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Hybrid Diphenylalkyne-Dipeptide Oligomers Induce Multi-Strand β-Sheet Formation**

Jonathan E. Ross, Peter C. Knipe, Sam Thompson* and Andrew D. Hamilton*

This paper is dedicated to Professor Sir Alan R. Battersby FRS in celebration of his 90th birthday.

Abstract: Functionalized diphenylalkynes provide a template for the presentation of protein-like surfaces composed of multi-strand β-sheets. The conformational properties of three-, four-, and seven-stranded systems have been investigated in the solid- and solution-state. This class of molecule may be suitable for the mediation of therapeutically relevant protein-protein interactions.

There is much current interest in the design of synthetic oligomers that mimic the recognition and folding properties of secondary structural domains on the surface of proteins.[1] These foldamers[2] have the potential to bind complementary protein targets and modulate their interactions with other proteins.[3] Considerable progress has been made in the design of various unnatural amino acid oligomers that are controlled by intramolecular hydrogen bonding interactions. In particular, β-peptides,[4][5] γ-peptides,[6] and sequences containing aminoquinolines,[7] anthranilamides,[8][9] and dialkylamino acids[10]–[14] have been shown to adopt helical conformations of varying pitch and dimensions. The β-sheet is also an important secondary structural protein element, motivating researchers to develop a range of synthetic templation strategies for two-stranded systems.[12]–[18] Macrocyclic peptides have frequently been used to stabilize the formation of sheet-like structures and have provided insights into their supramolecular properties.[19][20] However, there are a limited number of templates that take advantage of intramolecular hydrogen bonding to stabilize structures formed of three or more strands.[21] These include turn mimics that incorporate D-Pro,[22][23] oligomers of α/β-amino acids,[24][25] and the self-assembly of two-stranded β-sheet mimics[26] and macrocycles[27][28] into larger complexes.

Nature provides many examples of extended and stable sheet structures, as both components of soluble globular proteins and insoluble aggregates such as amyloid fibrils. These provide two functionalized surfaces, formed from the side-chain residues projected above and below the folded frame. The β-meander protein is an example of a super-secondary protein element formed of several short peptide strands occupying extended conformations, and is itself a common component of larger structures such as the β-barrel (Figure 1A).[29]

Figure 1. Multi-stranded β-sheets are key components of higher-order protein structures, and present large surface areas for interfacial protein-protein interactions: (A) the β-repeat (also known as a β-meander), exemplified by Koido’s protein self-assembly mimics (PDB 2HKD).[29] (B) extended β-meander motifs based upon the diphenylalkyne linker as a turn motif.

References:
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Our interest lay in creating oligomeric structures that stabilize extended β-sheet-like structures, with programmable surfaces projected above and below a common plane. Di-functionalized diphenylalkynes were introduced by Kemp as a non-peptidic anti-parallel β-turn inducer, and have been extended by Spivey to display peptide loops, and by our group to template bidirectional β-sheet formation. The motif also has the potential to stabilize large surfaces as a modular unit of a foldamer, in which multiple peptide strands are linked in an anti-parallel fashion (Figure 1B).

To explore the folding propensities of a series of anti-parallel two-peptide strands linked via diphenylalkynes we synthesized I on a multi-gram scale from readily available starting materials (see Supporting information). Alanine was selected as the amino acid for this proof-of-principle system due to its propensity to form NHs (labeling of NHs is shown in Figure 2, full data in the supporting information).

The Cα-Hs of mimics 5 and 6 show a pronounced downfield shift in the 1H-NMR spectra when compared to single-stranded control molecules; consistent with increasing β-sheet character. The non-terminal amino acids showed shifts of 0.78 – 1.04 (5) and 0.81 – 1.21 (6) ppm relative to the controls. Larger values in the latter case are likely due to the enforcement of greater sheet character by increased inter-strand H-bond cooperativity. The corresponding range for terminal amino acids was 0.12 – 0.32 ppm – indicative of less pronounced β-sheet structure (Figure 3a). The assignment of a secondary structural propensity score using the Foreman-Kay method allows contributions from different nuclei to be weighted according to their sensitivity to α- and β-structure. Mean β-sheet scores in excess of 94.0 and 99.8 % for three- (5), and four-stranded mimics (6) respectively, were consistent with significant β-sheet character, whilst control molecules 8-10 showed minimal β-sheet propensity (between 0 and 31.7 %, see supporting information).

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Figure 3. (a) Secondary structural behaviour: $^1$H NMR chemical shift values of the $\alpha$-hydrogens in (i) three-stranded 5; and (ii) four-stranded 6 mimics relative to single-stranded controls (8-10). Residue numbering indicated in Figure 2. (b) Amide $^1$H NMR behavior upon titration of (CD$_3$)$_2$SO into a CDCl$_3$ solution of (i) three-stranded 5; and (ii) four-stranded 6 mimics. Labeling of NHs indicated in Figure 2. 10 mM in 500 $\mu$L of CDCl$_3$; (CD$_3$)$_2$SO added in portions as follows: 10, 20, 20, 50, 50, 50 $\mu$L.

H$_b$/H$_g$ (of 5) and H$_b$/H$_j$ (of 6) shifted markedly downfield, suggesting greater exposure to solvent, whereas the other hydrogens shifted to an equal or greater degree upfield – behaviour consistent with adoption of a well-folded multi-strand sheet conformation (Figure 3b).$^{[43]}$ For mimics 5 and 6 a quantitative comparison of $^1$H NMR chemical shifts of the non-terminal amide NHs for spectra acquired in neat CDCl$_3$ and neat (CD$_3$)$_2$SO gave a high level of confidence for hydrogen-bonding networks consistent with well-folded multi-strand sheet conformation.$^{[44]}$ The hydrogens corresponding to the C- and N-terminal amino acids (H$_e$/H$_h$ for 5, H$_e$/H$_k$ for 6) gave intermediate values indicative of fraying, whilst the chemical shift change of the external hydrogens (H$_b$/H$_g$ for 5, H$_b$/H$_j$ for 6) suggested an absence of intramolecular H-bonding.$^{[45]}$

X-Ray crystallography of diffraction-quality single crystals of 5 was consistent with a structure formed of three intramolecularly hydrogen-bonded strands. In agreement with the solution-phase work, a network of six intramolecular hydrogen bonds places three side-chains above the plane and three below (coloured orange and purple, Figure 4a/b).$^{[46]}$ Average dihedral angles of $(\phi, \psi) = (−135 \pm 32^\circ, 136 \pm 37^\circ)$ are in excellent agreement with those of a canonical $\beta$-sheet $(−135, 135)$, see supporting information.$^{[47]}$

With solid- and solution-phase data consistent with the three- and four-stranded mimics adopting multi-stranded $\beta$-sheet conformation we sought to explore the preparation of extended systems with a greater number of strands. Further iteration of the synthetic route afforded seven-stranded meander mimic 7, with protected termini allowing further extension to larger surfaces (Figure 4c/d).$^{[38]}$

Figure 4. X-ray diffraction structure of three-stranded mimic 5: (a) top-; and (b) side-elevation. Side-chains highlighted in orange and purple (opposite face), diphenylalkyne turns in maroon, tert-butyl protecting groups in grey, dotted red lines represent hydrogen bonds, some hydrogens omitted for clarity. Mimicry of a seven-stranded $\beta$-meander 7: (c) structure; (d) schematic representation; (e) circular dichroism mean residue ellipticity $[\theta]$ of three- (5), four- (6), and seven-stranded (7) mimics relative to single-stranded control molecule (9); averages of ten acquisitions, 100 $\mu$M in trifluoroethanol.
Circular dichroism spectra of three- (5), four- (6), and seven-stranded mini-protein mimic (7) showed maxima at 253 nm and minima at 228 nm, consistent with β-sheet formation, whereas single-stranded control (9) gave a spectrum characteristic of random coil behavior. Maxima at 255 nm are due to chiral perturbations of the 2-amino-2′-diphenylalkyne chromophore (Figure 4e).[31][32]

In conclusion, we have shown that several short peptide strands may be linked in an anti-parallel arrangement by diphenylalkynes to give large surfaces presenting side-chains on two faces. The approach provides a template for the stabilization of multi-strand β-sheets and may find use in mediating protein-protein interactions. Work is underway to prepare extended systems containing longer sequences of amino acids per strand, and to incorporate hydrophilic side chains for functional studies in aqueous media.

**Keywords:** Peptidomimetic · foldamer · secondary structure · protein-protein interaction · β-sheet · protein surface

[17] Meander compounds 5, 6 and 9 exhibited sharp, well-dispersed 1H NMR spectra in CDC3d that were invariant across at least three orders of magnitude of concentration, consistent with no aggregation occurring, see the supporting information.
[24] Abraham “A”-values evaluate intramolecular H-bonding as follows: < 0.05 – strong, > 0.16 – absent. Intermediate values are indicative of an ensemble in which there is H-bond sampling. See the supporting information for calculation of the NHS in 5 and 6.
[25] CCDC 1049256 (5) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
[26] The relatively large errors in these values are due to fraying of the C- and N-terminal amino acids vide supra.
β-Sheets

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Hybrid Diphenylalkyne-Dipeptide Oligomers Induce Multi-Strand β-Sheet Formation

Multi-stranded β-sheets e.g. the β-meander

Strands linked by flexible loops

This work:
Extended β-surfaces stabilized by diphenylalkyne linkers

Functionalized diphenylalkynes provide a template for the presentation of protein-like surfaces composed of multi-strand β-sheets. The conformational properties of three-, four-, and seven-stranded systems have been investigated in the solid- and solution-state. This class of molecule may be suitable for the mediation of therapeutically relevant protein-protein interactions.