# Structural Aspects of the IL-1 Superfamily Investigated by Molecular Dynamics Simulations

by

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# ABSTRACT

Interleukin-1 (IL-1) is a master mediator of innate immunity and inflammation. There is a tight regulation by receptor antagonists, decoy and soluble receptors both extracellular and intracellular levels. Members of the IL-1 Receptor family include type I IL-1 receptor (IL-1RI), IL-1 receptor-like 1 (IL-1RPL1, also called as ST2) and IL-18 receptor as primary receptors, type II IL-1 (IL-1RII) receptor as a decoy receptor, IL-1 receptor accessory protein (IL-1RAcP) as a secondary receptor. One of the IL-1 family receptor ligands, IL-1$\beta$, has a major role in immune responses. It signals through binding its primary receptor IL-1RI and its secondary receptor IL-1RAcP. IL-1 has also a naturally occurring antagonist functions via binding to IL-1RI. Therefore, targeting IL-1R is a common therapeutic strategy for autoinflammatory diseases. However, structural basis of IL-1 antagonism is still unknown.

Here we used classical molecular dynamics to reveal key aspects of the signalling complex of IL-1$\beta$ and interaction between its primary and secondary receptors. We showed that high D3 domain fluctuation of IL-1RI when it is bound to antagonist can have an important role in inhibiting IL-1 signalling. In addition to IL-1 complexes, we have performed classical molecular dynamics simulations of single soluble forms of IL-1 receptors. We have found that in the absence of ligand, IL-1RI, ST2 and IL-18R take conformations that are dramatically different from their complex conformations. There are also experimental studies which support our findings on these different conformations. One of them is from a x-ray structure in which close form of IL-1RI is in complex with a short peptide. In addition, SAXS studies confirm that ligand-free ST2 has different conformers in solution. These different conformers can

not participate in ligand binding; therefore, this can be a new finding on inhibitory mechanism of IL-1 superfamily.

# ÖZETÇE

İnterlökin-1 (IL-1) doğuştan bağışıklığın ve enflamasyonun ana araçlarından biridir. Bağışıklık sistemi ve enflamasyon arasında sıkı bir düzenleme vardır. Bu düzen reseptör antagonisti, sahte reseptör ve inhibitörler ile sağlanıyor. IL-1 reseptör ailesinin birincil reseptörleri IL-1RI, ST2 ve IL-18R1 ünden, sahte reseptörü IL-1RII ve ikincil reseptörü IL-1RAcP inden oluşuyor. IL-1 ailesinin en önemli sitokinlerinden birisi olan IL-1$\beta$ bağışıklık tepki mekanizmasında en önemli rollerden birini oynar. IL-1$\beta$ öncelikle birincil reseptör IL-1RI e bağlanır sonrasında oluşan komplekse ikincil reseptör IL-1RAcP bağlanır ve bu şekilde etkili kompleksi oluşturmuş olurlar. IL-1 kendiliğinden oluşmuş olan bir antagoniste sahiptir. Bu antagonist de IL-1$\beta$ ile aynı yere IL-1RI e bağlanarak inhibitör görevini yerine getirir. Bu inhibisyon mekanizmas şu andaki otoimmün hastalıkların tedavisinde kullanılan ilaçların hedefledikleri mekanizmadır. Fakat hala IL-1 antagonizminin yapısal temeli bilinmemektedir.

Bu çalışmada IL-1$\beta$ nın sinyal veren kompleksinin klasik moleküler dinamik yöntemi kullanılarak birincil ve ikincil reseptörleriyle olan etkileşiminin ana hatları incelenmiştir. IL-1 reseptörünün D3 grubunun antagoniste bağlı olduğunda yüksek hareketlenmeye sahip olduğunu ve bu dalgalamann IL-1 sinyalleşmesini inhibe etmekte bir rolü olduğunu gösterdik. IL-1 komplekslerine ek olarak, IL-1 reseptörlerinin kendi başlarına solusyon içersindeki simulasyonu klasik MD kullanılarak gerekleştirildi. Bunun sonucunda IL-1RI, ST2 ve IL-18R1 reseptörlerinin fizyolojik koşullarda çok farklı konformasyonlar aldığı görüldü. Reseptörlerin bu yapısal değişimi herhangi bir ligandın reseptöre bağlanmasını engelleyici nitelikte olduğundan bu yeni bulmuş olduğumuz yapıların IL-1 ailesinin inhibisyon mekanizmasında yer aldığı söylenebilir.

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# LIST OF ACRONYMS & ABBREVIATIONS

| | |
|---|---|
| IL-1 | Interleukin-1 |
| IL-1RI or IL1R1 | Type I Interleukin-1 Receptor |
| sIL-1RI | Soluble type I Interleukin-1 Receptor |
| sIL-1RII | Soluble type II Interleukin-1 Receptor |
| IL-1RAcP or IL1R3 | Accessory receptor protein for Inerleukin-1 |
| sIL-1RAcP | Soluble accessory receptor protein for Inerleukin-1 |
| IL-1$\alpha$ | Interleukin-1 alpha |
| IL-1$\beta$ | Interleukin-1 beta |
| IL-1Ra | Interleukin-1 receptor antagonist |
| IL1RL1 | Interleukin-1 receptor Like 1 |
| IL18R1 | Interleukin 18 Receptor 1 |
| TM | transmembrane |
| RA | rheumatoid arthritis |
| CAPS | cryopyrin-associated periodic syndromes |
| MWS | Muckle-Wells syndrome |
| FCAS | familial cold autoinflammatory syndrome |
| TIR | The Toll/Interleukin-1 receptor |
| Ig-like | immunoglobulin-like |
| LRR | leucine-rich repeat |
| TLR | Toll-like receptor |

# Chapter 1

# INTRODUCTION

Immune system is made up by variety of effector cells and molecules to protect the human body from infectious agents and its damages. The first defense of the body is the innate responses that detect and destroy the cause within short period. Immune responses are mediated by binding of cytokines to specific receptors. Among the cytokines, one of the major mediators of innate immunity is IL-1.

IL-1 affects nearly every tissue and organ in the body; therefore, it is tightly regulated both extracellularly and intracellularly. IL-1 has a role in autoinflammatory diseases such as rheumatoid arthritis[2], inflammatory bowel disease and has a potential role in pathogenesis of type 2 diabetes and smoldering myeloma [3]. One of the IL-1 family members is IL-1$\beta$ which is a product of blood monocytes, tissue macrophages and dendritic cells. IL-1$\beta$ is first produced in an inactive form then caspase-1 cleaves amino-terminal of IL-1$\beta$ and mature form is released [4]. IL-1 family has a naturally occurring antagonist, IL-1Ra. Secretory IL-1Ra protein is produced by any cell synthesizing IL-1 except endothelial cells and hepatocytes [5]. Observations of elevated blood levels of IL-1Ra in patients with juvanile chronic arthritis, polymyositis, systemic lupus erythematosus; in synovial fluids of patients with rheumatoid arthritis; in plasma of acutely ill patients with inflammatory disease and in large intestine of patients with inflammatory bowel disease confirm IL-1Ra extensive role in host de-

fense [5].

The exact inhibition mechanism of IL-1Ra is still unknown. Therefore, the present study aims to investigate dynamics of the key members of IL-1 signalling in both complex and unbound forms in order to provide structural explanation on IL-1 inhibition and activation mechanism. This thesis is organized as follows. In chapter 2, we introduce the structural informations and activation mechanism of IL-1 family members. We also present the analysis on structural comparison of the IL-1 family. In chapter 3, we explain the methods that are used in this study. This chapter gives information about the Molecular Dynamics simulation parameters and the preparation of the structures that are used in this study. Chapter 4 presents the results from the MD Simulations to provide information on the stability of the Ig-like $3^{rd}$ Domain of the Interleukin-1 Receptor type I by comparing the binary complexes of receptor with IL-1$\beta$, IL-1Ra and mutant IL-1Ra, separately. In chapter 5, the role of IL-1RAcP on Ig-like $3^{rd}$ Domain of the IL-1RI stability is investigated by computational calculations. In chapter 6, the motions of the unbound form of IL-1 superfamily receptors will be presented with structural evidences. Chapter 7 concludes the thesis by discussing the results and analysis together with the suggestions on future research directions. Performed MD Simulations are outlined in 1.1.

Table 1.1: Outline of MD Simulations

| **Chapter 4**<br>**Stability of IL-1RI D3 Domain in binary complexes** | | |
|---|---|---|
| **cytokine** | **receptor** | |
| IL-1$\beta$ | IL-1RI | |
| IL-1Ra | IL-1RI | |
| C116Fmutant - IL-1Ra | IL-1RI | |
| **Chapter 5**<br>**Role of Accessory Protein on D3 domain stability** | | |
| **cytokine** | **receptor** | **accessory** |
| IL-1$\beta$ | IL-1RI | IL-1RAcP |
| IL-1Ra | IL-1RI | IL-1RAcP |
| IL-1$\beta$ | IL-1RII | IL-1RAcP |
| **Chapter 5**<br>**Motions of Unbound IL-1 Receptors** | | |
| IL-1RI | | |
| IL-1RII | | |
| IL-1RAcP | | |
| ST2 (IL-1RL1) | | |
| IL-18R | | |

# Chapter 2

# LITERATURE REVIEW

## 2.1 The IL-1 Family

IL-1 cytokines cause inflammation and increase in immune responses. Among these cytokines, IL-1$\beta$ is an important element of inflammatory and host defence through IL-1 receptor signalling assemblies. Overproduction of IL-1$\beta$ is associated with hereditary autoinflammatory disorders such as Muckle-Wells syndrome, familial cold autoinflammatory syndrome and cryopyrinopathies [3]. Moreover, deficient production or absence of IL-1 receptor antagonist (IL-1Ra) causes recently termed rare autoinflammatory disease DIRA (deficiency of IL-1Ra)[6]. Therefore, understanding the activation and inhibition mechanisms of IL-1 is crucial for developing a treatment for autoinflammatory diseases.

Use of the recombinant IL-1R antagonist anakinra in RA and other rheumatic diseases [3]; highly specific IL-1$\beta$ monoclonal antibodies canakinumab in cryopyrin-associated periodic syndromes (CAPS)[7]; dimeric fusion protein consisting of portions of IL-1 receptor and IL-1 receptor accessory rilonacept (IL-1 trap) in CAPS are all approved treatments that target IL-1 signalling. Also the treatment of gout, coronary atherosclerosis, RA and anemia with rilonacept is in phase 3, 2 and 1, respectively [8]. However, most of these agents fail in having relatively short half-life as they require frequent injections [9]. There are discoveries in orally deliverable IL-1 antagonist having lower molecular mass. AF10847 [10] and rytvela [11] are some of them.

## 2.2 IL-1 Mechanism of Activation and Regulation

IL-1 superfamily cytokines are key regulators of innate and adaptive immunity. The upper family is the the IL-1R/TLR superfamily, sharing a conserved sequence TIR domain which is responsible for the signalling. This superfamily is divided into two subfamilies: i) Immunoglobin domain members have extracellular Ig-like domains responsible for ligand binding and ii) TLR's have Leucine rich-repeats function in protein-protein interaction which are illustrated in Figure 2.1. The Immunoglobin domain is composed of IL-1, IL-18, IL-33 and the most recently added ones are IL-36 and IL-37 [12].

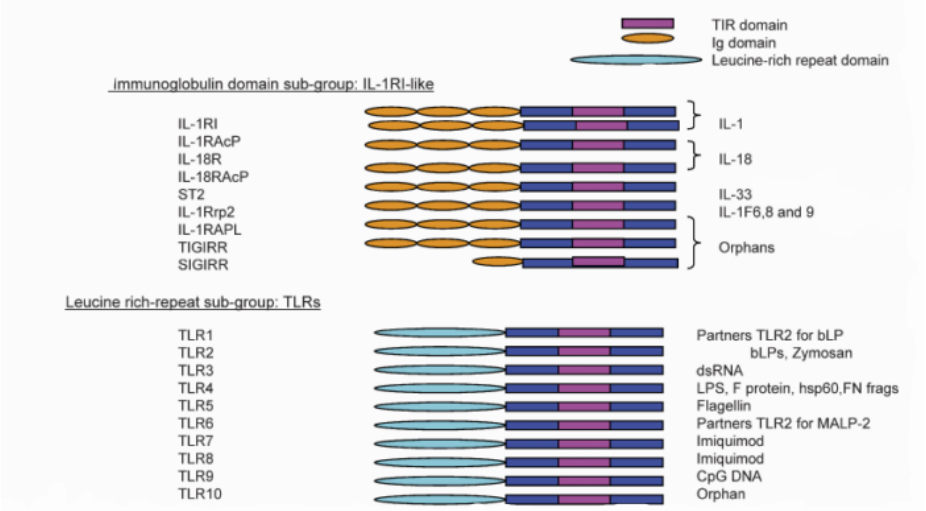


Figure 2.1: The IL1R/TLR superfamily; Immunoglobulin domain sub-group : IL-1RI like and Leucine rich-repeat sub-group: TLRs [1]

They share common receptor structure and signal transduction pathways. In this superfamily, signalling initiation requires two receptors: a primary and an accessory receptor. The primary receptor specifically binds its cytokine but the accessory receptor cannot bind the cytokine itself [13]. The response by IL-1 family cytokines is initiated by forming a binary complex consisting of the ligand and its primary

receptor subunit. The composite binding interface is formed for the recruitment of secondary or accessory protein receptor which is necessary to form a signalling complex [14]. Therefore, ligand, its primary receptor and its accessory receptor are the major components of heterotrimeric signalling complex. Upon binding of the accessory receptor to the binary complex, the TIR domains of both receptors juxtapose themselves. This dimerization results in recruitment of MyD88, IRAK-4, IRAK-1 and probably IRAK-2 [1]. Then, the activation of MAPK and NF-$\kappa$B occurs and intracellular signalling cascades take place [15].

Since IL-1$\beta$ is critical in inflammatory responses, the signalling cascade is controlled tightly with the existence of natural IL-1 inhibitors. IL-1Ra and the decoy receptor, IL-1RII, are naturally occurring inhibitors of IL-1 extracellular regulation. In addition, the soluble form of IL-1 receptors formed by the alternative splicing of cleavage from the membrane receptor, acts like an inhibition mechanism by capturing binary complexes to avoid forming signalling complexes [16]. As shown in the Figure 2.2, IL-1RI - IL-1Ra complex cannot recruit IL-1RAcP; therefore, signalling does not occur. Eventhough membrane-bound type II IL-1 receptor (IL-1R2) can recruit IL-1RAcP [13], the signal cannot be transduced because IL-1R2 lacks TIR domain in the intracellular region [15]. Also, soluble forms of IL-1 receptors can bind ligands in extracellular region; as a result, concentration of membrane receptor-ligand complexes decreases [17].

Understanding the function lies beneath the structure of the IL-1 family. For this purpose ligand and receptors are reviewed individually in the following subsections.

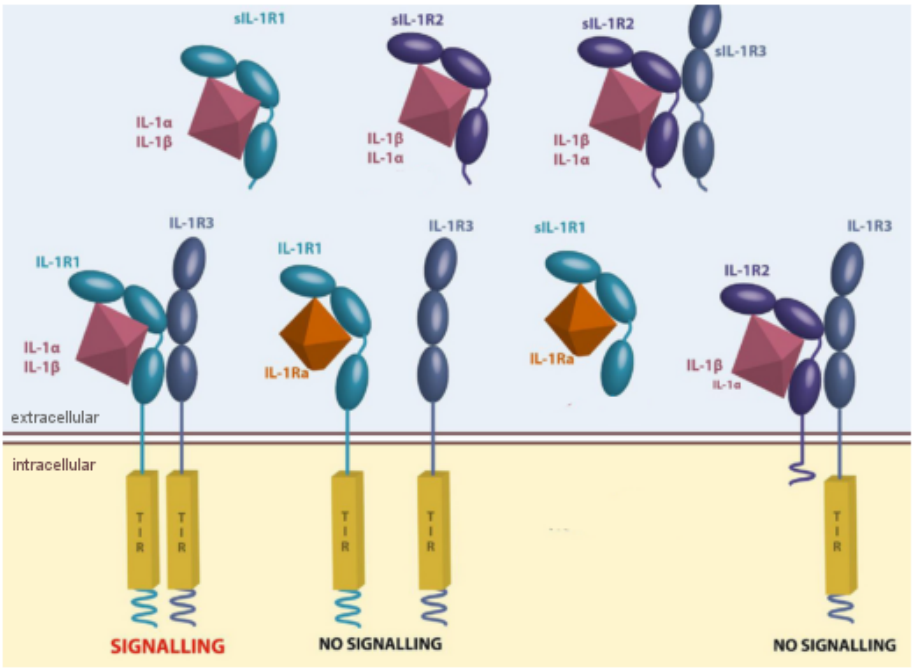


Figure 2.2: IL-1 receptor complexes at extracellular and intracellular region

### 2.2.1 IL-1 Ligands

Three naturally found ligands can bind to the IL-1 type I receptor (IL-1RI): two of them are agonists; IL-1$\alpha$ and IL-1$\beta$ and the other is an antagonist; IL-1Ra. IL-1$\alpha$ and IL-1$\beta$ are both transduce similar signal in terms of responses when bound to IL-1RI. However, IL-1Ra does not elicit any known signal responses upon binding [18].

All three ligands consist of 12 antiparallel $\beta$-strands; six of them are arranged structurally cooperative $\beta$-barrel and the other six strands are assembled to form $\beta$-sheets that caps one end of the barrel. Two receptors binding sites on IL-1$\beta$ are found by site-directed mutagenesis, buried in the receptor. Site A, one of the binding sites of the IL-1$\beta$ consists of residues: 11, 13-15, 20-22, 27, 29-36, 38, 126-131, 147 and 149 which are also common to all three IL-1 family members; IL-1$\alpha$, IL-1$\beta$ and IL-1ra.

Site B, the other binding site on IL-1$\alpha$ and IL-1$\beta$ but not on IL-1Ra is comprised of residues 1-4, 6, 46, 51, 53-54, 56, 92-94, 103, 105-106, 108, 109, 150 and 152. In Figure 2.3 Site A and B of IL-1$\beta$ is represented when it is in complexed with IL-1RI.

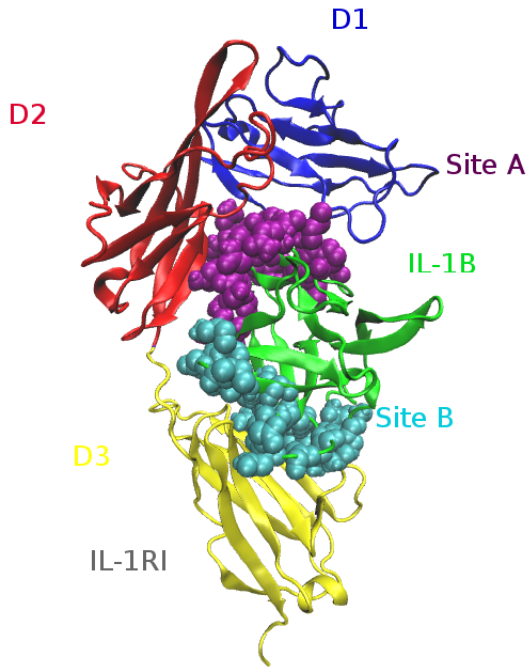


Figure 2.3: IL-1$\beta$ and IL-1RI complex is represented as a ribbon diagram with C-terminal end of receptor located at the bottom. Ig-like domains of IL-1RI is colored and labelled as D1, D2 and D3 in blue, red and yellow, respectively. Residues of Site A in IL-1$\beta$ represented as VDW in purple and Site C in cyan.

Studies on site-specific mutagenesis suggest that residues Arg 4, Leu 6, Phe 46, Ile 56, Lys 93, Lys 103 and Glu 105 on IL-1$\beta$ are essential for binding [19]. Gln15 is one of the most important residues in binding since its mutation to glycine causes 100 % loss of the binding activity [20]. An analysis suggests that His 30 is crucial for a high affinity binding with receptor through hydrogen bonding between loop 3 (Glu128) and turn 1 (Gln14) of the IL-1$\beta$ [21]. It is found that His 30 is responsible for organizing long-range interactions with site B (specifically V3, L10, M44, Y90, F99 and I104). It is an important study because different from IL-1$\beta$, which binds

IL-1RI Ig-like D1-D2 and D3 through its site A and B, IL-1Ra residues that are homologous to receptor site B on IL-1$\beta$ do not interact with D3 of IL-1RI. This site is proposed to activate a movement in domain 3 of IL-1RI [22]. According to site-directed mutagenesis studies on IL-1Ra [23], only site A residues affect receptor binding (specifically Trp16, Gln20, Tyr34, Gln36 and Tyr147). Therefore, the study suggests that communication between site A and site B is important for triggering a conformational change in the receptor for signalling [21].

### 2.2.2 IL-1 Primary and Secondary Receptors

Type I and II IL-1 receptors are well conserved in evolution and share the same chromosomal location. There are two types of IL-1 receptors; IL-1RI and IL-1RII, each having three extracellular Ig-like domains, limited sequence similarity (%28) and different pharmacological characteristics. The receptors exist both in transmembrane (TM) and soluble forms: the soluble IL-1 receptor is thought to be post-translationally derived from cleavage of the membrane receptors' extracellular portion [24]. IL-1RI contains three Ig domains in its extracellular domain and TIR domain in its cytoplasmic domain. Signalling TIR domain is composed of 200 amino acids and has similar homology with other members of IL-1R and TLR families [25]. After the binding of IL-$\alpha$ or IL-1$\beta$ to the extracellular domain of IL-1RI, IL-1RAcP is recruited. However, the type II receptor which lacks the TIR domain, is unable to transduce signal responses upon binding of IL-1$\beta$. Type II IL-1R has a higher affinity for IL-1$\beta$ than IL-1Ra. Also it has a scarce binding capacity for IL-1$\alpha$ [16]. Therefore, it acts as a competitive decoy receptor by inhibiting IL-1 activity. IL-1RI domain 1 and 2 are tightly joint with a disulfide linkage, at least 3 salt-bridges and a large domain of interaction between the side chains of both domains. Therefore, they behave as a single molecule rather than separate domain. On the contrary, domain 3 is connected

to domain 2 with a long flexible linker; consequently, it is able to move when no ligand is present [22]. Linkers and the $\beta$-bulge loop of IL-1$\beta$ and IL-1RI binary complex can be seen in Figure 2.4.

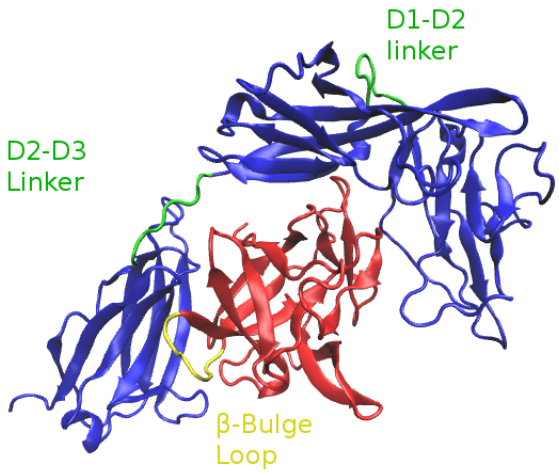


Figure 2.4: $\beta$-bulge loop members (colored in yellow); Gln 48 to Asp 54, 7 residue D2-D3 flexible linker members; 204-210, 7 residue D1-D2 rigid linker members; 100-107

There is a conformational change in the Ig-3 domain of IL-1RI after binding of IL-1 agonists but not in IL-1Ra, so this may help to develop an interaction between binary complex and IL-1RAcP [5]. At the beginning of the identification of IL-1AcP, some researchers claimed that IL-1Ra either does not permit the formation or disrupts the complex [26]. An additional finding shows that sIL-1AcP assists the antagonism

of IL-1 mechanism by increasing the binding affinity of IL-1$\beta$ to the soluble type II IL-1R, and by at the same time not changing the low binding affinity of IL-1Ra [27]. According to studies on IL-1 signalling cascades in liver cells, the deletion of a residue from the TIR domain of the IL-1RAcP results in failure of recruiting necessary elements in intracellular domain. Therefore, soluble IL-1RAcP is believed to form an inactive complex and therefore acts as an inhibitor [28].

# Chapter 3

# MATERIALS AND METHODS

## 3.1 Molecular Dynamics Simulations

Atomistic MD simulations were performed using GROMACS 4.5 simulation package[29] and [30] with the Charmm27 all atom force field (with CMAP). [31]. Leapfrog integrator [32] is used with a 2 fs time-step. Particle mesh Ewald electrostatics [33] were employed with fourth order interpolation, a maximum grid spacing of 0.12 nm and a cutoff of 1.0 nm. Water was modelled using the TIP3P water model [34]. A twin range van der Waals cutoff (1.0/1.4 nm) was used. Temperature is set to 310K via velocity-rescaling algorithm [35] with a coupling time constant of 0.1ps and pressure coupled at 1 bar using the Berendsen algorithm [36] with a coupling time constant of 1 ps. The initial structures for the simulations of IL-1$\beta$-IL-1RI, IL-1$\beta$-IL-1RI-IL-1RAcP, IL-1Ra-IL-1RI, IL-1$\beta$-IL-1RII-IL1-RAcP complexes were taken from the protein data bank with pdb ids; 1ITB [20], 4DEP [14], 1IRA [22] and 3O4O [15], respectively. Unbound type 1 and type 2 receptors were created from the x-ray structure of complexes (1ITB and 3O4O) by Chimera [37] for their individual simulations. In addition to these, for the unbound receptor simulations ST2, IL-18R1 and IL-1RAcP structures are taken from 4KC3, 4R6U and 4DEP respectively. Missing residues and atoms were added using Modeller [38] and Swiss-PdbViewer [39], respectively. After filling the gaps, energy minimization is applied by freezing native residues with 100 steps of steepest descent then 500 steps of limited-memory Broyden-Fletcher-Goldfarb-Shanno

(L-bfgs)[40]. After solvation to remove close contacts, stepwise minimization is applied by 200 steps l-bfgs with Protein-H frozen, 200 steps l-bfgs with Mainchain-H frozen, 500 steps l-bfgs with C-alpha frozen and 1000 steps unconstrained steepest descent. Then, to achieve desired temperature and pressure, system is equilibrated first under N,V,T ensemble then under N,P,T ensemble with leap-frog algorithm for both 200 ps. Production run were recorded for 200 ns and trajectories saved at every 10 ps. Visual Molecular Dynamics (VMD) package [41] is used for all molecular visualizations.

# Chapter 4

# STABILITY OF IL-1RI D3 DOMAIN

D1 and D2 domains of the IL-1RI act as a single module by forming a disulfide bond between C104 of D1-D2 linker and C147 of D2 domain. Also, as a result of 7 residue flexible linker between D2 and D3 domain, D3 domain acts independently. Because of the two main differences between the structure of the IL-1$\beta$ and IL-1Ra, they make distinct contacts with D3 domain of the IL-1RI. IL-1Ra lacks $\beta$-bulge loop ,which is usually functional [42], corresponding to residues 48 to 54 (QGEESND) in the IL-1$\beta$. When IL-1$\beta$ and IL-1Ra are aligned structurally, they show no significant difference with 1.7 Å RMSD. Aligned pairs sequence and the structures can be seen in Figures 4.1 and 4.2.

```
IL-1Ra:    8 SKMQAFRIWDVNQKTFYLRNN-QLVAGYLQGPNVNLEEKIDVVPIE------PHALFLGIHGGKMCLSCV

IL-1B :    3 VRSLNCTLRDSQQKSLVMSGPYELKALHLQGQDMEQQVVFSMSFVQGEESNDKIPVALGLKEKNLYLSCV
                                                                 49-54 (beta-bulge)
                                        91-94 (turn 6 bend 1)
IL-1Ra:   71 KSGDETRLQLEAVN---ITDLSENRKQDKRFAFIRSDSGPTTSFESAACPGWFLCTAMEADQPVSLTNMP

IL-1B :   73 LKDDKPTLQLESVDPKNYPKKKM----EKRFVFNKIEINNKLEFESAQFPNWYISTSQAENMPVFLGGTK
                              87-89 (turn 5)

                   140 (end of helix)
IL-1Ra:  138 DEGVMVTKFYFQED

IL-1B :  139 GG-QDITDFTMQFV
```

Figure 4.1: Sequences of aligned structures of IL-1$\beta$ and IL-1Ra. Regions that differ colored in red.

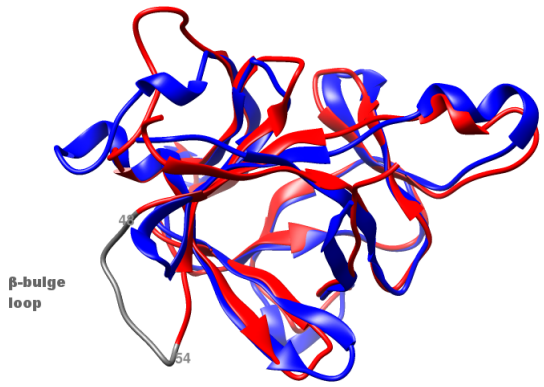


Figure 4.2: Aligned structures of IL-1$\beta$ and IL-1Ra colored in red and blue, respectively. $\beta$-bulge loop which is the major difference, colored in grey.

This causes weak interaction with the D3 domain of the IL-1RI as it can be seen from the interface representation in Figure 4.3.

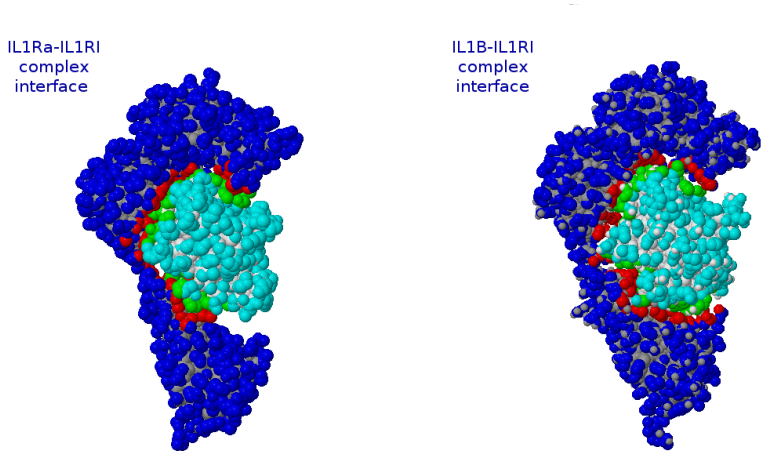


Figure 4.3: Absence of $\beta$-bulge loop in IL-1Ra creates less intimate interaction with the D3 domain of IL-1RI (left) compared to IL-1$\beta$ complex (right).

Another difference between the 3D structures of the IL1$\beta$ and IL-1Ra is that IL-1Ra has additional positively charged loop composed of Asn91, Arg92, Lys93 and Gln94 (90's loop) connecting $\beta$-12 and $\beta$-13 strands . This additional loop is distant

from the interface between the IL-1Ra and D3 domain of IL-1RI. Although it does not make direct contacts with IL-1RI, it interacts with Lys145 which is functionally an important residue. Asp145 of IL-1$\beta$ is a critical residue in recruiting IL-1RAcP, replacement of the Lys145 in IL-1Ra to Asp resulted in a partial agonist [43].

Despite IL-1Ra and IL-1$\beta$ three dimensional structural similarities, IL-1$\beta$ forms a signalling complex whereas IL-1Ra can not. In order to understand and quantify the effect of this structural difference between them, molecular dynamic simulations of the cytokines IL-1$\beta$, IL-1Ra and C116FmutantIL-1Ra in complex with IL-1RI are carried.

Mutagenesis experiments revealed important binding residues in both IL-1$\beta$ and IL-1Ra. However, since IL-1Ra - IL-1RI complex has a high fluctuation, the important residues can change and this might lead to new associations of residue pairs at the interface between cytokine and the receptor. For this reason, we take 5 snapshots from MD simulation of binary complexes and we determine their interfaces from PRISM [44]. In addition to residues which form the interface, hot spot's are found by using HotRegion [45]. These hot spots are small portions of interface residues which are critical for the majority of the binding energy. The method uses conservation, solvent accessible surface area and statistical pairwise residue potentials of interface residues [46].

Table 4.1: Clustered Hot Spot Predictions of binary complexes of IL-1$\beta$ and IL-1Ra with IL-1RI

| IL-1$\beta$-IL-1RI | | IL-1Ra-IL-1RI | |
|---|---|---|---|
| IL-1$\beta$ | IL-1RI | IL-1Ra | IL-1RI |
| 11,14,15,30,31,32,128 | 111,113,124,126,127 | 16,20,34,35,36,147 | 107,108,110 |
| 6,46,48,56 | 115,237,240,250,259,275 | 24,25,26 | 10,12,13 |
| | 13,15,16 | 51,56,102 | 6,112,113,121,124,297,233 |

In Table 6.7 and 6.8, first two column represents the native contacts at the interface between IL-1RI and IL-1$\beta$ in which coordinates are taken from the X-ray structure (1IRA). The second pair is the starting structure of the MD simulation which is at 310K. The other columns represent contacts at the interface of 5 different snapshots from MD production; 1, 2, 3, 4 and 5. (5 being last snapshot). Site I of IL-1$\beta$ interface is presented in Table 6.7 and site II in Table 6.8. The same procedure is applied for IL-1Ra - IL-1RI complex. Table 6.9 and Table 6.10 demonstrate site I and site II of the IL-1Ra interface, respectively.

Table 6.7 and 6.8 suggest that the binding sites that are found from mutagenesis and from X-ray structure do not change significantly. However, with motion of the complex, $\beta$-bulge members; 48 to 55 has changed. The shared site, siteI, between IL-1$\beta$-IL-1RI and IL-1Ra-IL-1RI complexes is stable in both of the simulations . As can be seen from Table 6.8, members of the $\beta$-bulge loop; Gly49, Ser52, Lys55, are forming contacts at the interface that are absent in the X-ray structure. Whereas, in Table 6.10, the absence of this loop creates weak interaction at the interface, thus interface members are changing. At first, Pro50, Ile51, Pro53 and His 54 are forming the interface with D3 domain and siteII of IL-1Ra, then it breaks with the rotation of the D3 domain. After the rotation, Asp104, Ser105, Gly106, Pro107, Gln149 and Glu150 are forming contacts. Many of these lastly formed contacts are made with the D2D3 linker of the IL-1RI.

IL-1Ra and IL-1RI complex is represented in 4.4 and native contacts at the interface is colored in yellow. Also in this Figure, the new contacts are depicted as green.

The change in these contact maps shows that IL-1Ra cannot make a stable contact with D3 domain of IL-1RI. In literature the cause of this change is the missing $\beta$-bulge loop. Therefore, in order to have a better understanding of the effect of these missing residues we concentrated on the D3 domain of the IL-1RI.

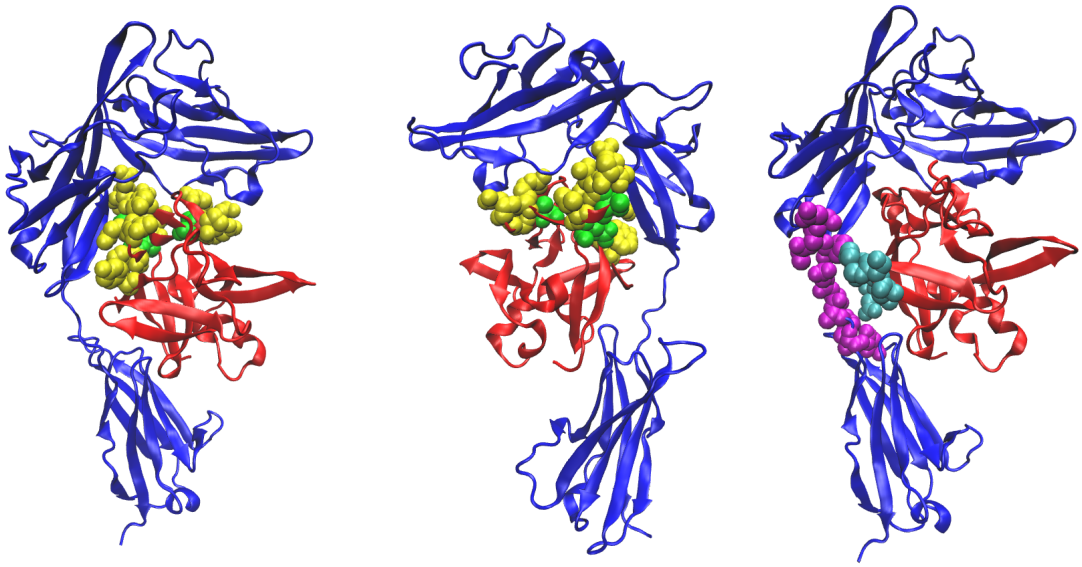

Figure 4.4: IL1Ra Site I members are represented as vdw with yellow and newly formed contacts in green from both front and back. IL-1Ra members of Asp104, Ser105, Gly106 ,Gln149 and Glu150 are forming contacts with D2D3 linker of IL-1RI represented as vdw in cyan and magenta respectively. In Table 6.10, the contacts with the D2D3 linker is written in bold

First, residue mean square fluctuations are calculated from trajectory which is aligned on the D1D2 module of IL-1RI. RMSF values of each binary complexes are analysed and residues of IL-1RI are presented in Figure 4.5.

As it can be seen from Figure 4.5, D3 domain of IL-1RI has much higher flexibility than D1D2 module. In addition to that, when it is complexed with the agonist IL-1$\beta$ and C116F-mutant-IL-1Ra, D3 domain flexibility is restricted. From RMSF values, it can be said that D3 domain becomes 3-fold flexible when it is bound to antagonist IL-1Ra. With this aligned trajectory, RMSF of ligands are plotted. The sequences of previously aligned structures of IL-1$\beta$ and IL-1Ra are used for comparison purposes. In Figure 4.6, it is seen that IL-1$\beta$ has high fluctuations at three parts in which members are residues 71Cys, 72Val, 73Leu; 93Lys, 94Lys, 95Met, 96Glu, 97Lys;

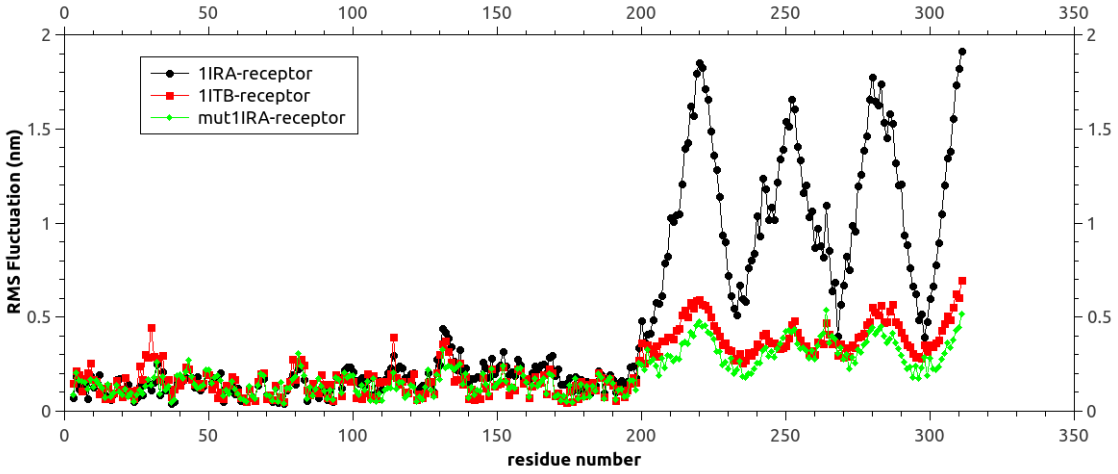


Figure 4.5: RMS Fluctuations of IL-1RI when it is in complex with agonist, antagonist, and C116F-mutant IL-1Ra, colored in red, black and green, respectively.

138Lys and 139Gly. These parts coincide with residues 69Cys, 70Val, 71Lys; 88Leu, 89Ser, 90Glu, 91Asn, 92Arg; 137Pro and 138Asp of IL-1Ra.

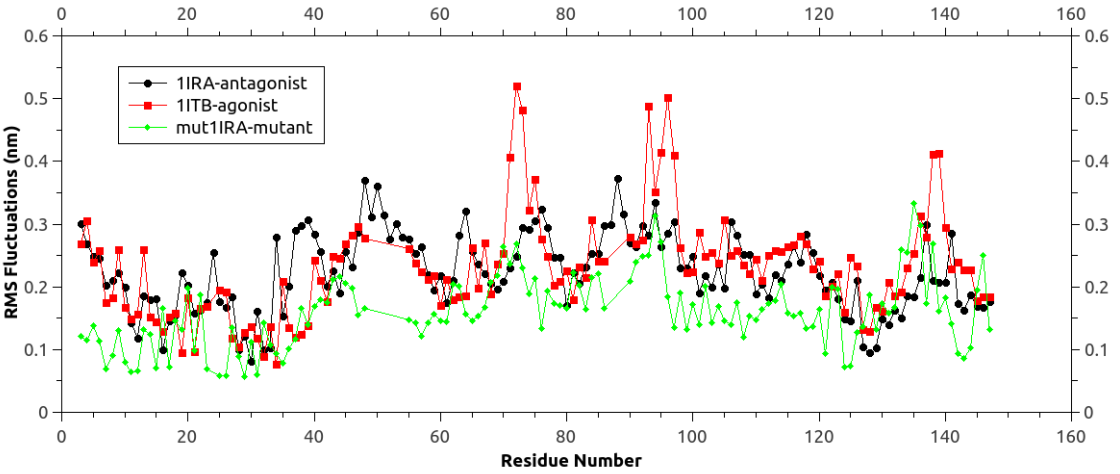


Figure 4.6: RMS Fluctuations of agonist, antagonist, and C116F-mutant IL-1Ra residues when it is in complex with IL-1RI, colored in red, black and green, respectively.

# Chapter 5

# ROLE OF D3 DOMAIN STABILITY ON IL-1RACP RECRUITMENT

IL-1 signalling is inhibited by IL-1ra and IL-1RII. While IL-1$\beta$ can recruit IL-1RAcP when it is bound to IL-1RI and is able to initiate signalling, bound form of IL-1Ra can not recruit IL-1AcP and signalling is inhibited. In order to understand the antagonism of IL-1Ra, we replace the IL-1$\beta$ with IL-1Ra in signalling complex which is IL-1$\beta$ - IL-1RI - IL-1RAcP. For that reason, we created a pseudo complex, a complex that can not be formed because of IL-1Ra. We used RCSB PDB Protein Comparison Tool with JCE structural alignment algorithm to create IL-1Ra - IL-1RI - IL-1RAcP pseudo complex.

In Chapter 4, we hypothesized that IL-1Ra - IL-1RI binary complex caused a high RMS fluctuation in D3 domain of IL-1RI when compared to IL-1$\beta$ - IL1RI. Consequently, IL-1RAcP can not form a stable complex with IL-1Ra - IL-1RI whereas with IL-1$\beta$ - IL-1RI it can. In this Chapter, we tried to analyze the differences between signalling complex, IL-1$\beta$ - IL-1RI - IL-1RAcP, and the complex that can not be stably formed, IL-1Ra - IL-1RI - IL-1RAcP. In addition to those, non-signalling complex that is formed with decoy receptor (IL-1RII) , IL-1$\beta$ and IL-1RAcP is simulated in order to compare the stability of D3 domain of IL-1RII which is structurally very similar to IL-1RII with RMSD of 2.8. Structural alignment of type 1 and 2 IL-1 receptors can be seen in Figure 5.1.

Figure 5.1: Aligned structures of type I and II IL-1 Receptors represented as ribbon in brown and blue respectively.

In order to compare the RMS fluctuation of receptor type I and II in ternary complex forms, first residues are aligned according to structural similarity. Trajectory is first aligned on D1D2 module of the receptor since it is known to be the most stable part. It can be said from the Figure 5.2 that D3 domain (residue 200-314) of the receptors has a high RMS fluctuation compared to D1D2 module. But most strikingly, created (pseudo) complex consisting of IL-1Ra - IL-1RI - IL-1RAcP has a much higher fluctuation in D3 domain of its receptor compared to decoy and signalling complexes. This fluctuation causes a loss of contacts compared to signalling and decoy complexes. Therefore, we showed that when IL-1RAcP tries to associate with the IL-1Ra - IL-RI complex there will be a high fluctuation in the D3 domain and this will cause an unstable structure.

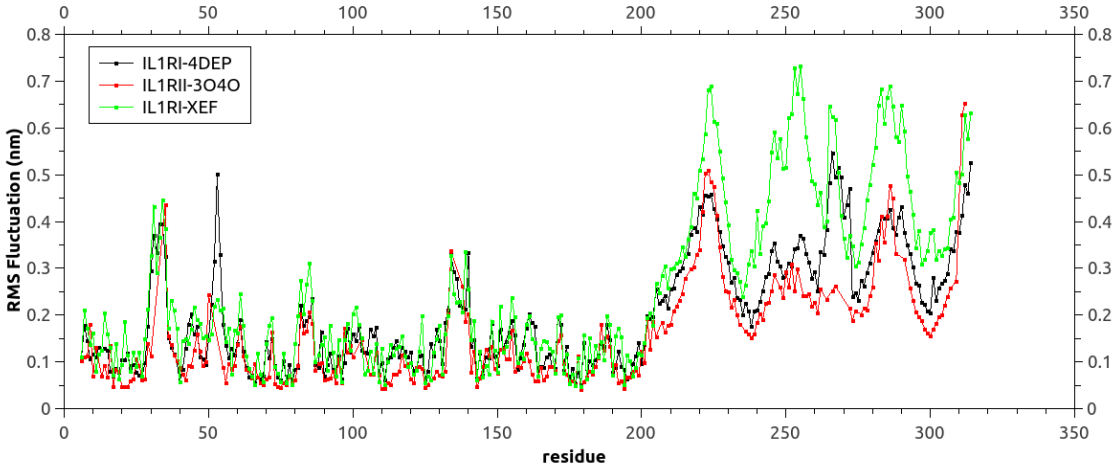


Figure 5.2: RMS fluctuation of IL-1 receptors. Green line represents the pseudo complex formed by IL-1Ra - IL-1RI - IL-1RAcP. Black line represents the signalling complex, IL-1$\beta$ - IL-1RI - IL-1RAcP. Red line represents the decoy complex, IL-1$\beta$ - IL-1RII - IL-1RAcP.

# Chapter 6

# UNBOUND FORM OF IL-1R SUPERFAMILY STRUCTURES AND MD RESULTS

## 6.1 Introduction

The conserved domain in a receptor family suggests common signalling pathways as in the IL-1 family [47]. IL-1 superfamily members are IL-1, IL-18 and IL-33. They have crucial roles in host defense against infection, inflammation, injury and stress. They share common origin, receptor structure and signalling pathways. Among the IL-1 family, the formation of a binary complex between the respective cytokine and its primary receptor is the first step of the initiating signalling. A second receptor is recruited and the alignment of the both receptor's TIR domains triggers intracellular proteins for a signalling. In spite of low sequence similarity with a 17 % score [48], IL-1 superfamily receptors share a high structure similarity with 56 % score [38]. Aligned structure sequences and sequence similarities are shown in Appendix. The structure and the importance of each member is discussed in detail below.

This chapter will focus on the structure of the IL-1 receptors (IL-1RI, IL-1RII, IL-1RAcP, IL-18R1, and IL-1RL1 also called ST2) and provide new conformations for the IL-1 Receptor family from Molecular Dynamics studies.

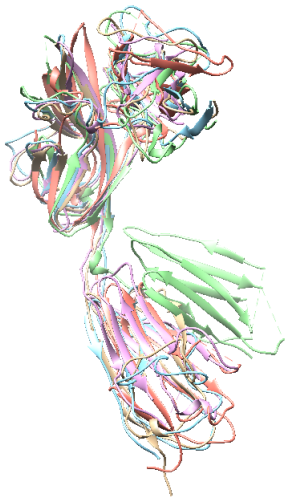

Figure 6.1: Structurally Aligned pdb's

## 6.2 MD Simulation Results of Unbound Form of Type I IL-1 Receptor

MD simulation of IL-1RI alone showed that after 50 ns it takes a compact form compared to its initial conformation which is taken from its complex structure with IL-1$\beta$. Dimensions of the open conformation is 102 Å × 63 Å × 46 Å . After taking this close conformation dimensions changed to 64 Å × 68 Å × 49 Å. For the remainder of the simulation IL-1RI conserves this state. Domains of open and close conformations are equivalent with each other whose members are 6-97, 107-201, 210-311 for D1, D2 and D3 respectively. D1 and D2 domains act as a whole and their structure is very similar to initial conformation with RMSD of 2.6 Å . Also when the D3 domain is aligned separately, it conserves rigidity with RMSD of 1.43 Å . As depicted in Figure 6.2, 3rd domain of IL-1RI rotates 160° compared to its initial open form with the help of a long flexible linker between D2 and D3, consisting of residues 202 - 209. With this rotation ligand binding SiteB is directing away from the interface between D1 and D3 domain.

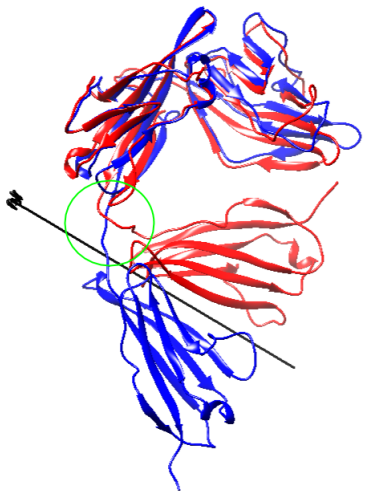

Figure 6.2: Rotation of D3 with respect to its initial conformation. Rotation axes is depicted as an arrow. Bending residues Glu 202 and Glu 203 are highlighted.

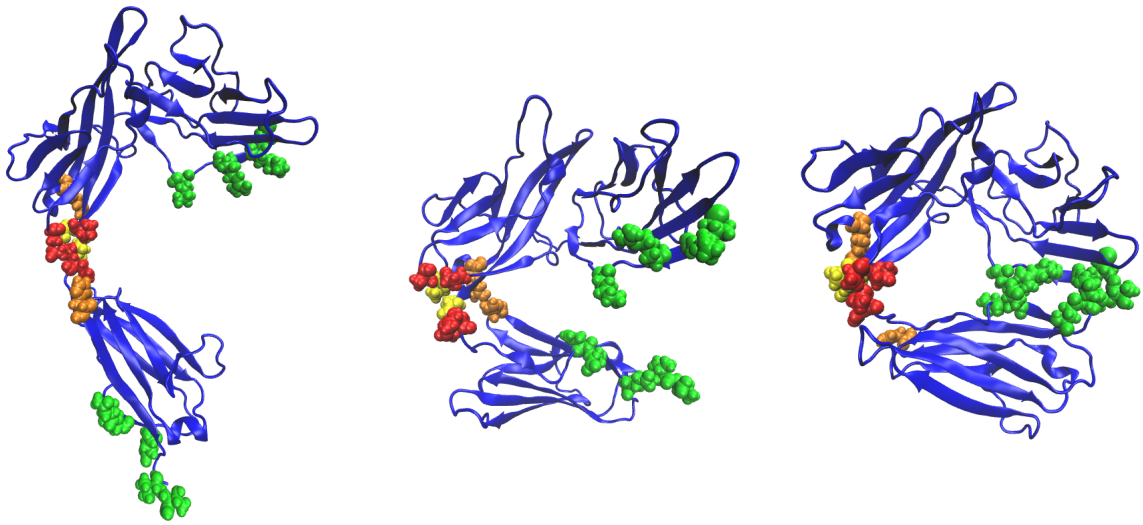

Figure 6.3: Important interface residues on IL-1RI in taking close conformation of IL-1RI

IL-1RI takes its close conformation first by making contacts with its flexible linker member Glu202 and D2 member Val117, depicted as yellow in Figure 6.3. Then, the hinge bending residue GLU 203 depicted as red makes contact with Glu201. After making this interaction, Arg174 and His301 make contacts (orange) and this contact forms a necessary part for the zipping movement of D3 domain towards D1D2 module. In the close conformation of IL-1RI there is a newly formed interface via extending of the beta sheets with D1 and D3 domain. Members of the interface are given in Table 6.1 below.

In the protein data bank, there is a complex structure of IL-1RI with a small antagonist peptide, 1g0y [10]. Complex structure of IL-1RI and small antagonist can be seen in Figure 6.6. This structure is very similar to the closed form of receptor when IL-1RI is simulated without any peptide. Alignment of the two receptor conformations, one from X-ray structure and the other from simulation gives 2.6 Å RMSD [49].

The x-ray structure also showed 170°rotation of D3 compared to the previous binary

Table 6.1: Interface residues of close conformation of IL-1RI colored in representative of Figure 6.3

| **interface residues** | |
|---|---|
| **D1 domain members** | **D3 domain members** |
| Cys6 | Thr315 |
| Lys7 | Glu220 |
| Arg9 | Glu217 |
| Lys12 | Asn216 |
| Pro116* | Asn204 |
| Ala118* | Asn204 |
| Arg174 | Glu202 |
| Arg174 | His301 |
| Thr200 | Glu202 |
| Val117 | Glu202 |

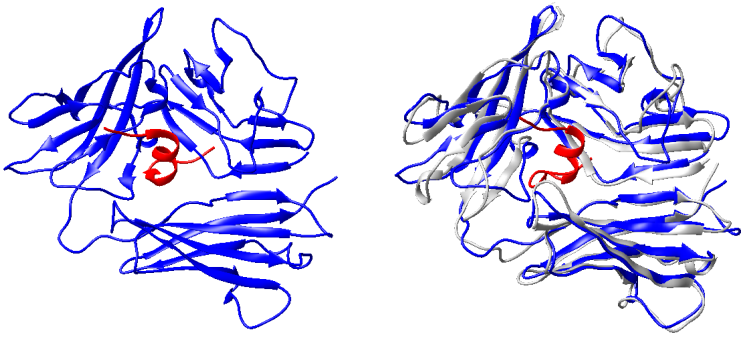

Figure 6.4: Crystal structure of IL-1RI with small antagonist (left) and aligned structures of close conformation of IL-1RI when simulated in unbound form (grey) with X-ray crystal structure (blue)

complexes of the IL-1RI [10]. In addition to its structure, they reported that GLU-203 is in the disallowed region of Ramachandran plot as a consequence of being a part of the linker of D2 and D3. According to our simulations, GLU-203 is the hinge-bending residue since its dihedral angle dramatically changes at the time where it takes the

close conformation. The change of the dihedral angle of Glu203 during MD simulation can be seen in Figure 6.5.

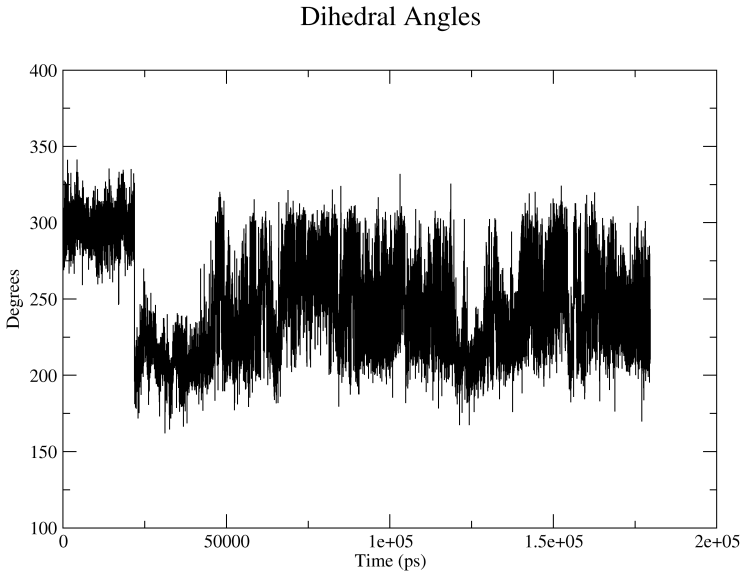


Figure 6.5: Change of Dihedral angle of the hinge residue Glu 203 along time.

Vigers et al. [10] suggest that this change in conformation is due to the binding of the small antagonist peptide to the IL-1RI. They tried to determine if this arrangement would occur in free receptor at physiological conditions with numerous experimental techniques but their attempts were unsuccessful. However, our studies show that it can take this compact conformation in the absence of a ligand.

While the receptor is taking this compact conformation, N-terminal residues 7-12 and D3 domain residues 216-220 forms an extended beta sheet. According to the simulation, 6 hydrogen bonds are formed by this new extended beta sheet. The x-ray structure of IL-1RI with small antagonist peptide also confirms this formation of an antiparallel beta sheet. Pairs that make hydrogen bonding with D1 domain and D3 members from both MD simulation and crystal structure are tabulated in Table 6.2

with 30° cutoff angle and 0.35 nm cutoff distance.

Table 6.2: Hydrogen Bonds between N-terminal residues of D1 domain of IL-1RI and D3 domain residues

| **Crystal Structure** | | | | **MD structure** | | | |
|---|---|---|---|---|---|---|---|
| **D1** | **Atom** | **D3** | **Atom** | **D1** | **Atom** | **D3** | **Atom** |
| Lys7 | N | Glu220 | OE1 | Lys7 | NZ | Glu220 | OE1 |
| Glu8 | O | Glu220 | N | Glu8 | O | Thr218 | OG1 |
| Glu8 | OE1 | Thr218 | OG1 | Arg9 | NH1 | Glu217 | OE1 |
| Arg9 | NH1 | Glu217 | OE1 | Arg9 | NH1 | Thr218 | O |
| Glu10 | N | Thr218 | O | Glu10 | N | Thr218 | OG1 |
| Glu10 | O | Thr218 | N | Glu10 | O | Thr218 | N |
| Lys12 | N | Asn216 | O | Lys12 | NZ | Asn216 | O |
| Ile13 | N | Asn216 | O | Lys12 | N | Glu217 | OE2 |
| Ile13 | N | Ala215 | O | Lys12 | NZ | Thr218 | OG1 |
| | | | | Lys12 | NZ | Ala215 | O |
| | | | | Cys6 | N | Thr315 | OT1 |
| | | | | Lys7 | NZ | Val314 | O |
| | | | | Lys7 | N | Thr315 | OT2 |
| | | | | Lys12 | NZ | Tyr307 | OH |
| | | | | Lys12 | NZ | Gln309 | OE1 |

We provide close conformation of free IL-1RI which is an inactive form since it prevents itself from the entry of its cytokine.

Extensive hydrogen bonding pattern is seen through out the IL-1 superfamily taking a close conformation. This arrangement can have a biological significance to prevent the binding of any unwanted IL-1 ligands, and can be another mechanism in modulating signalling.

Furthermore, the site which makes hydrogen bonding when the receptor takes an

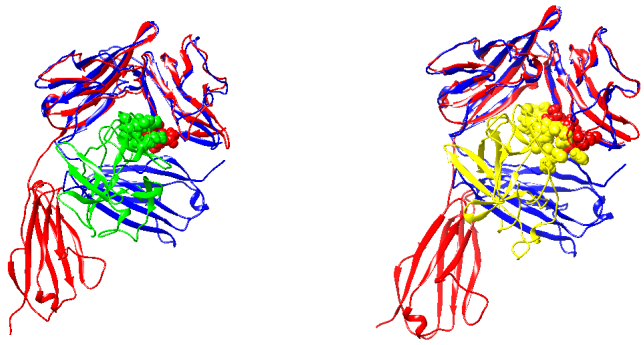

Figure 6.6: Close conformation of IL-1RI (blue) is aligned on IL-1$\beta$-IL-1RI (left) and IL-1Ra - IL-1RI (right) structures, contacts that are tabulated at 6.3 represented as VDW.

inactive form, also makes important contacts when it is bound to its ligands; IL-1$\beta$ and IL-1Ra. The contacts are comparatively demonstrated in Figure 6.6 and contact types of the residues are explained in Table 6.3.

Table 6.3: Important residues in close conformation of IL-1RI making contacts with IL-1$\beta$ and IL-1Ra

| **IL-1RI** | **Atom** | **IL-1$\beta$** | **Atom** | **Contact Type** | **IL-1RI** | **Atom** | **IL-1Ra** | **Atom** | **Contact Type** |
|---|---|---|---|---|---|---|---|---|---|
| Glu11 | OE1 | Asn129 | ND2 | HB | Arg9 | CZ | Asn27 | CB | HPH |
| Glu11 | OE2 | Lys27 | CE | Other | Arg9 | CZ | Arg26 | CG | HPH |
| Lys12 | NZ | Asp35 | OD1 | HB | Glu11 | OE1/OE2 | Arg26 | NH2/NE | HB |
| Lys12 | CG | Gln38 | CD | HPH | Glu11 | OE1 | Tyr24 | CE1 | Other |
| Ile13 | CB | Gln38 | CG | HPH | Lys12 | NZ | Leu25 | O | HB |
| Ile13 | CG1 | Asp35 | CG | HPH | Lys12 | CB/CD/CE/C | Leu42 | CD1/CD2 | HPH |
| Ile13 | CG1 | Leu31 | CD2 | HPH | Ile13 | CG1/CD1 | Tyr24 | CE1/CZ/CD1 | HPH |
| Ile13 | CG1 | Leu29 | CD1 | HPH | Ile13 | CG1/CD1 | Leu42 | CD2 | HPH |
| Ile13 | CG2 | Lys27 | CE | HPH | Ile13 | CG2/CD1 | Asn39 | CB | HPH |
| | | | | | Ile13 | CG2/CD1 | Leu35 | CD2/CA | HPH |
| | | | | | Ile13 | CA | Asn39 | OD1 | Other |

HB - Hydrogen Bond
HPH - Hydrophobic

## 6.3 MD Simulation Results of Unbound Form of IL-1 Receptor-Like 1

IL-33 is a recent member of the IL-1 cytokine superfamily. IL-1RAcP is an essential protein also for binary complex of IL-33 and its primary receptor, IL-1RPL1 (ST2). This ternary complex is needed for signalling. Ligand-free form of IL-1RPL1 MD simulation showed that this receptor is taking a compact conformation like IL-1RI with the dimensions changed from 72 Å × 90 Å × 49 Å to 56 Å × 63 Å × 54 Å. D1 and D2 domains act as a whole and their structure is very similar to initial conformation with RMSD of 2.9 Å . Also when D3 domain aligned separately, we see that it conserves rigidity with RMSD of 3 Å . As depicted in Figure 6.7, 3rd domain of IL-1RPL1 rotates 132° compared to its initial open form.

Figure 6.7: Rotation of D3 domain with respect to its initial conformation around its rotation axes depicted as black arrow and bending residues are circled; 181-190.

Flexibility comes from long D2 - D3 linker, consisting of 7 residues 183-190 and this flexibility make ST2 able to bind its cytokine, IL-33 [50]. Open and close conformations domains are similar with members of 1-86, 101-182 and 191-297 for D1, D2 and

D3 respectively.

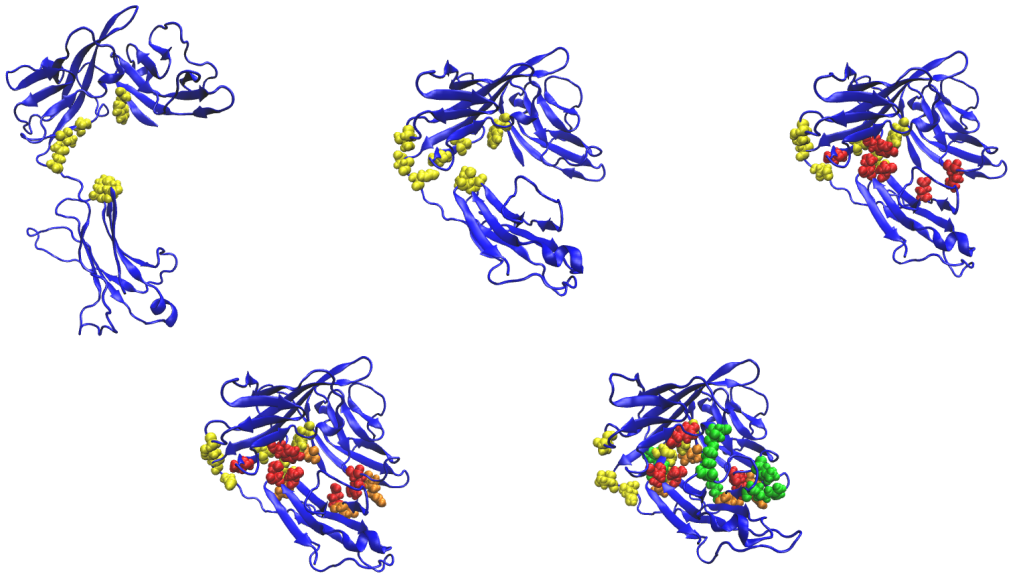

Figure 6.8: Conformations of IL-1RPL1 (ST2)

The formation of compact conformation is very much like IL-1RI. First, it makes contacts with its flexible linker member Gln186 and D2 member Val102, depicted as yellow in Figure 6.8. Also, D1-D2 linker member Ser100 makes contact with D3 member Leu288 and D1 member Trp5 with D3 member Phe225. Then, we see the same zipping movement of D3 domain towards the D1D2 module with order of formation of contacts colored in consequent manner yellow,red,orange and green respectively. In close conformation of IL-1RPL1 there is a newly formed interface via extending of the beta sheets with D1 and D3 domain. Members of the interface are given in Table 6.4 below.

There is also another study suggesting that ST2 takes a close conformation. This study uses both SAXS studies and BILBOMD program which uses constrained MD to characterize conformation changes of the receptor. They revealed that ST2 takes different conformers in solution which can be seen in Figure 6.10.

Table 6.4: Interface residues of close conformation of ST2 colored in representative of Figure 6.8

| **interface residues of ST2** | |
|---|---|
| **D1 domain members** | **D3 Domain members** |
| Ser1 | Glu250 |
| Lys21 | Arg245 |
| Tyr96 | Leu288 |
| Asp117 | Lys237 |
| Arg76 | Glu249 |
| Gln3 | Gln224 |
| Tyr99 | Leu288 |
| Arg18 | Glu250 |
| Val102 | Leu226 |
| Trp5 | Phe225 |
| Ser100 | Leu288 |
| Val102 | Gln186 |

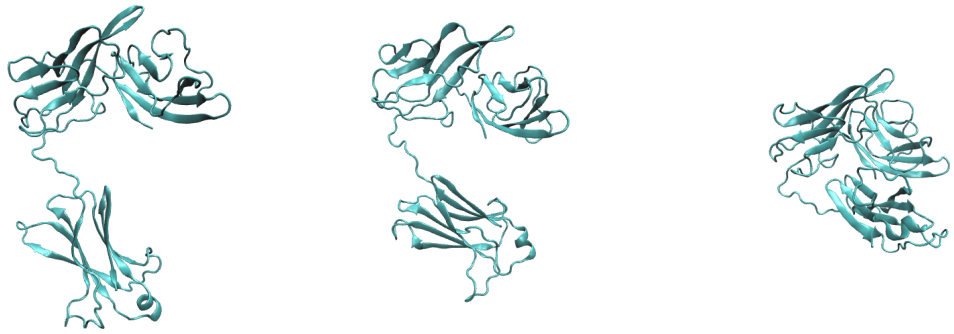

Figure 6.9: Conformations of IL1RPL1 (ST2)

Their structure is very similar to our findings. When their closed form of receptor and our structure from MD simulation is compared by aligning D1D2 module, it showed a resemblance with RMSD of 2.4 Å . Aligned structures can be seen in Figure 6.11.

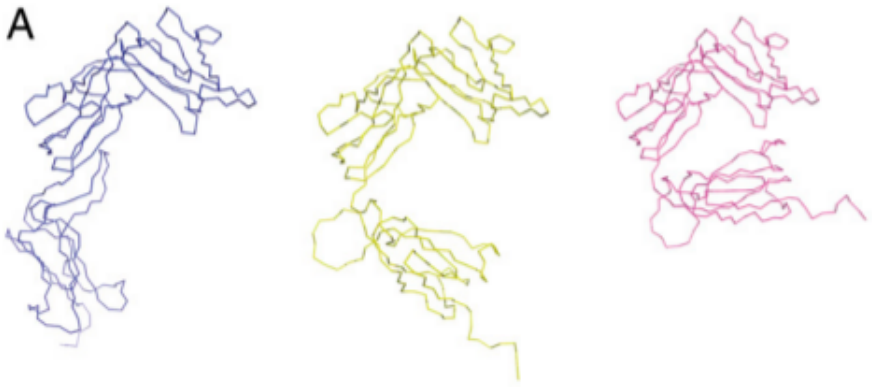


Figure 6.10: 3 conformers of ST2 from Liu et al.

Figure 6.11: ST2 conformers of Liu et al. (red) and our MD snapshot (blue) is aligned via D1D2 module.

## 6.4 MD Simulation Results of Unbound Form of IL-18 Receptor 1

IL-18 is involved in both innate and adaptive immune responses. This cytokine induces IFN-$\gamma$ production from T-helper lymphocytes cells and macrophages, and upregulates cytotoxic natural killer cells. Primary and accessory receptors for IL-18 signalling are IL-18R1 and IL-18RAcP. The structural comparison reveals that IL-18 binding sites of its primary receptor is structurally similar to IL-1RI site I and II on IL-1$\beta$ [12].

IL-18R1 is also similar to IL-1RI and IL-1RPL1 as it has a long flexible linker between D2 and D3 domain. Members of domains are 1-93, 106-186 and 194-298 for D1, D2 and D3 respectively. By the help of this linker, IL-18R1 takes a compact form like IL-1RI and IL-1RPL1. Dimension of the starting structure are 54 Å $\times$ 103 Å $\times$ 60 Å and the compact structure has dimensions of 58 Å $\times$ 64 Å $\times$ 71 Å. Close conformation showed 80°rotation of D3 compared to the starting structure of the IL-18R1 as depicted in Figure 6.12. These two conformations of IL-18R1 have 2.06Å RMSD when they are aligned according to their D1D2 module.

Figure 6.12: Rotation of the 3rd domain with respect to its initial conformation. Rotation axes is demonstrated as an arrow and close up is highlighted for bending residues Asp189, Arg190 and Ser191.

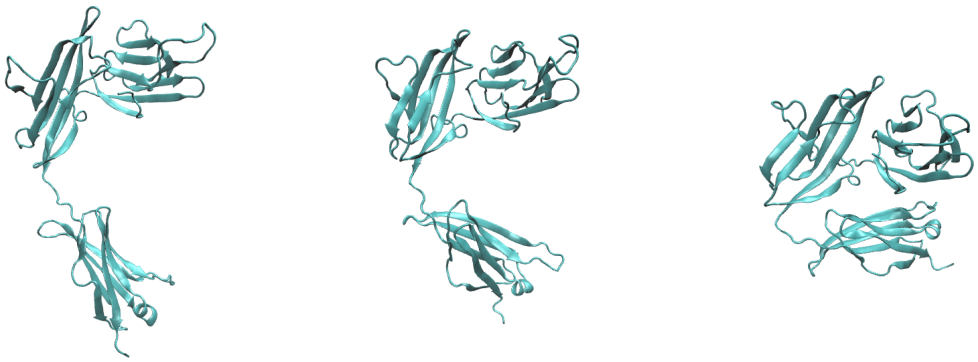

Figure 6.13: Conformations of IL-18R1

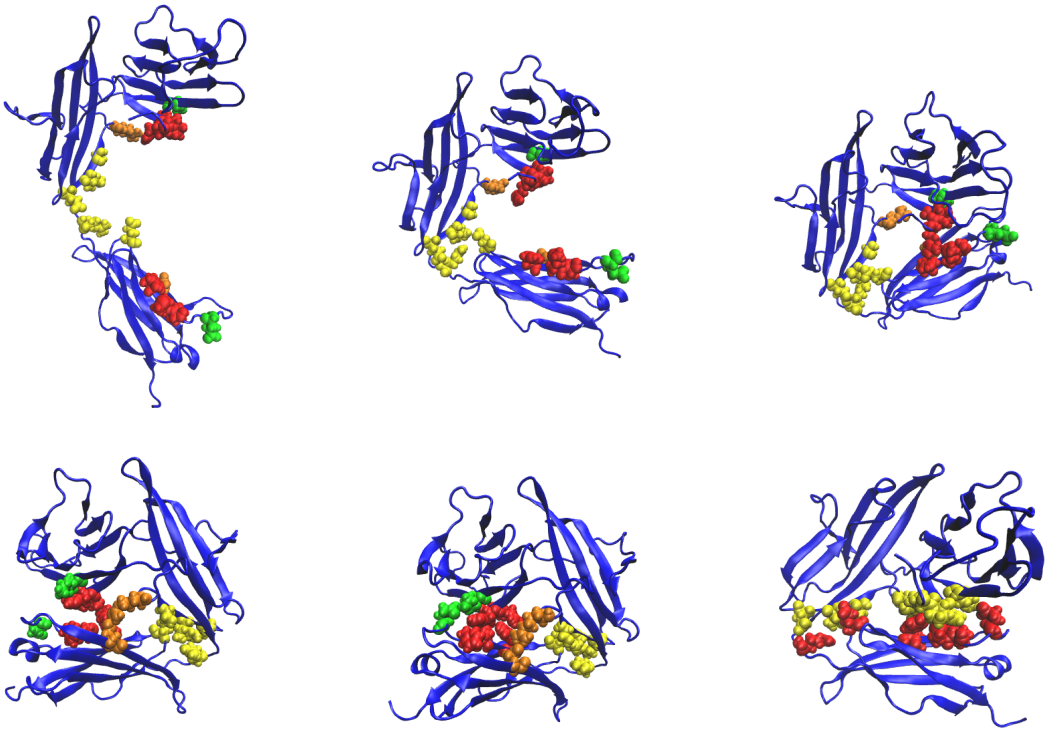

Figure 6.14: Formation of non-existing contact members are depicted as VDW with time evolution; yellow, red, orange and green respectively. Interface residues of close conformation of IL-18R1 is demonstrated at Table 6.5

Table 6.5: Interface residues of close conformation of IL-18R1 colored in representative of Figure 6.14

| **interface residues of IL-18R1** | |
|---|---|
| **D1 domain members** | **D3 Domain members** |
| Lys88 | Glu234 |
| Arg103 | Glu244 |
| Arg5 | Tyr228 |
| His7 | Met230 |
| Lys86 | Glu233 |
| Glu111 | Arg190 |
| Lys108 | Glu224 |
| Ile109 | Glu224 |
| Val187 | Arg190 |
| Glu188 | Arg190 |
| Asp189 | Arg190 |

Since D3 domains make a dramatic movement towards D1 domain of the receptors, the binding energy between D1 and D3 domains are calculated using Fiberdock [51]. In Table 6.6, the types of the contributing binding energies are demonstrated for IL-1RI, IL18R1 and ST2.

Table 6.6: Fiberdock energies of interfaces of inactive form of receptors

| **receptor** | **binding energy** | **aVdW** | **rVdW** | **ACE** | **HB** | **aElec** | **rElec** | **laElec** | **lrElec** |
|---|---|---|---|---|---|---|---|---|---|
| IL-1RI | -33.36 | -11.54 | 5.71 | 3.01 | -1.45 | -205.07 | 9.38 | -10.16 | 6.58 |
| IL-18R1 | -17.90 | -14.74 | 12.52 | 4.09 | -1 | -92.21 | 0 | -30.07 | 1.66 |
| ST2 | -38.19 | -21.91 | 15.73 | -1.65 | -0.22 | -204.31 | 47.97 | -26.36 | 21.15 |

aVdW, rVdW - softened attractive and repulsive van der Waals energy
ACE - atomic contact energy (ACE)
aElec,rElec - attractive and repulsive short-range Coulomb electrostatics
laElec, lrElec - attractive and repulsive long-range Coulomb electrostatics
HB - hydrogen and disulfide bonds

## 6.5 MD Simulation Results of Unbound Form of Type II IL-1 Receptor

IL-1RII cannot form the signalling complex because of its short TIR domain. Therefore, this decoy receptor has a role in IL-1 inhibition mechanism. Also, it binds IL-1$\beta$ with higher affinity than the IL-1Ra, showing another regulatory mechanism.

MD simulation of ligand-free form of IL-1RII revealed that it has a rigid form unlike IL-1RI, ST2 and IL-18R1. D1, D2 and D3 domain members of IL-1RII are 15-113, 116-216 and 222-330, respectively.

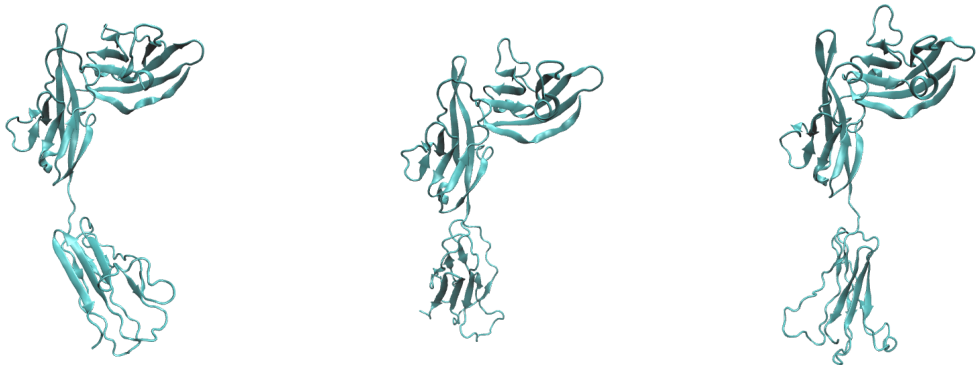

Figure 6.15: Snapshots from MD Simulation of IL-1RII

As presented in Figure 6.16, D2 member Asp183 is making contact with D3 member Glu290 from at the begining of the simulation. This contact also aids in forming strong contacts with D2-D3 linker member Lys217 , Lys219 and D3 member Glu290. Consequently, this contact restricts the movement of D3 domain.

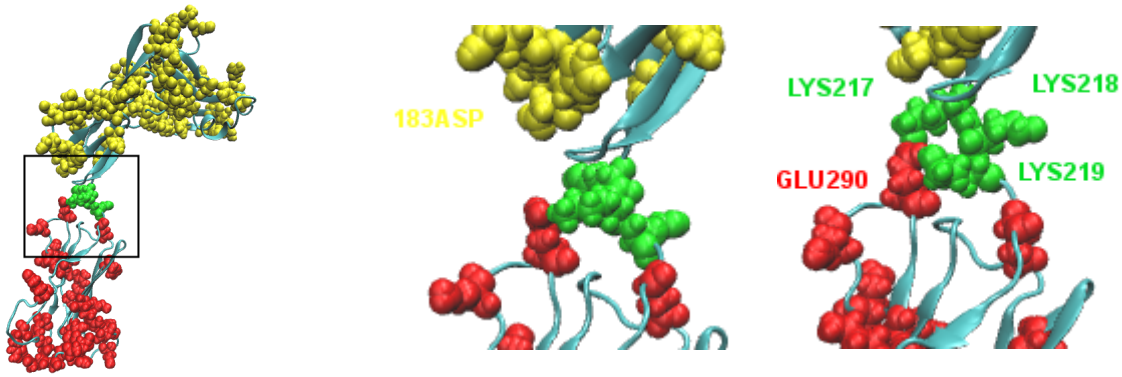


Figure 6.16: IL-1RII important contacts that disables D3 domain to gain high flexibility

## 6.6 MD Simulation Results Unbound Form of IL-1 Receptor Accessory Receptor

Both type I and type II receptors associate with IL-1RAcP but signalling occurs only with association of IL-1$\beta$ - IL-1RI complex. This activation by IL-1RAcP is essential for signalling. In addition to its activation role, there are also proposed inhibitory mechanisms of IL-1RAcP. First of all, IL-1RAcP is unable to recognize IL-1 cytokine by itself [13]. As a result, it does not decrease IL-1Ra concentration. Secondly, soluble form of IL-1RAcP can still bind the binary complex and inhibits IL-1 signalling [28]. Consequently, it decreases the concentration of available IL-1$\beta$ - IL-1RI complex and takes an inhibitory role.

200ns MD simulation of soluble IL-1RAcP showed that it has a single conformation and does not take a close form as primary receptors; IL-1RI, IL18R1 and ST2. This rigid behaviour is also supported by SAXS studies of unbound state IL-1RAcP in solution [50].

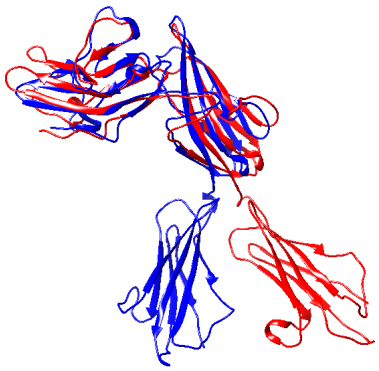

Figure 6.17: Starting structure and final structure is aligned through their D1D2 module represented as blue and red, respectively.

Domains of IL-1RAcP consist of residues: 5-112, 126-213 and 217-328 for D1, D2 and D3 respectively. D3 of IL-1AcP makes a swing through its short linker as it can be

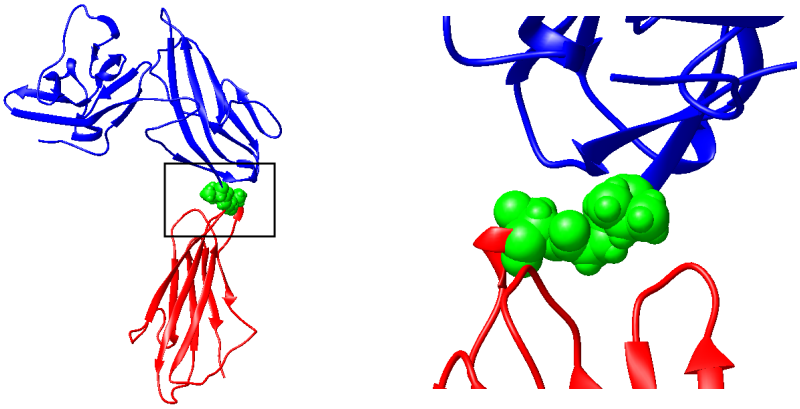

Figure 6.18: Short linker of sIL-1RAcP is represented as VDW with green.

seen from Figure 6.17. This short linker between D2 and D3 domain of IL-1RAcP consists of 3 members: Val124, Gly215 and Ser216 as depicted in Figure 6.18. Due to its shortness, D2 and D3 domain members are able to make contacts. Throughout the simulation Lys218 and Glu132; Glu288 and Lys128, Lys128; make strong contacts, illustrated in 6.19.

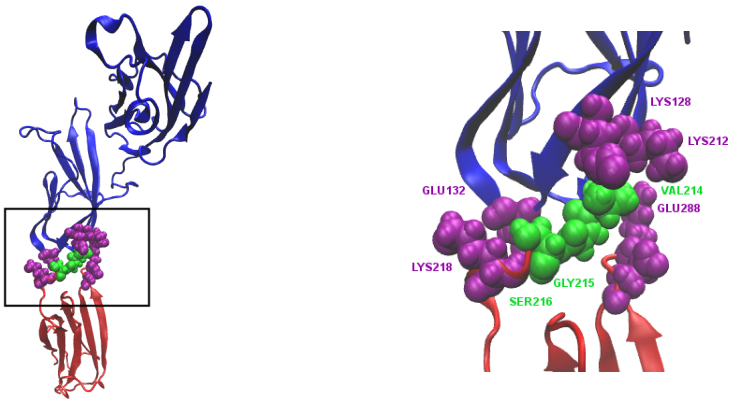


Figure 6.19: IL-1RAcP short linker stays rigid with its charged contacts

This study presents that sIL-1RAcP is rigid when it is unbound. This suggests another inhibitory role for sIL-1RAcP by not taking an inactive form and being ready for

forming non-signalling complexes. Furthermore, this low degree of flexibility can be a reason of sIL-1RAcP inability of directly engaging cytokines.

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# APPENDIX

**Interface residues of IL-1$\beta$-IL-1RI complex along trajectory**

Table 6.7: Interface residues and its contacts of IL-1$\beta$ Site I when it is in complex with IL-1RI during trajectory

| **IL-1$\beta$-IL-1RI Site I** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **X-ray structure (1ITB)** | | **starting structure** | | **1** | | **2** | | **3** | | **4** | | **5** | |
| **IL-1$\beta$** | **IL-1RI** | **IL-1$\beta$** | **IL-1RI** | **IL-1$\beta$** | **IL-1RI** | **IL-1$\beta$** | **IL-1RI** | **IL-1$\beta$** | **IL-1RI** | **IL-1$\beta$** | **IL-1RI** | **IL-1$\beta$** | **IL-1RI** |
| Arg11 | Lys114 | X | X | X | X | X | X | Arg11 | Lys114,Gln113 | Arg11 | Lys114,Pro116 | Arg11 | Lys114 |
| Gln14 | Val124,Arg163 | ✓ | ✓ | X | X | Gln14 | Val124 | Gln14 | Val124 | X | X | Gln14 | Val124 |
| Gln15 | Leu115,Gly122, Gln113,Lys114, | Gln15 | Gln113,Lys114, Gly122 | Gln15 | Gln113 | Gln15 | Gln113 | Gln15 | Leu115,Gln113, | Gln15 | Leu115,Gln113, | X | X |
| X | X | X | X | X | X | X | X | X | X | Val19 | Ile13 | X | X |
| X | X | Ser21 | Arg9 | Ser21 | Glu11 | Ser21 | Glu11 | Ser21 | Arg9,Glu11, Lys12 | Ser21 | Arg9,Glu11, Lys12 | Ser21 | Ile13 |
| X | X | X | X | X | X | Gly22 | Arg9 | ✓ | ✓ | ✓ | ✓ | Ser21 | Lys12 |
| X | X | X | X | Pro23 | Arg9 | Pro23 | Arg9 | ✓ | ✓ | X | X | X | X |
| Glu25 | Asn30,Pro31 | X | X | Glu25 | Arg9 | Glu25 | Arg9 | Glu25 | Arg9 | Glu25 | Arg9 | X | X |
| Lys27 | Glu11 | ✓ | ✓ | Lys27 | Glu11,Arg9 | Lys27 | Glu11 | Lys27 | ✓ | Lys27 | ✓ | Lys27 | Glu11 |
| X | X | Leu29 | Ile13 | Leu29 | Tyr127 | X | X | Leu29 | Ile13,Tyr127 | Leu29 | Ile13,Tyr127 | Leu29 | Ile13 |
| His30 | Phe111,Gln113, Val124,Pro126 | His30 | Tyr127,Gln113, Val124,Pro126 | His30 | Tyr127,Pro126 | His30 | Phe111,Val124, Pro126 | His30 | Phe111,Pro126, Tyr127 | His30 | Pro126,Tyr127, Val124 | His30 | Val124 |
| Leu31 | Phe111,Ile13 | ✓ | ✓ | Leu31 | Phe111 | Leu31 | Lys112,Phe111, Ile13 | Leu31 | Lys112,Phe111, Ile13 | Leu31 | Lys112,Phe111 | ✓ | ✓ |
| Gln32 | Le15,Val16, Gln108,Ala109, Ile110,Phe111 | Gln32 | Le15,Val16, Ala109,Phe111 | Gln32 | Le15,Val16, Ala109,Ile110 Phe111 | Gln32 | Le15,Val16, Gln108,Ala109, Ile110Phe111 | Gln32 | Le15,Val16, Ala109,Ile110, Phe111 | Gln32 | Val16,Gln108, Ala109,Ile110, Phe111 | Gln32 | Le15,Val16, Ala109,Ile110, Phe111 |
| Gly33 | Ile110,Lys112 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Gln34 | Gln108 | Gln34 | Val16,Gln108 | X | X | X | X | Gln34 | Gln108 | X | X | X | X |
| Asp35 | Ile13 | X | X | X | X | X | X | Asp35 | Ile13,Ile14 | X | X | X | X |
| X | X | Met36 | Lys112 | ✓ | ✓ | Met36 | Lys114,Lys112 | Met36 | Lys112 | Met36 | Lys114,Lys112 | ✓ | ✓ |
| X | X | X | X | X | X | Glu37 | Lys114,Lys112 | X | X | Glu37 | Lys114,Lys112 | ✓ | ✓ |
| Gln38 | Lys12 | ✓ | ✓ | ✓ | ✓ | X | X | ✓ | ✓ | X | X | X | X |
| X | X | X | X | Gln39 | Ile13 | X | X | X | X | X | X | X | X |
| X | X | Gln126 | Lys132 | X | X | X | X | Gln126 | Asn136 | X | X | X | X |
| Ala127 | Glu129 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Glu128 | Tyr127,Glu129 | ✓ | ✓ | ✓ | ✓ | Glu128 | Tyr127,Glu129, Pro126 | Glu128 | Tyr127,Pro126 | Glu128 | Tyr127,Pro126 | Glu128 | Tyr127,Glu129, Pro126 |
| X | X | X | X | X | X | X | X | X | X | X | X | Asn129 | Tyr127 |
| X | X | Met130 | Leu29 | X | X | X | X | Met130 | Phe130 | Met130 | Leu29 | X | X |
| X | X | X | X | X | X | Met148 | Ser238 | ✓ | ✓ | X | X | X | X |
| Gln149 | Leu201 | ✓ | ✓ | Gln149 | Lys114,Leu115, Leu201 | Gln149 | Asn204 | Gln149 | Pro116 | Gln149 | Pro116,Leu201 | Gln149 | Leu201 |

Table 6.8: Interface residues and its contacts of IL-1$\beta$ Site II when it is in complex with IL-1RI during trajectory

| **IL1$\beta$-IL1RI Site II** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **X-ray structure (1ITB)** | | **starting structure** | | **1** | | **2** | | **3** | | **4** | | **5** | |
| **IL-1$\beta$** | **IL-1RI** | **IL-1$\beta$** | **IL-1RI** | **IL-1$\beta$** | **IL-1RI** | **IL-1$\beta$** | **IL-1RI** | **IL-1$\beta$** | **IL-1RI** | **IL-1$\beta$** | **IL-1RI** | **IL-1$\beta$** | **IL-1RI** |
| Ala1 | Tyr261,Asp260 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Pro2 | Tyr261 | Pro2 | Tyr261,Glu252 | Pro2 | Asp260 | Pro2 | Tyr261,Glu252 | Pro2 | Tyr261 | Pro2 | Tyr261,Glu259 | Pro2 | Tyr261 |
| Val3 | Tyr261 | ✓ | ✓ | ✓ | ✓ | X | X | X | X | X | X | X | X |
| Arg4 | Ser263,Leu275, Asp239,Leu237 | Arg4 | Tyr261,Ser263, Ile240,Leu237 | Arg4 | Tyr261,Ile240, Leu275,Asp239 | Arg4 | Tyr261,Ile240, Leu275,Asp239 | Arg4 | Ile240,Asp239, Leu237 | Arg4 | Asp239,Leu237, Leu275 | Arg4 | Ile240,Asp239, Leu237 |
| Leu6 | Ser238,Leu237 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | Leu6 | Ser238 | Leu6 | Leu237 |
| Phe46 | Ile240 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | Phe46 | Ile240,Ser238 | Phe46 | Ser238 | Phe46 | Ile240,Ser238 |
| Gln48 | Tyr261,Val249 | Gln48 | Tyr242,Val249 | Gln48 | Tyr242,Val249 | ✓ | ✓ | Gln48 | Tyr242,Val249 | Gln48 | Val249,Ile250, Asp251,Glu252 | Gln48 | Val249,Asp251, Glu252 |
| X | X | Gly49 | Val249 | Gly49 | Val249 | Gly49 | Val249,Tyr242 | Gly49 | Val249 | Gly49 | Val249 | X | X |
| Glu51 | Lys298 | Glu51 | Lys298,Lys244, Tyr242 | Glu51 | Lys298,Lys244, Tyr242 | Glu51 | Val249,Lys244, Tyr242 | Glu51 | Lys244,Tyr242 | Glu51 | Val249,Lys244, Tyr242 | ✓ | ✓ |
| X | X | X | X | X | X | Ser52 | Lys298 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Asn53 | Ile303 | X | X | X | X | Asn53 | Lys298 | Asn53 | Lys298 | X | X | X | X |
| X | X | X | X | X | X | Lys55 | Lys298 | ✓ | ✓ | X | X | ✓ | ✓ |
| Ile56 | Lys298 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Lys92 | Glu252 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Lys93 | Glu259,Ile250, Glu252,Tyr261 | Lys93 | Glu259,Ile250, Glu252,Tyr261, Val249 | Lys93 | Glu259,Ile250, Glu252 | Lys93 | Glu259,Ile250, Glu252 | Lys93 | Glu259,Ile250, Glu252,Tyr261 | Lys93 | Glu259,Ile250, Glu252,Tyr261 | ✓ | ✓ |
| Lys94 | Asp251,Glu252 | Lys94 | Asp251,Glu252, Ile250,Val249 | Lys94 | Asp251,Glu252 | Lys94 | Asp251,Glu252, Asp253 | Lys94 | Asp251,Glu252, Asp253 | Lys94 | Asp251,Glu252, Asp253 | Lys94 | Asp251,Glu252, |
| X | X | X | X | Lys103 | Thr300 | Lys103 | Ser238 | Lys103 | Thr300 | X | X | X | X |
| Glu105 | Thr300 | ✓ | ✓ | ✓ | ✓ | Glu105 | Thr300,Pro206 | Glu105 | Thr300 | Glu105 | Thr300 | ✓ | ✓ |
| X | X | X | X | X | X | Asn107 | Pro206 | X | X | X | X | X | X |
| Asn108 | Asn204 | X | X | X | X | X | X | X | X | X | X | X | X |
| Phe150 | Ser238 | ✓ | ✓ | Phe150 | Leu237 | X | X | Phe150 | Ser238 | Phe150 | Ser238 | Phe150 | Asn204 |
| Ser152 | Glu265 | X | X | X | X | X | X | X | X | X | X | X | X |
| X | X | X | X | X | X | Ser153 | Arg271 | ✓ | ✓ | X | X | ✓ | ✓ |

**Interface residues of IL-1Ra-IL-1RI complex along trajectory**

Table 6.9: Interface residues and its contacts of IL-1Ra Site I when it is in complex with IL-1RI during trajectory

| **IL-1Ra-IL-1RI Site I** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **X-ray structure (1IRA)** | | **starting structure** | | **1** | | **2** | | **3** | | **4** | | **5** | |
| **IL-1Ra** | **IL-1RI** | **IL-1Ra** | **IL-1RI** | **IL-1Ra** | **IL-1RI** | **IL-1Ra** | **IL-1RI** | **IL-1Ra** | **IL-1RI** | **IL-1Ra** | **IL-1RI** | **IL-1Ra** | **IL-1RI** |
| Arg14 | Lys111,Leu198 | ✓ | Lys111 | ✓ | ✓ | X | X | X | X | ✓ | Leu198,Glu199 | ✓ | Leu198 |
| Trp16 | Leu198 | ✓ | Leu198,Lys111 | ✓ | Leu198,Lys111, Pro113 | ✓ | ✓ | ✓ | Leu198,Lys111 | ✓ | Lys111 | ✓ | Leu198,Lys111, Pro113 |
| Val18 | Arg160 | X | X | X | X | X | X | X | X | X | X | X | X |
| Asn19 | Val121 | ✓ | Val121,Arg160 | ✓ | Arg160 | ✓ | Val121,Arg160 | X | X | ✓ | Val121,Arg160 | ✓ | ✓ |
| Gln20 | Gly119,Lys111, Leu112 | ✓ | Gln110,Lys111, Leu112 | ✓ | Gln110,Lys111, Leu112,Gly119 | ✓ | ✓ | ✓ | Gln110,Lys111, Leu112,Val121 | ✓ | Gln110,Lys111, Leu112,Val121, Gly119 | ✓ | Gln110,Lys111, Leu112,Leu120, Gly119 |
| X | X | Thr22 | Lys111 | X | X | X | X | X | X | X | X | X | X |
| Tyr24 | Ile10 | X | X | X | X | X | X | X | X | Tyr24 | Ile19 | X | X |
| Leu25 | Lys9 | X | X | X | X | X | X | X | X | X | X | X | X |
| Arg26 | Asn27,Glu8 | ✓ | Asn27,Glu8, Arg6,Pro25 | ✓ | Asn27,Leu26, Glu8,Pro25 | ✓ | Asn27,Leu26, Glu8,Pro25, Arg6 | ✓ | Arg6,Glu8 | ✓ | Asn27,Ile10, Glu8,Pro25 | ✓ | Asn27,Glu8, Pro25 |
| Asn27 | Arg6 | ✓ | Arg6,Glu30, Asn27,Lys4 | ✓ | ✓ | ✓ | Arg6,Glu30, Asn27 | X | X | ✓ | ✓ | ✓ | Arg6 |
| X | X | Gln29 | Asn27 | ✓ | ✓ | ✓ | Asn27,Asn29 | ✓ | Asn27 | ✓ | ✓ | ✓ | Asn27 |
| X | X | X | X | X | X | Val31 | Asn27 | X | X | ✓ | ✓ | ✓ | ✓ |
| Tyr34 | Tyr124,Pro123, Phe108,Val121 | X | X | ✓ | Tyr124,Pro123, Phe108,Gln110 | ✓ | Tyr124,Pro123, Phe108 | ✓ | Val121,Phe108 | ✓ | Val121,Pro123 | ✓ | Val121,Pro123, Phe108 |
| Leu35 | Ile10 | Leu35 | Phe108,Lys109 | Leu35 | Ile10 | Leu35 | Phe108,Lys109 | ✓ | Phe108,Lys109, Lys111 | ✓ | Phe108,Lys109 | ✓ | ✓ |
| Gln36 | Leu12,Val13, Phe108,Ile107, Ala106 | ✓ | ✓ | ✓ | ✓ | ✓ | Leu12,Val13, Phe108,Ala106 | ✓ | Leu12,Val13, Phe108,Ile107, Ala106 | ✓ | Val13,Phe108, Ala106,Ile107 | ✓ | ✓ |
| Gly37 | Ile107 | ✓ | Ile107,Lys109 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | Ile107 | ✓ | Ile107 |
| X | X | Pro38 | Gln105 | ✓ | ✓ | ✓ | ✓ | Pro38 | Ile11 | ✓ | ✓ | ✓ | Ile11,Val13, Gln105 |
| Asn39 | Ile10,Ile11 | ✓ | Ile11 | ✓ | ✓ | ✓ | Ile10,Ile11 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| X | X | X | X | X | X | X | X | Val40 | Lys109 | X | X | ✓ | Lys109 |
| Leu42 | Lys9,Ile10 | ✓ | Lys9 | Leu42 | Ile10 | ✓ | ✓ | ✓ | Lys9 | ✓ | ✓ | ✓ | Ile10,Lys9 |
| X | X | X | X | X | X | X | X | Glu43 | Lys111 | ✓ | ✓ | ✓ | ✓ |
| X | X | Arg77 | Asn29 | X | X | ✓ | ✓ | ✓ | ✓ | X | X | ✓ | ✓ |
| X | X | X | X | X | X | X | X | Met125 | Arg160 | X | X | X | X |
| Glu126 | Glu126 | ✓ | ✓ | ✓ | ✓ | X | X | X | X | X | X | X | X |
| Ala127 | Tyr124,Glu126 | ✓ | ✓ | ✓ | Tyr124 | ✓ | ✓ | X | X | X | X | ✓ | ✓ |
| X | X | Asp128 | Tyr124 | ✓ | ✓ | ✓ | ✓ | Asp128 | Pro25 | X | X | X | X |
| X | X | X | X | X | X | X | X | Gln129 | Pro28 | X | X | ✓ | ✓ |
| Pro130 | Pro28 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | Asn27 | ✓ | Pro28,Asn27 | ✓ | Pro28 |
| Tyr147 | Leu112,Pro113 | ✓ | Pro113 | ✓ | Leu112,Pro113 | ✓ | Leu112,Pro113, Asp117 | ✓ | Leu112,Gly118 | ✓ | Leu112,Pro113 | ✓ | ✓ |

Table 6.10: Interface residues and its contacts of IL-1Ra Site II when it is in complex with IL-1RI during trajectory

| **IL-1Ra-IL-1RI Site II** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **X-ray structure (1IRA)** | | **starting structure** | | **1** | | **2** | | **3** | | **4** | | **5** | |
| **IL-1Ra** | **IL-1RI** | **IL-1Ra** | **IL-1RI** | **IL-1Ra** | **IL-1RI** | **IL-1Ra** | **IL-1RI** | **IL-1Ra** | **IL-1RI** | **IL-1Ra** | **IL-1RI** | **IL-1Ra** | **IL-1RI** |
| Ser8 | Ile237 | ✓ | Ser235,Ile237 | ✓ | ✓ | X | X | X | X | X | X | X | X |
| Lys9 | Ser235 | Lys9 | Ser235,Leu234 | ✓ | ✓ | ✓ | Leu234 | X | X | X | X | X | X |
| X | X | Met10 | Ser235 | X | X | X | X | X | X | X | X | X | X |
| X | X | Gln11 | Ser235,His298 | X | X | X | X | X | X | X | X | X | X |
| Pro50 | Ile237,Thr297, Asn296 | ✓ | Ser235,Thr297 | ✓ | ✓ | ✓ | Thr297 | ✓ | ✓ | X | X | X | X |
| Ile51 | Lys295 | ✓ | ✓ | ✓ | Lys295,Asn296, Thr297 | X | X | ✓ | Thr297 | X | X | X | X |
| X | X | X | X | X | X | Glu52 | Lys295 | ✓ | Thr297 | X | X | X | X |
| Pro53 | Ile300,Asn296, Thr297,His298, Gly299 | ✓ | Ile300,Asn296, Thr297,His298 | ✓ | Ile300,Asn296, Thr297,Gly299 | ✓ | Asn296,Thr297 | ✓ | Asn296,Thr297, Ile300 | X | X | X | X |
| His54 | Thr297 | ✓ | ✓ | ✓ | ✓ | X | X | X | X | X | X | ✓ | Leu234,Thr297, Ser235 |
| X | X | Ala55 | Thr297 | ✓ | ✓ | X | X | X | X | X | X | X | X |
| Leu56 | Thr297 | X | X | ✓ | ✓ | X | X | X | X | X | X | X | X |
| **X** | **X** | **X** | **X** | **Lys93** | **Glu249** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** |
| Arg102 | Thr297 | Arg102 | His298 | ✓ | Thr297,His298 | X | X | X | X | X | X | X | X |
| **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **Asp104** | **Thr297,His298** |
| **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **Ser105** | **Thr297,His298** |
| **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **Gly106** | **His298** |
| **X** | **X** | **X** | **X** | **Pro107** | **Pro113** | **Pro107** | **Gly116** | ✓ | **Gly116,Asp117** | **X** | **X** | ✓ | **Gly116,Ala115** |
| **X** | **X** | **Gln149** | **Asn201,Gln233** | ✓ | **Asn201** | **Gln149** | **Lys202** | **Gln149** | **Leu198,Pro113** | ✓ | ✓ | ✓ | **Leu198,Glu200** |
| **X** | **X** | **Glu150** | **Arg268,Asn201, Gln233** | ✓ | **Asn201,Arg268, Thr204** | ✓ | **Lys202,Arg268** | ✓ | **Lys202** | ✓ | **Lys202,Glu200** | ✓ | **Lys202** |
| **X** | **X** | **Asp151** | **Lys267,Arg268** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** | **X** |
| **X** | **X** | **Glu152** | **Lys267,Asn201** | **Glu152** | **Lys202** | **Glu152** | **Glu200,Lys202, Arg268** | ✓ | **Lys202** | ✓ | **Asn201,Arg268** | **X** | **X** |

**Interface residues of mutantIL-1Ra-IL-1RI complex along trajectory**

Table 6.11: Interface residues and its contacts of mutant IL-1Ra (differs in 1IRA with C116F and abesence of first 6 residues at the begining which are not determined in X-Ray structure) Site II when it is in complex with IL-1RI during trajectory

| **C116F_mut_IL1Ra-IL1RI Site II** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **starting structure (1IRA)** | | **starting structure (C116F_1IRA) mdrunmut** | | **1** | | **2** | | **3** | | **4** | | **5** | |
| **IL-1Ra** | **IL-1RI** | **mutIL-1Ra** | **IL1RI** | **mutIL-1Ra** | **IL-1RI** | **mutIL-1Ra** | **IL-1RI** | **mutIL-1Ra** | **IL-1RI** | **IL-1Ra** | **IL-1RI** | **IL-1Ra** | **IL-1RI** |
| X | X | Ser7 | Ile237 | Ser7 | Glu249,Glu256 | ✓ | ✓ | X | Glu249,Glu256, Tyr258 | ✓ | Ile247,Val246, Glu249,Glu256 | ✓ | Ile247,Tyr239, Glu256,Tyr258 |
| Ser8 | Ser235,Ile237 | X | X | X | X | ✓ | Ile237,Asp236 | Ser8 | Tyr258 | X | X | ✓ | ✓ |
| Lys9 | Ser235,Leu234 | Lys9 | Asp236,Ile237 | ✓ | Ile237,Leu272, Leu234,Ser235, Asp236 | ✓ | Leu234 | X | X | Lys9 | Ile237,Tyr258 | ✓ | ✓ |
| Met10 | Ser235 | ✓ | ✓ | X | X | ✓ | Leu234 | X | X | X | X | X | X |
| Gln11 | Ser235,His298 | ✓ | Ser235 | ✓ | Ser235,Asp236, Ile237,Asn296 | ✓ | Leu234,Ser235, Thr297 | ✓ | Ser235,Asp236, Ile237,Asn296 | ✓ | Ser235,Ile237 | ✓ | Ser235 |
| Pro50 | Ser235,Thr297 | ✓ | Thr297,Asn296 | ✓ | ✓ | ✓ | ✓ | ✓ | Thr297,Asn296, Lys295 | ✓ | Lys295 | ✓ | Asn296,Lys295 |
| Ile51 | Lys295 | ✓ | ✓ | ✓ | Ile300 | X | X | X | X | X | X | X | X |
| X | X | X | X | Glu52 | Ile300 | X | X | X | X | X | X | X | X |
| Pro53 | Ile300,Asn296, Thr297,His298 | ✓ | Ile300,Gly299, Thr297,Asn296 | ✓ | Thr297,Ile300 | ✓ | Ile300,Gly299, His298 | ✓ | Thr297,His298, Gly299 | ✓ | Ile300,Gly299 | ✓ | Gly299,His298, Ile300,Thr297 |
| His54 | Thr297 | ✓ | ✓ | ✓ | Thr297,His298 | ✓ | His298 | ✓ | His298,Thr297 | ✓ | ✓ | ✓ | ✓ |
| X | X | Glu90 | Lys295 | ✓ | ✓ | ✓ | ✓ | ✓ | Tyr239,Lys295, Lys241 | ✓ | Lys241 | ✓ | Tyr239,Lys295,Lys241 |
| X | X | X | X | X | X | X | X | Asn91 | Val246,Lys241, Gly244,Ser245 | X | X | X | X |
| Arg102 | Thr297 | X | X | X | X | X | X | Arg102 | Thr297 | ✓ | Thr297,His298 | ✓ | Thr297,His298, Lys202 |
| X | X | X | X | X | X | X | X | X | X | Asp104 | His298 | Asp104 | Lys202 |
| Tyr147 | X | Tyr147 | Leu112 | ✓ | ✓ | ✓ | Leu112,Pro113 | ✓ | Leu112 | ✓ | Leu112,Pro113 | ✓ | ✓ |
| Gln149 | Asn201,Gln233 | Gln149 | Glu199,Asn201, Pro113,Leu198 | ✓ | Glu199,Leu198 | ✓ | Pro113 | Gln149 | Glu199 | ✓ | Asn201,Glu199, Glu200 | ✓ | ✓ |
| Glu150 | Arg268,Asn201, Gln233 | ✓ | Lys267 | ✓ | Arg268 | ✓ | Arg268,Ser235, Gln233 | ✓ | ✓ | ✓ | Arg268,Ser235, Asn201 | ✓ | Arg268,Ser235 |
| X | X | X | X | X | X | Glu151 | Arg268 | ✓ | ✓ | ✓ | Arg268 | ✓ | ✓ |

Table 6.12: Interface residues and its contacts of mutant IL-1Ra (same with 1IRA except for only aminoacid at 116) Site II when it is in complex with IL-1RI during trajectory

| **C116F_mut_IL1Ra-IL1RI Site II** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **starting structure (1IRA)** | | **starting structure (C116F_1IRA) renewmut** | | **1** | | **2** | | **3** | | **4** | | **5** | |
| **IL-1Ra** | **IL-1RI** | **mutIL-1Ra** | **IL-1RI** | **mutIL-1Ra** | **IL-1RI** | **mutIL-1Ra** | **IL-1RI** | **mutIL-1Ra** | **IL-1RI** | **IL-1Ra** | **IL-1RI** | **IL-1Ra** | **IL-1RI** |
| | | Lys6 | Ile237 | ✓ | ✓ | X | X | X | X | X | X | X | X |
| X | X | Ser7 | Ile237 | ✓ | ✓ | ✓ | ✓ | X | X | X | X | ✓ | Lys295 |
| Ser8 | Ser235,Ile237 | Ser8 | Asp236,Ser235 | ✓ | ✓ | ✓ | Ile237,Asp236, Ser235 | Ser8 | Ser235 | X | X | ✓ | Ile237,Ser235 |
| Lys9 | Ser235,Leu234 | Lys9 | Ser235,Leu234 | ✓ | ✓ | ✓ | ✓ | X | X | Lys9 | Leu234,Aer235, Asp236 | ✓ | Leu234 |
| Met10 | Ser235 | ✓ | ✓ | ✓ | ✓ | ✓ | Leu234 | X | X | X | X | X | X |
| Gln11 | Ser235,His298 | ✓ | Ser235 | ✓ | ✓ | ✓ | Leu234,Ser235, Thr297 | X | X | ✓ | Thr297 | ✓ | ✓ |
| Pro50 | Ser235,Thr297 | ✓ | Thr297 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Ile51 | Lys295 | ✓ | ✓ | ✓ | ✓ | X | X | X | X | X | X | X | X |
| X | X | Glu52 | Lys295 | ✓ | ✓ | X | X | X | X | X | X | X | X |
| Pro53 | Ile300,Asn296, Thr297,His298 | ✓ | ✓ | ✓ | Thr297,Ile300, Gly299 | ✓ | Ile300,Gly299, His298 | X | X | X | X | ✓ | Gly299,His298 |
| His54 | Thr297 | ✓ | Thr297,His298 | ✓ | ✓ | ✓ | His298 | ✓ | His298,Pro203 | ✓ | His298 | ✓ | His298,Pro203 |
| X | X | X | X | X | X | X | X | X | X | Leu56 | Thr297 | X | X |
| X | X | Glu90 | Lys295 | ✓ | ✓ | X | X | X | X | X | X | X | X |
| X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Arg102 | Thr297 | X | X | X | X | ✓ | His298 | Arg102 | Pro203,His298, Asn201 | ✓ | Pro203,His298 | ✓ | Pro203,His298, Asn201 |
| X | X | X | X | X | X | X | X | X | X | Asp104 | Pro203 | ✓ | ✓ |
| X | X | X | X | X | X | Pro107 | Asn201 | Pro107 | Asp117 | Pro107 | Asn201 | ✓ | ✓ |
| X | X | X | X | X | X | X | X | Thr108 | Asn201 | ✓ | ✓ | ✓ | ✓ |
| X | X | X | X | X | X | X | X | Thr109 | Asn201 | ✓ | ✓ | ✓ | ✓ |
| X | X | X | X | Tyr147 | Leu112 | ✓ | Leu112,Pro113 | ✓ | Leu112,Pro113, Asn201 | ✓ | Leu112,Pro113 | ✓ | Leu112,Pro113, Asn201 |
| X | X | X | X | X | X | X | X | Phe148 | Asn201 | ✓ | ✓ | ✓ | ✓ |
| Gln149 | Asn201,Gln233 | Gln149 | Pro113,Leu198 | ✓ | ✓ | ✓ | ✓ | Gln149 | Glu199 | ✓ | Leu198,Glu199 | ✓ | ✓ |
| Glu150 | Arg268,Asn201, Gln233 | ✓ | Arg268,Lys267 | ✓ | ✓ | ✓ | Arg268,Gln233 | ✓ | ✓ | ✓ | Arg268,Thr204 | ✓ | Arg268,Ser235 |
| X | X | X | X | X | X | X | X | X | X | X | X | Asp151 | Arg268 |
| X | X | X | X | X | X | Glu152 | Arg268,Lys267 | ✓ | ✓ | ✓ | Arg268 | ✓ | ✓ |

### IL-1 Superfamily Unbound Form

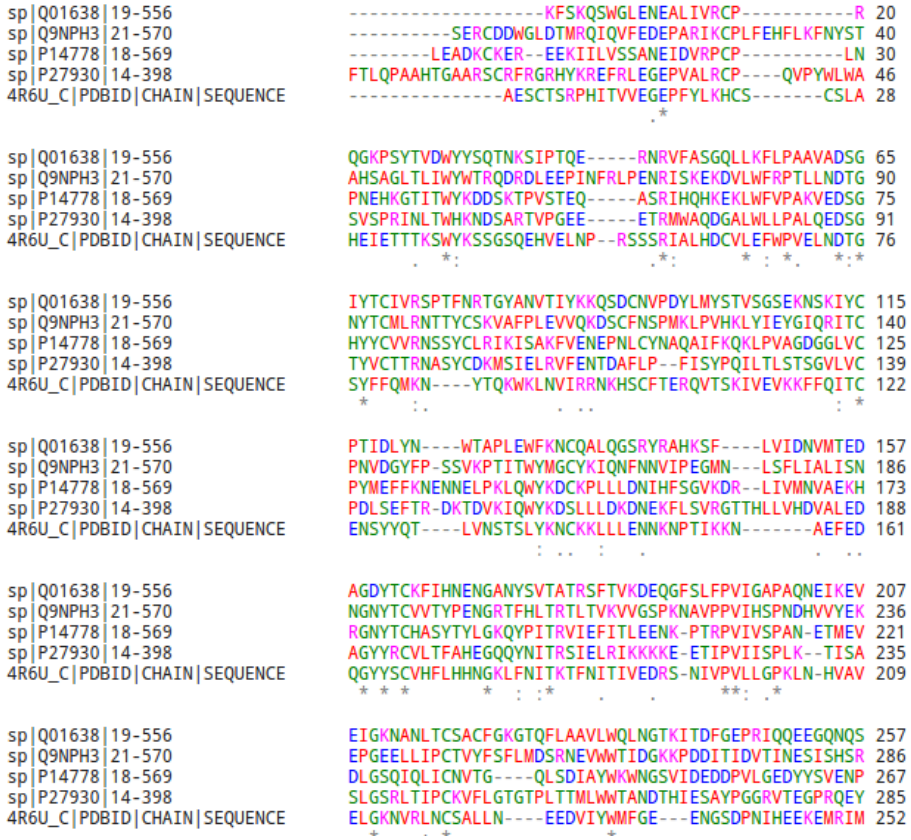


Figure 6.20: CLUSTALW2 Multiple Sequence Alignment 1 $Q01638 : ST2, Q9NPH3 : IL1RAcP, P14778 : IL1RI, P27930 : IL1RII, 4R6UchainC - IL18R$

4kc3B:ST2, 4depF:IL-1RAcP, 1itbB:IL-1RI, 3o4oC:IL-1RII,4r6uC-IL-18R1

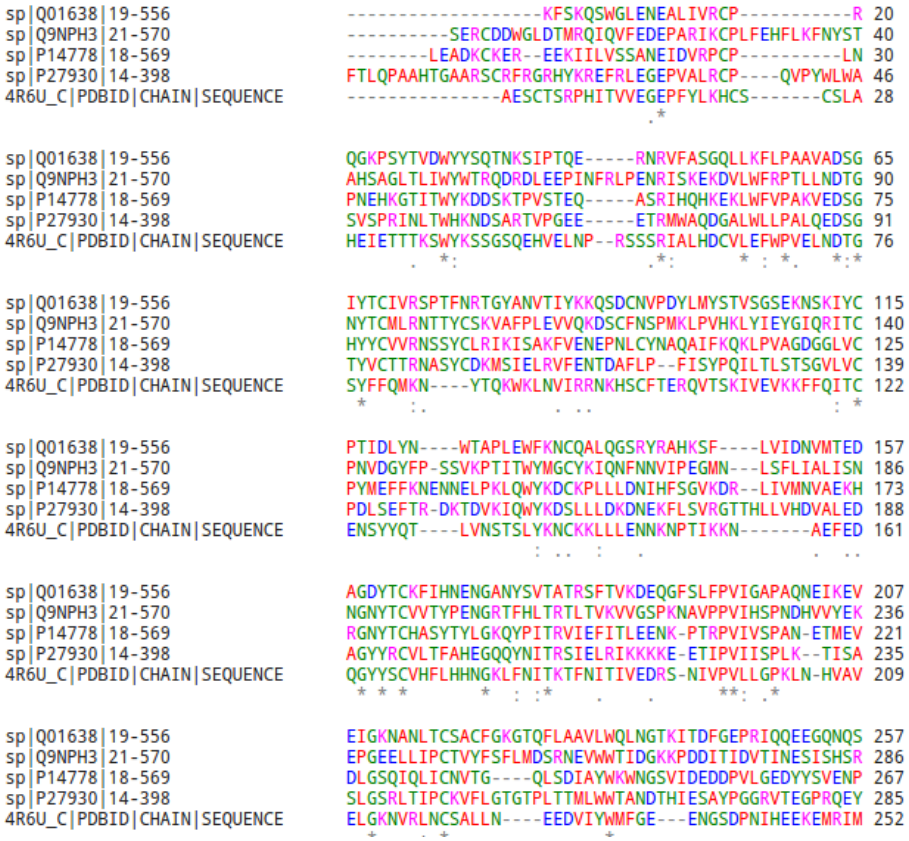


Figure 6.21: CLUSTALW2 Multiple Sequence Alignment 2 $Q01638 : ST2, Q9NPH3 : IL1RAcP, P14778 : IL1RI, P27930 : IL1RII, 4R6U_C - IL18R$

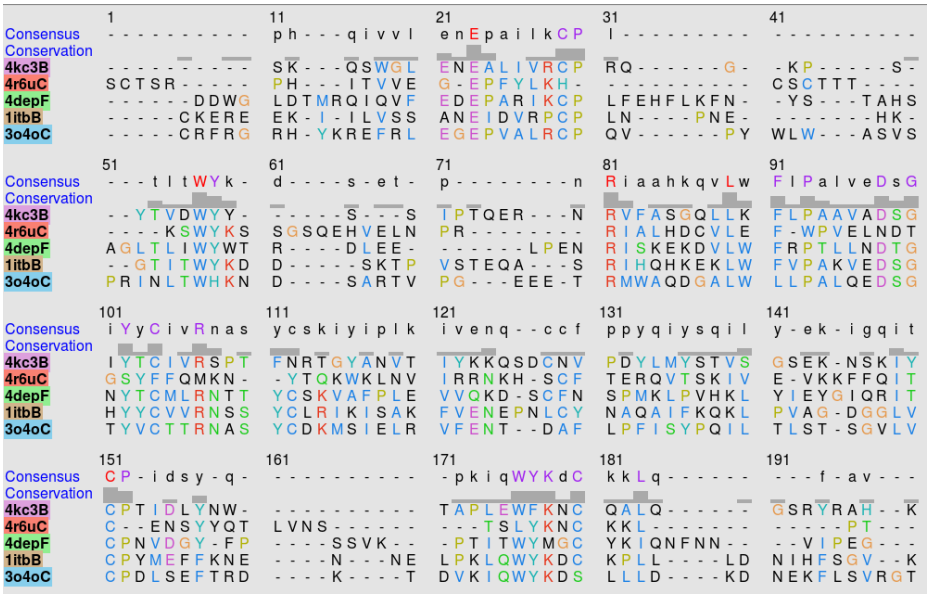


Figure 6.22: Multiple Structure Alignment 1

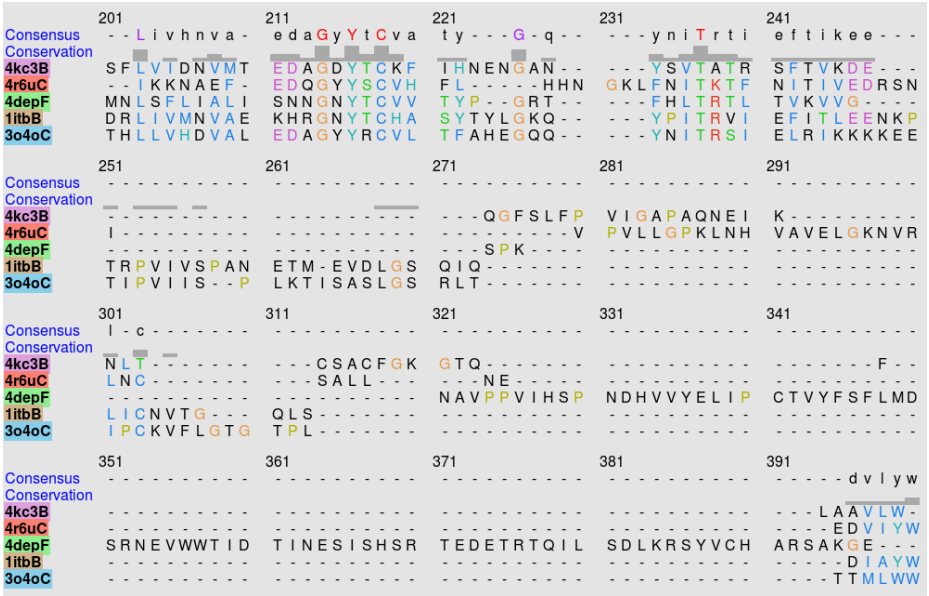


Figure 6.23: Multiple Structure Alignment 2

# CURRICULUM VITAE

Ayşegül TURUPCU was born in Tokat on November 8, 1989. She graduated from Beşiktaş Atatürk Anatolian High School in 2007. She received her B.S. degree in Chemical Engineering from Middle East Technical University (METU), Ankara, Turkey, in 2012. She received her M.S. degree under the supervision of Prof. Burak Erman and Asst. Prof. Mehmet Sayar in Chemical and Biological Engineering from Koç University, Istanbul, Turkey in 2015. At the same time, she was a research and teaching assistant in Koç University. Her current research interests include computational biophysics and drug design.