	Experiment title: Structural organization of plurimodular nanosomes	**Experiment number**: LS-1725
Beamline: ID02	**Date of experiment**: from: 17/11/2000 to: 20/11/2000	**Date of report**: 19/08/2002
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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 in order to elucidate . Unfortunately, their modularity, their size, their heterogeneity and their intrinsic flexibility prevent from obtaining a structural characterisation by means of structural techniques such as NMR and crystallography. However the three-dimensional structure of a number of isolated domains have been determined by recent studies. It is now possible to construct mini-scaffoldins with a restricted number of cohesin modules, the so-called nanosomes.

We have therefore studied several nanosomes of different size (composed of 2 to 5 domains) by small angle X-ray scattering on ID02 Beamline. During the experiment, we have encountered several difficulties. In particular, the smallest nanosomes presented some aggregation probably due to exposed surfaces normally buried in an interdomain interface and their scattering curves could not be exploited. Several buffers with different salt conditions were tested, without success. To solve this problem, we shall test a wide variety of buffers conditions with or without addition of detergents. Unfortunately, only SAXS experiments could tell us if the aggregation has been completely removed, as SAXS is the most sensitive technique to detect this kind of aggregation.

However data obtained on a double cellulase construct have been extremely prolific. The variant CBA6H1 is a fusion protein composed of two catalytic domain separated by a long linker (80 amino acids). Experiment at ESRF allowed us to determine a high quality scattering curve. The distance distribution function P(r) inferred from the scattering pattern (fig.1.) has revealed that the the solution contains a mixture of proteins in very different conformations and that the linker exhibits a very high flexibility. This very important result shows for the first time how flexible a cellulase linker is, and gives the first values of the amplitudes it can attain. A simulation of the scattering pattern is underway in order to determine the rough proportion of each intermediate and extreme conformations existing in solution.

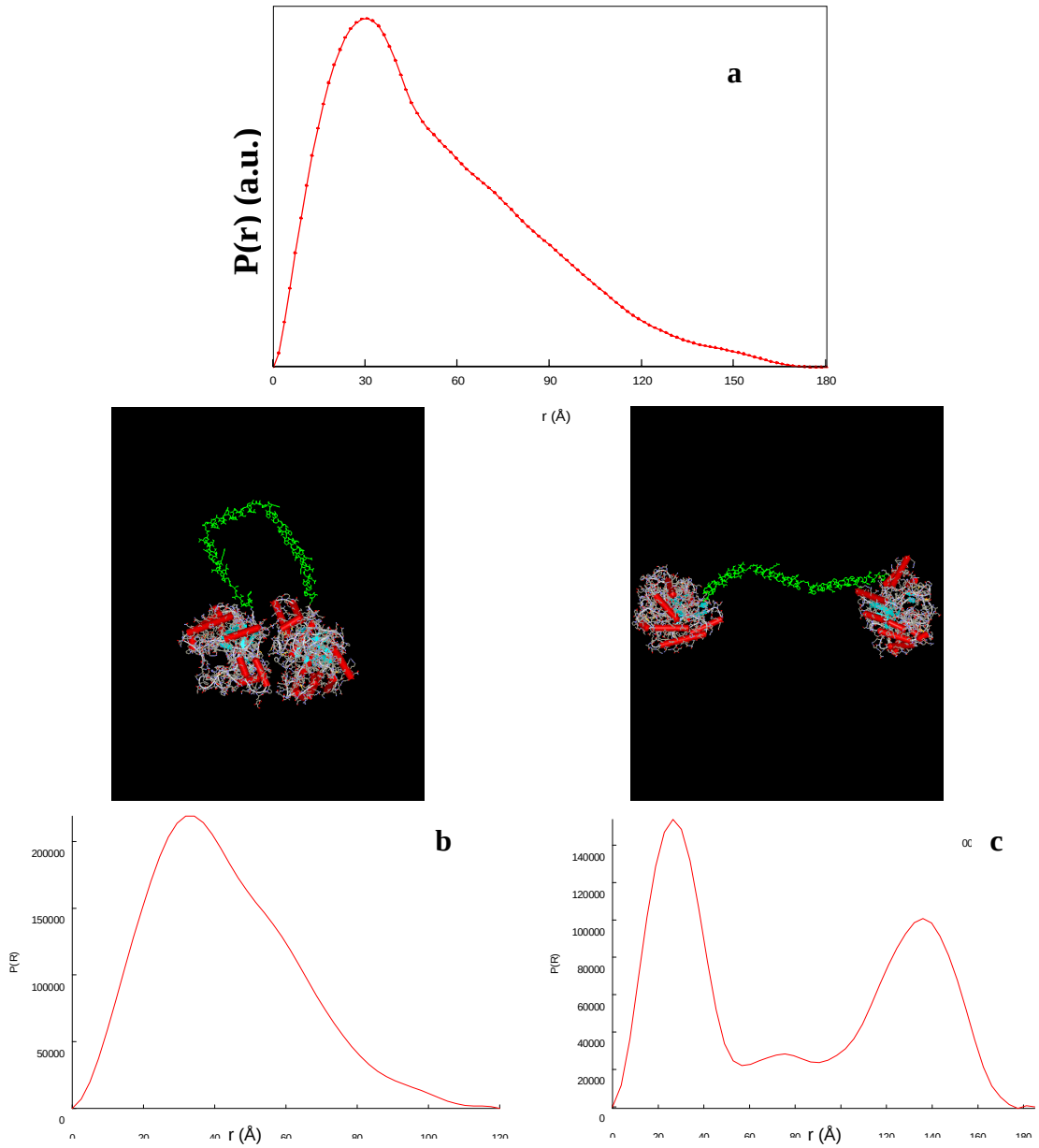

Figure 1. Distance distribution function of CBA6H1 variant(a) inferred from the experimental scattering curve and Distance distribution functions calculated for the models of CBA6H1 in a conformation where the two catalytic core lay at a minimal distance (b), and at a maximal distance of 90Å (c), necessary to interpret the experimental D_{max} obtained for CBA6H1. The experimental scattering curve arises from a mixture of conformations between these two extreme conformations in different proportions.