Design and structural characterisation of monomeric water-soluble α -helix and β -hairpin peptides: State-of-the-art

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Keywords

Peptide structure, α -helix, β -hairpin, peptide design, NMR

Highlights

α -helix and β -hairpin peptides are models for protein folding and stability

Guidelines to design stable α -helical and β -hairpin-forming peptides are available

Peptide structures are characterised by spectroscopic techniques, mainly CD and NMR

Peptide structures are modulated and even modified by the environment

Current peptide design aims to get improved bioactivity or novel biomaterials

Graphical abstract

Abstract

Peptides are not only useful models for the structural understanding of protein folding and stability but also provide promising therapeutic avenues for the treatment of numerous diseases, and as biomaterials. The field has been very active over the last decades, but the complex conformational behaviour of peptides still poses challenges to the characterization and rational design of defined structures. In this context, we aim to provide a comprehensive overview of linear water-soluble monomeric peptides able to form the two simplest structural motifs: α -helices and β -hairpins. For both structures, we describe the geometry features, and the main contributions to stability: intrinsic propensities, position dependence of specific residues, particular capping motifs and side chain interactions. They should be considered to design α -helical or β -hairpin peptides. Solvent influence on peptide stability and selected *in silico* design approaches are also discussed. Moreover, we provide guidelines for structural characterization of α -helical and β -hairpin-forming peptides by NMR and circular dichroism. We also highlight recently reported designed peptides and current strategies developed to improve their stability, bioactivity and bioavailability. The information gathered herein may aid peptide design and characterization of stable α -helical and β -hairpin motifs in the search of biological constructs or improved peptide therapeutics.

Abbreviation list:

Aib: α -aminoisobutyric; Abz: 2-aminobenzoic acid; DOSY: diffusion ordered spectroscopy; HB: hydrogen-bonded; HBS: hydrogen bond surrogate; HFIP: hexafluoroisopropanol; Hop: 5-(1-Hydroxy-pyridin-2(1H)-onyl)-l-alanine; NOE: nuclear Overhauser effects; pI: isoelectric point; RDC: residual dipolar coupling; TFE: trifluoroethanol; VCD: Vibrational circular dichroism; VEGF: Vascular endothelial growth factor.

1. Introduction

The diversity of protein three-dimensional structures arises from a few secondary structure elements, i.e. helices, strands and turns, which are organized in different numbers and spatial arrangements. Studies on the conformational properties of peptides, both protein fragments and designed, were initiated in the early 1980's to shed light into protein folding and stability. Moreover, peptides are not only interesting as minimalist models of protein secondary structures, but they fulfil many biologically relevant functions, such as cosmetics, hormones, antimicrobial, antiangiogenic, etc. In this context, peptide design is mostly aimed to enhance their applicability either by improving their bioactivity or decreasing their adverse effects. The knowledge derived from peptide structural studies has provided very valuable information about the principles underlying folding and stability of secondary structures, which paves the way towards the rational design of peptides able to form α -helical and β -sheet structural motifs.

In this review, we aim to give an overview of the state-of-the-art in the design of the simplest structural motifs formed by peptides in aqueous solution, that is, α -helices and β -hairpins. We are going to analyse how amino acid sequence determines α -helix and β -hairpin formation and stability. This knowledge is applicable as guidelines to design α -helical and β -hairpin-forming peptides. Moreover, we will describe some recent examples of designed peptides. Solvation effects and their importance in peptide structure determination will be also discussed. Moreover, we are going to explain the main methods used for structural characterisation of peptides in solution.

2. Geometry features of α -helices and β -hairpins

2.1. α -helices

A right-handed α -helix (Figure 1A), the most common helix in proteins, is formed by a series of consecutive residues whose ϕ and ψ dihedral angles are -62° and -41° , respectively (these are the mean values in globular proteins; the angles for an ideal α -helix are -57° and -47° ; [1]). Along the α -helix, the carbonyl oxygen of residue i is hydrogen-bonded to the amide H_N hydrogen of residue $i+4$, so that all carbonyl CO and amide H_N groups participate in hydrogen bonds except for those at the termini. This type of helix is also known as 3.6_{13} -helix, being 3.6 the average number of residues per helical turn and 13 the number of atoms involved in the hydrogen-bonded loop. Another characteristic of α -helices is that they have a helix dipole parallel to the helix axis, which is positively charged at the N-end, and negatively charged at the C-end. This is a consequence of two facts: (i) every peptide bond contains a dipole moment parallel to HN and CO bonds, and (ii) in an ideal α -helix, HN and CO bonds, as well as the hydrogen bonds that stabilize the helix, are nearly parallel to the helix axis (Figure 1A). Hence, the dipole moment from all peptide bonds in a α -helix sum up and give rise to a helix dipole [2]. As for side chains, they point outwards in order to reduce steric clashes (Figure 1B). Distribution of hydrophobic and polar/charged side chains may confer amphiphilic character to α -helices. Other helical structures are the 3_{10} -helix and the π -helix in which the carbonyl oxygen of residue i hydrogen bonds to the amide hydrogen of residue $i+3$ and $i+5$, respectively. Sometimes, the first and/or last helical turns exhibit 3_{10} -like conformations. These polypeptide secondary structures are beyond the scope of this review.

Figure 1. Ideal α -helix structure showing two helical turns (residues 2-8): (A) Lateral view showing backbone atoms. Hydrogen bonds are shown by blue dashed lines, and two $d_{\alpha N(i,i+3)}$ distances in magenta (approx. 3.4 Å). N- and C-termini are labeled N and C, respectively. Residues are labeled according to nomenclature shown in panel C. (B) Top-bottom view (C-termini looking upward the paper sheet). Side-chains are displayed in green. In both panels, H_N amide protons, H_α protons and oxygen atoms are shown as blue, white and red spheres, respectively. The $i,i+3$ and $i,i+4$ side chain interactions of residue 8 are highlighted by magenta lines. (C) Nomenclature of helical residues according to position [3].

2.2. β -hairpins

A β -hairpin, which is the simplest antiparallel β -sheet, is formed by two antiparallel hydrogen-bonded β -strands connected by a loop region of variable length and geometry (Figure 2). The ϕ and ψ dihedral angles in antiparallel β -strands are -139° and $+135^\circ$, respectively [4]. β -hairpin motifs are classified using a X:Y nomenclature [5], in which X is the total number of loop residues, and $Y=X$ if the CO and HN groups of the residues preceding and following the turn are both hydrogen-bonded (for example, in 2:2 and 4:4 β -hairpins; Figure 2A-D), and $Y=X+2$ if these residues form a single hydrogen bond (as in 3:5 β -hairpins; Figure 2E). Two kinds of β -strand sites can be distinguished in two-stranded β -sheets depending on whether the pair of facing residues are hydrogen-bonded (HB site), or not (nonHB site) (Figure 2A-E). The side chains of pairs of facing residues point outwards the same face of the β -sheet, but they are closer on nonHB sites than in HB sites (averaged distances are 2.4 Å and 2.8 Å, respectively; [6]). However, the side chains of residues at nonHB and HB sites point outwards opposite sides of the β -sheet plane, that is, side chains at one side belong to residues at nonHB sites, and at the other two HB sites (Figure 2B-C). Due to the right-handed twist of β -sheets (Figure 2F), the side chains of residues in two consecutive nonHB sites, but

at different strands (labelled as n4 and c2 in Figure 2), are also quite close (3.0 Å; [6]), this pair of side chains is denoted a diagonal interaction.

Figure 2. β-Hairpin structures. Schemes of the peptide backbones for: 2:2 (A), 4:4 (D), and 3:5 β-hairpins (E); and 2:2 β-hairpin formed by a 14-residue peptide in aqueous solution [7]: Backbone atoms for segment 3-12 are shown in panels B and C, and a ribbon representation in panel F. In panels A-E, turn residues are labelled (i to i+3), and N- and C-strand residues are numbered from the turn. In panels, B, C and E, the turn region is displayed in orange. Hydrogen bonds are indicated by dashed lines in panels A, C and D, and by blue lines in panels B and C. The 4:4 β-hairpin shown in panel D becomes a 4:6 if the encircled hydrogen bond is not formed. The nonHB and HB sites are coloured green and magenta, respectively. A light-green ellipse indicates the pair of diagonal residues, which has the spatially closest side chains. In panels B and C, oxygen atoms are displayed in red, amide H_N hydrogen atoms in blue, and the C_β of β-strand as spheres, which are coloured green for nonHB sites and magenta for HB sites. The β-hairpins shown in panels B and C correspond to a 180° rotation of the β-sheet plane. The double arrows in panels A, B, D and E link spatially close protons, which lead to observable NOEs. The average distances in protein antiparallel β-sheets are indicated in panel F.

Peptide design has focussed on β-hairpins with short loops, which mostly display regular β-turn topologies [5]. β-turns are four residues long and their first (i) and fourth (i+3) residues are spatially close (their C_α atoms are less than 7 Å far from each other), which leads to a change in the direction of the protein backbone. Frequently, the CO of residue i and the amide H_N of residue i+3 are hydrogen-bonded [8]. Different types of β-turns can be distinguished depending on the φ and ψ dihedral angles of the two central residues (i+1 and i+2). The β-turns in β-hairpins with short loops must have geometries

compatible with the characteristic right-handed twist of β -sheets (Figure 2F). In protein 2:2 β -hairpins, the most frequent loop conformation is a type I' β -turn followed by a type II'. This is in concordance with the right-handed twist displayed by these two types of β -turns, whose degree is larger in I' than II'. Their mirror images type I and II are left-handed twisted, and therefore unsuitable for β -hairpin structures. Indeed, type II β -turns, which are very common in proteins, are very rare in β -hairpins. Type I β -turns are very rarely found in 2:2 β -hairpins, but they are found in β -hairpins with slightly longer loops, such as 3:5 β -hairpins, which commonly have a type I + G1 loop (a type I β -turn in which the $i+3$ residue is usually a Gly, forming a sort of bulge), and 4:4 β -hairpins, which frequently have a type I β -turn. The inset in Figure 3 lists the ϕ and ψ dihedral angles for canonical β -turns of types I', II' and I. In 2:2 β -hairpins, residues i and $i+3$ of the type I' or type II' β -turn are hydrogen-bonded (Figure 3A-B).

Figure 3. Backbone structures of the β -turns types commonly present in β -hairpins: (A) Type I' β -turn for residues $^{37}\text{VAGQ}^{40}$ of a 2:2 β -hairpin in protein CarD from *M. xanthus* (pdb code: 2LT1); (B) Type II' β -turn for residues $^{67}\text{SGTD}^{70}$ of a 2:2 β -hairpin in the light chain of a crystallised VIH antibody (pdb code: 4WY7); (C) Type I β -turn for residues $^{25}\text{DRCE}^{28}$ of a 4:4 β -hairpin in human protein PHD (pdb code: 2M3H). H_N amide hydrogen, H_α hydrogen and oxygen atoms are displayed as blue, white and red spheres, respectively. Residues are labelled at the H_α atoms as i , $i+1$, $i+2$ and $i+3$. The H-bonds between the CO and H_N atoms of residues i and $i+3$ in the 2:2 β -hairpins (A & B panels) are shown by blue lines. The magenta arrows joint pairs of spatially closed protons, which give place to observable NOEs. The inset table lists the ϕ and ψ dihedral angles characteristic of ideal β -turns.

3. Experimental characterisation of peptide structures

3.1. State of association

Prior to structural characterisation, it is convenient to check the association state of the peptide under study. The simplest way to confirm that a particular peptide is monomeric, as aimed in most designs, and does not self-associate is to perform dilution experiments monitored by UV-CD (Ultraviolet circular dichroism) and/or ^1H -NMR. That UV-CD and 1D ^1H -NMR spectra acquired at two different peptide concentrations are identical is an indicator of monomeric states, whereas spectral changes evidence the presence of self-association, which will obviously grow upon peptide concentration increase. UV-CD spectra can be acquired at approximately 0.01-0.30 mM, and 1D ^1H -NMR spectra in cryoprobe-equipped NMR spectrometers can be recorded at concentrations as low as about 0.01 mM, with the high concentration being that employed for the structural characterisation (1-2 mM). But, if peptide solubility allows it, higher concentrations can be examined. The only caveat of dilution experiments is that the peptide might be already self-associated at the lowest examined concentration, and that the self-associated state remains invariable even at very high concentrations. To minimise this problem, the examined range of concentrations should be as broad as possible. In any case, spectral invariability upon dilution always discards the presence of non-specific aggregation. The monomeric or multimeric state of peptides can be confirmed by analytical ultracentrifugation [9-11], and by NMR diffusion ordered spectroscopy (DOSY) [12-15].

Since peptides, as proteins, are less water-soluble and more prone to self-associate at pH values close to their pI (isoelectric point), it is convenient to perform structural studies at pH values as far as possible from pI. Also, peptides with either a net positive charge or a net negative one are more likely to be water-soluble. Solubility criteria are taken into account for the design of both α -helical and β -hairpin forming peptides. A common strategy to increase peptide solubility in water is to incorporate N- or C-terminal tags composed of charged residues (usually Lys's), and sometimes separated by spacing linkers (usually Gly's) to avoid/minimise any interference of the tag to the peptide structure. In terms of helix stability, and because of the helix dipole, it is better to place Lys-tags at the C-end (section 4.1.1).

3.2. Conformational equilibrium

Once determined the association state, structural characterisation of peptides has to take into account that, in contrast to globular proteins, linear peptides do not have a single structure in solution, but they exist in equilibrium among different conformations [16, 17]. The "so-called" peptide structure is the preferred conformer within the equilibrium, which is usually fast. Hence, it is necessary to identify the preferred structure (α -helix, β -hairpin or any other), and the population or percentage of the structure within the conformational equilibrium.

The best technique to determine peptide structures in solution is NMR, but other spectroscopies, such as UV-CD, VCD (Vibrational circular dichroism) [18-22], FT-IR (Fourier-transform infra-red) [23-27] and fluorescence [28], provide structural data in peptides. Among them, UV-CD is the most extensively used since the first studies on α -helical and β -hairpin-forming peptides in the 80's and 90's until nowadays. Therefore, on the following we are going to describe how to characterise α -helices and β -hairpins by solution NMR and by UV-CD. Anyway, it should be mentioned that FT-IR is particularly useful in the case of β -hairpins [24, 26, 27], and that the use of VCD is increasing lately [18-22]. Concerning fluorescence, it requires the incorporation of

fluorophore groups, except in the case of Trp-containing peptides [28, 29]. Several designed peptides, usually hydrophobic, have been crystallised [30-32]. Crystal and NMR solution structures for some of these peptides were shown to be the same [31, 32].

3.3. Circular dichroism

α -Helices have very characteristic and intense bands in the far UV-CD [33]: a maximum about 192 nm, and two minima at 208 and 222 nm, so that the α -helix formation can be detected by UV-CD even at relatively low populations. A well-established method to quantify α -helix content (f_H ; [34]) is based on the intensity of the 222 nm band (experimental ellipticity denoted as $[\theta]_{222}^{\text{exp}}$, $\text{deg cm}^2 \text{dmol}^{-1}$) by applying the equation 1:

$$f_H = ([\theta]_{222}^{\text{exp}} - [\theta]_{222}^{0\% \text{helix}}) / ([\theta]_{222}^{100\% \text{helix}} - [\theta]_{222}^{0\% \text{helix}}) \quad \text{Eq. 1}$$

$$\text{in which } [\theta]_{222}^{100\% \text{helix}} = (-44000 + 250T) (1 - 3/n) \quad \text{Eq. 2}$$

$$\text{and } [\theta]_{222}^{0\% \text{helix}} = (2220 - 53T) \quad \text{Eq. 3}$$

being n the number of residues, and T the temperature in $^{\circ}\text{C}$.

The simplest equation 4, which assumes a $[\theta]_{222}^{0\% \text{helix}}$ equal to 0, and does not correct by temperature dependence, is applied in many CD studies [35-37]:

$$f_H = [\theta]_{222}^{\text{exp}} / (-39500(1 - 2.57/n)) \quad \text{Eq. 4}$$

in which n is the number of peptide bonds.

It should also be mentioned that molar ellipticity ($[\theta]$) depends on peptide concentration (Eq. 5):

$$[\theta] = \theta / (10 \times l \times C) \quad \text{Eq. 5}$$

in which θ is ellipticity in mdeg, l is the pathlength in cm, and C is molar concentration.

Hence, peptide concentrations have to be measured as accurate as possible for reliable quantification of α -helix content by CD. The best method to determine peptide concentrations is by UV absorbance using the molar extinction coefficient at 280 nm, which requires the presence of aromatic residues, particularly Tyr and Trp, in the sequence. Peptide concentrations are very poorly determined in aromatic-lacking sequences. In some cases, Tyr-tags at the N- or C-ends, usually separated by Gly residues from the sequence designed to be structured to avoid interference with it ([38, 39]. Experimental errors by weight, and by protocols based in UV absorbance at 205-215 nm [40] are large. Recently, a method to estimate the extinction coefficient at 205 nm from peptide sequence has been proposed as a way to get accurate concentrations in Trp- and Tyr-lacking peptides [41].

UV-CD spectra of β -hairpins are a combination of the bands from the two-stranded antiparallel β -sheet moiety plus the bands for the loop region (Figure 2). As α -helices, β -sheets exhibit characteristic bands [33]: a maximum about 198 nm, and a minimum at about 215 nm, but their intensities are lower than those of the helices. UV-CD bands for the loop region depend on its conformation, and they are not well established, even for canonical β -turns. Therefore, it is not always feasible to confirm β -hairpin formation only on the basis of UV-CD. In any peptide structure, far UV-CD spectra can be affected by Tyr and Trp side chains, and by disulphide bonds, which lead to positive bands in the 225-235 nm region. Since they are of opposite sign to the negative 222 nm band employed for quantification of α -helix populations, they might affect the resulting populations. The far UV-CD spectra of the β -hairpins, which contain edge-to-face Trp/Trp pairs at non-HB sites, display a maximum at 227-229 nm and a minimum at

213-215 nm, and their near UV-CD has well-defined bands around 290 nm (see [42] and references therein).

3.4. NMR parameters

Information about different structural characteristics of peptides can be derived from several NMR parameters: ^1H and ^{13}C chemical shifts (δ), J-couplings and NOEs. Prior to any NMR analysis, chemical shifts of the peptide under study have to be assigned. ^1H chemical shifts of peptides are assigned following the standard strategy developed by Wüthrich [43]. ^1H , ^1H -COSY and ^1H , ^1H -TOCSY spectra are analysed to identify and classify the spin systems, and ^1H , ^1H -NOESY or ^1H , ^1H -ROESY spectra for sequential assignment of the spin systems. Once assigned the ^1H chemical shifts, ^{13}C chemical shifts are straightforwardly assigned by analysis of ^1H , ^{13}C -HSQC spectra recorded at natural abundance of ^{13}C .

The J-coupling between the amide H_N and H_α protons ($^3J_{\text{HN-H}\alpha}$) is related to the ϕ dihedral angle, its value is different for ideal α -helices (≈ 4 Hz), antiparallel β -strands (≈ 9 Hz), and β -turns, in which depends on the β -turn type (≈ 3 -4 Hz for $i+1$ residue, and ≈ 6 -8 Hz for $i+2$ residue) [43]. However, in most peptides the type of secondary structure cannot be derived from J-couplings, because the experimentally observed $^3J_{\text{HN-H}\alpha}$ values are population-weighted averages among all the peptide conformations in equilibrium, and also the range of $^3J_{\text{HN-H}\alpha}$ values is small.

Chemical shifts for $^1\text{H}_\alpha$ protons, and for $^{13}\text{C}_\alpha$ and $^{13}\text{C}_\beta$ carbons can be used to qualitatively identify peptide structures, and also to quantify both α -helix and β -hairpin populations. The differences between the observed chemical shifts and reference values for the random coil ($\Delta\delta = \delta^{\text{observed}} - \delta^{\text{random coil}}$, ppm), which are denoted as chemical shift deviations (CSD) or conformational shifts, are related to the ϕ and ψ dihedral angles, and therefore analysis of the plots of $\Delta\delta_{\text{H}\alpha}$, $\Delta\delta_{^{13}\text{C}\alpha}$ and $\Delta\delta_{^{13}\text{C}\beta}$ as a function of peptide sequence is a fast way to determine whether a peptide forms α -helix or β -hairpin structures, and which residues of the peptide are structured. Several lists of reference values for the random coil ($\delta^{\text{random coil}}$) have been reported. They are derived by either in experimental measurements on short non-structure peptides [44-47] or on statistical analysis on protein's chemical shifts [48]. None of them has been established as the "best" one to be used. The use of different reference values leads to small changes in the profiles of conformational shifts, which do not affect the qualitative analysis described on the following. In our lab, we get consistent results by using a list derived from hexapeptides [46], which contains ^1H and ^{13}C reference values. α -Helices are delineated by stretches of consecutive residues having negative $\Delta\delta_{\text{H}\alpha}$ and $\Delta\delta_{^{13}\text{C}\beta}$ values, and positive $\Delta\delta_{^{13}\text{C}\alpha}$ values, but $\Delta\delta_{^{13}\text{C}\beta}$ are rarely used because their magnitudes are commonly small. The profiles of β -hairpins consist of two stretches of consecutive residues with positive $\Delta\delta_{\text{H}\alpha}$ and $\Delta\delta_{^{13}\text{C}\beta}$ values, and negative $\Delta\delta_{^{13}\text{C}\alpha}$ values corresponding to the β -strands, separated by a 2-4 segment displaying negative or very small in magnitude $\Delta\delta_{\text{H}\alpha}$, and at least one of these residues having a positive $\Delta\delta_{^{13}\text{C}\alpha}$ and a negative $\Delta\delta_{^{13}\text{C}\beta}$. The characteristic patterns for all the loop residues in 2:2 hairpins with I' and II' β -turns, 3:5 with a I + G1 bulge turn, and 4:4 with a I β -turn have been described [49]. $^1\text{H}_\text{N}$ conformational shifts ($\Delta\delta_{\text{HN}}$), which are negative in α -helices and positive in β -strands, can also be used to identify α -helices [50, 51], β -hairpins [52], and β -turns [52]. It should also be noticed that $\Delta\delta_{\text{H}\alpha}$ and $\Delta\delta_{\text{HN}}$ were shown to be useful to characterise α -helix distortions [50, 51]. Since δ_{HN} values are very sensitive to experimental conditions, such as temperature, ionic strength and pH, the recommended procedure to get $\Delta\delta_{\text{HN}}$ values is that proposed by Andersen et. al. [52-54]. $^{13}\text{C}'$ (carbonyl) conformational shifts are also different for α -helices and β -sheets, but their

measurement would require having ^{13}C -labelled peptides, which is very expensive for chemically synthesised peptides. It has to be noticed that anisotropy effects from the aromatic rings of Trp, Tyr, Phe and His can distort the plots of chemical shift deviations, in particular those of the H_α protons, which makes identification of the peptide structure less clear-cut.

Assuming a two-state transition, α -helix and β -hairpin populations can be quantified from the $\Delta\delta_{\text{H}_\alpha}$, $\Delta\delta_{^{13}\text{C}_\alpha}$ and $\Delta\delta_{^{13}\text{C}_\beta}$ values by applying the following general equation (Eq. 6):

$$\% \text{ folded structure} = \frac{\langle \Delta\delta \rangle}{\Delta\delta^{\text{Folded}}} \times 100 \quad \text{Eq. [6]}$$

where $\langle \Delta\delta \rangle$ is the $\Delta\delta$ value for the nuclei under consideration ($^1\text{H}_\alpha$, $^{13}\text{C}_\alpha$ or $^{13}\text{C}_\beta$) averaged for all the residues spanned by the α -helix or for all the β -strand residues in the case of β -hairpins. Reference values for $\Delta\delta$ of the folded state, which are taken from mean δ values in protein α -helices and β -sheet [55, 56], are listed in Table 1.

Table 1. $\Delta\delta^{\text{Folded}}$ values to be used for quantifying α -helix and β -hairpin populations from H_α protons, $^{13}\text{C}_\alpha$ and $^{13}\text{C}_\beta$ carbons by applying Equation 6.

	$^1\text{H}_\alpha$	$^{13}\text{C}_\alpha$	$^{13}\text{C}_\beta$
α -helix	− 0.39 ppm	+ 3.1 ppm	---
β -hairpin	+ 0.40 ppm	− 1.5 ppm	+ 2.2 ppm

In β -hairpins containing a Gly at the loop region, the Gly splitting (the difference in chemical shift between the two Gly H_α protons) has been used to quantify β -hairpin populations ([57] and references in [58]).

It should be noted that precision and accuracy of structure quantification in peptides by all the available procedures are limited because they are not free of caveats, i.e. validity of the two-state assumption, absence of good reference values for the fully folded (α -helix or β -hairpin) and random coil states (for discussions on this problem see: [49, 52, 59-63]).

^1H - ^1H NOEs (nuclear Overhauser effects) are the NMR parameters that provide the strongest structural evidences, since only spatially close protons, approximately less than 5.5 Å, give rise to NOEs. NOE intensity is inversely proportional to the sixth power of the inter-proton distance ($1/r^6$, where r is the proton-proton distance). It seems obvious that the inter-proton distances involving backbone protons will depend on the peptide structure. Thus, the distances between backbone protons of consecutive residues (i and $i+1$) are different in an α -helix than in a two-stranded β -sheet [44], i.e. the distance between the H_α proton of a residue and the H_N amide proton of the following residue, denoted as $d_{\alpha\text{N}(i,i+1)}$, is shorter in β -strands (2.2 Å) than in α -helices (3.5 Å). As a consequence, the corresponding sequential NOE is very intense (strong) in β -strands, and less intense in α -helices. In contrast, the distance between H_N amides protons of consecutive residues ($d_{\text{NN}(i,i+1)}$) is short in α -helices (2.8 Å) giving rise to intense NOEs, and longer (4.3 Å) in antiparallel β -sheets, so that the corresponding NOEs are weak and even unobserved. More interestingly, the spatially close backbone protons of non-consecutive residues are completely different in α -helices and β -sheets, so that different kinds of non-sequential NOEs are characteristics for α -helices and β -sheets. Because of the α -helix periodicity, the most intense NOEs of α -helix-characteristic NOEs are between residues placed at positions i and $i+3$ ($d_{\alpha\text{N}(i,i+3)}$; 3.4 Å, medium intensity; Figure

1A). Other non-sequential helix-characteristic NOEs are: $d_{NN(i,i+2)}$ (4.2 Å; weak), $d_{\alpha N(i,i+2)}$ (4.4 Å; weak), $d_{\beta(i,i+3)}$ (2.5-4.4 Å; variable intensity) and $d_{\alpha N(i,i+4)}$ (4.2 Å; weak). The NOEs observable in 3_{10} -helices are similar to those of α -helices, but $d_{\alpha N(i,i+4)}$ are only observed in α -helices. In the case of the antiparallel two-stranded β -sheet moiety of the β -hairpin, the shortest distance is between the H_α protons of facing residues in a nonHB site (2.3 Å; Figure 2E), which gives place to a very strong NOE. But, there are other inter-strand characteristics NOEs, $d_{\alpha N(i,j)}$ (3.2 Å; medium; relative position of residues i, j as c4 and n3 in Figure 2E) and $d_{NN(i,j)}$ (3.3 Å; medium; relative position of residues i, j as n3 and c3 in Figure 2E). Regarding β -turns, the shortest inter-proton distances for the turns present in β -hairpins are shown in Figure 3 [44]. Intensity of $d_{\alpha\alpha(i,j)}$ NOE (where i and j are residues facing each other in a nonHB site, as the pairs n2/c2 and n4/c4 in Figure 2A and D-E) has also been used to quantify β -hairpin populations ([64, 65] and references in [58]).

More interestingly, in peptides where the α -helix or β -hairpin populations are high, the three-dimensional structure can be calculated from distance restraints obtained from the full set of NOEs by applying protocols similar to those used in proteins (for details, see the procedures used in recent α -helix and β -hairpin peptides reported by our group; [66, 67]). It should be noticed that the N- and C-segments of both α -helical and β -hairpin peptides usually display some fraying, that is, are more flexible than the rest of the peptide chain, even in highly populated structures.

Information on the solvent protection of amide H_N protons, and hence on the hydrogen bonds, can be obtained from the H_N amide temperature coefficients, which are of small magnitude if the H_N is not accessible to solvent ($|\Delta\delta/\Delta T| \leq 4 \times 10^{-6}$ ppm K^{-1} in aqueous solutions) and from the rates of H/D exchange [17, 68].

Finally, it should be mentioned that residual dipolar couplings (RDCs) can also provide structural information on peptides [69]. However, their use has been limited mainly to cyclic peptides, and mostly to studies on non-aqueous solvents [70, 71]. The flexibility of linear peptides poses some problems to the application of RDCs [70-72]. Anyway, the use of RDCs in peptide structural studies might grow in the future.

3.5. Structure stability

α -helix and β -hairpin stabilities in peptides can be determined by applying the same methods that in protein structure stability. However, data analysis can be more complex than in proteins, because the plateaux for the fully folded and unfolded states are not reachable at experimentally available conditions, and also because the two-assumption state cannot be valid, especially in β -hairpins. Despite these problems, thermal unfolding has been studied in many α -helical and β -hairpin-forming peptides by using DSC (differential scanning calorimetry)[73, 74], FT-IR [27], CD [75, 76] and NMR chemical shifts [73, 77] (for further examples see reviews [58, 63, 78-80] and references therein). β -hairpin stability can be measured in disulphide-cyclised peptides by a non-spectroscopic method, which makes use of the changes suffered by the thiol/disulphide equilibrium constant upon residue substitutions [81-83].

4. α -helical peptides

4.1. Contributions to α -helix stability

Studies on the stability of α -helices were prompted at the early 1980's by almost simultaneous publications from the groups of Prof Baldwin (Stanford, USA; [84, 85]) and Prof Rico (Madrid, Spain; [86, 87]) showing that the 13- and 19-mer peptides derived from the N-terminal helix of Ribonuclease A (denoted as C- and S-peptide, respectively) form significant amounts of native-like helix in aqueous solution. These

findings confirmed a previous report on the C-peptide [88], that were not followed on at the 1970's because the earliest studies on protein fragments were unable to detect any helical formation. The initial question of whether other protein fragments might also form native-like helices or, if not, why C- and S-peptides were helical was solved in the subsequent years. Fragments from diverse proteins were shown to adopt native-like helices in aqueous solution, whose stabilities ranged from relatively high to almost marginal [89-100]. The field became very active during the latest 1980's, 1990's and early 2000's decades. As a result, the main factors contributing to α -helix stability in peptides were established on the basis of (i) structural studies on model peptides, generally Ala-rich peptides, (ii) statistical analysis of protein α -helices, and (iii) thermodynamic characterisation of proteins with mutations at α -helix residues. On the following, we are going to give a brief overview of the contributions to α -helix stability, which are useful as guidelines to design α -helical peptides. Further details can be found on previous reviews about α -helical peptides [34, 101-108] and the references therein.

4.1.1. Intrinsic α -helix propensities and their position-dependence

Once a few protein structures were determined, statistical analysis showed that the occurrence of certain amino acids is different in α -helices than in β -sheets. Based on this statistical data, Chou & Fasman [109-112] tabulated the α -helix and β -sheet intrinsic propensities, and classified amino acids as α -helix formers, α -helix indifferent, and α -helix breakers, and analogously for β -sheets, and established a series of rules to predict α -helix and β -strand from protein sequences with a 70-80 % predictive accuracy. According to statistical-based [112] and experimentally-derived scales [113], Glu, Met, Ala, Leu and Lys residues have high intrinsic α -helix propensities, and so they are considered strong α -helix formers, while Pro and Gly, which exhibit the lowest helix-forming tendencies, are classified as α -helix breakers (Table 2). But, not all the positions in a α -helix are equivalent. Thus, the H_N groups of the N-terminal residues and the CO groups of the C-terminal residues do not participate in intra-helical hydrogen bonds (Figure 1A), but they can be hydrogen-bonded to preceding or following residues. As a consequence, intrinsic helical propensities are position-dependent [3, 114-116]. Indeed, the helix-breaker residues Pro and Gly can favour α -helix formation at specific positions (see below). A nomenclature for helical residues, which takes into account the position differences [3], is shown in Figure 1C.

Because of the α -helix dipole (section 2.1) [2], positively charged residues (Lys, Arg, and also His; Table 2) are preferred at the C-terminal segment (positions C3, C2 and C1; Figure 1C), and disfavoured at the N-terminal region. Accordingly, negatively charged residues (Glu, Asp; Table 2) are stabilising at the α -helix N-terminus and destabilising at the C-terminus [117, 118]. Evidently, these electrostatic interactions between charged side chains and the α -helix dipole are pH dependent (section 6).

The α -helix breaker residues Pro and Gly merit further consideration. In the case of Pro, its lack of amide H_N hydrogen accounts for being the amino acid with the lowest intrinsic α -helix propensity. Also because of not having a H_N hydrogen, Pro residues disrupt the regularity of the α -helical backbone by either breaking or kinking α -helices. Pro kinks usually bend the helix axis about 30° [1, 119, 120]. This distortion is due to steric hindrance arising from its cyclic side chain with the backbone of the preceding α -helical turn (i to i-4). However, probably because of their rigidity, Pro is often the first N-terminal residue (N1 in Figure 1) in protein α -helices [3] and α -helical peptides [121]. Gly can also induce α -helical distortions [122, 123], but in contrast to Pro, it has a very high conformational flexibility. Hence, it confers additional flexibility to the peptide, which entropically disfavours the formation of a well-defined α -helical

structure around a Gly residue. However, Gly residues are frequently found as N- and/or C-caps (see section 4.1.2; Figure 1) [114, 116]. Besides Pro and Gly residues, the polar residues Ser and Thr can induce distortions and helical bending at specific α -helical positions. They can act as hinge residues precluding α -helicity, when their χ_1 dihedral angle is in a g- conformation ($\chi_1 = +60^\circ$). This is probably due to the formation of a hydrogen bond between the hydroxyl hydrogen of the side chain and the i-3 or i-4 carbonyl oxygen [124, 125].

Regarding Cys residues, most of them form disulphide bonds in proteins, which restricts the conformational space and contribute to structure stability. However, intra-helical disulphide bonds are rare. The most frequent of them is formed by the Cys-X-X-Cys motif, where X is any amino acid, but ϕ and ψ angles for one of the Cys deviate from those typical of regular α -helices [126].

Table 2. Summary of the main contributions to α -helix stability

^aPosition independent statistical data [112]. ^bThey can lead to kinked helices. ^cH⁺ = Protonated His; O = Orn, ornithine; X = Any aminoacid; Ac = Acetyl; Am = Amide; h = hydrophobic. ^dPairs written from N-to-C orientation ^ei, i+4 pairs more stabilising than i, i+3. ^fSee nomenclature in Figure 1. ^gPosition specific statistical data [3].

Intrinsic propensities (P_α) ^a						
α -helix formers ($P_\alpha > 1.1$)			E > M > A > L > K > F > Q			
α -helix breakers ^b ($P_\alpha < 0.7$)			P $\approx$ G < N < Y			
Stabilising pair-wise side chain interaction ^{c,d}						
Type of interaction		Residue pairs		i,i+3	i,i+4	
Ion pairs ^e		ER > EK, KE > RE, HD		✓	✓	
Hydrogen bonds		QN, QD ⁻ > QD		✗	✓	
Aromatic / His pairs		WH > WH ⁺ , FH > FH ⁺		✗	✓	
Aromatic / Basic pairs		WR $\approx$ FO > FR		✗	✓	
Aromatic / Aliphatic pairs		FL, YL, YV		✗	✓	
Aliphatic / Basic pairs		IK, IR, VK		✗	✓	
Aliphatic /Aliphatic pairs ^e		LL, VL, IL		✓	✓	
Propensities at the N-termini, and N-terminal motif sequence ^c						
Helix position ^g						
	N'	N-cap	N1	N2	N3	N4
High propensity ^g	---	Ac, N, D, S, T, C, G	P $\geq$ E	E, D	E, D	---
Capping box	---	S, T, N, D	X	X	E, Q, D	---
Capping box variant	---	S, N, D	X	X	T	---
Hydrophobic staple	h: L, I, V, M, F	X	X	X	X	h: L, I, V, M, F
Polar staple	----	S, T	X	X	X	K, R
N-terminal Pro-box	h: L, I, V, M, F	P	X	X	h: L, I, V, M, F	h: L, I, V, M, F
Propensities at the C-termini, and C-terminal motif sequences						
Helix position ^g						
	C3	C2	C1	C-cap	C'	C''
High propensity ^f	K	K, R	H, K, Q	G, Am	---	---
Schellman motif	h: A, L, I, V, M, F	X	X	X	G	h: A, L, I, V, M, F
C-terminal Pro-box	---	---	---	F, Y, W, H, C, N	P	---

4.1.2. N- and C-capping

Charged and polar residues are often found at N- and C-cap positions providing interactions with available side chains or unsatisfied backbone atoms (those not participating in the intra-helical $i, i+4$ hydrogen bonds; section 2.1; Figure 1). Their contributions to α -helical stability have been found to be more important in the case of N-caps than in C-caps [127].

Thus, the best N-cap residues are Asn, Asp, Ser and Thr [3, 118, 128] (Table 2), whose side chains can hydrogen bond to the amide H_N groups at the N-terminal helical turn, which are not hydrogen bonded to CO oxygen atoms of helical residues [129]. The side chains of these four residues can adopt the g^- rotamer, which allows them to hydrogen bond to the amide H_N of residue N3. In addition, the side chains of Asn and Asp can adopt the t rotamer and be hydrogen bonded to both the N2 and N3 amide H_N [129]. Free Cys [118] can also act as N-cap by its side chain adopting a g^- rotamer and hydrogen bonding to the N3 H_N amide [129]. Gly is also often found as an N-cap [3, 81, 128], because it allows better solvation of the amide H_N groups at the first helical turn [130], and statistically is the most frequent residue at the C-cap position [3].

Certain local motifs that involve amino acids outside the helix and pack against helical residues have been observed at the N- and C-termini. Their contribution to helix stability is not clear in some of them, in particular in the C-terminal motifs. They seem to act as stop signals, which define the limits of the α -helix. These motifs, which are summarised in Table 2, are the following:

- Capping box motif [127, 131, 132]: It consists of a good N-cap residue and a Glu, Gln or Asp at position N3 (Table 2). This motif contains an additional hydrogen bond to that typical of N-caps (see above), because the N3 side chain is hydrogen bonded to the backbone H_N of the N-cap residue. The most frequent capping box is: Ser/Thr-X-X-Glu, where X is any amino acid. A variant of this motif in which the side chain of a Thr at position N3 hydrogen bonds to the side chain of the N-cap residue has also been described [106].
- Hydrophobic staple [133]: It is formed by the interaction between the side chains of two hydrophobic residues at positions N' and N4 (Table 2). When placed in-phase (Table 2), the hydrophobic staple and the capping box are strongly cooperative [133].
- A polar staple [36]: In this motif, the charged side chain of a Lys or an Arg placed at position N4 hydrogen bonds to the CO oxygen of residue N', when the N-cap is Ser or Thr as N-cap residue (Table 2).
- N-terminal Pro-box [39]: A Pro residue in a N-cap position surrounded by hydrophobic residues at positions N' (mainly Ile and Leu), N3 (mainly Val) and N4. This motif likely acts as a stop signal, but hardly contribute to α -helix stability, because of Pro being the N-cap, where it is not favoured [3].
- Schellman motif [134, 135]: Two hydrophobic residues at positions C3 and C'', and a Gly at position C' (Table 2). It seems to contribute to helix stability in the presence of TFE, but its contribution in aqueous solution is unclear [135].
- C-terminal Pro-box motif [136]: It consists of an aromatic residue, as well as Cys or Asn at the C-cap followed by a Pro residue at position C' (Table 2).

Since N-terminal acetylation and C-terminal amidation also contribute to increase α -helix stability [128, 137], most synthetic α -helical peptides are acetylated and/or amidated.

4.1.3. Side-chain / side-chain interactions

Side chain-side chain interactions have also been shown to contribute to α -helix stability in peptides. Pairs of α -helix stabilising side chain interactions belong to residues spaced three ($i, i+3$), four ($i, i+4$) or seven ($i, i+7$) positions. As depicted in Figure 1B, these side chains lie on the same α -helix face. These interactions might also depend on its location along the helix [106]. They include the following types (note that residue pairs are denoted from N-to-C):

- Ion pairs: The contributions to α -helix stability of the interactions between the acidic (Glu, Asp) and the basic (Lys, Arg) residues have been extensively investigated [37, 138-144]. There is no doubt that they are helix stabilising, and it seems that $i, i+4$ pairs are better than $i, i+3$, and that Glu/Lys pairs are better in the N-to-C orientation than in the reverse C-to-N (Lys/Glu), as expected from helix dipole effects [138]. But, in some peptide models they have been reported to have similar contributions [142]. The $i, i+3$ and $i, i+4$ His/Asp pairs are α -helix stabilising only in the N-to-C orientation [143]. The fact that the strength of all these pairs are pH dependent (section 6), but they are hardly screened by salt, indicates that they are contributing mainly by hydrogen bonding effects [143]. More recently, the $i, i+3$ and $i, i+4$, Glu/Arg pairs have been found to be more stabilising than the correspondent Glu/Lys pairs, which are most commonly used in designed α -helical peptides [37].

- Hydrogen bonds: $i, i+4$ Gln-Asp higher for unprotonated Asp, but not $i, i+3$, and not reverse orientation [145]; $i, i+4$ Gln-Asn but not $i, i+3$ and not reverse [146].

- Aromatic/ His pairs: The $i, i+4$ Trp/His [147] and Phe/His [148] are stabilising, and stronger with protonated His (His^+). So, their stabilising efficacy is pH-dependent. Indeed, the $i, i+4$ Phe/His interaction was found to be responsible of the pH dependence of C-peptide [149].

- Hydrophobic and aromatic interactions: Most pairs of aliphatic residues (Leu/Leu, Val/Leu, Ile/Leu) and aromatic/aliphatic (Phe/Leu, Tyr/Leu and Tyr/Val) have been found to be more stabilising at positions $i, i+4$ than at $i, i+3$, and Leu is preferred as the C-residue [150]. The stabilising contribution of the $i, i+4$ Tyr/Leu is comparable to Phe/His⁺ pair [151]. Polar /non polar pairs such as Ile/Lys, Ile/Arg, and Val/Lys occur in protein helices more often than expected when spaced $i, i+4$, and so it can contribute to helix stability by hydrophobic interactions involving the aliphatic chains of the positively charged residues [152].

- Aromatic / basic pairs: The contributions to stability of theses pairs have received little attention in α -helical peptides. Nevertheless, the $i, i+4$ Trp/Arg pair was shown to contribute to helix stability in a model Ala-based peptide in the N-to-C, but not in the reverse C-to-N orientation [153]. The stabilising effect was proposed to be throughout a cation- π interaction. However, the contribution of the $i, i+4$ Phe/Arg pair was negligible. Later, the $i, i+4$ Phe/Arg and Phe/Lys pairs were found to be stabilising in both N-to-C and C-to-N orientations, but the stabilising effect was attributed to hydrophobic interactions between the phenyl ring and the aliphatic chain of Lys or Arg rather than by cation- π interactions [154]. The $i, i+4$ Phe/Orn pair (Orn = Ornithine) is more stabilising than the Phe/Arg pair, and as strong as the Trp/Arg pair [155]

A few side-chain interactions between residues farther apart have also been shown to stabilise α -helices. Indeed, the first side-chain interaction demonstrated to be helix stabilising was a Glu2/Arg10 salt-bridge in the C- and S-peptide helices [87, 101, 156].

4.2. *In-silico predictions of α -helix stability*

Theoretical predictions of α -helix stability in peptides require statistical mechanics approaches. An α -helix/coil transition algorithm was postulated by Zimm and Bragg in 1959 [157], decades before a peptide were experimentally shown to form α -helix structure in aqueous solution [88]. It made use of equilibrium constants characteristic of each amino acid for the nucleation and elongation of the helix. Since then, the theoretical α -helix/coil algorithms have been improved by incorporating terms for the experimentally and/or statistically identified contributions to α -helix stability (section 4.1). Current programs implementing these algorithms yield relatively reliable outputs, and are excellent guides to design α -helical peptides.

One of the most commonly used is AGADIR (<http://agadir.crg.es>), which is based on free energy contributions derived from experimental data [158-160], takes into account pH, temperature and ionic strength, and whose outputs can provide the helix percentage at residue or peptide level.

Other programs to be mentioned are: SeqOPT (<http://mml.spbstu.ru/services/seqopt/>), a AGADIR-based program able to optimize sequences of short peptides to increase their helix stability in water [107], by using statistical mechanical theory and the tunneling algorithm for global sequence optimization; SCINT2 [161], which calculate peptide α -helix tendency from sequence, and was optimized to account for the contribution of side-chain interactions; CAPS-DB, a database specially tailored to design α -helix terminal regions [162]. Moreover, certain *de novo* protein design methods, such as EGAD [163], RosettaDesign [164, 165] or Liang-Grishin [166] programs, may be applied for the design of α -helical peptides [167]. Furthermore, other computational methods, such as molecular modelling and molecular dynamics simulations, which predict conformational and dynamics properties of peptides, may aid to design α -helical, and also β -hairpin-forming (section 5) peptides. They have been reviewed elsewhere [168], and are beyond the scope of the current review.

4.3. *Current trends in the design of α -helical peptides*

Despite the well-established body on the knowledge about α -helix formation (sections 4.1-4.2), the success of α -helix peptide design is still unsure. Therefore, design of α -helical peptides remains a challenge. Last decades, it has been focused on the development of novel approaches to improve their structure stability, bioactivity and bioavailability [169, 170]. In many cases, the followed approaches make use of non-peptide surrogates or peptide-mimetics, which benefit from the recent advances in synthetic procedures, in particular the organometallic catalysis, and olefin metathesis [171-173].

A common strategy to stabilise α -helices consists in incorporating conformational restraints by linking side chains three ($i, i+3$), four ($i, i+4$) or seven ($i, i+7$) residues apart, that is, side chains that are close because of the α -helix periodicity, and whose interactions can contribute to α -helix stability (see section 4.1.3). This side chain cross-links are denoted “staples” [174]. The stapled-peptide approach began by employing lactam, disulphide, and metal-mediated bridges [175-178]. More recently, hydrocarbon-stapled peptides have emerged providing flexible linkers that not only confer α -helical stability, but also improve their drug-like properties. But, a lactam-stapled peptide showed better dimerization inhibitor capacity than its hydrocarbon-stapled counterpart [179]. Diverse stapled peptides have been successfully designed offering promising therapeutic avenues [169, 172, 180]. For instance, lately reported antimicrobial and antiparasitic hydrocarbon-stapled peptides show optimal protease resistance and cellular

penetrance [181-184]. Remarkably, two stapled peptides, a dual inhibitor of a protein/protein interaction and a growth hormone-releasing hormone inhibitor, have entered clinical trials [185].

An alternative strategy is the hydrogen bond surrogate (HBS) approach, in which one $i, i+4$ hydrogen bond (Figure 1) is replaced by a covalent linkage, such as a carbon-carbon bond, obtained via ring closing metathesis [186, 187]. This approach facilitates the stabilization of the α -helical conformation in short peptide sequences (7–12 amino acids). HBS helices can exhibit high thermal stability, be more resistant to proteases than their unconstrained counterparts [188, 189] and have potential as inhibitors of specific protein-protein interactions [188, 190].

Another interesting development is the incorporation of azobenzene cross-linkers, whose photo-induced *cis/trans* isomerization (extended /compact conformation) is reversibly triggered by light of different wavelengths [191, 192]. Since the helix content depends on the conformation of these cross-linkers, these photo-switches are being used as way to control α -helix formation [191, 192].

Finally, it should be mentioned that a cyclic peptapeptide (Ac-(cyclo-1,5)-[KAXAD]-NH₂; X=Ala or Arg) has been recently reported as the shortest peptide able to be α -helical in water [193]. It is stabilised by a ($i, i+4$) lactam bridge between the side chains of Lys and Asp, and likely to have the N- and C-termini protected contributes to its stability. It has been proposed as useful to nucleate α -helix formation in longer peptides.

5. β -hairpin peptides

5.1. Contributions to β -hairpin stability

The first peptide able to form a monomeric β -hairpin structure in aqueous solution was a 9-mer peptide reported in 1993 [194]. Since then the efforts of several groups have provided a large amount of information about the contributions to β -hairpin formation and stability (see previous reviews [58-61, 78-80, 106, 195-199] and references therein). On the following we are going to summarise them focussing on their applicability as guidelines for β -hairpin design.

The fact that the contributions of the loop region and the β -strands to β -hairpin stability seem to be independent and additive [82, 200] facilitates the design of β -hairpin peptides. Thus, β -hairpin-forming peptides can be designed from protein β -hairpins by optimising the loop sequences, the cross-strand side chain interactions or both. In the absence of β -hairpin suitable β -turns, β -hairpin formation depends on having stabilising side chain interactions [67]. An alternative design strategy was applied in the case of the β -hairpin-forming decapeptide, named chignolin [32, 76, 201]. The eight central residues correspond to the consensus sequence derived from the alignment of the structural homologues of the GB1₄₁₋₅₆ β -hairpin. The most stable variants contain the Glu/Lys, Ile/Ile or Tyr/Tyr pair at the terminal HB site.

5.1.1. Role of the β -turn region

The importance of the β -turn sequence for β -hairpin stability and also for determining the register between the β -strands was evidenced from the early β -hairpin designs. Thus, turn optimisation in a Tendamistat-derived peptide led to a β -hairpin with a non-native β -strand register [194], and in ubiquitin-derived peptides native and non-native registers were observed depending on the turn sequence [63, 202]. Further studies in diverse β -hairpin systems (see previous reviews and references therein; [58-61, 78-80, 106, 195-199]) have confirmed that the loop sequence is crucial in directing β -strand registers [203-205], and in determining the final β -hairpin stability [206]. However, a good loop

sequence is a necessary condition, but it does not suffice for a peptide being able to form a stable β -hairpin.

Since the most adequate β -turns in hairpins with short loops depend on the type of β -hairpin (section 2.2), the optimal β -turn sequences are different for 2:2, 3:5 and 4:4 β -hairpins. The statistical frequencies of the residues at each position of I', II' and I β -turns are listed in Table 3. Thus, the most common sequences for residues i+1 and i+2 in 2:2 β -hairpins (Figure 2) are AsnGly for I' β -turns, and GlyAsn or GlySer II' β -turns. Apart from them, it has been noted that certain two-residue sequences containing D-amino acids induce I' or II' β -turns [207]. Indeed, the use of DPro as the i+1 turn residue has proven to be an excellent way to get a stable β -hairpin-forming peptide (Haque and Gellman 1997), and leads to I' β -turns when is followed by DAla or Ala as the i+2 residue, and to II' if followed by Gly or Asn. It was reported that the sequence DProGly is better than AsnGly to get stable 2:2 β -hairpins [208]. However, the rigid DProGly turn might impose steric restraints, which affects side chain packing, and may impede optimal side chains' contacts of stabilising pairs and clusters (section 5.1.3). In contrast, the less rigid AsnGly turn allows side chains to optimise packing and interactions.

In the case of 3:5 β -hairpins, they show a characteristic loop denoted as I + G1 β -bulge, which always contains a Gly at position i+3. The most common sequences found on peptides forming this type of hairpin are: NPDGS [194, 209], NPDGT [64, 77, 204, 205, 210], NSDGT [64, 77, 204, 205, 210], and EPDGK [211] for residues (i, i+1, i+2, i+3, i+4 and c1; Figure 2E). The sequence of the I β -turn in the only reported 4:4 β -hairpin peptide is AKAG [203] (i, i+1, i+2, i+3, i+4; Figure 2D). And loop sequences in 4:6 β -hairpin peptides are DDATKT [75] and NPATGK [53] (n1, i, i+1, i+2, i+3, i+4 and c1; Figure 2D).

The importance of the turn sequence for β -hairpin formation has also been shown in nonpolar, and so mostly water-insoluble, peptides [212-215]. For instance, DProGly at the turn stabilises β -hairpin formation in an apolar octapeptide [213], and a hydrophobic dodecapeptide is α -helical when the two central residues are AibAib (Aib = α -aminoisobutyric), and β -hairpin forming if the central sequence is DProPro (II' β -turn) [215].

5.1.2. Intrinsic β -sheet propensities

Analogously to α -helices, based on statistical analysis in proteins and also experimental studies on *ad hoc* designed protein mutants, residues can be classified as β -sheet formers and β -sheet breakers. The β -branched (Val, Ile and Thr), and the aromatic (Tyr, Phe, Trp) residues are the best β -sheet formers [112], but the ranking by intrinsic β -sheet propensity varies from statistical (Table 3) to experimental data [216-219], and among different experimental data. β -sheet former residues are mainly hydrophobic. what explains the high tendency of β -sheet peptides to self-associate. Although Gly residues tend to destabilise β -sheet, or give rise to bulges, a cross-strand interaction between an aromatic residue and a facing cross-strand Gly can stabilise protein β -sheets [220].

5.1.3. Side chain / side-chain interactions

Side chain interactions between pairs or clusters of β -strand residues pointing outwards the same side of the β -sheet plane contribute to β -hairpin stability. According to location in the β -hairpin ([58] and references therein), pairs of interacting side chains can be classified as cross-strand, which is sub-divided into nonHB and HB sites, and diagonal (only that between residues n4 and c2; Figure 2). Their contributions are

position-dependent as a consequence of several factors, such as the side chain distances being different at nonHB and HB sites (section 2.2), and entropic effects depending on the closeness of any stabilising pair or cluster to the loop region. In general, the closer a pair or cluster is to the loop region, the higher is stabilisation efficacy [210, 221]. However, some stabilising pairs at the N- and C-ends can prevent fraying [200, 222]. Analogously to α -helices in which the effect of some side chain interactions depends on their N-to-C orientation (section 4.1.3), the contributions of side chain pairs to β -hairpin stability can be asymmetric [83, 223, 224]. That is, denoting residue pairs from N- to C-strand, the stabilisation due to X/Y and Y/X pairs may be different. For instance, cross-strand Glu/Lys and Lys/Glu pairs contribute differently to β -hairpin stability [225].

As in helices, side chain interactions can be classified according to the nature of the involved residues:

- Ion pairs: The ionic interaction between the side chains of cross-strand Glu/Lys pairs are stabilising at both nonHB [200, 222, 225, 226] and HB sites [32, 227] (Figure 2), but Glu/Lys salt bridges are better than Lys/Glu, at least at nonHB sites [225]. Ionic interactions between the N-terminal amino and the C-terminal carboxylate groups enhance β -hairpin stability [209]. In contrast to α -helices, N-terminal acetylation and C-terminal amidation decreases β -hairpin stability [209]. Recently, the effects of Asp/Arg and Glu/Arg pairs, as well as for pairs containing Glu and Arg analogues with different lengths of the aliphatic chain, have been examined at nonHB sites [228]. As detailed in section 6, these contributions are pH-dependent.

- Hydrophobic pairs and clusters: The roles of aliphatic/aliphatic, aliphatic/aromatic and aromatic/aromatic interactions in β -hairpin stability have been extensively examined using different model β -hairpin templates ([58] and references therein). In a general way, all of these cross-strand pairs are stabilising at both nonHB and HB sites. The most stabilising cross-strand pair is the Trp/Trp at a nonHB site [81, 82], in which the indole rings adopt an edge-to-face disposition. In a Vammin-derived peptide, this Trp/Trp pair was shown to be more stabilising than an equally placed covalent disulphide bond [229] (section 5.1.4), but it provided no stabilisation when located at a HB site [230]. Other aromatic-containing pairs shown to enhance β -hairpin stability are: Tyr/Trp [204], Tyr/Phe [231] and Phe/Phe [57] at non-HB sites. In a HB site adjacent to the turn region, the aromatic/aromatic pairs were less stabilising than the aromatic/aliphatic [232]. Aromatic interactions, in particular those involving Trp, have received great attention, and reviewed previously [42, 233].

The β -hairpin stabilising effect of hydrophobic clusters at nonHB sites was first evidenced for the Trp/Val//Tyr/Phe cluster present in the native β -hairpin of protein G B1 domain, since a peptide encompassing residues 41-56 of the GB1 domain (GB1₄₁₋₅₆) forms a native-like β -hairpin in aqueous solution [234]. Incorporation of this cluster into model dodecapeptides led to β -hairpin stabilisation [221, 235, 236]. Note that the two first residues of the cluster are at the N-strand, and the two last residues at the C-strand (such as positions n4/c4//n2/c2 in Figure 2A-E).

Taking together that hydrophobic clusters at nonHB sites are β -hairpin stabilisers and that a Trp/Trp pair is the most stabilising cross-strand interaction also at nonHB sites led to the design of peptides containing a Trp/Trp//Trp/Trp cluster at nonHB sites. The β -hairpins formed by these peptides, denoted Trpzip, are remarkably stable [75]. These Trpzip peptides have been used as models to further examination of β -hairpin stabilising effects cross-strand and diagonal pairs and as scaffolds for ligand binding (section 5.3.2). The rankings of the examined cross-strand interactions can be seen at Table 3.

The geometry of the cross-strand Trp/Tyr and Tyr/Tyr pairs is edge-to-face, as in Trp/Trp pairs [237, 238].

- Other aromatic-containing pairs also contribute to β -hairpin stability (Table 3), such as the aromatic / basic pairs, which have favourable cation- π interactions when they interact diagonally [239, 240](Figure 2), and diagonal aromatic / methionine pairs [241]. However, the interactions between the indole ring of Trp and negatively charged side chains (Glu, and phosphorylated Ser, Thr and Tyr) are repulsive and destabilise β -hairpin structures, at least at nonHB sites [242].

- Polar pairs: They have been scarcely examined. But, the Asn/Thr [203], Thr/Thr [204] and Ser/Thr [204, 210] at the HB site adjacent to the turn have been reported to increase β -hairpin stability.

5.1.4. Disulphide bonds

Because of entropic effects, disulphide bonds can contribute to β -hairpin stabilisation. Indeed, natural disulphide-stabilised β -hairpin peptides constitute a group of natural antimicrobial peptides [243, 244]. Moreover, disulphide-, as well as N-to-C-backbone-, cyclised β -hairpins have taken as references for the fully folded state [62, 81, 82]. In proteins, disulphide bonds linking adjacent antiparallel β -strands are more frequent at non-HB sites than at HB-sites [245-247]. In a designed model peptide, β -hairpin stability was stabilised by a disulphide bond when placed at a nonHB site, but not at a HB site [248]. No β -hairpin structure was formed by the incorporation of a disulphide bond at a HB site in a VEGF-derived peptide, though the β -turn was stabilised [249]. This can be explained by the geometry of the disulphide bond being more adequate at nonHB sites than at HB. An inadequate geometry might pose steric strain, and hence lead to unfavourable enthalpy, which might counterbalance the favourable entropic effect of the disulphide bond. Also, the rigidity of disulphide bonds may affect stabilising contributions from side chain interactions by impeding that they interact in an optimal way.

5.1.5. Capping motifs

In contrast to α -helices, capping motifs in β -hairpins have received little attention, and only two have been described:

- β -cap: It is really a cross-strand Trp/Trp pair, which is located at the nonHB position closest to the N- and C-ends and farthest from the β -turn, plus a C-terminal extension [250-252]. As in general Trp/Trp pairs, the two indole rings interact in an edge-to-face way. This motif decreases the fraying at the N- and C-end generally observed in β -hairpin peptides. More interestingly, it has been shown to stabilise β -hairpins with quite long and flexible loops [253].

- N-terminal aliphatic D-amino acids, which stabilise the β -hairpin structures formed by short nonpolar octapeptides [254, 255].

Table 3. Summary of the main contributions to β -hairpin stability

^a Statistical frequencies [256]. ^b Statistical data [112]. ^c Strong β -sheet formers and breakers are highlighted in bold. ^d For each pair, first and last residues belong to the N- and C-strands, respectively. ^e Pairs adjacent to the β -turn have been mostly examined. ^f For more details see [58] and references therein. ^g Any = All possible aromatic pairs, excluding His. ^h Aromatic pairs at HB sites are less stabilising than at non-HB sites, or even non-stabilising at all. ⁱ Aliphatic/aliphatic pairs are less stabilising than mixed aliphatic/aromatic pairs. ^j Most experimentally examined aliphatic/aliphatic pairs are β -hairpin stabilising, but their ranking depends on the peptide system and on their location (nonHB or HB site).

β -turn region				
Residues ranked by frequency at β -turn position ^a				
β -turn	i	i+1	i+2	i+3
I'	Y > H >> I $\approx$ V	N > H >> D > G	G	K >> N $\approx$ R $\approx$ E $\approx$ Q
II'	Y > V $\approx$ S $\approx$ H $\approx$ F	G	N > S > D > H	T > G > N $\approx$ R $\approx$ F $\approx$ K
I	D > N >> H $\approx$ C $\approx$ S > P	P >> E $\approx$ S	D > N >> T > S $\approx$ W	G >> C $\approx$ T $\approx$ D $\approx$ R $\approx$ N
Intrinsic β -sheet propensities ^{b,c}				
β -sheet formers ($P_\beta > 1.1$)		V > I > Y > F > W > L > C > T		
β -sheet breakers ($P_\beta < 0.8$)		E < D $\approx$ P < K < G < S		
Stabilising pair-wise side chain interactions ^d				
Type of interaction	Residue pairs	Type according to β -hairpin position		
		Cross-strand nonHB	Cross-strand HB	Diagonal nonHB
Ionic	E/K > K/E N-amino/C-carboxylate	✓	✓	---
Polar	N/T, T/T, ST	---	✓ ^{e,f}	---
Aromatic/Aromatic	W/W >> Any ^g	✓	✗ ^{f,h}	---
Aliphatic/Aliphatic ^{f,i}	Any ^{f,j}	✓	✓	---
Mixed Aliphatic/Aromatic ^{f,i}	I/W, Y/L, F/L	✓	✓	---
Aromatic / Basic ^f (cation- π)	W/R, W/K, Y/K, F/R, F/K	---	---	✓
Aromatic / Met ^f	W/M, F/M	✓	---	✓
Disulphide bond	C/C	✓	✗	---

5.1.6. β -sheet twist and cooperativity

Antiparallel β -sheets show a characteristic right-handed twist (Figure 2F; Section 2.2). In β -hairpins, the degree of this right-handed twist is related to stability, so that the most twisted β -hairpins are usually the most stable ones. For example, 3:5 β -hairpins are more twisted and stable than 4:4 [205]. Probably, the twist increases stability by enlarging the buried hydrophobic surface [205]. Furthermore, the fact that the side chains of the n4/c2 pair are the closest of the four possible diagonal pairs (n4/c2, n2/c4, n3/c1, and n1/c3; Figure 2A-C; [6]) is also a consequence of the right-handed β -hairpin twist.

Concerning cooperativity, its existence was indicated by the β -hairpin stability increasing upon strand lengthening in a model peptide [257]. Moreover, it has been found cooperativity among stabilising cross-strand side chain pairs, which are adjacent at the same side of the β -sheet plane. Thus, the contribution of two cross-strand Lys/Glu ion pairs was reported to be higher than the sum of their individual contributions [226].

5.2. *In-silico* evaluation of designed β -hairpins

Prediction methods for protein secondary structure from sequence are quite reliable for helices (section 4.2), but not for β -sheets. Analogously, there is no well-established program able to successfully predict β -hairpin formation and stability in peptides. We have developed the program BEHAIRPRED (BEta HAIRpin PREDiction; <http://triton.iqfr.csic.es/software/behairpredv1.0/behairpred.htm>), which indicates the probability of a sequence to form a β -hairpin and the most probable type (2:2; 3:5; 4:4) just by taking into account β -turn propensities, β -sheet propensities, number of H-bonds, and statistical data on favourable and unfavourable side-chain interactions. The non-natural residues Ornithine and DPro can be included in the input sequence. Programs developed for predicting β -turns [258-260] or β -hairpins [261, 262] in proteins from their sequences might also be helpful. In addition, as in helices (section 4.2), molecular modelling and molecular dynamics simulations may be useful to design β -hairpin-forming peptides.

5.3. Current trends in the design of β -hairpin peptides

5.3.1. Design strategies using non-peptide surrogates

As in α -helices, the use of non-peptidic elements as a novel strategy to design stable β -hairpins is increasing in the last years. In many cases, the proposed strategies are analogous to those applied in helices, and even developed from them.

One approach takes into account the essential role of the loop region (section 5.1.1) and incorporates non-natural sequences at the loop region able to induce β -hairpin formation [263, 264]. For instance, DPheAbz (Abz = 2-aminobenzoic acid) turn motif has been used to stabilise β -hairpin formation in an non-polar water-insoluble peptide, as well as in amphipathic peptides, which have antimicrobial activity [265]. Also, bulky amino acids at the turn are employed to increase resistance to proteolysis [266].

A different approximation explores the use of the HBS-approach, successfully applied to α -helical peptides (section 4.3), to increase β -hairpin stability in peptides by using a hydrogen bond surrogate for a HB site [267].

The other strategy consists in either linking side chains of facing residues (Figure 2) or replacing them by connectors, such as hydrocarbon- (prepared by synthetic procedures analogous to those used for the hydrocarbon-stapled helices) or triazol-bridges. It has been found that, when a Trp/Trp interaction in a non-HB is replaced by a triazol-bridge, β -hairpin stability depends on the length of the triazole-linker [268]. The stabilising

effect of triazol-bridges at HB positions also depends on the length of the triazole-linker [269].

Although it contains only natural amino acids, it is interesting to mention a non- β -hairpin two-stranded antiparallel β -strand [270]. This peptide has been recently designed by using a central disulphide to link two identical peptide chains, whose sequence has Trp residues at N- and C-ends. Once the disulphide bond is formed, the peptide chains adopt a symmetrical antiparallel orientation, in which the β -sheet is stabilised by the interactions between the N-terminal Trp of one chain and the C-terminal Trp of the other chain, and vice-versa, and the disulphide bond is at a non-HB site (Figure 2).

5.3.2. Applicability of β -hairpin peptides

As in helices, most β -hairpin peptides are currently designed because of their potential applications. The potential applicability of many designed β -hairpin peptides lies in their biological activities, which make them candidates as therapeutic agents: to inhibit amyloid formation [271], as inhibitors of antimalarial protein/protein target interactions [272], to inhibit or stimulate angiogenesis by targeting VEGF receptors ([273-275]), to modulate protein/protein interaction relevant in the immune response [276] and as antimicrobial [277-279]

β -hairpin peptides have also been used as scaffolds for ligand binding. To that aim, the designed β -hairpin contains pairs or clusters of residues with suitable side chains adequately positioned to interact with the target ligand. Thus, a β -hairpin containing a Trp/Lys//Trp/Lys cluster at a non-HB site can bind nucleotides (ATP, GTP, CTP, and FMN) at high affinity [280-282]. The nucleobase moiety intercalates at the cleft formed by the diagonal Trp/Trp pair and the nucleotide phosphates might favourably interact with the two Lys. Based on these nucleotide-binding peptides, β -hairpin dimers able to bind single- and double-stranded DNA and RNA were designed [283-285]. Also, a polyproline peptide binds at the Trp/Trp cleft of Trpzip-derived peptides containing a Trp/Trp//Trp/Trp cluster in a non-HB site, but not if the cluster is Trp/Lys//Trp/Lys [286]. Moreover, a heme-binding site has been achieved by linking two identical β -hairpin chains throughout a disulphide bond between the two N-terminal Cys [287]. A His from each β -hairpin binds the heme group. As stabilising features, the β -hairpin contains an AsnGly sequence at the turn region (section 5.1.1) and three Trp residues (section 5.1.3). It is also worth mentioning a β -hairpin peptide derived from a choline-binding protein, which was able to bind tri-methyl ammonium [288].

β -Hairpin stabilisation upon metal binding can be achieved by adequate placement of His or Cys residues, whose side chains chelate the metal. Thus, two or three His at non-HB sites bind Zn^{2+} [289], and two cross-strand Cys at a nonHB site for As^{3+} [290]. Also, three units of a disulphide-cyclised β -hairpin coordinate Ga^{3+} throughout the side chain of a non-natural aminoacid (Hop = 5-(1-Hydroxy-pyridin-2(1*H*)-onyl)-l-alanine) [291]. Iron-binding β -hairpins have also been reported [292].

β -Hairpin peptides have been shown to carry on proton-coupled electron transfer reactions between the side chains of cross-strand Tyr/His pairs [293-296] and between Tyr/Trp pairs [297].

As previously observed in helices, the introduction of an azo group can modify specific peptide properties (section 4.3). Thus, the turn of the β -hairpin decapeptide known as chignolin [32, 76, 201] has been replaced by an azo group to achieve a photo-switchable peptide, which forms a β -hairpin when the azo group is *cis* conformation and is disordered if the azo is *trans* [298].

Finally, although self-associating peptides are beyond the scope of this review, we should mention that the design of hydrogel-forming β -hairpins is a very active field nowadays [299-301].

6. Solvent effects on α -helix and β -hairpin formation

Up-to-now, we have examined structural stability effects mainly due to peptide sequence. But, solvent and media conditions, such as salt concentration and pH, also modulate α -helix and β -hairpin stabilities.

Ionic interactions have been shown to contribute to stability in both α -helical and β -hairpin peptides (sections 4.1.3 and 5.1.3). Evidently, the energetic contribution of these interactions depends on pH and on salt concentration. Indeed, contributions to α -helix stability of the interactions between $i, i+3$ and $i, i+4$ pairs of charged side chains, and between charged side chains with the helix dipole (sections 2.1 and 4.1.1) have been evidenced by analysis of pH dependence of peptide folding, and by salt screening [142, 143, 159, 302]. Salt and pH dependence of β -hairpin stability is also influenced by salts and pH throughout their effect on electrostatics interactions ([58] and references therein).

As in proteins [303, 304], the chemical environment directly influences the structure, thermodynamics and kinetics of peptides. In particular, solvation effects may directly impact the energetic state of the peptide consequently affecting its structure. Specific conformational properties shown by peptides under different solvation conditions depend on the nature of the solvent-peptide intermolecular interactions. Such interactions may stabilize the hydrophobic core and alter the exposure of side chains so that the peptide can adopt favourable energy states upon solvation. The effects of alcoholic solvents, such as methanol, trifluoroethanol (TFE) and hexafluoroisopropanol (HFIP), on the stability of peptide structures have been examined since the early years in the field [305, 306]. These alcoholic co-solvents strongly stabilise α -helix structures in peptides, so that TFE is generally known as a α -helix-inducer. However, some peptides do not become helical in the presence of TFE, and a segment, but not the full peptide length, becomes helical in others. Furthermore, β -hairpin structures are stabilised in the presence of TFE ([58, 307] and references therein). Hence, TFE should be considered a secondary structure enhancer. The physical-chemical bases underlying the effect of the alcoholic co-solvents on peptide structures are not well understood, and different mechanisms have been proposed [308-310]. Regarding methanol, it has been shown to stabilise β -hairpin structures in many peptides. Indeed, designed hydrophobic β -hairpin-forming peptides are usually studied in methanol solutions (Awasthi, Raghothama et al. 1995; Raghothama, Awasthi et al. 1998; Rai, Raghothama et al. 2006; Chandrappa, Madhusudana Reddy et al. 2015[254]).

Ionic liquids, molten salts typically composed of large-size organic cations, can also exert significant influence on peptide folding and structural stability. Current findings in peptide systems indicate that α -helical structures are stabilised by ionic liquids, but β -hairpins are destabilised [311, 312].

Micelles, which are commonly used as the simplest membrane-mimetic media (particularly for solution NMR studies [313, 314], can also affect α -helix stability. Many peptides, in particular antimicrobial, have been shown to be mainly random in pure aqueous solutions, and become helical in the presence of micelles (for some cases, see [66, 314]. Also, a 20-mer peptide, which lacks of any structure in aqueous solution, forms a native-like β -hairpin in alcoholic solvents [315] and in SDS micelles [316]. But, the most striking case it is a recent finding from our lab of a 14-mer peptide, which

forms a very stable β -hairpin in aqueous solution, and becomes α -helical in the presence of DPC and SDS micelles [7]. Amphipathic structures seem to be prevalent in micelle media [7, 67].

7. Concluding remarks and future challenges

A variety of structural features should be considered when designing α -helical and β -hairpin peptides. Similitudes can be found in the design of both types of motifs. For instance, the intrinsic propensities of residues are position-dependent in both structures. Likewise, the chemical-physical nature of stabilising side-chain interactions (hydrophobic, aromatic, electrostatics) is similar in α -helix and β -hairpin-forming peptides. H-bonds contribute to stability in both, but they are not useful as a design guideline, except that certain non-natural scaffolds are H-bond surrogates. In helices $i,i+3$ and $i,i+4$ interactions are relevant, and in hairpins are cross-strand between facing residues (different at HB and nonHB sites) and diagonal.

On the other hand, the interactions with the helix dipole are unique in helices, and no equivalent can be found in β -hairpins. Regarding N- and C-ends, capping motifs have been described for helices, and more recently some have been found in β -hairpins.

Guidelines to design α -helices and β -hairpins are well established at the qualitative level. However, even in the case of helices, it is not possible to quantitatively predict the percentage of folded structure that a particular sequence will acquire. Also, in terms of quantification of structure populations, the existent methods give good estimation of the population, but not accurate. Therefore, at the basic level, the challenge is at quantification, though it cannot be discarded that some stabilising motif could be found in the future.

The other point to respond in the future is to understand the characteristic of a sequence to be able to adopt completely different stable structures depending on the solvent conditions. Therefore, not only intrinsic properties but also solvent effects need to be taken into account when designing a peptide.

The recent advances in the field can anticipate remarkable developments in rational peptide design. Parametric computational peptide design is becoming increasingly used to successfully develop stable structures. As previously mentioned, this is still specially challenging for β -hairpin predictions, and thus, algorithms need to be fine-tuned. Moreover, new synthetic approaches are providing chemical tools that enable the stabilization of α -helices and β -hairpins. This may even provide access to novel structures involving features connected in previously unseen configurations. It is also worth mentioning that the progress in biophysical techniques will also aid the structural characterization of these novel peptides in a more efficient manner.

With these novel strategies we can expect a promising scenario in the development of stable α -helical and β -hairpin peptides with improved applications.

Acknowledgments

We are grateful to financial support from Spanish MCIU project CTQ2017-84371-P (co-financed by European FEDER funds). PM is a recipient of a “Juan-de-la-Cierva” post-doctoral fellowship FJCI-2016-29227 from Spanish MCIU.

References

- [1] D.J. Barlow, J.M. Thornton, Helix geometry in proteins, *J Mol Biol* 201 (1988) 601-19. [https://doi.org/10.1016/0022-2836\(88\)90641-9I](https://doi.org/10.1016/0022-2836(88)90641-9I)

- [2] W.G. Hol, The role of the alpha-helix dipole in protein function and structure, *Prog Biophys Mol Biol* 45 (1985) 149-95. [https://doi.org/10.1016/0079-6107\(85\)90001-XI](https://doi.org/10.1016/0079-6107(85)90001-XI)
- [3] J. Richardson, D. Richardson, G.D. Rose, Amino acid preferences for specific locations at the ends of alpha helices, *Science* 240 (1988) 1648-1652. <https://doi.org/10.1126/science.3381086I>
- [4] J.S. Richardson, The anatomy and taxonomy of protein structure, *Adv Protein Chem* 34 (1981) 167-339. [https://doi.org/10.1016/S0065-3233\(08\)60520-3I](https://doi.org/10.1016/S0065-3233(08)60520-3I)
- [5] B.L. Sibanda, T.L. Blundell, J.M. Thornton, Conformation of beta-hairpins in protein structures. A systematic classification with applications to modelling by homology, electron density fitting and protein engineering, *J Mol Biol* 206 (1989) 759-77. [https://doi.org/10.1016/0022-2836\(89\)90583-4I](https://doi.org/10.1016/0022-2836(89)90583-4I)
- [6] A.P. Cootes, P.M. Curmi, R. Cunningham, C. Donnelly, A.E. Torda, The dependence of amino acid pair correlations on structural environment, *Proteins* 32 (1998) 175-89. [https://doi.org/10.1016/0022-2836\(89\)90583-4I](https://doi.org/10.1016/0022-2836(89)90583-4I)
- [7] H. Zamora-Carreras, B. Maestro, E. Strandberg, A.S. Ulrich, J.M. Sanz, M.A. Jimenez, Micelle-Triggered beta-Hairpin to alpha-Helix Transition in a 14-Residue Peptide from a Choline-Binding Repeat of the Pneumococcal Autolysin LytA, *Chemistry* 21 (2015) 8076-89. <https://doi.org/10.1002/chem.201500447I>
- [8] G.D. Rose, L.M. Gierasch, J.A. Smith, Turns in peptides and proteins, *Adv Protein Chem* 37 (1985) 1-109. [https://doi.org/10.1016/S0065-3233\(08\)60063-7I](https://doi.org/10.1016/S0065-3233(08)60063-7I)
- [9] G.J. Howlett, A.P. Minton, G. Rivas, Analytical ultracentrifugation for the study of protein association and assembly, *Curr Opin Chem Biol* 10 (2006) 430-6. <https://doi.org/10.1016/j.cbpa.2006.08.017I>
- [10] J.L. Cole, J.W. Lary, P.M. T, T.M. Laue, Analytical ultracentrifugation: sedimentation velocity and sedimentation equilibrium, *Methods Cell Biol* 84 (2008) 143-79. [https://doi.org/10.1016/S0091-679X\(07\)84006-4I](https://doi.org/10.1016/S0091-679X(07)84006-4I)
- [11] T. Arakawa, T. Niikura, Y. Kita, F. Arisaka, Structure analysis of short peptides by analytical ultracentrifugation: Review, *Drug Discov Ther* 3 (2009) 208-14.
- [12] D.K. Wilkins, S.B. Grimshaw, V. Receveur, C.M. Dobson, J.A. Jones, L.J. Smith, Hydrodynamic radii of native and denatured proteins measured by pulse field gradient NMR techniques, *Biochemistry* 38 (1999) 16424-31. <https://doi.org/10.1021/bi991765qI>
- [13] A. Cypionka, O.R. de los Panos, M.G. Mateu, F.N. Barrera, E. Hurtado-Gomez, J. Gomez, M. Vidal, J.L. Neira, The isolated C-terminal domain of Ring1B is a dimer made of stable, well-structured monomers, *Biochemistry* 46 (2007) 12764-76. <https://doi.org/10.1021/bi701343qI>
- [14] T.D. Clark, L. Bartolotti, R.P. Hicks, The application of DOSY NMR and molecular dynamics simulations to explore the mechanism(s) of micelle binding of antimicrobial peptides containing unnatural amino acids, *Biopolymers* 99 (2013) 548-61. <https://doi.org/10.1002/bip.22215I>
- [15] Y. Wang, N.L. Truex, N.D.P. Vo, J.S. Nowick, Effects of charge and hydrophobicity on the oligomerization of peptides derived from IAPP, *Bioorg Med Chem* 26 (2018) 1151-1156. <https://doi.org/10.1016/j.bmc.2017.10.001I>
- [16] P.E. Wright, H.J. Dyson, R.A. Lerner, Conformation of peptide fragments of proteins in aqueous solution: implications for initiation of protein folding, *Biochemistry* 27 (1988) 7167-75. <https://doi.org/10.1021/bi00419a001I>
- [17] H.J. Dyson, P.E. Wright, Defining solution conformations of small linear peptides, *Annu Rev Biophys Biophys Chem* 20 (1991) 519-38. <https://doi.org/10.1146/annurev.bb.20.060191.002511I>

- [18] C. Zhao, P.L. Polavarapu, C. Das, P. Balaram, Vibrational Circular Dichroism of beta-Hairpin Peptides, *J Am Chem Soc* 122 (2000) 8228-8231. <https://doi.org/10.1021/ja000451j>
- [19] T.A. Keiderling, Protein and peptide secondary structure and conformational determination with vibrational circular dichroism, *Curr Opin Chem Biol* 6 (2002) 682-8. [https://doi.org/10.1016/S1367-5931\(02\)00369-1](https://doi.org/10.1016/S1367-5931(02)00369-1)
- [20] P. Bour, T.A. Keiderling, Vibrational spectral simulation for peptides of mixed secondary structure: method comparisons with the Trpzip model hairpin, *J Phys Chem B* 109 (2005) 23687-97. <https://doi.org/10.1021/jp054107q>
- [21] M.P. Hatfield, R.F. Murphy, S. Lovas, VCD spectroscopic properties of the beta-hairpin forming miniprotein CLN025 in various solvents, *Biopolymers* 93 (2010) 442-50. <https://doi.org/10.1002/bip.21356>
- [22] D. Kurouski, Advances of Vibrational Circular Dichroism (VCD) in bioanalytical chemistry. A review, *Anal Chim Acta* 990 (2017) 54-66. <https://doi.org/10.1016/j.aca.2017.08.014>
- [23] W.K. Surewicz, H.H. Mantsch, D. Chapman, Determination of protein secondary structure by Fourier transform infrared spectroscopy: a critical assessment, *Biochemistry* 32 (1993) 389-94. <https://doi.org/10.1021/bi00053a001>
- [24] J.L. Arrondo, F.J. Blanco, L. Serrano, F.M. Goni, Infrared evidence of a beta-hairpin peptide structure in solution, *FEBS Lett* 384 (1996) 35-7. [https://doi.org/10.1016/0014-5793\(96\)00261-X](https://doi.org/10.1016/0014-5793(96)00261-X)
- [25] A. Barth, C. Zscherp, What vibrations tell us about proteins, *Q Rev Biophys* 35 (2002) 369-430. <https://doi.org/10.1017/S0033583502003815>
- [26] A.W. Smith, A. Tokmakoff, Amide I two-dimensional infrared spectroscopy of beta-hairpin peptides, *J Chem Phys* 126 (2007) 045109. <https://doi.org/10.1063/1.2428300>
- [27] Y. Xu, D. Du, R. Oyola, Infrared study of the stability and folding kinetics of a series of beta-hairpin peptides with a common NPDG turn, *J Phys Chem B* 115 (2011) 15332-8. <https://doi.org/10.1021/jp2046867>
- [28] M.R. Liyanage, K. Bakshi, D.B. Volkin, C.R. Middaugh, Fluorescence spectroscopy of peptides, *Methods Mol Biol* 1088 (2014) 237-46. https://doi.org/10.1007/978-1-62703-673-3_16
- [29] A.T. Krueger, B. Imperiali, Fluorescent amino acids: modular building blocks for the assembly of new tools for chemical biology, *Chembiochem* 14 (2013) 788-99. <https://doi.org/10.1002/cbic.201300079>
- [30] I.L. Karle, S.K. Awasthi, P. Balaram, A designed beta-hairpin peptide in crystals, *Proc Natl Acad Sci U S A* 93 (1996) 8189-93. <https://doi.org/10.1073/pnas.93.16.8189>
- [31] S. Aravinda, N. Shamala, R. Rajkishore, H.N. Gopi, P. Balaram, A crystalline beta-hairpin peptide nucleated by a type I' Aib-D-Ala beta-turn: evidence for cross-strand aromatic interactions, *Angew Chem Int Ed Engl* 41 (2002) 3863-5. [https://doi.org/10.1002/1521-3773\(20021018\)41:20<3863::AID-ANIE3863>3.0.CO;2-A](https://doi.org/10.1002/1521-3773(20021018)41:20<3863::AID-ANIE3863>3.0.CO;2-A)
- [32] S. Honda, T. Akiba, Y.S. Kato, Y. Sawada, M. Sekijima, M. Ishimura, A. Ooishi, H. Watanabe, T. Odahara, K. Harata, Crystal structure of a ten-amino acid protein, *J Am Chem Soc* 130 (2008) 15327-31. <https://doi.org/10.1021/ja8030533>
- [33] N. Sreerama, R.W. Woody, Computation and analysis of protein circular dichroism spectra, *Methods Enzymol* 383 (2004) 318-51. [https://doi.org/10.1016/S0076-6879\(04\)83013-1](https://doi.org/10.1016/S0076-6879(04)83013-1)

- [34] N. Errington, T. Iqbalsyah, A.J. Doig, Structure and stability of the alpha-helix: lessons for design, *Methods Mol Biol* 340 (2006) 3-26. <https://doi.org/10.1385/1-59745-116-9:3I>
- [35] Y.H. Chen, J.T. Yang, K.H. Chau, Determination of the helix and beta form of proteins in aqueous solution by circular dichroism, *Biochemistry* 13 (1974) 3350-9. <https://doi.org/10.1021/bi00713a027I>
- [36] E. Lacroix, A.R. Viguera, L. Serrano, Elucidating the folding problem of alpha-helices: local motifs, long-range electrostatics, ionic-strength dependence and prediction of NMR parameters, *J Mol Biol* 284 (1998) 173-91. <https://doi.org/10.1006/jmbi.1998.2145I>
- [37] R.F. Sommese, S. Sivaramakrishnan, R.L. Baldwin, J.A. Spudich, Helicity of short E-R/K peptides, *Protein Sci* 19 (2010) 2001-5. <https://doi.org/10.1002/pro.469I>
- [38] A. Chakrabartty, T. Kortemme, S. Padmanabhan, R.L. Baldwin, Aromatic side-chain contribution to far-ultraviolet circular dichroism of helical peptides and its effect on measurement of helix propensities, *Biochemistry* 32 (1993) 5560-5. <https://doi.org/10.1021/bi00072a010I>
- [39] A.R. Viguera, L. Serrano, Stable proline box motif at the N-terminal end of alpha-helices, *Protein Sci* 8 (1999) 1733-42. <https://doi.org/10.1110/ps.8.9.1733I>
- [40] M.P. Tombs, F. Souter, N.F. MacLagan, The spectrophotometric determination of protein at 210 millimicrons, *Biochem J* 73 (1959) 167-71. <https://doi.org/10.1042/bj0730167I>
- [41] N.J. Anthis, G.M. Clore, Sequence-specific determination of protein and peptide concentrations by absorbance at 205 nm, *Protein Sci* 22 (2013) 851-8. <https://doi.org/10.1002/pro.2253I>
- [42] C.M. Santiveri, M.A. Jimenez, Tryptophan residues: scarce in proteins but strong stabilizers of beta-hairpin peptides, *Biopolymers* 94 (2010) 779-90. <https://doi.org/10.1002/bip.21436I>
- [43] K. Wuthrich, *NMR of proteins and nucleic acids*, 1986.
- [44] K. Wuthrich, M. Billeter, W. Braun, Polypeptide secondary structure determination by nuclear magnetic resonance observation of short proton-proton distances, *J Mol Biol* 180 (1984) 715-40. [https://doi.org/10.1016/0022-2836\(84\)90034-2I](https://doi.org/10.1016/0022-2836(84)90034-2I)
- [45] G. Merutka, H.J. Dyson, P.E. Wright, 'Random coil' ¹H chemical shifts obtained as a function of temperature and trifluoroethanol concentration for the peptide series GGXGG, *J Biomol NMR* 5 (1995) 14-24. <https://doi.org/10.1007/BF00227466I>
- [46] D.S. Wishart, C.G. Bigam, A. Holm, R.S. Hodges, B.D. Sykes, ¹H, ¹³C and ¹⁵N random coil NMR chemical shifts of the common amino acids. I. Investigations of nearest-neighbor effects, *J Biomol NMR* 5 (1995) 67-81. <https://doi.org/10.1007/BF00227471I>
- [47] M. Kjaergaard, F.M. Poulsen, Sequence correction of random coil chemical shifts: correlation between neighbor correction factors and changes in the Ramachandran distribution, *J Biomol NMR* 50 (2011) 157-65. <https://doi.org/10.1007/s10858-011-9508-2I>
- [48] L. Wang, H.R. Eghbalnia, J.L. Markley, Probabilistic approach to determining unbiased random-coil carbon-13 chemical shift values from the protein chemical shift database, *J Biomol NMR* 35 (2006) 155-65. <https://doi.org/10.1007/s10858-006-9022-0I>

- [49] C.M. Santiveri, M. Rico, M.A. Jimenez, $^{13}\text{C}(\alpha)$ and $^{13}\text{C}(\beta)$ chemical shifts as a tool to delineate beta-hairpin structures in peptides, *J Biomol NMR* 19 (2001) 331-45. <https://doi.org/10.1023/A:1011224625129I>
- [50] M.A. Jimenez, F.J. Blanco, M. Rico, J. Santoro, J. Herranz, J.L. Nieto, Periodic properties of proton conformational shifts in isolated protein helices. An experimental study, *Eur J Biochem* 207 (1992) 39-49. <https://doi.org/10.1111/j.1432-1033.1992.tb17017.xI>
- [51] F.J. Blanco, J. Herranz, C. Gonzalez, M.A. Jimenez, M. Rico, J. Santoro, J.L. Nieto, NMR Chemical Shifts: A Tool To Characterize Distortions of Peptide and Protein Helices, *J Am Chem Soc* 114 (1992) 9676-9677. <https://doi.org/10.1021/ja00050a067I>
- [52] R.M. Fesinmeyer, F.M. Hudson, K.A. Olsen, G.W. White, A. Euser, N.H. Andersen, Chemical shifts provide fold populations and register of beta hairpins and beta sheets, *J Biomol NMR* 33 (2005) 213-31. <https://doi.org/10.1007/s10858-005-3731-7I>
- [53] R.M. Fesinmeyer, F.M. Hudson, N.H. Andersen, Enhanced hairpin stability through loop design: the case of the protein G B1 domain hairpin, *J Am Chem Soc* 126 (2004) 7238-43. <https://doi.org/10.1021/ja0379520I>
- [54] N.H. Andersen, J.W. Neidigh, S.C. Harris, G.M. Lee, Z. Liu, H. Tong, Extracting Information from the Temperature Gradients of Polypeptide NH Chemical Shifts. 1. The Importance of Conformational Averaging, *J Am Chem Soc* 119 (1997) 8547-8561. <https://doi.org/10.1021/ja963250hI>
- [55] D.S. Wishart, B.D. Sykes, F.M. Richards, Relationship between nuclear magnetic resonance chemical shift and protein secondary structure, *J Mol Biol* 222 (1991) 311-33. [https://doi.org/10.1016/0022-2836\(91\)90214-QI](https://doi.org/10.1016/0022-2836(91)90214-QI)
- [56] S. Spera, A. Bax, Empirical Correlation between Protein Backbone Conformation and C-Alpha and C-Beta C-13 Nuclear-Magnetic-Resonance Chemical-Shifts, *J Am Chem Soc* 113 (1991) 5490-5492. <https://doi.org/10.1021/ja00014a071I>
- [57] C.D. Tatko, M.L. Waters, Selective aromatic interactions in beta-hairpin peptides, *J Am Chem Soc* 124 (2002) 9372-3. <https://doi.org/10.1021/ja0262481I>
- [58] M.A. Jimenez, Design of monomeric water-soluble beta-hairpin and beta-sheet peptides, *Methods Mol Biol* 1216 (2014) 15-52. https://doi.org/10.1007/978-1-4939-1486-9_2I
- [59] F. Blanco, M. Ramirez-Alvarado, L. Serrano, Formation and stability of beta-hairpin structures in polypeptides, *Curr Opin Struct Biol* 8 (1998) 107-11. [https://doi.org/10.1016/S0959-440X\(98\)80017-1I](https://doi.org/10.1016/S0959-440X(98)80017-1I)
- [60] S.H. Gellman, Minimal model systems for beta sheet secondary structure in proteins, *Curr Opin Chem Biol* 2 (1998) 717-25. [https://doi.org/10.1016/S1367-5931\(98\)80109-9I](https://doi.org/10.1016/S1367-5931(98)80109-9I)
- [61] E. Lacroix, T. Kortemme, M. Lopez de la Paz, L. Serrano, The design of linear peptides that fold as monomeric beta-sheet structures, *Curr Opin Struct Biol* 9 (1999) 487-93. [https://doi.org/10.1016/S0959-440X\(99\)80069-4I](https://doi.org/10.1016/S0959-440X(99)80069-4I)
- [62] F.A. Syud, J.F. Espinosa, S.H. Gellman, NMR-based quantification of beta-sheet populations in aqueous solution through use of reference peptides for the folded and unfolded states, *J Am Chem Soc* 121 (1999) 11578-11579. <https://doi.org/10.1021/ja992733tI>
- [63] M.S. Searle, Peptide models of protein b-sheets: design, folding and insights into stabilising weak interactions, *J Chem Soc, Perkin Trans 2* (2001) 1011-1020. <https://doi.org/10.1039/B008074HI>

- [64] E. de Alba, M.A. Jimenez, M. Rico, J.L. Nieto, Conformational investigation of designed short linear peptides able to fold into beta-hairpin structures in aqueous solution, *Fold Des* 1 (1996) 133-44. [https://doi.org/10.1016/S1359-0278\(96\)00022-3](https://doi.org/10.1016/S1359-0278(96)00022-3)
- [65] M. Ramirez-Alvarado, F.J. Blanco, L. Serrano, De novo design and structural analysis of a model beta-hairpin peptide system, *Nat Struct Biol* 3 (1996) 604-12. <https://doi.org/10.1038/nsb0796-604>
- [66] C.B. Falcao, C. Perez-Peinado, B.G. de la Torre, X. Mayol, H. Zamora-Carreras, M.A. Jimenez, G. Radis-Baptista, D. Andreu, Structural Dissection of Crotalicidin, a Rattlesnake Venom Cathelicidin, Retrieves a Fragment with Antimicrobial and Antitumor Activity, *J Med Chem* 58 (2015) 8553-63. <https://doi.org/10.1021/acs.jmedchem.5b01142>
- [67] H. Zamora-Carreras, B. Maestro, E. Strandberg, A.S. Ulrich, J.M. Sanz, M.A. Jimenez, Roles of Amphipathicity and Hydrophobicity in the Micelle-Driven Structural Switch of a 14-mer Peptide Core from a Choline-Binding Repeat, *Chemistry* 24 (2018) 5825-5839. <https://doi.org/10.1002/chem.201704802>
- [68] M.P. Williamson, Peptide structure determination by NMR, *Methods Mol Biol* 17 (1993) 69-85. <https://doi.org/10.1385/0-89603-215-9:69>
- [69] S. Ohnishi, D. Shortle, Observation of residual dipolar couplings in short peptides, *Proteins* 50 (2003) 546-51. <https://doi.org/10.1002/prot.10327>
- [70] C. Rigling, M.O. Ebert, RDC-enhanced structure calculation of a beta-heptapeptide in methanol, *Magn Reson Chem* 55 (2017) 655-661. <https://doi.org/10.1002/mrc.4569>
- [71] M.B. Schmid, M. Fleischmann, V. D'Elia, O. Reiser, W. Gronwald, R.M. Gschwind, Residual dipolar couplings in short peptidic foldamers: combined analyses of backbone and side-chain conformations and evaluation of structure coordinates of rigid unnatural amino acids, *Chembiochem* 10 (2009) 440-4. <https://doi.org/10.1002/cbic.200800736>
- [72] C.M. Thiele, V. Schmidts, B. Bottcher, I. Louzao, R. Berger, A. Maliniak, B. Stevensson, On the treatment of conformational flexibility when using residual dipolar couplings for structure determination, *Angew Chem Int Ed Engl* 48 (2009) 6708-12. <https://doi.org/10.1002/anie.200902398>
- [73] S. Honda, N. Kobayashi, E. Munekata, Thermodynamics of a beta-hairpin structure: evidence for cooperative formation of folding nucleus, *J Mol Biol* 295 (2000) 269-78. <https://doi.org/10.1006/jmbi.1999.3346>
- [74] A. Skwierawska, S. Oldziej, A. Liwo, H.A. Scheraga, Conformational studies of the C-terminal 16-amino-acid-residue fragment of the B3 domain of the immunoglobulin binding protein G from *Streptococcus*, *Biopolymers* 91 (2009) 37-51. <https://doi.org/10.1002/bip.21080>
- [75] A.G. Cochran, N.J. Skelton, M.A. Starovasnik, Tryptophan zippers: stable, monomeric beta-hairpins, *Proc Natl Acad Sci U S A* 98 (2001) 5578-83. <https://doi.org/10.1073/pnas.091100898>
- [76] S. Honda, K. Yamasaki, Y. Sawada, H. Morii, 10 residue folded peptide designed by segment statistics, *Structure* 12 (2004) 1507-18. <https://doi.org/10.1016/j.str.2004.05.022>
- [77] C.M. Santiveri, J. Santoro, M. Rico, M.A. Jimenez, Thermodynamic analysis of beta-hairpin-forming peptides from the thermal dependence of $(1)H$ NMR chemical shifts, *J Am Chem Soc* 124 (2002) 14903-9. <https://doi.org/10.1021/ja0278537>

- [78] M.S. Searle, B. Ciani, Design of beta-sheet systems for understanding the thermodynamics and kinetics of protein folding, *Curr Opin Struct Biol* 14 (2004) 458-64. <https://doi.org/10.1016/j.sbi.2004.06.001I>
- [79] R.M. Hughes, M.L. Waters, Model systems for beta-hairpins and beta-sheets, *Curr Opin Struct Biol* 16 (2006) 514-24. <https://doi.org/10.1016/j.sbi.2006.06.008I>
- [80] A. Lewandowska, S. Oldziej, A. Liwo, H.A. Scheraga, beta-hairpin-forming peptides; models of early stages of protein folding, *Biophys Chem* 151 (2010) 1-9. <https://doi.org/10.1016/j.bpc.2010.05.001I>
- [81] S. Russell, A.G. Cochran, Designing stable b-hairpins: energetics contributions from cross-strand residues, *J Am Chem Soc* 122 (2000) 12600-12601. <https://doi.org/10.1021/ja002085+I>
- [82] A.G. Cochran, R.T. Tong, M.A. Starovasnik, E.J. Park, R.S. McDowell, J.E. Theaker, N.J. Skelton, A minimal peptide scaffold for beta-turn display: optimizing a strand position in disulfide-cyclized beta-hairpins, *J Am Chem Soc* 123 (2001) 625-32. <https://doi.org/10.1021/ja003369xI>
- [83] S.J. Russell, T. Blandl, N.J. Skelton, A.G. Cochran, Stability of cyclic beta-hairpins: asymmetric contributions from side chains of a hydrogen-bonded cross-strand residue pair, *J Am Chem Soc* 125 (2003) 388-95. <https://doi.org/10.1021/ja028075II>
- [84] A. Bierzynski, P.S. Kim, R.L. Baldwin, A salt bridge stabilizes the helix formed by isolated C-peptide of RNase A, *Proc Natl Acad Sci U S A* 79 (1982) 2470-4. <https://doi.org/10.1073/pnas.79.8.2470I>
- [85] P.S. Kim, R.L. Baldwin, A helix stop signal in the isolated S-peptide of ribonuclease A, *Nature* 307 (1984) 329-34. <https://doi.org/307329a0I>
- [86] M. Rico, J.L. Nieto, J. Santoro, F.J. Bermejo, J. Herranz, E. Gallego, Low-temperature 1H-NMR evidence of the folding of isolated ribonuclease S-peptide, *FEBS Lett* 162 (1983) 314-9. [https://doi.org/10.1016/0014-5793\(83\)80779-0I](https://doi.org/10.1016/0014-5793(83)80779-0I)
- [87] M. Rico, E. Gallego, J. Santoro, F.J. Bermejo, J.L. Nieto, J. Herranz, On the fundamental role of the Glu 2- ... Arg 10+ salt bridge in the folding of isolated ribonuclease A S-peptide, *Biochem Biophys Res Commun* 123 (1984) 757-63. [https://doi.org/10.1016/0006-291X\(84\)90294-8I](https://doi.org/10.1016/0006-291X(84)90294-8I)
- [88] J.E. Brown, W.A. Klee, Helix-coil transition of the isolated amino terminus of ribonuclease, *Biochemistry* 10 (1971) 470-6. <https://doi.org/10.1021/bi00779a019I>
- [89] M.A. Jimenez, J.L. Nieto, J. Herranz, M. Rico, J. Santoro, 1H NMR and CD evidence of the folding of the isolated ribonuclease 50-61 fragment, *FEBS Lett* 221 (1987) 320-4. [https://doi.org/10.1016/0014-5793\(87\)80948-1I](https://doi.org/10.1016/0014-5793(87)80948-1I)
- [90] H.J. Dyson, M. Rance, R.A. Houghten, P.E. Wright, R.A. Lerner, Folding of immunogenic peptide fragments of proteins in water solution. II. The nascent helix, *J Mol Biol* 201 (1988) 201-17. [https://doi.org/10.1016/0022-2836\(88\)90447-0I](https://doi.org/10.1016/0022-2836(88)90447-0I)
- [91] M.A. Jimenez, M. Rico, J. Herranz, J. Santoro, J.L. Nieto, 1H-NMR assignment and folding of the isolated ribonuclease 21-42 fragment, *Eur J Biochem* 175 (1988) 101-9. <https://doi.org/10.1111/j.1432-1033.1988.tb14171.xI>
- [92] M.C. Pena, M. Rico, M.A. Jimenez, J. Herranz, J. Santoro, J.L. Nieto, Conformational properties of the isolated 1-23 fragment of human hemoglobin alpha-chain, *Biochim Biophys Acta* 957 (1988) 380-9. [https://doi.org/10.1016/0006-291X\(92\)91902-3I](https://doi.org/10.1016/0006-291X(92)91902-3I)

- [93] F.J. Blanco, A. Jimenez, M. Rico, J. Santoro, J. Herranz, J.L. Nieto, The homologous angiogenin and ribonuclease N-terminal fragments fold into very similar helices when isolated, *Biochem Biophys Res Commun* 182 (1992) 1491-8. [https://doi.org/10.1016/0006-291X\(92\)91902-3I](https://doi.org/10.1016/0006-291X(92)91902-3I)
- [94] H.J. Dyson, J.R. Sayre, G. Merutka, H.C. Shin, R.A. Lerner, P.E. Wright, Folding of peptide fragments comprising the complete sequence of proteins. Models for initiation of protein folding. II. Plastocyanin, *J Mol Biol* 226 (1992) 819-35. [https://doi.org/10.1016/0022-2836\(92\)90634-VI](https://doi.org/10.1016/0022-2836(92)90634-VI)
- [95] H.J. Dyson, G. Merutka, J.P. Waltho, R.A. Lerner, P.E. Wright, Folding of peptide fragments comprising the complete sequence of proteins. Models for initiation of protein folding. I. Myohemerythrin, *J Mol Biol* 226 (1992) 795-817. [https://doi.org/10.1016/0022-2836\(92\)90633-UI](https://doi.org/10.1016/0022-2836(92)90633-UI)
- [96] M.A. Jimenez, M. Bruix, C. Gonzalez, F.J. Blanco, J.L. Nieto, J. Herranz, M. Rico, CD and ¹H-NMR studies on the conformational properties of peptide fragments from the C-terminal domain of thermolysin, *Eur J Biochem* 211 (1993) 569-81. <https://doi.org/10.1111/j.1432-1033.1993.tb17584.xI>
- [97] M.A. Jimenez, C. Carreno, D. Andreu, F.J. Blanco, J. Herranz, M. Rico, J.L. Nieto, Helix formation by the phospholipase A2 38-59 fragment: influence of chain shortening and dimerization monitored by nmr chemical shifts, *Biopolymers* 34 (1994) 647-61. <https://doi.org/10.1002/bip.360340507I>
- [98] V. Munoz, L. Serrano, M.A. Jimenez, M. Rico, Structural analysis of peptides encompassing all alpha-helices of three alpha/beta parallel proteins: Che-Y, flavodoxin and P21-ras: implications for alpha-helix stability and the folding of alpha/beta parallel proteins, *J Mol Biol* 247 (1995) 648-69. [https://doi.org/10.1016/S0022-2836\(05\)80145-7I](https://doi.org/10.1016/S0022-2836(05)80145-7I)
- [99] M.T. Reymond, G. Merutka, H.J. Dyson, P.E. Wright, Folding propensities of peptide fragments of myoglobin, *Protein Sci* 6 (1997) 706-16. <https://doi.org/10.1002/pro.5560060320I>
- [100] S. Padmanabhan, M.A. Jimenez, M. Rico, Folding propensities of synthetic peptide fragments covering the entire sequence of phage 434 Cro protein, *Protein Sci* 8 (1999) 1675-88. <https://doi.org/10.1110/ps.8.8.1675I>
- [101] J.M. Scholtz, R.L. Baldwin, The mechanism of alpha-helix formation by peptides, *Annu Rev Biophys Biomol Struct* 21 (1992) 95-118. <https://doi.org/10.1146/annurev.bb.21.060192.000523I>
- [102] R.L. Baldwin, Alpha-helix formation by peptides of defined sequence, *Biophys Chem* 55 (1995) 127-35. [https://doi.org/10.1016/0301-4622\(94\)00146-BI](https://doi.org/10.1016/0301-4622(94)00146-BI)
- [103] A. Chakrabartty, R.L. Baldwin, Stability of alpha-helices, *Adv Protein Chem* 46 (1995) 141-76. [https://doi.org/10.1016/S0065-3233\(08\)60334-4I](https://doi.org/10.1016/S0065-3233(08)60334-4I)
- [104] V. Munoz, L. Serrano, Helix design, prediction and stability, *Curr Opin Biotechnol* 6 (1995) 382-6. [https://doi.org/10.1016/0958-1669\(95\)80066-2I](https://doi.org/10.1016/0958-1669(95)80066-2I)
- [105] C.A. Rohl, R.L. Baldwin, Deciphering rules of helix stability in peptides, *Methods Enzymol* 295 (1998) 1-26. [https://doi.org/10.1016/S0076-6879\(98\)95032-7I](https://doi.org/10.1016/S0076-6879(98)95032-7I)
- [106] L. Serrano, The relationship between sequence and structure in elementary folding units, *Adv Protein Chem* 53 (2000) 49-85. [https://doi.org/10.1016/S0065-3233\(00\)53002-2I](https://doi.org/10.1016/S0065-3233(00)53002-2I)
- [107] A. Yakimov, G. Rychkov, M. Petukhov, De novo design of stable alpha-helices, *Methods Mol Biol* 1216 (2014) 1-14. https://doi.org/10.1007/978-1-4939-1486-9_1I

- [108] A.P. Yakimov, A.S. Afanaseva, M.A. Khodorkovskiy, M.G. Petukhov, Design of Stable α -Helical Peptides and Thermostable Proteins in Biotechnology and Biomedicine, *Acta Naturae* 8 (2016) 70-81. <https://doi.org/PMC5199208I>
- [109] P.Y. Chou, G.D. Fasman, Prediction of protein conformation, *Biochemistry* 13 (1974) 222-45. <https://doi.org/10.1021/bi00699a002I>
- [110] P.Y. Chou, G.D. Fasman, Conformational parameters for amino acids in helical, beta-sheet, and random coil regions calculated from proteins, *Biochemistry* 13 (1974) 211-22. <https://doi.org/10.1021/bi00699a001I>
- [111] P.Y. Chou, G.D. Fasman, Empirical predictions of protein conformation, *Annu Rev Biochem* 47 (1978) 251-76. <https://doi.org/10.1146/annurev.bi.47.070178.001343I>
- [112] G.D. Fasman, The development of the prediction of protein structure, in: G.D. Fasman (Ed.), *Prediction of protein structure and the principles of protein conformation*, Plenum Press, New York, 1989, pp. 193-316.
- [113] C.N. Pace, J.M. Scholtz, A helix propensity scale based on experimental studies of peptides and proteins, *Biophys J* 75 (1998) 422-7. [https://doi.org/10.1016/S0006-3495\(98\)77529-0I](https://doi.org/10.1016/S0006-3495(98)77529-0I)
- [114] M. Petukhov, V. Munoz, N. Yumoto, S. Yoshikawa, L. Serrano, Position dependence of non-polar amino acid intrinsic helical propensities, *J Mol Biol* 278 (1998) 279-89. <https://doi.org/10.1006/jmbi.1998.1682I>
- [115] M. Petukhov, K. Uegaki, N. Yumoto, S. Yoshikawa, L. Serrano, Position dependence of amino acid intrinsic helical propensities II: non-charged polar residues: Ser, Thr, Asn, and Gln, *Protein Sci* 8 (1999) 2144-50. <https://doi.org/10.1110/ps.8.10.2144I>
- [116] M. Petukhov, K. Uegaki, N. Yumoto, L. Serrano, Amino acid intrinsic α -helical propensities III: positional dependence at several positions of C terminus, *Protein Sci* 11 (2002) 766-77. <https://doi.org/10.1110/ps.2610102I>
- [117] J. Aqvist, H. Luecke, F.A. Quirocho, A. Warshel, Dipoles localized at helix termini of proteins stabilize charges, *Proc Natl Acad Sci U S A* 88 (1991) 2026-30. <https://doi.org/10.1073/pnas.88.5.2026I>
- [118] A.J. Doig, R.L. Baldwin, N- and C-capping preferences for all 20 amino acids in α -helical peptides, *Protein Sci* 4 (1995) 1325-36. <https://doi.org/10.1002/pro.5560040708I>
- [119] R.H. Yun, A. Anderson, J. Hermans, Proline in α -helix: Stability and conformation studied by dynamics simulation, *Proteins: Structure, Function, and Genetics* 10 (1991) 219-228. <https://doi.org/10.1002/prot.340100306I>
- [120] D.-K. Chang, S.-F. Cheng, V.D. Trivedi, K.-L. Lin, Proline Affects Oligomerization of a Coiled Coil by Inducing a Kink in a Long Helix, *Journal of Structural Biology* 128 (1999) 270-279. <https://doi.org/10.1006/JSBI.1999.4182I>
- [121] M.K. Kim, Y.K. Kang, Positional preference of proline in α -helices, *Protein Sci* 8 (1999) 1492-9. <https://doi.org/10.1110/ps.8.7.1492I>
- [122] K.G. Strehlow, R.L. Baldwin, Effect of the substitution Ala---Gly at each of five residue positions in the C-peptide helix, *Biochemistry* 28 (1989) 2130-3. <https://doi.org/10.1021/bi00431a025I>
- [123] K. Imai, S. Mitaku, Mechanisms of secondary structure breakers in soluble proteins, *Biophysics (Nagoya-shi)* 1 (2005) 55-65. <https://doi.org/10.2142/biophysics.1.55I>
- [124] J.A. Ballesteros, X. Deupi, M. Olivella, E.E. Haaksma, L. Pardo, Serine and threonine residues bend α -helices in the $\chi(1) = g(-)$ conformation, *Biophys J* 79 (2000) 2754-60. [https://doi.org/10.1016/S0006-3495\(00\)76514-3I](https://doi.org/10.1016/S0006-3495(00)76514-3I)

- [125] X. Deupi, M. Olivella, A. Sanz, N. Dolker, M. Campillo, L. Pardo, Influence of the g- conformation of Ser and Thr on the structure of transmembrane helices, *J Struct Biol* 169 (2010) 116-23. <https://doi.org/10.1016/j.jsb.2009.09.009I>
- [126] A. De Simone, R. Berisio, A. Zagari, L. Vitagliano, Limited tendency of alpha-helical residues to form disulfide bridges: a structural explanation, *J Pept Sci* 12 (2006) 740-7. <https://doi.org/10.1002/psc.809I>
- [127] S. Dasgupta, J.A. Bell, Design of helix ends. Amino acid preferences, hydrogen bonding and electrostatic interactions, *Int J Pept Protein Res* 41 (1993) 499-511. <https://doi.org/10.1111/j.1399-3011.1993.tb00470.xI>
- [128] A. Chakrabartty, A.J. Doig, R.L. Baldwin, Helix capping propensities in peptides parallel those in proteins, *Proc Natl Acad Sci U S A* 90 (1993) 11332-6. <https://doi.org/10.1073/pnas.90.23.11332I>
- [129] A.J. Doig, M.W. MacArthur, B.J. Stapley, J.M. Thornton, Structures of N-termini of helices in proteins, *Protein Sci* 6 (1997) 147-55. <https://doi.org/10.1002/pro.5560060117I>
- [130] L. Serrano, A.R. Fersht, Capping and alpha-helix stability, *Nature* 342 (1989) 296-9. <https://doi.org/10.1038/342296a0I>
- [131] E.T. Harper, G.D. Rose, Helix stop signals in proteins and peptides: the capping box, *Biochemistry* 32 (1993) 7605-9. <https://doi.org/10.1021/bi00081a001I>
- [132] J.W. Seale, R. Srinivasan, G.D. Rose, Sequence determinants of the capping box, a stabilizing motif at the N-termini of alpha-helices, *Protein Sci* 3 (1994) 1741-5. <https://doi.org/10.1002/pro.5560031014I>
- [133] V. Munoz, F.J. Blanco, L. Serrano, The hydrophobic-staple motif and a role for loop-residues in alpha-helix stability and protein folding, *Nat Struct Biol* 2 (1995) 380-5. <https://doi.org/nsb0595-380I>
- [134] R. Aurora, R. Srinivasan, G.D. Rose, Rules for alpha-helix termination by glycine, *Science* 264 (1994) 1126-30. <https://doi.org/10.1126/science.8178170I>
- [135] A.R. Viguera, L. Serrano, Experimental analysis of the Schellman motif, *J Mol Biol* 251 (1995) 150-60. <https://doi.org/10.1006/jmbi.1995.0422I>
- [136] J. Prieto, L. Serrano, C-capping and helix stability: the Pro C-capping motif, *J Mol Biol* 274 (1997) 276-88. <https://doi.org/10.1006/jmbi.1997.1322I>
- [137] N.J. Greenfield, W.F. Stafford, S.E. Hitchcock-DeGregori, The effect of N-terminal acetylation on the structure of an N-terminal tropomyosin peptide and alpha alpha-tropomyosin, *Protein Sci* 3 (1994) 402-10. <https://doi.org/10.1002/pro.5560030304I>
- [138] S. Marqusee, R.L. Baldwin, Helix stabilization by Glu-...Lys+ salt bridges in short peptides of de novo design, *Proc Natl Acad Sci U S A* 84 (1987) 8898-902. <https://doi.org/10.1073/pnas.84.24.8898I>
- [139] A. Horovitz, L. Serrano, B. Avron, M. Bycroft, A.R. Fersht, Strength and cooperativity of contributions of surface salt bridges to protein stability, *J Mol Biol* 216 (1990) 1031-44. [https://doi.org/10.1016/S0022-2836\(99\)80018-7I](https://doi.org/10.1016/S0022-2836(99)80018-7I)
- [140] G. Merutka, E. Stellwagen, Effect of amino acid ion pairs on peptide helicity, *Biochemistry* 30 (1991) 1591-4. <https://doi.org/10.1021/bi00220a021I>
- [141] E. Stellwagen, S.H. Park, W. Shalongo, A. Jain, The contribution of residue ion pairs to the helical stability of a model peptide, *Biopolymers* 32 (1992) 1193-200. <https://doi.org/10.1002/bip.360320909I>
- [142] J.M. Scholtz, H. Qian, V.H. Robbins, R.L. Baldwin, The energetics of ion-pair and hydrogen-bonding interactions in a helical peptide, *Biochemistry* 32 (1993) 9668-76. <https://doi.org/10.1021/bi00088a019I>

- [143] B.M. Huyghues-Despointes, R.L. Baldwin, Ion-pair and charged hydrogen-bond interactions between histidine and aspartate in a peptide helix, *Biochemistry* 36 (1997) 1965-70. <https://doi.org/10.1021/bi962546xl>
- [144] H. Meuzelaar, J. Vreede, S. Woutersen, Influence of Glu/Arg, Asp/Arg, and Glu/Lys Salt Bridges on alpha-Helical Stability and Folding Kinetics, *Biophys J* 110 (2016) 2328-2341. <https://doi.org/10.1016/j.bpj.2016.04.015I>
- [145] B.M. Huyghues-Despointes, T.M. Klingler, R.L. Baldwin, Measuring the strength of side-chain hydrogen bonds in peptide helices: the Gln.Asp (i, i + 4) interaction, *Biochemistry* 34 (1995) 13267-71. <https://doi.org/10.1021/bi00041a001I>
- [146] B.J. Stapley, A.J. Doig, Hydrogen bonding interactions between glutamine and asparagine in alpha-helical peptides, *J Mol Biol* 272 (1997) 465-73. <https://doi.org/10.1006/jmbi.1997.1262I>
- [147] J. Fernandez-Recio, A. Vazquez, C. Civera, P. Sevilla, J. Sancho, The tryptophan/histidine interaction in alpha-helices, *J Mol Biol* 267 (1997) 184-97. <https://doi.org/10.1006/jmbi.1996.0831I>
- [148] K.M. Armstrong, R. Fairman, R.L. Baldwin, The (i, i + 4) Phe-His interaction studied in an alanine-based alpha-helix, *J Mol Biol* 230 (1993) 284-91. <https://doi.org/10.1006/jmbi.1993.1142I>
- [149] K.R. Shoemaker, R. Fairman, D.A. Schultz, A.D. Robertson, E.J. York, J.M. Stewart, R.L. Baldwin, Side-chain interactions in the C-peptide helix: Phe 8 ... His 12+, *Biopolymers* 29 (1990) 1-11. <https://doi.org/10.1002/bip.360290104I>
- [150] S. Padmanabhan, R.L. Baldwin, Tests for helix-stabilizing interactions between various nonpolar side chains in alanine-based peptides, *Protein Sci* 3 (1994) 1992-7. <https://doi.org/10.1002/pro.5560031111I>
- [151] S. Padmanabhan, R.L. Baldwin, Helix-stabilizing interaction between tyrosine and leucine or valine when the spacing is i, i + 4, *J Mol Biol* 241 (1994) 706-13. <https://doi.org/10.1006/jmbi.1994.1545I>
- [152] C.D. Andrew, S. Penel, G.R. Jones, A.J. Doig, Stabilizing nonpolar/polar side-chain interactions in the alpha-helix, *Proteins* 45 (2001) 449-55. <https://doi.org/10.1002/prot.1161I>
- [153] Z. Shi, C.A. Olson, N.R. Kallenbach, Cation-pi interaction in model alpha-helical peptides, *J Am Chem Soc* 124 (2002) 3284-91. <https://doi.org/10.1021/ja0174938I>
- [154] C.D. Andrew, S. Bhattacharjee, N. Kokkoni, J.D. Hirst, G.R. Jones, A.J. Doig, Stabilizing interactions between aromatic and basic side chains in alpha-helical peptides and proteins. Tyrosine effects on helix circular dichroism, *J Am Chem Soc* 124 (2002) 12706-14. <https://doi.org/10.1021/ja027629hI>
- [155] L.K. Tsou, C.D. Tatko, M.L. Waters, Simple cation-pi interaction between a phenyl ring and a protonated amine stabilizes an alpha-helix in water, *J Am Chem Soc* 124 (2002) 14917-21. <https://doi.org/10.1021/ja026721aI>
- [156] R. Fairman, K.R. Shoemaker, E.J. York, J.M. Stewart, R.L. Baldwin, The Glu 2- ... Arg 10+ side-chain interaction in the C-peptide helix of ribonuclease A, *Biophys Chem* 37 (1990) 107-19. [https://doi.org/10.1016/0301-4622\(90\)88012-HI](https://doi.org/10.1016/0301-4622(90)88012-HI)
- [157] B.H. Zimm, J.K. Bragg, Theory of the Phase Transition between Helix and Random Coil in Polypeptide Chains, *J Chem Phys* 31 (1959) 526-535. <https://doi.org/doi.org/10.1063/1.1730390I>
- [158] V. Munoz, L. Serrano, Elucidating the folding problem of helical peptides using empirical parameters, *Nat Struct Biol* 1 (1994) 399-409. <https://doi.org/nsb0694-399I>

- [159] V. Munoz, L. Serrano, Elucidating the folding problem of helical peptides using empirical parameters. III. Temperature and pH dependence, *J Mol Biol* 245 (1995) 297-308. <https://doi.org/10.1006/jmbi.1994.0024I>
- [160] V. Munoz, L. Serrano, Elucidating the folding problem of helical peptides using empirical parameters. II. Helix macrodipole effects and rational modification of the helical content of natural peptides, *J Mol Biol* 245 (1995) 275-96. <https://doi.org/10.1006/jmbi.1994.0023I>
- [161] B.J. Stapley, C.A. Rohl, A.J. Doig, Addition of side chain interactions to modified Lifson-Roig helix-coil theory: application to energetics of phenylalanine-methionine interactions, *Protein Sci* 4 (1995) 2383-91. <https://doi.org/10.1002/pro.5560041117I>
- [162] J. Segura, B. Oliva, N. Fernandez-Fuentes, CAPS-DB: a structural classification of helix-capping motifs, *Nucleic Acids Res* 40 (2012) D479-85. <https://doi.org/10.1093/nar/gkr879I>
- [163] N. Pokala, T.M. Handel, Energy functions for protein design I: efficient and accurate continuum electrostatics and solvation, *Protein Sci* 13 (2004) 925-36. <https://doi.org/10.1110/ps.03486104I>
- [164] Y. Liu, B. Kuhlman, RosettaDesign server for protein design, *Nucleic Acids Res* 34 (2006) W235-8. <https://doi.org/10.1093/nar/gkl163I>
- [165] L. Dai, Y. Yang, H.R. Kim, Y. Zhou, Improving computational protein design by using structure-derived sequence profile, *Proteins* 78 (2010) 2338-48. <https://doi.org/10.1002/prot.22746I>
- [166] S. Liang, N.V. Grishin, Effective scoring function for protein sequence design, *Proteins* 54 (2004) 271-81. <https://doi.org/10.1002/prot.10560I>
- [167] Z. Li, Y. Yang, J. Zhan, L. Dai, Y. Zhou, Energy functions in de novo protein design: current challenges and future prospects, *Annu Rev Biophys* 42 (2013) 315-35. <https://doi.org/10.1146/annurev-biophys-083012-130315I>
- [168] K. Kuczera, *Molecular Modeling of Peptides*, 2015, pp. 15-41.
- [169] L.K. Henchey, A.L. Jochim, P.S. Arora, Contemporary strategies for the stabilization of peptides in the alpha-helical conformation, *Curr Opin Chem Biol* 12 (2008) 692-7. <https://doi.org/10.1016/j.cbpa.2008.08.019I>
- [170] J.E. Bock, J. Gavenonis, J.A. Kritzer, Getting in shape: controlling peptide bioactivity and bioavailability using conformational constraints, *ACS Chem Biol* 8 (2013) 488-499. <https://doi.org/10.1021/cb300515uI>
- [171] G.H. Bird, W.C. Crannell, L.D. Walensky, Chemical synthesis of hydrocarbon-stapled peptides for protein interaction research and therapeutic targeting, *Curr Protoc Chem Biol* 3 (2011) 99-117. <https://doi.org/10.1002/9780470559277.ch110042I>
- [172] L.D. Walensky, G.H. Bird, Hydrocarbon-stapled peptides: principles, practice, and progress, *J Med Chem* 57 (2014) 6275-88. <https://doi.org/10.1021/jm4011675I>
- [173] Y.H. Lau, P. de Andrade, Y. Wu, D.R. Spring, Peptide stapling techniques based on different macrocyclisation chemistries, *Chem Soc Rev* 44 (2015) 91-102. <https://doi.org/10.1039/c4cs00246fI>
- [174] Y.S. Tan, D.P. Lane, C.S. Verma, Stapled peptide design: principles and roles of computation, *Drug Discov Today* 21 (2016) 1642-1653. <https://doi.org/10.1016/j.drudis.2016.06.012I>
- [175] J.C. Phelan, N.J. Skelton, A.C. Braisted, R.S. McDowell, A general method for constraining short peptides to an α -helical conformation, *J Am Chem Soc* 119 (1997) 455-460. <https://doi.org/10.1021/ja9611654I>

- [176] J.W. Taylor, The synthesis and study of side-chain lactam-bridged peptides, *Biopolymers* 66 (2002) 49-75. <https://doi.org/10.1002/bip.102031>
- [177] M.J. Kelso, R.L. Beyer, H.N. Hoang, A.S. Lakdawala, J.P. Snyder, W.V. Oliver, T.A. Robertson, T.G. Appleton, D.P. Fairlie, α -Turn Mimetics: Short Peptide α -Helices Composed of Cyclic Metallopentapeptide Modules, *J Am Chem Soc* 126 (2004) 4828-4842. <https://doi.org/10.1021/ja037980i1>
- [178] M.I. Garcia-Aranda, S. Gonzalez-Lopez, C.M. Santiveri, N. Gagey-Eilstein, M. Reille-Seroussi, M. Martin-Martinez, N. Inguibert, M. Vidal, M.T. Garcia-Lopez, M.A. Jimenez, R. Gonzalez-Muniz, M.J. Perez de Vega, Helical peptides from VEGF and Vammin hotspots for modulating the VEGF-VEGFR interaction, *Org Biomol Chem* 11 (2013) 1896-905. <https://doi.org/10.1039/c3ob27312a1>
- [179] P.A. Sánchez-Murcia, M. Ruiz-Santaquiteria, M.A. Toro, H. de Lucio, M.Á. Jiménez, F. Gago, A. Jiménez-Ruiz, M.J. Camarasa, S. Velázquez, Comparison of hydrocarbon-and lactam-bridged cyclic peptides as dimerization inhibitors of *Leishmania infantum* trypanothione reductase, *RSC Adv* 5 (2015) 55784-55794. <https://doi.org/10.1039/c5ra06853c1>
- [180] G.L. Verdine, G.J. Hilinski, *Stapled peptides for intracellular drug targets*, 1 ed., Elsevier Inc.2012.
- [181] J.C. Thomas, J.M. Cooper, N.S. Clayton, C. Wang, M.A. White, C. Abell, D. Owen, H.R. Mott, Inhibition of Ral GTPases using a stapled peptide approach, *Journal of Biological Chemistry* 291 (2016) 18310-18325. <https://doi.org/10.1074/jbc.M116.7202431>
- [182] V. Gaillard, M. Galloux, D. Garcin, J.-F. Eléouët, R. Le Goffic, T. Larcher, M.-A. Rameix-Welti, A. Boukadiri, J. Héritier, J.-M. Segura, E. Baechler, M. Arrell, G. Mottet-Osman, O. Nyanguile, A Short Double-Stapled Peptide Inhibits Respiratory Syncytial Virus Entry and Spreading, *Antimicrobial Agents and Chemotherapy* 61 (2017) 1-19. <https://doi.org/10.1128/AAC.02241-161>
- [183] D. Migoń, D. Neubauer, W. Kamysz, Hydrocarbon Stapled Antimicrobial Peptides, *Protein Journal* 37 (2018) 2-12. <https://doi.org/10.1007/s10930-018-9755-01>
- [184] M. Ruiz-Santaquiteria, S. de Castro, M.A. Toro, H. de Lucio, K.J. Gutiérrez, P.A. Sánchez-Murcia, M.Á. Jiménez, F. Gago, A. Jiménez-Ruiz, M.J. Camarasa, S. Velázquez, Trypanothione reductase inhibition and anti-leishmanial activity of all-hydrocarbon stapled α -helical peptides with improved proteolytic stability, *European Journal of Medicinal Chemistry* 149 (2018) 238-247. <https://doi.org/10.1016/j.ejmech.2018.02.0711>
- [185] F. Meric-Bernstam, M.N. Saleh, J.R. Infante, S. Goel, G.S. Falchook, G. Shapiro, K.Y. Chung, R.M. Conry, D.S. Hong, J.S.-Z. Wang, U. Steidl, L.D. Walensky, V. Guerlavais, M. Payton, D.A. Annis, M. Aivado, M.R. Patel, Phase I trial of a novel stapled peptide ALRN-6924 disrupting MDMX- and MDM2-mediated inhibition of WT p53 in patients with solid tumors and lymphomas, *Journal of Clinical Oncology* 35 (2017) 2505-2505. https://doi.org/10.1200/JCO.2017.35.15_suppl.25051
- [186] D. Wang, K. Chen, J.L. Kulp, P.S. Arora, Evaluation of biologically relevant short α -helices stabilized by a main-chain hydrogen-bond surrogate, *J Am Chem Soc* 128 (2006) 9248-9256. <https://doi.org/10.1021/ja062710w1>
- [187] J. Liu, D. Wang, Q. Zheng, M. Lu, P.S. Arora, Atomic structure of a short α -helix stabilized by a main chain hydrogen-bond surrogate, *J Am Chem Soc* 130 (2008) 4334-4337. <https://doi.org/10.1021/ja077704u1>

- [188] D. Wang, M. Lu, P.S. Arora, Inhibition of HIV-1 fusion by hydrogen-bond-surrogate-based α helices, *Angewandte Chemie - International Edition* 47 (2008) 1879-1882. <https://doi.org/10.1002/anie.200704227I>
- [189] S.T. Joy, P.S. Arora, An optimal hydrogen-bond surrogate for α -helices, *Chemical Communications* 52 (2016) 5738-5741. <https://doi.org/10.1039/C6CC01104GI>
- [190] S.E. Miller, P.F. Thomson, P.S. Arora, Synthesis of Hydrogen-Bond Surrogate α -Helices as Inhibitors of Protein-Protein Interactions, *Curr Protoc Chem Biol* 33 (2014) 101-116. <https://doi.org/10.1002/9780470559277.ch130202I>
- [191] C. Renner, L. Moroder, Azobenzene as conformational switch in model peptides, *Chembiochem* 7 (2006) 868-78. <https://doi.org/10.1002/cbic.200500531I>
- [192] A.A. Beharry, G.A. Woolley, Azobenzene photoswitches for biomolecules, *Chem Soc Rev* 40 (2011) 4422-37. <https://doi.org/10.1039/c1cs15023el>
- [193] H.N. Hoang, R.W. Driver, R.L. Beyer, T.A. Hill, D.d.A. A, F. Plisson, R.S. Harrison, L. Goedecke, N.E. Shepherd, D.P. Fairlie, Helix Nucleation by the Smallest Known α -Helix in Water, *Angew Chem Int Ed Engl* 55 (2016) 8275-9. <https://doi.org/10.1002/anie.201602079I>
- [194] F.J. Blanco, M.A. Jimenez, J. Herranz, M. Rico, J. Santoro, J.L. Nieto, NMR evidence of a short linear peptide that folds into a β -hairpin in aqueous solution, *J Am Chem Soc* 115 (1993) 5887-5888. <https://doi.org/10.1021/ja00066a092I>
- [195] M. Ramirez-Alvarado, T. Kortemme, F.J. Blanco, L. Serrano, β -hairpin and β -sheet formation in designed linear peptides, *Bioorg Med Chem* 7 (1999) 93-103. [https://doi.org/10.1016/S0968-0896\(98\)00215-6I](https://doi.org/10.1016/S0968-0896(98)00215-6I)
- [196] J. Venkatraman, S.C. Shankaramma, P. Balaram, Design of folded peptides, *Chem Rev* 101 (2001) 3131-52. <https://doi.org/10.1021/cr000053zI>
- [197] C.E. Stotz, E.M. Topp, Applications of model β -hairpin peptides, *J Pharm Sci* 93 (2004) 2881-94. <https://doi.org/10.1002/jps.20188I>
- [198] M.S. Searle, Insights into stabilizing weak interactions in designed peptide β -hairpins, *Biopolymers* 76 (2004) 185-95. <https://doi.org/10.1002/bip.10577I>
- [199] D. Pantoja-Uceda, C.M. Santiveri, M.A. Jimenez, De novo design of monomeric β -hairpin and β -sheet peptides, *Methods Mol Biol* 340 (2006) 27-51. <https://doi.org/10.1385/1-59745-116-9:27I>
- [200] Y. Wei, B.M. Huyghues-Despointes, J. Tsai, J.M. Scholtz, NMR study and molecular dynamics simulations of optimized β -hairpin fragments of protein G, *Proteins* 69 (2007) 285-96. <https://doi.org/10.1002/prot.21494I>
- [201] M.P. Hatfield, R.F. Murphy, S. Lovas, The CLN025 decapeptide retains a β -hairpin conformation in urea and guanidinium chloride, *J Phys Chem B* 115 (2011) 4971-81. <https://doi.org/10.1021/jp111076jI>
- [202] M.S. Searle, D.H. Williams, L.C. Packman, A short linear peptide derived from the N-terminal sequence of ubiquitin folds into a water-stable non-native β -hairpin, *Nat Struct Biol* 2 (1995) 999-1006. <https://doi.org/nsb1195-999I>
- [203] E. de Alba, M.A. Jimenez, M. Rico, Turn residue sequence determines β -hairpin conformation in designed peptides, *J Am Chem Soc* 119 (1997) 175-183. <https://doi.org/10.1021/ja962325el>
- [204] E. de Alba, M. Rico, M.A. Jimenez, Cross-strand side-chain interactions versus turn conformation in β -hairpins, *Protein Sci* 6 (1997) 2548-60. <https://doi.org/10.1002/pro.5560061207I>

- [205] E. de Alba, M. Rico, M.A. Jimenez, The turn sequence directs beta-strand alignment in designed beta-hairpins, *Protein Sci* 8 (1999) 2234-44. <https://doi.org/10.1110/ps.8.11.2234I>
- [206] J.M. Anderson, B. Jurban, K.N. Huggins, A.A. Shcherbakov, I. Shu, B. Kier, N.H. Andersen, Nascent Hairpins in Proteins: Identifying Turn Loci and Quantitating Turn Contributions to Hairpin Stability, *Biochemistry* 55 (2016) 5537-5553. <https://doi.org/10.1021/acs.biochem.6b00732I>
- [207] T.S. Haque, S.H. Gellman, Insights into b-hairpin stability in aqueous solution from peptides with enforced type I' and type II' b-turns, *J Am Chem Soc* 119 (1997) 2303-2304. <https://doi.org/10.1021/ja963653hI>
- [208] H.E. Stanger, S.H. Gellman, Rules for antiparallel b-sheet design: D-Pro-Gly is superior to L-Asn-Gly for b-hairpin nucleation, *J Am Chem Soc* 120 (1998) 4236-4237. <https://doi.org/10.1021/ja963653hI>
- [209] E. de Alba, F.J. Blanco, M.A. Jimenez, M. Rico, J.L. Nieto, Interactions responsible for the pH dependence of the beta-hairpin conformational population formed by a designed linear peptide, *Eur J Biochem* 233 (1995) 283-92. https://doi.org/10.1111/j.1432-1033.1995.283_1.xI
- [210] C.M. Santiveri, M. Rico, M.A. Jimenez, Position effect of cross-strand side-chain interactions on beta-hairpin formation, *Protein Sci* 9 (2000) 2151-60. <https://doi.org/10.1110/ps.9.11.2151I>
- [211] T. Blandl, A.G. Cochran, N.J. Skelton, Turn stability in beta-hairpin peptides: Investigation of peptides containing 3:5 type I G1 bulge turns, *Protein Sci* 12 (2003) 237-47. <https://doi.org/10.1110/ps.0228603I>
- [212] S.K. Awasthi, S. Raghothama, P. Balaram, A designed beta-hairpin peptide, *Biochem Biophys Res Commun* 216 (1995) 375-81. <https://doi.org/10.1006/bbrc.1995.2634I>
- [213] S.R. Raghothama, S.K. Awasthi, P. Balaram, b-Hairpin nucleation by Pro-Gly b-turns. Comparison of DPro-Gly and LPro-Gly sequences in an apolar octapeptide, *J Chem Soc, Perkin Trans 2* (1998) 137-143. <https://doi.org/10.1039/A703331AI>
- [214] R. Rai, S. Raghothama, P. Balaram, Design of a peptide hairpin containing a central three-residue loop, *J Am Chem Soc* 128 (2006) 2675-81. <https://doi.org/10.1021/ja056861vI>
- [215] S. Chandrappa, M.B. Madhusudana Reddy, R. Sonti, K. Basuroy, S. Raghothama, P. Balaram, Directing peptide conformation with centrally positioned pre-organized dipeptide segments: studies of a 12-residue helix and beta-hairpin, *Amino Acids* 47 (2015) 291-301. <https://doi.org/10.1007/s00726-014-1858-0I>
- [216] C.A. Kim, J.M. Berg, Thermodynamic beta-sheet propensities measured using a zinc-finger host peptide, *Nature* 362 (1993) 267-70. <https://doi.org/10.1038/362267a0I>
- [217] D.L. Minor, Jr., P.S. Kim, Context is a major determinant of beta-sheet propensity, *Nature* 371 (1994) 264-7. <https://doi.org/10.1038/371264a0I>
- [218] D.L. Minor, Jr., P.S. Kim, Measurement of the beta-sheet-forming propensities of amino acids, *Nature* 367 (1994) 660-3. <https://doi.org/10.1038/367660a0I>
- [219] C.K. Smith, J.M. Withka, L. Regan, A thermodynamic scale for the beta-sheet forming tendencies of the amino acids, *Biochemistry* 33 (1994) 5510-7. <https://doi.org/10.1021/bi00184a020I>
- [220] J.S. Merkel, L. Regan, Aromatic rescue of glycine in beta sheets, *Fold Des* 3 (1998) 449-55. [https://doi.org/10.1016/S1359-0278\(98\)00062-5I](https://doi.org/10.1016/S1359-0278(98)00062-5I)

- [221] J.F. Espinosa, V. Munoz, S.H. Gellman, Interplay between hydrophobic cluster and loop propensity in beta-hairpin formation, *J Mol Biol* 306 (2001) 397-402. <https://doi.org/10.1006/jmbi.2000.4349I>
- [222] B.M. Huyghues-Despointes, X. Qu, J. Tsai, J.M. Scholtz, Terminal ion pairs stabilize the second beta-hairpin of the B1 domain of protein G, *Proteins* 63 (2006) 1005-17. <https://doi.org/10.1002/prot.20916I>
- [223] K.M. Makwana, R. Mahalakshmi, Stereopositional Outcome in the Packing of Dissimilar Aromatics in Designed beta-Hairpins, *Chemistry* 22 (2016) 4147-56. <https://doi.org/10.1002/chem.201504428I>
- [224] K.M. Makwana, R. Mahalakshmi, Asymmetric contribution of aromatic interactions stems from spatial positioning of the interacting aryl pairs in beta-hairpins, *Chembiochem* 15 (2014) 2357-60. <https://doi.org/10.1002/cbic.201402340I>
- [225] M. Ramirez-Alvarado, F.J. Blanco, L. Serrano, Elongation of the BH8 beta-hairpin peptide: Electrostatic interactions in beta-hairpin formation and stability, *Protein Sci* 10 (2001) 1381-92. <https://doi.org/10.1110/ps.52901I>
- [226] B. Ciani, M. Jourdan, M.S. Searle, Stabilization of beta-hairpin peptides by salt bridges: role of preorganization in the energetic contribution of weak interactions, *J Am Chem Soc* 125 (2003) 9038-47. <https://doi.org/10.1021/ja030074II>
- [227] S.T. Phillips, G. Piersanti, P.A. Bartlett, Quantifying amino acid conformational preferences and side-chain-side-chain interactions in beta-hairpins, *Proc Natl Acad Sci U S A* 102 (2005) 13737-42. <https://doi.org/10.1073/pnas.0506646102I>
- [228] H.T. Kuo, S.L. Liu, W.C. Chiu, C.J. Fang, H.C. Chang, W.R. Wang, P.A. Yang, J.H. Li, S.J. Huang, S.L. Huang, R.P. Cheng, Effect of charged amino acid side chain length on lateral cross-strand interactions between carboxylate- and guanidinium-containing residues in a beta-hairpin, *Amino Acids* 47 (2015) 885-98. <https://doi.org/10.1007/s00726-015-1916-2I>
- [229] Y. Mirassou, C.M. Santiveri, M.J. Perez de Vega, R. Gonzalez-Muniz, M.A. Jimenez, Disulfide bonds versus TrpTrp pairs in irregular beta-hairpins: NMR structure of vamin loop 3-derived peptides as a case study, *Chembiochem* 10 (2009) 902-10. <https://doi.org/10.1002/cbic.200800834I>
- [230] C.M. Santiveri, M.J. Perez de Vega, R. Gonzalez-Muniz, M.A. Jimenez, Trp-Trp pairs as beta-hairpin stabilisers: hydrogen-bonded versus non-hydrogen-bonded sites, *Org Biomol Chem* 9 (2011) 5487-92. <https://doi.org/10.1039/c1ob05353aI>
- [231] N. Kobayashi, S. Honda, H. Yoshii, E. Munekata, Role of side-chains in the cooperative beta-hairpin folding of the short C-terminal fragment derived from streptococcal protein G, *Biochemistry* 39 (2000) 6564-71. <https://doi.org/10.1021/bi000013pI>
- [232] M.T. Pastor, A. Gimenez-Giner, E. Perez-Paya, The role of an aliphatic-aromatic interaction in the stabilization of a model beta-hairpin peptide, *Chembiochem* 6 (2005) 1753-6. <https://doi.org/10.1002/cbic.200500178I>
- [233] K. Madhusudan Makwana, R. Mahalakshmi, Implications of aromatic-aromatic interactions: From protein structures to peptide models, *Protein Sci* 24 (2015) 1920-33. <https://doi.org/10.1002/pro.2814I>
- [234] F.J. Blanco, G. Rivas, L. Serrano, A short linear peptide that folds into a native stable beta-hairpin in aqueous solution, *Nat Struct Biol* 1 (1994) 584-90.
- [235] J.F. Espinosa, S.H. Gellman, A Designed beta-Hairpin Containing a Natural Hydrophobic Cluster, *Angew Chem Int Ed Engl* 39 (2000) 2330-2333.

[https://doi.org/10.1002/1521-3773\(20000703\)39:13<2330::AID-ANIE2330>3.0.CO;2-Cl](https://doi.org/10.1002/1521-3773(20000703)39:13<2330::AID-ANIE2330>3.0.CO;2-Cl)

[236] J.F. Espinosa, F.A. Syud, S.H. Gellman, Analysis of the factors that stabilize a designed two-stranded antiparallel beta-sheet, *Protein Sci* 11 (2002) 1492-505. <https://doi.org/10.1110/ps.4140102I>

[237] L. Wu, D. McElheny, R. Huang, T.A. Keiderling, Role of tryptophan-tryptophan interactions in Trpzip beta-hairpin formation, structure, and stability, *Biochemistry* 48 (2009) 10362-71. <https://doi.org/10.1021/bi901249dl>

[238] L. Wu, D. McElheny, T. Takekiyo, T.A. Keiderling, Geometry and efficacy of cross-strand Trp/Trp, Trp/Tyr, and Tyr/Tyr aromatic interaction in a beta-hairpin peptide, *Biochemistry* 49 (2010) 4705-14. <https://doi.org/10.1021/bi100491sl>

[239] F.A. Syud, H.E. Stanger, S.H. Gellman, Interstrand side chain--side chain interactions in a designed beta-hairpin: significance of both lateral and diagonal pairings, *J Am Chem Soc* 123 (2001) 8667-77. <https://doi.org/10.1021/ja0109803I>

[240] C.D. Tatko, M.L. Waters, The geometry and efficacy of cation-pi interactions in a diagonal position of a designed beta-hairpin, *Protein Sci* 12 (2003) 2443-52. <https://doi.org/10.1110/ps.03284003I>

[241] C.D. Tatko, M.L. Waters, Investigation of the nature of the methionine-pi interaction in beta-hairpin peptide model systems, *Protein Sci* 13 (2004) 2515-22. <https://doi.org/10.1110/ps.04820104I>

[242] A.J. Riemen, M.L. Waters, Controlling peptide folding with repulsive interactions between phosphorylated amino acids and tryptophan, *J Am Chem Soc* 131 (2009) 14081-7. <https://doi.org/10.1021/ja9047575I>

[243] D. Andreu, L. Rivas, Animal antimicrobial peptides: an overview, *Biopolymers* 47 (1998) 415-33. [https://doi.org/10.1002/\(SICI\)1097-0282\(1998\)47:6<415::AID-BIP2>3.0.CO;2-DI](https://doi.org/10.1002/(SICI)1097-0282(1998)47:6<415::AID-BIP2>3.0.CO;2-DI)

[244] P.V. Panteleev, I.A. Bolosov, S.V. Balandin, T.V. Ovchinnikova, Structure and Biological Functions of beta-Hairpin Antimicrobial Peptides, *Acta Naturae* 7 (2015) 37-47. <https://doi.org/PMC4410394I>

[245] M.A. Wouters, P.M. Curmi, An analysis of side chain interactions and pair correlations within antiparallel beta-sheets: the differences between backbone hydrogen-bonded and non-hydrogen-bonded residue pairs, *Proteins* 22 (1995) 119-31. <https://doi.org/10.1002/prot.340220205I>

[246] K. Gunasekaran, C. Ramakrishnan, P. Balaram, Beta-hairpins in proteins revisited: lessons for de novo design, *Protein Eng* 10 (1997) 1131-41. <https://doi.org/10.1093/protein/10.10.1131I>

[247] E.G. Hutchinson, R.B. Sessions, J.M. Thornton, D.N. Woolfson, Determinants of strand register in antiparallel beta-sheets of proteins, *Protein Sci* 7 (1998) 2287-300. <https://doi.org/10.1002/pro.5560071106I>

[248] C.M. Santiveri, E. Leon, M. Rico, M.A. Jimenez, Context-dependence of the contribution of disulfide bonds to beta-hairpin stability, *Chemistry* 14 (2008) 488-99. <https://doi.org/10.1002/chem.200700845I>

[249] M.I. Garcia-Aranda, Y. Mirassou, B. Gautier, M. Martin-Martinez, N. Inguibert, M. Vidal, M.T. Garcia-Lopez, M.A. Jimenez, R. Gonzalez-Muniz, M.J. Perez de Vega, Disulfide and amide-bridged cyclic peptide analogues of the VEGF(8)(1)(-)(9)(1) fragment: synthesis, conformational analysis and biological evaluation, *Bioorg Med Chem* 19 (2011) 7526-33. <https://doi.org/10.1016/j.bmc.2011.10.032I>

- [250] L. Eidenschink, E. Crabbe, N.H. Andersen, Terminal sidechain packing of a designed beta-hairpin influences conformation and stability, *Biopolymers* 91 (2009) 557-64. <https://doi.org/10.1002/bip.21177I>
- [251] B.L. Kier, I. Shu, L.A. Eidenschink, N.H. Andersen, Stabilizing capping motif for beta-hairpins and sheets, *Proc Natl Acad Sci U S A* 107 (2010) 10466-71. <https://doi.org/10.1073/pnas.0913534107I>
- [252] B.L. Kier, N.H. Andersen, Captides: rigid junctions between beta sheets and small molecules, *J Pept Sci* 20 (2014) 704-15. <https://doi.org/10.1002/psc.2657I>
- [253] J.M. Anderson, A.A. Shcherbakov, B.L. Kier, J. Kellock, I. Shu, A.L. Byrne, L.A. Eidenschink, N.H. Andersen, Optimization of a beta-sheet-cap for long loop closure, *Biopolymers* 107 (2017). <https://doi.org/10.1002/bip.22995I>
- [254] K.M. Makwana, R. Mahalakshmi, Capping beta-hairpin with N-terminal d-amino acid stabilizes peptide scaffold, *Biopolymers* 106 (2016) 260-6. <https://doi.org/10.1002/bip.22837I>
- [255] K.M. Makwana, R. Mahalakshmi, NMR Analysis of Tuning Cross-Strand Phe/Tyr/Trp-Trp Interactions in Designed beta-Hairpin Peptides: Terminal Switch from L to D Amino Acid as a Strategy for beta-Hairpin Capping, *J Phys Chem B* 119 (2015) 5376-85. <https://doi.org/10.1021/acs.jpcb.5b00554I>
- [256] E.G. Hutchinson, J.M. Thornton, A revised set of potentials for beta-turn formation in proteins, *Protein Sci* 3 (1994) 2207-16. <https://doi.org/10.1002/pro.5560031206I>
- [257] H.E. Stanger, F.A. Syud, J.F. Espinosa, I. Gariat, T. Muir, S.H. Gellman, Length-dependent stability and strand length limits in antiparallel beta -sheet secondary structure, *Proc Natl Acad Sci U S A* 98 (2001) 12015-20. <https://doi.org/10.1073/pnas.211536998I>
- [258] H. Kaur, G.P. Raghava, An evaluation of beta-turn prediction methods, *Bioinformatics* 18 (2002) 1508-14. <https://doi.org/10.1093/bioinformatics/18.11.1508.I>
- [259] H. Kaur, G.P. Raghava, BetaTPred: prediction of beta-TURNS in a protein using statistical algorithms, *Bioinformatics* 18 (2002) 498-9. <https://doi.org/10.1093/bioinformatics/18.3.498I>
- [260] B. Petersen, C. Lundegaard, T.N. Petersen, NetTurnP--neural network prediction of beta-turns by use of evolutionary information and predicted protein sequence features, *PLoS One* 5 (2010) e15079. <https://doi.org/10.1371/journal.pone.0015079I>
- [261] X. de la Cruz, E.G. Hutchinson, A. Shepherd, J.M. Thornton, Toward predicting protein topology: an approach to identifying beta hairpins, *Proc Natl Acad Sci U S A* 99 (2002) 11157-62. <https://doi.org/10.1073/pnas.162376199I>
- [262] M. Kumar, M. Bhasin, N.K. Natt, G.P. Raghava, BhairPred: prediction of beta-hairpins in a protein from multiple alignment information using ANN and SVM techniques, *Nucleic Acids Res* 33 (2005) W154-9. <https://doi.org/10.1093/nar/gki588I>
- [263] R.V. Nair, S.B. Baravkar, T.S. Ingole, G.J. Sanjayan, Synthetic turn mimetics and hairpin nucleators: Quo Vadimus?, *Chem Commun (Camb)* 50 (2014) 13874-84. <https://doi.org/10.1039/c4cc03114hI>
- [264] J. Maity, U.I. Gerling, S. Vukelic, A. Schafer, B. Koksche, Proline-glutamate chimera's side chain conformation directs the type of beta-hairpin structure, *Amino Acids* 46 (2014) 177-86. <https://doi.org/10.1007/s00726-013-1610-1I>
- [265] A.J. Cameron, K.G. Varnava, P.J.B. Edwards, E. Harjes, V. Sarojini, Acyclic peptides incorporating the d-Phe-2-Abz turn motif: Investigations on antimicrobial

- activity and propensity to adopt beta-hairpin conformations, *J Pept Sci* 24 (2018) e3094. <https://doi.org/10.1002/psc.3094>
- [266] A. Jalan, D.W. Kastner, K.G.I. Webber, M.S. Smith, J.L. Price, S.L. Castle, Bulky Dehydroamino Acids Enhance Proteolytic Stability and Folding in beta-Hairpin Peptides, *Org Lett* 19 (2017) 5190-5193. <https://doi.org/10.1021/acs.orglett.7b02455>
- [267] N. Sawyer, P.S. Arora, Hydrogen Bond Surrogate Stabilization of beta-Hairpins, *ACS Chem Biol* 13 (2018) 2027-2032. <https://doi.org/10.1021/acschembio.8b00641>
- [268] D. Diana, C. Di Salvo, V. Celentano, L. De Rosa, A. Romanelli, R. Fattorusso, L.D. D'Andrea, Conformational stabilization of a beta-hairpin through a triazole-tryptophan interaction, *Org Biomol Chem* 16 (2018) 787-795. <https://doi.org/10.1039/c7ob02815f>
- [269] V. Celentano, D. Diana, C. Di Salvo, L. De Rosa, A. Romanelli, R. Fattorusso, L.D. D'Andrea, 1,2,3-Triazole Bridge as Conformational Constrain in beta-Hairpin Peptides: Analysis of Hydrogen-Bonded Positions, *Chemistry* 22 (2016) 5534-7. <https://doi.org/10.1002/chem.201600154>
- [270] B.L. Kier, J.M. Anderson, N.H. Andersen, Disulfide-Mediated beta-Strand Dimers: Hyperstable beta-Sheets Lacking Tertiary Interactions and Turns, *J Am Chem Soc* 137 (2015) 5363-71. <https://doi.org/10.1021/ja5117809>
- [271] A. Jha, M.G. Kumar, H.N. Gopi, K.M. Paknikar, Inhibition of beta-Amyloid Aggregation through a Designed beta-Hairpin Peptide, *Langmuir* 34 (2018) 1591-1600. <https://doi.org/10.1021/acs.langmuir.7b03617>
- [272] G. Wang, N. Drinkwater, D.R. Drew, C.A. MacRaild, D.K. Chalmers, B. Mohanty, S.S. Lim, R.F. Anders, J.G. Beeson, P.E. Thompson, S. McGowan, J.S. Simpson, R.S. Norton, M.J. Scanlon, Structure-Activity Studies of beta-Hairpin Peptide Inhibitors of the Plasmodium falciparum AMA1-RON2 Interaction, *J Mol Biol* 428 (2016) 3986-3998. <https://doi.org/10.1016/j.jmb.2016.07.001>
- [273] D. Diana, A. Basile, L. De Rosa, R. Di Stasi, S. Auriemma, C. Arra, C. Pedone, M.C. Turco, R. Fattorusso, L.D. D'Andrea, beta-hairpin peptide that targets vascular endothelial growth factor (VEGF) receptors: design, NMR characterization, and biological activity, *J Biol Chem* 286 (2011) 41680-91. <https://doi.org/10.1074/jbc.M111.257402>
- [274] L. De Rosa, D. Diana, A. Basile, A. Russomanno, C. Isernia, M.C. Turco, R. Fattorusso, L.D. D'Andrea, Design, structural and biological characterization of a VEGF inhibitor beta-hairpin-constrained peptide, *Eur J Med Chem* 73 (2014) 210-6. <https://doi.org/10.1016/j.ejmech.2013.12.016>
- [275] D. Diana, A. Russomanno, L. De Rosa, R. Di Stasi, D. Capasso, S. Di Gaetano, A. Romanelli, L. Russo, L.D. D'Andrea, R. Fattorusso, Functional binding surface of a beta-hairpin VEGF receptor targeting peptide determined by NMR spectroscopy in living cells, *Chemistry* 21 (2015) 91-5. <https://doi.org/10.1002/chem.201403335>
- [276] A. Gokhale, T.K. Weldeghiorghis, V. Taneja, S.D. Satyanarayanajois, Conformationally constrained peptides from CD2 to modulate protein-protein interactions between CD2 and CD58, *J Med Chem* 54 (2011) 5307-19. <https://doi.org/10.1021/jm200004e>
- [277] L. Xu, S. Chou, J. Wang, C. Shao, W. Li, X. Zhu, A. Shan, Antimicrobial activity and membrane-active mechanism of tryptophan zipper-like beta-hairpin antimicrobial peptides, *Amino Acids* 47 (2015) 2385-97. <https://doi.org/10.1007/s00726-015-2029-7>

- [278] K. Sivanesam, B.L. Kier, S.D. Whedon, C. Chatterjee, N.H. Andersen, Hairpin structure stability plays a role in the activity of two antimicrobial peptides, *FEBS Lett* 590 (2016) 4480-4488. <https://doi.org/10.1002/1873-3468.124771>
- [279] K. Sivanesam, B.L. Kier, S.D. Whedon, C. Chatterjee, N.H. Andersen, Biological consequences of improving the structural stability of hairpins that have antimicrobial activity, *J Pept Sci* 23 (2017) 899-906. <https://doi.org/10.1002/psc.30541>
- [280] S.M. Butterfield, M.L. Waters, A designed beta-hairpin peptide for molecular recognition of ATP in water, *J Am Chem Soc* 125 (2003) 9580-1. <https://doi.org/10.1021/ja03592541>
- [281] S.M. Butterfield, C.M. Goodman, V.M. Rotello, M.L. Waters, A peptide flavoprotein mimic: flavin recognition and redox potential modulation in water by a designed beta hairpin, *Angew Chem Int Ed Engl* 43 (2004) 724-7. <https://doi.org/10.1002/anie.2003525271>
- [282] S.M. Butterfield, M.M. Sweeney, M.L. Waters, The recognition of nucleotides with model beta-hairpin receptors: investigation of critical contacts and nucleotide selectivity, *J Org Chem* 70 (2005) 1105-14. <https://doi.org/10.1021/jo04911051>
- [283] S.M. Butterfield, W.J. Cooper, M.L. Waters, Minimalist protein design: a beta-hairpin peptide that binds ssDNA, *J Am Chem Soc* 127 (2005) 24-5. <https://doi.org/10.1021/ja045002o1>
- [284] A.L. Stewart, M.L. Waters, Structural effects on ss- and dsDNA recognition by a beta-hairpin peptide, *Chembiochem* 10 (2009) 539-44. <https://doi.org/10.1002/cbic.2008005241>
- [285] L.L. Cline, M.L. Waters, Design of a beta-hairpin peptide-intercalator conjugate for simultaneous recognition of single stranded and double stranded regions of RNA, *Org Biomol Chem* 7 (2009) 4622-30. <https://doi.org/10.1039/b913024a1>
- [286] D.J. Wilger, J.H. Park, R.M. Hughes, M.E. Cuellar, M.L. Waters, Induced-fit binding of a polyproline helix by a beta-hairpin peptide, *Angew Chem Int Ed Engl* 50 (2011) 12201-4. <https://doi.org/10.1002/anie.2011061771>
- [287] D. Nagarajan, S. Sukumaran, G. Deka, K. Krishnamurthy, H.S. Atreya, N. Chandra, Design of a heme-binding peptide motif adopting a beta-hairpin conformation, *J Biol Chem* 293 (2018) 9412-9422. <https://doi.org/10.1074/jbc.RA118.0017681>
- [288] B. Maestro, C.M. Santiveri, M.A. Jimenez, J.M. Sanz, Structural autonomy of a beta-hairpin peptide derived from the pneumococcal choline-binding protein LytA, *Protein Eng Des Sel* 24 (2011) 113-22. <https://doi.org/10.1093/protein/gzq0871>
- [289] G.W. Platt, C.-W. Chung, M.S. Searle, Design of histidin-Zn²⁺ binding sites within a b-hairpin peptide: enhancement of b-sheet stability through metal complexation, *Chem Commun* (2001) 1162-1163. <https://doi.org/10.1039/B100702P1>
- [290] D. Ramadan, D.J. Cline, S. Bai, C. Thorpe, J.P. Schneider, Effects of As(III) binding on beta-hairpin structure, *J Am Chem Soc* 129 (2007) 2981-8. <https://doi.org/10.1021/ja067068k1>
- [291] J. Reutzel, T.M. Diogo, A. Geyer, Reversible Folding of a beta-Hairpin Peptide by a Metal-Chelating Amino Acid, *Chemistry* 23 (2017) 8450-8456. <https://doi.org/10.1002/chem.2017006981>
- [292] G. Morris, I. Robertson, C.D. Tatko, Iron binding beta-hairpin peptides, *Biomaterials* 26 (2013) 667-75. <https://doi.org/10.1007/s10534-013-9638-yl>

- [293] R. Sibert, M. Josowicz, F. Porcelli, G. Veglia, K. Range, B.A. Barry, Proton-coupled electron transfer in a biomimetic peptide as a model of enzyme regulatory mechanisms, *J Am Chem Soc* 129 (2007) 4393-400. <https://doi.org/10.1021/ja068805f1>
- [294] C.V. Pagba, S.H. Chi, J. Perry, B.A. Barry, Proton-coupled electron transfer in tyrosine and a beta-hairpin maquette: reaction dynamics on the picosecond time scale, *J Phys Chem B* 119 (2015) 2726-36. <https://doi.org/10.1021/jp510171z1>
- [295] C.V. Pagba, T.G. McCaslin, S.H. Chi, J.W. Perry, B.A. Barry, Proton-Coupled Electron Transfer and a Tyrosine-Histidine Pair in a Photosystem II-Inspired beta-Hairpin Maquette: Kinetics on the Picosecond Time Scale, *J Phys Chem B* 120 (2016) 1259-72. <https://doi.org/10.1021/acs.jpcc.6b005601>
- [296] H. Hwang, T.G. McCaslin, A. Hazel, C.V. Pagba, C.M. Nevin, A. Pavlova, B.A. Barry, J.C. Gumbart, Redox-Driven Conformational Dynamics in a Photosystem-II-Inspired beta-Hairpin Maquette Determined through Spectroscopy and Simulation, *J Phys Chem B* 121 (2017) 3536-3545. <https://doi.org/10.1021/acs.jpcc.6b094811>
- [297] C.V. Pagba, T.G. McCaslin, G. Veglia, F. Porcelli, J. Yohannan, Z. Guo, M. McDaniel, B.A. Barry, A tyrosine-tryptophan dyad and radical-based charge transfer in a ribonucleotide reductase-inspired maquette, *Nat Commun* 6 (2015) 10010. <https://doi.org/10.1038/ncomms100101>
- [298] T. Podewin, M.S. Rampp, I. Turkanovic, K.L. Karaghiosoff, W. Zinth, A. Hoffmann-Roder, Photocontrolled chignolin-derived beta-hairpin peptidomimetics, *Chem Commun (Camb)* 51 (2015) 4001-4. <https://doi.org/10.1039/c4cc10304a1>
- [299] S. Sathaye, H. Zhang, C. Sonmez, J.P. Schneider, C.M. MacDermaid, C.D. Von Bargen, J.G. Saven, D.J. Pochan, Engineering complementary hydrophobic interactions to control beta-hairpin peptide self-assembly, network branching, and hydrogel properties, *Biomacromolecules* 15 (2014) 3891-900. <https://doi.org/10.1021/bm500874t1>
- [300] L.M. De Leon-Rodriguez, Y. Hemar, A.K. Mitra, M.A. Brimble, Understanding the metal mediated assembly and hydrogel formation of a beta-hairpin peptide, *Biomater Sci* 5 (2017) 1993-1997. <https://doi.org/10.1039/c7bm00512a1>
- [301] P. Worthington, S. Langhans, D. Pochan, beta-hairpin peptide hydrogels for package delivery, *Adv Drug Deliv Rev* 110-111 (2017) 127-136. <https://doi.org/10.1016/j.addr.2017.02.0021>
- [302] D.J. Lockhart, P.S. Kim, Electrostatic screening of charge and dipole interactions with the helix backbone, *Science* 260 (1993) 198-202. <https://doi.org/10.1126/science.84699721>
- [303] J.L. England, G. Haran, Role of solvation effects in protein denaturation: from thermodynamics to single molecules and back, *Annu Rev Phys Chem* 62 (2011) 257-77. <https://doi.org/10.1146/annurev-physchem-032210-1035311>
- [304] D.R. Canchi, A.E. Garcia, Cosolvent effects on protein stability, *Annu Rev Phys Chem* 64 (2013) 273-93. <https://doi.org/10.1146/annurev-physchem-040412-1101561>
- [305] M. Rico, J. Santoro, F.J. Bermejo, J. Herranz, J.L. Nieto, E. Gallego, M.A. Jimenez, Thermodynamic parameters for the helix-coil thermal transition of ribonuclease-S-peptide and derivatives from 1H-NMR data, *Biopolymers* 25 (1986) 1031-53. <https://doi.org/10.1002/bip.3602506051>
- [306] J.K. Myers, C.N. Pace, J.M. Scholtz, Trifluoroethanol effects on helix propensity and electrostatic interactions in the helical peptide from ribonuclease T1, *Protein Sci* 7 (1998) 383-8. <https://doi.org/10.1002/pro.55600702191>

- [307] C.M. Santiveri, D. Pantoja-Uceda, M. Rico, M.A. Jimenez, Beta-hairpin formation in aqueous solution and in the presence of trifluoroethanol: a ¹H and ¹³C nuclear magnetic resonance conformational study of designed peptides, *Biopolymers* 79 (2005) 150-62. <https://doi.org/10.1002/bip.20345>
- [308] P. Luo, R.L. Baldwin, Mechanism of helix induction by trifluoroethanol: a framework for extrapolating the helix-forming properties of peptides from trifluoroethanol/water mixtures back to water, *Biochemistry* 36 (1997) 8413-21. <https://doi.org/10.1021/bi9707133>
- [309] M. Buck, Trifluoroethanol and colleagues: cosolvents come of age. Recent studies with peptides and proteins, *Q Rev Biophys* 31 (1998) 297-355. <https://doi.org/10.1017/s003358359800345x>
- [310] P. Luo, R.L. Baldwin, Interaction between water and polar groups of the helix backbone: an important determinant of helix propensities, *Proc Natl Acad Sci U S A* 96 (1999) 4930-5. <https://doi.org/10.1073/pnas.96.9.4930>
- [311] J.L. Huang, M.E. Noss, K.M. Schmidt, L. Murray, M.R. Bunagan, The effect of neat ionic liquid on the folding of short peptides, *Chem Commun (Camb)* 47 (2011) 8007-9. <https://doi.org/10.1039/c1cc11527h>
- [312] Y. Yu, J. Wang, Q. Shao, J. Shi, W. Zhu, The effects of organic solvents on the folding pathway and associated thermodynamics of proteins: a microscopic view, *Sci Rep* 6 (2016) 19500. <https://doi.org/10.1038/srep19500>
- [313] C.R. Sanders, F. Sonnichsen, Solution NMR of membrane proteins: practice and challenges, *Magn Reson Chem* 44 Spec No (2006) S24-40. <https://doi.org/10.1002/mrc.1816>
- [314] L. Maler, Solution NMR studies of cell-penetrating peptides in model membrane systems, *Adv Drug Deliv Rev* 65 (2013) 1002-11. <https://doi.org/10.1016/j.addr.2012.10.011>
- [315] M.S. Searle, R. Zerella, D.H. Williams, L.C. Packman, Native-like beta-hairpin structure in an isolated fragment from ferredoxin: NMR and CD studies of solvent effects on the N-terminal 20 residues, *Protein Eng* 9 (1996) 559-65. <https://doi.org/10.1093/protein/9.7.559>
- [316] M.S. Searle, M. Jourdan, Templating peptide folding on the surface of a micelle: nucleating the formation of a beta-hairpin, *Bioorg Med Chem Lett* 10 (2000) 1139-42. [https://doi.org/10.1016/S0960-894X\(00\)00192-X](https://doi.org/10.1016/S0960-894X(00)00192-X)