Supplementary Discussion

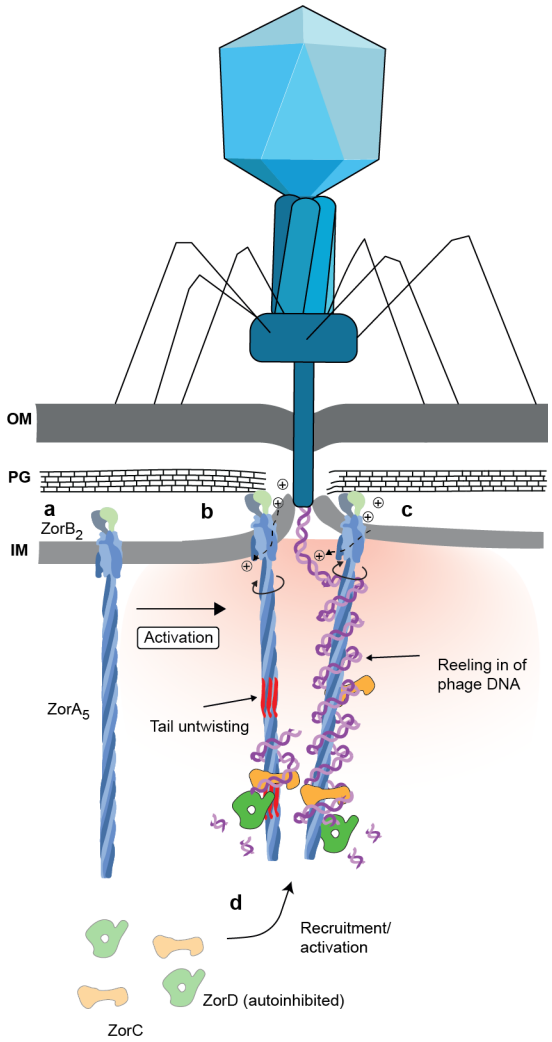
In addition to the mechanistic insights supported by our data, there are several aspects worthy of future investigations. First, future studies will need to determine whether Zorya functions through a true 'localized-response' model, where multiple ZorAB complexes diffuse to the site of phage penetration of the cell envelope to amplify the recruitment of ZorC/ZorD in a ZorAB rotation-dependent manner, or a more 'global' activation of ZorAB via perturbation of the IM-PG distance throughout the cell envelope. Interestingly, our model predicts that the 'reach' of the ZorB PGBD should be evolutionarily diversified to adapt to different bacterial cell envelope architectures, which appears to be the case (**Supplementary Discussion Figure 1**). Accumulated knowledge about phages and the mechanics phage-host physical interactions during adsorption and genome injection would deepen our understanding of the proposed role of ZorAB as a 'sensor' during phage infection, which is likely to be a universal mechanism of the Zorya defense system across diverse bacteria.

Another open question is the role of two striking features of the ZorAB structure: the rotary motor and the conserved long tail. One hypothesis for the role of rotation is in signal transduction from phage ingress at the cell envelope to activation of ZorC and ZorD. If the direction of the ZorAB motor is the same as for MotAB (clockwise rotation of A around B, as viewed from the outside of the cell), rotation could potentially induce untwisting of the pentameric ZorA tail super-helix, thereby altering the conformation of the tail to facilitate recruitment or activation of ZorC and/or ZorD (**Supplementary Discussion Figure 2a, b**). A somewhat similar sensory transmission mechanism occurs with bacterial chemosensory arrays and the long ZorA tail might serve to transmit the signal in a manner analogous to the long coiled-coil cytoplasmic signaling domains of methyl-accepting chemotaxis proteins[23]. A second hypothesis accounting for both rotation and a long ZorA tail is that phage DNA might be 'reeled in' around the tail, trapping the foreign DNA in an inactive state (e.g. preventing RNA polymerase access) prior to degradation by ZorD (**Supplementary Discussion Figure 2c**). A 'reeling in' mechanism could greatly enhance phage genome localization and sequester it from interactions required for host infection. A typical double-stranded DNA phage genome is 10s of μm long and would form a random coil with a radius of gyration similar to the size of the entire cell in the absence of constraints – thus negating any advantage of a localized nuclease defense response at the site of entry. Binding to multiple Zor complexes, perhaps via ZorC and/or D might contribute to localizing phage DNA to the entry site. Unless and until tail rotation is resisted, an almost inevitable consequence of tail rotation combined with DNA binding is that the DNA will wind around the tail like wire on a reel. If 'reeling in' of the phage DNA were an essential feature of the Zorya defense mechanism, this would explain the need for both rotation and a long ZorA tail. The length of the ZorA tail is similar to the diameter of a typical phage capsid into which an entire phage genome can be tightly packed, indicating that a single Zor complex may be sufficient to capture an entire genome. Rough calculations indicate that 100s of turns would be required to wind a 60 kb Bas24 genome onto a 70 nm tail, allowing the rotary ZorAB motor cumulatively to supply the necessary energy to wind

and compact the phage DNA. Reeling would also tighten any loops that might form between DNA sites bound to different ZorA tails, removing any freedom for further ZorA rotation - as required by the model of activation via untwisting of the ZorA tail.

Supplementary Discussion Figure 1. Structural prediction of the representative ZorAB complexes form different Zorya system types.

Representative predicted structures of $ZorA_5B_2$ complexes (omitting the ZorA tail region) for each ZorA and ZorB sequence family (protein sequence groups numbered above each predicted structure) for type I, II, and III Zorya systems. **a**, The predicted dimerized ZorB PGBDs. One ZorB subunit is colored in each structure. **b**, The predicted ZorAB transmembrane motor complex. One ZorA subunit is colored in each structure.



Supplementary Discussion Figure 2: Putative ZorA tail conformational change and phage DNA 'reeling in' mechanism in the activated Zorya defense system.

a, An inactive ZorAB embedded in the inner membrane. **b,** Phage invasion triggers ZorAB activation. The rotation of ZorA and its long intracellular tail around ZorB causes ZorA tail tip conformational change, which would recruit ZorC and ZorD**. c,** Reeling in of phage DNA around the long ZorA tail in the activated Zorya defense system.