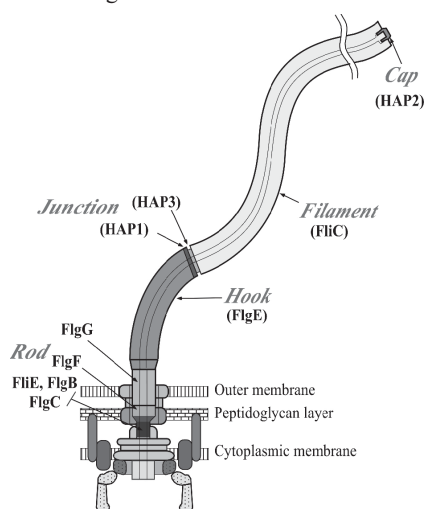


s1.m7.p27 **Twist and curvature formation of the hook: a two-domain movement.** F.A. Samatey¹, H. Matsunami¹, K. Imada^{1,2}, S. Nagashima¹, T.R. Shaikh³, D. Thomas³, D.J. DeRosier³, K. Namba^{1,2}. 1) *Dynamic NanoMachine Project, ICORP, JST*, 2) *Graduate School of Frontier Biosciences, Osaka University, Japan*, 3) *W.M.Keck Institute of Cellular Visualization, Brandeis University, U.S.A.* E-mail: alexis@fbs.osaka-u.ac.jp

Keywords: Protein structure; Bacteria motility; Molecular assembly

Bacteria swim by rotating long helical filaments called the flagellum. Each flagellum is rotated by a motor located at its base. The flagellum can be described as an assembly of 25 different proteins. HAP2 caps the flagellum at the distal end. Flc makes a long helical propeller, the filament, which makes the bacteria swim. FlgB, FlgC, FlgF, FlgG and FlgE form the rod, the driving shaft of the flagellum. Between the rod and the filament there is a short segment, the hook, that transmits the torque from the rod to the filament. The hook is connected with the filament by two junction proteins: HAP1 and HAP3. The hook is made by the polymerisation of a single protein, FlgE. The polymerisation occurs by interaction between the N-terminal and the C-terminal chains [1]. FlgE from *Salmonella typhimurium* is composed of 402 amino acid residues. The hook is not a rigid and straight structure. It is the most flexible segment in the flagellum. This flexibility assures a smooth swimming of bacteria and a coordination of all the flagella. To understand the function of the hook it is necessary to have its three dimensional structure. FlgE is very difficult to crystallize because of its high tendency to aggregate when put together with solutions used for crystallisation. We therefore prepared a fragment of FlgE by removing 70 residues and 33 residues in the N-terminal and the C-terminal chains, respectively. The fragment, named H32 (32 KDa), was successfully crystallised and the three-dimensional structure solved at 1.8 Å-resolution using X-ray diffraction. By combining the X-ray structure of H32 with low-resolution data of the hook obtained by electron microscopy we were able to build a high-resolution model of the hook. The hook and the filament have similar helical parameters but the structure of H32 shows a completely different folding from the structure of F41, a major fragment of the flagellin [2]. The detailed structure of H32, the model of the hook and a comparison between the hook and the filament will be shown and discussed during this conference.



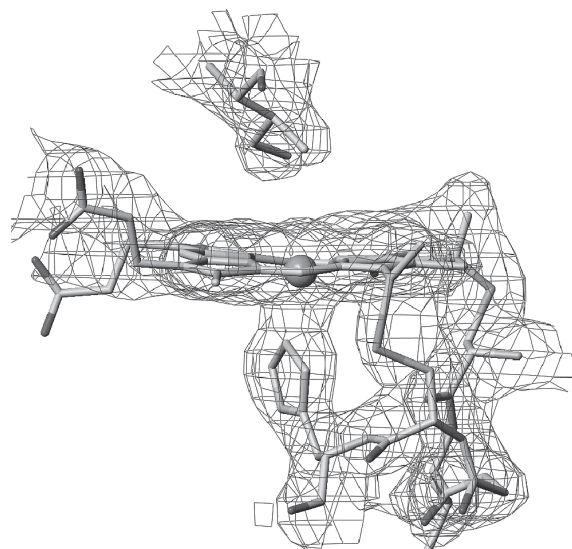
- [1] Vonderviszt *et al.*, *JMB*. 1995; **251**: 520-532
 [2] Samatey *et al.*, *Nature*. 2001; **410**: 331-337

s1.m7.p28 **Structure of a Sixteen Heme Cytochrome by X-Ray Crystallography.** Teresa Santos Silva^a, Joao M. Dias^a, Gleb Bourenkov^b, Hans Bartunik^b, Isabel Moura^a and Maria Joao Romao^a. ^a *REQUIMTE - Departamento de Química, CQFB, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Monte de Caparica, Portugal.* ^b *Max-Planck Research Unit for Structural Molecular Biology, MPG-ASMB c/o DESY, Notkestrasse 85, 22607 Hamburg, Germany.* E-mail: teresa.sss@dq.fct.unl.pt

Keywords: Cytochrome c; Multiheme; MAD

High molecular weight cytochromes (Hmc) belong to a large family of multiheme cytochromes in sulphate reducing bacteria and HmcA is the first cytochrome reported to have sixteen type c hemes arranged in its polypeptide chain. The function of this cytochrome is still unknown although it is clear that it is involved in electron transfer from the periplasm to the membrane [1].

HmcA from *Desulfovibrio gigas* (MM=67kDa) has been purified and successfully crystallized using the hanging-drop vapour-diffusion method. They diffracted beyond 2.07 Å and a MAD data set was collected. The structure was solved and the experimental (unbiased) electron density map around one of the hemes is shown in the picture. Refinement is now in progress.



- [1] Teresa Santos Silva, Joao M. Dias, Gleb Bourenkov, Hans Bartunik, Isabel Moura and Maria Joao Romao, *Acta Cryst. D* (submitted)