PNAS

Supporting Information for

The structured organization of Deinococcus radiodurans' cell envelope

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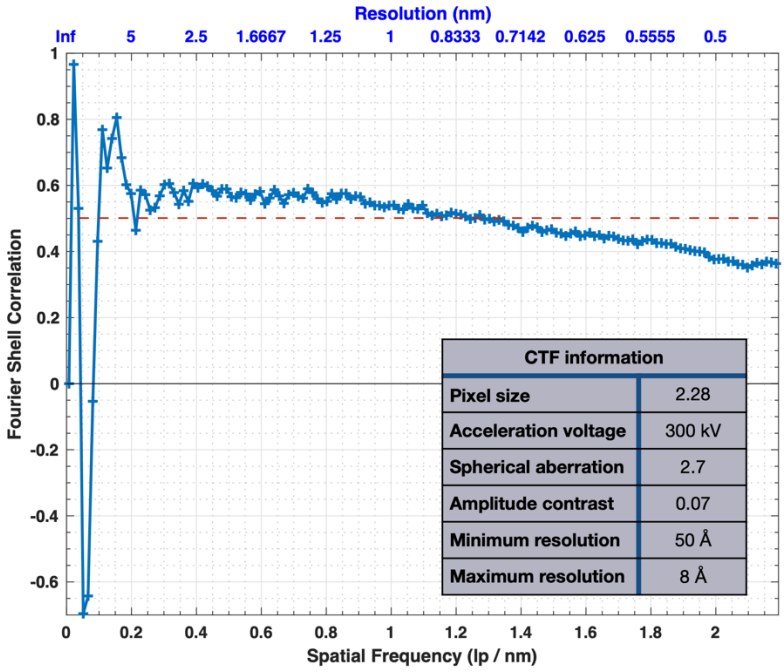
Other supporting materials for this manuscript include the following:

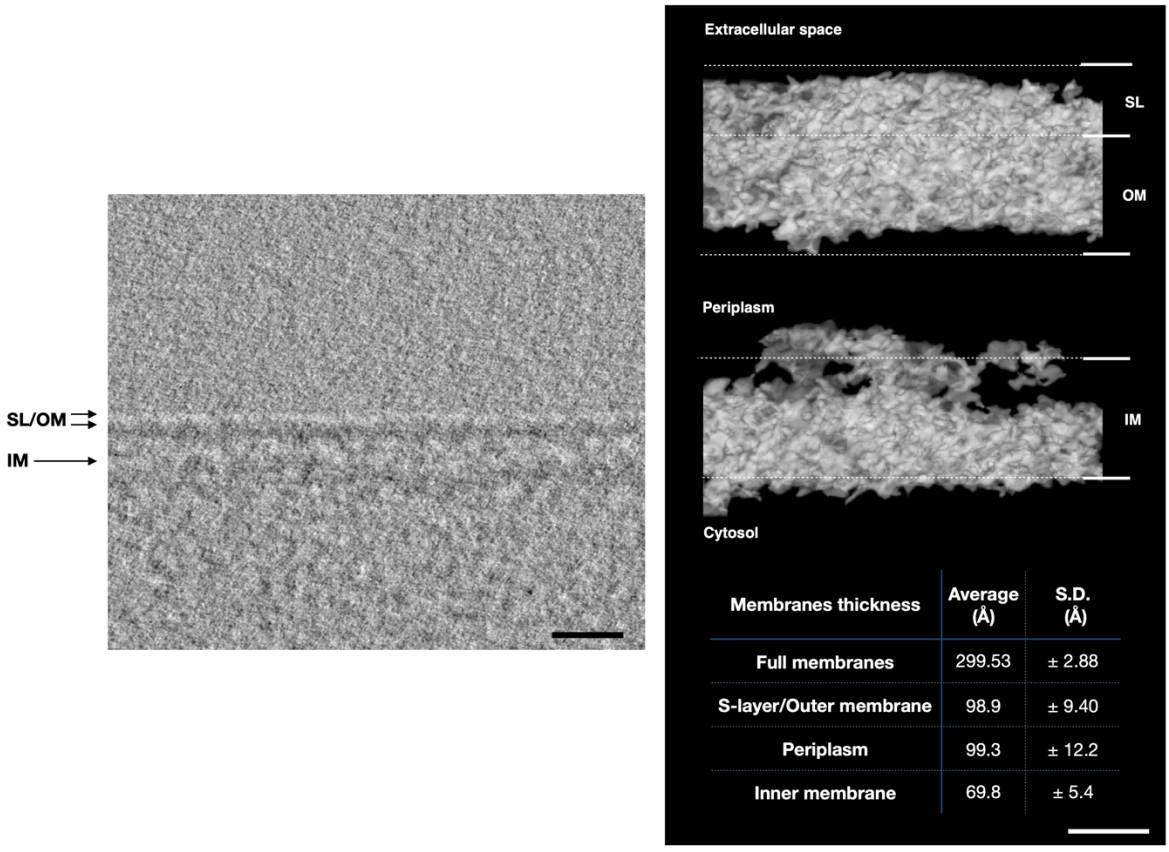
Supplementary Figure 1: Tomographic parameters and resolution data. In the image is reported the Fourier Shell Correlation (FSC) for the symmetrized subtomogram averaging; the dashed-red line represents the spatial frequency with cutoff at 0.5 (Nyquist frequency - gold standard). The inset table reports the CTF parameters. Related to Figure 6.

Figure S1



Supplementary Figure 2: Cell envelope layering and membranes localization. On the left is shown the tomographic reconstruction of a patch. The S-layer/outer membrane system (SL/OM) and the inner membranes (IM, black arrows) are highlighted with black arrows. The scale bar indicates 200 Å. On the right, it is shown a detail of the same cell envelope processed by subtomogram averaging with indicated cytosol, periplasm, and extracellular space. The scale bar indicates 50 Å. The inset table on the bottom summarizes the thickness values of the cell envelope and its regions. The values in the inset table represent the mean of 40 independent tomograms. Related to Figure 5.

Figure S2



Membranes thickness	Average (Å)	S.D. (Å)
Full membranes	299.53	± 2.88
S-layer/Outer membrane	98.9	± 9.40
Periplasm	99.3	± 12.2
Inner membrane	69.8	± 5.4

Supplementary Movie 1: Cryo-EM density map showing the features of the diffracting cell envelope fraction with a detail of its main complexes. The T4P-like (orange), the SDBC (pink), and the radial-dimeric complex (yellow) were extracted and refitted into the map. Related to Figure 3.

Supplementary Movie 2: Representative tomogram of a cell envelope patch reconstructed from a typical specimen. Related to Figure 5.

Supplementary Table 1: Key resources table for the Materials and Methods.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial strain		
Deinococcus radiodurans R1	ATCC (https://www.atcc.org/)	ATCC 13939
Chemicals, peptides, and recombinant proteins		
Tryptone	Becton, Dickinson and Company	Cat# 211705
Yeast extract	Becton, Dickinson and Company	Cat# 212750
Glucose	Carlo Erba	CAS n° 50-99-7
Sodium Phosphate dibasic dodecahydrate	Carlo Erba	CAS n° 10039-32-4
Sodium Phosphate monobasic monohydrate	Carlo Erba	CAS n° 10049-21-5
Dnase I	Roche	Art n° 11284932001
Lysozyme	Sigma Aldrich	CAS n° 12650-88-3
Bradford assay kit	ThermoFisher	Cat# 23246
n-dodecyl-β-D-maltoside	Glycon	Cat# D97002-C
Sodium chloride	Sigma Aldrich	CAS n° 7647-14-5
Potassium chloride	Carlo Erba	CAS n° 7447-40-7
HEPES	Roth	CAS n° 7365-45-9
Deposited data		
Subtomogram averaging of Deinococcus radiodurans' cell wall	This study	EMD-14095
Symmetrized subtomogram averaging of Deinococcus radiodurans' cell wall	This study	EMD-14096
3D map of Deinococcus radiodurans cell wall by electron crystallography	This study	EMD-14097
Software and algorithms		
CTFFIND3	Mindell *and* Grigorieff, 2003	https://grigoriefflab.umassmed.edu/ctf_estimation_ctffind_ctftilt
MotionCor2	Zheng *et al.,* 2017	https://emcore.ucsf.edu/ucsf-software
Focus package	Biyani *et al.,* 2017	https://lbem-focus.epfl.ch/about.php

2*dx* package	Gipson *et al.,* 2007	https://www.c-cina.org/stahlberg/research/tools/soft/2dx/
Chimera	Pettersen *et al.,* 2004	https://www.cgl.ucsf.edu/chimera/
etomo	Mastronarde, 2005	https://bio3d.colorado.edu/imod/
PEET	Heumann *et al.,* 2011	https://bio3d.colorado.edu/PEET/
Other		
anion-exchange chromatography column	Hiload HP, Amersham	n.a.
Quantifoil R2/1.3 holey carbon grids	Quantifoil	n.a.
autogrid	FEI, Eindhoven, Netherlands	n.a.