

# An exhaustive survey of regular peptide conformations using a new metric for backbone handedness ( $h$ )

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The Ramachandran plot is important to structural biology as it describes a peptide backbone in the context of its dominant degrees of freedom – the backbone dihedral angles  $\phi$  and  $\psi$  (Ramachandran *et al.*, 1963). Since its introduction, the Ramachandran plot has been a crucial tool to characterize protein backbone features. However, the conformation or twist of a backbone as a function of  $\phi$  and  $\psi$  has not been completely described for both *cis* and *trans* backbones. Additionally, little intuitive understanding is available about a peptide's conformation simply from knowing the  $\phi$  and  $\psi$  values of a peptide (e.g., is the regular peptide defined by  $\phi = \psi = -100^\circ$  left-handed or right-handed?). This report provides a new metric for backbone handedness ( $h$ ) based on interpreting a peptide backbone as a helix with axial or vertical displacement  $d$  and angular displacement  $\theta$ , both of which are derived from a peptide backbone's internal coordinates, especially dihedral angles  $\phi$ ,  $\psi$  and  $\omega$ . In particular,  $h = \sin(\theta)d/|d|$  with range  $[-1, 1]$  and negative (or positive) values indicating left(or right)-handedness. The metric  $h$  is used to characterize the handedness of every region of the Ramachandran plot for both *cis* ( $\omega = 0^\circ$ ) and *trans* ( $\omega = 180^\circ$ ) backbones, which provides the first exhaustive survey of twist handedness in Ramachandran ( $\phi, \psi$ ) space. These maps fill in the 'dead space' within the Ramachandran plot, which are regions that are not commonly accessed by globular (structured) proteins, but which may be accessible to intrinsically disordered proteins, short peptide fragments, and protein mimics such as peptoids. Finally, building on the work of Zacharias and Knapp (2013), this report presents a new plot based on  $d$  and  $\theta$  that serves as a universal and intuitive alternative to the Ramachandran plot. The universality arises from the fact that the co-inhabitants of such a plot include every possible peptide backbone including *cis* and *trans* backbones. The intuitiveness arises from the fact that  $d$  and  $\theta$  provide, at a glance, numerous aspects of the backbone including compactness, handedness, and planarity.

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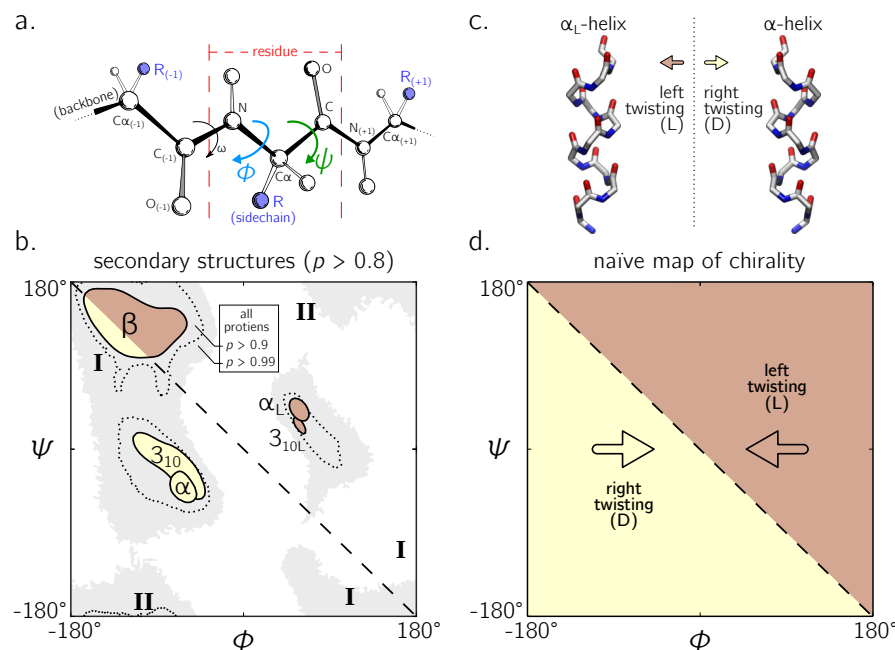
## ABSTRACT

The Ramachandran plot is important to structural biology as it describes a peptide backbone in the context of its dominant degrees of freedom – the backbone dihedral angles  $\phi$  and  $\psi$  (Ramachandran *et al.*, 1963). Since its introduction, the Ramachandran plot has been a crucial tool to characterize protein backbone features. However, the conformation or twist of a backbone as a function of  $\phi$  and  $\psi$  has not been completely described for both *cis* and *trans* backbones. Additionally, little intuitive understanding is available about a peptide's conformation simply from knowing the  $\phi$  and  $\psi$  values of a peptide (e.g., is the regular peptide defined by  $\phi = \psi = -100^\circ$  left-handed or right-handed?). This report provides a new metric for backbone handedness ( $h$ ) based on interpreting a peptide backbone as a helix with axial or vertical displacement  $d$  and angular displacement  $\theta$ , both of which are derived from a peptide backbone's internal coordinates, especially dihedral angles  $\phi$ ,  $\psi$  and  $\omega$ . In particular,  $h = \sin(\theta)d/|d|$  with range  $[-1, 1]$  and negative (or positive) values indicating left(or right)-handedness. The metric  $h$  is used to characterize the handedness of every region of the Ramachandran plot for both *cis* ( $\omega = 0^\circ$ ) and *trans* ( $\omega = 180^\circ$ ) backbones, which provides the first exhaustive survey of twist handedness in Ramachandran ( $\phi, \psi$ ) space. These maps fill in the 'dead space' within the Ramachandran plot, which are regions that are not commonly accessed by globular (structured) proteins, but which may be accessible to intrinsically disordered proteins, short peptide fragments, and protein mimics such as peptoids. Finally, building on the work of Zacharias and Knapp (2013), this report presents a new plot based on  $d$  and  $\theta$  that serves as a universal and intuitive alternative to the Ramachandran plot. The universality arises from the fact that the co-inhabitants of such a plot include every possible peptide backbone including *cis* and *trans* backbones. The intuitiveness arises from the fact that  $d$  and  $\theta$  provide, at a glance, numerous aspects of the backbone including compactness, handedness, and planarity.

## INTRODUCTION

The backbone of a protein (Fig. 1a) can twist and turn into numerous conformations (folds), in part due to the amino acid sequence that the protein displays. Understanding how a backbone twists is of great importance to the field of biochemistry, since understanding the structure of a protein goes a long way towards understanding how a protein functions (Alberts *et al.*, 2002; Berg *et al.*, 2010). While the conformation of a peptide backbone is dependent on a number of parameters (bond lengths, bond angles, and dihedral angles), Ramachandran *et al.* (1963) recognized that the twist of a peptide backbone can be described to a great degree by the dihedral angles  $\phi$  and  $\psi$  (Fig. 1a).

Today, two-dimensional ( $\phi, \psi$ ) plots are called Ramachandran plots (or 'maps'), and are introduced in undergraduate biology textbooks as a guide for understanding a peptide backbone's general conformational state or 'twistedness' at a glance (Bragg *et al.*, 1950; Pauling and Corey, 1951b; Pauling *et al.*, 1951; Linderstrøm-Lang, 1952; Laskowski *et al.*, 1993; Chothia *et al.*, 1997; Hooft *et al.*, 1997; Cooper and Hausman, 2013; Alberts *et al.*, 2002; Laskowski, 2003; Ho *et al.*, 2003; Eisenberg, 2003; Berg *et al.*, 2010; Mannige, 2014). The Ramachandran plot is especially useful because (stable) proteins are hierarchical in structure (Linderstrøm-Lang, 1952): the final (tertiary) conformation of a *structured* protein is composed of discrete secondary structures – *regular* structures – that interact with each other and are strung together by loops that are less regular (Alberts *et al.*, 2002; Berg *et al.*, 2010). Each regular secondary



**Figure 1.** The backbone of a single residue (a) can be described by its dihedral angles  $\phi$  and  $\psi$  (and in smaller part,  $\omega$ , which is predominantly *trans* or  $\sim 180^\circ$ ). For example, repeating a value for  $\phi$  and  $\psi$  for every residue within a peptide – i.e., selecting a point on the Ramachandran plot (b) – results in regular secondary structures that then combine to form most globular proteins in the protein databank (Berman *et al.*, 2000). The twist of each perfectly regular secondary structure possesses a distinct chirality: left-handed or right-handed; examples are shown in (c). all molecular representations in this text are shown in ‘licorice’ form, with the colors red, blue and white representing the oxygen, nitrogen and carbon atoms. As evidenced in (c), the -ve diagonal within the Ramachandran plot (dashed line described by  $\phi = \psi$ ) divides right-handed peptides from left-handed peptides, which leads to the naïve picture of handedness (d). Zacharias and Knapp (2013) showed that this picture is over simplistic, however an in-depth characterization of the backbone in all regions was not performed, and will be done here for both *cis* ( $\omega = 0$ ) and *trans* backbones ( $\omega = \pi$ ). Due to low count within the studied database (see Methods), the two left-handed helices in (b) are arbitrarily marked and have no statistical significance.

structure describes a backbone whose per-residue ( $\phi$ ,  $\psi$ ) values are generally the same, and therefore their ‘locations’ on the Ramachandran plot act as structural landmarks (Fig. 1b).

So far, our understanding of the Ramachandran plot has been limited mostly to ‘structured’ proteins that display stable conformations (Berman *et al.*, 2000; Alberts *et al.*, 2002). These types of proteins occupy only a limited region of the plot (dotted regions in Fig. 1b). The regular backbone conformations in these regions are well understood; for example, known regular structures that are to the right of the negatively sloping diagonal (dashed line in Fig. 1b; henceforth denoted as the ‘-ve diagonal’) are left-handed in backbone twist, while those that are to the left of the diagonal are right-handed (left- and right-handed regions are respectively shaded brown and gold<sup>1</sup>). For example, the position of the idealized left- and right-handed  $\alpha$ -helices (Fig. 1c) – respectively denoted as  $\alpha_L$  and  $\alpha$  in Fig. 1b – are on opposite sides of the -ve diagonal. The ‘naïve view’ of handedness, obtained from looking only at structured proteins, would be the expectation that the -ve diagonal neatly separates the Ramachandran plot into regions of left- and right-handedness (Fig. 1d).

However, structured proteins represent only a fraction of functional proteins. Indeed, up to 15% of mammalian proteins are completely disordered – they natively display multiple, often extended, conformations (Mannige, 2014) – and up to 50% of the mammalian proteins display large (> 30 residue) stretches of disorder (Iakoucheva *et al.*, 2002; Ward *et al.*, 2004; Orosz and Ovádi, 2011). Interestingly, when compared to structured backbones, structurally degenerate or disordered backbones occupy many

<sup>1</sup>Go Hillies!

more regions within the Ramachandran plot (Beck *et al.*, 2008).

Additionally, a number of peptide mimics – especially peptoids (Sun and Zuckermann, 2013) – display novel secondary structures that occupy regions that are strictly disallowed by proteins due to steric clashes. For example, a ‘higher-order’ peptoid secondary structure – the  $\Sigma$ -strand (Mannige *et al.*, 2015; Robertson *et al.*, 2016) – samples regions of the Ramachandran plot (‘I’ in Fig. 1b) that are not permitted within natural proteins (this is because of the lack of backbone hydrogen bond donors in peptoids). Another secondary structure – the ‘ $\omega$ -strand’ (Gorske *et al.*, 2016) – samples similarly historically uncharted regions of the Ramachandran plot (‘II’ in Fig. 1b). Importantly, backbone twist handedness plays a crucial part in explaining these new motifs: as one goes along the backbones of these secondary structures, alternating residues occupy equal but opposite backbone twists [for this reason, the  $\Sigma$ -strand is relatively linear, albeit meandering; Mannige *et al.* (2015); Mannige *et al.* (2016)].

Despite these recent discoveries of natively disordered proteins and novel peptidomimetic structures, a complete understanding of backbone conformations that stray from the ‘structured’ regions on the Ramachandran plot is missing, which impedes our ability to identify and explore such conformations. Towards filling this gap in understanding, this report outlines a detailed study of how regular backbones twist in every region of the Ramachandran plot. In particular, this report develops and explores a new metric for handedness ( $h$ ) based on modeling a regular backbone (described below) as a helix (Shimanouchi and Mizushima, 1955; Miyazawa, 1961; Zacharias and Knapp, 2013). The metric is used to exhaustively chart the handedness of regular backbones in every region of the Ramachandran plot. In doing so, this survey provides a new graphical format to explore new types of secondary structures being discovered (Mannige *et al.*, 2015; Gorske *et al.*, 2016), while dispelling the naïve view of handedness (Fig. 1d) by showing that the distribution of handedness as a function of  $\phi$  and  $\psi$  is more complicated than the distribution allowed by the naïve view. The results also show that the Ramachandran plot whose  $\phi$  and  $\psi$  values range between  $0^\circ$  and  $360^\circ$  is more intuitive and visually meaningful (compared to those that range between  $-180^\circ$  and  $180^\circ$ ), particularly for *cis* backbones. Finally, this work builds on a previous report (Zacharias and Knapp, 2013) and helps complete our understanding of the ways in which a peptide backbone twists, which is a basic component of structural biology.

## METHODS

While angular units in this report switch between radians and degrees, their units in any particular situation may be inferred by the presence or absence of the degree symbol ( $^\circ$ ). All methods and materials required to produce this manuscript are freely available at [https://github.com/ranjanmannige/backbone\\_chirality](https://github.com/ranjanmannige/backbone_chirality).

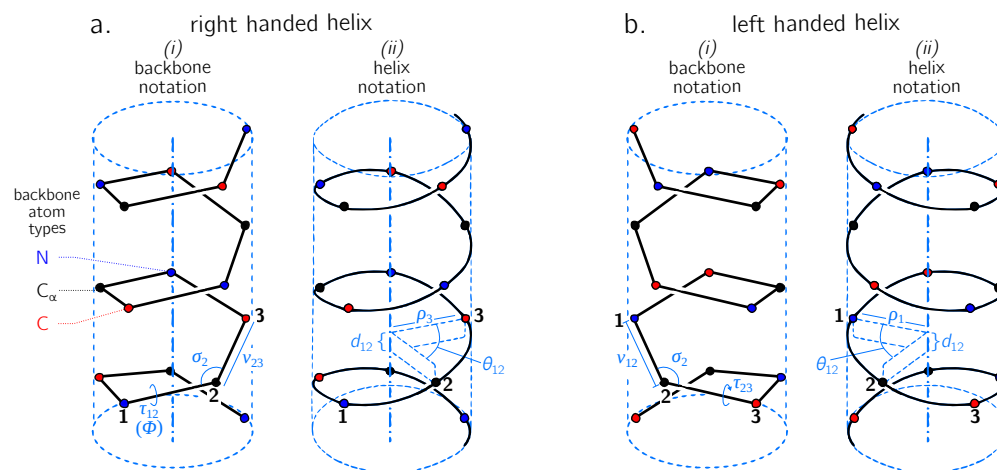
### Deriving measures for backbone handedness

Numerous metrics for molecular chirality and handedness have so far been discussed (Harris *et al.*, 1999). For example, metrics for chirality have been introduced that focus on vector orientations (Kwiecińska and Cieplak, 2005; Kabsch and Sander, 1983; Gruzziel *et al.*, 2013), optical activity (Osipov *et al.*, 1995), and molecular shape (Ferrarini and Nordio, 1998). However, this report will focus on a simpler metric for chirality associated with an idealized helix within which all (regular) backbone atoms of one type sit (Shimanouchi and Mizushima, 1955; Miyazawa, 1961; Zacharias and Knapp, 2013). Here, a ‘regular’ backbone indicates that each tunable parameter within a unit or ‘residue’ – say a particular dihedral angle – remains the same for all residues. Below, regular backbones are modeled in context of helical parameters that, when combined, form an intuitive metric for backbone handedness.

### Describing a regular backbone as a helix

Interest in how a backbone may be represented as a helix emerged shortly after the first secondary structures were introduced (Pauling *et al.*, 1951; Pauling and Corey, 1951b,a). In particular, Shimanouchi and Mizushima (1955) had derived a set of equations that fit a platonic helix to the atoms within a regular backbone. While the formalisms described by Shimanouchi and Mizushima (1955) [and later on by Miyazawa (1961), discussed below] apply to repeating linear polymers of arbitrary complexity, this report focuses specifically on how peptides may be modeled. Fig. 2 describes an arbitrary peptide backbone that may be represented either using internal coordinates (*i*) or helical coordinates (*ii*).

Internal coordinates are associated with stereochemical terms: bond lengths ( $v_{ij}$ ) between adjacent atoms  $i$  and  $j$ , bond angles ( $\sigma_i$ ) between the two backbone bonds adjacent to atom  $i$ , and dihedral or



**Figure 2.** Internal coordinate (i) and helical coordinate (ii) representations of right-handed (a) and left-handed (b) regular backbones. Internal coordinates are a function of bond lengths (e.g.,  $v_{12}$ ), angles ( $\sigma_2$ ), and dihedral angles ( $\tau_{12}$ ), while helical coordinates are a function of displacement along the helical axis ( $d_{12}$ ), angular displacement in the plane perpendicular to the helical axis ( $\theta_{12}$ ) and shortest distance of an atom of type  $i$  from the helical axis ( $\rho_i$ ). Representations are derived from Figs. 1 and 2 in Shimanouchi and Mizushima (1955).

torsion angles ( $\tau_{ij}$ ), which are associated with the bond  $i-j$  and the atoms preceding and succeeding these atoms. Helical coordinates (Fig. 2(ii)) are described using measures of vertical displacement ( $d$ ; this is related to the pitch of a platonic helix), angular displacement ( $\theta$ ) and the radius of the helix ( $\rho_i$ ) that hosts all backbone atoms of type  $i$ . Therefore, the single cylinder shown in Fig. 2 is too simplistic as there should be one distinct cylinder or radius per atom type.

Given that there are three atoms associated with a residue (Fig. 1a),  $d = d_{n,\alpha} + d_{\alpha,c} + d_{c,n}$  and  $\theta = \theta_{n,\alpha} + \theta_{\alpha,c} + \theta_{c,n}$ . Here,  $d_{i,j}$  and  $\theta_{i,j}$  respectively refer to the vertical and angular displacement between adjacent atoms  $i$  and  $j$ . Subscripts 'n', ' $\alpha$ ', and 'c' respectively refer to the backbone nitrogen,  $\alpha$ -carbon and carbonyl carbon atoms (Fig. 1a). The notation used by Shimanouchi and Mizushima (1955) was in terms of matrices, which were then simplified by Miyazawa (1961) into trigonometric terms. In particular, Miyazawa (1961) noted that the total residue-residue vertical displacement ( $d$ ) and angular displacement ( $\theta$ ) may be retrieved using the following two equations.

$$\begin{aligned} \cos\left(\frac{\theta}{2}\right) = & \cos\left(\frac{+\phi + \psi + \omega}{2}\right) \sin\left(\frac{\sigma_n}{2}\right) \sin\left(\frac{\sigma_\alpha}{2}\right) \sin\left(\frac{\sigma_c}{2}\right) \\ & - \cos\left(\frac{+\phi - \psi + \omega}{2}\right) \sin\left(\frac{\sigma_n}{2}\right) \cos\left(\frac{\sigma_\alpha}{2}\right) \cos\left(\frac{\sigma_c}{2}\right) \\ & - \cos\left(\frac{+\phi + \psi - \omega}{2}\right) \cos\left(\frac{\sigma_n}{2}\right) \sin\left(\frac{\sigma_\alpha}{2}\right) \cos\left(\frac{\sigma_c}{2}\right) \\ & - \cos\left(\frac{-\phi + \psi + \omega}{2}\right) \cos\left(\frac{\sigma_n}{2}\right) \cos\left(\frac{\sigma_\alpha}{2}\right) \sin\left(\frac{\sigma_c}{2}\right) \end{aligned} \quad (1)$$

$$\begin{aligned}
 d \sin\left(\frac{\theta}{2}\right) = & (+v_{n,\alpha} + v_{\alpha,c} + v_{c,n}) \sin\left(\frac{+\phi + \psi + \omega}{2}\right) \sin\left(\frac{\sigma_n}{2}\right) \sin\left(\frac{\sigma_\alpha}{2}\right) \sin\left(\frac{\sigma_c}{2}\right) \\
 & - (+v_{n,\alpha} - v_{\alpha,c} + v_{c,n}) \sin\left(\frac{+\phi - \psi + \omega}{2}\right) \sin\left(\frac{\sigma_n}{2}\right) \cos\left(\frac{\sigma_\alpha}{2}\right) \cos\left(\frac{\sigma_c}{2}\right) \\
 & - (+v_{n,\alpha} + v_{\alpha,c} - v_{c,n}) \sin\left(\frac{+\phi + \psi - \omega}{2}\right) \cos\left(\frac{\sigma_n}{2}\right) \sin\left(\frac{\sigma_\alpha}{2}\right) \cos\left(\frac{\sigma_c}{2}\right) \\
 & - (-v_{n,\alpha} + v_{\alpha,c} + v_{c,n}) \sin\left(\frac{-\phi + \psi + \omega}{2}\right) \cos\left(\frac{\sigma_n}{2}\right) \cos\left(\frac{\sigma_\alpha}{2}\right) \sin\left(\frac{\sigma_c}{2}\right)
 \end{aligned} \quad (2)$$

The ranges for  $d$  and  $\theta$ , respectively, are  $[-\lambda, +\lambda]$  and  $[0, 2\pi]$  (the positive limit  $\lambda$  is defined by allowed values for the various internal coordinates). As above, subscripts 'n', ' $\alpha$ ', and 'c' respectively refer to the backbone nitrogen,  $\alpha$ -carbon and carbonyl carbon atoms;  $v_{ij}$  refers to the distance between adjacent atoms  $i$  and  $j$ ;  $\sigma_i$  refers to the angle between the adjacent bonds that have  $i$  as the common atom. The dihedral angles  $\phi$ ,  $\psi$ , and  $\omega$  represent the traditional symbols for backbone dihedral angles, which may be otherwise denoted as  $\tau_{n,\alpha}$ ,  $\tau_{\alpha,c}$ , and  $\tau_{c,n(+1)}$ , respectively.

Finally, for any type of atom (say  $\alpha$ -carbons), the radius or distance from the helical axis  $\rho_\alpha$  is defined by

$$\begin{aligned}
 2\rho_\alpha^2 [1 - \cos(\theta)] + d^2 = & v_{\alpha,c}^2 + v_{c,n}^2 + v_{n,\alpha}^2 - 2v_{c,n} [v_{\alpha,c} \cos(\sigma_c) + v_{n,\alpha} \cos(\sigma_n)] \\
 & + 2v_{\alpha,c} v_{n,\alpha} [\cos(\sigma_c) \cos(\sigma_n) - \sin(\sigma_c) \sin(\sigma_n) \cos(\tau_{c,n})]
 \end{aligned} \quad (3)$$

Miyazawa (1961) noted that the right-hand side of Eqn. 3 is also the squared distance between adjacent atoms of the same type (which we denote as  $d_\alpha^2$  for  $\alpha$ -carbons), which allows for a more simplified form

$$\rho_\alpha = \sqrt{\frac{d_\alpha^2 - d^2}{2 - 2\cos(\theta)}} \quad (4)$$

The distance between adjacent  $\alpha$ -carbons ( $d_\alpha$ ) is  $\sim 3.8\text{\AA}$  for *trans* peptides and  $\sim 3.2\text{\AA}$  for *cis* peptides. Other radii ( $\rho_c, \rho_n$ ) can be obtained by cycling through ( $\alpha, c, n$ ) subscripts within Eqns. 3 and 4<sup>2</sup>. Note that all  $\rho_i$ 's are functions of  $\theta, d$  (along with other internal coordinates), and so one may use two of the three terms ( $d, \theta, \rho_i$ ) – to describe the helical state of a peptide. Since there is only one  $d$  and  $\theta$  per backbone (compared to three  $\rho_i$ 's, one per atom type), this report utilizes  $d$  and  $\theta$  as the two descriptors [discussions on this choice have also been made by Zacharias and Knapp (2013)].

Eqns. 1 and 2 may be substantially simplified (Miyazawa, 1961), given that backbone bond lengths and angles are much less 'tunable' when compared to dihedral angles (Ramachandran *et al.*, 1963; Improta *et al.*, 2015a; Esposito *et al.*, 2013; Improta *et al.*, 2015b). In particular, most backbone bond lengths and angles display one equilibrium value (Improta *et al.*, 2015a; Esposito *et al.*, 2013; Improta *et al.*, 2015b), while the backbone dihedral angles  $\phi$  and  $\psi$  occupy a range of possible values and minima, e.g., regions in the Ramachandran plot that describe  $\alpha$ -helices and  $\beta$ -sheets (Fig. 1b). With this in mind, Miyazawa (1961) set  $\omega = \pi$  (*trans*) and substituted average (equilibrium) values for bond angles and lengths into Eqns. 1 and 2 to arrive at a simpler equation for *trans* backbones. Zacharias and Knapp (2013) published an updated version of this set of equations, which follows<sup>3</sup>.

$$\cos\left(\frac{\theta}{2}\right) = -0.8235 \sin\left(\frac{\phi + \psi}{2}\right) + 0.0222 \sin\left(\frac{\phi - \psi}{2}\right), \quad (5)$$

$$d \sin\left(\frac{\theta}{2}\right) = 2.9986 \cos\left(\frac{\phi + \psi}{2}\right) - 0.6575 \cos\left(\frac{\phi - \psi}{2}\right). \quad (6)$$

This equation is especially relevant to peptides as they occur predominantly in *trans* conformations ( $\omega = \pi$ ). However, given the prevalence of *cis* backbones in peptide mimics such as peptoids (Mirijanian

<sup>2</sup> $\rho_c$  and  $\rho_n$  are obtained by the following subscript conversions: ( $\alpha \rightarrow c, c \rightarrow n, n \rightarrow \alpha$ ) and ( $\alpha \rightarrow n, c \rightarrow \alpha, n \rightarrow c$ ).

<sup>3</sup>Values used here are also used by Zacharias and Knapp (2013). These values are:

$v_{n,\alpha} = 1.459\text{\AA}$ ,  $v_{\alpha,c} = 1.525\text{\AA}$ ,  $v_{c,n(+1)} = 1.336\text{\AA}$ ,  $\sigma_\alpha = 111.0^\circ$ ,  $\sigma_c = 117.2^\circ$ , and  $\sigma_n = 121.7^\circ$ .  
For reference, Miyazawa (1961) originally used the following values:  
 $v_{n,\alpha} = 1.470\text{\AA}$ ,  $v_{\alpha,c} = 1.530\text{\AA}$ ,  $v_{c,n(+1)} = 1.320\text{\AA}$ ,  $\sigma_\alpha = 110.0^\circ$ ,  $\sigma_c = 114.0^\circ$ , and  $\sigma_n = 123.0^\circ$ .

*et al.*, 2014; Gorske *et al.*, 2016), for completeness, the corresponding relationships for a *cis* ( $\omega = 0$ ) backbone follows.

$$\cos\left(\frac{\theta}{2}\right) = 0.4052 \cos\left(\frac{\phi + \psi}{2}\right) - 0.4932 \cos\left(\frac{\phi - \psi}{2}\right), \quad (7)$$

$$d \sin\left(\frac{\theta}{2}\right) = 2.3093 \sin\left(\frac{\phi + \psi}{2}\right) + 0.0028 \sin\left(\frac{\phi - \psi}{2}\right). \quad (8)$$

Note that Eqns. 5 through 8 are simplifications of Eqns. 1 and 2, and are therefore prone to some limitations that are not present in Eqns. 1 and 2. For example, bond lengths (Improta *et al.*, 2015a) and bond angles (Esposito *et al.*, 2013; Improta *et al.*, 2015b) display some dependence on local backbone conformation. These subtle variations have great implications when dealing with a large number of residues, especially when considering bond angles. For example, when attempting to recreate a protein conformation from an original conformation's  $\phi$  and  $\psi$  values (ignoring deviations in  $\omega$ , bond angles, and lengths), the original and recreated conformations tend to deviate dramatically due to an accumulation of errors [by up to 22Å in root mean squared deviation; Tien *et al.* (2013)]. However, when studying changes in conformationally regular and *local* stretches of peptides, such deviations are not likely to change relevant features such as handedness and extent of twistedness. If circumstances indicate that the backbone values for bond angles and  $\omega$  of the peptide may be strained from their equilibrium values (e.g., due to bulky sidechains), only Eqns. 1 and 2 can be expected to faithfully (and perfectly) represent backbone features such as handedness of twist. However, the approximations of Eqns. 5 through 8 are sufficient for the purposes of this report, given that this report primarily discusses features within platonic regular backbones.

### On the one-to-one correspondence between $[\phi, \psi, \omega]$ and $[d, \theta]$

Given a particular *value* of  $\omega$ , every  $[\phi, \psi]$  pair points to exactly one  $[d, \theta]$ . However, when using Eqns. 1 and 2, one value of  $\omega$  can not be replaced with a *periodically equivalent* version of  $\omega$ . For example, using  $\omega = x + 2\pi$  instead of  $\omega = x$  will maintain the magnitude of  $d$  and  $\theta$ , but the signs will not remain conserved. This is because every summand in Eqns. 1 and 2 contains either a sine or cosine of  $[\pm\phi \pm \psi \pm \omega]/2$ . The issue arises because of the '2': even though the angle  $x$  is considered to be equivalent to the angle  $x + 2\pi$ , and even though  $\cos(x + 2\pi)$  equals  $\cos(x)$  (due to angle periodicity),  $\cos([x \pm 2\pi]/2) = \cos(x/2 \pm \pi) = -\cos(x/2)$  (note the negative sign). Similarly,  $\sin([x \pm 2\pi]/2) = -\sin(x/2)$ . Therefore, even though the angles  $\omega$  and  $\omega + 2\pi$  may be considered to be equivalent angles, expressions such as  $\cos([x - \omega + 2\pi]/2)$  and  $\cos([x - \omega]/2)$  are only equal in magnitude and not in sign. I.e., one-to-one correspondence between  $[\phi, \psi]$  and  $[d, \theta]$  is only possible if we insist on specific values for  $\omega$ s. For this reason, we propose to wrap the value of an amide backbone  $\omega'$  between  $[\Delta, \Delta + 360^\circ)$  using

$$\omega = (\omega' - \Delta) \% 360 + \Delta, \quad (9)$$

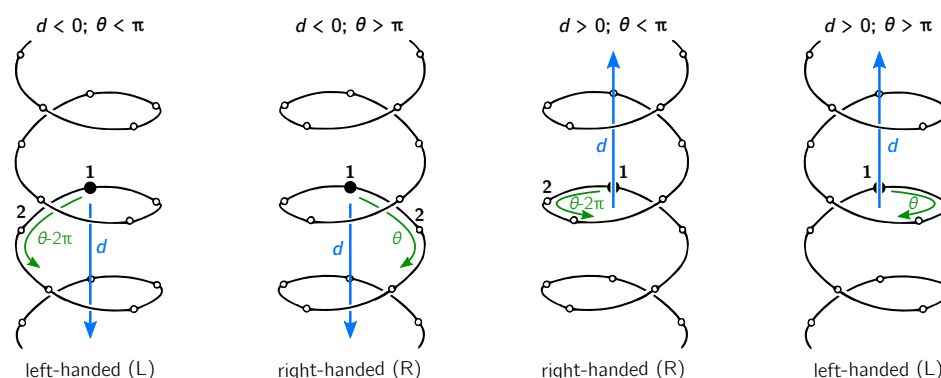
where  $\%$  represents the modulus function, and  $\Delta$  describes the start of the range. Choosing  $\Delta = -90^\circ$  would ensure that the distribution of both *cis* ( $\omega = 0 \pm 20^\circ$ ) and *trans* ( $\omega = 180 \pm 20^\circ$ ) will remain contiguous. Using this system, *cis* and *trans* backbones are respectively represented by  $\omega = 0$  (and not  $2\pi$ ) and  $\omega = \pi$  (not  $-\pi$ ) for *trans* backbones. The rest of this report assumes these values of  $\omega$  for *cis* and *trans* backbones.

These points lead to the conclusion that a *strict* one to one-to-one correspondence between  $(\phi, \psi, \omega)$  and  $(d, \theta)$  does not exist, since multiple sets of the former may be backmapped from the latter (by reconfiguring Eqns. 1 and 2). Yet, a one-to-one correspondence may be ensured by discarding as solutions all but the one set of  $(\phi, \psi, \omega)$ , whose  $\omega$  does not change after being wrapped by Eqn. 9.

### Introducing an equation for backbone handedness

The helical parameters  $d$  and  $\theta$  host a wealth of information, some of which is discussed in the Results section. For the purpose of developing an equation for backbone handedness, it is only important to recognize, as was done before (Zacharias and Knapp, 2013), that  $\theta$  and  $d$  together are instrumental in describing backbone handedness.

The relationship between  $d$ ,  $\theta$  and handedness is shown in Fig. 3. While  $\theta$  indicates the extent to which a regular backbone curves along a helical path, the handedness of a backbone is dependent on both



**Figure 3.** The handedness of a helix is a function of angular displacement perpendicular to the helical axis ( $\theta$ ; green curved arrows) and linear displacement along the helical axis  $d$  (blue, vertical arrows). Note that left- and right-handed backbone twists are respectively associated with the L and D chiralities within the Fisher Projection system and S and R chiralities within the Cahn–Ingold–Prelog system (Cross and Klyne, 2013); however, as discussed in the Methods section, this report makes a distinction between helix handedness and molecular chirality.

$\theta$  and  $d$ . This is because the sign of  $d$  provides a frame of reference for interpreting  $\theta$ . In particular, if  $d$  is positive, then  $0 < \theta < \pi$  indicates right-handedness, while  $\pi < \theta < 2\pi$  indicates a left-handed helix. However, if  $d$  is negative, then the manner in which the helix is ‘built’ reverses (Fig. 3), and  $0 < \theta < \pi$  indicates left-handedness, while  $\pi < \theta < 2\pi$  indicates right-handedness.

These relationships allow for a simple metric for backbone handedness that depends on the sign of  $d$  and the value of  $\theta$ :

$$h = \frac{d}{|d|} \sin(\theta). \quad (10)$$

Here,  $h \in \{-1, \dots, 1\}$  is negative (or positive) when the overall twist of the backbone is left(or right)-handed. Note that  $d/|d|$  is related to the traditional sign function  $\text{sgn}(d)$ , but deviates at  $d = 0$ , where the former term is undefined while the latter term is 0. Additionally,  $h$  will equal 0 if  $d = 0$  or if  $\theta = x\pi$  (where  $x$  is an integer); for more on the meaning of  $d$  and  $\theta$  in context of handedness and peptide geometry, please refer to the Results and Discussions section and Fig. 4 in particular.

### Alternative measures of handedness

Two estimates for chirality,  $\chi_1$  and  $\chi_2$ , used to validate the new measure of handedness  $h$  (Eqn. 10), were previously used by Kwiecińska and Cieplak (2005) and Kabsch and Sander (1983), respectively. The equations are:

$$\chi_1 = \frac{1}{N} \sum_{i=2}^{N-2} \frac{(\mathbf{v}_{i-1} \times \mathbf{v}_i) \cdot \mathbf{v}_{i+1}}{v_{i-1} v_i v_{i+1}}, \quad (11)$$

$$\chi_2 = \frac{1}{N} \sum_{i=2}^{N-2} \arctan 2(v_i \mathbf{v}_{i-1} \cdot \mathbf{v}_i \times \mathbf{v}_{i+1}, \mathbf{v}_{i-1} \times \mathbf{v}_i \cdot \mathbf{v}_i \times \mathbf{v}_{i+1}). \quad (12)$$

Here,  $N$  is the peptide length,  $i$  is the peptide residue number and the position of each  $\alpha$ -carbon is  $N_i$ , with vector  $\mathbf{v}_k \equiv N_{k+1} - N_k$ . The scalar component of the vector  $\mathbf{v}_i$  is denoted as  $v_i$ . Eqn. 11 has range  $[-1, 1]$ . Eqn. 12, also used by Gruzziel *et al.* (2013), is the dihedral angle associated with the four contiguous  $\alpha$ -carbons (one preceding and two succeeding the residue  $i$ ), and ranges between  $[-\pi, \pi]$  radians. For both metrics, values deviating more from 0 are more chiral (or ‘twisted’ or ‘handed’), and left-handed twists are negative while right-handed twists are positive. Only  $\alpha$ -carbon atom positions are used for the calculation.



Finally, a more backbone-agnostic metric of chirality has been introduced by Solymosi *et al.* (2002), which is replicated here purely for completeness:

$$\chi_3 = \frac{4!}{3N^4} \sum_{i,j,k,l \in N} \frac{((\mathbf{v}_{ij} \times \mathbf{v}_{kl}) \cdot \mathbf{v}_{il})(\mathbf{v}_{ij} \cdot \mathbf{v}_{jk})(\mathbf{v}_{jk} \cdot \mathbf{v}_{kl})}{(\mathbf{v}_{ij} \mathbf{v}_{jk} \mathbf{v}_{kl})^2 v_{il}}. \quad (13)$$

$\chi_3$ , of arbitrary range, is known as the chirality index  $G_{05}$  in Solymosi *et al.* (2002) and Neal *et al.* (2003).  $(i, j, k, l)$  are exhaustive permutations of  $\{1, 2, \dots, N\}$ . While unshown here, this metric qualitatively matches the values of Eqn. 11 and 12.

### Backbone structure generation

The metric  $h$  (Eqn. 10) is purely analytical and does not need structures to be computationally generated, since Eqns. 5 through 8 that provide  $d$  and  $\theta$  require only pairs of  $\phi$  and  $\psi$  angles. However, if values for bond angles, lengths and dihedral angles are expected to deviate greatly from equilibrium values,  $\theta$  and  $d$  can only be obtained from the more detailed Eqns. 1 and 2, whose parameters would likely be obtained from a structure. On the other hand, as  $\chi_1$  (Eqn. 11) and  $\chi_2$  (Eqn. 12) work explicitly with atom positions, these metrics explicitly need the generation of structures. In order to use these metrics, peptides (poly-glycines) of arbitrary length were generated using the Python-based PeptideBuilder library (Tien *et al.*, 2013). Analysis was performed using BioPython (Cock *et al.*, 2009) and Numerical Python (Van Der Walt *et al.*, 2011). Ramachandran plots that describe chirality (e.g., Fig. 5a) were generated using a grid spacing (in degrees) of  $\phi, \psi \in \{-180, -178, \dots, 178, 180\}$ .

### Backbone chirality $\neq$ backbone handedness

Finally, it is important to recognize the distinction between backbone (twist) handedness and backbone (molecular) chirality. Naïvely, chirality is a simple concept: a molecular conformation is achiral if its mirror image can be superimposed onto itself, otherwise that conformation is chiral (Gold *et al.*, 1997) (alternatively, and less commonly, achiral molecules possess inversion centers). Despite this intuitive definition, chirality has remained a confusing concept ever since its introduction (Bentley, 2010; Wallentin *et al.*, 2009), which is possibly due to the fact that ‘context’ is very important when discussing chirality (Mislow, 2002). For example, when looking at a peptide at the residue or ‘local’ level, every amino acid (excepting glycine) is chiral due to the presence of a chiral  $\alpha$ -carbon (its mirror image can not be superimposed onto itself). Yet, at the macromolecular level, even an all-glycine (and therefore locally achiral) peptide will display *conformations* that are not superimposable onto each other, and so such conformations would be chiral. Alternatively, when considering handedness, if a backbone is completely flat (say, a ring, where  $d = 0$ ), handedness ( $h$ ) will be undefined, and so one can not speak of handedness of the twist. Yet, the backbone may still remain chiral; e.g., cisplatin and transplatin are planar molecules that are nonetheless chiral opposites (Testa, 2013). It is for this reason that this report chooses to be careful to not claim that Eqn. 10 is a metric for peptide/backbone chirality, but of peptide backbone *twist* handedness. However, estimates for backbone chirality (e.g., Eqns. 11 and 12) may be used as surrogates for twist chirality to validate  $h$  (Eqn. 10), as both are related but not the same.

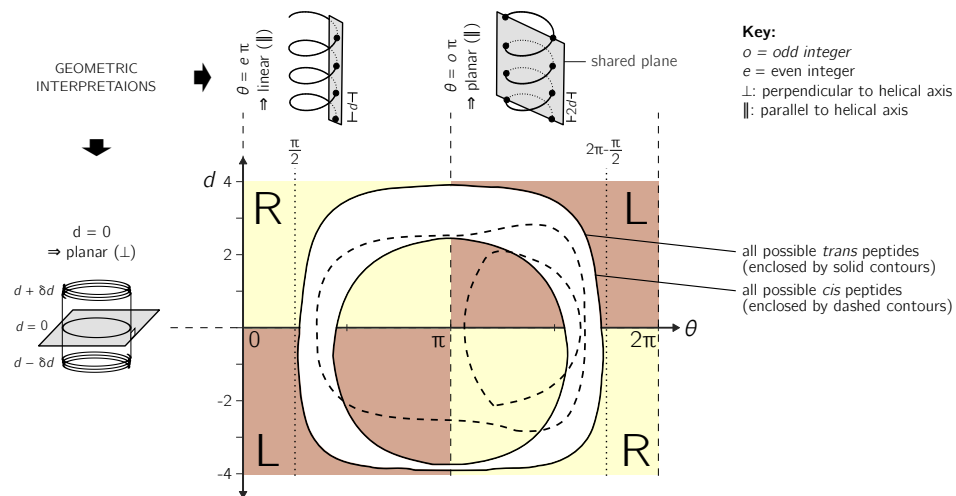
### Obtaining secondary structure statistics

Statistics about secondary structures – particularly  $\alpha$ -helices,  $3_{10}$ -helices and  $\beta$ -sheets – used in Figs. 1b and 9 were identified using the DSSP algorithm (Kabsch and Sander, 1983), although the STRIDE algorithm (Frishman and Argos, 1995) provides qualitatively identical distributions. This algorithm was applied to a database of 13,760 three-dimensional protein conformations (one domain per conformation) with lower than 40% sequence identity, obtained from the Structural Classification of Proteins or SCOPe website [Release 2.06; Fox *et al.* (2014)]. This database is currently available as: <http://scop.berkeley.edu/downloads/pdbstyle/pdbstyle-sel-gs-bib-40-2.06.tgz>.

## RESULTS AND DISCUSSION

### Relevance of $\theta$ and $d$

When discussing peptide backbones, two possible definitions of backbone ‘flatness’ (or linearity) are possible: flatness at a residue level and flatness at the atomic level. In the former, all atoms of the same *type* are coplanar (examples of atom types are the backbone nitrogens, carbonyl carbons,  $\alpha$ -carbons, or even



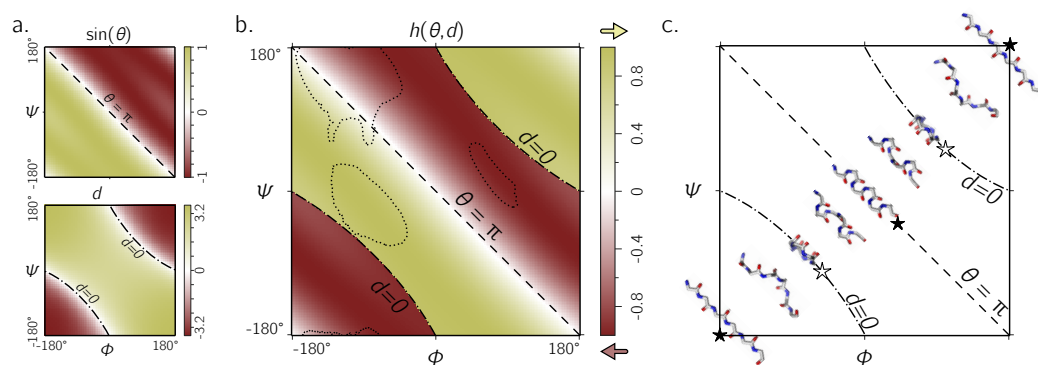
**Figure 4.** Further discussion on the meaning of  $d$  and  $\theta$ . As shown in Fig. 3, axial separation  $d$  and angular separation  $\theta$  between adjacent atoms of the same type combine to define handedness. The shaded regions within the graph show the distribution of handedness as a function of  $d$  and  $\theta$ . The relevant boundaries –  $\theta = x\pi$  (where  $x$  is a non-negative integer) and  $d = 0$  – separate the map into four quadrants of left- and right-twisting backbones ('L' and 'R', respectively). Geometric interpretations of various boundaries, discussed in the text, are shown to the top and left of the graph. The toroid enclosed by two solid lines (and shaded white) represents all possible conformations for *trans* peptides ( $\omega = 180 \pm 10^\circ$ ). Similarly, the region allowed for *cis* peptides are bound by the two dashed contours. As expected, *trans* and *cis* peptides behave very differently, further predicating the need to explore the two types of backbones as distinct.

sidechain  $\beta$ -carbons). In the latter, *all* atoms within the backbone are coplanar. For the discussions below, the former definition is chosen as the relevant scope for flatness (and 'linearity'), as residue-by-residue behavior of the peptide is of primary relevance.

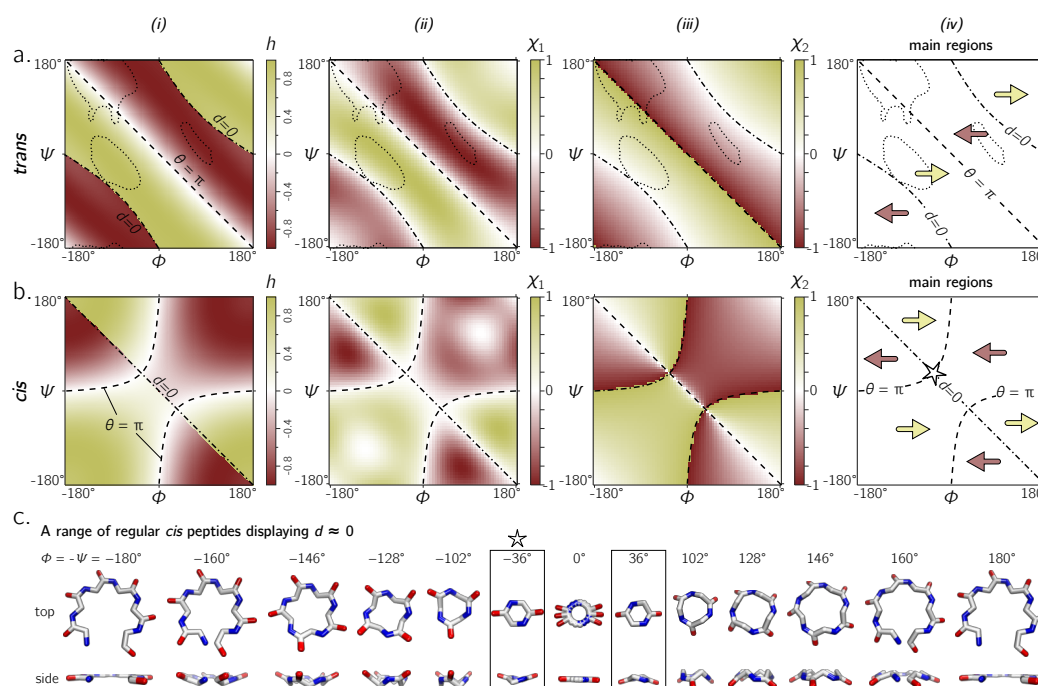
As described in Fig. 3, the helical parameters  $d$  and  $\theta$  respectively refer to a vertical displacement along the helical axis and an angular displacement in a plane perpendicular to the helical axis. For example,  $d = 0$  indicates a helix flattened along its helical axis (Fig. 4). This means that all regular peptides with  $d = 0$  will be ring-like at some peptide length (shown in a following figure for a range of peptides). As expected from Eqn. 10, at  $d = 0$ , one can not tell how the helix was built, since coplanar peptides can not be described as either left- or right-twisting. Therefore, even though  $d = 0$  indicates highly twisted peptides, these twists do not possess handedness. This shows up in the  $h$  metric because, at  $d = 0$ ,  $d/|d|$  is undefined.

Additionally Fig. 4 describes two important values for  $\theta$ :  $e\pi$  and  $o\pi$ , where  $e$  and  $o$  are even and odd non-negative integers. In particular, for any  $d$ ,  $\theta = e\pi$  indicates zero angular displacement along the axis, which puts all atoms of the same type on the same line parallel to the helical axis (Fig. 4). Similarly,  $\theta = o\pi$  indicates that every alternate atom (of the same type) along the backbone will be linear, and every adjacent atom will be diametrically opposite to each other (Fig. 4); i.e.,  $\theta = o\pi$  indicates that all atoms of the same type will lie on a plane that is parallel to the helical axis. In short,  $\theta = 0$  codes for backbones that are linear (optimally extended for a fixed  $d$ ) and  $\theta = \pi$  describe peptides that zig-zag along a plane perpendicular to the helical axis (for a fixed  $d$ ). Finally, as evident in Fig. 4,  $\theta = e\pi$  conformations are not available for peptide backbones; in these situations,  $\theta = o\pi$  (e.g.,  $\pi$  or  $180^\circ$ ) will be the most extended type of backbone (for a fixed  $d$ ). These ideas give us a sense, *a priori*, about the meaning of a backbone curve with particular  $d$  and  $\theta$ . These ideas, along with the combination rules for  $\theta$  and  $d$  (Fig. 3) that provide us with the handedness of a backbone ( $h$ ; Eqn. 10), provide a rich landscape onto which peptides can be discussed and explored (discussed further at the end of this section).

Finally,  $\theta$  may serve as an important single-number metric for describing backbone configurations. Mannige *et al.* (2016) developed one such number – a Ramachandran number ( $\mathcal{R}$ ) – that combines the values  $\phi$  and  $\psi$  to produce a structurally meaningful number. This number depends on the fact that



**Figure 5.** The chirality of an ordered *trans* peptide within the Ramachandran plot. Panel (a) displays the relationship between backbone parameters ( $\phi$ ,  $\psi$ ) and the associated helix parameters of curvature  $\sin(\theta)$  (top; Eqn. 1) and pitch  $d$  (bottom; Eqn. 2). The boundaries,  $\theta = \pi$  ('--') and  $d = 0$  ('-.-'), correspond to backbones that are equally flat, but which are respectively optimally extended and curved (see discussion in text). As shown in Fig. 3, the handedness of a helix is a function of these two variables ( $h$ ; Eqn. 10). Panel (b) is a map of backbone chirality ( $h$ ) as a function of  $\phi$  and  $\psi$ . This map shows that the naïve expectation of handedness in a Ramachandran plot (Fig. 1d) is inaccurate. Interestingly, our naïve expectations would be upheld if one were only to have sampled regions of the Ramachandran plot dominated by known proteins (a; regions enclosed by '.....' indicate 90% occupancy). An example of the behavior of one 'slice' of (b) is shown in (c). Each snapshot represents a peptide backbone that is either in a distinct region of handedness or at a boundary.



**Figure 6.** Panels (a) and (b) describe the handedness of backbone twists whose amide dihedral angles are *trans* ( $\omega = \pi$ ) and *cis* ( $\omega = 0$ ), respectively. Column (i) describes handedness calculated using  $h$  (Eqn. 10), which does not require structures to be computationally generated. Columns (ii) and (iii) respectively show vector-based estimates of backbone handedness –  $\chi_1$  (Eqn. 11) and  $\chi_2$  (Eqn. 12) – which are calculated from computationally generated peptides (see Methods). Regions of left- and right-handedness are identical for all measures (i–iii). This general map of how handedness is distributed within the Ramachandran plot is shown in (iv). Finally, Panel (c) displays a range of regular *cis* peptide backbones with  $d \approx 0$ . As explained by Fig. 4,  $d = 0$  indicates a flatness of the peptide perpendicular to the helical axis, which results in peptides that close in on themselves at varying radii. Interestingly, a point in the Ramachandran plot exists exclusively for *cis* peptides, where  $d = 0$  and  $\theta = \pi$ :  $\phi = -\psi = \pm 36^\circ$  [ $\star$  in Panels(b)-(iv) and (c)].

structural features of the backbone (e.g., radius of gyration) vary least when one slices through the *trans* Ramachandran plot along negative-sloping lines that conserve  $\phi + \psi$  (Ho *et al.*, 2003; Zacharias and Knapp, 2013; Mannige *et al.*, 2016). Interestingly,  $\theta$  follows that trend too, which – in combination with the fact that regions of the Ramachandran plot are sparse (Mannige *et al.*, 2016) – means that  $\theta$  and its derivatives (e.g.,  $h$ ) are *universal* Ramachandran numbers. The universality arises from the fact that *cis* Ramachandran plots do *not* conserve structure along lines that conserve  $\phi + \psi$  (and so  $\mathcal{R}$  only works for *trans* backbones), yet any two backbones with nearly identical  $\theta$ 's will also be conserved in structure (see, e.g., Fig. 5a, top). This feature of  $\theta$  will be true irrespective of the nature of the amide dihedral angle  $\omega$  (Eqn. 1).

### Handedness of *trans* backbones

Fig. 5a describes the behavior of  $d$  and  $\sin(\theta)$  as a function of  $\phi$  and  $\psi$  (assuming an all-*trans* backbone;  $\omega = \pi$  or  $180^\circ$ ). Fig. 5b describes the behavior of handedness ( $h$ ; Eqn. 10) as a function of  $\phi$  and  $\psi$ . This map is a complete description of the handedness of an all-*trans* (regular) peptide backbone. Fig. 5c describes some structures at various regions within the plot. As discussed above (Fig. 4),  $d = 0$  (‘☆’) indicates that each residue is at the same ‘altitude’, i.e., the helix is perfectly flat and maximally curved (at that particular  $\theta$ ). Note that any path on the Ramachandran plot that transitions from negative to positive  $d$  will encounter an infinitesimal region in its path that is  $d = 0$ : it therefore appears as a sharp transition from one handedness to the other. When  $\theta = \pi$ , then the backbone is also flat (see ‘★’ in Fig. 5c); however, atoms of the same type lie in a single plane that is perpendicular to the helical axis (Fig. 4). In short, within the Ramachandran plot,  $d = 0$  (‘-.-’) and  $h = \pi$  (‘--’) code for *flat* backbones that are respectively either optimally curved (at a given  $\theta$ ) or optimally extended (at a given  $d$ ). A future report will discuss how these simple rules may be combined to make conjectures about novel secondary and tertiary structures.

Fig. 6a shows that the equation for  $h$  match other metrics for handedness, as interpreted by other metrics for chirality (Kwiecińska and Cieplak, 2005; Kabsch and Sander, 1983; Gruzziel *et al.*, 2013). In particular, Fig. 6a displays the Ramachandran plot colored by  $h$  [(i); Eqn. 10] next to estimates calculated using  $\chi_1$  [(ii); Eqn. 11] and  $\chi_2$  [(iii); Eqn. 12]. Each panel describes identical regions of left- and right-handedness, which is shown as a cartoon in (iv). However, given that  $\chi_1$  and  $\chi_2$  are estimates of chirality and not backbone handedness, their exact values differ from the primary metric for handedness ( $h$ ) provided here.

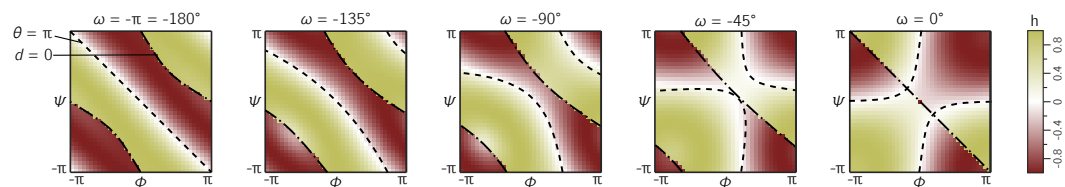
### Handedness of *cis* backbones

In the same vein as Fig. 6a, Fig. 6b displays  $h$ ,  $\chi_1$  and  $\chi_2$  as a function of  $\phi$  and  $\psi$  for all-*cis* backbones. This appears to be the first complete description of chirality of an all-*cis* backbone ( $\omega = 0$ ). Interestingly, the boundaries for  $d = 0$  and  $\theta = \pi$  switch in *cis* backbones, with the -ve diagonal and curved boundaries being caused by  $d$  and  $\theta$ , respectively. Additionally, Fig. 6a reiterates the idea that *cis* peptides are quite different when compared to *trans* peptides: the regions and boundaries of left- and right-handedness within the Ramachandran plot differ for *cis* versus *trans*. Finally, points on the *cis* map ( $\phi = \pm 36^\circ$ ,  $\psi = \mp 36^\circ$ ) exist where  $d = 0$  and  $\theta = \pi$ . An example of this, along with other  $d = 0$  configurations, is shown in Fig. 6c for a six-residue peptide.

At first, this appears to be contradiction, because  $d = 0$  indicates the most curved backbone at a fixed  $\theta$ , and  $\theta = \pi$  indicates the most linear backbone at a fixed  $d$ . Of course, this structure would only be possible for cyclic peptides with length two, given that any peptoid of length greater than two would result in many overlapping atoms. However, such a structure (one with  $d = 0$  and  $\theta = \pi$ ) is not possible in *trans* peptides, even in theory, because the boundaries associated with  $d = 0$  and  $\theta = \pi$  do not intersect (Fig. 6a); this is also evident in Fig. 4, where *trans* peptides are shown to not occupy regions of  $[d, \theta] = [0, \pi]$ , while *cis* peptides do.

### How does the amide backbone ( $\omega$ ) change the chirality landscape?

So far, only discrete values for  $\omega$  are discussed (either *cis* or *trans* peptides respectively corresponding to  $\omega = 0$  and  $\pi$ ). The picture that arises is that our naïve picture of chirality – that the -ve diagonal separates the right-twisting backbones from the left-twisting backbones (Fig. 1d) is wrong. In fact, the Ramachandran plots for both *cis* and *trans* backbones describe not two but four regions of distinct chirality (Fig. 6). However, deviations from  $\omega = 0$  or  $\pi$  are evident in the Protein Databank; see, e.g., discussions by Improta *et al.* (2011). This raises the question of how does varying  $\omega$  change the landscape? Fig. 7



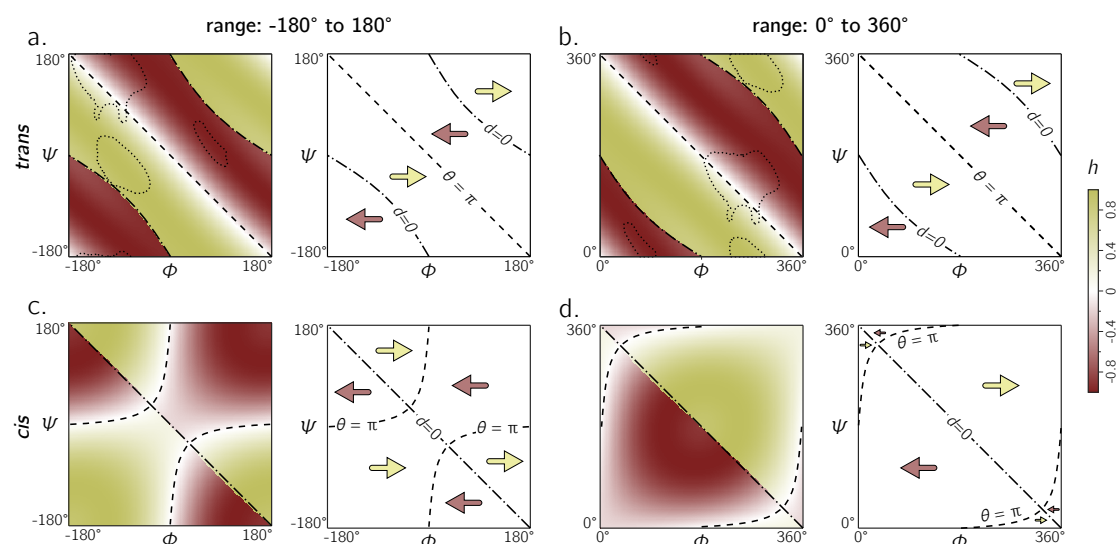
**Figure 7.** The landscape of backbone chirality as a function of amide dihedral angle  $\omega$ . As  $\omega$  is changed, the features of the landscape smoothly transform from the landscapes of  $\omega = \pm\pi$  to  $\omega = 0$ . For all values of  $\omega$ , it is evident that the naïve view of chirality (Fig. 1d) is wrong: at least four distinct regions of chirality [separated by boundaries  $d = 0$  and  $\sin(\theta) = \pi$ ] are evident in each scenario. Although only five snapshots (values of  $\omega$ ) are shown, all integer values of  $\omega$  were tested, which corroborates the fact that the naïve view of backbone handedness (Fig. 1d) is universally incorrect.

describes some Ramachandran plots that show chirality in terms of varying values for  $\omega$ . Fig. 7 shows that our naïve expectations of handedness (Fig. 1d) is too simple, irrespective of amide dihedral angle.

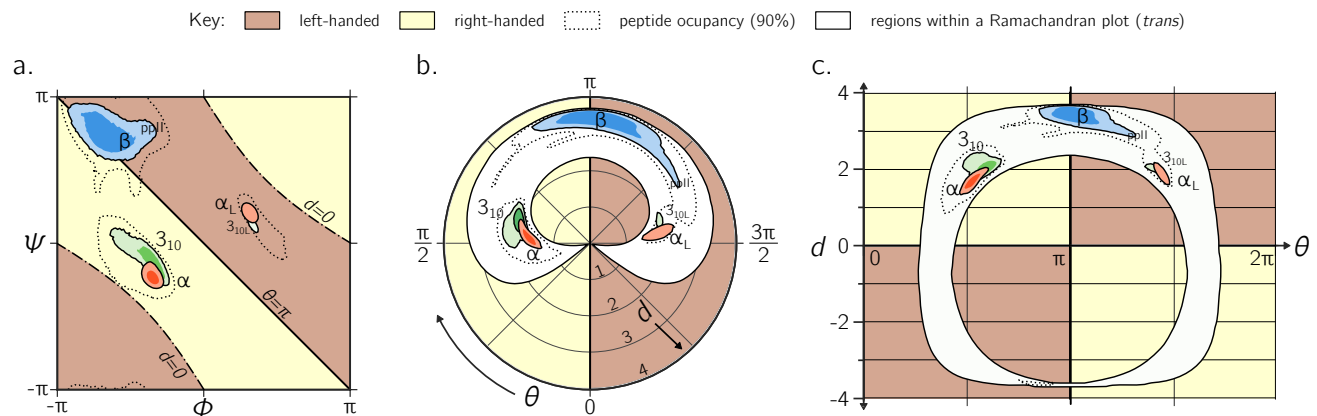
### **$[-\pi, \pi]$ or $[0, 2\pi]$ : which frame of reference to use?**

In structural biology,  $\phi$  and  $\psi$  within the Ramachandran plot has been historically set to range between the values  $[-\pi, \pi]$  radians [see, e.g., textbooks by Berg *et al.* (2010) and Alberts *et al.* (2002)]. However, Ramachandran *et al.* (1963) had originally used the range of  $[0, 2\pi]$ . Today, the range  $[-\pi, \pi]$  is used predominantly by structural biologists (Laskowski *et al.*, 1993; Laskowski, 2003; Zacharias and Knapp, 2013), while some have turned to  $[0, 2\pi]$  as the norm (Némethy *et al.*, 1966; Voelz *et al.*, 2011).

Given the periodicity of the Ramachandran plot, these differences do not make significant difference scientifically; however the value of the Ramachandran plot is undeniably relative to its boundaries: it is a *map* of important features of proteins relative to the various regions, quadrants, and diagonals in the map [see, e.g., discussions by Beck *et al.* (2008)]. The Ramachandran plot's value lies in being able to convey large amounts of information in easy to read logo- or picto-grams. For that reason, switching the map from one range to another means that two norms and two types of scientists will not be able to converse as seamlessly.



**Figure 8.** Each panel describes handedness data (right) and boundaries (left). Both frames of references  $[-\pi, \dots, \pi]$  and  $[0, \dots, 2\pi]$  yield similar trends for *trans* backbones (a,b); however, for *cis* backbones, the latter frame of reference (d) appears to more neatly apportion the handedness of the backbone rather than the traditional frame of reference (c). As in Figs. 5 and 6, ‘--’ and ‘- -’ respectively correspond to boundaries defined by  $\theta = \pi$  and  $d = 0$ . Also, regions bound by dotted contours indicate dominant regions within which proteins reside ( $p = 0.9$ ).



**Figure 9.** Alternative representations of the Ramachandran plot. While the Ramachandran plot is useful to map characteristics of secondary structures (a), it is not intuitive. For example, the relationship between the Ramachandran parameters ( $\phi$ ,  $\psi$ ) and the handedness of a backbone is not obvious (see, e.g., the non-obvious distribution of left- and right-handed peptides as a function of  $\phi$  and  $\psi$ ). For this reason, Zacharias and Knapp (2013) introduced a graphical format involving the helical parameters  $d$  and  $\theta$  in polar coordinate space (b), where the regions of left- and right-handedness are obvious [their format differs from (b) in that their  $\theta$  increases in counter-clockwise fashion]. Panel (c), which is an extension of Fig. 4, introduces another graphical representation of backbone degrees of freedom based on  $(\theta, d)$ , but in Cartesian space. While both (b) and (c) are equally useful in understanding regions available to a protein, the text discusses some benefits of (c) as a universal map for exploring new conformations and secondary structures. Excepting the left-handed helices ( $\alpha_L$ -,  $3_{10L}$ -helices), each secondary structure has two contours signifying  $p = 0.5$  and  $0.8$ .

Therefore, the following question must arise: which range  $[-\pi, \pi]$  or  $[0, 2\pi]$  – is able to convey more information with the least amount of effort? Fig. 8 shows the behavior of a *trans* backbone (a,b) and *cis* backbone (c,d) in the two frames of reference. From (a) and (b) it is evident that general trends in the map for *trans* backbones remain the same in both frames of reference: the negative diagonal ( $\sigma = \pi$ ) locally separates right-handed regions from left-handed regions, while the curved line ( $d = 0$ ) – which also separates handedness – also appears to be in generally the same regions (albeit inverted in curvature). The *cis* backbones, however, look dramatically different in the two frames of reference: the range  $[-\pi, \pi]$  separates handedness in a more complicated manner (c), while, for the most part, the -ve diagonal appears to meaningfully separate handedness when the plot ranges from  $0$  to  $2\pi$  (d). For this reason, purely when looking at handedness, and especially in the case of *cis* backbones, the Ramachandran plot that ranges between  $0$  and  $2\pi$  appears to be more meaningful.

### A universal alternative to the Ramachandran plot

While the Ramachandran plot is useful enough to earn a place in undergraduate-level biology textbooks, as discussed throughout this report, it is not easy to estimate features of a peptide backbone just from its  $(\phi, \psi)$  angles (Fig. 9a). This prompted Zacharias and Knapp (2013) to introduce a new representation for backbone degrees of freedom in the form of a polar graph. In this polar representation, the  $\theta$  is the angular coordinate (azimuth) and  $d$  is the radial coordinate. An example of one such representation is shown in Fig. 9b, with the direction of increasing  $\theta$  reversed (compared to the cited report) to maintain relative positions of secondary structures within the Ramachandran plot (Fig. 9a). Zacharias and Knapp (2013) stated an additional reason for the introduction of the polar representation (Fig. 9b):  $\theta$ , which is an angle and therefore periodic, can remain periodic as the angular coordinate in the graph.

However, the format proposed by Zacharias and Knapp (2013) (Fig. 9b) is incomplete for a few reasons: 1)  $d < 0$  peptides (the bottom-left and top-right regions of Fig. 5a, bottom) will never be observed in this map since only structures with  $d \geq 0$  are allowed; 2) all peptides with  $d = 0$  (marked by ‘- - -’ in every preceding Ramachandran plot) will be compressed into one point at the center, even though Fig. 6c shows a range of legitimate  $d = 0$  conformations); 3) while the graph is  $\theta$ -periodic, the values for  $\theta$  in peptides are constrained within one  $[0, 2\pi]$  period (peptides range between  $\theta = \pi/4$  and  $2\pi - \pi/4$ ; vertical dotted lines in Fig. 4); i.e., periodicity in  $\theta$  is not required for the faithful representation of peptides. Fortunately, even though this system is not universal (again, since  $d < 0$  structures are not

accommodated), most conformations in globular proteins display positive  $d$ , and so the representation presented by Zacharias and Knapp (2013) is a reasonable one for most proteins with known structure.

Interestingly, Fig. 9c – which arranges the parameters  $\theta$  and  $d$  along Cartesian axes – serves as both a universal *and* intuitive map for peptide backbone geometry. This is because: 1) as shown in Fig. 4, such maps reveal a wealth of information about the peptide backbone, 2) both positive and negative values of  $d$  are allowed (compared to Fig. 9b), due to the shift in the coordinate system from polar to Cartesian, and 3) this format accommodates *every* type of peptide conformation: any peptide (or its mimic) has a place in this map irrespective of whether the amide backbone is *cis* or *trans* or any other value; additionally, if the backbone is distorted, such distortions can also be accounted for since  $d$  and  $\theta$  includes such distortions (Eqns. 1 and 2). This is impossible to do using a single Ramachandran plot without making sweeping assumptions about backbone parameters that are not  $\phi$  and  $\psi$ . The  $(\theta, d)$  plot opens up the possibility for a new, intuitive, and *universal* kind of graphical representation as a supplement to the Ramachandran plot.

### A departure from perfect regularity

So far, this report has focused on *simple* regular backbone conformations: those that are formed from the same  $\phi$  and  $\psi$  angles repeated along the backbone. The text so far discusses the treatment of regular backbones particularly because a simple correspondence exists between a regular backbone (described by myriad internal coordinates) and a helix that is described simply by  $(d, \theta, \rho_i)$ . This simple correspondence allows for an intuitive understanding as to how to envision regular backbones as a function of  $d$  and  $\theta$ . There is a possibility that  $d$  and  $\theta$  are useful even in isolation, when the unreasonable constraint of perfect backbone regularity is lifted. Below we provide an example of such a departure from regularity, along with a guide regarding how distinct but consecutive  $(d, \theta)$ 's may be combined to great effect.

Some secondary structures are characterized by the regular combination of two or more sets of  $(\phi, \psi)$  pairs (Pauling and Corey, 1951b,a; Armen *et al.*, 2004; Daggett, 2006; Hayward and Milner-White, 2008; Mannige *et al.*, 2015, 2016). For example, the  $\Sigma$ -strand is constructed by alternating between two backbone states  $(\phi, \psi, \omega) = (-A, B, 180^\circ)$  and  $(A, -B, 180^\circ)$ , where  $A \approx 120$  and  $B \approx 90$  [Fig. 4h in Mannige *et al.* (2015)]. It was found that the two states are equal in the extent to which the backbone twists, but opposite in handedness, which allows for these secondary structures to remain linear, albeit in a meandering way (Mannige *et al.*, 2015). Interestingly, from Eqn. 10, we easily find that both states have handedness ( $h$ ) equal in magnitude but opposite in sign/handedness (the two  $h$ 's are approximately  $\pm 0.5144$ ). Similarly, the  $\alpha$ -sheet proposed by Pauling and Corey (1951a) is constructed by alternating between  $\alpha$  and  $\alpha_L$  backbone states, yet this motif is linear because each state describes equal but opposite handedness  $h = \pm 0.9979$ . These points raise the idea that, even in the absence of perfect backbone regularity, the values  $d$  and  $\theta$  may be considered to be residue-specific properties that may be combined to readily provide insights about higher order structures.

## CONCLUSIONS

This report introduces a metric for backbone handedness ( $h$ ) that is based on modeling the backbone as a helix [Fig. 2; Miyazawa (1961)]. In particular,  $h$ , which is a combination of the helical parameters  $\theta$  (angular displacement) and  $d$  (vertical displacement), ranges from -1 and 1, and is negative (or positive) when the backbone twist is left(or right)-handed (with larger values indicating greater extent of twistedness). This metric ( $h$ ) was used to characterize every regular backbone's twist, via Ramachandran plots, for both *cis* and *trans* peptides. In doing so, this report dispels a naïve view of handedness (Fig. 1d), which states that backbone handedness in the Ramachandran plot is separated by the negative-sloped (-ve) diagonal. Interestingly, the reason for the naïve view makes senses when considering only *trans* peptides: the -ve diagonal ('-' in Figs. 5a) separates  $D$  and  $L$  twists if we consider only the regions dominantly occupied by structured proteins ('.....' in Figs. 5a). Plotting the backbone handedness ( $h$ ) in the common frames of reference –  $\phi, \psi \in [-\pi, \pi)$  and  $[0, 2\pi)$  – indicates that the less commonly used frame  $[0, 2\pi)$  may be more appropriate for interpreting *cis* backbones (Fig. 8).

The behavior of a backbone in *cis* and *trans* Ramachandran plots look dramatically different (Fig. 6), and so scientists dealing with new structures having a combination of *cis* and *trans* backbones can not use one Ramachandran plot to faithfully describe these structures. Interestingly, the parameters  $\theta$  and  $d$  combine all features (internal coordinates) of a contorting backbone, including the amide dihedral angle  $\omega$ , which means that  $(\theta, d)$  can describe *any* peptide backbone, irrespective of  $\omega$ . The Cartesian plot with



$\theta$  and  $d$  as the x- and y-axis, respectively, serves as a unique plot for *any* peptide backbone (Fig. 9), with specific values and boundaries containing deep structural meaning (Fig. 4). These discussions, the author hopes, clarify a number of concepts associated with the Ramachandran plot, while providing new insights into how to interrogate the features of new protein and protein-like structures.

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