RUHR UNIVERSITÄT BOCHUM

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I
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Schwerpunkt

Proteine in der Biomedizin

Klaus Gerwert
Lehrstuhl für Biophysik
WWW.bph.rub.de

Protein alterations in disease

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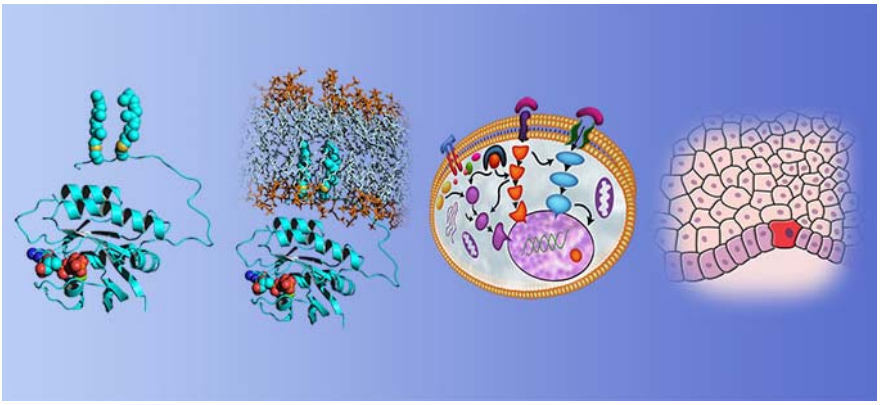
proteins **membranes** **cell** **tissue**



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Colon-cancer

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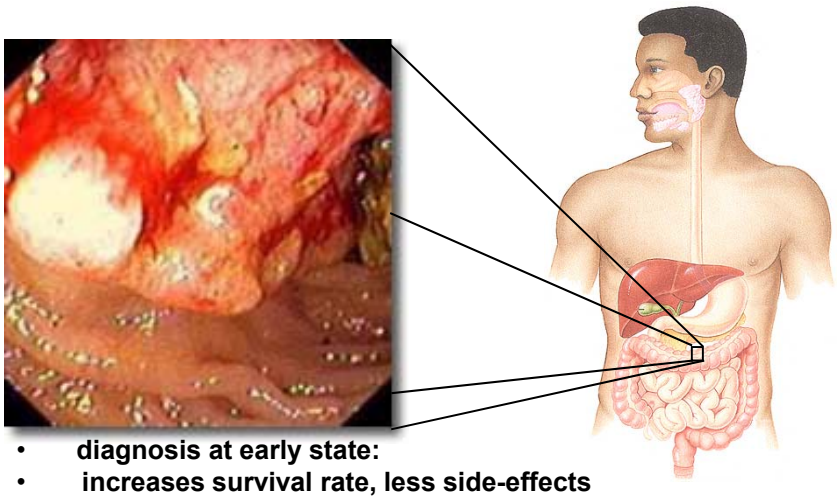
- molecular understanding of protein-interaction
- Protein alterations cause disease



- **diagnosis at early state:**
- **increases survival rate, less side-effects**
- **predictive diagnosis for targeted therapy**

3

Different states of colon-cancer (simplified)

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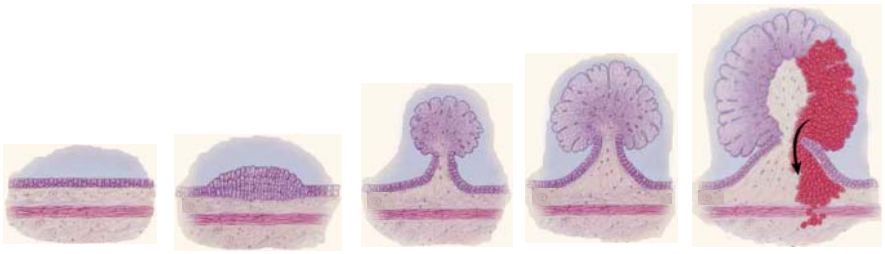


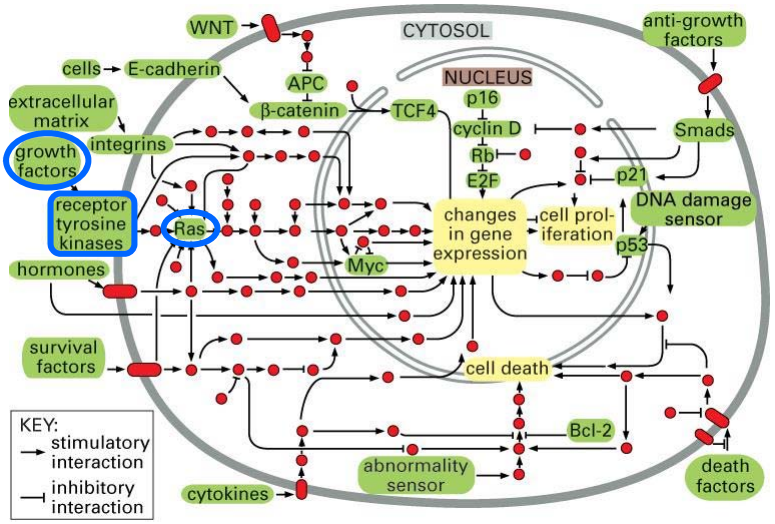
Normal → Hyperplasie → Small adenome → Large adenome → Carzinome

APC ras p53 Smad4

Adenoma-carcinoma sequence (Fearon u. Vogelstein, 1990)

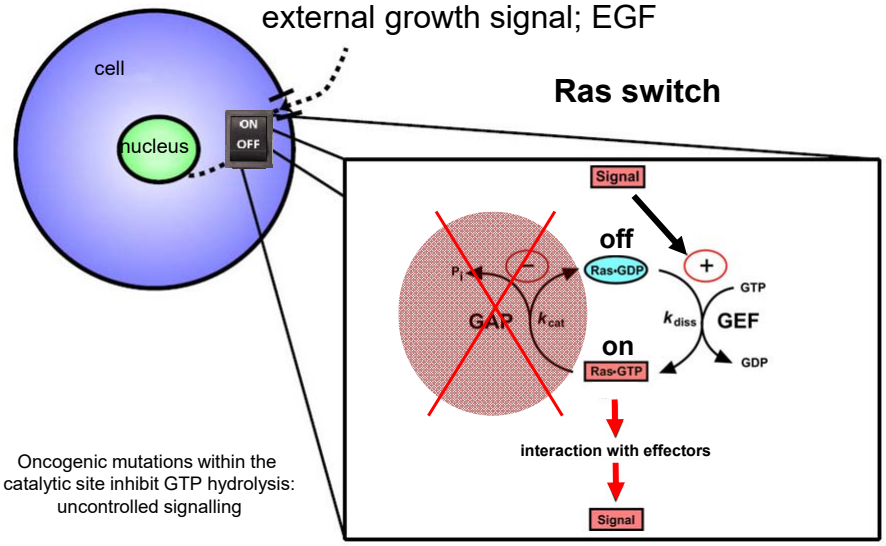
4

Proteins involved in cancer



5

Ras switches external growth signals



Oncogenic mutations within the catalytic site inhibit GTP hydrolysis: uncontrolled signalling

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Protein alterations in disease

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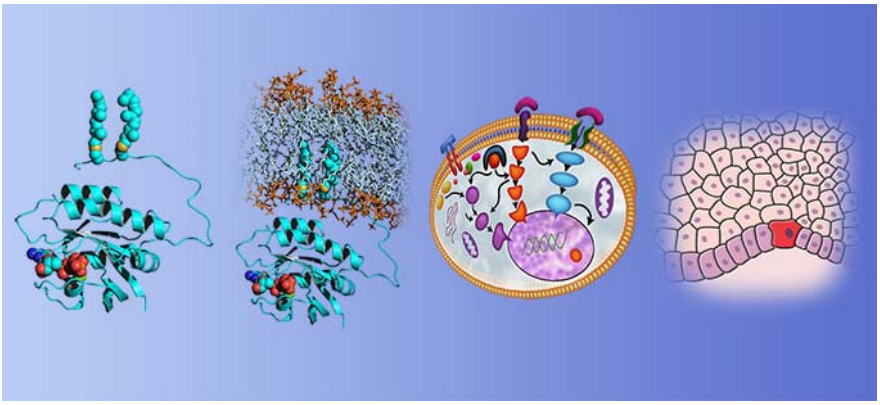
proteins membranes cell tissue



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Catalysis of GTP Hydrolysis

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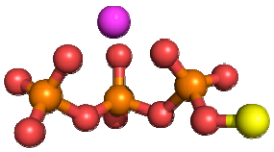

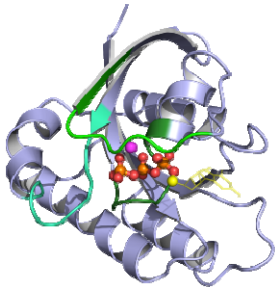

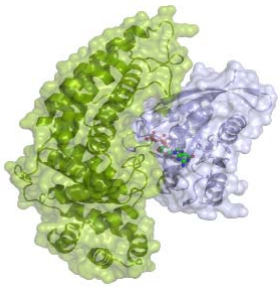

GTP + Mg^{2+} in water

rate of hydrolysis k_{water}

200 days

$\Delta G^{\ddagger}$ = 114 kJ/mol

GTP + Mg^{2+} + Ras

$k_{Ras} = 10^5 \times k_{water}$

30 min

$\Delta G^{\ddagger}$ = 92 kJ/mol

GTP + Mg^{2+} + Ras + RasGAP

$k_{Ras\text{-}GAP} = 10^5 \times k_{Ras}$

50 msec

$\Delta G^{\ddagger}$ = 66 kJ/mol

Kötting, Kallenbach, Suveyzdis,Wittinghofer, Gerwert, PNAS, 2008

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Key: orchestration of biophysical techniques which spatio-temporal resolution at the atomic level

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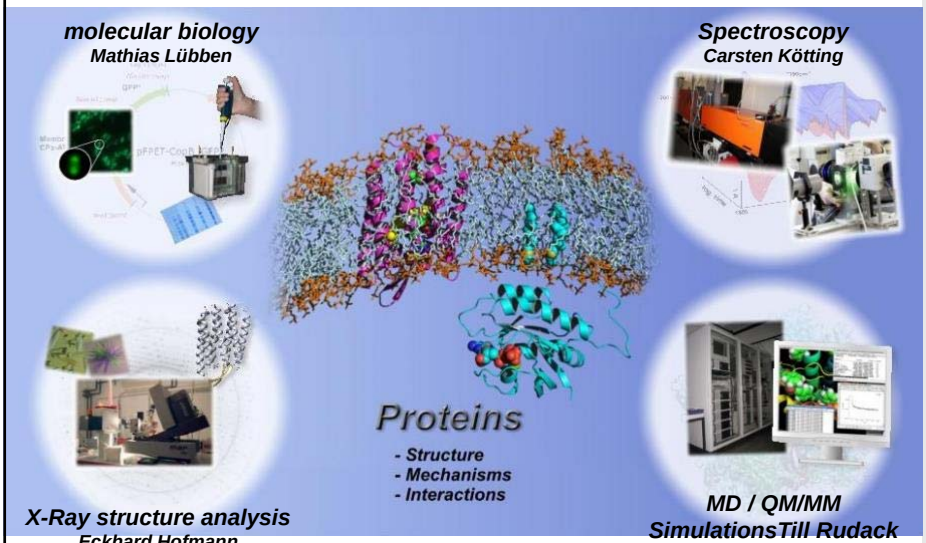

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GTP hydrolysis catalysed by GAP

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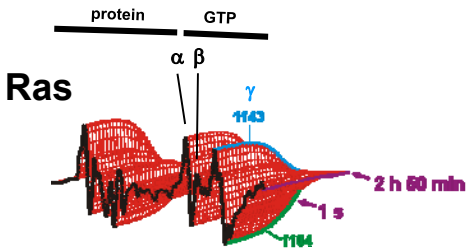
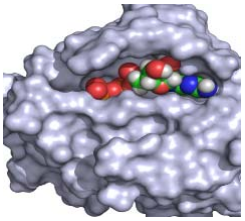
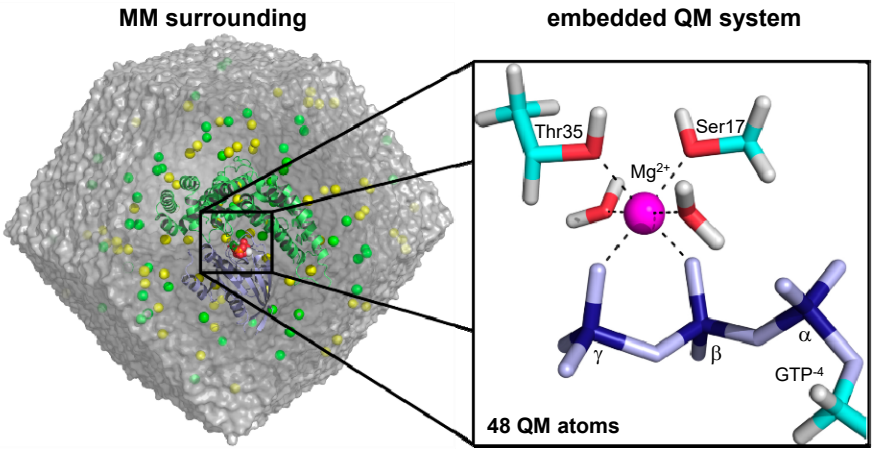




Kötting, Blessenohl, Suveyzdis, Goody, Wittinghofer, Gerwert, *PNAS* 103, 13911 (2006)

10

Decode IR spectral information by biomolecular QM/MM simulations



Simulation system: Ras·GTP·Mg^{2+}·RasGAP:
58058 water molecules, 162 Na^+, 162 Cl^-,
Total: 7855 substrate atoms

Rudack, Xia, Schlitter, Kötting, Gerwert, PNAS, 2012

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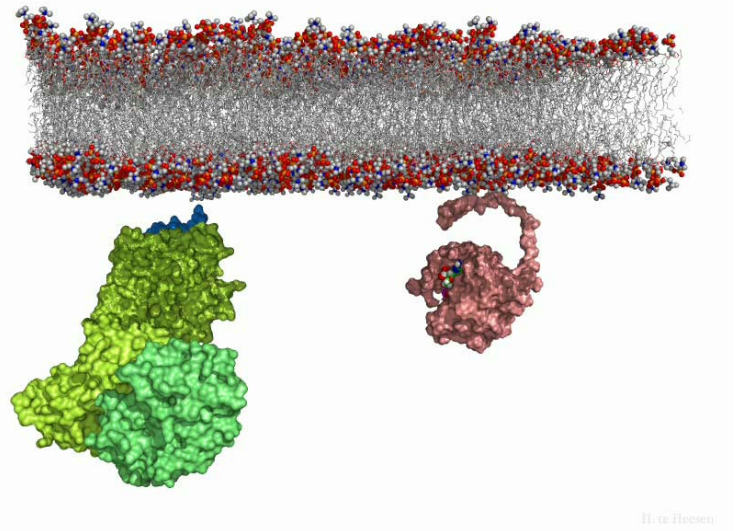
Ras Interaction network



Henrik te Heesen

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Protein-dynamics at different scales: Vibrational spectroscopy

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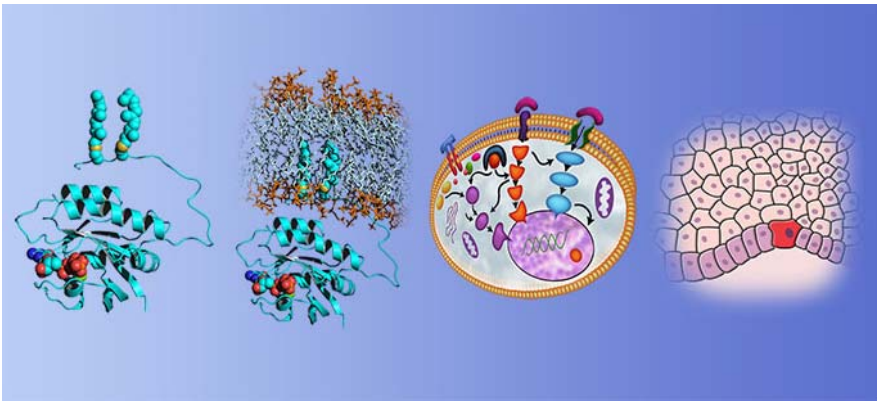
proteins membranes cell tissue



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The vibrational spectrum provides a fingerprint:label-free

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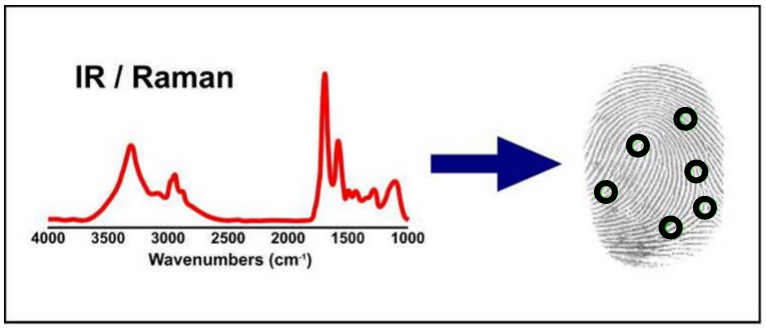

- integral signal of the biochemical status
- molecular information about proteom and genome
- need bioinformatics to distinguish between differnt patterns

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Annotation of index-colors

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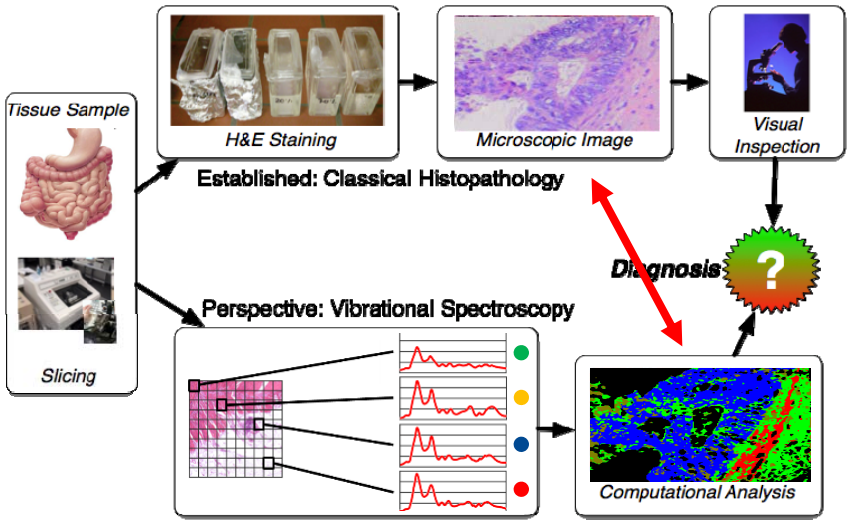

Mavarani, L., Petersen, D., El-Mashtoly, S.F., Mosig, A., Tannapfel, A., Kötting, C., Gerwert, K., Analyst, 2013, *138*, 4035-4039

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Image analysis: random forest

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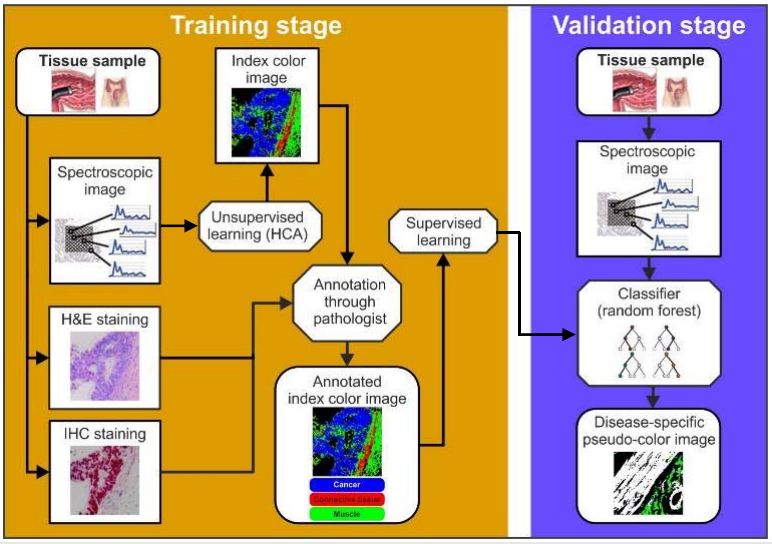

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colon tissue classification

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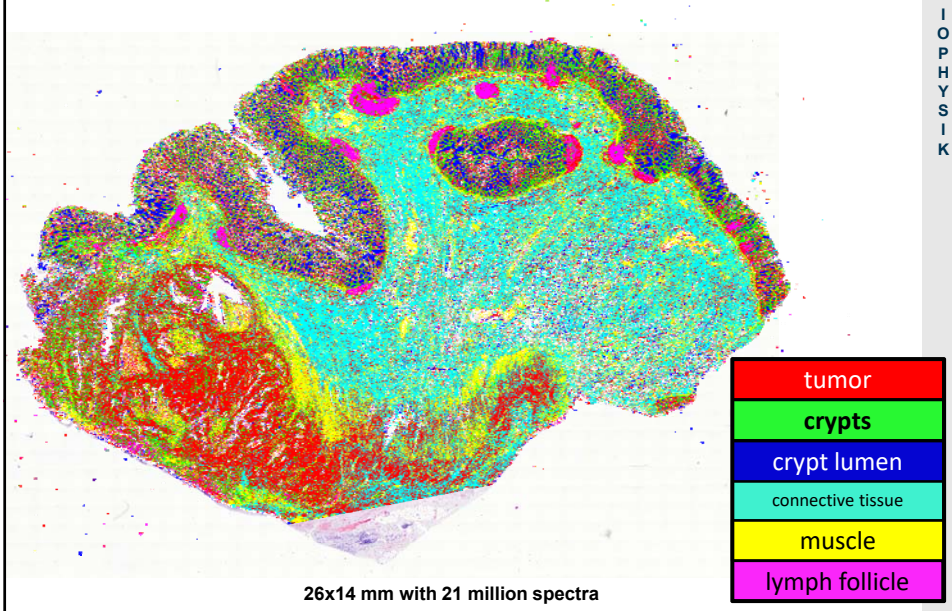

26x14 mm with 21 million spectra

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Integration of FTIR-Imaging and laser microdissection (LMD)

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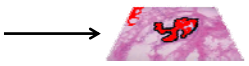
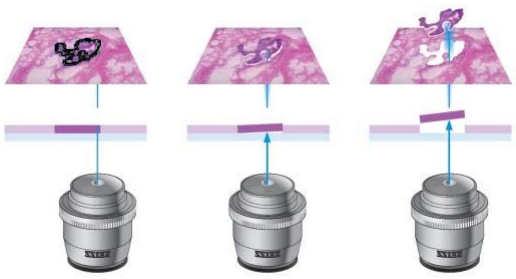
FTIR-Imaging



label-free annotation,

LMD

same thin section!



Laser microdissection

Großerueschkamp, Bracht, Diehl, Kuepper, Ahrens, Kallenbach-Thieltges, Mosig, Eisenacher, Marcus, Theegarten, Sitek, Gerwert, Scientific Reports, 2017, DOI: 10.1038/srep44829 open acces

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Thematische Kompetenzbereiche

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Vulcano Plot in proteome analysis:
epithelioid vs sarcomatoid diffuse malignant mesothelioma (DDM)

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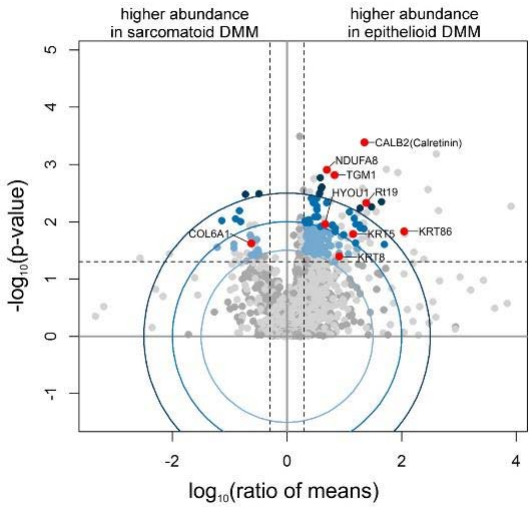

1869 proteins
(≥ 1 unique peptide)

142 differential abundant proteins
(≥ 2 or ≤ 2 fold change)
(p-value ≤ 0.05)

biomarkers 9

Großerueschkamp, Bracht, Diehl, Kuepper, …Gerwert, Scientific Reports, 2017, DOI: 10.1038/srep44829 open

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The American Journal of Pathology, Vol. 189, No. 3, March 2019

ELSEVIER

The American Journal of PATHOLOGY

ajp.amjpathol.org

MACHINE LEARNING, COMPUTATIONAL PATHOLOGY, AND BIOPHYSICAL IMAGING

Integrated Fourier Transform Infrared Imaging and Proteomics for Identification of a Candidate Histochemical Biomarker in Bladder Cancer

Kathrin E. Witzke,* Frederik Großerueschkamp,† Hendrik Jütte,† Melanie Horn,† Florian Roghmann,§ Nicolas von Landenberg,§ Thilo Bracht,* Angela Kallenbach-Thieltges,† Heiko Käfferlein,¶ Thomas Brüning,¶ Karin Schork,* Martin Eisenacher,* Katrin Marcus,* Joachim Noldus,§ Andrea Tannapfel,‡ Barbara Sitek,* and Klaus Gerwert†

From the Medizinisches Proteom-Center, the Department of Biophysics,† the Institute of Pathology,‡ the Department of Urology,§ Marien Hospital Herne, and the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance,¶ Ruhr University Bochum, Bochum, Germany*

Witzke, K.E., et al, *Integrated Fourier Transform Infrared Imaging and Proteomics for Identification of a Candidate Histochemical Biomarker in Bladder Cancer*, Am J Pathol. 2019 Mar;189(3):619-631

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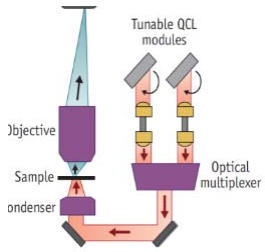
Translation of IR imaging to clinics

new light source: **QCL**



FTIR

Spero QT

Daylight Solutions, CA, USA

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Morbus Alzheimer

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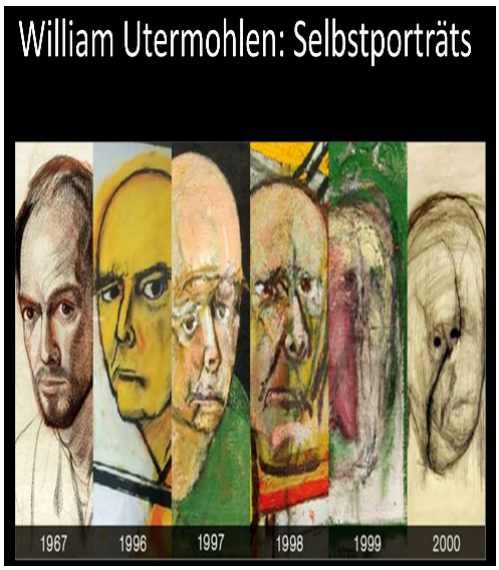
Morbus Alzheimer



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Morbus Alzheimer

- > 1.2 million cases in Germany / >40 million cases worldwide
- most common type of dementia ~70 %
- global costs ~ 640 billion US$ (care staff etc.)
- symptoms: dementia, speech disorders, movement disorders, depression, … (people often die of inanition or malnutrition)
- cureless

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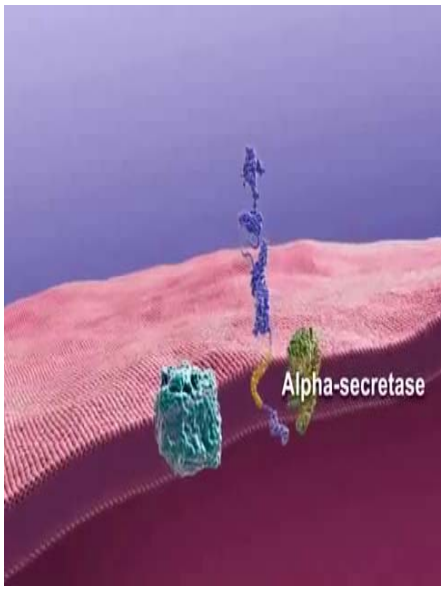
Aß Biomarker



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Aß Biomarker

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Alzheimer

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Amyloid-PET Scanning

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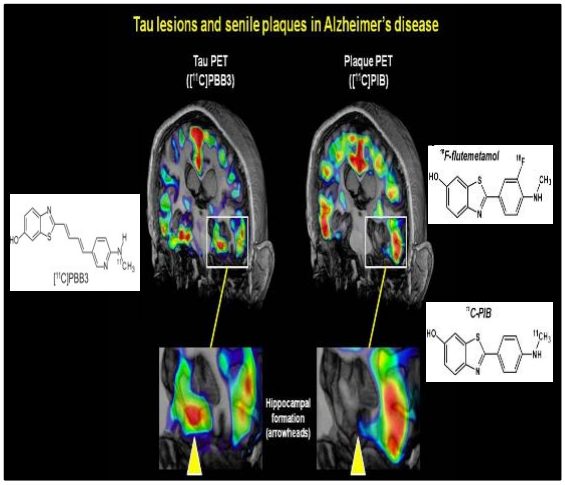

- e.g. injection of [18F]-flutemetamol and Pittsburgh compound B (Aß), or PBB derivates (Tau)
- direct detection of Aß and Tau protein deposition in the brain

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CSF by Lumbar Puncture

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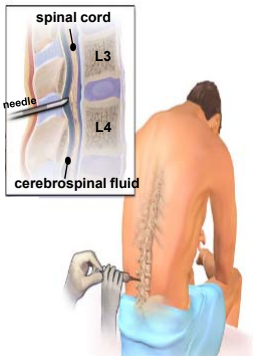

- invasive procedure
- spinal needle is inserted between 3rd and 4th lumbar vertebrae
- common side effects: dizziness, headache, sickness, pain (partly over several weeks)

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Blutuntersuchung

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- Routineuntersuchung; durchführbar bei jedem Hausarzt
- Minimal-invasiv
- Kosten pro Analyse ca. 90 €
- Nebenwirkungen sehr selten

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Detection of Aß pathological processes

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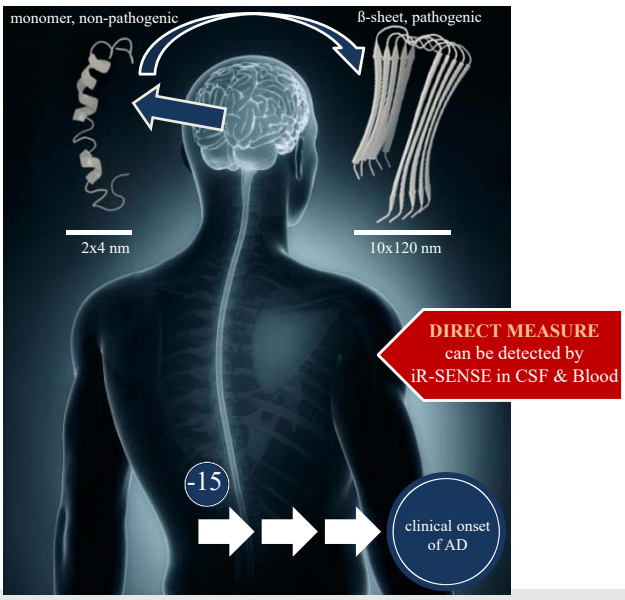

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Capture Antibody extracts monomers and fibrills out of the body fluid

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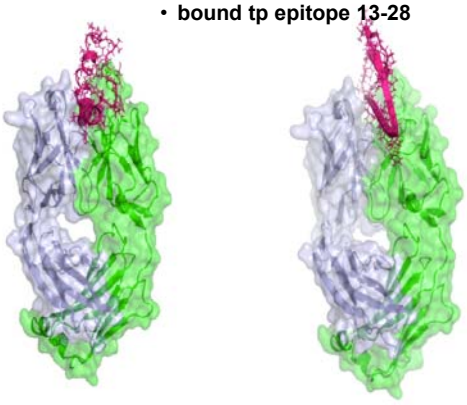
- bound tp epitope 13-28



Light Chain - Homology model on 3UJT.pdb
Heavy Chain – Homology model on 4OTX.pdb
Aβ in α-helix (1Z0Q.pdb) and β-sheet form (5OQV.pdb)

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Secondary structure distribution as biomarker

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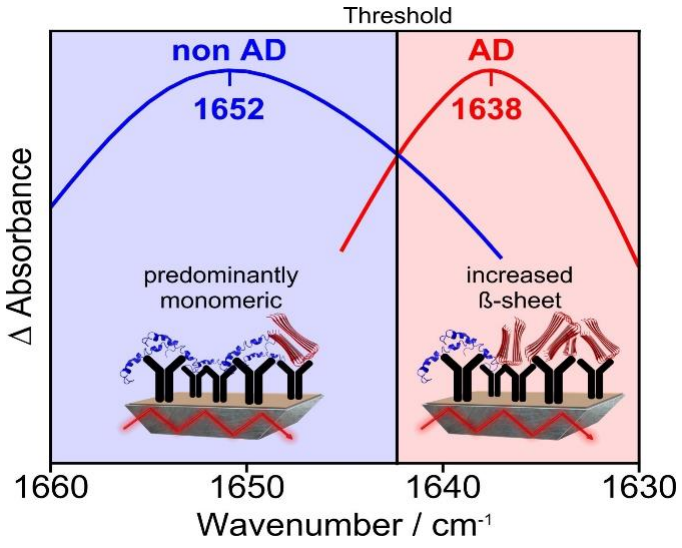

Nabers, Ollesch, Schartner, Kötting, Genius, Hafermann, Klafki, Gerwert, Wiltfang, *Analytical Chemistry*, 2016

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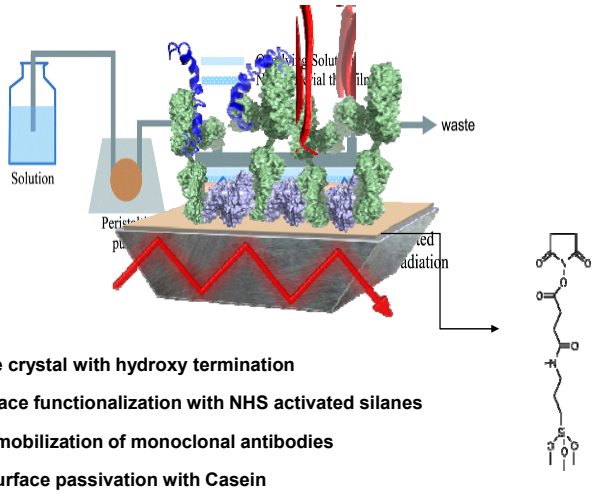
Immuno-Infrared-Sensor



1. Ge crystal with hydroxy termination
2. Surface functionalization with NHS activated silanes
3. Immobilization of monoclonal antibodies
4. Surface passivation with Casein
5. Capture of all Aß isoforms

esch, Schartner, Kötting, Genius, Haußmann, Klafki, Wiltfang, Gerwert, J. Biophotonics 2016

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klinische Studien

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Preclinical Alzheimer

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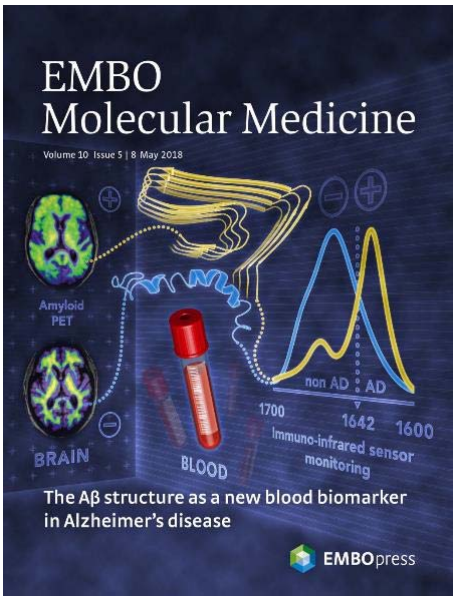

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preclinical longitudinal ESTHER over 15 years

(Prof. Dr. H. Brenner, DKFZ Heidelberg, healthy diagnosed by GP)

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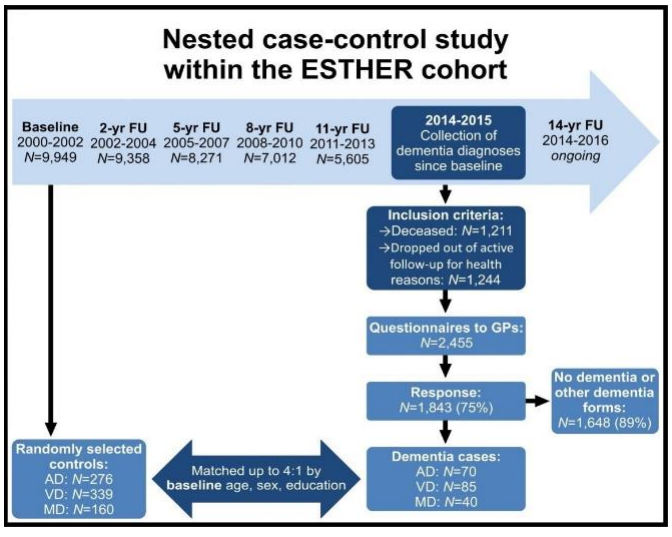

Nabers, Perna, Lange, Mons, Schartner, Güldenhaupt, Saum, Janelidze, Holleczek, Rujescu, Hansson, Gerwert, Brenner, *EMBO molecular medicine* 2018 May;10(5). pii: e8763

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ESTHER longitudinal study

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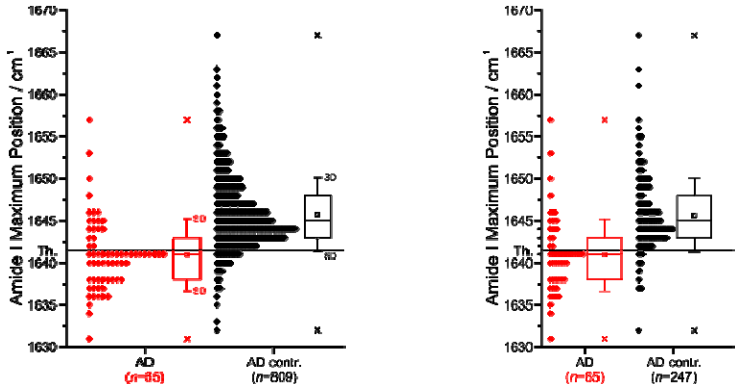
differentiation
AD ↔ DC

differentiation
AD ↔ age-, sex- , and education-matched controls



Healthy

≈ 8 years before clinical AD diagnosis

Acc. 88%
Sens. 71%
Spec. 91%

Clinical diagnosis
at baseline

on baseline blood

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ChemDetect (QCL spectrometer, Daylight solutions)

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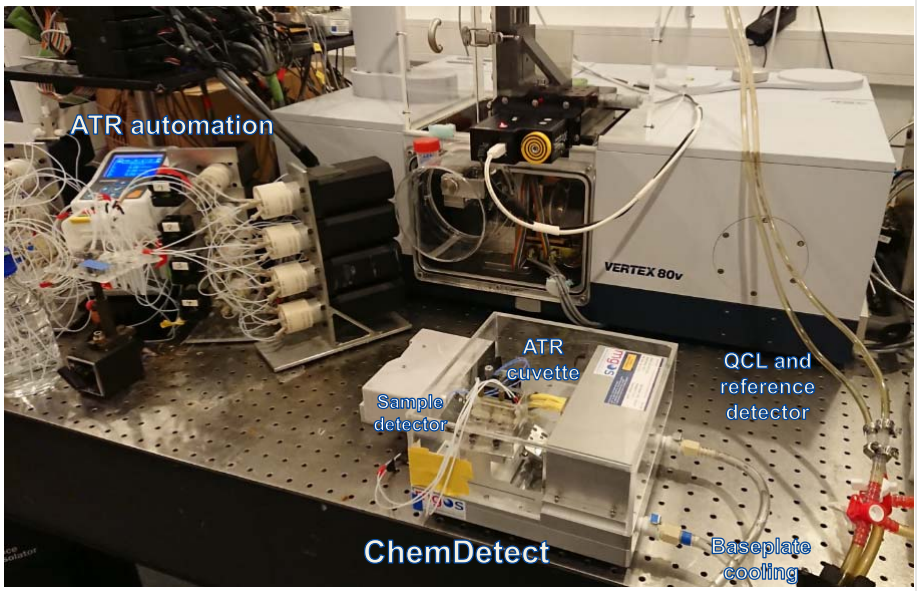

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Beteiligte Gruppen

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- Ruhr-Universität Bochum
  Fakultät für Biologie
  Lehrstuhl für Biophysik

- Ruhr-Universität Bochum
  Medizinisches Proteomcenter

- MPI für molekulare Physiologie
  Abteilung Strukturelle Biologie
  Dortmund

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Lehrstuhl für Biophysik

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Beteiligte Gruppen:

- **Klaus Gerwert**
- **Eckhard Hofmann**
- **Carsten Kötting**
- **Mathias Lübben**
- **Axel Mosig**

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Fakultät für Chemie und Biochemie

RUBiospek

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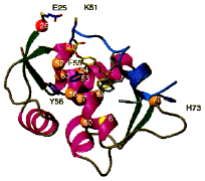
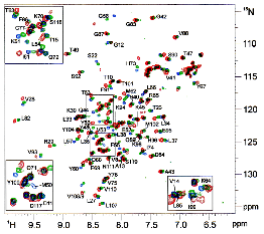
NMR-Spektroskopie an Proteinen

Raphael Stoll



Struktur und Modellierung



NMR Spektrum

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Medizinisches Proteom-Center (MPC)

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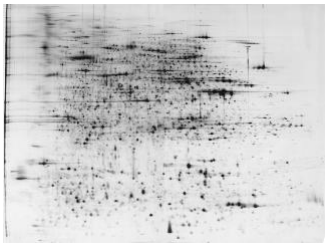
Prof. Dr. Katrin Marcus
Jun. Prof. Barabara Sitek

Ruhr-Universität Bochum



2D-Gel

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Lehrangebot
des Schwerpunkts
Proteine in der Biomedizin

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Angebot im 6. Semester

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- **Vorlesung SS**: „Aktuelle Methoden der Proteinbiochemie und Strukturbiologie“, 4 /5 CP, 2 SWS (mittwochs, 11. 15-12.45)
- **Praktikum**: Praktikum zur Spezialvorlesung Proteine in der Biomedizin, 4 CP, 5 SWS (freitags außerhalb der Vorlesungsstunden)
- **Bachelor Arbeit**: Thema aus einer Arbeitsgruppe des Schwerpunkts, 12 CP,
  8 Wochen, ganztägig

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Vorlesung 6. Semester: Verschiedene Dozenten

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Mathias Lübben	Klonierung, Expression in *Escherichia coli*, Proteinfaltung, Proteinaufreinigung, Protein-Quantifizierung
Klaus Gerwert	Einführung in die UV/Vis-, Raman- und FTIR Spektroskopie, Kinetische Analyse mit der FTIR
Carsten Kötting	Zeit- und ortsaufgelöste Spektroskopie
Ingrid Vetter, Eckhard Hofmann, Raphael Stoll	Grundlagen der Proteinstrukturbestimmung
Till Rudack	Protein Modelling
Katrin Marcus, Barbara Sitek	Protein- und Peptidtrennung Massenspektrometrie, Proteom-analyse

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Das Praktikum (6.Semester) des Schwerpunkts Proteine

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Proteine in der Biomedizin

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- **Spezial-Praktikum (4 CP)**:
- Kurspraktikum (incl. Seminar) mit 8-9 Versuchen am
  Lehrstuhl für Biophysik
  Medizinischem Proteomcenter
- Freitags von 10:15 – 17:00
- Nur Ergebnisprotokolle!
- Jedem Teilnehmer am Praktikum wird eine Stelle für die Bachelor-Arbeit in einer der am Schwerpunkt beteiligten Gruppen garantiert.

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Proteine in der Biomedizin

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Spezial-Praktikum (Konzept und Inhalte):

Methodenvorstellung am Beispiel des Bakteriorhodopsins auf zellulärer und molekularer Ebene:

- biochemische und biophysikalische Methoden- und Systemkenntnis
- Kennenlernen der Betreuer – Entscheidung für Bachelor-Arbeit erleichtern
- Aber keine obligate Voraussetzung für Bachelorarbeit!

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Vom Protein zur 3D-Struktur

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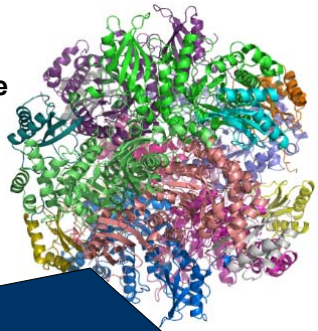
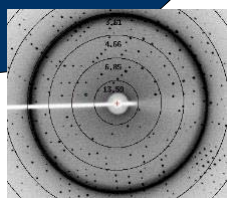
AG Proteinkristallographie
LS Biophysik

Photosynthese
Membrantransport
Pflanzenenzymologie





Prof. E. Hofmann

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RUBiospek, Raphael Stoll

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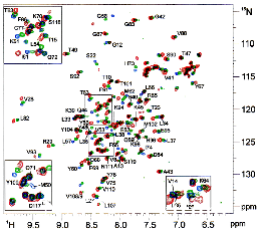
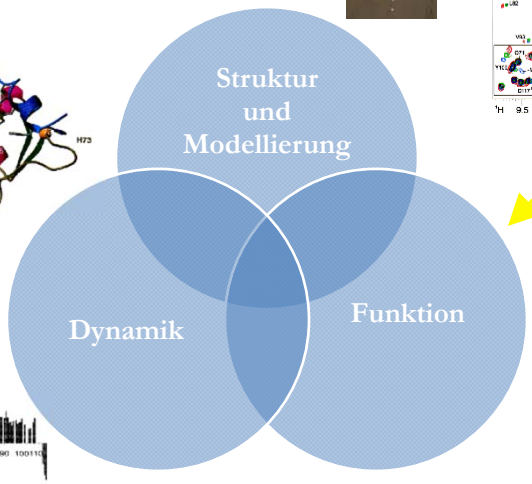
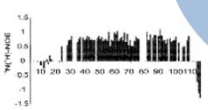
NMR-Spektroskopie an Proteinen







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Department of Biophysics: Leiter Klaus Gerwert

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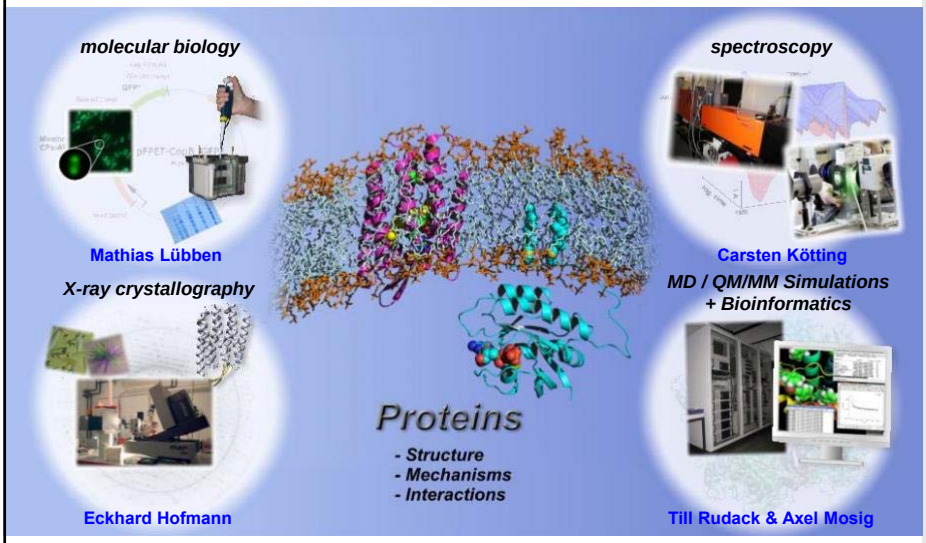

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Bakteriorhodopsin: Eine lichtgetriebene Protonenpumpe

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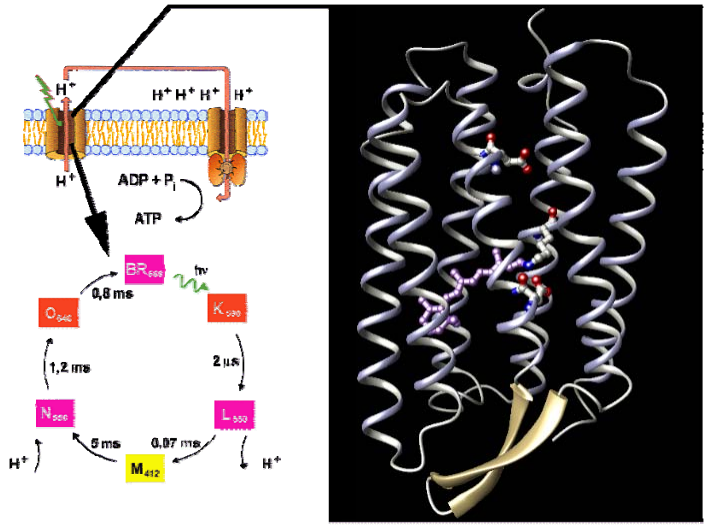

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Dioden Array Spektrometer

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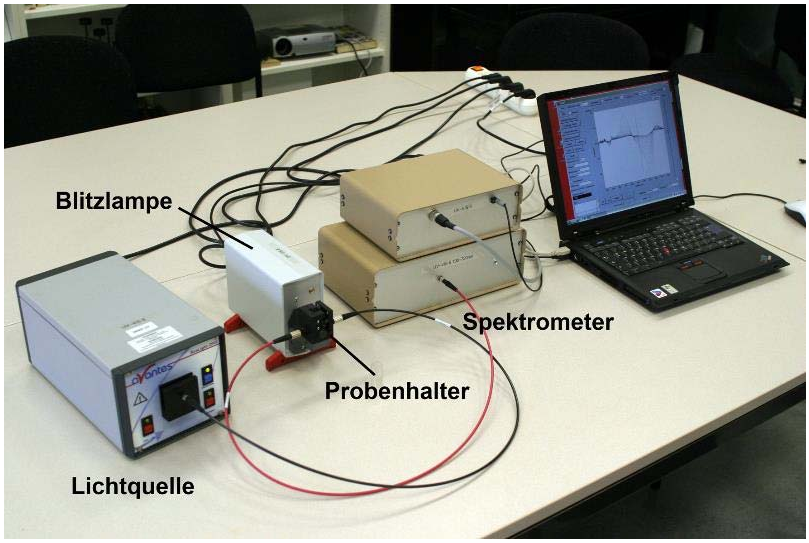

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Bioinformatik Raum ND04/99

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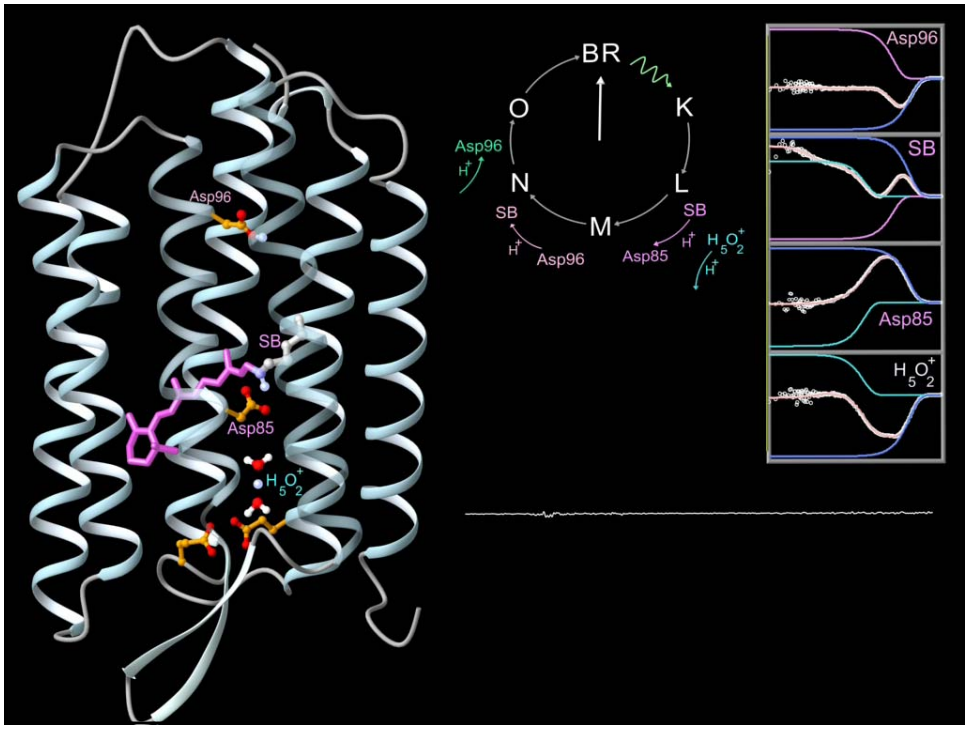

Bacteriorhodopsin, the movie

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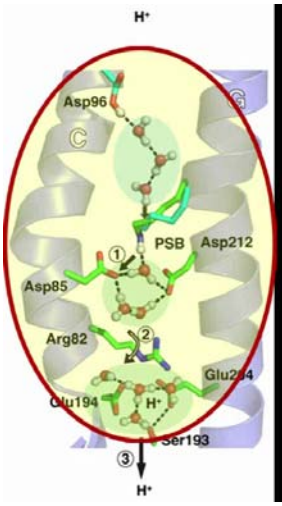

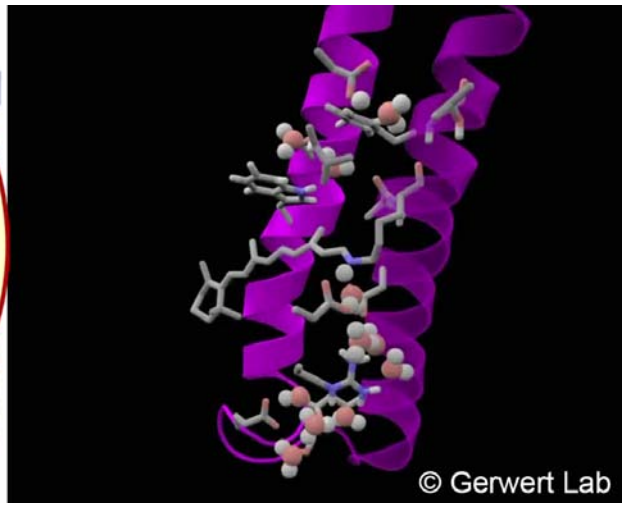

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Angebot im 6. Semester

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- **Bachelor-Arbeit**

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Angebot im 8. Semester

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- **Vorlesung**: „Proteine in der Signaltransduktion, 5 CP, 2 SWS (Freitags, 13.00-14.30 Uhr)
- **Spezial-Praktika des Schwerpunkts** 5 CP, 5 Wochen

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New application of microbial rhodopsins: *„Optogenetics“*

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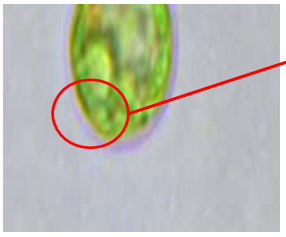

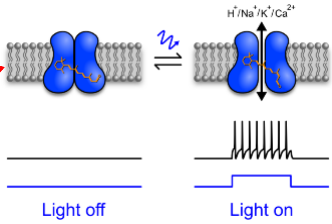

Nature Method of the year 2010

Optical control of excitable cells:

- minimally invasive
- genetically targeted
- millisecond-timescale rage

Quelle: www.optogenetics.org; www.harvesting-light.de/hegemanninfo.jpg; algaeforbiofuels.com/optogenetics-future-therapeutics/

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Medizinisches Proteom-Center (MPC)

RUB

I O P H Y S I K

Prof. Dr. Katrin Marcus

Prof. Barbara Sietek

Ruhr-Universität Bochum

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Ablauf eines 1-3-wöchigen Praktikums

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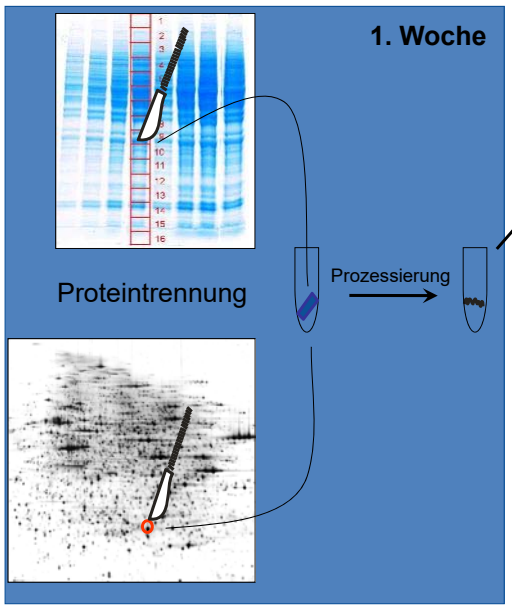
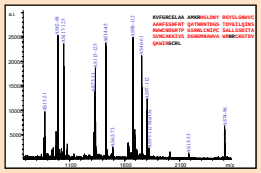


2. Woche

Massenspektrometrie



Automatische Datenbanksuche und Datenauswertung

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Angebot im 8. Semester

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IOPHYSIK

- **Masterarbeit**
- **(Doktorarbeit)**

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Alzheimer Sensor

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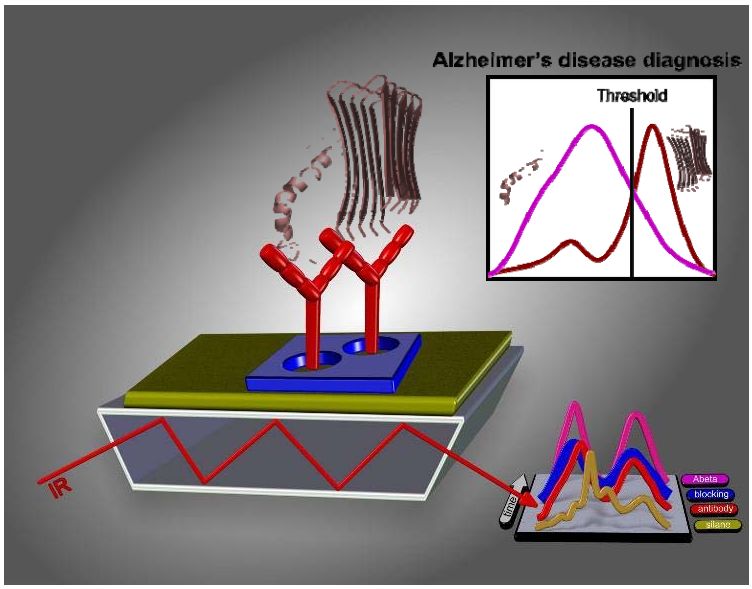

Nabers, Ollesch, Schartner, Kötting, Genius, Hafermann, Klafki, Gerwert, Wiltfang, *Analytical Chemistry*, 2016

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Schwerpunkt: Proteine

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Haben Sie Fragen zum Schwerpunkt

Proteine in der Biomedizin ?

Mathias Lübben, T: 24465
Mathias.Luebben@bph.rub.de

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