Rapid, B1-insensitive, dual-band quasi-adiabatic saturation transfer with optimal control for complete quantification of myocardial ATP flux

Online Supporting Information

1 Error propagation

1.1 Introduction

It is a well-known fact that phosphorus spectroscopy is unfortunately a fundamentally low SNR technique, owing to the Boltzmann distribution for the $^{31}$P nucleus, its lower gyromagnetic ratio than protons, and the lower natural biological concentration than, say, water protons. Furthermore, as outlined in the main text, the experiments performed on perfused hearts are subject to additional sources of biological variability: both individually, each heart will be different, and mechanistically, as their function throughout the perfusion experiment slowly deteriorates at a different rate.

As the work presented here encompasses both forward and reverse saturation-transfer experiments, which necessarily involve scanning a perfused heart for a comparatively long period of time in order to obtain a timecourse of spectral curves under varying saturation durations, it may well be the case that the physiological state of flux balance is slightly different between the forward and reverse experiments. An alternative hypothesis, explored here, is that the nonlinear propagation of uncertainty can combine unfavourably to make the uncertainty on the resulting point estimate potentially larger than it otherwise would be.

1.2 Cramér-Rao Lower Bounds and functions of a random variable

A result from information theory is that the minimum bound on an estimator in a statistical setting can be computed from the Hessian of the Fisher information matrix determined in that setting, known as the Cramér-Rao Lower Bound (CRLB). For the case of spectral fitting, particularly where estimates are noisy such as in phosphorus spectroscopy, it is appropriate to take an individual result of a spectral quantification algorithm as a point estimate, $x$, and the CRLB on $x$ as a measure of its uncertainty, $\sigma_x^2$. Typically $x$ is a peak amplitude of interest, though it may be, for example, spectral location.

Under these circumstances, it is appropriate to consider $x$ as a random variable; $x \sim N(\mu_x, \sigma_x^2)$, with the point estimate of $x$, $\mu_x$ that being returned from
a spectral fitting algorithm, and width its CRLB. The main point of this supplement is to briefly highlight that many nonlinear transformations are undertaken between spectral amplitudes, derived concentrations, and the ultimate recorded value of a rate constant.

Firstly, note that taking functions of two random variables, say, $x$ and $y$ and $f(x, y)$, produces a value that is itself randomly distributed. One can appeal to Taylor series expansions, and say that if $x$ is large in comparison to $x^2$, and similarly for $y$, then it will be the case that, if higher order terms are truncated,

$$f = f(x, y); \quad x \sim N(\mu_x, \sigma^2_x); \quad y \sim N(\mu_y, \sigma^2_y)$$

$$\hat{\sigma}^2_f \approx \left| \frac{\partial f}{\partial x} \right|^2 \sigma^2_x + \left| \frac{\partial f}{\partial y} \right|^2 \sigma^2_y + 2 \frac{\partial f}{\partial x} \frac{\partial f}{\partial y} \sigma_{xy},$$

where $\sigma_{xy}$ represents the covariance between $x$ and $y$, related to a correlation coefficient $\rho$ by $\sigma_{xy} = \sigma_x \sigma_y \rho$. The determination of this is ultimately an experimental question: it can be determined from the Fisher information matrix of the nonlinear spectral fit.

This expression, Eq. (3), is straightforward both analytically and computationally, but it is a simplified approximation. In particular, the truncation of the series expression can produce errors, and the assumptions of smoothness and differentiability that it predicts are not necessarily always true. If the uncertainty in $x$ and $y$ are small, it may well be appropriate to consider the ratio a Gaussian PDF with mean $f(x, y)$ and variance $\sigma^2_{xy}$, depending upon the choice of $f$. However, this relies upon the denominator being far from zero. A classic ‘pathological’ example would be the Cauchy distribution, i.e.

$$f(x, y) = \frac{x \sim N(0, 1)}{y \sim N(0, 1)}$$

which is a distribution with no well-defined mean and variance as the relevant generating moments are described by integrals that do not exist. The analytical form for the ratio distribution can be derived. It is a moderately complex expression: if we define the function of the ratio, $w = x/y$ where $x$ and $y$ have a correlation coefficient $\rho$, the ratio distribution is given by the expression:

$$f(w) = \frac{b(w) d(w)}{\sqrt{2 \pi} \sigma_x \sigma_y a^4(w)} \left( \Phi \left( \frac{b(w)}{\sqrt{1 - \rho^2} a(w)} \right) - \Phi \left( -\frac{b(w)}{\sqrt{1 - \rho^2} a(w)} \right) \right) + \frac{\sqrt{1 - \rho^2}}{\pi \sigma_x \sigma_y a^2(w)} \exp \left( -\frac{c}{2(1 - \rho^2)} \right),$$

(4)
where

\[ a(w) = \left( \frac{w^2}{\sigma_x^2} - \frac{2\rho w}{\sigma_x \sigma_y} + \frac{1}{\sigma_y^2} \right)^{\frac{1}{2}} \]

\[ b(w) = \frac{\mu_x w}{\sigma_x^2} - \frac{\rho (\mu_x + \mu_y w)}{\sigma_x \sigma_y} + \frac{\mu_y}{\sigma_y^2} \]

\[ c = \frac{\mu_x^2}{\sigma_x^2} + \frac{2\rho \mu_x \mu_y}{\sigma_x \sigma_y} + \frac{\mu_y^2}{\sigma_y^2} \]

\[ d(w) = \exp \left( \frac{b^2(w) - c(w) a^2(w)}{2(1 - \rho^2)a^2(w)} \right) ; \]

and

\[ \Phi(z) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{z} e^{-\frac{1}{2}u^2} \, du \]

\[ = \frac{1}{2} \left( 1 + \text{Erf} \left( \frac{z}{\sqrt{2}} \right) \right) , \]

where \( \text{Erf}(z) \) denotes the Gaussian error function, \( \text{Erf}(z) = \frac{2}{\sqrt{\pi}} \int_{0}^{z} e^{-t^2} \, dt \), and \( w \sim N(\mu_x, \sigma_x) \) and \( w \sim N(\mu_y, \sigma_y) \).

This matters in the context of phosphorus spectroscopy, because quite often we compute the ratios of peak amplitudes. In the case that quantities measured are far from zero, the Gaussian approximation is approximately correct, and quite useful: see elsewhere for a discussion of how properly incorporating such information about the expected distribution of the PCR/ATP ratio can improve statistical power when comparing groups of measured ratios.

### 1.3 Quantification

In this work, the developed saturation pulse was applied in forward and reverse schemes. Absolute quantification was performed on the basis of three separate measurements of the PPA balloon with differing volumes of PPA. This is because it is necessary to inflate the balloon appropriately to fill the LV cavum and this varies from heart to heart. At the end of the experiment, 100 µl of PPA was added, a hard pulse/acquire scan obtained, and then the process repeated once more. This then yields three PPA scouts with differing volumes (but known concentrations) of PPA, from which linear regression provides an estimate of the gradient of signal of PPA to µl. The CRLB on the PPA quantification is neglected, as it is a large peak. It is possible to perform robust linear regression taking into account uncertainty on the y-axis (i.e. incorporating this information), through means such as Deming regression, but that was not performed here. This regression yields a coefficient \( b \), of units au/µl, that converts from PPA to arbitrary units. There is a statistical regression error on this coefficient; its standard error, which is the standard deviation of its sampling distribution. Call this \( \sigma_b \).

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1 Miller, J. J., Cochlin, L., Clarke, K. & Tyler, D. J. Weighted averaging in spectroscopic studies improves statistical power. Magn. Reson. Med. 78, 2082-2094 (2017). doi:10.1002/mrm.26614
Additionally, in order to estimate concentrations of intracellular phosphorus compounds, it is necessary to calculate the approximate intracellular volume of the heart from its mass, \( m \). For this work, following on from others, we have determined it via reference to the specific gravity of myocardial tissue, \( s_g = 1.05 \text{ g/ml} \). An intracellular volume fraction of \( v_f = 0.48 \) additionally is required to correct for extracellular/intracellular fluid in the wet heart weight factors. Depending upon the scanner configuration, a final correction may be required to correct for averaging (as the final PPA spectra may be acquired with fewer averages, which may or may not change the absolute value of the acquired FID); or indeed gain. Ignoring these known constant scalings, the final ‘correction’ algorithm between derived 31P MRS peak amplitudes \( x \) and the concentration \( c \) of PPA (in units such as mM) becomes

\[
\text{Concentration} = \frac{x}{b/c} \times \frac{\text{Heart intracellular volume}}{s_g \frac{m}{n v_f}}.
\]  

(10)

This permits the determination of the absolute concentration of metabolites in fully-relaxed spectra.

For the propagation of uncertainty, note that those quantities that are determined on the basis of particularly uncertain things are \( b \) and \( x \) above. Other quantities are known to sufficient precision to avoid the need to propagate uncertainty quantitatively. This permits the approximate error on the concentration to be simply expressed as

\[
\Delta^2_{\text{Concentration}} \approx \left( \text{Concentration} \right)^2 \left( \frac{\sigma_x}{x} \right)^2 + \left( \frac{\sigma_b}{b} \right)^2
\]  

(11)

Detailed computations of this quantity for each of the datasets presented in this work are provided later, in Section 2.

1.4 Fluxes

For the computation of fluxes, i.e. the synthetic flux \( k_f [\text{PCr}] + k'_f [\text{Pi}] \) and degradative flux \( (k_r + k'_r) [\text{ATP}] \), we furthermore need to consider the uncertainty in the combined rate constants \( k \). The ideal analysis would again compute the CRLBs on fitted peak uncertainties, perform a form of weighted robust non-linear regression in the context of fitting kinetic model equations with weights of \( 1/CRLB^2 \). The estimate of the returned parameter of interest from such a fit, i.e. \( k_f, k'_f \) or \( (k_r + k'_r) \) is then simply used together with the expression Eq. (12) and derived estimates of uncertainty on concentration measurements. Taken together, this gives the results quoted in the main paper that the uncertainty on derived total forward and reverse fluxes are
2 Individual Results

Synth. Flux = \( k_f [\text{PCr}] + k'_f [\text{Pi}] \)

\[
\Rightarrow \frac{\sigma_{\text{Synth}}}{\text{Synth}} = \sqrt{\left( \frac{\sigma_{[\text{Pi}]} }{[\text{Pi}]} \right)^2 + \left( \frac{\sigma_{[\text{PCr}]} }{[\text{PCr}]} \right)^2 + \left( \frac{\sigma_{\kappa_f} }{\kappa_f} \right)^2 + \left( \frac{\sigma_{\kappa'_f} }{\kappa'_f} \right)^2 } \quad (12)
\]

Deg. Flux = \( (k_r + k'_r) [\text{ATP}] \)

\[
\Rightarrow \frac{\sigma_{\text{Deg.}}}{\text{Deg.}} = \sqrt{\left( \frac{\sigma_{k_r+k'_r} }{k_r+k'_r} \right)^2 + \left( \frac{\sigma_{[\text{ATP}]} }{[\text{ATP}]} \right)^2 } . \quad (13)
\]

We note further that while this truncated local-expansion based method is appropriate in the case where errors are small (as they are here), other methods are available and may be less subject to the bias. Although more computationally intensive, full Monte Carlo simulations or other methods (such as importance or interval analysis) may also be appropriate.

2 Individual Results

These uncertainties are now reported in full for each heart considered in the main manuscript. Whilst \(^3\)\(^1\)P MRS is inherently a low SNR technique, we highlight again how the main ‘point’ of the manuscript is the novel RF pulse design method designed for saturation transfer experiments; nevertheless, human experiments with similar pulses will be subject to uncertainty in much the same way.

Measurements and uncertainties computed during the determination of absolute concentrations from individual peak amplitudes are shown below, in Tables S2, S4, S6, S8, and S10, with a summary statement on the determined concentrations and uncertainties in Tables S3, S5, S7, S9, and S11. They are initially summarized in Table S2, and it is apparent that the relative error in inorganic phosphate is larger than that of other peaks (a one-way anova reveals a \( p \)-value < 0.05). This is arguably unsurprising, reflecting the greater uncertainty in quantifying smaller peaks.

Computing similar fluxes for all spectral time-courses returned in the process of estimating \( k_f, k'_f \) and \( (k_r + k'_r) \), we arrive at the figure quoted in the main manuscript. Detailed spectral information on each time-point is not included for reasons of space.

| Peak Name | \( \beta \)-ATP | \( \alpha \)-ATP | \( \gamma \)-ATP | [\text{PCr}] | [\text{Pi}] |
|-----------|---------------|---------------|---------------|-------------|-----------|
| Mean      | 0.167         | 0.136         | 0.103         | 0.0775      | 0.276     |
| SD        | 0.0703        | 0.0426        | 0.0455        | 0.0354      | 0.184     |
| SEM       | 0.0315        | 0.0190        | 0.0204        | 0.0158      | 0.0824    |

Tab. S1. Summary uncertainty values in derived concentrations.
2.1 Heart 1

| Quantity                        | Value  | Unit |
|--------------------------------|--------|------|
| Heart Mass                     | 1.916  | g    |
| PPA Concentration              | 100    | mM   |
| Specific gravity of tissue     | 1.05   | g/mL |
| Intracellular volume fraction  | 0.48   | %    |
| Average Correction Factor      | 4      |      |
| **PPA Signal Intensities**     |        |      |
| Baseline                       | 8.78   | 0.18 |
| +100 µl                        | 13.2   | 0.19 |
| +200 µl                        | 17.0   | 0.19 |
| Slope                          | 0.0409 | a.u. / ul |
| Regression uncertainty         | 0.00180 | a.u. / ul |
| PPA Contains                   | 0.0001 | mmol / ul |
| Conversion Factor              | 409    | a.u. / mmol |
| Heart Intracellular Volume     | 0.000876 | litres |

Tab. S2. Summary of parameters (Heart 1)

| Fitted Signals | Conc. (mM) | CRLB (Signals) (au) | Δ Conc (mM) |
|----------------|------------|---------------------|-------------|
| β-ATP          | 8.65       | 6.03                | 0.650       | 0.526 |
| α-ATP          | 10.2       | 7.14                | 0.897       | 0.700 |
| γ-ATP          | 10.8       | 7.56                | 0.542       | 0.503 |
| PCr            | 15.0       | 10.4                | 0.370       | 0.526 |
| Pi             | 9.93       | 6.93                | 1.12        | 0.836 |
| PCr/γ – ATP    | 1.38       | 1.38                | 0.0776      |      |
| PCr / ATP      | 1.51       | 1.51                | 0.0732      |      |

Tab. S3. Raw metabolite MRS peak amplitudes and CRLBs, and determined metabolite concentration and ΔConcentration. (Heart 1)
2.2 Heart 2

| Quantity                        | Value | Unit   |
|--------------------------------|-------|--------|
| Heart Mass                     | 1.261 | g      |
| PPA Concentration              | 100   | mM     |
| Specific gravity of tissue     | 1.05  | g/mL   |
| Intracellular volume fraction  | 0.48  | %      |
| Average Correction Factor      | 4     |        |
| PPA Signal Intensities         |       |        |
| Baseline                       | 10.7  | 0.0558 |
| +100 µl                        | 13.9  | 0.0634 |
| +200 µl                        | 16.4  | 0.0656 |
| Slope                          | 0.0286| a.u. / ul |
| Regression uncertainty         | 0.00190| a.u. / ul |
| PPA Contains                   | 0.0001| mmol / ul |
| Conversion Factor              | 286   | a.u. / mmol |
| Heart Intracellular Volume     | 0.000576| litres   |

Tab. S4. Summary of parameters (Heart 2)

| Fitted Signals | Conc. | CRLB (Signals) | Δ Conc |
|----------------|-------|----------------|--------|
| (au)           | (mM)  | (au)           | (mM)  |
| β-ATP          | 3.43  | 5.20           | 0.519  | 0.858  |
| α-ATP          | 4.71  | 7.13           | 0.663  | 1.11   |
| γ-ATP          | 4.64  | 7.02           | 0.463  | 0.841  |
| PCr            | 7.23  | 11.0           | 0.333  | 0.883  |
| Pi             | 4.63  | 7.01           | 0.812  | 1.31   |
| PCr/γ – ATP    | 1.56  | 1.56           | 0.172  |        |
| PCr / ATP      | 1.70  | 1.70           | 0.150  |        |

Tab. S5. Raw metabolite MRS peak amplitudes and CRLBs, and determined metabolite concentration and ΔConcentration. (Heart 2)
### 2.3 Heart 3

| Quantity                        | Value    | Unit |
|---------------------------------|----------|------|
| Heart Mass                      | 1.155    | g    |
| PPA Concentration               | 100      | mM   |
| Specific gravity of tissue      | 1.05     | g/mL |
| Intracellular volume fraction   | 0.48     | %    |
| Average Correction Factor       | 4        |      |
| **PPA Signal Intensities**      |          |      |
| Baseline                        | 9.39     | 0.188|
| +100 µl                         | 13.8     | 0.190|
| +200 µl                         | 16.7     | 0.194|
| Slope                           | 0.0368   | a.u. / ul |
| Regression uncertainty          | 0.00451  | a.u. / ul |
| PPA Contains                    | 0.0001   | mmol / ul |
| Conversion Factor               | 367      | a.u. / mmol |
| Heart Intracellular Volume      | 0.000528 | litres|

**Tab. S6.** Summary of parameters (Heart 3)

| Fitted Signals | Conc. | CRLB (Signals) | Δ Conc |
|----------------|-------|---------------|-------|
| (au)           | (mM)  | (au)          | (mM)  |
| β-ATP          | 4.17  | 5.37          | 0.583 | 0.998 |
| α-ATP          | 6.00  | 7.72          | 0.713 | 1.32  |
| γ-ATP          | 5.36  | 6.90          | 0.530 | 1.09  |
| PCr            | 7.88  | 10.2          | 0.385 | 1.34  |
| Pi             | 3.40  | 4.38          | 0.563 | 0.902 |
| PCr/γ − ATP    | 1.47  | 1.47          | 0.163 |       |
| PCr / ATP      | 1.52  | 1.52          | 0.129 |       |

**Tab. S7.** Raw metabolite MRS peak amplitudes and CRLBs, and determined metabolite concentration and ΔConcentration. (Heart 3)
### 2.4 Heart 4

| Quantity                           | Value | Unit  |
|------------------------------------|-------|-------|
| Heart Mass                         | 1.10  | g     |
| PPA Concentration                 | 100   | mM    |
| Specific gravity of tissue         | 1.05  | g/mL  |
| Intracellular volume fraction      | 0.48  | %     |
| Average Correction Factor          | 4     |       |

| PPA *Signal Intensities*           | Value  | CRLB  |
|------------------------------------|--------|-------|
| Baseline                           | 9.55   | 0.242 |
| +100 µl                            | 13.0   | 0.226 |
| +200 µl                            | 15.860 | 0.225 |
| Slope                              | 0.0315 | a.u. / ul |
| Regression uncertainty             | 0.00172| a.u. / ul |
| PPA Contains                       | 0.0001 | mmol / ul |
| Conversion Factor                  | 315    | a.u. / mmol |
| Heart Intracellular Volume         | 0.000504| litres |

Tab. S8. Summary of parameters (Heart 4)

| Fitted Signals | Conc. | CRLB (Signals) | Δ Conc |
|----------------|-------|----------------|--------|
| (au)           | (mM)  | (au)           | (mM)   |
| β-ATP          | 3.48  | 5.47           | 0.629  | 1.03   |
| α-ATP          | 4.14  | 6.51           | 0.696  | 1.15   |
| γ-ATP          | 5.81  | 9.12           | 0.545  | 0.991  |
| PCr            | 8.01  | 12.6           | 0.436  | 0.970  |
| Pi             | 3.32  | 5.21           | 0.624  | 1.02   |
| PCr/γ – ATP    | 1.79  | 1.38           | 0.151  |        |
| PCr / ATP      | 5.81  | 1.79           | 0.176  |        |

Tab. S9. Raw metabolite MRS peak amplitudes and CRLBs, and determined metabolite concentration and ΔConcentration. (Heart 4)
2.5 Heart 5

| Quantity                      | Value | Unit |
|-------------------------------|-------|------|
| Heart Mass                    | 1.2   | g    |
| PPA Concentration             | 100   | mM   |
| Specific gravity of tissue    | 1.05  | g/mL |
| Intracellular volume fraction | 0.48  | %    |
| Average Correction Factor     | 4     |      |

**PPA Signal Intensities**

| Value     | CRLB |
|-----------|------|
| Baseline  | 10.1 | 0.171|
| +100 µl   | 14.3 | 0.171|
| +200 µl   | 18.0 | 0.176|
| Slope     | 0.0393 | a.u. / ul |
| Regression uncertainty | 0.00158 | a.u. / ul |
| PPA Contains | 0.0001 | mmol / ul |
| Conversion Factor | 393 | a.u. / mmol |

Heart Intracellular Volume | 0.000549 litres

Tab. S10. Summary of parameters (Heart 5)

| Fitted Signals (au) | Conc. (mM) | CRLB (Signals) (au) | Δ Conc (mM) |
|---------------------|------------|---------------------|-------------|
| β-ATP               | 1.96       | 2.27                | 0.467       | 0.549       |
| α-ATP               | 3.66       | 4.24                | 0.720       | 0.852       |
| γ-ATP               | 5.14       | 5.96                | 0.542       | 0.672       |
| PCr                 | 11.9       | 13.8                | 0.330       | 0.672       |
| Pi                  | 2.57       | 2.98                | 0.675       | 0.791       |
| PCr/γ – ATP         | 2.31       | 2.31                | 0.253       |            |
| PCr / ATP           | 3.31       | 3.31                | 0.327       |            |

Tab. S11. Raw metabolite MRS peak amplitudes and CRLBs, and determined metabolite concentration and ΔConcentration. (Heart 5)
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