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Computational Design of a Self-Assembling β-Peptide Oligomer

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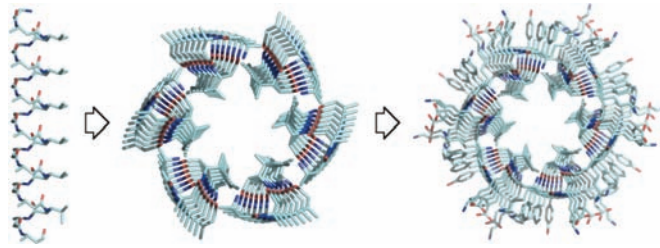
ABSTRACT



The first computationally designed self-assembling oligomer consisting of exclusively β-amino acids (βAAs) is presented. The packing of a β-3_{14} helix into coiled-coils of varying stoichiometries as a function of amino acid sequence is examined. β-Peptides with hVal repeating every third residue in the sequence appeared to have a strong propensity to pack into hexameric bundles. The designed sequence was synthesized and characterized with CD spectroscopy, NMR, and analytical ultracentrifugation, suggesting that the peptide adopts a well-folded hexameric structure.

Recently, there has been progress in the studies of the β-peptide class of foldamers.[1−4] The principles required for stabilization of secondary structure have been developed, and the design of protein-like tertiary structures is becoming feasible. Gellman and co-workers have studied the effects of alternating α and β amino acids (AAs)[5−9] and have established new rules regarding the stability and folding of these hybrid peptides. Zuckermann and Dill et al. have constructed nonbiological peptoids (*N*-alkyl-polyglycines) that assemble into water-soluble oligomeric bundles using amphiphilic sequence patterning.[10,11] Schepartz et al. recently demonstrated that sequence patterns similar to those found in αAA coiled-coils, adapted for βAA, allowed synthesis of an octameric β-peptide bundle.[12] Using the crystal structure of this bundle, the octamer was successfully converted into a tetramer by the inclusion of a linking loop region,[13] approaching natural protein-like properties.

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Thus, these previous studies have achieved considerable success by considering relatively coarse-grained concepts such as amphiphilicity and hydrophobic sequence patterning. More fine-grain computational methods should allow additional control, as recently demonstrated in the area of protein design. For example, computational methods have enabled design of new enzyme-like proteins,[14,15] sequence-specific transmembrane helix-binding peptides,[16] metalloproteins,[17−20] and self-assembling materials.[21,22]

In this work, we developed a new computational design algorithm, NAPOLI (Non-natural Automated Protein or LIgand), that accommodates a variety of backbones, secondary structure parametrizations, rotamer libraries, and energy functions. The name also recognizes early contributions to foldamer design made in Naples.[23] NAPOLI allows the use of arbitrary backbones (α, β, γ amino acids, cyclic structures, etc.) by indicating a branch point, where a variety of R-group atoms can be substituted.

The library of substitutable R-groups contains the 20 α-amino acid side chains as well as a subset of commercially available C_β-substituted derivatives. CHARMM[24,25] parameters for these atoms are applied at runtime. Side chain geometries are selected from either backbone-dependent or -independent rotamer libraries,[26,27] including one specifically calculated for various βAA helical structures.[28] NAPOLI allows users to select from a variety of energetic terms and weights, including different treatments of van der Waals interactions, solvation, and electrostatics.

Computational Design. Design of coiled-coils has played an important role in development of protein design and computational algorithms. Similarly, here we seek to design homo-oligomeric β-peptide coiled-coils. We used a variation of the Crick formula[29] to transform ideal β-3_{14} helices into radially symmetric parallel coiled-coils with different core residues (Figure 1).

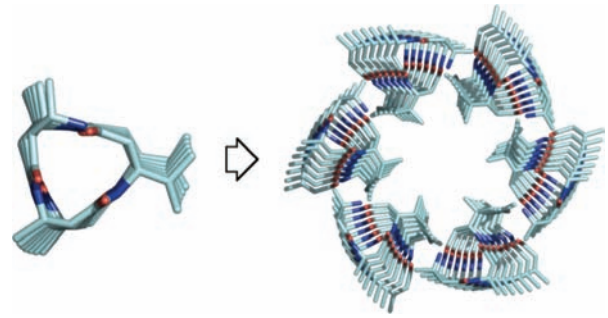

Figure 1. Scaffold design. From a β^3hV-peptide helical scaffold to a β^3hV hexameric bundle with optimized local helix parameters.

The packing of poly-β^3 homoAlanine (hA), poly-β^3 homoValine (hV), and poly-β^3 homoIsoleucine (hI) was examined. These sequences were modeled into different stoichiometries. The hA and hV sequences showed particularly good packing. The interhelical Lennard-Jones[30] and electrostatic energies on a per helix basis were systematically evaluated as a function of a bundle radius, helix phase, and supercoil pitch (Table S5, Supporting Information). The results were ranked by normalized interhelical interaction energy (energy per helix relative to the minimal energy computed for the dimer) (Figure 2, Table S6, Supporting Information). hV showed a particularly strong minimum at

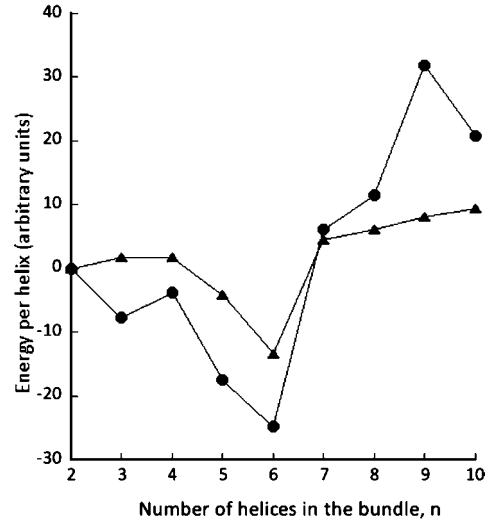

Figure 2. Interhelical packing energies of the poly-β^3 homoAlanine (hA, triangles) and poly-β^3 homoValine (hV, circles) bundles as a function of the bundle size *n*. The values represent the difference between the energies of the monomer and the packed *n*-mer and are normalized to those of the corresponding dimers that represent the minimal entity for a coiled-coil.

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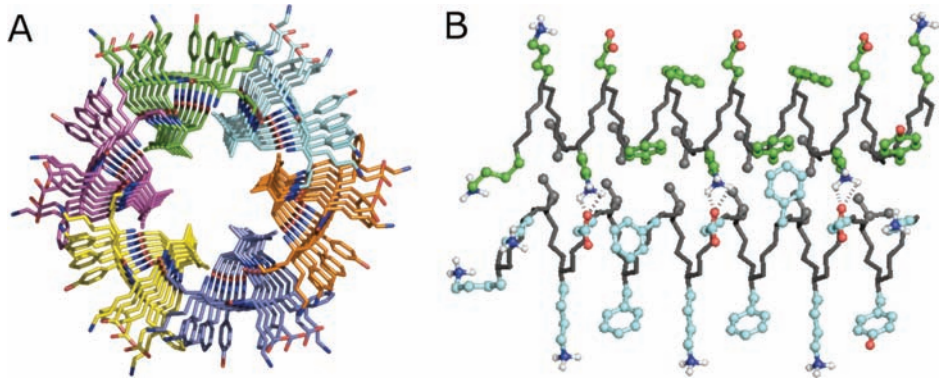

Figure 3. Final modeled β-peptide bundle. (A) N-terminal view of the designed bundle; chains shown in different colors. (B) Side view of the bundle showing packing of hydrophobic residues and designed electrostatic interactions. The backbone and the hV cores are shown in gray.

$n = 6$, associated with tight packing of every third hV side chain in the core. This finding suggested that β-peptides with hV in the core could show good geometric specificity for this hexameric association state.

To complete the design of a water-soluble bundle, the optimized hydrophobic hV core was fixed, while the identity and conformations of exterior residues were allowed to vary. We introduced water-solubilizing residues and stabilizing interhelical salt bridges at these positions. While it is possible to stabilize the 3_{14} helix through electrostatic interactions between residues one turn apart in the helix, such intrahelical interactions would not provide specificity for the desired tertiary structure versus other possible associations. Thus, to specifically stabilize the desired bundle rather than just the secondary structure, neutral residues were alternated with charged residues along a given face of the helices (Figure 3). Specifically, charged and neutral residues alternate at positions i, i + 3, i + 6, etc. in the sequence. A variety of sequence patterns consistent with this restraint were examined (Table S7, Figure S4, Supporting Information).

Experimental Characterization. The final sequence (hKhKhVhKhE-hVhFhFhVhK-hEhVhFhFhV-hKhEhVhY-hK), nicknamed **betaVhex**, contains C- and N-terminal β^3-homoLysine (hK) residues to improve solubility. To examine the role of the hydrophobic hV residues, we have also prepared a sequence with hA in the core: **betaAhex** (hKh-KhAhKhE-hAhFhFhAhK-hEhAhFhFhA-hKhEhAhYhK). Furthermore, we have synthesized a corresponding α-peptide, **alphaVhex** (KKVKE-VFFVK-EVFFV-KEVYK). Circular dichroism (CD) spectroscopy data show that the designed β-peptide **betaVhex** adopts a 3_{14}-helical conformation in a concentration-dependent manner as expected for a self-associating system in which secondary structure formation and association are thermodynamically linked. At high peptide concentrations (more than ca. 360 μM, Figure 4) the spectrum of **betaVhex** shows a minimum at 211 nm, consistent with that reported for β-3_{14} helices.[31−36] The

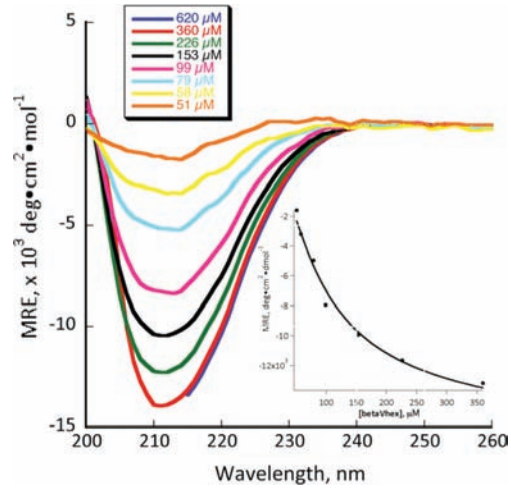

Figure 4. Mean residue ellipticity (MRE) of **betaVhex** at indicated concentrations in an aqueous environment at 20 °C. The coincidence of the spectra at 360 and 620 μM suggests saturation of helical content between the two concentrations. Inset: MRE_{215} (points) as a function of **betaVhex** concentration fit (soild line) to a monomer−hexamer equilibrium model.

concentration dependence of the MRE at 211 nm conforms well to a highly cooperative monomer−hexamer equilibrium with the free energy of association of 27.8 kcal/mol•hexamer (1 M standard state) (Figure 4). By contrast, the negative controls **betaAhex** as well as **alphaVhex** displayed CD spectra more typical of unordered polypeptides over the same concentration range (Figures S5 and S6, Supporting Information).

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The hexameric oligomerization state of **betaVhex** was confirmed by analytical ultracentrifugation (AUC). Equilibrium sedimentation data were well described by a monomer−hexamer equilibrium model. The free energy of hexamerization of 26.5 kcal/mol•hexamer obtained from the fit (Figure S7, Supporting Information) is in very good agreement with the CD data (27.8 kcal/mol•hexamer). Attempts to fit the data to other monomer−*n*-mer models ($n = 4, 5, 7, 8$) resulted in a poorer fit, confirming the hexameric state and the cooperativity of the assembly.

The thermal stability of the designed β^3-peptide was monitored using CD spectroscopy. A sigmoidal melting curve was observed indicative of a cooperative unfolding process. The designed bundle demonstrated an enthalpic unfolding transition, similar to other recently published β^3-peptides[12] (Figures S8 and S9, Supporting Information). The T_m (>96 °C) indicates a highly stable, well-folded β-peptide when compared to α-peptides (ubiquitin's $T_m = 90$ °C).

The conformation of **betaVhex** was also investigated by NMR. The 1D ^{1}H NMR spectrum of **betaVhex** shows (Figure S10, Supporting Information) peptide amide resonances well dispersed over the range of ca. 1.5 ppm, suggesting formation of a distinct quarternary structure that provides a differentiated electronic environment, similarly to previously reported β-peptide bundles.[12,37]

In summary, we have extended computational methods originally developed for α-peptides and proteins to β-peptides. Interestingly, while conformational search algorithms for α-peptides with core valines showed a strong preference for a trimeric association,[38,39] 3_{14} helical β-peptides showed optimal packing with six monomers. This reflects differences in shape and axial cross-sectional area between α- and β-peptides.

To test this computational result, we designed peptide **betaVhex**, which was indeed found to form a hexameric coiled-coil structure. The difference in the secondary structure has a large influence on the preferred association state for α- vs β-peptides. A tendency for higher-order association was also observed by Schepartz et al. who synthesized β-peptides with hydrophobic sequence repeats patterned after those seen in 2- and 3-stranded α-helical coiled-coils.[12] In related studies, Gellman et al.[9] have systematically introduced βAAs into α-peptide coiled-coils. Two interesting features were observed in this work. First, βAA residues could be inserted into an α-helix with minimal perturbation of its geometric parameters, and second, the packing patterns observed for the βAA core are quite different from those observed in α-helical coiled-coil quarternary structures. Therefore, we expect that utilizing intrinsic properties of βAA with this computational platform will pave a way to novel functional β-peptide-based molecules.

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Supporting Information Available: Detailed description of the NAPOLI platform as well as experimental procedures for preparation and characterization of peptides, along with CD and AUC data. Requests for NAPOLI should be sent directly to S.J.S. at scott.shandler@longevitybiotech.com. This material is available free of charge via the Internet at http://pubs.acs.org.

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