Online Sheath-Flow Liquid Chromatography - Surface Enhanced Raman Detection for Phosphorylated Carbohydrates

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Supporting Information

Summary:

This section contains supplemental figures and tables as described in the main article. The spontaneous Raman spectra of each analyte in solution is given. SERS spectra of glucose 1-phosphate, glucose 6-phosphate and fructose 6-phosphate obtained on bare silver SERS-active substrate and on 4-mercaptophenylboronic acid (4-MPBA) SAM silver substrate are reported. A Table of assignments for peaks observed in the SERS spectra is included. Details of PLS regression analysis for calibration model and cross-validation from experimental samples are also provided.

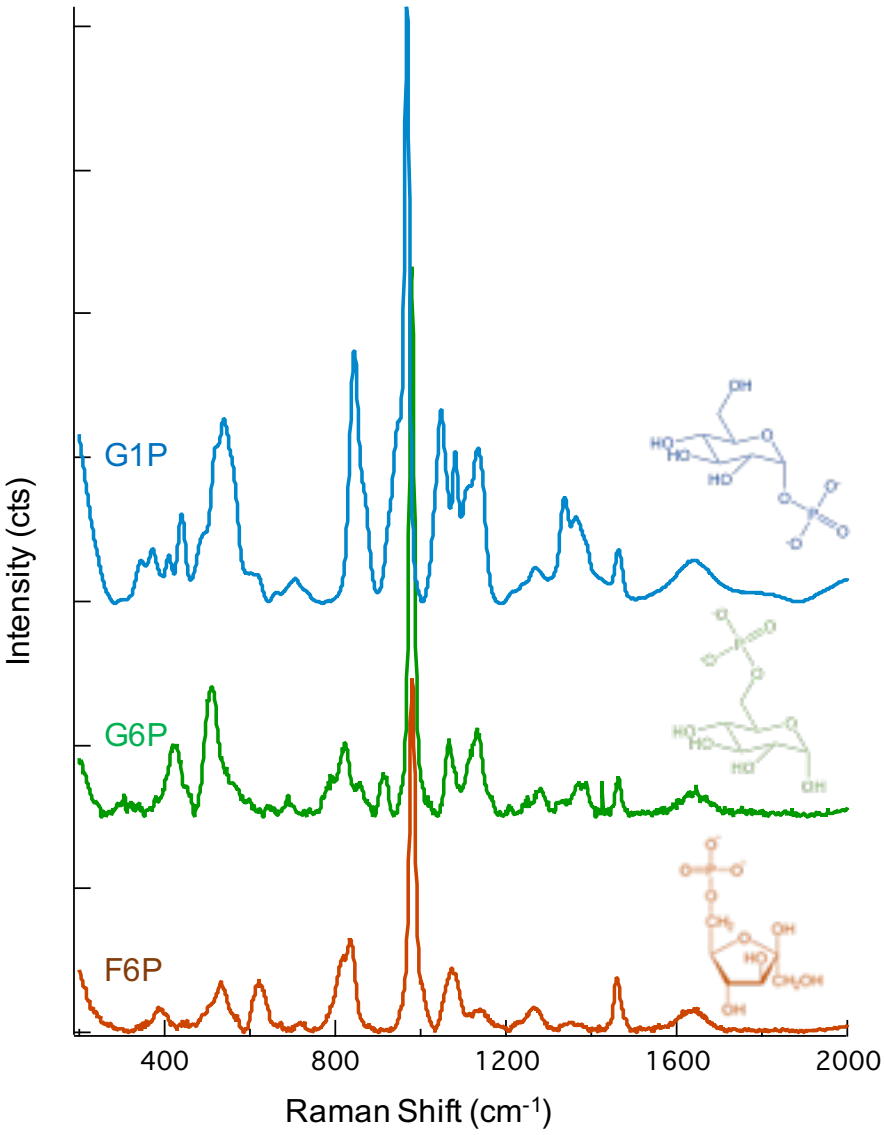

Figure S-1. The solution Raman spectrum of glucose-1-phosphate (blue) and glucose-6-phosphate (green) and fructose-6-phosphate (red) were acquired from 0.5 M solutions of each analyte in water. Differences associated with the structures of each molecule (shown) give rise to distinct Raman spectra that can be used for identification. The spectra are baseline subtracted and offset for clarity. The spectra were acquired using a Snowy Range Instruments Raman spectrometer with 638 nm excitation.

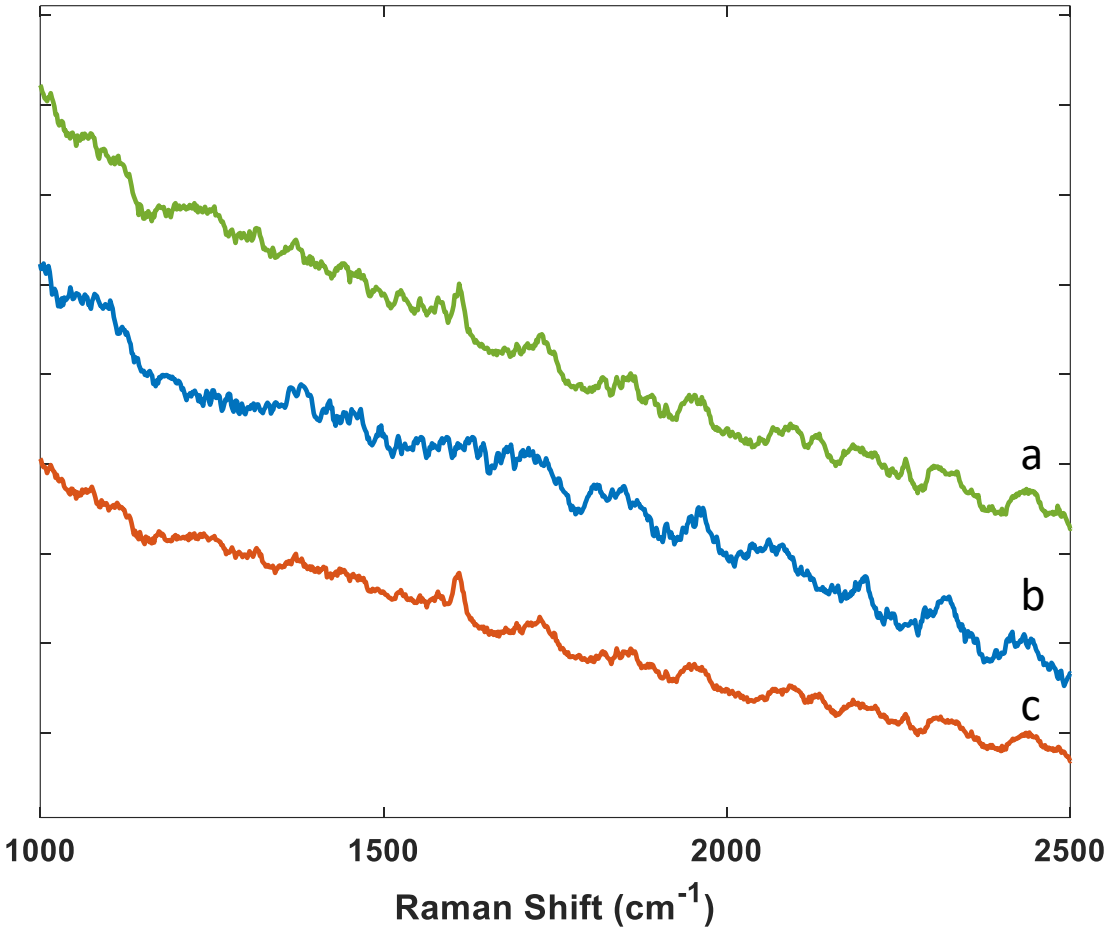

Figure S-2. The SERS spectra observed from (a) glucose 6-phosphate, (b) glucose 1-phosphate, and (c) fructose 6-phosphate using a bare silver SERS substrate are shown. The spectra show very low signal-to-noise ratio due to the low affinity of analytes to the metal substrate. The low signal to noise suggests that the sugars do not readily interact with the silver surface.

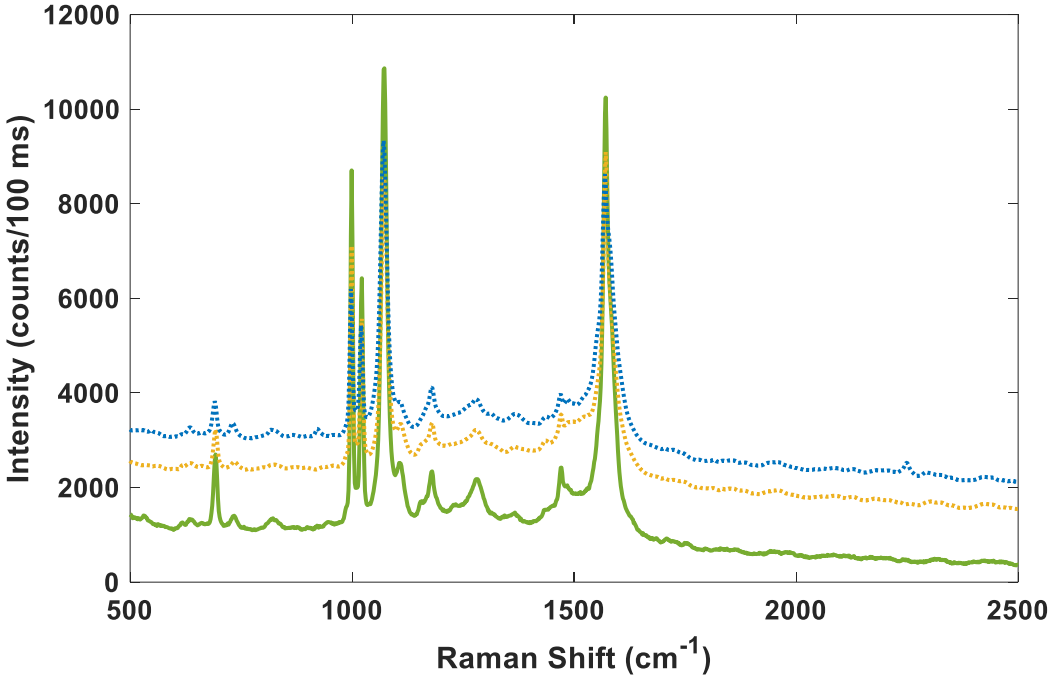

Figure S-3. The SERS spectra obtained from solutions of glucose-1-phosphate (blue dotted line) and glucose-6-phosphate (yellow dotted line) using a 4-mercaptophenylboronic acid (4-MPBA) SAM on silver substrate are shown. The spectrum of the background 4-MPBA acid monolayer (green) line is responsible for the observed peaks, without or without the sugar present. While 4-MPBA is reported to improve glucose detection, the analyte signals were difficult to measure over the the background due to charge repulsion between the boronic acid group on the monolayer and the phosphate group on the analytes at the pH where the boronate linkage will form.

SERS (cm-1)			Tentative Peak Assignments
Glucose 1-phosphate	**Glucose 6-phosphate**	**Fructose 6-phosphate**	
695	695	694	O-C-O bending
856			C-O-C stretching
921	920	921	Phosphate stretching
		994	C-O stretching
1070	1070	1069	C-O-C asymmetric/C-OH stretching
1120	1119	1120	C-O/C-C stretching
	1132		C-C vibration
		1281	C-C vibration
1345	1345	1343	C-C-H bending
		1394	CH2/CH3 vibrations
	1427		CH2/CH3 vibrations
		1523	C-C stretching
1565	1563		C-C stretching
		1618	H-O-H bending

Table S-1. Tentative SERS peaks assignments for glucose 1-phosphate, glucose 6-phosphate, and fructose 6-phosphate.[1-4]

The observation of peaks near 1600 cm^{-1} are not expected based on the powder spectra of the three sugars. These peaks are observed to be reproducible and concentration dependent. A plausible explanation for these peaks is the bending mode of water molecules hydrogen bonded to the sugars. The bending modes of water are evident in the solution Raman spectrum (Figure S-1).

PLS Model Information

The PLS regression calibration model was built by loading the spectra of G1P, G6P and F6P at different concentrations ranging from 0.25 μM to 20 μM, which were normalized against the intensity of acetonitrile at 2250 cm^{-1}. There is one calibration model for each analyte that is utilized to quantify the analyte concentration in observed in both simple mixtures and in cell culture media. Each data point is an average of the SERS spectra taken during the direct injection process, representing approximately 200 spectra in total. Details about how each model is constructed for each analyte together with their LVs are given below. The number of LVs was optimized using PLS toolbox to minimize the root mean square of cross-validation values. The reference SERS spectrum of each analyte is well decomposed into its LVs as shown below (Figure S5-7).

Part A. PLS regression analysis for calibration models (Figure 3 and 6 of main text)

Preprocess treatment of SERS spectra: Baseline (Automatic Weighted Least Squares), Smoothing (order: 0, window: 15 pt, incl only, tails: polyinterp), Mean Center

- **Glucose 1-phosphate**

Input data size: 26 (x-block) and 1228 (y-block) where x-block data represents average SERS spectra at different concentrations while y-block data corresponds to the Raman shift (cm^{-1})

RMSEC = 0.80635

RMSECV = 2.15481

Number of LVs = 3

R^2 Cal: 0.982831
R^2 CV: 0.9082266

Percent Variance Captured by Regression Model

	-----X-Block-----		-----Y-Block-----	
Comp	This	Total	This	Total
1	39.98	39.98	70.31	70.31
2	6.93	46.91	25.28	95.59
3	6.24	53.14	2.31	97.89
4	16.86	70.00	0.39	98.28

- **Glucose 6-phosphate**

Input data size: 34 (x-block) and 1228 (y-block) where x-block data represents average SERS spectra at different concentrations while y-block data corresponds to the Raman shift (cm^{-1})

RMSEC = 0.72537

RMSECV = 0.900078

Number of LVs = 3

R^2 Cal: 0.990772

R^2 CV: 0.985806

Percent Variance Captured by Regression Model

	-----X-Block-----		-----Y-Block-----	
Comp	This	Total	This	Total
1	75.69	75.69	82.86	82.86
2	14.10	89.79	15.32	98.18
3	3.64	93.44	0.90	99.08

- **Fructose 6-phosphate**

Input data size: 27 (x-block) and 1228 (y-block) where x-block data represents average SERS spectra at different concentrations while y-block data corresponds to the Raman shift (cm^{-1})

RMSEC = 0.8876922

RMSECV = 1.71236

Number of LVs = 3

R2 Cal: 0.984265

R2 CV: 0.940989

Percent Variance Captured by Regression Model

	-----X-Block-----		-----Y-Block-----	
Comp	This	Total	This	Total
1	86.11	86.11	90.07	90.07
2	4.28	90.38	7.40	97.46
3	3.73	94.11	0.96	98.43

Part B. PLS regression analysis for cross-validation of analytes concentrations in pure mixture and in cell culture media (Figure 3 and 6 of main text)

- **Glucose 1-phosphate**

Input data size for pure mixture: 3 (x-block) and 1228 (y-block) where x-block data represents average SERS spectra detected from different runs while y-block data corresponds to the Raman shift (cm^{-1})

Input data for cell culture media: 4 (x-block) and 1228 (y-block) where x-block data represents average SERS spectra detected from different runs while y-block data corresponds to the Raman shift (cm^{-1})

Cross-validation method: venetian blinds

RMSEP (pure mixture) = 0.84

RMSEP (cell media) = 2.48

- **Glucose 6-phosphate**

Input data size for pure mixture: 5 (x-block) and 1228 (y-block) where x-block data represents average SERS spectra detected from different runs while y-block data corresponds to the Raman shift (cm^{-1})

Input data for cell culture media: 4 (x-block) and 1228 (y-block) where x-block data represents average SERS spectra detected from different runs while y-block data corresponds to the Raman shift (cm^{-1})

Cross-validation method: venetian blinds

RMSEP (pure mixture) = 1.20

RMSEP (cell media) = 1.78

- **Fructose 6-phosphate**

Input data size for pure mixture: 3 (x-block) and 1228 (y-block) where x-block data represents average SERS spectra detected from different runs while y-block data corresponds to the Raman shift (cm^{-1})

Input data for cell culture media: 5 (x-block) and 1228 (y-block) where x-block data represents average SERS spectra detected from different runs while y-block data corresponds to the Raman shift (cm^{-1})

Cross-validation method: venetian blinds

RMSEP (pure mixture) = 0.30

RMSEP (cell media) = 1.95

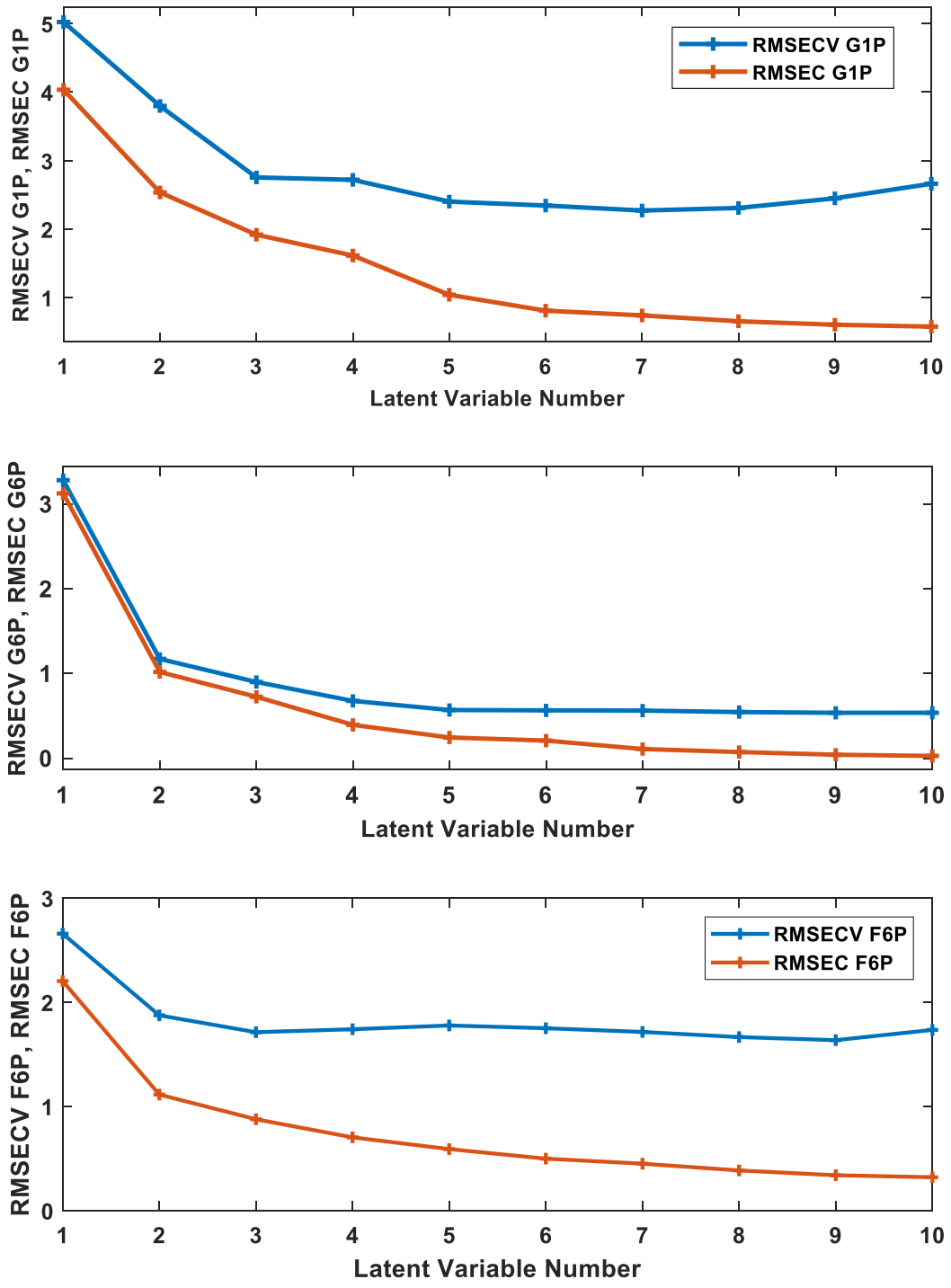

Figure S-4. The plots show how the model statistics RMSEC and RMSECV vary in terms of the number of LVs for the quantification of glucose 1-phosphate (G1P), glucose 6-phosphate (G6P), fructose 6-phoshate (F6P). The number of LV for each analyte was determined using Eigenvector softward to give an optimal RMSE values. 3 LVs were used for the analytes G1P, G6P and F6P.

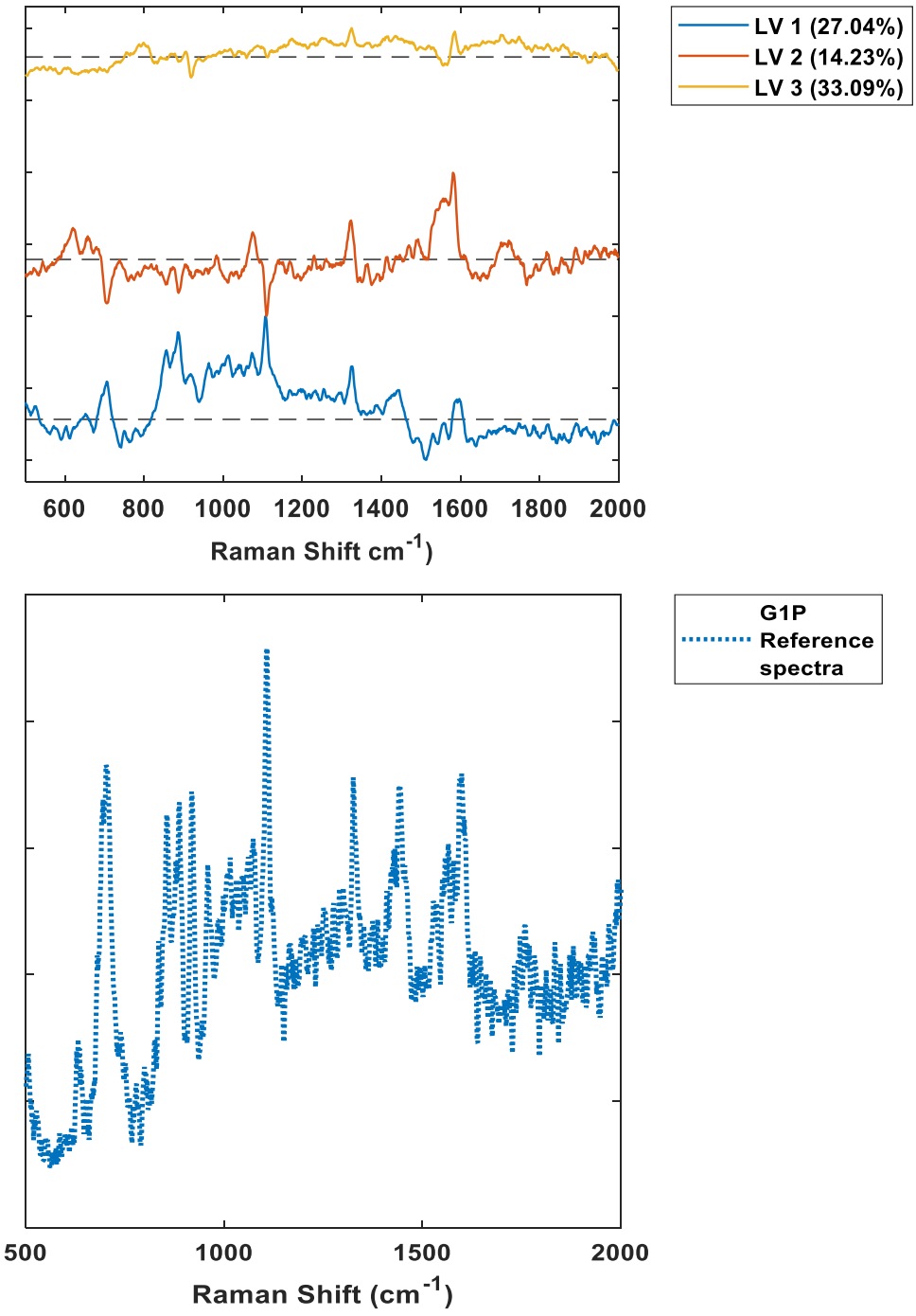

Figure S-5. Plots of the three LVs used in the PLS analysis of G1P (top figure) are compared to reference SERS spectra of G1P obtained from direct injection (bottom figure). The LVs well describe all the characteristic bands of G1P.

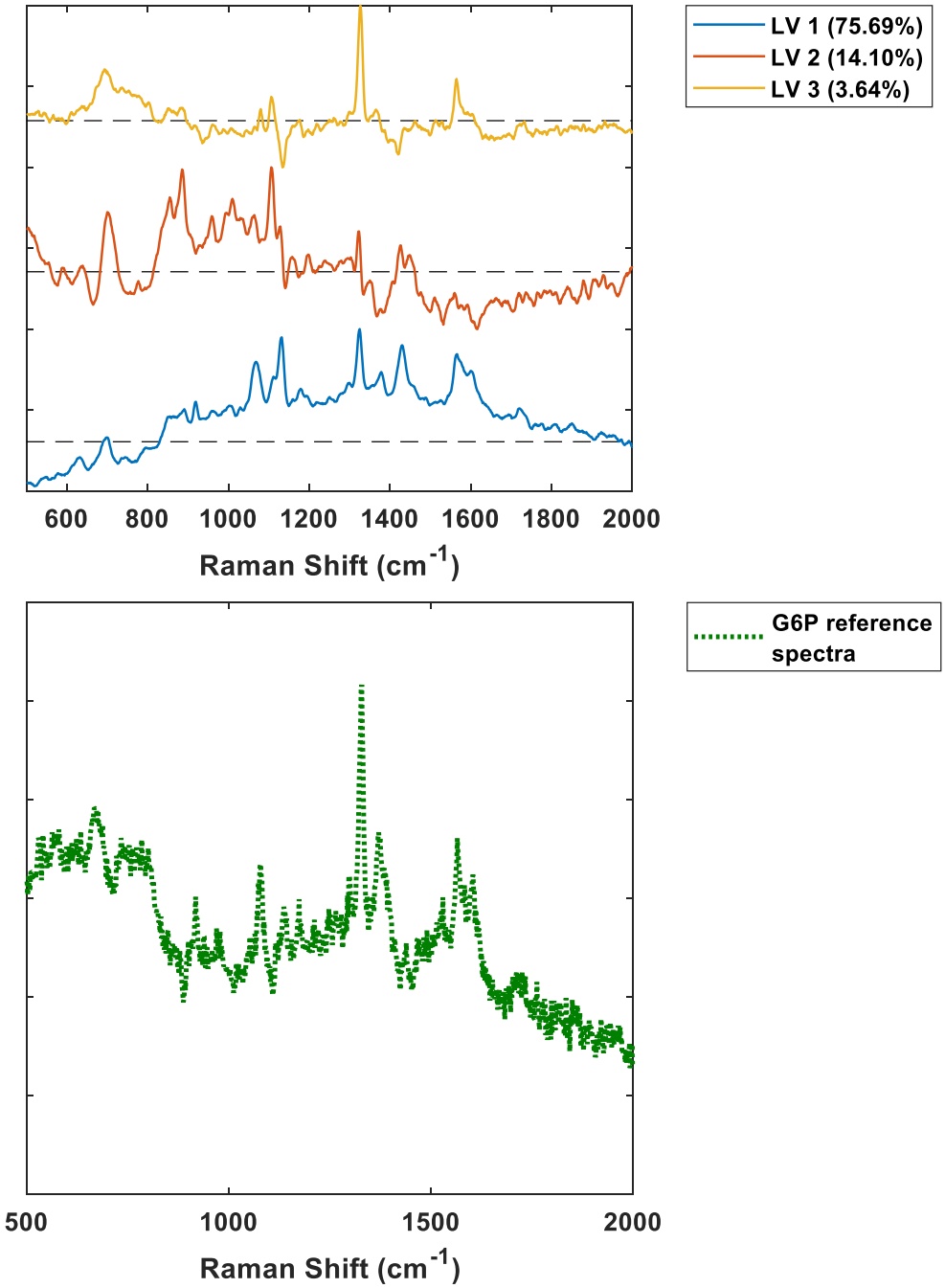

Figure S-6. Plots of the three LVs used in the PLS analysis of G6P (top figure) are compared to reference SERS spectra of G6P obtained from direct injection (bottom figure). The LVs well describe all the characteristic bands of G6P.

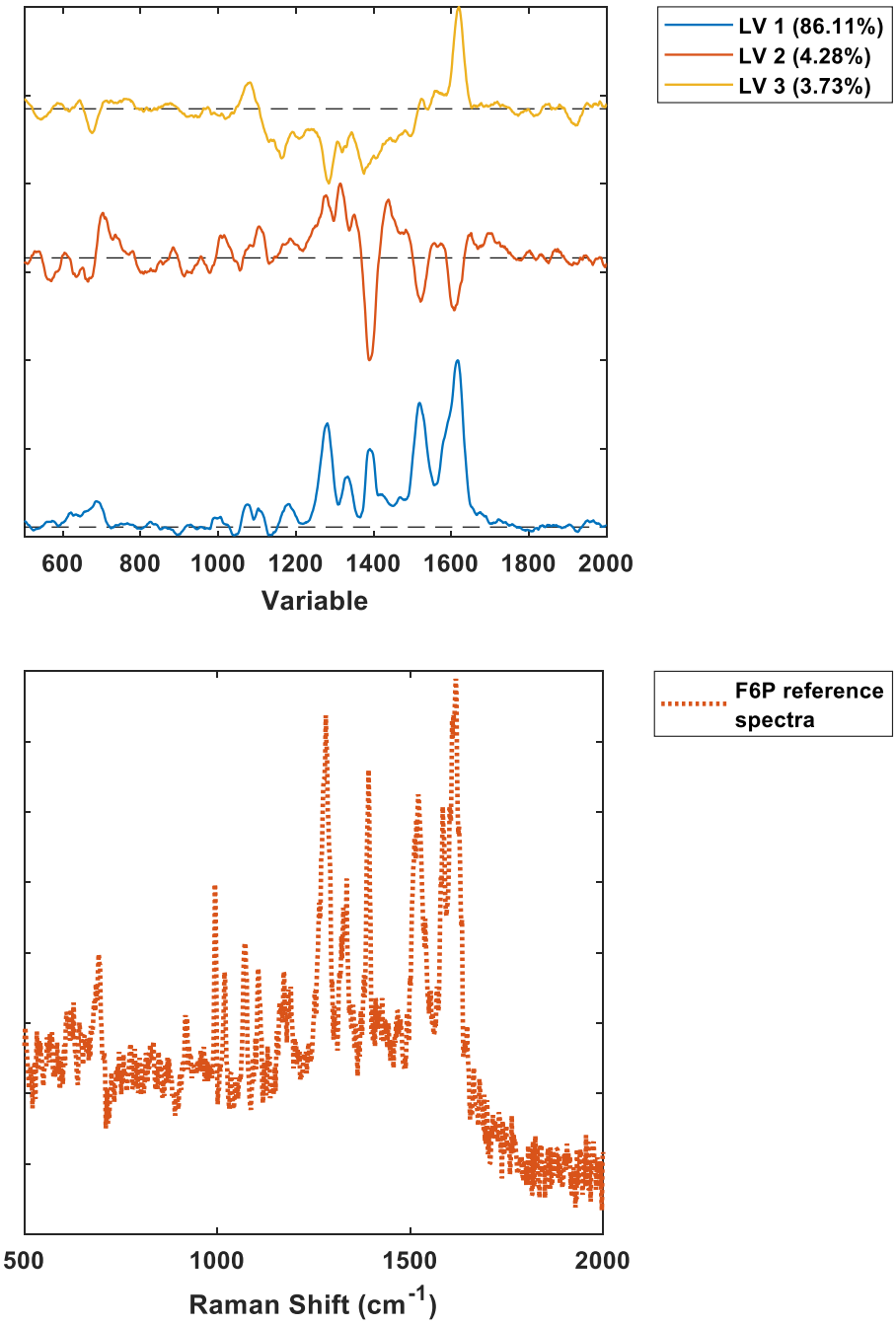

Figure S-7. Plots of the three LVs used in the PLS analysis of F6P (top figure) are compared to reference SERS spectra of F6P obtained from direct injection (bottom figure). The LVs well describe all the characteristic bands of F6P.

References

1. Söderholm, S.; Roos Yrjö, H.; Meinander, N.; Hotokka, M., Raman spectra of fructose and glucose in the amorphous and crystalline states. *Journal of Raman Spectroscopy* **1999,** *30* (11), 1009-1018.
2. Mathlouthi, M.; Vinh Luu, D., Laser-raman spectra of d-glucose and sucrose in aqueous solution. *Carbohydrate Research* **1980,** *81* (2), 203-212.
3. Goeller, L. J.; Riley, M. R., Discrimination of bacteria and bacteriophages by Raman spectroscopy and surface-enhanced Raman spectroscopy. *Appl Spectrosc* **2007,** *61* (7), 679-85.
4. Shafer-Peltier, K. E.; Haynes, C. L.; Glucksberg, M. R.; Van Duyne, R. P., Toward a Glucose Biosensor Based on Surface-Enhanced Raman Scattering. *Journal of the American Chemical Society* **2003,** *125* (2), 588-593.