SFB1032 Final Workshop at Schloss Lautrach

Book of Abstracts

Session 1:

From accurate FRET studies in proteins to systematic assay design

Presenting author: **Thorben Cordes**

Single-molecule FRET (smFRET) has become an established tool to study biomolecular structure and dynamics in vitro and in vivo. We recently performed an international blind study in collaboration with the Seidel and Lamb labs[1] to assess the uncertainty of FRET experiments for proteins with respect to the measured FRET efficiency histograms, determination of distances, and the detection and quantification of structural dynamics. While this provided confidence in the use of smFRET for both mechanistic biochemical studies and structural biology, the design of smFRET assays and the selection of suitable labelling positions remains rather unsystematic. There are sensible 'rules of thumb' for identification of fluorophore labelling-sites, however, there is no approach that allows systematic and quantitative prediction thereof in proteins. Based on a large literature screen and bioinformatics analysis, we identified a set of four parameters, which we combined into a label score to rank residues for their suitability to serve as label site. We show the predictive power of the score with literature data and new experiments. The "labelizer" package performs an analysis of a pdb-structure (or structural models), label score calculation, and FRET assay scoring in a script or via publicly available webserver (https://labelizer.bio.lmu.de/) to conveniently apply our approach.[2]

[1] Agam, Gebhardt, Popora et al., Nature Methods 20 (2023) 523-535
[2] Gebhardt, et al., https://www.biorxiv.org/content/10.1101/2023.06.12.544586.abstract

A02: A High-Throughput Nanomechanical Sensing Platform Based on Steric Hindrance

Presenting author: **Rui Yee Loke**

DNA origami self-assembly has enabled the manufacture of nanostructures with high throughput and precision for exploring molecular machine mechanisms. We began this journey by developing a self-assembled nanorobotic arm that, under electric field actuation, is dynamically controlled to perform directed motion, nanoparticle transport, and the application of piconewton forces. The electrical manipulation of the nanorobotic arm is further utilized to study electrokinetic torque generation under varying operational parameters and the storage of mechanical energy in the embedded joint functioning as a molecular torsion spring. The nanorobotic arm, attached to the base platform via a joint connection, manifests a rotor-stator construct. We studied the interactions between rotor

and stator and determined the underlying mechanical energy landscape. This insight, along with the development of a DNA origami ratchet motor, is employed to convert the rotor arm into a Brownian motor under a low amplitude switching external field combined with the intrinsic anisotropic energy landscape.

With further investigation into the energy landscape of DNA nanostructures, we propose a high-throughput nanomechanical sensing platform featuring a hinge-like arm restricted by target binding through steric hindrance. This platform, monitored by single-particle tracking and total internal reflection fluorescence microscopy, enables the detection of various target molecules, which is demonstrated by the detection of duplex DNA formation and the antigen/antibody interactions. Sensing based on steric hindrance is a promising single molecule sensor concept with potential applications in highly parallelized stochastic detection of protein-protein interactions or small molecule sensing based on structure-switching aptamers.

A10: Morphogenesis of pancreatic ductal adenocarcinoma-derived organoids

Presenting author: **Samuel Randriamanantsoa**

Pancreatic Ductal Adenocarcinoma (PDAC) is a highly lethal disease, for which the 5-year survival rate has remained staggeringly low during the past 40 years.
Pancreatic cancer-derived organoids capture key architectural features of the lesions in the diseased pancreas,
and can be monitored in real time, from their initial single cell state, to the appearance of a complex multicellular branched tissue bearing a lumen.
Through a combination of live imaging, chemical perturbations, immunostainings, and theoretical modelling, we investigate, in space and time, how cell motion, cell-cell interactions, extracellular matrix (ECM) interactions and protein expression combine to give rise to those final structures.

B11: Controlling cell behavior via the extracellular matrix

Presenting author: **Hanna Engelke**

Cells are strongly influenced by chemical and mechanical signals from the surrounding extracellular matrix. The matrix impacts crucial cellular processes including migration, proliferation and differentiation. In our project, we developed several techniques to manipulate cell-matrix interactions in order to gain an understanding of the underlying processes and to control cell behavior. These techniques include control of the mechanical properties of the matrix, as well as control of the mechanotransduction within the cell with light. I will provide an overview of the insight into cell-matrix interactions that we gained during the course of the project and continue to gain with these techniques.

A05: Non-Canonical Nucleosides and Click Chemistry on Nucleic Acids

Presenting author: **Thomas Carell**

DNA stores genetic information in the form of the sequence of the four canonical bases dA, dC, dG and dT and it contains epigenetic information, which is established by four modified cytidine bases 5-methylcytidine (mdC), 5-hydroxymethylcytidine (hmdC), 5-formylcytidine (fdC) and 5-carboxycytidine (cadC) (Fig. 1, left).[1] These bases are generated by Tet enzymes. The position and the kind of modified dC-base at a specific position in the genome

establishes an unknown 2nd code in our genetic system (Fig. 1, right). I am going to discuss results about the function and the distribution of the new epigenetic bases hmdC, fdC and cadC in the genome.[2] I am showing how metabolic states influence the chemistry by setting and erasing these modified bases. Particularly, isotope dilution and isotope tracing mass spectrometry will be discussed together with Click-Chemistry to understand the chemistry that occurs on these bases in the genome.[3] Interesting is the fact that base excision repair seems to play a central role during erasure of the bases and again mass spectrometry helped to quantify the repair processes involved epigenetics.[4] Finally, I am discussing potential präbiotic origins[5] of modified bases and how Click-Chemistry can help us to turn oligonucleotides into nanoagents to treat diseases.[6]

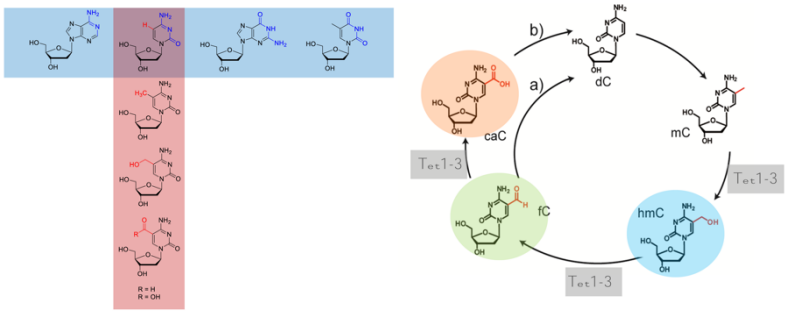

Figure 1. Illustrations of the 2nd orthogonal code that is present in DNA (left) and proposal of how the epigenetic bases are interconverted to establish dynamic changes of the epigenetic code during cellular development (right).

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[4] C.G. Spruijt & T. Carell, M. Vermeulen *Cell* **2013**, *152*, 1146-59.
[5] S. Becker & T. Carell *Science* **2016** & **2019**, *Nature* **2022**.
[6] A. J. Tölke and T. Carell *Angew. Chem. Int. Ed.* **2024**, 10.1002/anie.202405161

Session 2:

Controlling Mechanical Forces in Cells with Photoswitchable Molecules

Presenting author: **Dirk Trauner**

The actin cytoskeleton, in close conjunction with internal and external membranes, largely controls the shape and motility of cells. I will discuss how photoswitchable small molecules, as membrane lipids or inhibitors of actin dynamics. can shape the forces underlying cellular motility and remodeling.

A06, part 1: DNA-assembly for photonics, plasmonics and biosensing

Presenting author: **Tim Liedl**

Over the last decades, DNA self-assembly in general and DNA origami in particular have matured at a breathtaking pace and DNA architectures are today routinely used for the constructing of functional two- and three-dimensional nanomachines and materials. Our group has contributed complex and nanometer-precise assemblies of biomolecules, organic fluorophores and inorganic nanoparticles. In this talk I will report on our ongoing efforts to build functional plasmonic devices on the one hand and materials that are designed on the molecular level while reaching macroscopic dimensions on the other.

A06, Part 2: Nanoscale clustered FasL-DNA origami nanoagent induce death shock in large 3D tumoroid model

Presenting author: **Johann Moritz Weck**

Ligand-receptor interactions are taking place on the nanoscale. Precisely engaging and controlling these processes is key in the design of therapeutics. Potentially the most precise control over shape and functionality at the nanoscale is given through the DNA origami method. DNA origami nanostructures were already employed to deliver small molecules, drugs, nucleic acid or protein therapeutics, or to probe ligand-receptor interactions. We previously positioned FasL, an apoptosis inducing ligand, with nanometer precision on a DNA origami, creating a functional nanoagent. We found a most optimal configuration of the ligands resulting in a >100 fold increase in apoptosis efficacy. While this study was caried out in 2D cell culture, cancer in vivo forms large, solid tumors, changing cell behavior and microenvironment, as well as hindering distribution of the therapeutic in the tumor. Here, we created artificial, solid tumors of hundreds of µm in diameter to test nanoagent efficacy: we first studied nanoagent design and its effect on tumor penetration and found vastly different penetration behavior depending on DNA origami structure. Secondly, we studied the influence of nanoagent design on killing efficacy and found a most optimal architecture, which induces full apoptosis of the tumoroids and qualitatively a completely different phenotypical behavior of the tumoroids compared to delivery of the soluble ligand, paving the way for new therapeutics design.

A07: DNA origami crystals and switchable lipid membranes studied by x-rays and neutrons

Presenting author: **Bert Nickel**

We study molecular assemblies by scattering techniques. In the context of DNA origami, we find compaction of the DNA constructs in response to silicification and in turn a lattice constant reduction for silicified DNA crystals. Scattering contrast changes during silicification indicate complete fill-up of the DNA scaffold by silica. In-situ x-ray experiments during temperature ramps allow to understand annealing cycles. We observe a rather sharp melting behavior of the crystals for bare DNA crystals. The last generation of DNA origami crystals (provided by A6) are big enough for single crystal x-ray experiments such as in serial crystallography.

In the context of lipid photoswitches, we have established quantitative back-switching of photolipids by x-rays, and we have established ionic strength as required condition for efficient photo-control. The large membrane thickness changes of 1 nm observed by scattering suggest that also lateral area changes need to be huge with possible implications for the application of these switches in vesicles and supported membranes. Neutron reflectometry experiments indicated indeed formation of pores and water uptake during switching.

A08: Photoswitchable Nanoagents: From Lipids to Nanoparticles

Presenting author: **Theo Lohmüller**

This presentation will provide a brief overview of our research efforts to control and manipulate nanoagents with light, showcasing some of the main accomplishments from our project within the CRC. In particular, applications of photolipids for controlling lipid membrane systems and strategies for optical manipulation of plasmonic nanoparticles will be presented. Lastly, future directions will

be briefly outlined, focusing on our ongoing endeavor to enhance the capabilities and applications of photoswitchable nanoagents.

Session 3:

Genetically controlled nanocompartments in mammalian cells as reaction chambers or devices for information storage and transmission

Presenting author: **Gil Gregor Westmeyer**

Cellular metabolism and signaling are compartmentalized into a large number of organelles. In this talk, I will review our recent efforts to genetically control self-assembling compartments in mammalian cells to sequester enzymatic reactions or to regulate membrane budding to package biomolecules. I will then discuss some applications of such genetically controlled compartmentalization for patterning nano-reaction chambers or enabling cell-to-cell communication.

A09: Pattern forming proteins as active nanotransporters

Presenting author: **Petra Schwille**

I will talk about new exciting applications of using Min proteins to move large objects (DNA origami and vesicles) on membranes and to generate static patterns with and of origami structures.

A11: Ångstrom-resolution fluorescence microscopy

Presenting author: **Susanne Reinhardt**

Super-resolution fluorescence microscopy routinely reaches 15-20 nm resolution in intact cells. Recent approaches achieved localization precisions in the Ångström regime and – under in vitro conditions - resolved targets spaced 5 nm apart. However, Ångström resolution with optical microscopy has never been demonstrated in vitro or in cells. Thus, the study of direct molecular interactions at sub-10 nm distances has been inaccessible even with super-resolution microscopy.

We introduce Resolution Enhancement by Sequential Imaging (RESI), a DNA-barcoding method to expand the resolution of fluorescence microscopy down to the Ångström scale using a conventional inverted microscope. Labeling the target species with orthogonal DNA sequences allows sequential imaging of sparse molecule subsets via Exchange-PAINT resulting in label-size-limited resolution for biomolecules in whole intact cells.

A12: RNA aptamers as sensors for the miR200-family of micro RNAs

Presenting author: **Corinna Kersten**

MicroRNAs are fundamental regulators of protein expression in cells and play a critical role in disease development, like cancer. Dysregulation of specific miRNAs often comes together with poor patient prognosis. Especially one miRNA family stands out in terms of cancer progression: the miR200-family. MiR200 family members are interfering with epithelial to mesenchymal transition, cancer drug resistance, formation of metastasis and more. Thus, it is crucial to understand their spatiotemporal behavior and link changes in concentration and localization to certain cell behaviors. To enable spatiotemporal imaging of miR200C in living cells, Broccoli, a fluorescent light up aptamer, was turned into a switch for miR200C recognition. Experiments show that it is possible, to detect miR200C in vitro and in vivo, and

even distinguish it from other miR200 family members. Additionally, miR200C can be detected in total RNA isolates of human cancer cells. This enables fast an easy detection of enhanced miRNA levels in cells which is important for diagnostics in several disease.

A13: DNA origami sensing on lipid vesicles

Presenting author: **Philip Tinnefeld**

This project deals with the development of DNA origami sensors for different properties of lipid vesicles including membrane potentials and curvature. The sensors transduce the change of the physical property into a distance change that is read out by single-molecule FRET. Finally, we also introduce a DNA origami platform for transfer of single-molecules from the DNA origami to a lipid vesicle.

A03: Programmable pattern formation in systems of communicating subunits

Presenting author: **Ulrich Gerland, Valèria Ribelles Pérez**

How can systems consisting of communicating subunits form different patterns in a programmable way? Experimentally, such systems can be on the molecular scale, e.g. based on DNA or RNA nanotechnology or on the scale of cell-sized compartments. For our theoretical investigation, we assume that these `cells' have two or more internal states, and are able to perform simple local information-processing operations, the effects of which can be described in the framework of cellular automata models. Within this framework, we explore the general issue of controlling pattern formation processes in cellular systems. Our initial work was limited to a specific scenario, where a small number of local "organizer" subunits steers the patterning process of the entire system. We found that a small fraction of update rules indeed enables complete programmability of pattern formation in one-dimensional arrays. We then explored different scenarios for controlling pattern formation in one- and two-dimensional systems, where the patterning information is generated in a distributed way, without organizer cells. More specifically, we studied (i) pattern formation with asynchronous cell updates, (ii) the dynamics of irregularly arranged cells, (iii) the interplay of global and local signaling, (iv) the evolution of pattern formation rules, and (v) coupled pattern formation and growth/death of cells.

Session 4:

Self-organization and information flow in collective cell fate decisions

Presenting author: **David Brückner**

A key feature of many developmental systems is their ability to self-organize spatial patterns of functionally distinct cell fates. A spectacular example of this ability are artificial stem cell assemblies, which are paving the way towards a quantifiable self-organization of biological systems. However, while the relevant molecular processes are increasingly well understood, we lack conceptual theoretical frameworks for the dynamics and statistics of self-organized patterning. Specifically, it is unclear how to quantify the patterning performance of biological self-organizing systems, and how to identify the dynamical systems motifs that optimize this performance. Here, we develop an information-theoretic framework and use it to analyze a wide range of models of self-organization. Our approach can be used to define and measure the information content of observed patterns, to functionally assess the importance of

various patterning mechanisms, and to predict optimal operating regimes and parameters for self-organizing systems. I demonstrate the application of our framework using experimental observations of intestinal organoid symmetry breaking. This framework represents a unifying mathematical language to describe biological self-organization across diverse systems.

B01, part 1: From two to three cells: Are three-body interactions important in collective cell migration?

Presenting author: **Agathe Jouneau**

During collective cell migration, for example in embryo development or cancer invasion, cells coordinate their movement by actively interacting with each other. How cell-cell interactions shape the dynamics and emergent properties of the cell assembly is not fully understood. In recent work, we showed that the dynamics of two cells interacting on a dumbbell pattern can be captured by a particle model, including cell-cell interaction terms directly inferred from experimental data. However, we do not know if the collective dynamics of more than two cells can be described by pairwise interactions between cells, or if higher-order interactions come into play. To answer this question, we use time-lapse microscopy to record the dynamics of three cells interacting together in a tailored confinement. We collect a large number of cell trajectories and use them to infer the cell-cell interactions by adapting the framework of the two-cell study. Our work reveals that the pairwise interactions between cells appear to be preserved in the presence of a third cell. However, the superposition of the inferred pairwise interactions is not sufficient to fully capture the observed three-cell dynamics. This could indicate the presence of three-body interactions, with possible implications for large-scale collective behavior.

B01, part 2: The molecular motor Myosin-VI and cell migration

Presenting author: **Claudia Veigel**

Project B01 develops micro- and nanostructured arrays, such as fibronectin lanes and two-state patterns, that allow for the investigation of single migrating cells and two-cell interactions in confinement. A major goal is to describe dynamic phenotypes using mechanistic models of intracellular reaction-diffusion dynamics. The models need to be connected to the functions of the cytoskeleton in cell migration in health and disease, including the unknown mechanical properties and regulatory functions of the molecular motor myosin-VI. This motor protein was shown to be overexpressed in a myriad of metastasizing cancer cells and is now used as a marker for cancer aggressiveness. Here, we describe our recent findings on the basic mechanisms of myosin-VI using optical tweezers based single molecule technology.

B02: Deciphering the interface laws of Turing mixtures and foams

Presenting author: **Erwin Frey**

For cellular functions like cell division and polarization, protein pattern formation driven by NTPase cycles is a key spatial control strategy. However, it remains unclear how the properties of the macroscopic, highly nonlinear reaction–diffusion patterns can be systematically linked to the underlying reaction network. We show that a central concept from equilibrium physics---interfacial tension---arises effectively from distinct underlying physics in intracellular protein patterns: The cyclic, NTPase-driven attachment and

detachment of proteins at pattern interfaces. Based on this effective interfacial tension, we introduce “Turing mixtures” and “foams” by developing generalized Neumann and Plateau laws for interface junctions and foam vertices. In contrast to liquid foams, we demonstrate that interfacial-tension-driven coarsening can be interrupted in Turing foams, and an intrinsic pattern wavelength selected. Our theory describes central features of the stationary patterns formed by the Min system in vitro. Moreover, it allows the design of specific pattern morphologies with potential applications to the design of spatial control in synthetic cells.

Mechanical characterization of neural organoids

Presenting author: **Friedhelm Serwane**

Session 5:

Fluctuation dynamics and folding of heterogeneous active polymers

Presenting author: **Andriy Goychuk**

The spatiotemporal organization of chromatin—a large heteropolymer consisting of DNA in complex with many proteins—in the cell nucleus is tightly connected to gene expression and cell state. Longstanding research has elucidated basic physical principles underlying the folding and conformational dynamics of polymers through reciprocal monomer-monomer interactions. In the cell nucleus, however, active processes such as gene transcription, or the activity of energy-consuming nuclear enzymes, break detailed balance and lead to the violation of the fluctuation-dissipation theorem. Using analytical theory, I will show how a heterogeneous distribution of active processes along the backbone of the polymer can, through active kicks, statistically enhance or deplete monomer-monomer contacts by eliciting effective long-ranged attraction or repulsion. While the resulting folding statistics are indistinguishable from a passive polymer through structural data such as contact frequency maps alone, active polymers show enhanced dynamics and non-reciprocity. In closing, I will show how temporal patterns of active kicks—for example, due to chemically active cycles of protein (un)binding on chromatin, or due to self-propulsion of biomolecular condensates—lead to coherent motion and polymer compaction. This framework will help in further elucidating chromatin organization and dynamics, as well as potential feedback between chromatin activity and organization.

B03: Dynamic single-molecule FRET analyses established using DNA origami structures

Presenting author: **Pooyeh Asadiatouei**

Single-molecule techniques provide a great wealth of detailed knowledge about the structure, interactions and dynamics of the studied molecules. The data analysis step, which comes between state-of-the-art data collection and the results, is usually the most time-consuming and tricky as it can be easily influenced by user bias. To overcome such limitations, we introduce Deep-LASI (Deep-Learning Assisted Single-molecule Imaging analysis) as an easy and fast analysis tool empowered with neural networks. Deep-LASI can analyze single-molecule data especially produced from Förster Resonance Energy Transfer (FRET) experiments.

To establish and benchmark the software, L-shaped DNA origami structures were measured on a smTIRF microscope and analyzed with Deep-LASI. The dynamic origami structures were designed with a yellow dye (Cy3B) attached to a single strand DNA tether, which can

fluctuate between two complementary single-stranded binding sites. An acceptor fluorophore (two-color) and a blue dye (three-color) are placed close to the binding strands for two- and three-color experiments respectively. The energy transfer is expected to be distributed between a high FRET state and a low FRET state for the blue-yellow and yellow-red FRET pairs depending on the tether position. A range of different kinetic rates was introduced into the sample system by adjusting the number of complementary nucleotides between the tether and binding strands.

B04: Dynamic nanoagents for RNA delivery

Presenting author: **Ernst Wagner**

With Q1 2024, 32 gene therapies and 29 RNA therapies have been globally approved as medical drugs. Nevertheless, dynamic and targeted intracellular delivery of RNA remains a key requirement (1). We focus on a bio-inspired chemical evolution strategy. By incorporation of artificial amino acids such as tetraethylene pentamino succinic acid (Stp) or lipo amino fatty acids (LAFs) into xenopeptide (XP) sequences, novel double pH-responsive nucleic acid carriers have been designed that utilize intracellular delivery mechanisms of both cationizable lipids and polymers.

An endosomal pH-dependent polarity of LAF was implemented by a central tertiary amine, which disrupts the hydrophobic character once protonated, resulting in drastic pH-dependent change of XPs in the logarithmic (octanol/water) distribution logD from around +1 (pH 7.4) to zero (pH 6.5-~7.0) to -1 (pH 5.5). Enhanced endosomal escape turned out to be advantageous for potency of RNA delivery formulated as polyplexes (2) or LNPs (3). Transfer activity was maintained even in 90% serum and at extremely low dosage of 3 picogram mRNA (~2 nanoparticles/cell). Cell targeting and cellular uptake can be modulated by “chemical targeting” ´, i.e. physicochemical properties (size, surface properties) which influence protein corona formation or “biological targeting” via incorporation of receptor ligands. Of significance is functional *in vivo* delivery of mRNA, including CRISPER Cas9 mRNA/sgRNA genome editing (4), and an *in vivo* endothelial tropism of siRNA-LNPs (5) after systemic injection into mice, demonstrated by superior knockdown of liver sinusoidal endothelial cell-derived FVIII in comparison with standard LNPs. Optimizing LNPs following modification of siRNA-LNPs with integrin ligand cRGDfk efficiently silenced vascular endothelial growth factor receptor-2 in xenograft tumor endothelial cells.

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A04: Sequence self-selection by cyclic phase separation

Presenting author: **Dieter Braun**

A central mystery of the molecular origin of life is the emergence of oligonucleotides, such as RNA, that can self-replicate. In our work, we theoretically study and experimentally verify a simple though minimal mechanism capable of screening for such specific oligonucleotide sequences. This mechanism relies on two physical ingredients ubiquitous on early Earth: cycles of phase separation into oligonucleotide-dense and dilute phases and cyclic oligonucleotide exchange with a surrounding pool. We show that specific sequences can enrich oligonucleotide composition, evolving away from an initial pool. This non-equilibrium selection mechanism may provide the missing link in how specific short-chained peptides, RNA, and DNA sequences were recruited from prebiotic pools steering the assembly of self-replicating oligonucleotides at the molecular origin of life.

Session 6:

Photoswitchable Cholesterol Derivatives Substituted in the Lipophilic Side Chain

Presenting autho: **Michael Zott**

Lipids represent a broad class of biomolecules that encompass roles ranging from membrane formation to cell signaling. The Trauner Group is developing photoswitchable versions of lipids ("photolipids") that contain azobenzene moieties that can be selectively isomerized from *trans* to *cis* upon irradiation, thus providing optical control over lipid behavior. This talk will describe the application of this approach to controlled drug release from photoactivatable lipid nanoparticles formulated with AzoPC phosphatidylcholine photolipids as well as the development of cholesterol derived photolipids for use in studies of lipid nanoparticles and cell signaling.

B08: Artificial angiogenesis

Presenting author: **Stefan Zahler, Hong Liu**

In the previous funding periods, we used structured surfaces and various hydrogels to decipher and manipulate steps of angiogenesis in 1D, 2D and "2.5"D settings. We started from single cell phenomena and moved on to collective behaviour. The tools we developed in this context were also used to understand the impact of mechanics on cellular signalling. One of our most important findings was that endothelial cells remodel soft laminin based hydrogels in a way that enables subsequent pattern formation.
The last funding period we spent with analysing multicellular behaviour in fibrillary (collagen) gels. High-risk approaches, like the use of nucleic acid based intracellular and extracellular force sensors, did, unfortunately, not work out before the end of the CRC. Very recently, we focused on different approached for the vascularization of tumour spheroids including the use of blood vessel organoids, which turned out to be much more complex than it seemed. In addition to getting basic knowledge about vascularization processes, we aim to establish in vitro models for preclinical testing with a higher predictive value.

B09: Photons, Charges, Spins: from Photoswitches to aCoustics to Super-res

Presenting author: **Oliver Thorn-Seshold**

Chemical photoswitches have long been used "in molecular isolation," where the energy of absorbed photons only funnels into classical switching pathways. Through SFB1032 collaborations, we are exploring weak and strong coupling regimes to conditionally divert energy to or from photoswitches, reaching new pathways. These can radically (or tripletly) rewrite our expectations of how to convert light energy into sound or defined molecular motions in live animals, and how to design high-performance fluorophores for single-molecule techniques. These coupled systems can now find applications in membranes, gels, and cells; and they can be used for nanoscale imaging, switching, and control.

B12: Data-driven theory reveals universal cell-cell interactions across distinct motile cells

Presenting author: **Tom Brandstätter**

To drive various physiological processes like wound healing and cancer progression, motile cells exhibit a wide variety of interaction behaviors upon cell-cell contacts. These behaviors involve single cell motility and are critically determined by cell-cell interaction pathways that vary greatly across distinct cell types. While single cell motility arises from molecular migration mechanisms that are largely conserved across motile cell types, it remains unclear whether also cell-cell interactions arise from general biophysical mechanisms. Here, we study the two-cell collision behavior of a range of motile cells that exploit different interaction pathways. By employing a combination of a minimal confining two-state micropattern and a data-driven theoretical approach, we quantify behavior and infer a low-dimensional effective description of cell-cell interactions from experiments. Remarkably, while the behaviors and inferred cell-cell interactions of different cell types vary strongly, they can be described by a single interaction mechanism involving alignment interactions between cell polarity. These polarity alignment interactions are robust and cells control them via surface protein-mediated signaling to the cell's polarity machinery as revealed by molecular perturbations. Altogether, our data-driven characterization of cell-cell interactions reveals polarity alignment as a robust and general interaction mechanism, which may underlie the collective behavior of a wide range of motile cells.