

Supplementary Materials for
**Atomic structure and dynamics of the mechanosensitive channel MscL from
Escherichia coli by cryo-EM and solid-state NMR**

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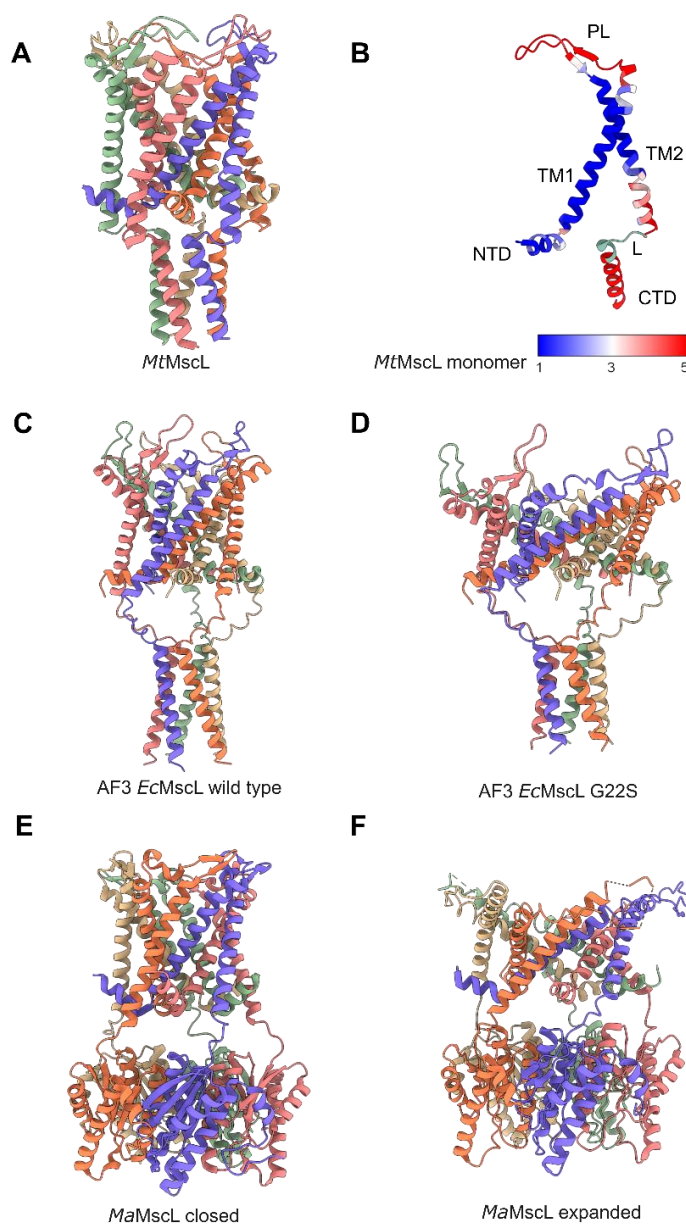


Fig. S1. Homologous structures and AlphaFold3 predictions. (A) The overall structure of closed-state *MtMscL* (PDB ID: 2OAR) from *Mycobacterium tuberculosis*. (B) Structural comparison of the crystal structure of *MtMscL* (PDB ID: 2OAR, chain A) with the G22S mutant of *EcMscL* (current study, PDB ID: 9TIV, chain A). Similarity is indicated by a red (different) to blue (similar) color scale. Missing residues (primarily in the ‘L’ region) are shown in green. (C) AlphaFold3 prediction of wild-type *EcMscL*. (D) AlphaFold3 prediction of the *EcMscL* G22S mutant. (E) The overall structure of closed-state *MaMscL* (PDB ID: 4Y7K) from *Methanosarcina acetivorans*. (F) The overall structure of expanded-state *MaMscL* (PDB ID: 4Y7J).

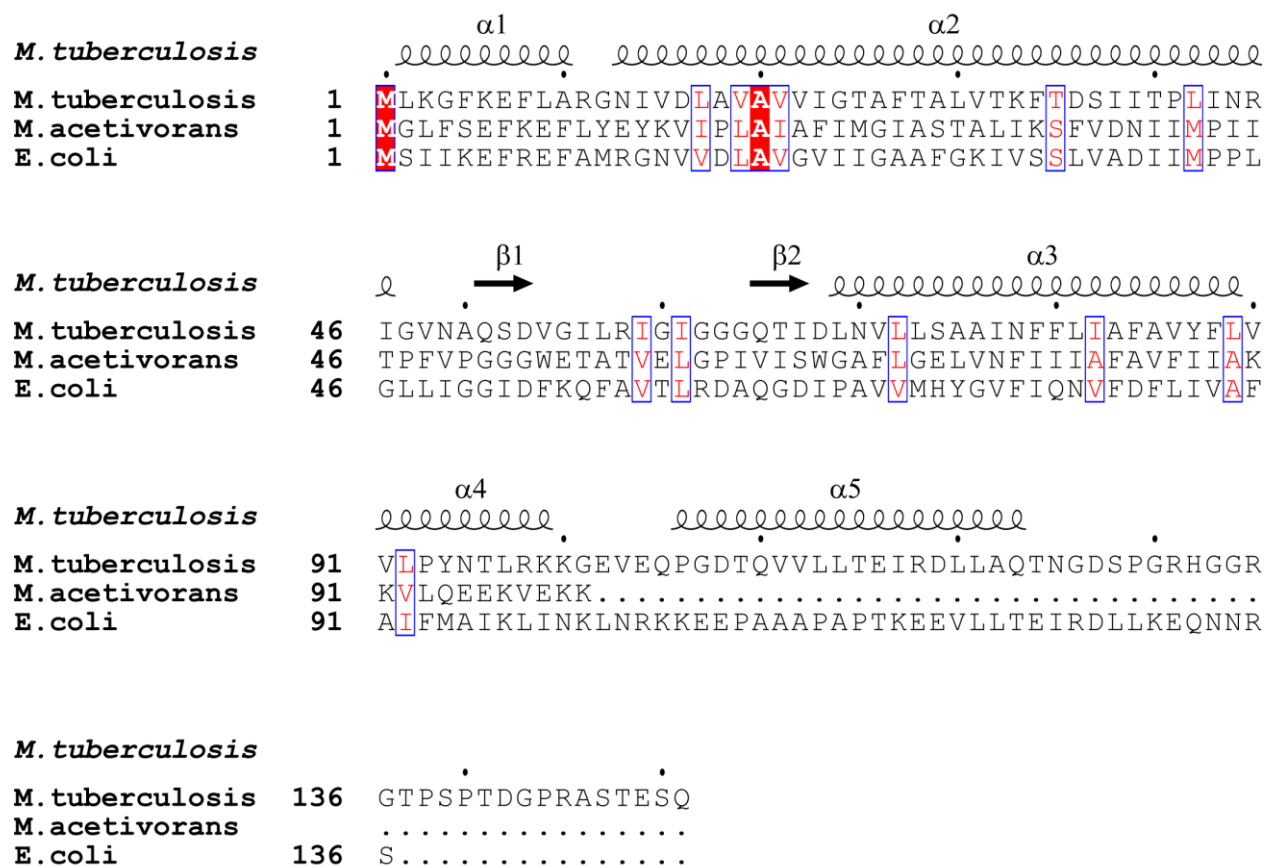


Fig. S2. Sequence alignment of *MtMscL*, *MaMscL*, and *EcMscL* by ESPript 3.2 (114).

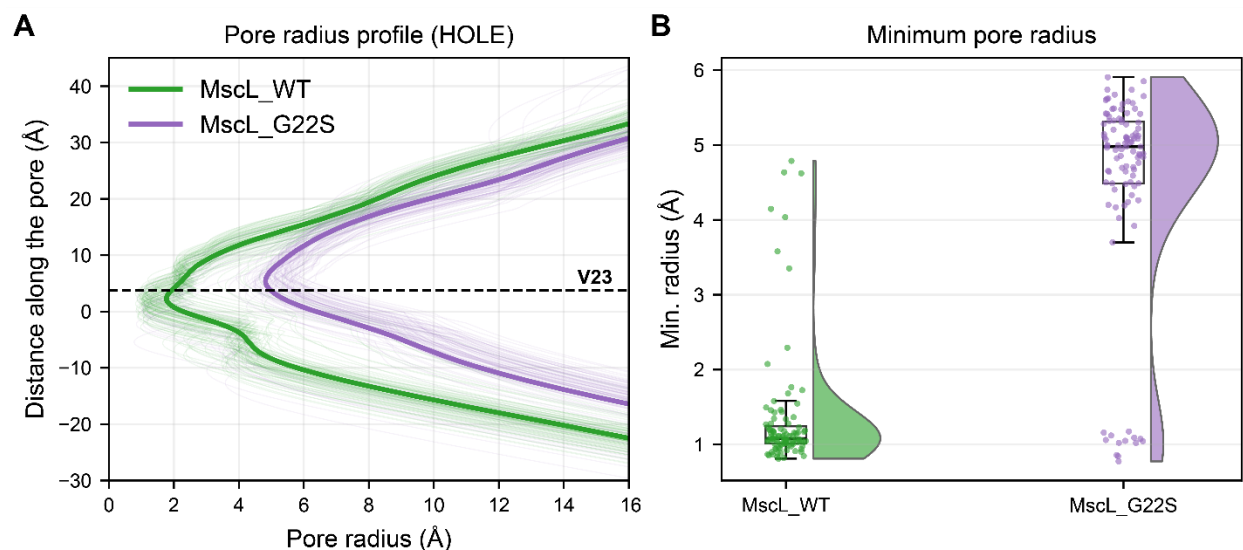


Fig. S3. Analysis of AlphaFold3 predicted structures. (A) Ion conduction pore profiles for wild-type *EcMscL* and its G22S mutant, predicted using AlphaFold3. For each sequence, 20 random seeds with five prediction samples per seed were generated and the pore was analyzed using the MDanalysis implementation of the HOLE algorithm. The position of V23 is indicated for reference. (B) Narrowest point in the pore according to the HOLE analysis. Individual predictions are shown as points; the box spans the second and third quartiles, the median is indicated by a horizontal line and the whiskers denote the maximum and minimum values, excluding outliers. The shaded area represents the kernel density of the individual minimum pore radii.

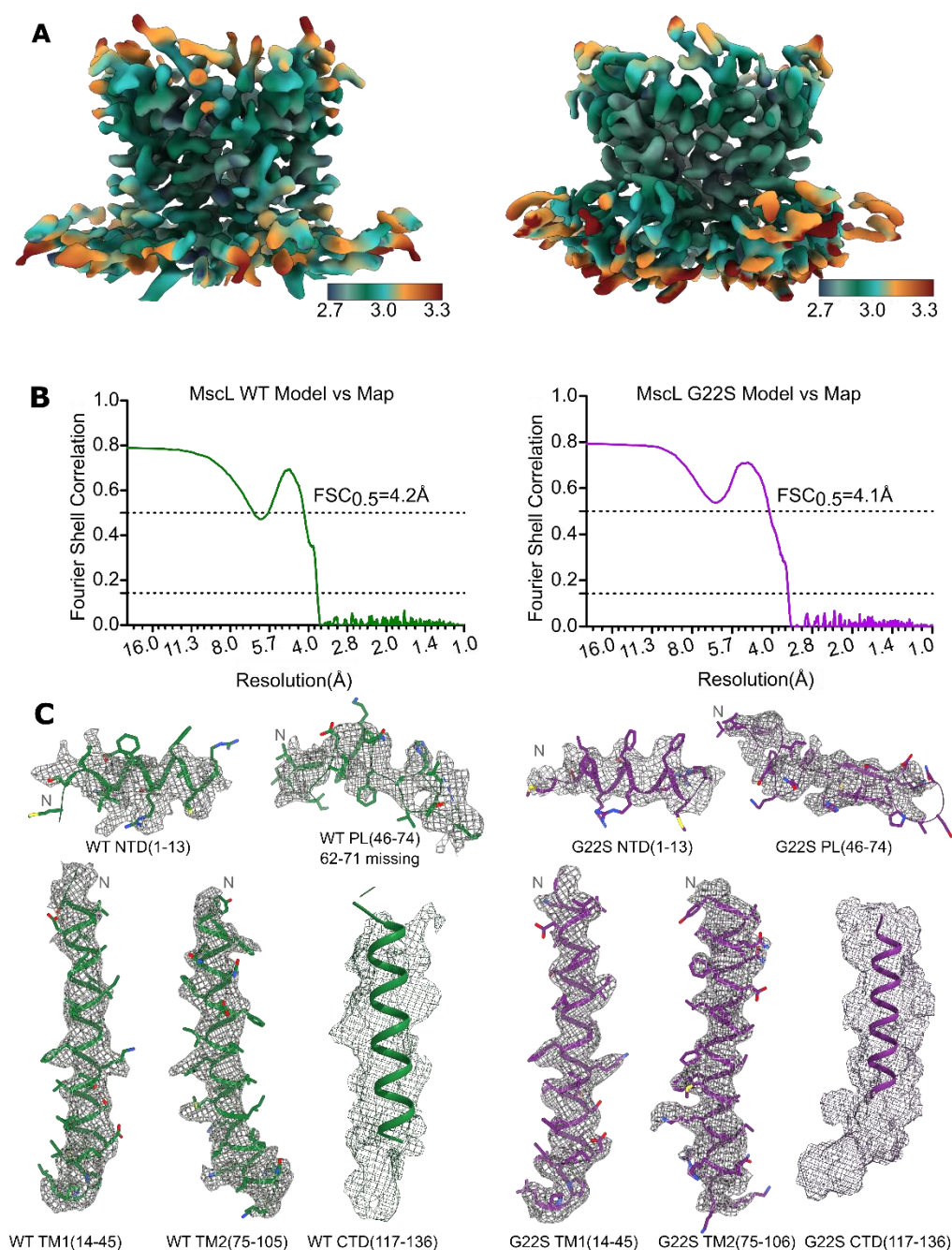


Fig. S4. Single-particle cryo-EM characterization of MscL wild-type and mutant. (A) Cryo-EM maps colored by local resolution, indicated values are in Å. (left: WT, right: G22S mutant). (B) FSC curves from cross validation between the atomic models against unmasked sum of both half-maps (left: WT, right: G22S mutant). (C) Segmented cryo-EM density maps (mesh) of WT and G22S mutant. The corresponding fitted atomic model is shown in green for WT and purple for the G22S mutant.

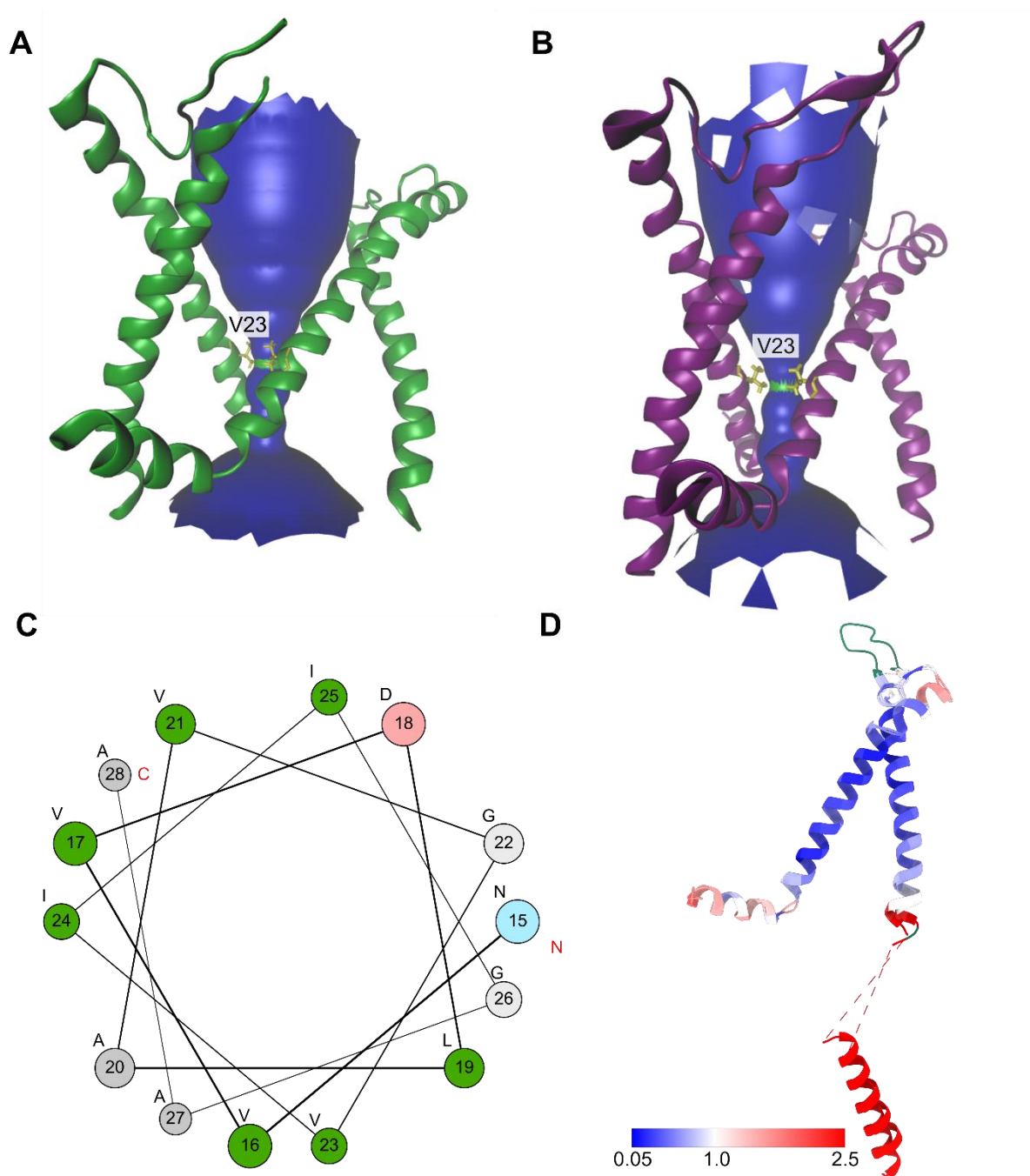


Fig. S5. Pore radius of the *EcMscL* channel as modelled by HOLE 2.2.005. (A) The ion conduction pore of wild-type *EcMscL*. Only two subunits are shown for clarity, and dots outline the central pore. The narrow position at V23 is highlighted. (B) The ion conduction pore of the *EcMscL* G22S mutant. (C) Helical wheel for residues N15-A28. (D) Structural comparison of wild-type *EcMscL* (PDB ID: 9TIU, chain A) with the G22S mutant of *EcMscL* (PDB ID: 9TIV, chain A). Similarity is indicated by a red (different) to blue (similar) color scale. Missing residues (primarily in the 'PL' region) are shown in green.

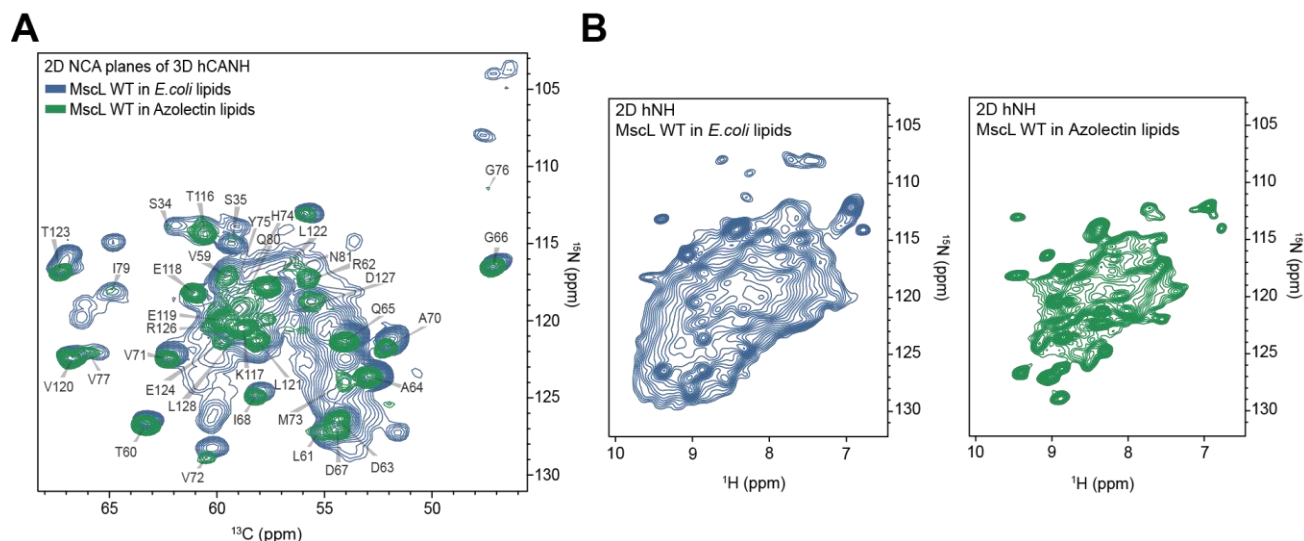


Fig. S6. Comparison of *EcMscL* WT in Azolectin and *E. coli* lipid extract. (A) Overlay of 2D planes extracted from the ^1H -detected 3D hCANH spectra of WT *EcMscL* reconstituted in Azolectin lipids (green; 1.3 mm rotor, 900 MHz) and in *E. coli* lipid extract (blue; 1.3 mm rotor, 600 MHz). Sample preparation of *EcMscL* in *E. coli* lipid extract was performed using the same protocol as described for the Azolectin-reconstituted samples in the Materials and Methods section. The indicated resonance assignments correspond to *EcMscL* in Azolectin lipids. **(B)** Comparison of the 2D hNH spectra of WT *EcMscL* reconstituted in Azolectin lipids (green, 900 MHz) and *E. coli* lipid extract (blue, 600 MHz). The major resonances show substantial overlap between the two samples, indicating that the overall protein structure is well preserved in both lipid environments.

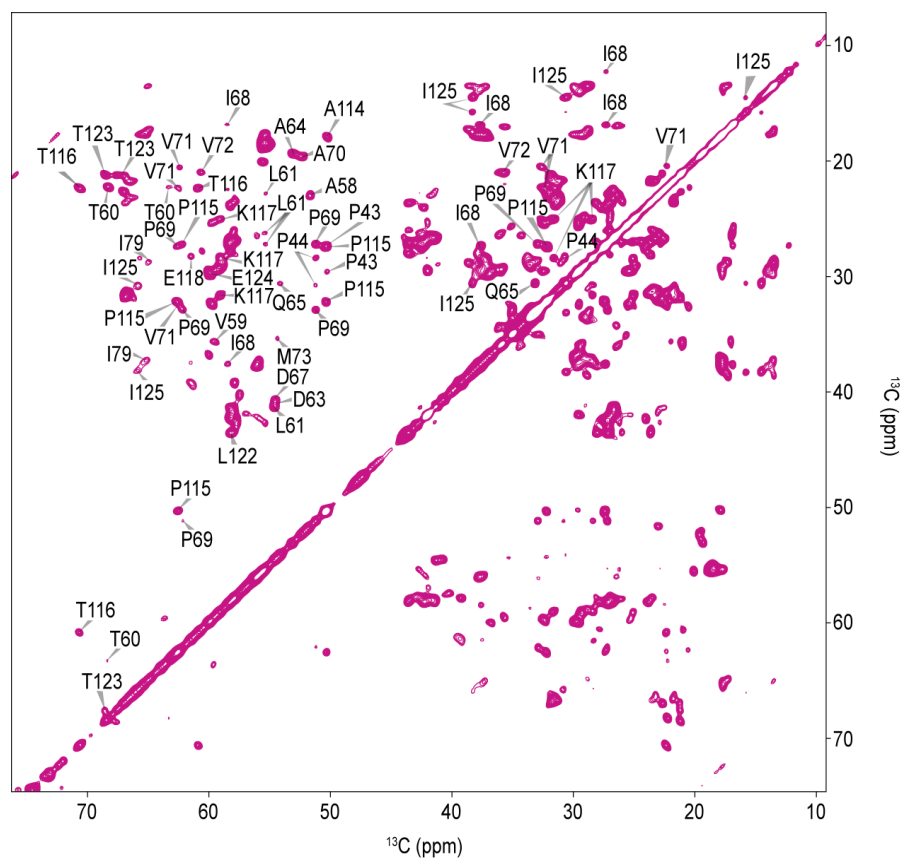


Fig. S7. 2D ^{13}C - ^{13}C PDSO spectrum. Spectrum of [^{13}C , ^{15}N]-labeled *EcMscL* WT in Azolectin proteoliposomes and with assigned residues labelled. The spectrum was recorded on a 700 MHz spectrometer at an MAS rate of 11 kHz.

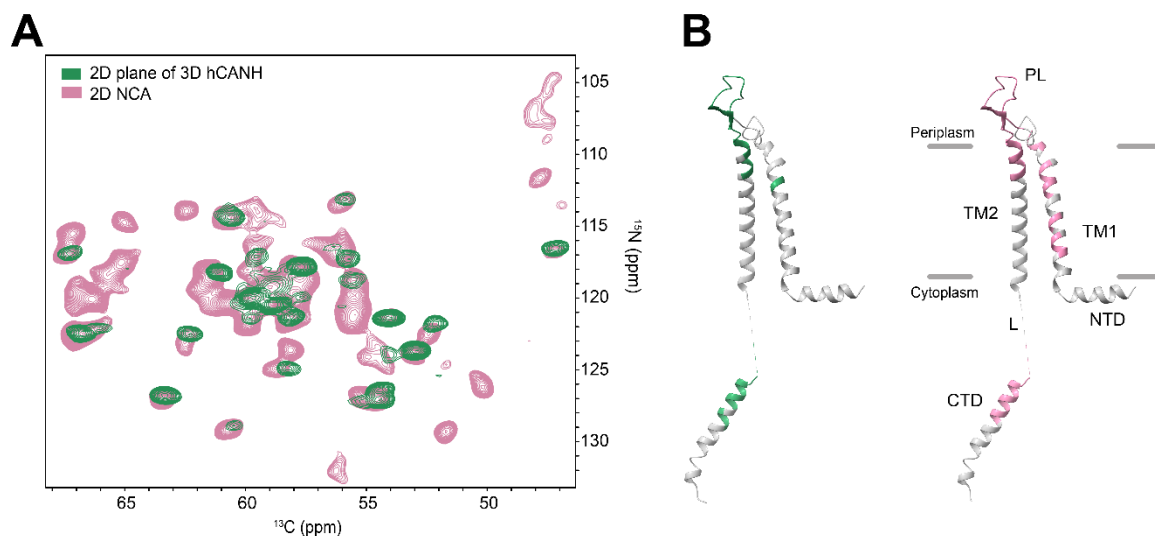


Fig. S8. Comparison of ^1H - and ^{13}C -detected spectra. (A). Overlay of ^{13}C -detected 2D NCA (3.2 mm, 700 MHz) and the 2D plane of a ^1H -detected 3D hCANH spectrum (1.3 mm, 900 MHz) of *EcMscL* WT in Azolectin. As a result of deuteration and subsequent H/D exchange, less peaks can be observed in the 2D plane of the ^1H detected spectrum (green), but the presented peaks coincide with the ^{13}C -detected 2D NCA spectrum (pink). (B). Assignments of WT *EcMscL* achieved using either ^1H -detected (green) or ^{13}C -detected (pink) spectra, and mapped onto a monomer of the here determined cryo-EM structure (PDB ID: 9TIU). PL = periplasmic loop, TM = transmembrane helix, L = loop, NTD = N-terminal domain, CTD = C-terminal domain. Missing residues in TM1 and TM2 in the ^1H -detected data could result from a lack of H/D back-exchange. In the case of the ^{13}C -detected data, however, all residues are fully protonated and thus the lack of peaks for TM2 likely results from conformational dynamics.

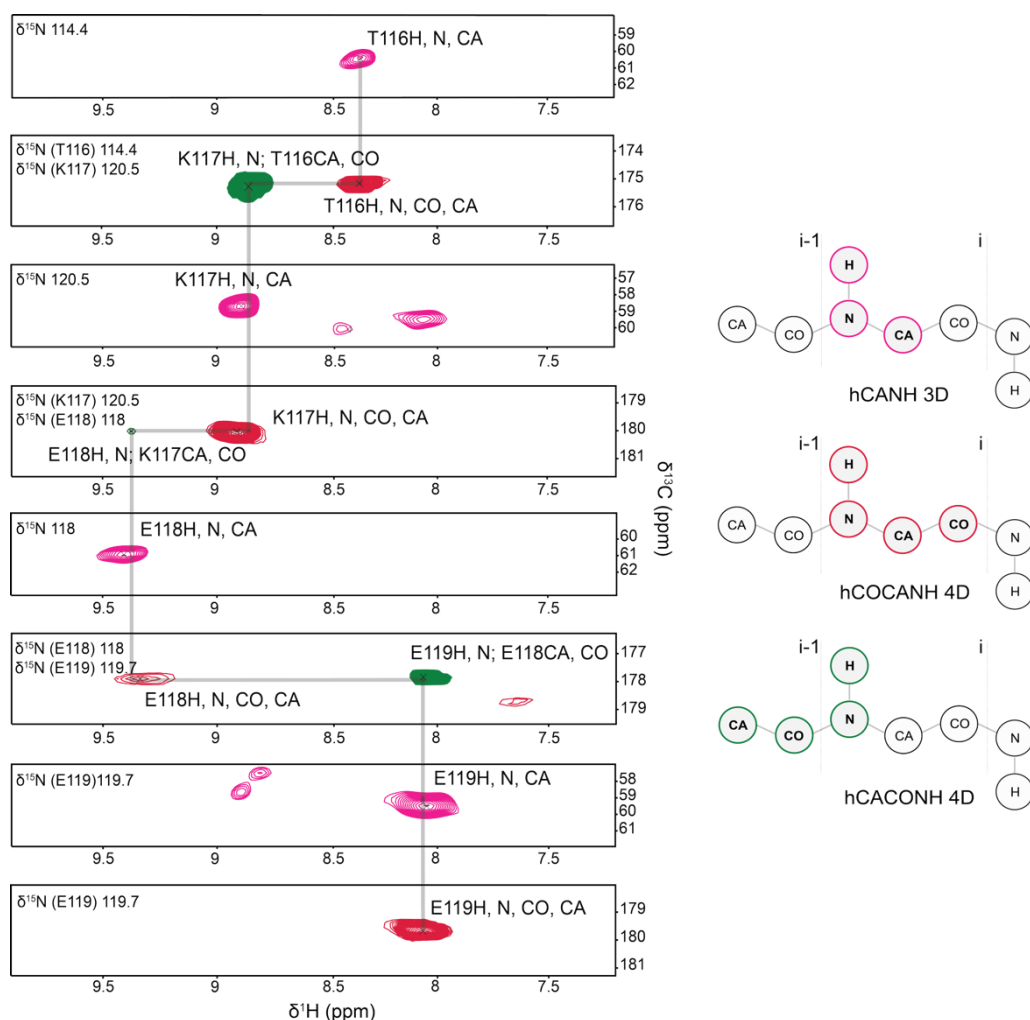


Fig. S9. Sequential residue walk for the amino acid stretch T116 – E119 of *EcMscL* WT by solid-state NMR. Peaks from the 3D hCANH spectrum are shown in pink, from the 4D hCACONH spectrum in green, and from the 4D hCOCANH spectrum in red. Gray lines represent the connections used for assignments.

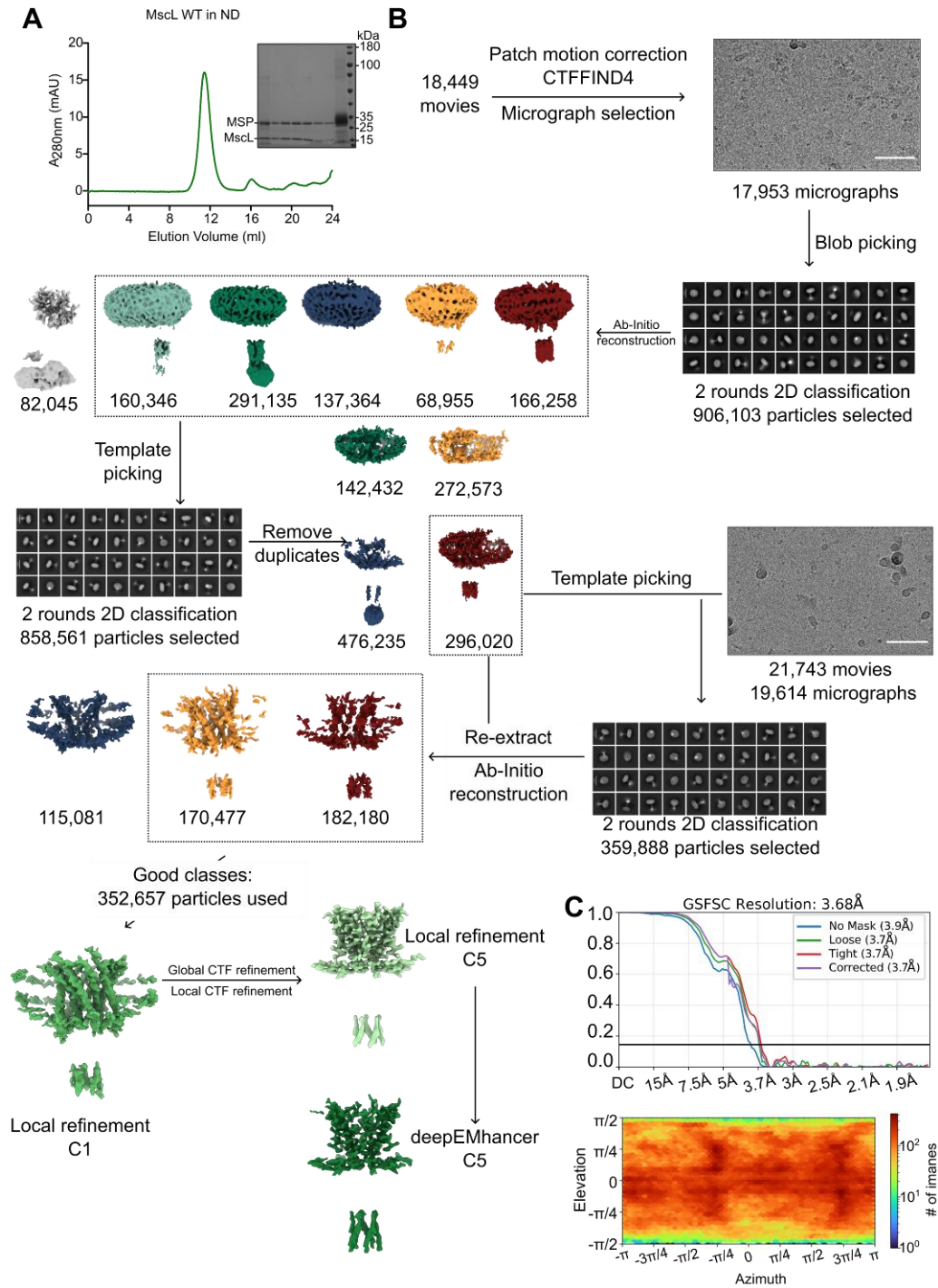


Fig. S10. Sample preparation and cryo-EM data processing of the *Ec*MscL wild type data using cryoSPARC v4.6.2. (A) Size-exclusion chromatography and SDS-PAGE of full-length wild-type *Ec*MscL reconstituted into nanodiscs. (B) Workflow of image processing. Scale bar = 100 Å (C) The FSC curves and particle orientation distribution of the final, refined cryo-EM map of *Ec*MscL at an estimated resolution of 3.7 Å.

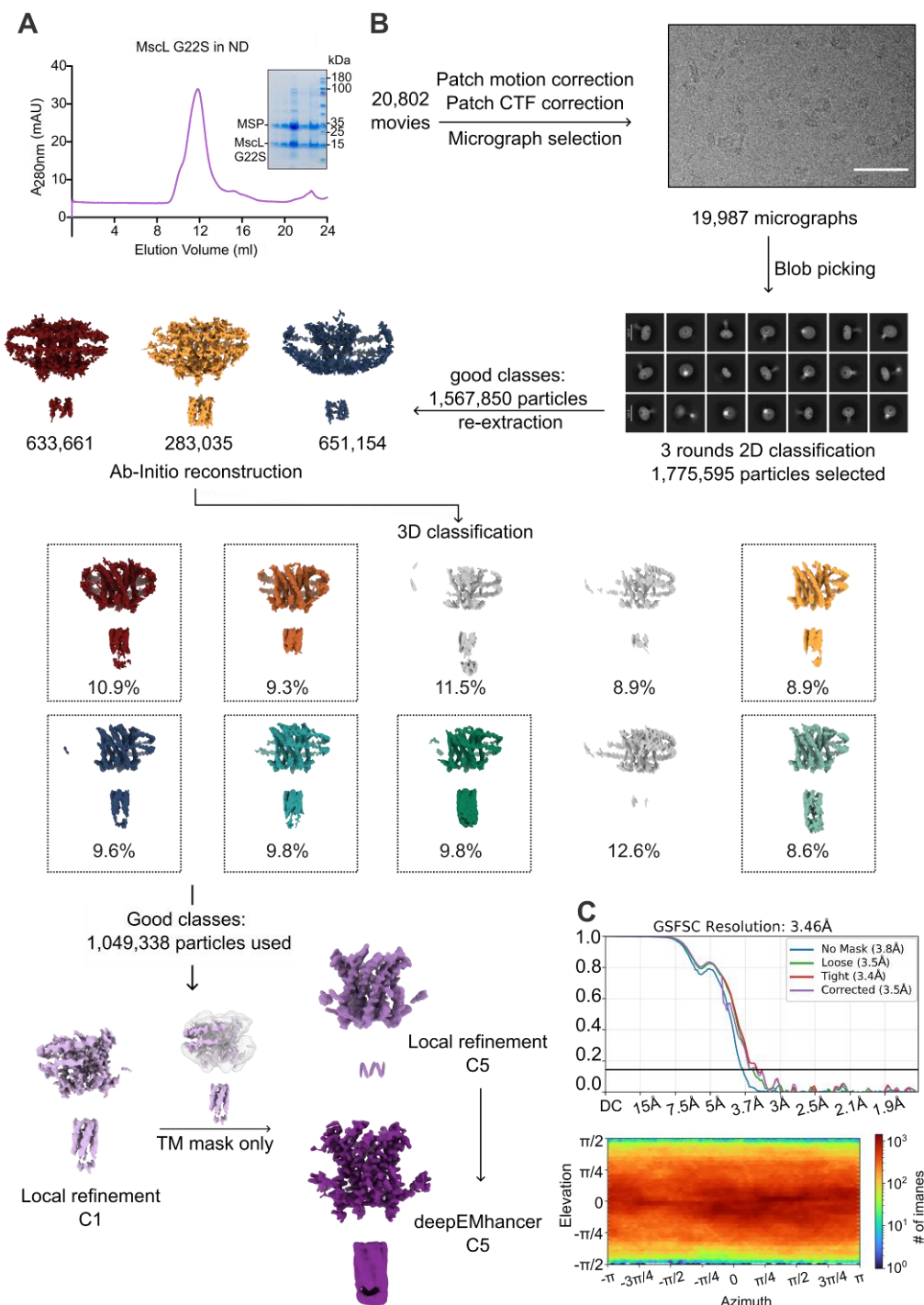


Fig. S11. Sample preparation and data processing of the *EcMscL* G22S mutant data using cryoSPARC v4.6.2. (A) Size-exclusion chromatography and SDS-PAGE of the full-length *EcMscL* G22S mutant reconstituted into nanodiscs. (B) Workflow of image processing. Scale bar = 100 Å (C) The FSC curves and particle orientation distribution of the final, refined cryo-EM map of *EcMscL* G22S at an estimated resolution of 3.5 Å.

Table S1. Assignment table for ^{13}C , ^{15}N -labeled WT *EcMscL*. Chemical shifts were referenced using internal DSS and are deposited in the BMRB (ID: 53470).

Number	Type	CA	CB	CO	N	CD	CD1	CG	CG1	CG2
20	Ala	55.58								
21	Val	67.1983	31.407	178.048	119.633					
22	Gly	47.8436			106.688					
23	Val	66.381								
26	Gly	47.1309								
27	Ala	54.5006								
33	Val	67.0065	31.2697	177.264	119.702					
34	Ser	62.4876	62.45		113.994					
35	Ser	59.5401			113.999					
37	Val	67.0382								
38	Ala	55.4019								
43	Pro	67.0022	29.637			50.2171		27.4136		
44	Pro	65.7797	30.7698		132.51	51.1705		28.402		
45	Leu	58.335								
46	Gly	47.245								
57	Phe	58.1965	38.286	174.238						
58	Ala	51.7073	23.032	175.437	129.468					
59	Val	59.7036	35.6563	173.737	116.608				22.0033	
60	Thr	63.4583	68.2939	173.663	127.08					22.2065
61	Leu	55.3151	42.7415	175.81	126.987		26.2357	27.2037		22.851
62	Arg	56.1113	34.0984	174.404	116.895					
63	Asp	54.6686	41.2893	177.049	126.936	179.526				
64	Ala	53.038	19.3187	177.4	124.049					
65	Gln	54.1328	30.6058		121.31			33.0882		
66	Gly	47.0853		175.185						
67	Asp	54.538	40.7796	175.822	127.202	180.525				
68	Ile	58.609	37.6049	174.452	124.97		12.2597		27.3276	16.8513
69	Pro	62.1598	32.9142	177.291	138.285	51.2099		27.2173		
70	Ala	52.2619	19.5317	177.495	122.695					
71	Val	62.4625	32.526	174.953	123.255				22.3023	20.5107
72	Val	60.6888	35.7539	174.037	129.213				20.9478	
73	Met	54.2939	35.1237		123.974					
74	His	56.0995								
75	Tyr	59.842	38.318	176.79	118.154					
76	Gly	47.686		175.663	111.607					
77	Val	66.6056	31.7693	177.716	122.383				23.074	21.702
78	Phe	61.8022	38.286	176.837	119.275					
79	Ile	65.161	37.1823	177.338	117.577		14.484		30.5938	17.644
80	Gln	59.0604		177.513	119.285					
81	Asn	56.1103	37.503		116.349					
82	Val	67.136								
113	Pro	62.6865		176.373				27.4073		
114	Ala	50.0559	17.888	175.498	126.186					
115	Pro	62.5781	32.1869	177.544	136.232	50.3593		27.4073		
116	Thr	60.8941	70.6838	175.293	114.439					22.255
117	Lys	59.1759	31.6332	180.154	120.576	28.4431		25.0677		
118	Glu	61.4975	28.2815	177.699	118.4			37.355		
119	Glu	60.0016	29.4967	179.788	120.874					
120	Val	67.1171	31.781	179.343	122.431				22.772	21.197
121	Leu	58.2372	43.5005	179.221	121.421					
122	Leu	57.9632	43.528	178.649	117.98					
123	Thr	67.4779	68.5458	174.853	117.008					21.1589
124	Glu	59.9993	29.8734	179.703	121.881	183.235		32.139		
125	Ile	66.2729	38.2351	176.34	120.147		14.484		30.5938	15.7079
126	Arg	60.533	27.555		120.402					
127	Asp	57.8565			119.839					

Table S2. Assignment table for ^2H , ^{13}C , ^{15}N labelled *EcMscL* WT. Chemical shifts were referenced using internal DSS and are deposited in the BMRB (ID: 53468)

Number	Type	H	N	CO	C α
34	SER	8.29	113.82	177.09	62.06
35	SER	7.59	114.96		59.33
59	VAL	8.32	117.01	175.25	59.37
60	THR	9.39	126.66	173.96	63.18
61	LEU	9.11	127.05	173.59	54.97
62	ARG	7.54	117.16	176.32	55.59
63	ASP	8.85	126.30	174.46	54.26
64	ALA	8.84	123.54	176.78	52.77
65	GLN	8.32	121.27	177.38	53.90
66	GLY	9.05	116.40	175.96	46.99
67	ASP	8.98	126.96	175.19	54.26
68	ILE	8.28	124.77	175.79	58.07
70	ALA	8.72	121.70	177.01	51.99
71	VAL	8.89	122.39	177.94	62.13
72	VAL	8.88	128.78	174.77	60.32
73	MET	9.11	123.81	173.85	53.85
74	HIS	8.22	117.89	176.29	57.20
75	TYR	7.87	118.20		59.47
76	GLY	7.80	111.33		47.24
77	VAL	7.51	121.99	175.41	65.97
79	ILE	8.40	117.84	177.09	64.75
80	GLN	8.16	119.37	178.59	58.88
81	ASN	7.96	116.34	177.31	56.19
113	PRO			176.36	
114	ALA	9.09	126.132	174.36	50.52
115	PRO			177.70	62.79
116	THR	8.35	114.27	177.72	60.40
117	LYS	8.92	120.36	175.22	58.69
118	GLU	9.44	118.10	180.05	60.93
119	GLU	8.09	119.87	177.76	59.50
120	VAL	8.36	122.35	179.82	66.60
121	LEU	8.02	121.19	179.34	57.92
122	LEU	8.83	117.71	179.35	57.53
123	THR	8.17	116.77	178.70	67.09
124	GLU	7.87	121.29	174.91	59.59
126	ARG	8.44	120.22	178.71	60.10
127	ASP	8.84	119.83	178.97	57.50
128	LEU	8.76	121.34	178.98	59.27

Table S3. Assignment table for ^2H , ^{13}C , ^{15}N labelled *EcMscL* G22S. Chemical shifts were referenced using internal DSS and are deposited in the BMRB (ID: 53469)

Number	Type	H	N	CO	C α	C β
33	VAL	8.31	119.05	177.05	66.94	
34	SER	8.22	113.82		61.76	
58	ALA	8.51	130.11		51.58	
62	ARG	8.38	117.40	177.25	55.70	
63	ASP	9.14	125.78	174.35	53.55	
64	ALA	8.92	122.00		54.17	
73	MET	9.15	124.51		54.01	
77	VAL	8.15	121.61	179.31	66.16	
78	PHE	8.27	119.02	178.88	61.24	42.31
79	ILE	8.41	118.03	177.46	64.074	
80	GLN	8.08	119.22	178.47	59.29	27.66
81	ASN	8.55	116.45		56.57	
113	PRO			176.36		
114	ALA	8.48	125.66		49.97	25.30
116	THR	8.41	113.85	177.63	60.40	
117	LYS	8.94	120.79	175.17	58.82	
118	GLU	9.18	117.63	179.67	60.57	
119	GLU	8.18	120.10		59.16	
120	VAL	8.44	122.66	179.74	66.61	
121	LEU	8.14	121.04	179.34	57.71	42.31
122	LEU	8.74	117.52	179.46	57.41	42.32
123	THR	8.17	117.12	178.56	67.12	
124	GLU	7.89	121.26	175.02	59.42	
125	ILE	8.57	119.85	179.59	65.80	
126	ARG	8.46	120.14		60.02	
127	ASP	8.82	119.93	178.89	57.47	38.73
128	LEU	8.73	121.21	179.06	59.49	

Table S4. Statistics for data collection and refinement. Statistics for 3D reconstruction (cryoSPARC 4.6.2), model refinement (Coot 0.9.6.2 and PHENIX 1.21.2), and validation (PHENIX 1.21.2) for the cryo-EM structural refinement of *EcMscL* wild type (WT) and G22S mutant.

	<i>EcMscL</i> WT	<i>EcMscL</i> G22S
	PDB ID: 9TIU	PDB ID: 9TIV
Data collection		
Microscope	Titan Krios G3i	Titan Krios G3i
Detector	Gatan K3	Gatan K3
Energy filter slit width (eV)	20	20
Magnification	105,000	105,000
Voltage (kV)	300	300
Electron exposure (e ⁻ /Å ²)	60	60
Defocus range (μm)	-2.8~-1	-2.4~-0.8
Pixel size (Å)	0.83	0.83
Symmetry imposed	C5	C5
Number of micrographs	37,567	19,987
Initial particle images (no.)	22,160,563	9,266,266
Final particle images (no.)	352,657	1,049,338
Map resolution (Å)		
FSC 0.5(unmasked/masked)	6.5/4.1	4.1/4.1
FSC 0.143(unmasked/masked)	3.7/3.6	3.5/3.4
B-factor (Å ²)	-116.2	-182.0
Model refinement		
Number of atoms		
All (hydrogens)	8355(4175)	9190(4595)
Protein residues	575	630
Ligand (sugars)	0	0
Model validation		
CC (mask) map versus model (%)	0.73	0.77
CC (box) map versus model (%)	0.62	0.65
RMSD		
Bond lengths (Å)	0.003	0.002
Bond angles (°)	0.598	0.568
Ramachandran statistics		
Favored regions (%)	98.35	98.03
Allowed regions (%)	1.65	1.97
Outliers (%)	0	0
Rotamer outliers (%)	6.41	1.38
Cβ outliers (%)	0.93	NA
CaBLAM outliers (%)	0.00	1.69
Clashscore	10.31	8.60
MolProbity overall score	2.15	1.57

Table S5. Parameters for all magnetization transfer steps. Sensitivity-enhanced 3D and 4D experiments used for assignments of *EcMscL* WT. The nutation frequency is given for the maximum amplitude of each pulse. Experiments were conducted at 900 MHz.

Experiment	hCANH	hCONH	hCACONH	hCOCANH
¹H-¹³C				
¹ H shape	ramp 90-100	ramp 90-100	ramp 90-100	ramp 90-100
¹ H power	82 kHz (8.2 W)	83 kHz (8.3 W)	82 kHz (8.2 W)	83 kHz (8.3W)
¹³ C shape	rectangular	rectangular	rectangular	rectangular
¹³ C power	20 kHz (1.8 W)	20 kHz (1.8 W)	20 kHz (1.8 W)	20 kHz (1.8 W)
duration (ms)	2.7	2.8	2.8	2.8
¹³C-¹³C (backbone)				
¹³ C shape	-	-	homo TROP	homo TROP
¹³ C power	-	-	68 kHz (21 W)	73 kHz (24 W)
duration (ms)	-	-	1.65	1.65
¹³C-¹⁵N				
¹³ C shape	TROP	TROP	TROP	TROP
¹³ C power	69 kHz (22 W)	69 kHz (22 W)	69 kHz (22 W)	69 kHz (22 W)
¹⁵ N shape	TROP	TROP	TROP	TROP
¹⁵ N power	46 kHz (50 W)	46 kHz (50 W)	46 kHz (50 W)	46 kHz (50 W)
duration (ms)	3.33	3.33	3.33	3.33
¹⁵N-¹H				
¹⁵ N shape	rectangular	rectangular	TROP	TROP
¹⁵ N power	14 kHz (4.5 W)	14 kHz (4.5 W)	52.6 kHz (65 W)	52.6 kHz (65 W)
¹ H shape	ramp 80-100	ramp 80-100	TROP	TROP
¹ H power	75 kHz (8 W)	75 kHz (8 W)	52 kHz (3.9 W)	52 kHz (3.9 W)
duration (ms)	0.7	0.7	0.7	0.7

Table S6. Parameters for all magnetization transfer steps. Sensitivity-enhanced 3D and 4D experiments used for assignments of *EcMscL* G22S. The nutation frequency is given for the maximum amplitude of each pulse. Experiments were conducted at 600 MHz, unless stated otherwise.

Experiment	hCANH	hCONH	hCAcoNH	hCOcaNH	hCXCANH (900 MHz)
^1H-^{13}C					
^1H shape	ramp 90-100	ramp 90-100	ramp 90-100	ramp 90-100	ramp 90-100
^1H power	79 kHz (3.6 W)	79 kHz (3.6 W)	80 kHz (3.7 W)	81 kHz (3.8 W)	80 kHz (9.2 W)
^{13}C shape	rectangular	rectangular	rectangular	rectangular	rectangular
^{13}C power	20 kHz (1.8 W)	20 kHz (1.8 W)	20 kHz (1.8 W)	20 kHz (1.8 W)	20 kHz (1.8 W)
duration (ms)	1.8	1.8	1.8	1.8	6
^{13}C-^{13}C (side-chains)					
^{13}C shape					DIPSI 3
^{13}C power					15.62 kHz (1.12 W)
duration (ms)					0.016
^{13}C-^{13}C (backbone)					
^{13}C shape	-	-	homo TROP	homo TROP	
^{13}C power	-	-	73 kHz (24 W)	73 kHz (24 W)	
duration (ms)	-	-	1.8	1.8	
^{13}C-^{15}N					
^{13}C shape	TROP	TROP	TROP	TROP	TROP
^{13}C power	67 kHz (20.2 W)	66 kHz (19.5 W)	66 kHz (19.5 W)	67 kHz (20.2 W)	70 kHz (22 W)
^{15}N shape	TROP	TROP	TROP	TROP	TROP
^{15}N power	42 kHz (21.3 W)	42 kHz (21.3 W)	42 kHz (21.3 W)	42 kHz (21.3 W)	46 kHz (50 W)
duration (ms)	3.64	3.64	3.64	3.64	3.33
^{15}N-^1H					
^{15}N shape	TROP	TROP	TROP	TROP	TROP
^{15}N power	55 kHz (36 W)	55 kHz (36 W)	55 kHz (36 W)	55 kHz (36 W)	52.6 kHz (65 W)
^1H shape	TROP	TROP	TROP	TROP	TROP
^1H power	61 kHz (2.1 W)	61 kHz (2.1 W)	61 kHz (2.1 W)	61 kHz (2.1 W)	52 kHz (3.9 W)
duration (ms)	0.8	0.8	0.8	0.8	0.733

Table S7. Acquisition parameters. Sensitivity-enhanced 3D and 4D experiments used for the assignments of *EcMscL* WT. SW = spectral width, NUS = Non-uniform sampling.

Experiment	hCANH	hCONH	hCACONH	hCOCANH
Scans	56	80	80	112
¹ H acquisition time (ms)	30.7	30.7	24	24
¹ H points	3072	3072	2400	2400
¹ H SW (ppm)	55.5	55.5	55.5	55.5
¹³ CA acquisition time (ms)	4.2	-	4.6	4.6
¹³ CA points	54	-	48	48
¹³ CA SW (ppm)	28	-	23	23
¹³ CO acquisition time (ms)	-	5.6	6.6	6.6
¹³ CO points	-	36	36	36
¹³ CO SW (ppm)	-	14	12	12
¹⁵ N acquisition time (ms)	11.3	7.8	9.8	9.8
¹⁵ N points	58	40	36	36
¹⁵ N SW (ppm)	28	28	20	20
NUS points	-	-	387	386
NUS %	-	-	5	5
total points	3132	1440	3096/62208	3088/62208
Experimental time (h)	65 h	43 h	140 h	196 h

Table S8. Acquisition parameters. Sensitivity-enhanced 3D and 4D experiments used for the assignments of *EcMscL* G22S. SW = spectral width, NUS = Non-uniform sampling.

Experiment	hCANH	hCONH	hCAcoNH	hCOcaNH	hCXCANH (900 MHz)
Scans	152	128	192	304	120
¹ H acquisition time (ms)	30	30	30	30	18.8
¹ H points	1438	1438	1438	1438	1344
¹ H SW (ppm)	40	40	40	40	39.7
¹³ CA acquisition time (ms)	5.3	-	4.6	-	4
¹³ CA points	48	-	42	-	40
¹³ C SW (ppm)	30	-	30	-	22
¹³ CO acquisition time (ms)	-	8.8	-	8.8	-
¹³ CO points	-	32	-	32	-
¹³ CO SW (ppm)	-	12	-	12	-
¹³ CX acquisition time (ms)	-	-	-	-	3.9
¹³ CX points	-	-	-	-	116
¹³ CX SW (ppm)	-	-	-	-	65
¹⁵ N acquisition time (ms)	14.6	12.7	12.7	12.7	9.2
¹⁵ N points	48	42	42	42	32
¹⁵ N SW (ppm)	27	27	27	27	19
NUS points	-	-	-	-	739
NUS %	-	-	-	-	4
total points	2304	1344	1764	1344	5 912/148480
Experimental time (h)	136 h	66	153	153	265 h

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