Structural Basis for APPTPPPLPP Peptide Recognition by the FBP11WW1 Domain

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WW domains are small protein–protein interaction modules that recognize proline-rich stretches in proteins. The class II tandem WW domains of the formin binding protein 11 (FBP11) recognize specifically proteins containing PPLPp motifs as present in the formins that are involved in limb and kidney development, and in the methyl-CpG-binding protein 2 (MeCP2), associated with the Rett syndrome. The interaction involves the specific recognition of a leucine side-chain. Here, we report on the novel structure of the complex formed by the FBP11WW1 domain and the formin fragment APPTPPPLPP revealing the specificity determinants of class II WW domains.

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Introduction

WW domains are protein–protein interaction modules forming three-stranded antiparallel β-sheet structures.1,2 They recognize peptides containing proline-rich motifs (Figure 1).5,9 A number of structures revealed the mechanism of proline recognition and specificity achievement.6–8 From these structural investigations and ligand binding studies employing peptide libraries it became apparent that peptides as short as five residues may bind with an affinity of 60 μM to WW domains,9 utilizing hydrophobic interactions and few hydrogen bonds. As initially observed for SH3 domains, the proline-rich ligands can bind in different orientations to WW domains.7,8

The WW domain containing formin binding proteins (FBPs) were originally identified by screening of mouse limb bud expression libraries for binders of a conserved proline-rich region present in formin isoforms. One of the candidates was formin binding protein 11 (FBP11), which is, as most FBPs, localized in the cell nucleus and assumed to be connected to pre-mRNA splicing. It possesses a
modular architecture comprising two WW domains followed by several FF domains.10,11 FBP11 WW domains are capable of binding the sequence APPTPPPLPP present in most formin isoforms, and further functional screening of cDNA expression libraries have identified eight putative ligands for FBP11 WW domains (WW domain binding proteins, WBP-3 to WBP-10). These proteins contain proline-rich sequences including leucine residues. Among these proteins, WBP-5 and WBP-8 comprise the longest poly-proline sequence, PPPPPPPLPP. Analysis of the proline-rich region within the identified WBPs resulted in the consensus sequence PPLP, defined as the binding motif of class II WW domains.12

The network of possible physiologically relevant interactions involving FBP11 is still emerging and in the past years the proteins Huntingtin,13,14 neural Wiskott–Aldrich syndrome protein (N-WASP),15 and MeCP216 (WBP-10 homologous protein) were shown to be able to interact with FBP11. Huntingtin and MeCP2 are proteins related to the human genetic disorders Huntington's disease and Rett syndrome, respectively. N-WASP plays a role in actin cytoskeleton reorganization. It was demonstrated that the FBP11 WW domains and proline-rich regions in these proteins are essential for their interactions. Formins and the protein MeCP2 contain the WW domain II binding motif PPLPp, whereas in N-WASP and Huntingtin the motif PPLPp is absent or replaced by long poly-P stretches. Substitution analysis of the peptide APPTPPPLPP has shown that the mutation L to P reduces the strength of the interaction significantly. In this case, specificity towards peptide ligands with the motif PPLPp is governed by hydrophobic interactions involving a residue other than proline.15 This result shows that specificity depends on the shape of a hydrophobic amino acid, a rare situation in the field of protein–peptide interactions.

Of special interest in this context is the complex of the FBP11 WW1 domain and the peptide APPTPPPLPP. The new complex structure reported here is the first involving a WW domain of the class II subfamily and expands our understanding about the mechanism of recognition of proline-rich motifs adopted by WW domains.

Results and Discussion

Binding constants and epitope mapping

To substantiate the contribution of WW domain interactions to the formation of complexes involving FBP11 we determined dissociation constants for the complex formed by FBP11 WW1 domain and the peptides GPPPPPPPLPP, GPPPPPPPP (WBP-5/WBP-8), APPTPPPLPP (formin) and the L to P mutants GPPPPPPPPP and APPTPPPPPP (Table 1 and Figure 2(a)). The micromolar constants obtained are in agreement with the constants recently determined by other authors using the surface plasmon resonance technique for the FBP11 WW1 domain and proline-rich peptides.17 The weak interactions determined here are typical for protein–protein interaction domains recognizing proline-rich motifs and could be related to the formation of transient complexes or may be supported by other interactions to form tight complexes.

Additionally, the dependence of the binding constants on pH was investigated. For the peptide

<table>
<thead>
<tr>
<th>Peptide</th>
<th>$K_D$ (µM)</th>
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<tbody>
<tr>
<td>GPPPPPPPLPP</td>
<td>145 ± 4</td>
</tr>
<tr>
<td>GPPPPPPPPP</td>
<td>242 ± 23</td>
</tr>
<tr>
<td>GPPPPPPPLPP</td>
<td>226 ± 7</td>
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<tr>
<td>APPTPPPLPP</td>
<td>371 ± 22</td>
</tr>
<tr>
<td>APPTPPPPPP</td>
<td>473 ± 54</td>
</tr>
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Table 1. Dissociation constants of complexes formed by the FBP11 WW1 domain and proline-rich peptides


The solution structure of the FBP11WW1 domain is shown in Figure 3(a) and the structure of its complex with the peptide APPTPPPLLP in Figure 3(b)–(d). The free domain structure was derived based on 851 experimental restraints and is well defined from residues 15–42. For detailed structure statistics see Table 2. FBP11WW1 shows the well-known WW fold. A hydrophobic cluster on one side of the β-sheet is formed by the highly conserved residues W17, Y29 and P42. On the other side W39, Y28 and S37 are conserved in most WW domains, and the variable residues T18, H20, S22, P23, D24 and Y30, form the binding surface for the proline-rich peptide.

Loop 1 is composed of residues P23, D24 and G25, and its structure is less conserved. It shows a backbone root-mean-square deviation (rmsd) value of 3.8 Å and 4.4 Å to the loops in the WW prototype and YAP65, respectively. In our structure, the position of loop 1 is defined by several nuclear Overhauser enhancements (NOEs) observed between W39 and loop residues P23 and D24. Equivalent NOEs were not observed for the class I WW domain of YAP65.

By addition of the ligand APPTPPPLLP, perturbation of W39 chemical shifts is observed and many
of the NOEs observed in the free state between W39 and residues P23 and D24 disappear. These results suggest that complex formation involves a smooth accommodation of the domain surface during ligand binding. This hypothesis was confirmed by the determination of the structure of the complex, where loop 1 no longer deviates significantly from other WW domain structures.

The calculation of the complex structure was supported by 40 intermolecular NOEs, 15 of them were manually assigned and a further 25 NOEs automatically using ARIA. The ligand was restrained to a poly-proline type II left-hand helical conformation (PPII), as observed for other complexes involving proline-rich ligands. However, similar results could be obtained without restraining the ligand conformation using only the 40 intermolecular NOEs (data not shown). Seven of the manually assigned NOEs occurred between L80 and Y28, W39, S22 and P23. These restraints define precisely the position and orientation of L80 with respect to the binding surface. Eight further NOEs assigned involved proline residues: between P5' and H20; between P6' and Y30; between P9' and S37/Y28; and between P10' and W39. The complete structure statistics can be found in Table 2.

Starting the inspection of the complex at the N-terminal residues of the ligand, we see that the ring of P6' is situated perpendicular to the β-sheet, equidistant from Y30, T18, Y28 and H20 (Figure 3(c)). These four residues form a hydrophobic groove on the surface of the domain where the ring of P6' is buried (Figure 3(d)). The hydrophobic nature of this interaction explains the dependence of the complex dissociation constant on pH: at low pH, His20 will be protonated, increasing the polar character of the recognition site, thus destabilizing the complex. The P6' carbonyl group is also

Figure 3. Structure of the FBP11 WW1 domain and of its complex with peptide APPTPPPLPP. (a) Stereo view of an ensemble of the 15 best structures selected for the FBP11 WW1 domain. β-Strand regions are colored blue and loop regions are orange. Selected side-chains are displayed and labeled. (b) Stereo view of an ensemble of the 15 best structures selected for the FBP11 WW1:APPTPPPLPP complex. The domain was colored as for (a) and the ligand was colored green. (c) Top view of a representative structure showing the residues involved in binding. (d) Ligand bound to the surface of the FBP11 WW1 domain. The surface was colored by hydrophobicity. Blue represents hydrophilic, green represents intermediate and brown represents hydrophobic regions. H-bonds are displayed in yellow.
Table 2. Structure statistics

<table>
<thead>
<tr>
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<th>WW alone</th>
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<tr>
<td>A. Number of restraints</td>
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<tr>
<td>Intra-residual</td>
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<tr>
<td>Intermolecular</td>
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<td>40</td>
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<td>40</td>
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<tr>
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<td>33</td>
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<tr>
<td>Total</td>
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<td>742</td>
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<tr>
<td>B. rmsd from experimental restraints</td>
<td>0.025 ± 0.001</td>
<td>0.016 ± 0.002</td>
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<tr>
<td>NOEs (Å)</td>
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<td>1.9 ± 0.1</td>
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<td>C. CNS potential energy (kcal mol⁻¹)</td>
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<td>328 ± 6</td>
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<td>E_vdw</td>
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<td>E_nonbonded</td>
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<td>E_vdw</td>
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<td>Residues in disallowed regions</td>
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<td>E. rmsd (Å) between average structure and selected set of structures</td>
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<tr>
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<td>0.89 ± 0.08</td>
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<td>WW domain residues 15–42+ligand residues 5'–10'</td>
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<tr>
<td>Backbone</td>
<td>0.37 ± 0.11</td>
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<tr>
<td>All non-H</td>
<td>0.82 ± 0.07</td>
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Involvement in H-bonding to the Y28 hydroxyl group. The ring of P7' is positioned parallel with the β-sheet, contacting the surface of the domain. The P7' carbonyl group forms a hydrogen bond involving the S37 hydroxyl group.

The second recognition site in FBP11WW1 is formed by Y28 and W39. It is conserved in all WW classes. As determined from the intermolecular NOEs, Leu8' of the class II binding motif PPLPp is placed at this binding site. The methyl groups of L8' contact S22 and P23 located in loop1 and Y28. One methyl group is directed to the surface of the domain. The P7' ring of P9' is positioned parallel with the β-sheet and parallel with the W39 indole ring. The carbonyl groups of P9' and P10' may form a bi-furcated H-bond involving the W39 indolic NH.

The structure of the complex FBP11WW1: APPTPPLPPPLP is in agreement with our binding constants measurements, epitope mapping and previous substitution analysis data. The residues that form the minimal ligand epitope are P6', P7', L8', P9' and P10'. From our structure we can see that all these five residues are involved in interactions with the WW domain, whereby P10' shows only contacts involving backbone atoms. The ring of P10' is directed towards the solution. P10' contributes mainly as an H-bond acceptor via its carbonyl group and is thus found to be essential for higher affinity in the length analysis, and in our binding constant measurements, but unspecific in the substitution analysis. P5' seems to be already outside the epitope. In the length analysis it does not seem to improve the affinity significantly, and the substitution analysis does not show specificity at this position. Accordingly, in the complex structure the ring of P5' is directed towards the solution and does not seem to contribute directly to the interaction. Residues P6', P7', L8' and P9' show high specificity in the interaction with the WW domain and in all cases the respective side-chains contact the WW domain surface. The clearest contributions to specificity are due to P6' and P9'. These residues are in equivalent positions of consecutive turns of the PPII helix. Their proline rings make extensive hydrophobic contacts to Y30 and/or Y28 in the case of P6' and to W39 in the P9' case.

The specificity for leucine at position 8' instead of Val, Ile or Ala is less obvious, but a possible factor contributing to this specificity is the shape of the
A proline side-chain at position 8 would not fit as perfectly as a leucine residue to the surface of the domain without distorting the PPII helix conformation or without changing other interactions, explaining the lower affinity shown by the L to P mutants (Table 1). A rationalization of the importance of proline in position 7 is less straightforward. The ring of P7 is positioned parallel with the β-sheet, contacting the surface of the domain. Its δ methylene group is close to the aromatic ring of Y30 making further hydrophobic contacts. This could be one contribution to the specificity for proline at this position, besides the stabilization of the PPII helix.

**Comparison of complexes of different WW families and SH3 domains**

Comparisons of the FBP11WW1:APPTPPLPP complex with other WW and SH3 complexes are shown in Figure 4(a)–(f). Several conserved features can be derived from these comparisons, and even more interesting are the differences between the complexes, which are responsible for specificity. The summary of these comparisons is shown in Figure 4(a). This Figure shows a ribbon representation of a typical WW domain fold, with a top view of the binding site. Conserved residues involved in binding are displayed with their side-chains in blue, while the position of key variable residues, responsible for subfamily...
specificity are in green, and only β-carbon atoms are shown.

Figure 4(b) compares the structure of the complex FBP11WW1:APPTPPPLPP with the complex dystrophinWW:SPPPY. FBP11WW1 makes use of two hydrophobic grooves for recognizing its proline-rich ligand. One groove is common to all WW domains, it is the one formed by W39 and Y28. These residues are very conserved in WW domains of all classes. The second hydrophobic groove is formed by T18, H20, Y30 and Y28, and is spatially distinct from the specificity recognition site of dystrophin, which uses residues I30, H32 and Q35 of the second and third WW domain β-strands for recognizing a tyrosine in the ligand. This Y recognition site is conserved in all domains that recognize the PPxY motif but is absent from the FBP11WW1 domain. Interestingly, the aromatic residue at position 30 superimposes well with the tyrosine of class I PPxY ligands, as seen in Figure 4(b). As a consequence, all WW domains possessing an aromatic residue at position 30 should be unable to bind to the PPxY motif, and should use instead a similar second hydrophobic recognition site as in FBP11WW1 for binding the ligand. This principle was suggested before as one of the rules that characterize differences between class I and class II WW domains. However, the binding studies for the tandem WW domains of the FBP11-homologous yeast protein PRP40, which also possess an aromatic residue at position 30, indicated that PRP40 WW domains can bind both PPLp and PpxY-containing peptides. Nevertheless, this study emphasizes that peptide ligands containing the class II motif seemed to have a higher affinity than those containing PPxY. Moreover, PRP40WW2 exhibited a more favorable interaction with PpxY-containing peptides than PRP40WW1. Figure 4(e) and (f) show the comparison of the FBP11WW1: APPTPPPLPP complex with the PRP40WW1 and WW2 domains, respectively. The difference in the affinity of the two PRP40 WW domains for the PpxY-containing peptides seems to correlate with the orientation of the aromatic residue at position 30. For PRP40WW1, the Y recognition site is more obstructed by the aromatic residue at position 30 compared with PRP40WW2 (Figure 4(e) and (f)). The overall poor affinity of the PRP40WW domains for peptides containing the PPxY motif can also be related to the lack of H32, previously determined to be important in the recognition of the Y-containing ligand.

Figure 4(c) compares the structure of the complex FBP11WW1:APPTTPPLLP with the complex Pin1 WW:(pS)PT(pS)P. It reveals two major differences: P23 in loop 1 and H20 in the first β-strand of FBP11WW1 are replaced by arginine residues in the Pin1 WW domain. Correspondingly P5′ and L8′ of the A1PPTTPPLLP ligand are replaced by phosphoserine residues in the Pin1 ligand. This emphasizes the importance of loop 1 for domain specificity in both cases, Pin1 and FBP11WW1. The N–C terminal orientation of the ligand peptide in the FBP11WW1 complex is the same as in Pin1 and opposite that in the dystrophin complex. Due to the pseudo-symmetry of the proline-rich ligand it is likely that some proteins could bind to FBP11 in the opposite orientation, depending on flanking interactions to the proline-rich region, as observed in SH3 domain complexes, where the orientation of the ligand is dependent on the presence of an arginine or lysine C or N-terminal to the proline-rich motif.

Figure 4(d) compares the structure of the complex FBP11WW1:APPTTPPLPP with the complex formed by the N-terminal SH3 domain of the proto-oncogene product c-Crk and peptide PpPLPPK in which the leucine plays a similar role, and because FBP11WW domains compete with SH3 domains for binding to similar ligands. W39 and H20 are the edging aromatics in the binding site of FBP11WW1. They superimpose nicely to the edging Trp and Phe of the SH3 domain. About halfway between these two edging aromatic residues in both cases there is a third aromatic residue, a tyrosine that divides the binding surface in two hydrophobic binding grooves. In these two grooves two turns of a PP1I helix of the ligand are fitted. However, from the comparison in Figure 4(d), it becomes obvious that there are many differences between these two classes of domains. The central aromatic ring is oriented differently and hence it serves different purposes in both domains. In SH3 it is perpendicular to the surface often serving as an H-bond donor and contact area for two proline residues that surround it forming a conserved umbrella-like structure, whereas in WW domains it is oriented along the surface providing a hydrophobic area for contacting only one proline ring. Hydrogen bonds to this aromatic residue are not always observed in WW domains.

Also, there is no equivalent for FBP11WW1 Y30 or loop 1 residues in the SH3 domain and there is no equivalent for the R/K recognition site of SH3 domains in the FBP11WW1 domain. Thus, although in some cases both domains can bind to the same proline-rich ligand, they have different ligand predilections.

Concluding remarks

The structure of the complex of FBP11WW1 with peptide APPTTPPLPP complements our previously derived structure–function relationships using screening of peptide libraries and binding constant measurements. The singular feature of this complex is the specificity invoked by a leucine residue. The new structure shows that this amino acid binds instead of proline in the conserved hydrophobic groove (Figure 4(a), blue). The topology of this groove is modulated by variable amino acid residues that compose loop 1 and which are responsible for the specificity for Leu. The rather flexible side-chain of leucine as compared to proline contributes to a perfect fit of the PP1I helix, allowing four consecutive residues (P6′–P9′) to have their
side-chains contacting the binding surface of the domain. We expect that FBP11WW1 interacts with the proline-rich regions of proteins formin and MeCP2 containing the PPLp motif in the way shown in our structure. For other proteins like Huntingtin and N-WASP, which were also shown to bind to FBP11WW1 but do not possess the PPLp motif, we could expect that the predicted lower affinity could be compensated to some extent by the very long all-proline stretches and the additive contributions of neighboring WW domains.

Considering the classification of WW domains according to ligand predilections, two different tendencies are presented in the recent literature.4,7,17 Katz et al.17 and Wiesner et al.24 have shown examples that some WW domains are promiscuous, displaying binding propensities of classes II and III or I and II, respectively. Based on these results it is suggested that borders between classes are not well defined. On the other hand, in the work by Otte et al.,3 different binding propensities were described for members of the same WW domain class, consequently class II was divided into domains preferring either PPLp or p/ψPPPPPP while class III was divided into domains binding preferably either (p/ψ)PPPP or (p/ψ)PPPpPp.

The structural comparisons of WW domains presented here show similarities in the binding modes of WW domains of different classes explaining the fragility of classification. On the other hand the present detailed structural investigation contributed to understanding the role of variable residues in recognition mechanisms and thus to understand differences in binding constants that can be important in tuning the complex net of interactions.

Materials and Methods

WW domain preparation

The FBP11WW1 sequence, residues W17 to D44 (Figure 1), was obtained in the expression plasmid pGEX-2TK (Amersham Pharmacia Biotech) encoding an N-terminal fusion protein of glutathione-S-transferase and FBP11WW1 domain (GST-WW). The N terminus of the FBP11WW1 domain was later extended by four residues, A13 to M16 from the FBP11WW1 gene sequence, by QuikChange site-directed mutagenesis (Stratagene).

GST-WW was expressed at 37°C in *Escherichia coli* strain BL21 (DE3) grown in shake flasks. To obtain 15C and 15N incorporation into the protein, cells were grown in mineral salt medium containing [15C]glucose as the only carbon source and [15N]ammonium chloride as nitrogen source. Unlabeled GST-WW was expressed in cells grown in LB medium. Protein induction was achieved at A600=0.7 by addition of IPTG to a final concentration of 1 mM in the culture medium. Recombinant GST-WW was purified from soluble cell lysate using glutathione-Sepharose 4B affinity resin (Amersham Pharmacia Biotech) following standard procedures involving a solvent system consisting of acetonitrile/water containing 0.05% (v/v) trifluoroacetic acid. Fractions containing the purified WW domain were pooled and lyophilized. Protein identity and homogeneity were confirmed by electrospray mass spectrometry.

Proline-rich peptide ligands

Peptide ligands (GPPPPPPPLLP, βGPPPPPPPLLP, βGPPPPPPPLPPP, CββAAAPPTPPLLPP, CββAAAPPTP PPLLPP, β=beta-alanine) were obtained from automated solid phase peptide synthesis on chlortriyl resin (Novabiochemtech) using FMOC strategy. The peptide N-terminal amino group and C-terminal carboxy group were retained unmodified. After cleavage of the peptides from the resin using hexa-fluoro-isopropanol/dichloromethane the peptides were purified by reversed phase HPLC using a Licrospher 100 RP column (Merck) following standard protocols involving a water/acetonitrile solvent system containing 0.05% trifluoroacetic acid. Pooled fractions of the pure peptides were lyophilized and analyzed by electrospray mass spectrometry to verify identity and homogeneity.

Epitope length analysis

The cellulose membrane-bound peptide library was prepared according to standard SPOT synthesis protocols26 using a Spot synthesizer (Abimed, Langenfeld, Germany) as described.28 The library was incubated with the peroxidase-labeled WW domain following the procedure described in the literature.4

Determination of *K*0 by fluorescence

Stock solutions of the WW domain and peptide ligands were prepared by dissolving the lyophilized powder in 10 mM potassium phosphate (pH 6.0), 100 mM NaCl, 0.1 mM EDTA, which allowed the WW domain to refold spontaneously (data not shown). Concentrations of the polyproline ligand stock solutions were determined gravimetrically; concentration of the WW domain was determined by measuring absorption at 280 nm and using the calculated absorption coefficient ε280=15,470 M⁻¹ cm⁻¹. For fluorescence measurements the WW domain was diluted into 2 ml of the corresponding buffer to a final concentration of 3.5 μM in a stirring quartz cuvette. Excitation of tryptophan fluorescence was carried out at 295 nm and change of fluorescence emission intensity at 340 nm upon ligand addition was monitored using a Fluoromax-2 spectrofluorometer (ISA SPEX; excitation and emission monochromator slits set to 2.5 nm, temperature-controlled by a cryostat). The emission intensity (*F*obs) was plotted as a function of total ligand concentration (*L*0) added to the sample and least-squares fitted according to equations (1) and (2) (assuming 1:1 complex formation) from which the dissociation constant *K*0 can be calculated:

\[ F_{\text{obs}} = F_{\text{free}} + (F_{\text{sat}} - F_{\text{free}}) \frac{[W_L]}{[W_L]} \]

(1)

where *F*free and *F*sat are the fluorescence intensity without ligand and with a saturating concentration of ligand, respectively, *W*0 is the total WW domain concentration.
Ligand chemical shift assignments were obtained by and the same spectra as described above were acquired. The 3D NOESY heteronuclear single quantum coherence (HSQC) measurement in the range of pH 7 to pH 8.5 using 50 mM Tris–HCl, 150 mM NaCl, 5 mM EDTA, respectively. Observed dissociation constants \( K \) were obtained according to the law of mass action:

\[
[W_L] = \frac{[W_0] + [L_0] + K_D}{2} - \sqrt{\left(\frac{[W_0] + [L_0] + K_D}{2}\right)^2 - [W_0][L_0]} (2)
\]

**pH titration of ligand binding**

Determination of dissociation constants \( K_D \) derived from binding of peptide ligand GPPPPPPPLP to FBP11WW1 domain was performed by fluorescence over a pH range of 5.5 to 8.5 at 25 °C involving different buffer systems. Titrations at pH < 7 were done using 50 mM sodium phosphate, 130 mM NaCl, 5 mM EDTA, measurements in the range of pH 7 to pH 8.5 using 50 mM Tris–HCl 150 mM NaCl, 5 mM EDTA, respectively. Observed dissociation constants \( K_{obs} \) were recorded as a function of pH and fitted to the sigmoidal curve described by equation (3):

\[
K_{obs} = \frac{K_{sh} + K_s 10^{pH-pK_a}}{1 + 10^{pH-pK_a}} (3)
\]

where \( K_{sh} \) represents the \( K_a \) value for the protonated species, \( K_s \) represents \( K_D \) displayed by the unprotonated species. The least-squares fit yields the \( pK_a \) value for the group, which changes ionization within the pH range probed.

**NMR spectroscopy**

**FBP11WW1 alone**

Multidimensional NMR experiments \(^{20,31}\) for the assignment of backbone and side-chain \(^{1}H,^{13}C\) and \(^{15}N\), chemical shifts were acquired in a 1.8 mM \([\text{H}-^{15}N,^{13}C]\)FBP11WW1 domain sample dissolved in 10 mM phosphate buffer (pH 6.0), 100 mM NaCl, 0.1 mM DTT, 0.1 mM EDTA, 90% \( \text{H}_2\text{O}, 10\% \text{D}_2\text{O} \). For the derivation of distance restraints a 3D \(^{13}C\) NOE spectroscopy (NOESY) heteronuclear multiple quantum coherence (HMOC) (100 ms mixing time), a 3D \(^{15}N\) NOESY heteronuclear single quantum coherence (HSQC) (150 ms) and a 2D NOESY (200 ms) were obtained.

**Complex**

For the complex structure determination unlabeled ligand was added in a molar ratio of 2:1 (ligand/ domain) and the same spectra as described above were acquired. Ligand chemical shift assignments were obtained by \(^{13}C/^{12}C\) filtered total correlated spectroscopy (TOCSY) and NOESY. All NMR spectra were acquired in a Bruker DRX600 spectrometer at 298 K, processed with XWINNMR (Bruker AG) and analyzed with Sparky (T.D. Goddard & D.G. Keenell, University of California, San Francisco).

**Structure calculations**

**FBP11WW1 alone**

The 3D NOESY spectra were analyzed manually for unambiguous \( \text{HN}^-\text{HN}^+, \text{H}^+\text{H}^a \) and \( \text{HN}^-\text{H}^a \) NOEs characteristic of secondary structure and other unambiguous NOEs that allowed the calculation of a preliminary 3D structure. Further assignments of NOEs were obtained automatically using ARIA \(^{20,21}\) version 1.2 and checked manually. Dihedral angle restraints (\( \phi, \psi \)) based on chemical shifts were obtained from CSI \(^{32}\) and inter-strand hydrogen-bond restraints based on NOEs characteristic of secondary structure pattern were used in the calculations with ARIA using CNS \(^{33}\) version 1.1.

**Atomic coordinates and NMR restraints**

The atomic coordinates, NMR restraints, and chemical shifts assignments for the FBP11WW1 and its complex were added to the RCSB Protein Data Bank, PDB codes 1YWJ and 1YWI, respectively.

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**References**


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