# Structural and mechanistic basis for protein glutamylation by the kinase fold

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Enzymes with a protein kinase fold transfer phosphate from adenosine 5′-triphosphate (ATP) to substrates in a process known as phosphorylation. Here, we show that the *Legionella* meta-effector SidJ adopts a protein kinase fold, yet unexpectedly catalyzes protein polyglutamylation. SidJ is activated by host-cell calmodulin to polyglutamylate the SidE family of ubiquitin (Ub) ligases. Crystal structures of the SidJ-calmodulin complex reveal a protein kinase fold that catalyzes ATP-dependent isopeptide bond formation between the amino group of free glutamate and the gamma-carboxyl group of an active-site glutamate in SidE. We show that SidJ polyglutamylation of SidE, and the consequent inactivation of Ub ligase activity, is required for successful *Legionella* replication in a viable eukaryotic host cell. [1]

Here we also present cryo-EM reconstructions of SidJ:CaM:SidE reaction intermediate complexes. We show that the kinase-like active site of SidJ adenylates an active site Glu in SidE resulting in the formation of a stable reaction intermediate complex. An insertion in the catalytic loop of the kinase domain positions the donor Glu near the acyl-adenylate for peptide bond formation. Our structural analysis led us to discover that the SidJ paralog SdjA is a glutamylase that differentially regulates the SidE-ligases during *Legionella* infection. Our results uncover the structural and mechanistic basis in which the kinase fold catalyzes non-ribosomal amino acid ligations and reveal an unappreciated level of SidE-family regulation. [2]

[1] Black, M. H., Osinski, A., Gradowski, M., Servage, K. A., Pawłowski, K., Tomchick, D. R., Tagliabracci, V. S. (2019). *Science* **364**, 787-792.

[2] Osinski, A., Black, M. H., Pawłowski, K., Chen, Z., Li, Y., Tagliabracci, V. S. (2021). *bioRxiv* **doi:** https://doi.org/10.1101/2021.04.13.439722

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