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Imaging in living cells using νB–H Raman spectroscopy: monitoring COSAN uptake

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The boron-rich cobaltabisdicarbollide (COSAN) and its 8,8′-I_2 derivative (I2-COSAN), both of purely inorganic nature, are shown to accumulate within living cells, where they can be detected using νB–H Raman microspectroscopy. This demonstrates an alternative method for cell labelling and detection.

The inorganic, boron-based molecule cobaltabisdicarbollide, $[3,3'\text{-Co}(1,2\text{-}C_2B_9H_{11})_2]^-$, commonly known as COSAN, comprises of a cobalt atom sandwiched by two carboranyl clusters.[1] This structure exhibits both electrostatic interactions, *via* a dispersed negative ionic charge covering the whole molecule,[2] and non-bonding intermolecular interactions between its weakly polarized B–H and C–H bonds.[3] This duality imparts the molecular property of being simultaneously hydrophobic and hydrophilic, and makes COSAN soluble in both water and oils. The polarized lipid molecules that make up biological membranes also possess amphiphilic properties, and can assemble into membranes and vesicles formed from lipid bilayers. COSAN has also been shown to form small nanovesicles and above a critical aggregation concentration ($cac_{vesicle} \approx$ 0.01 mM), begin to form micelles.[4] However, unlike lipid bilayer membranes, the membranes of COSAN vesicles are monolayered. Recently, $[3,3'\text{-Co}(8\text{-I-}1,2\text{-}C_2B_9H_{10})_2]^-$, I2-COSAN, has also been found to self-assemble into a lyotropic lamellar phase with sufficient curvature to create closed vesicles.[5] These similarities in physicochemical properties to biological lipids suggest that COSAN molecules may be compatible with living cells, and could be used to label cells in a similar manner to lipophilic dyes. Here, we use Raman microspectroscopy to show the accumulation of COSAN and I2-COSAN in living cells.

As a first step in this work, COSAN or I2-COSAN was added directly to Dulbecco's Modified Eagle medium (DMEM) with Human Embryonic Kidney (HEK293) cells. When the cells were concentrated by low speed centrifugation, washed and incubated in new DMEM medium, visual inspection showed accumulation of COSAN or I2-COSAN (orange in colour) within cell pellets (Fig. 1). This observation indicated that these COSAN molecules could be taken up by living cells.

Raman spectroscopy offers a useful tool for visualising COSAN uptake, as carboranyl clusters are rich in B–H bonds, which are not present in cells. Particularly conveniently, the B–H stretching frequency band at 2570 cm^{-1} is at a region of the vibrational spectrum at which no other frequencies seen in organic molecules can be found.[6] To establish the conditions for COSAN and I2-COSAN detection, Raman spectra were acquired for COSAN in solutions at the concentration range of 2–100 mM.

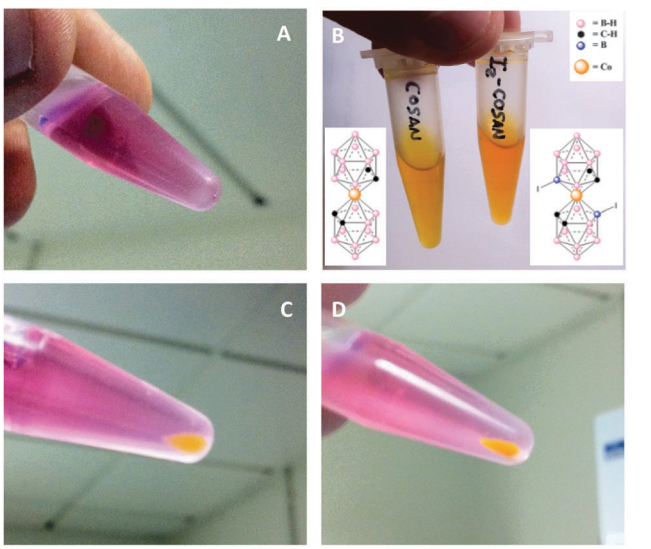

Fig. 1 (A) Untreated HEK293 cells (white dot at the bottom) in DMEM medium. (B) 25 mM solution of COSAN and I2-COSAN in PBS. (C) COSAN treated HEK293 cells after COSAN-DMEM removal, washing with DMEM medium and incubating in new DMEM medium. (D) The same as (C) but using I2-COSAN instead of COSAN.

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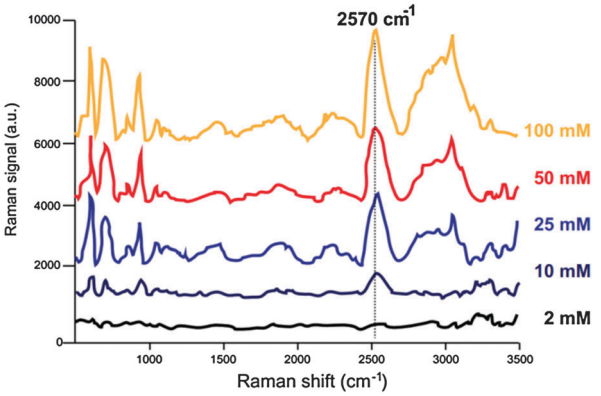

Fig. 2 Raman spectra calibration at different concentrations of I2-COSAN. The absorption at 2570 cm^{-1} that corresponds to νB–H is detectable above 10 mM.

Both COSAN and I2-COSAN produced a distinct spectral peak at a frequency near 2570 cm^{-1} (Fig. 2), which was detectable above a concentration of 10 mM.

Cells were incubated in DMEM that contained COSAN (25 mM) (or I2-COSAN) on poly-L-lysine coated glass microscope coverslips for 1 hour. Cells were then washed with Phosphate-Buffered Saline (PBS) and imaged using Raman microspectroscopy. For visualisation, cell samples were stimulated with a diode pumped solid-state (DPSS) laser operating at 532 nm and visualised with a Raman microscope system (Eclipse Ti-U, Nikon).[7] As a control, living HEK293 cells were analysed without COSAN (or I2-COSAN) by performing Raman imaging on the untreated cells (Fig. 3). A uniform distribution across the cell of C–H bond stretching, corresponding to an intense peak at 2950 cm^{-1}, but no COSAN signal at 2570 cm^{-1} was observed. In contrast, cells treated with 25 mM COSAN, or I2-COSAN, exhibited the distinctive B–H peak at 2570 cm^{-1} (Fig. 4). No such strong peak was observed outside the cell (Fig. 4A) indicating a high accumulation of COSAN inside the cells.

If cells that had been treated with COSAN (Fig. 4B) or I2-COSAN were washed and then re-incubated in the absence

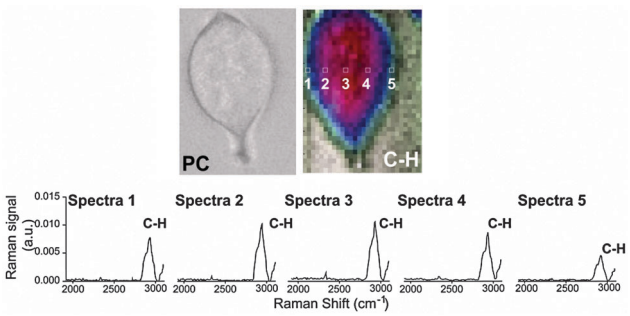

Fig. 3 Cellular imaging of COSANs untreated control HEK293. Images show phase contrast image (PC) and Raman chemical images at 2950 cm^{-1} (νC–H peak). Five Raman spectra (Spectra 1–5 below) show the spectral fingerprint of the cell at different positions across the cell. Pink zones in the C–H Raman image display high C–H content. The two weaker signals are at sites 1 and 5 at the cell, whereas the more intense are at 2–4 sites. No Raman signal at 2570 cm^{-1} (νB–H peak) was present.

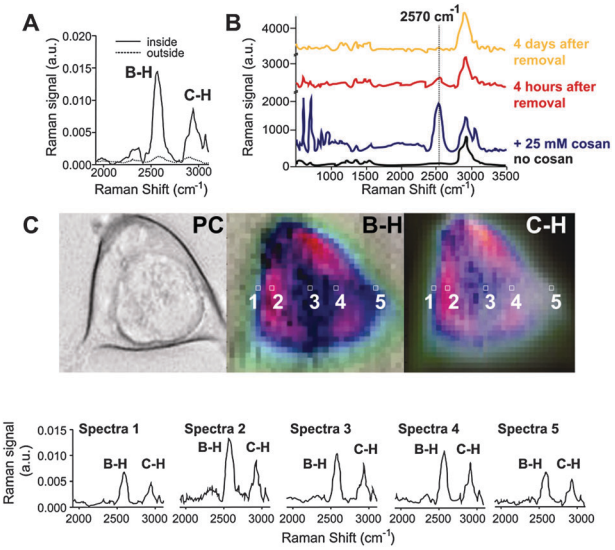

Fig. 4 (A) Comparison of spectral fingerprints of HEK293 cells treated with COSAN 25 mM for 1 hour. Spectra are normalised to the area under the νC–H peak. The amount of I2-COSAN (based on B–H as the reference peak) was ~85% higher inside the cell (solid line) than in the external medium (dotted line). (B) Spectra were obtained both inside and outside cells and 4 hours and 4 days after COSAN had been removed, the cells washed and then replaced in fresh medium. (C) Chemical imaging of HEK293 cell treated with 25 mM COSAN. PC shows phase contrast image while B–H and C–H display Raman chemical images at 2570 cm^{-1} (B–H peak) and 2950 cm^{-1} (C–H peak). Pink zones in the B–H Raman image show I2-COSAN accumulation inside the cell, whereas pink zones in the C–H Raman image display high C–H content. Five Raman spectra (spectra 1–5 below) show the spectral fingerprint of the cell at different positions across the cell.

of the compounds, the νB–H band was still observed inside the cell, but was then gradually lost over time. Raman imaging at points across the cell showed a loss of uniformity in the C–H distribution when the cells were exposed to COSAN, the presence of COSAN within the cytoplasm and to a lesser extent in the nucleus. Although dispersed throughout the cell, there appeared to be local subcellular COSAN accumulation within the cytoplasm. The identity of these regions is currently unclear; however areas of high νB–H signal match the areas of high C–H content (Fig. 4C), indicating that COSAN-rich areas are associated with cell components, and are unlikely to exclude major proportions of the cytoplasm contents.

This report demonstrates a novel means of cell labelling that uses the B–H rich molecules COSAN and I2-COSAN, both of purely inorganic nature, and their direct imaging using Raman microspectroscopy. We show that COSAN and I2-COSAN readily accumulate inside cells and are retained for at least four hours post-labelling, providing the opportunity to monitor labelled cells for prolonged periods. In general, this indicates that COSANs, but perhaps also other boron cluster molecules, offer a means of cell labelling and unequivocal detection against the background of all other cellular molecules, be they proteins, lipids, DNA or other biopolymers.

In the present study, we have examined the behaviour of COSAN and I2-COSAN alone; however improvements in the

derivatization chemistry of boron clusters makes it possible to functionalize molecules of biological interest.[8] For example, COSAN, and other borane–heteroborane clusters can be coupled to bioactive molecules, such as antibodies and protein ligands, and these protein-conjugated heteroboranes can be targeted to specific cell types.[9] Given the apparent ease with which COSAN molecules enter cells, it may be possible to tag molecules of biological interest and image them within subcellular localisations.

Medicinal boron chemistry is now attracting growing research interest[10] and investment from the pharmaceutical industry in the quest for novel drugs to tackle cancer and infectious diseases, potentially overcoming the limitations and side effects of the current products. These observations offer a direct approach to monitor and track Boron-based drugs at the cellular level.

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