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

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## Supporting information

**Table S1.** Training Set of Peptides with Measured MIC (*E. coli*) and $HC_{50}$ Values (μM).

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**Figure S1**. Helical Wheel Projections for Kiadin 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 | *Di*PGLa-H | KIAKVALKALKIAKVALKAL-NH2 | 1.5 | 270 | 180 | 93 | [1] |
| 2 | Brevinin-2Ej | GIFLDKLKNFAKGVAQSLLNKASCKLSGQC | 2.0 | | | 20 | [2] |
| 3 | Esculentin-1ARa | GIFSKINKKKAKTGLFNIIKTVGKEAGMDVIRAGIDTISCKIKGEC | 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 | GFKDWIKGAAKKLIKTVASAIANQ | 3.0 | >200 | >67 | 9 | UniProt[c] |
| 7 | Ascaphin 7-2 | GFKDWIKGAAKKLIKTVASSIANQ | 3.0 | >200 | >67 | 6 | [6] |
| 8 | CPF-MW1 | GLGSLLGKAFKFGLKTVGKMMGGAPREQ | 3.0 | 70 | 23 | 10 | [7] |
| 9 | Brevinin-2Ei | GILSTIKDFAIKAGKGAAKGLLEMASCKLSGQC | 3.0 | | | 55 | [2] |
| 10 | Brevinin-2SKb | GLFNVFKKVGKNVLKNVAGSLMDNLKCKVSGEC | 3.0 | | | 12 | [8] |
| 11 | Brevinin-2SKa | GLFSAFKKVGKNVLKNVAGSLMDNLKCKVSGEC | 3.0 | | | 15 | [8] |
| 12 | Brevinin-2PRe | GLLSVLKGVLKTAGKHIFKNVGGSLLDQAKCKISGQC | 3.0 | 80 | 27 | 15 | [9] |
| 13 | Brevinin-2PRb | GLMSLFRGVLKTAGKHIFKNVGGSLLDQAKCKITGEC | 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 | RIFSKIGGKAIKNLILKGIKNIGKEVGMDVIRTGIDVAGCKIKGEC | 3.1 | | | 95 | [10] |
| 17 | Dermaseptin-4 | ALWMTLLKKVLKAAAKAALNAVLVGANA | 4.0 | <1 | <0.25[d] | 11 | [4] |
| 18 | CPRF-Ec | GLGSFFKNAIKIAGKVGSTIGKVADAIGNKE | 4.0 | | | 55 | [2] |
| 19 | CPRF-Ea | GLGSILGKILNVAGKVGKTIGKVADAVGNKE | 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 | GLGSFLKNAIKIAGKVGSTIGKVADAIGNKE | 5.0 | | | 63 | [2] |
| 23 | Ranatuerin-2CSa | GILSSFKGVAKGVAKDLAGKLLETLKCKITGC | 5 | 160 | 32 | 22 | [12] |
| 24 | Ranatuerin-2AUa | GILSSFKGVAKGVAKNLAGKLLDELKCKITGC | 5 | 290 | 58 | 28 | [13] |
| 25 | Esculentin-2CHa | GFSSIFRGVAKFASKGLGKDLAKLGVDLVACKISKQC | 5 | 150 | 50 | 63 | [14] |
| 26 | Ascaphin-8 | GFKDLLKGAAKALVKTVLF-NH2 | 6.0 | 50 | 8 | 6 | [6] |
| 27 | Ascaphin-1 | GFRDVLKGAAKAFVKTVAGHIAN | 6.0 | >200 | >33 | 54 | [6] |
| 28 | Ascaphin-3 | GFRDVLKGAAKAFVKTVAGHIANI | 6.0 | >200 | >33 | 44 | [6] |
| 29 | RV-23 | RIGVLLARLPKLFSLFKLMGKKV | 6.0 | 35 | 6 | 12 | [15] |
| 30 | CPF-C1 | GFGSLLGKALRLGANVL-NH2 | 6.0 | 140 | 23 | 5 | [16] |
| 31 | Ranatuerin-2PRd | GILSSIKGVAKGVAKNVAAQLLDTLKCKITGC | 6 | >100 | >17 | 14 | [17] |
| 32 | Brevinin-2PRa | GLMSLFKGVLKTAGKHIFKNVGGSLLDQAKCKITGEC | 6 | 55 | 9 | 15 | [9] |
| 33 | Kassinatuerin-1 | GFMKYIGPLIPHAVKAISDLI-NH2 | 6.25 | 65 | 10 | 7 | [18] |
| 34 | Esculentin-1Isa | GIFSKFAGKGIKNLLVKGVKNIGKEVGMDVIRTGIDIAGCKIKGEC | 6.3 | | | 39 | [10] |

[a] SI=Selectivity index, calculated as $HC_{50}$/MIC when both vales are known from experiments for the peptide. [b] Predicted SI as the top output value of the MUTATOR algorithm[19]

[c] UniProt 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] [d] Estimated from the value for 100% haemolysis of red blood cells after one-hour incubation with peptide as reported in Ali et al.[3] [d] Polar face angle is equal to 110° for predicted 1-28 helical domain

[e] Estimated value for 100% haemolysis of red blood cells after one-hour incubation with the peptide dermaseptin B4[20]

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

| | $H_2O$ (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% |

[a]From molecular dynamics calculations.
[b]Estimated from CD spectra q intensity according to Chen et al.[1]
[c]Spectra deviate in shape from that of canonical a-helix so that % helix content cannot be estimated reliably

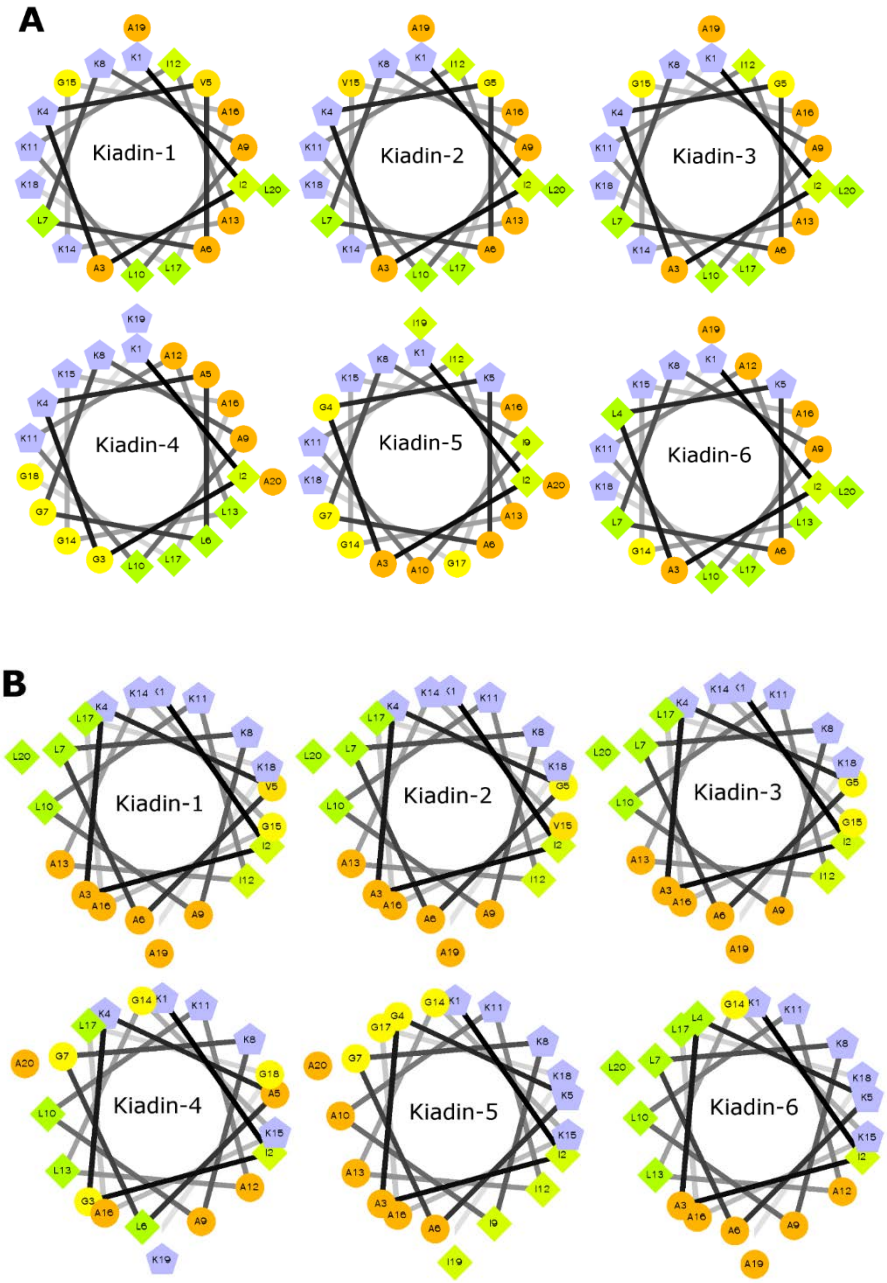


**Figure S1.** Helical Wheel Projections for Kiadin Peptides. Projections were obtained using the Helical Wheel Projections server at http://rzlab.ucr.edu/scripts/wheel/wheel.cgi. **A)** Per AA rotation = 100° (α-helix). **B)** Per AA rotation = 110° (intermediate between α and $3_{10}$ helix).

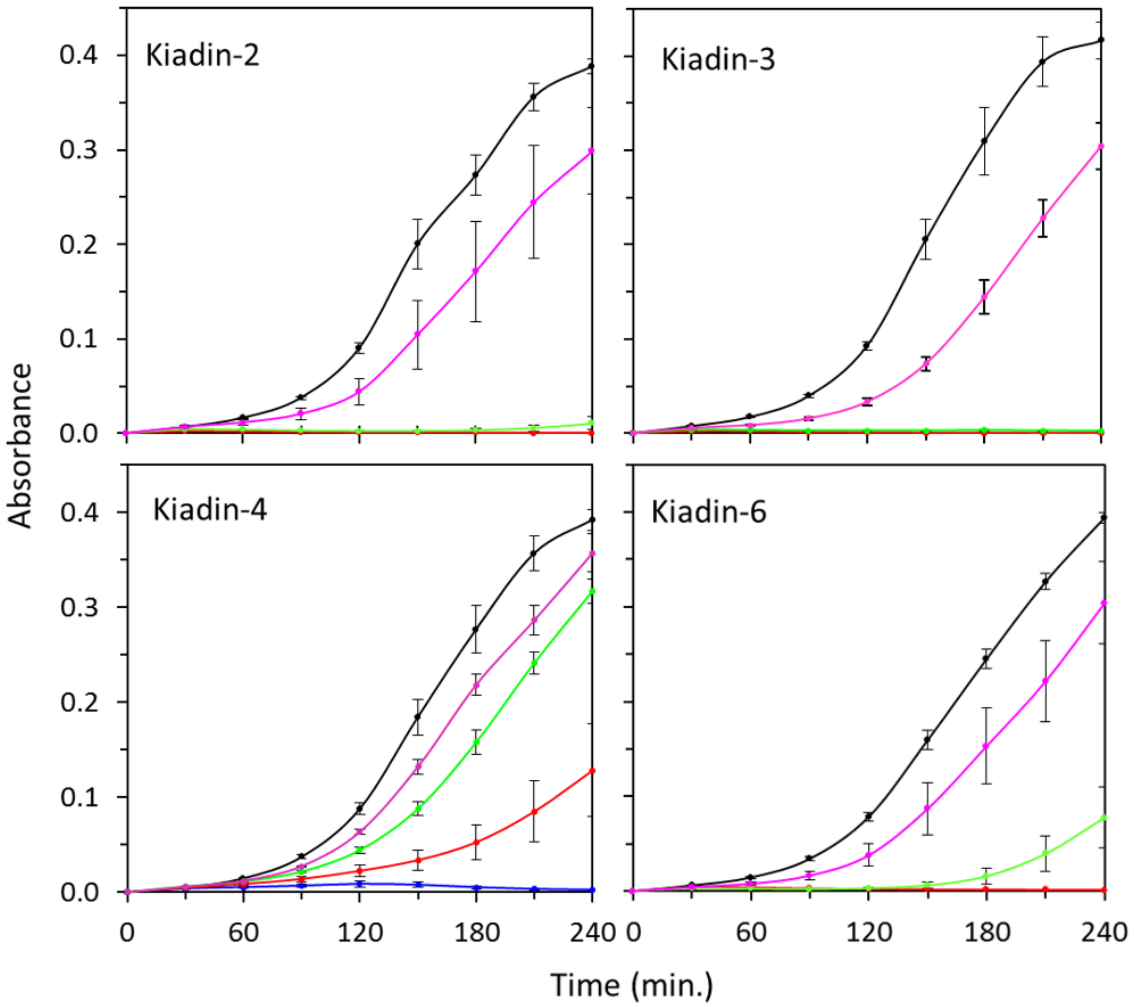


**Figure S2**. Effect of Kiadins on Bacterial Growth Kinetics. Growth curves for *E. coli* ATCC 25922 are shown after incubation with no peptide (—), 0.25 (—), 0.5 (—), 1 (—) and 2 (—) µM of Kiadins, depending on the MIC values. Results were obtained by measuring the absorbance at 620 nm for bacteria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

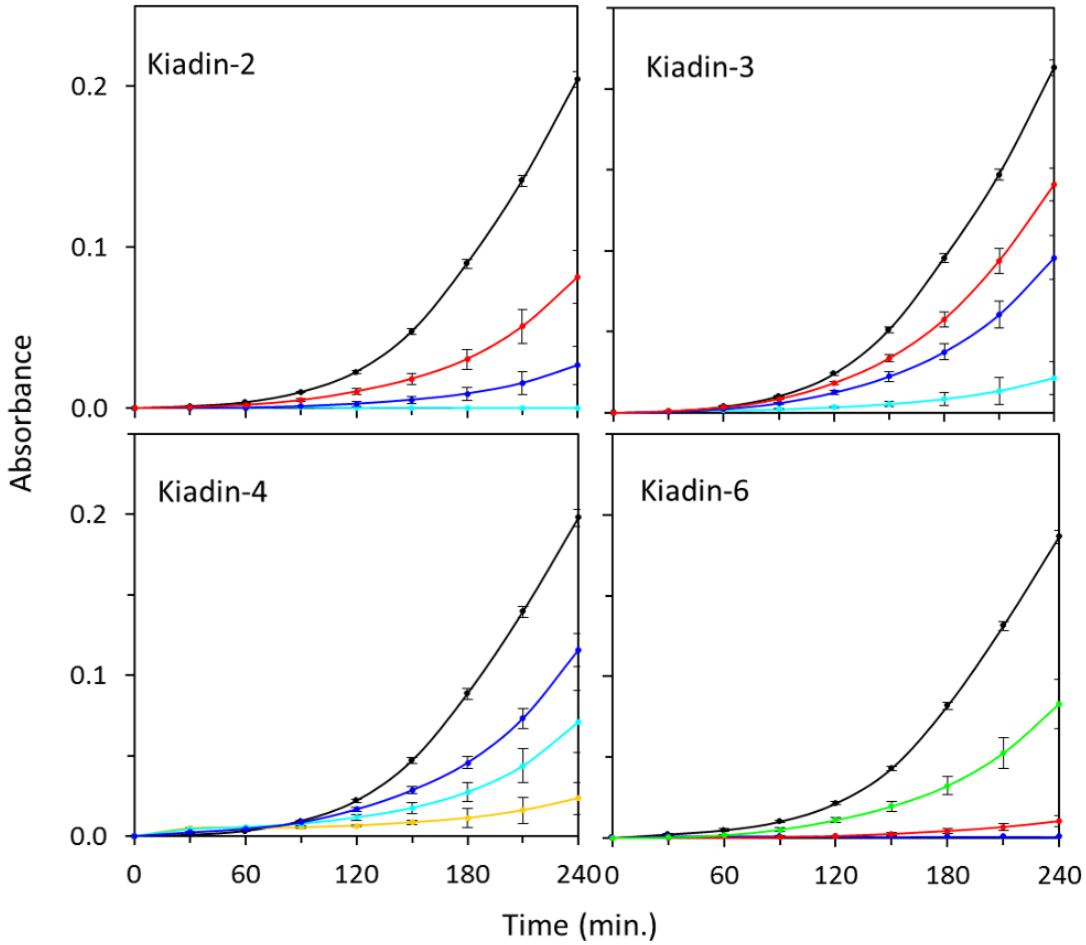


**Figure S3.** Effect of Kiadins on Bacterial Growth Kinetics**.** Growth curves for *S. aureus* ATCC 25923 are shown after incubation with no peptide (—),0.5 (—), 1 (—), 2 (—), 4 (—), and 8 (—) μM of Kiadins, depending on the MIC values. Results were obtained by measuring the absorbance at 620 nm for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

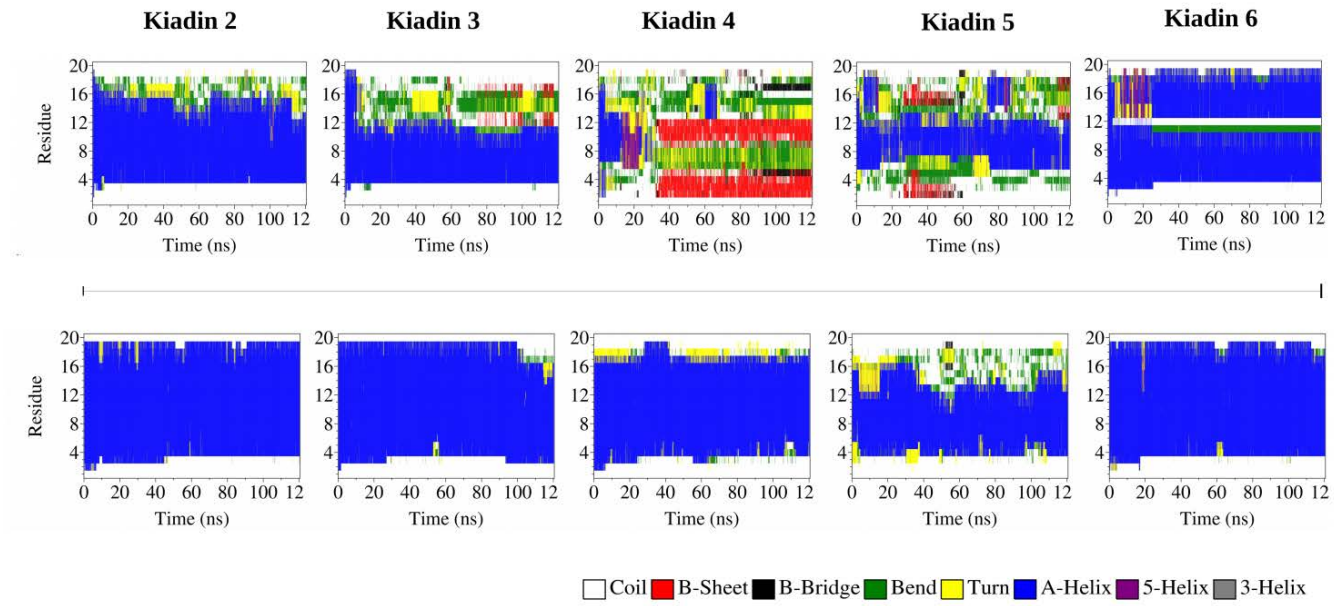


**Figure S4.** Molecular Dynamics Simulation of Peptides in an Aqueous Environment and in the Presence of 30% v/v TFE(bottom row). The plots show of residues along the sequence, evolving with time, calculated by DSSP program [2]. All peptide starting structures were helical structures as produced by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

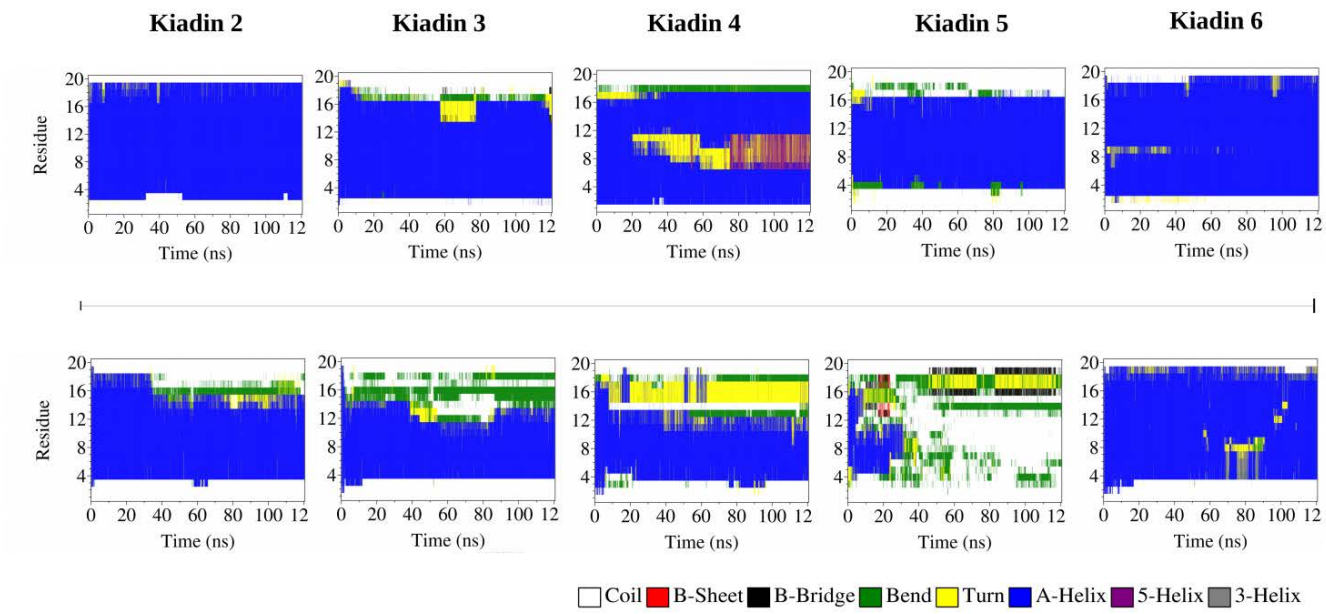


**Figure S5.** Molecular Dynamics Simulation of Kiadin Peptides Placed Within **(top row)** and on the Surface **(bottom row)** of the DLPC Membrane. The plots show of residues along the sequence evolving with time calculated by DSSP program [2]. All peptide starting structures were helical structures 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.

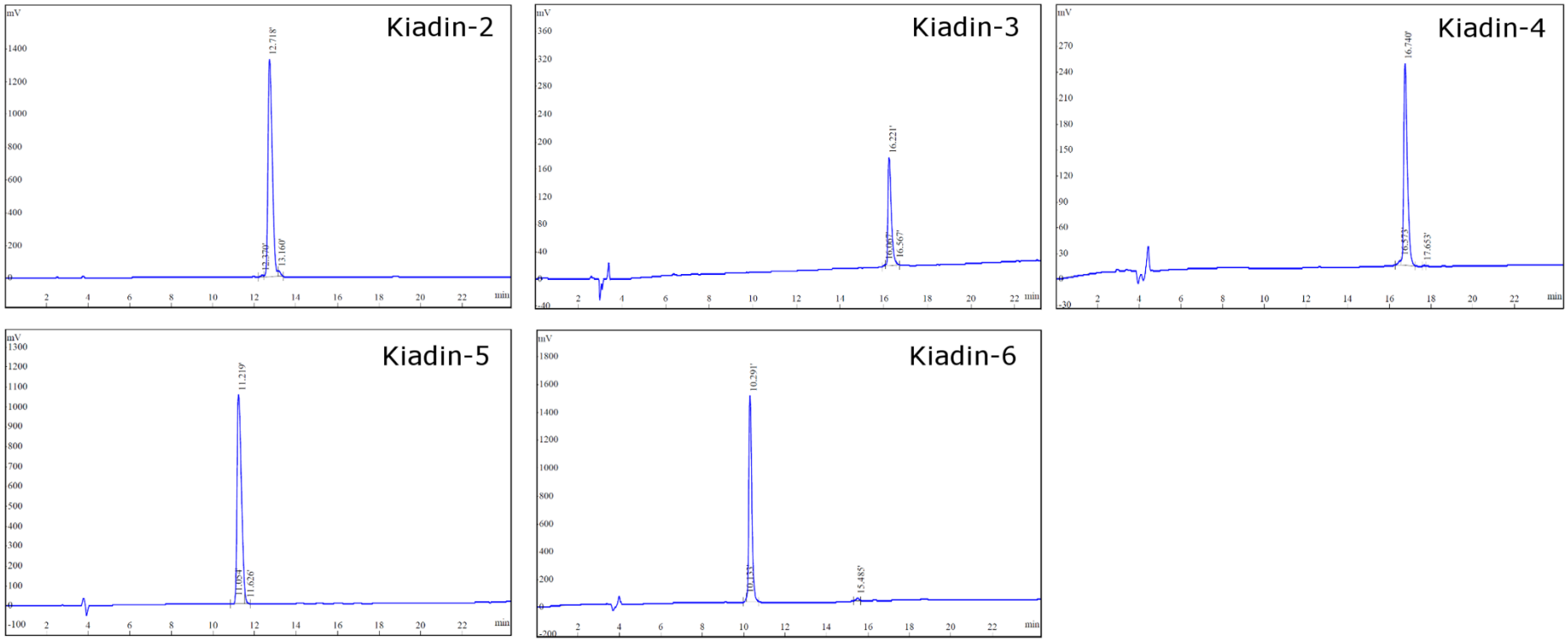


**Figure S6.** Analytical RP-HPLC of Kiadin Peptides**.** Phenomenex Gemini-NX analytical column (C18, 5 µm, 110 Å, 4.6 x 250 mm) was used with 25-50 % acetonitrile/0.1% TFA gradient in 25 min with flow of 1.0 mL/min.

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


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