Crossdisciplinary Applications of Microscopy Techniques, e.g. Physic-Life Science Interfaces

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Observing the kinetics of single enzyme molecules enclosed in femtoliter arrays by fluorescence microscopy

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The development of new technologies for the analysis of single enzyme molecules has considerably increased our understanding of biochemical processes, as the observation of single enzyme molecules uncovers subpopulations and kinetic details that remain hidden in traditional ensemble experiments. For example, single enzyme molecules exhibit a broad distribution of individual activities due to different protein conformations, which is known as static heterogeneity. A large number of single enzyme molecules are required to investigate the static heterogeneity within an enzyme population. However, most single molecule techniques only enable the investigation of a single or few molecules at a time. By contrast, so-called femtoliter arrays consist of thousands of reaction chambers each defining a volume of approximately 50 μm^3 (fL). Femtoliter arrays are suitable for isolating and analysing hundreds of individual enzyme molecules simultaneously. A highly diluted enzyme solution together with a large excess of a fluorogenic substrate is enclosed in the femtoliter chambers. In chambers that contain a single enzyme molecule, the activity is determined by the enzymatic turnover of the substrate into a fluorescent product. In the confinement of a femtoliter volume, a single enzyme molecule can generate a sufficiently high concentration of the fluorescent product to be detected by wide-field fluorescence microscopy [1]. In this way, we have shown that individual molecules of β-galactosidase [2] and horseradish peroxidase [3] possess constant turnover rates that are different for each enzyme molecule in the array. The large number or enzyme molecules observed in parallel provides excellent statistics on the activity distribution in an enzyme population. Furthermore, we have shown that enzyme inhibition at the single molecule level is a stochastic process because a single enzyme molecule is either free and catalytically active or an inhibitor has bound to the enzyme and no turnover is observed [4]. More classes of enzymes are currently under investigation to elucidate differences in their activity distribution. Additionally, femtoliter arrays can be used for the detection of single molecules of an analyte and thus reach the ultimate detection limit. For this so-called single molecule ELISA, the surface of femtoliter chambers is derivatized with a capture antibody, which for example is directed against a protein present in blood. After protein binding by the capture antibody, an enzyme-labeled detection antibody binds to the protein. As the enzyme activity can be detected on the single molecule level, also the protein is detectable at the single molecule level. The protein concentration can then be determined by counting the number of active wells that contain a single molecule of the protein [5].

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