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The Promiscuity of β-Strand Pairing Allows for Rational Design of β-Sheet Face Inversion

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The β-sheet is a fundamental structural element in proteins, and it is being exploited as a building block of nanomaterials.[1−3] A β-sheet consists of multiple β-strands that are linked by intervening segments such as turns and loops. The dominant role of the main-chain H-bonds in determining the β-sheet topology has recently emerged.[4,5] Although side chains are certainly important in providing factors that control β-sheet stability,[6] maximizing the main-chain H-bonds appears more important for determining β-strand register than side chain interactions. In other words, the main-chain edges of β-strands are naturally "sticky" and primed to zip up to form a β-sheet.[7]

The largely sequence-independent nature of main-chain H-bond formation suggests an interesting possibility in the design of β-sheet-based nanomaterial. The "β-meander" motif is one of the simplest antiparallel β-sheet architectures in which adjacent β-strands are connected with a turn, and thus the polypeptide takes on an overall zigzag topology (Figure 1a). This motif is an attractive template for constructing large "β-ribbon" nanostructures.[1] The dominant role of main-chain H-bonds in specifying β-sheet architecture suggests that it may be possible to rotate a large segment of a meander β-sheet relative to the rest by simply adding or removing a basic unit consisting of one strand and a turn in the middle of the β-sheet. Clearly, such perturbations would invert the face of one part of the β-sheet relative to the rest and thus enable large-scale changes in the conformation and the surface properties of the overall β-sheet (Figure 1a). They also would allow one to control the relative orientation of moieties attached on the surface of the β-sheet segments. The capability to rationally and precisely control these global and long-range conformational changes would significantly expand the scope of nanomaterial design based on β-ribbon-type structures.

Despite the simplicity of the concept and its interesting potentials, to our knowledge the possibility of inverting a large β-sheet segment has not been experimentally demonstrated, perhaps due to a lack of a suitable model system. β-Ribbon nanomaterials would not be a good system to test such design, because of the extreme difficulty in characterizing their atomic structures. Most of β-sheets in natural proteins are involved in extensive interactions through a hydrophobic core in addition to interactions between adjacent β-strands within the same β-sheet, and each β-strand often has amphiphilic character. Consequently, a deletion of an entire β-strand in the middle of a β-sheet would most likely lead to local structural adjustment with small global changes or destroy the core packing and denature the protein. Duplications of a strand-turn segment in T4 lysozyme resulted in displacement of a strand, but the perturbations were accommodated by looping out of an excess segment with minimal global structural changes, indicating the dominant role of core packing in specifying the global fold of amphiphilic β-sheets.[8]

Outer surface protein A (OspA) from *Borrelia burgdorferi* has served as an excellent model system for β-sheet design. It contains an unusual, single-layer β-sheet (SLB) with the β-meander topology that is exposed to the solvent on both faces. Despite its no association with a hydrophobic core, the SLB segment is highly stable in the context of the OspA protein. OspA provides a unique opportunity to test the fundamental properties of an isolated β-sheet unit in the absence of complications due to long-range interactions. Importantly, the OspA system allows for comprehensive characterization of stability and high-resolution structures, thus allowing critical evaluation of the design.[6,9]

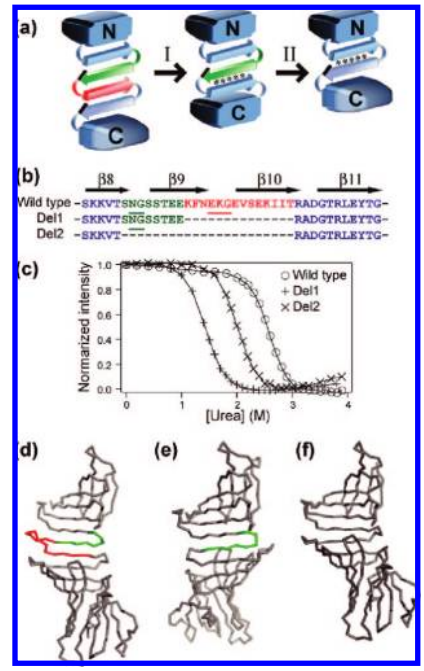

Figure 1. (a) Schematic representation of β-strand deletion. (I) Deletion of the red portion results flipping the β-sheet; (II) further deletion of the green portion restores the original orientation. Asterisks indicate non-native contacts. (b) The sequences of the deletion mutants at the SLB portion. (c) Urea-induced unfolding of the mutants. Normalized CD intensities are plotted as a function of urea concentration. (d−f) The crystal structures of the wild type, Del1 and Del2, respectively. The deletion parts are showing in red and green.

Here, we asked whether a β-sheet structure can be maintained when a large number of native contacts are removed and whether one can design a large-scale conformational transition of a β-sheet by exploiting the promiscuity of strand−strand interactions (Figure 1a). Specifically, we designed mutant SLBs by incrementally deleting a segment corresponding to an entire β-strand and a turn at the center of the SLB segment (note that the unit of manipulation here differs from that in our previous studies of hairpin insertions[10,11]). We used a mutant termed TR2 (ref 4) as the starting material, because it contains a more regularized turn than the wild type. For brevity, we will hereafter refer to it as "wild type".

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First, we deleted a 14-residue segment corresponding to strand-10 and β9/10-turn ("Del1", Figure 1b). Subsequently we produced another mutant by further deleting strand-9 and β8/9-turn (23 residues in total from the wild type; "Del2", Figure 1b). Because of the β-meander topology, Del1, which lacks strand-10 should create a mismatch in β-strand pairing between β-strands 9 and 11. If the newly exposed edges of these β-strands are indeed "sticky," the backbone H-bonds should be maximized between these β-strands and consequently a contiguous β-sheet should form in which the relative orientation of the two halves of the OspA molecule is inverted (Figure 1a). Otherwise, the sheet might be cut in halves connected with a disordered segment.

Del1 and Del2 were soluble and monomeric (data not shown). Their NMR spectra exhibited excellent peak dispersion, indicating the presence of a single, well-folded conformation in solution (Figure S1 in Supporting Information). Both mutants were substantially destabilized (Figure 1c). In particular, Del1 was destabilized by 6.1 kcal/mol, which is the largest destabilization among the OspA mutants we have constructed to date.[4,6] Still, the titration curve showed a clear cooperative transition with well-defined native baseline, a hallmark of a folded protein. The subsequent deletion of strand-9 recovered the stability by $\Delta\Delta G = -1.5$ kcal/mol, showing that the deletion of a β-hairpin is less damaging than a β-strand deletion, although the former removes more residues.

The crystal structures of Del1 and Del2 were determined at 1.80 Å (Del1) and 1.25 Å (Del2) resolutions, respectively. (Figure 1e,f; Table S1). Both terminal domains in these mutants had essentially identical structures to their respective counterparts in the wild type (Cα rmsd < 0.8Å). However, Del1 shows a large rearrangement in the relative orientation of the terminal domains. The deletion caused the segment from the point of deletion to the C-terminus to be rotated by ~180° about the axis perpendicular to the strand direction with respect to the N-terminal portion (i.e., an inversion of the β-sheet face), as our design intended (Figure 1d,e and Figure S2b). As a result, the spatial relationship between the two terminal domains was radically altered. For example, the distance between Cα atoms of Asp59 and Ser194 located on the opposite faces of the SLB in the wild type, was shortened by 22 Å in Del1. The newly formed β-sheet maintains the antiparallel, β-meander architecture (Figures 1e and 2). The number of the main-chain H-bonds is maximized in this new interface between the two β-strands, consistent with their dominant importance in determining β-sheet register.[4] The structure reveals that sheet inversion disrupts a continuous row of hydrophobic residues across one edge the SLB, rationalizing the large destabilization (Figure S2).

The two-strand deletion mutant, Del2, regained the interdomain orientation of the wild type (Figure 1f). Compared with the wild type, the interdomain distance was shortened by 9.4 Å, as expected for a deletion of two β-strands. Otherwise, Del2 showed minimal structural changes. The small structural and stability perturbations seen for Del2 can be attributed to the homology between strand 8 and 10 (K112/K135, V114/I137, T115/T138; Figure 2) that reduces the number of non-native contacts and restores the continuous hydrophobic row (Figure S2). The small impact of the hairpin deletion might have been expected from successful insertions of the 9/10 hairpin to the SLB,[9,10] which are conceptually similar to hairpin deletion. Together, these results demonstrate a remarkable level of malleability of the β-meander structure.

In summary, we showed the possibility of drastic redesign of β-sheets including unprecedented segmental face inversion of a large

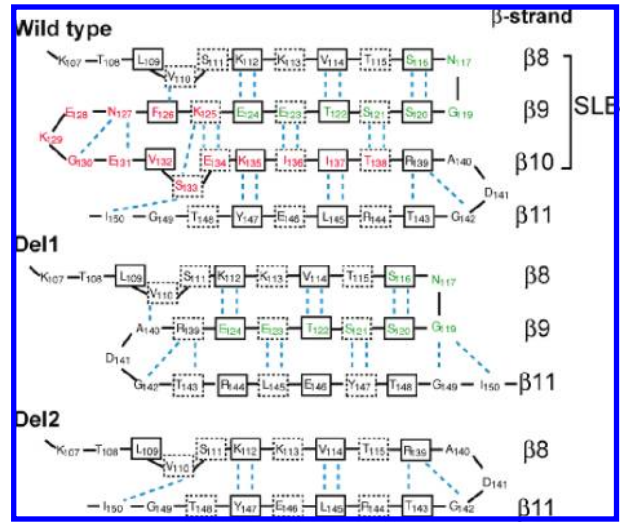

Figure 2. Schematic representation of the SLB segment. The main-chain H-bonds are shown in blue dashed line. Residues are colored in the same manner as in Figure 1.

β-sheet. Although our design principle was simple and did not take side chain interactions into account (Figure 2), the mutant SLBs folded as designed. The two globular "capping" domains probably serve as the templates to nucleate and maintain the structural integrity of the highly destabilized Del1 SLB. The success of our design suggests that β-sheet engineering based on the promiscuous nature of the β-sheet edge, maximizing main-chain H-bonds, and punctuating strands with strong turn motifs is effective in creating and controlling novel β-sheet topology. This principle may be applied to "bottom-up" design and control of nanomaterials based on β-rich peptide self-assembly, which can also be augmented with side-chain optimization.

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Supporting Information Available: Materials and methods, crystallography statistics, and supplementary figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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