Antibiotic Potential and Biophysical Characterization of Amphipathic β-Stranded $[XZ]_n$ Peptides With Alternating Cationic and Hydrophobic Residues

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Supporting Information

TABLE S1. CD results for $[XZ]_n$ peptides in different environments. (Abbreviations for secondary structures: α, α-helical structures; β, β-like structures; AP β, antiparallel β-sheet; Rc, random coil.)

Peptide	Environment							Reference
	Method	**Water**	**PB**	**Buffer**	**SDS micelles**	**Vesicles**		
						Neutral	Anionic	
$[KL]_8$	CD	β						(Baumruk et al. 1994)
$[KL]_{10}$	CD	Rc/β[a]						(Baumruk et al. 1994)
$[KL]_8$	IR	AP β						(Baumruk et al. 1994)
$[KL]_n$	CD	Rc/β[b]						(Brack and Spach 1981)
$[KL]_n$	IR	AP β						(Brack and Spach 1981)
Dansyl-$[KL]_4$K-NH_2	IR	AP β				AP β (DMPC monolayer)		(Castano et al. 2000)
Dansyl-$[KL]_5$K-NH_2	IR	AP β				AP β (DMPC monolayer)		(Castano et al. 2000)
Dansyl-$[KL]_6$K-NH_2	IR	AP β				AP β (DMPC monolayer)		(Castano et al. 2000)
Dansyl-$[KL]_7$K-NH_2	IR	AP β				AP β (DMPC monolayer)		(Castano et al. 2000)
C_{16}-$[VK]_4$	CD	β						(de Almeida et al. 2019)
L$[KL]_3$	CD			β (Tris)				(DeGrado and Lear 1985)
L$[KL]_3$	IR			β (Tris)				(DeGrado and Lear 1985)
$[KW]_3$	CD		Rc			Rc (EYPC/Chol)[e]	Rc+β (EYPE/EYPG)[f]	(Gopal et al. 2011)
$[WK]_3$	CD		Rc			Rc (EYPC/Chol)[e]	Rc+β (EYPE/EYPG)[f]	(Gopal et al. 2011)
$[KL]_4$K	CD	Rc		β (PBS)				(Hernández et al. 2006)
$[KL]_7$K	CD	Rc		β (PBS)				(Hernández et al. 2006)
RW-NH_2	CD		Rc			Rc (POPC in PB)[g]	Rc (POPG in PB)[h]	(Liu et al. 2007)
$[RW]_2$-NH_2	CD		Rc			Rc (POPC in PB)[g]	Rc (POPG in PB)[h]	(Liu et al. 2007)
$[RW]_3$-NH_2	CD		Rc			Rc (POPC in PB)[g]	Rc+β (POPG in PB)[h]	(Liu et al. 2007)
$[RW]_4$-NH_2	CD		Rc			Rc+β (POPC in PB)[g]	Rc+β (POPG in PB)[h]	(Liu et al. 2007)
$[RW]_5$-NH	CD		Rc			β (POPC in PB)[g]	β (POPG in PB)[h]	(Liu et al. 2007)
$[VRVK]_2$ -NH_2	CD	Rc			β			(Ong et al. 2013)
$[IRIR]_2$ -NH_2	CD	Rc			β			(Ong et al. 2013)
$[IKIK]_2$-NH_2	CD	Rc			β			(Ong et al. 2013)
$[IRIK]_2$-NH_2	CD	Rc			β			(Ong et al. 2013)
$[IRVK]_2$-NH_2	CD	Rc			β			(Ong et al. 2013)
$[FRFK]_2$-NH_2	CD	Rc			β			(Ong et al. 2013)
$[WRWK]_2$-NH_2	CD	Rc						(Ong et al. 2013)
$[VRVK]_3$-NH_2	CD	Rc			β			(Ong et al. 2013)
$[IRIK]_3$-NH_2	CD	Rc			β			(Ong et al. 2013)
$[IRVK]_3$-NH_2	CD	Rc			β			(Ong et al. 2013)

Ac-[KV]$_2$-NHCH$_3$	CD			Rc (Hepes)		Rc (DPPC)	Rc (DPPC/DPPG)[d]	(Ono et al. 1990)
Ac-[KV]$_3$-NHCH$_3$	CD			Rc (Hepes)		Rc (DPPC)	α (DPPC/DPPG)[d]	(Ono et al. 1990)
Ac-[KV]$_4$-NHCH$_3$	CD			Rc (Hepes)		Rc (DPPC)	β (DPPC/DPPG)[d]	(Ono et al. 1990)
[KL]$_3$-NH$_2$	CD	Rc					Rc (POPC/POPG)[c]	(Schweigardt 2020)
[KL]$_4$-NH$_2$	CD	Rc					β (POPC/POPG)[c]	(Schweigardt 2020)
[KL]$_4$K-NH$_2$	CD	Rc					β (POPC/POPG)[c]	(Schweigardt 2020)
L[KL]$_4$-NH$_2$	CD	Rc					β (POPC/POPG)[c]	(Schweigardt 2020)
[KL]$_5$-NH$_2$	CD	Rc					β (POPC/POPG)[c]	(Schweigardt 2020)
[LK]$_5$-NH$_2$	CD	Rc					β (POPC/POPG)[c]	(Schweigardt 2020)
[KL]$_5$K-NH$_2$	CD	Rc					β (POPC/POPG)[c]	(Schweigardt 2020)
L[KL]$_5$-NH$_2$	CD	Rc					β (POPC/POPG)[c]	(Schweigardt 2020)
[KL]$_6$-NH$_2$	CD	Rc					β (POPC/POPG)[c]	(Schweigardt 2020)
[KL]$_6$K-NH$_2$	CD	Rc					β (POPC/POPG)[c]	(Schweigardt 2020)
L[KL]$_6$-NH$_2$	CD	Rc					β (POPC/POPG)[c]	(Schweigardt 2020)
[KL]$_7$-NH$_2$	CD	Rc					β (POPC/POPG)[c]	(Schweigardt 2020)
[KL]$_7$K-NH$_2$	CD	Rc					β (POPC/POPG)[c]	(Schweigardt 2020)
L[KL]$_7$-NH$_2$	CD	Rc					β (POPC/POPG)[c]	(Schweigardt 2020)
[KL]$_8$-NH$_2$	CD	Rc					β (POPC/POPG)[c]	(Schweigardt 2020)
[KL]$_9$-NH$_2$	CD	Rc					β (POPC/POPG)[c]	(Schweigardt 2020)
[KL]$_{10}$-NH$_2$	CD	Rc					β (POPC/POPG)[c]	(Schweigardt 2020)
[KL]$_{11}$-NH$_2$	CD	Rc					β (POPC/POPG)[c]	(Schweigardt 2020)
[KL]$_{12}$-NH$_2$	CD	Rc					β (POPC/POPG)[c]	(Schweigardt 2020)
[KL]$_{13}$-NH$_2$	CD	Rc					β (POPC/POPG)[c]	(Schweigardt 2020)
[KL]$_3$-NH$_2$	CD	Rc	Rc			Rc (DMPC)	Rc (POPC/POPG)[c]	(Strandberg et al. 2020)
[KL]$_5$-NH$_2$	CD	Rc	β			Rc (DMPC)	β (POPC/POPG)[c]	(Strandberg et al. 2020)
[KL]$_7$-NH$_2$	CD	Rc	β			Rc (DMPC)	β (POPC/POPG)[c]	(Strandberg et al. 2020)
[KL]$_9$-NH$_2$	CD	Rc	β			Rc (DMPC)	β (POPC/POPG)[c]	(Strandberg et al. 2020)

[a] Random coil at low concentrations up to 3.55 mg/ml, β-structure at high concentrations from 7.1 mg/ml, no salt.
[b] Random coil in pure water, β-structure in 0.2 M NaCl.
[c] POPC/POPG (1/1 mol/mol) in water, pH ≈ 6
[d] DPPC/DPPG (3/1) in 5 mM Hepes buffer, pH 7.4
[e] Egg yolk-PC/cholesterol (10/1 w/w) in 10 mM sodium phosphate buffer
[f] Egg yolk-PE/egg yolk-PG (7/3 w/w) in 10 mM sodium phosphate buffer
[g] POPC in 10 mM phosphate buffer, 100 mM NaCl, pH 7.4. Strong Trp bands makes the secondary structure analysis uncertain. The stated secondary structures are based on our own analysis of the published spectra.
[h] POPG in 10 mM phosphate buffer, 100 mM NaCl, pH 7.4. Strong Trp bands makes the secondary structure analysis uncertain. The stated secondary structures are based on our own analysis of the published spectra.

TABLE S2. Overview of antimicrobial and antifungal assay results on XZ peptides. Exact values of MIC and details about the assay used can be found in the references.

Microorganism	**Antimicrobial activity (MIC values)**			**Reference**
	High (<10 μg/ml)	**Medium (10-100 μg/ml)**	**Low (>100 μg/ml)**	
Gram-negative bacteria				
E. coli DSM 30083		$[RW]_3$, $K[RW]_3$, $[RW]_3K$		(Albada et al. 2012)
A. baumannii DSM 30007		$[RW]_3$	$K[RW]_3$, $[RW]_3K$	(Albada et al. 2012)
P. aeruginosa DSM 50071			$[RW]_3$, $K[RW]_3$, $[RW]_3K$	(Albada et al. 2012)
A. laidlawii A-EF22	$[KL]_4K$	$[KL]_5K$, $[KL]_7K$		(Beven et al. 2003)
E. coli ATCC 25922			Acetyl-$[KL]_9$-NH_2	(Blondelle and Houghten 1992)
P. aeruginosa ATCC 27853			Acetyl-$[KL]_9$-NH_2	(Blondelle and Houghten 1992)
E. coli KCTC 1682		$[KW]_3$	$[WK]_3$	(Gopal et al. 2011)
S. typhimurium KCTC 1926		$[WK]_3$, $[KW]_3$		(Gopal et al. 2011)
P. aeruginosa KCTC 1637		$[WK]_3$, $[KW]_3$		(Gopal et al. 2011)
E. coli D31	$[RW]_4$, $[RW]_5$	$[RW]_3$	RW, $[RW]_2$	(Liu et al. 2007)
E. coli	$[IRIR]_2$	$[IKIK]_2$, $[IRIK]_2$, $[IRVK]_2$, $[FRFK]_2$, $[WRWK]_2$, $[VRVK]_3$, $[IRVK]_3$	$[VRVK]_2$, $[IRIK]_3$	(Ong et al. 2013)
P. aeruginosa	$[IRIR]_2$	$[IKIK]_2$, $[IRIK]_2$, $[IRVK]_2$, $[FRFK]_2$, $[VRVK]_3$, $[IRVK]_3$	$[VRVK]_2$, $[WRWK]_2$, $[IRIK]_3$	(Ong et al. 2013)
E. coli DSM 1116	$[KL]_5$, $[LK]_5$, $[KL]_5K$, $L[KL]_5$, $[KL]_6$, $[KL]_6K$, $L[KL]_6$, $[KL]_7$, $[KL]_7K$,	$[KL]_4$, $[KL]_4K$, $L[KL]_4$, $L[KL]_7$, $[KL]_8$, $[KL]_9$, $[KL]_{10}$, $[KL]_{11}$, $[KL]_{12}$, $[KL]_{13}$	$[KL]_3$	(Schweigardt 2020; Strandberg et al. 2020)
E. helveticus DSM 18390	$[KL]_4K$, $L[KL]_4$, $[KL]_5$, $[LK]_5$, $[KL]_5K$, $L[KL]_5$, $[KL]_6$, $[KL]_6K$, $L[KL]_6$, $[KL]_7$, $[KL]_7K$, $[KL]_8$	$[KL]_4$, $L[KL]_7$, $[KL]_9$, $[KL]_{10}$, $[KL]_{11}$, $[KL]_{12}$, $[KL]_{13}$	$[KL]_3$	(Schweigardt 2020; Strandberg et al. 2020)
E. coli 2592		$[LK]_7$, $[LK]_9$, $[LK]_{12}$, $[LR]_7$	$[LK]_8$, $[LK]_{10}$, $[LK]_{11}$, $[LR]_9$, $[LR]_{11}$	(Strom and Brondsema 2006)
P. aeruginosa 10145		$[LK]_9$, $[LK]_{11}$, $[LK]_{12}$	$[LK]_7$, $[LK]_8$, $[LK]_{10}$, $[LR]_7$, $[LR]_9$, $[LR]_{11}$	(Strom and Brondsema 2006)
E. coli ATCC 25922	$[RW]_3$	$[WR]_3$, $W[RW]_2$	$[RW]_2R$, $[WR]_2$, WRW, RWR	(Strøm et al. 2003; Strøm et al. 2002)
P. aeruginosa ATCC 27853		$[RW]_3$, $W[RW]_2$, $[RW]_2R$		(Strøm et al. 2002)
Gram-positive bacteria				
S. aureus DSM 20231		$[RW]_3$, $K[RW]_3$, $[RW]_3K$		(Albada et al. 2012)
S. aureus ATCC 43300	$[RW]_3$, $K[RW]_3$	$[RW]_3K$		(Albada et al. 2012)
B. subtilis 168 DSM 402	$[RW]_3$, $K[RW]_3$, $[RW]_3K$			(Albada et al. 2012)
M. gallisepticum S6	$[KL]_4K$	$[KL]_5K$, $[KL]_7K$		(Beven et al. 2003)

S. aureus ATCC 29213			Acetyl-$[KL]_9$-NH_2	(Blondelle and Houghten 1992)
S. aureus KCTC 1621			$[WK]_3$, $[KW]_3$	(Gopal et al. 2011)
B. subtilis KCTC 1918			$[WK]_3$, $[KW]_3$	(Gopal et al. 2011)
L. monocytogenes KCTC 3710		$[KW]_3$	$[WK]_3$	(Gopal et al. 2011)
S. aureus ATCC BAA-44	$[RW]_3$, $[RW]_4$, $[RW]_5$		RW, $[RW]_2$	(Liu et al. 2007)
S. epidermidis	$[IRIR]_2$, $[IKIK]_2$, $[IRIK]_2$, $[IRVK]_2$, $[FRFK]_2$, $[WRWK]_2$, $[VRVK]_3$, $[IRIK]_3$, $[IRVK]_3$	$[VRVK]_2$		(Ong et al. 2013)
S. aureus		$[IRIR]_2$, $[IRIK]_2$, $[WRWK]_2$, $[VRVK]_3$, $[IRIK]_3$, $[IRVK]_3$	$[IKIK]_2$, $[VRVK]_2$, $[IRVK]_2$, $[FRFK]_2$	(Ong et al. 2013)
B. subtilis DSM 347	$L[KL]_4$, $[KL]_5$, $[LK]_5$, $[KL]_5K$, $L[KL]_5$, $[KL]_6$, $[KL]_6K$, $L[KL]_6$, $[KL]_7$, $[KL]_7K$	$[KL]_4$, $[KL]_4K$, $L[KL]_7$, $[KL]_8$, $[KL]_9$, $[KL]_{10}$, $[KL]_{11}$, $[KL]_{12}$, $[KL]_{13}$	$[KL]_3$	(Schweigardt 2020; Strandberg et al. 2020)
S. xylosus DSM 20287	$[KL]_4$, $[KL]_4K$, $L[KL]_4$, $[KL]_5$, $[LK]_5$, $[KL]_5K$, $L[KL]_5$, $[KL]_6$, $[KL]_6K$, $L[KL]_6$, $[KL]_7$, $[KL]_7K$, $[KL]_8$, $[KL]_9$, $[KL]_{12}$	$L[KL]_7$, $[KL]_{10}$, $[KL]_{11}$, $[KL]_{13}$	$[KL]_3$	(Schweigardt 2020; Strandberg et al. 2020)
S. aureus ATCC 25923	$[WR]_3$, $[RW]_3$	$W[RW]_2$, $[RW]_2R$	$[WR]_2$, WRW, RWR	(Strøm et al. 2003; Strøm et al. 2002)
Fungal strains				
C. albicans KCTC 7270		$[WK]_3$, $[KW]_3$		(Gopal et al. 2011)
T. beigelli KCTC 7707	$[KW]_3$	$[WK]_3$		(Gopal et al. 2011)
F. solani KCTC 6326	$[KW]_4$, $[KW]_5$, $[RW]_4$, $[RW]_5$	$[KW]_3$, $[RW]_3$	$[KW]_2$, $[RW]_2$	(Gopal et al. 2012)
F. oxysporum KCTC 6076	$[KW]_5$, $[RW]_5$	$[KW]_3$, $[KW]_4$, $[RW]_3$, $[RW]_4$	$[KW]_2$, $[RW]_2$	(Gopal et al. 2012)
C. albicans	$[IKIK]_2$, $[IRIK]_2$, $[IRVK]_2$	$[VRVK]_2$, $[IRIR]_2$, $[FRFK]_2$, $[VRVK]_3$	$[WRWK]_2$, $[IRIK]_3$, $[IRVK]_3$	(Ong et al. 2013)
C. albicans 10231			$[LK]_7$, $[LK]_8$, $[LK]_9$, $[LK]_{10}$, $[LK]_{11}$, $[LK]_{12}$, $[LR]_7$, $[LR]_9$, $[LR]_{11}$	(Strom and Brondsema 2006)
Cr. Neoformans 32045	$[LK]_7$, $[LR]_7$	$[LK]_8$, $[LK]_9$, $[LK]_{10}$, $[LK]_{11}$, $[LK]_{12}$, $[LR]_9$, $[LR]_{11}$		(Strom and Brondsema 2006)
Resistant strains				
E. coli CCARM 1229		$[KW]_3$	$[WK]_3$,	(Gopal et al. 2011)
E. coli CCARM 1238			$[WK]_3$, $[KW]_3$	(Gopal et al. 2011)
C. albicans CCARM 14001		$[WK]_3$, $[KW]_3$		(Gopal et al. 2011)
C. albicans CCARM 14007		$[WK]_3$, $[KW]_3$		(Gopal et al. 2011)
C. albicans CCARM 14020		$[WK]_3$, $[KW]_3$		(Gopal et al. 2011)
S. aureus (methillicin resistant) 33591			$[LK]_7$, $[LK]_8$, $[LK]_9$, $[LK]_{10}$, $[LK]_{11}$, $[LK]_{12}$, $[LR]_7$, $[LR]_9$, $[LR]_{11}$	(Strom and Brondsema 2006)

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