Supporting Information

Optical Nanosensing of Lipid Accumulation due to Enzyme Inhibition in Live Cells

Vesna Živanović[†‡], Stephan Seifert[§], Daniela Drescher[†], Petra Schrade[⊥], Stephan Werner[∥], Peter Guttmann[∥], Gergo Peter Szekeres[†‡], Sebastian Bachmann[⊥], Gerd Schneider[∥], Christoph Arenz[†‡], Janina Kneipp[†‡]*

[†]Department of Chemistry, Humboldt-Universität zu Berlin, Brook-Taylor-Str. 2, 12489 Berlin, Germany

[‡]School of Analytical Sciences Adlershof SALSA, Humboldt-Universität zu Berlin, Albert-Einstein-Str. 5 -9, 12489 Berlin, Germany

[§]Institute of Medical Informatics and Statistics, Kiel University, University Hospital Schleswig-Holstein, 24105 Kiel, Germany

[⊥]Department of Anatomy, Charité Universitätsmedizin Berlin, Berlin, Germany

[∥]Helmholtz-Zentrum Berlin für Materialien und Energie, BESSY II, Albert-Einstein-Str. 15, 12489 Berlin, Germany

* e-mail: janina.kneipp@chemie.hu-berlin.de

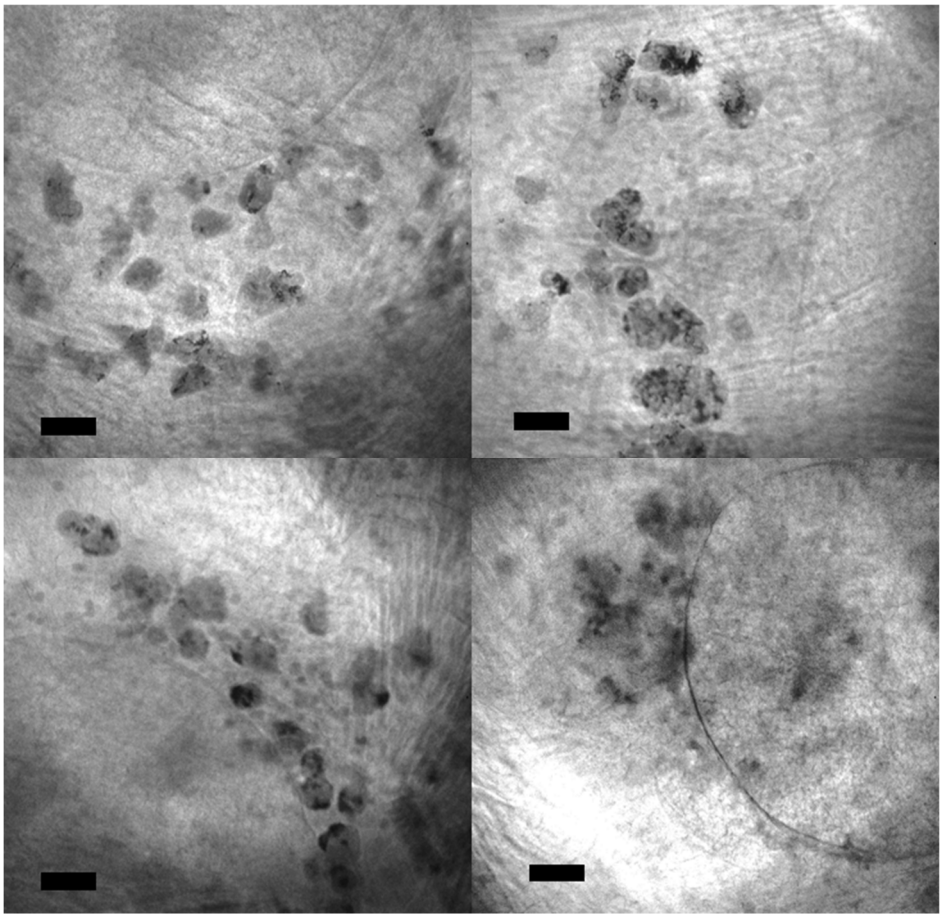

Figure S1. Cryo-X ray images obtained from cells treated with gold nanoparticles and desipramine for 24 h. Scale bars: 1 µm.

Table S1. Raman Shifts and Tentative Assignments of Important Bands in the SERS Spectra from Cells. Assignments Based on Refs [1-8].

Raman shift /cm^{-1}	Tentative assignments
410	Phosphoinositol, CH_2 bend
420	Sterols, CH_2 bend
429/440	Cholesterol
475	TCA
505	Proteins, S–S str
532	Choleterol
580	TCA
620	TCA
630 / 650	Proteins, C–S str
670	TCA
685	TCA
713	Sterols, ring def
730	Lipids, Choline, C–N str
750	Proteins, Trp
770/786	TCA
806	Lipids, O–P–O str
812/818	Lipids, PO_2 str
835	Tyr, ring breathing
845	TCA
885	TCA
920/930	Proteins, proline
940/950/960	TCA
990	Phosphorylated proteins , PO_2 str
1000	Proteins, Phe
1030	Proteins, Tyr, ring def
1086/1097	Phospholipids, C–C str
1105	TCA
1130	Proteins, Tyr, C–C str
1140	Lipids, C–C str
1155	Proteins, Lipids, C–C, C–N
1165	Lipids, ester
1185	Lipids, phosphate
1205	TCA
1211	Tyr, Phe, C-C_6H_5 str
1220/1225/1230	Proteins, Amide III, PO_2^- str
1252	Amide III
1270	Lipids, fatty acids
1278	Lipids, Proteins, PO_4^{3-}, amide III
1295	Lipids, Fatty acids, CH_2 twist
1310	Lipids, CH_2/CH_3 def
1320	Proteins, CH_2, CH_3 def
1336/1350	Proteins, Trp
1370	Lipids, CH_2
1380	Lipids, CH_3 def
1418	Amino acids, CO_2^- str
1440-1465	Lipids, CH_2 def
1452	Lipids, proteins, CH_2 def
1482	Lipids, proteins, CH_2 def
1495	Proteins, Amino acids
1530	TCA,
1550-1570	Proteins, Trp, Amide II
1595	
1602	Ergosterol, C=C str
1608	Tyr, Trp
1618	Trp
1630	Amide I
1650	Lipids, C=C str
1670	Cholesterol
1690	Proteins, Amide I
1700	Proteins, Amino acids
1725-1754	Lipids, ester

TCA, tricyclic antidepressants; Tyr, tyrosine; Trp, tryptophan; Phe, phenylalanine; def, deformation; str, stretching; bend, bending; twist, twisting.

Table S2. Sensitivity and specificity of the random forest classification for all data sets. The incubation sequences are displayed in Scheme 1 in the manuscript text.

incubation sequence	Ami		Des		Imi	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Scheme A	0.79	0.91	0.78	0.89	0.79	0.82
Scheme B	0.71	0.72	0.92	0.86	0.95	0.87
Scheme C	0.83	0.88	0.94	0.92	0.80	0.78

Table S3. Important bands assigned to the respective antidepressant and their related bands obtained by the application of SMD on SERS spectra of cells treated 24 h with TCA prior to 6 h incubation with gold nanoparticles. Bands assigned to antidepressants are marked in red, to lipids in yellow, and to proteins in green. Bands without color are unassigned modes.

Ami											
Important bands	Related bands										
961	776	866	880	892	928	1135	1272	1290	1302	1315	1329
1028	652	1128	1138	1208	1219	1309	1609	1624			
1537	515	835	1460	1573	1585	1593					
Des											
Important bands	Related bands										
780	581	589	722	729	882	897	964	1123	1273	1608	1632
870	579	903	938	1125	1270	1306	1325				
1034	641	669	715	746	753	1475	1493	1504			
1538	1446	1460	1488	1573							
Imi											
Important bands	Related bands										
1032	616	655	661	681	754	846	1055	1479	1530		
1532	514	1487	1576	1593	1605						

Table S4. Important bands assigned to lipids and their related bands obtained by the application of SMD on SERS spectra of cells treated with 24 h TCA prior to 6 h incubation with AuNP. Bbands assigned to antidepressants are marked in red, to lipids in yellow, and to proteins in green. Bands without color are unassigned modes.

Ami												
Important bands	Related bands											
1018	501	653	837	1128	1223	1274	1310	1320	1636			
1061	415	653	828	834	1129	1309	1609	1624				
1138	1316	1341	1661									
1381	1168	1193	1243	1415								
1458	1397	1492	1530	1547	1576							
Des												
Important bands	Related bands											
818	492	518	545	626	650	665	1072	1399	1520	1530	1595	
1013	506	834	1010	1127	1271	1312	1486	1504	1530	1608	1632	1614
1130	1274	1316	1698	1651	1681							
1149	1339	1364	1347	1355	1806							
1456	1492	1515	1530	1545								
Imi												
Important bands	Related bands											
1014	501	510	655	837	1125	1315	1480	1495	1532	1541	1748	
1151	1315	1328	1340	1355								
1458	1495	1515	1533	1540	1556	1581						
1487	1532	1542	1577	1594	500							

Table S5. Important bands assigned to proteins and their related bands obtained by the application of SMD on SERS spectra of cells treated with 24 h TCA prior to 6 h incubation with AuNP. Bands assigned to antidepressants are marked in red, to lipids in yellow, and to proteins in green. Bands without color are unassigned modes.

Ami									
Important bands	Related bands								
505	415	501	654	837	1128	1271	1311	1640	
746	580	590	599	703	883	1112			
833	415	461	507	654	1128	1531	1598	1641	
Des									
Important bands	Related bands								
505	415	459	452	656	834	1130	1503	1608	1632
1125	909	1272	1316	1297	1680	1650	1702		
1541	1487	1468	1461	1575	1590				
Imi									
Important bands	Related bands								
505	381	373	460	655	836	1127	1620	1637	1478
1125	502	1127	1272	1293	1315	1657	1698	1733	
1548	511	518	645	1487	1497	1593	1605		
1607	376	417	459	510	657	1576	1644	1729	1741

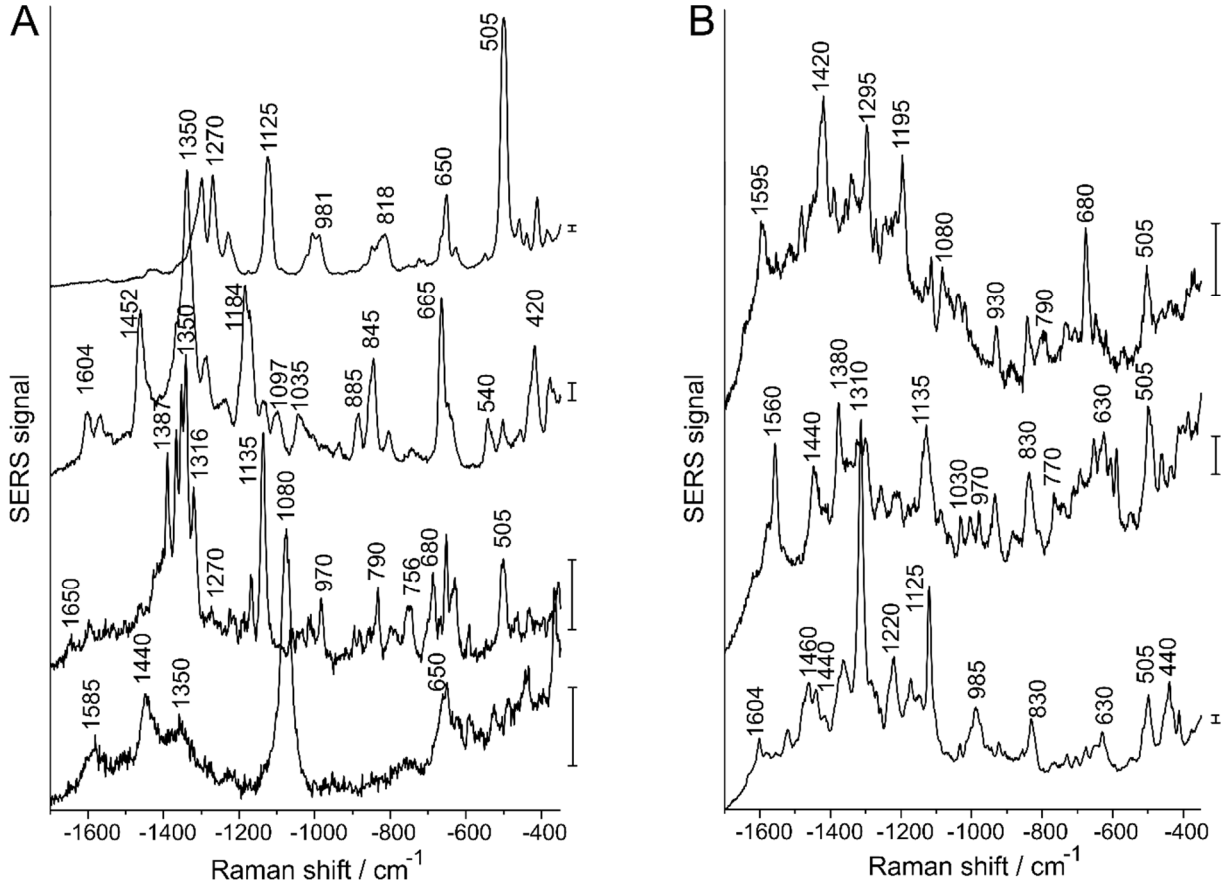

Figure S2. Single SERS spectra (examples) extracted from cells treated with: (A) desipramine and (B) imipramine for 24 h prior to treatment with gold nanoparticles for 6 h. Excitation wavelength: 785 nm, acquisition time: 1 s, excitation intensity: 2×10^5 W cm^{-2}. Scale bars: (A) 20 cps, (B) 100 cps.

Table S6. Important bands assigned to antidepressants and their related bands obtained by the application of SMD on SERS spectra of cells incubated for 6 h with gold nanoparticles prior to the treatment with TCA for 24 h. Bands) assigned to antidepressants are marked in red, to lipids in yellow, and to proteins in green. Bands without color are unassigned modes.

Ami																
Imp. bands	Related bands															
620	371	429	441	478	522	536	666	672	685							
691	544	570	577	598	609	753	883									
783	568	722	731	879	894	913	1216	1261	1283	1323	1364	1351	1312			
869	716	772	792	924	962	966	1274	1279	1286	1292	1303	1330	1366			
960	715	725	732	737	768	776	875	888	900	931	1368	1373	1381	1426		
1028	842	998	1446	1486	1500	1505	1514	1523	1530	1538						
Des																
Imp. bands	Related bands															
1026	1498	584	663	668	678	688	733	749	771	789	849	868	890	911	970	1259
1036	565	584	672	705	698	722	735	749	767	784	861	900	929	948	970	1267
1533	1205	1432	1455	1479	1494	1563	1593									
Imi																
Imp. bands	Related bands															
1028	510	493	653	838	1128	1135	1623									
1111	1219	1255	1266	1290	1304	1313	1663	1704	1715							

Table S7. Important bands assigned to lipids and their related bands obtained by the application of SMD onSERS spectra of cells incubated for 6 h with gold nanoparticles prior to the treatment with TCAs for 24 h. Bands) assigned to antidepressants are marked in red, to lipids in yellow, and to proteins in green. Bands without color are unassigned modes.

Ami

Imp.	Related bands																
721	564	580	768	792	879	890	907	1259	1281	1324							
1082	1162	1184	1191	1548	1564	1575	1173										
1090	1158	1166	1175	1191	1202	1754											
1138	1278	1316	1709	1659													
1165	1220	1238	1284	1373	1703	1687	1774	1799									
1180	1219	1234	1243	1272	1677	1693	1704										
1253	1153	1167	1174	1223	1288	1307	1688	1702									
1276	1127	1137	1225	1235	1300	1322	1686	1704									
1420	1155	1166	1176	1203	1219	1231	1237	1241	1258	1271	1279	1374	1695				
540	585	599	711	749	762												
610	540	585	675	695	715	728	762										
1188	1234	1241	1684	1693	1702	1711											
886	714	731	753	765	780	792	918	924	961	972	1259	1277	1309	1328	1370	1374	1427
1580	1192	1428	1436	1455	1467	1485	1532	1548	1580								

Des

Imp	Related bands																
1277	719	780	770	872	892	903	913	924	1130	1144	1223	1306	1679	1689			
1325	568	724	783	794	868	904	915	923	942	1133	1287	1355	1420	1663	1690	1701	1706
1380	781	793	869	896	905	920	925	1177	1250	1279	1336	1351	1410	1690			
1432	566	730	768	780	791	808	1191	1211	1236	1246	1286	1357	1372	1388	1398	1462	1521
1437	730	771	781	797	807	818	1162	1172									
1458	1198	807	816	1190	1509	1521	1532	1547									
1468	810	816	1191	1198	1207	1520	1542	1557									
1477	660	809	818	1505	1546												

Imi

Imp	Related bands											
1093	1172	1161	1189	1203	1220	1244						
1087	1170	1191	1415									
1098	1158	1191	1202	1210	1244	1244	1250	1420	1427	1693	1702	1168

Table S8. Important bands assigned to proteins and their related bands obtained by the application of SMD on SERS spectra of cells incubated for 6 h with gold nanoparticles followed by treatment with TCA for 24 h. Bands) assigned to antidepressants are marked in red, to lipids in yellow, and to proteins in green. Bands without color are unassigned modes.

Ami														
Important	Related bands													
829	504	655	1000	1014	1022	1127	1505	1521	1544	1487				
1607	515	520	548	1461	1484	1523	1539	1561						
1618	389	416	463	490	507	650								
640	357	367	428	441	466	479	660	683						
669	523	535	619	638	701	737	747							
853	701	710	718	739	766	802	881	911	925	962	973	1379	1390	
1340	1139	1272	1286	1300	1681	1704								
Des														
Important	Related bands													
837	505	655	1053	1067	1074	1129	1137	1315	1323	1510	1605	1619	1631	1667
1237	1147	1151	1277	1636	1693	1693	1076	1721	1777					
750	542	565	572	579	588	606	679	695	704	861	870	884	915	933
1505	816	1449	1553	1593	1591									

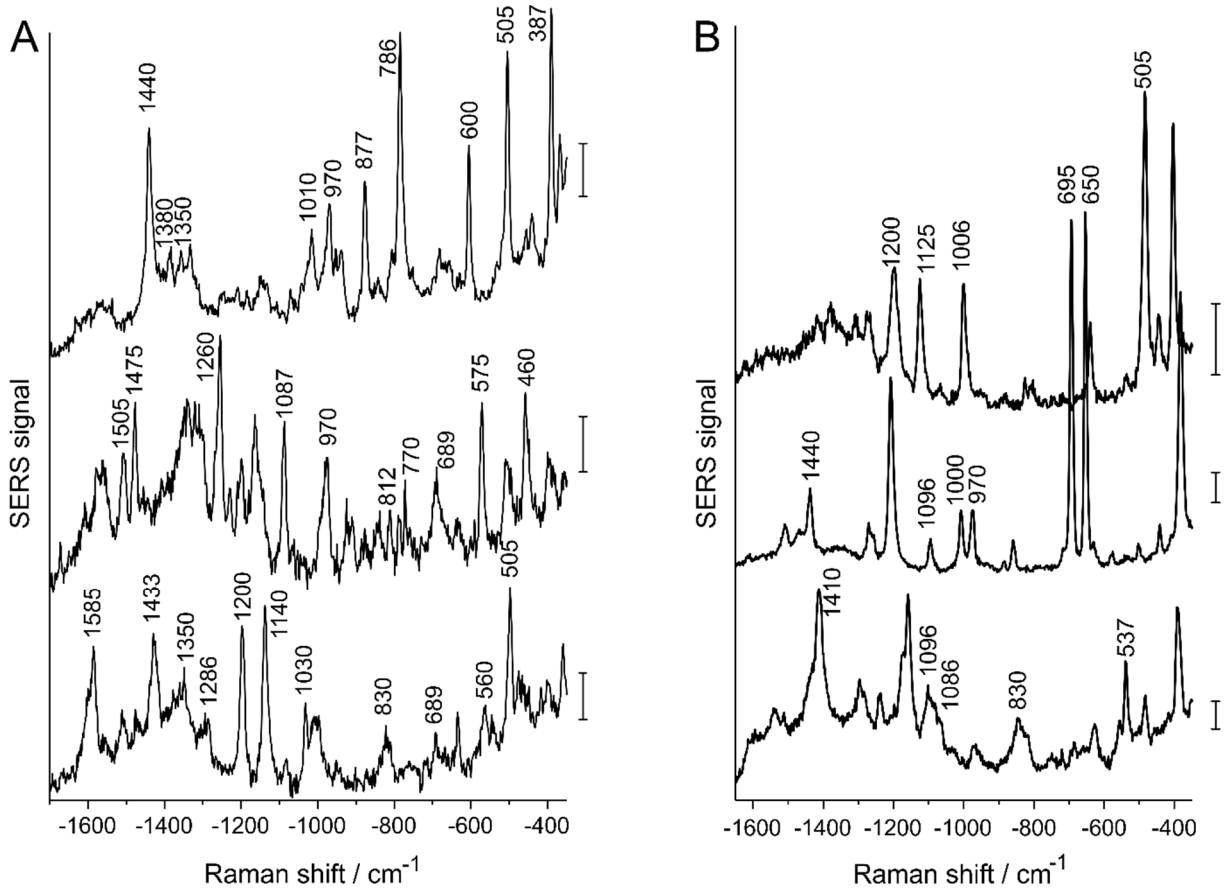

Figure S3. Single SERS spectra (examples) extracted from cells treated with gold nanoparticles for 6 h prior to treatment with (A) desipramine and (B) imipramine for 24 h Excitation wavelength: 785 nm, acquisition time: 1 s, excitation intensity: 2×10^5 W cm^{-2}. Scale bars: (A) 20 cps, (B) 50 cps.

Table S9. Important bands assigned to antidepressants and their related bands obtained by the application of SMD on SERS spectra of cells incubated with TCA molecules and gold nanoparticles for 24 h. With red color are marked bands assigned to antidepressants, with yellow bands to lipids and with green bands to proteins. (Bands without color are unassigned modes).

Ami

Imp.	Related bands														
1037	970	883	980	874	862	1531	1516	1541	1466	1492	1503	1483	1453	761	730
868	886	925	768	792	719	724	1274	1267	1286	1362	1367	1376	1333	962	
1205	1240	1251	1701	1711	1689	1288	1166	1155	1300	1277	1309				
383	1642	1651	1666	1729	453	438	483	478							

Des

Imp.	Related bands														
446	461	400	472	379	356	363	393	484	520	538	645	663	634		
1037	874	900	911	970	748	921	584	742	720	784	773	565	1262	1408	704
1205	1242	1228	1251	1175	1155	1284	1277	1711	1701	1112	1121				

Imi

Imp.	Related bands														
591	568	720	727	770	774	874	893	1253	1241	1086	1275	1548	1438		
1037	747	757	687	683	968	977	990	675							
1205	1193	1769	1228	1703	1239	1253	1751	1717	1735	1693	1264				
1207	1228	1239	1706	1765	1251	1688	1718	1264	1172						
1544	1508	1495	1489	1474	1450	1463	1439								
384	364	400	450	411	461	421	431	1650	1636	481	1668	651	671	664	
1111	1264	1722	1715	1704	1257	1289	1303	1219	1666	1313					

Table S10. Important bands assigned to lipids and their related bands obtained by the application of SMD on SERS spectra of the cells incubated with TCA molecules and gold nanoparticles for 24 h. With red color are marked bands that are assigned to antidepressants, with yellow bands that are assigned to lipids and with green bands that are assigned to proteins. (Bands without color are unassigned modes).

Ami

Imp.	Related bands																
1084	1064	1191	1184	1166	1200	1175	1548	1577									
1151	1704	1697	1681	1690	1675	1237	1229	1104									
532	1112	1226	1192	1124	1106	1268	1235	1245	1253	1316	1306	1278	1089	1184			
1167	1704	1692	1679	1372	1239	1228	1219	1247	1367	1211	1384						

Des

Imp.	Related bands																
1061	1046	1087	502	654	1618	1597	1128	972	827	900	956	949	933	941	1272	415	
1082	1058	503	827	832	1193	1184	1179	1591	1584	1171	1200	654	1163	1573	1607	1617	415
1084	1057	1191	1184	1180	502	1591	1585	1200	1173	1162	827	834	654	416	1156	1572	1565
1090	1071	1057	1191	1173	1200	502	1162	655	827	1590	1481	1154	415				
1160	1170	1820	1770	1705	1728	1241	1231	1688	1677	1251							
1044	900	948	932	964	911	874	756	709	719	727	585	773	1130	502	1330	1309	1408

Imi

Imp.	Related bands												
410	357	1634	375	384	460	488	493	1620	502	1679	1737		
1060	498	759	705	975	965	744	753	956					
1084	1061	1192	1184	1200	1279	1383	1173						
1095	1191	1174	1201	1166	1211	1422							
1167	1708	1691	1241	1211	1679	1255	1224						
1178	1196	1703	1693	1237	1211	1715	1782	1746	1734	1250	1262	1272	
1463	1440	1549	1538	1559	1522	1576	1388	1376	768	726	1190	1240	
1488	1526	1548	1452	1576	1438								
1044	748	758	972	962	901	741	728	913	895	924	949	700	720

Table S11. Important bands assigned to proteins and their related bands obtained by the application of SMD on SERS spectra of the cells incubated with TCA molecules and gold nanoparticles for 24 h. With red color are marked bands) that are assigned to antidepressants, with yellow bands that are assigned to lipids and with green bands that are assigned to proteins. (Bands without color are unassigned modes).

Ami

Imp.	Related bands																
1561	1528	1518	1467	1474	1485	1498	1454	1437	1434	1198	1191	1575	1587				
1511	1548	1478	1573	1458	1442	1440	1191	1181	1187	1198							
1213	1237	1250	1285	1702	1711	1259	1277	1307	1184	1174	1166	1155					
948	895	872	906	1336	1345	1327	1315	779	1280	730	721	1135					
933	894	906	880	1331	1338	1358	1344	1297	1311	1279	1288	779	794	1135			
1101	1205	1192	1218	1182	1175	1427	1434	1252	1244	1264	527						
356	405	454	1648	1654	1659	1666	1747	1735	1727								

Des

Imp.	Related bands									
1591	1559	1533	1524	1515	1493	1485	1476	1470	1458	1437
1007	1088	1454	1120	829	657					

Imi

Imp.	Related bands																
1560	1535	1529	1517	1495	1482	1475	1468	1451	1445	1431							
1591	1556	1529	1517	1506	1488	1475	1456	845	515								
1248	1217	1699	1292	1280	1369	1192	1177	1116	1154								
948	927	901	872	781	1280	1338	1355	1361	720	730	767	1272	793	1309	1296	1258	1135
1700	1693	1707	1774	1728	1669	1674	1680										
901	920	878	781	774	789	1281	1296	1336	1327	1259	1361	1351	730	724			
919	895	1283	1329	1340	1315	780	1361	1290	1270	870	796	768	1133	721			
933	911	899	1280	1313	1335	1327	1296	1275	1365	877	773	1261	1254	1135	722	794	
356	383	460	1650	1659	1666	1636	432	1735	1744	1725	1776						
375	364	400	440	432	451	425	1652	1637	672	660	479						
532	1277	1114	1245	1123	588	614	564	579	755	712	766	1305	1315	1252	1089	1198	1287
1516	1548	1565	1576	1459	1450												
1192	1231	1238	1245	1156	1702	1696	1264	1378	1369	1323	1331	1710	1688				

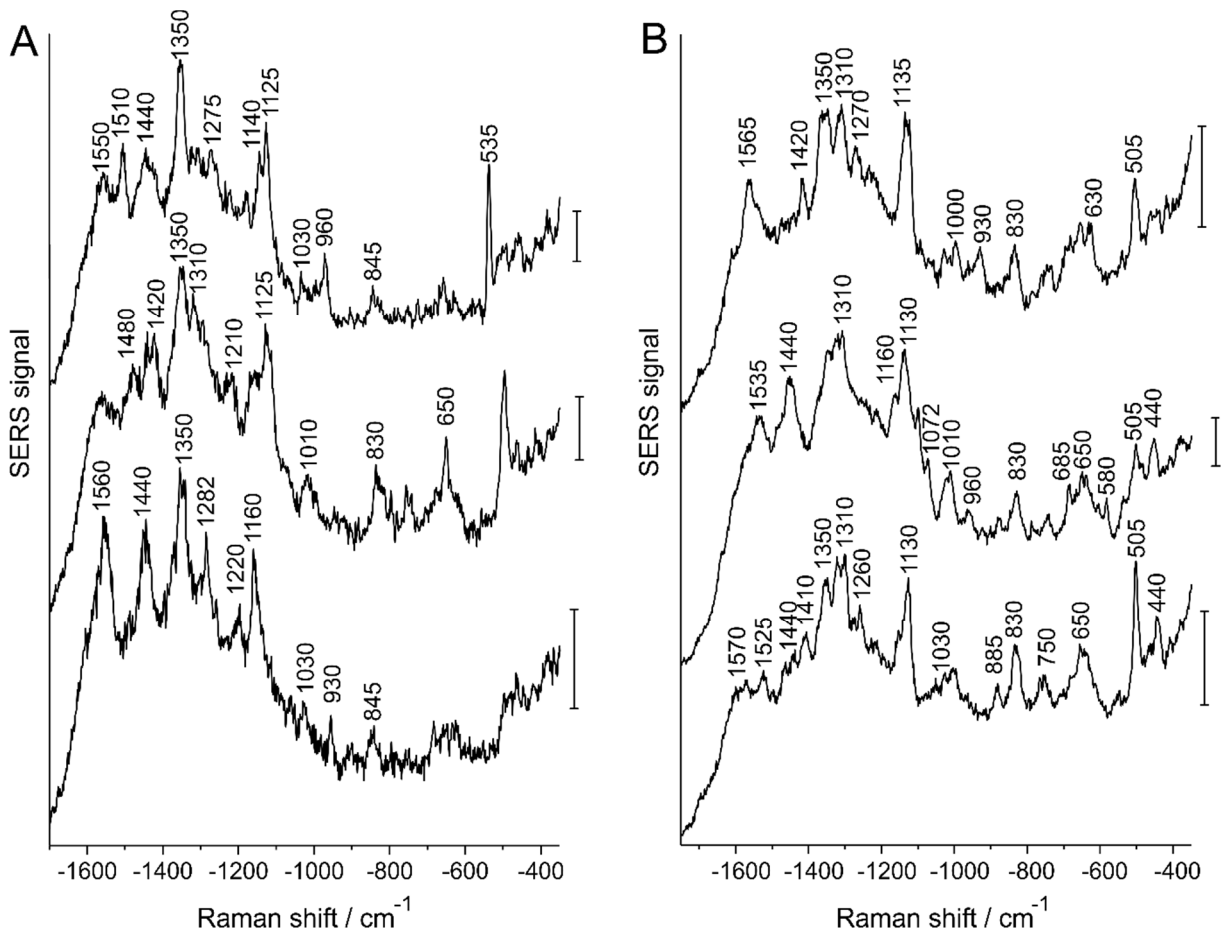

Figure S4. Single SERS spectra (examples) extracted from cells treated with gold nanoparticles and with (A) desipramine and (B) imipramine simultaneously for 24 h. Excitation wavelength: 785 nm, acquisition time: 1 s, excitation intensity: 2×10^5 W cm^{-2}. Scale bars: (A) 50 cps, (B) 200 cps.

Table S12. Results of the classification of spectra with SERS signals (non-empty) and spectra without a SERS signal (empty) by random forest. Spectra without SERS signals occur due to absence of gold nanoprobe containing endolysosomes in the respective focal volumes. The incubation sequences are displayed in Scheme 1 in the manuscript text.

incubation sequence	Ami		Des		Imi	
	Non-empty	Empty	Non-empty	Empty	Non-empty	Empty
Scheme A	886	928	875	605	897	926
Scheme B	232	2058	205	2799	321	1484
Scheme C	1736	555	1556	565	897	822

Table S13. Number of spectra in the training and test sets used in the classification according to treated and control spectra by random forest for the different TCA incubations. The spectra are obtained from the non-empty spectra identified in Table S1. The incubation sequences are displayed in Scheme 1 in the manuscript text.

incubation sequence	Ami		Des		Imi		Controls	
	Training	Test	Training	Test	Training	Test	Training	Test
Scheme A	150	736	100	747	150	725	150	142
Scheme B	150	132	100	247	150	221	100	105
Scheme C	150	1586	100	729	150	1395	150	525

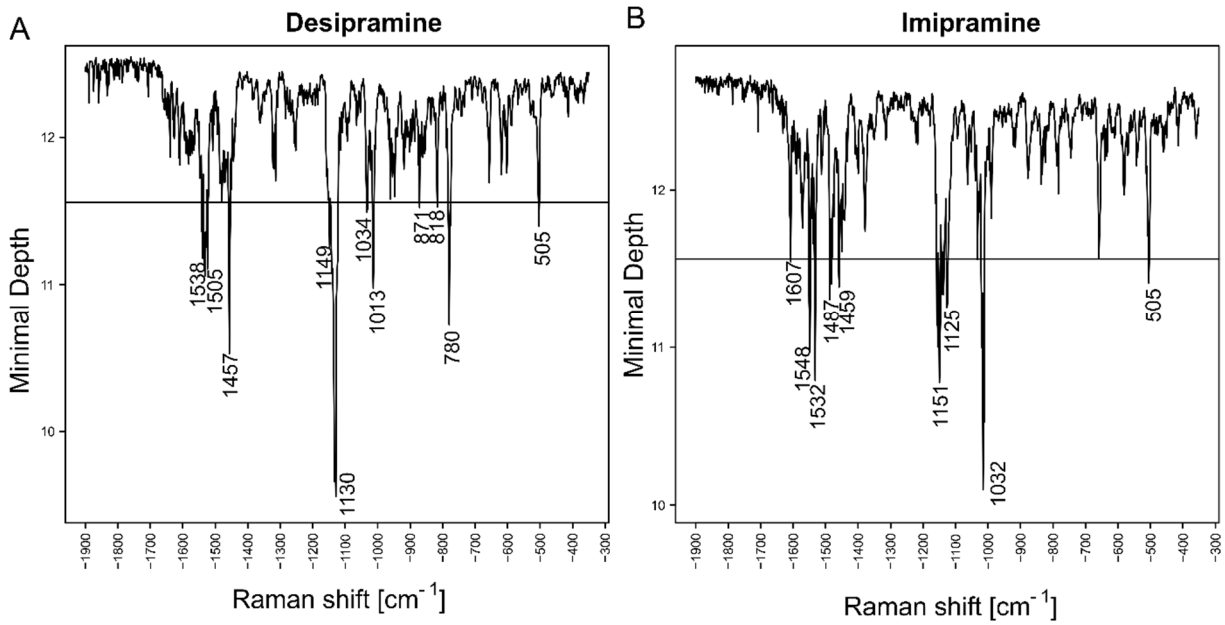

Figure S5. An example of results from minimal depth (MD) analysis of SERS spectra. Graphs show selection of important bands for the cells treated with desipramine (A) and imipramine (B) for 24 h prior to treatment with gold nanoparticles for 6 h. Important bands are labelled.

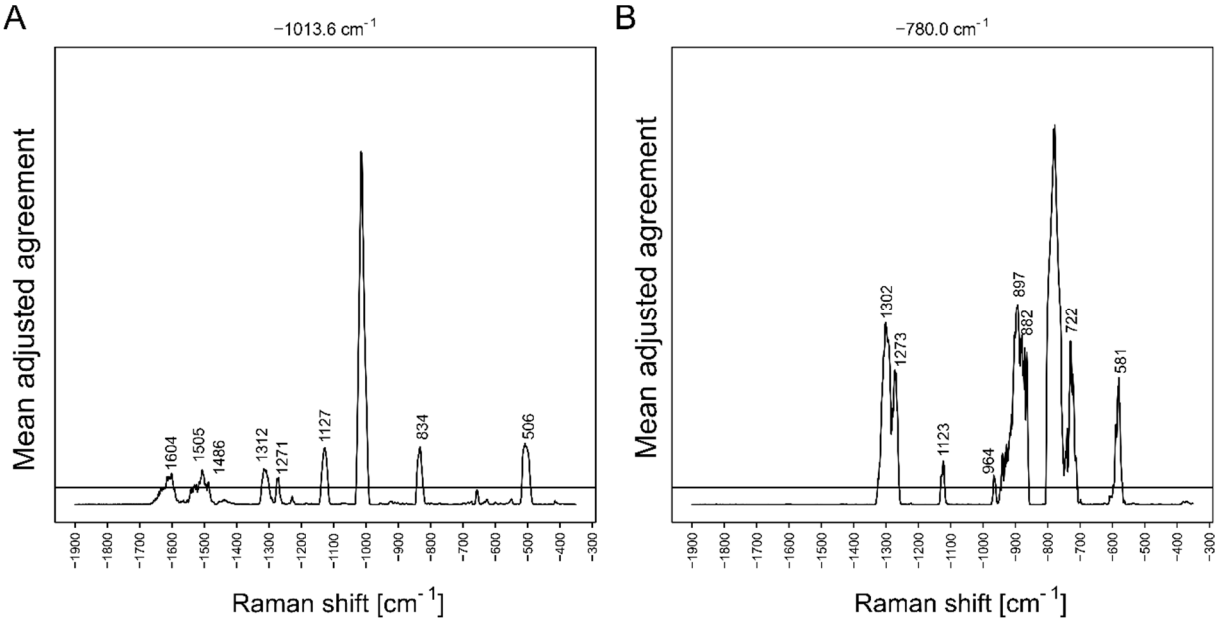

Figure S6. An example of results from surrogate minimal depth (SMD) analysis of SERS spectra from cells treated with desipramine for 24 h prior to treatment with gold nanoparticles for 6h. Graphs show bands (variables) related to the important bands at: (A) 1013 cm^{-1}, and (B) 780 cm^{-1} together with the threshold (horizontal line).

References

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