Supporting information

Unprecedented specific staining of polar lipids by a luminescent rhenium species revealed by FTIR microscpectroscopy in adipocytes

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Methods

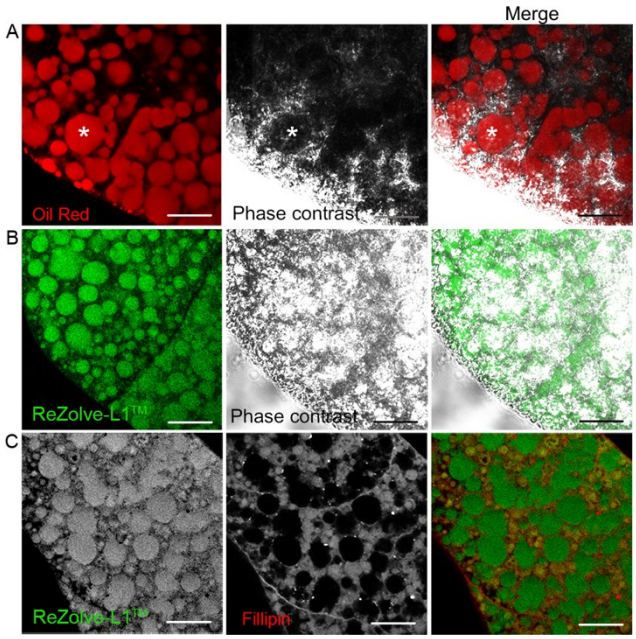
ReZolve-L1 is amenable to both live or fixed sample imaging consistent with methods used for the vast majority of molecular probes. Importantly, the staining pattern is unchanged regardless of method chosen. Imaging of live samples is achieved by incubating the cells in a solution of the complex and is followed by a washing step. These treated cells are then ideally imaged on a live cell stage to ensure optimum cell conditions. For FTIR experiments cell were required to be fixed as a live cell stage was not available with the equipment available, in addition the long collection time required to collect cellular maps is not compatible with live cell imaging. We chose to fix the cells first then incubate with the Re(I) complex rather than the alternate method (incubate with live cells, wash and then fix) using rapid fixation with cold methanol; rapid fixation with cold methanol eliminates biochemical changes in the cell. Fixation prior to incubation allowed for overnight incubation (overnight cell incubation with a live cell may alter the health of the cell) this was done with the aim of increasing the concentration of the complex for detection by FTIR.

Cell culture

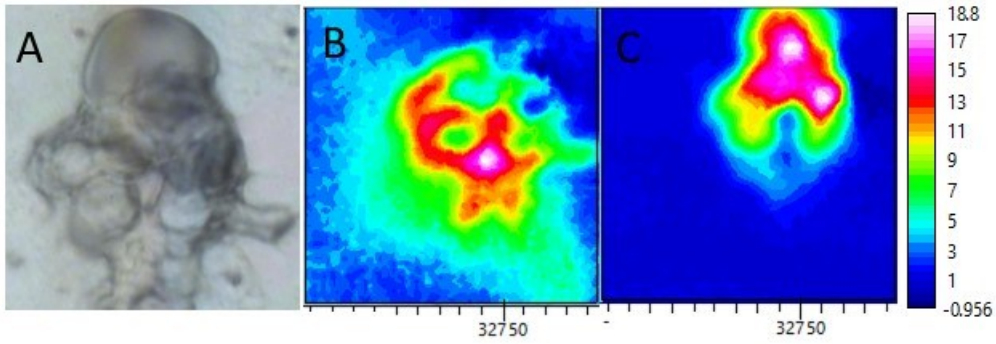
Murine 3T3-L1 adipocytes were grown and differentiated on a silicon nitride substrate as described previously[1,2]. Silicon nitride membrane windows (Silson Ltd) were sterilised in 70% ethanol, washed in PBS followed by growth media containing serum, L-glutamine and antibiotics and placed in a dry sterile 24 welled cell culture plate. Cells were seeded directly on to the substrate at a density of $1x10^4$ cells per well and maintained at 37°C with 5% CO_2. Before imaging cells were fixed in cold methanol and air dried. For cell staining dried cell were incubated with 10µM of ReZolve-L1™ in PBS and 0.1% DMSO overnight. Cell were then dried to allow for IR analysis.

FTIR spectroscopy

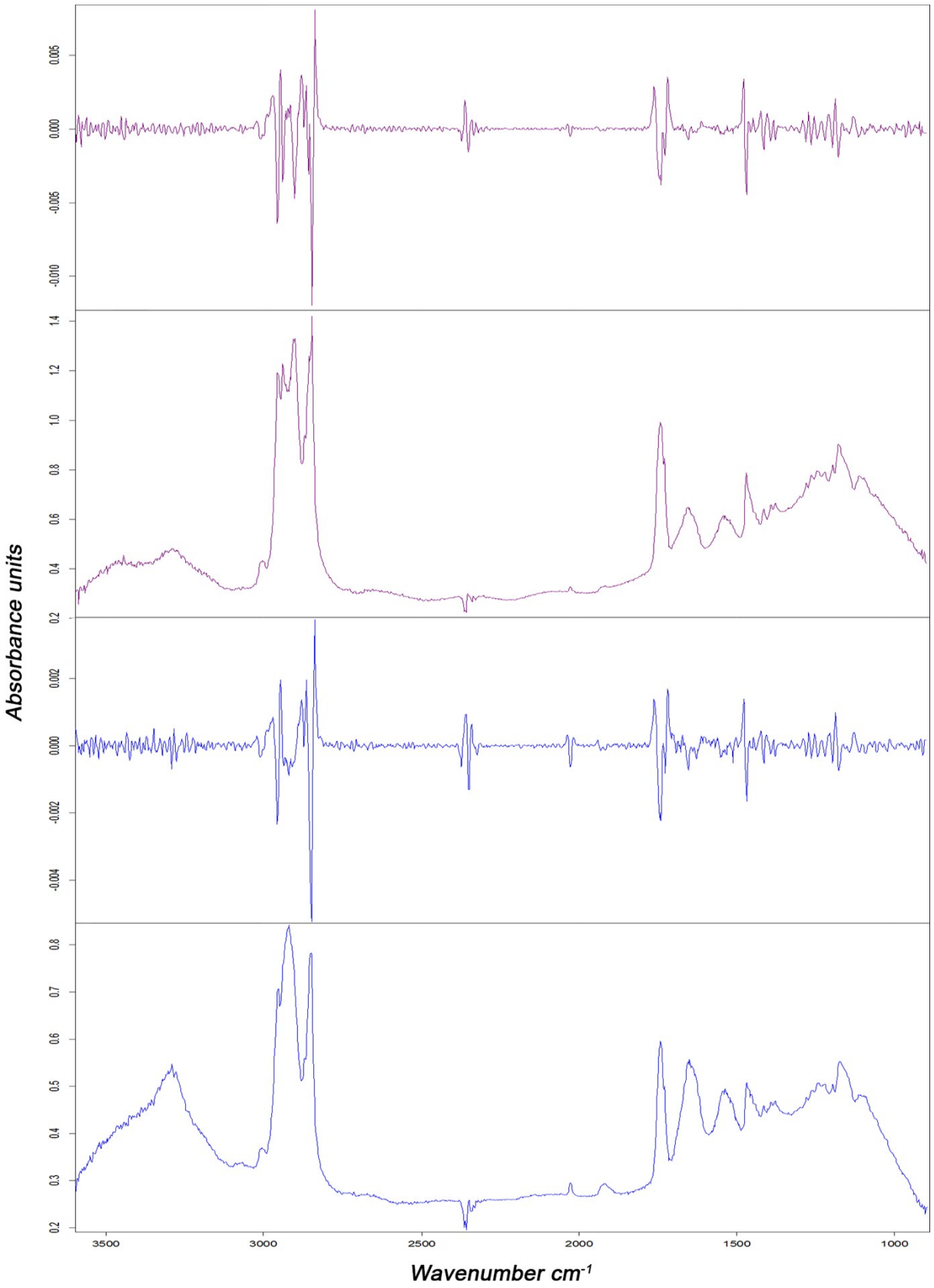
FTIR spectra and maps were collected using a Bruker Tensor 27 FTIR spectrometer coupled with a Hyperion 300 microscope. Reference spectra for lipids and ReZolve-L1™ were collected with attenuated total reflection sampling x20 objective in the mid IR range. For adipocyte maps were collected with a x36 objective and focal plane array (FPA) detector, as a culmination of 512 scan with a 0.32ms exposure time and a step size of 2.5µm. Four control cells and four cells incubated with ReZolve-L1™ were mapped and maps. Data analysis and IR maps were generated in OPUSversion 7.2 (Bruker). Atmospheric compensation was performed on all spectra to account for water and carbon dioxide, prior to data analysis of mapping.



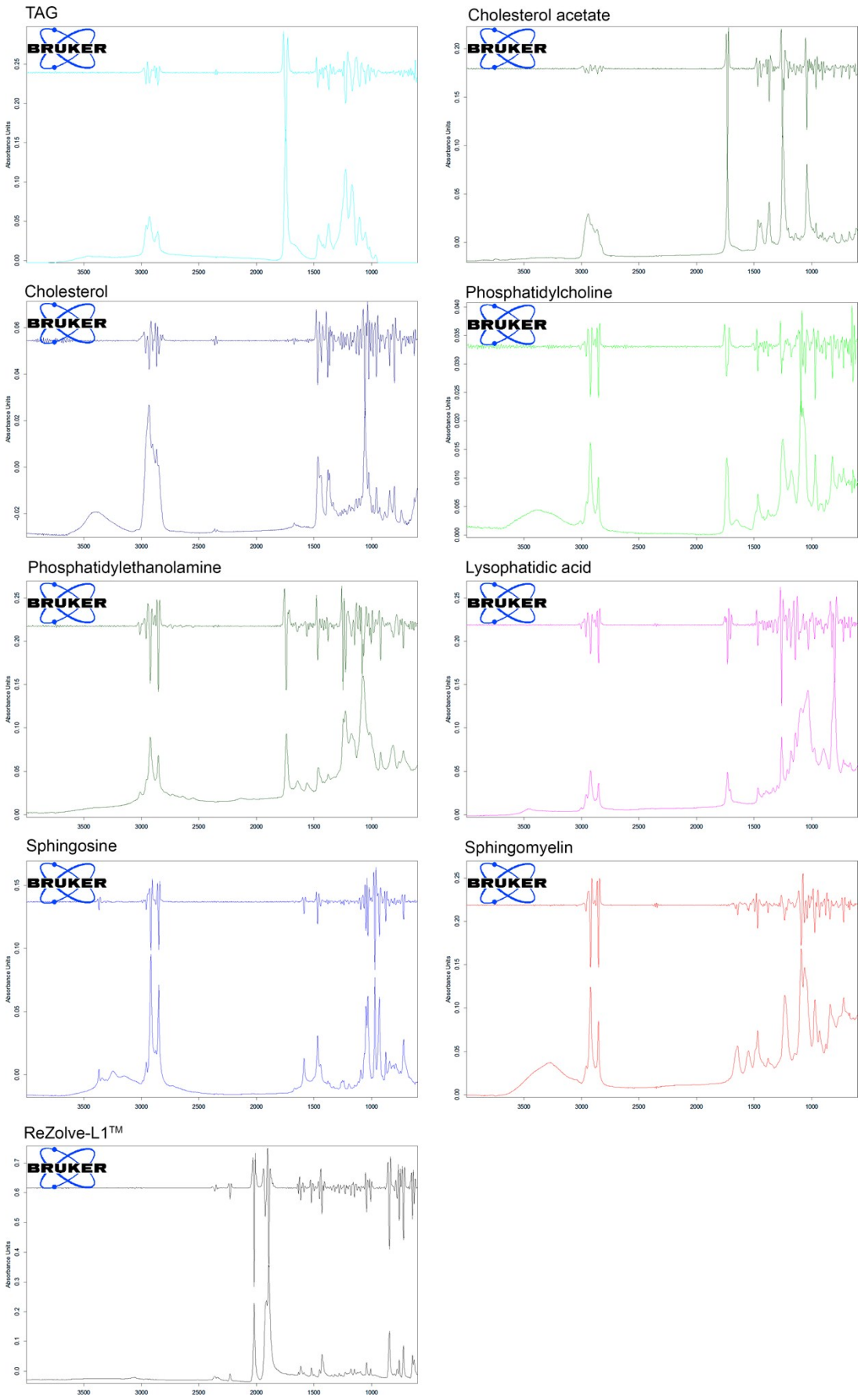
Supplementary Figure S1. A-C) Confocal micrographs previously published in Bader *et al.*[3]. A) Fixed *drosophila* fat body tissue stained with neutral lipid stained Oil Red O. B) Live *drosophila* fat body tissue stained with ReZolve-L1™. C) Fixed *drosophila* fat body tissue stained with ReZolve-L1™ and counter-stained with fillipin-III which detects free cholesterol.



Supplementary Figure S2. A) Bright Field image of control adipocytes grown silicon nitride substrate, cells were then fixed using cold methanol and air dried before imaging; B) IR image of protein amide I band; C) IR image of lipid □□C=O) band.



Supplementary Figure S3. Averaged IR spectra from region X (purple) and region Y (blue) with 2nd derivatives below.



Supplementary Figure S4. Reference IR spectrum with second derivatives shown above of pure lipid as designated

Lipid (Company/Code)	O-H, N-H	C-H	C=O	C=C	N-H	CH_2	C-O	P=O	C-O, C-C, C-O-H, N-C	C=C, O-H, N-H	Aro-matic Ring	CH_2
Triglyceride Mix C2 - C10 (Sigma/17810)		2957, 2928, 2857	1745			1459, 1371	1225, 1168, 1103, 1052					
Cholesteryl acetate (Sigma/151114)		2941, 2912, 2863	1731	1675		1465, 1441, 1367	1251		1040		961, 905, 805	736
Cholesterol (Scharlau/CO0180)	3394	2933, 2902, 2867, 2851		1671		1465, 1439, 1377, 1365			1056, 1023		956, 842, 800	
L-α-Phosphatidylcholine (Sigma/P3556)		2955, 2922, 2852	1736, 1650			1467, 1377	1250	1175	1092, 1075	967, 816, 760		721
L-α-Phosphatidylethanolamine (Sigma/P7943)	3010	2955, 2922, 2853	1739, 1642		1559	1494, 1377		1245, 1225, 1172	1071, 1014	919, 811		721
oleoly-L-α-lysophosphatidic acid (Sigma/L7260)		2958, 2921, 2851	1733, 1706			1465	1260	1210, 1178, 1139	1093, 1031	975, 893, 800		
D-Sphingosine (Sigma/S7049)	3368, 3246	2956, 2917, 2849		1668	1585	1468			1046, 1030	970, 931, 875		720
Sphingomyelin (Sigma/S0756)	3270	2958, 2921, 2851		1643	1548	1468, 1378		1231	1089, 1061	971, 930, 837		720

Supplementary Figure S5. Lipid peak assignment table. Peaks determined from 2nd derivatives using 9 smoothing points.

References

(1) Carter, E. A.; Rayner, B. S.; McLeod, A. I.; Wu, L. E.; Marshall, C. P.; Levina, A.; Aitken, J. B.; Witting, P. K.; Lai, B.; Cai, Z.; Vogt, S.; Lee, Y.-C.; Chen, C.-I.; Tobin, M. J.; Harris, H. H.; Lay, P. A. *Mol Biosyst* **2010**, *6*, 1316.

(2) Govers, R.; Coster, A. C.; James, D. E. *Molecular and cellular biology* **2004**, *24*, 6456.

(3) Bader, C. A.; Brooks, R. D.; Ng, Y. S.; Sorvina, A.; Werrett, M. V.; Wright, P. J.; Anwer, A. G.; Brooks, D. A.; Stagni, S.; Muzzioli, S.; Silberstein, M.; Skelton, B. W.; Goldys, E. M.; Plush, S. E.; Shandala, T.; Massi, M. *Rsc Adv* **2014**, *4*, 16345.