Supp Table 1. Table of the conserved positions that define the binding sphere of NM102 in the Mfd of the ESKAPE group. Hydrophobic and polar residues are colored in green and cyan, respectively, Aspartate and Glutamate residues are salmon, Lysine and Arginine residues are blue and Glycine residues are wheat. The residues which are not strictly conserved are highlighted with black stars

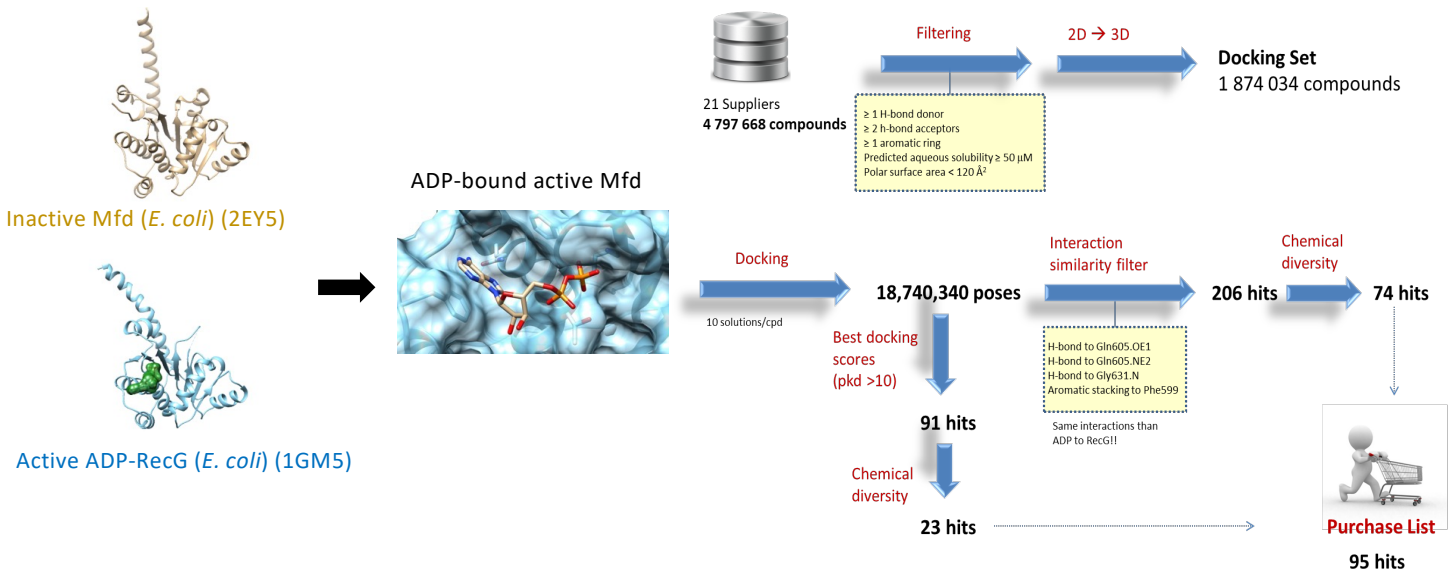
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20	P21
E.coli	F597	F599	E600	T602	Q605	D629	V630	G631	F632	G633	K634	T635	E636	Q664	H665	N668	E730	P780	A781	D876	R905
E. faecium	F618	Y620	S621	T623	Q626	D650	V651	G652	Y653	G654	K655	T656	E657	Q685	H686	T689	E751	P801	A802	D897	R926
S.aureus	F615	Y617	E618	T620	Q623	D647	V648	G649	Y650	G651	K652	T653	E654	Q682	H683	T686	E748	P798	E799	D894	R923
K.pneumoniae	F597	F599	E600	T602	Q605	D629	V630	G631	F632	G633	K634	T635	E636	Q664	H665	N668	E730	P780	A781	D876	R905
A. baumanii	F602	Y604	E605	E606	T607	D634	V635	G636	F637	G638	K639	T640	E641	Q669	H670	S673	E735	P785	A786	D881	R910
P.aeruginosa	F598	F600	E601	E602	T603	D630	V631	G632	F633	G634	K635	T636	E637	Q665	H666	N668	E731	P781	A782	D877	R906
E. cloacae	F597	F599	E600	T602	Q605	D629	V630	G631	F632	G633	K634	T635	E636	Q664	H665	N668	E730	P780	A781	D876	R905
S.flexneri	F597	F599	E600	T602	Q605	D629	V630	G631	F632	G633	K634	T635	E636	Q664	H665	N668	E730	P780	A781	D876	R905
		*	*	*					*							*			*		

Supp Table 2. The solubility of NM102 in some common solvents

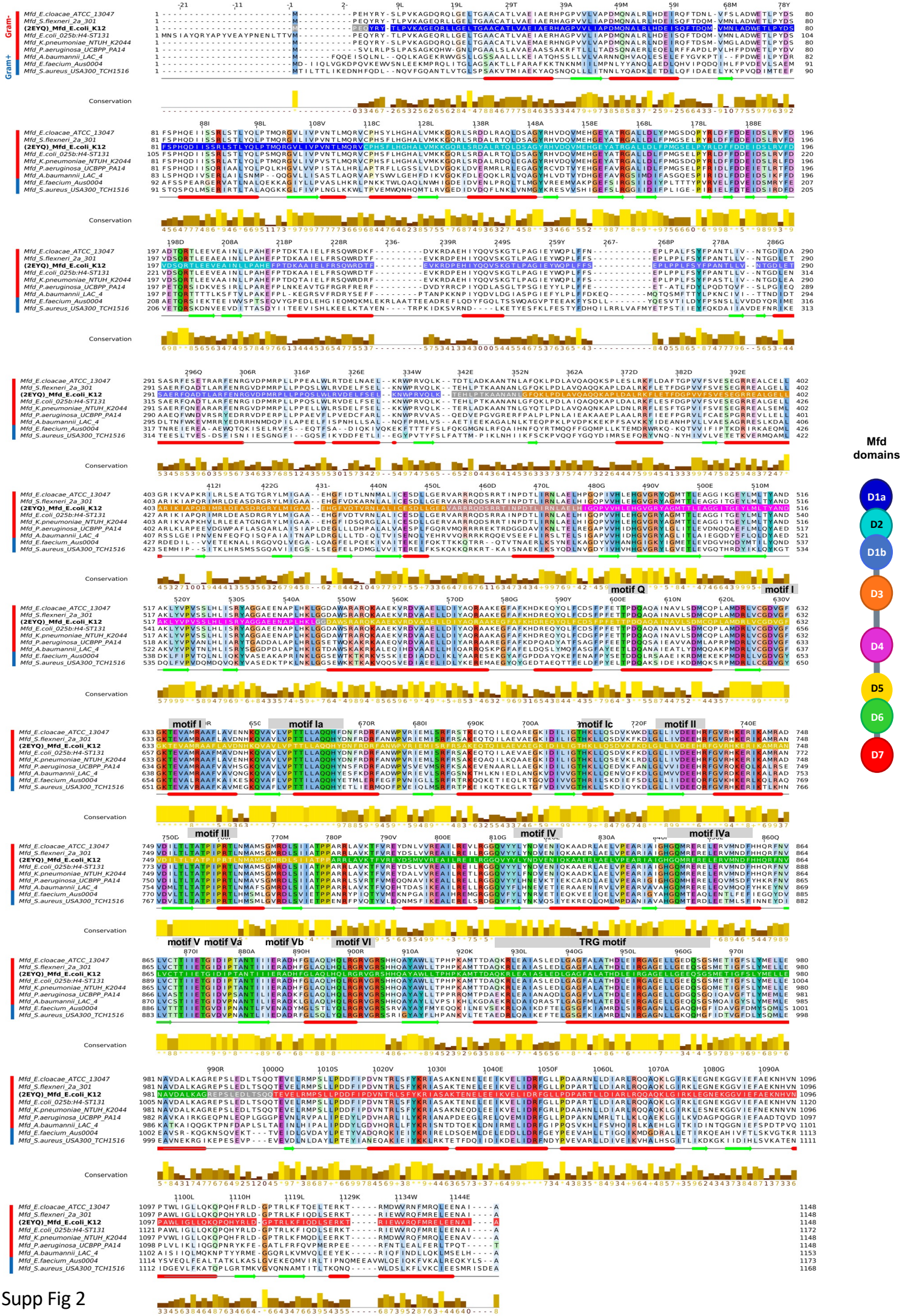
Excipient	Maximal solubility of NM102 after 24 h at 20°C, quantified by HPLC (mg/mL)
Water	≤ 0.001
Tween 80	0.09
Intralipid 20%	0.05
Soya oil	< 0.002
Corn oil	< 0.002
Capmul® MCM	0.01
Mygliol 912	0.002
NMP	≥ 10
DMSO	≈ 6.7

Supp Table 3: ESKAPE strains used in this study and their resistance to antibiotics. The MIC for each antibiotic tested is in µg/mL**.**

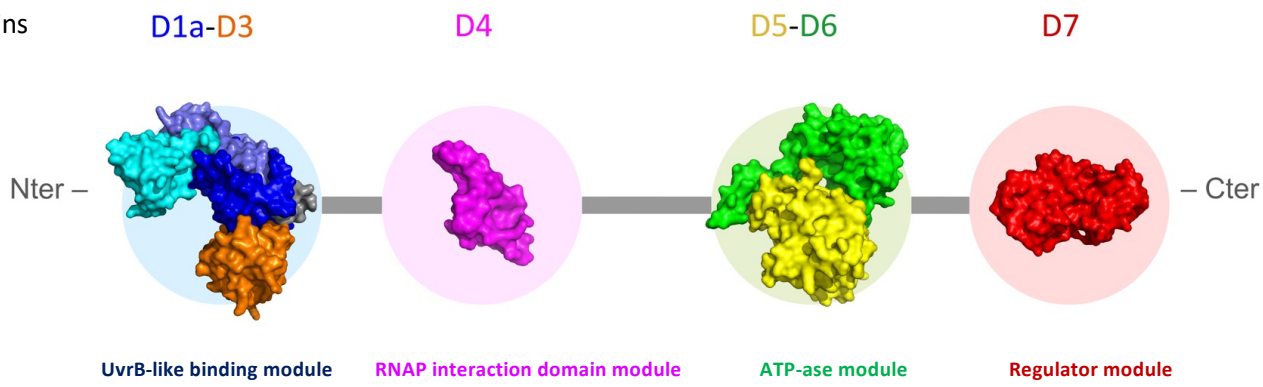
	K. pneumoniae ATCC 700603	*K. pneumoniae* ALE CTXM-15+	*K. pneumoniae* DOU CTXM-15	*P. aeruginosa* CIP 27853	*E. coli* ATCC 25922
Ciprofloxacin	>512	>512	>512	>512	4
Streptomycin	>512	>512	>512	>512	2
Oxacillin	8	>512	>512	16	<0.5
Cefotaxin	<0.5	8	8	<0.5	<0.5
Meropenem	2	>512	>512	16	<0.5
Ceftriaxone	>512	>512	>512	>512	16
Ampicilin	8	4	>512	64	4
Penicillin	16	>512	>512	32	1


Inactive Mfd (E. coli) (2EY5)
Active ADP-RecG (E. coli) (1GM5)
ADP-bound active Mfd
21 Suppliers
4 797 668 compounds
Filtering
≥ 1 H-bond donor
≥ 2 h-bond acceptors
≥ 1 aromatic ring
Predicted aqueous solubility ≥ 50 µM
Polar surface area < 120 Å²
2D → 3D
Docking Set
1 874 034 compounds
Docking
10 solutions/cpd
18,740,340 poses
Best docking scores (pkd >10)
91 hits
Chemical diversity
23 hits
Interaction similarity filter
H-bond to Gln605.OE1
H-bond to Gln605.NE2
H-bond to Gly631.N
Aromatic stacking to Phe599
Same interactions than ADP to RecG!!
206 hits
Chemical diversity
74 hits
Purchase List
95 hits

Supp Fig 1


Gram-
Gram+
Mfd_E.cloacae_ATCC_13047
Mfd_S.flexneri_2a_301
(2EYQ)_Mfd_E.coli_K12
Mfd_E.coli_025b:H4-ST131
Mfd_K.pneumoniae_NTUH_K2044
Mfd_P.aeruginosa_UCBPP_PA14
Mfd_A.baumannii_LAC_4
Mfd_E.faecium_Aus0004
Mfd_S.aureus_USA300_TCH1516
Conservation
motif Q
motif I
motif Ia
motif Ic
motif II
motif III
motif IV
motif IVa
motif V
motif Va
motif Vb
motif VI
TRG motif
Mfd domains
D1a
D2
D1b
D3
D4
D5
D6
D7

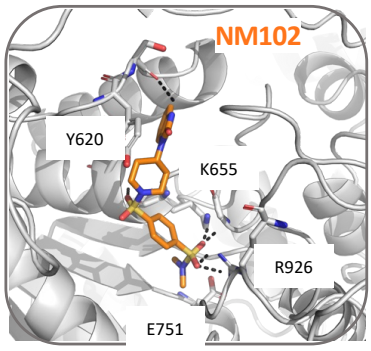
Supp Fig 2



Average sequence identity in ESKAPE	47 %	58 %	68 %	44 %

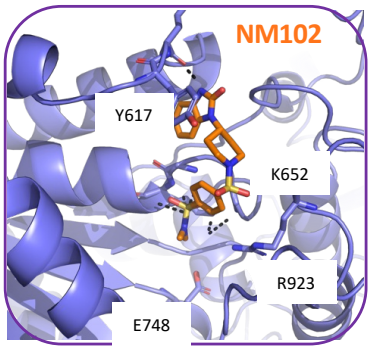
Suppl Fig. 3

E. faecium



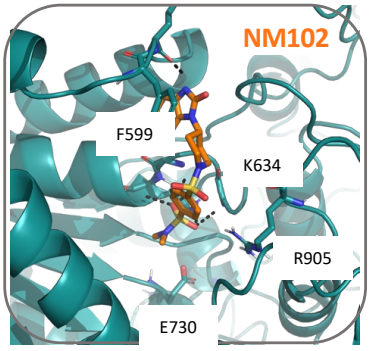
BE = -10.2 kcal.mol^{-1}

S. aureus



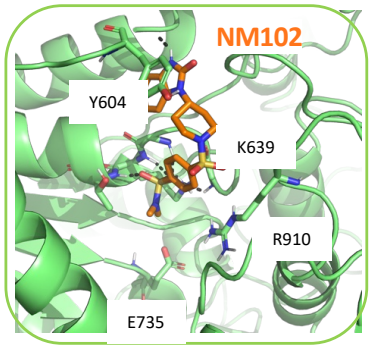
BE = -10.3 kcal.mol^{-1}

K. pneumoniae



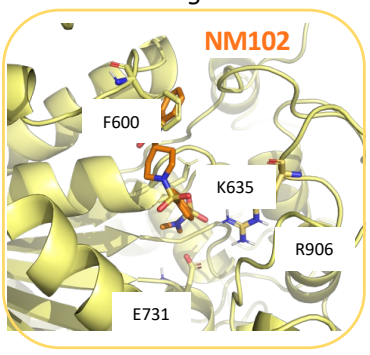
BE = -9.3 kcal.mol^{-1}

A. baumannii



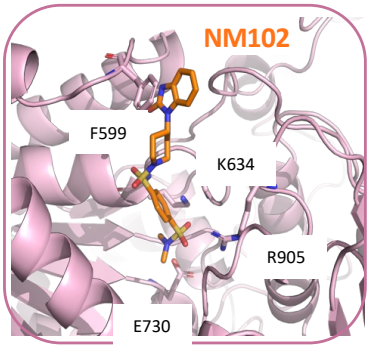
BE = -10.0 kcal.mol^{-1}

P. aeruginosa



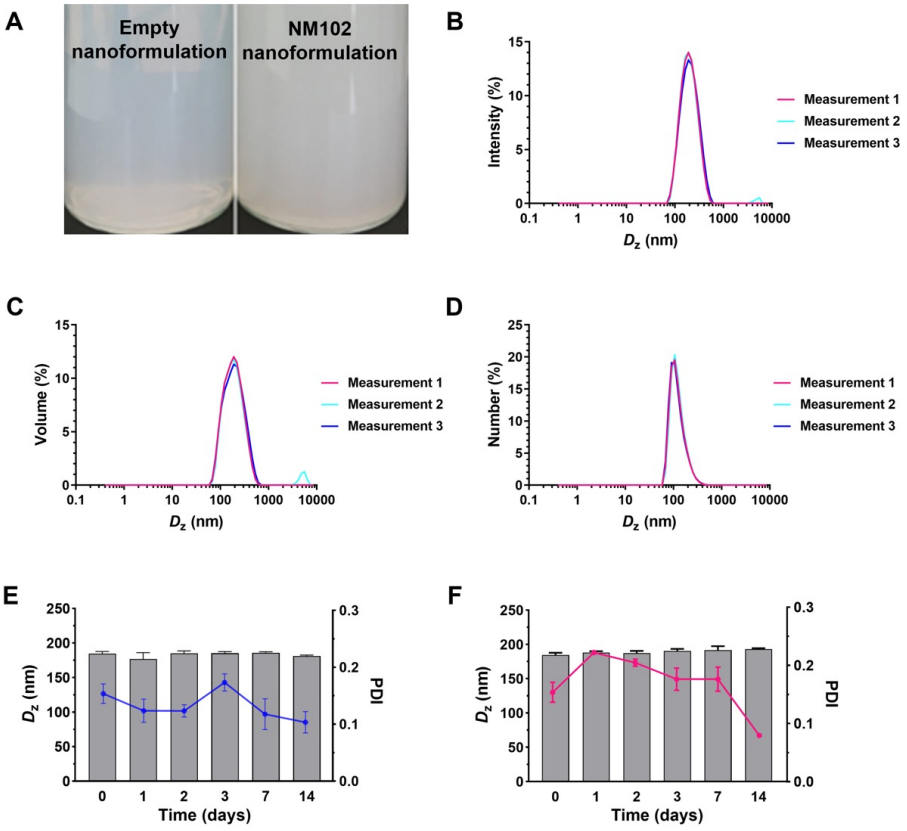
BE = -9.1 kcal.mol^{-1}

E. cloacae

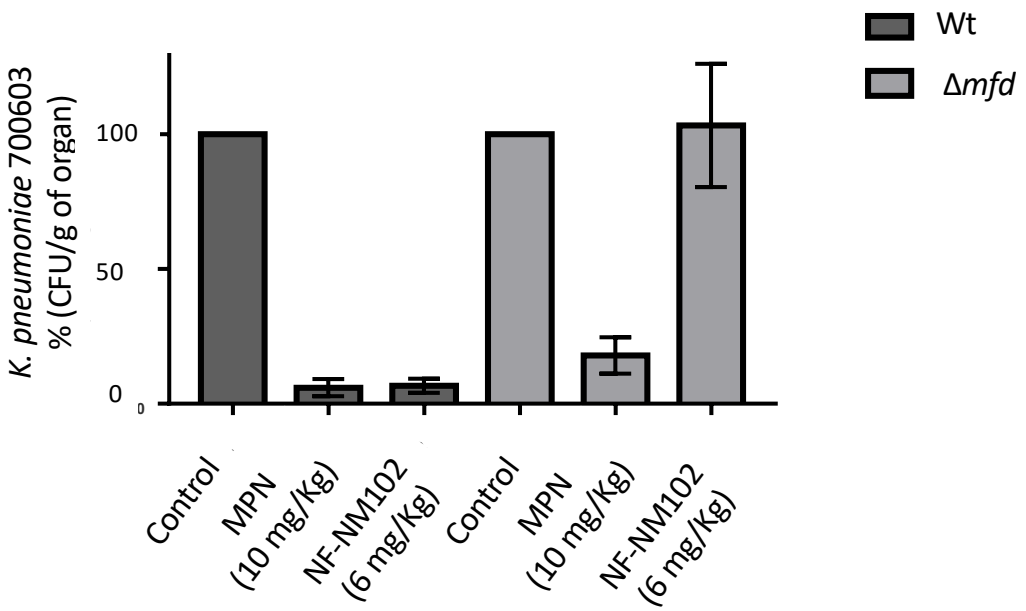


BE = -9.0 kcal.mol^{-1}

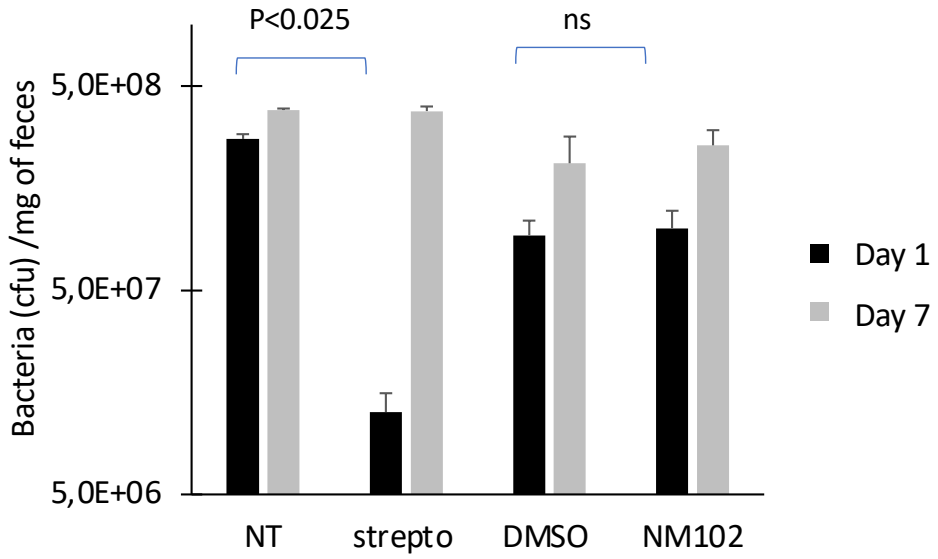
Supp Fig. 4


A
Empty nanoformulation
NM102 nanoformulation
B
Intensity (%)
Measurement 1
Measurement 2
Measurement 3
D_z (nm)
C
Volume (%)
Measurement 1
Measurement 2
Measurement 3
D_z (nm)
D
Number (%)
Measurement 1
Measurement 2
Measurement 3
D_z (nm)
E
D_z (nm)
PDI
Time (days)
F
D_z (nm)
PDI
Time (days)

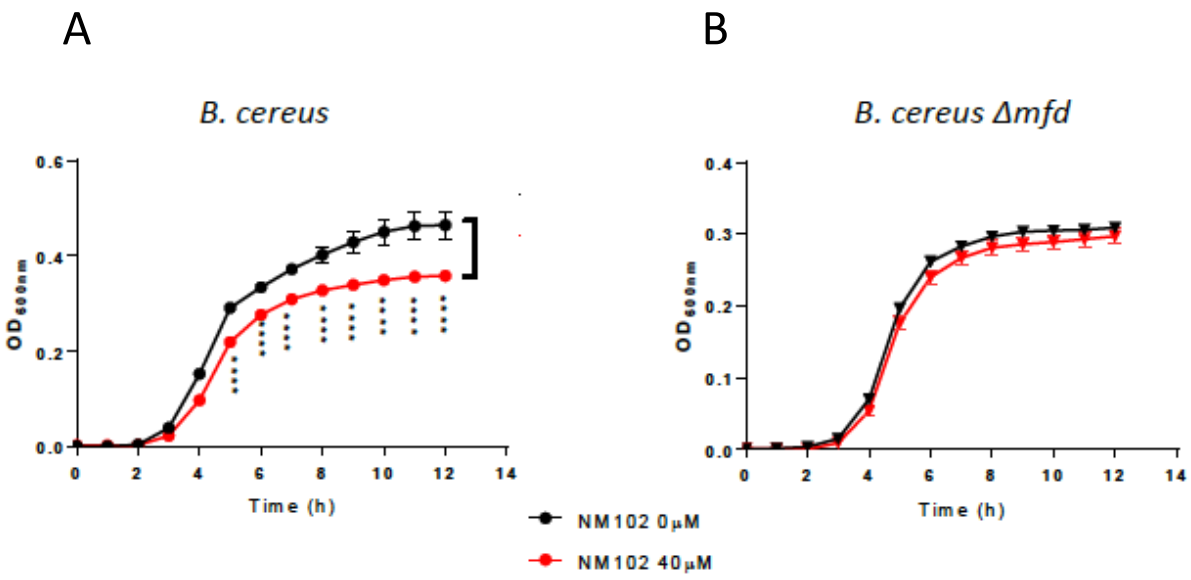
Supp Fig. 5


Wt
Δmfd
K. pneumoniae 700603
% (CFU/g of organ)
100
50
0
Control
MPN
(10 mg/Kg)
NF-NM102
(6 mg/Kg)
Control
MPN
(10 mg/Kg)
NF-NM102
(6 mg/Kg)

Supp Fig 6

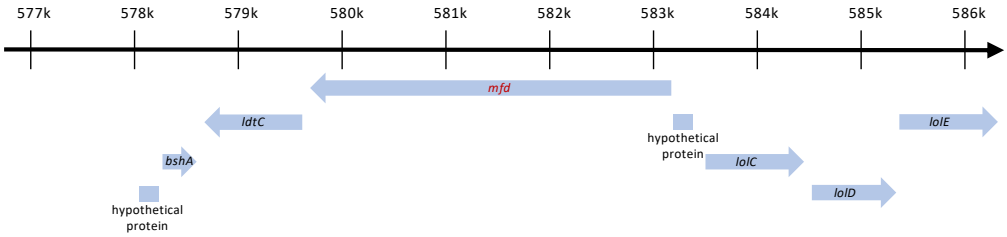

P<0.025
ns
5,0E+08
5,0E+07
5,0E+06
Bacteria (cfu) /mg of feces
NT
strepto
DMSO
NM102
Day 1
Day 7

Supp Fig 7



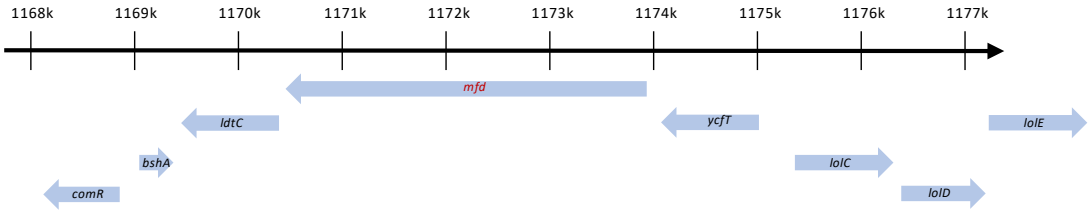
Supp Fig 8

Klebsiella pneumoniae ATCC 700603



bshA: DUF1471 domain-containing multiple stress resistance outer membrane protein BhsA
ldtC: L,D-transpeptidase LdtC
mfd: mutation frequency decline/ transcription-repair coupling factor
lolC: lipoprotein release complex - inner membrane subunit
lolD:lipoprotein release complex - ATP binding subunit
lolE: lipoprotein release complex - inner membrane subunit

Escherichia coli str. K-12 substr. MG1655



comR: DNA-binding transcriptional repressor ComR
bshA: DUF1471 domain-containing multiple stress resistance outer membrane protein BhsA
ldtC: L,D-transpeptidase LdtC
mfd: mutation frequency decline/ transcription-repair coupling factor
ycfT: inner membrane protein YcfT
lolC: lipoprotein release complex - inner membrane subunit
lolD:lipoprotein release complex - ATP binding subunit
lolE: lipoprotein release complex - inner membrane subunit

Supp Fig 9

Supp Fig. 1. Modelling of active Mfd of *E. coli* and high throughput screening of compounds library. Left: 3D model of active *E. coli* Mfd allows the docking of ADP and molecules. Right: Virtual screening workflow to select 95 commercially-available potential Mfd inhibitors.

Supp Fig. 2. Multiple sequence alignment (MSA) of Mfd from ESKAPE plus *E. coli*, using MAFFT. The vertical stripe refers to the color code of Mfd domains. This color code is highlighted horizontally in the MSA along $Mfd_{E.\ coli}$ sequence. The motifs, typical of ATP-ase located in D5 and D6, are highlighted in black.

Supp Fig. 3. Functional Mfd modules are rendered by surface, with respect to the color code used in Supp Fig. 2. The function of each module and the average identity in sequence between the ESKAPE modeled Mfd is noted below each module.

Supp Fig. 4. Homology models of Mfd from ESKAPE bacteria in complex with NM102. Inserted are close-views of their respective active site with residues involved in the binding of NM102 highlighted in white squares. The NM102 is shown as bold color-coded stick. The carbon, nitrogen, oxygen, sulfur atoms are orange, blue, red and yellow, respectively.

Supp Fig. 5. NM102 nanoformulation. (A) Representative images of empty and NM102 nanoformulations. Size distribution of NM102 nanoformulation by (B) intensity, (C) volume and (D) number. Evolution with time of intensity-averaged diameter of NM102 nanoformulation at (E) 4°C and (F) room temperature up to 14 days.

Supp Fig. 6. Antimicrobial effect of NM102 *in vivo. K. pneumoniae* ATCC 700603 wt and Δ*mfd* were administered intranasally through slow instillation of 20 µL of bacterial suspension (1x 10^7 CFU). 200 µL of NM102 formulation (NF-NM102 6 mg/kg) or empty formulation (control) or MPN (10 mg/kg) were administrated in mice *via* the i.p. route. Mice were sacrificed by cervical dislocation after 24 h and the CFU in the lung was calculated per gram of organ. For each strain (wt and Δ*mfd*), the CFU values were normalized to the controls. Data represent the mean values with standard deviation. For wt: CTR n=9, NM102 6 mg/kg n=6, MPN 10 mg/kg n=9; for Δ*mfd*: CTR n=10, NM102 6 mg/kg n=10, MPN 10 mg/kg n=10.

Supp Fig. 7. NM102 does not impact the host microbiome. The quantity of total bacteria present in the mice feces was quantified by qPCR at day 1 and day 7 post-administration of NM102. The graph shows the mean ± SD of two independent samples.

Supp Fig. 8. NM102 decreases *B. cereus* survival in the context of NO-stress. *B. cereus* wt (A) and Δ*mfd* (B) strains were exposed to NM102 (40 µM) and NOC-5 and bacterial growth was followed by measuring the $OD_{600\,nm}$ for 12 h. The results reported are mean ± SD of three independent experiments each in triplicates, P values are calculated against the condition without NM102, using One Way ANOVA (**** P<0.0001)

Supp Fig. 9. Genetic environment of the *mfd* gene in *E. coli* and *K. pneumoniae*. The sequences of the *mfd* genes and their respective environment were retrieved from ncbi using the following references: *Escherichia coli* str. K-12 substr. MG1655 (GCF_000005845.2) and *Klebsiella quasipneumoniae* strain ATCC 700603 (CP014696.2)