Scanning probe and fluorescence microscopy – a joint approach to analyze the structure of intermediate filaments and the affinity of Protein DNA complexes.

Atomic force as well as optical microscopy are potent tools for biophysical experiments on the single molecule level. Simultaneous fluorescence and AFM measurements open up various new opportunities to investigate the interplay of single biomolecules:

The desmin intermediate filament is a vital structural component of the cytoskeleton in myocytes. Mutations of the DES gene can cause severe muscle diseases like arrhythmogenic right ventricular cardiomyopathy (ARVC), which is a major cause of sudden cardiac death in adolescent and athletes. Commonly, only one of the diploid genes is mutated, therefore wild type and mutated desmin co-exist. This raises the question if and how the mutated protein is integrated in the filament. We aim to use apertureless scanning near-field optical microscopy (SNOM) to analyze the structure of the filament and the localization of both protein species.

Furthermore, atomic force spectroscopy based dynamic force spectroscopy (AFM-DFS) is a widely used tool to evaluate the affinity of (supra-) molecular complexes. Yet, the analysis of force distance curves still demands substantial knowledge and experience to rule out artifacts and unspecific dissociation events. We use fluorescently labeled binding partners to identify specific complexation and dissociation of receptor ligand pairs by fluorescence resonant energy transfer (FRET).