VIP Protein Topology

DOI: 10.1002/ange.201107846

Design, Total Chemical Synthesis, and X-Ray Structure of a Protein Having a Novel Linear-Loop Polypeptide Chain Topology**

*Kalyaneswar Mandal, Brad L. Pentelute, Duhee Bang, Zachary P. Gates, Vladimir Yu. Torbeev, and Stephen B. H. Kent**

Proteins found in nature usually consist of a linear polypeptide chain folded into a defined three-dimensional structure that gives rise to the functional properties of the molecule. In mathematical terms, these proteins have a linear polypeptide chain topology. There is increasing interest in rare protein molecules that contain more complex polypeptide chain topologies such as knots[1,2] and catenanes.[3,4] Recently, two types of ribosomally translated polypeptides of unusual topology have been described: "cyclotides" are miniproteins that have a peptide bond connecting the N- and C-terminal amino acids of their polypeptide chain to form a cyclic covalent structure;[5,6] and "lasso peptides", in which the N terminus of the polypeptide chain connects to the side chain carboxylate of an internal Asp or Glu residue through an amide bond, and the linear C-terminal portion of the polypeptide threads through the resulting macrolactam ring.[7–9] Since the biological activity of lasso peptides arises from this folded (tertiary) structure, strictly these molecules should be considered to be microproteins. The exceptional stability of cyclotides and lasso peptides is thought to originate in the cyclic topology of their polypeptide chains.[10] Cyclotides have been prepared by modern chemical ligation methods,[11] but to date lasso peptide molecules of correct folded structure have not been prepared by chemical synthesis.

As part of our continuing investigations of chirality[12] and topology[13] in protein molecules, here we report the design, chemical synthesis, and structural characterization of a novel topological analogue of the protein crambin. Crambin is a small, disulfide-rich, membrane-associated protein natural product[14] that has been widely used for model studies,[15] but that has proved refractory to recombinant DNA-based protein engineering methods.[15,16] The X-ray structure of native crambin has been determined by using protein isolated from plant seeds (Figure 1 A).[17] We have previously established total syntheses of the native crambin molecule by modern ligation methods.[18,19] Furthermore, by total chemical synthesis of a linear crambin polypeptide as the α carboxamide, we demonstrated that a salt bridge between the δ guanidinium of the Arg10 side chain and the α carboxylate of Asn46 at the C terminus of the crambin molecule guides the formation of the correct disulfide bonds and contributes to the tightly folded overall structure and stability of the native protein molecule,[20] as originally suggested by Arnold.[21]

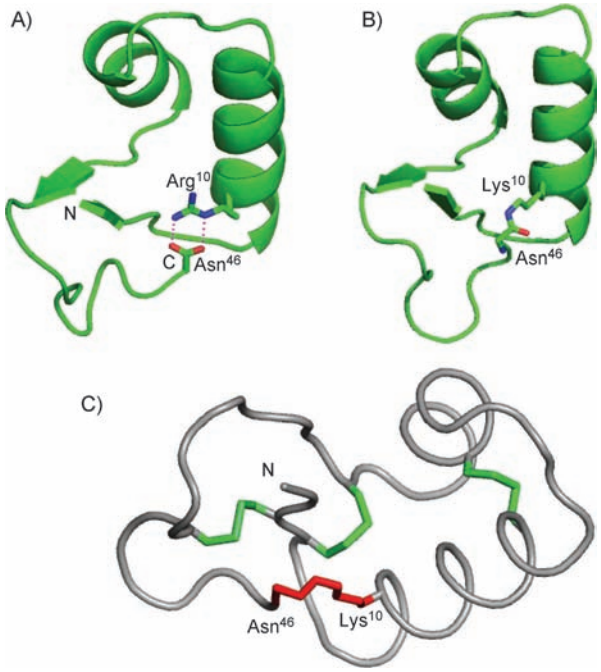

Figure 1. Structures of crambin and a novel topological analogue protein. A) A cartoon representation of the X-ray structure of crambin, as isolated from plant seeds.[17] The salt bridge between Arg10 and the C-terminal α carboxylate of Asn46 is highlighted. B) Concept for a novel topological analogue of crambin, showing the replacement of Arg10 by Lys10 and formation of an amide covalent bond between the ε amino group of Lys10 and the α carboxyl group of Asn46. C) Diagram of the polypeptide backbone (gray) in relation to the disulfide bonds (green) in the topological analogue protein. As a consequence of the amide bond between the C terminus and the ε amino group of the side chain of Lys10 (red), the N-terminal residues thread through a macrolactam ring and are held in position by disulfide bonds.

The critical role of the Asn46–Arg10 ion pair interaction prompted us to consider replacing that salt bridge by a covalent linkage: if the C terminus of the crambin polypeptide chain were to be connected to the ε amino group of a Lys10 (thus replacing Arg10; Figure 1 B) while the tertiary structure of the protein was retained, the resulting novel topology would be fascinating in two respects. First, the hypothetical protein molecule would contain a polypeptide

[*] K. Mandal, B. L. Pentelute, D. Bang, Z. P. Gates, V. Y. Torbeev, Prof. S. B. H. Kent
Department of Biochemistry and Molecular Biology
Department of Chemistry, Institute for Biophysical Dynamics
The University of Chicago, Chicago, IL 60637 (USA)
E-mail: skent@uchicago.edu

[**] This research was supported by the Office of Science (BER), U.S. Department of Energy (grant no. DE-FG02-07ER64501 to S.B.H.K.).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201107846.

chain with an N terminus but no C terminus. Second, the polypeptide chain would have an extraordinary linear-loop topology in which two disulfide bonds would lock the N-terminal nine residues so that this linear stretch of peptide chain would penetrate through the covalent macrolactam ring (Figure 1C). The novel polypeptide topology shown in Figure 1B and Figure 1C is reminiscent of, but not identical to, both the naturally occurring cyclotides[5,6] and lasso peptides.[7–9]

Preparation and characterization of this unique protein molecule required the development and use of novel synthetic and structure determination methods. In order to achieve an efficient synthesis of the topological analogue protein, we developed the kinetically controlled ligation method.[22] Here, kinetically controlled ligation enabled us to prepare a key branched Cys-peptide-thioester intermediate in a convergent fashion. Then, a final intramolecular native chemical ligation[23] gave a polypeptide with the desired linear-loop covalent structure, that was folded in near-quantitative yield with concomitant formation of three disulfide bonds. To determine the structure of the resulting topological analogue protein, we used quasi racemate crystallization, a novel method of obtaining crystals of recalcitrant proteins for X-ray structure determination.

It proved to be a significant challenge to develop an efficient convergent synthesis of a crambin-derived topological analogue protein ("topologue", from topological analogue), in which the ε amino group of a Lys at position 10 of the polypeptide chain is acylated by the α carboxyl group of Asn46 (Figure 1B). Our initial retrosynthetic analysis (see Figure S1 in the Supporting Information, strategy 1) envisioned preparation of the key 46-residue branched polypeptide intermediate Thr1–Lys10(Asn46–Cys16)–Val15-α-thioalkylester (**A**) by highly optimized stepwise solid phase peptide synthesis (SPPS),[24] followed by intramolecular native chemical ligation of the Val15-α-thioester and Cys16 to give the linear-loop polypeptide intermediate **B**. This intermediate can then be folded with concomitant formation of disulfides to give the topologue **1**. Although this first strategy is potentially the simplest one, the necessary stepwise synthesis of the full-length branched 46-residue intermediate was not feasible; stepwise synthesis of even a linear 31-residue crambin peptide had proved impractical in our hands.[18] In a second design (Figure S1, strategy 2), we considered a convergent synthetic approach to key intermediate **A**, with subsequent cyclization of the polypeptide by native chemical ligation to give polypeptide **B**. However, reaction of Thz16–Gly31-α-thioalkylester with Thr1–Lys10(Asn46–Cys32)–Val15-α-thioalkylester under native chemical ligation reaction conditions[25] was not sufficiently selective, presumably because faster reaction of the less hindered thioester[26] was offset by the intramolecular nature of the competing undesired ligation reaction. We were able to obtain small amounts of a product with the expected mass (Figure S2), but the complex product composition resulted in low synthetic yields and led us to abandon this route.

The strategy we ultimately devised for the chemical synthesis of the topologue **1** is shown in Figure 2. Disconnection of the target peptide chain was redesigned to simultaneously take advantage in the key ligation step of both the more rapid reaction of an Ala thioester than a hindered Thr thioester and the greater inherent reactivity of a thioarylester compared with a thioalkylester. The branched peptide Thr1–Lys10(Asn46–Thz40)–Ala15-α-thioarylester (**I**) was reacted at pH 6.3 with Cys16–Thr39-α-thioalkylester (**II**), under kinetically controlled ligation reaction conditions,[22] that is, *in the absence of an exogenous thiol catalyst*, to give the branched 46-residue polypeptide Thr1–Lys10(Asn46–Thz40)–Thr39-α-thioalkylester (**III**). The thiazolidine Thz40 was converted to Cys40 by treatment with methoxylamine·HCl at pH 4.0 in the same pot. After dilution, the product mixture was adjusted to pH 6.8 and intramolecular native chemical ligation in the presence of added thiol catalyst gave the target polypeptide **V**. Side-chain thiol groups of the Cys residues not at a ligation site (i.e., Cys3, Cys4, Cys26, and Cys32) were protected as Cys(Acm) (Acm = acetamidomethyl), because earlier test reactions with unprotected internal Cys residues resulted in lower reaction yields because of thiolactone formation.[27] Removal of the Cys(Acm) protecting groups and purification by reverse-phase HPLC gave the cyclized polypeptide **VII** in reduced form (see Figure 3). Reduced peptide **VII** was folded, with concomitant formation of disulfides, at pH 7.9 in an aqueous buffer containing guanidine·HCl (2 M) and a cysteine/cystine redox

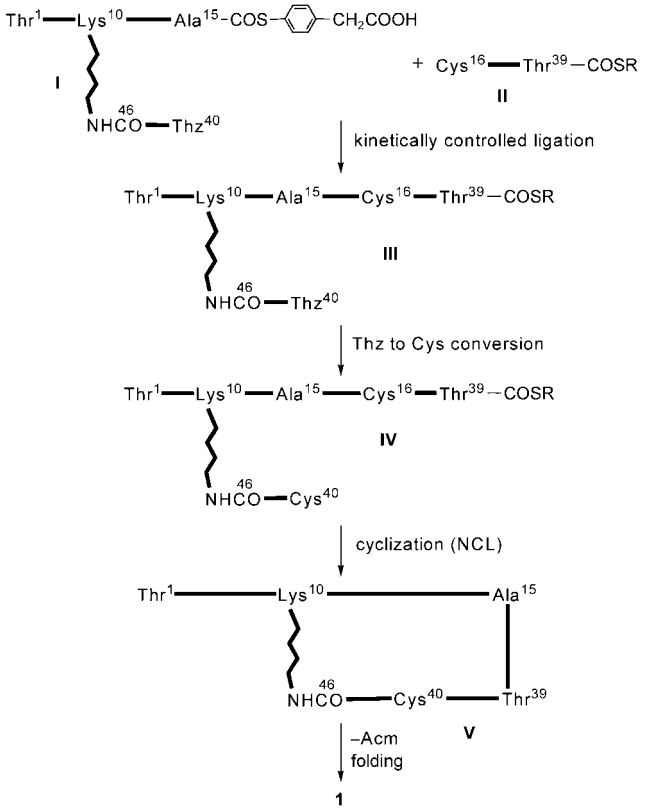

Figure 2. Optimized synthetic strategy used in the total chemical synthesis of the novel topological analogue protein. Strategy 3; kinetically controlled ligation[22] was used to prepare the key intermediate **III** (R = $CH_2CH_2CO(Arg)_4$-Ala-COOH). Synthetic steps are described in detail in the main text. The amino acid sequence of (Lys10,Ala15)crambin is given in the Supporting Information.

couple. Analytical data for all steps in the synthesis are shown in Figure 3.

We had anticipated that the folding of the linear-loop polypeptide **VII** might be difficult because conformational constraints imposed by the novel covalent topology of the polypeptide chain could interfere with the productive folding pathway. Surprisingly, folding proceeded smoothly to give a single product, crambin-derived topologue **1**: folding and concomitant disulfide bond formation were essentially complete within 30 minutes, as evidenced by a mass decrease of 6.0 Daltons (compare the $[M+3H]^{3+}$ charge states in Figure 3E and Figure 3F), which corresponds to the formation of three disulfides. Purification by HPLC gave the topologue **1** in approximately 20% overall yield from the starting peptide segments. The synthetic crambin topologue **1** was characterized by HPLC/electrospray mass spectrometry (Figure S6A); it had the expected mass, and had a single defined tertiary fold, as determined by 2D TOCSY proton NMR spectroscopy (Figure S7).

In order to experimentally verify that the topology of the polypeptide chain in the synthetic topologue **1** was as predicted, we set out to determine the high resolution structure of the product by X-ray crystallography. However, crystallization trials with synthetic topologue **1** over several months did not produce crystals under the conditions examined. Recently, we have shown that use of a racemic mixture of protein enantiomers, that is, (L-protein + D-protein), can facilitate the crystallization of recalcitrant proteins,[28,29] as had earlier been predicted on theoretical grounds.[30] Herein, we extended this idea, and explored the possibility of more facile crystal formation from a quasi-racemic mixture that consisted of crambin-derived topologue **1** and the D-enantiomer of native crambin. In dramatic contrast to the failure to obtain crystals under standard screening conditions with the topologue protein alone, a solution that contained equal amounts of (synthetic L-topologue **1** + synthetic D-crambin) produced microcrystals within 4 days in 40–50% of the conditions examined in a standard Hampton Research Index screen.

For structure determination, crystals of the quasi racemate were grown under optimized conditions (see Supporting Information). Synchrotron diffraction data were collected at the Advanced Photon Source, Argonne National Laboratory. We obtained diffraction data to 1.08 Å resolution, and solved the structure by molecular replacement with the MolRep software[31] using the reported crambin structure (PDB ID 1CRN) as a search model. The structure was refined to crystallographic R-factor and R-free values of 0.144 and 0.176 respectively. X-ray data collection and refinement statistics for the final dataset are summarized in Table S1. The quasi-racemate crystal belonged to the monoclinic space group $P2_1$ with two molecules in the asymmetric unit (one D-crambin, one L-topologue) arranged in a (pseudo)centrosymmetric fashion (see Figure S10A). The arrangement of the crambin L-topologue and the D-crambin protein molecules in the unit cell is shown in Figure 4A. A cartoon representation of the X-ray structure of the topologue **1** is shown in Figure 4B: the new amide bond between the α carboxylate of Asn46 and the ε amino group of Lys10 was clearly evident in the 2Fo-Fc electron density map (Figure 4C).

Quasi-racemate crystals of small organic molecules were first described by Pasteur in 1853,[32] and their properties have more recently been explored by Curran[33] and Wheeler.[34] The quasi enantiomers that were used to form these crystals are related to each other by an almost mirror-image relationship and can be converted to the true enantiomers by slightly changing the chemical composition of one or more atoms or functional groups. Although small-molecule quasi-racemates are widely used in asymmetric organic synthesis and for organizing supramolecular arrays, utilization of macromolecular quasi-racemates was demonstrated only recently when we found that the incorporation of an additional -Se-CH_2- in only the L enantiomer of snowflea antifreeze protein still facilitated crystallization with the D-protein molecule as was seen for the true racemate.[28] In the current work, we have made use of near-mirror-image protein molecules with more substantial differences in their covalent structures. The unusual quasi-enantiomeric protein molecules described here are related by replacement of a noncovalent bond (salt-bridge interaction) in one enantiomer by a covalent linkage in the other (quasi-)enantiomer, while maintaining an almost mirror-image relationship between the two protein molecules. Apparently, molecular shape is more important than the precise chemical identity for regular packing of these quasi-enantiomeric protein molecules, which thus retain the propensity to form highly ordered crystals in which the quasi-enantiomeric proteins are related through a (pseudo)center of inversion (Figure S10A).

Comparison of the D-crambin structure determined here inverted to the L form, with previous X-ray structures of L-crambin[17] showed no significant differences in the backbone fold of the polypeptide chain of crambin (Figure S10B). Thus, co-crystallization with the topological analogue protein has not induced conformational changes in D-crambin. The crambin-derived topologue **1** itself shares a similar fold with native crambin, with facile formation of correct disulfide bonds in spite of the presence of the artificial covalent linkage and the resulting novel topology of the synthetic polypeptide chain. Superposition of the X-ray structure of the inverted D-crambin and topologue **1** (Figure 4D) shows that, through residue 41, both polypeptide main chain conformations are the same (Cα root mean square deviation 0.48 Å). Interestingly, there were no significant differences in the crystallographic B factors of the two protein molecules (see Figure S11), although the crambin topologue **1** has a somewhat different structure in the flexible loop region (residues 42–46) compared with the native crambin molecule.

In the work reported here, we have designed a novel protein analogue in which the C terminus of the polypeptide chain is covalently cyclized to the side chain of an internal amino acid, and we have developed and applied robust, modular chemical strategies to enable the efficient total synthesis of the novel polypeptide chain topology. The synthetic challenge presented by this complex molecule led us to develop and use kinetically controlled ligation for convergent synthesis.[22] Kinetically controlled ligation chemistry allows more flexible, fully convergent synthetic

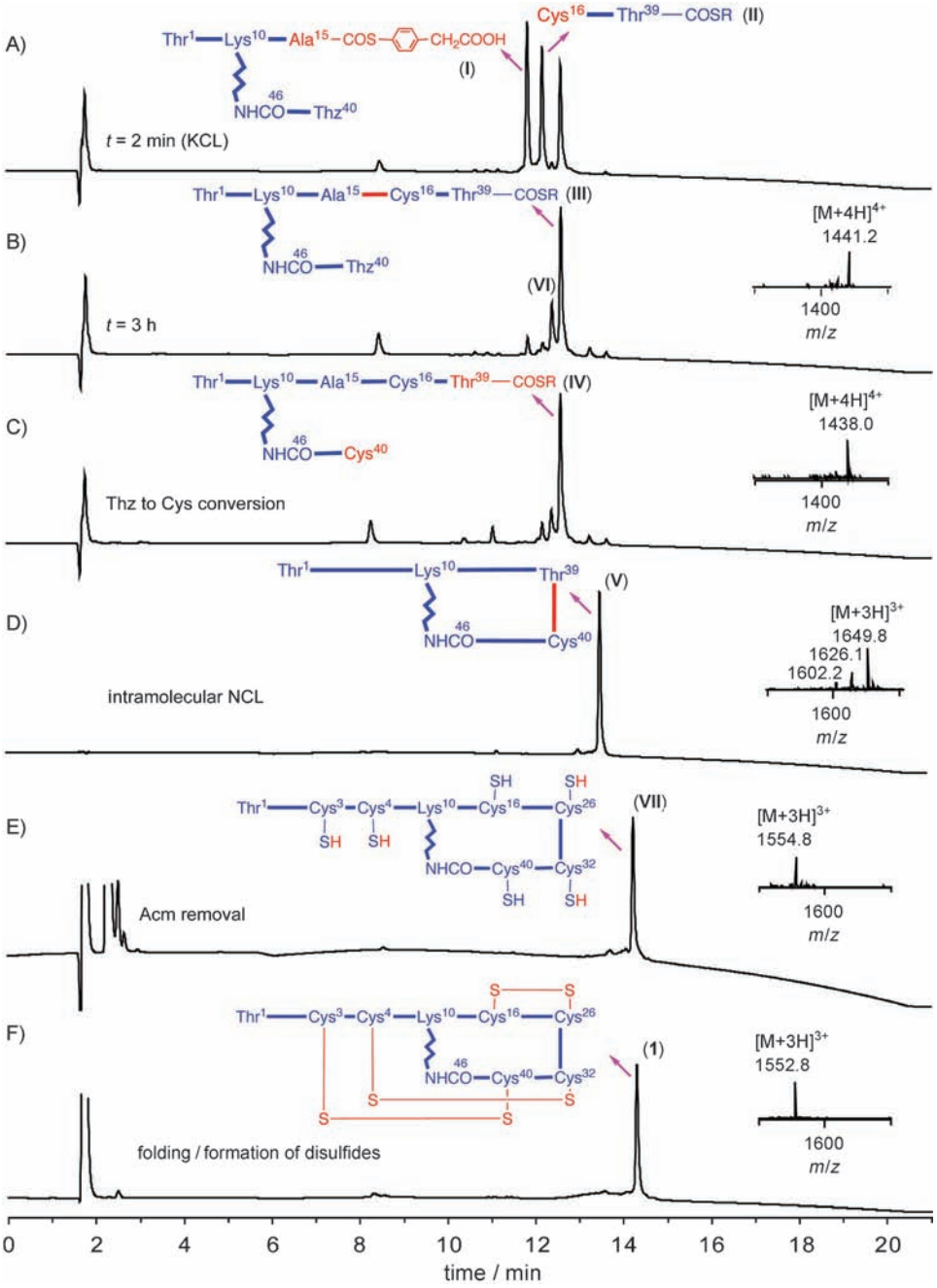

Figure 3. LCMS analytical data for the total chemical synthesis of the novel topological analogue protein. Analytical HPLC profiles ($\lambda = 214$ nm) are shown, together with online electrospray ionization mass spectrometry (MS) data (insets correspond to major products). A) Kinetically controlled ligation (KCL) between **I** (42 mg, 16 μmol) and **II** (48 mg, 14 μmol) at $t = 2$ min. B) Kinetically controlled ligation reaction after 3 h: **III** is the ligation product; co-product **VI** is a branched thioester from reaction of excess **I** with the Cys side chain at the ligation site in product **III**; addition of methoxylamine·HCl in the next step partially converted the branched thioester **VI** to additional desired **III**. C) Crude reaction mixture after conversion of Thz to Cys by using methoxylamine·HCl (35 mM) at pH 4: formation of the desired Cys-peptide **IV** was shown by a mass decrease of 12 Da. D) Purified intramolecular native chemical ligation (NCL) product **V** (21 mg, 4.5 μmol, 32.1% based on limiting peptide segment **II**; observed mass: 4946.4 Da; calcd mass: 4946.7 Da; the additional peaks seen in the MS data (see inset) are artefacts from Acm loss because of fragmentation in the MS). E) Crude reaction mixture after removal of the Acm group. Compound **V** (16 mg, 3.2 μmol) was used for the Acm removal and after solid-phase extraction gave the desired reduced polypeptide **VII** (13 mg, 2.8 μmol). Observed mass: 4661.4 Da; calcd mass (high point of isotope envelope): 4661.2 Da. F) Folding of the crude linear-loop polypeptide **VII** obtained after Acm removal, with concomitant disulfide formation. The folded topological analogue protein **1** showed a mass decrease of 6.0 Da ($[M+3H]^{3+}$ charge states: 1554.8 m/z-1552.8 $m/z = 2.0$ m/z units, corresponding to 6.0 Da) that confirmed the formation of three disulfides. Preparative HPLC purification gave topological analogue protein **1** (9 mg, 1.9 μmol, 59% over two steps). Observed mass: 4655.4 Da; calcd mass (high point of isotope envelope): 4655.2 Da. Analytical HPLC: a linear gradient (5–65%) of buffer B in buffer A over 15 min (buffer A = 0.1% trifluoroacetic acid (TFA) in water; buffer B = 0.08% TFA in acetonitrile) on a C-3 (Agilent), 4.6×150 mm column at 40 °C (flow rate = 1 mL min^{-1}).

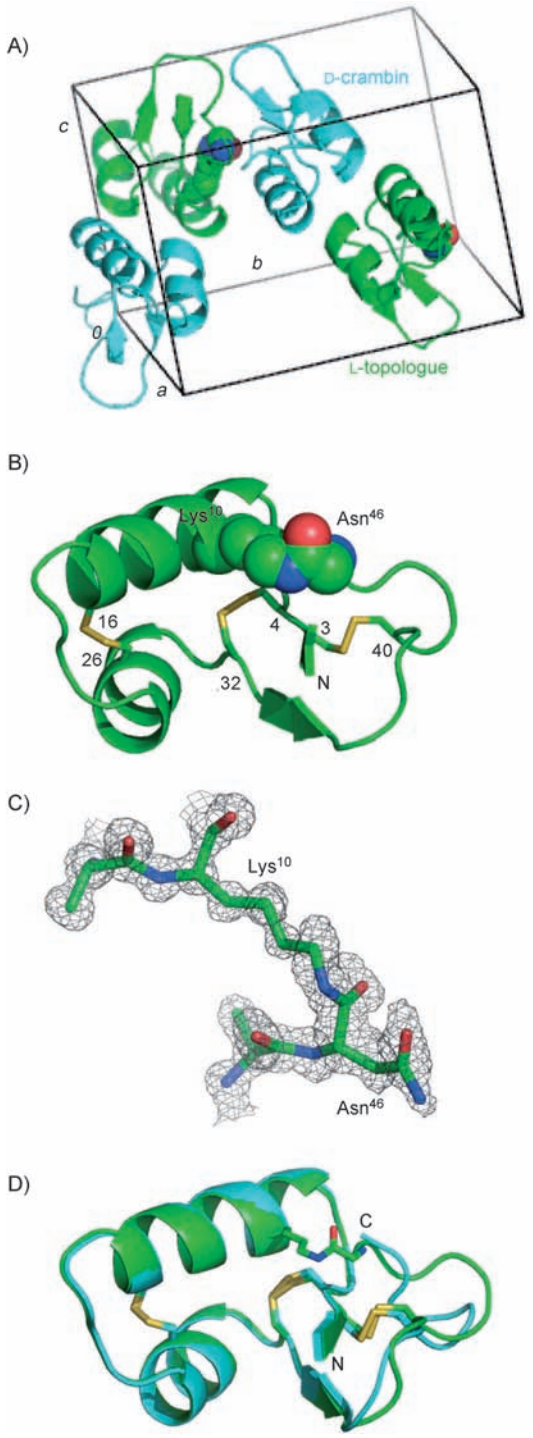

Figure 4. X-ray structure of the quasi-racemate (L-crambin topologue + D-crambin) in space group $P2_1$. A) Unit cell composed of four molecules, two L-crambin topologue proteins (shown as green ribbons) and two D-crambins (shown as cyan ribbons). Note that pairs of (L-topologue + D-crambin) are related by a pseudocenter of symmetry. B) Ribbon representation of the molecular structure of crambin-derived topologue **1**; the disulfides are shown as yellow sticks, and the covalent bridge between Lys10 and Asn46 is shown as CPK spheres. C) SigmaA-weighted $2F_o-F_c$ electron density map of topologue **1** contoured at 1σ encompassing the covalent bridge between Lys10 and Asn46. D) Superposition of the X-ray crystal structure of crambin (i.e., inverted D-crambin, in cyan) and the X-ray crystal structure of the crambin-derived topologue **1** (in green). Coordinates and structure factors have been deposited in the Protein Data Bank with accession code 3UE7.

strategies and has already proved to be useful for the total chemical synthesis of a variety of other challenging proteins in our laboratory.[27,35,36]

The high-resolution X-ray structure determination reported here for the novel synthetic topological analogue protein is a vivid demonstration of the utility of the quasi-racemate crystallization technique. In our hands, quasi-racemic protein crystallization is proving to be as effective as racemic crystallization for structure determination of recalcitrant proteins. We predict that this technique will be particularly useful for the structural characterization of a series of analogue proteins that can be individually crystallized with the D-enantiomer corresponding to the native protein.

The novel topology of the crambin-derived protein **1**, in which the linear N terminus of the polypeptide is threaded through a macrolactam ring that is formed at the C terminus of the same polypeptide chain, has yet to be discovered in natural proteins. The biochemical machinery for making this type of polypeptide chain topology certainly exists, so we can expect that this linear-loop topology will in due course be discovered in the world of natural protein molecules.

Received: November 8, 2011
Published online: December 30, 2011

Keywords: chemical ligation · protein structures · proteins · quasi racemates · topology

[1] C. Liang, K. Mislow, *J. Am. Chem. Soc.* **1995**, *117*, 4201 – 4213.
[2] W. R. Taylor, *Nature* **2000**, *406*, 916 – 919.
[3] L. Z. Yan, P. E. Dawson, *Angew. Chem.* **2001**, *113*, 3737 – 3739; *Angew. Chem. Int. Ed.* **2001**, *40*, 3625 – 3627.
[4] J. W. Blankenship, P. E. Dawson, *Protein Sci.* **2007**, *16*, 1249 – 1256.
[5] M. Trabi, D. J. Craik, *Trends Biochem. Sci.* **2002**, *27*, 132 – 138.
[6] D. J. Craik, N. L. Daly, T. Bond, C. Waine, *J. Mol. Biol.* **1999**, *294*, 1327 – 1336.
[7] K. A. Wilson, M. Kalkum, J. Ottesen, J. Yuzenkova, B. T. Chait, R. Landick, T. W. Muir, K. Severinov, S. A. Darst, *J. Am. Chem. Soc.* **2003**, *125*, 12475 – 12483.
[8] K. J. Rosengren, R. J. Clark, N. L. Daly, U. Goransson, A. Jones, D. J. Craik, *J. Am. Chem. Soc.* **2003**, *125*, 12464 – 12474.
[9] M. J. Bayro, J. Mukhopadhyay, G. V. Swapna, J. Y. Huang, L. C. Ma, E. Sineva, P. E. Dawson, G. T. Montelione, R. H. Ebright, *J. Am. Chem. Soc.* **2003**, *125*, 12382 – 12383.
[10] C. W. Gruber, M. Cemazar, M. A. Anderson, D. J. Craik, *Toxicon* **2007**, *49*, 561 – 575.
[11] S. Gunasekera, N. L. Daly, M. A. Anderson, D. J. Craik, *IUBMB Life* **2006**, *58*, 515 – 524.
[12] R. C. deLisle-Milton, S. C. F. Milton, S. B. H. Kent, *Science* **1992**, *256*, 1445 – 1448.
[13] L. E. Canne, A. R. Ferré-D'Amaré, S. K. Burley, S. B. H. Kent, *J. Am. Chem. Soc.* **1995**, *117*, 2998 – 3007.
[14] C. H. VanEtten, H. C. Nielsen, J. E. Peters, *Phytochemistry* **1965**, *4*, 467 – 473.
[15] H. C. Ahn, N. Juranic, S. Macura, J. L. Markley, *J. Am. Chem. Soc.* **2006**, *128*, 4398 – 4404.
[16] L. Lobb, B. Stec, E. K. Kantrowitz, A. Yamato, V. Stojanoff, O. Markman, M. M. Teeter, *Protein Eng.* **1996**, *9*, 1233 – 1239.
[17] M. M. Teeter, *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 6014 – 6018.

[18] D. Bang, N. Chopra, S. B. H. Kent, *J. Am. Chem. Soc.* **2004**, *126*, 1377 – 1383.
[19] D. Bang, S. B. H. Kent, *Angew. Chem.* **2004**, *116*, 2588 – 2592; *Angew. Chem. Int. Ed.* **2004**, *43*, 2534 – 2538.
[20] D. Bang, V. Tereshko, A. A. Kossiakoff, S. B. H. Kent, *Mol. Biosyst.* **2009**, *5*, 750 – 756.
[21] F. H. Arnold, *Protein Eng.* **1988**, *2*, 21 – 25.
[22] D. Bang, B. L. Pentelute, S. B. H. Kent, *Angew. Chem.* **2006**, *118*, 4089 – 4092; *Angew. Chem. Int. Ed.* **2006**, *45*, 3985 – 3988.
[23] P. E. Dawson, T. W. Muir, I. Clark-Lewis, S. B. H. Kent, *Science* **1994**, *266*, 776 – 779.
[24] M. Schnölzer, P. F. Alewood, A. Jones, D. Alewood, S. B. H. Kent, *Int. J. Pept. Res. Ther.* **2007**, *13*, 31 – 44.
[25] P. E. Dawson, M. Churchill, M. R. Ghadiri, S. B. H. Kent, *J. Am. Chem. Soc.* **1997**, *119*, 4325 – 4329.
[26] T. M. Hackeng, J. H. Griffin, P. E. Dawson, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 10068 – 10073.
[27] T. Durek, V.Yu. Torbeev, S. B. H. Kent, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 4846 – 4851.
[28] B. L. Pentelute, Z. P. Gates, V. Tereshko, J. L. Dashnau, J. M. Vanderkooi, A. A. Kossiakoff, S. B. H. Kent, *J. Am. Chem. Soc.* **2008**, *130*, 9695 – 9701.
[29] K. Mandal, B. L. Pentelute, V. Tereshko, A. A. Kossiakoff, S. B. H. Kent, *J. Am. Chem. Soc.* **2009**, *131*, 1362 – 1363.
[30] S. W. Wukovitz, T. O. Yeates, *Nat. Struct. Biol.* **1995**, *2*, 1062 – 1067.
[31] A. Vagin, A. Teplyakov, *J. Appl. Crystallogr.* **1997**, *30*, 1022 – 1025.
[32] L. Pasteur, *Ann. Chim. Phys.* **1853**, *38*, 437 – 483.
[33] Q. Zhang, D. P. Curran, *Chem. Eur. J.* **2005**, *11*, 4866 – 4880.
[34] K. A. Wheeler, R. C. Grove, R. E. Davis, W. S. Kassel, *Angew. Chem.* **2008**, *120*, 84 – 87; *Angew. Chem. Int. Ed.* **2008**, *47*, 78 – 81.
[35] V. Y. Torbeev, S. B. H. Kent, *Angew. Chem.* **2007**, *119*, 1697 – 1700; *Angew. Chem. Int. Ed.* **2007**, *46*, 1667 – 1670.
[36] S. Liu, B. L. Pentelute, S. B. H. Kent, *Angew. Chem.* **2011**, DOI: 10.1002/ange.201106060; *Angew. Chem. Int. Ed.* **2011**, DOI: 10.1002/anie.201106060.

