

## Supporting information for:

# Glycine Substitution Reduces Antimicrobial Activity and Helical Stretch of diPGLa-H in Lipid Micelles

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## Supporting Information includes:

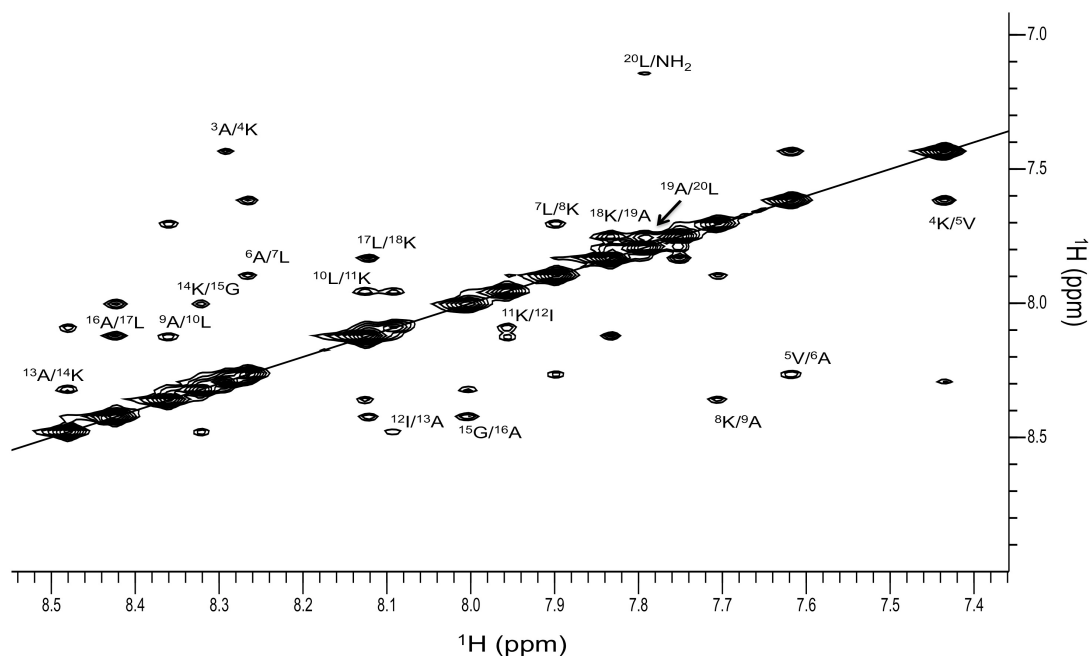
Figure S1. NOESY spectra of Kiadin

Figure S2. NOESY spectra of DiPGLa-H

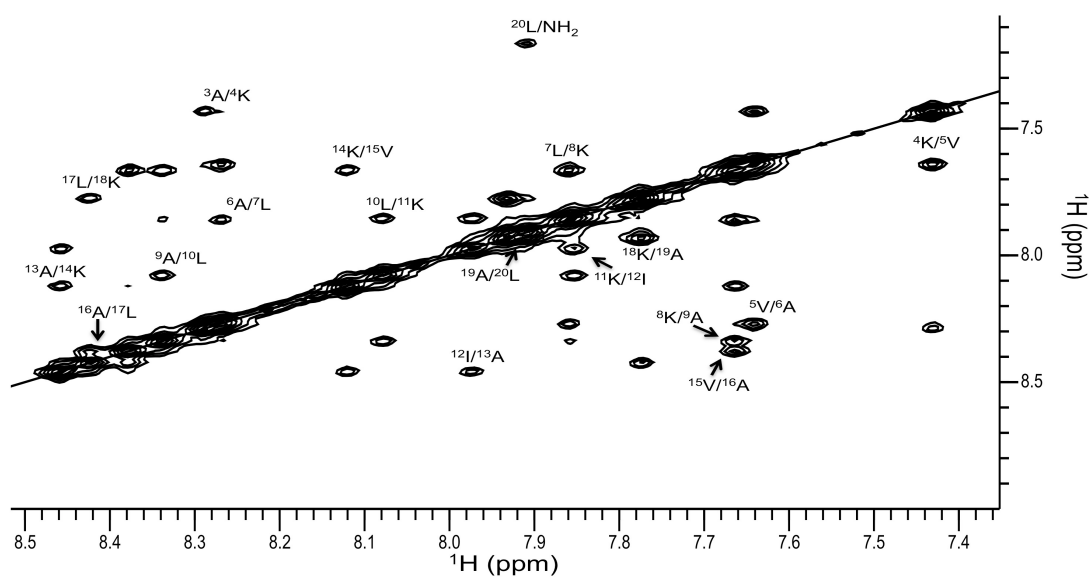
Figure S3. HSQC spectra of Kiadin and DiPGLa-H

Table S1 and S2. Chemical shifts (ppm), hydrogen bond strengths and temperature coefficients of Kiadin and diPGLa-H in SDS micelles

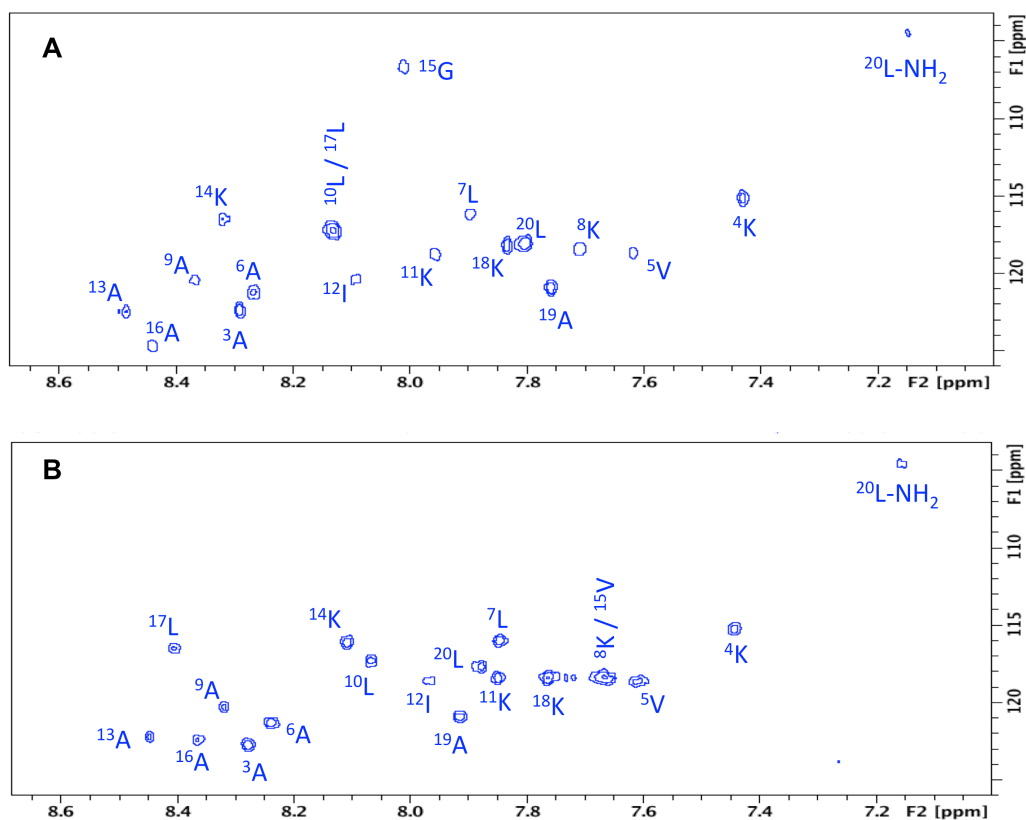
Table S3. Statistical information for the Kiadin and diPGLa-H NMR structures in SDS micelles



**Figure S1** Expanded NH region of a NOESY spectrum ( $\tau_{\text{mix}} = 150$  ms) for the 2 mM Kiadin in 150 mM SDS micelles (20 mM phosphate pH 7.4, 50 mM KCl, 0.05 mM DSS, 10% v D<sub>2</sub>O) at 50°C. Annotations represent the sequential walk from *i* to *i*+1 residue.



**Figure S2** Expanded NH region of a NOESY spectrum ( $\tau_{\text{mix}} = 150$  ms) for the 2 mM diPGLa-H in 150 mM SDS micelles (20 mM phosphate pH 7.4, 50 mM KCl, 0.05 mM DSS, 10%v D<sub>2</sub>O) at 50°C. Annotations represent the sequential walk from residue *i* to *i*+1 residue.



**Figure S3** <sup>15</sup>N <sup>1</sup>H HSQC spectra of: (A) Kiadin, and (B) DiPGLa-H in 150 mM SDS micelles (20 mM phosphate pH 7.4, 50 mM KCl, 0.05 mM DSS, 10%v D<sub>2</sub>O) at 50°C. Annotations represent the sequential walk from residue *i* to *i*+1 residue.

**Table S1:** Chemical shifts, hydrogen bond strengths and temperature coefficients of Kiadin in SDS micelles

|        | $H_N$<br>(ppm) | $H_\alpha$<br>(ppm) | $H_\beta$<br>(ppm) | $H_\gamma$<br>(ppm) | $H_\delta / H_\epsilon$<br>(ppm) | Hydrogen<br>bond<br>strength | $\Delta\delta/\Delta T$<br>(ppb/K) |
|--------|----------------|---------------------|--------------------|---------------------|----------------------------------|------------------------------|------------------------------------|
| 1 Lys  | -              | 4.10                | 2.15               | 1.94                | 1.59                             | W                            |                                    |
| 2 Ile  | -              | 3.95                | 2.01               | -                   | -                                | W                            |                                    |
| 3 Ala  | 8.30           | 3.98                | 1.48               |                     |                                  | W                            | -5.6                               |
| 4 Lys  | 7.44           | 4.01                | 1.95               | -                   | -                                | W                            | 0                                  |
| 5 Val  | 7.62           | 3.70                | 2.2                | 0.97                |                                  | W                            | -1.8                               |
| 6 Ala  | 8.27           | 3.95                | 1.42               |                     |                                  | W                            | -5.3                               |
| 7 Leu  | 7.90           | 4.05                | 1.81               | -                   | -                                | W                            | -4.1                               |
| 8 Lys  | 7.71           | 4.01                | 2.04               | -                   | -                                | W                            | -2.4                               |
| 9 Ala  | 8.36           | 3.98                | 1.46               |                     |                                  | W-M                          | -4.4                               |
| 10 Leu | 8.13           | 3.99                | 1.94               | -                   | -                                | S                            | -5.5                               |
| 11 Lys | 7.96           | 3.93                | 2.02               | -                   | -                                | W                            | -5.1                               |
| 12 Ile | 8.10           | 3.82                | 2.01               | -                   | -                                | S                            | -5.6                               |
| 13 Ala | 8.48           | 3.98                | 1.47               |                     |                                  | S                            | -5.6                               |
| 14 Lys | 8.32           | 3.92                | 2.00               | -                   | -                                | W                            | -3.9                               |
| 15 Gly | 8.01           | 3.88                |                    |                     |                                  | W                            | -2.4                               |
| 16 Ala | 8.43           | 4.14                | 1.48               |                     |                                  | S                            | -6.2                               |
| 17 Leu | 8.13           | 4.05                | 1.87               | -                   | -                                | S                            | -5.5                               |
| 18 Lys | 7.84           | 4.07                | 1.94               | -                   | -                                | W                            | -4.9                               |
| 19 Ala | 7.76           | 4.22                | 1.50               |                     |                                  | W                            | -2.6                               |
| 20 Leu | 7.80           | 4.20                | 1.83               | 1.63                | -                                | W                            | -5.0                               |
|        |                |                     |                    |                     |                                  |                              |                                    |

**Table S2:** Chemical shifts (ppm), hydrogen bond strengths and temperature coefficients of diPGLa-H in SDS micelles

|        | H <sub>N</sub><br>(ppm) | H <sub>α</sub><br>(ppm) | H <sub>β</sub><br>(ppm) | H <sub>γ</sub><br>(ppm) | Hydrogen<br>bond<br>strength | Δδ/ΔT<br>(ppb/K) |
|--------|-------------------------|-------------------------|-------------------------|-------------------------|------------------------------|------------------|
| 1 Lys  | -                       | 4.09                    | 2.13                    | -                       |                              |                  |
| 2 Ile  | -                       | 3.94                    | 2.04                    | -                       |                              |                  |
| 3 Ala  | 8.28                    | 4.00                    | 1.48                    |                         | W                            | -5.9             |
| 4 Lys  | 7.43                    | 4.03                    | 1.94                    | -                       | W                            | 0                |
| 5 Val  | 7.63                    | 3.69                    | 2.20                    | 1.08, 0.96              | W                            | -2.6             |
| 6 Ala  | 8.26                    | 3.94                    | 1.43                    |                         | W                            | -5.9             |
| 7 Leu  | 7.85                    | 4.05                    | 1.81                    | -                       | W                            | -4.6             |
| 8 Lys  | 7.66                    | 4.02                    | 2.06                    | -                       | W                            | -1.9             |
| 9 Ala  | 8.33                    | 3.97                    | 1.45                    |                         | M                            | -4.8             |
| 10 Leu | 8.07                    | 3.98                    | 1.93                    | -                       | S                            | -4.9             |
| 11 Lys | 7.85                    | 3.94                    | 2.04                    | -                       | W                            | -4.6             |
| 12 Ile | 7.97                    | 3.73                    | 2.01                    | 1.48, 0.89              | S                            | -5.5             |
| 13 Ala | 8.45                    | 3.92                    | 1.47                    |                         | S                            | -6.2             |
| 14 Lys | 8.11                    | 3.89                    | 2.05                    | -                       | W                            | -5.3             |
| 15 Val | 7.66                    | 3.71                    | 2.27                    | 1.15, 1.00              | W                            | -1.9             |
| 16 Ala | 8.37                    | 3.99                    | 1.44                    |                         | M-S                          | -4.3             |
| 17 Leu | 8.42                    | 4.00                    | 1.85                    | -                       | S                            | -7.5             |
| 18 Lys | 7.77                    | 4.07                    | 1.96                    | -                       | W                            | -3.7             |
| 19 Ala | 7.93                    | 4.22                    | 1.50                    |                         | W                            | -2.2             |
| 20 Leu | 7.90                    | 4.22                    | 1.82                    | -                       | W                            | -5.5             |
|        |                         |                         |                         |                         |                              |                  |

**Table S3:** Statistical information for the Kiadin and diPGLa-H NMR structures in SDS micelles

|                                                |                       | Kiadin     | diPGLa-H    |
|------------------------------------------------|-----------------------|------------|-------------|
| <b>Distance restraints</b>                     | Total                 | 154        | 156         |
|                                                | Intra residue         | 59         | 62          |
|                                                | Inter                 | 95         | 94          |
|                                                | Sequential            | 63         | 61          |
|                                                | Short range (seq)     | 95         | 94          |
|                                                | Short range (non-seq) | 32         | 33          |
| <b>Atomic RMSD (Å) from average structure</b>  |                       |            |             |
| Backbone (1-20)                                |                       | 0.52       | 0.47        |
| Backbone (3-18)                                |                       | 0.39       | 0.32        |
| <b>Violations from experimental restraints</b> |                       |            |             |
| NOE violations (> 0.3 Å)                       |                       | 1          | 0           |
| Average violation (Å)                          |                       | 0.57       | -           |
| <b>Molprobit statistics</b>                    |                       |            |             |
| Clashes (> 0.4 Å / 1000 atoms)                 |                       | 8.13±4.3   | 12.90±2.99  |
| Poor rotamers                                  |                       | 0.60±0.49  | 0.30±0.46   |
| Ramachandran outliers (%)                      |                       | 0          | 0           |
| Ramachandran favoured (%)                      |                       | 99.44±1.67 | 99.44± 1.67 |
| MolProbit score                                |                       | 1.80±0.48  | 1.84± 0.31  |
| MolProbit score percentile                     |                       | 79.5±19.23 | 81.1±15.01  |