**Supporting Information**

# Quasiracemate Crystal Structures of Magainin 2 Derivatives Support the Functional Significance of the Phenylalanine Zipper Motif

Zvi Hayouka[†], Nicole C. Thomas[†], David E. Mortenson[†], Kenneth A. Satyshur[§], Bernard Weisblum[‡], Katrina T. Forest[§]* and Samuel H. Gellman[†]*

[†]Department of Chemistry, [‡]Department of Medicine, and [§]Department of Bacteriology, University of Wisconsin-Madison, Wisconsin 53706, United States

## Chemicals

Fmoc-protected D- and L-α-amino acids with acid-labile side-chain protecting groups were purchased from Novabiochem. N-Hydroxybenzotriazole (HOBt), N,N-dimethylformamide (DMF), and N,N-diisopropylethylamine (DIEA) were purchased from Sigma-Aldrich. 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) was purchased from Anaspec. (*S*,*S*)-*trans*-2-Aminocyclopentanecarboxylic acid (ACPC) was purchased from Chemimpex. Rink Amide resin was purchased from Novabiochem. Dehydrated LB culture medium (244610) was obtained from BD (Franklin Lakes, NJ). All other chemicals were purchased from Sigma-Aldrich and used without purification.

## Peptides synthesis

Peptides were synthesized using microwave irradiation on Rink Amide resin in Alltech filter tubes. The appropriate D or L Fmoc-protected amino acid (4 equiv. in DMF) was delivered to the reaction vessel and activated with 4 equiv. HBTU, 4 equiv. HOBt and 8 equiv. of DIEA. The activated amino acid solution was added to the solid-phase synthesis resin, and the reaction mixture was heated to 70 °C in a MARS V multimode microwave (2 minute ramp to 70 °C, 4 minute hold 70°C) with stirring. Fmoc deprotection reactions used 20 % piperidine in DMF. Reaction solutions were heated to 80 °C in the microwave (2 minute ramp to 80 °C, 2 minute hold 80 °C) with stirring. After each coupling/deprotection cycle the resin was washed 3 times with DMF. **The coupling of the β-amino acid (ACPC) was performed at room temperature overnight rather than with microwave irradiation**. Upon completion of the synthesis, the peptide was cleaved from the resin by stirring the resin in a solution containing 92.5%

trifluoroacetic acid (TFA), 2.5% water, and 2.5% triisopropylsilane and 2.5% 1,2-ethanedithiol for 3 hours. The peptide was precipitated from the TFA solution by addition of cold ether. The precipitated peptide was collected by centrifugation. Ether was removed, and the pellet was dried under a stream of nitrogen over night. Purification by preparative reverse phase HPLC and lyophilization afforded the final peptides. Purity, determined by analytical HPLC, was greater than 95 % for all peptides. Solvents used for HPLC purifications were A: 100:0.1 $H_2O$:TFA and B: 100:0.1 acetonitrile:TFA.

**Ala$^{8,13,18}$-Magainin 2 amide (1)**: Gradient of 30-50% B solvent over 20 min.; retention time 16.5 min. MALDI-TOF mass spectrum m/z = 2478.3 [(M+H)+; calcd 2477.4].

**ACPC$^{8}$-Ala$^{13,18}$-Magainin 2 amide (2)**: Gradient of 40-60% B solvent over 20 min.; retention time 7.5 min. MALDI-TOF mass spectrum m/z = 2539.4 [$(M+Na)^+$; calcd 2539.5].

**Ala$^{8,18}$-ACPC$^{13}$-Magainin 2 amide (3)**: Gradient of 40-60% B solvent over 20 min.; retention time 7.0 min. MALDI-TOF mass spectrum m/z = 2517.8 [$(M+H)^+$; calcd 2517.9].

## Crystallization Conditions

Crystals of quasiracemic mixtures [L-**2** + D-**1**] and [L-**3** + D-**1**] suitable for X-ray diffraction were grown using a precipitant condition containing 0.1 M sodium citrate tribasic dihydrate pH 5.6 and 35% v/v *tert*-butanol (Index, Hampton Research). In both cases, crystals were grown using the hanging drop vapor diffusion method at room temperature.[1] For each drop, 1 μL of a stock solution of the quasiracemate (2.5 mg/mL of each peptide) was combined with 1 μL of preciptant solution on a silanized glass slide and equilibrated against a reservoir of 500 μL of precipitant solution. Because of the high concentration of *tert*-butanol in the precipitant solution (35% v/v), crystals of the quasiracemic mixtures were vitrified directly without the need for additional cryoprotection. Crystals were harvested with pin-mounted nylon loops and plunged into liquid nitrogen prior to collection of diffraction data.

## Data Processing and model refinement

Diffraction data for the [L-**2** + D-**1**] structure were collected at the Advanced Photon Source (λ=0.97872 Å, LS-CAT21-ID-F) at 100 K using a MarCCD 225 detector at a distance of 150

mm. Diffraction data for the [L-**3** + D-**1**] structure was collected at the Advanced Photon Source (λ=0.97872 Å, LS-CAT21-ID-G) at 100 K using a MarCCD 300 detector at a distance of 120 mm. Integration and scaling for both quasiracemic structures were carried out in the XDS program.[2] Five percent of reflections randomly distributed across resolution shells were used for the calculation of $R_{free}$.[3]

Both quasiracemate structures were solved using molecular replacement in Phaser[4], using the L-dimer of the previously-reported structure of racemic **1** (PDB 4MGP)[5] as an initial search model. Following molecular replacement, manual rebuilding of the models was carried out in Coot[6] followed by model refinement in Refmac5 (version 5.8.0123)[7], with unrestrained isotropic temperature factors and hydrogens at riding positions. For the refining of the D amino acids, we created a library that had identical bond length and angle restraints as the respective L amino acids; the only change was the chirality of the alpha carbon atom. This new library treats D amino acid peptide bonds identically to L amino acids peptide bonds instead of nonbonded ligands. Model refinement converged at $R_{work}/R_{free}$ values of 0.182/0.247 for the [L-**2** + D-**1**] structure and 0.172/0.239 for the [L-**3** + D-**1**] structure. Data processing and refinement statistics are reported in Table S1.

**Root Mean Square Deviation (RMSD) Calculations**

For RMSD calculations between the Ala-Magainin derivatives, coordinates from refined structures were separated into files containing a dimer of each polypeptide. For the racemic **1** and [L-**2** + D-**1**] structures, the C-terminal serine residue was omitted from the refined models; therefore, this residue was removed from the coordinates of the L-**3** and D-**3** polypeptides for consistency. The atoms belonging to the unnatural ACPC residues in L-**2** and L-**3** along with the atoms of their corresponding alanine residue in L-**1** were not included in the RMSD calculations. RMSD calculations of backbone atoms only were carried out in the program Pymol 1.5.0.4 using the "align" command with "cycles=0" to prevent atom rejections. **Table S2** summarizes RMSD values reported in this paper.

**Figure S1.** Crystals of quasiracemate [L-**2** + D-**1**]:

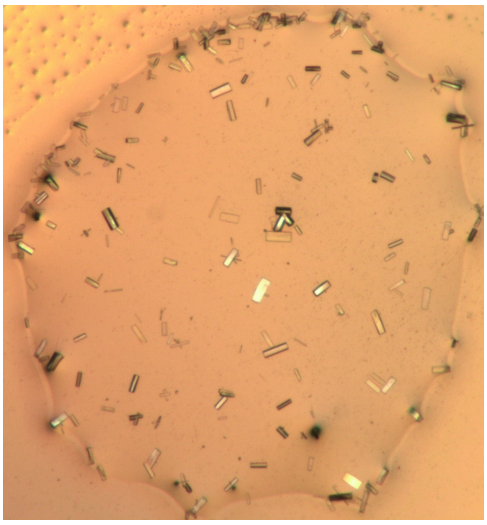

**Figure S2.** Crystals of quasiracemate [L-**3** + D-**1**]:

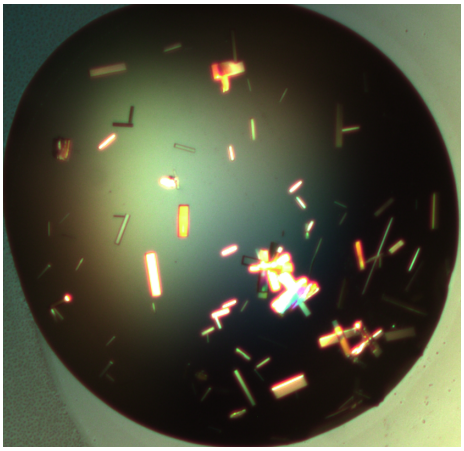

**Figure S3.** Structure of quasiracemic mixture [L-**2** + D-**1**] in space group $P2_12_12$. The unit cell contains 16 copies of L-**2** (green) and 16 copies of D-**1** (yellow).

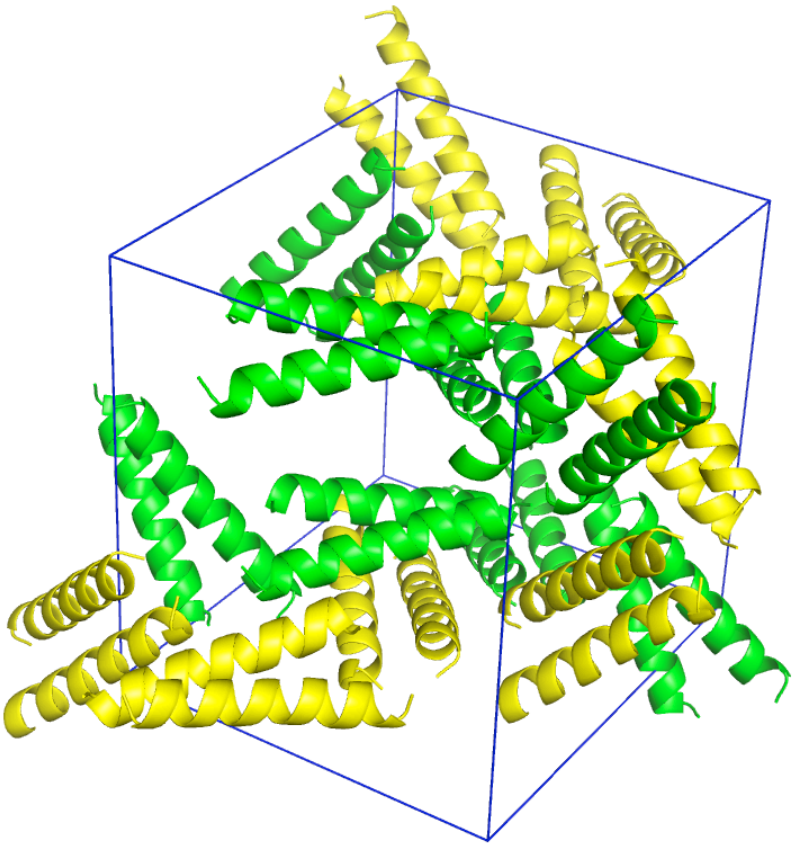

**Figure S4.** $2F_o$-$F_c$ electron density surrounding one asymmetric unit in the structure of quasiracemic mixture [L-**2** + D-**1**] contoured at 1.5σ.

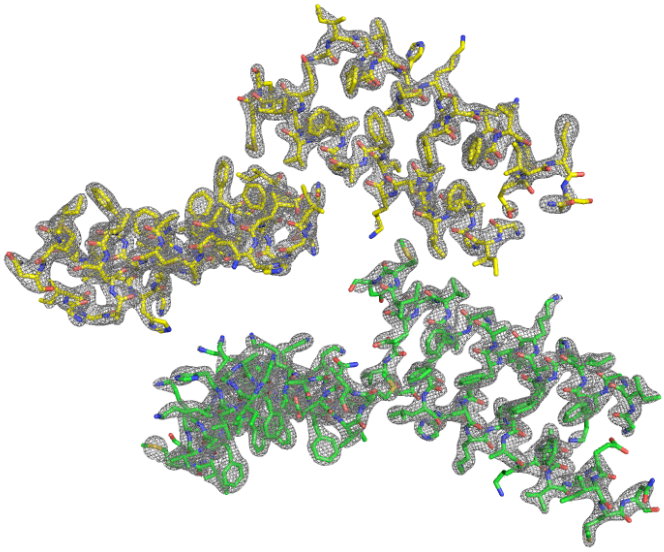

**Figure S5.** Structure of quasiracemic mixture [L-**3** + D-**1**] in space group P1. The unit cell contains 2 copies of L-**2** (red) and 2 copies of D-**1** (yellow).

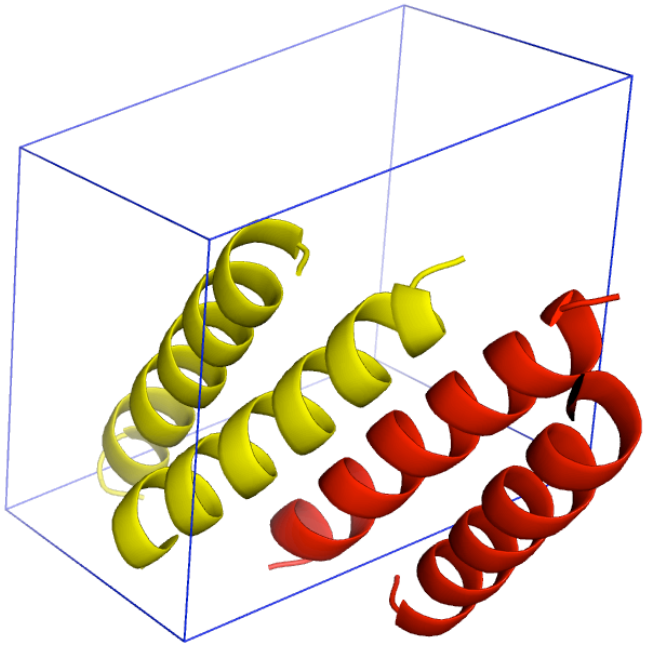

**Figure S6.** $2F_o$-$F_c$ electron density surrounding one asymmetric unit in the structure of quasiracemic mixture [L-**3** + D-**1**] contoured at 1.5σ.

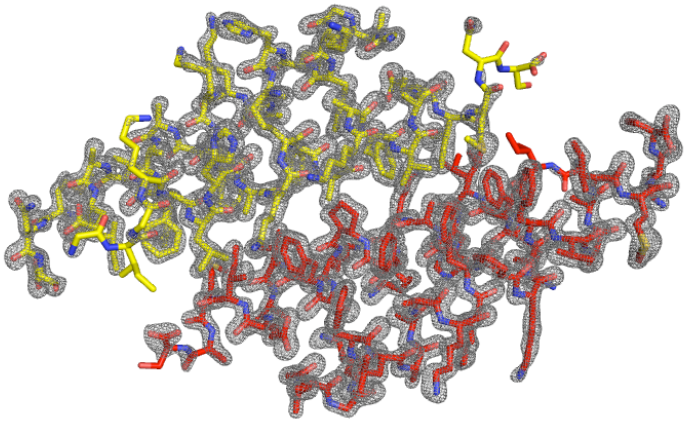

**Table S1.** Data processing and refinement statistics. Values in parentheses are for reflections in the highest resolution shell.

| **Data collection** | **ACPC8-Ala-Magainin 2** | **ACPC13-Ala-Magainin 2** |
|---|---|---|
| PDB code | 5CGN | 5CGO |
| X-ray source | APS 21-ID-F | APS 21-ID-G |
| X-ray detector | MARCCD225 | MARCCD300 |
| Data Collection Temp. (K) | 100 | 100 |
| Wavelength (Å) | 0.97872 | 0.97872 |
| Space group | $P2_12_12$ | P1 |
| a / b / c (Å) | 56.69 / 56.69 / 51.65 | 24.85 / 27.00 / 29.96 |
| α / β / γ (°) | 90.0 / 90.0 / 90.0 | 109.76 / 102.35 / 90.23 |
| Volume ($Å^3$) | 165990.5 | 18417.0 |
| Matthews coefficient ($Å^3$/Da) | 2.10 | 1.78 |
| Solvent content (%) | 41.35% | 30.92% |
| # Peptides in a.u. | 8 | 4 |
| Resolution range (Å) | 40.09-2.20 (2.30-2.20) | 27.45-1.50 (1.60-1.50) |
| Unique Reflections | 8711 (1081) | 10964 (1869) |
| Completeness | 97.6% (99.8%) | 96.1% (94.4%) |
| Redundancy | 12.9 (14.6) | 3.75 (3.75) |
| Mean $I$ / $\sigma_{(I)}$ | 12.2 (2.90) | 14.9 (5.0) |
| $R_{sym}$ | 0.111 (1.36) | 0.052 (0.310) |
| Wilson $B$-factor ($Å^2$) | 43.7 | 17.6 |
| **Refinement statistics** | | |
| Refinement program | REFMAC 5.8.0123 | REFMAC 5.8.0123 |
| Non-hydrogen protein atoms refined | 1336 | 750 |
| Resolution range (Å) | 40.09-2.20 (2.26-2.20) | 27.45-1.50 (1.54-1.50) |
| No. of reflections used in refinement | 8271 | 10395 |
| Reflections in cross-validation set | 440 | 566 |
| R-value (work) | 0.182 (0.162) | 0.172 (0.156) |
| R-value (free) | 0.247 (0.310) | 0.239 (0.249) |
| Coordinate error (ML, Å) | 0.15 | 0.062 |
| RMSD | | |
| Bond lengths (Å) | 0.018 | 0.009 |
| Bond angles (°) | 1.94 | 1.77 |

**Table S2.** Summary of reported RMSD values of backbone atoms only. When comparing L-**2** and L-**3** to L-**1**, the unnatural β-amino acid, ACPC, was omitted from the calculation along with the corresponding alanine residue in L-**1**. The ACPC residue was included in the RMSD calculation between L-**2** and L-**3**. The final two entries represent the comparison of inverted coordinates for L-dimers with the D-dimers in the same crystal.

| Pairing | RMSD (Å) |
|---|---|
| L-ACPC8 & L-Ala-Mag | 1.1 |
| L-ACPC13 & L-Ala-Mag | 1.4 |
| L-ACPC8 & L-ACPC13 | 1.2 |
| D-ACPC8 & D-Ala-Mag | 1.1 |
| D-ACPC13 & D-Ala-Mag | 1.4 |
| D-ACPC8 & D-ACPC13 | 1.1 |
| $L_{inv}$-ACPC8 & D-ACPC8 | 0.8 |
| $L_{inv}$-ACPC13 & D-ACPC13 | 0.6 |

**REFERENCES**


1. McPherson, A. *Preparation and analysis of protein crystals*, John Wiley and Sons.

2. Kabsch, W. Acta Cryst. D 2010, 66, 125-132.

3. Brünger, A. T. Nature 1992, 355, 472-475.

4. McCoy, A. J.; Grosse-Kunstleve, R. W.; Adams, P. D.; Winn, M. D.; Storoni, L. C.; Read, R. J. J Appl Cryst 2007, 40, 658-674.

5. Hayouka, Z.; Mortenson, D. E.; Kreitler, D. F.; Weisblum, B.; Forest, K. T.; Gellman, S.H. JACS 2013, 135, 15738-15741.

6. Emsley, P.; Cowtan, K. Acta Cryst D 2004, 60, 2126-2132.

7. Murshudov, G. N.; Vagin, A. A.; Dodson, E. J. Acta Cryst D 1997, 53, 240-255.