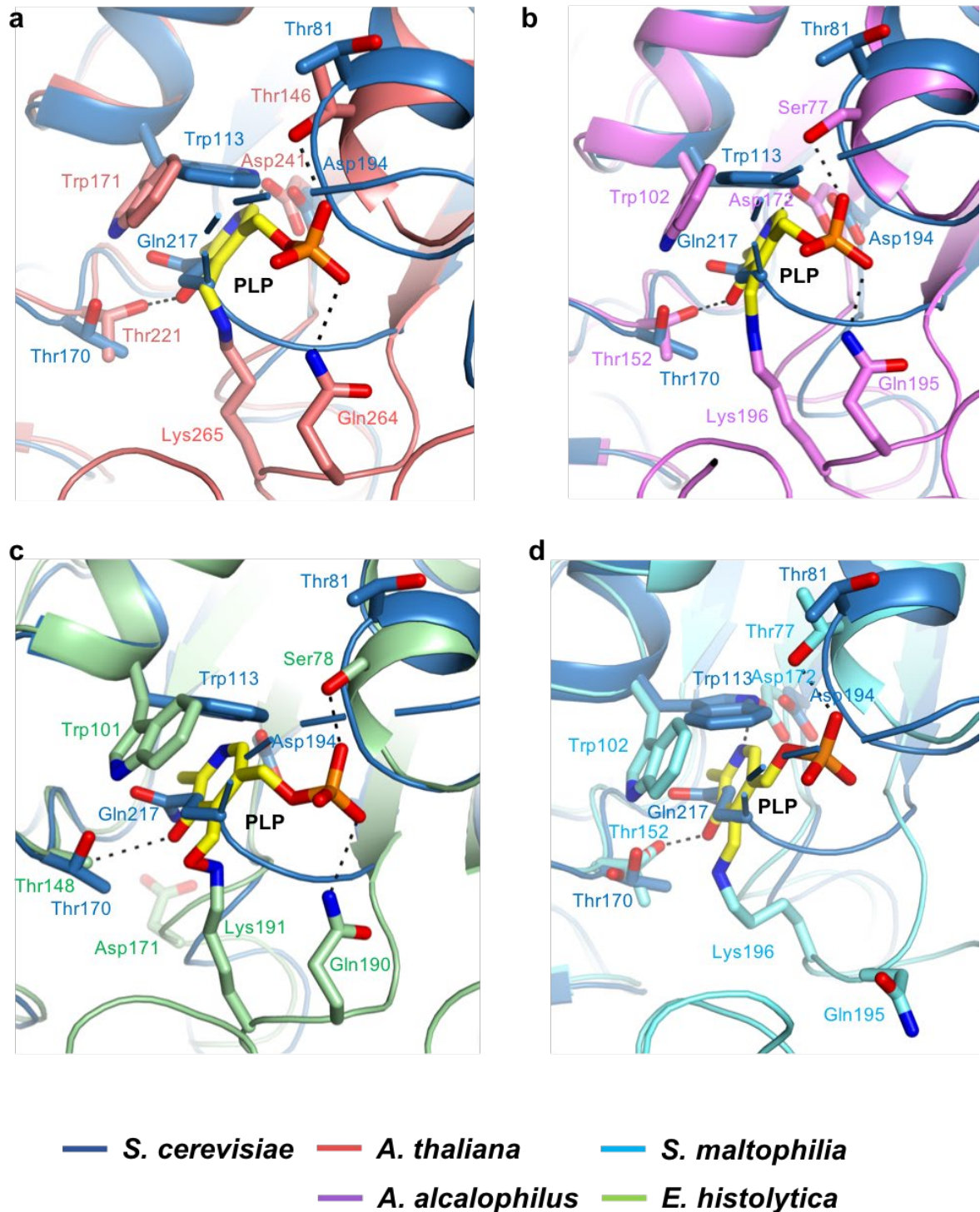
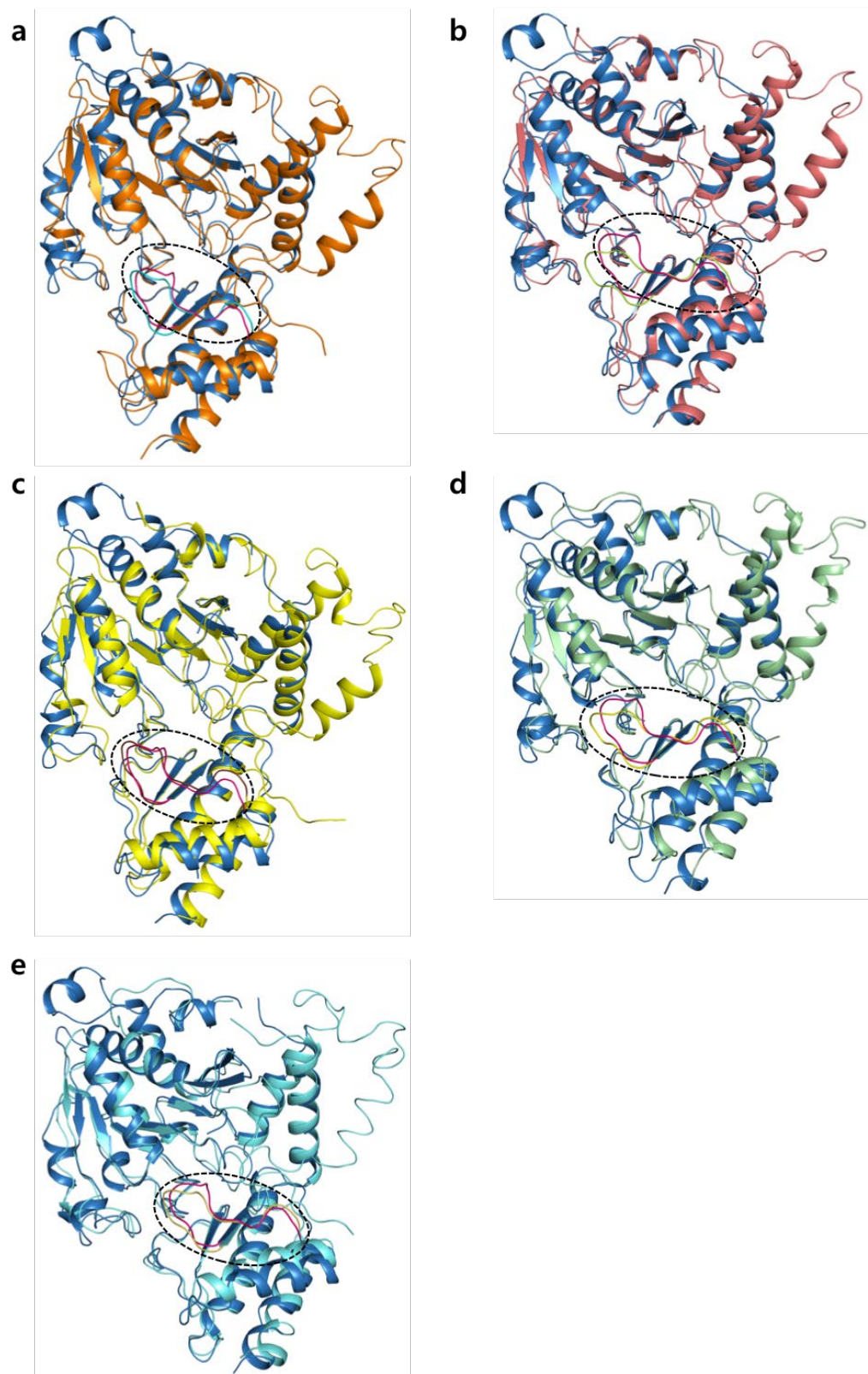


**Figure S1. SDS-PAGE analysis of dissolved crystals.** Crystals of ScPSAT protein (left panel) were dissolved by a buffer containing 20 mM Tris (pH 7.5), 150 mM NaCl, and 2 mM dithiothreitol, and it was compared with the purified protein by using SDS-PAGE analysis (right panel). The sizes of standard marker were shown on the gel.

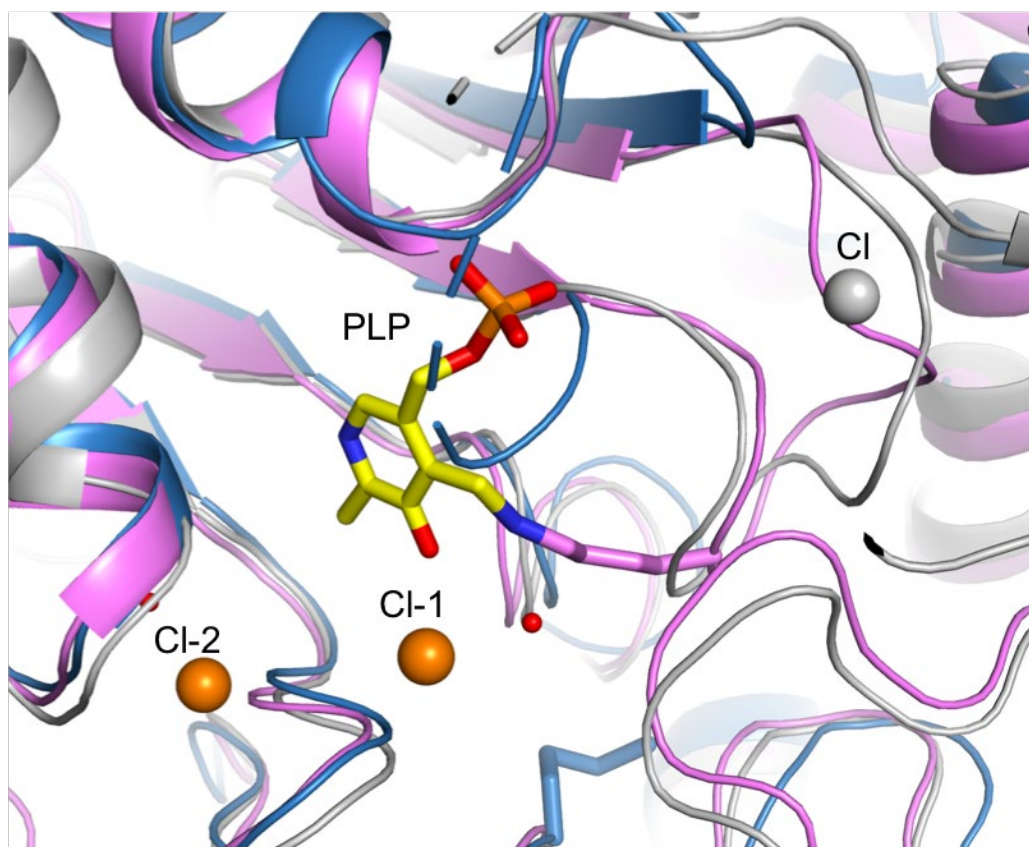


**Figure S2. Comparison of the active site of ScPSAT with its homologs:** (a) Detailed view of the active site by superimposed structures of ScPSAT and AtPSAT-PLP complex. The ScPSAT and AtPSAT structures are colored blue and pink, respectively. Residues His44 and Arg45 are from a neighboring molecule of HsPSAT. Covalently linked PLP with Lys265 bound in the AtPSAT is colored in yellow; (b) Detailed view of the active site by superimposed structures of ScPSAT and AaPSAT-PLP complex. The ScPSAT and AaPSAT structures are colored blue and purple, respectively. Covalently linked PLP with Lys196 bound in the AaPSAT is colored in yellow; (c) Detailed view of the active site by superimposed structures of ScPSAT and EhPSAT-PLP complex. The ScPSAT and EhPSAT structures are colored blue and green, respectively. Covalently linked PLP with Lys191 bound in the EhPSAT is colored in yellow; (d) Detailed view of the active site by superimposed structures of ScPSAT and SmPSAT-PLP complex. The ScPSAT and SmPSAT structures are colored blue and cyan, respectively. Covalently linked PLP with Lys196 bound in the SmPSAT is colored in yellow; Abbreviations: Aa, *Alkalihalobacillus alcalophilus*. At, *Arabidopsis thaliana*. Eh, *Entamoeba histolytica*. Hs, *Homo sapiens*. PLP, pyridoxal-5-phosphate. PSAT, 3-phosphoserine aminotransferase. Sc, *Saccharomyces cerevisiae*. Sm, *Stenotrophomonas maltophilia*.



**Figure S3. Structural comparison of the gate-keeping loop of ScPSAT with its homologs:** (a) Superposition of ScPSAT (blue) and HsPSAT (PDB ID: 3E77; orange) structures. The gate-keeping loops of ScPSAT (red) and HsPSAT (cyan) are highlighted by a black-dashed oval; (b) Superposition of the ScPSAT (blue) and AtPSAT (PDB ID: 6CZX; pink) structures. The gate-keeping loops of ScPSAT (red) and AtPSAT (green) are highlighted by a black-dashed oval; (c) Superposition of the ScPSAT (blue) and AaPSAT (PDB ID: 1W23; yellow) structures. The gate-keeping loops of ScPSAT (red) and AaPSAT (brown) are highlighted by a black-dashed oval; (d) Superposition of the ScPSAT (blue) and EhPSAT (PDB ID: 5YB0; light green) structures. The gate-keeping loops of ScPSAT (red) and EhPSAT (yellow) are highlighted by a black-dashed oval; (e) Superposition of the ScPSAT (blue) and SmPSAT (PDB ID: 6XDK; cyan) structures. The gate-keeping loops of ScPSAT (red) and SmPSAT (yellow) are highlighted by a black-dashed oval.





**Figure S4. Distinct chloride ion binding in *Stenotrophomonas maltophilia* PSAT.** The halide binding site of SmPSAT (grey) is different to ScPSAT (blue) and HsPSAT-PLP complex. The chloride ions of HsPSAT-PLP and SmPSAT are colored orange and grey, respectively. PLP-bound in HsPSAT is colored yellow.