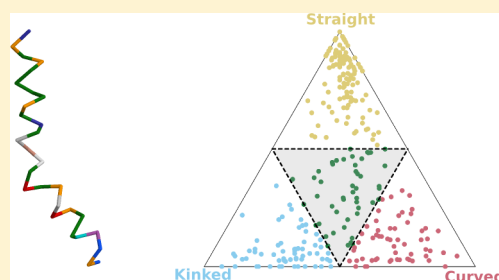


# Crowdsourcing Yields a New Standard for Kinks in Protein Helices

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## S Supporting Information

**ABSTRACT:** Kinks are functionally important structural features found in the  $\alpha$ -helices of proteins. Structurally, they are points at which a helix abruptly changes direction. Current kink definition and identification methods often disagree with one another. Here we describe a crowdsourcing approach to obtain a reliable gold standard set of kinks. Using an online interface, we collected more than 10 000 classifications of 300 helices into straight, curved, or kinked categories. We found that participants were better at discriminating between straight and not-straight helices than between kinked and curved helices. Surprisingly, more obvious kinks were not necessarily identified as more localized within the helix. We present a set of 252 helices where more than 50% of the participants agree on a classification. This set can be used as a reliable gold standard to develop, train, and compare computational methods. An interactive visualization of the results is available online at [http://opig.stats.ox.ac.uk/webapps/ahah/php/experiment\\_results.php](http://opig.stats.ox.ac.uk/webapps/ahah/php/experiment_results.php).



## INTRODUCTION

Helices are important secondary structures in proteins. Kinks are regions in  $\alpha$ -helices where the helix direction changes abruptly; however, the specific definition varies from study to study. Many terms have been used to describe this type of helix distortion, with kink<sup>1–8</sup> being the most popular, but bend,<sup>9</sup> hinge,<sup>10</sup> alteration,<sup>11,12</sup> and cusp<sup>13</sup> have also been used. Such helix kinks are a common feature of  $\alpha$ -helical membrane proteins<sup>2,3,5,6,9,14–16</sup> as well as long helices in soluble proteins.<sup>2,7,17</sup> They have been implicated in the function of G protein-coupled receptors,<sup>18–20</sup> in the conformational change of ion channels,<sup>21–23</sup> in heme binding in cytochrome *b*<sub>559</sub>,<sup>24</sup> and in the function of many other proteins.<sup>10,25,26</sup> Accurate determination of kinks is important to the modeling of membrane proteins,<sup>3</sup> to computational docking studies of medically relevant compounds to membrane proteins,<sup>27</sup> and to the general understanding of protein structure.

**Computational Methods To Identify Kinks.** A large number of methods to identify kinks in proteins have been published.<sup>4–6,8,9,28</sup> Such structure-based kink identification methods use the three-dimensional atomic coordinates of the C $\alpha$  atoms as a basis for kink identification. Examples include ProKink,<sup>28</sup> TMKink,<sup>5</sup> Helanal-Plus,<sup>4,8</sup> MC-Helan,<sup>9</sup> and Kink Finder.<sup>2</sup> These methods identify the  $\alpha$ -helices within a protein using a variety of approaches<sup>29,30</sup> and then analyze these  $\alpha$ -helices to identify kinks in various ways.

Helanal-Plus<sup>4,8</sup> fits axes to four residue windows of a helix using the fitting method of Sugeta and Miyazawa.<sup>31</sup> All possible windows are considered. The angle between each pair of adjacent windows is calculated. The method also calculates a trace of the helix axis, which is fitted to a line and the surface of

a sphere. The helix is classified as kinked, curved, or linear on the basis of the angles and the quality of the fit to the line and sphere.

Werner and Church<sup>3</sup> fit axes to four residue sliding windows of the helix using Kahn's method.<sup>32</sup> The angle between each pair of axes that are separated by a residue is calculated. Helices are classified as kinked if they contain an angle of  $\geq 13^\circ$ .

ProKink<sup>28</sup> only identifies kinks at proline residues in helices. Proline is often implicated in kinks because it lacks the ability to form one of the standard backbone hydrogen bonds of an  $\alpha$ -helix. ProKink fits axes to the pre- and post-proline sections of the helix using cylinders. The angle between these two axes is the kink angle.

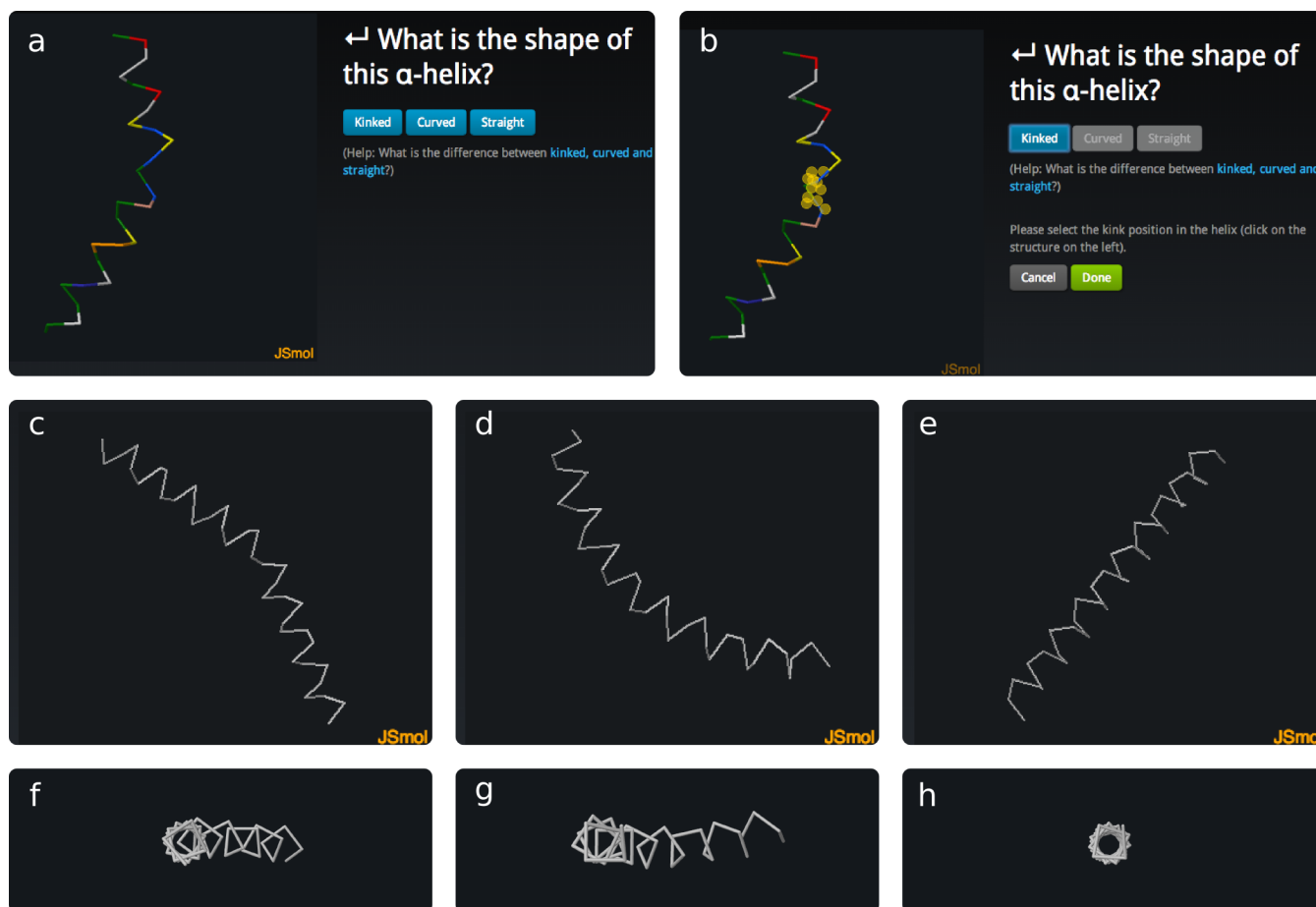
TMKink<sup>5</sup> fits axes to six residue windows of the helix using cylinders. As with Helanal-Plus, all possible windows are considered. The angle between each pair of adjacent windows is calculated. Kinks are identified if a helix contains an angle of  $\geq 24^\circ$  or if the average over four consecutive angles is  $\geq 13^\circ$ .

Kink Finder<sup>2</sup> fits cylinders to a sliding window of six residues using cylinders fitted to all backbone atoms. Local angles are calculated from the axes of adjacent windows. Kinks are identified where the local angle is  $\geq 20^\circ$ . A residue on the inside of the kink is selected as the kink residue.

MC-Helan<sup>9</sup> identifies straight helix sections in a protein. Axes are fitted to these strictly defined straight helix sections. Helices are classified as bent or distorted if they contain two or more straight helix sections; otherwise, the helix is classified as "good".

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**Figure 1.** Representation of helices in AHAH. (a) AHAH user interface. Participants identify the helix as kinked, curved, or straight. (b) Where they identify a helix as kinked, they select a residue at the kink position. (c–h) Idealized helices shown to participants in the tutorial: (c) and (f) are two representations of a kinked helix, (d) and (g) are two representations of a curved helix, and (e) and (h) show a straight helix.

Hischenhuber and co-workers<sup>11,12</sup> identify helix alterations using a polynomial fit to the  $C\alpha$  coordinates in the helix and use the curvature and torsion of this polynomial to determine whether and where a kink is present.

Kneissl et al.<sup>6</sup> took a completely different approach. Instead of using a computational algorithm, they manually inspected a set of 1014 helices and classified them as kinked, curved, or straight.

The methods described above use a variety of definitions for kinks and approaches to identify them. Differences in algorithm design and parameter choice have led to large discrepancies among the kinks identified by various methods.

**Crowdsourcing Science.** To address this challenge, we present a crowdsourcing approach for protein kink determination. Crowdsourcing is a technique that employs the human intelligence of a large number of participants to solve computationally challenging problems.<sup>33–35</sup> There have been a number of recent examples of crowdsourcing. For example, the Galaxy Zoo Project<sup>36,37</sup> uses volunteers to classify images that would otherwise take individual researchers years. Foldit<sup>38,39</sup> invites volunteers to fold protein structures via an interactive game. Similarly, Phylo<sup>40</sup> uses an online game to solve difficult alignment problems.

In this article, we describe Alpha Helices Assessed by Humans (AHAH), a crowdsourcing experiment to identify protein helix kinks. More than 300 volunteers provided over 10 000 annotations of 300  $\alpha$ -helices through an interactive 3D

Web interface. We show that the consensus classifications from the participants differ from those of previous approaches. On the basis of our crowdsourcing approach, we provide a high-quality “gold standard” set of 252 helix classifications that can act as a reliable training set for future computational classifiers.

## MATERIALS AND METHODS

**Helix Set.** The helices used in this study were extracted from the set of 1014 helices manually annotated by Kneissl et al.<sup>6</sup> We randomly selected 300 of these helices while maintaining the same proportion of kinked, curved, and straight helices as annotated by Kneissl et al. A full list of these is given in the Supporting Information and at <http://www.stats.ox.ac.uk/proteins/resources#AHAH>.

**Technical Implementation.** The crowdsourcing application was implemented as a Web application using PHP (version 5.4.4) for middleware and MySQL (version 9.1.13) for the database backend. Results were analyzed using Python (2.7.5) and R (3.0.2).<sup>41</sup> A schematic diagram of the participants’ interaction with the Web application is shown in Figure S1 in the Supporting Information.

**Helix Data Representation.** The helices were displayed to participants using the JSmol viewer in a  $C\alpha$ -only ribbon representation (Figure 1a,b). Residues were colored by their type, and participants were able to freely rotate them and zoom. The helices rotated slowly by default.

**Participants.** A randomly selected subset of 30 helices was shown to each participant. A total of 310 people with diverse backgrounds (Table 1) and education levels (Table 2) registered and took part.

**Table 1. Proportions of Participants with Given Backgrounds<sup>a</sup>**

background	% of participants
structural biology	14.8
chemistry/biochemistry	22.9
physics/maths	18.7
computer science/IT	24.8
other science	20.0
other nonscience	11.0
none	27.4

<sup>a</sup>Participants were able to indicate multiple backgrounds, and the “none” category included 77 school pupils (24.8%).

**Table 2. Participant Education Levels<sup>a</sup>**

education level (achieved to date)	% of participants
postdoctoral and above	17.7
undergraduate and above	48.4
secondary	5.2
other	26.1
none indicated	2.6

<sup>a</sup>The “other” category includes 77 school pupils (24.8%).

**Training of Participants.** Participants were trained in three stages. First, written descriptions of the classification definitions were given, along with idealized examples of each helix type (Figure 1c–h). The definitions were as follows:

- **Kinked:** “There is a clear location where the direction of the helix changes. Only a small part of the helix is involved in this.”
- **Curved:** “There is a slow but steady change of the direction of the helix. This can happen over a large part or even all of the helix.”
- **Straight:** “There is no change in the overall direction of the helix.”

Second, examples of three real helices were illustrated. In addition, we stated that such real helices might be more ambiguous than the idealized examples shown previously. Finally, participants had to annotate three idealized helices correctly before they could continue. For the idealized kinked helix, this included identifying a residue close to the site of the kink. This step attempted to ensure that only those participants who understood the concept of kinked, curved, and straight could participate in the project.

**Crowdsourcing Survey.** After successfully completing the tutorial, participants could annotate one helix before being required to register. Participants were then asked to classify 30 randomly selected helices. After 30 annotations, participants were thanked and shown a comparison between their responses and those of other participants. The participants were then given an option to either stop annotating helices or continue until a maximum of 300 helices were classified. Each helix was shown only once to the respective participant, and corrections after the initial assessment were not possible. Participants were not able to skip a helix.

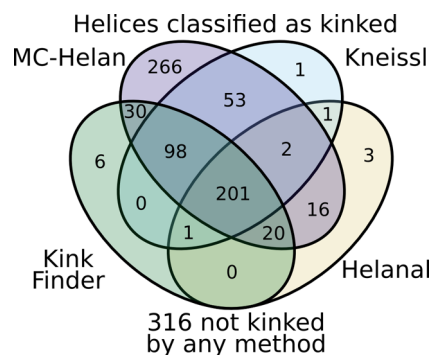
For each helix, participants were shown the helix, displayed in JSmol as described above, and had to indicate whether it was kinked, curved, or straight by clicking the appropriate button (see Figure 1a). If the participant indicated that the helix was kinked, then he/she had to select a residue as the kink point by clicking on it in the JSmol viewer. This residue was then highlighted, and they could change their choice of kink residue or confirm it by clicking a button (see Figure 1b).

**Response Consistency.** In order to assess the annotations, we considered response consistency. The consistency of a subset of results was calculated by taking the number of annotations that agreed with the majority view. Annotations were divided into subcategories on the basis of features such as time taken and the backgrounds and education levels of the participants. The time taken for each annotation was calculated as the difference between the time at which the annotation was made and the time at which the previous annotation was made. There were 138 annotations that took less than 2 s, which were removed from the analysis. These were outliers with respect to time taken and were disproportionately provided by a small number of participants (79 from the same five participants).

**Other Kink Identification Methods.** We compared the results of our crowdsourcing approach against four previous approaches. These were Kink Finder,<sup>2</sup> MC-Helan,<sup>9</sup> Helanal-Plus,<sup>4</sup> and the Kneissl annotation.<sup>6</sup> These methods use different terminology (i.e., “kink”, “bend”, or “distortion” and “straight”, “linear”, or “good”). In this work, we treat bent and distorted (MC-Helan) as synonymous with kinked. Linear (Helanal-Plus) and good (MC-Helan) are synonymous with straight.

## RESULTS

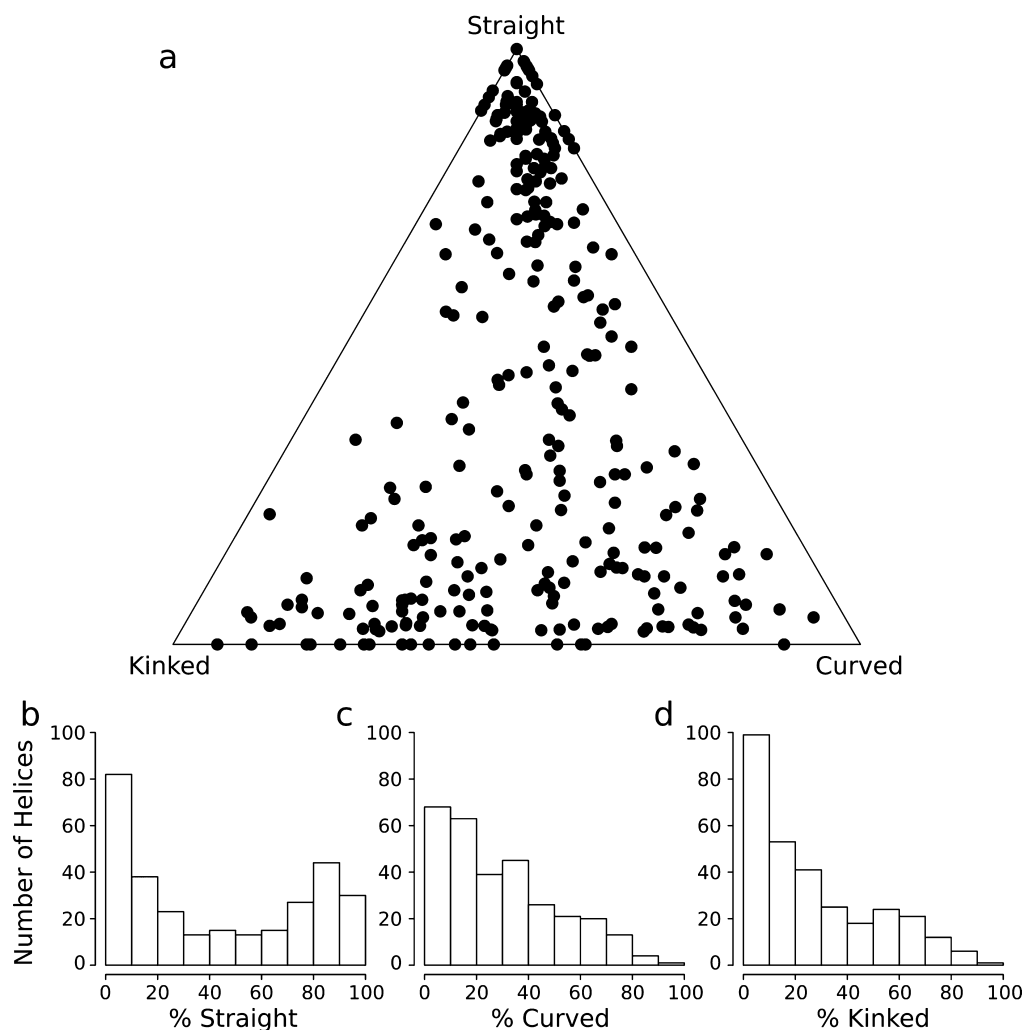
A large number of helix classification methods have been published.<sup>4–6,8,9,28</sup> The agreement between these is relatively poor. Figure 2 shows the 1014 helices in the Kneissl et al. data



**Figure 2.** Numbers of helices classed as kinked (or bent by MC-Helan) by four helix classification methods. All four methods agree in only 517 (201 kinked, 316 not-kinked) of 1014 cases.

set,<sup>6</sup> annotated as kinked by three computational methods and by the manual Kneissl et al. approach. The methods agree on only half of the helices (517 out of 1014). The percentage of kinked helices varies from 24.1% to 67.7%. Of the 201 helices that are classified as kinked by all four methods, there are none for which all four approaches identify the same kink residue within the helix.

Although all of the methods identify “kinks”, the precise definition varies from method to method. The computational methods all also rely on one or more thresholds and assumptions to discriminate between kinked and not-kinked



**Figure 3.** (a) Ternary diagram showing the responses for each helix. Each point represents a helix. The perpendicular distance from each side is the proportion of responses of each type. For example, those points on the bottom line represent helices that were not annotated as straight by any participants, and those points in the top corner represent helices that were annotated as straight by all participants. (b–d) Frequencies of helices with given percentages of (b) straight, (c) curved, and (d) kinked responses.

helices. These differences lead to the large discrepancies among the methods. The problem of classifying helices is computationally difficult and ambiguous. However, there is no objective basis to identify the correct or ideal threshold(s). In order to address this problem, we built Alpha Helices Assessed by Humans (AHAH), a crowdsourcing experiment to identify helix kinks.

**Participants and Annotations.** A total of 310 participants registered to take part in AHAH (Tables 1 and 2), of which 290 annotated at least 30 helices. In addition, there were 928 annotations by unregistered participants. This yielded a total of 10 665 helix annotations. The average number of annotations was 35.6 per helix and 34.4 per registered participant.

**Helix Classification by Our Participants.** Figure 3 illustrates the responses of our participants for each helix. It shows that many helices do not fall obviously into one of the three possible classifications. For only 86 of the 300 helices (28.7%) is the majority view held by more than 80% of the participants who classified it; for 194 (64.7%), the majority view is held by  $\geq 60\%$  of the participants, and for 252 (83.7%), the majority view is held by  $\geq 50\%$  of participants.

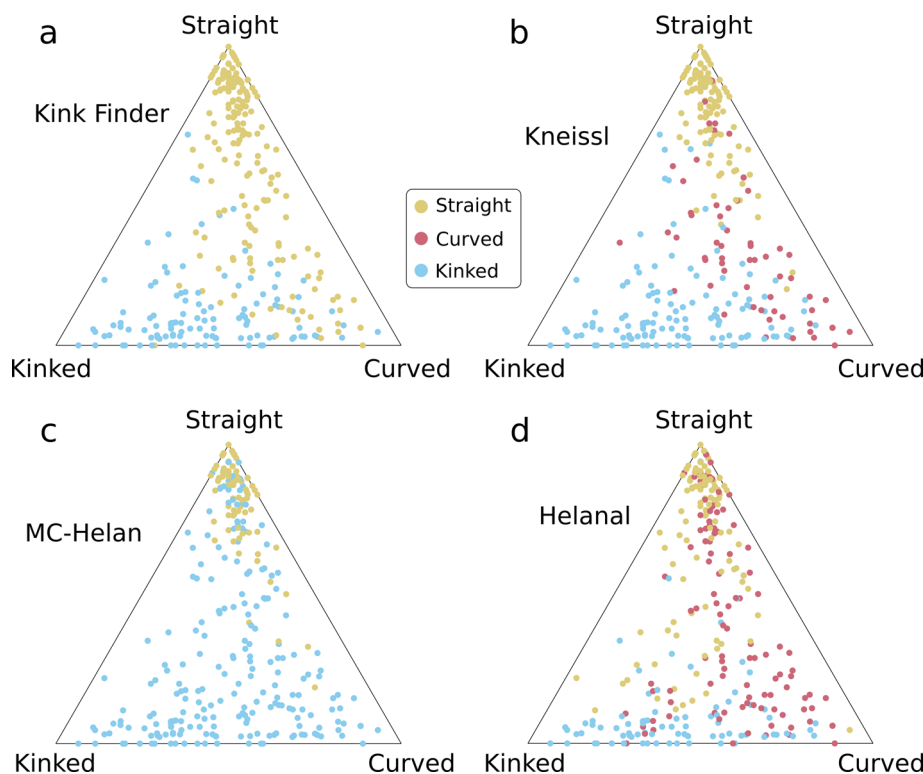
It is not clear from previous studies whether a binary or tertiary classification of helices is most appropriate. Of the

possible binary classifications (straight/not-straight, curved/not-curved, or kinked/not-kinked), straight/not-straight provides the clearest separation. There are 191 (63.7%) helices where more than 80% of participants agreed on a straight/not-straight classification (Figure 3b), compared with 134 (44.7%) for curved/not-curved (Figure 3c) and 155 (51.7%) for kinked/not-kinked (Figure 3d). This indicates that it is easier for people to identify whether helices are straight but more difficult to differentiate between kinked and curved.

**Consistency.** The ambiguity of the results could be caused by poor-quality responses from subgroups among our participants. We tested this hypothesis in two ways. First, we investigated whether the consistency of a response was related to the time taken by the participant to make that classification. Second, we compared the consistency of participant groups to ensure that no group was significantly worse than any other.

Divergence from the majority view did not vary with time taken to classify a helix (Figures S2 and S3 in the Supporting Information). There were a small number of annotations that took under 2 s, which were less consistent. As described in Materials and Methods, these were removed from the analysis. The annotations from unregistered participants were only slightly less consistent than those of the registered participants.





**Figure 4.** Ternary diagrams showing the responses for each helix, colored by annotation, for four helix classification methods: (a) Kink Finder; (b) Kneissl manual annotation; (c) MC-Helan; (d) Helanal-Plus.

We divided the participants into background and education-level groups on the basis of their responses during registration. Although there was some difference between the consistencies of the groups, no group was sizeably worse than any other. Structural biologists appeared to be the best group (73.5% agreement with the majority view) and other scientists the worst group (69% agreement) in terms of backgrounds (Figures S4 and S5 in the Supporting Information). When the data were divided by education level, the postdoctoral and above group had the best agreement with the majority view (71.2% agreement), whereas the school pupils had the worst agreement (64.6% agreement) except for the unregistered annotations (62.5% agreement).

The differences between the groups of participants were small. Therefore, we excluded only those annotations that took less than 2 s but kept all of the other data.

**Comparison with Other Methods.** In Figure 4 we compare the results of our crowdsourcing approach with those of other methods from the literature. Two of these methods (Kink Finder and MC-Helan) only split helices into straight and kinked. None of the individual methods have strong agreement with the AHAH participants. The Kink Finder and Kneissl classifications are more consistent with the crowdsourced classifications than the other two methods (Figure 4a,b). Kink Finder correctly annotated all but one of the helices that  $\geq 60\%$  of participants classified as kinked. However, there are a number of helices toward the curved corner that Kink Finder classified as straight (Figure 4a). The Kneissl annotation broadly agrees with the crowdsourced data, although helices classified by Kneissl as curved are distributed across most of the ternary diagram (Figure 4b).

MC-Helan appears to overclassify helices into the kinked category, classifying the majority of helices as kinked, except

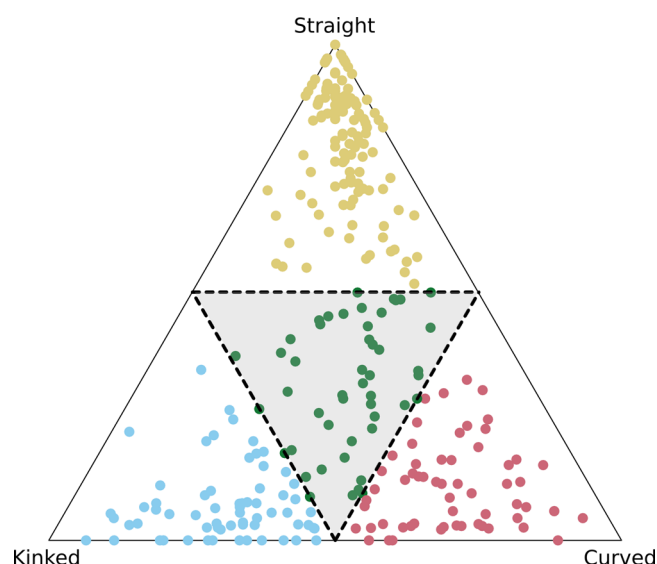
those that nearly all participants annotate as straight (Figure 4c). Helanal-Plus classified far fewer helices as kinked than any of the other methods (Figure 4d). The helices classified as curved by Helanal-Plus are distributed across the diagram, like the curved helices of Kneissl. The helices annotated as linear (straight) by Helanal-Plus are distributed across the straight and kinked regions.

All of the methods annotate a number of the helices in the curved corner of the ternary plot as kinked, particularly helices that are annotated as straight by very few, if any, participants.

**Gold Standard.** A major aim of this study is to provide a reliable gold standard set to be used in the development of future computational annotation approaches. Figure 5 shows the cutoffs we have used to produce our gold standard set. A helix falls into a class if more than 50% of the manual annotations agree. Otherwise the helix is not assigned to any class. The gold standard set is shown in full in Table S1 in the Supporting Information. A total of 64 (21.3%) helices are classified as kinked, 59 (19.7%) as curved, and 129 (43.0%) as straight. The remaining 48 (16.0%) helices were not assigned a classification. Table 3 shows the overlap between this gold standard set and the other kink identification methods.

The previous methods typically overestimate the number of kinked helices, annotating 38.0% (Kink Finder), 40% (Kneissl), 67.3% (MC-Helan), and 26.7% (Helanal-Plus) of the helix set as kinked (Table 3). For each method, there are between 22 (7.3%) and 57 (19.0%) helices that are annotated as kinked but are classified as curved in our gold standard.

The Kink Finder annotation agrees well with the straight and kinked classifications, with only five helices (1.7%) annotated as kinked by the gold standard or Kink Finder and straight by the other. The helices classified as curved in our gold standard are roughly evenly split between kinked and straight by Kink



**Figure 5.** Ternary diagram showing the thresholds used to get the gold standard. Helices within the gray area were not classified into any group. Color scheme: blue, kinked; red, curved; yellow, straight; green, unassigned. An interactive version of this figure is available online.

Finder. Similarly, for the Kneissl annotation, only six helices (2.0%) are classified as straight by either Kneissl or our gold standard but kinked by the other (Table 3).

All but five of the 88 helices annotated as good by MC-Helan are in the straight group in the gold standard. However, the helices that MC-Helan identified as bent are split roughly evenly between the kinked, curved, and straight groups in the gold standard (Table 3). Many helices annotated as curved by Helanal-Plus are annotated as straight in the gold standard set (Table 3).

On average, the Kneissl annotation agrees with 61.3% of our participants responses, Kink Finder 58.1%, Helanal-Plus 53.1%, and MC-Helan 48.3%. Of the 48 helices (16.0%) that AHAH is unable to classify, there are only four cases where all the other methods give the same classification, and in over half of the cases (25), they give all three classifications (kinked, curved, and straight).

**Effect of Helix Length on Classification.** In our gold standard set, shorter helices are far more likely to be annotated as straight: of the 63 helices with fewer than 20 residues, 50 (79.3%) are annotated as straight (Figure 6). From 20 to 27 residues, the proportion of straight helices falls to nearly zero. Only four of the 64 helices longer than 27 residues (6.3%) are classified as straight. This relationship is also seen in the classifications of the four previous methods Kink Finder, Kneissl, MC-Helan, and Helanal-Plus (Figures S6–S9 in the Supporting Information).

The agreement with the majority view is also higher for shorter helices (Figure S10 in the Supporting Information). In particular, helices shorter than 20 residues are classified with very good agreement among participants. This suggests that straight helices are easier to classify.

**Kink Positions.** It is unclear from previous work whether a single kink residue is appropriate or if kinks are localized over several residues or even several turns of the helix. Among the 64 helices classified as kinked in the gold standard set, the selected kink residue varies from participant to participant. The most popular residue was selected by more than half of the participants for only three helices. The size of the variation of the chosen position differs between the helices. Looking at the standard deviation of the kink positions identified by the participants gives us a rough indication of the degree of localization of the kink.

The standard deviation of the kink position selected by the participants varies between 0.6 and 5.9 residues (Figure 7a). The distribution of standard deviations is unimodal with a median of 1.9, but it is skewed toward 0 and has a tail to the right. This indicates that there is no archetypal kink and that the degree of localization is not constant. The “average” kink is localized over a turn (3.6 residues) or thereabouts, and most are localized over no more than two turns. There is no correlation ( $r^2 = 0.08$ ) between the percentage of participants annotating the helix as kinked and the standard deviation (Figure 7b). This shows that more obvious kinks are not necessarily localized over a smaller region of the helix.

## DISCUSSION

### Disagreement among Current Helix Characterization Tools.

Kinks are widely thought to be important features of membrane<sup>2,3,5,6,9,14–16</sup> and soluble<sup>2,7,17</sup> proteins. However, their specific definition is subjective and changes from study to study. The four helix classifiers Kink Finder,<sup>2</sup> Kneissl et al.,<sup>6</sup> MC-Helan,<sup>9</sup> and Helanal-Plus<sup>4</sup> considered in this article provide a range of approaches to kink identification. They agree on the classification of only half of the helices in our tests. This disagreement is likely to be caused by a lack of a gold standard, which makes a performance comparison impossible. In this article, we provide a crowdsourcing-based gold standard aimed to be used in the training and comparison of computational methods.

**Crowdsourcing Can Tackle Difficult Problems.** Crowdsourcing has been used by a number of researchers to provide classifications for computationally difficult tasks that require a large amount of time. Researchers found for micro<sup>42,43</sup> and mega<sup>38–40</sup> tasks that a large number of citizens provide responses that are as good as, if not better than, expert classifications. Studies rarely need to apply acceptance criteria for the crowd data,<sup>44,45</sup> and weighting user responses has little effect on the classifications.<sup>42,43</sup> In agreement with this, we

**Table 3.** Percentage Overlaps between Our Gold Standard and Other Kink Identification Methods<sup>a</sup>

gold standard annotation <sup>b</sup>	Kink Finder		Kneissl			MC-Helan		Helanal-Plus		
	K	S	K	C	S	K	S	K	C	S
K	21.0	0.7	21.0	0.3	0.0	21.3	0.0	15.7	3.3	2.3
C	10.3	9.3	10.3	8.7	0.7	19.0	0.7	7.3	12.0	0.3
S	1.0	42.0	2.0	4.0	37.0	16.7	26.3	0.7	14.0	28.3
U	5.7	10.3	6.7	7.0	2.3	15.0	1.0	3.0	7.0	6.0

<sup>a</sup>Bold numbers indicate where the methods agree with our gold standard. <sup>b</sup>K, kinked; C, curved; S, straight; U, unassigned.

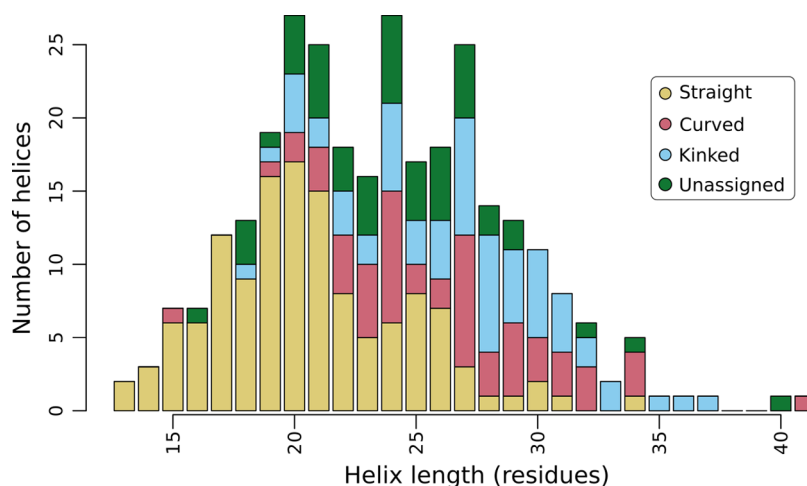


Figure 6. Helix annotations in the gold standard set, grouped by helix length.

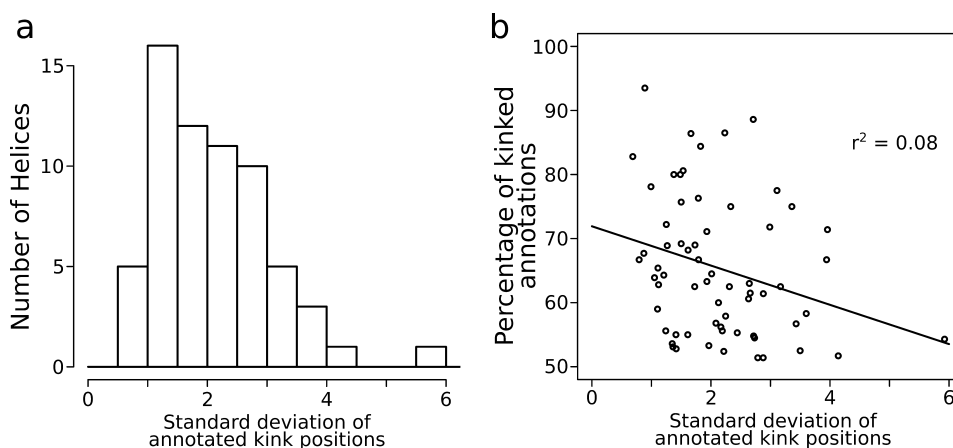


Figure 7. Variation of the standard deviation of kink positions identified by participants in helices: (a) distribution of standard deviations; (b) relationship between standard deviation and the percentage of participants that classified the helix as kinked.

found that specialists (i.e., structural biologists) are only slightly more consistent at classifying the helices than untrained individuals.

**Discrimination between Straight and Not-Straight Helices.** The classifications provided by our participants indicate that many helices do not fall into the clear classes of kinked, curved, and straight. There are a number of helices that are consistently classified as straight. There are also many helices which are very rarely classified as straight. However, there is no clear dividing line between a set of “kinked” helices and a set of “curved” helices. This could be due to the participants or our chosen definitions, but in view of the disagreements in the published computational methods, it is perhaps more likely to be due to the inherent nature of the helices.

Helix length has a strong influence on helix classification. In our gold standard set, short helices ( $\leq 20$  residues) are generally straight, while longer helices ( $\geq 27$  residues) are generally either kinked or curved. This is a common feature of all of the classification methods discussed here and replicates one of the findings of our recent study,<sup>2</sup> indicating that this is most likely the case because short helices are typically straight rather than being an artifact of the method used to identify kinks.

**Little Agreement in Exact Kink Position.** The difficulty in identifying kinks is also demonstrated by the kink positions

annotated by the participants. For some helices, the position is regularly annotated within a few residues, while in others it is identified over a larger range of residues. It is very rare for a single residue to be consistently identified as the site of a kink by either our participants or the previous methods. It is perhaps surprising that the variation in the kink position is not correlated ( $r^2 = 0.08$ ) with the proportion of participants that annotated the helix as kinked (i.e., a more obviously kinked helix is no indication of a more localized kink). This suggests a cause for the different residue patterns identified around kinks in previous studies.<sup>3,5,6,9</sup> For example, some studies have identified an enrichment in glycine residues at specific positions relative to the kink position,<sup>2,6,7,15</sup> but others have not.<sup>3,5,9</sup> It is likely that some of these contrasting results are due to small differences in the position of the kink identified by each method. In our recent study,<sup>2</sup> we suggested identifying a residue on the inside of the kink as the kink residue in order to improve the consistency of the choice of kink residue.

**A New Gold Standard Training Set.** Our participants classified a total of 300 helices. In 252, the agreement was above 50%. We suggest this subset as a high-quality gold standard set for the testing of future computational approaches. This set has a lower proportion of kinked helices than previous methods, which suggests that previous methods have been overly sensitive to helix distortions. We also provide the raw data

from our survey to allow researchers to produce their own gold standards with different criteria if they so wish.

## ■ ASSOCIATED CONTENT

### ■ Supporting Information

The gold standard data set (Table S1) and Supporting Figures S1–S10 mentioned in the text. This material is available free of charge via the Internet at <http://pubs.acs.org>. An interactive version of Figures 4 and 5 is available online at [http://opig.stats.ox.ac.uk/webapps/ahah/php/experiment\\_results.php](http://opig.stats.ox.ac.uk/webapps/ahah/php/experiment_results.php). A plain-text version of the gold standard data set and the raw annotations of the participants are available online at <http://www.stats.ox.ac.uk/research/proteins/resources#AHAAH>.

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### Notes

The authors declare no competing financial interest.

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