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Synthesis and Single-Molecule Studies of a Well-Defined Biomimetic Modular Multidomain Polymer Using a Peptidomimetic β-Sheet Module

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One fundamental challenge in polymer science is to design polymers that have a combination of mechanical strength, fracture toughness, and elasticity.[1] These properties require different molecular mechanisms and, hence, are generally considered exclusive to each other.[2] Nature, on the other hand, has evolved many biopolymers, such as the skeletal muscle protein titin, cell adhesion proteins, and connective proteins in both soft and hard tissues, which have a remarkable combination of these three important properties.[3−7] Single-molecule nanomechanical studies have revealed that their combined mechanical properties originate from their unique modular multidomain structure, which appears to be a general mechanism used in nature to achieve advanced materials.[7] Inspired by nature, we intend to mimic this modular domain design in the pursuit of synthetic biomaterials with advanced properties. We have previously reported the design of polymers containing folded loops held by the strong hydrogen-bonding (H-bonding) motif, 2-ureido-4-pyrimidone (UPy).[8] Single-molecule force-extension studies revealed the sequential unfolding of loops as a polymer chain was stretched. The excellent correlation between the single-molecule and the bulk properties demonstrated our biomimetic concept of using a modular domain structure to achieve advanced polymer properties.

While demonstrating our biomimetic concept, the UPy system had a few limitations. The structure of the polymer had nonuniformity that arose from the polydispersed poly(tetramethylene oxide) loop and the different enchainment of the UPy units (head-to-head, head-to-tail, and tail-to-tail).[8] The UPy units could also randomly bind to each other within a chain or between different chains. Finally, the binding strength of UPy is not tunable. For further exploration of modular biomimetic materials with high strength and toughness, more uniform and higher-ordered polymer systems would be desirable. Herein, we report the use of a peptidomimetic β-sheet motif to construct a modular polymer that has a better-defined structure (Figure 1). The module in this system is composed of a β-sheet-like duplex that is connected at both ends with hydrocarbon loops. As a module is stretched, the force will shear the hydrogen bonds in the duplex, and the loops will be extended. After releasing the force, the double-closed loop topology should ensure the strands rebind to their original pairs.

We envision that this system should overcome the limitations of our previously reported UPy system. First, the double-closed loop (DCL) topology will enhance the possibility for each H-bonding unit to bind to its original counterpart, therefore, minimizing dimerization of nonadjacent units on the same chain or from different chains. Second, by choosing a monodispersed alkyl linker in the monomer synthesis, the loop size is monodispersed. Last, by choosing the sequence and adjusting the length, the binding strength between β-sheets can be easily tuned.

Among the many peptidomimetic β-sheets reported in the literature,[9] we chose the quadruple H-bonding duplex developed by Gong and co-workers for its simplicity in synthesis.[10] Construction of the DCL monomer began with the synthsis of the self-complementary H-bonding dimer **1**.[11] Covalent capture of the H-bonded dimer via ring-closing metathesis (RCM) provided the protected DCL monomer as a mixture of *E*/Z double bond isomers.[12] Hydrogenation reduced the double bonds and removed the benzyl protecting groups simultaneously, affording the DCL monomer **2** (Scheme 1). Polymerization reactions were performed by mixing equimolar amounts of the DCL monomer and 4,4'-methylenebis-(phenyl-isocyanate) (MDI) in chloroform at 45 °C. GPC analysis showed number average molecular weights for the DCL polymers as high as 89 000 g/mol (Scheme 2).

The DCL polymers were subjected to single-molecule force-extension experiments using atomic force microscopy (AFM) following the literature protocols.[8,13] Sawtooth patterns were consistently observed in the force-extension curves and are similar to those seen in both natural and synthetic modular polymers (Figure

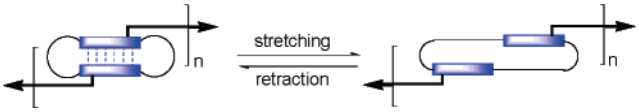
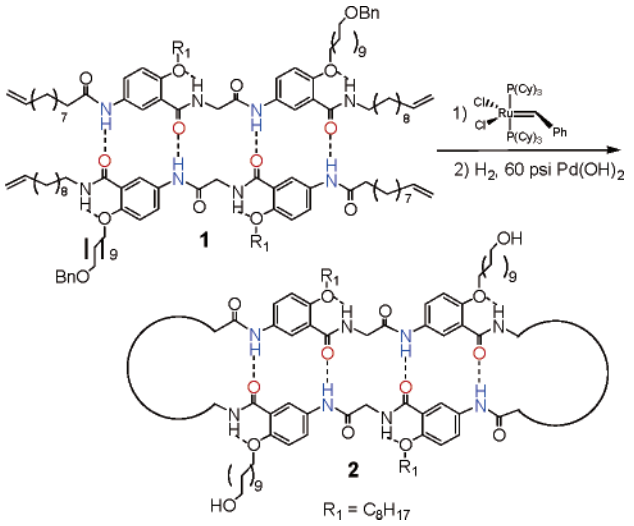
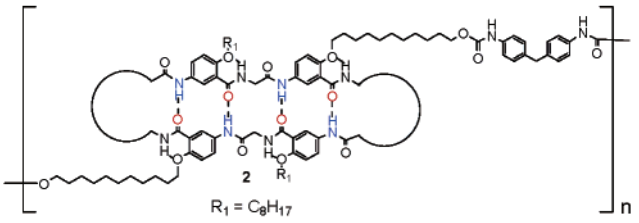


Figure 1. Schematic representation of domain unfolding of a modular polymer composed of double-closed loop monomers.

Scheme 1. Synthesis of the DCL Monomer by RCM



Scheme 2. Structure of the DCL Modular Polymer



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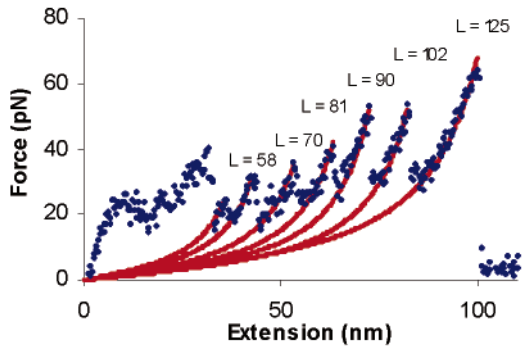

Figure 2. AFM single-molecule force-extension curve for DCL modular polymer. The solid line is the fitting with WLC model at a 0.55 nm persistence length (L is the contour length during stretching).

2). The patterns in the force-extension curves were more uniform for the DCL modular polymer than for the UPy system, which was attributed to its more uniform structure. The number of peaks in the stretching curves ranges from 2 to 7 with the most probable range being 3−5. The chain detaches from the surface typically after 60−120 nm stretching. The most probable peak force for unfolding each module is ∼ 50 pN (see the histograms in the Supporting Information). This force value is lower than that of our UPy modular polymers which had an unfolding force of ∼100−200 pN. This is consistent with the binding strengths of the two modules: the dimerization constants (K_{dim}) measured in chloroform for the UPy and the current peptidomimetic β-sheet units are $\sim 10^7$ and 10^4, respectively.[10,14]

The consistent sawtooth pattern in the single-molecule force-extension curves suggests the DCL monomer units unfold sequentially as the polymer is stretched. As the AFM tip picks up a single chain and is retracted from the surface, tension builds up on the bridging polymer chain. Once the force builds up enough to unfold one of the DCL units, a rupturing event occurs and the force drops. This sequence of events can happen repeatedly as the polymer chain is stretched, resulting in the sawtooth pattern seen in Figure 2. Further evidence for single-chain stretching comes from fitting the single-molecule data to the wormlike chain (WLC) model,[5] which is used to predict the relationship between the extension of a polymer chain and its entropic restoring force. Our force-extension data fit nicely to this model with a persistence length (b) of 0.55 nm (Figure 2). This value is reasonable as compared to the 0.4 nm values for proteins.[4] The persistence length for DCL polymer is larger than for the UPy polymer or natural proteins because the DCL polymer has a more rigid backbone than these systems.

Biological modular polymers such as *titin* show a constant increase in contour length (ΔL) between consecutive peaks in their force-extension curves due to the sequential unfolding of identical modules as the polymer is stretched.[4] For DCL polymers, the ΔL ranges from 5 to 21 nm with the most probable value of 12.5 nm (see the histograms in the Supporting Information). We propose that this nonconstant peak spacing is due to the formation of tertiary structures by $\pi-\pi$ stacking between adjacent DCL modules. Computer modeling shows that the four phenyl rings on each DCL module are coplanar. Strong $\pi-\pi$-stacking interactions between phenyl rings on adjacent DCL modules drive them to form small clusters along polymer chains (see Supporting Information). Similar π-stacking interactions have been observed in other aromatic oligomers and polymers.[15−17] The association energy between the four phenyl rings on two DCL modules is comparable to that for the four hydrogen bonds within each DCL module. As the force builds to the threshold, both the π-stacking and hydrogen bonding within each cluster unfold simultaneously. Clusters with varying number of DCL modules will gain different length upon unfolding, resulting in a distribution of ΔL. Computer modeling also shows that the large alkyl loops on both sides of a DCL module repel each other due to sterics as DCL modules π-stack with each other, making the smallest cluster formed between two DCL modules most favorable (see Supporting Information). The unfolding of the smallest cluster containing two DCL modules should gain 12 nm in contour length, which agrees well with the most probable ΔL value observed experimentally (Figure 2).

In conclusion, a new class of well-defined modular polymers has been synthesized using a double-closed-loop peptidomimetic β-sheet module. Single-molecule nanomechanical studies on these polymers show more uniform sawtooth patterns, suggesting that the DCL modular polymer undergoes sequential unfolding upon stretching. Further studies toward the bulk mechanical properties and the design of well-defined modules having varying H-binding strength are currently underway in our laboratory.

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Supporting Information Available: Synthesis and characterization. This material is available free of charge via the Internet at http://www.pubs.acs.org.

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