Supporting Information for

A Crystallographic Investigation of Phosphoantigen Binding to Isopentenyl Pyrophosphate/Dimethylallyl Pyrophosphate Isomerase

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Experimental Methods. Crystals of *E.coli* IPPI were grown at room temperature by the hanging drop method. Protein (10 mg/mL) was equilibrated against a reservoir containing PEG2000 (16%), ammonium sulfate (100 mM), $MgCl_2$ (10 mM) and $MnCl_2$ (10 mM) buffered with Tris/maleate at pH 5.5. Complexes with **3** and **4** were obtained by soaking crystals of the protein with solutions (about 25 mM) of the inhibitors in Tris/maleate (100 mM, pH 5.5), PEG2000 (16%), ammonium sulfate (100 mM), $MnCl_2$ (10 mM), $MgCl_2$ (10 mM), and glycerol (25 %). After soaking, crystals were flash frozen and diffraction data collected. Data for both complexes were collected by using a Mar345 imaging plate system from Marresearch, (Marresearch GmbH) equipped with Osmic optics and using an FR591 rotating anode generator (41 mA, 120 kV). Diffraction data were processed with the MarFLM suite of programs. Data for the Phosphostim™ complex were also collected on a Marresearch CCD detector on beam line BM30A at the European Synchrotron Radiation Facility (ESRF) (Grenoble), and were processed with the HKL suite of programs (Otwinowski, Z.; Minor, W. *Methods Enzymol.* **1997**, *276*, 307-326).

Figure S1. Active site of the CYS-67 thioether complex of *rac*-**3** and w.t. IPPI. Cyan sphere = Mg^{2+}; purple sphere = Mn^{2+}.

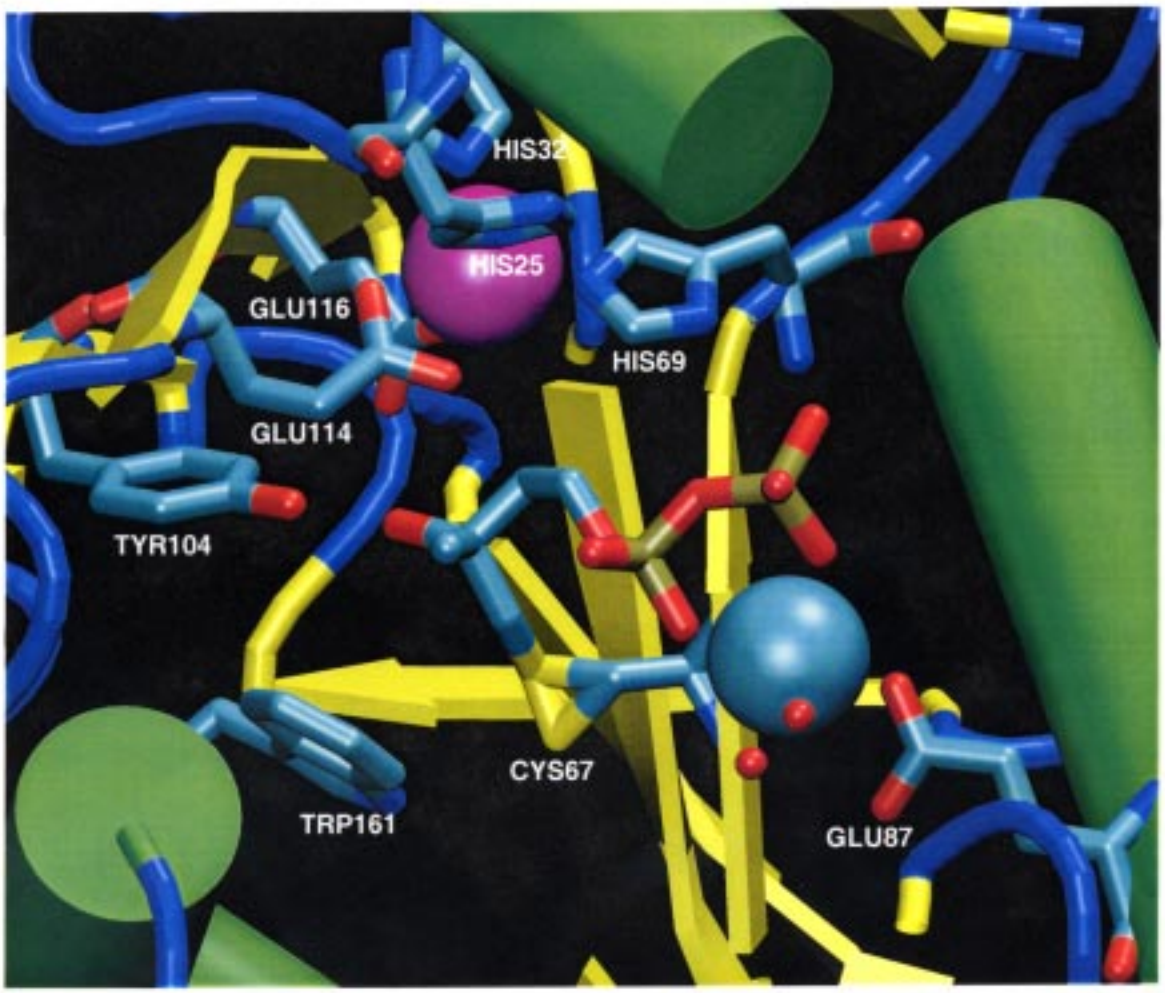

Figure S2. Active site of the GLU-116 ester complex of *rac*-**3** and w.t. IPPI. Cyan sphere = Mg^{2+}; purple sphere = Mn^{2+}.

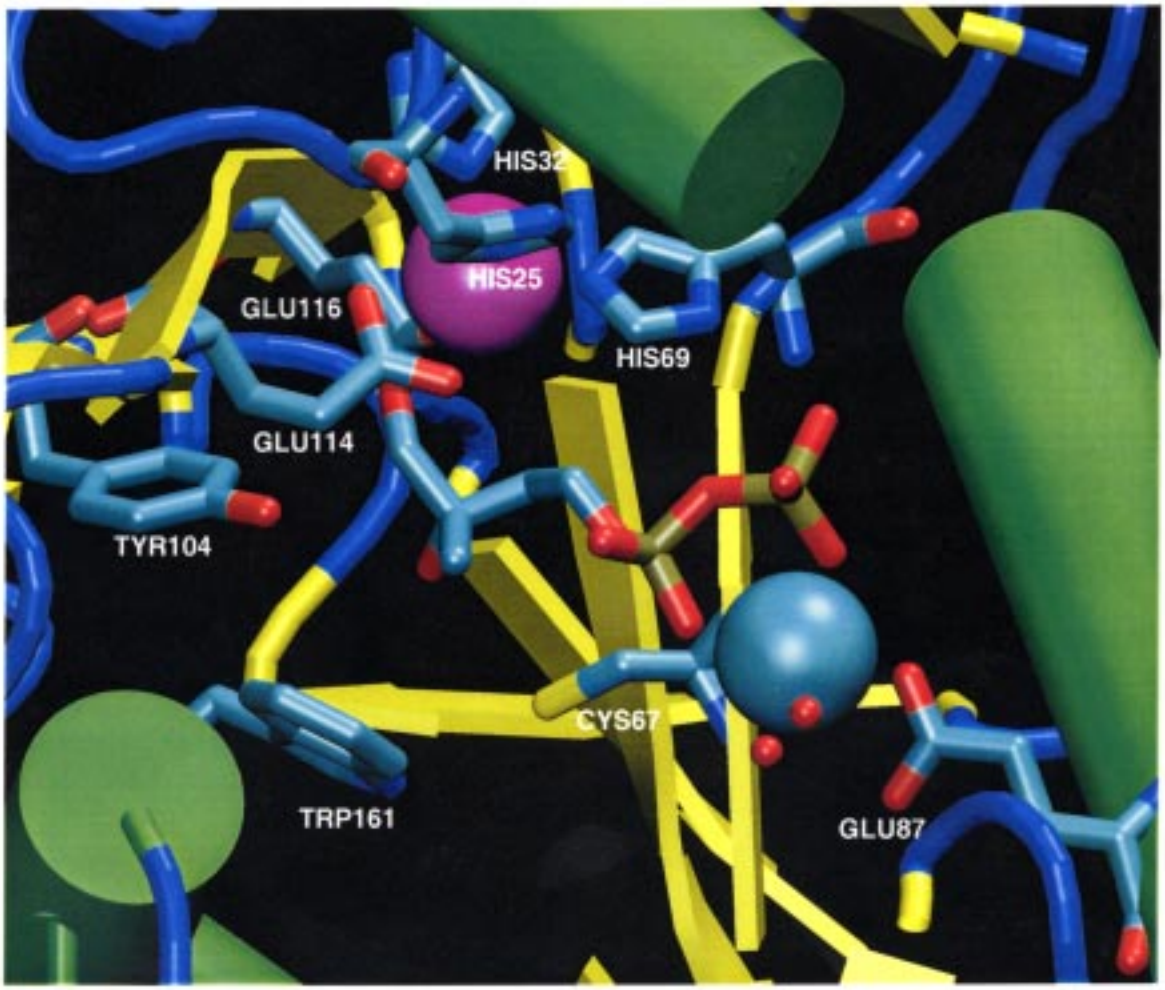

Figure S3. Active site of the GLU-116 ester complex of *S*-**3** and w.t. IPPI. Cyan sphere = Mg^{2+}; purple sphere = Mn^{2+}.

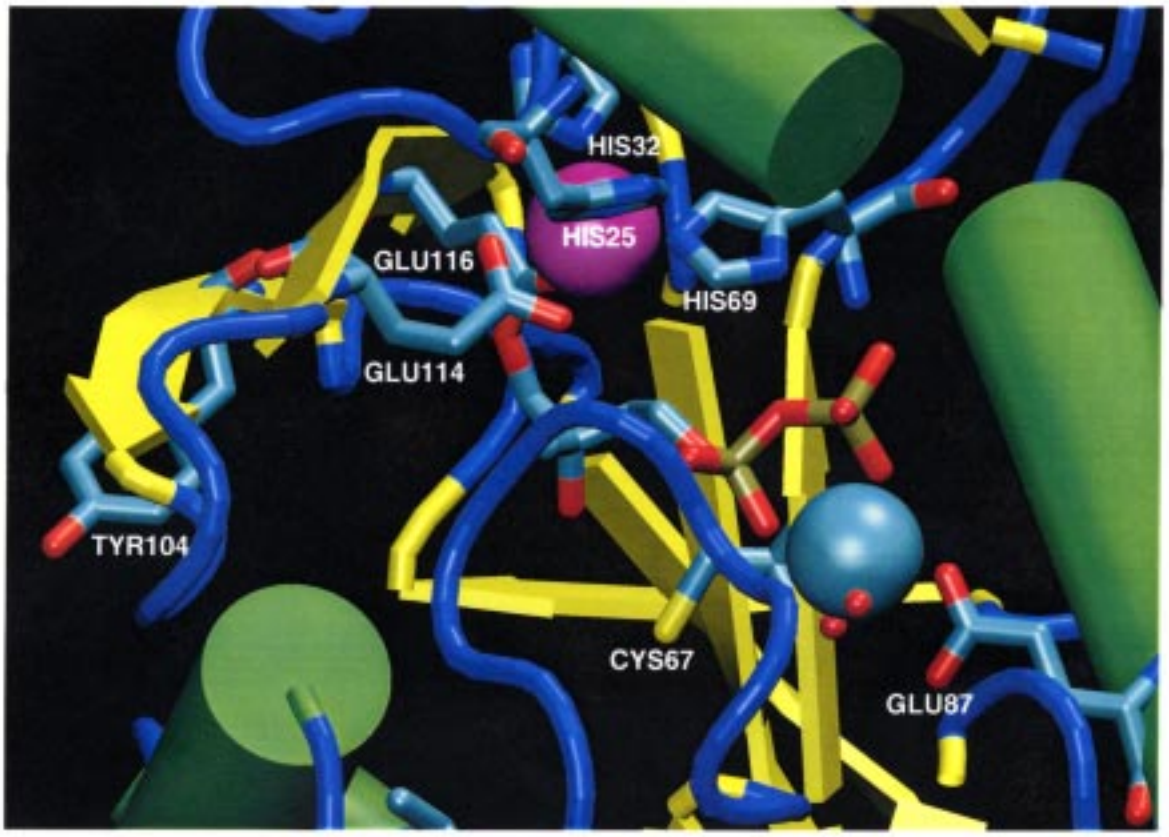

Figure S4. Active site of the *S*-**3** and Y104F IPPI. **A**, one molecule of *S*-3 binds to C67 and **B**, the Mg^{2+} site binds free diphosphate in one molecule of the asymmetric unit. Cyan spheres = Mg^{2+}; purple spheres = Mn^{2+}.

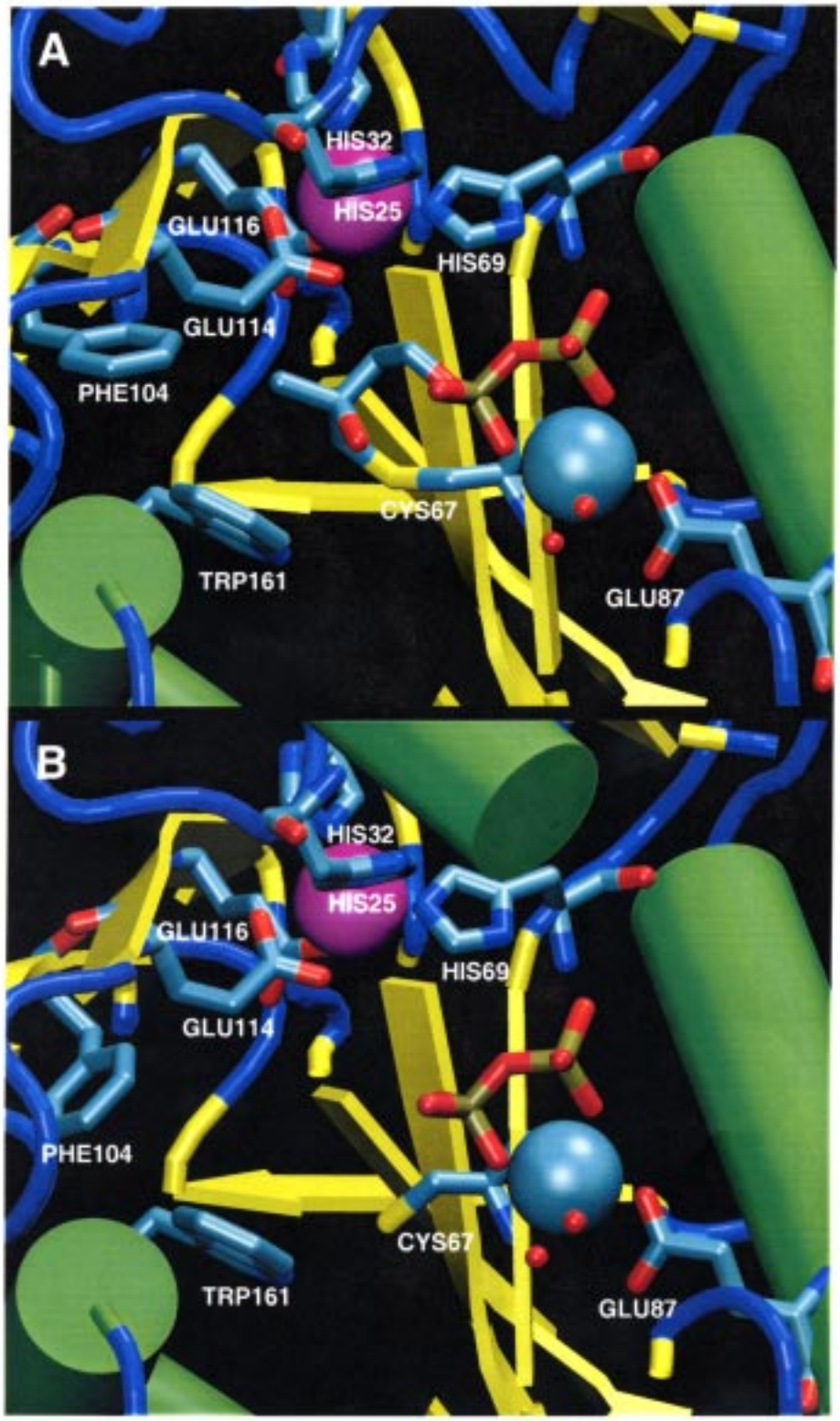

Figure S5. Active site of the complex of **4** and w.t. IPPI. Cyan sphere = Mg^{2+}; purple sphere = Mn^{2+}.