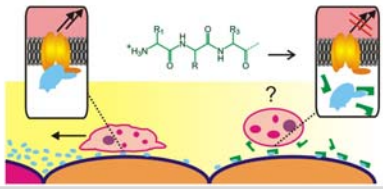
Small molecules to manipulate leukocyte behavior

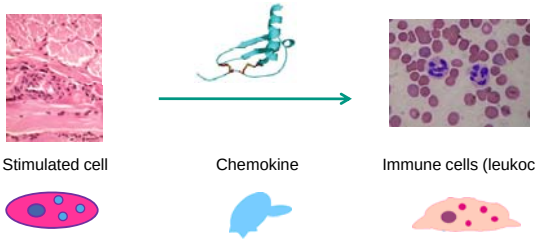
Dr. Katja Schmitz
Eggenstein-Leopoldshafen, 18.01.10

INSTITUT FÜR ORGANISCHE CHEMIE, FAKULTÄT FÜR CHEMIE UND LEBENSWISSENSCHAFTEN, RESEARCH GROUP „RECEPTOR-LIGAND-INTERACTIONS“



Intercellular communication by chemokines

Sender	Message	Receiver
Stimulated cell	Chemokine	Immune cells (leukocytes)

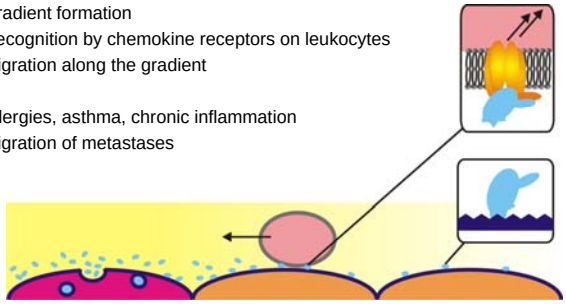


Images: http://anatomy.iupui.edu; Allen, Ann. Rev. Immunol. 2007; http://fr.wikipedia.org

Chemokines control leukocyte migration

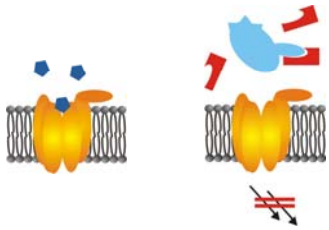
- Secretion
- Glycosaminoglycan binding
- Gradient formation
- Recognition by chemokine receptors on leukocytes
- Migration along the gradient

- Allergies, asthma, chronic inflammation
- Migration of metastases

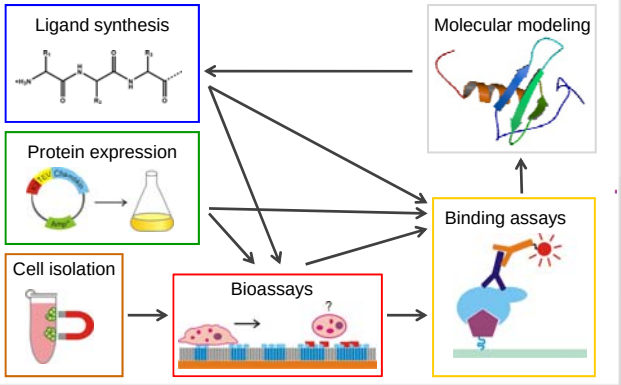


Blocking chemokine-receptor interactions

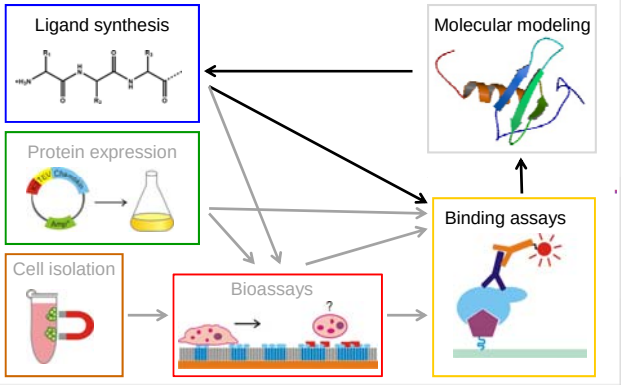
Receptor-based **Chemokine-based**



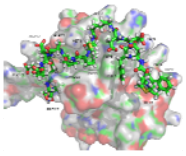
Design, test and synthesis of inhibitors

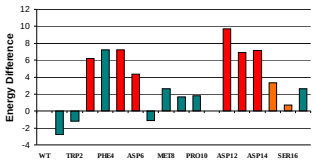


Molecular modeling and binding studies



Modeling of interleukin-8 interaction with receptor peptides





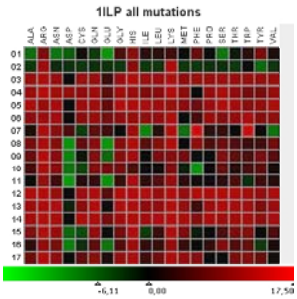
Protein structure of IL-8 with receptor peptide from PDB

Computational alanine scan

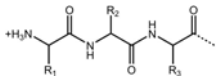
Irene Meliciani

Meliciani *et al.* (2009), *J. Chem. Phys.*, 131, 034114

Modeling chemokine-receptor complexes



Full peptide exchange scan

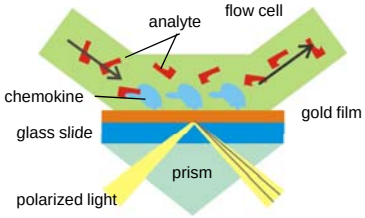


MWDFDDGMPPADEDYSP
MWDFDDGMPFADEDYSP
MWDFDDGMPPDDEDYSP

Selected peptides ordered for binding assays

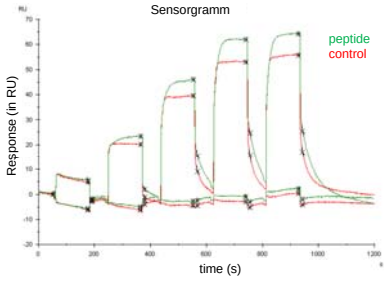
Irene Meliciani

Determination of binding constants by surface plasmon resonance (SPR)



Principle:
Resonance angle of total internal reflexion depends on load on gold film.

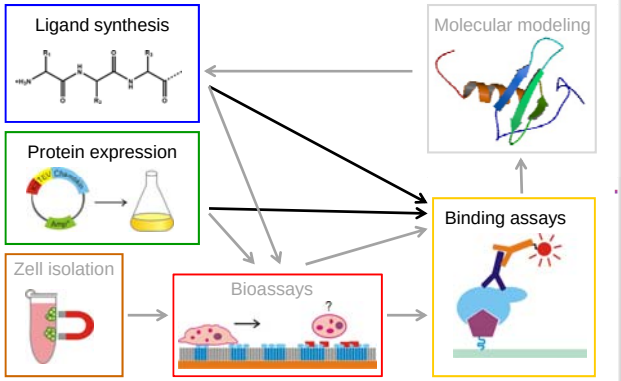
Determination of binding constants by SPR



Binding constant of IL-8-receptor peptide: 8 µM

Bianca Stolzenberger

Combinatorial chemistry and binding assays

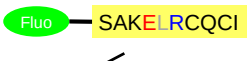


Peptides that bind the IL-8 N-terminus

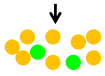
- IL-8 N-terminal ELR motif is essential for receptor activation

Combinatorial library

N-terminal 10 amino acids labelled with fluoresceine

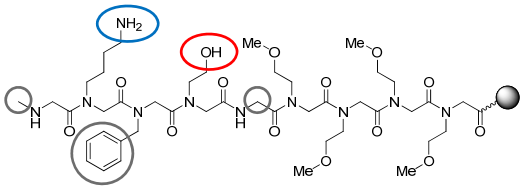


incubate and screen



Elena Heidenreich, Dorothea Helmer

Peptoids as chemokine ligands



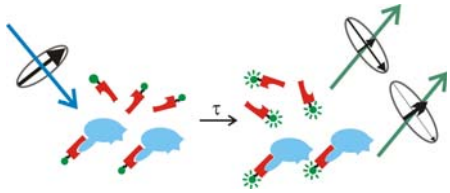
- Libraries of pentapeptoides with the sequence:
  hydrophobic – acidic – hydrophobic – basic - hydrophobic

- on-bead screening
- re-synthesis of candidates

Dorothea Helmer

Fluorescence Polarization

- Polarization of fluorescent light depends on molecular motility
- Fluorescence Polarization reflects ratio of bound/unbound ligand



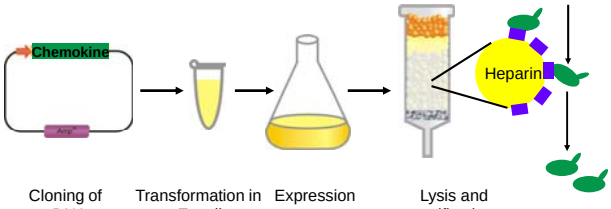
- Homogeneous assay
- Requires ligand labeling
- Can be automated

FP-Competition assay for lead structure 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

Proteins for binding experiments



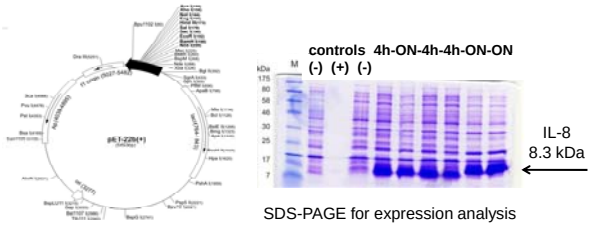
Cloning of cDNA | Transformation in *E. coli* | Expression | Lysis and purification

Advantages: Production of proteins in-house permits mutations and tags

Dana Wiese

Expression of recombinant chemokines

- Optimized cDNA cloned into expression vectors for
  - Tag-free expression
  - expression with bacterial export signal
  - Expression with His_6-tag

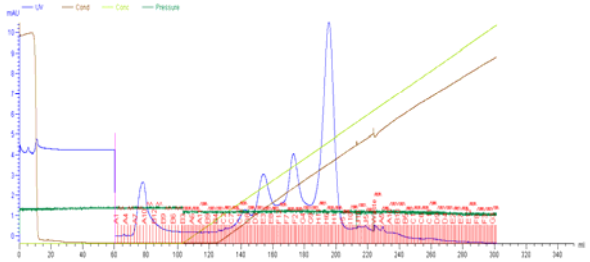


SDS-PAGE for expression analysis

Dana Wiese

Proteins for binding experiments

Example for chromatographic trace in cation exchange chromatography (Gradient of buffered NaCl, protein detection at 280nm)



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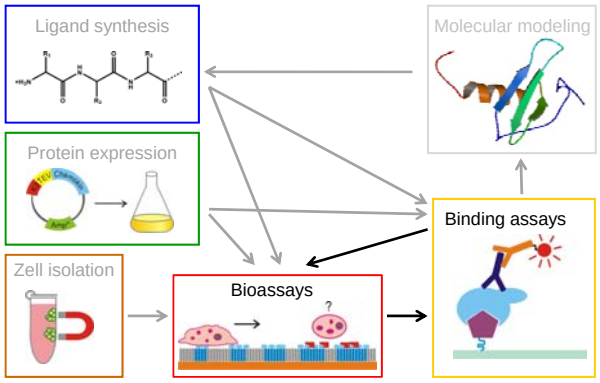
Microarrays and bioassays

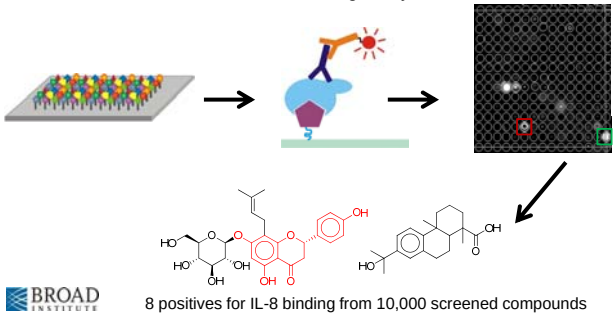


Binding experiments on microarrays

10,000 immobilized small molecules

Fluorescence-based binding assay

Potential ligands



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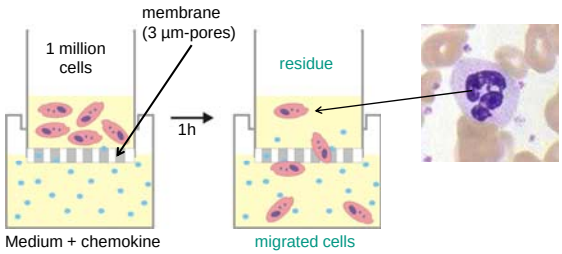
8 positives for IL-8 binding from 10,000 screened compounds

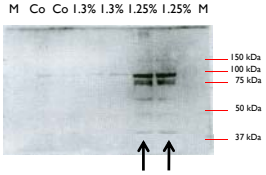
Chemotaxis assay to test bioactivity



- differentiated HL-60-cells
- chemokine +/- inhibitor
- cell count
- Total migration = total cell number - residue

Nicole Niemeier, Maria Braun

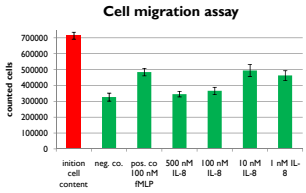
Model system: Differentiated HL-60 cells expressing IL-8 receptors



HL-60 cells, differentiated with 1.25% DMSO and G-CSF (Granulocyte-Colony Stimulating Factor)

IL-8 Receptor A: ~70 kDa
IL-8 Receptor B: ~45 kDa

Nicole Niemeier, Maria Braun

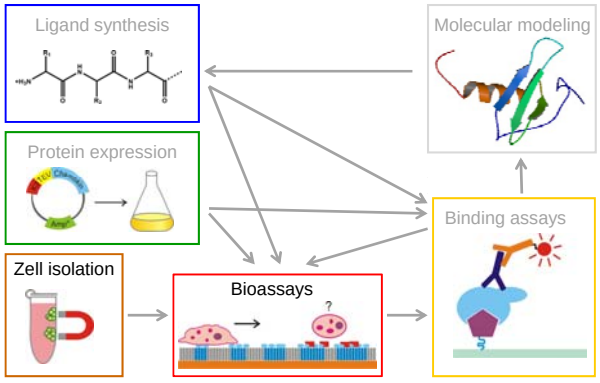


Optimum chemotaxis response at 10 nM IL-8 after 30 min incubation

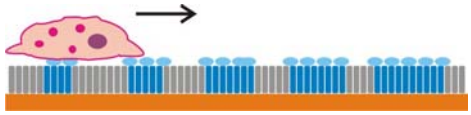
Z'-value = 0.87

10 µM inhibitor 1 reduces migration by 31%.

Other projects



Microstructured surfaces for leukocyte migration



Microcontact printing (µCP)

direct „stamping“ of IL-8 on surface printing of alkane thiols, incubation with IL-8

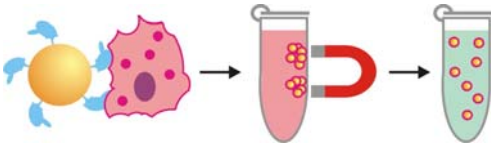
- bad resolution
- good resolution

Chemokine patterns:

Collaboration with Martin Bastmeyer (CFN)
Nicole Niemeier, Maria Braun

Immobilized chemokines for leukocyte isolation and binding assays

- Magnetic microparticles to immobilize arginine-rich proteins
- Binding of leukocytes or small molecules to immobilized chemokines
- Magnetic separation of micro particles



- Can be extended into a binding assay, too

Dorothea Helmer

Acknowledgement

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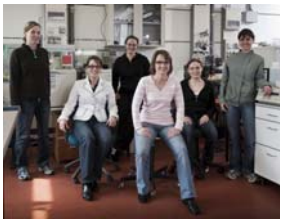
Christian Mink



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INT: Wolfgang Wenzel
ITG: Ute Schepers
IBL: Bianca Stolzenberger
Jürgen Hubbuch
CFN: Martin Bastmeyer

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