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REVIEW

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Intramolecular backbone···backbone hydrogen bonds in polypeptide conformations. The other way around: ε-turn

Claudio Toniolo[1,2] | Marco Crisma[2] | Fernando Formaggio[1,2] | Carlos Alemán[3] | Chandrasekharan Ramakrishnan[4] | Neha Kalmankar[5] | Padmanabhan Balaram[4]

[1] Department of Chemistry, University of Padova, Padova 35131, Italy

[2] Institute of Biomolecular Chemistry, Padova Unit, CNR, Padova 35131, Italy

[3] Departament d'Enginyeria Quimica, ETSEIB, Universitat Politècnica de Catalunya, Barcelona 08028, Spain

[4] Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560012, India

[5] National Centre for Biological Sciences (TIFR), GKVK Campus, Bangalore 560065, India

Correspondence
Claudio Toniolo, Department of Chemistry, University of Padova, via Marzolo 1, 35131 Padova, Italy.
Email: claudio.toniolo@unipd.it

Abstract

In this study, we performed a detailed literature survey of the ε-turn in peptides and proteins. This three-dimensional structural feature is characterized by an eleven-membered pseudo-cycle closed by an intramolecular backbone. . .backbone H-bond. Interestingly, in this motif the direction of the N-H. . .O = C H-bond runs opposite to that of the much more popular and extensively investigated α-, β-, and γ-turns. We did not authenticate unequivocally the ε-turn main-chain reversal topology in any linear short peptide. However, it is frequently observed in small cyclic peptides formed by four, five, and six amino acid residues with stringent geometric requirements. Rather surprisingly, ε-turns do occur in proteins, although to a relatively moderate extent, as an isolated feature or in the turn segment of hairpin motifs based on two antiparallel, pleated β-strands. Moreover, the ε-turn may also host not only the seven-membered, intramolecularly H-bonded, pseudo-cycle termed γ-turn, either of the classic or inverse type, but also one (or even two) *cis* peptide bond(s) or a β-bulge conformation. Based on their ϕ, ψ backbone torsion angles, we were able to classify the protein ε-turns in six different families. Conformational energy computations using the DFT methodology were also performed on the ε-turns adopted by the amino acid triplet -Gly-Gly-Gly- (Gly is the most commonly found residue at each of the three positions in our analysis of proteins). Again, in this computational study, six families of turns were identified, but only some of them resemble rather closely those extracted from our investigation on proteins.

KEYWORDS

conformational energy calculations, NMR, peptide turns, solution conformation, X-ray diffraction

1 | INTRODUCTION

The architecture of polypeptide three-dimensional (3D) structures is characterized by well-defined basic elements such as turns, helices, and sheets. The diversity of turn types and conformations has been revealed by over half a century of theoretical, crystallographic and spectroscopic studies on proteins and peptides, the latter both acyclic and cyclic. An overwhelming number of them are stabilized by intramolecular C = O. . .H-N or N-H. . .O = C backbone. . .backbone H-bonds. Commonly accepted geometric cut-off criteria for the occurrence of H-bonds between peptide N-H and C = O groups (although somewhat arbitrary because the nature of such H-bonds is largely electrostatic and the energy of a dipole-dipole interaction diminishes only slowly as $-r^{-3}$, rather than vanishing abruptly)[1] are that the H. . .O distance should be ≤ 2.5 Å, the N. . .O distance ≤ 3.2–3.5 Å, and the N-H. . .O angle larger than 110–120°.[2–4]

An intramolecular H-bond between the *m*th N-H of an α-amino acid sequence and the *n*th C = O is designated as *m*

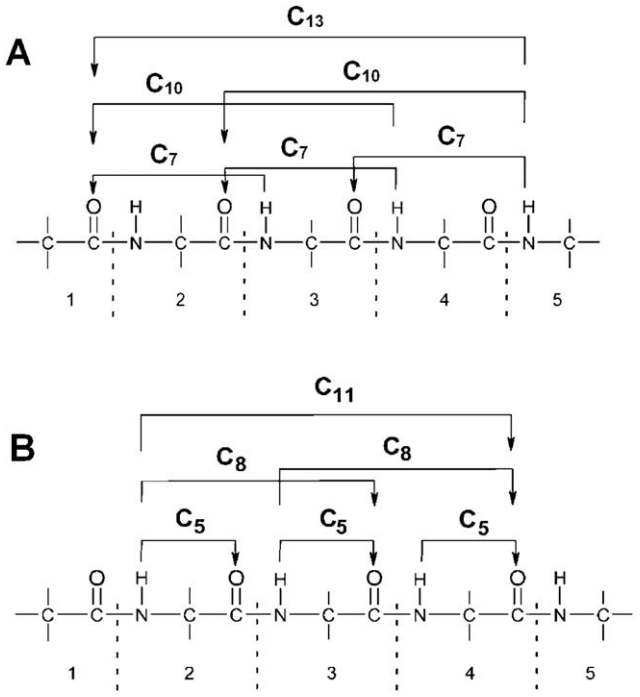

FIGURE 1 Possible intramolecularly H-bonded sets of conformations in a system of four covalently linked peptide units. **A**: the common way. **B**: the other way around

→ *n* (H-bonding donor → H-bonding acceptor). As a consequence, the possible intramolecularly H-bonded sets of 3D structures in a system of four covalently linked peptide units (Figure 1) include (**A**): the 3 → 1 (or 4 → 2, or 5 → 3, or C_7-, or γ-turn);[5–24] the 4 → 1 (or 5 → 2, or C_{10}-, or β-turn);[5,6,13–15,22–30] and the 5 → 1 (or C_{13}-, or α-turn)[5,13,31–34] conformations. (**B**): the 2 → 2 (or 3 → 3, or 4 → 4, or C_5, or fully extended);[13,14,35,36] the 2 → 3 (or 3 → 4, or C_8-, or δ-turn);[6,37] the 2 → 4 (or C_{11}-, or ε-turn) conformations. Note that the capital letter C stands for (pseudo)-cyclic and the subscript indicates the number of atoms closed by the intramolecular H-bond in that specific annular structure. As for those conformations: (i) all of them are folded, except C_5, and (ii) all of them, except C_5 and C_7, are forced to (C_8) or may (C_{10}, C_{13}, C_{11}) involve an internal peptide bond in the *cis* conformation. A large body of review articles, some of them published recently, discussed in detail occurrence and properties of the C_7, C_{10}, C_{13}, C_5, and C_8 conformations in peptides and proteins (see references mentioned above). The conformations in part **A** of Figure 1 represent the "common way" for the direction of intramolecular H-bond formation between an upstream backbone carbonyl oxygen and a downstream N-H hydrogen. Conversely, those in part **B** are disposed "the other way around," with an upstream N-H hydrogen and a downstream carbonyl oxygen. These alternative sets of intramolecularly H-bonded polypeptide conformations were first discussed as early as 1950 by Bragg, Kendrew and Perutz (BKP)[6] and termed types **A** and **B**, respectively. Notably, the BKP paper did not recognize the planarity of the peptide unit. At this point, it is worth pointing out that by purpose in this review we did not take into consideration possible intramolecularly H-bonded 3D structures occurring in longer systems of (five or six) covalently linked peptide units, for example, the C_{16}− (or π-) turn for the "common way" and the C_{14}− and C_{17}− turns for "the other way around" H-bonding (for a selection of articles, see Refs. 38–47). We planned to investigate these three types of wider turns in peptides and proteins in the near future.

To date, among those mentioned above in a system of four covalently linked peptide units, only the C_{11}−(hereafter termed ε-) turn peptide motif (Figure 2) has not been considered as the main target of a review article. Here, results from published studies on the ε-turn, as obtained from a literature survey on peptides and proteins, accompanied by data on our recent conformational energy computations on a model peptide, are presented and discussed.

2 | LITERATURE SURVEY ON PEPTIDES

As early as in the two decades 1935–1955, the principles and basic elements underlying polypeptide 3D structures were discussed extensively. In particular, it was proposed that not only fully extended (C_5) or nearly extended (pleated β-sheets) conformations, but also folded or helical conformations were sterically possible, all of them (except some β-pleated sheet types) being characterized by intramolecular H-bonds which link backbone N-H to backbone C = O groups. In this connection, also the ε-turn was repeatedly

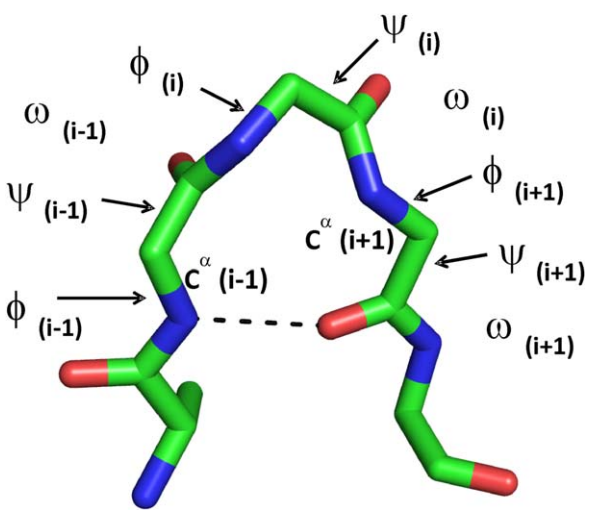

FIGURE 2 The ε (or C_{11} or 2 → 4) intramolecularly H-bonded turn, one of the possible "the other way around" sets of conformations in a system of four covalently linked peptide units. The various sub-sets of the ε-turn conformation are determined by the ϕ, ψ, and ω torsion angles for each residue $i - 1$, i, and $i + 1$

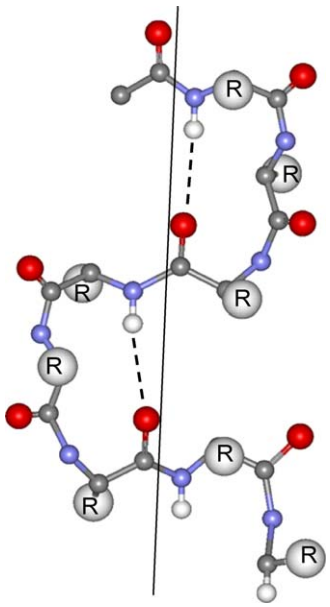

FIGURE 3 The 11-atom ring polypeptide helix. Adapted from Bragg et al.[6]

mentioned as a potential constituent.[6–8] In 1952, Huggins[7] even calculated the atomic coordinates of the 11-atom ring polypeptide helix, first mentioned by Bragg et al.[6] (Figure 3). However, the occurrence of this long-range conformation was heavily challenged by Pauling and Corey[8] in a side-by-side communication in the same J. Am. Chem. Soc. issue and labeled as unacceptable because of its very large total strain energy of amide bond deformation.

A number of ε-turn types can exist, depending upon the conformation of the two peptide groups (ω torsion angles) internal to the annular structure and the ϕ, ψ torsion angles of the three α-amino acid residues involved. Almost 20 years after Huggins, from their theoretical computations De Santis and coworkers[48,49] were the first to suggest the occurrence of a folded form of the ε-turn type [with two consecutive *cis* peptide bonds involving the D-Val-L-Pro and L-Pro-Sar (Sar is sarcosine or MeGly) tertiary amides and an intramolecular H-bond between the D-Val N-H and Sar C = O functionalities] in the *isolated* cyclopentapeptide lactone part of actinomycin D (Figure 4). This antibiotic is known to bind to double helical DNA. The following backbone torsion angles were proposed: $\phi = -50°$, $\psi = -92°$ for D-Val; $\phi = -68°$, $\psi = 157°$ for L-Pro; $\phi = -86°$, $\psi = 164°$ for Sar. It is evident that the conformation of the L-Pro-Sar dipeptide closely parallels that of a short type-I poly(Pro)$_n$ segment.[50] This conformation agrees well with the results of an earlier ^{1}H NMR experiment on the same peptide (in particular, with the slow proton exchange rate of the D-Val amide NH proton and the splitting of the Sar CH_2 protons).[51] The X-ray diffraction structure of the crystalline 1:2 complex containing a full actinomycin D molecule (consisting of two cyclopentapeptide lactone rings arranged almost perpendicularly to a phenoxazone moiety linker on both sides) and two deoxyguanosine molecules was published at the same time.[52,53] Between the two neighboring cyclopentapeptides, a pair of *inter*-annular H-bonds is formed connecting the D-Val N-H to the O = C of the opposite D-Val. No other H-bonds stabilize the actinomycin 3D structure, either of the inter- or intra-annular type (in other words, the ε-turn H-bond is missing in this complex).

In their classical paper, where the γ-turn is described for the first time, Némethy and Printz[16] proposed that this reversal of the peptide backbone can connect two strands of an antiparallel pleated β-sheet conformation with the stabilizing participation of *two* intramolecular H-bonds. One of them (γ-turn) is a strongly bent bond, while the other (ε-turn) is nearly straight and of optimal strength (Figure 5A). The same research group developed a model of this tight γ-/ε- turn also for the central tripeptide sequence -Val^4-Tyr^5-Val^6- of the peptide hormone angiotensin II on the basis of experimental evidence and conformational energy computations.[18] However, the proof for the existence of this 180° polypeptide chain reversal was first reported by Matthews[17] who solved the X-ray diffraction structure of the enzyme thermolysin and showed that the tripeptide sequence Ser^{25}-Thr^{26}-Tyr^{27} is indeed folded in the γ-/ε- turn (Figure 5B). The following backbone torsion angles were observed: $\phi = -148°$, $\psi = 92°$ for L-Ser^{25}, $\phi = 86°$, $\psi = -57°$ for L-Thr^{26}, and $\phi = -114°$, $\psi = 148°$ for L-Tyr^{27} (the positive value for the ϕ torsion angle of the central L-Thr^{26} residue,

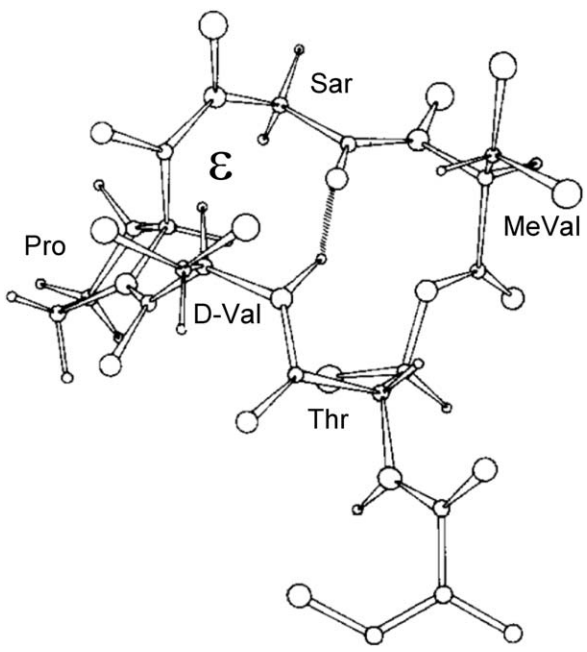

FIGURE 4 The 3D structure of the *isolated* cyclopentapeptide lactone fragment of actinomycin D (adapted from De Santis and coworkers[48,49]) on the basis of energy calculations. The N-H. . .O = C intramolecular H-bond is represented by a dashed line

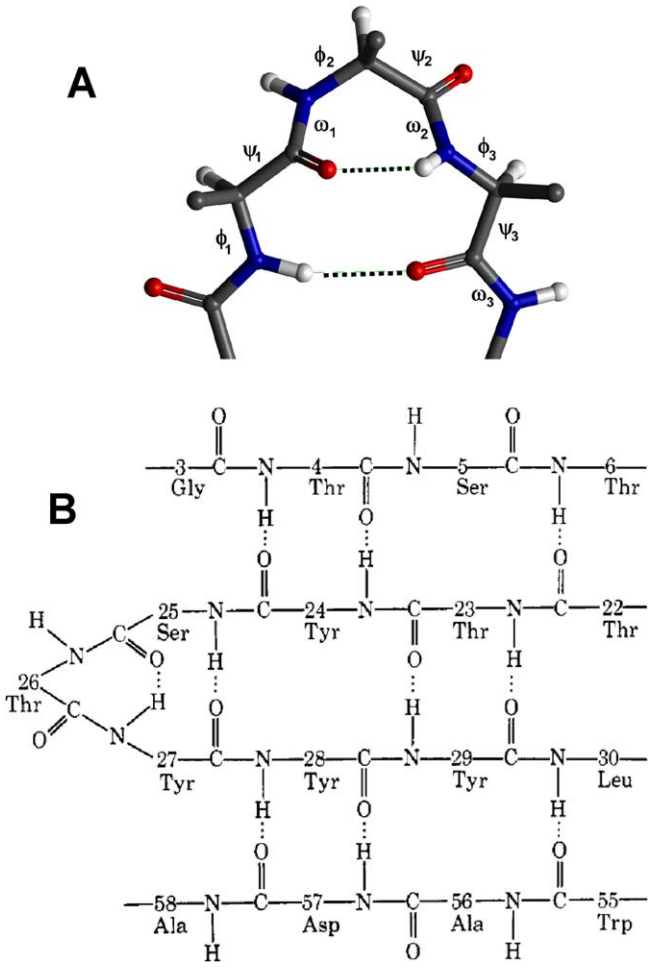

FIGURE 5 **A**: Perspective drawing of a tripeptide in an ε-turn which incorporates a smaller γ-turn.[16] The two intramolecular H-bonds are marked by dashed lines. The backbone torsion angles for each residue are indicated next to each respective bond. **B**: Secondary structure of the enzyme thermolysin in the region of the γ-/ε-turn spanning the tripeptide sequence Ser^{25}-Thr^{26}-Tyr^{27}. Adapted from Ref. [17]

typical of a "classic" γ-turn,[17] is worth noting). Interestingly, this unexpected finding was correctly interpreted without previous knowledge of the Némethy and Printz's prediction.[16] The Thr^{26} side chain is at an apex of the protein molecule. The ε-turn H-bond is at least partially protected from the solvent by the side chains of three neighboring Tyr residues. A subsequent high resolution (1.6 Å) refinement of the crystallographic 3D structure of thermolysin unambiguously confirmed the aforementioned unusual conformational feature.[19]

Before 1976, two additional papers on the ε-turn deserve mention. Schwyzer, Wüthrich, and coworkers[54] used ^{1}H NMR to suggest that two conformers occur in the homodetic cyclopentapeptide *c*-(Gly-L-Ala-Gly-Gly-L-Pro) in DMSO (dimethylsulfoxide) solution at room temperature. The Gly-L-Pro tertiary amide bond is *cis* (in the major conformer) or *trans* (in the minor conformer). The major conformer has two intramolecular H-bonds (each giving rise to an ε-turn), whereas the minor conformer has only one such H-bond, involving the Gly(3) N-H and Pro(5) C = O groups (Figure 6). It should be noted that in a cyclopentapeptide the ε-turn is always accompanied by a β- (or C_{10}-) turn in the other part of the molecule. On the basis of only a chemical structure—bioactivity relationship study and a CD analysis of eledoisin and some C-terminal segments thereof, Miyoshi and Sugano[55] proposed that the hexapeptide amide backbone of this hormone would have a turning point at the -L-Ile^3-Gly^4-L-Leu^5- sequence, with the Ile N-H and the Leu C = O groups making an intramolecular H-bond (ε-turn). All peptide bonds are *trans*.[55]

In summary, in the 40 year-period 1935–1975 the ε-turn was authenticated without ambiguity only in the crystal structure of thermolysin.[17,19] This scenario changed drastically when X-ray diffraction crystallography began to be extensively applied to cyclopeptides. In our search, including the Cambridge Crystallographic Database[56] (CSD version 5.36, updated to November 2014, organics only) by using a generic tripeptide backbone as the search fragment and the criteria N(1)...O(3) 2.5–3.6 Å and H(1)...O(3) 1.5–2.7 Å, 14 examples of ε-turns in cyclopeptides were identified: one in a cyclotetra-, 11 in cyclopenta- (including one cyclodepsi), and two in cyclohexapeptides (Table 1).[57–69] Only one tertiary amide bond (entry 10 in Table 1), internal to the ε-turn, is disposed in the *cis* conformation (the related ω_1 Gly-Pro bond has a value of −8.2°). As stated (in part) earlier in the text, in a cyclo*tetra*peptide the ε-turn always generates a γ-

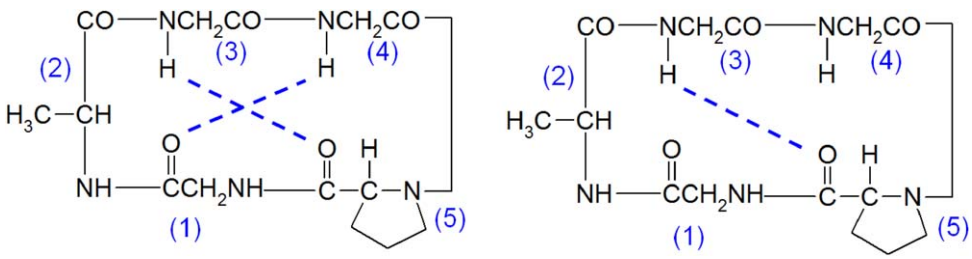

FIGURE 6 The cyclopentapeptide *c*-(Gly-L-Ala-Gly-Gly-L-Pro) with two (**M**) or one (**m**) intramolecular H-bonds (dashed lines). **M** is the major conformer and **m** is the minor conformer. Adapted from Ref. [54]

TABLE 1 ε-Turns reported in X-ray diffraction structures of cyclic peptides

Entry	Name of peptide	Type of ring	Type of turn(s)	ϕ/ψ Values (°)[a]	Structural features	CSD code	Ref.
1	*c*-(Aib-L-Phe-D-Pro-L-Gly*) · H_2Odihydrochlamydocin monohydrate	*cyclo*-4-peptide	two ε/γ	83.0/−72.8 (Aib) 71.9/−63.7 (D-Pro)	Gly* has a complex, *bis*-chiral side chain	DHCMYD10	[57]
2	*c*-(D-Phe-L-Pro-D-Phe-L-Pro-L-Hyv) alternaramide	*cyclo*-5-peptide (3 independent molecules)	one ε/γ	−80.1/75.3 (L-Pro) −83.7/70.4 (L-Pro) −82.8/67.5 (L-Pro)	cyclopenta-*depsi*peptide (1 backbone ester bond)	ASOSAD	[58]
3	*c*-[L-Thr(Bzl)-D-Val-L- Pro-Sar-L-MeAla]	*cyclo*-5-peptide (2 independent molecules)	one ε	120.0/−88.2 (D-Val) 126.3/−72.7 (D-Val)	L-Thr is side-chain protected	BEHTEN	[59]
4	*c*-(Gly-L-Pro-Gly-D-Ala-D-Pro)	*cyclo*-5-peptide	one ε/γ	−86.0/70.4 (L-Pro)		CGPGAP	[60]
5	*c*-(Gly-L-Pro-L-Ser-D-Ala-L-Pro) · CH_2Cl_2	*cyclo*-5-peptide	one ε	85.8/−122.6 (D-Ala)		CGPSAQ	[61]
6	*c*-(D-Phe-L-Pro-Gly-D-Ala-L-Pro)	*cyclo*-5-peptide	one ε/γ	−82/59 (L-Pro)		PAPGAP	[62]
7	*c*-(Gly-L-Pro-D-Phe-Gly-L-Val) · 2 H_2O	*cyclo*-5-peptide	one ε	−70.4/−39.5 (L-Val)		FUDWIK	[63]
8	*c*-(Gly-L-Pro-D-Phe-D-Ala-L-Pro)	*cyclo*-5-peptide	one ε/γ	not available (L-Pro)		–	[64]
9	*c*-(Gly-L-Pro-D-Phe-Gly-L-Ala)	*cyclo*-5-peptide	one ε	58/65 (L-Ala)		FIVSAE	[65]
10	*c*-[Gly-(αMe)Phe-Aib-Aib-Gly]	*cyclo*-5-peptide	one ε	−57.7/−25.3 (Aib)		MUCYIT	[66]
11	*c*-(D-Ala-L-Pro-Gly-Gly-Δ^ZPhe)	*cyclo*-5-peptide	one ε	53/35 (Δ^ZPhe)		–	[64]
12	*c*-[L-Phe-L-Ser(Bzl)-L- Ser(Bzl)-L-Phe-MeAib]	*cyclo*-5-peptide	one ε	77.4/5.1 [L-Ser(Bzl)]	the two L-Ser are side-chain protected	XACJAM	[67]
13	*c*-(L-Ala-D-Ala-L-MeTyr*-L-Ala-L-Me-Tyr*-L- MeTyr*) · $CHCl_3$ · EtOH · 0.5 H_2O	*cyclo*-6-peptide	one ε/(distorted) γ	−93.1/52.1 (L-MeTyr*)	MeTyr* residues are O-and ring-substituted	OHUXAQ	[68]
14	*c*-(L-Pro-Gly-L-Pro-Gly-L-Pro-Gly) · 1.5 H_2O	*cyclo*-6-peptide	one ε	−68.1/−12.0 (L-Pro)	ω −8.2° (*cis*)	PROGLY20	[69]

Notes: Hyv: α-hydroxyvaleric acid. Bzl: benzyl. MeAla: N-methyl Ala. (αMe)Phe: C^{α}-methyl Phe. Δ^ZPhe: α,β-didehydro Phe (*Z*-configurational isomer). MeAib: N-methyl Aib. MeTyr: N-methyl Tyr.

[a]These values refer to those of the central residue of the ε-turn amino acid triplet.

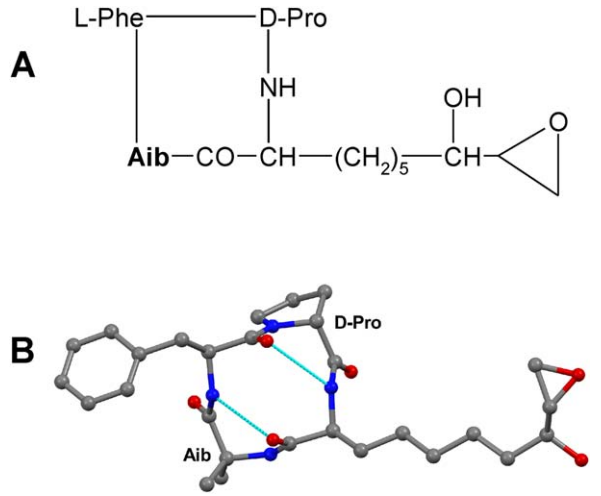

FIGURE 7 **A**: The chemical structure and **B**: the X-ray diffraction structure of the cyclotetrapeptide dihydrochlamydocin (entry **1** in Table 1). Adapted from Ref. [57]

(or C_7-) turn in the other part of the molecule, whereas in a cyclo*penta*peptide it is accompanied by a β- (or C_{10}-) turn and in a cyclohexapeptide by an α- (or C_{13}-) turn.

The ε-turns and their 3D structural details found in our investigation on cyclic peptides are listed in Table 1. Particularly significant examples are shown in Figures 7–9 for cyclotetra-, cyclopenta-, and cyclo*hexa*peptides, respectively. In the much less active dihydro-derivative of the keto-epoxide, cytostatic metabolite chlamydocin from *D. chlamydosporia* (with a Gly* side-chain secondary alcohol function replacing the ketone group), entry **1**, two ε-turns were observed each containing a smaller γ-turn at the Aib (α-aminoisobutyric acid) and D-Pro corners (Figure 7).[57] In addition to establishing the configurations of the two side-chain chiral carbon atoms, this crystallographic structure allowed the authors to highlight two features first observed at that time (1976) in a cyclotetrapeptide: an all-*transoid* (although with significantly nonplanar ω torsion angles: ≅ ±160°) conformation and intramolecular H-bonds of the ε-/γ- turn types. Interestingly, at variance with Pro that is relatively often seen in an (*inverse*) γ-turn,[8,16,17] the Aib residue is forced to being in a γ-turn, a unique conformation for this C^{α}-tetrasubstituted α-amino acid.[70] More recently, the X-ray diffraction structure of an analog of the natural chlamydocin where the Aib residue was replaced by L-Ala,[71] did not exhibit any ε-/γ- turn feature. Here, the L-Phe-D-Pro tertiary amide is *cis* (the related ω torsion angle is 3.4°, 10.9°, and 7.9° in the three independent molecules). Not surprisingly, these two conformations of chlamydocin and a variety of its analogs [(i) all-*transoid*, *bis*-turn intramolecularly H-bonded, and (ii) *cis*, *trans*, *trans*, *trans* without any intramolecular H-bond] are identical to those reported by NMR for these molecules in different solvents.[72–74]

The most extensively investigated peptide ring size is that formed by 15 atoms (cyclo*penta*peptides; Table 1). In a cyclopeptide (entry **2**), one of the amide bonds is replaced by an ester (L-Pro-L-Hyv) (Hyv, α-hydroxyvaleric acid) (cyclo*depsi*peptide). Two crystallographic structures are illustrated in Figure 8. Four of them (entries **2**, **4**, **6**, and **8**) show an ε-turn which includes an *inverse* γ-turn. All have an L-Pro residue in the central position of the ε-turn amino acid triplet. In entries **7**, **9**, and **10** the central residue (L-Val, L-Ala, and Aib, respectively) is helical (this type of main-chain reversal is termed *H-turn* in Ref. 53), whereas in entry **12** the central L-Ser(Bzl) (Bzl, benzyl) is in the "bridge" region[75] of the conformational space. Some of the peptide bonds (ω torsion angles) are remarkably non planar. Considering that in entries **3** and **5**, the central residue (D-Val and D-Ala, respectively) is almost *semi*-extended, it is remarkable that a constrained three-residue segment could adopt several very different conformations depending on amino acid sequence

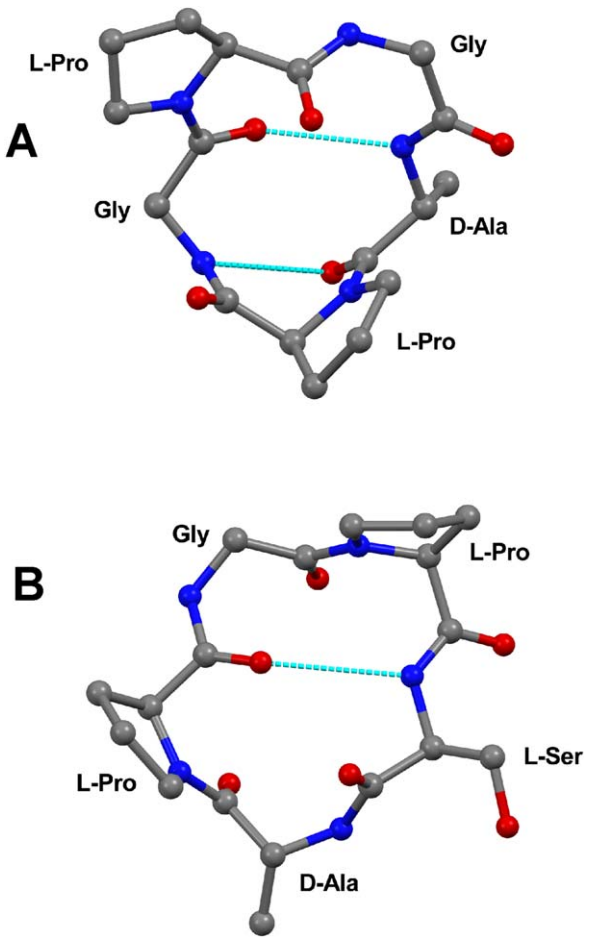

FIGURE 8 The X-ray diffraction structures of **A**: the cyclopentapeptide *c*-(Gly-L-Pro-Gly-D-Ala-D-Pro) (entry **4** in Table 1) (adapted from Ref. [60]) and **B**: the cyclopentapeptide *c*-(Gly-L-Pro-L-Ser-D-Ala-L-Pro) (entry **5** in Table 1) (adapted from Ref. [61]). In both crystallographic structures the intramolecular H-bonds are marked

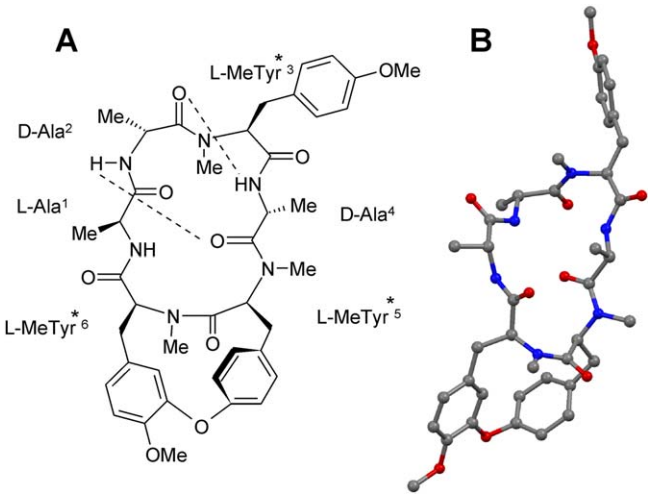

FIGURE 9 **A**: The chemical structure and **B**: the X-ray diffraction structure of a synthetic epimer of the natural cyclohexapeptide RA-VII (entry **13** in Table 1). Adapted from Ref. 68. In part **A**, the intramolecular H-bonds are marked

and, perhaps, driven by crystal packing influences. Obviously, in all 11 structures of cyclopentapeptides solved by X-ray diffraction the ε-turn is accompanied by a β-turn in the other part of the molecule. The observed β-turns are typically type-II (II′) depending on the order (L-D or D-L) of the heterochiral dipeptide sequence involved (clearly, one chiral amino acid can be replaced by an achiral one). This all-*trans*, ε-turn conformation (with an *internal* γ-turn) was also documented for cyclopentapeptides in solution by use of detailed NMR experiments.[76,77] An extremely careful analysis on all-*trans* cyclopentapeptides was also conducted by conformational energy calculations.[78] It was found that the minimum energy conformations fall into six families. In general, two large categories of conformations were identified: (i) with ε-/γ- turns and (ii) with the ε-turn only. Only some of these conformations have formed good crystals. Surprisingly, at variance with the X-ray diffraction crystal data, in solution the accompanying β-turn can easily be accommodated in the type-I (I′) disposition as well, as first reported by Kessler.[79]

The backbone of the older of the only two available crystal structures of cyclohexapeptides,[59] *c*-(L-Pro-Gly-L-Pro-Gly-L-Pro-Gly) · 1.5 H_2O (entry **14**), was surprisingly found to be non symmetric as it is formed by five *trans* peptide groups and one *cis* (the ω torsion angle value for one Gly-Pro bond is −8.2°). The only intramolecular H-bond observed (joining a Gly N-H and the C = O of the next Gly) generates an ε-turn conformation, accompanied by an α-(C_{13}-) turn in the other part of the molecule. The central residue (L-Pro) of the amino acid triplet participating in the ε-turn is distorted helical ($\phi = -68.1°$, $\psi = -12.0°$). The *cis* tertiary peptide bond is that *internal* to the ε-turn. The water molecule forms a bridge between two oxygen atoms separated by two intervening residues.

In the crystal state the D-Ala[2] synthetic epimer of the naturally occurring, antitumoral cyclohexapeptide RA-VII (Figure 9**B** and entry **13**), exhibits the *trans*, *trans*, *trans*, *trans*, *cis*, *trans* series for the amide conformations.[68] The chemical structure of this more recently studied, rather exotic compound (Figure 9**A**) is actually characterized by a *bi*cyclic system wherein the second ring is generated by a highly strained, 14-membered cycloisodityrosine dipeptide unit containing a *cis* L-MeTyr*-L-MeTyr* tertiary amide bond. The -D-Ala[2]-L-MeTyr*[3]-D-Ala[4]- tripeptide segment is involved in two intramolecular H-bonds, which generate an ε-turn and an enclosed distorted, *inverse* γ-turn (the ϕ, ψ values for the central L-MeTyr*[3] residue are −93.1°, 52.1°). The -L-MeTyr*[5]-L-MeTyr*[6]-L-Ala[1]- tripeptide sequence forms an α-(C_{13}-) turn characterized by the aforementioned internal *cis* peptide bond. A close relationship between the conformation in $CDCl_3$ solution and that in the crystalline state was strongly supported by NOESY correlations in the NMR spectra.

As a final corollary for this section, more than 30 years ago an intriguing proposal was published for the conformation of a synthetic heterodetic peptide in $CDCl_3$ solution based on an NMR analysis (Figure 10).[80] The γ/ε-turn motif of this N-terminally protected trimer methylamide, Boc-L-Cys-L-Ala-L-Cys-NHMe (Boc, *tert*-butyloxycarbonyl; NHMe, methylamino), is stabilized by an intramolecular side-chain to side-chain cystine disulfide bridge. Hopefully, the feasibility of all three conformational constraints at the same time in a linear peptide will be carefully checked in the near future by computer modeling. However, in a more polar environment (DMSO) the ε-turn H-bond is broken. A potential example in proteins of this double turn system possessing an extra 11-membered disulfide loop might occur in the -L-Cys[82]-L-His-L-Cys[84]- sequence of the α-subunit of human chorionic gonadotropin.[81]

3 | ANALYSIS ON PROTEINS

We surveyed the Protein Data Bank to July 2015 for the occurrence of the ε- (C_{11}−) turn, a 3D structural element

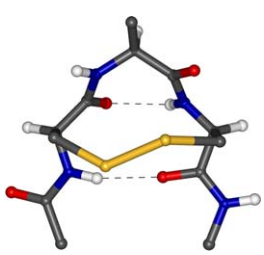

FIGURE 10 The γ-/ε-turn conformation comprising an intramolecular cystine disulfide proposed for the tripeptide Boc-L-Cys-L-Ala-L-Cys-NHMe (adapted from Ref. 80). The intramolecular H-bonds are marked

TABLE 2 Families of ε-turns in proteins with all-*trans* ω torsion angles based on the sets of the ϕ, ψ torsion angle signs for the $i-1$, i, and $i+1$ residues[a,b]

		$i-1$ residue		i residue		$i+1$ residue	
Family	**No. of members**	**ϕ**	**ψ**	**ϕ**	**ψ**	**ϕ**	**ψ**
1	59	−	−	−	0	−	+
2	14	+	−	−	0	−	+
3	14	−	+	+	−	−	+
4	10	−	+	+	+	+	+/−
5	8	−	−	−	−	−	−
6	2	−	+	−	0	−	+

[a]"0" indicates 0 ± 45°; "+" indicates a positive value > 45°; " − "indicates a negative value < −45°.
[b]Only families with at least two examples of ε-turns were classified. Four different ε-turns were labeled as unclassified.

with the directionality of the H-bond, as stated earlier in the text, running counter to that typically observed for α-, β-, and γ-turns. Only the structures determined by X-ray (including synchrotron radiation) and neutron diffractions were taken up. The N. . .O distance and ∠HNO angle in the 11-membered *pseudo*-cycles were fixed at ≤ 3.5 Å and < 40°, respectively. This procedure resulted in a redundant dataset of 4027 PDB codes in which at least one C_{11}-type H-bond was found. After applying a resolution cut-off of 1.5 Å, 459 structures remained. After removal of various types of identical examples (≤ 40% identity cut-off and single representative chain selection), the analyzed dataset consisted of as many as 120 unique examples of ε-turns (see Supporting Information), 111 of which are characterized by all-*trans* amide bonds, while nine contain one (eight examples) or two (one example) *cis* amide bonds in the amino acid triplet. In six out of the eight examples of ε-turns with a single *cis* amide bond, this uncommon feature joins residues $i-1$ and i. Among the 111 all-*trans* amide peptides, 41 exhibit "isolated" ε-turns, while the remaining 70 contribute in generating a hairpin motif connecting two, antiparallel, pleated β-strands. We classified 107 out of the 111 all-*trans* amide ε-turns into six families based on the ϕ, ψ torsion angles for the $i-1$, i, and $i+1$ residues of the triplet, and with at least two examples each (Table 2) (using these parameters, four ε-turns remain "unclassified").

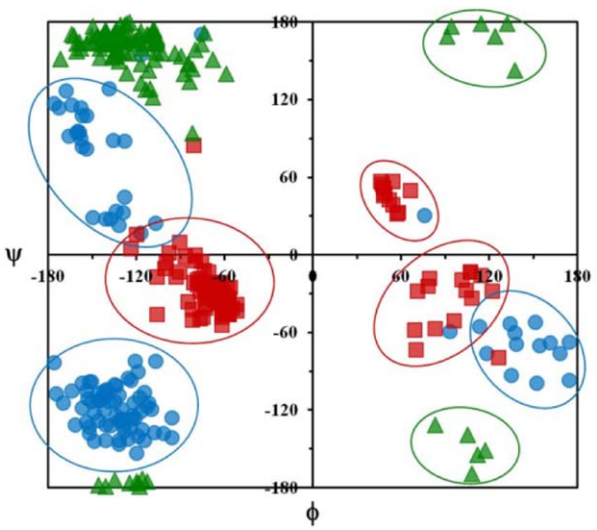

FIGURE 11 Scatter plot of the ϕ, ψ torsion angles for residues $i-1$ (blue circles), i (red squares), and $i+1$ (green triangles) in the intramolecularly H-bonded, all-*trans* ε-turns found in proteins

In Figure 11, which shows a scatter plot of the ϕ, ψ torsion angles for the three residues in each of the all-*trans* amide ε-turns, it is worth noting that as many as 13%, 23%, and 9% of the $i-1$, i, and $i+1$ residues have positive ϕ values, an unusual observation for L-configured residues. Interestingly, the percentages of the screw-sense neutral Gly (the most extensively occurring amino acid in each position of the triplet) are 41%, 17%, and 23%, respectively. Curiously, the population of Gly is least at position i where the highest percentage of residues with the positive ϕ value is found. Other commonly (≥ 8% or more) observed amino acids at the three positions of the ε-turns are: Ser (10%) at position $i-1$; Asp (8%), Glu (9%), His (8%), and Thr (9%) at position i; Ser (9%) and Thr (11%) at position $i+1$. It is particularly intriguing that residues with potentially H-bonding active side chains (Asp, Glu, and His) are relatively much more frequently seen in the central position i of the triplet.

Figures 12–18 illustrate representative examples of ε-turns in proteins, each with a different emblematic feature. In particular, Figures 12 and 13 show a selection of ε-turns extracted from the by far most populated family (**1** in Table 2) covering about 50% of the total examples found. Specifically, the two cases reported in Figure 12 are characterized by all-*trans* amide bonds, the central residue (Gly or Leu) of

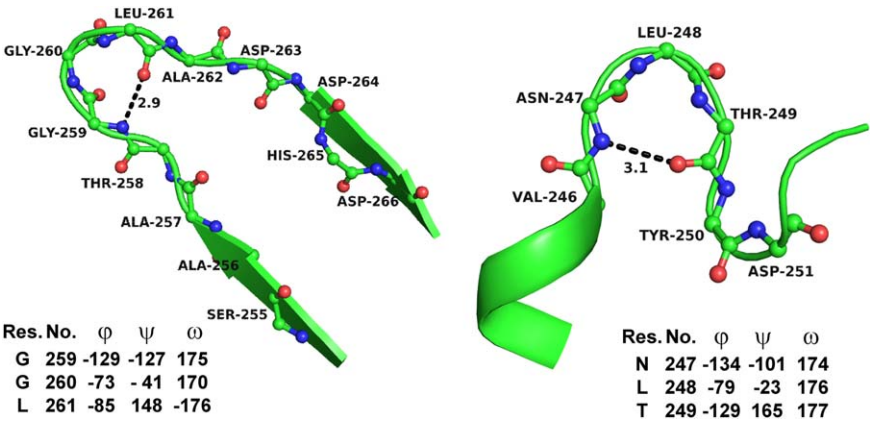

FIGURE 12 Two representative examples of the most abundant family (**1** in Table 2) of "isolated" ε-turns in proteins [left, domain of P58/ergic-53, PDB ID: 1GV9; right, GDSL-like lipase/acylhydrolase, PDB ID: 4Q7Q]. Here, the central residue of each of the two triplets is (distorted) helical

the triplet accommodated in a (distorted) helical conformation, and an "isolated" (where other main chain-to-main chain N-H. . .O = C H-bonds are absent) ε-turn. The protein segment in Figure 13 presents the same two features mentioned above for both examples of Figure 12, but here the ε-turn is nucleating an overall hairpin conformation with two long pleated β-strands running antiparallel. We also found that the "isolated" and hairpin ε-turns constitute about 37%–38% and 62%–63% of all ε-turns, respectively.

We observed an additional feature incorporated in some ε-turns, namely the occurrence of a γ-(C_7-) turn with an 1 ← 3 intramolecular H-bond. Figure 14 shows the classic Némethy/Matthews[16,17,19] γ-turn inserted in a hairpin ε-turn (same situation as in Figure 5). The residue entirely involved in this tight turn is Asp. This situation is not rare in ε-turns, as authenticated in the 14 examples (8%) of family **3** in Table 2, where the signs of the ϕ, ψ torsion angles of residue *i* are +, −. A much less common case is that referring to the Matthews[17,19] *inverse* γ-turn, exemplified again by Asp, with the −, + signs of the ϕ, ψ torsion angles (Figure 15).

Among the eight examples of ε-turns with one *cis* amide bond, in six of them the residue involved is located at position $i-1$. Not surprisingly, the following residue is Pro, so that the *cis* bond is always a tertiary amide (e.g., the -Ala-Pro- bond shown in Figure 16). The sets of ϕ, ψ angles of all these Pro residues fall in the "bridge" region ($-22° < \phi < 12°$)[75] of the Ramachandran map. In the additional two examples, the residue with the *cis* amide bond is seen at position *i*. Their dipeptide sequences are -Glu-Pro- and -Asp-Cys-. Moreover, the unique ε-turn forming triplet with two consecutive *cis* amide bonds is -His-Ala-Pro- (Figure 17) with the values of the ω_{i-1} and ω_i torsion angles −10° and 0°. This bizarre hairpin conformation is stabilized by (a) a side chain (His $N^{\pi}H$)-to-main chain (Ala C = O) intramolecular H-bond forming an eight-membered ring system within

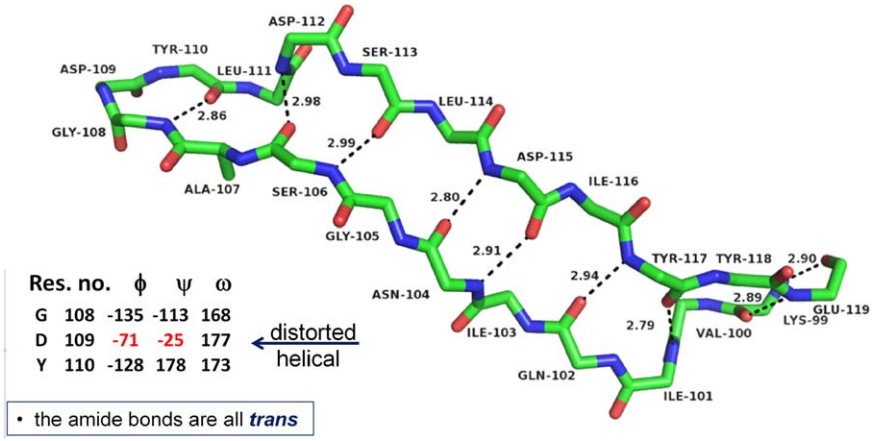

FIGURE 13 A representative example of the most abundant family (**1** in Table 2) of ε-turns in proteins [griffithsin, PDB ID: 2GUD]. It exhibits an overall hairpin conformation, with the ε-turn at the -Gly-Asp-Tyr- triplet and two long antiparallel, pleated β-strands. Here, the central residue of the triplet is (distorted) helical

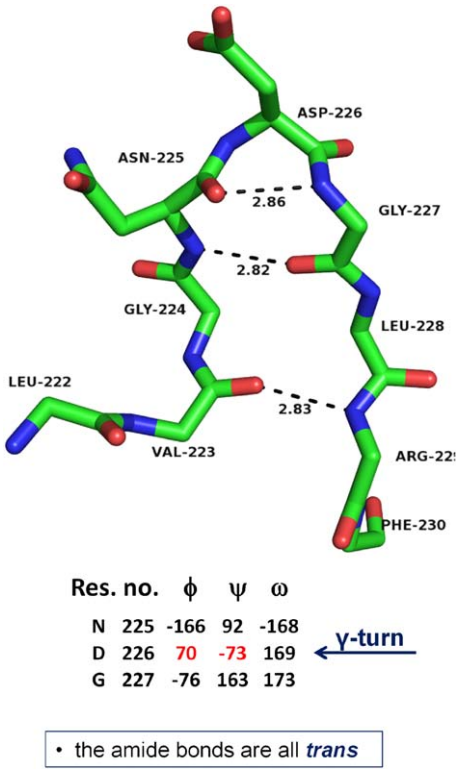

FIGURE 14 An example of an ε-turn in proteins containing the central Asp residue of the triplet in the Némethy[16]/Matthews[17,19] γ-turn conformation [β-N-acetylhexosaminidase, PDB ID: 4AZ6]

the ε-turn, and (b) a phenyl...phenyl interaction between two Phe residues located on the two adjacent antiparallel strands.

In the last Figure 18 of this section, we describe an ε-turn with a non-Gly (here, Glu) residue characterized by an uncommon Ramachandran conformation (*left*-handed, *semi*-extended) at position $i-1$ of the triplet. The ϕ, ψ set of torsion angles of the central residue (Pro) can be classified as intermediate between a helical and a bridge conformation. The tertiary amide bond Glu-Pro is accommodated in the *cis* conformation. A Coulombic interaction between the oppositely charged Glu and Arg (the latter far away in the sequence but spatially proximal) side chains exerts an energy compensatory effect.

Last but not least, two additional intriguing examples of ε-turns (not shown) deserve to be mentioned: (a) An ε-turn, Trp-Gly-Trp (endo-1,4-β-glucanase, PDB ID: 3AMN, residues 176–178), with two Trp residues at positions $i-1$ and $i+1$ in formally disallowed conformations (the corresponding sets of torsion angles are $\phi_{i-1} = -116°$, $\psi_{i-1} = -135°$ and $\phi_{i+1} = -150°$, $\psi_{i+1} = 177°$, respectively). The central (*i*) Gly adopts a conformation intermediate between helical and bridge. Here, energy compensatory effects are provided by aromatic...aromatic and aromatic...peptide interactions. (b) A Gly-Gln-Gly ε-turn (methionine aminopeptidase, PDB ID: 4A6W, residues 174–176) nucleating an antiparallel β-strand motif and accommodating a β-bulge, immediately preceding (Ile) and following (dipeptide sequence -Phe-His-) the ε-turn, stabilized by a Phe...His aromatic...aromatic interaction.

4 | CONFORMATIONAL ENERGY CALCULATIONS ON MODEL PEPTIDES

Based on the results from our analysis on proteins discussed above, which demonstrated that Gly is the residue most extensively populating each of the three positions of the ε-turn, we studied the preferred conformations of the terminally blocked tripeptide Ac-Gly-Gly-Gly-NHMe (Ac, acetyl) using conformational energy calculations. Density functional theory (DFT) computations were performed using the Gaussian 09[82] computer package at the B3LYP/6-31 + G(d, p)[83,84] level. All calculations were carried out *in vacuo*.

To this end, starting conformations were constructed by varying the sets of the ϕ, ψ torsion angles of the three Gly residues in steps of 20°, while the ω torsion angles were fixed in the *trans* (180°) disposition. In addition, selected ε-turn conformations with a *cis* ω torsion angle were

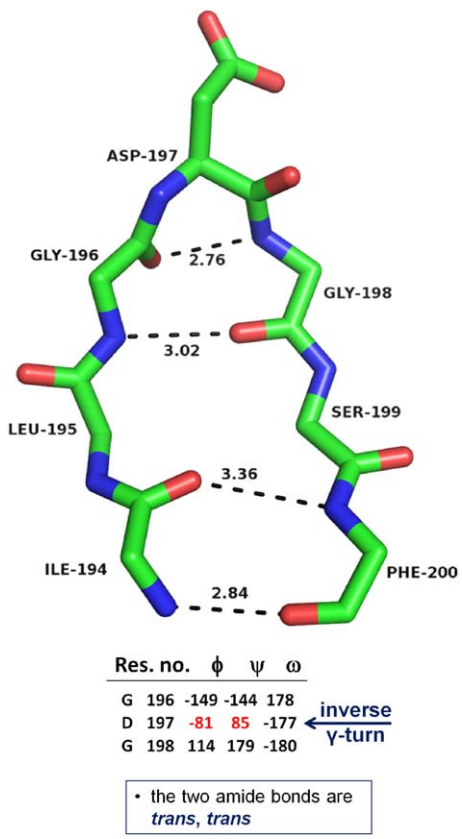

FIGURE 15 An example of an ε-turn in proteins containing the central Asp residue of the triplet in the Matthews *inverse* γ-turn[17,19] conformation [biotin protein ligase, PDB ID: 2E10]

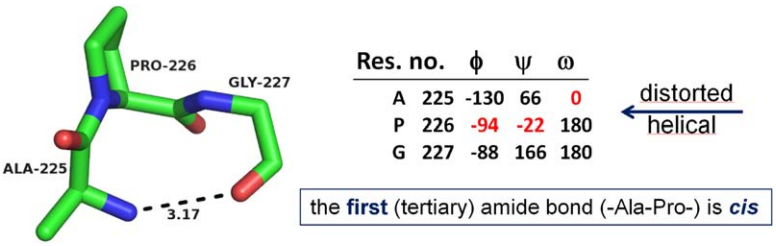

FIGURE 16 An example of an ε-turn in proteins characterized by a single *cis* (tertiary) amide unit at the -Ala-Pro- sequence of the triplet [hydroxynitrile lyase, PDB ID: 1JU2]

constructed using the torsion angles extracted from our literature survey on proteins. All such initial conformations were submitted to geometry optimization. Frequency analyses were carried out to verify the nature of the minimum state of selected stationary points (i.e., those involving ε- and β-turns and that derived from the all-*trans* ϕ, ψ, ω conformation) and to determine the zero-point vibrational energies as well as the thermal and entropic corrections. These terms were used to compute the ΔG values at 298 K. This analysis, which was performed using the standard expressions for an ideal gas in the canonical ensemble, treats all modes, other than the free rotations and translations, as harmonic vibrations.

Calculations of the simple and flexible tripeptide amide Ac-Gly-Gly-Gly-NHMe, lacking any side chain, and in the gas phase, thus avoiding any influence of the environment, provide information on the intrinsic stabilities of the various types of ε-turns. The relative stabilities of the ε-turns identified were calculated with respect to the all-*trans* ϕ, ψ, ω, fully extended conformation,[13,14,35] which is stabilized by three, consecutive, C_5 interactions. The ϕ, ψ, ω torsion angles and the relative energies in the gas phase (ΔG_{gp}) for all ε-turn minima are listed in Table 3. Only six ε-turn minima (**A**–**F**) were identified. Since the model tripeptide investigated is achiral, each of the enantiomeric conformers obtained by inverting the signs of all torsion angles of minima **A**–**F** is isoenergetic to its counterpart. To investigate the effect of solvent, we also calculated the free energy of hydration for each conformation obtained in the gas phase. Appropriate combination of the free energies of hydration with the ΔG_{gp} values allowed us to estimate the relative free energies in aqueous solution (ΔG_{wat} values), also given in Table 3.

From the results of these calculations, we conclude that the intrinsic stability of the ε-turn is relatively low, as deduced from the observation that the most favored ε-turn in the gas phase, **A** in Table 3 and (**b**) in Figure 19, is 3.5 kcal/mole less stable with respect to the all-*trans* ϕ, ψ, ω conformation. Also, the two most stable ε-turn minima, **A** and the related **B**, (**c**) in Figure 19, are characterized by a co-existing γ-turn in the central position, of the classic type in **A** while of the *inverse* type in **B**[16,17,19] [this intramolecular H-bond provides an extra stability with respect to structure **C**, (**d**) in Figure 19, which does not show any γ-turn]. Two of the minima observed (**D** and **E**) exhibit a single peptide bond (the N-terminal one) arranged in the *cis* conformation, while one minimum (**F**) presents two (the N-terminal and the

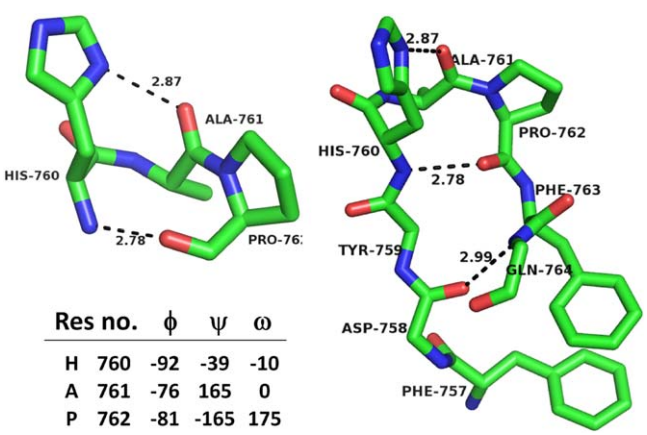

FIGURE 17 A rare and bizarre example of two consecutive *cis* amide units in an ε-turn in proteins [1,2-α-L-fucosidase, PDB ID: 2EAB]. Two additional uncommon features are seen: (1) a side chain (His $N^{\pi}H$)-to-main chain (Ala C = O) intramolecularly H-bonded C_8 local conformation within the ε-turn, and (2) an aromatic (Phe^{757}). . .aromatic (Phe^{763}) stabilizing interaction in the protein antiparallel β-strand region

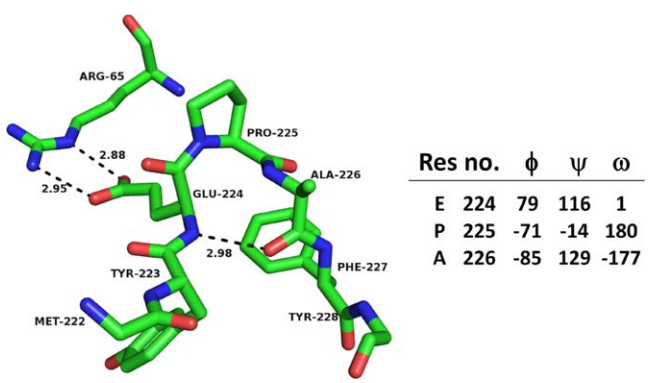

FIGURE 18 An example of an ε-turn with a non-Gly residue (here, Glu) having an unusual set of ϕ, ψ torsion angles at position $i - 1$ [L-rhamnose isomerase, PDB ID: 3M0M]. The -Glu-Pro- tertiary amide is *cis*. The energy compensatory effect of an Arg^{+}. . .Glu^{-} salt bridge is also observed

TABLE 3 Detailed description of the ε-turn forming Ac-Gly-Gly-Gly-NHMe minima (**A–F**) calculated in the gas phase and comparison with the all-*trans* ϕ, ψ ,ω (fully extended) conformation and the most stable (type II) β-turn conformation[a]

Conformations	ϕ_1	ψ_1	ω_1	ϕ_2	ψ_2	ω_2	ϕ_3	ψ_3	ω_3	ΔG_{gp}	ΔG_{wat}	Type of H-bonding interactions
all-*trans* ϕ, ψ, ω	180.0	180.0	180.0	180.0	180.0	180.0	180.0	180.0	180.0	0.0	0.0	three consecutive C_5
A	−133.0	43.9	−166.5	80.1	−70.1	161.9	−67.9	152.0	179.3	3.5	6.1	ε-turn (2.08 Å, 159.2°) + γ-turn
B	−137.6	−118.7	169.5	−78.7	72.9	−164.2	73.3	−145.6	−179.7	4.8	5.9	ε-turn (2.07 Å, 169.7°) + γ-turn
C	−135.9	−127.5	175.6	−72.7	−16.8	162.9	−129.7	164.0	179.3	6.3	4.3	ε-turn (2.18 Å, 166.2°)
D	107.6	145.3	−4.2	−75.9	−11.8	168.6	−108.4	165.1	178.4	8.1	6.2	ε-turn (2.19 Å, 146.8°)
E	−121.1	37.6	25.7	−95.9	−8.8	171.3	−105.7	−170.5	177.1	8.3	8.3	ε-turn (2.04 Å, 165.3°)
F	−85.3	−36.4	−17.2	−64.0	−178.5	−16.2	−79.6	−145.0	−175.4	17.4	11.3	ε-turn (2.11 Å, 161.8°)
β-turn (type II)	−173.7	171.3	173.1	−61.7	132.9	−174.1	98.5	−13.3	−176.8	3.4	2.3	β-turn (2.20 Å, 163.1°)

[a]ϕ, ψ, ω torsion angles in (°), relative free energy in the gas-phase (ΔG_{gp}) in kcal/mol, relative free energy in aqueous solution (ΔG_{wat}) in kcal/mol, H-bonding parameters (H. . .O distance in Å and N-H. . .O angle in °) for the ε- and β-turns. The additional γ-turn incorporated into the ε-turn, if formed at the level of the central residue, is mentioned.

central) peptide bonds in *cis* [structures **D**, **E**, and **F** are labeled (**a**), (**b**), and (**c**), respectively, in Figure 20]. Peptide bonds arranged in *cis* do not contribute to the stabilization of

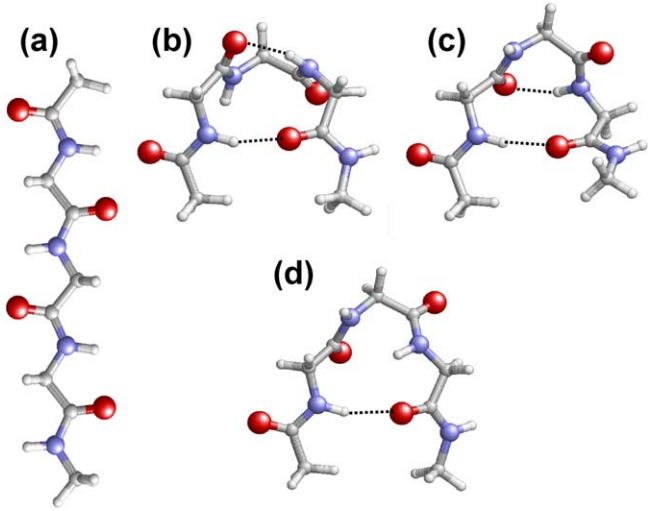

FIGURE 19 Minimum energy structures calculated for Ac-Gly-Gly-Gly-NHMe in the gas phase: (**a**) corresponds to the structure all-*trans* in Table 3, (**b**) to structure **A**, (**c**) to structure **B**, and (**d**) to structure **C**. Intramolecular H-bonds are indicated by dotted lines

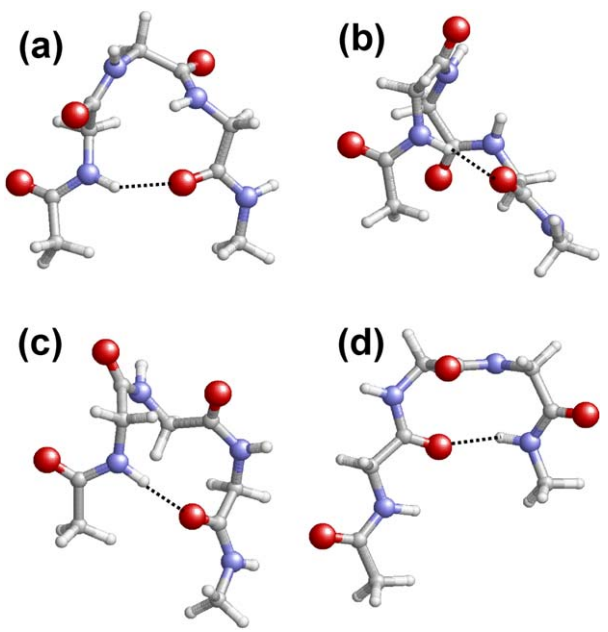

FIGURE 20 Minimum energy structures calculated for Ac-Gly-Gly-Gly-NHMe in the gas phase: (**a**) corresponds to structure **D** in Table 3, (**b**) to structure **E**, (**c**) to structure **F**, and (**d**) to the type II β-turn structure. Intramolecular H-bonds are indicated by dotted lines

the corresponding ε-turns. Table 3 and Figure 20 (**d**) also provide information on the most stable β-turn (C_{10}), type II, involving residues i and $i+1$, adopted by this tripeptide amide. Its stability is quite close to that of the most stable, type **A**, ε-turn. Formation of an ε-turn induces some strain in the tripeptide backbone, as it is evident from the relatively large deformations of the ω torsion angles from the ideal *trans* and *cis* values (180° and 0°, respectively). From the ΔG_{wat} values, the effect of solvent (water) is not immediately apparent in these calculations. In any case, it is clear that, in the absence of intramolecular interactions involving the side-chain functionalities, the solvent is not active enough to stabilize the ε-turn structures in peptides more than the all-*trans* ϕ, ψ, ω conformation.

If one compares the families of the *six* ε-turns identified by conformational energy calculations on the Ac-Gly-Gly-Gly-NHMe model triplet [only three (**A**, **B**, **C**) with all *trans* peptide bonds, Table 3] with the *six* ε-turn categories found in proteins (all with *trans* peptide groups, Table 2), it turns out that the correspondence is only partial. Specifically, one family (**C**) does correspond to the most abundant category from proteins (**1** in Table 2); one other (**A**) does, reflecting the Némethy[16]/Matthews[17,19] ε-/γ-turn (**3** in Table 2; Figure 14); the third (**B**) resembles only the uncommon Matthews[17,19] ε-/*inverse* γ-turn (Figure 15). On the other hand, the three families remaining (**D**, **E**, **F**) do not correspond closely to any of the six categories from proteins, the main difference being the occurrence of one (in **D** and **E**) or even two (in **F**) *cis* peptide bonds [however, very rare ε-turns with one or two *cis* peptide bonds do occur in proteins (Figures 16–18)].

5 | SUMMARY AND OUTLOOK

At variance with the common and well known α-, β-, and γ-turns, cases with H-bonded peptide backbone reversals where the H-bonding direction runs opposite (δ- and ε-turns) were supposed to be unusual. However, the results of our recent review article convincingly validate the conclusion that δ-turns, albeit not frequent nor remarkably stable, are not as rare as had been expected, at least in proteins, nor are energetically unstable.[37] In the present work, we extended our analysis to the immediately larger member of this family of turns (from the eight atoms characterizing the ring of a δ-turn to the eleven atoms typical of an ε-turn).

The present literature survey conclusively showed that the ε-turn is a rather common feature of small cyclic peptides, particularly of cyclopentapeptides, in that case being accompanied by the widespread β-turn in the other part of the molecule. The available results from the X-ray diffraction and NMR techniques unambiguously proved this conclusion. However, only very limited attention was paid by structural biochemists to the onset of this motif in published linear oligopeptides in which the stringent geometric requirements typical of their ring-forming counterparts are missing. Moreover, in our view synthetic peptide/peptidomimetic specialists, spectroscopists, and X-ray crystallographers have not shown so far significant interest to the discovery and utilization of coded and non-coded, α-amino acid ε-turn inducers.

Our results more exciting and hopefully prone to intriguing future developments in this area of structural biochemistry are those extracted from the analysis of ε-turns in proteins and from the conformational energy computations of their most representative triplet -Gly-Gly-Gly-. In particular, we grouped in six families both the ε-turns in proteins (based on their ϕ, ψ torsion angles similarities) and the most energy stable ε-turn types theoretically calculated. However, only some of the families are represented in each set. The reasons for this apparent discrepancy remain to be investigated more deeply. Finally, a still open, important issue is the role of interactions involving amino acid side chains on ε-turn stability. In this connection, appropriate emphasis should be given to our observation that all amino acid residues most extensively occurring at each $i-1$, i, and $i+1$ position of the ε-turn forming triplets (after Gly in each position) are characterized by polar side chains (containing aliphatic alcohol, carboxylic acid, or phenol functionalities) known to easily participate in H-bonding interactions.

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