



(51) International Patent Classification:

A61P 37/00 (2006.01) C07K 16/28 (2006.01)
A61K 39/395 (2006.01)

(21) International Application Number:

PCT/EP2012/060248

(22) International Filing Date:

31 May 2012 (31.05.2012)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

11168327.2 31 May 2011 (31.05.2011) EP
61/492,990 3 June 2011 (03.06.2011) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available):

AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available):

ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))



WO 2012/164021 A1

(54) Title: IL-21 EPITOPE AND IL-21 LIGANDS

(57) Abstract: The present invention relates to IL-21 ligands, such as e.g. antibodies, as well as use thereof.

IL-21 EPITOPE AND IL-21 LIGANDS

The present invention is concerned with a discontinuous epitope present on IL-21, and ligands which bind to this epitope.

5 IL-21 is a type I cytokine, which exerts pleiotropic effects on both innate and adaptive immune responses. It is mainly produced by activated CD4⁺ T cells, follicular T cells and Natural killer cells (NKT). In addition, recent evidence suggests that Th17 cells can produce high amount of IL-21.

10 IL-21 increases the cytotoxicity of CD8⁺ T cells and can promote proliferation of CD8⁺ cells in the presence of antigens. IL-21 is induced by IL-6, a cytokine known to promote development of Th17 cells. IL-21 acts on T helper cells in an autocrine manner promoting its own production and supporting differentiation of T-helper cells into Th17 cells. In agreement with this, IL-21 deficient mice show an impaired Th17 response. IL-21 also acts on B-cells and increases antibody production; however, IL-21 is not essential for production of functional antibodies, whereas IL-21R α negative mice exhibit both reduced proliferation as well as impaired cytotoxicity of CD8⁺ cells. A recent set of studies suggests that IL-21 produced by CD4⁺ cells is critical for the ability of CD8⁺ T cells to control viral infection.

20 Mature IL-21 is a 133 amino acid polypeptide (residues 30-162 of SEQ ID No. 1, Figure 2) featured by four helical segments, arranged in an up-up-down-down topology. IL-21 signals through a heterodimeric receptor complex consisting of the private IL-21 receptor alpha chain (IL-21R α and the common gamma chain (γ C) (residues 23-369 of SEQ ID No. 8). IL-21 comprises two binding sites, binding site 1 (BS1) and 2 (BS2), via which it interacts with IL-21R α and γ C, respectively. IL-21 binds via BS1 to IL-21R α with high affinity, but receptor activation and signaling requires constructive interaction between IL-21 and γ C via BS2 as well, hereby forming a ternary complex. IL-21 variants which bind IL-21R α with high affinity, but lack the ability to interact constructively with γ C will occupy the IL21 receptor without inducing signaling, and, thus, function as IL-21 receptor antagonists.

30 The ability of IL-21 to augment immunity has spurred substantial interest in the therapeutic use of IL-21. It is currently evaluated in clinical trials against metastatic melanoma types and renal cancer. Animal studies have demonstrated a synergistic effect between IL-21 and tumor specific antibodies, which could suggest a future therapeutic use of IL-21 as a potentiator of anti-tumor antibodies. Furthermore, IL-21

plays a complex role in autoimmune diseases. The ability of IL-21 to downregulate IgE production suggests that it could be used therapeutically against asthma and allergy. Results from animal studies support this view. On the other hand, the ability of IL-21 to promote Th17 development makes it a pro-inflammatory cytokine and a number of
5 different IL-21 and IL-21R α antagonists/inhibitors are currently investigated for potential use in treatment of a range of different autoimmune diseases.

Monoclonal antibodies specific for IL-21 are known in the art, for example from WO2007111714 and WO2010055366 (Zymo-Genetics, Inc.). In particular, WO2010055366 describes an IL-21 antibody, designated by clone number
10 366.328.10.63 (herein referred to as "mAb14") which has high affinity for its cognate antigen, and other desirable properties, showing specificity for human and cynomolgus monkey IL-21. This antibody was shown not to compete with neither IL-21R α nor γ C binding of IL-21 using either a homodimeric IL-21R α -Fc construct or a heterodimeric IL-21R α / γ C-Fc construct.

15 SUMMARY OF THE INVENTION

We herein define a novel epitope on IL-21. Binding of a IL-21 ligand, e.g. an antibody, to this epitope competes or interferes with binding of γ C to IL-21 via BS2, but does not interfere with binding of IL-21R α to IL-21 via BS1.

We also describe IL-21 ligands, such as antibodies, which bind specifically to the epitope
20 according to the invention, provided that the ligand is not mAb14, and not γ C, as well as methods for making and using such ligands. We also describe how binding of mAb14 to IL-21 interferes with the binding of γ C to IL-21.

Distinctive features of IL-21 ligands according to the invention are their ability to compete or interfere with binding of γ C to IL-21, while IL-21 complexed with the ligand will maintain
25 an IL-21R α binding competent BS1. Accordingly, ligands of the present invention will in the presence of IL-21 form ligand:IL-21 complexes having the ability to bind specifically, and with high affinity, to IL-21R α present on cell surfaces.

IL-21 variants which retain the ability to bind to IL-21R α with high affinity via BS1, but have a BS2 lacking the ability to interact with γ C will occupy the IL-21R α receptor and
30 function as IL-21R α receptor antagonists. One way of compromising BS2 binding is the introduction of one or more point mutations of IL-21 residues critically involved in the interaction with γ C. Another way is to block BS2 by binding a BS2 ligand to IL-21. Thus,

IL-21 ligands effectively blocking BS2, but leaving BS1 unaffected, essentially as described for ligands of the present invention, are in the presence of IL-21 expected to act as IL-21R α receptor antagonists *in vivo*.

5 Commonly, monoclonal antibodies are used therapeutically to “neutralize” soluble targets, such as pro-inflammatory molecules in autoimmune and chronic inflammatory disease. Binding of a IL21 ligand interfering with BS2 on an IL-21 molecule in solution will result in “neutralization” of that particular IL-21 molecule. However, as the formed ligand:IL-21 complex acquires antagonistic properties, it will additionally be able to block and “neutralize” the function of one IL-21R α molecule on a IL-21R α bearing cell. This
10 dual mode of action, i.e. neutralization of soluble IL-21 and blockade of membrane bound IL-21R α , will potentially improve the potency of such BS2 blocking/interfering IL-21 ligands, as compared with ligands interfering with IL-21 BS1, where the ligand:IL-21 complex formed will not acquire IL-21R α antagonistic properties.

Ligands of the invention may thus have improved potency due to the combined
15 neutralizing and receptor blocking properties.

Generally, a ligand of the invention will bind to IL-21 and form a ligand:IL-21 complex which retains a competent BS1 and thereby the ability to bind with high affinity to IL-21R α . Therefore, the ligand:IL-21 complex is capable of binding to soluble fragments of IL-21R α (e.g. its extra cellular domain) or membrane bound IL-21R α present on cell
20 surfaces. In other words ligands according to the invention may in the presence of IL-21 have the ability to bind specifically to IL-21R α bearing cells.

In case the ligand is an antibody comprising a Fc domain capable of inducing ADCC and/or CDC, such ligand may, by virtue of its high affinity and specific binding to IL-21R α bearing cells, possess the ability to kill such IL-21R α bearing cells.

25 Thus, in another aspect ligands of the invention, e.g. antibodies comprising an Fc domain with built in effector functions, may mediate specific depletion of cells carrying IL-21R α on their surfaces.

Depletion of specific cellular sub-sets, e.g. T cells and macrophages in the gut of patients with Crohn's disease (CD), has been shown to be an important component in the mode
30 of action in current anti-TNF α therapy in CD (MacDonald, Nature Medicine, **16** (2010), p. 1194-1195, and references therein). Thus, depletion of specific inflammatory cells may be advantageous in the treatment of some inflammatory diseases.

The effector functions of antibodies are dependent on the isotype and can be modulated by several methods known in the art, including introduction of mutations in the Fc domain which will alter the binding of the antibody to Fc receptors. Ligands of the present invention include such ligands with modified effector functions.

5 IL-21 ligands binding to the epitope of the invention competes or interferes with γ C binding to IL-21. Using experimental and homology modelling methods we predicted the location of the binding interface between IL-21 and γ C and the specific amino acid residues in IL-21 which are involved in the interaction, and, thus, are targets for IL-21 ligands designed to inhibit the activity of IL-21 through disruption of the interaction
10 between IL-21 and γ C.

The following IL-21 amino acids, or a sub set thereof (with reference to SEQ ID NO 1) are bound by antibodies having CDR sequences similar to those of mAb14 (referred to as antibody 366.328.10.63 in WO2010055366): Glu 65, Asp 66, Val 67, Glu 68, Thr 69, Asn 70, Glu 72, Trp 73, Lys 117, His 118, Arg 119, Leu 143, Lys 146, Met 147, His 149,
15 Gln 150 and His 151 as shown herein by X-ray crystallographic data.

DETAILED DESCRIPTION OF THE INVENTION

Unless otherwise stated, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. The practice of the present invention employs, unless otherwise
20 indicated, conventional methods of chemistry, biochemistry, biophysics, molecular biology, cell biology, genetics, immunology and pharmacology, known to those skilled in the art.

Brief Description of the Drawings

Figure 1: The amino acid sequences referred to herein.

25 **Figure 2:** The mature IL-21 amino acid sequence (residues 30-162 of SEQ ID NO 1) is shown with helix A, B, C and D (corresponding to amino acids 34-50 of SEQ ID NO 1 (SEQ ID NO 2), 72-82 of SEQ ID NO 1 (SEQ ID NO 3), 93-103 of SEQ ID NO 1 (SEQ ID NO 4) and 133-152 of SEQ ID NO 1 (SEQ ID NO 5), respectively) appearing bold and underlined. Residues belonging to BS1, BS2 and the epitopes of mAb14 and mAb5
30 (Epitope14 and Epitope5, respectively) are marked below the amino acid sequence by "X". The Mab5 epitope is indicated as "epitope5" in the figure. The Mab14 epitope is indicated as "epitope14" in the figure.

Figure 3: HX monitored by mass spectrometry identifies regions in hIL-21 involved in mAb binding. For all panels the upper spectrum shows the non-deuterated control, the lower panel shows the deuterated control, i.e. hIL-21 in the absence of mAbs after 30 sec incubation in D₂O. The middle panels show the peptide after 30 sec in-exchange in the presence of mAbs as indicated.

(A) Mass/charge spectra corresponding to the peptide fragment 29-44, MQGQDRHMIRMRLID ($m/z = 676.68$, $z = 3$) situated in helix A. mAb5 result in exchange protection in this region.

(B) Mass/charge spectra corresponding to the peptide fragment 67-76, VETNCEWSAF ($m/z = 1185.49$, $z = 1$) situated in a loop and helix B. mAb14 result in exchange protection in this region.

(C) Mass/charge spectra corresponding to the peptide fragment 93-98, ERIINV ($m/z = 743.47$, $z = 1$) situated in helix C. mAb5 result in exchange protection in this region.

(D) Mass/charge spectra corresponding to the peptide fragment 138-162, ERFKSLQKMIHQHLSSRTHGSEDS ($m/z = 738.63$, $z = 4$) situated in helix D. mAb14 result in exchange protection in this region.

Figure 4: Hydrogen exchange time-plots of representative peptides of hIL-21 in the absence or presence of mAb5 or mAb14. Deuterium incorporation (Da) of hIL-21 peptides is plotted against time on a logarithmic scale in the absence (black diamonds, ◆) or presence of mAb5 (white triangles, △) or mAb14 (white circles, ○).

Figure 5: Sequence coverage of HX analyzed peptides of hIL-21 in the presence and absence of mAb14. The primary sequence is displayed above the HX analyzed peptides (shown as horizontal bars). Peptides showing similar exchange patterns both in the presence and absence of mAb14 are displayed in white whereas peptides showing reduced deuterium incorporation upon mAb14 binding are coloured black. Boxed sequence regions define the epitope.

Figure 6: Modelled hIL-21 residues in the X-ray structures of the different hIL-21/Fab complexes. Fab35 (From Example 1) is added for comparison.

Figure. 7: Summary of the Fab56, Fab57, Fab59 and Fab60 hIL-21 epitopes on hIL-21 identified by running the CONTACT software of the CCP4 program suite (Bailey, 1994). '=' denotes a 4.0 Å distance cut-off between the Fab fragment and the hIL-21 molecule. '-' denotes distances between 4.0 and 5.0 Å between the Fab fragment and the hIL-21

molecule.

Definitions

IL-21 refers, unless otherwise specifically stated, to human IL-21. The amino acid
5 sequence of IL-21, including its signal sequence, is shown in fig. 1 (SEQ ID NO 1). The
mature IL-21 polypeptide corresponds to residues 30-162 of SEQ ID NO 1. IL-21 is
featured by four helical segments, arranged in an up-up-down-down topology typical for
the class I cytokines. IL-21 signals through a heterodimeric receptor complex consisting
of the private chain IL-21R α and γ C the latter being shared by IL-2, IL-4, IL-7, IL-9, and
10 IL-15. IL-21R α binds IL-21 with high affinity via binding site 1 (BS1) on IL-21. The
interaction between IL-21 and γ C is, on the other hand, of a relatively low affinity. IL-21
binds to γ C via its binding site 2 (BS2). IL-21 binding to both IL-21R α and γ C is required
for signaling. Thus, IL-21 variants having high affinity for IL-21R α and no or strongly
reduced affinity for γ C are expected to bind to IL-21R α on the surface of IL-21R
15 expressing cells and thereby block intracellular IL-21 induced signaling.

The structure of human IL-21 has previously been determined by NMR spectroscopy
(Bondensgaard et. al J. Biol. Chem. (2007), 282, 23326-23336). The crystal structure of
IL-21, free or in complex with receptor chains, has not yet been published but the
structurally related IL-2 molecule in complex with its three receptor chains (IL-2:IL2R α :IL-
20 2R β : γ C) determined by X-ray crystallography has been published and its coordinates
have been deposited in a publicly available database (Protein Data Bank).

Ligands interfering with γ C binding to IL-21: Ligands according to the invention that have
the ability to interfere with binding of γ C to IL-21 does in this context mean ligands that
bind to IL-21 and in doing so either directly compete with γ C for binding to IL-21 or
25 reduce its ability to bind to/affinity for IL-21. Such ligands will furthermore not interfere
with binding of IL-21R α to IL-21. This means that ligands according to the invention may
bind to an epitope that either overlaps with or is situated close enough to BS2 to provide
sterical hindrance for γ C-binding and thereby reducing its ability to bind to IL-21 by at
least 25%, preferably at least 50%, preferably at least 60%, preferably at least 70%,
30 preferably at least 75%, preferably at least 80%, preferably at least 90%, and most
preferably at least 95%. It follows that the epitope on IL-21 of the ligand according to the
invention is well separated from BS1 because binding of the ligands according to the
invention does not significantly interfere with IL-21R α binding to IL-21. Interference with

γ C binding can be detected by e.g. Surface Plasmon Resonance (SPR) as shown in the examples.

The term "treatment", as used herein, refers to the medical therapy of any human or other animal subject in need thereof. Said subject is expected to have undergone
5 physical examination by a medical or veterinary medical practitioner, who has given a tentative or definitive diagnosis which would indicate that the use of said specific treatment is beneficial to the health of said human or other animal subject. The timing and purpose of said treatment may vary from one individual to another, according to the status quo of the subject's health. Thus, said treatment may be prophylactic, palliative,
10 symptomatic and/or curative.

In terms of the present invention, prophylactic, palliative, symptomatic and/or curative treatments may represent separate aspects of the invention.

The present invention concerns an epitope which has been discovered on human IL-21. Polypeptides having this epitope, therefore, are polypeptides which share at least part of
15 the three-dimensional structure of human IL-21.

A fragment of a polypeptide is a polypeptide which is truncated at the C or N terminus, or which has had one or more amino acids removed from its sequence. In the context of the present invention, a fragment should retain sufficient three-dimensional structure to define the epitope or paratope of the invention.

20 Screening for binding activity (or any other desired activity) is conducted according to methods well known in the art, for instance SPR (Surface Plasmon Resonance), FACS, ELISA, etc. Screening allows selection of members of a repertoire according to desired characteristics.

As used herein, an "isolated" compound is a compound that has been removed from its
25 natural environment.

IL-21 variants: IL-21 mimics/variants according to the present invention comprises the discontinuous epitope comprising at least one amino acid residue from at least two of the following IL-21 peptide segments: Glu 65 to Phe 73, Lys 117 to Arg 119, and Leu 143 to His 151, as set forth in SEQ ID No 1. Such mimics/variants may be produced in a
30 number of ways, one of which is the mutation of native IL-21 by insertion, substitution or deletion of amino acids. The insertion, substitution or deletion may vary in size and extent, largely as a function of its position in the molecule. For example, large N or C-

terminal insertions may be tolerated without modifying the epitope of the invention, as can C-terminal deletions. Elsewhere, smaller insertions, deletions or substitutions may be better tolerated.

Antibodies: The term "antibody" as referred to herein refers to a poly-peptide derived from a germline immunoglobulin sequence. The term includes full-length antibodies and any antigen binding fragment as e.g. Fab fragments, and other monovalent antibodies. The term "antibody", "monoclonal antibody" and "mAb" as used herein, is intended to refer to immunoglobulin molecules and fragments thereof that have the ability to specifically bind to an antigen. A sub-class of the immunoglobulins of particular pharmaceutical interest are those belonging to the IgG family, which can be sub-divided into the iso-types IgG1, IgG2, IgG3 and IgG4. IgG molecules are composed of two heavy chains interlinked by two or several disulfide bonds and two light chains, one attached to each of the heavy chains by a disulfide bond. The IgG heavy chain is composed of four Ig-domains, including the variable domain (VH) and three constant domains (CH1, CH2, and CH3). Each light chain is comprised of a light chain variable region (VL) and a light chain constant region (CL). The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

Examples of antigen-binding fragments include Fab, Fab', F(ab)2, F(ab')2, F(ab)S, Fv (typically the VL and VH domains of a single arm of an antibody), single-chain Fv (scFv; see e.g., Bird et al., Science 1988; 242:42S-426; and Huston et al. PNAS 1988; 85:5879-5883), dsFv, Fd (typically the VH and CHI domain), and dAb (typically a VH domain) fragments; VH, VL, VhH, and V-NAR domains; monovalent molecules comprising a single VH and a single VL chain; minibodies, diabodies, triabodies, tetrabodies, and kappa bodies (see, e.g., Ill et al. Protein Eng 1997;10:949-57); camel IgG; IgNAR; as well as one or more isolated CDRs or a functional paratope, where the isolated CDRs or antigen-binding residues or polypeptides can be associated or linked together so as to form a functional antibody fragment. Various types of antibody fragments have been described or reviewed in, e.g., Holliger and Hudson, Nat Biotechnol 2005;2S:1126-1136; WO2005040219, and published U.S. Patent Applications 20050238646 and 20020161201.

The Fc domain of an antibody according to the invention may be modified in order to modulate certain effector functions such as e.g. complement binding and/or binding to certain Fc γ receptors. The Fc domain may furthermore be modulated in order to increase affinity to the neonatal Fc receptor (FcRn). Mutations in positions 234, 235 and 237
5 (residue numbering according to the EU index) in an IgG1 Fc domain will generally result in reduced binding to the Fc γ RI receptor and possibly also the Fc γ RIIa and the Fc γ RIII receptors. These mutations do not alter binding to the FcRn receptor, which promotes a long circulatory half life by an endocytic recycling pathway. Preferably, a modified IgG1 Fc domain of an antibody according to the invention comprises one or more of the
10 following mutations that will result in decreased affinity to certain Fc γ receptors (L234A, L235E, and G237A) and in reduced C1q-mediated complement fixation (A330S and P331S), respectively (residue numbering according to the EU index). Alternatively, the Fc domain may be an IgG4 Fc domain optionally comprising the S241P/S228P mutation (S241P denotes residue numbering according to Kabat, S228P denotes residue
15 numbering according to the EU numbering system (Edelman G.M. et AL., Proc. Natl. Acad. USA 63, 78-85 (1969)).

The term "human antibody", as used herein, means antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human
20 germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human
25 framework sequences, e.g. the so-called "humanized antibodies" or human/mouse chimera antibodies.

The term "chimeric antibody" or "chimeric antibodies" refers to antibodies whose light and heavy chain genes have been constructed, typically by genetic engineering, from immunoglobulin variable and constant region genes belonging to different species. For
30 example, the variable segments of genes from a mouse monoclonal antibody may be joined to human constant segments.

Half life extending moiety: The ligand according to the invention may be modified in order to increase its serum half-life, for example, by adding molecules - such as fatty acids or fatty acid derivatives, PEG (poly ethylene glycol) or other water soluble polymers, including

polysaccharide polymers to increase circulatory half-life. "Protractive groups"/"half life extending moiety" is herein understood as one or more chemical groups attached to one or more amino acid site chain functionalities such as -SH, -OH, -COOH, -CONH₂, -NH₂, or one or more N- and/or O-glycan structures and that can increase in vivo circulatory half life of a number of therapeutic proteins/peptides when conjugated to these proteins/peptides. Examples of protractive groups/half life extending moiety include but not limited to are: Biocompatible fatty acids and derivatives thereof, Hydroxy Alkyl Starch (HAS) e.g. Hydroxy Ethyl Starch (HES), Poly Ethylen Glycol (PEG), Poly (Glyx-Sery)_n (HAP), Hyaluronic acid (HA), Heparosan polymers (HEP), Phosphorylcholine-based polymers (PC polymer), Fleximers, Dextran, Poly-sialic acids (PSA), an Fc domain, Transferrin, Albumin, Elastin like peptides, XTEN polymers, Albumin binding peptides, a CTP peptide, and any combination thereof.

Binning/competition binding: Antibodies binding to the same antigen can be characterized with respect to their ability to bind to their common antigen simultaneously. Antibodies may be subjected to "binning", which term in the present context refers to a method of grouping antibodies that bind to the same antigen. "Binning" of antibodies may be based on competition binding of two antibodies to their common antigen in assays based on standard techniques such as surface plasmon resonance (SPR), ELISA or flow cytometry.

A "bin" is defined by a reference antibody. If a second antibody is unable to bind to the antigen at the same time as the reference antibody, the second antibody is said to belong to the same "bin" as the reference antibody. In this case the reference and the second antibody are competing for binding to the antigen, thus the pair of antibodies is termed "competing antibodies". If a second antibody is capable of binding to the antigen at the same time as the reference antibody, the second antibody is said to belong to a separate "bin". In this case the reference and the second antibody are not competing for binding to the antigen, thus the pair of antibodies is termed "non-competing antibodies".

Antibody "binning" does not provide direct information about the epitope. Competing antibodies, i.e. antibodies belonging to the same "bin" may have identical epitopes, overlapping epitopes or even separate epitopes. The latter is the case if the reference antibody bound to its epitope on the antigen takes up the space required for the second antibody to contact its epitope on the antigen ("steric hindrance"). Non-competing antibodies have separate epitopes.

Epitope, paratope and antigen: The term "epitope", as used herein, is defined in the context of a molecular interaction between an "antigen binding molecule", such as an antibody (Ab), and its corresponding "antigen" (Ag). The term antigen (Ag) may refer to the molecular entity used for immunization of an immunocompetent vertebrate to produce the antibody (Ab) that recognizes the Ag. Herein, Ag is termed more broadly and is generally intended to include target molecules that are specifically recognized by the Ab, thus including fragments or mimics of the molecule used in the immunization process for raising the Ab. Generally, "epitope" refers to the area or region on an Ag to which an Ab specifically binds, i.e. the area or region in physical contact with the Ab. Physical contact may be defined through distance criteria (e.g. a distance cut-off of 4 Å) for atoms in the Ab and Ag molecules.

A "discontinuous epitope" is an epitope which is formed by two or more regions of a polypeptide which are not adjacent to each other in the linear peptide sequence, but which are arranged in the three-dimensional structure of the polypeptide to form a structural epitope. Other types of epitopes include: linear peptide epitopes, conformational epitopes which consist of two or more non-contiguous amino acids located near each other in the three-dimensional structure of the antigen; and post-translational epitopes which consist, either in whole or part, of molecular structures covalently attached to the antigen, such as carbohydrate groups.

The epitope for a given antibody (Ab)/antigen (Ag) pair can be defined and characterized at different levels of detail using a variety of experimental and computational epitope mapping methods. The experimental methods include mutagenesis, X-ray crystallography, Nuclear Magnetic Resonance (NMR) spectroscopy and Hydrogen deuterium eXchange Mass Spectrometry (HX-MS), methods that are known in the art. As each method relies on a unique principle, the description of an epitope is intimately linked to the method by which it has been determined. Thus, depending on the epitope mapping method employed, the epitope for a given Ab/Ag pair will be described differently.

At its most detailed level, the epitope for the interaction between the Ag and the Ab can be described by the spatial coordinates defining the atomic contacts present in the Ag-Ab interaction, as well as information about their relative contributions to the binding thermodynamics. At a less detailed level, the epitope can be described by the spatial coordinates defining the atomic contacts between the Ag and Ab. At an even less detailed level the epitope can be described by the amino acid residues that it comprises as defined by a specific criteria such as the distance between atoms in the Ab and the

Ag. At a further less detailed level the Ab-Ag interaction can be characterized through function, e.g. by competition binding with other Abs and “binning” although competition binding does not provide any structural information about the epitope.

5 In the context of an X-ray derived crystal structure defined by spatial coordinates of a complex between an Ab, e.g. a Fab fragment, and its Ag, the term epitope is herein, unless otherwise specified or contradicted by context, specifically defined as IL21 residues characterized by having a heavy atom (i.e. a non-hydrogen atom) within a distance of about 3.5 to about 5.0 Å, such as e.g. 4 Å from a heavy atom in the Ab.

10

From the fact that descriptions and definitions of epitopes, dependant on the epitope mapping method used, are obtained at different levels of detail, it follows that comparison of epitopes for different Abs on the same Ag can similarly be conducted at different levels of detail.

15

Epitopes described on the amino acid level, e.g. determined from an X-ray structure, are said to be identical if they contain the same set of amino acid residues. Epitopes are said to overlap if at least one amino acid is shared by the epitopes. Epitopes are said to be separate (unique) if no amino acid residue are shared by the epitopes.

20

The definition of the term “paratope” is derived from the above definition of “epitope” by reversing the perspective. Thus, the term “paratope” refers to the area or region on the Ab to which an Ag specifically binds, i.e. with which it makes physical contact to the Ag.

25

In the context of an X-ray derived crystal structure, defined by spatial coordinates of a complex between an Ab, such as a Fab fragment, and its Ag, the term paratope is herein, unless otherwise specified or contradicted by context, specifically defined as Ab residues characterized by having a heavy atom (i.e. a non-hydrogen atom) within a distance of about 4 Å (3.5 to 5.0 Å) from a heavy atom in IL21.

30

The epitope and paratope for a given antibody (Ab)/antigen (Ag) pair may be described by routine methods. For example, the overall location of an epitope may be determined by assessing the ability of an antibody to bind to different fragments or variants of IL21. The specific amino acids within IL21 that make contact with an antibody (epitope) and
35 the specific amino acids in an antibody that make contact with IL21 (paratope) may also be determined using routine methods. For example, the Ab and Ag molecules may be

combined and the Ab/Ag complex may be crystallised. The crystal structure of the complex may be determined and used to identify specific sites of interaction between the Ab and Ag.

5 Binding affinity between two molecules, e.g. an antibody, or fragment thereof, and an antigen, through a monovalent interaction may be quantified by determination of the equilibrium dissociation constant (KD). In turn, KD can be determined by measurement of the kinetics of complex formation and dissociation, e.g. by the SPR method. The rate constants corresponding to the association and the dissociation of a monovalent complex
10 are referred to as the association rate constant k_a (or k_{on}) and dissociation rate constant k_d (or k_{off}), respectively. KD is related to k_a and k_d through the equation $KD = k_d / k_a$. Following the above definition, binding affinities associated with different molecular interactions, such as comparison of the binding affinity of different antibodies for a given antigen, may be compared by comparison of the KD values for the individual
15 antibody/antigen complexes.

Non-Antibody Ligands: Ligands specific for the epitope according to the present invention can also encompass antibody mimics comprising one or more IL-21 binding portions built on a molecular scaffold (such as a protein or carbohydrate scaffold) specific for the
20 epitope described herein. Proteins having relatively defined three-dimensional structures, commonly referred to as protein scaffolds, may be used as templates for the design of antibody mimics. These scaffolds typically contain one or more regions which are amenable to specific or random sequence variation, and such sequence randomization is often carried out to produce libraries of proteins from which desired products may be
25 selected. For example, an antibody mimic can comprise a chimeric non-immunoglobulin binding polypeptide having an immunoglobulin-like domain containing scaffold having two or more solvent exposed loops containing a different CDR from a parent antibody inserted into each of the loops and exhibiting selective binding activity toward a ligand bound by the parent antibody. Non-immunoglobulin protein scaffolds have been
30 proposed for obtaining proteins with novel binding properties.

Structure of ligands: As described above, a ligand as referred to herein may be an antibody (for example IgG, IgM, IgA, IgE) or fragment thereof (for example Fab, Fv, disulphide linked Fv, scFv, diabody) which comprises at least one heavy and a light chain variable domain which are complementary to one another and thus can associate with
35 one another to form a VH/VL pair. It may be derived from any species naturally producing

an antibody, or created by recombinant DNA technology; whether isolated from serum, B-cells, hybridomas, transfectomas, mammalian cells, yeast or bacteria.

Therapeutic Applications: IL-21 is involved in T-cell mediated immunity, and has been shown to promote a number of inflammatory cytokines. Accordingly, the ligands according to invention can be used in the treatment of diseases involving an inappropriate or undesired immune response (immunological disorders), such as inflammation, autoimmunity, conditions involving such mechanisms as well as graft vs. host disease. In one embodiment, such disease or disorder is an autoimmune and/or inflammatory disease. Examples of such autoimmune and/or inflammatory diseases are Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA) and inflammatory bowel disease (IBD) (including ulcerative colitis (UC) and Crohn's disease (CD)), multiple sclerosis (MS), scleroderma and type 1 diabetes (T1 D), and other diseases and disorders, such as PV (pemphigus vulgaris), psoriasis, atopic dermatitis, celiac disease, kol, hashimoto's thyroiditis, graves' disease (thyroid), Sjogren's syndrome, guillain-barre syndrome, goodpasture's syndrome, additon's disease, Wegener's granulomatosis, primary biliary sclerosis, sclerosing cholangitis, autoimmune hepatitis, polymyalgia rheumatica, paynaud's phenomenon, temporal arteritis, giant cell arteritis, autoimmune hemolytic anemia, pernicious anemia, polyarteritis nodosa, behcet's disease, primary biliary cirrhosis, uveitis, myocarditis, rheumatic fever, ankylosing spondylitis, glomerulonephritis, sarcoidosis, dermatomyositis, myasthenia gravis, polymyositis, alopecia areata, type I diabetes, Colitis-Associated Tumorigenesis, and vitilgo.

In one embodiment, such disease or disorder is SLE, RA or IBD. In one embodiment, such disease or disorder is MS.

The IL-21 ligands of the present invention may be administered in combination with other medicaments as is known in the art.

The present invention further includes pharmaceutical compositions/formulations, comprising a pharmaceutically acceptable carrier and a polypeptide/ligand/antibody according to the invention as well as kits comprising such compositions. The pharmaceutical composition according to the invention may be in the form of an aqueous formulation or a dry formulation that is reconstituted in water/an aqueous buffer prior to administration.

Pharmaceutical compositions comprising ligands/antibodies/polypeptides according to the invention may be supplied as a kit comprising a container that comprises the

compound according to the invention. Therapeutic polypeptides can be provided in the form of an injectable solution for single or multiple doses, or as a sterile powder that will be reconstituted before injection. Pharmaceutical compositions comprising compounds according to the invention are suitable for subcutaneous and/or IV administration.

- 5 Combinatiton treatment: antibodies according to the invention may be co-administered with one or other more other therapeutic agents or formulations. The other agent may be intended to treat other symptoms or conditions of the patient. For example, the other agent may be an analgesic, an immunosuppressant or an anti-inflammatory agent.

10 Combined administration of two or more agents may be achieved in a number of different ways. In one embodiment, the antibody and the other agent may be administered together in a single composition. In another embodiment, the antibody and the other agent may be administered in separate compositions as part of a combined therapy. For example, the modulator may be administered before, after or concurrently with the other agent.

- 15 The antibodies/proteins according to the present invention may be administered along with other drugs (e.g. methotrexate, dexamethasone, and prednisone) and/or other biological drugs. Agents already in use in autoimmunity include immune modulators such as IFNbeta, Orencia (CTLA4-Ig), Humira (anti-TNF), Cimzia (anti-TNF, PEG Fab), Tysabri (a4-integrin mAb), Simponi, Rituxan/MabThera, Actemra/RoActemra, Kineret,
- 20 Non-steroidal anti-inflammatory drugs (NSAIDS) like Aspirin, Ibuprofen etc, Corticosteroids, disease-modifying antirheumatic drugs (DMARDS) like Plaquenil, Azulfidine, Methotrexate etc, Copaxone (glatirimer acetate), Gilneya (fingolimod), Antibiotics like Flagyl, Cipro, Topical (skin applied) medications including topical corticosteroids, vitamin D analogue creams (Dovonex), topical retinoids (Tazorac),
- 25 moisturizers, topical immunomodulators (tacrolimus and pimecrolimus), coal tar, anthralin, and others, Raptiva, Ustekimumab, light therapy like PUVA, UVB, CellCept (mycophenolate mofetil).

Embodiments

30 The following list of embodiments represents examples of embodiments of the present invention and should thus not be understood as limiting the invention.

1. An IL-21 mimic comprising an epitope comprising the following amino acids: Glu 65, Asp 66, Val 67, and His 149 as set forth in SEQ ID No.1.

2. The mimic according to embodiment 1, wherein the epitope of said mimic further comprises one or more of the following amino acids: Arg 40, Lys 50, Glu 129, Glu 135, Glu 138, Arg 139, Lys 141, Ser 142, and Gln 145 as set forth in SEQ ID NO 1.
3. The mimic according to embodiment 1, wherein the epitope of said mimic further
5 comprises one or more of the following amino acids: Glu 68, Thr 69, Asn 70, Glu 72, Trp 73, Lys 117, His 118, Arg 119, Leu 143, Lys 146, Met 147, Gln 150, and His 151.
4. The mimic according to any one of embodiments 1 to 3, wherein the epitope of said mimic further comprises the following amino acids: Glu 68, Thr 69, Asn 70, Glu 72, Trp 73, Lys 117, His 118, Arg 119, Leu 143, Lys 146, Met 147, Gln 150, and His 151.
- 10 5. A method for selecting a ligand which binds to IL-21, comprising screening one or more libraries of ligands with an IL-21 mimic according to any one of embodiments 1-4, and isolating one or more ligands which bind to said epitope.
6. Use of an IL-21 mimic according to any one of embodiments 1-4, for selecting a ligand which binds selectively to IL-21.
- 15 7. A ligand, wherein said ligand is preferably an antibody, which ligand binds specifically to the epitope of the IL-21 mimic according to any one of embodiments 1-4, provided that the ligand is not: (i) naturally occurring common γ C (SEQ ID No. 8), and not (ii) the monoclonal antibody mAb14, the light and heavy chains of which are set forth in SEQ ID No. 6 and SEQ ID No. 7, respectively. If the ligand is an antibody, the antibody is not the
20 monoclonal mAb14 antibody.
8. A ligand, wherein said ligand is preferably an antibody, which ligand binds to an epitope on IL-21, wherein said epitope comprises one or more of the Arg 40 to Val 67 amino acids as well as one or more of the Glu 129 to His 149 amino acids, as set forth in SEQ ID No.1, provided that the ligand is not: (i) naturally occurring common gamma
25 chain (SEQ ID No. 8), and not (ii) mAb14, the light and heavy chains of which are set forth in SEQ ID No. 6 and SEQ ID No. 7 respectively. Said ligand preferably comprises one or more of the Glu 65 to Val 67 amino acids and one or more of the Glu 129 to His 149 amino acids. If the ligand is an antibody, the antibody is not the monoclonal mAb14 antibody.
- 30 9. A ligand which binds to IL-21, wherein said ligand is preferably an antibody, wherein said ligand binds to at least one of the Arg 40, Lys 50, Glu 65, Asp 66, Val 67, Glu 129, Glu 135, Glu 138, Arg 139, Lys 141, Ser 142, Gln 145, and His 149 amino acids as set

forth in SEQ ID NO 1, provided that the ligand is not: (i) naturally occurring common γ C (SEQ ID No. 8), and not (ii) mAb14, the light and heavy chains of which are set forth in SEQ ID No. 6 and SEQ ID No. 7, respectively.

5 10. A ligand according to embodiment 9, wherein the said ligand binds to the Arg 40, Lys 50, Glu 65, Asp 66, Val 67, Glu 129, Glu 135, Glu 138, Arg 139, Lys 141, Ser 142, Gln 145, and His 149 amino acids as set forth in SEQ ID NO 1.

10 11. A ligand which binds to IL-21, wherein said ligand is preferably an antibody, wherein said ligand binds to at least one of the amino acids Glu 72 to Ala 82 in IL-21 (SEQ ID NO 1) provided that the ligand is not mAb14, the light and heavy chains of which are set forth in SEQ ID No. 6 and SEQ ID No. 7 respectively. Preferably, said ligand binds to at least one of the amino acids Glu 65 to Trp 73, provided that the ligand is not naturally occurring common γ C (SEQ ID No. 8) and not mAb14, the light and heavy chains of which are set forth in SEQ ID No. 6 and SEQ ID No. 7, respectively. If the latter ligand is an antibody, the antibody is not the monoclonal mAb14 antibody.

15 12. A ligand according to any one of embodiments 7-11, wherein said ligand is preferably an antibody, wherein said ligand binds to amino acids Asn 70, Glu 72, and Trp 73 in IL-21 (SEQ ID NO 1).

20 13. A ligand according to any one of embodiments 7-12, wherein said ligand is preferably an antibody, wherein said ligand furthermore binds one or more of amino acids Glu 65, Asp 66, and Val 67 as set forth in SEQ ID NO 1.

14. A ligand according to any one of embodiments 7-13, wherein said ligand is preferably an antibody, wherein said ligand furthermore binds amino acid His 149 as set forth in SEQ ID NO 1.

25 15. A ligand according to any one of embodiments 7-14, wherein said ligand is preferably an antibody, wherein said ligand binds amino acids Glu 65, Asp 66, Val 67, and His 149 as set forth in SEQ ID NO 1.

30 16. A ligand which binds to IL-21, wherein said ligand is preferably an antibody, wherein said ligand binds to an epitope comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 of the following amino acids: Arg 40, Lys 50, Glu 65, Asp 66, Val 67, Glu 129, Glu 135, Glu 138, Arg 139, Lys 141, Ser 142, Gln 145, and His 149 as set forth in SEQ ID No. 1, provided that the ligand is not: (i) naturally occurring common gamma chain (SEQ ID No. 8), and not (ii) mAb14, the light and heavy chains of which are set forth in SEQ ID No. 6

and SEQ ID No. 7, respectively. Preferably the ligand binds to the following amino acids: Arg 40, Lys 50, Glu 65, Asp 66, Val 67, Glu 129, Glu 135, Glu 138, Arg 139, Lys 141, Ser 142, Gln 145, and His 149 as set forth in SEQ ID No. 1.

17. A ligand according to embodiment 16, wherein said ligand is preferably an antibody,
5 wherein said ligand binds to an epitope comprising the following amino acids: Arg 40, Lys 50, Glu 65, Asp 66, Val 67, Glu 129, Glu 135, Glu 138, Arg 139, Lys 141, Ser 142, Gln 145, and His 149 as set forth in SEQ ID No. 1.

18. A ligand according to any one of embodiments 7-15, wherein said ligand is preferably
10 an antibody, wherein said ligand binds to an epitope comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 of the following amino acids: Glu 65, Asp 66, Val 67, Glu 68, Thr 69, Asn 70, Glu 72, Trp 73, Lys 117, His 118, Arg 119, leu 143, Lys 146, Met 147, His 149, Gln 150, and His 151.

19. A ligand which binds to IL-21, wherein said ligand is preferably an antibody, wherein
15 said ligand binds to an epitope comprising the following amino acids: Glu 65, Asp 66, Val 67, Glu 68, Thr 69, Asn 70, Glu 72, Trp 73, Lys 117, His 118, Arg 119, leu 143, Lys 146, Met 147, His 149, Gln 150, and His 151, provided that the ligand is not: (i) naturally occurring common γ C (SEQ ID No. 8), and not (ii) mAb14, the light and heavy chains of which are set forth in SEQ ID No. 6 and SEQ ID No. 7, respectively.

20. A ligand according to any one of embodiments 7-19, wherein said ligand is preferably
20 an antibody, wherein said ligand comprises one, two, or three of CDR1, CDR2 and CDR3 as set forth in SEQ ID No. 6, and one, two, or three of CDR1, CDR2 and CDR3 as set forth in SEQ ID No. 7, provided that the ligand is not mAb14, the light and heavy chains of which are set forth in SEQ ID NO 6 and SEQ ID NO 7, respectively. The mAb14 antibody is the same antibody which is disclosed in WO2010/055366, designated
25 therein by hybridoma clone number 366.328.10.63.

21. A ligand according to any one of embodiments 7-20, wherein said ligand is preferably
an antibody, wherein said ligand interferes with binding of IL-21 to common γ C.

22. A ligand according to any one of embodiments 7-21, wherein said ligand is an
30 antibody. The antibody can be an antibody, a monoclonal antibody, an antigen binding fragment of an antibody, a monovalent antibody, a divalent antibody. The antibody may be a human or humanized form of any of these.

23. A ligand according to embodiment 22, wherein said antibody is an IgG1 antibody. The ligand may alternatively be an IgG4 antibody.

24. A ligand according to any one of embodiments 22-23, wherein said antibody comprises an Fc domain, which mediates antibody effector functions.

5 25. A ligand according to embodiment 24, wherein said ligand comprises an Fc domain having reduced effector functions.

26. A ligand according to embodiment 25, wherein said ligand comprises an IgG1 Fc domain comprising one, two, three, four or all of the following mutations that result in decreased affinity to certain Fc receptors (L234A, L235E, and G237A) and in reduced
10 C1q-mediated complement fixation (A330S and P331S), respectively (residue numbering according to the EU index). Such ligands will retain a relatively long *in vivo* half life and significantly reduced effector functions.

27. A ligand according to embodiment 20, wherein said ligand is an antibody that is a variant of mAb14, the light and heavy chains thereof which are set forth in SEQ ID No. 6
15 and SEQ ID No. 7 respectively, wherein said ligand comprises one or more mutations in the CDR sequences, wherein said mutations are selected from one or more from the list consisting of: A61S (SEQ ID NO 7), D62E (SEQ ID NO 7), V64I (SEQ ID NO 7), and K65R (SEQ ID NO 7), R24K (SEQ ID NO 6), S26T (SEQ ID NO 6), Q27N (SEQ ID NO 6), D30E (SEQ ID NO 6), S53T (SEQ ID NO 6), and S56T (SEQ ID NO 6). Each of these
20 mutations thus represents separate embodiments. Any combination thereof also represents separate embodiments.

28. An antibody which binds to an epitope on IL-21, wherein said epitope comprises one or more of the following amino acids: Glu 65, Asp 66, Val 67, Glu 68, Thr 69, Asn 70, Glu 72, Trp 73, one or more of the following amino acids Lys 117, His 118, Arg 119, and one
25 or more of the following amino acids: Leu 143, Lys 146, Met 147, His 149, Gln 150, and His 151 as set forth in SEQ ID No.1, provided that the antibody is not the monoclonal antibody mAb14, the light and heavy chains of which are set forth in SEQ ID No. 6 and SEQ ID No. 7, respectively. The antibody may alternatively bind to an epitope on IL-21, wherein said epitope comprises one or more of the following amino acids: Glu 65, Asp
30 66, Val 67, Glu 68, Thr 69, Asn 70, Glu 72, Trp 73, Lys 117, His 118, and Arg 119, and one or more of the following amino acids: Leu 143, Lys 146, Met 147, His 149, Gln 150, and His 151 as set forth in SEQ ID No.1. The antibody may alternatively bind to an epitope on IL-21, wherein said epitope comprises one or more of the following amino

acids: Glu 65, Asp 66, Val 67, Glu 68, Thr 69, Asn 70, Glu 72, and Trp 73, and one or more of the following amino acids: Lys 117, His 118, and Arg 119, Leu 143, Lys 146, Met 147, His 149, Gln 150, and His 151 as set forth in SEQ ID No.1.

29. An antibody which binds to an epitope on IL-21, wherein said epitope comprises one
5 or more of the following amino acids: Glu 65 to Trp 73, one or more of the following amino acids: Lys 117 to Arg 119, and one or more of the following amino acids: Leu 143 to His 151 as set forth in SEQ ID No.1, provided that the antibody is not the monoclonal antibody mAb14, the light and heavy chains of which are set forth in SEQ ID No. 6 and SEQ ID No. 7, respectively. The antibody may alternatively bind to an epitope on IL-21,
10 wherein said epitope comprises one or more of the following amino acids: Glu 65 to Trp 73, and one or more of the following amino acids: Leu 143 to His 151 as set forth in SEQ ID No.1.

30. An antibody which binds to an epitope on IL-21, wherein said epitope comprises one or more of the Arg 40 to Val 67 amino acids as well as one or more of the Glu 129 to His
15 149 amino acids, as set forth in SEQ ID No.1, provided that the antibody is not mAb14, the light and heavy chains of which are set forth in SEQ ID No. 6 and SEQ ID No. 7, respectively.

31. An antibody which binds to an epitope on IL-21, wherein said epitope comprises one or more of the Glu 65 to Trp 73 amino acids in IL-21 (SEQ ID NO. 1) provided that the
20 antibody is not mAb14, the light and heavy chains of which are set forth in SEQ ID No. 6 and SEQ ID No. 7, respectively.

32. An antibody which binds to an epitope on IL-21, wherein said epitope comprises one or more of the Glu 65, Asp 66, Val 67, and His 149 amino acids as set forth in SEQ ID
25 NO. 1, provided that the antibody is not mAb14, the light and heavy chains of which are set forth in SEQ ID No. 6 and SEQ ID No. 7, respectively.

33. A pharmaceutical composition comprising a ligand/antibody according to any one of embodiments 7-32 and optionally one or more pharmaceutically acceptable excipients. Such excipients/carriers are well known in the art. Such pharmaceutical compositions are preferably intended for IV administration and/or subcutaneous administration.

30 34. A kit comprising a ligand/antibody according to any one of embodiments 7-32.

35. Use of a ligand/antibody according to any one of embodiments 7-32 as a medicament.

36. Use of a ligand/antibody according to any one of embodiments 7-32 for treating an immunological disorder.

37. Use of a ligand/antibody according to any one of embodiments 7-32 for treating an autoimmune disease.

5 38. Use of a ligand/antibody according to any one of embodiments 7-32 for treating SLE.

39. Use of a ligand/antibody according to any one of embodiments 7-32 for treating RA.

40. Use of a ligand/antibody according to any one of embodiments 7-32 for treating IBD.

41. Use of a ligand/antibody according to any one of embodiments 7-32 for treating CD.

10 42. A method of treating an immunological disorder, wherein said method comprises administering to a person in need thereof an appropriate dosis of a ligand/antibody according to any one of embodiments 7-32.

15 The provision herein of the detailed 3-dimensional structural knowledge of the complex between the Fab fragment of mAb14 (Fab35) and IL-21, including their binding interface, can form the basis for rationally designing variants of the interacting molecules with desired properties. Properties that might be desirable to improve for antibodies may be chemical or physical properties e.g. solubility, viscosity and stability. Other properties that might be desirable to modulate are the antigenic properties of the antibodies and their ability to be bound by anti-antibodies.

EXAMPLES

20 Example 1

Crystal structure of IL-21 in complex with a Fab fragment of mAb14 (Fab35)

25 The 3-dimensional structure of IL-21 in complex with the Fab fragment (Fab35) of the human anti-IL-21 monoclonal antibody mAb14 was solved and refined to 1.64 Å resolution using X-ray crystallography. The results demonstrate that the Fab35 (representing mAb14) epitope on IL-21 is situated on a completely different part of the IL-21 molecule as compared with that of mAb5, and binds with a different binding mode. "mAb5" corresponds to an IgG1 version of the clone 362.78.1.44 antibody disclosed in WO2010055366, the Fc region of mAb5 carrying the L234A, L235E, and G237A (reduced Fc receptor binding) and A330S and P331S mutations (reduced C1q-mediated

complement fixation). While mAb5 binds to the surface exposed faces of helix A and C on IL-21 Fab35 (mAb14) binds more towards one end of the four-helix bundle, interacting with the exposed loops but also penetrating in to the IL-21 molecule by inserting the side chain of a Tryptophane residue, W102 of the heavy chain, between helices B and D, and thereby slightly distorting the C-terminal part of helix D. Fab35 (representing mAb14) will, instead of competing with binding of IL-21R α to IL-21 as mAb5, compete with, and due to its high binding affinity, block the binding of γ C to IL-21. Hence, mAb14 will inhibit the biological effects mediated by IL-21 through γ C.

The epitope described was characterized using the structure of the complex between Fab35 and IL-21. However, the conclusions regarding the epitope of Fab35 on IL-21 will also apply to the interaction between IL-21 and the corresponding full antibody, mAb14, from which Fab35 was derived.

hIL-21 (expressed in *E. coli* as the mature peptide; residues 30-162 of SEQ ID NO: 1 with an added N-terminal Methionine residue) in 10 mM histidine buffer, pH 5.3, and anti-IL-21 Fab35 (comprising a light chain corresponding to SEQ ID NO. 9 and a heavy chain fragment corresponding SEQ ID NO. 10), formulated in PBS buffer, pH 7.4 (4 tablets in 2 liter of water, GIBCO Cat.No. 18912-014 Invitrogen Corporation), were mixed in a molar ratio of 1:1. The final concentration of the complex was 10.3 mg/ml. Crystals were grown with the sitting drop technique in 30% w/v PEG1000 and 200 mM magnesium formate mixed in a ratio of 1:1 (precipitant solution volume:protein solution volume). Total drop size was 0.2 μ l. A crystal was prepared for cryo-freezing by transferring 3 μ l of a cryo-solution containing 75 % of the precipitant solution and 25 % glycerol to the drop containing the crystal, and soaking was allowed for about half a minute. The crystal was then flash frozen in liquid N₂ and kept at a temperature of 100 K during data collection by a cryogenic N₂ gas stream. Crystallographic data were collected to 1.64 Å resolution at beam-line BL911-2 (1) at MAX-lab, Lund, Sweden. Space group determination, integration and scaling of the data were made by the XDS software package (2). Cell parameters for the data were determined to be 89.4, 65.2, 106.7 Å, 90°, 111.57° and 90°, respectively, and the space group C2. R-sym to 1.64 Å resolution was 6.4 % and completeness 98.2 %. The molecular replacement technique, using the PHASER software program (3;4) of the CCP4 suite (5) was used for structure determination. The X-ray structure of the anti-IL-21 Fab9 (corresponding to mAb5), in complex with IL-21 (unpublished results), was used as input model for the PHASER software. The IL-21 molecule from the Fab9:IL-21 complex structure was also used, independently from the Fab, as input for the PHASER software. The software ARP/wARP (6) was subsequently

used for an initial round of model building and was then followed by crystallographic refinements, using the software programs REFMAC5 (7) of the CCP4 software package and PHENIX.REFINE (8) of the PHENIX software package (9) and by computer graphics inspection of the electron density maps, model corrections and building using the Coot software program (10). The procedure was cycled until no further significant improvements could be made to the model. Final R- and R-free for all data were 0.179 and 0.211, respectively, and the model showed a root-mean-square deviation (RMSD) from ideal bond lengths of 0.022 Å.

10 Results

The binding site of Fab35 will compete with, and due to its high binding affinity, block the binding of γ C to IL-21. Hence, it will inhibit the biological effects mediated by IL-21 through γ C.

Calculation of the areas excluded in pair-wise interactions by the software program Areaimol (11;12) of the CCP4 program suite (5) gave for the IL-21/Fab35 molecular complex in the crystal structure 1082 Å² for IL-21 and 1041 Å² for anti-IL-21, respectively. The average areas excluded in pair-wise interaction between the IL-21 molecule and Fab35 were calculated to be 1061 Å².

The direct contacts between the IL-21 and Fab35 were identified by running the contacts software of the CCP4 program suite (5) using a cut-off distance of 4.0 Å between Fab35 and the IL-21 molecules. The results from the IL-21/Fab35 complex crystal structure are shown in Table 1. The resulting IL-21 epitope for Fab35 (representing mAb14) was found to comprise the following residues of IL-21 (SEQ ID NO. 1): Glu 65, Asp 66, Val 67, Glu 68, Thr 69, Asn 70, Glu 72, Trp 73, Lys 117, His 118, Arg 119, Leu 143, Lys 146, Met 147, His 149, Gln 150 and His 151.

Thus, the Fab35 (mAb14) epitope comprise residues in the N-terminal part of helix B (residues 72-73), and residues in the C-terminal part of helix D (residues 143-151). Additionally, several contact residues were identified in the loop segment proceeding helix B (residues 65-70), and in the loop between helix C and helix D (residues 117-119). This epitope has a partial overlap with the predicted binding site for γ C to IL-21.

The Fab35 (representing mAb14) paratope for IL-21 included residues Ser 31, Asp 50, Phe 91, Asn 92 and Tyr 94 of the light (L) chain (SEQ ID NO. 9, Table 2), and residues Ile 28, Ser 30, Ser 31, Tyr 32, Ser 33, Thr 52, Ser 53, Gly 54, Ser 55, Tyr 56, Tyr 57, His

59, Glu 99, Arg 100, Gly 101, Trp 102, Gly 103, Tyr 104 and Tyr 105 of the heavy (H) chain (SEQ ID NO. 10, Table 2). The epitope for the Fab35 fragment/mAb14 antibody is shown in figure 2

5 **Table 1.** Results from the X-ray model refinement to the observed data of the IL-21/Fab35 complex by the software program Refmac5 (7) of the CCP4 program software package (5).

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REMARK 3 REFINEMENT.
REMARK 3 PROGRAM : REFMAC 5.6.0085
REMARK 3 AUTHORS : MURSHUDOV,VAGIN,DODSON
10 REMARK 3
REMARK 3 REFINEMENT TARGET : MAXIMUM LIKELIHOOD
REMARK 3
REMARK 3 DATA USED IN REFINEMENT.
REMARK 3 RESOLUTION RANGE HIGH (ANGSTROMS) : 1.64
15 REMARK 3 RESOLUTION RANGE LOW (ANGSTROMS) : 27.23
REMARK 3 DATA CUTOFF (SIGMA(F)) : NONE
REMARK 3 COMPLETENESS FOR RANGE (%) : 98.27
REMARK 3 NUMBER OF REFLECTIONS : 65231
REMARK 3
20 REMARK 3 FIT TO DATA USED IN REFINEMENT.
REMARK 3 CROSS-VALIDATION METHOD : THROUGHOUT
REMARK 3 FREE R VALUE TEST SET SELECTION : RANDOM
REMARK 3 R VALUE (WORKING + TEST SET) : 0.18040
REMARK 3 R VALUE (WORKING SET) : 0.17877
25 REMARK 3 FREE R VALUE : 0.21100
REMARK 3 FREE R VALUE TEST SET SIZE (%) : 5.1
REMARK 3 FREE R VALUE TEST SET COUNT : 3487
REMARK 3
REMARK 3 FIT IN THE HIGHEST RESOLUTION BIN.
30 REMARK 3 TOTAL NUMBER OF BINS USED : 20
REMARK 3 BIN RESOLUTION RANGE HIGH : 1.640
REMARK 3 BIN RESOLUTION RANGE LOW : 1.682
REMARK 3 REFLECTION IN BIN (WORKING SET) : 4786
REMARK 3 BIN COMPLETENESS (WORKING+TEST) (%) : 97.49
35 REMARK 3 BIN R VALUE (WORKING SET) : 0.293
REMARK 3 BIN FREE R VALUE SET COUNT : 267
REMARK 3 BIN FREE R VALUE : 0.302
REMARK 3
40 REMARK 3 NUMBER OF NON-HYDROGEN ATOMS USED IN REFINEMENT.
REMARK 3 ALL ATOMS : 4812
REMARK 3
REMARK 3 B VALUES.
REMARK 3 FROM WILSON PLOT (A**2) : NULL
REMARK 3 MEAN B VALUE (OVERALL, A**2) : 27.871
45 REMARK 3 OVERALL ANISOTROPIC B VALUE.
REMARK 3 B11 (A**2) : -0.34
REMARK 3 B22 (A**2) : 0.81
REMARK 3 B33 (A**2) : 0.23
REMARK 3 B12 (A**2) : 0.00
50 REMARK 3 B13 (A**2) : 0.96
REMARK 3 B23 (A**2) : 0.00
REMARK 3
REMARK 3 ESTIMATED OVERALL COORDINATE ERROR.
REMARK 3 ESU BASED ON R VALUE (A) : 0.097
55 REMARK 3 ESU BASED ON FREE R VALUE (A) : 0.096
REMARK 3 ESU BASED ON MAXIMUM LIKELIHOOD (A) : 0.072
REMARK 3 ESU FOR B VALUES BASED ON MAXIMUM LIKELIHOOD (A**2) : 4.258
REMARK 3
60 REMARK 3 CORRELATION COEFFICIENTS.
REMARK 3 CORRELATION COEFFICIENT FO-FC : 0.967
REMARK 3 CORRELATION COEFFICIENT FO-FC FREE : 0.951
REMARK 3

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REMARK 3 RMS DEVIATIONS FROM IDEAL VALUES          COUNT    RMS    WEIGHT
REMARK 3 BOND LENGTHS REFINED ATOMS          (A): 4425 ; 0.024 ; 0.022
REMARK 3 BOND ANGLES REFINED ATOMS    (DEGREES): 6019 ; 2.001 ; 1.957
5  REMARK 3 TORSION ANGLES, PERIOD 1    (DEGREES): 571 ; 6.320 ; 5.000
REMARK 3 TORSION ANGLES, PERIOD 2    (DEGREES): 185 ; 35.306 ; 24.000
REMARK 3 TORSION ANGLES, PERIOD 3    (DEGREES): 760 ; 14.206 ; 15.000
REMARK 3 TORSION ANGLES, PERIOD 4    (DEGREES): 25 ; 15.286 ; 15.000
REMARK 3 CHIRAL-CENTER RESTRAINTS    (A**3): 671 ; 0.156 ; 0.200
REMARK 3 GENERAL PLANES REFINED ATOMS    (A): 3325 ; 0.012 ; 0.021
10  REMARK 3
REMARK 3 ISOTROPIC THERMAL FACTOR RESTRAINTS.    COUNT    RMS    WEIGHT
REMARK 3
REMARK 3 NCS RESTRAINTS STATISTICS
REMARK 3 NUMBER OF NCS GROUPS : NULL
15  REMARK 3
REMARK 3 TWIN DETAILS
REMARK 3 NUMBER OF TWIN DOMAINS : NULL
REMARK 3
REMARK 3
20  REMARK 3 TLS DETAILS
REMARK 3 NUMBER OF TLS GROUPS : 3
REMARK 3 ATOM RECORD CONTAINS SUM OF TLS AND RESIDUAL B FACTORS
REMARK 3
REMARK 3 TLS GROUP : 1
25  REMARK 3 NUMBER OF COMPONENTS GROUP : 2
REMARK 3 COMPONENTS          C SSSEQI    TO C SSSEQI
REMARK 3 RESIDUE RANGE :    L    1          L    109
REMARK 3 RESIDUE RANGE :    H    1          H    122
REMARK 3 ORIGIN FOR THE GROUP (A): 9.3480 52.1830 33.9230
30  REMARK 3 T TENSOR
REMARK 3 T11: 0.0431 T22: 0.0196
REMARK 3 T33: 0.0276 T12: 0.0114
REMARK 3 T13: 0.0100 T23: -0.0020
REMARK 3 L TENSOR
35  REMARK 3 L11: 1.2847 L22: 0.6769
REMARK 3 L33: 2.3566 L12: 0.2152
REMARK 3 L13: 0.4752 L23: 0.5847
REMARK 3 S TENSOR
40  REMARK 3 S11: 0.0830 S12: -0.0041 S13: -0.0198
REMARK 3 S21: 0.0073 S22: -0.0057 S23: -0.0459
REMARK 3 S31: 0.0390 S32: 0.1660 S33: -0.0773
REMARK 3
REMARK 3 TLS GROUP : 2
45  REMARK 3 NUMBER OF COMPONENTS GROUP : 2
REMARK 3 COMPONENTS          C SSSEQI    TO C SSSEQI
REMARK 3 RESIDUE RANGE :    L   110          L   250
REMARK 3 RESIDUE RANGE :    H   123          H   250
REMARK 3 ORIGIN FOR THE GROUP (A): 27.2190 42.4690 5.5720
REMARK 3 T TENSOR
50  REMARK 3 T11: 0.0288 T22: 0.0170
REMARK 3 T33: 0.0255 T12: 0.0116
REMARK 3 T13: -0.0108 T23: -0.0068
REMARK 3 L TENSOR
REMARK 3 L11: 1.9851 L22: 2.0128
55  REMARK 3 L33: 1.0452 L12: 0.5265
REMARK 3 L13: -0.3061 L23: -0.2683
REMARK 3 S TENSOR
REMARK 3 S11: -0.0438 S12: -0.0112 S13: -0.0403
REMARK 3 S21: -0.0720 S22: -0.0072 S23: 0.0391
60  REMARK 3 S31: -0.0593 S32: 0.0626 S33: 0.0510
REMARK 3
REMARK 3 TLS GROUP : 3
REMARK 3 NUMBER OF COMPONENTS GROUP : 1
REMARK 3 COMPONENTS          C SSSEQI    TO C SSSEQI
65  REMARK 3 RESIDUE RANGE :    I    1          I    200
REMARK 3 ORIGIN FOR THE GROUP (A): -7.7370 51.3830 61.1860
REMARK 3 T TENSOR

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REMARK 3 T11: 0.1110 T22: 0.1464
 REMARK 3 T33: 0.0970 T12: -0.0398
 REMARK 3 T13: -0.0116 T23: -0.0399
 REMARK 3 L TENSOR
 5 REMARK 3 L11: 2.1367 L22: 1.7294
 REMARK 3 L33: 3.9727 L12: 0.4565
 REMARK 3 L13: -2.2072 L23: -1.0335
 REMARK 3 S TENSOR
 10 REMARK 3 S11: 0.0766 S12: -0.3405 S13: 0.1642
 REMARK 3 S21: 0.2556 S22: -0.0443 S23: -0.0496
 REMARK 3 S31: -0.1361 S32: 0.1334 S33: -0.0323
 REMARK 3
 REMARK 3
 15 REMARK 3 BULK SOLVENT MODELLING.
 REMARK 3 METHOD USED : MASK
 REMARK 3 PARAMETERS FOR MASK CALCULATION
 REMARK 3 VDW PROBE RADIUS : 1.20
 REMARK 3 ION PROBE RADIUS : 0.80
 REMARK 3 SHRINKAGE RADIUS : 0.80
 20 REMARK 3
 REMARK 3 OTHER REFINEMENT REMARKS:
 REMARK 3 U VALUES : WITH TLS ADDED
 REMARK 3
 25 SSBOND 1 CYS L 88 CYS L 23
 LINKR SG ACYS L 194 SG CYS L 134 SS
 LINKR SG BCYS L 194 SG CYS L 134 SS
 SSBOND 2 CYS H 134 CYS L 214
 SSBOND 3 CYS H 96 CYS H 22
 30 LINKR SG ACYS H 203 SG ACYS H 147 SS
 LINKR SG BCYS H 203 SG BCYS H 147 SS
 SSBOND 4 CYS I 71 CYS I 122
 SSBOND 5 CYS I 78 CYS I 125
 CISPEP 1 SER L 7 PRO L 8 0.00
 CISPEP 2 TYR L 94 PRO L 95 0.00
 35 CISPEP 3 TYR L 140 PRO L 141 0.00
 CISPEP 4 PHE H 153 PRO H 154 0.00
 CISPEP 5 GLU H 155 PRO H 156 0.00
 LINKR LYS I 106 ARG I 114 gap
 LINKR CYS I 78 SER I 86 gap
 40 CRYST1 89.410 65.160 106.690 90.00 111.57 90.00 C 1 2 1

Table 2. IL-21, chain I, (SEQ ID NO. 1) interactions with the the heavy chain (chain H) of Fab35 (SEQ ID NO. 10) and light chain (chain L) of Fab35 (SEQ ID NO. 9). A distance cut-off of 4.0 Å was used. The contacts were identified by the CONTACT computer software program of the CCP4 suite (5). In the last column "****" indicates a strong possibility for a hydrogen bond at this contact (distance < 3.3 Å) as calculated by CONTACT, " *" indicates a weak possibility (distance > 3.3 Å). Blank indicates that the program considered there to be no possibility of a hydrogen bond. Hydrogen-bonds are specific between a donor and an acceptor, are typically strong, and are easily identifiable.

IL-21			Fab35			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
Glu	65 I	OE1	Tyr	56 H	OH	3.69	*
Asp	66 I	CB	Tyr	56 H	CB	3.76	
			Tyr	56 H	CG	3.81	
			Tyr	56 H	CD2	4.00	
Asp	66 I	CG	Tyr	56 H	CB	3.92	
			Gly	54 H	N	3.31	
			Gly	54 H	CA	3.33	
			Thr	52 H	CB	3.96	

IL-21			Fab35			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Thr	52 H	OG1	3.40	
			Gly	54 H	C	3.79	
Asp	66 I	OD1	Ser	53 H	OG	3.56	*
			Gly	54 H	N	3.07	***
			Gly	54 H	CA	3.56	
			Thr	52 H	CB	3.66	
			Thr	52 H	OG1	3.57	*
			Ser	53 H	N	3.79	*
Asp	66 I	OD2	Gly	54 H	O	3.71	*
			Tyr	56 H	CB	3.23	
			Tyr	56 H	CG	3.93	
			Gly	54 H	N	3.18	***
			Gly	54 H	CA	3.11	
			Ser	55 H	C	3.89	
			Thr	52 H	CB	3.57	
			Thr	52 H	OG1	2.64	***
			Gly	54 H	C	3.08	
			Ser	55 H	N	3.16	***
			Tyr	56 H	N	2.87	***
			Tyr	56 H	CA	3.57	
Asp	66 I	C	Tyr	57 H	CE2	3.76	
Asp	66 I	O	Tyr	57 H	OH	3.96	*
			Tyr	57 H	CE2	3.46	
Val	67 I	N	Tyr	57 H	CE2	3.86	
Val	67 I	CA	Tyr	57 H	CE2	3.83	
Val	67 I	C	Tyr	57 H	CE2	3.74	
			Tyr	57 H	CD2	3.69	
Val	67 I	O	Thr	52 H	CB	3.86	
			Thr	52 H	CG2	3.44	
Glu	68 I	N	Tyr	57 H	CE2	3.64	
			Tyr	57 H	CD2	3.60	
Glu	68 I	CG	Tyr	57 H	CG	3.76	
			Tyr	57 H	CD1	3.59	
			Tyr	57 H	CE1	3.83	
Glu	68 I	CD	Tyr	57 H	CB	3.91	
			Tyr	57 H	CG	3.71	
			Tyr	57 H	CD1	3.45	
			His	59 H	NE2	3.77	
Glu	68 I	OE1	Tyr	57 H	CB	3.69	
			Tyr	57 H	CG	3.95	
			His	59 H	NE2	3.07	***
			His	59 H	CD2	3.89	
Glu	68 I	OE2	Tyr	57 H	CD1	3.36	
			Tyr	57 H	CE1	3.92	
			His	59 H	NE2	3.69	*
Thr	69 I	N	Thr	52 H	CG2	3.85	
Thr	69 I	CB	Ser	33 H	OG	3.73	
			Tyr	94 L	OH	3.61	
Thr	69 I	OG1	Ser	33 H	CB	3.43	
			Ser	33 H	OG	2.70	***
			Thr	52 H	CG2	3.66	
			Tyr	94 L	OH	3.79	*
Thr	69 I	CG2	Ser	33 H	OG	3.68	
			Glu	99 H	CD	3.55	

IL-21			Fab35			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Glu	99 H	OE1	3.82	
			Glu	99 H	OE2	3.50	
Asn	70 I	CB	Tyr	105 H	CE1	3.72	
Asn	70 I	CG	Gly	103 H	N	3.87	
			Tyr	105 H	CD1	3.74	
			Tyr	105 H	CE1	3.49	
Asn	70 I	OD1	Arg	100 H	O	3.96	*
			Gly	101 H	CA	3.25	
			Gly	101 H	C	3.20	
			Gly	101 H	O	3.73	*
			Trp	102 H	N	3.42	*
			Trp	102 H	C	3.93	
			Gly	103 H	N	2.83	***
			Tyr	104 H	N	3.77	*
			Gly	103 H	CA	3.40	
			Gly	103 H	C	3.96	
Asn	70 I	ND2	Glu	99 H	OE2	3.87	*
			Tyr	105 H	CD1	3.49	
			Tyr	105 H	CE1	3.55	
Glu	72 I	CB	Trp	102 H	NE1	3.82	
			Trp	102 H	CE2	3.31	
			Trp	102 H	CD2	3.33	
			Trp	102 H	CE3	3.61	
			Trp	102 H	CZ3	3.86	
			Trp	102 H	CH2	3.82	
			Trp	102 H	CZ2	3.57	
			Trp	102 H	CG	3.89	
Glu	72 I	CG	Trp	102 H	NE1	3.75	
			Trp	102 H	CE2	3.73	
Glu	72 I	CD	Trp	102 H	N	3.51	
Glu	72 I	OE1	Gly	101 H	CA	3.65	
			Gly	101 H	C	3.61	
			Trp	102 H	N	2.68	***
			Trp	102 H	CA	3.53	
Glu	72 I	OE2	Gly	101 H	CA	3.89	
Glu	72 I	C	Trp	102 H	CZ3	3.91	
Glu	72 I	O	Trp	102 H	CZ3	3.78	
Trp	73 I	CG	Trp	102 H	CE3	3.94	
Trp	73 I	CD1	Trp	102 H	CE3	3.95	
			Trp	102 H	CA	3.79	
			Trp	102 H	C	3.63	
			Trp	102 H	O	3.31	
Trp	73 I	NE1	Trp	102 H	CE3	3.72	
			Trp	102 H	CA	3.98	
			Trp	102 H	CB	3.75	
			Trp	102 H	C	3.82	
			Trp	102 H	O	3.15	***
Trp	73 I	CE2	Trp	102 H	CE3	3.49	
Trp	73 I	CD2	Trp	102 H	CE3	3.67	
			Trp	102 H	CZ3	3.94	
Trp	73 I	CE3	Trp	102 H	CZ3	3.97	
Trp	73 I	CZ2	Trp	102 H	CE3	3.95	
Lys	117 I	CD	Ser	31 L	OG	3.81	
			Asp	50 L	OD1	3.65	

IL-21			Fab35			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Asp	50 L	OD2	3.81	
Lys	117 I	CE	Asp	50 L	OD1	3.92	
			Asp	50 L	OD2	3.34	
Lys	117 I	NZ	Ser	31 L	CB	3.94	
			Ser	31 L	OG	3.22	***
			Asp	50 L	CG	3.54	
			Asp	50 L	OD1	3.44	*
			Asp	50 L	OD2	2.84	***
Lys	117 I	O	Trp	102 H	C	3.94	
			Gly	103 H	N	3.73	*
			Gly	103 H	CA	3.60	
His	118 I	CA	Tyr	105 H	OH	3.82	
His	118 I	C	Tyr	105 H	OH	3.46	
His	118 I	O	Tyr	105 H	OH	3.73	*
Arg	119 I	N	Tyr	105 H	OH	3.56	*
Arg	119 I	CG	Tyr	105 H	OH	3.87	
Arg	119 I	CD	Phe	91 L	O	3.46	
			Asn	92 L	C	3.96	
			Asn	92 L	O	3.28	
Arg	119 I	NH2	Tyr	94 L	CE1	3.92	
Leu	143 I	CG	Trp	102 H	CH2	3.62	
			Trp	102 H	CZ2	3.79	
Leu	143 I	CD1	Trp	102 H	CZ2	3.77	
Leu	143 I	O	Trp	102 H	CH2	3.71	
			Trp	102 H	CZ2	3.33	
Lys	146 I	CG	Ser	31 H	OG	3.69	
Lys	146 I	CE	Ser	30 H	O	3.79	
			Ser	53 H	OG	3.62	
Met	147 I	N	Trp	102 H	NE1	3.77	*
			Trp	102 H	CE2	3.77	
			Trp	102 H	CZ2	3.59	
Met	147 I	CA	Trp	102 H	NE1	3.75	
			Trp	102 H	CE2	3.62	
			Trp	102 H	CZ2	3.76	
Met	147 I	CB	Trp	102 H	CE2	3.66	
			Trp	102 H	CD2	3.99	
			Trp	102 H	CZ3	3.89	
			Trp	102 H	CH2	3.58	
			Trp	102 H	CZ2	3.49	
Met	147 I	CG	Trp	102 H	CD2	3.93	
			Trp	102 H	CE3	3.78	
			Trp	102 H	CZ3	3.83	
His	149 I	CB	Ser	31 H	OG	3.99	
His	149 I	CG	Ile	28 H	CG1	4.00	
			Ser	31 H	OG	3.93	
His	149 I	ND1	Ile	28 H	CB	3.89	
			Ile	28 H	CG1	3.98	
			Ile	28 H	CG2	3.71	
			Ser	31 H	OG	3.07	***
His	149 I	CE1	Ile	28 H	CG1	3.86	
			Ile	28 H	CG2	3.81	
His	149 I	NE2	Ile	28 H	CG1	3.68	
His	149 I	CD2	Ile	28 H	CG1	3.83	
His	149 I	O	Tyr	32 H	OH	3.60	*

IL-21			Fab35			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
Gln	150 I	CA	Tyr	32 H	OH	3.52	
Gln	150 I	CG	Tyr	32 H	CE1	3.74	
			Tyr	32 H	CZ	3.81	
Gln	150 I	CD	Ser	31 H	O	3.78	
			Tyr	32 H	CD1	3.94	
			Tyr	32 H	CE1	3.95	
			Gly	101 H	N	3.94	
			Trp	102 H	CD1	3.90	
Gln	150 I	OE1	Arg	100 H	CB	3.51	
			Arg	100 H	CG	3.79	
			Arg	100 H	CA	3.52	
			Arg	100 H	C	3.73	
			Gly	101 H	N	2.99	***
			Gly	101 H	CA	3.97	
			Trp	102 H	CD1	3.53	
Gln	150 I	NE2	Ser	31 H	C	3.76	
			Tyr	32 H	CA	3.82	
			Ser	31 H	O	2.70	***
			Tyr	32 H	CG	3.93	
			Tyr	32 H	CD1	3.70	
Gln	150 I	C	Arg	100 H	NH2	3.84	
Gln	150 I	O	Arg	100 H	NE	3.47	*
			Arg	100 H	CZ	3.14	
			Arg	100 H	NH1	3.57	*
			Arg	100 H	NH2	3.19	***
His	151 I	CG	Arg	100 H	NH2	3.70	
His	151 I	CE1	Trp	102 H	CB	3.73	
			Trp	102 H	CG	3.99	
His	151 I	NE2	Arg	100 H	NH2	3.52	*
His	151 I	CD2	Arg	100 H	NH2	3.30	

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15

Example 2

Description and comparison of BS1, BS2, mAb14 and mAb5 epitope

20 Binding sites and epitopes provided in this example are based on three experimental (crystal/X-ray) structures and one homology model. The three crystal structures are:

- (i) the IL-21:IL-21R α complex,
- (ii) the IL-21:Fab35 complex ("Fab35" is the Fab fragment corresponding to mAb14), and
- (iii) the IL-21:Fab9 complex ("Fab9" is the Fab fragment corresponding to mAb5
25 referred to as the 362.78.1.44 antibody disclosed in WO2010/055366).

The crystal structure of IL-21:IL-21R α (PDB, 3TGX) provided the basis for building a model of the ternary IL-21:IL-21R α : γ C complex. The homology model of the IL-21:IL-21R α : γ C complex was built using the IL-21:IL-21R α , IL-2:IL-2RA:IL-2RB: γ C and IL-4:IL-4R: γ C complexes as templates. It should be noted that there may be minor inaccuracies
30 in this model, and that such inaccuracy will affect the accuracy of the prediction of the IL-21 residues belonging to BS2.

Receptor binding sites and epitopes are determined from the experimental and model
35 structures using a 4Å distance cut-off.

IL-21 BS1 residues (SEQ ID NO. 1) determined from the crystal structure of the IL-21:IL21R α complex comprises the following residues:

IL-21 residues in BS1	#
ARG	34
ILE	37
ARG	38
ARG	40
GLN	41
ASP	44
ILE	45
GLN	48
TYR	52
ILE	95
VAL	98
SER	99
LYS	102
ARG	105
LYS	106
PRO	107
PRO	108
SER	109

5

IL-21 BS2 residues determined from the homology model structure of the IL-21:IL21R α : γ C complex comprises the following residues:

IL-21 residues in BS2	#
ARG	40
LYS	50
GLU	65
ASP	66
VAL	67
GLU	129
GLU	135
GLU	138
ARG	139
LYS	141
SER	142
GLN	145
HIS	149

10

IL-21 epitope residues (mAb14) determined from the crystal structure of the IL-21:Fab35 complex (Example 1) comprises the following residues:

IL-21 residues in mAb14 epitope	#
GLU	65
ASP	66
VAL	67
GLU	68
THR	69
ASN	70
GLU	72
TRP	73
LYS	117
HIS	118
ARG	119
LEU	143
LYS	146
MET	147
HIS	149
GLN	150
HIS	151

IL-21 epitope residues (mAb5) determined from the crystal structure of the IL-21:Fab9 complex (unpublished results) comprises the following residues:

IL-21 residues in mAb5 epitope	#
Ile	37
Arg	38
Gln	41
Asp	44
Ile	45
Asp	47
Gln	48
Asn	51
Tyr	52
Asn	92
Arg	94
Ile	95
Asn	97
Val	98
Val	98
Ser	99
Lys	101
Lys	102
Arg	105
Lys	106
Pro	107
Pro	108

BS1, BS2, mAb14 and mAb5 epitope residues are mapped on to the primary sequence of IL-21 in Figure 2. Overlap between the predicted BS2 and the mAb14 epitope is observed for amino acid residues **E65, D66, V67** and **H149**.

5 **Example 3**

Co-binding studies of human IL-21 to anti-IL-21 mAbs and IL-21R α / γ C subunits by Surface Plasmon Resonance (SPR)

10 Binding studies were performed on a Biacore T100 instrument that measures molecular interactions in real time through surface plasmon resonance. Experiments were run at 25°C. The signal (RU, response units) reported by the Biacore is directly correlated to the mass on the individual sensor chip surfaces in four serial flow cells.

15 Anti-IL-21 monoclonal antibodies mAb6, mAb14 and mAb19 were immobilized directly onto flow cells of a CM5 sensor chip according to the manufacturer's instructions. "mAb6" corresponds to an IgG1 version of the clone 362.78.1.44 antibody disclosed in WO2010055366, the Fc region of mAb6 carrying the L234A, L235E, and G237A for reduced Fc receptor binding and A330S and P331S mutations for reduced C1q-mediated complement fixation), i.e mAb6 is the same antibody as mAb5. Only difference between
20 the two antibodies is the mammalian expression host used for mAb production. "mAb19" is the antibody produced by the clone "272.21.1.13.4.2"/ "272.21.1.3.4.2" disclosed in WO2007111714. The final immobilization level of antibody was approximately 500-800 RU in one experiment. Capture of IL-21 was conducted by diluting the protein to 100 nM into running buffer (10 mM Hepes, 0.15 M NaCl, 3mM EDTA, 0.05% surfactant P20, pH
25 7.4) and injected at 30 μ l/min for 120s in flow cell 2, creating a reference surface in flow cell 1 with only respective anti-IL-21 antibody immobilized. This typically resulted in final capture levels of IL-21 of approximately 40 to 140 RU. Binding of the extra cellular domains of hIL-21R α , hIL21R α -ECD or γ C-ECD was conducted by injecting analyte over all flow cells to allow for comparative analyses of binding to IL-21 captured by different
30 anti-IL21 antibodies relative to binding to the reference flow cell. IL-21R α -ECD or γ C-ECD protein was diluted serially 1:2 to 0.3-10 or 625 nM-10 μ M into running buffer, injected at 30 μ l/min for 120 s and allowed to dissociate for 300 s. The CM5 surface was regenerated after each injection cycle of analyte via two 8s injections of 1M Formic acid at 30 μ l/min. This regeneration step removed the IL-21 and any bound hIL-21R α -ECD or
35 γ C-ECD chain from the immobilized capture antibody surface, and allowed for the

subsequent binding of the next interaction sample pair. The regeneration procedure did not remove the directly immobilized anti-IL-21 capture antibody from the chip surface.

Data analysis was performed using the Biacore T100 evaluation software 2.0.3. No significant non-specific binding to the reference control surface was observed. Binding curves were processed by double referencing (subtraction of reference surface signals as well as blank buffer injections over captured IL-21). This allowed correction for instrument noise, bulk shift and drift during sample injections.

IL-21 captured by immobilized mAb6 was not able to simultaneously interact with hIL-21R α -ECD, demonstrating that this antibody bind in or close to BS1 on IL-21 and thus compete for binding of the hIL-21R α receptor subunit to this site. In contrast, IL-21 captured by mAb14 could form a stable complex with IL-21R α -ECD demonstrating that mAb14 does not compete for binding of the receptor subunit to BS1 and thus bind to a separate epitope on IL-21.

The same competition study was performed with mAb14 and mAb6 together with γ C-ECD. IL-21 captured by immobilized mAb14 was not able to simultaneously interact with γ C-ECD, demonstrating that this antibody binds in or close to BS2 on IL-21 and thus compete for binding of the γ C receptor subunit to this site. In contrast, IL-21 captured by mAb6 could bind weakly to γ C-ECD demonstrating that mAb6 does not compete for binding of the receptor subunit to BS2 and thus bind to a separate epitope on IL-21. IL-21 captured by mAb19 was not able to bind simultaneously to neither IL-21R α -ECD nor γ C-ECD but the mechanism for this is not clear.

Table 3 Ability of different antibodies to bind simultaneously to (+) or to compete with (-) binding of different receptor subunits to IL-21.

IL-21 captured by mAb	hIL21R α	γ C
mAb6	-	+
mAb14	+	-
mAb19	-	-

The SPR binding competition studies clearly demonstrate that mAb6 and mAb14 interfere with the binding of the different receptor subunits of the IL-21 receptor complex to their respective binding sites on IL-21 and that these antibodies thus operate by

separate mechanisms. Further mAb/IL-21/IL-21 receptor studies are described in Example 16.

Example 4

5

Study of interaction kinetics for anti-IL-21 antibody mAb37 to IL-21 by Surface Plasmon Resonance (SPR)

10 Binding studies were performed on a Biacore T200 instrument that measures molecular interactions in real time through surface plasmon resonance. Experiments were run at 25°C and the samples were stored at 10 °C in the sample compartment. The signal (RU, response units) reported by the Biacore is directly correlated to the mass on the individual sensor chip surfaces in four serial flow cells.

15 Anti-human Fc monoclonal antibody from Biacore human Fc capture kit was immobilized onto flow cells of a CM4 sensor chip according to the manufacturer's instructions. The final immobilization level of capture antibody was approximately 2,000 RU in one experiment. Kinetic studies were performed with a variant of mAb14, mAb37 containing a single point mutation, S241P (numbering according to Kabat) in the IgG4 hinge region,
20 which prevents formation of half antibodies, but does not affect binding to the antigen. Capture of the human anti-IL-21 antibody mAb37 was conducted by diluting the antibody to 0.1 µg/ml into running buffer (10 mM Hepes 0.3 M NaCl, 5 mM CaCl₂, 0.05% surfactant P20, pH 8.0 containing 1 mg/ml BSA) and injected at 10 µl/min for 180s in one of flow cells 2-4, creating a reference surface in flow cell 1 with only anti-Fc antibody
25 immobilized. This typically resulted in final capture levels of test antibodies of approximately 30-50 RU and R_{max} values of analyte of 6-8 RU. Binding of IL-21 protein was conducted by injecting analyte over all flow cells to allow for comparative analyses of binding to different captured anti-IL-21 antibodies relative to binding to the reference flow cell. IL-21 protein was diluted serially 1:3 to 0.2-54 nM into running buffer, injected at 100
30 µl/min for 210 s and allowed to dissociate for 600 or 14000 s. The CM4 surface was regenerated after each injection cycle of analyte via two injections of 3M MgCl₂ at 50µl/min. This regeneration step removed the anti-IL-21 antibody and any bound IL-21 from the immobilized capture antibody surface, and allowed for the subsequent binding of the next interaction sample pair. The regeneration procedure did not remove the
35 directly immobilized anti-Fc capture antibody from the chip surface.

In order to obtain kinetic data, such as k_a (association rate), k_d (dissociation rate) and K_D (equilibrium dissociation constant), data analysis was performed using the Biacore T200 evaluation software 1.0, fitting data to 1:1 Langmuir model. No significant non-specific binding to the reference control surface was observed. Binding curves were processed by double referencing (subtraction of reference surface signals as well as blank buffer injections over captured anti-IL-21 antibodies). This allowed correction for instrument noise, bulk shift and drift during sample injections.

Human IL-21 dissociates from mAb37 with an off-rate less than what can be accurately measured by the currently used assay ($k_d < 1E-5 \text{ s}^{-1}$), an average $k_a 6E +5 \text{ (Ms)}^{-1}$ resulting in a K_D of $< 20 \text{ pM}$. Results are based on triplicate measurements. Individual relative standard errors of parameters k_a and k_d were $< 0.6 \%$. These data clearly demonstrates that mAb37 bind to human IL-21 with high affinity.

Table 4 Results from triplicate measurements of binding constants k_a (association rate), k_d (dissociation rate) and K_D (equilibrium dissociation constant) for the interaction of human IL-21 to mAb37 and mAb19.

Antibody	K_a (1/Ms)	k_d (1/s)	K_D (M)	RSE k_a (%)	RSE k_d (%)
mAb37	6.4E+05	4.5E-06	7.0E-12	0.5	0.5
mAb37	6.0E+05	4.5E-06	7.5E-12	0.4	0.4
mAb37	6.0E+05	6.4E-06	1.1E-11	0.3	0.5

Antibody	k_a (1/Ms)	k_d (1/s)	K_D (M)	RSE k_a (%)	RSE k_d (%)
mAb19	2.00E+06	1.25E-05	6.26E-12	0.2	0.3
mAb19	1.68E+06	1.03E-05	6.12E-12	0.4	0.3
mAb19	1.93E+06	1.01E-05	5.22E-12	0.4	0.3

Example 5

B cell proliferation and maturation assays

To test the effect of the anti-IL-21 antibodies in a biologically relevant setting three functional assays were established where relevant IL-21 biology was studied in primary human cells.

Stimulation with a combination of Anti-CD40 antibody and recombinant IL-21 induces proliferation of primary B cells and B cell maturation as measured by the frequency of plasma blasts with a $CD19^+CD27^{\text{high}}CD38^{\text{high}}$ phenotype. The Anti-IL-21 antibody(ies) were able to prevent both proliferation and maturation.

The relevance of B cells to chronic inflammatory disease has been described in the literature as well as by the clinical effect of B-cell depletion with Rituximab in e.g. rheumatoid arthritis. In the literature, B cells were shown to play an important role in driving chronic inflammation (Dörner T et al (2009) Arthritis Res. Therapy), both as antigen presenting cells as well as producers of (auto)antibodies. IL-21 induces B cell proliferation (when combined with CD40 co-stimulation), immunoglobulin (Ig) class switching to particular IgG1 and IgG3, and differentiation of activated B cells to Ig-producing plasma cells (Ozaki, K. et al., Science, 2002; Ettinger R. J. et al., J Immunol, 2005; Kuchen, S., et al., J Immunol, 2007; Ettinger, R. et al., Immunol Rev, 2008; Leonard, W. J. et al.. Nat. Rev. Immunol. 2005). Neutralization of IL-21 activity is therefore expected to reduce B cell differentiation and thus potentially decrease B cell immune-stimulating properties and autoantibody production in autoimmune patients.

Blood bags were obtained from healthy human volunteers and PBMCs were isolated from 50 ml of heparinised peripheral blood by Ficoll-Paque™ Plus (GE Healthcare) gradient centrifugation. Blood was diluted to 100 ml in phosphate-buffered saline (PBS) at room temperature and 35 ml aliquots were distributed into 50 ml conical tubes carefully overlaying 14 ml of Ficoll-Paque™ Plus (Ge Healthcare) at room temperature. The tubes were spun for 25 minutes at 1680 rpm (600 x g) at room temperature without brake. The PBMC interface layer was removed carefully and washed twice with PBS containing 2% FCS. B cells were isolated by negative selection using EasySep human B Cell enrichment Kit (StemCell Technologies SERL, Grenoble, France). A small sample of the purified B cells was tested for purity by FACS analysis and found to be > 95-97% pure in all experiments.

B cells were cultured in RPMI-1640 media (Invitrogen) supplemented with heat inactivated foetal calf serum (FCS) (Gibco) or Healthy human serum (HS) (Sigma), and Penicillin/Streptomycin (Gibco). Purified human B cells were plated at 50,000 cells/well in a 96-well U-bottom tissue culture plate (BD Biosciences). The cells were treated with or without 0.1 µg/ml anti-CD40 (goat anti-human CD40 polyclonal; R&D Systems), plus a titration of recombinant human IL-21 (Novo Nordisk A/S) prepared as a 1:3 serial dilution. The plate of cells was then incubated for 3 days at 37 °C and 5% CO₂ in a humidified incubator. After three days, the cells were pulsed with 1 µCi/well of [³H]-Thymidine (Perkin Elmer Life Sciences). After 16 hours, the cells were harvested onto UniFilter-96 GF/C filter plates (Packard, Perkin Elmer) and the amount of [³H]-Thymidine

incorporation was quantitated using a TopCount NXT (Perkin Elmer Life Sciences). The effective concentration of IL-21 required for induction of 50% and 90% maximum proliferation (EC_{50} and EC_{90} , respectively) were calculated using the GraphPad Prism v5.0 software (GraphPad Inc) and the sigmoidal dose-response (variable slope) equation.

The two anti-IL-21 antibodies mAb14 and mAb37 were tested and compared for their ability to neutralise recombinant human IL-21 in the B cell proliferation assay.

Human B cells were isolated from 2 individual donors. The B cells were plated at 50,000 cells per well in a 96-well U-bottom tissue culture plate. The cells were treated with 0.1 μ g/ml anti-CD40 (R&D Systems), 50 ng/ml (3.21 nM) recombinant human IL-21. The cells were incubated for 3 days at 37 °C and 5% CO₂ in a humidified incubator. The antibodies were 3-fold titrated and after three days, the cells were pulsed with 1 μ Ci/well of [³H]-Thymidine (Perkin Elmer Life Sciences) for the last 20 hours. The cells were harvested onto UniFilter-96 GF/C filter plates (Packard Instruments, Perkin Elmer) and the amount of [³H]-thymidine incorporation was quantified using a TopCount NXT (Perkin Elmer). The inhibitive concentration of each antibody required for reducing proliferation by 50% (IC_{50}) was calculated using the GraphPad Prism v5.0 software (GraphPad Inc.) and the sigmoidal dose-response (variable slope, 4-parameters) equation.

The IC_{50} for both antibodies was determined to be in the low nanomolar range but mAb37 was slightly more efficient in neutralizing IL-21 compared to mAb14, this is most likely due to the increased stability in the mAb37 molecule due the stabilizing S241P hinge mutation.

Table 5
 IC_{50} values for mAb14 and mAb37 in B cell proliferation assay

	Donor 1	Donor 2	Donor 1	Donor 2
	Exp 1	Exp 1	Exp 2	Exp 2
mAb14	0.138	0.142	-	-
mAb37	-	-	0.085	0.067

30

Example 6**Design of antibodies according to the invention**

In order to design mutants of mAb14 which bind to the epitope described herein, the Kabat defined CDR-loops for mAb14 were analysed.

5

CDR-regions in the mAb14 heavy chain and light chain comprise the following residues (CDR-residues) according to SEQ ID NO 7 and 6, respectively:

10 CDR_H1: S31, Y32, S33, M34, N35

CDR_H2: S50, I51, T52, S53, G54, S55, Y56, Y57, I58, H59
Y60, A61, D62, S63, V64, K65, G6615 CDR_H3: E99, R100, G101, W102, G103, Y104, Y105, G106,
M107, D108, V109CDR_L1: R24, A25, S26, Q27, D28, I29, D30, S31, A32, L33,
A34

20 CDR_L2: D50, A51, S52, S53, L54, E55, S56

CDR_L3: Q89, Q90, F91, N92, S93, Y94, P95, Y96, T97

25 The paratope defined using a 4 Å distance cut-off was determined from the crystal structure of the Fab35:IL-21 complex. Fab35 is the Fab fragment corresponding to mAb14. The paratope is determined to comprise the following residues:

In CDR_H1: I28, S30, S31, Y32, S33

30 In CDR_H2: T52, S53, G54, S55, Y56, Y57, H59

In CDR_H3: E99, R100, G101, W102, G103, Y104, Y105

In CDR_L1: S31

35

In CDR_L2: D50

In CDR_L3: F91, N92, Y94

40 Thus, CDR-residues not included in the paratope are the following (in total 38):

In CDR_H1: M34, N35

In CDR_H2: S50, I51, I58, Y60, A61, D62, S63, V64,

K65, G66

In CDR_H3: G106, M107, D108, V109

5 In CDR_L1: R24, A25, S26, Q27, D28, I29, D30, A32, L33, A34

In CDR_L2: A51, S52, S53, L54, E55, S56

In CDR_L3: Q89, Q90, S93, P95, Y96, T97

10

Among the 38 non-paratope CDR-residues 10 were selected as potential mutation sites. The selection was based on inspection of the crystal structure. Extensively buried residues and residues for which the side chains appeared to be involved in several important interactions were deselected. The identified potential mutation sites are listed in Table 6. Specific mutations (Table 6) at these sites were chosen such that no or minimal effect on the protein structure would result.

Table 6 Selected mutation sites and suggested mutations of the mAb14 antibody. Each of the individual mutations shown in this table represents different embodiments of the present invention, i.e. monoclonal antibodies having the ability interfere with binding of γ C to IL-21. Antibodies according to the invention may also comprise two or more of the mutations shown in this table. It follows that variant antibodies according to the invention can only comprise one mutation in a specific position.

20

Residue	CDR-loop	Mutation
A61	H2	A61S
D62	H2	D62E
V64	H2	V64I
K65	H2	K65R
R24	L1	R24K
S26	L1	S26T
Q27	L1	Q27N
D30	L1	D30E
S53	L2	S53T
S56	L2	S56T

25 This example describes one method applicable for designing antibodies according to the invention based on the information contained in the crystal structure of Fab35:IL-21. It follows that several other approaches can be taken in designing ligands according to the invention.

One approach could be e.g. to design a ligand essentially comprising the paratope of mAb14 except that one or more conservative substitutions can be made.

Another approach could be to design an IL-21 ligand based on the structure of the binding interface between IL-21 and γ C. This ligand could be in the form of e.g. an antibody or a γ C variant/mimic that essentially retains the structure of said γ C binding interface.

It follows that one or more of such approaches can be combined.

Autoimmune disorders and other immune related disorders can be treated with e.g. therapeutic human monoclonal antibodies. However, said monoclonal antibodies may be immunogenic and give rise to the formation of anti-antibodies, also referred to as HAHA (human anti-human antibodies). It is conceivable that HAHA bind to areas of the therapeutic antibodies that will affect the binding of the therapeutic antibody to its antigen, i.e. the HAHA is a neutralizing antibody. If such potentially immunogenic sites, leading to development of anti-antibodies against mAb14, are recognized and characterized, the detailed description of the paratope for the antibody mAb14 derived from the 3-dimensional structure of the Fab35:IL-21 complex provides a possibility for rationally designing variants of mAb14 that will retain high-affinity binding to IL-21, but potentially are less immunogenic. Alternatively, variants of mAb14 may be designed in such a way that unwanted binding to specific anti-antibodies is reduced or prevented. It is thus possible to use the crystal structure information to provide improved versions of mAb14.

The provision of the crystal structure of this Fab fragment as well as its paratope also provides the possibility of e.g. replacing residues therein that could potentially result in antibodies improved with respect to stability, solubility or other chemical or physical properties of a molecule comprising this paratope while maintaining its biological functionality including high-affinity binding to IL-21. Stability can e.g. be improved by reducing aggregation, self association, fragmentation, and disulfide formation/exchange. Other properties, such as viscosity, may also be altered by introduction of one or more mutations.

The provision of the Fab35:IL-21 crystal structure furthermore provides a possibility of providing variants of mAb14 having reduced risk of e.g. deamidation, isomerization and/or oxidation and thereby improving the physical/chemical stability of a molecule

comprising this paratope while maintaining its biological functionality including high-affinity to IL-21.

One example of potential stability improving mutations in the antibody mAb14 is the elimination of potential oxidation sites by mutation of Methionine residues. One specific
5 example of such a mutation is the change of the Methionine in position 83 in the heavy chain (SEQ ID No. 7) to an amino acid with similar properties, e.g. Isoleucine. A second specific example of such a mutation is the change of the Methionine in position 107 in the heavy chain (SEQ ID No. 7) to an amino acid with similar properties, e.g. Isoleucine.

One example of potential stability improving mutations in the antibody mAb14 is
10 elimination of potential hot-spots (DX-motifs, e.g. DG- and DS-motifs) for isomerisation of Aspartate residues. Such potentially labile DX-motifs can be eliminated by appropriate mutation of one or both of the constituent D or X residues. One specific example of such a mutation is the change of the Aspartate (present in a DS motif) in position 62 in the heavy chain (SEQ ID No. 7) to an amino acid with similar properties, e.g. Glutamate. A
15 second specific example of such a mutation is the change of the Aspartate (present in a DS motif) in position 206 in the heavy chain (SEQ ID No. 7) to an amino acid with similar properties, e.g. Glutamate. A third specific example of such a mutation is the change of the Aspartate (present in a DS motif) in position 167 in the light chain (SEQ ID No. 6) to an amino acid with similar properties, e.g. Glutamate. A fourth specific example of such a
20 mutation is the change of the Aspartate (present in a DS motif) in position 170 in the light chain (SEQ ID No. 6) to an amino acid with similar properties, e.g. Glutamate.

One example of potential stability improving mutations in the antibody mAb14 is
25 elimination of potential hot-spots (NX-motifs, e.g. NG- or NS-motifs) for deamidation of Asparagine residues. Such potentially labile NX-motifs can be eliminated by appropriate mutation of one or both of the constituent N or X residues. One specific example of such a mutation is the change of the Asparagine (present in a NS motif) in position 77 in the heavy chain (SEQ ID No. 7) to an amino acid with similar properties, e.g. Glutamine. A
30 second specific example of such a mutation is the change of the Asparagine (present in a NS motif) in position 84 in the heavy chain (SEQ ID No. 7) to an amino acid with similar properties, e.g. Glutamine. A third specific example of such a mutation is the change of the Asparagine (present in a NS motif) in position 158 in the light chain (SEQ ID No. 6) to an amino acid with similar properties, e.g. Glutamine.

Example 7

Epitope mapping by HX-MS of mAb14 and mAb5

Introduction to HX-MS

5 The HX-MS technology exploits that hydrogen exchange (HX) of a protein can readily be followed by mass spectrometry (MS). By replacing the aqueous solvent containing hydrogen with aqueous solvent containing deuterium, incorporation of a deuterium atom at a given site in a protein will give rise to an increase in mass of 1 Da. This mass increase can be monitored as a function of time by mass spectrometry in quenched
10 samples of the exchange reaction. The deuterium labelling information can be sub-localized to regions in the protein by pepsin digestion under quench conditions and following the mass increase of the resulting peptides.

One use of HX-MS is to probe for sites involved in molecular interactions by identifying regions of reduced hydrogen exchange upon protein-protein complex formation. Usually,
15 binding interfaces will be revealed by marked reductions in hydrogen exchange due to steric exclusion of solvent. Protein-protein complex formation may be detected by HX-MS simply by measuring the total amount of deuterium incorporated in either protein members in the presence and absence of the respective binding partner as a function of time. The HX-MS technique uses the native components, ie protein and antibody or Fab
20 fragment, and is performed in solution. Thus HX-MS provides the possibility for mimicking the *in vivo* conditions (for a recent review on the HX-MS technology, see Wales and Engen, Mass Spectrom. Rev. 25, 158 (2006)).

Materials

25 *Protein batches used were:*

hIL-21: human recombinant IL-21 (expressed in *E. coli* as the mature peptide; residues 30-162 of SEQ ID NO: 1 with an added N-terminal Methionine residue). Antibodies were mAb5 and mAb14.

30 All proteins were buffer exchanged into PBS pH 7.4 before experiments.

Methods: HX-MS experiments

Instrumentation and data recording

35 The HX experiments were automated by a Leap robot (H/D-x PAL; Leap Technologies Inc.) operated by the LeapShell software (Leap Technologies Inc.), which performed

initiation of the deuterium exchange reaction, reaction time control, quench reaction, injection onto the UPLC system and digestion time control. The Leap robot was equipped with two temperature controlled stacks maintained at 20 °C for buffer storage and HX reactions and maintained at 2 °C for storage of protein and quench solution, respectively.

5 The Leap robot furthermore contained a cooled Trio VS unit (Leap Technologies Inc.) holding the pre- and analytical columns, and the LC tubing and switching valves at 1 °C. The switching valves of the Trio VS unit have been upgraded from HPLC to Microbore UHPLC switch valves (Cheminert, VICI AG). For the inline pepsin digestion, 100 µL quenched sample containing 200 pmol hIL-21 was loaded and passed over a

10 Poroszyme® Immobilized Pepsin Cartridge (2.1 × 30 mm (Applied Biosystems)) placed at 20 °C using a isocratic flow rate of 200 µL/min (0.1% formic acid:CH₃CN 95:5). The resulting peptides were trapped and desalted on a VanGuard pre-column BEH C18 1.7 µm (2.1 × 5 mm (Waters Inc.)). Subsequently, the valves were switched to place the pre-column inline with the analytical column, UPLC-BEH C18 1.7 µm (2.1 × 100 mm (Waters

15 Inc.)), and the peptides separated using a 9 min gradient of 15-35% B delivered at 200 µl/min from an AQUITY UPLC system (Waters Inc.). The mobile phases consisted of A: 0.1% formic acid and B: 0.1% formic acid in CH₃CN. The ESI MS data, and the separate data dependent MS/MS acquisitions (CID) and elevated energy (MS^E) experiments were acquired in positive ion mode using a Q-TOF Premier MS (Waters Inc.). Leucine-

20 enkephalin was used as the lock mass ([M+H]⁺ ion at *m/z* 556.2771) and data was collected in continuum mode (For further description of the set-up, see Andersen and Faber, Int. J. Mass Spec., 302, 139–148(2011)).

Data analysis

25 Peptic peptides were identified in separate experiments using standard CID MS/MS or MS^E methods (Waters Inc.). MS^E data were processed using BiopharmaLynx 1.2 (version 017). CID data-dependent MS/MS acquisition was analyzed using the MassLynx software and in-house MASCOT database.

HX-MS raw data files were subjected to continuous lock mass-correction. Data analysis, i.e., centroid determination of deuterated peptides and plotting of in-exchange curves, was performed using prototype custom software (HDX browser, Waters Inc.) and HX-Express ((Version Beta); Weis et al., J. Am. Soc. Mass Spectrom. 17, 1700 (2006)). All data were also visually evaluated to ensure only resolved peptide isotopic envelopes were subjected to analysis.

35

Epitope mapping experiment

Amide hydrogen/deuterium exchange (HX) was initiated by a 16-fold dilution of hIL-21 in the presence or absence of mAb5 or mAb14 into the corresponding deuterated buffer (*i.e.* PBS prepared in D₂O, 96% D₂O final, pH 7.4 (uncorrected value)). All HX reactions were carried out at 20°C and contained 4 µM hIL-21 in the absence or presence of 2.4
5 µM mAb thus giving a 1.2 fold molar excess of mAb binding sites. At appropriate time intervals ranging from 10 sec to 10000 sec, 50 µl aliquots of the HX reaction were quenched by 50 µl ice-cold quenching buffer (1.35M TCEP) resulting in a final pH of 2.5 (uncorrected value). Examples of raw data identifying the mAb5 and the mAb14 epitopes are shown in figure 3.

10

Results and Discussion

Epitope Mapping of mAb5 and mAb14

The epitope of mAb5 has previously been mapped (example 2 and fig. 2).

15

The HX time-course of 34 peptides, covering 100% of the primary sequence of hIL-21, were monitored in the absence or presence of mAb5 or mAb14 for 10 to 10000 sec (Figs 1 and 2). Exchange protection observed in the early time-points, *e.g.* <300 sec, relate to surface exposed amide protons and thus also relate to protein interfaces. In contrast,
20 effects observed late in the time course are related to slow exchanging amide hydrogens and thus related to the structural core of the protein. Therefore, epitope effects appear in the early time points whereas structural stabilization effects will manifest as exchange reduction in late time points (Garcia, Pantazatos and Villareal, *Assay and Drug Dev. Tech.* 2, 81 (2004); Mandell, Falick and Komives, *Proc. Natl. Acad. Sci. USA*, 95, 14705
25 (1998)).

Epitope Mapping of mAb14

The observed exchange pattern in the early timepoints (< 300 sec) in the presence or absence of mAb14 can be divided into two different groups: One group of peptides
30 display an exchange pattern that is unaffected by the binding of mAb14. In contrast, another group of peptides in hIL-21 show protection from exchange upon mAb14 binding (Figs 3B, 3D and 4). For example at 30 sec exchange with D₂O, more than 1 amide is protected from exchange in the region V67-F76 upon mAb14 binding (Figs 3B, and 4). The regions displaying protection upon mAb14 binding encompass peptides covering
35 residues V67-F76 and A112-S162 (Figs. 4 and 5). However, by comparing the relative amounts of exchange protection within each peptide upon binding mAb14 and the lack of

epitope effects in several other and smaller peptides in these regions, the epitope can be narrowed to residues V67-S74 and L143-K146. Furthermore, the epitope effects in peptide A112-L127 could arise from two different regions within this long peptide. Of these two, only region R115-L120 is in close proximity in the 3D structure of the other two epitope regions and thus the epitope effects are assigned to this region (Fig. 5).

The mAb5 and the mAb14 epitopes are not overlapping

As can be seen from the examples in figure 5 and the exchange plots in figure 4, the epitopes for mAb5 and mAb14 are completely separated and not overlapping.

10

Example 8

Crystal structures of hIL-21 in complex with CDR-loop mutated Fab fragments of mAb14

The 3-dimensional structures of hIL-21 in complex with four different Fab fragments, Fab56, Fab57, Fab59 and Fab60 were solved and refined to high resolution using X-ray crystallography. The Fabs are all variants of the Fab35 fragment of anti-IL-21 human monoclonal antibody mAb14 and were designed and generated as described in example 6 and 14, respectively. Fab56, Fab57, Fab59 and Fab60 correspond to Fab fragments of mAb61, mAb62, mAb64 and mAb65, respectively. The results demonstrate that Fab56, Fab57, Fab59 and Fab60 share the epitope on hIL-21 with Fab35. Therefore the binding sites of Fab56, Fab57, Fab59 and Fab60 will, as for Fab35, according to comparative studies/modelling, Example 2, compete with, and due to its high binding affinity, block the binding of the γ C receptor chain to hIL-21. Hence, they will inhibit the biological effects mediated by hIL-21 through γ C.

Fab59 form a different crystal packing compared to the other mutants, and Fab35, resulting in an epitope including 4 additional residues, when using a 4.0 Å cut-off in the calculation of the epitope, as compared to the other mutants.

The epitopes described were characterized using the 3-dimensional structure of the complexes between Fab56, Fab57, Fab59 or Fab60 and hIL-21, respectively. The conclusions regarding the epitopes of Fab56, Fab57, Fab59 or Fab60 on hIL-21 will, moreover, also apply to the interaction between hIL-21 and the full antibody, mAb14, from which Fab56, Fab57, Fab59 or Fab60, via Fab35, were derived.

30

Materials and Methods

IL-21 (expressed in *E. coli* as the mature peptide; residues 30-162 of SEQ ID NO: 1 with an added N-terminal Methionine residue), in PBS buffer, pH 7.4 (4 tablets in 2 liter of water, GIBCO Cat. No. 18912-014 Invitrogen Corporation), and anti-IL-21 Fabs (comprising light chains and heavy chains corresponding to WT or mutants of SEQ ID No.9 and 10, respectively, see example 6 and 14) formulated in PBS buffer, pH 7.4, were mixed in a 1:1 molar ratio. The final concentrations of the complexes are shown in Table 7. Crystals were grown with the sitting drop-technique with volumes according to Table 7. Total drop sizes were 0.2 or 0.3 μ l, depending on the mixing ratio. Crystals were prepared for cryo-freezing by transferring of 3 μ l of a cryo-solution, containing 75 % of the precipitant solution and 25 % glycerol, to the drop containing the crystal. Soakings were allowed for about one minute. The crystals were then fished into a MiTeGen MicroLoop™, flash frozen in liquid N₂ and kept at a temperature of 100 K during data collection by a cryogenic N₂ gas stream. Crystallographic data were collected at beam-line BL911-3 (Ursby et al., 2004) at MAX-lab, Lund, Sweden, to resolutions indicated in Table 8. Space group determination, integration and scaling of the data were made with the XDS software package (Kabsch, 2010). A summary of obtained cell parameters, space groups, resolutions, R-sym and completeness are shown in Table 8. For the crystal complexes between hIL-21 and Fab56, Fab57 or Fab60, respectively, the Fab35/hIL-21 crystal structure were used as starting models for rigid body refinements in the Refmac5 software (Murshudov et al., 2011) of the CCP4 crystallography software suite (Bailey, 1994). Rigid body refinements were then followed by restrained crystallographic refinements, using the software programs Refmac5 and by computer graphics inspection of the electron density maps, model corrections and building using the Coot software program (Emsley et al., 2010). The procedure was cycled until no further significant improvements could be made to the model. Table 10,11 and 13.

Table 7. Summary of protein samples and conditions used for crystallizations of the different mutant-Fab/hIL-21 complexes. Mut: chain name H (heavy chain), L (Light chain) and amino acid mutation relative to the corresponding WT light or heavy chain reference (ref) sequence from Fab35.

Protein	Ref SEQ ID NO	Mut	Concentration [mg/ml]		Crystallization Precipitant Solution	Protein:precipitant solution mix [nL]
			Before Mix	1:1 Complex		
Soluble hIL-21	Residues 30-162 of SEQ ID No. 1	-	10.5			
Fab56	10	H-D62E	3.5	4.4	20 % PEG4000 200 mM Sodium Chloride	200:100
Fab57	10	H-K65R	7.6	8.6	30 % PEG 1000, 20 mM Diammonium tartrate	100:100
Fab59	9	L-Q27N	9.4	9.7	30 % PEG 1000 150 mM Sodium Chloride	200:100
Fab60	9	L-D30E	9.0	9.3	20 % PEG 4000, 20 mM Calcium Acetate	200:100

5

Table 8. Some crystallographic data and model statistics for the different mutant-Fab/hIL-21 complexes. Fab35 data (From Example 1) are added for comparison.

Fab complex with hIL-21	a [Å]	b [Å]	c [Å]	β [°]	Space group	Resol [Å]	R-sym [%]	Compl [†] [%]	R [‡] [%]	R-free [‡] [%]	RMSD [§] ideal bond-lengths [Å]	SSM [§] to Fab35	
												RMSD [§] [Å]	# Resid [‡]
Fab35	89.4	65.2	106.7	111.6	C2	1.64	6.4	98.2	17.9	21.1	0.024	-	
Fab56	89.4	65.2	106.9	111.7	C2	1.65	2.8	99.2	17.7	21.4	0.022	0.144	548
Fab57	89.7	65.1	107.1	111.6	C2	1.63	2.5	98.7	17.2	20.6	0.024	0.144	548
Fab59	86.5	65.6	106.7	113.8	C2	1.65	3.1	97.6	16.7	20.7	0.019	0.557	533
Fab60	89.4	65.0	106.7	111.4	C2	1.75	3.7	99.3	17.1	21.5	0.020	0.120	548

^{*)}To the specified resolution observed diffraction data completeness according to XSCALE (Kabsch, 2010)

10 ^{†)} $R_{\text{sym}} = \frac{\sum_i |I(h,i) - \langle I(h) \rangle|}{\sum_i I(h,i)}$, where $I(h,i)$ is the intensity of the i th measurement of h and $\langle I(h) \rangle$ is the corresponding average value of all i measurements.

^{‡, §)} $R = \frac{\sum_h | |F(h)_o| - |F(h)_c| |}{\sum_h |F(h)_o|}$, where $F(h)_c$ is the calculated structure factor of reflection h , R_{free} is equivalent to R_{cryst} , but calculated for randomly chosen 5% of reflections that were omitted from the refinement process.

15 ^{§)}Root-mean-square deviation

^{§)}Secondary Structure Matching (Krissinel & Henrick, 2004)

^{‡)}Number of amino acid residues used during structure superimpositioning

20 For the crystal complex between hIL-21 and Fab59 the complex Fab35/hIL-21 crystal structure was used as starting model for structure determination using molecular replacement technique by the Molrep software (Vagin & Teplyakov, 1997) of the CCP4 software suit. It was followed by restrained refinements using the software program Refmac5 and by computer graphics inspection of the model and electron density maps,

using the Coot software program (Emsley, Lohkamp, Scott, & Cowtan, 2010). The model needed modifications to the N-terminal part of helix A and to part of the loop-structure between helix C and D. The software ARP/wARP (Perrakis et al., 1999) was used for an initial round of automated model building which was followed by crystallographic refinements, again using the software programs Refmac5 and the Coot software for computer graphic inspections of the electron density maps, model corrections and building. The procedure was cycled until no further significant improvements could be made to the model. The model was then subject to twin-refinement (using the twin-law h, -k, -h-l) in Phenix.Refine (Afonine et al., 2005) of the Phenix software package (Adams et al., 2010). The twin fraction was refined to 0.03 and the resulting R and R-free were 0.166 and 0.201, respectively. Finally the structure was transferred to the CCP4 software system again where a final round of restrained refinements were carried out in Refmac5 followed by structure interpretations, Table 12.

Final R- and R-free, root-mean-square deviation (RMSD) from ideal bond lengths and Secondary Structure Matching (Krissinel & Henrick, 2004) results for the superimpositions of Fab35-hIL-21 onto each of the Fab56-, Fab57-, Fab59- and Fab60-hIL-21 complexes, respectively, are shown in Table 8.

Results

The results demonstrate that Fab56, Fab57, Fab59 and Fab60 share the epitope on hIL-21 with Fab35. The Fab59/hIL-21 structure shows a minor difference in inter-molecular interactions within the crystal (crystal packing) compared to the other Fab variants though. The reason for the difference in crystal packing is that the Fab light chain Gln 27 residue is involved in crystal packing (forming a hydrogen bond to Asp 44 of a symmetry related hIL-21 molecule) in the Fab35, Fab56, Fab57 and Fab60 crystals while that residue is mutated to Asn in Fab59 and cannot form the same inter-molecular contacts (crystal packing interactions) as the other variants, but a slightly different type. The difference results in a closer packing for two symmetry related Fab/hIL21-complex molecules in Fab59 relatively to the equivalent symmetry related packing in Fab35. The distance between the two complexes is reduced about 2.3 Å for Fab59/hIL-21 relative to Fab39/hIL-21 (calculated as the distances between the first axis of the principal moment of inertia for the two systems) and the average areas excluded in pairwise interactions increase from 738 Å² for the Fab35/hIL-21 crystal to 967 Å² in the Fab59/hIL-21 crystal, respectively (calculated by the software program Areaimol (Lee & Richards, 1971, Saff & Kuijlaars, 1997)). That, locally, tighter crystals packing of the Fab59/hIL-21 crystals result in that the missing residues of the loop between helices C and D of hIL-21, unobserved

in the Fab35/hIL-21 crystal, forms a stable conformation in the Fab59/hIL-21 crystal and are clearly seen in the electron density maps. Moreover the conformation of part of the loop between the hIL-21 helices C and D is, by the symmetry related molecule which is closer in Fab59/hIL21, driven in the direction towards helix A of hIL-21. This force the first
5 part of helix A in hIL-21 to become unstructured and not seen in the electron density maps in the Fab59/hIL-21 complex. Moreover, the ordering and movement of residues 105 to 119 in the loop between helices C and D of hIL-21 make 4 additional residues of hIL-21 (Phe 76, Ala 112, Gly 113, and Gln 116: SEQ ID NO. 1) fall within a 4 Å distance cut-off from the heavy chain of Fab59 as compared to the Fab56, Fab57, Fab60 and
10 Fab35 hIL-21 complexes (See Figure 6). The hIL-21 binding properties of Fab59 are, however, not different from the other Fab-variants. The binding sites of Fab56, Fab57, Fab59 and Fab60 will all, as for Fab35, instead of competing with the private hIL-21 receptor chain (IL-21R α), according to comparative studies/modelling, Example 2, compete with, and due to its high binding affinity, block the binding of the γ C receptor
15 chain to hIL-21. Hence, it will inhibit the biological effects mediated by hIL-21 through γ C.

Table 9 show the calculated (by the software Areaimol (Lee & Richards, 1971, Saff & Kuijlaars, 1997)), average areas excluded in pair-wise interactions for the hIL-21/Fab56, hIL-21/Fab57, hIL-21/Fab59 and hIL-21/Fab60 complexes, respectively. Corresponding calculations for the Fab35/hIL-21 crystal complex show a very similar value (see
20 Example 1), included in the table.

The direct contacts between the hIL-21 and Fab56, Fab57, Fab59 or Fab60, respectively, were identified by running the Contacts software of the CCP4 program suite (Bailey, 1994) using a cut-off distance of 4.0 and 5.0 Å between the anti-IL-21 Fab and the hIL-21 molecules. The results from the hIL-21/Fab56, hIL-21/Fab57, hIL-21/Fab59,
25 hIL-21/Fab60 complex crystal structure are shown in Tables 14, 15, 16 and 17, respectively. The resulting hIL-21 epitopes for Fab56, Fab57, Fab59 and Fab60 were found to comprise the residues of hIL-21 (SEQ ID No. 1) as shown in Table 9 and Figure 6. Those epitopes agrees very well with the hIL-21 epitope of Fab35, from Example 1, included in Table 9 and Figure 7.

30

Table 9. Epitopes and paratopes for the different Fab fragments (Fab56, Fab57, Fab59 and Fab60) using a 4.0 Å distance cut-off between hIL-21 and each of the Fab fragments. The calculated average areas excluded in pair-wise interactions between hIL-21 and each of the Fab fragments are also shown. The Seq ID No. for WT light / heavy chain reference (ref) sequence from Fab35 are listed and mutation (Mut) as also listed in Table 7.

Antibody fragment complex	Ref. SEQ ID No. including mutations	Epitope to shIL-21 (4.0 Å cut-off) SEQ ID No.1	Paratope		Avr Area [#] [Å ²]
			Heavy chain	Light chain	
Fab35/hIL-21 (From Example 1)	SEQ ID No. 9 LC/10 HC	Glu 65, Asp 66, Val 67, Glu 68, Thr 69, Asn 70, Glu 72, Trp 73, Lys 117, His 118, Arg 119, Leu 143, Lys 146, Met 147, His 149, Gln 150, His 151	Ile 28, Ser 30, Ser 31, Tyr 32, Ser 33, Thr 52, Ser 53, Gly 54, Ser 55, Tyr 56, Tyr 57, His 59, Glu 99, Arg 100, Gly 101, Trp 102, Gly 103, Tyr 104, Tyr 105	Ser 31, Asp 50, Phe 91, Asn 92, Tyr 94	1061
Fab56/hIL-21	SEQ ID No. 9 LC/10 H D62E	Glu 65, Asp 66, Val 67, Glu 68, Thr 69, Asn 70, Glu 72, Trp 73, Lys 117, His 118, Arg 119, Leu 143, Lys 146, Met 147, His 149, Gln 150, His 151	Ile 28, Ser 30, Ser 31, Tyr 32, Ser 33, Thr 52, Ser 53, Gly 54, Ser 55, Tyr 56, Tyr 57, His 59, Glu 99, Arg 100, Gly 101, Trp 102, Gly 103, Tyr 104, Tyr 105	Ser 31, Asp 50, Phe 91, Asn 92, Tyr 94	1068
Fab57/hIL-21	SEQ ID No. 9 LC/10 H K65R	Glu 65, Asp 66, Val 67, Glu 68, Thr 69, Asn 70, Glu 72, Trp 73, Lys 117, His 118, Arg 119, Leu 143, Lys 146, Met 147, His 149, Gln 150, His 151	Ile 28, Ser 30, Ser 31, Tyr 32, Ser 33, Thr 52, Ser 53, Gly 54, Ser 55, Tyr 56, Tyr 57, His 59, Glu 99, Arg 100, Gly 101, Trp 102, Gly 103, Tyr 104, Tyr 105	Ser 31, Asp 50, Phe 91, Asn 92, Tyr 94	1067
Fab59/hIL-21	SEQ ID No. 9 L Q27N /10 HC	Glu 65, Asp 66, Val 67, Glu 68, Thr 69, Asn 70, Glu 72, Trp 73, Phe 76, Ala 112, Gly 113, Gln 116, Lys 117, His 118, Arg 119, Leu 143, Lys 146,	Ile 28, Ser 31, Tyr 32, Ser 33, Thr 52, Ser 53, Gly 54, Ser 55, Tyr 56, Tyr 57, His 59, Glu 99, Arg 100, Gly 101, Trp 102,	Asp 30, Ser 31, Asp 50, Phe 91, Asn 92, Tyr 94	1169

Antibody fragment complex	Ref. SEQ ID No. including mutations	Epitope to hIL-21 (4.0 Å cut-off) SEQ ID No.1	Paratope		Avr Area# [Å ²]
			Heavy chain	Light chain	
		Met 147, His 149, Gln 150, His 151	Gly 103, Tyr 104, Tyr 105		
Fab60/hIL-21	SEQ ID No. 9 L D30E /10 HC	Glu 65, Asp 66, Val 67, Glu 68, Thr 69, Asn 70, Glu 72, Trp 73, Lys 117, His 118, Arg 119, Leu 143, Lys 146, Met 147, His 149, Gln 150, His 151	Ile 28, Ser 30, Ser 31, Tyr 32, Ser 33, Thr 52, Ser 53, Gly 54, Ser 55, Tyr 56, Tyr 57, His 59, Glu 99, Arg 100, Gly 101, Trp 102, Gly 103, Tyr 104, Tyr 105	Ser 31, Asp 50, Phe 91, Asn 92, Tyr 94	1103

#) Average areas excluded in pairwise interactions

Thus, the Fab56/Fab57/Fab59/Fab60 hIL-21 epitopes comprise residues (SEQ ID No. 1) in the N-terminal part of helix B, residue 72-76, and residues in the C-terminal part of helix D, residues 143-151. Additionally, several contact residues are identified in the loop segment proceeding helix B, residues 65-70, and in the loop between helix C and helix D, residues 112-119, Figure 7. These contact areas agrees well with what has been determined as the binding site for γ C, Example 2.

The Fab56, Fab57, Fab59 and Fab60 paratopes for hIL-21 are shown in Table 9. The hIL-21 paratopes, and the residues involved in hydrogen-binding, are also indicated in Tables 14, 15, 16, and 17.

Table 10. Results from the X-ray model refinement to the observed data of the hIL-21/Fab56 complex by the software program Refmac5 (Murshudov, Skubak, Lebedev, Pannu, Steiner, Nicholls, Winn, Long, & Vagin, 2011) of the CCP4 program software package (Bailey, 1994).

```

REMARK 3 REFINEMENT.
REMARK 3 PROGRAM : REFMAC 5.6.0119
REMARK 3 AUTHORS : MURSHUDOV,VAGIN,DODSON
REMARK 3
20 REMARK 3 REFINEMENT TARGET : MAXIMUM LIKELIHOOD
REMARK 3
REMARK 3 DATA USED IN REFINEMENT.
REMARK 3 RESOLUTION RANGE HIGH (ANGSTROMS) : 1.65
REMARK 3 RESOLUTION RANGE LOW (ANGSTROMS) : 99.39
25 REMARK 3 DATA CUTOFF (SIGMA(F)) : NONE
REMARK 3 COMPLETENESS FOR RANGE (%) : 99.29
REMARK 3 NUMBER OF REFLECTIONS : 64936
REMARK 3
REMARK 3 FIT TO DATA USED IN REFINEMENT.
30 REMARK 3 CROSS-VALIDATION METHOD : THROUGHOUT
REMARK 3 FREE R VALUE TEST SET SELECTION : RANDOM
REMARK 3 R VALUE (WORKING + TEST SET) : 0.17902

```

REMARK 3 R VALUE (WORKING SET) : 0.17716
 REMARK 3 FREE R VALUE : 0.21406
 REMARK 3 FREE R VALUE TEST SET SIZE (%) : 5.1
 REMARK 3 FREE R VALUE TEST SET COUNT : 3463
 5 REMARK 3
 REMARK 3 FIT IN THE HIGHEST RESOLUTION BIN.
 REMARK 3 TOTAL NUMBER OF BINS USED : 20
 REMARK 3 BIN RESOLUTION RANGE HIGH : 1.650
 10 REMARK 3 BIN RESOLUTION RANGE LOW : 1.693
 REMARK 3 REFLECTION IN BIN (WORKING SET) : 4541
 REMARK 3 BIN COMPLETENESS (WORKING+TEST) (%) : 96.05
 REMARK 3 BIN R VALUE (WORKING SET) : 0.290
 REMARK 3 BIN FREE R VALUE SET COUNT : 247
 15 REMARK 3 BIN FREE R VALUE : 0.316
 REMARK 3
 REMARK 3 NUMBER OF NON-HYDROGEN ATOMS USED IN REFINEMENT.
 REMARK 3 ALL ATOMS : 4831
 REMARK 3
 20 REMARK 3 B VALUES.
 REMARK 3 FROM WILSON PLOT (A**2) : NULL
 REMARK 3 MEAN B VALUE (OVERALL, A**2) : 28.072
 REMARK 3 OVERALL ANISOTROPIC B VALUE.
 25 REMARK 3 B11 (A**2) : 0.52
 REMARK 3 B22 (A**2) : 0.13
 REMARK 3 B33 (A**2) : -0.11
 REMARK 3 B12 (A**2) : 0.00
 REMARK 3 B13 (A**2) : 0.73
 REMARK 3 B23 (A**2) : 0.00
 30 REMARK 3
 REMARK 3 ESTIMATED OVERALL COORDINATE ERROR.
 REMARK 3 ESU BASED ON R VALUE (A) : 0.097
 REMARK 3 ESU BASED ON FREE R VALUE (A) : 0.098
 REMARK 3 ESU BASED ON MAXIMUM LIKELIHOOD (A) : 0.070
 35 REMARK 3 ESU FOR B VALUES BASED ON MAXIMUM LIKELIHOOD (A**2) : 4.166
 REMARK 3
 REMARK 3 CORRELATION COEFFICIENTS.
 REMARK 3 CORRELATION COEFFICIENT FO-FC : 0.967
 REMARK 3 CORRELATION COEFFICIENT FO-FC FREE : 0.950
 40 REMARK 3
 REMARK 3 RMS DEVIATIONS FROM IDEAL VALUES COUNT RMS WEIGHT
 REMARK 3 BOND LENGTHS REFINED ATOMS (A) : 4470 ; 0.022 ; 0.020
 REMARK 3 BOND ANGLES REFINED ATOMS (DEGREES) : 6091 ; 2.181 ; 1.958
 REMARK 3 TORSION ANGLES, PERIOD 1 (DEGREES) : 587 ; 6.416 ; 5.000
 45 REMARK 3 TORSION ANGLES, PERIOD 2 (DEGREES) : 187 ; 37.025 ; 24.064
 REMARK 3 TORSION ANGLES, PERIOD 3 (DEGREES) : 771 ; 15.116 ; 15.000
 REMARK 3 TORSION ANGLES, PERIOD 4 (DEGREES) : 25 ; 18.592 ; 15.000
 REMARK 3 CHIRAL-CENTER RESTRAINTS (A**3) : 681 ; 0.166 ; 0.200
 REMARK 3 GENERAL PLANES REFINED ATOMS (A) : 3365 ; 0.013 ; 0.021
 50 REMARK 3
 REMARK 3 ISOTROPIC THERMAL FACTOR RESTRAINTS. COUNT RMS WEIGHT
 REMARK 3
 REMARK 3 NCS RESTRAINTS STATISTICS
 REMARK 3 NUMBER OF NCS GROUPS : NULL
 55 REMARK 3
 REMARK 3 TWIN DETAILS
 REMARK 3 NUMBER OF TWIN DOMAINS : NULL
 REMARK 3
 REMARK 3
 60 REMARK 3 TLS DETAILS
 REMARK 3 NUMBER OF TLS GROUPS : 3
 REMARK 3 ATOM RECORD CONTAINS SUM OF TLS AND RESIDUAL B FACTORS
 REMARK 3 ANISOU RECORD CONTAINS SUM OF TLS AND RESIDUAL U FACTORS
 REMARK 3
 65 REMARK 3 TLS GROUP : 1
 REMARK 3 NUMBER OF COMPONENTS GROUP : 2
 REMARK 3 COMPONENTS C SSSEQI TO C SSSEQI
 REMARK 3 RESIDUE RANGE : L 1 L 109
 REMARK 3 RESIDUE RANGE : H 1 H 122
 70 REMARK 3 ORIGIN FOR THE GROUP (A) : 9.2840 52.2740 33.9990
 REMARK 3 T TENSOR
 REMARK 3 T11: 0.0540 T22: 0.0428
 REMARK 3 T33: 0.0291 T12: 0.0084
 REMARK 3 T13: 0.0134 T23: -0.0009
 75 REMARK 3 L TENSOR
 REMARK 3 L11: 1.3802 L22: 0.7593
 REMARK 3 L33: 2.3791 L12: 0.2416

REMARK 3 L13: 0.4577 L23: 0.6954
 REMARK 3 S TENSOR
 REMARK 3 S11: 0.0958 S12: -0.0684 S13: -0.0101
 REMARK 3 S21: 0.0525 S22: -0.0192 S23: -0.0359
 5 REMARK 3 S31: 0.0395 S32: 0.1578 S33: -0.0767
 REMARK 3
 REMARK 3 TLS GROUP : 2
 REMARK 3 NUMBER OF COMPONENTS GROUP : 2
 10 REMARK 3 COMPONENTS C SSSEQI TO C SSSEQI
 REMARK 3 RESIDUE RANGE : L 110 L 214
 REMARK 3 RESIDUE RANGE : H 123 H 221
 REMARK 3 ORIGIN FOR THE GROUP (A): 27.1960 42.5590 5.5800
 REMARK 3 T TENSOR
 15 REMARK 3 T11: 0.0131 T22: 0.0125
 REMARK 3 T33: 0.0096 T12: 0.0048
 REMARK 3 T13: -0.0100 T23: -0.0061
 REMARK 3 L TENSOR
 20 REMARK 3 L11: 1.7376 L22: 1.9299
 REMARK 3 L33: 1.0465 L12: 0.3656
 REMARK 3 L13: -0.2327 L23: -0.2852
 REMARK 3 S TENSOR
 25 REMARK 3 S11: -0.0367 S12: 0.0026 S13: -0.0209
 REMARK 3 S21: -0.0592 S22: -0.0009 S23: 0.0273
 REMARK 3 S31: -0.0484 S32: 0.0552 S33: 0.0375
 REMARK 3
 REMARK 3 TLS GROUP : 3
 REMARK 3 NUMBER OF COMPONENTS GROUP : 1
 30 REMARK 3 COMPONENTS C SSSEQI TO C SSSEQI
 REMARK 3 RESIDUE RANGE : I 33 I 152
 REMARK 3 ORIGIN FOR THE GROUP (A): -7.8680 51.4100 61.2480
 REMARK 3 T TENSOR
 35 REMARK 3 T11: 0.1909 T22: 0.2025
 REMARK 3 T33: 0.1246 T12: -0.0519
 REMARK 3 T13: -0.0005 T23: -0.0452
 REMARK 3 L TENSOR
 REMARK 3 L11: 2.1894 L22: 1.8121
 REMARK 3 L33: 4.6135 L12: 0.6326
 REMARK 3 L13: -2.5769 L23: -1.2482
 40 REMARK 3 S TENSOR
 REMARK 3 S11: 0.1031 S12: -0.3514 S13: 0.1365
 REMARK 3 S21: 0.3341 S22: -0.0740 S23: -0.1003
 REMARK 3 S31: -0.1958 S32: 0.2196 S33: -0.0292
 REMARK 3
 45 REMARK 3 BULK SOLVENT MODELLING.
 REMARK 3 METHOD USED : MASK
 REMARK 3 PARAMETERS FOR MASK CALCULATION
 REMARK 3 VDW PROBE RADIUS : 1.20
 50 REMARK 3 ION PROBE RADIUS : 0.80
 REMARK 3 SHRINKAGE RADIUS : 0.80
 REMARK 3
 REMARK 3 OTHER REFINEMENT REMARKS:
 REMARK 3 U VALUES : WITH TLS ADDED
 55 REMARK 3 HYDROGENS HAVE BEEN USED IF PRESENT IN THE INPUT
 REMARK 3
 SSBOND 1 CYS L 88 CYS L 23
 SSBOND 2 CYS H 134 CYS L 214
 SSBOND 3 CYS H 96 CYS H 22
 60 SSBOND 4 CYS I 71 CYS I 122
 SSBOND 5 CYS I 78 CYS I 125
 LINKR SG ACYS L 194 SG CYS L 134 SS
 LINKR SG BCYS L 194 SG CYS L 134 SS
 LINKR SG ACYS H 203 SG ACYS H 147 SS
 65 LINKR SG BCYS H 203 SG BCYS H 147 SS
 LINKR LYS I 106 ARG I 114 gap
 LINKR CYS I 78 SER I 86 gap
 CISPEP 1 SER L 7 PRO L 8 0.00
 CISPEP 2 TYR L 94 PRO L 95 0.00
 CISPEP 3 TYR L 140 PRO L 141 0.00
 70 CISPEP 4 PHE H 153 PRO H 154 0.00
 CISPEP 5 GLU H 155 PRO H 156 0.00
 CRYST1 89.370 65.220 106.940 90.00 111.67 90.00 C 1 2 1

Table 11. Results from the X-ray model refinement to the observed data of the hIL-21/Fab57 complex by the software program REFMAC5 (Murshudov, Skubak, Lebedev, Pannu, Steiner, Nicholls, Winn, Long, & Vagin, 2011) of the CCP4 program software package (Bailey, 1994).

```

5      REMARK 3 REFINEMENT.
      REMARK 3 PROGRAM      : REFMAC 5.6.0119
      REMARK 3 AUTHORS     : MURSHUDOV, VAGIN, DODSON
      REMARK 3
10     REMARK 3 REFINEMENT TARGET : MAXIMUM LIKELIHOOD
      REMARK 3
      REMARK 3 DATA USED IN REFINEMENT.
      REMARK 3 RESOLUTION RANGE HIGH (ANGSTROMS) : 1.63
      REMARK 3 RESOLUTION RANGE LOW  (ANGSTROMS) : 99.59
15     REMARK 3 DATA CUTOFF          (SIGMA(F)) : NONE
      REMARK 3 COMPLETENESS FOR RANGE (%) : 98.79
      REMARK 3 NUMBER OF REFLECTIONS : 67154
      REMARK 3
      REMARK 3 FIT TO DATA USED IN REFINEMENT.
      REMARK 3 CROSS-VALIDATION METHOD : THROUGHOUT
20     REMARK 3 FREE R VALUE TEST SET SELECTION : RANDOM
      REMARK 3 R VALUE (WORKING + TEST SET) : 0.17343
      REMARK 3 R VALUE (WORKING SET) : 0.17173
      REMARK 3 FREE R VALUE : 0.20563
25     REMARK 3 FREE R VALUE TEST SET SIZE (%) : 5.0
      REMARK 3 FREE R VALUE TEST SET COUNT : 3567
      REMARK 3
      REMARK 3 FIT IN THE HIGHEST RESOLUTION BIN.
      REMARK 3 TOTAL NUMBER OF BINS USED : 20
30     REMARK 3 BIN RESOLUTION RANGE HIGH : 1.630
      REMARK 3 BIN RESOLUTION RANGE LOW : 1.672
      REMARK 3 REFLECTION IN BIN (WORKING SET) : 4231
      REMARK 3 BIN COMPLETENESS (WORKING+TEST) (%) : 87.29
      REMARK 3 BIN R VALUE (WORKING SET) : 0.273
35     REMARK 3 BIN FREE R VALUE SET COUNT : 232
      REMARK 3 BIN FREE R VALUE : 0.289
      REMARK 3
      REMARK 3 NUMBER OF NON-HYDROGEN ATOMS USED IN REFINEMENT.
      REMARK 3 ALL ATOMS : 4888
      REMARK 3
40     REMARK 3 B VALUES.
      REMARK 3 FROM WILSON PLOT (A**2) : NULL
      REMARK 3 MEAN B VALUE (OVERALL, A**2) : 24.862
      REMARK 3 OVERALL ANISOTROPIC B VALUE.
45     REMARK 3 B11 (A**2) : -0.19
      REMARK 3 B22 (A**2) : 0.04
      REMARK 3 B33 (A**2) : 0.48
      REMARK 3 B12 (A**2) : 0.00
      REMARK 3 B13 (A**2) : 0.45
50     REMARK 3 B23 (A**2) : 0.00
      REMARK 3
      REMARK 3 ESTIMATED OVERALL COORDINATE ERROR.
      REMARK 3 ESU BASED ON R VALUE (A) : 0.092
      REMARK 3 ESU BASED ON FREE R VALUE (A) : 0.092
55     REMARK 3 ESU BASED ON MAXIMUM LIKELIHOOD (A) : 0.062
      REMARK 3 ESU FOR B VALUES BASED ON MAXIMUM LIKELIHOOD (A**2) : 3.580
      REMARK 3
      REMARK 3 CORRELATION COEFFICIENTS.
      REMARK 3 CORRELATION COEFFICIENT FO-FC : 0.967
60     REMARK 3 CORRELATION COEFFICIENT FO-FC FREE : 0.951
      REMARK 3
      REMARK 3 RMS DEVIATIONS FROM IDEAL VALUES COUNT RMS WEIGHT
      REMARK 3 BOND LENGTHS REFINED ATOMS (A) : 4497 ; 0.024 ; 0.020
      REMARK 3 BOND ANGLES REFINED ATOMS (DEGREES) : 6140 ; 2.346 ; 1.958
      REMARK 3 TORSION ANGLES, PERIOD 1 (DEGREES) : 599 ; 6.782 ; 5.000
65     REMARK 3 TORSION ANGLES, PERIOD 2 (DEGREES) : 186 ; 35.335 ; 23.925
      REMARK 3 TORSION ANGLES, PERIOD 3 (DEGREES) : 776 ; 14.615 ; 15.000
      REMARK 3 TORSION ANGLES, PERIOD 4 (DEGREES) : 26 ; 17.500 ; 15.000
      REMARK 3 CHIRAL-CENTER RESTRAINTS (A**3) : 692 ; 0.178 ; 0.200
70     REMARK 3 GENERAL PLANES REFINED ATOMS (A) : 3386 ; 0.013 ; 0.021
      REMARK 3
      REMARK 3 ISOTROPIC THERMAL FACTOR RESTRAINTS. COUNT RMS WEIGHT
      REMARK 3
      REMARK 3 NCS RESTRAINTS STATISTICS
  
```

REMARK 3 NUMBER OF NCS GROUPS : NULL
REMARK 3
REMARK 3 TWIN DETAILS
REMARK 3 NUMBER OF TWIN DOMAINS : NULL
5 REMARK 3
REMARK 3
REMARK 3 TLS DETAILS
REMARK 3 NUMBER OF TLS GROUPS : 3
10 REMARK 3 ATOM RECORD CONTAINS SUM OF TLS AND RESIDUAL B FACTORS
REMARK 3 ANISOU RECORD CONTAINS SUM OF TLS AND RESIDUAL U FACTORS
REMARK 3
REMARK 3 TLS GROUP : 1
REMARK 3 NUMBER OF COMPONENTS GROUP : 2
15 REMARK 3 COMPONENTS C SSSEQI TO C SSSEQI
REMARK 3 RESIDUE RANGE : L 1 L 109
REMARK 3 RESIDUE RANGE : H 1 H 122
REMARK 3 ORIGIN FOR THE GROUP (A): 9.3170 52.1750 34.1280
REMARK 3 T TENSOR
20 REMARK 3 T11: 0.0709 T22: 0.0696
REMARK 3 T33: 0.0055 T12: 0.0119
REMARK 3 T13: 0.0124 T23: -0.0033
REMARK 3 L TENSOR
REMARK 3 L11: 1.2847 L22: 0.7320
25 REMARK 3 L33: 2.0359 L12: 0.2717
REMARK 3 L13: 0.4247 L23: 0.5557
REMARK 3 S TENSOR
REMARK 3 S11: 0.0711 S12: -0.0092 S13: -0.0205
REMARK 3 S21: 0.0265 S22: -0.0078 S23: -0.0337
30 REMARK 3 S31: 0.0283 S32: 0.1350 S33: -0.0633
REMARK 3
REMARK 3 TLS GROUP : 2
REMARK 3 NUMBER OF COMPONENTS GROUP : 2
REMARK 3 COMPONENTS C SSSEQI TO C SSSEQI
35 REMARK 3 RESIDUE RANGE : L 110 L 214
REMARK 3 RESIDUE RANGE : H 123 H 220
REMARK 3 ORIGIN FOR THE GROUP (A): 27.2680 42.5310 5.6470
REMARK 3 T TENSOR
REMARK 3 T11: 0.0368 T22: 0.0396
40 REMARK 3 T33: 0.0051 T12: 0.0215
REMARK 3 T13: -0.0045 T23: -0.0093
REMARK 3 L TENSOR
REMARK 3 L11: 1.5197 L22: 1.6538
REMARK 3 L33: 0.8328 L12: 0.2355
45 REMARK 3 L13: -0.2583 L23: -0.2416
REMARK 3 S TENSOR
REMARK 3 S11: -0.0261 S12: -0.0122 S13: -0.0176
REMARK 3 S21: -0.0321 S22: -0.0010 S23: 0.0237
REMARK 3 S31: -0.0309 S32: 0.0316 S33: 0.0272
50 REMARK 3
REMARK 3 TLS GROUP : 3
REMARK 3 NUMBER OF COMPONENTS GROUP : 1
REMARK 3 COMPONENTS C SSSEQI TO C SSSEQI
REMARK 3 RESIDUE RANGE : I 33 I 152
55 REMARK 3 ORIGIN FOR THE GROUP (A): -7.7920 51.3210 61.3990
REMARK 3 T TENSOR
REMARK 3 T11: 0.1553 T22: 0.1892
REMARK 3 T33: 0.0740 T12: -0.0298
REMARK 3 T13: -0.0119 T23: -0.0378
60 REMARK 3 L TENSOR
REMARK 3 L11: 1.8425 L22: 1.8733
REMARK 3 L33: 3.8411 L12: 0.4705
REMARK 3 L13: -1.9837 L23: -1.1584
REMARK 3 S TENSOR
REMARK 3 S11: 0.0700 S12: -0.3050 S13: 0.1406
65 REMARK 3 S21: 0.2310 S22: -0.0397 S23: -0.0781
REMARK 3 S31: -0.1358 S32: 0.1565 S33: -0.0302
REMARK 3
REMARK 3
70 REMARK 3 BULK SOLVENT MODELLING.
REMARK 3 METHOD USED : MASK
REMARK 3 PARAMETERS FOR MASK CALCULATION
REMARK 3 VDW PROBE RADIUS : 1.20
REMARK 3 ION PROBE RADIUS : 0.80
REMARK 3 SHRINKAGE RADIUS : 0.80
75 REMARK 3
REMARK 3
REMARK 3 OTHER REFINEMENT REMARKS:

```

REMARK 3 U VALUES      : WITH TLS ADDED
REMARK 3 HYDROGENS HAVE BEEN USED IF PRESENT IN THE INPUT
REMARK 3
5  SSBOND 1 CYS L   88   CYS L   23
   SSBOND 2 CYS H  134   CYS L  214
   SSBOND 3 CYS H   96   CYS H   22
   SSBOND 4 CYS I   71   CYS I  122
   SSBOND 5 CYS I   78   CYS I  125
10  LINKR   SG ACYS L 194           SG CYS L 134           SS
   LINKR   SG BCYS L 194           SG CYS L 134           SS
   LINKR   SG ACYS H 203           SG ACYS H 147          SS
   LINKR   SG BCYS H 203           SG BCYS H 147          SS
   LINKR   LYS I 106                ARG I 114              gap
   LINKR   CYS I 78                 SER I 86                gap
15  CISPEP 1 SER L   7   PRO L   8           0.00
   CISPEP 2 TYR L   94   PRO L   95           0.00
   CISPEP 3 TYR L  140   PRO L  141           0.00
   CISPEP 4 PHE H  153   PRO H  154           0.00
   CISPEP 5 GLU H  155   PRO H  156           0.00
20  CRYST1 89.670 65.120 107.130 90.00 111.62 90.00 C 1 2 1

```

Table 12 Results from the X-ray model refinement to the observed data of the hIL-21/Fab59 complex by the software program REFMAC5 (Murshudov, Skubak, Lebedev, Pannu, Steiner, Nicholls, Winn, Long, & Vagin, 2011) of the CCP4 program software package (Bailey, 1994).

```

REMARK 3 REFINEMENT.
REMARK 3 PROGRAM      : REFMAC 5.6.0119
REMARK 3 AUTHORS     : MURSHUDOV,VAGIN,DODSON
REMARK 3
30  REMARK 3 REFINEMENT TARGET : MAXIMUM LIKELIHOOD
   REMARK 3
   REMARK 3 DATA USED IN REFINEMENT.
   REMARK 3 RESOLUTION RANGE HIGH (ANGSTROMS) : 1.65
   REMARK 3 RESOLUTION RANGE LOW  (ANGSTROMS) : 97.67
35  REMARK 3 DATA CUTOFF          (SIGMA(F)) : NONE
   REMARK 3 COMPLETENESS FOR RANGE (%) : 97.62
   REMARK 3 NUMBER OF REFLECTIONS : 61004
   REMARK 3
40  REMARK 3 FIT TO DATA USED IN REFINEMENT.
   REMARK 3 CROSS-VALIDATION METHOD : THROUGHOUT
   REMARK 3 FREE R VALUE TEST SET SELECTION : RANDOM
   REMARK 3 R VALUE (WORKING + TEST SET) : 0.16868
   REMARK 3 R VALUE (WORKING SET) : 0.16667
45  REMARK 3 FREE R VALUE : 0.20681
   REMARK 3 FREE R VALUE TEST SET SIZE (%) : 5.1
   REMARK 3 FREE R VALUE TEST SET COUNT : 3247
   REMARK 3
50  REMARK 3 FIT IN THE HIGHEST RESOLUTION BIN.
   REMARK 3 TOTAL NUMBER OF BINS USED : 20
   REMARK 3 BIN RESOLUTION RANGE HIGH : 1.650
   REMARK 3 BIN RESOLUTION RANGE LOW : 1.693
   REMARK 3 REFLECTION IN BIN (WORKING SET) : 3989
   REMARK 3 BIN COMPLETENESS (WORKING+TEST) (%) : 88.41
55  REMARK 3 BIN R VALUE (WORKING SET) : 0.279
   REMARK 3 BIN FREE R VALUE SET COUNT : 228
   REMARK 3 BIN FREE R VALUE : 0.349
   REMARK 3
60  REMARK 3 NUMBER OF NON-HYDROGEN ATOMS USED IN REFINEMENT.
   REMARK 3 ALL ATOMS : 4862
   REMARK 3
   REMARK 3 B VALUES.
   REMARK 3 FROM WILSON PLOT (A**2) : NULL
   REMARK 3 MEAN B VALUE (OVERALL, A**2) : 29.931
65  REMARK 3 OVERALL ANISOTROPIC B VALUE.
   REMARK 3 B11 (A**2) : -0.79
   REMARK 3 B22 (A**2) : 0.32
   REMARK 3 B33 (A**2) : -0.20
   REMARK 3 B12 (A**2) : 0.00
   REMARK 3 B13 (A**2) : -0.83
70  REMARK 3 B23 (A**2) : 0.00
   REMARK 3

```

REMARK 3 ESTIMATED OVERALL COORDINATE ERROR.
REMARK 3 ESU BASED ON R VALUE (A): 0.096
REMARK 3 ESU BASED ON FREE R VALUE (A): 0.099
REMARK 3 ESU BASED ON MAXIMUM LIKELIHOOD (A): 0.072
5 REMARK 3 ESU FOR B VALUES BASED ON MAXIMUM LIKELIHOOD (A**2): 4.204
REMARK 3
REMARK 3 CORRELATION COEFFICIENTS.
REMARK 3 CORRELATION COEFFICIENT FO-FC : 0.972
10 REMARK 3 CORRELATION COEFFICIENT FO-FC FREE : 0.955
REMARK 3
REMARK 3 RMS DEVIATIONS FROM IDEAL VALUES COUNT RMS WEIGHT
REMARK 3 BOND LENGTHS REFINED ATOMS (A): 4455 ; 0.019 ; 0.020
REMARK 3 BOND ANGLES REFINED ATOMS (DEGREES): 6106 ; 2.007 ; 1.957
15 REMARK 3 TORSION ANGLES, PERIOD 1 (DEGREES): 603 ; 6.728 ; 5.000
REMARK 3 TORSION ANGLES, PERIOD 2 (DEGREES): 181 ; 36.592 ; 24.254
REMARK 3 TORSION ANGLES, PERIOD 3 (DEGREES): 760 ; 14.368 ; 15.000
REMARK 3 TORSION ANGLES, PERIOD 4 (DEGREES): 22 ; 15.959 ; 15.000
REMARK 3 CHIRAL-CENTER RESTRAINTS (A**3): 686 ; 0.156 ; 0.200
20 REMARK 3 GENERAL PLANES REFINED ATOMS (A): 3376 ; 0.012 ; 0.021
REMARK 3
REMARK 3 ISOTROPIC THERMAL FACTOR RESTRAINTS. COUNT RMS WEIGHT
REMARK 3
REMARK 3 NCS RESTRAINTS STATISTICS
25 REMARK 3 NUMBER OF NCS GROUPS : NULL
REMARK 3
REMARK 3 TWIN DETAILS
REMARK 3 NUMBER OF TWIN DOMAINS : NULL
REMARK 3
30 REMARK 3
REMARK 3 TLS DETAILS
REMARK 3 NUMBER OF TLS GROUPS : 3
REMARK 3 ATOM RECORD CONTAINS SUM OF TLS AND RESIDUAL B FACTORS
REMARK 3 ANISOU RECORD CONTAINS SUM OF TLS AND RESIDUAL U FACTORS
REMARK 3
35 REMARK 3
REMARK 3 TLS GROUP : 1
REMARK 3 NUMBER OF COMPONENTS GROUP : 2
REMARK 3 COMPONENTS C SSSEQUI TO C SSSEQUI
REMARK 3 RESIDUE RANGE : L 1 L 109
40 REMARK 3 RESIDUE RANGE : H 1 H 122
REMARK 3 ORIGIN FOR THE GROUP (A): 29.0754 47.2942 15.5475
REMARK 3
REMARK 3 T TENSOR
REMARK 3 T11: 0.0323 T22: 0.0800
REMARK 3 T33: 0.0367 T12: 0.0301
45 REMARK 3 T13: -0.0119 T23: -0.0453
REMARK 3
REMARK 3 L TENSOR
REMARK 3 L11: 2.8773 L22: 0.5802
REMARK 3 L33: 1.2720 L12: -0.7786
REMARK 3 L13: -0.4675 L23: 0.2500
50 REMARK 3
REMARK 3 S TENSOR
REMARK 3 S11: -0.1120 S12: -0.4390 S13: 0.2056
REMARK 3 S21: 0.1209 S22: 0.1658 S23: -0.0721
REMARK 3 S31: -0.0327 S32: 0.0715 S33: -0.0538
REMARK 3
55 REMARK 3
REMARK 3 TLS GROUP : 2
REMARK 3 NUMBER OF COMPONENTS GROUP : 2
REMARK 3 COMPONENTS C SSSEQUI TO C SSSEQUI
REMARK 3 RESIDUE RANGE : L 110 L 214
REMARK 3 RESIDUE RANGE : H 123 H 220
60 REMARK 3 ORIGIN FOR THE GROUP (A): 47.8498 56.3361 43.1198
REMARK 3
REMARK 3 T TENSOR
REMARK 3 T11: 0.1330 T22: 0.0092
REMARK 3 T33: 0.0336 T12: 0.0037
REMARK 3 T13: -0.0270 T23: -0.0126
65 REMARK 3
REMARK 3 L TENSOR
REMARK 3 L11: 2.6436 L22: 1.7178
REMARK 3 L33: 2.2083 L12: -1.0715
REMARK 3 L13: 0.2707 L23: 0.3686
REMARK 3
70 REMARK 3 S TENSOR
REMARK 3 S11: -0.0461 S12: -0.0631 S13: 0.0549
REMARK 3 S21: 0.1094 S22: -0.0586 S23: 0.0461
REMARK 3 S31: -0.1437 S32: -0.0273 S33: 0.1046
REMARK 3
REMARK 3
75 REMARK 3
REMARK 3 TLS GROUP : 3
REMARK 3 NUMBER OF COMPONENTS GROUP : 1
REMARK 3 COMPONENTS C SSSEQUI TO C SSSEQUI
REMARK 3 RESIDUE RANGE : I 41 I 152

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REMARK 3 ORIGIN FOR THE GROUP (A): 10.7692 48.3487 -11.1611
REMARK 3 T TENSOR
REMARK 3 T11: 0.0629 T22: 0.1001
REMARK 3 T33: 0.0340 T12: 0.0146
5 REMARK 3 T13: 0.0170 T23: -0.0336
REMARK 3 L TENSOR
REMARK 3 L11: 4.9770 L22: 3.1372
REMARK 3 L33: 3.1444 L12: -1.0585
10 REMARK 3 L13: 1.7375 L23: -0.9936
REMARK 3 S TENSOR
REMARK 3 S11: 0.1327 S12: 0.6864 S13: -0.1705
REMARK 3 S21: -0.4090 S22: -0.1371 S23: -0.0276
REMARK 3 S31: 0.0681 S32: 0.1554 S33: 0.0044
REMARK 3
15 REMARK 3
REMARK 3 BULK SOLVENT MODELLING.
REMARK 3 METHOD USED : MASK
REMARK 3 PARAMETERS FOR MASK CALCULATION
20 REMARK 3 VDW PROBE RADIUS : 1.20
REMARK 3 ION PROBE RADIUS : 0.80
REMARK 3 SHRINKAGE RADIUS : 0.80
REMARK 3
REMARK 3 OTHER REFINEMENT REMARKS:
25 REMARK 3 U VALUES : WITH TLS ADDED
REMARK 3
SSBOND 1 CYS L 23 CYS L 88
SSBOND 2 CYS L 214 CYS H 134
SSBOND 3 CYS H 22 CYS H 96
30 SSBOND 4 CYS I 71 CYS I 122
SSBOND 5 CYS I 78 CYS I 125
LINKR SG CYS L 134 SG ACYS L 194 SS
LINKR SG CYS L 134 SG BCYS L 194 SS
LINKR SG ACYS H 147 SG CYS H 203 SS
35 LINKR SG BCYS H 147 SG CYS H 203 SS
CISPEP 1 SER L 7 PRO L 8 0.00
CISPEP 2 TYR L 94 PRO L 95 0.00
CISPEP 3 TYR L 140 PRO L 141 0.00
CISPEP 4 PHE H 153 PRO H 154 0.00
40 CISPEP 5 GLU H 155 PRO H 156 0.00
CRYST1 86.510 65.580 106.720 90.00 113.77 90.00 C 1 2 1

```

45 **Table 13** Results from the X-ray model refinement to the observed data of the hIL-21/Fab60 complex by the software program REFMAC5 (Murshudov, Skubak, Lebedev, Pannu, Steiner, Nicholls, Winn, Long, & Vagin, 2011) of the CCP4 program software package (Bailey, 1994).

```

REMARK 3 REFINEMENT.
REMARK 3 PROGRAM : REFMAC 5.6.0119
REMARK 3 AUTHORS : MURSHUDOV,VAGIN,DODSON
50 REMARK 3
REMARK 3 REFINEMENT TARGET : MAXIMUM LIKELIHOOD
REMARK 3
REMARK 3 DATA USED IN REFINEMENT.
REMARK 3 RESOLUTION RANGE HIGH (ANGSTROMS) : 1.75
REMARK 3 RESOLUTION RANGE LOW (ANGSTROMS) : 99.36
55 REMARK 3 DATA CUTOFF (SIGMA(F)) : NONE
REMARK 3 COMPLETENESS FOR RANGE (%) : 99.32
REMARK 3 NUMBER OF REFLECTIONS : 54297
REMARK 3
REMARK 3 FIT TO DATA USED IN REFINEMENT.
60 REMARK 3 CROSS-VALIDATION METHOD : THROUGHOUT
REMARK 3 FREE R VALUE TEST SET SELECTION : RANDOM
REMARK 3 R VALUE (WORKING + TEST SET) : 0.17370
REMARK 3 R VALUE (WORKING SET) : 0.17150
65 REMARK 3 FREE R VALUE : 0.21523
REMARK 3 FREE R VALUE TEST SET SIZE (%) : 5.0
REMARK 3 FREE R VALUE TEST SET COUNT : 2873
REMARK 3
REMARK 3 FIT IN THE HIGHEST RESOLUTION BIN.
70 REMARK 3 TOTAL NUMBER OF BINS USED : 20
REMARK 3 BIN RESOLUTION RANGE HIGH : 1.750
REMARK 3 BIN RESOLUTION RANGE LOW : 1.795

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REMARK 3 REFLECTION IN BIN (WORKING SET) : 3872
 REMARK 3 BIN COMPLETENESS (WORKING+TEST) (%) : 99.66
 REMARK 3 BIN R VALUE (WORKING SET) : 0.261
 5 REMARK 3 BIN FREE R VALUE SET COUNT : 218
 REMARK 3 BIN FREE R VALUE : 0.326
 REMARK 3
 REMARK 3 NUMBER OF NON-HYDROGEN ATOMS USED IN REFINEMENT.
 REMARK 3 ALL ATOMS : 4853
 REMARK 3
 10 REMARK 3 B VALUES.
 REMARK 3 FROM WILSON PLOT (A**2) : NULL
 REMARK 3 MEAN B VALUE (OVERALL, A**2) : 31.261
 REMARK 3 OVERALL ANISOTROPIC B VALUE.
 15 REMARK 3 B11 (A**2) : 0.15
 REMARK 3 B22 (A**2) : 0.44
 REMARK 3 B33 (A**2) : -0.27
 REMARK 3 B12 (A**2) : 0.00
 REMARK 3 B13 (A**2) : 0.45
 20 REMARK 3 B23 (A**2) : 0.00
 REMARK 3
 REMARK 3 ESTIMATED OVERALL COORDINATE ERROR.
 REMARK 3 ESU BASED ON R VALUE (A) : 0.114
 REMARK 3 ESU BASED ON FREE R VALUE (A) : 0.115
 25 REMARK 3 ESU BASED ON MAXIMUM LIKELIHOOD (A) : 0.084
 REMARK 3 ESU FOR B VALUES BASED ON MAXIMUM LIKELIHOOD (A**2) : 5.263
 REMARK 3
 REMARK 3 CORRELATION COEFFICIENTS.
 REMARK 3 CORRELATION COEFFICIENT FO-FC : 0.969
 30 REMARK 3 CORRELATION COEFFICIENT FO-FC FREE : 0.949
 REMARK 3
 REMARK 3 RMS DEVIATIONS FROM IDEAL VALUES COUNT RMS WEIGHT
 REMARK 3 BOND LENGTHS REFINED ATOMS (A) : 4472 ; 0.020 ; 0.020
 REMARK 3 BOND ANGLES REFINED ATOMS (DEGREES) : 6096 ; 2.047 ; 1.958
 35 REMARK 3 TORSION ANGLES, PERIOD 1 (DEGREES) : 587 ; 6.658 ; 5.000
 REMARK 3 TORSION ANGLES, PERIOD 2 (DEGREES) : 186 ; 35.861 ; 24.032
 REMARK 3 TORSION ANGLES, PERIOD 3 (DEGREES) : 771 ; 15.436 ; 15.000
 REMARK 3 TORSION ANGLES, PERIOD 4 (DEGREES) : 25 ; 16.721 ; 15.000
 REMARK 3 CHIRAL-CENTER RESTRAINTS (A**3) : 685 ; 0.156 ; 0.200
 40 REMARK 3 GENERAL PLANES REFINED ATOMS (A) : 3361 ; 0.012 ; 0.021
 REMARK 3
 REMARK 3 ISOTROPIC THERMAL FACTOR RESTRAINTS. COUNT RMS WEIGHT
 REMARK 3
 REMARK 3 NCS RESTRAINTS STATISTICS
 45 REMARK 3 NUMBER OF NCS GROUPS : NULL
 REMARK 3
 REMARK 3 TWIN DETAILS
 REMARK 3 NUMBER OF TWIN DOMAINS : NULL
 REMARK 3
 50 REMARK 3 TLS DETAILS
 REMARK 3 NUMBER OF TLS GROUPS : 3
 REMARK 3 ATOM RECORD CONTAINS SUM OF TLS AND RESIDUAL B FACTORS
 REMARK 3 ANISOU RECORD CONTAINS SUM OF TLS AND RESIDUAL U FACTORS
 REMARK 3
 55 REMARK 3 TLS GROUP : 1
 REMARK 3 NUMBER OF COMPONENTS GROUP : 2
 REMARK 3 COMPONENTS C SSSEQUI TO C SSSEQUI
 REMARK 3 RESIDUE RANGE : L 1 L 109
 REMARK 3 RESIDUE RANGE : H 1 H 122
 60 REMARK 3 ORIGIN FOR THE GROUP (A) : 9.4500 52.1010 34.0400
 REMARK 3 T TENSOR
 REMARK 3 T11: 0.0551 T22: 0.0460
 REMARK 3 T33: 0.0379 T12: 0.0189
 65 REMARK 3 T13: 0.0116 T23: -0.0013
 REMARK 3 L TENSOR
 REMARK 3 L11: 1.0795 L22: 0.6600
 REMARK 3 L33: 2.3184 L12: 0.1843
 REMARK 3 L13: 0.5068 L23: 0.5570
 70 REMARK 3 S TENSOR
 REMARK 3 S11: 0.0616 S12: -0.0144 S13: -0.0581
 REMARK 3 S21: 0.0275 S22: 0.0169 S23: -0.0385
 REMARK 3 S31: 0.0405 S32: 0.1344 S33: -0.0786
 REMARK 3
 75 REMARK 3 TLS GROUP : 2
 REMARK 3 NUMBER OF COMPONENTS GROUP : 2
 REMARK 3 COMPONENTS C SSSEQUI TO C SSSEQUI

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REMARK 3 RESIDUE RANGE : L 110 L 214
REMARK 3 RESIDUE RANGE : H 123 H 220
REMARK 3 ORIGIN FOR THE GROUP (A): 27.2030 42.4080 5.6610
5 REMARK 3 T TENSOR
REMARK 3 T11: 0.0234 T22: 0.0148
REMARK 3 T33: 0.0247 T12: 0.0097
REMARK 3 T13: -0.0073 T23: -0.0075
REMARK 3 L TENSOR
10 REMARK 3 L11: 1.9247 L22: 1.8682
REMARK 3 L33: 0.9227 L12: 0.2905
REMARK 3 L13: -0.2738 L23: -0.3214
REMARK 3 S TENSOR
15 REMARK 3 S11: -0.0453 S12: -0.0032 S13: -0.0157
REMARK 3 S21: -0.0520 S22: -0.0019 S23: 0.0018
REMARK 3 S31: -0.0496 S32: 0.0461 S33: 0.0471
REMARK 3
REMARK 3 TLS GROUP : 3
REMARK 3 NUMBER OF COMPONENTS GROUP : 1
20 REMARK 3 COMPONENTS C SSSEQI TO C SSSEQI
REMARK 3 RESIDUE RANGE : I 33 I 152
REMARK 3 ORIGIN FOR THE GROUP (A): -7.5740 51.3910 61.2800
REMARK 3 T TENSOR
25 REMARK 3 T11: 0.1810 T22: 0.1966
REMARK 3 T33: 0.1218 T12: -0.0274
REMARK 3 T13: 0.0045 T23: -0.0317
REMARK 3 L TENSOR
30 REMARK 3 L11: 2.0882 L22: 2.1201
REMARK 3 L33: 4.3804 L12: 0.4268
REMARK 3 L13: -2.3533 L23: -1.0835
REMARK 3 S TENSOR
REMARK 3 S11: 0.0647 S12: -0.3084 S13: 0.1729
REMARK 3 S21: 0.3921 S22: -0.0441 S23: -0.0698
REMARK 3 S31: -0.1554 S32: 0.0224 S33: -0.0206
REMARK 3
35 REMARK 3
REMARK 3 BULK SOLVENT MODELLING.
REMARK 3 METHOD USED : MASK
REMARK 3 PARAMETERS FOR MASK CALCULATION
40 REMARK 3 VDW PROBE RADIUS : 1.20
REMARK 3 ION PROBE RADIUS : 0.80
REMARK 3 SHRINKAGE RADIUS : 0.80
REMARK 3
REMARK 3 OTHER REFINEMENT REMARKS:
45 REMARK 3 U VALUES : WITH TLS ADDED
REMARK 3 HYDROGENS HAVE BEEN USED IF PRESENT IN THE INPUT
REMARK 3
SSBOND 1 CYS L 88 CYS L 23
SSBOND 2 CYS H 134 CYS L 214
50 SSBOND 3 CYS H 96 CYS H 22
SSBOND 4 CYS I 71 CYS I 122
SSBOND 5 CYS I 78 CYS I 125
LINKR SG ACYS L 194 SG CYS L 134 SS
LINKR SG BCYS L 194 SG CYS L 134 SS
55 LINKR SG ACYS H 203 SG ACYS H 147 SS
LINKR SG BCYS H 203 SG BCYS H 147 SS
LINKR LYS I 106 ARG I 114 gap
LINKR CYS I 78 SER I 86 gap
CISPEP 1 SER L 7 PRO L 8 0.00
60 CISPEP 2 TYR L 94 PRO L 95 0.00
CISPEP 3 TYR L 140 PRO L 141 0.00
CISPEP 4 PHE H 153 PRO H 154 0.00
CISPEP 5 GLU H 155 PRO H 156 0.00

```

Table 14.

65 hIL-21, chain I, (SEQ ID NO: 1) interactions with the the heavy chain (chain H) of Fab56 (SEQ ID NO: 10, D62E mutation) and light chain (chain L) of anti-IL-21 Fab56 (SEQ ID NO: 9). A distance cut-off of 5.0 Å was used. The contacts were identified by the CONTACT computer software program of the CCP4 suite (Bailey, 1994). In the last column "****" indicates a strong possibility for a hydrogen bond at this contact (distance < 3.3 Å) as calculated by CONTACT, "*" indicates a weak possibility (distance > 3.3 Å).
70 Blank indicates that the program considered there to be no possibility of a hydrogen

bond. Hydrogen-bonds are specific between a donor and an acceptor, are typically strong, and are easily identifiable.

hIL-21			aIL-21 Fab56(Fab35 with H: D62E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
Met	39I	CE	Trp	102H	CZ3	4.46	
			Trp	102H	CH2	4.76	
Glu	65I	CB	Tyr	56H	CZ	4.90	
			Tyr	56H	OH	4.71	
			Tyr	56H	CE2	4.59	
Glu	65I	CG	Tyr	56H	CZ	4.91	
			Tyr	56H	OH	4.64	
			Tyr	56H	CE2	4.98	
Glu	65I	CD	Tyr	56H	CZ	4.44	
			Tyr	56H	OH	3.82	
			Tyr	56H	CE2	4.86	
Glu	65I	OE1	Tyr	56H	CZ	4.38	
			Tyr	56H	OH	3.46	*
			Tyr	56H	CE2	4.79	
Glu	65I	OE2	Tyr	56H	CZ	4.66	
			Tyr	56H	OH	4.10	*
Asp	66I	N	Tyr	56H	CE2	4.44	
			Tyr	56H	CD2	4.40	
Asp	66I	CA	Tyr	56H	CG	4.95	
			Tyr	56H	CD2	4.58	
			Tyr	57H	CE2	4.71	
Asp	66I	CB	Gly	54H	O	4.81	
			Tyr	56H	CG	3.66	
			Tyr	56H	CD1	4.32	
			Tyr	56H	CE1	4.92	
			Tyr	56H	CZ	4.87	
			Tyr	56H	CE2	4.31	
			Tyr	56H	CD2	3.64	
			Gly	54H	N	4.56	
			Gly	54H	CA	4.20	
			Thr	52H	OG1	4.74	
			Gly	54H	C	4.64	
			Tyr	56H	N	4.69	
			Tyr	56H	CA	4.87	
			Tyr	56H	CB	3.75	
			Tyr	57H	CE2	4.29	
			Tyr	57H	CD2	4.57	
Asp	66I	CG	Gly	54H	O	4.38	
			Tyr	56H	CG	4.26	
			Tyr	56H	CD1	4.82	
			Tyr	56H	CD2	4.68	
			Ser	53H	OG	4.81	
			Ser	53H	C	4.61	
			Gly	54H	N	3.35	
			Gly	54H	CA	3.37	
			Thr	52H	CB	3.95	
			Thr	52H	OG1	3.38	
			Thr	52H	C	4.73	
			Ser	53H	N	4.55	
			Gly	54H	C	3.83	

hIL-21			aIL-21 Fab56 (Fab35 with H: D62E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Ser	55H	N	4.21	
			Tyr	56H	N	4.06	
			Tyr	56H	CA	4.60	
			Tyr	56H	CB	3.88	
			Tyr	57H	CE2	4.52	
			Tyr	57H	CD2	4.32	
			Thr	52H	CA	4.99	
			Thr	52H	CG2	4.99	
Asp	66I	OD1	Ser	53H	CA	4.41	
			Ser	53H	CB	4.66	
			Ser	53H	OG	3.73	*
			Ser	53H	C	4.19	
			Gly	54H	N	3.10	***
			Gly	54H	CA	3.57	
			Thr	52H	CB	3.66	
			Thr	52H	OG1	3.56	*
			Thr	52H	C	4.31	
			Ser	53H	N	3.83	*
			Gly	54H	C	4.37	
			Ser	55H	N	4.66	*
			Tyr	57H	CD2	4.92	
			Thr	52H	CA	4.61	
			Thr	52H	CG2	4.70	
Asp	66I	OD2	Gly	54H	O	3.69	*
			Tyr	56H	CG	3.80	
			Tyr	56H	CD1	4.27	
			Tyr	56H	CD2	4.57	
			Ser	53H	C	4.47	
			Gly	54H	N	3.22	***
			Gly	54H	CA	3.14	
			Ser	55H	CA	4.09	
			Thr	52H	CB	3.56	
			Thr	52H	OG1	2.61	***
			Thr	52H	C	4.27	
			Thr	52H	O	4.55	*
			Ser	53H	N	4.42	*
			Gly	54H	C	3.12	
			Ser	55H	N	3.26	***
			Ser	55H	C	3.91	
			Tyr	56H	N	2.87	***
			Tyr	56H	CA	3.56	
			Tyr	56H	CB	3.18	
			Tyr	57H	N	4.08	*
			Tyr	56H	C	4.35	
			Tyr	57H	CE2	4.52	
			Tyr	57H	CD2	4.06	
			Thr	52H	CA	4.49	
			Thr	52H	CG2	4.69	
Asp	66I	C	Tyr	56H	CD2	4.96	
			Tyr	57H	CZ	4.93	
			Tyr	57H	CE2	3.86	
			Tyr	57H	CD2	4.49	
Asp	66I	O	Tyr	56H	CD2	4.66	

hIL-21			aIL-21 Fab56 (Fab35 with H: D62E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Tyr	57H	CZ	4.33	
			Tyr	57H	OH	4.19	*
			Tyr	57H	CE2	3.57	
			Tyr	57H	CD2	4.54	
Val	67I	N	Tyr	57H	CE2	4.00	
			Tyr	57H	CD2	4.32	
Val	67I	CA	Tyr	57H	CE2	3.98	
			Tyr	57H	CD2	4.31	
Val	67I	C	Thr	52H	CB	4.77	
			Tyr	57H	CG	4.60	
			Tyr	57H	CZ	4.94	
			Tyr	57H	CE2	3.85	
			Tyr	57H	CD2	3.70	
			Thr	52H	CG2	4.20	
Val	67I	O	Thr	52H	CB	3.80	
			Thr	52H	OG1	4.37	*
			Tyr	57H	CG	4.87	
			Tyr	57H	CE2	4.46	
			Tyr	57H	CD2	4.00	
			Thr	52H	CG2	3.30	
Glu	68I	N	Tyr	57H	CB	4.80	
			Tyr	57H	CG	4.11	
			Tyr	57H	CD1	4.84	
			Tyr	57H	CZ	4.51	
			Tyr	57H	CE2	3.71	
			Tyr	57H	CD2	3.55	
			Thr	52H	CG2	4.52	
Glu	68I	CA	Tyr	57H	CB	4.43	
			Tyr	57H	CG	4.16	
			Tyr	57H	CD1	4.92	
			Tyr	57H	CE2	4.59	
			Tyr	57H	CD2	4.06	
			Thr	52H	CG2	4.07	
Glu	68I	CB	Tyr	57H	CB	4.90	
			Tyr	57H	CG	4.56	
			Tyr	57H	CD1	4.93	
			Tyr	57H	CD2	4.77	
Glu	68I	CG	Tyr	57H	CB	4.31	
			Tyr	57H	CG	3.78	
			Tyr	57H	CD1	3.78	
			Tyr	57H	CE1	4.16	
			Tyr	57H	CZ	4.54	
			Tyr	57H	CE2	4.56	
			Tyr	57H	CD2	4.26	
Glu	68I	CD	Tyr	57H	CB	3.78	
			Tyr	57H	CG	3.67	
			Tyr	57H	CD1	3.59	
			Tyr	57H	CE1	4.36	
			Tyr	57H	CD2	4.59	
			His	59H	CE1	4.29	
			His	59H	NE2	3.64	
			His	59H	CD2	4.79	
Glu	68I	OE1	Tyr	57H	CA	4.92	

hIL-21			aIL-21 Fab56 (Fab35 with H: D62E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Tyr	57H	CB	3.61	
			Tyr	57H	CG	3.98	
			Tyr	57H	CD1	4.22	
			Tyr	57H	CD2	4.90	
			Tyr	94L	CD1	4.89	
			Tyr	94L	CZ	4.72	
			His	59H	CE1	3.85	
			His	59H	NE2	2.92	***
			His	59H	CD2	3.86	
			Tyr	94L	CE1	4.13	
			Tyr	94L	OH	4.54	*
Glu	68I	OE2	Tyr	57H	CB	4.12	
			Tyr	57H	CG	3.93	
			Tyr	57H	CD1	3.41	
			Tyr	57H	CE1	4.09	
			His	59H	CE1	3.89	
			His	59H	NE2	3.60	*
			His	59H	CD2	4.91	
Glu	68I	C	Thr	52H	CG2	4.19	
Thr	69I	N	Ser	33H	OG	4.69	*
			Thr	52H	CG2	3.73	
			Tyr	94L	OH	4.41	*
Thr	69I	CA	Ser	33H	OG	4.25	
			Thr	52H	CG2	4.60	
			Tyr	94L	OH	4.65	
Thr	69I	CB	Ser	33H	CB	4.32	
			Ser	33H	OG	3.64	
			Thr	52H	CG2	4.69	
			Tyr	94L	OH	3.70	
			Glu	99H	CD	4.56	
			Glu	99H	OE1	4.45	
			Glu	99H	OE2	4.43	
			Tyr	96L	OH	4.29	
Thr	69I	OG1	Thr	52H	CB	4.73	
			Ser	50H	OG	4.39	*
			Ser	33H	CA	4.76	
			Ser	33H	CB	3.34	
			Ser	33H	OG	2.63	***
			Thr	52H	CA	4.83	
			Thr	52H	CG2	3.55	
			Tyr	94L	OH	3.81	*
			Glu	99H	CD	4.84	
			Glu	99H	OE1	4.39	*
			Tyr	96L	OH	4.98	*
Thr	69I	CG2	Ser	33H	CB	4.05	
			Ser	33H	OG	3.62	
			Tyr	94L	OH	4.69	
			Glu	99H	CG	4.21	
			Glu	99H	CD	3.64	
			Glu	99H	OE1	3.88	
			Glu	99H	OE2	3.56	
			Arg	100H	C	4.76	
			Arg	100H	O	4.46	

hIL-21			aIL-21 Fab56(Fab35 with H: D62E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Gly	101H	N	4.59	
			Gly	101H	CA	4.12	
			Tyr	96L	OH	4.23	
Thr	69I	O	Tyr	94L	OH	4.55	*
Asn	70I	N	Gly	101H	CA	4.06	
			Gly	101H	C	4.65	
			Trp	102H	N	4.32	*
Asn	70I	CA	Gly	101H	CA	4.46	
			Gly	101H	C	4.60	
			Trp	102H	N	4.08	
			Trp	102H	CA	4.94	
			Gly	103H	N	4.33	
Asn	70I	CB	Gly	101H	CA	4.90	
			Trp	102H	N	4.84	
			Gly	103H	N	4.35	
			Gly	103H	CA	4.81	
			Tyr	105H	CE1	3.70	
			Tyr	105H	CZ	4.37	
			Tyr	105H	OH	4.41	
			Tyr	105H	CD1	4.40	
Asn	70I	CG	Arg	100H	O	4.53	
			Gly	101H	CA	4.02	
			Gly	101H	C	4.27	
			Gly	101H	O	4.82	
			Trp	102H	N	4.40	
			Gly	103H	N	3.85	
			Gly	103H	CA	4.27	
			Tyr	105H	CE1	3.48	
			Tyr	105H	CZ	4.34	
			Tyr	105H	OH	4.82	
			Gly	103H	C	4.89	
			Tyr	104H	N	4.83	
			Tyr	105H	N	4.56	
			Tyr	105H	CA	4.92	
			Tyr	105H	CD1	3.69	
			Tyr	105H	CG	4.70	
Asn	70I	OD1	Arg	100H	C	4.63	
			Arg	100H	O	3.98	*
			Gly	101H	N	4.42	*
			Gly	101H	CA	3.24	
			Gly	101H	C	3.21	
			Gly	101H	O	3.65	*
			Trp	102H	N	3.41	*
			Trp	102H	CA	4.25	
			Trp	102H	C	3.93	
			Gly	103H	N	2.83	***
			Gly	103H	CA	3.43	
			Tyr	105H	CE1	4.00	
			Tyr	105H	CZ	4.65	
			Gly	103H	C	3.99	
			Tyr	104H	N	3.80	*
			Tyr	104H	CA	4.79	
			Tyr	104H	C	4.95	

hIL-21			aIL-21 Fab56 (Fab35 with H: D62E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Tyr	105H	N	4.06	*
			Tyr	105H	CA	4.77	
			Tyr	105H	CD1	4.06	
			Tyr	105H	CG	4.75	
Asn	70I	ND2	Glu	99H	CD	4.86	
			Glu	99H	OE2	3.88	*
			Arg	100H	O	4.27	*
			Gly	101H	CA	4.49	
			Phe	91L	CB	4.58	
			Phe	91L	CD1	4.56	
			Phe	91L	O	4.91	*
			Tyr	96L	OH	4.28	*
			Tyr	105H	CE1	3.60	
			Tyr	105H	CZ	4.76	
			Tyr	105H	N	4.45	*
			Phe	91L	CG	4.89	
			Tyr	105H	CA	4.46	
			Tyr	105H	CD1	3.49	
			Tyr	105H	CG	4.62	
Asn	70I	C	Trp	102H	N	4.89	
Asn	70I	O	Gly	103H	N	4.97	*
			Tyr	105H	OH	4.66	*
Glu	72I	N	Trp	102H	N	4.92	*
Glu	72I	CA	Trp	102H	CE2	4.68	
			Trp	102H	CZ3	4.56	
			Trp	102H	CH2	4.62	
			Trp	102H	CZ2	4.72	
			Trp	102H	CD2	4.59	
			Trp	102H	CE3	4.55	
Glu	72I	CB	Trp	102H	NE1	3.77	
			Trp	102H	CE2	3.29	
			Trp	102H	CZ3	3.87	
			Trp	102H	CH2	3.78	
			Trp	102H	CZ2	3.53	
			Trp	102H	N	4.37	
			Trp	102H	CA	4.49	
			Trp	102H	CB	4.83	
			Trp	102H	CG	3.96	
			Trp	102H	CD1	4.11	
			Trp	102H	CD2	3.36	
			Trp	102H	CE3	3.68	
Glu	72I	CG	Trp	102H	NE1	3.70	
			Trp	102H	CE2	3.73	
			Trp	102H	CH2	4.74	
			Trp	102H	CZ2	4.08	
			Trp	102H	N	4.44	
			Trp	102H	CG	4.43	
			Trp	102H	CD1	4.10	
			Trp	102H	CD2	4.17	
			Trp	102H	CE3	4.86	
Glu	72I	CD	Trp	102H	NE1	4.23	
			Trp	102H	CE2	4.57	
			Gly	101H	CA	4.08	

hIL-21			aIL-21 Fab56(Fab35 with H: D62E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Gly	101H	C	4.25	
			Trp	102H	N	3.53	
			Trp	102H	CA	4.45	
			Trp	102H	CG	4.51	
			Trp	102H	CD1	4.16	
			Trp	102H	CD2	4.72	
Glu	72I	OE1	Trp	102H	NE1	4.62	*
			Trp	102H	CE2	4.84	
			Gly	101H	N	4.94	*
			Gly	101H	CA	3.65	
			Gly	101H	C	3.56	
			Gly	101H	O	4.75	*
			Trp	102H	N	2.65	***
			Trp	102H	CA	3.53	
			Trp	102H	CB	4.52	
			Trp	102H	CG	4.24	
			Trp	102H	CD1	4.21	
			Trp	102H	C	4.63	
			Gly	103H	N	4.65	*
			Trp	102H	CD2	4.60	
Glu	72I	OE2	Ser	033H	OG	4.96	*
			Trp	102H	NE1	4.78	*
			Gly	101H	N	4.82	*
			Gly	101H	CA	3.94	
			Gly	101H	C	4.55	
			Trp	102H	N	4.15	*
			Trp	102H	CD1	4.71	
Glu	72I	C	Trp	102H	CE2	4.95	
			Trp	102H	CZ3	3.92	
			Trp	102H	CH2	4.31	
			Trp	102H	CZ2	4.84	
			Trp	102H	CD2	4.57	
			Trp	102H	CE3	4.08	
Glu	72I	O	Trp	102H	CZ3	3.76	
			Trp	102H	CH2	3.99	
			Trp	102H	CZ2	4.76	
			Trp	102H	CE3	4.35	
Trp	73I	N	Trp	102H	CZ3	4.22	
			Trp	102H	CH2	4.98	
			Trp	102H	CA	4.93	
			Trp	102H	CD2	4.72	
			Trp	102H	CE3	4.08	
Trp	73I	CA	Trp	102H	CZ3	4.41	
			Trp	102H	CE3	4.45	
Trp	73I	CB	Trp	102H	CE3	4.82	
Trp	73I	CG	Trp	102H	CZ3	4.42	
			Trp	102H	CA	4.84	
			Trp	102H	C	4.91	
			Trp	102H	O	4.65	
			Trp	102H	CD2	4.97	
			Trp	102H	CE3	3.93	
Trp	73I	CD1	Trp	102H	CZ3	4.82	
			Trp	102H	CA	3.82	

hIL-21			aIL-21 Fab56(Fab35 with H: D62E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Trp	102H	CB	4.12	
			Trp	102H	CG	4.87	
			Trp	102H	C	3.64	
			Trp	102H	O	3.26	
			Gly	103H	N	4.48	
			Trp	102H	CD2	4.70	
			Trp	102H	CE3	3.93	
Trp	73I	NE1	Trp	102H	CZ3	4.61	
			Trp	102H	CA	3.99	
			Trp	102H	CB	3.77	
			Trp	102H	CG	4.56	
			Trp	102H	C	3.85	
			Trp	102H	O	3.15	***
			Gly	103H	N	4.95	*
			Trp	102H	CD2	4.42	
			Trp	102H	CE3	3.64	
Trp	73I	CE2	Trp	102H	CZ3	4.04	
			Trp	102H	CB	4.59	
			Trp	102H	O	4.50	
			Trp	102H	CD2	4.52	
			Trp	102H	CE3	3.44	
Trp	73I	CD2	Trp	102H	CZ3	3.89	
			Trp	102H	CD2	4.88	
			Trp	102H	CE3	3.63	
Trp	73I	CE3	Trp	102H	CZ3	3.96	
			Trp	102H	CE3	4.18	
Trp	73I	CZ3	Trp	102H	CZ3	4.14	
			Trp	102H	CE3	4.48	
Trp	73I	CH2	Trp	102H	CZ3	4.31	
			Trp	102H	CE3	4.36	
Trp	73I	CZ2	Trp	102H	CZ3	4.26	
			Trp	102H	CD2	4.91	
			Trp	102H	CE3	3.86	
Phe	76I	CB	Trp	102H	CZ3	4.37	
			Trp	102H	CH2	4.74	
Phe	76I	CG	Trp	102H	CZ3	4.98	
Lys	117I	N	Trp	102H	O	4.87	*
Lys	117I	CA	Gly	103H	CA	4.79	
Lys	117I	CB	Gly	103H	CA	4.27	
			Tyr	105H	CZ	4.83	
			Tyr	105H	OH	4.43	
			Gly	103H	O	4.83	
			Tyr	105H	CE2	4.28	
Lys	117I	CG	Tyr	105H	CE2	4.97	
Lys	117I	CD	Ser	31L	OG	3.88	
			Asp	50L	CG	4.18	
			Asp	50L	OD1	3.74	
			Asp	50L	OD2	3.96	
			Gly	103H	O	4.45	
			Tyr	105H	CE2	4.31	
			Tyr	105H	CD2	4.75	
Lys	117I	CE	Ser	31L	OG	4.08	
			Asp	50L	CG	4.13	

hIL-21			aIL-21 Fab56 (Fab35 with H: D62E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Asp	50L	OD1	3.99	
			Asp	50L	OD2	3.51	
Lys	117I	NZ	Ser	31L	OG	3.17	***
			Asp	50L	CG	3.58	
			Asp	50L	OD1	3.41	*
			Asp	50L	OD2	2.96	***
			Ser	31L	CB	3.99	
Lys	117I	C	Gly	103H	N	4.70	
			Gly	103H	CA	4.31	
			Tyr	105H	OH	4.44	
Lys	117I	O	Trp	102H	CA	4.70	
			Trp	102H	C	3.91	
			Trp	102H	O	4.04	*
			Gly	103H	N	3.67	*
			Gly	103H	CA	3.58	
			Tyr	105H	OH	4.75	*
			Gly	103H	C	4.99	
His	118I	N	Tyr	105H	OH	4.06	*
His	118I	CA	Tyr	105H	OH	3.86	
His	118I	C	Tyr	105H	CZ	4.81	
			Tyr	105H	OH	3.46	
His	118I	O	Tyr	105H	OH	3.73	*
Arg	119I	N	Tyr	105H	CZ	4.78	
			Tyr	105H	OH	3.50	*
Arg	119I	CA	Tyr	105H	OH	4.04	
Arg	119I	CB	Tyr	105H	OH	4.66	
			Asn	92L	O	4.54	
Arg	119I	CG	Phe	91L	O	4.26	
			Tyr	105H	CE1	4.44	
			Tyr	105H	CZ	4.70	
			Tyr	105H	OH	3.99	
			Asn	92L	CA	4.96	
			Asn	92L	C	4.89	
			Asn	92L	O	4.15	
Arg	119I	CD	Phe	91L	C	4.58	
			Phe	91L	O	3.47	
			Asn	92L	N	4.94	
			Asn	92L	CA	4.32	
			Asn	92L	C	3.89	
			Asn	92L	O	3.15	
			Ser	93L	N	4.83	
Arg	119I	NE	Tyr	94L	CE1	4.82	
			Phe	91L	O	4.07	*
			Asn	92L	C	4.90	
			Asn	92L	O	4.21	*
Arg	119I	CZ	Tyr	94L	CD1	4.94	
			Tyr	94L	CE1	4.31	
			Asn	92L	O	4.69	
Arg	119I	NH1	Tyr	94L	CE1	4.79	
			Asn	92L	O	4.30	*
Arg	119I	NH2	Tyr	94L	CD1	4.71	
			Tyr	94L	CZ	4.61	
			Tyr	94L	CE1	3.83	

hIL-21			aIL-21 Fab56 (Fab35 with H: D62E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Tyr	94L	OH	4.39	*
Thr	121I	OG1	Asn	92L	O	4.98	*
Tyr	128I	CE1	Tyr	57H	OH	4.78	
Glu	129I	CD	Tyr	56H	OH	4.89	
Glu	129I	OE1	Tyr	56H	OH	4.79	*
Glu	129I	OE2	Tyr	56H	OH	4.68	*
Leu	143I	CA	Trp	102H	CZ2	4.82	
Leu	143I	CB	Trp	102H	CH2	4.85	
			Trp	102H	CZ2	4.89	
Leu	143I	CG	Trp	102H	CZ3	4.82	
			Trp	102H	CH2	3.61	
			Trp	102H	CZ2	3.72	
Leu	143I	CD1	Trp	102H	CE2	4.72	
			Trp	102H	CH2	4.01	
			Trp	102H	CZ2	3.69	
Leu	143I	CD2	Trp	102H	CZ3	4.86	
			Trp	102H	CH2	3.96	
			Trp	102H	CZ2	4.49	
Leu	143I	C	Trp	102H	CH2	4.40	
			Trp	102H	CZ2	4.19	
Leu	143I	O	Trp	102H	NE1	4.96	*
			Trp	102H	CE2	4.42	
			Trp	102H	CH2	3.78	
			Trp	102H	CZ2	3.32	
Gln	145I	O	Ser	31H	OG	4.83	*
Lys	146I	N	Ser	31H	OG	4.77	*
Lys	146I	CA	Ser	31H	OG	4.04	
			Ser	31H	CB	4.40	
			Ser	31H	O	4.94	
			Trp	102H	NE1	4.95	
Lys	146I	CB	Ser	31H	OG	4.34	
			Ser	31H	CA	4.81	
			Ser	31H	CB	4.52	
			Ser	31H	O	4.37	
			Trp	102H	NE1	4.44	
			Trp	102H	CE2	4.89	
			Trp	102H	CZ2	4.61	
Lys	146I	CG	Ser	30H	O	4.77	
			Ser	31H	OG	3.67	
			Ser	31H	C	4.66	
			Ser	31H	CA	4.08	
			Ser	31H	CB	4.07	
			Ser	31H	O	4.27	
Lys	146I	CD	Ser	30H	O	4.61	
			Ser	53H	OG	4.20	
			Ser	31H	OG	4.83	
			Ser	31H	C	4.91	
			Ser	31H	CA	4.58	
			Ser	31H	O	4.53	
Lys	146I	CE	Ser	30H	O	3.69	
			Ser	53H	CB	4.39	
			Ser	53H	OG	3.67	
			Ser	31H	OG	4.94	

hIL-21			aIL-21 Fab56(Fab35 with H: D62E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Ser	30H	C	4.77	
			Ser	31H	CA	4.53	
Lys	146I	NZ	Ser	30H	O	4.61	*
Lys	146I	C	Trp	102H	NE1	4.12	
			Trp	102H	CE2	4.53	
			Trp	102H	CZ2	4.56	
Lys	146I	O	Ser	31H	OG	4.93	*
			Ser	31H	CB	4.74	
			Ser	31H	O	4.93	*
			Trp	102H	NE1	4.35	*
			Trp	102H	CE2	4.99	
			Trp	102H	CD1	4.93	
Met	147I	N	Trp	102H	NE1	3.83	*
			Trp	102H	CE2	3.84	
			Trp	102H	CH2	4.55	
			Trp	102H	CZ2	3.70	
			Trp	102H	CD1	4.77	
			Trp	102H	CD2	4.85	
Met	147I	CA	Trp	102H	NE1	3.81	
			Trp	102H	CE2	3.70	
			Trp	102H	CZ3	4.92	
			Trp	102H	CH2	4.45	
			Trp	102H	CZ2	3.84	
			Trp	102H	CG	4.65	
			Trp	102H	CD1	4.40	
			Trp	102H	CD2	4.28	
			Trp	102H	CE3	4.85	
Met	147I	CB	Trp	102H	NE1	4.29	
			Trp	102H	CE2	3.67	
			Trp	102H	CZ3	3.92	
			Trp	102H	CH2	3.60	
			Trp	102H	CZ2	3.48	
			Trp	102H	CG	4.77	
			Trp	102H	CD1	4.92	
			Trp	102H	CD2	4.01	
			Trp	102H	CE3	4.13	
Met	147I	CG	Trp	102H	NE1	4.91	
			Trp	102H	CE2	4.18	
			Trp	102H	CZ3	3.80	
			Trp	102H	CH2	4.04	
			Trp	102H	CZ2	4.25	
			Trp	102H	CG	4.62	
			Trp	102H	CD2	3.95	
			Trp	102H	CE3	3.75	
His	149I	CB	Ile	28H	CB	4.63	
			Ile	28H	CG1	4.77	
			Ser	31H	OG	3.88	
			Ser	31H	CB	4.06	
His	149I	CG	Ile	28H	CB	4.09	
			Ile	28H	CG1	3.98	
			Ile	28H	CG2	4.37	
			Ser	31H	OG	3.82	
			Ser	31H	CB	4.44	

hIL-21			aIL-21 Fab56 (Fab35 with H: D62E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
His	149I	ND1	Ile	28H	CB	3.88	
			Ile	28H	CG1	4.01	
			Ile	28H	CG2	3.68	
			Ser	31H	OG	2.97	***
			Ser	31H	CB	3.96	
His	149I	CE1	Ile	28H	CD1	4.64	
			Ile	28H	CB	4.10	
			Ile	28H	CG1	3.89	
			Ile	28H	CG2	3.71	
			Ser	31H	OG	3.93	
His	149I	NE2	Ile	28H	CD1	4.45	
			Ile	28H	CB	4.43	
			Ile	28H	CG1	3.76	
			Ile	28H	CG2	4.41	
His	149I	CD2	Ile	28H	CD1	4.89	
			Ile	28H	CB	4.48	
			Ile	28H	CG1	3.86	
			Ile	28H	CG2	4.83	
His	149I	C	Tyr	32H	OH	4.01	
			Tyr	32H	CZ	4.79	
His	149I	O	Tyr	32H	OH	3.54	*
			Tyr	32H	CZ	4.60	
Gln	150I	N	Tyr	32H	OH	4.10	*
			Tyr	32H	CZ	4.66	
Gln	150I	CA	Tyr	32H	CE2	4.95	
			Tyr	32H	OH	3.50	
			Tyr	32H	CE1	4.46	
			Tyr	32H	CZ	4.10	
			Arg	100H	NE	4.69	
			Arg	100H	CZ	4.90	
			Arg	100H	NH2	4.83	
Gln	150I	CB	Tyr	32H	OH	4.22	
			Tyr	32H	CE1	4.34	
			Tyr	32H	CZ	4.40	
			Arg	100H	CG	4.75	
			Trp	102H	CD1	4.81	
			Arg	100H	CD	4.93	
			Arg	100H	NE	4.19	
			Arg	100H	CZ	4.68	
			Arg	100H	NH2	4.57	
Gln	150I	CG	Ser	31H	C	4.95	
			Tyr	32H	CD2	4.71	
			Ser	31H	CB	4.97	
			Ser	31H	O	4.10	
			Trp	102H	NE1	4.86	
			Tyr	32H	CE2	4.31	
			Tyr	32H	OH	4.06	
			Tyr	32H	CG	4.66	
			Tyr	32H	CD1	4.11	
			Tyr	32H	CE1	3.67	
			Tyr	32H	CZ	3.77	
			Arg	100H	CG	4.89	
			Trp	102H	CD1	4.64	

hIL-21			aIL-21 Fab56(Fab35 with H: D62E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
Gln	150I	CD	Ser	31H	C	4.86	
			Tyr	32H	CA	4.97	
			Ser	31H	O	3.87	
			Trp	102H	NE1	4.32	
			Tyr	32H	CE2	5.00	
			Tyr	32H	CG	4.54	
			Tyr	32H	CD1	3.92	
			Tyr	32H	CE1	3.91	
			Tyr	32H	CZ	4.47	
			Arg	100H	CB	4.46	
			Arg	100H	CG	4.41	
			Arg	100H	CA	4.25	
			Arg	100H	C	4.67	
			Gly	101H	N	3.97	
			Gly	101H	CA	4.94	
			Trp	102H	CD1	3.90	
Gln	150I	OE1	Ser	31H	O	4.98	*
			Trp	102H	NE1	4.34	*
			Tyr	32H	CD1	4.50	
			Tyr	32H	CE1	4.44	
			Glu	99H	O	4.97	*
			Arg	100H	CB	3.55	
			Arg	100H	CG	3.81	
			Arg	100H	N	4.88	*
			Arg	100H	CA	3.50	
			Arg	100H	C	3.75	
			Arg	100H	O	4.94	*
			Gly	101H	N	3.08	***
			Gly	101H	CA	4.05	
			Gly	101H	C	4.11	
			Gly	101H	O	4.06	*
			Trp	102H	N	4.92	*
			Trp	102H	CB	4.87	
			Trp	102H	CG	4.53	
			Trp	102H	CD1	3.54	
			Arg	100H	CD	4.67	
			Arg	100H	NE	4.23	*
Gln	150I	NE2	Ser	31H	C	3.83	
			Tyr	32H	N	4.34	*
			Tyr	32H	CA	3.88	
			Tyr	32H	CB	4.43	
			Tyr	32H	CD2	4.60	
			Tyr	32H	C	4.97	
			Ser	33H	N	4.94	*
			Ser	31H	O	2.79	***
			Trp	102H	NE1	4.42	*
			Tyr	32H	CE2	4.93	
			Tyr	32H	CG	4.03	
			Tyr	32H	CD1	3.75	
			Tyr	32H	CE1	4.15	
			Tyr	32H	CZ	4.72	
			Glu	99H	O	4.70	*
			Arg	100H	CA	4.51	

hIL-21			aIL-21 Fab56 (Fab35 with H: D62E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Arg	100H	C	4.83	
			Gly	101H	N	4.06	*
			Gly	101H	CA	4.96	
			Trp	102H	CD1	4.25	
Gln	150I	C	Tyr	32H	OH	4.30	
			Arg	100H	NE	4.30	
			Arg	100H	CZ	4.10	
			Arg	100H	NH1	4.71	
			Arg	100H	NH2	3.86	
Gln	150I	O	Tyr	32H	OH	4.24	*
			Arg	100H	CD	4.34	
			Arg	100H	NE	3.59	*
			Arg	100H	CZ	3.25	
			Arg	100H	NH1	3.64	*
			Arg	100H	NH2	3.28	***
His	151I	N	Arg	100H	CZ	4.89	
			Arg	100H	NH2	4.33	*
His	151I	CA	Arg	100H	CZ	4.89	
			Arg	100H	NH2	4.12	
His	151I	CB	Arg	100H	NH2	4.46	
His	151I	CG	Arg	100H	CZ	4.97	
			Arg	100H	NH2	3.76	
His	151I	ND1	Arg	100H	NH2	4.17	*
His	151I	CE1	Trp	102H	CB	3.87	
			Trp	102H	CG	4.10	
			Trp	102H	CD1	4.57	
			Trp	102H	CD2	4.75	
			Arg	100H	NH2	4.11	
His	151I	NE2	Trp	102H	CB	4.38	
			Trp	102H	CG	5.00	
			Arg	100H	CZ	4.83	
			Arg	100H	NH2	3.57	*
His	151I	CD2	Arg	100H	CZ	4.65	
			Arg	100H	NH2	3.32	

Table 15

hIL-21, chain I, (SEQ ID NO: 1) interactions with the the heavy chain (chain H) of Fab57 (SEQ ID No 10, K65R mutation) and light chain (chain L) of anti-IL-21 Fab57 (SEQ ID No 9). A distance cut-off of 5.0 Å was used. The contacts were identified by the CONTACT computer software program of the CCP4 suite (Bailey, 1994). In the last column "****" indicates a strong possibility for a hydrogen bond at this contact (distance < 3.3 Å) as calculated by CONTACT, "*" indicates a weak possibility (distance > 3.3 Å). Blank indicates that the program considered there to be no possibility of a hydrogen bond. Hydrogen-bonds are specific between a donor and an acceptor, are typically strong, and are easily identifiable.

hIL-21			aIL-21 Fab57 (Fab35 with H: K65R)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
Met	39I	CE	Trp	102H	CZ3	4.39	
			Trp	102H	CH2	4.71	
Glu	65I	CB	Tyr	56H	CE2	4.83	
Glu	65I	CD	Tyr	56H	OH	4.73	
Glu	65I	OE1	Tyr	56H	CZ	4.36	
			Tyr	56H	OH	3.75	*
			Tyr	56H	CE2	4.53	
Asp	66I	N	Tyr	56H	CE2	4.77	
			Tyr	56H	CD2	4.49	
Asp	66I	CA	Tyr	56H	CD2	4.76	
			Tyr	57H	CE2	4.75	
Asp	66I	CB	Gly	54H	O	4.76	
			Tyr	56H	CG	3.82	
			Tyr	56H	CD1	4.65	
			Tyr	56H	CE2	4.65	
			Tyr	56H	CD2	3.84	
			Gly	54H	N	4.45	
			Gly	54H	CA	4.10	
			Thr	52H	OG1	4.69	
			Gly	54H	C	4.56	
			Tyr	56H	N	4.63	
			Tyr	56H	CA	4.85	
			Tyr	56H	CB	3.74	
			Tyr	57H	CE2	4.44	
			Tyr	57H	CD2	4.66	
Asp	66I	CG	Gly	54H	O	4.38	
			Tyr	56H	CG	4.42	
			Tyr	56H	CD2	4.87	
			Ser	53H	OG	4.67	
			Ser	53H	C	4.53	
			Gly	54H	N	3.27	
			Gly	54H	CA	3.33	
			Thr	52H	CB	3.90	
			Thr	52H	OG1	3.26	
			Thr	52H	C	4.61	
			Ser	53H	N	4.46	
			Gly	54H	C	3.80	
			Ser	55H	N	4.14	
			Tyr	56H	N	3.99	

hIL-21			aIL-21 Fab57 (Fab35 with H: K65R)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Tyr	56H	CA	4.56	
			Tyr	56H	CB	3.87	
			Tyr	57H	CE2	4.57	
			Tyr	57H	CD2	4.32	
			Thr	52H	CA	4.90	
			Thr	52H	CG2	4.95	
Asp	66I	OD1	Ser	53H	CA	4.31	
			Ser	53H	CB	4.58	
			Ser	53H	OG	3.59	*
			Ser	53H	C	4.12	
			Gly	54H	N	3.06	***
			Gly	54H	CA	3.57	
			Thr	52H	CB	3.64	
			Thr	52H	OG1	3.50	*
			Thr	52H	C	4.22	
			Thr	52H	O	4.96	*
			Ser	53H	N	3.76	*
			Gly	54H	C	4.38	
			Ser	55H	N	4.65	*
			Tyr	57H	CD2	4.93	
			Thr	52H	CA	4.56	
			Thr	52H	CG2	4.71	
Asp	66I	OD2	Gly	54H	O	3.67	*
			Tyr	56H	CG	3.88	
			Tyr	56H	CD1	4.48	
			Tyr	56H	CD2	4.67	
			Ser	53H	C	4.46	
			Gly	54H	N	3.21	***
			Gly	54H	CA	3.13	
			Ser	55H	O	4.89	*
			Thr	52H	CB	3.59	
			Thr	52H	OG1	2.56	***
			Thr	52H	C	4.23	
			Thr	52H	O	4.51	*
			Ser	53H	N	4.43	*
			Gly	54H	C	3.10	
			Ser	55H	N	3.22	***
			Ser	55H	CA	4.03	
			Ser	55H	C	3.81	
			Tyr	56H	N	2.78	***
			Tyr	56H	CA	3.48	
			Tyr	56H	CB	3.12	
			Tyr	56H	C	4.29	
			Tyr	57H	N	4.05	*
			Tyr	57H	CE2	4.59	
			Tyr	57H	CD2	4.10	
			Thr	52H	CA	4.48	
			Thr	52H	CG2	4.73	
Asp	66I	C	Tyr	57H	CZ	4.83	
			Tyr	57H	CE2	3.80	
			Tyr	57H	CD2	4.45	

hIL-21			aIL-21 Fab57 (Fab35 with H: K65R)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Tyr	57H	OH	4.82	
Asp	66I	O	Tyr	56H	CD2	4.84	
			Tyr	57H	CZ	4.31	
			Tyr	57H	CE2	3.58	
			Tyr	57H	CD2	4.55	
			Tyr	57H	OH	3.99	*
Val	67I	N	Tyr	57H	CZ	4.95	
			Tyr	57H	CE2	3.81	
			Tyr	57H	CD2	4.19	
Val	67I	CA	Tyr	57H	CZ	4.66	
			Tyr	57H	CE2	3.73	
			Tyr	57H	CD2	4.18	
			Tyr	57H	OH	4.83	
Val	67I	C	Thr	52H	CB	4.79	
			Tyr	57H	CG	4.55	
			Tyr	57H	CZ	4.60	
			Tyr	57H	CE2	3.62	
			Tyr	57H	CD2	3.59	
			Thr	52H	CG2	4.24	
Val	67I	O	Thr	52H	CB	3.78	
			Thr	52H	OG1	4.39	*
			Tyr	57H	CG	4.83	
			Tyr	57H	CE2	4.27	
			Tyr	57H	CD2	3.91	
			Thr	52H	CG2	3.33	
Glu	68I	N	Tyr	57H	CB	4.88	
			Tyr	57H	CG	4.14	
			Tyr	57H	CD1	4.74	
			Tyr	57H	CE1	4.77	
			Tyr	57H	CZ	4.17	
			Tyr	57H	CE2	3.52	
			Tyr	57H	CD2	3.50	
			Tyr	57H	OH	4.79	*
			Thr	52H	CG2	4.55	
Glu	68I	CA	Tyr	57H	CB	4.54	
			Tyr	57H	CG	4.19	
			Tyr	57H	CD1	4.81	
			Tyr	57H	CZ	4.97	
			Tyr	57H	CE2	4.41	
			Tyr	57H	CD2	3.99	
			Thr	52H	CG2	4.07	
Glu	68I	CB	Tyr	57H	CB	4.98	
			Tyr	57H	CG	4.55	
			Tyr	57H	CD1	4.76	
			Tyr	57H	CE2	4.95	
			Tyr	57H	CD2	4.67	
Glu	68I	CG	Tyr	57H	CB	4.34	
			Tyr	57H	CG	3.71	
			Tyr	57H	CD1	3.56	
			Tyr	57H	CE1	3.79	
			Tyr	57H	CZ	4.13	

hIL-21			aIL-21 Fab57 (Fab35 with H: K65R)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Tyr	57H	CE2	4.31	
			Tyr	57H	CD2	4.11	
			Tyr	57H	OH	4.93	
Glu	68I	CD	Tyr	57H	CB	3.80	
			Tyr	57H	CG	3.59	
			Tyr	57H	CD1	3.34	
			Tyr	57H	CE1	4.01	
			Tyr	57H	CZ	4.74	
			Tyr	57H	CE2	4.96	
			Tyr	57H	CD2	4.45	
			His	59H	CE1	4.34	
			His	59H	NE2	3.64	
			His	59H	CD2	4.73	
Glu	68I	OE1	Tyr	57H	CA	4.91	
			Tyr	57H	CB	3.65	
			Tyr	57H	CG	3.92	
			Tyr	57H	CD1	4.00	
			Tyr	57H	CE1	4.92	
			Tyr	57H	CD2	4.80	
			Tyr	94L	CD1	4.93	
			His	59H	CG	4.98	
			His	59H	CE1	3.86	
			His	59H	NE2	2.89	***
			His	59H	CD2	3.79	
			Tyr	94L	CE1	4.13	
			Tyr	94L	CZ	4.78	
			Tyr	94L	OH	4.58	*
Glu	68I	OE2	Tyr	57H	CB	4.15	
			Tyr	57H	CG	3.85	
			Tyr	57H	CD1	3.17	
			Tyr	57H	CE1	3.74	
			Tyr	57H	CZ	4.77	
			Tyr	57H	CD2	4.89	
			His	59H	CE1	3.98	
			His	59H	NE2	3.61	*
			His	59H	CD2	4.87	
Glu	68I	C	Thr	52H	CG2	4.24	
Thr	69I	N	Ser	33H	OG	4.83	*
			Thr	52H	CG2	3.83	
			Tyr	94L	OH	4.38	*
Thr	69I	CA	Ser	33H	OG	4.32	
			Thr	52H	CG2	4.64	
			Tyr	94L	OH	4.65	
Thr	69I	CB	Ser	33H	CB	4.38	
			Ser	33H	OG	3.76	
			Thr	52H	CG2	4.73	
			Tyr	94L	OH	3.71	
			Glu	99H	CD	4.54	
			Glu	99H	OE1	4.45	
			Glu	99H	OE2	4.42	
			Tyr	96L	OH	4.33	

hIL-21			aIL-21 Fab57 (Fab35 with H: K65R)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
Thr	69I	OG1	Thr	52H	CB	4.72	
			Ser	50H	OG	4.45	*
			Ser	33H	CA	4.81	
			Ser	33H	CB	3.41	
			Ser	33H	OG	2.77	***
			Thr	52H	CA	4.86	
			Thr	52H	CG2	3.57	
			Tyr	94L	OH	3.82	*
			Glu	99H	CD	4.85	
			Glu	99H	OE1	4.43	*
Thr	69I	CG2	Ser	33H	CB	4.10	
			Ser	33H	OG	3.72	
			Tyr	94L	OH	4.71	
			Glu	99H	CG	4.19	
			Glu	99H	CD	3.61	
			Glu	99H	OE1	3.85	
			Glu	99H	OE2	3.55	
			Arg	100H	C	4.73	
			Arg	100H	O	4.39	
			Gly	101H	N	4.58	
			Gly	101H	CA	4.08	
			Tyr	96L	OH	4.28	
Thr	69I	C	Tyr	94L	OH	4.98	
Thr	69I	O	Tyr	94L	OH	4.46	*
Asn	70I	N	Gly	101H	CA	4.06	
			Gly	101H	C	4.65	
			Trp	102H	N	4.36	*
Asn	70I	CA	Gly	101H	CA	4.49	
			Gly	101H	C	4.63	
			Trp	102H	N	4.14	
			Gly	103H	N	4.39	
Asn	70I	CB	Gly	101H	CA	4.90	
			Trp	102H	N	4.86	
			Gly	103H	N	4.40	
			Gly	103H	CA	4.83	
			Tyr	105H	CE1	3.67	
			Tyr	105H	CZ	4.34	
			Tyr	105H	OH	4.39	
			Tyr	105H	CD1	4.41	
Asn	70I	CG	Arg	100H	O	4.51	
			Gly	101H	CA	3.99	
			Gly	101H	C	4.25	
			Gly	101H	O	4.85	
			Trp	102H	N	4.42	
			Gly	103H	N	3.92	
			Gly	103H	CA	4.31	
			Tyr	105H	CE1	3.46	
			Tyr	105H	CZ	4.31	
			Tyr	105H	OH	4.81	
			Tyr	105H	CA	4.97	
			Gly	103H	C	4.89	

hIL-21			aIL-21 Fab57 (Fab35 with H: K65R)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Tyr	104H	N	4.81	
			Tyr	105H	N	4.59	
			Tyr	105H	CG	4.72	
			Tyr	105H	CD1	3.72	
Asn	70I	OD1	Arg	100H	C	4.56	
			Arg	100H	O	3.92	*
			Gly	101H	N	4.36	*
			Gly	101H	CA	3.20	
			Gly	101H	C	3.17	
			Gly	101H	O	3.65	*
			Trp	102H	N	3.43	*
			Trp	102H	CA	4.30	
			Trp	102H	C	3.96	
			Gly	103H	N	2.90	***
			Gly	103H	CA	3.45	
			Tyr	105H	CE1	3.98	
			Tyr	105H	CZ	4.59	
			Tyr	105H	CA	4.77	
			Gly	103H	C	3.95	
			Tyr	104H	N	3.73	*
			Tyr	104H	CA	4.75	
			Tyr	104H	C	4.91	
			Tyr	105H	N	4.03	*
			Tyr	105H	CG	4.72	
			Tyr	105H	CD1	4.06	
Asn	70I	ND2	Glu	99H	CD	4.85	
			Glu	99H	OE2	3.85	*
			Arg	100H	O	4.29	*
			Gly	101H	CA	4.50	
			Phe	91L	CB	4.56	
			Phe	91L	CD1	4.56	
			Phe	91L	O	4.91	*
			Tyr	96L	OH	4.25	*
			Tyr	105H	CE1	3.53	
			Tyr	105H	CZ	4.69	
			Tyr	105H	CA	4.51	
			Tyr	105H	N	4.47	*
			Phe	91L	CG	4.93	
			Tyr	105H	CG	4.61	
			Tyr	105H	CD1	3.47	
Asn	70I	C	Trp	102H	N	4.95	
Asn	70I	O	Tyr	105H	OH	4.64	*
Glu	72I	N	Trp	102H	N	4.98	*
Glu	72I	CA	Trp	102H	CE2	4.69	
			Trp	102H	CZ3	4.56	
			Trp	102H	CH2	4.62	
			Trp	102H	CZ2	4.74	
			Trp	102H	CD2	4.60	
			Trp	102H	CE3	4.57	
Glu	72I	CB	Trp	102H	NE1	3.75	
			Trp	102H	CE2	3.29	

hIL-21			aIL-21 Fab57 (Fab35 with H: K65R)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Trp	102H	CZ3	3.86	
			Trp	102H	CH2	3.77	
			Trp	102H	CZ2	3.56	
			Trp	102H	N	4.37	
			Trp	102H	CA	4.49	
			Trp	102H	CB	4.87	
			Trp	102H	CG	4.00	
			Trp	102H	CD1	4.12	
			Trp	102H	CD2	3.36	
			Trp	102H	CE3	3.68	
Glu	72I	CG	Trp	102H	NE1	3.67	
			Trp	102H	CE2	3.71	
			Trp	102H	CH2	4.72	
			Trp	102H	CZ2	4.09	
			Trp	102H	N	4.45	
			Trp	102H	CG	4.45	
			Trp	102H	CD1	4.10	
			Trp	102H	CD2	4.15	
			Trp	102H	CE3	4.83	
Glu	72I	CD	Trp	102H	NE1	4.23	
			Trp	102H	CE2	4.57	
			Gly	101H	CA	4.08	
			Gly	101H	C	4.26	
			Trp	102H	N	3.55	
			Trp	102H	CA	4.47	
			Trp	102H	CG	4.55	
			Trp	102H	CD1	4.17	
			Trp	102H	CD2	4.71	
Glu	72I	OE1	Trp	102H	NE1	4.69	*
			Trp	102H	CE2	4.92	
			Gly	101H	N	4.97	*
			Gly	101H	CA	3.69	
			Gly	101H	C	3.63	
			Gly	101H	O	4.87	*
			Trp	102H	N	2.74	***
			Trp	102H	CA	3.64	
			Trp	102H	CB	4.66	
			Trp	102H	CG	4.37	
			Trp	102H	CD1	4.31	
			Trp	102H	C	4.71	
			Gly	103H	N	4.70	*
			Trp	102H	CD2	4.67	
Glu	72I	OE2	Ser	33H	OG	4.96	*
			Trp	102H	NE1	4.69	*
			Gly	101H	N	4.72	*
			Gly	101H	CA	3.85	
			Gly	101H	C	4.46	
			Trp	102H	N	4.10	*
			Trp	102H	CD1	4.63	
Glu	72I	C	Trp	102H	CE2	4.98	
			Trp	102H	CZ3	3.93	

hIL-21			aIL-21 Fab57 (Fab35 with H: K65R)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Trp	102H	CH2	4.32	
			Trp	102H	CZ2	4.88	
			Trp	102H	CD2	4.60	
			Trp	102H	CE3	4.12	
Glu	72I	O	Trp	102H	CZ3	3.80	
			Trp	102H	CH2	4.02	
			Trp	102H	CZ2	4.80	
			Trp	102H	CE3	4.41	
Trp	73I	N	Trp	102H	CZ3	4.22	
			Trp	102H	CH2	4.98	
			Trp	102H	CA	4.97	
			Trp	102H	CD2	4.76	
			Trp	102H	CE3	4.12	
Trp	73I	CA	Trp	102H	CZ3	4.39	
			Trp	102H	CE3	4.48	
Trp	73I	CB	Trp	102H	CE3	4.82	
Trp	73I	CG	Trp	102H	CZ3	4.38	
			Trp	102H	CA	4.80	
			Trp	102H	C	4.91	
			Trp	102H	O	4.69	
			Trp	102H	CD2	4.97	
			Trp	102H	CE3	3.93	
Trp	73I	CD1	Trp	102H	CZ3	4.78	
			Trp	102H	CA	3.77	
			Trp	102H	CB	4.13	
			Trp	102H	CG	4.87	
			Trp	102H	C	3.63	
			Trp	102H	O	3.30	
			Gly	103H	N	4.42	
			Trp	102H	CD2	4.68	
			Trp	102H	CE3	3.92	
			Gly	103H	CA	5.00	
Trp	73I	NE1	Trp	102H	CZ3	4.64	
			Trp	102H	CA	3.97	
			Trp	102H	CB	3.81	
			Trp	102H	CG	4.61	
			Trp	102H	C	3.84	
			Trp	102H	O	3.16	***
			Gly	103H	N	4.88	*
			Trp	102H	CD2	4.48	
			Trp	102H	CE3	3.70	
Trp	73I	CE2	Trp	102H	CZ3	4.02	
			Trp	102H	CA	4.98	
			Trp	102H	CB	4.57	
			Trp	102H	O	4.48	
			Trp	102H	CD2	4.51	
			Trp	102H	CE3	3.44	
Trp	73I	CD2	Trp	102H	CZ3	3.85	
			Trp	102H	CD2	4.86	
			Trp	102H	CE3	3.62	
Trp	73I	CE3	Trp	102H	CZ3	3.89	

hIL-21			aIL-21 Fab57 (Fab35 with H: K65R)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Trp	102H	CE3	4.15	
Trp	73I	CZ3	Trp	102H	CZ3	4.14	
			Trp	102H	CE3	4.48	
Trp	73I	CH2	Trp	102H	CZ3	4.34	
			Trp	102H	CE3	4.38	
Trp	73I	CZ2	Trp	102H	CZ3	4.32	
			Trp	102H	CD2	4.96	
			Trp	102H	CE3	3.92	
Phe	76I	CB	Trp	102H	CZ3	4.38	
			Trp	102H	CH2	4.71	
Phe	76I	CG	Trp	102H	CZ3	4.99	
Lys	117I	N	Trp	102H	O	4.83	*
			Gly	103H	CA	4.98	
Lys	117I	CA	Gly	103H	CA	4.74	
Lys	117I	CB	Gly	103H	CA	4.16	
			Tyr	105H	CZ	4.93	
			Tyr	105H	OH	4.52	
			Gly	103H	C	4.97	
			Gly	103H	O	4.72	
			Tyr	105H	CE2	4.33	
Lys	117I	CD	Gly	103H	O	4.47	
			Ser	31L	OG	3.85	
			Asp	50L	CG	4.15	
			Asp	50L	OD1	3.73	
			Asp	50L	OD2	3.91	
			Tyr	105H	CE2	4.40	
			Tyr	105H	CD2	4.86	
Lys	117I	CE	Ser	31L	OG	4.04	
			Asp	50L	CG	4.16	
			Asp	50L	OD1	4.02	
			Asp	50L	OD2	3.52	
Lys	117I	NZ	Ser	31L	OG	3.25	***
			Asp	50L	CB	4.96	
			Asp	50L	CG	3.48	
			Asp	50L	OD1	3.38	*
			Asp	50L	OD2	2.80	***
			Ser	31L	CB	4.00	
			Asp	50L	O	4.84	*
Lys	117I	C	Gly	103H	N	4.64	
			Gly	103H	CA	4.25	
			Tyr	105H	OH	4.40	
Lys	117I	O	Trp	102H	CA	4.71	
			Trp	102H	C	3.92	
			Trp	102H	O	4.05	*
			Gly	103H	N	3.64	*
			Gly	103H	CA	3.55	
			Tyr	105H	OH	4.71	*
			Gly	103H	C	4.97	
His	118I	N	Tyr	105H	OH	3.96	*
His	118I	CA	Tyr	105H	CZ	5.00	
			Tyr	105H	OH	3.81	

hIL-21			aIL-21 Fab57 (Fab35 with H: K65R)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
His	118I	C	Tyr	105H	CZ	4.82	
			Tyr	105H	OH	3.45	
His	118I	O	Tyr	105H	OH	3.70	*
Arg	119I	N	Tyr	105H	CZ	4.82	
			Tyr	105H	OH	3.55	*
Arg	119I	CA	Tyr	105H	OH	4.10	
Arg	119I	CB	Tyr	105H	OH	4.72	
			Asn	92L	O	4.67	
Arg	119I	CG	Phe	91L	O	4.23	
			Tyr	105H	CE1	4.41	
			Tyr	105H	CZ	4.71	
			Tyr	105H	OH	4.04	
			Asn	92L	CA	4.97	
			Asn	92L	C	4.90	
			Asn	92L	O	4.14	
Arg	119I	CD	Phe	91L	C	4.63	
			Phe	91L	O	3.51	
			Asn	92L	CA	4.33	
			Asn	92L	C	3.90	
			Asn	92L	O	3.12	
			Ser	93L	N	4.81	
Arg	119I	NE	Tyr	94L	CE1	4.87	
			Phe	91L	O	4.09	*
			Asn	92L	C	4.92	
			Asn	92L	O	4.21	*
Arg	119I	CZ	Tyr	94L	CD1	5.00	
			Tyr	94L	CE1	4.33	
			Asn	92L	O	4.73	
Arg	119I	NH1	Tyr	94L	CE1	4.84	
			Asn	92L	O	4.41	*
Arg	119I	NH2	Tyr	94L	CD1	4.76	
			Tyr	94L	CE1	3.83	
			Tyr	94L	CZ	4.62	
			Tyr	94L	OH	4.33	*
Thr	121I	OG1	Asn	92L	O	4.93	*
Tyr	128I	CD1	Tyr	57H	OH	4.88	
Tyr	128I	CE1	Tyr	57H	OH	4.33	
Glu	129I	OE2	Tyr	56H	OH	4.71	*
Leu	143I	CA	Trp	102H	CZ2	4.82	
Leu	143I	CB	Trp	102H	CH2	4.85	
			Trp	102H	CZ2	4.89	
Leu	143I	CG	Trp	102H	CZ3	4.83	
			Trp	102H	CH2	3.61	
			Trp	102H	CZ2	3.75	
Leu	143I	CD1	Trp	102H	CE2	4.77	
			Trp	102H	CH2	3.98	
			Trp	102H	CZ2	3.70	
Leu	143I	CD2	Trp	102H	CZ3	4.88	
			Trp	102H	CH2	3.97	
			Trp	102H	CZ2	4.51	
Leu	143I	C	Trp	102H	CH2	4.39	

hIL-21			aIL-21 Fab57 (Fab35 with H: K65R)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Trp	102H	CZ2	4.17	
Leu	143I	O	Trp	102H	CE2	4.44	
			Trp	102H	CH2	3.74	
			Trp	102H	CZ2	3.27	
Leu	144I	N	Trp	102H	CH2	4.98	
Leu	144I	CA	Trp	102H	CH2	4.97	
Gln	145I	O	Ser	31H	OG	4.91	*
Lys	146I	N	Ser	31H	OG	4.81	*
Lys	146I	CA	Ser	31H	OG	4.04	
			Ser	31H	CB	4.39	
			Ser	31H	O	4.96	
			Trp	102H	NE1	4.95	
Lys	146I	CB	Ser	31H	OG	4.33	
			Ser	31H	CA	4.82	
			Ser	31H	CB	4.49	
			Ser	31H	O	4.37	
			Trp	102H	NE1	4.42	
			Trp	102H	CE2	4.85	
			Trp	102H	CZ2	4.56	
Lys	146I	CG	Ser	30H	O	4.78	
			Ser	31H	OG	3.68	
			Ser	31H	CA	4.13	
			Ser	31H	CB	4.08	
			Ser	31H	C	4.73	
			Ser	31H	O	4.32	
Lys	146I	CD	Ser	30H	O	4.53	
			Ser	53H	CB	4.97	
			Ser	53H	OG	4.16	
			Ser	31H	OG	4.76	
			Ser	31H	CA	4.50	
			Ser	31H	CB	4.93	
			Ser	31H	C	4.84	
			Ser	31H	O	4.45	
Lys	146I	CE	Ser	30H	O	3.72	
			Ser	53H	CB	4.49	
			Ser	53H	OG	3.75	
			Ser	31H	OG	4.87	
			Ser	30H	C	4.79	
			Ser	31H	CA	4.57	
Lys	146I	NZ	Ser	30H	O	4.76	*
Lys	146I	C	Trp	102H	NE1	4.11	
			Trp	102H	CE2	4.49	
			Trp	102H	CZ2	4.52	
			Trp	102H	CD1	4.99	
Lys	146I	O	Ser	31H	OG	4.95	*
			Ser	31H	CB	4.71	
			Ser	31H	O	4.86	*
			Trp	102H	NE1	4.28	*
			Trp	102H	CE2	4.91	
			Trp	102H	CD1	4.86	
Met	147I	N	Trp	102H	NE1	3.87	*

hIL-21			aIL-21 Fab57 (Fab35 with H: K65R)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Trp	102H	CE2	3.86	
			Trp	102H	CH2	4.58	
			Trp	102H	CZ2	3.71	
			Trp	102H	CD1	4.79	
			Trp	102H	CD2	4.88	
Met	147I	CA	Trp	102H	NE1	3.79	
			Trp	102H	CE2	3.68	
			Trp	102H	CZ3	4.93	
			Trp	102H	CH2	4.48	
			Trp	102H	CZ2	3.84	
			Trp	102H	CG	4.59	
			Trp	102H	CD1	4.36	
			Trp	102H	CD2	4.27	
			Trp	102H	CE3	4.83	
Met	147I	CA	Trp	102H	NE1	3.73	
			Trp	102H	CE2	3.65	
			Trp	102H	CZ3	4.97	
			Trp	102H	CH2	4.52	
			Trp	102H	CZ2	3.84	
			Trp	102H	CG	4.55	
			Trp	102H	CD1	4.30	
			Trp	102H	CD2	4.26	
			Trp	102H	CE3	4.85	
Met	147I	CB	Trp	102H	NE1	4.33	
			Trp	102H	CE2	3.72	
			Trp	102H	CZ3	4.02	
			Trp	102H	CH2	3.71	
			Trp	102H	CZ2	3.54	
			Trp	102H	CG	4.80	
			Trp	102H	CD1	4.94	
			Trp	102H	CD2	4.09	
			Trp	102H	CE3	4.21	
Met	147I	CB	Trp	102H	NE1	4.01	
			Trp	102H	CE2	3.42	
			Trp	102H	CZ3	3.85	
			Trp	102H	CH2	3.58	
			Trp	102H	CZ2	3.36	
			Trp	102H	CG	4.41	
			Trp	102H	CD1	4.57	
			Trp	102H	CD2	3.75	
			Trp	102H	CE3	3.93	
Met	147I	CG	Trp	102H	NE1	4.84	
			Trp	102H	CE2	4.15	
			Trp	102H	CZ3	3.89	
			Trp	102H	CH2	4.12	
			Trp	102H	CZ2	4.26	
			Trp	102H	CG	4.55	
			Trp	102H	CD2	3.95	
			Trp	102H	CE3	3.81	
Met	147I	CG	Trp	102H	NE1	4.25	
			Trp	102H	CE2	3.64	

hIL-21			aIL-21 Fab57 (Fab35 with H: K65R)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Trp	102H	CZ3	3.67	
			Trp	102H	CH2	3.96	
			Trp	102H	CZ2	3.97	
			Trp	102H	CB	4.49	
			Trp	102H	CG	3.81	
			Trp	102H	CD1	4.36	
			Trp	102H	CD2	3.33	
			Trp	102H	CE3	3.34	
Met	147I	SD	Trp	102H	CZ3	4.15	
			Trp	102H	CH2	4.66	
			Trp	102H	CD2	4.64	
			Trp	102H	CE3	4.15	
His	149I	CB	Ile	28H	CB	4.70	
			Ile	28H	CG1	4.85	
			Ser	31H	OG	3.93	
			Ser	31H	CB	4.05	
His	149I	CG	Ile	28H	CB	4.14	
			Ile	28H	CG1	4.03	
			Ile	28H	CG2	4.47	
			Ser	31H	OG	3.83	
			Ser	31H	CB	4.42	
His	149I	ND1	Ile	28H	CB	3.91	
			Ile	28H	CG1	4.03	
			Ile	28H	CG2	3.77	
			Ser	31H	OG	2.95	***
			Ser	31H	CB	3.91	
His	149I	CE1	Ile	28H	CD1	4.79	
			Ile	28H	CB	4.07	
			Ile	28H	CG1	3.83	
			Ile	28H	CG2	3.75	
			Ser	31H	OG	3.90	
His	149I	NE2	Ile	28H	CD1	4.60	
			Ile	28H	CB	4.38	
			Ile	28H	CG1	3.69	
			Ile	28H	CG2	4.44	
His	149I	CD2	Ile	28H	CD1	5.00	
			Ile	28H	CB	4.42	
			Ile	28H	CG1	3.81	
			Ile	28H	CG2	4.84	
His	149I	C	Tyr	32H	OH	4.03	
			Tyr	32H	CZ	4.82	
His	149I	O	Tyr	32H	OH	3.58	*
			Tyr	32H	CZ	4.66	
Gln	150I	N	Tyr	32H	OH	4.14	*
			Tyr	32H	CZ	4.70	
Gln	150I	CA	Tyr	32H	OH	3.57	
			Tyr	32H	CE1	4.46	
			Tyr	32H	CZ	4.16	
			Arg	100H	NE	4.70	
			Arg	100H	CZ	4.91	
			Arg	100H	NH2	4.85	

hIL-21			aIL-21 Fab57 (Fab35 with H: K65R)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
Gln	150I	CB	Tyr	32H	OH	4.29	
			Tyr	32H	CE1	4.35	
			Tyr	32H	CZ	4.46	
			Arg	100H	CG	4.64	
			Trp	102H	CD1	4.73	
			Arg	100H	CD	4.89	
			Arg	100H	NE	4.18	
			Arg	100H	CZ	4.67	
			Arg	100H	NH2	4.59	
Gln	150I	CG	Tyr	32H	CD2	4.78	
			Ser	31H	CB	4.99	
			Ser	31H	C	4.97	
			Ser	31H	O	4.06	
			Trp	102H	NE1	4.79	
			Tyr	32H	CE2	4.43	
			Tyr	32H	OH	4.13	
			Tyr	32H	CG	4.70	
			Tyr	32H	CD1	4.15	
			Tyr	32H	CE1	3.72	
			Tyr	32H	CZ	3.85	
			Arg	100H	CG	4.82	
			Trp	102H	CD1	4.55	
Gln	150I	CD	Tyr	32H	CA	4.97	
			Ser	31H	C	4.86	
			Ser	31H	O	3.82	
			Trp	12H	NE1	4.28	
			Tyr	32H	CG	4.57	
			Tyr	32H	CD1	3.94	
			Tyr	32H	CE1	3.95	
			Tyr	32H	CZ	4.51	
			Arg	100H	CB	4.55	
			Arg	100H	CG	4.37	
			Arg	100H	CA	4.24	
			Arg	100H	C	4.65	
			Gly	101H	N	3.93	
			Gly	101H	CA	4.90	
			Trp	102H	CD1	3.85	
Gln	150I	OE1	Ser	31H	O	4.94	*
			Trp	102H	NE1	4.33	*
			Tyr	32H	CD1	4.49	
			Tyr	32H	CE1	4.47	
			Glu	99H	O	4.93	*
			Arg	100H	CB	3.63	
			Arg	100H	CG	3.74	
			Arg	100H	N	4.81	*
			Arg	100H	CA	3.46	
			Arg	100H	C	3.71	
			Arg	100H	O	4.89	*
			Gly	101H	N	3.01	***
			Gly	101H	CA	3.99	
			Gly	101H	C	4.07	

hIL-21			aIL-21 Fab57 (Fab35 with H: K65R)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Gly	101H	O	4.01	*
			Trp	102H	N	4.89	*
			Trp	102H	CB	4.86	
			Trp	102H	CG	4.50	
			Trp	102H	CD1	3.51	
			Arg	100H	CD	4.70	
			Arg	100H	NE	4.28	*
Gln	150I	NE2	Tyr	32H	N	4.30	*
			Tyr	32H	CA	3.82	
			Tyr	32H	CB	4.39	
			Tyr	32H	CD2	4.59	
			Tyr	32H	C	4.87	
			Ser	33H	N	4.84	*
			Ser	31H	CA	4.95	
			Ser	31H	CB	4.98	
			Ser	31H	C	3.78	
			Ser	31H	O	2.71	***
			Trp	102H	NE1	4.44	*
			Tyr	32H	CE2	4.96	
			Tyr	32H	CG	4.01	
			Tyr	32H	CD1	3.73	
			Tyr	32H	CE1	4.17	
			Glu	99H	O	4.64	*
			Tyr	32H	CZ	4.72	
			Arg	100H	CA	4.54	
			Arg	100H	C	4.86	
			Gly	101H	N	4.08	*
			Gly	101H	CA	4.97	
			Trp	102H	CD1	4.28	
Gln	150I	C	Tyr	32H	OH	4.36	
			Arg	100H	NE	4.31	
			Arg	100H	CZ	4.10	
			Arg	100H	NH1	4.75	
			Arg	100H	NH2	3.88	
Gln	150I	O	Tyr	32H	OH	4.33	*
			Arg	100H	CG	4.99	
			Arg	100H	CD	4.26	
			Arg	100H	NE	3.53	*
			Arg	100H	CZ	3.18	
			Arg	100H	NH1	3.62	*
			Arg	100H	NH2	3.22	***
His	151I	N	Arg	100H	CZ	4.88	
			Arg	100H	NH2	4.33	*
His	151I	CA	Arg	100H	CZ	4.82	
			Arg	100H	NH2	4.07	
His	151I	CB	Arg	100H	NH2	4.39	
His	151I	CG	Arg	100H	CZ	4.91	
			Arg	100H	NH2	3.71	
His	151I	ND1	Trp	102H	CB	4.95	
			Trp	102H	CG	4.94	
			Arg	100H	NH2	4.13	*

hIL-21			aIL-21 Fab57 (Fab35 with H: K65R)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
His	151I	CE1	Trp	102H	CB	3.73	
			Trp	102H	CG	3.99	
			Trp	102H	CD1	4.50	
			Trp	102H	CD2	4.70	
			Arg	100H	NH2	4.09	
His	151I	NE2	Trp	102H	CB	4.23	
			Trp	102H	CG	4.89	
			Trp	102H	O	4.90	*
			Arg	100H	CZ	4.82	
			Arg	100H	NH2	3.58	*
His	151I	CD2	Arg	100H	CZ	4.64	
			Arg	100H	NH2	3.31	

Table 16.

hIL-21, chain I, (SEQ ID No 1) interactions with the the heavy chain (chain H) of Fab59 (SEQ ID No 10) and light chain (chain L) of anti-IL-21 Fab59 (SEQ ID No 9, mutation Q27N). A distance cut-off of 5.0 Å was used. The contacts were identified by the CONTACT computer software program of the CCP4 suite (Bailey, 1994). In the last column "****" indicates a strong possibility for a hydrogen bond at this contact (distance < 3.3 Å) as calculated by CONTACT, "*" indicates a weak possibility (distance > 3.3 Å). Blank indicates that the program considered there to be no possibility of a hydrogen bond. Hydrogen-bonds are specific between a donor and an acceptor, are typically strong, and are easily identifiable.

hIL-21			aIL-21 Fab59 (Fab35 with L, Q27N mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
Glu	65I	N	Tyr	56H	CE2	4.80	
Glu	65I	CA	Tyr	56H	OH	4.99	
			Tyr	56H	CE2	4.73	
Glu	65I	CB	Tyr	56H	CD2	4.93	
			Tyr	56H	CZ	4.04	
			Tyr	56H	OH	3.62	
			Tyr	56H	CE2	3.94	
Glu	65I	CG	Tyr	56H	CZ	4.72	
			Tyr	56H	OH	3.90	
			Tyr	56H	CE2	4.78	
Glu	65I	CD	Tyr	56H	CZ	4.31	
			Tyr	56H	OH	3.18	
			Tyr	56H	CE2	4.77	
Glu	65I	OE1	Tyr	56H	CZ	4.59	
			Tyr	56H	OH	3.50	*
Glu	65I	OE2	Tyr	56H	CZ	4.20	
			Tyr	56H	OH	2.93	***
			Tyr	56H	CE2	4.70	
Glu	65I	C	Tyr	56H	CE2	4.78	
Asp	66I	N	Tyr	56H	CD2	4.39	
			Tyr	56H	CE2	4.35	
Asp	66I	CA	Tyr	56H	CD2	4.55	
			Tyr	56H	CE2	4.91	
Asp	66I	CB	Tyr	56H	CD2	3.61	
			Tyr	56H	CG	3.74	
			Tyr	56H	CE1	4.82	
			Tyr	56H	CZ	4.71	
			Tyr	56H	CE2	4.15	
			Tyr	56H	CD1	4.31	
			Tyr	57H	CE2	4.71	
			Tyr	57H	CD2	4.82	
			Tyr	56H	CA	4.98	
			Tyr	56H	CB	3.97	
			Thr	52H	OG1	4.85	
			Gly	54H	N	4.62	
			Gly	54H	CA	4.31	
			Gly	54H	C	4.75	
			Tyr	56H	N	4.75	
Asp	66I	CG	Tyr	56H	CD2	4.62	

hIL-21			aIL-21 Fab59 (Fab35 with L, Q27N mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Tyr	56H	CG	4.30	
			Gly	54H	O	4.52	
			Tyr	56H	CD1	4.73	
			Tyr	57H	CE2	4.93	
			Tyr	57H	CD2	4.59	
			Tyr	56H	CA	4.72	
			Tyr	56H	CB	4.12	
			Thr	52H	CB	4.03	
			Thr	52H	OG1	3.51	
			Thr	52H	C	4.86	
			Ser	53H	N	4.65	
			Gly	54H	N	3.37	
			Gly	54H	CA	3.44	
			Gly	54H	C	3.91	
			Ser	55H	N	4.25	
			Tyr	56H	N	4.12	
			Ser	53H	OG	4.67	
			Ser	53H	C	4.62	
			Thr	52H	CG2	5.00	
Asp	66I	OD1	Thr	52H	CB	3.80	
			Thr	52H	OG1	3.73	*
			Thr	52H	C	4.40	
			Ser	53H	N	3.89	*
			Gly	54H	N	3.07	***
			Gly	54H	CA	3.55	
			Gly	54H	C	4.38	
			Ser	55H	N	4.63	*
			Ser	53H	CB	4.51	
			Ser	53H	OG	3.53	*
			Ser	53H	CA	4.33	
			Ser	53H	C	4.11	
			Thr	52H	CA	4.77	
			Thr	52H	CG2	4.81	
Asp	66I	OD2	Tyr	56H	CD2	4.42	
			Tyr	56H	CG	3.76	
			Gly	54H	O	3.91	*
			Tyr	56H	CD1	4.16	
			Tyr	57H	CE2	4.70	
			Tyr	57H	CD2	4.13	
			Tyr	56H	CA	3.66	
			Tyr	56H	CB	3.32	
			Tyr	56H	C	4.41	
			Tyr	57H	N	4.14	*
			Thr	52H	CB	3.57	
			Thr	52H	OG1	2.68	***
			Thr	52H	C	4.44	
			Thr	52H	O	4.76	*
			Ser	53H	N	4.57	*
			Gly	54H	N	3.31	*
			Gly	54H	CA	3.33	

hIL-21			aIL-21 Fab59 (Fab35 with L, Q27N mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Gly	54H	C	3.32	
			Ser	55H	N	3.42	*
			Ser	55H	CA	4.24	
			Ser	55H	C	4.02	
			Tyr	56H	N	2.96	***
			Ser	53H	C	4.57	
			Thr	52H	CA	4.61	
			Thr	52H	CG2	4.59	
Asp	66I	C	Tyr	56H	CD2	4.77	
			Tyr	57H	CE2	3.95	
			Tyr	57H	CD2	4.49	
Asp	66I	O	Tyr	56H	CD2	4.32	
			Tyr	56H	CE2	4.91	
			Tyr	57H	CZ	4.40	
			Tyr	57H	OH	4.25	*
			Tyr	57H	CE2	3.56	
			Tyr	57H	CD2	4.46	
Val	67I	N	Tyr	57H	CE2	3.90	
			Tyr	57H	CD2	4.19	
Val	67I	CA	Tyr	57H	CZ	4.47	
			Tyr	57H	OH	4.76	
			Tyr	57H	CE2	3.57	
			Tyr	57H	CD2	4.00	
Val	67I	CA	Tyr	57H	CZ	4.71	
			Tyr	57H	OH	4.99	
			Tyr	57H	CE2	3.81	
			Tyr	57H	CD2	4.21	
Val	67I	CB	Tyr	57H	CE2	4.91	
Val	67I	C	Tyr	57H	CZ	4.58	
			Tyr	57H	CE2	3.67	
			Tyr	57H	CD2	3.61	
			Tyr	57H	CG	4.49	
			Thr	52H	CB	4.97	
			Thr	52H	CG2	4.36	
Val	67I	O	Tyr	57H	CE2	4.26	
			Tyr	57H	CD2	3.83	
			Tyr	57H	CB	5.00	
			Tyr	57H	CG	4.67	
			Thr	52H	CB	3.92	
			Thr	52H	OG1	4.43	*
			Thr	52H	CG2	3.40	
Glu	68I	N	Tyr	57H	CE1	4.68	
			Tyr	57H	CZ	4.20	
			Tyr	57H	OH	4.87	*
			Tyr	57H	CE2	3.63	
			Tyr	57H	CD2	3.60	
			Tyr	57H	CB	4.86	
			Tyr	57H	CG	4.13	
			Tyr	57H	CD1	4.64	
			Thr	52H	CG2	4.68	

hIL-21			aIL-21 Fab59 (Fab35 with L, Q27N mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
Glu	68I	CA	Tyr	57H	CE1	4.96	
			Tyr	57H	CZ	4.89	
			Tyr	57H	CE2	4.45	
			Tyr	57H	CD2	4.02	
			Tyr	57H	CB	4.43	
			Tyr	57H	CG	4.09	
			Tyr	57H	CD1	4.58	
			Thr	52H	CG2	4.18	
Glu	68I	CB	Tyr	57H	CE1	4.80	
			Tyr	57H	CD2	4.76	
			Tyr	57H	CB	4.92	
			Tyr	57H	CG	4.49	
			Tyr	57H	CD1	4.53	
Glu	68I	CG	Tyr	57H	CE1	3.51	
			Tyr	57H	CZ	4.03	
			Tyr	57H	OH	4.83	
			Tyr	57H	CE2	4.34	
			Tyr	57H	CD2	4.18	
			Tyr	57H	CB	4.29	
			Tyr	57H	CG	3.65	
			Tyr	57H	CD1	3.30	
Glu	68I	CD	Tyr	57H	CE1	3.87	
			Tyr	57H	CZ	4.77	
			Tyr	57H	CD2	4.66	
			Tyr	57H	CB	3.92	
			Tyr	57H	CG	3.71	
			Tyr	57H	CD1	3.24	
			His	59H	CE1	4.25	
			Tyr	94L	CE2	4.87	
			His	59H	NE2	3.54	
			His	59H	CD2	4.63	
Glu	68I	OE1	Tyr	57H	CE1	4.67	
			Tyr	57H	CD2	4.86	
			Tyr	57H	CA	4.79	
			Tyr	57H	CB	3.56	
			Tyr	57H	CG	3.83	
			Tyr	57H	CD1	3.74	
			Tyr	94L	CD2	4.69	
			His	59H	ND1	4.96	*
			His	59H	CE1	3.83	
			Tyr	94L	CZ	4.61	
			Tyr	94L	OH	4.58	*
			Tyr	94L	CE2	3.97	
			His	59H	CG	4.90	
			His	59H	NE2	2.81	***
			His	59H	CD2	3.67	
Glu	68I	OE2	Tyr	57H	CE1	3.86	
			Tyr	57H	CZ	4.99	
			Tyr	57H	CB	4.52	
			Tyr	57H	CG	4.23	

hIL-21			aIL-21 Fab59 (Fab35 with L, Q27N mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Tyr	57H	CD1	3.38	
			His	59H	CE1	3.79	
			His	59H	NE2	3.47	*
			His	59H	CD2	4.76	
Glu	68I	C	Thr	52H	CG2	4.29	
Thr	69I	N	Tyr	94L	OH	4.53	*
			Ser	33H	OG	4.78	*
			Thr	52H	CG2	3.80	
Thr	69I	CA	Tyr	94L	OH	4.69	
			Ser	33H	OG	4.30	
			Thr	52H	CG2	4.67	
Thr	69I	CB	Tyr	94L	OH	3.73	
			Ser	33H	CB	4.38	
			Ser	33H	OG	3.68	
			Thr	52H	CG2	4.73	
			Tyr	96L	OH	4.41	
			Glu	99H	CD	4.63	
			Glu	99H	OE1	4.57	
			Glu	99H	OE2	4.55	
Thr	69I	OG1	Thr	52H	CB	4.69	
			Tyr	94L	OH	3.93	*
			Ser	50H	OG	4.64	*
			Ser	33H	CA	4.76	
			Ser	33H	CB	3.37	
			Ser	33H	OG	2.64	***
			Thr	52H	CA	4.77	
			Thr	52H	CG2	3.59	
			Glu	99H	CD	4.91	
			Glu	99H	OE1	4.55	*
Thr	69I	CG2	Tyr	94L	OH	4.69	
			Ser	33H	CB	4.12	
			Ser	33H	OG	3.66	
			Tyr	96L	OH	4.28	
			Arg	100H	C	4.70	
			Arg	100H	O	4.37	
			Gly	101H	N	4.53	
			Gly	101H	CA	4.09	
			Glu	99H	CG	4.21	
			Glu	99H	CD	3.63	
			Glu	99H	OE1	3.93	
			Glu	99H	OE2	3.57	
Thr	69I	O	Tyr	94L	OH	4.54	*
Asn	70I	N	Trp	102H	N	4.38	*
			Gly	101H	CA	4.14	
			Gly	101H	C	4.75	
Asn	70I	CA	Trp	102H	N	4.20	
			Gly	103H	N	4.46	
			Gly	101H	CA	4.56	
			Gly	101H	C	4.74	
Asn	70I	CB	Gly	103H	CA	4.91	

hIL-21			aIL-21 Fab59 (Fab35 with L, Q27N mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Tyr	105H	CE1	3.59	
			Tyr	105H	CZ	4.07	
			Tyr	105H	OH	4.04	
			Trp	102H	N	4.95	
			Gly	103H	N	4.56	
			Tyr	105H	CD1	4.33	
Asn	70I	CG	Gly	103H	CA	4.36	
			Tyr	105H	CE1	3.37	
			Tyr	105H	CZ	4.07	
			Tyr	105H	OH	4.54	
			Trp	102H	N	4.48	
			Gly	103H	N	4.05	
			Arg	100H	O	4.62	
			Gly	101H	CA	4.10	
			Gly	101H	C	4.37	
			Gly	101H	O	4.98	
			Tyr	105H	CA	4.92	
			Tyr	105H	CG	4.55	
			Tyr	105H	CD1	3.61	
			Tyr	105H	CE2	4.95	
			Gly	103H	C	4.97	
			Tyr	104H	N	4.91	
			Tyr	105H	N	4.64	
Asn	70I	OD1	Gly	103H	CA	3.39	
			Tyr	105H	CE1	3.90	
			Tyr	105H	CZ	4.36	
			Tyr	105H	OH	4.95	*
			Trp	102H	N	3.47	*
			Trp	102H	CA	4.34	
			Trp	102H	C	4.02	
			Gly	103H	N	2.93	***
			Arg	100H	C	4.69	
			Arg	100H	O	4.08	*
			Gly	101H	N	4.50	*
			Gly	101H	CA	3.34	
			Gly	101H	C	3.29	
			Gly	101H	O	3.78	*
			Tyr	105H	CA	4.70	
			Tyr	105H	CG	4.54	
			Tyr	105H	CD1	3.94	
			Tyr	105H	CE2	4.93	
			Tyr	105H	CD2	4.98	
			Gly	103H	C	3.95	
			Tyr	104H	N	3.78	*
			Tyr	104H	CA	4.86	
			Tyr	104H	C	4.95	
			Tyr	105H	N	4.05	*
Asn	70I	ND2	Tyr	105H	CE1	3.46	
			Tyr	105H	CZ	4.49	
			Phe	91L	CB	4.55	

hIL-21			aIL-21 Fab59 (Fab35 with L, Q27N mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Phe	91L	CD2	4.42	
			Tyr	96L	OH	4.15	*
			Arg	100H	O	4.35	*
			Gly	101H	CA	4.55	
			Glu	99H	CD	4.80	
			Glu	99H	OE2	3.81	*
			Tyr	105H	CA	4.49	
			Tyr	105H	CG	4.49	
			Tyr	105H	CD1	3.39	
			Phe	91L	CG	4.84	
			Tyr	105H	N	4.53	*
Asn	70I	O	Tyr	105H	OH	4.32	*
Glu	72I	CA	Trp	102H	CE2	4.68	
			Trp	102H	CD2	4.57	
			Trp	102H	CE3	4.51	
			Trp	102H	CZ3	4.52	
			Trp	102H	CH2	4.59	
			Trp	102H	CZ2	4.72	
Glu	72I	CB	Trp	102H	NE1	3.78	
			Trp	102H	CE2	3.36	
			Trp	102H	CD2	3.35	
			Trp	102H	CE3	3.66	
			Trp	102H	CZ3	3.93	
			Trp	102H	CH2	3.90	
			Trp	102H	CZ2	3.68	
			Trp	102H	N	4.30	
			Trp	102H	CA	4.36	
			Trp	102H	CB	4.71	
			Trp	102H	CG	3.87	
			Trp	102H	CD1	4.10	
Glu	72I	CG	Trp	102H	NE1	3.76	
			Trp	102H	CE2	3.82	
			Trp	102H	CD2	4.18	
			Trp	102H	CE3	4.86	
			Trp	102H	CH2	4.83	
			Trp	102H	CZ2	4.22	
			Trp	102H	N	4.50	
			Trp	102H	CG	4.40	
			Trp	102H	CD1	4.17	
Glu	72I	CD	Trp	102H	NE1	4.30	
			Trp	102H	CE2	4.68	
			Trp	102H	CD2	4.79	
			Trp	102H	N	3.68	
			Trp	102H	CA	4.59	
			Trp	102H	CG	4.54	
			Trp	102H	CD1	4.25	
			Gly	101H	CA	4.13	
			Gly	101H	C	4.42	
Glu	72I	OE1	Trp	102H	NE1	4.76	*
			Trp	102H	CD2	4.77	

hIL-21			aIL-21 Fab59 (Fab35 with L, Q27N mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Trp	102H	N	2.85	***
			Trp	102H	CA	3.77	
			Trp	102H	CB	4.68	
			Trp	102H	CG	4.38	
			Trp	102H	CD1	4.38	
			Trp	102H	C	4.87	
			Gly	103H	N	4.82	*
			Gly	101H	N	4.90	*
			Gly	101H	CA	3.62	
			Gly	101H	C	3.72	
			Gly	101H	O	4.94	*
Glu	72I	OE2	Trp	102H	NE1	4.74	*
			Ser	33H	OG	4.98	*
			Trp	102H	N	4.29	*
			Trp	102H	CD1	4.69	
			Gly	101H	N	4.83	*
			Gly	101H	CA	4.01	
			Gly	101H	C	4.70	
Glu	72I	C	Trp	102H	CE2	4.90	
			Trp	102H	CD2	4.55	
			Trp	102H	CE3	4.02	
			Trp	102H	CZ3	3.82	
			Trp	102H	CH2	4.20	
			Trp	102H	CZ2	4.76	
Glu	72I	O	Trp	102H	CD2	4.94	
			Trp	102H	CE3	4.27	
			Trp	102H	CZ3	3.64	
			Trp	102H	CH2	3.82	
			Trp	102H	CZ2	4.59	
Trp	73I	N	Trp	102H	CD2	4.69	
			Trp	102H	CE3	3.99	
			Trp	102H	CZ3	4.07	
			Trp	102H	CH2	4.84	
			Trp	102H	CA	4.92	
Trp	73I	CA	Trp	102H	CE3	4.35	
			Trp	102H	CZ3	4.23	
Trp	73I	CB	Trp	102H	CE3	4.73	
			Trp	102H	CZ3	4.93	
Trp	73I	CG	Trp	102H	CE3	3.91	
			Trp	102H	CZ3	4.35	
			Trp	102H	CA	4.76	
			Trp	102H	C	4.85	
			Trp	102H	O	4.73	
Trp	73I	CD1	Trp	102H	CD2	4.81	
			Trp	102H	CE3	3.97	
			Trp	102H	CZ3	4.80	
			Gly	103H	CA	4.80	
			Trp	102H	CA	3.80	
			Trp	102H	CB	4.27	
			Trp	102H	C	3.61	

hIL-21			aIL-21 Fab59 (Fab35 with L, Q27N mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Trp	102H	O	3.38	
			Gly	103H	N	4.31	
Trp	73I	NE1	Trp	102H	CD2	4.59	
			Trp	102H	CE3	3.76	
			Trp	102H	CZ3	4.68	
			Trp	102H	CA	3.94	
			Trp	102H	CB	3.93	
			Trp	102H	CG	4.74	
			Trp	102H	C	3.73	
			Trp	102H	O	3.11	***
			Gly	103H	N	4.71	*
Trp	73I	CE2	Trp	102H	CD2	4.62	
			Trp	102H	CE3	3.51	
			Trp	102H	CZ3	4.09	
			Trp	102H	CA	4.92	
			Trp	102H	CB	4.64	
			Trp	102H	C	4.96	
			Trp	102H	O	4.38	
Trp	73I	CD2	Trp	102H	CD2	4.94	
			Trp	102H	CE3	3.64	
			Trp	102H	CZ3	3.85	
Trp	73I	CE3	Trp	102H	CE3	4.12	
			Trp	102H	CZ3	3.84	
Trp	73I	CZ3	Trp	102H	CE3	4.47	
			Trp	102H	CZ3	4.10	
Trp	73I	CH2	Trp	102H	CE3	4.44	
			Trp	102H	CZ3	4.40	
Trp	73I	CZ2	Trp	102H	CE3	3.98	
			Trp	102H	CZ3	4.39	
Phe	76I	CB	Trp	102H	CZ3	3.95	
			Trp	102H	CH2	4.19	
Phe	76I	CG	Trp	102H	CZ3	4.48	
			Trp	102H	CH2	4.78	
Phe	76I	CD1	Trp	102H	CZ3	4.59	
			Trp	102H	CH2	4.62	
Ala	112I	C	Trp	102H	O	4.12	
Ala	112I	O	Trp	102H	CB	4.62	
			Trp	102H	C	4.57	
			Trp	102H	O	3.39	*
Gly	113I	N	Trp	102H	O	4.18	*
Gly	113I	CA	Gly	103H	CA	4.35	
			Trp	102H	C	4.50	
			Trp	102H	O	3.38	
			Gly	103H	N	4.93	
			Asp	50L	OD2	4.85	
			Gly	103H	O	4.10	
			Gly	103H	C	4.30	
Gly	113I	C	Gly	103H	CA	4.66	
			Trp	102H	C	4.76	
			Trp	102H	O	3.81	

hIL-21			aIL-21 Fab59 (Fab35 with L, Q27N mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Gly	103H	O	4.98	
Gly	113I	O	Gly	103H	CA	4.14	
			Trp	102H	C	4.62	
			Trp	102H	O	3.94	*
			Gly	103H	N	4.76	*
			Gly	103H	O	4.76	*
			Gly	103H	C	4.81	
Arg	114I	N	Trp	102H	O	4.66	*
Gln	116I	CG	Asp	50L	OD1	4.81	
			Tyr	105H	CE2	4.89	
Gln	116I	CD	Gly	103H	CA	4.60	
			Ser	31L	CB	4.98	
			Asp	50L	CG	3.93	
			Asp	50L	OD1	3.39	
			Asp	50L	OD2	3.74	
			Gly	103H	O	4.02	
			Tyr	105H	CE2	3.87	
			Tyr	105H	CD2	4.25	
			Gly	103H	C	4.77	
Gln	116I	OE1	Ser	31L	N	4.89	*
			Ser	31L	CA	4.73	
			Ser	31L	CB	3.74	
			Asp	50L	CG	3.31	
			Asp	50L	OD1	2.49	***
			Asp	50L	OD2	3.40	*
			Gly	103H	O	4.30	*
			Ser	31L	C	4.80	
			Ser	31L	O	4.75	*
			Asp	50L	CB	4.75	
			Tyr	105H	CE2	3.72	
			Tyr	105H	CD2	3.89	
			Ser	31L	OG	4.34	*
Gln	116I	NE2	Gly	103H	CA	3.44	
			Tyr	105H	CZ	4.94	
			Trp	102H	O	4.87	*
			Gly	103H	N	4.76	*
			Asp	50L	CG	3.83	
			Asp	50L	OD1	3.59	*
			Asp	50L	OD2	3.54	*
			Gly	103H	O	2.94	***
			Tyr	105H	CE2	3.83	
			Tyr	105H	CD2	4.05	
			Gly	103H	C	3.58	
			Tyr	104H	N	4.86	*
Lys	117I	CA	Asp	30L	CG	4.74	
			Asp	30L	OD2	4.33	
Lys	117I	CB	Asp	30L	OD1	3.90	
			Asp	30L	CB	4.08	
			Asp	30L	CG	3.56	
			Asp	30L	OD2	3.38	

hIL-21			aIL-21 Fab59 (Fab35 with L, Q27N mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
Lys	117I	CG	Asp	30L	OD1	4.25	
			Asp	30L	CB	4.79	
			Asp	30L	CG	4.31	
			Asp	30L	OD2	4.47	
Lys	117I	CD	Asp	30L	OD1	3.48	
			Asp	30L	CB	4.68	
			Asp	30L	CG	3.98	
			Asp	30L	OD2	4.38	
Lys	117I	CE	Asp	30L	OD1	4.56	
Lys	117I	NZ	Asp	30L	OD1	4.47	*
			Ser	67L	OG	4.88	*
Lys	117I	C	Asp	30L	CB	4.88	
			Asp	30L	CG	4.71	
			Asp	30L	OD2	4.04	
Lys	117I	O	Tyr	105H	OH	4.71	*
			Asp	30L	CB	4.39	
			Asp	30L	CG	4.60	
			Asp	30L	OD2	4.07	*
			Tyr	105H	CE2	4.96	
His	118I	N	Asp	30L	OD2	4.47	*
His	118I	CA	Tyr	105H	OH	4.54	
			Asp	30L	OD2	4.88	
His	118I	ND1	Gly	103H	CA	4.99	
			Tyr	105H	OH	4.33	*
His	118I	CE1	Gly	103H	CA	4.46	
			Gly	103H	N	4.81	
His	118I	C	Tyr	105H	OH	4.74	
			Asp	30L	OD2	4.49	
His	118I	O	Asp	30L	OD2	3.90	*
Arg	119I	N	Tyr	105H	OH	4.16	*
Arg	119I	CA	Tyr	105H	OH	4.92	
Arg	119I	CB	Asn	92L	O	4.54	
			Tyr	105H	OH	4.55	
Arg	119I	CG	Asn	92L	CA	4.83	
			Asn	92L	ND2	4.87	
			Asn	92L	O	4.24	
			Phe	91L	O	4.82	
			Asn	92L	C	4.98	
			Tyr	105H	CE1	4.63	
			Tyr	105H	CZ	4.39	
			Tyr	105H	OH	3.20	
Arg	119I	CD	Asn	92L	CA	4.04	
			Asn	92L	O	3.46	
			Phe	91L	C	4.43	
			Phe	91L	O	3.47	
			Asn	92L	N	4.73	
			Asn	92L	C	4.06	
			Tyr	105H	CE1	4.13	
			Tyr	105H	CZ	4.34	
			Tyr	105H	OH	3.53	

hIL-21			aIL-21 Fab59 (Fab35 with L, Q27N mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
Arg	119I	NE	Asn	92L	O	4.22	*
			Phe	91L	O	3.94	*
			Asn	92L	C	4.88	
			Tyr	105H	CE1	4.56	
			Tyr	105H	CZ	4.93	
			Tyr	105H	OH	4.18	*
Arg	119I	CZ	Tyr	94L	CE2	4.85	
			Asn	92L	O	4.03	
			Phe	91L	O	4.18	
			Asn	92L	C	4.78	
Arg	119I	NH1	Tyr	94L	CD2	4.96	
			Tyr	94L	CE2	4.69	
			Asn	92L	CA	4.71	
			Asn	92L	O	2.95	***
			Phe	91L	O	4.00	*
			Asn	92L	C	3.81	
			Ser	93L	N	4.48	*
			Ser	93L	CA	4.59	
			Ser	93L	CA	4.42	
Arg	119I	NH2	Tyr	94L	OH	4.96	*
			Tyr	94L	CE2	4.33	
Pro	123I	CG	Tyr	57H	OH	4.85	
Tyr	128I	CD1	Tyr	57H	OH	4.87	
Tyr	128I	CE1	Tyr	57H	OH	4.32	
Leu	143I	CA	Trp	102H	CZ2	4.90	
Leu	143I	CB	Trp	102H	CZ2	4.87	
Leu	143I	CG	Trp	102H	CE2	4.93	
			Trp	102H	CH2	3.72	
			Trp	102H	CZ2	3.64	
Leu	143I	CD1	Trp	102H	CE2	4.85	
			Trp	102H	CH2	4.25	
			Trp	102H	CZ2	3.86	
Leu	143I	CD2	Trp	102H	CZ3	4.97	
			Trp	102H	CH2	3.91	
			Trp	102H	CZ2	4.32	
Leu	143I	C	Trp	102H	CH2	4.73	
			Trp	102H	CZ2	4.21	
Leu	143I	O	Trp	102H	NE1	4.65	*
			Trp	102H	CE2	4.35	
			Trp	102H	CH2	4.16	
			Trp	102H	CZ2	3.38	
Leu	144I	CD2	Trp	102H	CH2	4.96	
Gln	145I	C	Ser	31H	OG	4.98	
Gln	145I	O	Ser	31H	OG	4.67	*
Lys	146I	N	Ser	31H	OG	4.68	*
Lys	146I	CA	Ser	31H	CA	4.90	
			Ser	31H	CB	4.13	
			Ser	31H	OG	3.91	
			Ser	31H	O	4.74	
Lys	146I	CB	Trp	102H	NE1	4.78	

hIL-21			aIL-21 Fab59 (Fab35 with L, Q27N mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Ser	31H	CA	4.55	
			Ser	31H	CB	4.26	
			Ser	31H	OG	4.21	
			Ser	31H	C	4.83	
			Ser	31H	O	4.15	
Lys	146I	CG	Ser	30H	O	4.72	
			Ser	53H	OG	4.92	
			Ser	31H	CA	3.95	
			Ser	31H	CB	3.98	
			Ser	31H	OG	3.65	
			Ser	31H	C	4.56	
			Ser	31H	O	4.24	
Lys	146I	CD	Ser	30H	O	4.76	
			Ser	53H	CB	4.79	
			Ser	53H	OG	3.86	
			Ser	31H	CA	4.58	
			Ser	31H	OG	4.92	
			Ser	31H	C	4.89	
			Ser	31H	O	4.50	
Lys	146I	CE	Ser	30H	O	4.19	
			Ser	53H	CB	4.29	
			Ser	53H	OG	3.31	
			Ser	31H	CA	4.91	
Lys	146I	NZ	Ser	53H	OG	4.68	*
Lys	146I	C	Trp	102H	NE1	4.31	
			Trp	102H	CE2	4.94	
			Ser	31H	CB	4.75	
			Ser	31H	OG	4.86	
			Ser	31H	O	4.98	
Lys	146I	O	Trp	102H	NE1	4.67	*
			Ser	31H	CB	4.33	
			Ser	31H	OG	4.68	*
			Ser	31H	O	4.62	*
Met	147I	N	Trp	102H	NE1	3.83	*
			Trp	102H	CE2	4.14	
			Trp	102H	CZ2	4.15	
			Trp	102H	CD1	4.77	
Met	147I	CA	Trp	102H	NE1	3.62	
			Trp	102H	CE2	3.78	
			Trp	102H	CD2	4.55	
			Trp	102H	CH2	4.94	
			Trp	102H	CZ2	4.05	
			Trp	102H	CG	4.77	
			Trp	102H	CD1	4.25	
Met	147I	CA	Trp	102H	NE1	3.62	
			Trp	102H	CE2	3.76	
			Trp	102H	CD2	4.53	
			Trp	102H	CH2	4.88	
			Trp	102H	CZ2	4.00	
			Trp	102H	CG	4.78	

hIL-21			aIL-21 Fab59 (Fab35 with L, Q27N mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Trp	102H	CD1	4.27	
Met	147I	CB	Trp	102H	NE1	3.84	
			Trp	102H	CE2	3.45	
			Trp	102H	CD2	4.08	
			Trp	102H	CE3	4.51	
			Trp	102H	CZ3	4.43	
			Trp	102H	CH2	3.91	
			Trp	102H	CZ2	3.38	
			Trp	102H	CG	4.70	
			Trp	102H	CD1	4.55	
Met	147I	CB	Trp	102H	NE1	3.98	
			Trp	102H	CE2	3.56	
			Trp	102H	CD2	4.23	
			Trp	102H	CE3	4.63	
			Trp	102H	CZ3	4.48	
			Trp	102H	CH2	3.90	
			Trp	102H	CZ2	3.39	
			Trp	102H	CG	4.90	
			Trp	102H	CD1	4.74	
Met	147I	CG	Trp	102H	NE1	4.22	
			Trp	102H	CE2	3.69	
			Trp	102H	CD2	3.73	
			Trp	102H	CE3	3.92	
			Trp	102H	CZ3	4.10	
			Trp	102H	CH2	4.11	
			Trp	102H	CZ2	3.92	
			Trp	102H	CG	4.26	
			Trp	102H	CD1	4.50	
Met	147I	CG	Trp	102H	NE1	4.16	
			Trp	102H	CE2	3.47	
			Trp	102H	CD2	3.58	
			Trp	102H	CE3	3.67	
			Trp	102H	CZ3	3.65	
			Trp	102H	CH2	3.58	
			Trp	102H	CZ2	3.50	
			Trp	102H	CG	4.32	
			Trp	102H	CD1	4.59	
Met	147I	SD	Trp	102H	CE2	4.84	
			Trp	102H	CD2	4.70	
			Trp	102H	CE3	4.34	
			Trp	102H	CZ3	4.13	
			Trp	102H	CH2	4.33	
			Trp	102H	CZ2	4.69	
Met	147I	SD	Trp	102H	CE2	4.48	
			Trp	102H	CD2	4.50	
			Trp	102H	CE3	4.01	
			Trp	102H	CZ3	3.46	
			Trp	102H	CH2	3.49	
			Trp	102H	CZ2	4.02	
Met	147I	C	Trp	102H	NE1	4.97	

hIL-21			aIL-21 Fab59 (Fab35 with L, Q27N mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
His	149I	CB	Ser	31H	CB	3.80	
			Ser	31H	OG	3.77	
			Ile	28H	CB	4.57	
His	149I	CG	Ile	28H	CG1	4.68	
			Ser	31H	CB	4.25	
			Ser	31H	OG	3.71	
			Ile	28H	CB	4.	
His	149I	ND1	Ile	28H	CG1	3.94	
			Ile	28H	CG2	4.41	
			Ser	31H	CB	3.82	
			Ser	31H	OG	2.88	***
His	149I	CE1	Ile	28H	CB	3.88	
			Ile	28H	CG1	3.96	
			Ile	28H	CG2	3.70	
			Ile	28H	CD1	4.82	
His	149I	NE2	Ser	31H	OG	3.92	
			Ile	28H	CB	4.15	
			Ile	28H	CG1	3.87	
			Ile	28H	CD1	4.82	
His	149I	CD2	Ile	28H	CG2	3.80	
			Ile	28H	CB	4.55	
			Ile	28H	CG1	3.84	
			Ile	28H	CD1	4.72	
His	149I	O	Ile	28H	CG2	4.57	
			Ser	31H	OG	4.93	
			Ile	28H	CB	4.52	
			Ile	28H	CG1	3.86	
His	149I	C	Ile	28H	CG2	4.90	
			Tyr	32H	CE1	4.99	
			Tyr	32H	CZ	4.67	
His	149I	O	Tyr	32H	OH	3.97	
			Tyr	32H	CE1	4.96	
			Tyr	32H	CZ	4.48	
Gln	150I	N	Tyr	32H	OH	3.48	*
			Tyr	32H	CZ	4.58	
			Tyr	32H	OH	4.13	*
Gln	150I	CA	Tyr	32H	CE1	4.87	
			Tyr	32H	CZ	4.03	
			Tyr	32H	CE2	4.32	
			Arg	100H	NE	4.67	
			Tyr	32H	OH	3.57	
Gln	150I	CB	Trp	102H	CD1	4.90	
			Tyr	32H	CZ	4.48	
			Tyr	32H	CE2	4.37	
			Arg	100H	CG	4.58	
			Arg	100H	CD	4.86	
			Arg	100H	NE	4.22	
			Tyr	32H	OH	4.44	
			Arg	100H	NH2	4.88	
Gln	150I	CG	Arg	100H	CZ	4.86	
			Ser	31H	CB	4.93	

hIL-21			aIL-21 Fab59 (Fab35 with L, Q27N mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Ser	31H	C	4.81	
			Ser	31H	O	3.93	
			Tyr	32H	CD1	4.77	
			Trp	102H	CD1	4.82	
			Tyr	32H	CG	4.62	
			Tyr	32H	CE1	4.46	
			Tyr	32H	CZ	3.94	
			Tyr	32H	CE2	3.79	
			Tyr	32H	CD2	4.13	
			Arg	100H	CG	4.74	
			Tyr	32H	OH	4.34	
Gln	150I	CD	Trp	102H	NE1	4.57	
			Ser	31H	C	4.85	
			Ser	31H	O	3.79	
			Trp	102H	CD1	4.02	
			Arg	100H	CA	4.16	
			Arg	100H	CB	4.42	
			Arg	100H	C	4.56	
			Gly	101H	N	3.86	
			Gly	101H	CA	4.81	
			Tyr	32H	CG	4.73	
			Tyr	32H	CZ	4.79	
			Tyr	32H	CE2	4.26	
			Tyr	32H	CD2	4.20	
			Arg	100H	CG	4.44	
Gln	150I	OE1	Trp	102H	NE1	4.52	*
			Ser	31H	O	4.95	*
			Trp	102H	N	4.84	*
			Trp	102H	CB	4.81	
			Trp	102H	CG	4.56	
			Trp	102H	CD1	3.60	
			Arg	100H	N	4.84	*
			Arg	100H	CA	3.48	
			Arg	100H	CB	3.57	
			Arg	100H	C	3.68	
			Arg	100H	O	4.86	*
			Gly	101H	N	2.98	***
			Gly	101H	CA	3.92	
			Gly	101H	C	4.05	
			Gly	101H	O	4.00	*
			Tyr	32H	CE2	4.81	
			Tyr	32H	CD2	4.81	
			Arg	100H	CG	3.91	
			Arg	100H	CD	4.81	
			Arg	100H	NE	4.42	*
Gln	150I	NE2	Trp	102H	NE1	4.55	*
			Ser	33H	N	4.95	*
			Ser	31H	C	3.92	
			Ser	31H	O	2.78	***
			Tyr	32H	N	4.50	*

hIL-21			aIL-21 Fab59 (Fab35 with L, Q27N mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Tyr	32H	CA	4.08	
			Tyr	32H	CB	4.72	
			Trp	102H	CD1	4.27	
			Arg	100H	CA	4.54	
			Arg	100H	C	4.80	
			Gly	101H	N	3.98	*
			Gly	101H	CA	4.82	
			Glu	99H	O	4.79	*
			Tyr	32H	CG	4.45	
			Tyr	32H	CE2	4.67	
			Tyr	32H	CD2	4.25	
Gln	150I	C	Arg	100H	NE	4.26	
			Tyr	32H	OH	4.29	
			Arg	100H	NH2	4.15	
			Arg	100H	CZ	4.24	
			Arg	100H	NH1	4.92	
Gln	150I	O	Arg	100H	CG	4.80	
			Arg	100H	CD	4.11	
			Arg	100H	NE	3.37	*
			Tyr	32H	OH	4.31	*
			Arg	100H	NH2	3.33	*
			Arg	100H	CZ	3.21	
			Arg	100H	NH1	3.73	*
His	151I	N	Arg	100H	NH2	4.64	*
His	151I	CA	Arg	100H	NH2	4.29	
			Arg	100H	CZ	4.94	
His	151I	CB	Arg	100H	NH2	4.60	
His	151I	CG	Arg	100H	NH2	3.85	
			Arg	100H	CZ	4.95	
His	151I	ND1	Trp	102H	CB	4.97	
			Trp	102H	CG	4.92	
			Trp	102H	CD1	4.97	
			Arg	100H	NH2	4.43	*
His	151I	CE1	Trp	102H	CD2	4.70	
			Trp	102H	CB	3.75	
			Trp	102H	CG	3.98	
			Trp	102H	CD1	4.26	
			Arg	100H	NH2	4.25	
His	151I	NE2	Trp	102H	CB	4.27	
			Trp	102H	CG	4.89	
			Trp	102H	O	4.92	*
			Arg	100H	NE	4.92	*
			Arg	100H	NH2	3.45	*
			Arg	100H	CZ	4.61	
His	151I	CD2	Arg	100H	NE	4.97	
			Arg	100H	NH2	3.09	
			Arg	100H	CZ	4.35	

Table 17

hIL-21, chain I, (SEQ ID NO: 1) interactions with the the heavy chain (chain H) of Fab60 (SEQ ID No 10) and light chain (chain L) of anti-IL-21 Fab60 (SEQ ID No 9, mutation D30E). A distance cut-off of 5.0 Å was used. The contacts were identified by the CONTACT computer software program of the CCP4 suite (Bailey, 1994). In the last column "****" indicates a strong possibility for a hydrogen bond at this contact (distance < 3.3 Å) as calculated by CONTACT, "*" indicates a weak possibility (distance > 3.3 Å). Blank indicates that the program considered there to be no possibility of a hydrogen bond. Hydrogen-bonds are specific between a donor and an acceptor, are typically strong, and are easily identifiable.

hIL-21			aIL-21 Fab60 (Fab35 with L, D30E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
Met	39I	CE	Trp	102H	CZ3	4.48	
			Trp	102H	CH2	4.79	
Glu	65I	CB	Tyr	56H	CE2	4.78	
Glu	65I	CD	Tyr	56H	CZ	4.81	
			Tyr	56H	OH	4.36	
			Tyr	56H	CE2	4.95	
Glu	65I	OE1	Tyr	56H	CE1	4.70	
			Tyr	56H	CZ	3.74	
			Tyr	56H	OH	3.16	***
			Tyr	56H	CE2	3.94	
Asp	66I	N	Tyr	56H	CE2	4.78	
			Tyr	56H	CD2	4.53	
Asp	66I	CA	Tyr	56H	CD2	4.78	
			Tyr	57H	CE2	4.70	
Asp	66I	CB	Gly	54H	O	4.87	
			Tyr	56H	CA	4.78	
			Tyr	56H	CB	3.71	
			Tyr	56H	CG	3.78	
			Tyr	56H	CD1	4.58	
			Tyr	56H	CE2	4.61	
			Tyr	56H	CD2	3.82	
			Gly	54H	N	4.51	
			Gly	54H	CA	4.14	
			Thr	52H	OG1	4.69	
			Gly	54H	C	4.59	
			Tyr	56H	N	4.60	
			Tyr	57H	CE2	4.33	
			Tyr	57H	CD2	4.56	
Asp	66I	CG	Gly	54H	O	4.38	
			Tyr	56H	CA	4.52	
			Tyr	56H	CB	3.90	
			Tyr	56H	CG	4.38	
			Tyr	56H	CD2	4.86	
			Ser	53H	OG	4.68	
			Ser	53H	C	4.54	
			Gly	54H	N	3.27	
			Gly	54H	CA	3.29	
			Thr	52H	CA	4.98	

hIL-21			aIL-21 Fab60 (Fab35 with L, D30E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Thr	52H	CB	3.92	
			Thr	52H	OG1	3.32	
			Thr	52H	C	4.70	
			Ser	53H	N	4.55	
			Gly	54H	C	3.75	
			Ser	55H	N	4.05	
			Ser	55H	C	4.98	
			Tyr	56H	N	3.96	
			Tyr	57H	N	4.99	
			Tyr	57H	CE2	4.58	
			Tyr	57H	CD2	4.34	
			Thr	52H	CG2	4.96	
Asp	66I	OD1	Ser	53H	CA	4.36	
			Ser	53H	CB	4.63	
			Ser	53H	OG	3.61	*
			Ser	53H	C	4.08	
			Gly	54H	N	2.99	***
			Gly	54H	CA	3.46	
			Thr	52H	CA	4.57	
			Thr	52H	CB	3.59	
			Thr	52H	OG1	3.45	*
			Thr	52H	C	4.24	
			Thr	52H	O	4.88	*
			Ser	53H	N	3.80	*
			Gly	54H	C	4.25	
			Ser	55H	N	4.46	*
			Tyr	56H	N	4.92	*
			Tyr	57H	CD2	4.90	
			Thr	52H	CG2	4.68	
Asp	66I	OD2	Ser	55H	O	4.93	*
			Gly	54H	O	3.68	*
			Tyr	56H	CA	3.51	
			Tyr	56H	CB	3.23	
			Tyr	56H	CG	3.92	
			Tyr	56H	CD1	4.42	
			Tyr	56H	CD2	4.72	
			Ser	53H	C	4.41	
			Gly	54H	N	3.16	***
			Gly	54H	CA	3.10	
			Thr	52H	CA	4.51	
			Thr	52H	CB	3.58	
			Thr	52H	OG1	2.61	***
			Thr	52H	C	4.26	
			Thr	52H	O	4.47	*
			Ser	53H	N	4.44	*
			Gly	54H	C	3.05	
			Ser	55H	N	3.12	***
			Ser	55H	CA	3.95	
			Ser	55H	C	3.78	
			Tyr	56H	N	2.78	***

hIL-21			aIL-21 Fab60 (Fab35 with L, D30E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Tyr	56H	C	4.25	
			Tyr	57H	N	4.01	*
			Tyr	57H	CE2	4.66	
			Tyr	57H	CD2	4.15	
			Thr	52H	CG2	4.71	
Asp	66I	C	Tyr	57H	CZ	4.81	
			Tyr	57H	CE2	3.79	
			Tyr	57H	CD2	4.46	
			Tyr	57H	OH	4.78	
Asp	66I	O	Tyr	57H	CZ	4.25	
			Tyr	57H	CE2	3.54	
			Tyr	57H	CD2	4.55	
			Tyr	57H	OH	3.91	*
Val	67I	N	Tyr	57H	CZ	4.97	
			Tyr	57H	CE2	3.85	
			Tyr	57H	CD2	4.26	
Val	67I	CA	Tyr	57H	CZ	4.64	
			Tyr	57H	CE2	3.74	
			Tyr	57H	CD2	4.24	
			Tyr	57H	OH	4.77	
Val	67I	C	Thr	52H	CB	4.81	
			Tyr	57H	CG	4.60	
			Tyr	57H	CZ	4.54	
			Tyr	57H	CE2	3.59	
			Tyr	57H	CD2	3.64	
			Thr	52H	CG2	4.22	
Val	67I	O	Thr	52H	CB	3.83	
			Thr	52H	OG1	4.45	*
			Tyr	57H	CG	4.86	
			Tyr	57H	CE2	4.24	
			Tyr	57H	CD2	3.94	
			Thr	52H	CG2	3.35	
Glu	68I	N	Tyr	57H	CB	4.89	
			Tyr	57H	CG	4.11	
			Tyr	57H	CD1	4.68	
			Tyr	57H	CE1	4.62	
			Tyr	57H	CZ	4.02	
			Tyr	57H	CE2	3.40	
			Tyr	57H	CD2	3.46	
			Tyr	57H	OH	4.59	*
			Thr	52H	CG2	4.51	
Glu	68I	CA	Tyr	57H	CB	4.55	
			Tyr	57H	CG	4.15	
			Tyr	57H	CD1	4.71	
			Tyr	57H	CE1	4.98	
			Tyr	57H	CZ	4.78	
			Tyr	57H	CE2	4.28	
			Tyr	57H	CD2	3.94	
			Thr	52H	CG2	4.07	
Glu	68I	CB	Tyr	57H	CG	4.52	

hIL-21			aIL-21 Fab60 (Fab35 with L, D30E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Tyr	57H	CD1	4.66	
			Tyr	57H	CE1	4.81	
			Tyr	57H	CZ	4.90	
			Tyr	57H	CE2	4.83	
			Tyr	57H	CD2	4.64	
Glu	68I	CG	Tyr	57H	CB	4.36	
			Tyr	57H	CG	3.69	
			Tyr	57H	CD1	3.46	
			Tyr	57H	CE1	3.58	
			Tyr	57H	CZ	4.00	
			Tyr	57H	CE2	4.25	
			Tyr	57H	CD2	4.11	
			Tyr	57H	OH	4.77	
Glu	68I	CD	Tyr	57H	CB	3.98	
			Tyr	57H	CG	3.75	
			Tyr	57H	CD1	3.44	
			Tyr	57H	CE1	4.01	
			Tyr	57H	CZ	4.81	
			Tyr	57H	CD2	4.60	
			His	59H	CE1	4.40	
			His	59H	NE2	3.62	
			His	59H	CD2	4.67	
Glu	68I	OE1	Tyr	57H	CA	4.94	
			Tyr	57H	CB	3.66	
			Tyr	57H	CG	3.88	
			Tyr	57H	CD1	3.94	
			Tyr	57H	CE1	4.79	
			Tyr	57H	CD2	4.76	
			Tyr	94L	CD1	4.92	
			Tyr	94L	CZ	4.75	
			His	59H	CE1	4.02	
			His	59H	NE2	2.96	***
			His	59H	CD2	3.77	
			Tyr	94L	CE1	4.13	
			Tyr	94L	OH	4.55	*
Glu	68I	OE2	Tyr	57H	CB	4.55	
			Tyr	57H	CG	4.27	
			Tyr	57H	CD1	3.58	
			Tyr	57H	CE1	4.05	
			His	59H	CE1	3.90	
			His	59H	NE2	3.49	*
			His	59H	CD2	4.75	
Glu	68I	C	Thr	52H	CG2	4.22	
Thr	69I	N	Ser	33H	OG	4.82	*
			Thr	52H	CG2	3.80	
			Tyr	94L	OH	4.48	*
Thr	69I	CA	Ser	33H	OG	4.33	
			Thr	52H	CG2	4.62	
			Tyr	94L	OH	4.70	
Thr	69I	CB	Ser	33H	CB	4.44	

hIL-21			aIL-21 Fab60 (Fab35 with L, D30E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Ser	33H	OG	3.80	
			Thr	52H	CG2	4.71	
			Tyr	94L	OH	3.68	
			Glu	99H	CD	4.60	
			Glu	99H	OE1	4.44	
			Glu	99H	OE2	4.47	
			Tyr	96L	OH	4.32	
Thr	69I	OG1	Thr	52H	CA	4.86	
			Thr	52H	CB	4.74	
			Ser	50H	OG	4.40	*
			Ser	33H	CA	4.82	
			Ser	33H	CB	3.45	
			Ser	33H	OG	2.82	***
			Thr	52H	CG2	3.60	
			Tyr	94L	OH	3.76	*
			Glu	99H	CD	4.85	
			Glu	99H	OE1	4.35	*
			Tyr	96L	OH	4.97	*
Thr	69I	CG2	Ser	33H	CB	4.11	
			Ser	33H	OG	3.72	
			Tyr	94L	OH	4.69	
			Glu	99H	CG	4.24	
			Glu	99H	CD	3.63	
			Glu	99H	OE1	3.80	
			Glu	99H	OE2	3.54	
			Arg	100H	C	4.73	
			Arg	100H	O	4.37	
			Gly	101H	N	4.55	
			Gly	101H	CA	4.17	
			Tyr	96L	OH	4.27	
Thr	69I	O	Tyr	94L	OH	4.61	*
Asn	70I	N	Gly	101H	CA	3.98	
			Gly	101H	C	4.63	
			Trp	102H	N	4.32	*
Asn	70I	CA	Gly	101H	CA	4.39	
			Gly	101H	C	4.62	
			Trp	102H	N	4.11	
			Trp	102H	CA	4.97	
			Gly	103H	N	4.35	
Asn	70I	CB	Gly	101H	CA	4.86	
			Trp	102H	N	4.85	
			Gly	103H	N	4.37	
			Gly	103H	CA	4.77	
			Tyr	105H	CD1	4.32	
			Tyr	105H	CE1	3.65	
			Tyr	105H	CZ	4.35	
			Tyr	105H	OH	4.45	
Asn	70I	CG	Arg	100H	O	4.46	
			Gly	101H	CA	3.97	
			Gly	101H	C	4.27	

hIL-21			aIL-21 Fab60 (Fab35 with L, D30E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Gly	101H	O	4.86	
			Trp	102H	N	4.43	
			Gly	103H	N	3.90	
			Gly	103H	C	4.83	
			Tyr	104H	N	4.79	
			Gly	103H	CA	4.26	
			Tyr	105H	CD1	3.65	
			Tyr	105H	CE1	3.49	
			Tyr	105H	CZ	4.37	
			Tyr	105H	OH	4.90	
			Tyr	105H	N	4.50	
			Tyr	105H	CA	4.90	
			Tyr	105H	CG	4.65	
Asn	70I	OD1	Arg	100H	C	4.50	
			Arg	100H	O	3.86	*
			Gly	101H	N	4.30	*
			Gly	101H	CA	3.13	
			Gly	101H	C	3.15	
			Gly	101H	O	3.65	*
			Trp	102H	N	3.41	*
			Trp	102H	CA	4.29	
			Trp	102H	C	3.97	
			Gly	103H	N	2.89	***
			Gly	103H	C	3.94	
			Tyr	104H	N	3.73	*
			Gly	103H	CA	3.44	
			Tyr	105H	CD1	4.05	
			Tyr	105H	CE1	4.05	
			Tyr	105H	CZ	4.71	
			Tyr	104H	CA	4.72	
			Tyr	104H	C	4.86	
			Tyr	105H	N	3.99	*
			Tyr	105H	CA	4.75	
			Tyr	105H	CG	4.73	
Asn	70I	ND2	Glu	99H	CD	4.88	
			Glu	99H	OE2	3.87	*
			Arg	10H	O	4.26	*
			Gly	11H	CA	4.53	
			Phe	91L	CB	4.53	
			Phe	91L	CD1	4.48	
			Phe	91L	O	4.88	*
			Tyr	96L	OH	4.34	*
			Tyr	105H	CD1	3.42	
			Tyr	105H	CE1	3.56	
			Tyr	105H	CZ	4.73	
			Tyr	105H	N	4.36	*
			Phe	91L	CG	4.83	
			Tyr	105H	CA	4.42	
			Tyr	105H	CG	4.52	
Asn	70I	C	Trp	102H	N	4.93	

hIL-21			aIL-21 Fab60 (Fab35 with L, D30E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
Asn	70I	O	Gly	103H	N	4.98	*
			Tyr	105H	OH	4.65	*
Glu	72I	N	Trp	102H	N	4.97	*
Glu	72I	CA	Trp	102H	CE2	4.68	
			Trp	102H	CD2	4.58	
			Trp	102H	CE3	4.49	
			Trp	102H	CZ3	4.52	
			Trp	102H	CH2	4.62	
			Trp	102H	CZ2	4.71	
Glu	72I	CB	Trp	102H	NE1	3.78	
			Trp	102H	CE2	3.27	
			Trp	102H	CD2	3.34	
			Trp	102H	CE3	3.61	
			Trp	102H	CZ3	3.82	
			Trp	102H	CH2	3.76	
			Trp	102H	CZ2	3.51	
			Trp	102H	N	4.39	
			Trp	102H	CA	4.47	
			Trp	102H	CB	4.82	
			Trp	102H	CG	3.96	
			Trp	102H	CD1	4.13	
Glu	72I	CG	Trp	102H	NE1	3.70	
			Trp	102H	CE2	3.68	
			Trp	102H	CD2	4.13	
			Trp	102H	CE3	4.77	
			Trp	102H	CH2	4.69	
			Trp	102H	CZ2	4.02	
			Trp	102H	N	4.50	
			Trp	102H	CG	4.41	
			Trp	102H	CD1	4.11	
Glu	72I	CD	Trp	102H	NE1	4.20	
			Trp	102H	CE2	4.52	
			Trp	102H	CD2	4.67	
			Gly	101H	CA	4.04	
			Gly	101H	C	4.31	
			Trp	102H	N	3.58	
			Trp	102H	CA	4.46	
			Trp	102H	CG	4.47	
			Trp	102H	CD1	4.13	
Glu	72I	OE1	Trp	102H	NE1	4.70	*
			Trp	102H	CE2	4.90	
			Trp	102H	CD2	4.67	
			Gly	101H	N	4.92	*
			Gly	101H	CA	3.65	
			Gly	101H	C	3.69	
			Gly	101H	O	4.92	*
			Trp	102H	N	2.79	***
			Trp	102H	CA	3.65	
			Trp	102H	CB	4.61	
			Trp	102H	CG	4.33	

hIL-21			aIL-21 Fab60 (Fab35 with L, D30E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Trp	102H	CD1	4.32	
			Trp	102H	C	4.73	
			Gly	103H	N	4.73	*
Glu	72I	OE2	Ser	1033H	OG	4.93	*
			Trp	102H	NE1	4.64	*
			Gly	101H	N	4.64	*
			Gly	101H	CA	3.86	
			Gly	101H	C	4.54	
			Trp	102H	N	4.15	*
			Trp	102H	CD1	4.57	
Glu	72I	C	Trp	102H	CE2	4.97	
			Trp	102H	CD2	4.60	
			Trp	102H	CE3	4.05	
			Trp	102H	CZ3	3.90	
			Trp	102H	CH2	4.31	
			Trp	102H	CZ2	4.86	
Glu	72I	O	Trp	102H	CE3	4.33	
			Trp	102H	CZ3	3.77	
			Trp	102H	CH2	4.01	
			Trp	102H	CZ2	4.79	
Trp	73I	N	Trp	102H	CD2	4.70	
			Trp	102H	CE3	4.02	
			Trp	102H	CZ3	4.16	
			Trp	102H	CH2	4.94	
			Trp	102H	CA	4.93	
Trp	73I	CA	Trp	102H	CE3	4.42	
			Trp	102H	CZ3	4.38	
Trp	73I	CB	Trp	102H	CE3	4.79	
Trp	73I	CG	Trp	102H	CD2	4.96	
			Trp	102H	CE3	3.93	
			Trp	102H	CZ3	4.43	
			Trp	102H	CA	4.73	
			Trp	102H	C	4.82	
			Trp	102H	O	4.54	
Trp	73I	CD1	Trp	102H	CD2	4.74	
			Trp	102H	CE3	3.98	
			Trp	102H	CZ3	4.86	
			Trp	102H	CA	3.75	
			Trp	102H	CB	4.17	
			Trp	102H	CG	4.90	
			Trp	102H	C	3.56	
			Trp	102H	O	3.15	
			Gly	103H	N	4.37	
			Gly	103H	CA	4.92	
Trp	73I	NE1	Trp	102H	CD2	4.47	
			Trp	102H	CE3	3.72	
			Trp	102H	CZ3	4.67	
			Trp	102H	CA	3.91	
			Trp	102H	CB	3.82	
			Trp	102H	CG	4.59	

hIL-21			aIL-21 Fab60 (Fab35 with L, D30E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Trp	102H	C	3.76	
			Trp	102H	O	3.03	***
			Gly	103H	N	4.83	*
Trp	73I	CE2	Trp	102H	CD2	4.55	
			Trp	102H	CE3	3.50	
			Trp	102H	CZ3	4.09	
			Trp	102H	CA	4.96	
			Trp	102H	CB	4.63	
			Trp	102H	O	4.38	
Trp	73I	CD2	Trp	102H	CD2	4.86	
			Trp	102H	CE3	3.63	
			Trp	102H	CZ3	3.88	
Trp	73I	CE3	Trp	102H	CE3	4.12	
			Trp	102H	CZ3	3.89	
Trp	73I	CZ3	Trp	102H	CE3	4.45	
			Trp	102H	CZ3	4.08	
Trp	73I	CH2	Trp	102H	CE3	4.34	
			Trp	102H	CZ3	4.27	
Trp	73I	CZ2	Trp	102H	CD2	4.92	
			Trp	102H	CE3	3.92	
			Trp	102H	CZ3	4.30	
Phe	76I	CB	Trp	102H	CZ3	4.38	
			Trp	102H	CH2	4.73	
Phe	76I	CG	Trp	102H	CZ3	4.96	
Lys	117I	N	Trp	102H	O	4.82	*
Lys	117I	CA	Gly	103H	CA	4.80	
Lys	117I	CB	Gly	103H	CA	4.30	
			Tyr	105H	CZ	4.86	
			Tyr	105H	OH	4.50	
			Gly	103H	O	4.83	
			Tyr	105H	CE2	4.32	
Lys	117I	CD	Ser	31L	OG	3.88	
			Asp	50L	CG	4.01	
			Asp	50L	OD1	3.64	
			Asp	50L	OD2	3.69	
			Gly	103H	O	4.55	
			Tyr	105H	CE2	4.53	
			Tyr	105H	CD2	4.92	
Lys	117I	CE	Ser	31L	OG	4.06	
			Asp	50L	CG	4.16	
			Asp	50L	OD1	4.04	
			Asp	50L	OD2	3.45	
Lys	117I	NZ	Ser	31L	CB	4.05	
			Ser	31L	OG	3.21	***
			Asp	50L	CB	4.94	
			Asp	50L	CG	3.46	
			Asp	50L	OD1	3.37	*
			Asp	50L	OD2	2.77	***
			Asp	50L	O	4.85	*
Lys	117I	C	Gly	103H	N	4.71	

hIL-21			aIL-21 Fab60 (Fab35 with L, D30E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Gly	103H	CA	4.29	
			Tyr	105H	OH	4.45	
Lys	117I	O	Trp	102H	CA	4.72	
			Trp	102H	C	3.95	
			Trp	102H	O	4.00	*
			Gly	103H	N	3.72	*
			Gly	103H	CA	3.61	
			Tyr	105H	OH	4.80	*
His	118I	N	Tyr	105H	OH	4.01	*
His	118I	CA	Tyr	105H	CZ	4.98	
			Tyr	105H	OH	3.85	
His	118I	C	Tyr	105H	CZ	4.72	
			Tyr	105H	OH	3.39	
His	118I	O	Tyr	105H	CZ	4.95	
			Tyr	105H	OH	3.58	*
Arg	119I	N	Tyr	105H	CZ	4.79	
			Tyr	105H	OH	3.55	*
Arg	119I	CA	Tyr	105H	OH	4.03	
Arg	119I	CB	Tyr	105H	OH	4.64	
			Asn	92L	O	4.73	
Arg	119I	CG	Phe	91L	O	4.25	
			Tyr	105H	CE1	4.34	
			Tyr	105H	CZ	4.63	
			Tyr	105H	OH	3.94	
			Asn	92L	C	5.00	
			Asn	92L	O	4.25	
Arg	119I	CD	Phe	91L	C	4.52	
			Phe	91L	O	3.41	
			Tyr	105H	CE1	4.96	
			Asn	92L	N	4.95	
			Asn	92L	CA	4.38	
			Asn	92L	C	3.96	
			Asn	92L	O	3.24	
			Ser	93L	N	4.87	
Arg	119I	NE	Tyr	94L	CE1	4.94	
			Phe	91L	O	4.05	*
			Asn	92L	C	4.98	
			Asn	92L	O	4.30	*
Arg	119I	CZ	Tyr	94L	CD1	4.89	
			Tyr	94L	CE1	4.32	
			Asn	92L	O	4.67	
Arg	119I	NH1	Tyr	94L	CD1	4.88	
			Tyr	94L	CE1	4.66	
			Asn	92L	O	4.15	*
Arg	119I	NH2	Tyr	94L	CD1	4.70	
			Tyr	94L	CZ	4.72	
			Tyr	94L	CE1	3.87	
			Tyr	94L	OH	4.48	*
Leu	120I	CG	Glu	30L	OE2	4.89	
Leu	120I	CD1	Glu	30L	OE2	4.27	

hIL-21			aIL-21 Fab60 (Fab35 with L, D30E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
Leu	120I	CD2	Glu	30L	OE2	4.49	
Thr	121I	OG1	Asn	92L	O	4.82	*
Pro	123I	CG	Tyr	57H	OH	4.92	
Tyr	128I	CD1	Tyr	57H	OH	4.80	
Tyr	128I	CE1	Tyr	57H	OH	4.22	
Glu	129I	OE2	Tyr	56H	OH	4.96	*
Leu	143I	CA	Trp	102H	CZ2	4.82	
Leu	143I	CB	Trp	102H	CH2	4.81	
			Trp	102H	CZ2	4.88	
Leu	143I	CG	Trp	102H	CZ3	4.78	
			Trp	102H	CH2	3.56	
			Trp	102H	CZ2	3.71	
Leu	143I	CD1	Trp	102H	CE2	4.73	
			Trp	102H	CH2	3.96	
			Trp	102H	CZ2	3.68	
Leu	143I	CD2	Trp	102H	CZ3	4.87	
			Trp	102H	CH2	3.96	
			Trp	102H	CZ2	4.52	
Leu	143I	C	Trp	102H	CH2	4.37	
			Trp	102H	CZ2	4.16	
Leu	143I	O	Trp	102H	NE1	4.93	*
			Trp	102H	CE2	4.40	
			Trp	102H	CH2	3.75	
			Trp	102H	CZ2	3.28	
Leu	144I	N	Trp	102H	CH2	4.96	
Leu	144I	CA	Trp	102H	CH2	4.98	
Gln	145I	O	Ser	31H	OG	4.76	*
Lys	146I	N	Ser	31H	OG	4.74	*
Lys	146I	CA	Ser	31H	OG	4.01	
			Ser	31H	CB	4.33	
			Ser	31H	O	4.87	
			Trp	102H	NE1	4.91	
Lys	146I	CB	Ser	31H	OG	4.33	
			Ser	31H	CA	4.84	
			Ser	31H	CB	4.49	
			Ser	31H	O	4.36	
			Trp	102H	NE1	4.44	
			Trp	102H	CE2	4.91	
			Trp	102H	CZ2	4.63	
Lys	146I	CG	Ser	30H	O	4.92	
			Ser	31H	OG	3.78	
			Ser	31H	CA	4.27	
			Ser	31H	CB	4.19	
			Ser	31H	C	4.84	
			Ser	31H	O	4.44	
Lys	146I	CD	Ser	30H	O	4.75	
			Ser	53H	OG	4.25	
			Ser	31H	OG	4.86	
			Ser	31H	CA	4.67	
			Ser	31H	C	4.97	

hIL-21			aIL-21 Fab60 (Fab35 with L, D30E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Ser	31H	O	4.58	
Lys	146I	CE	Ser	30H	C	5.00	
			Ser	30H	O	3.92	
			Ser	53H	CB	4.44	
			Ser	53H	OG	3.63	
			Ser	31H	CA	4.69	
Lys	146I	NZ	Ser	30H	O	4.78	*
			Ser	53H	OG	4.97	*
Lys	146I	C	Trp	102H	NE1	4.06	
			Trp	102H	CE2	4.52	
			Trp	102H	CZ2	4.57	
			Trp	102H	CD1	4.94	
Lys	146I	O	Ser	31H	OG	4.94	*
			Ser	31H	CB	4.68	
			Ser	31H	O	4.78	*
			Trp	102H	NE1	4.27	*
			Trp	102H	CE2	4.98	
			Trp	102H	CD1	4.84	
Met	147I	N	Trp	102H	NE1	3.79	*
			Trp	102H	CE2	3.87	
			Trp	102H	CD2	4.88	
			Trp	102H	CH2	4.64	
			Trp	102H	CZ2	3.75	
			Trp	102H	CD1	4.73	
Met	147I	CA	Trp	102H	NE1	3.70	
			Trp	102H	CE2	3.67	
			Trp	102H	CD2	4.27	
			Trp	102H	CE3	4.90	
			Trp	102H	CZ3	4.99	
			Trp	102H	CH2	4.53	
			Trp	102H	CZ2	3.86	
			Trp	102H	CG	4.60	
			Trp	102H	CD1	4.29	
Met	147I	CB	Trp	102H	NE1	4.04	
			Trp	102H	CE2	3.48	
			Trp	102H	CD2	3.84	
			Trp	102H	CE3	4.05	
			Trp	102H	CZ3	3.90	
			Trp	102H	CH2	3.58	
			Trp	102H	CZ2	3.38	
			Trp	102H	CG	4.56	
			Trp	102H	CD1	4.65	
Met	147I	CG	Trp	102H	NE1	4.66	
			Trp	102H	CE2	4.03	
			Trp	102H	CD2	3.79	
			Trp	102H	CE3	3.72	
			Trp	102H	CZ3	3.84	
			Trp	102H	CH2	4.08	
			Trp	102H	CZ2	4.20	
			Trp	102H	CG	4.40	

hIL-21			aIL-21 Fab60 (Fab35 with L, D30E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Trp	102H	CD1	4.89	
His	149I	CB	Ile	28H	CB	4.66	
			Ile	28H	CG1	4.89	
			Ser	31H	OG	3.98	
			Ser	31H	CB	4.04	
His	149I	CG	Ile	28H	CB	4.10	
			Ile	28H	CG1	4.06	
			Ile	28H	CG2	4.39	
			Ser	31H	OG	3.87	
			Ser	31H	CB	4.40	
His	149I	ND1	Ile	28H	CB	3.96	
			Ile	28H	CG1	4.13	
			Ile	28H	CG2	3.77	
			Ser	31H	OG	3.01	***
			Ser	31H	CB	3.92	
His	149I	CE1	Ile	28H	CD1	4.90	
			Ile	28H	CB	4.17	
			Ile	28H	CG1	3.98	
			Ile	28H	CG2	3.81	
			Ser	31H	OG	3.95	
His	149I	NE2	Ile	28H	CD1	4.68	
			Ile	28H	CB	4.42	
			Ile	28H	CG1	3.79	
			Ile	28H	CG2	4.44	
His	149I	CD2	Ile	28H	CB	4.45	
			Ile	28H	CG1	3.90	
			Ile	28H	CG2	4.83	
His	149I	C	Tyr	32H	OH	4.03	
			Tyr	32H	CZ	4.82	
His	149I	O	Tyr	32H	OH	3.59	*
			Tyr	32H	CZ	4.66	
Gln	150I	N	Tyr	32H	OH	4.13	*
			Tyr	32H	CZ	4.70	
Gln	150I	CA	Tyr	32H	CE2	4.96	
			Tyr	32H	OH	3.57	
			Tyr	32H	CE1	4.46	
			Tyr	32H	CZ	4.16	
			Arg	100H	NE	4.61	
			Arg	100H	CZ	4.84	
			Arg	100H	NH2	4.80	
Gln	150I	CB	Tyr	32H	OH	4.33	
			Tyr	32H	CE1	4.41	
			Tyr	32H	CZ	4.49	
			Arg	100H	CG	4.62	
			Arg	100H	CD	4.88	
			Arg	100H	NE	4.14	
			Trp	102H	CD1	4.72	
			Arg	100H	CZ	4.64	
			Arg	100H	NH2	4.56	
Gln	150I	CG	Tyr	32H	CG	4.67	

hIL-21			aIL-21 Fab60 (Fab35 with L, D30E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Tyr	32H	CD2	4.73	
			Ser	31H	C	4.91	
			Ser	31H	O	4.00	
			Trp	102H	NE1	4.83	
			Tyr	32H	CE2	4.39	
			Tyr	32H	OH	4.18	
			Tyr	32H	CD1	4.19	
			Tyr	32H	CE1	3.77	
			Tyr	32H	CZ	3.90	
			Arg	100H	CG	4.76	
			Trp	102H	CD1	4.56	
Gln	150I	CD	Tyr	32H	CA	4.99	
			Tyr	32H	CG	4.59	
			Ser	31H	C	4.90	
			Ser	31H	O	3.86	
			Trp	102H	NE1	4.36	
			Tyr	32H	CD1	4.02	
			Tyr	32H	CE1	4.02	
			Tyr	32H	CZ	4.57	
			Arg	100H	CB	4.33	
			Arg	100H	CG	4.31	
			Arg	100H	NE	4.98	
			Arg	100H	CA	4.12	
			Arg	100H	C	4.52	
			Gly	101H	N	3.85	
			Gly	101H	CA	4.83	
			Trp	102H	CD1	3.88	
Gln	150I	OE1	Ser	31H	O	4.98	*
			Trp	102H	NE1	4.38	*
			Tyr	32H	CD1	4.61	
			Tyr	32H	CE1	4.57	
			Glu	99H	O	4.95	*
			Arg	100H	CB	3.48	
			Arg	100H	CG	3.76	
			Arg	100H	CD	4.67	
			Arg	100H	NE	4.24	*
			Arg	100H	N	4.82	*
			Arg	100H	CA	3.43	
			Arg	100H	C	3.65	
			Arg	100H	O	4.85	*
			Gly	101H	N	3.00	***
			Gly	101H	CA	3.96	
			Gly	101H	C	4.04	
			Gly	101H	O	3.95	*
			Trp	102H	N	4.85	*
			Trp	102H	CB	4.79	
			Trp	102H	CG	4.51	
			Trp	102H	CD1	3.53	
Gln	150I	NE2	Tyr	32H	N	4.32	*
			Tyr	32H	CA	3.86	

hIL-21			aIL-21 Fab60 (Fab35 with L, D30E mutation)			Distance [Å]	Possibly H-bond
Res. Type	Res. # and Chain	Atom name	Res. Type	Res. # and Chain	Atom name		
			Tyr	32H	CB	4.42	
			Tyr	32H	CG	4.04	
			Tyr	32H	CD2	4.61	
			Tyr	32H	C	4.94	
			Ser	33H	N	4.85	*
			Ser	31H	C	3.87	
			Ser	31H	O	2.82	***
			Trp	102H	NE1	4.54	*
			Tyr	32H	CE2	4.98	
			Tyr	32H	CD1	3.79	
			Tyr	32H	CE1	4.22	
			Glu	99H	O	4.54	*
			Tyr	32H	CZ	4.78	
			Arg	100H	CB	4.98	
			Arg	100H	CA	4.37	
			Arg	100H	C	4.68	
			Gly	101H	N	3.93	*
			Gly	101H	CA	4.88	
			Trp	102H	CD1	4.30	
Gln	150I	C	Tyr	32H	OH	4.38	
			Arg	100H	NE	4.20	
			Arg	100H	CZ	4.00	
			Arg	100H	NH1	4.63	
			Arg	100H	NH2	3.80	
Gln	150I	O	Tyr	32H	OH	4.32	*
			Arg	100H	CG	4.93	
			Arg	100H	CD	4.22	
			Arg	100H	NE	3.46	*
			Arg	100H	CZ	3.12	
			Arg	100H	NH1	3.53	*
			Arg	100H	NH2	3.21	***
His	151I	N	Arg	100H	CZ	4.75	
			Arg	100H	NH2	4.21	*
His	151I	CA	Arg	100H	CZ	4.69	
			Arg	100H	NH2	3.95	
His	151I	CB	Arg	100H	NH2	4.31	
His	151I	CG	Arg	100H	CZ	4.84	
			Arg	100H	NH2	3.63	
His	151I	ND1	Trp	102H	CB	4.98	
			Arg	100H	NH2	3.97	*
His	151I	CE1	Trp	102H	CD2	4.84	
			Trp	102H	CB	3.82	
			Trp	102H	CG	4.13	
			Trp	102H	CD1	4.58	
			Arg	100H	NH2	3.91	
His	151I	NE2	Trp	102H	CB	4.41	
			Arg	100H	CZ	4.72	
			Arg	100H	NH2	3.45	*
His	151I	CD2	Arg	100H	CZ	4.56	
			Arg	100H	NH2	3.23	

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- 35

Example 9**Comparison of interaction kinetics for anti-hIL-21 mAb37, mAb61, mAb62 and mAb65 to hIL-21 by Surface Plasmon Resonance (SPR)**

5 Binding studies were performed on a Biacore T200 instrument that measures molecular interactions in real time through surface plasmon resonance. Experiments were run at 25°C and the samples were stored at 10 °C in the sample compartment. The signal (RU, response units) reported by the Biacore is directly correlated to the mass on the individual sensor chip surfaces in four serial flow cells.

10

Anti-human Fc monoclonal antibodies from Biacore human Fc capture kit was immobilized onto flow cells of a CM4 sensor chip according to the manufacturer's instructions. The final immobilization level of capture antibody was approximately 2,500 RU in one experiment. Capture of the human anti-hIL21 antibodies mAb37, mAb61, 15 mAb62, mAb65 was conducted by diluting the antibody to 0.125 µg/ml into running buffer (10 mM Hepes 0,3 M NaCl, 5 mM CaCl₂, 0.05% surfactant P20, pH 8.0 containing 1 mg/ml BSA) and injected at 10 µl/min for 180s in one of flow cells 2-4, creating a reference surface in flow cell 1 with only anti-Fc antibody immobilized. This typically resulted in final capture levels of test antibodies of approximately 50-85 RU and Rmax 20 values of analyte of 10-16 RU. Binding of hIL-21 protein was conducted by injecting analyte over all flow cells to allow for comparative analyses of binding to different captured anti-IL-21 antibodies relative to binding to the reference flow cell. hIL-21 protein was diluted serially 1:3 to 2-162 nM into running buffer, injected at 100 µl/min for 210 s and allowed to dissociate for 600 or 14000 s. The CM4 surface was regenerated after 25 each injection cycle of analyte via two injections of 3M MgCl₂ at 50µl/min. This regeneration step removed the anti-IL-21 antibody and any bound IL-21 from the immobilized capture antibody surface, and allowed for the subsequent binding of the next interaction sample pair. The regeneration procedure did not remove the directly immobilized anti-Fc capture antibody from the chip surface.

30

In order to obtain kinetic data, such as k_a (association rate), k_d (dissociation rate) and KD (equilibrium dissociation constant), data analysis was performed using the Biacore T200 evaluation software 1.0, fitting data to 1:1 Langmuir model. No significant non-specific binding to the reference control surface was observed. Binding curves were 35 processed by double referencing (subtraction of reference surface signals as well as

blank buffer injections over captured anti-IL-21 antibodies). This allowed correction for instrument noise, bulk shift and drift during sample injections.

Human IL-21 dissociates from mAb37, mAb61, mAb62 and mAb65 with off-rates less than what can be accurately measured by the currently used assay ($k_d < 1E-5 \text{ s}^{-1}$) and average k_a values of $5-7 E +5 (\text{Ms})^{-1}$ resulting in K_D of $< 20 \text{ pM}$. Results are based on two different experiments. Individual relative standard errors (RSE) of parameter k_a were $< 1.1 \%$. Results are shown in Table 18.

These data clearly demonstrates that the four different antibodies tested share similar binding properties to human IL-21.

Table 18

Results from individual experiments of binding constants k_a (association rate), k_d (dissociation rate) and calculated K_D (equilibrium dissociation constant) for the interaction of human IL21 to monoclonal antibodies mAb37, mAb61, mAb62 and mAb65.

Antibody	Exp no	k_a (1/Ms)	RSE k_a (%)	k_d (1/s)	K_D calc. (M)
mAb37	1	5.7E+05	0.1	$< 1 E-5$	$< 1.8E-11$
mAb37	1	5.9E+05	0.3	$< 1 E-5$	$< 1.7E-11$
mAb37	1	5.8E+05	0.1	$< 1 E-5$	$< 1.7E-11$
mAb37	2	6.6E+05	0.4	$< 1 E-5$	$< 1.5E-11$
mAb37	2	6.2E+05	0.2	$< 1 E-5$	$< 1.6E-11$
mAb61	2	6.6E+05	0.4	$< 1 E-5$	$< 1.5E-11$
mAb61	2	6.7E+05	0.7	$< 1 E-5$	$< 1.5E-11$
mAb62	1	5.6E+05	0.2	$< 1 E-5$	$< 1.8E-11$
mAb62	1	5.8E+05	0.3	$< 1 E-5$	$< 1.7E-11$
mAb62	1	6.0E+05	0.9	$< 1 E-5$	$< 1.7E-11$
mAb65	1	5.2E+05	0.1	$< 1 E-5$	$< 1.9E-11$
mAb65	1	5.3E+05	0.7	$< 1 E-5$	$< 1.9E-11$
mAb65	1	5.4E+05	0.2	$< 1 E-5$	$< 1.9E-11$

Example 10

20 Inhibitory effect of anti-IL-21 mAb37 variants on human B cell proliferation

The neutralising potential of 6 anti-IL-21 antibodies was compared in a B cell proliferation assay. The 6 antibodies include mAb37 and the 5 variants, mAb61, mAb62, mAb63, mAb64 and mAb65 described in example 12. The antibodies were tested for their ability to neutralise the recombinant human IL-21 in the B cell proliferation assay.

Blood bags were obtained from healthy human volunteers and PBMCs were isolated from 50 ml of heparinised peripheral blood by Ficoll-Paque™ Plus (GE Healthcare) gradient centrifugation. Blood was diluted to 100 ml in phosphate-buffered saline (PBS) at room temperature and 35 ml aliquots were distributed into 50 ml conical tubes
5 carefully overlaying 14 ml of Ficoll-Paque™ Plus (Ge Healthcare) at room temperature. The tubes were spun for 25 minutes at 1680 rpm (600 x g) at room temperature without brake. The PBMC interface layer was removed carefully and washed twice with PBS containing 2% FCS. B cells were isolated by negative selection using EasySep human B Cell enrichment Kit (StemCell Technologies SERL, Grenoble, France). A small sample of
10 the purified B cells was tested for purity by FACS analysis and found to be > 95-97% pure in all experiments.

B cells were cultured in RPMI-1640 media (Invitrogen) supplemented with heat inactivated foetal calf serum (FCS) (Gibco) or Healthy human serum (HS) (Sigma), and Penicillin/Streptomycin (Gibco). To test the inhibitory effect of mAb37 variants, human B
15 cells were isolated from 2 individual donors, donor 1 and 2.

The B cells were plated at 50.000 cells per well in a 96-well U-bottom tissue culture plate. Cells were treated with 0.1 µg/ml anti-CD40 (R&D Systems), 50 ng/ml (3.21 nM) recombinant human IL-21. The cells were incubated for 3 days at 37 °C and 5% CO₂ in a
20 humidified incubator. The antibodies were titrated and after three days, the cells were pulsed with 1 µCi/well of [³H]-Thymidine (Perkin Elmer Life Sciences) for the last 20 hours. The cells were harvested onto UniFilter-96 GF/C filter plates (Packard Instruments, Perkin Elmer) and the amount of [³H]-thymidine incorporation was quantified using a TopCount NXT (Perkin Elmer). The concentration of anti-IL-21 mAb required for
25 reducing proliferation by 50% (IC₅₀) was calculated using the GraphPad Prism v5.0 software (GraphPad Inc.) and the sigmoidal dose-response (variable slope, 4-parameters) equation.

The IC₅₀ for the WT mAb37 and the 5 variants were all found to be very similar, with IC₅₀
30 values in the sub-nanomolar range. All antibodies were tested on B-cells from both donors and the data is listed in table 19 below. Due to technical issues a full data set for mAb62 was only obtained for donor 2.

Table 19**IC₅₀ values for mAb37, mAb61, mAb62, mAb63, mAb64 and mAb65 in B cell proliferation assay**

	IC ₅₀ (nM)	IC ₅₀ (nM)
	Donor 1	Donor 2
mAb37	0.14	0.18
mAb61	0.22	0.21
mAb62	N/A	0.23
mAb63	0.16	0.25
mAb64	0.80	0.74
mAb65	0.49	0.19

5

Example 11**Bioactivity of anti-IL-21 antibodies in NK-92 assay.**

10 The antibodies were tested for their ability to neutralise the recombinant human IL-21 in the NK-cell based bioassay. The anti-IL-21 mAb37 was included as reference material.

The NK-cell based bioassay was used for *in vitro* determination of the bioactivity of anti-IL-21 antibodies. The NK-92 cell line (ATCC/LGC Promochem) is a human suspension lymphoblast derived from peripheral blood mononuclear cells. Cells express the IL-21 receptor endogenously and are dependent on IL-2 or IL-21 for cell proliferation. The neutralization of IL-21 by anti-IL-21 is measured by growth inhibition via addition of alamarBlue® (a cell viability indicator).

20 During maintenance the NK-92 cells were kept proliferating by addition of IL-2. For assay, NK-92 cells were washed and plated out in 96 well plates (Matrix Technology) at a density of 1.6×10^5 cells/ml (equal to 12,800 cells per well). The cells were stimulated with recombinant human IL-21 at a fixed concentration of 5431 pg/ml. Serial dilutions of Anti-IL-21 antibodies prepared in assay media, ranging from 0-12,800 pg/ml, was added in triplicates in three different positions in the 96-well plate. The cells were incubated for 3 days at 37 °C and 5% CO₂ in a humidified incubator. On day three 10 µl alamarBlue® (Biosource) was added and fluorescence was measured after 5 hours of incubation on a Synergy instrument (Bio Tek).

30 Data was analyzed in BioCalc (MicroLex) in a four-parameter logistic curve model. Results are given as percentage (%) of reference material mAb37, based on single determinations.

The bioactivity measured for the 5 mutated antibodies (table 20) were all found to be very similar when compared relative to the bioactivity of the reference material mAb37.

5

Table 20 Bioactivity for mAb61, mAb62, mAb63, mAb64 and mAb65 NK-92 assay relative to mAb37

Antibody	Bioactivity as % of RM (mAb37)
mAb63	92.1
mAb64	116.4
mAb61	86.2
mAb62	73.2
mAb65	96.8

10 **Example 12**

Cloning and sequencing of anti-IL-21 mAb14

This example describes cloning and sequencing of the human heavy chain and light chain sequences of anti-IL-21 mAb14 from hybridoma 366.328.10.63

15 Total RNA was extracted from hybridoma cells using the RNeasy-Mini Kit from Qiagen and used as template for cDNA synthesis. cDNA was synthesized in a 5'-RACE reaction using the SMARTer™ RACE cDNA amplification kit from Clontech. Subsequent target amplification of HC and LC sequences was performed by PCR using Phusion Hot Start polymerase (Finnzymes) and the universal primer mix (UPM) included in the SMARTer™

20 RACE kit as forward primer. Reverse primers specific for human IgG constant regions or the human Kappa constant region were used for PCR amplification of the HC and LC sequences, respectively. The PCR products were separated by gel electrophoresis, extracted using the GFX PCR DNA & Gel Band Purification Kit from GE Healthcare Bio-Sciences and cloned for sequencing using a Zero Blunt TOPO PCR Cloning Kit and

25 chemically competent TOP10 *E.coli* (Invitrogen). Colony PCR was performed on selected colonies using an AmpliTaq Gold® FAST Master Mix from Applied Biosystems and M13uni/M13rev primers. Colony PCR clean-up was performed using the ExoSAP-IT enzyme mix (USB). Sequencing was performed at MWG Biotech, Martinsried Germany using either M13uni(-21)/M13rev(-29) or T3/T7 sequencing primers. Sequences were

30 analyzed and annotated using the VectorNTI program. All kits and reagents were used according to the manufacturer's instructions.

A single unique human kappa type LC and a single unique human HC, subclass IgG4 were identified.

Example 13

Generation of expression vectors for transient expression of anti-IL-21 mAb14 antibody and Fab fragment variants.

To enable epitope mapping and binding analyses, a series of CMV promotor-based expression vectors (pTT vectors) were generated for transient expression of mAb14 variants in the HEK293-6E EBNA-based expression system developed by Yves Durocher (Durocher *et al.* Nucleic Acid Research, 2002). In addition to the CMV promoter, the vectors contain a pMB1 origin, an EBV origin and the Amp resistance gene.

The region corresponding to the anti-IL-21 mAb14 VH domain was cloned into a linearized pTT-based vector containing the sequence of an engineered human IgG4 CH domain using standard PCR and restriction-based cloning methods. As part of the PCR amplification, the sequence for the native IgG signal peptide was exchanged by standard overlapping PCR with the signal peptide sequences derived from human CD33. The PCR template used was a topo-vector generated as described in Example 12. The engineered human IgG4 CH domain contains a single amino acid substitution: S241P in the hinge region. The proline mutation at position 241 (S241P residue numbering according to Kabat, S228P residue numbering according to the EU numbering system (Edelman G.M. et AL., Proc. Natl. Acad. USA 63, 78-85 (1969) and S228P numbering in SEQ ID No. 7) was introduced in the IgG4 hinge region to eliminated formation of monomeric antibody fragments, i.e. "half-antibodies" comprising of one LC and one HC.

Vector constructs were transformed into *E. coli* for selection. The sequence of the final construct was verified by DNA sequencing. The stabilizing S241P mutation in the human IgG4 hinge region constitutes the only difference between mAb14 and mAb37, i.e. mAb37 is the hinge stabilized version of mAb14. The amino acid of HC mAb37 corresponds to SEQ ID No 7 with an S228P substitution at residue 228. The mAb14 and mAb37 nomenclature is used interchangeably, but for all recombinantly produced mAb variants the IgG4 constant region contains the stabilizing S241P mutation.

A pTT-based vector was also generated for transient expression of the mAb37 Fab fragment; Fab35. The region corresponding to the VH domain was cloned into a linearized pTT-based vector containing the sequence of a truncated human IgG4 constant domain. The IgG4 CH domain is terminated in the hinge region – generating a truncated HC, constituting amino acid residues 1-221 of the full HC listed as SEQ ID No. 7. The VH domain was swapped into the Fab expression vector by restriction-based cloning and transformed into *E. coli* for selection. The sequence of the final construct was verified by DNA sequencing. The Fab35 HC amino acid sequence is listed as SEQ ID No. 10. The Fab35 LC corresponds to the mAb37 LC, the amino acid sequence is listed as SEQ ID No. 9 (and SEQ ID No. 6).

The region corresponding to the mAb37 VL domain was cloned into a linearized pTT-based vector containing the sequence for a human kappa CL domain using the standard PCR methods for amplification and signal peptide exchange described for mAb37 HC above and standard restriction-based cloning methods. The PCR template used was a topo-vector generated as described in Example 12. Vector constructs were transformed into *E. coli* for selection. The sequence of the final construct was verified by DNA sequencing. The mAb37 LC amino acid sequence corresponds to mAb14 LC and is listed as SEQ ID No 6 (and SEQ ID No. 9).

Recombinant expression of mAb37 and Fab35 were performed as described in Example 14.

Example 14

Site-directed mutagenesis of anti-IL-21 mAb37

Site-directed mutagenesis was performed to generate the variants of anti-IL-21 mAb37/Fab35 listed in table 21. The mutations are listed according to numbering on reference sequences corresponding to mAb14 LC SEQ ID 6, mAb14 HC SEQ ID No. 7, Fab35 LC SEQ ID 9, Fab35 HC SEQ ID No. 10. Mutations were introduced in the HC or LC by standard site directed mutagenesis using the QuikChange™ Site-Directed mutagenesis kit from Stratagene and specific mutagenic primers were used to introduce point mutations. The kit was used according to the manufacturer's protocol. The pTT-based expression plasmid for WT mAb37/Fab35 LC described in Example 13 was used

as template for the LC mutagenesis. The HC mutants were generated using the truncated HC expression plasmid for WT Fab35 described in Example 13 as template. Subsequently the plasmid for expression of full length HC mutants were generated by swapping the mutated VH domains into the linearized pTT-based vector containing the sequence of the human IgG4(S241P) CH domain. Domain swapping was done by standard restriction-based cloning methods. Vector constructs were transformed into *E. coli* for selection. The sequences of all final constructs were verified by DNA sequencing.

Table 21 Variants of mAb37/Fab35

mAb	Fab	mutation ID	CDR	Chain	LC Reference SEQ ID No. mAb/Fab	HC reference SEQ ID No. mAb/Fab
mAb37	Fab35	WT			6/9	7/10
mAb61	Fab56	D62E	H2	H	6/9	7/10
mAb62	Fab57	K65R	H2	H	6/9	7/10
mAb63	Fab58	R24K	L1	L	6/9	7/10
mAb64	Fab59	Q27N	L1	L	6/9	7/10
mAb65	Fab60	D30E	L1	L	6/9	7/10

10

To express mAb37 mutants, HEK293-6E cells were co-transfected with LC plasmids (WT or mutants) and HC plasmids (WT or mutant) as described below. To express mAb37 Fab fragment, HEK293-6E cells were co-transfected with LC plasmids (WT or mutants) and truncated HC plasmids (WT or mutant).

15 **Recombinant expression of mAb variants**

Variants of mAb37 including variants of Fab35 were expressed by co-transfection of HEK293-6E cells with pTT-based HC and LC vectors according to the generic antibody expression protocol listed below.

20 • **Cell maintenance:**

HEK293-6E cells were grown in suspension in FreeStyle™ 293 expression medium (Gibco) supplemented with 25 µg/ml Geneticin (Gibco), 0.1% v/v of the surfactant Pluronic F-68 (Gibco) & 1% v/v Penicillin-Streptomycin (Gibco). Cells were cultured in Erlenmeyer shaker flasks in shaker incubators at 37°C, 8 % CO₂ and 125 rpm and maintained at cell densities between 0.1-1.5 x 10⁶ cells/ml.

25

• DNA Transfection:

- *The cell density of cultures used for transfection was $0.9-2.0 \times 10^6$ cells/ml.*
- A mix of 0.5 µg LC vector DNA + 0.5 µg HC vector DNA was used per ml cell culture.
- 5 • The DNA was diluted in Opti-MEM media (Gibco) 30µl media/µg DNA, mixed and incubated at room temperature (23-25 °C) for 5 min.
- *293Fectin™ (Invitrogen) was used as transfection reagent at a concentration of 1µl per µg DNA.*
- The 293Fectin™ was diluted 30X in Opti-MEM media (Gibco), mixed and
10 incubated at room temperature (23-25 °C) for 5 min.
- The DNA and 293Fectin solutions were mixed and left to incubate at room temperature (23-25 °C) for 25 min.
- The DNA-293Fectin mix was then added directly to the cell culture.
- The transfected cell culture was transferred to a shaker incubator at 37°C, 8 %
15 CO₂ and 125 rpm.
- 5 days post transfection, cell culture supernatants were harvested by centrifugation, followed by filtration through a 0.22 µm PES filter (Corning).
- Quantitative analysis of antibody production was performed by BioLayer
20 Interferometry directly on clarified cell culture supernatants using the FortéBio Octet system or by SDS-PAGE analysis.

Purification of mAb and Fab fragment variants

mAb37 variants were purified by standard affinity chromatography using MabSelectSuRe resin from GE Healthcare. The purified antibodies were buffer exchanged to PBS buffer
25 pH7.2.

Fab fragments were purified by standard affinity chromatography using KappaSelect resin from GE Healthcare. The purified Fab fragments were buffer exchanged to PBS buffer pH7.2.

Quality assessment and concentration determination was done by SEC-HPLC, endotoxin
30 levels were measured by the standard Kinetic Turbidimetric LAL method.

Abbreviations

Aa: amino acid

mAb: monoclonal antibody

5 HC: heavy chain

LC: light chain

VH: variable domain – heavy chain

VL: variable domain – light chain

CH: constant region – heavy chain

10 CL: constant region – light chain

PCR: polymerase chain reaction

WT: wild type

Example 15

15

Epitope mapping by HX-MS of mAb37 and variants mAb61, mAb62 and mAb65 on hIL-21 (see also example 7)

Materials

20 *Protein batches used were:*

hIL-21: human recombinant IL-21 (expressed in E. coli as the mature peptide; residues 30-162 of SEQ ID NO: 1 with an added N-terminal Methionine residue), mAb37 and variants mAb61, mAb62 and mAb65, sequences as described in example 14

25 All proteins were buffer exchanged into PBS pH 7.4 before experiments.

Methods: HX-MS experiments

Instrumentation and data recording

The HX experiments were performed on a nanoACQUITY UPLC System with HDX
30 Technology (Waters Inc.) coupled to a Synapt G2 mass spectrometer (Waters Inc.). The Waters HDX system contained a Leap robot (H/D-x PAL; Waters Inc.) operated by the LeapShell software (Leap Technologies Inc/Waters Inc.), which performed initiation of the deuterium exchange reaction, reaction time control, quench reaction, injection onto the UPLC system and digestion time control. The Leap robot was equipped with two

temperature controlled stacks maintained at 20 °C for buffer storage and HX reactions and maintained at 2 °C for storage of protein and quench solution, respectively. The Waters HDX system furthermore contained a temperature controlled chamber holding the pre- and analytical columns, and the LC tubing and switching valves at 1 °C. A
5 separately temperature controlled chamber holds the pepsin column at 25 °C. For the inline pepsin digestion, 100 µL quenched sample containing 100 pmol hIL-21 was loaded and passed over a Poroszyme® Immobilized Pepsin Cartridge (2.1 × 30 mm (Applied Biosystems)) placed at 25°C using a isocratic flow rate of 100 µL/min (0.1% formic acid:CH₃CN 95:5). The resulting peptides were trapped and desalted on a VanGuard
10 pre-column BEH C18 1.7 µm (2.1 × 5 mm (Waters Inc.)). Subsequently, the valves were switched to place the pre-column inline with the analytical column, UPLC-BEH C18 1.7 µm (1 × 100 mm (Waters Inc.)), and the peptides separated using a 9 min gradient of 10-40% B delivered at 200 µl/min from the nanoAQUITY UPLC system (Waters Inc.). The mobile phases consisted of A: 0.1% formic acid and B: 0.1% formic acid in CH₃CN. The
15 ESI MS data, and the separate elevated energy (MS^E) experiments were acquired in positive ion mode using a Synapt G2 mass spectrometer (Waters Inc.). Leucine-enkephalin was used as the lock mass ([M+H]⁺ ion at *m/z* 556.2771) and data was collected in continuum mode (For further description, see Andersen and Faber, Int. J. Mass Spec., 302, 139–148(2011)).

20

Data analysis

Peptic peptides were identified in separate experiments using standard MS^E methods where the peptides and fragments are further aligned utilizing the ion mobility properties of the Synapt G2 (Waters Inc.). MS^E data were processed using ProteinLynx Global
25 Server version version 2.5 (Waters Inc.). The HX-MS raw data files were processed in the DynamX software (Waters Inc.). DynamX automatically performs the lock mass-correction and deuterium incorporation determination, i.e., centroid determination of deuterated peptides. Furthermore, all peptides were inspected manually to ensure correct peak and deuteration assignment by the software.

30

Epitope mapping experiment

Amide hydrogen/deuterium exchange (HX) was initiated by a 10-fold dilution of hIL-21 in the presence or absence of mAb37, mAb61, mAb62 or mAb65 into the corresponding deuterated buffer (*i.e.* PBS prepared in D₂O, 96% D₂O final, pH 7.4 (uncorrected value)).
35 All HX reactions were carried out at 20°C and contained 2 µM hIL-21 in the absence or presence of 1.2 µM mAb thus giving a 1.2 fold molar excess of mAb binding sites. At

appropriate time intervals ranging from 10 sec to 3000 sec, 50 µl aliquots of the HX reaction were quenched by 50 µl ice-cold quenching buffer (1.35M TCEP) resulting in a final pH of 2.5 (uncorrected value).

5 Results and Discussion

Epitope Mapping mAb37, mAb61, mAb62 and mAb65

The epitope mapping of mAb14 on hIL-21 is described in example 7. However, mAb14, in the form of mAb37 (see example 12-13), was also included in these experiments for
10 reference.

The HX time-course of 29 peptides, covering 97% of the primary sequence of hIL-21 were monitored in the absence or presence of mAb37, mAb61, mAb62 or mAb65 for 10 to 3000 sec (table 22).

15

Epitope mapping

The observed exchange pattern in the early timepoints (< 300 sec) in the presence or absence of mAb37, mAb61, mAb62 or mAb65 can be divided into different groups: One group of peptides display an exchange pattern that is unaffected by the binding of these
20 mAbs in the early timepoints. In contrast, another group of peptides in hIL-21 show protection from exchange upon mAb37, mAb61, mAb62 or mAb65 binding in the very early timepoints (Table 22, fx peptide F76-L84 at less than 1 min exchange). Interestingly, the same group of hIL-21 derived peptides were affected by binding of these mAbs thus the epitopes for mAb37, mAb61, mAb62 or mAb65 appear identical and
25 thus identical to the epitope for mAb14 as determined in example 7. A group of peptides showed weak protection at slightly longer timelines. These could be secondary effects of mAb binding, e.g. stabilization effects (Table 22, e.g. peptide I45-D55).

Conclusion

30 Upon binding of either mAb37, mAb61, mAb62 or mAb65 all regions of hIL-21 showed similar responses. The same group of peptides were affected by mAb binding in the early time-points thus the epitopes for mAb37, mAb61, mAb62 or mAb65 appear identical to the epitope for mAb14 determined in example 7.

35

Table 22: HXMS analysis of hIL-21 yielding epitope information for mAb molecules.

After the deuterium exchange reaction, IL-21 was digested with pepsin yielding the following peptic peptide regions that were analyzed.

Compound	mAb37	mAb61	mAb62	mAb65
<i>Sequence</i>				
M29-M39	N	N	N	N
M29-D44	N	N	N	N
Q30-M39	N	N	N	N
G31-M39	N	N	N	N
R40-D44	N	N	N	N
I45-N51	W	W	W	W
I45-D55	W	W	W	W
L56-D66	W	W	W	W
P58-D66	W	W	W	W
L61-D66	N	N	N	N
N70-F76	EX	na	na	na
F76-L84	EX	EX	EX	EX
S77-L84	EX	EX	EX	EX
Q80-V98	N	N	N	N
K85-V98	N	N	N	N
E93-V98	N	N	N	N
E93-S127	N	N	N	N
R94-V98	N	N	N	N
S127-S162	EX	EX	EX	EX
F136-L143	W	W	W	W
F136-L144	W	W	W	W
F136-S162	EX	EX	EX	EX
L137-L143	W	W	W	W
L137-L144	W	W	W	W
E138-L144	W	W	W	W
E138-S162	EX	EX	EX	EX
K141-S162	W	W	W	W
L144-S162	N	N	N	N
Q145-S162	N	N	N	N

- 5 EX: exchange protection upon mAb binding indicating epitope region (> 0.6 Da at both two timepoints below 1 min exchange time).
W: Weak exchange protection upon mAb binding (>0.6 Da at more than two timepoints below 10 min exchange time).
N: No exchange protection upon mAb binding (< 0.2 Da).
na: Not analyzable in respective experiment.
- 10

Example 16**Co-binding studies of human IL-21 to anti IL-21 and IL-21R α / γ C subunits by Surface Plasmon Resonance (SPR) with mAb6, mAb37 and mAb24**

- 5 Binding studies were performed on a Biacore T200 as described in Example 3 but in the current example, anti-human IL-21 monoclonal antibodies mAb6, mAb37 and mAb24 (binding to IL-21 but not competing with mAb6 or mAb37), were immobilized directly onto flow cells of a CM5 sensor chip. mAb24 is the antibody produced by the hybridoma clone 338.28.6.3 / 338.28.6 disclosed in WO2010055366. Another difference from Example 3
- 10 was that individual IL-21 receptor chains IL-21R α -ECD and common γ C-ECD protein were injected in series, creating a stepwise binding of (mAb)/IL-21/IL-21R α / γ C. In this setup, any lack of common γ C protein binding was not dependent on absence of IL-21R α but on competing antibody used to capture IL-21.
- Data analysis was performed as described in Example 3, but using the Biacore T200
- 15 evaluation software 1.0.

In the current example it was shown that binding of IL-21R α to captured IL-21 is a prerequisite for binding of common γ C. It was also concluded that mAb37 prevents interaction of γ C to IL-21/IL-21R α complex. Hence, mAb37 will inhibit the biological

20 effects mediated by IL-21 through γ C and form ligand:IL-21 complexes having the ability to bind specifically to IL-21R α present on cell surfaces.

When IL-21 was captured by a control antibody, binding to a separate site on IL-21 compared to both mAb6 and mAb37, sequential binding of both individual IL-21 receptor chains IL-21R α and common γ C protein was observed.

- 25 These results also explain why IL-21 captured by mAb19, as described in Example 3, was not able to bind simultaneously to neither IL-21R α -ECD nor γ C-ECD.

Table 23 Ability of different antibodies to bind simultaneously to (+) or to compete with (-) binding of different receptor subunits to IL-21. Injection number indicate sequence of injections. Y/N indicates whether receptor subunits were injected or not.

Immobilized mAb	Injection 1	Injection 2		Injection 3	
	Capture	IL-21R α injected	IL-21R α binding	γ C injected	γ C binding
mAb6	100nM hIL-21	N	n/a	Y	(+)
mAb6	100nM hIL-21	Y (50 nM)	-	Y	(+)
mAb24	100nM hIL-21	N	n/a	Y	-
mAb24	100nM hIL-21	Y (50 nM)	+	Y	+
mAb37	100nM hIL-21	N	n/a	Y	-
mAb37	100nM hIL-21	Y (50 nM)	+	Y	-

5

CLAIMS

1. An antibody which binds to an epitope on IL-21, wherein said epitope comprises one or more of the following amino acids: Glu 65, Asp 66, Val 67, Glu 68, Thr 69, Asn 70, Glu 72, Trp 73, one or more of the following amino acids Lys 117, His 118, Arg 119, and
5 one or more of the following amino acids: Leu 143, Lys 146, Met 147, His 149, Gln 150, and His 151 as set forth in SEQ ID No.1, provided that the antibody is not the monoclonal antibody mAb14, the light and heavy chains of which are set forth in SEQ ID No. 6 and SEQ ID No. 7, respectively.
2. An antibody which binds to an epitope on IL-21, wherein said epitope comprises one
10 or more of the following amino acids: Glu 65 to Trp 73, one or more of the following amino acids: Lys 117 to Arg 119, and one or more of the following amino acids: Leu 143 to His 151 as set forth in SEQ ID No.1, provided that the antibody is not the monoclonal antibody mAb14, the light and heavy chains of which are set forth in SEQ ID No. 6 and SEQ ID No. 7, respectively.
- 15 3. An antibody which binds to an epitope on IL-21, wherein said epitope comprises one or more of the Arg 40 to Val 67 amino acids as well as one or more of the Glu 129 to His 149 amino acids, as set forth in SEQ ID No.1, provided that the antibody is not mAb14, the light and heavy chains of which are set forth in SEQ ID No. 6 and SEQ ID No. 7, respectively.
- 20 4. An antibody which binds to an epitope on IL-21, wherein said epitope comprises one or more of the Glu 65 to Trp 73 amino acids in IL-21 (SEQ ID NO. 1) provided that the antibody is not mAb14, the light and heavy chains of which are set forth in SEQ ID No. 6 and SEQ ID No. 7, respectively.
5. An antibody according to any one of the preceding claims, wherein said antibody
25 binds one or more of Glu 65, Asp 66, and Val 67 as set forth in SEQ ID NO. 1.
6. An antibody according to any one of the preceding claims, wherein said antibody binds His 149 as set forth in SEQ ID NO. 1.
7. An antibody which binds to an epitope on IL-21, wherein said epitope comprises
30 one or more of the Glu 65, Asp 66, Val 67, and His 149 amino acids as set forth in SEQ ID NO. 1, provided that the antibody is not mAb14, the light and heavy chains of which are set forth in SEQ ID No. 6 and SEQ ID No. 7, respectively.

8. An antibody which binds to an epitope on IL-21, wherein said epitope comprises one or more of the following amino acids: Arg 40, Lys 50, Glu 65, Asp 66, Val 67, Glu 129, Glu 135, Glu 138, Arg 139, Lys 141, Ser 142, Gln 145, and His 149 as set forth in SEQ ID No. 1, provided that the antibody is not mAb14, the light and heavy chains of which are set forth in SEQ ID No. 6 and SEQ ID No. 7, respectively.
9. An antibody which binds to an epitope on IL-21, wherein said epitope comprises one or more of the following amino acids: Glu 65, Asp 66, Val 67, Glu 68, Thr 69, Asn 70, Glu 72, Trp 73, Lys 117, His 118, Arg 119, leu 143, Lys 146, Met 147, His 149, Gln 150, and His 151, provided that the antibody is not mAb14, the light and heavy chains of which are set forth in SEQ ID No. 6 and SEQ ID No. 7, respectively.
10. An antibody according to claim 9, wherein said antibody comprises a light chain comprising at least one of CDR1, CDR2, and CDR3 as set forth in SEQ ID No. 6, and a heavy chain comprising at least one of CDR1, CDR2, and CDR3 as set forth in SEQ ID No. 7.
11. An antibody according to any one of claims 1-10, wherein said antibody interferes with the binding of common γ C chain to IL-21.
12. An antibody according to claim 10, wherein said antibody is a variant of mAb14, the light and heavy chains thereof which are set forth in SEQ ID No. 6 and SEQ ID No. 7 respectively, wherein said antibody comprises one or more mutations in the CDR sequences, wherein said mutations are selected from one or more from the list consisting of: A61S (SEQ ID NO 7), D62E (SEQ ID NO 7), V64I (SEQ ID NO 7), and K65R (SEQ ID NO 7), R24K (SEQ ID NO 6), S26T (SEQ ID NO 6), Q27N (SEQ ID NO 6), D30E (SEQ ID NO 6), S53T (SEQ ID NO 6), and S56T (SEQ ID NO 6).
13. A pharmaceutical composition comprising an antibody according to any one of the preceding claims, and optionally one or more pharmaceutically acceptable excipients.
14. Use of an antibody according to any one of claims 1-12 for treating an immunological disorder.
15. A method for selecting a ligand which binds to IL-21, wherein said method comprises screening one or more libraries of ligands with an IL-21 mimic, wherein said IL-21 mimic comprises an epitope comprising the following amino acids: Glu 65, Asp 66, Val 67, and His 149 as set forth in SEQ ID No.1, and isolating one or more ligands which bind to said discontinuous epitope.

Fig. 1

SEQ ID No 1: IL-21

MRSSPGNMERIVICLMVIFLGLTLVHKSSSQGQDRHMIRMRLIDIVIDQLKKNYVNDLVPPEFLPAPEDVETNCEWSA
FSCFQKAQLKSANTGNNERIINVSIIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLERFKSLLQKMIHQ
HLSSRTHGSEDS

SEQ ID No 2: Helix A of IL-21:

RHMIRMRLIDIVIDQLKKNY

SEQ ID No 3: Helix B of IL-21:

EWSAFSCFQKA

SEQ ID No 4: Helix C of IL-21:

ERIINVSIKKL

SEQ ID No 5: Helix D of IL-21:

PKEFLERFKSLLQKMIHQHL

SEQ ID No 6: mAb14 light chain (signal peptide omitted) (**variable region shown in UPPERCASE letters, CDR sequences shown in bold/underline, constant region shown in lowercase letters**)

AIQLTQSPSSLSASVGDVRTITC**RASQDIDSALAWYQQKPGKAPKILIH****DASSLES**GVPSRFSGSGSGTDFTLTI
SSLQPEDFATYYC**QQFN****SYPYT**FGQGTKLEIKRtvaapsvfifppsdeqlksgtasvvc1lnnfyreakvqwk
dnalqsgnsqesvteqdkdstylsstltlksadyekhkvacevthqglsspvtksfnrgec

SEQ ID No 7: mAb14 heavy chain (signal peptide omitted) (**CDR sequences shown in bold/underline, constant region shown in lowercase letters**)

EVQLVESGGGLVQPGGSLRLSCAASGFIFD**SYSMN**WVRQAPGKGLEWVS**SITSGSYIHYADSVKGR**FTISRDNA
KNSLYLQMNLSRAEDTAVYYCVR**ERGWGYYGMDV**WGQGTTVTVSSastkqpsvfplapcsrstsestaalgclvk
dyfpepvtvswngaltsgvhtfpav1qssgylsslsvvtvpssslgktytcnvdhkpsntkvdkrveskygpp
cpscpapeflggpsvflfppkpkdtlmsirtpevtcvvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnsty
rvsvltvlhqdwlngkeykckvsnkg1pssiectiskakgpprepqvytlppsqeemtknqvsltclvkgfyps
diavewesngqpennykttppvldsdgsfflysr1tvdksrwqegnfvfscsvmhealhnhytqks1slslgk

SEQ ID No 8: Common gamma chain (γC). The sequence is the full length incl. Signal sequence)

MLKPSLPFTSLLFLQLPLLVGLNNTTILTPNGNEDTTADFFLTTMPTDSLVSSTLPLPEVQCFFVFNVEYMNCTWN
SSSEPOPTNLTLYHYWYKNSDNDKVQKCSHYLFSEEITSGCQLQKKEIHLYQTFVVQLQDPREPRRQATQMLKLN
LVIPWAPENLTLHKLSESQLELNWNNRFLNHCLEHLVQYRTDWDHWSWTEQSDYRHKFSLPSVDGQKRYTFRVRS
RFNPLCGSAQHWSEWSHPIHWGNSNTSKENPFLFALEAVVISVGSMLIISLLCVYFWLERTMPRIPTLKNLEDLV
TEYHGNFSAWSGVSKGLAESLQPDYSERLCLVSEIIPKGGALGEGPGASPCNQHSPYWAPPCYTLKPEP

SEQ ID No 9: Fab35 light chain (Fab35 is a Fab fragment of mAb14) (signal peptide omitted) (**variable region shown in UPPERCASE letters, CDR sequences shown in bold/underline, constant region shown in lowercase letters**)

AIQLTQSPSSLSASVGDVRTITC**RASQDIDSALAWYQQKPGKAPKILIH****DASSLES**GVPSRFSGSGSGTDFTLTI
SSLQPEDFATYYC**QQFN****SYPYT**FGQGTKLEIKRtvaapsvfifppsdeqlksgtasvvc1lnnfyreakvqwk
dnalqsgnsqesvteqdkdstylsstltlksadyekhkvacevthqglsspvtksfnrgec

SEQ ID No 10: Fab35 heavy chain (Fab35 is a Fab fragment of mAb14) (signal peptide omitted) (**CDR sequences shown in bold/underline, constant region shown in lowercase letters**)

EVQLVESGGGLVKPGGSLRLSCAASGFI F S SYSMNWVRQAPGKGLEWVS SITSGSYIHYADSVKGRFTISRDN
KNSLYLQMNSLRAEDTAVYYCVR ERGWGYGMDVWGQGT T V T V S Sastkgpsvfplapcsrstsestaalgclvk
dyfpepvtvswngaltsgvhtfpavlgssglyslssvvtvpssslgktytcnvdhkpsntkvdkrvesk

Fig. 2

hIL21: QGQDRHMIRMQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEEWSAFSCFQKAQLKSANT
BS1: X XX XX XX X X
BS2: X X XXX
Epitope14: XXXXXX XX
Epitope5: XX X XX XX XX

hIL-21: GNNERIINVSIKKLKRKPPSTNAGRROKHRLTCPCSDSYEKKPPPKEFLERFKSLLQKMIH
BS1: X XX X XXXXX
BS2: X X XX XX X X
Epitope14: XXX X XX X
Epitope5: X XX XXX XX XXXX

hIL-21: QHLSSRTHGSEDS
BS1:
BS2:
Epitope14: XX
Epitope5:

Fig.3

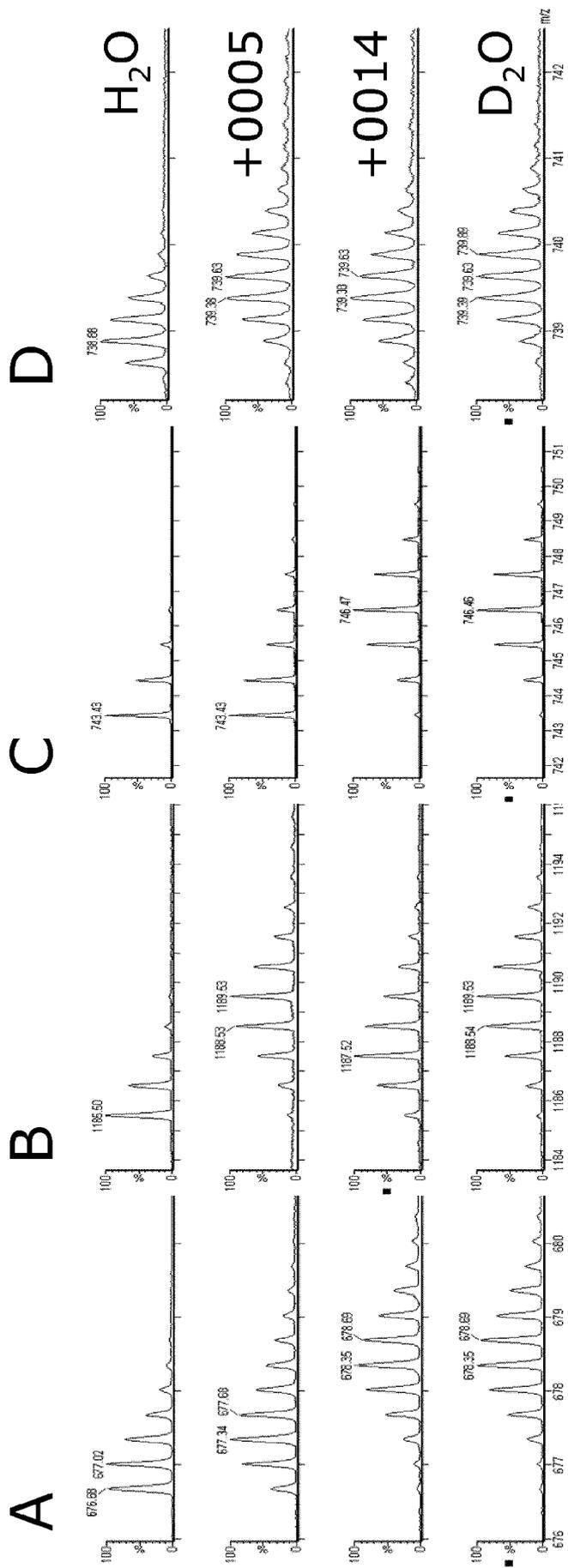


Fig. 4a

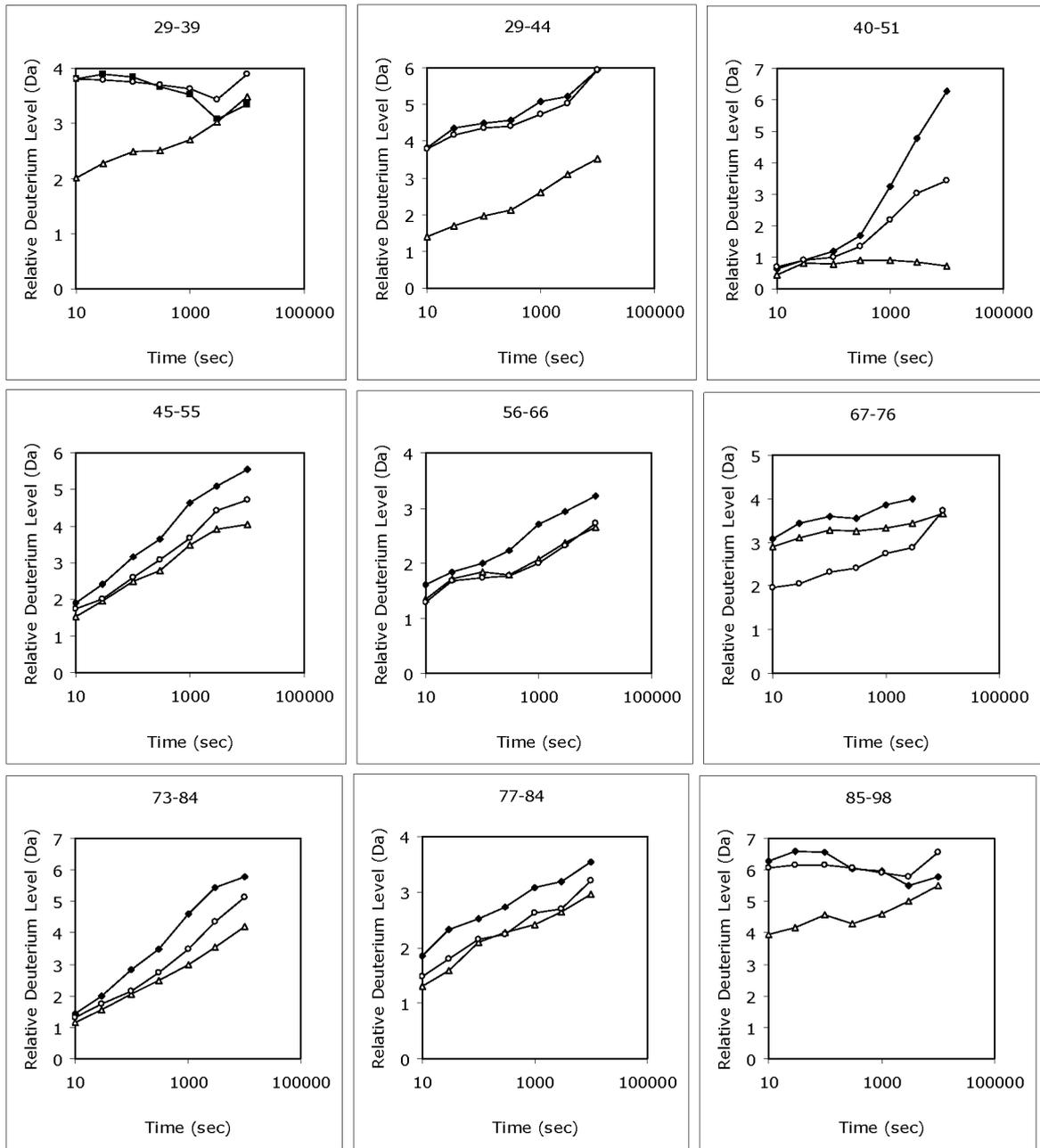


Fig. 4b

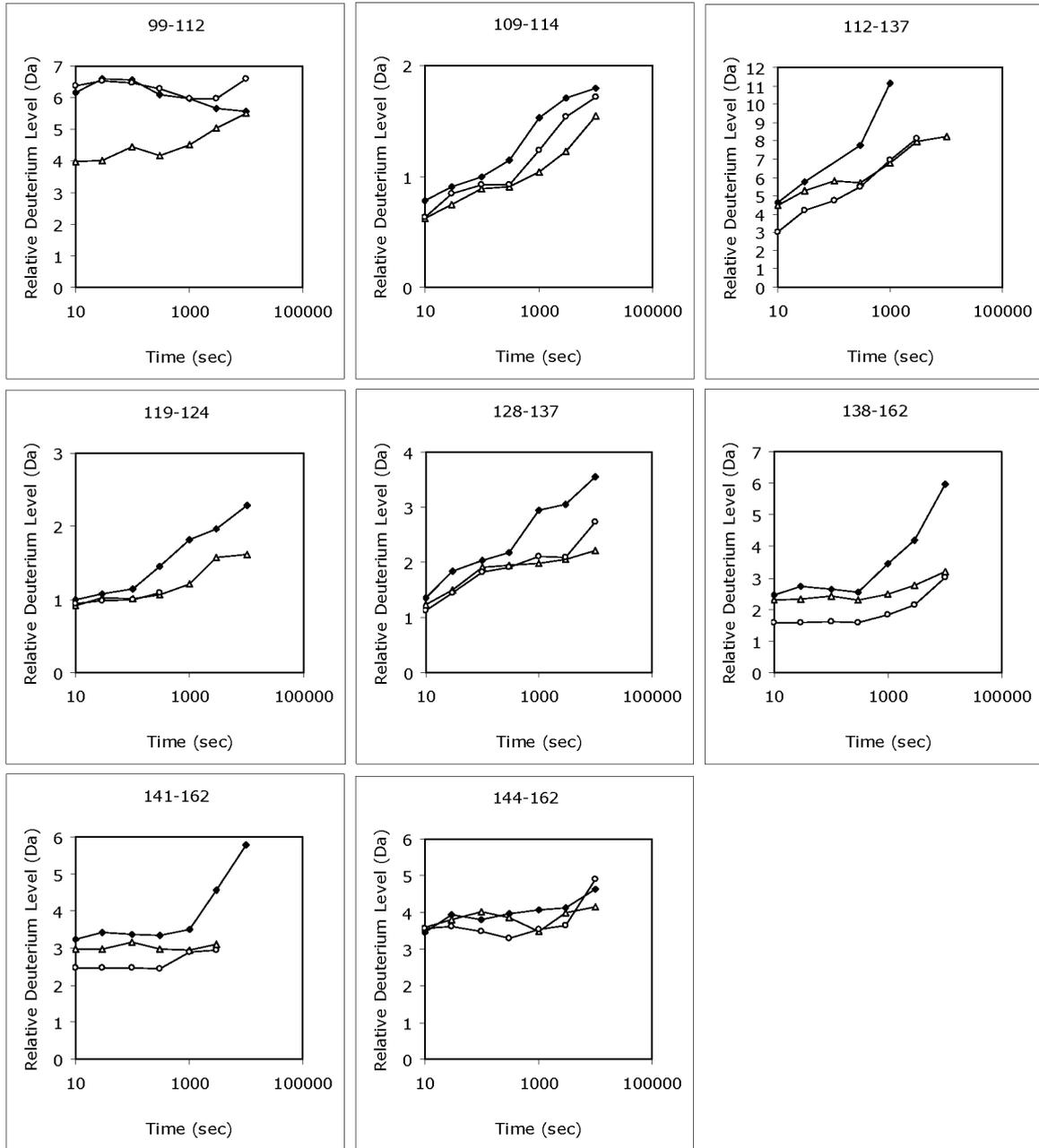
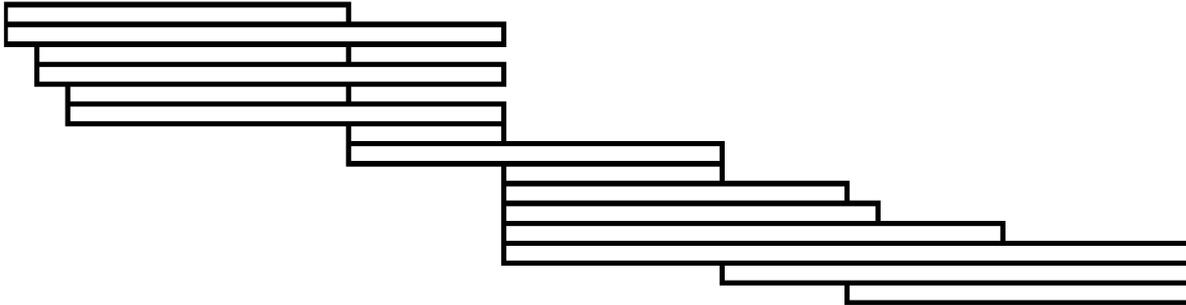
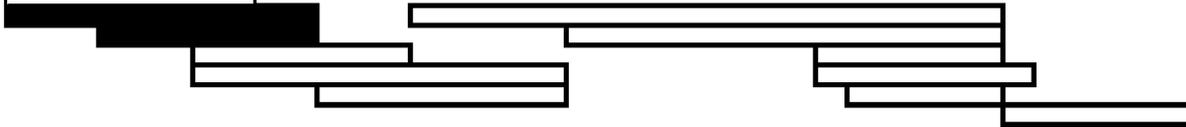


Fig. 5

29 66
MQGQDRHMI RMRQL I D I VDQLKNYVNDLVPEFLPAPED



67 74 103
VETNCEWS AFSCFQKAQLKSANTGNNERI INVS IKKL



104 115 120 137
KRKPPSTNAGR RQKHRL T CPSCDSYEKKPPKEFL



138 143 146 162
ERFKS LLQK MI HQHLSRTHGSEDS



Fig. 6

```

SEQ ID NO:1
33                                     82
hIL-21_Fab35 DRHMIRMRLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKA
hIL-21_Fab56 DRHMIRMRLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKA
hIL-21_Fab57 DRHMIRMRLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKA
hIL-21_Fab59 -----QLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKA
hIL-21_Fab60 DRHMIRMRLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKA

83                                     132
hIL-21_Fab35 QLKSANTGNNERIINVSIKKLRK-----RRQKHRLTCPSCDSYEKKP
hIL-21_Fab56 QLKSANTGNNERIINVSIKKLRK-----RRQKHRLTCPSCDSYEKKP
hIL-21_Fab57 QLKSANTGNNERIINVSIKKLRK-----RRQKHRLTCPSCDSYEKKP
hIL-21_Fab59 QLKSANTGNNERIINVSIKKLRKPPSTNAGRRQKHRLTCPSCDSYEKKP
hIL-21_Fab60 QLKSANTGNNERIINVSIKKLRK-----RRQKHRLTCPSCDSYEKKP

133                                     152
hIL-21_Fab35 PKEFLERFKSLLQKMIHQHL
hIL-21_Fab56 PKEFLERFKSLLQKMIHQHL
hIL-21_Fab57 PKEFLERFKSLLQKMIHQHL
hIL-21_Fab59 PKEFLERFKSLLQKMIHQHL
hIL-21_Fab60 PKEFLERFKSLLQKMIHQHL
    
```

Fig. 7

```
SEQ ID 3.....4.....5.....6.....7.....
NO: 1 0.....0.....0.....0.....0.....
      QQQDRHMIRMRLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCF
Fab35 .....-.....=====,==,-...
Fab56 .....-.....=====,==,-...
Fab57 .....-.....=====,==,-...
Fab59 .....-.....=====,==,-...
Fab60 .....-.....=====,==,-...

      8.....9.....10.....11.....12.....
      0.....0.....0.....0.....0.....
      QKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRQKHRLTCPSCDSYE
Fab35 .....=====,-.....-...
Fab56 .....=====.....--...
Fab57 .....=====,-.....--...
Fab59 .....==-,=====,-.....-...
Fab60 .....=====,-.....--...

      13.....14.....15.....16.....17
      0.....0.....0.....0.....0
      KKPPKEFLERFKSLQKMIHQHLSSRTHGSEDS
Fab35 .....=-==,====,.....
Fab56 .....=-==,====,.....
Fab57 .....=-==,====,.....
Fab59 .....=-==,====,.....
Fab60 .....=-==,====,.....
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/060248

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61P37/00 A61K39/395 C07K16/28
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C.

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Date of the actual completion of the international search 22 August 2012	Date of mailing of the international search report 31/08/2012
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Ulbrecht, Matthias

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/060248

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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Information on patent family members

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