-Supporting Information-

Electrofluidynamic Patterning of Tailorable Nanostructured Substrates for Surface-Enhanced Raman Scattering

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S1 Representative Average Surface Roughness in the Centre of EFDP Fabricated Pillar Topology

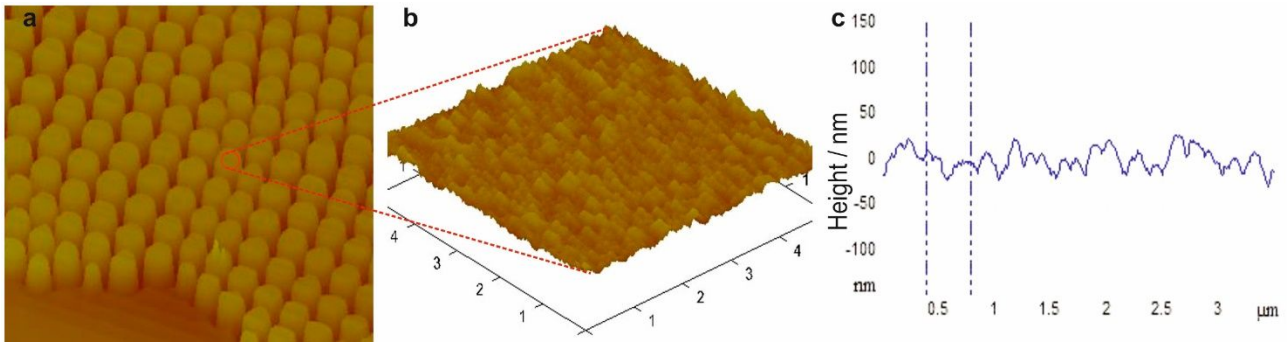

Figure S1. (a) Representative three-dimensional AFM height image of the EFDP fabricated pillar structures with the higher magnification **(b)** AFM image of the average surface roughness. **(c)** The calculated arithmetic average of the height deviations from the centre plane of the surface in the central pillar region was on average of 30.0±12.0nm per 5µm surface area.

S2 Calculation of the Enhancement Factor and Reproducibility Coefficient

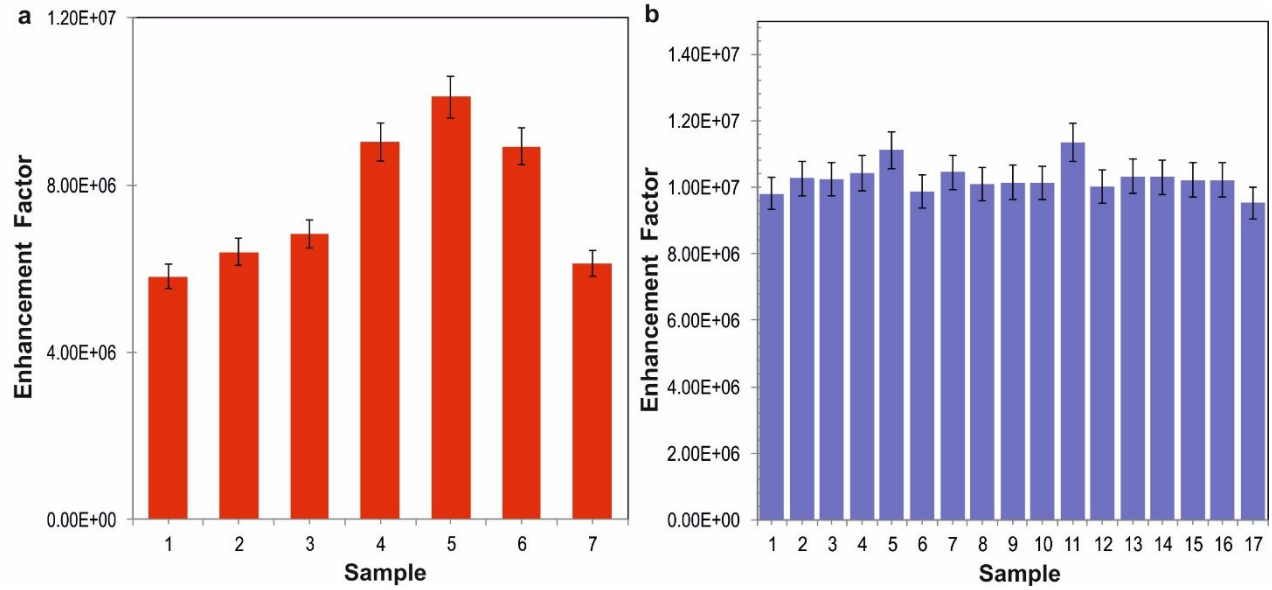

Figure S2. (a) Absolute enhancement factor values of the seven representative pillars in Figure 2. **(b)** For the enhancement factors from the 17 reproducible substrates fabricated *via* the micronanolithographic EFDP apparatus , the reproducibility coefficient and the confidence interval of the mean EF were found to be: Standard Deviation$_{Relative\ EF}$×2.8×100%=8.55%: (9.4x10^6, 1.1x10^7). All of the achieved EFs are on the order of magnitude of x10$^{6\text{-}7}$ with the small variation in the pre-factor values, with the largest difference in the SERS EFs of less than two fold for the between the highest and the lowest substrate, indicating a good signal reproducibility. This is in agreement with the conventional SERS platforms, considered highly reproducible even when showing nearly one order of magnitude difference. [22] The confidence interval limits for the enhancement factor were calculated based on the mean difference of -1.96×stanadrd deviation (differences) and mean difference of +1.96× standard deviation (differences).

S3 Reproducibility of EFDP-SERS Substrates - Uniform Platforms for Reliable SERS

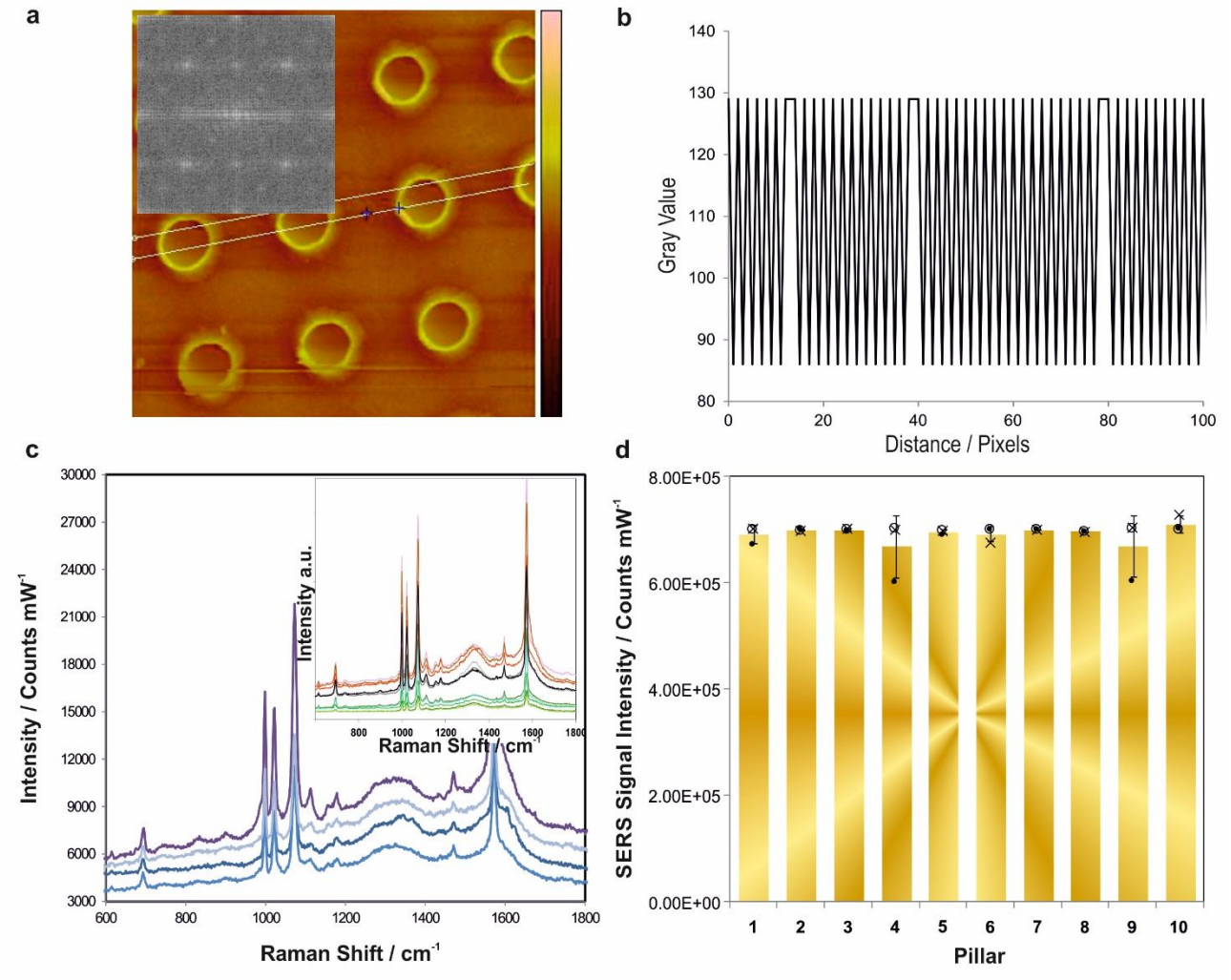

Figure S3. (a) AFM image of the uniform periodic EFDP-fabricated nanoplatforms and the corresponding fast Fourier transform (FFT) image **(inset)**. **(b)** Ingrained by the plot profile of the AFM image the grey value distribution as function of distance across the image, demonstrating highly-consistent structural units with low and high frequencies containing similar image information with two dominating directions in the FFT image passing through the centre, both originating from the uniform structures in the background of the original image. **(c)** These structural units provide a highly-consistent SERS signal over the entire substrate surface area (200x200μm^2) with pillars distribution at the surface in regards to the laser spot size (diameter: ~1.0μm) yielding uniform signal. SERS spectra acquired from four random locations across each substrate as well as from **(inset)** three different substrates at average of five locations (*i.e.,* pillars) each. **(d)** Representative SERS spectra of BT on uniform substrates across ten random pillars on each substrate, for the 1070cm^{-1} peak. Repeatable SERS response was obtained from the pillars with relative standard deviation values of less than 3.0% with an average variation of less than 7.5% in terms of the relative peak intensities in the framework of one substrate (error bars) and between the different samples (height of the bars).

S4 Stability Analysis

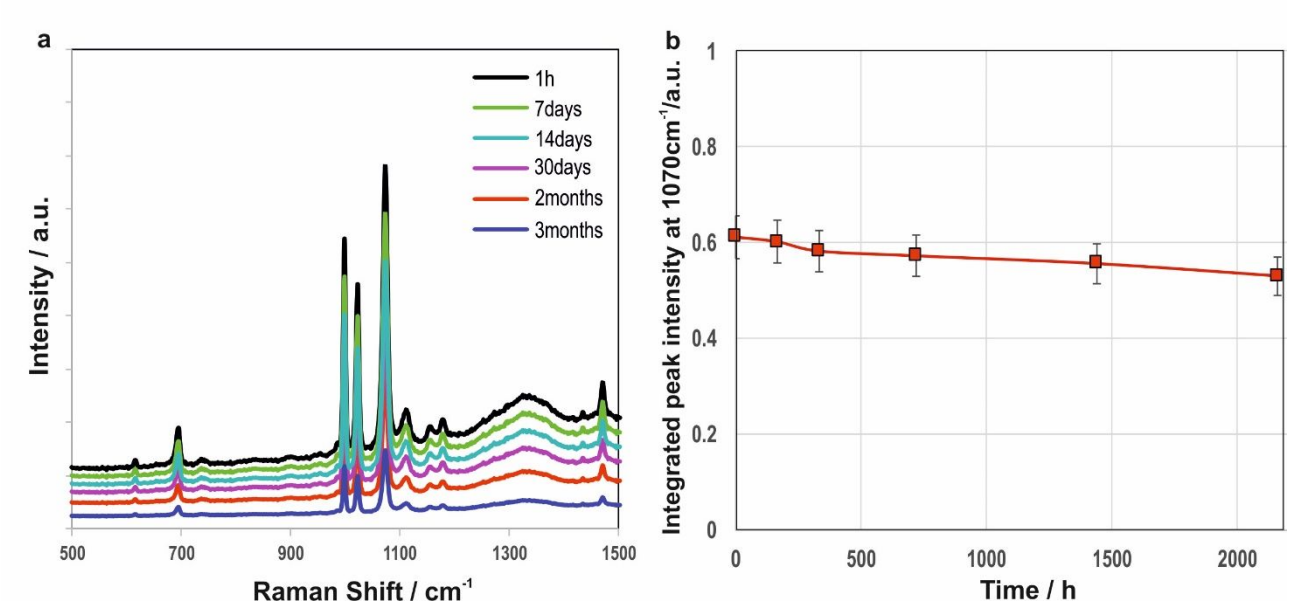

Figure S4. (a) Characterization of SERS activity with the representative Raman spectra of BT on the EFDP-SERS substrates. **(b)** Temporal evolution of the signal intensity of BT at 1070 cm^{-1} with a very minor variation of less than 1% within the first 2 weeks and overall of 5.7% over the period of 3 months. Within the experimental error, the substrates are stable over long periods of time.

S5 Spike-and-Recovery (S&R) Proof-of-Concept Assay of the EFDP SERS Substrate TBI Biomarker

a

Sample	No spike (0 pg.mL)	Spiked (~50 pg/mL)
Control (diluent)	0	53
1	0	51.5
2	0.7	48.9
3	0	51.7
4	0	47.1
5	0	50.9
6	0.7	49.3
7	0.5	52.8
8	2.3	51.5
9	0	49.8
10	0.3	53
Average		50.65
		0.044339623
Mean Recovery %		95.56603774
(+/- S.D.)		(+/-3.4%)

b

Raman Band/cm^{-1}	Peak Assignment
682m	γ (C=O) τ (O-H) in *N*-acetyl bending mode of C=O·O-
717m	$\sigma(C_\alpha C)$, *def*(O-H)
930s	ν(C-C) and N-C symmetric stretching
1010w	out of plane $\rho(CH_3)$, $\tau(CH_2)$ γ (C=O) amides
1168m	γ (C=O) and δ(C-OH)
1420m	$\sigma(COO^-)$, acetylamide in the CH_3-NH-C=O of the *N*-acetyl asymmetric *def*(C_tH_3) and $\delta_s(CH_3)$
1517s	δ(N–H) in (NH-C=O-CH_3) group

Figure S5. (a) S&R validation assay of the EFDP-SERS substrates establish a difference between the diluent and the analytical sample matrix. 10μL of analyte spiked in the 50μL test sample matrix showed a mean recovery (%) response± standard deviation (S.D.) of 95.5±3.4 in the assay relative to a duplicate spike in the standard diluent. **(b)** Representative SERS peak assignments of the N-acetylaspartate.

S6 Characteristic Fingerprint SERS Spectra and Barcode of Peaks of the N-acetylaspartate Marker

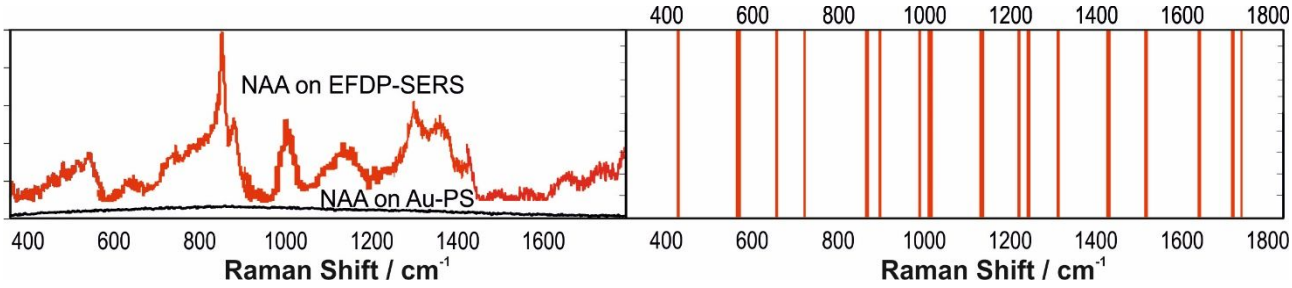

Figure S6. Left: Average Raman spectra of the N-acetylaspartate using the EFDP-SERS substrates (red) and using the reference gold-nanocoated PS film of the same thicknesses used for the EFDP patterning but with no structures on the surfaces (black) recorded at 785nm excitation wavelength, 3mW laser power, with the EFDP substrates, used as fingerprint references. **Right:** The corresponding barcode for the marker. The characteristic finger-prints include the peak at 682cm^{-1}, attributed to the C=O *wagging* and *twisting* of –OH bonds in the *N*-acetyl, the 717cm^{-1} peak originating from the *stretching* of $C_{\alpha}C$ bonds as well as the deformation of the O-H groups. The peaks at and between 1010cm^{-1}, 1168cm^{-1} are from the out-of-plane CH_3 *rocking* mode, *twisting* of the CH_2, the *wagging* of the C=O amides and the N-H *vibrations* in the NH-C=O-CH_3 group at 1517cm^{-1} in the N-acetyl. The peak at 1420cm^{-1} originates from the acetylamide in the CH_3-NH-C=O of the asymmetric *vib* CH_3 deformations.

S7 Roughness of the Au-PS film

The average roughness (*R*a) of the pristine as-spun polymer films and Au coated polymer substrates were analysed using AFM. The mean roughness value represents the arithmetic average of the surface height deviations measured from the centre plane of the sample calculated from five independent measurements.

The AFM analysis indicates that the roughness of the pristine polymer film is slightly lower compared to those after the deposition of the gold nano-layer. The overall roughness after gold deposition is only of the order of ~nm which is not enough to generate any SERS signal under our experimental conditions.

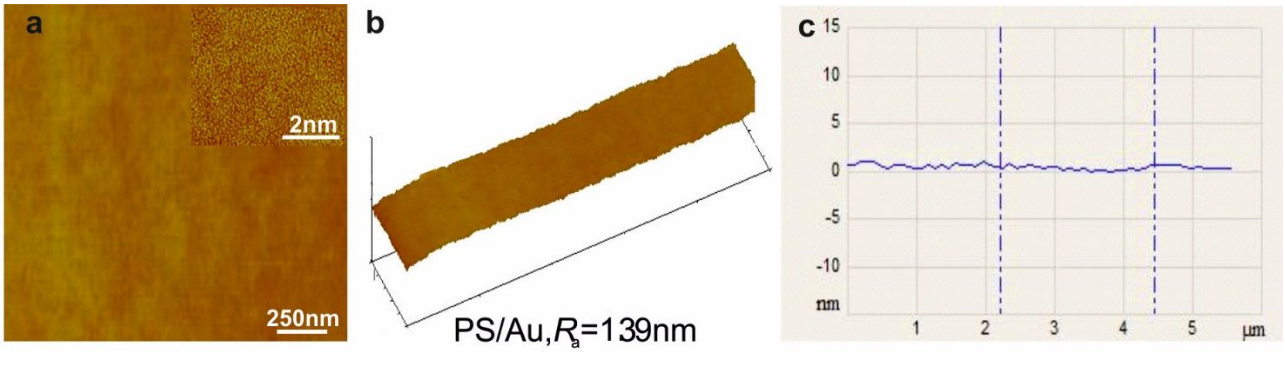

Figure S7. AFM height (left) and three-dimensional morphology (middle) images of (a) as-spun polymer film and of gold layers (b) deposited after two cycles of 30s long sputtering at 70mA on the polymer coated substrate. The cross-sections of the corresponding AFM scans (right hand side) in (a and b) show an average roughness of pristine polymer film and the gold.

S8 Quantitative Detection of Representative Biomarker *via* EFDP-SERS

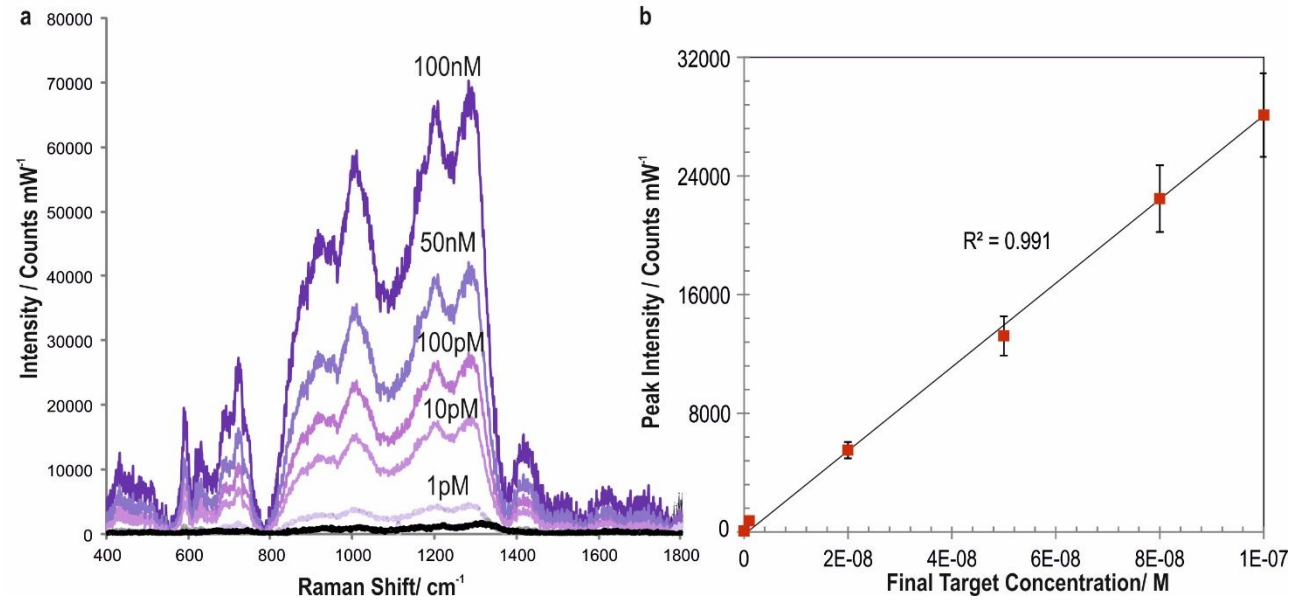

Figure S8. SERS substrates were used as an assay to perform dilutions using the specified concentration range for the biomarker. **(a)** SERS spectra detected from dilutions at 100nM to1fM concentrations and the **(b)** corresponding calibration curve used to calculate the limits of detection, which is three times the standard deviation of the blank divided by the gradient of the linear curve. Error bars denote the standard deviation.

S9 Detectable Biochemical Probes

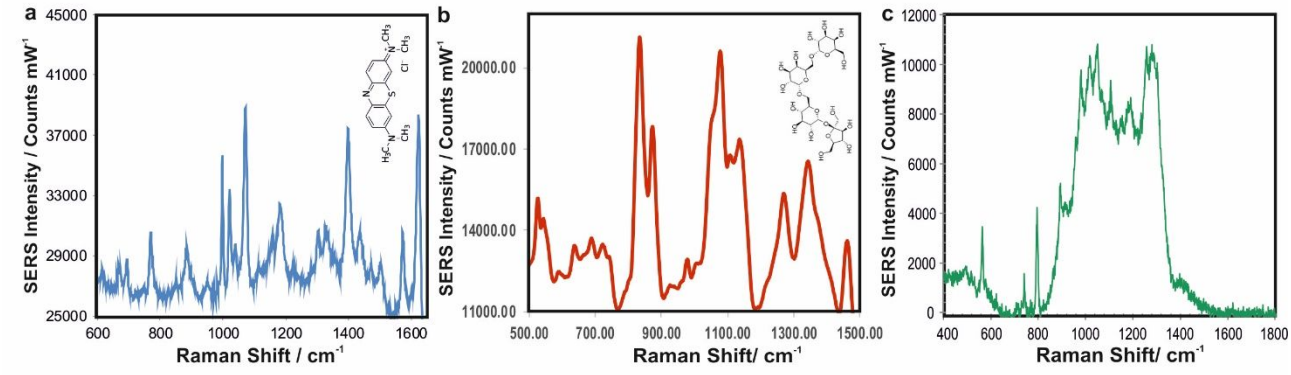

Figure S9. Representative fingerprint SERS spectra for **(a)** Methylene Blue, **(b)** Stachyose and **(c)** S-one-hundred calcium-binding protein B on EFDP-SERS substrates.