Supporting Information

Gräwert et al. 10.1073/pnas.0913045107

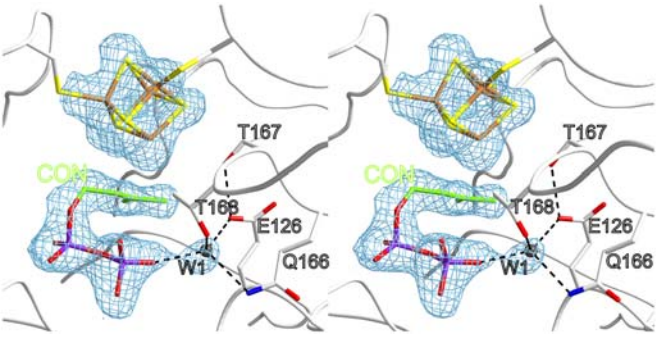

Fig. S1. Close-up stereoview of the active site of IspH with bound converted substrate interacting with the [4Fe–4S] cluster. The hydrogen bonding network at the IspH active site including protein, ligand and the W1 water molecule are indicated by dashed black lines. Electron densities represented in blue are contoured at 1.0° with 2 F_0–F_c coefficients; ligand has been omitted for the electron densities calculations.

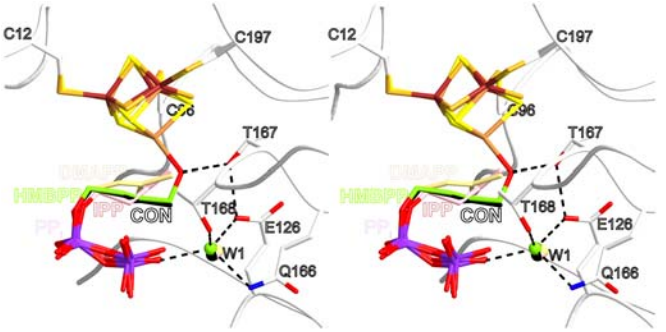

Fig. S2. Superposition of IspH active site with either covalently bound substrate, one of the products (IPP / DMAPP) or pyrophosphate. Notably, the pyrophosphate as well as water molecule W1 occupy the same position within the active site in all four structures. The conserved amino acids involved in the hydrogen-bonding network (*Dashed black lines*) show identical orientation in the respective structures.

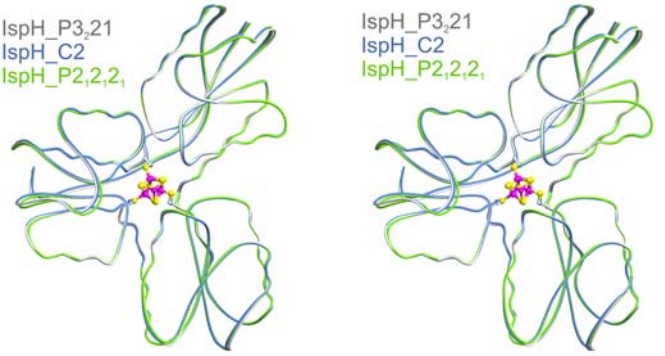

Fig. S3. Superposition of IspH protein crystallized in different space groups: Complex of IspH and pyrophosphate yielded space group $P3_221$ when using 1.8 M potassium phosphate as crystallization buffer (reference 5 in the main text); complex of IspH and IPP yielded space group C2; IspH:DMAPP, IspH:HMBPP, IspH:CON(-verted substrate) and IspH:PP_i (BIS-Tris buffer, see methods section) complexes were crystallized in space group $P2_12_12_1$.

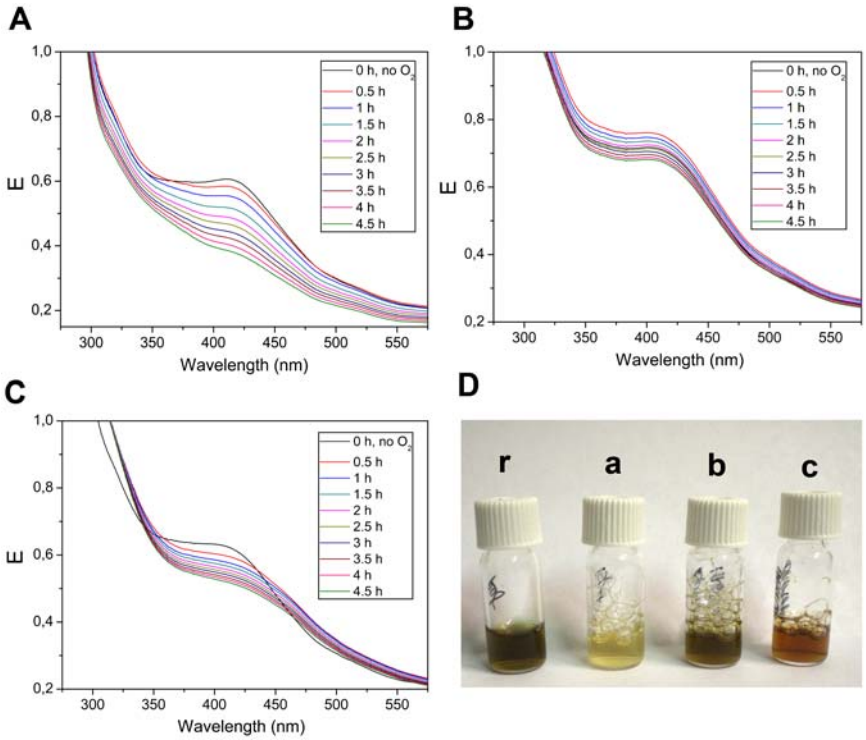

Fig. S4. UV-Vis spectra of IspH protein reveal influence of oxygen. (*A*) Wavelength scans of IspH show a shoulder at 410 nm typical for iron-sulfur clusters. After exposure to oxygen, the local maximum starts to vanish. (*B*) Wavelength scans of IspH with 50–mM substrate. The shoulder remains after exposure to oxygen. (*C*) Wavelength scans of IspH with 50 mM product (DMAPP). The shoulder vanishes after exposure to oxygen, but not as quickly as without any additive. (*D*) The effect of oxygen on IspH can be observed visually. All samples after incubation for 20 h at room temperature. r: IspH with no oxygen added. *A*: IspH with oxygen added. IspH lost its typical color. *B*: IspH in presence of 50 mM substrate with oxygen added. Protein retained its brown color. *C*: IspH in presence of 50 mM product (DMAPP) with oxygen added. IspH changed its color from brown to reddish.

Table S1. Data collection and refinement statistics

	IspH:HMBPP	IspH:CON	IspH:IPP	IspH:DMAPP	IspH:PP_i
Crystal parameter					
Space group	$P2_12_12_1$	$P2_12_12_1$	C2	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions					
a, *b*, *c* (Å)	70.9; 80.7; 111.2	69.6; 80.8; 112.7	112.5; 80.6; 70.2	70.1; 80.4; 110.8	71.2; 80.7; 111.4
α, β, γ (°)			β=94.7		
Molecules per AU[a]	2	2	2	2	2
Data collection					
Beam line	SLS, PX06SA	SLS, PX06SA	CuK_α	SLS, PX06SA	CuK_α
Wavelength (Å)	1.0	1.0	1.5418	1.0	1.5418
Resolution range (Å)[b]	10–1.7 (1.8–1.7)	10–1.9 (2.0–1.9)	65–2.0 (2.1–2.0)	65–1.7 (1.8–1.7)	71–1.8 (1.9–1.8)
Unique reflections[c]	70059	50648	39891	68998	60002
Completeness (%)[b]	99.6 (99.4)	99.9 (99.9)	100 (100)	99.3 (99.3)	100 (100)
R_{merge} (%)[bd]	6.8 (29.4)	5.2 (40.9)	4.8 (24.3)	5.5 (27.7)	5.3 (28.2)
I/σ (I)[b]	17.1 (5.8)	21.8 (5.4)	17.6 (4.0)	21.9 (6.6)	15.6 (3.4)
Refinement					
Resolution (Å)	10–1.7	10–1.9	15–2.0	10–1.7	10–1.8
R_{work}/R_{free}[e]	0.241/0.269	0.214/0.248	0.226/0.272	0.216/0.237	0.234/0.278
Number atoms					
Protein	4773	4773	4773	4773	4773
Ligand	30	28	28	28	18
FeS-Cluster	16	16	14	14	14
Water	603	484	456	460	631
B factors	22.9	35.8	20.1	22.1	15.1
rms deviations[f]					
Bond lengths (Å)	0.007	0.007	0.006	0.005	0.006
Bond angles (°)	1.2	1.2	1.3	1.4	1.3
Ramachandran (%)[g]	98.2/1.8/0.0	98.7/1.3/0.0	98.5/1.5/0.0	98.7/1.3/0.0	98.0/2.0/0.0
PDB accession code	3KE8	3KE9	3KEM	3KEF	3KEL

[a]Asymmetric unit.

[b]Values in parenthesis of resolution range, completeness, R_{merge} and I/σ (I) correspond to the last resolution shell.

[c]Friedel pairs were treated as identical reflections.

[d]$R_{merge}(I) = \Sigma\Sigma|[I(\mathrm{hkl})_j - I(\mathrm{hkl})]|/[\Sigma I_{\mathrm{hkl}}\mathrm{hkl}_j\mathrm{hkl}$, where $I(\mathrm{hkl})_j$ is the jth measurement of the intensity of reflection hkl and $<I(\mathrm{hkl})>$ is the average intensity.

[e]$R = \Sigma||F_{\mathrm{obs}}| - |F_{\mathrm{calc}}||/\Sigma|F_{obs}|\mathrm{hkl}$ hkl, where R_{free} is calculated without a sigma cutoff for a randomly chosen 5% of reflections, which were not used for structure refinement, and R_{work} is calculated for the remaining reflections.

[f]Deviations from ideal bond lengths/angles.

[g]Number of residues in favored region/allowed region/outlier region