Table S1. Initial ASO^- transport rates for WT and mutant UCP2s, related to Figure 5 and 6

ASO^- Transport Assay	Initial Slope	Standard Error	Number of replicates	% ASO transport inhibition
W.T.	-169.71	9.24	4	0.0
W.T+GDP	-9.96	8.62	3	94.1
Mutations affecting ASO^- transport but not GDP inhibition				
R60S	-42.03	7.32	3	75.2
R60S+GDP	-17.91	8.44	2	89.4
K271S	-76.81	9.10	3	54.7
K271S+GDP	-10.91	10.33	2	92.0
R60S,K271S	-36.19	14.92	2	78.7
R60S,K271S+GD			2	93.6
P	-13.58	10.00		
R279T	-124.89	6.12	3	26.4
R279T+GDP	-11.20	7.24	3	93.4
K241S	-123.85	9.26	3	27.0
K241S+GDP	-9.86	4.57	2	94.1
K16S	-97.63	12.49	3	43.4
K16S+GDP	-13.40	6.76	3	92.1
Mutation affecting GDP inhibition but not ASO^- transport				
R185T	-159.18	9.74	3	6.2
R185T+GDP	-86.04	5.45	3	49.3
Mutations affecting ASO^- transport and GDP inhibition				
K141T	-103.56	6.07	3	38.9
K141T+GDP	-41.67	8.29	3	75.4
R88T	-69.04	7.43	3	59.3
R88T+GDP	-42.03	7.32	3	75.2
Mutations with no significant effect in ASO^- transport or GDP inhibition				
R76S	-178.02	9.27	3	-4.9
R76S+GDP	-10.55	7.74	2	93.8
I30A	-158.66	6.54	3	6.5
I30A+GDP	-2.90	7.05	3	98.3
L278A	-174.64	8.52	3	-2.9
L278A+GDP	-12.63	5.26	3	92.5

Supplementary Experimental Procedures

Expression and Purification of UCP2

DNA encoding residues 14-309 of mouse UCP2 was sub-cloned into the pET-21a vector and overexpressed in *E. Coli* Rossetta DE3 cells with a C-terminal 6His tag in LB media at 37 °C. When the culture reached OD600 = 1.0, the expression was induced with 0.6 mM IPTG at 30 °C. Cell pellets were collected 6 hrs after induction and washed twice in 20 mM potassium phosphate (pH 8.5). The cell mass was recorded, and cells were re-suspended at 100 mg/ml and lysed in a buffer consisting of 20 mM potassium phosphate (pH 7.4), 20 μg/ml lysosime, 5 μg/ml DNAse I, 10 mM Mg^{2+}, and protease inhibitors for 1 hr at 37 °C. Triton X-100 was added to the lysis solution at 10 μl per gram of cells and the buffer was supplemented with 10 μM GDP and 0.2% BME, and stirred for 2 hr at 4 °C. The suspension was then homogenized using a dounce homogenizer. Unlysed cells and debris were then removed by centrifugation at 1500g for 10 minutes. To the supernatant, 10 mg of 1,2-Dimyristoyl-sn-Glycero-3-Phosphocholine (PC), 2 mg 14:0 cardiolipin (CL) and 0.1 mg 1,2-diphytanoyl-sn-glycero-3-phosphate (PL) was added per gram of cells. Lipids were added as stock solutions solubilized in a 10% detergent mixture (1:1 molar ratio of decyl-maltoside (DM):octly-glucoside (OG)), followed by stirring for 2 hrs at 4 °C. Membranes in the supernatant were collected by centrifugation at 50,000g for 2 hrs. The pellet was re-suspended in Buffer A (40 mM potassium phosphate, 250 mM NaCl, 10 mM BME, and 10 μM GDP, pH 8.0) with addition of 0.5 mg of PL per gram of cells followed by incubation at 4 °C for 2 hrs. This suspension was homogenized using a dounce homogenizer and debris was discarded. The supernatant was then centrifuged at 50,000g for 2 hrs. The pellet was washed by re-suspension in Buffer A followed by incubation for 4 hrs in Buffer A (10x the initial re-suspension volume) with 0.2% Foscholine-12 (DPC) detergent. Solubilized UCP2 was loaded to a Ni-NTA column and washed with Buffer B (40 mM potassium phosphate, 250 mM NaCl, 0.2% DPC, and 10 mM BME, pH 8.0) and eluted in Buffer B plus 0.6 M imidazole. The buffer was exchanged to low salt condition (10 mM Tris, 50 mM NaCl, and 0.2% DPC, pH 8.0) and the sample was applied to a MonoQ column. The pass-through fraction was incubated with CybaBlue resin at 1 g per gram cells for 1 hr, eluted with 100 mM GDP, concentrated and loaded onto a Sephadex S-200 column equilibrated in 50 mM potassium phosphate (pH 6.5), 100 mM NaCl and 1 μM GDP, 0.01% DPC for a final round of size exclusion purification. Fractions containing UCP2 were pooled and the protein concentrated to ~50 μM. The buffer composition was adjusted to 5 mM GDP, 50 μM CL and 100 μM DMPC, and then the protein was further concentrated to ~10 mg/ml and the buffer exchanged into the measurement conditions. In addition to the lipids carried over from the purification, a typical NMR UCP2-GDP sample contains 0.8

mM UCP2, 100 mM DPC, 5 mM GDP, 5 mM BME, 30 mM potassium phosphate (pH 6.5), and 80 mM NaCl. The mutants were purified following the same protocol.

NMR RDC Measurement

RDC measurements were conducted at 33 °C using a 600 MHz spectrometer equipped with cryogenic TXI probes (Bruker). The RDCs were obtained by subtracting J of the isotropic sample from $J+D$ of the aligned sample containing 20 mg/ml DNA nanotubes (Douglas et al., 2007) (all other components same as in the isotropic sample). The sign of the RDC follows the convention that $|{}^1J_{NH} + {}^1D_{NH}| < 92$ Hz when ${}^1D_{NH}$ is positive. The ${}^{15}N–{}^1H$ J or $J+D$ were measured using the J-scaled TROSY-HNCO experiment with two interleaved 3D spectra, the regular TROSY-HNCO spectrum and a modified TROSY-HNCO spectrum with J_{NH} evolution during the ${}^{15}N$ chemical shift evolution scaled to zero (Kontaxis et al., 2000). The ${}^{13}C'–{}^{13}C^{\alpha}$ J or $J+D$ couplings were measured using the 3D TROSY-HNCO experiment with quantitative $J_{C'C\alpha}$ modulations of 0 and 28 ms (Jaroniec et al., 2004).

Supplementary Reference

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