	Experiment title: ***Crystal structure of the integrin beta4-plectin primary complex***	**Experiment number**: MX 565
Beamline: ID23.1	**Date of experiment**: from: July 17th 2006 to: July 18th 2006	**Date of report**:
Shifts: 2	**Local contact(s)**: Dr Stéphanie MONACO	*Received at ESRF:*

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Report:

Experiment objective.

The aim of this experiment was the collection of high resolution data from crystals of the complex formed between the first pair of fibronectin type III (FnIII) domains of integrin β4 and the actin binding domain (ABD) of plectin. These data would allow the solution and refinement of the complex structure. Previous measurements of these crystals using an in house rotating anode system were limited to ca. 3.8 Å resolution.

Data collection

The data collected during the experiment included 6 datasets of the integrin β4-plectin native complex, each collected from a single crystal. In addition 2 datasets were measured from crystals of the complex in which the plectin ABD protein was labeled with the fluorophor probe 1,5-I-AEDANS. Data from the crystals measured extend typically to a maximal resolution of around 3 Å. The usable resolution of each dataset was only fully assessed during detailed analysis of the images after the measurement time, hence the collection of multiple datasets. The best quality dataset measured of the integrin β4-plectin complex extends to 2.7 Å resolution (Table 1), while the best quality dataset of the fluorescence labeled complex reach 3.1 Å (Table 1). Based on the previously available low resolution data the space group initially identified as $P3_2$, yet the current data allowed the identification of the actual space group as $P3_221$.

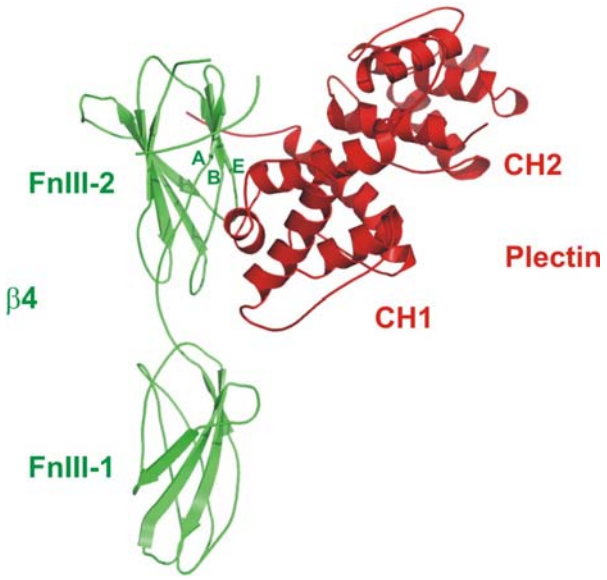
Table 1. Data collection statistics of integrin β4-plectin complex crystals.		
Dataset	integrin β4-plectin complex	integrin β4-plectin(AEDANS) complex
Space group	$P3_221$	$P3_221$
Unit cell	a=b=107.3 Å c=204.0 Å α=β=90º γ=120º	a=b=107.1 Å c=203.1 Å α=β=90º γ=120º
Wavelength (Å)	1.0723	1.0723
Resolution (Å)	102 - 2.7 (2.85 - 2.7)[a]	93 - 3.1 (3.27 - 3.1)[a]
Unique reflections	37987	24569
Multiplicity	9.6 (9.7)[a]	10.3 (10.6)[a]
Completeness (%)	99.8 (100)[a]	98.1 (97.9)[a]
Rmeas (%)	8.4 (70.3)[a]	12.3 (69.0)[a]
<<I>/<σI>>	19.4 (4.0)[a]	18.5 (3.1)[a]
[a] data in parenthesis correspond to the higher resolution shell.		

Structure solution

The structure of the integrin β4-plectin complex has been solved by using molecular replacement methods as implemented in the program Phaser [1]. The structures of the first pair of FnIII domains of β4 [2] (PDB code 1QG3) and the ABD of plectin [3] (PDB code 1MB8), previously solved by our group, were used as search models. Initially two copies of the plectin ABD and two copies of the integrin β4 fragment were located; no other copies of the ABD were found in the molecular replacement search. Visual inspection of $2f_{obs}$-f_{cal} and f_{obs}-f_{calc} sigmaA maps showed clear density for an additional copy of the β4 molecule which was located by an additional search with Phaser. Thus the asymmetric unit contains two copies of the integrin b4-plectin complex plus an unligated copy of the β4 molecule, corresponding to a solvent content of 57%. At this moment the model is still under refinement, being the current Rwork 23.6% and Rfree 28.5%. Once that this structure will be completely refined it will be used as starting point for the solution and refinement of the structure of the AEDANS labeled complex.

The structure reveals the details of the macromolecular recognition between integrin β4 and plectin (fig 1). The binding interface in β4 is located in the second FnIII domain and involves the A-B-E β-sheet; additional contacts are provided by the sequence downstream the second FnIII domain. In plectin the interaction occurs via the first calponin homology (CH) domain that forms the ABD.

Figure 1. Structure of the integrin β4-plectin complex. Ribbon representation of the first pair of FnIII domains of integrin β4 (green) bound to the ABD of plectin (red). The interaction occurs primarily between the second FnIII domain of β4 and the first CH domain of plectin.



Additional measurements.

Crystals of a 27 kD plectin fragment corresponding to the region of the plakin domain extending downstream the ABD were also measured during this beamtime allocation. A native dataset reaching 2.2 Å resolution was collected (table 2). Sequence analysis suggests that this region is build up of two spectrin repeats. Yet it has not been possible to solve the structure using molecular replacement methods, most likely due to two circumstances: the sequence identity with spectrin repeats of know structure is less than 19%, in addition the relative orientation of adjacent spectrin repeats in the currently available structures of tandem pairs is highly variable. Thus the solution of this structure will require the experimental determination of phases (ie. by MAD methods) and will be the subject of a future beam time application.

Table 2. Data collection statistics of plakin domain crystals

Space group	$P2_12_12$
Unit cell	a=155.0 Å b=26.5 Å c=58.2 Å $\alpha=\beta=\gamma=90^o$
Wavelength (Å)	1.0723
Resolution (Å)	78 - 2.2 (2.35 - 2.2)[a]
Unique reflections	11870
Multiplicity	5.4 (4.0) [a]
Completeness (%)	91.6 (53.7) [a]
Rmeas (%)	5.3 (27.5) [a]
$<<I>/<\sigma I>>$	23.2 (6.1) [a]

[a] data in parenthesis corresponds to the higher resolution shell.

Summary.

The experiments carried out during this beamtime have been highly successful. The main objective, the collection of high resolution data of crystals of the integrin β4-plectin complex, has been achieved and has allowed the solution and refinement of the structure. In addition a 3.1 Å dataset of a fluorophor derivatized form of the complex was collected. Finally, native data was collected from crystals of another region of plectin involved in integrin β4 binding, paving the way for the future structure solution by experimental phasing methods.

References.

[1] McCoy, AJ, Grosse-Kunstleve, RW, Storoni, LC and Read RJ (2005) *Acta Cryst.* D61, 458-464.
[2] de Pereda JM, Wiche G, and Liddington RC. (1999) *EMBO J.* 18(15):4087-95.
[3] Garcia-Alvarez B, Bobkov A, Sonnenberg A, de Pereda JM. (2003) *Structure* 11(6):615-25.