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Supporting Information

Raman-DIP (Deuterium Isotope Probing) Reveals Microbial Metabolism at the Single-Cell Level by Using Deuterated Carbon Sources

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This file includes details on control Raman spectra, control growth kinetics, biological mechanism, phenylalanine structure and computational calculations.

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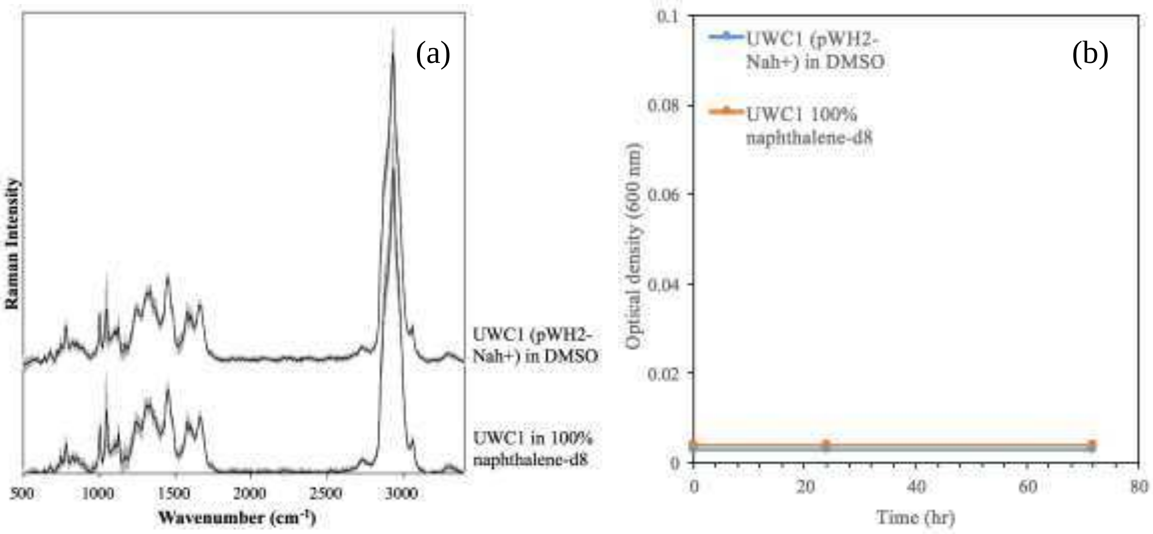

Figure S1. (a) Raman spectra of P. putida UWC1 (pWH2-Nah$^+$) grown in DMSO without any naphthalene and P. putida UWC1 grown in 100% naphthalene-d_8, both of which have a silent Raman window between 2070 - 2300 cm^{-1}. Each spectrum is an average of 30 single-cell Raman spectra and the shaded area represents the standard deviation. (b) The OD_{600} remains the same during 72 hours for P. putida UWC1 (pWH2-Nah$^+$) grown in DMSO without any naphthalene and P. putida UWC1 grown in 100% naphthalene-d_8.

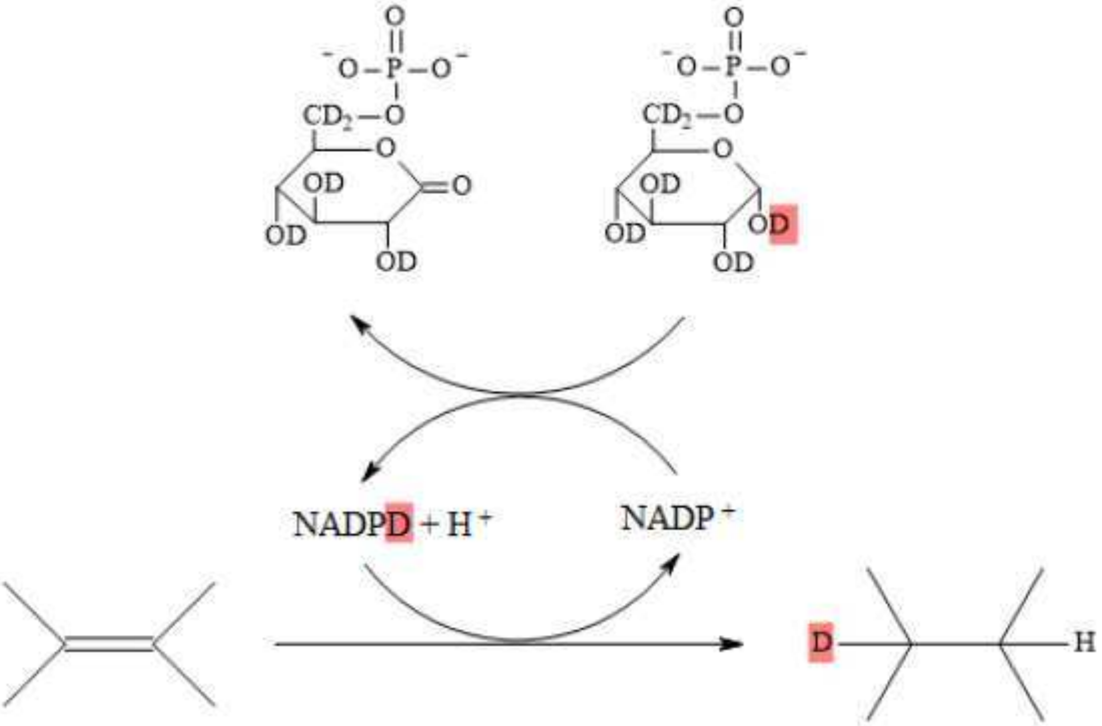

Figure S2. Biological mechanism of incorporation of deuterium from deuterated carbon source, using glucose-d$_{12}$ as an example. Deuterium in the deuterated carbon source contributes to a new C-D bond during the reduction reactions to synthesise biomolecules via the action of NADP$^+$/NADPH.

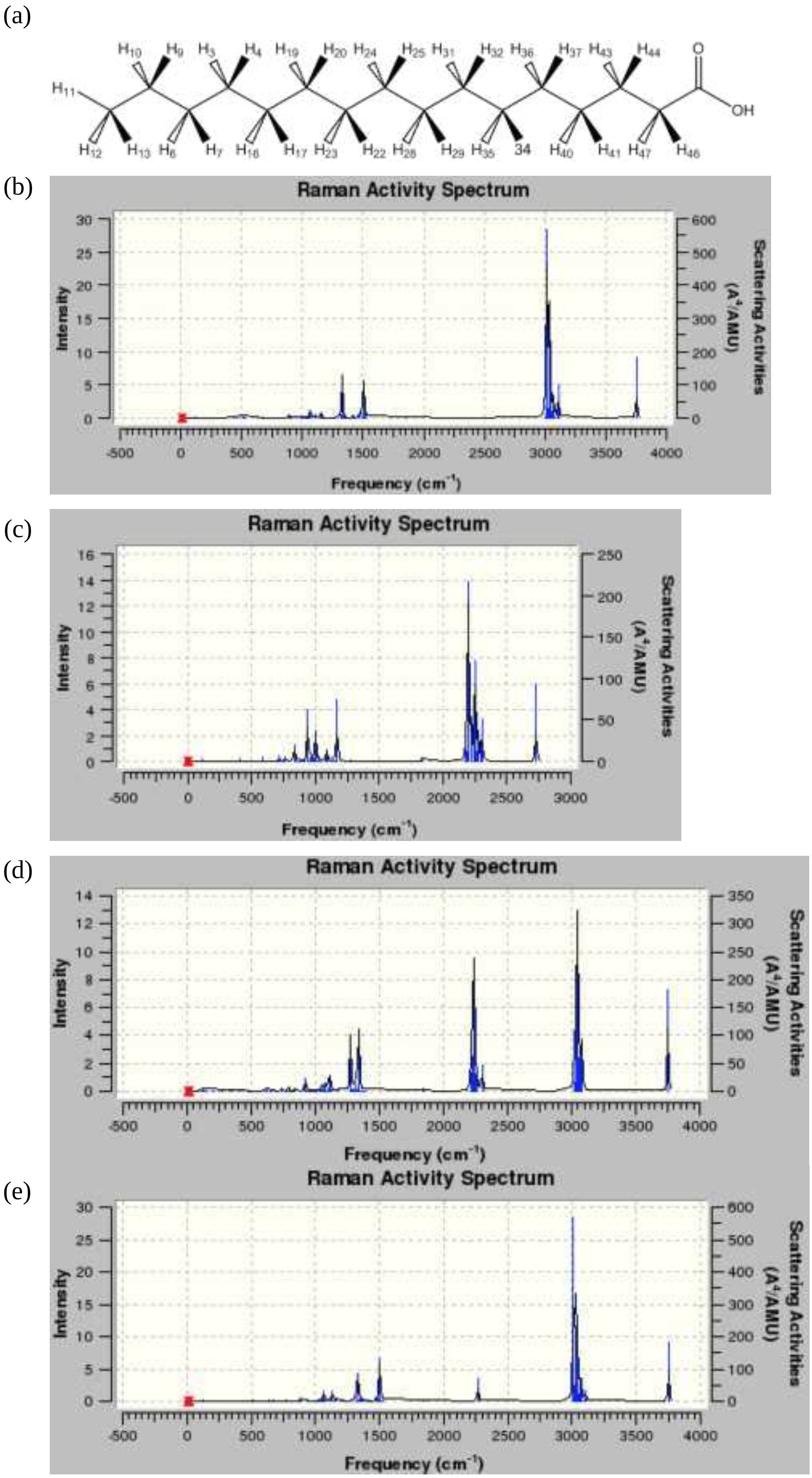
(a)
H10 H9 H3 H4 H19 H20 H24 H25 H31 H32 H36 H37 H43 H44
O
H11
OH
H12 H13 H6 H7 H16 H17 H23 H22 H28 H29 H35 34 H40 H41 H47 H46
(b)
Raman Activity Spectrum
Intensity
Scattering Activities (A4/AMU)
Frequency (cm-1)
-500 0 500 1000 1500 2000 2500 3000 3500 4000
0 5 10 15 20 25 30
0 100 200 300 400 500 600
(c)
Raman Activity Spectrum
Intensity
Scattering Activities (A4/AMU)
Frequency (cm-1)
-500 0 500 1000 1500 2000 2500 3000
0 2 4 6 8 10 12 14 16
0 50 100 150 200 250
(d)
Raman Activity Spectrum
Intensity
Scattering Activities (A4/AMU)
Frequency (cm-1)
-500 0 500 1000 1500 2000 2500 3000 3500 4000
0 2 4 6 8 10 12 14
0 50 100 150 200 250 300 350
(e)
Raman Activity Spectrum
Intensity
Scattering Activities (A4/AMU)
Frequency (cm-1)
-500 0 500 1000 1500 2000 2500 3000 3500 4000
0 5 10 15 20 25 30
0 100 200 300 400 500 600

Figure S3. a) Molecular structure of palmitic acid. The optimisation yielded a fully extended conformer, with mirror symmetry. Simulated Raman spectra for palmitic acid as a simplified model for aliphatic CH environments in lipids. The spectra from top to bottom are calculated for (b) the all-H form of the molecule, (c) the all-D form, (d) a form in which all H positions to one side of the mirror plane are replaced by D and (e) a sample single-D substitution on the end methyl group.

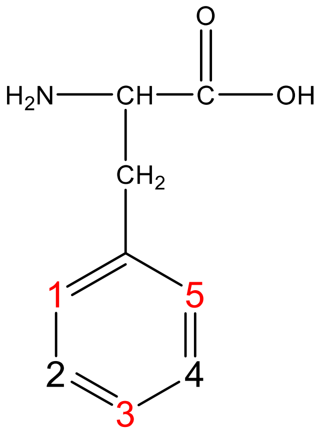

Figure S4. Phenylalanine molecular formula with labelled ring C-H positions. Only the red-labelled 1, 3 and 5 sites are responsible for observed phenylalanine shifts in wavenumber of the symmetric ring-breathing mode.

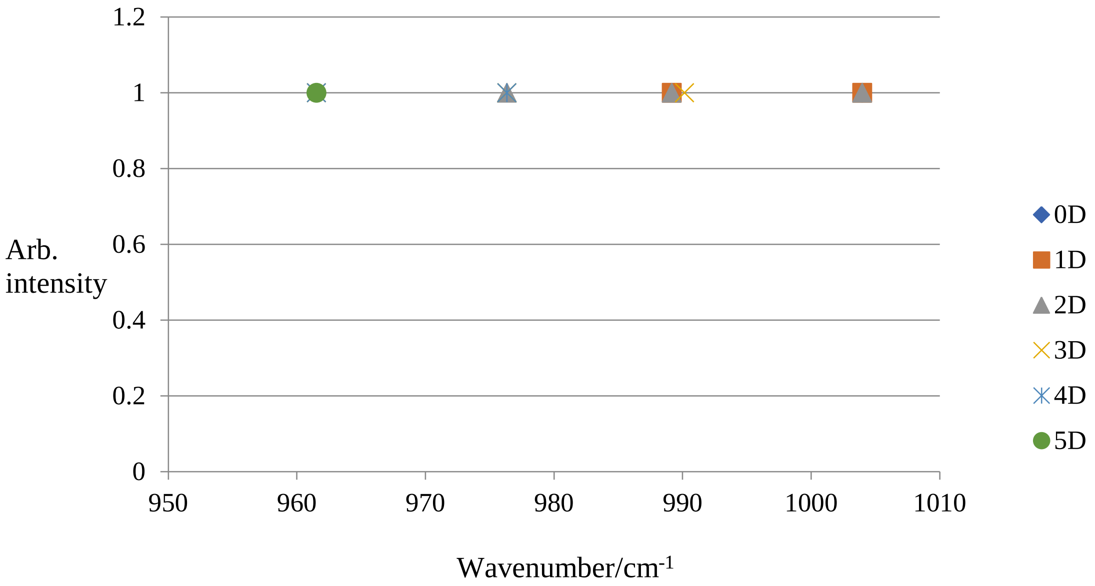

Figure S5. Only four distinct Raman-band positions are found, at 1004, 989-990, 976 and 962-963 cm^{-1}, for the 32 possible isotopomers of phenylalanine with different H/D ratios and distributions around the aromatic ring.

Table S1. The equilibrium geometry of the phenylalanine molecule was calculated at the B3LYP/6 31G** level of theory. Raman wavenumbers of 32 possible isotopomers of phenylalanine were calculated, ignoring symmetry redundancies. The averaged wavenumber represents an average from two wavenumbers generated and the collected peaks rule out equivalents from free rotations along symmetric axis. The 'ring breathing' vibration at 1017 cm^{-1} in the 'all-H' structure was identified as the match for the experimental peak at 1004 cm^{-1}. Raman-band wavenumbers are scaled by a factor of 1004/1017 for comparison with experiment and listed as scaled peaks in the table.

sites	**12345**	**wavenumber**	**wavenumber2**	**intensity**	**intensity2**	**number of D**	**free rotation equivalent**	**averaged wavenumber**	**collected peaks**	**scaled peaks**
1	HHHHH	1017		24.33		0	-	1017	1017	1004
2	DHHHH	1002		24.4		1	6	1002	1002	989.1917404
3	HDHHH	1017		24.68		1	5	1017	1017	1004
4	HHDHH	1003	1001	21.3	12.17	1	-	1002		
5	HHHDH	1017		25.9		1				
6	HHHHD	1001		23.97		1				
7	DDHHH	1002		25.76		2	16	1002	1002	989.1917404
8	DHDHH	988		29.92		2	15	989	989	976.3579154
9	DHHDH	1002		23.74		2	13	1002	1017	1004
10	DHHHD	988	988	17.65	8.87	2	-	988		
11	HDDHH	1003	1001	21.07	10.15	2	14	1002		
12	HDHDH	1017		26.26		2	-	1017		
13	HDHHD	1001		23.67		2				
14	HHDDH	1002		31.12		2				
15	HHDHD	989		28.36		2				
16	HHHDD	1002		26.28		2				
17	DDDHH	988		29.44		3	26	989	989	976.3579154
18	DDHDH	1002		26.21		3	25	1002	974	961.5496559
19	DDHHD	989		27.51		3	22	989	1003	990.1789577

20	DHDDH	989		28.55		3	24	989		
21	DHDHD	974		28.93		3	-	974		
22	DHHDD	988		26.61		3				
23	HDDDH	1003		29.77		3	-	1003		
24	HDDHD	989		28.06		3				
25	HDHDD	1001		26.19		3				
26	HHDDD	989		28.29		3				
27	DDDDH	988	992	24.37	8.49	4	31	990	989	976.3579154
28	DDDHD	974		27.78		4	30	974	974	961.5496559
29	DDHDD	988		26.81		4	-	988		
30	DHDDD	974		29.49		4				
31	HDDDD	989		28.12		4				
32	DDDDD	974		28.8		5	-	974	974	961.5496559

Table S2. Calculated band positions correlate with the experimental observation of phenylalanine Raman shifts. Theoretical Raman band frequencies have been scaled by a factor of 1004/1017. Black crosses represent theoretical calculation of vibrational wavenumbers and red crosses represent experimental Raman shifts observed in SCRS using E. coli as an example.

%D/band position (cm^{-1})	**1004**	**989-990**	**976**	**962**
0	x			
5	x			
10	x	x		
20	x	x		
25	x	x		
40	x	x	x	
50	x	x	x	
60	x	x	x	x
75	x	x	x	x
80		x	x	x
100		x	xx	xx