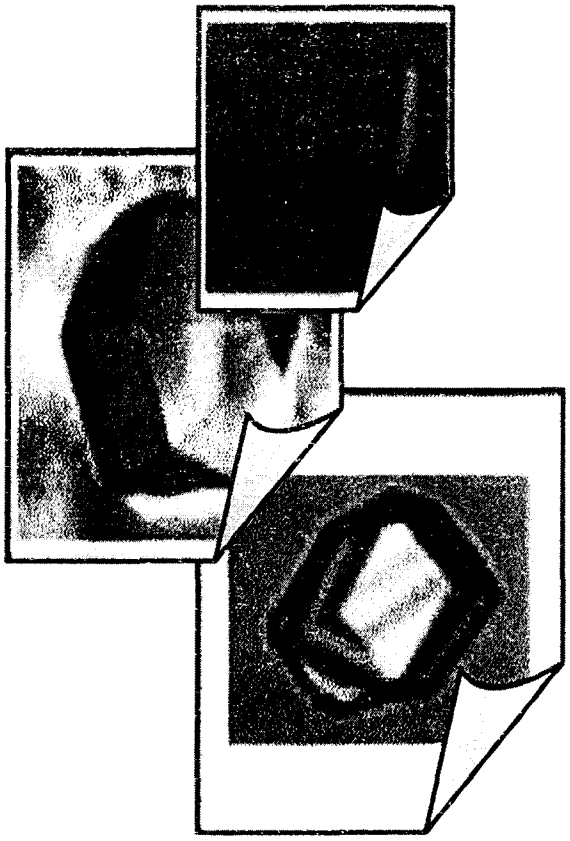
PROTEIN STRUCTURE

NEW APPROACHES TO DISEASE AND THERAPY

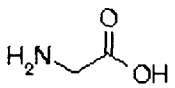


선임연구원 차선신

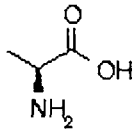
PAL 포항가속기연구소
POHANG ACCELERATOR LABORATORY

DNA => Protein

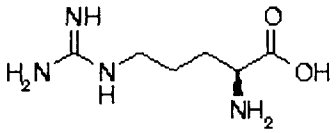
Proteins are composed of 20 amino acids that are connected chemically through amide bonds



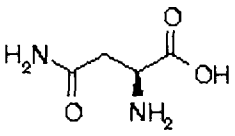
gly g Glycin



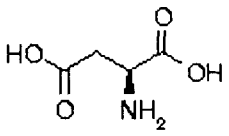
ala a Alanin



arg r Arginin



asn n Asparagin



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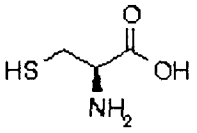
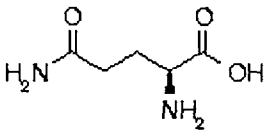
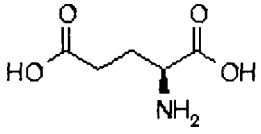


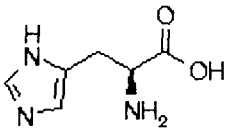
cys c Cystein



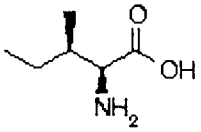
gln q Glutamin



glu e Glutaminsaeure



his h Histidin



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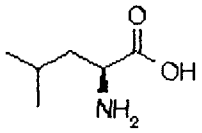
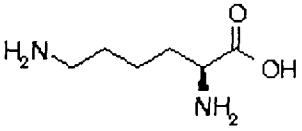
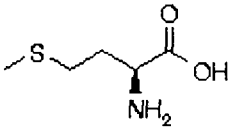


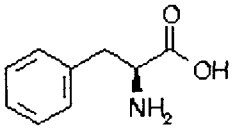
leu l Leucin



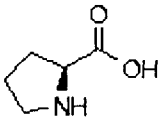
lys k Lysin



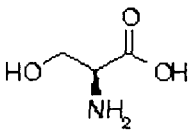
met m Methionin



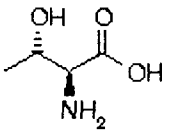
phe f Phenylalanin



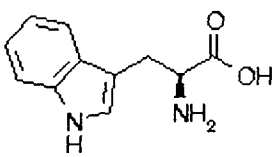
pro p Prolin



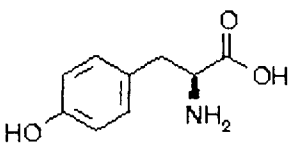
ser s Serin



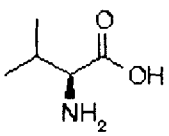
thr t Threonin



trp w Tryptophan



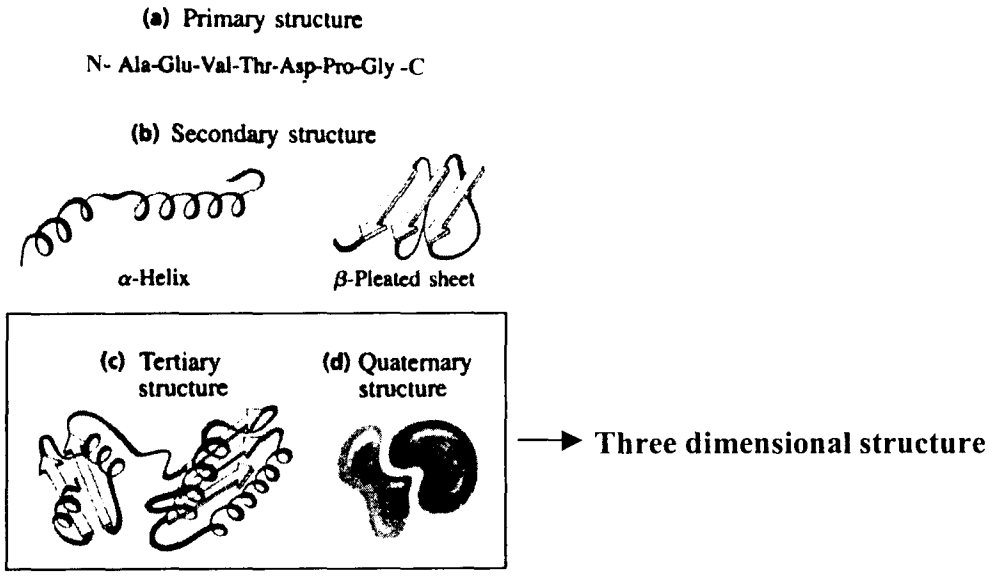
tyr y Tyrosin



val v Valin

Basic Knowledge

The Structure of Proteins is Hierarchical



Protein Engineering

- change amino acids at specific positions in proteins

  Asp => Asn : point mutation

  Insertion of one to several amino acids

  Deletion of one or several amino acids

- make mutant proteins with designed biological characteristics

APOPTOSIS

Two kinds of death in cells

Death by injury – serious effects on life

Cells that are damaged by injury, such as by
•mechanical damage
•exposure to toxic chemicals

Death by suicide (programmed cell death or **PCD. Apoptosis)**

1. proper development.
2. destroy cells that represent a threat to the integrity of the organism

Examples-

Cells infected with viruses
Cells of the immune system
Cells with DNA damage
Cancer cells

Cancer Immunotherapy

1. Various immune responses to tumor cells

2. Not sufficient to prevent tumor growth

3. Augment or supplement natural defense mechanisms <= safe cancer treatment

Cytokine Therapy

(Many of the bio-venture companies)

1. Interferons

   - strengthen immune defense system

2. Tumor Necrosis Factor-α (TNF-α)

   - directly kills cancer cells : *today's topic*

Limitations of TNF-α therapy

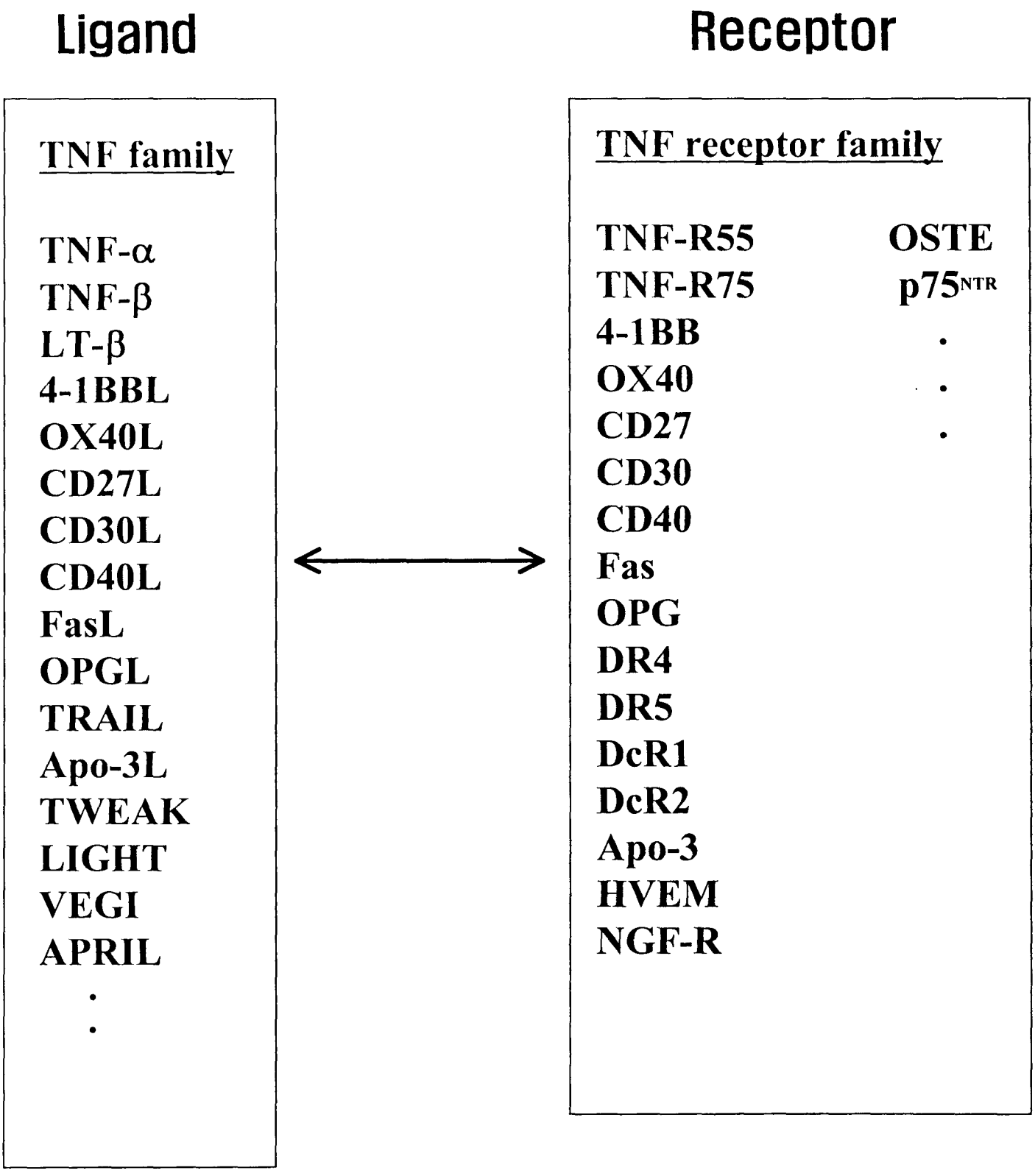
1. Short half-life necessitates frequent injections

2. Adverse side effects: fever, chills, blood-pressure changes, and decreased counts of white blood cells
   => death of normal cells

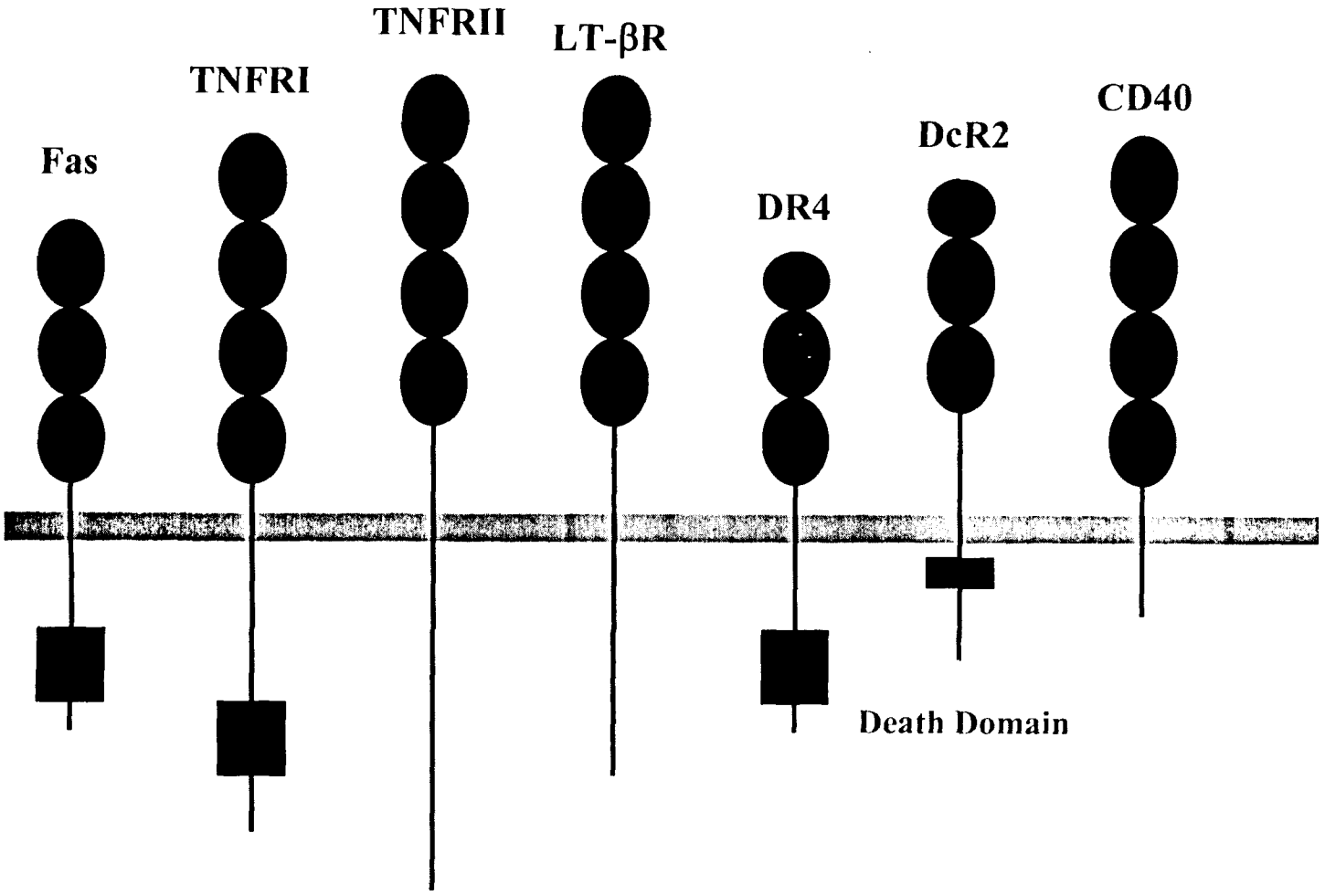
The search for a cytokine that **selectively affects tumor survival** in humans without significant toxicity : ***TRAIL***

Generation of thousands of TNF mutants with low toxicity and longer half-life : ***M3S***

TRAIL belongs to TNF family, a rapidly growing superfamily that interact with a corresponding superfamily of receptors on cell surface


Ligand
Receptor
TNF family
TNF-α
TNF-β
LT-β
4-1BBL
OX40L
CD27L
CD30L
CD40L
FasL
OPGL
TRAIL
Apo-3L
TWEAK
LIGHT
VEGI
APRIL
TNF receptor family
TNF-R55
TNF-R75
4-1BB
OX40
CD27
CD30
CD40
Fas
OPG
DR4
DR5
DcR1
DcR2
Apo-3
HVEM
NGF-R
OSTE
p75NTR

Signals are transduced through receptors
from exterior to interior of cells after binding of ligand



From the structural analyses of TRAIL, TRAIL/DR5 complex, and a TNF-α mutant (M3S)

1. Insights into the specific recognition between proteins
   - *all the biological processes are mediated by specific interactions between biomolecules*

2. Rational design of mutants that are more suitable for safe anticancer agents

3. Structure-Function relationship
   => ***structural genomics***

Human TRAIL (Apo-2L)

TNF-Related Apoptosis Inducing Ligand.

Discovered in 1995 (Immunex, Genentech).

Immunex and Genentech have patent on TRAIL gene.

Induces rapid apoptosis in a variety of tumor cell lines.

But, normal cells are not killed by TRAIL.

Administration of TRAIL suppresses transplanted tumors in mice.

No cytotoxicity to normal tissues in vivo mouse and primate models

"Magic bullet" so far.

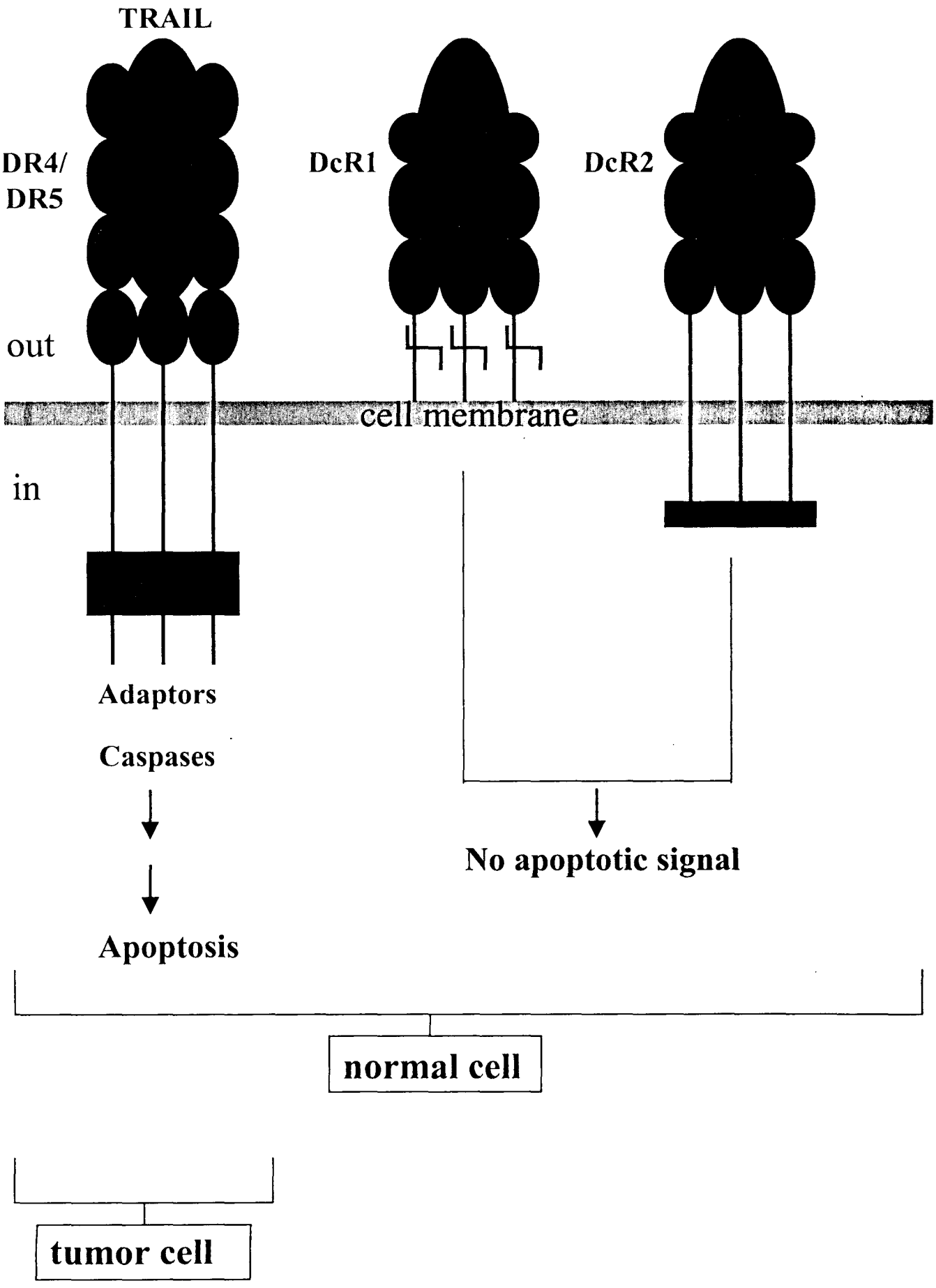
Under a clinical demonstration in U.S.A. (Immunex, Genentech).

Two signalling receptors are identified.

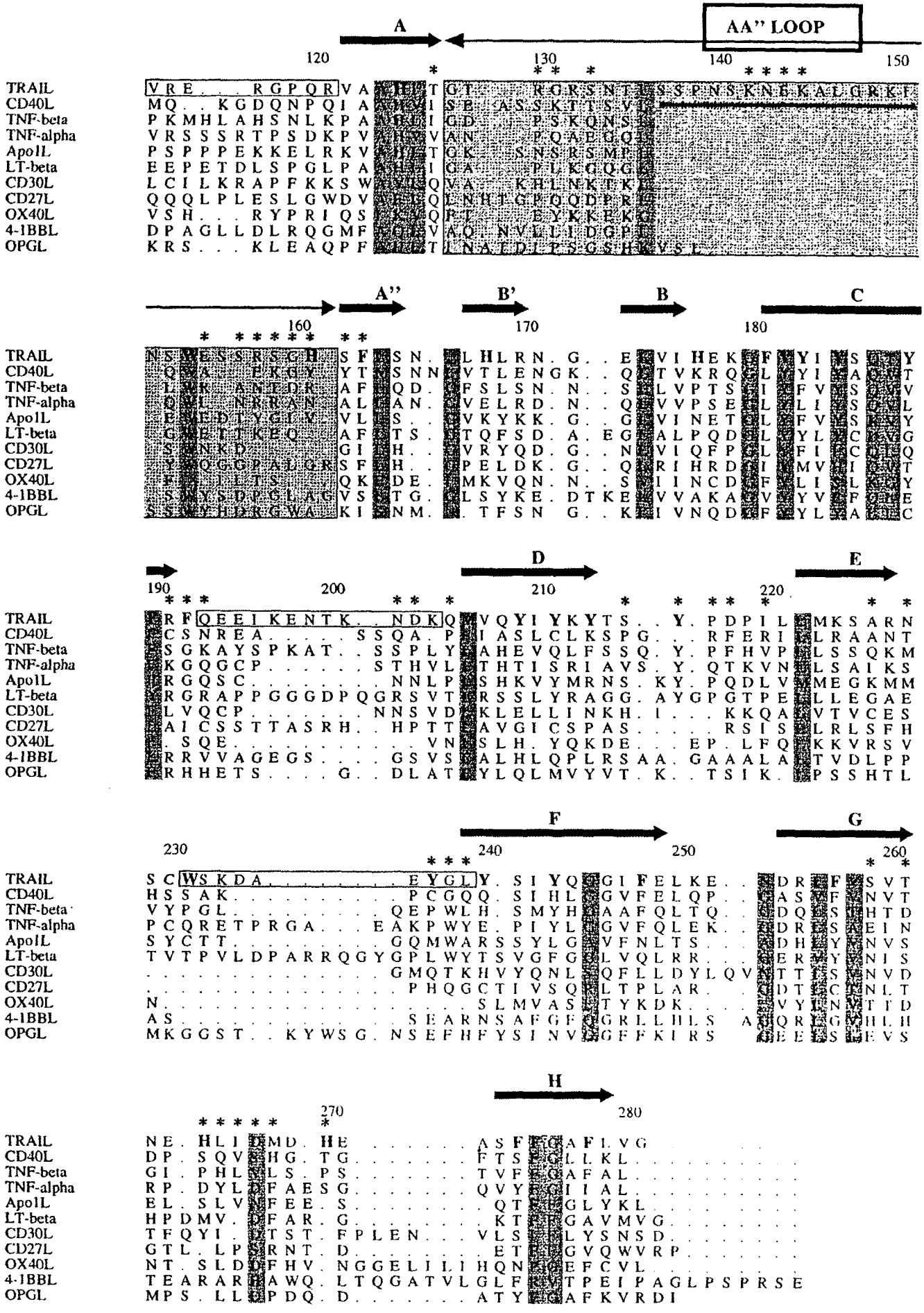
Two non-signalling decoy receptors are identified.

Decoys are mainly expressed in normal cells.

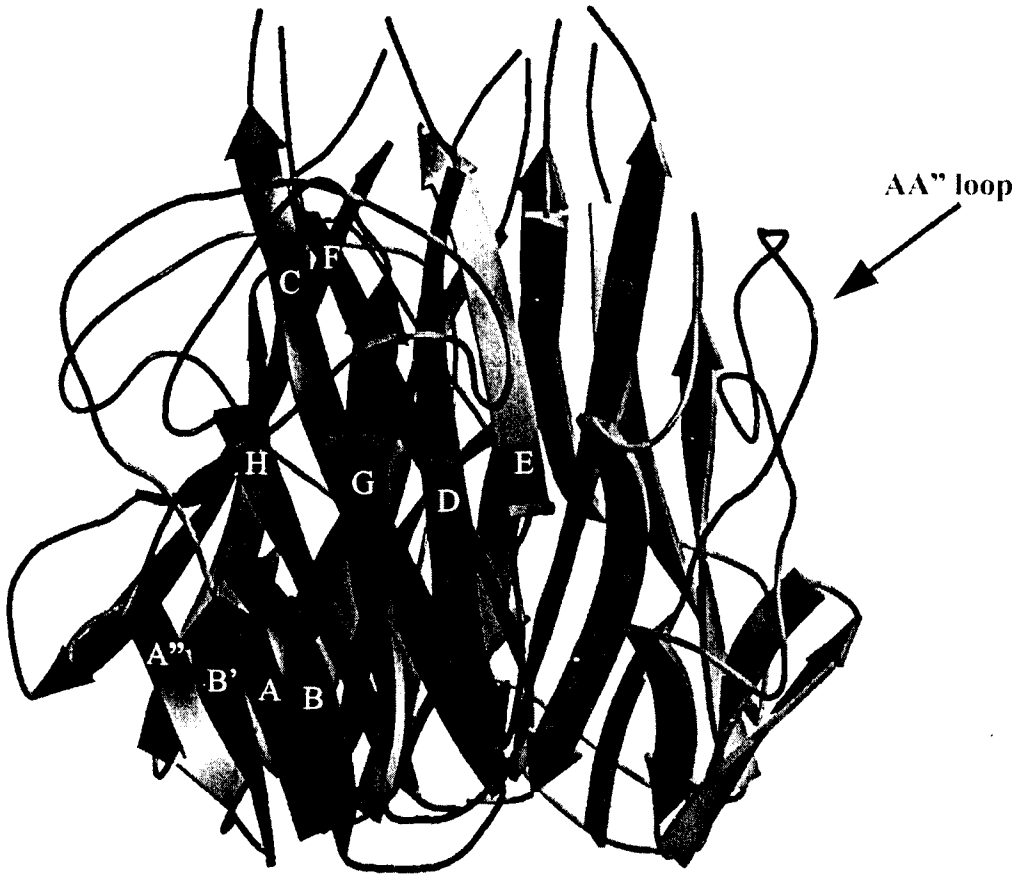
How does TRAIL selectively kills tumor cells



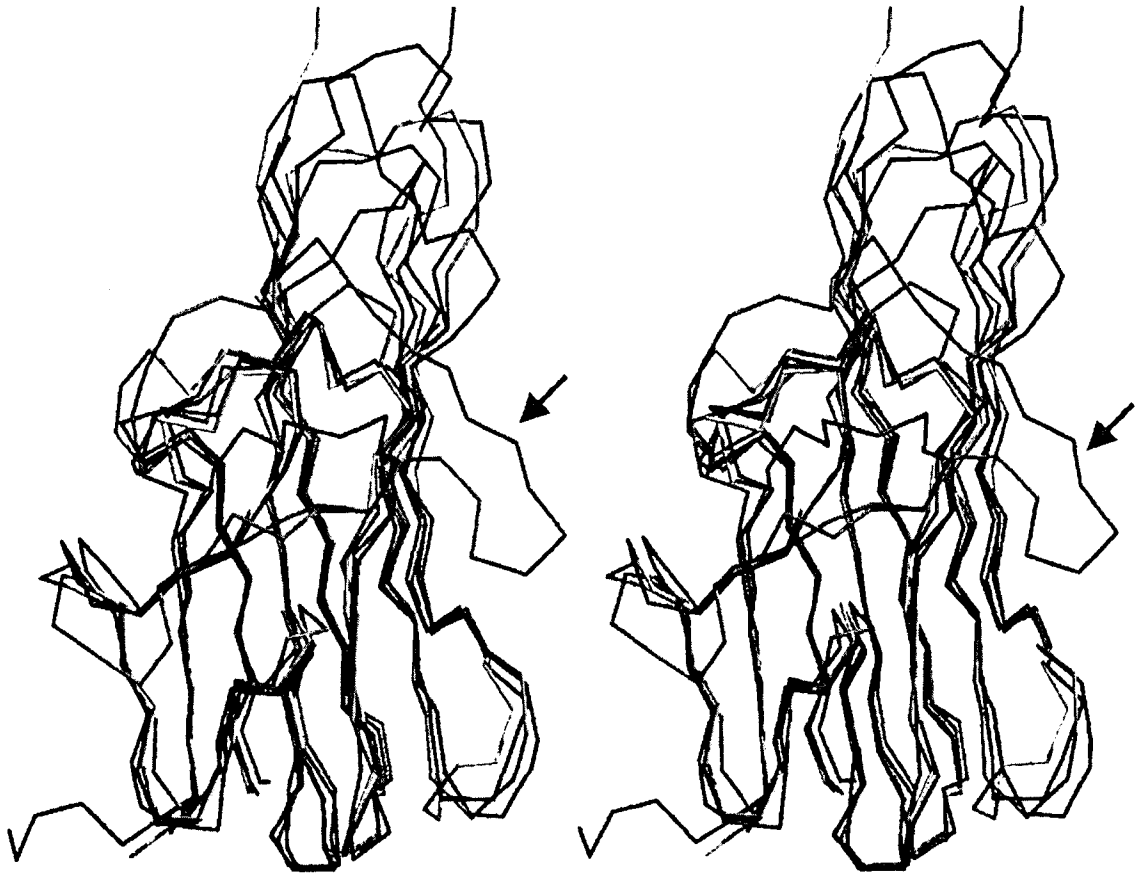
Primary structure of TRAIL



Tertiary Structure of TRAIL



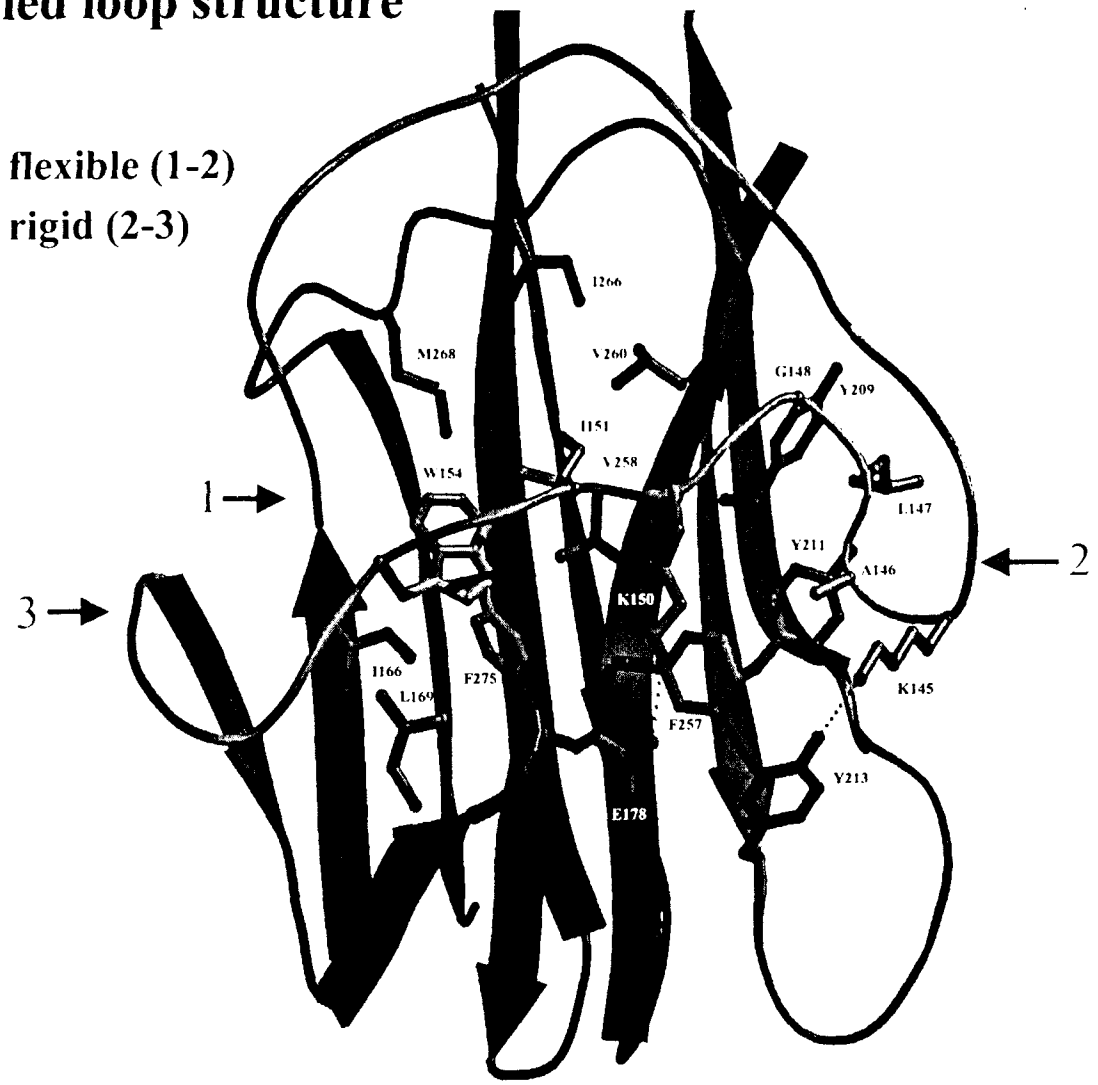
Homotrimeric structure



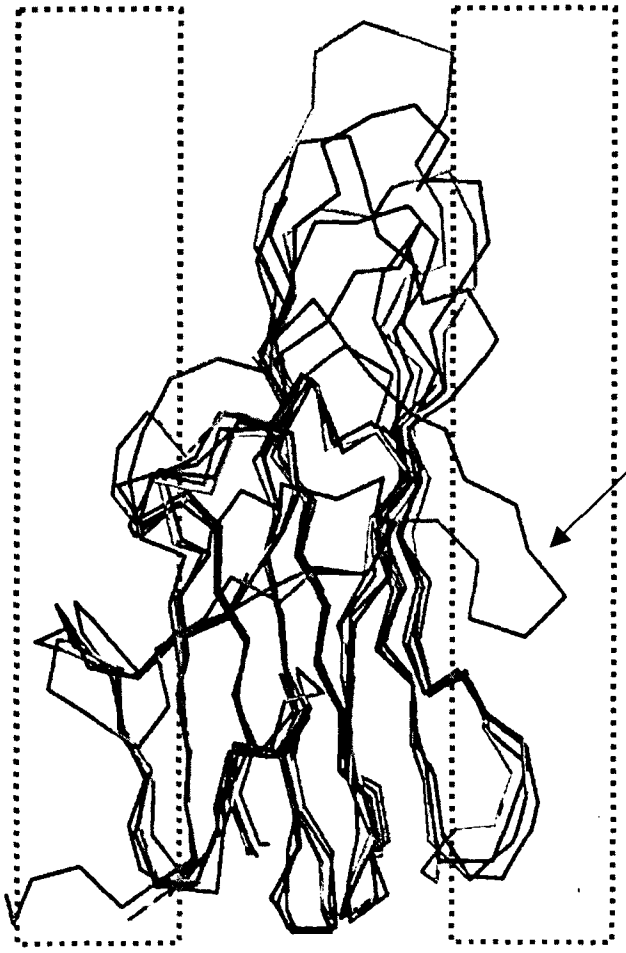
Detailed loop structure

Half : flexible (1-2)

Half : rigid (2-3)



Putative receptor binding region



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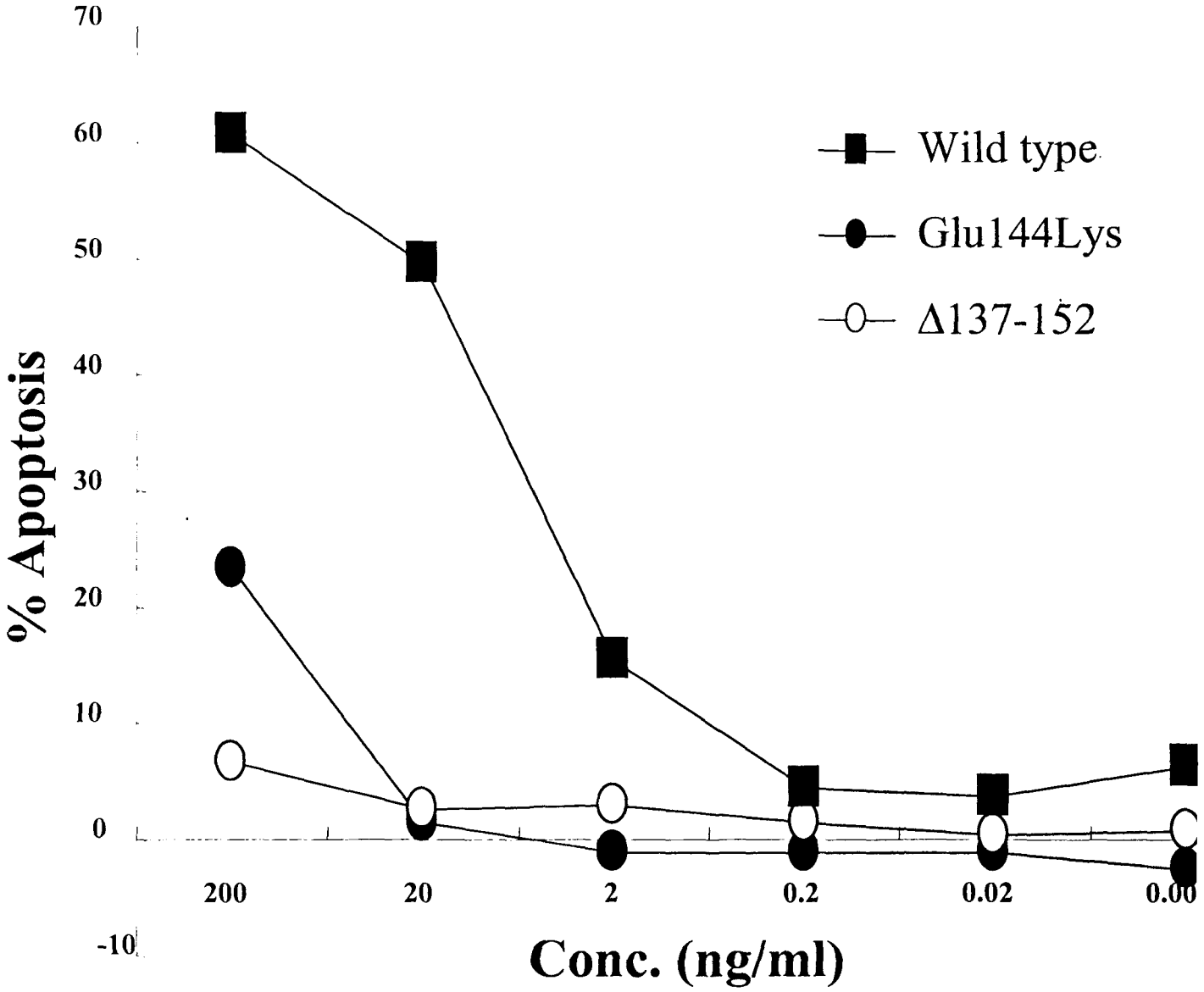
AA" loop Receptor binding

Mutations on AA" loop

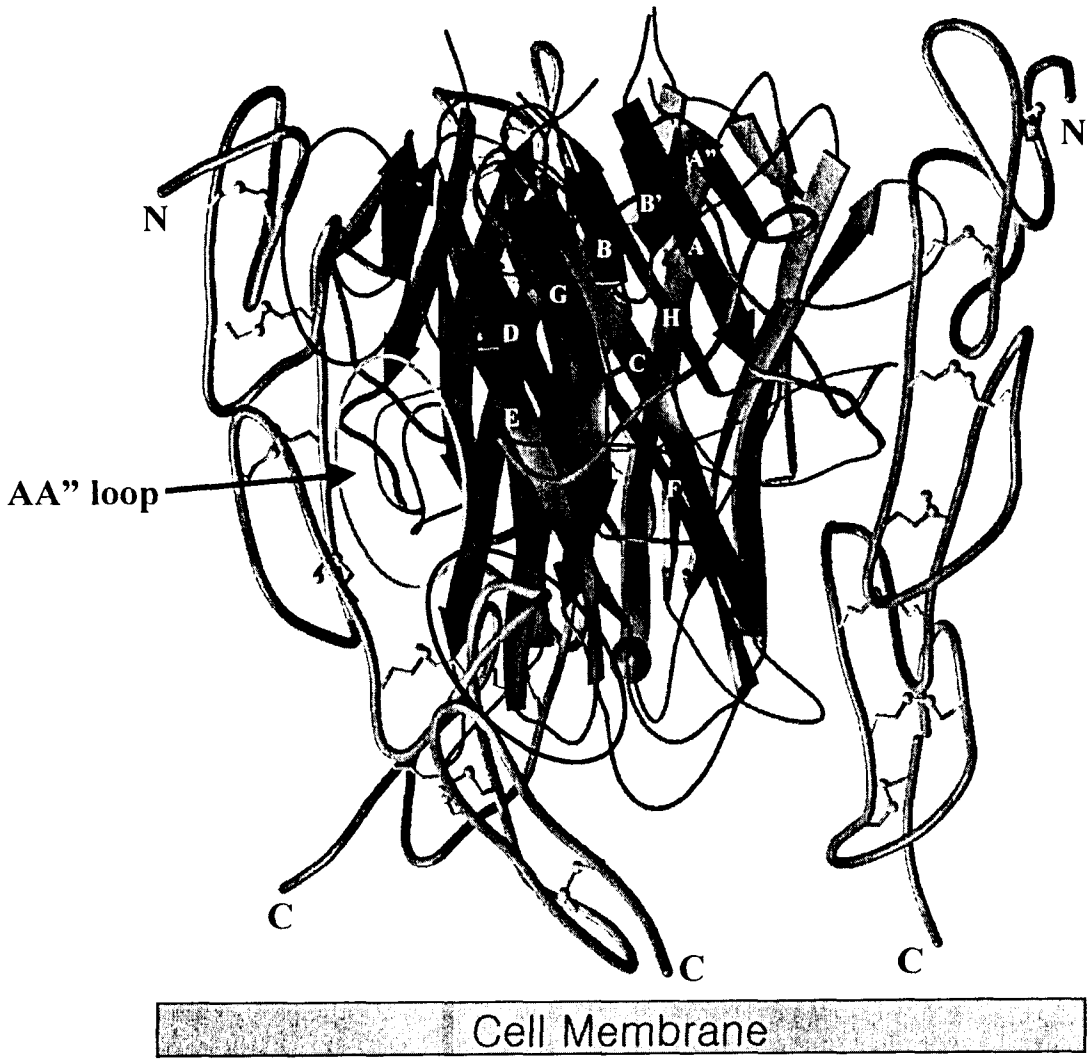


Indirect evidence of the engagement of the loop on the complex formation

Complete understanding of complex formation needs three dimensional structure of the complex

Crystallization of TRAIL/DR5 complex

Tertiary Structure of TRAIL/DR5 complex



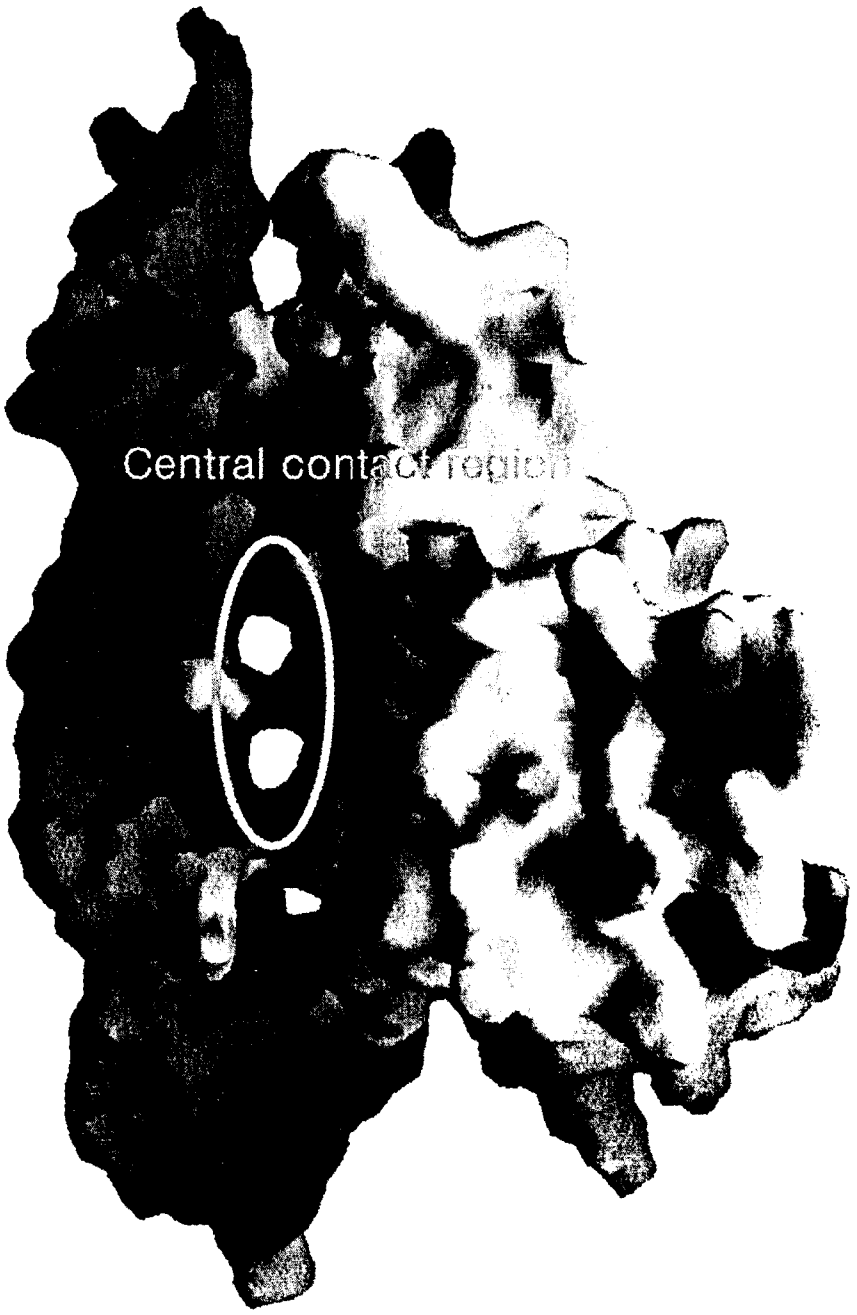
Contact region can be divided into three parts

- Upper, Central, and Lower contact region

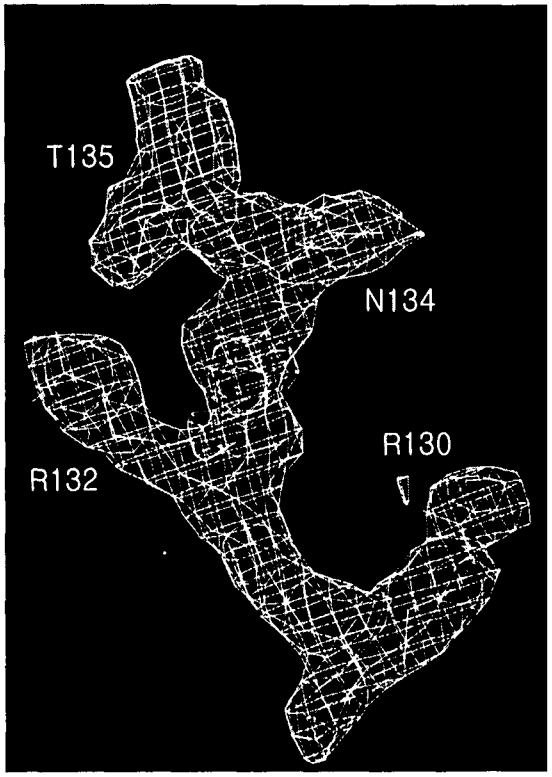
Central Contact Region

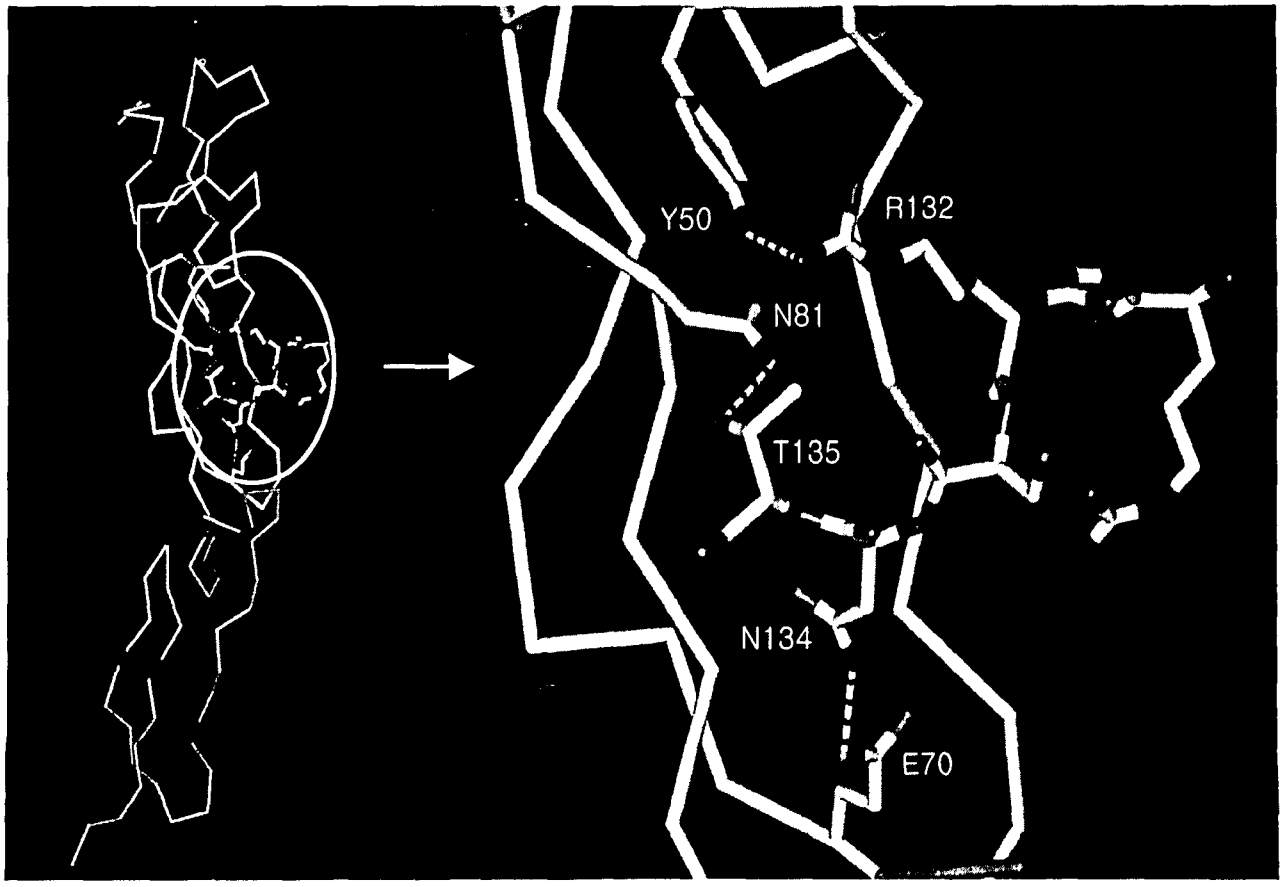
- Involvement of AA" loop in complex formation

Central Contact Region

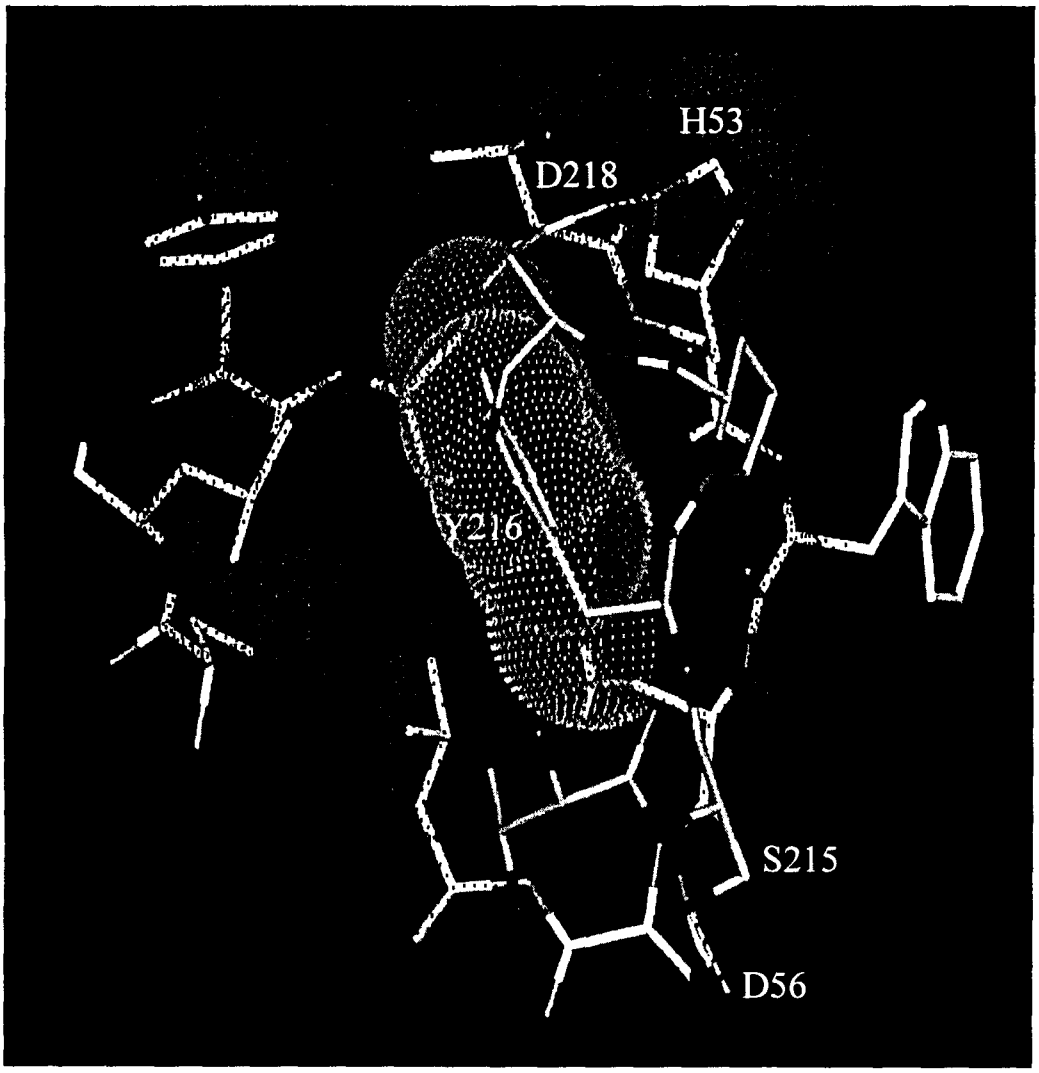



Residues 130-135


T135
N134
R130
R132


Y50
R132
N81
T135
N134
E70

Upper Contact Region



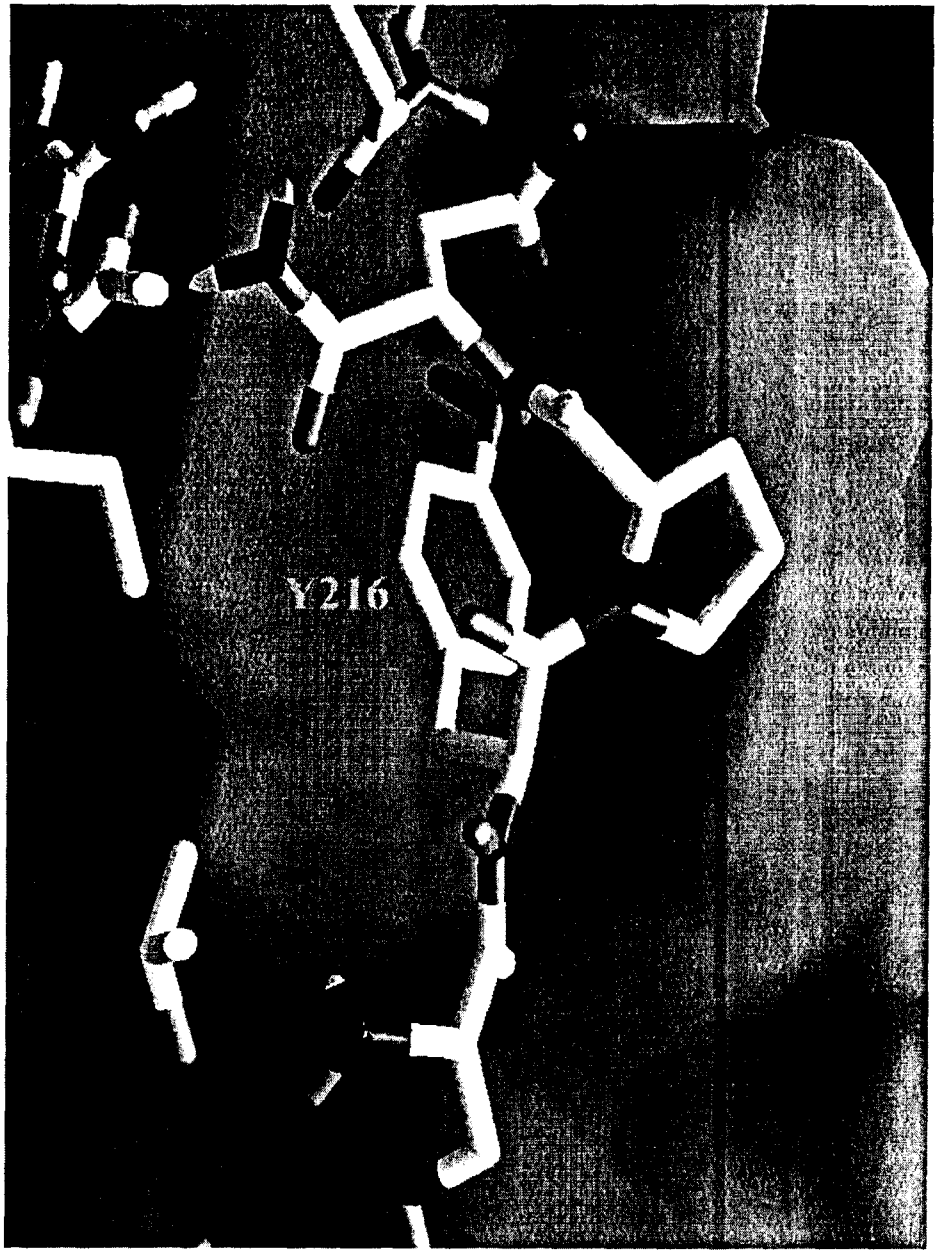
Polar Interactions:

S215 (TRAIL)- D56 (sDR5)
D218 (TRAIL)- H53 (sDR5)

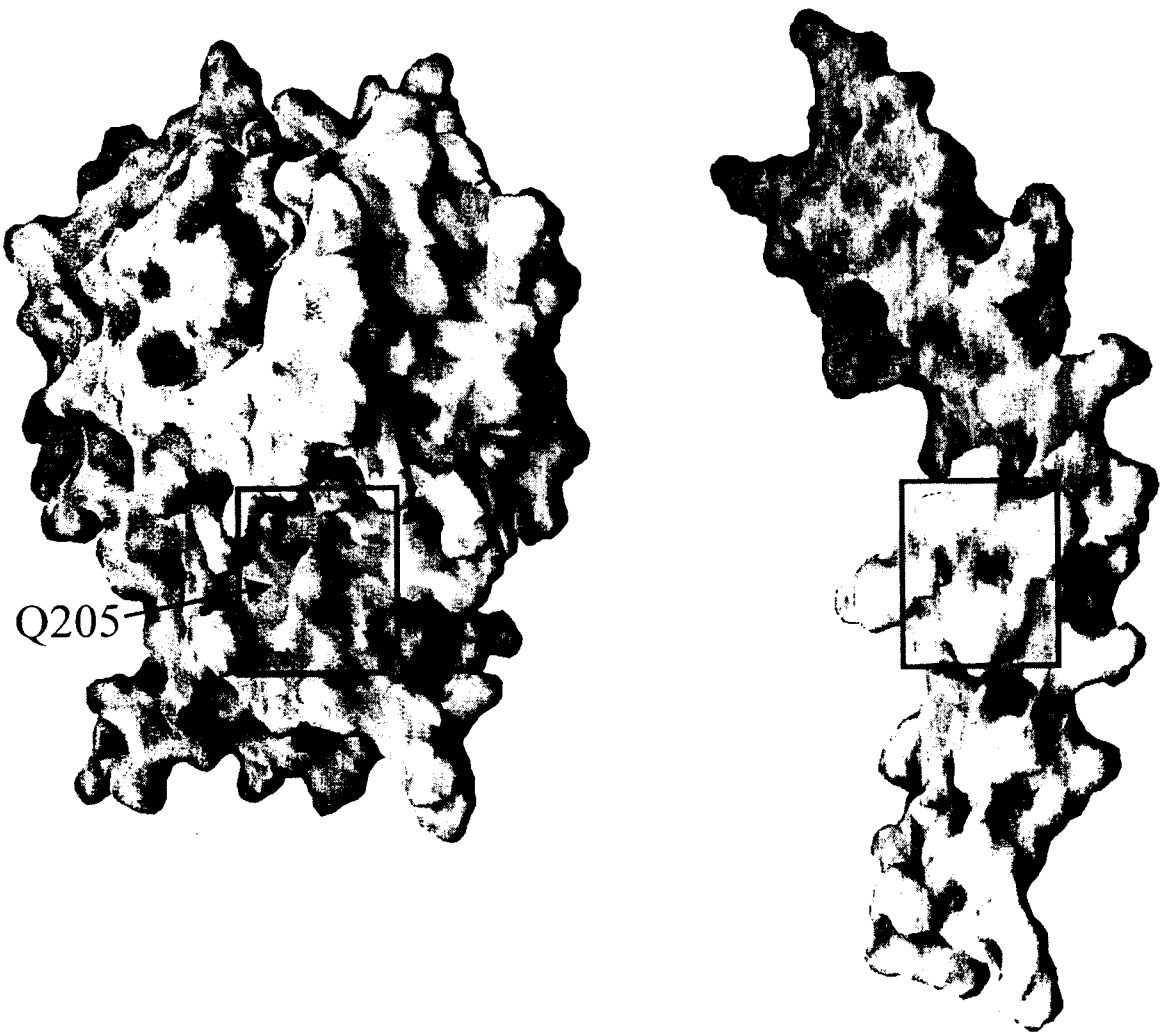
Van der Waals surfaces:

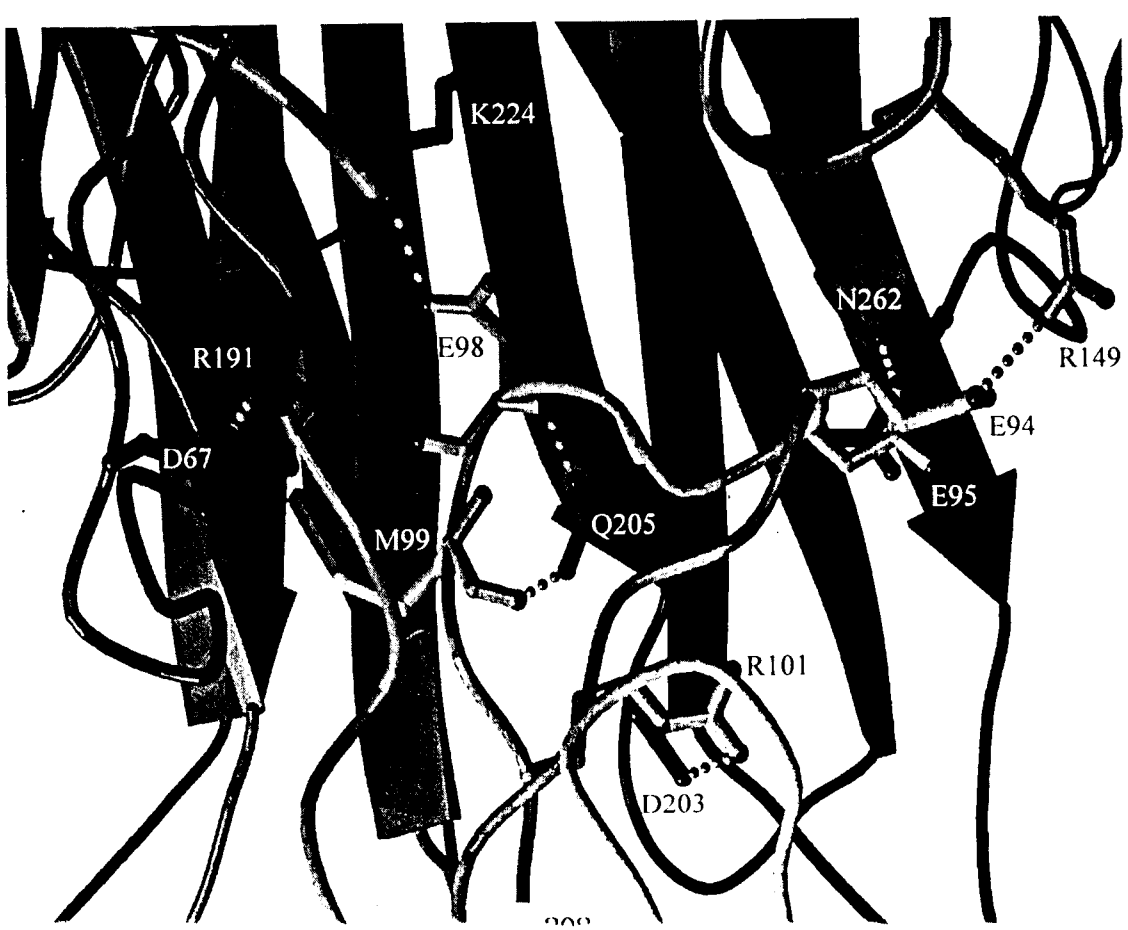
Y216 (green dot) fits snugly into a cavity of sDR5 (violet dots)

The protrusion of Y216 into a Small Cavity on DR5



Lower Contact Region





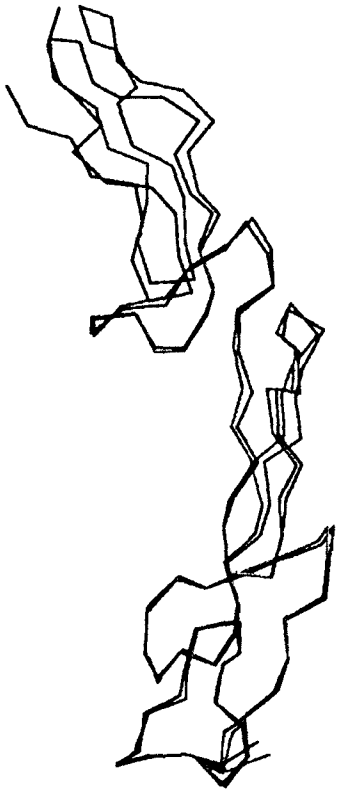
Structural similarity between members

1. Structural similarity between TNF members

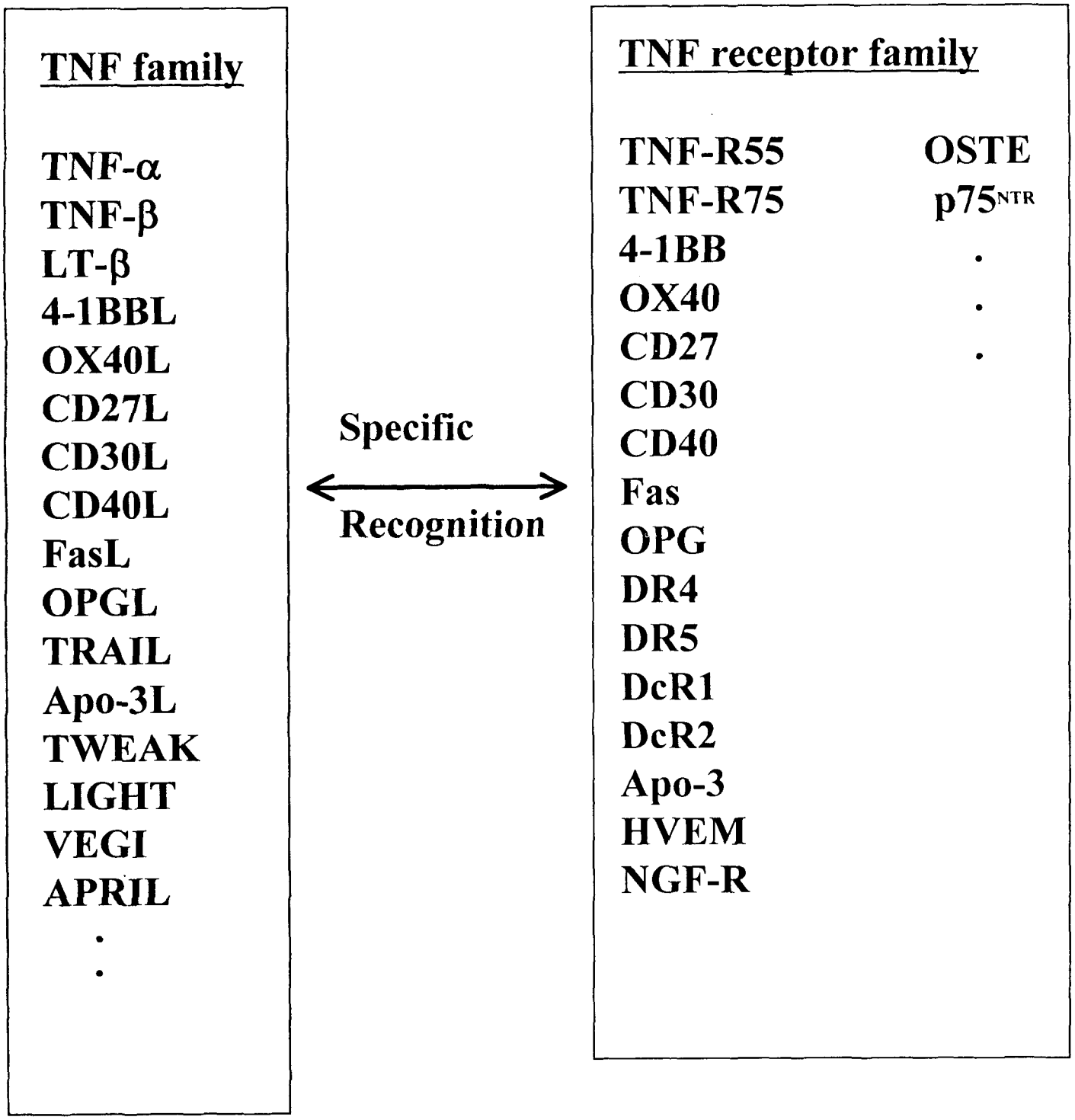
TNF-α
TNF-β
CD40L
TRAIL

2. Structural similarity between TNF members

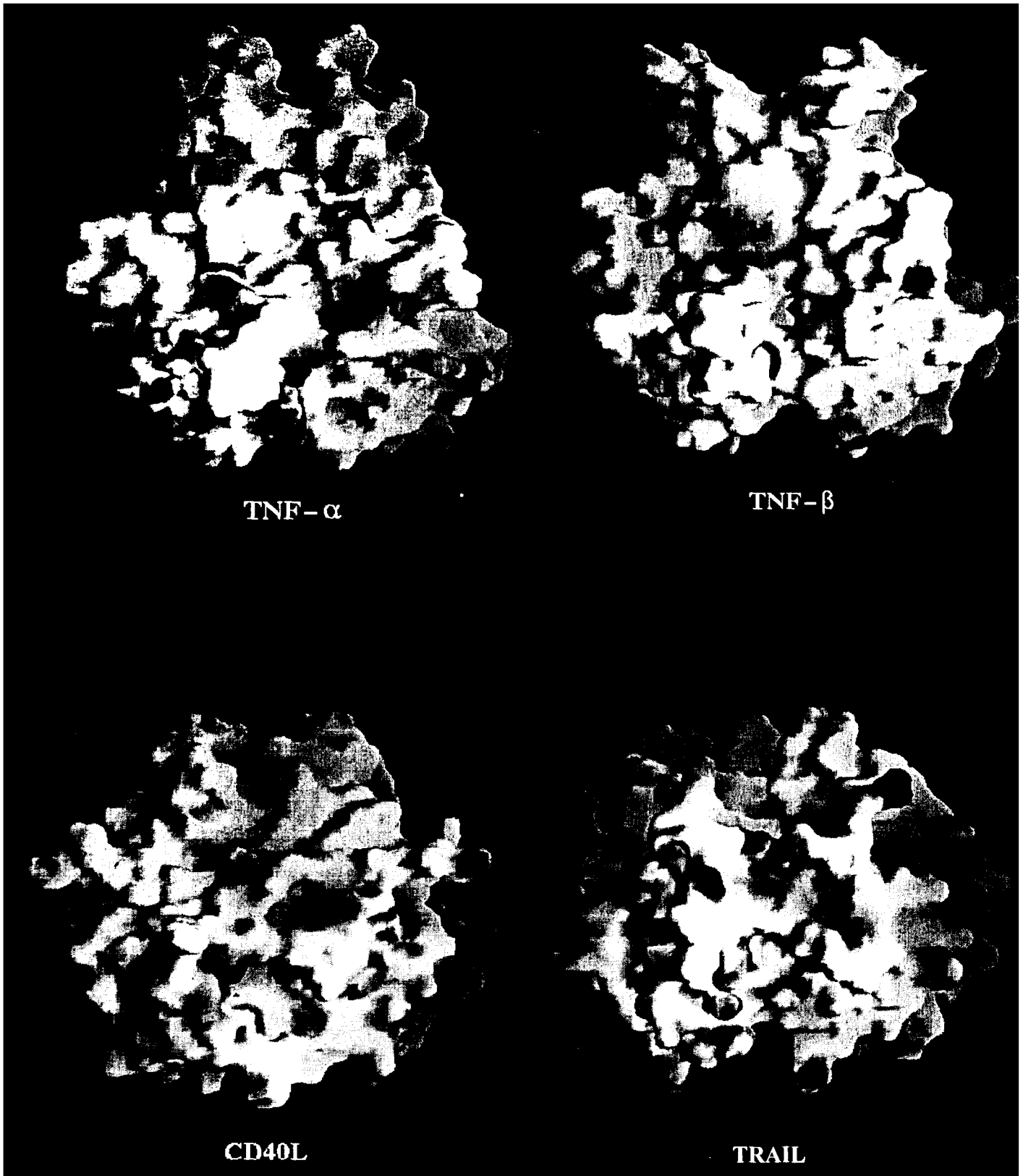
TNFRI
DR5



Then, how does specific recognition occur ?



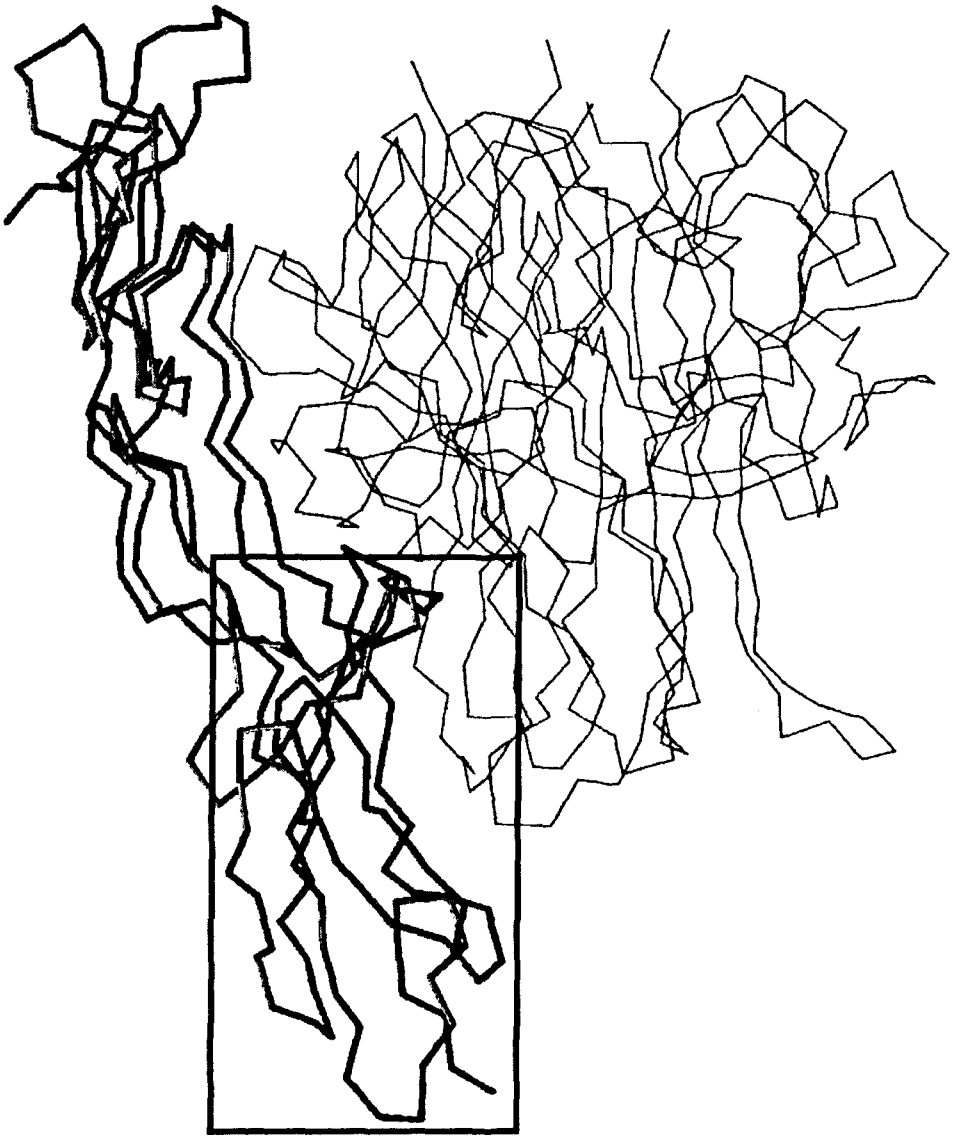
Insights from the structures



1. surface charge differences in receptor binding site

2. flexibility of receptor structure

Superposition of TNF/TNFRI and TRAIL/DR5 structures



After complex formation, this region Undergoes conformational change

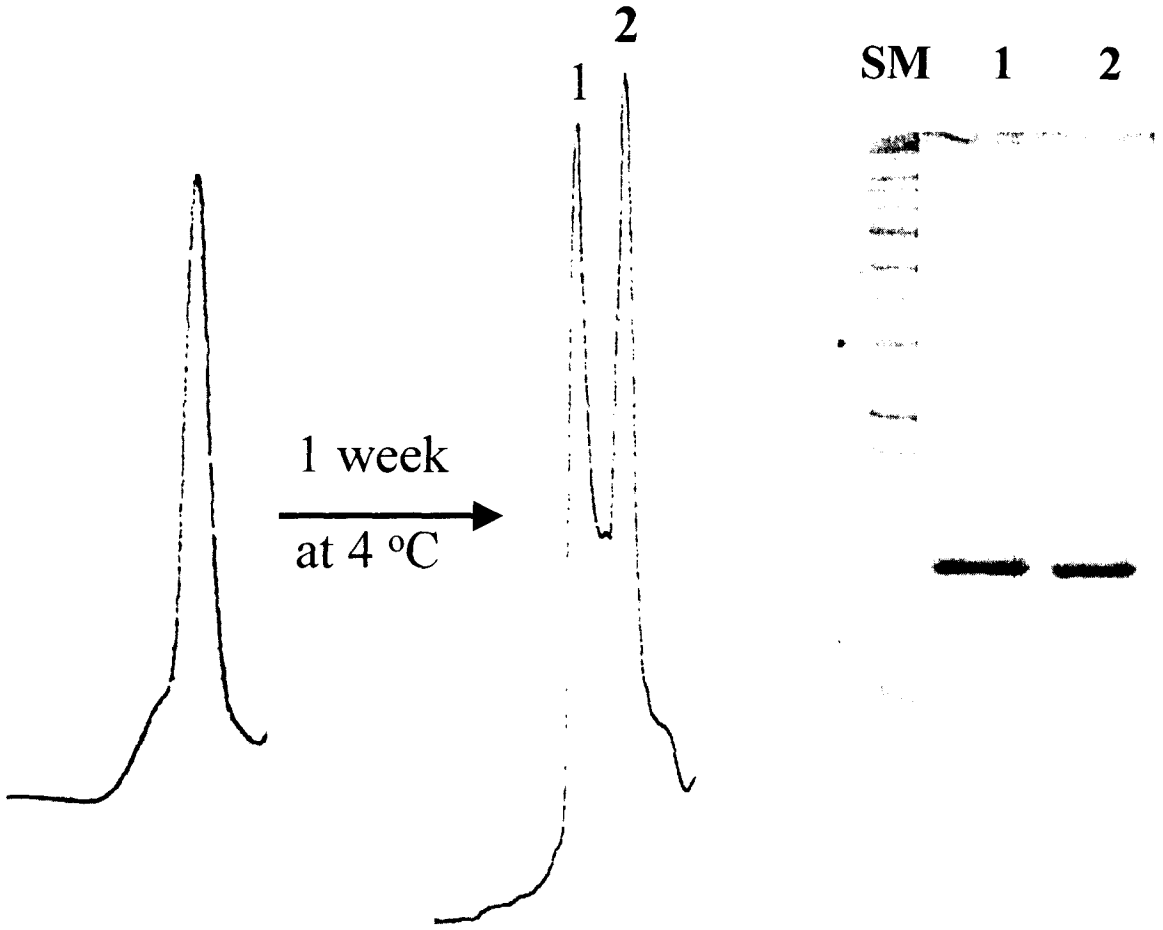
3. Insertion of several amino acids into receptor binding site – generation of new interaction

<u>As a results of 1,2, and 3, each pair exhibits exellent electrostactic and geometric complementarity</u>

Ex) TRAIL/DR5 complex

Structures provided ideas in designing mutants that are more suitable for anticancer drugs

Instability of TRAIL trimer



Change of oligomeric state of TRAIL

Trimer => not Trimer
(active) (inactive)

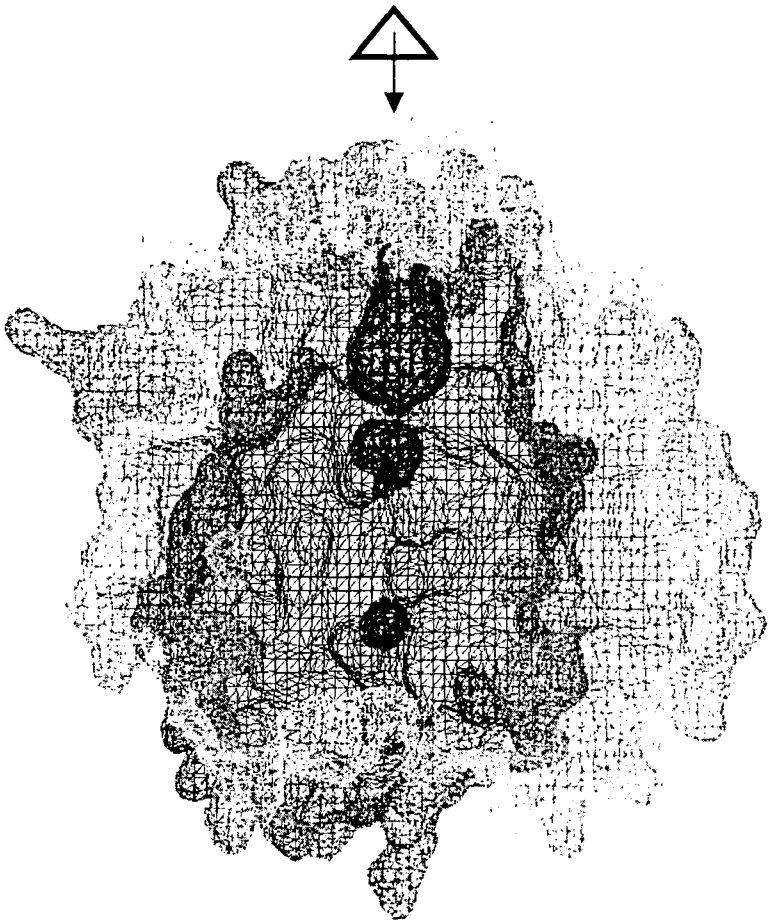
Retention of trimeric conformation of TRAIL is important in cancer treatment

In vivo efficacy test

Immunex
Leucine zipper-TRAIL
- stabilization of trimer

Genentech
Native TRAIL
injection of 15 mg/kg

Cavities along the 3-fold axis



Cavity =>

no contact between monomeric TRAILs =>

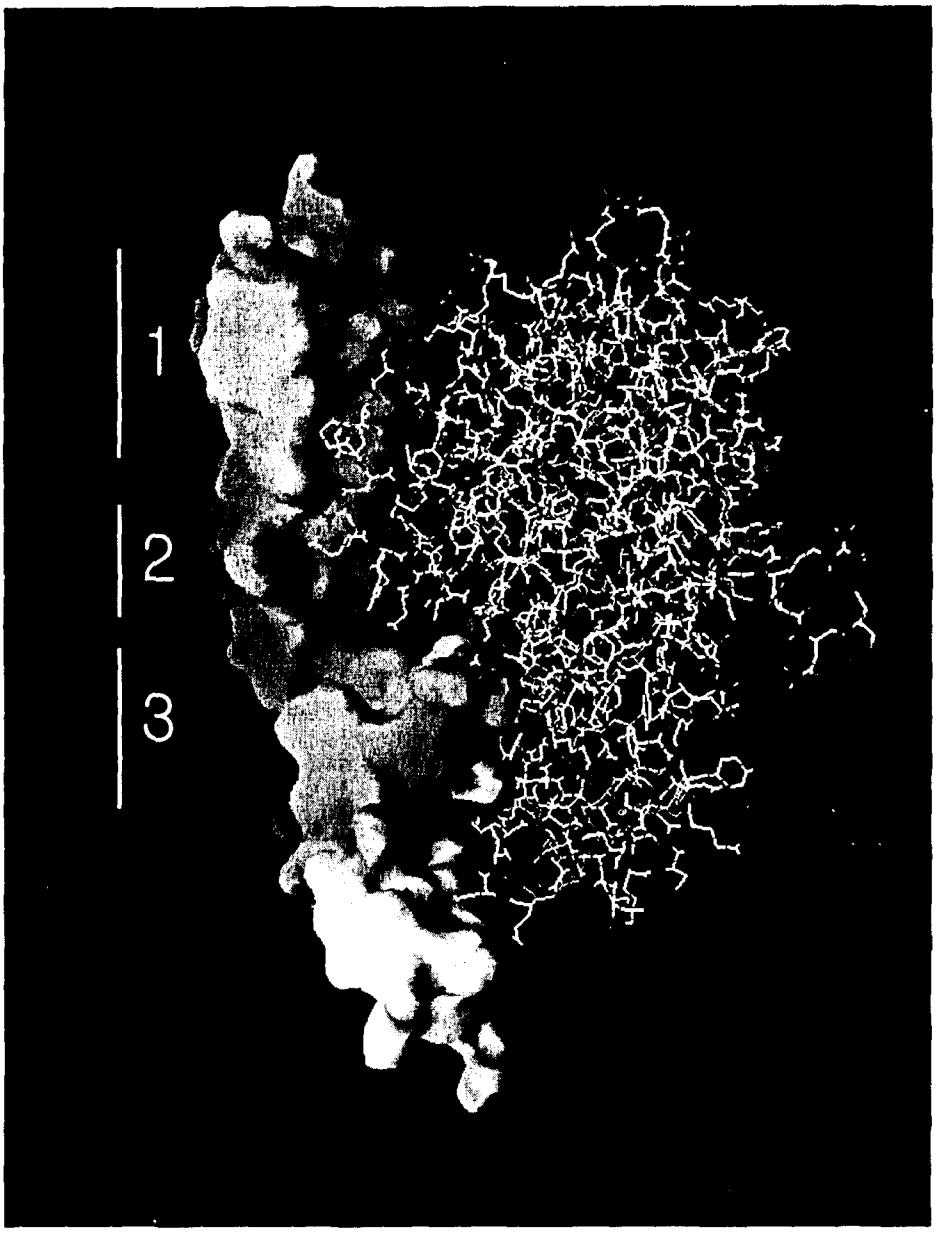
decreased stability of trimeric TRAIL

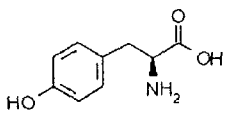
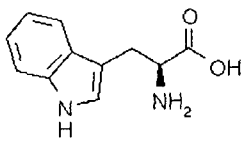
Mutations that can fill the cavities

⇒ stabilize trimeric conformation

⇒ Increased therapeutic efficacy

Mutants that are more active
=> strong binding to receptor

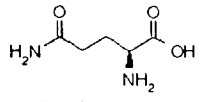
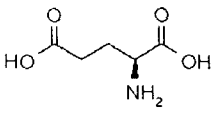


1. Tyr  **=> Trp** 

tyr y Tyrosin

trp w Tryptophan

2. elongation of AA' loop by insertion or cutting the loop

3. Gln  **=> Glu** 

gln q Glutamin

glu e Glutaminsaeure