Supplementary Appendix 2

Characterizing metabolic stress-induced phenotypes of *Synechocystis* PCC6803 with Raman spectroscopy

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Note: **Figure S1-S4** are represented in Supplementary Appendix 1.

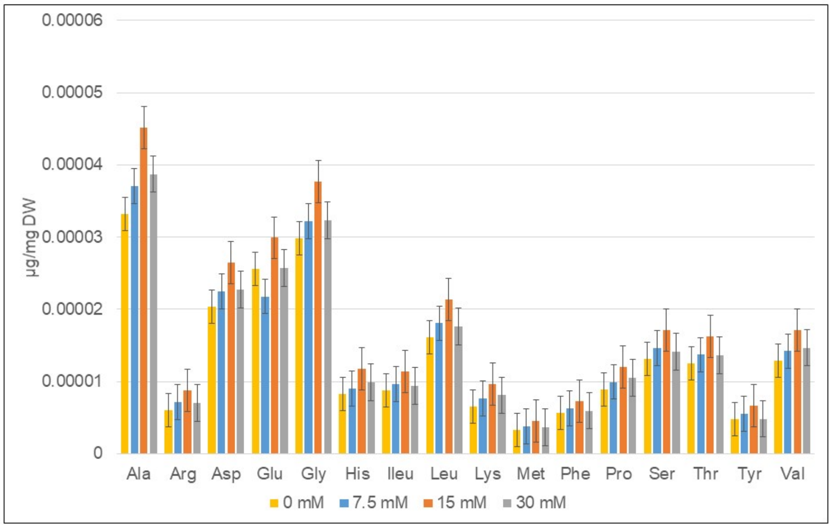
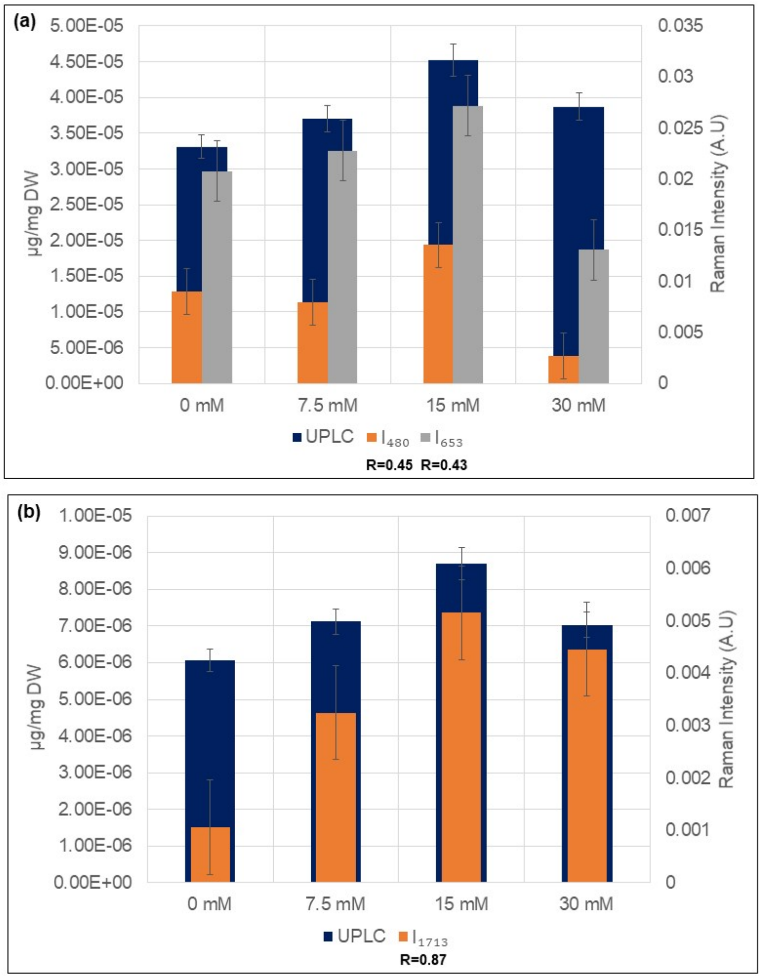
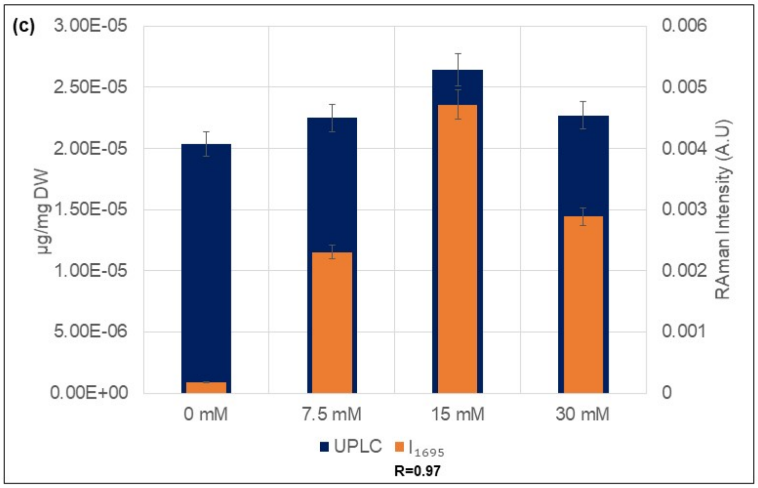
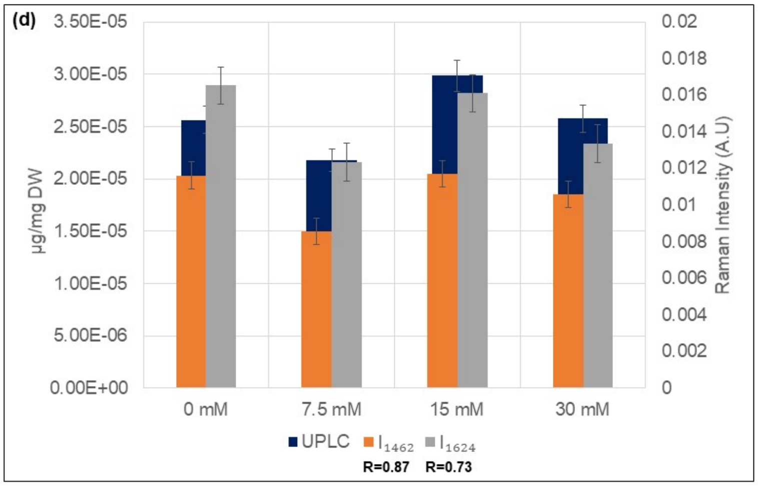
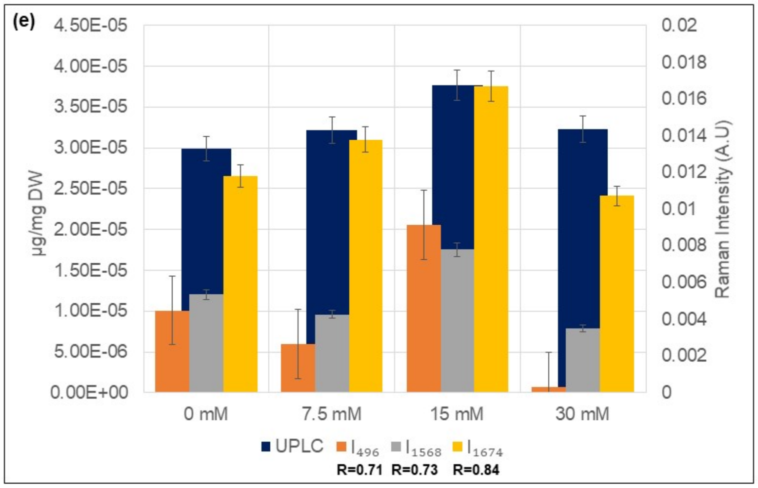


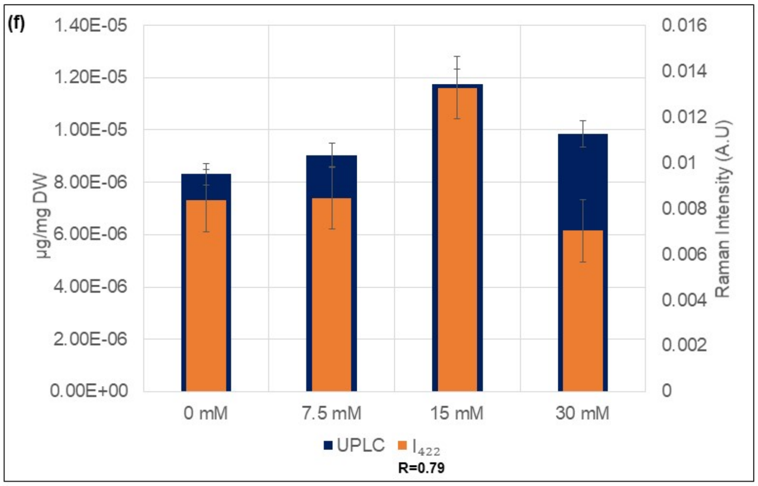
Figure S5. Amino acids analysis for acetate study. Data represent means ± standard deviation (SD) of values from three independent experiments.



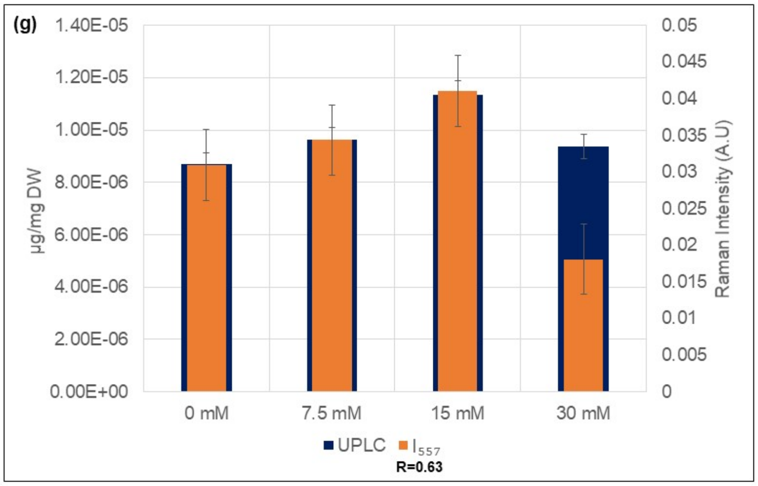

(c)
3.00E-05
2.50E-05
2.00E-05
1.50E-05
1.00E-05
5.00E-06
0.00E+00
µg/mg DW
0.006
0.005
0.004
0.003
0.002
0.001
0
RAman Intensity (A.U)
0 mM
7.5 mM
15 mM
30 mM
UPLC
I_{1695}
R=0.97


(d)
3.50E-05
3.00E-05
2.50E-05
2.00E-05
1.50E-05
1.00E-05
5.00E-06
0.00E+00
µg/mg DW
0.02
0.018
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
Raman Intensity (A.U)
0 mM
7.5 mM
15 mM
30 mM
UPLC
I_{1462}
I_{1624}
R=0.87
R=0.73

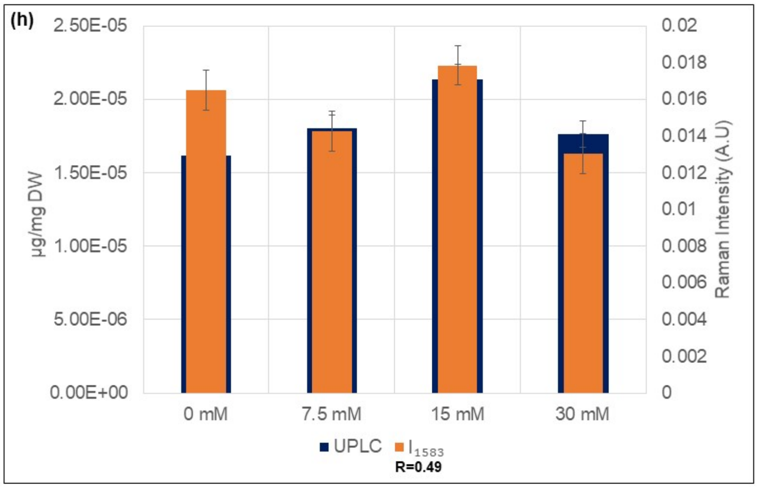

(e)
4.50E-05
4.00E-05
3.50E-05
3.00E-05
2.50E-05
2.00E-05
1.50E-05
1.00E-05
5.00E-06
0.00E+00
µg/mg DW
0.02
0.018
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
Raman Intensity (A.U)
0 mM
7.5 mM
15 mM
30 mM
UPLC
I_{496}
I_{1568}
I_{1674}
R=0.71
R=0.73
R=0.84



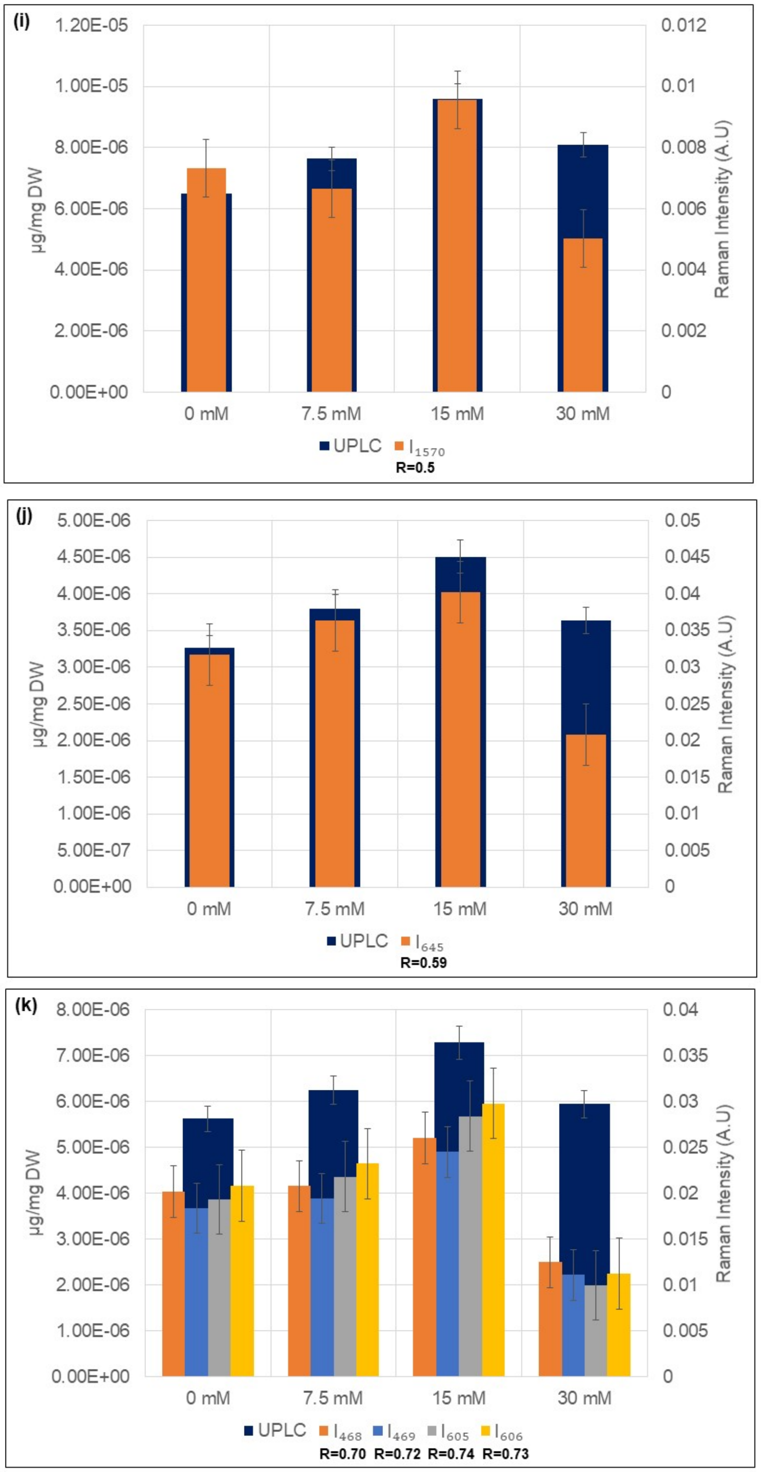
R=0.79

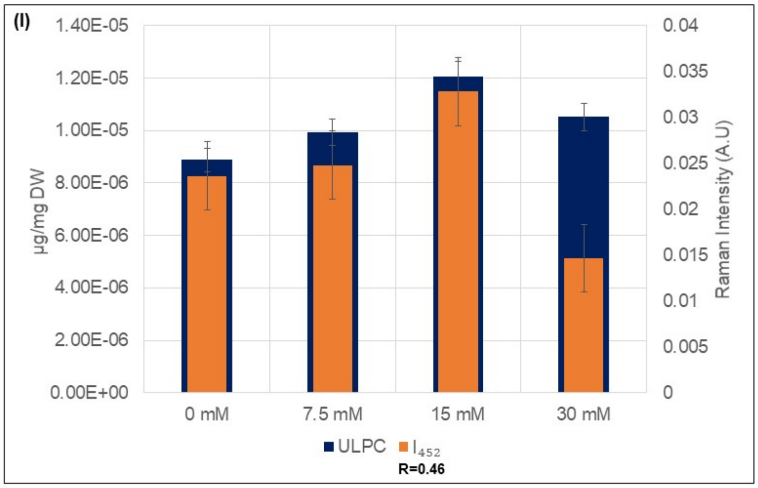


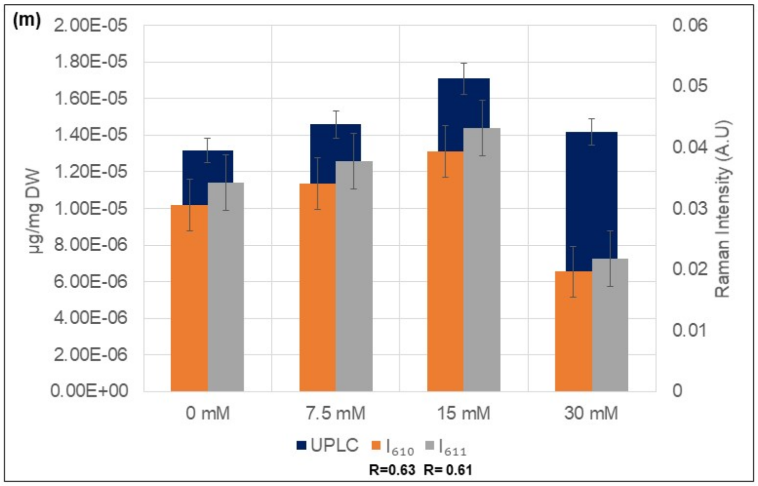
R=0.63

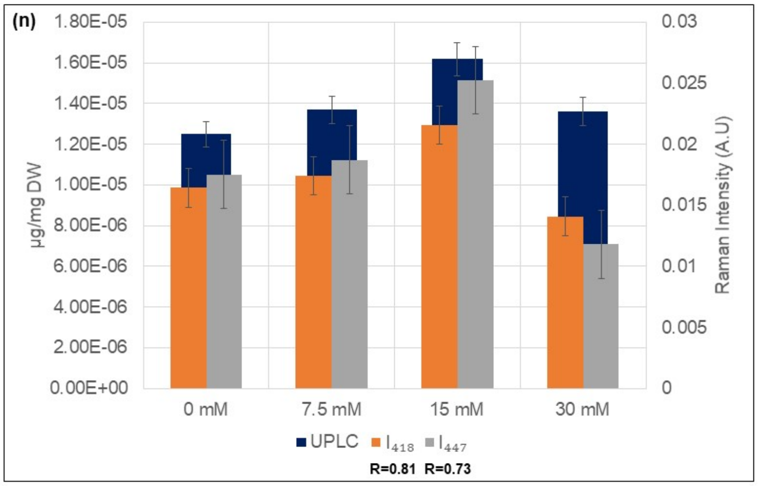


R=0.49


(i)
µg/mg DW
Raman Intensity (A.U)
1.20E-05
1.00E-05
8.00E-06
6.00E-06
4.00E-06
2.00E-06
0.00E+00
0.012
0.01
0.008
0.006
0.004
0.002
0
0 mM
7.5 mM
15 mM
30 mM
UPLC
I_{1570}
R=0.5
(j)
µg/mg DW
Raman Intensity (A.U)
5.00E-06
4.50E-06
4.00E-06
3.50E-06
3.00E-06
2.50E-06
2.00E-06
1.50E-06
1.00E-06
5.00E-07
0.00E+00
0.05
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
0 mM
7.5 mM
15 mM
30 mM
UPLC
I_{645}
R=0.59
(k)
µg/mg DW
Raman Intensity (A.U)
8.00E-06
7.00E-06
6.00E-06
5.00E-06
4.00E-06
3.00E-06
2.00E-06
1.00E-06
0.00E+00
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
0 mM
7.5 mM
15 mM
30 mM
UPLC
I_{468}
I_{469}
I_{605}
I_{606}
R=0.70 R=0.72 R=0.74 R=0.73


(l)
1.40E-05
1.20E-05
1.00E-05
8.00E-06
6.00E-06
4.00E-06
2.00E-06
0.00E+00
µg/mg DW
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
Raman Intensity (A.U)
0 mM
7.5 mM
15 mM
30 mM
■ ULPC ■ I_{452}
R=0.46


(m)
2.00E-05
1.80E-05
1.60E-05
1.40E-05
1.20E-05
1.00E-05
8.00E-06
6.00E-06
4.00E-06
2.00E-06
0.00E+00
µg/mg DW
0.06
0.05
0.04
0.03
0.02
0.01
0
Raman Intensity (A.U)
0 mM
7.5 mM
15 mM
30 mM
■ UPLC ■ I_{610} ■ I_{611}
R=0.63 R= 0.61


(n)
1.80E-05
1.60E-05
1.40E-05
1.20E-05
1.00E-05
8.00E-06
6.00E-06
4.00E-06
2.00E-06
0.00E+00
µg/mg DW
0.03
0.025
0.02
0.015
0.01
0.005
0
Raman Intensity (A.U)
0 mM
7.5 mM
15 mM
30 mM
■ UPLC ■ I_{418} ■ I_{447}
R=0.81 R=0.73

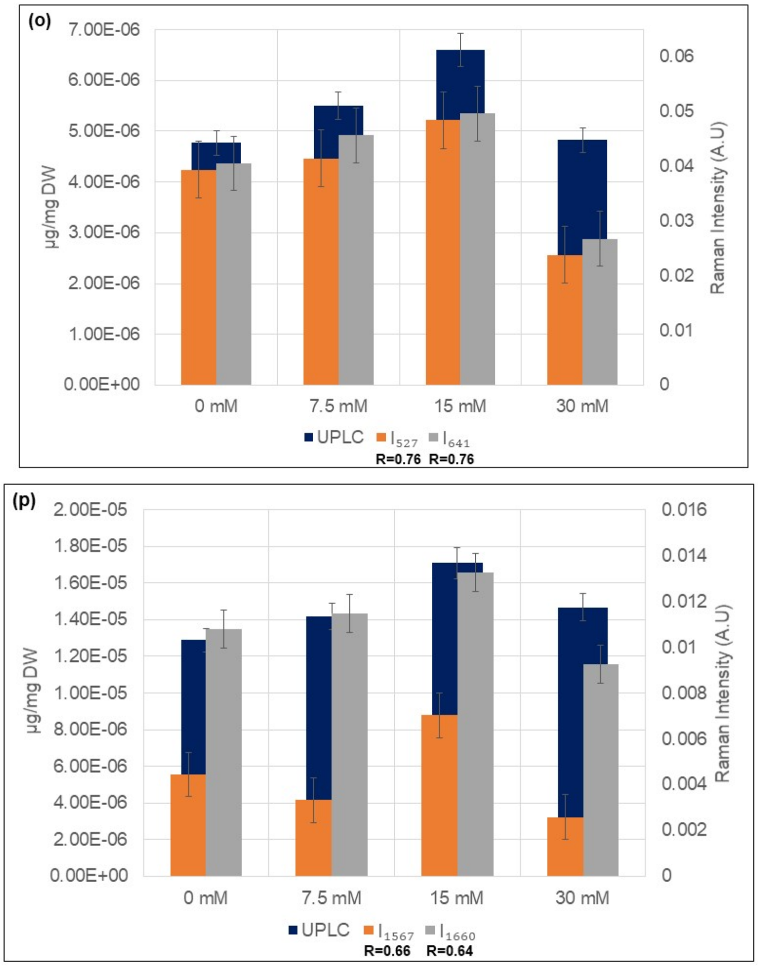
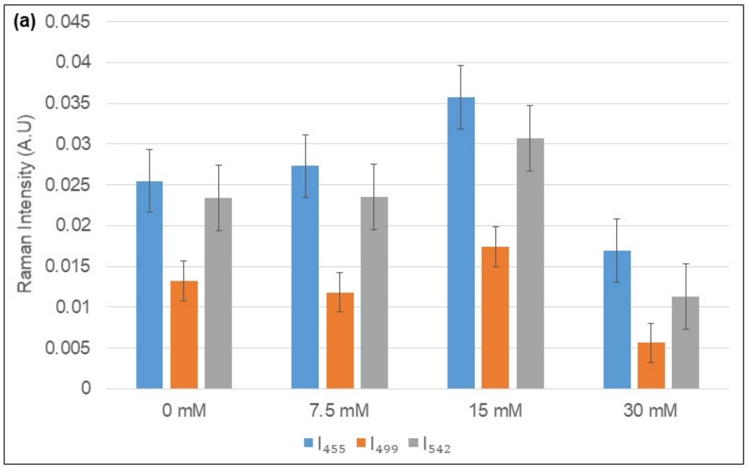


Figure S6. Correlation of Raman data with UPLC analysis of amino acids in the acetate study. (a) Alanine, (b) Arginine, (c) Aspartate/Asparagine, (d) Glutamate, (e) Glycine, (f) Histidine, (g) Isoleucine, (h) Leucine, (i) Lysine, (j) Methionine, (k) Phenylalanine, (l) Proline, (m) Serine, (n) Threonine, (o) Tyrosine, (p) Valine. Correlation coefficients (R) for each Raman band are represented.



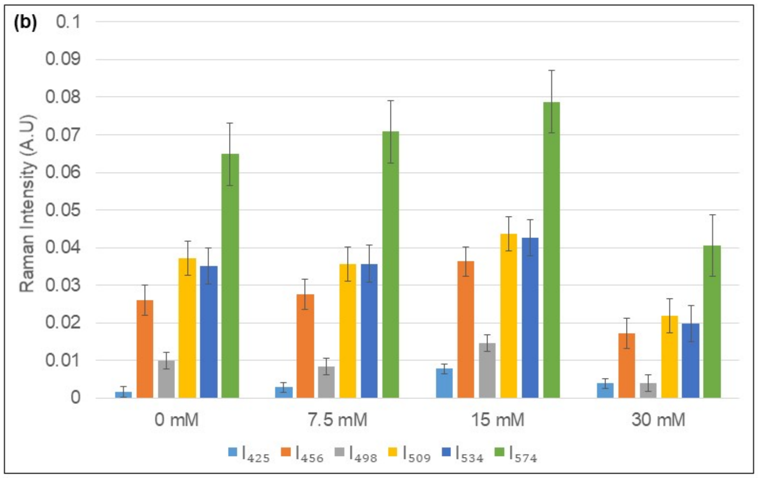


Figure S7. Acetate study: Raman bands predictions of amino acids non-resolved with UPLC. (a) Cysteine predictions, (b) Tryptophan predictions.

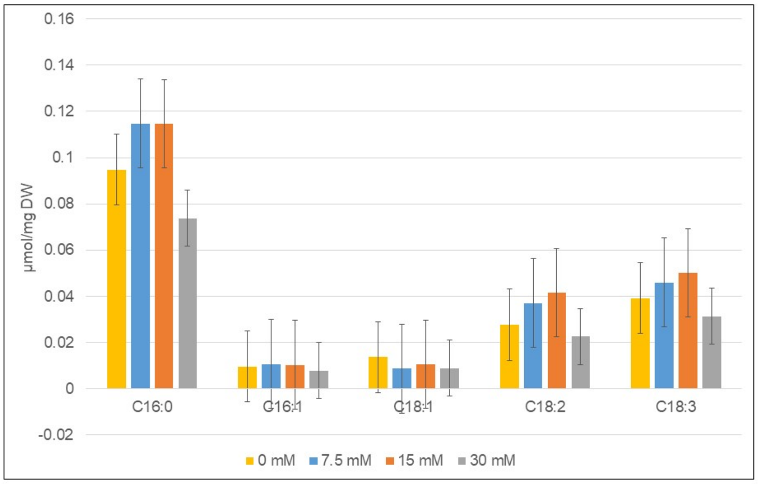
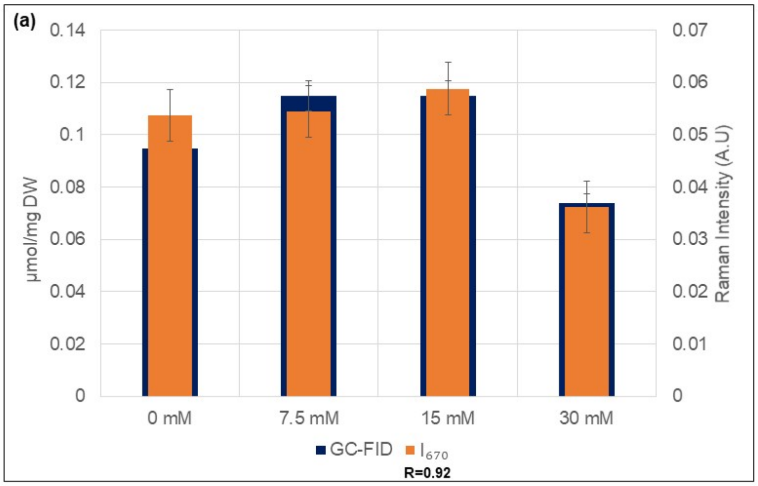
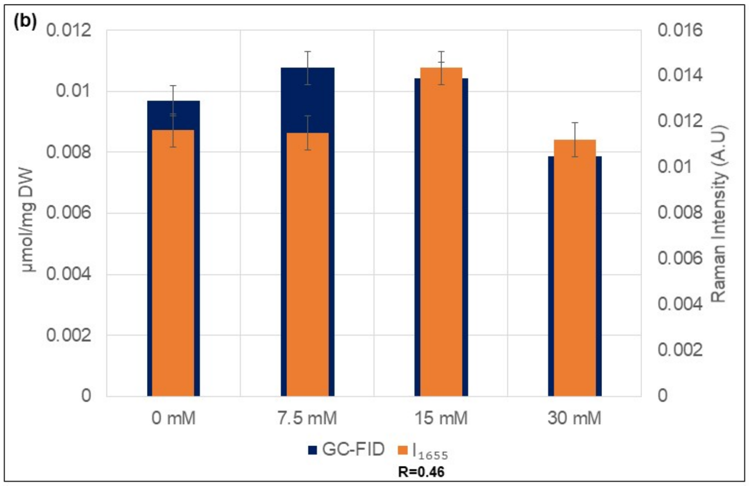
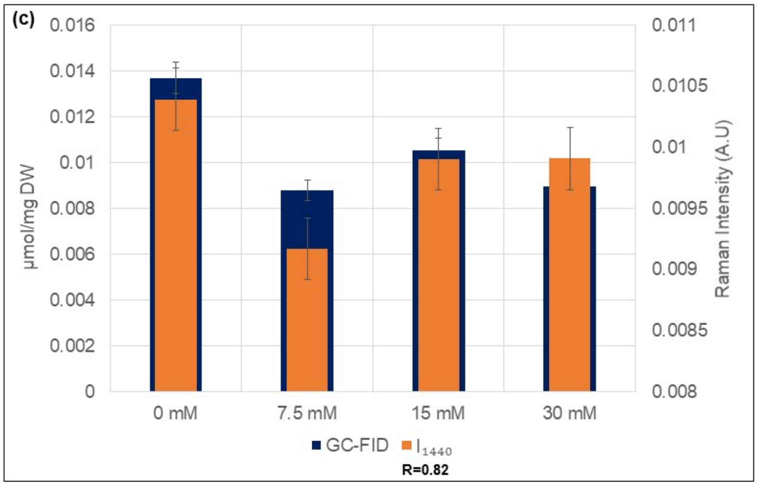


Figure S8. Levels of fatty acids for acetate study. Data represent means ± standard deviation (SD) of values from three independent experiments.


(a)
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
μmol/mg DW
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Raman Intensity (A.U)
0 mM
7.5 mM
15 mM
30 mM
■ GC-FID ■ I_{670}
R=0.92


(b)
0.012
0.01
0.008
0.006
0.004
0.002
0
μmol/mg DW
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
Raman Intensity (A.U)
0 mM
7.5 mM
15 mM
30 mM
■ GC-FID ■ I_{1655}
R=0.46


(c)
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
μmol/mg DW
0.011
0.0105
0.01
0.0095
0.009
0.0085
0.008
Raman Intensity (A.U)
0 mM
7.5 mM
15 mM
30 mM
■ GC-FID ■ I_{1440}
R=0.82

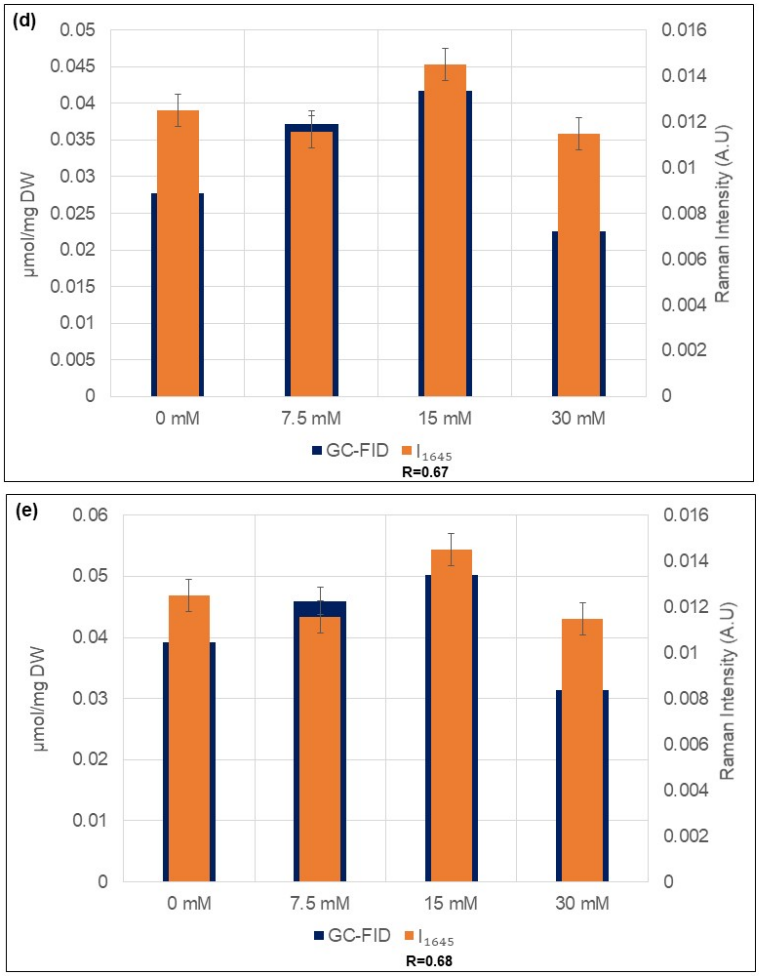


Figure S9. Correlation between Raman spectroscopy and GC-FID data of fatty acids for acetate study. (a) Palmitic acid, (b) Palmitoleic acid, (c) Oleic acid (d) Linoleic acid, (e) Linolenic acid. Correlation coefficients (R) for each Raman band are represented.

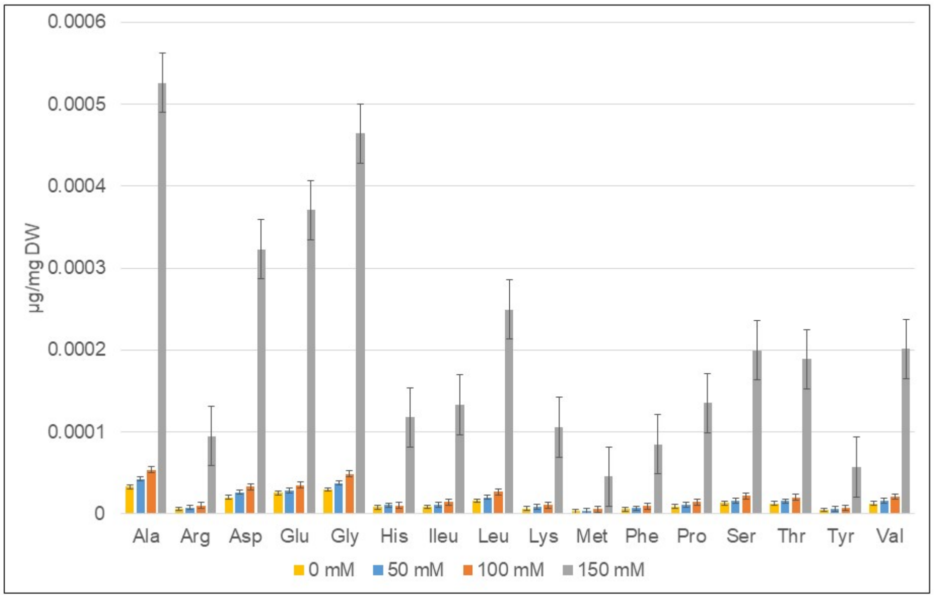
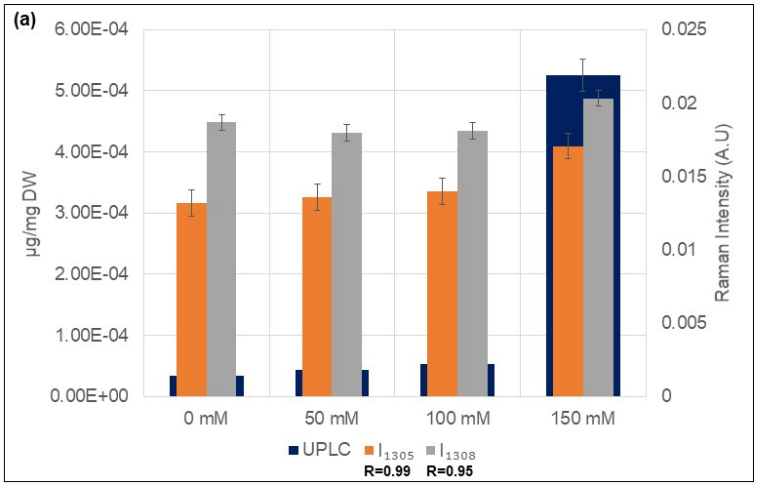
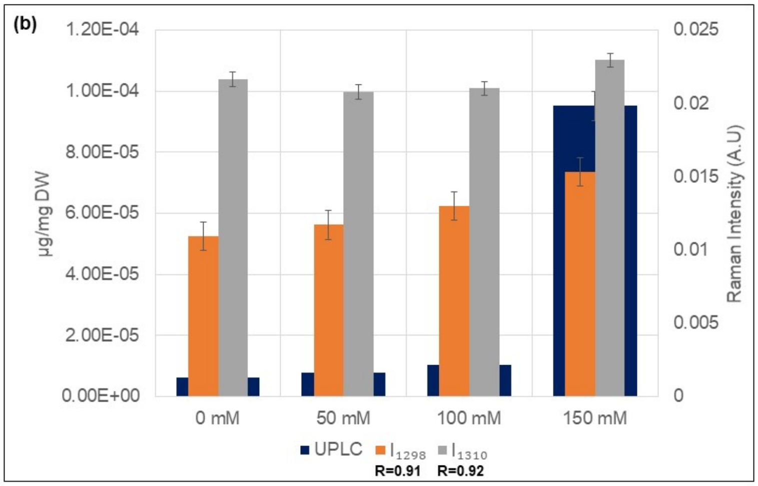
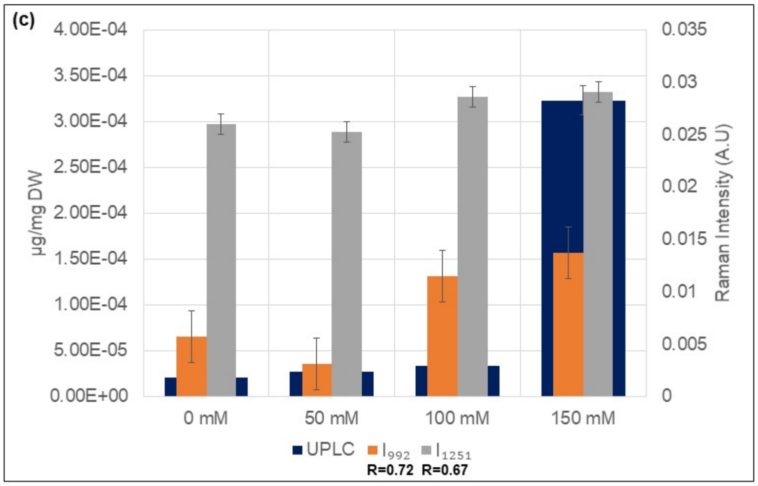
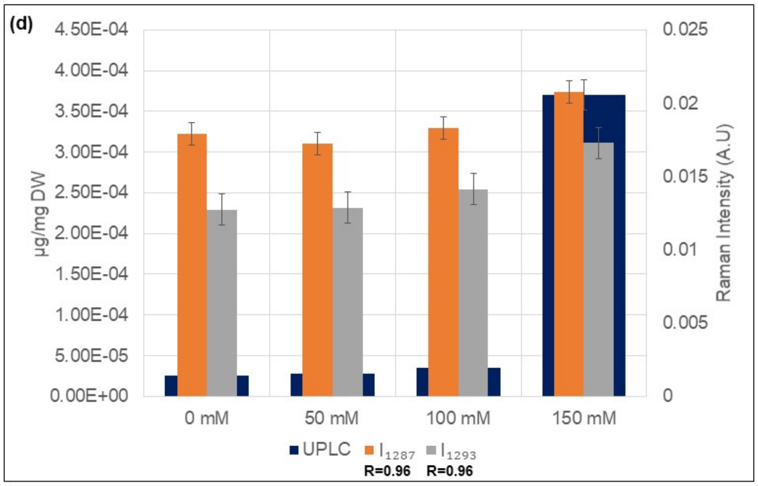


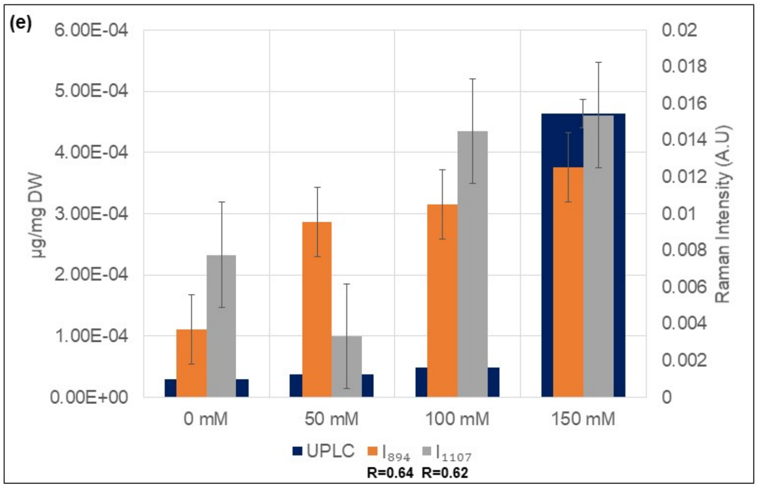
Figure S10. Amino acids analysis for NaCl study. Data represent means ± standard deviation (SD) of values from three independent experiments.

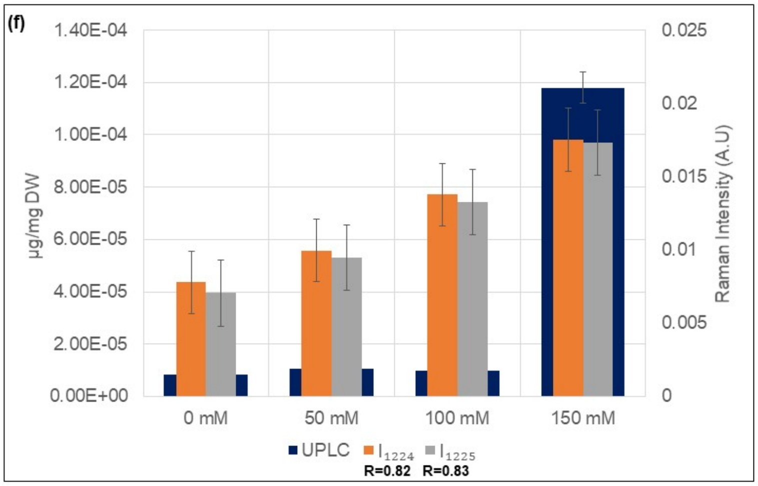


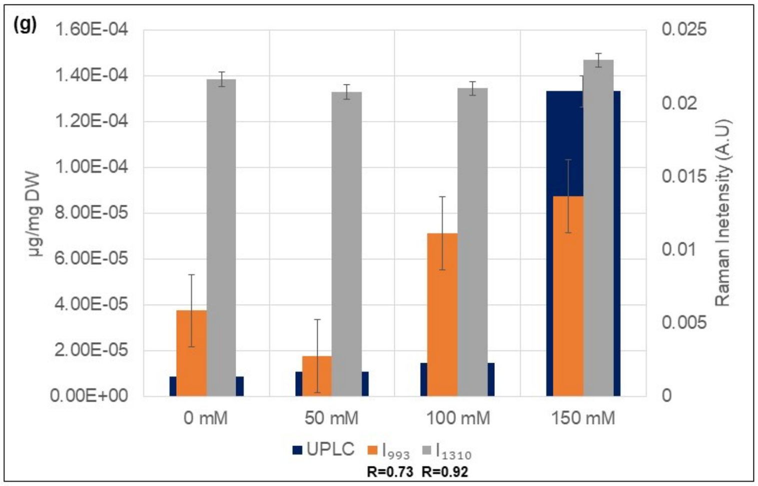

(b)
µg/mg DW
1.20E-04
1.00E-04
8.00E-05
6.00E-05
4.00E-05
2.00E-05
0.00E+00
Raman Intensity (A.U)
0.025
0.02
0.015
0.01
0.005
0
0 mM
50 mM
100 mM
150 mM
UPLC
I_{1298}
I_{1310}
R=0.91
R=0.92

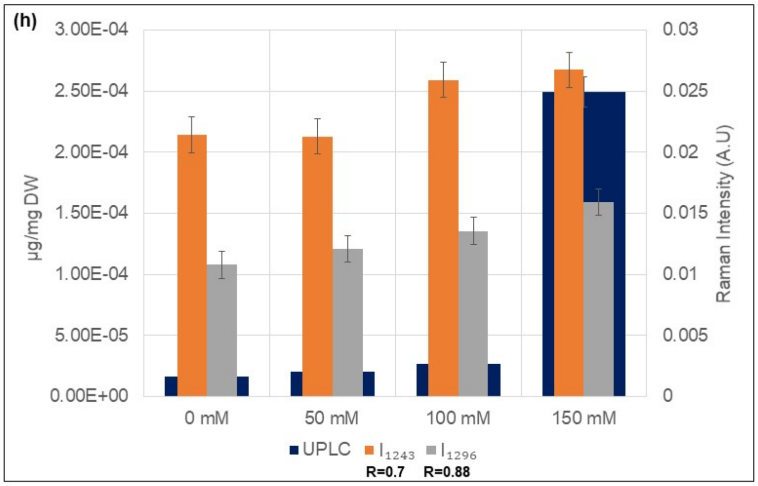

(c)
µg/mg DW
4.00E-04
3.50E-04
3.00E-04
2.50E-04
2.00E-04
1.50E-04
1.00E-04
5.00E-05
0.00E+00
Raman Intensity (A.U)
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
0 mM
50 mM
100 mM
150 mM
UPLC
I_{992}
I_{1251}
R=0.72
R=0.67

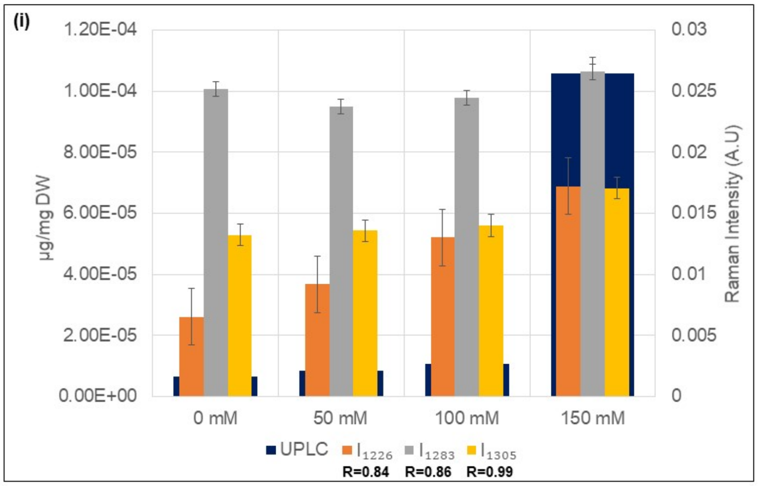

(d)
µg/mg DW
4.50E-04
4.00E-04
3.50E-04
3.00E-04
2.50E-04
2.00E-04
1.50E-04
1.00E-04
5.00E-05
0.00E+00
Raman Intensity (A.U)
0.025
0.02
0.015
0.01
0.005
0
0 mM
50 mM
100 mM
150 mM
UPLC
I_{1287}
I_{1293}
R=0.96
R=0.96

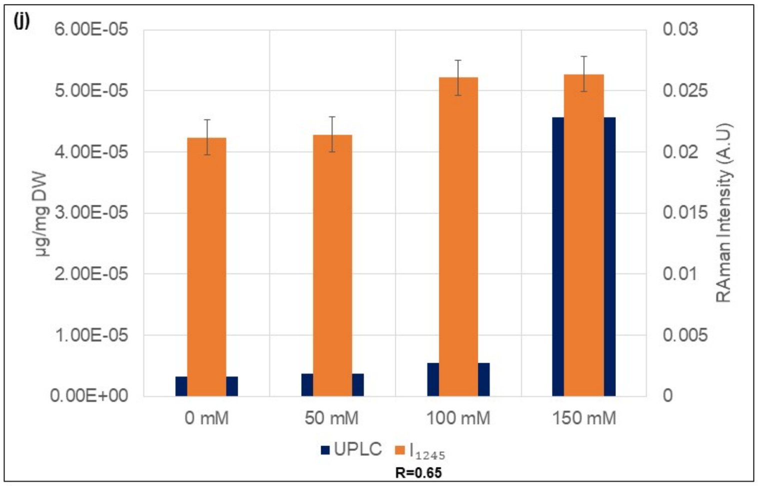

(e)
6.00E-04
5.00E-04
4.00E-04
3.00E-04
2.00E-04
1.00E-04
0.00E+00
µg/mg DW
0.02
0.018
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
Raman Intensity (A.U)
0 mM
50 mM
100 mM
150 mM
UPLC
I_{894}
I_{1107}
R=0.64
R=0.62

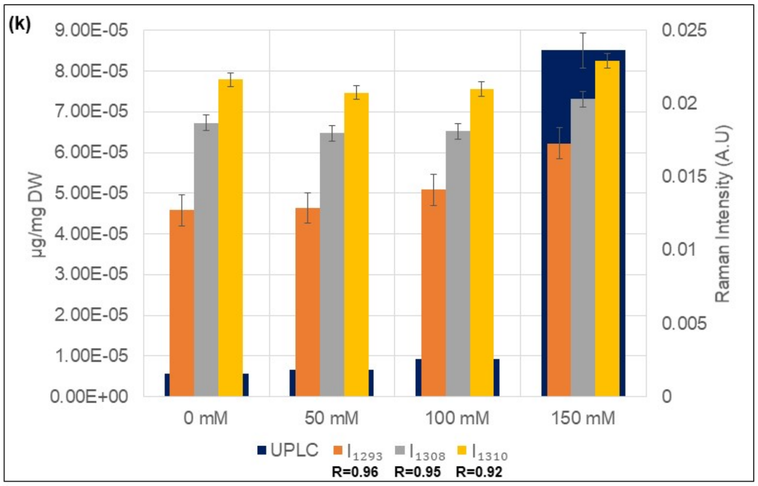

(f)
1.40E-04
1.20E-04
1.00E-04
8.00E-05
6.00E-05
4.00E-05
2.00E-05
0.00E+00
µg/mg DW
0.025
0.02
0.015
0.01
0.005
0
Raman Intensity (A.U)
0 mM
50 mM
100 mM
150 mM
UPLC
I_{1224}
I_{1225}
R=0.82
R=0.83

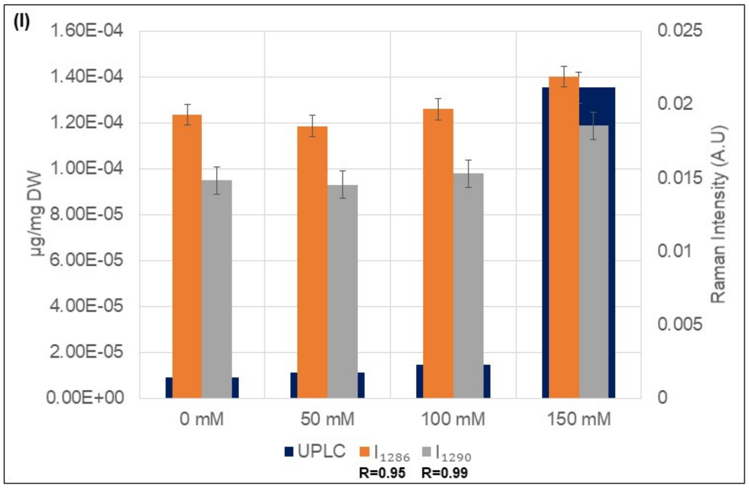

(g)
1.60E-04
1.40E-04
1.20E-04
1.00E-04
8.00E-05
6.00E-05
4.00E-05
2.00E-05
0.00E+00
µg/mg DW
0.025
0.02
0.015
0.01
0.005
0
Raman Inetensity (A.U)
0 mM
50 mM
100 mM
150 mM
UPLC
I_{993}
I_{1310}
R=0.73
R=0.92

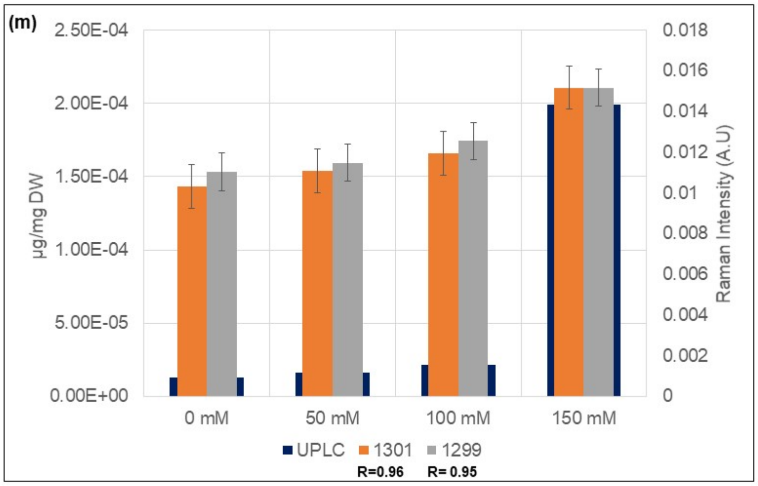

(h)
µg/mg DW
Raman Intensity (A.U)
3.00E-04
2.50E-04
2.00E-04
1.50E-04
1.00E-04
5.00E-05
0.00E+00
0.03
0.025
0.02
0.015
0.01
0.005
0
0 mM
50 mM
100 mM
150 mM
■ UPLC ■ I_{1243} ■ I_{1296}
R=0.7 R=0.88


(i)
µg/mg DW
Raman Intensity (A.U)
1.20E-04
1.00E-04
8.00E-05
6.00E-05
4.00E-05
2.00E-05
0.00E+00
0.03
0.025
0.02
0.015
0.01
0.005
0
0 mM
50 mM
100 mM
150 mM
■ UPLC ■ I_{1226} ■ I_{1283} ■ I_{1305}
R=0.84 R=0.86 R=0.99


(j)
µg/mg DW
RAman Intensity (A.U)
6.00E-05
5.00E-05
4.00E-05
3.00E-05
2.00E-05
1.00E-05
0.00E+00
0.03
0.025
0.02
0.015
0.01
0.005
0
0 mM
50 mM
100 mM
150 mM
■ UPLC ■ I_{1245}
R=0.65


(k)
µg/mg DW
Raman Intensity (A.U)
9.00E-05
8.00E-05
7.00E-05
6.00E-05
5.00E-05
4.00E-05
3.00E-05
2.00E-05
1.00E-05
0.00E+00
0.025
0.02
0.015
0.01
0.005
0
0 mM
50 mM
100 mM
150 mM
UPLC
I_{1293}
I_{1308}
I_{1310}
R=0.96
R=0.95
R=0.92


(l)
µg/mg DW
Raman Intensity (A.U)
1.60E-04
1.40E-04
1.20E-04
1.00E-04
8.00E-05
6.00E-05
4.00E-05
2.00E-05
0.00E+00
0.025
0.02
0.015
0.01
0.005
0
0 mM
50 mM
100 mM
150 mM
UPLC
I_{1286}
I_{1290}
R=0.95
R=0.99


(m)
µg/mg DW
Raman Intensity (A.U)
2.50E-04
2.00E-04
1.50E-04
1.00E-04
5.00E-05
0.00E+00
0.018
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
0 mM
50 mM
100 mM
150 mM
UPLC
1301
1299
R=0.96
R= 0.95

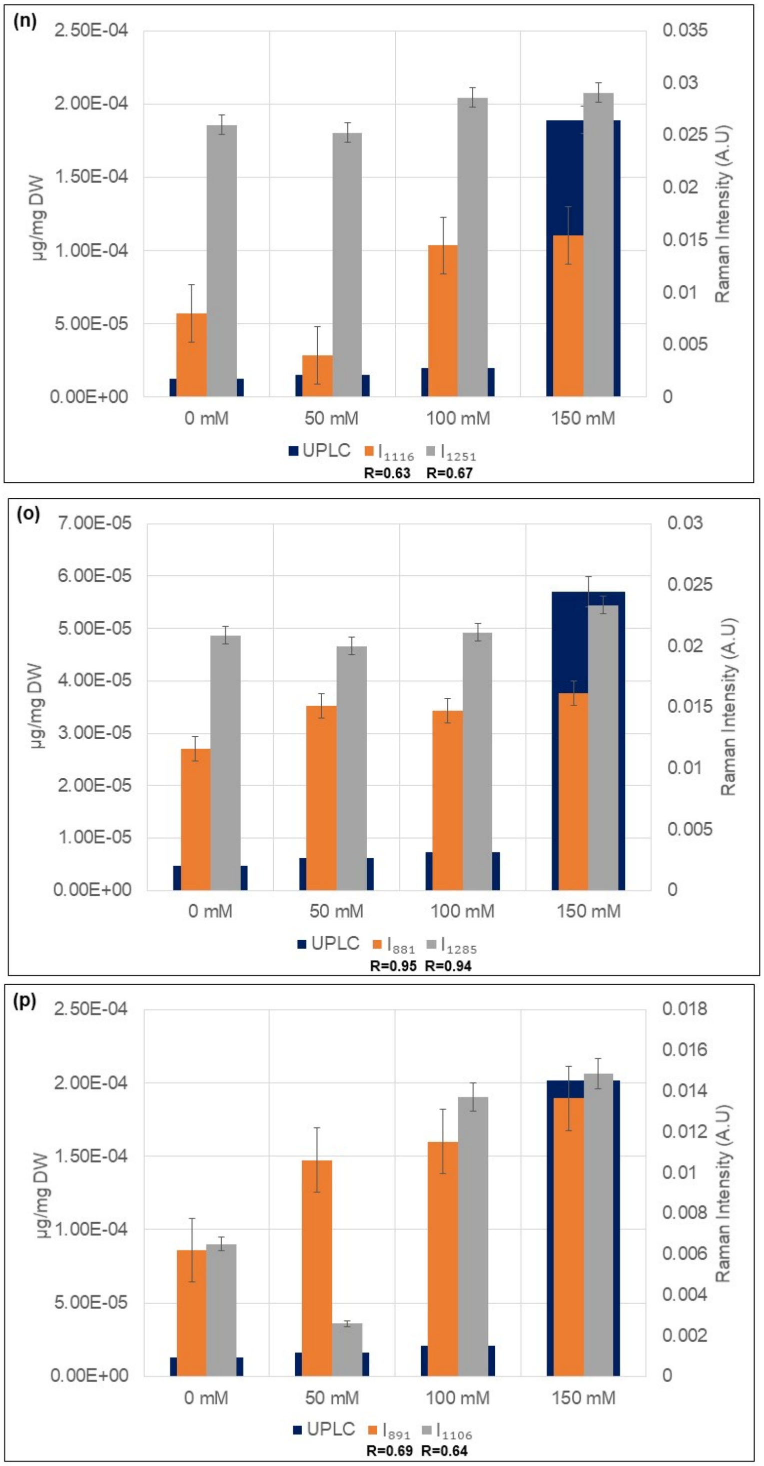


Figure S11. Correlation of Raman data with UPLC analysis of amino acids in the NaCl study. (a) Alanine, (b) Arginine, (c) Aspartate/Asparagine, (d) Glutamate, (e) Glycine, (f) Histidine, (g) Isoleucine, (h) Leucine, (i) Lysine, (j) Methionine, (k) Phenylalanine, (l) Proline, (m) Serine, (n) Threonine, (o) Tyrosine, (p) Valine. Correlation coefficients (R) for each Raman band are represented.

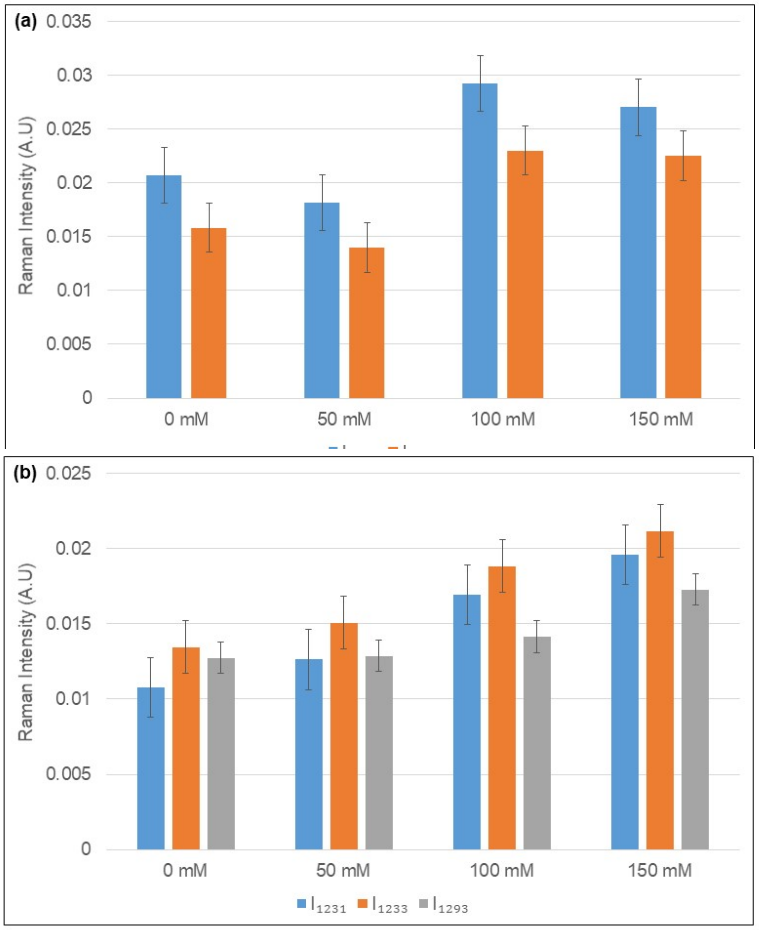


Figure S12. NaCl study: Raman bands predictions of amino acids non-resolved with UPLC. (a) Cysteine predictions, (b) Tryptophan predictions.

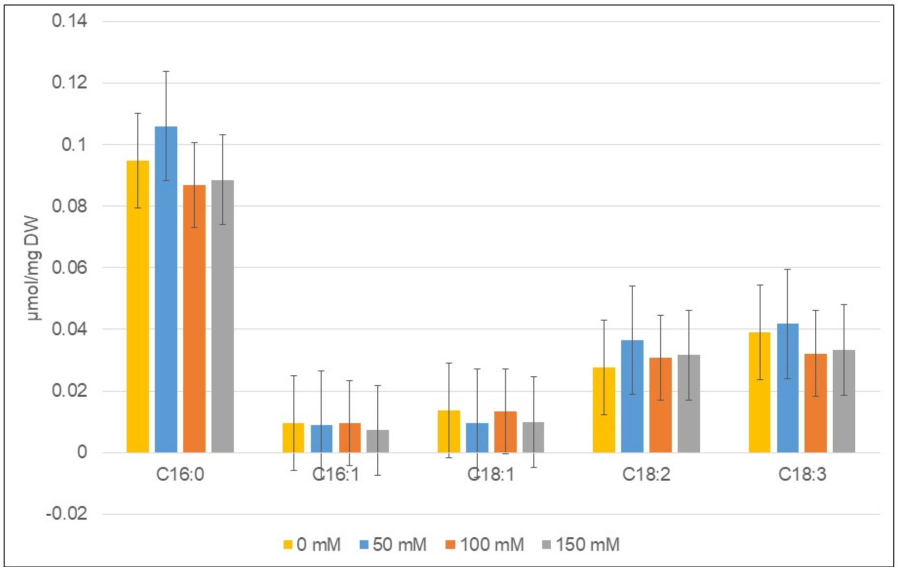
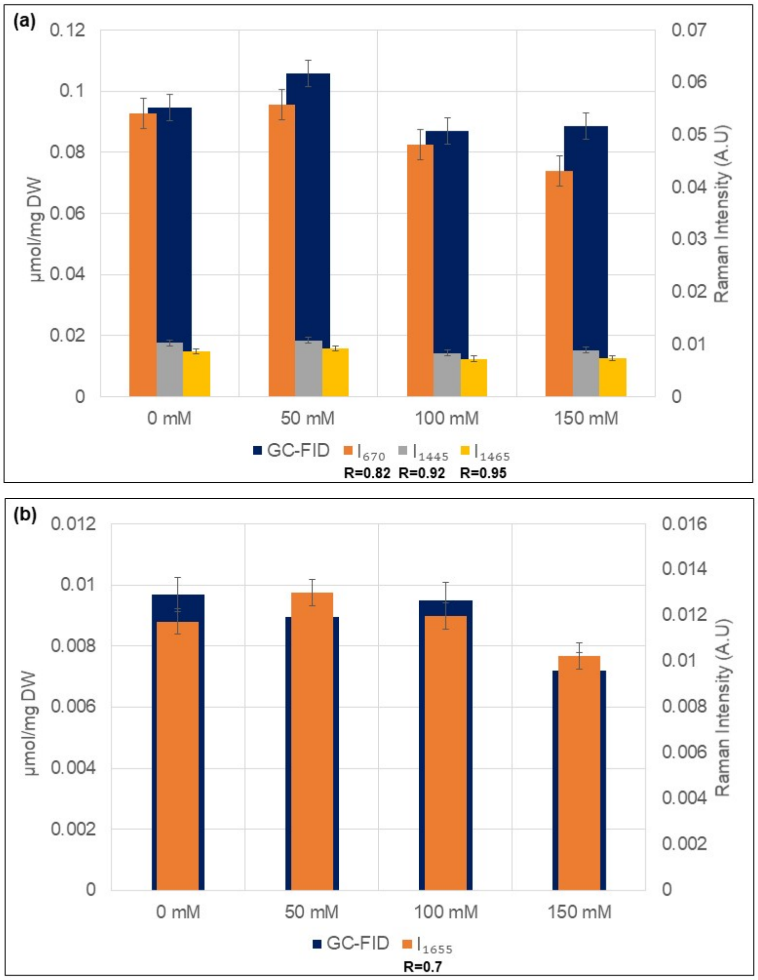


Figure S13. Levels of fatty acids for NaCl study. Data represent means ± standard deviation (SD) of values from three independent experiments.



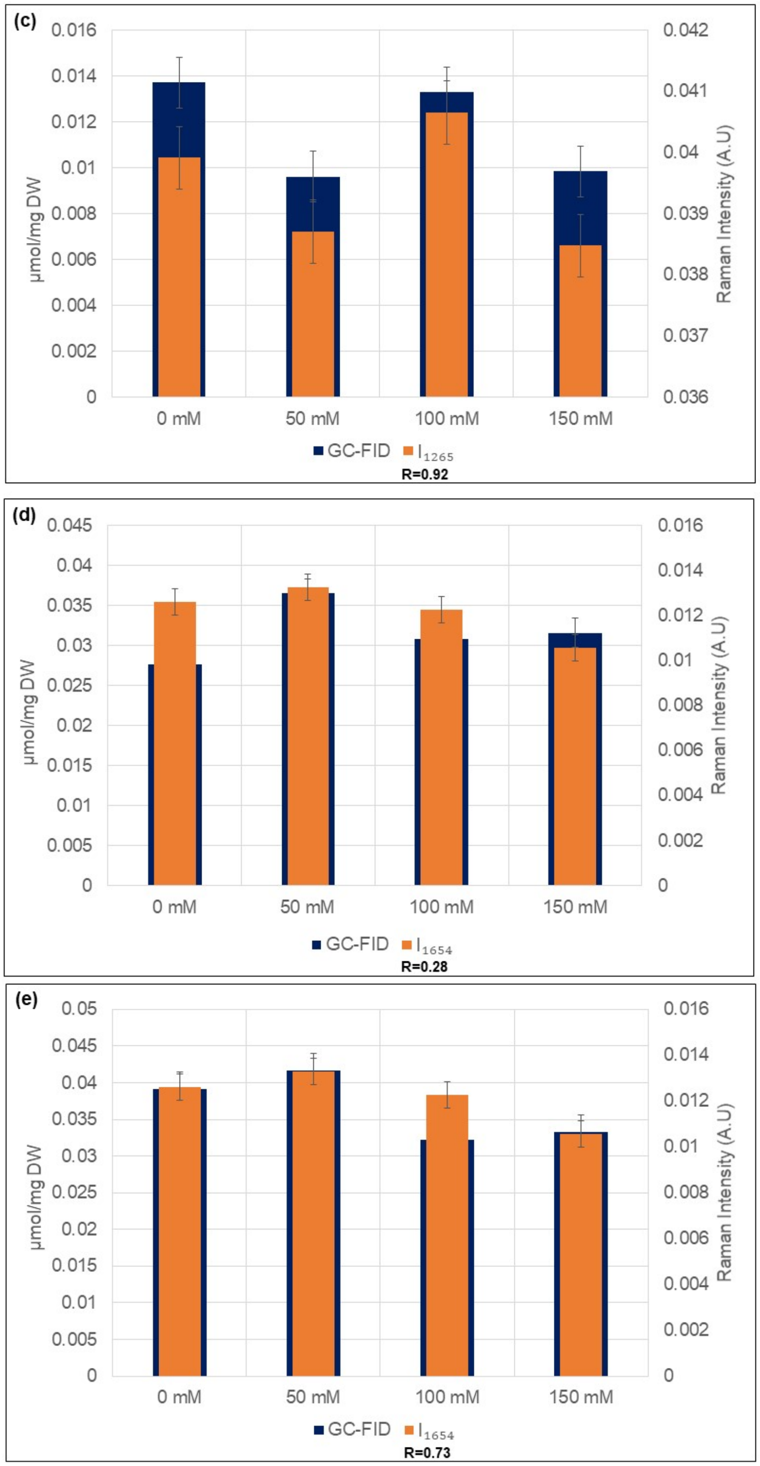


Figure S14. Correlation between Raman spectroscopy and GC-FID data of fatty acids for the NaCl study. (a) Palmitic acid, (b) Palmitoleic acid, (c) Oleic acid (d) Linoleic acid, (e) Linolenic acid. Correlation coefficients (R) for each Raman band are represented.

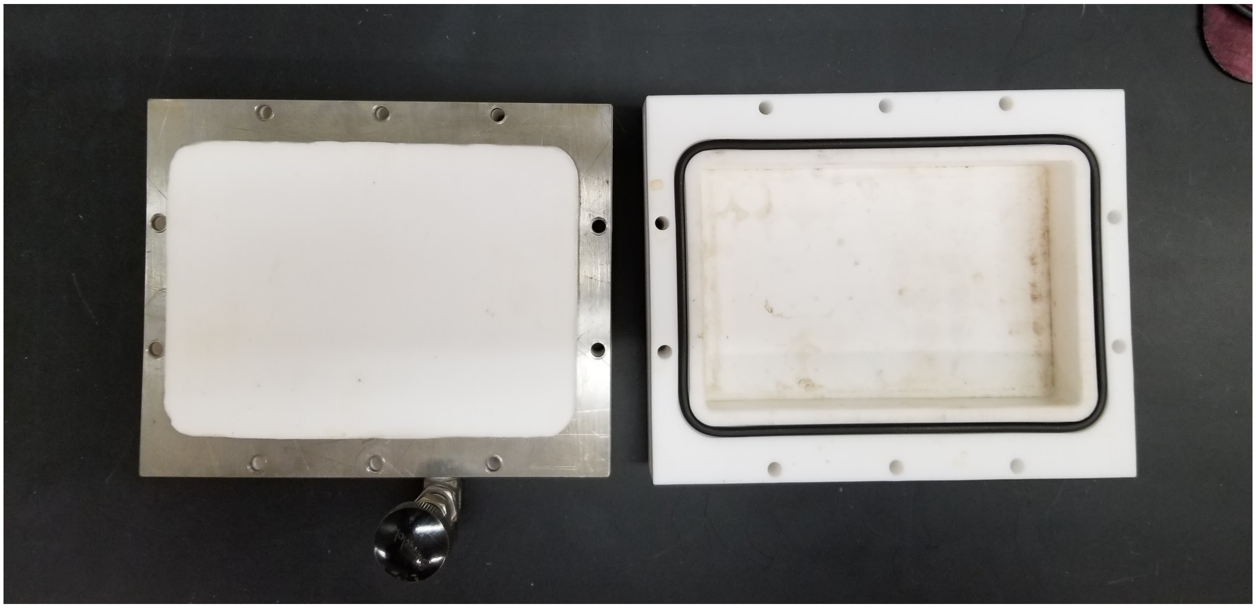


Figure S15. The fabricated teflon hydrolysis chamber.

Table S1. All Raman bands cited and tested for amino acids and fatty acids.

Acetate Study		
Biomolecules	**Raman bands (cm^{-1}) and Correlation Coefficient (R)**	**References**
Ala	480 (R = 0.45), 653 (R = 0.43)	(1,2)
Arg	1713 (R = 0.87)	(1)
Asp/Asn	1695 (R = 0.97)	(2)
Cys*	455, 499, 542	(2)
Glu/Gln	1462 (R = 0.87), 1624 (R = 0.73)	(1,2)
Gly	496 (R = 0.71), 1568 (R = 0.73), 1674 (R = 0.84)	(2)

His	422 (R = 0.79)	(1)
Ile	557 (R = 0.63)	(2)
Leu	1583 (R = 0.49)	(2)
Lys	1570 (R = 0.5)	(2)
Met	645 (R = 0.59)	(2)
Phe	468 (R = 0.70), 469 (R = 0.72), 0.74 (R = 605), 606 (R = 0.73)	(1,2)
Pro	452 (R = 0.46)	(1)
Ser	610 (R = 0.63), 611 (R = 0.61)	(1,2)
Thr	418 (R = 0.81), 447 (R = 0.73)	(2)
Trp*	425, 456, 498, 509, 509, 574	(1)
Tyr	527 (R = 0.76), 641 (R = 0.76)	(1,2)
Val	1567 (R = 0.66), 1660 (R = 0.64)	(1)
Palmitic acid (C16:0)	670 (R = 0.92)	(1)
Palmitoleic acid (C16:1)	1655 (R = 0.46)	(3)
Oleic acid (C18:1)	1440 (R = 0.82)	(1)
Linoleic acid (C18:2)	1645 (R = 0.67)	(3)
Linolenic acid (C18:3)	1645 (R = 0.68)	(3)
Sodium chloride Study		

Biomolecules	**Raman bands (cm^{-1}) and Correlation Coefficient (R)**	**References**
Ala	1305 (R = 0.99), 1308 (R = 0.95)	(1,2)
Arg	1298 (R = 0.91), 1310 (R = 0.92)	(1,2)
Asp/Asn	992 (R = 0.72), 1251 (R = 0.67)	(2)
Cys*	1041, 1092	(2)
Glu/Gln	1287 (R = 0.96), 1293 (R = 0.96)	(1,2)
Gly	894 (R =0.64), 1107 (R = 0.62)	(1,2)
His	1224 (R = 0.82), 1225 (R = 0.83)	(1,2)
Ile	993 (R = 0.73), 1310 (R = 0.92)	(2)
Leu	1243 (R = 0.7), 1296 (R = 0.88)	(2)
Lys	1226 (R = 0.84), 1283 (R = 0.86), 1305 (R = 0.99)	(2)
Met	1245 (R = 0.65)	(2)
Phe	1293 (R = 0.96), 1308 (R = 0.95), 1310 (R = 0.92)	(1,2)
Pro	1286 (R = 0.95), 1290 (R = 0.99)	(1)
Ser	1299 (R = 0.95), 1301 (R = 0.96)	(1,2)
Thr	1116 (R = 0.63), 1251 (R = 0.67)	(2)
Trp*	1231, 1233, 1293	(1,2)
Tyr	881 (R = 0.95), 1285 (R = 0.94)	(2)

Val	891 (R = 0.69), 1106 (R = 0.64)	(1)
Palmitic acid (C16:0)	670 (R = 0.82), 1445 (R = 0.92), 1465 (R = 0.95)	(1)
Palmitoleic acid (C16:1)	1655 (R = 0.7)	(3)
Oleic acid (C18:1)	1265 (R = 0.92)	(1)
Linoleic acid (C18:2)	1654 (R = 0.28)	(3)
Linolenic acid (C18:3)	1654 (R = 0.73)	(3)

* Values for Cys and Trp could not be obtained by the UPLC method used in this research. Only Raman predictions are provided.

References

1. De Gelder J, De Gussem K, Vandenabeele P, Moens L. Reference database of Raman spectra of biological molecules. J Raman Spectrosc. 2007;38(9):1133–47.
2. Zhu G, Zhu X, Fan Q, Wan X. Raman spectra of amino acids and their aqueous solutions. Spectrochim Acta Mol Biomol Spectrosc. 2011;78(3):1187–95.
3. Czamara K, Majzner K, Pacia MZ, Kochan K, Kaczor A, Baranska M. Raman spectroscopy of lipids: a review. J Raman Spectrosc. 2015;46(1):4–20.