Dear Prof. Wilke,

Thank you for your consideration of this manuscript, and for your review. I would also like to thank the reviewer for equally useful comments.

Please find below a point-by-point response to the requested changes by you and the reviewer. All editor and reviewer statements below are shown italicized and in *red*. All author responses below are shown as normal font (black). All excerpts from the manuscript are enclosed in double inverted commas, and additions made to the manuscript are colored blue (captions, however, are not placed in inverted commas).

I believe that the revised manuscript is clearer than the original, thanks to the suggestions made, and I hope that you find this revision suitable for publication in PeerJ.

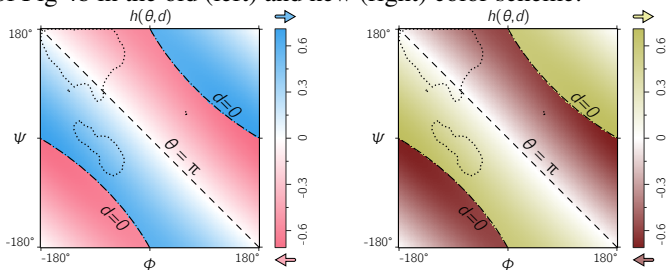
Finally, please note that I have just moved from LBNL, and so my present address is: The Multiscale Institute, Redwood City, CA U.S.A.

Sincerely,

Ranjan Mannige

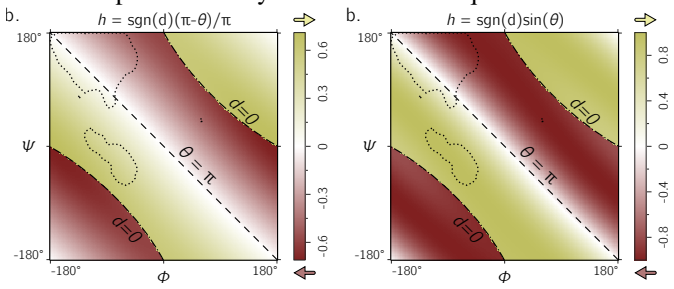
Unsolicited changes

Change in heatmap color scheme. The original colors used for the numerous heat maps in the paper have been changed from pink/blue to brown/gold, particularly because the original color combination, while pleasing in color, does not distinguish between the two color types when printed in black and white. Here is an example of Fig 4b in the old (left) and new (right) color scheme:



Changed the symbol for handedness. I changed the symbol for backbone handedness from χ to h, since backbone handedness is more specific a metric than chirality.

Change in the equation for handedness. Originally, I had provided two equations for backbone chirality/twistedness:
$h = \frac{d}{|d|}\frac{\pi-\theta}{\pi}$ and, within a footnote, $h = \frac{d}{|d|}\sin(\theta)$.
However, during the course of creating a new figure (Fig 4; discussed below), it has become evident that the latter equation is the most appropriate, since all values of $\theta = x\pi$ (x being an integer) *correctly* show up as untwisted in this equation (while only $\theta = \pi$ shows up as untwisted in the former equation, which is incorrect). For that reason, I have changed the primary equation for twistedness to the latter one. I have changed all figures accordingly, which does not change the outcome of the study. For example, the following are the *trans* landscapes colored by the old and new equation.



Editor's Decision

Your manuscript has been carefully reviewed by one reviewer and myself. In general, the reviewer and I have similar comments. We think that overall your work is interesting and fits with PeerJ. However, we are requesting some revisions to presentation and style of your manuscript that will markedly improve it.

In particular, neither the title nor the introduction of your manuscript make it particularly clear what it is that you're doing in this paper. Your paper will be much more impactfull if you can address this issue. In particular, by the end of the introduction, I should have a better sense of what the problem is you're addressing and what your answer is going to be. As an example, you touch on the naive map of chirality in Fig. 1d, but leave the reader hanging on why it is incorrect. Giving some more intuitive motivation here will help.

I have changed the title to "An exhaustive survey of regular peptide conformations using a new metric for backbone handedness (h)"

In addition, I have reworded the abstract, and the last portion of the introduction – which describes problems that I address – is new:

(line 76) "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 ϕ and ψ is more complicated than the distribution allowed by the naïve view. The results also show that the Ramachandran plot whose ϕ and ψ values range between 0° and 360° is more intuitive and visually meaningful (compared to those that range between -180° and 180°), 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."

Other comments follow below.

- p. 3: "samples regions of the Ramachandran plot ('I' in Fig. 1d)" Do you mean Fig. 1b? Also note a similar problem three lines down.

Yes. Thank you for pointing that out.

"We do this because any regular backbone can be associated with a specific helix" You're using the concept "regular backbone" before you define it (in the next section).

I introduce the idea of a regular backbone twice. One reference lies between lines 57 and 60:

(line 43) "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 structure describes a backbone whose per-residue (ϕ, ψ) values are generally the same, and therefore their 'locations' on the Ramachandran plot act as structural landmarks (Fig. 1b)."

Additionally, an explicit definition of the idea 'regular backbone' has been introduced in the following (lines 119–124):

(line 101) “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.”

- p. 5: “Given that backbone bond lengths and angles are generally less tunable when compared to dihedral angles, we can keep them fixed.” My lab has previously found that backbone bond angles actually do fluctuate, see e.g. `https://peerj.com/articles/80/` (already cited in the paper). You may want to comment on this or soften your statement.

OK, that statement was a little too sweeping. For the sake of completeness, I have added two paragraphs to that section: one which motivates why, in some cases, average values for bond angles and lengths may be used, and another which raises the caveats of this assumption. These two paragraphs precede and succeed the equations in the excerpt below:

(following line 139) “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 ϕ and ψ occupy a range of possible values and minima, e.g., regions in the Ramachandran plot that describe α-helices and β-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.”

...

(line 141) “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 ϕ and ψ values (ignoring deviations in ω, 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 ω 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.”

- p. 6: “while $\sin(\theta) < \pi$ indicates right-handedness.” Do you mean $\theta < \pi$? Also, please proof-read the entire paragraph. As far as I can tell, you’re stating the same inequalities for $d < 0$ and $d > 0$, when they actually should be reversed.

My apologies, and thank you for that excellent detective work. I have gone through all paragraphs in that section and fixed a number of typos. The relevant portions of that section follow (starting from line 189):

(line 171) “The relationship between d, θ and handedness is shown in Fig. 3. While θ indicates the extent to which a regular backbone curves along a helical path, the handedness of a backbone is dependent on both θ and d. This is because the sign of d provides a frame of reference for interpreting θ. In particular, if d is negative, then $0 < \theta < \pi$ indicates left-handedness (Fig. 3a), while $\pi < \theta < 2\pi$ indicates a right-handed helix (Fig. 3b). However, if d is positive, then the manner in which the helix is ‘built’ reverses, and $0 < \theta < \pi$ indicates right-handedness (Fig. 3c), while $\pi < \theta < 2\pi$ indicates left-handedness

(Fig. 3d).

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

$$h = \frac{d}{|d|}\sin(\theta). \tag{1}$$

Here, $h \in \{-1, \ldots, 1\}$ 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 $\mathrm{sgn}(d)$, 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 θ in context of handedness and peptide geometry, please refer to the Results and Discussions section and Fig. 4 in particular."

- p. 6: Instead of $d/|d|$, please use the sign function $sgn(d)$.

I believe that $d/|d|$ is more appropriate a term than $\mathrm{sgn}(d)$ because the former expression is undefined when $d = 0$, which is desirable because handedness is undefined when $d = 0$, as is discussed in the second paragraph of the new subsection (bold font used to highlight the relevant point):

(line 232) "**Relevance of θ 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, α-carbons, or even sidechain β-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 θ 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.**"

- p. 10: Note that you have a typo in the PeptideBuilder reference. The first author should be listed as Tien MZ, not Matthew Z. Also, you're missing the middle initials for all authors.

Thank you. The changes have been made.

- The image shown between lines 179 and 180 needs to be converted into a figure with a figure number and a caption. It is also really difficult to figure out what's going on. I would suggest to show a series of images with decreasing d, so that we can see how the structure collapses onto itself as $d = 0$ is approached.

I have now added a new figure to explain how to interpret what $d = 0$ and $\theta = 0, \pi$ mean. That figure (Fig. 4) and caption follows this page. Additionally, I have added a panel (c) to the sixth figure that displays a range of structures with $d \approx 0$. The new Fig. 6 and its modified caption also follows.

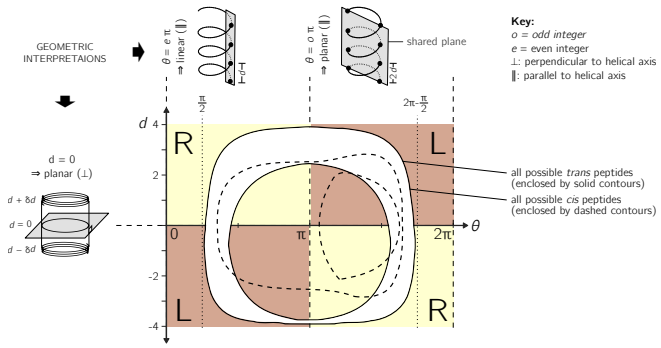


Figure 4. Further discussion on the meaning of d and θ. As shown in Fig. 3, axial separation d and angular separation θ between adjacent atoms of the same type combine to define handedness. The shaded regions within the graph show the distribution of handedness as a function of d and θ. The relevant 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 ($\omega = 180 \pm 10°$). 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.

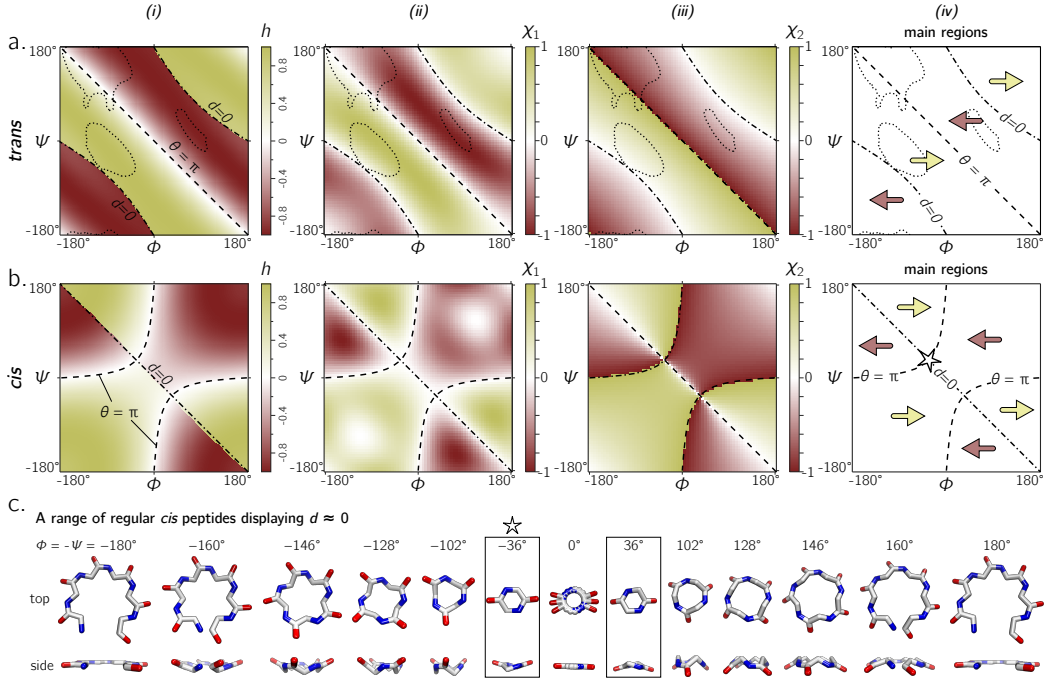


Figure 6. Panels (a) and (b) describe the handedness of backbone twists whose amide dihedral angles are *trans* ($\omega = \pi$) and *cis* ($\omega = 0$), respectively. Column *(i)* describes handedness calculated using h (Eqn. 10), which does not require structures to be computationally generated. Columns *(ii)* and *(iii)* respectively show vector-based estimates of backbone handedness – χ_1 (Eqn. 11) and χ_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 $\theta = \pi$: $\phi = -\psi = \pm 36°$ ['☆' in Panels(b)-*(iv)* and (c)].

- l. 195: Capitalize Ramachandran.

Thank you. The change has been made.

- l. 204-207: There are several typos in these lines. Please copy-edit carefully.

The following typos were fixed in those lines: (line 336) "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 2π (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 2π appears to be more meaningful."

- "Discussion" should be renamed into "Results", or possibly "Results and Discussion".

The "Discussion" section has been changed to the "Results and Discussion" section.

- The Conclusions section should be written in prose form, not as an itemized list.

I was wondering whether I could get away with that :)
The Conclusions section is now in paragraph form:

(line 395) "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 θ (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 θ and d combine all features (internal coordinates) of a contorting backbone, including the amide dihedral angle ω, which means that (θ,d) can describe *any* peptide backbone, irrespective of ω. The Cartesian plot with θ 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."

Also, the figure needs to have a figure number and caption. And finally, please don't introduce new results in the Conclusions. The figure you're showing in Conclusions is a new result and hence should be shown in the Results section.

I have created a new figure and a new section that describes these results. The section is titled below. The accompanying figure follows.

(line 308) "**How does the amide backbone (ω) change the chirality landscape?**
So far, only discrete values for ω are discussed (either *cis* or *trans* peptides respectively corresponding to $\omega = 0$ and π). 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 π are evident in the Protein Databank; see, e.g., discussions by Improta *et al.* (2011). This raises the question of how does varying ω change the landscape? Fig. 7

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

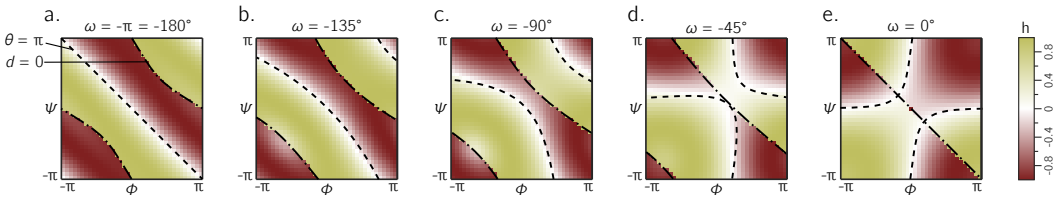


Figure 7. The landscape of backbone chirality as a function of amide dihedral angle ω. As ω is changed, the features of the landscape smoothly transform from the landscapes of $\omega = \pm\pi$ to $\omega = 0$. For all values of ω, it is evident that the naïve view of chirality (Fig. 1d) is wrong: at 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 ω) are shown, all integer values of ω were tested, which corroborates the fact that the naïve view of backbone handedness (Fig. 1d) is universally incorrect.

Comments from the reviewer 1

I would like to thank the reviewer for a thoughtful review and useful comments that, I believe, has helped greatly improve the manuscript. All editor and reviewer statements below are shown italicized and in *red*. All author responses below are shown as normal font (black). All excerpts from the manuscript are enclosed in double inverted commas, and additions made to the manuscript are colored blue (captions, however, are not placed in inverted commas).

Basic reporting

This paper is generally well written and clear. It contains a few typos (see specific comments below) that can be easily corrected. Background is well introduced and references seem to be appropriate. There seems to be an almost 40 year gap between the earliest references and most recent ones. I assume this gap arises from a renewed interest in this topic that was left behind. I would still recommend the author to investigate if developments in the 80s and early 90s are important to reference. . . .

I have gone through every citation to Miyazawa's paper reported in the Web of Knowledge and Google Scholar, but have found relatively few papers focusing primarly on peptides during that fourty-year timeframe. The gap of *relevant* references during that fourty year gap is likely due to the following points: 1) Miyazawa's also focuses on spectroscopy, and most citations to this paper discuss developments in spectroscopy, and 2) the paper is extremely general/diverse and refers to polymers of all kinds. For example, Miyazawa's paper discusses, among other systems, diverse molecules such as polyethylene, polytetrafluoroethylene, fibrous sulfur, selenium, tellurium, polypropylene, polyisobutylene, and, finally, polypeptides. Indeed, out of the 136 citations to this paper (reported by Google Scholar), only 13 citations are matched by the search term 'peptide' and only 3 citations are matched by the search terms 'peptide' and 'backbone'. In order to indicate how expansive Miyazawa's paper is, I have provided the following in the introduction preceeding the Miyazawa equations (Eqns. 1 and 2):

(line 111) "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."

. . . Figures are clear and well made, please check the use of punctuation in Figure 1 when refereeing to the panel labels (a) – (d).

Thank you. All labels were checked and, where appropriate, corrected.

- The use of inline figures like the ones in 180 and 214 is not standard and might lead to confusion. I suggest to label them and reference them in the traditional way.

The two inline figures are now Fig. 6c and Fig. 7. Also, the figure Fig. 7 has a new accompanying section titled "How does the amide backbone (ω) change the chirality landscape?" (starting on line 307).

- Although the title is quite catchy, I think the audience could benefit from a more descriptive title that reflects the content of the paper, for instance the fact that the paper introduces a simplified metric of chirality

Agreed. I have changed the title to:

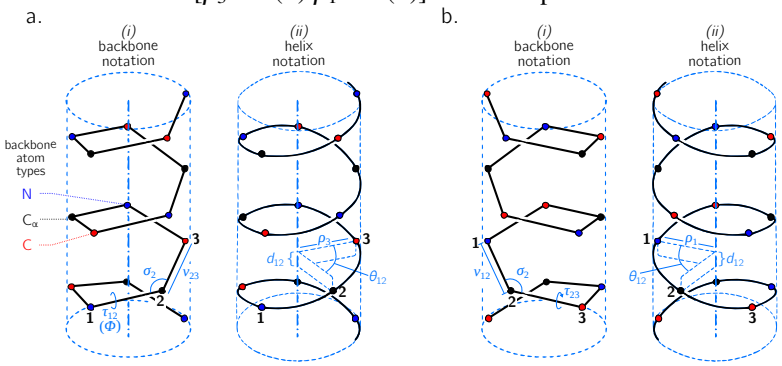
"An exhaustive survey of regular peptide conformations using a new metric for backbone handedness (h)"

- I assume that PeerJ allows the use of footnotes. In my opinion the use of footnotes is anachronic. In any case, the author uses too many of them which can be distracting to the reader. I suggest reducing them to the bare minimum and move such information in the main text.

The present manuscript contains ony three footnotes (which has been brought down from from 11). Most of the removed footnotes are subsumed the text, unless I found them to be unnecessary, in which case I deleted them. I believe that these footnotes are revelant to only a small portion of the readers, and so I hope to keep them as footnotes in order to reduce distractions from the paper's narrative.

- In figure 2, the helix [radius] is not shown but it is described in the manuscript. I suggest to incorporate an illustration of the radius rho in the figure.

I have newly annotated two radii [ρ_3 in a(ii) ρ_1 in b(ii)]. The new picture follows.



Additionally, I have added to the manuscript the equation (Eqns. 3 & 4) for the helix radius, for the sake of completeness.

Experimental design

The motivation for this research is well stated and the methods presented to resolve the question are sound. It would be beneficial is the author could clarify if there is an strict one-to-one correspondence between the use of internal coordinates and the helical coordinate approximation.

I have added the following the introduction of Equations 1 and 2:

(line 156) "**On the one-to-one correspondence between $[\phi, \psi, \omega]$ and $[d, \theta]$**
Given a particular *value* of ω, every $[\phi, \psi]$ pair points to exactly one $[d, \theta]$. However, when using Eqns. 1 and 2, one value of ω can not be replaced with a *periodically equivalent* version of ω. For example, using $\omega = x + 2\pi$ instead of $\omega = x$ will maintain the magnitude of d and θ, 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 ω 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 ωs. For this reason, we propose to wrap the value of an amide backbone ω' between $[\Delta, \Delta + 360°)$ using

$$\omega = (\omega' - \Delta)\%360 + \Delta, \tag{2}$$

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

These points lead to the conclusion that a *strict* one to one-to-one correspondence between (ϕ, ψ, ω) and (d, θ) 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 (ϕ, ψ, ω), whose ω does not change after being wrapped by Eqn. 9."

Also, it would be important to incorporate a description or a reference discussing the maximal error when applying this transformation.

I was unable to find a reference regarding the maximal error, and I believe that a thorough analysis would be beyond the scope of the paper, especially since the transformation has been around since the 1960's.

However, I have added the following paragraph to indicate how some estimates the paper makes may adversely affect the transformations:

(line 141) "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 ϕ and ψ values (ignoring deviations in ω, 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 ω 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."

Validity of the findings

Since the metric introduced in this study is used strictly for "regular" backbones a discussion is needed on how accurate is this assumption for most structures in the PDB and especially for peptoids and cis-conformations that is the main focus of this study.

I have added the following subsection before the conclusion section, which discusses how, even though we use regular backbones to best describe how to interpret d and θ, these two parameters can be used on a per-residue-basis, where the 'constraint' of regularity is lifted.

(line 372) "**A departure from perfect regularity**
So far, this report has focused on *simple* regular backbone conformations: those that are formed from the same ϕ and ψ 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, θ, ρ_i). This simple correspondence allows for an intuitive understanding as to how to envision regular backbones as a function of d and θ. There is a possiblity that d and θ 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, θ)'s may be combined to great effect.

Some secondary structures are characterized by the regular combination of two or more sets of (ϕ, ψ) 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 Σ-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 ± 0.5144). Similarly, the α-sheet proposed by Pauling and Corey (1951a) is constructed by alternating between α and α_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 θ may be considered to be residue-specific properties that may be combined to readily provide insights about higher order structures."

My understanding is that the fact that this chirality metric is not dependent on structural computations lies from the fact that the author is making the "regular backbone" assumption, however it is not clear how general is this assumption. The author should provide arguments why this is generally true and make sure it states that this is an important underlying assumption of the method.

(line 193) "**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 θ require only pairs of ϕ and ψ angles. However, if values for bond angles, lengths and dihedral angles are expected to deviate greatly from equilibrium values, θ 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 χ_1 (Eqn. 11) and χ_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 $\phi, \psi \in \{-180, -178, \ldots, 178, 180\}$."

In line 170, the author claims that the discrepancies between the metrics based on internal coordinates and the presented metric is due to the fact that these metrics are "estimates". This seems to be misleading since the metric presented by the author is also an estimate given that we have the perfect helix approximation and the assumption of regular backbones. It would be probably more appropriate to [state] that all these metrics are estimates that only deviate minimally and reach essentially the same conclusions.

I believe that a few misunderstandings emerge from the fact that I did not distinguish between backbone (twist) handedness and molecular chirality. In particular, the metric proposed in this manuscript is for twist handedness, while the other metrics discussed in the paper are for backbone (molecular) chirality. While the latter measure often corresponds to twist handedness, only helical parameters meaningfully describe the nature of the twist, the granular form of which is the handedness. It is for this that the two measures for chirality were considered to be *estimates* for handedness. To distinguish between the handedness and chirality, I have added the following new subsection in the Methods section.

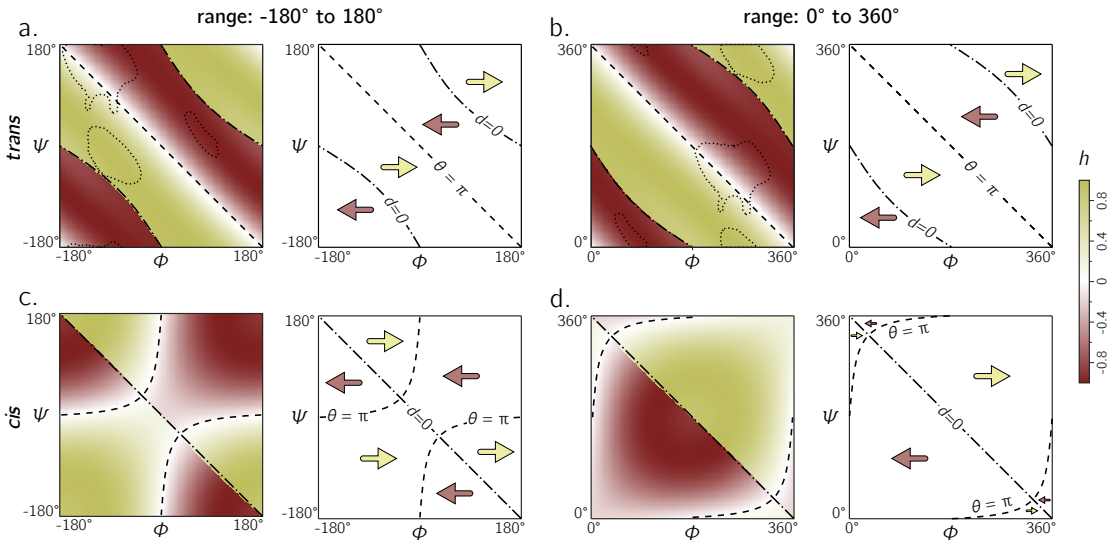
(line 204) "**Backbone chirality $\neq$ backbone handedness**
Finally, it is important to recognize the distinction between backbone (twist) handedness and backbone (molecular) chirality. Naïvely, chirality is a simple concept: a molecular conformation is achiral if its mirror image can be superimposed 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 α-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."

One conclusion of Fig. 6d, is that if we use the range 0-360 degrees we could have a simplified description of chirality for cis backbones. I agree with this claim and I believe it is an interesting one, however, the figure does show small but non-negligible values of handedness at the upper left and lower right corners. Why did the author decide to exclude those regions at the time of labeling the handedness? If there is a sensible argument, the author should explicitly state it otherwise the arrows should also be placed in those regions even if this weakens the author's claims.

I excluded the arrows of those regions as they were very small (and so the arrows would be tiny). I have added those arrows, and made the following changes to the discussion (in particular, I applied the qualifier "for the most part" when discussing how well the negative diagonal apportions the alternative frame of reference):

(line 336) “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 2π (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 2π appears to be more meaningful.”

The corresponding (modified) figure is below:



Comments for the author

I believe this work could be a useful reference for the study of chirality in secondary structure but even more applicable to the growing field of biologically inspired molecule design. Although its applicability is reduced in common biological systems, I do believe it can be beneficial to the scientific community. If the author clarifies the questions and claims of this study, the paper would benefit and might be acceptable for publication.

The following changes help clarify the questions and claims of the paper: 1) the title is changed to be more descriptive, 2) the abstract has been changed to clearly outline the question and outcomes of the paper, 3) the last paragraph of the introduction is mostly new, and otulines the progression of the paper, and 4) the new conclusion, written in paragraph form, reiterates all of the discussions.

Specific comments:

- Abstract: Please replace “In these lines” with “Along these lines”

Done.

- Line 34, replace “… or peptide backbone is dependent on the dihedral angles …” with “… or peptide backbone can be described by the dihedral angles …”

Done.

- Line 64, when you say “not well characterized” do you mean “not populated” ?

This part of the paper has been modified and does not contain this line anymore. However, its general spirit lies in the following lines of the final introductory paragraph.

(line 76) “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.”

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THIS PAGE CONTAINS THE CORRECT ORDER OF (DUMMY) FIGURE AND EQUATION NUMBERS SO THAT THE REFERENCES USED ABOVE POINT TO THE RIGHT NUMBERS.

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.

Figure 7.

Figure 8.

Figure 9.

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