Karlsruhe Institute of Technology

BIO Interfaces
International Graduate School
(BIF-IGS)

GRK 2039

General Information & Abstract Booklet

Retreat

in Haus Lutzenberg, Althütte

July 4 – 6, 2016

BIF-IGS meets GRK

Preface

Dear fellow Graduate Students, Dear Administration and PIs,

We are happy to welcome you to the Retreat 2016!

This year for the first time, the Retreat is a joined venture of BIF-IGS and GRK2039 – thus, promising many new faces and interesting interdisciplinary discussions beyond the individual programmes. So, we can surely look forward to even more stimulating and inspiring talk and poster sessions.

We want to encourage you to be an active participant at the talk and poster sessions and to take this chance to get in contact with other PhD students. The annual Retreat meeting is a great opportunity to obtain an overview of what other PhD students at KIT do and maybe to get new ideas inspired by the versatile talks and posters. Take advantage of the unforced atmosphere and get to know the other participants and you will surely benefit from these three days in Haus Lutzenberg.

We would also like to take the opportunity to thank the Administration and the PIs for spending their time on participating in this Retreat. Joining the event (and awarding the prices) are **Kersten Rabe, Markus Reischl, Nick Foulkes, Sepand Rastegar and Thomas Dickmeis, as well as Larissa Kaufmann, Alexandra Schade, Hans-Achim Wagenknecht and Stefan Bräse**. We really appreciate this and will be happy to welcome you maybe again next year.

We hope, our efforts to put a satisfying and enjoyable programme together were successful and we look forward to having a great time with great science.

The Organisation Committee

Bernadett Bösze (ITG), Caroline Schweigert (IPC), Eva Zittel (ITG/IOC), Manfred Maier (IBG-1), Vanessa Gerber (ITG), Vanessa Kappings (ITG/IOC)

General Information

Departure & Journey

For our Retreat, we will meet **on Monday, 04th July at 8 am sharp** at the **KIT Shuttle Bus Stop at Campus South** (Engesser Straße, in front of Chemistry building / AKK). There will be two buses from **KASPER** taking us to the accommodation and we expect **everyone to be on time** as they will leave. Depending on traffic, the ride roughly takes 2 hours.
We will be returning to Karlsruhe (Campus South) on Wednesday, 06th July. The buses will depart from Ludwigsburg at 3.30 pm and will arrive back at approx. 5 pm.

It is mandatory to travel by the provided buses. If there are important reasons for you to travel by yourself, you have to inform Larissa in time!

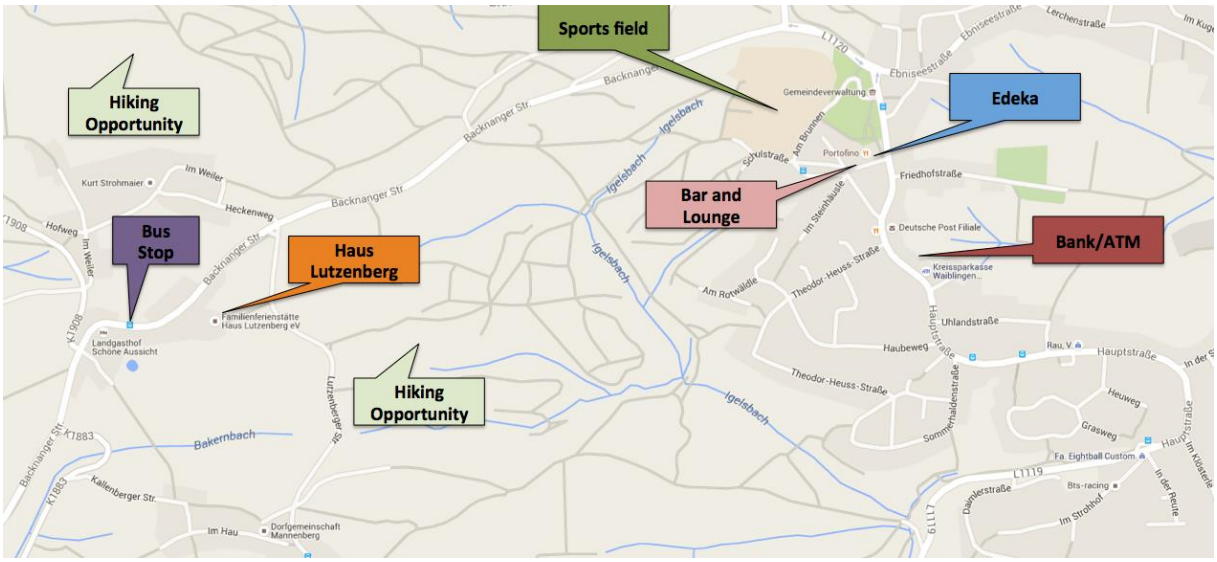
Accommodation

This year's Retreat takes place in Haus Lutzenberg, Althütte (Schwäbischer Wald). Our travelling costs, accommodation and meals are completely covered by BIF-IGS and the GRK. This includes breakfast, lunch and dinner as well as beverages (soft drinks during the day, beer and (sparkling) wine in the evening) and coffee/ tea breaks. Depending on the weather, we will also have a barbecue on Tuesday evening.
In the accommodation, all linen and towels are provided, also WiFi is available. All rooms are equipped with their own bathroom. For your entertainment, you will find bowling, billiards, table tennis, table football, a climbing wall, pianos, TV and DVD player. Additionally, there are several common rooms for our use.
Please be advised that smoking is prohibited inside the premises and that it is not allowed to bring external food and beverages.
Also remember that if you cancel your participation short-term or do not turn up without an excuse you/ your group will have to pay the full price for the Retreat (approximately 400 € per student)!

Vicinity

Besides the activities offered by Haus Lutzenberg, one could find additional free time activities. Althütte offers a great opportunity for nature-lovers. There are also several hiking roads in close vicinity to the accommodation. (www.schwaebischerwald.com, www.althuette.de/gemeinde-althuette-wanderrouten.html)

Below you find the map of Althütte with some major points: supermarkets, public transport, ATM, etc. in the neighbourhood.



Excursion

At the end of our three day Retreat 2016, we will have a joint excursion to visit one of the largest baroque palaces in Germany. (Fees will be taken over by BIF-IGS and GRK!)

The Ludwigsburg Residential Palace is one of the few Baroque buildings to have survived the tumultuous history of the last centuries almost unscathed. In order to get an impression of this impressive part of history, we will have a guided tour. In general, there are several possible parts of the palace and the surrounding buildings (museum, theatre…) which could be viewed. What we selected for you is a guided tour through the splendid apartments of either Queen Mathilde, or King Friedrich. Both tours will start at the same point and will take about one hour and will give you an insight of a unique combination of three quite different architectural styles: Baroque, Rococo and Neoclassicism.

In view of our large number of people we will be split in three groups which start their guided tour at 15 minute intervals. All three guides will give their tour in English. The free time between the arrival in Ludwigsburg and start of your tour through the palace, or after your tour and the travel back to Karlsruhe, can be used for a little walk through Ludwigsburg or a coffee break in one of the surrounding cafes and bars**. If you still have a valid student card please take it with you** for a reduced tour ticket and conserving the budget.

Talks & Posters – general information and guidelines

When you have to do a poster or a talk, you should always keep in mind that the audience (PhD-students and PIs) is very versatile in their background knowledge. So try to not loose attention by doing it too specific and complex.

Also, we would like to encourage everybody to actively participate and profit from the given presentations. Therefore, there is supposed to be **at least one (student) audience question for the discussion** after each talk. Use the event to improve your own interdisciplinary understanding and help the others to clarify their ways of outlining and expressing their work.

The best talks and posters will be awarded by the PIs. They will consider comprehensibility, presentation style and time of your talk/ poster presentation. Also, they will take into account, if there was a clear 'take home message' about what you are doing.

This year, the prices are sponsored by the company Carl Roth. We are very grateful for that.

Talks

You should put your talk on the laptop at least 15 min before your session starts.

Your speaking time should not be longer than **12 minutes**, followed by 5 minutes for discussion. You can use laptop and laser pointer which are provided by us.

Posters

You should put your poster on the provided walls at least 15 min before your session starts.

As the PIs have to look at all posters for assessing them, you should be able to present your poster within **5 minutes**.

Schedule

	Monday 4th July		**Tuesday 5th July**		**Wednesday 6th July**
Time		**Time**		**Time**	
	Travel to Ludwigsburg: Departure from Campus South	**8:00**	Breakfast	**8:00**	Breakfast and check out
	Arrival and unpacking	**9:15 – 11:45**	Talks 5 - 10	**9:15 – 10:45**	Poster session III
11:15 – 12:00	Welcome speech + get to know each other			**11:30**	Group photo election Retreat Team 2017 Feedback Award Ceremony
12:00	Lunch	**12:00**	Lunch	**11:30**	Lunch
13:15 – 15:00	Talks 1 - 4	**13:30 – 15:00**	Poster Session II	**12:30**	Travel to Ludwigsburg
				13:45	Visit of the " Ludwigsburg Residential Palace "
15:30 – 17:00	Poster Session I	**15:30 – 17:45**	Talks 11 – 15	**15:30**	Travel back to Karlsruhe
18:00	Dinner	**18:30**	Dinner (Barbecue)		
	Free time ☺		Free time ☺		

Talks: 12 min + 5 min discussion

Poster Presentations: 5 min

Sessions Monday

Talks 1 - 4:

Time	Speaker
13:15 - 13:35	Bäcker, Anne
13:35 - 13:55	Donati, Alessia
13:55 - 14:15	Break
14:15 - 14:35	Fischer, Andrea
14:35 – 14:55	Gralla, Robert

Speakers have to put their presentations on the laptop at 13:00 !

Posters I:

Time	Presenter
15:30 – 17:00	Raic, Annamarija
	Schneider, Ann-Kathrin
	Schneider, Violetta
	Schweigert, Caroline
	Steinmeyer, Jeannine
	Tronser, Tina
	Varadharajan, Divya
	Walter, Romina
	Yu, Xiaobing
	Zhao, Haiyu

Sessions Tuesday

Talks 5 - 10:

Time	Speaker
09:15 – 09:35	Hansen, Silla
09:35 – 09:55	Keller, Dominic
09:55 – 10:15	Kemler, Denise
10:15 – 10:40	Break
10:40 – 11:00	Kossmann, Katja
11:00 – 11:20	Marcato, Daniel
11:20 – 11:40	Ntim, Emmanuel Amankwah

Speakers have to put their presentations on the laptop at 09:00 !

Posters II:

Time	Presenter
13:30 - 15:00	Kabbeck, Tobias
	Kowoll, Thomas
	Lehmann, Benjamin
	Leidner, Arnold
	Li, Ying
	Maier, Manfred
	Mattes, Benjamin
	Münch, Stephan
	Olshausen, Bettina
	Peschke, Theo

Talks 11 - 15:

Time	Speaker
15:30 - 15:50	Salama, Mohammed
15:50 – 16:10	Schmieg, Barbara
16:10 – 16:30	Srivastava, Tanu
16:30 – 16:55	
16:55 – 17:15	Susanto, Steven
17:15 – 17:35	Weber, Laura

Speakers have to put their presentations on the laptop at 15:15 !

Session Wednesday

Posters III:

Time	Presenter
09:15 – 10:45	Becker, Katharina
	Bichelberger, Mathilde
	Cruz Sánchez, Daniela
	Ebrahimi Khonachah, Mojtaba
	Fanselau, Katharina
	Garrecht, Ruben
	Gerber, Vanessa
	Hurrle, Thomas

Abstracts of all Participants

Name: George Ainooson

Group: Prof. Dr. Andrew Cato

Title: PEST-domain enriched tyrosine phosphatase (PEP) in the regulation of mast cell signalling

Abstract: Protein tyrosine phosphatases (PTP) are targets in immune cell-mediated diseases. PTP dephosphorylate cellular proteins which generally downregulates immune cell function. PEP, a cytoplasmic PTP, is one of such phosphatases. PEP is expressed and is up regulated by the antiallergic glucocorticoid (GC) dexamethasone in mast cells. Surprisingly, gene knock-out studies showed that inhibition of PEP protects against mast cell-mediated allergic response, suggesting that PEP may be a positive regulator of mast cell action. Thus, RNA sequencing experiment was carried out with PEP wild-type (PEP+/+) and PEP deficient (PEP-/-) bone marrow derived mast cells to understand the mechanism by which PEP modulates mast cell signalling, and to clarify its contribution to the antiallergic action of GC. The results showed that deletion of PEP did not significantly change the gene expression profile of resting mast cells, but greatly altered the pattern of gene expression induced by antigen and GC. Gene Ontology analysis showed that deletion of PEP affected a wide range of biological processes with protein binding and cell differentiation, cytokine and immune cell response as some of the most significantly affected processes. Further analysis is being carried-out to unravel the mechanism of PEP in mast cell and its role in GC action.

Name: Anne Bäcker

Group: Friederike J. Gruhl (IMT)

Title: Impact of adjustable cryogel properties on the performance of prostate cancer cells in 3D

Abstract: Biochemical and physical characteristics of extracellular environment play a key role in assisting cell behavior. In this study, we investigated how the presence of binding sites, incorporated within the scaffold, the pore network and the stiffness of designed scaffolds affected the epithelial prostate cancer cell line LNCaP. Growth, spheroid formation and localization of androgen receptor (AR) were measured to evaluate cell response. A blend of poly hydroxyethyl methacrylatealginate-gelatin (pHAG) scaffold was synthesized by cryogelation process using polyethylenglycol diacrylate (PEGda) and glutaraldehyde as cross linking agents. The chemical and mechanical scaffold properties were varied by concentration of gelatin and PEGda, respectively. Whereas the pore network was modified by applying different 'freezing time' and ‘freezing temperature’. Spheroid size was reduced by insufficient porosity, elasticity as well as by the absence of RGD. Localization of AR indicates its activity and should be under normal culture conditions in the nucleus. But in this study, we could investigate for the first time that AR remains in the cytoplasm when LNCaP cells are cultured in scaffolds without RGD as well as in case of an insufficient pore network (total porosity under 10%) and a too less stiffness of around 10 kPa.

Name: Katharina Becker

Group: Institute of Biological Interfaces (IBG-2), Prof. Anne S. Ulrich

Title: Structure-function analysis of the small toxin BsrG using anisotropy-based methods

Abstract: The small 38 amino acid peptide BsrG from *B. subtilis* is part of a toxin-antitoxin type I system and leads to cell lysis in absence of the antagonizing antitoxin SR4. Until now, no details are known about the mechanism and the structure of BsrG. We used solid-state NMR measurements on macroscopically aligned samples to investigate its alignment and dynamical behavior in model membranes and found an α-helical conformation and a transmembrane orientation. Although solid-state NMR is a powerful method to investigate the molecular structure and orientation of proteins in membranes, the method is restricted to artificial lipid bilayers and does not allow investigations in natural membranes of living systems. On the other hand, fluorescence measurements are highly sensitive and can be done in living systems. Our aim is therefore to establish a fluorescence-anisotropy based method to investigate the orientation of membrane proteins under natural conditions. To prove the feasibility of this new approach, we synthesized analogues of the well-studied peptide PGLa containing rigid fluorescent amino acids with well-defined transition dipolar moments. This should allow studying the orientation of peptides like PGla and BsrG in real membranes of living cells.

Name: Franziska Beyle

Group: Prof. Dr. Marcus Elstner

Title: Getting a Trajectory in the Excited State of the Fluorescent Molecule Flugi2

Abstract: We investigate the solvatochromic shifts in absorption and emission for the Flugi-2 molecule in DMSO by simulations. The solvatochromic shifts in absorption is negligible. This effect cannot explain the large experimentally measured solvent effect on the Stoke's shifts, however it can be explained by the solvatochromism in emission. We would like to obtain a set of snapshots (100-300) for a thermally equilibrated system, which comprises Flugi-2 in the lowest excited state and the solvent (DMSO). The geometry of the chromophore should be allowed to fluctuate around the equilibrium excited state geometry. On this snapshots the energy gap between excited state and ground state will be calculated by quantum mechanic methods and give the distribution of the fluorescence spectrum.

Name: Mathilde Bichelberger

Group: Prof. G. U. Nienhaus

Title: Photophysical characterization of novel pyridinium azobenzenes

Abstract: Azobenzenes are light sensitive molecules widely used to control molecular motions. When exposed to light of a particular wavelength the molecules switch from the *trans* form to the *cis* form. Over time, the molecules relax back into the thermodynamically stable *trans* state. This back relaxation happens on the time scale of hours and even days for non-substituted azobenzene molecules, whereas substituted molecules may relax within seconds or even nanoseconds. Nowadays, efforts are concentrated on synthesizing water-soluble and biocompatible azobenzene probes with absorption bands in the red region.

Here, we have synthesized and characterized water-soluble and fast switching azo compounds. Their short lived *cis* forms with half-life times in the microsecond to millisecond time range could only be studied with nanosecond flash photolysis. We investigated the effect of cylic and acyclic substituents as well as the bromine atom on the half-life of the *cis* form in phosphate buffered saline (PBS) and in a solvent with higher viscosity. Moreover, we have observed irreversible photoconversion of disubsituted azo compounds. The photoproducts featured reversible isomerization. These disubstituted azo compounds also showed pH-dependent isomerization kinetics.

Name: Bernadett Bösze

Group: Dr. Steffen Scholpp

Title: Pcdh18a$^+$ tip cells orchestrate notochord formation

Abstract: Collective cell migration is a fundamental process required for tissue development, wound repair and cancer invasion. The most impressive example of cohort migration is the formation of the germ layers during vertebrate gastrulation. Mesoderm cells involute to form the prechordal plate and subsequently the notochord, the major axial skeletal element of the developing embryo. The cellular and molecular basis of collective migration regulating the formation of axial and paraxial mesoderm is unknown.

We found Pcdh18a, a member of the cadherin superfamily as a novel regulator of cell motility in gastrulating zebrafish. We show that Pcdh18a is induced and its expression is maintained in the notochord tip cells (NTCs). We find that Pcdh18a enhances adhesiveness and cell migration in NTCs *in vivo*, and in E-cadherin$^+$ mouse fibroblasts and in HeLa cells *in vitro*. On a molecular level, Pcdh18a interacts with the E-cadherin complex and leads to dynamic remodeling of cell-cell contacts by endocytosis. Upon endocytic blockade, Pcdh18a$^+$ cells showed decreased migration but maintain enhanced adhesiveness. In zebrafish, we find that increased migration and adhesiveness of the NTCs are prerequisite for elongation of the axial mesoderm. We propose that Pcdh18a$^+$ NTCs orchestrate the shape of the forming notochord during vertebrate gastrulation.

Name: Özgül Demir Bulut

Group: Sven Ulrich IAM-AWP, STN /Group leader

Title: Surface Engineering of Biocompatible a-C based Coatings

Abstract: The physical and chemical composition of the samples interacts with their surroundings through their surfaces. Therefore, the first regulatory of the surface chemistry of materials determines the nature of the interactions. Despite the undoubted importance of surfaces, only a very small proportion of the regulators of most solids will influence such factors as corrosion rates, catalytic activity, adhesive properties, wettability, contact potential, and failure mechanisms. Although only a very small proportion of the atoms of most coatings are found at the surface, they are the first regulatory of the biocompatible materials. This project focuses on combined plasma chemical of amorphous carbon thin film surfaces and intends to provide insight into the interactions of proteins with these biomaterials. These interactions are of importance since they are the first regulatory events controlling the interactions of cells and tissues with any biomaterial surface. The protein adsorbate formed on these surfaces and elaborate correlations between structure, surface properties and bio functional properties of as-deposited will be analysed in detail by Osteoblast Cell Adhesion, Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), Raman, Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS), Nanoindendation, X-Ray Photoelectron Spectroscopy (XPS) and Contact Angle Measurement. It is planned to investigate, if and

Name: Teresa Burgahn

Group: Prof. Dr. C. M. Niemeyer

Title: Assembly of functionalized Proteins on DNA nanostructures

Abstract: Structural DNA nanotechnology uses DNA as basis molecules to create structures in nanometer scale. Due to the high specificity of Watson Crick base pairing the DNA structures can be designed in a discretionary form. Among others the DNA origami is one example of a self-assembly DNA nanostructure. A DNA origami is built up by a long single stranded DNA plasmid (Scaffold strand) folded by various complementary short synthetic oligonucleotides (staple strands). Such DNA nanostructures are used to immobilize proteins in an accurate order in nanometer scale. The arrangement of the biomolecules (e.g. biotechnical interesting enzymes) on the surface of the DNA nanostructures takes place by immobilization methods like functionalized linkers (e.g. fusion proteins).

The resulting assembly of protein-DNA-nanostructures delivers a technique to analyze the interactions between multi-enzyme complexes at distinct positions. Analysis of Protein-DNA-nanostructures produced by this evolved method gain to characterize single protein and multi-enzyme complexes concerning their activity along with the control of different arrangements of various proteins at the nanometer length scale.

Name: Daniela Sánchez

Group: Prof. A. Guber

Title: Automated microfluidic system for gRNA cloning

Abstract: Scaling fluid volumes from milliliters to the microliters or nanoliters scale and automating workflows are two of the main reasons for the increasing attempt to prove the microfluidic technologies successful for molecular biology applications. Molecular cloning is a basic technique in the molecular biology, thus several microfluidic devices have been developed to automate its workflow. Each step has been previously carried out in a microfluidic device; however, those systems are only partially automated or use pipetting systems, which are very expensive and can be very cumbersome.

The integration and complete automation of the molecular cloning workflow is, to the best of my knowledge, still unsuccessful. Therefore, three steps of this workflow, namely, digestion, ligation and transformation, are being integrated as a modular automated microfluidic system. The module digestion consists of a mixer, a reaction zone with a constant temperature, where the digestion takes place, and size separation by capillary electrophoresis. The module ligation enables a high-throughput ligation with several identical mixers and reaction zones. Lastly, the module transformation is also for high-throughput transformation and consists of several identical mixers and reactions zones that enable a heat shock-transformation or an electroporation-transformation.

Name: Yonglong Dang

Group: Scholpp Lab

Title: Identification of Genes Controlling the Wnt Cytoneme Formation

Abstract: Cytonemes are signaling filopodia. Recent work in my lab showed that cytonemes are used to transport Wnt8a to pattern the neural plate during zebrafish gastrulation. The study of cytonemes as signaling filopodia can provide people insights into how signaling molecules travel between cells.

To find regulators that are involved in cytoneme formation, we will do a screening in vitro. The screening include cell transfection, imaging and cytoneme measurement, etc. We tested Hela, HEK293T and Pac2 cells for cytoneme study. Preliminary data showed Pac2 cells have much longer filopodia, which makes it much easier for further analysis. So, Pac2 cells will be used to finish the screening.

The screening will be performed in 96-well plate and on the microdroplet array in collaboration with the Levkin lab. Cytoneme measurement will be done automatically by using the Filopodia Segmentation GUI, a software designed by the Mikut lab from IAI. A kinase Medaka cDNA library will be firstly used to finish the screening. Then we will use a whole genome library from Medaka to go on the screening to find more genes that regulate Wnt cytoneme formation.

Name: Alessia Donati

Group: Dr. Ilya Reviakine, IFG in collaboration with Dr.Aldo Jesorka, Chalmers University

Title: Towards Analyzing Platelet Secretion at the Single Platelet Level

Abstract: Platelets are 2-4 um anuclear cell fragments circulating in blood. They are the main actors in hemostasis and thrombosis, on the one hand limiting the traumatic blood loss in case of injury but on the other causing complications of cardiovascular disorders such as heart attacks and strokes. They are involved in a multitude of other physiological functions such as wound healing, inflammation, immune response, angiogenesis, and the development of cancer metastases.The diversity of the functional roles implies a diversity of activation states. Since the secretion of active substances and expression of specific markers are key processes by which platelets carry out their functions, their functional diversity should be reflected in the expression and secretion patterns. To identify these patterns, single cell approaches to studying platelet activation are needed.

Here, we describe for the first time an approach to addressing single platelets. It brings together two advances. Firstly, we developed a model system where the secretion and the expression pattern of the surface-adherent platelets can be triggered at will. Secondly, an open-volume microfluidic pipette was used to deliver agonists and antibodies to individual adhering platelets. In this way, we are able to specifically trigger the secretion and analyze the surface

Name: Manuel Drefahl

Group: Christof Niemeyer

Title: Nanoscale and multifunctional cell- substrate interfaces reveal receptor architectures inside and outside of receptor clusters

Abstract: Project outline: Adherent cells are grown on several nano-functionalized substrates while changes in shape and phosphorylation pattern are recorded. Strong deviations indicate that receptors on the cell membrane are facing a matching pattern on the structured substrate.The pattern on the substrate is scalable down to 5 nm, because it is built from DNA-origami. DNA-origamis are 3-dimensional objects consisting of interconnected DNA double helices. We choose a DNA-origami design with lateral dimensions of 100 nm and a height of 2 nm that serves as a molecular pegboard for the immobilization of cell receptor ligands. The ligands are placed on the origami in various numbers and distances with the help of DNA-oligomers, whose position on the origami is exactly known.The conjugation of DNA-oligomer and receptor ligand is achieved by fusing the ligand with a HaloTag and the oligomer with a chlorohexane moiety. Expression of the fusion proteins in *E. coli* and chemical modification of the oligomers is followed by the assembly of DNA origami with chlorohexane modified oligomers and the conjugation to the fusion proteins. The modified origamis are anchored to surfaces by DNA- directed immobilization.Cells grown on origami surfaces are analyzed by immunocytochemistry and high resolution microscopy.

Name: Mojtaba Ebrahimi

Group: Dr. Thomas Dickmeis

Title: Imaging the spatiotemporal distribution of glucose in zebrafish using genetically encoded FRET-based sensors, and Biomarker discovery of metabolic diseases using 1H NMR spectroscopy

Abstract: Using a genetically encoded protein FRET-based glucose sensor enables us to get information regarding spatiotemporal distribution of glucose. Glucose activates *mondo* signaling pathway, where this pathway plays an important role in one of the embryonic movement so-called "epiboly" which is engulfment of the large ventral yolk cell by a cap of embryonic cells. In the first attempt to visualize glucose distribution in very early stage in the zebrafish development *in vivo*, we stablished a zebrafish transgenic line which stably and homogenously express the glucose sensor. With the recent preliminary data, it has been shown that genetically encoded glucose sensor is functional in the very early stage of zebrafish development 6 hpf and it was possible to show changes in the level of FRET by providing external glucose in the 24 hpf larvae.

Metabolomics is regarded as a "chemical fingerprint" of the organism, reflecting for example the developmental or circadian state, malnutrition or disease. Results of the past years indicate that it can be exploited as a diagnostic tool for disease recognition. It was possible to identify a list of metabolites which were significantly changed in the β-cell ablation zebrafish model using chemically induced cell ablation approach.

Name: Katharina Fanselau

Group: Inorganic Chemistry, Group of Prof. Dr. Peter Roesky

Title: Lanthanide Hydroxy Clusters and Lanthanide - functionalized Nanoparticles

Abstract: Lanthanide ions offer remarkable opportunities regarding the formation of bioimaging agents. Especially Eu(III) compounds dominate the optical microscopy applications of lanthanide systems. In 2013 our group reported the synthesis of novel luminescent lanthanide clusters [{$Ln_{15}(\mu^3$-$OH)_{20}(PepCO_2)_{10}(DBM)_{10}Cl\}Cl_4$] (Ln = Eu, Tb). The hydroxy cluster with an europium core showed intense red fluorescence. *In vitro* investigations on HeLa tumor cells showed an accumulation of the clusters in the endosomal-lyosomal system. The current focus is set on further functionalization of the cluster's coordination sphere to create novel, water - soluble clusters. Another objective is the combination of magnetic nanomaterials with luminescent lanthanide compounds to combine their unique properties and create new hybrid materials. Magnetic Fe_3O_4 nanoparticles have been studied extensively for biological imaging and therapeutic applications. More recently they were coupled with various functional components, so multifunctionality can be present within one nanostructure for dual imaging applications. Via combining magnetic Fe_3O_4 nanoparticles with fluorescent europium complexes, this multifunctionality is given. My aim is to synthesize and characterize such new superparamagnetic hybrid materials, especially to increase the magnetic and fluorescent properties of these compounds. Those new hybrid nanomaterials can serve for example as MPI (Magnetic particle imaging) or confocal microscopy contrast agents.

Name: Andrea Fischer

Group: Alexander Nesterov-Müller

Title: Development of a microfluidic based assay for the investigation of peptide-antibody interactions in a high-density array format

Abstract: Peptide arrays can be employed in the search for linear epitopes in the immune response to diseases or vaccines. Displaying the whole amino acid sequence of a toxoid as overlapping peptides, the disease specific antibodies in a patient serum can be analyzed and characterized.

The adsorption and desorption process of an antibody can be described in terms of kinetic rate constants. Therefore it is also necessary to determine the concentration of antibodies which bind certain peptides.

For concentration and kinetic analysis a continuous flow of sample over the sensing area is necessary. This is realized with a microfluidic channel onto which the peptide array can be mounted.

For the real-time detection of peptide-antibody interaction fluorescence is applied due to its high sensitivity. In order to convert the fluorescence signal into the initial concentration of antibodies a standard antibody will be used for calibration.

Name: Ruben Garrecht

Group: Prof. Dr. Christof M. Niemeyer

Title: Establishing Multifunctional Surfaces for the Investigation of Complex Processes in Cells

Abstract: Natural Killer (NK) cells are at the junction of the innate and the adaptive immune response and serve a very important role in host defense against viral infections and cancer. They have numerous cell surface receptors which activate or inhibit various intracellular signaling cascades that are integrated to determine the functional activity of these cells. In my dissertation I use a surface-based approach which aims at tackling the largely unknown molecular mechanisms of signal integration. I use DNA microarrays containing capture oligonucleotides for the DNA-directed immobilization (DDI) of oligonucleotide-tagged αCD16 antibodies as ligands for NK cells. I could already demonstrate that the resulting surfaces can be gradually tuned in terms of ligand density to trigger the activation of living NK cells.

Furthermore, with the site-directed sorting of differently encoded, protein-decorated DNA origami structures on DNA microarrays, I will combine the advantages of the bottom-up self-assembly of protein-DNA nanostructures and top-down micropatterning of solid surfaces to create multiscale origami structures as interface for cells (MOSAIC). This holds the potential to present to adhered cells well-defined arrangements of ligands with full control over their absolute number, stoichiometry and precise nanoscale orientation.

Name: Vanessa Gerber

Group: Strähle research group

Title: Investigation of V2 Interneuron Specification and Subtypes in the Spinal Cord of Zebrafish Embryos

Abstract: Different neuronal subtypes form at determined positions along the dorsal-ventral axis of the spinal cord through the combinatorial action of transcription factors. V2 interneurons are located in the ventral half of the spinal cord above the motor neuron domain. In mouse three V2 interneuron subtypes exist: V2a, V2b and V2c. While zebrafish have V2a and V2b interneurons, it has not been resolved yet whether V2c interneurons exist also in the spinal cord of zebrafish. In an expression screen for transcriptional regulators in zebrafish, we found the two closely related *sox1a* and *sox1b* genes expressed in KA", KA' interneurons and a third more dorsal class of cells in the V2 domain of the spinal cord. These *sox1a/b* positive cells do not co-express the markers for the V0, V1 interneurons (*dbx1b*), the motoneuron progenitors (*olig2*) and V2a cells (*vsx2*). A proportion of *sox1a/b* positive cells were found to co-express *gata2* and GABA, markers of V2b cells, while other *sox1a/b* positive cells in the V2 domain did not express these markers. These findings suggest that V2c neurons exist in the spinal cord of zebrafish embryos. We speculate that *sox1a* expression marks the V2b/c precursor state and mature V2c cells.

Name: Robert Gralla-Koser

Group: Cornelia Lee-Thedieck (IFG)

Tunable synthetic polymer coatings for mechanosensitivity studies of hematopoietic stem cells

Abstract: Hematopoietic stem cells (HSC) play an important role in the regeneration of the blood and the function of the immune system as they possess the ability to differentiate into every cell type of the blood. However, a systematic multiplication of HSCs is not possible yet with today's cell culturing methods. Thus new materials with further regulating parameters are needed.

Recent publications show the mechanosensitivity of stem cells. The proliferation of undifferentiated HSCs can be enhanced on Tropoelastin (the most elastic known biomaterial) coated surfaces (Holst 2010) whereby the elasticity of Tropoelastin was shown to be the critical factor. Though for biomolecules, it is hard to distinguish between the influence of mechanical properties and biological activity.

Here we introduce extensible synthetic polymer coatings developed via surface-initiated RAFT polymerization of HEMA with subsequent biofunctionalization using bioactive peptide sequences to mimic the extensibility and adhesiveness of Tropoelastin. The mechanical characterization of the polymer substrates is performed by AFM single molecule force spectroscopy and imaging. In the future, this novel fully synthetic xeno-free material will be compared to natural ECM derived surface coatings in regard to its effect on the proliferation of undifferentiated HSCs in order to establish the applicability of this approach.

Name: Bianka Grosshäuser

Group: Ute Schepers

Title: 3-dimensional neural tissue engineering to model systems for neural disease development

Abstract: The brain is the most complex organ of the body. The intertwined and complex network explains why damage to the circuit may manifest itself in various forms of developmental diseases, psychiatric disorders, or neurodegenerative diseases. In order to study defined molecular pathways, *in vitro* studies are indispensable. In order to create *in vitro* models, bioengineering tools can be leveraged to accelerate our understanding of for example neural circuits that may lead to the development of new drugs, drug targets, and therapies. In my research, I have developed an innovative tool to grow neurons in 3D cultures to build a brain surrogate model. Here, cortical mouse primary neurons were encapsulated in a synthesized hydrogel. I was able to demonstrate 3D bio-fidelity of neural networks over the course of more than four weeks. The functional 3D neural network tissue constructs can be used to model brain illness and identify disease mechanisms and biomarkers of for instance Alzheimer´s disease. These engineered tissues could further be used to engineer other organs in vitro due to its facile hydrogel creation and easily adjustable gel stiffness and composition. This has the potential to shape our future of creating broadly applicable platforms for scientific discovery, providing clinical

Name: Zhao Haiyu

Group: Prof. Nicholas S. Foulkes

Title: Exploring light-driven DNA repair mechanisms using fish models

Abstract: DNA stability is of utmost importance for proper functioning and existence of all living systems. Increases in ultraviolet (UV) radiation at the earth's surface have fuelled interest in the physiological mechanisms of DNA damage repair in respond to sunlight.

The goal of this project is to explore the mechanisms underlying the light-driven repair of UV damaged DNA using the genetic model zebrafish as well as a novel model Somalian cavefish. Our main biological question is: what effect does evolution under complete isolation from UV radiation of sunlight for around 2.5 million years have on the function of DNA repair pathways?

Based on our preliminary evidences that show abnormalities of light-driven DNA repair system in cavefish, we are doing systematic comparative studies of zebrafish and cavefish in the transcription regulation (light inducibility), protein coding (gene truncation) and RNA splicing level and using the zebrafish model to test functional predictions made with the cavefish. This project will allow a uniquely in depth study of the function and evolution of DNA damage repair pathways in response to light and provide evidence for future studies of DNA damage prevention and cure.

Name: Silla Hansen

Group: Prof. Christof M. Niemeyer

Title: Microfluidic bioreactors for biofilm studies

Abstract: Biofilms are one of the most abundant forms of microbial life in our ecosystem. This extremely robust and vital class of living systems affects various environmental and industrial processes as well as human health. On the one hand biofilms are causing many severe problems, like biofouling or infectious diseases, but on the other hand they are also offering promising possibilities for innovative applications in biotechnology. By taking advantage of their unique properties biofilms could be used for bioproduction of new pharmaceuticals or the selective removal of unwanted substances from waste. Successfully pursuing this strategy will need a deep understanding of the biology of biofilm formation and growth as well as the interactions within the community.

Microfluidic bioreactors are a promising tool for biofilm studies, since they offer the possibility to better control growth conditions. However there is urgent need for analytical methods compatible with microfluidic bioreactor systems.

A novel platform was developed for cultivation and analysis of multi-species biofilms under flow. The system allows the control of the flow rate, temperature and gaseous environment. The flexible design allows the use of various flowcell geometries, from microfluidic channels to millimeter scaled channels. An automatic sampling system as well as offline assays

Name: Patrick Hodapp

Group: Prof. Stefan Bräse / Dr. Nicole Jung

Title: Synthesis of novel Wnt-active N-heterocyclic compounds via SPOS

Abstract: Quinoxaline and quinoxalinone derivatives display diverse biological activity including antibiotic, antidiabetic, antithrombic, antiviral, anti-inflammatory, antimicrobial and antitumor activity. Additionally their ability to inhibit the Wnt signaling pathway – important for cell differentiation and proliferation – gives these compounds great importance as research tools and as potential treatments for various diseases arising from dysregulated Wnt signaling. Almost all patients suffering from colorectal carcinomas, for example, have mutations in Wnt-related genes.

During the course of my work a library of compounds based on the quinoxaline and quinoxalinone scaffolds is to be synthesized and screened for biological activity. Substances found to be Wnt-active are to be further investigated and their structures will be optimized to increase activity and selectivity.

The synthetic route chosen to efficiently create a diverse library, in a modular route, is via solid phase organic synthesis (SPOS). The synthesis of quinoxalines via SPOS is to be established and optimized to allow for on-bead modification of the substrate - to maximize derivatization – before a cyclative cleavage with diamines to obtain the N-heterocyclic target products.

Name: Yu-Chieh Huang

Group: IOC, Steven Bräse

Title: Implementation of new structure- and calculation models in chemistry research and their effects on established information systems and databases

Abstract: There are software tools helping us to manage daily activities, like schedule and notes. However, for chemical and biological researchers, most of time we still record data and ideas on traditional paper lab notebooks. My main target is to build an electronic lab notebook which focus on chemical and biological applications.

This electronic lab notebook is a web-based application. Compared to previous chemical and biological software, it has several advantages: (1) the information contained can be better secured and stored, (2) activities can be standardized, (3) the data is available from several locations, (4) the information can be easily shared on request with others and (5) an export of data or use of the information will be significantly simplified.

With my software experience of web-based applications, I am now studying a newer framework which can result in a better user experience and interaction. Base on previous works and input from researchers, I will add and modify functions for the electronic lab notebook. Meanwhile, I am also learning basic chemical and biological knowledge for a better communication with others.

Name: Julia Hümmer

Group: Cornelia Lee-Thedieck / Hartmut Gliemann

Title: SURGEL thin films as a potential drug release platform for stem cell applications

Abstract: Chemical versatility, controlled structure and high porosity are among the advantages of Metal-Organic Frameworks (MOFs), which can be grown on functionalized substrates yielding thin oriented films. By subsequent crosslinking of the organic ligands and removal of the coordinated metal ions “ideal network polymer” thin films (SURGELs) can be obtained, that combine the advantages of MOFs with those of biocompatible coatings. Upon functionalization of the SURGEL with photosensitive groups a drug-release system can be obtained triggered by light irradiation.

Here, the second messenger Nitric Oxide (NO) will be employed as drug, since it regulates the metabolic state of hematopoietic stem cell (HSC). This feature renders NO an interesting candidate for the *in vitro* expansion of HSC, one of the most relevant challenges in stem cell biology. Today, these are the only stem cells commonly used to treat patients, but their number is limited. To match supply and demand, *in vitro* multiplication of HSC could be a solution, however this cannot be achieved without differentiation and loss of stem cell potential. Since the targets are highly dependent on NO concentration, SURGELs as well-defined, porous material systems could ensure controlled administration due to homogenous distribution of NO donating moieties and unhindered diffusion.

Name: Thomas Hurrle

Group: AK Bräse

Title: Novel Cannabinoids; Synthesis, biological Evaluation and targeted delivery

Abstract: Over the last few decades, cannabinoids have been a source of debate. While their recreational use is still mostly a taboo, medicinal applications increased in numbers.

The possible applications cover a lot of disease patterns such as nausea, overweight, inflammation, pain, abnormal blood pressure, MS, mental and motoric disorders. Since cannabinoids operate in the endocannabinoid system, they often address all receptors belonging to this class, especially the cannabinoid receptors CB_1 and CB_2. This often renders active compounds useless for certain applications since a desired effect is most commonly accompanied by unwanted side effects.

Aim of this work is to find novel compounds, based on 3-benzyl coumarins that have higher activity and selectivity to certain receptors and should therefore have fewer side effects.

Further optimization is investigated in cooperation with the Wischke Group in Teltow, where encapsulation of the most effective compounds into polymeric carriers and their controlled release is evaluated.

Name: Swarnima Joshi

Group: Prof. Uwe Strähle / Dr. Sepand Rastegar

Title: Investigating the role of Apobec2 gene in Zebrafish

Abstract: Determining genes involved in the development of zebrafish muscles and discovering novel genes implicated in muscle development or maintenance will greatly contribute to comprehending muscle diseases in vertebrates. Unc45b is a chaperone protein that mediates the folding of myosin thick-filaments during the formation of sarcomere and has been shown to be essential for muscle development. In our lab, a two hybrid screen assay was carried out where Apobec2 protein was identified as an interacting partner of the myosin chaperon Unc45b. Apobec2a and 2b exhibit a structural role in maintenance of muscle integrity but their function is unknown. Amongst the Apobec family of proteins, Apobec1 exhibits C to U deaminase activity and since the structure Apobec2 is very close to Apobec1, thus it is very likely that Apobec2 also plays an enzymatic role. The aim of the project is to use CRISPR/Cas9 method to knockout Apobec2 generating mutants which will then be characterized and will help to understand the function of this gene. The differences between mutant and wild type transcriptome will be examined using RNA sequencing.

Name: Kabbeck, Tobias

Group: Prof. Dr. Johannes Gescher

Title: From microfluidic biofilm formation to the isolation of so far unculturable microorganisms

Abstract: Under laboratory conditions the vast majority of microorganisms (>99%) remain unculturable to date. Thus, the understanding of function and ecological niches of an enormous part of organisms inhabiting our planet remains enigmatic. Moreover, we are unable to harvest their biochemical and physiological potential in manifold directions of applied sciences.

Many key-characteristics regarding growth under environmental conditions are based on interspecies interactions. To investigate the detailed processes and interactions in multispecies biofilms, we developed and designed a novel microfluidic chip-based system for the formation of biofilms enabling co-culture growth. Biofilms are matrix-enclosed communities mostly adhered to surfaces, harboring a multitude of environmental niches in their architecture.

Our idea focusses on the fractionation of a native three-dimensional biofilm into a two-dimensional structure to enable separated co-culture enrichment of previously uncultivable bacteria as well as archaea.

Name: Vanessa Kappings

Group: Prof. Dr. Ute Schepers

Title: µ3DVasc: An artificial blood vessel scaffold for complex 3D applications

Abstract: The field of Tissue Engineering has recently become of great interest of various research groups. The major goal of Tissue Engineering is a replication of specific biological tissues, which can be used alternatively to expensive, time-consuming animal testing. A great advantage of Tissue Engineering is the possibility to perform organ-specific high-throughput experiments in vitro with cells of human origin. It is also possible to define and vary specific culture conditions and to perform real-time observations. Therefore, our research group recently developed a microfluidic bioreactor µ3DVasc which consists of an artificial blood vessel and an adjacent compartment permitting the growth of three-dimensional organotypic cell cultures. With µ3DVasc, different 3D in vitro models like vascularized liver, intestine, skin and brain can be established and combined with microfluidic enabling drug testing experiments. Besides drug testing applications, this novel system can be used to examine complex processes like immune responses or the pre-metastatic niche formation. We are aiming to reconstruct this complex process by using µ3DVasc. Therefore we intend to combine a model of a vascularized tumor (skin-melanoma) and a metastasis-typical tissue (brain) providing the appropriate environment for immune cells within the microfluidic system. The connection of these both systems via microfluidics enables substrate

Name: Dominic Keller

Group: Guillaume Delaittre

Title: Nitrilotriacetic Acid-Functionalized Nanoparticles via PISA for Enzyme Immobilization

Abstract: Enzyme immobilization is a common method to increase stability under a range of reaction conditions. In addition, it provides a means to more easily recycle the biocatalysts or even to employ them in continuous processes. Nanoparticles (NPs) based on amphiphilic block copolymers are poorly explored systems for enzyme immobilization. We develop nanoparticles (NPs) containing surface-displayed nitrilotriacetic acid (NTA), able to bind specifically to His-tagged enzymes. For this purpose, novel initiators bearing the NTA moiety are synthesized and used in nitroxide-mediated polymerization (NMP) to obtain well-defined hydrophilic polymers. Afterwards, polymerization-induced self-assembly (PISA) is used which leads to the desired amphiphilic block copolymer NPs with various NTA contents in the corona. The resulting NPs are then fully characterized. Protein/enzyme immobilization and activity are finally investigated.

Name: Denise Kemler

Group: Olivier Kassel

Title: The role of nTrip6 in myoblast differentiation and fusion

Abstract: Skeletal muscle regeneration is carried out by resident adult stem cells, the so-called satellite cells. Upon muscle damage these cells get activated and start to proliferate as myoblasts, which then further differentiate and fuse to form new myofibres. One key regulator required for late differentiation and fusion is the transcription factor MEF2C. However, MEF2C is already expressed in proliferating myoblasts. Thus, its activity must be controlled. Previous work of the lab has identified the transcriptional co-regulator nTRIP6 as a putative regulator of MEF2C activity. I showed that nTRIP6 interacts with MEF2C and is recruited to the promoter of MEF2C target genes, where it represses MEF2C activity. When nTRIP6 function was blocked in myoblasts the expression of differentiation markers was increased, although the cells were still proliferating. This was associated with a reduced fusion. Thus, nTRIP6 appears to prevent premature differentiation, which is necessary for a proper temporal control of fusion. Furthermore, in a model of muscle regeneration in the mouse, newly formed fibres were smaller in size upon the loss of nTRIP6 in the satellite cells, illustrating the *in vivo* relevance of my findings. In conclusion, I uncovered a novel function for nTRIP6 in the temporal regulation of myoblast differentiation.

Name: Katja Koßmann

Group: Prof. C. M. Niemeyer

Title: HOBbing proteins with DNA

Abstract: DNA has proven as an advantageous construction material for nanotechnology. The desired structures allow for precise arrangement of other molecules (e.g. proteins) at nanometer scale using a broad spectrum of (bioorthogonal) immobilization methods (e.g. fusion protein tags).

The HaloTag® fusion protein is genetically fused to the protein to be immobilized on the DNA nanostructure and reacts with a chlorohexyl ligand (CH) attached to the DNA. By rational engineering of the HaloTag® binding interface an optimized linker for DNA nanostructures was obtained. Five amino acids positioned around the active site entry channel of the HaloTag® protein were exchanged against positively charged lysine amino acids to produce the HOB (Halo-based oligonucleotide binder) protein.

HOB was genetically fused with the enzymes Cytochrome P450 BM3 as well as with BMR, the separated reductase domain of BM3. The resulting HOB-fusion proteins revealed significantly improved rates in the ligation with CH-modified oligonucleotides and DNA origami nanostructures. These results suggest that the efficient self-assembly of protein-decorated DNA structures can be largely improved by fine tuning of the electrostatic interactions between proteins and the negatively charged nucleic acid nanostructures.

Name: Thomas Kowoll

Group: Dagmar Gerthsen

Title: Scanning Electron Microscope (SEM) investigations of nanoparticles in and on cells

Abstract: In studies regarding the toxicity of nanoparticles (NP), the toxicity is usually determined in terms of the incubation concentration, but more crucial is the amount of NPs actually arriving at the cell surface, the *direct cellular dose*. Our goal is to utilize SEM for a quantitative determination of this *direct cellular dose*. The biggest advantage of SEM over bulk techniques is the capability to detect single particles spatially-resolved and hence to distinguish between intercellular and cellular regions. In cooperation with the ITG we investigate the sedimentation of NPs onto human lung epithelial cells (A549). In *in vitro* experiments these cells are seeded on substrates, incubated with NPs and investigated with SEM. Measured quantities of NPs are compared with simulated values obtained by the *In vitro Sedimentation, Diffusion and Dosimetry model* (ISDD). The results indicate that considerably fewer NPs sediment onto the cells than in intercellular areas, while on the other hand intercellular measurements are consistent with simulations. Since almost no NP uptake has been observed, we suspect protein dependent interactions to have a severe impact on NP sedimentation behavior, which is subject of current investigations.

Name: Benjamin Lehmann

Group: Prof. Dr. Hans-Achim Wagenknecht

Title: Investigations of the "Photoclick"-reaction

Abstract: 1,3-dipolar cycloaddition of nitrilimines and electron-deficient alkenes was first described by *Huisgen et al* about 50 years ago. Development of new long-wavelength photoactivatable diaryltetrazoles and high reaction rates make this reaction a very attractive tool for *in vivo* visualization of biomolecules. Therefore this method has been widely used as a bioorthogonal reaction to label proteins and nucleic acids in the last few years.

As we know from previous experiments the "photoclick"-reaction does not give quantitative yields if oligonucleotides are involved. We want to determine the suitability of natural nucleotides as dienophiles for the "photoclick"-reaction. Furthermore the reactivity will be quantified by kinetic measurements. We aim to expand our toolbox for labeling nucleic acids by developing tetrazoles with new optical properties.

Recent studies have shown that nitrilimines readily react with different nucleophiles as well as with some solvents. Additionally we developed two new tetrazoles. The first diaryltetrazole features an electron-rich dimethylaminophenyl-substituent on the C^5-position and an electron-deficient methoxygroup on the N^2-position. This tetrazole only shows 1,3-dipolar cycloaddtions in solvents equally or more unpolar than dichloromethane. The second tetrazole is based on 2'-deoxyuridine and shows an absorption maximum at 305 nm.

Name: Arnold Leidner

Group: Prof. C.M. Niemeyer

Title: Nanoparticle reporters for imaging metabolites during zebrafish development

Abstract: The aim of this work focuses on the development of novel biosensors for the detection of small metabolites *in vivo*. For this purpose we primarily aim to synthesize and characterize multifunctional silica-nanoparticles, which are accessible by a reverse microemulsion method. This method enables the synthesis of monodisperse particles in the size-range between 20 and 100 nm. Herein, we will take advantage of their non-toxic properties and the well-adjustable silane-chemistry, which allows for subsequent modification of the particle surface with a large variety of functional groups (e.g. amines, thiols, carboxylates etc.). The latter enables the biorthogonal coupling of many biomolecules, which will be used as recognition and sensing elements.

As biomolecules of choice, we focus on genetically engineered metabolite binding proteins, in particular glucose binding proteins. Members of this protein family have been used for the construction of small-molecule biosensors based on autofluorescent protein FRET pairs or site-specifically ligated solvatochromic dyes, which enable the selective detection of small metabolites.

The functionality of the synthesized biosensors will finally be tested in *in vitro* and *in vivo* studies, by microinjection in developing zebrafish embryos.

Name: Ying Li

Group: Prof. Dr. Nicholas S. Foulkes

Title: Unraveling multiple clock mechanisms in vertebrates

Abstract: It is well established that zebrafish has a light entrainable oscillator (LEO) present in most tissues and even cell lines, however, like other vertebrates, they also possess a food entrainable oscillator (FEO). Links between food and feeding regulated clocks and the FEO are likely to involve metabolic signals. Thus, understanding how changes in the environment influence metabolism represents a crucial issue. My project involves studying light and food regulated cyclic changes in the metabolome of zebrafish. By performing a hydrogen nuclear magnetic resonance (^{1}H NMR) spectroscopy analysis, we have identified most circadian metabolites in the fish liver. NAD+, which occupies a central position connecting most circadian metabolic pathways, has been revealed under the control of both LEO and FEO. Then we have focused on rhythmic expression and regulation of genes encoding elements of the NAD+ metabolic pathway in order to explore the molecular basis of FEO. We have identified several circadian genes and factors could not only establish links between energy metabolism and circadian function, but also constitute connections between the LEO and FEO. The key goal of this study will be to answer fundamental questions regarding the existence and clock mechanisms of FEO in zebrafish.

Name: Manfred Maier

Group: Dr. Kersten S. Rabe

Title: Protein evolution to improve biocatalysts for use in thermophiles

Abstract: Though cloning and expression in well-studied organisms such as *E. coli* or Yeast has become common practice in academic and industrial settings, their field of industrial application is physically limited due to their physiological bias. These organisms deal poorly with harsher reaction conditions which can be encountered in industrial processes, especially high temperatures. Enzymes from extremophiles or engineered variants from mesophilic sources have been established as a means to deal with these challenges, offering an improvement of existing processes or the possibility to establish new pathways to bio-based products. The expression of thermostable enzymes in extremophile host systems for example could so enable the use of extremophiles as high performance, robust whole cell bio-catalysts in biotechnology.

Using both, rational protein engineering and random mutagenesis were able to find single amino acid mutations (and combinations of them), for the enzyme α-ketoisovalerate decarboxylase, which contributed markedly to an enhanced stability without compromising the enzymatic activity. Furthermore, the variants also had a much higher stability compared to the wildtype when performing reactions at lower temperatures. The *in vivo* analysis of these enzyme variants is currently underway.

Name: Daniel Marcato

Group: ITG: Ravindra Peravali – IAI: Christian Pylatiuk

Title: Design and Development of Imaging Platforms for Phenotypic Characterization of Early Zebrafish

Abstract: One of today's challenging questions in biology is the understanding of physical and biochemical traits of an organism and the influence the environment can have on them. The zebrafish (*Danio rerio*) has emerged as a well-established model organism for this type of studies due to the numerous advantages over traditional models. Their small size, transparent eggs, high fecundity and simpler neuronal organization also make them an ideal resource for high-throughput screening. In this project we develop automated and high-throughput imaging platforms to invoke and measure behavioral responses of zebrafish to different stimuli like light, vibration and touch. Firstly we developed a Photomotor Response Platform (PMR) that is able to give readouts for changing motility after administering a light-flash to embryos (30-42hpf) that have previously been accustomed to darkness. A newly developed vibrational setup then allows for examination of the startle response of hatched fish (4-7dpf). Together with an automated touch response system this forms a comprehensive platform for the analysis of multiple early zebrafish behaviors. The results can then be used to, for example, characterize mutant phenotypes and identify neuroactive compounds by comparing behavioral changes to the normal wild type controls.

Name: Benjamin Mattes

Group: Steffen Scholpp

Title: Filopodia-mediated transport of Wnt8a in zebrafish

Abstract: Signaling molecules are fundamental to orchestrate early events of diversification by generating cellular responses depending on its local concentration. Communication of cells by paracrine Wnt/β-catenin signaling is essential for developmental processes such as cell fate specification, regeneration and stem cell regulation. Wnt morphogens are transported from a local signaling source to exert its function to pattern the central nervous system during gastrulation. To fulfill this paracrine function, a defined propagation mechanisms to distribute these molecules from the source to the determined target tissue is required.

Recently we have demonstrated that Wnt8a is transported on cytonemes of signal-transmitting cells to contact responding neural plate cells to induce Wnt8a/Lrp6 ligand-receptor complexes and activate the Wnt/ß-catenin transduction cascade. We identified the receptor tyrosine kinase Ror2 as a regulator of the Cdc42-dependent Wnt cytonemes. The classically canonical-assigned ligand Wnt8a can interact with the non-canonical receptor Ror2 to activate signaling. We found that autocrine Ror2 signaling regulates specifically Wnt cytoneme formation in the source cells and thus enhances paracrine Wnt/β-catenin activation in the neighboring tissue. The further characterization of this contact-mediated mechanism reveals insight into molecular processes controlling spatiotemporal dynamics of Wnt signaling during vertebrate tissue patterning.

Name: Stephan Münch

Group: Prof. Dr. Stefan Bräse

Title: Functionalized peptoids

Abstract: Selective transport of active compounds, or cargo molecules in general, in specific organs, cells or cell organelles is not a trivial problem due to the fact that the mechanism of cellular uptake is still not fully understood. Cell penetrating peptides (CPPs) are known for a long time, but have their limitations regarding in vivo applications because of their decomposition through enzymes like proteases. Peptidomimetics like peptoids are an alternative, as they are stable against enzymatic decomposition as a result of their secondary amine in the peptoic backbone. Due to this feature and the broad scope of application, they are very promising compounds and interesting for pharmaceutics and biological assays.

Our peptoids can be conjugated to a wide scope of fluorophores and cargos via copper catalyzed CuAAC click chemistry or even copper free with our strain promoted cyclooctyne linkers. The internalization process is typically observed due to fluorophore conjugation. Specifically designed peptoids allow targeted delivery to specific organs or cellular compartments.

Name: An Nguyen

Group: Nicole Jung

Title: Optimization of scientific research processes through modern electronic data processing in chemistry and biology sciences

Abstract: Typical modern chemistry lab, particularly in academia, still have many missing correlation of chemical structures and data. We introduce an integration platform between LIMS (Laboratory Information Management System), ELN (Electronic Laboratory Notebook) with data repository. The solution facilitates new ways to analyze, share and reuse research data outputs, with libraries, and other service units within academic institutions. With the combination between ELN and data repository, the platform provides structured data, makes it available for reuse and also allows intra and inter-group collaboration across the university. This software infrastructure, which is high flexibility and scalability, is available in an open source and modular format that is easy to use and deploy ensure the.

Name: Emmanuel A. Ntim

Group: Prof. Dr. A.C.B. Cato

Title: Regulation of Bag-1L-Androgen receptor action by the histone chaperone NPM1

Abstract: The androgen receptor (AR) is a ligand activated transcription factor that contributes to prostate cancer development. Various factors regulate the action of AR, one of which is the co-chaperone Bag-1L. To determine how Bag-1L regulates AR action, mass spectrometry was used to determine the Bag-1L interactome. Among the proteins that bind to Bag-1L is the histone chaperone nucleophosmin 1 (NPM1) that is also implicated in the regulation of AR action. The aim of this study is therefore to investigate whether nucleophosmin 1 plays a role in Bag-1L-AR regulation. NPM1 was knocked down and its effect on Bag-1L - AR regulation was investigated. We could show that NPM1 knockdown significantly decreased Bag-1 protein levels and AR mediated gene expression as well as androgen mediated prostate tumour cell growth. Bag-1L rescue restores AR mediated gene expression and partly restored androgen dependent prostate cancer cell growth. Thus it appears that NPM1 plays a pivotal role in the regulation of AR action by regulating the level of Bag-1.

Name: Bettina Olshausen

Group: Prof. Ute Schepers

Title: Novel fluorescent compounds for the early detection of melanoma

Abstract: With more than 50.000 cases of death worldwide per year, melanoma belong to the most dangerous types of skin cancer. While healing chances are promising when the disease is detected in early stages, they are rapidly decreasing for the detection in advanced cases. In addition to an increasing incidence during the last years, this circumstance emphasizes the significance of an efficient early detection. However, the methods are limited. A visual inspection of the skin is usually performed by a dermatologist. In case of suspicion, degenerated tissue is removed surgically and examined histologically. This comes along with a lot of effort and allows only random examinations of patients. The discovery of a fluorescent cyclooctyne-compound that was formerly used to label cell surface glycans to be selectively taken up in melanoma cells, promises new efficient possibilities for diagnostics. It is now the aim of my PhD thesis to synthesize a combinatorial library of derivatives of the lead structure and to investigate them for melanoma-specific uptake *in vitro* in 2D cell cultures and 3D melanoma skin cultures as well as *in vivo* in a melanoma-zebrafish model and a melanoma-mouse model. These screenings should allow conclusions concerning the structure-function-relationship of the tissue specificity.

Name: Dorothea Paulssen

Group:

Title: Application of slippery surfaces in Bio-Engineering

Abstract: Wettability patterning of surfaces finds wide ranging applications in bioengineering, including as microfabrication strategy in High-Throughput Screening, Bioreactors, Analytics, as well as in Medical Gear. Extreme repellency against water and aqueous solutions is currently achieved by two strategies: the air-cushioning of a hydrophobic surface rising the contact angle of water on it to superhydrophobicity; the stable infusion of a surface by a lubricant film rendering it chemically highly homogenous and slippery against impacting fluids and particles. Besides mode of repellency, slippery surfaces possess further properties that distinct them from 'dry' superhydrophobic surfaces, e.g. they broaden the liquid unit operations possible on surfaces analogous to open-wall microfluidics. In this context, dry and slippery surfaces are not mutually exclusive; in our lab we developed a porous polymer HEMA-EDMA, that can equally serve as matrix for a dry or slippery superhydrophobic surfaces. Oil infusion is stable but can be reversed. HEMA-EDMA can be patterned to save as base for nano-droplet arrays. One aim of this project is to characterize how cell growth can benefit from turning the surface slippery, in terms of liquid handlings and maintainance, mass and charge transport between compartments, as well as cell and substance recovery.

Name: Theo Peschke

Group: AG Niemeyer (IBG1)

Title: Immobilization of Biocatalysts for Microfluidic Reaction Cascades

Abstract: Nature has evolved a highly efficient metabolic network in the form of fine-tuned enzymatic multistep reactions that ensure life. Mimicking this natural system, for example in a microfluidic bioreactor, could enable the biotechnological accessibility of molecules, which are not readily available by conventional approaches. One key aspect to develop artificial microfluidic enzyme cascades concerns the immobilization of accessible and active biocatalysts. In the most often used approaches, the enzyme is non-specifically immobilized (e.g. physically absorbed, chemically cross-linked or entrapped) thus leading to random orientations of the proteins with respect to the solid support. Therefore, these non-specific immobilizations often decrease the active site's accessibility and lead to a loss in catalytic activity.

In a proof of concept study, we have adopted a previously developed one-pot enzymatic cascade for the highly stereoselective synthesis of *meso*-compounds, for a compartmentalized microfluidic reactor. To this end, we genetically modified 3 different enzymes with peptide tags for the site-specific immobilization on superparamagnetic microparticles. The enzyme-functionalized microparticles were sequentially loaded in a microfluidic packed bed reactor, thereby enabling the fluidic production of the *meso*-compound in high yields (82%) with excellent stereoselectivity (e.r. >99:1 and d.r. >91:9).

Name: Annamarija Raic

Group: Dr. Cornelia Lee-Thedieck

Title: Protein based three-dimensional scaffolds for expansion of hematopoietic stem cells

Abstract: At the top of the hierarchy of hematopoiesis are hematopoietic stem cells (HSCs). The so-called stem cell niche, in the red part of the bone marrow, hosts these cells and regulates their maintenance, differentiation and proliferation. Nowadays, marrow transplantations have become established as a standard therapy for the cure of hematological diseases. In the current study, three dimensional bone marrow analogs were constructed for the multiplication of HSCs to overcome the lack of donors. In order to mimic the extracellular matrix (ECM), cationized BSA (cBSA) was used as scaffold substrate. With this cell-adhesive protein a macroporous bulk scaffold, mimicking the spongy architecture of trabecular bone was build up by cross linking the protein in a cryogelation approach. In a second approach, a fibrous scaffold with an ECM-like architecture was constructed by electrospinning of the cBSA. First cell culture experiments showed that both scaffolds were not cytotoxic and were able to maintain CD34 positive cells to a similar or even better extent than tissue culture plate. In conclusion, the developed scaffolds are suitable to mimic different aspects/features of the 3D architecture of HSC niches.

Name: Gennaro Ruggiero

Group: Prof. Nicholas S. Foulkes

Title: Regulation of the circadian clock by the environment

Abstract: The Synchronization or “entrainment” of the endogenous circadian clocks with the environment occurs on a daily basis, primarily by light. The mechanisms underlying light-entrainment are not fully understood. We propose to utilize the zebrafish and light-sensitive zebrafish cells, powerful genetic models in circadian clock research, as well as artificially reconstituted light-sensitive cells from several species including human, to explore light-entrainment and its evolutionary conservation by focusing on an important gene in this pathway, *period2*.

A newly developed *period2* knockout fish line and derived cell lines will be used to investigate the role of *period2* in light-entrainment of rhythmic and light-induced gene expression leading to clock entrainment. Moreover, knock-in zebrafish lines expressing a mutant form of *period2* will be developed. Characterization of these mutated *period2* fish and their derived cell lines will provide insight into the conservation of *period2* function, its regulation and mode of action.

Finally, to study the conservation of the clock entraining pathways we propose to artificially reconstitute light-responsiveness in otherwise ‘blind’ cell lines of different species and monitor the effect of light on *period2* activation. This approach will provide insight into the extent of conservation of clock entrainment pathways throughout almost half a billion years of evolution.

Name: Farid Behboodi Sadabad

Group: Dr. Levkin

Title: UV-triggered polymerization and deposition of plant polyphenols

Abstract: Surface modification by multifunctional coatings is one of the most important techniques to control surface properties and develop functional materials for diverse applications, such as drug delivery, antifouling, catalysis, and Water-dispersible and biocompatible nanoparticles. Advances in materials design are highly dependent on the development of versatile thin-film and particle engineering strategies. Recently it was shown that plant polyphenols, which are rich in catechol and pyrogallol moieties, could form coatings on various substrates, followed by polymerization using enzymes, coordination complexes and using mildly alkaline pH solutions in the presence of dissolved oxygen. In this project, UV-induced polymerization and deposition of plant polyphenols have been demonstrated for the first time. The reaction was investigated using UV-Vis spectroscopy. Our first results show that UV-induced polymerization of dopamine and polydopamine deposition is a general phenomenon and can be applied to other types of polyphenols like pyrogallol, tannic acid, caffeic acid, and gallic acid. This strategy was used to modify PE, PTFE, and PMMA surfaces with pyrogallol. Static water contact angle measurements and XPS analysis indicate covering the surface with polymer based on pyrogallol layer.

Name: Mohammed Salama

Group: Christine Blattner

Regulation of the tumor suppressor protein p53 by FAM83F.

Abstract: p53 is one of the most important tumor suppressor proteins. The most common biological consequences of p53 activation are cell cycle arrest, induction of apoptosis and cellular senescence. In the cell p53 is kept under tight control by its negative regulator Mdm2, an ubiquitin ligase which targets p53 for degradation. Although p53 is inactivated in about half of human cancers through mutation, it is functional in most other cases of cancer. Because of its high anti-proliferative activity, p53 could be used for therapy for these cases of cancer. It is therefore important to determine how p53 is regulated. In our lab we identified FAM83F as a novel regulator of p53 by performing a cell culture overexpression screen using cDNA Medaka library. For my PhD I am investigating the molecular mechanism by which Fam83F regulates p53. I will show that FAM83F stabilizes p53 in a proteasome-dependent manner by reducing p53 ubiquitination. Fam83F interacts with the C-terminus of p53 which holds the main ubiquitination sites. The increase in p53 abundance caused by Fam83F is also reflected by an increase in p53 activity. In response to DNA damage, FAM83F is induced suggesting that it may contribute to the activation of p53 under these

Name: Andrea Schink

Group: Thomas Dickmeis

Title: Glucose signaling and metabolism during vertebrate embryo development

Abstract: My PhD project explores the zebrafish as a model organism to study energy metabolism in development and disease. I will engineer and apply sensor lines for metabolites and metabolite signaling pathways to understand metabolic contributions to early zebrafish development. Specifically, I want to generate a transgenic zebrafish line expressing a fluorescent sensor for pyruvate. This line will serve to examine the spatio-temporal distribution of this central metabolite during normal development and thereby detect changes e.g. in glycolysis during early development. I will also examine potential connections of such changes to the activity of the glucose sensing Mondo pathway, which the Dickmeis lab has recently linked with the regulation of early embryonic morphogenetic movements. An intriguing perspective of the project is the application of the sensor to measure changes in pyruvate dynamics in various zebrafish lines exhibiting endocrine defects with the aim to better understand metabolic changes in these disease models. Application of the sensors will involve methods in advanced bioimaging techniques including laser-scanning confocal time-lapse microscopy and digital scanned light sheet microscopy. Furthermore, genetic approaches such as morpholinos and CRISPR-Cas9 will be applied to test the contribution of MondoA signaling targets identified by the Dickmeis lab to vertebrate development.

Name: Barbara Schmieg

Group: Prof. Matthias Franzreb, Institute of Functional Interfaces, KIT

Title: Processing of technical relevant biomonomers in scalable components produced by rapid prototyping

Abstract: In order to obtain a pure product (while processing of compounds produced by biotechnological means) from biotechnologically produced mixture, a combination of several process steps is necessary. For early stages of process development with low amounts of substances or for the production of compounds with varying annual outputs flexible production methods are attractive: scalable systems that can be attached to standard laboratory equipment.

In our group, a reactor system produced by rapid prototyping was developed, which enables the production of technical relevant compounds in a scalable setup. This system is used as a platform for the integration of further process steps.

An application of the system is the development of a fixed-bed reactor. To that, porous hydrogel structures containing catalysts are produced. They are set into the reactor before use manually. As a next step, the production of the whole system by rapid prototyping will be automated. Another assignment is the construction of a filtration module for the retention of larger particles within the system. Aim of the project is the production of tailor-made modules to set up a complex process with a modular organization.

Name: Ann-Kathrin Schneider

Group: Prof. Christof M. Niemeyer

Title: Development of Microbioreactors for Cultivation of Eukaryotic Cells

Abstract: The establishment of precisely defined microenvironments is essential to investigate in specific cellular processes like differentiation or receptor dimerization. For this purpose appropriate 3D structures can be created by well-established methods like thermoforming of thermoplastic polymers or the soft lithographic replication of polydimethylsiloxane (PDMS) structures. Subsequently implemented surface modifications of the built structure via the DNA-directed immobilization (DDI) technique enable specific cell adhesion as well as stimulation intentions.

There are two projects related to the establishment of bioreactors mimicking suitable microenvironments for different applications. The first project is focused on the development of a microbioreactor for specific cell stimulation and analyzation purposes of processes like axonal guidance.

The second project aims the improvement of the hemocompatibility of polymer-based implants which are directly in contact with the blood stream. The approach to tackle this problem is the induction of rapid endothelialization of implant surfaces after the implantation procedure. To facilitate this anti vascular endothelial growth factor receptor 2 (VEGF-R2) antibodies will be immobilized on the implants surface enabling the specific trapping of VEGF-R2 positive endothelial progenitor cells (EPCs) from the blood stream. To investigate this further a fluidic channel system, which functions as a stent model, has to be developed.

Name: Violetta Schneider

Group: Prof. Dr. Marcus Elstner – Theoretical Chemical Biology

Title: MD Simulations of Membrane Peptides- First Investigations of the Toxin BsrG from Bacillus Subtilis

Abstract: The 38 amino acid long hydrophobic protein BsrG is part of a temperature-dependent type 1 toxin-antitoxin system BsrG/SR4 in *Bacillus subtilis*. Accumulation of the toxin BsrG leads in cell lysis. Recent mircobiological studies have shown that this type I toxin is membrane associated but does not interfere with the permeability function of the membrane. Despite there is no structural information about BsrG available we want to elucidate the structure and the membrane interactions of BsrG via classical all-atom MD-Simulations and with enhanced sampling methods. The main objective is the prediction of a possible mechanism for cell lysis. These studies are in cooperation with the experimental work group of Anne Ulrich (KIT, Germany) they perform solid state NMR and (oriented) circular dichroism measurements on the BsrG toxin.

Name: Benjamin Schott

Group: Arbeitsgruppe Automatisierte Bild- und Datenanalyse / Group leader: Prof. Ralf Mikut

Title: Challenges of Integrating A Priori Information Efficiently in the Discovery of patio-Temporal Objects in Large Databases

Abstract: Recent data acquisition techniques and new developments in tracking technologies enable the collection of a tremendous amount of spatio-temporal 2D+t or 3D+t object data. This leads to an exponential growth of the amount of spatiotemporal data describing movements of various kinds of objects (e.g cells in developmental biology). Therefore, applications in various domains need to extract information out of the collected data (e.g. identify and analyze groups of 3D+t objects) to gain knowledge about domain-specific behavior. Often, automated cluster methods are used to access this problem and to gain knowledge about the objects. However, objects within large datasets with a complex behavior can not be analyzed fully automated due to their heterogeneous behavior patterns. To cope with the highly heterogeneous objects, the application-specific a priori knowledge of a human expert can help to extract the desired information. A reasonably approach to implement human prior knowledge is to use a stepwise cluster framework driven by a human analyst through interacting with a visual interface. As a result the human analyst can navigate and control the use of different cluster algorithms applied to the datasets of complex 3D+t objects. Therefore, we provide a framework reaching from data acquisition, object detection and tracking

Name: Christian Schwechheimer

Group: Prof. Dr. Hans-Achim Wagenknecht

Title: Synthesis, Spectroscopic Studies and Applications of Novel Cyanine-Styryl Dyes

Abstract: MicroRNAs (miRNA, miR) are single stranded non-coding oligonucleotides (19-24 nt) which play an important role in many biological processes, e. g. RNA interference (RNAi). Therefore miRNA-imaging offers valuable information to miRNA-involving cellular developments and diseases like cancer (oncomir). Low transfection of fluorescent probes, low miRNA expression and poor optical properties of fluorophores mainly limit the fluorescence brightness in cells. Furthermore a high photostability enables long-term observations and reduces phototoxicity.

Cyanine-styryl dyes are suitable tools for nucleic acid probes due to their high fluorescence intensity enhancement in presence of nucleic acids, an outstanding optical behavior and an improved photostability.

In this study, the combination of synthetically optimized fluorophores and covalently bound cyclooctatetraene enabled a significant increase in photostability by more than 300%, as well as far improved optical properties. Hereby novel, powerful and photostable fluorophores were developed for nucleic acid detection and opened up a wide range of applications.

This will now be further investigated in hybridization probes to detect the oncomir miR-155-5p, which is commonly overexpressed in tumors, e. g. breast cancer. In all probability the overexpression is detectable by these hybridization probes and also long-term studies within the zerbrafish cancer model (*Danio rerio*) are planned.

Name: Caroline Schweigert

Group: PD Dr. Andreas Neil-Unterreiner

Title: Photoswitching molecules studied by Transient Absorption (TA) Spectroscopy

Abstract: Photochromic molecular switches are systems that can be reversibly shifted between two or more stable electronic configurations by isomerization after resonant irradiation.

TA pump-probe spectroscopy can be used for a better understanding of the dynamical processes competing during photoisomerization.

Two different photoswitching molecules were examined at an excitation wavelength of 305 nm and probed in the visible region: (1) diarylethene and (2) pyrrole derivatives.

Diarylethene derivatives are particularly stable photoswitches shifting between open and closed states. Both configurations have overlapping and non-overlapping absorption bands in the UV-Vis spectra. In this study both configurations were excited simultaneously for the first time. Dynamical processes of both isomers could be observed identified by different time constants in a 1-3 ps regime. The investigated pyrrole derivative is known for isomerization between E and Z configurations via a conical intersection (CI). Excitation of the system led to a biexponential decay originating from vibrational relaxation followed by ground state recovery within several ps, presumably over a CI. Unexpectedly, indications for a solvent dependence were found in terms of a varying second time constant indicating channel branching due to solvent-solute interactions.

Name: Tanu Srivastava

Group Leader: Dr. Ilya Reviakine

Title: Investigating the role of adsorbed protein layer properties in platelet adhesion and activation at biomaterial surfaces.

Abstract: Biomaterials are implanted into body to treat diseases such as cardiovascular diseases (CVDs). Implantations today evoke defensive responses; include blood coagulation and immune reactions which lead to thrombosis. The inability, over the last 70 years, to create a material compatible with the environment of the blood is a major medical problem. Thrombosis is caused by platelets that become activated at the implant surface. The mechanism underlying platelet activation involves adsorption of plasma proteins to the implant surface. Considerable differences in composition and conformation have been found from surface to surface with increasing residence time. There is diverse evidence that surface properties influence protein adsorption which influences platelet behavior at surfaces, but there is no coherent picture or a mechanistic link between the three parameters (surface properties, protein film properties, platelet behavior). Our objective is to establish a relationship between these three parameters. Plasma proteins adsorbed on different surfaces are analyzed by SIMS and MALDI-ToF, incubated with the platelets for immunofluorescence and SEM studies. We observed difference in adsorbed protein layer properties. No obvious correlation with platelet activation is observed. It has previously been noted by our group that platelets activation has different profiles on different surfaces.

Name: Jeannine Steinmeyer

Group: Prof. Dr. Hans-Achim Wagenknecht

Title: Synthesis and optical properties of cyanine dyes as wavelength-shifting fluorescent DNA and siRNA probes

Abstract: To design better delivery systems for synthetic siRNA, it would be of utmost importance to track the cellular processing of siRNA by fluorescence in real time. The broadly applied method is based on a single emission color readout that bears the risk of false positive or false negative readout due to undesired fluorescence quenching by cellular components. In contrast, wavelength-shifting siRNA probes should overcome these drawbacks, as their readout focus relies on two distinct emission colors.

Our group recently worked out a concept of wavelength-shifting DNA/siRNA probes by the combination of thiazole orange (TO) and thiazole red (TR) as an interstrand energy transfer pair in DNA/siRNA. A distinct color change by an efficient energy transfer was obtained, because both dyes are forced as base surrogates in close proximity by the surrounding DNA/siRNA architecture. To allow a long-term cellular imaging it is necessary to incorporate photostable cyanine dyes as base surrogates. We incorporate photostable fluorescent DNA/siRNA probes with a sensitive and reliable readout that do not significantly interfere with the silencing efficacy and that are able to discriminate between single and double stranded condition by their emission color using a single excitation wavelength.

Name: Steven Susanto

Group: Prof. Stefan Bräse / Dr. Nicole Jung

Title: Synthesis of 9-deoxysibiromycin, a DNA interacting agent

Abstract: Targeting cancer cells specifically by chemotherapeutics remains a difficult task even with modern therapeutics. Currently available therapeutics are not specific enough and harm healthy cells as well. Present research studies focus on the DNA of cancer cells, which is a favored target for the elimination of cancer cells and the prevention of cancer growth. For this, a suitable compound needs to be found that bind covalently to the DNA and interrupts the metabolism of the cancer cell. Recently a novel antitumor compound, 9-deoxysibiromycin, has been identified showing promising binding activity to the minor groove of DNA. The main aim of this project is the development of a synthesis protocol for 9-deoxysibiromycin and derivatives thereof. By varying the different building blocks of the target compound, a medium sized library should be prepared, which will be tested for their anticancer activity.

Name: Christina Thiemann

Group: Thomas Dickmeis

Title: Risk assessment of high consumption chemical effects in water ecosystems using zebrafish *in vivo* assays – with focus on artificial sweeteners, antidiabetics, and antidepressants

Abstract: One of the major goals of the EU Water Framework Directive is the achievement of a good ecological status of surface waters. However, many chemical compounds from urban wastewaters are not fully removed during the wastewater treatment process and end up in water ecosystems.

The present project aims to identify hazards and to evaluate risks posed by high consumption chemicals such as artificial sweeteners, antidiabetics, and antidepressants. A total of 4 *in vivo* assays will be applied: First, the **G**lucocorticoid **R**esponsive **I**n vivo **Z**ebrafish **L**uciferase activit**Y (**GRIZLY) assay to test impacts on glucocorticoid signaling using the established GRE:Luc zebrafish line. Second, a similar assay will be developed to analyze the effects of test compounds regarding the glucose-sensing Mondo-pathway. Third, a larval feeding assay with fluorescently labeled plankton food will measure community relevant effects. Additionally, a photomotor response assay evaluates the movement profile of embryos exposed to test substances after two light flashes. Also, a combined evaluation of feeding and movement related behaviours will be attempted with an image analysis program developed at the ITG screening centre.

The PhD project is embedded within the project network *Eff-Net*, funded by the Water Research Funding Program of the State of Baden-Württemberg.

Name: Tina Tronser

Group: Pavel Levkin

Title: Investigation of mouse embryonic stem cell (mESC) differentiation using the DropletMicroarray

Abstract: Over the past decades stem cells gained interest in developmental and clinical research, due to their ability of self-renewal and of differentiating into various cell types. But nevertheless the long-term maintenance of these properties and the inhibition of spontaneous stem cell differentiation still remain challenging and are not fully understood. For further characterisation and to overcome limitations in cell availability, stem cell screens using miniaturized platforms are highly in focus. In this project a miniaturized array composed of a superhydrophobic-superhydrophilic-pattern on a nanoporous HEMA-EDMA polymer (DropletMicroarry) was used to investigate mouse embryonic stem cell (mESC) development, differentiation and stemness maintenance. The mESC showed an increased cell growth on the DropletMicroarray compared to conventional culture method. Besides that an inhibition of spontaneous differentiation and maintenance of mESC stemness on the DropletMicroarray over time was observed indicated by the Oct4 (pluripotency-gene) expression level and visualized through stable fusion of GFP to Oct4. The observed difference in behaviour of mESCs on the HEMA-EDMA polymer was attributed to the different surface physical properties of the polymer layer. The results demonstrate that the DropletMicroarray possesses promising properties to establish a facile miniaturized platform for screening of mESC while avoiding spontaneous differentiation of the cells.

Name: Divya Varadharajan

Group:

Title: Nanostructured polymeric nanoparticles for controlled enzyme immobilization

Abstract: Enzyme cascades are diversely used in nature and industry to catalyze several processes. Immobilization of these enzymes improves their working efficiency by making them more resistant to external conditions like temperature, pH, solvents etc. Using block copolymers as substrates gives better spatial resolution than what can be achieved currently using photolithographic techniques and an added advantage in terms of industrial scale production. With an aim to immobilize enzymes on such substrates to efficiently mimic enzyme cascades, herein, the synthesis of a library of functional block copolymers based on two polymer systems, namely, PS-*b*-PMMA (poly(styrene)-*b*poly(methylmethacrylate)) and PS-*b*-PBMA (poly(styrene)-*b*-poly(n-butylmethacrylate)) has been described. Reversible addition fragmentation chain-transfer (RAFT) polymerization was used to obtain the block copolymers with precise control over molecular weight (M_n = 40,000 g/mol) and quantity of functional momomers (approx.. 8%) incorporated. Further, using self-organized precipitation (SORP), nanoparticles in water were made in order to investigate their nano-structuration on surface.

Name: Sanamjeet Virdi

Group: Uwe Straehle/Sepand Rastegar

Title: Rapid Identification of ENU Mutants by High-throughput Sequencing

Abstract: Among vertebrates *Danio Rerio* (zebrafish) is uniquely suited to identifying the molecular functions of genes by mutagenesis screening. A major obstacle in this approach is the molecular identification of the affected gene.

For most mutations a rough genetic map position (within several tens of megabases) can be obtained in a few days by microsatellite mapping (Geisler et al., 2007). However, fine mapping and molecular cloning of the affected gene is still a major undertaking that requires on the order of a person-year of work by a qualified scientist as well as facilities for breeding of thousands of mutant offspring, with no guarantee of success.

Use of next generation sequencing to identify affected genes in mutants could be done in more systematic and efficient manner. The aim of the project is to focus on development of a software pipeline for data analysis, and validating it by sequencing of 3 previously uncharacterized myogenesis mutants which mimic myopathies and neuropathies of humans. The indentified mutations will be further characterized by transcriptional profiling using RNA sequencing. Confirmation of the results by biological means will be performed in collaboration with researchers working on myogenesis mutations in the laboratory of Prof. Uwe Strähle.

Name: Romina Walter

Group: Véronique Orian-Rousseau

Title: The role of CD44 in Wnt/ β-catenin signaling

Abstract: Due to its ability to regulate stem cell and progenitor cell renewal the Wnt signaling pathway is crucial for embryonic development and for the maintenance of tissue homeostasis at the adult stage. Consequently it is not surprising that dysregulation of this particularly complex pathway results in developmental defects and various diseases amongst others, cancer. CD44 is known as a target gene of the Wnt signaling pathway in the intestine. It belongs to the cell adhesion molecules and designates a family of alternatively spliced members that control normal cellular processes but als pathological processes such as tumor progression and metastasis. Recently our group has shown that CD44 is not only a Wnt target gene but acts as a positive regulator in this pathway. Indeed, downregulation respectively upregulation of CD44 affects Wnt-induced TCF/LEF transcriptional activity. The regulatory function of CD44 occurs at the level of LRP6 co-receptor at the plasma membrane. Furthermore several indications suggest a role for CD44 in the LRP6-signalosome, a complex that is assembled at the plasma membrane after Wnt stimulation. Therefore the aim of my thesis focuses on the molecular function of CD44 in the activation of LRP6 and the possible role of CD44 in the signalosome formation.

Name: Wawryszyn Mirella

Group: Prof. Dr. Stefan Bräse group

Title: Fluorescently Labeled Glycostructures as Tools for Imaging Techniques

Abstract: We developed novel chemical tools for the visualization of biological systems based on fluorescently labeled glycostructures.

The general concept is to combine biocompatible labeling strategies on glycostructures and link them afterwards to fluorescently dyes. Bioorthogonal functionalized saccharides react with their biocompatible fluorescently dyes either in *vitro* or in *vivo* to form fluorescently labeled glycostructures. To reach the desired modified saccharides we started with an orthogonal set of protecting groups first and proceeded with glycosylation steps from di- up to pentasaccharides. The glycostructures are based on D-glucopyranose as well as D-mannopyranose, the dyes are cyanine-, tetrazine-, or BOBIPY-based.

We utilized labeled saccharides either for cellular uptake, incorporation onto cell surfaces or for the linkage to proteins as well as peptoids.

With these shortly represented chemical tools we were able to visualize various cancer cell-lines *via* fluorescence microscopy techniques. The biosensors showed internalization into the cells and moreover accumulation in mitochondria. These candidates may turn out to be promising novel tools for fluorescence imaging.

Name: Laura Weber

Group: Frank Breitling

Title: Investigation of antibody signatures in Lyme disease patients with high density peptide arrays

Abstract: Lyme disease is the most common tick-borne infection in Europe and the United States. In most cases it can be treated very efficiently with antibiotics however in some cases it entails a multisystem disorder involving the skin, nervous system, joints, and heart. Peptide arrays enable the investigation of antibody-peptide interactions in a highly parallel fashion and require a minimum of sample volume. They allow the sensing of immune responses towards linear epitopes relating to a vaccination or disease. In this study we investigate the antibody response to the VlsE protein, the Variable Surface Antigen of *Borrelia Burgdorferi*, in patients who suffer from Lyme disease. We carry out epitope mapping of the whole protein with 15-mer peptides and a lateral shift of only one amino acid. The aim is the investigation of differences in the antibody response in patients and the elucidation of the role of every single amino acid.

Name: Ilona Wehl

Group: Ute Schepers

Title: High-throughput screenings of a cell penetrating peptoid library to isolate organ specific transporter molecules

Abstract: In recent research an organ specific drug delivery is of high interest, as it can increase the efficiency and decrease the toxicity of drugs and therefore improve their overall activity. A promising approach to reach this aim is the development of carrier molecules. Over the last years, cell penetrating peptides (CPPs) have been well established as molecular transporters. Although CPPs have many advantages, they display a poor bioavailability because of their proteolysis in the presence of serum. Cell penetrating peptoids (CPPos), which are modified peptides, display improved stability and pharmacokinetic properties. For high-throughput screenings in biological systems highly diverse compound libraries are needed. A completely permutated peptoid library could be synthesized, by the submonomer-based solid phase synthesis, and analyzed, by using high-throughput screenings, on cells and zebrafish embryos. The aim of this project is to find correlations between the physicochemical properties of the peptoids and their biodistribution in a whole organism. In cells, lipophilic peptoids show an accumulation in mitochondria, whereas cationic candidates concentrate in endosomes. In zebrafish embryos the tested peptoids accumulate in the digestive system, lateral line, kidney, caudal vein and olfactory system. For further investigation, we plan to do high-throughput screenings of peptoids on adult zebrafish.

Name: Anna-Lena Winkler

Group: Stem Cell-Material Interactions, Dr. Cornelia Lee-Thedieck

Title: Biomimetic Surface Engineering for Hematopoietic Stem Cell Expansion

Abstract: Hematopoietic stem cells (HSCs) are located in niches in the bone marrow. They continuously form new blood cells. This property makes them useful in the treatment of patients with diseases such as leukemia. However, the number of HSCs from one cord blood unit is insufficient to treat adult patients. Therefore proliferation of these cells would be a great benefit for many patients. Proliferation of HSCs outside of their natural microenvironment results in loss of their stem cell character. Since this niche offers ideal conditions for maintenance and differentiation of HSCs, the development of an artificial stem cell niche seems to be a promising approach, to effectively reproduce the HSCs. Beside biological and chemical factors there are also physical properties, which play an important role in the niche. The aim of the project is to develop nanostructured, multifunctional biomaterials, on which several relevant biomolecules are presented to HSCs. To create these biomaterials, a technique called BCML is used. Cellular proteins such as DLL1 and RGD peptides which are present in the bone marrow niche are provided to the cells. The developed materials are used to study the impact of nanostructure in synergy with biological signaling molecules on HSCs.

Name: Nicolai Wippert

Group: Prof. Stefan Bräse / Dr. Nicole Jung

Title: Solid phase synthesis of Pyr-3 analogues to investigate the interaction of small molecules with ion channels

Abstract: Ethyl-1-(4-(2,3,3-trichloroacrylamide)phenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylate (Pyr-3) is claimed to be used as immunosuppressive agent as already reported for several Bis(trifluoromethyl)pyrazole compounds (BTPs).

Pyr-3 was previously shown to selectively inhibit “transient receptor potential C3” (TRPC3) channels, while other TRPC channels remain unaffected. The selectivity was ascribed to the trichloroacrylamide building block of the molecule. Furthermore,Pyr-3 is a potential treatment for cardiac hypertrophy. These, among other studies, resulted in two patents of Pyr-3.
Similar activity was shown on TRPN channels, which are present in the cnidocydes of hydra and enable the prey capture process of these species, which makes hydra a good model system for screening the synthesized molecules.

The synthetic sequence will be performed on solid phase using the T1-triazene linker to which pyrazole compound are coupled via CN-cross coupling followed by cleavage off the solid phase to yield the target molecules. In addition the molecules will be modified before and/or after cleavage. This approach enables a high diversity of target molecules will be synthesized.

The first batch of molecules was already sent to our collaboration partner Suat Özbek in Heidelberg and is currently being screened for their activity.

Name: Xiaobing Yu

Group: Véronique Orian-Rousseau

Title: CD44 involvement in homing and engraftment of leukemic cells in their niche

Abstract: Interactions with the microenvironment are known to be crucial for proliferation and maintenance of leukemic cells. Many questions remain however on how circulating leukemic cells hijack and reprogram their specific niche. Cell Adhesion Molecules (CAMs) play an important role in cell-cell and cell-matrix crosstalk. There are accumulating data demonstrating that either the expression pattern of CAMs amongst which CD44 isoforms or its main ligand hyaluronan(HA), is altered in most cases of leukemia.

The CAM CD44 is a transmembrane glycoprotein family that controls activation and signaling of receptor tyrosine kinases and G-protein coupled receptors. We have shown that the binding of CD44 to high molecular weight HA enhances CXCL12-induced CXCR4 signaling while small HA fragments inhibit CXCL12-induced signaling. CD44 isoforms have also been shown to cooperate with the other CAMs family amongst which the integrin α4β1 in the leukemic cells multistep homing process. In vivo, Cd44 germline knockout mice show less engraftment rates in a chronic lymphocytic leukemia (CLL) adoptive transfer model. Since CXCL12 is a crucial chemoattractant for leukemic cell, we are currently investigating the interplay between CD44 and integrin α4β1 in the CXCL12- triggered homing of leukemic cells to their niche and their engraftment in this favorable environment.

Name: Jiaojiao Zhang

Group: Véronique Orian-Rousseau

Title: The role of CD44 in Wnt signaling

Abstract: CD44 is a Wnt target gene in the intestine. Our previous research identified a positive feedback control mechanism exerted by CD44 on Wnt signaling at the level of Wnt membrane receptors, specifically LRP6. CD44 physically associates with LRP6 upon Wnt treatment and modulates its phosphorylation as well as the correct localization of LRP6 at the membrane.

My PhD project aims at investigating the role of CD44 in the maturation and localization of LRP6, as well as in Wnt-dependent processes in the intestine. So far, we found that the amout of LRP6 is less after silencing CD44, but the ratio of matured/immature LRP6 was not changed. This indicates that CD44 regulates the stability of LRP6 by playing an role of the maturation of LRP6.

On the other hand, we conducted DSS-induced Inflammatory Bowel Disease (IBD) model in $CD44^{fl/fl}$VillinCreER^{T2} mice. We found that mice without CD44 in the epithelia exhibited more serious colon inflammation and worse regeneration. This suggests that CD44 may play an role in preventing the mice from inflammation and promoting regeneration of the colon.

Name: Eva Zittel

Group: Prof. Dr. Ute Schepers

Title: A novel blood vessel scaffold for the reconstruction of 3d-tissues *in vitro*

Abstract: Despite a lot of progress in the synthesis of potential drugs and in the treatment of various diseases, there are still severe problems in characterising new substances and their effects. When evaluating and testing such substances, there is often a poor transfer obtained from the results in cell culture experiments to those in the animal model. For example, different dose-effect relations or certain resistances in immortalized cell lines can be observed. Usually, the natural surroundings of the cells and their *in vivo* differentiation (like cell-cell interactions or gene expression) do not exist *in vitro*. For an improved *in vitro* testing of potential drugs, it is therefore necessary to create an organotypic micro environment that allows a three-dimensional arrangement and interactions between different tissues. The development of such tissue substitutes that enable organospecific experiments *in vitro*, is the aim in many 'Tissue Engineering' research projects. Our approach involves the microfluidic model *µ3DVasc*, which consists of a curved porous capillary for embedding an endothelial layer and a surrounding cavity for the cultivation of adjacent tissue. The aim is to establish specific organotypic tissues, such as liver, skin or tumour tissue, to allow an easy and fast screening of substances within the system.