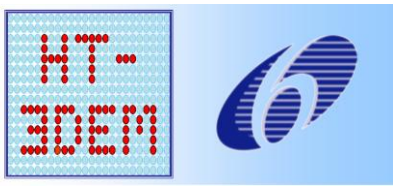

Project no. LSHG-CT-2005-018811

SPECIFIC TARGETED RESEARCH PROJECT

HIGH THROUGHPUT THREE DIMENSIONAL ELECTRON MICROSCOPY

PUBLISHABLE FINAL ACTIVITY REPORT

Period covered: from 01/10/2005 to 30/09/2008

Start date of project: 01/10/2005 Duration: 48 Months

Date of preparation: October 31, 2008

Project coordinator name: Prof. Andreas Engel
Project coordinator organisation: University of Basel, Maurice E. Mueller Institute, Biozentrum, Basel (CH)

SPECIFIC TARGETED RESEARCH PROJECT
HIGH THROUGHPUT THREE DIMENSIONAL ELECTRON MICROSCOPY
HT-3DEM, LSHG-CT-2005-018811

FINAL PUBLISHABLE SUMMARY

The general aim of HT3DEM was to enhance the European leadership in three-dimensional electron microscopy (3D EM). Specifically, HT-3DEM developed an automated platform to enable high throughput screening and analysis of native protein complexes and two-dimensional (2D) membrane protein crystals by EM. For this purpose, HT-3DEM brought together European experts in EM, 2D crystallisation, machine vision and robotics with leading manufacturers of electron microscopes and micro systems technologies. The innovative technology platform includes (i) the preparation of 2D membrane protein crystals, (ii) automated EM-sample grid preparation and transfer to the EM, (iii) automated image acquisition and sample analysis by EM, and (iv) management of all data produced in the pipeline.

Each of these modules represents a substantial advance of the state of the art, and greatly enhances the throughput in 3D EM. With the successful realisation of the high throughput chain, the reduction of processing time and cost for individual samples opens new opportunities of applications, among others, in health care (screening of patient materials for infective agents) and pharmaceutical industry (acceleration of rational drug design, improving drug formulations).

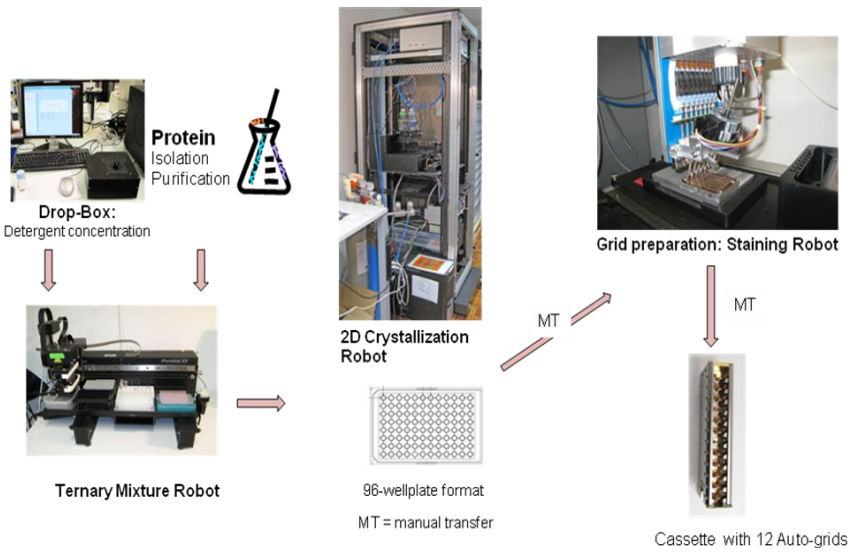

Figure 1: Schematic overview: Sample Preparation, 2DX-robot, Staining robot, Grid holder

After extensive testing and crystallisation of several membrane proteins the 2DX robot has reached high operational reliability and robustness over days. Purified protein is mixed in a 96-wellplate with the desired variation of buffers, salts, lipids, and detergents on the easily programmable ternary mixture robot. The detergent concentration is measured in the in-house developed so called Drop-Box [1]. The crystallisation method used with the present 2D crystallisation robot is based on the neutralisation of detergent by the controlled addition of cyclodextrins [2]. Features like temperature control of the sample plate, light scattering device for monitoring sample aggregation in the wells, liquid level control and a user-friendly graphic interface allow maximum degrees of variability in the crystallisation process.

With the gantry robot equipped with eight dispensers [3] combined with an automatic blotting system, the high-throughput preparation of negatively stained samples is reliably achieved. This high-quality staining leaves reproducibly up to 95% of the carbon-coated grid surfaces undamaged.

To screen the large amounts of grids, the room-temperature version of the autoloader developed by FEI Company for the Titan KRIOS was adapted and fitted on a Tecnai 12 electron microscope. A cassette revolver accommodating eight standard twelve grid-autoloader cartridges designed by FEI and fabricated at the Biozentrum, University of Basel is mounted onto the autoloader. This combination allows the automatic loading of 96 grids to the Tecnai 12 and their processing without breaking the vacuum. The demonstration Tecnai 12/autoloader system is operated at the Centre of Cellular Imaging and Nanoanalytics (C-CINA) at the Biozentrum, University of Basel, Basel (Switzerland). The software for the EM, the autoloader, the cassette revolver operation and the picture acquisition has been adapted in collaboration between Partners BIOZ, MIPS and FEI.

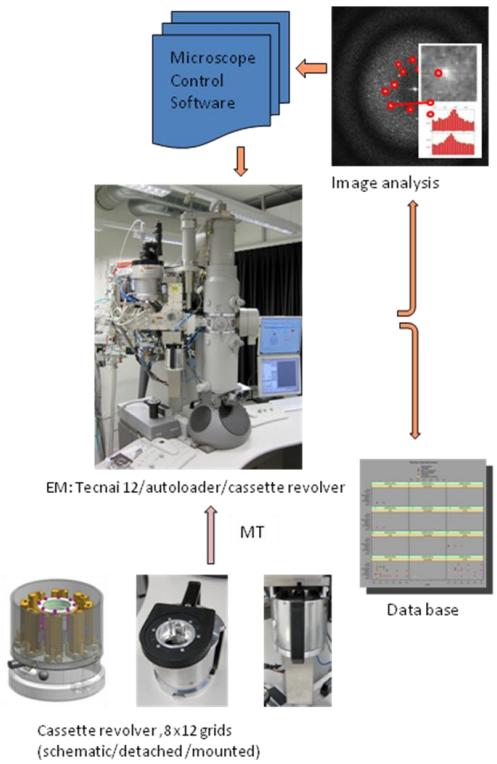

Figure 2: Overview of automatic screening of 96 grids.

Image processing tools developed at the University of Haute Alsace, Mulhouse analyse the EM-pictures at (i) low magnification for area identification, (ii) at medium magnification for particle/crystal localisation and (iii) at high magnification for crystal analysis. Practical testing and acquisition of large image series allow the continuous optimisation of the image processing tools. The alpha-version of the image processing software has been integrated into the TOM toolbox used for microscope control, and successfully tested at the Institute of Biochemistry in Martinsried, Germany. Further adaptations to both the imaging software and the TOM toolbox [4] are needed to offer an alternative to the present software for instrument control and the integrated image analysis software.

Based on extensive tests a redesigned beta-version of the database has been completed. Users can design crystallisation experiments, assign a unique identifier to each sample, upload a crystallisation program in the 2DX robot and link EM data (magnification, micrographs etc) to their crystallization screens. Search and statistical analysis engines are being developed with the growing collection of representative data sets.

After four years of intensive development within the frame of the FP6 LSHG-CT-2005-011881 project we have achieved a fully operational high throughput chain for the 2D-crystallisation of membrane proteins. Extensive applications are under way and undoubtedly will lead to further improvements. However, with the first and most important step demonstrating not only the rationalised high throughput crystallisation process but more importantly also the adequate sample preparation and image analysis process, done, the way has been opened for the development of further applications in health care (e.g. screening of patient materials for infective agents) and pharmaceutical industry (e.g. acceleration of rational drug design, improving drug formulations).

[1] Kaufmann TC, Engel A, Rémigy HW. A novel method for detergent concentration determination. *Biophys J.* 90; 310-7 (2006)

[2] Signorell GA, Kaufmann TC, Kukulski W, Engel A, Remigy HW, Controlled 2D crystallization of membrane proteins using methyl-beta-cyclodextrin. *J Struct Biol* 2007, 157:321-32

[3] Seyonic SA, CH-2000 Neuchâtel Switzerland. http://www.seyonic.com/

[4] http://www.biochem.mpg.de/baumeister/tom_e/index.html /