Supplementary Information for

Intracellular Redox Potential is correlated with miRNA expression in MCF7 cells under Hypoxic Conditions

Hannah Johnston[1], Paul Dickinson[2], Alasdair Ivens[2], Amy Buck[2],
R. D. Levine[3], Francoise Remacle[4], Colin J. Campbell[1]

[1] The University of Edinburgh School of Chemistry, Joseph Black Building, West Mains Road, Edinburgh, EH9 3FJ.
[2] Institute of Infection and Immunology Research, Ashworth Building, West Mains Road Edinburgh EH9 3FL.
[3] Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel.
[4] Theoretical Physical Chemistry, Research Unit MOLSYS, University of Liège, B4000 Liège, Belgium.

Corresponding Author: Colin Campbell
Email: colin.campbell@ed.ac.uk

This PDF file includes:

> Supplementary text
> Figures S1 to S4
> Tables S1 to S4
> SI References

Estimating the error on the Lagrange multipliers $\lambda_\alpha(p)$

The Lagrange multipliers $\lambda_\alpha(p)$ is the weight of constraint $\alpha, \alpha = 0,1,2,..,$ at the oxygen

pressure p. See equation [1] of the main text., reproduced here

$$\ln X_i(p) = \ln X_i^o(p) + \sum_{\alpha = 1,2,..} G_{i\alpha} \lambda_\alpha(p)$$

$$= \sum_{\alpha = 0,1,2,..} G_{i\alpha} \lambda_\alpha(p)$$

S.1

$X_i(p)$ is the level of miRNA i at oxygen pressure p. $\alpha = 0$ is the stable state. We compute the

weight $G_{i\alpha}$ of miRNA i in constraint α by the method of SVD. This means that the different

vectors G_α are orthogonal,

$$\sum_i G_{i\alpha} G_{i\beta} = \delta_{\alpha,\beta} \; \cdot$$

S.2

It follows from equation [1] that an explicit result for the weight $\lambda_\alpha(p)$ is

$$\lambda_\alpha(p) = \sum_i G_{i\alpha} \ln X_i(p)$$

S.3

and an uncertainty in the weight due a measurement error of each miRNA is

$$\delta\lambda_\alpha(p) = \sum_i G_{i\alpha} \delta\left(\ln X_i(p)\right)$$

S.4
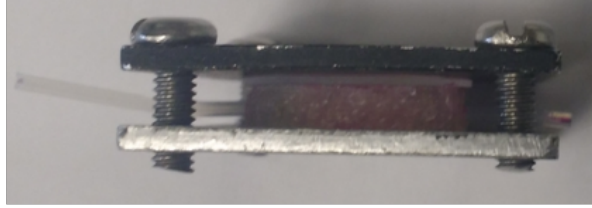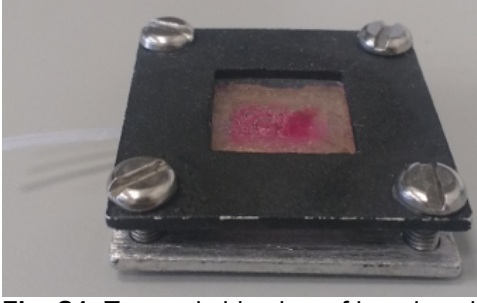
The schwarz inequality of vector algebra yields an upper bound

$$\delta\lambda_\alpha(p) \le \left(\sum_{i=1}^N G_{i\alpha}{}^2\right)^{1/2} \left(\sum_{i=1}^N \left(\frac{\delta X_i^p}{X_i^p}\right)^2\right)^{1/2}$$

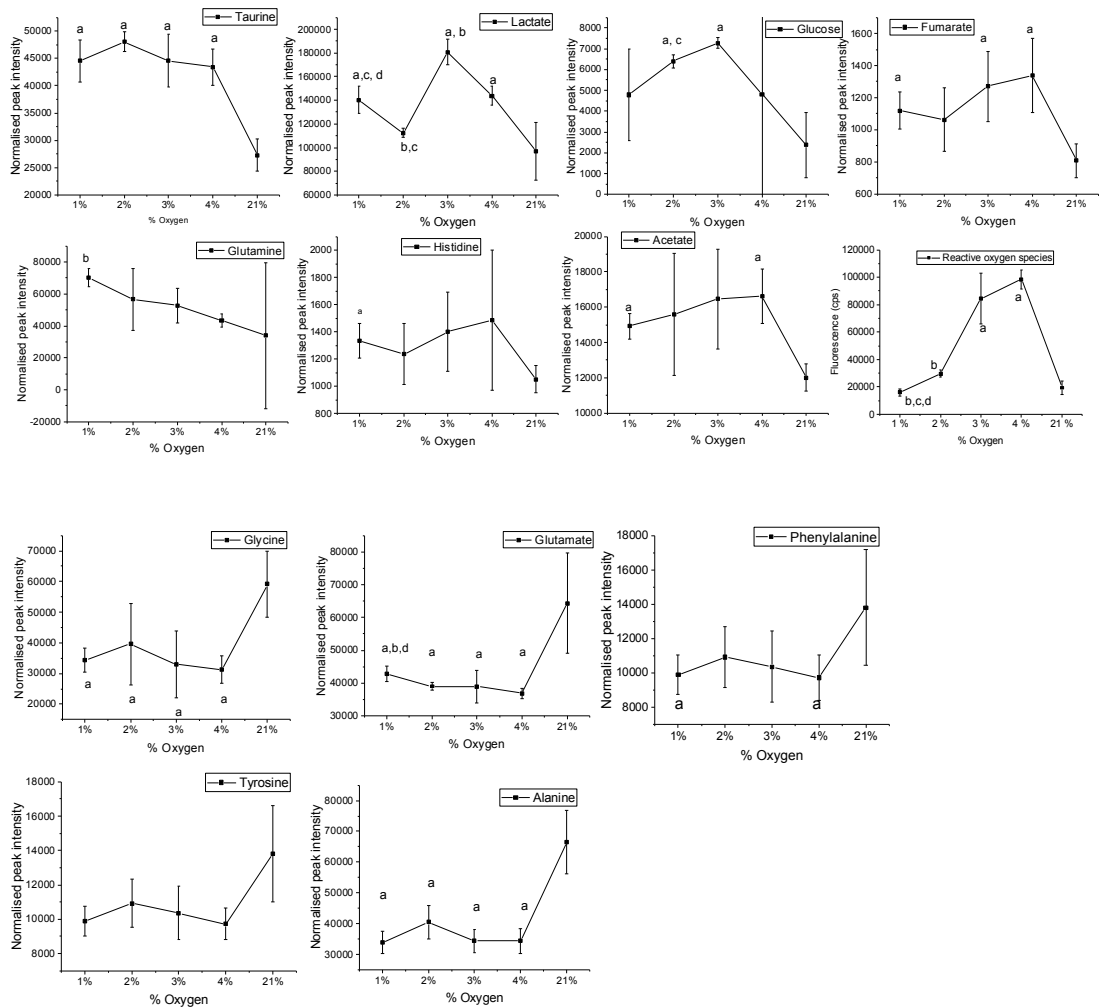From equation S.2 the first sum is unity and the second sum can be approximated as

$\left(\delta X/X\right)\left(N\right)^{1/2}$ where $\left(\delta X/X\right)$ is he mean error per reading and N is the number of readings.

We have N=69, so $\left(N\right)^{1/2}$ =8.31 and for the six replicates we get
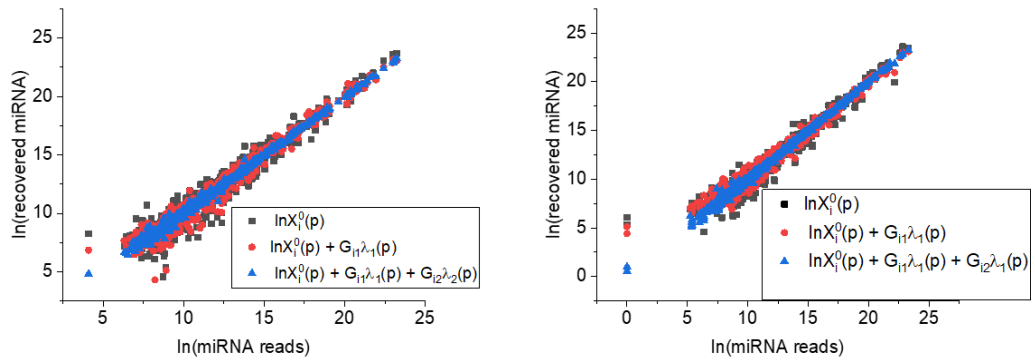
PO$_2$	1%	2%	3%	4%	21%
$av\left(\delta X/X\right)$	0.12	0.12	0.16	0.16	0.6
$\delta\lambda_\alpha(p)$	1	1	1.32	1.32	1.32

Fig. S1. Top and side view of imaging chamber.
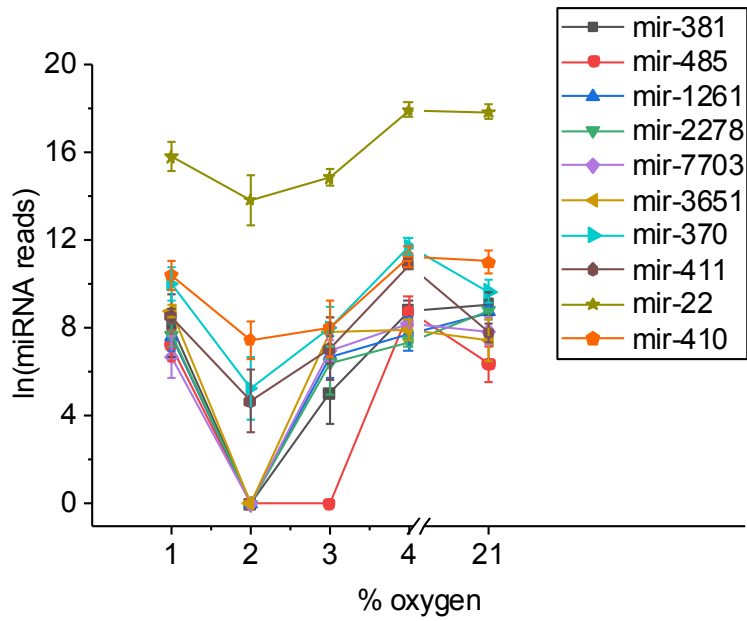
Fig. S2. Concentration profile of a panel of measured metabolites. Error bars represent the standard deviation of 5 independent measurements. Letters as labels signify a p-value < 0.05 for a paired t-test versus 21% (a); 4% (b); 3% (c); 2%(d).

Fig. S3. Addition of λ_1 and λ_2 to the stable state improves the fit of the data at A - 4% O_2 and B – 21% O_2.

Fig. S4. The top 10 miRNAs for which $G_{i,\alpha=1} < 0$. Error bars show the standard deviation of 3 independent measurements.

Table S1. Cellular pH at the oxygen concentrations used.

O_2 of cell, %	Average pH of all replicates	STDEV of pH of all replicates
1	6.3	±0.12
2	6.1	±0.03
3	6.7	±0.24
4	6.6	±0.24
21	6.5	±0.05

Table S2. Pearson correlation coefficient measuring the correlation between miRNA reads and ROS.

miRNA	Correlation Coefficient
mir-548p	0.9520
mir-769	0.9342
mir-6747	0.9130
mir-1306	0.8978
let-7c	0.8893
let-7g	0.8776
mir-767	0.8753
mir-340	0.8678
mir-1303	0.8588
mir-24	0.8552
mir-6807	0.8546
mir-6764	0.8428

Table S3. Pearson correlation coefficient measuring the correlation between ln(miRNA) and E.

miRNA	Correlation coefficient
mir-381	0.8188
mir-485	0.2237
mir-1261	0.8882
mir-2278	0.8869
mir-7703	0.8781
mir-3651	0.7577
mir-370	0.6649
mir-411	0.5421
mir-22	0.7799
mir-410	0.6519

Table S4. miRNA roles and known transcription factors for the top 10 miRNAs for which $G_{i,\alpha=1} < 0$.

miRNA	Role	Transcription factors (Genecards.org)
mir-381	Neural SC differentiation and proliferation. Upregulates Nestin. HES 1 target.[1] Tumor repressor Inhibitor of metastasis.[2]	Several Zn fingers identified
mir-485	Low levels poor prognostic for glioblastoma[3] Suppresses breast cancer metastasis by inhibiting PGC1a[4] Suppresses proliferation and invasion in hepatocellular carcinoma – targets stanniocalcin[5]	GATA (zn finger) ZNF680 (zn finger) ZFP3 (zn finger) MNT
mir-1261	Regulated by YAP member of mir29 family Inhibits PTEN expression.[6] Repressor. "Sponging" is associated with proliferation and invasion.[7,8]	YAP
mir-2278	Tumor suppressor targets STAT5A and induces apoptosis.[9]	ZNF169
mir-7703	Very little information	GLIS1(zn finger)
mir-3651	Down regulated in squamous cell carcinoma.[10,11] Repressor	C/EBP Beta (redox regulated)[12] ZNF348 (zn finger)
mir-370	TF (Ets) had disulfide in dna binding domain. Regulates cyp2d6 (drug metabolism) Inhibits fibrogenesis Supresses proliferation.[13] Downregulates FOX01 and regulates redox.[14]	ELF3 SOX TFAP4 (two Cysteines) BCOR(zn finger)
mir-411	Inhibits proliferation and metastasis in breast cancer. Targets GRB2[15] Targets SPRY4(a kinase inhibitor) inhibits proliferation in vitro and in vivo.[16]	No information
mir-22	Inhibits tumor growth in AML.[17]	C/EBP Beta (redox regulated)[12]
mir-410	Tumor suppressor in breast cancer.[18] Tumor enhancer in liver cancer.[19]	NFE2U, AP1 (redox regulated)[20]

References

1 X. Shi, C. Yan, B. Liu, C. Yang, X. Nie, X. Wang, J. Zheng, Y. Wang and Y. Zhu, *PLoS One*, 2015, **10**, 1–10.

2 Y. Xue, W. Xu, W. Zhao, W. Wang, D. Zhang and P. Wu, 2017, **86**, 426–433.

3 Z.-Q. Wang, M.-Y. Zhang, M.-L. Deng, N.-Q. Weng, H.-Y. Wang and S.-X. Wu, *PLoS One*, 2017, **12**, e0184969.

4 C. Lou, M. Xiao, S. Cheng, X. Lu, S. Jia, Y. Ren and Z. Li, *Cell Death Dis.*, 2016, **7**, e2159.

5 G. X. Guo, Q. Y. Li, W. L. Ma, Z. H. Shi and X. Q. Ren, *Int. J. Clin. Exp. Pathol.*, 2015, **8**, 12292–12299.

6 K. Tumaneng, K. Schlegelmilch, R. C. Russell, D. Yimlamai, H. Basnet, N. Mahadevan, J. Fitamant, N. Bardeesy, F. D. Camargo and K. L. Guan, *Nat. Cell Biol.*, 2012, **14**, 1322–1329.

7 J. H. He, B. X. Li, Z. P. Han, M. X. Zou, L. Wang, Y. B. Lv, J. Bin Zhou, M. R. Cao, Y. G. Li and J. zhi Zhang, *Tumor Biol.*, 2016, **37**, 16163–16176.

8 H. Wei, L. Pan, D. Tao and R. Li, *Biochem. Biophys. Res. Commun.*, 2018, **503**, 56–61.

9 B. T. Kaymaz, N. S. Günel, M. Ceyhan, V. B. Çetintaş, B. Özel, M. K. Yandım, S. Kıpçak, Ç. Aktan, A. A. Gökbulut, Y. Baran and B. K. Can, *Tumor Biol.*, 2015, **36**, 7915–7927.

10 C. Wang, S. Guan, X. Chen, B. Liu, F. Liu, L. Han, E. Un Nesa, Q. Song, C. Bao, X. Wang and Y. Cheng, *Biochem. Biophys. Res. Commun.*, 2015, **465**, 30–34.

11 C. Wang, S. Guan, X. Chen, B. Liu, F. Liu, L. Han, E. Un, Q. Song, C. Bao, X. Wang and Y. Cheng, *Biochem. Biophys. Res. Commun.*, 2015, **465**, 30–34.

12 W. C. Su, H. Y. Chou, C. J. Chang, Y. M. Lee, W. H. Chen, K. H. Huang, M. Y. Lee and S. C. Lee, *J. Biol. Chem.*, 2003, **278**, 51150–51158.

13 J. Zhu, B. Zhang, W. Song, X. Zhang, L. Wang, B. Yin, F. Zhu, C. Yu and H. Li, *Gene Reports*, 2016, **4**, 37–44.

14 L. Klotz, C. Sánchez-ramos, I. Prieto-arroyo, P. Urbánek, H. Steinbrenner and M. Monsalve, *Redox Biol.*, 2015, **6**, 51–72.

15 Y. Zhang, G. Xu, G. Liu, Y. Ye, C. Zhang, C. Fan, H. Wang, H. Cai, R. Xiao, Z. Huang and Q. Luo, *Biochem. Biophys. Res. Commun.*, 2016, **476**, 607–613.

16 M. Sun, F. Huang, D. Yu, Y. Zhang, H. Xu, L. Zhang, L. Li, L. Dong, L. Guo and S. Wang, *Cell Death Dis.*, 2015, **6**, 1–13.

17 X. Jiang, C. Hu, S. Arnovitz, J. Bugno, M. Yu, Z. Zuo, P. Chen, H. Huang, B. Ulrich, S. Gurbuxani, H. Weng, J. Strong, Y. Wang, Y. Li, J. Salat, S. Li, A. G. Elkahloun, Y. Yang, M. B. Neilly, R. A. Larson, M. M. Le Beau, T. Herold, S. K. Bohlander, P. P. Liu, J. Zhang, Z. Li, C. He, J. Jin, S. Hong and J. Chen, *Nat. Commun.*, 2016, 11452.

18 H. Wu, J. Li, E. Guo, S. Luo and G. Wang, *Cell. Physiol. Biochem.*, 2018, **48**, 461–474.

19 Y. Wang, J. Fu, M. Jiang, X. Zhang, L. Cheng, X. Xu, Z. Fan, J. Zhang, Q. Ye and H. Song, *PLoS One*, 2014, **9**, 1–7.

20 Z. Yin, M. Machius, E. J. Nestler and G. Rudenko, *Nucleic Acids Res.*, 2017, **45**, 11425–11436.