Supporting information

Turning cationic antimicrobial peptide KR-12 into Self-assembled nanobiotics with potent bacterial killing and LPS neutralizing activities

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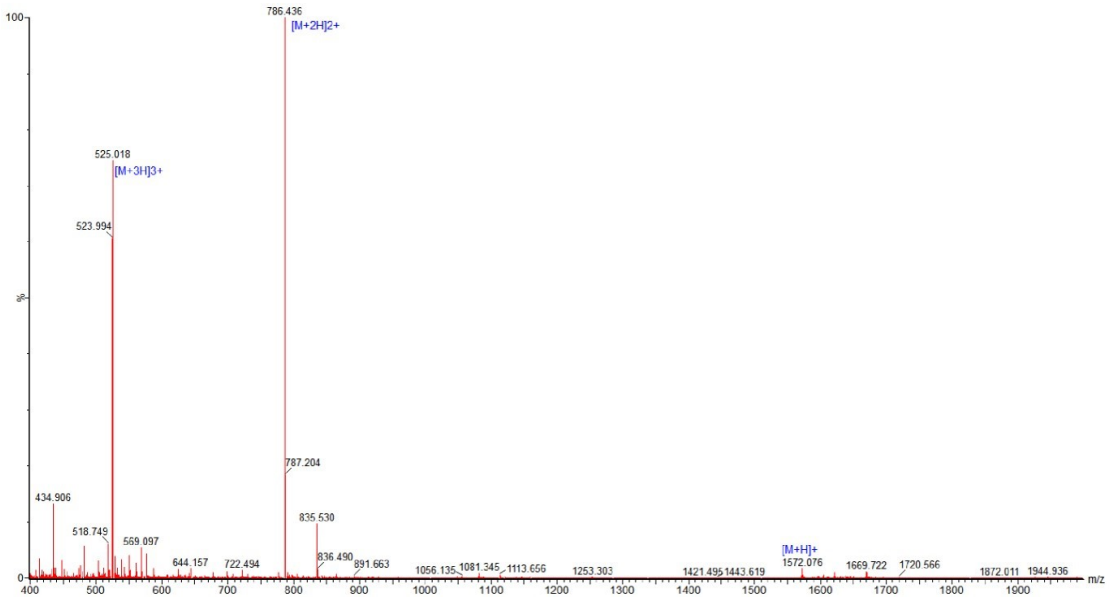


Figure S1. Electrospray ionization (ESI) mass spectrum (MS) of KR-12. The calculated molecular weight (MW) is 1571.95, and the observed MW is 1572.05.

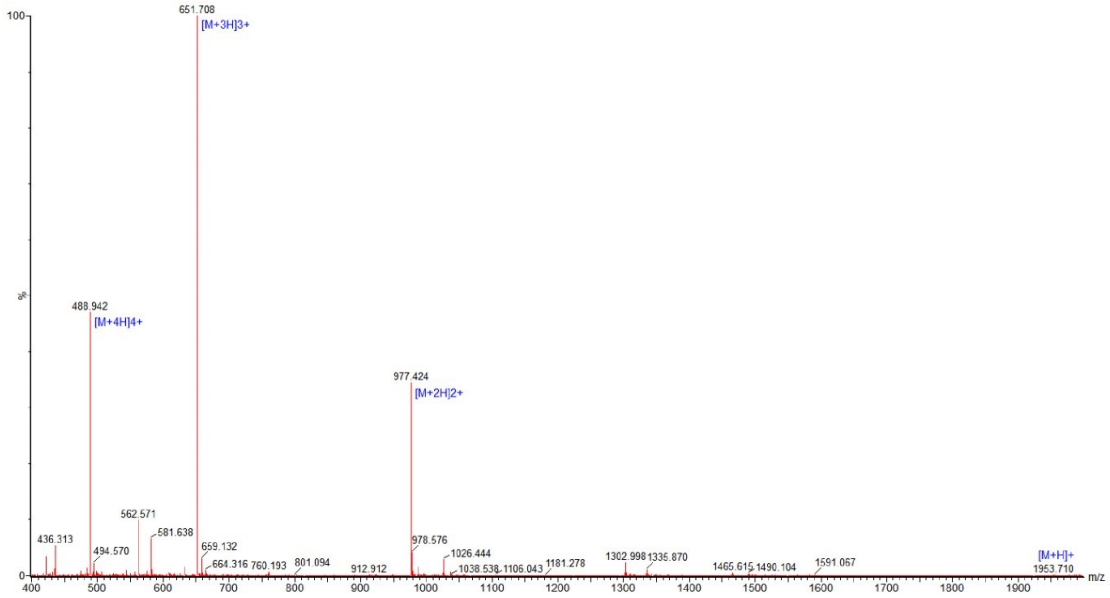


Figure S2. Electrospray ionization (ESI) mass spectrum (MS) of Myr-KR-12N. The calculated molecular weight (MW) is 1952.48, and the observed MW is 1952.12.

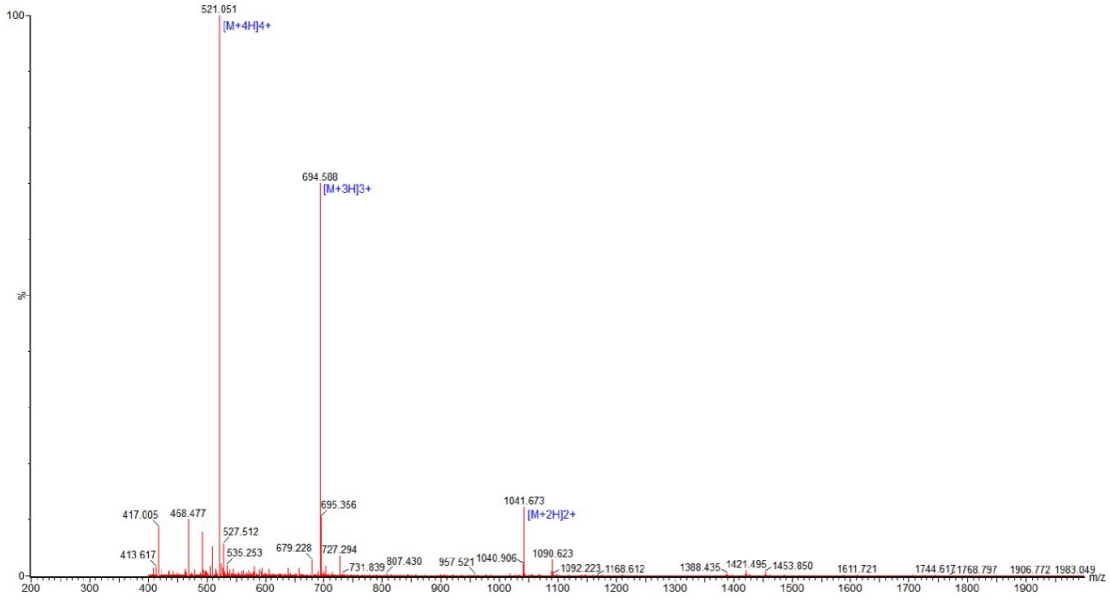
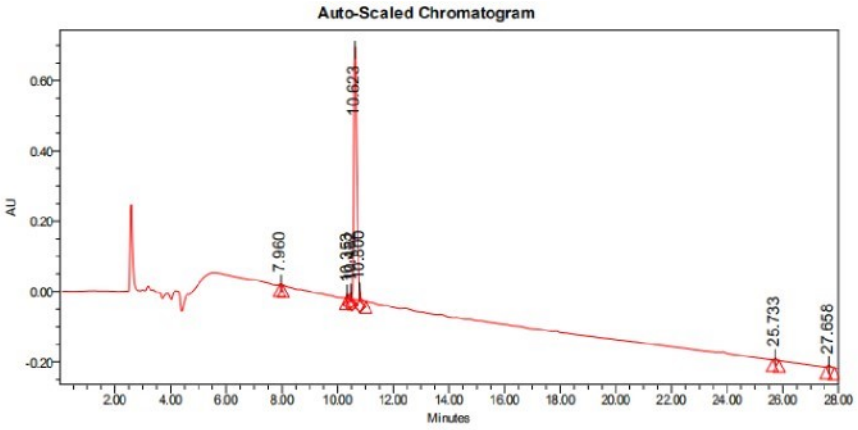


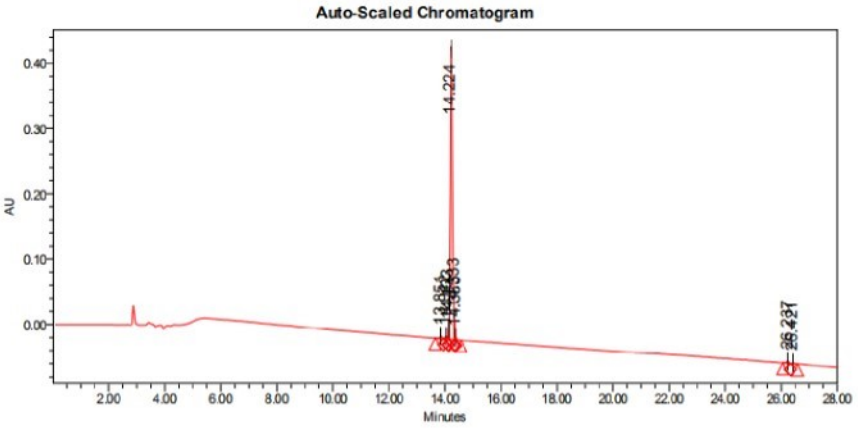
Figure S3. Electrospray ionization (ESI) mass spectrum (MS) of Myr-KR-12C. The calculated molecular weight (MW) is 2081.65, and the observed MW is 2081.76.



Peak Results

	RT	Area	Height	% Area
1	7.960	12442	3114	0.22
2	10.353	54054	12329	0.95
3	10.482	78729	15905	1.39
4	10.623	5396589	723778	95.07
5	10.800	56279	24390	0.99
6	25.733	33481	5500	0.59
7	27.658	44618	5366	0.79

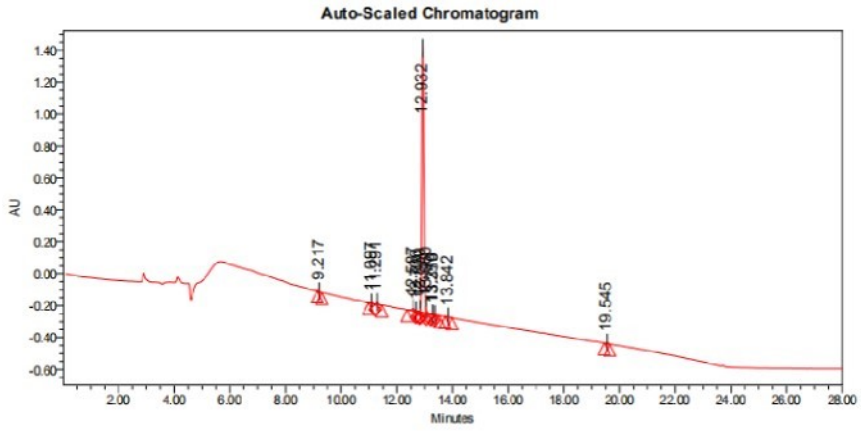
Figure S4. High-performance liquid chromatography (HPLC) analysis of KR-12. HPLC analysis of KR-12 was performed to confirm its purity of 95.07%.



Peak Results

	RT	Area	Height	% Area
1	13.851	20898	3631	0.74
2	14.042	23571	4249	0.83
3	14.133	11766	13868	0.42
4	14.224	2695392	451391	95.48
5	14.333	40225	31249	1.42
6	14.383	4784	3462	0.17
7	26.237	16970	3020	0.60
8	26.421	9526	1613	0.34

Figure S5. High-performance liquid chromatography (HPLC) analysis of Myr-KR-12N. HPLC analysis of Myr-KR-12N was performed to confirm its purity of 95.48%.



Peak Results

	RT	Area	Height	% Area
1	9.217	5773	1252	0.07
2	11.097	16934	3779	0.19
3	11.291	59175	13140	0.67
4	12.587	118391	17802	1.34
5	12.700	23127	4907	0.26
6	12.850	15318	18284	0.17
7	12.932	8461260	1649238	95.73
8	13.050	62699	36146	0.71
9	13.276	24641	4625	0.28
10	13.359	17204	2633	0.19
11	13.842	13107	2789	0.15
12	19.545	21403	3448	0.24

Figure S6. High-performance liquid chromatography (HPLC) analysis of Myr-KR-12C. HPLC analysis of Myr-KR-12C was performed to confirm its purity of 95.73%.

Table S1 Secondary structure of KR-12, Myr-KR-12N and Myr-KR-12C based on CD spectrum

	Helix 1	Helix2	Strand 1	Strand 2	Turns	Unordered	Total
KR-12	-0.001	0.028	0.295	0.196	0.229	0.253	0.999
Myr-KR-12N	-0.001	0.028	0.294	0.195	0.228	0.252	0.997
Myr-KR-12C	-0.001	0.028	0.295	0.196	0.229	0.252	0.998

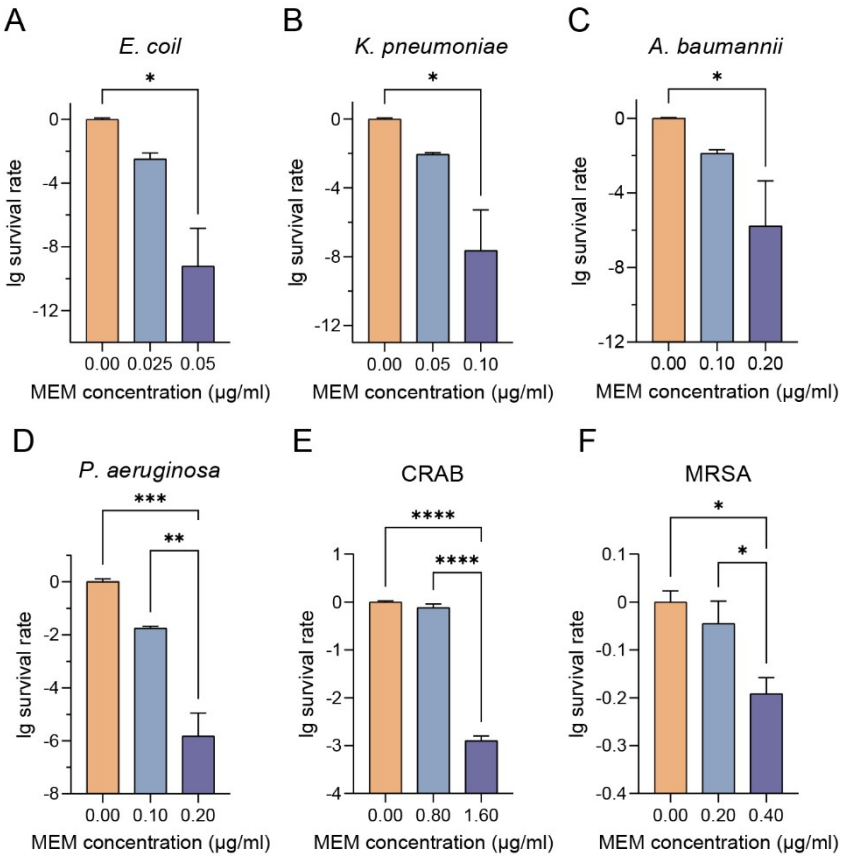


Figure S7. Antimicrobial efficacy of meropenem (MEM) against *E. coli*, *K. pneumoniae, A. baumannii*, *P. aeruginosa*, carbapenem-resistant *A. baumannii* (CRAB), and methicillin-resistant *S. aureus* (MRSA) was assessed. Bacterial cultures (2×10^6 CFU/ml) were exposed to MEM at different concentrations, and the percentage of bacterial survival (represented on a logarithmic scale) was determined by comparing colony counts from treated samples to those from mock-treated vehicle samples (0.00 μg/ml). The results are presented as the mean value with accompanying SEM. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons test, with significance levels denoted by asterisks as follows: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

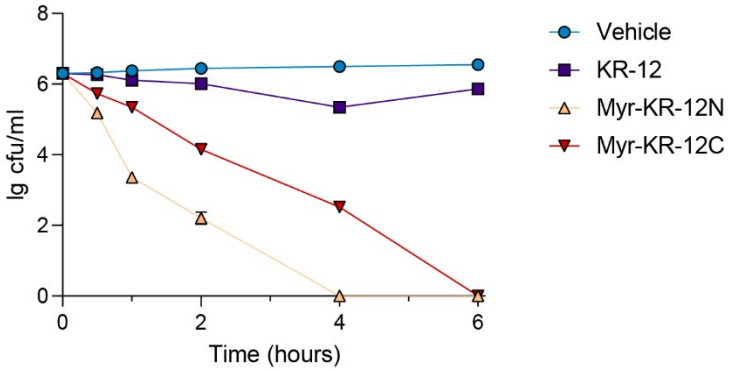


Figure S8. Time-kill kinetics of KR-12, Myr-KR-12N, and Myr-KR-12C at a concentration of 64 μg/ml against carbapenem-resistant *A. baumannii* (CRAB). Data presented here are the average values from three independent experiments, accompanied by SD.

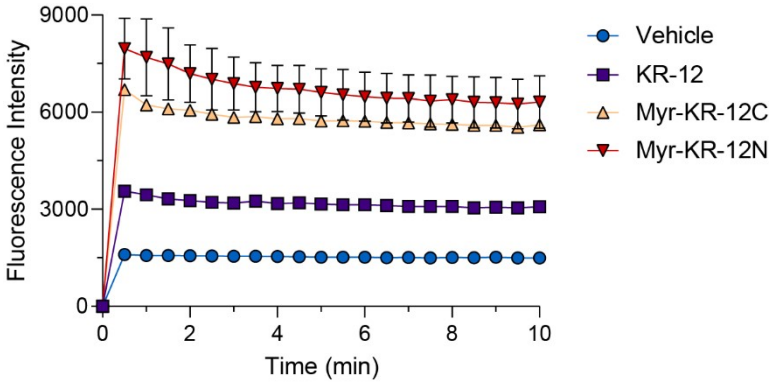


Figure S9. Evaluation of the outer membrane (OM) permeability of carbapenem-resistant *A. baumannii* (CRAB) was conducted through the NPN uptake method, employing a peptide concentration of 128 μg/ml. Data presented here are the average values from three independent experiments, accompanied by SD.

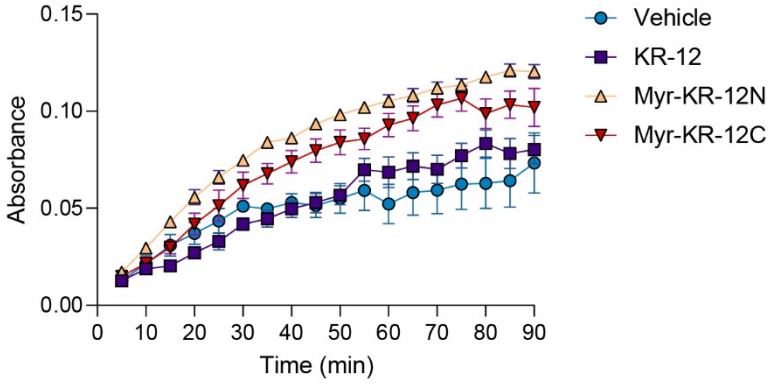


Figure S10. Evaluation of the inner membrane (IM) permeability of carbapenem-resistant *A. baumannii* (CRAB) using the ONPG reagent at a peptide concentration of 128 μg/ml. Data presented here are the average values from three independent experiments, accompanied by SEM.

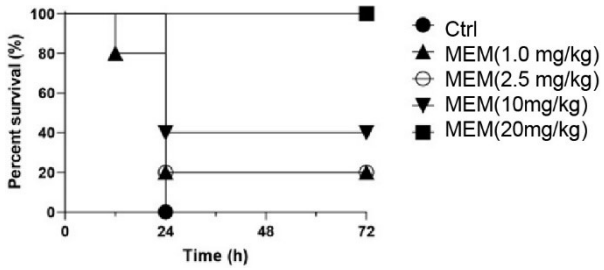


Figure S11. Therapeutic efficacy of MEM in bacterial sepsis. 5 mice per group were intraperitoneally challenged with 1×10^7 CFU of *E. coli*, and different doses of MEM were then administered as single-dose treatments.

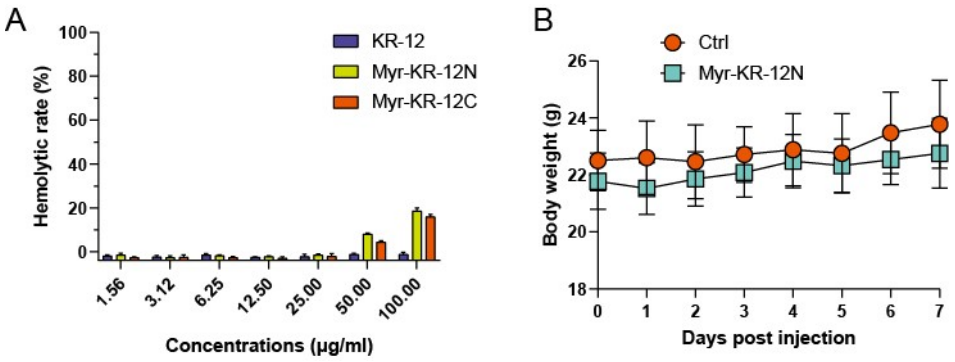


Figure S12. Toxicity of KR-12, Myr-KR-12N, and Myr-KR-12C in vitro and *in vivo*. (A) The dose-dependent hemolytic activity of KR-12, Myr-KR-12N, and Myr-KR-12C was examined in mouse red blood cells. Percent hemolysis was calculated as described in the experimental section. The data are presented as mean ± SD from five independent experiments. (B) Toxicity of Myr-KR-12N *in vivo*. 5 mice per group were intraperitoneally injected with 5 mg/kg of Myr-KR-12N per experimental animal or PBS as control. Body weight was monitored every day for 7 days. No difference was observed between the 2 groups.

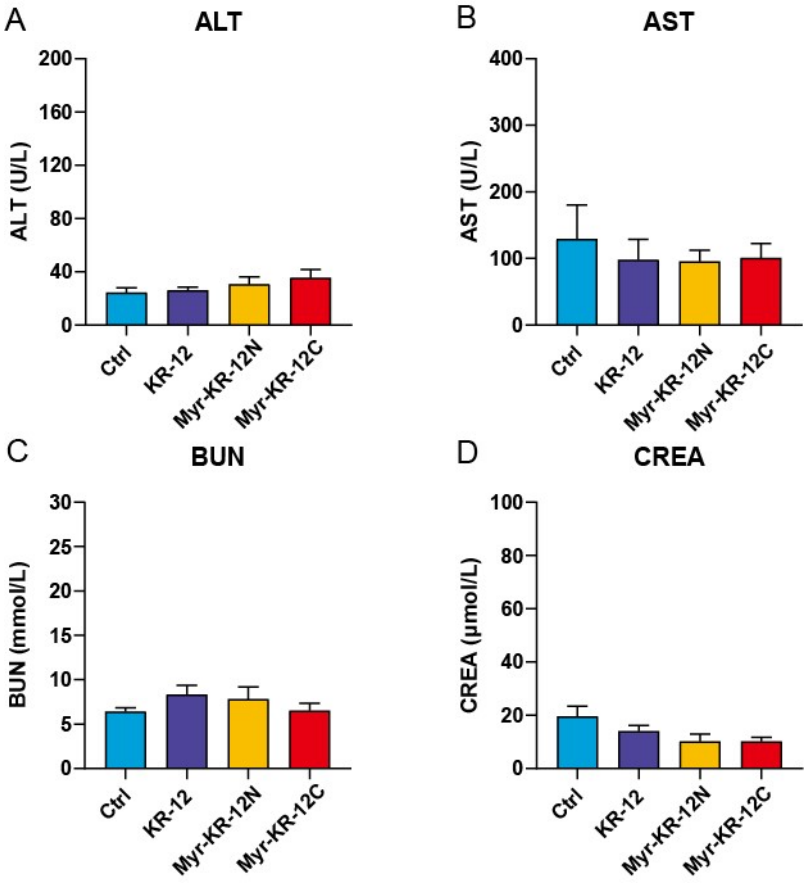


Figure S13. Toxicity of KR-12, Myr-KR-12N, and Myr-KR-12C *in vivo*. four groups of mice (6 mice per group) were subcutaneously injected with 12 mg/kg of KR-12, Myr-KR-12N, or Myr-KR-12C or PBS every 2 h for 6 times injection. At 20 h after the last injection of the drug, liver, kidney and blood were collected from the mice. Serum was taken for liver function (A-B) and kidney function (C-D) tests. No liver or kidney dysfunction was observed after treating with these nanobiotics.

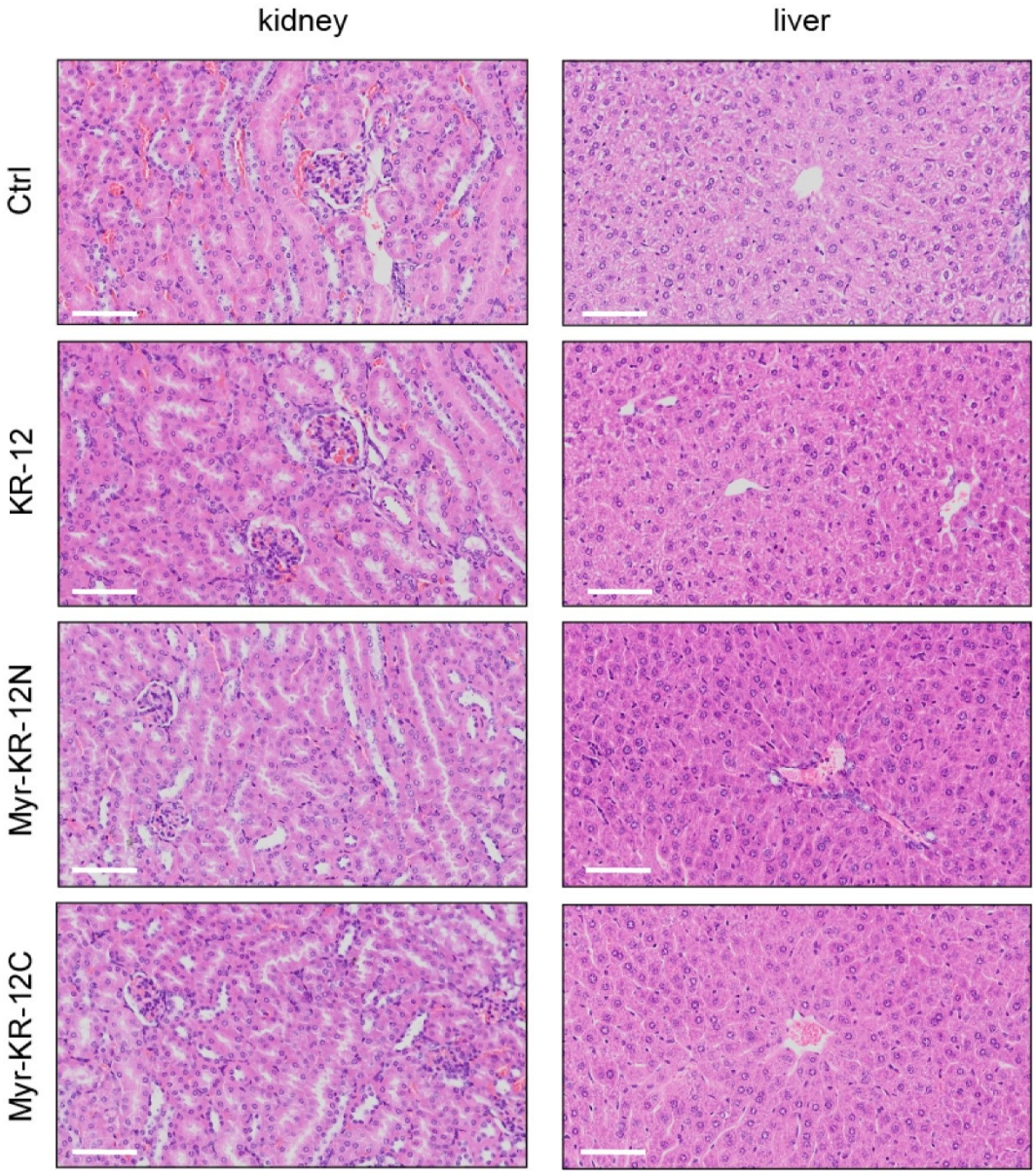


Figure S14. Toxicity of KR-12, Myr-KR-12N, and Myr-KR-12C *in vivo*. 4 groups of mice (6 mice per group) were subcutaneously injected with 12 mg/kg of KR-12, Myr-KR-12N, or Myr-KR-12C or PBS every 2 h for 6 times. At 20 h after the last injection of the drug, liver, kidney, and blood were collected from the mice. The liver and kidney were fixed in formalin and then subjected to histopathological examination; scale bar = 100 μm. No histopathological changes were observed after treating with these peptides.