Paving the way to single-molecule protein sequencing

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Proteins are major building blocks of life. The protein content of a cell and an organism provides key information for the understanding of biological processes and disease. Despite the importance of protein analysis, only a handful of techniques are available to determine protein sequences, and these methods face limitations, for example, requiring a sizable amount of sample. Single-molecule techniques would revolutionize proteomics research, providing ultimate sensitivity for the detection of low abundance proteins and the realization of single-cell proteomics. In recent years, novel single-molecule protein sequencing schemes that use fluorescence, tunneling currents and nanopores have been proposed.

I will present the first experimental efforts from my group towards the realization of single-molecule protein sequencing. First, I will present a proof of concept of single-molecule FRET-based protein fingerprinting. My group harnessed the AAA+ protease ClpXP to scan peptides. By using donor fluorophore-labeled ClpP, we sequentially read out FRET signals from acceptor-labeled amino acids of peptides. The repurposed ClpXP exhibits unidirectional processing with high processivity and has the potential to detect low abundance proteins. In the second part, I will present our new effort of using nanopores for single-molecule sequencing of proteins – similar to nanopore-based sequencing of DNA but facing multiple challenges, including unfolding of the complex tertiary structure of the proteins and enforcing their unidirectional translocation through nanopores. We combine molecular dynamics simulations with single-molecule experiments to investigate the utility of nanopores in protein sequencing and posttranslational modification detection.