ESRF	**Experiment title:** Structural organization of simple plurimodular nanosomes	**Experiment number**: LS-1725
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Report:

Cellulosomes are multienzymatic assemblies synthesized by anaerobic bacteria hydrolysing crystalline cellulose. They are composed of a scaffolding protein containing several "receptor" modules (cohesins) on which hydrolytic enzymes are anchored (up to 10). Many studies have dealt with the catalytic activity and the modular organisation of *Clostridium cellulolyticum* cellulosomes and showed that these assemblies were highly synergistic compared to the isolated enzymes. 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..

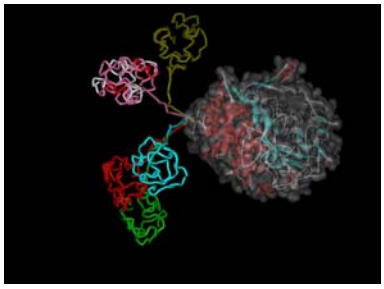
We have therefore studied several nanosomes of different size (composed of 2 to 5 domains) by small angle X-ray scattering on ID02 Beamline by proceeding step by step, with nanosomes more and more complex. We improved our first trials and found the best buffer and ionic strength conditions leading to homogeneous solution without any aggregation, as assessed by the excellent Guinier fits obtained. The very high quality data measured in ID2 allowed us to readily elucidate the structural arrangement of these simple nanosomes and obtained some wonderful results.

We tested several combination of nanosomes using 2 kinds of catalytic domains and cohesins from *Clostridium cellulolyticum* and from *Clostridium thermocellum.* Our best results were obtained on the following samples (from *Clostridium cellulolyticum*) :

-cellulase (catalytic domain) with its dockerin 

We could show that the dockerin when unbound to the cohesin, is folded, while several studies suggested that it might be unfolded when free, and fold upon binding to the cohesin.

Thanks to the program package of D. Svergun (GASBOR, CREDO), we could restore the shape of this protein, and determine the structure of the linker separating the dockerin and the catalytic domain. We could also show the high flexibility of this linker. The fit to the data are extremely good.



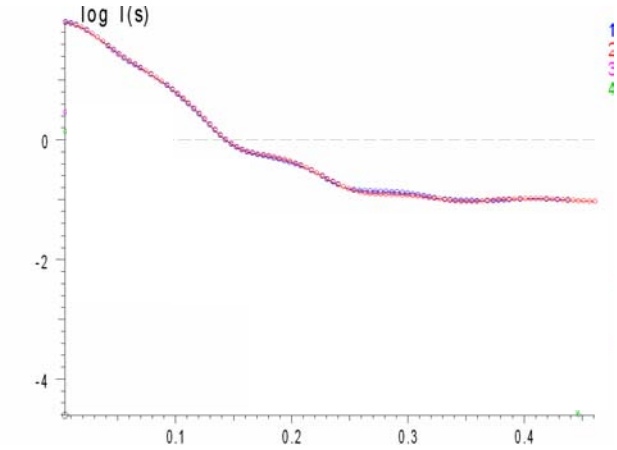


Fig. 1. left : shape and structure of cellulase CelF from *clostridium cellulolyticum* with its dockerin in different conformations as inferred from the SAXS data. Right : experimental SAXS data and corresponding fit by CREDO.

-cellulase (catalytic domain) with its dockerin, bound to a cohesin 

By restoring the shape and conformation of this small nanosome with the same method as previously, we saw that the *linker* between the catalytic domain and the dockerin folds upon binding to the cohesin. This complex is more rigid than the free cellulase-dockerin. The catalytic domains have therefore less degree of liberty than expected when anchored to the scaffoldin (fig. 2).

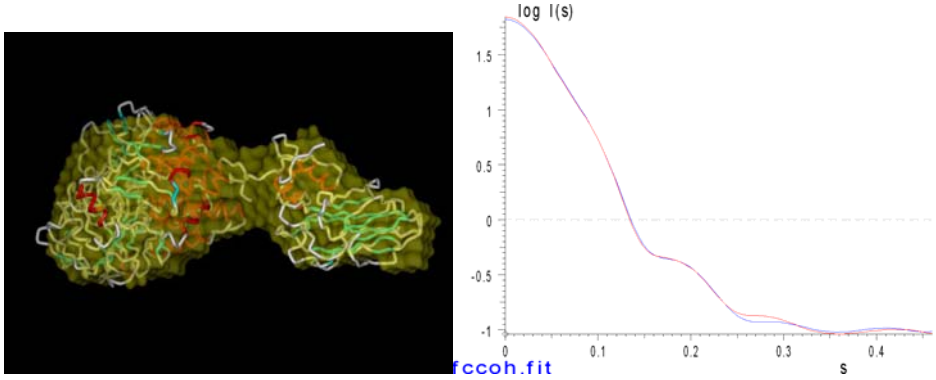
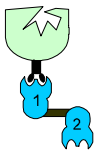


Fig. 2. left : shape and structure of cellulase CelF from *clostridium cellulolyticum* with its dockerin bound to a cohesin as inferred from the SAXS data. Right : experimental SAXS data and corresponding fit by CREDO.



-cellulase (catalytic domain) with its dockerin, bound to a scaffoldin made of 2 cohesins

Using the same methodology, and thanks to the models obtained on smaller complexes, we could infer the spatial arrangement of this more complex nanosome. We saw that the real flexibility of this nanosome arises from the flexible linker between the two cohesins, and not from the linker separating the cellulase and the dockerin.

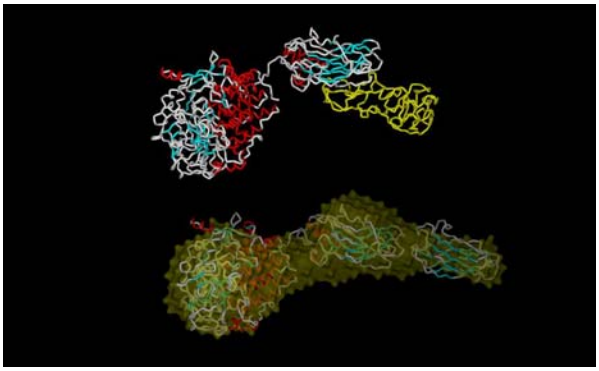


Fig. 2. bottom : shape and structure of cellulase CelF from *clostridium cellulolyticum* with its dockerin bound to a scaffoldin made of two cohesincohesin as inferred from the SAXS data. Top : The cohesin in a different conformation due to the flexibility of the linker separating the two cohesins.

A paper gathering all these results is already in preparation.

These first results are extremely encouraging. We need now, to understand the structural mecanism of the synergy of cellulosomes to characterise the structure of more complex nanosomes, made of several cohesins, on which different cellulases are anchored *via* their respective dockerin, and exhibiting different synergy between each other.