19p-D4-5

Watching bioactive small molecules in live cells by alkyne-tag Raman imaging

Almar Palonpon[1,3], Hiroyuki Yamakoshi[1,2], Kosuke Dodo[1,2], Jun Ando[1,3], Satoshi Kawata[2,3], Katsumasa Fujita[1,3], and Mikiko Sodeoka[1,2]

[1] Sodeoka Live Cell Chemistry Project, JST-ERATO, [2] RIKEN, [3] Department of Applied Physics, Osaka University
E-mail: almar@ap.eng.osaka-u.ac.jp

1. Introduction

Visualization of biomolecules in live cells plays a major role in advancing the field of life sciences. To this end, many bioimaging tools have been developed, among which fluorescence microscopy is most widely used. However, fluorescence labeling has problems with imaging small molecules because the larger size of the label often perturbs the biological activity and localization of the target small molecule. To detect small bioactive molecules, we use a small chemical tag called alkyne and detect its unique vibrational signature by Raman microscopy.

2. Alkyne-tag Raman imaging

Alkyne emits a unique Raman signal in a spectrally silent region of the cell (1800-2700 cm^{-1}). Since alkyne consists of two carbons connected by a triple bond, this tag is sufficiently small which has minimal influence on the target small molecule. As a proof-of-concept, recently we successfully imaged the DNA synthesis of a live cell using the alkyne-tagged analog of thymidine, EdU, which is easily incorporated in the DNA of the cell [1,2]. To further expand the utility of this technique to a wide range of small molecules, we studied the Raman signals of various alkynyl structures and came up with guidelines for tag design usable for different targets [3].

3. Results

Fig. 1 shows the structure-Raman intensity relationships of various candidate Raman tags that are active in the silent region of the cell such as alkynes, nitriles, and deuterium. Since alkynes give the highest Raman intensities among the Raman tags, we investigated further the Raman signals from various alkynyl structures and found that alkynes conjugated to an aromatic ring and diynes (two alkynes joined together) produce the most intense Raman signals. Moreover, the results of the structure-Raman intensity relationship studies could be used as a guideline for designing alkyne tags that are suitable for a particular target molecule.

Multicolor Raman imaging is also possible by choosing alkyne tags that are well separated in the Raman spectra for different targets. Figure 2 shows a multicolor Raman image of live cell treated with two different alkyne-tagged bioactive small molecules, EdU and CoQ analogue, obtained simultaneously together with the image of endogenous protein, cytochrome c [3].

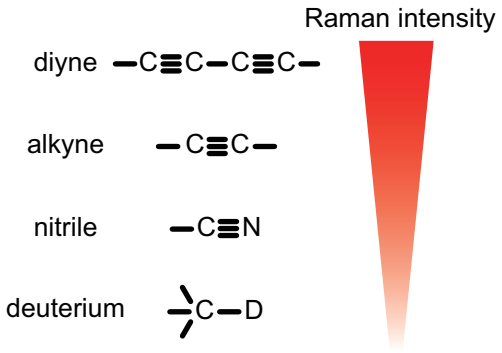

Figure 1. Structure-Raman intensity relationship of various candidate Raman tags

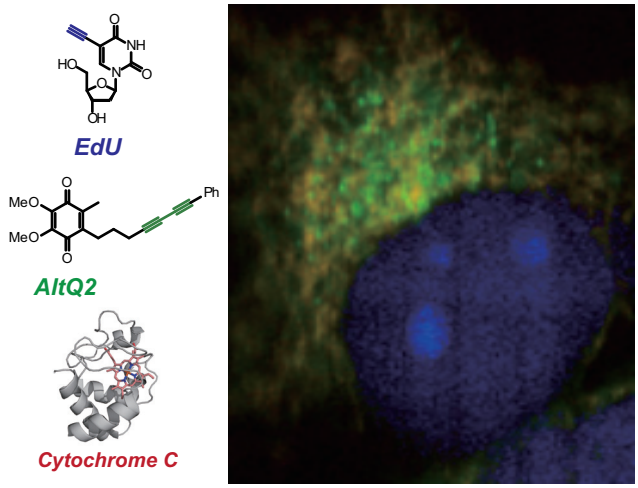

Figure 2. Multicolor alkyne-tag Raman imaging of two small alkyne-tagged bioactive molecules (blue and green) and an endogenous protein (red) within a live cell.

4. Conclusions

Alkynyl structures especially alkynes conjugated to a ring and diynes were found to be the most intense Raman tags suitable for tagging small molecules in live cells. Multicolor Raman imaging of two alkyne-tagged bioactive molecules was demonstrated.

References

[1] Yamakoshi et al., J. Am. Chem. Soc. **133** (2011), 6102.
[2] Palonpon et al., Nat. Protoc. **8** (2013), 677.
[3] Yamakoshi et al., J. Am. Chem. Soc. **134** (2012), 20681.