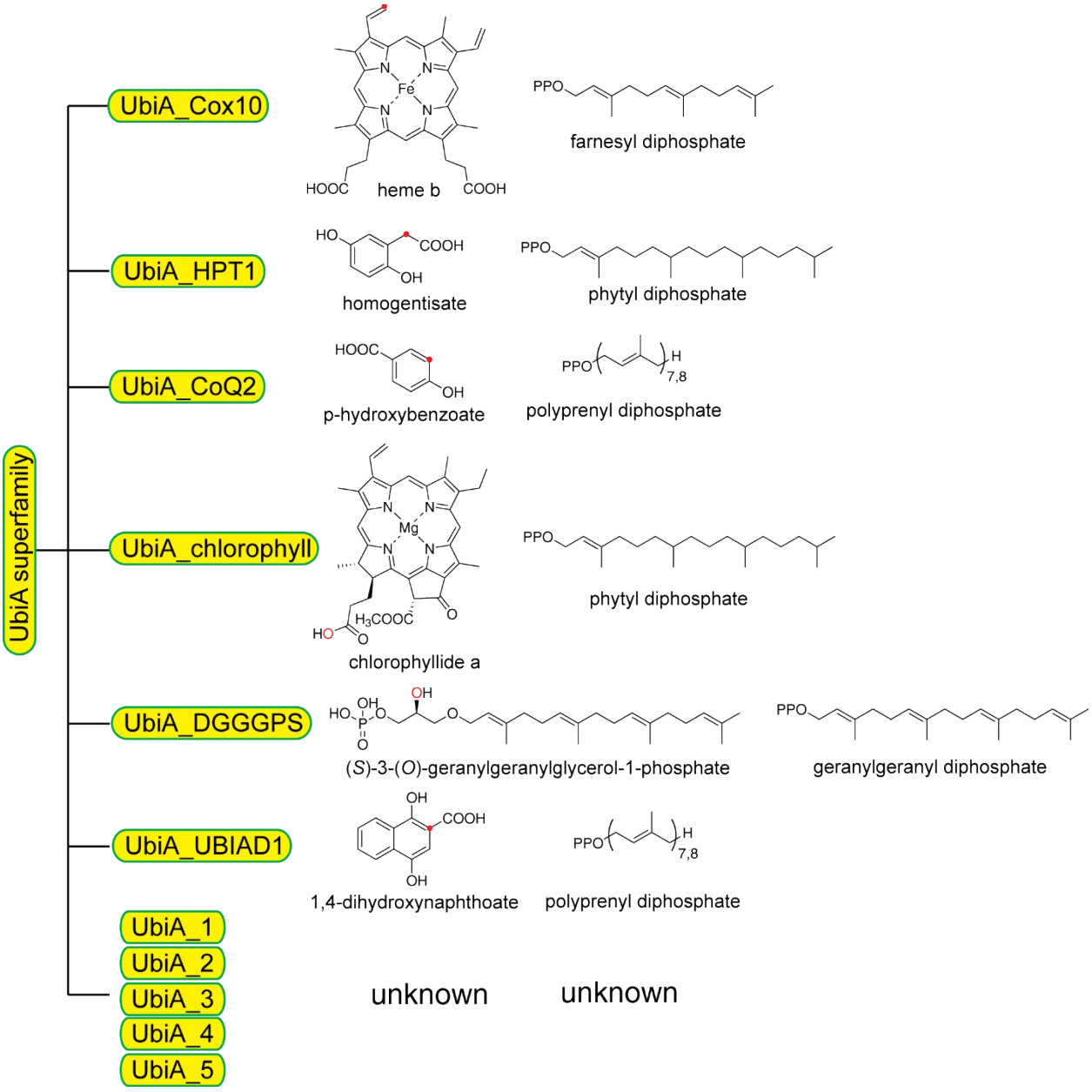

Figure S2. The UbiA superfamily. The UbiA superfamily has a very diverse range of substrates for prenylation and the substrates/products in several cases are not known. The sub-groups of UbiA and their substrates are listed. Atoms in red indicate the sites of prenylation. The UbiA in the IspH-UbiA hybrid belongs to the UbiA_5 category with unknown function.

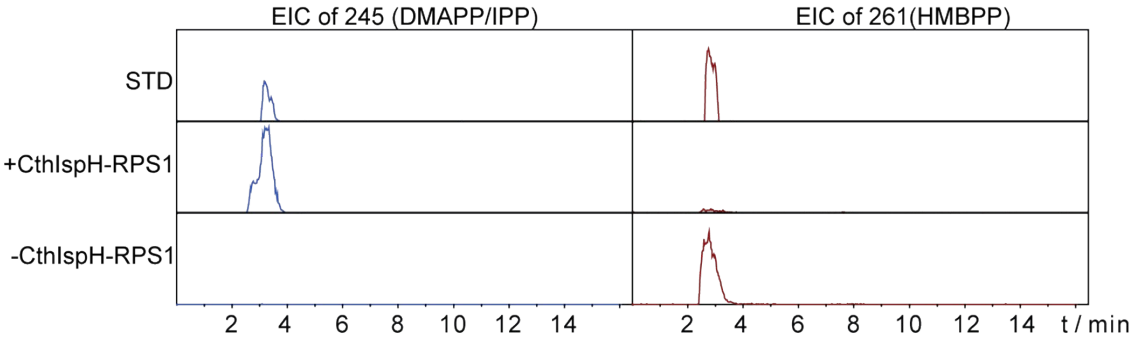

Figure S3. LC-MS of CthIspH-RPS1 catalyzed reaction confirmed the production of DMAPP/IPP and consumption of HMBPP. DMAPP and IPP were not separated in this chromatogram.

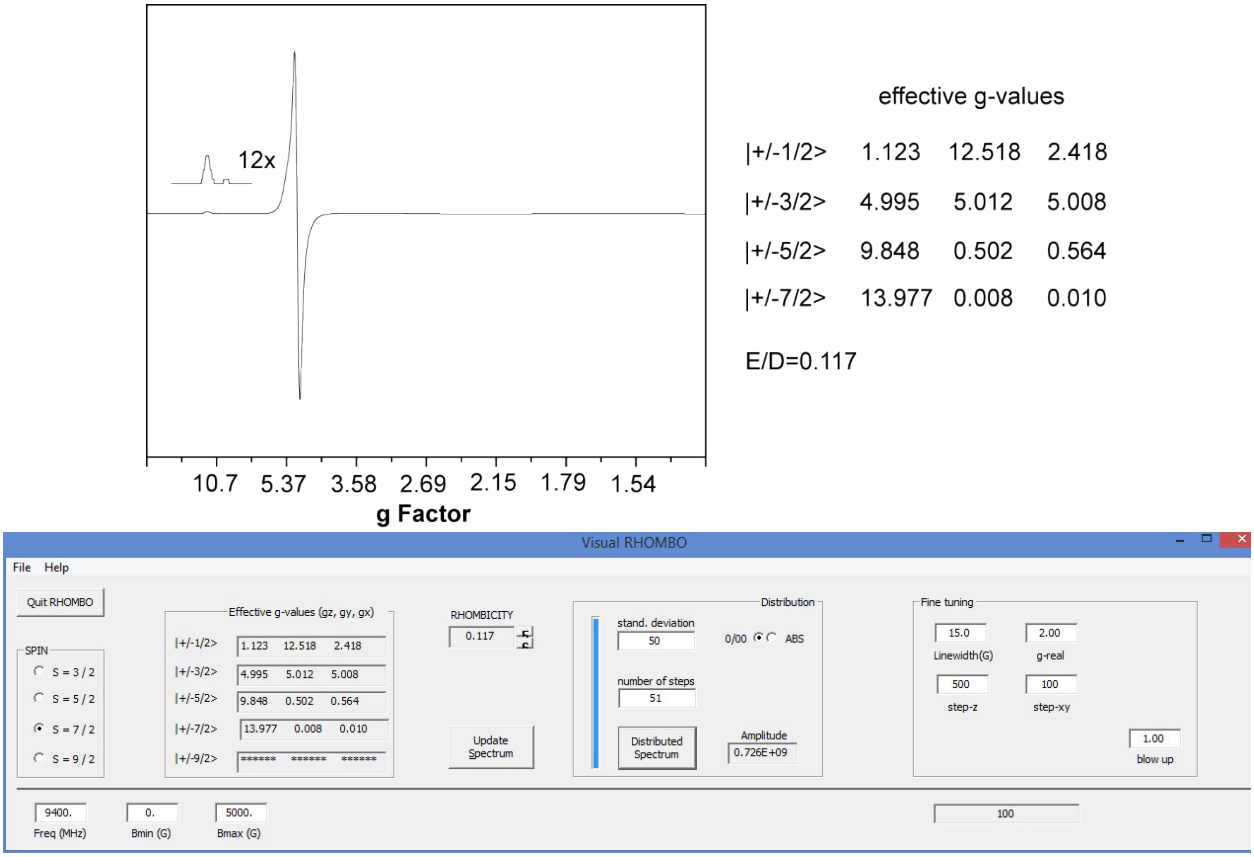

Figure S4. Simulation of the g=5.0 signal from the EPR spectrum of reduced CthIspH-RPS1 with an S=7/2 spin system and E/D=0.117. The total effective g-values are listed. Simulation was carried out using Visual RHOMBO (http://www.tnw.tudelft.nl/nl/over-faculteit/afdelingen/biotechnology/data-software/visual-rhombo/)

A

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  1 MNLTELKNTP VSELITLGEN MGLENLARMR KQDIIFAILK QHAKSGEDIF
 51 GDGVLEILQD GFGFLRSADS SYLAGPDDIY VSLSQIRRFN LRTGDTISGK
101 IRPPKEGERY FALLKVNEVN FDKPENARNK ILFENLTPLH ANSRLRMERG
151 NGSTEDLTAR VLDLASPIGR GQRGLIVAPP KAGKTMLLQN IAQSIAYNHP
201 DCVLMVLLID ERPEEVTEMQ RLVKGEVVAS TFDEPASRHV QVAEMVIEKA
251 KRLVEHKKDV IILLDSITRL ARAYNTVVPA SGKVLTGGVD ANALHRPKRF
301 FGAARNVEEG GSLTIIATAL IDTGSKMDEV IYEEFKGTGN MELHLSRKIA
351 EKRVFPAIDY NRSGTRKEEL LTTQEELQKM WILRKIIHPM GEIDAMEFLI
401 NKLAMTKTND DFFEMMKRS
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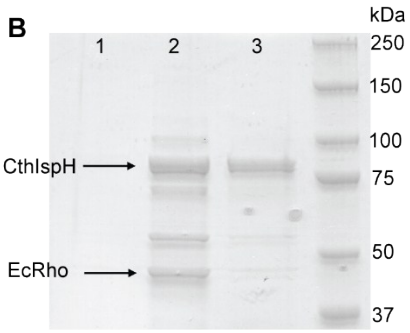
B



1. 50 mM Imidazole wash
2. 0.5 mM (CT)4 wash
3. 250 mM Imizadole elution

Figure S5. (A) *E. coli* Rho transcription termination factor MS fingerprint. Covered peptide fragments are shown in red. (B) SDS-PAGE gel showing that EcRho can be dissociated from IspH-RPS1 with a $(CT)_4$ DNA oligomer.