



	Experiment title: Structural features of the intracellular domains of the CFTR by small angle x ray scattering	Experiment number: MX- 991
Beamline: ID14 3	Date of experiment: from: 02-Oct-2009 to: 05-Oct-2009	Date of report: 10-Feb-2010
Shifts: 9	Local contact(s):	<i>Received at ESRF:</i>
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Report:

Nucleotide binding domains (NBD1 and NBD2) of the cystic fibrosis transmembrane conductance regulator (CFTR), the defective protein in cystic fibrosis, are responsible for controlling the gating of the chloride channel, and are the putative binding site for several candidate drugs for the treatment of the disease. We have studied the structural properties of recombinant NBD1, NBD2 and an equimolar NBD1/NBD2 mixture in solution by small-angle X-ray scattering. Structural parameters obtained from the SAXS experiments are shown in Table 1. We demonstrated that the sole NBD1 or NBD2 have an overall structure similar to that observed for NBD1 crystals. Application of 2 mM ATP induces a dimerisation of the NBD1, but does not modify the NBD2 monomeric conformation. The NBD1/NBD2 mixture spontaneously forms dimers, that becomes tighter in the presence of ATP. To our knowledge, this is the first direct observation of a conformational change of the NBD1-NBD2 interaction by ATP. Additional experiments indicate that application of CFTR potentiator, at concentration that strongly activate the CFTR mediated transport, seems to destabilize the NBDs polypeptides.

Table 1. Structural parameters of the CFTR nucleotide binding domains in solution, obtained from SAXS experiments. MM and MM_{exp} are the molecular mass calculated from the amino acid sequence and from the experimental data, respectively. MM_{exp} and R_g , the gyration radius, were calculated from the Guinier plot. The maximum length of the particle D_{max} was estimated from the distance distribution function.

	NBD1		NBD2		NBD1 + NBD2	
	control	2 mM ATP	control	2 mM ATP	control	2 mM ATP
MM (KDa)	32.2		33.6		64.7	
MM_{exp} (KDa)	34.3	75.41	35.1	34.24	64.52	69.5
R_g (nm)	1.77	2.06	1.79	1.97	2.79	2.11
D_{max} (nm)	4.89	5.51	5.02	5.29	7.35	7.35

Low resolution molecular models of the NBD-polypeptides, in the absence and in the presence of ATP, were obtained by *ab initio* reconstruction from SAXS data. Both NBD1 and NBD2 in solution show a globular conformation that is equivalent to that reported for the NBD1 structure by crystallography. *Ab initio* reconstruction of the shape results to an envelope that can host the crystallographic structure of NBD1, or the equivalent homology model of NBD2 (Fig 1A). In the presence of 2 mM ATP, NBD1 undergoes to a tight dimerisation (Fig 1B), in a conformation very similar to the head-to-tail crystallographic conformation reported for this domain. Differently, there are not evidences of a dimerisation of NBD2 in 2 mM ATP (Fig. 1B).

The NBD1–NBD2 equimolar mixture in solution reveals a conformation that cannot be ascribed as the superposition of the single NBDs. The *ab initio* reconstruction of the NBD1/NBD2 dimer shows a globular structure, with two

divergent "wings" (Fig. 1C). The NBD1/NBD2 dimer becomes tighter in the presence of 2 mM ATP, and the medium resolution model obtained from the SAXS data is compatible with the expected head-to-tail conformation (Fig. 1C).

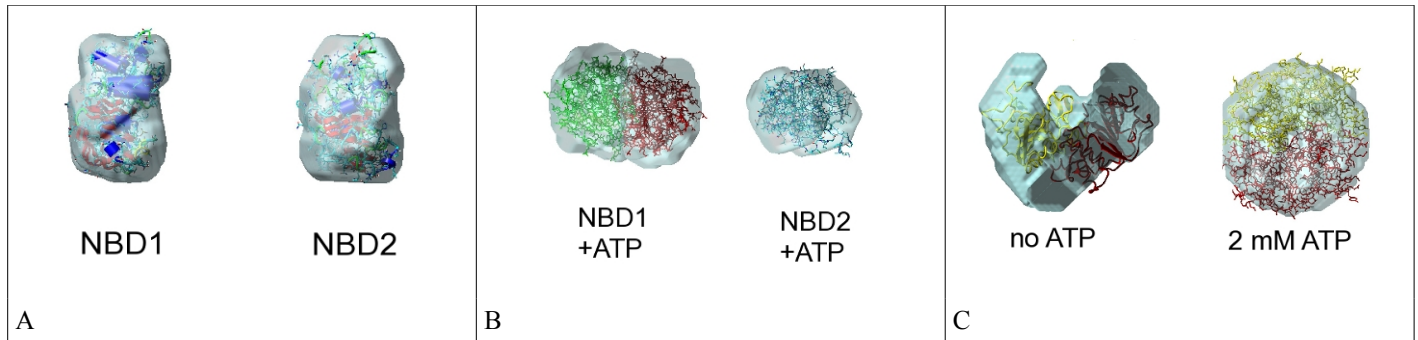


Figure 2. Ab initio rigid model of the CFTR NBD1 NBD2 and NBD1/NBD2 equimolar mixture in solution, without ATP or in presence of 2 mM ATP, as indicated.

These data may be useful to understand the physiopathology and pharmacology of the cystic fibrosis.

We thank the ESRF for provision of synchrotron radiation facilities and we would like to thank Petra Pernod for assistance in using beamline ID14-EH3. This project was partially supported by the Fondazione per la Ricerca sulla Fibrosi Cistica (grant #2/2008), Mille bambini a Via Margutta - onlus, Blunotte and Lega Italiana FC - Associazione Toscana Onlus.