Toward nanoscopic cellular imaging by X-ray

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X-ray spectroscopy has nanometer spatial resolution, long penetration depth and excellent elemental specificity. The power of synchrotron-based X-ray microscopy (XRM) has been demonstrated in assessing the structure and function of cells at the subcellular level [1]. However, currently available contrast agents or probes for XRM, such as metal or semiconductor nanoparticles, generally lack specificity and biocompatibility and thus limit its utility [2]. Newly developed genetic tags for electron microscopy (EM) provide EM contrast in cellular compartments in situ and enable intracellular specific protein imaging and spatially resolved proteomic mapping [3,4], but use of EM for whole cell imaging remains challenging because of low penetration capability.

In a research article recently published in NSR [5], Fan, Zhu and coworkers reported a genetically encoded tagging system that could generate XRM reporters intracellularly, which were used for imaging organelles and specific proteins in cells at an ultra-high resolution of about 30 nm (Fig. 1). They firstly confirmed that an engineered ascorbate peroxidase (APEX2) could provide contrast enhancement for XRM by catalyzing local polymerization of diaminobenzidine (DAB). Polymerized DAB showed much higher contrast to the water background compared to DAB monomers, as shown in images obtained by synchrotron-based scanning transmission X-ray microscopy (STXM). To substantiate the utility of the APEX2 system for XRM imaging in mammalian cells, they constructed plasmids expressing APEX2 fused to different proteins. The proteins fused with APEX2 were either components of subcellular structures, such as nexin-43, α-tubulin and β-actin, or markers of organelles, including nuclear localization sequence (NLS) and galactosyltransferase. After staining with DAB monomers, the STXM images of transfected cells showed distinctly high-contrast structures with typical shapes of corresponding subcellular structures, namely gap junctions, microtubules, microfilaments, cell nucleus and Golgi apparatus, compared to cells

![Image of diagram showing genetically encoded tagging and X-ray cellular imaging for protein localization.](image-url)

**Figure 1.** Schematic of genetically encoded tagging and X-ray cellular imaging for protein localization. Protein localization with XRM is achieved by (1) constructing fusion expression plasmids including APEX2 and biotargets, (2) transfecting cells with plasmids, (3) specific expressing of fusion proteins containing APEX2 and biotargets, (4) catalyzing the polymerization of DAB in situ into localized X-ray visible dense DAB polymers and (5) XRM imaging.

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without tagging. Of note, the X-ray tag showed enhanced photostability (almost no photo-bleaching after 10 frames of scans) compared to endogenous fluorescent tags (up to 28% decrease), which allowed repetitive scanning at high density and thus enabled imaging with high spatial and energy resolution. Furthermore, the XRM cell imaging based on this tagging system had approximately an order of magnitude improved spatial resolution (about 30 nm) when compared to the resolution of classic optical microscopy (about 200 nm).

The authors demonstrated two important applications of this genetic tagging and X-ray imaging system: it can be used to image subtle changes and refined structures of intercellular connections, which remains challenging for optical microscopy and electron microscopy because of their resolution limits; and it can achieve multi-color X-ray imaging by introducing different peroxidase tags and DAB substrates containing elements with distinguishable adsorption energies.

The work by the Fan and Zhu group provides a highly versatile platform technique for imaging targeted molecules and structures in cells with high specificity and resolution. Importantly, as part of the ‘Synchrotron for Neuroscience—an Asia Pacific Strategic Enterprise’ (SYNAPSE) project, aiming to comprehensively map the neuronal network of the entire human brain, this genetically encoded X-ray tagging system can be adapted readily to visualize the neuron network at a subcellular level resolution.

Conflict of interest statement. None declared.

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National Science Review  
B: nwa206, 2021  
doi: 10.1093/nsr/nwaa206  
Advance access publication 8 September 2020

MATERIALS SCIENCE

Confined nanospace for enhanced photocatalysis

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The world today is very different from how it was. The consumption rate of fossil fuels compared with the available resources and the calamity of global warming frighten people. The entire planet is at risk, but these are our crises. Mankind is the cause and so mankind should find sustainable and green energy solutions to satisfy the needs of society.

Chemical reactions driven by solar energy can not only diminish consumption of fossil fuels and pollution from such consumption, but also achieve high selectivity through exact energy exchange between photons and electrons. Since the pioneering work on electrochemical photocatalysis of water in 1972, semiconductors and other light-absorbing materials such as plasmonic metals have been widely explored [1–3]. For a long time, scientists have focused on enhancing photocatalytic performance through optimization of the three crucial processes in photocatalysis: light harvesting, electron-hole separation, and surface redox reactions. With the development of nanotechnology, hollow nanostructures have been found to efficiently promote light absorption and charge separation [4]. Not only that, the confined nanospace in the hierarchically hollow structures can provide a specific microenvironment for the reactions and affect the mass transfer of substrate to achieve a maximized efficiency [5–7].

To demonstrate the vital role of confined nanospace, a recent cooperative research group led by Prof. Jian Liu from the Dalian Institute of Chemical Physics, Chinese Academy of Sciences and Prof. Jun Huang from the University of Sydney investigated the effect of hierarchically hollow-structured nano-photoreactors loaded with bimetallic catalysts at different positions for photocatalytic oxidation of cinnamyl alcohol [8]. Hierarchically hollow nanostructures can be obtained through a nicely designed spontaneous phase transformation of core-shell structured zeolitic imidazolate frameworks (ZIF)-8@SiO2 to hollow mesoporous zinc silica composites (HMZS) under mild hydrothermal conditions. The research traces the transformation process under different conditions via semi-in situ technique, and an ‘adhesive-contraction’ mechanism of hollow structure formation is proposed. Meanwhile, the synthesis strategy ensures controllability of the spatial location of active metals. The situation of AuPt bimetallic nanoparticles inside/on the surface (AuPt@HMZS and AuPt/HMZS) of the hollow structures depends on the order of metal loading and shell coating.