BIOLOGICAL
CRYSTALLOGRAPHY

Volume 70 (2014)

Supporting information for article:

Structure-based identification of inositol polyphosphate 1-phosphatase from *Entamoeba histolytica*

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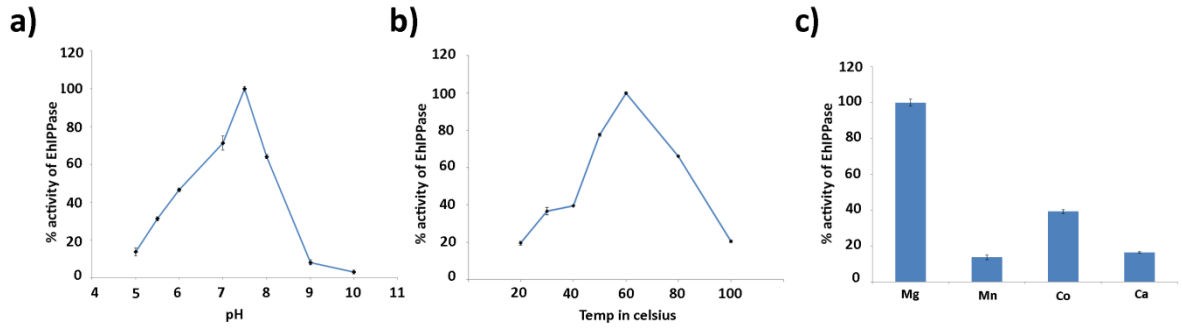

Figure S1 Effects of pH, temperature, and divalent metal ions on EhIPPase activity. Phosphatase activity of EhIPPase as a function of **(a)** pH, **(b)** temperature **(c)** and various metal ions. For EhIPPase, the standard 100µl reaction mixture volume contained 50 mM Tris pH 7.5, 1 mM PAP, 5 mM $MgCl_2$ and 2 µg of purified EhIPPase protein. To determine the nature of divalent metal ions as a cofactor the activity was separately checked in the presence of 5mM $MgCl_2$, $CoCl_2$, $MnCl_2$ or $CaCl_2$. For pH variations 50 mM each of sodium acetate pH5.0, Bis Tris in the range of pH5.5-6.0, Tris in the range of pH7-9 and Glycine pH10 were used. Activities are expressed as the percentage of the maximum observed activities. The data shown represent the average of two independent experiments. Error bars represent the corresponding standard deviation.

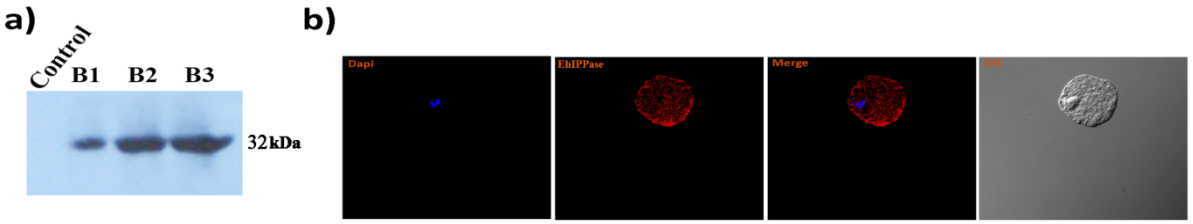

Figure S2 **(a)** Western blots of amoebic cell lysates. A thirty-microgram sample of the lysate was loaded in each lane and the blot was probed with anti-EhIPPase generated in mouse. The EhIPPase antibody stains endogenous EhIPPase band at 32 kDa as shown in lanes B1 to B3. **(b)** Confocal image of immunofluorescently labeled *E. histolytica* cells show EhIPPase (red colored due to Alexa flour 594) distributed throughout the cytoplasm. Nucleus is stained with DAPI (shown in blue colour) while DIC corresponds to differential interference contrast.

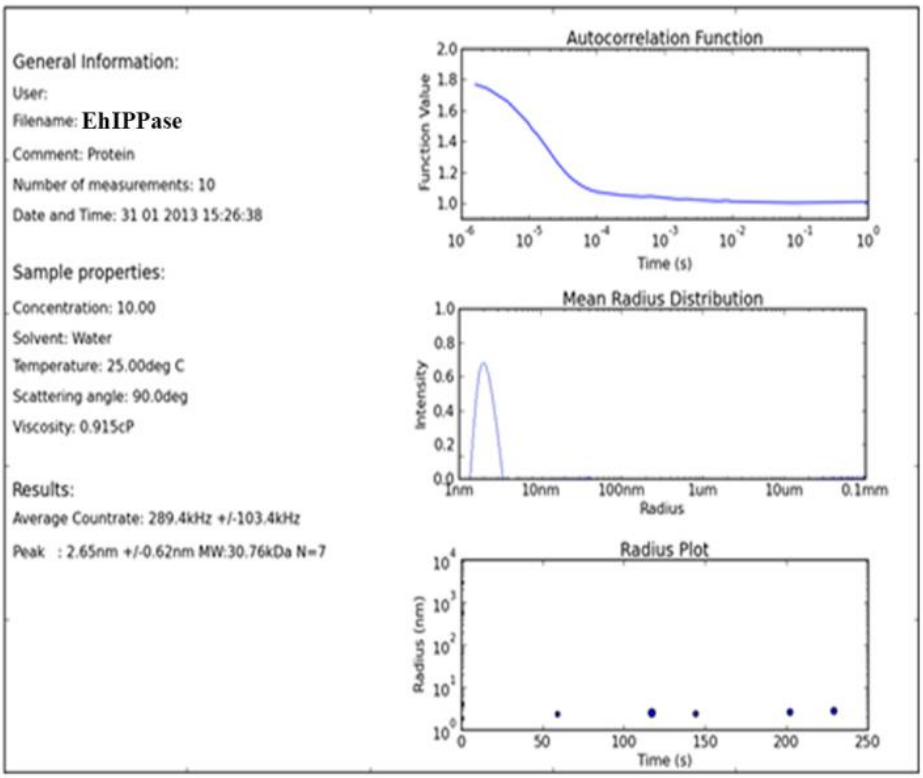

Figure S3 The dynamic light scattering (DLS) measurements were performed on SpectroSize300 from NanoBiochemTechnology, Hamburg. The DLS experiments show the homogeneity and monomeric state of EhIPPase.

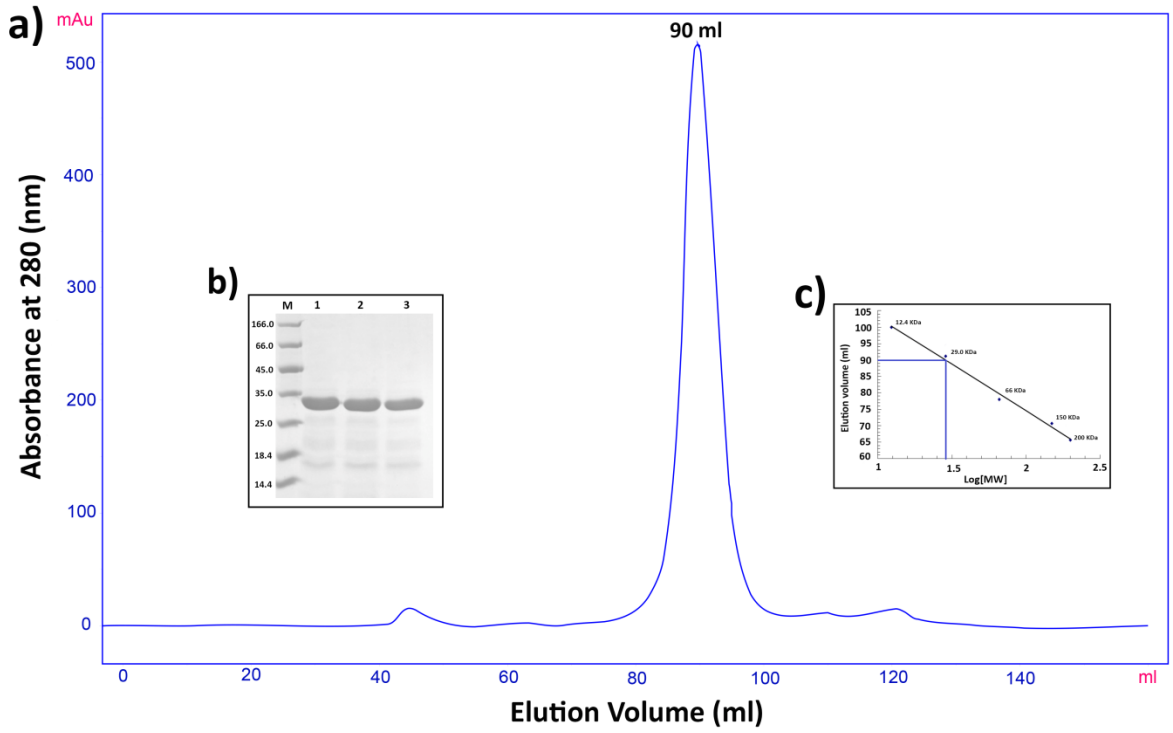

Figure S4 Gel filtration profile**. (a)** EhIPPase is a monomer in solution according to size-exclusion chromatography. The protein was collected after being passed through a HiLOAD 16/60 Superdex 200 column. The elution volume (90ml) and elution pattern of the protein are displayed. **(b)** SDS-PAGE showing fractions purified by gel filtration. The proteins are separated on 12.5 % SDS-PAGE and stained with Coomassie Brilliant Blue. Lane M shows the molecular markers; lanes 1, 2, 3, and 4 are gel-filtration fractions. **(c)** The molecular weight of the eluted EhIPPase, deduced from a standard plot, is about ~32 kDa, and corresponds to the monomeric state of the protein.

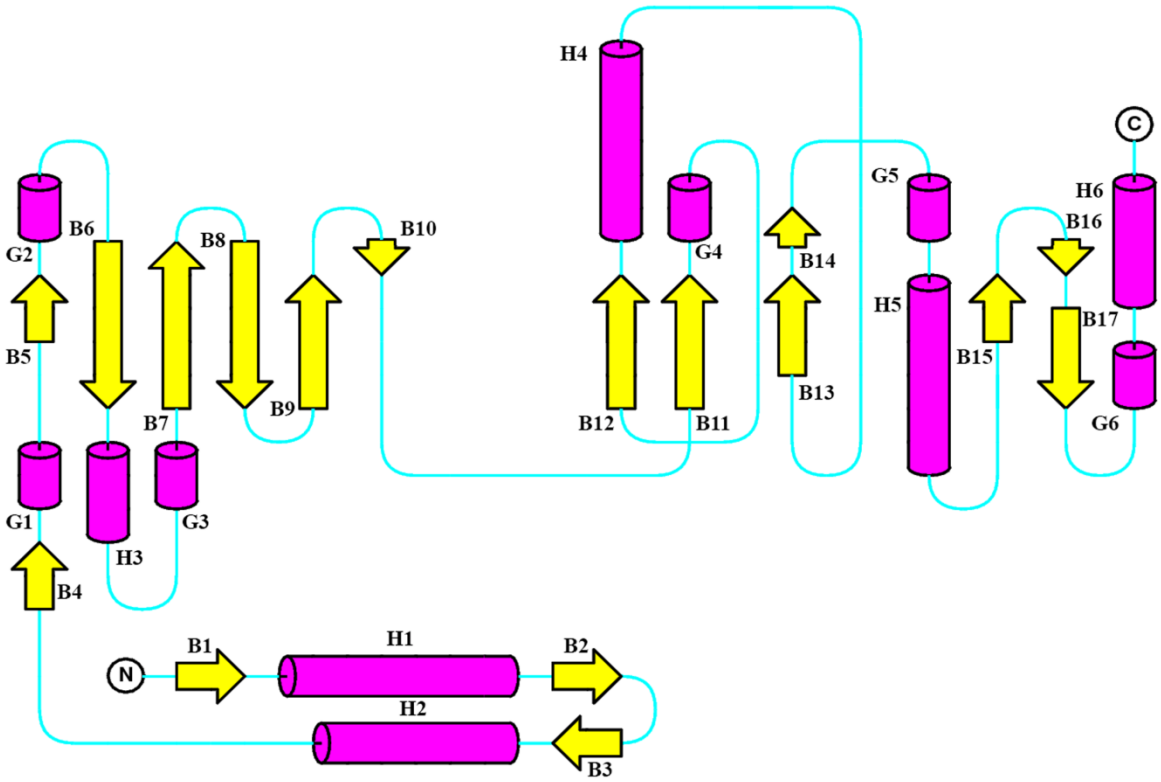

Figure S5 Topology of the secondary structural elements of EhIPPase. The N terminal domain forms an approximate α + ß fold, while the C-terminal domains belongs to the α/ß class. Yellow arrows represent ß strands, pink cylinders represent α helices, and short pink cylinders labelled with a "G" are 3_{10} helices.

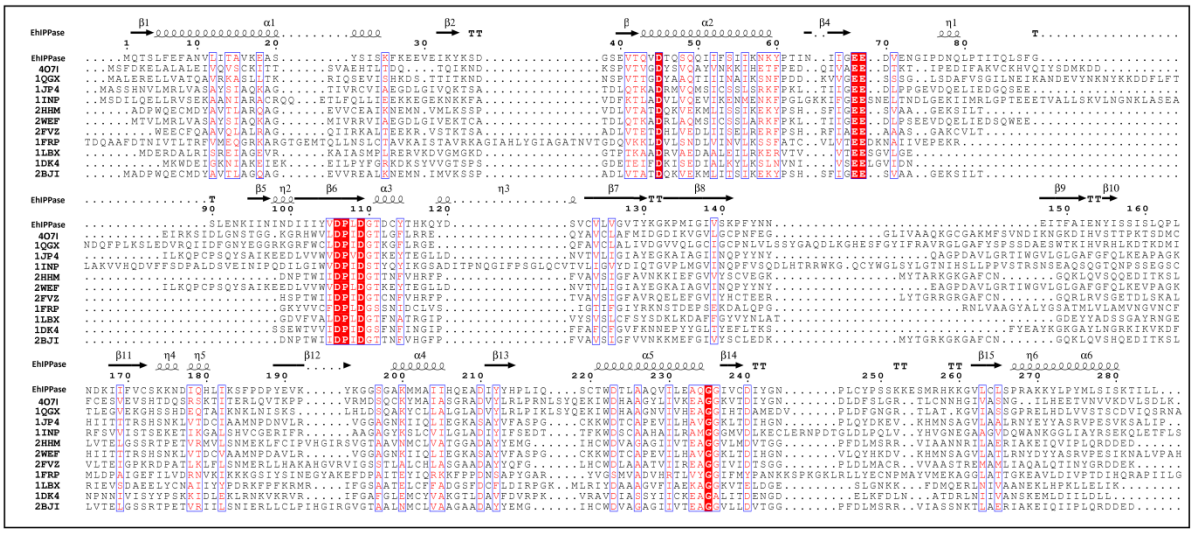

Figure S6 Multiple sequence alignment. Multiple sequence alignment of EhIPPase with other members of the Li^{+}/Mg^{2+} phosphatase superfamily found in the PDB. The alignment was generated by ESPript (Gouet *et al.*, 2003) with clustalW (McWilliam *et al.*, 2013). Secondary structural elements of EhIPPase as determined by DSSP are shown above the sequences (α-helices, ß-strands, η-3_{10} helices and TT- ß turn). All members of this superfamily share a similar core structure and conserved residues essential for metal binding and substrate hydrolysis, i.e., D-Xn-EE-Xn-DP(I/L)DG(S/T)-Xn-WD-Xn-GG (red: totally conserved and pink: partially conserved).

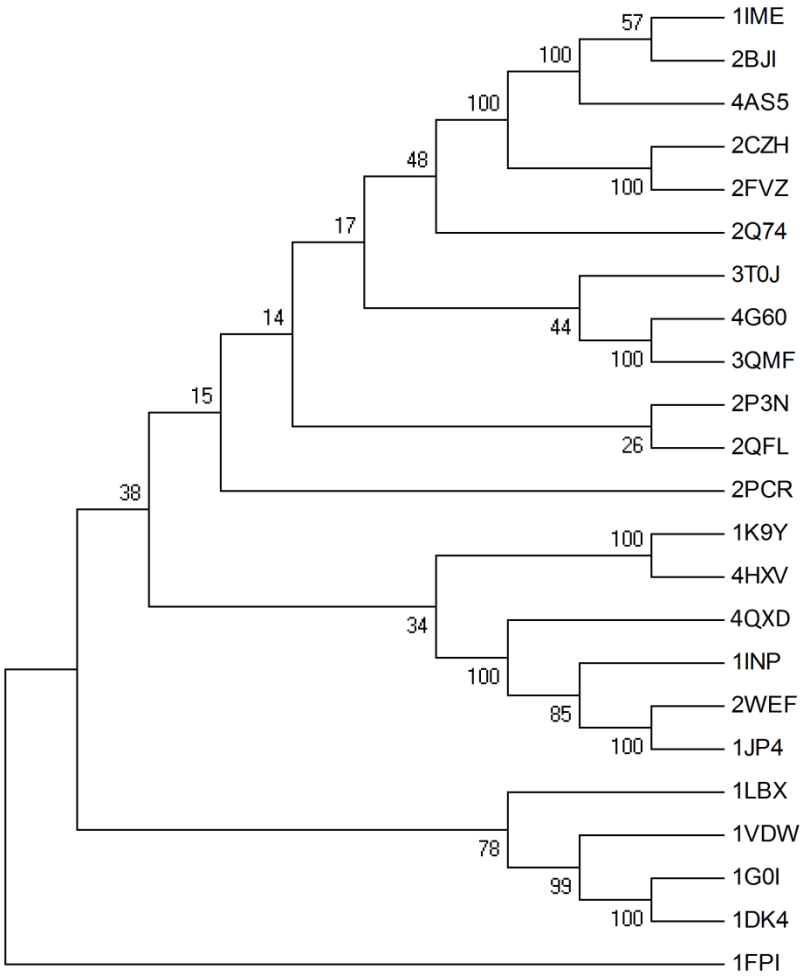

Figure S7 Phylogenetic analysis of members of the IMPase superfamily. Representative structures of various proteins of this IMPase superfamily were obtained from the PDB and a phylogenetic tree was constructed. The tree shows a distinct branch for IPPase and PAP phosphatase enzymes. It is clearly visible that EhIPPase is distinct from other PAP phosphatase enzymes. The enzymes that correspond to the displayed PDB ids are listed in Table 3 legend.

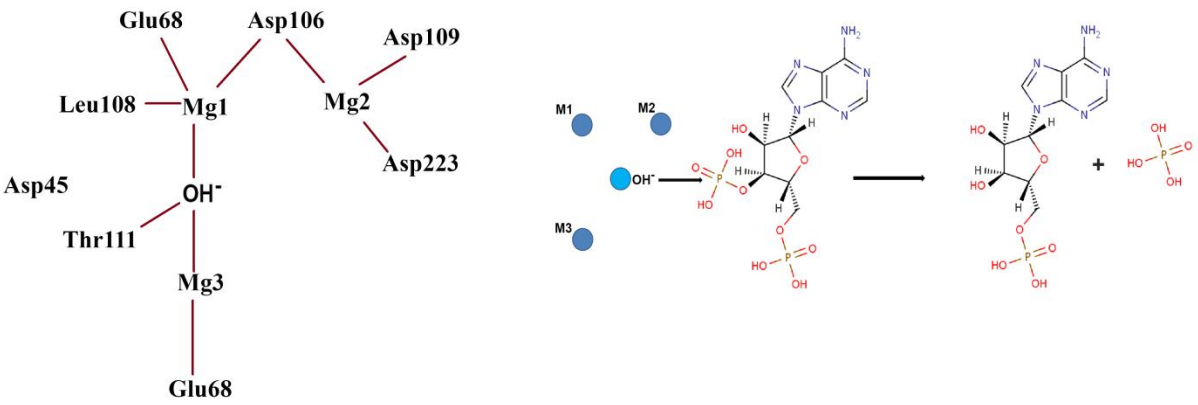

Figure S8 Mechanistic scheme for EhIPPase activity. Spheres M1, M2 and M3 shown in pure cyan blue colors are Mg^{2+} ions. Here the reaction would start with the interaction of Glu68 with Mg^{2+} at M1 and M3. Then a nucleophilic water molecule coordinated by these metal ions, and activated through a conserved Thr111 side chain, will subsequently lead to an in-line attack at the phosphorus moiety of Ins(1,4)P2, leading to the formation of a trigonal bipyramidal transition state. This state is stabilized by Mg^{2+} at M2, which disperses the developed negative charge on the phosphorus group. The substrate hydrolysis would then lead to the inversion of the phosphate molecule configuration at the active site, thereby releasing the product.

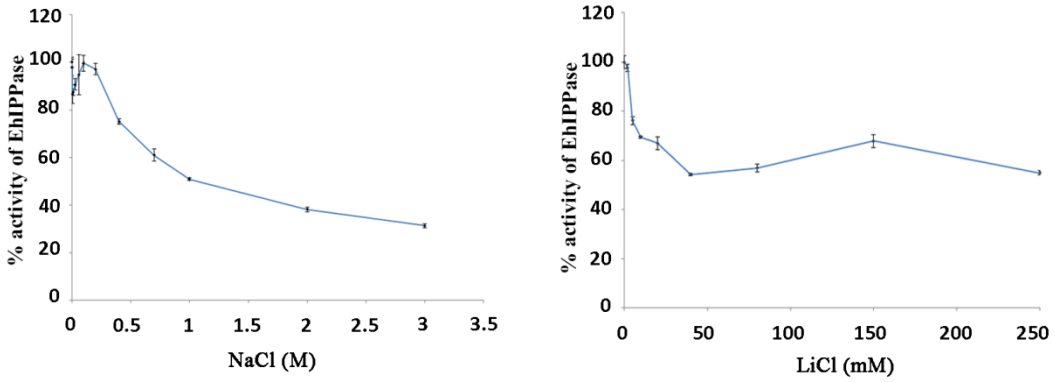

Figure S9 Salt sensitivity of EhIPPase with PAP as the substrate. EhIPPase activity in the presence of **(a)** NaCl and **(b)** LiCl. While there is minimal effect on the enzyme's activity even at high NaCl concentration ($IC_{50=}$1M), EhIPPase gets moderately inhibited by LiCl concentration (IC_{50}=40mM).

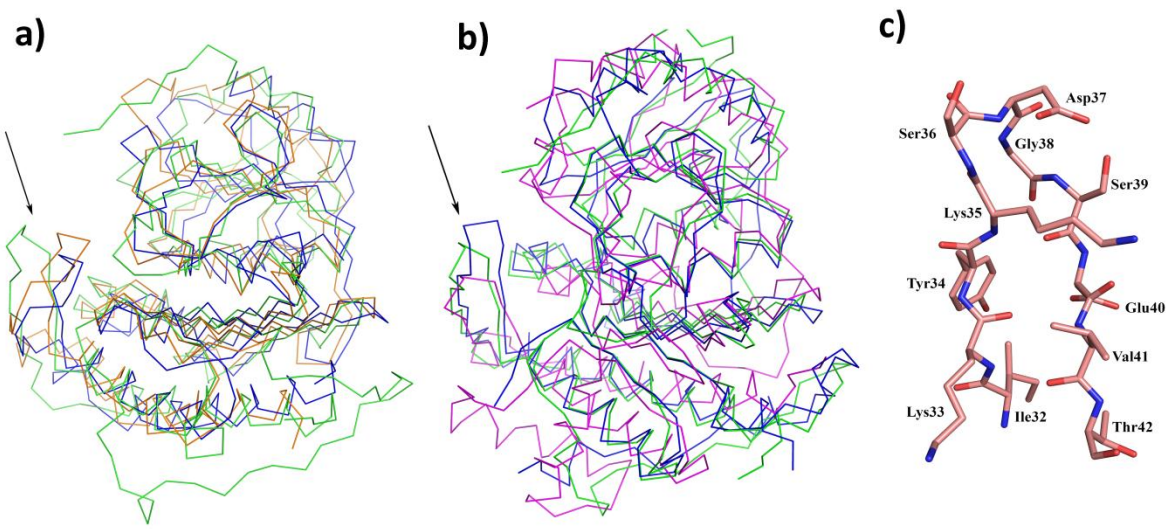

Figure S10 Analysis of the mobile loop conformation across the IMPase superfamily. Superposition of EhIPPase (green) with **(a)** homologous Li^+-insensitive enzymes from *A. fulgidus* and *M. jannaschi* (purple and orange) and **(b)** with Li^+-sensitive enzymes from yeast and rat (magenta and purple). The conformation of the loop is shown with an arrow. **(c)** Residues Lys33 to Val41 constitute the active mobile loop in EhIPPase.