Supplemental Material

Specific protein-membrane interactions promote packaging of metallo-β-lactamases into outer membrane vesicles

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Table S1.

Supplementary Table 1. MIC values of imipenem (IPM) and ceftazidime (CAZ) for *E. coli* carrying the empty vector (EV) or expressing $bla_{\text{NDM-1}}$, $bla_{\text{NDM-1 C26A}}$ (NDM-1 C26A), $bla_{\text{NDM-1 R45E/R52E}}$ (NDM-1 2RE), $bla_{\text{IMP-1}}$, $bla_{\text{IMP-1 K87E/K89E/K145E/K147E}}$ (IMP-1 4KE) at 20 μM of IPTG. Data correspond to mean values from three independent experiments.

MIC values (μg ml^{-1}) of imipenem (IPM) and ceftazidime (CAZ) for *E. coli* expressing MBLs at 20 μM of IPTG		
	IPM	CAZ
EV	<0.06	0.03
NDM-1	2	1024
NDM-1 C26A	2	1024-2048
NDM-1 2RE	2	1024
IMP-1	1	258
IMP-1 4KE	1	126
VIM-2	1-2*	16*

*at 10 μM of IPTG

Fig. S1

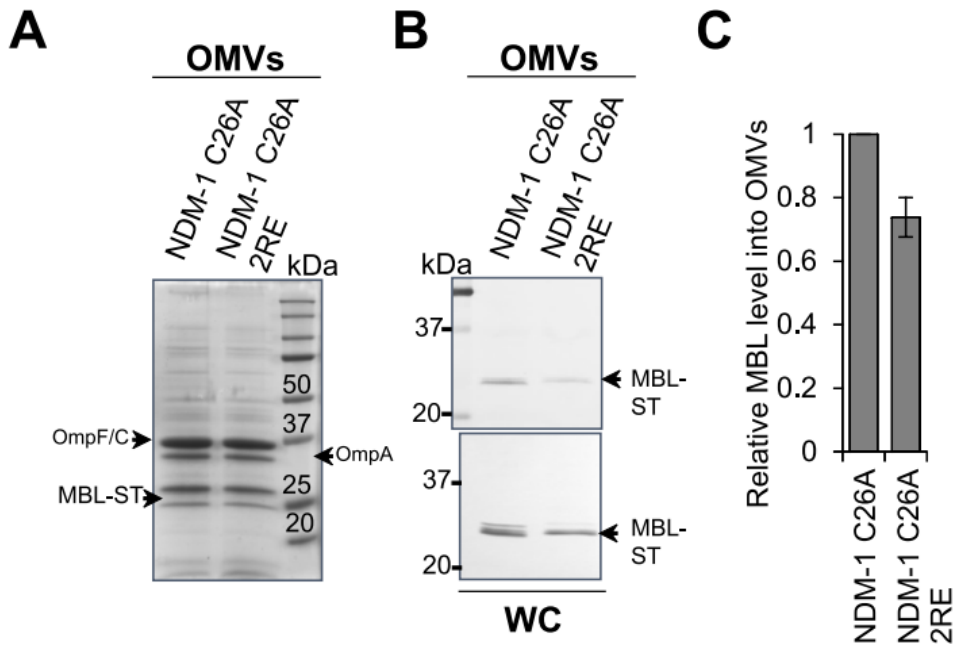

Figure S1. The two Arg residues also play a role in the level of incorporation of the soluble variant NDM-1 C26A into vesicles. (A) SDS-PAGE of the OMVs purified from *E. coli* expressing $bla_{\text{NDM-1 C26A}}$ or $bla_{\text{NDM-1 C26A 2RE}}$.(B) Immunoblotting detection of NDM-1 C26A and NDM-1 C26A 2RE (fused to a C-terminal Strep-tag sequence, -ST) in OMVs and in whole cells (WC) from *E. coli* strains expressing each MBL. (C) Protein levels of NDM-1 C26A and NDM-1 C26A 2RE into OMVs. The plotted values were calculated as described in the materials and methods section. Data correspond to two independent experiments and are shown as the mean value. Error bars represent the standard deviation (SD).

Fig. S2

A

	Replica 1	Replica 2	Replica 3	Replica 4	Replica 5
IMP-1	ν	ν	ν	ν	ν
NDM-1 (lip)	ν	ν	ν	ν	ν
IMP-1 (4KE)	χ	χ	χ	ν	ν
VIM-2	χ	χ	χ	χ	χ

ν = binding event occurred
χ = binding event did not occur

B

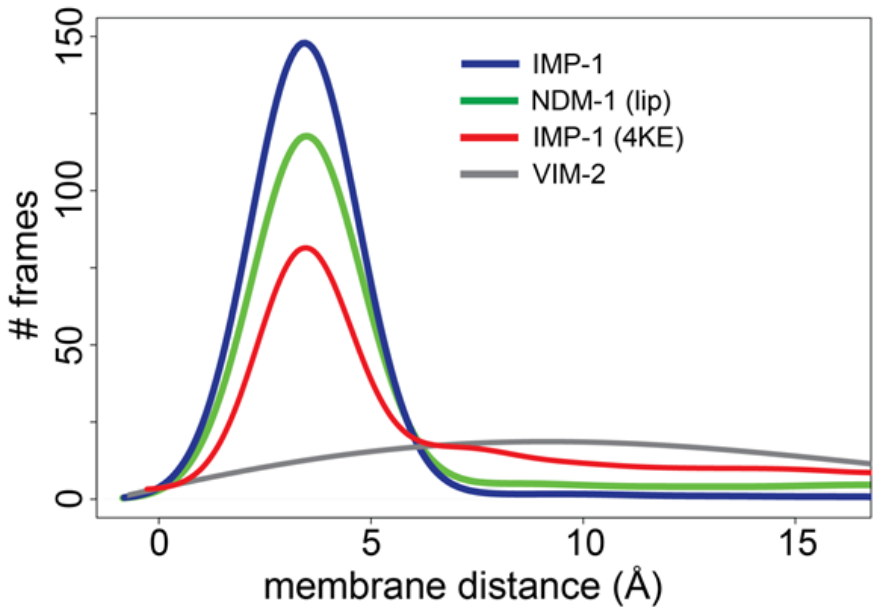

Figure S2. MBLs membrane association. (A) Binding events observed for WT IMP-1, NDM-1, mutated IMP-1 (IMP-1 4KE) and VIM-2 in the different CG MD replicas. (B) Distribution of CG MD trajectory frames collected every 750 ps for all available replicas with respect to protein-membrane distance.

Fig. S3

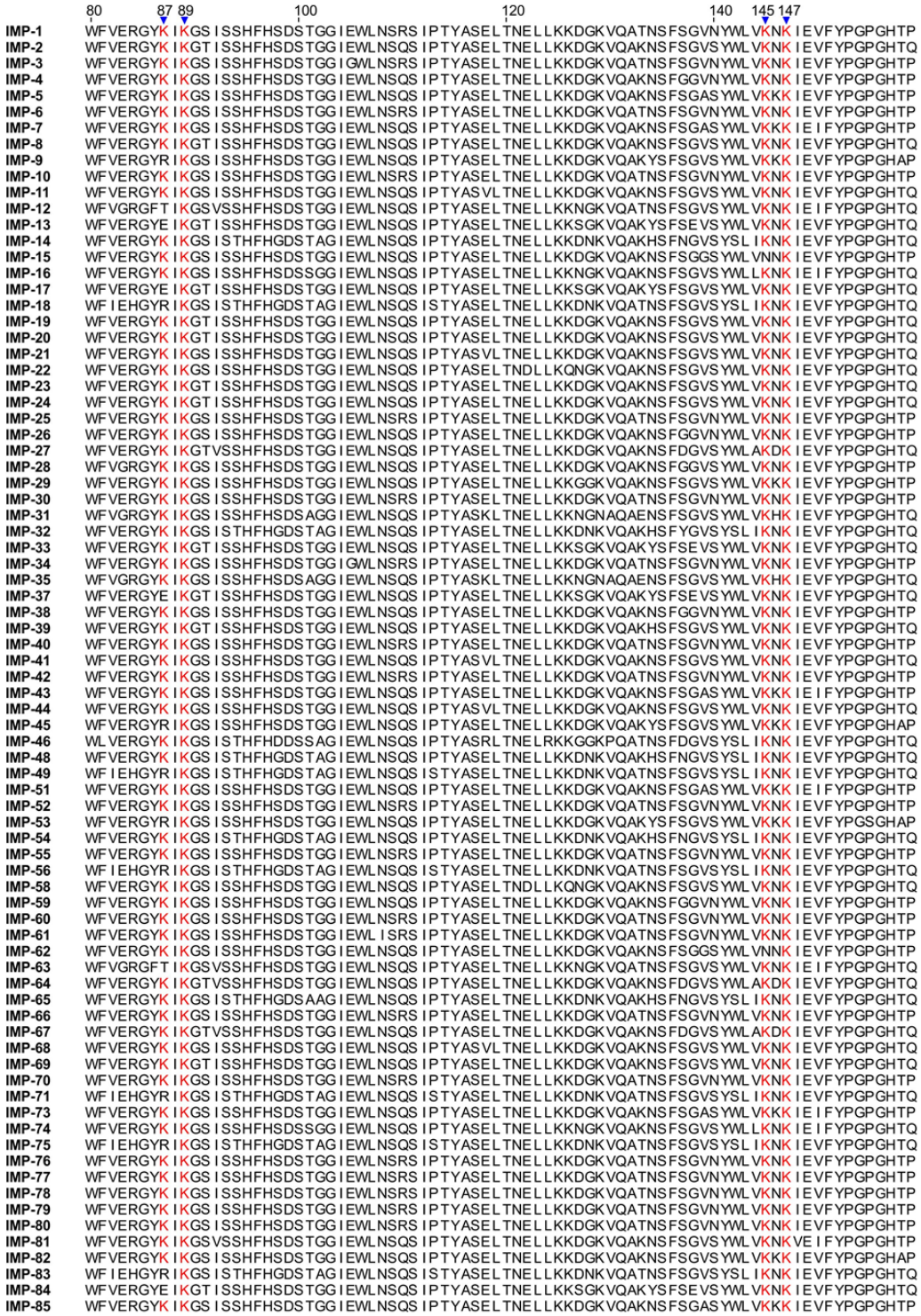

Figure S3. Sequence alignment of known 80 IMP allelic variants (only the region of interest is shown, from amino acid 80 to 159). Conserved Lys residues (K) at positions

87, 89, 145 and 147 are shown in red. Alignment was performed with the T-Coffee tool, available at www.tcoffee.org.