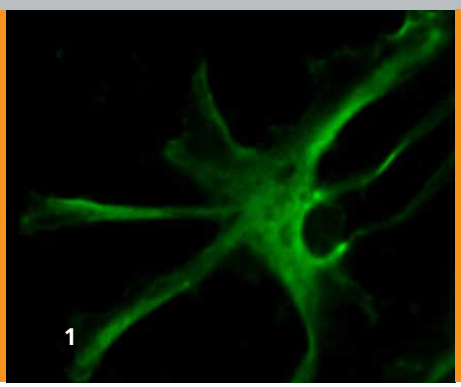
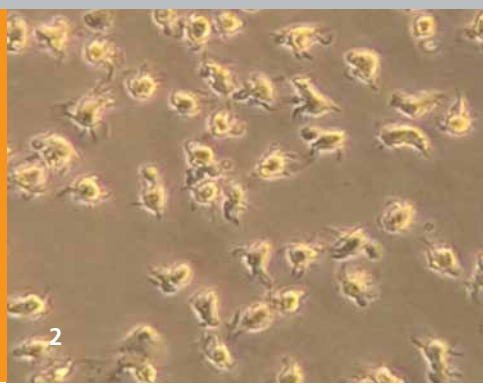
Fraunhofer
IME

FRAUNHOFER INSTITUTE FOR MOLECULAR BIOLOGY AND APPLIED ECOLOGY IME





1 *Oligodendrocytes © Fraunhofer IME / Marina Henke.*

2 *Dendritic cells © Fraunhofer IME / Thomas Ulshöfer.*

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ASSAYS FOR COMPOUND SCREENING AND CHARACTERIZATION

A wide variety of compound screening assays are available within the Fraunhofer IME, branch for Translational Medicine and Pharmacology. Emphasis is laid on cellular signaling processes, lipid metabolism, neuronal cell responses and immune cell activity. State-of-the-art immunological, cellular, genomic, proteomic and biochemical assays as well as modern techniques for metabolome analysis are employed.

Cell culture techniques

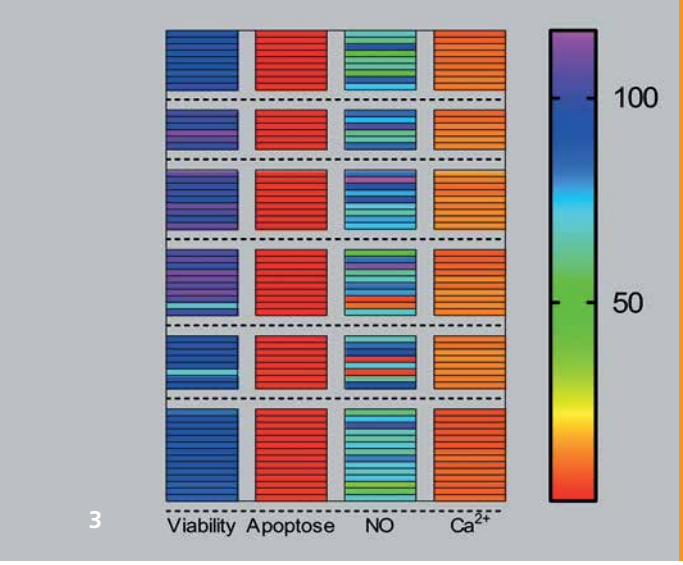
- Generation of plasmids and lenti virus expressing specific target genes
- Transient and stable transfection/ transduction of plasmids/lenti virus
- Apoptosis assay, proliferation assay, migration assay, adhesion assay, cytotoxicity assay, Ca^{2+} imaging, wound healing assay, metabolic function assay, cell cycle assay, cell barrier assay

Available cell types

- Fibroblast cell line, endothelial cell line, epithelial cell line (e.g. cell barrier assay)
- Cancer cell line, macrophage cell line, embryonal kidney cell line (e.g. apoptosis assay)
- Primary microglia, astroglia, oligodendrocytes, neurons (e.g. cytotoxicity assay, functional assays)
- Primary immune cells (e.g. immune cell function tests)

Imaging methods

- Imaging of proteins and mRNA in murine tissue sections (e.g. spinal cord, brain, lymph nodes) by in-situ hybridisation and immunohistochemistry
- Imaging of up to 15 proteins on one tissue section (Multi-Epitope-Ligand-Cartography)



- Imaging of proteins in human and murine cells by flow cytometry and fluorescence microscopy
- Confocal imaging of proteins in live or fixed cells (ImageXpress device)

Analysis of lipid metabolites and expression of target and effector proteins

- LC-MS/MS of lipid mediators such as sphingolipids, eicosanoids, endocannabinoids, lysophosphatidic acids
- Flow cytometry or ELISA based analysis of target proteins
- Fluorescence-activated cell sorting
- Quantitative PCR based analysis of mRNA of transcription factors and target proteins
- Western blot analysis of proteins
- Metabolic studies using Seahorse

Protein biochemical techniques and assays

- Design and cloning of plasmids for heterologous expression of target proteins w/o fusion partners
- Optimization of expression including co-factors or isotopic enrichment
- Purification using state of the art chromatography (affinity, ion exchange, size exclusion)
- Ligand binding assays (surface plasmon resonance technologies, Homogeneous Time Resolved FRET (HTRF))
- Thermodynamic characterization of ligand binding by e.g. isothermal titration calorimetry (ITC)

Activity/Signaling assays

- Activity assays of proteins (e.g. NOX4-assay, BLT2-assay)
- Receptor-binding assays (e.g. G-protein linked, eicosanoid receptors)
- Reporter-Gene assays (e.g. PPARgamma-dependent transactivation assay)
- Flow cytometry-based FRET assay to identify and analyze protein-protein interactions in living cells
- HTRF based assay for co-regulator recruitment of nuclear receptors (e.g. HTRF assay for PPARgamma)
- Development of customer specific assays

Selected Publications

- Knape T, Flesch D, Kuchler L, Sha LK, Giegerich AK, Labocha S, Ferreirós N, Schmid T, Wurglics M, Schubert-Zsilavecz M, Proschak E, Brüne B, Parnham MJ, von Knethen A. Identification and characterisation of a prototype for a new class of competitive PPARγ antagonists. Eur J Pharmacol. 2015;755:16-26. doi: 10.1016/j.ejphar.2015.02.034.
- Kurz J, Brunkhorst R, Foerch C, Blum L, Henke M, Gabriel L, Ulshöfer T, Ferreirós N, Parnham M, Geisslinger G, Schiffmann S. The relevance of ceramides and their synthesizing enzymes for multiple sclerosis. Clin Sci. 2018; 132(17):1963-1976. doi: 10.1042/CS20180506.
- Blum L, Tafferner N, Spring I, Kurz J, deBruin N, Geisslinger G, Parnham M, Schiffmann S. Dietary phytol reduces clinical symptoms in experimental autoimmune encephalomyelitis at least partially by modulating NOX2 expression. J Mol Med. 2018; 96(10):1131-1144. doi: 10.1007/s00109-018-1689-7.

3 *Heat Map of substance screening with various in vitro assays © Fraunhofer IME / Susanne Schiffmann.*

4 *Seahorse device used for metabolic studies © Fraunhofer IME / Susanne Schiffmann.*