A Coiled Coil with a Fluorous Core

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Abstract: The design, synthesis, and structural characterization of a highly fluorinated peptide system based on the coiled coil region of the yeast transcription factor GCN4 is described. All four leucine residues (*a* position) and three valine residues (*d* position) were replaced by the unnatural amino acids 5,5,5-trifluoroleucine and 4,4,4-trifluorovaline, respectively. The peptide is highly α-helical at low micromolar concentrations as judged by circular dichroism spectra, sediments as a dimeric species in the 5−30 μM concentration range, and exhibits a dimer melting temperature that is 15 °C higher than a control peptide with a hydrocarbon core. Furthermore, the apparent free energy of unfolding as calculated from guanidinium hydrochloride denaturation experiments is larger by ∼1.0 kcal/mol for the fluorinated peptide than its hydrocarbon counterpart. We conclude that additional stability is derived from sequestering the more hydrophobic trifluoromethyl groups from aqueous solvent. These studies introduce a new paradigm in the design of molecular self-assembling systems, one based on orthogonal solubility properties of liquid phases.

Introduction

Formation of defined structures in biology is frequently accompanied by separation of immiscible phases into distinct domains.[1,2] Indeed, the energetic advantage of getting nonpolar substances to minimize their contacts with water is evident in the structure of the plasma membrane and of most proteins, which in essence form intramolecular micelles. Phase separation is therefore a structural imperative in biology. Recently, the orthogonal solubility of fluorous phases has been exploited in catalysis,[3a] reaction acceleration,[4] combinatorial chemistry,[3b] and organic separation methodology.[5] However, structural use of this phase separation paradigm has been, at best, limited.[6,7]

Herein, we report the design, chemical synthesis, and structural characterization of a self-assembling peptide with a highly fluorinated core based on the coiled coil domain of yeast bZIP transcriptional activator GCN4. We hypothesized that immiscibility of fluorous phases in both water and many organic solvents should provide enough driving force for fluorinated side chains to collapse in a predetermined fashion to yield a fluorous core.[8] We expect such altered cores to have desirable properties for use in high-temperature catalysis,[9] stabilization of native protein structures in organic solvents,[9b] selective protein−protein interactions,[10] and the design of membrane spanning peptides.[11]

Results and Discussion

Design Principles. The coiled coil domain is a wide-spread structural pattern found in fibrous as well as globular proteins.[12] It is estimated that greater than 5% of all open reading frames in genomes that have been sequenced contain the coiled coil motif.[13] These motifs are well-suited to protein design studies, as the interactions governing structure are relatively well understood and represent the simplest case for which computational tools are sufficiently sophisticated to allow rational design. Therefore, it is not surprising that coiled coils have been extensively used in de novo peptide and protein design.[14] Coiled coils self-assemble into oligomers, the primary driving force being hydrophobic interactions. The sequences exhibit a 4−3

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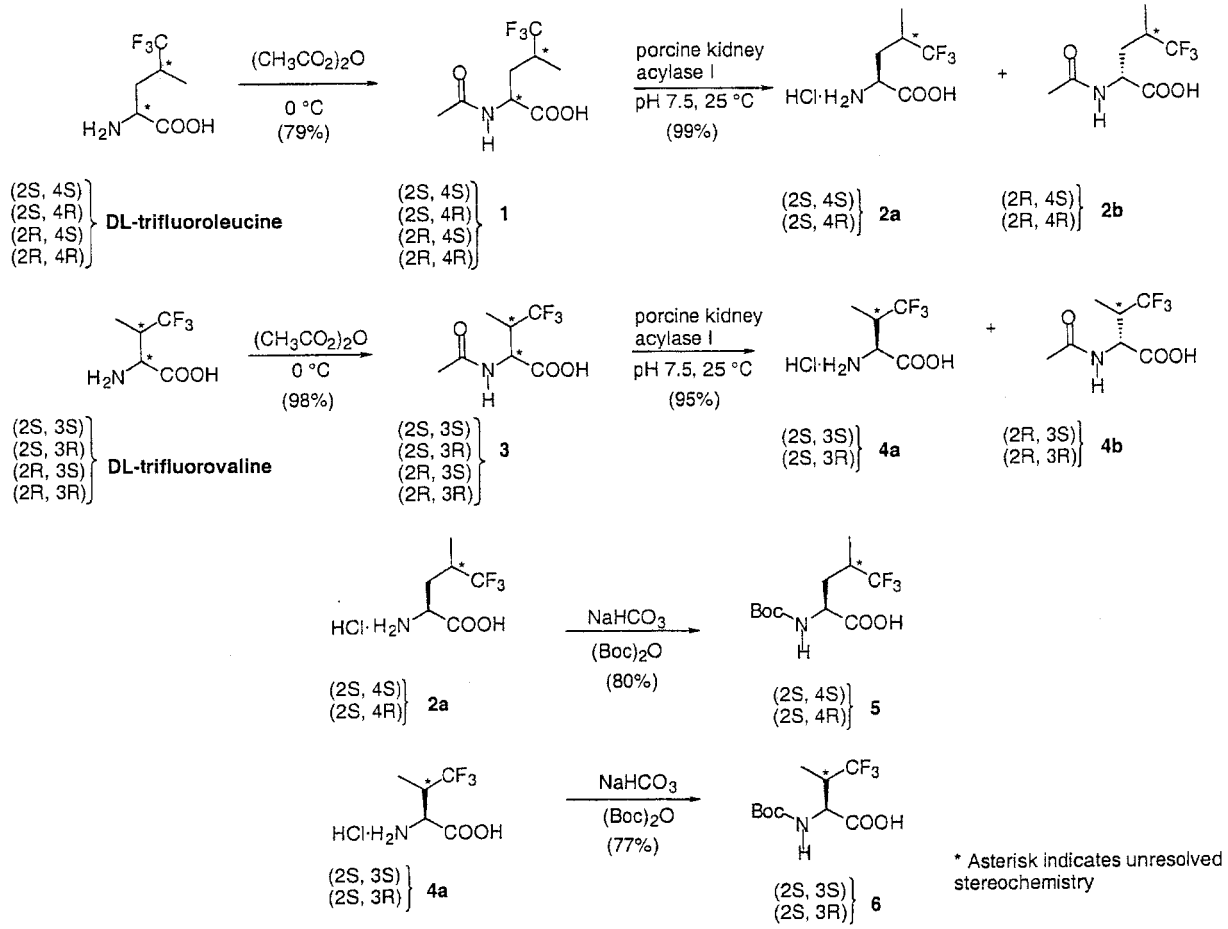
Scheme 1



hydrophobic repeat $(abcdefg)_n$, with the *a* and *d* positions predominantly occupied by amino acid residues with hydrophobic side chains and charged residues occurring frequently at the *e* and *g* positions. Residues at these four positions participate in interhelical hydrophobic and electrostatic interactions. The solvent-exposed positions *b*, *c*, and *f* are not directly involved in the self-assembly process and can tolerate a wide variety of amino acid substitutions. Studies of peptide models have suggested that four heptad repeats in the sequence are sufficient to yield a stable dimer.[15,23a] We hypothesized that extensive fluorination of the core found in these small proteins would provide greater driving force for structure formation. The coiled coil domain of GCN4 is a 33 residue peptide that forms a parallel homodimer.[23b] It contains leucine residues in *d* positions and valine in *a* positions with the exception of an asparagine at position 16. We sought to replace the core residues by trifluoromethyl-containing amino acid residues. It is well-known that replacement of CH_3 groups with CF_3 groups results in an increase in hydrophobicity due to the low polarizibility of the fluorine atoms.[16,17] This is also evident when one compares the estimated partial molal heat capacities for the process of (aqueous) solution[18,19] (98 cal mol^{-1} K^{-1} for CF_4 and 173 cal mol^{-1} K^{-1} for C_2F_6 which are considerably more positive than the values of 50 cal mol^{-1} K^{-1} for CH_4 and 72 cal mol^{-1} K^{-1} for C_2H_6). The higher values for the fluorocarbon gases are consistent with much larger perturbations of water structure,[20] based on the classical analysis of Frank and Evans.[21] Furthermore, although the trifluoromethyl group has twice the molar volume of a methyl group, it can statistically replace the latter in crystals[16b,22] (solid solutions). The designed peptides **1** and **2** (Figure 1) are similar to the coiled coil region of GCN4 with minor modifications.[23] Four leucine and three valine residues in the hydrophobic core of peptide **1** were replaced by 5,5,5-trifluoroleucine and 4,4,4-trifluorovaline respectively to yield peptide **2**.[24] In this manner, an α-helical coiled coil dimer

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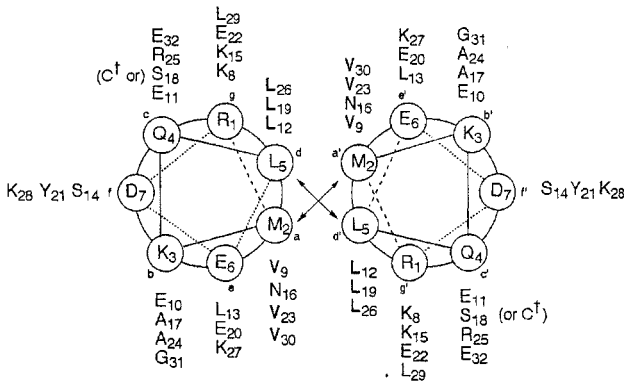
(24) Amino acids were mixtures of *N*-Boc-(2*S*,4*S*)- and (2*S*,4*R*)-5,5,5-trifluoroleucine (*d* position) or *N*-Boc-(2*S*,3*S*)- and (2*S*,3*R*)-4,4,4-trifluorovaline (*a* position).



1: Ar·HN·RMKQLEDKVEELLSKNAC†LEYEVARLKKLVGE·CO·NH$_2$

2: Ac·HN·RMKQLEDKVEELLSKNASLEYEVARLKKLVGE·CO·NH$_2$

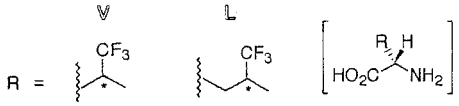


Figure 1. Helical wheel diagram and peptide sequences **1** and **2**. Abbreviations, Ar = 4-acetamidobenzoic acid, Ac = acetyl, C†= acetamidocysteine. The asterisk indicates unresolved stereochemistry.

was obtained with a very highly fluorinated core shielding 14 trifluoromethyl groups from aqueous solvent. Since the population of multiple oligomerization states by self-assembly is possible in these systems, a design feature that restricts the oligomerization to a dimeric state was needed to facilitate biophysical studies. Earlier studies have established that a single polar residue in the hydrophobic core can impart structural uniqueness in designed coiled coil systems by formation of an inter-strand hydrogen bond.[14d,25] Therefore, we retained the single conserved asparagine residue (*a* position) in the core to dictate oligomerization and orientation specificity.[23c,26]

Synthesis. Fluorine-containing building blocks for peptide synthesis were resolved by enzymatic deacylation of trifluoromethyl-containing *N*-acetyl amino acids with porcine kidney acylase I to deliver *S* stereochemistry at the C_α position.[27] The stereochemical purity of the enzyme-resolved amino acids was ascertained by chiral HPLC.[28] All four diastereomers of 4,4,4-trifluorovaline and 5,5,5-trifluoroleucine were separable. Only those diastereomers that had an *S* configuration at the C_α position could be detected after enzymatic resolution, judged by comparison to the mixture of all four diastereomers in each case. Peptides were assembled by manual solid-phase peptide synthesis by using the in situ neutralization protocol[29] on commercially available methylbenzhydrylamine [MBHA, co-poly(styrene, 1% DVB)] resin, using *t*-Boc chemistry, and purified by a combination of reversed-phase (C_4 and C_{18}) HPLC (Figure 2) and ion exchange chromatography. While the C_α position was exclusively *S* stereochemistry for all residues, the secondary stereocenters at C_β (trifluorovaline) and C_γ (trifluoroleucine) positions were left unresolved. Given the presence of two diastereomeric amino acids at each fluorinated core

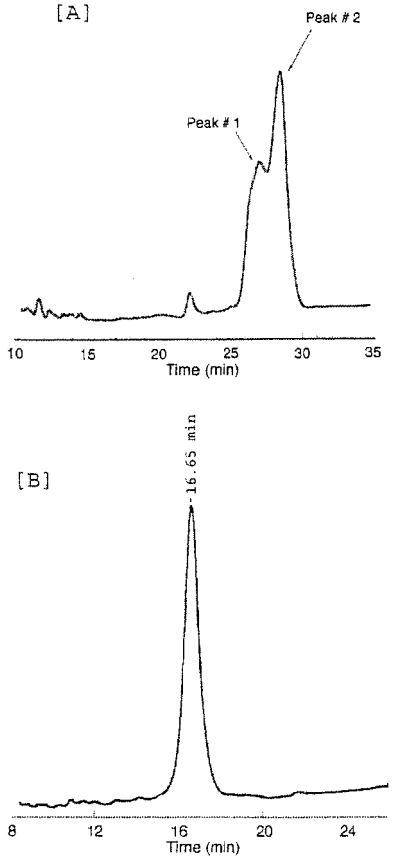


Figure 2. [A] Reversed phase HPLC trace (Vydac C_{18} column; 22 × 250 mm, 300 Å size, 10−15 μm) showing the fluoro peptide before final purification. Both peaks correspond to fluoro peptide **2**. The peaks were separated and the studies reported here were conducted exclusively with peak 2. [B] Analytical run of the purified peak 2 eluting at 16.65 min used in all structural characterization experiments. Gradient for [B]: 32% to 50% acetonitrile/H_2O/0.1% TFA at 2.5 mL/min (over 20 min). Detection: absorbance at 230 nm.

residue that was substituted, peptide **2** is a mixture of several diastereomers.[30]

Structural and Biophysical Characterization. The MALDI-TOF mass spectrum of the purified fluorinated peptide **2** showed a single peak at 4167.3 Da (calculated, 4165.9 Da) consistent with the proposed structure (Figure 3). Circular dichroism spectra of purified peptides **1** and **2** at 5 °C showed that both peptides are extremely α-helical in aqueous solution at pH 7.40 in the low micromolar concentration regime with characteristic minima at 222 and 208 nm. The magnitude of the minima at 222 and 208 nm for **2** is much larger than those for **1**, indicating a much higher level of helicity in the peptide. Both the hydrocarbon peptide **1** and fluoro peptide **2** exhibit cooperative unfolding transitions as a function of temperature (Figure 4). Thus, it would appear that both trifluoroleucine and trifluorovaline residues are incorporated in the hydrophobic core without large structural distortions. Apparent molecular masses of the self-assembled coiled coils in solution were determined by sedimentation equilibrium experiments. Data obtained were fit to an ideal single species model plot of ln(absorbance) versus radial distance squared. Both peptides **1** and **2** sediment as dimers in the 5−30 μM concentration range (Figure 5), thus validating the comparison of melting temperatures. Dimer melting temperatures obtained by monitoring the molar ellip-

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(30) A maximum of $2^7 = 128$ diastereomers could have been produced as seven of the core residues have been replaced by fluorinated amino acids.

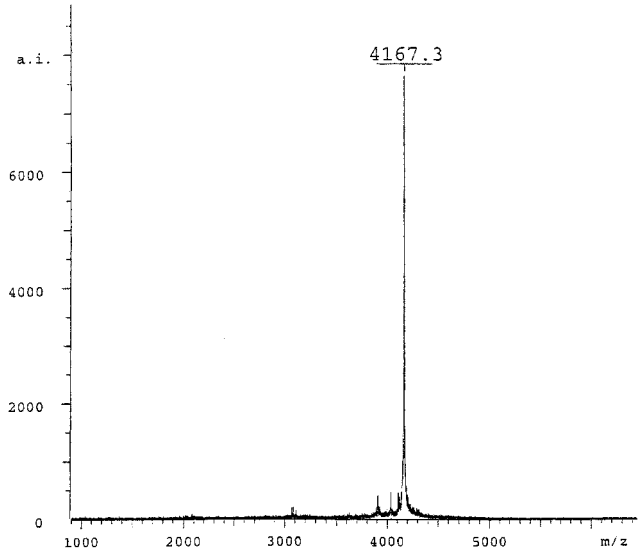

Figure 3. MALDI-TOF MS of purified fluorinated peptide **2** (MW_{calc} = 4165.9 Da, found = 4167.3 Da).

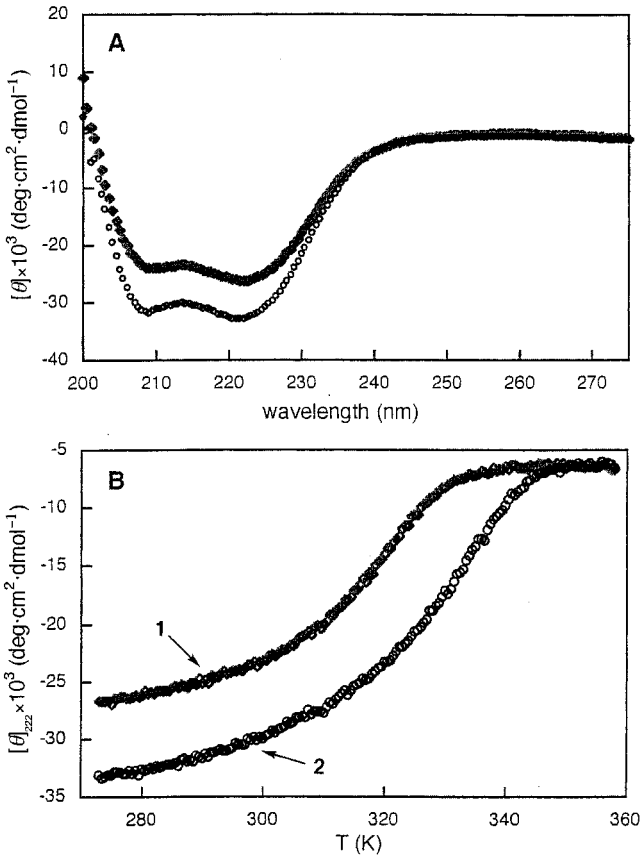

Figure 4. [A] Circular dichroism spectra of peptides **1** (◆) and **2** (○) in PBS (10 mM phosphate, 137 mM NaCl, 2.7 mM KCl, pH 7.40) at 5 °C ([**1**] = [**2**] = 5 μM). The minima at 222 and 208 nm and the large negative $[\theta]_{222}$ indicate that both peptides are highly α-helical. [B] Thermal denaturation of peptides **1** and **2** monitored by CD ($[\theta]_{222}$) yielded a T_m of 47 (**1**) and 62 °C (**2**). The T_m values were determined from the minima of the first derivative of $[\theta]_{222}$ with T^{-1}, where T is in K. Melting curves were reversible (folding and unfolding curves were superimposable, with ≥97% of the initial signal regained upon cooling).

ticity at 222 nm as a function of temperature show that peptide **2** is more stable than peptide **1** by ~15 °C (T_m^1 = 47 ± 1 °C, T_m^2 = 62 ± 1 °C), establishing the higher thermal stability of the fluorinated core in aqueous solution (Figure 4).

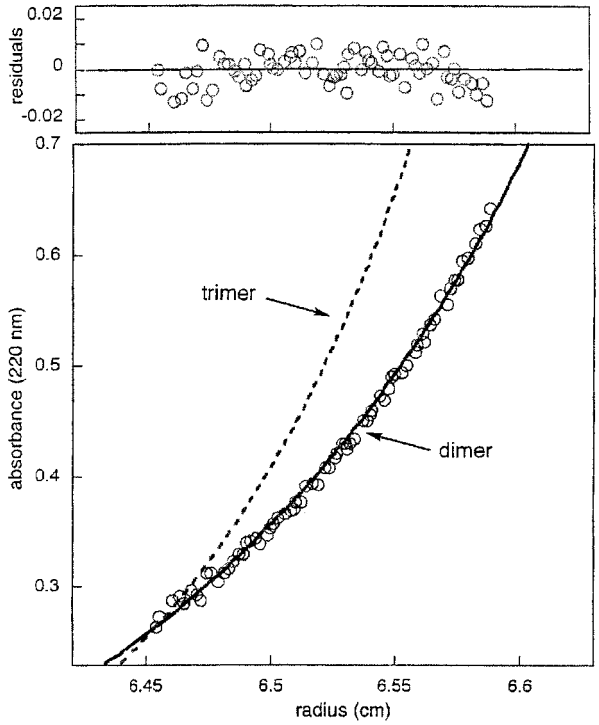

Figure 5. Sedimentation equilibrium trace for peptide **2** (14 μM in PBS at 10 °C at pH 7.40) confirming a dimeric species in the concentration regime of the CD experiments. Likewise, control peptide **1** sediments as a dimer (Supporting Information).

The stability of the peptides toward chaotropic reagents was determined by monitoring the molar ellipticity at 222 nm as a function of Gdn·HCl concentration (Figure 6). The apparent free energy of unfolding as calculated from these experiments is modestly larger for peptide **2** than peptide **1** (~1.0 kcal/mol at 5 °C, Table 1). The sensitivity of peptides to the denaturant value *m*, given by $d(\Delta G)/d[\text{Gdn·HCl}]$, is believed to be proportional to the amount of hydrophobic surface area exposed upon denaturation.[31] The *m* value for fluoro peptide **2** extracted from nonlinear fitting is 3.08 ± 0.22 kcal mol^{-1} M^{-1}, which is much larger than the 2.40 ± 0.24 kcal mol^{-1} M^{-1} obtained for **1**. This is consistent with the burial of 14 voluminous trifluoromethyl instead of methyl groups. Once some of the structural features of packing in the fluorinated cores are elucidated in greater detail, optimization of the packing interactions in fluorinated cores should provide additional stability. On the basis of the denaturation and thermal stability data, we conclude that the additional stability of the fluorinated peptide is derived from sequestering the more hydrophobic trifluoromethyl groups from aqueous solvent.

The helix stabilizing effects of certain alcohol solvents, especially 2,2,2-trifluoroethanol (TFE), have been employed frequently in the study of equilibrium[32] and kinetic[33] studies of protein folding. Given the large number of trifluoromethyl groups buried in the hydrophobic core of peptide **2**, we sought to examine the effect of TFE on peptide **2** in relation to the effects observable on **1**. The data are shown in Figure 7 where the percentage decrease in molar ellipticity at 222 nm ($[\theta]_{222}$)

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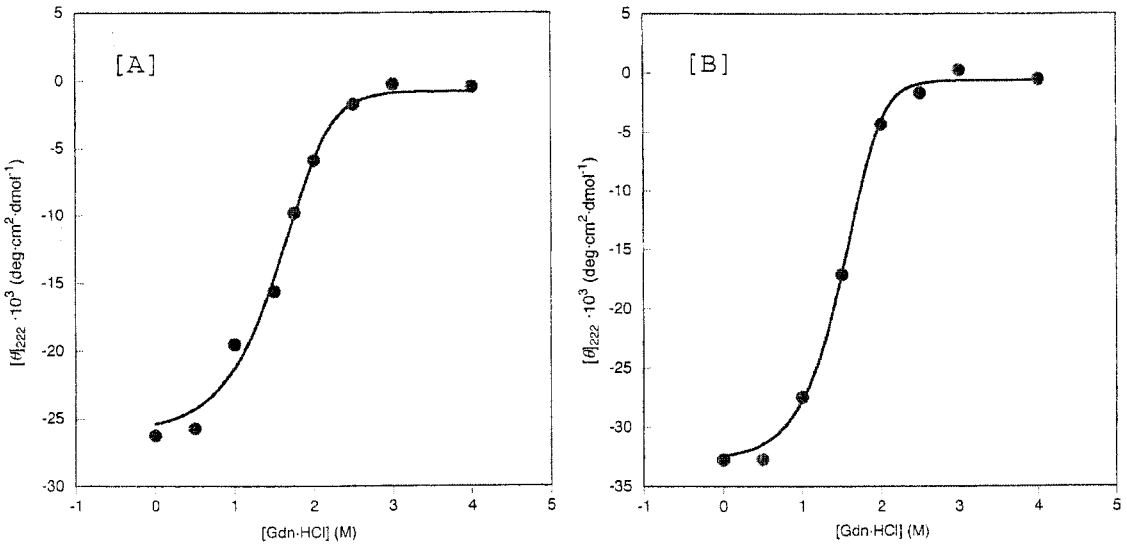

Figure 6. Guanidinium denaturation of control peptide **1** (5 μM, [Panel A]) and fluorinated peptide **2** (5 μM, [Panel B]) at 5 °C (pH 7.40) monitored by the circular dichroism at 222 nm. The solid line is a curve generated from the parameters listed in Table 1.

Table 1. Fitting Parameters for Gdn·HCl Denaturation Experiments[a]

peptide	$\Delta G^{\circ}_{H_2O}$ (5 °C) (kcal mol^{-1})	m (kcal mol^{-1} M^{-1})
1	10.50 ± 0.42	2.40 ± 0.24
2	11.43 ± 0.34	3.08 ± 0.22

[a] The parameters were obtained by nonlinear least-squares fitting of the data in Figure 6.

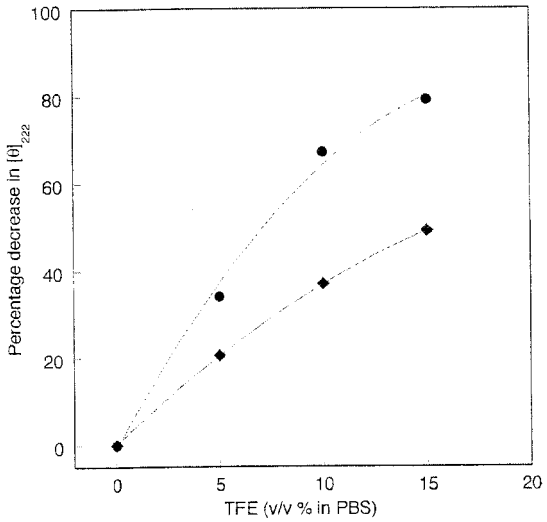

Figure 7. Effect of 2,2,2-trifluoroethanol (TFE) on the helical content of peptide **1** (5 μM, ●) and peptide **2** (5 μM, ◆) monitored by circular dichroism at 222 nm.

in circular dichroism experiments is plotted with TFE concentration. The percentage increase in helicity is much less pronounced for **2** than for control peptide **1**. There are two possible explanations. The first possibility is that this difference is due to the intrinsically higher helical content of the fluoro peptide **2** at 0% TFE. At low concentrations, the helix-enhancing ability of TFE in coiled coil systems has been attributed to an increase in the structure of the binary alcohol/water solvent and a concomitant increase in the energetic cost of polypeptide backbone solvation.[34] This view is consistent with minimal involvement of the side chains of the hydrophobic core residues in the mechanism of helical induction by TFE, and could be sufficient to explain our observations given the higher intrinsic helical content of peptide **2**. A second possibility is that TFE may effectively solvate the fluorinated residues, thereby disrupting dimerization. In this case, the competing effects of denaturation of the fluorous core (decrease in helicity) and amide backbone desolvation (increase in helicity) with increasing TFE are responsible for the smaller increase observed for **2**. Our data do not allow us to distinguish between these mechanisms. It would be informative to explore the effects of fluorinated alcohol cosolvents in systems with even more highly fluorinated cores.

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Conclusions

We have outlined a strategy for the design and construction of peptides with highly fluorinated interiors by solid-phase peptide synthesis. Characterization of such peptides by a wide variety of biophysical techniques reveals higher stability compared to hydrocarbon counterparts. The intriguing possibility of creating a binary patterning scheme for protein design based on hydrocarbon/fluorocarbon phase separation seems within reach. With the recent introduction of protein ligation techniques, the size range of proteins accessible by chemical synthesis and semisynthesis has been greatly expanded.[35] The site-specific incorporation of fluorinated amino acids onto hydrophobic folds of larger proteins is therefore feasible with current technology. In our laboratories, further biophysical studies aimed at probing the nature of fluorous interactions in the core, synthesis, and characterization of coiled coils with hexafluoroleucine and fluorinated membrane spanning peptides are currently in progress. With this study, we introduce a new paradigm for molecular design, one based on orthogonal solubility properties of liquid phases.

Experimental Methods

Chemicals. Acetonitrile (optima grade), dichloromethane (ACS grade), and dimethylformamide (DMF, sequencing grade) were purchased from Fisher Scientific and used without further purification. *N*,*N*-Diisopropylethylamine (DIEA, Aldrich, biotech grade), trifluoroacetic acid (TFA, New Jersey Halocarbon), guanidine hydrochloride

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(Gdn·HCl, Fisher Scientific, electrophoresis grade), 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, Quantum Biotechnologies), 4-methylbenzhydrylamine resin (MBHA, Novabiochem), anisole (anhydrous, Aldrich), and trifluoroethanol (99%, Acros) were used without further purification. *N*-Boc-α-amino acids were used as obtained from Novabiochem, Advanced Chemtech or American Peptide Company. Hydrogen fluoride was purchased from Matheson Gas.

Preparation of Fluorinated Amino Acids for Peptide Synthesis. DL-Trifluoroleucine and DL-trifluorovaline were purchased from Oakwood Chemicals or prepared by literature procedures.[36] The *N*-acetyl derivatives of both 5,5,5-trifluoroleucine[36c] and 4,4,4-trifluorovaline[36b] are known. The *N*-acetyl amino acids were then deacylated by porcine kidney acylase I, using a modification of a known literature procedure.[27a] Details of acetylation and deacylation reactions are given in the Supporting Information. The hydrochloride salts of the amino acids with *S* stereochemistry at C_α were *t*-Boc protected on the amino terminus by using a mild protection method[37] and after purification were used directly in solid-phase peptide synthesis.

***N*-(*t*-Boc)-(2*S*,4*S*),(2*S*,4*R*)-trifluoroleucine (5).** (2*S*,4*S*),(2*S*,4*R*)-5,5,5-trifluoroleucine HCl salt (1.00 g, 4.5 mmol), $NaHCO_3$ (1.14 g, 13.5 mmol), and $(Boc)_2O$ (1.08 g, 5.0 mmol) were suspended in 22 mL of MeOH and sonicated for 1 h. The reaction mixture was filtered. Rotary evaporation of the filtrate gave a white solid. Further purification of this material by C_{18} reversed phase column chromatography (30% CH_3CN/H_2O, 0.25% HOAc eluant) provided pure N-*t*-Boc-5,5,5-(2*S*,4*S*),(2*S*,4*R*)-trifluoroleucine in 80% yield (1.05 g). ^{1}H NMR (300 MHz, D_2O) [mixture of two diastereomers] δ 1.13 (d, 3H, $J = 6.8$ Hz), 1.42 (s, 9H), 1.58−2.13 (m, 2H), 2.30−2.48 (m, 1H), 3.90−4.02 (m, 1H). GC-MS (CI, CH_4): calcd for $C_{11}H_{18}F_3NO_4$ 285.28, found 286 ($[M + 1]^+$).

***N*-(*t*-Boc)-(2S,3S),(2S,3R)-trifluorovaline (6).** (2*S*,3*S*),(2*S*,3*R*)-Trifluorovaline hydrochloride salt (1.00 g, 4.8 mmol) was *t*-Boc protected following the protocol given for trifluoroleucine above to yield the desired product in 82% yield (1.07 g). ^{1}H NMR (300 MHz, D_2O) [23% (2*S*,3*S*); 77% (2S,3R)] δ [1.06 (d, $J = 7.2$ Hz), (2*S*,3*R*), 1.16 (d, $J = 7.1$ Hz), (2*S*,3*S*)] (3H), 1.41 (s, 9H), 2.90−2.95 (m, 1H), [4.12 (d, $J = 5.1$ Hz), (2*S*,3*S*), 4.43 (d, $J = 2.6$ Hz), (2*S*,3*R*)] (1H). GC-MS (CI, CH_4): calcd for $C_{10}H_{16}F_3NO_4$ 271.25, found 272 ($[M + 1]^+$).

Peptide Synthesis. Peptides were prepared by using the *N*-*tert*-butyloxycarbonyl (*t*-Boc) amino acid derivatives for Merrifield manual solid-phase synthesis (MBHA resin), using the in situ neutralization/HBTU protocol typically on a 0.5 mmol scale.[29] *N*-α-Boc-α-*S*-amino acids were used with the following side chain protecting groups: Arg(Tos), Asp(OBzl), Asn(Xan), Gln(Xan), Cys(Acm), Glu(OBzl), Lys(2-Cl-Z), Ser(Bzl), and Tyr(2-Br-Z). Peptide coupling reactions were carried out with a 4-fold excess (2.0 mmol) of activated amino acid for at least 15 min. Peptides were cleaved from the resin by using high HF conditions (90% anhydrous HF/10% anisole at 0 °C for 1.5 h).[38]

Purification. Peptides were desalted by reversed phase HPLC [Vydac C_4 column with a 30 min linear gradient of 20−40% acetonitrile/H_2O/0.1% TFA at 8.0 mL/min]. Further purification of **2** was carried out by ion exchange chromatography by using reverse organic conditions [PolyWAX A column from PolyLC, Inc., Columbia, MD (200 × 9.4 mm, 5 μm, 300 Å)]. Following purification by ion-exchange chromatography, **2** was again subjected to purification and desalting by reversed phase HPLC (Vydac C_{18} column, 22 mm × 250 mm, 300 Å size, 10−15 μm). There were two peaks in the reversed phase that could be separated (Figure 2). The studies reported in this paper were all carried out with the peak eluting at 16.65 min [Figure 2B]. The other peak eluted at 15.69 min (under the conditions of Figure 2B), gave similar CD spectra at 5 μM concentration, and also shows similar T_m values. However, in sedimentation equilibrium experiments, the residuals were large and non-random for material obtained from this peak (even after repeated purification), preventing us from assigning a unique oligomerization state.

Circular Dichroism. CD spectra were obtained on a JASCO J-715 spectropolarimeter fitted with a PTC-423S single position Peltier temperature controller. Buffer conditions were 10 mM phosphate (pH 7.40), 137 mM NaCl, 2.7 mM KCl unless otherwise noted. The spectrometer was calibrated with an aqueous solution of recrystallized d_{10}-(+)-camphorsulfonic acid at 290.5 nm. The concentrations of the peptide stock solutions were determined by amino acid analysis (average of 3 runs) or by integration of the tyrosine absorbance on analytical HPLC relative to standard *N*-acetyltyrosinamide ($\epsilon = 1490\ M^{-1}\ cm^{-1}$). Mean residue ellipticities (deg cm^2 $dmol^{-1}$) were calculated by using the relation:

$$[\theta] = \theta_{obs} \times MRW/10 \cdot l \cdot c \quad (1)$$

where θ_{obs} is the measured signal (ellipticity) in millidegrees, l is the optical path length of the cell in cm, c is concentration of the peptide in mg/mL, and MRW is the mean residue molecular weight (molecular weight of the peptide divided by the number of residues).

Thermal denaturation studies were carried out at the concentrations indicated by monitoring the change in $[\theta]_{222}$ as a function of temperature. Temperature was increased in steps of 0.5 °C with an intervening equilibration time of 120 s. Data were collected over 16 s per point. The T_m was determined from the minima of the first derivative of $[\theta]_{222}$ with T^{-1}, where T is in K. All thermal melts were reversible with ≥97% of the starting signal regained upon cooling.

Gdn·HCl Denaturation. Denaturation experiments were carried out assuming a two-state unfolding transition.[39]

$$D \rightleftharpoons 2M$$

where $K_d = [M]^2/[D]$ (M = monomer and D = dimer). Given that the total peptide concentration, P_t, in terms of monomers is $P_t = 2[D] + [M]$, the CD signal Y_{obs} can be described in terms of folded and unfolded baselines, Y_{fol} and Y_{unfol}, respectively, by the following expression.[40]

$$Y_{obs} = (Y_{unfol} - Y_{fol})\frac{\sqrt{K_d^2 + 8K_dP_t} - K_d}{4P_t} + Y_{fol} \quad (2)$$

Furthermore, K_d can be expressed in terms of the free energy of unfolding,

$$K_d = \exp(-\Delta G^\circ/RT) \quad (3)$$

and assuming that the apparent free energy difference between folded dimer and unfolded monomer states is linearly dependent on the Gdn·HCl concentration, ΔG° can be written as:

$$\Delta G^\circ = \Delta G^\circ_{H_2O} - m[\text{Gdn·HCl}] \quad (4)$$

where $\Delta G^\circ_{H_2O}$ is the free energy difference in the absence of denaturant and m is the dependency of the unfolding transition with respect to the concentration of Gdn·HCl.[41] The data were fit for two parameters, namely $\Delta G^\circ_{H_2O}$ and m, by nonlinear least-squares fitting (KaliedaGraph v3.5).

Analytical Ultracentrifugation. Apparent molecular masses were determined by sedimentation equilibrium on a Beckman XL-A ultracentrifuge. Loading peptide concentrations were 5−30 μM in 10 mM phosphate (pH 7.40), 137 mM NaCl, 2.7 mM KCl. The samples were centrifuged at 32 000 and 26 000 rpm for 18 h at 10 °C before absorbance scans were performed. Data obtained at 10 °C were fit globally to an equation that describes the sedimentation of a homoge-

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neous species.

$$\text{Abs} = A' \exp(H \times M[x^2 - x_0{}^2]) + B \quad (5)$$

where Abs = absorbance at radius x, A' = absorbance at reference radius x_0, $H = (1 - \bar{V}\rho)\omega^2/2RT$, $\bar{V}$ = partial specific volume = 0.75531 mL/g, ρ = density of solvent = 1.0017 g/mL, ω = angular velocity in radians/s, M = apparent molecular weight, and B = solvent absorbance (blank). We estimated partial specific volume of the peptides using amino acid composition, substituting leucine and valine for 5,5,5-trifluoroleucine and 4,4,4-trifluorovaline respectively in peptide **2** for lack of available data.[42]

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Supporting Information Available: Experimental details, HPLC data, MALDI mass spectrum, and sedimentation equilibrium trace for peptide **1** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(43) **Note Added in Proof:** While this manuscript was under review, another study detailing a similar approach to ours has been reported: Tang, Y.; Ghirlanda, G.; Vaidehi, N.; Kua, J.; Mainz, D. T.; Goddard III, W. A.; DeGrado, W. F.; Tirrell, D. A. *Biochemistry* **2001**, *40*, 2790−2796.