Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information for

Quantification of an Exogenous Cancer Biomarker in Urinalysis by Raman Spectroscopy

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1. Surface characterization of the Klarite functionalization

We observed the surface of Klarite Raman substrate before and after *β*-CD functionalized using scanning electron microscope (SEM), atomic force microscope (AFM) and surface enhanced Raman spectroscopy (SERS). CD encapsulation process was performed with the same way described in the main manuscript.

1.1 SEM images

The SEM images were taken at the Advanced Microscope Facility, University of Victoria. The images were obtained using a S-4800 Semi in-lens type field emission scanning electron microscope (Hitachi High-Tech, Japan) operating at 1.0 kV. Figure S1a shows the SEM images of Klarite prior to functionalization. Figure S1b shows the SEM images of Klarite Raman substrate after the thiol-terminated *β*-CD functionalization.

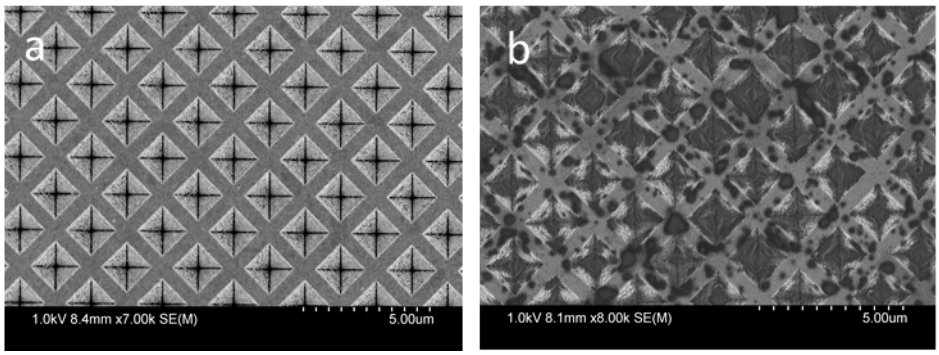

Fig S1. SEM images of Klarite Raman substrate with and without *β*-CD encapsulation. (a) SEM image of bare Klarite. (b) SEM images of Klarite with *β*-CD functionalization.

1.2 AFM images

The AFM scans were performed using an Atomic Force Microscope (Agilent 5500). Figure S2 shows the AFM images of Klarite before and after β-CD functionalization. Clear surface coverage is seen.

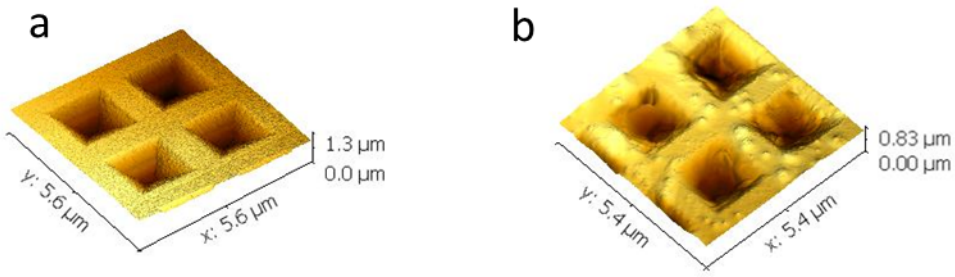

Fig S2. AFM images of Klarite Raman substrate (a) before and (b) after *β*-CD functionalization.

1.3 SERS measurements

SERS measurements of Klarite substrate were performed using the same fiber-coupled low-cost Raman system. The specifications of the system were described in the main manuscripts. Figure S3 show the SERS spectra of Klarite before and after β-CD functionalization. From the Figure, we can see peaks at 534, 674, and 948 cm^{-1} from *β*-cyclodextrin.

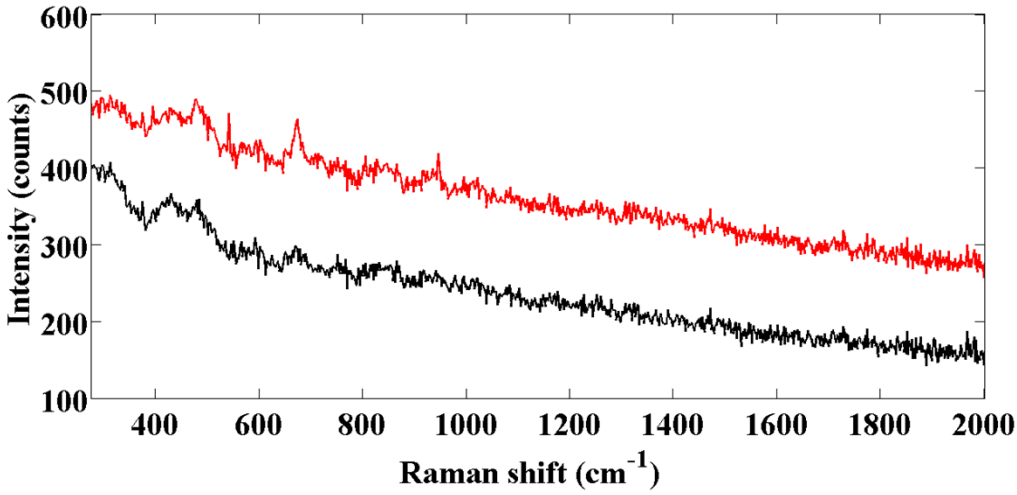

Fig S3. SERS spectra of Klarite with (red) and without (black) *β*-CD functionalization.

2. Incubation experiment

Incubation experiments were using the same approach described in the manuscript. We tried with different incubation times for Klarites with CD encapsulation to capture the AcAm in artificial urine. The Figure S4 shows the summed intensity of spectra with different incubation times. From the figure, we verified that the 4 hours is sufficient.

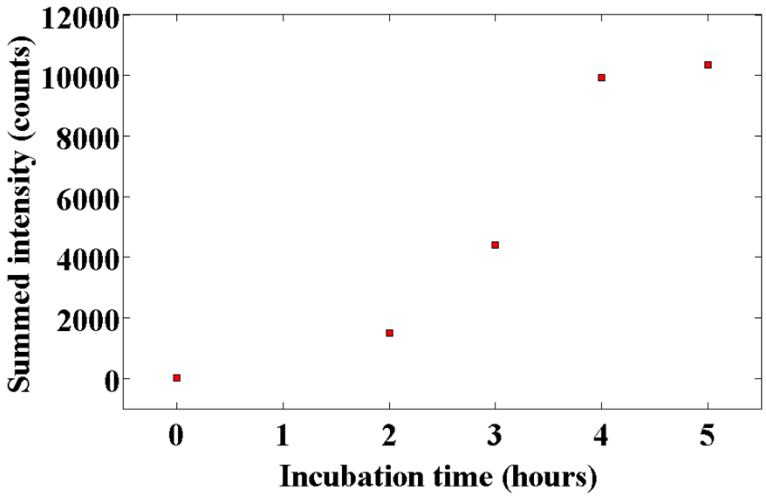

Fig S4. Summed intensity of AcAm versus incubation time.

3. Reproducibility

Figure S5 shows the reproducibility of this method. We did the experiment 5 times and calculated the summed intensity of AcAm for each time. The Figure S5 shows the summed intensity versus the concentrations for the repeated measurement. The error bars were calculated based on the standard error on the mean. For the reproducibility experiment, five spectra were taken for each sample with 30 s integration time instead of twenty spectra. We changed the y-axis to linear scale instead of log scale in the manuscript to make the error bars more easily to read.

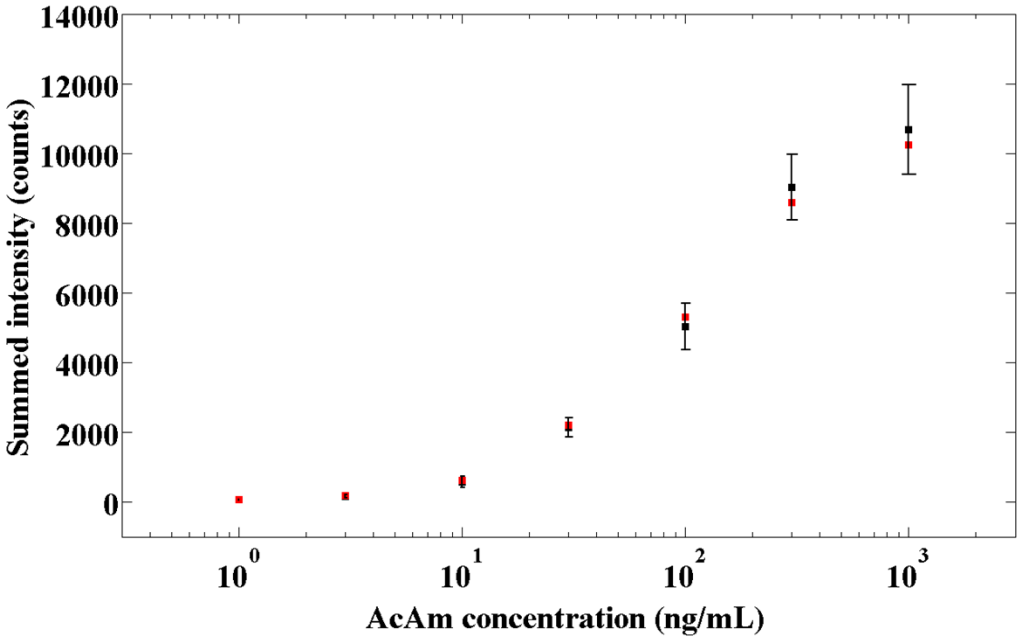

Fig S5. Summed intensity of AcAm versus concentration. The red squares are from the data in main manuscript and the black squares with error bars are from the reproducibility experiment.