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Supporting Information

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Fluoroproline Flip-Flop: Regiochemical Reversal of a Stereoelectronic Effect on Peptide and Protein Structure.

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Contents

Experimental Section

Materials and Methods.

All chemical reagents were purchased from either Fisher Scientific, Inc. (Pittsburgh, PA) or Sigma Chemical Co. (St. Louis, MO) unless otherwise noted. *N-tert*-butoxycarbonyl-(2*R*,3*S*)-3-fluoroproline and *N-tert*-butoxycarbonyl-(2*R*,3*R*)-3-fluoroproline were synthesized from (2*S*,3*S)*-3-hydroxyproline (Acros Organics, Inc.) using a modification of the method of Demange, et al.[1] The preparation of the *E. coli* expression strain CAG18515(*proA*3096::Tn10Kan)[pWK1] and the plasmid, pAG2, that encodes the elastin-mimetic polypeptide sequence have been previously described.[2] NMM medium was prepared according to the protocol of Budisa, et al,[3] with the exception that proline was not added to the medium prior to cell culture. TALON metal affinity resin was purchased from BD Biosciences, Inc. Protein electrophoresis was performed on 10-15% gradient discontinuous SDS polyacrylamide gels on a PhastSystem from Amersham Pharmacia Biotech and was visualized via a silver staining procedure. Aqueous solutions of **elastin**-(**1**) and **elastin**-(**2**) were prepared from lyophilized specimens of the purified proteins that were dissolved at the appropriate concentration in distilled, deionized water at 4 °C. Quantitative amino acid analysis was performed on aliquots of protein stock solutions (circa 0.2–0.3 mg/mL) at the W. M. Keck Foundation Biotechnology Resource Laboratory of Yale University to provide accurate concentrations for CD spectroscopic experiments and DSC measurements.

Physical and Analytical Measurements.

Suitable crystals of (**1**) and (**2**) were coated with Paratone N oil, suspended in small fiber loops and placed in a cooled nitrogen gas stream at 173 K on a Bruker D8 APEX II CCD sealed tube diffractometer with graphite monochromated CuK_{α} (1.54178 Å) radiation. Data were measured using a series of combinations of phi and omega scans with 10 s frame exposures and 0.5° frame widths. Data collection, indexing and initial cell refinements were all carried out using APEX II[4] software. Frame integration and final cell refinements were done using SAINT[5] software. The final cell parameters were determined from least-squares refinement on 3071 and 2727 reflections, respectively. The structures were solved using Direct methods and difference Fourier techniques (SHELXTL, V6.12).[6] Hydrogen atoms were placed at their expected chemical positions using the HFIX command and were included in the final cycles of least

squares with isotropic U_{ij} 's related to the atom's ridden upon. All non-hydrogen atoms were refined anisotropically. Scattering factors and anomalous dispersion corrections are taken from the *International Tables for X-ray Crystallography.*[7] Structure solution, refinement, graphics and generation of publication materials were performed by using SHELXTL, V6.12 software. The crystallographic data for (**1**) and (**2**) were deposited to the Cambridge Crystallographic Data Centre (CCDC) with registration numbers 288114 and 288115. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033. Crystal data for (**1**): $C_8H_{12}FNO_3$, M = 189.18; orthorhombic, space group P2(1)2(1)2(1); a = 7.0907(2) Å, b = 9.8318(4) Å, c = 12.9324(5) Å; V = 901.57(6) $Å^3$; T = 173(2) K; Z = 4; CuKα 1.54178 Å; reflections: total = 4224, independent = 1201 (R_{int} = 0.0198); R_1 = 0.0216, wR_2 = 0.0532 for 1201 observed data [$I > 2\sigma(I)$]. Crystal data for (**2**): $C_8H_{12}FNO_3$, M = 189.18, a = 7.555(2) Å, b = 9.550(2) Å, c = 13.080(3) Å; V = 943.8(4) $Å^3$; T = 173(2) K; Z = 4; CuKα 1.54178 Å ; reflections: total = 3638, independent = 1128 (R_{int} = 0.2104); R_1 = 0.0830, wR_2 = 0.2187 for 1128 observed data [$I > 2\sigma(I)$].

ESI mass spectra were acquired on a JEOL JMS-SX 102/SX 102 A/E mass spectrometer. Molar masses of elastin analogues were determined by MALDI-TOF MS on an Applied Biosystems Voyager System 428 mass spectrometer in the positive linear mode. The matrices, 2-(4-hydroxyphenylazo)benzoic acid (HABA) or 4-hydroxy-3-methoxycinnamic acid, were used at a concentration of 10 mg/ml in a 50:50 mixture of water and 2-propanol. The protein solution (1 mg/ml in distilled water) was mixed with the matrix solution in a ratio of 1:10 and dried under vacuum or air. Bovine serum albumin was used as a standard for external calibration.

The inverse temperature transitions of the elastin polypeptides were monitored as a function of temperature using an ultra-sensitive differential scanning calorimeter (VP-DSC MicroCal, LLC, Northampton, MA). Proteins samples were dissolved in distilled, de-ionized water at 4 °C at concentrations ranging from 0.5 to 2 mg/mL, degassed under dynamic vacuum and scanned from 5-60

°C at a rate of 60 deg/hr. DSC data were processed using the program Origin (MicroCal, LLC, Northampton, MA).

Circular dichroism (CD) spectra were recorded on a Jasco J-810 spectropolarimeter equipped with a PFD-425S Peltier temperature control unit in 1 mm sealed quartz cells. Proteins were dissolved in distilled, deionized water at a concentration of 5.4 μM for **elastin-**(**1**) and 9.1 μM for **elastin-**(**2**). Temperature/wavelength CD-scans were performed within the temperature range from 5 °C to 65 °C with equilibration for 5 min at each temperature. Spectra were obtained from 260 to 190 nm at a resolution of 0.2 nm and at a scanning rate of 50 nm per min. The CD curves represented the average of five measurements and were smoothed using the means-movement method on the interval analysis of the spectral manager program. CD data are reported as mean residue ellipticity ([Θ], deg cm^2 $dmol^{-1}$) in which the molar masses of the polypeptides, **elastin-**(**1**) and **elastin-**(**2**), were calculated on the basis of on complete substitution of the canonical proline residues with the respective amino acid analogue.

NMR spectra were acquired on either a Varian INOVA 400 (^{1}H, 399.94 MHz; ^{13}C, 100.57 MHz), a Varian Unity (^{19}F, 564.044 MHz) or a Varian INOVA 600 (^{1}H, 599.74 MHz; ^{13}C, 150.82 MHz). Chemical shifts for ^{1}H NMR and ^{13}C spectra in organic solvents were reported in ppm and were referenced to the solvent signals and reported relative to tetramethylsilane (0.0 ppm). Chemical shifts for ^{1}H NMR spectra in aqueous solution were reported in ppm and were referenced and reported relative to internal sodium 2,2-dimethyl-2-silapenta-5-sulfonate (0.0 ppm). Protein samples were prepared for NMR analysis by dissolving the polypeptide in a H_2O/D_2O (70:30) mixture at a concentration of 10 mg/mL. The pH of the specimens was adjusted to 2.7 to retard amide proton exchange on the NMR time scale. The NMR spectra of polypeptide samples were acquired at 4 ºC. Standard solvent suppression techniques were employed to reduce signal due to the residual protons of H_2O in the ^{1}H NMR of aqueous solutions of the polypeptides. Chemical shifts for the ^{19}F NMR spectra are referenced and reported relative to external sample of aqueous (10 % v/v) trifluoroacetic acid (0.0 ppm). Two-dimensional ^{1}H-^{1}H NOESY NMR spectra were acquired in phase-sensitive mode using the hypercomplex method with a mixing time of 200 ms at a spectral width of 6799.8 Hz. Spectra were collected with 512 t_1 increments and 2048 complex

data points with 32 scans. The two-dimensional NMR data were further processed using the program NutsPro (Acorn NMR, Inc.). The equilibrium constants, $K_{trans/cis}$, were calculated from ration of integration of the well-resolved resolved H^{β} resonances in the ^{1}H NMR spectra of (**1**) and (**2**).

Chemical Synthesis of Model Compounds (1) and (2) and Free Amino Acids

Synthesis of *N*-tert-butoxycarbonyl-(2*R*,3*R*)-3-fluoroproline methyl ester**:** *N*-tert-butoxycarbonyl-(2*R*,3*R*)-3-fluoroproline (0.80 g, 3.43 mmol) was dissolved in 15 mL of methanol. A solution of diazomethane (16.6 mmol) in diethyl ether was added dropwise to the reaction mixture, which was stirred for 30 min at ambient temperature. The solvent was removed by rotary evaporation and crude product was purified by flash chromatography (ethyl acetate:hexanes, 1:1) to give 0.82 g (96.8 %) of the product as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.44 and 1.49 (s, 9H), 1.96-2.3 (m, 2H), 3.58-3.78 (m, 2H), 3.77 (s, 3H), 4.51 (dd, *J* = 26.8 Hz, 0.6H), 4.58 (dd, *J* = 27.6 Hz, 0.4H), 5.29-5.31 and 5.43-5.46 (m, 1H); ESI-MS (m/z): Calc. for $C_{11}H_{18}FNO_4$, 247.26; Obs., 248.13 (M+H).

Synthesis of *N*-tert-butoxycarbonyl-(2*R*,3*S*)-3-fluoroproline methyl ester: *N*-tert-butoxycarbonyl-(2*R*,3*S*)-3-fluoroproline (1.13 g, 4.8 mmol) was converted to the methyl ester using the procedure described above. Flash chromatography (ethyl acetate:hexanes, 1:1) afforded 1.19 g (99 %) of the product as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ: 1.42 and 1.48 (s, 9H), 2.1-2.24 (m, 2H), 3.48-3.81 (m, 2H), 3.77 (s, 3H), 4.48 (d, *J* = 23.2 Hz, 0.6H), 4.60 (d, *J* = 22.8 Hz, 0.4H), 5.1 and 5.23 (br s, 1H); ESI-MS (m/z): Calc. for $C_{11}H_{18}FNO_4$, 247.26; Obs., 248.13 (M+H).

Synthesis of (2*R*,3*R*)-3-fluoroproline methyl ester: *N*-tert-butoxycarbonyl-(2*R*,3*R*)-3-fluoroproline methyl ester (0.82 g, 3.3 mmol) was dissolved in 10 mL of trifluoroacetic acid/methylene chloride (1:2, v/v), and stirred for 2 h at ambient temperature. The compound was concentrated by rotary evaporation and dried under high vacuum to afford the product as a dark red oil (0.84 g, 96.4 %). ^{1}H NMR(400 MHz, $CDCl_3$): δ 2.33-2.57 (m, 2H), 3.64-3.87 (m, 2H), 3.92 (s, 3H), 4.84 (dd, *J* = 31.6 Hz, 1H), 5.5 and 5.64 (br s, 1H); ESI-MS (m/z): Calc. for $C_6H_{10}FNO_2$, 147.15; Obs., 148.08 (M+H).

Synthesis of (2*R*,3*S*)-3-fluoroproline methyl ester: This compound was synthesized from N-tert-butoxycarbonyl-(2*R*,3*S*)-3-fluoroproline methyl ester (1.19 g, 4.8 mmol) utilizing the procedure described above for (2*R*,3*R*)-3-fluoroproline methyl ester. The product was obtained as a dark red oil (1.22 g, 97 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.08-2.56 (m, 2H), 3.8-3.89 (m, 2H), 3.92 (s, 3H), 4.79 (d, *J* = 23.2 Hz, 1H), 5.51 (d, *J* = 52.0 Hz, 1H); ESI-MS (m/z): Calc. for $C_6H_{10}FNO_2$, 147.15; Obs., 148.08 (M+H)

Synthesis of N-acetyl-(2*R*,3*R*)-3-fluoroproline methyl ester (**1**): (2*R*,3*R*)-3-fluoroproline methyl ester (0.60 g, 4.1 mmol) was dissolved in neat acetic anhydride (10 mL) and the mixture was stirred for 5 h at ambient temperature. The solvent was removed rotary evaporation to afford a crude product that was purified by flash chromatography (ethyl acetate:hexane, 2:1, followed by 100% ethyl acetate). The product was obtained as a colorless oil (0.55 g, 70.0%) that crystallized upon drying under high vacuum. Crystals suitable for x-ray diffraction analysis were grown from ethyl acetate solution by slow evaporation at ambient temperature. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.89 and 2.08 (s, 3H), 2.02-2.35 (m, 2H), 3.64-3.79 (m, 2H), 3.73 and 3.77 (s, 3H), 4.61 (dd, *J* = 25.2 Hz, 0.2H), 4.65 (dd, *J* = 22.0 Hz, 0.8H), 5.26-5.29 and 5.39-5.43 (m, 0.8H), 5.35-5.38 and 5.48-5.52 (m, 0.2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.25 (21.92), 32.61 ($J_{C\text{-}F}$ = 21.9 Hz) (30.82 ($J_{C\text{-}F}$ = 21.5 Hz)), 44.17 (45.76), 52.49 (52.89), 63.56 ($J_{C\text{-}F}$ = 22.1 Hz) (64.80 ($J_{C\text{-}F}$ = 22.9 Hz)), 91.80 ($J_{C\text{-}F}$ = 183.2 Hz) (93.70 ($J_{C\text{-}F}$ = 185.2 Hz)), 167.21 (168.0), 169.46 (169.7); ESI-MS (m/z): Calc. for $C_8H_{12}FNO_3$, 189.18; Obs., 190.09 (M+H); Elemental analysis: Calc. for $C_8H_{12}FNO_3$, C: 50.79, H: 6.39, N: 7.40, found. C: 50.51, H: 6.41, N: 7.31. $[\alpha]_D^{20^\circ C}$: -180 (c = 1, H_2O).

Synthesis of *N*-acetyl-(2*R*,3*S*)-3-fluoroproline methyl ester, (**2**): This compound was synthesized from (2*R*,3*S*)-3-fluoroproline methyl ester (0.65 g, 2.5 mmol) using the procedure described above for *N*-acetyl-(2*R*,3*R*)-3-fluoroproline methyl ester. The product was obtained as a pale yellow oil (0.40 g, 84%) that crystallized upon drying under high vacuum. Crystals suitable for x-ray diffraction analysis were grown by slowly cooling a concentrated solution of (**2**) in ethyl acetate to 4 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.23 and 2.14 (s, 3H), 2.05-2.39 (m, 2H), 3.68-3.83 (m, 2H), 3.77 and 3.81 (s, 3H), 4.60 (d, *J* = 19.2 Hz, 0.2H), 4.78 (d, *J* = 25.2 Hz, 0.8H), 5.19 (dd, *J* = 51.2 Hz, 0.8H), 5.33 (dd, *J* = 51.6 Hz, 0.2H); ^{13}C NMR (100

MHz, $CDCl_3$): δ 22.26 (22.48), 31.73 ($J_{C\text{-}F}$ = 21.5 Hz) (29.81 ($J_{C\text{-}F}$ = 21.1 Hz)), 45.53 (44.40), 52.97 (53.34), 65.74 ($J_{C\text{-}F}$ = 24.4 Hz) (67.19 ($J_{C\text{-}F}$ = 24.4 Hz)), 93.60 ($J_{C\text{-}F}$ = 185.3 Hz) (95.42 ($J_{C\text{-}F}$ = 185.6 Hz)), 169.30 (169.47), 170.02; ESI-MS (m/z): Calc. for $C_8H_{12}FNO_3$, 189.18; Obs., 190.09 (M+H); Elemental analysis: Calc. for $C_8H_{12}FNO_3$, C: 50.79, H: 6.39, N: 7.40, found. C: 50.80, H: 6.36, N: 7.38. $[\alpha]_D^{20^oC}$: -86 (c = 1, H_2O).

Synthesis of (2*R*,3*R*)-3-fluoroproline: *N*-tert-butoxycarbonyl-(2*R*,3*R*)-3-fluoroproline (1.0 g, 4.2 mmol) was dissolved in 24 mL of TFA/CH_2Cl_2 (1:2, v/v), and stirred for 1 h at ambient temperature. The progress of the reaction was monitored by thin-layer chromatography (ethyl acetate:methanol:acetic acid, 99:1:0.01) and visualized by staining with ninhydrin. After disappearance of the starting material, the mixture was concentrated by rotary evaporation and triturated with diethyl ether. The residual solid was filtered and washed with ethyl ether and dried under vacuum. The solid was recrystallized from ethyl acetate and MeOH affording the product as colorless needles (0.70 g, 2.8 mmol, 65.8%) that corresponded to a 3:1 adduct of amino acid to trifluoroacetic acid on the basis of elemental analysis. ^{1}H NMR (400 MHz, D_2O): δ 2.24-2.54 (m, 2H), 3.53-3.67 (m, 2H), 4.39 (dd, *J* = 32.8 Hz, 1H), 5.48-5.50 and 5.61-5.63 (m, 1H); ^{13}C NMR (150 MHz,D_2O): δ 31.36 (31.49), 43.86, 66.38 (66.54), 92.54 (93.71), 168.49; DIOS MALDI-TOF MS (m/z): Calc. for $C_5H_8FNO_2$, 133.12; Obs., 133.50 Elemental analysis (3:1, amino acid:trifluoroacetic acid adduct): Calc. for $C_{17}H_{25}F_6N_3O_8$, C: 39.77, H: 4.91, N: 8.18, found. C: 40.07, H: 4.96, N: 8.27. $[\alpha]_D^{20^oC}$: -73 (c = 1, H_2O).

Synthesis of (2*R*,3*S*)-3-fluoroproline: *N*-tert-butoxycarbonyl-(2*R*,3*S*)-3-fluoroproline (0.90 g, 3.8 mmol) was deprotected using the procedure described above. The solid was recrystallized from ethyl acetate and MeOH affording colorless needles of the product (0.77 g, 3.1 mmol, 81.6 %). The product was isolated as a 1:1 adduct with a molecule of trifluoroacetic acid on the basis of elemental analysis. ^{1}H NMR (400MHz, D_2O): δ 2.02-2.48 (m, 2H), 3.52-3.60 (m, 1H), 3.67-3.73 (m, 1H), 4.64 (d, *J* = 22.0 Hz, 1H), 5.59 (dd, *J* = 51.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O): δ 30.23 (30.37), 44.27, 67.15 (67.30), 94.81 (96.01), 169.13; DIOS MALDI-TOF MS (m/z): Calc. for $C_5H_8FNO_2$, 133.12; Obs., 133.44; Elemental analysis (1:1,

amino acid:trifluoroacetic acid adduct): Calc. for $C_7H_9F_4NO_4$ C: 34.02, H: 3.67, N: 5.67, found. C: 34.23, H: 3.62, N: 5.67. $[\alpha]_D^{20°C}$: -7 (c = 1.2, H_2O).

Protein Expression and Purification. The expression vector pAG2–encoding elastin-mimetic protein sequence–was transformed into the auxotrophic *E. coli* strain CAG18515[pWK1] to generate the expression host employed for these studies. Single colonies of the expression strains were inoculated into sterile LB broth (50 mL) supplemented with the appropriate antibiotics (100 μg/mL ampicillin and 34 μg/mL chloramphenicol) as required for plasmid maintenance. The overnight culture was centrifuged at 4000*g* for 10 min to isolate the cells, which were re-suspended in sterile NMM medium (1 L) supplemented with the appropriate antibiotics. The proline concentration was adjusted to 0.3 mM from a sterile 100× stock solution. The culture was incubated with agitation (225 rpm) at 37 °C until the OD_{600} reached between 0.8 and 1.0 absorbance units, the cells were collected by centrifugation at 4000*g* for 10 min. The cell pellet was washed with cold (4 °C), sterile 0.9% aqueous NaCl twice (2 × 100 mL) and resuspended in sterile NMM containing antibiotics but without proline supplementation. After incubation at 37 °C for 30 min, the proline analogues were added to a final concentration of 0.5 mM from sterile 100× stock solutions. An aliquot of aqueous 1.0 M IPTG was added to the cultures to a final concentration of 1 mM to induce expression of the elastin-mimetic protein. After a 3 h induction period, the cells were harvested by centrifugation at 4000*g* and 4 °C for 20 min. The cell pellets were resuspended in lysis buffer (50 mL, 50 mM sodium phosphate, 300 mM NaCl, pH 7.0) and stored at -80 °C. The frozen cells were lysed by three freeze/thaw cycles. Lysozyme (1 mg/mL), EDTA-free protease inhibitor cocktail, benzonase (25 units/mL), and $MgCl_2$ (1 mM) were added to the lysate and the mixture was incubated with shaking at 4 °C overnight. The cell lysate was centrifuged at 40,000*g* for 30 min at 4 °C. Supernatant and pellet were separated and analyzed by SDS-PAGE to determine the location of the target protein. **Elastin**-(**1**) was localized in the supernatant fraction and was loaded directly onto cobalt charged TALON resin (5 mL) and washed of lysis buffer (50 mL) containing 20 mM imidazole. The target protein was eluted with elution buffer (20 mL, 50 mM sodium phosphate, 300 mM NaCl, 250 mM imidazole, pH 7.0) and dialyzed (MWCO = 10 kDa) against distilled deionized water (5 × 4 L). The dialysate was lyophilized to produce a white spongy solid. **Elastin**-(**2**) was located in the cell pellet and was solubilized in

denaturing lysis buffer (100 mL, 50 mM sodium phosphate, 300 mM NaCl, 6 M urea, pH 7.0). The resulting mixture was centrifuged at 40,000*g* and 4 °C for 30 min. SDS-PAGE analysis indicated that the majority of the target protein dissolved under these conditions. The soluble fraction was loaded onto cobalt-charged TALON resin (10 mL) that had been previously equilibrated with denaturing lysis buffer. The target protein was washed with denaturing lysis buffer (100 mL) containing 20 mM imidazole and eluted with denaturing lysis buffer (40 mL) containing 250 mM imidazole. The eluted target protein was dialyzed (MWCO = 10 kDa) against a diminishing urea step gradient from 6 M to 1 M, and, subsequently, against distilled water (5 × 4 L). Lyophilization of the dialysate produced the elastin-mimetic polypeptide as a white spongy solid.

Elastin-(**1**): Yield, 53.5 mg/L culture. MALDI-TOF MS (m/z): Calc., 37305.60; Obs., 37286.3.

Elastin-(**2**): Yield, 48.7 mg/L culture. MALDI-TOF MS (m/z): Calc., 37305.60; Obs., 37231.9.

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Figure 1. SDS-PAGE analysis of whole-cell lysates derived from expression cultures of *E. coli* CAG18515[pAG2][pWK1] in NMM media supplemented with the indicated proline derivative. Samples were removed after a 3 h induction period with 1 mM IPTG. Lane 1, proline deficient (negative) control; lane 2, uninduced control; lane 3, proline supplemented (positive) control; lane 4, (2*R*,3*R*)-3-fluoroprolineproline; lane 5, (2*R*,3*S*)-3-fluoroproline; and lane 6, molecular weight standards (35, 50, and 75 kDa).

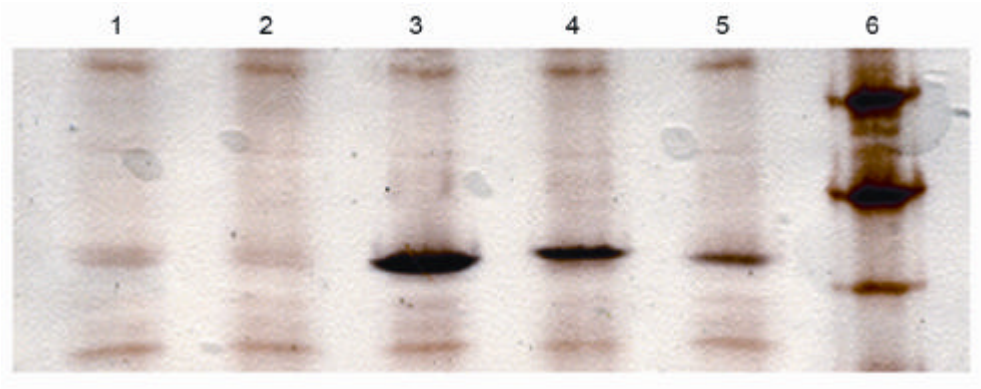
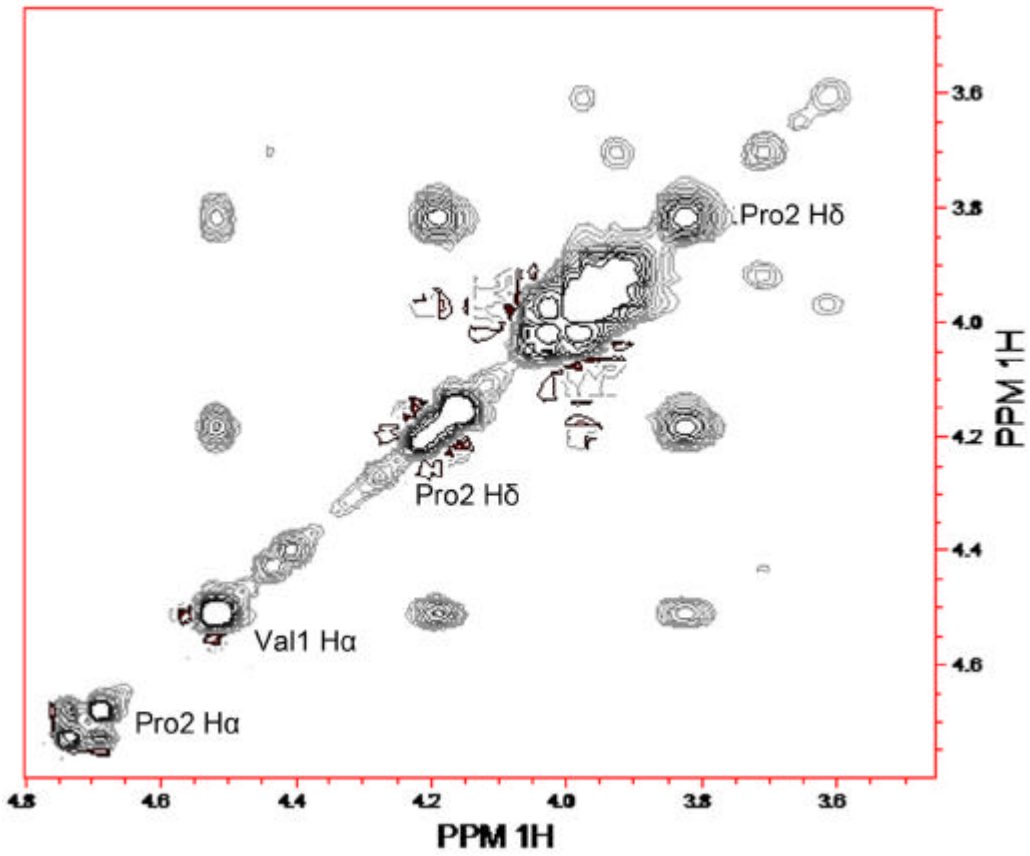


Figure 2. Expansion of the two-dimensional ^{1}H-^{1}H NOESY NMR spectra of **elastin-(1),** (**A**), and **elastin-(2),** (**B**). The spectral windows depict the Val(αH)-Pro(δH) cross-peaks associated with the *trans* configuration of the Val-Pro peptidyl bond. Note the absence of a strong Val(αH)-Pro(αH) cross-peak corresponding to a *cis* Val-Pro peptidyl bond configuration.

A.



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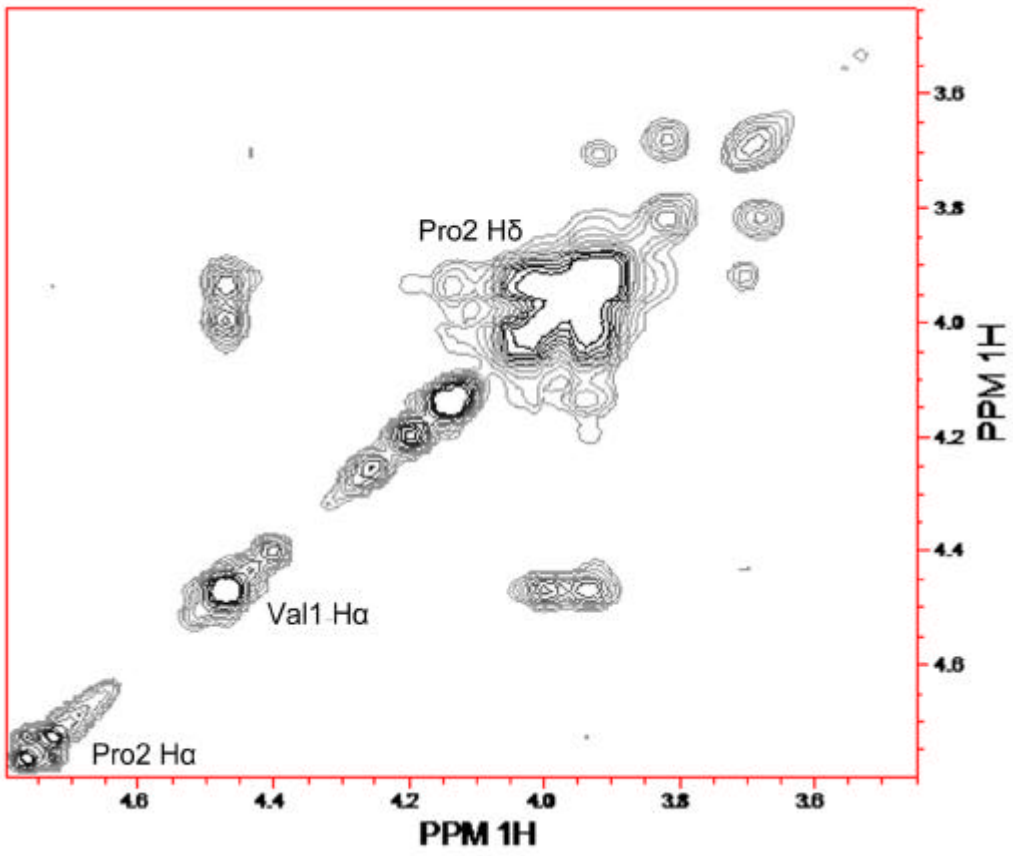

Figure 3. ^{19}F NMR spectra of **elastin-**(**1**), (**A**), and **elastin-**(**2**), (**B**), indicating the incorporation of (2*R*,3*R*)-3-fluoroproline and (2*R*,3*S*)-3-fluoroproline, respectively, into the recombinant polypeptide. The symbols **t** and **c** refer to the *trans* and *cis* isomers, respectively, of the Val-Pro peptide bonds within the repeat units. These spectroscopic assignments were based on comparison with ^{19}F NMR chemical shift values for the corresponding 3-fluoroproline model compounds, (**1**) and (**2**). The greater relative abundance of the *cis* prolyl-peptidyl bond isomer in **elastin-**(**2**) versus **elastin-**(**1**) reflects the increased thermodynamic preference for this configuration in (2*R*,3*S*)-3-fluoroproline derivatives.

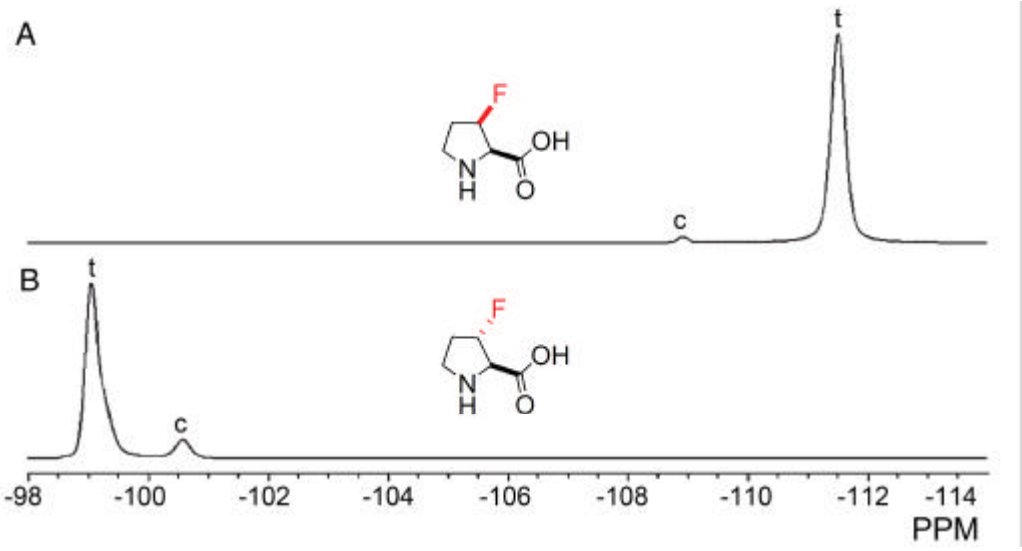

Figure 4. Raw, unadjusted differential scanning calorimetry data for dilute aqueous solutions (1.0 mg/mL) of **elastin-**(**1**) (red), and **elastin-**(**2**) (blue). The maxima for the endothermic transitions for **elastin**-(**1**) and **elastin**-(**2**) occur at temperatures of 35.9 ºC and 13.6 ºC, respectively.