

S3 Figure XcpX exhibits calcium-binding activity. (A) ITC results show that XcpX's K_d of calcium binding is 26.04 μM . (B) Phylogenetic trees of GspK^(XcpX) proteins in different Gram-negative bacteria illustrate the evolutionary relationship. Left panel: phylogenetic trees of full-length GspKs with the first main clade boxed. Right panel: phylogenetic trees of the sequence of the new calcium-binding site in different GspKs shows that this additional calcium binding site only exists in limited Gram-negative bacteria (highlighted by the dotted box). (C) The alignment of the sequences of canonical calcium binding sites in different species implies that several species do not possess the ability of binding calcium. All the necessary residues for calcium binding are labeled.