Online SERS Detection of the 20 Proteinogenic L-Amino Acids Separated by Capillary Zone Electrophoresis

Supporting Information

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Abstract

This supplement contains additional experimental methods regarding the SERS substrate nanofabrication, details on the CZE-SERS setup used for the experiments, Figure S-1 and S-2, as well as Table S-1, S-2, S-3, S-4, S-5, and S-6. Figure S-1 shows a diagram of the experimental CZE-SERS setup. Figure S-2 shows the averaged SERS spectra extracted from the SERS electropherogram of the twenty proteinogenic amino acids. Tables summarizing the observed vibrational bands with assignments in the SERS spectra of six groups of structurally-related amino acids are provided in Table S-1, S-2, S-3, S-4, S-5, and S-6. They include the aromatic side chain amino acids (Table S-1), the acidic and amide side chain amino acids (Table S-2), the basic side chain amino acids (Table S-3), the sulfur side chain amino acids (Table S-4), the aliphatic side chain amino acids (Table S-5), and the alcoholic side chain amino acids (Table S-6).

Experimental Methods

Substrate Fabrication. Silver was vapor deposited onto the anodized aluminum oxide filter at a constant rate of 1.0-1.5 Å/s until a quartz crystal microbalance (QCM) registered a final nominal thickness of 500 nm. Prior to deposition, the Anodisc filters were cleaned for 5 minutes in an Ar^+ plasma (Model PDC-32G, Harrick Plasma, Ithaca, NY) to remove any surface contamination. Following deposition, the substrates were allowed to cool to room temperature under vacuum inside the deposition chamber for half an hour. The deposited metal films were then removed from the deposition chamber and stored in a vacuum desiccator to prevent oxidation and surface contamination.

CZE-SERS Setup. The homebuilt flow cell consists of a FEP plastic base plate, a SERS substrate, and a 250 μm thick silicone gasket with a 2 mm slit to define the flow channel, as well as a stainless steel top plate as shown in Figure S-1. The end of a 50 cm bare fused silica capillary (Polymicro Technologies, Phoenix, AZ) with 72 μm i.d., 143 μm o.d. was tightly clamped in between the gasket and the substrate to deliver the sample into the detection region. The capillary dimensions were chosen so that the ratio of the o.d. to i.d. is as close to one as possible. Under these conditions, the distance between the molecules eluting from the capillary and the SERS-active substrate is minimized, which increases detection sensitivity.[1] The sample injection was pressure driven through the capillary at a flow rate of 1 μL/min using a custom-made injection block.[2] Hydrodynamic focusing of the sample stream inside the flow chamber was achieved by pumping the sheath liquid (15 mM sodium tetraborate buffer, pH 9.4) continuously at a flow rate of 10 μL/min through the flow chamber via the inlet port located on the base plate. The sheath liquid flow rate was controlled using a syringe pump (Model NE-500 OEM, New Era Pump Systems Inc., Farmingdale, NY) controlled by LabView (National Instruments, Austin, TX). The

liquid was drained out of the flow chamber via the outlet channel connected to the waste reservoir. The flow channel was sealed with a standard cover glass, pressed by the top plate, and secured using 4 tensioning screws. The system was grounded directly from the SERS substrate during the CZE separations.

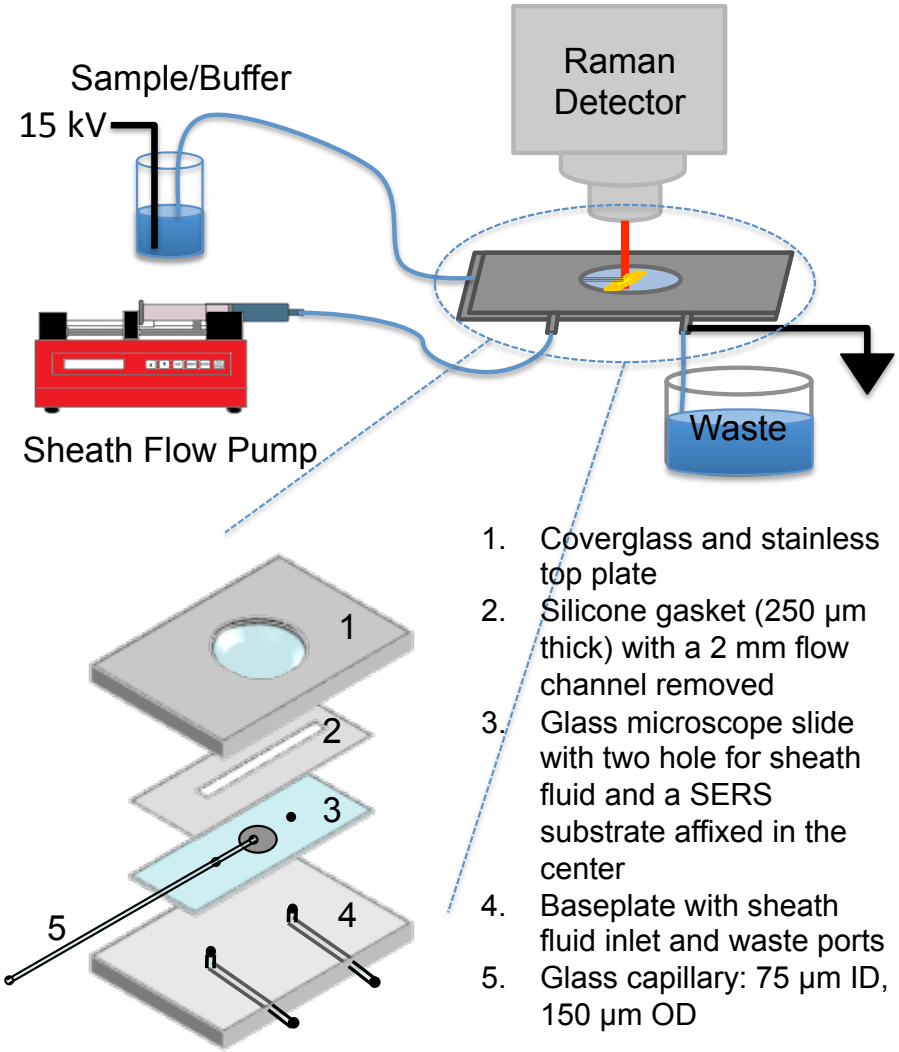

Figure S-1. The instrument layout is shown, illustrating how the sheath-flow SERS detector is incorporated into a capillary electrophoresis experiment. The components of the detector (blow-up) are numbered and described.

Table S-1. Observed vibrational bands with assignments in the SERS spectra of the aromatic side chain amino acids (Trp, Phe, and Tyr). Bands allowing discrimination and identification of the amino acids are highlighted in red.

Peak Position (cm^{-1})			Proposed Band Assignment
Trp	Phe	Tyr	
677			Ring C-H deformation
	813		CH_2 rocking
1004		1004	Symmetric ring C-C stretching
1134	1142		NH_3^+ rocking
1165			Ring C-H bending
		1193	C_β-C_γ stretching
	1244		Ring stretching
1272	1272	1272	CH_2 wagging
1356			Pyrrole ring stretching
1463		1463	CH_2 scissoring
	1516		NH_3^+ deformation
1552	1564	1564	Ring stretching

Table S-2. Observed vibrational bands with assignments in the SERS spectra of the acidic and amide side chain amino acids (Gln, Asn, Glu, and Asp). Bands allowing discrimination and identification of the amino acids are highlighted in red.

Peak Position (cm^{-1})				Proposed Band Assignment
Gln	Asn	Glu	Asp	
		692	692	COO^- deformation
		755	755	NH_3^+ deformation
		795	795	COO^- bending
		893	893	C-C stretching
1123	1123			NH_2 rocking
		1151	1151	NH_3^+ deformation
		1182	1182	C-H bending
1248	1248			CH_2 bending
1346	1346	1346		C-H bending
1407	1407			COO^- symmetric stretching
		1437	1437	CH_2 scissoring
1465	1465	1473	1473	CH_2 scissoring
1575	1575			COO^- deformation

Table S-3. Observed vibrational bands with assignments in the SERS spectra of the basic side chain amino acids (Arg, Lys, and His). Bands allowing discrimination and identification of the amino acids are highlighted in red.

Peak Position (cm^{-1})			Proposed Band Assignment
Arg	Lys	His	
		1123	NH_3^+ rocking
1151	1155		N-H wagging
1175			NH_3^+ deformation
1282	1244		CH_2 wagging
		1306	CH_2 wagging
		1349	C-H bending
1417		1410	COO^- symmetric stretching
		1463	CH_2 scissoring
		1482	Ring stretching + C_1-H in-plane bending
	1486		CH_2 wagging
1523	1536		NH_3^+ deformation
1564	1569	1564	COO^- anti-symmetric stretching

Table S-4. Observed vibrational bands with assignments in the SERS spectra of the sulfur-containing side chain amino acids (Met and Cys). Bands allowing discrimination and identification of the amino acids are highlighted in red.

Peak Position (cm^{-1})		Proposed Band Assignment
Met	Cys	
673	673	C-S stretching
	875	C-C stretching
995		C-C stretching
1155	1155	NH_3^+ deformation
1244	1244	CH_2 wagging
	1349	C-H bending
1443		CH_2 scissoring
	1496	CH_2 scissoring
1539		NH_3^+ deformation

Table S-5. Observed vibrational bands with assignments in the SERS spectra of the aliphatic side chain amino acids (Leu, Ile, Val, Pro, Ala, and Gly). Bands allowing discrimination and identification of the amino acids are highlighted in red.

Leu	Ile	Val	Pro	Ala	Gly	
				692		NH_3^+ wagging
				989		C-C stretching
1112	1108			1106		C-H deformation
1148			1152		1130	NH_3^+ deformation
	1186				1165	NH_3^+ deformation
		1227	1226			C-N stretching
1278	1274					CH_2 wagging
			1366			CH_2 scissoring
1413	1397		1393			COO^- symmetric stretching
			1472			CH_2 scissoring
1493	1493	1493		1490		COO^- symmetric stretching
1572				1564	1543	C=O stretching
1614	1624	1621		1614		COO^- symmetric stretching

Table S-6. Observed vibrational bands with assignments in the SERS spectra of the alcoholic side chain amino acids (Thr and Ser). Bands allowing discrimination and identification of the amino acids are highlighted in red.

Peak Position (cm^{-1})		Proposed Band Assignment
Thr	Ser	
1151		NH_3^+ deformation
1184	1179	C-H bending
	1417	COO^- symmetric stretching
1456		CH_3 asymmetric bending
	1496	COO^- symmetric stretching
1539		NH_3^+ deformation

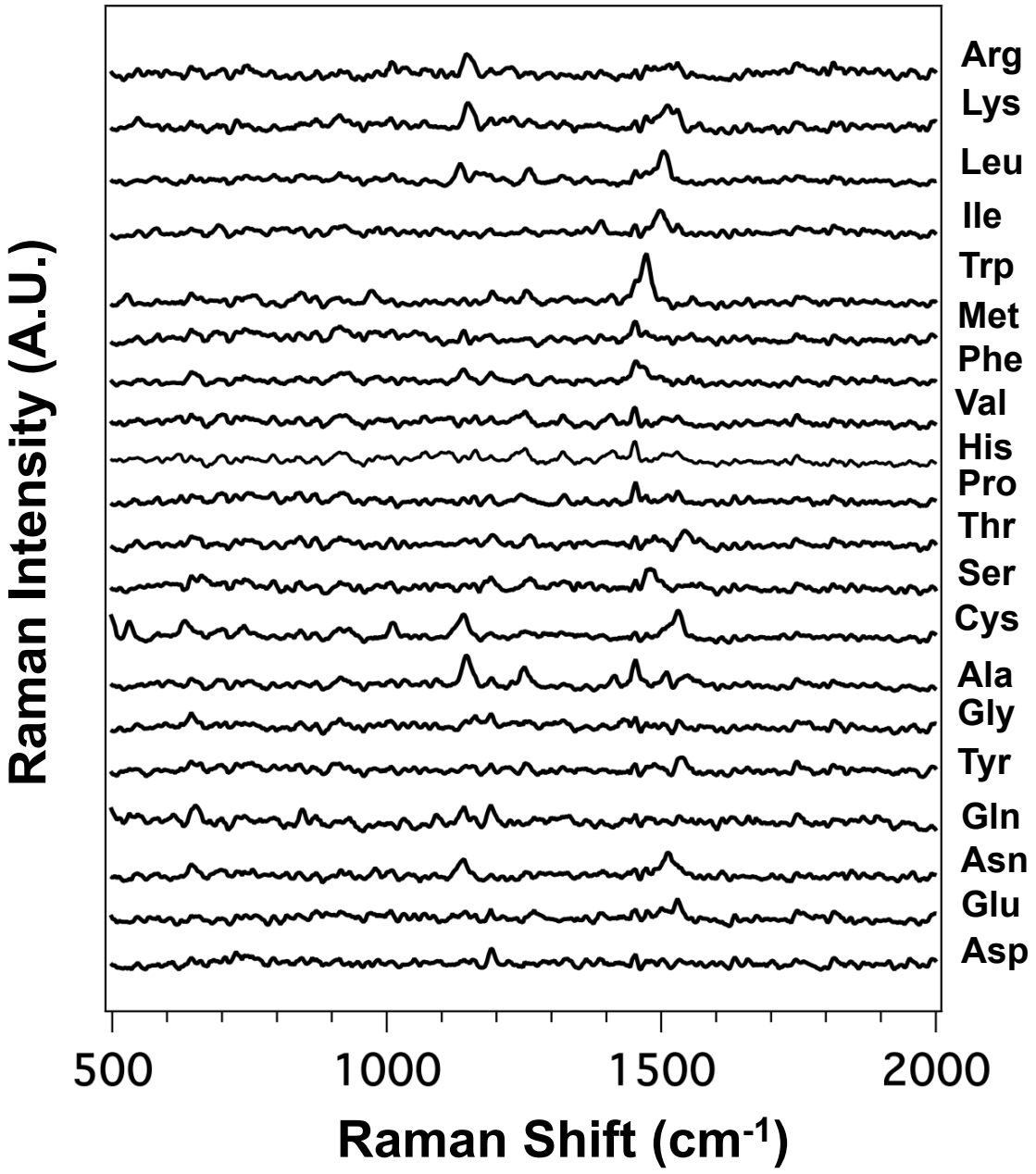

Figure S-2: Averaged SERS spectra of all twenty proteinogenic amino acids extracted from the heatmap shown in Figure 7A. The amino acids were assigned based on the spectral agreement observed in the text and listed in the tables above.

Assignment of Migration Signals in 20 Amino Acid Separation.

Arginine, at t_m= 108.8 s, exhibits bands at 1045 (C-H stretching), 1148 (N-H wagging), 1477 (CH_2 scissoring), and 1530 cm^{-1} (NH_3^+ deformation).

Lysine, at t_m= 110.2 s, shows the bands assigned to the wagging mode of the N-H group (1148 cm^{-1}), the bending mode of the C-H group (1339 cm^{-1}), the scissoring mode of the CH_2 group (1477 cm^{-1}), the deformation of the NH_3^+group (1528 cm^{-1}), and the COO^- anti-symmetric stretch of the carbonyl group (1569 cm^{-1}).

Leucine and its structural isomer isoleucine closely elute at t_m= 112.1 and t_m= 113.1 s. Assignment is made based on bands attributed to stretching mode of the C-H group at 1042 cm^{-1} and the stretching deformation of the COO^- group at 1505 cm^{-1}. As reported in the main text, the band assigned to the CH_2 wagging mode at 1265 cm^{-1} is more intense in the SERS spectrum of leucine than that of isoleucine. Further differentiation between the isomers was made by the deformation mode of the NH_3^+ at 1158 cm^{-1} group of leucine; whereas the spectrum of isoleucine displays the deformation mode of the NH_3^+group at 1189 cm^{-1}.

Tryptophan migrates at t_m= 116.9 s. The main features shown in Figure 7 allowing identification of tryptophan are the bands attributed to the methylene vibrations at 1258 (CH_2 wagging) and 1458 cm^{-1} (CH_2 scissoring). The aromatic contributions present in the SERS spectrum of tryptophan are the two bands at 1178 (ring C-H bending) and 1565 cm^{-1} (ring stretching). The only feature present in the SERS spectrum shown in Figure 7 attributed to the pyrrole ring is the symmetric indole N-H stretching at 1616 cm^{-1}.

Methionine migrates at t_m= 119.7 s and is easily identifiable by the band at 669 cm^{-1} attributed to the C-S stretching motion. The other features present in the spectrum of methionine

shown in Figure 7 are the bands at 1053 (C-H stretching), 1142 (NH_3^+rocking), and 1448 cm^{-1} (CH_2 scissoring), as reported in the text.

Phenylalanine is detected at t_m= 121.0 s by the features attributed to the aromatic constituents of phenylalanine, more specifically, the bands at 642 (skeletal ring stretching), 1193 (C_β-C_γ stretching), 1238 (ring stretching), and 1558 cm^{-1} (ring stretching). The other prominent features present in the spectrum of phenylalanine are the bands at 1137 (NH_3^+rocking) and 1442 cm^{-1} (CH_2 scissoring).

Valine is observed at t_m= 124.3 s. The spectrum is characterized by the C-N stretching mode at 1236 cm^{-1} and the symmetric stretch of the COO^- group at 1488 cm^{-1}, as reported in the article. Again, the absence of bands attributed to methylene vibrations provides further evidence for the identification of valine.

Histidine migrates at t_m= 126.7 s and is readily identified by the bands attributed to the imidazole ring deformation (1194 cm^{-1}), the CH_2 bending (1248 cm^{-1}) and scissoring (1458 cm^{-1}) modes, as well as the symmetric stretch of the COO^- group (1413 cm^{-1}).

Proline is observed at 127.6 s. The features allowing identification of threonine are the deformation of the NH_3^+ group at 1189 cm^{-1} and the C-N stretching motion of the α carbon at 1226 cm^{-1}. In addition, modes of the methylene groups in the cyclic hydrocarbon chain of proline were observed at 1363 (CH_2 wagging) and 1462 cm^{-1} (CH_2 scissoring).

Threonine and serine are detected at t_m= 129.1 and t_m= 131.5 s. In addition to common spectral bands (deformation of the NH_3^+ group at 1189 and 1548 cm^{-1}), the features that distinguish threonine are the bands attributed to the C-N stretching mode at 1265 cm^{-1} and the

band at 1450 cm^{-1} assigned to the CH_3 symmetric bend in threonine, while serine is identified by the band at 1275 cm^{-1} attributed to the methyl group (CH_2 wagging).

Cysteine is observed at t_m= 132.2 s and is easily identifiable by the characteristic C-S stretching mode at 673 cm^{-1}. Other features allowing identification of cysteine include the C-H stretching mode at 1041 cm^{-1}, the deformation of the NH_3^+ group at 1141 cm^{-1}, and the CH_2 scissoring mode at 1496 cm^{-1}, in agreement with the spectrum of cysteine reported in the text.

The SERS spectrum of alanine is detected at t_m= 134.8 by bands characteristic of the carboxyl and amine group, as well as a distinct contribution from the aliphatic side chain. Contributions of the amine group are observed at 1250 (C-N bending mode) and 1144 cm^{-1} (deformation of the NH_3^+ group), while the asymmetric CH_3 deformation mode at 1453 cm^{-1} is attributed to the aliphatic side chain of alanine.

Glycine is observed at t_m= 136.5 s and identified by the bands assigned to the NH_3^+ wagging (662 cm^{-1}) and deformation (1165 and 1543 cm^{-1}) modes of the amine group. The feature allowing identification of glycine is the band 1112 cm^{-1} assigned to the C-H stretching modes of the aliphatic side chain, as reported in the text.

Tyrosine is observed at t_m= 176.2. The SERS spectrum of tyrosine is characterized by a low signal to noise (S/N) ratio. However, two features allow identification of tyrosine. The first is the band at 1463 cm^{-1} attributed to the scissoring mode CH_2. The second is a ring stretching mode at 1560 cm^{-1}, a feature intrinsic to the ring in tyrosine.

Glutamine (t_m= 180.7 s) and asparagine (t_m= 194.6 s) have similar SERS spectra. As reported above, these amino acids can be distinguished from the others amino acids by the bands attributed to the carboxiamide group intrinsic to these two amino acids. The amine bands

observed at 1132 (NH_3^+ rocking) and 1516 cm^{-1} (NH_3^+ deformation), as well as the carboxyl band at 647 cm^{-1} (COO^- wagging) indicate that both the amine and carboxyl groups are interacting with the surface. The band attributed to the scissoring mode of CH_2 at 1465 cm^{-1} is only observed in the SERS spectrum of glutamine, enabling differentiation of glutamine from asparagine.

Glutamic acid (t_m= 207.6 s) and aspartic acid (t_m= 214.2 s) are the last two amino acids to migrate off the capillary. The spectra share some common features, such as the NH_3^+ deformation of the protonated amine terminus at1151 cm^{-1}, the C-H bending motion of the α carbon at 1189 cm^{-1} and the CH_2 scissoring mode at 1440 cm^{-1}. The distinct features allowing identification of the SERS spectrum of glutamic acid are the band at 1265 cm^{-1} assigned to the CH_2 bending mode of the methylene group and the band at 1529 cm^{-1} attributed to the NH_3^+ deformation. These bands are not observed for aspartic acid.

References:

1. P. Negri, K. T. Jacobs, O. O. Dada and Z. D. Schultz, *Anal Chem*, 2013, 85, 10159-10166.
2. S. N. Krylov, D. A. Starke, E. A. Arriaga, Z. Zhang, N. W. Chan, M. M. Palcic and N. J. Dovichi, *Anal Chem*, 2000, 72, 872-877.