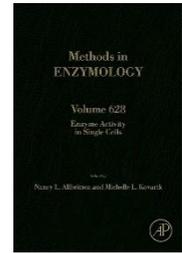


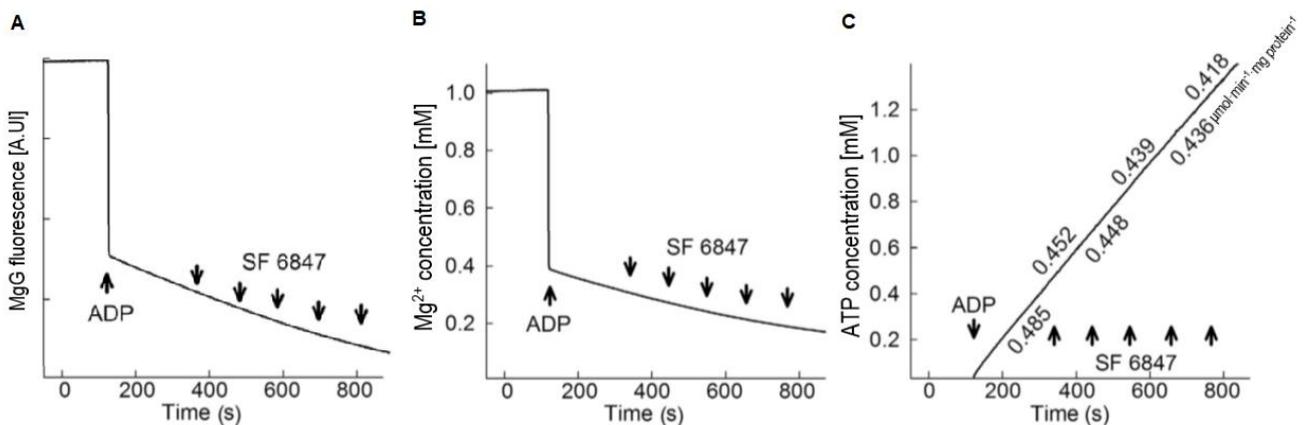
## Measurement of ADP–ATP Exchange in Relation to Mitochondrial Transmembrane Potential and Oxygen Consumption

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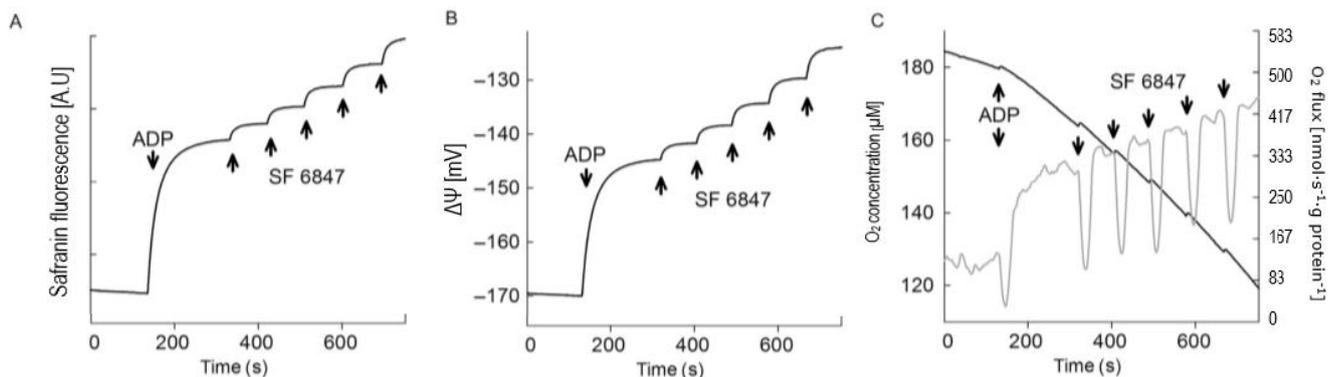
### Extramitochondrial Mg<sup>2+</sup> concentration and conversion to ATP



**Figure 1.** **A)** Reconstructed time recording of MgG raw fluorescence from permeabilized HEK293 cells upon addition of 2 mM ADP, followed by incremental 10 nM additions of the uncoupler SF 6847. **B)** Corrected calibrated trace of extramitochondrial Mg<sup>2+</sup> concentration obtained from panel A. **C)** ATP concentration calculated from panel B.

**The rate of ATP appearing in the medium can be calculated by making a linear regression for the ATP values as a function of time**

### Mitochondrial membrane potential and oxygen flux determination in permeabilized cells



**Figure 2.** **A)** Reconstructed time recording of safranin raw fluorescence in permeabilized HEK293 cells upon addition of 2 mM ADP, followed by incremental 10 nM additions of the uncoupler SF 6847. **B)** Calibrated time recording of  $\Delta\Psi$  obtained from panel A. **C)** Reconstructed time recording of oxygen concentration in the medium (black trace) and oxygen flux (gray trace) recorded simultaneously with either MgG signal (Figure 1, A) or safranin signal (Figure 2, A).

**ATP rate production is obtained as a function of  $\Delta\Psi$  or oxygen flux**

Reference: Chinopoulos C, Kiss G, Kawamata H, Starkov AA (2014) Measurement of ADP-ATP exchange in relation to mitochondrial transmembrane potential and oxygen consumption. *Methods Enzymol* 542:333-48.

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