Supporting Information

A Systematic Study of Fundamentals in α-Helical Coiled Coil Mimicry by Alternating Sequences of β- and γ-Amino Acids

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Materials

N-Fmoc-(*S*)-β3-leucine, was purchased from Fluka and *N*-Fmoc-(*R*)-γ4-aspartic acid and *N*-Fmoc(*R*)-γ4-leucine from RareChemicals (Gettorf, Germany). The synthesis of *N*-Fmoc-(*S*)-β3-Homoleucine was carried out according to reported methodologies which were slightly modified (Müller et al. 1998; Cesar and Sollner Dolenc 2001). *N*-Fmoc-homoLeu was purchased from Polypeptide (Strasbourg, France). Fmoc-Arg-NovaSyn®-TGA resin (0.2 mmol g^{-1}) was purchased from Novabiochem. Fmoc-L-amino acids, and HOBt were purchased from Fa. Gerhardt (Wolfhagen, Germany). The following chemicals were used as purchased: acetonitrile (HPLC gradient grade, Acros), dimethylformamide (p.a., Acros), dichloromethane (Fisher), HOAt (Iris Biotech), DIC (99%, Acros), TIS (99%, Acros), piperidine (99% extra pure, Acros), 1,8 diazabicyclo[5.4.0]undec-7-ene (Merck), disodium hydrogenphosphate dihydrate (p.a., Merck), and sodium dihydrogenphosphate dehydrate (ultra >99%, Fluka), acetic acid (p.a. 100%, Roth), Thioanisole (99%, Acros), DDT (98%, Acros), TFA (Uvasol®, Merck), GndHCl (99.5%, Acros), glutathione and glutathione-oxidized (Aldrich) and H_2O (MilliQ-AdvantageA10 Millipore).

Synthesis of N-9-Fluorenylmethoxycarbonyl-β-homoLeucine (N-Fmoc-β-hLeu)

Isobutyl chloroformate (144 µl; 1.1 mmol) was added to a flask containing a solution of *N*-Fmoc-HomoLeu (367 mg; 1.0 mmol) and diisopropylethylamine (DIEA; 182 µl; 1.1 mmol) in anhydrous THF (5 ml) at -15 °C under inert atmosphere. The reaction mixture was stirred at this temperature for 15 min. Then, the solution was warmed up to 0 °C. Afterwards, anhydrous acetonitrile (3.0 ml) and $TMSCHN_2$ (2.0 M solution in diethyl ether; 2.0 mmol) were sequentially added into the flask and the reaction left under a bath ice overnight. After that, diethyl ether was added and the mixture was extracted with 10% aq. citric acid, saturated solution of $NaHCO_3$, and saturated solution of NaCl. The organic layer was then dried over anhydrous Na_2SO_4 and the solvents evaporated to give crude diazoketone. This material was purified via flash chromatography (ethyl acetate/hexane 1:1) to afford a yellow oilish material.

Diazoketone (220 mg; 0.56 mmol) was dissolved in dioxane/water (30 ml; 5:1) followed by addition of silver benzoate (15 mg; 0.066 mmol). The reaction mixture was then sonicated for about 40 min. The reaction progress was monitored via TLC (ethyl acetate/hexane 1:1). When the reaction was complete, the solution was acidified to pH ~2 with HCl (1.0 M) and extracted with diethyl ether (4x). The organic layers were then pooled, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The resulting residue was finally purified by flash chromatography on a silica gel column using a mixture of ethyl acetate/hexane (3:2) and 0.3% AcOH as the eluent affording 180 mg of product in 47.2 % overall yield; *m/z* (ESI+) 382.2006 $[M+H]^+$; ^{1}H-NMR (250 MHz; d_6-Acetone) δ = 7.86 (2 H, d, *J*= 7.15 Hz, 2x fluorenyl CH), 7.68 (2 H, d, *J*= 7.15 Hz, 2x fluorenyl CH), 7.41 (2 H, t, *J*= 7.15 Hz, 2x fluorenyl CH), 7.31 (2 H, t, *J*= 7.15 Hz, 2x fluorenyl CH), 6.43 (1 H, d, *J*= 8.8 Hz, NH), 4.17−4.42 (3 H, m, fluorenyl CH-CH_2O), 3.90−4.05 (1 H, m, $(CH_3)_2CHCH_2CH_2$-C<u>H</u>), 2.58 (1 H, dd, *J*= 15.4 and 6.6 Hz, <u>H</u>CH-COOH), 2.49 (1 H, dd, *J*= 15.4 and 6.6

Hz, HC<u>H</u>-COOH), 1.48−1.65 and 1.21− 1.33 (5 H, m, $(CH_3)_2C\underline{H}C\underline{H}_2C\underline{H}_2$-CH), 0.87 (6 H, d, *J*= 6.6 Hz, $(C\underline{H}_3)_2$-CH); ^{13}C-NMR (100 MHz; d_6-Acetone): δ = 23.69, 24.05, 29.49, 34.08, 36.86 $(CH_3)_2CHCH_2CH_2$-), 41.14, 49.08, 50.39 (fluorenyl CH-$\underline{C}H_2O$; $\underline{C}HCH_2$-COOH), 67.68 (fluorenyl CH-$\underline{C}H_2O$), 121.75, 127.04 , 127.08, 128.85, 128.87, 129.44, 129.45 (8x fluorenyl CH), 143.06, 146.06, 146.16 (4x fluorenyl quartenary C), 157.60 (N-CO-O), 173.91 (COOH).

NMR spectroscopy

^{1}H-NMR spectra were measured using a *Bruker* AC 250 spectrometer operating at 250 MHz, whereas ^{13}C-NMR was measured using a *JEOL* ECX 400 operating at 100 MHz. All chemical shifts (δ) are reported in parts per million (ppm) and are quoted relative to the residual proton peak of d_6-Acetone. Spectral coupling patterns are designated as follows; d: doublet; dd: doublet of doublets; t: triplet; m: multiplet.

Analytical Ultracentrifugation (AUC)

A) Sedimentation equilibrium measurements for αβγ1 and αβγ2

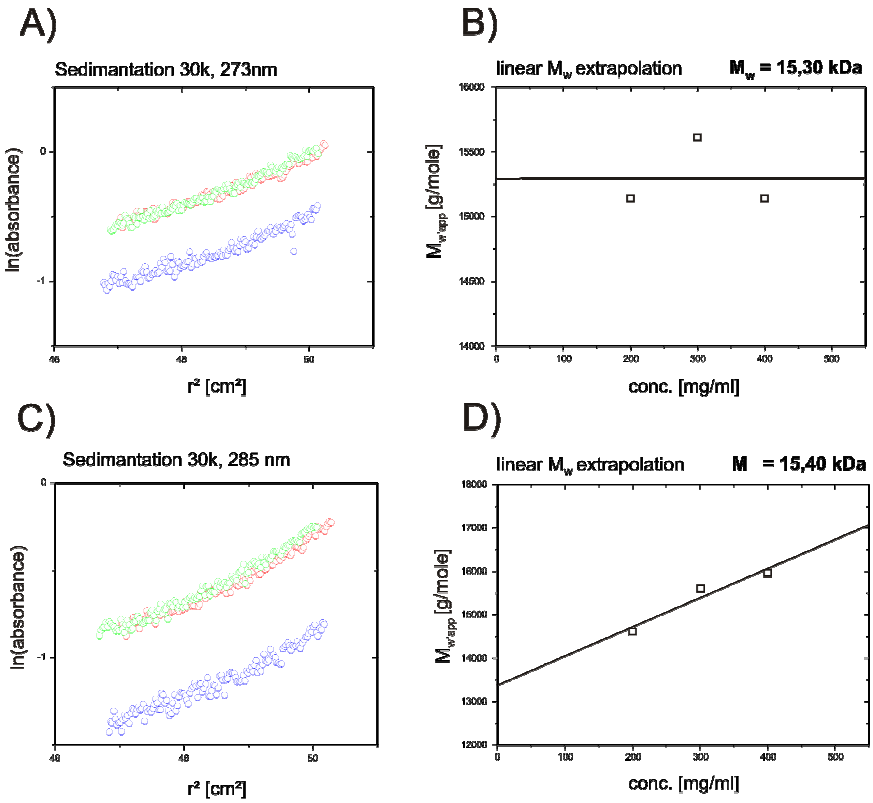

Fig. S1 Sedimentation equilibrium experiments for αβγ-1. A, C) Classical MSTAR equilibrium evaluation for tree different peptide concentrations: 400 µM (red), 300 µM (green), 150 µM (blue). B, D) Apparent molecular masses, derived from extrapolation of M* towards the cell bottom, as a function of concentration. A linear regression is automatically performed; the resulting estimates for M_w values are given in the figure. A) and B) are according to a speed of 30000 and an absorbance at 273 nm, C) and D) correspond to 30000 rpm and Absorption at 285 nm

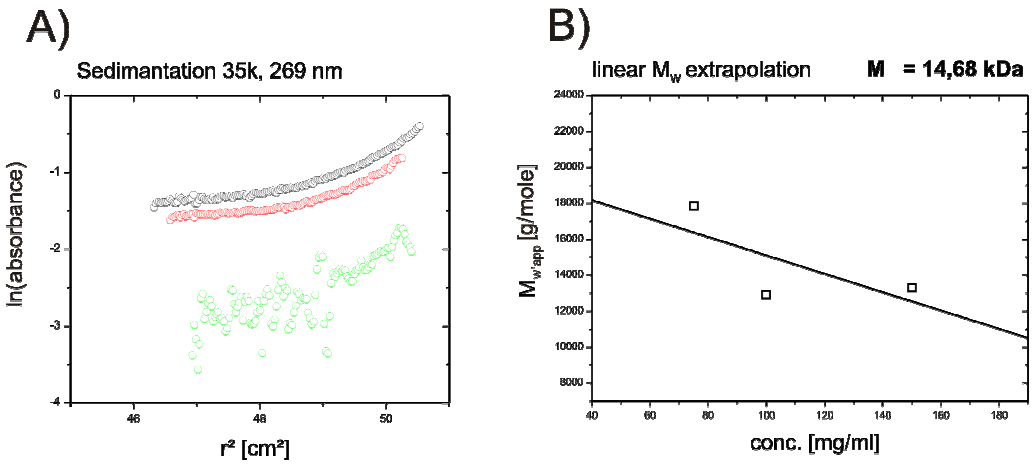

Fig. S2 Sedimentation equilibrium experiments for αβγ-2. A) Classical MSTAR equilibrium evaluation for tree different peptide concentrations: 150 µM (black), 100 µM (red), 75 µM (grren). B) Apparent molecular masses, derived from extrapolation of M* towards the cell bottom, as a function of concentration. A linear regression is automatically performed; the resulting estimate for M_w value is according to a speed of 35000 and an absorbance at 269 nm

The obtained ratio (observed MW/monomer MW) for αβγ1 and αβγ2 are 3.9 ± 0.1 and 3.7 ± 0.1, respectively. The tetrameric state of these chimeras was further evaluated by size exclusion chromatography (Fig. S4).

B) Velocity analysis

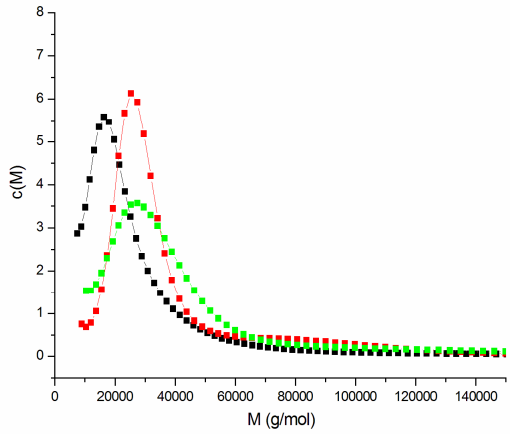

Fig. S3 Molecular weight distribution from the sedimentation velocity analysis of αβγ3 (red), αβγ4 (green) compared to αβγ1(black). The samples were prepared in 50mM phosphate buffer, ph 7.4 and total peptide concentration of 150 µM

Size Exclusion Chromatography (SEC)

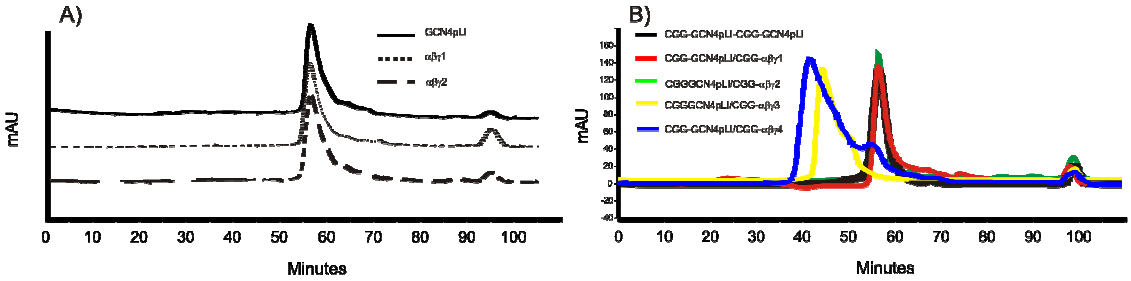

Fig. S4 Size exclusion chromatograms. A) Similar retention time as literature-reported tetrameric GCN4pLI (Harbury et al. 1993) was observed by αβγ1 and αβγ2 at total peptide concentration 150 μM in 50 mM phosphate buffer, pH 7.4 B). Similar retention time as literature-reported disulfide-bonded tetrameric GCN4pLI (Harbury et al. 1993) was observed by hetero-oxidized species CGG-GCN4pLI/CGG-αβγ1 and CGG-GCN4pLI/CGG-αβγ1 at total peptide concentration 50 μM

Molecular dynamics simulations

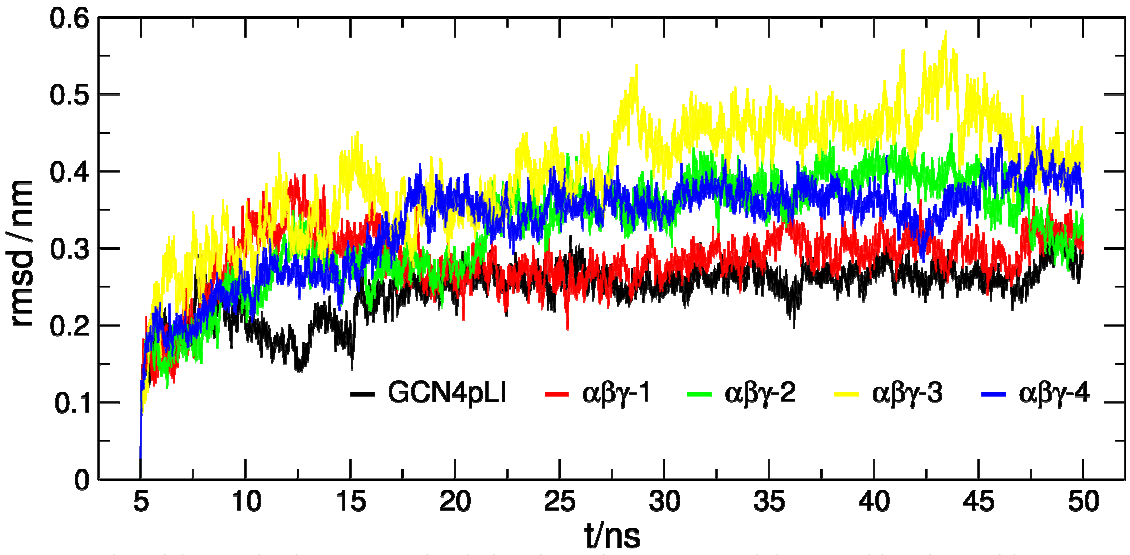

Fig. S5 Plot of the rmsd values versus simulation time of GCN4pLI and the αβγ-chimeric peptides

References

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