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Supporting information for article:

Structure activity and thermostability investigations of OXA-163, OXA-181 and OXA-245 using biochemical, crystal structures and differential scanning calorimetry analysis

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Table S1 Macromolecule production information for the cloning and production of OXA-163, OXA-181 and OXA-245.

Source organism	<i>Klebsiella pneumoniae</i>	
DNA source	Genomic DNA	
	Native construct with signal peptide:	His-tagged construct with TEV-protease cleavage site:
Forward primer	OXAs: ATAATTTGTTAACCTTAAGA AGGAGATATACATATGCGTGT ATTAGCCTTATC GG	TEV-cleavage site^a: ACCATCACCTCGAACAAAGTTG TACGGTGAGAACATCTTATTTTC GGGTT
Reverse primer	OXAs: GGCTTGTTAGCAG CCTCGAACACTAGGAAATAA TTTTTCCTGTTGAG	TEV-cleavage site^a: GGCTTGTTAGCAGCCTCGAAC CCTGAAAATAAAAGATTCTCACCG
EMP R2 primer	TTCTAGAGGGAAACCGTTGT GTCT	OXAs: GGCTTGTTAGCAGCCTCGAAC AGGAAATAATTTCCTGTTGAG
Cloning vector	pDEST17	
Expression vector	pDEST17	
Expression host	<i>E. coli</i> BL21 (DE3) STAR pRARE	
Complete amino acid sequence of the construct produced^a		
OXA-163^b	(tOXA-163)	
	HHHHHHENLYFQGKEWQENKSWNAHF TEHKSGVVVLWNENKQQGFTNNLKR ANQAFLPASTFKIPNSLIALDLGVVKDE HQVFKWDGQTRDIATWNRDHNLITAM KYSVVPVYQEFAQRQIGEARMSKMLHAF DYGNEDISGNVDSFWLDGGIRISATEQIS FLRKLYHNKLHVRSQRIVKQAMLTE ANGDYIIRAKTGYDTKIGWWVGWVEL DDNVWFFAMNMDMPTSDGLGLRQAIT	

KEVLKQEKEIIP**OXA-181****(nOXA-181)****(tOXA-181)**

<u>MRVLALSAVFLVASIIGMPAVA</u>	<u>MSYYHHHHHLESTSILYGENLYFQ</u>
KEWQENKSWNAHFTEHKSGQ	GKEWQENKSWNAHFTEHKSGVV
VVVLWNENKQQGFTNNLKRA	VLWNENKQQGFTNNLKRNQAFPLP
NQAFLPASTFKIPNSLIALDLGV	ASTFKIPNSLIALDLGVVKDEHQVF
VKDEHQVFKWDGQTRDIAAW	KWDGQTRDIAAWNRDHDLITAMK
NRDHDLITAMKYSVVPVYQEF	YSVVPVYQEFARQIGEARMSKMLH
ARQIGEARMSKMLHAFDYGNE	AFDYGNEDISGNVDSFWLDGGIRIS
DISGNVDSFWLDGGIRISATQQI	ATQQIAFLRKLYHNKLHVSERSQRI
AFLRKLYHNKLHVSERSQRIVK	VKQAMLTEANGDYIIRAKTGYSTRI
QAMLTEANGDYIIRAKTGYSTR	EPKIGWWVGWVELDDNVWFFAMN
IEPKIGWWVGWVELDDNVWFF	MDMPTSDGLGLRQAITKEVLKQEKI
AMNMDMPTSDGLGLRQAITKE	IP
VLKQEKEIIP	

OXA-245**(nOXA-245)****(tOXA-245)**

<u>MRVLALSAVFLVASIIGMPAVA</u>	<u>MSYYHHHHHLESTSILYGENLYFQ</u>
KEWQENKSWNAHFTEHKSGQ	GKEWQENKSWNAHFTEHKSGVV
VVVLWNENKQQGFTNNLKRA	VLWNENKQQGFTNNLKRNQAFPLP
NQAFLPASTFKIPNSLIALDLGV	ASTFKIPNSLIALDLGVVKDEHQVF
VKDEHQVFKWDGQTRDIATW	KWDGQTRDIATWNRDHNLITAMK
NRDHNLITAMKYSVVPVYQYF	YSVVPVYQYFARQIGEARMSKMLH
ARQIGEARMSKMLHAFDYGNE	AFDYGNEDISGNVDSFWLDGGIRIS
DISGNVDSFWLDGGIRISATEQI	ATEQISFLRKLYHNKLHVSERSQRIV
SFLRKLYHNKLHVSERSQRIVK	KQAMLTEANGDYIIRAKTGYSTRIE
QAMLTEANGDYIIRAKTGYSTR	PKIGWWVGWVELDDNVWFFAMN
IEPKIGWWVGWVELDDNVWFF	MDMPTSDGLGLRQAITKEVLKQEKI
AMNMDMPTSDGLGLRQAITKE	IP
VLKQEKEIIP	

^a The nucleotides in the TEV protease cleavage site sequence is in italics. Underlined residues in the amino acid sequence are cleaved off by signal peptide peptidases in transport to the periplasm for the native construct or by an in-house TEV-protease during purification for the His-tagged construct.

^b The gene for OXA-163 was synthesized with optimized codon usage and subcloned into the expression vector.

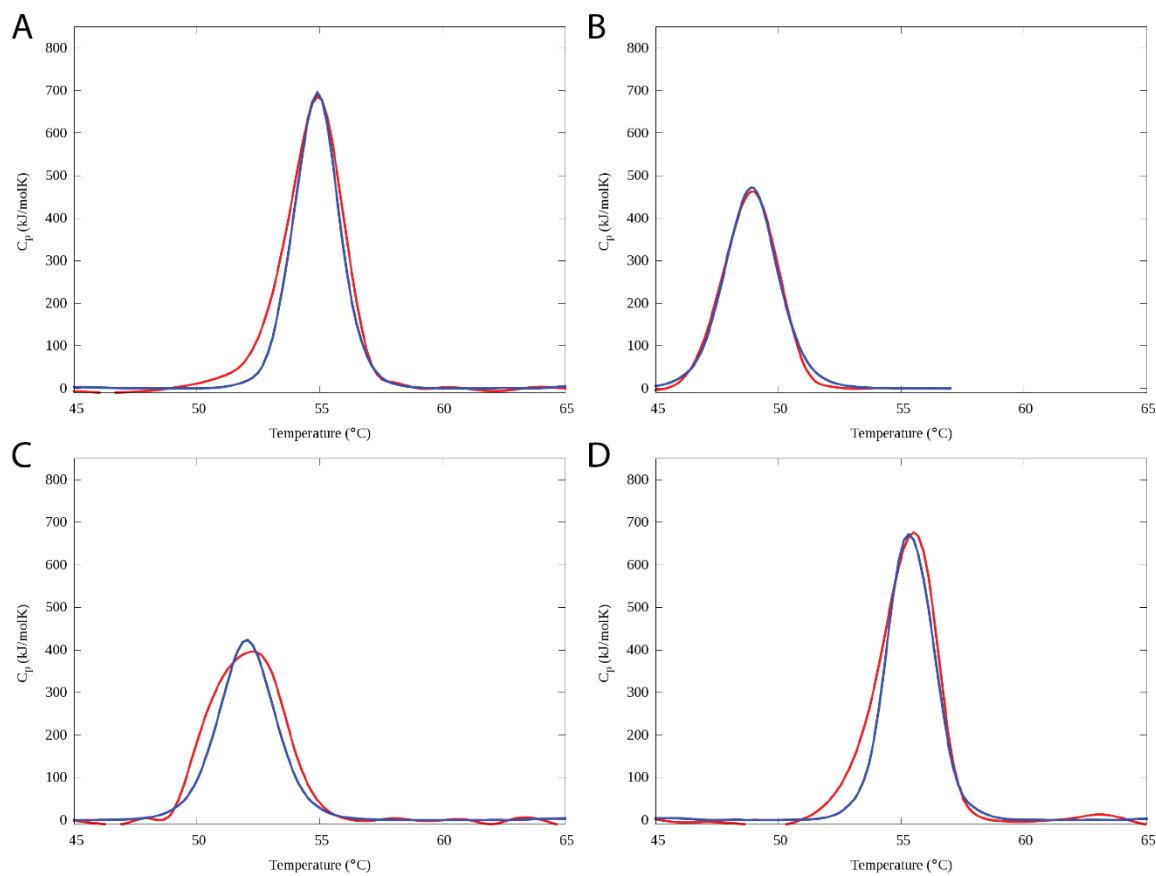


Figure S1 Differential scanning calorimetry curves (red) for (A) OXA-48, (B) OXA-163, (C) OXA-181 and (D) OXA-245 with the theoretical two-state model fitted (blue) for the respective dimers to calculate the ΔH of unfolding.