The structure of the extracellular domains of human interleukin 11 α-receptor reveals mechanisms of cytokine engagement

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Running title: The structure of human interleukin 11 α-receptor

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Abstract
Interleukin 11 (IL-11) activates multiple intracellular signalling pathways by forming a complex with its cell surface α-receptor, IL-11Rα, and the β-subunit receptor, gp130. Dysregulated IL-11 signalling has been implicated in several diseases, including some cancers and fibrosis. Mutations in IL-11Rα that reduce signalling are also associated with hereditary cranial malformations. Here we present the first crystal structure of the extracellular domains of human IL-11Rα, and a structure of human IL-11 that reveals previously unresolved detail. Disease-associated mutations in IL-11Rα are generally distal to putative ligand binding sites. Molecular dynamics simulations showed that specific mutations destabilise IL-11Rα and may have indirect effects on the cytokine binding region. We show that IL-11 and IL-11Rα form a 1:1 complex with nanomolar affinity and present a model of the complex. Our results suggest that the thermodynamic and structural mechanisms of complex formation between IL-11 and IL-11Rα differ substantially from those previously reported for similar cytokines. This work reveals key determinants of the engagement of IL-11 by IL-11Rα that may be exploited in the development of strategies to modulate formation of the IL-11/IL-11Rα complex.
Interleukin 11 (IL-11) is a member of the IL-6 family of cytokines, which includes IL-6, leukemia inhibitory factor (LIF), oncostatin M, ciliary neutrophilic factor, IL-27, IL-31, cardiotrophin-1, cardiotrophin-like cytokine, and neuropoietin (1). Activation of downstream signalling pathways by these cytokines is generally initiated via the formation of oligomeric receptor complexes that include the β-subunit signalling receptor, gp130, and one or more cytokine-specific co-receptors (2,3). The majority of our structural and mechanistic understanding of this cytokine family is based on structural information available for IL-6 and LIF and their receptors (4-6).

Characterisation of the in vivo source of IL-11 has only recently begun, as a result of emerging links to multiple pathologies. IL-11 has classically been associated with haematopoiesis (7); however, it has more recently been identified as the major cytokine involved in gastrointestinal tumorigenesis, and is a promising therapeutic target (8). IL-11 also has emerging roles in cardiovascular and liver fibrosis (9,10). Mutations in the IL-11 specific alpha-receptor, IL-11Rα, have gained increased interest as a result of their causative role in hereditary diseases that are typified by craniosynostosis and delayed tooth eruption (11-14). Several of these mutations have been shown to impair IL-11 signalling in vitro (11).

Following secretion, IL-11 is believed to interact with IL-11Rα, which is expressed on tissue specific cell populations (15). This binary complex is thought to subsequently engage gp130 (6,16). Previous mutagenesis and structural studies indicate that IL-11 interacts with its receptors through three independent sites on its surface (17). Site-I is responsible for IL-11Rα binding; Site-II binds a gp130 molecule and contributes to the formation of a trimeric complex; and Site-III engages with a second gp130 molecule, resulting in the cooperative formation of a hexameric signalling complex containing two copies of each component (16).

Upon formation of the signalling complex, Janus kinases (JAK) associated with the cytoplasmic regions of gp130 are activated, although the exact mechanisms of activation remain unclear (18). As IL-11Rα does not bind JAKs at its cytoplasmic domain, signalling is thought to result from trans-activation of JAK molecules bound to the cytoplasmic domains of the two gp130 molecules of the hexameric signalling complex. JAK activation then leads primarily to phosphorylation and activation of signal transducer and activator of transcription (STAT) 3. Activation of other signalling pathways, including the ERK/MAPK pathway and the PI(3)K pathway, is less well understood.

The structural basis of IL-6 signalling has been well studied and the structure of the hexameric IL-6 signalling complex has been solved (6). Low-resolution electron microscopy studies of the IL-11 signalling complex suggest that the overall arrangement is likely similar to that of IL-6 (19). We previously reported the first crystal structure of human IL-11 (17) and showed that while the topology is similar to IL-6, IL-11 is significantly elongated, suggesting different geometry of the signalling complex. Despite the growing biological importance of IL-11 signalling, molecular understanding of the structure and assembly of the IL-11 signalling complex remains in its infancy.

Here, we present the first crystal structure of human IL-11Rα and a new, more complete structure of IL-11 that reveals structural details of functionally important regions. Disease-associated mutations in IL-11Rα are generally located distal to putative binding
surfaces of the receptor. Molecular dynamics simulations reveal the mechanisms by which several of these mutations disrupt the structure of IL-11Rα and thereby prevent signalling. We present a model of the IL-11/IL-11Rα complex, and in combination with biophysical and mutagenic characterisation of the cytokine/receptor interaction show that IL-11Rα and IL-6Rα engage their cognate cytokines with similar affinities, but use surprisingly different thermodynamic and structural mechanisms. Our work provides structural and mechanistic detail of the first step of formation of the IL-11 signalling complex that may be exploited in the development of molecules that can modulate complex formation.

Results and Discussion

The structure of the extracellular domains of the interleukin 11 α-receptor

The complete extracellular region of IL-11Rα (IL-11RαEC; residues 1-341 of the mature protein after signal peptide cleavage) was expressed in the insect cell line, Sf21, and purified from the cell culture supernatant. To reduce formation of disulfide-linked dimers, the C226S mutation (20) was present in all IL-11Rα constructs described in this work. Crystals of IL-11RαEC were in space group P6522. Initial phase estimates were obtained by molecular replacement using domains from unpublished Fab-bound structures of IL-11Rα, and the structure was refined at a resolution of 3.43 Å (PDB ID: 6O4P). Data and refinement statistics are presented in Table 1 and representative electron density is shown in Figure S1A, B.

The structure of IL-11RαEC consists of an N-terminal Ig-like domain (D1) and two fibronectin-type III (FnIII) domains (D2, D3) that form the cytokine-binding homology region (CHR) (Figure 1A). By homology to other cytokine receptors, IL-11 likely binds to the loops present at the D2/D3 junction (Figure 1A). The receptor is ‘L-shaped’, with D2 and D3 forming the CHR. The arrangement of the three domains is similar to other IL-6 family cytokine receptors (Figure S1C). The α-carbon RMSD between IL-11Rα and IL-6Rα (PDB ID: 1N26) is 5.5 Å, and that between IL-11Rα and D1-D3 of gp130 (PDB ID: 111R) is 5.8 Å, indicating moderate structural similarity. The primary deviations between the three structures are in the position of D1. The putative cytokine binding region in IL-11Rα shows less surface charge than that of IL-6Rα (Figure S1D).

Two protein molecules are present in the asymmetric unit (α-carbon RMSD of 2.0 Å), forming a crystallographic dimer in a ‘head-to-head’ configuration through an interaction between D2 of each receptor molecule (Figure 1B). The C-terminus of the receptor is more complete in chain A, forming a crystal contact with a protein molecule in a neighbouring asymmetric unit. The absence of density for the complete C-terminus may be a result of disorder, or due to the presence of endoprotease Glu-C during the crystallization experiment. N-linked glycans are observed at N105 and N172.

D1 of IL-11Rα forms an Ig-like domain with an unusual s-type topology (22) (Figure 1C). Strand A in the β sandwich forms a non-canonical mixed parallel/anti-parallel β sheet with strands G, F, C and D. This is a similar overall topology to D1 of IL-6Rα, in both cases the Ig-fold is distorted (21). Two disulfide bonds are present in D1, one between C26 in the strand B/strand C linker and C72 in strand F. A disulfide bond in a similar position is present in the D1 of IL-6Rα (21). A second disulfide bond is present between C4 and C25 in strand B, which was not predicted from sequence analysis or homology to other receptors. The disulfide bond is well-supported in the electron density and confirmed in a simulated-annealing omit
The unusual fold of D1 may be a consequence of these disulfides, with the C4-C25 disulfide serving to sterically constrain strand A, preventing the formation of a typical anti-parallel β-sheet with strands B and E.

D2 of IL-11Rα contains the two disulfide bonds expected for this domain (between C98 and C108, and between C148 and C158). D3 of IL-11Rα contains the conserved tryptophan-arginine ladder (comprising tryptophan residues 246, 282, and 285 and arginine residues 235, 239, 270 and 274), which includes the strongly conserved WSXWS sequence motif. Like other cytokine receptors, the sequence containing the WSXWS motif forms a short polyproline type-II helix that is stabilised by sidechain-mainchain interactions and the tryptophan-arginine ladder.

The interface formed between the two IL-11Rα monomers in the asymmetric unit of the crystal structure has a buried surface area of 1088 Å² (Figure 1B) (23). To establish whether IL-11RαEC self-associates in solution, we used sedimentation velocity-analytical ultracentrifugation (SV-AUC) at protein concentrations of 6.5 μM-19.5 μM, (0.25 - 0.75 mg/mL) (Figure 1D; Figure S1Ei). These experiments show that IL-11RαEC is predominantly monomeric in solution with a standardised sedimentation coefficient (s_20,w) of 2.70 at 13.0 μM. This represents a molecular mass of 41.3 kDa, with a frictional ratio (f/f₀) of 1.57 calculated from the fit to the SV data (Figure S1Ei), in good agreement with the expected molecular mass from the sequence (38.2 kDa). The theoretical sedimentation coefficient, calculated from the crystal structure coordinates of chain A using HYDROPRO (24) was 2.92, consistent with the experimental value. A small, concentration-dependent increase in weight-average sedimentation coefficient was observed, from 2.67 S at 6.5 μM to 2.72 S at 19.5 μM, likely indicating the formation of a weak-affinity dimer. It is possible that any weak-affinity dimerisation is increased at the cell membrane, where the receptor may be concentrated in lipid rafts, analogous to other cytokine receptors (25,26), and can diffuse in only two dimensions, increasing its effective concentration.

To study the solution properties of IL-11Rα without the C-terminal extension, we generated a construct comprising only domains D1-D3 (IL-11RαD1-D3; residues 1-297 of the mature protein). The standardised sedimentation coefficient of IL-11RαD1-D3 measured at a protein concentration of 15.5 μM (0.5 mg/mL) was 2.62 S (Figure 1E; Figure S1Eii), corresponding to a molecular weight of 34.9 kDa, with f/f₀ of 1.47, in good agreement with the sequence molecular weight (32.1 kDa). Similar to IL-11RαEC, a small concentration-dependent increase in weight average sedimentation coefficient was observed from 2.62 S at 7.8 μM to 2.69 S at 23.3 μM, suggesting that possible weak dimerization is mediated by the structured, extracellular domains of IL-11Rα and is not a consequence of the disordered C-terminus. The small-angle X-ray scattering (SAXS) profile of IL-11RαD1-D3 agrees well with the monomer of the crystal structure coordinates (χ² = 1.05) (Figure 1F; Table S1; Figure S1F), confirming that the crystal structure accurately represents the solution conformation of the structured, extracellular domains of IL-11Rα.

Pathogenetic mutations disrupt the structure of IL-11Rα

A number of pathogenic mutations have been identified in the gene for IL-11Rα, IL11RA, resulting in point-substitution mutations in IL-11Rα that cause a genetic disease featuring craniosynostosis and delayed tooth
eruption (11-14). Mapping the disease-associated mutations onto our structure of IL-11Rα indicates that very few of the mutations are in the putative IL-11 or gp130 binding sites (Figure 2A). The P178T, P199R and R274W mutations have previously been studied in vitro (11), however the lack of structural information on IL-11Rα has hindered understanding of the molecular impact of the mutations.

To investigate the effects of the mutations on the structure of IL-11Rα, we ran a series of short (50 ns) all-atom molecular dynamics (MD) simulations on IL-11Rα (Figure 2B) and several of the disease mutants. In IL-11Rα, the Cα RMSD and backbone amide bond order parameters (S²) calculated from the MD trajectory indicate a low level of local disorder, and overall local rigidity within each of the three domains (Figure 2B; Figure S2A). However, the three domains were dynamic with respect to each other throughout the simulation (Figure 2B; Figure S2B). The loops comprising the putative IL-11 binding site were relatively rigid and do not undergo large motions on the timescale of the simulation.

MD simulations of IL-11Rα with the disease-associated mutations suggest that several of these destabilise key structural elements in the receptor or destabilise inter-domain interfaces. One mutation, C72F, removes a disulfide bond in D1, which likely has a role in stabilizing the unusual Ig-fold of D1. Introducing this mutation to D1 resulted in the loop joining strands F and G adopting a markedly different conformation, which may alter the stability of the domain (Figure 2C; Figure S2C; Movie S1).

A second mutation, P178T, is located in a loop in D2 that faces D1. This mutation resulted in a shift in the relative pose of D1 and D2, likely due to removal of the interaction of P176 with a pocket on D1 that stabilises the D1/D2 interface. However, in each replicate simulation the final relative orientation of D1 and D2 differed. (Figure 2D; Figure S2D; Figure S2E; Movie S2).

The R274W mutation is situated within the tryptophan-arginine ladder in D3 of the receptor. This mutation destabilised the tryptophan-arginine ladder, and resulted in the destabilization of the membrane-distal region of D3. R274 also contributes to a hydrogen-bonding network at the D2/D3 interdomain interface in the wild-type receptor (Figure 2E; Figure S2F; Movie S3). The mutation thus destabilizes the D2/D3 linker, and results in an increase in flexibility at the D2/D3 interface, potentially disrupting the IL-11 binding interface and reducing cytokine affinity.

The P199R mutation is located in the D2-D3 inter-domain linker. The mutation causes a slight increase in the D2-D3 interdomain distance but does not otherwise greatly alter the inter-domain pose or dynamics of IL-11Rα (Figure 2F; Figure S2G; Movie S4).

Several other pathogenic mutations have little appreciable impact on the structural dynamics within the timescale of the simulation. For example, P43T does not greatly alter the flexibility of the affected loop in D1, C108S does not appear to significantly alter D2 through the simulation, nor does R239C destabilize D3 or the tryptophan-arginine ladder in which it is situated (Figure S2H-J). One mutation (H276R) is close to the putative gp130 binding region of D3, and thus may act by directly altering signalling complex formation at this interface.

Together our simulations show that the effect of a subset of the craniosynostosis mutations in IL-11Rα is to destabilise the structure of
IL-11Rα. The P176T, R274W and P199R mutations have previously been shown to result in incomplete glycosylation leading to retention in the endoplasmic reticulum and poor cell surface expression, contributing to reduced IL-11 mediated STAT3 activation (11). Our results suggest that destabilisation of the structure due to the P176T and R274W mutations is sufficient to stall correct trafficking of the receptor. D1 of IL-6Rα has previously been shown to be involved in intracellular trafficking of the receptor (27). Thus, destabilisation of D1 or the D1/D2 interface in IL-11Rα by the P176T mutation may result in a lack of correct processing of the receptor. In the case of the R274W and P199R mutations, destabilisation of the cytokine binding surface at the junction between D2 and D3 may also reduce IL-11 binding capacity of mutant IL-11Rα that is correctly expressed at the cell surface, further reducing the potential for formation of the active signaling complex. The apparently minor structural effects of some mutations, such as P199R, that are positioned distal to the putative cytokine and gp130 binding regions of the receptor, suggest alternative mechanisms that impair IL-11 signalling, for example, disruption of protein expression or global receptor folding.

The high-resolution structure of IL-11

In our previous structure of human IL-11 parts of the long loops between helices A and B and between helices C and D were poorly defined (17). Mutagenesis suggests that the AB loop is involved in binding IL-11Rα (28), and in the structure of the IL-6 signalling complex, the AB loop forms contacts to both IL-11Rα and gp130 (6). To gain insight into these loops, we solved a higher-resolution structure of IL-11.

To facilitate growth of crystals that diffracted to high resolution we truncated IL-11 by ten residues at the N-terminus. We named this new construct IL-11Δ10 (residues 11-178 of the mature protein) and the ‘full-length’ protein IL-11FL. Both IL-11FL and IL-11Δ10 have similar high thermal stability, as measured by differential scanning fluorimetry (Figure S3A) (29). Stimulation of human colon cancer cell line, DLD1 with either IL-11Δ10 or IL-11FL results in similar levels of activation of STAT1 and STAT3 (Figure 3A) indicating that they have similar biological activity. We note that N-terminally truncated IL-11 constructs have been used previously with no reported alteration in biological activity (30,31).

Crystals of IL-11Δ10 were rod-like plates in space group P21212. Initial phase estimates were obtained by molecular replacement using our previous structure of IL-11 (PDB ID: 4MHL) and the new structure was refined at a resolution of 1.62 Å (PDB ID: 6O4O; see summary statistics in Table 1 and representative electron density in Figure S3B). Overall, the structure of IL-11Δ10 is similar to our previously solved structure of IL-11 (RMSD 1.5 Å), forming a typical cytokine four-α-helical bundle (Figure 3B). The three receptor binding sites of the cytokine are not significantly altered in the structure (Figure 3C) (17). A cis proline (P103) is observed at the C-terminal end of the 310 helical section of helix C. The equivalent proline in our previous structure of IL-11 is in the trans configuration (Figure S3D). Both proline isomers are strongly supported by electron density in their respective structures, suggesting that P103 can adopt either the cis or trans isomer and that the 310 helix is dynamic in solution.

Our high-resolution structure of IL-11Δ10 allows the extended loops joining helices A and B and helices C and D to be included in the model. The AB loop is formed by 26 residues between F43 and L69 (Figure 3D). The position of the loop is stabilised by a...
hydrogen bond between S53 and H86 in helix B, this region of the loop is thus well-defined in the electron density. Mutagenesis has previously implicated the C-terminal end of the loop in receptor binding (28). This portion of the loop is adjacent to site-I, and poorly defined in the electron density.

The CD loop of IL-11 forms an unusually long polyproline type II (PP2) helix (Figure 3E), comprising 14 residues. The CD loop is stabilised by several contacts between the loop and the core of the cytokine (Figure 3E). To our knowledge, an equivalently long polyproline helix has not been observed in the structure of any other cytokine. The role of the PP2 helix is likely structural, to efficiently join the C-terminal end of helix C and the N-terminal end of helix D, which are 44 Å apart, with a relatively short sequence of 21 residues.

To further study the dynamic nature of the loops of IL-11, we ran a series of short (100 ns) molecular dynamics simulations on IL-11 (Figure 3F). In the timescale of the simulation, the four-α-helical bundle was stable and did not undergo large movements (Figure 3F; Figure S3E). In agreement with NMR studies of other IL-6 family cytokines, the α-helices showed ‘helical fraying,’ and were more dynamic at the ends of the helices, compared to the core (Figure 3F) (32,33). The PP2 helix structure of the CD loop was preserved throughout the simulation, although the loop underwent lateral movements. The AB loop was generally highly dynamic on the timescale of the simulation, although the loop underwent lateral movements. The AB loop was generally highly dynamic on the timescale of the simulation, although the central portion of the loop was stabilised by interactions with the α-helical core. The C-terminal end of the loop, which is implicated in IL-11α binding, was highly dynamic on the timescale of the simulation.

We also used SV-AUC to show that IL-11Δ10 is monomeric, with no concentration-dependent increase in sedimentation coefficient. The sedimentation coefficient was measured at 1.7 S, representing a molecular mass of 17.2 kDa (f/f₀ 1.28), in agreement with the sequence molecular mass (18.2 kDa). The theoretical sedimentation coefficient calculated from the crystal structure was 1.8 S, in good agreement with the experimental value. SAXS data for IL-11Δ10 also agrees well with the theoretical scattering profile calculated for the crystal structure coordinates (χ² = 1.43) (Figure 3H; Table S1; Figure S3G). These experiments confirm that IL-11Δ10 is monomeric in solution. Similar experiments show that IL-11FL is monomeric in solution (Figure S4A-D).

**IL-11 and IL-11Ra interact with nanomolar affinity**

We used SV-AUC to investigate the interaction between IL-11 and IL-11Ra. For these experiments, the complex was formed by mixing 5 μM IL-11Δ10 and 5 μM IL-11RaEC immediately prior to the experiment, with no further purification. The appearance of a peak in the c(s20,w) distribution with a sedimentation coefficient of 3.2 S, larger than either IL-11Δ10 and IL-11RaEC alone, indicated formation of a complex between IL-11RaEC and IL-11Δ10 (Figure 4A). The estimated molecular weight of this species was 60.8 kDa, with f/f₀ of 1.71, consistent with a complex forming with 1:1 stoichiometry (Figure S5A). A similar complex was formed between IL-11Δ10 and IL-11RaΔD1-D3 (Figure 4Aii: sedimentation coefficient 3.3, molecular weight 55.8 kDa, f/f₀ 1.61), IL-11FL and IL-11RaEC (sedimentation coefficient 3.2, molecular weight 60.5 kDa, f/f₀ 1.71) and between IL-11FL and IL-11RaΔD1-D3 (Figure S6A and S7D; sedimentation coefficient 3.3, molecular weight 55.9 kDa, f/f₀ 1.61).
We used multi-angle light scattering coupled with size-exclusion chromatography (SEC-MALS) to provide additional evidence for the formation of a 1:1 complex between IL-11 and IL-11Rα. We measured the absolute molecular weight ($M_w$) of IL-11Δ10 as 21.0 kDa, IL-11RαD1-D3 as 36.3 kDa (Figure S6B), and the IL-11Δ10/IL-11RαD1-D3 complex as 51.1 kDa (Figure 4C), consistent with a 1:1 complex.

To determine the dissociation constant for the IL-11/IL-11Rα interaction, we used fluorescence-detected SV-AUC (FD-AUC), which can accurately measure proteins present at nanomolar and picomolar concentrations (34). We expressed IL-11FL N-terminally fused to a monomeric, ultrastable (μ) green fluorescent protein (GFP) (35). SV-AUC showed that μGFP-IL-11 is monomeric across a wide concentration range (Figure S6C, Figure S8E) and forms a complex with IL-11RαEC in a 1:1 stoichiometry at concentrations of 5 µM of each component (Figure 4C; Figure S5B). Complex formation was apparent at concentrations of IL-11RαEC in the nanomolar range, with two peaks observed in $c(s20,w)$ distributions corresponding to free μGFP-IL-11 and μGFP-IL-11 in complex with IL-11RαEC (Figure 4D; Figure S5C). We generated a sedimentation coefficient isotherm for the titration of IL-11RαEC against μGFP-IL-11, which, when fit to a 1:1 binding model, gave a $K_D$ of 22 nM ($68\%$ confidence interval 14-35 nM] (Figure 4E; Figure S5C). This is consistent with the dissociation constant for similar site I cytokine/α-receptor interactions. For example, IL-6 and IL-6Rα interact with a $K_D$ of 9 nM (6), IL-2 and IL-2Rβ interact with a $K_D$ of 144 nM (36) and IL-7 interacts with IL-7Rα with a $K_D$ of approximately 50 nM (37). In each of these cases, the complete signalling complex is formed by further high-affinity interactions between the cytokine/α-receptor complex and other receptors. These experiments show that the IL-11/IL-11RαEC interaction also fits into this paradigm; an initial low-nanomolar affinity step to form the complex between IL-11 and IL-11Rα occurs first, allowing subsequent engagement by gp130.

The tendency of GFP to form weakly-associating dimers with a $K_D$ of approximately 100 µM has previously limited the use of GFP in quantitative biophysical experiments (38). The monomeric, ultrastable GFP used here does not detectably dimerise (35), allowing it to be used as a genetically-encoded fluorescent tag for biophysical experiments. Previous efforts to use FD-AUC to measure high-affinity protein-protein interactions have generally relied on covalent modification of one of the interacting partners with a fluorescent dye, with previous studies noting that the use of covalent dyes as fluorescent labels alters the binding properties of the proteins under investigation (39). The use of a genetically encoded, monomeric fluorescent fusion tag overcomes this limitation, allowing the accurate measurement of nanomolar-affinity dissociation constants in the analytical ultracentrifuge, without requiring the covalent modification of one of the proteins involved in the interaction.

The IL-11/IL-11Rα interaction is entropically driven

We used isothermal titration calorimetry (ITC) to complement our FD-AUC binding experiments above, and to examine the thermodynamic basis of cytokine-receptor engagement (Table 2). ITC showed that IL-11Δ10 interacts with IL-11RαEC and IL-11RαD1-D3 with similar affinities, with $K_D$ values of 40 ± 20 nM and 23 ± 3 nM respectively (n=3, standard error; Figure 5Ai and 5Aii). These values are consistent with our AUC experiments and show that the C-
terminal extension of IL-11Ra does not affect IL-11 binding. We also measured the affinity for the interaction between IL-11FL and IL-11RαEC, $K_D$ of 55 ± 14 nM (n=3, standard error; Figure S6D), showing that deletion of the N-terminus of IL-11 does not significantly alter affinity for IL-11Rα ($p = 0.58$). The thermodynamics of the IL-11Δ10/IL-11RαD1-D3 interaction are strongly driven by entropy ($ΔH = -25 ± 2$ kJ/mol, $ΔS = 66 ± 7$ J/(mol·K)). We also measured the IL-11Δ10/IL-11RαΔ1-1-D3 interaction using ITC at two additional temperatures (283 and 298 K) to determine the heat capacity of the reaction, $ΔC_p$ (Figure S6Ei-iii, Table 2). The heat capacity was measured as $-3.3 ± 0.07$ kJ/(mol·K) (mean ± standard error). An empirical relationship exists between heat capacity and total buried surface area, a large negative $ΔC_p$ being consistent with a large buried surface area (40-42). This suggests that the IL-11/IL-11Rα interaction is hydrophobic in nature, resulting in the burying of a large hydrophobic surface.

The cytokine binding site of IL-11RαEC lacks large charged or hydrophilic regions, consistent with a hydrophobic interaction that is primarily driven by a positive change in entropy. This contrasts strongly with the IL-6/IL-6Rα interaction, which is strongly exothermic, with a corresponding unfavourable entropy change ($ΔH = -100$ kJ/mol, $ΔS = -192$ J/(mol·K) at 10 °C), a consequence of the structural differences between the two cytokines and receptors (6). Thus, in spite of apparent structural similarity, IL-6Rα and IL-11Rα employ different thermodynamic mechanisms to engage their cognate cytokines.

A model of the IL-11/IL-11Rα binary complex provides detail of the structural mechanism of engagement.

Cytokines generally bind to the CHR surface at the junction between FnIII-type domains D2 and D3, with D3 also involved in interacting with other receptors comprising the complete signalling complex (1). This region of IL-11Rα is made up of four loops, formed by residues 98-106 (between strands A and B), 129-145 (between strands C and D) and 160-169 (between strands D and E) in D2, and residues 220-232 (between strands B and C) in D3. Part of the loop between strands C and D of D2 (residues 132-139 of chain A and 132-141 of chain B) was not defined in the electron density. To our knowledge, a similar large and disordered loop in the CHR has not yet been described for any other cytokine receptor. The membrane-proximal region of D3 serves to engage gp130, to complement the Site-II interaction on the cytokine. This region is similar in topology and surface charge in both IL-6Rα and IL-11Rα, suggesting that the mechanism of α-receptor engagement with gp130 is similar between the two receptors.

The configuration of the CHR differs between IL-11Rα and IL-6Rα (Figure S1Ci). In IL-11Rα, the relative positioning of D2 and D3, which is more similar to that of gp130 (Figure S1Cii), creates a smaller cytokine binding surface than IL-6Rα. The electrostatic surface potential in the cytokine binding sites also differ between the two proteins (Figure S1D). The IL-6 binding site in IL-6Rα is noticeably more charged than that of IL-11Rα, with a negatively charged patch formed by several acidic residues in the loop formed between strands F and G in D3, which mediate a number of electrostatic contacts to IL-6 in the IL-6 signalling complex (Figure S1D) (6,21). These structural differences suggest that IL-11Rα employs different structural mechanisms from IL-6Rα to engage its cognate cytokine at Site-I.

To investigate the structural mechanism of IL-11 binding by IL-11Rα, we constructed a
model of the IL-11/IL-11Rα complex. Using the structure of the IL-6 signalling complex (PDB ID: 1P9M (6)), we aligned IL-11 and IL-11Rα to their homologous chains in the IL-6 complex, and refined this model using RosettaDock of the Rosie server (43,44). Models were scored using RosettaDock and the top-scoring model was taken as the representative model (Figure 5B). An overlay of the initial model and the final model is shown in Figure S9A, the top-ten scoring models are shown in Figure S9B. Relative to the initial model, the docked model shows a significant rotation of the pose of cytokine with respect to the binding site on the receptor. The model shows that the missing CD loop in D2 of IL-11Rα, which we did not include in the model, is in close proximity to the binding site.

Our model has a buried surface area of 567 Å² at the interface between IL-11 and IL-11Rα, similar to that of the IL-6/IL-6Rα interface in the IL-6 signalling complex (706 Å²). This is consistent with the initial cytokine-receptor interaction forming a transiently stable complex. The pose of D2 with respect to D3 of IL-11Rα is different to that of IL-6Rα resulting in a differently-shaped cytokine binding surface (Figure S9C), which may account for the small difference in buried surface area. The binding mode of the cytokine is overall similar, consistent with previous mutagenesis on IL-11, and our structure of IL-11 (17,45-47).

SAXS analysis of the IL-11/IL-11Rα complex supports the docked model. The complex was formed by mixing IL-11RΔD1-D3 and IL-11Δ10 at an equimolar ratio, prior to SAXS measurement. The molecular mass was measured as 50.1 kDa, consistent with a 1:1 complex, and in agreement with the mass and stoichiometry determined by SV-AUC and SEC-MALS (Table S1). Theoretical scattering for the docked model fits the experimental SAXS data well ($\chi^2 = 1.03$) (Figure 5C; Table S1; Figure S7A), showing that the model accurately represents the overall shape of the binary IL-11Rα/IL-11 complex. Similarly, the model agrees well with an ab initio model of the complex, generated using DAMMIN (Figure S9D, E). Likewise, the theoretical sedimentation coefficient (3.3 S) matches the experimentally determined sedimentation coefficient of the IL-11RΔD1-D3/IL-11Δ10 complex (3.3 S) (Figure 4Aii), further supporting the 1:1 stoichiometry of the complex.

We used the PISA server to analyse the interactions formed between the two proteins in the docked model. The major interacting residues of IL-11 are R33, M59, A61, G62, and several residues in the C-terminus of the cytokine, particularly R169 (Figure 5B). R33, in the N-terminal helix of the cytokine, and H182 helix D, both form hydrophobic interactions with F276, L277 and D278 in the FG loop in D3 of the receptor. Similar contacts are formed in the five top scoring models. An extensive contact is formed between the C-terminal region of the cytokine and the receptor in the model. IL-11 residues D165, W166, R169, L172 and L173 form an extensive hydrophobic interaction with H229 and F230 in the BC loop of D3 of the receptor, with a small contribution from Y103 in the AB loop of D2. A contact is also formed by M59, A61 and G62 in the AB loop of IL-11 with Y166 in the EF loop of D2 of the receptor.

As R169 of IL-11 makes a key intermolecular contact in our model, we constructed and purified the IL-11Δ10/R169A mutant. SV-AUC analysis of 5 μM IL-11RΔD1-D3 in the presence of 5 μM IL-11Δ10 R169A results in a peak in the sedimentation coefficient distribution of approximately 2.7 S (Figure 5D; Figure S7A), less than that for the IL-
IL-11/IL-11RαD1-D3 complex (3.3 S), showing that the R169A mutation substantially decreases affinity for IL-11Rα. Stimulation of DLD1 cells with IL-11Δ10/R169A showed greatly reduced potency in activation of STAT3 than the wild-type cytokine (Figure S7B), consistent with the reduction in IL-11Rα binding leading to impaired formation of the active signalling complex. Residues important for biological activity of IL-11 have previously been identified by site-directed mutagenesis of human and mouse IL-11 (45-47), and these mutagenesis experiments further support our model (Figure S9F). Substitution of R33, D165, W166, R169, L172 and L173 all reduce the biological activity of IL-11 (45-47). The N-terminal region of the AB loop of IL-11, containing the interacting residues M59, A61 and G62 has previously been targeted by phage-display and mutagenesis to alter the binding of IL-11 to IL-11Rα, thus, this region has also been shown to be key for the interaction.

Our model predicts that IL-11 forms interfaces of 225 Å² and 369 Å² with D2 and D3 respectively. Previously, the isolated D3 of IL-11Rα was reported to bind IL-11 with an affinity of 48 nM (20). We expressed, purified and re-folded D3 of IL-11Rα (IL-11RαD3; residues 192-315 of the mature protein) from E. coli inclusion bodies. SV-AUC analysis of IL-11RαD3 showed a single, narrow peak in the c(s20,w) distribution with sedimentation coefficient of 1.5 S (calculated from the fit to the data at 28 μM) and no concentration dependent change (Figure S7C; Figure S8F), indicating a homogenous product that did not self-associate in the concentration range measured. Circular dichroism spectra of the re-folded protein showed a characteristic all-β spectrum, with a positive peak at 230 nm, likely due to π-stacking interactions in the WSXWS motif (48) (Figure S7D). 15N-1H heteronuclear single quantum coherence (HSQC) spectra from the purified, re-folded IL-11RαD3 were well-dispersed and showed seven resolved tryptophan indole NH resonances of different intensities and line-widths, indicating that the protein is folded (Figure S7E).

SV-AUC analysis of IL-11RαD3 (5 μM) with increasing concentrations of IL-11Δ10 showed no concentration dependent increase in sedimentation coefficient, with weight average S20,w of 1.67 S at 10 μM IL-11Δ10, 1.66 S at 20 μM, and 1.62 S at 40 μM (Figure 5E; Figure S8B). As the theoretical sedimentation coefficient of the IL-11/IL-11RαD3 complex is 2.62 S, these data suggest that IL-11RαD3 does not bind IL-11 with high affinity.

An apparently unique feature of the IL-11 binding site in IL-11Rα is a dynamic loop between strands C and D in D2 of the receptor. Our model of the binary complex suggests that this loop may contact bound cytokine and, therefore, could have a role in binding IL-11, through the formation of polar contacts between the loop and cytokine or by providing additional buried surface area. To investigate this, we generated a construct, IL-11RαD1-D3/Δloop, in which residues 132 to 140 in the loop were removed and replaced by two glycine residues. SV-AUC showed that IL-11RαD1-D3/Δloop is monomeric in solution and formed a complex with IL-11Δ10 with the expected 1:1 stoichiometry (Figure 5F; Figure S7C). ITC showed that the KD of the interaction between IL-11Δ10 and IL-11RαD1-D3/Δloop is 8 ± 4 nM (n=3; Figure 5Aiii), not significantly different from that of IL-11Δ10 and IL-11RαD1-D3 (p = 0.21). Thus, removal of the loop does not significantly alter the affinity for the interaction between IL-11Rα and IL-11Δ10, suggesting that the loop does not participate directly in cytokine binding.
It is possible that the dynamic loop functions to partially shield the hydrophobic regions of the cytokine binding surface in the absence of cytokine, thereby reducing the potential of this region to participate in deleterious, non-specific interactions. This function would be consistent with our observation that other cytokine receptors that have more hydrophilic character at their cytokine binding regions, such as IL-6Rα, do not possess this dynamic loop structure.

Conclusion
The increasing identification of roles for IL-11 in a broad range of diseases underscores the need to thoroughly understand the structure of IL-11, its receptors, and the overall molecular mechanism of IL-11 signalling complex formation. Here, we have solved the crystal structure of human IL-11Rα and a new structure of human IL-11 that reveals detail of functionally important loop regions. We show that several mutations in IL-11Rα that are associated with disease act to disrupt key structural elements in IL-11Rα, for example through disrupting interdomain interfaces, or conserved structural motifs within the receptor. We present a model of the complex and support this model through biophysical and mutagenic analysis. We propose that a dynamic loop proximal to the cytokine binding region of IL-11Rα functions to protect this region from nonspecific interactions. Our data elucidate the structural and thermodynamic mechanisms of IL-11 binding by IL-11Rα and show that this engagement is mediated by both D2 and D3 of the receptor. Together, this work reveals key structural determinants of cytokine engagement by IL-11Rα on the pathway to formation of the active signalling complex. This molecular detail can be exploited in future development of agents that can modulate this process.

Experimental procedures
Protein expression and purification
Human IL-11RαEC with N-terminal honeybee-melittin signal peptide and C-terminal 8x His tag, was expressed in SF21 insect cells. Recombinant protein was purified using nickel-affinity chromatography and gel filtration chromatography. IL-11RαD1-D3 and IL-11RαD1-D3/Δloop with N-terminal honeybee-melittin signal peptide, 8x His tag, and TEV cleavage site were expressed in SF21 cells. Recombinant protein was purified from conditioned media using nickel-affinity chromatography and gel filtration chromatography. Cleavable tags were removed using TEV protease. IL-11RαD3 was refolded and purified from bacterial inclusion bodies as previously described (20). All IL-11Rα constructs contained the C226S mutation to reduce formation of disulfide crosslinked dimers (20).

IL-11FL, IL-11Δ10, and IL-11Δ10/R169A with N-terminal 6x His tag, maltose binding protein, and a TEV protease cleavage site (MBP-IL-11FL or MBP-IL-11Δ10) were expressed in BL21(DE3) E. coli cells. All constructs were purified by nickel-affinity chromatography, followed by cation exchange chromatography, and gel-filtration chromatography. Tag removal was achieved using TEV protease. muGFP-IL-11 was expressed and purified as above with no TEV cleavage.

Crystallization and X-ray diffraction data collection
IL-11RαEC was crystallized using the sitting-drop vapor diffusion method. Initial crystals were obtained at 293 K in the precipitant 28% PEG 400, 0.2 M calcium chloride and 0.1 M sodium HEPES pH 7.5. Crystallization drops were produced by mixing 1.1 mg/mL IL-11RαEC in a ratio of 1:0.9:0.1 with the precipitant and the endoproteinase Glu-C.
Spherulites appeared after 24 hours, these were used to prepare a micro-seed stock (49). Subsequent seeding gave needle clusters in the condition 20% PEG 3350, 0.2 M lithium citrate. Seeding using these needle crystals produced single crystals in the condition 0.1 M HEPES pH 8, 20 mM sodium chloride, 1.6 M ammonium sulfate, 67 mM NDSB-195. Crystallization drops were produced by mixing 1.5 μL IL-11RαEC (1 mg/mL), 0.65 μL precipitant, 0.4 μL NDSB-195, 0.15 μL Glu-C and 0.3 μL seed. Spindle-like crystals appeared after 48 hours and grew to approximate dimensions 20 μm × 7 μm × 7 μm.

IL-11Δ10 was crystallised using the sitting-drop vapor diffusion method. Crystals were obtained at 293 K in the precipitant 30% PEG 3350, 0.2 M ammonium sulfate, 0.1 M Tris pH 8.5. Crystals appeared after 24 hours as thick bundles of two-dimensional plates. These crystals were used for micro-seeding, providing single crystals in the precipitant 18% PEG 3350, 0.1 M Bis-Tris Propane pH 9, 0.2 M ammonium sulfate, 5 mM praseodymium chloride. Crystallisation drops were produced by mixing 1.5 μL IL-11Δ10 (5 mg/mL), 1.5 μL precipitant and 0.5 μL seed. Plates appeared overnight and grew to approximate dimension 500 × 20 × 5 μm after equilibration against precipitant for one week.

Crystals were flash-cooled in liquid nitrogen directly from crystallization drops, and X-ray diffraction data were collected at 100 K at the Australian Synchrotron MX2 beamline (50).

**X-ray diffraction data processing and structure refinement**

Diffraction data were indexed, integrated and scaled using XDS (51), analysed using POINTLESS (52) and merged using AIMLESS (53) from the CCP4 suite. Initial phase estimates for IL-11Rα were obtained by molecular replacement with Phaser (54), using individual domains of IL-11Rα from unpublished Fab-bound structures as the search models. Refinement was performed using phenix.refine with non-crystallographic symmetry torsion restraints (55), followed iteratively by manual building using Coot (56). Several cycles of simulated annealing were performed early in the refinement to reduce potential model bias. Translation/libration/screw (TLS) refinement was performed in the final rounds, with each domain defined as a separate TLS group. Simulated annealing composite omit maps were calculated using Phenix.

Initial phase estimates for IL-11Δ10 were obtained using molecular replacement with Phaser (54), using our previous structure of IL-11 (PDB ID:4MHL) (17) as the search model. Auto-building with simulated annealing was performed in phenix.autobuild to reduce phase bias from the search model. Refinement was performed in phenix.refine (55) with iterative manual building using Coot (56). TLS refinement was performed using a single TLS group containing all protein atoms. Explicit riding hydrogens were used throughout refinement and included in the final model, the atomic position and B factors for hydrogens were not refined. Structures were visualised in Pymol, and aligned with the CE (57) algorithm in Pymol. Buried surface area was determined using the PISA server (23).

Residues of both structures are numbered according to the mature protein sequence after cleavage of signal peptides.

**Absorbance-detected sedimentation velocity analytical ultracentrifugation**

Absorbance-detected SV-AUC experiments were conducted using a Beckman Coulter XL-I analytical ultracentrifuge, equipped with UV-visible scanning optics. Reference
and sample solutions were loaded into double-sector 12 mm cells with quartz windows and centrifuged using an An-60 Ti or An-50 Ti rotor at 50,000 rpm (201,600 g) and 20 °C. Radial absorbance data were collected at 280 nm, in continuous mode. All experiments were conducted in TBS (20 mM Tris, 150 mM sodium chloride) pH 8 or 8.5. IL-11Δ10 and muGFP-IL-11 was centrifuged at concentrations of 0.8, 0.4 and 0.2 mg/mL. IL-11Rα was centrifuged at concentrations of 0.75, 0.5 and 0.25 mg/mL. Complexes of IL-11 and IL-11Rα were prepared by mixing 5 μM each of IL-11 and IL-11Rα and centrifuged without further purification. Sedimentation data were fitted to a continuous sedimentation coefficient, c(\text{s}), model, and the frictional ratio (f/f₀) was fit using SEDFIT (58). Buffer density, viscosity and the partial specific volume of the protein samples were calculated using SEDNTERP (59). For the complexes between IL-11 and IL-11Rα, and muGFP-IL-11 and IL-11Rα, the partial specific volume used was 0.73 mL/g. The theoretical sedimentation coefficients of IL-11Δ10 and IL-11Rα were calculated using HYDROPRO, using standard conditions (water, 20 °C) (24).

**Fluorescence-detected sedimentation velocity analytical ultracentrifugation**

Fluorescence-detected SV experiments were conducted using a Beckman XL-A analytical ultracentrifuge, equipped with an Aviv Biomedical fluorescence detection system. Sample solutions were loaded into double-sector 12 mm cells with quartz windows and centrifuged using an An-50 Ti rotor. Experiments were conducted at 50,000 rpm (201,600g) and 20 °C. muGFP-IL-11 was centrifuged at a concentration of 150 nM (0.007 mg/mL).

To generate the sedimentation coefficient isotherm, the concentration of muGFP-IL-11 was 150 nM, and 1.5-fold serial dilution series of IL-11Rα was prepared starting from a concentration of 1 μM in TBS pH 8.0. To prevent non-specific absorption of muGFP-IL-11 to cell components, 0.2 mg/mL κ-casein (Sigma-Aldrich) was added to the samples (34). Sedimentation velocity data were processed in SEDFIT as above. c(s) distributions were integrated between 1.0 S and 6.0 S. The isotherm was fitted to a 1:1 hetero-association model in SEDPHAT, with Kₐ and the sedimentation coefficients of muGFP-IL-11 and the complex floated in the analysis (60). The 68% confidence interval was estimated using SEDPHAT.

**Small-angle X-ray scattering**

SAXS experiments were conducted at the Australian Synchrotron SAXS/WAXS beamline, using co-flow to limit radiation damage and allow higher X-ray flux onto the sample, and an optimised chromatography system to limit sample dilution (61-63). The X-ray beam energy was 11,500 eV (λ = 1.078 Å). For IL-11Δ10 and IL-11FL, the sample-to-detector distance used was 2038 mm, providing a total q range of 0.007-0.664 Å⁻¹, q=(4πsinθ)/λ. For IL-11RαD1-D3 and the IL-11RαD1-D3/IL-11Δ10 complex, the sample-to-detector distance used was 2539 mm, providing a total q range of 0.006-0.534 Å⁻¹. Data were collected following fractionation with an in-line size-exclusion chromatography column (Superdex 200 5/150 Increase, GE Healthcare,) pre-equilibrated in TBS pH 8.5, 0.2 % sodium azide. The IL-11RαD1-D3/IL-11Δ10 complex was prepared by mixing IL-11RαD1-D3 and IL-11Δ10 in a 1:1.5 molar ratio. Data were collected from a 1.5 mm capillary under continuous flow, with frames collected every second. Data reduction and was performed using the Scatterbrain software, SEC-SAXS analysis using CHROMIXS (64) and the ATSAS suite (64,65). Theoretical scattering profiles from the crystal structure coordinates were calculated and fit to the experimental
scattering data using CRYSOl (66). Ab initio models were calculated using DAMMIF (67) and DAMMIN (68,69). Ten models were calculated using DAMMIF, the models averaged using DAMAVER, the averaged model was used as a starting model for DAMMIN. A summary of the SAXS data acquisition and processing is given in Table S1.

Molecular-dynamics simulations
All MD simulations were performed using NAMD 2.1.3b1 (70) and the CHARMM22 forcefield (70,71) at 310 K in a water box with periodic boundary conditions. Simulations were analysed in VMD 1.9.3 (72). A model of IL-11Rα was created based on chain B of the crystal structure. The missing loop (residues 132 to 141) was rebuilt using the PHYRE2 server (73). The missing loop was excluded from all representations of the trajectories and the analysis. The disordered C-terminus was not simulated in the model. The structures were solvated (box size 88.8 × 126.6 × 53.9 Å), and ions were added to an approximate final concentration of 0.15 M NaCl. Simulations of IL-11Rα was carried out with 10 ps minimisation, followed by 50 ns MD. Mutations were introduced to this equilibrated model, and a further simulation was carried out with 10 ps minimization, then 50 ns MD. An additional 50 ns MD was also performed for the un-mutated IL-11Rα. The interdomain distance distributions were calculated using a script in VMD, which defined a centroid for each of the three domains and measured the change in distance through the MD simulation. A model of the complete IL-11Δ10 structure was created based on the crystal structure. For residues with multiple orientations, only one orientation was selected. The structure was solvated (box size 53.6 × 53.1 × 74.9 Å), and ions added to approximate final concentration of 0.15 M NaCl. A MD simulation was performed using a 10 ps minimisation time, followed by 100 ns MD.

Differential scanning fluorimetry
Protein samples were analysed by DSF at a concentration of 0.1 mg/mL in TBS pH 8.5, with 2.5 × SYPRO Orange dye (Sigma Aldrich). 20 μL of the sample was loaded into 96-well qPCR plate (Applied Biosystems), and four technical replicates of each sample were analysed. The plates were sealed, and samples heated in an Applied Biosystems StepOne Plus qPCR instrument, from 4 °C to 95 °C, with a 1% gradient. Unfolding data were analysed using a custom script in MATLAB r2016a. The temperature of hydrophobic exposure (Tₘ), was defined as the minimum point of the first derivative curve, and used to compare the thermal stability of different proteins (29).

Isothermal titration calorimetry
Protein samples were buffer exchanged into TBS pH 8.5 using gel filtration before analysis by ITC. ITC data were collected at 303 K using a MicroCal iTC200 (GE Healthcare). Titrations were performed using 15 2.5 μL injections of IL-11, after an initial injection of 0.8 μL. IL-11Rα was present at a concentration of 10 μM and the concentration of IL-11 was 10-fold greater than the concentration of IL-11Rα. Titration data were integrated using NITPIC (74,75), and analysed in SEDPHAT using a 1:1 interaction model (60). Each titration was conducted in triplicate, values stated are the mean ± standard error of the mean.

In vitro cell culture
DLD1 cells were grown in RPMI+10% foetal calf serum, in a 5% CO₂ atmosphere. Cells were grown to confluency in 6-well plates, the media was removed and cells were treated with IL-11Δ10 or IL-11FL at a concentration of 50 ng/mL in RPMI, or RPMI as a vehicle control, and incubated for one hour. Cells
were then washed with cold PBS and lysed in RIPA buffer. Protein concentration was determined by the bicinchoninic acid (BCA) assay. Lysates were diluted with SDS-PAGE loading buffer, resolved on a 10% polyacrylamide gel and wet transferred to a nitrocellulose membrane. The membranes were blocked, incubated with the indicated primary antibodies (for phospho-STAT3 CST cat 9145; for phospho-STAT1 CST cat 9167, for total STAT3 CST cat 4904 for total STAT1 CST cat 9172, for actin Sigma cat A1978), then detected using conjugated fluorescent secondary antibodies (Odyssey cat 926-32211/926-68072), and visualised using the Odyssey Infrared Imaging System (LI-COR Biosciences).

Docking

*In silico* docking was performed using the Docking2.0 algorithm, part of the ROSIE server (43,44,76). An initial approximation of the complex orientation was generated by overlaying the IL-11Rα and IL-11 structures with IL-6Rα and IL-6 in the IL-6 signalling complex structure (6). This model was used as input to the ‘docking local refine’ protocol of RosettaDock, which limits rotations/transitions of the complex components. Models were scored by RosettaDock. The top-scoring model was taken as the representative model. Buried surface area and interacting residues were determined using the PISA server (23).

Circular dichroism spectroscopy

Circular dichroism (CD) experiments were conducted using an Aviv CD spectrometer (410-SF). Spectra were collected for 12 μM IL-11RαD3 at 20 °C, in 50 mM sodium phosphate pH 8.0 over a wavelength range of 260-190 nm in 1 nm steps with an averaging time of 4 s, using a 1 mm path length quartz cuvette. Each measurement (sample and blank) was collected in triplicate. Buffer signal was subtracted, and data were converted to mean residue ellipticity.

Multi-angle light scattering

SEC-MALS data were collected using a Shimadzu LC-20AD HPLC, coupled to a Shimadzu SPD-20A UV detector, Wyatt Dawn MALS detector and Wyatt Optilab refractive index detector. Data were collected following in-line fractionation with a Zenix-C SEC-300 4.6 × 300 mm SEC column (Sepax Technologies), pre-equilibrated in 20 mM Tris, 150 mM sodium chloride pH 8.5, running at a flow rate of 0.35 mL/min. 10 μL of sample was applied to the column at a concentration of approximately 2 mg/mL. The IL-11RαD1-D3/IL-11Δ10 complex was prepared by mixing equimolar concentrations of the components prior to the experiment. MALS data were analysed using ASTRA v. 7.3.2.19 (Wyatt). The detector response was normalised using monomeric bovine serum albumin (BSA) (Pierce, cat no. 23209). Protein concentration was determined using differential refractive index, using a dn/dc of 0.184.

Nuclear magnetic resonance spectroscopy

15N-IL-11RαD3 was expressed using the method of Marley *et al.* (77), purified and refolded as previously described (20), and successful incorporation of 15N was confirmed using electrospray ionisation-time of flight (ESI-TOF) mass spectrometry (MS). 15N-1H heteronuclear single quantum coherence (HSQC) spectra were collected on an 18.8 T Bruker Avance II spectrometer (1H resonance frequency 800 MHz), at 283 K, on 130 μM 15N-IL-11RαD3, 20 mM bis-tris, 50 mM arginine, 10% 2H2O pH 7. Spectra were processed using NMRPipe (78) and visualised using NMRFAM-SPARKY (79).
**Statistical analysis**
Statistical significance was determined using a 2-tailed, paired t test in Microsoft Excel v. 16.27 for Mac OSX.

**Data availability**
Coordinates and structure factors for IL-11RαEC and IL-11Δ10 have been deposited in the Protein Data Bank with accession codes 6O4P and 6O4O, respectively. SAXS data and models for IL-11Δ10, IL-11FL, IL-11RαΔD3, and the IL-11Δ10/IL-11RαΔD3 complex have been deposited in the SASBDB with accession codes SASDGH2, SASDGJ2, SASDGG2 and SASDGK2, respectively. All other data are contained within the manuscript and Supporting Information.
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## Tables:

### Table 1. X-ray data collection and structure refinement statistics for IL-1Rα and IL-11Δ10. Values for the highest resolution shell are given in parentheses.

|                      | IL-11Ra                     | IL-11Δ10                    |
|----------------------|-----------------------------|-----------------------------|
| **Data collection**  |                             |                             |
| Space group          | $P6_{5}22$                  | $P2_12_12$                  |
| Wavelength (Å)       | 0.9537                      | 0.9537                      |
| Number of images     | 60                          | 3600                        |
| Oscillation range per image (°) | 1.0                      | 0.1                          |
| Detector             | ADSC Quantum 315r           | Eiger 16M                   |
| **Cell dimensions**  |                             |                             |
| $a, b, c$ (Å)        | 171.46, 171.46, 107.94      | 39.02, 133.76, 27.18        |
| $\alpha, \beta, \gamma$ (°) | 90, 90, 120               | 90, 90, 90                  |
| Resolution (Å)       | 45.67-3.43 (3.70-3.43)      | 37.46-1.62 (1.68-1.62)      |
| $R_{\text{sym}}^{\dagger}$ | 0.575 (1.770)               | 0.0774 (1.031)              |
| $R_{\text{meas}}^{\dagger}$ | 0.611 (1.901)               | 0.0808 (1.071)              |
| $R_{\text{pim}}^{\dagger}$ | 0.307 (0.952)               | 0.0227 (0.286)              |
| $CC_{1/2}$           | 0.904 (0.436)               | 0.999 (0.764)               |
| $I/\sigma(I)$        | 3.9 (1.1)                   | 17.79 (2.08)                |
| **Total observations** | 92918                      | 244140                      |
| **Unique reflections** | 12990                      | 18927                       |
| **Completeness (%)** | 99.5 (98.5)                 | 99.95 (99.89)               |
| **Multiplicity**     | 7.2 (7.3)                   | 12.9 (13.6)                 |
| Wilson $B$-factor (Å$^2$) | 65.0                       | 24.0                        |
| **Refinement**       |                             |                             |
| Resolution (Å)       | 45.67-3.43 (3.55-3.43)      | 37.5-1.62 (1.72-1.62)       |
| Reflections used in refinement | 12962 (1243)               | 18925 (1845)                |
| $R_{\text{free}}$    | 0.244 (0.318)               | 0.1739 (0.2515)             |
| $R_{\text{work}}$    | 0.298 (0.342)               | 0.1926 (0.2742)             |
| **Protein molecules in asymmetric unit** | 2                        | 1                            |
| **Total non-hydrogen atoms** | 4580                      | 1470                        |
| **Protein**          | 4433                        | 1319                        |
| **Ligand/ion**       | 147                         | 6                            |
Solvent 0 145
Mean $B$-factor ($\text{Å}^2$) 65.9 36.02
Protein 64.9 35.33
Ligand/ion 97.4 47.58

RMSD
Bond lengths (Å) 0.002 0.010
Bond angles (°) 0.58 1.36

Ramachandran Plot
Favoured (%) 95.10 98.80
Allowed (%) 4.55 1.20
Outliers (%) 0.35 0.00

† $R_{\text{sym}} = \frac{\sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl}\sum_i I_i(hkl)}$
§ $R_{\text{meas}} = \frac{\sum_{hkl} [N/(N-1)]^{1/2} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl}\sum_i I_i(hkl)}$
‡ $R_{\text{pim}} = \frac{\sum_{hkl} [1/(N-1)]^{1/2} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl}\sum_i I_i(hkl)}$

$CC_{1/2}$ = Pearson correlation coefficient between independently merged half data sets.
Table 2: Isothermal titration calorimetry (ITC) data. Values shown are mean ± standard error of the mean, $n = 3$ for all.

|                | $K_D$ (nM) | $\Delta H$ (kJ/mol) | $\Delta S$ (J/molK) | $\Delta G$ (kJ/mol) | Incompetent receptor fraction $^d$ | T (K) |
|----------------|------------|---------------------|---------------------|--------------------|-----------------------------------|-------|
| **IL-11Δ10**   |            |                     |                     |                    |                                   |       |
| IL-11RαEC      | 40 ± 20    | -24 ± 0.6           | 65 ± 7              | -44 ± 2            | 0.30 ± 0.03                      | 303   |
| IL-11RαD1-D3   | 23 ± 3     | -25 ± 2             | 66 ± 7              | -45 ± 0.3          | 0.28 ± 0.06                      | 303   |
| IL-11RαD1-D3   | 25 ± 2     | -10 ± 0.4           | 120 ± 10            | -46 ± 2.6          | 0.09 ± 0.04                      | 298   |
| IL-11RαD1-D3   | 130 ± 20   | 41 ± 1              | 280 ± 5             | -38 ± 0.4          | 0.05 ± 0.02                      | 283   |
| IL-11RαD1-D3Δloop | 8 ± 4 | -26 ± 0.9           | 70 ± 6              | -47 ± 1            | 0.06 ± 0.05                      | 303   |
| **IL-11 FL**   |            |                     |                     |                    |                                   |       |
| IL-11RαEC      | 55 ± 14    | -25 ± 1             | 59 ± 6              | -44 ± 0.7          | 0.27 ± 0.03                      | 303   |

$^d$ Similar to ‘n’ See (60).
Figure 1: The crystal structure of IL-11RαEC. A) Two views of the structure of IL-11RαEC. Each of the domains and the section of the C-terminus that is defined in the electron density are indicated. The transmembrane domain is at the C-terminal region of the receptor. B) The
asymmetric unit of the IL-11Rα crystal structure, formed by two IL-11Rα molecules, with an extensive contact between D2 of the two molecules. C) The structure (left) and topology (top right) of D1 from chain B of IL-11RαEC with disulfide bonds indicated. Loops are coloured pink, the two strands contributing to the smaller, anti-parallel β-sheet blue, and the five strands contributing to the larger, mixed parallel/anti-parallel β-sheet orange. A topology diagram of the typical s-type Ig domain is also shown (bottom right). D) Continuous sedimentation coefficient (c(s)) distributions for IL-11RαEC at three concentrations, showing that IL-11RαEC is primarily monomeric in solution under the conditions tested. Slight concentration dependence in the sedimentation coefficient suggests the formation of a transient oligomer. E) c(s) distributions for IL-11RαD1-D3 at several concentrations. F) Small-angle X-ray scattering data for IL-11RαD1-D3, overlaid with the theoretical scattering profile calculated from molecule A of the crystal structure of IL-11RαEC (χ^2 =1.05).
Figure 2: Craniofacial disease associated mutations in IL-11Rα. A) Disease associated mutations that have been identified in IL-11Rα. These mutations are shown mapped onto the structure and
primary occur in D1, inter-domain turns and D3. **B)** Structural dynamics from a 50 ns MD simulation of IL-11Rα. i) Superposition of frames from the simulation. Five frames are shown, coloured by simulation time. Coordinates were aligned to D2 in IL-11Rα. Frames are shown coloured by Ca RMSD in ii). **C)** Frames from a 50 ns MD simulation of the C72F mutant. Frames are shown overlaid through the simulation. The WT IL-11Rα simulation is shown for direct comparison. **D)** The structural impact of the P178T mutation, i) showing the location of P178 at the D1/D2 interface, and ii) showing frames from the MD simulation, showing that the P178T mutation destabilizes the native position of the D1. **E)** The structural impact of the R274W mutation, i) showing the position of R274, at the extreme end of the tryptophan-arginine ladder in D3. R274 also forms a hydrogen-bond network, stabilising the D2/D3 interface, ii) frames from an MD simulation, showing that the R274W mutation disrupts the tryptophan-arginine ladder and the D2/D3 interface. **F)** Frames from a 50 ns MD simulation of the P199R mutant. Frames are shown overlaid through the simulation, with the WT IL-11Rα simulation shown for direct comparison.
Figure 3: Biological activity and crystal structure of IL-11Δ10. A) Western blot, showing activation of STAT1 and STAT3 by IL-11FL and IL-11Δ10 in the colon cancer cell line, DLD1. B) Two views of the structure of IL-11Δ10. The four helices in the structure are labelled. C) Regions previously implicated in binding the IL-11 receptors. Site I is involved in binding IL-11Ra, site II and III subsequently interact with the shared receptor gp130. D) i) The loop between the A and B helices (AB loop; blue). The residues mutated in the IL-11 antagonist are indicated. The interaction between the loop and core 4-helix bundle structure is shown in ii), with H105 and S75 forming a hydrogen bond. E) The CD loop (green), part of which forms a polyproline helix. Two views of the helix are shown in i). The interactions stabilising the N and C-terminal parts of the polyproline helix are shown in ii). F) 100 ns MD simulation of IL-11Δ10. Frames are overlaid at 20 ns intervals.
coloured by alpha carbon (Ca) RMSD. The α-helical core is stable through the simulation, while the loops undergo dynamic motions. **G)** c(s) distributions for IL-11Δ10, at three concentrations, showing that it is monomeric in solution. **H)** Small-angle X-ray scattering data for IL-11Δ10, overlaid with the theoretical scattering profile calculated from the crystal structure coordinates ($\chi^2 = 1.43$).
Figure 4: SV-AUC analysis of the IL-11/IL-11Rα complex. A) Continuous sedimentation coefficient (c(s)) distributions for the complex between i) IL-11RαEC and IL-11Δ10, ii) and IL-11RαD1-D3 and IL-11Δ10. The complex was formed by mixing 5 μM IL-11 and 5 μM IL-11Rα prior to the experiment, with no further purification. The c(s) distribution for 5 μM IL-11RαEC or IL-11RαD1-D3 is shown in all panels. B) SEC-MALS chromatograms (showing light scattering at 90 °
against elution volume) for the IL-11Δ10/IL-11RαD1-D3 complex (Mₘ 51.1 kDa). C) The c(s) distribution for muGFP-IL-11, and muGFP-IL-11 in complex with IL-11Rα. The complex was formed by mixing 5 μM muGFP-IL-11 and IL-11Rα prior to the experiment, with no further purification. The c(s) distribution for 5 μM IL-11RαEC is also shown. D) Fluorescent-detected c(s) distributions for the muGFP-IL-11/IL-11Rα complex at concentrations close to the Kᵰ of the interaction. IL-11Rα concentrations are indicated in the figure, muGFP-IL-11 was at a constant concentration of 150 nM. E) Sedimentation coefficient isotherm for muGFP-IL-11 binding to IL-11Rα. The concentration of muGFP-IL-11 was 150 nM, titrated with increasing concentrations of IL-11Rα. The best-fit to the data yielded a Kᵰ of 22 nM [68% CI 14-35 nM].
Figure 5: Thermodynamics and molecular model of the interaction between IL-11 and IL-11Rα.  

A) Isothermal titration calorimetry isotherms for the interaction between i) IL-11Δ10 and IL-11RαEC (KD = 40 ± 20 nM), ii) IL-11Δ10 and IL-11RαD1-D3 (KD = 23 ± 3 nM), and iii) IL-11Δ10 and IL-11RαD1-D3/Δloop (KD = 8 ± 4 nM). A representative titration of three replicates is shown for each. All experiments were conducted at 30 °C (303 K) with approximately 10 μM IL-11Rα in the cell, and a 10-fold molar excess of IL-11Δ10 in the syringe.  

B) Model of the IL-11RαEC /IL-11Δ10 complex. i) Two views of the complex. ii) Details of the interface with residues previously implicated in receptor binding highlighted.  

C) The experimental SAXS profile for the IL-11Rα/IL-11Δ10 complex overlaid with the theoretical scattering profile calculated from the model.
coordinates ($\chi^2 = 1.03$). An *ab initio* model is presented in Figure S9D. **D)** Continuous sedimentation coefficient (c(s)) distributions for the complex between IL-11Rα_{D1-D3} and IL-11Δ_{10/R169A}. The broad peak in the c(s) distribution suggests that the complex formed is lower-affinity compared to IL-11Δ_{10}. **E)** Continuous sedimentation coefficient (c(s)) distributions showing that IL-11Rα_{D3} does not interact with IL-11Δ_{10} at high affinity. No significant complex formation was observed with increasing concentrations of IL-11Δ_{10} in the presence of 5 μM IL-11Rα_{D3}. **F)** Continuous sedimentation coefficient (c(s)) distributions for the complex between IL-11Rα_{D1-D3/Δloop} and IL-11Δ_{10}. The complex was formed by mixing 5 μM IL-11Rα_{D1-D3/Δloop} with 5 μM IL-11Δ_{10} and centrifuged without further purification.
The structure of the extracellular domains of human interleukin 11 α-receptor reveals mechanisms of cytokine engagement

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