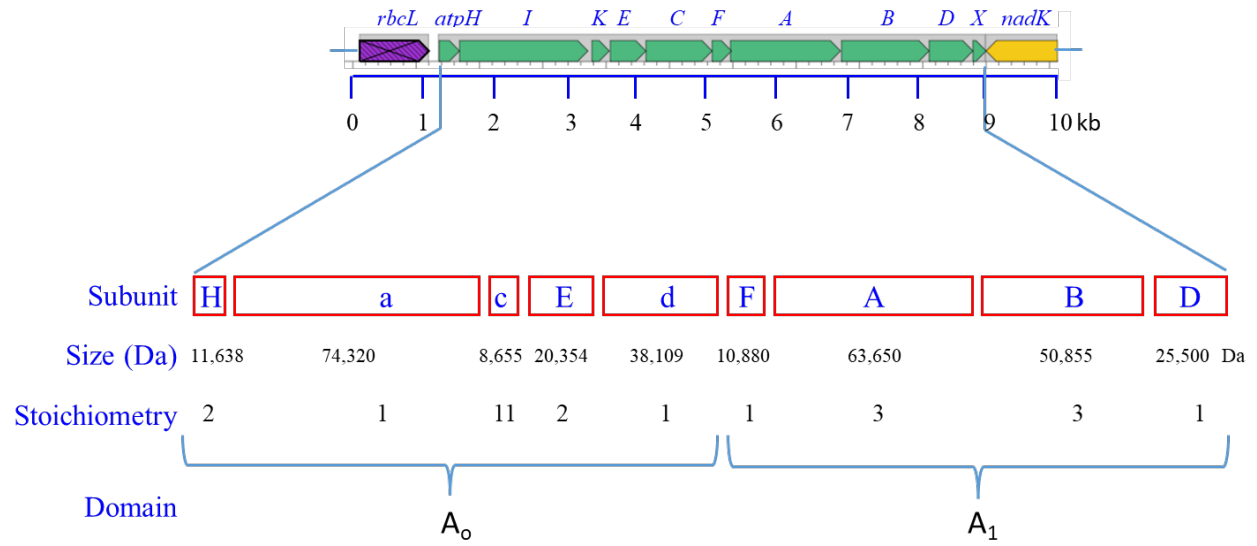


Supplementary Figure

Figure S1 | The *M. concilii* A1Ao gene locus and encoded ATP synthase subunit proteins.



Supplementary Tables

Table S1 | Summary of *M. concilii* GP6 genes encoding the ATP synthase A₁A_o.

Gene Name	Subunit	Copy #	NCBI protein ID	Size (aa)	Description
atpH/G	H	2	WP_013719984.1	108	peripheral arm subunit
atpI	a	1	WP_013719983.1	677	A _o domain a subunit
atpK/L	c	11	WP_013719982.1	88	A _o domain ring subunit
atpE	E	2	WP_013719981.1	182	peripheral arm subunit
atpC	d	1	WP_048132365.1	337	A ₁ rotor subunit
atpF	F	1	WP_013719979.1	99	A ₁ rotor subunit
atpA	A	3	WP_013719978.1	576	A ₁ catalytic subunit
atpB	B	3	WP_013719977.1	461	A ₁ allosteric subunit
atpD	D	1	WP_013719976.1	231	A ₁ rotor subunit

Table S2 | Summary of *M. concilii* GP6 genes encoding the USP paralogs.

IMG-JGI locus tag	NCBI protein ID	Size (aa)	USP domain #
Mcon_0159	WP_013718124.1	149	1
Mcon_0171	WP_013718135.1	145	1
Mcon_1429	WP_013719135.1	291	2
Mcon_1433	WP_013719137.1	287	2
Mcon_2453	WP_013719936.1	314	2
Mcon_2630	WP_013720073.1	285	2
Mcon_2642	WP_013720083.1	143	1

Table S3 | CryoET data collection (ATP γ S-free).

Data collection	
Microscope	Titan Krios G4
Voltage (kV)	300
Total electron exposure (e ⁻ /Å ²)	120
Slit width (eV)	20
Detector	Falcon 4i
Defocus range (μm)	-2 to -5
Pixel size (Å)	1.573
Tilt-series range	±45°
Tilt-series increment	±3°
Tilt-series scheme	dose symmetry
Tilt-series used	79
Data processing	
Software: tilt-series alignment	AreTomo
Software: particle picking	pytom-match-pick
Software: final reconstruction	Relion-5
Initial particle images	99,942
Final particle images	30,809 (A ₁ A ₀); 23,027 (A ₁)
Map resolution (Å)	5.70 (A ₁ A ₀); 3.97 (A ₁)
Symmetry imposed	C1
Gold-standard FSC threshold	0.143

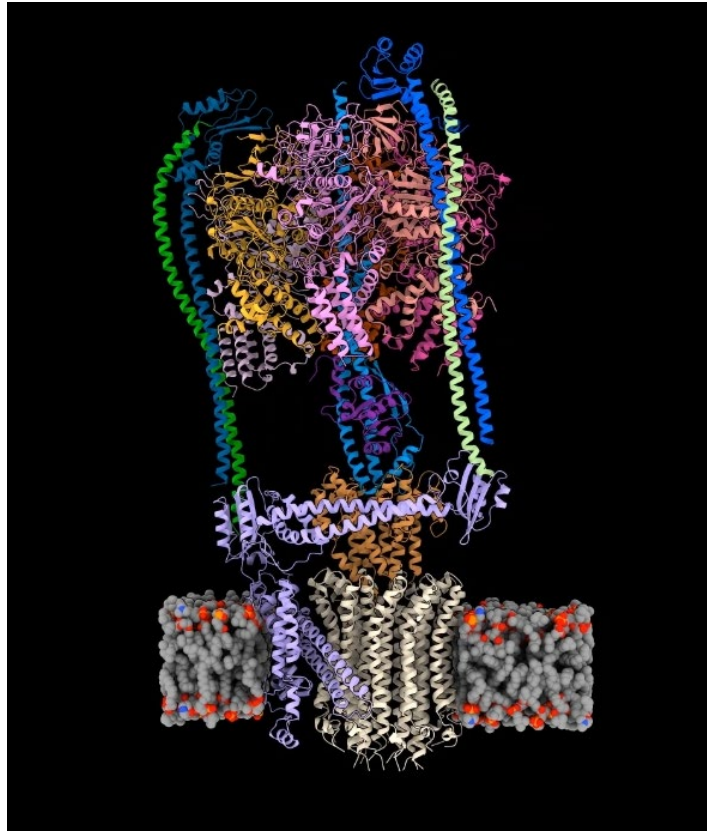
Table S4 | CryoET data collection (ATPyS-treated).

Data collection	
Microscope	Titan Krios G4
Voltage (kV)	300
Total electron exposure ($e^-/\text{\AA}^2$)	120
Slit width (eV)	20
Detector	Falcon 4i
Defocus range (μm)	-2 to -5
Pixel size ($\text{\AA}$)	1.235
Tilt-series range	$\pm 45^\circ$
Tilt-series increment	$\pm 3^\circ$
Tilt-series scheme	dose symmetry
Tilt-series used	495
Data processing	
Software: tilt-series alignment	AreTomo
Software: particle picking	pytom-match-pick
Software: final reconstruction	Relion-5
Initial particle images	607,200
Final particle images (State 0)	94,608 (A_1A_0); 29,959 (A_1)
Map resolution ($\text{\AA}$) (State 0)	4.20 (A_1A_0); 3.64 (A_1)
Final particle images (State 1)	13,432 (A_1A_0); 13,432 (A_1)
Map resolution ($\text{\AA}$) (State 1)	7.44 (A_1A_0); 4.80 (A_1)
Final particle images (State 2)	15,038 (A_1A_0); 15,038 (A_1)
Map resolution ($\text{\AA}$) (State 2)	6.94 (A_1A_0); 4.46 (A_1)
Final particle images (State 3)	35,402 (A_1A_0); 35,402 (A_1)
Map resolution ($\text{\AA}$) (State 3)	4.17 (A_1A_0); 3.57 (A_1)
Symmetry imposed	C1
Gold-standard FSC threshold	0.143

Table S5 | CryoEM data validation statistics (ATP γ S-treated).

CryoEM data validation statistics		
Model refinement		
Model composition		
Non-hydrogen atoms		42,062
Protein residues		3,673
Water		0
<i>B</i> factors (Å ²)		
Protein		58.24
Water		NA
Map CC		0.80
R.m.s. deviations		
Bond lengths (Å)		0.006
Bond angles (°)		0.858
MolProbity score		2.99
Clash score		25.12
Ramachandran plot statistics (%)		
Preferred		90.79
Allowed		8.69
Outlier		0.52
Rotamers outliers (%)		3.97
PDB code		xxx
EMDB code		xxx

Supplementary Movie



Supplementary Video 1 | Atomic-model morph from State 1 to the USP-bound State 0 of the A_1A_0

ATP synthase.