Imaging

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Imaging Bioorthogonal Groups in Their Ultrastructural Context with Electron Microscopy

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Bioorthogonal chemistry has proven to be a powerful technique for imaging biomolecules, especially those that are not under direct genetic control, such as glycans, lipids, bacterial cell wall components and, one could argue, DNA itself.[1]

Recently, the drive for ever higher imaging resolutions has been applied to the labeling of bioorthogonal groups. For example, the imaging of bioorthogonal analogues of mitochondrial lipids using STED microscopy[2] and the imaging of cell-surface glycans using dSTORM[3] have been reported. However, one downside of super-resolution is the limited number of fluorophores which can be imaged in parallel in a single experiment. This aspect leaves the cellular context in which the bioorthogonal groups reside invisible.[4]

Electron microscopy (EM) is the preferred technique for obtaining morphological information on (sub)cellular structures with nanometer-scale resolution.[5] Nonetheless, information obtained with EM is both difficult to obtain and interpret, as sample preparation is non-trivial and the revealed biomolecules and structures are hard to define. Traditionally, antibodies have been used to reveal the nature of the biomolecules upon conjugation with electron-dense labels, such as gold particles.[6] However, for this strategy formaldehyde fixation and permeabilization of the cells is required to allow antibodies to enter the cell or tissue. This use of fixative and detergent results in a greatly compromised cellular ultrastructure. Alternatively, antibody labeling after sample preparation in electron microscopy is an elaborate process, the success rate of which is notoriously low because of a lack of functional antibodies.[7]

EM-compatible visualization of non-templated molecules is even more difficult: staining of carbohydrates using lectins has been reported, but suffers from limited specificity and limited availability of lectins.[8] The development of EM-compatible bioorthogonal imaging techniques would thus be of great benefit to provide facile ultrastructural information of the context in which these non-templated molecules reside.[9] This technique has, however, been a long time waiting, as many EM sample preparation techniques are based on the thermal/radical polymerization of resins within the samples, a technique with which bioorthogonal chemical functionalities are not compatible.

Recently, Ngo et al.[10] elegantly solved the issue of instability of the bioorthogonal group during resin-based EM sample preparation. By performing the bioorthogonal modification and visualization before going through EM sample preparation they have circumvented this problem. To achieve this, Ngo et al. used a photoconversion strategy: they ligated fluorophores to their bioorthogonal groups that are capable of photoconverting triplet oxygen to singlet oxygen, which in turn can be used to polymerize diaminobenzidine (DAB) near the site of the fluorophore into an insoluble precipitate. The resulting polymer has a very high affinity for osmium tetraoxide making the site of the original bioorthogonal group show up dark in an EM image (Figure 1 A).[11] This technique was previously developed for visualizing genetic tags, such as mini-SOG[12] and the tetracysteine-tag-based FLASH/ReASH tags.[11] By introducing the fluorophore before the fixation, dehydration and resin polymerization, the incompatibility of bioorthogonal groups with this workflow is overcome. A further advantage is that it precludes the need of permeabilization of the cells as conventional fixation and the auxiliary reagents required for the generation of contrast are small molecules which readily permeate across fixed cell membranes. This means the ultrastructure of the cells is preserved. The resolution of imaging is also very good, as it has been shown that photooxidation-based polymerization of DAB occurs within four nanometers of the original photoconversion reaction, thus outlining the labelled structures with the osmiophilic product.

Ngo et al. use this technique to image newly synthesized DNA by imaging the incorporation of a bioorthogonal DNA analogue, ethynyl deoxyuridine (EdU).[13] They exploit the resin-compatible nature of the technique by combining it with block-faced scanning EM (SBEM), an approach whereby sequential 60 nm sections are taken from a sample block for EM imaging. The series of images generated in this approach is then used to reconstruct a three-dimensional image of the cell. They use this approach to beautifully show DNA separation in the anaphase of a dividing cell (Figure 1 B).

They further highlight the breadth of the approach by labeling and imaging choline-containing lipids and bioorthog-

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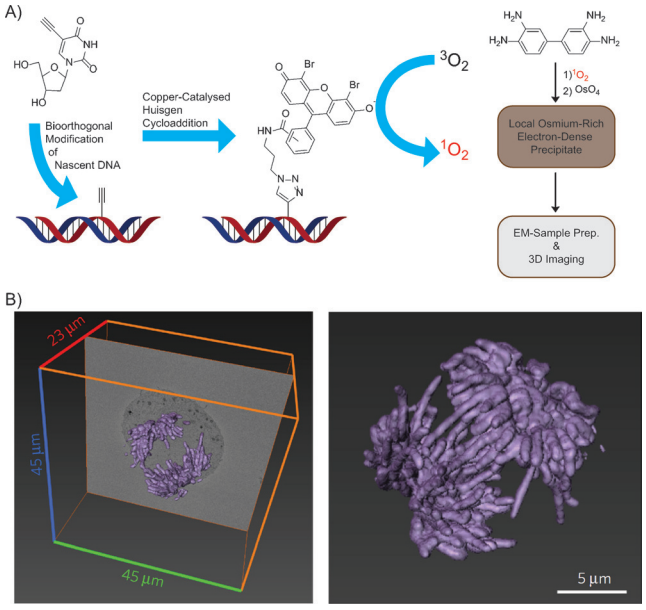

Figure 1. Detection of bioorthogonal groups in resin embedded EM samples: A) First, a bioorthogonal uridine analogue is metabolically incorporated in nascent DNA. This handle was modified at the end of the experiment with dibromofluorescein, which can efficiently convert triplet oxygen into singlet oxygen. This singlet oxygen can then convert a 3,3′-diaminbenzidine substrate into an insoluble polymer with high affinity for osmium. This osmium is electron dense and can thus be used to visualize the presence of the original bioorthogonal group. B) An example, taken from Ref. [10], of the three-dimensional reconstruction of bioorthogonally labelled DNA in the nucleus of a dividing cell.

onally labeled cell wall of *Listeria monocytogenes*. In the latter experiment they exploit the resolution of the technique to distinguish extracellular peptidoglycan from its intracellular intermediates (which are only separated by 7 nm).

With this approach Ngo et al. confirm the power of bioorthogonal chemistry to image non-templated molecules and these new EM techniques finally provide ultrastructural context to the study of these functional groups. The approach complements another recently reported approach which bioorthogonal handles were visualized in EM sections after sample preparation using correlative light-electron microscopy, a technique in which a fluorescent image of a 100 nm section is overlaid on an EM micrograph of the same section. Here the problem of chemical incompatibility was solved by using the only EM-sample preparation that does not use a polymer resin for sample preparation, but instead relies on plunge-freezing the samples and cutting them at ultra-low temperatures.[14] Together these papers have finally put electron microscopy imaging on the map for imaging bioorthogonal groups in their cellular context.

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