Helical Structures

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Proteomorphous Objects from Abiotic Backbones**

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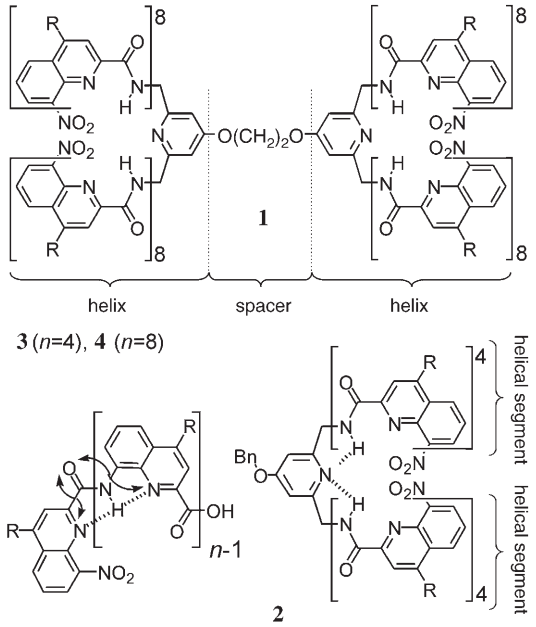
The functions of proteins rest on the ability of peptidic sequences to fold into well-defined conformations. Previously, molecular folding was believed to belong mainly to biological macromolecules but, over the last decade, chemists have shown that numerous families of synthetic oligomers (foldamers) also adopt folded structures,[1–4] some of which display biological activities.[3–6] Foldamers have opened the path to mimic not only protein structure but also protein function in artificial systems. However, they have thus far been limited in size and mostly consist of simple analogues of secondary structural elements as, for example, isolated helical or linear strands. Herein, we present a large foldamer (>8 kDa) that compares to a modest-sized protein tertiary fold in terms of its dimension and structural complexity. Our results show that design and stepwise synthetic strategies can be devised to access abiotic, very large, yet conformationally defined architectures that resemble proteins somewhat more closely than previously described sizeable non-peptidic structures, such as discrete self-assemblies[7,8] or dendrimers.[9,10] These proteomorphous objects represent novel platforms to envisage molecular recognition and enzymelike catalysis on a different scale.

In designing a large artificial folded structure, one must take into account the feasibility of the synthesis. Considering the difficulty of the stepwise preparation of long peptides in significant quantities, we determined that linear sequences of monomers may not be the best target for practical purposes. Alternatively, large branched architectures have been prepared by using efficient divergent or convergent synthetic schemes.[9,10] Branched structure **1** (8.2 kDa, $C_{464}H_{464}N_{70}O_{74}$) was thus chosen as our first design (Scheme 1). It consists of two helices connected by a short ethylene glycol spacer that imposes proximity, and thus interactions, between their peripheral residues, as in protein tertiary motifs. Each helix comprises two identical octameric quinoline oligoamides linked to a bis(aminomethyl)pyridine connector. Quinoline oligoamides derived from 8-amino-2-quinolinecarboxylic acid



Scheme 1. Structures and helical folding of oligoamide sequences. Compound **1** possesses a branched architecture made of two helices each comprising 17 units (8+1+8) separated by an ethylene glycol spacer. Oligomer **2** consists of a single helix of nine units (4+1+4). The formula of helical tetrameric and octameric precursors[12] **3** and **4**, respectively, show hydrogen bonds (dashed lines) and electrostatic repulsions (double headed arrows) that, along with intramolecular aromatic stacking, stabilize the helically folded conformers. The helical conformations have been characterized both in the solid state and in solution.[11–15] R = isobutoxy residues ($(CH_3)_2CHCH_2O$-) that protrude from the helices toward the solvent.

adopt particularly robust helical conformations in a wide range of nonprotic ($CDCl_3$, DMSO)[11–14] and protic (MeOH, H_2O)[15] solvents and thus represent versatile "building blocks" to construct proteinlike objects in a modular fashion.

The multistep synthesis of **1** includes two key reactions (Scheme 2): the divergent attachment of two pyridine units to the central ethylene glycol spacer to give intermediate **8** and the subsequent simultaneous ligation of four quinoline octameric amides to give **1** (see the Supporting Information). The synthesis has been completed on a 50-mg scale but could easily be scaled up to produce larger amounts.

As detailed below, an essential feature of the design of **1**, which greatly facilitated the study of its structure, is that it contains no chiral group to favor a right (P) or left (M) handedness of the helices.[14] The proposal that **1** consists of two distinct helices relies on the premise that each bis(aminomethyl)pyridine connector that links a pair of quinoline oligomers inserts itself in the quinoline helical motif. This was validated by a conformational study of the shorter

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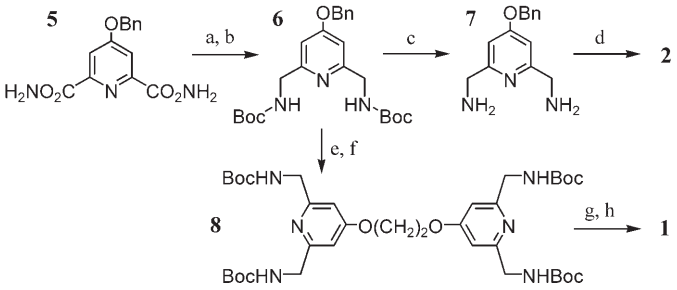

Scheme 2. Synthesis of folded oligomeric sequences **1** and **2**. a) BH_3, THF, quant.; b) BocOBoc, THF, 75%; c) TFA, CH_2Cl_2, quant.; d) **3**, HBTU, HOBT, Et*i*Pr$_2$N, DMF, 65%; e) H_2, Pd black, MeOH/EtOAc, 73%; f) HO-$(CH_2)_2$OH, DIAD, PPh_3, THF, 89%; g) TFA, CH_2Cl_2, quant.; h) **4**, HBTU, HOBT, Et*i*Pr$_2$N, DMF, 44%. Boc = *tert*-butoxycarbonyl, TFA = trifluoroacetic acid, HBTU = *O*-(benzotriazol-l-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOBT = hydroxybenzotriazole, DMF = *N*,*N*-dimethylformamide, DIAD = diisopropylazodicarboxylate, Bn = benzyl.

oligomer **2** (Scheme 1). The two quinoline helical segments of **2** may, in principle, have P or M helicity. Thus, **2** may exist as a mixture of a racemic P-P/M-M and a *meso* P-M species that interconvert through the handedness inversion of one helical segment. This process is slow on the NMR time scale and should give rise to two sets of signals on the ^{1}H NMR spectrum of **2** if the two species coexist.[15,16] However, the spectrum of **2** shows that it consists of a single set of signals (Figure 1a). A crystal structure of **2** confirmed that this species corresponds to the racemic P-P/M-M mixture for which the two helical segments of each molecule always possess the same handedness (Figure 2a). This preference presumably arises from favorable interactions between the endocyclic nitrogen of the pyridine unit and the two neighboring amide protons that set the orientation of the

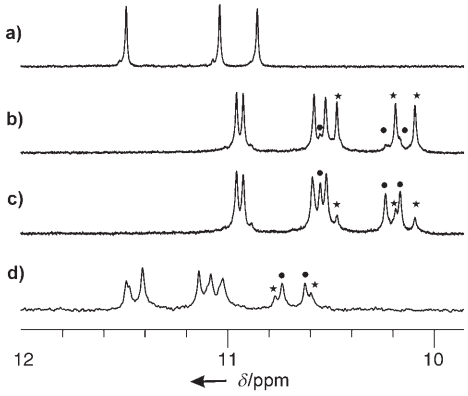

Figure 1. Selected region of the 400 Mhz ^{1}H NMR spectra of **1** and **2** at 25 °C showing amide resonances. a) Compound **2** at equilibrium in $CDCl_3$. b) Branched structure **1** at equilibrium in $CDCl_3$. c) Compound **1** freshly dissolved in $CDCl_3$ after one week of equilibration in toluene. d) Compound **1** at equilibrium in [D_8]toluene. The stars and circles indicate signals assigned individually to the P-M and P-P/M-M conformers of **1**, respectively. Other signals of the two species overlap. Full spectra and experiments that led to the assignment of the signals to P-P/M-M and P-M conformers based on the monitoring of chemical-shift variations in solvent mixtures are reported in the Supporting Information.

relative quinoline helical segments so that they have the same handedness. In contrast, an analogue of **2**, in which the pyridine ring is replaced by a phenyl group, shows little preference for the *meso* or the racemic species, and undergoes faster helix inversion than **2**: the temperature of coalescence of the ^{1}H NMR signals of diasterotopic motifs of isobutoxy side chains protons in $CDCl_3$ is above 55 °C for **2** (see the Supporting Information) compared with 25 °C for its C analogue.[16]

The same reasoning applies to the structure of **1**: each of its two large helices may have P or M helicity and thus **1** may exist as a mixture of P-P/M-M and P-M species. Importantly, the two helices of **1** are connected side by side and not end to end anymore as in the two helical segments of **2**. Thus, any preference for the racemic or *meso* species in **1** should reveal interactions between the side chains of the helices, which amount to tertiary interactions in proteins. Taking into account the size of **1**, its NMR spectrum in $CDCl_3$ is strikingly simple (Figure 1b). The symmetrical branched architecture is conserved in the solution conformation, giving rise to a degenerate spectroscopic signature. Essentially, one set of signals can be observed in the spectrum, which implies a strong preference for either the *meso* or the racemic conformer and thus significant helix–helix interactions. Small signals amounting to about 7% can also be observed and were eventually assigned to the other conformer (see below).

The unambiguous assignment of the preferred conformation of **1** in $CDCl_3$ to the *meso* P-M species was made possible by a crystal structure (see the Supporting Information) that showed that the two helices have opposite handedness (Figure 2b–d) and by a NMR spectrum of the same freshly dissolved crystals, which showed that the crystals correspond to the major species in $CDCl_3$. The structure validates the design strategy and provides a clear illustration of the size of **1**, which compares with that of a 75-residue protein. This structure is significant in that it represents a rare case of genuine tertiary, albeit primarily steric, interactions in an abiotic system. The very fact that **1** easily crystallizes (overnight) suggests a well-defined three-dimensional conformation. By comparison, only very small dendrimers can crystallize.[18] The structure reveals a perpendicular orientation of the two helices. This conformation is apparently imposed by steric repulsions between the isobutoxy groups in position 4 of each helical segment (Figure 2c), which would otherwise clash if the helices were oriented parallel. Presumably, residues in position 6 and 7 of each octameric sequence may also contribute to contacts between the two helices and participate in the stabilization of the P-M species at the expense of the P-P/M-M form.

The characterization of the *meso* helix underlines the usefulness of not controlling handedness. If identical chiral residues had been introduced in the helices, they would have favored one of the two homochiral species (P-P or M-M),[14] therefore playing against the natural tendency of the system. Induction of helix handedness by a neighboring helix has rarely been observed.[19,20] As shown herein, it represents a novel, reliable, and quantitative probe of helix–helix interactions. In contrast, previous studies of synthetic peptidic

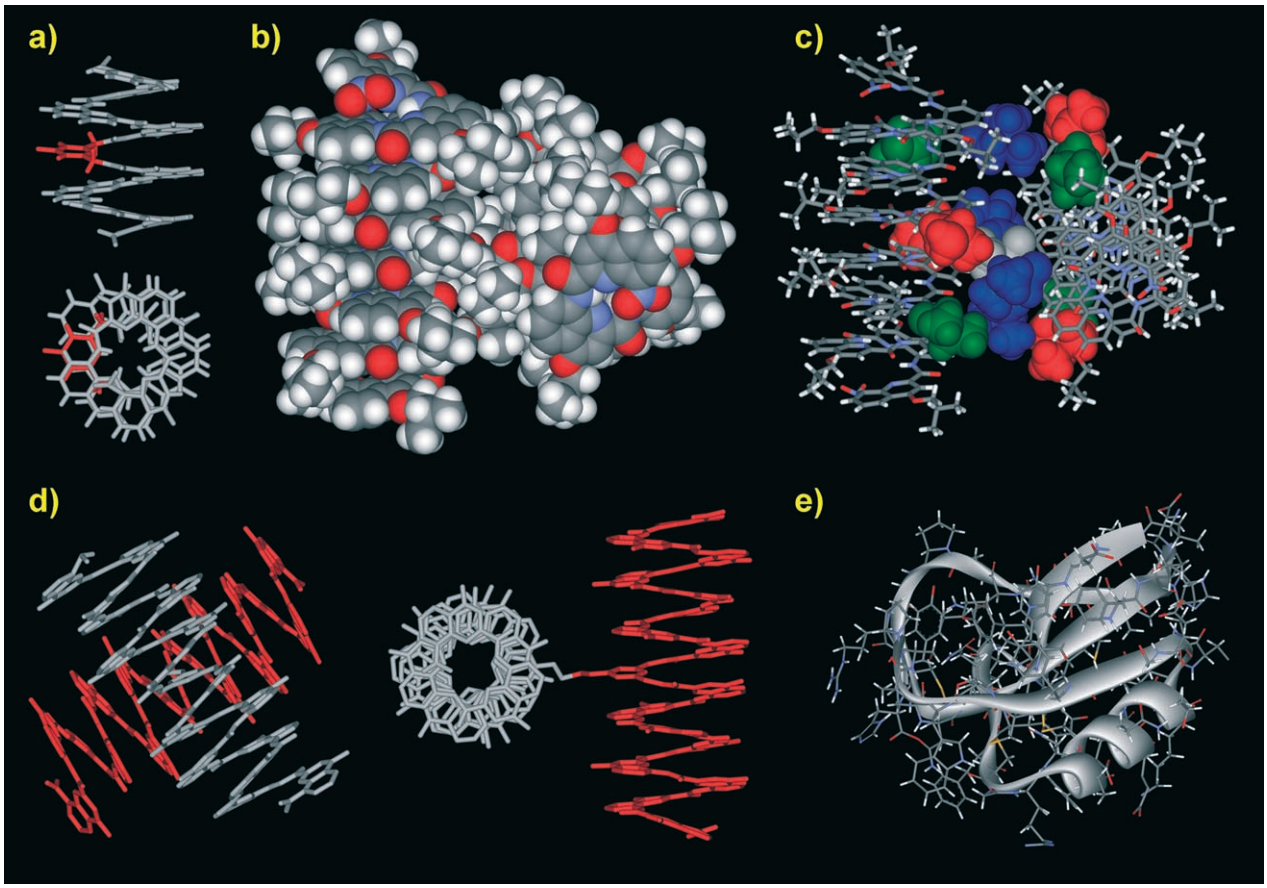

Figure 2. Crystal structures of helix **2** and of the branched structure **1**. a) Side view and top view of the right-handed helix of **2** in the solid state (quinoline segments in grey and pyridine unit in red). Alkoxy residues have been omitted for clarity. b) View of the structure of **1** as van der Waals spheres (carbon in gray, hydrogen in white, oxygen in red, nitrogen in blue). c) Same view of **1**, however, the structure is represented as tubes except for the residues that are responsible for intramolecular helix–helix interactions that are shown as van der Waals spheres (ethylene glycol spacer in gray, isobutoxy chains in position 4, 6, and 7 of each four subhelix in blue, green, and red, respectively; numbering begins at each of the four N termini). Residues in position 4 clearly lie at the helix–helix interface. d) Two views of the folded structure of **1** in which residues have been omitted to show the perpendicular arrangement of the right-handed helix (gray) and of the left-handed helix (red). e) For the purpose of size comparison, the crystal structure of the scorpion toxin protein is shown at the same scale as all other structures in this figure.[17] The scorpion toxin protein contains 65 amino acids (7.1 kDa, $C_{311}H_{423}N_{86}O_{95}S_8$) and is 15% smaller than **1**.

helix bundles showed that handedness is determined by chiral centers on each amino acid and interactions between helices are probed by the onset of helicity.[21]

Furthermore, we observed that helix–helix interactions and the preference for the *meso* species of **1** much depends on the solvent. A complete shift of equilibrium occurs in aromatic solvents in which the racemic P-P/M-M form dominates (70% compared with 7% in $CDCl_3$) at the expense of the *meso* P-M form (30% compared with 93% in $CDCl_3$). Upon dissolving crystals grown in chlorinated solvents into deuterated toluene or benzene, or crystals grown in an aromatic solvent into $CDCl_3$, the slow interconversion of each species into the other can be monitored and be fitted to a single exponential decay curve. Interestingly, helix inversion is faster in toluene (characteristic time of 3.5 h) than in chloroform (characteristic time of 10 h) at 25 °C. The exact mechanism by which the solvent influences the proportions between the two species and the rates at which they interconvert is not well established. However, the presence of a dichloroethane molecule in a cavity between the two helices in the crystal structure of **1** suggests that solvent is intimately associated with helix–helix interactions (see the Supporting Information).

This study demonstrates that is possible to design, synthesize, and characterize folded molecules that, by their size (> 8 kDa) and structural complexity, mimic the tertiary fold of a small protein yet only consist of non-natural units. Additionally, it shows that purposely not controlling helical handedness within secondary motifs allows the observation of tertiary interactions between helical modules through induction of helix–helix side-by-side handedness.

Although the abiotic proteomorphous foldamer presented herein is the first of its kind, it has a large potential for development. Its structure is amenable to specific changes to promote attractive interactions between the helices. For example, tuning the spacer length and level of branching, the size of each helical module, and the nature of the side chains may allow us to define binding sites and position catalytic

groups within the folded structure or onto its exterior, which may be decorated so as to interact over large surfaces with specific targets.

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