International Journal of Molecular Sciences

Supplementary Material

Structure-Function Relationship Study of an Amoebic Secretory Phosphatase: A Computational-Experimental Approach

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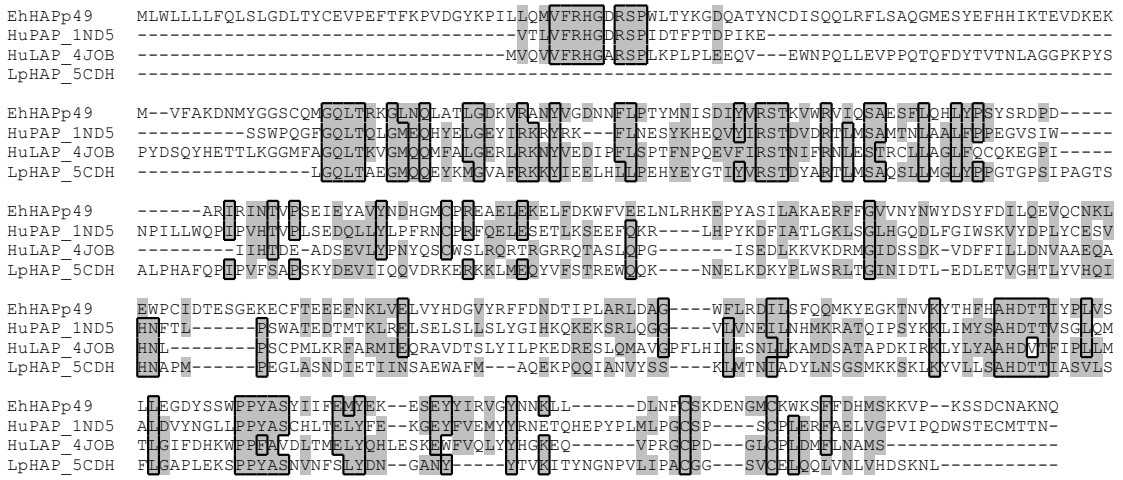


Figure S1. Multiple sequence alignment of the protein sequences of *Eh*HAPp49 and three structural homologs: HuPAP, human prostatic acid phosphatase (PDB Entry 1ND5); HuLAP, human lysophosphatidic acid phosphatase type 6 (PDB Entry 4JOB); and LpHAP, *Legionella pneumophila* HAP (PDB Entry 5CDH). The alignment was performed using Chimera's Multalign Viewer tool. Similar residues are shaded gray, while identical residues are boxed.

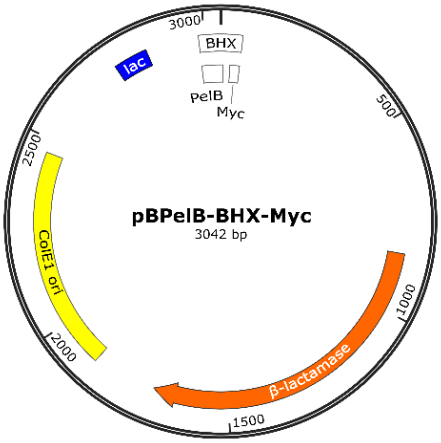
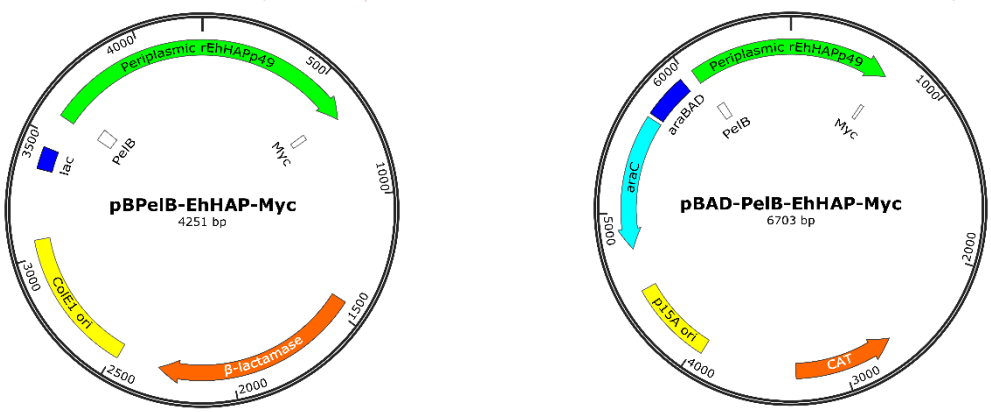


Figure S2. Schematic depiction of pBPelB-BHX-Myc, a pBluescript-based plasmid allowing gene fusion of a periplasmic targeting sequence (PelB) and a molecular tag peptide (Myc) to the N- and C-termini ends of recombinant proteins. BHX represents a mini MCS, which includes the BamHI, HindIII, and XhoI restriction sites. While ColE1 ori (yellow) functions as the autonomous replication sequence, encoded β-lactamase (orange) as the selection marker (Amp^R), and lac promoter (blue) as the regulatory region.



(**a**) (**b**)

Figure S3. (**a**) Schematic depiction of pBPelB-EhHAP-Myc, a pBluescript-based plasmid encoding the *Eh*HAPp49 as a Myc-tagged periplasmic protein under the control of lac promoter (blue). ColE1 ori (yellow) and β-lactamase (orange) function as described for the parental plasmid, pBPelB-BHX-Myc (Supp. Fig. 1). (**b**) Schematic depiction of pBAD-PelB-EhHAP-Myc, a pBAD33-based plasmid encoding the *Eh*HAPp49 as a Myc-tagged periplasmic protein under the control of araBAD promoter (blue). While p15A ori (yellow) functions as the autonomous replication sequence, encoded chloramphenicol acetyl-transferase, CAT (orange), as the selection marker (Cm^R), and AraC (turquoise) as the regulatory protein.

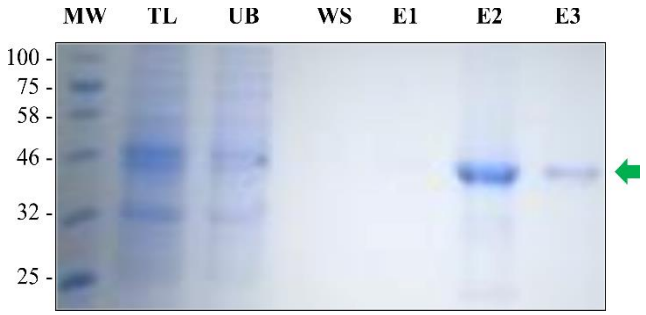


Figure S4. Analysis of IMAC fractions by 13.5% SDS-PAGE. Lanes: MW, molecular weight markers (kDa; on the left side); TL, total lysate; UB, unbound; WS, wash; E1-E3, elutions. Gel stained with Coomassie Brilliant Blue. On the right side, a green arrow indicates the relative mobility of r*Eh*HAPp49.