ESRF	**Experiment title:** Structure determination of acetylcholine binding protein	**Experiment number:** LS1351/LS1495
Beamline: ID14-4	Date of experiment: ID14-4 27-2-99 – 1-3-99 ; 19-4-1999 - 21-4-1999; from: 7.00 am to: 7.00 am	**Date of report:** 24-aug-1999
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Report:
The time mentioned above has been shared with projects LS1351(=LS1495)/LS1494(=LS1348)/LS1492/LS1491/

Acetylcholine binding protein is a protein cloned from the central nervous system of the mollusc *Lymnea stagnalis*. This protein binds acetylcholine and appears to be involved in a unique acetylcholine scavenging system of the molluscs. In a collaboration with Guus Smit at the Free University of Amsterdam we are studying the acetylcholine binding properties and biological function of this interesting new class of proteins.
The protein is expressed and purified from *Pichia pastoris*. We have crystallized it in a tetragonal and an orthorhombic spacegroup. Native data have been collected to 2.8 A on beam line X11 in Hamburg. We plan to solve the structure by MIR, and because it is a homo-oligomer, by averaging.

The following data sets have been collected on Abp:

	cell parameters	mosaicity	complete ness	Rmerge	Rmerge last shell		Resol
$P4_22_12$							
nati	141.4 141.4 120.161	0.487	99.6	0.110	0.753		3.5
ertl	143.6 143.6 120.897		99.9	0.120	0.784		4.0
ajd	144.0 144.0 121.207	0.483	99.2	0.096	0.848		3.3
ghb	142.0 142.0 121.117	0.71	85.8	0.113	0.489		
hdo	143.5 143.5 120.854	0.886	99.9	0.130	0.688		
lifb	143.1 143.1 120.812	1.221	98.6	0.090	0.697		
lifa	143.5 143.5 120.939	0.949	98.9	0.144	0.000		4.0
pb	142.1 142.1 120.260	0.528	99.7	0.114	0.927		
lig	143.9 143.9 121.569	0.813	86.6	0.137	0.806		3.80
pt	140.5 140.5 119.878	0.892	95.9	0.098	0.421		3.3
$P2_12_12_1$							
hg1	160.2 136.4 120.9	0.908	98.8	13.2	55.9	2.0	3.9
pt1	161.5 133.5 120.7	0.628	96.6	11.7	49.5	2.2	4.1
nati	161.4 133.5 120.5	0.446	99.3	10.5	49.7	2.5	3.9
lu2	160.3 134.4 121.1	1.248	93.8	11.6	47.7	2.4	3.7
lu3	161.1 133.6 120.6	0.772	98.0	10.3	53.0	2.1	3.6
pb2	160.3 135.9 120.5	0.841	98.1	12.4	65.2	2.1	3.9

Many data sets have been collected on the ABP project, both native and potential derivatives. The tetragonal data suffer from considerable non-isomorphism, as was obvious from the difference with the original 2.8 A. native data set in this space group.

In the tetragonal spacegroup a set of potential sites was identified in a gadolinium data set, but due to a pseudocentrosymmetric arrangement of the molecules these could not be refined and/or used.

An initiative was started to exploit a second, related crystal form, with space group $P2_12_12_1$, but no successful derivatives were identified in these crystals yet. The non-isomorphism between these crystals however seems to be limited to the changes in cell-parameters observed. This gives us some hope of finding a suitable pair of native and derivative crystals, which are isomorphous to eachother. Since we cannot make selenomethionine protein (too few methionines, as well as problems synthesizing on a minimal medium using the *Pichia pastoris* strain) we need to identify an isomorphous derivative. In addition we plan to use the best derivatives from the tetragonal experiments in a MAD experiment using the orthorhombic crystals.