SFB 1078

Protonation Dynamics in Protein Function

Mon, **Apr. 29**, 2019

**15:15 – 17:30**

Freie Universität Berlin
Physics Department
Lecture Hall B

(Arnimallee 14, 14195 Berlin-Dahlem)

# Colloquium

## **Prof. Henrike Müller-Werkmeister** – Universität Potsdam

### *Time resolved serial crystallography: Watching proteins in action*

Sample delivery tools originally developed for studies of protein dynamics with serial femtosecond crystallography at XFELs on the ultrafast time scale [1] start to inspire similar experiments at synchrotrons [2], thereby enabling time-resolved serial synchrotron crystallography (TR-SSX), which allows to study biological time-scales (> ns) at ambient conditions. We have recently demonstrated the hit-and return (HARE) approach [3], which uses fixed targets for sample delivery of up to 25.000 protein crystals on an individual crystallography chip and allows the data collection for several time points during a single synchrotron beamtime. Using the HARE approach, we were able to follow the full reaction cycle of an enzyme, fluoroacetate dehalogenase, and captured 18 time points from 30 milliseconds to 30 seconds during the non-reversible turnover. Crucial for timing of this experiment was the efficient triggering of the enzymatic reaction using a photocaged substrate, demonstrating the importance of strong integration of spectroscopic and crystallographic methods for dynamic structural biology.

[1] Acta Cryst D **75**,160 (2019)
[2] Acta Cryst. D **73**, 373 (2017)
[3] Nat. Methods **15**, 901 (2018)

## **Prof. Michael Börsch** – Friedrich-Schiller-Universität Jena

### *Fast subunit rotation in $F_oF_1$-ATP synthase by single-molecule FRET in an ABELtrap*

Since 20 years we apply intensity-based and time-resolved single-molecule FRET measurements to study subunit rotation and regulatory conformational changes of individual $F_oF_1$-ATP synthases in liposomes, either driven by ATP hydrolysis or during ATP synthesis. However, observation times of freely diffusing proteoliposomes in a confocal microscope are limited by Brownian motion. In addition, arbitrary trajectories through the confocal detection volume cause large intensity fluctuations in smFRET traces. Intensity fluctuations result in varying precision for the smFRET distance measurement within the data for a single enzyme at work. To counteract diffusive motion actively in real time we have built a fast anti-Brownian electrokinetic trap (ABELtrap, invented by A. E. Cohen and W. E. Moerner at Stanford). The ABELtrap utilizes a laser focus pattern controlled by a programmable FPGA. The FPGA estimates the position of a fluorescent molecule in the trapping region and applies voltages onto electrodes to push back the molecule into the center of the laser pattern. We recorded surprisingly fast subunit rotation of $F_oF_1$-ATP synthases at different ATP concentrations and could analyze Michaelis-Menten kinetics as extracted from single-molecule FRET traces of individual membrane enzymes hold in solution by the ABEL trap.

*Coffee and tea are ready at 15:00 and during the break from 16:15 – 16:30.*

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