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Antibody elbow angles are influenced by their light chain class

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Abstract

We have examined the elbow angles for 365 different Fab fragments, and observe that Fabs with lambda light chains have adopted a wider range of elbow angles than their kappa-chain counterparts, and that the lambda light chain Fabs are frequently found with very large (>195°) elbow angles. This apparent hyperflexibility of lambda-chain Fabs may be due to an insertion in their switch region, which is one residue longer than in kappa chains, with glycine occurring most frequently at the insertion position. A new, web-based computer program that was used to calculate the Fab elbow angles is also described.

Introduction

Antibodies are composed of two light (L; MW~25,000) and two heavy (H; MW~50,000) polypeptide chains that combine to form one Fc and two Fab modules that can be isolated as functional fragments by proteolytic cleavage of the intact immunoglobulin. Within each Fab fragment are two types of distinct structural domains termed variable (V_L,V_H) and constant (C_L,C_H1), with the amino acids linking V_L to C_L and V_H to C_H1 called 'switch' residues. Since the early 1970's, when Fab and light-chain dimer structures first became available, it was noted that these fragments displayed a variability in the angle between their variable and constant domains[1], referred to as the 'elbow bend' or 'elbow angle' and defined as the angle between the pseudo 2-fold axes relating V_L to V_H, and C_L to C_H1 (Fig. 1). While these early antibody structures sparked speculation that the elbow angle might change in response to ligand binding[2], no

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convincing data have since been found to support this theory. Rather, it appears that

the elbow angle may simply serve to increase Fab flexibility, thus enhancing the ability

of an antibody to bind bivalently to ligands arranged on a pathogen surface, such as a

virus or bacteria.

The Fab elbow angle (Fig. 1) is a useful descriptor of the overall topology of the Fab

fragment, serving as a measure for the relative disposition of the variable versus the

constant domains. The elbow angle is almost always cited in Fab structure reports that

include comparison of liganded versus unliganded Fab structures, and assessment of

Fab switch region flexibility. In order to simplify the elbow angle calculation, we have

developed a web-based program to more readily calculate the elbow angle for PDB-

formatted Fab coordinates. We have tested this method by calculating elbow angles for

536 Fab fragments from the PDB (of which 365 are non-redundant). The elbow angle

calculations are consistent with previous compilations but now clearly demonstrate that

the propensity for lambda (λ) light chains to assume elbow angles above 180° and

beyond 195° is significant compared to kappa (κ) light chains.

Materials and methods

Calculation of elbow angles

The elbow angle of a Fab antibody fragment is defined as the angle between the two

not necessarily intersecting pseudo-dyad axes (PDAs) relating the light (V_L) and heavy

(V_H) chain variable domains, and the light (C_L) and heavy (C_H) chain constant domains.

Small deviations in the exact locations of the PDAs arise depending on which residues

are used for their calculation; however, the resultant deviations for the values of the

elbow angles are limited usually to only a few degrees. It is standard practice to use only structurally-conserved residues from the antibody framework region for this calculation to eliminate errors due to differences in conformations of the complementarity determining regions (CDR). If the Fab coordinates used for the calculation have been numbered in a consistent way (such as the Kabat and Wu convention[3]), these structurally-equivalent residues are easily defined.   The calculation is then easy; however, most of the Fab structures deposited in the RCSB Protein Databank [4] are not numbered or labeled consistently, making such comparisons more difficult. However, the program we use for the V_L-V_H and C_L-C_H1 superposition (LGA[5]) also refines the sequence alignment between the domains, and, thus the superposition geometry does not critically depend on the numbering system used for the Fab.

However, there are additional fine points in the elbow angle calculation that need to be addressed. The elbow angle is calculated as the dot product of the V_L-V_H and C_L-C_H1 pseudo-dyad angle, and always computes between 0 and 180°. Although one could readily determine the absolute value in mathematical terms through the sign of the determinant of the basis matrix formed by the two pseudo-dyad vectors and their cross product vector, this approach does not overcome the problem. Due to the reduction of the information to a single scalar angle value, the relative orientation of the axes is lost, and the solutions become degenerate (imaginable as located on a cone) between 90° to 180°.   To regain the domain orientation on an absolute scale and to solve the complement ambiguity, one needs to compare each Fab to a 'standard' Fab with a defined elbow angle. In this case, we use unliganded Fab 8F5 (PDB code = 1bbd) as

the standard, with an elbow angle of 127°. The Fab to be tested is first superimposed via its variable domain (V_L-V_H) onto the variable domain of the standard Fab. From this V-aligned orientation, the constant domain (C_L-C_H1) of the test Fab is aligned with the constant domain of the standard Fab, yielding the rotation relative to 1bbd. The sum of the standard Fab's elbow angle (127°) plus the θ_3 domain rotation angle is then used to resolve the degeneracy and to determine whether the elbow angle calculated by the dyad dot product is direct or complementary (> 180°). This method has been extensively used in previous reviews and compilations[6].

Limitations and accuracy

The underlying assumption of the definition of the elbow angle is that the superposition of V_L onto V_H, as well as C_L onto C_H1, is predominantly a two-fold rotation. In this case, the directional cosine matrix resembles a pure two-fold rotation with relatively small non-diagonal components and the Euler axis is dominated by a correspondingly large principal component. In more than 95% of the Fabs that we examined, the assumption of near-twofold superposition operations is justified, and the elbow angle concept fully applicable.

If additional rotational components become significant in the domain superpositions, the Euler axes can deviate from the 2-fold to a point where the two axes become non-opposing and the calculated elbow angle is less than 90°. Even in these cases, a complement (180 - α) can be interpreted as the elbow angle, but such Fabs need to be individually examined to decide whether the elbow angle definition is still meaningful.

Although elbow angles tend to be reported to a precision of one decimal point, the choice of domain limits and superposition procedure places limitations on the absolute accuracy of the elbow angles. The superposition results can vary depending how the domain limits are defined, and depend on the alignment procedure used. With default domain limits ($V_L \leq L107 < C_L$ and $V_H \leq H113 < C_H1$, residues in Kabat numbering), we compared the elbow angles obtained using Kabat-renumbered Fab coordinates, and a fixed set of structurally-conserved residues for the superposition (using the program OVRLAP[7]) with those computed using our web application based on automated LGA alignments [5] and original numbering from the PDB. The angles computed using the different methods (n=167) agreed with a difference in their mean of -0.12° and a standard deviation of no more than 1.1°. It is interesting to note that molecular dynamics simulations of Fab domain movement in solution show periodic hinge bending fluctuations, with a 2-3° root mean square deviation (r.m.s.d.) in elbow angle[8]. The presence of such dynamic fluctuations indicates that the reported precision to a tenth of a degree in the elbow angles is, indeed, overly optimistic, whereas the differences in calculated elbow angles based on different domain superposition techniques are well within the range of the dynamic elbow flexibility. It would, therefore, seem reasonable that any difference in elbow angles below 2-3° should not be considered significant.

In addition, the extent to which crystal packing additionally affects or limits the observed elbow angles is uncertain. Several examples exist where two Fabs in the same crystallographic asymmetric unit display significantly different elbow angles, for example

1jnh (27° difference), 1s78 (22°) and 1ots (21°). However, even within the limits of accuracy discussed above, the elbow angles are still useful as measures of hinge flexibility and for classification purposes.

Analysis of elbow angles

Elbow angles for a total of 536 Fab fragments in 416 PDB entries have been calculated using the automated procedure described above. A non-redundant data set was created by omitting repetitions (such as one antibody in complex with many similar haptens), similar to a procedure used previously[9]. Fabs crystallizing in the same space group with cell constants within 1% and their elbow angles within +/-3° were considered equivalent, resulting in a non-redundant set containing 365 unique Fab structures (Table S1, supplemental material). The distributions of elbow angles (Fig. 2) are distinctly different depending on the Fab light chain type. Most of the elbow angles larger than 190° belong to the group of 33 unique λ chains in the test set (Table 1, Fig. 2). No correlation is apparent with heavy chain type (Table 1). The most frequent space groups for Fab crystal structures follow the general distribution analyzed previously for proteins [9; 10] with $P2_12_12_1$ (32%), $P2_1$ (29%), P1 (24%) and C2 (13%) the dominant space groups.

Implementation

The program RBOW is implemented on a Linux platform (Apache web server) using a combination of Perl scripts and standard Fortran90 code. The alignment program used is the local-global alignment program LGA[5], which assures minimal dependence of the

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results on the Fab numbering convention used. The program allows upload of PDB format coordinate files, selection of heavy and light chain identifiers, and input of domain boundaries, for which reasonable default values are provided. During the calculation as described above, the program checks for format errors and issues warnings at several levels for unexpected or borderline behavior. Warnings include large coordinate r.m.s.d.'s on superposition (>3.5Å), significant deviations from pseudo-twofold rotation axes, occurrence of parallel superposition axes, and swapped annotation of L and H chains. The superposition files are available for upload and visual inspection, if desired. Coordinates may be downloaded to http://as2ts.llnl.gov/AS2TS/RBOW/ for the elbow angle calculation.

Discussion

The majority of Fab fragment structures in the RCSB Protein Database (PDB)[4] have kappa (κ) light chains, with only 37 lambda (λ)-chain Fab PDB depositions (as of May 2005), half of which have been submitted since the year 2000. This paucity of λ chain Fab structures reflects their lower abundance, particularly in mice where the antibody light chain repertoire is about 5% λ and 95% κ[11], whereas in humans about 40% of the repertoire is λ. Also, the number of mouse structures far outweighs their human counterparts (of 416 PDB entries, 305 were murine and 51 human, with the rest chimeric or humanized, rat(4) or hamster(1). Our results show that of the 12 unique human and 21 unique murine or hamster Fab structures with λ light chains, about 60% have elbow angles greater than 180°, with 11 instances of elbow angles greater than 195° (Table 1). The largest elbow angle found was 227°. In contrast, the vast majority

of the κ light chains have elbow angles less than 180°, with a maximum elbow angle in this group of 196° (Table 1). Note that while a large percentage of λ chain Fab structures have elbow angles greater than 180º, λ chain Fabs can also display elbow angles as small as 117°, the smallest elbow angle found in this study. Thus, λ chain Fabs are not restricted to elbow angles >180°, but rather they are more able to assume larger (>195º) elbow angles compared to κ chain Fab fragments (Table 1, S1). A 2-way Analysis of Variance (ANOVA) of light chain type and heavy chain class reveals only the previously described significant correlation of elbow angle preference with light chain type, and no correlation with heavy chain class. There are insufficient data to allow a discrimination by species, as this distribution is predominantly represented by mouse Fabs.

Fab elbow angles were also examined to see if differences existed between the elbow angles of liganded and unliganded Fabs (Table S2). There are 61 Fabs in the dataset with structures for both their liganded and unliganded forms, many of these determined with multiple haptens, or in multiple crystal forms. Of these 61 Fabs, 38 show differences of less than 5° in elbow angle between the liganded and unliganded forms, with another 15 Fabs showing differences of between 5-20°. The largest elbow angle deviation was seen for the germline 48G7 (1AJ7 and 2RCS) with a 66° difference between the liganded and unliganded Fab. Since not all Fabs have different elbow angles for their free and bound forms, it seems likely that such changes are due to the inherent flexibility of the Fab, or to different crystal packing environments, as liganded and unliganded Fabs frequently crystallize as different crystal forms.

Amino acid sequence differences arise in the 'switch' regions of λ versus κ light chains, including an inserted residue (L106a; Kabat numbering) in the sequence of λ light chains (Fig. 3, Table 2). However, comparison of switch region structures from λ and κ light chains shows that residues L106a(λ) and L107(κ) are in fact structurally equivalent, as are L108(λ) and L108(κ), with residue L107(λ) corresponding to the insertion in the λ chain (Fig. 3, Table 2), as defined by structural analysis. In λ chains, residue L107 is usually a Glycine (78.9% Gly, 13.4% Ser, 4.5% Arg from 960 λ sequences; 34 Gly, 1 Ser, 2 Arg in the 37 λ PDB entries). Switch regions are usually well ordered in Fab crystal structures, with only a few exceptions in the Fabs analyzed here, including the Fab with the smallest elbow angle (1jnh) which has no visible electron density for the switch region in any of the four Fabs in its asymmetric unit. The distributions of main-chain torsion angles for residues L105-L109 in the switch region are displayed in Fig. 4 for all (λ and κ) Fab structures studied. Torsion angles are clustered fairly tightly for residues L105, L106, and L109, with more spread for L106a, L107, and L108, indicating that most of the elbow variation can be attributed to these residues. Comparison of κ and λ chain switch regions from Fabs with extreme elbow angle values (Fig. 5) shows that large elbow angle differences in κ chains are manifested through consecutive small, sequential torsion angle changes around residues L105-L107, while λ chain Fabs exhibit a more abrupt torsion angle change centered around residues L106a, L107 and L108. In this particular example, the largest difference between the two λ structures is at residue L107.

Early pioneering studies by Lesk and Chothia [12] based on the available Fab crystal structures (only five at that time) had led to a proposal that Fab fragments could not achieve elbow angles greater than 180°. In these five Fab crystal structures (New, McPC603, KOL, J539, and HyHEL5)[13-17], five conserved residues in the heavy chain were involved in key contacts between the V_H and C_H1 domains. These residues formed what was termed a flexible 'ball-and-socket' joint, with Phe^{H148} and Pro^{H149} (Kabat numbering) serving as the 'ball' that inserted into a socket formed by residues Leu/Val^{H11}, Thr^{H110}, and Ser^{H112} (Fig. 6). No such conserved contacts or interactions were found between the V_L and C_L domains of these structures. Despite different elbow angles in the Fabs, the five ball-and-socket residues always maintained physical contact, although they changed position slightly with respect to one another. Lesk and Chothia proposed that Fabs could not attain elbow angles of greater than 180° because the ball-and socket contacts would be lost in these extreme conformations[12]. However, in 1993, two crystal structures of an anti-chelate Fab (1ind, 1ine) [18] unexpectedly revealed that Fab fragments could indeed adopt elbow angles greater than 180°, which in this case were ~194° for the two different ligand complexes. Examination of the ball-and-socket region from structures of Fabs with vary large elbow angles shows that, as predicted, the ball-and-socket residues are not in contact (Fig. 6d), although they remain fairly close in space. Thus, maintenance of the 'ball-and-socket' contacts are not required, at least for large elbow angle Fabs.

Conclusions

We have developed an easy to use, web-based service to calculate the elbow angle of

a Fab fragment in a PDB format, and demonstrated its utility by rapidly calculating

elbow angles for 536 Fab fragments in the PDB. The results show excellent agreement

with previous compilations of Fab elbow angles[6]. The distribution of elbow angles is

bimodal (Fig. 2), with the largest elbow angles (>195°) only found in Fabs with λ light

chains (the largest elbow angle seen for a κ light chain is 196°). These structural

differences may be due to an additional residue (L107) in the λ chain switch region that

perhaps allows for more flexibility. The L107 insertion is usually a glycine, which also

can provide more conformational freedom. It is not clear whether Fabs with λ light

chains bend or flex around their elbow angle in solution more than other Fabs.   Indeed,

it is not certain how much elbow angle flexibility any particular Fab exhibits in solution.

Molecular dynamics studies would indicate that elbow angle fluctuations of several

degrees are common at least for κ-chain Fabs in solution[8]. The availability of additional

λ-chain Fab structures in the future will likely allow for further refinement of our analysis.

From a practical standpoint, the knowledge that λ chain Fabs tend towards large elbow

angles may be useful to consider for the crystallographer carrying out molecular

replacement structure determinations of these Fabs.

Acknowledgements

Table 1. Descriptive statistics* of non-redundant elbow angle distributions in kappa and lambda chain type Fabs.

antibody light chain type	N	< 180º	> 180º	> 195º	mean	median	skew
κ	332	290 (87%)	42 (13%)	1 (0%)	156º	150º	+0.4
λ	33	14 (42%)	19 (58%)	11 (33%)	178º	185º	- 0.4
antibody heavy chain class							
IgG1	239	203 (85%)	36 (15%)	8 (4%)	156º	150º	+0.7
IgG2a	79	63 (79%)	16 (21%)	0	158º	154º	+0.1
IgG2b	22	19 (86%)	3 (14%	0	158º	151º	+0.8

* in addition to the moment-based statistics listed above, F-tests, 2-factor ANOVA (light chain type, heavy chain class) and non-parametric Spearman rank tests confirm that no significant correlations in the data exist beyond the preference of λ type light chains to adopt large elbow angles.

Table 2. Amino acid preferences in elbow regions of kappa and lambda light chains. Lambda chains bulge out at residue L107, so that kappa L107 and lambda L106a are structurally-equivalent positions.

kappa	lambda
Leu/Val L104	Leu/Val L104
Glu/Asp L105	Thr L105
Ile/Leu/Val L106	Val L106
Lys L107	Leu L106a
	Gly L107
Arg L108	Gln L108
Ala/Thr L109	Pro L109

Figure legends

Figure 1. Superposition of a variety of Fabs with different elbow angles. Fabs from PDB files 1bbd (red), 7fab (yellow), 1dba (green), 1plg (cyan), and 1nl0 (blue) have been superimposed on their variable light chain regions. The range of elbow angles is shown from small (1bbd, 127°; 7fab, 132°;) to around 180° (1dba, 183°) to large (1plg, 190°; 1nl0, 220°).

Figure 2. Distribution of elbow angles for κ and λ chain type Fabs. The distinct preference of λ chains to adopt large elbow angles is clearly displayed. Bin numbers indicate the highest value of the corresponding bin.

Figure 3. Switch regions from λ and κ light chains. The switch residues from λ (1ggc, light blue) and κ (1nj9, pink) light chains with similar elbow angles have been superimposed. λ chains have an extra residue in the region (L106a by Kabat numbering); however, residue L107 (λ) is in fact the structural insertion, with residues L107(κ) and L106a(λ), and L108(κ) and L108(λ) being structurally equivalent.

Figure 4. Ramachandran plots for residues in the light chain switch regions.   Plots for residues L105 (a), L106 (b), lambda L106a/kappa L107 (c), lambda L107 (d), L108 (e), and L109 (f). These plots combine both λ (pink) and κ (blue) chains, except for (d), where L107 is a structural insertion found only found in λ chains.

Figure 5. Comparison of extreme elbow angles from κ and λ chains. (a) The switch region residues from κ light chain Fabs 1bbd (127°, light blue) and 1plg (190°, blue) have been superimposed. This 63° difference is achieved by small movements around residues L105, L106, and L107. (b) Switch region residues from λ light chain Fabs 7fab (130°, light pink) and 1nl0 (220°, pink). This 90° difference is characterized by differences around residues L106a, L107 and L108, with the largest difference at residue L107. 1bbd and 1plg (mouse, IgG2a), and 7fab and 1nl0 (human, IgG1) have the same light and heavy chain constant region types.

Figure 6. Ball-and-socket joint. The entire Fab fragment and a close-up of the residues that contribute to the heavy chain ball-and socket joint are shown for Fabs with extreme elbow angles from both the κ and λ light chain class. (a) 1bbd; 127° elbow angle, murine IgG2a, κ (b) 1plg; 190° elbow angle, murine IgG2a, κ (c) 7fab; 130° elbow angle, human IgG1, λ (d) 1nl0; 220° elbow angle, human IgG1, λ.
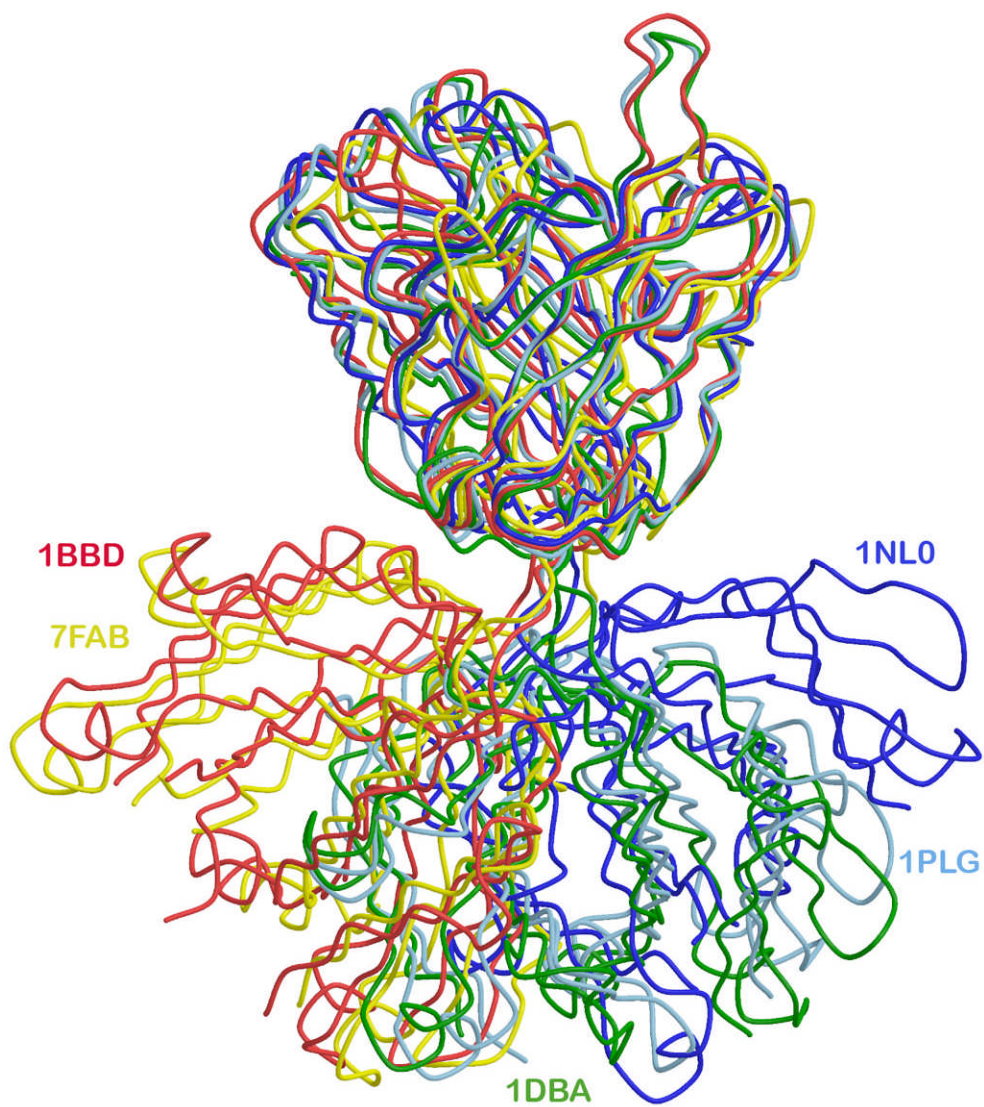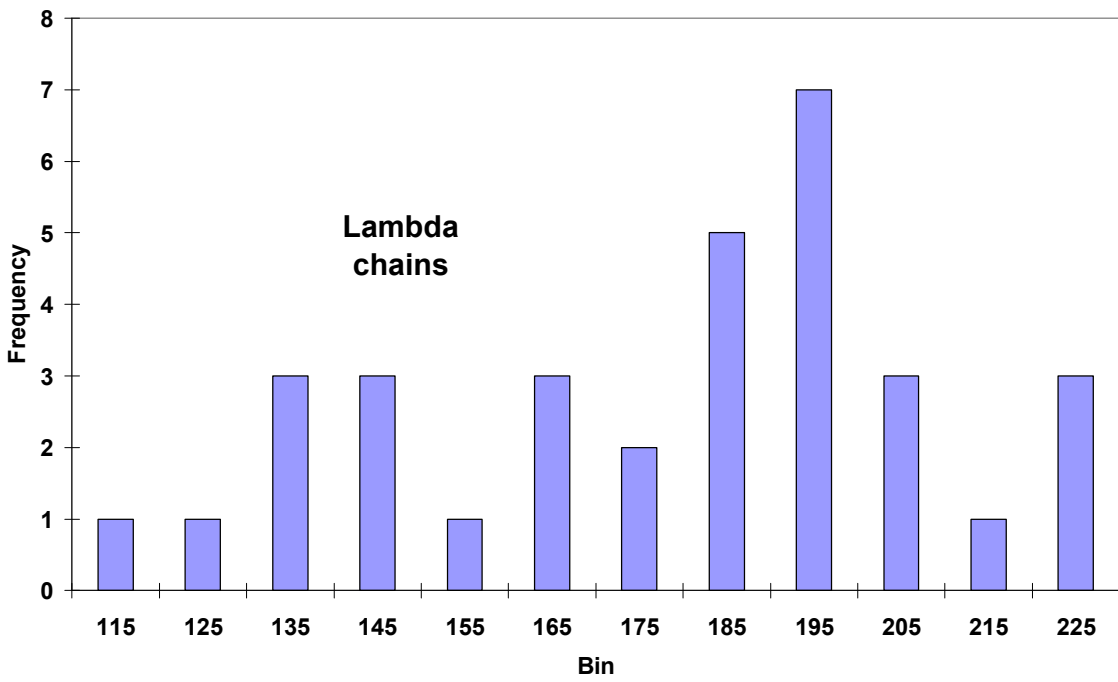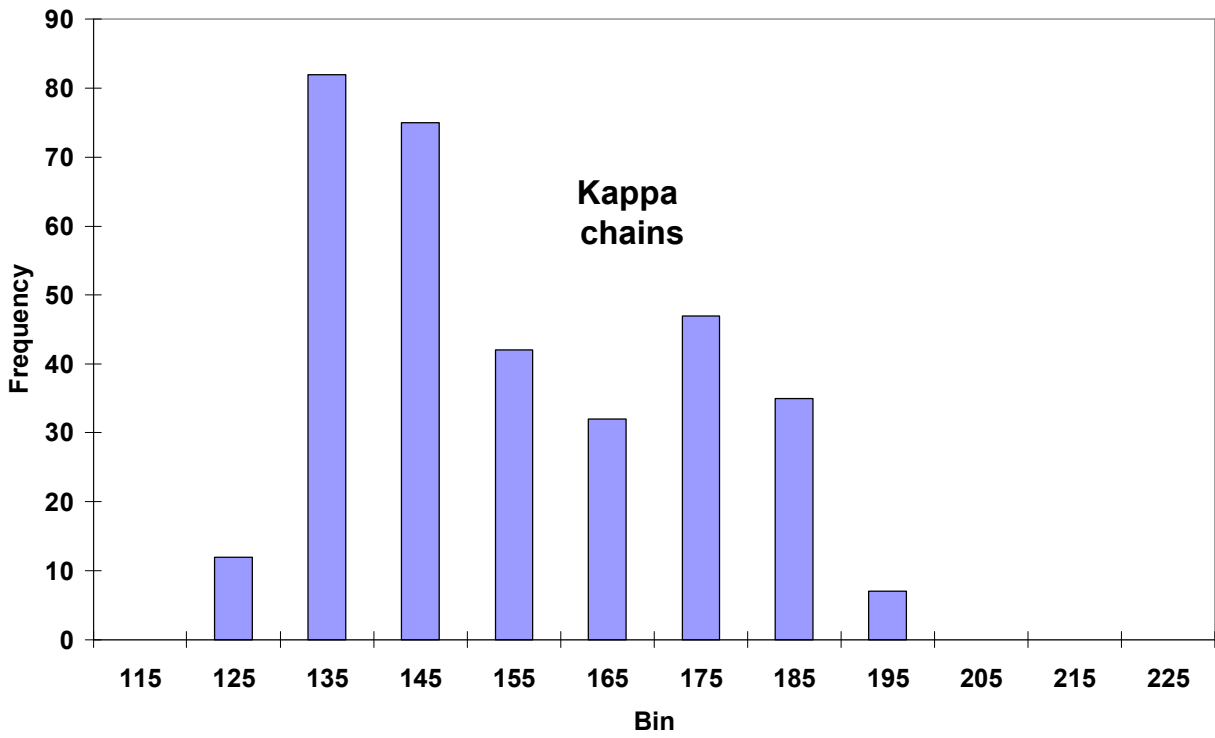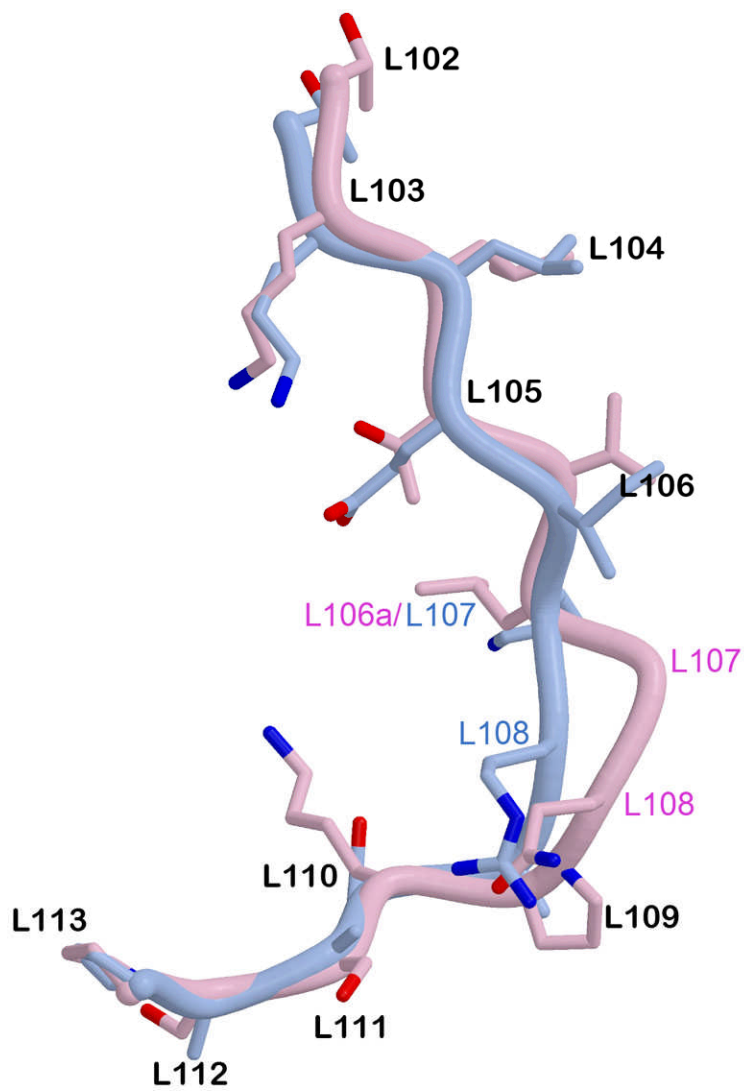
Figure 1

Figure 2

18

Figure 3

Figure 4

Figure 5

1bbd - 127°

1plg - 190°

7fab - 130°

1nl0 - 220°

a.

b.

c.

d.

T110
L11
S112
P149
F148

T110
L11
S112
F148
P149

T110
L11
S112
P149
F148

T110
V11
S112
F148
P149

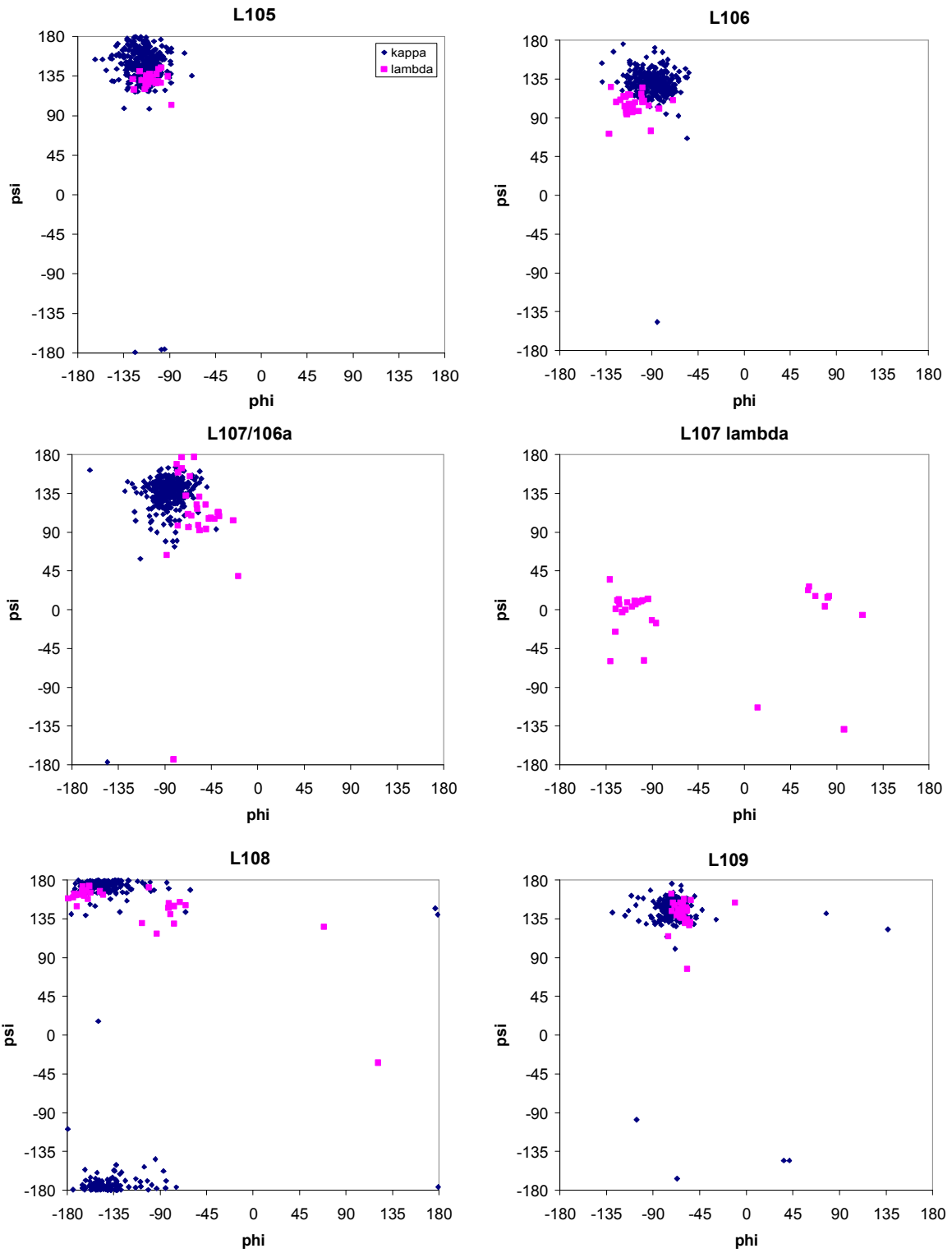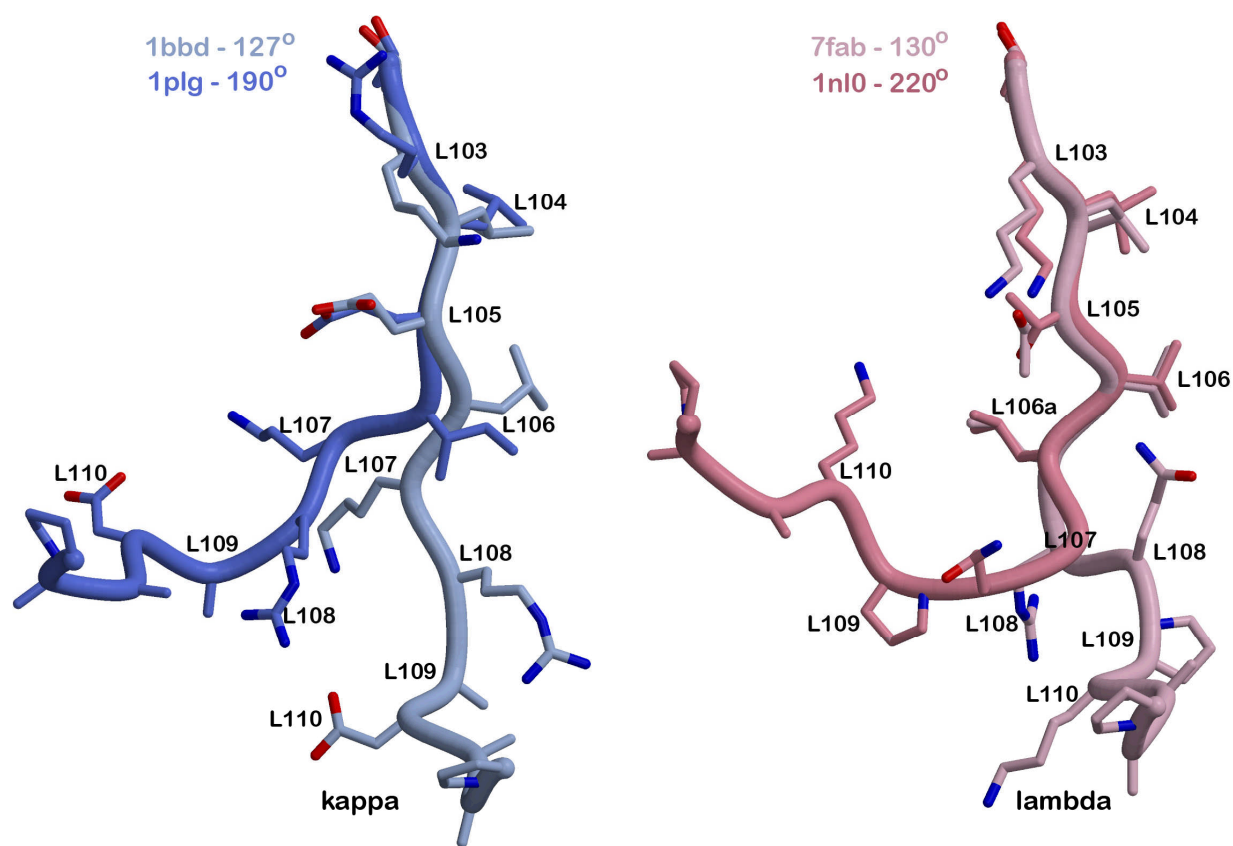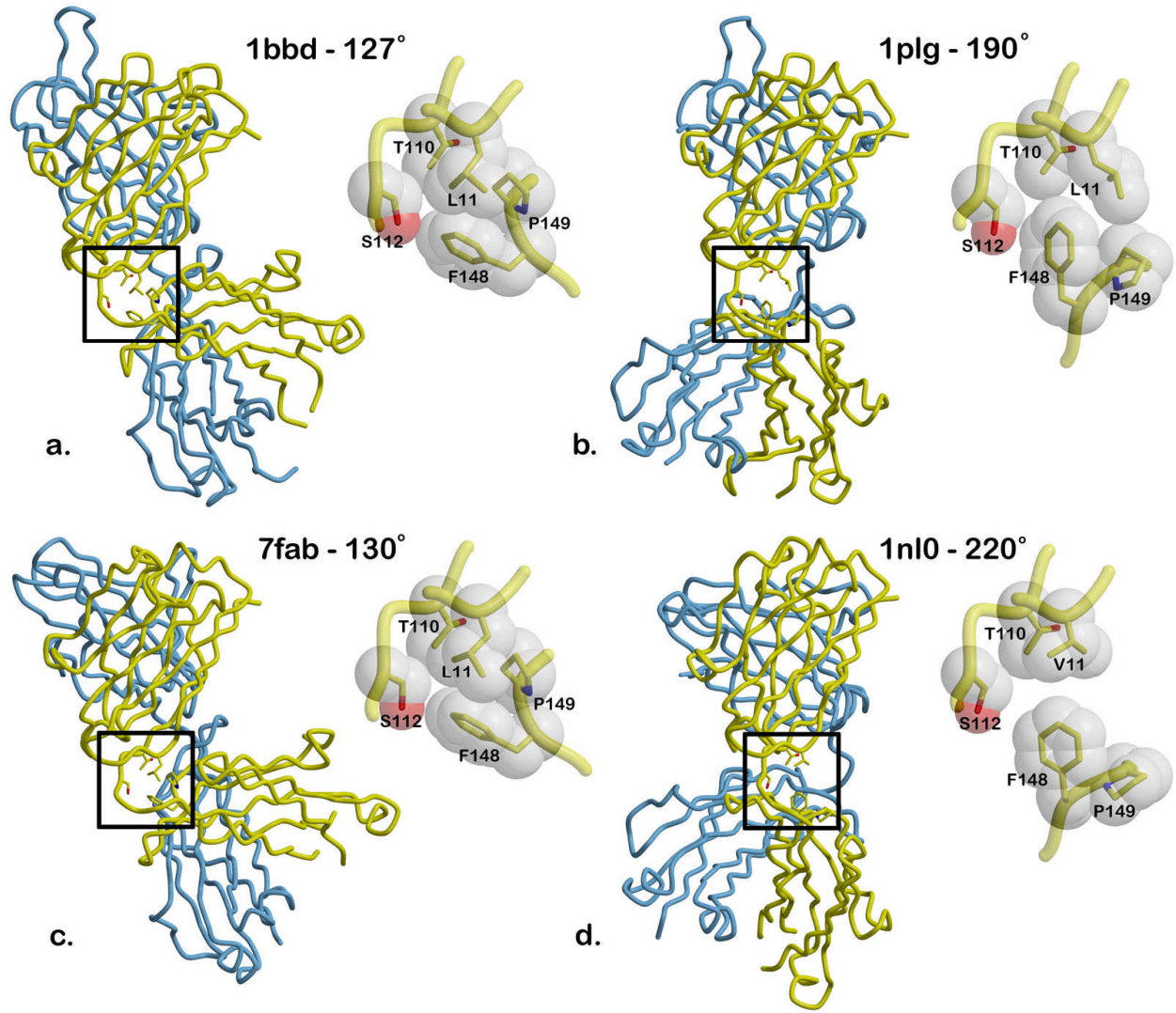**Figure 6**

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