METHODS

Sample preparation Mouse UCP2 (residues 14-309 with a C-terminal His_6 tag) was expressed using a pET-21 vector in *E. Coli* Rossetta DE3 cells. After cell lysis, Triton X-100 was added to the lysate at 10 μL per gram of cell pellet, followed by stirring at 4 °C for 2 hrs in the presence of 10 μM GDP and 0.2% BME. The suspension was homogenized and centrifuged at 1500 g to remove insoluble debris. To adjust the lipid composition of the membrane fraction, 10 mg dimyristoyl-phosphatidylcholine (DMPC), 2 mg cardiolipin, and 0.1 mg phytanoyl lipid per gram of cell pellet were solubilized at 20 mg/ml in 10% octyl-glucoside and then added to the supernatant, followed by 2 hrs of stirring at 4 °C. The membranes were collected by centrifugation at 50,000 g for 2 hrs, and UCP2 was extracted in 40 mM potassium phosphate (pH 8.0), 250 mM NaCl, 50 mM BME, 10 μM GDP and 0.2% dodecyl-phosphocholine (DPC). The solubilized UCP2 was adsorbed on a Ni-NTA resin in the absence of GDP and eluted with 300 mM imidazole. The sample was then exchanged by dialysis into low salt buffer and applied to a MonoQ column. The MonoQ flow-through was incubated with Ciba Blue nucleotide-analog resin (Sigma) for 1 hr, eluted with 100 μM GDP, and finally loaded onto a Sephadex S-200 column for size exclusion purification in 50 mM potassium phosphate (pH 6.5), 100 mM NaCl, 5 mM DPC. The eluted UCP2 sample was supplemented with GDP, detergent and lipids such that the final NMR sample contained 0.8 mM UCP2, 5 mM GDP, 150 mM DPC, 2 mM DMPC, 1 mM cardiolipin, 5 mM BME, 30 mM potassium phosphate (pH 6.5), and 80 mM NaCl.

In addition to UCP2, we have tested expression and purification of mouse UCP1 and UCP3. UCP1 could be expressed and purified as described for UCP2, and showed FFA dependent proton translocation activity and GDP inhibition. UCP3 could be expressed but presented serious solubility problems during purification.

GDP binding We used FRET to test GDP binding to UCP2 under the NMR conditions. The intrinsic tryptophan florescence (λ_{ex} 280 nm, λ_{em} 350 nm) of UCP2 was the resonance energy transfer donor and MANT-GDP (λ_{ex} 356 nm, λ_{em} 440 nm), the acceptor. We recorded the emission intensity at 440 nm (λ_{ex} 280 nm) of 10 μM UCP2 / MANT-GDP complex for increasing GDP concentrations. The graph shows the FRET response as a percent relative to 1:0 MANT-GDP:GDP. Data were fit to the standard equilibrium binding equation.

NMR spectroscopy NMR experiments were conducted at 33 °C on spectrometers equipped with cryogenic TXI probes (Bruker). Sequence specific assignment of backbone $^{1}H^{N}$, ^{15}N, $^{13}C^{\alpha}$, $^{13}C^{\beta}$, and $^{13}C'$ chemical shifts were accomplished using three pairs of triple resonance experiments and a double ^{15}N-edited NOESY, recorded on a (^{15}N-, ^{13}C, ^{2}H) labeled protein sample. The triple resonance experiments include the TROSY versions of HNCA, HN(CO)CA, HNCACB, HN(CO)CACB, HN(CA)CO, and HNCO[1,2]. The ^{15}N-edited NOESY is a high

resolution 3D (H^N,H^N)-HMQC-NOESY-TROSY with ^{15}N, ^{15}N, and $^1H^N$ evolution in the t_1, t_2, and t_3 dimensions, respectively.

The RDCs were obtained by subtracting J of the isotropic sample above from $J + D$ of the aligned sample containing 0.5 mM protein and 20 mg/ml DNA nanotube (all other components same as in the isotropic sample). Details of preparing DNA nanotubes and aligned protein samples are in **Supplementary Information**. The sign of the RDC follows the convention that $|^1J_{NH} + {}^1D_{NH}| < 92$ Hz when $^1D_{NH}$ is positive. The ^1H-^{15}N couplings were measured at 600 MHz (^1H frequency) using the J-scaled TROSY-HNCO experiment to exploit the favorable relaxation property of the TROSY transitions[3]. In this experiment, two interleaved spectra were recorded, the regular TROSY-HNCO and also a modified TROSY-HNCO with J_{NH} evolution during the ^{15}N chemical shift evolution scaled to zero. The $^{13}C'$-$^{13}C^\alpha$ couplings were measured at 600 MHz using the 3D TROSY-HNCO experiment with quantitative-$J_{C'C\alpha}$ modulations of 0 and 28 ms[4]. The $^{13}C'$-^{15}N couplings were measured at 750 MHz using the 3D TROSY-HNCO experiment with quantitative-$J_{NC'}$ modulations of 33 and 66 ms[5].

For introducing a single paramagnetic site for PRE measurement, the 5 cysteines of UCP2 were all mutated to alanine or serine (C25A, C191S, C217S, C227S, C256S); the Cys-free UCP2 had GDP binding properties similar to those of wild-type protein. A single cysteine was introduced into the protein based on the known secondary structures from MFR segments. The nitroxide spin label, *S*-(2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)methyl methanesulfonothioate (MTSL), was then attached at the cysteine position by adding 7-fold excess label to 10 μM UCP2 in the NMR buffer at pH 8.0, and incubating at 25 °C for 4 hrs. Excess label was removed to avoid nonspecific broadening. The pH was changed back to 6.5 for NMR measurements. To quantify residue-specific broadening of backbone $^1H^N$, two TROSY-HNCO spectra were recorded, one after nitroxide labeling and another after reducing the nitroxide free electron with a 5x molar ascorbic acid. The same protocol was used to measure broadening of UCP2 resonances by the spin-labeled GDP (**Fig. 3c**; custom synthesized by Shanghai Chempartner Co., Shanghai, China).

Structure determination The procedure had two stages: 1) determining local structural segments by RDC-based MFR and 2) determining the spatial arrangement of the MFR-derived segments in the tertiary fold using PRE distance restraints. In Stage 1, we fitted RDCs of every 7-residue stretch along the UCP2 sequence to the fragments in the database using the program PALES[6]. Only those 7-residue stretches with on average more than 2 RDCs/residue were used for fragment searching. In the end, 5520 fragments with $Q_{free} < 0.25$ were collected, where Q_{free} = For each candidate fragment (residue *n*-*m*) evaluated in the *fragment assignment*, *gap filling*, or *end extension* operations (described in the main text), only the ϕ and ψ angles of residue (*n*+1)-(*m*-1) were used, because the end residues do not have the ^{15}N-$^{13}C'$ dipolar coupling. During *fragment assignment*, we found that for each 7-residue stretch, the best 5-10 fragments typically have very similar Q_{free} (differences less than 10%). Therefore, the best fragment was assigned to the region only if these fragments have backbone r.m.s. deviation <

0.5 Å. Similarly, there are multiple fragments that score equally well in *gap filling* or *end extension*. In those cases, we assigned the best-scoring fragment only if the final merged or extended segments have backbone r.m.s. deviation < 1 Å. Examples of fragment convergence are shown in **Supplementary Fig. 9**.

In Stage 2, we calculated structures using XPLOR-NIH[7] with the assigned structured segments, RDCs and PREs. The ϕ and ψ values of the segments in **Fig. 2c** were strongly enforced by a harmonic potential with force constant ramped from 10 to 1000 kcal mol^{-1} rad^{-2}. All RDCs used for determining the segments were applied, and the RDC force constant was ramped from 0.01 to 1.5 kcal mol^{-1} Hz^{-2}. PRE restraints (uncertainties in **Supplementary Table 2**) were enforced with flat-well harmonic potentials, with the force constant ramped from 1 to 40 kcal mol^{-1} $Å^{-2}$. In addition to experimental restraints, a weak database-derived 'Rama' potential function[8] was ramped from 0.02 to 0.2 (dimensionless force constant) for the general treatment of sidechain rotamers. A total of 30 monomer structures were calculated using a simulated annealing protocol in which the bath temperature was cooled from 2000 to 200 K. Fifteen low energy structures were selected as the structural ensemble (**Supplementary Table 1**).

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