Supplementary Material



Supplementary Figure S1

A.12% SDS-PAGE profile of YwfH purified using cobalt-affinity chromatography. Lane 1.Recombinant YwfH purified with a N-terminal poly-histidine tag, Lane2: Molecular weight marker.B. Crystals of YwfH in the monoclinic form C. Crystals of the YwfH-NADPH complex.



Supplementary Figure S2

Sequence alignment of *B. subtilis* YwfH with representative Short Chain Reductases (SDR).



Supplementary Figure S3

Size exclusion chromatographic profile of YwfH and the YwfH-NADPH complex



Supplementary Figure S4

In silico docking of different conformations of 3E-ex-H₂HPP (blue) and 3Z-ex-H₂HPP (red) at the catalytic site of YwfH. Docking analysis was performed using the crystal structure of the YwfH-NADPH complex.



Supplementary Figure S5

Enantiomeric preferences evaluated by docking analysis. These studies reveal a preference for the minor form (E-ex- H_2HPP).



Supplementary Figure S6

LC-ESI/MS spectra of deuterated H_4HPP in negative ionization mode.