

*Discussion Letter*

## Depicting topology and handedness in jellyroll structures

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The jellyroll structure is a special case of the Greek key topology and, to date, has only been observed in complete form in one of its four possible arrangements. Like other elements of super-secondary structure involving the  $\beta$ -strand (e.g. the  $\beta\alpha\beta$  unit) the known structure forms a right-handed superhelix. The possibility of losing such tertiary information and other problems associated with representing these structures by two-dimensional topology diagrams are discussed. A series of rules are presented which allow this three-dimensional information to be represented in two-dimensional topology diagrams from which the handedness of a jellyroll structure can be determined.

Greek key; Protein topology; Jellyroll protein

### 1. INTRODUCTION

The majority of proteins which are largely made up of  $\beta$ -strands and sheets contain a unit of four strands which when represented in two dimensions by a flat 'topology diagram', resembles a design often seen on Greek vases [1,2] (Fig. 1). This element of super-secondary structure has become known as the Greek key [1]. Displaying the Greek key proteins in two-dimensional topology diagrams is problematic as information about the spatial relationship of the four strands can be lost. For example, in two layer structures, which predominate in the protein structural data base [3], there are three possible three-dimensional arrangements of the Greek key structure ([4,5], E.G.H. and J.M.T., manuscript in preparation). One of these, the *abcd* unit, appears to be common to all  $\beta$  sandwich type structures [2]. Further, it only appears as one of its two possible hands (Fig. 2). The right-handed type of the *abcd* unit is a feature of the jellyroll protein structure, or fold, which is common to a large number of proteins, notably those forming icosahedral viral capsids.

### 2. DISCUSSION

The jellyroll is most accurately described as an eight-stranded  $\beta$ -sandwich rather than a barrel as it has been frequently interpreted. A barrel implies that the hydro-

gen bonding pattern between adjacent strands remains unbroken around the entire structure; in effect, there is only one  $\beta$ -sheet. This is seen in the  $\alpha\beta$ , or TIM barrels and in the trypsin fold. However, in the jellyroll structures the hydrogen bonding pattern between the strands is broken in two places and, as a consequence, the structure comprises of two four stranded sheets. This is depicted in the triangle and topology diagrams of Fig. 3. Both sheets are purely anti-parallel, with strands adjacent in sequence appearing in different sheets with the exception of the fourth and fifth strands which are in the same sheet. This leads to a structure with only one hairpin, all other  $\beta\beta$  connections being arches (Fig. 3). A simple folding model has been suggested which is consistent with these structural features: the sequence is folded in two, producing a long hairpin which then folds upon itself to form a right-handed superhelix following the preferred twist of the  $\beta$ -sheet [1]. When such a structure is depicted in two dimensions the origin of its name becomes apparent; the structure resembles a swiss roll or (in North America) a jellyroll. The 'swirl' of the swiss roll is defined as the direction which the chain follows when being traced from the N-terminus in towards the central hairpin and is a property only of the two-dimensional diagrams (Fig. 4). The nomenclature of the strands is not intuitive, being based on the strand arrangement of the S-domain of Tomato Bushy Stunt virus; the first jellyroll structure to be solved [6]. In this structure an extra strand (strand a) precedes the N-terminal strand of the jellyroll and the jellyroll strands are consequentially labelled from b to i with strands e and f forming the hairpin.

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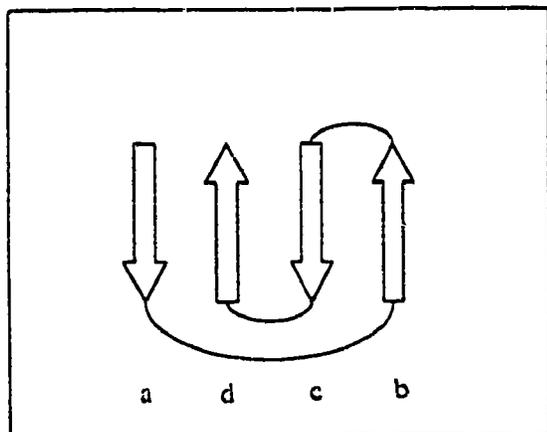


Fig. 1. Flat or topological representation of four  $\beta$ -strands linked with the Greek key topology. The strands are represented by arrows, loops and turns by curved lines.

### 3. DIFFERENT TYPES OF JELLYROLL

In theory there are four possible jellyroll folds [5]. Two of these have the observed right-handed superhelical twist (class I and class II) and others are left-handed (class I' and class II') (Figs. 2 and 3). The difference between the class I and II structures is in the positioning of the N-terminal strand. In the more common jellyroll (class I), this strand forms the edge of the first sheet, while in the class II structures it forms the second strand in the sheet and is thus hydrogen bonded on both sides, the C-terminal strand taking up the edge position (Fig. 3). In consequence, the class I and class II structures are not superposable [5]. To our knowledge, the class II structure has so far only been observed in an incomplete form in the P domain of Tomato Bushy Stunt virus [6] and the recently determined dUTPase structure [7]. In

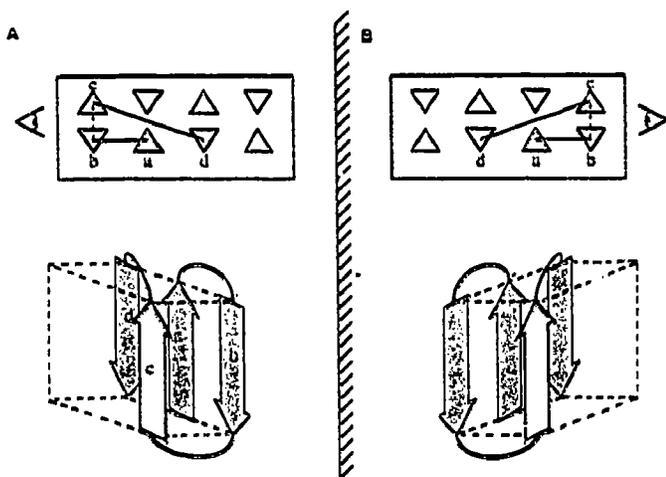


Fig. 2. This diagram relates the handedness of the *abcd* unit [2] to the triangle diagrams. The handedness is defined by the screw of the path of the chain through the *b*, *c*, and *d* strands. Case A is right-handed and is the one seen in protein structures.

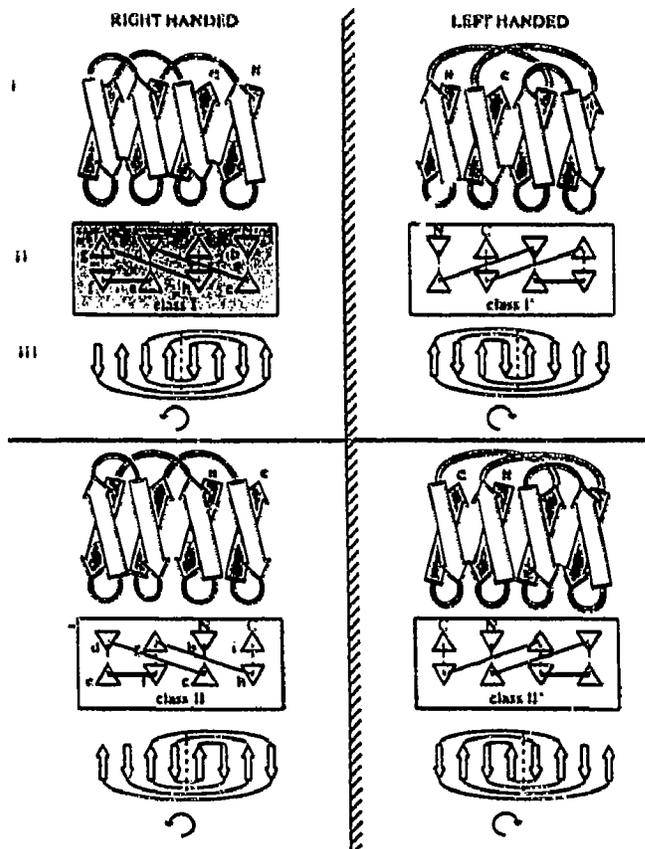


Fig. 3. The four possible jellyroll topologies. (i) Cartoons of the  $\beta$ -sandwich viewed from the side with arrows representing the strands. The hairpin is marked in black. (ii) Schematic representations of the  $\beta$ -sandwich viewed from above. The triangles represent the strands. Those with their apex pointing up represent strands which run out of the plane of the paper towards the observer. Connecting loops and turns between the strands are represented by straight lines. The solid ones are for connections that are at the top of the molecule and thus nearest to the observer and those that are broken are for connections at the bottom of the molecule. The N- and C-termini are marked accordingly. These diagrams depict the sandwich rather than the barrel nature of the jellyroll. (iii) Topology diagrams of the type introduced by Richardson [10]. In these the protein is viewed from the side. Rules for generating these diagrams are presented in the text and if followed the swirl of the jellyroll relates to the handedness of the three-dimensional structure. The swirl of these structures is represented by the curved arrow. The right-handed class I structure predominates in biology. Hence the box containing the structure is shaded.

these structures only six of the eight strands of the classic jellyroll structure are present. As a result of the different topology it is meaningless to align the sequences of the two types of structure as has been attempted by McKenna and co-workers [8].

### 4. THE HANDEDNESS OF JELLYROLL FOLDS

As with the  $\beta c \beta$  and  $\beta$ -coil- $\beta$  units only right-handed Greek key and, thus, jellyroll structures are observed in globular proteins. Fig. 2 describes how the handedness

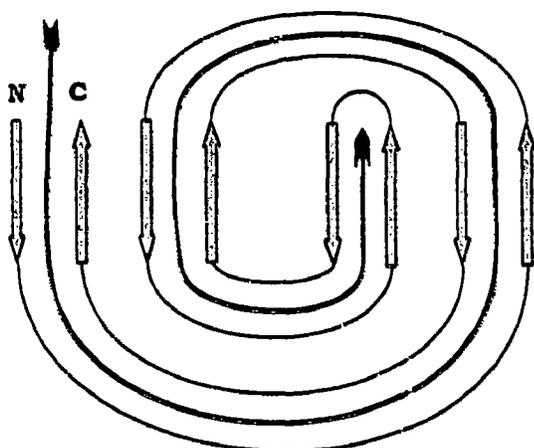


Fig. 4. A flat topology diagram of a jellyroll structure with the grey arrows representing the strands and the 'swirl' of the jellyroll shown by the dark arrow.

of an *abcd* Greek key structure can be determined: the principle is the same for other supersecondary units. First two parallel strands from the same sheet are chosen, in this case strands *b* and *d*. The intervening strand *c* is in the other sheet. Viewing this arrangement end on, as shown in Fig. 2, shows that the chain can follow a right-handed screw (right-handed superhelix) or left-handed screw (left-handed superhelix) running into the paper and through the structure. Note that, although the three-dimensional representation of the structures aids in the identification of handedness it is not necessary and handedness can be identified using the more schematic 'triangle' diagrams after Sternberg and Thornton [9]. Once the handedness of one of the units of a two layer  $\beta$ -structure has been defined, that of the whole structure is also defined. Thus, it is trivial to determine this handedness from three-dimensional diagrams or schematics of the type shown in Fig. 2. However, the question is: can this information be reliably and consistently translated into the popular two-dimensional topology diagrams of Richardson [1,10] (Fig. 3)?

## 5. DEPICTING JELLYROLL FOLDS

In three recent publications [7,11,12], the hydrogen bonding patterns between the strands seem to have been ignored when preparing the flat topology diagrams. This could be rationalised if the structure were a barrel and hydrogen bonding were contiguous around the whole structure. However, this is not the case in the observed jellyrolls which, as discussed above are two-layer structures. In our opinion, hydrogen bonding in the two sheets is important and the strands from the

same sheet are best drawn in a contiguous way across the paper in the topology diagrams. This has been done in Fig. 3, where the sandwich, rather than the barrel nature of the structure, is emphasised further by a broken vertical line drawn between the sheets. Although three-dimensional diagrams are always more accurate, the application of a few simple rules, listed below, allows reliable two-dimensional topology diagrams to be constructed which retain the salient features of the jellyroll protein fold.

(i) The sandwich should always be opened such that the strands are viewed from their outside, or hydrophilic, faces [1]. Although the choice is arbitrary, in the past this convention has usually been adopted but rarely explicitly stated.

(ii) The hairpin of the structure should be at the 'hinge' point of the opening and thus appears nearer to the centre of the topology diagram rather than at an edge.

(iii) All hydrogen bonded pairs of  $\beta$ -strands should appear next to each other in the topology diagram.

If these rules are followed for the jellyroll structures the relationship between the overall swirl of the topology diagram and the handedness of the structure is such that an anticlockwise swirl is equivalent to a right-handed superhelix in three dimensions. From observations made in the protein structural database to date this is the handedness expected in Greek key and jellyroll motifs to come.

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