Rituximab is a widely used monoclonal antibody drug for treating certain lymphomas and autoimmune diseases. To understand the molecular mechanism of recognition of human CD20 by Rituximab, we determined the crystal structure of the Rituximab Fab in complex with a synthesized peptide comprising the CD20 epitope (residues 163–187) at 2.6-Å resolution. The combining site of the Fab consists of four complementarity determining regions that form a large, deep pocket to accommodate the epitope peptide. The bound peptide assumes a unique cyclic conformation that is constrained by a disulfide bond and a rigid proline residue (Pro172). The 170ANPS173 motif of CD20 is deeply embedded into the pocket on the antibody surface and plays an essential role in the recognition and binding of Rituximab. The antigen–antibody interactions involve both hydrogen bonds and van der Waals contacts and display a high degree of structural and chemical complementarity. These results provide a molecular basis for the specific recognition of CD20 by Rituximab as well as valuable information for development of improved antibody drugs with better specificity and higher affinity.

CD20 is a pan-B cell marker expressed from pre-B cells until B cells are differentiated into plasma cells (1). It is a tetraspan membrane protein that is predicted to contain a large extracellular loop (about residues 142 to 182) and to form oligomers on the cell surface (2–4). Although the precise function of CD20 remains unclear, biochemical and cell biological data have shown that it seems to form or regulate a voltage-independent calcium channel (3, 5). Despite the limited knowledge about its function, several lines of evidence have clearly demonstrated that CD20 is an ideal target for passive immunotherapy of B-cell lymphoma: it is highly expressed in more than 80% of the B-cell lymphomas but not in stem cells, pro-B cells, normal plasma cells, or other normal tissues; it remains on the cell surface without substantial internalization after cross-linking with antibodies; and it is not shed to the circulation to inhibit the antibody therapy (6–8).

The CD20-targeted chimeric monoclonal antibody (mAb)3 Rituximab (Rituxan®, IDEC-C2B8) was the first Food and Drug Administration approved mAb drug for the treatment of malignancy. Although it was originally used for treating low-grade non-Hodgkin lymphoma, Rituximab has been proven to be also effective against other types of lymphomas (9, 10) and some autoimmune diseases (11–13). Multiple mechanisms have been proposed for the action of Rituximab in the depletion of B cells including its ability to mediate complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity, and to induce cell apoptosis (reviewed in Refs. 14 and 15). With the expansion of the clinical application of Rituximab in the treatment of lymphoproliferative diseases, it has been noticed that intensity of CD20 expression on B cells varies in patients that may affect the binding and efficacy of Rituximab therapy (16, 17). Therefore, it is rational to expect that new antibodies with higher affinity and better specificity developed based on Rituximab might be beneficial in clinical use, especially for patients who have low expression levels of CD20.

Besides Rituximab and Zevalin® (the prototype of Rituximab 288 attached by a radioactive substance90Y), another mAb specific to human CD20, namely B1, in both native and radiolabeled forms (Bexxar®, Tositumomab and 131I-Tositumomab), was approved by the Food and Drug Administration for the treatment of non-Hodgkin lymphoma in 2003 (18). These antibody drugs along with some other mAbs against CD20 such as 1F5, AT80, and 2H7 were suggested to most likely recognize the same region (Tyr122 to Tyr125) of the large extracellular loop of human CD20 with fine specificities (2, 19). However, these antibodies vary considerably in their functional activities. For example, treatment of B cells with most mAbs promotes segregation of CD20 into detergent-insoluble...
ble lipid raft, whereas B1 is the exception. This property seems to be correlated with the ability of the antibodies to mediate complement-dependent cytotoxicity but irrespective of activation of cell apoptosis pathways (20–22).

To understand the functional diversity of CD20 mAbs and the underlying mechanisms, identification of the epitope of CD20 recognized by Rituximab and other CD20-targeted mAbs has raised great interest in recent years. Sequence comparison of human and murine CD20 reveals that although the two species share 73% sequence identity, the large extracellular loop is less conserved as 16 of the approximate 43 amino acids are different (19). Mutagenesis studies by exchanging variant residues of the large extracellular loop between human CD20 and mouse CD20 at the equivalent position indicate that residues Ala170 and Pro172 of human CD20 are critical determinants for the CD20 epitope (19). Using a process of biopanning of a phage display peptide library consisting of randomized 7-mer cyclic peptides, it has been shown that an NPS motif corresponding to 171NPS173 of human CD20 is essential for Rituximab binding and Ala170 can be substituted by Ser (4). Similar studies have also defined a discontinuous epitope that comprises 170ANPS173 and 187YCYS185 of CD20 joined together spatially by a disulfide bond between Cys187 and Cys183 (23). However, the underlying mechanism of the recognition and binding of Rituximab with the CD20 epitope remains unclear.

We report here the crystal structure of the Rituximab Fab fragment in complex with an epitope peptide of the large extracellular loop (residues 163–187) of CD20 that provides the molecular basis for the antigen-antibody recognition and binding. Analysis of the complex structure explains very well biochemical data from the epitope-mapping studies and provides useful hints for the design and development of improved antibody drugs.

EXPERIMENTAL PROCEDURES

Preparation of Antibody and Peptide—Rituximab was purchased from Roche. The Fab fragment was obtained by papain digestion of Rituximab and purified by cation exchange chromatography using SP-Sepharose FF (GE Healthcare) followed by hydrophobic interaction chromatography using phenyl-Sepharose HP (GE Healthcare). The purity and homogeneity of the Fab fragment was characterized by SDS-PAGE and dynamic light scattering analyses. The protein sample was concentrated to 8 mg/ml and then exchanged into a stock buffer (150 mM NaCl and 10 mM Tris-HCl, pH 8.0) for crystallization. The protein sample was concentrated to 8 mg/ml and then exchanged into a stock buffer (150 mM NaCl and 10 mM Tris-HCl, pH 8.0) for crystallization. The protein sample was concentrated to 8 mg/ml and then exchanged into a stock buffer (150 mM NaCl and 10 mM Tris-HCl, pH 8.0) for crystallization. The protein sample was concentrated to 8 mg/ml and then exchanged into a stock buffer (150 mM NaCl and 10 mM Tris-HCl, pH 8.0) for crystallization. The protein sample was concentrated to 8 mg/ml and then exchanged into a stock buffer (150 mM NaCl and 10 mM Tris-HCl, pH 8.0) for crystallization.

Crystallization and Diffraction Data Collection—Initial crystallization trials of the Rituximab Fab fragment itself yielded large crystals that, however, did not diffract x-ray beyond 7-Å resolution and could not be used for structure determination. For co-crystallization experiments, the purified Rituximab Fab and the epitope peptide were mixed at a molar ratio of 1:5 at 4 °C for 12 h. Co-crystallization was carried out using the hanging drop vapor diffusion method by mixing equal volumes of the protein/peptide mixture solution and a reservoir solution (0.2 M calcium acetate, 0.1 M sodium cacodylate, pH 6.5, and 18% PEG8000). Square shaped crystals grew to a maximum size of 0.1 × 0.1 × 0.2 mm³ at 4 °C in 2 weeks. For diffraction data collection, crystals were cryostabilized by Paratone-N (Hampton Research) and then flash-cooled to −170 °C. Diffraction data were collected to 2.6-Å resolution at beamline NW12 of Photon Factory, Japan, and processed using suite HKL2000 (24). Statistics for diffraction data are summarized in Table 1.

Structure Determination, Refinement, and Analysis—The structure of the Rituximab Fab in complex with human CD20 epitope peptide was solved using the molecular replacement method as implemented in Phaser (25). The structure of the Fab fragment of human mAb A5B7 (26) (Protein Data Bank code 1AD0) was used as the search model. Structure refinement was carried out by CNS using standard protocols consisting of conjugate-gradient energy minimization, torsion-constrained molecular dynamics simulated annealing, group B factor refinement, and individual B factor refinement (27). Free R factor was calculated using 5% randomly selected reflections. After several cycles of manual model building using program O (28), the electron density was further improved and clear enough for tracing the epitope peptide without ambiguity. The stereochemistry of the structure model was analyzed with Procheck (29). Statistics of the structure refinement are also summarized in Table 1. Structural analysis was mainly performed using CNS (27) and programs in the CCP4 suite (29). The elbow angle of the Fab fragment was calculated with the method described by Stanfield et al. (30). Figures were prepared using programs Ribbons (31) and Pymol (32).

RESULTS AND DISCUSSION

Overall Structure of the Rituximab Fab-CD20 Epitope Peptide Complex—Recent studies have identified the epitope of CD20 recognized by Rituximab being a sequence motif located at the large extracellular loop of CD20 consisting of 170ANPS173 (4, 19, 23). To understand the structural basis of the recognition of CD20 by Rituximab, we synthesized a 25-mer peptide mimic of the epitope of CD20 comprising the CD20 sequence from Asn162 to Gln187 (numbered according to the CD20 sequence). To mimic the conformation of the CD20 epitope, an intrachain disulfide bond was introduced between residues Cys167 and Cys183 of the synthesized peptide because such linkage has been found in human CD20 expressed in Escherichia coli and probably exists naturally (33). This disulfide linkage has also been implicated to play an important role in the recognition and binding of the epitope by Rituximab because disruption of the disulfide bond abolishes the binding of CD20 to Rituximab and reconstruction of the disulfide bond can partially restore the binding (33). The peptide was conjugated to a protein carrier keyhole limpet hemocyanin and shown to have similar reactivity with Rituximab as the keyhole limpet hemocyanin-
Conjugated cyclic peptides Rp15-C and Rp3-C (4) by the enzyme-linked immunosorbent assay binding assay (supplementary Fig. S1A). Specificity of the reactivity was also confirmed by peptide blocking assays with immunofluorescence and complement-dependent cytotoxicity using Raji cells (supplemented by peptide blocking assays with immunofluorescence). The CD20 epitope peptide was co-crystallized in complex A (side chains of Fab and epitope peptide) (Fig. 1). The CD20 epitope peptide was co-crystallized in complex A (side chains of Fab and epitope peptide) (Fig. 1). Due to the better electron density quality, the structure model of complex A will be used for further structural analysis and discussion. The CDR loops L3, H1, H2, and H3 of the Rituximab Fab (Fig. 2A), which is consistent with the observation that the heavy chain CDRs usually make more contributions than the light chain CDRs in antigen binding especially when Fab binds with a small antigen (38, 39). The binding of the epitope peptide with the Fab buries a solvent accessible surface area of about 440 Å² (calculated with a probe radius of 1.4 Å) which is about 23% of the peptide surface (1911 Å²) or 2.3% of the Fab surface (19403 Å²). Although the buried surface area is within the average range of the protein-peptide complexes (400–700 Å²) (40), the peptide fits the CDR regions of the Rituximab Fab quite well with a high degree of structural and chemical complementarity (Fig. 2B) as indicated by the high shape complementarity value (Sc) of 0.83 (calculated with default parameters) compared with the average Sc value of 0.64–0.68 for antibody-antigen complexes (41).

Overall, the Rituximab Fab has very good electron density, except residues from SerH135 to GlyP148 of a loop in the constant region that have high B factors above 80 Å² compared with the average B factor of 49 Å² for the whole model. The Rituximab Fab has a canonical immunoglobulin fold consisting of four β-barrel domains (Fig. 1A). The light chain comprises residues Leu1 to Leu213 that fold into the V_L and C_L domains, and heavy chain residues His1 to His224 that fold into the V_H and C_H domains. The elbow angle made by the two pseudo 2-fold axes that define the relative disposition of V_H to V_L and C_H to C_L is about 139°. There are four intra-domain disulfide bonds between CysL223 and CysL187, CysL133 and CysL193, CysH196, and CysH1148 and CysH1209, and one inter-domain disulfide bond between CysL213 and CysH224. Like other Fab structures, residue Thr50 of the light chain (ThrL50) lies in the disallowed region of the Ramachandran plot and forms part of the classic γ-turn (34). The complementarity determining regions (CDRs) of the Rituximab Fab have ordinary length without unusual residues according to Kabat sequence data base searching (35). The CDR loops L1, L2, L3, H1, and H2 belong to Chothia canonical classes (34) 1, 1, 1, 1, and 2, respectively. The CDR loops L3, H1, H2, and H3 together form a large, deep pocket to accommodate the epitope peptide, whereas loops L1 and L2 are located behind loops L3 and H3 (Fig. 1A).

The electron density for the bound epitope peptide is well defined without ambiguity in the positioning of the main chains and side chains (Fig. 1B). Residues CysP167 and CysP183 of the peptide form a disulfide bond that drags the termini of the peptide together covalently and makes the peptide to adopt a unique cyclic conformation (Fig. 1C). The C-terminal part of the peptide (CysP167 to AsnP171) forms a short coil that is stabilized by the tenuous restraints of the disulfide bond and the hydrogen-bonding interactions between residues of the coil and the CDRs of the Rituximab Fab. The middle part of the peptide forms a short 3_10 helix (ProP172 to GluP174) and a small loop (LysP175 to SerP177) that are stabilized by hydrogen-bonding interactions with the other regions (Table 2 and Fig. 1C). The C-terminal part of the peptide (ProP172 to TyrP174) forms a short α-helix of hydrophobic nature (TyrP172, TyrP174, and IleP175) (38, 39). Considering it is at the end of the extracellular loop and must be close to the cell membrane, this short α-helix might be the extension of a long transmembrane α-helix of CD20 as predicted by PredictProtein Server (36).

Interactions between the Rituximab Fab and the Epitope Peptide—Rituximab can bind to CD20 on B cells with a binding affinity of 5 nM (37). In the complex structure, the epitope peptide of human CD20 is bound at the large pocket formed by CDR loops L3, H1, H2, and H3 of the Rituximab Fab (Fig. 2A), which is consistent with the observation that the heavy chain CDRs usually make more contributions than the light chain CDRs in antigen binding especially when Fab binds with a small antigen (38, 39). The binding of the epitope peptide with the Fab buries a solvent accessible surface area of about 440 Å² (calculated with a probe radius of 1.4 Å) which is about 23% of the peptide surface (1911 Å²) or 2.3% of the Fab surface (19403 Å²). Although the buried surface area is within the average range of the protein-peptide complexes (400–700 Å²) (40), the peptide fits the CDR regions of the Rituximab Fab quite well with a high degree of structural and chemical complementarity (Fig. 2B) as indicated by the high shape complementarity value (Sc) of 0.83 (calculated with default parameters) compared with the average Sc value of 0.64–0.68 for antibody-antigen complexes (41).

The CDR loops L3, H1, H2, and H3 of the Fab participate in interactions primarily with four residues, 170ANPS173, of the epitope peptide that have been shown to be a critical motif on
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A

B

C
the CD20 surface for antibody recognition (2, 4, 23). Residues of the motif form a network of hydrogen bonds with residues of the surrounding CDR loops (Table 3 and Fig. 2, C and D). Specifically, the side chain of AsnP171 forms two hydrogen bonds with the side chains of SerH99 and TrpH106 of the H3 loop, respectively. The main carboxyl of ProP172 forms a hydrogen bond with the side chain of AsnH33 of loop H2 via a water molecule; and the main chain amide and side chain O\(^{-}\) of SerP173 make two hydrogen bonds with the side chain of AsnH33 of loop H1, respectively. Moreover, the residues flanking the motif including GluP168, ProP169, and LysP175 also contribute to the interactions of the peptide with the Fab by forming hydrogen bonds with AsaL93, SerH59, and ThrH58, respectively (Table 3 and Fig. 2, C and D). In addition to these hydrogen-bonding interactions, extensive van der Waals contacts are observed between residues 168 and 179 of the peptide and the Fab (Table 4). In particular, the 170ANPS173 motif contributes 79 of the 97 van der Waals contacts of the Rituximab Fab contacts between the peptide and the Fab (Table 4). These interactions can partially explain the high affinity of Rituximab with human CD20.

In addition, ProP172 of the 170ANPS173 motif that has been shown to play an essential role in the antigen-antibody recognition (4, 19), is located at the bottom of the CDR pocket (Fig. 2B) formed by residues AlaL50, TyrH52, AspH57, and SerH59 of loop H2 of the Fab and residue AsnH33 of loop H1, and has both hydrophilic and hydrophobic interactions with the surrounding residues of the Fab. The special position of ProP172 at the turn of the 3\(_{10}\) helix and the rigid conformation of proline might play an important role in maintaining the unique conformation of the peptide and hence in the recognition and binding of Rituximab.

**Structural Basis for the Specificity of Rituximab—Human CD20 is an important drug target for the treatment of lymphomas. Rituximab is the first Food and Drug Administration approved mAb drug against CD20 for the treatment of B cell non-Hodgkin lymphoma, and now has been used to treat autoimmune diseases and reduce the alloreaction in organ transplantations. Our crystal structure of the Rituximab Fab epitope peptide complex, with a 25-mer peptide mimicking the epitope on the large extracellular loop of human CD20 and provided a molecular basis for understanding the underlying mechanisms of the recognition and binding of CD20 with its antibodies and will be valuable in the modification of Rituximab for development of more effective mAb drugs against non-Hodgkin lymphoma and other diseases.

Screenings of phage display peptide libraries that can express 7-mer cyclic and 7-/12-mer linear peptides have shown that the sequence motif A(S)NPS corresponding to 170ANPS173 of the large extracellular loop of human CD20 is the most important region for Rituximab recognition and binding (4, 23). In particular, Ala170 and Pro172 are the most critical ones determined by means of phage display, mutagenesis, and peptide scanning (2, 4, 19). In our structure of the Rituximab Fab-CD20 epitope-peptide complex, four residues 170ANPS173 of the motif are deeply buried in the pocket formed by four CDR loops (L3, H1, H2, and H3). Ala170 is located in a hydrophobic cavity formed by TrpL47, TrpH90, AsnH93, and ProH95 of the Fab. The space of the cavity is too narrow to accommodate other residues with a large side chain except serine due to steric conflict (Fig. 2B), providing an explanation for the result that only serine is interchangeable with Ala170 (4). Similarly, Pro172 is positioned at the tip of the 3\(_{10}\) helix and is bound at the bottom of the CDR pocket (Fig. 2B). Modeling study indicates that a serine residue could fit at the same site. However, the relaxation of the rigid conformation of ProP172 might disrupt the conformational constraint of the 3\(_{10}\) helix and hence reduce the specificity and binding affinity, which explains the result that when ProP172 was substituted by serine in the cyclic 7-mer peptide, the mutant peptide could not bind to Rituximab (4).

The importance and requirement of the two other residues, Asn171 and Ser173, of the motif are also supported by our crystal structure showing that each residue makes two of total eight hydrogen bonds as well as extensive van der Waals contacts with the Rituximab Fab. In the structure model, six residues (Glu168, Pro169, Asn171, Pro172, Ser173, and Lys175) of the epitope peptide make hydrogen-bonding interactions, and additionally Ala170, Glu174, Asn176, and Ser179 make van der Waals contacts with the Rituximab Fab. Although only 170ANPS173 of CD20 were documented to be involved in the Rituximab binding, the other residues might also play some roles in the recognition and binding of CD20 by Rituximab.

Based on phage display results, it was suggested that fragment 182YCYSI186 at the C terminus of the large extracellular loop of CD20 is also involved in Rituximab binding (23). In our structure, this region forms part of the C-terminal \(\alpha\)-helix and has no direct interaction with the Fab. However, residues CysP163 and CysP167 of the epitope peptide form a disulfide bond that makes the peptide adopt a unique cyclic conformation. In search for the epitope of CD20 with phage display peptide libraries, a series of cyclic and linear peptides were obtained; however, only the cyclic peptides match the sequence of human CD20 (4). Biochemical data have shown that disruption of the disulfide bond on the large extracellular loop of

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**TABLE 2**

| Hydrogen-bonding interactions between the middle part of the peptide and the other regions of the peptide |
|---|
| **Donor** | **Acceptor** | **Distance** |
| Glu174-N | Asn171-O | 3.08 |
| Ser173-O | Glu174-O | 2.93 |
| Lys175-N | Pro172-O | 3.15 |
| Ser177-N | Glu174-O | 2.69 |
| Ser177-O | Thr176-O | 2.76 |

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**FIGURE 1.** Overall structure of the Rituximab Fab-CD20 epitope-peptide complex. A, overall structure of the complex. The Rituximab Fab is colored with the light chain in yellow and the heavy chain in green, and the CD20 epitope peptide in cyan. B, a stereoview of a composite-omit electron density map at 2.6-Å resolution for the bound epitope peptide contoured at 1.0-σ level. The atomic coordinates of the peptide residues are shown in ball and stick models. C, structure of the bound epitope peptide. The peptide consists of a short N-terminal coil (residues 167–171), a 3\(_{10}\) helix (residues 172–174), a small loop (residues 175–177), and a short C-terminal \(\alpha\)-helix (residues 178–184). The intra-peptide hydrogen-bonding interactions between residues of the middle part (the 3\(_{10}\) helix and the small loop) and the other parts of the peptide are indicated with dashed lines.
FIGURE 2. Interactions between the Rituximab Fab and the epitope peptide. A, an overview showing the interactions of the epitope peptide with the Rituximab Fab. The Fab CDRs are shown with the H1 loop in orange, H2 in green, H3 in tinted green, L1 in gold, L2 in pink, and L3 in purple. The peptide is colored in cyan. The four CDR loops (H1, H2, H3, and L3) of the Fab form a pocket to accommodate the epitope peptide. B, an electrostatic potential surface of the Rituximab Fab in the region of the epitope peptide binding pocket showing the structural and chemical complementarity between the Fab and the bound peptide. The residues of the epitope peptide involved in interactions with the Fab are shown with ball and stick models. The ANPS motif of the CD20 epitope is located in a pocket formed by CDR loops H1, H2, H3, and L3 of the Fab. C, a stereoview showing the hydrogen-bonding interactions between residues of the epitope peptide and CDR loops H1 and H3 of the Fab. D, a stereoview showing the hydrogen bonding between the epitope peptide and CDR loops H2 and L3 of the Fab.
GluP174 and/or hydrophilic interaction with the side chain of such as Lys might form a salt bridge with the side chain of AsnP176. Similarly, substitution of TyrH102 with a basic residue might form potential hydrogen bonds with the side chain of AsnP171 and GluP174.

AsnP171 and GluP174. Change of AspH57 to Glu could generate more hydrophilic interactions with the side chain of LysP175 and/or AsnP176. Mutation of AspH55 to Gln might form potential hydrogen bonds with the side chain of AsnP176. Similarly, substitution of TyrH102 with a basic residue such as Lys might form a salt bridge with the side chain of GluP174 and/or hydrophilic interaction with the side chain of SerP179. Substitution of TrpP172 with a basic residue like Lys might form new hydrophilic interactions with the side chain of GluP168 and/or LysP175. Change of AspH57 to Glu could generate more favorable interactions binding affinity. Changes of residues on the CDR loops of the Fab and recognized by residues of the CDR loops through a network of hydrogen-bonding interactions and extensive van der Waals contacts. The unique cyclic conformation of the epitope peptide, which is attributed to the formation of a disulfide bond between CysP176 and CysP178 and the presence of a rigid ProP172 forms the basis of the specificity of Rituximab. Our structural results also provide useful hints for the development of new therapeutic antibodies with higher binding affinity and better specificity for the treatment of non-Hodgkin lymphoma.

Table 3: Hydrogen-bonding interactions between the Rituximab Fab CDRs and the epitope peptide

| Peptide atom | Fab atom | CDR loop | Distance |
|--------------|----------|----------|----------|
| GluP168-O    | AsnL93-N | L3       | 2.94     |
| ProP169-O    | SerH59-O  | H2       | 2.75     |
| AsnP171-O    | SerH59-O  | H3       | 2.79     |
| AsnP171-N    | TrpH59-N  | H3       | 3.10     |
| ProP172-O    | AsnH53-N  | H2       | 2.66/2.97 |
| SerP173-N    | AsnH33-N  | H1       | 2.93     |
| SerP173-O    | AsnH33-N  | H1       | 3.06     |
| LysP175-N    | ThrH58-O  | H2       | 3.12     |

* Hydrogen bond mediated by water molecule. The number before the slash is the distance between the Fab atom and the water molecule, and the number after the slash is the distance between the water molecule and the peptide atom.

Table 4: van der Waals contacts between the Rituximab Fab CDRs and the epitope peptide (≤4.0 Å)

| Peptide residues | Rituximab Fab residues |
|------------------|------------------------|
| GluP168          | AsnH33 (4), SerH59 (1) |
| ProP169          | AsnH33 (2), SerH59 (2) |
| AlaP170          | TrpH59 (1), AsnH33 (4), ProH59 (1), HisH53 (1), TrpH57 (2), AlaH50 (1), SerH59 (3) |
| AsnP171          | TrpH59 (6), AsnH33 (8), HisH53 (2), SerH59 (2), TrpH56 (18) |
| ProP172          | AsnH33 (4), AlaH50 (4), IleH51 (2), TyrH52 (1), AspH57 (3), ThrH58 (1), SerH59 (2) |
| SerP173          | AsnH33 (7), TyrH52 (6) |
| GluP174          | TyrH52 (6), TrpH56 (2) |
| LysP175          | AspH57 (1) |

* Numbers in parentheses refer to the number of van der Waals contacts.

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