## **Supplemental Material**

## Specific protein-membrane interactions promote packaging of metalloβ-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*<sub>NDM-1</sub>, *bla*<sub>NDM-1</sub> <sub>C26A</sub> (NDM-1 C26A), *bla*<sub>NDM-1</sub> <sub>R45E/R52E</sub> (NDM-1 2RE), *bla*<sub>IMP-1</sub>, *bla*<sub>IMP-1</sub> <sub>K87E/K89E/K145E/K147E</sub> (IMP-1 4KE) at 20 μM of IPTG. Data correspond to mean values from three independent experiments.

MIC values (µg ml <sup>-1</sup> ) of imipenem (IPM) and ceftazidime (CAZ) for <i>E. coli</i> 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*<sub>NDM-1 C26A</sub> or *bla*<sub>NDM-1 C26A 2RE</sub>.(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).

Α

в

	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)	x	x	x	ν	ν
VIM-2	x	x	x	x	x

v = binding event occurred

x = binding event did not occur



**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.

	80 87	89	100	120	140	145 147
IMP-1	WEVERGYK	IKGS	ISSHFHSDSTGG IEWLNSRS	IPTYASELTNEL	LKKDGKVQATNSFSGVNYWL	VKNKIEVFYPGPGHTP
IMP-2	WFVERGYK	IKGT	ISSHFHSDSTGGIEWLNSQS	IPTYASELTNEL	LKKDGK VQAKNSFSGVS YWL	VKNKIEVFYPGPGHTQ
IMP-3	WFVERGYK	IKGS	ISSHFHSDSTGGIGWLNSRS	IPTYASELTNEL	LKKDGKVQATNSFSGVNYWL	VKNK IEVFYPGPGHTP
IMP-4	WEVERGYK	IKGS	ISSHFHSDS IGG IEWLNSQS	IPTYASELINEL		
IMP-6	WEVERGYK	IKGS	ISSHFHSDSTGG1EWLNSRS	IPTYASELTNEL	LKKDGKVQATNSFSGVNYM	VKNKIEVFYPGPGHTP
IMP-7	WEVERGYK	IKGS	ISSHFHSDSTGGIEWLNSQS	IPTYASELTNEL	LKKDGK VQAKNSFSGAS YWL	VKKKIEIFYPGPGHTP
IMP-8	WEVERGYK	IKGT	ISSHFHSDSTGGIEWLNSQS	IPTYASELTNEL	LKKDGKVQAKNSFSGVSYWL	.VKNKIEVFYPGPGHTQ
IMP-9	WEVERGYR	IKGS	ISSHFHSDSTGG IEWLNSQS	IPTYASEL TNEL	LKKDGKVQAKYSFSGVSYWL	
IMP-10	WEVERGYK	IKGS	ISSHFHSDSTGGIEWLNSQS	IPTYASVLTNEL	LKKDGKVQAKNSFSGVSYML	
IMP-12	WFVGRGFT	KGS	VSSHFHSDSTGG1EWLNSQS	IPTYASELTNEL	LKKNGKVQATNSFSGVSYWL	VKNKIEIFYPGPGHTQ
IMP-13	WFVERGYE	IKGT	ISSHFHSDSTGGIEWLNSQS	IPTYASELTNEL	LKKSGKVQAKYSFSEVSYWL	.VKNKIEVFYPGPGHTQ
IMP-14	WEVERGYK	IKGS	ISTHFHGDSTAG IEWLNSQS	IPTYASELTNEL	LKKDNKVQAKHSFNGVSYSL	
IMP-16	WEVERGYK	IKGS	ISSHFHSDSSGG IEWLNSQS	IPTYASELTNEI	LKKNGKVOAKNSESGVSYM	
IMP-17	WFVERGYE	IKGT	ISSHFHSDSTGG1EWLNSQS	IPTYASELTNEL	LKKSGKVQAKYSFSGVSYWL	VKNKIEVFYPGPGHTQ
IMP-18	WFIEHGYR	IKGS	ISTHFHGDSTAGIEWLNSQS	ISTYASELTNEL	LKKDNKVQATNSFSGVSYSL	. IKNKIEVFYPGPGHTQ
IMP-19	WEVERGYK	IKGT	I SSHFHSDSTGG I EWLNSQS	IPTYASELTNEL	LKKDGKVQAKNSFSGVSYWL	
IMP-20	WEVERGYK	IKGS	ISSHEHSDSTGGTEWLNSQS	IPTYASEL INEL	LKKDGKVQAKNSFSGVS WIL	VKNK LEVEYPGPGHTQ
IMP-22	WEVERGYK	IKGS	ISSHFHSDSTGGIEWLNSQS	IPTYASELTNDI	LKQNGK VQAKNSFSGVS YWL	VKNKIEVFYPGPGHTQ
IMP-23	WFVERGYK	IKGT	ISSHFHSDSTGGIEWLNSQS	IPTYASELTNEL	LKKDGKVQAKNSFSGVSYWL	.VKNKIEVFYPGPGHTQ
IMP-24	WEVERGYK	IKGT	I SSHFHSDSTGG I EWLNSQS	IPTYASELTNEL	LKKDGKVQAKNSFSGVSYWL	VKNK IEVFYPGPGHTQ
IMP-25 IMP-26	WEVERGYK	IKGS	ISSHEHSDSTGGTEWLNSRS	IPTYASELINEL		
IMP-27	WEVERGYK	KGT	VSSHFHSDSTGGIEWLNSQS	IPTYASELTNEL	LKKDGKVQAKNSFDGVSYWL	AKDKIEVFYPGPGHTQ
IMP-28	WFVGRGY <mark>K</mark>	I KGS	ISSHFHSDSTGGIEWLNSQS	IPTYASELTNEL	LKKDGK VQAKNS FGG VS YWL	VKNK I E V FYPGPGHTP
IMP-29	WEVERGYK	IKGS	ISSHFHSDSTGGIEWLNSQS	IPTYASELTNEL	LKKGGKVQAKNSFSGVSYWL	VKKK IEVFYPGPGHTP
IMP-30 IMP-31	WEVGROVK	IKGS	ISSHEHSDSAGG IEWLNSRS	IPTYASELINEL		
IMP-32	WEVERGYK	IKGS	ISTHFHGDSTAG I EWLNSQS	IPTYASELTNEL	LKKDNKVQAKHSFYGVSYSL	IKNKIEVFYPGPGHTQ
IMP-33	WFVERGYK	I <mark>K</mark> GT	ISSHFHSDSTGG IEWLNSQS	IPTYASELTNEL	LKKSGKVQAKYSFSEVSYWL	VKNK I E V FYPGPGHTQ
IMP-34	WEVERGYK	IKGS	ISSHFHSDSTGGIGWLNSRS	IPTYASELTNEL	LKKDGKVQATNSFSGVNYWL	VKNK I EVFYPGPGHTP
IMP-35 IMP-37	WEVERGYK	IKGS	ISSHEHSDSAGGTEWLNSQS	IPIYASKLINEL		
IMP-38	WEVERGYK	IKGS	ISSHFHSDSTGG IEWLNSQS	IPTYASELTNEL	LKKDGKVQAKNSFGGVNYWL	VKNKIEVFYPGPGHTP
IMP-39	WFVERGYK	IKGT	ISSHFHSDSTGGIEWLNSQS	IPTYASELTNEL	LKKDGKVQAKHSFSGVSYWL	VKNK I EVFYPGPGHTQ
IMP-40	WEVERGYK	IKGS	ISSHFHSDSTGGIEWLNSRS	IPTYASELTNEL	LKKDGKVQATNSFSGVNYWL	.VKNKIEVFYPGPGHTP
IMP-41 IMP-42	WEVERGYK	IKGS	ISSHEHSDSTGGTEWLNSQS	IPTYASVLINEL		
IMP-43	WEVERGYK	IKGS	ISSHFHSDSTGGIEWLNSQS	IPTYASELTNEL	LKKDGKVQAKNSFSGASYWL	VKKKIEIFYPGPGHTP
IMP-44	WFVERGYK	IKGS	ISSHFHSDSTGGIEWLNSQS	IPTYASVLTNEL	LKKDGKVQAKNSFSGVSYWL	.VKNKIEVFYPGPGHTQ
IMP-45	WEVERGYR	IKGS	I SSHFHSDSTGG I EWLNSQS	IPTYASELTNEL	LKKDGKVQAKYSFSGVSYML	VKKK IEVFYPGPGHAP
IMP-46	WEVERGYK	IKGS	ISTHEHODSTAG LEWENSOS	IPTYASEL TNEL		
IMP-49	WFIEHGYR	IKGS	ISTHFHGDSTAGIEWLNSQS	ISTYASELTNEL	LKKDNKVQATNSFSGVSYSL	IKNKIEVFYPGPGHTQ
IMP-51	WFVERGYK	IKGS	ISSHFHSDSTGGIEWLNSQS	IPTYASELTNEL	LKKDGK VQAKNS FSGAS YWL	.VKKKIEIFYPGPGHTP
IMP-52	WEVERGYK	IKGS	ISSHFHSDSTGG IEWLNSRS	IPTYASEL TNEL		
IMP-54	WEVERGYK	IKGS	ISTHFHGDSTAG IEWLNSQS	IPTYASELTNEL	LKKDNKVQAKHSFNGVSYSL	IKNKIEVFYPGPGHTQ
IMP-55	WFVERGYK	IKGS	ISSHFHSDSTGGIEWLNSRS	IPTYASELTNEL	LKKDGKVQATNSFSGVNYWL	VKNKIEVFYPGPGHTP
IMP-56	WFIEHGYR	IKGS	ISTHFHGDSTAGIEWLNSQS	ISTYASELTNEL	LKKDNKVQATNSFSGVSYSL	. IKNK IEVFYPGPGHTQ
IMP-58	WEVERGYK	IKGS	ISSHEHSDSTGGTEWLNSQS	IPTYASEL INDU	LKQNGKVQAKNSFSGVSYWL	
IMP-60	WEVERGYK	IKGS	ISSHFHSDSTGG IEWLNSRS	IPTYASELTNEL	LKKDGKVQATNSFSGVNYWL	VKNKIEVFYPGPGHTP
IMP-61	WFVERGYK	IKGS	ISSHFHSDSTGG1EWLISRS	IPTYASELTNEL	LKKDGKVQATNSFSGVNYWL	VKNK I E V FYPGPGHTP
IMP-62	WEVERGYK	IKGS	ISSHFHSDSTGGIEWLNSQS	IPTYASELTNEL	LKKDGKVQAKNSFSGGSYWL	VNNK IEVFYPGPGHTP
IMP-63	WEVERGYK	KGT	VSSHEHSDSTGGTEWLNSQS	IPTYASEL INEL	I KKDGK VQATNSFSGVS IVIL	
IMP-65	WEVERGYK	IKGS	ISTHFHGDSAAGIEWLNSQS	IPTYASELTNEL	LKKDNKVQAKHSFNGVSYSL	IKNKIEVFYPGPGHTQ
IMP-66	WFVERGYK	IKGS	ISSHFHSDSTGGIEWLNSRS	IPTYASELTNEL	LKKDGKVQATNSFSGVNYWL	.VKNKIEVFYPGPGHTP
IMP-67	WEVERGYK	IKGT	VSSHFHSDSTGGIEWLNSQS	IPTYASELTNEL	LKKDGKVQAKNSFDGVSYWL	
IMP-66	WEVERGYK	IKGT	ISSHEHSDSTGGTEWLNSQS	IPTYASEL TNEL	LKKDGKVQAKNSFSGVS WIL	VKNK LEVEYPGPGHTQ
IMP-70	WFVERGYK	IKGS	ISSHFHSDSTGGIEWLNSRS	IPTYASELTNEL	LKKDGKVQATNSFSGVNYWL	VKNKIEVFYPGPGHTP
IMP-71	WFIEHGYR	IKGS	ISTHFHGDSTAG IEWLNSQS	ISTYASELTNEL	LKKDNKVQATNSFSGVSYSL	. IKNK IEVFYPGPGHTQ
IMP-73	WEVERGYK	IKGS	ISSHEHSDSIGGIEWLNSQS	IPTYASELTNEL		
IMP-75	WFIEHGYR	IKGS	ISTHEHGDSTAGIEWLNSOS	ISTYASELTNEI	LKKDNKVQATNSFSGVSYSI	IKNK IEVFYPGPGHTO
IMP-76	WEVERGYK	IKGS	ISSHFHSDSTGG IEWLNSRS	IPTYASELTNEL	LKKDGKVQATNSFSGVNYWL	VKNKIEVFYPGPGHTP
IMP-77	WEVERGYK	IKGS	ISSHFHSDSTGG IEWLNSRS	IPTYASELTNEL	LKKDGKVQATNSFSGVNYWL	VKNK I EVFYPGPGHTP
IMP-78	WEVERGYK	IKGS	ISSHEHSDSTCCLEM/ NCC	IPIYASELTNEL		
IMP-80	WEVERGYK	IKGS	ISSHFHSDSTGG IEWLNSRS	IPTYASELTNEL	LKKDGKVQATNSFSGVNYM	VKNKIEVFYPGPGHTP
IMP-81	WEVERGYK	KGS	VSSHFHSDSTGGIEWLNSQS	IPTYASELTNEL	LKKDGKVQAKNSFSGVSYWL	VKNKVE I FYPGPGHTP
IMP-82	WEVERGYK	IKGS	ISSHFHSDSTGG IEWLNSQS	IPTYASELTNEL	LKKDGKVQAKNSFSGVSYWL	VKKK IEVFYPGPGHAP
IMP-83	WEVERGVE	IKGT	ISSHEHSDSTGGTEWINSOS	IPTYASEL INEL	LKKSGKVOAKYSESEVSYSL	
IMP-85	WEVERGYK	IKGS	ISSHFHSDS TGG IEWLNSQS	IPTYASELTNEL	LKKDGK VQAKNSFSGAS YWL	VKKKIEVFYPGPGHTP

**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.