A "Build and Retrieve" methodology to simultaneously solve cryo-EM structures of a variety of membrane proteins

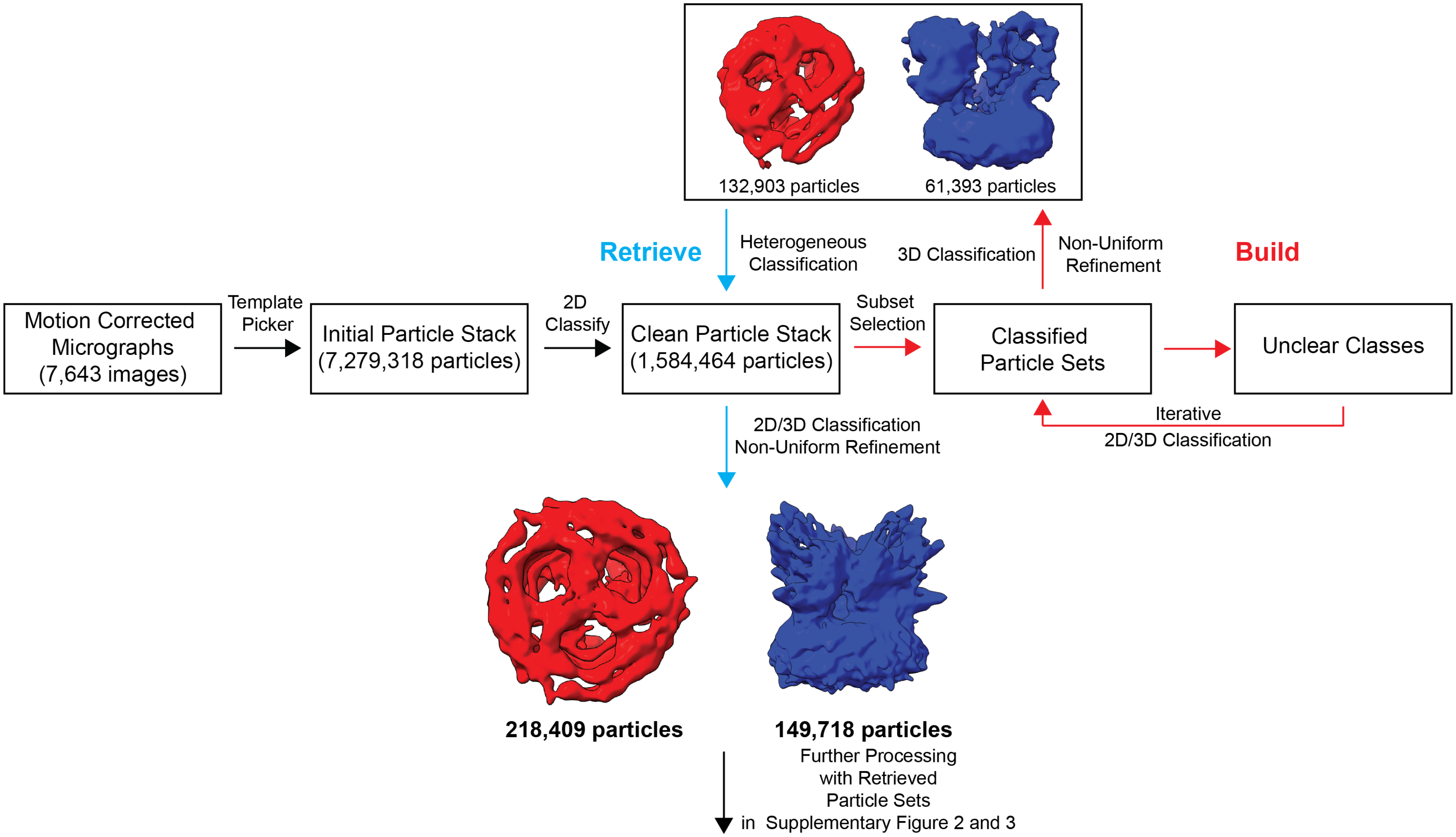
Chih-Chia Su[1,Ψ], Meinan Lyu[1,Ψ], Christopher E. Morgan[1,Ψ], Jani Reddy Bolla[2], Carol V. Robinson[2] and Edward W. Yu[1,*]

[1]Department of Pharmacology, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA.

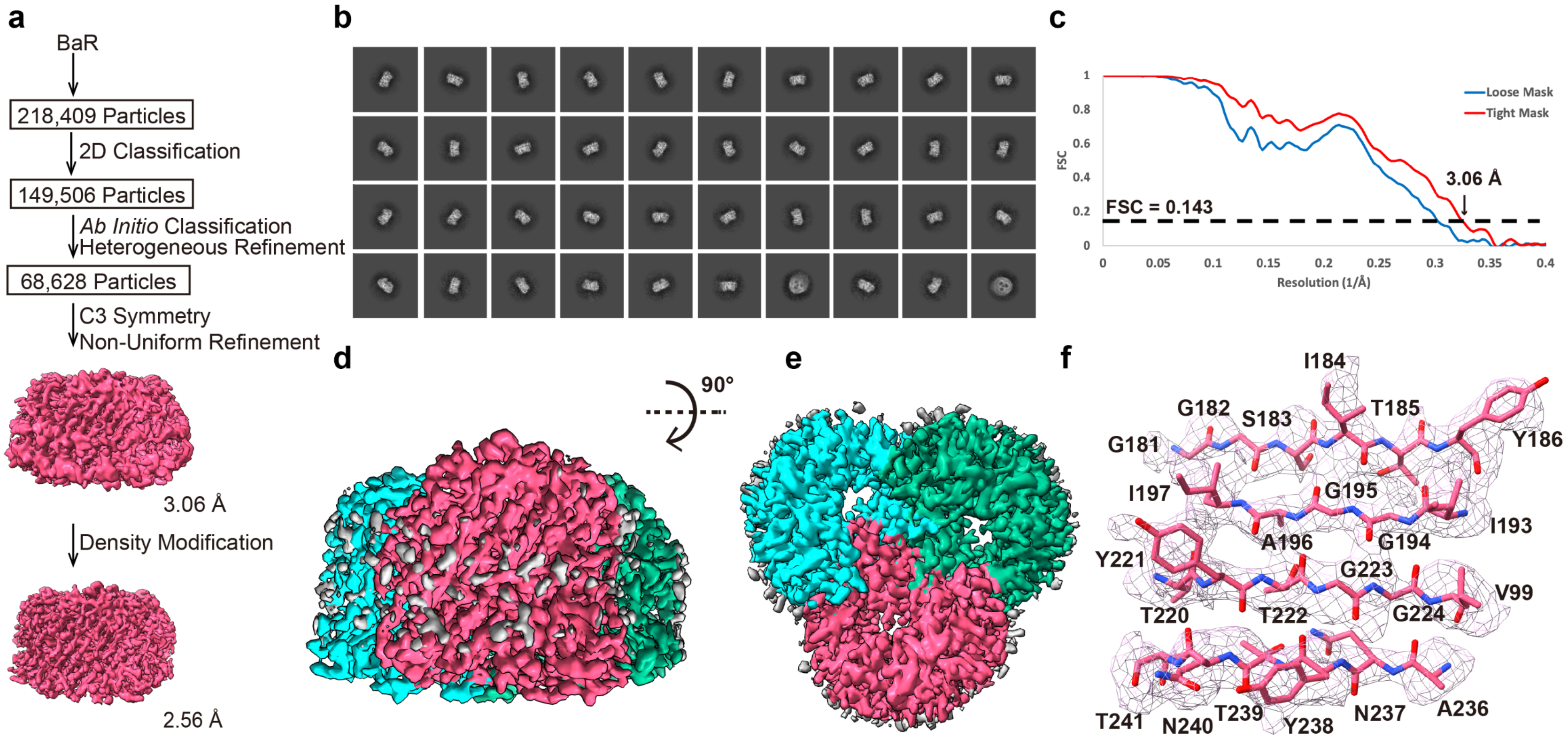
[2]Department of Chemistry, University of Oxford, South Parks Road, Oxford, OX1 3QZ, UK.

[Ψ]C.S., M.L. and C.E.M. contributed equally to this work.

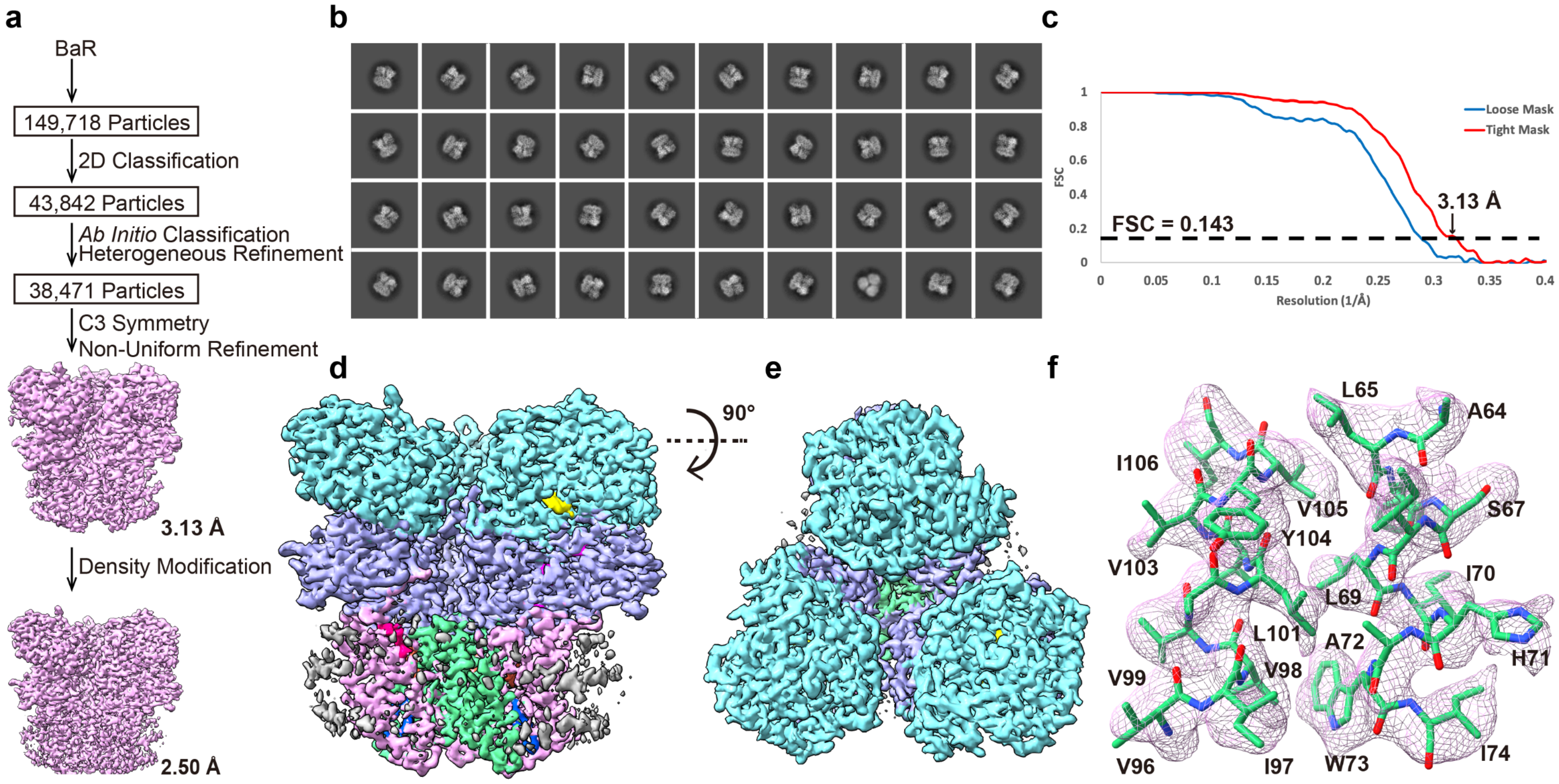
[*]To whom correspondence should be addressed. Email: edward.w.yu@case.edu



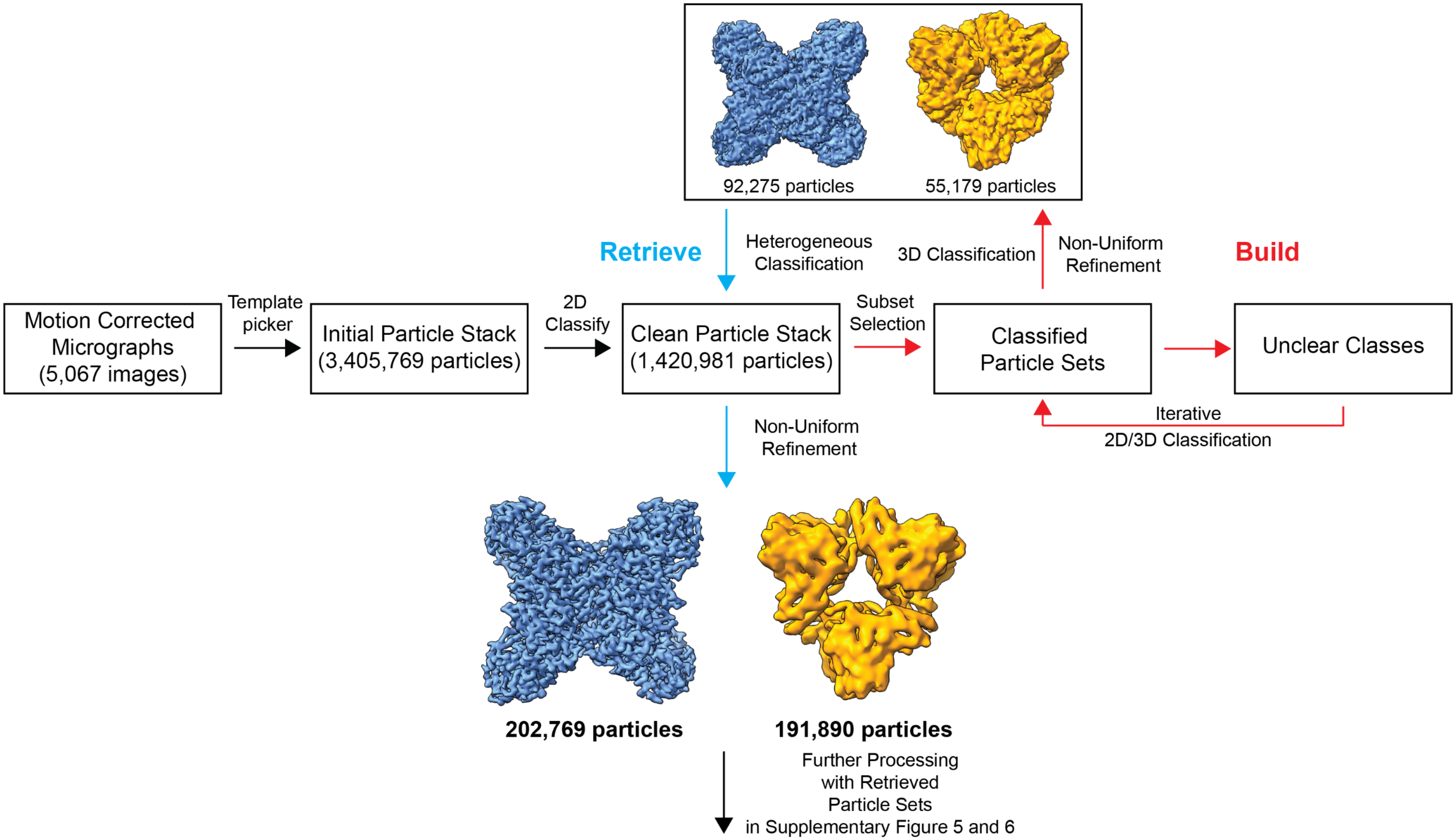
Supplementary Figure 1. Flowchart of the “Build and Retrieve” (BaR) iterative method.



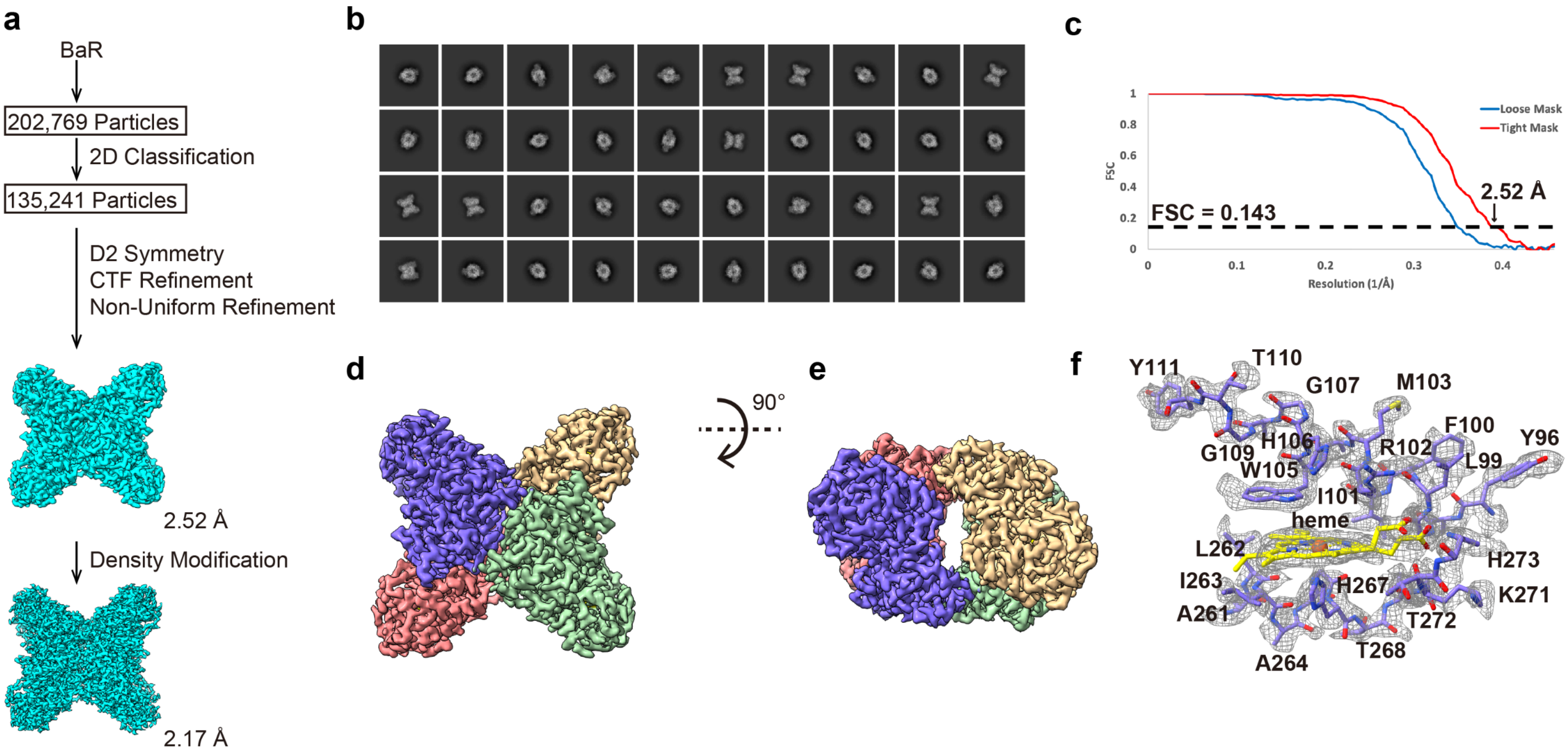
Supplementary Figure 2. Cryo-EM analysis of the *E. coli* OmpC porin channel. (a) BaR processing flowchart. (b) Representative 2D classes. (c) Fourier Shell Correlation (FSC) curves. (d) Sharpened cryo-EM map of the OmpC porin viewed in the membrane plane and from the periplasmic side. (e) Sharpened cryo-EM map of the OmpC viewed from the exterior side. (f) Local EM density map of OmpC.



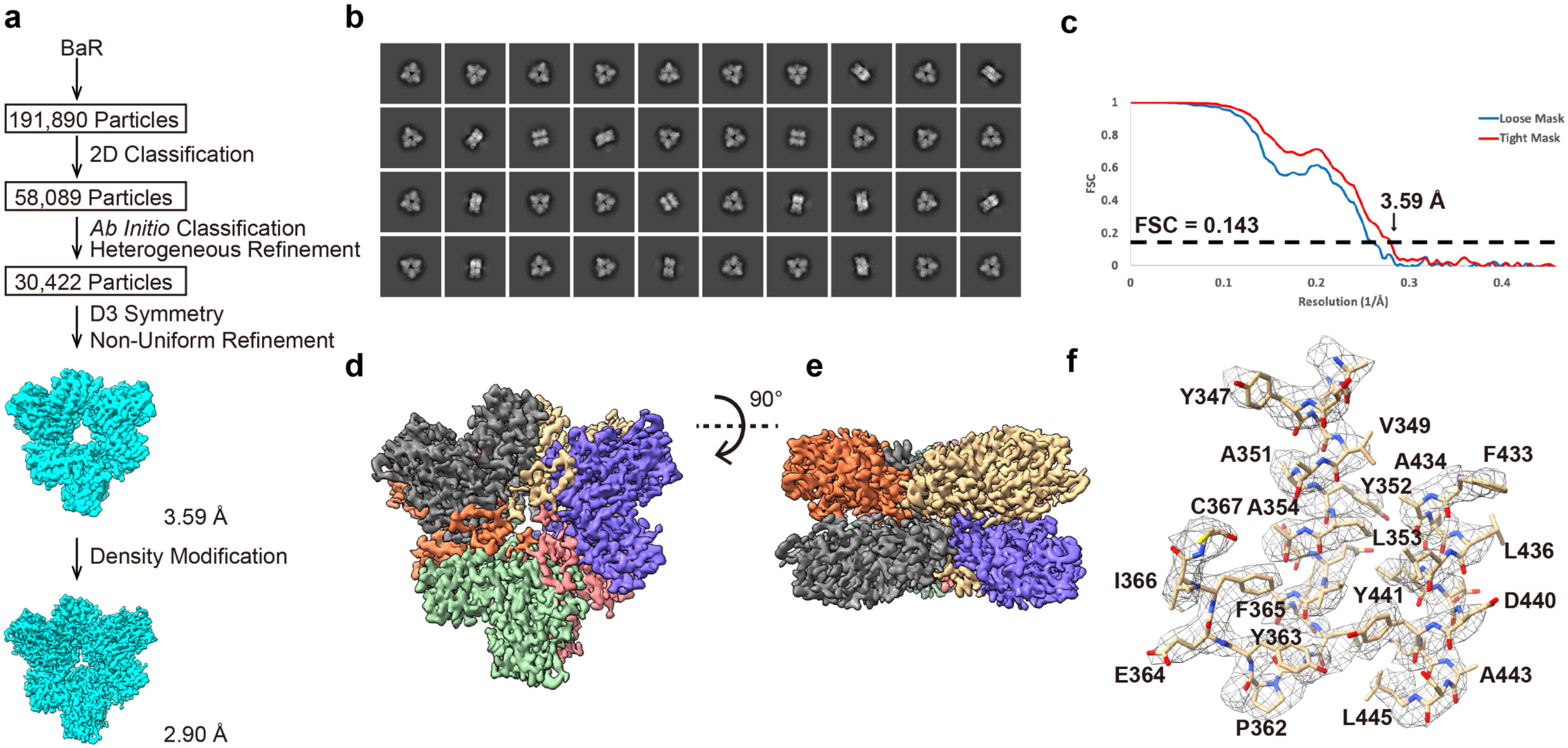
Supplementary Figure 3. Cryo-EM analysis of the *E. coli* SQR complex. (a) BaR processing flowchart. (b) Representative 2D classes. (c) Fourier Shell Correlation (FSC) curves. (d) Sharpened cryo-EM map of the SQR complex viewed in the membrane plane. (e) Sharpened cryo-EM map of the SQR complex viewed from the cytoplasmic side. (f) Local EM density map of SQR.



Supplementary Figure 4. Flowchart of the "Build and Retrieve" (BaR) iterative method.



Supplementary Figure 5. Cryo-EM analysis of the *E. coli* KatG complex. (a) BaR processing flowchart. (b) Representative 2D classes. (c) Fourier Shell Correlation (FSC) curves. (d) Sharpened cryo-EM map of the KatG complex. (e) Sharpened cryo-EM map of the KatG complex rotated 90° compared with the orientation of (d). (f) Local EM density map of KatG.



Supplementary Figure 6. Cryo-EM analysis of the *E. coli* GadB complex. (a) BaR processing flowchart. (b) Representative 2D classes. (c) Fourier Shell Correlation (FSC) curves. (d) Sharpened cryo-EM map of the GadB complex. (e) Sharpened cryo-EM map of the GadB complex rotated 90° compared with the orientation of (d) . (f) Local EM density map of GadB.

Supplementary Table 1. Cryo-EM data collection, processing, and refinement statistics.

Data set	Proteins from the BpHpnN sample reconstituted in nanodiscs (1E3D1)			
	Cytochrome bo_3	**BpHpnN**	**OmpF**	**SQR**
Data collection and processing				
Magnification	81,000			
Voltage (kV)	300			
Electron Microscope	Krios-GIF-K3			
Defocus range (μm)	-1.0 to -2.5			
Total exposure time (s)	3.2			
Energy filter width (eV)	20			
Pixel size (Å)	1.08			
Total dose (e^-/Å^2)	50			
Number of frames	40			
Does rate (e^-/phys. Pixel/s)	18.2			
No. of initial micrographs	13,093			
No. of initial particles	9,955,108			
No. of final particles	334,222	63,910	43,793	12,706
Symmetry	C1	C1	C3	C3
GSFSC Resolution (Å)	2.38	4.04	3.15	4.06
FSC threshold (0.143)				
Density modification resolution[41]	2.20	3.59	2.54	3.60
Refinement				
Model resolution cut-off (Å)	2.20	3.59	2.54	3.60
Model composition				
No. of Protein residues	1,207	854	1,020	2,784
No. ligands	14	0	0	18
RMSD[a]				
Bond lengths (Å)	0.009	0.003	0.004	0.006
Bond angles (°)	1.611	0.634	0.834	0.836
Validation				
MolProbity score	2.54	1.94	2.96	2.48
Ramachandran plot (%)				
Favored (%)	95.50	94.00	93.49	86.52
Allowed (%)	4.50	6.00	6.51	13.48
Disallowed (%)	0	0	0	0
CC[b] Mask	0.74	0.64	0.70	0.63

[a] root mean square deviation

[b] correlation coefficient

Supplementary Table 2. Cryo-EM data collection, processing, and refinement statistics.

Data set	*E. coli* K12 membrane proteins reconstituted in nanodiscs (1E3D1)	
	OmpC	**SQR**
Data collection and processing		
Magnification	81,000	
Voltage (kV)	300	
Electron Microscope	Krios-GIF-K3	
Defocus range (µm)	-1.0 to -2.5	
Total exposure time (s)	4.2	
Energy filter width (eV)	None	
Pixel size (Å)	1.08	
Total dose (e^-/Å^2)	40	
Number of frames	42	
Does rate (e^-/phys. Pixel/s)	11.97	
No. of initial micrographs	7,643	
No. of initial particles	7,279,318	
No. of final particles	68,628	38,471
Symmetry	C3	C3
GSFSC Resolution (Å)	3.06	3.13
FSC threshold (0.143)		
Density modification resolution[41]	2.56	2.50
Refinement		
Model resolution cut-off (Å)	2.56	2.50
Model composition		
No. of Protein residues	1,038	2,871
No. ligands	0	18
RMSD[a]		
Bond lengths (Å)	0.002	0.002
Bond angles (°)	0.570	0.427
Validation		
MolProbity score	1.89	1.75
Ramachandran plot (%)		
Favored (%)	94.38	96.44
Allowed (%)	5.62	3.56
Disallowed (%)	0	0
CC[b] Mask	0.64	0.73

[a] root mean square deviation
[b] correlation coefficient

Supplementary Table 3. Proteomic analysis of the *E. coli* K12 cell membrane.
*please note that OmpC was not present in the top 25 list

Uniprot ID|Fasta header	Peptides	Sequence coverage [%]	Mol. weight [kDa]	Score	Intensity	iBAQ	MS/MS count
P0ADB7|ECNB_ECOLI Entericidin B	1	39.6	4.8095	296.09	8.23E+09	4.11E+09	5
P0ADB1|OSME_ECOLI Osmotically-inducible putative lipoprotein OsmE	4	27.7	12.021	114.43	5.6E+09	1.12E+09	15
P0ABA0|ATPF_ECOLI ATP synthase subunit b	13	49.4	17.264	272.28	9.74E+09	9.74E+08	49
P0ABJ9|CYDA_ECOLI Cytochrome bd-I ubiquinol oxidase subunit 1	20	22.6	58.204	323.31	1.38E+10	8.61E+08	48
P00363|FRDA_ECOLI Fumarate reductase flavoprotein subunit	27	38.7	65.971	323.31	2.82E+10	8.53E+08	62
P0ABB4|ATPB_ECOLI ATP synthase subunit beta	35	80	50.325	323.31	1.84E+10	7.67E+08	94
P0ABB0|ATPA_ECOLI ATP synthase subunit alpha	43	65.3	55.221	323.31	2.06E+10	7.37E+08	91
P65292|YGDI_ECOLI Uncharacterized lipoprotein YgdI	2	36	8.1741	71.771	2.86E+09	7.14E+08	7
P0ADZ7|YAJC_ECOLI Sec translocon accessory complex subunit YajC	4	37.3	11.887	38.796	2.85E+09	7.12E+08	8
P64581|YQJD_ECOLI Uncharacterized protein YqjD	5	65.3	11.051	174.4	2.61E+09	6.52E+08	19
P0AC47|FRDB_ECOLI Fumarate reductase iron-sulfur subunit	7	39.3	27.123	235.32	6.35E+09	6.35E+08	13
P36672|PTTBC_ECOLI PTS system trehalose-specific EIIBC component	9	14.2	51.08	46.31	5.95E+09	5.95E+08	14
P0A6E6|ATPE_ECOLI ATP synthase epsilon chain	4	23.7	15.068	250.24	1.87E+09	4.68E+08	15
P11349|NARH_ECOLI Respiratory nitrate reductase 1 beta chain	32	53.7	58.066	323.31	1.22E+10	4.54E+08	70
P07014|SDHB_ECOLI Succinate dehydrogenase (SQR) iron-sulfur subunit	6	20.6	26.77	39.201	6.92E+09	4.33E+08	14
P0ADW3|YHCB_ECOLI Inner membrane protein YhcB	13	72	14.961	323.31	3.76E+09	4.17E+08	31
P63235|GADC_ECOLI Probable glutamate/gamma-aminobutyrate antiporter	3	6.5	55.076	211.68	4.43E+09	4.03E+08	6
P0AFC3|NUOA_ECOLI NADH-quinone oxidoreductase subunit A	2	17.7	16.457	24.272	1.9E+09	3.8E+08	4
P0A8Q3|FRDD_ECOLI Fumarate reductase subunit D	1	5.9	13.107	3.4712	2.17E+09	3.62E+08	7
P37636|MDTE_ECOLI Multidrug resistance protein MdtE	14	50.1	41.19	249.02	7.96E+09	3.62E+08	24
P0AG99|SECG_ECOLI Protein-export membrane protein SecG	2	27.3	11.365	24.703	2.13E+09	3.55E+08	4
P0ADN6|YIFL_ECOLI Uncharacterized lipoprotein YifL	2	34.3	7.1771	49.849	1.57E+09	3.15E+08	8
P0AEH5|ELAB_ECOLI Protein ElaB	5	68.3	11.306	7.2983	1.84E+09	3.07E+08	11
P09152|NARG_ECOLI Respiratory nitrate reductase 1 alpha chain	84	64.2	140.49	323.31	1.95E+10	3E+08	182
P0ABA6|ATPG_ECOLI ATP synthase gamma chain	27	67.9	31.577	323.31	4.4E+09	2.59E+08	62
*P06996|OMPC_ECOLI Outer membrane porin C (OmpC)	1	3		0.003663	1.1014	4732300	1

Supplementary Table 4. Cryo-EM data collection, processing, and refinement statistics.

Data set	***E. coli* K12 cell lysate proteins**	
	KatG	**GadB**
Data collection and processing		
Magnification	81,000	
Voltage (kV)	300	
Electron Microscope	Krios-GIF-K3	
Defocus range (µm)	-1.0 to -2.5	
Total exposure time (s)	4.2	
Pixel size (Å)	1.08	
Total dose (e^-/Å^2)	40	
Number of frames	42	
Does rate (e^-/phys. Pixel/s)	11.97	
No. of initial micrographs	5,067	
No. of initial particles	3,405,769	
No. of final particles	135,241	30,422
Symmetry	D2	D3
GSFSC Resolution (Å)	2.52	3.59
FSC threshold (0.143)		
Density modification resolution[41]	2.17	2.90
Refinement	2.17	2.90
Model resolution cut-off (Å)		
Model composition		
No. of Protein residues	2,648	2,562
No. ligands	4	0
RMSD[a]		
Bond lengths (Å)	0.002	0.006
Bond angles (°)	0.651	0.970
Validation		
MolProbity score	2.03	3.13
Ramachandran plot (%)		
Favored (%)	95.17	91.45
Allowed (%)	4.83	8.55
Disallowed (%)	0	0
CC[b] Mask	0.65	0.73

[a] root mean square deviation

[b] correlation coefficient

Supplementary Table 5. Proteomic analysis of the *E. coli* K12 cell lysate.

Uniprot ID|Fasta header	Peptides	Sequence coverage [%]	Mol. weight [kDa]	Score	Intensity	iBAQ	MS/MS count
P69910|DCEB_ECOLI Glutamate decarboxylase beta (GadB)	32	67.4	52.668	323.31	5.74E+10	2.39E+09	186
P0ACF0|DBHA_ECOLI DNA-binding protein HU-alpha	5	61.1	9.5349	162.75	7.66E+09	1.91E+09	10
P0A991|ALF1_ECOLI Fructose-bisphosphate aldolase class 1	38	75.7	38.109	323.31	3.15E+10	1.85E+09	108
P0A6Y8|DNAK_ECOLI Chaperone protein DnaK	57	81.5	69.114	323.31	6.14E+10	1.75E+09	171
P0ABH7|CISY_ECOLI Citrate synthase	25	56.9	48.014	323.31	3.49E+10	1.66E+09	60
P13029|KATG_ECOLI Catalase-peroxidase (KatG)	49	68.5	80.023	323.31	4.53E+10	1.22E+09	130
P09373|PFLB_ECOLI Formate acetyltransferase 1	60	66.3	85.356	323.31	4.01E+10	9.54E+08	144
P0A6F5|CH60_ECOLI 60 kDa chaperonin	62	74.3	57.328	323.31	1.75E+10	5.66E+08	118
P0ADG7|IMDH_ECOLI Inosine-5-monophosphate dehydrogenase	25	58.4	52.022	323.31	9.78E+09	5.15E+08	58
P00957|SYA_ECOLI Alanine--tRNA ligase	58	64.5	96.031	323.31	2.29E+10	4.4E+08	125
P0A9Y6|CSPC_ECOLI Cold shock-like protein CspC	4	78.3	7.4023	136.17	1.74E+09	4.35E+08	8
P0AD61|KPYK1_ECOLI Pyruvate kinase I	26	51.5	50.729	323.31	1.16E+10	4.31E+08	55
P08312|SYFA_ECOLI Phenylalanine--tRNA ligase alpha subunit	24	78.9	36.831	323.31	8.24E+09	4.12E+08	60
P0A6Z3|HTPG_ECOLI Chaperone protein HtpG	42	65.4	71.422	323.31	1.37E+10	3.5E+08	73
P68066|GRCA_ECOLI Autonomous glycyl radical cofactor	14	70.9	14.284	178.54	3.19E+09	3.19E+08	20
P07395|SYFB_ECOLI Phenylalanine--tRNA ligase beta subunit	32	44.3	87.377	323.31	1.02E+10	2.69E+08	61
P0AEJ6|EUTB_ECOLI Ethanolamine ammonia-lyase heavy chain	6	12.4	49.403	49.133	5.48E+09	2.61E+08	6
P69908|DCEA_ECOLI Glutamate decarboxylase alpha	31	67.2	52.685	42.178	6.2E+09	2.58E+08	7
P0A9G6|ACEA_ECOLI Isocitrate lyase	18	52.8	47.521	323.31	5.34E+09	2.43E+08	36
P31057|PANB_ECOLI 3-methyl-2-oxobutanoate hydroxymethyltransferase	8	41.3	28.237	132.91	1.66E+09	2.38E+08	11
P76143|LSRF_ECOLI 3-hydroxy-5-phosphonooxypentane-2,4-dione thiolase	15	50.2	31.892	306.48	3.72E+09	2.19E+08	31
P0ABB4|ATPB_ECOLI ATP synthase subunit beta	22	68.9	50.325	323.31	5.21E+09	2.17E+08	42
P0AC38|ASPA_ECOLI Aspartate ammonia-lyase	16	39.7	52.356	323.31	4.87E+09	2.12E+08	35
P0AD33|YFCZ_ECOLI UPF0381 protein YfcZ	2	27.7	10.318	26.966	9.93E+08	1.99E+08	3
P0A9P0|DLDH_ECOLI Dihydrolipoyl dehydrogenase	23	50	50.688	323.31	4.18E+09	1.9E+08	36

References

41. Terwilliger, T. C., Ludtke, S. J., Read, R. J., Adams, P. D. & Afonine, P. V. Improvement of cryo-EM maps by density modification. *Nature methods* **17**, 923-927 (2020).