

Antibacterial Activity Affected by the Conformational Flexibility in Glycine-Lysine-Based α -Helical Antimicrobial Peptides

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Table S1: Training Set of Peptides with Measured MIC (*E. coli*) and HC50 Values (µM).

No.	Name	Sequence	MIC	HC ₅₀	SI(exp) ^a	SI(pred) ^b	Table S1 References
1	DiPGLa-H	KIAKVALKALKIAKVALKAL-NH2	1.5	270	180	93	1
2	Brevinin-2Ej	GIFLDKLNFAKGV AQSLLNKASCKLSGQC	2.0			20	2
3	Esculentin-1ARa	GIFSKINKKAKTGLFNIIKTGVKEAGMDVIRAGIDTISCKIKGEC	2.0	180	90	93	3
4	Dermaseptin-3	ALWKNMLKGIGKLAGKAALGAVKKLVGAES	2.5	32	<80 ^d	69	4
5	Dermaseptin-B3	ALWKNMLKGIGKLAGQAALGAVKTLVGA	2.6			14	5
6	Ascaphin 7	GFKDWIKGAAKLIKTVASAIANQ	3.0	>200	>67	9	UniProt ^c
7	Ascaphin 7-2	GFKDWIKGAAKLIKTVASSIANQ	3.0	>200	>67	6	6
8	CPF-MW1	GLGSLGKAFKFLKTVGKMMGGAPREQ	3.0	70	23	10	7
9	Brevinin-2Ei	GILSTIKDFAIKAGKGAAGLLEMASCKLSGQC	3.0			55	2
10	Brevinin-2SKb	GLFNVFKKVGNV LKNVAGSLMDNLKCKVSGEC	3.0			12	8
11	Brevinin-2SKa	GLFSAFKKVGNV LKNVAGSLMDNLKCKVSGEC	3.0			15	8
12	Brevinin-2Pre	GLLSVLKGVLKTAGKHIFKNVGGSLLDQAKCKISGQC	3.0	80	27	15	9
13	Brevinin-2PRb	GLMSLFRGV LKTAGKHIFKNVGGSLLDQAKCKITGEC	3.0	65	22	15	9
14	Brevinin-2PRc	GLMSVLKGVLKTAGKHIFKNVGGSLLDQAKCKISGQC	3.0	125	42	17	9
15	Brevinin-2PRd	GLMSVLKGVLKTAGKHIFKNVGGSLLDQAKCKITGQC	3.0	100	33	15	9
16	Esculentin-1ISb	RIFSKIGGKAIKNLILKGIKNIGKEVGM DVIRTGIDVAGCKIKGEC	3.1			95	10
17	Dermaseptin-4	ALWMTLLKVLKAAAKAALNAV LVGANA	4.0	<1	<0.25 ^d	11	4
18	CPRF-Ec	GLGSFFKNAIKIAGKVGSTIGKVAD AIGNKE	4.0			55	2
19	CPRF-Ea	GLGSILGKILNVAGKVGKTIGKVAD AVGNKE	4.0			46	2
20	Temporin-SHd	FLPAALAGIGGILGKLF-NH2	5.0	44	9	6	11
21	Dermaseptin-B4	ALWKDILKNVGKAAGKAVLNTVTDMVNQ	5.0	<200 ^e	<40	79	5
22	CPRF-Eb	GLGSFLKNAIKIAGKVGSTIGKVAD AIGNKE	5.0			63	2
23	Ranatuerin-2CSa	GILSSFKGVAKGVAKDLAGK LLET LKCKITGC	5	160	32	22	12
24	Ranatuerin-2AUa	GILSSFKGVAKGVAKNLAGK LLD ELKCKITGC	5	290	58	28	13
25	Esculentin-2CHa	GFSSIFRGVAKFASKGLGKDLAKLGVDLVACKISKQC	5	150	50	63	14
26	Ascaphin-8	GFKDLLKGAALVKT VLF-NH2	6.0	50	8	6	6
27	Ascaphin-1	GFRDVLKGAAKAFVKT VAGHIAN	6.0	>200	>33	54	6
28	Ascaphin-3	GFRDVLKGAAKAFVKT VAGHIANI	6.0	>200	>33	44	6
29	RV-23	RIGVLLARLPKLFSLFKLMGKKV	6.0	35	6	12	15
30	CPF-C1	GFGSLGKALRLGANV L-NH2	6.0	140	23	5	16
31	Ranatuerin-2PRd	GILSSIKGVAKGVAKNVAQLD TLKCKITGC	6	>100	>17	14	17
32	Brevinin-2PRa	GLMSLFRGV LKTAGKHIFKNVGGSLLDQAKCKITGEC	6	55	9	15	9
33	Kassinatuerin-1	GFMKYIGPLIPHAVKAISDLI-NH2	6.25	65	10	7	18
34	Esculentin-1Isa	GIFSKFAGKGIKNLLVKG VKNIGKEVGM DVIRTGIDIAGCKIKGEC	6.3			39	10

^aSI=Selectivity index, calculated as HC₅₀/MIC when both values are known from experiments for the peptide. ^bPredicted SI as the top output value of the MUTATOR algorithm¹⁹

^cUniProt sequence for the ascaphin-7 is used, although that sequence is not identical to the sequence cited for the ascaphin-7 in the paper by Conlon et. al.⁶ ^dEstimated from the value for 100% haemolysis of red blood cells after one-hour incubation with peptide as reported in Ali et al.³ ^ePolar face angle is equal to 110° for predicted 1-28 helical domain

^eEstimated value for 100% haemolysis of red blood cells after one-hour incubation with the peptide dermaseptin B4²⁰

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Table S2. Helical Content of Kiadin Peptides in Different Environments.

	H ₂ O (MD) ^a	SPB (CD) ^b	SDS (CD) ^b	50% TFE (CD) ^b	30% TFE (MD) ^a	LUV (CD) ^b	DLPC (MD) ^a
kiadin-2	58%	<5%	45%	40%	85%	(>100%) ^c	85%
kiadin-3	42%	<5%	55%	90%	80%	(>100%) ^c	70%
kiadin-4	6%	5%	60%	75%	70%	^c (60%) ^c	65%
kiadin-5	32%	<5%	20%	60%	50%	65%	65%
kiadin-6	64%	20%	>90%	>90%	80%	^c (60%)	80%

^aFrom molecular dynamics calculations.

^bEstimated from CD spectra q intensity according to Chen et al.¹

^cSpectra deviate in shape from that of canonical α -helix so that % helix content cannot be estimated reliably

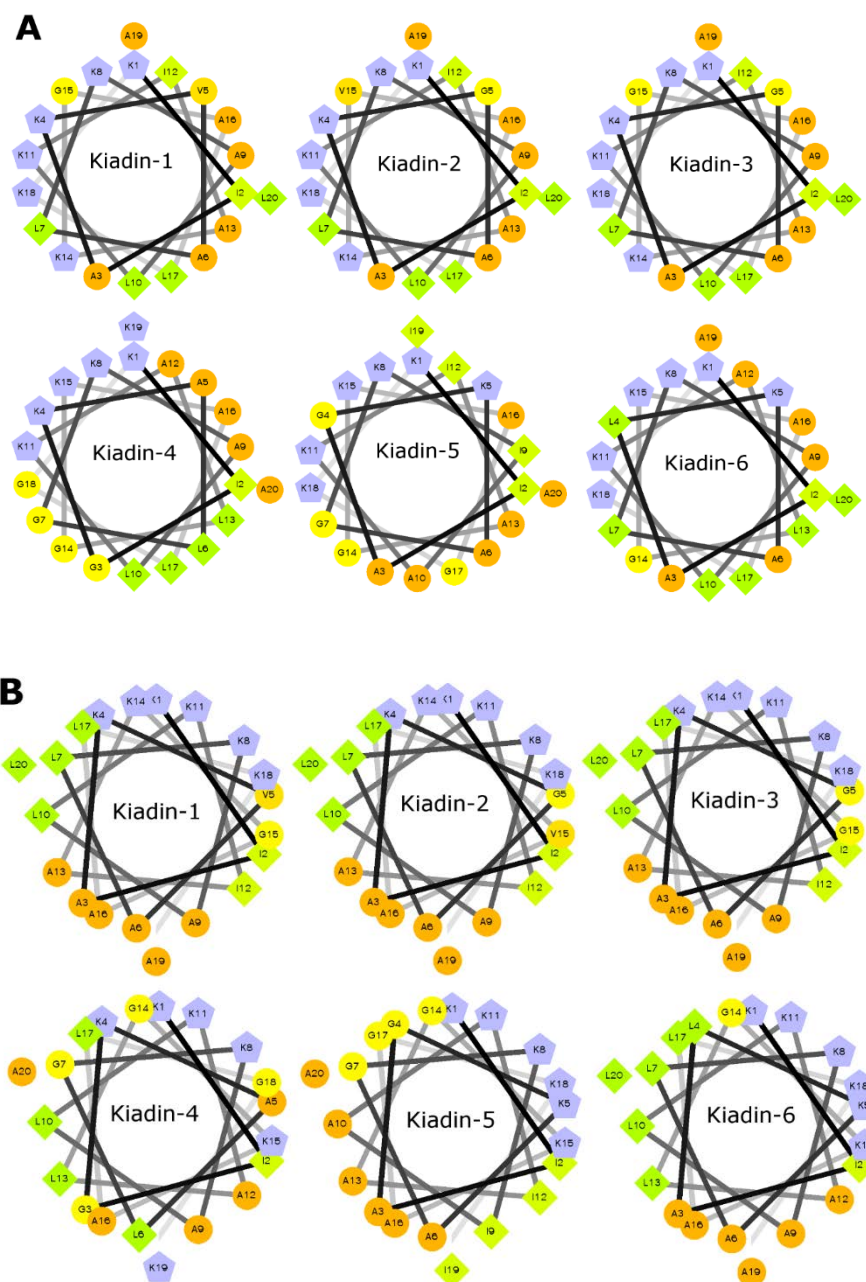


Figure S1. Helical Wheel Projections for Kiadin Peptides. Projections were obtained using the Helical Wheel Projections server at <http://rzlab.ucr.edu/scripts/wheel/wheel.cgi>. **A**) Per AA rotation = 100° (α -helix). **B**) Per AA rotation = 110° (intermediate between α and 3_{10} helix).

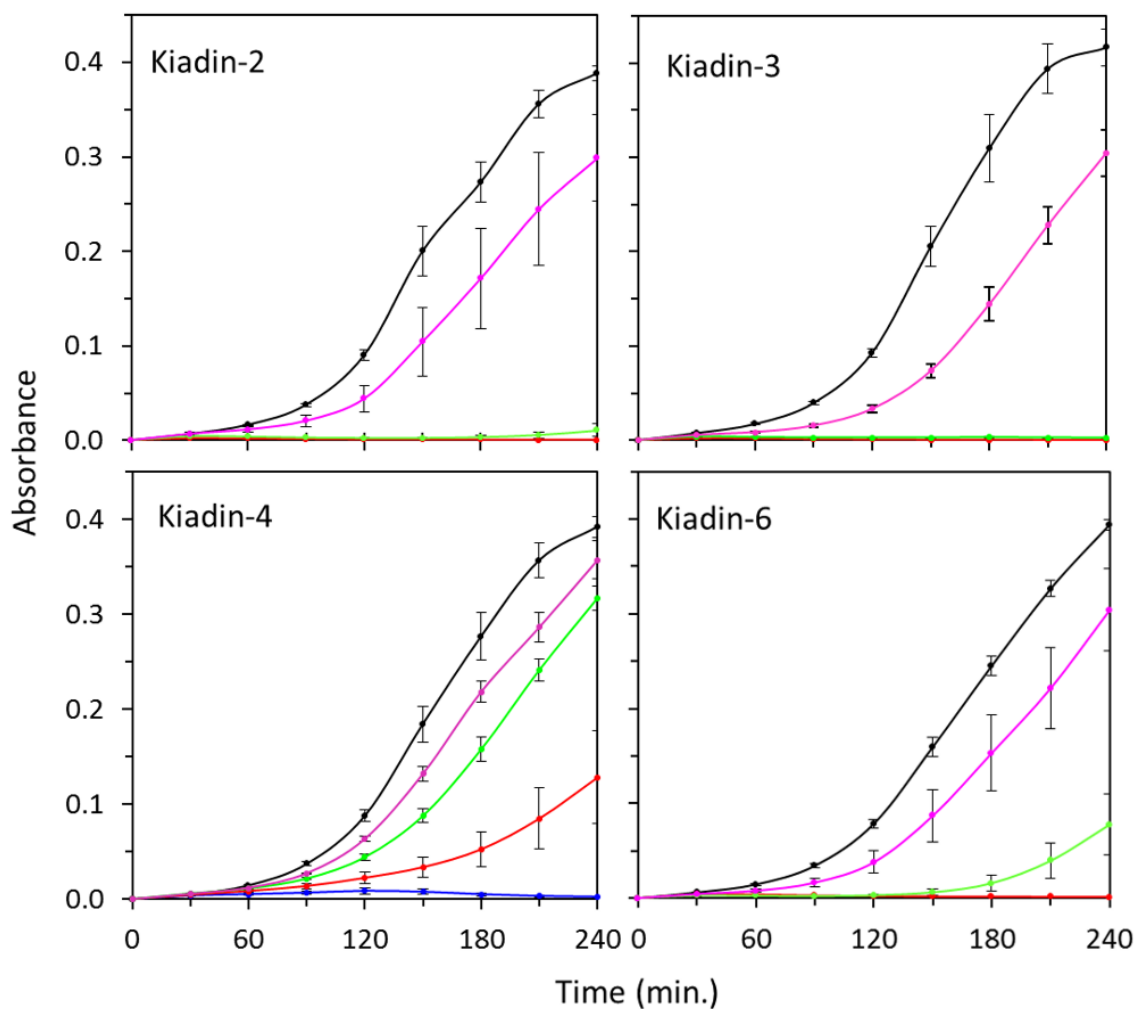


Figure S2. Effect of Kiadins on Bacterial Growth Kinetics. Growth curves for *E. coli* ATCC 25922 are shown after incubation with no peptide (—), 0.25 (—), 0.5 (—), 1 (—) and 2 (—) μM of Kiadins, depending on the MIC values. Results were obtained by measuring the absorbance at 620 nm for bacteria grown in full MHB.

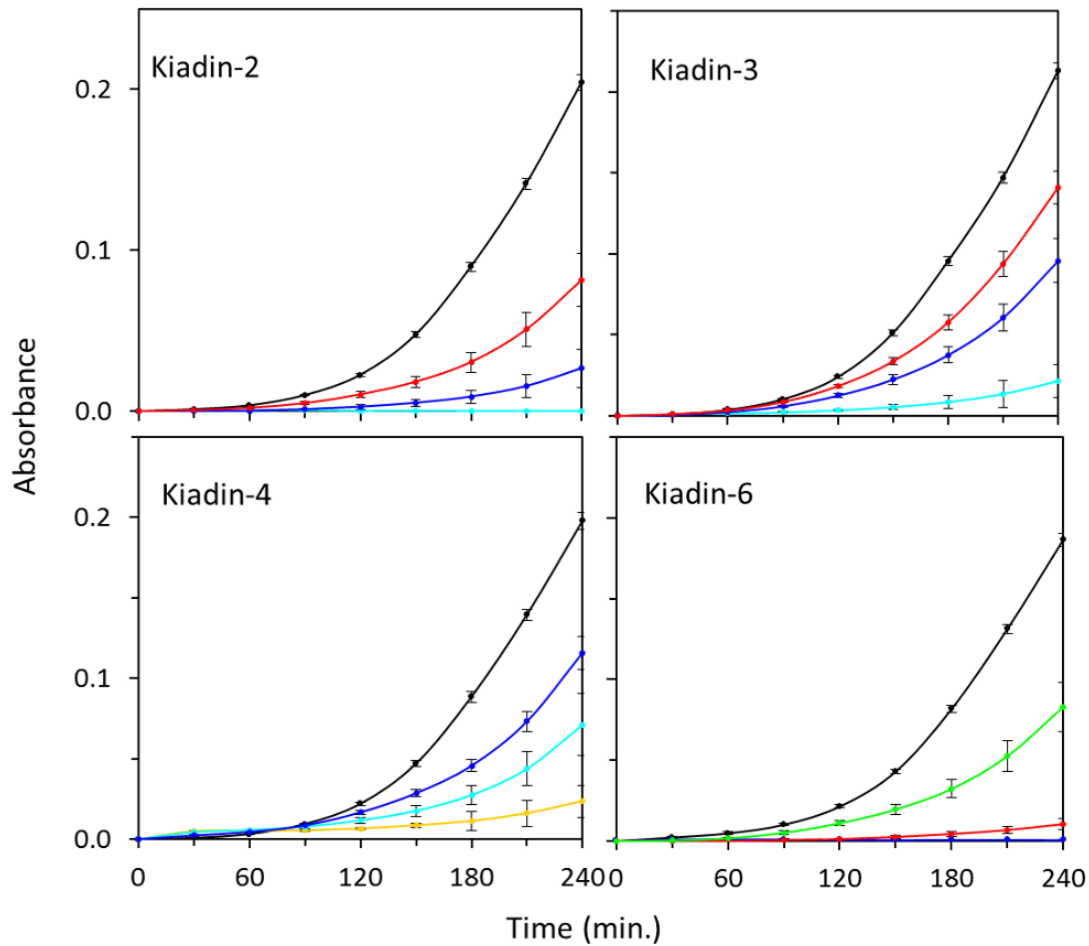


Figure S3. Effect of Kiadins on Bacterial Growth Kinetics. Growth curves for *S. aureus* ATCC 25923 are shown after incubation with no peptide (—), 0.5 (—), 1 (—), 2 (—), 4 (—), and 8 (—) μM of Kiadins, depending on the MIC values. Results were obtained by measuring the absorbance at 620 nm for bacteria grown in full MHB.

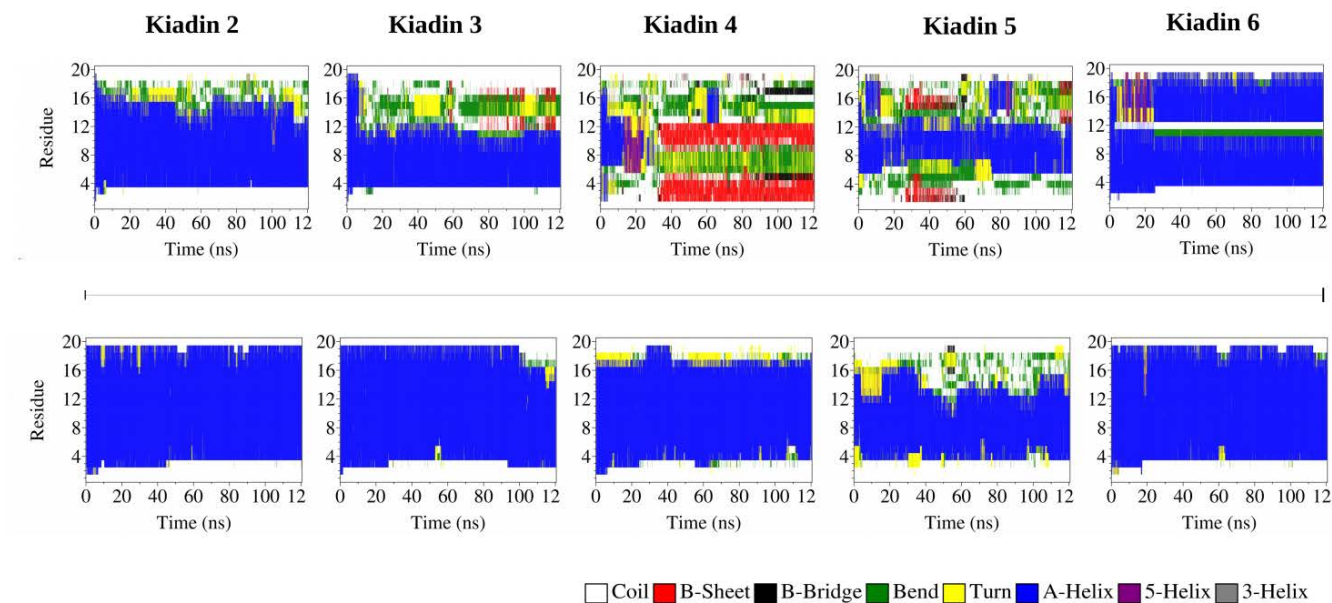


Figure S4. Molecular Dynamics Simulation of Peptides in an Aqueous Environment and in the Presence of 30% v/v TFE(bottom row). The plots show of residues along the sequence, evolving with time, calculated by DSSP program ². All peptide starting structures were helical structures as produced by the Quark tool ³. The peptides were then subjected to 120 ns of MD run using Gromacs package ⁴ as described in the paper.

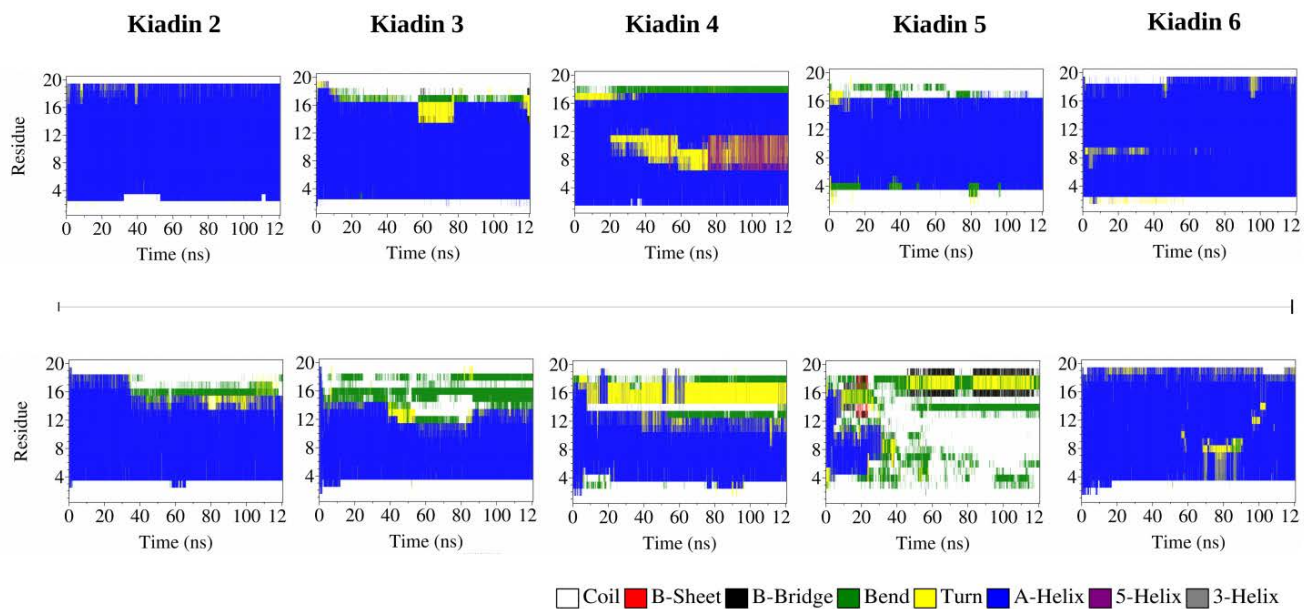


Figure S5. Molecular Dynamics Simulation of Kiadin Peptides Placed Within (**top row**) and on the Surface (**bottom row**) of the DLPC Membrane. The plots show of residues along the sequence evolving with time calculated by DSSP program ². All peptide starting structures were helical structures as produced by the Quark tool ³. The peptides were then subjected to 120 ns of MD run using Gromacs package ⁴ as described in the paper.

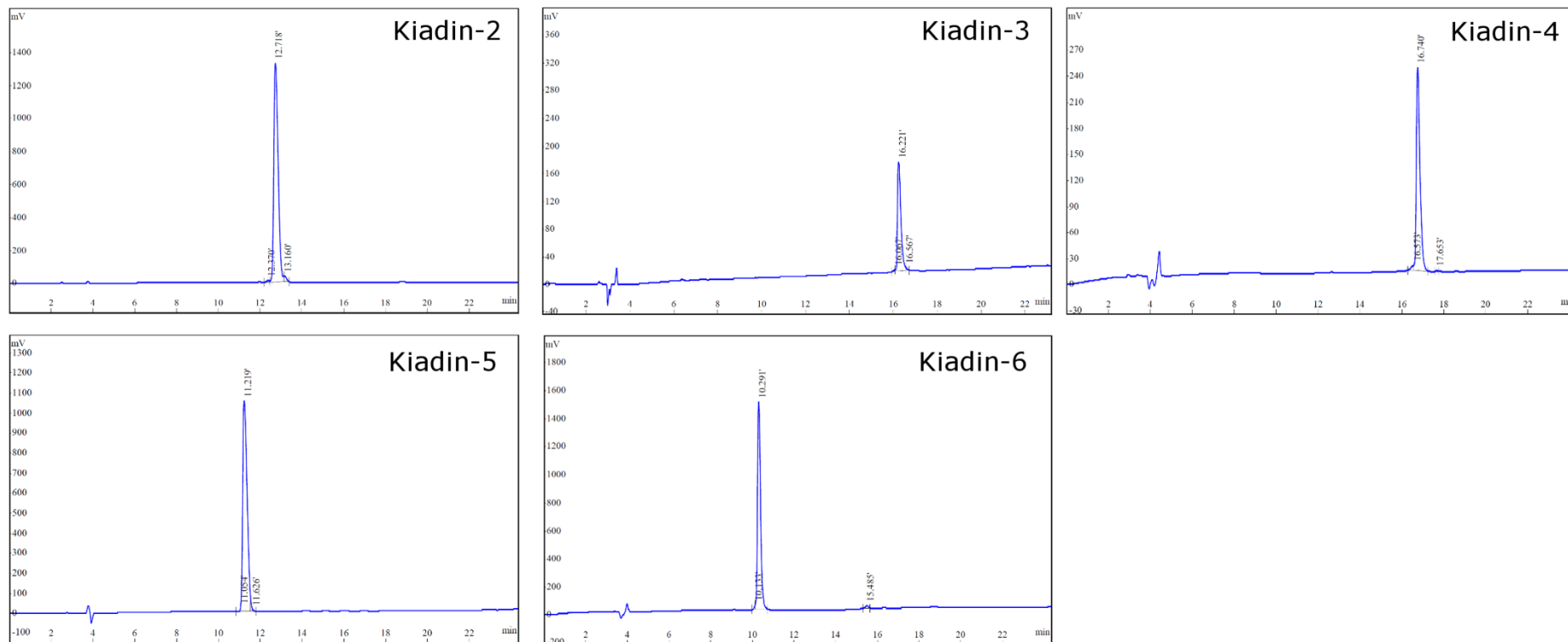


Figure S6. Analytical RP-HPLC of Kiadin Peptides. Phenomenex Gemini-NX analytical column (C18, 5 μm , 110 \AA , 4.6 x 250 mm) was used with 25-50 % acetonitrile/0.1% TFA gradient in 25 min with flow of 1.0 mL/min.

References associated with Figure S1, Table S2, Figure S4, and Figure S5:

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