Supporting information

Monitoring bacterial spore metabolic activity using heavy water-induced Raman peak evolution

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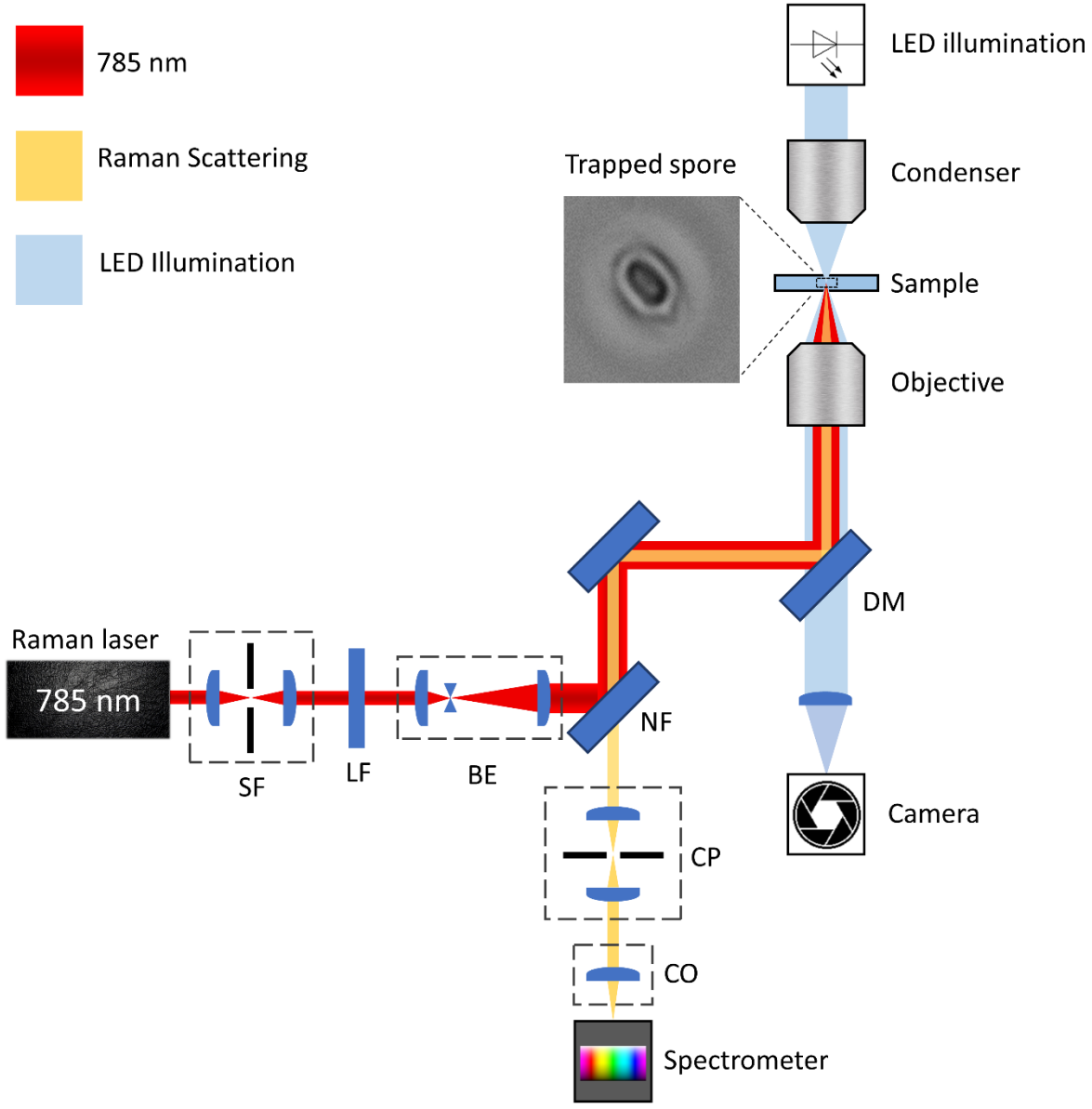

Figure S1. Illustration of our LTRS setup measure Raman spectra of spores. It comprises a spatial filter (SF), beam expander (BE), 785 nm line filter (LF), 785 nm notch filter (NF), dichroic 650 shortpass mirror (DM), confocal pinhole (CP), coupling optics for spectrometer (CO). We illuminate the sample using a LED and acquire images using a CMOS camera.

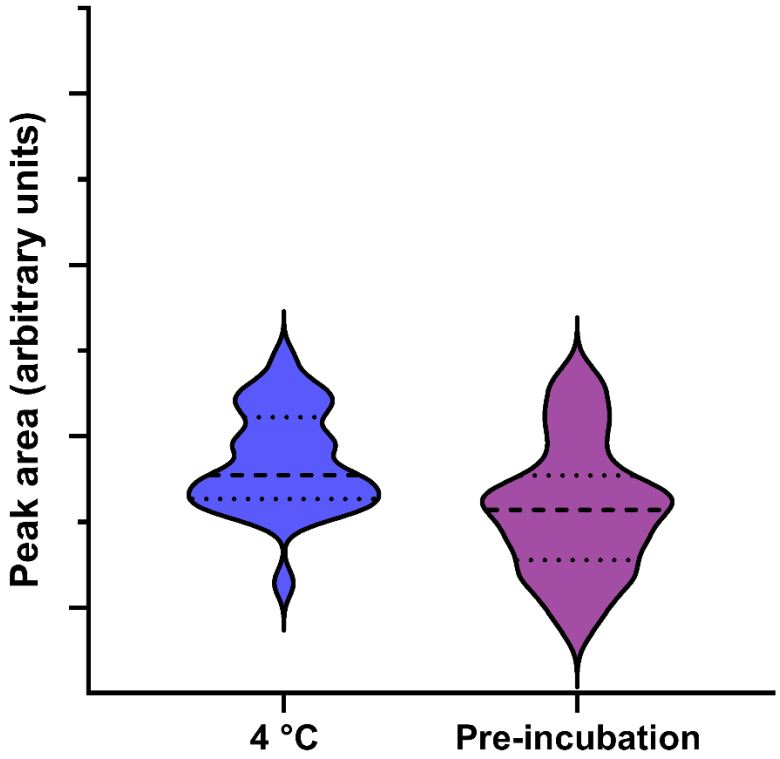

Figure S2. The area of the C-D Raman peak (2100 – 2300 cm^{-1}) in *B. cereus,* incubated at 4 °C for 24 h, compared to non-incubated spores. The dashed lines show the median and the upper and lower quartiles in the distribution. No change in the peak area was observed ($p > 0.41$).

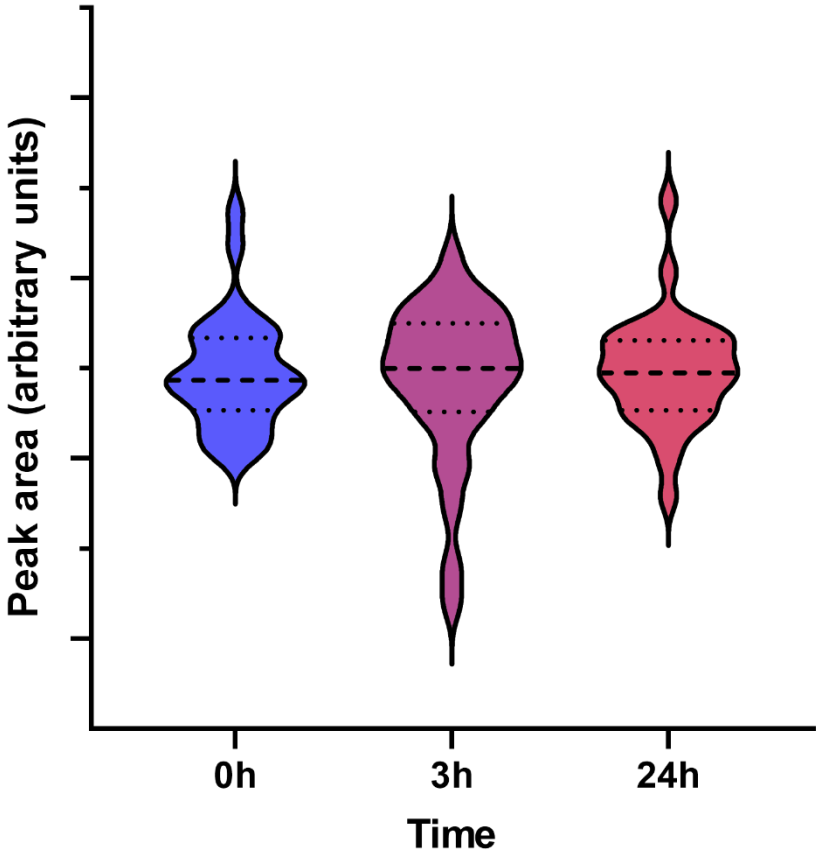

Figure S3. The area of the C-D Raman (2100 – 2300 cm^{-1}) peak in *B. cereus*, incubated at 37 °C after a 10 min treatment with 150 ppm chlorine dioxide (DK-DOX 1500). The dashed lines show the median and the upper and lower quartiles in the distribution. Spores were inactivated and no change in the peak area was observed ($p > 0.99$).

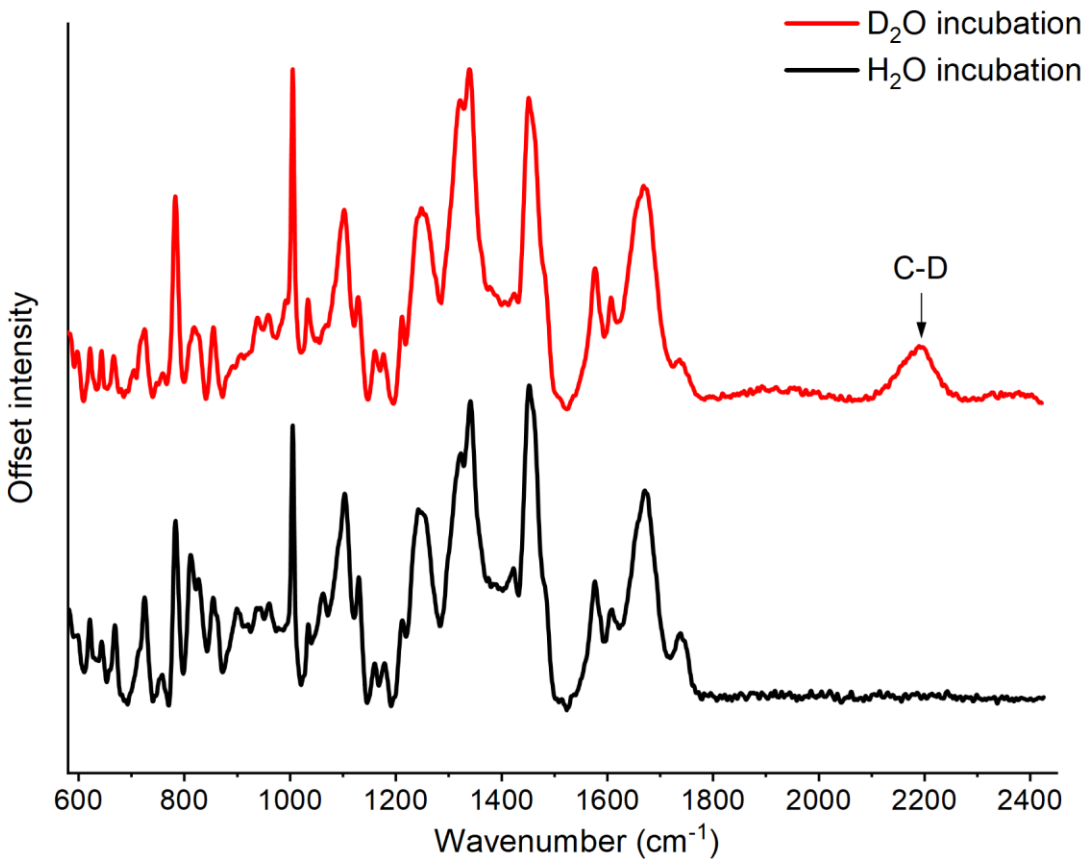

Figure S4. Averaged Raman spectra of *B. cereus* after 24 hour incubation in normal and D_2O enriched media. Each spetrum is a composite of 3 sets (n=30 spores for each set), covering overlapping ranges of 600 – 1400 cm^{-1}, 1200 – 1950 cm^{-1} and 1800 – 2450 cm^{-1}, for a total of n=90 for each spectrum. In the more Raman active region of 600 – 1900 cm^{-1}, it is not possible to clearly assign any peak to deuterium bonds, while the C-D peak is easily detectable even a lower incubabtion times.