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Design of Three-Dimensional, Millimeter-Scale Models for Molecular Folding

Thomas D. Clark, Mila Boncheva, Jennifer M. German, Marcus Weck, and George M. Whitesides*

Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138

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This communication describes the fabrication of three-dimensional (3-D), millimeter-sized structures using principles of design inspired by protein folding. Our approach is based on mesoscale self-assembly (MESA)[1,2] and uses capillary interactions to mimic the hydrophobic forces that are important in biomolecular self-assembly.[3a] In previous work, we demonstrated the crystallization of millimeter-sized components into ordered 3-D arrays;[1b] here, using concepts derived from the study of molecules—including hydrophobicity, shape complementarity, and conformational constraint—we show that, by tethering the components with flexible linkers, we can control their interactions and relative placement in the resulting assemblies.

We designed the self-assembling systems described here ("models") to fold into structures having shapes and topographies reminiscent of natural proteins or protein fragments. The models consist of rigid polyhedral components ("microdomains"), representing α-helical and β-sheet secondary structures, connected by flexible linkers, denoting loops or turns (Figure 1).[4,5] We cast the models from polyurethane (PU), using molds derived from masters prepared via photolithography, and patterned the surfaces of the microdomains into hydrophobic and hydrophilic regions by protecting the areas to remain hydrophobic and exposing the models to an oxygen plasma (Figure 2a). Self-assembly occurred in water through coalescence of thin films of a hydrophobic liquid (a photocurable adhesive) precipitated selectively onto the hydrophobic areas of the microdomains; rotation of the "reaction" vessel enabled the microdomains to move with respect to each other and provided the agitation that permitted the self-assembly to proceed (Figure 2b). When the adhesive-coated regions came into contact, they adhered through capillary attraction and aligned themselves to minimize the exposed hydrophobic area. Curing the adhesive with ultraviolet light locked the structures in place.

Figure 3 shows the models designed in this study.[6] We drew inspiration for the design of our simplest model, a single helix tethered to a single preformed sheet, from the ubiquitous interaction of α-helices and β-sheets in proteins (Figure 3a).[3a] As expected for a model containing only two components, we found that even long linkers (10−12 mm) allowed the microdomains to assemble in the correct orientation. The introduction of linkers ∼4 mm in length, however, led to a model, **1**, that folded more quickly (≤5 min) than those containing longer linkers.

Next we constructed a model in which three microdomains assembled in a predetermined manner. Our design was inspired by the structure of the peptide-binding domain of the class I major histocompatibility protein[3b] and consists of two helices that bind to distinct sites on a preformed sheet (Figure 3b). The use of linkers ∼6 mm in length resulted in unwanted interactions, such as the binding of a helix to the wrong site on the sheet (e.g. helix **1** to site **2′**) or the association of the two helices with each other. By constraining the system with linkers ∼4 mm long, however, we obtained model **2** in which the helices bound selectively to the correct sites (Figure 3b).

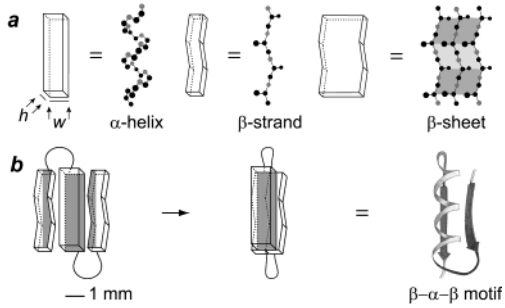

Figure 1. (a) In the folding models, α-helices are depicted by rectangular boxes ($h = w = 1$ mm), while β-strands ($h = 0.7$ mm, $w = 0.7$ mm) and sheets ($h = 0.7$ mm, $w = 2.1$ mm) are represented by zigzag-shaped polyhedra.[5] (b) A representative folding model. Shaded areas are hydrophobic and coated with a nonpolar photocurable adhesive. In water, the two β-strands converge to provide a hydrophobic area complementary in shape to the hydrophobic face of the α-helix. After irradiation with UV light, the resulting β-α-β assembly can be removed and examined (Figure 3c).

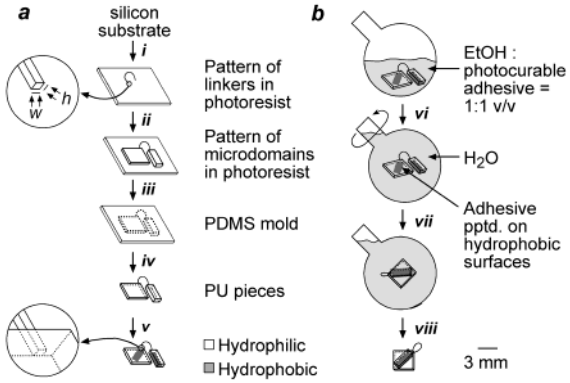

Figure 2. (a) Fabrication of the folding models. (i) Exposure and development of SU-8 photoresist spun on a silicon wafer produced a pattern of linkers. For all models except **4** (Figure 3d), $h = w = 50$ μm. (ii) We spun additional layers of photoresist, exposed them using photomasks for the microdomains, and developed the wafer to give a master. (iii) Casting poly(dimethylsiloxane) (PDMS) against the master produced molds. (iv) Casting polyurethane (PU) in the molds gave PU models. (v) We patterned the models into hydrophobic and hydrophilic regions by covering the regions to remain hydrophobic and exposing the models to an oxygen plasma. (b) Self-assembly of the models. (vi) We placed a patterned PU model in a 100 mL Morton flask containing ∼300 μL of 1:1 (v/v) ethanol:nonpolar photocurable adhesive (96:2:2 w/w/w dodecyl methacrylate/benzoin isobutyl ether/1,6-hexanediol diacrylate).[1b] Dilution of the ethanol with water precipitated the adhesive selectively onto the hydrophobic areas. (vii) Rotation of the flask enabled the adhesive-coated regions to contact each other, cohere through capillarity, and align themselves to minimize the interfacial energy of the system. (viii) Irradiation with UV light locked the assemblies in place.

We also designed a model in which the sheet self-assembled from individual strands (Figure 3c). This system is based on the β-α-β motif, a supersecondary structure found in nearly all proteins

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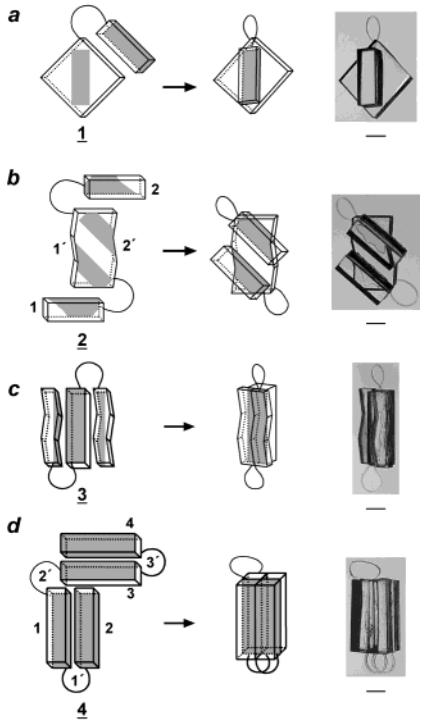

Figure 3. The folding models examined in this study. The shaded areas are hydrophobic and coated with a photocurable adhesive; the scale bars refer to 1 mm. (a) Model **1**, consisting of a single helix that associates with a single, preformed sheet. (b) Model **2**, comprising two helices that bind to selected sites on a preformed sheet. (c) Model **3**, composed of two strands flanking a central helix, folds to give a β-α-β structure. (d) Model **4**, consisting of four helices connected by three linkers, folds into a four-helix bundle. In **4**, the cross-sectional dimensions of linkers **1′** and **3′** are $h = w = 100$ μm; those of linker **2′** are $h = w = 50$ μm.

containing parallel β-sheets.[3a] The model comprises three microdomains: a central helix connected through a loop at either end to two separate strands (Figures 1b and 3c). From our prior experience with model **2**, we chose short linkers (~3 mm) for the design of **3**. The resulting model folded successfully into the target structure.

We based our last design on the four-helix bundle, the most common domain structure found in α-helical proteins.[3a] The model consists of four helical microdomains connected by three linkers (Figure 3d). Here the linkers served to ensure the formation of a unique structure: there are $W = (4 - 1)! = 6$ different bundle structures that can form from the association of four distinguishable helices patterned as shown in Figure 3d; if we further assume that each helix has a directionality analogous to the N → C polarity of an α-helix, the number of possible structures increases to $W^2 = 36$.[7]

To simplify the problem, we envisioned the folding occurring in two steps: first, the binding of helix **1** to helix **2**, and of helix **3** to helix **4**, to form helical pairs **[1+2]** and **[3+4]**, respectively, and, second, the association of these pairs to give the final four-helix bundle. We sought to favor the initial formation of the correct helical pairs by choosing short linkers (~3 mm) to join helix **1** to helix **2** (linker **1′**), and helix **3** to helix **4** (linker **3′**); we also sought to disfavor the formation of unwanted intra-pair contacts by placing the two groups of helices perpendicular to one another and joining them with a somewhat longer linker (linker **2′**; ~4 mm). Despite these design features, the resulting models frequently gave misfolded structures in which, for example, helices **2** and **3** bound to one another through the hydrophobic faces that were intended to bind to helices **1** and **4**, respectively. Having determined that this behavior arose from unanticipated twisting of linkers **1′** and **3′**, we further constrained the system by increasing the cross-sectional dimensions, and therefore the stiffness, of linkers **1′** and **3′** ($h = w = 100$ μm) relative to those of linker **2′** ($h = w = 50$ μm). The result was model **4**, which folded consistently into the desired four-helix bundle.

The results described here strongly support the notion that concepts derived from the study of molecules—in this case the folding of proteins—can find fruitful application in the fabrication of small, 3-D structures.[1a] In contrast to those techniques in current use, including micromachining,[8a] stereolithography,[8b] and 3-D printing,[8c] the present method is simple to perform and provides rapid access to a range of constructs. The experiments described here were performed serially, using one model per reaction vessel, to avoid unwanted "intermolecular" contacts; we believe, however, that appropriate modifications to the system—for example, immobilization of an array of models on a supporting substrate—will ultimately allow us to carry out these processes in parallel. Furthermore, because the models are derived from photolithography, they can, in principle, be miniaturized to the micron- or submicron-size scale.[8] In other work, we have already demonstrated the self-assembly of 10-μm-sized objects into crystalline arrays,[9a] and the folding of millimeter-sized components to form electronic devices,[9b] using MESA. One current goal is to extend the concepts described here to the self-assembly of small, 3-D structures that display a range of interesting functions.

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Supporting Information Available: Details of the fabrication and self-assembly of the models used here (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(4) We note that the design of our folding models is reminiscent of the diffusion-collision theory of protein folding, in which preorganized secondary structural elements ("microdomains") are thought to play an important role in guiding the formation of tertiary structure. While this and related theories await definitive experimental verification, we chose to represent protein secondary structures as rigid components in order to simplify the fabrication of our models. For leading references, see: Kirshenbaum, K.; Zuckermann, R. N.; Dill, K. A. *Curr. Opin. Struct. Biol.* **1999**, *9*, 530−535.

(5) We chose a zigzag shape for the design of the sheet and strand microdomains to suggest the pleated conformation of a β-sheet.[3a] We note, however, that the direction of the pleating in natural proteins is approximately perpendicular to the direction of the zigzags in our models.

(6) We obtained the intended structure in all cases in at least four separate repetitions of the same experiment. The experiments were typically complete within 5 min, a time frame similar to that reported previously for the formation of a crystalline array from eight PU cubes.[1b] Models that gave misfolded structures or that failed to fold within 5 min were redesigned by placing the microdomains closer together and/or by making the linkers shorter and varying their radii of curvature.

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