SUPPLEMENTARY INFORMATION

Fermentation and Cost-Effective $^{13}C/^{15}N$-Labeling of the Non-Ribosomal Peptide Gramicidin S for NMR Structure Analysis

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Running title: Efficient production of $^{13}C/^{15}N$-labeled gramicidin S

SUPPLEMENTARY METHODS

Chromatographic purification of GS and determination of the extent of labeling. The identity of the peptide was confirmed by time-of-flight mass spectrometry (MALDI-TOF). MALDI samples were co-crystallized either with α-cyano-4-hydroxy-cinnamic acid or 3,5-dihydroxy-benzoic acid (Sigma-Aldrich) as matrices. Ethanol/water (1/1 *v/v*) or 0.1% trifluoroacetic acid:acetonitrile (TFA:MeCN, 1:2, *vol:vol*) mixtures were used as matrix/analyte co-solvents for the extracts and RP-HPLC-fractions, respectively. Measurements were made in the positive ion reflector mode on Bruker Daltonics (Bremen, Germany) MALDI-TOF instruments Biflex IV and Autoflex III.

Solid-state nuclear magnetic resonance. Solid-state NMR experiments were performed on an Avance II spectrometer from Bruker Biospin (Karlsruhe, Germany), equipped with a wide-bore ultra-shielded 14.1 Tesla magnet (600 MHz ^{1}H frequency). All experiments were conducted using the ^{13}C/^{15}N-GS reconstituted in multilamellar lipid vesicles of 1,2-di-O-tetradecyl-*sn*-glycero-3-phosphocholine (DM-O-PC, Avanti polar Lipids, Alabaster, USA) at a peptide/lipid ratio of 1/20 (mol/mol). The actual temperature at the sample was maintained at 25°C throughout the experiments.

One-dimensional solid-state ^{13}C-NMR spectra were recorded with direct acquisition of 256 scans. The ^{15}N-NMR spectra were recorded (i) with direct acquisition of 14336 scans, and (ii) using ramped-amplitude (from 38.5 to 30.8 kHz) cross-polarization with a contact time of 400 μs, acquired for 10800 scans. Two-dimensional ^{13}C-^{13}C chemical shift correlation spectra were acquired using dipolar assisted rotational resonance experiments (1) (DARR at N=1; mixing time of 60 ms), and by experiments with magnetization transfer enhanced by a phase-alternated recoupling irradiation scheme (2) (PARIS at N=0.5; mixing times of 20 ms, 35 ms and 50 ms). All two-dimensional experiments were performed with direct ^{13}C excitation, no ^{1}H-^{13}C cross-polarization was applied. During the ^{13}C chemical shift evolution period in the indirect dimension, a 60 kHz two-

pulse-phase-modulation (TPPM) ^{1}H decoupling was used. Irradiation of 10 kHz was applied to ^{1}H during the mixing period. Typical settings for two-dimensional ^{13}C-^{13}C spectra were: a spectral width of 15 kHz in both dimensions (128 t_1 transients) with a total acquisition of 720 scans (PARIS with mixing time of 35 ms), and a spectral width of 30 kHz in both dimension (256 t_1 transients) with a total acquisition of 544 scans (DARR with mixing time of 60 ms).

All spectra were acquired using 10 kHz magic angle spinning (MAS) and ^{1}H decoupling during the acquisition time (TPPM decoupling sequence with effective 30 kHz decoupling for the ^{15}N spectra, and 60 kHz for the ^{13}C spectra, respectively) on a standard triple-resonance probe from Bruker Biospin (Ettlingen, Germany). ^{13}C chemical shifts were referenced against 2,2-dimethylsilapentane-5-sulfonic acid (DSS), using adamantane as external referencing standard (assuming the chemical shift of its most deshielded carbon to be 38.4 ppm with respect to TMS; 1.7 ppm was subsequently added to adjust the reference scale to DSS (3).

Dihedral angles prediction. Dihedral angle estimates for the GS backbone were predicted from $^{13}C_{\alpha}$ and $^{13}C_{\beta}$ chemical shifts using the TALOS+ program (4). The primary structure of GS was entered as a linear sequence [Phe-Pro-Val-Lys-Leu]$_2$ in the TALOS+ input file (TALOS+ is not designed for cyclic sequences and does not accept non-standard amino acids, hence the native D-Phe in GS was replaced by L-phenylalanine, and ornithine was replaced by lysine, given the side chain similarity. The chemical shifts in the TALOS+ input file were referenced with respect to TSP (0.12 ppm were added to the chemical shift values referenced against DSS (3). The dihedral angles were predicted (i) automatically excluding proteins with an amino acid sequence identical to the GS sequence given in the TALOS+ input file from the TALOS+ database, and (ii) without TALOS+ automated chemical shift offset correction. The predictions are identical for each symmetric pair of amino acid residues in the sequence. The signs of the dihedral angles estimates predicted for L-Phe were changed in order to provide the corresponding estimates for D-Phe.

Table S1. ^{13}C solid-state chemical shifts of GS given with respect to TMS (δ_{TMS}) and DSS (δ_{DSS}) and the corresponding characteristic chemical shift regions as provided by the Biological Magnetic Resonance Data Bank (average chemical shift $\overline{\delta}_{DSS}$ and standard deviation SD).

Residue	δ_{TMS}, ppm	δ_{DSS}, ppm	$\overline{\delta}_{DSS}$ ± SD, ppm
D-Phe[1] at positions 1 & 6			
C_α	59.9	61.6	58.2±2.6 (L-Phe C_α)
C_β	39.5	41.2	40.0±2.1 (L-Phe C_β)
C_γ	139.2	140.9	138.6±2.0 (L-Phe C_γ)
$C_\delta1$ & $C_\delta2$	132.2	133.9	131.6±1.2 (L-Phe $C_\delta1$)
$C_\varepsilon1$ & $C_\varepsilon2$	130.3	132.0	130.7±1.3 (L-Phe $C_\varepsilon1$)
Pro at positions 2 & 7			
C_α[2]	63.1	64.8	63.4±1.5
C_β[2]	32.0	33.7	31.9±1.2
Val at positions 3 & 8			
C_α	61.0	62.7	62.6±2.9
C_β	33.4	35.1	32.7±1.8
$C_\gamma1$ & $C_\gamma2$	21.0	22.7	21.5±1.4 (Val $C_\gamma1$)
Orn[1] at positions 4 & 9			
C_α	54.3	56.0	57.0±2.2 (Lys C_α)
C_β	31.6	33.3	32.8±1.8 (Lys C_β)
C_γ	26.0	27.7	29.0±1.1 (Lys C_δ)
C_δ	41.8	43.5	42.9±0.8 (Lys C_ε)
Leu at positions 5 & 10			
C_α	52.2	53.9	55.7±2.1
C_β	42.9	44.6	42.3±1.9
C_γ[2]	27.0	28.7	26.8±1.1
$C_\delta1$ & $C_\delta2$[2]	25.0	26.7	24.7±1.6 (Leu $C_\delta1$)

[1] No chemical shift statistics is available for D-Phe and Orn; the corresponding statistics for L-Phe and Lys, respectively, are given.

[2] Tentative assignment.

Table S2. Dihedral angles in the GS backbone predicted by TALOS+ program.

Residue	φ, deg	ψ, deg
D-Phe	75	-128
Pro	-65	144
Val	-117	140
Orn	-102	131
Leu	-120	145

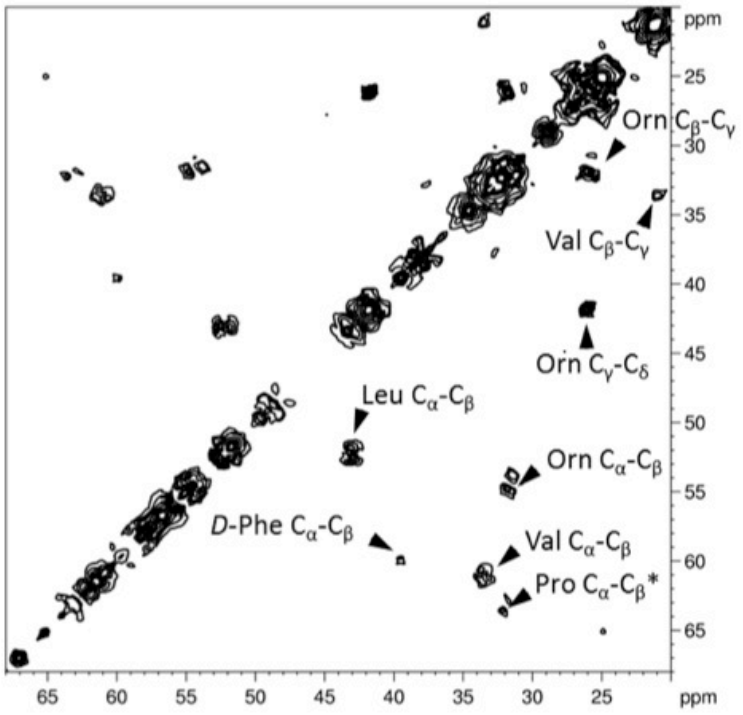

FIG S1. Two-dimensional ^{13}C-^{13}C PARIS spectrum of membrane-bound GS with a mixing time of 35 ms. The spectrum shows resonances corresponding to one-bond intra-residue ^{13}C-^{13}C correlations. Arrows denote the assigned resonances.

SUPPLEMENTARY REFERENCES:

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