

# Calculation of effective ATP/O ratio

This protocol describes the steps needed to extract and process the raw oxygen and fluorescence signals and the steps required to complete the Excel template file as done in Salin *et al.* (2016). If you have any feedbacks or questions, please don't hesitate to contact me – [salin.karine@gmail.com](mailto:salin.karine@gmail.com).

## 1/ Calculation of the calibration curve for the fluorescence signal.

- In Datlab, select the layout showing “Amp-Raw (V)”.
- Mark the 11 points of calibration: 1 before the first MgCl<sub>2</sub> addition and 10 after each of the MgCl<sub>2</sub> addition (Figure 1).

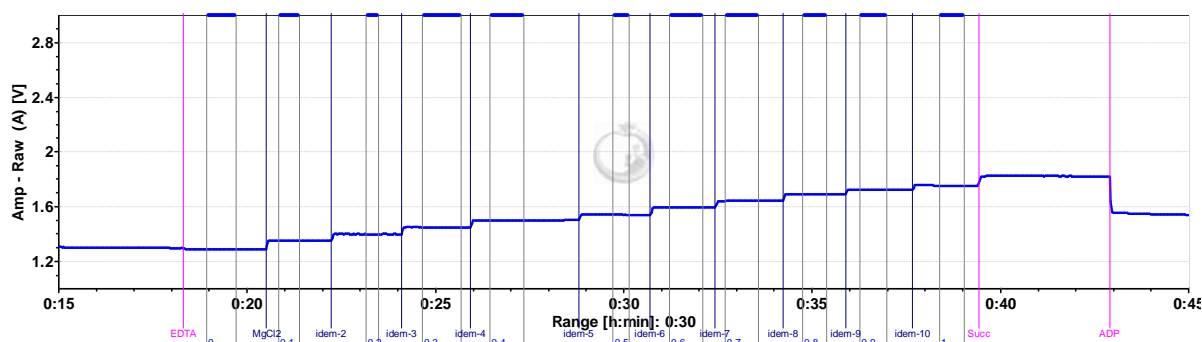


Figure 1.

- Export the data from Datlab: “Mark” > “Statistics” > Select Marks in “Amp-raw A or B” > “Copy to Clipboard”.
- Past in Excel file in the sheet called “raw data Mg Calibration”.
- Duplicate the “template ATP-O ratio”. Name it as “name of the sample”.
- In the Calibration curve section, copy/past-transpose (ctrl+T) the Amp(V) in cell Q2 (Figure 2).

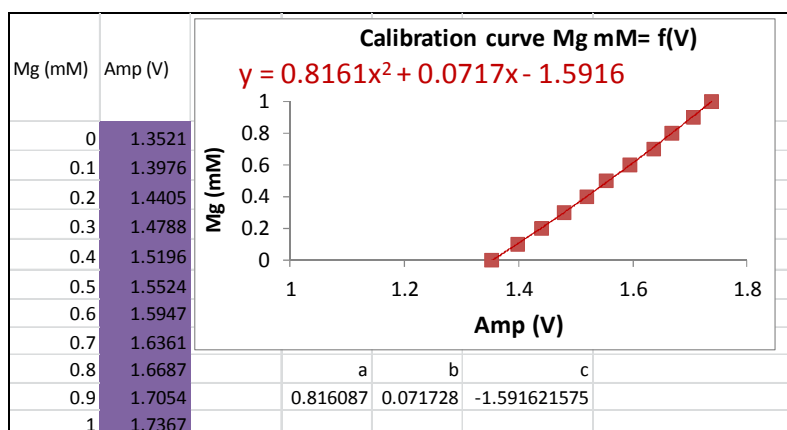


Figure 2.

- Repeat the same steps for the other chamber.

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## 2/Extract the values for the raw oxygen and fluorescence signals for State 3 and State 4.

This will allow the calculation of the raw JO<sub>2</sub> and raw JATP values. Data from both chambers are extracted at the same time.

- In the datlab file, select layout with both Y1 as “Amp-Raw (V)” and Y2 as “O<sub>2</sub> Concentration[nmol/ml]” (Figure 3)

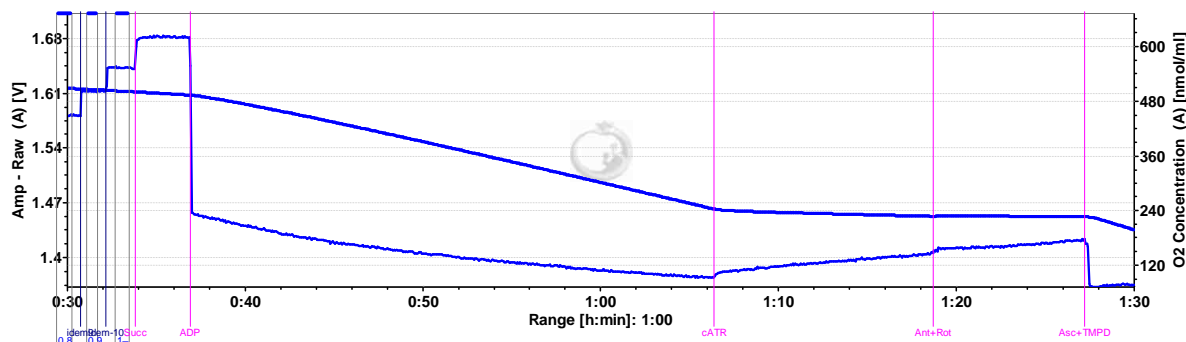


Figure 3.

- > “file”> “export”> “data to text file” (Figure 4)

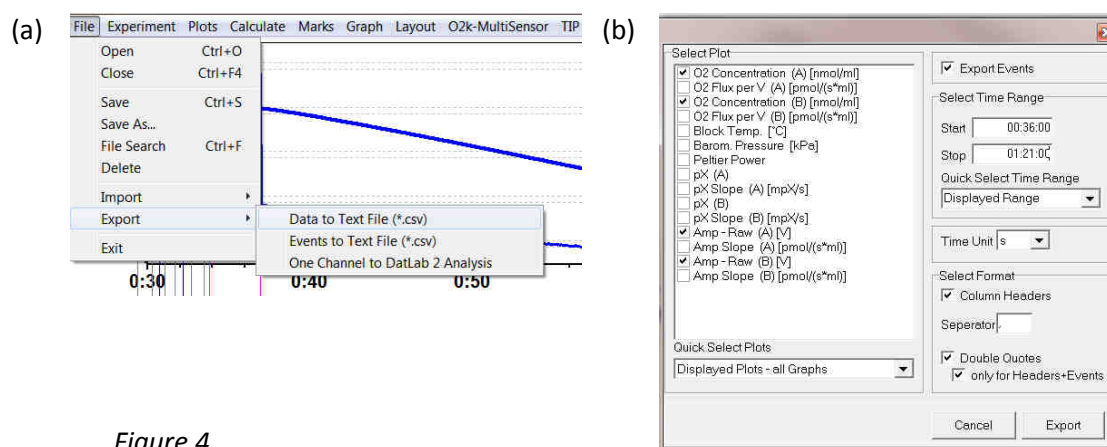


Figure 4.

- Select time range:
  - delete the time written in “Start” and “Stop” by selecting the number with the mouse and press “delete” on the keyboard.
  - introduce the “Start” time as one minute before the addition of ADP to the chamber.
  - introduce the “Stop” time where antimycin A and rotenone were added to the chamber.
- Select seconds (s) as “Time Unit”.
- Click on “Export” and “save”. This gives a csv file.

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## 3/ Clean the raw data

- Open the csv file.
- Duplicate the sheet.
- Name them by the “name of the sample ChA or ChB” (Figure 5).

| 1  | Time [s] | Event Name              | Chamber     | Event Text | O2 Concentration (A) [nmol/ml] | O2 Concentration (B) [nmol/ml] | Amp - Raw (A) [V] | Amp - Raw (B) [V] |
|----|----------|-------------------------|-------------|------------|--------------------------------|--------------------------------|-------------------|-------------------|
| 2  | 2160     | O2, Pyr+Mal, Both, Left | Injected mo |            | 495.6869                       | 487.8157                       | 1.6837            | 1.7631            |
| 3  | 2162     |                         |             |            | 495.629                        | 487.7285                       | 1.6826            | 1.7638            |
| 4  | 2164     |                         |             |            | 495.4843                       | 487.6413                       | 1.6835            | 1.7628            |
| 5  | 2166     |                         |             |            | 495.4119                       | 487.5396                       | 1.6828            | 1.7624            |
| 6  | 2168     |                         |             |            | 495.3974                       | 487.496                        | 1.6814            | 1.7619            |
| 7  | 2170     |                         |             |            | 495.2817                       | 487.4233                       | 1.6824            | 1.7622            |
| 8  | 2172     |                         |             |            | 495.2238                       | 487.3216                       | 1.6814            | 1.763             |
| 9  | 2174     |                         |             |            | 495.1659                       | 487.2198                       | 1.6829            | 1.7624            |
| 10 | 2176     |                         |             |            | 495.0501                       | 487.1181                       | 1.6821            | 1.7629            |
| 11 | 2178     |                         |             |            | 494.9199                       | 487.06                         | 1.6834            | 1.7625            |
| 12 | 2180     |                         |             |            | 494.8476                       | 487.0018                       | 1.6823            | 1.7635            |
| 13 | 2182     |                         |             |            | 494.8042                       | 486.9146                       | 1.6826            | 1.7628            |
| 14 | 2184     |                         |             |            | 494.7897                       | 486.8855                       | 1.6823            | 1.7629            |
| 15 | 2186     |                         |             |            | 494.7173                       | 486.7983                       | 1.6835            | 1.7626            |
| 16 | 2188     |                         |             |            | 494.6884                       | 486.7111                       | 1.6828            | 1.7636            |
| 17 | 2190     |                         |             |            | 494.5871                       | 486.6094                       | 1.6816            | 1.7622            |
| 18 | 2192     |                         |             |            | 494.4134                       | 486.5222                       | 1.6816            | 1.7632            |
| 19 | 2194     |                         |             |            | 494.3122                       | 486.4495                       | 1.6818            | 1.7626            |
| 20 | 2196     |                         |             |            | 494.2109                       | 486.3768                       | 1.6828            | 1.7629            |
| 21 | 2198     |                         |             |            | 494.1674                       | 486.3187                       | 1.6825            | 1.763             |
| 22 | 2200     |                         |             |            | 494.0517                       | 486.246                        | 1.682             | 1.7629            |
| 23 | 2202     |                         |             |            | 493.9649                       | 486.1588                       | 1.6838            | 1.7618            |
| 24 | 2204     |                         |             |            | 493.8315                       | 486.0731                       | 1.6824            | 1.7626            |

Figure 5.

- Delete the columns that do not correspond with the chamber being evaluated.
- Locate the rows where ADP has been added.
- Delete the extra rows above this row such as 29 rows preceding ADP addition are kept and the row for ADP addition is located line 30.
- Delete all rows below Ant-Rot.
- Delete columns “Event Name” “Chamber” “Event Text” (Figure 6).

| 1  | Time [s] | O2 Concentration (B) [nmol/ml] | Amp - Raw (B) [V] |
|----|----------|--------------------------------|-------------------|
| 2  | 2216     | 485.6501                       | 1.7627            |
| 3  | 2218     | 485.5484                       | 1.764             |
| 4  | 2220     | 485.5048                       | 1.7621            |
| 5  | 2222     | 485.403                        | 1.7617            |
| 6  | 2224     | 485.3449                       | 1.7631            |
| 7  | 2226     | 485.2722                       | 1.7632            |
| 8  | 2228     | 485.1996                       | 1.7627            |
| 9  | 2230     | 485.0833                       | 1.7625            |
| 10 | 2232     | 484.967                        | 1.7624            |
| 11 | 2234     | 484.8943                       | 1.7617            |

Figure 6.

- Repeat all the steps above for the other chamber.

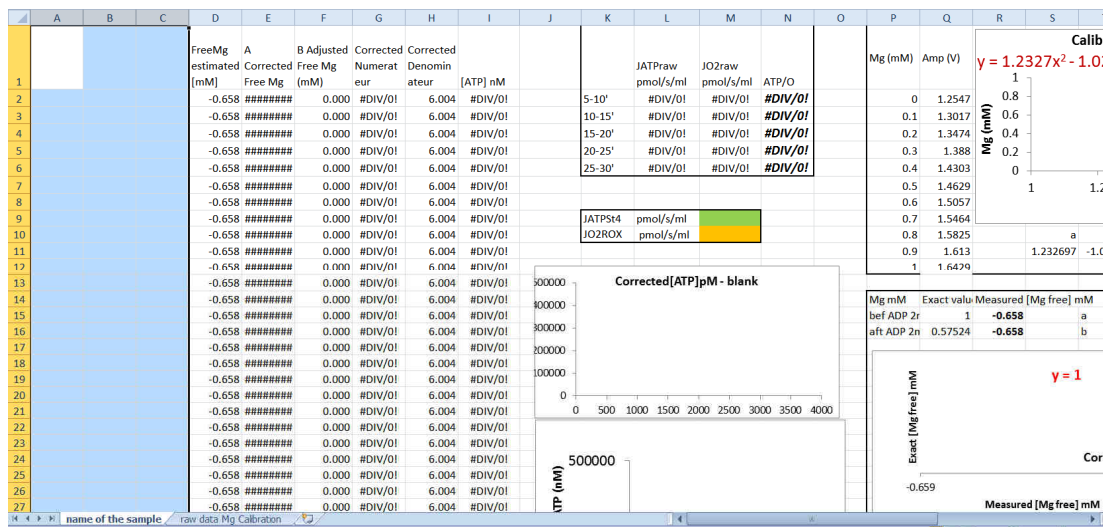
# Calculation of effective ATP/O ratio

## 4/ Calculation of effective ATP/O ratio

Concentrations ADP and  $Mg^{2+}$  and  $K_d$  of ATP and ADP for free magnesium for your experimental conditions should be determined as in Chinopoulos *et al.* (2014). In the Excel sheet "template ATP-O Ratio", complete parameters for your experimental conditions in M33, M34, M35 and M36.

- Copy the three columns from the .csv file and paste them into columns A, B and C of the Excel template file (Figure 7).

Before:



After:

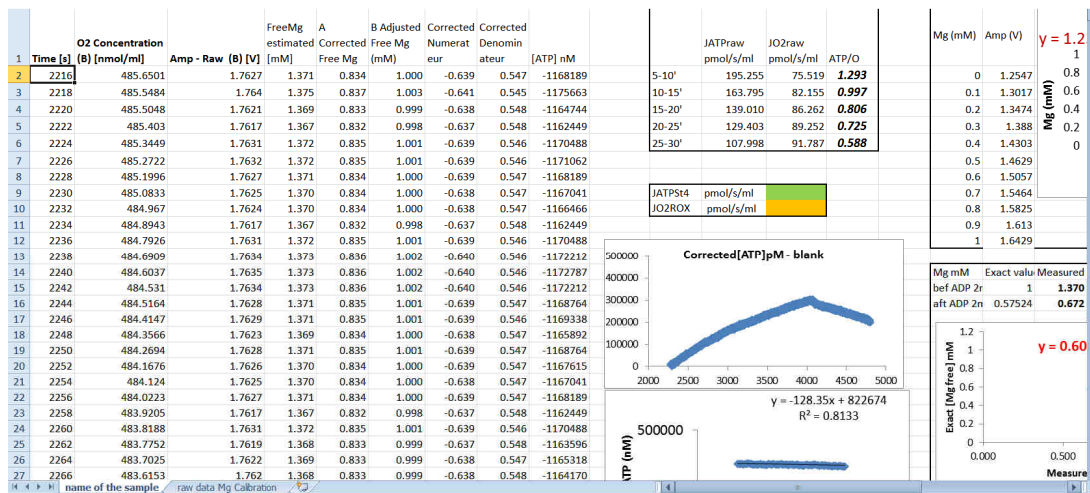


Figure 7.

# Calculation of effective ATP/O ratio

- Click on the points of the graph entitled “corrected[ATP]nM - St4” to selected the data set.
- Drag the selection to the bottom of the data set (Figure 8).

|      | A    | B        | C      | D      | E      | F      | G      | H       | I      | J | K | L |
|------|------|----------|--------|--------|--------|--------|--------|---------|--------|---|---|---|
| 1277 | 4766 | 321.543  | 1.4869 | 0.548  | 0.333  | 0.500  | 0.242  | 1.149   | 210742 |   |   |   |
| 1278 | 4768 | 321.4558 | 1.4858 | 0.545  | 0.332  | 0.498  | 0.249  | 1.153   | 215758 |   |   |   |
| 1279 | 4770 | 321.4413 | 1.487  | 0.548  | 0.334  | 0.500  | 0.242  | 1.149   | 210286 |   |   |   |
| 1280 | 4772 | 321.4122 | 1.4866 | 0.547  | 0.333  | 0.499  | 0.244  | 1.150   | 212110 |   |   |   |
| 1281 | 4774 | 321.4122 | 1.4871 | 0.548  | 0.334  | 0.500  | 0.241  | 1.148   | 209830 |   |   |   |
| 1282 | 4776 | 321.3686 | 1.487  | 0.548  | 0.334  | 0.500  | 0.242  | 1.149   | 210286 |   |   |   |
| 1283 | 4778 | 321.325  | 1.4875 | 0.549  | 0.334  | 0.501  | 0.239  | 1.147   | 208006 |   |   |   |
| 1284 | 4780 | 321.2814 | 1.4886 | 0.552  | 0.336  | 0.502  | 0.232  | 1.143   | 202989 |   |   |   |
| 1285 | 4782 | 321.2378 | 1.4871 | 0.548  | 0.334  | 0.500  | 0.241  | 1.148   | 209830 |   |   |   |
| 1286 | 4784 | 321.1942 | 1.4872 | 0.549  | 0.334  | 0.500  | 0.240  | 1.148   | 209374 |   |   |   |
| 1287 | 4786 | 321.1651 | 1.4874 | 0.549  | 0.334  | 0.500  | 0.239  | 1.147   | 208462 |   |   |   |
| 1288 | 4788 | 321.1506 | 1.4883 | 0.552  | 0.336  | 0.502  | 0.234  | 1.144   | 204357 |   |   |   |
| 1289 | 4790 | 321.1215 | 1.4872 | 0.549  | 0.334  | 0.500  | 0.240  | 1.148   | 209374 |   |   |   |
| 1290 | 4792 | 321.0779 | 1.4873 | 0.549  | 0.334  | 0.500  | 0.240  | 1.148   | 208918 |   |   |   |
| 1291 | 4794 | 321.0343 | 1.4885 | 0.552  | 0.336  | 0.502  | 0.233  | 1.143   | 203445 |   |   |   |
| 1292 | 4796 | 321.0052 | 1.4884 | 0.552  | 0.336  | 0.502  | 0.233  | 1.144   | 203901 |   |   |   |
| 1293 | 4798 | 320.9762 | 1.4892 | 0.554  | 0.337  | 0.503  | 0.228  | 1.141   | 200253 |   |   |   |
| 1294 |      |          |        | -0.658 | -0.400 | -0.234 | -6.322 | -13.066 | 483866 |   |   |   |
| 1295 |      |          |        | -0.658 | -0.400 | -0.234 | -6.322 | -13.066 | 483866 |   |   |   |
| 1296 |      |          |        | -0.658 | -0.400 | -0.234 | -6.322 | -13.066 | 483866 |   |   |   |
| 1297 |      |          |        | -0.658 | -0.400 | -0.234 | -6.322 | -13.066 | 483866 |   |   |   |
| 1298 |      |          |        | -0.658 | -0.400 | -0.234 | -6.322 | -13.066 | 483866 |   |   |   |
| 1299 |      |          |        | -0.658 | -0.400 | -0.234 | -6.322 | -13.066 | 483866 |   |   |   |
| 1300 |      |          |        | -0.658 | -0.400 | -0.234 | -6.322 | -13.066 | 483866 |   |   |   |
| 1301 |      |          |        | -0.658 | -0.400 | -0.234 | -6.322 | -13.066 | 483866 |   |   |   |
| 1302 |      |          |        | -0.658 | -0.400 | -0.234 | -6.322 | -13.066 | 483866 |   |   |   |
| 1303 |      |          |        | -0.658 | -0.400 | -0.234 | -6.322 | -13.066 | 483866 |   |   |   |
| 1304 |      |          |        | -0.658 | -0.400 | -0.234 | -6.322 | -13.066 | 483866 |   |   |   |
| 1305 |      |          |        | -0.658 | -0.400 | -0.234 | -6.322 | -13.066 | 483866 |   |   |   |
| 1306 |      |          |        | -0.658 | -0.400 | -0.234 | -6.322 | -13.066 | 483866 |   |   |   |
| 1307 |      |          |        | -0.658 | -0.400 | -0.234 | -6.322 | -13.066 | 483866 |   |   |   |

Figure 8.

- Enter the value of the slope in the cell M9 using the opposite symbol as the slope displays (Figure 9).

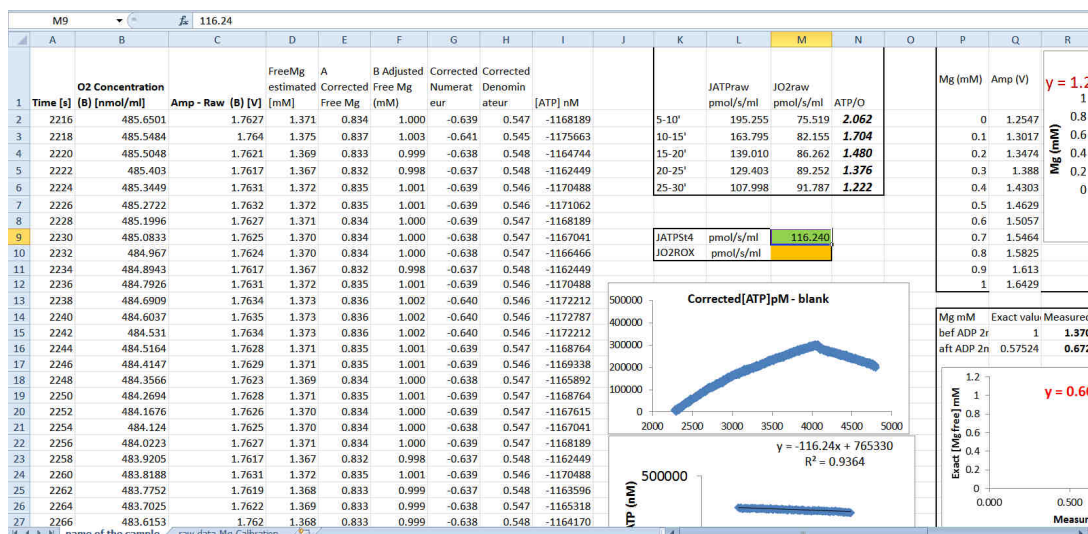


Figure 9.

- Introduce the value for JO<sub>2</sub>-ROX (in “pmol/s.ml”) obtained in Datlab into cell M10:  
- In Datlab: set the Mark.

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- Go to "Marks">"Statistics" (Figure 10).
- Click on the value > Ctrl c.

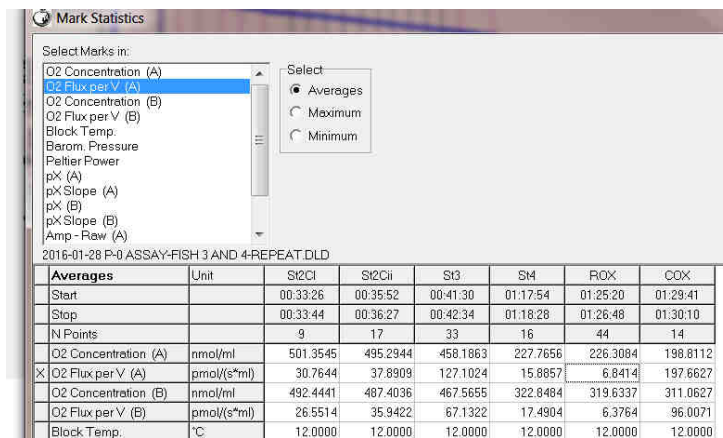


Figure 10.

- In excel, Ctrl v in M10 (Figure 11).

|    | A        | B                              | C                 | D                     | E                   | F                       | G                     | H                       | I        | J | K                 | L                | M       | N            | O |
|----|----------|--------------------------------|-------------------|-----------------------|---------------------|-------------------------|-----------------------|-------------------------|----------|---|-------------------|------------------|---------|--------------|---|
|    |          | O2 Concentration (B) [nmol/ml] | Amp - Raw (B) [V] | FreeMg estimated [mM] | A Corrected Free Mg | B Adjusted Free Mg (mM) | Corrected Numerat eur | Corrected Denomin ateur | [ATP] nM |   | JATPraw pmol/s/ml | JO2raw pmol/s/ml | ATP/O   |              |   |
| 1  | Time [s] |                                |                   |                       |                     |                         |                       |                         |          |   |                   |                  |         |              |   |
| 2  | 2216     | 485.6501                       | 1.7627            | 1.371                 | 0.834               | 1.000                   | -0.639                | 0.547                   | -1168189 |   | 5-10'             | 195.255          | 75.519  | <b>2.268</b> |   |
| 3  | 2218     | 485.5484                       | 1.764             | 1.375                 | 0.837               | 1.003                   | -0.641                | 0.545                   | -1175663 |   | 10-15'            | 163.795          | 82.155  | <b>1.859</b> |   |
| 4  | 2220     | 485.5048                       | 1.7621            | 1.369                 | 0.833               | 0.999                   | -0.638                | 0.548                   | -1164744 |   | 15-20'            | 139.010          | 86.262  | <b>1.607</b> |   |
| 5  | 2222     | 485.403                        | 1.7617            | 1.367                 | 0.832               | 0.998                   | -0.637                | 0.548                   | -1162449 |   | 20-25'            | 129.403          | 89.252  | <b>1.490</b> |   |
| 6  | 2224     | 485.3449                       | 1.7631            | 1.372                 | 0.835               | 1.001                   | -0.639                | 0.546                   | -1170488 |   | 25-30'            | 107.998          | 91.787  | <b>1.320</b> |   |
| 7  | 2226     | 485.2722                       | 1.7632            | 1.372                 | 0.835               | 1.001                   | -0.639                | 0.546                   | -1171062 |   |                   |                  |         |              |   |
| 8  | 2228     | 485.1996                       | 1.7627            | 1.371                 | 0.834               | 1.000                   | -0.639                | 0.547                   | -1168189 |   |                   |                  |         |              |   |
| 9  | 2230     | 485.0833                       | 1.7625            | 1.370                 | 0.834               | 1.000                   | -0.638                | 0.547                   | -1167041 |   | JATPSt4           | pmol/s/ml        | 116.240 |              |   |
| 10 | 2232     | 484.967                        | 1.7624            | 1.370                 | 0.834               | 1.000                   | -0.638                | 0.547                   | -1166466 |   | JO2ROX            | pmol/s/ml        | 6.841   |              |   |
| 11 | 2234     | 484.8943                       | 1.7617            | 1.367                 | 0.832               | 0.998                   | -0.637                | 0.548                   | -1162449 |   |                   |                  |         |              |   |

Figure 11.

→ In the ATP/O column (N2 to N6), the JATP and  $JO_2$  are automatically corrected for rates of non-mitochondrial ATP hydrolyse ( $JATP_{St3} = JATP_{raw} - JATP_{St4}$ ) and respiration ( $JO_{2-St3} = JO_{2-raw} - JO_{2-ROX}$ ) respectively; the ATP/O ratio is calculated as the ratio of  $JATP_{St3}$  to 2-fold  $JO_{2-St3}$ .

Chinopoulos, C, Kiss, G, Kawamata, H, Starkov, AA (2014) Chapter seventeen - **Measurement of ADP-ATP exchange in relation to mitochondrial transmembrane potential and oxygen consumption**. In 'Methods in enzymology.' (Eds G Lorenzo, K Guido.) Vol. 542 pp. 333-348. Elsevier academic press inc, San Diego). (<http://dx.doi.org/10.1016/B978-0-12-416618-9.00017-0>)

Salin, K, Villasevil, EM, Auer, SK, Anderson, GJ, Selman, C, Metcalfe, NB, Chinopoulos, C (2016) **Simultaneous measurement of mitochondrial respiration and ATP production in tissue homogenates and calculation of effective P/O ratios**. *Physiological Reports* 4, e13007. (<http://dx.doi.org/10.14814/phy2.13007>)