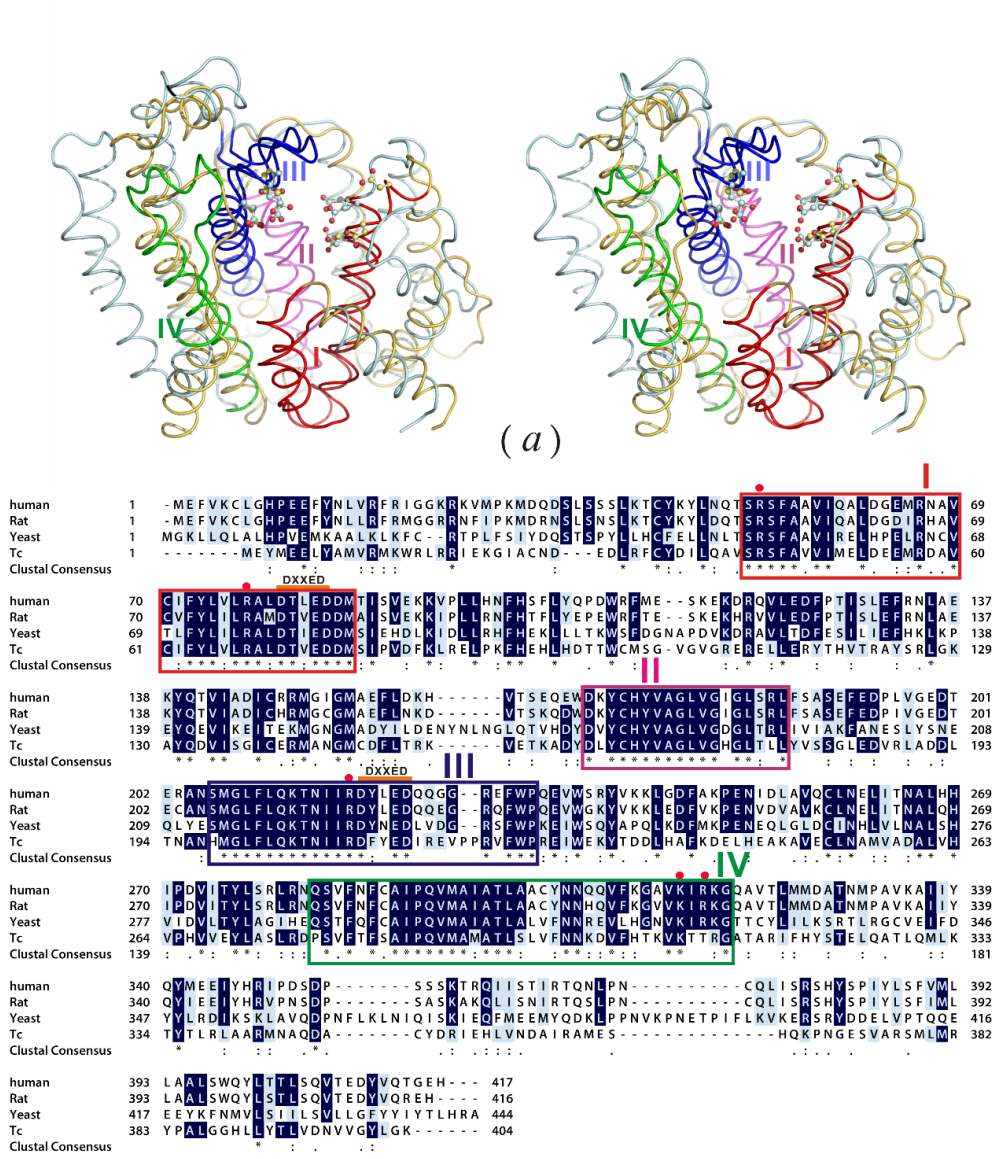
## Supplementary Material

**Supplementary Table S1.** Primers used to construct human SQS mutants in this study.

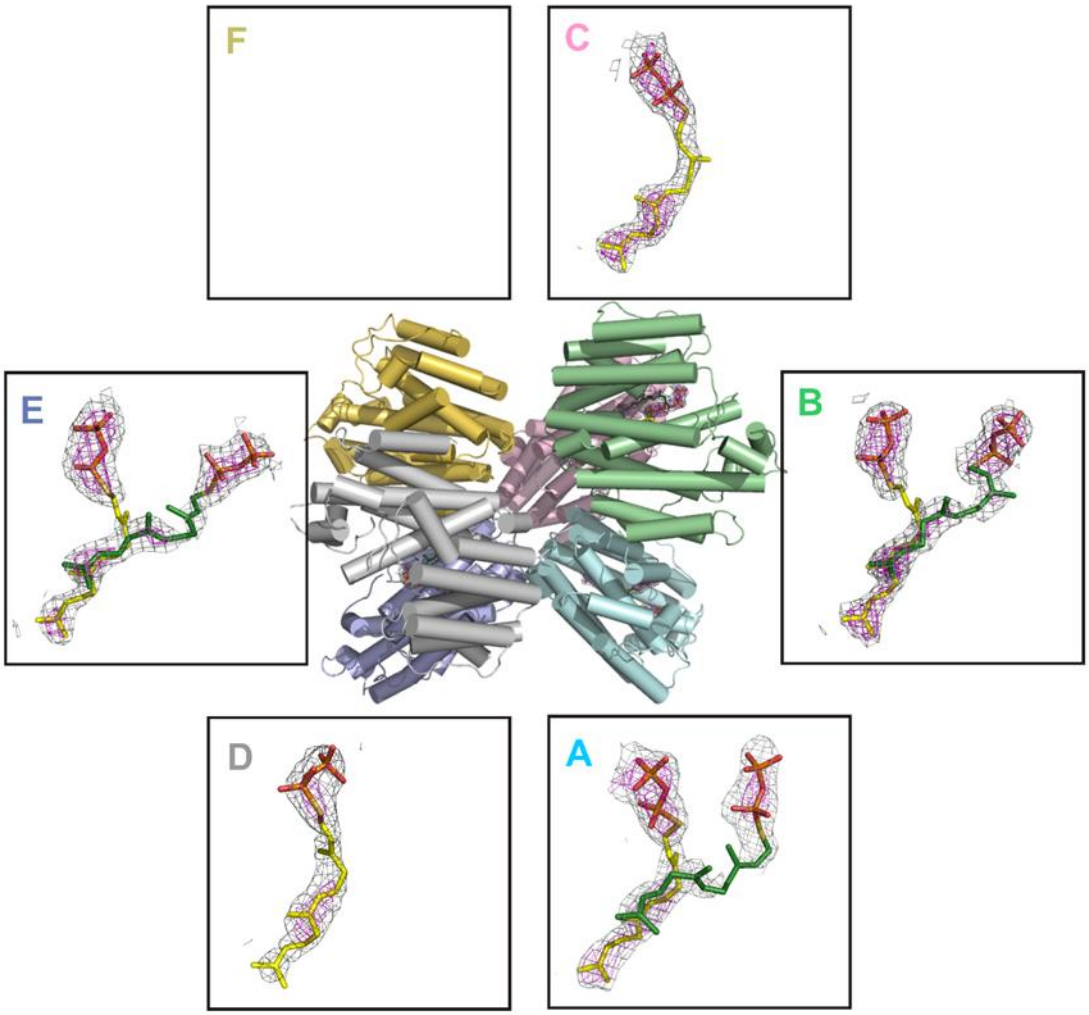
| Mutant name | Primer sequence |
|---|---|
| **R52A** | 5’-GTATCTCAATCAGACCAGTGCCAGTTTCGCAGCTGTTATCC-3’ |
| **R52E** | 5’-GTATCTCAATCAGACCAGTGAGAGTTTCGCAGCTGTTATCC-3’ |
| **Y73A** | 5’-CGCAACGCAGTGTGCATATTTGCTCTGGTTCTCCGAGCTCTGGA-3’ |
| **R77A** | 5’-ATATTTTATCTGGTTCTCGCGGCTCTGGACACACTGGA-3’ |
| **K117A** | 5’-GAGAGCAAGGAGGCAGATCGCCAGGTGCT-3’ |
| **Q212L** | 5’-TCTATGGGCCTGTTTCTGCTGAACACA AACATCATCCG-3’ |
| **Q212N** | 5’-TCTATGGGCCTGTTTCTGAATAAAACAAACATCATCCG-3’ |
| **Q212E** | 5’-TCTATGGGCCTGTTTCTGGAGAAAACAAACATCATCCG-3’ |
| **R218A** | 5’-CAGAAAACAAACATCATCGCGGACTATCTGGAAGACCA -3’ |
| **R228A** | 5’-GAAGACCAGCAAGGAGGAGCAGAGTTCTGGCCTCAAGA-3’ |
| **F288A** | 5’-GAAACCAGAGTGTGTTTAACGCCTGTGCTATTCCACAGGTG-3’ |
| **F288L** | 5’-GAAACCAGAGTGTGTTTAACCTCTGTGCTATTCCACAGGTGATG-3’ |
| **K315E** | 5’-GGTGTTCAAAGGGGCAGTGGAGATTCGGAAAGGGCAAGC -3’ |
| **R317E** | 5’-CAAAGGGGCAGTGAAGATTGAGAAAGGGCAAGCAGTGACCC -3’ |
| **K315E+R317E** | 5’-GGTGTTCAAAGGGGCAGTGGAGATTGAGAAAGGGCAAGCAGTGACCC-3’ |
| **K318E** | 5’-GGGGCAGTGAAGATTCGGGAAGGGCAAGCAGTGACCCTG -3’ |
| **K315E+K318E** | 5’-GGTGTTCAAAGGGGCAGTGGAGATTCGGGAAGGGCAAGCAGTGACCCTG -3’ |

**Supplementary Table S2.** Effects of mutation on retained activity of *h*SQS in this study.

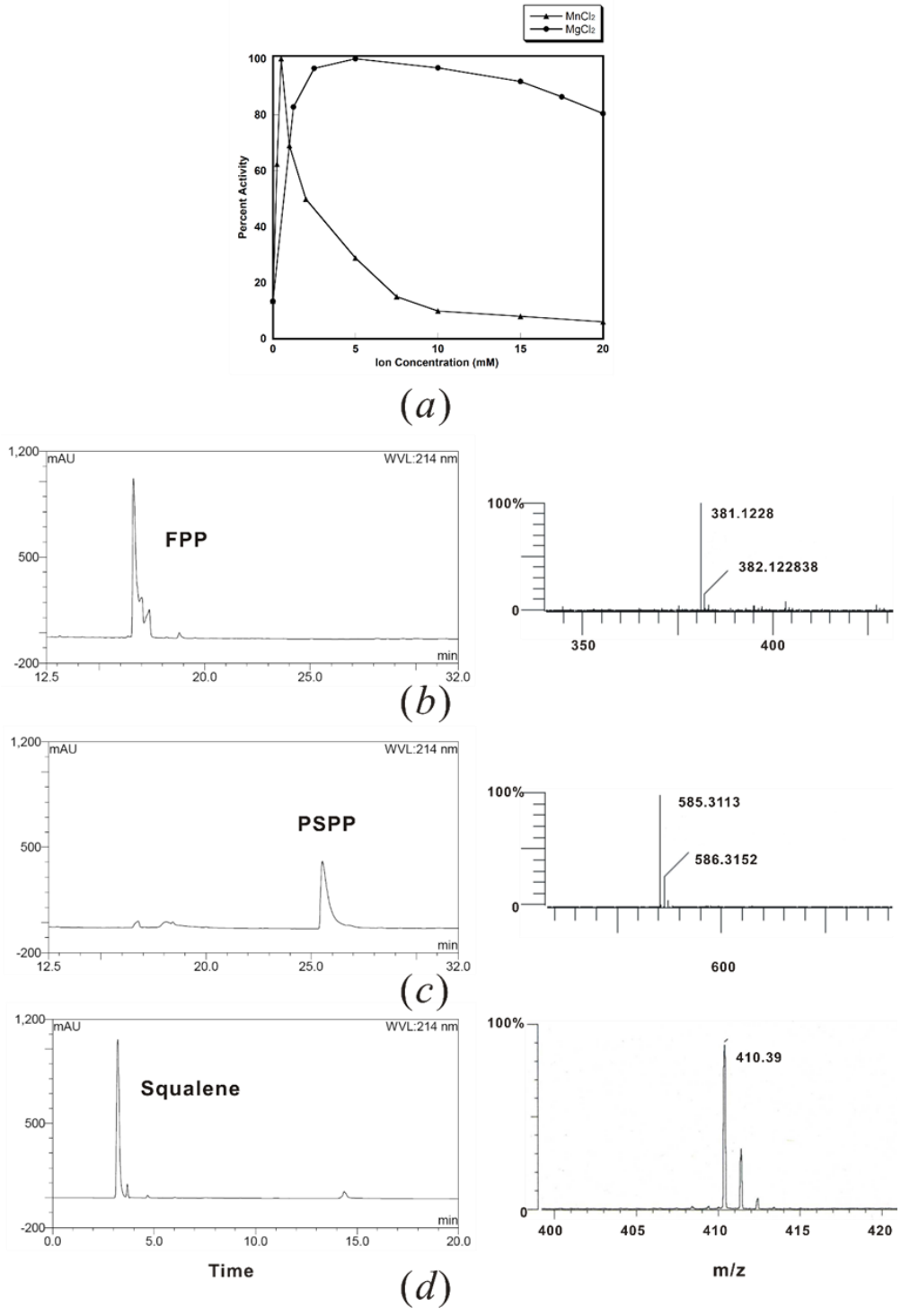
| Human SQS | % | |
|---|---|---|
| | First-step | Second-step |
| **Wild type** | 100 | 100 |
| **R52A** | 59.7 | 37.8 |
| **R52E** | 91.7 | 9.1 |
| **Y73A** | N/A | 9.7 |
| **R77A** | 55.5 | 14.5 |
| **K117A** | N/A | 100 |
| **Q212L** | N/A | 4 |
| **Q212N** | 53.1 | 8.1 |
| **Q212E** | N/A | 85.4 |
| **R218A** | 93.6 | 22.4 |
| **R228A** | 85.3 | 70.2 |
| **F288L** | N/A | 53.2 |
| **F288A** | N/A | 22.7 |
| **K315E** | 89.6 | 40.5 |
| **R317E** | N/A | 82.6 |
| **K318E** | 93.6 | 86.9 |
| **K315E/R317E** | N/A | 9.2 |
| **K315E/K318E** | 94.1 | 7.4 |



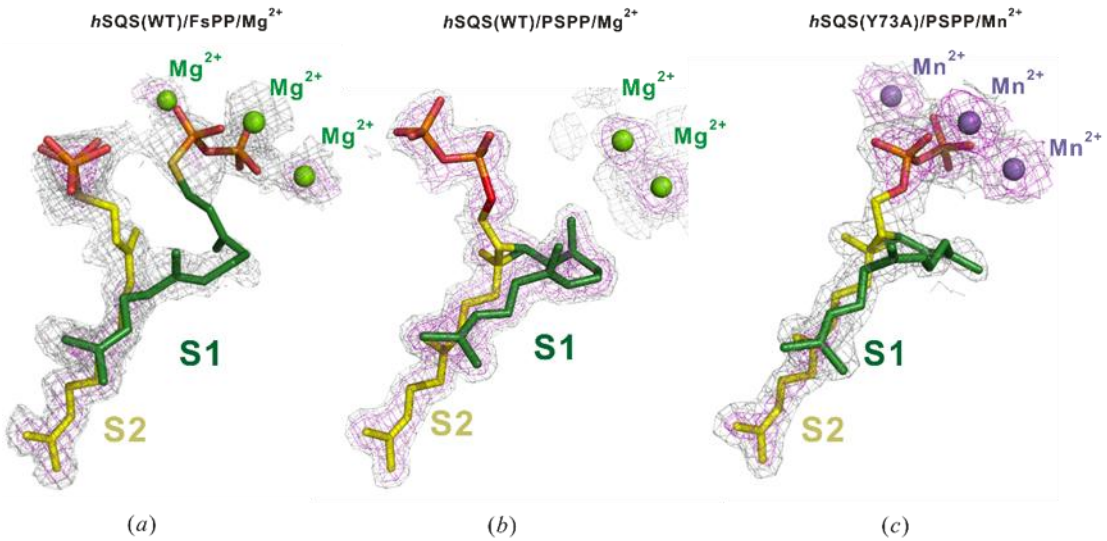

**Supplementary Fig. S1.** (*a*) Stereo view of the alignment of *h*SQS with *Sa*CrtM, indicating the highly conserved regions are located at Regions I (red), II (magenta), and III (blue). Region IV (green), constituted NAD(P)H binding site, is an unique and conserved feature among SQSs and absent in *Sa*CrtM. The negative residues on both asp-rich motifs are shown in ball and stick. *h*SQS and *Sa*CrtM are presented in palecyan and yellow, respectively. (*b*) Alignment of SQS protein sequences. The protein sequences for human (AAP36671), rat (AAA42179), yeast (CAA42583), and *T. cruzi* (XP806809) were aligned using Clustal program with manual adjustment. Identities among the five species are marked by (*) while homologous are indicated by (.) and conserved substitution by (:). Four conserved regions are labeled in boxes. Two DXXED motifs as the substrate binding sites are shown. The circles indicate the residues involved in NADPH recognition.



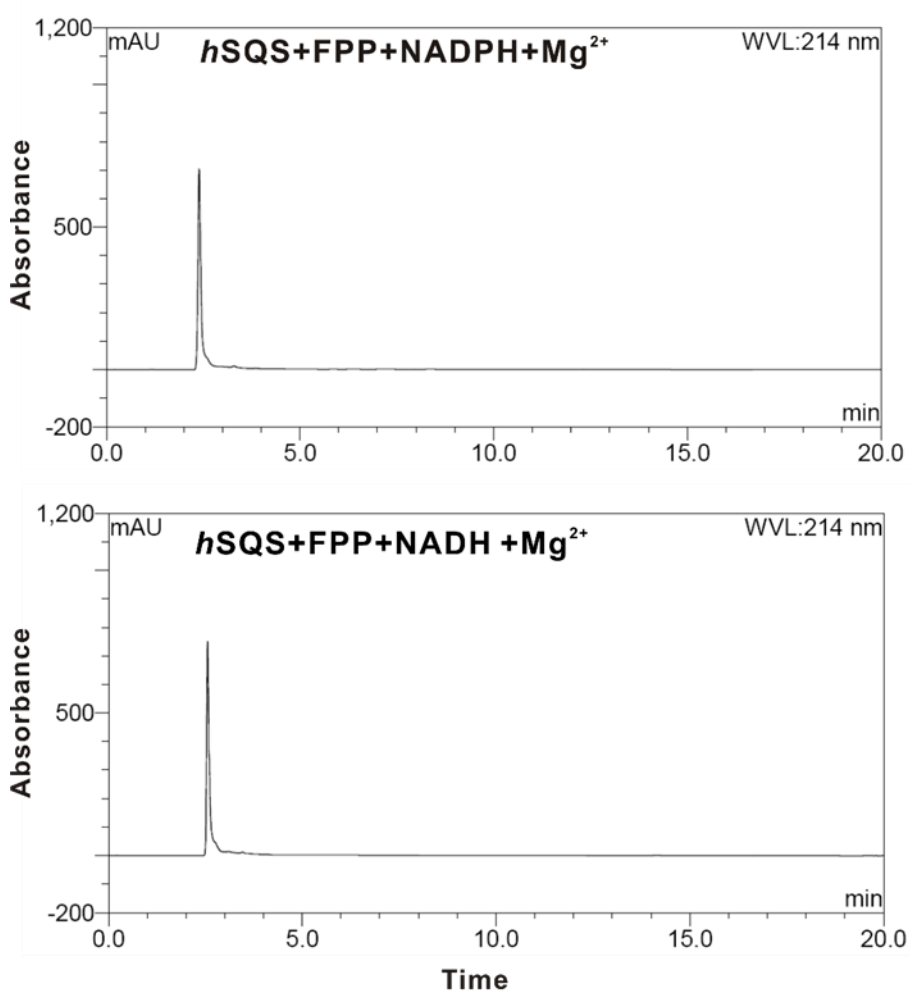

**Supplementary Fig. S2.** The final 2*Fo-Fc* electron densities map (gray contoured at 1.0σ, red at 2.0σ) for FsPP without $Mg^{2+}$ in the active site. FsPP in S1 site is labeled in green, FsPP in S2 site is labeled in yellow. In their active sites, two $C_{15}$-FsPP molecules are in chains A, B and E; one molecule in chains C and D; and chain F is empty.



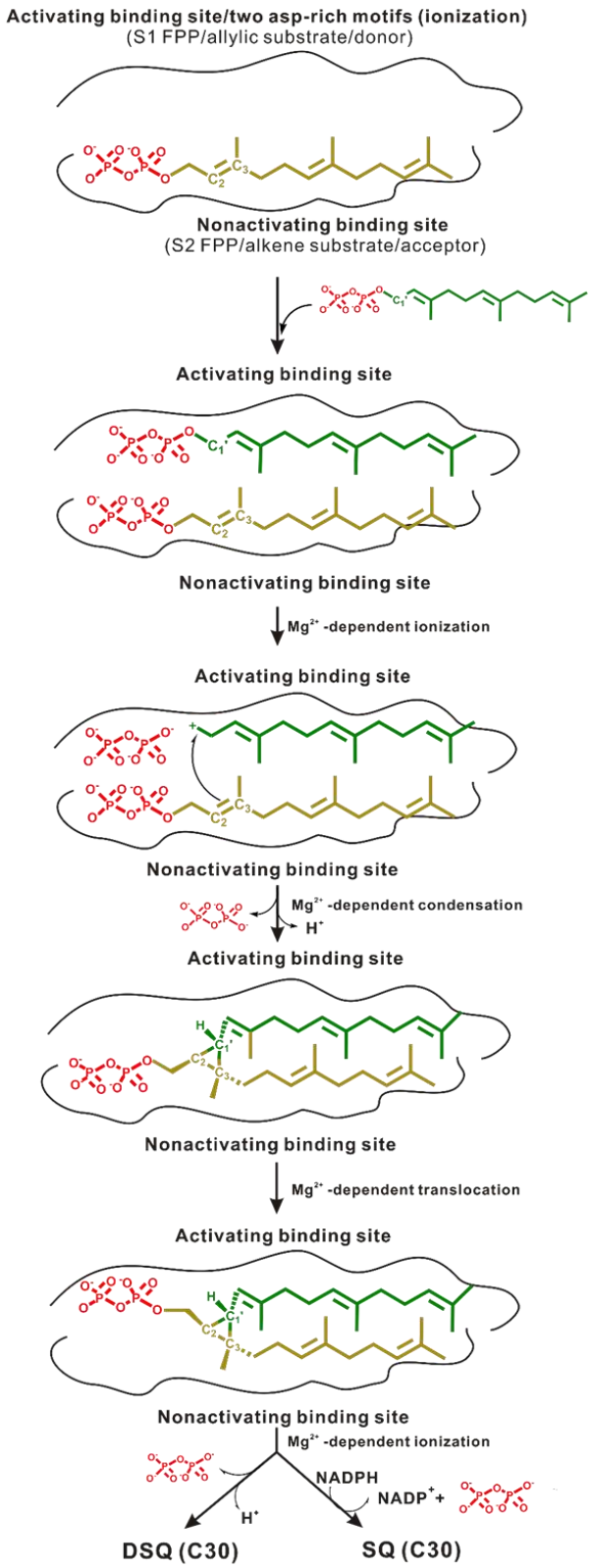

**Supplementary Fig. S3.** Activity Assays. (*a*) Both $Mg^{2+}$ and $Mn^{2+}$ stimulate enzyme activity. Reverse phase HPLC trace of FPP (*b*) and PSPP (*c*). The retention times of FPP and PSPP were 17.5 and 27.0 min. (*d*) Normal phase HPLC trace of squalene. The retention time of squalene was 3 min. Mass spectral analysis of FPP, PSPP and squalene are shown the molecular ions at *m/z* 381, 585 and 410, respectively.




**Supplementary Fig. S4.** Substrate models in the active site. The $2F_o$-$F_c$ electron density maps for all ligands are contoured 1σ (in gray) and 3σ (in red). (*a*) FsPP with $Mg^{2+}$. (*b*) PSPP with $Mg^{2+}$. (*c*) PSPP with $Mn^{2+}$. The catalytic $Mg^{2+}$ and $Mn^{2+}$ are shown as green and purple balls, respectively.




**Supplementary Fig. S5.** *h*SQS not only uses NADPH but NADH as reducing agents.




**Supplementary Fig. S6**. Proposed cartoon model of ordered incorporation of substrates in *h*SQS and *Sa*CrtM.