The Conformational Manifold of α-Aminoisobutyric Acid (Aib) Containing Alanine-Based Tripeptides in Aqueous Solution Explored by Vibrational Spectroscopy, Electronic Circular Dichroism Spectroscopy, and Molecular Dynamics Simulations.

Reinhard Schweitzer-Stenner[†*], *Widalys González*,[†] *Gregory T. Bourne*[‡], *Jianwen A. Feng*[‡], *and Garland R. Marshal*,[‡]

[†]Department of Chemistry, Drexel University, 3141 Chestnut Street, Philadelphia, Pennsylvania 19104 and [‡]Department of Biochemistry and Molecular Biophysics, Washington University, St. Louis, Missouri 63110

Supporting Information

Materials and Method.

NMR Spectroscopy. Nuclear Magnetic Resonance spectra were recorded at 300 MHz (^{1}H)/75 MHz (^{13}C) on a Varian Gemini-300 and NMR spectrometers. ^{1}H and ^{13}C chemical shifts (δ) are given in parts per million (ppm) using residual protonated solvent as an internal standard. Coupling constants are given in Hertz (Hz). The following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet. Low resolution mass spectral data were recorded on a Micromass LCT (TOF MS ES+) instrument.

Melting points were determined on a Bausch and Lomb hot stage. Analytical reversed-phase HPLC was performed on a Phenenomex C_{18} Jupiter column (4.6 x 250 mm). Preparative reversed phase HPLC was performed on a Phenonemex Gemini C_{18} column (22 x 250 mm) Separations were achieved using linear gradients of buffer B in A (A = aqueous 0.045 % HCl; B = 90 % CH_3CN, 10 % H_2O, 0.045 % aqueous HCl) at a flow rate of 1 mL/min (analytical) and 15 mL/min (preparative). Abbreviations: MeCN, acetonitrile; HCl, hydrogen chloride; DCM, dichloromethane; petrol, petroleum spirit (bp 40 – 60 °C); EtOAc, ethyl acetate; DIEA, diisopropylethyl amine; AcOH, acetic acid; MeOH, methanol; Et_2O, diethyl ether; DMSO, dimethylsulfoxide; DMF, *N*,*N*-dimethylformamide; HBTU, *O*-benzotriazol-1-yl-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate.

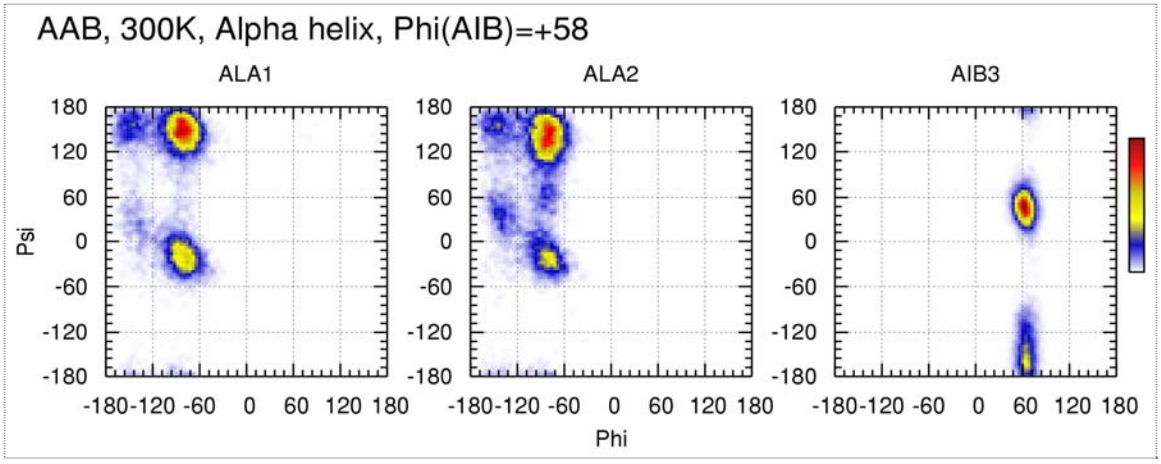
Synthesis of Ac-Aib-OH. H-Aib-OH (1.03 g, 10 mmol) was dissolved with stirring in boiling acetic acid to give a 10% w/v solution. After allowing the solution to cool slightly, acetic anhydride (1.53 g, 15 mmol, 1.5 equiv.) was added in portions over a 10-minute period. The mixture was refluxed for 5-10 min, allowed to cool overnight, and the solvent evaporated to give a syrup, to which water (50 mL) was added and the solution

was re-evaporated. This process was repeated until a solid was obtained. The solid was washed with ice-water, then dried under vacuum. The solid, free from acetic acid was recrystallized from H_2O to give the desired product. (0.25 g, 40 %). ^{1}H NMR (300 MHz, d^6-DMSO) δ 1.22 (s, 6H, $C(CH_3)_2$), 1.68 (s, 3H, CH_3CO), 7.92 (s, NH) 11.98(br s, 1H, OH); ^{13}C NMR (75 MHz, d^6-DMSO) δ 23.1, 25.6, 55.2, 169.2, 176.2. ES-MS *Mr* 145.2, calcd for $C_6H_{11}NO_3$, 145.1 (monoisotopic).[1]

Synthesis of linear peptides. All linear peptides were chemically synthesised on chlorotrityl resin stepwise using Fmoc protecting groups and *in situ* HBTU activation protocols as previously described.[2,3] Coupling efficiencies were determined by the quantitative ninhydrin test[4] and recoupled where necessary to obtain >99.5% efficiency. The linear peptides were cleaved from resin using 95% TFA with 5% H_2O. The product was purified by reverse-phase HPLC. No TFA was used in the preparative HPLC. The procedure yielded the following results. Ac-Ala-Ala-Aib-OH: Yield after cleavage and purification was 142 mg (71%); ES-MS *Mr* 287.2, calculated for $C_{12}H_{21}N_3O_5$, 287.2 (monoisotopic); Ac-Ala-Aib-Ala-OH: Yield after cleavage and purification was 156 mg (78%); ES-MS *Mr* 287.3, calculated for $C_{12}H_{21}N_3O_5$, 287.2 (monoisotopic); Ac-Aib-Ala-Ala-OH: Yield after cleavage and purification was 171 mg (86%); ES-MS *Mr* 287.3, calculated for $C_{12}H_{21}N_3O_5$, 287.2 (monoisotopic).

General method for methyl ester formation. The linear peptides were dissolved in 2 mL EtOH and diazomethane in ether (20 mL of 0.4 M solution) was added dropwise at room temperature. The solution was left standing for 60 minutes, 1 mL of HOAc added to

neutralize the excess diazomethane and solvents removed under vacuum. The residue was taken up in 3 mL aqueous acetonitrile (50%) and lyophilized. Preparative HPLC gave the desired products. No TFA was used in the preparative HPLC. The procedure yielded the following results. Ac-Ala-Ala-Aib-OCH_3: Yield after cleavage and purification was 103 mg (80%); ES-MS *Mr* 301.2, calcd for $C_{13}H_{23}N_3O_5$, 301.2 (monoisotopic); Ac-Ala-Aib-Ala-OCH_3: Yield after cleavage and purification was 122 mg (87%); ES-MS *Mr* 301.3, calculated for $C_{13}H_{23}N_3O_5$, 301.2 (monoisotopic); Ac-Aib-Ala-Ala-OCH_3: Yield after cleavage and purification was 147 mg (91%); ES-MS *Mr* 301.3, calculated for $C_{13}H_{23}N_3O_5$, 301.2 (monoisotopic).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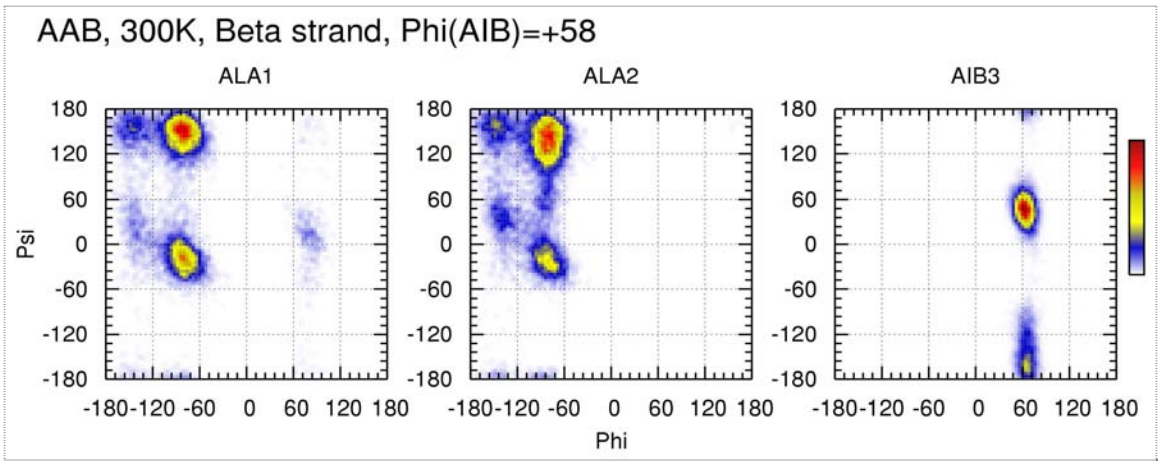
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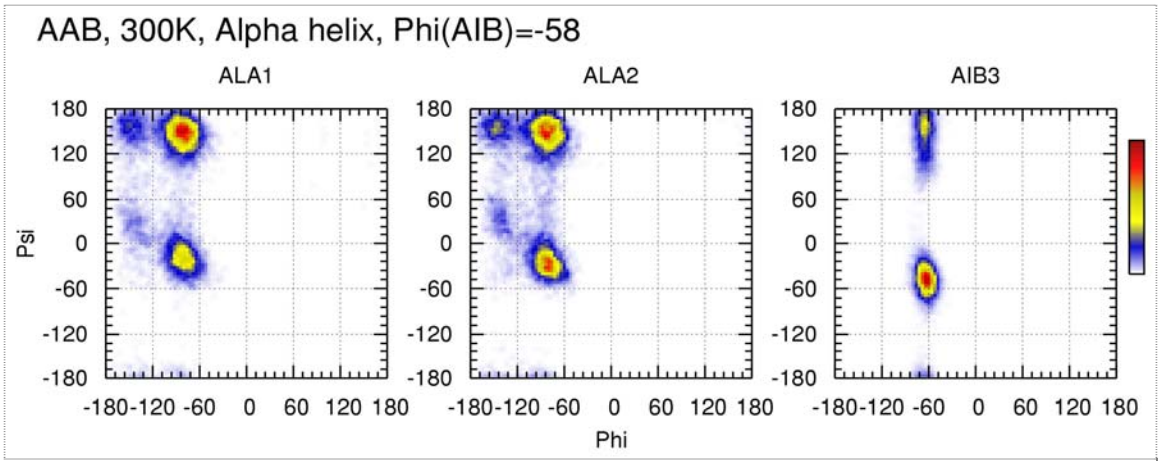
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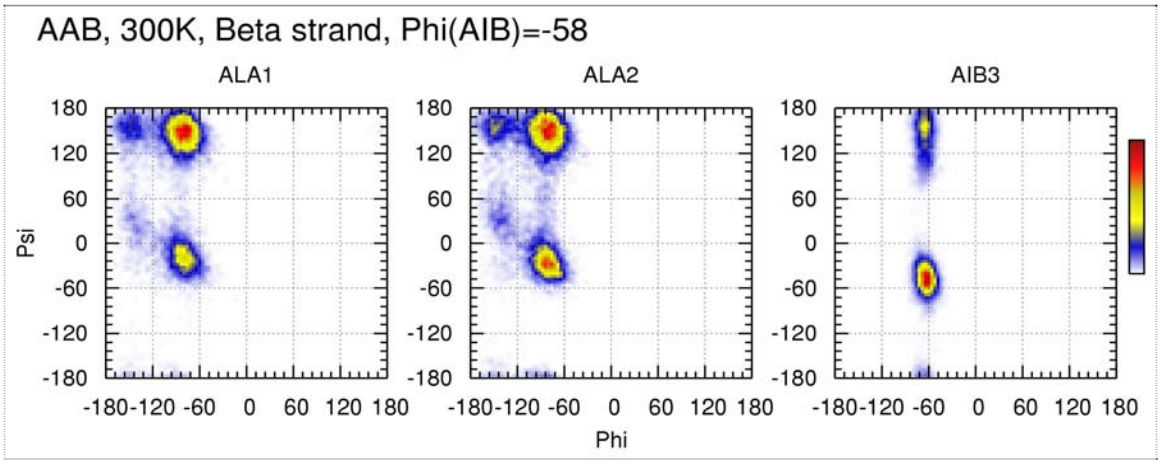
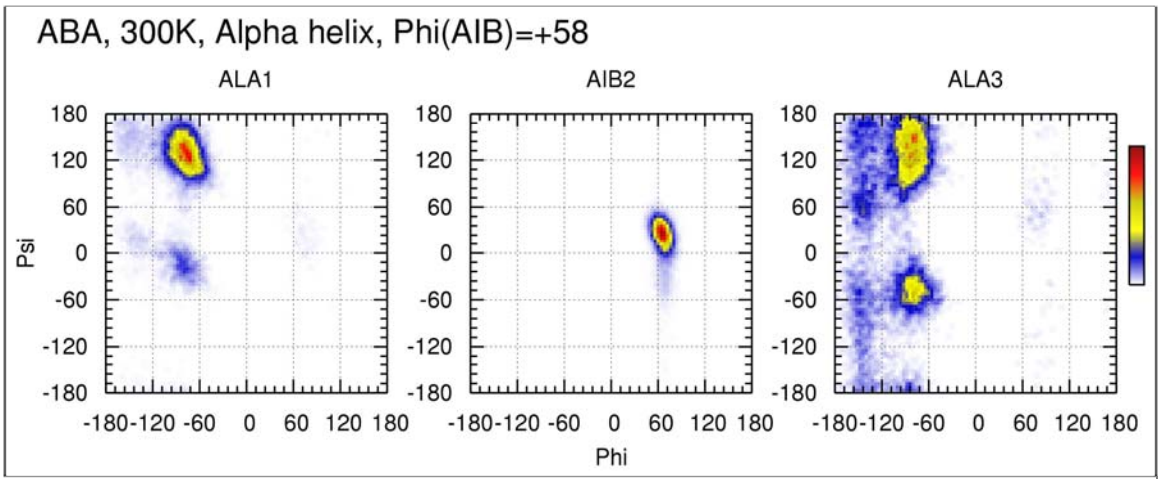


Figure S1: Summary Ramachandran plots of four Ac-AlaAlaAib-OMe (AAB) simulations at 300^{o} K.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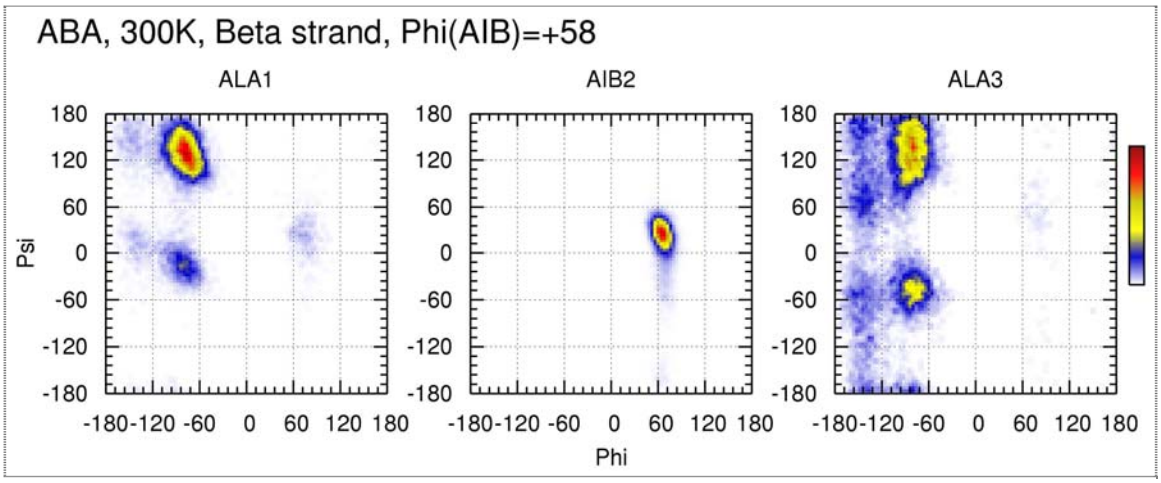
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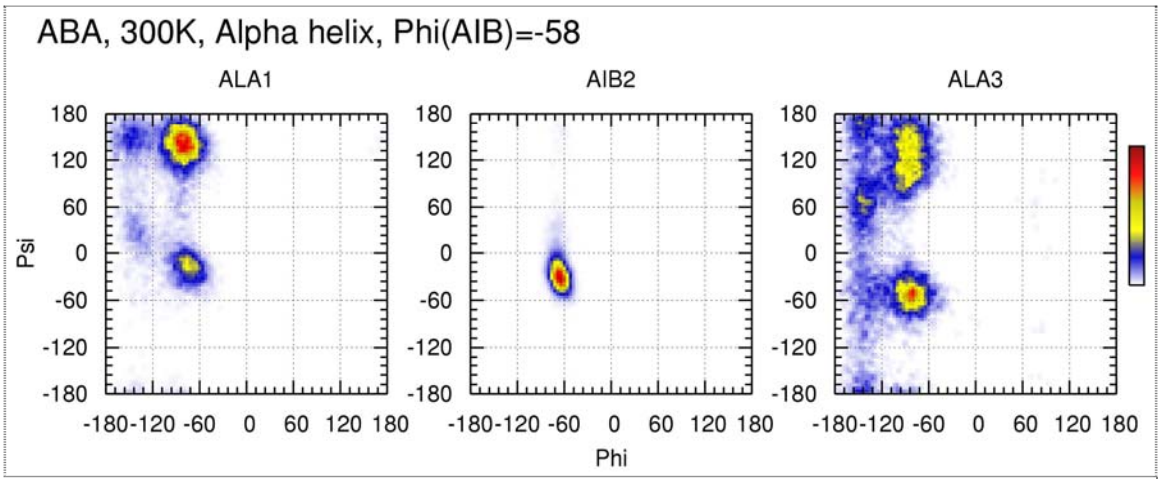
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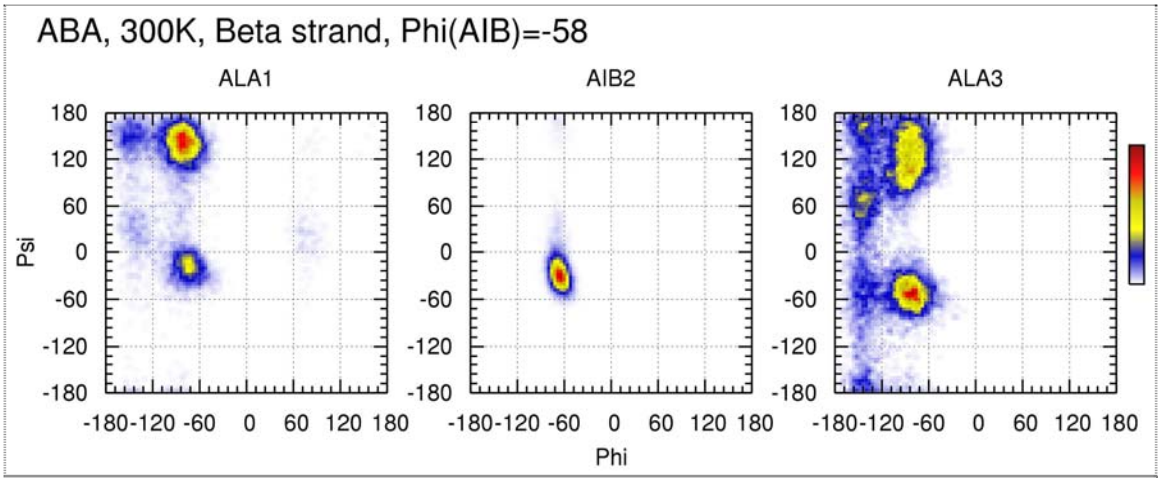
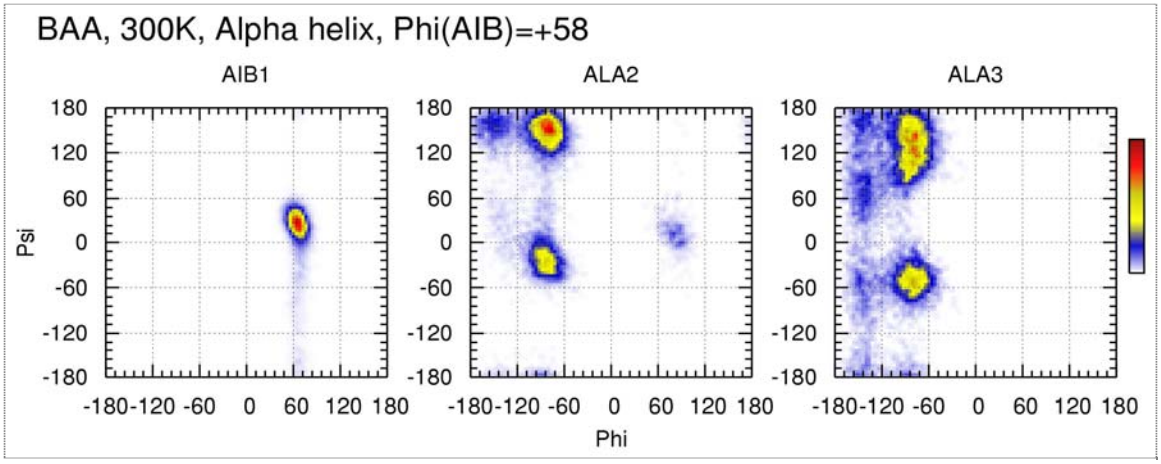


Figure I2: Summary Ramachandran plots of four Ac-AlaAibAla-OMe (ABA) simulations at 300^{o} K.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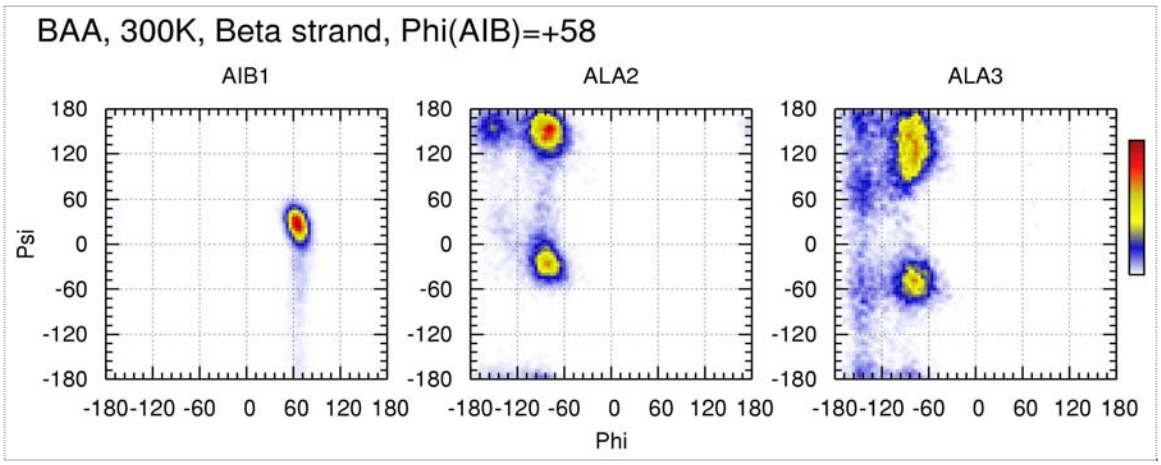
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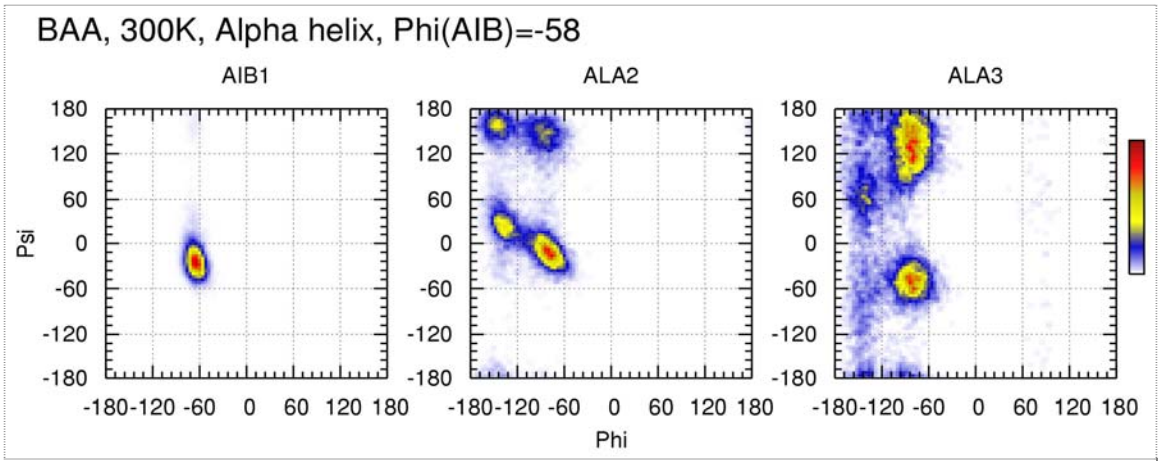
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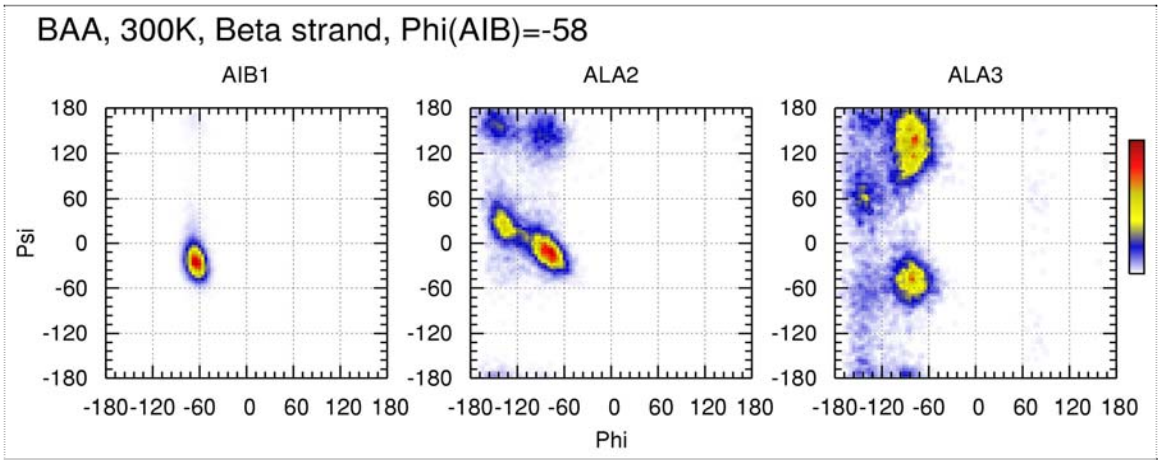

Figure 13: Summary Ramachandran plots of four Ac-AibAlaAla-OMe (BAA) simulations at 300^{o} K.

References

1. Cotton, R., Hardy, P. M.; Langran-Goldsmith, A. E., *Int. J. Peptide Protein Res.,* **1986**, *28*, 230-244.

2. Schnölzer, M.; Alewood, P. F.; Jones, A.; Alewood, D.; Kent, S. B. H.; *Int. J. Pept. Protein Res.*, **1992**, *40*, 180-93.

3. Alewood, P.; Alewood, D.; Miranda, L.; Love, S.; Meutermans W.; Wilson, D. *Methods Enzymol.*, 1997, **289**, 14-29.

4. Sarin, V. K.; Kent, S. B. H.; Tam J. P.; Merrifield R. B.; *Anal. Biochem.*, **1981**, **117**, 147-157.