

## **ANT1 regulation supports the fatty acid cycling mechanism for the proton transport**

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## **Supplementary Tables**

**Supplementary Table 1: Overview of ADP/ATP exchange rate of ANT reconstituted into liposomes described in literature.**

Protein	$k_{\text{ADP/ATP}}$ mmol/min/g	Nucleotides	T, K	Technique	Ref.
Isolated ANT from beef heart mitochondria	0.05 – 0.2	ADP/ATP <sub>internal</sub> = 5 mM ADP/ATP <sub>external</sub> = 10 - 100 $\mu$ M	296	Radioactivity	[1]
Yeast ANT2	3.9 – 8.3	ADP/ATP <sub>internal</sub> = 20 mM ADP/ATP <sub>external</sub> = 100 $\mu$ M	283	Radioactivity	[2]
Yeast ANT2	10.7	ADP <sub>internal</sub> = 5 mM ADP <sub>external</sub> = 1.5 $\mu$ M	296	Radioactivity	[3]
Recombinant murine ANT1	3.49 $\pm$ 0.41	ADP <sub>internal</sub> = 2 mM ADP <sub>external</sub> = 2 mM	296	Fluorescence	[4]
	2.90 $\pm$ 0.47			Radioactivity	
Recombinant murine ANT1	5.53 $\pm$ 0.74	ADP <sub>internal</sub> = 2 mM ADP <sub>external</sub> = 2 mM	296	Radioactivity	In this study

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**Supplementary Table 2: Total membrane conductance at 0, 130 and 190 mV.**

Respective values as shown in Suppl. Fig. 3 and 4.

$\Delta\Phi_m$ , mV	$G_m$ , nS/cm <sup>2</sup>					
	None	ANT	ANT + (ADP,ATP)	ANT +(ADP,ATP) + CATR	AA	ANT
<b>0</b>	8.41 ± 2.31	10.0 ± 2.0	11.8 ± 5.5	8.78 ± 2.69	30.8 ± 0.3	93.3 ± 5.1
<b>130</b>	16.3 ± 3.1	14.0 ± 6.8	27.8 ± 6.4	17.2 ± 5.2	204 ± 106	584 ± 97
<b>190</b>	20.4 ± 3.4	22.0 ± 4.8	39.5 ± 4.9	18.1 ± 2.7	440 ± 135	1750 ± 220

**Supplementary Table 3: Overview of fatty acid activated H<sup>+</sup> turnover number of UCP and ANT reconstituted into proteoliposomes**

Fatty acids are: AA – arachidonic acid (C20:4), LA – lauric acid (C12:0), OA – oleic acid (C18:1) and PA - palmitic acid (C16:0). \* Values are recalculated; \*\* 6-methoxy-N-(3-sulfopropyl)quinolinium

Protein	$k_{H^+}$ , s <sup>-1</sup>	Fatty acid	T, K	Measurement system	Ref.
Isolated hamster UCP1	2 - 10	n.a.	284	Proteoliposomes subjected to a pH electrode	[1]
Recombinant rat UCP1, human UCP2 and human UCP3	5.3 – 15.9*	40 μM PA	n.a.	Proteoliposomes loaded with SPQ** fluorescence dye	[2]
Isolated hamster UCP1	14 ± 5	14.5 mol% OA	295	Planar lipid bilayer with transmembrane pH gradient	[3]
Recombinant human UCP2	4.8	15 mol% AA	310		[4]
Recombinant human UCP2	0.85 – 2.08*	100 μM LA	298	Proteoliposomes loaded with SPQ** fluorescence dye	[5]
Recombinant human UCP4	0.35 – 0.58*	100 μM LA	298		
Recombinant human UCP5	1.39 – 2.67*	100 μM LA	298		
Recombinant murine UCP3	2.56 ± 0.79	15 mol% AA	305	Planar lipid bilayer with transmembrane pH gradient	[6]
Recombinant murine UCP1	5.56 ± 0.38	15 mol% AA	305		[7]
Recombinant murine UCP3	4.10 ± 0.71	15 mol% AA	305		
Recombinant murine ANT1	14.6 ± 2.5	15 mol% AA	305	Planar lipid bilayer with transmembrane pH gradient	In this study

## References:

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**Supplementary Table 4: IC50 and maximum inhibition of the interaction of CATR, BA and PN with ANT.**

Respective values deduced as fitting parameters in Fig. 4 and 5.

<b>Inhibitor</b>	<b>EC50, mM</b>	<b>Maximum inhibition, %</b>
CATR	0.019 ± 0.002	64.2 ± 2.8
BA	0.032 ± 0.011	44.3 ± 5.7
ATP	0.250 ± 0.034	95.7 ± 5.7
ADP	1.310 ± 0.537	107 ± 15
AMP	2.657 ± 0.817	63.7 ± 8.7
GTP	3.324 ± 0.093	48.4 ± 0.1
GDP	5.408 ± 2.008	65.0 ± 20.0
GMP	> 10	n.a.