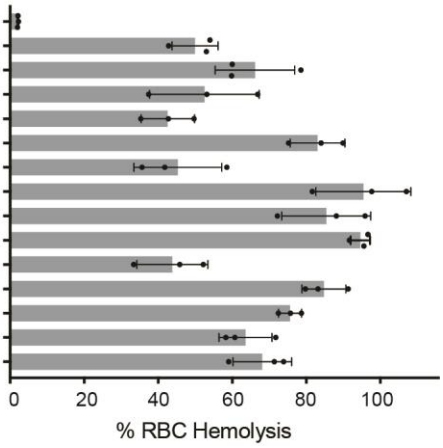
Peptide	Sequence	Antimicrobial Activity (MIC in μg/ml)*				RBC Hemolysis at 25 μg/mL
		E .coli	*B. cereus*	*P. aeruginosa*	*S. aureus*	
Magainin II	GIGKFLHSAKKFGKAFVGEIMNS	29.73	59.46	>50	>50	
Mag(i+7)1	G**8**GKFLHS**X**KKFGKAFVGEIBNS	2.63	4.42	3.72	10.51	
Mag(i+7)2	GI**8**KFLHSA**X**KFGKAFVGEIBNS	4.42	8.84	21.02	12.50	
Mag(i+7)3	GIG**8**FLHSAK**X**FGKAFVGEIBNS	10.51	12.50	21.02	14.87	
Mag(i+7)4	GIGK**8**LHSAKK**X**GKAFVGEIBNS	2.21	5.26	6.25	21.02	
Mag(i+7)5	GIGKF**8**HSAKKF**X**KAFVGEIBNS	3.13	3.13	3.13	8.84	
Mag(i+7)6	GIGKFL**8**SAKKFG**X**AFVGEIBNS	3.13	4.42	14.87	6.25	
Mag(i+7)7	GIGKFLH**8**AKKFGK**X**FVGEIBNS	3.13	3.72	5.26	3.72	
Mag(i+7)8	GIGKFLHS**8**KKFGKA**X**VGEIBNS	2.21	3.72	3.72	6.25	
Mag(i+7)9	GIGKFLHSA**8**KFGKAF**X**GEIBNS	3.72	4.42	5.26	3.72	
Mag(i+7)10	GIGKFLHSAK**8**FGKAFV**X**EIBNS	3.13	3.72	7.43	4.42	
Mag(i+7)11	GIGKFLHSAKK**8**GKAFVG**X**IBNS	2.21	2.63	2.21	2.63	
Mag(i+7)12	GIGKFLHSAKKF**8**KAFVGE**X**BNS	2.21	3.72	3.13	7.44	
Mag(i+7)13	GIGKFLHSAKKFG**8**AFVGEI**X**NS	2.21	4.42	6.25	7.44	
Mag(i+7)14	GIGKFLHSAKKFGK**8**FVGEIB**X**S	1.86	1.56	5.26	2.63	

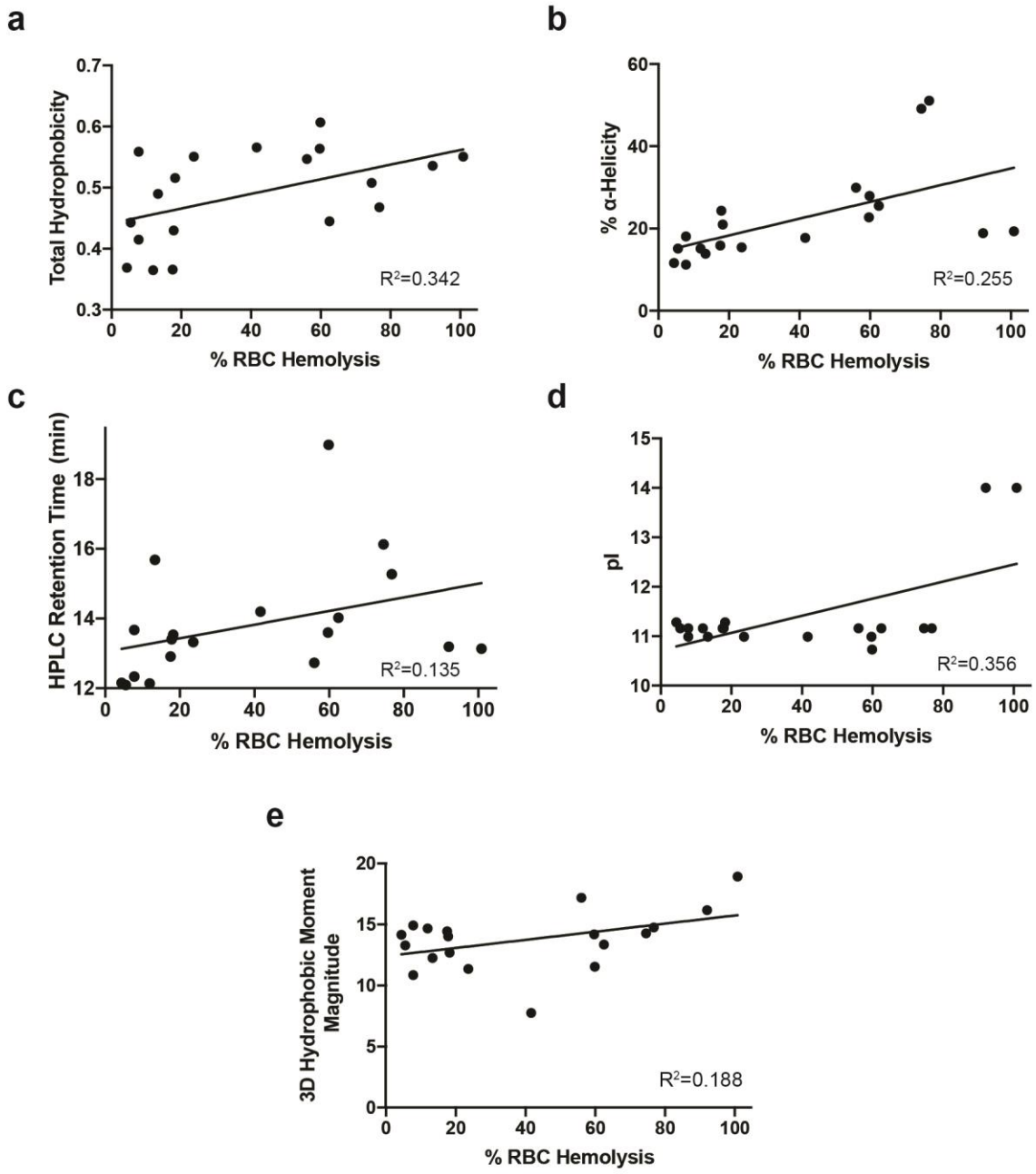
*Geometric mean



Supplementary Figure 1

Antimicrobial and hemolytic activities of an *i, i+7*-stapled Mag2 library

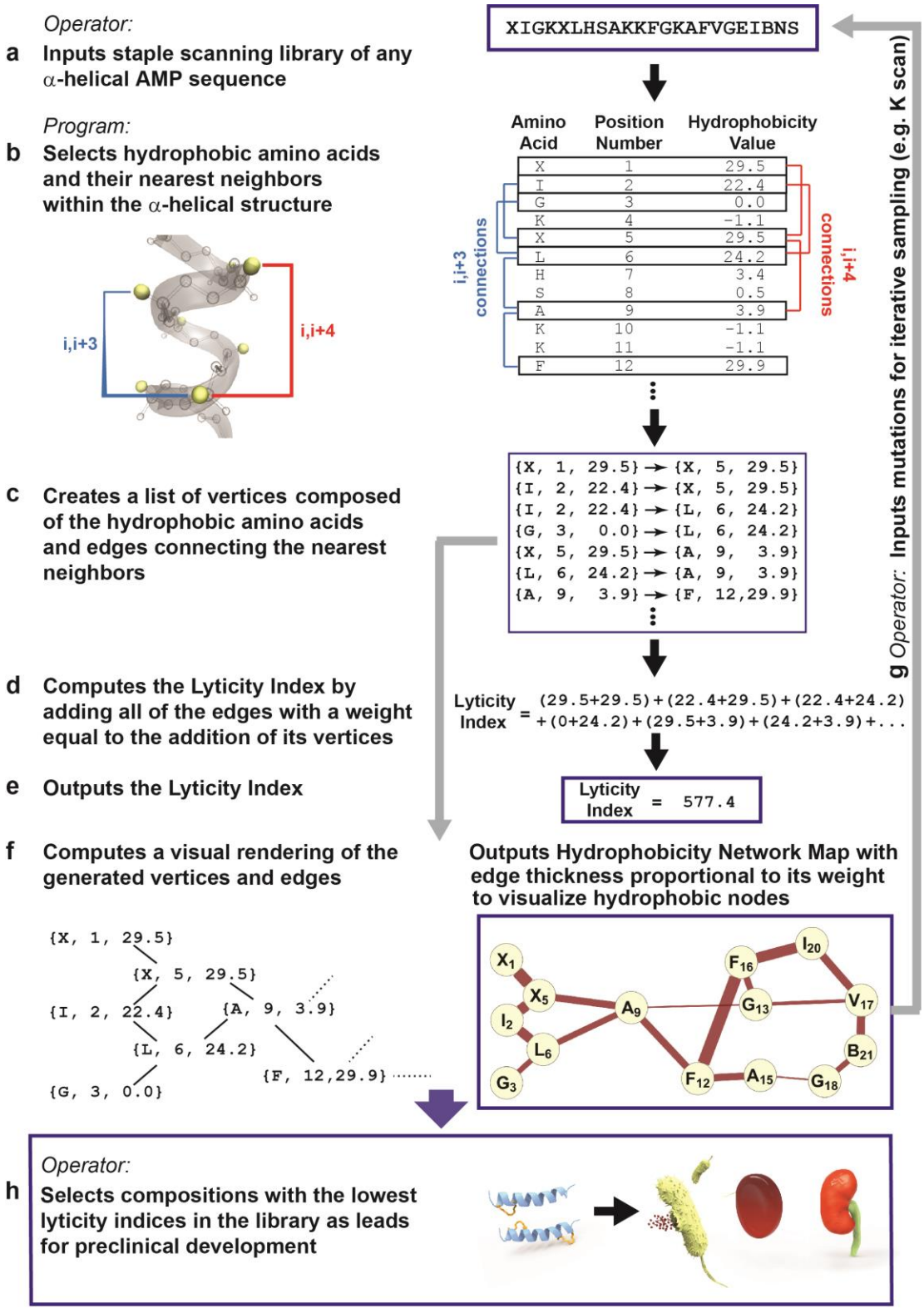
The antimicrobial activity (MIC in μg/mL) and percent RBC hemolysis at 25 μg/mL were determined for a library of *i, i+7* stapled peptides based on the Mag2 sequence. The MIC is the geometric mean of four independent experiments. Percent hemolysis data are the mean of three independent experiments (shown as dots). X, S5 stapling amino acid; 8, R8 stapling amino acid; B, norleucine (substituted for methionine to maximize the efficiency of ruthenium-catalyzed olefin metathesis).



Supplementary Figure 2

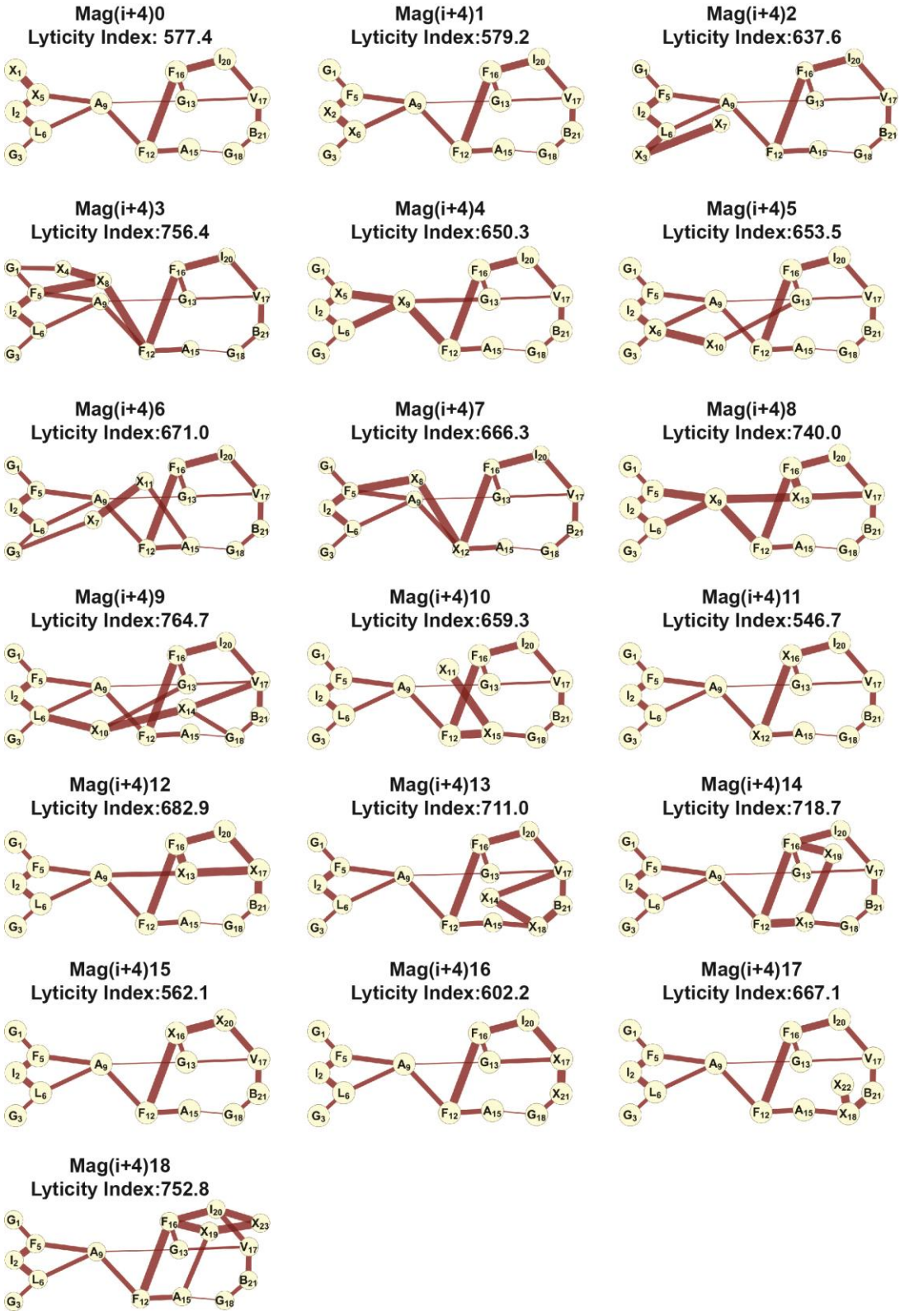
Relationships between StAMP biophysical parameters and hemolysis

a-e, No direct correlations were observed between percent RBC hemolysis and the total hydrophobicity (**a**), percent α-helicity (**b**), HPLC retention time (**c**), pI (**d**), or 3D hydrophobic moment magnitude (**e**) of StAMPs (n=19), as calculated by Pearson correlation.



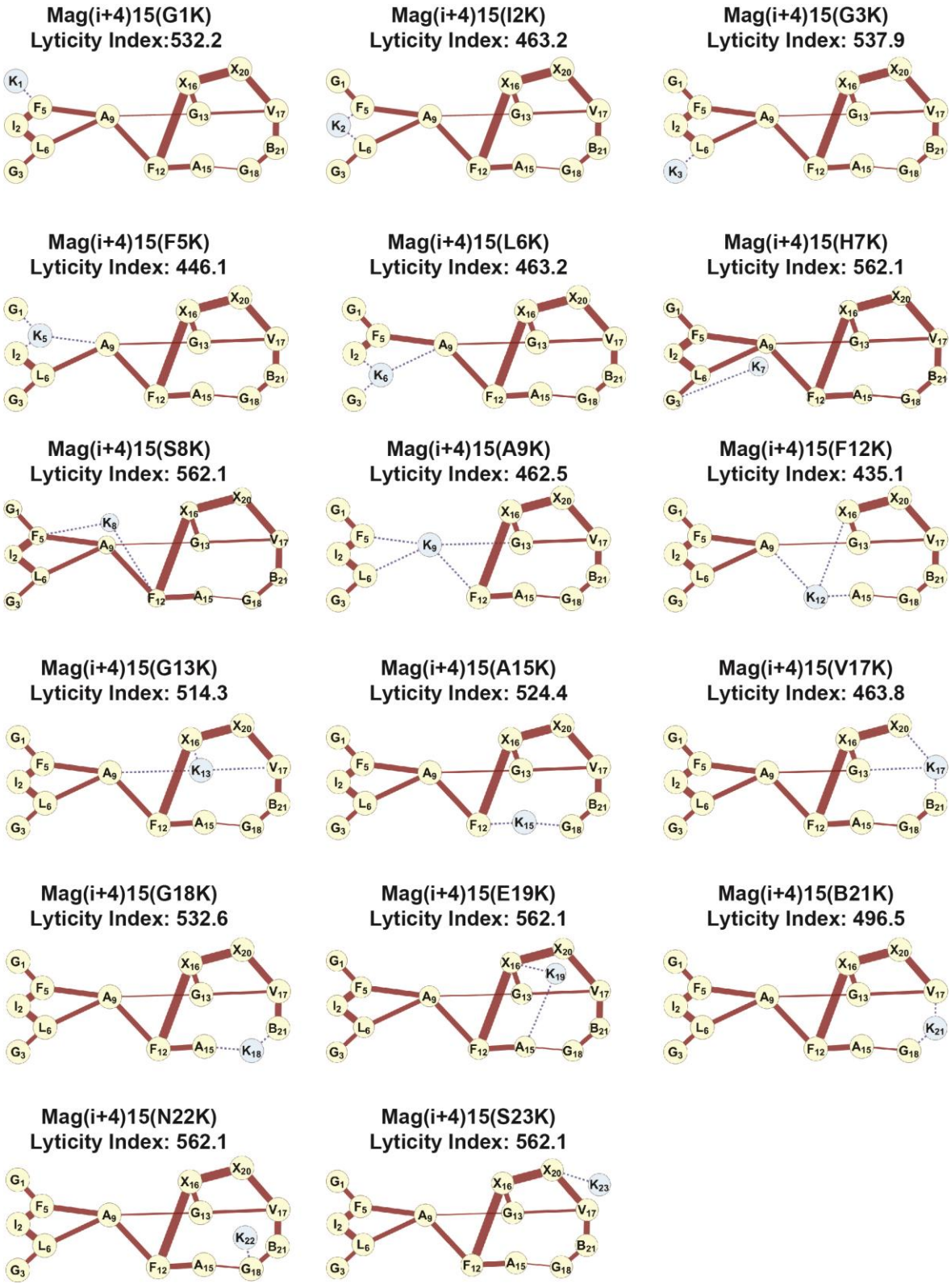
Supplementary Figure 3

A computational algorithm yields HNMs and lyticity indices for StAMPs



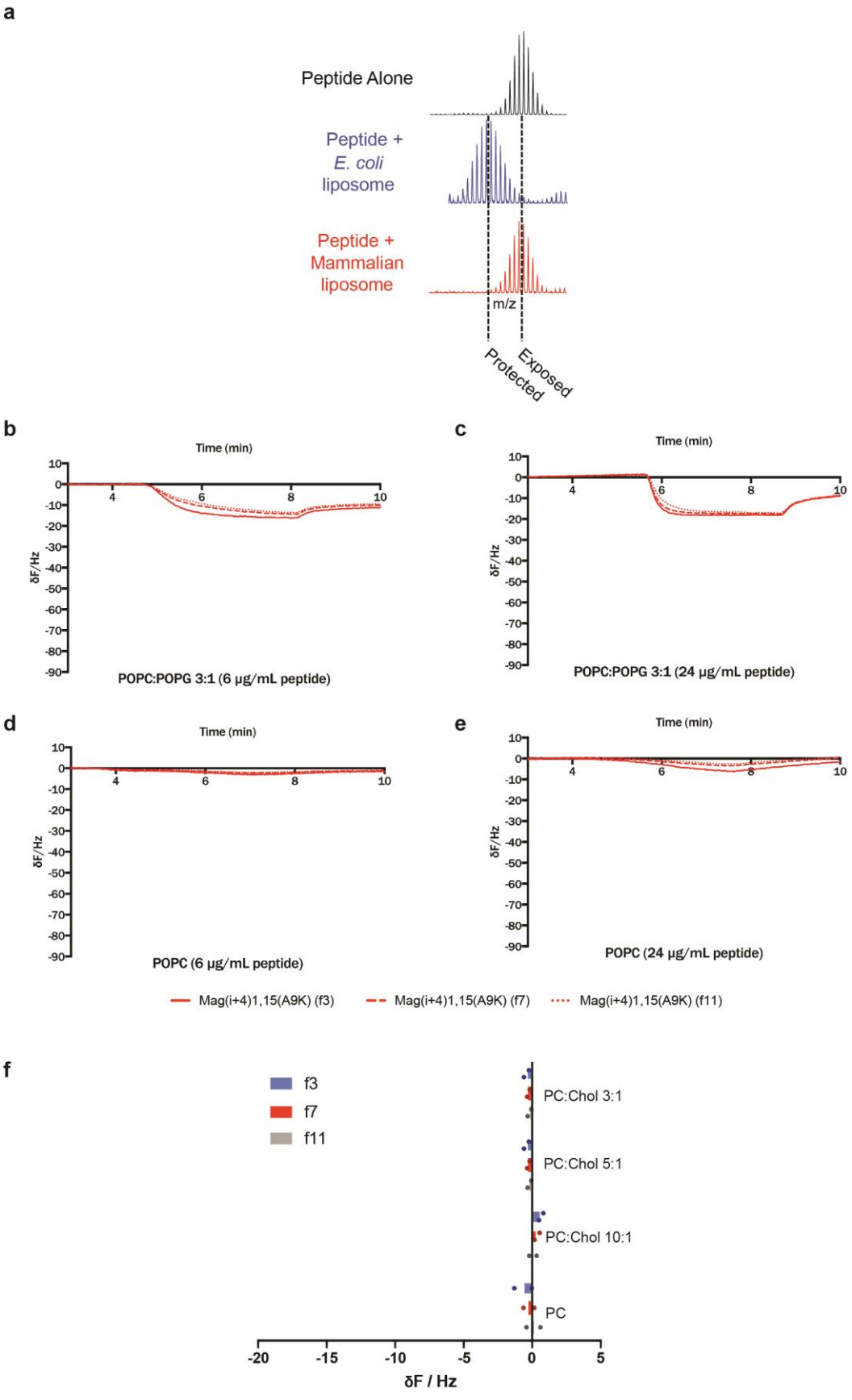
Supplementary Figure 4

HNMs and lyticity indices of a staple-scanning Mag(i+4) library



Supplementary Figure 5

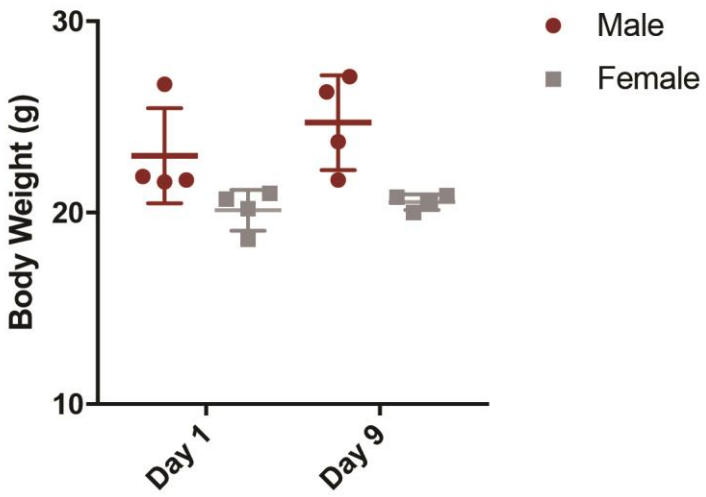
HNMs and lyticity indices of a lysine-scanning Mag(i+4)15 library


a
Peptide Alone
Peptide +
E. coli
liposome
Peptide +
Mammalian
liposome
m/z
Protected
Exposed
b
Time (min)
δF/Hz
POPC:POPG 3:1 (6 μg/mL peptide)
c
Time (min)
δF/Hz
POPC:POPG 3:1 (24 μg/mL peptide)
d
Time (min)
δF/Hz
POPC (6 μg/mL peptide)
e
Time (min)
δF/Hz
POPC (24 μg/mL peptide)
Mag(i+4)1,15(A9K) (f3)
Mag(i+4)1,15(A9K) (f7)
Mag(i+4)1,15(A9K) (f11)
f
f3
f7
f11
PC:Chol 3:1
PC:Chol 5:1
PC:Chol 10:1
PC
δF / Hz

Supplementary Figure 6

Membrane selectivity and insertion mechanism of Mag(i+4)15(A9K)

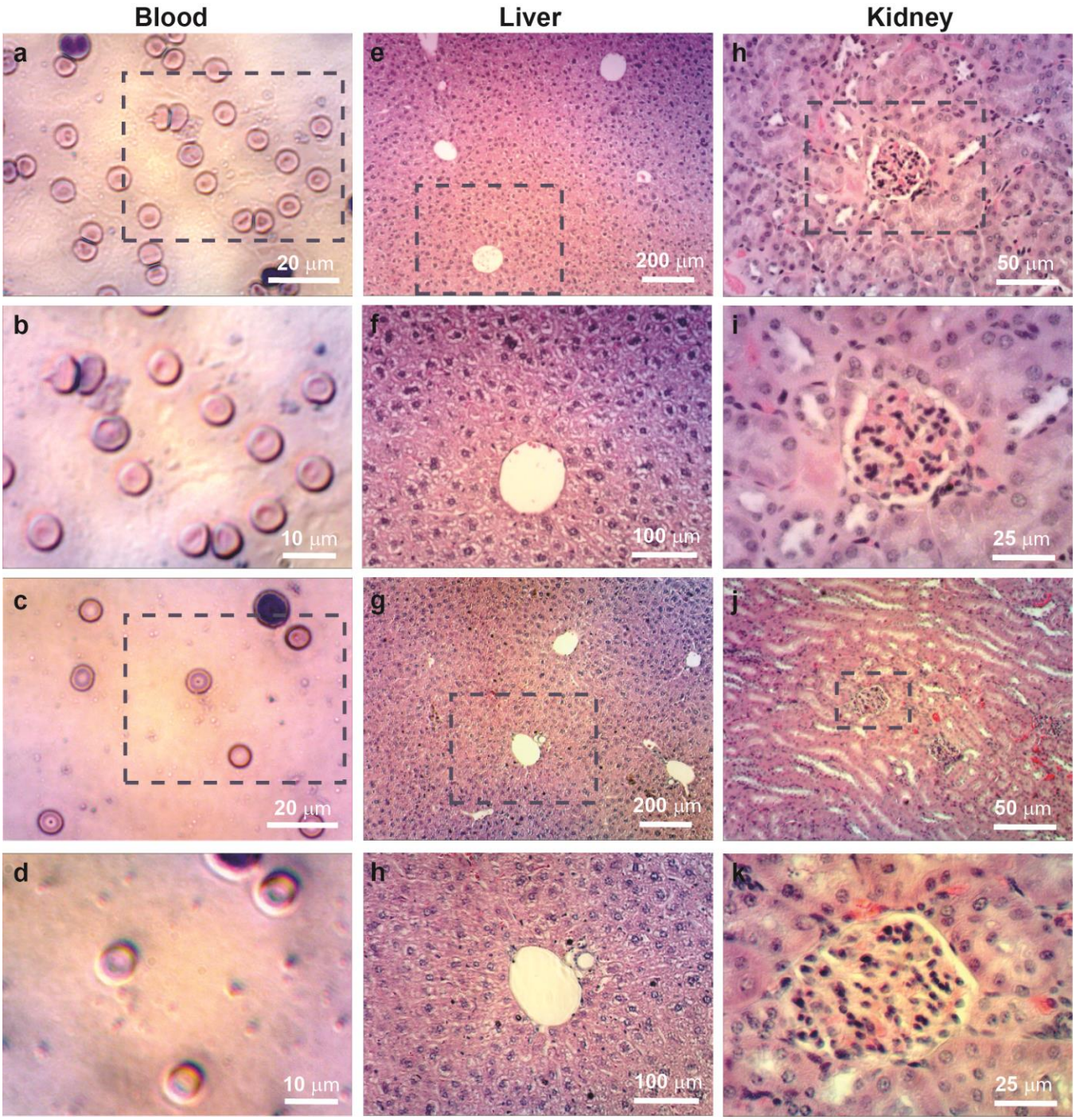
a, Incubation of Mag(i+4)1,15(A9K) with anionic liposomes that mimic *E. coli* membranes (POPC:POPG) reduces deuterium exchange, whereas exposure to zwitterionic liposomes that mimic the mammalian membrane condition (POPC:Cholesterol) has no such effect and is identical to the peptide's deuterium exchange profile in aqueous solution alone. HX-MS experiments were performed independently three times with similar results. **b-e,** QCM sensorgrams demonstrate uniformity of changes in resonant frequency across harmonics for SLBs mimicking bacterial membranes (POPC:POPG) upon exposure to 6 (**b**) and 24 (**c**) μg/mL of Mag(i+4)1,15(A9K), consistent with transmembrane insertion. In contrast, little to no interaction is reflected in the QCM profiles of POPC mammalian-type membranes at low or high peptide dosing (**d-e**). For each condition, an exemplary sensorgram is shown for experiments performed independently two times with similar results. **f**, Accordingly, there were no changes in resonant frequency across the third (blue), seventh (red), and eleventh (black) harmonics in response to Mag(i+4)1,15(A9K) treatment (8 μg/mL) of SLBs composed of POPC and increasing concentrations of cholesterol. Data are mean of two independent experiments (shown as dots).



Supplementary Figure 7

Body weights of mice treated intravenously with Mag(i+4)1,15(A9K)

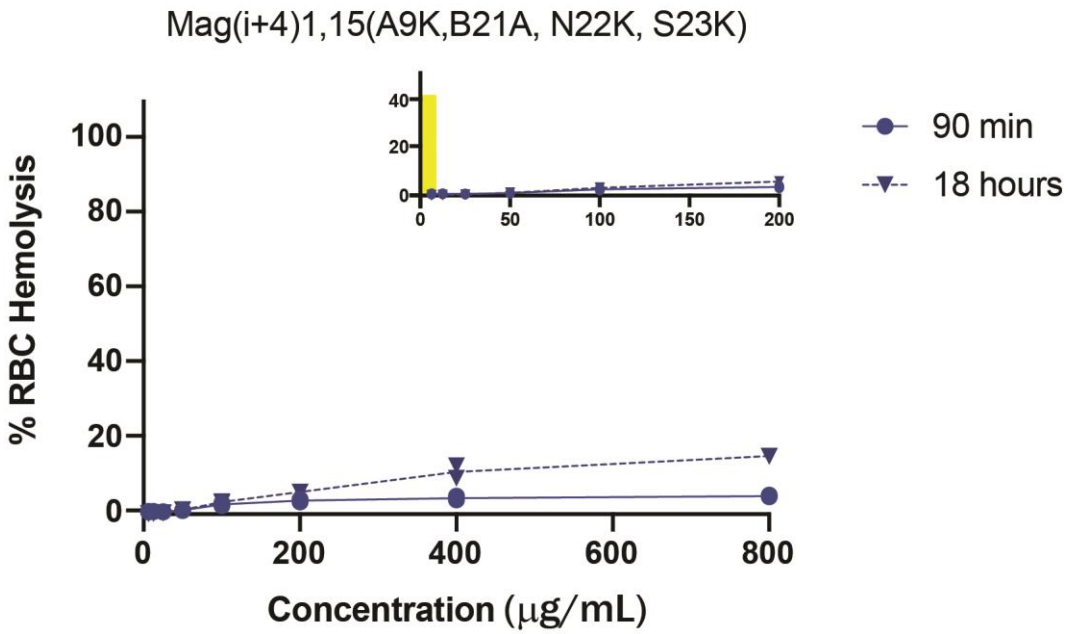
Mice were treated with Mag(i+4)15(A9K) at an intravenous dose of 5 mg/kg twice daily for 8 days. Body weights were measured before (day 1) and after 8 days of treatment (day 9). Data are mean ± s.d. for n=4 mice per sex.



Supplementary Figure 8

Histology of murine tissues after IV treatment with Mag(i+4)1,15(A9K)

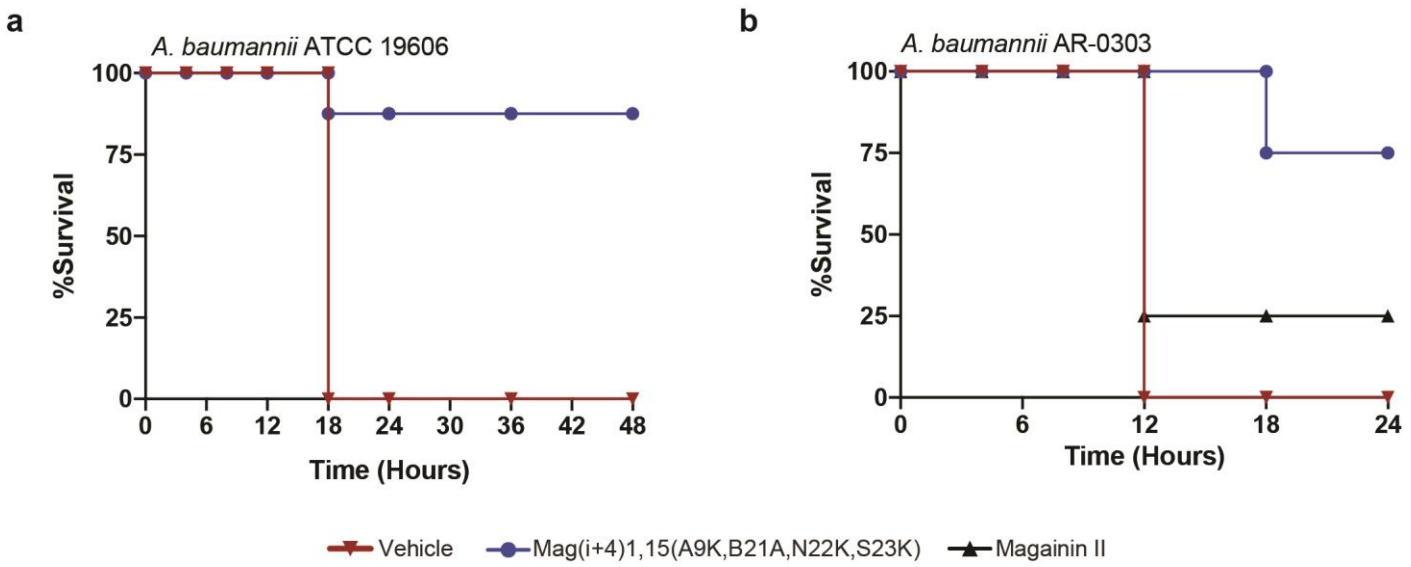
Peripheral blood smears (**a-d**) and H&E stained sections of liver (**e-h**) and kidney (**h-k**) from n=8 mice (4 male, 4 female) treated with Mag(i+4)15(A9K) (5 mg/kg IV BID x 8 d). Each vertical pair (**a-b**, **c-d**, **e-f**, **g-h**, **h-i**, **j-k**) represents a low and higher power view (enlargement of boxed image) of the indicated tissue. For each tissue, specimens from two different mice are shown. RBC morphology and liver histology are normal. Whereas some of the treated mice showed predominantly normal kidney histology (**h-k**), others manifested regions of mild-to-moderate tubular degeneration, as shown in Fig. 4j and Supplementary Fig. 12b.



Supplementary Figure 9

RBC response to Mag(i+4)15(A9K,B21A,N22K,S23K) treatment

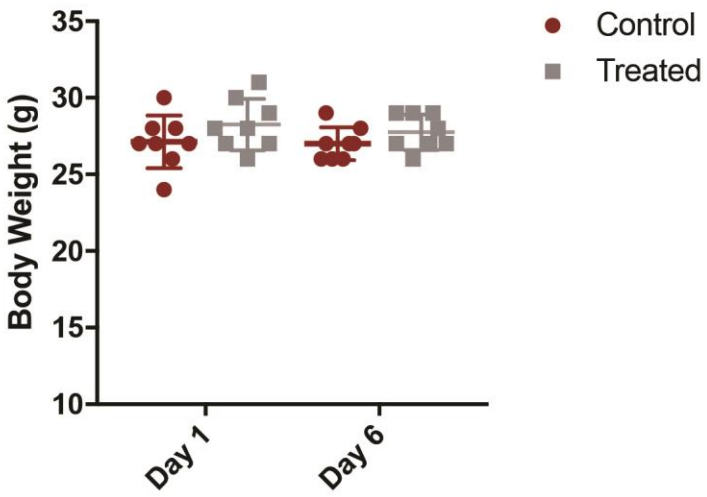
Mag(i+4)15(A9K,B21A,N22K,S23K) shows little to no RBC hemolytic activity across a broad dose-effective range, and even when dosed as high as 800 μg/mL for 90 minutes or 18 hours. The data from two independently performed experiments are shown as dots, with several pairs of replicate data points overlapping. The Gram-negative bactericidal dosing range is highlighted in yellow in the inset.



Supplementary Figure 10

Comparative activity of linear and stapled Mag2 peptides in a peritonitis-sepsis mouse model

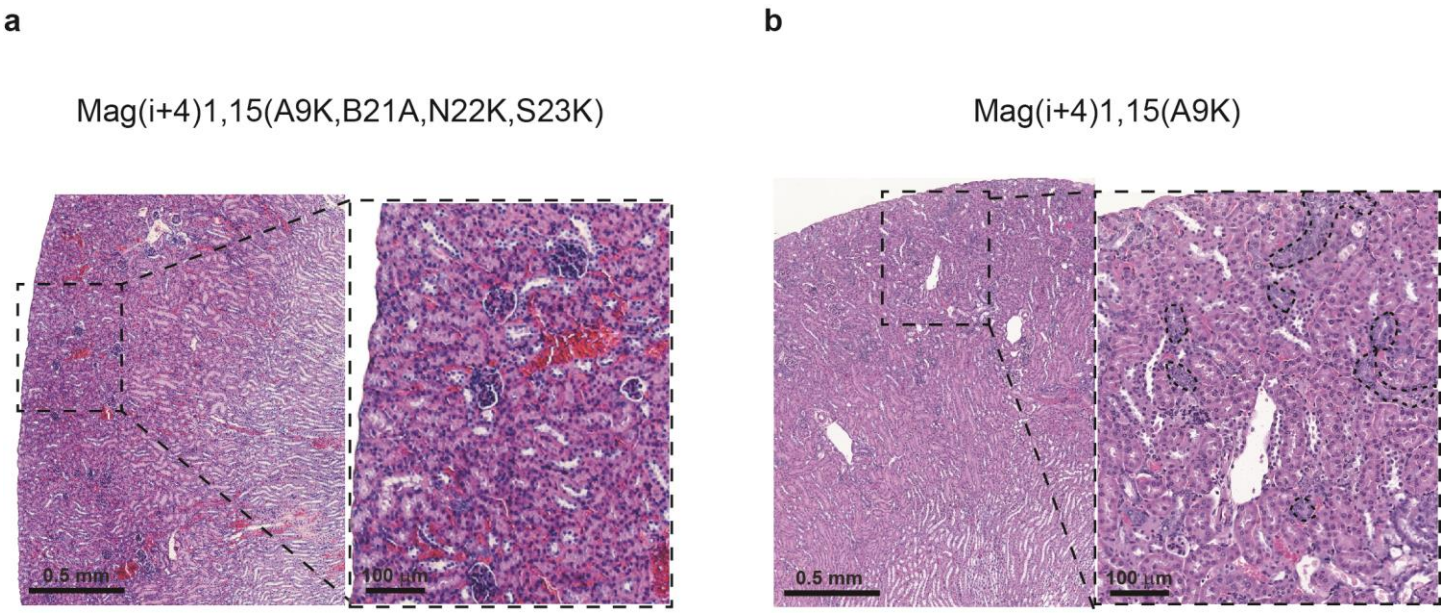
a, Kaplan Meier survival curves of neutropenic mice (n=8 per arm) infected with *A. baumannii* (ATCC 19606) intraperitoneally and treated with either vehicle (saline) or two 5 mg/kg IP doses of Mag(i+4)15(A9K,B21A,N22K,S23K). p=0.0006 for StAMP vs. vehicle by log rank test (two-sided). **b,** Kaplan Meier survival curves of neutropenic mice (n=8 per arm) infected with *A. baumanii* (AR-0303) intraperitoneally and treated with either vehicle (saline) or two 5 mg/kg IP doses of Mag2 or Mag(i+4)15(A9K,B21A,N22K,S23K). p=0.0001 for StAMP vs. Vehicle; p=0.02 for StAMP vs. Mag2; and p=0.1432 (n.s.) for Mag2 vs. Vehicle, as calculated by log rank test (two-sided).



Supplementary Figure 11

Body weights of StAMP-treated mice

Mice were treated with vehicle or Mag(i+4)15(A9K,B21A,N22K,S23K) at an intravenous dose of 5 mg/kg twice daily for 5 days. Body weights were measured before (day 1) and after 5 days of treatment (day 6). Data are mean ± s.d. for n=8 female mice per arm.



Supplementary Figure 12

Histology of murine kidney after intravenous treatment with StAMPs

a-b, Mice (n=8 per arm) were treated with vehicle, Mag(i+4)15(A9K,B21A,N22K,S23K), or Mag(i+4)15(A9K) at an intravenous dose of 5 mg/kg twice daily for 5 days. H&E stained sections of kidney tissue from mice treated with Mag(i+4)15(A9K,B21A,N22K,S23K) showed normal histology (**a**), whereas select mice treated with Mag(i+4)15(A9K) manifested mild-to-moderate renal tubule degeneration (**b**) (affected regions outlined in dashed black lines). For each image pair, the boxed tissue on the left is enlarged in the image to the right.

Peptide	Total Hydrophobicity	% α-Helicity	C18 Column HPLC Retention Time (min)	pI	3D Hydrophobic Moment Magnitude	3D Hydrophobic Moment Angle
Mag(i+4)0	0.443	15	12.093	11.16	13.297	104.145
Mag(i+4)1	0.369	12	12.154	11.28	14.153	102.544
Mag(i+4)2	0.516	21	13.537	11.28	12.687	99.6603
Mag(i+4)3	0.566	18	14.198	10.99	7.762	121.614
Mag(i+4)4	0.430	24	13.402	11.16	14.028	103.384
Mag(i+4)5	0.490	14	15.684	10.99	12.253	91.951
Mag(i+4)6	0.559	18	13.672	10.99	10.861	97.766
Mag(i+4)7	0.445	26	14.021	11.16	13.359	104.697
Mag(i+4)8	0.508	49	16.127	11.16	14.275	103.744
Mag(i+4)9	0.607	28	18.986	10.73	11.545	78.655
Mag(i+4)10	0.551	15	13.320	10.99	11.350	100.438
Mag(i+4)11	0.366	16	12.907	11.16	14.435	102.540
Mag(i+4)12	0.468	51	15.271	11.16	14.754	101.222
Mag(i+4)13	0.564	23	13.596	10.99	14.184	87.685
Mag(i+4)14	0.536	19	13.188	14.00	16.177	103.941
Mag(i+4)15	0.365	15	12.133	11.16	14.682	102.505
Mag(i+4)16	0.415	11	12.333	11.16	14.922	101.059
Mag(i+4)17	0.547	30	12.726	11.16	17.185	95.744
Mag(i+4)18	0.551	19	13.135	14.00	18.917	103.915

Supplementary Table 1 Measured and calculated biophysical parameters for a staple-scanning Mag(i+4) library.

Strain	Microorganism	Drug Sensitivity								Mag(i+4)1,15(A9K) MIC (μg/ml)*
		Amp	Ceftaz	CTX	Cipro	Doxy	Gent	Mero	TMP/SMX	
RB001	*E. coli*	S	S	S	S	I	S	S	S	1.56
RB002	*E. coli*	R	R	R	R	R	-	-	I	1.56
RB019	*P. aeruginosa*	-	S	-	S	-	-	S	-	3.12
RB020	*P. aeruginosa*	-	R	-	I	-	-	-	-	1.56
RB040	*K. pneumoniae*	-	S	-	S	-	S	-	-	6.25
RB013	*K. pneumoniae*	-	R	-	R	-	I	-	-	3.12
RB197	*A. baumannii*	-	-	-	S	-	S	S	S	1.56
RB206	*A. baumannii*	-	-	-	R	-	R	R	R	1.56

S=Susceptible, I=Intermediate, R=Resistant; Amp=Ampicillin, Ceftaz=Ceftazidime, CTX=Ceftriaxone Cipro=Ciprofloxacin, Doxy=Doxycycline, Gent=Gentamicin, Mero=Meropenem, TMP/SMX=Trimethoprim/sulfamethoxazole. *Geometric mean.

Supplementary Table 2 Activity of Mag(i+4)1,15(A9K) against drug-resistant Gram-negative clinical isolates from the Massachusetts General Hospital.

Peptide	Sequence	MW	(MW+3)/3	Figures/Tables
Magainin II	GIGKFLHSAKKFGKAFVGEIMNS	2466	823	1,2,4,5,S1, S10b
Mag(i+4)0	XIGKXLHSAKKFGKAFVGEIBNS	2493	832	1,2,S2,S3,S4,Table S1
Mag(i+4)1	GXGKFXHSAKKFGKAFVGEIBNS	2471	825	1,2,S2,S3,S4,Table S1
Mag(i+4)2	GIXKFLXSAKKFGKAFVGEIBNS	2503	835	1,2,3,S2,S3,S4,Table S1
Mag(i+4)3	GIGXFLHXAKKFGKAFVGEIBNS	2482	828	1,2,S2,S3,S4,Table S1
Mag(i+4)4	GIGKXLHSXKKFGKAFVGEIBNS	2479	827	1,2,S2,S3,S4,Table S1
Mag(i+4)5	GIGKFXHSAXKFGKAFVGEIBNS	2456	820	1,2,S2,S3,S4,Table S1
Mag(i+4)6	GIGKFLXSAKXFGKAFVGEIBNS	2432	812	1,2,S2,S3,S4,Table S1
Mag(i+4)7	GIGKFLHXAKKXGKAFVGEIBNS	2463	822	1,2,3,S2,S3,S4,Table S1
Mag(i+4)8	GIGKFLHSXKKFXKAFVGEIBNS	2569	857	1,2,3,S2,S3,S4,Table S1
Mag(i+4)9	GIGKFLHSAXKFGXAFVGEIBNS	2441	815	1,2,S2,S3,S4,Table S1
Mag(i+4)10	GIGKFLHSAKXFGKXFVGEIBNS	2498	834	1,2,S2,S3,S4,Table S1
Mag(i+4)11	GIGKFLHSAKKXGKAXVGEIBNS	2403	802	1,2,S2,S3,S4,Table S1
Mag(i+4)12	GIGKFLHSAKKFXKAFXGEIBNS	2541	848	1,2,S2,S3,S4,Table S1
Mag(i+4)13	GIGKFLHSAKKFGXAFVXEIBNS	2512	838	1,2,S2,S3,S4,Table S1
Mag(i+4)14	GIGKFLHSAKKFGKXFVGXIBNS	2497	833	1,2,3,S2,S3,S4,Table S1
Mag(i+4)15	GIGKFLHSAKKFGKAXVGEXBNS	2437	813	1,2,S2,S3,S4,Table S1
Mag(i+4)16	GIGKFLHSAKKFGKAFXGEIXNS	2485	829	1,2,3,S2,S3,S4,Table S1
Mag(i+4)17	GIGKFLHSAKKFGKAFVXEIBXS	2526	843	1,2,S2,S3,S4,Table S1
Mag(i+4)18	GIGKFLHSAKKFGKAFVGXIBNX	2481	828	1,2,3,S2,S3,S4,Table S1
Mag(i+4)15(G1K)	KIGKFLHSAKKFGKAXVGEXBNS	2508	837	2,S5
Mag(i+4)15(I2K)	GKGKFLHSAKKFGKAXVGEXBNS	2452	818	2,S5
Mag(i+4)15(G3K)	GIKKFLHSAKKFGKAXVGEXBNS	2508	837	2,S5
Mag(i+4)15(F5K)	GIGKKLHSAKKFGKAXVGEXBNS	2418	807	2,S5
Mag(i+4)15(L6K)	GIGKFKHSAKKFGKAXVGEXBNS	2452	818	2,S5
Mag(i+4)15(H7K)	GIGKFLKSAKKFGKAXVGEXBNS	2428	810	2,S5
Mag(i+4)15(S8K)	GIGKFLHKAKKFGKAXVGEXBNS	2478	827	2,S5
Mag(i+4)15(A9K)	GIGKFLHSKKKFGKAXVGEXBNS	2494	832	2,S5
Mag(i+4)15(F12K)	GIGKFLHSAKKKGKAXVGEXBNS	2418	807	2,S5
Mag(i+4)15(G13K)	GIGKFLHSAKKFKKAXVGEXBNS	2508	837	2,S5
Mag(i+4)15(A15K)	GIGKFLHSAKKFGKKXVGEXBNS	2494	832	2,S5
Mag(i+4)15(V17K)	GIGKFLHSAKKFGKAXKGEXBNS	2466	823	2,S5
Mag(i+4)15(G18K)	GIGKFLHSAKKFGKAXVKEXBNS	2508	837	2,S5
Mag(i+4)15(E19K)	GIGKFLHSAKKFGKAXVGKXBNS	2436	813	2,S5
Mag(i+4)15(B21K)	GIGKFLHSAKKFGKAXVGEXKNS	2452	818	2,S5
Mag(i+4)15(N22K)	GIGKFLHSAKKFGKAXVGEXBKS	2451	818	2,S5
Mag(i+4)15(S23K)	GIGKFLHSAKKFGKAXVGEXBNK	2478	827	2,S5
Mag(i+4)1,15(A9K)	GXGKFXHSKKKFGKAXVGEXBNS	2518	840	4, 5,S5,S6,S7,S8,S12,Table S2
Mag(i+7)1	G8GKFLHSXKKFGKAFVGEIBNS	2555	853	S1
Mag(i+7)2	GI8KFLHSAXKFGKAFVGEIBNS	2554	852	S1
Mag(i+7)3	GIG8FLHSAKXFGKAFVGEIBNS	2483	829	S1
Mag(i+7)4	GIGK8LHSAKKXGKAFVGEIBNS	2445	816	S1
Mag(i+7)5	GIGKF8HSAKKFXKAFVGEIBNS	2569	857	S1
Mag(i+7)6	GIGKFL8SAKKFGXAFVGEIBNS	2474	826	S1
Mag(i+7)7	GIGKFLH8AKKFGKXFVGEIBNS	2581	861	S1
Mag(i+7)8	GIGKFLHS8KKFGKAXVGEIBNS	2521	841	S1
Mag(i+7)9	GIGKFLHSA8KFGKAFXGEIBNS	2512	838	S1
Mag(i+7)10	GIGKFLHSAK8FGKAFVXEIBNS	2554	852	S1
Mag(i+7)11	GIGKFLHSAKK8GKAFVGXIBNS	2463	822	S1
Mag(i+7)12	GIGKFLHSAKKF8KAFVGEXBNS	2569	857	S1
Mag(i+7)13	GIGKFLHSAKKFG8AFVGEIXNS	2498	834	S1
Mag(i+7)14	GIGKFLHSAKKFGK8FVGEIBXS	2554	852	S1
Mag(i+4)1,15 (K4R, A9K)	GXGRFXHSKKKFGKAXVGEXBNS	2546	850	5
Mag(i+4)1,15 (K4R, A9K, K10R)	GXGRFXHSKRKFGKAXVGEXBNS	2574	859	5
Mag(i+4)1,15 (K4H, A9K)	GXGHFXHSKKKFGKAXVGEXBNS	2527	843	5
Mag(i+4)1,15 (K4H, A9K, K10H)	GXGHFXHSKHKFGKAXVGEXBNS	2536	846	5
Mag(i+4)1,15 (K4H, A9K, K10H, K11H)	GXGHFXHSKHHFGKAXVGEXBNS	2545	849	5
Mag(i+4)1,15 (A9K, B21A)	GXGKFXHSKKKFGKAXVGEXANS	2476	826	5
Mag(i+4)1,15 (A9K, B21A, N22K, S23K)	GXGKFXHSKKKFGKAXVGEXAKK	2531	845	5, 6,S9,S10,S11,S12
Pleurocidin	GWGSFFKKAAHVGKHVGKAALTHYL	2709	904	6
Pleu(i+4)12	GWGSFFKKAAHVXKHVXKAALTHYL	2845	949	6
Pleu(i+4)1,15	GXGSFXKKAAHVGKHXGKAXLTHYL	2706	903	6
Pleu(i+4)1,15(A9K)	GXGSFXKKKAHVGKHXGKAXLTHYL	2763	922	6
CAP(i+4)1,23(L17K)	GXRKRXRKFRNKIKEKKKIGQKXQGLXPKLA	3862	1288	6
Esc(i+4)1,14(A7K)	GXFSKXKGKKIKNLXISGXKG	2289	764	6

X, S5 stapling amino acid; 8, R8 stapling amino acid; B, norleucine; peptide N-termini are NH_2, and C-termini are $CONH_2$ for stapled peptides and COOH for Mag2.

Supplementary Table 3 Stapled peptide compositions and experimental applications.

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