Abstract

A close connection exists between the structure of biological matter (e.g. biomolecules, cells, and tissues) and their function. Structural studies can hardly be overestimated as they influence all aspects of biology (and medicine), from understanding fundamental cellular processes to the development of therapeutic agents.
Nowadays, numerous advanced methods and approaches are applied to determine structures. My thesis presents that a spatial architecture can be revealed, quite unobvious, by analysing the molecules' motion. Studying the processes associated with changes in the mobility of the molecules gave me insight into their structure or the architecture of the microenvironment in which they diffuse. The diffusion was examined by fluorescence correlation spectroscopy (FCS). FCS is a method of analysing the fluctuations in the fluorescence signal from a small detection volume, providing quantitative information about, e.g. the diffusion properties, concentration, and interactions of analytes. A wealth of information is gained from these parameters, which I used to determine the structure of biological matter with varying complexity levels, as I demonstrated in the following examples:

1. Proteins (chapter 3). Measuring the diffusion of proteins can reveal factors influencing their conformation and, as a result, identify agents which can be treated as disease markers. Using the example of trimethylamine, I showed that it is a factor causing protein degradation. Since proteins play a central role in virtually all biological processes, defining the disturbing effect of factors on proteins can explain their toxic effect on humans.

2. The cytoplasm of cells cultured in a tissue-like architecture (chapter 4). I established a procedure for FCS measurements inside the cells in a three-dimensional tissue model. Based on the mobility measurements, I determined cytoplasmic viscosity, an essential structural parameter that governs intracellular reaction rates and strongly affects intracellular transport. Furthermore, by using FCS inside cells in a tissue model, I demonstrated the increased accumulation of olaparib, an anticancer drug, compared to adherent cells. As a result, I noted some differences in the intracellular structure between cells cultured on the flat surface and tissue-like architecture.

3. The extracellular matrix (ECM) within tumour models (chapter 5). I proved that FCS is a method that permits exploring the tumour

environment – its extracellular space. The application of FCS provided information about the architecture of the main physical barrier that inhibits the penetration of anticancer drugs into the tissue and, consequently, their effectiveness. The study of vary-sizing nanoprobes mobility in the ECM has led to the discovery of the length-dependent viscosity of the ECM. As a result, the probes with a radius of up to 10 nm freely diffuse in the complex network of the extracellular areas - a few times faster than it would appear from the Stokes-Einstein relation.

The presented applications of FCS, first and foremost, prove that FCS is a powerful tool that enables the probing of complex systems in terms of their structural studies, revealing valuable information in biological or medical research.

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