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Supporting information for article:

Structural elucidation of a dual active PAP phosphatase-1 from *Entamoeba histolytica*: capable of hydrolysing both 3'-phosphoadenosine 5′-phosphate and inositol 1,4-bisphosphate

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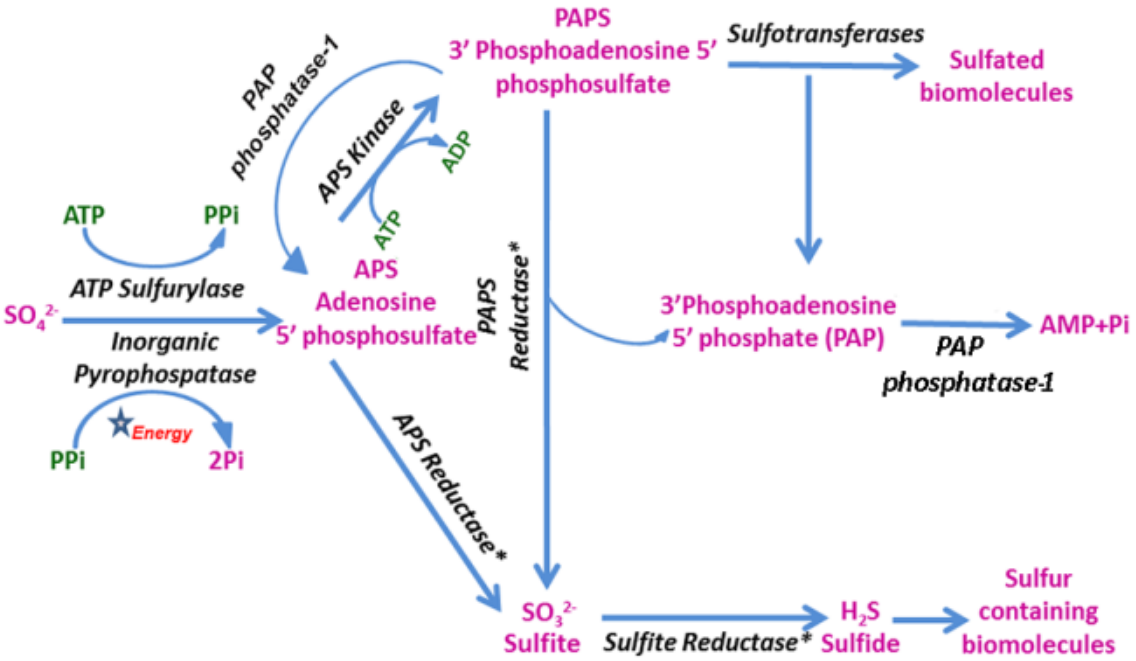

Figure S1 Sulfate activation pathway. Schematic representation of the sulfate assimilation pathway in living organisms. Arrows designate enzymatic reactions specific to each step. Steps showing enzymes with asterisk are reportedly absent in *Entamoeba histolytica*.

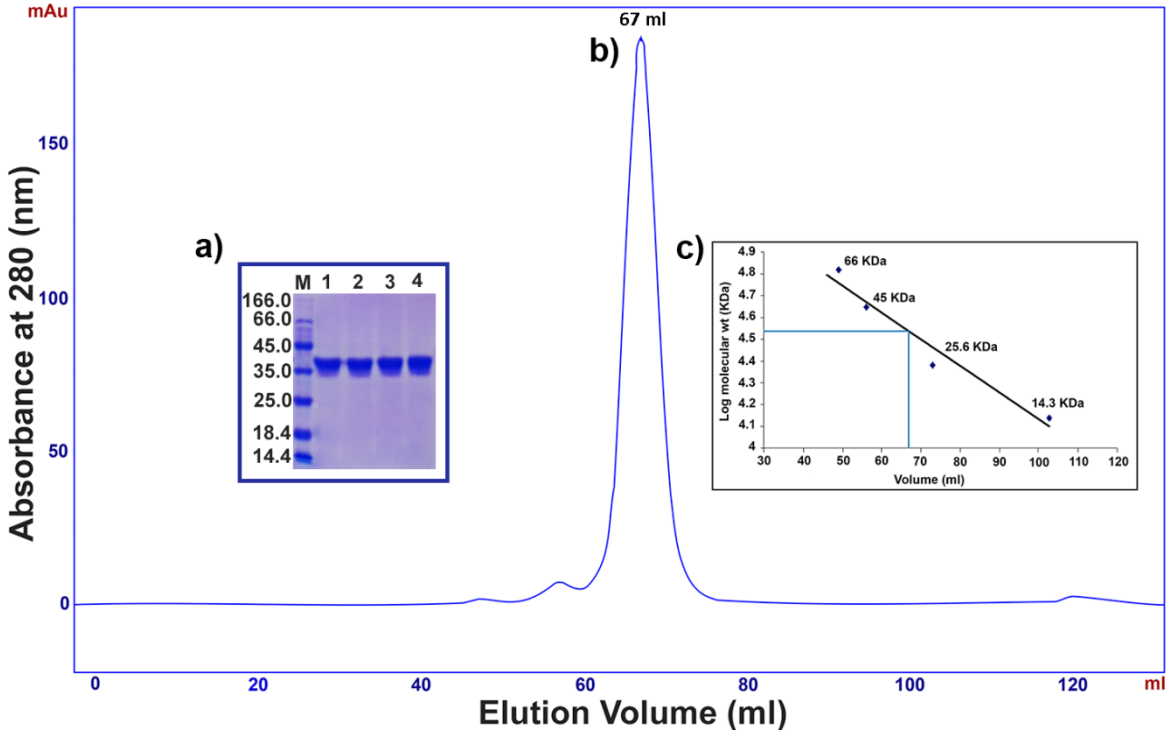

Figure S2 Over expression and Purification of PAP phosphatase-1. **(a)** SDS-PAGE showing fractions purified by gel filtration. The proteins are separated on 12.5 % SDS-PAGE and stained with Coomassie Brilliant Blue. Lane M shows the molecular markers; lanes 1,2,3,4 are gel-filtration fractions. **(b)**The protein was collected after being passed through a HiLOAD 16/60 Superdex 75 column. The elution volume (67ml) and elution pattern of the protein are displayed. PAP phosphatase-1 is a monomer in solution according to size exclusion chromatography. **(c)** The molecular weight of the eluted PAP phosphatase-1, deduced from a standard plot, is about ~35kDa, and corresponds to the monomeric state of the protein.

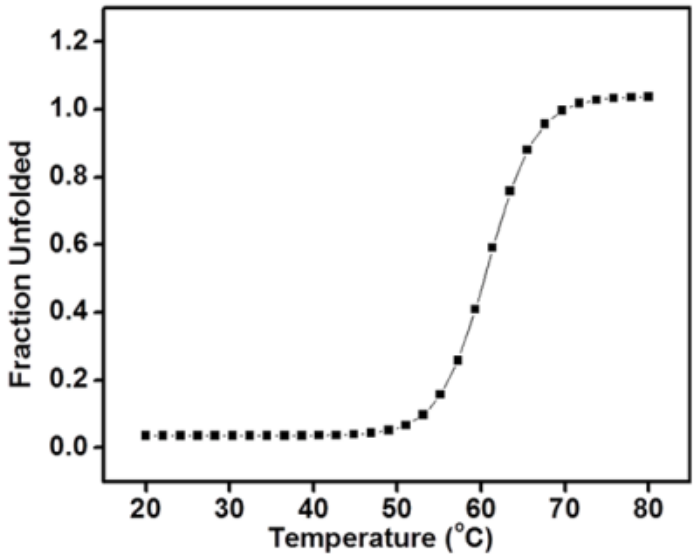

Figure S3 Thermal denaturation. Thermal denaturation of PAP phosphatase-1 was measured by CD spectroscopy and the curve was plotted using Origin software. Tm of the enzyme was found to be about 61°C

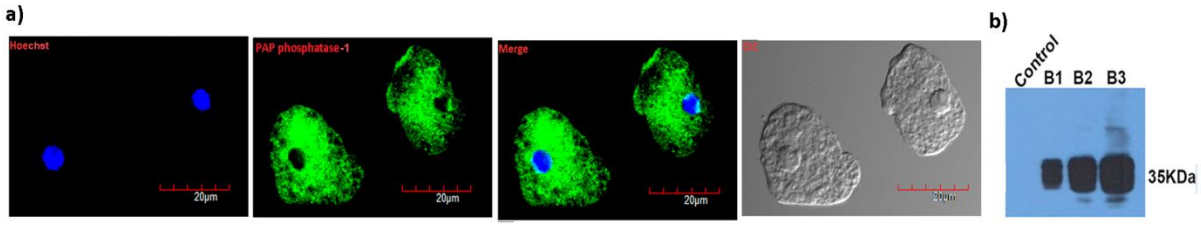

Figure S4 Immunolocalization and western blots of amoebic cell lysates. **(a)** Confocal image of immunofluorescencently labelled *E. histolytica* cells show PAP phosphatase-1 distributed throughout the cytoplasm. **(b)** A thirty microgram sample of the lysate was loaded in each lane and the blot was probed with anti-PAP phosphatase-1 generated in mouse. The PAP phosphatase-1 antibody stains endogenous PAP phosphatase-1 band at 35 kDa respectively as shown in lanes B1 to B3.

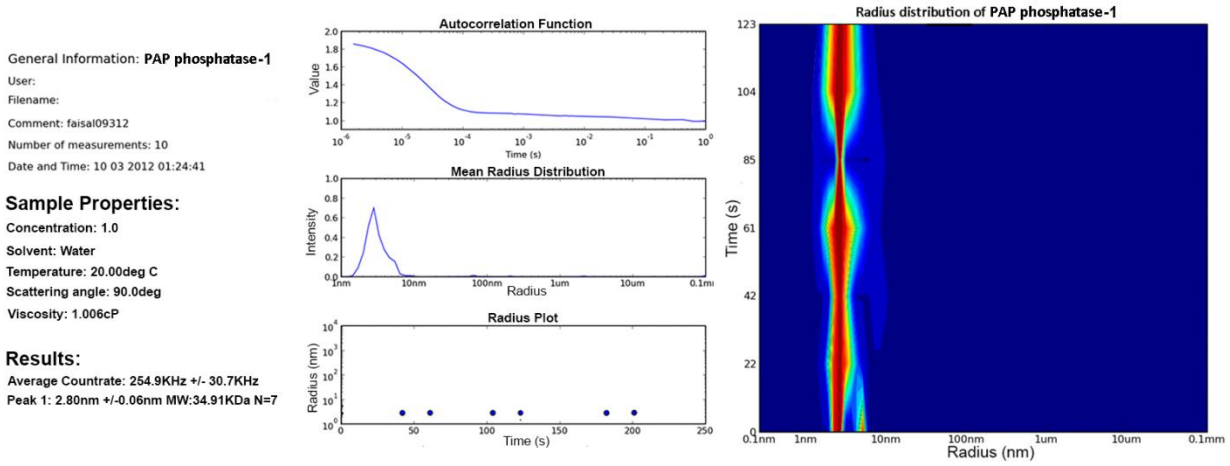

Figure S5 The dynamic light scattering. (DLS) measurements were performed on SpectroSize300 from Nano BiochemTechnology, Hamburg. The DLS experiments show the homogeneity and monomeric state of PAP phosphatase-1.

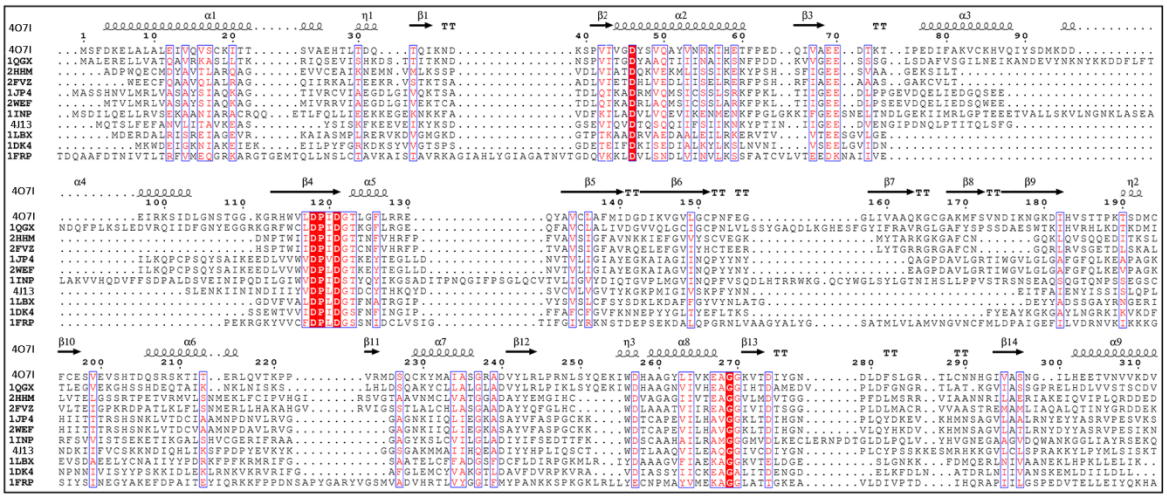

Figure S6 Multiple sequence alignment of PAP phosphatase-1 with other members of the Li^{+}/Mg^{2+} phosphatase superfamily picked from PDB. The alignment was generated by ESPript (Gouet *et al.*, 2003) with clustalW (McWilliam *et al.*, 2013). Secondary structural elements of PAP phosphatase-1 as determined 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 colour). The PDB codes of the respective proteins used for the MSA. **1K9Y**: 3'(2')5" bisphosphatase of Yeast (Hal2P), **2HHM**: Inositol Monophosphatase of homosapiens **2FVZ**: Human Inositol Monophosphophatase 2, **1JP4**: Inositol-Polyphosphate 1-Phosphatase (IPP) and 3'-Phosphoadenosine-5'-Phosphate Phosphatase from rat, **2WEF**: 3'(2')5'bisphosphatase of human, **1INP** :Inositol Polyphosphate 1-Phosphatase(IPP) from bovine, **1LBX**: Dual activity FBPase/IMPase from Archaeoglobus fulgidus, **1FRP**: Fructose-1,6-bisphosphatase (FBPase) from Pig, **1DK4:** inositol monophosphatase and the 'missing' archaeal fructose-1,6-bisphosphatase from *Methanococcus jannaschi*, **4J13:** PAP phosphatase-2 of *E.histolytica*.

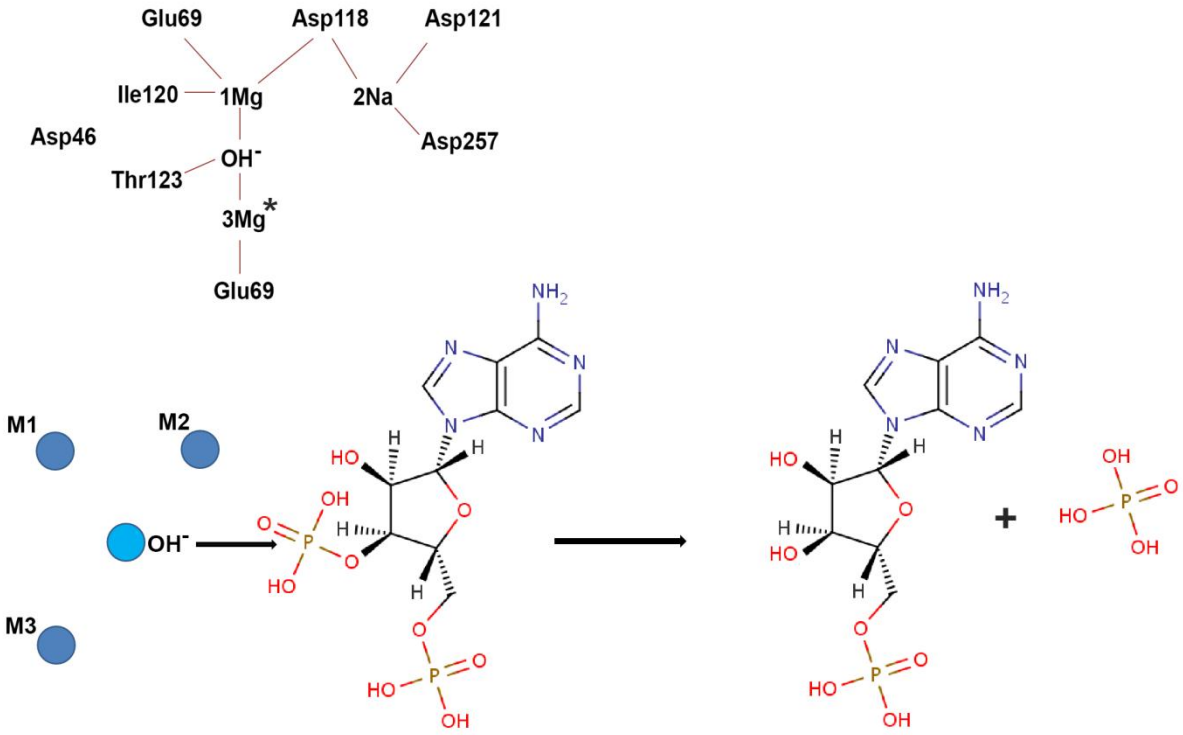

Figure S7 Mechanistic scheme for PAP phosphatase-1 activity. Spheres M1, M2 and M3 (with asterisks) shown in dark cyan colours are Mg^{2+}, Na^{+} and another probable Mg^{2+}. Activation of a hydroxide nucleophile (light cyan sphere) through a charge network of Asp46 and Thr123, leads to subsequent inline attack on the phosphate moiety of PAP and its hydrolysis into AMP and PO_4^{3-}.

Table S1 Interactions and interatomic distance of Pap phosphatase-1 with metal ions and product.

Interactions	**Distance (Å)**
Mg--OE1Glu69	2.39
Mg--OD1Asp118	2.74
Mg--OIle120	2.2
Mg--Water (W1)	2.70
Mg--Water (W49)	2.30
Na--OD1Asp257	2.93
Na--OD2Asp118	2.49
Na--O3'AMP	2.04
Na--Water(W1)	2.78
AMP-O1P--OGSer198	2.46
AMP-O1P--NZLys230	2.55
AMP-O1P--NH1Arg244	3.00
AMP-O2P--NSer227	2.62
AMP-O3P--ND1His203	2.57
AMP-O5'--NH2Arg244	3.09
AMP-O4'--NH2Arg244	2.68
AMP-N3--NH2Arg244	2.82
AMP-O2'--OD2Asp257	3.05