Toxic, aggregation-prone proteins can induce neuronal damages, which are a characteristic of many neurodegenerative disorders such as polyglutamine (polyQ) diseases. One notorious polyQ disease is Huntington’s disease, whose typical symptoms include memory deficit and depression. Huntington’s patients carry mutations in the protein called Huntingtin (mHtt). The mHtt protein contains polyQ with 40–250 glutamine repeating units and tends to aggregate in the brain. It is crucial to understand the molecular composition, structure, and kinetics of mHtt aggregates. However, it remains challenging to visualize the protein aggregates in vivo. Now, a research team led by Lu Wei at California Institute of Technology provides a noninvasive way to visualize and quantify polyQ protein aggregates in living cells.

Live-cell fluorescence microscopy of polyQ protein aggregates requires labeling them with large fluorophores that may induce unwanted perturbations. Alternatively, Raman microscopy provides a means to visualize biomolecules in vivo by mapping the characteristic vibrational signals of chemical bonds of biomolecules, which in principle can be used in a label-free manner. In particular, stimulated Raman scattering (SRS) microscopy, with improved sensitivity and imaging speed, has found broad applications in bioimaging. One limitation of this approach is that Raman signals of various biomolecules are very similar and often identical because proteins are not quite different from nucleic acids and glycans in terms of chemical composition. To overcome this issue, one can metabolically label biomolecules with short chemical bonds such as alkyne and carbon-deuterium (C–D) bonds, whose Raman signals in cells fall into the so-called “Raman-silent region.” Spectroscopically, these bioorthogonal Raman tags do not show any interference with naturally occurring biomolecules. Among them, C–D tags are the most attractive because they only introduce an isotopic substitution.

In this issue of ACS Central Science, Miao and Wei exploited bioorthogonal SRS imaging using C–D tags for live-cell visualization, quantification, and spectral analysis of polyQ aggregates. To this end, they replaced glutamine in the medium with deuterium-labeled glutamine (Gln-d5), which was metabolically incorporated into the long polyQ tail of mHtt protein. The vibrational frequency of C–D bonds enables superb selectivity and sensitivity for subcellular mapping of mHtt aggregates in live cells by SRS imaging (Figure 1). Importantly, the background signals from the free Gln-d5 and other incorporated proteins are much weaker than the signal from aggregates and can be easily subtracted.
By coupling SRS microscopy with stable isotope labeling of glutamine (Q), bioorthogonal Raman imaging in live cells enables specific detection and ratiometric quantification of native polyQ aggregates, the possible cause of Huntington’s disease.

By using Gln-\(d_5\) labeling and SRS imaging, the authors visualized polyQ aggregates in live cells and revealed that aggregation of mHtt was indeed affected by GFP tagging. Through ratiometric quantification (i.e., by the C–H/C–D ratio), they reported absolute concentrations for sequestered mHtt and non-mHtt proteins within the same aggregates. Furthermore, the authors showed that hyperspectral SRS analysis contains comprehensive spectroscopic information and ensures the spectral specificity, differentiating the aggregates from other puncta-like structures (e.g., nucleoli, stress granules, and lipid droplets). These results provide valuable information for understanding the mechanism of aggregate formation and may pave the way for further therapeutic intervention in Huntington’s diseases.

Wei’s imaging method can be readily applied to study other polyQ diseases as well as other poly(amino acid) diseases. The potential of this method is exciting for researchers in the field of neurodegenerative diseases. On the other hand, the sensitivity of Raman imaging, even with SRS, is still nowhere near the sensitivity of the fluorescence microscopy. Further improvements may just be on the way.

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**Notes**

The authors declare no competing financial interest.

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