

Crystal Structure of FlgD from *Xanthomonas campestris*: Insights into the Hook Capping Essential for Flagellar Assembly

Wei-Ting Kuo (郭威庭)¹, Ko-Hsin Chin (秦可欣)¹, Wen-Ting Lo (羅文廷)¹,
Andrew Wang (王惠鈞)², and Shan-Ho Chou (周三和)¹

¹Institute of Biochemistry, National Chung Hsing University, Taichung, Taiwan

²Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan

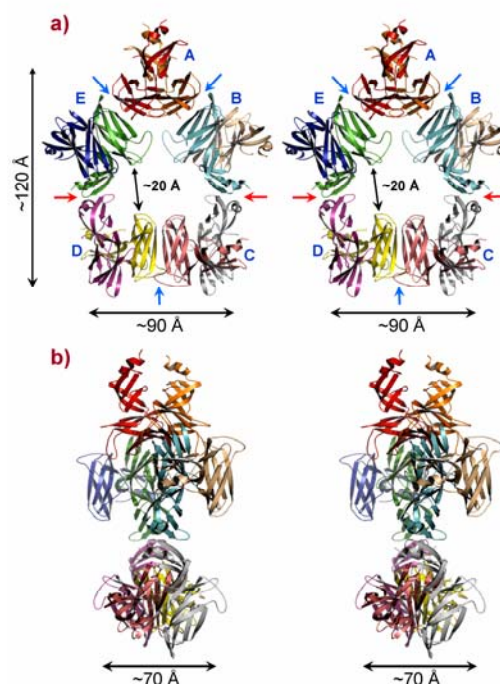
The first crystal structure of a hook-capping protein FlgD from the plant pathogen *Xanthomonas campestris* has been determined using X-ray crystallography. The monomer comprises 221 amino acids with a MW of 22.7kD, but the disordered N-terminus is cleaved for up to 75 residues during crystallization automatically. The final core structure reveals a novel hybrid comprising a tudor-like domain interdigitated with a fibronectin type III domain. In the crystal, the monomers form an annular pentamer of dimers of pseudo five-fold symmetry, due to different dimer-dimer interactions employed. The resulting asymmetrical star-like complex has a outer dimensions of approximately 110 Å x 90 Å x 65 Å, and a shortest diameter of approximately 20 Å in the center. The outer dimensions of the atomic *Xanthomonas* hook-capping FlgD complex turn out to be very similar to those of the *Salmonella* filament cap complex observed by electron microscopy. This atomic hook cap structure may help understand hook protein – cap protein interactions, hook protein insertion, and hook length control, the three features that are crucial for understanding the bacterial flagellar biogenesis.

The bacterial flagellum is a very complicated nano-structure assembled from more than 40 gene products. It comprises the basal body, hook, filament, and other labile structures such as motor, switch, and export apparatus. The flagellum is sequentially assembled from simpler to more complex structures. In a swimming cell, flagella rotate at high speed in the order of 100 Hz. However, it is important to note that flagella are attached on an immobile basal body. Hence an intermediate substructure is necessary for a cell to withstand the enormous torsional force caused by the highly rotating flagella. Such a “buffer” is served by a hook substructure of around 55 Å. However, to form such an intermediate hook structure, a cap is necessary to prevent the leakage of hook monomers into the medium. Furthermore, the hook cap should not be permanently present and need be removed when hook structure is complete to continue the flagellar biogenesis. The scaffolding protein FlgD is believed to be the protein to form the hook cap structure. Besides its obvious role as a cap, it also serves the active roles of productive hook monomer polymerization, and determination of correct hook length, along with the help of FliK protein. Thus, although FlgD is not present in a mature flagellum, it plays diverse roles in forming a functional hook structure.

Flagellum is important to a bacterial viability and pathogenicity, and has been the target of extensive studies over the past two decades. However, until to date, no atomic structure for the hook cap or filament cap has been determined. The determined hook cap structure

reported in this manuscript shows that 1) the hook cap complex has a inner diameter of around 20 Å, leaving room for holding the N-terminus of FliK protein to help block the leakage of FlgE monomer into the medium; 2) the interaction between the hook cap subunits is not very stable, which may be necessary due to its transient presence; 3) the hook cap complex has shape and dimension similar to those of the filament cap from electron microscopy data.

Figure below shows the decamer structure of XcFlgD possibly existing in vivo.



Beamline:

13B2, 12B2

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PI Email: Dr. Shan-Ho Chou, shchou@nchu.edu.tw