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ESI

High-resolution NMR-based metabolic detection of microgram biopsies using a 1 mm HRμMAS probe

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Sample preparation

Chicken and pig livers were purchased from a local supermarket. The rat [3-^{13}C]lactate infused brain biopsy[1] and [1-^{13}C]glucose infused brain extract[2] were obtained by Dr. Anne-Karine Bouzier-Sore (Centre RMSB, Bordeaux). The sample preparation for HRμMAS was performed under a stereomicroscope. A small biopsy was cut from the frozen liver using a surgical scalpel, and placed directly at the entrance of the Kel-F rotor (see Fig S1). Using a clean micro-drill bit with a diameter of 400 μm, gently pushed the frozen biopsy into the rotor. Filled the rotor with D_2O using a 20 μl GELoader® tip (Eppendorf, US), and closed with the Kel-F inserts. The entire sample-preparation period was about 5-10 minutes.

1 D. Sampol, E. Ostrofet, M-L. Jobin, G. Raffard, S. Sanchez, V. Bouchaud, J-M. Franconi, G. Bonvento and A-K. Bouzier-Sore, *Front. Neuroenerg.*, 2013, **5**, 5.
2 A-K. Bouzier-Sore, P. Voisin, V. Bouchaud, E. Bezancon, J.M. Franconi and L. Pellerin, *Eur. J. Neurosci.*, 2006, **24**, 1687–1694.

NMR experimental details

^{1}H NMR experiments were carried out on a JEOL spectrometer operating at 600.17 MHz (B_0 = 14.1 T) with a 1 mm HRμMAS probe (more detailed in text) using the ^{1}H-channel for detection and X-channel for ^{2}H-lock. A costume-made 1 mm Kel-F MAS rotor was used. The MAS frequency was manually adjusted to 2000 – 2500 Hz with stability of ±10 Hz. All ^{1}H NMR experiments were recorded under a ^{2}H-lock (expected for HMQC) with a continuous-wave (CW) pulse applied during the 1 s recycle-delay for suppressing the HDO resonance. The π/2-pulse length was set to 1.2 μs at 20 W. The B_0 shimming was performed on a sucrose-D_2O solution prior to the specimens. ^{1}H chemical shifts were internally referenced to the alanine $-CH_3-$ doublet at δ = 1.47 ppm.

The 1D ^{1}H NMR spectra were carried out with t_2-edited CMPG pulse-experiment. A spin-echo delay of 0.4 ms with 250 of echo loops were used, resulting a total echo time of 100 ms. A total of 8192 data points was acquired using a spectral width ranging from 10 ppm to 18.6 ppm rendering a scan repetition time of 1.7 s to 3.2 s. The total experimental times were in the range of 10 to 30 minutes depending on the number of scans acquired.

The 2D ^{1}H-^{1}H TOCSY spectrum of brain biopsy, which had been infused with [3-^{13}C]lactate, was acquired with a DIPSI2 train pulses during the spin-lock. A mixing time of 50 ms was used. A spectral dimension of 5 ppm × 5 ppm was applied with 256 data point in t_1 and 2048 in t_2. A total of 64 scans was collected per t_1, resulting a total experimental time of 7 hours.

The 2D ^{1}H-^{13}C HMQC spectrum of [3-^{13}C]lactate infused brain biopsy was recorded without the ^{2}H-lock, as the X-channel was used for the ^{13}C resonance. A ^{13}C-decoupled CW pulse was applied during the ^{1}H data acquisition. A spectral dimension of 15 ppm (^{1}H) × 15 ppm (^{13}C) was applied with 128 data point in t_1 and 8192 in t_2. A total of 448 scans was collected per t_1, resulting a total experimental time of 27.5 hours.

The 2D ^{1}H-^{1}H TOCSY spectrum of [1-^{13}C]glucose infused brain extract was carried out with a DIPSI2 train pulses during the spin-lock. A mixing time of 50 ms was used. A spectral dimension of 4 ppm × 4 ppm was applied with 196 data point in t_1 and 2048 in t_2. A total of 64 scans was collected per t_1, resulting a total experimental time of 5.8 hours.

The 2D ^{1}H-^{1}H COSY spectrum of [1-^{13}C]glucose infused brain extract was acquired with a phase sensitive COSY pulse experiment. A spectral dimension of 4 ppm × 4 ppm was applied with 512 data point in t_1 and 1280 in t_2. A total of 16 scans was collected per t_1, resulting a total experimental time of 3.2 hours.

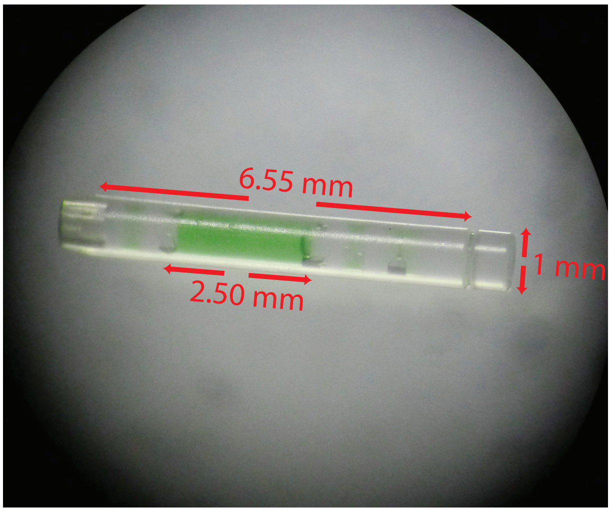

Fig. S1. A photo-illustration of a Kel-F MAS rotor. It was taken under the stereomicroscope. The rotor is filled with a green inorganic solution for showing the sample region. The rotor dimensions: 6.55 mm in length, 1 mm in outer diameter and 0.5 mm in inner diameter. The total NMR detectable sample volume is about 490 ηl.

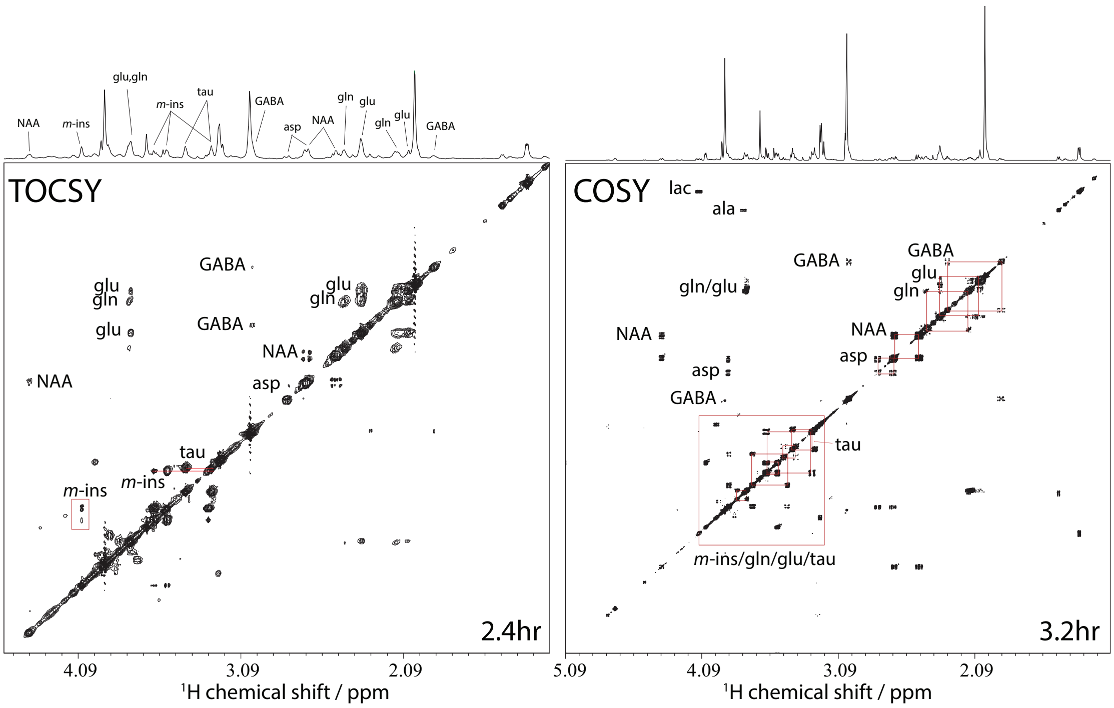

Fig. S2. ^{1}H-^{1}H TOCSY and ^{1}H-^{13}C HMQC HRμMAS spectrum of a [1-^{13}C]glucose infused brain extract.

Tab. S1. A comparison of the probe properties and NMR performances between 4mm HRMAS, 1mm HRμMAS and 840μm HRMACS

	HRMAS (Bruker)[a]	**HRμMAS prototype (JEOL)**	**HRMACS**[b]
Coil diameter	Fixed with 4 mm	Fixed with 1 mm	Flexible[b]
Probe durability	Strong	Strong	Weak
Detection volume	30 μl with a Kel-F insert	~490 nl	~250 nl
^{1}H frequency	500 MHz	600 MHZ	500 MHz
Unloaded coil quality factor	120	150	30 – 40
B_1 homogeneity ($I_{450°}/I_{90°}$)	0.9	0.95	0.70 – 0.75
Probe efficiency ($B_1/P^{0.5}$)[c]	0.15 mT/W	1.10 mT/W	1.00 mT/W
Resolution	0.002 ppm	~0.002 ppm	0.001 ppm
Sample holder	Disposable Kel-F insert	Disposable Kel-F rotor	Disposable glass capillary
MAS frequency	Up to 10,000 Hz	Up to 10,000 Hz	<500 Hz
1D experiment	Flexible	Flexible	Spinning sideband suppression
2D experiment	Flexible	Flexible	Only ^{1}H-detected experiments; caution with spinning sidebands
Data repeatability	Good	Good	Modest

a) A $^{1}H/^{31}P/^{13}C$ elongated HRMAS probe adapts to a widebore magnet.
b) The values and properties stated in the table correspond to the current design of a 840μm HRMACS resonator[1] with a frequency of 500 ± 20 MHz.
c) Based on the reciprocity principle,[2] the probe efficiency $B_1/P^{0.5}$ (where B_1 is the RF magnetic field at a given transmitter power P) is directly proportional to the detection sensitivity of a probe.

1. A. Wong, X. Li and D. Sakellariou, *Anal. Chem.*, 2013, **85**, 2021–2026.
2. D. I. Hoult and R. E. Richards, *J. Magn. Reson.*, 1976, **24**, 71–85.

Tab. 1S. The coefficient values (%) on each of the chemical shift bucket (0.005ppm) intensity (average sum) between 3.4 – 4.3 ppm of the 24 overlay spectra shown in Fig. 1b. These values are plotted as a column representation in Fig. 1b.

Chemical shift bucket (ppm)	Mean intensity of 24 spectra	SD of 24 spectra	CV(%) of 24 spectra
3.405	0.1566	0.03225	20.59386973
3.41	0.1714	0.03229	18.83897316
3.415	0.1855	0.03292	17.74663073
3.42	0.1942	0.03204	16.4984552
3.425	0.2102	0.03284	15.62321598
3.43	0.2255	0.03176	14.08425721
3.435	0.242	0.03317	13.70661157
3.44	0.2539	0.03247	12.78849941
3.445	0.2711	0.03397	12.53043158
3.45	0.2895	0.03382	11.68221071
3.455	0.32	0.03446	10.76875
3.46	0.3872	0.03732	9.638429752
3.465	0.8557	0.0399	4.662849129
3.47	0.4033	0.02379	5.898834614
3.475	0.4571	0.0365	7.985123605
3.48	1.316	0.1011	7.682370821
3.485	0.6242	0.09423	15.09612304
3.49	0.3364	0.01632	4.85136742
3.495	0.653	0.07704	11.79785605
3.5	0.5979	0.08055	13.47215253
3.505	0.2243	0.01974	8.80071333
3.51	0.2142	0.02244	10.47619048
3.515	0.2162	0.02439	11.28122109
3.52	0.2193	0.02613	11.91518468
3.525	0.2239	0.02728	12.18401072
3.53	0.2337	0.02856	12.22079589
3.535	0.2478	0.03097	12.49798224
3.54	0.2664	0.03223	12.09834835
3.545	0.2956	0.03348	11.32611637
3.55	0.3465	0.03719	10.73304473
3.555	0.4762	0.04545	9.544309114
3.56	0.9841	0.03856	3.918300986
3.565	0.9918	0.03602	3.631780601
3.57	0.4828	0.01592	3.297431649
3.575	1.049	0.03934	3.750238322
3.58	0.7846	0.07519	9.583227122
3.585	0.5779	0.09877	17.09119225
3.59	0.2183	0.01887	8.644067797
3.595	0.211	0.02255	10.68720379
3.6	0.2494	0.02634	10.56134723
3.605	0.3094	0.02435	7.870071105
3.61	0.3243	0.02403	7.409805735

3.615	0.3386	0.0253	7.471943296
3.62	0.3456	0.02581	7.468171296
3.625	0.3482	0.02779	7.981045376
3.63	0.358	0.02801	7.824022346
3.635	0.3717	0.02871	7.723970944
3.64	0.3836	0.0311	8.107403545
3.645	0.4047	0.03202	7.912033605
3.65	0.4242	0.03572	8.420556341
3.655	0.4374	0.03766	8.609967993
3.66	0.463	0.03978	8.591792657
3.665	0.5085	0.04402	8.656833825
3.67	0.5891	0.04843	8.221015108
3.675	0.6762	0.05769	8.531499556
3.68	0.8912	0.08042	9.023788151
3.685	1.752	0.1829	10.43949772
3.69	5.117	0.2876	5.62048075
3.695	0.6757	0.09391	13.89817967
3.7	0.4161	0.03048	7.325162221
3.705	0.3815	0.01483	3.887287025
3.71	0.3909	0.009875	2.52622154
3.715	0.359	0.01258	3.504178273
3.72	0.3654	0.01367	3.741105638
3.725	0.3454	0.01923	5.56745802
3.73	0.3504	0.02306	6.581050228
3.735	0.3921	0.02823	7.199693956
3.74	0.4787	0.03124	6.526007938
3.745	0.5734	0.03503	6.109173352
3.75	0.6822	0.04143	6.07299912
3.755	1.273	0.05653	4.44069128
3.76	0.7254	0.0253	3.487730907
3.765	0.7734	0.04029	5.209464701
3.77	1.573	0.1066	6.776859504
3.775	1.043	0.08736	8.375838926
3.78	0.7621	0.02732	3.584831387
3.785	1.009	0.06804	6.743310208
3.79	1.107	0.06205	5.605239386
3.795	0.8568	0.0361	4.213352007
3.8	1.024	0.05168	5.046875
3.805	1.082	0.05303	4.901109057
3.81	1.224	0.06942	5.671568627
3.815	1.506	0.09576	6.358565737
3.82	2.857	0.277	9.695484774
3.825	4.364	0.2318	5.311640697
3.83	5.241	0.2897	5.527571074
3.835	3.264	0.2453	7.515318627
3.84	1.152	0.1024	8.888888889

3.845	1.318	0.08766	6.650986343
3.85	1.125	0.08792	7.815111111
3.855	0.8034	0.0622	7.742096092
3.86	0.9371	0.05798	6.187173194
3.865	0.9922	0.06826	6.879661359
3.87	0.6144	0.05974	9.723307292
3.875	0.3825	0.04918	12.85751634
3.88	0.4034	0.03701	9.174516609
3.885	0.7154	0.03284	4.590438915
3.89	0.6372	0.04529	7.107658506
3.895	0.7231	0.03817	5.278661319
3.9	0.884	0.04492	5.081447964
3.905	0.8033	0.04796	5.970372215
3.91	0.5836	0.05183	8.881082934
3.915	0.4316	0.05038	11.67284523
3.92	0.2789	0.05934	21.27644317
3.925	0.1776	0.04382	24.67342342
3.93	0.1705	0.04069	23.86510264
3.935	0.1729	0.03799	21.97223829
3.94	0.1893	0.03465	18.30427892
3.945	0.1845	0.03313	17.95663957
3.95	0.175	0.03361	19.20571429
3.955	0.1688	0.03249	19.24763033
3.96	0.17	0.03044	17.90588235
3.965	0.1754	0.03079	17.55416192
3.97	0.1746	0.02992	17.13631157
3.975	0.1888	0.02702	14.31144068
3.98	0.2001	0.02587	12.92853573
3.985	0.1983	0.02558	12.899647
3.99	0.2086	0.02589	12.41131352
3.995	0.2141	0.02375	11.09294722
4	0.2201	0.02401	10.90867787
4.005	0.2272	0.02323	10.22447183
4.01	0.2354	0.02232	9.48173322
4.015	0.2451	0.02085	8.506731946
4.02	0.2549	0.02017	7.912907022
4.025	0.2655	0.01935	7.288135593
4.03	0.2782	0.01777	6.387491014
4.035	0.2969	0.01535	5.17009094
4.04	0.3371	0.01404	4.164936221
4.045	0.5801	0.0568	9.791415273
4.05	0.7229	0.06259	8.658182321
4.055	0.3903	0.01396	3.576735844
4.06	1.11	0.1361	12.26126126
4.065	0.7345	0.1511	20.57181756
4.07	0.2748	0.03073	11.18267831

4.075	0.7438	0.03649	4.90588868
4.08	0.2514	0.05894	23.44470963
4.085	0.1507	0.03657	24.26675514
4.09	0.1462	0.03336	22.81805746
4.095	0.154	0.03079	19.99350649
4.1	0.147	0.03093	21.04081633
4.105	0.1461	0.02948	20.1779603
4.11	0.1503	0.02869	19.08848969
4.115	0.1449	0.0287	19.80676329
4.12	0.1399	0.0281	20.08577555
4.125	0.1417	0.02859	20.17642908
4.13	0.1497	0.02748	18.35671343
4.135	0.1634	0.02584	15.81395349
4.14	0.1647	0.02484	15.08196721
4.145	0.1754	0.02391	13.63169897
4.15	0.189	0.02366	12.51851852
4.155	0.1885	0.02466	13.08222812
4.16	0.1946	0.02428	12.47687564
4.165	0.2001	0.02394	11.96401799
4.17	0.2064	0.0222	10.75581395
4.175	0.213	0.02121	9.957746479
4.18	0.2272	0.02172	9.559859155
4.185	0.2314	0.02026	8.755401901
4.19	0.2434	0.0197	8.093672966
4.195	0.2585	0.01814	7.017408124
4.2	0.2742	0.01814	6.615609044
4.205	0.3037	0.01669	5.495554824
4.21	0.3632	0.01583	4.358480176
4.215	0.6533	0.0563	8.617786622
4.22	1.303	0.08166	6.267075979
4.225	0.3189	0.02341	7.340859204
4.23	0.6032	0.07763	12.86969496
4.235	0.9297	0.09634	10.36248252
4.24	0.164	0.03871	23.60365854
4.245	0.151	0.0338	22.38410596
4.25	0.1507	0.031	20.57067021
4.255	0.1534	0.02965	19.3285528
4.26	0.1611	0.02832	17.57914339
4.265	0.1724	0.02688	15.59164733
4.27	0.1884	0.02491	13.22186837
4.275	0.2064	0.02536	12.28682171
4.28	0.2279	0.02209	9.69284774
4.285	0.2494	0.02142	8.58861267
4.29	0.2551	0.02289	8.972951784
4.295	0.2624	0.02067	7.877286585
4.3	0.2589	0.02107	8.138277327