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Highlights of Analytical Chemistry in Switzerland

Label-Free Detection of Single Native Proteins: Ultimate Sensitivity and Convenience

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Proteins are the workhorses in nature. Cellular physiology, intercellular communication, and regulation depend on the diversity and the reliable activity of proteins.

Hence, the analysis of these processes has a strong impact in many areas, such as drug discovery, medical diagnosis, food processing, *etc.* Analytical chemistry methods, *e.g.* immunodiagnostics, proteomics, DNA diagnosis and many other fields are based on powerful and sensitive observation techniques. In order to achieve appropriate sensitivity, different labeling methods have been developed, *e.g.* labeling with enzymes, radio nuclides, and fluorescent dyes. However, the labeling procedure is usually time consuming, expensive and due to purification steps substance can be lost.

Direct and sensitive detection of proteins without any labeling, use of surfaces or other additives is therefore a challenging goal for analytical technology development. Recently, we could show for the first time the detection of a single native protein molecule without any labeling. For detection, a pulsed picosecond laser system is used to excite the native autofluorescence of β-galactosidase from *Escherichia coli* (*Ecβ Gal*) in a tiny volume of a few femtoliter (10^{-15} l) with a wavelength of only 266 nm. A time gate filters scattered light from delayed fluorescence emission of the protein to enhance the signal-to-noise ratio.

Although single molecule detection is not essential in many analytical tasks, the method offers high sensitivity in general. Low background signal, small sample volume, fast response time *etc.* are obvious advantages for the user.

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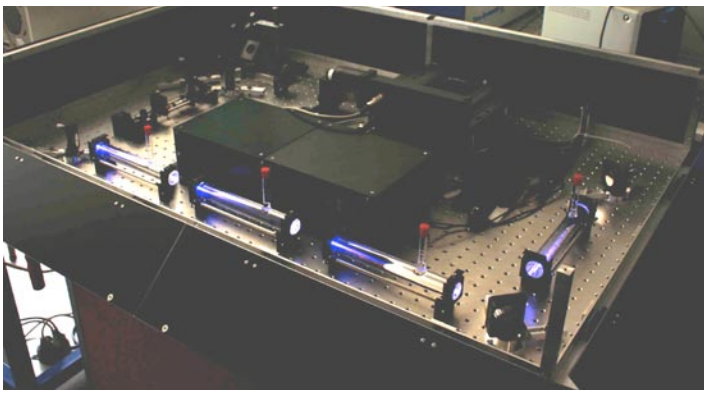
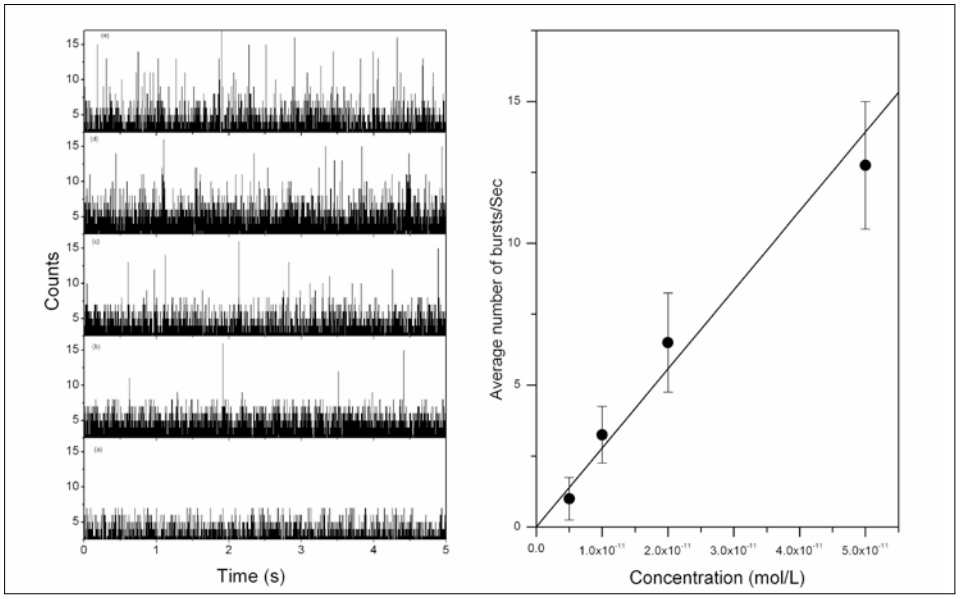


Experimental setup for the deep UV laser-based fluorescence lifetime microscopy system. In order to see the UV laser beam path, quartz tubes filled with fluorescence dye POPOP (1,4-bis(5-phenyl-2-oxazolyl)benzene) were placed into the beam path for visualization.



Left: The fluorescence photon bursts observed from (a) sodium phosphate buffer solution, (b) 5×10^{-12} mol/l, (c) 1×10^{-11} mol/l, (d) 2×10^{-11} mol/l, and (e) 5×10^{-11} mol/l Ecβ Gal solution, respectively. Data acquisition was performed at a speed of 1000 data points per second (1 ms integration time). Right: The dependence of number of fluorescence bursts on lower concentrations of Ecβ Gal solutions.

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