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Electronic Supplementary Information

Co-exposure of C₆₀ fullerene with benzo[*a*]pyrene results in enhanced biological effects in cells as determined by Fourier-transform infrared spectroscopy

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Supporting Information

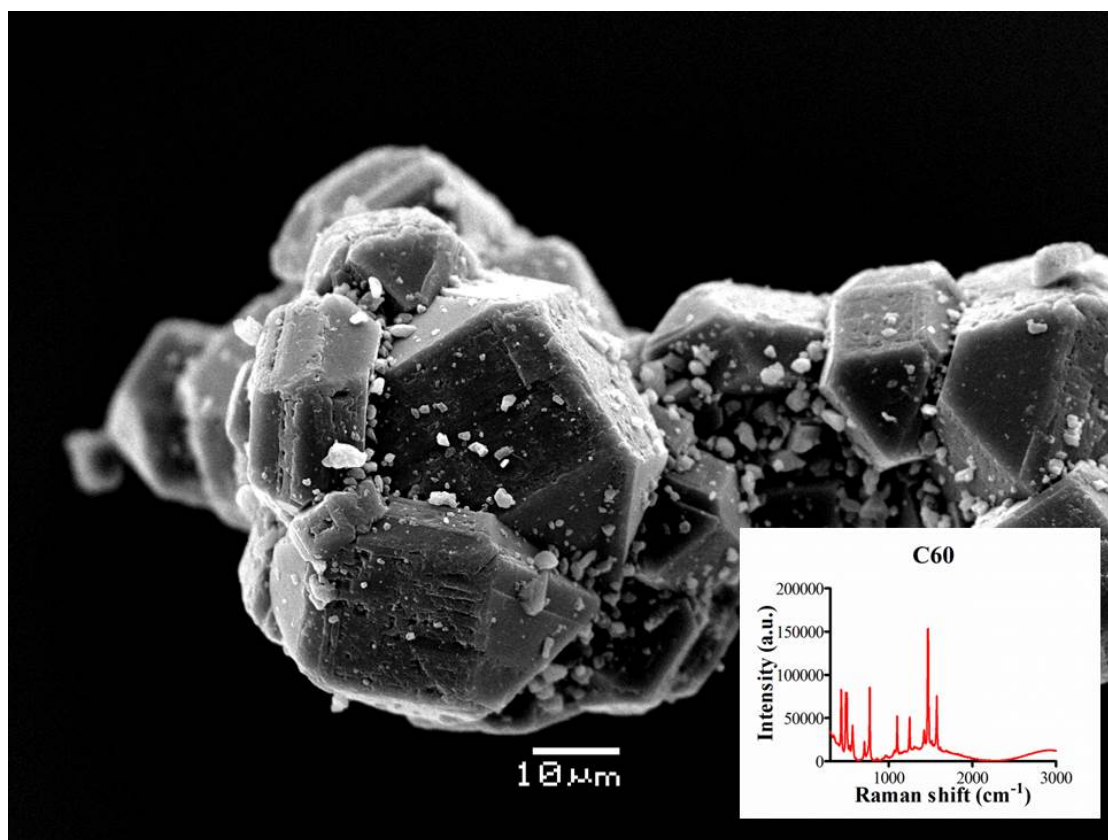


Figure S1 Scanning electron microscopy (SEM) images of C₆₀ fullerene with Raman spectra of characterization.

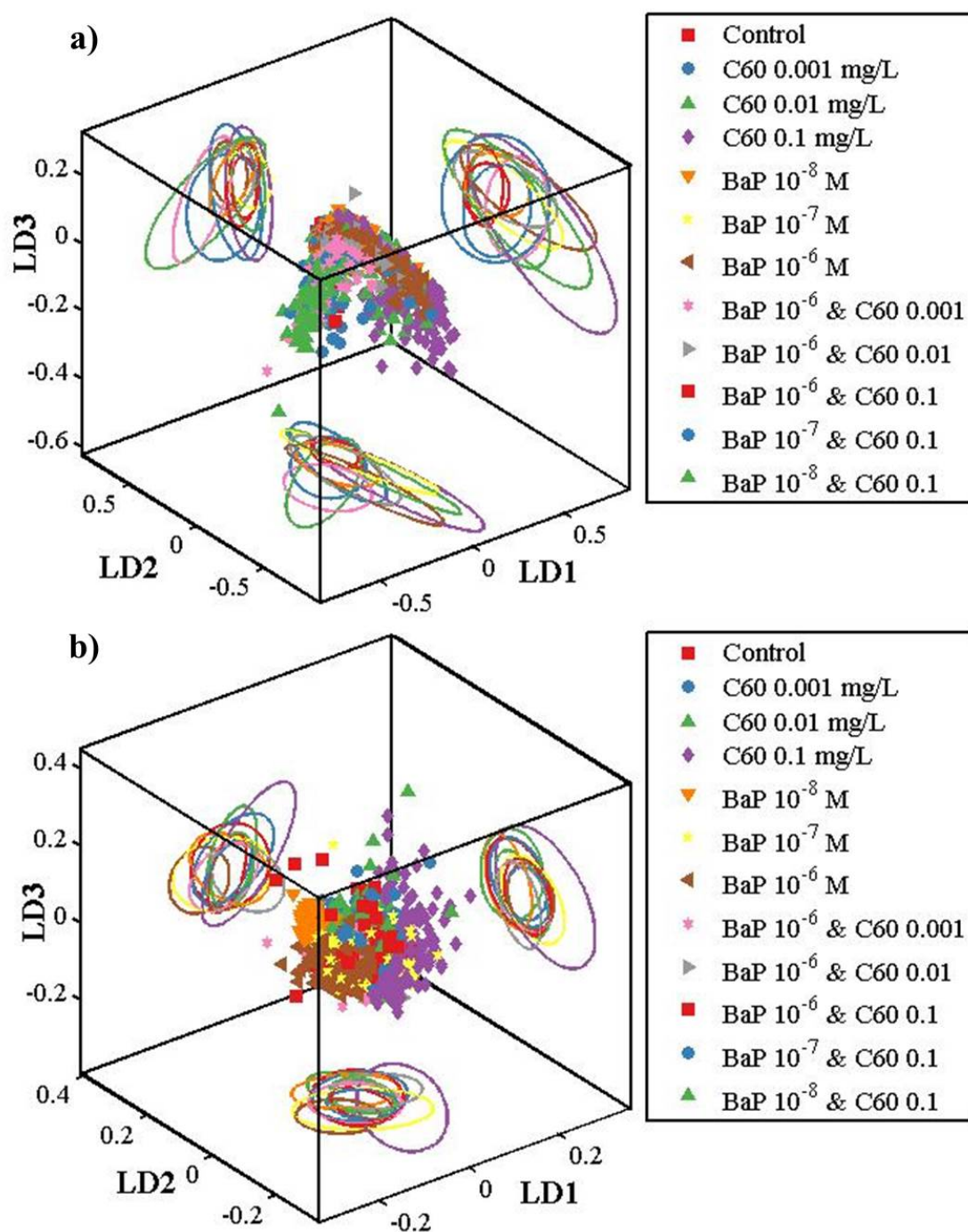


Figure S2 Three-D PCA-LDA scores plots (95% Confidence ellipsoids projected on walls) derived from post-exposure cells interrogated by FTIR: **a)** Gill cells; and, **b)** MCF-7 cells.

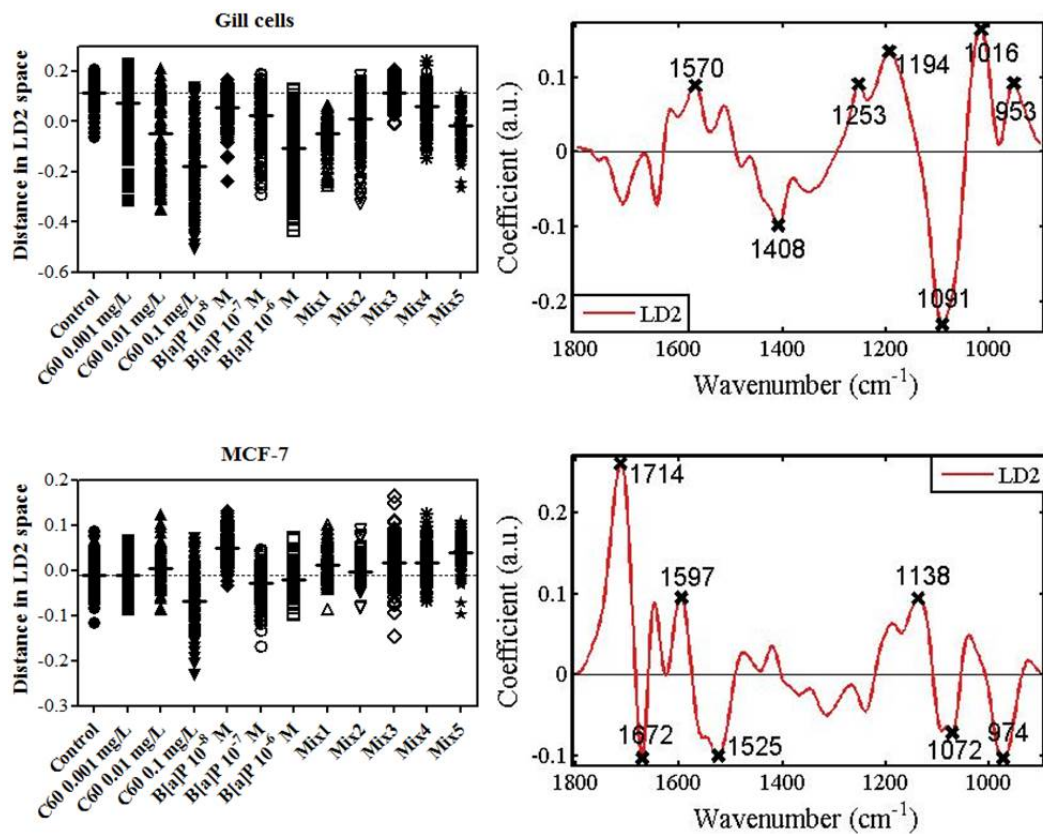


Figure S3 One-D scores plot in LD2 derived from PCA-LDA of spectral dataset (Dataset Total), with corresponding loadings plot: **a)** Gill cells; and, **b)** MCF-7 cells.

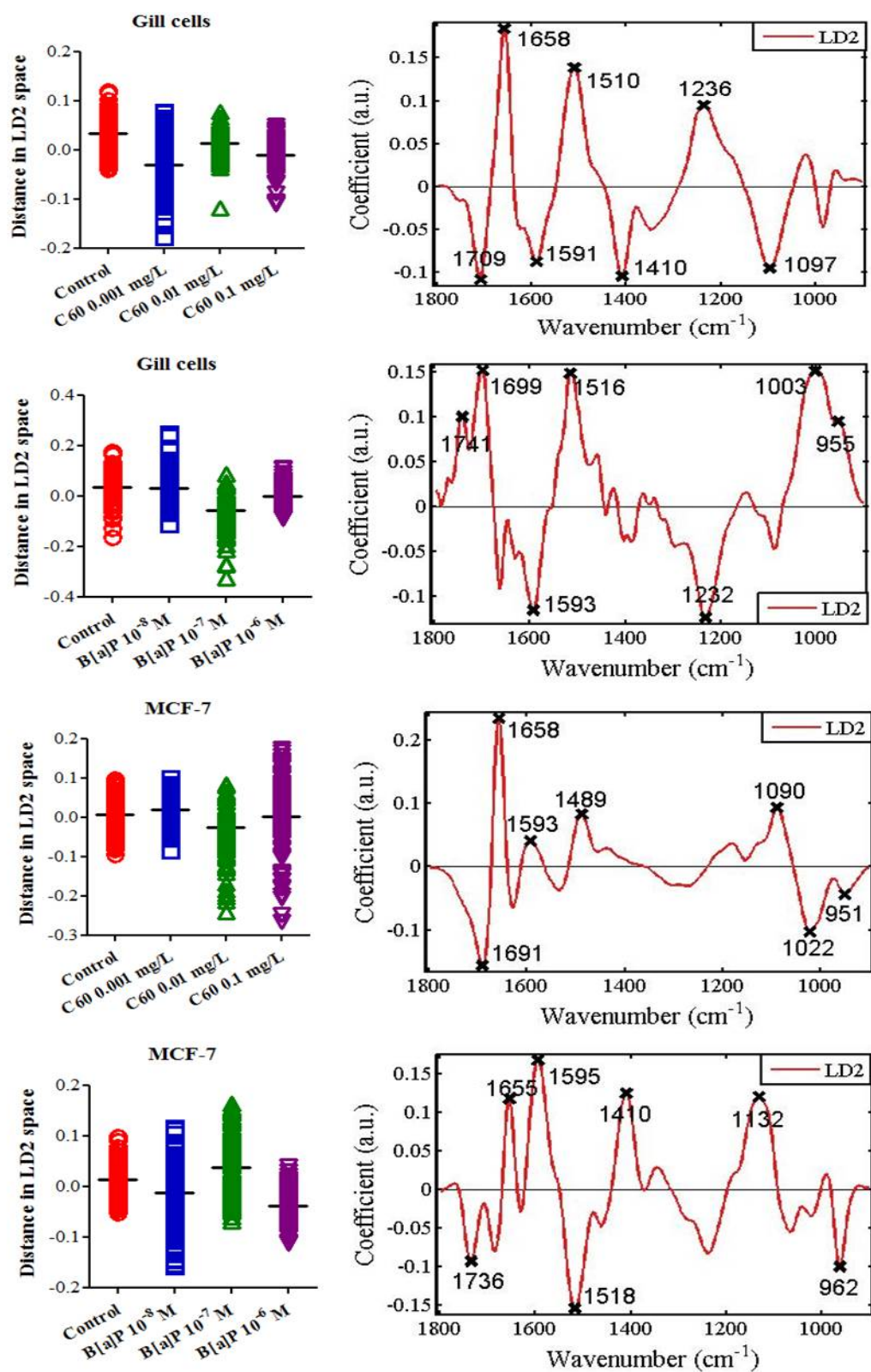


Figure S4 One-D scores plot in LD2 derived from PCA-LDA of spectral dataset for single treatment, with corresponding loadings plot.

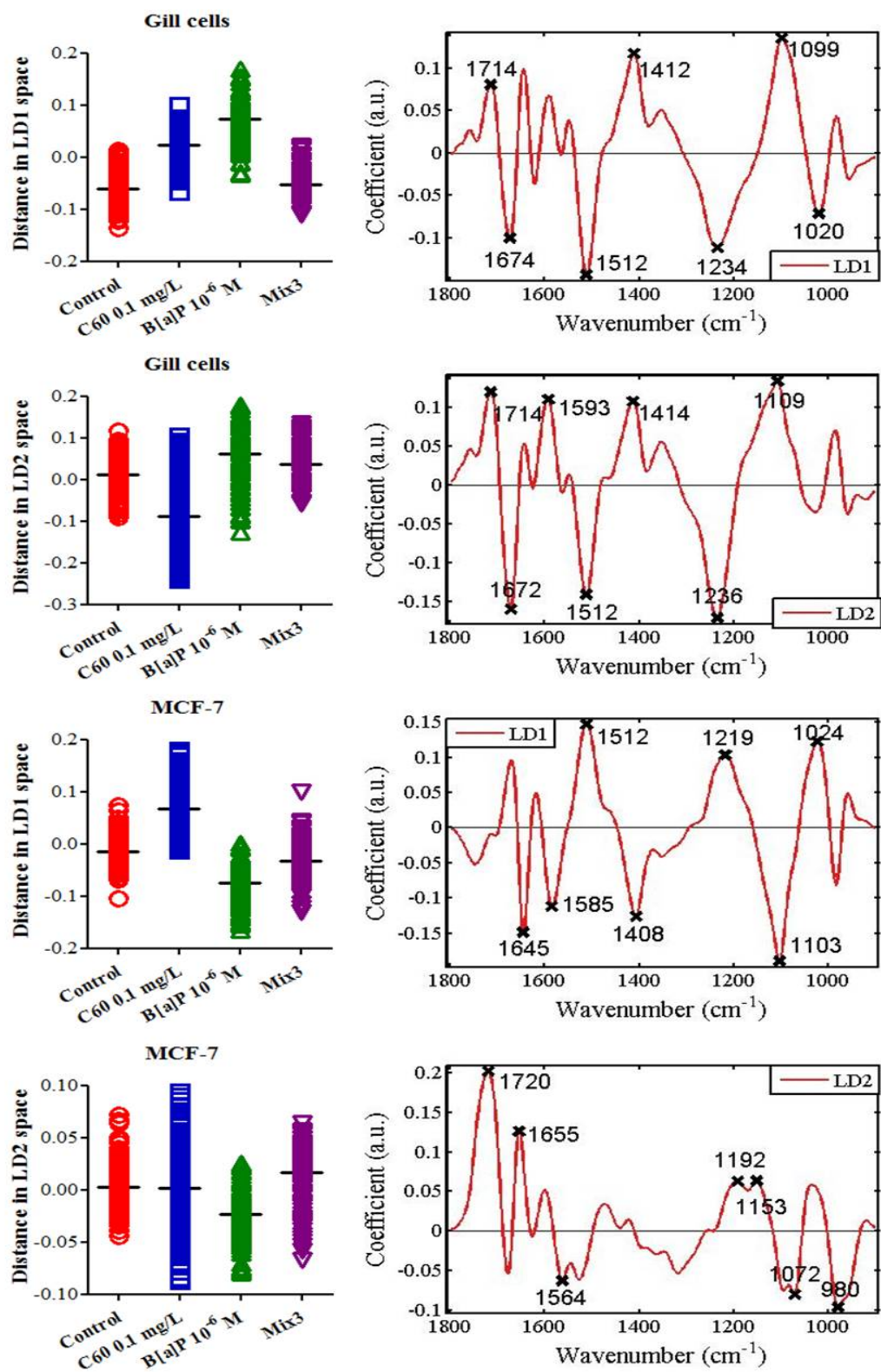


Figure S5 One-D scores plot derived from PCA-LDA of spectral dataset (Dataset mix), with corresponding loadings plot.

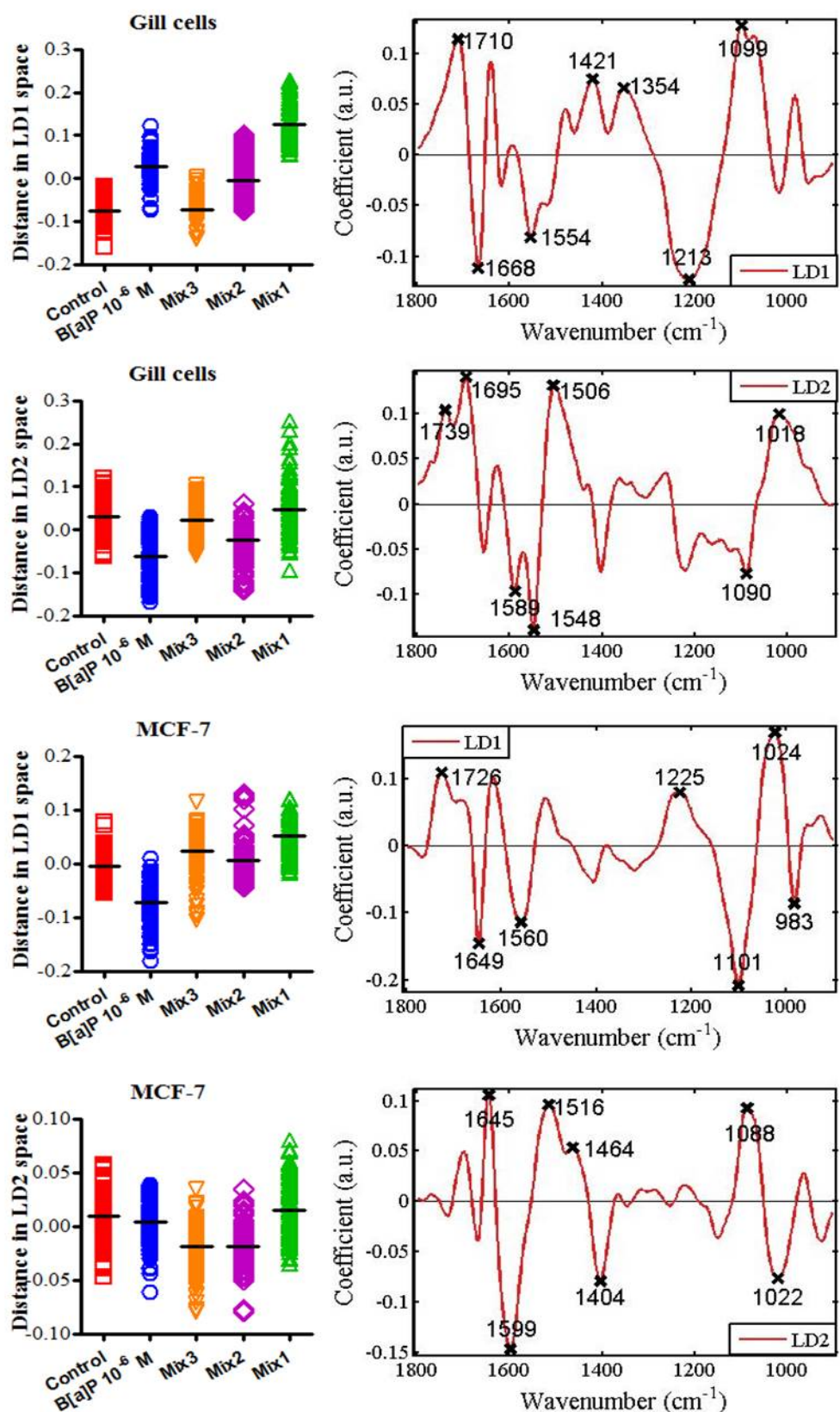


Figure S6 One-D scores plot derived from PCA-LDA of spectral dataset (Dataset B[a]P mix), with corresponding loadings plot.

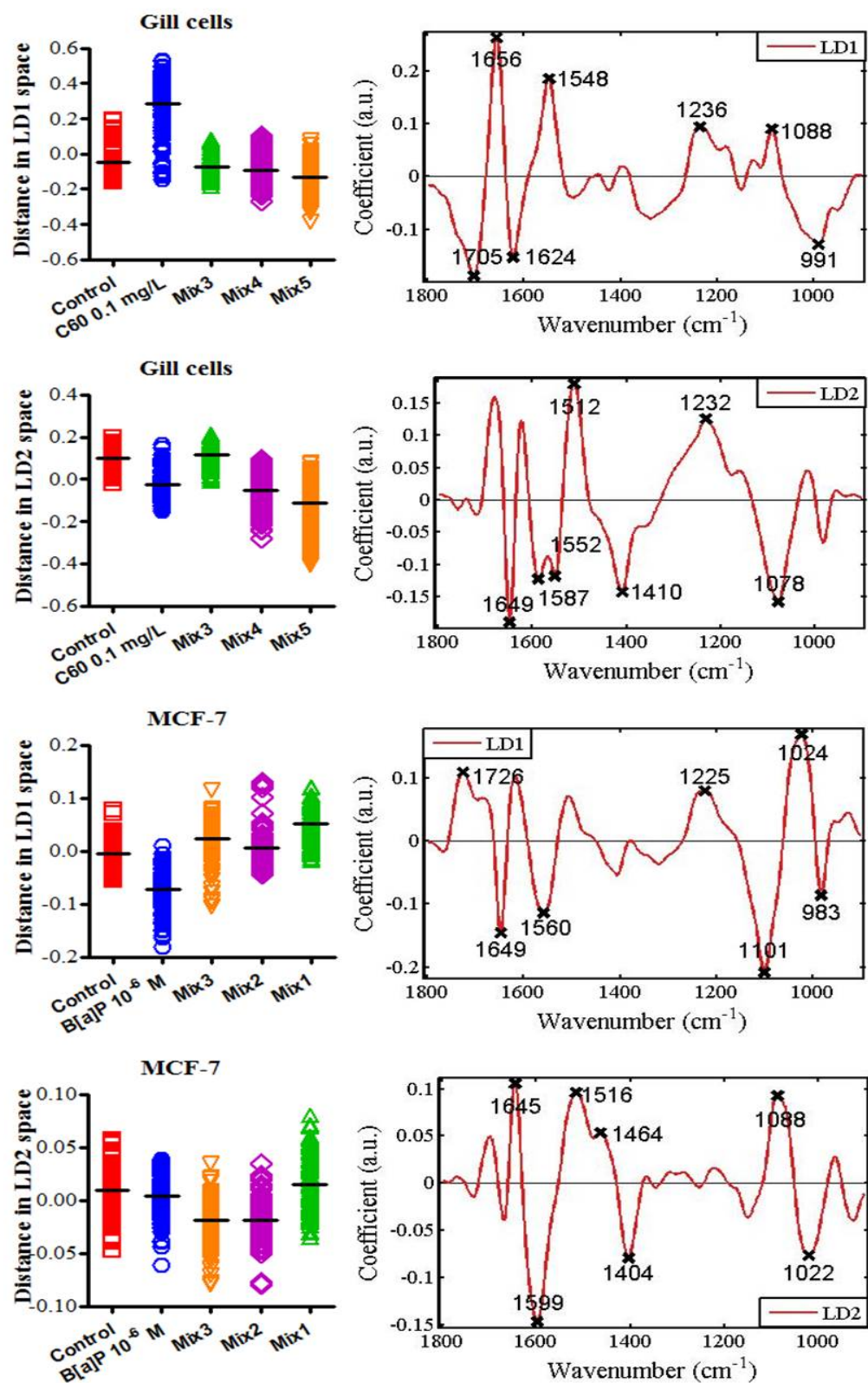


Figure S7 One-D scores plot derived from PCA-LDA of spectral dataset (Dataset C₆₀ mix), with corresponding loadings plot.

Table S1 Ratio of Protein-to-Lipid derived from FTIR spectra of cells

One-way ANOVA with Dunnett's Multiple Comparison Test	Gill cells	MCF-7 cells
Control vs C ₆₀ 0.001 mg/L	$P > 0.05$	$P > 0.05$
Control vs C ₆₀ 0.01 mg/L	$P < 0.05$	$P > 0.05$
Control vs C ₆₀ 0.1 mg/L	$P < 0.001$	$P > 0.05$
Control vs B[a]P 10 ⁻⁸ M	$P < 0.001$	$P > 0.05$
Control vs B[a]P 10 ⁻⁷ M	$P < 0.001$	$P > 0.05$
Control vs B[a]P 10 ⁻⁶ M	$P < 0.0001$	$P > 0.05$
Control vs Mix 1	$P > 0.05$	$P > 0.05$
Control vs Mix 2	$P < 0.05$	$P > 0.05$
Control vs Mix 3	$P > 0.05$	$P > 0.05$
Control vs Mix 4	$P > 0.05$	$P < 0.001$
Control vs Mix 5	$P > 0.05$	$P > 0.05$

Table S2 Ratio of Protein-to-Nucleic acid derived from FTIR spectra of cells

One-way ANOVA with Dunnett's Multiple Comparison Test	Gill cells	MCF-7 cells
Control vs C ₆₀ 0.001 mg/L	$P > 0.05$	$P > 0.05$
Control vs C ₆₀ 0.01 mg/L	$P < 0.0001$	$P > 0.05$
Control vs C ₆₀ 0.1 mg/L	$P < 0.0001$	$P < 0.0001$
Control vs B[a]P 10 ⁻⁸ M	$P < 0.0001$	$P > 0.05$
Control vs B[a]P 10 ⁻⁷ M	$P < 0.0001$	$P > 0.05$
Control vs B[a]P 10 ⁻⁶ M	$P < 0.0001$	$P > 0.05$
Control vs Mix 1	$P < 0.0001$	$P < 0.05$
Control vs Mix 2	$P < 0.0001$	$P > 0.05$
Control vs Mix 3	$P < 0.0001$	$P > 0.05$
Control vs Mix 4	$P < 0.0001$	$P < 0.05$
Control vs Mix 5	$P < 0.0001$	$P < 0.05$

Table S3 Ratio of RNA-to-DNA derived from FTIR spectra of cells

One-way ANOVA with Dunnett's Multiple Comparison Test	Gill cells	MCF-7 cells
Control vs C ₆₀ 0.001 mg/L	$P > 0.05$	$P > 0.05$
Control vs C ₆₀ 0.01 mg/L	$P < 0.0001$	$P > 0.05$
Control vs C ₆₀ 0.1 mg/L	$P < 0.0001$	$P > 0.05$
Control vs B[a]P 10 ⁻⁸ M	$P < 0.05$	$P > 0.05$
Control vs B[a]P 10 ⁻⁷ M	$P > 0.05$	$P < 0.05$
Control vs B[a]P 10 ⁻⁶ M	$P > 0.05$	$P < 0.0001$
Control vs Mix 1	$P < 0.001$	$P > 0.05$
Control vs Mix 2	$P > 0.05$	$P > 0.05$
Control vs Mix 3	$P > 0.05$	$P > 0.05$
Control vs Mix 4	$P < 0.0001$	$P < 0.05$
Control vs Mix 5	$P < 0.0001$	$P > 0.05$

Table S4 Scores plots derived from PCA-LDA of FTIR spectra of cells

One-way ANOVA with Dunnett's Multiple Comparison Test		Gill cells		MCF-7 cells	
		LD1	LD2	LD1	LD2
Dataset C₆₀	Control vs. C ₆₀ 0.001 mg/L	<i>P</i> < 0.05	<i>P</i> < 0.0001	<i>P</i> > 0.05	<i>P</i> > 0.05
	Control vs. C ₆₀ 0.01 mg/L	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> > 0.05	<i>P</i> < 0.0001
	Control vs. C ₆₀ 0.1 mg/L	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> > 0.05
Dataset B[a]P	Control vs. B[a]P 10 ⁻⁸ M	<i>P</i> < 0.0001	<i>P</i> > 0.05	<i>P</i> < 0.0001	<i>P</i> < 0.0001
	Control vs. B[a]P 10 ⁻⁷ M	<i>P</i> < 0.0001	<i>P</i> < 0.001	<i>P</i> < 0.0001	<i>P</i> < 0.0001
	Control vs. B[a]P 10 ⁻⁶ M	<i>P</i> < 0.0001	<i>P</i> < 0.001	<i>P</i> < 0.0001	<i>P</i> < 0.0001
Dataset total	Control vs. C ₆₀ 0.001 mg/L	<i>P</i> < 0.0001	<i>P</i> < 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05
	Control vs. C ₆₀ 0.01 mg/L	<i>P</i> < 0.001	<i>P</i> < 0.0001	<i>P</i> < 0.001	<i>P</i> < 0.0001
	Control vs. C ₆₀ 0.1 mg/L	<i>P</i> < 0.001	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001
	Control vs. B[a]P 10 ⁻⁸ M	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> > 0.05	<i>P</i> < 0.0001
	Control vs. B[a]P 10 ⁻⁷ M	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.05	<i>P</i> < 0.0001
	Control vs. B[a]P 10 ⁻⁶ M	<i>P</i> < 0.0001	<i>P</i> < 0.001	<i>P</i> < 0.0001	<i>P</i> > 0.05
	Control vs. Mix1	<i>P</i> < 0.0001	<i>P</i> < 0.001	<i>P</i> < 0.0001	<i>P</i> < 0.0001
	Control vs. Mix2	<i>P</i> < 0.001	<i>P</i> < 0.0001	<i>P</i> > 0.05	<i>P</i> > 0.05
	Control vs. Mix3	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> < 0.0001
	Control vs. Mix4	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001
	Control vs. Mix5	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.05	<i>P</i> < 0.0001

Table S5 Scores plots derived from PCA-LDA of FTIR spectra of cells

One-way ANOVA with Dunnett's Multiple Comparison Test		Gill cells		MCF-7 cells	
		LD1	LD2	LD1	LD2
Dataset B[a]P mix	Control vs. B[a]P 10^{-6} M	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P > 0.05$
	Control vs. Mix 1	$P < 0.0001$	$P < 0.001$	$P < 0.0001$	$P < 0.05$
	Control vs. Mix 2	$P < 0.0001$	$P < 0.0001$	$P > 0.05$	$P < 0.0001$
	Control vs. Mix 3	$P > 0.05$	$P > 0.05$	$P < 0.0001$	$P < 0.0001$
Dataset C₆₀ mix	Control vs. C ₆₀ 0.1 mg/L	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
	Control vs. Mix 3	$P > 0.05$	$P > 0.05$	$P < 0.0001$	$P < 0.0001$
	Control vs. Mix 4	$P < 0.001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
	Control vs. Mix 5	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Dataset mix	Control vs. C ₆₀ 0.1 mg/L	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P > 0.05$
	Control vs. B[a]P 10^{-6} M	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
	Control vs. Mix 3	$P > 0.05$	$P < 0.05$	$P < 0.0001$	$P < 0.0001$

Table S6 Primary wavenumbers in loadings plots derived from PCA-LDA of FTIR spectral dataset (Gill cells)

	LD1		LD2	
	Wavenumber (cm ⁻¹)	Tentative assignment	Wavenumber (cm ⁻¹)	Tentative assignment
Dataset C₆₀	1656	Amide I	1658	Amide I
	997	Glycogen	1510	Amide II
	1240	$\nu_{\text{as}}\text{PO}_2^-$	1709	Lipid, $\nu(\text{C}=\text{O})$
	1709	Lipid, $\nu(\text{C}=\text{O})$	1410	$\nu(\text{COO}^-)$
	951	Protein phosphorylation	1236	$\nu_{\text{as}}\text{PO}_2^-$
	1597	Amide I	1097	$\nu_{\text{s}}\text{PO}_2^-$
	1090	$\nu_{\text{s}}\text{PO}_2^-$	1591	Amide I
Dataset B[a]P	1103	Carbohydrates	1699	Lipid, $\nu(\text{C}=\text{O})$
	1246	$\nu_{\text{as}}\text{PO}_2^-$	1003	Glycogen
	1558	Amide II	1516	Amide II
	1645	Amide I	1232	$\nu_{\text{as}}\text{PO}_2^-$
	1712	Lipid, $\nu(\text{C}=\text{O})$	1593	Amide I
	1026	Glycogen	1741	Lipid, $\nu(\text{C}=\text{O})$
	1421	$\nu(\text{COO}^-)$	955	Protein phosphorylation
Dataset Total	1232	$\nu_{\text{as}}\text{PO}_2^-$	1091	$\nu_{\text{s}}\text{PO}_2^-$
	1709	Lipid, $\nu(\text{C}=\text{O})$	1016	Glycogen
	1664	Amide I	1194	Collagen
	1070	$\nu_{\text{s}}\text{PO}_2^-$	1408	$\nu(\text{COO}^-)$
	985	Protein phosphorylation	953	Protein phosphorylation
	1417	$\nu(\text{COO}^-)$	1253	$\nu_{\text{as}}\text{PO}_2^-$
	1556	Amide II	1570	Amide II

Table S7 Primary wavenumbers in loadings plots derived from PCA-LDA of FTIR spectral dataset (Gill cells)

	LD1		LD2	
	Wavenumber (cm ⁻¹)	Tentative assignment	Wavenumber (cm ⁻¹)	Tentative assignment
Dataset C₆₀ mix	1656	Amide I	1649	Amide I
	1705	Lipid, $\nu(\text{C=O})$	1512	Amide II
	1548	Amide II	1078	$\nu_s\text{PO}_2^-$
	1624	Amide I	1410	$\nu(\text{COO}^-)$
	991	Protein phosphorylation	1232	$\nu_{as}\text{PO}_2^-$
	1236	$\nu_{as}\text{PO}_2^-$	1587	Amide I
	1088	$\nu_s\text{PO}_2^-$	1552	Amide II
Dataset B[a]P mix	1099	$\nu_s\text{PO}_2^-$	1695	Lipid, $\nu(\text{C=O})$
	1212	$\nu_{as}\text{PO}_2^-$	1548	Amide II
	1710	Lipid, $\nu(\text{C=O})$	1506	Amide II
	1668	Amide I	1739	Lipid, $\nu(\text{C=O})$
	1554	Amide II	1018	Glycogen
	1421	$\nu(\text{COO}^-)$	1588	Amide I
	1354	Amide III	1090	$\nu_s\text{PO}_2^-$
Dataset mix	1512	Amide II	1236	$\nu_{as}\text{PO}_2^-$
	1099	$\nu_s\text{PO}_2^-$	1672	Amide I
	1412	$\nu(\text{COO}^-)$	1512	Amide II
	1234	$\nu_{as}\text{PO}_2^-$	1109	Carbohydrates
	1674	Amide I	1714	Lipid, $\nu(\text{C=O})$
	1714	Lipid, $\nu(\text{C=O})$	1593	Amide I
	1020	Glycogen	1414	$\nu(\text{COO}^-)$

Table S8 Primary wavenumbers in loadings plots derived from PCA-LDA of FTIR spectral dataset (MCF-7 cells)

	LD1		LD2	
	Wavenumber (cm ⁻¹)	Tentative assignment	Wavenumber (cm ⁻¹)	Tentative assignment
Dataset C₆₀	1649	Amide I	1658	Amide I
	1718	Lipid, $\nu(\text{C=O})$	1691	Amide I
	1516	Amide II	1022	Glycogen
	1132	Carbohydrates	1090	$\nu_s\text{PO}_2^-$
	1244	$\nu_{as}\text{PO}_2^-$	1489	Protein, CH ₂
	960	Protein phosphorylation	951	Protein phosphorylation
	1095	$\nu_s\text{PO}_2^-$	1593	Amide I
Dataset B[a]P	1710	Lipid, $\nu(\text{C=O})$	1595	Amide I
	1097	$\nu_s\text{PO}_2^-$	1518	Amide II
	1037	Glycogen	1410	$\nu(\text{COO}^-)$
	982	Protein phosphorylation	1132	Carbohydrates
	1602	Amide I	1655	Amide I
	1533	Amide II	962	Protein phosphorylation
	1317	Amide III	1736	Lipid, $\nu(\text{C=O})$
Dataset Total	1101	$\nu_s\text{PO}_2^-$	1714	Lipid, $\nu(\text{C=O})$
	1508	Amide II	1672	Amide I
	1026	Glycogen	974	Protein phosphorylation
	1566	Amide II	1525	Amide II
	983	Protein phosphorylation	1597	Amide I
	1406	$\nu(\text{COO}^-)$	1138	Carbohydrates
	1712	Lipid, $\nu(\text{C=O})$	1072	$\nu_s\text{PO}_2^-$

Table S9 Primary wavenumbers in loadings plots derived from PCA-LDA of FTIR spectral dataset (MCF-7 cells)

	LD1		LD2	
	Wavenumber (cm ⁻¹)	Tentative assignment	Wavenumber (cm ⁻¹)	Tentative assignment
Dataset C₆₀ mix	1716	Lipid, $\nu(\text{C}=\text{O})$	1647	Amide I
	1595	Amide I	1606	Amide I
	1516	Amide II	1093	$\nu_s\text{PO}_2^-$
	1132	Carbohydrates	1026	Glycogen
	1674	Amide I	974	Protein phosphorylation
	1068	$\nu_s\text{PO}_2^-$	1196	Collagen
	962	Protein phosphorylation	1437	$\nu(\text{COO}^-)$
Dataset B[a]P mix	1101	$\nu_s\text{PO}_2^-$	1599	Amide I
	1024	Glycogen	1645	Amide I
	1649	Amide I	1516	Amide II
	1560	Amide II	1088	$\nu_s\text{PO}_2^-$
	1726	Lipid, $\nu(\text{C}=\text{O})$	1404	$\nu(\text{COO}^-)$
	983	Protein phosphorylation	1002	Glycogen
	1225	$\nu_{as}\text{PO}_2^-$	1464	Protein, CH ₂
Dataset mix	1103	$\nu_s\text{PO}_2^-$	1720	Lipid, $\nu(\text{C}=\text{O})$
	1645	Amide I	1655	Amide I
	1512	Amide II	980	Protein phosphorylation
	1408	$\nu(\text{COO}^-)$	1072	$\nu_s\text{PO}_2^-$
	1024	Glycogen	1153	Carbohydrates
	1585	Amide I	1192	Collagen
	1219	$\nu_{as}\text{PO}_2^-$	1564	Amide II

Table S10 Primary wavenumbers in cluster vectors plots for PCA-LDA derived from IR spectra

	Gill cells		MCF-7 cells	
	Wavenumber (cm ⁻¹)	Tentative assignment	Wavenumber (cm ⁻¹)	Tentative assignment
C₆₀ 0.1 mg/L	1020	Glycogen	1647	Amide I
	1097	$\nu_s\text{PO}_2^-$	1514	Amide II
	956	Protein phosphorylation	1107	Carbohydrates
	1518	Amide II	1410	$\nu(\text{COO}^-)$
	1658	Amide I	1736	Lipid, $\nu(\text{C=O})$
	1595	Amide I	1226	$\nu_{as}\text{PO}_2^-$
	1411	$\nu(\text{COO}^-)$	960	Protein phosphorylation
B[a]P 10⁻⁶ M	1512	Amide II	1098	$\nu_s\text{PO}_2^-$
	1099	$\nu_s\text{PO}_2^-$	1024	Glycogen
	1412	$\nu(\text{COO}^-)$	1581	Amide II
	1236	$\nu_{as}\text{PO}_2^-$	1405	$\nu(\text{COO}^-)$
	1714	Lipid, $\nu(\text{C=O})$	983	Protein phosphorylation
	1672	Amide I	1214	$\nu_{as}\text{PO}_2^-$
	1022	Glycogen	1666	Amide I
B[a]P 10⁻⁶ & C₆₀ 0.1	958	Protein phosphorylation	1724	Lipid, $\nu(\text{C=O})$
	1022	Glycogen	1591	Amide I
	1110	Carbohydrates	1672	Amide I
	1518	Amide II	1629	Amide I
	1599	Amide I	1124	Carbohydrates
	1412	$\nu(\text{COO}^-)$	1022	Glycogen
	1685	Amide I	960	Protein phosphorylation

Table S11 Primary wavenumbers cluster vectors plots for PCA-LDA derived from IR spectra

	Gill cells		MCF-7 cells	
	Wavenumber (cm ⁻¹)	Tentative assignment	Wavenumber (cm ⁻¹)	Tentative assignment
B[a]P 10⁻⁶ M	1095	$\nu_s\text{PO}_2^-$	1098	$\nu_s\text{PO}_2^-$
	1020	Glycogen	1556	Amide II
	1671	Amide I	1648	Amide I
	1512	Amide II	1031	Glycogen
	1410	$\nu(\text{COO}^-)$	983	Protein phosphorylation
	1593	Amide I	1504	Amide II
	1252	$\nu_{as}\text{PO}_2^-$	1230	$\nu_{as}\text{PO}_2^-$
B[a]P 10⁻⁶ & C₆₀ 0.001	1710	Lipid, $\nu(\text{C=O})$	1097	$\nu_s\text{PO}_2^-$
	1101	Carbohydrates	1022	Glycogen
	1223	$\nu_{as}\text{PO}_2^-$	1730	Lipid, $\nu(\text{C=O})$
	1664	Amide I	1648	Amide I
	1550	Amide II	1689	Amide I
	1423	$\nu(\text{COO}^-)$	1571	Amide II
	1352	Amide III	948	Protein phosphorylation
B[a]P 10⁻⁶ & C₆₀ 0.01	1095	$\nu_s\text{PO}_2^-$	1022	Glycogen
	1020	Glycogen	1091	$\nu_s\text{PO}_2^-$
	1512	Amide II	1668	Amide I
	1674	Amide I	1498	Amide II
	1597	Amide I	1552	Amide II
	1408	$\nu(\text{COO}^-)$	1705	Lipid, $\nu(\text{C=O})$
	956	Protein phosphorylation	1155	Collagen
B[a]P 10⁻⁶ & C₆₀ 0.1	1649	Amide I	1648	Amide I
	1545	Amide II	1726	Lipid, $\nu(\text{C=O})$
	1612	Amide I	1606	Amide I
	1712	Lipid, $\nu(\text{C=O})$	1095	$\nu_s\text{PO}_2^-$
	1024	Glycogen	1542	Amide II
	1103	Carbohydrates	1020	Glycogen
	958	Protein phosphorylation	929	Protein phosphorylation

Table S12 Primary wavenumbers cluster vectors plots for PCA-LDA of IR spectra

	Gill cells		MCF-7 cells	
	Wavenumber (cm ⁻¹)	Tentative assignment	Wavenumber (cm ⁻¹)	Tentative assignment
C₆₀ 0.1 mg/L	1655	Amide I	1648	Amide I
	1550	Amide II	1716	Lipid, $\nu(\text{C=O})$
	1699	Lipid, $\nu(\text{C=O})$	1514	Amide II
	1086	$\nu_{\text{s}}\text{PO}_2^-$	1128	Carbohydrates
	1012	Glycogen	1588	Amide I
	1508	Amide II	1411	$\nu(\text{COO}^-)$
	953	Protein phosphorylation	1023	Glycogen
B[a]P 10⁻⁶ & C₆₀ 0.1	1651	Amide I	1597	Amide I
	1545	Amide II	1716	Lipid, $\nu(\text{C=O})$
	1616	Amide I	1070	$\nu_{\text{s}}\text{PO}_2^-$
	960	Protein phosphorylation	964	Protein phosphorylation
	1024	Glycogen	1140	Carbohydrates
	1712	Lipid, $\nu(\text{C=O})$	1637	Amide I
	1346	Amide III	1521	Amide II
B[a]P 10⁻⁷ & C₆₀ 0.1	1514	Amide II	1604	Amide I
	1591	Amide I	1545	Amide II
	1676	Amide I	1650	Amide I
	1412	$\nu(\text{COO}^-)$	1712	Lipid, $\nu(\text{C=O})$
	1232	$\nu_{\text{as}}\text{PO}_2^-$	1041	Glycogen
	1076	$\nu_{\text{s}}\text{PO}_2^-$	970	Protein phosphorylation
	985	Protein phosphorylation	1415	$\nu(\text{COO}^-)$
B[a]P 10⁻⁸ & C₆₀ 0.1	1514	Amide II	1598	Amide I
	1232	$\nu_{\text{as}}\text{PO}_2^-$	1716	Lipid, $\nu(\text{C=O})$
	1673	Amide I	1070	$\nu_{\text{s}}\text{PO}_2^-$
	1413	$\nu(\text{COO}^-)$	1643	Amide I
	1074	$\nu_{\text{s}}\text{PO}_2^-$	1515	Amide II
	1590	Amide I	1136	Carbohydrates
	985	Protein phosphorylation	964	Protein phosphorylation