Micro- and Nanostructured Biointerfaces

682. WE-Heraeus-Seminar

November 25 - 28, 2018
Physikzentrum Bad Honnef/Germany

WILHELM UND ELSE
HERAEUS-STIFTUNG

Subject to alterations!

Introduction

The Wilhelm und Else Heraeus-Stiftung is a private foundation that supports research and education in science with an emphasis on physics. It is recognized as Germany's most important private institution funding physics. Some of the activities of the foundation are carried out in close cooperation with the German Physical Society (Deutsche Physikalische Gesellschaft). For detailed information see https://www.we-heraeus-stiftung.de.

Scope of the 682. WE-Heraeus-Seminar:

Micro-and Nanostructured Biointerfaces hold great promise as tools for many biological applications ranging from the detailed understanding of complex signaling processes to massively parallel live-cell microarrays. As such, the interest in biointerfaces in cutting edge science and technology has grown rapidly in recent years.

With this seminar we aim at providing a forum for scientists who work on engineering micro- or nanostructured functional biointerfaces and their application in cell biology, immunology, and drug discovery. The seminar will include 14 invited contributions covering the following areas:

- micropatterning to address cell biological questions
- manipulating proteins on the nanoscale

A particular focus of the seminar is the discussion of novel approaches for solving common problems and for addressing novel challenges. WE-Heraeus-Seminars are typically smaller meetings with approx. 60 participants and organized in a workshop-like fashion, with an informal atmosphere and a lot of room for personal contacts. We believe that this is the perfect setting to bring together researchers from different fields to create a prolific environment where common interests can be discussed, challenges can be communicated and solutions developed in international collaborations, and a community can be shaped.

Scientific Organizers:

Prof. Dr. Sebastian Springer
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Program

Program

Sunday, November 25, 2018

16:00 – 21:00	Registration	
from 18:00	*BUFFET SUPPER / Informal get together*	
19:00 – 19:15	Scientific organizers	**Opening and welcome**
19:15 – 20:00	Joachim Rädler	**Cell migration and gene expression - single cell studies on artificial micro-pattern**

Monday, November 26, 2018

08:00	*BREAKFAST*	
Micro/Nanostructuring of surfaces (I)		
09:00 – 09:45	Pascal Jonkheijm	**Cell-instructive biointerfaces with dynamic complexity**
09:45 – 10:30	Christof Niemeyer	**Multiscale DNA systems to investigate living cells**
10:30 – 11:00	*COFFEE BREAK*	
Micropatterns for cell biology (I)		
11:00 – 11:45	Shalini Low-Nam	**Molecular binding events, phase transitions, and signal integration during T cell activation**
11:45 – 12:30	Kheya Sengupta	**T cells on engineered substrates: The impact of T cell receptor clustering is enhanced by integrin engagement**
12:30	**Conference Photo** (in the foyer of the lecture hall)	
12:35	*LUNCH*	

Program

Monday, November 26, 2018

Micro/Nanostructuring of surfaces (II)

14:00 – 14:45	Shalom Wind	**Nanoscale architectures for probing cellular mechanoresponse**
14:45 – 15:00	Agnes Dobos	**High-resolution 3D printing of biomimetic microenvironments to study T-cell activation**
15:00 – 15:15	Mario Mairhofer	**Functionalized nanostructures for cell culture and cell-based assays**
15:15 – 16:00	**Poster flash talks** (1 min each / 1 slide)	
16:00 – 16:30	*COFFEE BREAK*	

Manipulating proteins on the nanoscale

16:30 – 17:15	Ana Teixeira	**Spatial signalling in cell-to cell communication**
17:15 – 17:30	Joschka Hellmeier	**DNA origami as a nanoscale platform for T-cell activation**
17:30 – 17:45	Elena Ambrosetti	**Characterization of nanoscale protein clusters at the cell membrane with DNA nanotechnology**
17:45 – 18:00	Martin Humenik	**Hybrid spider silk nanostructures**
18:15	*DINNER*	
19:15 – 21:15	**Poster session**	

Program

Tuesday, November 27, 2018

08:00	*BREAKFAST*	
Cells on structured biointerfaces (I)		
09:00 – 09:45	Josep Samitier Martí	**Nano-patterns distributions for tissue engineering**
09:45 – 10:30	Raghavendra Palankar	**Dissecting platelet function and dynamics using nano- and micropatterned arrays**
10:30 – 11:00	*COFFEE BREAK*	
Cells on structured biointerfaces (II)		
11:00 – 11:45	Elena Martínez	**Nanopatterns of surface-bound EphrinB1 ligand to tune EphB2 receptor clustering**
11:45 – 12:00	Marga Lensen	**Micropatterned, nanocomposite biomaterials based on bioinert PEG-hydrogels and bioactive nanohydroxyapatite and gold nanoparticles**
12:00 – 12:15	María Teresa Alameda	**Design smart multifunctional surfaces by using micro-nano hierarchical topographies**
12:15 – 12:30	Jordi Comelles	**Hydrogels with independently controlled topography and stiffness**
12:30	*LUNCH*	

Program

Tuesday, November 27, 2018

14:00	**Trip / „NetWalking“** (Leisurely hike in the vicinity with a visit to a coffeehouse.)	

Micropatterns for cell biology (II)

16:30 – 17:15	Jacob Piehler	**Surface micropatterning for quantitative protein interaction analysis in cellular signaling**
17:15 – 17:30	Noemi Linden	**Conformation-specific MHC I protein clusters detected by a two-hybrid antibody micropattern assay**
17:30 – 17:45	Peter Lanzerstorfer	**Analysis of EGFR downstream signaling by live cell micropatterning**
17:45 -18:00	Gergő Fülöp	**Probing lipid interactions of plasma membrane proteins: A micropatterning approach**
18:15	*HERAEUS DINNER at the Physikzentrum (cold & warm buffet, free beverages)*	
19:15 – 20:30	**Interactive session**	

Program

Wednesday, November 28, 2018

08:00	*BREAKFAST*	
Micro/Nanostructuring of surfaces (III)		
09:00 – 09:45	Karen Martinez	**Arrays of high-aspect ratio nanostructures for biological applications**
09:45 – 10:30	Graham Leggett	**Quantum optical proteins: Nanofabrication, strong coupling and synthetic biology**
10:30 – 10:55	*COFFEE BREAK*	
10:55 – 11:15	**Poster awards and poster award talk**	
Micro/Nanostructuring of surfaces (VI)		
11:15 – 11:30	Yoojin Oh	**Force spectroscopy and recognition imaging: Quantifying binding strength and affinity on the single-molecule level**
11:30 – 12:15	Jose Moran Mirabal	**Lipids at micro- and nanostructured interfaces – from phase segregation to biosensing**
12:15 – 12:30	Scientific organizers	**Summary and closing remarks**
12:30	*LUNCH*	

End of the seminar *and FAREWELL COFFEE* ***/ Departure***

Please note that there will be ***no*** *dinner at the Physikzentrum on Wednesday evening for participants leaving the next morning.*

Posters

Posters

1.	Sami Alalawi	**Fabrication of macroscopic arrays of metal nanostructures for studies of strong plasmon-exciton coupling**
2.	Lion Augel	**Plasmonic enhanced group-IV nanopillar photo-detectors for refractive index sensing and potential molecular fluorescence detection**
3.	Victor Bamieh	**Nanoscale organization and mobility of ligands direct T-cell activation**
4.	Tonia Bargmann / Zeynep Hein	**Cell surface dynamics of spondyloarthropathy related HLA Class I molecules**
5.	Alessandro Bosco	**The role of the cortical actin in the regulation of Eph receptor signaling**
6.	Evelin Csányi	**Quantum-optical polymers**
7.	Veronika Dockalova	**Probing the membrane environment of palmitoylated transmembrane proteins: A micropatterning approach**
8.	Trixy Fang	**Spatial regulation in the immunological synapse**
9.	Maria Gräber / Ruth Herbst	**Using micropatterning to develop a novel in vitro muscle cell model to study development of the neuromuscular junction**
10.	Theresia Gutmann	**The activation mechanism of the insulin receptor reconstituted in lipid nanodiscs**
11.	Jann Haberts	**Culturing and patch clamping cells on nanowire arrays**
12.	Nouria Jantz	**Micropatterned assays of peptide-receptive MHC class I proteins**

Posters

13.	Enara Larrañaga Carricajo	**A micropatterned platform to analyze the effects of ephrin ligands on the compartmentalization of organoid-derived intestinal cells**
14.	Anna Lindemann	**Antibody micropatterning on surfaces for single molecule investigation of protein-protein interaction in membranes**
15.	Anna Lishchuk	**Coupling of light-harvesting chlorophyll–protein complex II to nanoarrays of gold fabricated by interferometric lithography**
16.	Sara Löchte	**Synthetic STAT signaling modules for studying Jak/STAT activation and regulation**
17.	Markus Lunzer	**A modular approach to sensitized two-photon micropatterning of photolabile hydrogels**
18.	Christian Niederauer	**Direct characterization of the evanescent field in objective-type total internal reflection fluorescence microscopy**
19.	Mahlaqua Noor	**Functionalized polyelectrolyte microcapsules for T-cell activation**
20.	Sokunthearath (Kevin) Saem	**Detection of membrane disrupting agents via electrochemical biosensors**
21.	Anna Vila Giraut	**Engineering 3D intestinal Mucosa models using hydrogel co-polymers**
22.	Themistoklis Zisis	**Controlling cellular function by structured surfaces: "Artificial angiogenesis"**

Abstracts of Lectures

(in chronological order)

Cell Migration and Gene Expression - single cell studies on artificial micro-pattern

Joachim O. Rädler

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Micro-structured arrays of protein surface-pattern provide a standardized platform for the investigation of cell migration at the single cell level. We present time-lapse microscopy studies of cells on various defined geometries, including short stripes, ring-shaped micro-lanes, ring-shaped micro-lanes, as well as dumbbell pattern. Cell motion is analyzed by automated tracking and compared to analytical models and Potts Model computer simulations. On larger circular micro-pattern, small groups of cells exhibit spontaneous emergence of rotational migration with survival times of the rotation depending on cell number. Secondly we use automated time-lapse microscopy in combination with micro-patterned surfaces to monitor the time courses of fluorescent reporter signals from many individual cells in parallel. We show that measurement of single cell gene expression kinetics yields access to mRNA translation efficiency, mRNA lifetime and delivery delay times.

References

[1] FJ Segerer, F Thüroff, AP Alberola, E Frey, and JO Rädler. "Emergence and Persistence of Collective Cell Migration on Small Circular Micropatterns." Physical Review Letters **114, no. 22** 228102 (2015).

[2] C Schreiber, FJ Segerer, E Wagner, A Roidl, and JO Rädler. „Ring-Shaped Microlanes and Chemical Barriers as a Platform for Probing Single-Cell Migration." Scientific Reports **6** 26858 (2016).

[3] M. Ferizi, C. Leonhardt, C. Meggle, M.K. Aneja, C. Rudolph, C. Plank, and J.O. Rädler. "Stability Analysis of Chemically Modified mRNA Using Micropattern-Based Single-Cell Arrays." *Lab on a Chip* 15, no. 17 (2015): 3561–71.

Cell-instructive biointerfaces with dynamic complexity

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Supramolecular chemistry provide nowadays an excellent prospect to construct reversible biological interfaces that can be employed for supramolecular cell manipulation experiments.[1] Making use of supramolecular chemistry is rewarding to develop functional materials and devices. Knowing the limitations involved in ordering proteins at different length scales will surely hasten developing future applications, supramolecular bionanotechnology being the most prominent. The construction of synthetic supramolecular assemblies of proteins provides an excellent tool to fabricate organized bioactive components at surfaces. I will present new synthetic procedures for site-specific noncovalent anchoring of proteins to surfaces and polymers.[2, 3] Special attention is paid to orientational and conformational aspects at the surface and will be demonstrated. Using concepts of multivalency the interactions between proteins and surfaces can be modulated by design. Many of the protein complexes were patterned on surfaces using microcontact printing or nanolithography and visualized using fluorescence microscopy. Furthermore, supramolecular linkers that are sensitive to remote electrochemical stimuli will be presented, using cucurbituril (CB) and cyclodextrin (CD)-modified surfaces.[2, 4] Electrochemical switching was studied using surface embedded electrodes.[4] Cell release was studied in detail in the case of cell-adhesive peptides and growth factors. Lastly, supramolecular linkers were compared to reversible covalent linkers, providing insight in the cell receptor signaling pathway.[5] With the development of reversible bioactive platforms on surfaces serving as a reversible dynamic interfaces to cells, improved scaffolds for tissue regeneration will become in hand. First steps into this directions will be introduced as well.

References

[1] J. Brinkmann, et al., Chem. Soc. Rev. **43**, 4449 (2014).
[2] L. Yang, et al., J. Am. Chem. Soc. **134**, 19199 (2012).
[3] D. Wasserberg, et al., ACS Nano, **11**, 9068 (2017).
[4] Q. An, et al., Angew. Chem. Int. Ed. **51**, 12233 (2012).
[5] S. Sankaran, et al., ACS Nano 11, 3867 (2017).

Multiscale DNA systems to investigate living cells

Christof M. Niemeyer

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The advent of DNA microarray technology in the course of the human genome project in the late 1980s has led to the evolution of sophisticated DNA-functionalized solid substrates, which are nowadays routine tools for fundamental and applied research in biology and medicine. However, the versatility of DNA biochips goes far beyond the established applications in genotyping and expression profiling, because their capability for highly parallel, site-directed immobilization of complementary nucleic acids can be harnessed to assemble complex surface architectures comprised of colloidal materials and proteins. While this approach enables novel sensor platforms for protein and small-molecule analysis, the full potential of DNA surfaces can be exploited by implementation of structural DNA nanotechnology.

We have previously applied this DNA chip-based approach to investigate cell adhesion and the recruitment of transmembrane proteins in living cells. By combining DNA micro- and nanostructures, we have now developed "multiscale origami structures as interface for cells" (MOSAIC). This tool allows one to present ligands to living cells on surfaces with a full control over their stoichiometry and nanoscale orientation, thereby enabling to address fundamental questions in cell signalling which cannot be tackled by conventional technologies.

Gandor, S., Reisewitz, S., Venkatachalapathy, M., Arrabito, G., Reibner, M., Schroder, H., Ruf, K., Niemeyer, C. M., Bastiaens, P. I., Dehmelt, L. (2013) A Protein-Interaction Array Inside a Living Cell. *Angew. Chem. Int. Ed. Engl.* **52**, 4890–4894

Meyer, R., Giselbrecht, S., Rapp, B. E., Hirtz, M., Niemeyer, C. M. (2014) Advances in DNA-directed immobilization. *Curr Opin Chem Biol* **18C**, 8-15

Angelin, A., Weigel, S., Garrecht, R., Meyer, R., Bauer, J., Kumar, R. K., Hirtz, M., Niemeyer, C. M. (2015) Multiscale Origami Structures as Interface for Cells. *Angew Chem Int Ed Engl* **54**, 15813-15817

Molecular binding events, phase transitions, and signal integration during T cell activation

S.T. Low-Nam, J.J.Y. Lin, D.B. McAffee, and J. T. Groves

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Using single molecule imaging techniques [1, 2], we map the spatial locations and duration of each individual pMHC:TCR binding event while simultaneously monitoring the signaling state of the cell during the process of antigen discrimination by T cells. This strategy enables direct connection between the specific stochastic sequence of binding events experienced by a cell and that particular cell's decision to activate. We observe a discrete condensation events of LAT in response to individual pMHC:TCR interactions. Although triggered by pMHC:TCR binding, the size and lifetime of the LAT assemblies are decoupled from the duration of the originating pMHC:TCR binding event. Taken together, these observations reveal a delocalized mechanism of antigen discrimination that relies on a phospho-tyrosine driven phase transition in the assembly and disassembly of LAT at the membrane [3].

References

[1] ***eLife* 2013** 2: e00778, "Direct single molecule measurement of TCR triggering by agonist pMHC in living primary T cells", Geoff P. O'Donoghue, Rafal M. Pielak, Alexander A. Smoligovets, Jenny J. Lin, Jay T. Groves.

[2] ***Proc. Natl. Acad. Sci. USA* 2017**, 114, 46: 12190-12195: "Early T cell receptor signals globally modulate ligand-receptor affinities during antigen discrimination", Rafal M. Pielak, Geoff P. O'Donoghue, Jenny J. Lin, Kate Alfieri, Nicole C. Fay, Shalini T. Low-Nam, Jay T. Groves.

[3] ***Proc. Natl. Acad. Sci. USA* 2016**, 113, 29: 8218-8223: "Phosphotyrosine-mediated LAT assembly on membranes drives kinetic bifurcation in the recruitment dynamics of the Ras activator SOS", William Y. C. Huang, Qingrong Yan, Wan-Chen Lin, Jean K. Chung, Scott D. Hansen, Sune M. Christensen, Hsiung-Lin Tua, John Kuriyan, and Jay T. Groves.

T cells on engineered substrates: the impact of T cell receptor clustering is enhanced by integrin engagement

Kheya Sengupta

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The interface between an Antigen Presenting Cell (APC) and a T-lymphocyte (T cell), sometimes called a synapse, plays a key role in sensitivity and precision of antigen recognition by T-cells and therefore the subsequent immune response. T cell receptor (TCR) clusters are believed to be very important in this process of amplification of the recognition signal to a response at cellular level. Recently, it was recognized that TCR-ligands on APCs are also displayed as submicronic clusters. We aim to study how the clustering of ligands on APCs influences T-cell membrane and actin organization. To achieved this, a new technical approach based on size-tunable organic nano dot arrays was use to created synthetic substrates which mimic the APC-membrane by presenting molecules of anti-CD3 (targeting the TCR-complex), in the form of an array of sub micrometric dots surrounded by a fluid supported lipid bilayer (SLB), which is optionally functionalized with ICAM-1 (ligand for the T-cell integrin-LFA). We find a dual scale of T cell response: locally, the cell responds at the nano-scale and restructures its membrane and actin according to local cues; globally, it integrates the signal and responds to an average dose. The local membrane and actin organization are however strongly modified in presence of ICAM-1. Interestingly, presence of ICAM-1 amplifies the effect of TCR-clustering. No such effect is evident for co-engagement of CD28. These results indicate complementary role for LFA-1 and CD28 in the regulation and putative coupling of TCR micro-clusters to actin. The engineered substrates have the potential to act as platform for fundamental research in immune cell biology, as well as translational analyses in immunotherapy, for example to screen molecules for their role in T cell adhesion/activation.

Nanoscale Architectures for Probing Cellular Mechanoresponse

H. Cai[1], J. Liao[1], M. Schvartzman[1], M. J. P. Biggs[1], M. Palma[1], M. L. Dustin[2], M. P. Sheetz[1] and S. J. Wind[1]

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Faithful devotion to Moore's Law over the past several decades has led to the manufacture of advanced electronic circuits with unprecedented complexity and with near-perfect order down to the nanoscale - critical features of transistors are now around the same size as many proteins and other biomolecules. This has created new opportunities whereby the tools and processes originally developed for the semiconductor industry may be exploited for biomedical purposes.

This talk describes the development and application of two biomimetic platforms designed to probe how cells sense and respond to physical features in their environment and whose fabrication is adapted from transistor technology. One platform consists of hierarchical nanoarrays of single molecule binding sites[1]. Virtually any geometric arrangement is possible, ranging from isolated, individual sites to small (3 – 7) and large (> 1000) clusters, with inter-site spacings as small as 40 nm. Ligands of specific cellular receptors are immobilized at these sites, with precise control over the number of ligands at each site[2]. The background can be passivated against non-specific binding, and it can present additional ligand species, either statically or dynamically, using, e.g., a supported lipid bilayer. A second platform consists of elastomeric substrates with controlled, patterned rigidity ranging in size from the micro- to the nanoscale over a wide range of stiffnesses[3].

The utility of these surfaces is demonstrated with T lymphocytes, where careful engineering of the nanoarray platform reveals how both lateral and axial spacing affect T cell receptor triggering via a balance between inhibitor segregation and close packing of the T cell receptor[4]. Similarly, heterogeneous rigidity surfaces[3] illuminate the role of local stiffness in the interplay between adhesion and T cell receptor activation. These results provide unique insight into the mechanisms governing T cell function, and they may have significant implications for future therapeutic applications.

References

[1] M. Schvartzman et al., Nano. Lett. **11**, 1306 (2011); H. Cai et al., Biophys. J. **108**, 631a (2015); H. Cai et al., in *Methods in Molecular Biology, The Immune Synapse* (2016).
[2] H. Cai et al., ACS Nano **10**, 4173 (2016).
[3] M. J. P. Biggs et al, Adv. Mater. **29**, 1702119 (2017).
[4] H. Cai et al., Nat. Nano. **13**, 610 (2018).

High-resolution 3D printing of biomimetic microenvironments to study T-cell activation

Agnes Dobos[1,2], Peter Gruber[1,2], Wolfgang Steiger[1,2], Gergo Fulop[3], Eva Sevcsik[3], Gerhard Schutz[3], and Aleksandr Ovsianiov[1,2]

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T-cell activation plays a crucial role in adaptive immunity. Antigen presenting cells present peptide bound major histocompatibility complexes (pMHC) to which T-cells can bind to via their specific T-cell antigen receptors (TCRs), which in turn it recruits other molecules to initiate a signaling cascade. T-cell activation processes, including immunological synapse formation, cellular polarization, receptor sequestration and signaling are regulated by the cytoskeleton motility. [1] However, most of the studies concerning T-cell antigen recognition and kinetics were mainly performed on 2D systems. Additive manufacturing technology (AMT) allow the reproducible production of complex 3D constructs in accordance to computer-aided design (CAD) models.[2] However, conventional AMTs such as stereolithography and extrusion based bioprinting technologies require a layer-by-layer deposition of the resin, which is often results in low resolution. Two photon-polymerization (2PP) is a state-of the-art 3D printing technique, where nonlinear absorption of a femtosecond-pulsed infrared laser leads to the crosslinking of a very small voxel within the volume of the photosensitive material or photoresist. High resolution 3D structures with feature sizes down to 100 nm can be produced directly within the bulk of the sample.[3] With the help of 2PP it is possible to generate complex high resolution 3D architectures which make it possible to perform in situ experiments to study single cell response.

References

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Functionalized nanostructures for cell culture and cell-based assays

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Currently, most cell culture models are cultivated in 2D. Alternative 3D cell culturesrely on the self-organization capability of isolated cells (e.g. organoids), or on de-cellularized tissue scaffolds, which are re-populated with cells.

To advance our knowledge on organ formation and cell differentiation, fully synthetic scaffolds with tunable surface properties are intensively sought for. We have developed bio-compatible, functional polymers to fabricate such scaffolds by multi-photon lithography (MPL) [1, 2]. In MPL, a femtosecond-pulsed laser is focused into a photosensitive resin solution and initiates polymerization solely within the focal volume of the laser beam. Consequently, sub-micrometer resolution in two or three dimensions is achieved. For fast fabrication of high aspect ratio structures with flexible mechanical properties, a combination of thiolen/methacrylate resins has been used.

For biocompatibility testing, the scaffolds were seeded with different cell types and analyzed by immunofluorescence microscopy [3]. Further, the surface properties of the scaffolds were optimized to enable optimal cell adhesion and growth. For functionalization of the surface, monomers with orthogonal functional groups have been used. This way, we can immobilize different peptides/proteins/growth factors on freely designed scaffolds to improve cell adhesion, attract certain cell types or locally activate/stimulate the cells. These developments pave the way for new cultivation systems and new cell-based assays [4] based on MPL-printed, functionalized nanostructures.

References

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Spatial signalling in cell-to cell communication

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Membrane proteins are key sensor components in cells and form the largest class of proteins that are targeted by drugs. The dominance of membrane proteins as drug targets is set to continue as antibodies and other biological molecules that cannot easily cross the cell membrane are increasingly used as drugs. Therefore, biological drug development relies largely on understanding the key factors that regulate membrane protein functions. The biophysical context of ligands and receptors at the membrane is one such factor and its study requires methods to control and analyse membrane protein microenvironments at the nanoscale.

DNA origami is a nanofabrication technology that uses DNA self-assembly to drive the precise formation of nanostructures. We have shown that DNA origami can be used to tailor the spatial distribution of protein assemblies and regulate signaling mediated by membrane receptors (1). This tool allows for display of well-defined protein nanoclusters in solution and is therefore amenable for *in vitro* and *in vivo* experiments.

We are analyzing receptor activation with single cell resolution using in situ proximity ligation assay (in situ PLA). We have validated this method for the analysis of EphA2 receptor activation and showed that the levels of EphA2 receptor activation depend on the spatial distribution of ephrinA5 ligands at the nanoscale. To investigate the morphological and dynamical aspects of the assembly of the receptor on the cell membrane, we are using superresolution microscopy. These studies are complemented with RNA-sequencing and functional assays to identify the downstream signaling pathways modulated by membrane receptor clustering.

DNA origami/ligand nanoclusters form a nanotool that is well suited to investigate the roles of the biophysical properties of ligand/receptor interactions on downstream signaling and cellular outcomes. We are applying these tools to understand signaling mediated by cell-cell contact in neural stem cells, cancer cells and immune cells.

References

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DNA ORIGAMI AS A NANOSCALE PLATFORM FOR T-CELL ACTIVATION

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In the human immune system, the recognition of an antigen by the T-cell receptor (TCR) takes place within the contact area between the T-cell and the antigen-presenting cell (APC. It is thought that the nanoscale spatial distribution of proteins within this contact zone plays an essential role in the initiation of an immune response. Despite extensive studies, the molecular details of this process, in particular the structural requirements for TCR triggering and how nanoscale events are translated into T-cell activation, are still poorly understood.

Here, we use DNA origami decorated with TCR ligands anchored to a planar glass-supported lipid bilayer to assess the effects of local ligand density and arrangement on T-cell activation. Thus, our experimental setup allows for the precise nanoscale arrangement of TCR ligands on the DNA origami scaffold, while at the same time permitting the re-organization of ligand and TCR during T-cell activation. We used either fluorescently labeled CD3 antibody or recombinant TCRβ-reactive single chain antibody fragment (scF_v) as stimulatory ligands that were placed on the DNA origami at one to 20 engineered capture sites in different layouts and densities.

The actual number of ligands per origami was determined using several single molecule fluorescence microcopy methods and atomic force microscopy. The activation of T-cells interfaced with the APC-mimicking surfaces was measured using a Ca^2-sensitive fluorescent dye and the effects of local ligand density, nanoscale ligand arrangement as well as the nature of the ligand were assessed. Further, the rearrangement of TCR and ligand in the process of T-cell activation was monitored by single molecule microscopy.

Characterization of nanoscale protein clusters at the cell membrane with DNA nanotechnology

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The interplay and clustering between proteins on the cell surface is a key component in regulating membrane protein function. Protein assemblies regulate biological activity and have emerged as being of therapeutic importance: the nanoscale spatial ordering and the dynamic distribution of proteins in the cell membrane fine tune signalling pathways critical to various normal physiological processes and in diseases [1,2]. The study of protein nanoscale environments at cell surface will shed light on the spatial regulation of receptor signalling and of the complex molecular networks downstream of receptor activation. Therefore, there is a huge need to find high-throughput methods to study how proteins organize at molecular level and how this determines the cell phenotypes.

In this work, we present the development of a non-microscopy based super-resolution method for unbiased analysis of protein nanoclusters at the cell membrane, studying the frequency in which specific types of protein appear in the proximity of the protein of interest. We setup a DNA nanotechnology-based approach, using DNA nanostructures to decipher the position and the identity of proteins within the assembly. STORM-TIRF super-resolution microscopy analysis and advanced biochemical assays were combined to validate the methodology and to obtain exhaustive characterization of protein clusters. We focused here on clustering of the membrane receptor Her2. Although Her2 has well known roles in driving tumor progression, the molecular mechanisms by which Her2 can lead to diverse cellular outcomes are still unclear [3]. Since Her2 forms homo- and hetero-dimers and oligomers, we aim to provide a precise portrait of the spatial organization of Her2-containing clusters and to understand how it correlates with downstream signaling and cellular outcomes. Our approach has the potential to contribute to developing a new paradigm in targeting receptor signaling at the nanoscale, focusing not on the membrane receptors per se, but on their spatial organization.

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Hybrid spider silk nanostructures

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Self-assembly of recombinant spider silk proteins into nanofibrils is specifically triggered by low phosphate ions concertation [1]. Thus, such fibrils represent a nanoscaffold suitable for incorporation of functional bio-macromolecules.
We prepared DNA-spider silk conjugates, in which self-assembly properties of a recombinant spider silk protein and hybridization properties of DNA were combined in one chemical entity. Corresponding self-assembled silk fibrils exposed nucleic acid strands suitable for specific fibril labeling [2]. Moreover, hybridization of the DNA-silk hybrids into linear or branched constructs allowed controlled hierarchical self-organization of the conjugate fibrils into nano-ribbons and microscopic rafts using controlled temperature conditions [3].
The functionality of DNA was also exploited in DNA directed immobilization of respective spider silk hybrids onto complementary modified surfaces using soft lithography. This strategy enabled initialization of the protein nucleation and fibrils self-assembly on predestined spots and enabled pattering of nanofibrils with high- or low-densities across multiple length scales.
In another approach, a recombinant spider silk protein was genetically combined with either the hydrolytic enzyme Esterase 2 or green fluorescent protein GFP. Respective catalytic and light emitting properties of the functional moieties in the fusions were comparable to that of the unmodified precursors in solution as well as they further maintained their activities upon self-assembly of the spider silk domain into fibrils and hydrogels [4].

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Nano-patterns distributions for Tissue Engineering

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Cell adhesion onto bioengineered surfaces is affected by several variables, including the former substrate derivatization process. The information arising from this environmental sensing is integrated into the cell machinery through receptor proteins located at the cell membrane. We have studied the correlation between cell adhesion and cell–adhesive ligand surface gradient concentration. Similar procedure has been used to systematic in vitro screening of the effects of different concentrations of immobilized Bone Morphogenetic Protein (BMP-2). For this purpose, gradient surfaces were created on poly(methyl methacrylate) substrates by continuous hydrolysis and were then grafted with biotin-PEG-RGD molecules or BMP-2 molecules. The experimental results obtained allow the evaluation at early stages of osteogenesis in C2C12 cells, indicating the potential of continuous gradients for in vitro screening applications. To analyze in detail the nanoclustering effects in the cell adhesion and differentiation process, we have created large-scale uneven nanopatterns of arginine-glycine-aspartic acid (RGD)-functionalized dendrimers that permit the nanoscale control of local RGD surface density changing the initial dendrimer concentration. We have used this model to study the first steps of chondrogenesis differentiation of mesenchymal stem cells (MSCs). Transplantation of mesenchymal stem cells (MSCs), which have a vast proliferative capacity and differentiation potential, has emerged as an attractive strategy to treat widespread joint defects. However, direct implantation of undifferentiated MSCs without any preconditioning lead to calcification of the implanted cells, fibrogenesis and heterotopic tissue formation in the cartilage. Results show that in vitro stem cell preconditioning is required to address cells to a specific differentiation pathway prior to implantation.

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Dissecting Platelet Function and Dynamics Using Nano- and Micropatterned Arrays

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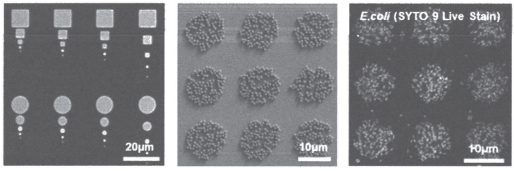
Platelets are discoidal multifunctional cellular fragments (3-5µm in diameter) generated from bone marrow megakaryocytes. Circulating platelets are essential for haemostasis and thrombosis, as they "survey" the integrity of the vascular system. Upon vascular injury, platelets rapidly (within few seconds) adhere to the exposed extracellular matrix, undergo activation and form a haemostatic plug to seal the wound. In addition, platelets contribute to crucial physiological functions in human health and onset of diseases as they interact with a wide variety of cells such as those from the adaptive and innate immune system, cells of vasculature and as well as bacterial pathogens via specific receptors. These dynamic interactions occur at length scales from nanometers to several microns and at time scales of milliseconds up to minutes. Currently, in platelet research, to decipher such interactions *in vitro*, assays in large volumes (e.g. aggregometry) and end-point readout (e.g. FACS) are performed albeit with several limitations. Recent developments in lithography and microfabrication techniques in combination with live imaging modalities have made it possible to further our understanding of platelet dynamics at interfaces. We use electron beam lithography to fabricate nano- and micropatterned arrays[1]. Such arrays can be functionalized with platelet adhesive ligands, immune complexes[2], bacterial proteins[3], "bacteriamimetic" microbeads[4] and live bacteria[5]. Currently, we are using micropatterned arrays to visualize and assess platelet function and dynamics to stimuli and their biological significance up to single platelet level. I will present some of the micropatterned tools developed by our lab and their applications in platelet research. In addition, I will also outline the limitations in setting up platelet function assays on micropatterned arrays.



Micropatterned Arrays | Bacteriamimetic Microbead Arrays | Live Bacteria

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Nanopatterns of surface-bound EphrinB1 ligand to tune EphB2 receptor clustering

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Eph-ephrin signaling is critical for tissue patterning, cancer progression and regeneration, yet the activation mechanism remains largely unknown [1]. Eph receptor activation and downstream signaling requires Eph receptor clustering upon multivalent stimulation with ephrin ligands [2]. Because of its s diffusivity in highly dynamic membrane environments, the observation of oligomerization processes in real-time is beyond the reach of live-imaging tools [3] This fact limits the characterization and optimization of the ephrin/Eph multivalent interactions.

We in here have engineered a strategy to present surface-bound ephrin ligands eliciting multivalent effects on Eph receptor oligomerization through a nanopatterned surface [4]. Nanopatterns of ephrin ligands are visualized by atomic force microscopy (AFM) and direct stochastic optical reconstruction microscopy (dSTORM). To quantitatively track the EphB2 receptor oligomerization process in real time we used a statistically enhanced version of the Number and Brightness technique (eN&B), which can discriminate the oligomeric states of diffusive species [9]. Such a unique set-up combination has allowed us to assess the stronger effects and accelerated kinetics of ephrin-Eph multivalent interactions produced by the nanopatterned surfaces. Although previous studies had hypothesized about the possibility of stimulating oligomerization and signaling of Eph receptors through surface-bound ligands, [3] our study represents the first experimental confirmation of this hypothesis.

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Micropatterned, Nanocomposite Biomaterials based on Bioinert PEG-Hydrogels and Bioactive Nanohydroxyapatite and Gold Nanoparticles

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Hydroxyapatite (HAp) is one of the most important bioceramics for medical and dental applications, as it possesses excellent biocompatibility and is osteoconductive. However, pure HAp is difficult to shape in the complex forms required for tissue engineering applications such as bone repair because of its hardness and brittleness. Therefore, we have developed novel nanocomposite hydrogels based on HAp and poly(ethylene glycol) (PEG) hydrogels. The gel properties (soft, hydrated and moldable) allow the fabrication of 2D-patterned substrates and 3D-structured scaffolds, which can be further biomineralized to exhibit increased bioactivity and functionality to support the growth of osteoblast cells.

Depending on the requirement of the patterning method (vide infra), several PEG-based macromonomers can be selected from our library of multifunctional building blocks, e.g. linear or star-shaped, liquid or solid, with a low or high molecular weight and with the desired functional groups for crosslinking the gels.

Simple mixing of hydroxyapatite nanoparticles (HAp NPs) with the hydrogel precursors yields novel nanocomposite materials with interesting properties. Nevertheless, even more finely dispersed HAp can be obtained when the PEG-precursors are mixed with salt solutions containing calcium and phosphate ions; in that case, nHAp is formed in situ. The nanocomposite precursor mixtures can be further crosslinked to create stable, new hydrogels with tunable mechanical and swelling properties. Interestingly, the shear mixing of our 8-arm PEG-prepolymer ($M_n \approx 15{,}000$ Da) with salt solutions already resulted in spontaneous gelation, which is attributed to an amine Michael type addition reaction taking place.

Fluid precursor mixtures containing minimal amounts of water can be processed by our unique Fill-Molding In Capillaries (FIMIC) method to fabricate surface patterns of bioactive composite materials verses bio-inert regions of pure PEG, which were demonstrated to induce selective cell adhesion.

It was found that cells adhere preferentially to PEG-hydrogels with gold nanoparticles (Au NPs). Micropatterns of such Au NPs were fabricated on PEG-hydrogels to control cell adhesion and migration.

Design smart multifunctional surfaces by using micro-nano hierarchical topographies

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The extensive use of antibiotics and biocides has led to the development of multi-drug resistant bacteria also called superbugs. As such, there is an urgent need to use alternative antibacterial mechanisms that do not cause resistance. In this context, bioinspired nanostructured surfaces have shown a potential to preclude bacteria colonization avoiding the use of antibacterial chemical means responsible for the rise of resistant bacteria. For instance, moth-eye mimetic topographies have demonstrated a bactericidal activity by rupturing bacteria adhered on their surfaces [1].

From the point of view of clinical implants, an additional challenge is to identify topographical features that not only prevent bacterial colonization but also promote the development of mammalian cells to obtain a more favourable host tissue response and implant integration. Previous studies have shown that mammalian cells sense and respond to topographical features in the mesoscopic range modulating their biological response [2].

In this work, a convergent design of nano and micro hierarchical topographies has been developed to attain biocidal surface properties and, at the same time, an appropriate niche for mammalian cell growth. The fabrication process combines sequential nanoimprint lithography (NIL) with optical lithography steps giving rise to micro-nano hierarchical topographies. This process allows for a simple and well-controlled fabrication of hierarchical structures, which is typically very challenging. Hierarchical micro-nano structures with different geometrical designs covered by moth-eye type of nanocones have been developed. On these surfaces, the response of mammalian cells and bacteria is being studied. Preliminary results have shown a mechanical disruption of bacterial cell wall after incubating these on the surfaces (see Figure 1).

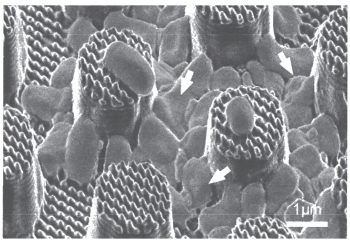

Figure 1. *Escherichia coli* wall disruption (white arrows) when attaches on hierarchical micro-nano topography.

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Hydrogels with independently controlled topography and stiffness

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The physical properties of the extracellular matrix (ECM) play a key role in the regulation of cell processes that determine their fate and function. For example, it is well documented that cells can sense the stiffness of their environment, and change their differentiation lineage accordingly [1]. In a similar way, cells exposed to topographical features can also change their differentiation profile [2]. Other important cell functions, such as migration, are also affected by stiffness [3] and topography [4]. Although the mechanisms by which cells sense stiffness have been extensively studied [5], much less is known about how cells sense the topography of their surroundings. The current view suggests that cells have conserved pathways for both stiffness and topography sensing. However, most of the works done on topographical patterns are carried out on stiff substrates, thus both cues are not disentangled. Here, we present a simple method to obtain topographical structures of biologically-relevant sizes (5-10 μm) on polyacrylamide (PA) hydrogels of biologically-relevant stiffness (2-200 kPa). Our method is based on the capillary force lithography (CFL) approach using flexible materials. The microstructures are further characterized by optical microscopy, following the changes in size and shape upon swelling. Mechanical properties of the hydrogel are also characterized by atomic force microscopy (AFM) measurements. The topographical structures can be fully covered by extracellular matrix proteins and sustain the growth of both cell lines (fibroblasts and myoblasts) and primary cells (mouse intestinal epithelial cells). The method here described allow the fabrication in a very simple manner of topographically patterned substrates with all the advantages of PA hydrogels such as tunable stiffness and coupling of proteins, and traction force microscopy compatible.

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Surface micropatterning for quantitative protein interaction analysis in cellular signaling

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Signal transduction across the plasma membrane critically depends on the assembly of multi-protein complexes that is highly regulated in time and space. Mechanistic understanding of signal activation and propagation requires unraveling the intricate interplay of protein-protein interactions involved in the assembly of signaling complexes in the context of the specific features provided by the membrane. We have developed microstructured surface functionalization for capturing proteins in the plasma membrane of living cells into micropatterns to explore the assembly of cytokine receptor signaling complexes that activate the JAK/STAT pathway. For this purpose, we employ poly-L-lysine/polyethylene glycol (PLL-PEG) co-polymers functionalized with the HaloTag ligand (HTL) to capture HaloTag fusion proteins in the plasma membrane. Cell attachment was controlled using PLL-PEG functionalized with RGD. Based on these surfaces, we have established diverse assays for probing protein interactions that include two-dimensional interactions between transmembrane receptors and binding of effector proteins [1, 2] as well as in cell pulldown and in situ pulldown from lysed cells [3]. These assays were used to verify protein interactions and to probe for competition as well as cooperation of interactions. Furthermore, we quantified complex stabilities and stoichiometries in cells and by single cell pulldown experiments. In combination with single molecule imaging and molecular dynamics simulations, these studies were instrumental to develop mechanistic models of cytokine receptor activation and downstream signal propagation as well as its regulation by negative feedback inhibitors.

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Conformation-Specific MHC I Protein Clusters Detected by a Two-Hybrid Antibody Micropattern Assay

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We fabricate geometric antibody micropatterns on glass coverslips with PDMS stamps that are inked with fluorescently labeled antibody solutions. Using anti-tag and conformation-specific antibodies, we demonstrated the differential capture of distinct protein conformations of cell surface proteins. Based on such antibody micropatterns, we then developed a two-hybrid assay to monitor conformation-specific protein-protein interactions of major histocompatibility complex (MHC) class I at the cell surface. With our two-hybrid assay, we test for *in cis* interactions of an HA-tagged bait protein (HA-MHC class I) captured on anti-HA antibody micropatterns with a prey protein fused to the green fluorescent protein (GFP) (MHC class I-GFP) (Dirscherl *et al.*, 2018). The redistribution of the MHC class I-GFP prey protein into the geometric shapes of the antibody pattern elements indicates the formation of MHC class I clusters and is read out by confocal laser scanning microscopy. We discovered that homotypic *in cis* interactions occur exclusively between one specific conformation of MHC class I proteins, namely free heavy chains (Dirscherl *et al.*, 2018). For further investigation of the functional role of these MHC class I free heavy chain clusters, we are currently screening other MHC class I allotypes for homo- and heterotypic protein-protein interactions.

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Analysis of EGFR downstream signaling by live cell micropatterning

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The epidermal growth factor receptor (EGFR) is expressed on the surface of numerous cell types. When in its active state, the EGFR transduces signals to the cell interior that instigate key cellular processes, such as growth, differentiation, proliferation and motility. It has recently been shown that different ligands as well as phosphorylation patterns regulate the preferred association of EGFR signaling molecules as well as further downstream pathway activation [1, 2]. However, the precise properties of the respective downstream molecule interaction with the EGFR remain elusive. By using the micropatterning (MPT) approach [3, 4] in combination with total internal reflection fluorescence (TIRF microscopy), we investigated putative differences in membrane recruitment of various EGFR downstream proteins upon ligand stimulation, as well as the formation of cytosolic downstream protein complexes in HeLa cells. Grb2 was found to be highly pre-coupled to the EGFR in the basal state as indicated by significant corecruitment into EGFR-enriched micropatterns. Additionally, Grb2 exhibited accelerated and more extensive EGFR-induced membrane recruitment than compared to other downstream molecules such as Shc1, SOS1, Gab1 and PLCγ. Whereas EGFR corecruitment remained quite stable for Grb2, Shc and PLCγ over a time period of 30 min, Gab1 and SOS1 signals steadily declined after reaching the maximum (~7 - 10 min) without agonist washout. Based on fluorescence recovery after photobleaching (FRAP) experiments, significant differences in receptor exchange rates could be found, with Grb2 and SOS1 showing the most transient receptor interaction. Interestingly, Gab1 exhibited by far the most stable interaction properties. In addition, we could show the formation of large EGFR-mediated downstream protein complexes in a live-cell context by using artificial transmembrane proteins that facilitate the transfer of the micrometer-scale antibody surface pattern into an ordered array of cytosolic bait proteins in the plasma membrane. By utilizing the MPT technology, this work probes large variances in EGFR downstream protein recruitment behavior, which might contribute to the regulation of different signaling pathways.

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Probing lipid interactions of plasma membrane proteins: a micropatterning approach

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It is well established that lipids and proteins are not just independent components of the plasma membrane of eukaryotic cells but that their arrangement, dynamics and function are interdependent.
Besides direct lipid-protein interaction, transmembrane proteins are thought to bind a shell of annular lipids, which are more or less tightly associated with the proteins. Furthermore, highly ordered nanoscopic membrane domains have been proposed to act to compartmentalize proteins and their interactions, but have thus far not been directly observed.
In this study, we use protein micropatterning combined with single-molecule tracking to directly measure lipid-protein interactions in the plasma membrane of living cells: different fluorescently labelled transmembrane proteins of interest (POIs) were captured and enriched within well-defined areas in the plasma membrane, leaving regions depleted of POI, which function as reference areas. From the distribution and diffusion behaviour of lipids and proteins with respect to the POI patterns, we were able to conclude on the local membrane environment of the POI. We found that a palmitoylated protein based on the transmembrane domain of hemagglutinin (HA-mGFP) influences its membrane environment well beyond the size of the transmembrane helix. The same effect was observed for a palmitoylation-deficient mutant allowing us to rule out formation of a more ordered membrane domain around HA-mGFP as the cause for this apparently increased protein size.

Arrays of high-aspect ratio nanostructures for biological applications

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The endeavour of exploiting arrays of vertical high-aspect-ratio nanostructures (NSs) for biological applications is experiencing a pronounced surge of activity. The interest is primarily rooted in their dimensions (diameter 100- 500 nm and height of several microns), which make them relevant for a very broad range of applications with biological samples due to their size compatibility with proteins and cells. Furthermore, their intrinsic optical properties are also relevant for enhanced detection of biological events.

The combination of theoretical and experimental studies on high-aspect ratio nanostructures provided a detailed understanding of their intrinsic optical properties as well as their interface with proteins and living cells. We will review a series of examples of new biological applications with perspectives in diagnostics and drug discovery.

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Quantum Optical Proteins: Nanofabrication, Strong Coupling and Synthetic Biology

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The organization of proteins into functional nanostructured assemblies presents formidable challenges. It is necessary to control not only the spatial organization of the biomolecules, but also their orientation. Moreover, proteins have fragile structures and to be able to address biological questions it is vital to ensure that the protein activity is maintained during the fabrication process. We have found that nanophotonic techniques are powerful for the organization of proteins on nanometre length scales. Aminosilanes with photoremovable protecting groups that have oligo(ethylene glycol) adducts can be patterned on sub-wavelength scales by exposure using a near-field probe and a simple interferometer. Near-field methods allow the fabrication of arbitrary structures containing multiple protein components, while interferometric methods allow patterning over macroscopic regions in fast, simple processes. Integration of polymer brushes into the fabrication process allows more complex, functional structures to be assembled. Binary brush microstructures may be fabricated using photolithographic techniques, and used to form corrals in which a lipid bilayer containing functional transmembrane proteins rests on a brush "cushion" and is surrounded by polymer walls. Incorporation of a ratiometric pH dye into the walls allows the quantitative investigation of transmembrane proton transport. Metal nanostructures may also be incorporated into biological systems using photolithographic approaches. Using interferometric lithography, arrays of gold nanostructures may be formed. By using a double exposure process and rotating the sample through a variable angle between exposures it is possible to assemble combinatorial libraries of structures with different periods and feature sizes. When light-harvesting proteins from bacteria and plants are attached to these arrays, their plasmon bands are split. This splitting is attributed to strong coupling of the plasmon mode to excitons in the light harvesting proteins, leading to the creation of hybrid light-matter states called plexcitons. This strong coupling is a quantum optical phenomenon: the plasmon couples collectively to an array of excitons, so that changes to the arrangement of pigment molecules alters the coupling energy. Using synthetic maquette proteins it is possible to manipulate the strong coupling to yield new optical states and transitions. We demonstrate ultra-fast exchange of energy between synthetic chlorins via the plasmon mode in strongly coupled systems, to yield dimer states with large transition dipole moments not seen in absorption spectra of the proteins.

Force Spectroscopy and Recognition Imaging: Quantifying Binding Strength and Affinity on the Single-Molecule Level

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Single-molecule and single-cell force spectroscopy are appropriate tools for retrieving accurate dynamic and statistical information about the nanomechanical behavior of molecular bonds involved in adhesion to biotic and abiotic surfaces. In biochemistry, the determination of the equilibrium dissociation constant is key for quantifying the interaction between biological molecules. Despite the wide range of approaches in increasing the measurement sensitivity for minute sample amounts, critical limitations with respect to labelling, fluorescence tags, and low detection signals combined with high noise are difficult to overcome. This intimately leads to requirements of new measurement tools that combine high sensitivity with nano-scale spatial resolution. In recent years, the topography and recognition (TREC) imaging technique, based on force spectroscopy in resonance, has been utilized for mapping bio-molecular recognition events to localize bio-molecules at the nano-scale. In the present work, we fabricated DNA arrays on glass or silicon substrates as platforms capable for sensing single molecular interactions. We employed TREC to characterize the DNA array and quantified the equilibrium dissociation constant K_d of DNA duplexes from recognition images, yielding $K_d = 2.4 \times 10^{-10}$ M. Using TREC we developed an affinity sensing assay, which can be directly assessed without any labelling or secondary binding for detection.

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Lipids at micro- and nanostructured interfaces – from phase segregation to biosensing

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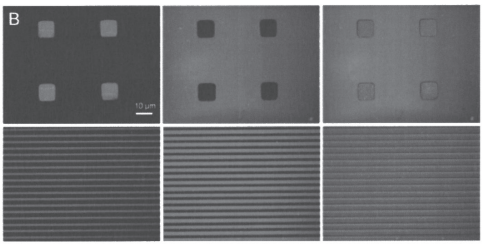
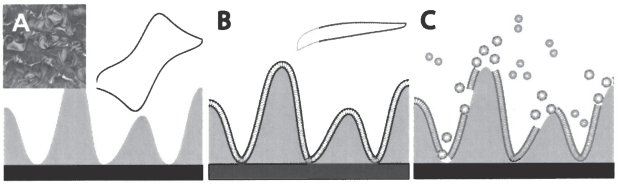
Biomembranes are arguably the most important structural elements in nature. The plasma membrane not only delimits the cell body and organelles, but serves as a barrier through which ions, small molecules, biopolymers and proteins are selectively transported. As such, biomembranes are the focus of intense fundamental and translational research. In this presentation, I will describe our efforts at understanding the way lipids behave at the interface of micro- and nanostructured materials. Early work in the Moran-Mirabal research group on patterning model membranes to understand the impact of confinement and boundary conditions on phase segregation will be discussed. I will also describe a benchtop structuring approach, based on the thermal shrinking of shape-memory polymers, that our group has developed to obtain tuneable topographies from a wide range of thin films. The application of such surfaces for the study of immune cell membrane receptor mobility, and for the development of a "membrane-on-a-chip" platform will also be presented. Furthermore, the integration of this platform with electrochemical sensing techniques will be described in the framework of the detection of membrane disrupting agents. The talk will conclude with a brief overview of ongoing work focused on the detection of pathogen-derived hemolytic factors.





Abstracts of Posters

(in alphabetical order)

Fabrication of Macroscopic Arrays of Metal Nanostructures for Studies of Strong Plasmon-Exciton Coupling

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In strong plasmon-exciton coupling, localised surface plasmon resonances (LSPRs) associated with the surface of gold nanoparticles are coupled to excitonic states in nearby molecules to produce new hybrid states that combine the properties of light and matter. Recently work in Sheffield has provided the first demonstration of strong coupling to excitonic states in biological molecules. It has been shown that the coupling can be modelled as coupled harmonic oscillators. Conventional plasmonic sensors exhibit small red shifts after binding of biological molecules. However, in strong coupling a pronounced splitting of the plasmon band is observed, that has a precise quantitative relationship to the amount of bound analyte. This makes strongly coupled systems highly attractive for biological sensing and medical diagnostics. We have investigated the suitability of methods based on interferometric lithography (IL) for the fabrication of macroscopically extended arrays of gold nanostructures for studies of strong plasmon-exciton coupling. Conventionally, electron-beam lithography is used but this method is expensive and typically enables fabrication of structures only in microscopic areas. By using IL, our goal is to fabricate arrays over areas large enough to enable analysis by techniques such as ellipsometry, which allow determination of optical constants for the system. A systematic study has been carried out of the influence of the fabrication conditions on array morphology. We use a double-exposure method, in which the sample is rotated through a variable angle between exposures. The method is fast and requires only simple apparatus. We demonstrate that the pitch and geometry of the array (eg square vs hexagonal) and the nanoparticle dimensions (full width at half maximum height) can be controlled over large areas, enabling optimisation of the localized surface plasmon resonance energy and refractive index sensitivity. Studies of plasmon-exciton coupling have been carried out using light-harvesting complexes from purple bacteria.

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Plasmonic enhanced group-IV nanopillar photodetectors for refractive index sensing and potential molecular fluorescence detection

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Structuring metals and semiconductors down to nanometer dimensions enables tuning of plasmonic and photonic features. In vertical Ge PIN nanopillar photodiodes (NP-PD, fig. 1) this structuring allows to tailor the spectral absorption behavior based on the photonic modes which are inherent in such structures[1]. Plasmonic modes with their high sensitivity to refractive index changes are already used for biosensors[2]. Typically, plasmonic resonances and active device structures are spatially separated, only sensing plasmonic effects on the far-field. In NP-PD the coupling between plasmonic and photonic modes can be improved and the use of near-field effects can be facilitated.

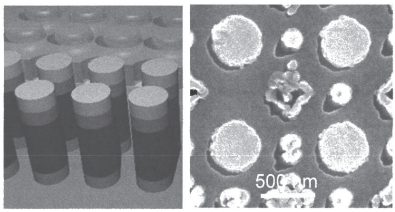

Fig. 1 Left: 3D sketch of the nanopillar device with partly removed oxide and metallization. Right: Top SEM view of the NP-PD.

A possible application of these type of devices could be single molecule refractive index sensing or molecule fluorescence detection. Highly confined, localized plasmonic resonances (fig. 2) allow to excite the investigated molecules[3]. The tailored photonic modes offer a spectral cut-out function that can be used to detect wavelength of interest e.g. fluorescence bands. This can help to replace spectrometers and hereby enhancing integrated sensing platforms.

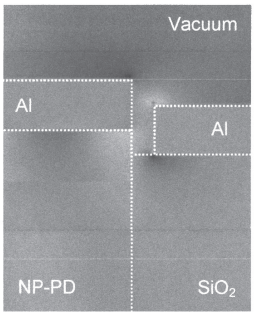

Fig. 2: Simulation of the electric field E_z in the device cross-cut showing the localized plasmonic modes near the aperture.

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Nanoscale Organization and Mobility of Ligands Direct T-Cell Activation

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The interaction of T-cells with Antigen Presenting Cells (APC) plays a central role in the adaptive immune system. However, the molecular mechanisms by which binding of the T-Cell Receptor (TCR) to its ligand triggers a signal within the cell is still debated. To investigate how pre-clustering and rearrangement of TCR ligands on the APC affect the activation of T-cells we designed a nanoscale platform for T cell activation based on DNA origami. Within the DNA origami TCR ligands can be arranged with nanometer precision. To mimic an APC, the DNA origami were anchored to a glass supported lipid bilayer featuring adhesion (ICAM-1) and costimulatory (B7-1) molecules.

A fluorescently labeled recombinant TCRβ-reactive single chain antibody fragment (scFv) served as T-cell stimulating ligand. DNA platforms were characterized using single molecule fluorescence microscopy and AFM. The effect of the nanoscale arrangement of ligands on early T-cell activation was determined by Ca^{2+} imaging. By varying the lipid bilayer composition, we altered the ability of T-cells to reorganize the platforms on the membrane, allowing us to assess the effect this has on activation.

Cell surface dynamics of spondyloarthropathy related HLA Class I molecules

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Human Leukocyte Antigens (HLA) bind and present peptides derived from microbial or tumor antigens to cytotoxic T cells. In some cases, native peptides that are not of pathogenic origin are recognized by the immune system, leading to autoimmune diseases. HLA-B*27:05 (B27) is a subtype of HLA Class I molecules that confers strong genetic association with autoimmune spondyloarthopathies. Under certain cellular conditions (such as when antigen processing and loading machinery is perturbed), peptide supply to the B27 molecule is altered and the molecule starts to form non-natural homodimers/oligomers at the cell surface. We made use of antibody-micropatterned surfaces to study the molecular interactions between B27 monomers, under various cellular landscapes.

The role of the cortical actin in the regulation of Eph receptor signaling

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Actin cytoskeleton filaments form a mesh lying in close proximity to membrane (cortical actin) that is able to compartmentalize receptors and other membrane proteins. It has been previously reported that the spatial organization of Eph receptors and their ligands (ephrin) at the cell-cell contact interface modulate signaling cascades in breast cancer cell lines, impacting tumor aggressiveness. In particular, it has been suggested that the formation of clusters of Eph receptors on the membrane correlates with an invasive phenotype.

Following this evidence, we hypothesize that cortical actin regulates Eph receptor signaling by tuning the organization of receptor at the membrane.

By employing ephrin decorated DNA nanostructures rendered from polyhedral flat sheets, we are studying the role of the cortical actin in the stimulation of Eph-A2 receptor with their ligands. The shape and size of the nanostructure will impact on the lateral association of the ligands and hence the receptors. DNA flat sheets are functionalized with monomeric ephrin-A5 conjugates following two designs that allow or not the dimerization of ligand bound receptor, depending on the proximity between ligands on the nanostructures. Proximity Ligation Assay is used to monitor the phosphorylation levels of the receptor upon modulation of the size of the cortical actin mesh with drugs (Latrunculin A, Jasplakinolide). High-resolution microscopies (AFM and STORM) are used to monitor the mesh size and the clustering of the receptors. All together this approach will point out molecular mechanisms of spatial organization of ligands and receptors during clustering and the effects of cytoskeleton on membrane receptor-mediated signaling.

Quantum-Optical Polymers

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The growing world energy crisis and concern about the consequences of climate change have spurred efforts to develop alternative energy sources. Organic semiconductors are attractive for the production of photovoltaic (PV) devices because they are formed from earth-abundant elements in processes that consume comparatively small amounts of energy. However, organic PV devices show much lower power conversion efficiencies than equivalent silicon-based devices, in large measure because their small exciton diffusion lengths currently necessitate the use of thermodynamically unstable multiple heterojunction architectures. [1] In biological light-harvesting systems this process has been perfected through millions of years of evolution. Taking inspiration from nature and the recent discovery that biological photosynthetic protein membranes are strongly coupled to plasmon modes when bound to nanostructured gold, we have developed a new approach to the design of materials for solar energy capture that aims to exploit strong plasmon-exciton coupling for light-harvesting and long-range transport of energy. [2] Strong plasmon-exciton coupling is a quantum optical phenomenon in which a plasmon mode is mixed with an exciton to yield new hybrid light-matter states. The strength of coupling depends upon the density of pigment molecules within the plasmon mode and the transition dipole moments of the excitons. In strong coupling to light-harvesting proteins, the protein structure functions as a scaffold that organizes the excitons in space. We hypothesized that a synthetic polymer could perform the same function. Poly(cysteine methacrylate) (polyCysMA) brushes were grown via surface-initiated atom transfer radical polymerization (SI ATRP) from plasmonically active gold nanoparticle surfaces and were derivatized with modified chlorophyll a molecules. A pronounced splitting of the plasmon band was observed, consistent with strong coupling of the plasmon mode to the chlorophyll excitons. The splitting energy increased with the density of chlorophyll a attachment, reaching energies of ca. 0.35 eV. Strong new resonances were additionally observed at low energies that are thought to result from enhanced evanescent coupling between nanostructures that results from strong plasmon-exciton coupling. These results provide a positive outlook for future work on the development of biologically-inspired materials for solar energy capture.

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Probing the membrane environment of palmitoylated transmembrane proteins: A micropatterning approach

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Protein palmitoylation refers to the post-translational covalent lipid modification of a cysteine with palmitic acid. While palmitoylation provides a means to anchor peripheral membrane proteins to the plasma membrane, its role is far less clear in the case of integral membrane proteins. Palmitoylation increases the hydrophobicity of such proteins and has been implicated in controlling the conformation of transmembrane segments and protein-protein interactions. Further, palmitoylated proteins (PPs) are generally found in detergent-resistant membrane fractions and often partition into liquid-ordered domains in phase-separated giant unilamellar vesicles, thus an association to "membrane rafts" was proposed.

Here we investigate whether PPs can influence their membrane environment to co-enrich or nucleate ordered membrane domains. As a model PP we used the transmembrane domain of hemagglutinin fused to mGFP (HA-mGFP).

In order to observe the effect of palmitoylation on the protein's membrane environment, they were immobilized on a micropatterned surface; subsequently single molecule trajectories of different fluorescent tracer lipids and proteins were recorded and their distribution and mobility within and outside of PP patterns was compared.

The effect of palmitoylation on the membrane environment was monitored by comparing the results with palmitoylation-deficient mutants. We find no pronounced effect of protein palmitoylation on the interaction between the immobilized proteins and the tracer molecules indicating that palmitoylation does not significantly influence the membrane environment of transmembrane proteins.

Spatial regulation in the immunological synapse

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The programmed cell death-1 (PD-1) receptor is expressed on effector T cells to negatively regulate T cell effector functions. Although how PD-1 regulates T cell signaling has been studied, our understanding of the molecular mechanisms of PD-1 suppression in the immunological synapse remains incomplete. The formation of the immunological synapse between a T cell and an antigen-presenting cell is spatially regulated. It is characterized by the initial formation of T cell receptor (TCR) - peptide-major histocompatibility complexes (pMHC) nanoclusters which then migrate and accumulate to the core of the synapse. Activated PD-1 colocalizes with TCR nanoclusters and induces dephosphorylation of TCR signaling proteins. However, the mechanisms of regulation of TCR activation by the spatial distribution of PD-1 and TCR at the nanoscale level are unclear. We are investigating the role of nanoscale spatial distribution of TCR and PD-1 by using DNA origami nanostructures that display PD-L1 proteins and anti-CD3ε at defined distances to constrain the spatial distributions of their respective receptors into defined configurations. These nanostructures not only offer precise control in positioning ligands in nanoscale distances but also the ability to induce ligand-receptor clustering in cells. We have used these DNA nanostructures to study PD-1-inhibition of T cell signalling in PD-1-expressing Jurkat T cells with a NFAT-luciferase reporter. The use of customized ligand-DNA origami methods to provide defined physical signals to cells has immense potential to contribute to our understanding of the molecular mechanisms of PD-1 regulation on TCR signalling.

Using micropatterning to develop a novel in vitro muscle cell model to study development of the neuromuscular junction

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The neuromuscular junction (NMJ) - the synapse between a motor neuron and a muscle fiber - is formed by the exchange of signals between nerve and muscle. NMJ formation is characterized by the dense accumulation of acetylcholine receptors (AChRs) at the synaptic site. The formation and integrity of AChR clusters are maintained by neuron-derived Agrin, its receptor Lrp4 and the receptor tyrosine kinase MuSK (= muscle specific kinase), both localized at the postsynaptic muscle membrane. Agrin is deposited into the basal lamina and binds to Lrp4, which induces MuSK dimerization and a subsequent signaling cascade, leading to clustering of AChRs at the synaptic site.

Current *in vitro* methods stimulate myotubes with soluble variants of Agrin, effectively triggering random AChR cluster at the cell surface. Agrin stably anchored to the cell substrate in a distinct pattern would lead to spatially defined AChR clustering, resembling the *in vivo* NMJ. We intend to create a model system where Agrin is micro-patterned onto a surface substrate for *in vitro* differentiated myotubes, mimicking the locally limited distribution of Agrin in the synaptic cleft of the NMJ. In combination with muscle cell lines expressing GFP-tagged MuSK this will allow us to image and study focal delivery of MuSK to the NMJ by a combination of FRAP and TIRF microscopy. This would allow studying how MuSK facilitates localized AChR clustering at the NMJ.

Understanding MuSK transport dynamics and its impact on NMJ formation may also produce new insights into degenerative diseases of the NMJ such as Myasthenia gravis.

The activation mechanism of the insulin receptor reconstituted in lipid nanodiscs

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Insulin is a critical hormone in the regulation of metabolism and growth. It acts on cells by binding to the ectodomain of the insulin receptor, a dimeric membrane-spanning receptor tyrosine kinase, promoting autophosphorylation of its cytoplasmic kinase domains and initiating downstream signaling. The overall architecture of the intact membrane-embedded insulin receptor and its mode of activation, i.e., how the extracellular signal of insulin binding is transmitted across the membrane, is incompletely understood [1,2]. Here, glycosylated full-length human insulin receptor was produced in human embryonic kidney cells, affinity-purified, and reconstituted in membrane-mimetic nanodiscs of a ternary lipid composition. We show by negative stain electron microscopy and single-particle analysis that insulin binding to the dimeric receptor converts its ectodomain from an inverted U-shaped conformation to a T-shaped conformation [3]. This structural rearrangement of the ectodomain propagates to the transmembrane domains, which are well separated in the inactive conformation but come close together upon insulin binding, facilitating autophosphorylation of the cytoplasmic kinase domains. Even though the insulin receptor is unusual among receptor tyrosine kinases in that it exists as a covalent dimer at the cell surface, mounting evidence suggests that other receptor tyrosine kinases also form dimers prior to ligand binding. Therefore, the activation mechanism described here might also be applicable to other receptor tyrosine kinases.

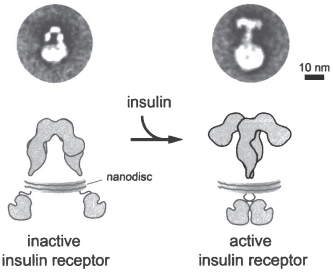

Fig. 1: Proposed mechanism underlying insulin receptor activation. Adapted from [3].

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Culturing and patch clamping cells on nanowire arrays

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Nanowire substrates play an increasingly important role for cell cultures as an approach for hybrid bio-semiconductor junctions. We investigate Jurkat T cells and neurons from mice cultured on Al_2O_3 coated ordered and randomly distributed nanowires. Cell viability was examined by life/membrane staining reporting comparable viability on planar and nanowire substrates. Imaging the hybrid interface reveals a wrapping of the cell membrane around the very nanowire tip. Patch clamp recordings show similar electrophysiological responses on each type of nanowires compared to planar control substrates. We demonstrate that the morphological characteristic of the nanowire substrate plays a subordinate role which opens up the arena for a large range of nanowire substrates in a functionalized application such as stimulation or sensing.

Micropatterned assays of peptide-receptive MHC class I proteins

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Micropatterned surfaces are promising tools for screening applications. For the screening of cytotoxic T cells, arrays of major histocompatibility complex (MHC) class I molecules have been suggested. Current approaches generally describe the spotting of individual MHC class I molecules complexed with antigenic peptide (pMHC I) on surfaces. Instead of printing the pMHC I complex, we demonstrate the fabrication of peptide-free MHC class I arrays which can be loaded individually with test peptides. In our approach, we use microcontact printing to immobilize empty MHC class I molecules in geometric structures on glass surfaces. We demonstrate that the printed MHC class I molecules are functional and can be specifically loaded with antigenic peptides. The ulterior goal is to develop a ready-to-use microfluidic MHC class I array which can be loaded with candidate peptide and serve as a point-of-care T cell screen of tumor patients.

A micropatterned platform to analyze the effects of ephrin ligands on the compartmentalization of organoid-derived intestinal cells

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The small intestine is lined by a monolayer of polarized epithelial cells organized in protrusions called villi and invaginations named crypts.[1] The intestinal epithelium is completely replaced every 3-6 days, making this tissue the most actively self-renewing tissue in the body. The epithelial intestine regeneration is driven by a pool of stem cells localized at the crypt base. Stem cell division gives rise to transit amplifying cells that proliferate and differentiate into absorptive and secretory cells while migrate up to the villi tip where undergo apoptosis and exfoliate.[1] *In vivo* experiments have shown that gradients of ephrin ligand and Eph receptors are key in maintaining such cellular compartmentalization in the intestinal crypt *in vivo*.[2,3] However, direct experimental manipulation of these gradients and the concentration range that promote these functions has been hampered by limitations of the *in vitro* systems available.

In the current work, we report the development of an *in vitro* setup to investigate the effects of ephrin ligands on intestinal stem cells positioning and differentiation. The experimental setup is a 2-dimensional culture platform that employs micropatterns of ephrin ligands on substrates coated by thin layers of Matrigel, a cell-derived protein mixture. On these substrates, we culture single cells derived from small intestinal organoids. Ephrin patterns are created by microcontact printing on freeze-dried Matrigel layers.[4] Micropatterned substrates support the growth of organoid-derived cells, which create an epithelial monolayer within days. The monolayer on the substrate exhibits compartmentalization of proliferative and differentiated cells until day 5 (theirself-renewing rate *in vivo*). Our hypothesis is that our platform will contribute to understand more accurately the compartmentalization effect of specific ephrin concentrations on the primary intestinal epithelia and stem cell behavior.

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Antibody micropatterning on surfaces for single molecule investigation of protein-protein interactions in membranes

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Even though protein-protein interactions are a major research topic for many decades now, a lot is still unknown. Mainly membrane proteins with their hydrophobic character as well as short lived, weak interactions are difficult to investigate.
Aim of this work is to establish a method to observe protein-protein interactions in membranes at the single molecule level by combining micropatterning with fluorescence microscopy. The method is based on the work of Michaela Schwarzenbacher et al. (2008) [1] and Eva Sevcsik et al. (2015) [2], who used it to study Lck-CD4 interaction and the organization of GPI anchored proteins in the plasma membrane, respectively.
Antibody micropatterning of the substrate enables the arrangement of a transmembrane protein in an adherent cell in a defined pattern. A second transmembrane protein is freely diffusing in the membrane. The proteins contain fluorescent domains to be detectable via Total Internal Reflection Fluorescence (TIRF) microscopy. By observing single molecules, the degree of co-localization provides information about interaction strength and duration.
We demonstrated that fluorescent proteins and antibodies can be organized in micropatterns on glass coverslips. Adherent CHO (Chinese Hamster Ovary) cells were seeded on coverslips and transfected with membrane proteins. Membrane proteins bound to the pattern on the substrate could be shown to interact with the freely defusing protein via synthetic leucine zipper domains. The specificity of the micropattern needs to be further improved. Single molecules have not yet been observed because of too high expression levels of the fluorescent constructs.

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Coupling of light-harvesting chlorophyll–protein complex II to nanoarrays of gold fabricated by interferometric lithography

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Interferometric lithography of self-assembled monolayers on gold enables the fabrication of arrays of gold nanostructures over square cm areas. The dimensions and morphology of nanostructures obtained were characterized by AFM. After annealing, the nanostructures are highly crystalline and yield strong plasmon absorptions. UV-vis absorption spectra of fabricated gold nanostructures showed a characteristic peak at ca. 570 – 670 nm associated with localized surface plasmons. Both the position and intensity of the surface plasmon band depend on the spacing and dimensions of the nanostructures.

Gold nanostructure arrays exhibit surface plasmon resonances that split after attaching light-harvesting chlorophyll–protein complex LHCII from chloroplasts. That phenomenon results from coupling between surface electronic excitations and excitons in the chlorophyll pigments. An enhancement of the fluorescence spectra for LHCII strongly coupled to gold nanostructures was observed. To determine the energy of the excitonic state to which the plasmon couples and the coupling energy, a model based on two coupled harmonic oscillators was used. Fitting of spectra revealed that the LSPR is coupled to an exciton with energy of 2.24 ± 0.04 eV with a coupling energy of 0.24 ± 0.01 eV.

At metal particle separations less than 25 nm, evanescent coupling occurs between the plasmon modes of neighbouring metal particles. Preliminary data obtained by us support this hypothesis. Using randomly polarised light, we observe resonances in extinction spectra of arrays of gold nanoparticles coupled to LHCs that have negligible intensity for clean gold arrays. We see strong resonances at the same energy when lines of clean, closely packed nanostructures are formed. We hypothesise that these are due to evanescent coupling between nanostructures; the observation of these coupled resonances with randomly polarised light when LHCs are present is attributed to strong plasmon-exciton coupling, which causes increased spatial coherence. Our data suggest that the energies of these coupled resonances are controlled by the energies and arrangement of excitons within the optical field and by the properties of the nanostructure arrays.

Synthetic STAT signaling modules for studying Jak/STAT activation and regulation

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Cytokine receptor signaling via the Jak/STAT plays an important role in our innate and adaptive immune system. The cytokine receptor family comprises a plenty of different transmembrane proteins which associate with one of four different Janus kinases and activate different homo- or heterodimeric complexes of six members of STAT proteins. The transcription factor STAT2 is specifically involved in signal transduction of the type I and III IFN system. It is recruited via a constitutive binding site but also in response to signal activation in a phosphorylation dependent manner.[1]

We could demonstrate that STAT2 recruits the negative feedback regulator USP18 to the receptor subunit IFNAR2 which leads to destabilization of signaling complexes.[2] It was suggested that USP18 interferes with kinase-receptor association or interaction between the kinases which further stabilize signaling complexes.[3,4] However, the exact mechanism by which USP18 regulates signaling remains still elusive. Furthermore, the exact mechanism of STAT activation and the role of the constitutive binding site are not solved. By implementing live cell micropatterning techniques, we have mapped the constitutive binding site and analyzed signal activation. To this end, we created receptor truncations and re-engineered IFNAR2 constructs containing STAT2 binding fragments. To further elucidate the importance of the constitutive binding site for signal activation and regulation we implemented a synthetic binding module on IFNAR2. The STAT2 binding site was replaced by a GFP-nanobody which allows affinity controlled recruitment of STAT proteins revealing that even strong binding allowed activation of STAT proteins.

To validate the determinants of IFNAR2 responsible for STAT activation we transferred the STAT2 binding site to another receptor system creating a chimeric IL4 receptor variant. Monitoring STAT activation demonstrated that a type I IFN specific response and negative regulation by USP18 could be transferred to a different receptor system.

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A Modular Approach to Sensitized Two-Photon Micropatterning of Photolabile Hydrogels

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Multiphoton lithography is a powerful approach that permits the processing of light-sensitive materials by the use of fs-pulsed NIR-laser light. Owing to its unique features, including free-form 3D-patterning and the non-invasiveness to areas outside the focal volume, this method is particularly suited for micropatterning of hydrogels in presence of cells. Besides two-photon (2P) polymerization where scaffolds-like constructs are built in a bottom-up approach from a macromer solution, 2P-degradation of pre-formed photoactive hydrogels allows for localized micro-channel formation or in-situ modification of cell surroundings.[1] Nevertheless, due to the high resolution this techniques offers and the resulting small feature sizes of created constructs, maximizing the achievable writing speed, with the aim to push the feasible spatial dimensions in the cubic cm regime, is a current key challenge.[2]

In this contribution, an approach for the improvement of the 2P-induced degradation of o-nitrobenzyl ester (oNB) containing hydrogels is presented. oNB derivatives are photocleavable groups commonly included in photosensitive materials for tissue engineering applications and mechanobiology studies.[1] However, as the 2P-absorption cross-sections δ_a of these functionalities are usually rather low, relatively high laser intensities and long irradiation times are required for their cleavage that can harm living cells encapsulated in the material. To increase the reactivity towards pulsed laser light, we developed a modular system permitting the sensitization of the 2P induced oNB photocleavage. By adding the water-soluble small molecule 2P sensitizer P2CK,[3] we demonstrate that the efficiency of the oNB photo-scission can be effectively promoted in a concentration dependent manner and demonstrate the efficacy of this method in the presence of encapsulated stem cell spheroids (ASC/TERT1).

The presented approach provides a useful tool for two-photon micropatterning of photodegradable hydrogels at moderate laser powers in presence of cells.

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Direct characterization of the evanescent field in objective-type total internal reflection fluorescence microscopy

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Total internal reflection fluorescence (TIRF) microscopy is a commonly used method for studying fluorescently labeled molecules in close proximity to a surface. Usually, the TIRF axial excitation profile is assumed to be single-exponential with a characteristic penetration depth, governed by the incident angle of the excitation laser beam towards the optical axis. However, in practice, the excitation profile does not only comprise the theoretically predicted single-exponential evanescent field, but also an additional non-evanescent contribution, supposedly caused by scattering within the optical path or optical aberrations. We developed a calibration slide to directly characterize the TIRF excitation field. Our slide features ten height steps ranging from 25 to 550 nanometers, fabricated from a polymer with a refractive index matching that of water. Fluorophores in aqueous solution above the polymer step layers sample the excitation profile at different heights. The obtained excitation profiles confirm the theoretically predicted exponential decay over increasing step heights as well as the presence of a non-evanescent contribution.

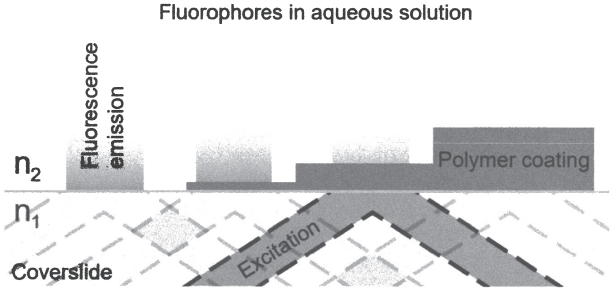

Figure 1: Calibration slide concept. The TIRF excitation profile is axially sampled by imaging the fluorescent emission of freely diffusing fluorophores in aqueous solution above water refractive index matched polymer step layers of different height on a coverslide.

Functionalized Polyelectrolyte Microcapsules for T-cell Activation

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For the timely diagnosis and personalized treatment of diseases, rapid and sensitive detection of biomarkers is crucial[1]. Polyelectrolyte microcapsules offer great variability in physiochemical properties and surface modifications making them a promising biomedical tool for the rapid quantitative detection of a broad range of analytes at very low concentrations[2]. Here, we designed polyelectrolyte microcapsules fabricated on various templates such as silica beads or $CaCO_3$ cores using the Layer-by-Layer (LbL) technique. The microcapsules are then functionalized with streptavidin to allow for the attachment of diverse biotinylated detectors such as oligonucleotides or proteins. As proof of principle, we used biotinylated oligonucleotides to detect complementary analyte oligonucleotides. Upon addition of a fluorescently labeled detector oligonucleotide, binding of the analyte oligonucleotide to the polyelectrolyte microcapsule is read out by flow cytometry.

The functionalization of capsules with biotinylated major histocompatibility complex (MHC) class I proteins is currently being tested as alternative method for T cell staining, activation, and/or isolation. The sensitivity and affinity of T-cell binding by MHC class I coated microcapsules is compared with existing T-cell staining methods such as MHC class I tetramers. In the future, MHC class I functionalized capsules may be used to monitor the immune responses.

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Detection of membrane disrupting agents via electrochemical biosensors

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There is increasing concern about the danger that water borne pathogens and pollutants pose to the public.[1] Of particular concern are those that disrupt the plasma membrane, since this structure is central to the integrity of the cell and any injury to it can lead to cell death. Currently, most diagnostics for membrane disrupting (lytic) agents are done offsite, leading to longer response times and higher cost. Furthermore, existing portable solutions are colorimetric, which sacrifice accuracy and sensitivity. Thus, inexpensive, sensitive, and portable solutions are needed for the detection of lytic agents for applications in precision medicine, agriculture, and health.[2]

In this work, a lipid-based electrochemical biosensor is introduced to detect membrane disrupting agents (*e.g.,* polymyxin-b sulfate (PmB), and intermedilysin (ILY)) in aqueous solutions. A benchtop micro structuring approach for electrode miniaturization and electrochemical signal enhancement (up to 6-fold) was used in combination with a model membrane composed of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC). Cyclic voltammetry (CV) was used to sense membrane damage via electrochemistry. A DMPC membrane stack was placed on the micro structured electrodes, which acts as a passivation layer that prevents electron transfer between the electrode and the working solution. Once lytic agents in solution degrade the membrane, the underlying gold surface can interact with a redox reporter molecule, generating an electrical signal proportional to the concentration of lytic agent. The electrical signal appears as a prominent peak with respect to the flat passive signal on a current vs voltage plot. To quantify, we integrated the cathodic peak in the voltammogram and subtracted out the non-Faradaic current background. We exposed the DMPC biosensors to increasing concentrations of lytic agents and found detection limits for SDS and PmB of 10 and 1 ppb, respectively.

Ongoing work in our lab focuses on employing this technique with membranes of increasing complexity, with the aim of eventually using real human plasma membranes for the detection of pathogens and harmful pesticides.

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Engineering 3D Intestinal Mucosa Models using Hydrogel Co-polymers

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Microengineered tissues represent a new paradigm in the field of cell-based *in vitro* assays. They combine microfabrication and tissue engineering components to provide *in vitro* models with tissue-like characteristics. Tissue-like structures have been mimicked using hydrogels due to their biocompatibility and properties similar to the extracellular matrix. There are two main types of hydrogels (i) natural hydrogels which are biodegradable and present cell adhesion motifs and (ii) synthetic hydrogels which are non-biodegradable and can withstand long-term cell cultures but do not have bioadhesion sequences[1]. Both types present complementary benefits. We fabricated a hydrogel composed of gelatin methacrylate (GelMA) co-polymerized with poly(ethylene glycol) diacrylate (PEGDA)[2]. We employed a single-step, moldless, UV- photolithography technique to fabricate scaffolds mimicking the 3D architecture of the small intestinal mucosa[3]. Using this approach, we obtained villi-like microstructures in the hydrogel co-polymers, with roundness and dimension found *in vivo*. Mechanical and physicochemical properties of the hydrogels co-polymers have been characterized. Adding PEGDA to GelMA hydrogel allows to have a hydrogel with lower degradation and higher Young's modulus. Furthermore, following the same fabrication method we are able to embed stromal cells, to mimic the lamina propria compartment, and culture epithelial cells on the surface, to mimic the epithelial layer, up to 21 days[4]. Our preliminary results showed that the co-culture of Caco-2 cells with 3T3-NIH fibroblasts favours the epithelial cell growth and improves their barrier function. Taking all together, we have generated an intestinal mucosa model that allows for the co-culture of different intestinal cells distributed in compartments, mimicking the spatial-physiological features of the intestinal mucosa. We believe that our model better recapitulates cell-cell crosstalk and cell-matrix interactions found *in vivo*, being an improved alternative for cell-base *in vitro* assays.

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Controlling cellular function by structured surfaces: "Artificial angiogenesis"

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Angiogenesis, the growth and formation of novel blood vessels from preexisting vessels, is an important physiological and pathophysiological process involved in wound healing but also in cancer progression [1]. However, dynamics of angiogenesis in general and the impact of physical factors on those dynamics, are barely understood. Additionally, cellular communication during angiogenesis plays an important part in tissue morphogenesis and function. In this respect, endothelial cells are of particular importance, since they play a crucial role as a transport network, and can regulate organ morphogenesis, maintenance and regeneration [2]. Our first aim is to model different cellular processes, which play an important role in angiogenesis, of primary endothelial cells using micro-structured coated surfaces [3] and hydrogel substrates. By means of either plasma-initiated protein patterning [4] or light activated molecular patterning [5], we bring HUVECs, HeLa and T24 cell lines into defined cellular shapes and stress conditions, in order to model tip-stalk cell formation and collective/single cell migration [6]. In parallel, we use molecular biosensors and immunostaining to track and record the expression level of specific gene targets and proteins related to tip-stalk cell morphology, cell proliferation and migration. Furthermore, as our second aim, we use different synthetic hydrogel substrates coated with Matrigel or Matrigel components to study endothelial tube formation and mechanical cell communication using a number of different techniques including Traction force and Atomic force microscopy [7].

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