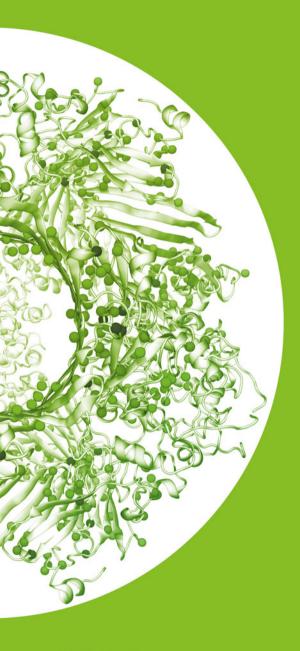
RESEARCH REPORT



LEIBNIZ FORSCHUNGSINSTITUT FÜR MOLEKULARE PHARMAKOLOGIE





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RESEARCH REPORT

2017/2018



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WELCOME!

WILLKOMMEN!



Prof. Dr. Dorothea Fiedler Director at Leibniz-Forschungsinstitut für Molekulare Pharmakologie

→ DEAR READERS,

For almost three decades, the FMP has successfully dedicated itself to basic research in the field of molecular pharmacology; in 1992 the Institute was founded in a unique historical situation from the former Institute of Drug Research of the Academy of Sciences of the GDR. Group leaders who had been working at the Institute from the very beginning left for a well-deserved retirement: Margitta Dathe, Eberhard Krause, Burkhard Wiesner and Ingolf Blasig. A big thank you for years of successful research!

The technology platforms will be continued. In 2017 Fan Liu already started as group leader and head of the proteomics platform. Her group "Structural Interactomics" has tested a new, promising method to decipher protein networks. The group "Cellular Imaging" is led by a new group leader Martin Lehmann and the senior scientist Dmytro Puchkov. They will further develop the technologies of light and electron microscopy and dedicate themselves to new projects.

An exciting phase is now beginning at EU-OPENSCREEN, as the European Commission has awarded the consortium the prestigious status of European Research Infrastructure Consortium (ERIC). The FMP as initiator remains connected to EU-OPENSCREEN as a partner site and is delighted about its stay on the Campus Berlin-Buch.

The campus is thus becoming even more attractive for research at the moment: several new buildings are being constructed right on our doorstep. The

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planning and construction work for the Cryo-Electron Microscopy building and the NMR III building has already started. It will be equipped with a new spectrometer with the highest field strength currently available at 1.2 gigaherz. In addition, the construction of an Optical Imaging Center of the Max Delbrück Center for Molecular Medicine (MDC) has begun, with access for FMP scientists.

Our goal of identifying new bioactive molecules and characterizing the interactions with their biological targets in cells or organisms continues to be pursued on a daily basis. Read more about our current research and its highlights in this report!

I hope you enjoy reading it! Dorothea Fiedler

→ LIEBE LESER*INNEN,

seit fast drei Jahrzehnten widmet sich das FMP erfolgreich der Grundlagenforschung im Bereich der Molekularen Pharmakologie; entstanden ist das Institut 1992 in einer einzigartigen historischen Situation aus dem ehemaligen Institut für Wirkstofforschung der Akademie der Wissenschaften der DDR. Gruppenleiter*innen, die von Beginn an dabei waren, verabschiedeten sich nun in den wohl verdienten Ruhestand: Margitta Dathe, Eberhard Krause, Burkhard Wiesner und Ingolf Blasig. Ein großes Dankeschön für jahrelange erfolgreiche Forschung!

Die Technologieplattformen werden weitergeführt, und bereits 2017 startete Fan Liu als Gruppenleiterin und Chefin der Proteomik-Plattform. Ihre Gruppe "Strukturelle Interaktomik" hat ein neues, vielversprechendes Verfahren erprobt, um Protein-Netzwerke zu entschlüsseln. Die Gruppe "Zelluläre Bildgebung" wurde mit Martin Lehmann als Gruppenleiter und Dmytro Puchkov als Senior Scientist neu besetzt, sie werden die Technologien der Licht-und Elektronenmikroskopie weiterentwickeln und sich neuen Projekten widmen.

Eine spannende Phase beginnt jetzt bei EU-OPENSCREEN, denn die Europäische Kommission verlieh dem Konsortium den begehrten Status European Research Infrastructure Consortium (ERIC). Das FMP als Initiator bleibt als Partnersite weiterhin mit EU-OPENSCREEN verbunden und freut sich über dessen Verbleib auf dem Campus Berlin-Buch.

Insgesamt wird der Campus im Moment für die Forschung noch attraktiver: Gleich mehrere neue Gebäude entstehen direkt vor unserer Haustür. Die Planungen und Bauarbeiten für das Gebäude der Cryo-Elektronenmikrokopie und für das NMR III-Gebäude starteten bereits. Letzteres wird ein neues Spektrometer beherbergen, mit der zurzeit größten verfügbaren Feldstärke von 1,2 Gigaherz. Zudem hat die Errichtung eines Optical Imaging Center des Max-Delbrück-Centrum für Molekulare Medizin (MDC) begonnen, mit Zugang für FMP Wissenschaftler*innen.

Unserem Ziel, neue bioaktive Moleküle zu identifizieren und die Wechselwirkungen mit ihren biologischen Zielstrukturen in Zellen oder Organismen zu charakterisieren, sind wir weiterhin täglich auf der Spur. Lesen Sie in diesem Bericht mehr über unsere aktuelle Forschung und deren Highlights!

Ich wünsche Ihnen viel Freude dabei! Dorothea Fiedler

FORSCHUNGSBERICHT 2017/2018 VORWORT 05

RESEARCH HIGHLIGHTS

In 2017/2018, our researchers published 224 articles, including 191 peer-reviewed articles in scientific journals. In the following we are presenting some highlights.

In den Jahren 2017/2018 veröffentlichten unsere Forschenden 224 Publikationen, darunter 191 referierte Beiträge in Fachzeitschriften. Im Folgenden stellen wir Ihnen einige Highlights vor.

VRACS INCREASE SUGAR SENSITIVITY OF INSULIN-PRODUCING CELLS

Insulin-producing β -cells of the pancreas have sensory capabilities and monitor the blood glucose level. When the concentration of glucose increases, these cells secrete insulin to adjust blood glucose levels. A team led by Thomas J. Jentsch now demonstrated that upon exposure to high glucose levels β -cells swell, leading to opening of volume-regulated anion channels (VRACs), depolarization and increased insulin secretion. These results were confirmed in a mouse model in which his team specifically deleted VRAC in β -cells.

VRACS ERHÖHEN ZUCKEREMPFINDLICHKEIT INSULIN PRODUZIERENDER ZELLEN

Insulin produzierende Zellen, β-Zellen des Pankreas, messen den Glukosespiegel im Blut. Steigt er an, schütten sie entsprechend viel Insulin aus, um den Blutzuckerspiegel anzupassen. Das Team um Thomas J. Jentsch konnte nun zeigen, dass erhöhte Glukosekonzentration zur Schwellung von β-Zellen, Depolarisation, und erhöhter Insulinsekretion führt. Diese Resultate wurden in vivo in einem Mausmodell bestätigt, in dem die Gruppe den VRAC Kanal spezisch in β-Zellen eliminiert hatte.

WHEN BACTERIA BUILD SHELTERS

Biofilms are a potential hazard for people because inside these protective shelters, bacteria are safe from attack by the immune system or antibiotics. To hinder the formation of biofilms, scientists need to know the structure of the building blocks. A team led by Hartmut Oschkinat watched the structural transition of the involved protein (TasA) upon biofilm formation. Further studies will help scientists to reveal the secret of biofilm stability. The next results may offer new approaches to fighting pathogens.

WENN BAKTERIEN EIGENE GERÜSTE BAUEN

Biofilme können für den Menschen eine Gefahr darstellen, da sie Krankheitserregern Schutz vor den Waffen des Immunsystems und vor Antibiotika bieten. Um die Ausbildung von Biofilmen zu hemmen, ist es notwendig, die Struktur der Bausteine zu kennen. Ein Team um Hartmut Oschkinat konnte nun strukturelle Umwandlungen des Proteins TasA bei der Bildung der Biofilme detektieren. Weitere Untersuchungen sollen dazu beitragen, die Stabilität der Biofilme besser zu verstehen und vielleicht sogar neue Ansätze im Kampf gegen Krankheitserreger zu finden.

Diehl, A., Roske, Y., Ball, L., Chowdhury, A., Hiller, M., Moliere, N., Kramer, R., Stoppler, D., Worth, C.L., Schlegel, B., Leidert, M., Cremer, N., Erdmann, N., Lopez, D., Stephanowitz, H., Krause, E., van Rossum, B.J., Schmieder, P., Heinemann, U., Turgay, K., Akbey, U., and Oschkinat, H. (2018). Structural changes of TasA in biofilm formation of Bacillus subtilis. Proc Natl Acad Sci U S A. 115, 3237-3242.

OFF-SWITCH FOR MTOR KINASE

As the cell's molecular control center, the mTor kinase regulates cellular metabolism, growth and division. In cells affected by pathological change, this regulation goes astray. Volker Haucke's team now succeeded in identifying a crucial off-switch for the mTOR complex that sheds light on one of the riddles in basic cell biology. In addition, the new insights are of relevance for clinical research as they bolster the hopes of patients waiting for new effective therapies against diabetes, obesity, cancer, and a rare congenital muscular disease.

EIN AUSSCHALTER FÜR DIE MTOR-KINASE

Die mTor-Kinase ist eine molekulare Schaltzentrale in der Zelle, die Stoffwechsel, Zellteilung und Zellwachstum reguliert. Bei krankhaften Veränderungen ist eine normale Regulation jedoch nicht mehr gegeben. Das Team von Volker Haucke hat nun einen entscheidenden "Aus"-Schalter für den mTor-Komplex gefunden und damit ein großes Rätsel der Grundlagenforschung gelöst. Zudem sind die neuen Erkenntnisse für die klinische Forschung relevant. Sie lassen auf neue Therapien gegen Diabetes, Fettleibigkeit, Krebs und eine seltene angeborene Muskelkrankheit hoffen.

Marat, A.L., Wallroth, A., Lo, W., Müller, R., Norata, G.D., Falsaca, M., Schultz, C., Haucke, V. (2017) mTORC1 activity repression by late endosomal phosphatidylinositol 3,4-bisphosphate. **Science**, **356**, **968**-**972**.

Stuhlmann, T., Planells-Cases, R., Jentsch, T.J. (2018) LRRC8/VRAC anion channels enhance β -cell glucose sensing and insulin secretion. Nat Commun. 9, 1974.

MAKING HALOGEN-AROMATICS INTERACTIONS MEASURABLE FOR THE FIRST TIME

Halogens - such as chlorine, bromine or iodine - occur in nature and in medicines and interact strongly with aromatic amino acids. This interaction, which until recently attracted little attention, plays a decisive role in enabling the receptor to recognise and bind the molecules. The team led by Marc Nazaré and Han Sun has now been able to use molecular "scales" to measure the strength of this individual interaction independently of the protein environment and finally better understand it. The new findings can be used to design active substances for new drugs.

HALOGEN-AROMATEN WECHSELWIRKUNGEN ERSTMALS MESSBAR MACHEN

Halogene - etwa Chlor, Brom oder auch Jod - kommen in der Natur und in Medikamenten vor und interagieren stark mit aromatischen Aminosäuren. Diese bis vor kurzem nur wenig beachtete Wechselwirkung trägt entscheidend dazu bei, dass die Moleküle vom Rezeptor erkannt und gebunden werden. Das Team um Marc Nazaré und Han Sun konnte nun mithilfe molekularer "Waagen" erstmalig die Stärke dieser einzelnen Wechselwirkung unabhängig von der Proteinumgebung messen und sie so besser verstehen. Die neuen Erkenntnisse lassen sich für das Wirkstoffdesign für neue Medikamente nutzen.

Sun, H., Horatscheck, A., Martos, V., Bartetzko, M., Uhrig, U., Lentz, D., Schmieder, P., Nazaré, M. (2017), Direct Experimental Evidence for Halogen-Aryl n Interactions in Solution from Molecular Torsion Balances. Angew Chem Int Ed Engl. 2017 Jun 1;56 (23):6454-6458.

DECIPHERING PROTEIN NETWORKS

The cell cannot function without the smooth functioning of highly complex protein structures. But how do proteins exchange information? The team around Fan Liu has made a contribution to solving this question. Using cross-linked mass spectrometry, she investigated the protein network in mitochondria, the cell's "power plant", in order to find out how the proteins are arranged there. The method, which was used for the first time, turned out to be very efficient and reliable - and will be used in the next step to reveal changes in mitochondrial protein structures.

PROTEIN-NETZWERKE ENTSCHLÜSSELN

Ohne das reibungslose Funktionieren hochkomplexer Protein-Strukturen geht nicht viel in der Zelle. Doch wie tauschen Proteine Informationen aus? Einen Beitrag zur Lösung dieser Frage liefert das Team um Fan Liu. Sie untersuchte mittels vernetzter Massenspektrometrie das Protein-Netzwerk in Mitochondrien, dem "Kraftwerk" der Zelle, um herauszufinden, wie die Proteine dort angeordnet sind. Die erstmals angewandte Methode zeigte sich dabei sehr effizient und verlässlich - und soll im nächsten Schritt genutzt werden, um Veränderungen in mitochondrialen Protein-Strukturen aufzudecken.

Liu, F., Lössl, P., Rabbitts, B.M., Balaban, R.S., Heck, A.J. (2018) The interactome of intact mitochondria by cross-linking mass spectrometry provides evidence for co-existing respiratory supercomplexes. Mol Cell Proteomics 17(2), 216-232.

EU-OPENSCREEN RECEIVES ERIC STATUS

The European Commission awards the EU-OPENSCREEN consortium the coveted status of European Research Infrastructure Consortium (ERIC). In the future partner laboratories can use the 140,000 chemical substances from the drug library for extensive biological tests with cell lines in which thousands of experiments are conducted in parallel. In the EU Horizon 2020 calls for proposals in 2018, all four submitted applications by the ERIC were evaluated positively. Over the next four years, EU-OPENSCREEN will coordinate a Europe-wide project with 33 partners and a total volume of five million euros. Initiatior and now partner institute FMP congratulates to this success!

EU-OPENSCREEN ERHÄLT ERIC-STATUS

Die Europäische Kommission verlieh 2018 dem Konsortium EU-OPENSCREEN den begehrten Status European Research Infrastructure Consortium (ERIC). Zukünftig können Partnerlabore die 140.000 chemischen Substanzen aus der Wirkstoffbibliothek für umfangreiche biologische Tests mit Zell-Linien nutzen, bei denen tausende Experimente parallel ablaufen. Bei den EU-Horizon 2020 Ausschreibungen in 2018 wurden die vier eingereichten Anträge des ERIC positiv begutachtet. So wird EU-OPEN-SCREEN in den nächsten vier Jahren unter anderem ein europaweites Projekt mit 33 Partnern und einem Volumen von fünf Millionen Euro koordinieren. Initiatior und jetzt Partnerinstitut FMP gratuliert zum Erfolg!

AWARDS

2017

→ Thomas Jentsch is named Honorary Doctor by the University Medical Center Hamburg-Eppendorf (UKE). From 1988 to 2006, he was based at the UKE's Center for Molecular Neurobiology Hamburg (ZMNH), and headed the institute for several years.

Thomas Jentsch ist zum Ehrendoktor der Medizinischen Fakultät des Universitätsklinikums Hamburg-Eppendorf (UKE) ernannt worden. Thomas Jentsch war von 1988 bis 2006 am Zentrum für Molekulare Neurobiologie Hamburg (ZMNH) des UKE tätig und leitete das Zentrum zeitweise auch.

→ ERC Advanced Grant: Thomas Jentsch receives for the second time the prestigious award from the European Research Council. Jentsch will examine the importance of the VRAC channel, which has been molecularly identified by his group a few years ago.

Thomas Jentsch erhält einen weiteren ERC Advanced Grant. In seinem zweiten geförderten ERC-Projekt wird Jentsch die Bedeutung des erst vor wenigen Jahren von seiner Gruppe molekular identifizierten VRAC-Kanals für den Körper genauer untersuchen.

→ Tatiana Korotkova receives an ERC Consolidator Grant.

Tatiana Korotkova erhält den ERC Consolidator Grant.

→ Volker Haucke receives the Avanti Award in Lipids from the American Society for Biochemistry and Molecular Biology (ASBMB) for his research on the role of membrane lipid homoeostasis during cell transport.

Volker Haucke erhält den Avanti Award in Lipids für seine Forschung zur Rolle der Membran-Lipid-Homöostase während des Zelltransports von der American Society for Biochemistry and Molecular Biology (ASBMB).

- → In recognition of his scientific achievements Volker Haucke was elected as a member of the National Academy of Sciences Leopoldina in June 2017. In Anerkennung seiner wissenschaftlichen Leistungen wurde Volker Haucke im Juni 2017 zum Mitglied der Nationalen Akademie der Wissenschaften Leopoldina gewählt.
- → Volker Haucke is a newly elected member of the Berlin-Brandenburg Academy of Sciences and Humanities.

Die Berlin-Brandenburgische Akademie der Wissenschaften (BBAW) hat Volker Haucke als ordentliches Mitglied neu in die Akademie gewählt.

→ Volker Haucke is granted funding in the Reinhart Koselleck program of the DFG for highly innovative research on the mechanisms of neuronal communication.

Volker Haucke erhält eine Förderung von 900.000 Euro im Reinhart Koselleck-Programm der DFG für hochinnovative Forschung an Mechanismen der neuronalen Kommunikation.

→ A team of researchers from the Ludwig Maximilian Universität München (LMU Munich) and the FMP receives the recipients of one of five "m4 Awards" provided by the Bavarian Ministry of Economic Affairs. The team receives 500,000 Euro for the project "Tubulis Therapeutics". The German Leibniz Association honored the joint project with the Leibniz Entrepreneur Award 2018. The prize is endowed with 50,000 Euro.

Ein Team aus Forschenden der Ludwig-Maximilians-Universität München (LMU) und des FMP hat einen der fünf "m4 Awards" des bayerischen Wirtschaftsministeriums gewonnen und wird mit 500.000 Euro für sein Projekt "Tubulis Therapeutics" gefördert. Das Gründungsvorhaben ist zudem mit dem Gründerpreis der Leibniz-Gemeinschaft 2018 ausgezeichnet worden. Der Preis ist mit 50.000 Euro dotiert.

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2018

- → The European Peptide Society honors scientist Christian Hackenberger with the Leonidas Zervas Award 2018 for his outstanding success in the peptide research.
 - Christian Hackenberger erhält für seine Erfolge im Bereich der Peptidforschung den Leonidas Zervas Award 2018 von der European Peptide Society.
- → The "Society Needs Science" award, sponsored by Stifterverband, goes to two separate winners this year: Thomas J. Jentsch from the FMP and Macartan Humphreys from the WZB Berlin Social Science Center. The award, worth 50,000 Euro in total, is conferred biannually to outstanding scientists whose research results bear high application potential and are of fundamental societal relevance.
- Thomas J. Jentsch erhält gemeinsam mit Macartan Humphreys vom Wissenschaftszentrum Berlin für Sozialforschung (WZB) den diesjährigen Wissenschaftspreis des Stifterverbandes "Gesellschaft braucht Wissenschaft". Der Preis wird alle zwei Jahre an Forscherinnen und Forscher vergeben, deren Arbeiten sich durch besondere gesellschaftliche Relevanz und gute Umsetzbarkeit auszeichnen. Er ist mit insgesamt 50.000 Euro dotiert.
- → Volker Haucke receives the Feldberg Prize 2020, which is awarded annually by the Feldberg Foundation for anglo-german scientific exchange. Volker Haucke erhält den Feldberg-Preis 2020. Die Auszeichnung wird jährlich von der Feldberg Foundation for anglo-german scientific exchange verliehen.

APPOINTMENTS

BERUFUNGEN

2018

- → Fan Liu's proposal for the Leibniz Programme for Women Professors was successful. An appointment procedure for a joint appointment of Fan Liu as W2-S Professor of Structural Interactomics together with the Charité is in progress.
 - Der Antrag im Leibniz-Professorinnenprogramm für Fan Liu war erfolgreich. Ein Berufungsverfahren für eine gemeinsame Berufung von Fan Liu als W2-S Professorin für Strukturelle Interaktomik gemeinsam mit der Charité ist eröffnet.
- Alexey Ponomarenko has accepted a call on a W2 professorship for Physiology at the Medical Faculty in Erlangen.
 - Alexey Ponomarenko hat einen Ruf auf eine W2-Professur für Physiologie an der Medizinischen Fakultät in Erlangen angenommen.
- → Tatiana Korotkova holds a chair (W3) in Physiology at the Medical Faculty of the University of Cologne. Tatiana Korotkova ist Inhaberin eines Lehrstuhls (W3) für Physiologie an der Medizinischen Fakultät an der Universität zu Köln.



→ Since October 2018, Philipp Selenko is professor at the Weizmann Institute in Rehovot, Israel. Philipp Selenko ist seit Oktober 2018 Professor am Weizmann-Institut in Rehovot, Israel.

2017

→ Andrew Plested was appointed Heisenberg professor at the Humboldt-Universität zu Berlin. Andrew Plested ist Heisenberg-Professor an der Humboldt-Universität zu Berlin.

MOLECULAR PHYSIOLOGY AND CELL BIOLOGY

BEREICH

MOLEKULARE PHYSIOLOGIE UND ZELLBIOLOGIE

→ Life is based on complex cellular and physiological mechanisms and their well-orchestrated interplay. In the case of disease, this interplay becomes unbalanced. Research in the "Molecular Physiology and Cell Biology' section aims at understanding such mechanisms in molecular detail, as well as their dysfunction in disease. Cellular targets for pharmaceutical intervention, many of them membrane proteins such as ion channels and G-protein-coupled receptors, are identified, studied in their physiological environment, and their modulation by bioactive compounds explored. According to our mission to create a broader basis for pharmacology, we focus on the study of less-explored membrane proteins and of molecules of key importance for intracellular trafficking. We largely focus on neurobiology, with several groups working on trafficking and signal transduction at the synapse. Other research topics include protein aggregation and degradation, processes relevant for many neurodegenerative diseases, as well as ion channels and signaling receptors whose importance extends to tissues beyond the nervous system.

We employ a broad arsenal of techniques, ranging from molecular and cellular biology, to biochemistry and biophysics, to whole-animal physiology using genetically modified mice, often with links to human disease. Our projects have benefitted greatly from interactions with other sections of the FMP, including those concerned with structural biology and modeling, drug and siRNA screening, as well as chemical biology.

→ Leben gründet sich auf komplexe zelluläre und physiologische Mechanismen und deren optimal abgestimmtes Zusammenspiel. Gerät dieses aus dem Gleichgewicht, entstehen Krankheiten. Das Verständnis dieser Mechanismen im molekularen Detail sowie deren Störung bei Krankheit ist Ziel der Forschung im Bereich "Molekulare Physiologie und Zellbiologie". Zelluläre Zielmoleküle (Targets) für eine pharmakologische Einflussnahme, darunter viele Ionenkanäle und G-Proteingekoppelte Rezeptoren, werden identifiziert und in ihrer physiologischen Umgebung untersucht. Zudem werden bioaktive Substanzen, die diese Targets modulieren können, gesucht. Im Sinne unserer Mission, die Basis für pharmakologische Einflussnahme zu vergrößern, ist unsere Forschung darauf ausgerichtet, wenige charakterisierte Membranproteine und Schlüsselmoleküle des intrazellulären Membrantransportes zu untersuchen. Ein wichtiger Fokus unserer Arbeit ist die Neurobiologie, mit mehreren Gruppen, die Transportprozesse und Informationsverarbeitung an Synapsen untersuchen. Des Weiteren erforschen wir Proteinaggregation und -degradation, Prozesse von hoher Relevanz für viele neurodegenerative Erkrankungen sowie Ionenkanäle und Rezeptoren, deren Bedeutung über das Nervensystem hinausgehen.

Dazu setzen wir eine breite Palette von Techniken und Methoden aus Molekular- und Zellbiologie, Biochemie, Biophysik und Physiologie an Tiermodellen, in der Regel genetisch veränderten Mäusen, ein. Die untersuchten Tiermodelle sind oft mit menschlichen Krankheiten verknüpft. Die Projekte des Bereiches profitieren hier sehr von der Zusammenarbeit mit den anderen Bereichen des FMP, insbesondere mit Strukturbiologie und Modellierunng, Wirkstoff-, siRNA-Screening und chemischer Biologie.

PHYSIOLOGY AND PATHOLOGY OF ION TRANSPORT

PHYSIOLOGIE UND PATHOLOGIE DES IONENTRANSPORTS



GROUP LEADER (at the FMP since 2006) Prof. Dr. Thomas J. Jentsch

GROUP MEMBERS

Carolin Backhaus, Dr. Sandy Blin, Carlo Barbini*, Tony Daubitz, Corinna Göppner, Petra Göritz, Anika Günther*, Dr. Maja Hoegg-Beiler*, Deborah Knecht (née Elger), Janet Liebold, Dr. Karen López Cayuqueo, Jennifer Lück, Dr. Norma Nitschke, Dr. Anna Oliveras Martínez, Dr. Ian Orozco, Dr. Rosa Planells-Cases, Maya Polovitskaya, Katrin Räbel*, Patrick Seidler, Judith von Sivers*, Audrey Soria*, Dr. Florian Ullrich, Dr. Felizia Voss*, Dr. Joanna Ziomkowska

- * part of the period
- → leibniz-fmp.de/jentsch

- → We aim to understand ion transport processes from the molecular to the subcellular and cellular levels, up to the level of the organism. The latter levels are addressed through an investigation of genetic mouse models and the analysis of human genetic diseases. We are focusing on anion channels, in particular CLC Cl- channels and transporters and more recently volume-regulated VRAC channels. Key research areas are structure/function analysis, cellular neurobiology, extracellular signaling, volume regulation, and the endosomal-lysosomal system. We study many organs, including the brain, inner ear, olfactory epithelium, kidney, and endocrine organs such as the adrenal gland and endocrine pancreas. For example, we found that CIC-2 mutations can cause hypertension and that VRAC modulates insulin secretion. After our breakthrough in identifying the long-sought volume-regulated anion channel VRAC in 2014, we developed research programs to define its diverse functions. The analyses of a large collection of mouse models have already provided novel biological and medically important insights.
- → Unser Ziel ist es, Ionentransportprozesse von der molekularen über die subzelluläre und zelluläre Ebene bis zur Rolle im gesamten Organismus zu verstehen. Letzteres versuchen wir durch Untersuchung der Phänotypen von genetisch veränderten Mäusen und durch Analyse menschlicher Erbkrankheiten zu erreichen. Unser Fokus liegt dabei auf Anionenkanälen, insbesondere CLC Cl- Kanälen und -Transportern und zunehmend Volumen-regulierten VRAC-Kanälen. Wir studieren unter anderem ihre Struktur/Funktions-Beziehungen, ihre Rolle bei Signaltransduktion und zellulärer Volumenregulation und ihre Funktion in Lysosomen. Dies führt uns zu verschiedenen Organsystemen wie Gehirn, Niere und Innenohr sowie zu endokrinen Organen wie Nebenniere und Bauchspeicheldrüse. Hier konnten wir zum Beispiel nachweisen, dass CIC-2-Mutationen zu Bluthochdruck führen können und dass VRAC die Insulinsekretion moduliert. Nachdem uns 2014 der Durchbruch mit der molekularen Identifizierung des schwell-aktivierten Anionenkanals VRAC gelungen ist, haben wir breite Forschungsprogramme zur Aufklärung seiner vielfältigen Rollen entwickelt. Die Analyse einer großen Anzahl von Mausmodellen erlaubt uns schon jetzt wichtige Einblicke in bisher unbekannte physiologische Prozesse und ergibt medizinisch relevante Erkenntnisse.

→ DESCRIPTION OF PROJECTS

SIGNAL TRANSDUCTION BY VOLUME-REGULATED VRAC/LRRC8 CHANNELS

We found previously that VRACs are heteromers of up to five different LRRC8 proteins. The specific subunit composition not only determines biophysical properties, but also the range of substrates that can permeate this channel. Intriguingly, VRACs not only transport small ions such as Cl-, but also a broad range of small molecules such as amino-acids and anti-cancer drugs. We now showed that VRACs, depending on their subunit composition, transport a broad range of neurotransmitters, suggesting an important role in extracellular signal transduction (3).

A role of VRAC in (intra)cellular signaling is evident from its role in insulin secretion (Fig. 1). We found that glucose-induced swelling of pancreatic β -cells activates VRAC/LRRC8 channels. The resulting efflux of Cl⁻ ions depolarizes β -cells, thereby opening voltage-dependent Ca²⁺ channels and stimulating insulin secretion *in vitro* an *in vivo* (1).

ROLE OF THE CLC-2 CL⁻ CHANNELS IN HYPERTENSION AND OTHER PATHOLOGIES

CIC-2 is a widely expressed Cl⁻ channel that is only partially open at resting conditions. We previously showed that disruption of CIC-2 in mice leads to blindness, male infertility, and leukodystrophy (a degeneration of the white matter in brain), symptoms that were later found also in patients with CIC-2 mutations. Collaborating with M.C. Zennaro (Paris) we found that mutations activating CIC-2 lead to primary aldosteronism, a disease in which autonomous overproduction of salt-retaining aldosterone causes severe hypertension (2). The patient's mutation affects a protein region we had shown to be crucial for channel gating. Like other mutations in that region, it drastically increased Cl-currents. As Cl⁻ concentration is high in aldosterone-producing glomerulosa cells of the adrenal gland, mutant CIC-2 depolarizes these cells, causing Ca²⁺ influx and increased aldosterone synthase levels.

VRAC in insulin secretion glucose Matp metabolism osmolytes VRAC a_{inolerization} Ca²⁺

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A gain-of-function mutation in the CLCN2 chloride channel gene causes primary aldosteronism. Nat Genet. 50, 355-361.

(* corresponding)

(3) Lutter, D., Ullrich, F., Lueck, J.C., Kempa, S., Jentsch, T.J. (2017) Selective transport of neurotransmitters and modulators by distinct volume-regulated LRRC8 anion channels. J. Cell Sci. 130, 1122-1133.

SELECTED EXTERNAL FUNDING

Deutsche Forschungsgemeinschaft, SFB 740/3, TP C05, "Modules involved in endosomal/ lysosomal ion homeostasis and function", 01.2015 - 12.2018, 417.667 Euro

Deutsche Forschungsgemeinschaft, "Volumen-regulierter Anionen-Kanal VRAC und seine Rolle im Gehirn", JE 164/12-1, 01.2016 - 06.2019, 433.500 Euro

European Research Council (Horizon 2020), "Volume regulation and extracellular signaling by anion channels (VOLSIGNAL)", ERC-2016-ADG, # 740537, 10.2017 - 09.2022, 2.021.112 Euro

← FIG. 1

Model for role of VRAC in insulin secretion. Besides the canonical pathway in which glucose-derived ATP inhibits $K_{\rm ATP}$ $K^{\rm t}$ channels, VRAC modulates insulin secretion by mediating depolarizing Cl efflux in response to an increase of glucose-derived intracellular osmolytes.

MOLECULAR PHARMACOLOGY AND CELL BIOLOGY

MOLEKULARE PHARMAKOLOGIE UND ZELLBIOLOGIE



GROUP LEADER (at the FMP since 2012)
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GROUP MEMBERS

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→ HOW DO CELLS COMMUNICATE WITH EACH OTHER?

Cells communicate with each other by secreting signaling molecules and recognizing those from other cells. For example, nerve cells release neurotransmitters contained in small membrane-bounded vesicles at synaptic cell contacts to elicit responses in neighbouring cells. We are studying how nerve cells form synapses and how synaptic vesicles containing messenger substances are recycled to keep synapses up to speed in fractions of a second. If these processes are disrupted, neurological and neurodegenerative diseases such as Alzheimer's disease may occur. Not only neurons, but almost all cells in our body release or respond to signaling molecules. Growth factors such as insulin and nutrients promote cell growth and division while suppressing the degradation of metabolites. In cancer cells, this nutrient signaling pathway is often disturbed. We have deciphered cellular mechanisms that regulate nutrient signaling and thus the balance between cell growth and metabolite degradation. Understanding these mechanisms is essential for a better understanding of diseases such as cancer and diabetes. In addition, we use this knowledge to develop new pharmacological approaches for the treatment of such diseases.

→ WIE KOMMUNIZIEREN ZELLEN MITEINANDER?

Zellen kommunizieren miteinander, indem sie Signalmoleküle abgeben und solche wiederum von anderen Zellen erkennen. Nervenzellen beispielsweise setzen ihre Botenstoffe aus kleinen membranumhüllten Vesikeln bzw. Bläschen an Synapsen frei, um Antworten in ihren Nachbarzellen auszulösen. Wir studieren, wie Nervenzellen Synapsen bilden und wie synaptische Vesikel, welche die Botenstoffe enthalten, recycelt werden, um Synapsen in Bruchteilen von Sekunden auf dem Laufenden zu halten. Sind diese Abläufe gestört, kann dies zu neurologischen und neurodegenerativen Krankheiten wie der Alzheimerschen Krankheit führen. Nicht nur Neuronen, nahezu alle Zellen unseres Körpers setzen Signalmoleküle frei oder antworten auf solche. So fördern Wachstumsfaktoren wie Insulin und Nährstoffe das Zellwachstum und die Zellteilung, während sie den Abbau von Metaboliten unterdrücken. In Krebszellen ist dieser Nährstoffsignalweg oftmals gestört. Wir haben zelluläre Mechanismen entschlüsselt, welche den Nährstoffsignalweg und damit die Balance zwischen Zellwachstum und Abbau von Metaboliten regulieren. Diese Mechanismen zu verstehen, ist grundlegend für ein besseres Verständnis von Krankheiten wie Krebs und Diabetes. Zudem nutzen wir dieses Wissen, um neue pharmakologische Wege der Behandlung solcher Krankheiten zu entwickeln.

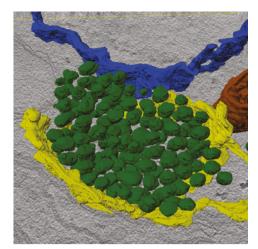


FIG. 1

False color image of a presynaptic transport vesicle packet (green) inside the cell body of a motor neuron of the fruit fly Drosophila Photography: Dr. Dmytro Puchkov, FMP. See Vukoja et al (2018).

→ DESCRIPTION OF PROJECTS

Research within the department covers two major areas: (i) the role of exo-endocytic membrane dynamics in synapse function and neuronal development and (ii) the regulation of membrane homeostasis and cell signaling by phosphoinositides. We also develop and use super-resolution light (e.g. multi-color STORM and 3D-gSTED, TIRF-SIM) and electron microscopy approaches for studying these processes.

i Exo-endocytic membrane dynamics in the functioning of synapses and in neuronal development

Neurotransmission involves the exocytic release of neurotransmitters from synaptic vesicles (SVs) at presynaptic active zones, followed by their endocytic recycling. Using mouse knockout technology, stem cell-derived human neurons, and RNA interference in combination with optical imaging including optogenetics and electrophysiology, we aim to dissect the pathways and molecular mechanisms of SV recycling, regeneration, and axonal transport of SV precursor organelles. We found that the recycling of SV membranes occurs via formin-mediated endocytosis independent of clathrin, whereas clathrin/AP-2 are required for SV reformation from internal structures. In recent work we have also discovered a novel type of lysosome-related organelle that delivers presynaptic vesicle and active zone proteins to forming synapses (Vukoja et al., 2018; Figure 1). Understanding this pathway may aid in the development of therapies against nerve injury.

ii Regulation of endocytic and endolysosomal membrane homeostasis and cell signaling by phosphoinositides

Eukaryotic cells internalize nutrients, antigens, growth factors, pathogens, ion channels and receptors via endocytosis. We found that membrane remodeling in endocytosis is tightly linked to the regulated synthesis and turnover of phosphoinositide signaling lipids (PIs) (Lo et al., 2017). Other major efforts are directed at elucidating how PI conversion along the endolysosomal pathway is linked to cell signaling processes, e.g. nutrient signaling at lysosomes. In recent studies we have discovered a mechanism for the repression of nutrient signaling by local lipid synthesis via a growth factor repressed PI 3-kinase isoform (Marat et al., 2017). As many PI-metabolizing enzymes are implicated in cancer, diabetes, or hereditary disorders such as Charcot-Marie-Tooth disease, we also seek to identify novel pharmacological and chemical inhibitors of select PI-metabolizing enzymes.

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SELECTED EXTERNAL FUNDING

Bundesministerium für Bildung und Forschung (BMBF), "Die neurobiologischen Grundlagen Polyamin-induzierter Protektion gegen altersassoziierte Einschränkungen der Gedächtnisfunktionen (SMARTAGE)", V. Haucke, 07.2015 - 06.2020, 438.924 Euro

Deutsche Forschungsgemeinschaft, Reinhart-Koselleck-Award (HA 2686/ 13-1), 1.2017 - 12.2021, 750.000 Euro

Deutsche Forschungsgemeinschaft, SFB 958, projects A01 ("Structural and functional organization of endocytic scaffolds within the periactive zone", with T. Maritzen) and A07 ("Regulation of SH3 domain-containing scaffolds in synaptic vesicle clustering"), with C. Freund), 07.2015 – 06.2019, 942.800 Euro

MOLECULAR NEUROSCIENCE AND BIOPHYSICS

MOLEKULARE NEUROWISSEN-SCHAFTEN UND BIOPHYSIK



GROUP LEADER (at the FMP since 2008) Prof. Dr. Andrew J.R. Plested

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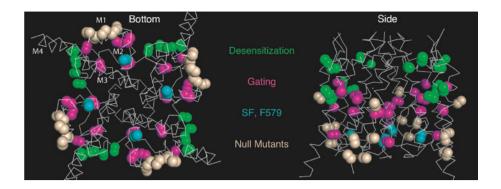
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- → Our principal research interests are glutamate receptors and the excitatory synapses in which they reside. These fine connections between nerve cells are implicated in cognition and brain disease. We aim to understand the molecular basis of fast excitatory transmission, and to develop methods to observe and alter synapse activity. To achieve these goals, we study receptor activation with a range of biophysical techniques, including electrophysiology, single-channel recording, fluorescence microscopy and computer simulations. We complement these approaches with investigations of ion channel structure and composition using chemical biology, X-ray crystallography and biochemistry. We employ computational approaches to build novel insights into receptor activation and develop our own software to analyze single receptor activity. A further aspect of our research is to extend these studies to other important components of fast signaling in the brain, such as enzymes and other ion channels.
- → Unsere Forschungsschwerpunkte sind die Glutamatrezeptoren und die exzitatorischen Synapsen, in denen sie sich befinden. Diese feinen Verbindungen zwischen Nervenzellen spielen eine Rolle bei Kognitions- und Gehirnerkrankungen. Wir wollen die molekularen Grundlagen der schnellen exzitatorischen Übertragung verstehen und Methoden zur Beobachtung und Veränderung der Synapsenaktivität entwickeln. Um diese Ziele zu erreichen, untersuchen wir die Rezeptoraktivierung mit einer Reihe biophysikalischer Techniken, darunter Elektrophysiologie, Einkanalaufzeichnung, Fluoreszenzmikroskopie und Computersimulationen. Wir ergänzen diese Ansätze durch Untersuchungen der Ionenkanalstruktur und -zusammensetzung mittels chemischer Biologie, Röntgenkristallographie und Biochemie. Wir verwenden computergestützte Ansätze, um neue Erkenntnisse über die Rezeptoraktivierung zu gewinnen, und entwickeln unsere eigene Software zur Analyse der einzelnen Rezeptoraktivitäten. Ein weiterer Aspekt unserer Forschung ist es, diese Studien auf andere wichtige Komponenten der schnellen Signalübertragung im Gehirn auszuweiten, wie Enzyme und andere Ionenkanäle.

→ DESCRIPTION OF PROJECTS

STRUCTURAL AND COMPUTATIONAL STUDIES OF ION PERMEATION IN GLUTAMATE RECEPTORS

Recently, open state structures of the AMPA-type glutamate receptor were released. These structures were in complex with the auxiliary protein Stargazin and



← FIG. 1
Gating Modules of the AMPA receptor pore domain.

Using kinetic modeling and unnatural amino acid mutagenesis, we identified three separate activation processes in the transmembrane domain (helices M1-M4). Views from beneath the channel ("Bottom", left) and the membrane ("Side", right). Residues involved in desensitization (green) and the opening of the bundled crossing in the channel core ("Gate", magenta) form contiguous groups. Peripheral residues ("Null Mutants", wheat) are not involved. Surprisingly, the region that selects for permeant ions (SF, F579; blue) has a distinct, unexpected coupling to channel gating and desensitization

included the membrane and extracellular domains of the receptor. AMPA receptors are known to open to different configurations, and some measurements of the pore geometry were estimated previously from experiments with large permeant cations. We modified these structures for all-atom simulations of ion permeation by removing the extracllular domains and restraining the loose ends to maintain an open pore. In these computational electrophysiology experiments, we could recapitulate a number of known features of the permeation of cations, including the conductance, the ion selectivity and the role of water molecules. The results were robust across different transmembrane voltages, simulation forcefields and temperatures. These simulations show that the open configuration captured in these structural experiments is a good congener of the full open conductance.

In complementary experiments, we solved crystal structures of a related cation channel from bacteria (NaK) with its selectivity filter modified to resemble that of an AMPA receptor. These structures, solved in the presence of monovalent and divalent cations, reveal distinct mechanisms of ion permeation and a conversion to two-fold symmetry. We are pursuing similar all atom simulations based on these structures to investigate ion permeation in the simplest possible system, with the eventual goal of generating and validating simulations of divalent cation permeation.

STRUCTURAL DYNAMICS OF GLUTAMATE RECEPTOR ACTIVATION

Various structures of receptors with auxiliary proteins were published in the past five years but there was limited valiadation of the unusual geometries that were found in some experiments. We examined in detail the state-dependence of the LBD layer movement in receptors with and without auxiliary proteins using bifunctional Cysteine crosslinkers. Contrary to previous reports, we found that the receptor is more compact when it is in complex with auxiliary proteins, and that these complexes are quite stable.

In the membrane domain, cysteine mutagenesis is problematic because few sites have the solution access needed to complete the experiment. To address the structural dynamics of the membrane segment, we turned to unnatural amino acids that act as UV-triggered crosslinkers. Using kinetic modeling of the UV modification we could identify the selectivity filter as an element that gates ion flow, in multiple states. Most surprisingly of all, the selectivity filter is coupled to the desensitized state.

SELECTED PUBLICATIONS

Baranovic, J., Plested, A.J. (2018) Auxiliary subunits keep AMPA receptors compact during activation and desensitization. Elife 7. pii: e40548. doi: 10.7554/eLife.40548.

Yu A.†, Salazar H.†, Plested A.J.R.*, Lau A.Y.* (2018) Neurotransmitter funnelling optimises glutamate receptor kinetics. Neuron 97, 139–149. †These authors contributed jointly. *Corresponding authors.

Riva, I.#, Eibl, C.#, Volkmer, R., Carbone, A.L.*, Plested, A.J.* (2017) Control of AMPA receptor activity by the extracellular loops of auxiliary proteins. Elife 6. pii: e28680. doi: 10.7554/eLife.28680. †These authors contributed jointly. *Corresponding authors.

SELECTED EXTERNAL FUNDING

Deutsche Forschungsgemeinschaft SFB/TRR 186 AO7 "Optical Control of Calcium Switches that Orchestrate Fast Signaling in the Brain" (with Peter Hegemann, HU Berlin) 2016 - 2020, 295.000 Euro

Deutsche Forschungsgemeinschaft DFG Research Group "Dynlon" FG 2518, P3 "Dynamics of permeation and activation of AMPA Receptors" (with Han Sun, FMP) 2017 - 2020, 193.000 Euro

European Research Council, ERC CoG 647895 "GluActive", 2015 - 2020, 1,980.000 Euro

MEMBRANE TRAFFIC AND CELL MOTILITY

MEMBRANTRANSPORT UND ZELLULÄRE MOTILITÄT



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→ HOW AND WHY CELLS REGULATE THE UPTAKE OF PROTEINS

To interact with the extracellular environment, cells rely to a large extent on cell surface-localized proteins. The binding of extracellular signaling molecules to these cell surface receptors triggers diverse intracellular signaling cascades that determine, for example, whether cells start to proliferate, to differentiate or to migrate, and also whether neurons store memories. Accordingly, the number of these signal receptors at the cell surface has to be tightly regulated to ensure the correct response to a specific extracellular cue. The internalization of specifically selected receptors via the remodeling of the surrounding cell membrane into an intracellular hollow sphere (vesicle) in the process of endocytosis constitutes a powerful mechanism for this (Figure 1). However, for many cell surface proteins it is still unclear whether they are in fact internalized by endocytosis, how they interact with the endocytic machinery, and how their internalization is regulated. We investigate the role of endocytosis proteins in the physiological function of cell surface proteins by combining cell biological, microscopic and genetic methods.

→ WIE UND WOZU ZELLEN DIE AUFNAHME VON PROTEINEN REGULIEREN Zellen interagieren mit ihrer Umgebung hauptsächlich über Proteine, die sich an der Zelloberfläche befinden. Die Bindung von Signalmolekülen an solche Zelloberflächenrezeptoren löst vielfältige Signalkaskaden in der Zelle aus. Diese veranlassen Zellen etwa, sich zu teilen, sich in einen anderen Zelltyp zu verwandeln oder sich in Bewegung zu setzen. Bei Nervenzellen bestimmen Signalkaskaden unter anderem auch, ob Erinnerungen gespeichert werden. Um in jeder Situation angemessen auf die jeweiligen Signale zu reagieren, muss die Zelle die Anzahl ihrer Signalempfänger an der Zelloberfläche exakt regulieren. Ein wichtiger Mechanismus dafür ist die Aufnahme ausgewählter Rezeptoren mittels Endozytose. Dabei werden die aufzunehmenden Proteine mithilfe von Adaptoren an eine Maschinerie geknüpft, die die Zellmembran um die Rezeptoren herum zu einem Bläschen umbaut. Dieses Transportbläschen trägt die Rezeptoren dann nach seiner Abschnürung in die Zelle (Abb. 1).

Für viele Zelloberflächenproteine ist noch nicht bekannt, ob sie tatsächlich durch Endozytose in die Zelle aufgenommen werden, wie sie mit der Endozytosemaschinerie interagieren und wie ihre Internalisierung in die Zelle gesteuert wird. Wir untersuchen die Rolle von Endozytoseproteinen für die physiologische Funktion von Zelloberflächenproteinen, indem wir zellbiologische, mikroskopische und genetische Methoden kombinieren.

→ DESCRIPTION OF PROJECTS

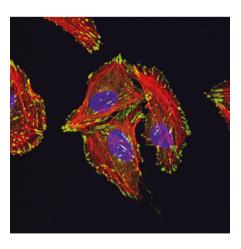
IMPORTANCE OF ENDOCYTIC PROTEINS FOR BRAIN FUNCTION

To enable us to sense our environment, execute actions and form memories, our neurons have to communicate with each other. They do so by releasing signaling molecules from membranous vesicles via their fusion with the cell membrane. These molecules then modulate receptors on the receiving neuron. For the sending neuron, endocytosis is crucial to retrieve vesicle proteins after vesicle fusion because these proteins are needed to generate new vesicles for continued signaling. For the receiving neuron, endocytosis is essential to adjust the amount of surface receptors which relay the signal into the cell because an adaptable signaling strength is the basis for learning and memory. We investigate the importance of specific endocytic proteins for efficient neuronal signaling. We have shown, for example, that the adaptor protein AP180 is crucial for the retrieval of the vesicle protein VAMP2 and thereby protects against seizures. Currently, we for example dissect the importance of the endocytic protein CALM for the uptake of neurotransmitter receptors and thus for learning.

MECHANISMS UNDERLYING FOCAL ADHESION DYNAMICS AND CELL MIGRATION

Our cells adhere to their environment via cell surface proteins which are organized together with additional factors in complex focal adhesions (FAs, Figure 2). By being transient traction points during cell migration and also signaling platforms, FAs play a role in numerous processes including the development of our organism, wound healing and immune defense. In addition, altered FAs contribute to diseases like cancer. In spite of the importance of FAs, our knowledge regarding their disassembly is still fragmentary. While we have studied the importance of endocytosis for FA disassembly, it is clear that additional processes must contribute. To identify these, we have, one-by-one, depleted all known proteins and determined the effect of their loss on FA number and size. Currently, we are investigating the function of those proteins whose loss affected FAs to unravel the mechanisms underlying FA disassembly.

Cargo Clathrin



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Jakob, B., Kochlamazashvili, G., Jäpel, M., Gauhar, A., Bock, H., Maritzen, T., Haucke, V. (2017) Intersectin 1 is a component of the Reelin pathway to regulate neuronal migration and synaptic plasticity in the hippocampus. Proc Natl Acad Sci U S A, May 23; 114(21):5533-5538.

Gerth, F., Jäpel, M., Pechstein, A., Kochlamaszashvili, G., Lehmann, M., Puchkov, D., Onofri, F., Benfenati, F., Nikonenko, A. G., Maritzen, T., Freund, C., Haucke, V. (2017) Intersectin associates with synapsin and regulates its nanoscale localization and function. Proc Natl Acad Sci U S A. Nov 7; 114(45):12057-12062.

SELECTED EXTERNAL FUNDING

Deutsche Forschungsgemeinschaft,

"Untersuchung der Bedeutung von Stonin1 für die Dynamik fokaler Adhäsionen und die Unterdrückung von Tumoren", MA 4735/2-1, 2017 – 2019, 428.330 Euro

Deutsche Forschungsgemeinschaft, "Untersuchung der Funktion der Gerüstproteine Intersectin 1 und Intersectin 2 bei der Insulinsekretion", MA 4735/3-1, SCHU 750/9-1, with A. Schürmann, 2019 – 2021, 366.830 Euro

Deutsche Forschungsgemeinschaft, "Neuronale Funktion des Endozytose-Adaptors CALM bei der Sortierung von SNAREs und AMPARs", MA 4735/1-2, HA 2686/8-2 with V. Haucke, 2019 - 2021, 387.610 Euro

← FIG. 1 (LEFT)

To take up surface-localized cargo proteins, cells employ clathrin-mediated endocytosis. In this process endocytic adaptors select cargo proteins for uptake and recruit the coat protein clathrin. Together with additional endocytic proteins clathrin reshapes a patch of cell membrane into a spherical vesicle which finally is pinched off and carries the cargo proteins into the cell.

← FIG. 2 (RIGHT)

Cells adhere to their environment via complex protein assemblies, so-called focal adhesions (green), which have to be dynamically remodelled to allow for cell migration (cell nuclei in blue, actin cytoskeleton in red).

PROTEOSTASIS IN AGING AND DISEASE

PROTEINHOMÖOSTASE IM ALTERN UND IN KRANKHEITEN



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\rightarrow UNDERSTANDING INCORRECTLY FOLDED PROTEINS IN AGING PROCESSES AND ALZHEIMER'S DISEASE

Proteins are central components of every cell. However, errors in protein folding are more frequent in humans as they grow older or suffer from neurodegenerative diseases: Proteins are often misfolded and tend to clump together and are therefore no longer functional. We want to gain a better understanding of this protein folding and focus in particular on the complexity, function and dynamics of molecular chaperones. These special proteins help in folding newly produced proteins, enable the folding of unfolded and denatured proteins and can also dissolve protein aggregates. Our group investigates the function of chaperones during aging and in neurodegenerative diseases. These diseases are characterized by the accumulation of amyloid protein fibrils in cells, i.e. long protein chains that often lead to cell death. We reproduce such scenarios in cell cultures and in the nematode C. elegans using targeted synthesis of disease-causing peptides and proteins. In addition, we have established a series of *in vitro* experiments that allow us to investigate how individual chaperones or chaperone complexes influence the structure of amyloid protein fibrils and their stability.

→ FEHLERHAFT GEFALTETE PROTEINE BEI ALTERUNGSPROZESSEN UND ALZHEIMER VERSTEHEN

Proteine sind zentrale Bausteine jeder Zelle. Bei Menschen, die älter werden oder an neurodegenerativen Erkrankungen leiden, treten häufiger Fehler bei der Protein-Faltung auf: Proteine sind oft fehlgefaltet, neigen dann zu Verklumpungen und sind somit nicht mehr funktional. Wir wollen diese Protein-Faltung besser verstehen und konzentrieren uns dabei insbesondere auf die Komplexität, Funktion und Dynamik molekularer Chaperone. Diese speziellen Proteine helfen bei der Faltung neu erzeugter Proteine, ermöglichen die Faltung ungefalteter und denaturierter Proteine und können auch Proteinaggregate wieder auflösen. Unsere Gruppe erforscht die Funktion der Chaperone während des Alterns und in neurodegenerativen Krankheiten. Diese zeichnen sich dadurch aus, dass sich in Zellen amyloide Proteinfibrillen anhäufen, also lange Proteinketten, die meist zum Zelltod führen. Solche Szenarien stellen wir in Zellkulturen und im Fadenwurm C. elegans durch gezielte Synthese der krankheitserzeugenden Peptide und Proteine nach. Zusätzlich haben wir eine Reihe von in vitro-Versuchsanordnungen etabliert, die es uns erlauben zu untersuchen, wie einzelne Chaperone oder Chaperonkomplexe die Struktur von amyloiden Proteinfibrillen und deren Stabilität beeinflussen.

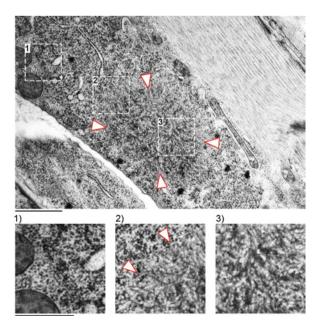
→ DESCRIPTION OF PROJECTS

COMPLETE SUPPRESSION OF HTT AMYLOID FORMATION AND DISAGGREGATION OF HTT FIBRILS BY HSP70-110-J CHAPERONE COMPLEX

Huntington's disease (HD) is a neurodegenerative disorder that is caused by expanded CAG trinucleotide repeats within the huntingtin gene (HTT) that form amyloid deposits in the cell (Figure 1). We have identified a trimeric chaperone complex composed of a member each of the Hsp70, Hsp110, and type II J-protein families that can completely suppress the formation of amyloid fibrils by HttQ. We could also demonstrate that the same chaperone complex disaggregates HttExon1Q₄₈ fibrils (Scior et al., 2018). Depletion of these chaperones in HD patient-derived neuronal progenitor cells (NPCs) lead to a pronounced increase in aggregation of the endogenous Htt protein (Q_{44}). Consequently, overexpression of DNAJB1 in HEK cells expressing robustly aggregating HttExon1Q₀₇ rescued the aggregation phenotype. Currently, we are investigating the functional spectrum of this trimeric chaperone complex. We have already demonstrated that the same chaperone complex disaggregates fibrils formed by a-synuclein. In addition, we also aim to identify additional chaperones that can remodel polyQ proteins, and have identified HSP-17 (Figure 2) as a potential aggregase facilitating the formation of aggregates. The aggregase activity could provide a beneficial role to store aggregation-prone proteins in a more inert aggregate structure.

INTERPLAY BETWEEN DISAGGREGATION AND PROTEOLYTIC CLEARANCE PATHWAYS

To eliminate protein aggregates, the cell could employ molecular chaperones to disaggregate and thereby resolubilize the sequestered proteins or degrade the aggregated proteins by the ubiquitin proteasome system (UPS) or autophagy. Little is known regarding if and how the clearance pathways communicate with each other to maintain a balanced proteostasis with the progression of aging or in disease. We observed that a depletion of disaggregating chaperones leads to an induction of autophagy. Autophagy appears to compensate for the lack of disaggregation activity as a means to cope with the accumulation of misfolded and aggregated proteins (Feleciano et al., 2019). It is noteworthy that the UPS activity and the abundance of 20S subunits decreased upon knockdown of chaperones that mediate disaggregation. Disease proteins such as $A\beta_{1.42}$ and polyQ inhibited autophagy as well the UPS capacity across tissues in a systemic manner.



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SELECTED EXTERNAL FUNDING

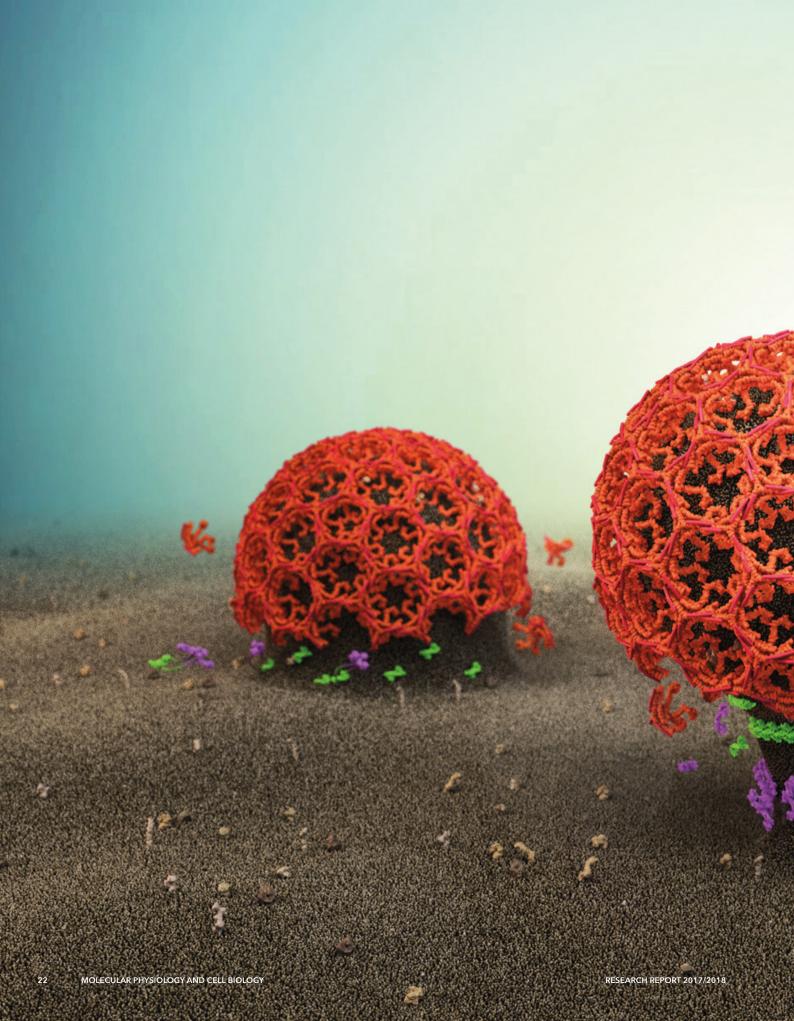
Deutsche Forschungsgemeinschaft, SFB740 TP B8, Identification and characterization of a novel eukaryotic disaggregation complex, 01/2015 - 12/2018, 222.000 Euro

Deutsche Forschungsgemeinschaft, SPP1623 KI-1988/2-1 Chemoselective Staudinger induced Michael-addition of antibodies for the analysis of protein homeostasis in C. elegans, 01/2016 -12/2018, 217.500 Euro

Deutsche Forschungsgemeinschaft, KI-1988/3-1, Aging, synapses and the neuronal cytoskelet: analyses of age-dependent regulation of the actin-binding protein Drebrin, 01/2015 - 12/2018, 203.200 Euro

- FIG. 1

Electron micrograph of a polyQ aggregate within muscle tissue of C. elegans. The image depicts a muscle cell of C. elegans with an amyloid aggregate formed by polyQ proteins. The boundaries of the aggregate are indicated by red triangles. Inset 1 depicts an area outside of the aggregate showing the presence of ribosomes and mitochondria. Inset 2 shows the interface of the aggregate and the immediate surrounding and it is apparent that the aggregate is not enclosed by a membrane. Inset 3 shows an area within the aggregate depicting the fibrils and the complete absence of ribosomes.





MOLECULAR AND THEORETICAL NEUROSCIENCES

MOLEKULARE UND THEORETISCHE NEUROWISSENSCHAFTEN



GROUP LEADER (at the FMP since 2015)
Dr. Alexander Matthias Walter

GROUP MEMBERS Andreas Till Grasskamp, Anthony McCarthy, Gabriela Pimenta Dos Reis, Dr. Mathias Böhme, Meida Jusyte, Sabine Hahn

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→ EXPLAINING THE SIGNAL TRANSDUCTION OF NERVE CELLS AT MOLECULAR LEVEL

Communication between neurons is the basis of survival, cognition and behaviour. Synaptic dysfunction is linked to many neurological diseases. We aim to understand the molecular mechanisms of synaptic transmission. We are particularly interested in understanding how synaptic molecules function together to produce the complex features of neurotransmission, which is not only optimized for speed but which can also plastically adapt to evade interference or to store information. To do so, we combine experimental and theoretical approaches. Our experimental system is the neuromuscular junction of the fruit fly Drosophila melanogaster, a powerful genetic model system. Despite its simplicity, human and Drosophila synaptic genes are remarkably similar. We measure synaptic transmission by electrophysiology and live cell imaging. In addition, super-resolution imaging enables us to uniquely define the topology of the molecular machinery driving transmission on the nanometre scale. To arrive at a conceptual framework of how synaptic molecules enable, control, and adapt synaptic transmission, we generate mathematical models based on parameters derived from our experiments. These are used to generate hypotheses which are then tested experimentally.

→ DIE SIGNALÜBERTRAGUNG VON NERVENZELLEN AUF MOLEKULARER EBENE ERKLÄREN

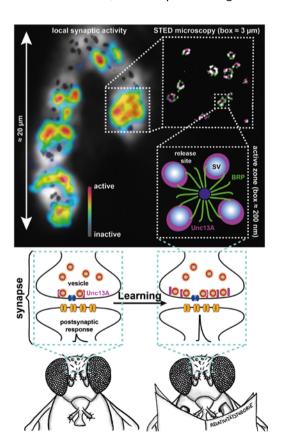
Die Kommunikation zwischen Nervenzellen ist Grundlage von Überleben, Kognition und Verhalten. Sind Synapsenfunktionen fehlerhaft, führt dies zu verschiedenen neurologischen Erkrankungen. Wir möchten die molekularen Mechanismen synaptischer Signalübertragung untersuchen. Insbesondere versuchen wir zu verstehen, wie die einzelnen Moleküle zusammenarbeiten und dazu beitragen, Informationen schnell und verlässlich zu übertragen. Darüber hinaus erforschen wir, wie Synapsen sich anpassen, um störenden Einflüssen entgegenzuwirken und um Informationen zu speichern. Wir untersuchen diese Eigenschaften mithilfe experimenteller und theoretischer Methoden an der Fruchtfliege Drosophila melanogaster, deren Synapsen den menschlichen sehr ähnlich sind. Dort messen wir Signalübertragung mit elektrophysiologischen Methoden oder visualisieren diese mit Mikroskopie. Wir benutzen hochauflösende Mikroskopie, um die "Nanomaschinerie" zu visualiseren (ein Nanometer ist ein Millionstel Millimeter), mit der Botenstoffe übertragen werden. Um zu verstehen, wie diese Komponenten zur Funktion beitragen, konstruieren wir mathematische Modelle auf Grundlage experimenteller Daten und nutzen diese, um Hypothesen aufzustellen und experimentell zu testen.

NEUROTRANSMITTER RELEASE SITES - MOLECULAR COMPOSITION AND TOPOLOGY

Neurotransmitters are released from highly specialized locations in a synapse, called release sites, where evolutionarily conserved proteins orchestrate synaptic vesicle (SV) fusion. Action potentials lead to the opening of voltage gated Ca²⁺ channels, and local Ca²⁺ influx subsequently triggers SV fusion. Therefore, the precise positioning of release sites with respect to these channels is crucial for synaptic information processing. In our lab, we investigate which molecules (proteins and signaling lipids) generate release sites and which protein-protein or protein-lipid interactions are required for their correct placement. Using physiology, genetic interference, live imaging and super-resolution STED microscopy and mathematical modeling we have begun to identify the molecular components of release sites and the interactions between synaptic proteins that operate in precise release site positioning on the nanometer scale, thereby critically determining neural function.

MOLECULAR PRINCIPLES TO REGULATE SYNAPTIC STRENGTH

A remarkable feature of synapses is their capacity to adapt their transmission. This so-called synaptic plasticity allows the nervous system to maintain communication if transmission is challenged, or to store information. In our lab, we therefore investigate the molecular components that allow adaptive transmission on multiple timescales. We particularly focus on how these plasticity mechanisms operate on the level of release sites, for example by changing release site number, or by increasing their efficacy. We investigate plasticity phenomena with physiological readouts and by imaging the changes in the local release site environment using light microscopy. We furthermore address the role of these mechanisms in paradigms to assess how different plasticity mechanisms encode animal behaviour, for example learning and memory.



SELECTED PUBLICATIONS

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SELECTED EXTERNAL FUNDING

Deutsche Forschungsgemeinschaft, TRR 186 "Molecular switches in the spatiotemporal regulation of cellular signal transduction", 06.2016 - 05.2020, 237.120 Euro

Deutsche Forschungsgemeinschaft, Emmy Noether funding, "Investigating how the active zone cytomatrix orchestrates neuronal exo- and endocytosis", 03.2015 - 02.2020, 2.051.700 Euro

FIG. 1

(Top) Illustration of synaptic activity and structure in different scale factors. Single synapse activity was determined and shown in different colours according to strength (blue = low activity, red = high activity). The sites where SVs are released (release sites) can be visualized by super resolution STED microscopy. The function of these sites requires the presence of Unc13A (magenta).

(Bottom) Simplified cartoon of the learning process, during which synaptic signal transmission changes. The release site generating molecule Unc13A plays a key role here.

Transmission can become stronger if more Unc13A is incorporated into the synapse (right), because more neurotransmitter can be released. Illustration credits: Mathias Böhme, Meida Jusyte, Andreas Grasskamp, Alexander Walter, FMP.

PROTEIN TRAFFICKING

PROTEINTRANSPORT



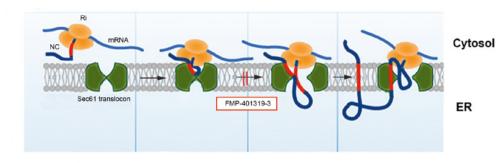
GROUP LEADER (at the FMP since 1997)
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- ightarrow INFLUENCING THE TRANSPORT OF MEMBRANE PROTEINS BY NOVEL DRUGS G protein-coupled receptors (GPCRs) are the most important drug targets. The receptors must reach their correct subcellular locations, usually the plasma membrane, in order to function. Their transport is enabled by the secretory pathway and with a signal sequence-mediated insertion of the receptors into the membrane of the endoplasmic reticulum (ER) by the Sec61 translocon complex. The aim of the Protein Trafficking group is to find novel substances that influence ER insertion of GPCRs and other integral membrane proteins at the level of the Sec61 translocon. For this purpose, a new screening assay was developed and a high-throughput screen was performed, with the help of which new inhibitors of the Sec61 translocon could be identified. These substances could represent compounds against tumors. A similar screen for the homologous bacterial SecYEG translocon could lead to the identification of novel antibiotics. Furthermore, the Protein Trafficking group is interested in the functional interaction of GPCRs with unrelated membrane proteins to identify novel mechanisms which influence GPCR signaling.
- → BEEINFLUSSUNG DES TRANSPORTS VON MEMBRANPROTEINEN DURCH NEUE WIRKSTOFFE

G-Protein-gekoppelte Rezeptoren (GPCR) sind die wichtigsten Zielproteine für Arzneimittel. Um korrekt zu funktionieren, müssen die Rezeptoren in das richtige Zielkompartiment der Zelle transportiert werden, normalerweise in die Plasmamembran. Dieser Transport wird über den sekretorischen Weg ermöglicht: Der Rezeptor wird, mit Hilfe einer Signalsequenz, über den Sec61-Translokon-Komplex in die Membran des endoplasmatischen Retikulums (ER) eingebaut. An diesem Punkt setzt unsere Arbeitsgruppe an. Wir wollen neue Substanzen finden, die die Integration von GPCR und anderer Membranproteine in die ER-Membran auf der Ebene des Sec61-Translokons beeinflussen. Hierfür haben wir ein neues Screening-Verfahren entwickelt und einen Hochdurchsatz-Screen durchgeführt, mit dessen Hilfe wir neue Inhibitoren des Sec61-Translokons identifizieren konnten. Diese Substanzen könnten neue Mittel gegen Tumoren darstellen. Ein ähnlicher Screen für das homologe, bakterielle SecYEG-Translokon könnte zur Identifizierung neuer Antibiotika führen. Darüber hinaus sind wir an der funktionellen Interaktion von GPCR mit nicht verwandten Membranproteinen interessiert, um neue Mechanismen zu identifizieren, die Einfluss auf die GPCR-Signalisierung haben.



← FI(

Mechanism of action of compound FMP-401319-3. During the early steps of the transport of a membrane protein, a complex of the ribosome (Ri, orange), mRNA (blue), and the nascent peptide chain (NC, dark blue) with its signal sequence (red) is targeted to the Sec61 translocon at the ER membrane (strongly simplified scheme without signal recognition particle, SRP receptor and other accessory components). The signal sequence engages the Sec61 translocon and mediates its opening. Transmembrane domains leave the complex laterally and are embedded into the ER membrane; later on extracellular domains are translocated through a transversal pore. Our novel compound FMP-4013193-3 blocks this translocation in a low micromolar concentration

→ DESCRIPTION OF PROJECTS

IDENTIFICATION OF INHIBITORS OF THE SEC61 TRANSLOCON BY HIGH-THROUGHPUT SCREENING

Setting up a high-throughput screening assay for inhibitors of the Sec61 translocon is notoriously difficult since the complex has no own enzymatic activity, is expressed only in the ER membrane, and is difficult to isolate. Our novel whole cell screening assay was established and performed using a primary and secondary screen in cooperation with the Screening Unit. The resulting hit compounds were analysed by substructure-based back screening in cooperation with the Medicinal Chemistry group. The hit compounds were validated using cellular assays and *in vitro* assays (reconstituted Sec61 translocon pathway). Finally, we identified a novel inhibitor (FMP-401319-3) which interferes with a step after targeting of nascent chains to the Sec61 translocon, but before the nascent chain has been translocated to the luminal side of the ER (see Fig. 1 above).

INTERACTION OF THE G PROTEIN-COUPLED TYROTROPIN RECEPTOR (TSHR) WITH THE MONOCARBOXYLATE TRANSPORTER 8 (MCT8)

Only a handful of papers suggest that GPCRs can form complexes with non-GPCR integral membrane proteins, while such interaction has not been demonstrated at a single molecule level so far. We investigated the potential interaction between the TSHR and MCT8 which are co-expressed endogenously on the basolateral plasma membrane of thyrocytes and are involved in stimulation of thyroid hormone production and release. Indeed, we demonstrated strong interaction of both proteins at the single molecule level using fluorescence cross correlation spectroscopy (FCCS). The interaction suppresses one of the TSHR signaling pathways, namely Gq-mediated signaling. Signaling by Gs, in contrast, remains unaffected. Thus, we provided not only evidence for a novel interaction between the TSHR and MCT8, we could also verify that this interaction forces biased signaling at the TSHR.

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CORE FACILITY

CELLULAR IMAGING

ZELLULÄRE BILDGEBUNG





GROUP LEADER (at the FMP since 2018)
Dr. Martin Lehmann, Light Microscopy
SENIOR SCIENTIST
Dr. Dmytro Puchkov, Electron Microscopy

GROUP MEMBERS
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Svea Hohensee, Martina Ringling

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→ EXPLORING CELLS WITH MICROSCOPY

As a technology platform, we support over 100 researchers with advanced light and electron microscopy technology, and we develop new imaging techniques for studying living and fixed cells, small organisms and tissue organization. We continuously improve our super-resolution microscopes, develop correlative light and electron microscopy to study dynamic processes in the nanometer range and measure molecular interactions by means of Förster resonance energy transfer (FRET).

THE LIGHT MICROSCOPY CORE FACILITY

The light microscopy facility supports all research groups within the FMP with fluorescence imaging technology, including labeling and analysis expertise. We apply single cell and molecular imaging techniques such as FRET, FRAP, FLIM, TIRFM, FCS, as well as lon measurements and caged compounds. Since 2018, Christopher Schmied consults and trains users in applying image analysis techniques to extract meaningful quantitative measurements. Together we establish automated imaging and quantitative image and data analysis workflows.

THE ELECTRON MICROSCOPY (EM) CORE FACILITY

The EM facility provides support in the visualisation of cellular ultrastructure and in localising individual proteins at the subcellular level. The lab provides specimen preparation techniques, EM imaging, quantitative analysis, and interpretation for all biological applications. Immunogold labeling, correlative light and electron microscopy (CLEM), and tomographical 3D reconstruction can then be applied in advanced projects. In addition, we assist with the visualization of *in vitro* structures such as proteins, fibril structures, and liposomes.

→ ZELLEN MIT HILFE DER MIKROSKOPIE ERFORSCHEN

Als Technologieplattform unterstützen wir über 100 Forschende am FMP bei Licht- und Elektronenmikroskopiemessungen und verbessern kontinuierlich unser Angebot zur Superauflösungsmikroskopie für lebende Zellen und Gewebe sowie korrelative Licht- und Elektronenmikroskopie. Ein wichtiger Anwendungsbereich unserer Arbeit ist auch, neue Zielmoleküle für pharmakologische Wirkstoffe zu identifizieren: Dafür setzen wir molekulare Interaktionsmessungen in Zellen mittels Förster-Resonanzenergietransfer (FRET) und automatisierte Mikroskopie ein.

LICHTMIKROSKOPIE

Die Lichtmikroskopie-Gruppe unterstützt alle Forschungsgruppen des FMP mit Fluoreszenz-Technologien, Beratung bei der optischen Markierung sowie Datenanalyse beim Studium lebender und fixierter Zellen, kleiner Organismen sowie von Geweben und Lösungen. Dazu etablieren wir Einzelzelltechniken basierend auf Weitfeld-, konfokaler und superauflösender Mikroskopie: FRET, FRAP, FLIM, TIRFM, FCS. Seit 2018 erhalten unsere Nutzerinnen und Nutzer Unterstützung von Christopher Schmied bei der automatischen Bildanalyse. Er etabliert automatisierte Mikroskopie und quantitative Bild- und Datenworkflows und schult Nutzerinnen und Nutzer in der Anwendung von Bildanalyse-Software.

ELEKTRONENMIKROSKOPIE

Die FMP-Service-Gruppe Elektronenmikroskopie unterstützt die Visualisierung zellulärer Ultrastrukturen und die Lokalisierung einzelner Proteine auf subzellulärer Ebene. Das Labor stellt Probenpräparationstechniken, mikroskopische Aufnahmen und quantitative Analysen für alle biologische Applikationen bereit. Immunogold-Markierungen, Korrelative Licht- und Elektronenmikroskopie (KLEM) sowie tomographische 3D-Rekonstruktionen werden bei Projekten mit vielversprechenden Phänotypen angewendet. Zusätzlich helfen wir bei der Visualisierung von *in vitro-*Strukturen bzw. Proteinen, fibrillären Strukturen und Liposomen.

SELECTED PUBLICATIONS

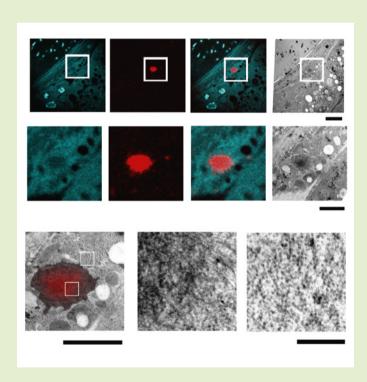
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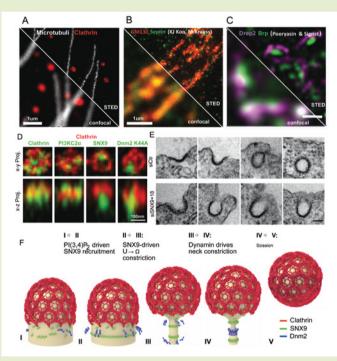
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SELECTED EXTERNAL FUNDING

Deutsche Forschungsgemeinschaft, GRK 2318 " Tight junctions and their proteins: molecular features and actions in health and disease" 10.2017 - 10.2022, 348.000 Euro







↑ FIG. 2

STED Microscopy offers increased resolution A) Microtubules and Clathrin coated pits are visualized in a fixed Cos7 cell. B) Septin and Golgimarker GM130 stained in a fixed HeLa cell, C) Pre and postsynaptic of Brp and Drep2, respectively in D.melanogaster fly brain (with AG Sigrist), scale bar 1 µm. D) 3D STORM images from Cos7 cells showing PI3KC2a, SNX9 or Dynamin localization at clathrin coated pits E) Electron micrographs of clathrin coated pits show an arrest of membrane constriction in siSNX9/18 treated cells compared to siControl, Scale bar 100nm F) Model of membrane constriction at clathrin coated Pits via PI3,4P2 mediated recruitment of SNX9

CORE FACILITY

ANIMAL FACILITY

TIERHALTUNG



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→ leibniz-fmp.de/animal_facility

- → The Animal Facility takes care of management and organization of breeding and keeping laboratory animals for use in scientific projects. Animal welfare legislation and the highest scientific standards are enforced to obtain highly relevant scientific results. We support and give advice to scientists in all questions of planning and performing experiments involving animals. We also provide practical support, for example by taking samples and in keeping proper documentation. Furthermore, we organize worldwide export and import of laboratory animals as well as embryonal stem cells.
- → Unsere Aufgabe besteht darin, Versuchstiere zu züchten und zu halten, die im Rahmen der Bearbeitung wissenschaftlicher Fragestellungen eingesetzt werden. Die Anforderungen des Deutschen Tierschutzgesetzes werden dabei umfassend berücksichtigt. Wir unterstützen und beraten die Forschenden des FMP bei allen Fragen zur Planung und Durchführung von Tierversuchen. Dazu gehört auch praktische Unterstützung, etwa bei der Probengewinnung und der Dokumentation. Zudem organisieren wir den Export und Import von Tieren und Embryonalzellen weltweit.
- → The team of the Animal Facility supports the scientists using animals for experiments in compliance with the Animal Welfare Act and with an animal welfare officer.

It is a service supplying animal environment housing and management of genetically modified mice, and also frogs and rats under performance standards, veterinary care, qualified animal care staff, using a standard operation procedure and related documentation. The service also includes also education and training of personnel responsible for animal care, of personnel carrying out experimental procedures (research technicians), and also for scientists involved in experimental trials with animals.

The aim of the team is to establish a well-planned, well-designed, well-constructed, and properly maintained laboratory animal facility that is responsible for animal care in compliance with applicable laws and regulations.

→ INTERESTS AND FOCUS

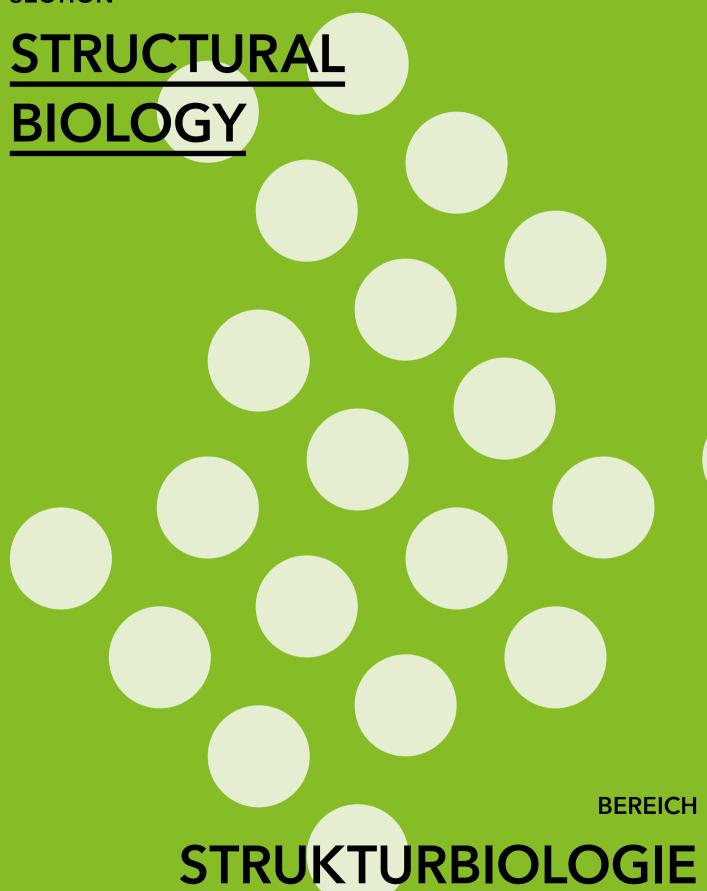
The Animal Facility helps oversee all research at the Institute that involves animals, including:

- Management and organization of breeding and care of a variety of genetically modified mice and frogs, in compliance with animal welfare regulations.
- Organization of veterinary care and health monitoring in accordance with FELASA guidelines.
- Implementation of European guidelines on animal welfare in high-quality research.
- Organization of imports and exports of animals, embryos, stem cells, and related materials.
- Supporting and consulting scientists in the design of experiments that use animals.
- Supporting scientists in implementing experiments using animals.
- Participation in training courses for scientists and other staff involved in animal experimentation.

SELECTED PUBLICATIONS

Zhao, X., Zhang, K., Daniel, P., Wisbrun, N., Fuchs, H., Fan, H. (2018) **Delayed allogeneic skin graft rejection in CD26-deficient mice.** Cell Mol Immunol. 2018 Mar 23. doi: 10.1038/ s41423-018-0009-z.

Pohlmann, A., Karczewski, P., Ku, M.C., Dieringe, B., Waiczies, H., Wisbrun, N., Kox, S., Palatnik, I., Reimann, H.M., Eichhorn, C., Waiczies, S., Hempel, P., Lemke, B., Niendorf, T., Bimmler, M. (2014) Cerebral blood volume estimation by ferumoxytol-enhanced steady-state MRI at 9.4T reveals microvascular impact of a1-adrenergic receptor antibodies. NMR Biomed. 27, 1085-1093.



- → Molecular pharmacology requires three-dimen- → In der molekularen Pharmakologie ist die Kenntnis sional representations of supramolecular arrangements within the cell, which are controlled in vivo by temporal and spatial coordination of protein expression, degradation, and post-translational modification. The dynamic nature of these phenomena challenges static structure determination techniques and implies a strong incentive to employ Nuclear Magnetic Resonance spectroscopy (NMR). In this spirit, the "Structural Biology" section develops and applies solution and solid-state NMR techniques to investigate pharmacologically relevant proteins in native environments or even in intact biological systems such as cells or functional modules. Furthermore, the insights gained for molecular interactions are used to develop NMR reporters for diagnostic imaging purposes where fluorescence detection fails. The departments of Adam Lange and Hartmut Oschkinat apply solid-state NMR to membrane proteins in native lipid bilayers, for example, and Philipp Selenko detects proteins and their modifications in live cells by solution NMR. Leif Schröder images cells or organisms by means of molecule-specific contrast agents. In concert with molecular modeling and structure function studies provided by the groups of Gerd Krause and Ronald Kühne, atomic-resolution structural data is derived that is indispensable on the path to pharmacological interference, typically supported by the "Solution NMR" group of Peter Schmieder. With regard to the design of bioactive molecules, the development of protein interaction inhibitors is a major theme of the section.
- der drei-dimensionalen Struktur supramolekularer Komplexe innerhalb der Zelle, die in vivo durch die zeitliche und räumliche Koordination von Proteinexpression, -abbau und posttranslationaler Modifikation kontrolliert wird, von entscheidender Bedeutung. Die diesen Prozessen innewohnende Dynamik ist eine große Herausforderung für statische Strukturbestimmungsmethoden und legt es nahe, Kernspinresonanzmethoden (NMR) anzuwenden. Daher entwickeln und nutzen die Forschenden des Bereichs "Strukturbiologie" Lösungs- und Festkörper-NMR-Techniken, um pharmakologisch relevante Proteine in ihrer natürlichen Umgebung oder sogar in intakten biologischen Systemen wie Zellen oder funktionellen Modulen zu untersuchen. Darüber hinaus werden die Erkenntnisse über molekulare Wechselwirkungen eingesetzt, um daraus NMR-Reporter für solche diagnostische Bildgebungszwecke zu entwickeln, bei denen Fluoreszenz-Bildgebung nicht anwendbar ist. Die Abteilungen von Hartmut Oschkinat und von Adam Lange nutzen beispielsweise Festkörper-NMR für Membranproteine in nativen Lipidmembranen, und Philipp Selenko detektiert Proteine und Proteinmodifikationen in lebenden Zellen. Leif Schröder arbeitet an der Bildgebung von Zellen und Organismen mittels spezifischer Kontrastmittel. Zusammen mit molekularem Modelling und Struktur-Funktions-Untersuchungen der Arbeitsgruppen von Gerd Krause und Ronald Kühne werden Strukturdaten mit atomarer Auflösung erarbeitet, die unverzichtbar sind, um Wege zu einer pharmakologischen Einflussnahme auf Zielproteine zu finden. Diese Untersuchungen werden gewöhnlich von der "Lösungs-NMR"-Gruppe unter der Leitung von Peter Schmieder unterstützt. Hinsichtlich des Designs von biologisch aktiven Wirkstoffen ist insbesondere die Entwicklung von Inhibitoren von Protein-Wechselwirkungen ein Leitthema des Bereichs.

FORSCHUNGSBERICHT 2017/2018 **STRUKTURBIOLOGIE**

MOLECULAR BIOPHYSICS

MOLEKULARE BIOPHYSIK



GROUP LEADER (at the FMP since 2014)
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Öster, Dr. Oxana Krylova, Dr. Reiner Haseloff,
Kitty Hendriks, Claudia Bohg, Maximilian
Zinke, Songhwan Hwang, Heike Nikolenko,
Dagmar Michl, Susanne Bischoff, Marleen van
Rossum, Stefanie Schneider, Julia Ruta, René
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→ DETECTING THE STRUCTURE OF PROTEINS

Proteins are long-chain macromolecules and usually folded three-dimensionally into a complex architecture. In order to understand the function of a protein, it is helpful to know its structure. Researchers usually study proteins in soluble or crystalline form. However, not all proteins can be investigated in this way. We also use solid-state nuclear magnetic resonance spectroscopy (solid-state NMR) to analyze the structure and dynamics of proteins. This technique allows the investigation of chemical details, interaction with water and lipid molecules, and functionally relevant protein dynamics. The last aspect is important because proteins are not rigid structures with a fixed architecture, but have moving parts similar to machines. For solid-state NMR investigations, we place the samples in a strong superconducting magnet (external field up to 20 T, i.e. ~400,000 times stronger than the earth's magnetic field), rotate them rapidly (up to 100,000 revolutions per second; magic-angle spinning) and investigate them spectroscopically using radio waves. Important applications of our work are the analysis of membrane proteins in their natural lipid environment and the 3D structure determination of molecular machines in the cell.

→ DIE STRUKTUR VON PROTEINEN ERKENNEN

Proteine sind langkettige Makromoleküle und meist dreidimensional zu einer komplexen Architektur gefaltet. Um die Funktion eines Proteins zu verstehen, ist es hilfreich, seine Struktur zu kennen. Dazu nutzen Forschende in der Regel Verfahren, bei denen die Proteine gelöst sind oder kristallisiert werden. Nicht alle Proteine lassen sich jedoch auf diese Weise untersuchen. Wir nutzen zusätzlich Festkörper-Kernspinresonanzspektroskopie (Festkörper-NMR), um die Struktur und Dynamik von Proteinen zu analysieren. Diese Technik erlaubt die Untersuchung chemischer Details, der Wechselwirkung mit Wasser- und Lipidmolekülen und der funktionell relevanten Proteindynamik. Der letzte Aspekt ist wichtig, da Proteine keine starren Gebilde mit einer festen Architektur sind, sondern ähnlich wie Maschinen bewegliche Teile besitzen. Für Festkörper-NMR Untersuchungen bringen wir die Proben in einen starken, supraleitenden Magneten (mit Feldstärken bis zu ~20 Tesla und damit ca. 400.000-mal so stark wie das Magnetfeld der Erde), versetzen sie in eine schnelle Rotation (bis zu 100.000 Umdrehungen pro Sekunde; magic-angle spinning) und untersuchen sie spektroskopisch mittels Radiowellen. Wichtige Anwendungen unserer Arbeit sind die Analyse von Membranproteinen in ihrer natürlichen Lipidumgebung und die 3D-Strukturbestimmung molekularer Maschinen in der Zelle.

STRUCTURAL BIOLOGY RESEARCH REPORT 2017/2018

→ DESCRIPTION OF PROJECTS

MEMBRANE PROTEINS

In our group, a major focus lies on membrane proteins. Distinct from other methods in structural biology, solid-state NMR makes it possible to study membrane proteins in native-like lipid bilayers at room temperature and under physiological buffer conditions. Current projects involve for instance nonselective cation channels such as NaK that are able to conduct both sodium (Na+) and potassium (K+) with equally high efficiency (see Figure). In contrast to previous crystallographic results, we could recently show that the selectivity filter of NaK in native-like lipid membranes adopts two distinct conformations that are stabilized by either Na⁺ or K⁺ ions. The atomic differences of these conformations were resolved by solid-state NMR spectroscopy and molecular dynamics (MD) simulations. We propose that structural plasticity within the selectivity filter and the selection of these conformations by different ions are key molecular determinants for highly efficient conduction of different ions in non-selective cation channels (Shi et al., Nature Communications 2018). Other membrane proteins of interest comprise the human voltage-dependent anion channel (VDAC), the histidine kinase CitA (Salvi et al., PNAS 2017), and rhomboid proteases, that initiate signal cascades by cleaving proteins within the membrane and thus releasing signal proteins that are no longer anchored.

SUPRAMOLECULAR ASSEMBLIES

Furthermore, we characterize structure and dynamics of bacterial supramolecular assemblies. For example, we have determined the structure of the bactofilin BacA by solid-state NMR. Bactofilins are a new class of cytoskeletal proteins that are involved in key cellular processes. For instance, in the human pathogen Helicobacter pylori, they are responsible for maintaining its characteristic helical cell shape, a feature required for cells to efficiently colonize the gastric mucus. We discovered that bactofilins adopt a β -helical architecture, which has not been observed before for other cytoskeletal filaments. Interestingly, however, the structure bears similarities to that of the fungal prion protein HET-s. We also introduced a general hybrid approach for determining the structures of supramolecular assemblies. Cryo-electron microscopy (cryo-EM) data define the overall envelope of the assembly and rigid-body orientation of the subunits while solid-state NMR chemical shifts and distance restraints define the local secondary structure, protein fold and inter-subunit interactions. Using this approach we could determine the structure of the type-III secretion system needle of Shigella flexneri to a very high precision.

SELECTED PUBLICATIONS

Fricke, P., Chevelkov, V., Zinke, M., Giller, K., Becker, S. and Lange, A. (2017) Backbone assignment of perdeuterated proteins by solid-state NMR using proton-detection and ultrafast magic-angle spinning. Nat Protoc., 764–782.

Salvi, M., Schomburg, B., Giller, K., Graf, S., Unden, G., Becker, S., Lange*, A., and Griesinger*, C. (2017) Sensory domain contraction in histidine kinase CitA triggers transmembrane signaling in the membrane-bound sensor. Proc Natl Acad Sci U S A., 114, 3115-3120.

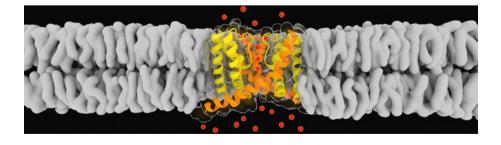
Zinke, M., Fricke, P., Samson, C., Hwang, S., Wall, J. S., Lange, S., Zinn-Justin, S. and Lange, A. (2017) **Bacteriophage tail tube** assembly studied by proton-detected 4D solid-state NMR. Angew Chem Int Ed Engl., 56, 9497-9501

SELECTED EXTERNAL FUNDING

European Research Council, ERC Starting Grant "3D structures of bacterial supramolecular assemblies by solid-state NMR" (Project acronym: assemblyNMR; Grant agreement no.: 337490); 2014-2019, 1.456.000 Euro

Deutsche Forschungsgemeinschaft,
DFG-eigene Stelle (Dr. Stamatios Liokatis)
"Post-translationale Protein Modifikationen,
Querregulierung und Auswirkungen auf
Histone H3 Tail Struktur und Dynamik: Eine
strukturelle und mechanistische Studie an
intakten Nukleosomen mittels hochauflösender Kernresonanzspektroskopie" (DFG GZ.:
LI 2402/2-2); 04/2017 - 09/2019;
281.000 Euro

Unifying Concepts in Catalysis - Cluster of Excellence, now: Unifying Systems in Catalysis (UniSysCat; DFG) "Regulated catalysis by intra-membrane proteases"; 11/2017 - 12/2022; 59.000 Euro (HU Berlin) + 422.000 Euro (FMP)



← FIG. 1

The non-selective cation channel NaK was studied by solid-state NMR and MD simulations (Shi et al., Nature Communications 2018). Artwork by Barth van Rossum, FMP.

FORSCHUNGSBERICHT 2017/2018 STRUKTURBIOLOGIE

NMR-SUPPORTED STRUCTURAL BIOLOGY

NMR-UNTERSTÜTZTE STRUKTURBIOLOGIE



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→ INVESTIGATING HIGHLY COMPLEX STRUCTURAL INFORMATION Magic-angle-spinning (MAS) solid-state NMR spectroscopy provides highresolution structural information on heterogeneous samples, independent of the molecular weight of the investigated biological macromolecules such as proteins, carbohydrates or chains of nucleic acids. It is an attractive method for structural investigations on "difficult" systems such as small proteins embedded in lipid bilayers, large polydisperse complexes (Fig. 1) or biological macromolecules in their natural environment such as in live biofilms. As special benefit, NMR enables a direct view onto protons, the connected exchange processes, and it allows for a distinction of rapidly interconverting structural states. In the long run, we aim to carry out structural investigations within the "real space" of a cell, capitalizing on a 20-100-fold increase in the signal-to-noise ratio afforded by the use of dynamic nuclear polarization (DNP). For this purpose, we have been improving and testing DNP methods on biological samples. Furthermore, we apply very fast MAS for studying protein structure. At spinning frequencies of 100,000 rotations per second, high-resolution proton spectra can be obtained using a minimal amount of sample. Fast MAS at 100 kHz was used for instance to study binding of small molecules to the neonatal Fc receptor. Finally, we investigate large dynamic and polydisperse protein systems involved in protein

homeostasis, including small heat shock proteins, and biofilms.

→ HOCHKOMPLEXE STRUKTURINFORMATIONEN UNTERSUCHEN Die Festkörper-NMR-Spektroskopie mit Magic-angle-spinning (MAS) ist in der Lage, hochauflösende Strukturinformationen von komplexen Proben biologischer Makromoleküle zu liefern, unabhängig von deren Molekulargewicht. Die MAS-NMR-Spektroskopie ist eine attraktive Methode für strukturelle Untersuchungen an kleinen Proteinen, die in Lipiddoppelschichten eingebettet sind, großen polydispersen Komplexen (Abb. 1) oder Proteinen in ihrer natürlichen Umgebung, beispielsweise in lebenden Biofilmen. Als besondere Vorteile kann NMR die Position von Protonen sowie ihren chemischen Austausch erfassen und schnell interkonvertierende Zustände charakterisieren. Da die MAS-NMR-Spektroskopie die Untersuchung heterogener Proben ermöglicht, wollen wir Strukturuntersuchungen im "realen Raum" einer Zelle durchführen, wobei wir langfristig von einer 20-100-fachen Erhöhung des Signal-Rausch-Verhältnisses durch den Einsatz der dynamischen Kernpolarisation (DNP) profitieren. Zu diesem Zweck haben wir DNP-Methoden an biologischen Proben verbessert und wenden sehr schnelle Rotation in der MAS-NMR (100.000 Rotationen pro Sekunde) zur Untersuchung

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biologischer Proben an. Bei hohen MAS-Geschwindigkeiten können mit einer minimalen Probenmenge hochauflösende Protonenspektren erhalten werden. Schnelle MAS bei 100 kHz wurde beispielsweise zur Untersuchung der Bindung kleiner Moleküle an den neonatalen Fc-Rezeptor verwendet. Desweiteren untersuchen wir große dynamische und polydisperse Proteinsysteme, die an der Proteinhomöostase beteiligt sind, einschließlich kleiner Hitzeschockproteine, sowie Biofilme.

→ DESCRIPTION OF PROJECTS

STRUCTURAL STUDIES OF TASA IN LIVE BIOFILMS

Biofilms are surface-attached communities formed by microorganisms that can serve as protection against host immune reactions or antibiotics. Bacillus subtilis biofilms contain TasA as major proteinaceous component in addition to exopolysaccharides. We characterized different oligomeric forms of TasA *in vitro* by NMR, EM, X-ray diffraction, and analytical ultracentrifugation experiments. We found by MAS NMR on live biofilms that a swift structural change toward only one of these forms occurs, consisting of homogeneous and protease-resistant, β -sheet-rich fibrils. We discovered a reorientation of flexible TasA sections when the TasA building blocks assemble into fibrils. Further studies of the fibrils will help scientists to reveal the secret of biofilm stability. The next results may offer new approaches to fighting pathogens.

INSIGHT INTO SMALL MOLECULE BINDING TO THE NEONATAL FC RECEPTOR

The neonatal Fc receptor plays a crucial role in regulation of Immunoglobulin G and serum albumin catabolism and is a clinically validated drug target for the treatment of autoimmune diseases caused by pathogenic antibodies via the inhibition of its interaction with IgG. As part of a multidisciplinary research, we used fast-MAS (100 kHz) to study the binding of small molecules to heterodimers of the neonatal Fc receptor. We were able to detect ligand-induced chemical-shift perturbations for residues in the binding pocket and allosteric changes close to the interface of the two receptors in the asymmetric unit as well as potentially in the albumin interaction site. Our investigation establishes a method to characterize structurally small molecule binding to non-deuterated large proteins by NMR, even in their glycosylated form, which may prove highly valuable in structure-based drug discovery campaigns.

SELECTED PUBLICATIONS

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Stöppler, D., Macpherson, A., Smith-Penzel, S., Basse, N., Lecomte, F., Deboves, H., Taylor, R.D., Norman, T., Porter, J., Waters, L.C., Westwood, M., Cossins, B., Cain, K., White, J., Griffin, R., Prosser, C., Kelm, S., Sullivan, A.H., Fox, D. 3rd, Carr, M.D., Henry, A., Taylor, R., Meier, B.H., Oschkinat, H., Lawson, A.D. (2018) Insight into small molecule binding to the neonatal Fc receptor by X-ray crystallography and 100 kHz magic-angle-spinning NMR. PLoS Biol 16(5): e2006192.

Retel, J.S., Nieuwkoop, A.J., Hiller, M., Higman, V.A., Barbet-Massin, E., Stanek, J., Andreas, L.B., Franks, W.T., van Rossum, B.-J., Vinothkumar, K.R., Handel, L., de Palma, G.G., Bardiaux, B., Pintacuda, G., Emsley, L., Kühlbrandt, W., Oschkinat, H. (2017) Structure of outer membrane protein G in lipid bilayers. Nat Commun, 8(1), 2073.

SELECTED EXTERNAL FUNDING

Deutsche Forschungsgemeinschaft,

SFB 740, TP B07, "Strukturuntersuchungen an Modulen der Proteinhomöostase", 01.2015 - 12.2018, 515.520 Euro

Deutsche Forschungsgemeinschaft, SFB 1078, TP B01, "Strukturelle Dynamik von Kanalrhodopsinen", 01.2017 - 12.2020, 699.121 Euro

European Commission,

EU 8. RP / H2020 iNext, Integrating Infrastructures for Structural Biology, 09.2015 - 08.2019, 438.609 Euro

← FIG.

Visualization of paramagnetic relaxation effect (PRE). A free radical attached to the C-terminus of aB-crystallin disturbs the NMR signals. This can be used to study the interaction of the C-terminus with the a-crystallin core domain in oligomers. Red balls indicate a strong PRE effect, orange an intermediate and green no effect. Cyan balls indicate the stretch where the radical is attached.

FORSCHUNGSBERICHT 2017/2018 STRUKTURBIOLOGIE

MOLECULAR IMAGING

MOLEKULARE BILDGEBUNG



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→ BIOSENSORS FOR IMPROVED MRI DIAGNOSTICS

Magnetic resonance imaging (MRI) is an indispensable tool for diagnostic imaging in drug development and therapy monitoring. Its limited sensitivity makes conventional MRI rely on the detection of tissue water. There is still the unmet need for precision medicine of visualizing the distribution of dilute molecular markers in live tissue within the context of a whole opaque organism. Thus, the leading biomedical research institutions have initiated programs to translate the potential of spin hyperpolarization techniques into diagnostic imaging. By optimizing the steps of preparation, manipulation, and encoding of spin magnetization, our group strives to design ultra-sensitive NMR reporters. A key concept is the development of biosensors that are only loaded in situ with hyperpolarized nuclei and thus significantly expand the application range for this artificially prepared state with a limited lifetime. To explore this potential, we successfully engage in highly competitive programs supporting highly innovative research. Through the Reinhart Koselleck program of the DFG and the Human Frontiers Science Program we develop methodologies to obtain MRI contrast from molecular targets at extremely low concentrations and sense molecular interactions within a few minutes where conventional MRI protocols would require thousands of years.

→ BIOSENSOREN FÜR EINE VERBESSERTE MRT-DIAGNOSTIK

Die Magnetresonanztomographie (MRT) ist ein unverzichtbares Werkzeug für die diagnostische Bildgebung bei der Arzneimittelentwicklung und für die Therapieüberwachung in der Präzisionsmedizin. Aufgrund ihrer begrenzten Empfindlichkeit ist die herkömmliche MRT auf die Detektion von Gewebswasser angewiesen. Es besteht nach wie vor die Notwendigkeit, die Verteilung verdünnter molekularer Marker in lebendem Gewebe im Gesamtkontext eines opaken Organismus zu visualisieren. Daher haben die führenden biomedizinischen Forschungseinrichtungen Programme initiiert, um das Potenzial von Spin-Hyperpolarisierungstechniken für die diagnostische Bildgebung umzusetzen. Durch die Optimierung der Schritte zur Vorbereitung, Manipulation und Kodierung der Spin-Magnetisierung arbeitet unsere Gruppe daran, ultraempfindliche NMR-Reporter zu entwickeln. Ein Schlüsselkonzept ist die Entwicklung von Biosensoren, die erst *in situ* mit hyperpolarisierten Kernen beladen werden und somit den Anwendungsbereich für diesen künstlich vorbereiteten Zustand mit einer begrenzten Lebensdauer erheblich erweitern. Um dieses Potenzial auszuloten, engagieren wir uns erfolgreich in hoch kompetitiven Programmen, die hoch innovative Forschung unterstützen. Aus Mitteln des Reinhart Koselleck-Programms

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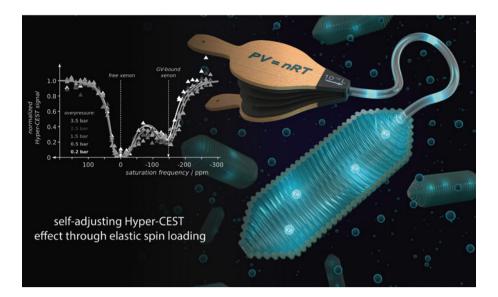


FIG. 1
 Elastic loading of self-assembling hollow protein structures following the ideal gas law yields optimized CEST agents with superior contrast behaviour.

der DFG und des Human Frontiers Science-Programms entwickeln wir Methoden, um MRT-Kontrast von molekularen Markern bei extrem niedrigen Konzentrationen zu erhalten und molekulare Wechselwirkungen innerhalb weniger Minuten zu erfassen, für die herkömmliche MRT-Protokolle Tausende von Jahren erfordern würden.

→ DESCRIPTION OF PROJECTS

OPTIMIZED XENON MRI REPORTER SYNTHESIS

Xenon biosensors are an emerging tool for different molecular imaging applications. Their development requires peptide synthesis steps, followed by the selective installation of a xenon host onto the peptide backbone in solution. In this project, different routes were pursued for generating the entire Xe biosensor on the solid support. Notably, one strategy involving CryA-da was beneficial by directly integrating this host into the growing construct. Functionalizing CryA-da on both sides with multiple labels did not alter significantly its NMR capabilities compared to uncoupled CrA-ma. Hence, this work demonstrated the successful and complete synthesis of a CryA-da-based xenon biosensor on the solid support while avoiding previously described notable side reactions and without the necessity of multiple purification steps.

ELASTIC CEST AGENTS FOR OPTIMIZED DIAGNOSTIC IMAGING

A mechanism widely used for MRI signal enhancement is chemical exchange saturation transfer (CEST) of nuclei between a dilute sensing pool and an abundant detection pool. However, the dependence of CEST amplification on the relative size of these spin pools confounds easy improvements expected for a larger detection pool: increasing the detection pool typically reduces the CEST efficiency and is counter-productive. We could demonstrate that genetically encoded nanoscale reporters for ¹²⁹Xe magnetic resonance overcome this fundamental limitation through an elastic binding capacity for NMR-active nuclei. The xenon fraction that partitions into the reporter follows the ideal gas law, allowing the signal transfer under hyperpolarized xenon chemical exchange saturation transfer (Hyper-CEST) imaging to scale linearly with the total xenon ensemble. This conceptually distinct elastic response will foster emerging biochemical and *in vivo* applications of hyperpolarized NMR and magnetic resonance imaging.

SELECTED PUBLICATIONS

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Lakshmanan, A., Lu, G.J., Farhadi, A., Nety, S.P., Kunth, M., Lee-Gosselin, A., Maresca, D., Bourdeau, R.W., Yin, M., Yan, J., Witte, C., Malounda, D., Foster, F.S., Schröder, L., Shapiro, M.G. (2017) Preparation of biogenic gas vesicle nanostructures for use as contrast agents for ultrasound and MRI. Nat Protoc. 12(10), 2050-2080.

SELECTED EXTERNAL FUNDING

International Human Frontiers Science Program Organization, "Imaging Cellular Function Non-invasively with genetically Engineered Reporters for Hyperpolarized MRI", Program Grant, joint with Mikhail Shapiro; 05.2016 - 04.2020; 750.000 USD

Deutsche Forschungsgemeinschaft, "Multivalent Hosts for Hyperpolarized Xenon Enabling in vivo MRI Visualization of Tumor Cell Surface Glycans", reinhard Koselleck-Förderung (first of its kind for the Leibniz Association); 07.2017 - 06.2022; 1.525.000 Euro

Deutsche Forschungsgemeinschaft, Research Training Group "BIOQIC - Biophysical Quantitative Imaging Towards Clinical Diagnosis"; administered through Charité -Universitätsmedizin Berlin; 04.2017 -09.2021; 4.497.810 Euro

COMPUTATIONAL CHEMISTRY/ DRUG DESIGN

WIRKSTOFF-DESIGN



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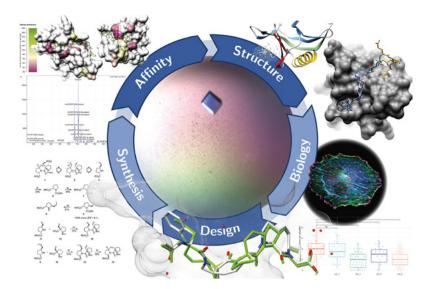
→ DEVELOPING USEFUL SUBSTANCES AND SOFTWARE

Development of chemical probes that bind to biologically important target proteins is the major task of the Drug Design group. Our drug design strategy integrates *in silico* ligand design, structural biology (X-ray, NMR), and cell biology. In recent years, we have focused on targeting protein-protein interactions mediated by protein domains specifically recognizing proline-rich motifs. These domains are involved in many disease-relevant signal transduction cascades and in cytoskeleton remodeling. Design of chemical libraries for high-throughput screening is another focus of research in our group. Our library design software is directly linked to our regularly updated database of commercially available chemical compounds.

→ NÜTZLICHE SUBSTANZEN UND SOFTWARE ENTWICKELN

Unser zentrales Forschungsziel besteht darin, Substanzen zu entwickeln, die an biologisch wichtige Zielproteine binden. Dabei kommen in unserer Gruppe computergestütztes Ligandendesign, strukturbiologische Verfahren (Röntgenstrukturanalyse, NMR) und zellbiologische Methoden zur Anwendung. Wir wollen neuartige Inhibitoren von Protein-Protein-Wechselwirkungen entwickeln, die durch Prolin-reiche Motive vermittelt werden. Solche Wechselwirkungen finden sich in etlichen mit Krankheiten assoziierten Signalübermittlungswegen sowie in der strukturellen Organisation des Zytoskeletts. Einen weiteren Schwerpunkt unserer Arbeit bildet das Design chemischer Bibliotheken für das Hochdurchsatz-Screening. Die von uns entwickelte Software ist direkt mit unserer regelmäßig aktualisierten Datenbank kommerziell verfügbarer chemischer Verbindungen verknüpft.

STRUCTURAL BIOLOGY RESEARCH REPORT 2017/2018



← FIG.

Workflow of structure-guided drug design as followed in the group of Ronald Kühne: High-resolution crystal structures serve as a basis to design scaffolds that mimic proline-rich segments. New synthesis routes are being found by our collaborators and substances subsequently tested *in vitro*, in cellular and *in vivo* context and scaffolds re-designed based on new complex structures.

→ DESCRIPTION OF PROJECTS

ENA/VASP EVH1 DOMAINS AS TARGETS TO INHIBIT CANCER CELL EXTRAVASATION

Metastasis is the major lethal attribute of cancer. Yet, the progress of metastasis-directed drug development efforts is limited, making new approaches in drug design essential. We identified a novel Ena/VASP EVH1 interacting protein. Interruption of this protein-protein interaction by CRISPR/Cas9 knockout of the interaction partner yields inhibition of cancer cell invasion in the Boyden chamber assay as well as extravasation of the knockout cells in zebrafish xenograft. To address the identified protein-protein interaction, we developed based on an integrated strategy of *in silico* design and X-ray crystallography high affinity inhibitors of the Ena/VASP EVH1 domain. Treatment of highly invasive triple negative breast cancer cells with our novel chemical entities abrogated both, cell invasion in the boyden chamber assay and extravasation in zebrafish xenograft.

LIBRARY DESIGN

Cheminformatics, bioinformatics, and molecular modeling are disciplines important for supporting the rational design of chemical probes. In combination with experimental chemical biology, these *in silico* tools improve the probability of success for ligand development. We have established a wide range of methods covering library design, ligand optimization, and calculation of ADMET parameters. Our in-house library design toolbox utilizes an innovative fragment-based concept to select chemically diverse screening compounds with sufficient solubility, low toxicity, and low chemical reactivity. Within the Helmholtz initiative "Wirkstoffforschung" we developed a biannually updated, web-based database of commercially available compounds (DACS) containing more than 80 million compounds. The combination of our toolbox and DACS allows fast design of hitor target-focused libraries, as well as of new screening libraries. Target-based ligand optimization is another focus of our work. In particular, we support selected screening projects of the FMP Screening Unit.

SELECTED PUBLICATIONS

Chiha, S., Soicke, A., Barone, M., Müller, M., Bruns, J., Opitz, R., Neudoerfl, J.-M., Kühne, R., Schmalz, H.-G. (2018) Design and Synthesis of Building Blocks for PPII-Helix Secondary-Structure Mimetics: A Stereoselective Entry to 4-Substituted 5-Vinylprolines. Eur. J. Org. Chem., 4, 455-460.

Hinderlich, S., Neuenschwander, M., Wratil, P.R., Oder, P., Lisurek, M., Nguyen, L.D., von Kries, J-P, Hackenberger, C.P.R. (2017) Small Molecules Targeting Human N-Acetylmannosamine Kinase. ChemBio-Chem.18.1279 - 1285.

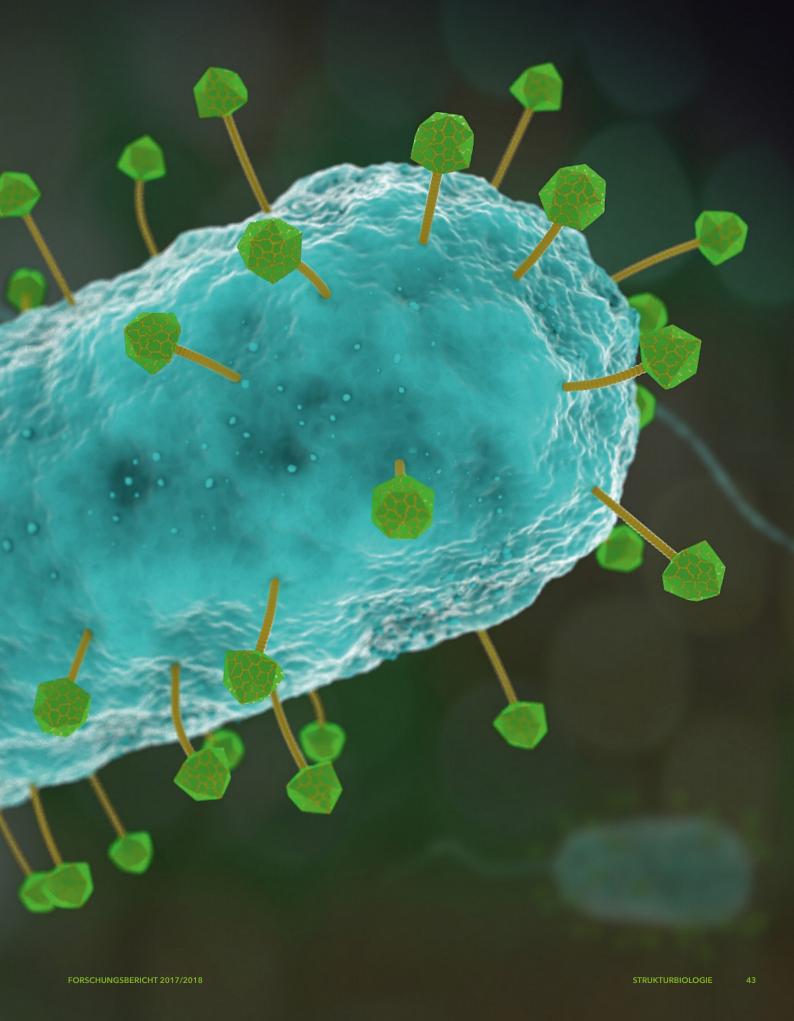
SELECTED EXTERNAL FUNDING

Validierung von Ena/VASP als Zielproteine zur Therapie metastasierender Krebserkrankungen (EnVision), Rahmenprogramm Gesundheitsforschung des BMBF, Förderkennzeichen: 16GW0186K MDC-Kooperationsvertrag Chemische Biologie Plattform

FORSCHUNGSBERICHT 2017/2018 STRUKTURBIOLOGIE

VIRUSES INSTEAD OF ANTIBIOTICS! VIREN STATT ANTIBIOTIKA! Quite a while ago, the WHO declared that resistance to antibiotics created a health crisis. This crisis is so much the worse because the search for new antibiotics hits many snags. Phages may be an alternative. Phages occur naturally. They attack and feed on individual bacteria, hence their long name bacteriophage. In experiments, they turned out to be "little helpers" in the fight against multi-resistant bacteria. Adam Lange and his team developed a new method to elucidate the complex phage structure in atomic detail. Fricke, P. et al., Nat Protoc. 2017 Die WHO hat Antibiotikaresistenzen längst zu einer globalen Gesundheitskrise erklärt. Doch die Suche nach neuen Antibiotika gestaltet sich schwierig. Eine Alternative könnten Phagen sein. Das sind natürlich vorkommende Viren, die einzelne Bakterien angreifen und fressen und darum auch "Bakteriophagen" heißen und sich in Experimenten bereits als wirksam gegen multiresistente Bakterien erwiesen haben. Das Team um Adam Lange konnte eine neue Methode entwickeln, mit der sich die komplexe Struktur bis ins atomare Detail aufklären lässt. Artist's impression of phages (green and yellow) attacking a bacterium (blue). Künstlerische Darstellung von Phagen (grün und gelb), die ein Bakterium (blau) angreifen. Visualisation: Barth van Rossum, FMP

STRUCTURAL BIOLOGY RESEARCH REPORT 2017/2018



STRUCTURAL BIOINFORMATICS AND PROTEIN DESIGN

STRUKTUR-ORIENTIERTE BIOINFORMATIK UND PROTEINDESIGN



GROUP LEADER (at the FMP since 1998)
Dr. Gerd Krause

GROUP MEMBERS
Dr. Jonas Protze, Dr. Katrin M. Hinz, Patrick
Marcinkowski, Sandro Mendieta, Paul Becker

→ leibniz-fmp.de/gkrause

→ INVESTIGATING STRUCTURE AND FUNCTION OF MEMBRANE PROTEINS
Membrane proteins play important roles in several biological processes,
such as transport of small molecules, establishing cell-cell contacts, or hormone
recognition. Our group focuses on analyzing the relationship between the
sequences and structures of membrane proteins using structural bioinformatics,
combined with experimental studies of altered protein sequences. Our aim
is to reveal structure-function relationships of proteins and their potential
interaction partners. A major activity of ours is the development of bioinformatic
tools to investigate these structure-function relationships; another is to apply
said tools to molecular biological projects of protein-ligand, protein-substrate,
or protein-protein interactions. To verify our structure-function hypotheses we
use models to guide site-directed mutagenesis of specific residues and analyze
available mutation data. Bioinformatic tools/database development and molecular
biology applications mutually support one another.

The main aims of the group are to achieve: (1) a detailed understanding of the intramolecular mechanisms of membrane proteins; (2) the rational discovery of molecular mechanisms and sites for protein-protein interactions and protein-ligand or protein-substrate interactions; and (3) prediction of small molecules or modifications of biosimilar molecules for potential pharmacological interventions.

→ STRUKTUR UND FUNKTION VON MEMBRANPROTEINEN UNTERSUCHEN
Wir beschäftigen uns mit der Frage, wie Membranproteine funktionieren,
genauer: wie Sequenz und Struktur dieser wichtigen Zellbausteine miteinander
in Beziehung stehen. Membranproteine haben vielfältige Aufgaben inne, etwa
den Transport von Substanzen oder die Herstellung von Zell-zu-Zell-Kontakten,
können aber auch als Rezeptoren für Hormone oder Wirkstoffe fungieren.
Wir wollen aufklären, in welchen Struktur-Funktionsbeziehungen Proteine und
potenzielle Interaktionspartner zueinander stehen. Dafür nutzen wir Methoden
der strukturellen Bioinformatik in Kombination mit experimentellen Funktionsuntersuchungen gezielt veränderter Proteinsequenzen. Ferner entwickeln wir
auch bioinformatische Werkzeuge und Datenbanken zur Untersuchung solcher
Struktur-Funktionsbeziehungen und setzen diese "Tools" bei spezifischen
molekularbiologischen Projekten ein, in denen wir uns etwa mit der Bindung von
Liganden an G-Protein-gekoppelte Rezeptoren und der Modulation von Tight
Junction-Proteinen beschäftigen.

STRUCTURAL BIOLOGY RESEARCH REPORT 2017/2018

Um von uns aufgestellte Struktur-Funktions-Hypothesen zu überprüfen, führen wir zielgerichtete *in vitro*-Mutationen spezifischer Aminosäuren experimentell durch und analysieren die funktionalen Effekte. Auf diese Weise lassen sich anschließend sowohl niedermolekulare Moleküle identifizieren oder biologisch verfügbare Moleküle so abwandeln, dass sie zur gezielten pharmakologischen Beeinflussung genutzt werden können.

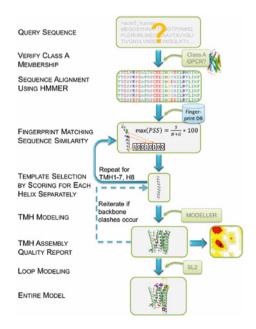
→ DESCRIPTION OF PROJECTS

MODULATION OF G-PROTEIN-COUPLED RECEPTORS (GPCRS)

Pathogenic activation of the human thyroid-stimulating hormone receptor (TSHR) by autoantibodies results in dysregulation of the thyroid hormone status. In order to block such pathogenic TSHR activation we developed negative allosteric small molecule modulators (NAM) by i) high throughput screening of the FMP compound library (in cooperation with J. von Kries), followed ii) by synthetic modifications (by E. Specker) and iii) functional characterization (in cooperation with R. Schülein) (Marcinkowski P, Hoyer I et al. Thyroid 2018). Structure-function studies of other GPCRs revealed the ligand-binding site of the human odorant receptor OR1A1 (Geithe et al. Cell Mol Life Sci 2017). For the generation of the required molecular models, a web server (www.ssfa-7tmr.de/ssfe2) for fragment-based homology modeling of class A GPCRs has been developed (Worth et al. NAR 2017). Workflow see figure 1.

REVERSIBLE OPENING OF THE BLOOD BRAIN BARRIER BY MODIFIED CLOSTRIDIUM PERFRINGENS ENTEROTOXIN

The blood-brain barrier (BBB) prevents entry of neurotoxic substances but also that of drugs into the brain. The paracellular barrier is formed by tight junctions (TJs) with claudin-5 being the main sealing constituent. Improving paracellular drug delivery, we demonstrate a reversible BBB opening targeting exclusively claudin-5. The non-toxic C-terminal domain of Clostridium perfringens enterotoxin (cCPE) binds to a distinct subset of claudins, for example Cldn3 and -4. Homology model guided mutagenesis was used to generate Cldn5-binding variants of cCPE (Y306W/S313H and N218Q/Y306W/S313H) that decreased transendothelial electrical resistance in a concentration-dependent and reversible manner. The results demonstrate that cCPE-based biologics designed to bind Cldn5 improve paracellular drug delivery across the BBB.



SELECTED PUBLICATIONS

Worth, C.L., Kreuchwig, F., Tiemann, J., Kreuchwig, A., Ritschel, M., Kleinau, G., Hildebrand, P.W., Krause, G. (2017) **GP-CR-SSFE 2.0** - a fragment-based molecular modeling web tool for Class A G-protein coupled Receptors. Nucleic Acid Res. 2017, 45:W1, W408-W415, doi: 10.1093/nar/ gkx399.

Neuhaus, W.*, Piontek, A.*, Protze, J., Eichner, M., Mahringer, A., Sibileau, E.A., Lee, I.M., Schulzke, J., Krause, G.#, Piontek, J. (2018) Reversible opening of the blood-brain barrier by claudin-5-binding variants of Clostridium perfingens enterotoxin's claudin binding domain. Biomaterials 161:129-143, doi.org/10.1016/j.biomaterials. 2018.01.028.

Marcinkowski, P.*, Hoyer, I.*, Specker, E., Furkert, J., Rutz, C., Neuenschwander, M., Sobotka, S., Sun, H., Nazare, M., Berchner Pfannschmidt, U., von Kries, J., Eckstein, A., Schuelein, R., Krause, G. (2018) A new highly thyrotropin receptor-selective antagonist with potential for the treatment of Graves' orbitopathy. Thyroid 2018 Oct 23. doi: 10.1089/thy.2018.0349.

* equal contribution; # corresponding author if not last author

SELECTED EXTERNAL FUNDING

Deutsche Forschungsgemeinschaft, Neue TSHR-Antagonisten als potenzieller Ansatz zur Schließung der therapeutischen Lücke bei der Endokrinen Orbitopathie, KR 1273/4-2, 10/2015 - 09/2019, 310.650 Euro

Deutsche Forschungsgemeinschaft, SPP1629, Thyroid Trans Act: Molekulare Determinanten unterschiedlicher Mechanismen des Schilddrüsenhormon-Imports/ -Exports der L-type Aminosäuretransporter KR1273/5-2; 6/2016 - 5/2019, 193.850 Euro

Deutsche Forschungsgemeinschaft, Suche nach Transportproteinen für TRIAC oder DITPA welche als T3/TH-Substituenten wirken, KR1273 9-1, PR1616/2-1, 12/2017 -11/2020, 352.850 Euro

← FIG.

GPCR-SSFE 2.0 workflow for template selection and homology modeling of class A GPCR (http://www.ssfa-7tmr.de/ssfe2/). A transmembrane helices (TMHs) sequence alignment of a query is used to identify matching fingerprint motifs from our database. Template selection is performed separately for each helix using a fingerprint scoring strategy and sequence similarity score. Once template selection has been carried out for the 7TMHs and helix 8 (H8), modeling is performed using Modeller. Super-Looper 2 (Ismer, NAR 2016) is used to perform loop modeling interactively by the user. Both the TMH models and entire models are available for download by the user. Figure taken from Worth et al., NAR 2017.

FORSCHUNGSBERICHT 2017/2018 STRUKTURBIOLOGIE

CORE FACILITY

<u>NMR</u>

NMR



GROUP LEADER (at the FMP since 1995)
Dr. Peter Schmieder

GROUP MEMBERS Monika Beerbaum, Brigitte Schlegel, Nils Trieloff

→ leibniz-fmp.de/nmr_facility

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY AT THE FMP

The NMR facility maintains the NMR spectrometers of the FMP and is thus able to support researchers from inside and outside the FMP in using NMR spectroscopy, either solid state or solution state.

NMR spectroscopy allows us to study the electronic environment of individual atoms and their interactions with neighbouring atoms, for example to determine the structure and dynamics of molecules. Our facility provides the research groups with two Open Access spectrometers (300 MHz and 600 MHz) for their own use. For more complex problems, we collaborate with the scientists using our NMR instruments equipped with cryoprobes. A probe suitable for 31P-NMR spectroscopy is of particular importance because the phosphorylation of different molecules is intensively studied in the departments for chemical biology.

The solid-state spectrometers are primarily used by the departments for structural biology to determine the structures of membrane proteins or larger aggregates. For this purpose, a wide range of field strengths and probes with different sample diameters is used. DNP experiments (Dynamic Nuclear Polarization) can also be performed on two spectrometers. Furthermore, the facility receives requests from groups outside of the FMP, as well as from commercial companies located nearby the institute. These requests are fulfilled in the form of collaborations or as a NMR service provided by the facility. It also participates in the German DFG-funded G-NMR network of facilities, the iNEXT initiative, and worldwide NMR studies such as the Fab-NMR-study by NIST.

→ KERNSPINRESONANZSPEKTROSKOPIE AM FMP

Wir betreuen die Kernspinresonanz- bzw. NMR-Spektrometer des FMP und unterstützen Forschende des FMP und anderer Institutionen bei der Nutzung der NMR-Spektroskopie – ob im Festkörper oder in Lösung.

NMR-Spektroskopie macht es möglich, die elektronische Umgebung einzelner Atome und ihrer Wechselwirkungen mit Nachbaratomen zu untersuchen, etwa um Struktur und Dynamik von Molekülen zu bestimmen. Unsere Facility stellt den Forschungsgruppen zwei Open-Access-Spektrometer (300 MHz und 600 MHz) für die Eigennutzung zur Verfügung. Bei komplexeren Problemen arbeiten wir mit den Forschenden zusammen und setzen hierfür unsere mit Kryoköpfen ausgestatteten NMR-Geräte ein. Besonders von Bedeutung ist dabei ein für die

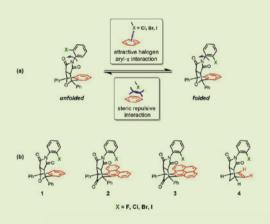
STRUCTURAL BIOLOGY RESEARCH REPORT 2017/2018

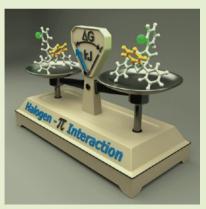
31P-NMR-Spektroskopie geeigneter Kopf, weil die Phosphorylierung von unterschiedlichen Molekülen in den Abteilungen für Chemische Biologie intensiv studiert wird.

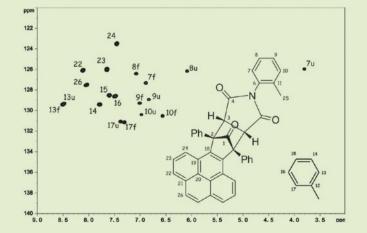
Die Festkörper-Spektrometer werden vorrangig von den Abteilungen für Strukturbiologie genutzt, um Strukturen von Membranproteinen oder größeren Aggregaten zu bestimmen. Dazu setzen wir eine große Breite von Feldstärken und Probenköpfen mit unterschiedlichen Probendurchmessern ein, an zwei Spektrometern können zudem DNP-Experimente (Dynamische Kernpolarisation) durchgeführt werden. Daneben erhält die NMR-Facility viele Anfragen von anderen akademischen Gruppen oder von Firmen aus der Nähe des FMP. Diese Anfragen werden nach Möglichkeit entweder durch Kooperationen oder als NMR-Service beantwortet. Darüber hinaus sind wir Teil des von der DFG geförderten G-NMR-Facility-Netzwerks und an der iNEXT (Infrastructure for NMR, EM and X-rays for Translational Research)-Initiative sowie weltweiten Ringversuchen beteiligt.

→ DESCRIPTION OF PROJECTS

One example of an in-house collaboration is shown in Figure 1. Together with the groups of Han Sun and Marc Nazare we investigated halogen-aryl π interactions that have received increasing attention with respect to molecular recognition in chemical and biological systems, using a bicyclic N-arylimide based molecular torsion balances system. Through comparison of balances modulated by higher halogens with fluorine balances, we determined the magnitude of the halogen-aryl π interactions and provided direct experimental evidence of halogen-aryl π interactions in solution.







SELECTED PUBLICATIONS

Diehl, A., Roske, Y., Ball, L., Chowdhury, A., Hiller, M., Moliere, N., Kramer, R., Stoppler, D., Worth, C.L., Schlegel, B., Leidert, M., Cremer, N., Erdmann, N., Lopez, D., Stephanowitz, H., Krause, E., van Rossum, B.J., Schmieder, P., Heinemann, U., Turgay, K., Akbey, U., and Oschkinat, H. (2018). Structural changes of TasA in biofilm formation of Bacillus subtilis. Proc Natl Acad Sci U S A. 115. 3237–3242.

Sama, S., Jerz, G., Schmieder, P., Joseph, J.F., Melzig, M.F., and Weng, A. (2018). Plant derived triterpenes from Gypsophila elegans M.Bieb. enable non-toxic delivery of gene loaded nanoplexes. J. Biotechnol. 284, 131-139.

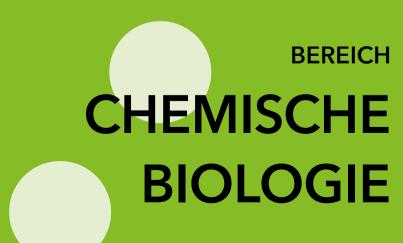
Sun, H., Horatscheck, A., Martos, V., Bartetzko, M., Uhrig, U., Lentz, D., Schmieder, P., and Nazare, M. (2017). Direct Experimental Evidence for Halogen-Aryl π Interactions in Solution from Molecular Torsion Balances. Angew. Chem. Int. Ed. Engl. 56, 6454–6458.

← FIG.

Halogen-aryl π interactions were investigated using a molecular torsion balances system. A set of 16 balance molecules was synthesized, the NMR spectra were assigned and the energy of isomerization was determined using NMR. By comparing balances incorporating a methyl group or halogens the magnitude of the halogen-aryl π interactions in our unimolecular systems was determined to be larger than -5.0 kJmol-1

FORSCHUNGSBERICHT 2017/2018 STRUKTURBIOLOGIE

CHEMICAL BIOLOGY



- → Chemical Biology utilizes chemical methods to advance the understanding and modulation of cellular function, especially in the context of pharmacological targets and the development of novel approaches in the medicinal sciences. Research projects in this section are devoted to the synthesis and identification of novel bioactive small molecules and protein conjugates of high pharmacological potency as well as to the development of new chemical and analytical tools to study biologically relevant pathways. In addition to the two departments "Chemical Biology 1 and 2", headed by Dorothea Fiedler and Christian Hackenberger, important research and technologies are provided within this section for the whole institute. A central component in this aspect is the "Mass Spectrometry" unit, headed by Fan Liu, which develops new proteomic methods for the analysis of proteinprotein interactions. Furthermore, this section constitutes the "Chemical Biology Platform", which is devoted to the validation and chemical optimization of small molecule screening hits of pharmacological targets, thereby serving as an essential partner site in several institutional national and international research networks, for example within EU-OPENSCREEN. Within this platform, the "Screening Unit", led by Jens von Kries, provides high-throughput screening of RNAi libraries and small molecules from the unique FMP compound library. The "Medicinal Chemistry" group led by Marc Nazaré is developing new chemical tools using strategies like fragment growing, re-scaffolding approaches, and structure-based design to chemically optimize the initial screening hits. Finally, the group "Drug Design" (led by Ronald Kühne and imbedded in the section Structural Biology) gives further support in computational methods.
- → Die Chemischen Biologie nutzt chemische Methoden, um das Verständnis und die Modulation der Zellfunktion voranzutreiben, insbesondere im Zusammenhang mit pharmakologischen Zielen und der Entwicklung neuer Ansätze in den Medizinwissenschaften. Die Projekte der Abteilung widmen sich der Synthese und Identifizierung neuartiger bioaktiver kleiner Moleküle und Proteinkonjugate, die eine hohe pharmakologische Wirksamkeit aufweisen. Darüber hinaus entwickeln die Forschenden neue chemische und analytische Werkzeuge zur Untersuchung biologisch relevanter Signalwege. Neben den beiden Abteilungen "Chemische Biologie 1 und 2" unter der Leitung von Dorothea Fiedler und Christian Hackenberger werden in diesem Bereich wichtige Forschung und Technologien für das gesamte Institut bereitgestellt. Ein zentraler Bestandteil ist dabei die Massenspektrometrie unter der Leitung von Fan Liu, in der neue proteomische Methoden zur Analyse von Protein-Protein-Interaktionen entwickelt werden. Darüber hinaus gehört auch die Chemische Biologie Plattform zum Bereich. Die Plattform widmet sich der Validierung und chemischen Optimierung kleinmolekularer Screening-Treffer pharmakologischer Targets, sogenannte Hits, und agiert damit auch als unverzichtbarer Partnerstandort in mehreren nationalen und internationalen Forschungsnetzwerken, beispielsweise im EU-OPENSCREEN Netzwerk. Innerhalb dieser Plattform bietet die "Screening Unit" unter der Leitung von Jens von Kries ein Hochdurchsatz-Screening von RNAi-Bibliotheken und kleinen Molekülen aus der einzigartigen FMP eigenen Wirkstoffbibliothek. Die von Marc Nazaré geführte Gruppe "Medizinische Chemie" erforscht neue chemische Werkzeuge, und nutzt hierbei Strategien wie Fragmentwachstum und strukturbasiertes Design, um die initialen Hits chemisch zu verbessern. Zudem unterstützt die Gruppe "Wirkstoff-Design", unter der Leitung von Ronald Kühne (aus dem Bereich der Strukturbiologie) mit computergestützten Methoden.

FORSCHUNGSBERICHT 2017/2018 CHEMISCHE BIOLOGIE 49

CHEMICAL BIOLOGY II

CHEMISCHE BIOLOGIE II



GROUP LEADER (at the FMP since 2012) Prof. Dr. Christian P.R. Hackenberger

GROUP MEMBERS

Dr. Antoine Wallabregue, Dr. Philipp Ochtrop, Dr. Reihaneh Safavi-Sohi, Dr. Dominik Schumacher, Sergej Schwagerus, Marc-André Kasper, Anett Hauser, Alice Baumann, Anselm Schneider, Lutz Adam, Alec Michels, Sebastián Florez Rueda, Don Shenal Munasinghe, Jacob Gorenflos, Dagmar Krause, Kristin Kemnitz-Hassanin, Beate Kindt, Ines Kretzschmar, Katrin Wittig, Jennifer Trümpler

→ leibniz-fmp.de/hackenbe

→ HOW PROTEIN MODIFICATIONS IN CELLS CAN TRIGGER DISEASES
In the cell, protein modifications control many signaling pathways that support healthy functioning and that are disrupted in disease. Such modifications can act as "toggle switches" between health and disease. Chemical biologists want to control these protein modifications in the cell, both to study the biological role of such modifications and to decorate proteins with fluorescent moieties that permit their visualization.

Our laboratory aims to identify new chemical approaches that allow the modification of peptides and proteins, both on isolated biomolecules as well as in living cells and organisms. In this, our main objective is to apply these highly selective bioconjugation reactions to study the functional consequences of natural protein modifications, as well as to generate novel peptide- and protein-conjugates, in particular antibody-drug conjugates (ADCs), for pharmaceutical and medicinal applications.

→ WIE PROTEIN-VERÄNDERUNGEN IN ZELLEN KRANKHEITEN AUSLÖSEN KÖNNEN

In der Zelle werden viele Signalwege, die normales Leben steuern und bei Krankheit gestört sind, durch Veränderungen an Proteinen reguliert. Solche Modifikationen können wie "Wechselschalter" zwischen Gesundheit und Krankheit wirken. Forschende in der Chemischen Biologie versuchen deshalb zunehmend, die Modifizierung von Proteinen in der Zelle zu kontrollieren, um die biologische Rolle solcher Modifikationen zu erforschen oder Proteine mit fluoreszierenden Gruppen zu versehen, die ihre Visualisierung ermöglichen.

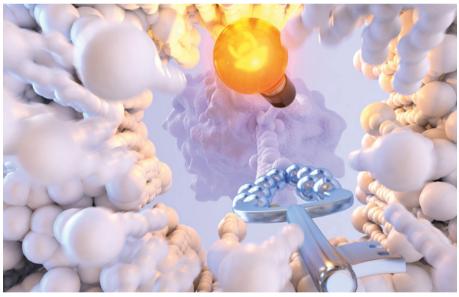
Wir haben uns zum Ziel gesetzt, neue chemische Verfahren zu entwickeln, mit denen sich Proteine und kleinere Moleküle sowohl in isolierter Form als auch in lebenden Zellen oder Organismen gezielt funktionalisieren, d.h. für bestimmte Aufgaben einsetzen lassen. Insbesondere wollen wir hochselektive organischchemische Methoden für die Biokonjugation, also der Verknüpfung von einem Protein oder einem Antikörper mit einem synthetischen Molekül, entwickeln. Damit können wir die Auswirkungen natürlich vorkommender Modifikationen auf die Funktion von Proteinen untersuchen und neue medizinische und pharmakologische Anwendungen ermöglichen, beispielsweise durch Antikörper-Wirkstoff-Konjugate.

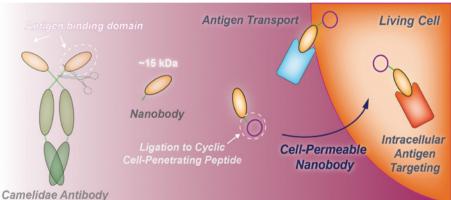
CHEMICAL BIOLOGY RESEARCH REPORT 2017/2018

→ DESCRIPTION OF PROJECTS

Advancing the cellular uptake of molecules holds promise for the engineering of unprecedented tools for the life sciences as well as the development of new drugs. In 2017 we succeeded to transport small antigen recognizing proteins, so-called nanobodies, into living cells. Key to success for the site-specific ligation of cyclic cell penetrating peptides to nanobodies using methods developed in the Hackenberger laboratory (Herce, Schumacher *et al*, Nature Chem. 2017). In collaboration with the Cardoso group at the TU Darmstadt, we demonstrated that these proteins were transported in the cytosol or nucleus depending on the intracellular localization of the antigen. Using this protocol, we also shuttled pharmacologically relevant proteins into living cells, which opens the door for further application for the targeted delivery of biopharmaceuticals into cells.

Engineering novel therapeutics to fight influenza infection is one of the most challenging tasks in drug development due to the multivalent binding of a virus during infection. In order to break this multivalent interaction, we designed a peptides-polymer conjugate, which showed picomolar binding to the viral surface protein hemagglutinin and prevented influenza infections in living cells and *in vivo* (Lauster, Glanz *et al.*, Angew. Chem. Int Ed. 2017). Our study, which was performed in close collaboration with the Herrmann group at the HU Berlin, used for the first time peptides as ligands in the engineering of multivalent influenza inhibitors, which offers significant advantages in terms of synthetic accessibility and the development of inhibitors against mutated influenza strains.





SELECTED PUBLICATIONS

Herce, H.D., Schumacher, D., Schneider A.F.L., Ludwig A.K., Mann, F.A., Fillies, M., Kasper M.A., Reinke, S., Krause, E., Leonhardt, H., Cardoso, M.C., Hackenberger, C.P.R. (2017). Cell-permeable nanobodies for targeted immunolabelling and antigen manipulation in living cells. Nat Chem. 2017, 9, 762–771.

Schumacher, D., Lemke, O., Helma, J., Gerszonowicz, L., Walle, r V., Stoschek, T., Durkin, P.M., Budisa, N., Leonhardt, H., Keller, B.G., Hackenberger, C.P.R. (2017). Broad substrate tolerance of tubulin tyrosine ligase enables one-step site-specific enzymatic protein labeling. Chem Sci. 8, 3471–3478.

Lauster, D., Glanz, M., Bardua, M., Ludwig, K., Hellmund, M., Hoffmann, U., Hamann, A., Böttcher, C., Haag, R., Hackenberger, C.P.R., Herrmann, A. (2017). Multivalent Peptide-Nanoparticle-conjugates for Influenza Virus Inhibition. Angew Chem Int Ed Engl. 56(21), 5931-5936.

SELECTED EXTERNAL FUNDING

Deutsche Forschungsgemeinschaft,

Priority Programme SPP 1623 "Chemoselective reactions for the synthesis and application of functional proteins":

- Funds for the coordination of the Priority Programme, 2012 - 2019, 612.500 Euro,
- Funds for joint project with H. Leonhardt (LMU München) and C. Cardoso (TU Darmstadt), "Site-specific functionalization of nanobodies: From labelling to cellular uptake", 2012 - 2019, 412.800 Euro
- Funds for joint project with J. Kirstein (FMP), "Chemoselective Staudinger-induced Michael-additions to antibodies to analyze protein homeostasis in C.elegans", 2016 -2019, 217.150 Euro SPP 1623 funding in total: 1.242.450 Euro

Deutsche Forschungsgemeinschaft,

SFB 765 B05, "Site-specific functionalization of proteins for the acquisition of multivalent glycoconjugtes", 3rd funding period 2016 - 2019, jointly with N. Budisa (TU Berlin), 428.600 Euro

Leibniz Gemeinschaft e.V.,

Senatsausschuss Wettbewerb (SAW), "Cystein-selective bioconjugation for next generation antibody drug conjugates", jointly with H. Leonhardt (LMU München), J. von Kries (FMP), C. Hertweck (HKI Jena) and L. Wessjohann (IPB Halle), 864.183 Euro

← FIG. 1 & 2

Cyclic cell penetrating peptide-conjugates: The key to cellular entry of nanobodies! (refers to publication 1) Graphic designed by Christoph Hohmann, LMU München (Christoph.Hohmann@physik. uni-muenchen.de).

CHEMICAL BIOLOGY I

CHEMISCHE BIOLOGIE I



GROUP LEADER (at the FMP since 2015)
Prof. Dr. Dorothea Fiedler

GROUP MEMBERS

Tim Kröber, Minh Nguyen Trung, Sandra Schlomach, David Furkert, Robert Harmel, Robert Puschmann, Jeremy Morgan, Sarah Hostachy, Lena von Oertzen, Katy Franke, Jennifer Trümpler

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\rightarrow TO ELUCIDATE AND CONTROL THE FUNCTION OF MESSENGER MOLECULES IN SIGNALING PROCESSES

Our group seeks to develop a better understanding of the multiple ways in which nature utilizes phosphate in protein signaling cascades and metabolic networks. As deregulation of cellular information transfer is associated with a wide range of diseases, a detailed annotation of signaling events in healthy and diseased states can highlight new avenues for therapeutic intervention.

One group of messengers of particular interest to our group are the inositol pyrophosphates (PP-InsPs). These molecules have emerged as central regulators of cell homeostasis, and genetic studies in mice and humans implicate PP-InsPs in a host of processes including weight gain, fertility, longevity, and tumor metastasis. However, how these molecules exert their effects at the molecular level is not well understood. Using a multi-disciplinary approach that employs techniques from organic chemistry, chemical genetics and genetics, molecular biology, and proteomics, it is our goal to decipher the concrete signaling functions of PP-InsPs and ultimately to guide the development of new therapeutic strategies against cancer, diabetes, and obesity.

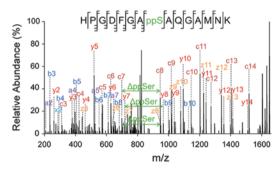
\rightarrow DIE ROLLE VON BOTENSTOFFEN IN SIGNALPROZESSEN AUFKLÄREN UND BEEINFLUSSEN

Unsere Gruppe will ein besseres Verständnis dafür entwickeln, wie die Natur Phosphat in Protein-Signalkaskaden und metabolischen Netzwerken verwendet. Ist der Informationstransfer in Zellen gestört, kommt es zu einer Vielzahl von Krankheiten. Wir wollen Signalereignisse in gesunden und kranken Zuständen ausführlich aufklären und so dazu beitragen, neue therapeutische Ansätze zu entwickeln.

Von besonderem Interesse ist eine Gruppe von Botenstoffen, die Inositolpyrophosphate (PP-InsPs). Diese Moleküle sind als zentrale Regulatoren der Zellhomöostase bekannt. Genetische Untersuchungen an Mäusen und Menschen zeigen, dass PP-InsPs an einer Vielzahl von Prozessen beteiligt sind, darunter Gewichtszunahme, Fruchtbarkeit, Langlebigkeit und Tumormetastase. Doch wie diese Moleküle ihre Wirkung auf molekularer Ebene ausüben, ist nur ansatzweise zu erahnen. Mit einem multidisziplinären Ansatz, der Techniken aus der organischen Chemie, der chemischen Genetik und der Genetik, der Molekularbiologie und der Proteomik einsetzt, wollen wir die konkreten Signalfunktionen

CHEMICAL BIOLOGY RESEARCH REPORT 2017/2018

EThcD mass spectrometry for definitive assignment of pyrophosphosites



von PP-InsPs entschlüsseln und letztlich die Entwicklung neuer therapeutischer Strategien gegen Krebs, Diabetes und Fettleibigkeit ermöglichen.

→ DESCRIPTION OF PROJECTS

CHEMICAL TOOLS FOR ANNOTATING AND CHARACTERIZING THE PYROPHOSPHOPROTEOME

In addition to the traditional binding mechanism, a covalent protein modification termed "protein pyrophosphorylation" has been proposed for PP-InsPs. Since pyrophosphorylation expands our view of classical phosphorylation-based signaling, it constitutes an intriguing additional layer of regulation. Efforts to characterize this modification have exclusively relied on *in vitro* labeling strategies; consequently, many questions about the regulation of pyrophosphorylation *in vivo* still linger. Our group has now implemented a set of new methods to elucidate the effects of pyrophosphorylation on protein structure and function, and to annotate pyrophosphorylation sites from complex samples.

To determine the biochemical function of pyrophosphorylation at the protein level, the modification has to be introduced stoichiometrically and site-specifically onto the protein substrate of interest. To do so, our group relied on an amber codon suppression system for installing a phosphoserine at the relevant site, followed by selective chemical pyrophosphorylation using novel phosphoimidazolide reagents.

Complementary to the selective phosphoprotein derivatization, we recently developed a mass spectrometry (MS)-based method to directly detect and identify pyrophosphopeptides (in collaboration with E. Krause/F. Liu, FMP) using a neutral-loss-triggered EThcD acquisition method. This strategy permitted reliable identification of synthetic pyrophosphopeptides, spiked into complex samples, without compromising speed and sensitivity.

← FIG.

Chemical approaches to elucidate the function of protein pyrophosphorylation.

a) A new "on-the-fly" mass spectrometry approach enables detection of pyrophosphopeptides.

b) Stoichiometrically pyrophosphorylated protein are obtained using phosphorimidazolide reagents.

SELECTED PUBLICATIONS

Marmelstein, A.M., Morgan, J.A.M., Penkert, M., Rogerson, D.T., Chin, J.W., Krause, E., Fiedler, D. (2018) Pyrophosphorylation via selective phosphoprotein derivatization. Chem Sci. 9(27), 5929-5936.

Harmel, R., Fiedler, D. (2018) Features and regulation of non-enzymatic post-translational modifications. Nat Chem Biol. 14(3), 244-252

Penkert, M., Yates, L.M., Schümann, M., Perlman, D., Fiedler, D., Krause, E. (2017) Unambiguous Identification of Serine and Threonine Pyrophosphorylation Using Neutral-Loss-Triggered Electron-Transfer/ Higher-Energy Collision Dissociation. Anal Chem. 89(6), 3672–3680.

SELECTED EXTERNAL FUNDING

Swiss National Science Foundation, Sinergia. Joint with A. Mayer, S. Hiller, M. Hothorn. "Discovery and mechanistic dissection of novel signaling pathways controlling phosphate homeostasis in eukaryotes" 2016 - 2020, 2.405.000 CHF

Leibniz Gemeinschaft, Leibniz Wettbewerb.Joint with H. Oschkinat, V. Haucke. "Systems level analysis of inositol messengers in nutrient signaling" 2017 - 2020, 1.063.000 Euro

Exzellenzcluster UniCat. "Development of photoswitchable small molecule kinase inhibitors." 2017 - 2018, 120.000 Euro

FORSCHUNGSBERICHT 2017/2018 CHEMISCHE BIOLOGIE 5

STRUCTURAL INTERACTOMICS

STRUKTURELLE INTERAKTOMIK



GROUP LEADER (at the FMP since 2017)
Dr. Fan Liu

GROUP MEMBERS Dr. Michal Nadler-Holly, Dr. Antoine Wallabregue, Lennart Schnirch, Thomas Stellwag, Ying Zhu, Heike Stephanowitz

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→ BETTER UNDERSTANDING OF PROTEIN INTERACTIONS

Nearly every process in the living cell is based on proteins. To precisely execute this plethora of tasks, proteins are highly organized in a variety of assemblies, ranging from stable protein complexes, well-regulated pathways, to extended protein interaction networks. Perturbation of these well-balanced systems is linked to many different physiological and pathological conditions.

Our group is interested in developing and applying tools to characterize the complexity of protein interactions within the cell. Using state-of-the-art mass spectrometric technologies, in particular cross-linking mass spectrometry, we aim to gain a better understanding of protein interactomes in complex biological systems. These studies will offer enormous opportunities to elucidate the fundamental organization principles of proteins and discover previously unrecognized protein interactions in health and disease.

→ WECHSELWIRKUNGEN VON PROTEINEN BESSER VERSTEHEN

Eiweiße sind an nahezu jedem Prozess in der lebenden Zelle beteiligt. Um diese Fülle von Aufgaben präzise auszuführen, schließen sich die Eiweiße zu zahlreichen organisierten Komplexen zusammen und bilden so zum Beispiel gut regulierte Signalwege und weitreichende Protein-Interaktionsnetzwerke. Veränderungen oder Störungen dieser ausbalancierten Systeme haben Auswirkungen auf eine Vielzahl verschiedener physiologischer und pathologischer Zustände. Unsere Arbeitsgruppe forscht an der Entwicklung und Anwendung von Technologien, die es ermöglichen, die Komplexität dieser Proteinwechselwirkungen in der Zelle besser zu verstehen. Mithilfe modernster massenspektrometrischer Technologien, insbesondere der quervernetzenden Massenspektrometrie ("crosslinking MS"), wollen wir die Wechselwirkungen von Proteinen in komplexen biologischen Systemen besser verstehen. Diese Untersuchungen bieten hervorragende Möglichkeiten, um die grundlegenden Prinzipien der Organisation von Proteinen aufzuklären und bisher nicht bekannte Proteinwechselwirkungen in der gesunden Zelle und bei Krankheiten aufzudecken.

CHEMICAL BIOLOGY RESEARCH REPORT 2017/2018

→ DESCRIPTION OF PROJECTS

DEVELOPING HIGH-THROUGHPUT PROTEOME-WIDE XL-MS STRATEGY

To comprehensively understand the protein interactome, our group has developed a novel XL-MS method to characterize the structures and interactions of various protein complexes in a high-throughput manner. This approach allows us to handle highly complex samples and simultaneously investigate stable and dynamic protein assemblies by capturing their residue-residue connectivities *in vivo*. In the future, we aim to unveil the full potential of this technique by designing novel cross-linkers, implementing creative approaches for cross-link enrichment, developing a cutting-edge data analysis pipeline, and applying state-of-the-art MS technology. We integrate expertise from synthetic chemistry, analytical chemistry, mass spectrometry and informatics, aiming to reach unprecedented analytical depth, complexity and precision in interactome profiling.

ESTABLISHING THE STRUCTURAL INTERACTOMES OF ORGANELLES

Organelles are specialized compartments within the cell, in which proteins are selectively imported to work cooperatively to conduct a variety of cellular functions. Although many organelles were discovered decades ago and found to play essential roles for the cell, questions regarding to which extent protein complexes cooperate within and between organelles remain completely elusive. Our group studies the structural interactome of proteins in their organellular environment using the newly developed cross-linking mass spectrometry approach. We focus on two biological systems, the neuronal synapses and the mitochondria, the functions of which are linked to a wide variety of physiological and pathological conditions. Our aim is to obtain comprehensive protein interaction maps which will provide crucial insights into the interaction patterns, the binding interfaces, and the three-dimensional organization of protein complexes in their native cellular context.

SELECTED PUBLICATIONS

Liu, F., Lössl, P., Rabbitts, B.M., Balaban, R.S., Heck, A.J.R. (2018) The interactome of intact mitochondria by cross-linking mass spectrometry provides evidence for coexisting respiratory supercomplexes. Mol Cell Proteomics. 17(2), 216-232.

Liu, Q., Remmelzwaal, S., Heck, A.J.R., Akhmanova, A., Liu, F. (2017) Facilitating identification of minimal protein binding domains by cross-linking mass spectrometry. Sci Rep. 7(1), 13453.

Liu, F., Lössl, P., Scheltema, R., Viner, R., Heck, A.J.R. (2017) **Optimized fragmentation** schemes and data analysis strategies for proteome-wide cross-link identification. Nat Commun. 8. 15473.

SELECTED EXTERNAL FUNDING

Leibniz Gemeinschaft, Professorinnenprogramm (Leibniz-Wettbewerb), "Cellular Interactomics in Health and Disease" 2020 - 2025, 808.055 Euro

FIG. 1
 Cellular functions are highly regulated through protein-protein interactions.

FORSCHUNGSBERICHT 2017/2018 CHEMISCHE BIOLOGIE

INTRODUCING NANO ANTIBODIES INTO LIVING CELLS

NANO-ANTIKÖRPER IN LEBENDE ZELLEN EIN-SCHLEUSEN

Antibodies are one of the main weapons of our immune system. They dock to viruses, bacteria and other invaders that course through our blood, and thereby render them harmless. Antibodies also play a key role in basic and applied research, for instance in the diagnosis and treatment. So far, due to their size antibodies were unable to permeate living cells. In a collaborative effort, the teams of Christian Hackenberger and Cristina Cardoso at the TU Darmstadt have managed to introduce tiny antibodies into living cells, and examined their benefits. This opens up a new door to treatments for diseases that have so far been untreatable.

Herce, H.D., et al. Nat Chem. 2017

Antikörper zählen zu den Hauptwaffen unseres Immunsystems. Sie docken an Viren, Bakterien und andere Eindringlinge an, die in unserem Blut kursieren, und machen sie so unschädlich. Auch in der Therapie und Diagnostik von Krankheiten sowie in der Forschung spielen Antikörper eine entscheidende Rolle, konnten aber bislang wegen ihrer Größe nicht in lebende Zellen gelangen. In einer Kooperation der Teams von Christian Hackenberger und Cristina Cardoso (TU Darmstadt) ist es erstmals gelungen, winzige Antikörper in lebende Zellen zu schleusen und ihren Nutzen zu untersuchen. Damit könnte sich eine Tür zu neuen Therapien für bislang unheilbare Krankheiten öffnen.

PCNA*
PCNA fused with GFP

fusion linker

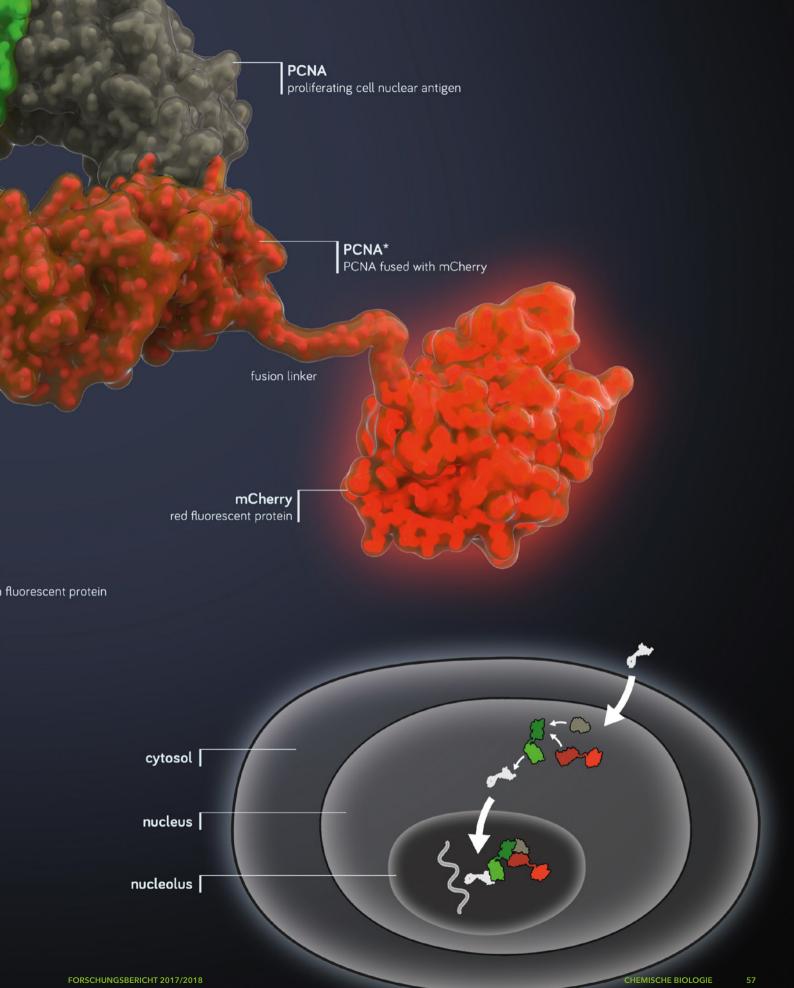
GBP nanobodyGFP-binding protein

cCPP

cyclic cell-penetrating peptide structure currently unknown

RNA

GFP greer



MEDICINAL CHEMISTRY

MEDIZINISCHE CHEMIE



GROUP LEADER (at the FMP since 2013)
Dr. Marc Nazaré

GROUP MEMBERS

Dr. Rana Alsalim, Benjamin Brennecke, Thais Gazzi, Dr. Murat Kücükdisli, Dr. Peter Lindemann, Keven Mallow, Sandra Miksche, Dr. Yelena Mostinski, Dr. Yelha Phani Kumar Nekkanti, Jérôme Paul, Dr. Lioudmila Perepelittchenko, Dr. Edgar Specker, Marie Weise

 $\rightarrow leibniz\text{-}fmp.de/nazare$

- → DESIGN AND SYNTHESIS OF CHEMICAL PROBES FOR THE PHARMACOLOGICAL INVESTIGATION OF BIOLOGICAL SYSTEMS Small molecules can be used as research tools to investigate protein functions and elucidate molecular mechanisms or to influence signal transduction pathways. They can also be employed to validate hypotheses from genetic studies such as knock-down- and loss-of-function approaches. Moreover, these substances can serve as starting points for new therapeutic approaches and new drugs. Goal of our research is the discovery and development of highly active, selective chemical probes for the specific modulation of protein-ligand or protein-protein interactions. We want to discover and develop highly active and selective chemical probes for the specific modulation of protein-ligand and protein-protein interactions. We secondly focus on the development and enhancement of the FMP compound collection with currently 62,000 commercial compounds and 9,000 small molecules from academic research. A carefully thought-out extension of the collection should guarantee optimal coverage of the biological space being screened, along with high screening hit rates as well as suitable starting points for chemical optimization.
- → DESIGN UND SYNTHESE VON CHEMISCHEN WERKZEUGEN ZU PHARMAKOLOGISCHEN UNTERSUCHUNG BIOLOGISCHER SYSTEME Kleine Moleküle lassen sich als Forschungswerkzeuge nutzen, um damit Proteinfunktionen zu untersuchen und molekulare Mechanismen aufzuklären oder ganze Signaltransduktionswege mit Hilfe dieser Substanzen zu beeinflussen. Zudem können sie genutzt werden, um Hypothesen aus genetischen Studien zu validieren, die aus Knock-Down- oder Loss-of-Function-Ansätzen gewonnen wurden. Oft dienen die Substanzen als Vorläufersubstanzen von Pharmaka oder sind gar ein Startpunkt für neuartige Therapien. Ziel unserer Forschung ist die Entdeckung und Entwicklung hochaktiver, selektiver chemischer Sonden für eine spezifische Modulation von Protein-Liganden- oder Protein-Protein Wechselwirkungen. Wir wollen ganz bestimmte Werkzeuge - hochaktive, selektive chemische Sonden - finden und weiter entwickeln, um mit ihnen Protein-Liganden- oder Protein-Protein-Wechselwirkungen spezifisch zu modulieren. Ein zweiter Fokus liegt auf der Erweiterung und Entwicklung der FMP-Substanzsammlung mit derzeit 62.000 kommerziellen Verbindungen und 9.000 kleinen Molekülen aus der akademischen Forschung. Eine gut durchdachte Erweiterung der Sammlung soll hier eine optimale Abdeckung des zu untersuchenden biologischen Raumes, hohe Trefferraten beim Screening sowie geeignete Ansatzpunkte für die chemische Optimierung gewährleisten.

CHEMICAL BIOLOGY RESEARCH REPORT 2017/2018

→ DESCRIPTION OF PROJECTS

DEVELOPMENT OF SPECIFIC INHIBITORS OF THE TYROSINE PHOSPHATASE SHP-2

In stark contrast to their validated significance in signal transduction and disease pathology, phosphatases are notoriously difficult to inhibit using small molecules. The protein tyrosine phosphatase Shp-2 plays a critical role in growth factor-mediated processes, primarily by promoting the activation of the RAS/ERK signaling pathway. Aberrant gain-of-function mutations are associated with several metastatic cancers. In collaboration with Walter Birchmeier (MDC), a re-scaffolding approach that involves replacing the former framework of a tyrosine phosphatase Shp2 inhibitor (W. Birchmeier et al., PNAS, 2008), led to the discovery of novel structural classes and eliminated several chemical liabilities, i.e. unfavourable structural features. These novel compounds are not only active in a sub-micromolar range in the Shp2-enzyme assay, but are also effective in the low micromolar range on hepatocyte growth factor (HGF)-stimulated canine MDCK-C cells, as well as human pancreatic tumor cells for epithelial-mesenchymal transition (EMT), a hallmark of cancer cell dissemination.

DISCOVERY OF A HIGHLY SELECTIVE, LEAD STAGE TANKYRASE INHIBITOR BY A HYBRIDIZATION APPROACH

Based on a screening of the FMP library using a Wnt/ß-catenin signaling reporter assay, and a subsequent target deconvolution, tankyrase was identified as the target enzyme of the new the hit series. Further classical optimization led to G007-LK as a first lead structure. However, albeit being highly potent and selective, distinct structural features of G007-LK were unfavorably influencing the overall molecular properties such as solubility and light stability. In addition, G007-LK had a poor pharmacokinetics in rats and rabbits. Using a structure-guided hybridization approach with two privileged substructures gave instant access to a new series of tankyrase inhibitors. The identified inhibitor OD336 displays much higher affinity and cellular activity on tankyrase with an IC50 value of 6 nM and 19 nM respectively and an outstanding selectivity over the entire PARP family of homologous enzymes and was also active in pharmacologically relevant in vivo tumor models. Critical for this approach was the utilization of the appropriate linking moiety, whereby a cyclobutyl linker displayed superior affinity compared to a cyclohexane linker. A favorable overall in vitro ADME as well as pharmacokinetic properties make this series suitable as a chemical tool for pharmacological investigations and further optimization.

SELECTED PUBLICATIONS

Ruess, D.A., Heynen, G.J., Ciecielski, K.J., Ai, J., Berninger, A., Kabacaoglu, D., Görgülü, K., Dantes, Z., Wörmann, S.M., Diakopoulus, K.N., Karpathaki, M.K., Kowalska, M., Kaya-Aksoy, E., Song, L., Zeeuw van der Laan, E.A., López-Alberca, M.P., Nazaré, M., Reichert, M., Saur, D., Erkan, M.M., Hopt, U.T., Sainz Jr, B., Birchmeier, W., Schmid, R.M., Lesina, M., Algül, Mutant, H. (2018) KRAS-driven cancers depend on PTPN11/SHP2 phosphatase. Nat. Med., 24, 954-960.

Anumala, U.R., Waaler, J., Nkizinkiko, Y., Ignatev, A., Lazarow, K., Lindemann, P., Olsen, P.A., Murthy, S., Obaji, E., Majouga, A.G., Leonov, S., von Kries, J.P., Lehtiö, L., Krauss, S., Nazaré, M. (2017) Discovery of a Novel Series of Tankyrase Inhibitors by a Hybridization Approach. J. Med. Chem., 60, 10013 -10025.

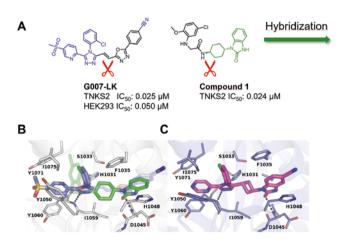
Sun, H., Horatscheck, A., Martos, V., Bartetzko, M., Uhrig, U., Lentz, D., Schmieder, P., Nazaré, M. (2017) Direct Experimental Evidence for Halogen-Aryl n Interactions in Solution from Molecular Torsion Balances. Angew Chem Int Ed Engl., 129, 6554-6558.

SELECTED EXTERNAL FUNDING

EU-DRIVE Consortium, 2018-2021, 258.211 Euro

Philipp Schwartz-Initiative der Alexander von Humboldt-Stiftung "Development of Novel Transition Metal Catalyzed Strategies to Access 2H-Azaindazoles and 2H-Indazoles as Privileged Heterocyclic Scaffolds", 2017-2019, 108.000 EURO

Deutsche Forschungsgemeinschaft, Sino-German Center for Research Promotion (DFG), GZ 1271, "Tumor-targeting SMART Imaging Agents" together with Prof. Haiyu-Hu, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China. 2016-2019, 207.845 Euro



- N CI NH
- New Hybrid OD336 TNKS2 IC₅₀: 0.006 μM HEK293 IC₅₀: 0.019 μM
- · Specific vs other ARTDs
- Good ADME profile
- Good PK in mouse, rat and dog
- Efficacious in COLO 320DM and leukemic p388 mouse model

FIG. 1

Structure-guided chemical hybridization approach toward a new specific tankyrase inhibitor (A) Structures of G007-LK and compound 1. Hybridization logic to yield OD336 from the deconstructed parent inhibitors. (B) Superposition of G007-LK (blue) and 1 (green) co-crystal structures (PDB: 4HYF and 4K4E). Only TNK52 protein is shown for clarity. (C) Co-crystal structure of OD336 (magenta) with TNK52 (PDB: 5NOB).

CORE FACILITY

SCREENING UNIT

SCREENING UNIT



GROUP LEADER (at the FMP since 2003)
Dr. Jens Peter von Kries

GROUP MEMBERS

Dr. Katina Lazarow, Dr. Silke Radetzki, Dr. Martin Neuenschwander, M.Sc. Marc Wippich (-2018), Sabrina Kleissle, Caroly Seyffarth, Romy Leu (-2018), Andreas Oder

→ leibniz-fmp.de/screening_unit

→ TESTING SUBSTANCES AND FINDING NEW TARGETS

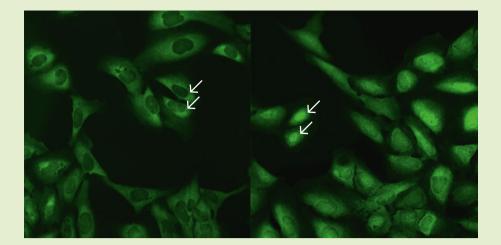
The Screening Unit serves as an open access technology platform for automated screening, using either compound libraries (~70,000 compounds) or genome-wide RNAi libraries (human, mouse, nematodes). The platform is typically a part of scientific collaborations, and its primary aim is to make possible the use of drugs in academic research for analysis of molecular mechanisms in disease and development. Besides supporting assay development, process automation, screening and automated data analysis, the Unit engages in the identification of novel screening technologies that may prove useful in its services. The Unit currently supports compound screening projects in assay development and optimization of High-Throughput Screening (HTS, Silke Radetzki), and in process automation (Martin Neuenschwander), including automated data documentation and analysis. Genome-wide RNAi and CRISPR-CAS screening (HTS, Katina Lazarow) has been also established for identification of novel cellular targets.

The Unit is building a central core facility for drug screening on the Campus Berlin-Buch for the Helmholtz-Initiative für Wirkstoffforschung, the Berlin Institute of Health (BIH), and EU-OPENSCREEN.

→ SUBSTANZEN TESTEN UND NEUE "TARGETS" FINDEN

Die Screening Unit ist eine frei zugängliche Technologieplattform für automatisierte systematische Testungen ("Screenings"). Wir verwenden entweder Substanzbibliotheken (~70.000 chemische Substanzen) oder genomweite RNA-Interferenz (RNAi)-Bibliotheken (Mensch, Maus, Nematoden), die spezifische RNA-Moleküle zur Hemmung der Übersetzung jedes einzelnen Gens in sein entsprechendes Protein enthalten. Die Forschenden des FMP und anderer, kooperierender Institutionen unterstützen wir bei der Testentwicklung, Prozessautomatisierung, beim Screening und bei der automatischen Datenanalyse. Darüber hinaus identifizieren wir neue Screening-Techniken und implementieren diese für den Einsatz. Derzeit unterstützen wir Screening-Projekte, die chemische Verbindungen bei der Entwicklung und Optimierung von Testverfahren zum Hochdurchsatz-Screening (Silke Radetzki), bei der Prozessautomatisierung (Martin Neuenschwander) und bei der automatisierten Datendokumentation und Analyse nutzen. Zudem haben wir das genomweite RNAi- und CRISPR-CAS-Screening (Katina Lazarow) als Service-Einheit etabliert, um die Identifizierung neuer zellulärer Zielstrukturen ("Targets") zu ermöglichen, die entweder durch Substanzwirkung, RNA-Interferenz oder CRISPR-CAS sichtbar werden.

CHEMICAL BIOLOGY RESEARCH REPORT 2017/2018



← FIG.

Without DNA damage (left image) NFkB localizes in the cytoplasm of cells (green fluorescence). Compound mediated damage (right) results into activation of the signaling and translocation of the transcription factor to the nucleus.

→ DESCRIPTION OF PROJECTS

DNA-DAMAGE INDUCED NF-KB SIGNALING

The research team of Claus Scheidereit is interested in mechanisms and factors contributing to NF-kB activation by DNA lesions. Finding pathway-selective inhibitors may help to overcome therapy resistance in cancer treatment. Therefore, we started with set-up of robust assays for high-content-screening using automated microscopes to identify drugs, which specifically interfere with DNA-damage induced NF-kB signaling but not with other kinds of pathway activation. We used quantification of translocation of the transcription factor from cytoplasm to nucleus, identified by fluorescently labeled antibodies, as a measure of activation and inhibition. We identified compounds, which interfere selectively with DNA damage induced NF-kB activation. These were also used for SILAC based target identification.

In a next step we suggested to apply genome-wide RNA-interference with a human RNAi library to identify novel factors involved in this specific type of NF-kB activation. For this purpose we applied a luciferase reporter cell line and performed about 70.000 RNAi transfections to test for about 23.000 cellular proteins. 1.000 primary hits, modulating NF-kB signaling, were selected for a second round of validation in the Screening Unit.

In a third approach we wanted to use the CRISPR-CAS technology for application in automated high-throughput screening. Therefore, we purchased a library with guide-oligonucleotides for editing about 1.200 genes for factors involved in proteasomal degradation. In contrast to RNA-interference, which often results in partial reduction of mRNA and corresponding cellular protein levels, CRISPR-CAS technology provides a complete removal of specific gene products and allows for systematic modification by targeted mutation. This approach demonstrated excellent robustness and data quality.

In summary, all three different NF-kB screens supported showed an exciting partial overlap of validated targets plus novel targets, which make sense in their contribution from already published involvement of these factors in cellular processes like nuclear transport or communication.

SELECTED PUBLICATIONS

Otten, C., Knox, J., Boulday, G., Eymery, M., Haniszewski, M., Neuenschwander, M., Radetzki, S., Vogt, I., Hähn, K., De Luca, C., Cardoso, C., Hamad, S., Igual Gil, C., Roy, P., Albiges-Rizo, C., Faurobert, E., von Kries, J. P., Campillos, M., Tournier-Lasserve, E., Derry, W. B., and Abdelilah-Seyfried, S. (2018)

Systematic pharmacological screens uncover novel pathways involved in cerebral cavernous malformations. EMBO Mol Med, vol. 10, no. 10, p. e9155, Oct. 2018.

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Du, J., Neuenschwander, M., Yu, Y., Däbritz, JH., Neuendorff, NR., Schleich, K., Bittner, A., Milanovic, M., Beuster, G., Radetzki, S., Specker, E., Reimann, M., Rosenbauer, F., Mathas, S., Lohneis, P., Hummel, M., Dörken, B., von Kries, J. P., Lee, S., Schmitt, CA. (2017). Pharmacological restoration and therapeutic targeting of the B-cell phenotype in classical Hodgkin lymphoma. Blood. 129(1):71–81.

SELECTED EXTERNAL FUNDING

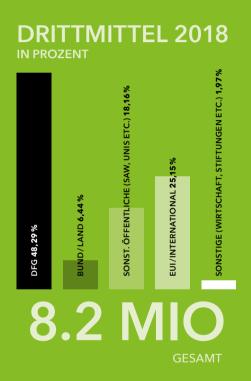
Bundesministerium für Bildung und Forschung, Helmholtz-Wirkstoffinitiative, ab 28.09.2011, 1.632.000 Euro

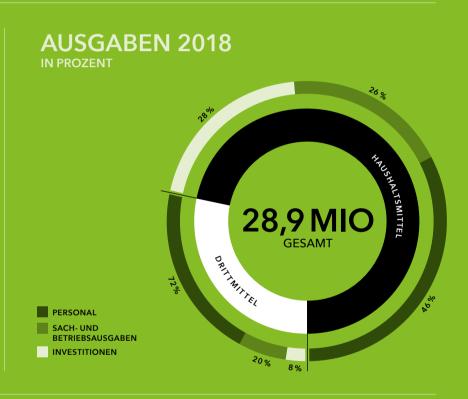
Bundesministerium für Bildung und Forschung, CCMCURE, E-RARE 2014, 01.06.2015 - 31.05.2018. 102.040.80 Euro

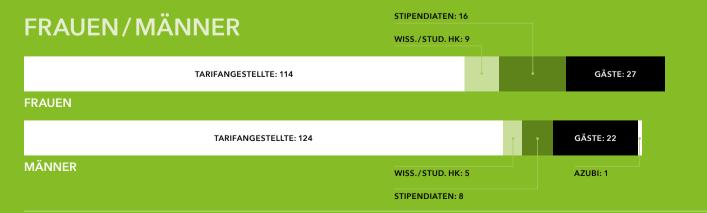
Bundesministerium für Bildung und Forschung, Berlin Institute of Health,
05.08.2015 - 31.12.2019, 1.225.000 Euro

FORSCHUNGSBERICHT 2017/2018 CHEMISCHE BIOLOGIE

FACTS AND FIGURES



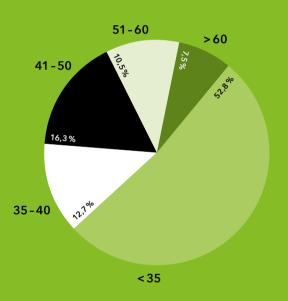




FACTS AND FIGURES RESEARCH REPORT 2017/2018

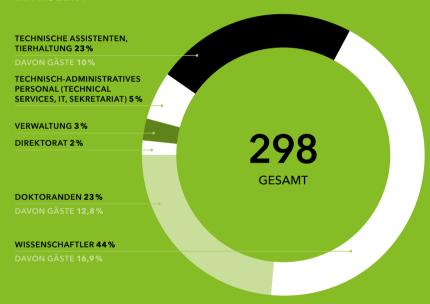
ALTERSSTRUKTUR

IN JAHREN



MITARBEITER*INNENSTRUKTUR

IN PROZENT



NATIONALITÄTEN

BRASILIEN (1), CHINA (7), DEUTSCHLAND (231), FRANKREICH (6), GEORGIEN (1), GREECE (2), HOLLAND (4), INDIEN (4), IRAN (2), IRLAND (1), ISRAEL (3), ITALIEN (5), KOLUMBIEN (1), MEXIKO (2), ÖSTERREICH (4), POLEN (1), PORTUGAL (1), RUSSLAND (3), SCHWEDEN (1), SCHWEIZ (2), SERBIEN (1), SPANIEN (4), SYRIEN (1), TÜRKEI (2), UK (3), UKRAINE (1), USA (3), ZYPERN (1)



FORSCHUNGSBERICHT 2017/2018 ZAHLEN UND FAKTEN

TECHNOLOGY TRANSFER

TECHNOLOGIETRANSFER

HEAD/LEITUNG Dr. Birgit Oppmann

Technology transfer at the FMP supports scientists in translating their research results into applications. This can be done, for example, by licensing out patent families to industrial partners or by supporting spin-off projects. The following start-ups are currently in preparation:

TUBULIS

As part of a scientific cooperation between the laboratories of Christian Hackenberger, FMP, and Heinrich Leonhardt, Ludwig Maximilian University Munich, two processes were developed to provide proteins with site-specific functionalities. These can be active agents, fluorophores, tracers, small chemical molecules, proteins and peptides, respectively. The so-called Tub-tag® and P5 technology form the basis for the spin-off project Tubulis which will be founded by Leonhardt and Hackenberger as well as their (former) employees Jonas Helma-Smets and Dominik Schumacher. The spin-off aims to market technologies for the production of diagnostic and therapeutic protein conjugates and to use these technologies to produce its own diagnostic and therapeutic products.

The founders are currently supported by the EXIST Research Transfer I Programme of the BMWi, the m4 Award of the Bavarian Ministry of Economic Affairs and the Leibniz Competition of the Leibniz Association. On 16th March 2018, they also received the Leibniz Start-up Award [Fig. 1] for the advancement of their corporate concept and ranked 10th among the top 50 start-ups of 2018 in 2019 (www.fuer-gruender.de).

PROSION

The foundations of the PROSION spin-off project were laid in many years of cooperation between the laboratories of Ronald Kühne, FMP and Hans-Günther Schmalz, Department of Chemistry, University of Cologne. The business area of the future company will initially comprise the development, manufacture and marketing of novel secondary structure mimetic building blocks as well as chemical libraries derived thereof for use in drug research.

ICHTHYOSIS THERAPEUTICS

The groups of Margitta Dathe, FMP, and Heiko Traupe, Department of Dermatology at the Westfälische Wilhelms-Universität Münster, have developed the basis for an enzyme replacement therapy for a rare skin disease. Affected patients lack the enzyme transglutaminase 1, which crosslinks proteins in the upper skin layers and thus enables normal skin function by forming the skin barrier.

The team received financial support from patients for the advancement of the therapeutic approach: the parents of affected twins started a crowdfunding campaign, and the first funds were forwarded to the FMP in September 2018. (https://www.comitatouffi.org).

Der Technologietransfer am FMP unterstützt Wissenschaftlerinnen und Wissenschaftler darin, ihre Forschungsergebnisse in die Anwendung zu bringen. Das kann etwa durch Auslizensierung von Patentfamilien an Industriepartner geschehen oder durch Unterstützung von Ausgründungsvorhaben. Aktuell befinden sich die folgenden Gründungen in Vorbereitung:

TUBULIS

Im Rahmen einer wissenschaftlichen Kooperation der Labore von Christian Hackenberger, FMP, und Heinrich Leonhardt, Ludwig-Maximilians-Universität München, wurden Verfahren entwickelt, um Proteine ortsspezifisch mit Funktionalitäten auszustatten. Dies können etwa Wirkstoffe, Fluorophore, Tracer, kleine chemische Moleküle, Proteine und Peptide sein. Die sog. Tub-tag® und P5-Technologie bildet die Grundlage für das Ausgründungs-

TECHNOLOGY TRANSFER RESEARCH REPORT 2017/2018



← FIG. 1

Bettina Böhm, Jonas Helma-Smets,

Christian Hackenberger,

Dominik Schumacher, Matthias Kleiner

vorhaben Tubulis, welches von den AG-Leitern Leonhardt und Hackenberger sowie deren (ehemaligen) Mitarbeitern Jonas Helma-Smets und Dominik Schumacher gegründet wird. Die Ausgründung hat das Ziel, Technologien für die Herstellung von diagnostischen und therapeutischen Protein-Konjugaten zu vermarkten und mit diesen Technologien eigene diagnostische und therapeutische Produkte herzustellen.

Zur Zeit werden die Gründer durch das EXIST Forschungstransfer I Programm des BMWi, den m4-Award des bayerischen Wirtschaftsministeriums sowie über den Leibniz-Wettbewerb der Leibniz-Gemeinschaft gefördert. Am 16.03.2018 erhielten sie außerdem den Leibniz-Gründerpreis [Fig. 1] für die weitere Entwicklung ihres Unternehmenskonzepts sowie 2019 Platz 10 unter den Top 50 Start-ups des Jahres 2018 (www.fuer-gruender.de).

PROSION

Die Grundlagen des Ausgründungsvorhabens PROSION wurden in einer langjährigen Zusammenarbeit zwischen den Laboren von Ronald Kühne, FMP, und Hans-Günther Schmalz, Department für Chemie, Universität zu Köln erarbeitet. Das Geschäftsfeld der künftigen Firma wird zunächst die Entwicklung, Herstellung und Vermarktung neuartiger Sekundärstruktur-mimetischer Bausteine

umfassen sowie davon abgeleitete chemische Bibliotheken zum Einsatz in der Wirkstoffforschung.

ICHTHYOSIS THERAPEUTICS

Die Arbeitsgruppen von Margitta Dathe, FMP, und Heiko Traupe, Hautklinik der Westfälischen Wilhelms-Universität Münster, haben die Grundlagen für eine Enzymersatztherapie einer seltenen Hautkrankheit erforscht. Den Patienten fehlt das Enzym Transglutaminase 1, das in den oberen Hautschichten Proteine quervernetzt und so durch Bildung der Hautbarriere eine normale Hautfunktion ermöglicht.

Finanzielle Hilfe zur weiteren Entwicklung des Therapieansatzes bekam das Team von Seite der Patienten: Die Eltern von betroffenen Zwillingen haben eine Crowdfunding-Kampagne gestartet, die ersten Gelder wurden im September 2018 an das FMP weitergeleitet. (https://www.comitatouffi.org).

FORSCHUNGSBERICHT 2017/2018 TECHNOLOGIETRANSFER

PUBLIC RELATIONS

ÖFFENTLICHKEITSARBEIT

HEAD/LEITUNG
Silke Oßwald M.A., M.Sc.

Public relations activities cover many aspects, including brochures, flyers and an internal newsletter (FMP interview) as well as press releases, websites and social media. Every year we receive visitors and organize several events:

Die Öffentlichkeitarbeit hat viele Aspekte, zu nennen wären Broschüren, Flyer und ein interner Newsletter (FMP Interview) sowie Pressemitteilungen, Website und Social Media. Jedes Jahr empfangen wir Besucher*innen oder nehmen an Events teil:



FMP CHEMLAB

The ChemLab is the student laboratory of the FMP. Since 2010, scientists have been teaching chemistry methods in a vivid and unconventional way. These courses are available: caffeine, dyes, plastics, fragrances and proteins. In 2017 and 2018, a total of 800 students attended the ChemLab.

FMP CHEMLAB

Das ChemLab ist das Schülerlabor des FMP. Hier vermitteln seit 2010 Wissenschaftler*innen Methoden der Chemie anschaulich und unkonventionell. Zur Wahl stehen diese Kurse: Koffein, Farbstoffe, Kunststoffe, Duftstoffe und Proteine. 2017 und 2018 besuchten insgesamt 800 Schüler*innen das ChemLab.



LONG NIGHT OF SCIENCES

Every year, thousands of visitors come to the Campus Buch to experience the latest in research in lectures and laboratory tours. More than 100 program items attract visitors to the institutes.

Visits 2017 (10,200); 2018 (7,200)

LANGE NACHT DER WISSENSCHAFTEN

Jedes Jahr nehmen Tausende von Besucher*innen am großen Experimentieren auf dem Campus Buch teil und erleben in Shows, Vorlesungen oder Laborführung Aktuelles aus der Forschung in mehr als 100 Programmpunkten.

Besuche 2017 (10.200); 2018 (7.200)

CHEMISTRY COURSE FROM BERLIN-LICHTERFELDE SYNTHESIZES "LOVE POWDER"

Students synthesized the hormone oxytocin by coupling amino acids using the SPPS method (Solid Phase Peptide Synthesis). This provided them an insight into scientific research practice and the working environment at a research institute.

CHEMIE-LEISTUNGSKURS AUS BERLIN- LICHTERFELDE SYNTHETISIERT "LIEBESPULVER"

Schüler*innen synthetisierten das Hormon Oxytocin durch Kopplung von Aminosäuren mit der SPPS-Methode (Solid Phase Peptide Synthesis). So konnte ein Einblick in die naturwissenschaftliche Forschungspraxis und Arbeitsathmosphäre an einem Forschungsinstitut gewonnen werden.



PUBLIC RELATIONS RESEARCH REPORT 2017/2018



André Lampe und Janine Kirstein

BERLIN SCIENCE WEEK

Exploring Berlin as a "Brain City" has been possible at Science Week since 2016. For ten days, Berlin is transformed into a showcase for science. The FMP moved closer to the city centre with a format combining "Science in the Pub" and a podcast. Janine Kirstein was the first guest at André Lampe's new podcast "mal ganz grundsätzlich". (mgg.andre-lampe.de.)

BERLIN SCIENCE WEEK

Berlin als "Brain City" erkunden, das kann man seit 2016 jährlich auf der Science Week. Zehn Tage lang wird Berlin in ein Schaufenster der Wissenschaft umfunktioniert. Das FMP rückte hierfür näher in die City mit einem Format, das "Wissenschaft in der Kneipe" und Podcast verbindet. Die erste Folge des Podcasts "mal ganz grundsätzlich" wurde live in einer Kneipe in Berlin Wedding aufgenommen. Zum Thema Proteine war Janine Kirstein zu Gast bei André Lampe. (mgg.andre-lampe.de.)

Follow us/Folgen Sie uns auf:

☑ @LeibnizFMP

@ @fmp.berlin

GIRLS' DAY

Schoolgirls from different grades are guests in the research groups at the FMP every year. For one day, they can look over the researchers' shoulders and try out small experiments for themselves.

GIRLS' DAY

Schülerinnen aus unterschiedlichen Klassenstufen sind jährlich in den Arbeitsgruppen am FMP zu Gast und dürfen für einen Tag den Forschenden über die Schulter schauen und kleine Experimente selber ausprobieren.



Wing Ying Chow im Sony Center

SOAPBOX EVENT

Women scientists present their topics in public places in an entertaining and understandable way, for example in 2017 at the Tempelhofer Feld in Berlin or in the Sony Center.

SOAPBOX EVENT

Wissenschaftlerinnen stellen an öffentlichen Plätzen ihre Themen auf lehrreiche und verständliche Weise vor. So auch bei der Veranstaltung 2017 auf dem Tempelhofer Feld in Berlin, oder im Sony Center.

→ WSR007 Moleküle
Atom für Atom
untersuchen mit
NMR-Spektroskopie Interview mit Dr. Peter



INDEPENDENT PROJECT

WIRKSTOFFRADIO (EPISODES ONLY IN GERMAN)

Regardless of where Bernd Rupp and André Lampe meet, whether in Bernd's living room or in a laboratory visiting scientists - the topics always focus on active substances of drugs. With great enthusiasm for science, research and a lot of curiosity, new episodes are produced for the Podcast "Wirkstoffradio" every second week. You are invited to listen to the episodes and to subscribe via the usual "Podcatcher" or directly via the website wirkstoffradio.de.

EIGENSTÄNDIGES PROJECT

FORSCHENDEN ZUHÖREN - DAS WIRKSTOFFRADIO

Egal, wo sich Bernd Rupp und André Lampe treffen, ob in Bernds Wohnzimmer oder in einem Labor zu Gast bei Wissenschaftler*innen in einem Forschungsinstitut – die Themen drehen sich immer um Wirkstoffe. Mit großer Begeisterung für die Wissenschaft, eigener Recherche und einer Portion Neugier entstehen alle 14 Tage neue Folgen für den Podcast "Wirkstoffradio", den man über die üblichen "Podcatcher" sowie direkt über die Website wirkstoffradio.de anhören und abonnieren kann.

FORSCHUNGSBERICHT 2017/2018 ÖFFENTLICHKEITSARBEIT

EDUCATION AND TRAINING AUSBILDUNG

Sharing knowledge! The promotion of young, talented people is a central concern at the FMP:

STUDENT LABORATORY FMP-CHEMLAB

In the ChemLab, located in the Gläsernen Labor, students from upper and secondary schools slip into the role of chemists and carry out experiments that are impossible to implement in school laboratories. The courses are available in caffeine, dyes, plastics, fragrances and proteins.

APPRENTICESHIPS

From school desk to lab bench! Our trainees as animal keepers or biology laboratory assistants can expect a comprehensive program in the working groups at the FMP. The training is supplemented by courses in the MDC learning laboratory together with the trainees of the Campus Berlin-Buch. The theoretical training takes place in the Berufsschulen Lise-Meitner Schule (for biology laboratory assistants) and Peter-Lenné-Schule (for animal keepers).

ACADEMIC STUDIES

Students are welcome to do the experimental work for their bachelor and master theses in our institute. We offer the following courses as an introduction to working here:

- Seminar and Practical Course Molecular Pharmacology and Cellular Signal Transduction (Ralf Schülein, Volker Haucke)
- Biological NMR spectroscopy (Hartmut Oschkinat)
- NMR Spectroscopy and Imaging (Hartmut Oschkinat)
- Functional analysis of molecular chaperones and their role in neurodegenerative diseases (Janine Kirstein)
- NMR School (Peter Schmieder)

PHD - THE FMP GRADUATE SCHOOL

For a successful PhD, doctoral students should not only be well supervised in their project, but should also get to know the research questions and methods of other groups and finally be able to build networks. In order to structure and improve training and supervision, the FMP Graduate School was opened in 2013 and accessible to all PhD students. The Graduate School is headed by Christian Hackenberger, the administration is in the hands of Katrin Wittig.

NETWORKING ON THE BERLIN-BUCH CAMPUS

During the doctoral period, the Graduate School will hold a joint annual retreat with the Max Delbruck Center for Molecular Medicine (MDC) as well as postdoctoral retreats, which are jointly organized by the two institutes.

Wissen weitergeben! Die Förderung junger, talentierter Menschen ist ein zentrales Anliegen am FMP.

SCHÜLERLABOR FMP-CHEMLAB

Im ChemLab im Gläsernen Labor, schlüpfen Schüler*innen der Ober- und Sekundarstufe in die Rolle von Chemiker*innen und führen Experimente durch, wie sie in Schullaboren nicht umsetzbar sind. Zur Wahl stehen die Kurse: Coffein, Farbstoffe, Kunststoffe, Duftstoffe und Proteine.

AUSBILDUNGSBERUFE

Von der Schulbank zur Lab bench! Unsere Auszubildenden als Tierpfleger*innen oder Biologielaborant*innen erwartet ein umfassendes Programm in den Arbeitsgruppen am FMP. Die Ausbildung wird ergänzt durch Kurse im MDC Lernlabor gemeinsam mit den Auszubildenden des Campus Berlin-Buch. Die theoretische Ausbildung erfolgt in den Berufsschulen Lise-Meitner Schule (für Biologielaborant*innen) und Peter-Lenné-Schule (für Tierpfleger*innen).

STUDIUM

Student*innen können bei uns die Experimente für ihre Bachelor-/Masterarbeit durchführen. Als Einstieg in die Arbeiten in unserem Institut bieten wir folgende Kurse an:

- Seminar und Praktikum Molekulare Pharmakologie und zelluläre Signaltransduktion (Ralf Schülein, Volker Haucke)
- Biologische NMR-Spektroskopie (Hartmut Oschkinat)
- NMR-Spektroskopie und Imaging (Hartmut Oschkinat)
- Funktionelle Analyse von molekularen Chaperonen und ihrer Rolle in neurodegenerativen Krankheiten (Janine Kirstein)
- NMR School (Peter Schmieder)

PROMOTION - DIE FMP GRADUATE SCHOOL

Für eine erfolgreiche Promotion sollten Doktorand*innen nicht nur in ihrem Projekt gut betreut werden, sondern auch die Fragestellungen und Methoden anderer Gruppen kennenlernen und Netzwerke bilden können. Um die Ausbildung und Betreuung zu strukturieren und zu verbessern, ist daher 2013 die Graduiertenschule des FMP eröffnet worden, die allen Doktoranden des Instituts offensteht. Geleitet wird die Graduiertenschule von Christian Hackenberger, die Verwaltung liegt in den Händen von Katrin Wittig.

VERNETZUNG AUF DEM CAMPUS BERLIN-BUCH

Während der Promotion findet im Rahmen der Graduate School ein gemeinsames jährliches Retreat mit dem Max Delbruck Centrum für Molekulare Medizin (MDC) statt sowie auch Postdoc Retreats, die beide Institute gemeinsam veranstalten.

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ALL RESEARCH GROUPS

ALLE FORSCHUNGSGRUPPEN

MOLECULAR PHYSIOLOGY AND CELL BIOLOGY

→ PHYSIOLOGY AND PATHOLOGY OF ION TRANSPORT

Thomas J. Jentsch

→ MOLECULAR PHARMACOLOGY AND CELL BIOLOGY

Volker Haucke

- → MOLECULAR NEUROSCIENCE AND BIOPHYSICS
 Andrew Plested
 Heisenberg Guest Group
- → MEMBRANE TRAFFIC AND CELL MOTILITY Tanja Maritzen Junior Research Group
- → PROTEOSTASIS IN AGING AND DISEASE Janine Kirstein Junior Research Group
- → MOLECULAR AND THEORETICAL
 NEUROSCIENCES
 Alexander Walter
 Emmy Noether Junior Group,
 Liaison Group Neuroscience
- → PROTEIN TRAFFICKING Ralf Schülein
- → CORE FACILITY: CELLULAR IMAGING Martin Lehmann
- → CORE FACILITY: ANIMAL FACILITY Natali Wisbrun

STRUCTURAL BIOLOGY

- → MOLECULAR BIOPHYSICS Adam Lange
- → NMR-SUPPORTED STRUCTURAL BIOLOGY Hartmut Oschkinat
- → MOLECULAR IMAGING Leif Schröder Junior Research Group
- → COMPUTATIONAL CHEMISTRY/ DRUG DESIGN Ronald Kühne
- → STRUCTURAL BIOINFORMATICS AND PROTEIN DESIGN Gerd Krause
- → CORE FACILITY: NMR Hartmut Oschkinat/ Peter Schmieder

CHEMICAL BIOLOGY

- → CHEMICAL BIOLOGY II Christian Hackenberger
- → CHEMICAL BIOLOGY I Dorothea Fiedler
- → STRUCTURAL INTERACTOMICS
 Fan Liu
- → MEDICINAL CHEMISTRY Marc Nazaré
- → CORE FACILITY: SCREENING UNIT Jens-Peter von Kries
- → CORE FACILITY: MASS SPECTROMETRY Fan Liu

FORSCHUNGSBERICHT 2017/2018 ORGANIGRAM 69

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IMPRESSUM

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RESEARCH REPORT 2017/2018

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3-D IllustrationBarth van Rossum

Further Visualisation

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Design and Layout

KRAUT & KONFETTI, Berlin

Print

Pinguin Druck GmbH, Berlin

Berlin, 2019

