MicroPRO

Advanced phase microscopy for protein detection

Programm / Ausschreibung	FORPA, Forschungspartnerschaften NATS/Ö-Fonds, FORPA NFTE2018	**Status**	abgeschlossen
Projektstart	01.10.2018	**Projektende**	30.09.2021
Zeitraum	2018 - 2021	**Projektlaufzeit**	36 Monate
Keywords	Optics Microscopy		

Projektbeschreibung

Proteins perform a variety of tasks within organisms, ranging from DNA replication to molecular transport. Knowledge about their structure and dynamics are of utmost importance in the life sciences and in biomedical research. While methods like NMR, electron microscopy or X-ray diffraction yield information about protein structure, this information is static, and often averaged over ensembles of proteins. For protein binding studies, it is thus often preferable to use optical techniques, which provide high temporal resolution and single particle sensitivity at the expense of lower spatial resolution. For these studies, proteins are typically labelled with fluorescent dyes. However, the addition of a label may change the behavior of the proteins, may cause photo-toxic effects, and limits the signal that can be extracted from a single molecule (bleaching).

Within the project ‚microPRO', we will develop a new, label-free, optical technique for protein detection and characterization. The technique will combine two recently published techniques, iSCAT and multi-pass microscopy, to enable unprecedented sensitivity and temporal resolution. While the interferometric detection scheme of iSCAT has been shown to allow for the detection of proteins as small as 50 kDa, the cavity enhancement offered by multi-pass microscopy is expected to further increase sensitivity by a factor of 5-10x. This will enable the detection of smaller protein complexes, as well as dynamic studies at high frame rates.

In phase 1 and 2 of the project, a first prototype of this worldwide unique microscope will be developed. A careful choice of imaging modality and the optical components, and a thorough minimization of noise sources will be necessary to reach the envisioned sensitivity. In phase 3 and 4 of the project, the prototype will then be used to detect small proteins (e.g. Bovine Serum Albumin (BSA)) and perform dynamic binding studies to optimize the setup for biological applications. After proof of concept experiments, we will study intrinsically disordered proteins (IDPs) in cooperation with the Konrat group (Max F. Perutz Laboratories) and proteasome regulation in cooperation with the Haselbach group (IMP Vienna). This will demonstrate the broad applicability of the method to study biologically relevant questions and pave the path for future collaborations with various research groups at the VBC.

Finally, the setup shall be made accessible at the VBCF for the core facility Advanced Microscopy, where it can then be used

by researchers from the Vienna BioCenter, as well as by external customers.

Projektpartner

- Vienna Biocenter Core Facilities GmbH