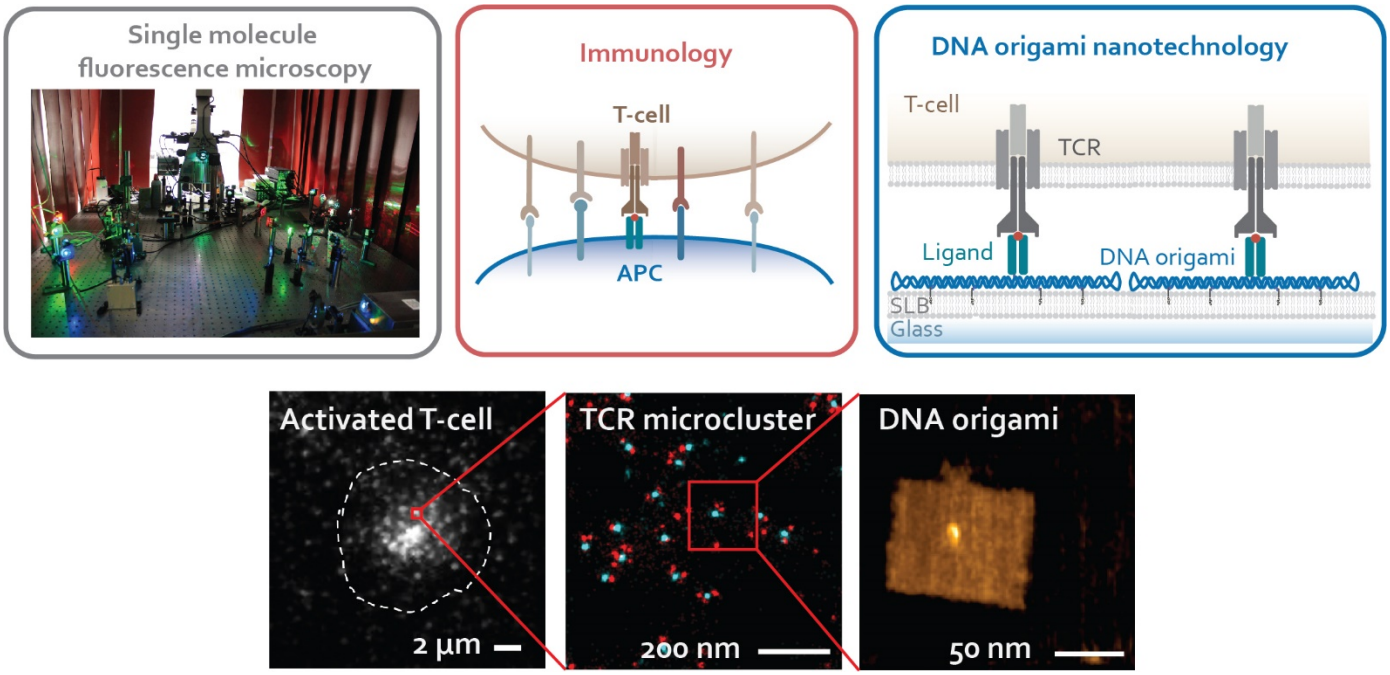
STIMULI-RESPONSIVE NANOSTRUCTURED BIOINTERFACES FOR T-CELL ACTIVATION



Supervisory Team[1]

Primary Supervisor: *Eva Sevcsik, Institute of Applied Physics, TU Wien*

TU Wien project partners: *Stefan Baudis, Marko Mihovilovic, Institute of Applied Synthetic Chemistry*

External academic partners: *Johannes Huppa, Medical University of Vienna; Ralf Jungmann, MPI Martinsried*

Project Description

Recreating biological phenomena in model systems with defined components and properties has proven to be a powerful means for dissecting molecular mechanisms. Recent advances in fluorescence microscopy and DNA nanotechnology have made it possible to **reconstitute, observe and physically manipulate cellular processes down to the single molecule level.** We have recently developed a cell-responsive biomimetic interface based on laterally mobile **ligand-functionalized DNA origami platforms** that allows to manipulate the organization of signaling molecules in a live cell environment (https://doi.org/10.1073/pnas.2016857118).

[1] The Early Stage Researchers (ESRs) will be accompanied during their thesis by an individual "Thesis Advisory Committee" (TAC), which will guide the ESR through the graduate studies. The TAC will consist of the thesis primary supervisor, and two additional members of the Supervisory Team selected by the ESR.

Using this system to mimic an antigen-presenting cell (APC), we were able to determine the spatial requirements for ligand and receptor organization that control **T-cell activation**.

In this PhD project, we will use and refine this biointerface to **probe the molecular mechanisms of early T-cell signaling**. Generating **3D DNA origami structures** will allow to manipulate the axial position of ligands; **heterobifunctional DNA origami structures** will be employed to decipher the effect of co-receptors and antagonists. Finally, we propose to endow DNA origami structures with **stimuli-responsive elements** to make them "smart", by introducing photo-switchable or thermo-responsive linkers so that their interactions with T-cells can be modified in a user-controllable manner.

Key Goals and Tasks

Design, production and characterization of DNA origami structures. The DNA origami technique is based on the programmable folding of a long DNA scaffold into a defined secondary structure by addition of short oligonucleotides. This requires careful software-aided design, purification of the self-assembled structure, functionalization and, finally, characterization via e.g. gel electrophoresis, single-molecule fluorescence microscopy and AFM imaging.

Monitoring the T-cell response. Primary mouse T-cells will be interfaced with the generated biointerfaces and their response will be assessed using different experimental approaches. For determining the T-cell activation state, we will use ratiometric calcium imaging, total internal reflection fluorescence microscopy, and immunostaining. These experiments will be complemented by DNA-PAINT (DNA points accumulation for imaging in nanoscale topography) super-resolution imaging to resolve the nanoscale organization of signaling molecules at the cell interface.

The ultimate aim of this project to obtain a more complete understanding of the earliest molecular events that trigger T-cell activation. The DNA nanotechnology tools developed in this project will, however, be also useful to study receptor-mediated signaling processes in other cellular contexts.

Project-specific Requirements

- Completed master studies in (bio)physics, (bio)chemistry, biomedical engineering or a related discipline
- Knowledge on microscopy, tissue culture
- Interest in advanced fluorescence microscopy, nanotechnology, immunology
- Willingness to travel to project meetings and scientific conferences
- Enthusiasm for science and problem-solving
- Excellent English language skills
- Personal skills: independence, ability to work in a team, communication