

# Structure-guided fusion-protein designs using *Bacillus* flagellin as a vaccine adjuvant

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Flagellin is a major component of the flagellar filament that provides bacterial motility and pathogenicity. In the host, flagellin is an active agonist and stimulates the innate immune response by directly interacting with Toll-like receptor 5 (TLR5). Thus, flagellin has been widely studied as a vaccine adjuvant. Moreover, there are numerous efforts to use a recombinant flagellin-fused protein as a vaccine to prevent various infectious diseases. Despite the widespread applications of flagellin, the biophysical and structural integrity of various flagellin-antigen fusion proteins has never been assessed, and the optimal fusion design that assures vaccine efficacy remains to be elucidated. Furthermore, flagellin from *Salmonella* species has been extensively used for vaccine application but contains hypervariable domains that are dispensable for TLR5 binding but could induce an unexpected immune response. To eliminate any unwanted problems caused by the hypervariable domains of *Salmonella* flagellin, a natural and minimal flagellin protein that activates TLR5 would be of great value. In addition, it is desirable to ensure the integrity of the flagellin-fused protein. We will present our recent data that identified the smallest flagellin protein [*Bacillus cereus* flagellin (BcFlg)] that fully satisfies the need of TLR5 stimulation as a flagellin fusion adjuvant. Using the BcFlg, crystal structure was determined to provide as a template for flagellin-conjugated fusion vaccine designs. Based on cellular and biophysical analyses of the fusion proteins, the optimal fusion site will be proposed.