Cell-Permeant Fluorescent Nanocomposites

New Tools for Fluorescent Contrast in Microscopy

Different types of molecular and nanoscale fluorophores are being developed for fluorescent imaging of live cells. Here we present their novel types and novel compositions. The dyes incorporated into nanostructures and their metal coordination complexes as well as carbon nanodots penetrate easily into the cell with the lack of toxicity, forming stable images. They are prospective for further functionalization for targeting and contrasting cell organelles and for biosensing.

Introduction

New fluorescent nanomaterials attract increasing interest in the field of biosensing and bioimaging offering superior brightness and resistance to photobleaching as well as low cytotoxicity and good biocompatibility. Their diversity offers broad choice of possibilities covering both traditional fluorescence microscopy and its new versions such as two-photon excitation, lifetime imaging and super-resolution. The research activities of our team were focused on designing new fluorophores composed of organic dyes stabilized by metal atoms or included into nanostructures and also on spontaneously formed carbon nanodots (CNDs).

Ag&Thioflavin Composites
The few-atom clusters formed of silver and gold are known to exhibit the light absorption and photoluminescence properties, but the protein, DNA or synthetic polymer support is needed for their stabilization. When they were formed by photoreduction on polymer scaffolds, we were able to demonstrate their bright Stokes-shifted and strongly polarized emission [1,2]. These properties were similar and, regarding photostability, superior to that of organic dyes. However, due to heterogeneity and the absence of well-defined composition they were not very attractive for cellular applications. The solution was found in obtaining the smallest brightly fluorescent metal-dye composites by coupling the silver atoms to organic dye Thioflavin T. In our technology Thioflavin T works both as the photoreducer and the structure-former, so that two its molecules and two silver atoms shape the composite.

The spectroscopic properties of such Ag &Thioflavin composites are unique. In the excited state they behave as the metal-to-ligand charge transfer complexes, which results in dramatic Stokes-shifted emission.

Their well-separated excitation and emission spectra are solvent-independent. They are observed at 340 and 450 nm correspondingly and their overlap is practically absent. The most prospective applications for Ag &Thioflavin composites are expected in the live cell imaging, since they penetrate easily into the cells after the short-term incubation. They can be used both in single-photon and two-photon excitation modes (fig. 1). Been excited by 380 nm UV light, Ag&Thioflavin composites in U2OS cells demonstrated fine staining of cytoplasm without penetration into the cell nucleus. Similar features were observed with two-photon excitation on HeLa cells with the advantage of relatively lower background signal. Importantly, these composites are safe in production and applications possessing an attractive set of properties including subnanometer size, high brightness and photostability.

**Carbon Nanodots**

Fluorescent nanoparticles known as carbon nanodots (CNDs) become popular in cellular research. We demonstrated different approaches for their synthesis from alanine, citric acid, urea, etc. by hydrothermal treatment and studied their structures and optical features [3]. These materials are brightly fluorescent in the visible region, soluble in various organic solvents, stable at different pH and are non-toxic for cell cultures [4]. We tested different approaches for cell labeling by CNDs, providing live cell and fixed cell imaging (fig. 2). For live cells they stain the intracellular vesicles and lysosomes. In fixed cells the nucleus is labeled also. Recent studies showed the possibility of CNDs application for super-resolution
fluorescence microscopy without any additional modifications [5]. Been applied in localization-based techniques, their excellent contrasting and stochastic blinking properties provide strongly increased resolution of cellular components. An example of combined application of CNDs with cell-impermeable dye NR12S is presented in figure 3. Whereas NR12S stains selectively all the membranous structures associated with outer leaflet of plasma membrane [6], the nanoparticles penetrating through plasma membrane are distributed in all areas of cytoplasm.

**Conclusions**

Nanomaterials demonstrate great potential in the frontiers of biological sciences and medical research. When applied to cell fluorescent imaging they can provide visual information on cell conditions and disease, as it was demonstrated for the detection of apoptosis with our CNDs [4]. Their cell permeation is a very attractive property. Its mechanism is not fully clear and may change in size-dependent manner from passive diffusion through cell membrane to clathrin-mediated endocytosis. In practical sense, their versatility and ample possibilities for their chemical modifications allow targeting to different cellular sites and organelles, carrying metabolic modulators and drugs. Future work should focus on utilizing the full potential of these nanomaterials.
References

Authors
Alexander Demchenko¹, Kyrylo Pyrshev¹, Mykola Kanyuk¹, Mariia Dekaliuk¹

Affiliation
¹ Laboratory of NanoBiotechnologies, O.V. Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine (NASU), Kyiv, Ukraine

Contact
Prof. Dr. Alexander Demchenko
Laboratory of NanoBiotechnologies
O.V. Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine (NASU)
Kyiv, Ukraine