Targeted single cell proteomics using magnetic nanoparticles to study prion-like spreading of amyloid nanoparticles

This is a highly interdisciplinary project, combining biomedical research questions and method development (microfabrication, instrument & software development, electron microscopy (EM), image processing, biochemistry and cell biology).

Biomedical background: Stereotypic spreading of protein aggregation through the nervous system is a hallmark of many neurodegenerative diseases. This was demonstrated for Alzheimer's disease (AD, amyloid-β & tau protein) and Parkinson's disease (PD, α-synuclein, or α-syn). α-syn is a natively unfolded, presynaptic protein of unknown function and unusual conformational plasticity. Evidence accumulates that progression of synucleinopathies not only involves transmission of simple "protein misfolding" but rather specific "structural information" from one cell to the next, leading to the progressive proliferation of "structural α-syn strains". It is now suspected, that different forms of α-syn inclusions lead to different phenotypes of neurodegeneration, i.e., lead to different synucleinopathy diseases, such as PD, or Dementia with Lewy body disease. To date the patho-mechanisms are unknown, but a prion-like transmission via an intrusion of protein nanoparticles imprinting their specific folding onto native host proteins is most likely.

Project goals: Today's biophysical (e.g., mass spectrometry) methods can trace the presence of proteins, but do not allow detecting and monitoring the structural arrangement of the involved proteins. In this project we will develop a novel method for single cell analyses, not only detecting proteins, but also providing structural information. This approach is geared for the study of "structural strains" of neurodegerative diseases. Project goals are:

- **Development of a targeted proteomics and electron microscopy (EM) labelling method for single cell analysis using magnetic nano particles.** During recent years, we developed a novel method for single-cell proteomics ("visual proteomics") combining micro-fluidics, nanotechnology and EM: This includes cultivation and monitoring of adherent eukaryotic cells by light microscopy, their subsequent lysis and sample conditioning and preparation for EM. Furthermore, we developed a method for targeted proteomics using magnetic composite materials and photo cleavage from minute volumes. The latter was successfully applied for detection and initial structural analysis of weakly interacting large protein complexes using only ~40'000 cells. **However, new magnetic nanoparticles can significantly improve this technology towards targeted proteomics in single cells:** They are small enough to be directly visualized by EM. Coupled to cognitive proteins, e.g., antibodies, they will fulfill two purposes: First, we will use them to extract the target protein by a "magnetic trap" in microfluidics employing magnetic field gradients. Second, they will serve as electron dense labels aiding the identification of specific parts constituting the protein complexes.
- **Study the prion-like mechanism of the spreading of α-syn nanoparticles from diseased cells to healthy cells. Identify determinants defining different structural strains of α-syn.**

The new method optimally complements technologies for **visual proteomics** we developed during recent years:

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3. Giss, D., Kemmerling, S., Dandey, V., Stahlberg, H. & Braun, T. Anal. Chem. 86, 4680–4687 (2014).