Structural basis of ligand binding modes at the human formyl peptide receptor 2

Tong Chen1,2,3,8, Muya Xiong1,3,8, Xin Zong1,2,3, Yunjun Ge4, Hui Zhang1,2,3, Mu Wang1,5, Gye Won Han6, Cuiying Yi1, Limin Ma2, Richard D. Ye7, Yechun Xu1,3,8, Qiang Zhao2,3,8 & Beili Wu1,3,5

The human formyl peptide receptor 2 (FPR2) plays a crucial role in host defense and inflammation, and has been considered as a drug target for chronic inflammatory diseases. A variety of peptides with different structures and origins have been characterized as FPR2 ligands. However, the ligand-binding modes of FPR2 remain elusive, thereby limiting the development of potential drugs. Here we report the crystal structure of FPR2 bound to the potent peptide agonist WKYMVm at 2.8 Å resolution. The structure adopts an active conformation and exhibits a deep ligand-binding pocket. Combined with mutagenesis, ligand binding and signaling studies, key interactions between the agonist and FPR2 that govern ligand recognition and receptor activation are identified. Furthermore, molecular docking and functional assays reveal key factors that may define binding affinity and agonist potency of formyl peptides. These findings deepen our understanding about ligand recognition and selectivity mechanisms of the formyl peptide receptor family.
Three formyl peptide receptors (FPR1, FPR2, and FPR3), which belong to the GPCR superfamily, participate in many physiological processes in humans. These receptors bind a vast array of structurally diverse agonists, including N-formyl peptides from microbes and mitochondria, and non-formyl peptides of microbial and host origins. FPR2, also known as the lipoxin A4 receptor (LXA4R, ALX), plays important roles in chemotaxis, cell proliferation, wound healing, migration, and vessel growth, and is involved in the pathogenesis of chronic inflammatory diseases such as rheumatoid arthritis, colitis, Alzheimer's disease, systemic amyloidosis and atherosclerosis. Although FPR1 and FPR2 share 69% sequence identity, FPR2 shows low affinity binding to the prototypical formyl peptide, N-formyl-Met-Leu-Phe (fMLF) and many potent formyl peptide agonists for FPR1. However, FPR2 can recognize a wider array of ligands with different structures and functions, which include not only bacterially derived formyl peptides but also non-formyl peptides, lipid mediators such as lipoxin A4 (LXA4), small molecules and proteins, making this receptor one of the most promiscuous GPCRs characterized to date. How FPR2 is able to recognize and bind these ligands and transduce both proinflammatory and anti-inflammatory signals remains a mystery. Some FPR2 ligands have shown therapeutic potential for the treatment of inflammation, diabetic wounds and Alzheimer’s disease. However, the lack of a three-dimensional structure of FPR2 has hampered the understanding of the potential therapeutic mechanism as well as their clinical applications. Trp-Lys-Tyr-Met-Val-D-Met-NH₂ (WKYMVm), a highly potent FPR2 agonist isolated through a library screening of synthetic peptides, shows therapeutic effects on cutaneous wound healing, and vessel growth, and is involved in the pathogenesis of chronic inflammatory diseases such as rheumatoid arthritis, colitis, Alzheimer’s disease, systemic amyloidosis and atherosclerosis. Although FPR1 and FPR2 share 69% sequence identity, FPR2 shows low affinity binding to the prototypical formyl peptide, N-formyl-Met-Leu-Phe (fMLF) and many potent formyl peptide agonists for FPR1. However, FPR2 can recognize a wider array of ligands with different structures and functions, which include not only bacterially derived formyl peptides but also non-formyl peptides, lipid mediators such as lipoxin A4 (LXA4), small molecules and proteins, making this receptor one of the most promiscuous GPCRs characterized to date. How FPR2 is able to recognize and bind these ligands and transduce both proinflammatory and anti-inflammatory signals remains a mystery. Some FPR2 ligands have shown therapeutic potential for the treatment of inflammation, diabetic wounds and Alzheimer’s disease.

### Results

The FPR2-WKYMVm structure adopts an active conformation. To facilitate crystal packing, the N-terminal residues M1-E2 of FPR2 were replaced with a thermostable apocytochrome b562RIL (bRIL) fusion protein and five residues at C terminus were truncated. A single mutation S2115.48L (superscript indicates residue numbering using the Ballesteros Weinstein nomenclature) was introduced to further improve protein quality. It was designed by switching the hydrophilic residue to a hydrophobic counterpart presented in several class A GPCRs with known structures, including chemokine receptors CXCR4 and CCR5, C5a receptor, μ-opioid receptor (μOR) and prostanoid receptor DP2, which share high sequence similarity with FPR2 (35–45%) and share high sequence similarity with FPR2 (35–45%). This mutation may introduce extra hydrophobic interactions with its neighboring residues on the external surface of the receptor, aiming for better protein stability. Functional assays indicate that the above modifications have little effect on ligand binding and receptor activation (Supplementary Tables 1, 2). The modified FPR2 protein was co-purified and co-crystallized with the peptide agonist WKYMVm. The FPR2-WKYMVm complex structure was determined at 2.8 Å resolution (Supplementary Table 3).

The FPR2 structure exhibits a canonical seven-transmembrane helical bundle conformation (helices I–VII) (Fig. 1). Some conserved GPCR structural features are observed in the extracellular region of the receptor, including a disulfide bridge connecting helix III and the second extracellular loop (ECL2) and a β-hairpin conformation of ECL2, which is shared by other solved peptide class A GPCR structures. The extracellular region, mainly including the N terminus, the first extracellular loop (ECL1) and ECL2, forms a “lid” conformation that stacks on top of the ligand-binding pocket of FPR2 (Fig. 1b). However, the structure does not rule out the possibility that the conformation of the receptor N terminus was affected by the N-terminal bRIL fusion protein, which is involved in mediating crystal packing (Supplementary Fig. 1).

The WKYMVm-bound FPR2 structure exhibits an outward shift of helix VI that is not seen in the inactive μOR structure. This movement of helix VI, however, is similar to that observed in the active μOR structure (Fig. 2a), suggesting that the FPR2 structure adopts an active conformation. The “ionic lock"
The conformational change of W6.48, which represents the conserved residue W6.48, which is involved in receptor activation16,22,28. The above structural features demonstrate that the receptor-ligand interaction, destabilization of ligand-binding pocket conformation, and/or impairment of global conformational rearrangement required for receptor activation. In contrast to the substantial effect of most of the residues within the two hydrophobic clusters, the alanine mutation of the N-terminal residue F5 displays little effect in both assays (Supplementary Tables 1, 2), suggesting that the interaction between the receptor N terminus and the peptide agonist is either not important for ligand recognition or introduced by crystal packing (Supplementary Fig. 1).

Two polar residues D3.33 and R5.38 in FPR1 have been suggested to form hydrogen-bond interactions with the N-terminal formyl group of fMLF31. In the FPR2-WKYVMv structure, the corresponding residues in FPR2 establish a hydrogen-bond network with the side chain hydroxyl of Y3, the main chain carbonyl of M4, the main chain nitrogen of m6 and the C-terminal amide group in the peptide agonist (Fig. 3f), thereby greatly contributing to the receptor-ligand interaction and stabilizing the peptide in a conformation favoring its binding to the receptor. Consistent with the importance of these two polar residues, the mutations D1063.33A and R2015.38A significantly reduce the coupling of Gαi-bound receptor to the phospholipase C signaling pathway. The results show that the alanine replacements of Val3.32, Ile3.36, Val3.40, Leu3.55, Val3.56, and Met3.61 reduce the EC50 of WKYMVm-induced IP production by over 65-fold (Fig. 4e, f and Supplementary Table 1). The effect of these mutations on ligand binding and receptor signaling could be explained by direct disruption of the receptor-ligand interaction, destabilization of ligand-binding pocket conformation, and/or impairment of global conformational rearrangement required for receptor activation.

Binding mode of FPR2 to WKYMVm. The peptide agonist WKYMVm binds to FPR2 in a pocket bordered by N terminus, ECL1, ECL2, ECL3, and helices III, V, VI, and VII of the receptor (Figs. 1b, 3a, b, and Supplementary Fig. 2). The peptide penetrates into the binding cavity with its C terminus occupying a deeper site within the receptor transmembrane helical bundle compared to the binding sites in other known peptide-bound GPCR structures (Fig. 3c and Supplementary Fig. 3), while the N terminus of the peptide approaches the extracellular surface of the receptor and forms contacts with the extracellular loops. A close inspection of the ligand-binding pocket has found two hydrophobic clusters that play critical roles in ligand recognition and receptor activation. The residues F5, L164, F178, and L198 are suggested to form hydrogen-bond interactions with the N-terminal amide group in the peptide agonist (Fig. 3d). At the bottom of the ligand-binding pocket, another hydrophobic cluster, including Val3.32, Leu3.36, Val3.40, Leu3.55, Val3.56, and Met3.61, forms close contacts with the C-terminal residues V5 and m6 (D-Met6) of the peptide agonist (Fig. 3e).}

between the conserved class A GPCR residue R3.50 in the D/ER3.50Y motif and D/E6.30 in helix VI, which is observed in some inactive GPCR structures and has been suggested to stabilize the receptor in an inactive state20,21, breaks in the FPR2-WKYVMv structure due to the outward shift of helix VI. Instead, the residue R1233.50 makes a hydrogen-bond interaction with Y2215.38 (Fig. 2b). This interaction was also observed in other active GPCR structures and has been suggested to stabilize the receptor helix V in an active orientation19,22–26. Moreover, the highly conserved residue W6.48, which represents the "toggleswitch"27, and the P5.50/I/V3.40p6.44 motif display rotamer conformational changes in the WKYMv-bound FPR2 structure relative to the inactive µOR structure, and adopt similar conformations to those in the active µOR structure (Fig. 2c, d). These two "micro-switches" have been reported to be involved in receptor conformational rearrangement, which is required for GPCR activation6,22,28. The above structural features demonstrate that the FPR2-WKYVMv structure is in an active conformation.

**Fig. 2 Active conformation of the FPR2-WKYVMv complex.** a The movement of helix VI. The transmembrane helical bundles in the structures of FPR2-WKYVMv (cyan), µOR-BU72 (pink, PDB code: 5C1M) and µOR-naloxone (dark red, PDB code: 4DKL) are shown in cartoon representation at an intracellular view. The red arrows indicate the movements of helices V, VI, and VII in the FPR2-WKYVMv and µOR-BU72 (active) structures relative to the µOR-naloxone (inactive) structure. b The hydrogen bond interaction between R3.50 and Y5.58 in FPR2. The FPR2 residues R3.50 and Y5.58 are shown as cyan sticks. The inactive A1AR-DU172 structure (PDB code: 5UEN) is shown in yellow cartoon representation. The A1AR residues Y5.58 are shown as cyan sticks. The inactive A1AR-DU172 structure (PDB code: 5UEN) is shown in yellow cartoon representation. The A1AR residues Y5.58 are shown as cyan sticks. The inactive A1AR-DU172 structure (PDB code: 5UEN) is shown in yellow cartoon representation. 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impaired binding of WK(FITC)YMVm and the ability of WKYMVm in stimulating Gdagon4myr-mediated IP production (Fig. 4c, g and Supplementary Tables 1, 2). To further stabilize the binding of the peptide C terminus to the receptor, another polar residue R2055.42 forms two hydrogen bonds with the main chain carbonyls of M4 and V5 (Fig. 3f). This was reflected by a complete loss of WK(FITC)YMVm binding and a significant reduction of the agonistic potency of WKYMVm for the mutant R2055.42A (Fig. 4c, g and Supplementary Tables 1, 2). Moreover, a water molecule establishes a “bridge” between the main chain of the peptide residue M4 and the receptor residues R2015.38 and R2055.42, further stabilizing the peptide conformation and strengthening the receptor-peptide binding (Fig. 3f).

In addition to the above polar interactions involving the C terminus of WKYMVm, the receptor-ligand binding is facilitated by three polar interactions between the N terminus of the peptide and the extracellular region of helix VII and ECL1 in FPR2. The negatively charged residue D2817.32 forms a salt bridge with the N-terminal NH2 group of WKYMVm, while the only charged residue in the peptide, K2, is engaged in a salt bridge with the residue E89 in ECL1 and a hydrogen bond with N2857.36 in helix VII (Fig. 3f). However, in contrast to the significant impairment of WK(FITC)YMVm binding and IP production due to the D1063.33A, R2015.38A, and R2055.42A substitutions, the mutants E89A/G, D2817.32A, and N2857.36A had much less impact on ligand recognition and receptor activation (Fig. 4d, h and Supplementary Tables 1, 2). These data suggest that these polar interactions with the N terminus of WKYMVm are less critical for recognition of the peptide ligand and its agonistic potency.

WKYMVM-NH2, a derivative of WKYMVm with the substitution of L-methionine at the C terminus, is less effective on activating FPR2 than WKYMVm with an over 100-fold reduction in EC50 in stimulating phosphoinositide hydrolysis29. Molecular docking of WKYMVM-NH2 (Supplementary Data 1) revealed a rotation of the C-terminal amide relative to the binding pose of WKYMVm due to the alteration of chiral carbon. This movement breaks the hydrogen bonds between the C-terminal NH2 group of the peptide and the receptor residues D1063.33 and R2015.38, and disrupts the polar interaction network established by the peptide C terminus (Supplementary Fig. 4), which is important for stabilizing receptor-peptide binding.

Fig. 3 Ligand binding mode of FPR2 to WKYMVm. a Binding pocket of WKYMVm in FPR2. The receptor in the FPR2-WKYMVm structure is shown in cyan cartoon representation. The ligand WKYMVm is shown as orange sticks. b Schematic representation of interactions between FPR2 and WKYMVm analyzed using the LigPlot+ program44. Salt bridges and hydrogen bonds are shown as red and green dashed lines, respectively. The stick drawings of FPR2 and WKYMVm are colored blue and orange, respectively. c Comparison of the peptide ligand-binding sites in peptide-bound GPCR structures. WKYMVm in FPR2 (orange), sAngII in AT2R (red, PDB code: 5XJM), CVX15 in CXCR4 (purple, PDB code: 3OEO), DAMGO in μOR (yellow, PDB code: 6DDF), NTSp13 in NTSR (pink, PDB code: 4GRV), and NNC1702 in GCGR (blue, PDB code: 5YQZ) are shown as sticks. Only the receptor in the FPR2-WKYMVm structure is shown in cyan cartoon representation for calibration. d Hydrophobic cluster that forms interactions with W1 and Y3 of WKYMVm. The receptor residues and ligand are shown as blue and orange sticks, respectively. e Hydrophobic cluster that forms interactions with V5 and m6 of WKYMVm. f Polar interactions between FPR2 and WKYMVm. Salt bridges and hydrogen bonds are shown as red and green dashed lines, respectively.
Molecular docking of formyl peptides. The E. coli-derived chemotactic peptide iMLF is the shortest formyl peptide that exhibits full agonistic activity. However, it acts as a weak agonist for FPR2 with a 2000-fold lower potency in inducing Ca2+ mobilization and an over 400-fold lower binding affinity compared to those for FPR1. To investigate the binding mode of formyl peptide in FPR2, molecular docking of this tripeptide to the FPR2 crystal structure was performed (Supplementary Data 2). Similar to the C-terminal residue m6 of WKYMVm in the crystal structure, the formylated methionine at the N terminus of iMLF reaches deep into the ligand-binding pocket with the N-formyl group hydrogen bonding with the FPR2 residues D1063.33 and R2015.38 in the docking model (Fig. 5a), while the two hydrophobic residues L2 and F3 in iMLF occupy similar binding sites to those of V5 and Y3 in WKYMVm (Fig. 5b and Supplementary Fig. 5a, b). Furthermore, two basic residues in receptor helix V, R2015.38 and R2055.42, which provide the only positively charged binding interface in the ligand-binding cavity of FPR2, anchor the C-terminal COO− group of iMLF through two salt bridges (Fig. 5a).

To provide a structural basis for different behaviors of iMLF at FPR1 vs. FPR2, molecular docking of iMLF to a FPR2-based model of FPR1 was also carried out (Supplementary Data 3). Comparison of the FPR1-iMLF and FPR2-iMLF models revealed major difference in the interaction mode between the C-terminal COO− group of iMLF and the receptor. Unlike FPR2, in which the COO− group of the peptide forms salt bridges with R2015.38 and R2055.42, this C-terminal acidic group of iMLF may form ionic interactions with the residues R842.63 and K852.64 at the extracellular tip of helix II in FPR1 (Fig. 5c). These interactions are not possible in FPR2 as these two basic residues are replaced with non-charged residues, S842.63 and M852.64. The FPR1 residues R842.63 and K852.64 have been suggested to play critical roles in iMLF binding and receptor function of FPR1. In contrast to the binding mode of iMLF in FPR2, where the residues D1063.33, R2015.38, and R2055.42 provide major polar contacts with the peptide, the extra ionic interactions between the peptide COO− group and R842.63 and K852.64 in FPR1 may contribute to the high binding affinity of iMLF at FPR1. This agrees with previous data showing that the FPR2 mutants S842.63R and M852.64K displayed greatly increased binding affinity for [3H]iMLF. These data support that these two residues in helix II are key factors governing iMLF recognition by FPR1, which align well with our molecular docking results.

It has been suggested that the length of the formyl peptide and its C-terminal charge are determinants for optimal agonistic activity at FPR2. The tetrapeptide fMLFK and pentapeptide fMLFII showed increased binding affinity and agonist potency over iMLF at FPR2. It was also reported that the polar residue D2817.32 was crucial for the interaction of FPR2 with certain formyl peptides. It was proposed that this negatively charged residue in FPR2 was repulsive with the C-terminal COO− group of iMLF and the negatively charged glutamate residue in fMLF, but forms a stable interaction with the positively charged lysine in fMLF. In our docking models of the formyl peptides, the tetrapeptide fMLFK binds to FPR2 with its C-terminal residue K4 forming a salt bridge with either E89 or D2817.32 (Fig. 5d, Supplementary Fig. 5c, and Supplementary Data 4). This interaction was verified by a competition binding assay of fMLFK with WK(FITC)YMVm, showing that the mutation E89G decreased the binding affinity for fMLFK by about 4-fold compared to the wild-type receptor while the mutant D2817.32G displayed a slightly higher binding affinity to this tetrapeptide than the wild type (Fig. 4i and Supplementary Table 1). These data suggest that E89 plays a more important role in recognizing fMLFK and is likely the binding partner for the positively charged lysine at the C terminus of the peptide. In addition to this ionic interaction, K4 may also form a hydrogen bond with N2857.36 (Fig. 5d). These polar interactions most likely contribute to the increased binding affinity and agonist potency of fMLFK relative to iMLF. Likewise, molecular docking
identified extensive interactions between the two isoleucine residues at the C terminus of the pentapeptide fMLFII and the receptor ECL2, ECL3 and helices V and VI (Fig. 5e and Supplementary Data 5), a similar binding site to that of the residue W1 in WKYMVm (Fig. 5b and Supplementary Fig. 5a, d), which may lead to the improved activity of the pentapeptide. In contrast to the different behaviors of various formyl peptides at FPR2, peptide length and the composition of the peptide C terminus are not critical to FPR1 binding4. This may arise from a broader and less negative charged binding cavity on the extracellular side of the ligand-binding pocket in FPR1, which accommodates the peptide C terminus with fewer contacts and no preference for charges (Supplementary Fig. 6).

**Discussion**

Among the FPR2 ligands with diverse structures, WKYMVm is by far the most potent peptide agonist for FPR2. It exhibits stronger potency in activating FPR2 than FPR1 and FPR3, displaying a 40–300-fold higher EC50 in mobilizing intracellular calcium (FPR2, 75 pM and FPR1, 25 nM34; FPR2, 75 pM and FPR3, 3 nM35). Sequence alignment of the three FPRs reveals that most of the key residues involved in WKYMVm binding are conserved except for F5, E89, L1644.64, L1985.35, R2015.38, and D2817.32 (Supplementary Fig. 7), suggesting that these six residues may be determinants for binding selectivity of WKYMVm. In FPR1, the substitutions of F5 with serine and E89 with glycine may disrupt the interaction between the peptide and the extra-cellular loops of the receptor, leading to a decreased binding ability of the peptide. In FPR3, the bulky residue W4.64 would most likely cause a spatial clash with the peptide residue Y3 to decrease the binding affinity, while the replacement of E89 with glycine may disrupt the interaction between the peptide and the extra-cellular loops of the receptor, leading to a decreased binding ability of the peptide. In FPR3, the bulky residue W4.64 would most likely cause a spatial clash with the peptide residue Y3 to decrease the binding affinity, while the replacement of L198 5.35A reduced the agonist potency of WKYMVm by about 70-fold (Fig. 4a, e, f and Supplementary Tables 1, 2). Furthermore, instead of a polar arginine, the residue at position 5.38 in FPR3 is a hydrophobic phenylalanine, which disturbs the polar interaction network between the receptor helices III and V and the peptide, and probably mediates

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**Fig. 5 Molecular docking of formyl peptides.** **a** Docking model of FPR2-fMLF. The receptor is shown in cyan cartoon representation. The peptide fMLF and the FPR2 residues that may form interactions with fMLF are shown as sticks and colored magenta and blue, respectively. Salt bridges and hydrogen bonds are shown as red and green dashed lines, respectively. **b** Docking poses of fMLF, fMLFK, and fMLFII in comparison with the binding pose of WKYMVm in FPR2. The peptides WKYMVm, fMLF, fMLFK, and fMLFII are shown as orange, magenta, yellow, and gray sticks, respectively. The receptor residues that may form polar interactions with the peptide residues I4 and I5 are shown as sticks and colored magenta and blue, respectively. Salt bridges and hydrogen bonds are shown as red (salt bridge) and green (hydrogen bond) dashed lines. **c** Comparison of the fMLF docking poses in FPR1 and FPR2. The model of FPR1-fMLF is colored light green (FPR1) and green (fMLF), while the model of FPR2-fMLF is in cyan (FPR2) and magenta (fMLF). The residues that may form salt-bridge interactions with the C-terminal COO− group of fMLF are shown as sticks and colored light green (R842.63 and K852.64 in FPR1) and blue (R2015.38 and R2055.42 in FPR2). The salt bridges are displayed as black and red dashed lines in FPR1 and magenta (R852.63 and K852.64 in FPR1) and blue (R2015.38 and R2055.42 in FPR2). The polar interactions are shown as red (salt bridge) and green (hydrogen bond) dashed lines. **d** Docking pose of fMLFK in FPR2. fMLFK is shown as yellow sticks. The receptor residues that may form polar interactions with the peptide residues I4 and I5 are shown as sticks and colored magenta and blue, respectively. Salt bridges and hydrogen bonds are shown as red (salt bridge) and green (hydrogen bond) dashed lines. **e** Docking pose of fMLFII in FPR2. fMLFII is shown as gray sticks. The receptor residues that may form hydrophobic interactions with the peptide residues I4 and I5 are shown as blue sticks.
selectivity. This agrees with the fact that the mutation R2013.38F substantially reduced the WK(FITC)YMVM binding and WKYMVM-induced IP accumulation (Fig. 4c, g and Supplementary Tables 1, 2). Similarly, the FPR2 residue D7.32 is replaced by glycine and leucine in FPR1 and FPR3, respectively, preventing the salt-bridge interaction with the N-terminal NH2- group of WKYMVM. Indeed, the FPR2 mutants D281.2L and D281.3L exhibited decreased binding of WK(FITC)YMVM and an impaired ability to induce IP production (Fig. 4d, h and Supplementary Tables 1, 2). In addition, although the FPR2 residue F257.51A is substituted with a similar aromatic tyrosine residue in FPR1 and FPR3, the extra hydroxyl group may form a spatial clash with the peptide ligand, which is supported by a 7-fold reduction of binding affinity of WK(FITC)YMVM for the mutant F257.51Y (Fig. 4b and Supplementary Table 1). These insights gained from the FPR2-WKYMVm structure will facilitate the development of selective drug molecules by targeting the variable regions of the ligand-binding pocket.

It was reported that the related peptide MKYMPM-NH2 was inactive, while the residue M6 was either eliminated or replaced by glycine.29 Furthermore, previous studies of D-type amino acid-containing peptide analogs of MKYMPM-NH2 and WKYMVM-NH2 revealed that none of the peptides with D-type amino acid substitutions was as effective as the original peptides, except for the ones with the D-Met6 substitution.29 These data suggest that the peptide C terminus is a determinant of its biological activity. Indeed, among the alanine mutations of FPR2 tested in the GsA646myr-mediated IP accumulation assay, D106.33A, L109.36A, V113.34A, R205.42A, W254.48A, and F257.51A exhibited the largest effect on receptor signaling, showing a significantly impaired agonistic potency of WKYMVM (Fig. 4e-g and Supplementary Table 2). In the FPR2-WKYMVm structure, these six residues all locate at the bottom of the ligand-binding pocket and mainly form interactions with the C-terminal residue m6 of the peptide (Fig. 3e, f). The highly conserved class A GPCR residues I7.34 and W6.48 have been suggested to be involved in stimulating receptor activation through their conformational changes.16,22 The interactions between the WKYMVM residue m6 and the FPR2 residues V113.40 and W254.48 are most likely crucial for triggering the conformational rearrangement of these conserved motives to relay the agonist-induced conformational changes in the ligand-binding pocket to the cytoplasmic domain, while the other key residues within the sub-pocket may play a critical role in mediating receptor-ligand binding and/or stabilizing the receptor active conformation.

Although the formyl peptides and WKYMVM adopt different binding modes to FPR2, these peptide agonists may activate the receptor using a similar mechanism. This is supported by the fMLFK-induced IP accumulation assay, showing that the alanine replacements of L106.33, L109.36, V113.40, R201.38, R205.42, W254.48, and F257.51 abolished FPR2-mediated cell signaling (Fig. 4) and Supplementary Table 2). In the docking models of FPR2 bound to the formyl peptides, these residues all interact with the N-terminal formylated M1 of the peptides, suggesting that the N terminus of the formyl peptides activates the receptor in a similar manner to the C terminus of WKYMVM. These data indicate that the bottom region of the ligand-binding pocket in FPR2 plays an important role in regulating receptor activation, and can be considered as a drug target site for drug molecule design.

Collectively, the FPR2-WKYMVm complex structure provides molecular details regarding ligand recognition by FPR2 and other formyl peptide receptors. It is expected that understanding of the structural basis for FPR2 interaction with a variety of ligands will enable structure-based drug discovery targeting this physiologically important GPCR family.
Inositol phosphate accumulation assay. Flag-tagged wild-type and mutant FPR2s were cloned into the expression vector pT75 (Invitrogen) and expressed in HEK293 cells (Invitrogen) along with the chimeric Ga protein \( \text{Ga}_{\alpha\Delta} \text{M WKYMVm} \), showing a docking pose similar to the binding pose observed in the crystal structure of FPR2-WKYMVm. For comparison, we also applied the same docking algorithm on the FPR1-WKYMVm complex. To verify the above method, we also performed using PHENIX38 and BUSTER39, and manual examination and rebuilding of the refined coordinates were carried out in COOT30 with both \( \{\text{E}^\alpha_{\text{E}}\} \) and \( \{\text{E}^\alpha_{\text{E}}\} \) maps. The final model includes 320 residues (T3-E322) of FPR2 and residues A1-L106 of \( \text{BRIL} \).

Ligand binding assay. Flag-tagged wild-type and mutant FPR2s were cloned into the pT75 vector and expressed in HEK293F cells. The cells were harvested, washed with Hanks’ Balanced Salt Solution (HBSS) buffer supplemented with 0.5% bovine serum albumin and 20 mM HEPES, pH 7.4. The cells were then resuspended in the same buffer to the final concentration of \( 1 \times 10^5 \) cells per ml. The cell-surface expression was measured as mentioned above. For saturation binding of WKYMVm, cells were plated in 96-well plates (100,000 cells per well) and incubated with increasing concentrations of fluorescein isothiocyanate (FITC)-conjugated peptide WK(FITC)YMVm (1 nM–1 μM) on ice for 1 h. Mean fluorescent intensity of each well was then read by a FCM (flow cytometry) reader (Millipore). Total binding and nonspecific binding were measured in the absence and presence of unlabeled ligand (200 μM WKYMVm), respectively. For competitive binding of MIFLKM, cells were plated in 96-well plates (100,000 cells per well), and incubated with WK(FITC)YMVm at 4 °C for 1 h. The following concentrations of WK(FITC)YMVm were used: 10 nM (wild type, \( \text{L}_{42}^\alpha \text{E}^\alpha_{\text{E}} \), \( \text{E}^\alpha_{\text{E}} \), and \( \text{V}_{105}^\alpha \text{S}, \alpha^\alpha \) 32A), 30 nM (D281T^E2G, 40 nM (H102^T^E2F, \( \text{V}_{164}^\alpha \text{H}^\alpha_{\text{E}}, \text{L}_{268}^\alpha \text{C}^\alpha_{\text{E}}, \text{N}_{285}^\alpha \text{R}^\alpha_{\text{E}} \), and F292^4A), and 100 nM (L164^A^A and T177^A). Then increasing concentrations of MIFLKM (100 nM–1 μM) were added, and incubated for another 1 h on ice. Mean fluorescent intensity values were measured by flow cytometry. Data were analyzed using Prism 7.0.

Molecular docking of peptide ligands. The structure of FPR1 was modeled using the crystal structure of FPR2-WKYMVm as a template and re

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Author contributions

T.C. optimized the construct, purified the FPRL2 protein, performed crystallization trials, solved the structure, performed ligand binding and signaling assays, and helped with manuscript preparation. M.X. performed molecular docking. X.Z. helped with protein sample optimization and functional assays. Y.G. helped with functional assays. H.Z. and M.W. collected X-ray diffraction data. G.W.H. helped with structure determination. C.Y. and L.M. expressed the protein. R.D.Y. helped with data analysis and interpretation, and edited the manuscript. Y.X. oversaw the molecular docking and edited the manuscript. B.W. and Q.Z. initiated the project, planned and analysed experiments, supervised the research, and wrote the manuscript with input from all co-authors.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to Y.X., Q.Z. or B.W.

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