

SEMINAIRE

*Auditorium Fernand Gallais (bât. LCC)
Campus CNRS, 205 route de Narbonne TOULOUSE*

Jeudi 18 septembre 2008 à 11 h.

Dr. Frank Bernhard - Goethe-University Frankfurt
" Cell-free expression strategies for the high-level production of membrane proteins "

Cell-free expression techniques have emerged in recent times as powerful tools for the fast and efficient production of membrane proteins. Most central problems associated with conventional cellular expression systems are eliminated and the direct synthesis into defined artificial hydrophobic environments like detergents or liposomes enables completely new strategies for membrane protein production. In addition, reaction protocols of cell-free expression systems can be individualized according to the specific requirements of particular target proteins. This exceptional versatility guarantees high success rates for the production of even complex membrane proteins.

We have established throughput strategies for the optimization of cell-free reaction protocols for the expression of membrane proteins as integrated processes with robotic platforms. Different reaction modes have been developed for the optimized production of functionally folded membrane proteins. The efficiencies of our techniques are demonstrated by the expression of representative sets of > 100 membrane proteins involved in transport, efflux, signalling, metabolism or biosynthesis in mg amounts in a single ml of cell-free reaction mixtures. The quality of selected membrane proteins including eukaryotic solute carriers, G-protein coupled receptors and diverse transporters is evaluated by a number of complementary techniques comprising functional assays as well as structural and biophysical approaches. We demonstrate the preparative scale production of pharmaceutical important targets such as G-protein coupled receptors or Alzheimer's disease related proteins in high quality in less than 24 hours and we present new strategies for their specific labelling and their functional as well as structural evaluation in particular by NMR spectroscopy.

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