



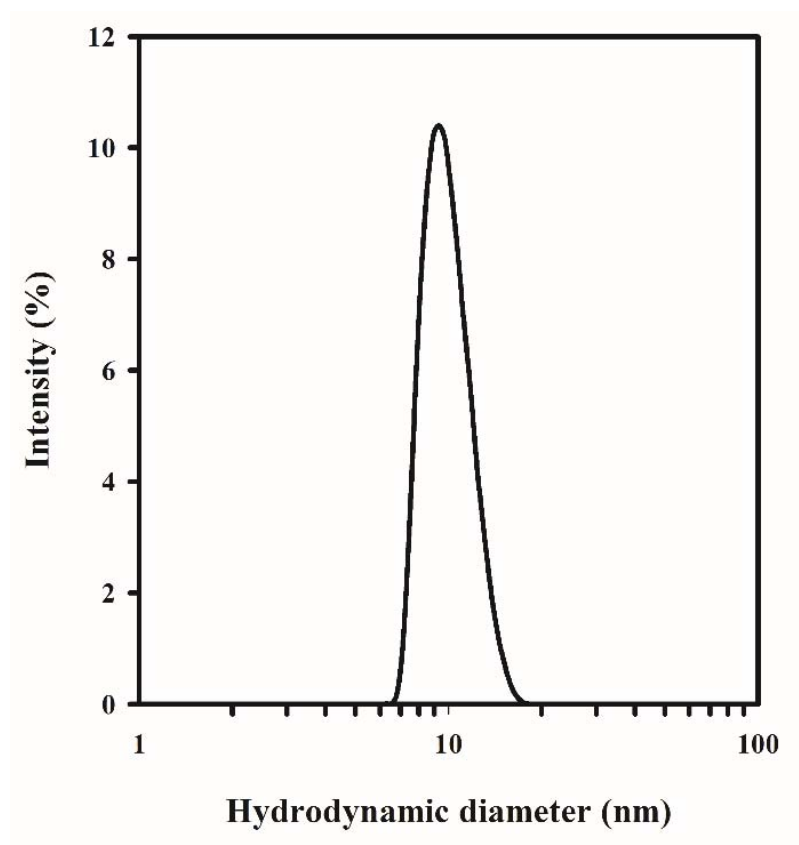
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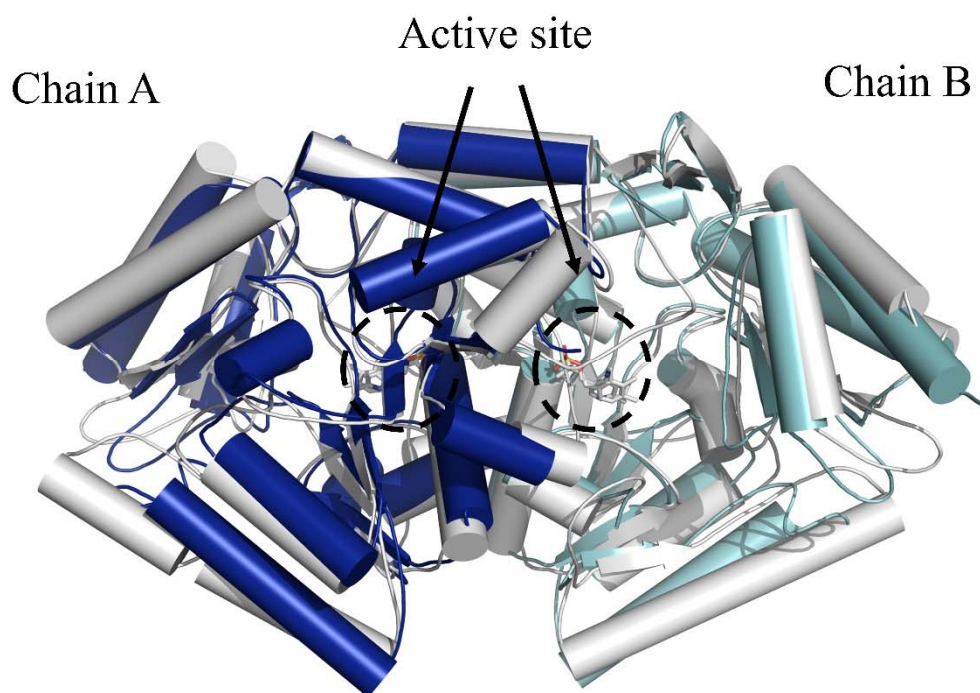
**Supporting information for article:**

**Structural characterization of human O-phosphoethanolamine phospho-  
lyase**

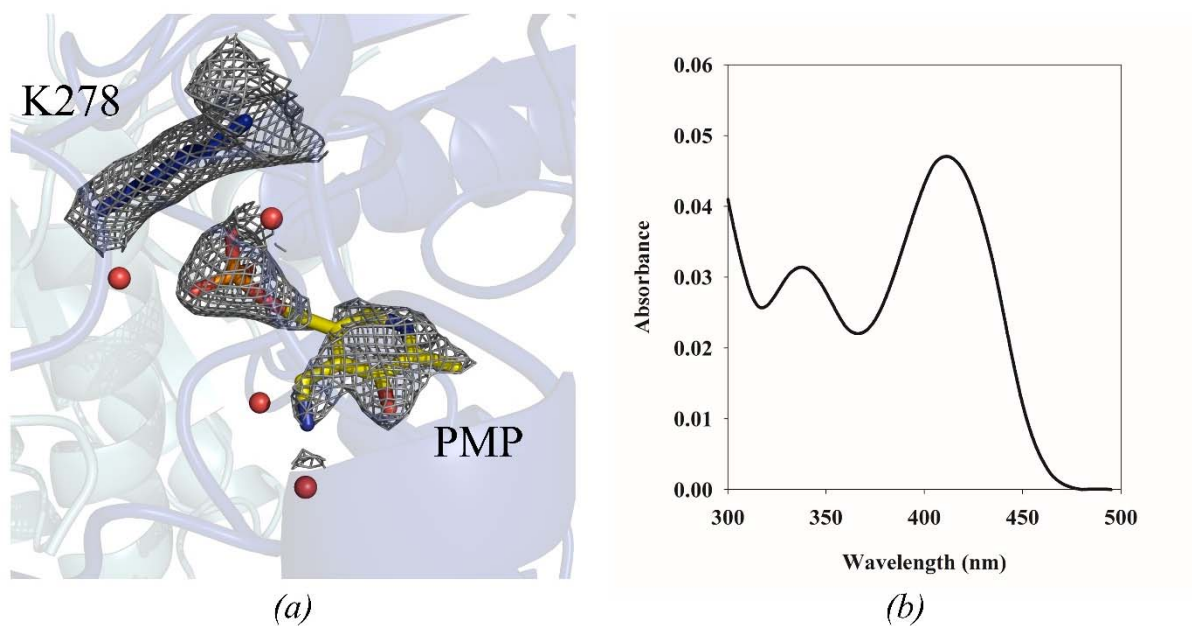
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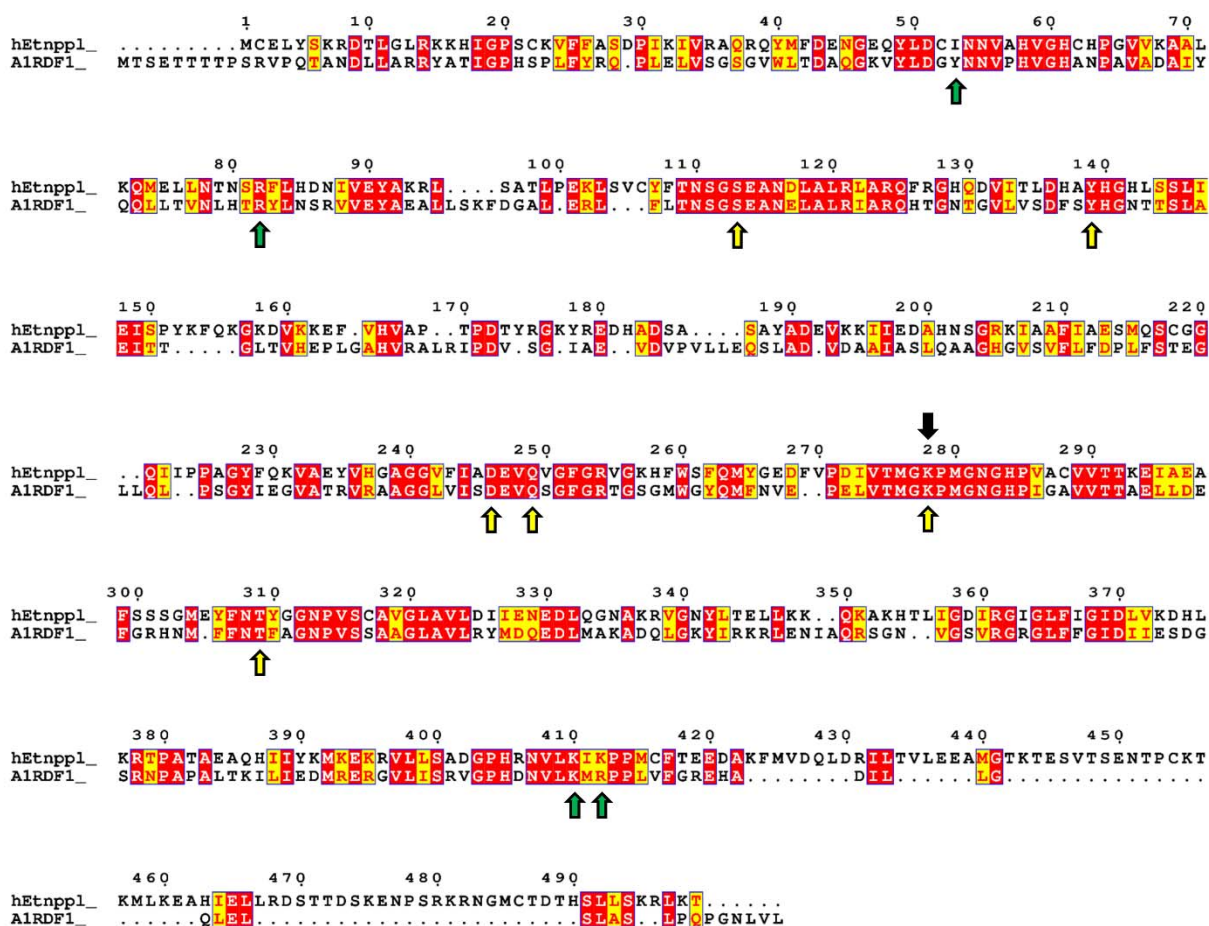
**Figure S1** DLS analysis of hEtnppl. The enzyme in solution features a hydrodynamic diameter (Size = 10 nm) that is consistent with a homodimer.



**Figure S2** Comparison of the hEtnppl dimer (blue/cyan) and A1RDF1 from *Arthrobacter aurescens* TCI (white) (PDB 5g4i). The  $\alpha$ -helices and the  $\beta$ -strands are depicted as cylinders and arrows, respectively. The bound cofactor (PLP or PMP) molecule is shown as grey sticks.



**Figure S3** PMP cofactor in the active site. (a) PMP molecule (0.7 occupancy) and K278 residue are represented in yellow and blue sticks, respectively. The 2Fo-Fc electron density map is depicted as gray mesh at 1  $\sigma$ . Water molecules are shown as red spheres. (b) The absorption spectrum of hEtnppl shows a prominent peak at 410 nm, typical of bound PLP, and a second peak centered at approximately 330 nm region is indicative of the presence of bound pyridoxamine-5'-phosphate (PMP).



**Figure S4** Alignment of hEtnppl with A1RDF, identical and similar residues are boxed in red and yellow background, respectively. A black arrow indicates the catalytic lysine residue. Yellow and green arrows indicate the residues involved in binding the cofactor and in the PEA phosphate recognition, respectively.

**Table S1** Purification table for wild type hEtnppl.

<b>Operation step</b>	<b>Total protein</b>	<b>Total Activity</b>	<b>Specific activity</b>	<b>Fold Purification</b>	<b>Yield (%)</b>
Crude Lysate	2350	282	0.12	1	100
NiNTA	167	20	0.12	1	7
S100	98.1	75	0.77	6.4	26.5