How Can Folded Biopolymers and Synthetic Foldamers Recognize Each Other?

3 **AUTHORS**, INCLUDING:

Benoit Baptiste

University Joseph Fourier - Grenoble 1

11 PUBLICATIONS **160** CITATIONS

SEE PROFILE

Frédéric Godde

Université Bordeaux 1

18 PUBLICATIONS **485** CITATIONS

SEE PROFILE

How Can Folded Biopolymers and Synthetic Foldamers Recognize Each Other?

Benoit Baptiste,[b] Frédéric Godde,[a] and Ivan Huc*[a]

In the last decade, chemists have synthesized numerous families of oligomeric foldamers by using a variety of backbones.[1] In terms of their chemical composition, foldamers may be quite remote from biopolymers, yet their folded motifs—helices, turns, linear strands—often resemble those of their natural counterparts. Such a resemblance opens great perspectives for the future development of foldamers. Indeed, the control of molecular structure and dynamics through folding is the corner stone of biopolymers' extraordinary functions. The use of artificial backbones to invent new functions is thus expected to give access to countless applications. Some long-term objectives, such as artificial enzymes from non-α-peptidic backbones or molecular systems that would store and copy information as efficiently as DNA, are being considered but remain far from being achieved, even though the first artificial tertiary and quaternary structures[2] and even catalytic activities[3] have recently been reported. Nonetheless, other foldamer properties have been intensely investigated in recent years, in particular their potential to interfere with biological functions.[4]

At first glance, the idea of using synthetic foldamers as potential therapeutic agents is not obvious, because they are generally much larger—typically between 0.5 and 5 kDa—than conventional drugs. However, size can also help solve problems that are hard to tackle with smaller molecules. Besides, the rapid development of biologics shows that entire proteins and even cells and tissues may be used as medicinal products. Moreover, foldamers tend to show high resistance to enzyme degradation and may advantageously replace α-peptides or oligonucleotides as active substances. So why wouldn't foldamers find their own niche? One area where a large size and the plasticity of a folded structure become an advantage is in binding to protein or nucleic acid surfaces and the subsequent inhibition of interactions between biopolymers.[5] The sites at which these interactions take place have long been recognized as valid targets, but efficient binding at biopolymer surfaces with small molecules has met limited success so far. On the other hand, foldamers have been shown to fold into tertiary and quaternary structures in which artificial secondary motifs bind to other artificial secondary motifs.[2] Foldamers thus also emerge as suitable candidates for binding to the surfaces of protein or nucleic acid folded conformations.

A growing number of publications validate this concept. Examples that date from even before the term "foldamer" was coined[6] include numerous mimics of oligonucleotidic backbones, such as peptide nucleic acids (PNA),[7] which were designed in the context of the so-called antisense and antigene strategies; peptoids, which are α-peptidic oligomers whose side chains have been shifted to the amide nitrogen atoms;[8] and oligoamides of pyrrole and imidazole, which bind to the minor groove of duplex DNA.[9] In the past few years, aromatic oligoamide foldamers with helical or linear conformations,[10, 11] artificial α-peptidic constructs, such as mini-proteins,[12] branched structures,[13] or macrocycles,[14, 15] β-peptide helices,[16–18] and helical α/β-peptide hybrids[19–21] have all been shown to bind to protein or nucleic acid structures and are presumed to cover a substantial part of the surface areas of their targets.

Two approaches may be envisaged to identifying synthetic folded oligomers that recognize biopolymer targets. The first consists of eliciting arrays of interactions that do not exist in nature, and creating de novo new recognition motifs. Although there is no fundamental obstacle to this approach, examples of the sort are quite rare. Quinoline-derived helical oligoamides have been serendipitously found to bind to G-quadruplexes,[10] and their mode of interaction is likely to be new but it has not yet been elucidated. Pyrrole and imidazole oligoamides that bind in the minor groove of DNA were initially inspired by the binding mode of the natural product distamycin A, but were thereupon extended by design to an artificial extensive recognition mode of nucleotidic sequences.[9] In fact, most artificial folded structures capable of recognizing biopolymers through new recognition schemes are not synthetic foldamers but non-natural peptidic or nucleotidic sequences, such as aptamers,[22] that are produced by in vitro evolution techniques using molecular biology tools.

The second strategy for identifying synthetic folded oligomers that recognize biopolymer targets consists of mimicking arrays of interactions that already exist in nature, for example protein epitopes.[23] This generally amounts to creating mimics of the secondary-folded motifs of proteins or nucleic acids. Some of these mimics follow quite obvious designs: PNAs as DNA analogues[7] and peptoids as peptide analogues.[8] Yet the task is far from easy. For example, a central issue that has proven to be very challenging is the design of α-helical peptide mimics. This problem is often re-

[a] Dr. F. Godde, Dr. I. Huc
Institut Européen de Chimie et Biologie
Université de Bordeaux–CNRS UMR 5248
2 rue Robert Escarpit, 33607 Pessac (France)
Fax: (+33) 540-002-215
E-mail: i.huc@iecb.u-bordeaux.fr

[b] B. Baptiste
Laboratoire de Pharmacochimie
Université de Bordeaux
146 rue Léo Saignat, 33076 Bordeaux (France)

duced to finding scaffolds that display an array of proteinogenic residues at distances identical to those found at the *i*, *i*+4 and *i*+7 positions of α-helices. This has been achieved by Hamilton and co-workers, who have shown that aromatic oligoamides or oligoureas can mimic an α-helical domain of the protein BH3 and bind into the hydrophobic cleft of the Bcl-x$_L$ protein with good affinities,[11] although these affinities remain smaller than those of α-peptidic constructs.[12, 14]

Helical β-peptides can adopt a variety of conformations (Figure 1), and it has been hoped that some of them could

These doubts and difficulties arise from the fact that none of the various kinds of β-peptide helix accurately reproduces the spatial arrangement of residues found in α-helices (Figure 1). The distribution of the side chains in space varies, helical handedness can be *P* or *M*, and the orientation of the helix macrodipole with respect to the C and N termini can also be inverted.

A successful refinement of this approach has emerged in the past two years and consists of tuning the position of residues at the helix surface through a combination of α- and β-amino acids in

slow? One critical element is a sheer lack of accurate structural information and, in particular, of crystallographic data at atomic resolution on the recognition interface between biopolymers and synthetic foldamers. Two early examples are the crystal structures of a peptoid bound to Src homology 3 (SH3) protein[8b] and that of an oligoamide of pyrrole and imidazole bound to the complementary sequence of duplex DNA.[9b]

In this context, the recent publication[21] by Fairlie, Gellman and colleagues of the crystal structure of an α/β + α chimeric peptide bound to the BH3-recognition cleft of the protein Bcl-x$_L$ represents a highly important contribution (Figure 2). This structure validates (and provides a structural basis for) a number of sequence–affinity relationships previously established in solution studies. It confirms that the α/β decameric segment adopts a 14/15 helical conformation (Figure 1), whereas the C-terminal α-peptidic segment is α-helical. It shows why either side-chain modifications or residue homologation at some α-peptidic positions of the foldamer in close contact with Bcl-x$_L$ result in a substantial decrease of foldamer binding. On the other hand, some solvent-exposed residues may be modified with greater tolerance. The structure validates the initial concept that a foldamer helix can mimic an α-helix if it can (very) accurately reproduce the spatial organization of the
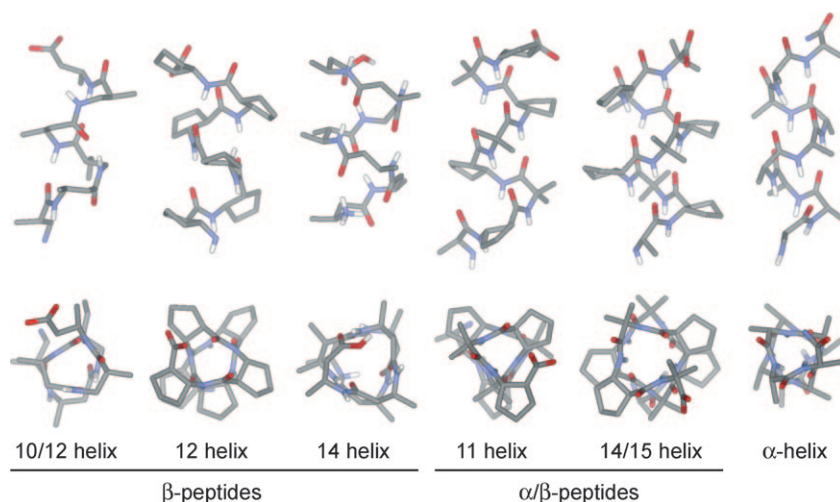
Figure 1. Side and top views of various β- and α/β-peptide helices and of an α-helix illustrating the global common appearance of these structures yet clear differences in the spatial organization of side chains.

mimic α-helices. Several works tend to validate this hypothesis: β-peptidic helices have been used to bind to the hydrophobic groove of the protein hDM2 and to inhibit interactions with the α-helix of the protein p53;[18] they have been shown to bind to proteins of the brush border membrane and inhibit cholesterol absorption by the small intestine;[16] they have even served to replace an entire α-helical domain of a human interleukin 8 that then remains sufficiently well folded to be active.[17] However, in all three cases, the β-peptide helix only mimics the overall amphipathicity of the natural α-helix. It is unclear whether interactions between the side chains of the target protein and its natural α-helical ligand/fragment are mimicked in all their intricacy by the β-peptide helix.

the same sequences, either in periodic arrangements (e.g., αβαβ, ααββ, etc.) or in nonperiodic arrangements (e.g. α/β + α).[19, 20] Definite progress was shown by the identification of α/β + α chimeric peptides that inhibit the interaction between Bcl-x$_L$ and its proapoptotic partner with K_d values in the 2 nM range. It is striking, however, that despite intensive efforts, these sequences were not derived from a structure-based design, but from a systematic variation of α and β residues, a method named "sequence-based design" by the authors.[20] Since the design of synthetic foldamers rests primarily on structural considerations, it is reasonable to hope that their interactions with biopolymers would also be structure-based. Why have developments in this direction remained so
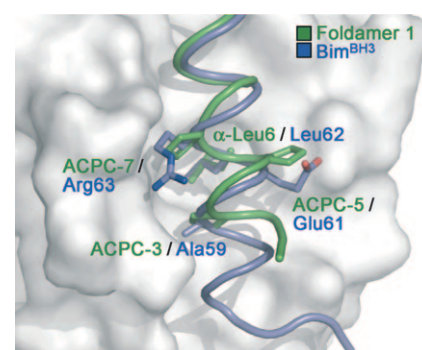
Figure 2. Overlay of the crystal structures of a Bim^{BH3} α-peptide (blue ribbon) and of an α/β + α chimeric peptide foldamer (green ribbon) bound to the BH3-recognition cleft of Bcl-x$_L$ (in gray). Details of the ligand–protein interfaces, in particular at β-peptidic residues, and the analogously positioned side chains on Bim^{BH3} are shown. Reproduced with permission from ref. [21]. Copyright: Wiley-VCH, 2009.

α-peptide side chains. A careful examination of the foldamer–Bcl-x$_L$ complex structure also suggests that cyclic β-peptide residues as well (ACPC: *trans*-2-aminocyclopentane-carboxylic acid) can establish intimate new contacts with the protein surface and significantly contribute to complex stability (Figure 2).

Structural studies of foldamer–protein interactions thus greatly enhance our knowledge of how binding occurs and are expected to improve our ability to design foldamers targeted to specific proteins or nucleic acids. Even though examples have been rare thus far, more are expected to follow in the coming years. There are indeed many reasons to believe that foldamers, be they chemically similar or remote from biopolymers, might constitute adequate scaffolds for creating stable complexes based on exquisite surface complementarity or even chimeric tertiary structures involving protein, nucleic acid and foldamer fragments.

Acknowledgements

[1] *Foldamers: Structure, Properties and Applications* (Eds.: S. Hecht, I. Huc) Wiley-VCH, Weinheim, **2007**; C. M. Goodman, S. Choi, S. Shandler, W. F. DeGrado, *Nat. Chem. Biol.* **2007**, *3*, 252–262; D. J. Hill, M. J. Mio, R. B. Prince, T. S. Hughes, J. S. Moore, *Chem. Rev.* **2001**, *101*, 3893–4012.

[2] a) D. S. Daniels, E. J. Petersson, J. X. Qiu, A. Schepartz, *J. Am. Chem. Soc.* **2007**, *129*, 1532–1533; b) W. S. Horne, J. L. Price, J. L. Keck, S. H. Gellman, *J. Am. Chem. Soc.* **2007**, *129*, 4178–4180; c) J. L. Price, W. S. Horne, S. H. Gellman, *J. Am. Chem. Soc.* **2007**, *129*, 6376–6377; d) E. J. Petersson, A. J. Schepartz, *J. Am. Chem. Soc.* **2008**, *130*, 821–823; e) O. Khakshoor, B. Demeler, J. S. Nowick, *J. Am. Chem. Soc.* **2007**, *129*, 5558–5569; f) B. C. Lee, R. Zuckermann, K. A. Dill, *J. Am. Chem. Soc.* **2005**, *127*, 10999–11009; g) N. Delsuc, J.-M. Léger, S. Massip, I. Huc, *Angew. Chem.* **2007**, *119*, 218–221; *Angew. Chem. Int. Ed.* **2007**, *46*, 214–217.

[3] M. M. Müller, M. A. Windsor, W. C. Pomerantz, S. H. Gellman, D. Hilvert, *Angew. Chem.* **2009**, *121*, 940–943; *Angew. Chem. Int. Ed.* **2009**, *48*, 922–925.

[4] M. Koyack, R. Cheng in *Foldamers: Structure, Properties and Applications* (Eds.: S. Hecht, I. Huc), Wiley-VCH, Weinheim, **2007**, pp. 229–265.

[5] H. Yin, A. D. Hamilton, *Angew. Chem.* **2005**, *117*, 4200–4235; *Angew. Chem. Int. Ed.* **2005**, *44*, 4130–4163; T. Berg, *Angew. Chem.* **2003**, *115*, 2566–2586; *Angew. Chem. Int. Ed.* **2003**, *42*, 2462–2481.

[6] S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173–180.

[7] P. E. Nielsen, M. Egholm, R. H. Berg, O. Buchardt, *Science* **1991**, *254*, 1497–1500; P. E. Nielsen, *Acc. Chem. Res.* **1999**, *32*, 624–630.

[8] a) J. A. Patch, K. Kiershenbaum, S. L. Seurynck, R. N. Zuckermann, A. E. Barron in *Pseudo-Peptides in Drug Discovery* (Ed.: P. E. Nielsen), Wiley-VCH, Weinheim, **2004**, pp. 1–31; b) J. T. Nguyen, C. W. Turck, F. E. Cohen, R. N. Zuckermann, W. A. Lim, *Science* **1998**, *282*, 2088–2092.

[9] a) P. B. Dervan, *Bioorg. Med. Chem.* **2001**, *9*, 2215–2235; b) C. L. Kielkopf, S. White, J. W. Szewczyk, J. M. Turner, E. E. Baird, P. B. Dervan, D. C. Rees, *Science* **1998**, *282*, 111–115.

[10] P. S. Shirude, E. R. Gillies, S. Ladame, F. Godde, K. Shin-ya, I. Huc, S. Balasubramanian, *J. Am. Chem. Soc.* **2007**, *129*, 11890–11891.

[11] J. T. Ernst, J. Becerril, H. S. Park, H. Yin, A. D. Hamilton, *Angew. Chem.* **2003**, *115*, 553–557; *Angew. Chem. Int. Ed.* **2003**, *42*, 535–539; J. M. Rodriguez, A. D. Hamilton, *Angew. Chem.* **2007**, *119*, 8768–8771; *Angew. Chem. Int. Ed.* **2007**, *46*, 8614–8617.

[12] J. W. Chin, A. Schepartz, *Angew. Chem.* **2001**, *113*, 3922–3925; *Angew. Chem. Int. Ed.* **2001**, *40*, 3806–3809.

[13] S. Fournel, S. Wieckowski, W. Sun, N. Trouche, H. Dumortier, A. Bianco, O. Chaloin, M. Habib, J.-C. Peter, P. Schneider, B. Vray, R. E. Toes, R. Offringa, C. J. M. Melief, J. Hoebeke, G. Guichard, *Nat. Chem. Biol.* **2005**, *1*, 377–382.

[14] D. L. Walensky, A. L. Kung, I. Escher, T. J. Malia, S. Barbuto, R. D. Wright, G. Wagner, G. L. Verdine, S. J. Korsmeyer, *Science* **2004**, *305*, 1466–1470.

[15] R. Fasan, R. L. A. Dias, K. Moehle, O. Zerbe, J. W. Vrijbloed, D. Obrecht, J. A. Robinson, *Angew. Chem.* **2004**, *116*, 2161–2164; *Angew. Chem. Int. Ed.* **2004**, *43*, 2109–2112.

[16] M. Werder, H. Hauser, S. Abele, D. Seebach, *Helv. Chim. Acta* **1999**, *82*, 1774–1783.

[17] R. David, R. Günther, L. Baumann, T. Lühmann, D. Seebach, H.-J. Hofmann, A. G. Beck-Sickinger, *J. Am. Chem. Soc.* **2008**, *130*, 15311–15317.

[18] J. A. Kritzer, J. D. Lear, M. E. Hodsdon, A. Schepartz, *J. Am. Chem. Soc.* **2004**, *126*, 9468–9469.

[19] J. D. Sadowsky, W. D. Fairlie, E. B. Hadley, H. S. Lee, N. Umezawa, Z. Nikolovska-Coleska, S. Wang, D. C. Huang, Y. Tomita, S. H. Gellman, *J. Am. Chem. Soc.* **2007**, *129*, 139–154; J. D. Sadowsky, J. K. Murray, Y. Tomita, S. H. Gellman, *ChemBioChem* **2007**, *8*, 903–916.

[20] W. S. Horne, M. D. Boersma, M. A. Windsor, S. H. Gellman, *Angew. Chem.* **2008**, *120*, 2895–2898; *Angew. Chem. Int. Ed.* **2008**, *47*, 2853–2856.

[21] E. F. Lee, J. D. Sadowsky, B. J. Smith, P. E. Czabotar, K. J. Peterson-Kaufman, P. M. Colman, S. H. Gellman, W. D. Fairlie, *Angew. Chem.* **2009**, *121*, 4382–4386; *Angew. Chem. Int. Ed.* **2009**, *48*, 4318–4322.

[22] H. Shi, X. Fan, A. Sevilimedu, J. T. Lis, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 3742–3746; P. Parekh, J. Martin, Y. Chen, D. Colon, H. Wang, W. Tan, *Adv. Biochem. Eng./Biotechnol.* **2008**, *110*, 177–194.

[23] J. A. Robinson, S. DeMarco, F. Gombert, K. Moehle, D. Obrecht, *Drug Discovery Today* **2008**, *13*, 944–951; M. J. Perez de Vega, M. Martín-Martínez, R. González-Muñiz, *Curr. Top. Med. Chem.* **2007**, *7*, 33–62.