Supplementary material for:

Goldilocks Dilemma: LPS Works Both as the Initial Target and a Barrier for the Antimicrobial Action of Cationic AMPs on *E. coli*

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Peptide chemical analysis

The crude cyclic peptides were purified by preparative reverse phase-HPLC using gradients from 15 to 40 % buffer B (buffer A: water/0.1% TFA, buffer B: acetonitrile/0.1% TFA) over 50 minutes (11 ml/min flow rate). c(LWwNKr) was isolated as a white fluffy solid. **HRMS** (ESI): Calculated for: $C_{44}H_{62}N_{13}O_7^+$ $[M+H]^+$ 884.4890; found; 884.4885. c(WWWRRR) was isolated as a white solid. **HRMS** (ESI): Calculated for: $C_{51}H_{68}N_{18}O_6^{2+}$ $[M+H]^{2+}$ 514.2779; found; 514.2775. c(WRWRWR) was isolated as a white solid. **HRMS** (ESI): Calculated for: $C_{51}H_{68}N_{18}O_6^{2+}$ $[M+H]^{2+}$ 514.2779; found; 514.2773. c(WWWKKK) was isolated as a white solid. **HRMS** (ESI): Calculated for: $C_{51}H_{68}N_{12}O_6^{2+}$ $[M+H]^{2+}$ 472.2687; found; 472.2682. c(WKWKWK) was isolated as a white solid. **HRMS** (ESI): Calculated for: $C_{51}H_{68}N_{12}O_6^{2+}$ $[M+H]^{2+}$ 472.2687; found; 472.2681.

Table S1: K_P and k_{off} from Figure 2.

AMP	K_P			k_{off} (s^{-1})		
	DMPC	***DMPC:PG***	***DMPC:LPS***	***DMPC***	***DMPC:PG***	***DMPC:LPS***
LWwNKr	278 ± 8	401 ± 19	1145 ± 99	1.76 ± 0.12	1.75 ± 0.16	1.01 ± 0.18
WKWKWK	531 ± 10	630 ± 33	1191 ± 166	0.90 ± 0.24	1.32 ± 0.05	0.95 ± 0.12
WRWRWR	1299 ± 94	3160 ± 15	4520 ± 215	0.87 ± 0.19	0.48 ± 0.05	0.70 ± 0.16
WWWKKK	2534 ± 80	5156 ± 34	3943 ± 218	0.48 ± 0.07	0.32 ± 0.05	0.60 ± 0.15
WWWRRR	6649 ± 80	12705 ± 16	17040 ± 1150	0.22 ± 0.02	0.19 ± 0.01	0.25 ± 0.03

Table S2: γB/γF-1 ratios.

AMP	γB/γF-1						
	DMPC	*DMPC:LPS*	*E. coli ATCC 25922 lysate*	*E. coli ATCC 25922*	*E. coli CCUG 70662-*	*E. coli CCUG 70662+*	*E. coli NR 698*
LWwNKr	7.830	2.620	0.035	0.106	5.202	4.990	0.186
WKWKWK	7.127	3.915	0.044	0.083	0.170	0.295	0.180
WRWRWR	2.900	3.475	0.275	0.143	0.549	1.062	3.059
WWWKKK	0.297	0.402	0.225	0.289	0.317	0.197	0.370
WWWRRR	0.247	0.199	0.216	0.239	0.473	0.238	0.819

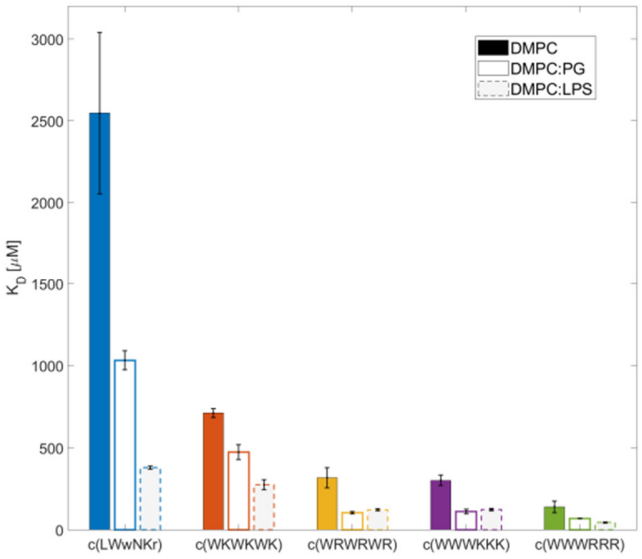

Figure S1: K_D extracted from steady state SPR analysis

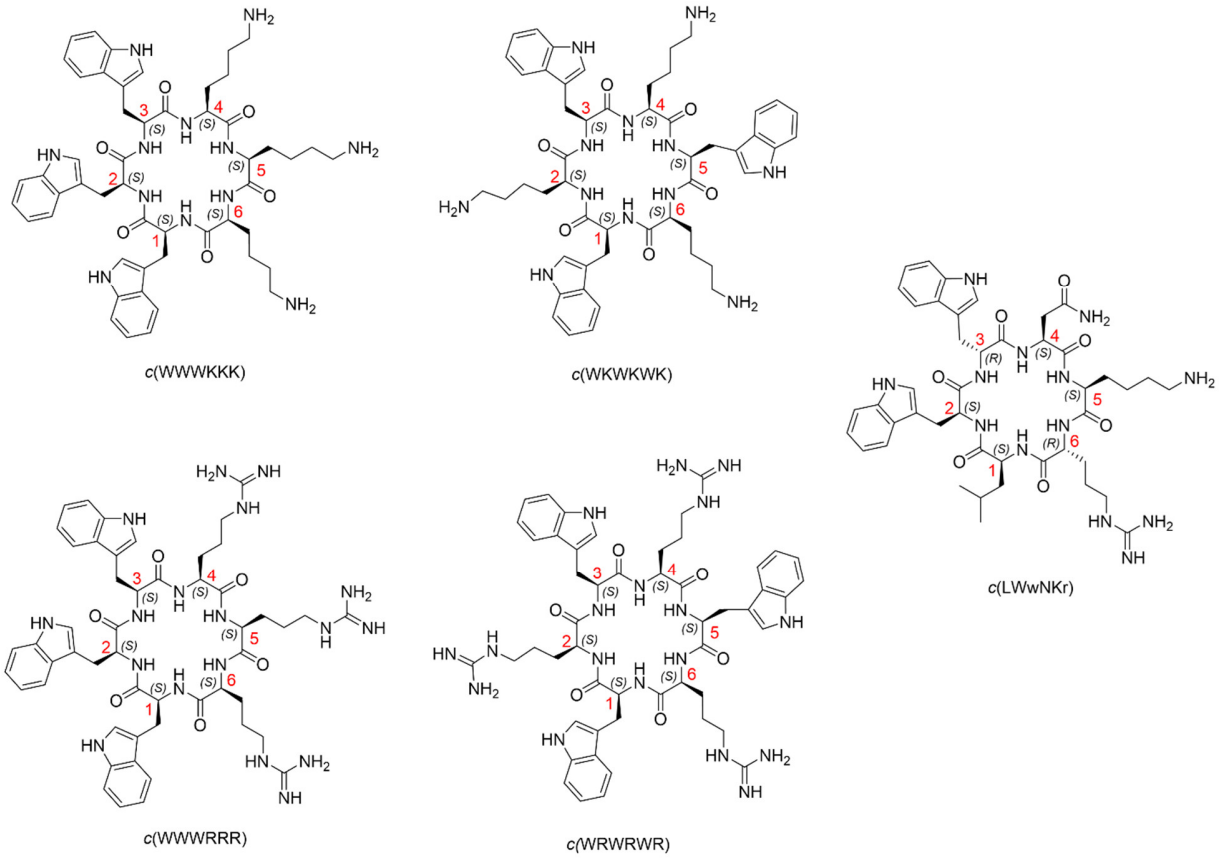

Figure S2: The five peptides with numbered amino acids for structure elucidation.

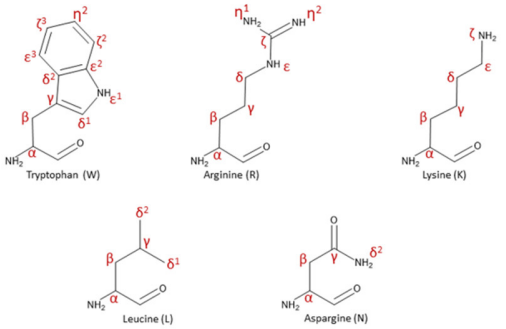

Figure S3: Labelled amino acids.

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Table S3: Full assignment of c(WWWKKK) according to Figure S2

c(WWWKKK)														
Residue #	Amino acid	Chemical shift (δ)												
	W - Tryptophan	NHα	CO	α	β	ε1	δ1	γ	δ2	ε3	ζ3	η2	ζ2	ε2
1	^{1}H	7.85	-	4.48	2.99/2.86	10.10	7.06	-	-	7.46	7.09	7.17	7.41	-
	^{13}C/^{15}N	118.01	173.41	54.70	26.00	129.28	124.45	108.96	127.01	118.48	119.41	122.04	111.98	136.34
	W - Tryptophan	NHα	CO	α	β	ε1	δ1	γ	δ2	ε3	ζ3	η2	ζ2	ε2
2	^{1}H	7.33	-	4.51	3.11/2.87	10.05	6.95	-	-	7.30	7.05	7.15	7.39	-
	^{13}C/^{15}N	119.00	172.67	54.55	26.36	130.00	124.76	108.30	127.06	118.30	119.59	122.16	112.04	136.20
	W - Tryptophan	NHα	CO	α	β	ε1	δ1	γ	δ2	ε3	ζ3	η2	ζ2	ε2
3	^{1}H	7.55	-	4.45	3.06/2.76	10.07	6.92	-	-	7.30	7.02	7.11	7.33	-
	^{13}C/^{15}N	121.25	172.67	55.19	26.06	129.56	124.19	108.54	126.86	118.30	119.44	122.05	111.94	136.24
	K -Lysine	NHα	CO	α	β	γ	δ	ε	ζ					
4	^{1}H	7.84	-	3.75	1.49/1.67	0.66	1.30/1.58	2.26	7.41	-	-	-	-	-
	^{13}C/^{15}N	120.75	173.08	54.90	30.07	21.86	26.19	39.24	96.51	-	-	-	-	-
	K -Lysine	NHα	CO	α	β	γ	δ	ε	ζ					
5	^{1}H	8.03	-	4.10	1.51	1.68/1.50	1.32/1.24	2.88	7.46	-	-	-	-	-
	^{13}C/^{15}N	119.47	173.29	53.60	28.83	30.06	22.34	39.43	96.73	-	-	-	-	-
	K -Lysine	NHα	CO	α	β	γ	δ	ε	ζ					
6	^{1}H	7.77	-	3.88	1.22	0.85/0.74	1.33	2.61	7.37	-	-	-	-	-
	^{13}C/^{15}N	119.35	173.76	54.55	29.43	21.86	X	39.24	96.52	-	-	-	-	-

Table S4: Full assignment of c(WKWKWK) according to Figure S2.

c(WKWKWK)														
Residue #	Amino acid	Chemical shift (δ)												
1	W - Tryptophan	NHα	CO	α	β	ε1	δ1	γ	δ2	ε3	ζ3	η2	ζ2	ε2
	^{1}H	7.99	-	4.54	3.09	10.17	7.16	-	-	7.52	7.10	7.17	7.37	-
	^{13}C/^{15}N	119.292	173.01	54.66	25.99	129.67	124.35	108.77	127.06	118.37	119.46	122.03	112.04	136.21
2	K -Lysine	NHα	CO	α	β	γ	δ	ε	ζ	-	-	-	-	-
	^{1}H	7.62	-	3.89	1.42	0.67	1.28	2.55	7.35	-	-	-	-	-
	^{13}C/^{15}N	120.24	172.91	54.55	29.59	21.59	26.21	39.25	134.12/95.83	-	-	-	-	-

Table S5: Full assignment of c(WRWRWR) according to Figure S2.

c(WRWRWR)														
Residue #	Amino acid	Chemical shift (δ)												
1	W - Tryptophan	NHα	CO	α	β	ε1	δ1	γ	δ2	ε3	ζ3	η2	ζ2	ε2
	^{1}H	8.07	-	4.54	3.13	10.13	7.16	-	-	7.53	7.10	7.16	7.37	-
	^{13}C/^{15}N	119.43	172.84	54.84	26.04	129.65	124.44	108.78	126.96	118.23	119.39	122.07	111.97	136.23
2	R - Arginine	NHα	CO	α	β	γ	δ	ε	ζ	η1	η2	-	-	-
	^{1}H	7.65	-	3.90	1.41	0.82	2.71	6.77	-	X	X	-	-	-
	^{13}C/^{15}N	119.96	172.88	54.39	27.28	23.71	40.44	84.78	153.71	X	X	-	-	-

Table S6: Full assignment of c(WWWRRR) according to Figure S2.

c(WWWRRR)

Residue #	Amino acid	Chemical shift (δ)												
	W - Tryptophan	NHα	CO	α	β	ε1	δ1	γ	δ2	ε3	ζ3	η2	ζ2	ε2
1	^{1}H	7.88	-	4.52	2.85/3.04	10.03	7.04	-	-	7.46	7.07	7.15	7.38	-
	^{13}C/^{15}N	118.00	173.20	54.68	26.04	129.05	124.44	109.20	126.79	118.31	119.29	122.02	111.89	136.27
	W - Tryptophan	NHα	CO	α	β	ε1	δ1	γ	δ2	ε3	ζ3	η2	ζ2	ε2
2	^{1}H	7.42	-	4.54	2.98/3.03	10.05	7.02	-	-	7.38	7.08	7.16	7.40	-
	^{13}C/^{15}N	119.05	172.55	54.36	26.68	130.00	124.76	108.40	127.37	118.30	119.44	122.03	112.02	136.22
	W - Tryptophan	NHα	CO	α	β	ε1	δ1	γ	δ2	ε3	ζ3	η2	ζ2	ε2
3	^{1}H	7.64	-	4.43	2.85/3.05	10.03	6.94	-	-	7.36	7.04	7.12	7.33	-
	^{13}C/^{15}N	121.23	173.10	55.49	26.04	129.50	124.27	108.40	126.82	118.17	119.34	122.03	111.87	136.23
	R - Arginine	NHα	CO	α	β	γ	δ	ε	ζ	η1	η2	-	-	-
4	^{1}H	7.94	-	3.72	1.58/1.50	0.74/0.87	2.75/2.73	6.77	-	X	X	-	-	-
	^{13}C/^{15}N	120.23	172.89	54.85	26.37	24.11	40.38	85.03	156.63	X	X	-	-	-
	R - Arginine	NHα	CO	α	β	γ	δ	ε	ζ	η1	η2	-	-	-
5	^{1}H	8.15	-	4.18	1.48/1.75	1.49	3.11	7.12	-	X	X	-	-	-
	^{13}C/^{15}N	119.02	172.78	53.24	27.98	24.43	40.54	84.97	156.89	X	X	-	-	-
	R - Arginine	NHα	CO	α	β	γ	δ	ε	ζ	η1	η2	-	-	-
6	^{1}H	7.87	-	3.85	1.29/1.24	0.85/0.93	2.78/2.72	6.80	-	X	X	-	-	-
	^{13}C/^{15}N	119.50	173.54	54.83	27.01	23.76	40.21	84.97	156.57	X	X	-	-	-

Table S7: Full assignment of c(LWwNKr) according to Figure S2.

c(LWwNKr)														
Residue #	Amino acid	Chemical shift (δ)												
	L - Leucine	NHα	CO	α	β	γ	δ							
1	1H	8.597	-	4.01	1.32	1.43	0.80/0.72	-	-	-	-	-	-	-
	13C/15N	126.00	173.54	53.33	39.17	24.35	22.25/19.98	-	-	-	-	-	-	-
	W - Tryptophan	NHα	CO	α	β	ε1	δ1	γ	δ2	ε3	ζ3	η2	ζ2	ε2
2	1H	7.45	-	4.70	3.09/3.13	10.01	7.10	-	-	7.49	7.07	7.19	7.43	-
	13C/15N	117.50	172.00	53.34	28.21	129.07	124.68	108.88	127.14	118.34	119.17	121.87	113.01	136.17
	w - (*R*)-Tryptophan	NHα	CO	α	β	ε1	δ1	γ	δ2	ε3	ζ3	η2	ζ2	ε2
3	1H	8.35	-	3.90	2.82/2.91	10.00	7.00	-	-	7.36	7.08	7.16	7.40	-
	13C/15N	126.00	174.22	56.72	25.70	129.48	124.58	108.26	126.50	118.15	119.35	122.01	112.05	136.25
	N - Aspargine	NHα	CO	α	β	γ	δ2							
4	1H	7.68	-	4.28	1.70/2.36	-	6.39/7.03	-	-	-	-	-	-	-
	13C/15N	124.00	172.41	49.63	34.71	174.06	110.50	-	-	-	-	-	-	-
	K - Lysine	NHα	CO	α	β	γ	δ	ε	ζ					
5	1H	7.67	-	4.33	1.52/1.72	1.24/1.15	1.52	2.86	X		-	-	-	-
	13C/15N	119.00	172.23	52.56	31.03	21.62	26.24	39.49	X		-	-	-	-
	r - (*R*)-Arginine	NHα	CO	α	β	γ	δ	ε	ζ	η1	η2			
6	1H	8.11	-	4.21	1.60	1.36/1.49	3.08	7.11	-	X	X	-	-	-
	13C/15N	124.00	174.05	53.66	27.34	24.51	40.61	84.49	156.81	X	X	-	-	-