



Published in final edited form as:

Cytokine Growth Factor Rev. 2010 October ; 21(5): 325–330. doi:10.1016/j.cytogfr.2010.08.003.

Structural analysis of cytokines comprising the IL-10 family

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Abstract

Interleukin-10 (IL-10) family of cytokines includes a number of its viral homologs, and eight cellular cytokines (IL-19, IL-20, IL-22, IL-24, IL-26, IL-28A, IL-28B, and IL-29). The latter three proteins are also known as IFN- λ 2, IFN- λ 3, and IFN- λ 1 and are recognized as type III (or λ) interferons. Most of the cellular homologs of IL-10 are monomeric in solution, whereas IL-10 and its viral homologs are intercalated dimers consisting of two helical bundle domains topologically similar to the monomeric members of the family. A classical four-helix bundle, a signature element of all helical cytokines, is always found as part of the domain of each member of the IL-10 family. The only crystal structures of the cytokine receptors that have been determined to date are for their extracellular domains (ECDs). Each ECD consists of two β -sandwich domains connected in the middle by a linkage. Signal transduction occurs when a cytokine binds to its two appropriate receptor chains. IL-10 and its viral homologs use the same IL-10 receptor system, whereas the cellular homologs of IL-10 use their own receptors, which in some cases may overlap and be used in different pairwise combinations. The known structures of binary complexes allowed marking of the receptor binding site, which always includes helix A, loop AB and helix F (IL-10 notations) on the side of a ligand, and loops of the N-terminal and C-terminal domains directed toward the ligand, and the interdomain linkage of the ECD. An analysis of the published structures of both the binary and ternary complexes of all helical cytokines allowed generation of a model of the signaling complex of IL-10. The receptor binding site I of the high affinity receptor IL-10R1 is exactly the same as in the crystal structure of the binary IL-10/sIL-10R1 complex, whereas the receptor binding site II is located on the surface of the first and the third helices of the four-helix bundle. The receptor/receptor interface, or site III, is formed between the C-terminal domains of IL-10R1 and IL-10R2.

Keywords

Class II cytokines; interleukin-10; ligand/receptor interactions; helix bundle; signal transduction

Introduction

The structure-function relationships are the key factors to our understanding of how cytokines play their biological role in communication between cells on molecular level. This review is exclusively devoted to discussing the known structures of cellular cytokines belonging to the IL-10 family. Since the other chapters have described the biological activity of each protein in great detail, it is unnecessary to repeat such information here. It is

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important to understand that the IL-10 family is only a subfamily of a large group of proteins that are collectively named cytokines, unifying interleukins, interferons, and some growth factors. The modern classification of cytokines is far from perfect, even though a number of different approaches, functional and structural, have been attempted [1–5]. It is now accepted that helical cytokines can be divided on two classes by the structure of their receptors. The whole IL-10 family belongs to class II cytokines, identified in early nineties [1,2,6] (reviewed in ref. [7–12]). This classification is mainly based on the primary structural features of the ECDs of the receptors, characterized by the presence of a single, mostly conserved disulfide bridge in each (N- and C-terminal) subdomain of the ECD, and the lack of the intact “WSXWS” motif in the proximity of the cell membrane [1]. The family of Class II cytokines consists of type I IFNs, including IFN- α , IFN- β , IFN- ω , IFN- ϵ , IFN- κ , IFN- τ , IFN- ζ /lumitin, IFN- δ and IFN- ν [13–19]; IFN- γ , the single type II IFN (reviewed in [19,20]); and the IL-10 family of cytokines [21] that includes four viral homologs (Epstein-Barr virus [22–24], equine herpesvirus type 2 [25], Orf parapoxvirus [26,27], simian cytomegalovirus [28,29]), and eight cellular homologs (IL-19 [30], IL-20 [31], IL-22 [32,33], IL-24 [34,35], IL-26 [36]) (reviewed in [5,8,37–42]), and also including type III IFNs [43,44]: IFN- λ 1 (IL-29), IFN- λ 2 (IL-28A), IFN- λ 3 (IL-28B) (reviewed in [45]). While the sequence similarity between IL-10 and some of its viral homologs may be very high, the cellular homologs are much less homologous and differ from both IL-10 and from each other in their biological functions.

Signal initiation

All class II cytokines, including members of the IL-10 family [46], initiate their biological signals by association with two appropriate membrane receptors. Both receptors have topologically similar ECDs, composed of two fibronectin type III domains [47,48] folded in the conformation of a seven stranded β -sandwich, a short transmembrane domain, and intracellular/cytoplasmic domains of different lengths (Fig. 1). The chain with a long cytoplasmic domain is called receptor chain one, and the one with a short cytoplasmic domain is receptor chain two (Fig.1). The dimerization of receptor chains upon ligand binding leads to engagement of the intracellular Jak/STAT pathways [49,50] that eventually initiates transcription of appropriate genes. Besides their sizes, the receptor chains also differ by their affinity toward cytokines. Chain one exhibits usually, although not always [51], higher affinity, and, with the exception of IL-26 which signals through two low affinity receptor chains [52], all other signaling complexes always include one high and one low affinity chain. In addition, the receptor chains can also be used by different cytokines in different combinations. IL-10 and its viral homologs use high affinity IL-10R1 [53] and low affinity IL-10R2 [54], whereas the cellular homologs of IL-10 use combinations of different receptor chains: IL-20R1, IL-20R2 [31,55,56], IL-22R1 [33,57,58], IFN- λ R [43,44] and IL-10R2 (Fig.1).

Some of these cytokines have the ability to bind to a few different combinations of the receptor chains. For example, IL-20 and IL-24 form stable complexes with either a pair IL-20R1/IL-20R2, or with IL-22R1/IL-20R2 [55,56,59]. There are altogether six known different membrane-bound receptor chains and one naturally occurring soluble receptor called IL-22 binding protein (IL-22BP) [60–62] that are utilized by the IL-10 family of cytokines.

Structures of the cytokines and receptors deposited in the PDB

Table I lists all structural information about members of IL-10 family deposited to date in the Protein Bank. Most of the crystal structures represent free ligands determined at medium-to-high resolution, along with a few structures of binary complexes. Unfortunately,

there is as yet no available experimentally-determined structure of a ternary or signaling complex. Whereas such complexes have been prepared in a soluble form necessary for crystallization [51], some remaining technical problems must be overcome before crystals will be obtained.

Structures of the ligands

Helical cytokines are very compact molecules made of five to seven amphipathic helices, with a total helical content of 70–90%. All of the cellular homologs of IL-10 are monomers [51,63–65] (Table 1), whereas IL-10 and its viral homologs are intercalated dimers (Fig. 2) [66,67] composed of two identical domains. Each domain is a topological equivalent of a monomeric cytokine [64] (Fig. 2,3,4). The amphipathic helices are arranged in such a way that most of the hydrophobic amino acid residues are involved in the formation of the extended internal hydrophobic core inside the five-, six- or seven-helix bundle, stabilized by one to three disulfide bridges. These structures always include the ‘classical’ left-handed four-helix bundle [68], a signature element of all helical cytokines. An example of such a bundle can be seen in the structure of IL-10 [67] (helices A, C, D, F’ and A’, C’, D’, F) (Fig. 2). Crystal structures of IL-19 [64] and IL-22 [65,69,70] showed new structural features (Fig. 3), which marked a new IL-19 subfamily of cytokines [64,71]. Helix A becomes short and is connected to helix B by a short β -strand, which in the structure of IL-19 (Fig. 3) participates, along with the C-terminal β -strand, in formation of a two strand β -sheet, never before seen in helical cytokines. An analysis of the amino acid sequence of human IL-20 strongly suggests that its structure must be very close to that of IL-19 [5,64], although it lacks the C-terminal β -sheet. It is also likely that IL-26 exists and is active both as a dimer [36,40,72,73] and a monomer (data are not shown). Crystal structure of IFN- λ 3 [74] (Fig. 4) has shown complete lack of short helix A, found in IL-19 and IL-22 structures, even though the general topology is that of a member of the IL-10 family. Structural superposition of IFN- λ 3 with IL-19 and IL-22 showed that it fits better to IL-22. That may be the reason why IFN- λ 3 and IL-22 possess parallel functions, protecting epithelial tissue against viral and bacterial infections [74]. Members of IL-10 family of cytokines also belong to long-chain subfamily of helical cytokines, characterized by the conformation and the location of crossover polypeptide chain between the first and the second helix of the four-helix bundle [75,76]. The known structures (Table I) of the binary complexes of cytokines with their appropriate soluble receptors showed that the first receptor chain binding site on the surface of the ligand includes the first and the last helix, as well as the loop between the first and the second helix of the four-helix bundle [68]. For example, in the case of IL-10 (Fig. 3), the receptor binding site includes [77,78]: helix A, loop AB, and helix F’ of one domain, and helix A’, loop A’B’ and helix F of another domain. In the case of IL-19 (Fig. 4), an equivalent site includes helix B, loop BC, and helix G.

Receptors and binary complexes

No structures of the unbound receptor chains, nor of the whole receptor complex or have been determined to date. However, structures of the binary complexes of IL-10R1 [78], a number of viral homologs, IL-22/IL22R1 [79,80], IL-22/IL-22BP [81] and IFN- λ 1/IFN- λ R1 (data not shown) are available (Table I). These structures showed that an ECD of class II cytokine receptor chains consist of two domains, each having fibronectin type III-like topology [47,48,82]. Fig. 5 shows the crystal structure of the complex of IL-10 with its soluble receptor chain, sIL-10R1 [78]. The molecule of the complex is a tetramer made up of an intercalated dimer of IL-10 and two sIL-10R1 molecules attached to each domain of IL-10. Two parts of the complex, including one domain of IL-10 and bound to it sIL-10R1, are related by a crystallographic two-fold axis, which is approximately perpendicular to the plane of Fig. 5 (its position is shown by a cross). Therefore, a 180° rotation around this axis

perfectly superimposes both parts of the complex. Each domain of sIL-10R1 consists of one 3-stranded and one 4-stranded β -sheets (Fig. 5), packed one against another in the form of a sandwich. The domains are linked together by a short linkage consisting of one helical turn and a short β strand. Out of 14 loops connecting the β strands, five that are located in the vicinity of the receptor inter-domain junction were found to be the most important [78] because of their involvement in ligand-receptor interactions. The structure of the complex is quite rigid, with no conformational changes found between sIL-10R1 molecules bound to either IL-10 or to viral IL-10s [83,84]. In spite of the low amino acid sequence identity (27%) between IL-10 and CMVIL-10, the interaction of either one with the receptor involves essentially the same areas on the surface of both the ligand and the receptor. In fact, a superposition of the CMVIL-10 domain bound to the sIL-10R1 with the structure of the IL-10 domain bound to the sIL-10R1 gives an r.m.s. deviation only 1.2 Å for 331 C α pairs. Most of the ligand/receptor contacts are of polar character and can be clustered into two interacting sites [78,83]. Binary complexes of other members of the IL-10 family have in general a similar topology, although the details of ligand/receptor interface are quite unique [79,81,85]. These specific differences in the interactions must define their high specificity.

Ternary complexes

As mentioned before, no crystal structure of a ternary or signaling complex has been determined so far for any member of the IL-10 family. However, an analysis of all known crystal structures of ternary complexes of other helical cytokines [46,86] allows to mark the putative second receptor chain binding site as the first and the fourth helices of the four-helix bundle. In the case of IL-10 these are helices A and D of one domain and helices A', D' of another domain. Based on this information and on the assumption that the molecule should keep its two-fold symmetry, a homology model [86] of the ternary IL-10/sIL-10R1/sIL-10R2 complex was generated (Fig. 6). This model was later found to be in good agreement with both peptide scans [87] and with mutagenesis/surface plasmon resonance studies [88]. The receptor chains bind adjacent sides on the surface of each IL-10 domain. sIL-10R1 binding site or site I is exactly the same as in the binary IL-10/sIL-10R1 complex, sIL-10R2 binding site II is located on the surface of helices A and D, while sIL-10R1 and sIL-10R2, bound to the same IL-10 domain, interact with each other by their C-terminal domains, creating site III (Fig. 6). Most of the interactions found in all three interfaces are of polar nature, although hydrophobic interactions are also important, constituting 20%–35% of the total number of intermolecular contacts. The details of the ligand/receptor and receptor/receptor interactions can be found in the original papers [86] and in many reviews [5,46,89–91]. Both IL-10 receptor chains have a few glycosylation sites, which were found not to interfere with an overall organization of the IL-10/sIL-10R1/sIL-10R2 complex, and since the binary complexes of both IL-10 and its viral homologs with deglycosylated mutant of sIL-10R1 were also found quite stable [78,83,84,92], it is clear that N-glycosylation is not that important for the formation of a biologically relevant ternary complex of IL-10/IL-10R1/IL-10R2. However, in some other instances the N- and O-linked oligosaccharides may be quite important [46]. In general, we may conclude that even though the role of glycosylation is not completely understood, it is likely that, in some cases, it may enhance the biological activity, as well as change the solubility and thermostability of both the cytokine and its receptor.

Discussion and concluding remarks

The IL-10 family of cytokines includes IL-10, viral homologs of IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, and three IFN- λ s, the latter forming a unique subfamily of type III IFNs. Biologically active IL-10 and its viral homologs exist as intercalated dimers, whereas IL-19, IL-20, IL-22, and IL-24 are active as monomers. IL-26 is active as either a dimer or a

monomer. The three-dimensional structures of the monomeric members of IL-10 family are topologically similar to the structure of a single domain of IL-10, which consists of a six-helix bundle that includes a classical four-helix bundle found in the structures of all helical cytokines. Published crystal structures of the complexes of IL-10/IL-10R1, its viral homologs, along with the recently solved structures of IL-22/IL-22R1, IL-22/IL-22BP and IFN- λ 1/IFN- λ 1R1 (data not shown) allowed detailed description of the ligand/receptor interface. The areas of the ligand creating the interface include helix A, loop AB and helix F (IL-10 notation), whereas the receptor utilizes three-four loops of domain D1, the interdomain junction, and two-three loops of domain D2. Topologically, structural elements involved in the interaction are similar, but at atomic level they are quite unique for each IL-10 family member, which certainly is necessary to provide high specificity of ligand/receptor interactions. The known crystal structures of the binary and ternary complexes allowed not only marking the receptor binding sites on the surface of IL-10 domains, but also provided guidance for generating a ternary model of IL-10/sIL-10R1/sIL-10R2 complex. This model is likely not very accurate in details, but this is the only model of this signaling complex which is currently available that shows how it could look on the surface of the cell.

Acknowledgments

This research was supported by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research. Special thanks go to Prof. Alexander Wlodawer for accurately proofreading the manuscript.

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Biography



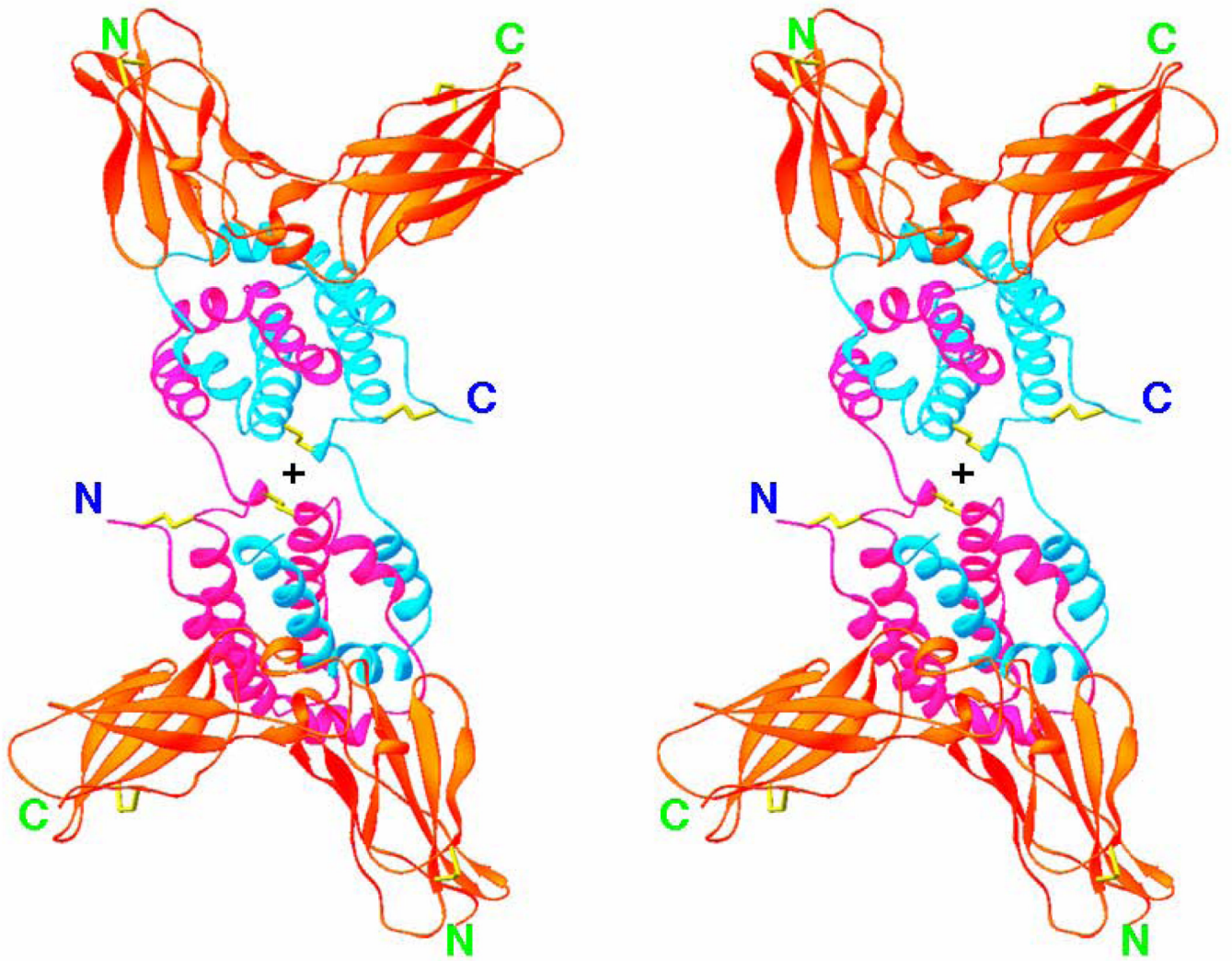


Fig.1. Schematic representation of the IL-10 family of cytokines. Different combinations of receptor chains are shown. The brown plate represents a hypothetical cell membrane.

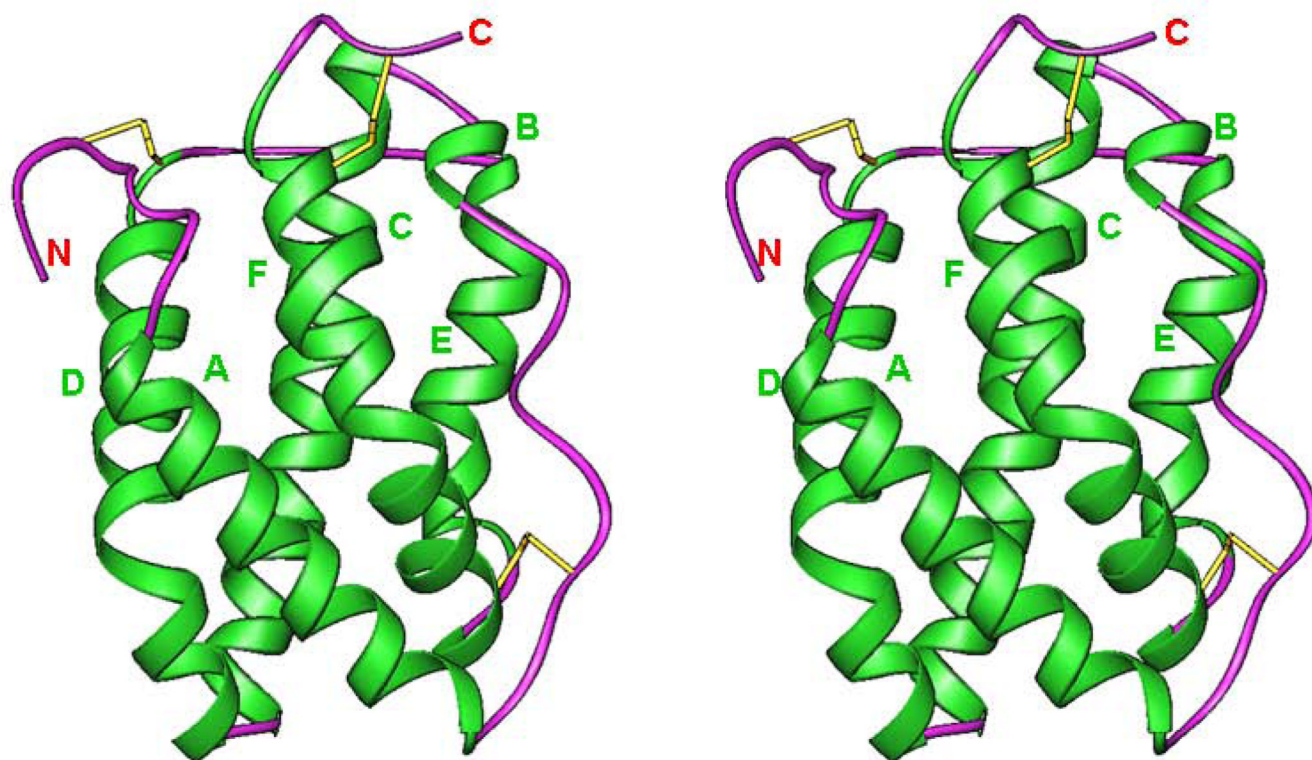


Fig.2. Stereo diagram of the structure of IL-10 (PDB code 2ILK). The two monomers that form a domain-swapped dimer are shown in violet and green, respectively, and the disulfide bridges are in yellow. Figures 2–6 were generated with the program RIBBONS [93].

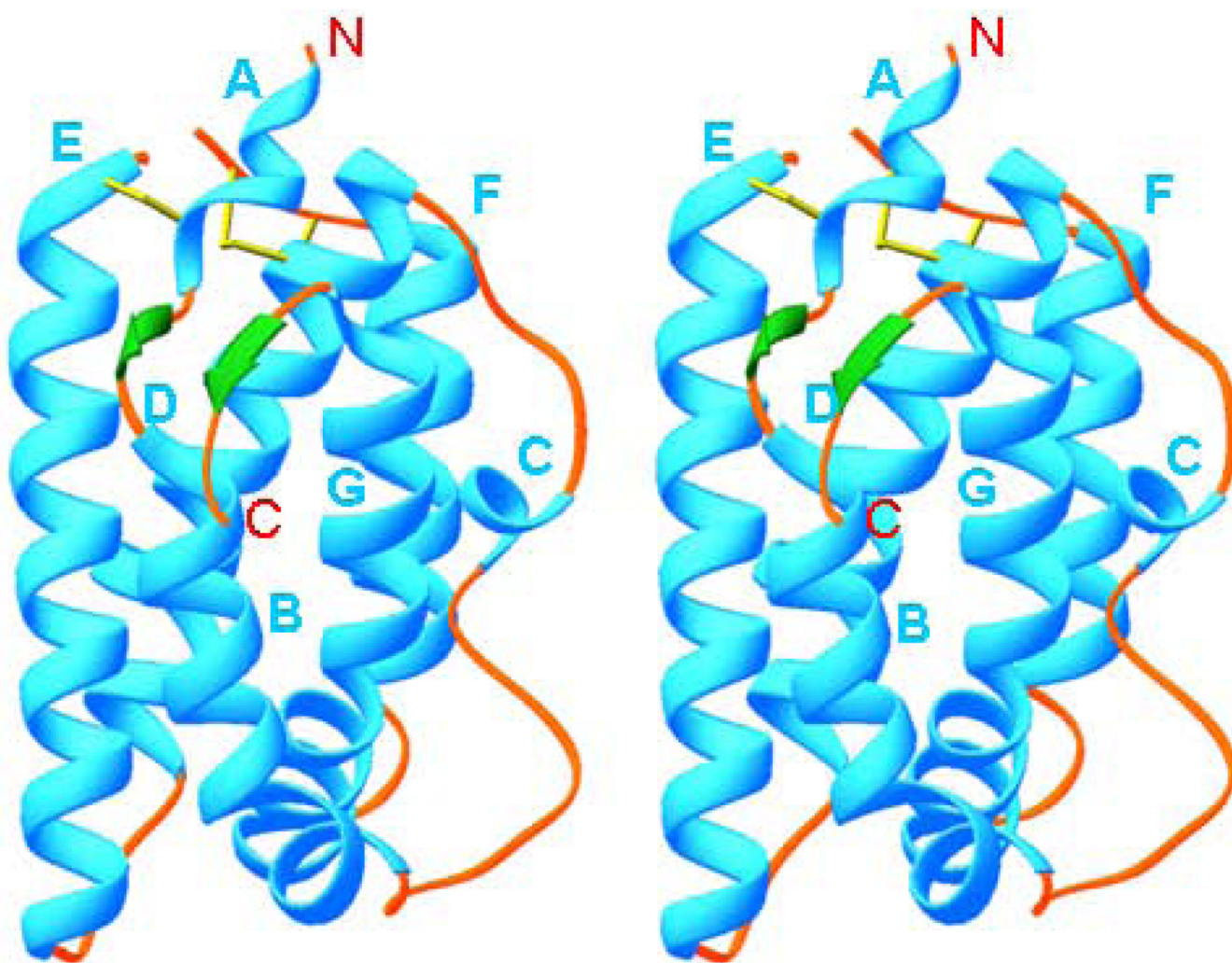


Fig. 3. Stereo diagram of IL-19 (PDB code 1N1F). The helices are shown in cyan, loops in light-brown, β -strands in green, and disulfide bridges in yellow. The helices are labeled in cyan and the N- and C-termini are labeled in red.



Fig. 4. Stereo diagram of IFN- λ 3 (PDB code 3HHC). The helices shown in green, loops in magenta, and the disulfide bridges in yellow. The helices and the N-, C-termini are marked in green and red, respectively.

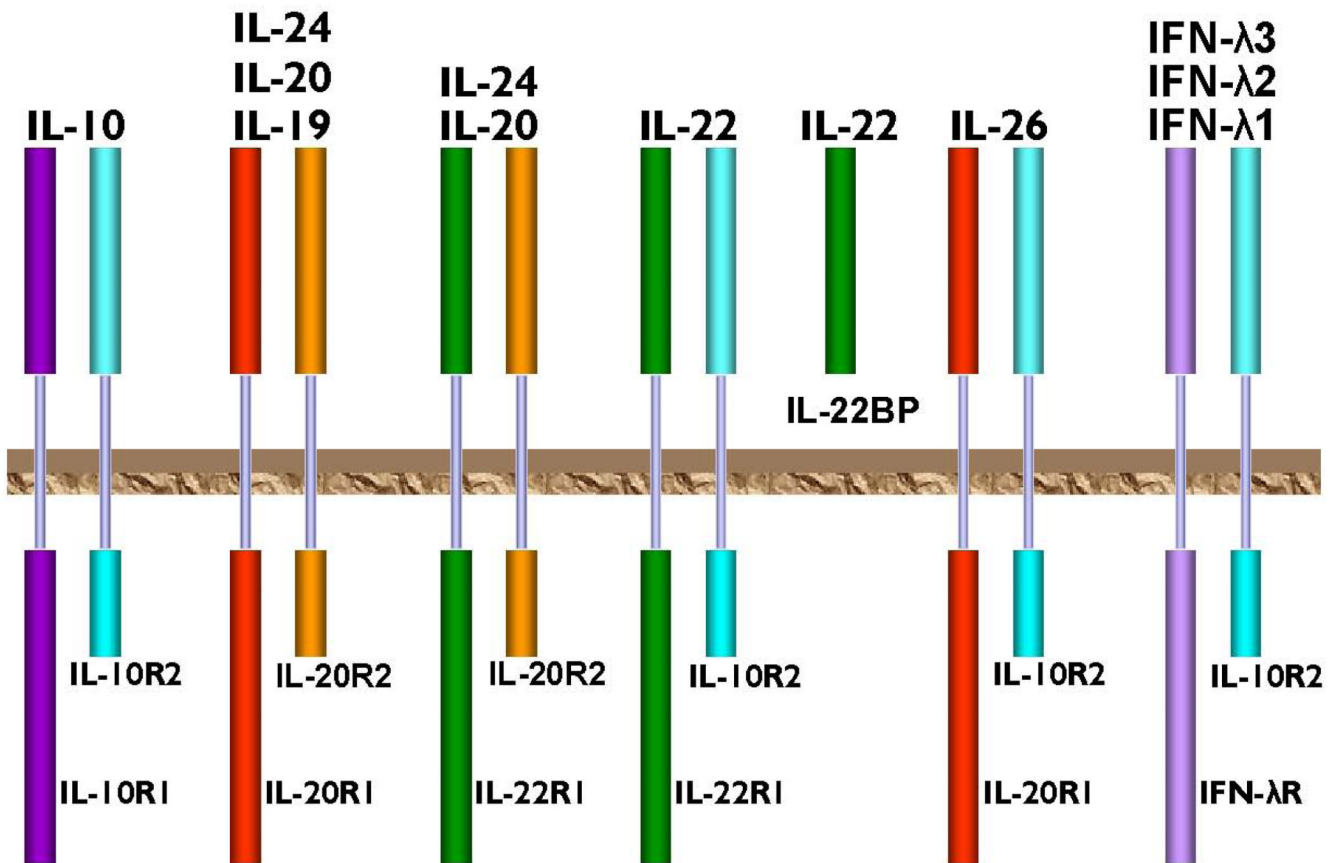


Fig. 5. Stereo diagram of IL-10/sIL-10R1 complex (PDB code 1J7V) viewed approximately along the crystallographic two-fold axis (its position is marked by cross). IL-10 is shown in violet and blue, the receptor chains are red, and the disulfide bridges are yellow.

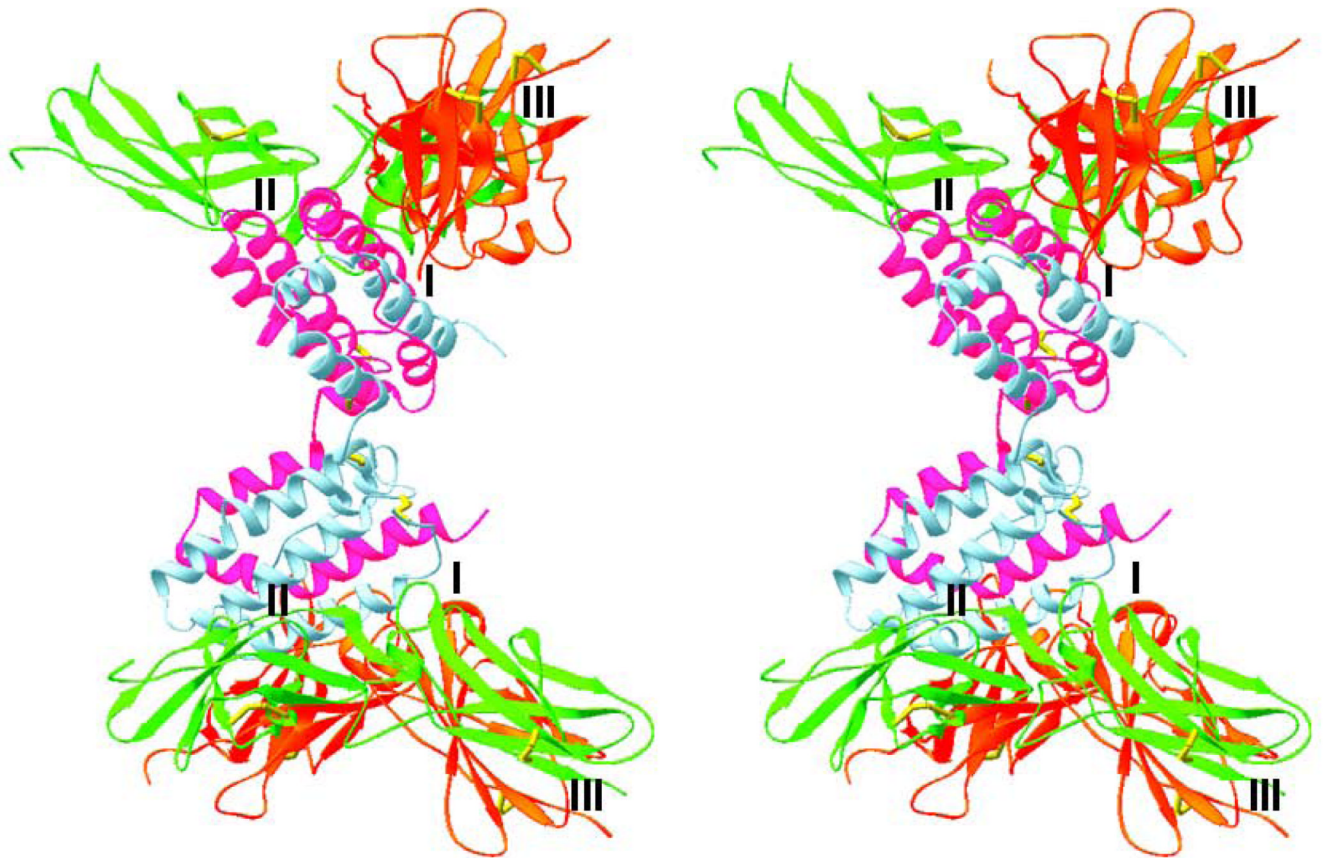


Fig. 6. Stereo diagram of a model of the ternary complex of IL-10/sIL-10R1/sIL-10R2. IL-10 is shown in violet and blue, sIL-10R1 in red, sIL-10R2 in green, and the disulfide bridges are in yellow. A postulated position of the cell membrane is on the right hand side of the figure. The ligand/receptor and receptor/receptor interfaces are marked I–III in each half of the complex.

Table I

Structures of the proteins that belong to the Class II cytokines family deposited in the PDB.

Protein	Biol. relev. aggregation	Species	Expression system	PDB code/Reference	Resolution/Remarks
IL-10	Dimer	Human	<i>E. coli</i>	1ILK/[66]	1.8 Å, room temp
IL-10	Dimer	Human	<i>E. coli</i>	2ILK/[67]	1.6 Å, 100 K.
IL-10	Dimer	Human	<i>E. coli</i>	1INR/[94]	2.0 Å
IL-10	Dimer	Human	<i>E. coli</i>	2H24/[88]	2.0 Å
Epstein-Barr IL-10	Dimer	EBV	<i>E. coli</i>	1VLK/[95]	1.9 Å
IL-19	Monomer	Human	DES ²	1N1F/[64]	1.95 Å
IL-22	Monomer	Human	<i>E. coli</i>	1MR4/[65]	2.0 Å
IL-22	Monomer	Human	DES ²	1YKB/[96]	2.6 Å
IFN-λ3	Monomer	Human	<i>E. coli</i>	3HHC/[74]	2.8 Å
IL-10/sIL-10R1	Tetramer	Human	<i>E. coli</i> , DES ²	1J7V/[78]	2.9 Å
IL-10/sIL-10R1mut	Tetramer	Human	<i>E. coli</i> , DES ²	1Y6K/not publ.	2.52 Å
EBV IL-10/sIL-10R1, and mut.	Tetramer	EBV	<i>E. coli</i> , DES ²	1Y6M,1Y6N/[84]	2.8 Å, 2.7 Å
CMV IL-10/sIL-10R1	Tetramer	CMV	<i>E. coli</i> , DES ²	1LQS/[83]	2.7 Å
IL-10 monomer/9D7 Fab	Trimer	Human Rat	<i>E. coli</i>	1LK3/[97]	1.91 Å
IL-22/IL-22R1	Dimer	Human	<i>E. coli</i>	3DLQ/[79]	1.9 Å
IL-22/IL-22R1	Dimer	Human	DES ²	3DGC/[80]	2.5 Å
IL-22/ILBP	Dimer	Human	<i>E. coli</i>	3G9V/[81]	2.76 Å

¹ Chinese hamster ovary cells;

² Drosophila expression system, Sf9 cells.