ESRF	**Experiment title:** Structural organization of complex nanosomes	**Experiment number**: SC1517
Beamline: ID02	**Date of experiment**: from: 15/12/2004 to: 18/06/2003	**Date of report**: 01/01/2006
Shifts: 8	**Local contact(s)**: Stéphanie Finet	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Véronique RECEVEUR-BRECHOT*, Mirjam CZJZEK*, Michal HAMMEL*

AFMB-CNRS – 163 avenue de Luminy, case 932 - 13288 Marseille cedex 9, France

Henri-Pierre FIEROBE*

BIP-CNRS- 31, Chemin Joseph Aiguier – 13402 Marseille cedex 20, France

Report:

Cellulose, the main structural component of plant cell walls, is the most abundant carbohydrate polymer in nature. To break down plant cell walls, anaerobic microorganisms have evolved a large extracellular enzyme complex termed cellulosome. This megadalton catalytic machinery organizes an enzymatic assembly, tenaciously bound to a scaffolding protein via specialized intermodular "cohesin-dockerin" interactions that serve to enhance synergistic activity among the different catalytic sub-units. The three-dimensional structure of a number of isolated domains have been determined by recent studies. Unfortunately, the modularity of cellulosomes, their size, their heterogeneity and their intrinsic flexibility prevent from obtaining a structural characterisation by means of structural techniques such as NMR and crystallography and to understand their mechanisms of synergy. It is now possible to construct mini-scaffoldins with a restricted number of cohesin modules, the so-called nanosomes, with the desired cellulase attached on it. Our previous studies made on ID02, ESRF **(SC-1159)** provided the first clues to the mechanisms of complexation of cellulases onto the scaffoldin, and led to the publication of an article (1)

In the present study, we examined cellulosome-like assemblies of higher complexity to gain further insight into the mechanism by which the cellulosomes enhance activity on recalcitrant substrates. For this purpose, a hybrid scaffoldin (S4), bearing a pair of divergent cohesins from *C. cellulolyticum* and *C. thermocellum*, was combined with one or two enzymes, each of which contained a dockerin of appropriate specificity.

We studied the chimeric scaffoldin S4 that contains a *C. cellulolyticum* cohesin fused to a *C. thermocellum* cohesin, in complex with the *C. cellulolyticum* cellulases Cel48F appended with either its native

C. cellulolyticum dockerin or a *C. thermocellum* dockerin, and Cel5A appended with a *C. thermocellum* dockerin. These three cellulase constructs are termed hereafter Fc, Ft and At respectively. We studied the different complexes Fc-S4, Fc-S4-Ft and Fc-S4-At.

The experimental SAXS data for all samples were linear in a Guinier plot of the low q region, indicating that the proteins did not undergo aggregation. The radii of gyration RG, calculated for different protein concentrations, displayed a slight concentration-dependence arising from particle interferences in solution. The programs GNOM was used to compute the pair-distance distribution functions, P(r) (Figure 1).

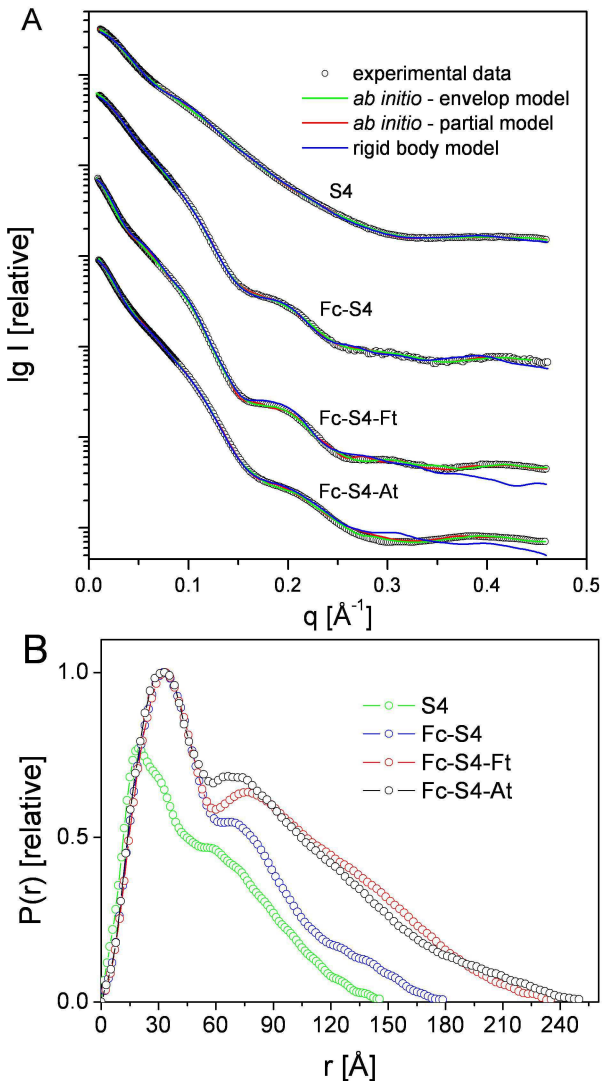

Fig. 1. Experimental SAXS curves and scattering profiles computed from the models of the cellulosome constructs (S4, Fc-S4, Fc-S4-Ft, Fc-S4-At). (A) Black circles: experimental data; green line: scattering from *ab initio* model using the program GASBOR; red line: scattering from the partial *ab initio* model using the program CREDO; blue line: average scattering from three best-fitting atomic models obtained by rigid body modeling. (B) Distance distribution functions of the cellulosome constructs computed from experimental SAXS data. Green circles: S4; blue circles: Fc-S4; red circles: Fc-S4-Ft; black circles: Fc-S4-At; the P(r) functions are normalized to unity at their maximum, except for the P(r) function of S4 which is plotted in offset for clarity.

The overall shapes of the entire assemblies were restored from the experimental data using the program GASBOR. The program package CREDO was then used to add missing domains (from 250 residues for S4,

to 910 residues for Fc-S4-Ft) by fixing the known atomic structures and building the unknown regions to fit the experimental scattering data obtained from the entire particle. This procedure was applied in all cases to restore the low-resolution shapes of missing linker regions.

We then positioned the atomic structures of the individual modules into the low resolution model obtained by CREDO by using the program SUPCOMB (Figure 2).

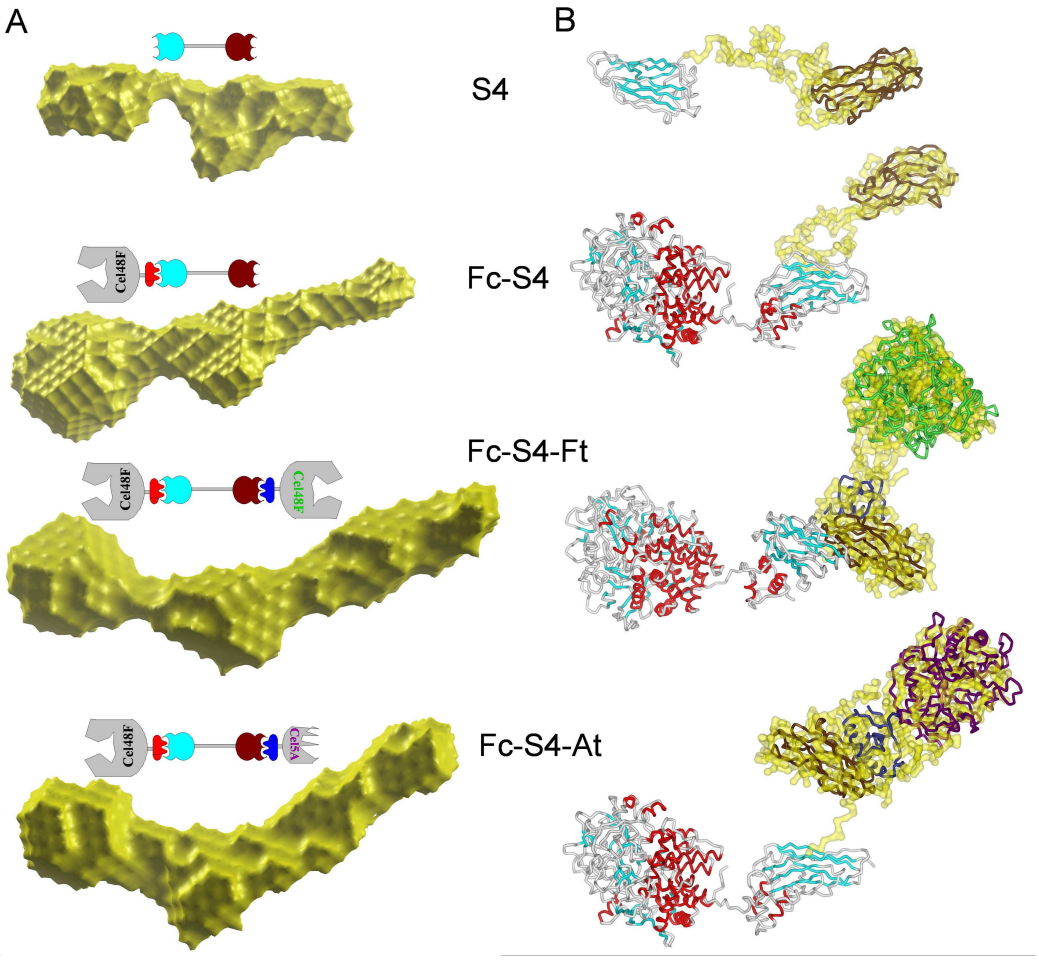

Fig. 2. ***Ab initio* models.** (A) The average envelope shape of different cellulosome constructs calculated with GASBOR (yellow transparent surface). The corresponding modules are schematically represented on the top of each shape.
(B) Restored partial *ab intio* models of the different cellulosome constructs calculated with CREDO. The CREDO models are displayed in surface representation. The secondary structural elements of fixed known atomic structures are in C_α tube representation with cyan β-sheets and red α-helices. The secondary structural elements of superimposed atomic structures are in colored C_α tube representation; brown: cohesin from *C. thermocellum*; blue: dockerin from *C. thermocellum; green*: catalytic module of Cel48F; magenta: catalytic module of Cel5A.

Thorough inspection of the best-fitting structures suggests an elongated arrangement of the protein, but with a more compact overall form. The cohesins of the scaffoldin are in close proximity indicating that the scaffoldin linker explores a smaller conformational space, although no direct inter-cohesin interaction is observed. The data suggest that the motional freedom of the scaffoldin allows precise positioning of the complexed enzymes according to the topography of the substrate, whereas short-scale motions permitted by

residual flexibility of the enzyme linkers allow “fine-tuning” of individual catalytic domains. An article reporting these results has been published in 2005 (2)

References

(1) Hammel M., Fierobe HP, Czjzek M., Finet S., Receveur-Bréchot V. (2004) Structural Insights into the Mechanism of Formation of Cellulosomes probed by Small Angle X-ray Scattering, *J. Biol. Chem.*, **279,** 55985-55994).

(2) Hammel M., Fierobe H.P., Czjzek M., Kurkal V., Smith J.C., Bayer EA, Finet S., Receveur-Bréchot V. Structural basis of cellulosome efficiency explored by small angle X-ray scattering, J. Biol. Chem., 280: 38562-38568.)