Imaging glucose uptake inside single cells

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Stimulated Raman scattering is used together with a novel, small-alkyne-tagged glucose analogue to visualize glucose uptake activity at the single-cell level.

Almost all living organisms—from bacteria to humans—require glucose as an energy source, i.e., cells constantly take up glucose to satisfy organisms’ energetic needs for survival, maintenance, and proliferation. However, in many diseases (such as obesity, diabetes, and cancer), glucose uptake activity becomes poorly regulated. Visualization of this important process, in both physiological and pathological conditions, is thus highly desired, and is indispensable for understanding energy metabolism and cellular activity. Over the past few decades several prominent techniques have been developed for the visualization of glucose uptake. For instance, radioactive fluorine-18-labeled 2-deoxyglucose (FDG)—see Figure 1(a)—is widely used in clinical tumor diagnostics, with positron emission tomography (PET), to locate hot spots of glucose uptake.\(^1\) In addition, complex signal amplification techniques in magnetic resonance imaging (MRI) have recently been demonstrated for glucose imaging in mouse tumors.\(^2\) Although both PET and MRI methods have many uses in clinical applications, they have limited spatial resolution. Moreover, energy requirements and glucose uptake inherently vary between cells, which means PET and MRI are not ideally suited to the visualization of glucose uptake at the cellular level.

To image glucose uptake activity down to single-cell resolution, glucose analogues that are labeled with fluorescent dyes have thus been developed (for use in fluorescence microscopy, with optical resolution). 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose—2-NBDG, shown in Figure 1(b)—is one of the most commonly used fluorescent probes for these purposes.\(^3\) Labeling glucose with fluorescent dyes, however, alters the physical and chemical properties of the molecule. Furthermore, the tagged fluorophores (molecular structures that emit fluorescence) are generally larger than the glucose itself. Most fluorescent glucose analogues therefore exhibit perturbed behaviors and undesired interactions in cells and tissues, i.e., which can bias the true glucose uptake activity and distribution. To overcome these problems, we have thus developed a new approach—based on stimulated Raman scattering (SRS) microscopy—for the visualization of glucose uptake activity, with single-cell resolution and minimal perturbation.\(^4\) SRS microscopy is an emerging multiphoton optical imaging technique,

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based around the Raman scattering of chemical bond vibrations, which offers sub-micrometer spatial resolution and a 3D optical-sectional capability. Through stimulated emission of targeted chemical bonds, we can use SRS imaging to produce a quantitative concentration spatial map that has high sensitivity and specificity. Indeed, we have previously reported the coupling of SRS with vibrational tags as a general strategy for imaging small biomolecules in living systems.

In this work, we designed, synthesized, and characterized a new, vibrationally labeled glucose analogue. We also used SRS microscopy (with single-cell resolution) to visualize the uptake of this analogue as an energy source in live cancer cells, tumor xenograft tissues, primary neurons, and mouse brain tissues. Specifically, we labeled glucose with a small alkyne (i.e., a carbon–carbon triple bond) tag at the third oxygen position. The resulting molecule—3-O-propargyl-D-glucose (3-OPG), shown in Figure 1(c)—exhibits a strong and characteristic Raman scattering signal at 2129 cm$^{-1}$. This signal occurs within an otherwise cell-silent spectral window—see Figure 1(d)—and it can thus be imaged sensitively and specifically with SRS microscopy.

We verified the glucose transporter dependence of 3-OPG uptake by conducting glucose competition, small-molecule inhibitor, and RNA interference experiments. In addition, we obtained quantitative kinetic measurements that showed the uptake of 3-OPG by cellular glucose transporters is fast and efficient. Moreover, our simultaneous imaging of 2-NBDG and 3-OPG showed that there was little non-specific interaction of 3-OPG inside cells (unlike 2-NBDG). This result is thus evidence that 3-OPG can be used as a more truthful probe for glucose uptake activity and distribution than 2-NBDG.

In our study, we also used 3-OPG to visualize glucose uptake activity in live tumor and brain tissues. By imaging the 3-OPG uptake, we could use SRS microscopy to distinguish cancer cell lines that had different metabolic activities. We also observed the distinct uptake of 3-OPG inside mouse xenograft tumor tissues. For example, we could clearly image (down to single-cell resolution) the border between the tumor’s proliferating and necrotic regions, i.e., by the sharp contrast in glucose uptake activity: see Figure 2(a). In addition, we observed heterogeneous uptake patterns in a primary hippocampal neuron culture—see Figure 2(b)—and mouse brain tissues, with clear cell-to-cell variations.

In summary, we have developed a new SRS-based technique for single-cell resolution imaging of glucose uptake activity within a variety of live cells. With our use of a new alkyne-labeled glucose analogue, we can achieve sub-cellular resolution and avoid the undesirable influence of fluorescent dyes. Our approach can thus be used to complement FDG in clinical PET imaging, i.e., for visualizing glucose uptake activity at the cellular level with minimal perturbation. We are currently working to apply this technique to in vivo imaging of glucose uptake dynamics. We expect that our new method will become an attractive tool for the study of energy activity (at the single-cell level) in living systems (i.e., in brain and malignant tumors that have high energy demands).

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References