

Duplex Foldamers from Assembly Induced Folding

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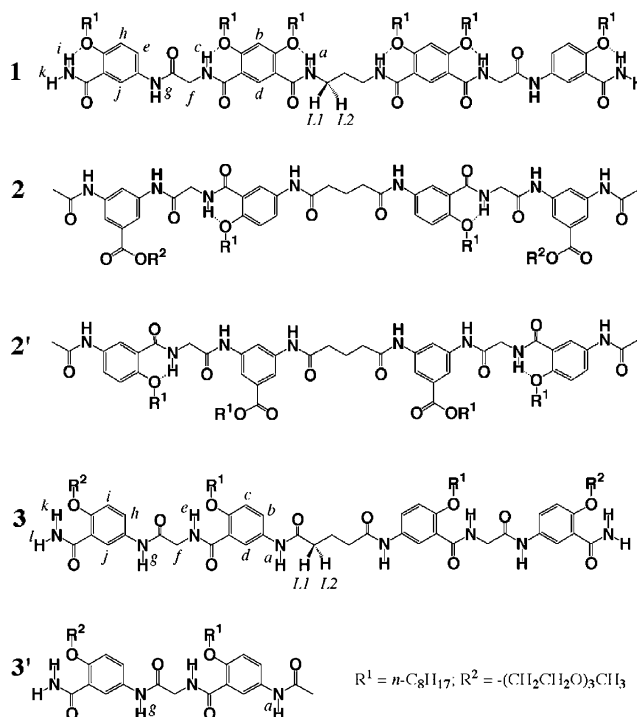
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The association of biomacromolecules is often accompanied by conformational changes. In many cases, an otherwise poorly defined structure folds into a well-defined conformation upon associating with another molecule. Such an assembly coupled folding process is best exemplified by the formation of double-stranded DNA: The zippering of two flexible single strands leads to the formation of a well-defined, double helical structure. Association-induced folding is also found in DNA-binding^{1,2} and protein–protein^{3,4} interactions. To control intermolecular associations, various strategies have been described. Most of these strategies are based on the design of molecular modules carrying linear arrays of hydrogen bond donors (D) and acceptors (A).^{5–8} The design of modules for directing molecular association has focused on the preorganization or rigidification of the corresponding molecular components.^{9–15} On the other hand, the development of unnatural folding oligomers, or foldamers, with well-defined secondary structures, has focused on constructing backbones that undergo specific intramolecular interactions.^{16–18} Few unnatural systems that combine specific intermolecular association with subsequent formation of folded structures are known.^{19,20} In this paper, we would like to report oligoamide strands that not only sequence-specifically associate (self-assemble) into H-bonded duplexes but also adopt folded conformations in the final assembled architectures.

Single strand **2**, formed by linking two 4-H-bonding DDAD units in a head-to-head way, was originally designed to associate with single strand **1**, consisting of two AADA units linked in a head-to-head fashion. If strands **1** and **2** adopted extended conformations, they would only partially overlap with each other due to their unsymmetrical 4-H-bonding units, leading to the formation of noncovalent copolymers. On the other hand, strand **2'**, if it adopted an extended conformation, would be completely complementary to an extended strand **1**, leading to the formation of an 8-H-bonded, extended duplex. Similar to the design of **1** and **2**, linking two self-complementary ADAD units in a head-to-head fashion leads to oligoamide strand **3**. Extended strand **3** would self-associate into homopolymeric aggregates.

Examining a 1:1 mixture of **1** and **2** by one-dimensional (1D) ¹H NMR (CDCl₃, 500 MHz) indicated that these two strands indeed associated via H-bonds.²¹ The signal of proton *g* appeared at 9.66 ppm in the spectrum of single strand **1** (1 mM, 23 °C in CDCl₃). In contrast, proton *g* moved to 10.03 ppm in the spectrum of the 1:1 mixture of **1** and **2** (1 mM, 23 °C in CDCl₃). When the 1D ¹H NMR spectrum of **3** was compared to that of **3'** whose ADAD array was shown to dimerize into a self-complementary, 4-H-bonded duplex,¹³ the signal of proton *a* of **3** appeared at 10.59 ppm, while that of proton *a* of **3'** appeared at 9.89 ppm. These results suggest that the molecules of **3** associated through much stronger H-bonding interactions than those of **3'**. This was not expected because if the



molecules of **3** adopted an extended conformation and thus associated in a staggered fashion as required by its ADAD units, the strength of its intermolecular H-bonds would be similar to those of the 4-H-bonded self-dimer **3'·3'**.

Further 1D ¹H NMR experiments confirmed that the intermolecular H-bonding interactions between **1** and **2** and among the molecules of **3** were very strong. At 1 mM, the aniline NH signals of the 1:1 mixture of **1** and **2** and those of **3** showed insignificant shifts with increasing percentage (up to 20%) of DMSO-*d*₆ in CDCl₃. Diluting a sample of **1** and **2** (1:1), or that of **3** in a mixed solvent containing 10% DMSO-*d*₆ (down to 10 μM), in CDCl₃ did not lead to any apparent change in the chemical shifts of the aniline NH signals that are involved in intermolecular H-bonding. The stabilities of the H-bonds of **1** and **2**, and those of **3**, are in sharp contrast to those of the corresponding 4-H-bonded (i.e., DDAD/AADA and DADA/ADAD) duplexes,^{13,22} whose association constants were previously shown to be in the 10⁴ M⁻¹ range in chloroform and could be easily determined by NMR dilution experiments.

The appearance of the 1D ¹H NMR spectra of the 1:1 mixture of **1** and **2**, and **3** was not consistent with the formation of polymeric aggregates: several sets of well-resolved, sharp signals were observed.²¹ Furthermore, in CDCl₃, the signal of the methylene protons *L1* and *L2*, which appeared at 3.54 ppm as a singlet in the spectrum of single strand **1**, split into two peaks (3.11 and 4.55

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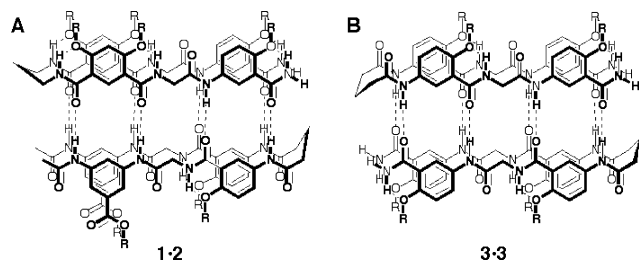


Figure 1. Illustration of duplex foldamers (A) **1·2** and (B) **3·3**.

ppm) in the spectrum of the 1:1 mixture of **1** and **2**. The 1D ^1H NMR spectrum of **3** demonstrated similar features: in CDCl_3 , the methylene protons *L1* and *L2* split into two signals at 2.50 and 2.81 ppm, respectively; in $\text{DMSO}-d_6$, on the other hand, methylene protons *L1* and *L2* appeared as one signal (a triplet at 2.36 ppm). These results suggest that the rotational freedom of the trimethylene linker of **1** or **3** was restricted when **1** and **2**, or the molecules of **3**, associates through intermolecular H-bonds. The best explanation for these observations is that, instead of being extended, strands **1** and **2**, and **3**, adopt folded conformations when associated into their corresponding H-bonded assemblies.

Cross-strand NOEs from 2D NMR (NOESY) studies indicate that the 1:1 mixture of **1** and **2**, and the self-complementary **3**, sequence-specifically associate through their 4-H-bonding units.²¹ This and the above results, combined with the facts that the ^1H NMR spectra of **1** and **2**, and that of **3**, contained the same number of aniline NH signals as the corresponding 4-H-bonded duplexes, suggested that the two 4-H-bonding units in **1**, **2**, or **3** were indistinguishable in their H-bonded assemblies. These 4-H-bonded units acted cooperatively, leading to enhanced intermolecular H-bonding strengths between **1** and **2** and between the molecules of **3**. In other words, a molecule of **1** mostly likely associates with another molecule of **2** through two 4-H-bonded units, leading to the formation of an 8-H-bonded heterodimer **1·2**. Similarly, single strand **3** very likely self-dimerizes into a homodimer **3·3**.

Examining the H-bonding sequences of **1** and **2** leads to only one possibility for the formation of an 8-H-bonded dimer: **1** and **2** can only adopt the folded (stacked) conformations as shown in Figure 1A. On the basis of the same analysis, the molecules of **3** can associate into a homodimer only by adopting the similar folded conformation (Figure 1B). This model of self-assembling foldamers is fully consistent with the above experimental results.

The formation of these folded duplexes has a strict sequence requirement: a single strand such as **2'** whose two 4-H-bonded units are linked in a "wrong" way should not form an 8-H-bonded, folded duplex with **1**. Indeed, the 1D ^1H NMR spectrum of the 1:1 mixture of strands **1** and **2'** was not consistent with a discrete species: poorly defined, broad ^1H NMR signals, which prevent further 2D NMR studies, were observed.²¹ Thus, linking the same 4-H-bonded units in different ways can result in different molecular strands that assemble into completely different architectures.

To confirm the dimeric nature of the aggregates formed by **1** and **2**, and by **3**, vapor pressure osmometry (VPO) measurements²¹ were carried out (room temp in CHCl_3). Using polystyrene as molecular weight standards and at the concentration range of 15–50 mM, apparent molecular weights of $2862 \pm 6\%$ corresponding to a heterodimer **1·2** and $2590 \pm 5\%$ corresponding to homodimer **3·3** were obtained.

The formation of discrete dimers by **1** and **2**, and by **3**, was further confirmed by mass spectrometry.²¹ The ESI spectrum showed that for the 1:1 mixture of **1** and **2**, the doubly charged $[\mathbf{1} + \mathbf{2} + 2\text{Cl}]^{2-}$ ($m/z = 1454.3$) is the most abundant ion detected. For **3**, $[\mathbf{3} + \mathbf{3} + \text{Cl}]^-$ ($m/z = 2636.3$), corresponding to the self-

dimer **3·3**, and $[\mathbf{3} + \text{Cl}]^-$ ($m/z = 1335.6$), corresponding to single strand **3**, were the two major ions detected.

In summary, we have designed and discovered molecular duplexes whose formation is characterized by the combination of sequence-specific self-assembly and folding of the component strands. Our evidence indicates that an initial assembly process is followed by the folding of the component strands, which leads to the final folded, dimeric species. Such assembly induced folding systems are reminiscent of biomacromolecules such as duplex DNA and DNA-recognizing proteins. The assembled and folded structures are stabilized by both H-bonding and aromatic stacking interactions. The modular nature of this system allows the convenient design of longer oligomers which should assemble and fold into duplex foldamers with multiple stacks of H-bonded units. Similar to the stacking of Watson–Crick base pairs in duplex DNA, the stacking of the H-bonded units in our duplex foldamers not only provides additional stabilization to the assembled structure but also shields the H-bonds away from solvent molecules. On the basis of this novel assembling and folding motif, sequence-specifically formed duplex foldamers that are stable in competitive media such as aqueous solution should become available.

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Supporting Information Available: Synthetic procedures, NMR spectra, VPO experiments, MS spectra, and other details (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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