ESRF	**Experiment title:** Human LDL subfractions	**Experiment number**: LS-1282
Beamline: ID14-3	**Date of experiment**: from: 14.4.99 to: 15.4.99	**Date of report**: 7.6.99
Shifts: 3	**Local contact(s)**: Wim Burmeister	*Received at ESRF:*

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Report:

LDL particles play a major role in the development of coronary heart disease. A certain subclass of LDL particles, (small, dense LDL) has been identified as a major determinant of the severity and progression of atherosclerosis in humans [1]. Solving the structure of LDL, respectively its protein component apoB, is of basic relevance as it represents a novel kind of macromolecular assembly, for which no high resolution structures are known. In addition, the analysis of the three-dimensional structure of apoB is an important approach to explain the mechanisms involved in the development of atherosclerosis on a molecular basis. Of special interest would be to compare the structure of apoB in LDL particles from LDL subfractions of different atherogenic potential [1].

Currently, we are able to crystallize four of six subfractions of LDL-particles prepared from different human donors. Best crystals are obtained from LDL-2 (d=1.031-1.034 mg/ml) and LDL-3 (d=1.034-1.037 mg/ml). We observe at least 3 morphologically different crystal forms, that grow to sizes of up to 1500x250x100µm³.

At ID-14-3 reflections can be measured down to 500Å, due to the low divergence of the beam, and a special setup of the beamstop build by Wim Burmeister based on a prototype developed by us [2] . Low order Fourier coefficients are very important for the calculation of low resolution structures, especially if ab inito methods [3] are used for phasing. Primary aim of our experiment was therefore to collect complete datasets, with all inner reflections resolved. Six such datasets (4 native, 2 derivative) were collected at a detector distance of 520 mm under cryogenic conditions. For each dataset 2 passes were necessary (10s, $\Delta\varphi=1°$ and 0.5s $\Delta\varphi=2°$) to collect the inner reflections with a few overloaded pixels only.

All crystals (LDL-2, LDL-3, native and labeled with Au) diffracted to a resolution of 29Å, and up to 15Å in one direction. The diffraction data could be autoindexed and integrated by XDS. The Bravais lattice of the observed diffraction pattern indicates space group C2 with unit cell dimensions of a = 180, b = 415, c = 379Å, $\alpha = \gamma = 90°$, $\beta \approx 90°$. Measured diffraction intensities at the current resolution are also consistent with $C222_1$, producing similar merging statistics as data reduction in C2. Packing considerations [2] show, that in spacegroup $C222_1$ the LDL particles are located at a special position in the unit cell, implying an approximate internal 2-fold symmetry of the particle. Evaluation of the derivative datasets confirmed an measurable anomalous signal due to the presence of the gold label. This suggests that phasing by MAD could be a successful strategy to get experimental phases even in the case of these low resolution datasets.

Further efforts will focus on the evaluation of the collected low resolution datasets, a major point being the assignment of phases by ab initio methods [3]. The next experiments will focus on experimental methods to collect phase information (MAD, MASC [4]), and the improvement of resolution by various strategies.

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