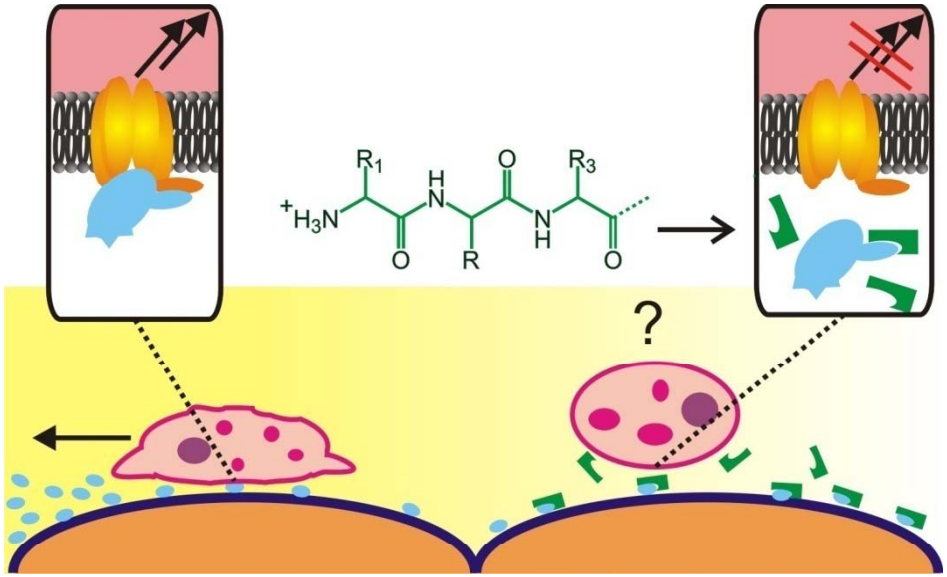
Research Group
„Receptor-Ligand-Interactions“

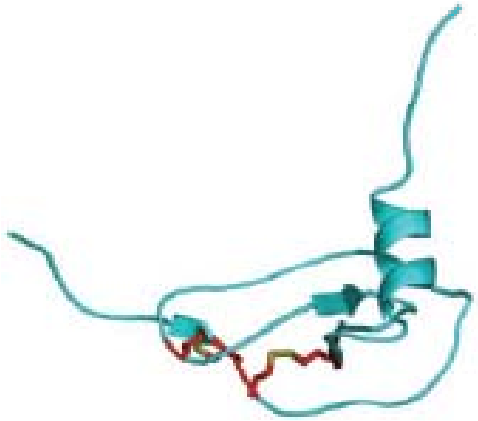
Towards small-molecule ligands to modulate leukocyte behavior



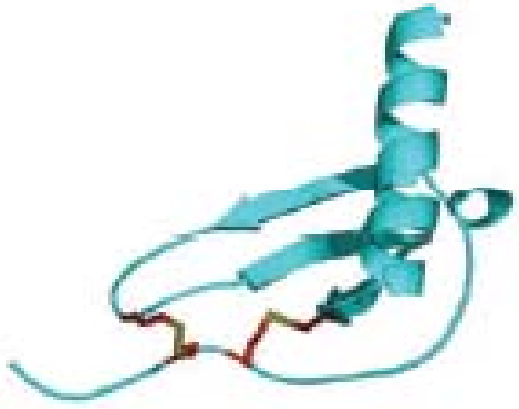
Dr. Katja Schmitz, 28.07.09

Chemokines and chemokine receptors

- Chemotactic cytokines
- Small, soluble proteins (80-120 aa)
- about 50 human chemokines known
- 20 different receptors



CCL2 Chemokine (MCP-1)

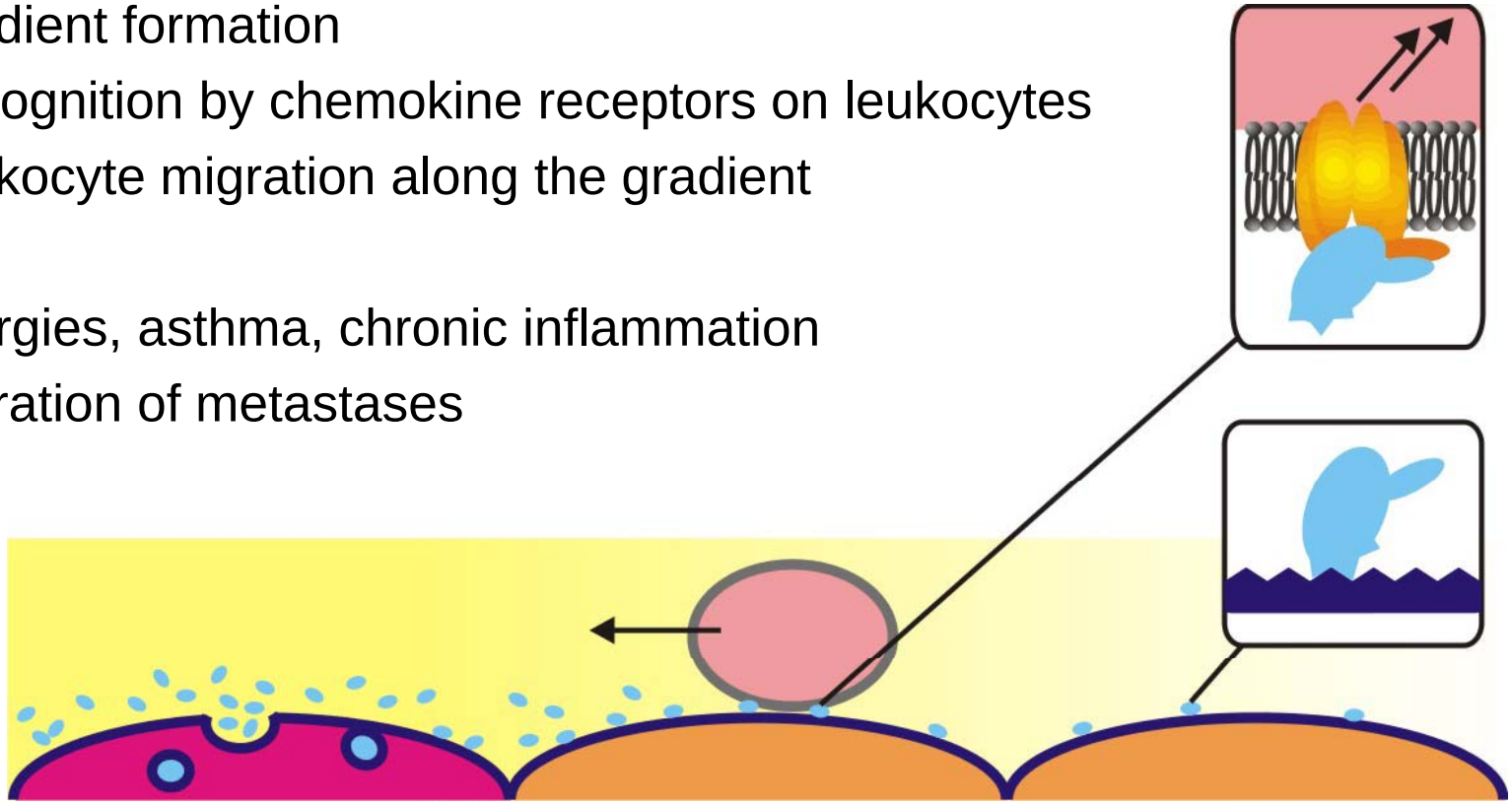


CXCL8 Chemokine (IL-8)

Chemokines control leukocyte migration

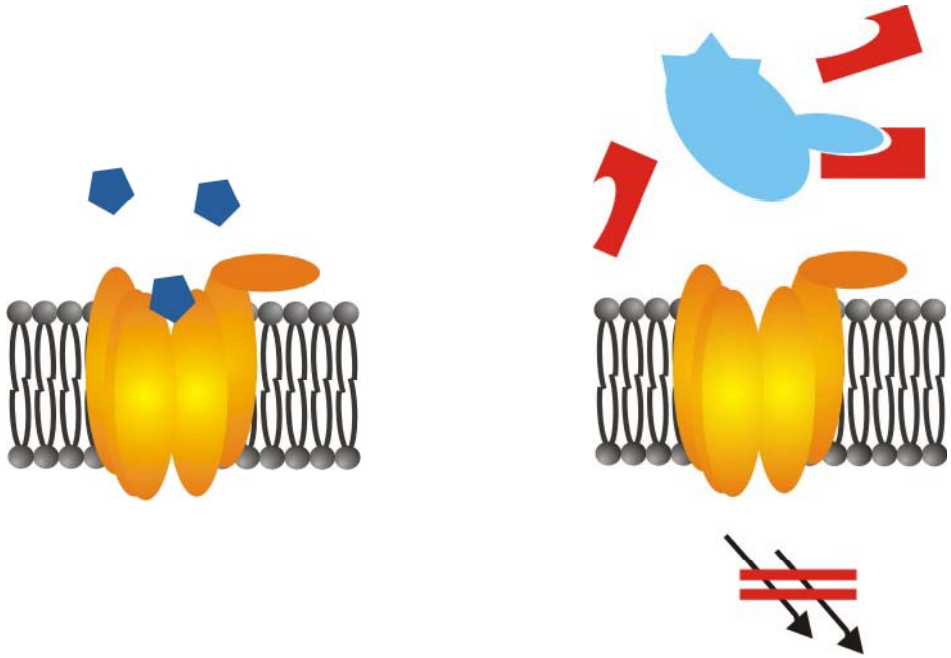
- Secretion
- Glycosaminoglycan binding
- Gradient formation
- Recognition by chemokine receptors on leukocytes
- Leukocyte migration along the gradient

- Allergies, asthma, chronic inflammation
- Migration of metastases



Blocking chemokine-receptor interactions

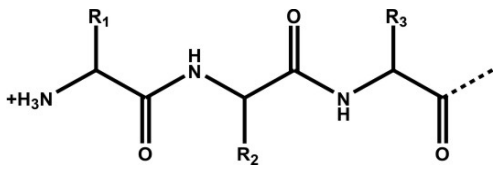
Receptor-based **Chemokine-based**



Design, test and synthesis of ligands

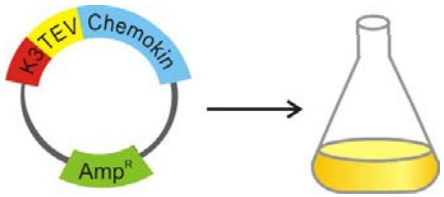
Karlsruhe Institute of Technology

Ligand synthesis

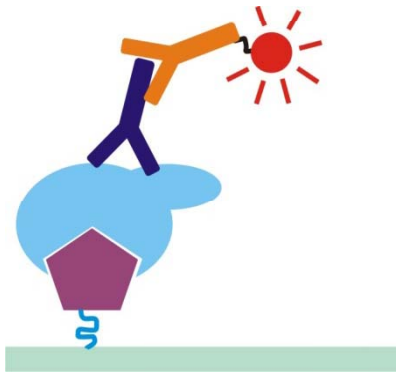


Molecular modeling

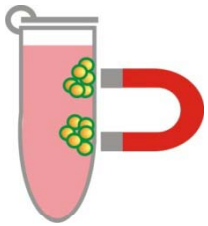
Protein expression



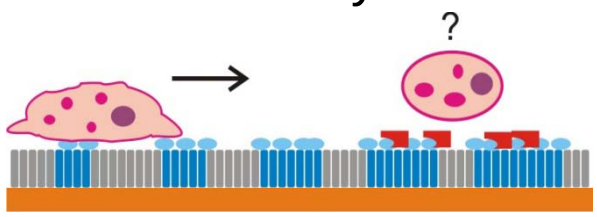
Binding assays



Cell isolation



Bioassays



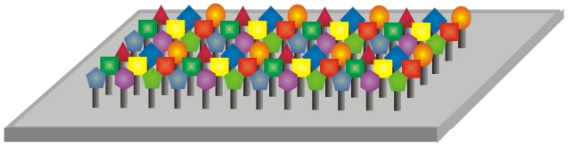
Sources of lead structures

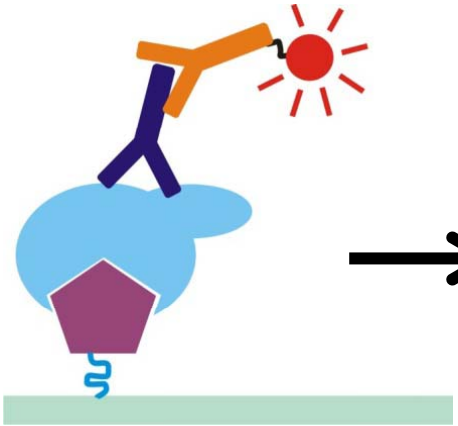
Small-molecule microarrays

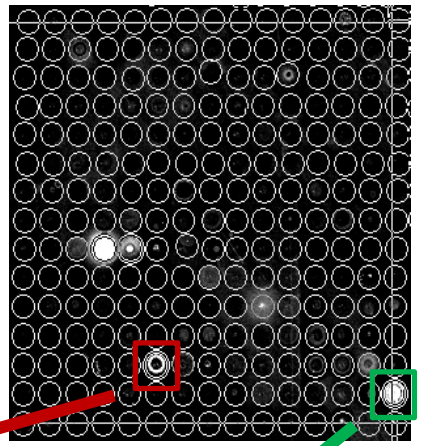
10,000 immobilized small molecules

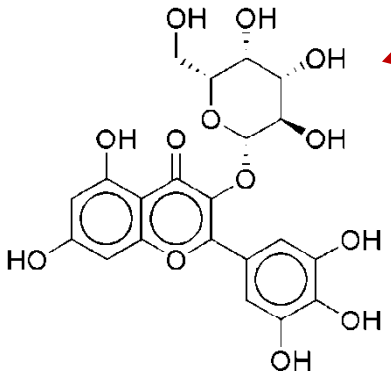
Fluorescence-based binding assay

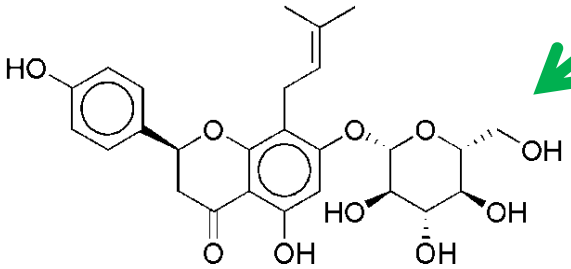
Potential ligands





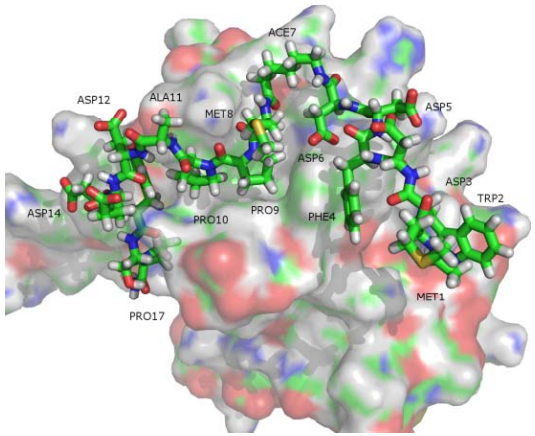


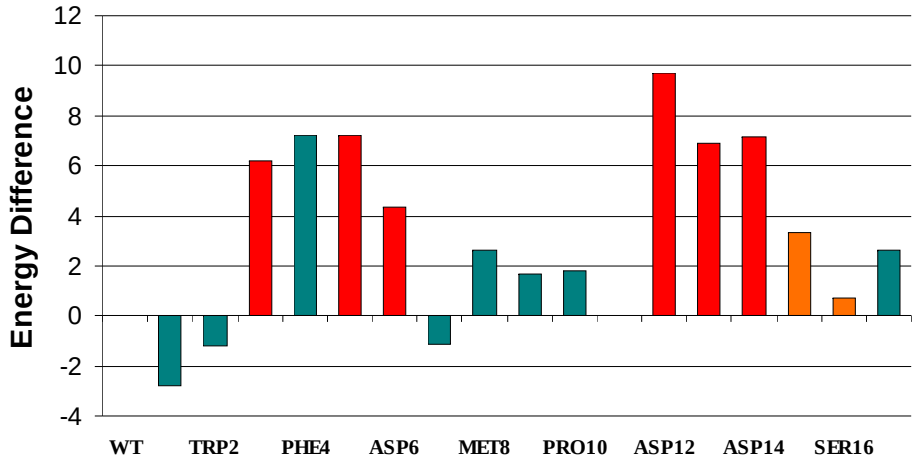




BROAD INSTITUTE

Simulation of interleukin-8 receptor peptides





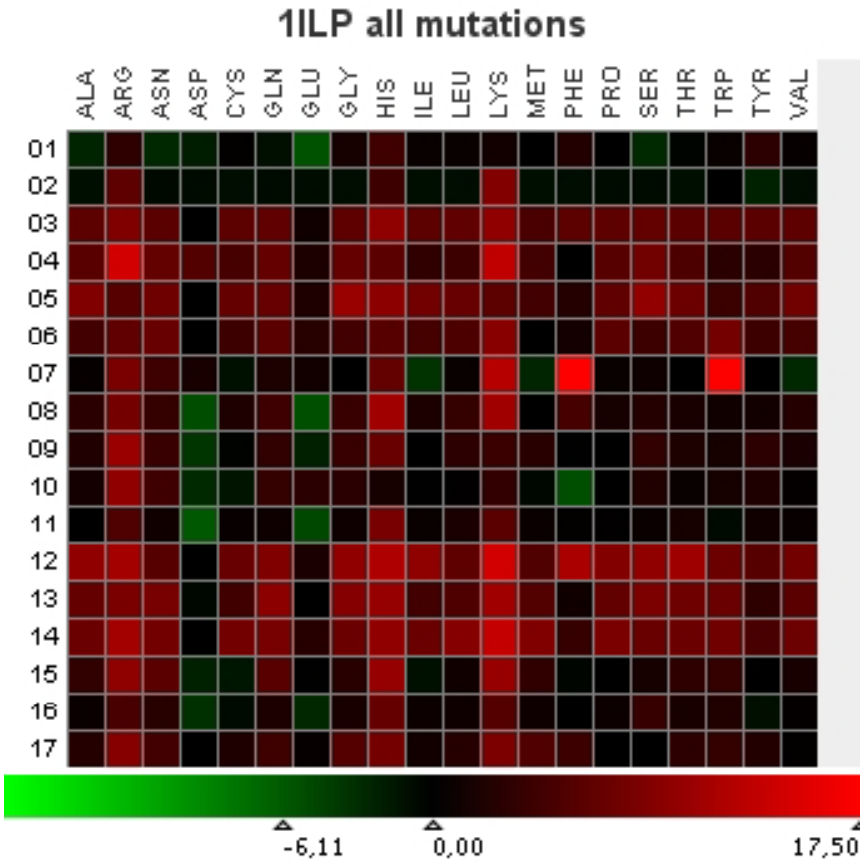
Protein structure from PDB

Computational Alanine Scan

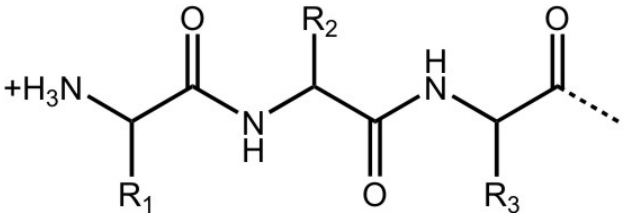
Irene Meliciani

Meliciani *et al.* (2009), *J. Chem. Phys.*, in press

Simulation of interleukin-8 receptor peptides



Computational full peptide exchange scan



MWDFDDGMPPADEDYSP

MWDFDDGMPFADEDYSP

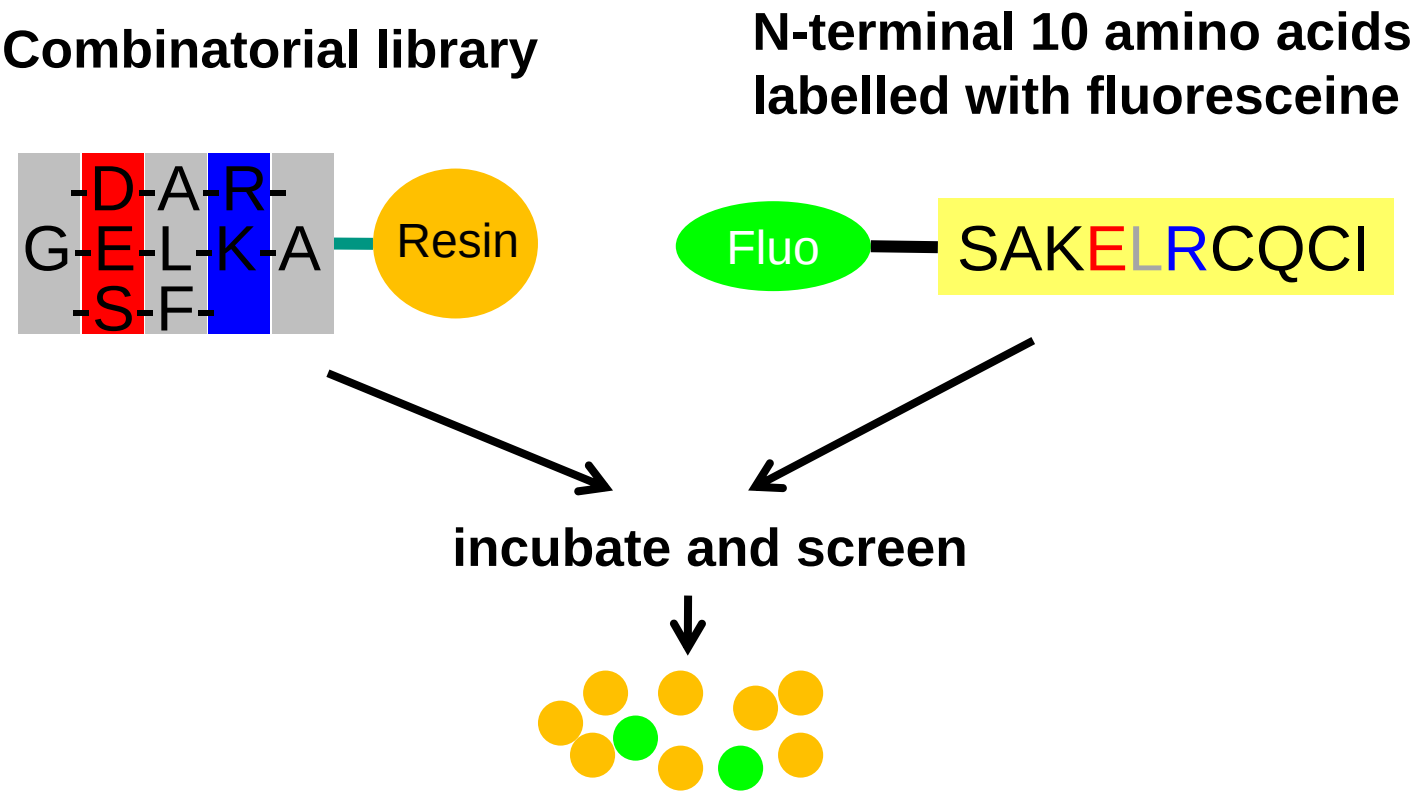
MWDFDDGMPPDDEDYSP

Peptide synthesis for experimental verification

Irene Meliciani

Peptides and peptide mimetics that bind the IL-8 N-terminus

- IL-8 N-terminal ELR motif is essential for receptor activation.



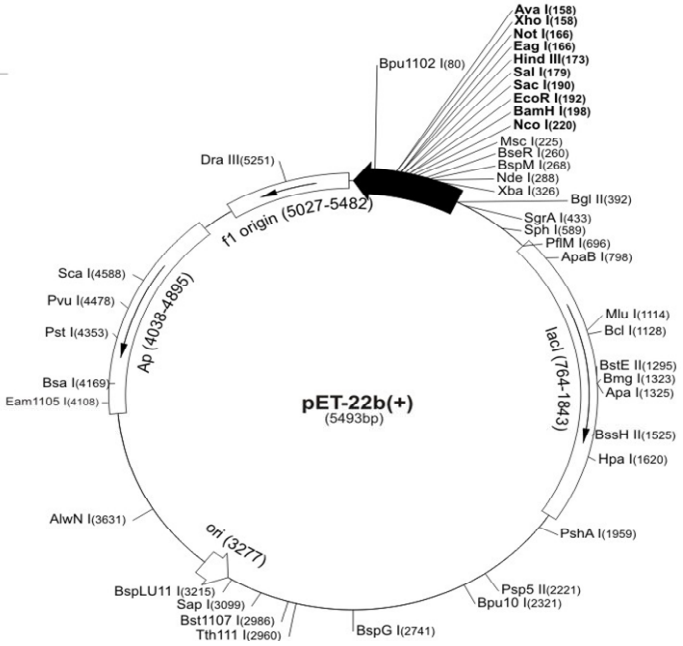
Elena Heidenreich

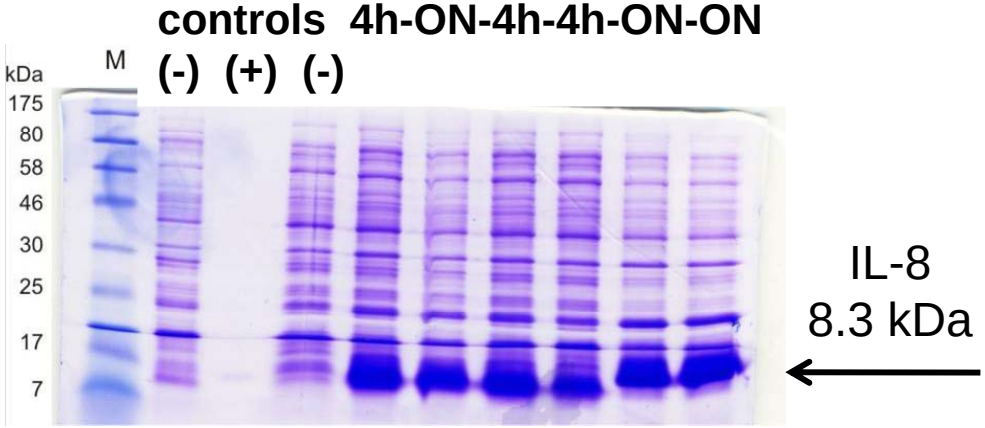
Colaboration with Ute Schepers and Esther Birthalan

Validation of lead structures

Expression of recombinant chemokines

- Cloning of optimized cDNA into an expression vector
- Tag-free expression or expression with bacterial export signal



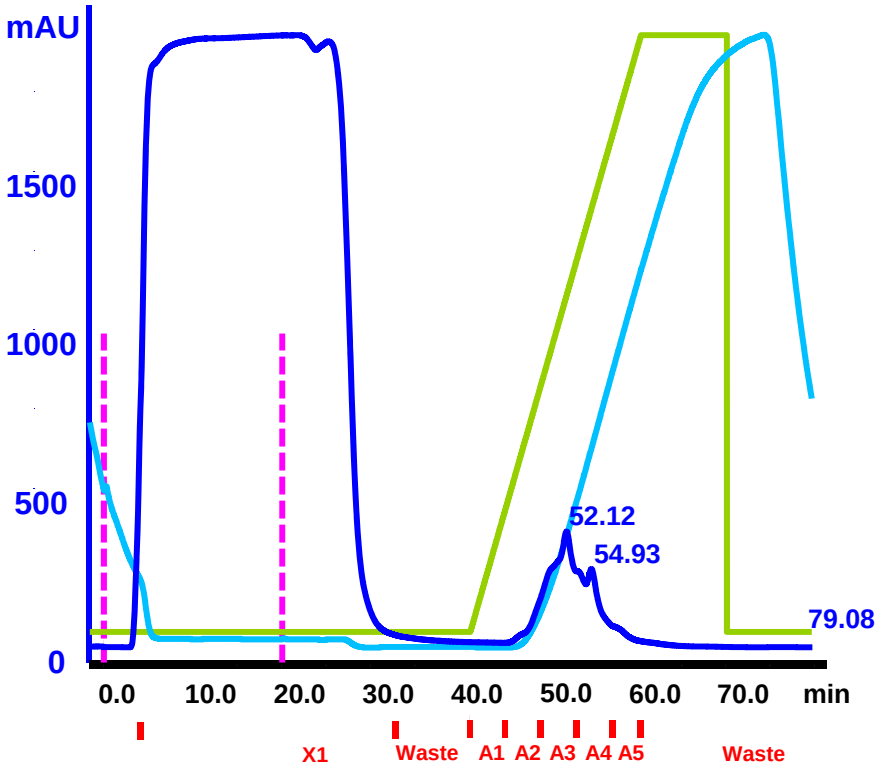


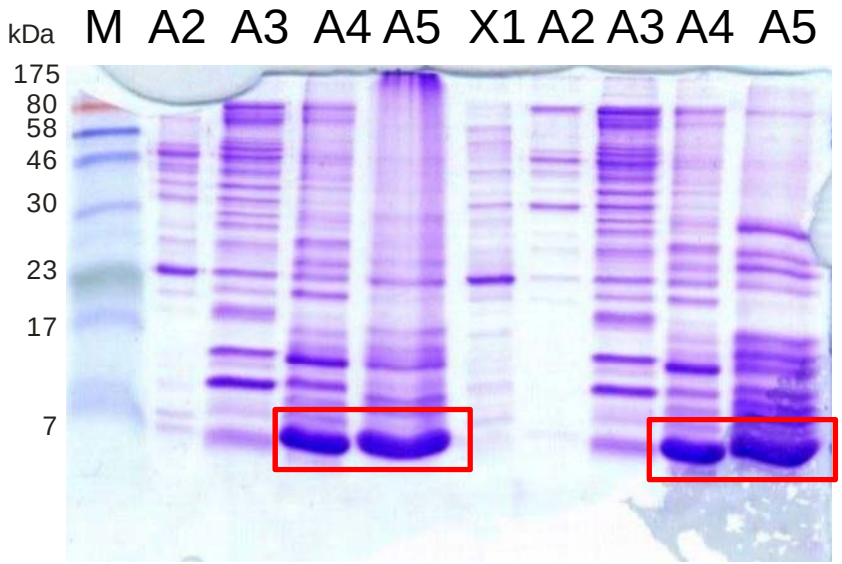
SDS-PAGE for expression analysis

Dana Wiese

Purification of recombinant chemokines

- Affinity chromatography on Hi-Trap heparin resin
- Analysis of fractions by SDS-PAGE, Western blot and MALDI
- Further purification on CIEX cation exchange chromatography

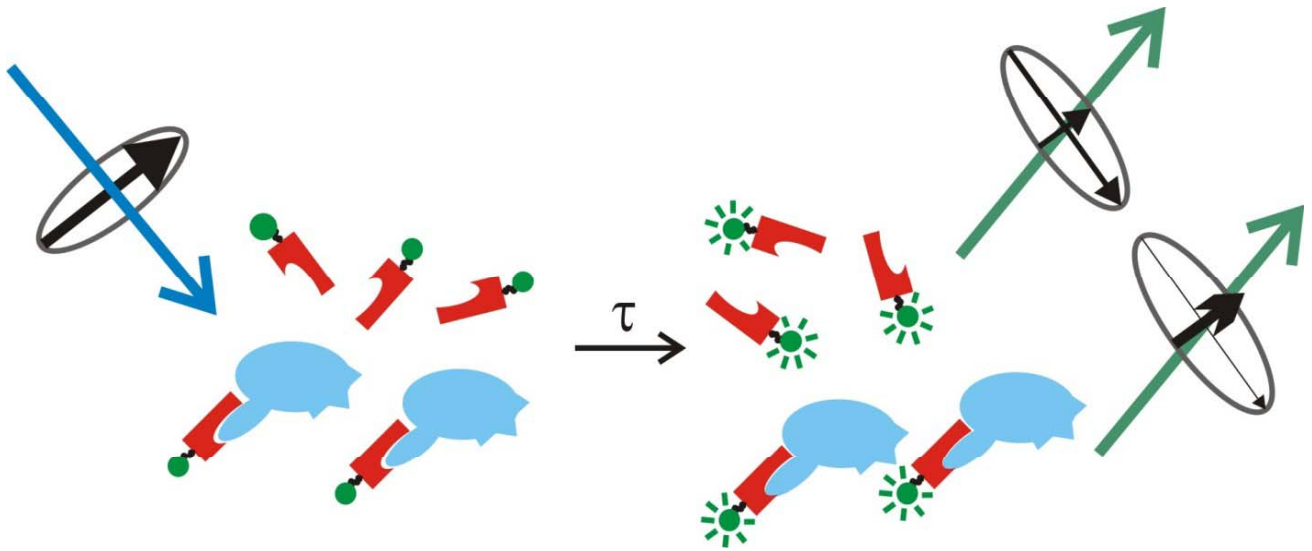




Dana Wiese

Fluorescence Polarization

- Polarization of fluorescent light depends on molecular motility
- Free tracer = high flexibility = low polarization
- Protein-bound tracer = low flexibility = high polarization
- Fluorescence Polarization reflects ratio of bound/unbound ligand

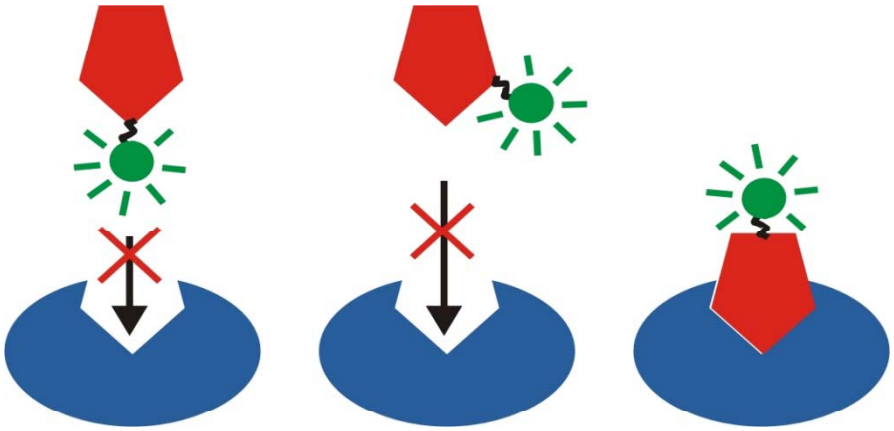


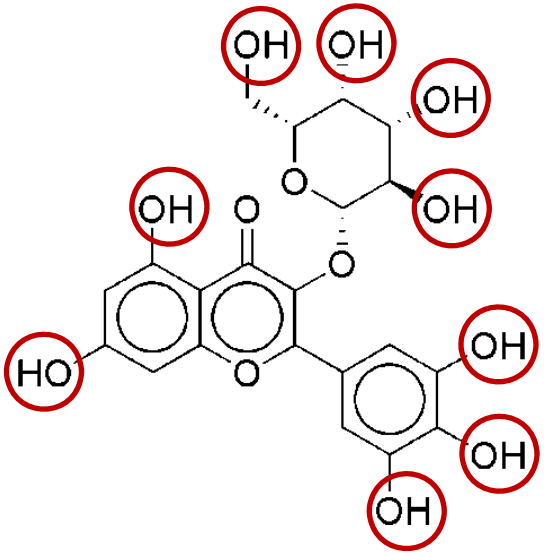
SAR studies by Fluorescence Polarization

If functional groups essential for binding are labelled, binding is prevented

⇒ All derivatives need to be tested

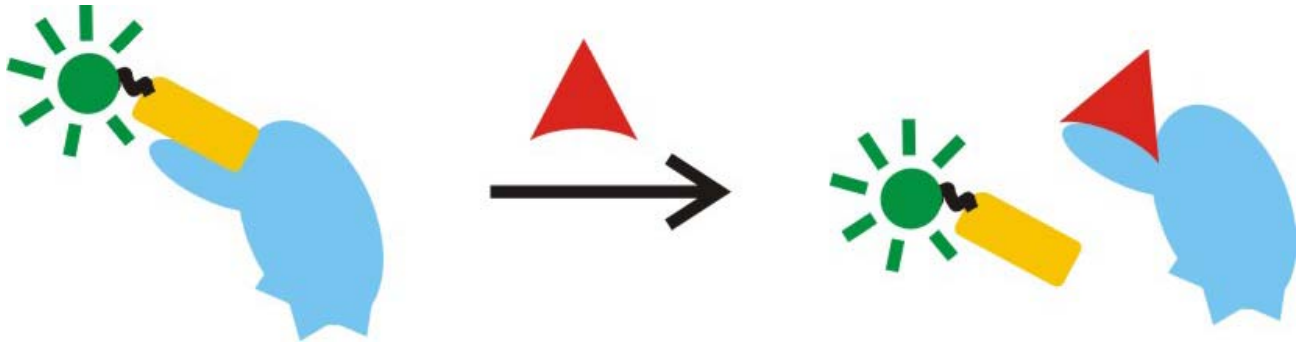
⇒ …and synthesized





FP-Competition assay for lead validation

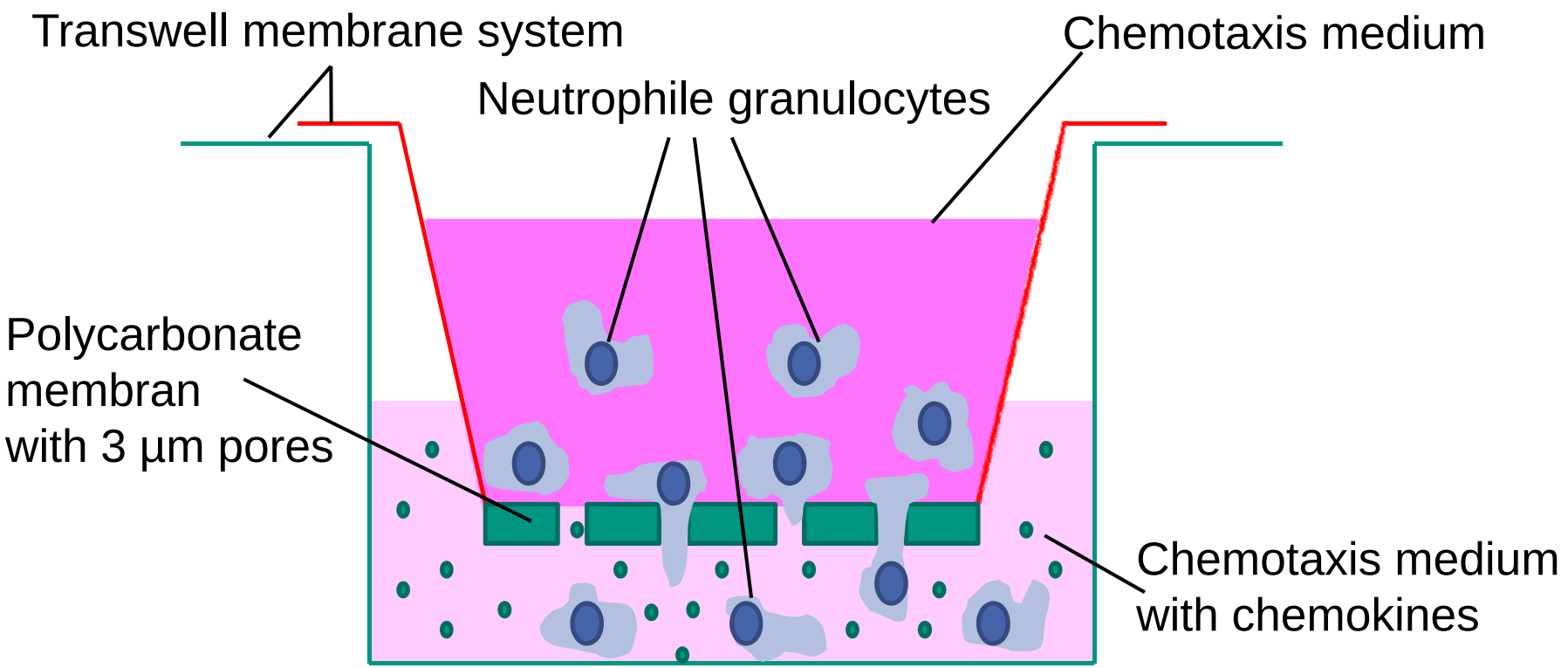
- N-terminal binding peptide with fluorescent label as tracer



- Suitable for comparison of affinities of unlabeled ligands
- K_d of tracer needs to be known
- Only one binding mode is considered

Colaboration with Bianca Stolzenberger

Establishing a Cell Migration Assay

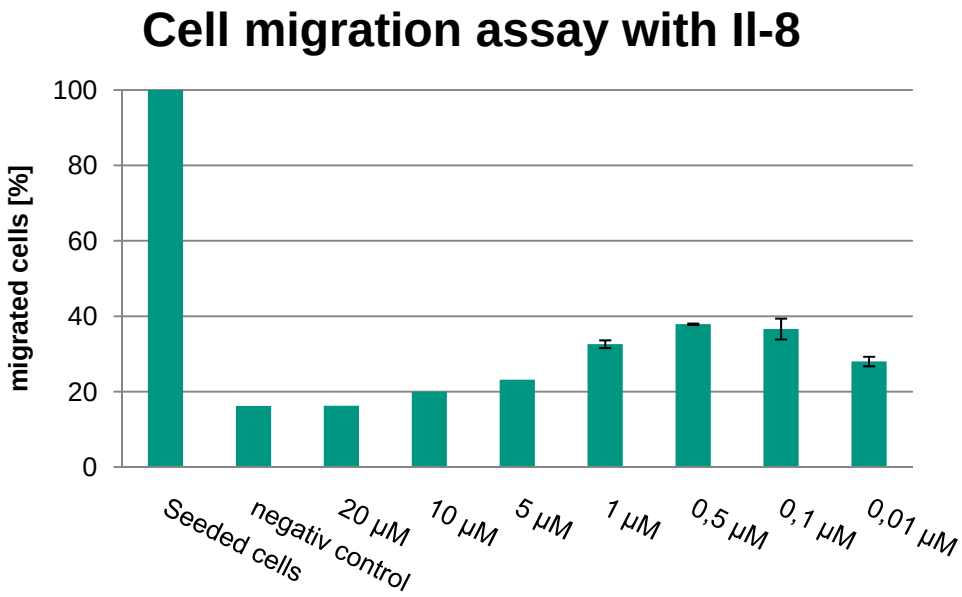


Nicole Niemeier

Cell migration evaluated with CASY® 1 Cell Counter

1. Quantification of granulocytes migrating along a chemokine gradient
2. Evaluation of the „best“ chemokine concentration for migration
3. Test system of inhibitory peptides and positives from microarrays

- Differentiated HL-60 cells = neutrophile granulocytes
- 1 million cells seeded
- Incubation time 1h



Nicole Niemeier

Acknowledgement

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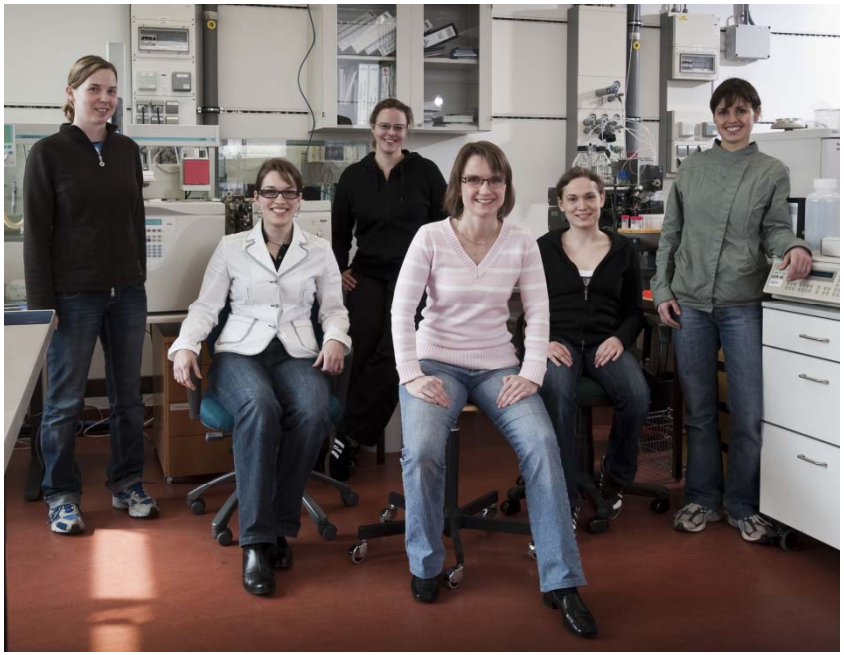
Dana Wiese

Irene Meliciani

Dorothea Helmer



Ebru Diler

Elena Heidenreich

Katharina Güse

Alisha Oster

Ilona Schmelcher

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IOC: Stefan Bräse **INT:** Wolfgang Wenzel

ITG: Ute Schepers **IBL**: Bianca Stolzenfelder, Jürgen Hubbuch

‚Concept for the future' of KIT (German Excellence Initiative)