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BAG Beam time Progress Report

This represents a summary of the BAG progress in the reporting period, and is **in addition to** the standard ESRF report sheet for each project which will be used for the Review of the BAG.

BAG title: Block allocation: Structural Biology Programme EMBL, Heidelberg (LS-1662)

Allocation Period: February 2000 – July 2000

List of publications resulting from ESRF beam time

- Montoya G, Kaat K, Moll R, Schafer G, Sinning I. (2000). The crystal structure of the conserved GTPase of SRP54 from the archaeon Acidianus ambivalens and its comparison with related structures. Structure Fold Des. 2000 May 15;8(5):515-25.
- Scheffzek, K., Stephan, I., Jensen, O.N., Illenberger, D., Gierschik, P. (2000). The Rac-RhoGDI complex and the structural basis for the regulation of Rho proteins by RhoGDI. Nature Struct. Biol. 7, 122-126.
- Djinovic-Carugo, K., Young, P., Gautel, M. and Saraste, M. (1999) Structure of the alpha-actinin rod: Molecular basis for crosslinking of actin filaments. Cell 98, 537-546.
- Raaijmakers, H., Vix, O., Törö, I., Golz, S., Kemper, B. & Suck, D. (1999). Crystal structure of T4 endonuclease VII - a DNA junction resolvase with a novel fold and unusual domain-swapped dimer architecture. EMBO J. 18, 1447-1458.
- Baraldi, E., Djinovic Carugo, K., Hyvönen, M., Lo Surdo, P., Riley, A.M., Potter, B.V.L., O'Brien, R., Ladbury, J.E. and Saraste, M. (1999) Structure of the PH domain from Bruton's tyrosine kinase in complex with inositol-(1,3,4,5)-tetrakisphosphate. Structure, 7, 449-460.
- Suck, D., Buchholz, F., Dreher, M., Stewart, F. & Meyer, J.E.W. (1999). Crystal structure of a synaptic Cre recombinase – loxP complex. Biochemie 81, s279.

Global Summary

During the allocation period we have collected various data sets that brought the respective projects closer to completion (α-actinin, TAP, SRP mutants, SAND domain). Others gave important clues for further experimental strategies which we are pursuing (SopE, esportin, SAND-domain-DNA complex).

1. Protein Name α-actinin (Saraste, Scheffzek, Ylänne)

• α-Actinin fragment: Data to 3 Å resolution, Rsym 4% (11%), completeness 98% (98%); I/σ 11.8 (2.6). (BM30, 5 shifts). Refinement is in progress.

2. Protein Name cbb3 cytochrome oxidase (Saraste, Scheffzek, Urbani)

• Tested cryoconditions. Problems with the 700 Å cell axis.

3. Protein Name SRP signal recognition particle (Sinning, Montoya, Groves, Rosendal, teKaat)

• T331A mutant of FtsY from *E. coli.* Data to1.7 resolution, completeness 89%, Rsym 8.4%
The structure was solved by molecular replacement, refinement in progress, current Rcryst=23.6%.
• Full length FtsY from *A. ambivalens,* an Archaebacterium. Data to 3.0 Å resolution, completeness 99.7%, Rsym: 6.1%.
The structure could be solved using the NG-domain from FtsY from *E. coli* as a search model, building of the extra, N-terminal domain is in progress.
• FtsY/-46 construct from *E. coli.* Data from ID13 at 3 Å resolution, completeness 96%, Rsym 11%.
This construct of the receptor is very interesting since the GTPase is not activated upon membrane interaction (in contrary to the full length protein), but it's the shortest form of the receptor that is still able to complement an FtsY deficient strain. The structure was solved by molecular replacement with AMoRe using the FtsY NG-domain as a model.
• Diffraction tests were performed on a number of small crystals, these were: full length Ffh from *A. ambivalens,* bc1 complex from *R. sulf.*, human SRP19/RNA complex and human SRb/SRa complex, cpSRP43. None of them diffracted.

4. Protein Name TAP (Conti, Liker)

• SeMet MAD experiment at ID14-4 (February 2000).
Data at the peak (0.9789 Å) to 3.5 Å, Rsym 10.7 (31.2), completeness 99..8 (98.9), redundancy 6.4.
Data at the inflection (0.9793 Å) to 3.5Å, Rsym 10.7 (34.9), completeness 99.8 (99.9), redundancy 6.0
Data at the high-energy remote (0.9393 Å) to 3.15Å, Rsym 9.9 (28.0), completeness 99.6 (99.9), redundancy 6.4.
Despite the relatively poor accuracy of the data, the low resolution and the small signal (12 ordered methionines in the 120 kDa in the asymmetric unit), the Se sites could be found with the program SOLVE.
The structure is currently refined to Rfree of 30.3 at 3.15 Å resolution.

5. Protein Name Exportin (Conti, Fernandez)

new project – needle-like crystals obtained after submission of the LS1662 proposal.
• Tested needle crystals at ID14 and ID13. No diffraction.

5. Protein Name SopE (Scheffzek, Buchwald)

new project – crystals obtained after submission of the LS1662 proposal.
• Data set to 3.5 Å resolution, completeness 98% (98%), Rsym 3.2 (12%), I/σ 13.5 (4.5) at BM14.
• Derivative testing, no real progress made, focus on other crystal form(s) -> ID13

7. Protein Name SAND (Saraste, Scheffzek, Lo Surdo)

new project – crystals obtained after submission of the LS1662 proposal.
• Data set to 1.55 Å resolution, completeness 89% (100%), Rsym 4.7% (18%), I/σ 29 (7) at BM14.
• Data set to 1.2 Å resolution, completeness 99% (99%), Rsym 12% (32%), I/σ 7.8 (2.9) at ID14-2.
• SAND-DNA complex. Diffraction to 5 Å at most at ID14-2. Testing of cryoconditions, but no breakthrough. Broad improvement required.