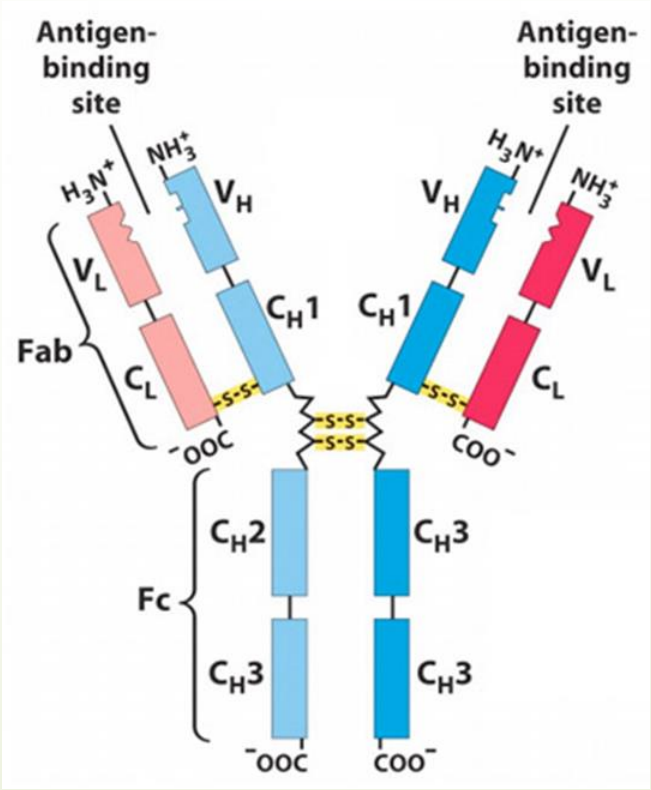

بسم الله الرحمن الرحیم

In-silico pseudo affimer selection

Affinity reagents

- **Affinity reagents** are compounds that bind specifically to the target molecule.
- **Monoclonal antibodies (mAbs)** are the most well-known biological affinity reagents.



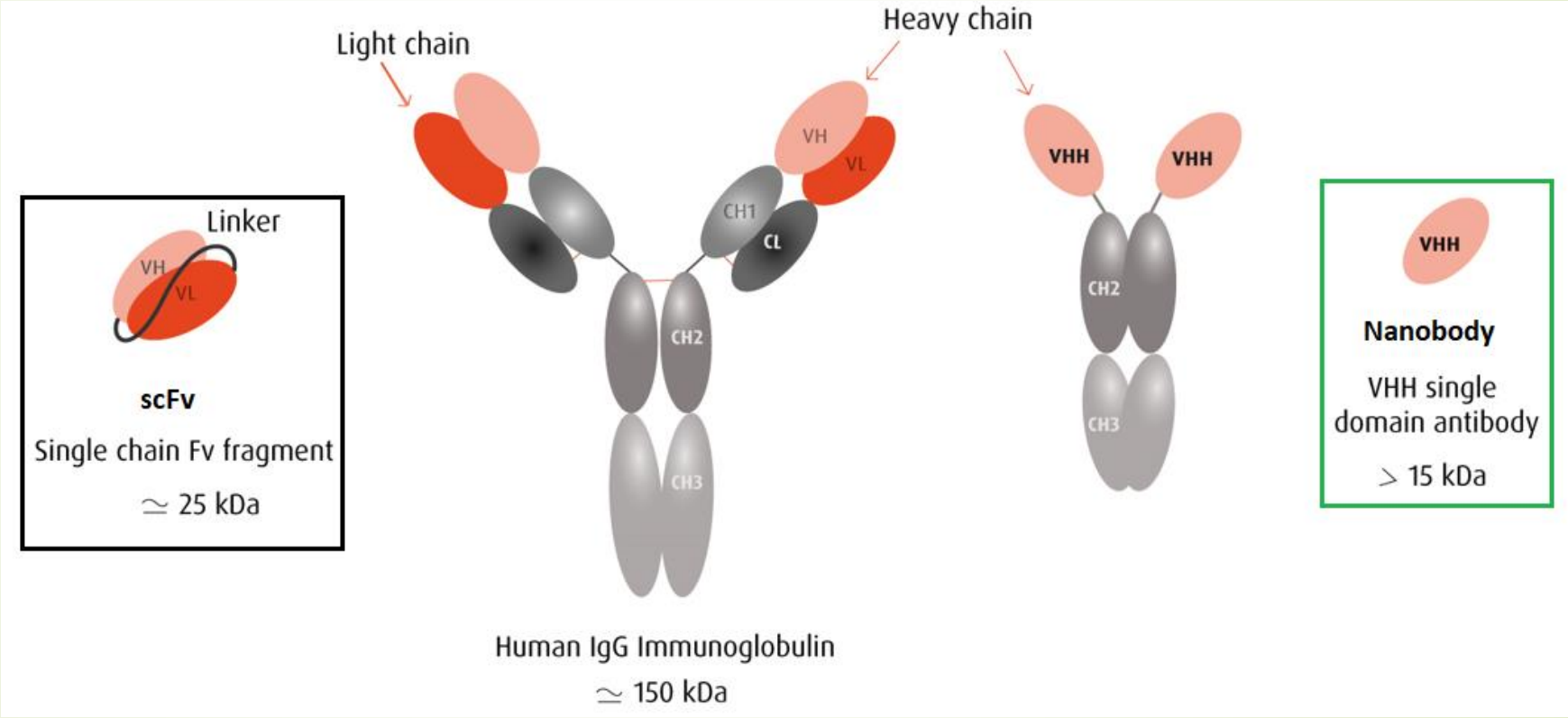
Antibody limitations

- Establish an immune response in human.
- Costly and time consuming (Specially the humanized form).
- Cell penetration limitation.
- Ethical problems (Killing animals).

mAb alternatives

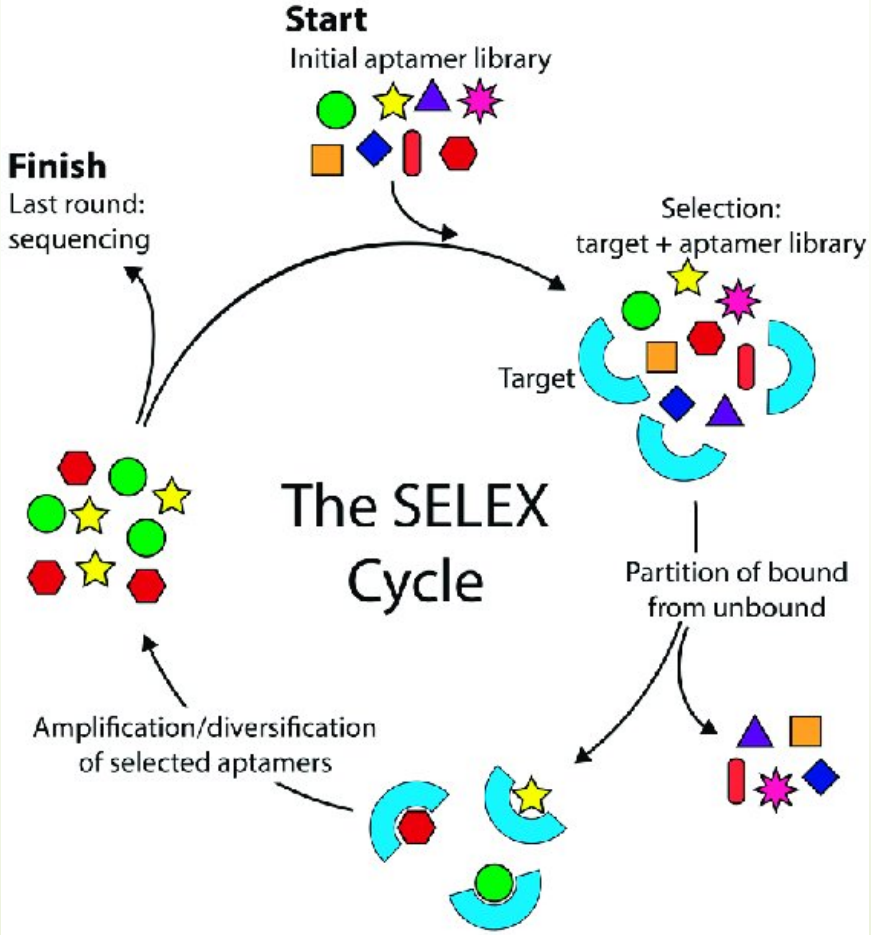
- Single-chain fragment variable (scFv)
- Nanobody
- Aptamer
- Affimer

scFv and Nanobody

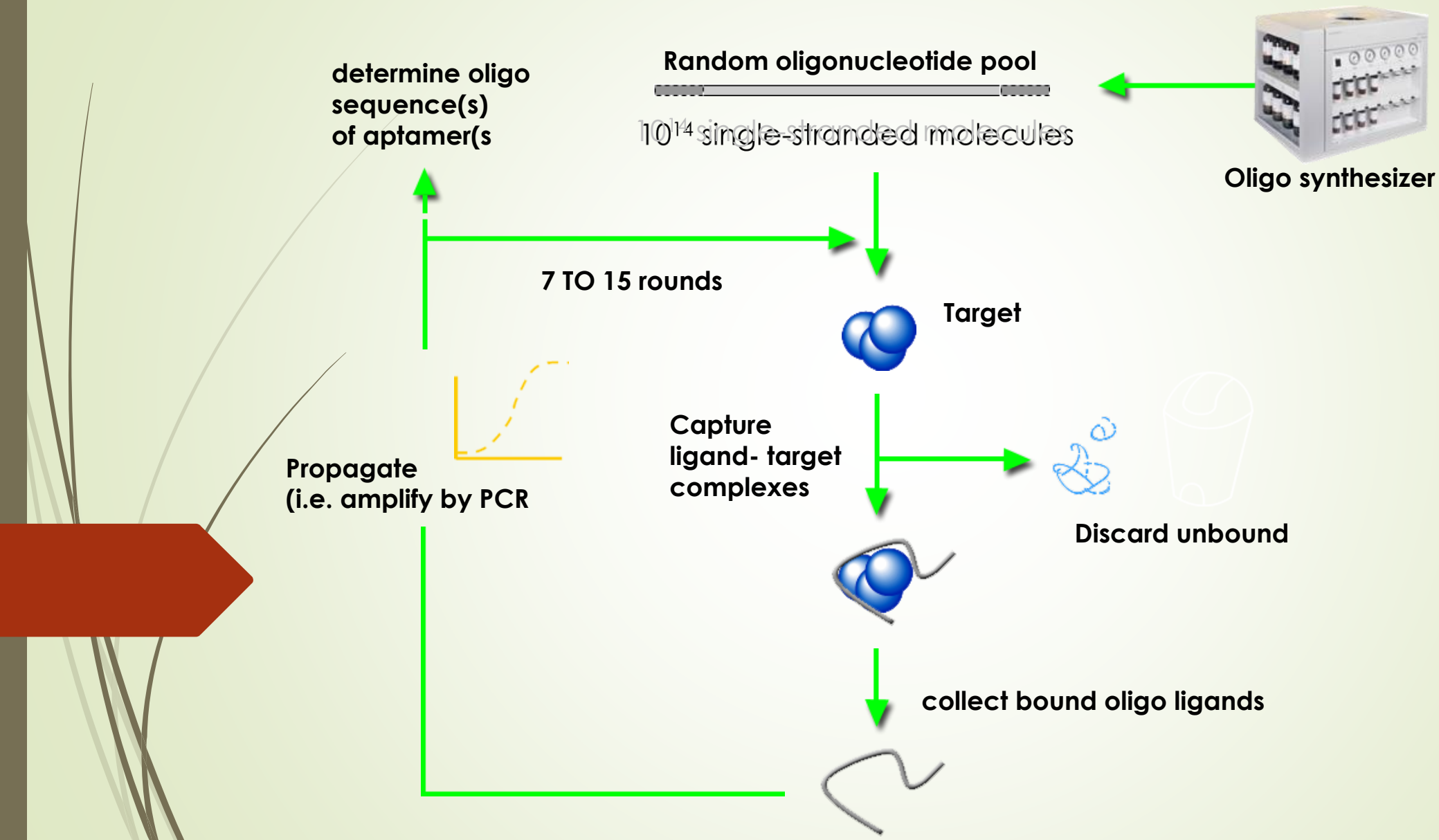


Aptamer

- Aptamers are single-stranded oligonucleotides (**RNA** and **ssDNA**) or **peptide** molecules that bind to the antigen with high affinity.
- The **SELEX** method is used to produce aptamers.
- Oligonucleotide aptamers are amplified by **PCR**.

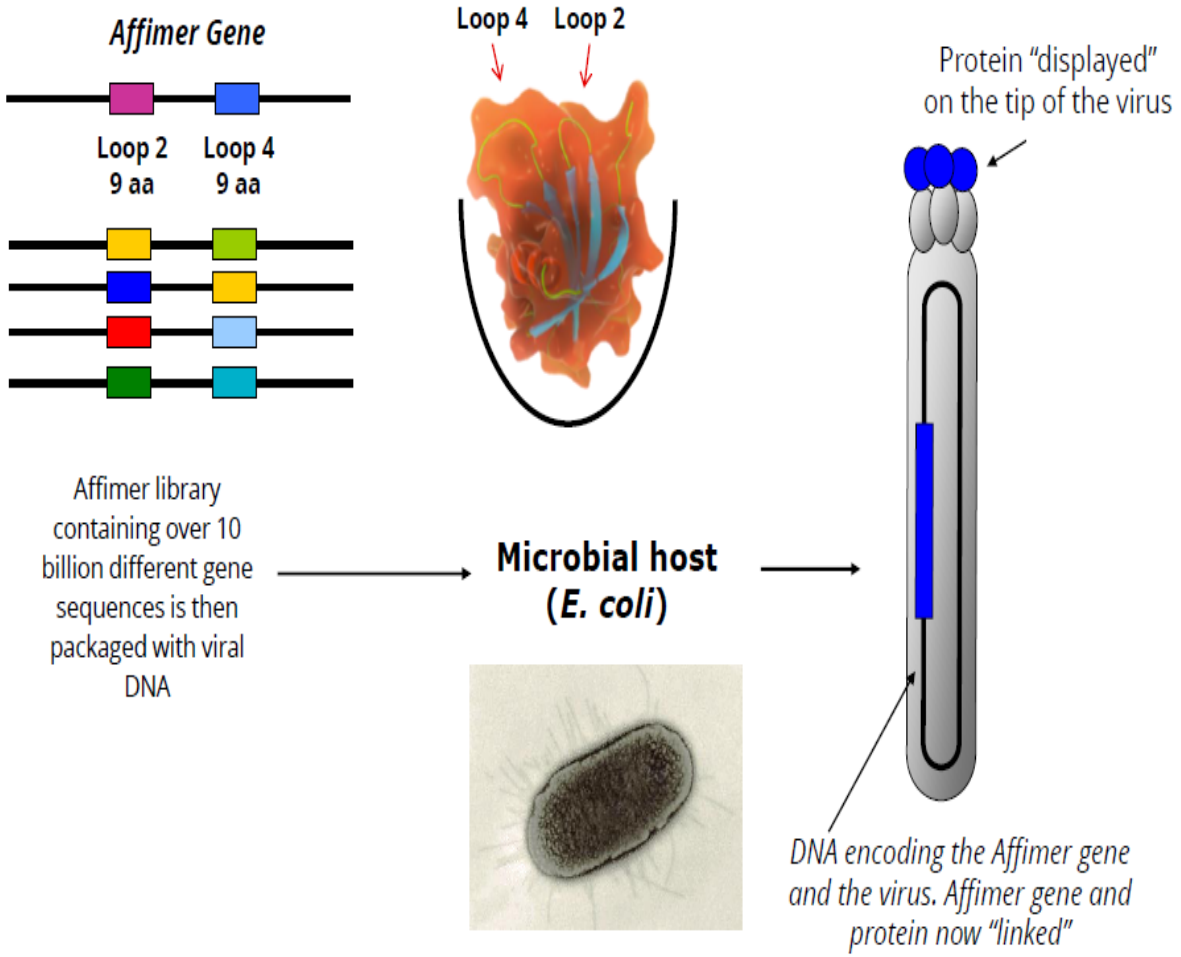


General Aptamer Selection Scheme

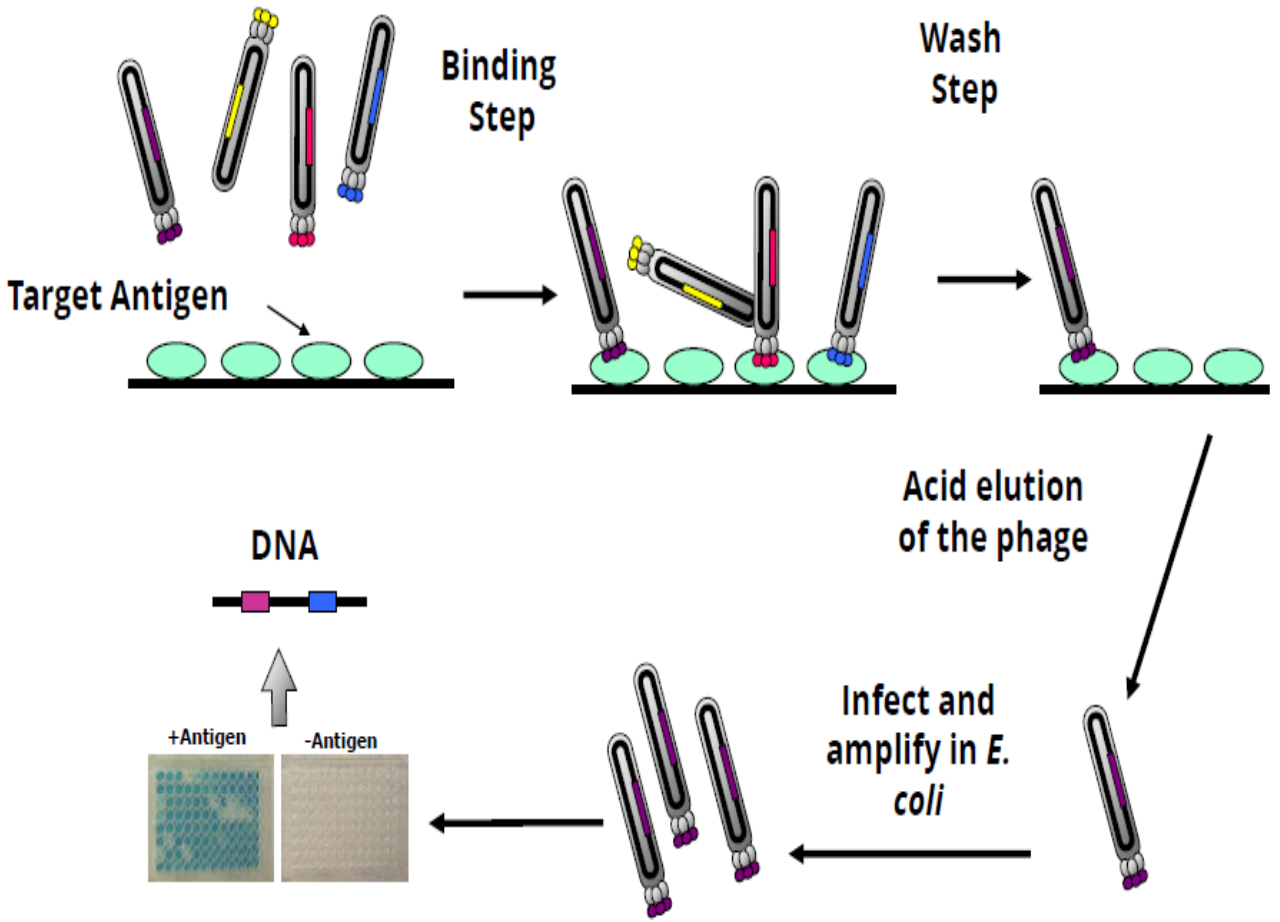


Affimer: Phage display and selection

Library Generation: Phage Display



Lead Identification: Phage Selections



In silico design of pseudo affimers

- Pseudo Affimers can be designed by **computational methods.**

- The designed pseudo affimer can be produced by **recombination method.**

CEA specific pseudo affimer

- Carcinoembryonic antigen (CEA) is normally produced in gastrointestinal tissue during fetal development, but stops before birth. CEA is present at very low levels in the blood of healthy adults and raised in some types of cancer. So, it can be used as a tumor marker in clinical tests.

- Dr adhesin of escherichia coli (E. coli) is able to bind to CEA.

- The CEA pseudo affimer was designed by selecting the amino acid sequence of Dr adhesin that interacts with CEA representing a high binding strength like a complete protein.

Materials

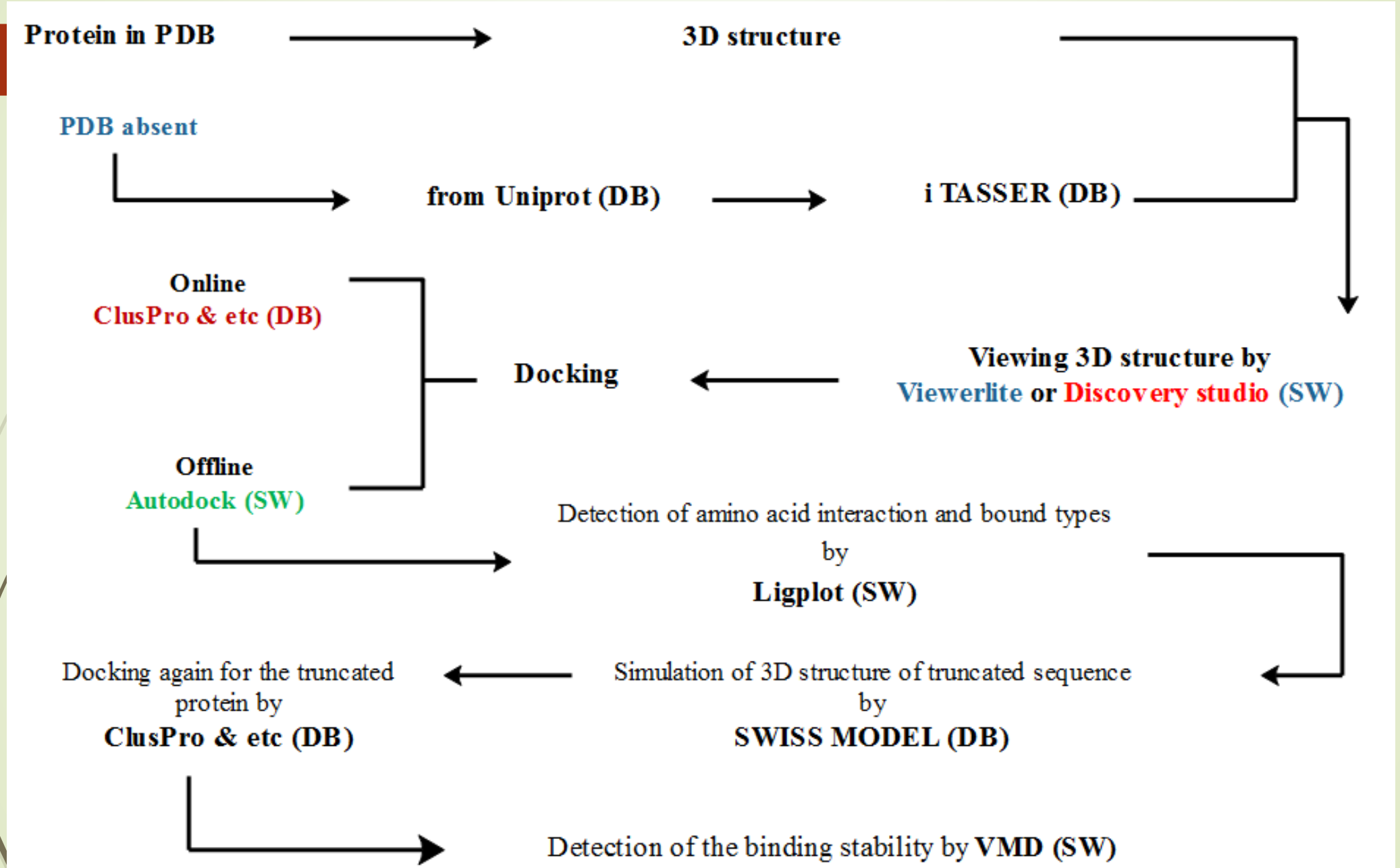
Softwares (SW)

- **Viewerlite**
- **Ligplot**
- **VMD**
- **Discovery studio**
- **Autodock**

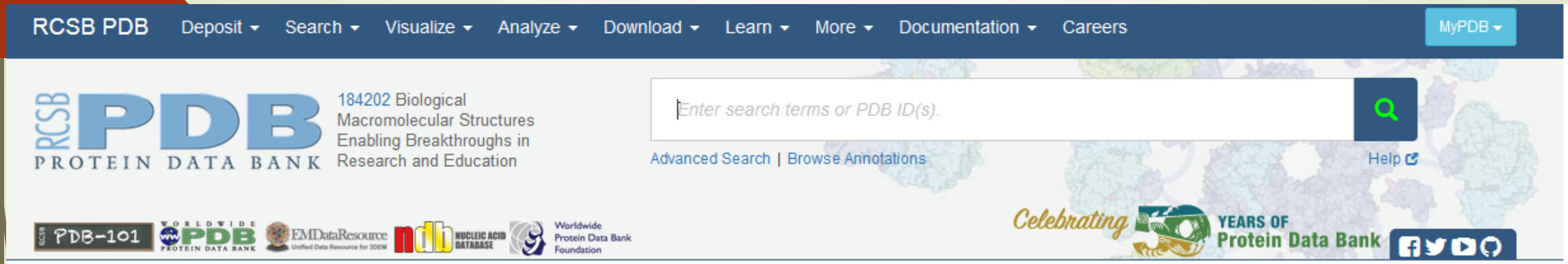
Databases (DB)

- **PDB**
- **Uniprot**
- **I- TASSER**
- **ClusPro**
- **EMBOSS Seqret**
- **SWISS- MODEL**

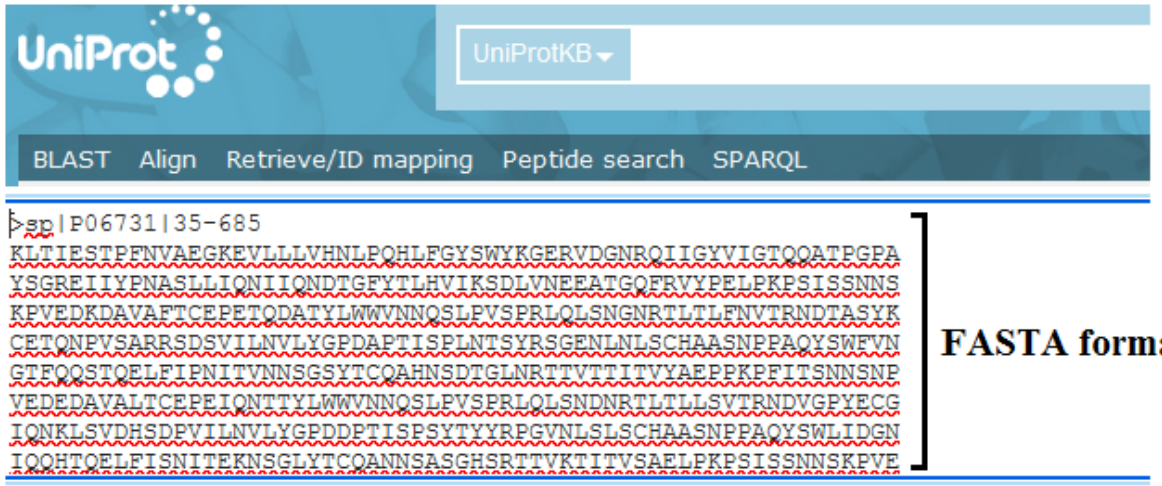
Methods


Protein in PDB
3D structure
PDB absent
from Uniprot (DB)
i TASSER (DB)
Online
ClusPro & etc (DB)
Docking
Viewing 3D structure by
Viewerlite or Discovery studio (SW)
Offline
Autodock (SW)
Detection of amino acid interaction and bound types
by
Ligplot (SW)
Docking again for the truncated
protein by
ClusPro & etc (DB)
Simulation of 3D structure of truncated sequence
by
SWISS MODEL (DB)
Detection of the binding stability by VMD (SW)

Selection of 3D structure of proteins



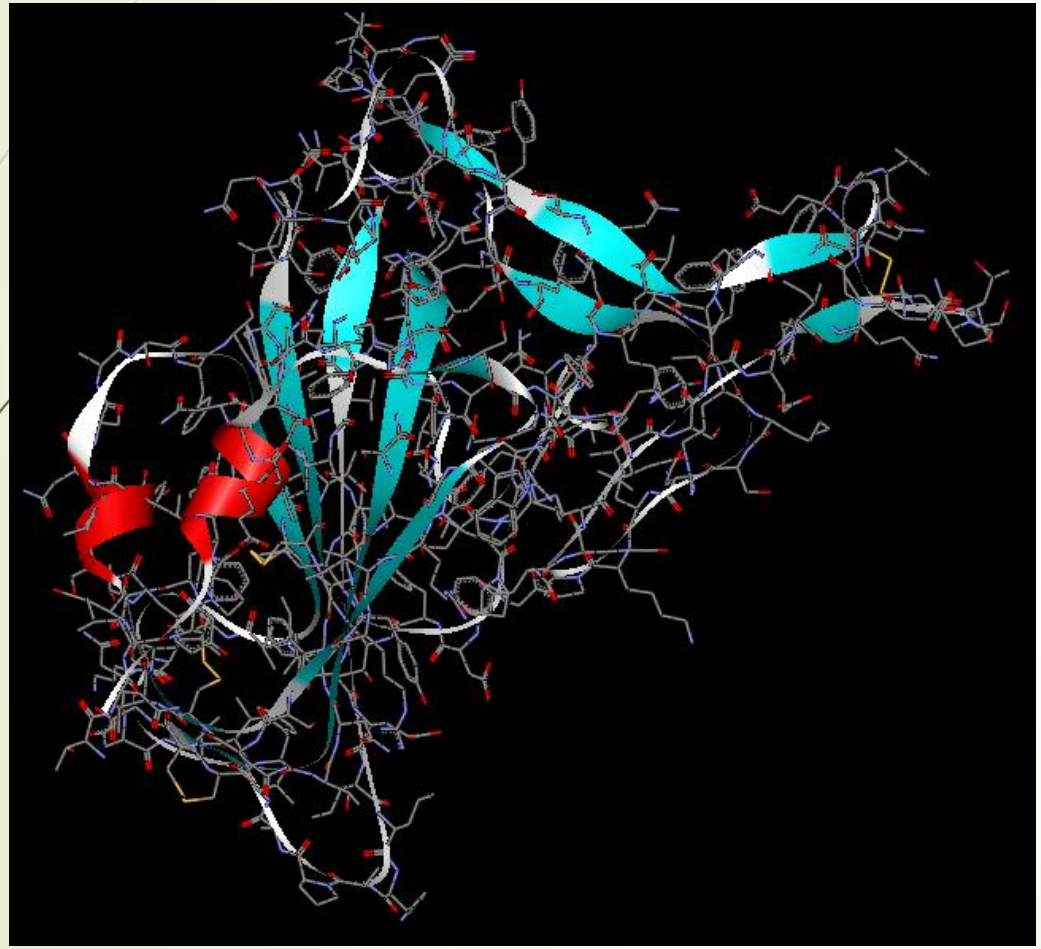
Absent of protein structure in PDB



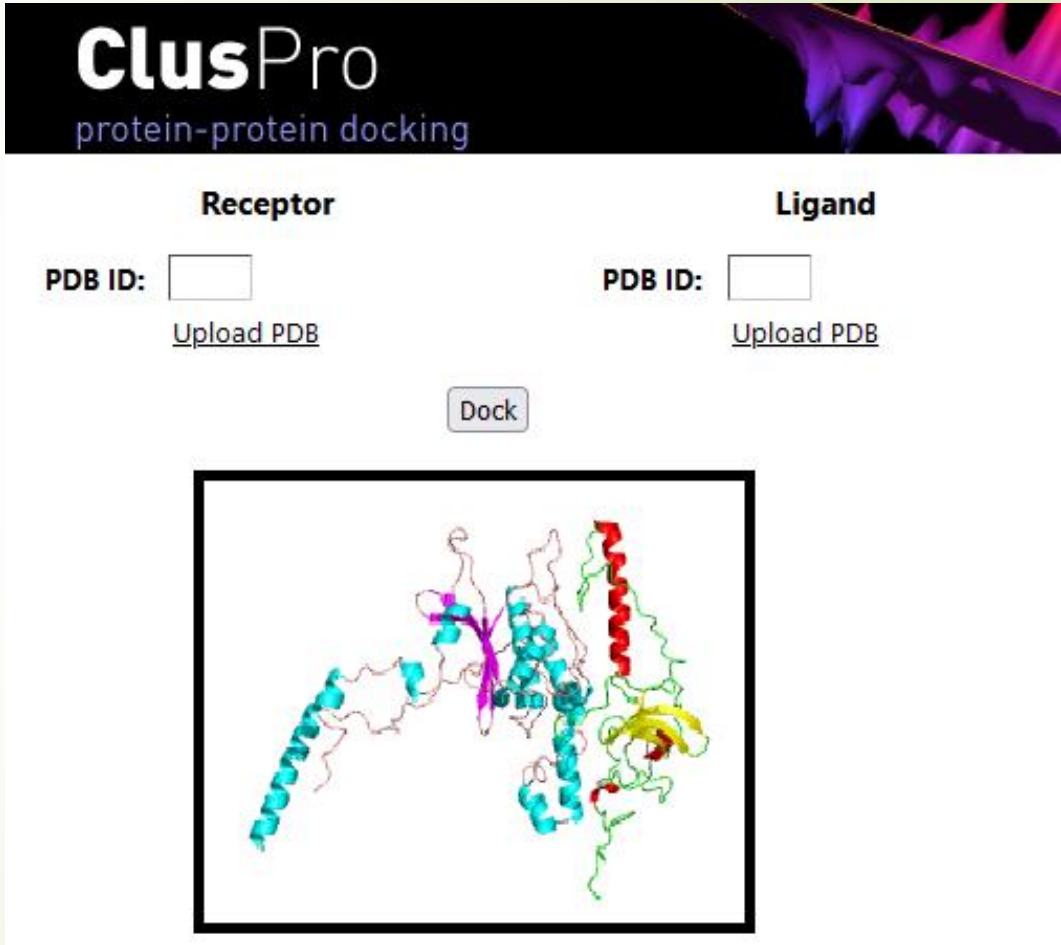
FASTA format

Viewing and Docking

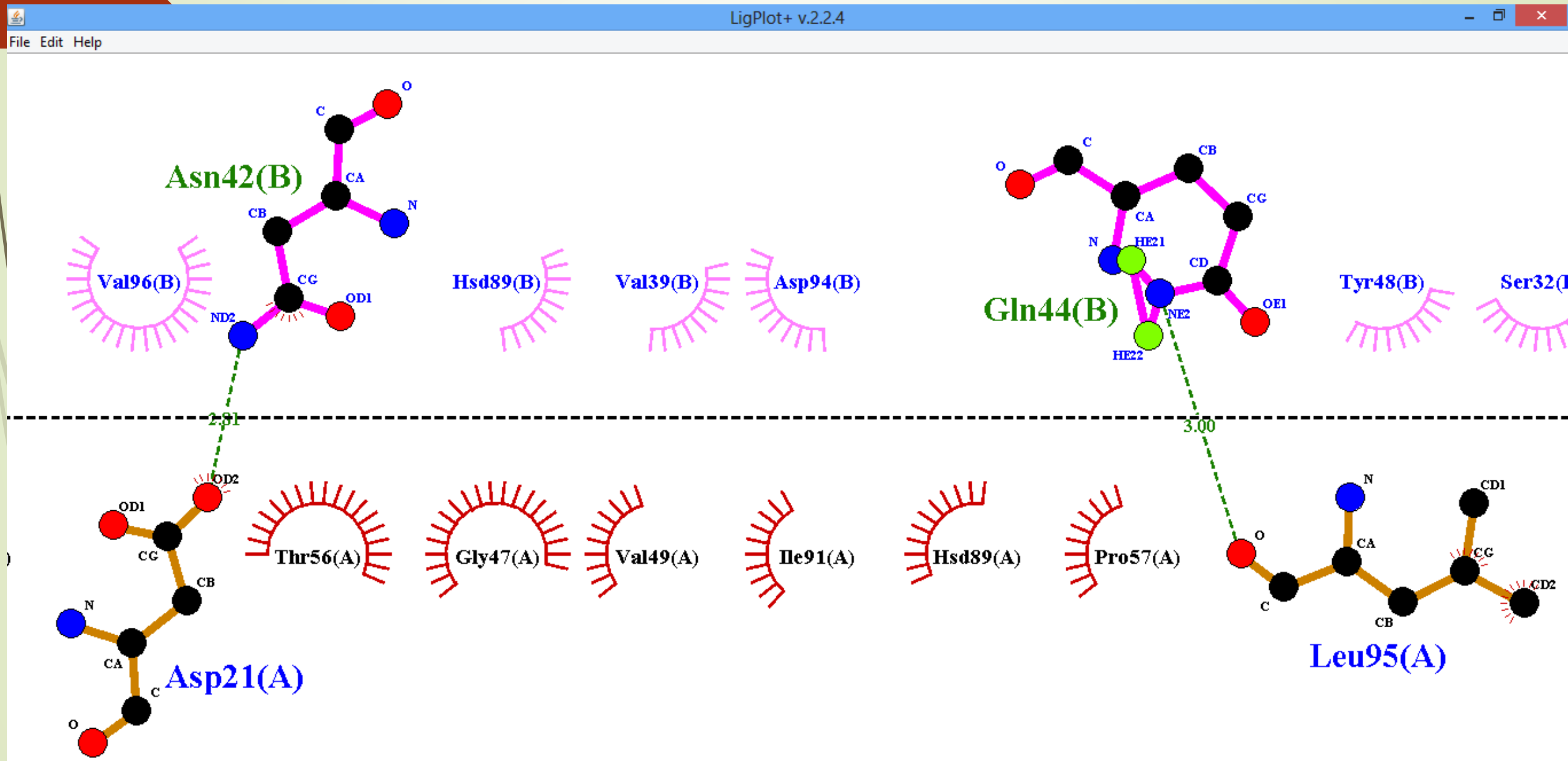
View 3D structures



Docking



Determination of amino acids interaction with Ligplot



Simulation of 3D structure of truncated sequence

BIOZENTRUM
University of Basel
The Center for Molecular Life Sciences

SWISS-MODEL

Modelling | Reposit

Start a New Modelling Project

Target Sequence(s):
(Format must be FASTA, Clustal, plain string, or a valid UniProtKB AC)

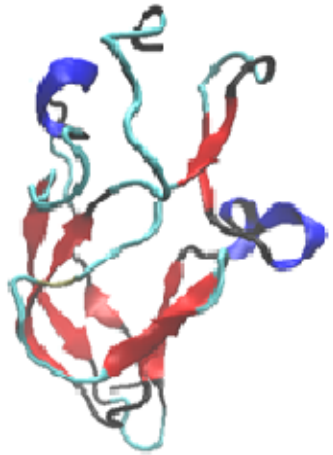
Paste your target sequence(s) or UniProtKB AC here

+ Upload Target Sequence File...

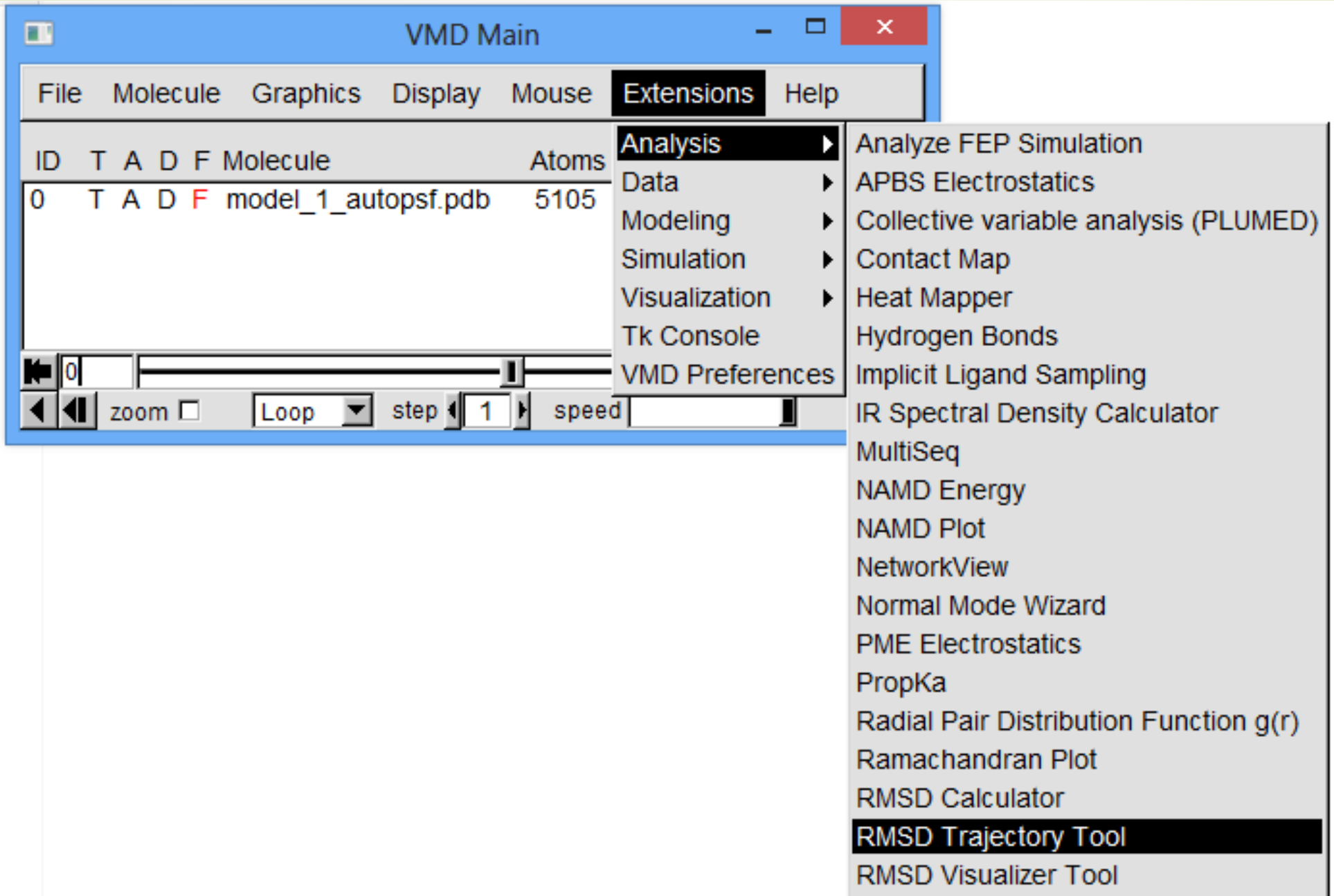
Validate

Search For Templates

Build Model



Detection of binding stability

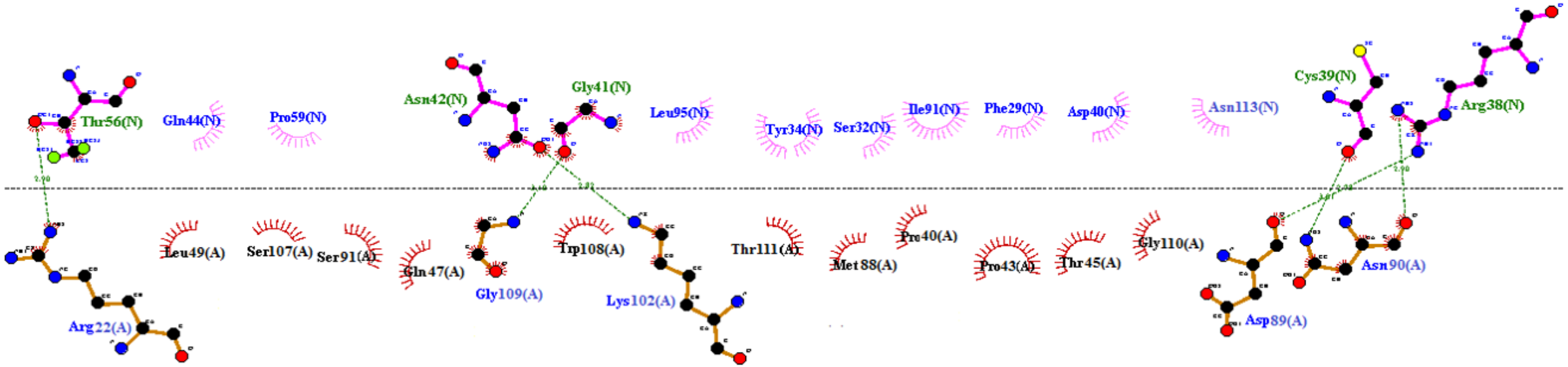


Results

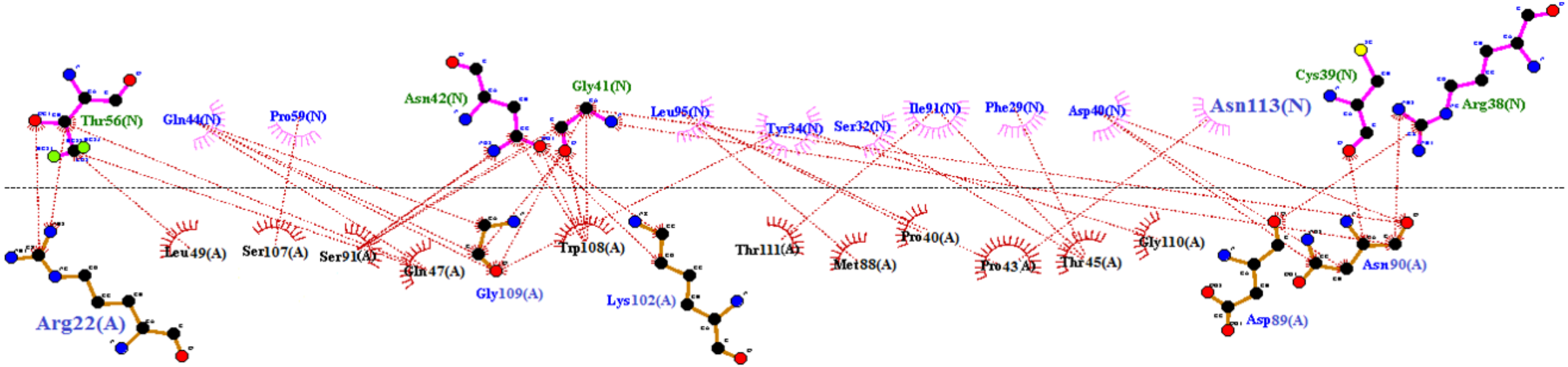
- 3D structures of CEA with code 1e07 and Dr adhesin with code 2ver were downloaded in .pdb format.

- Study of the interaction between the two proteins showed the binding of the residues in sequences 22 to 111 of Dr adhesin to the N domain of the CEA protein.

CEA & Dr adhesin interactions



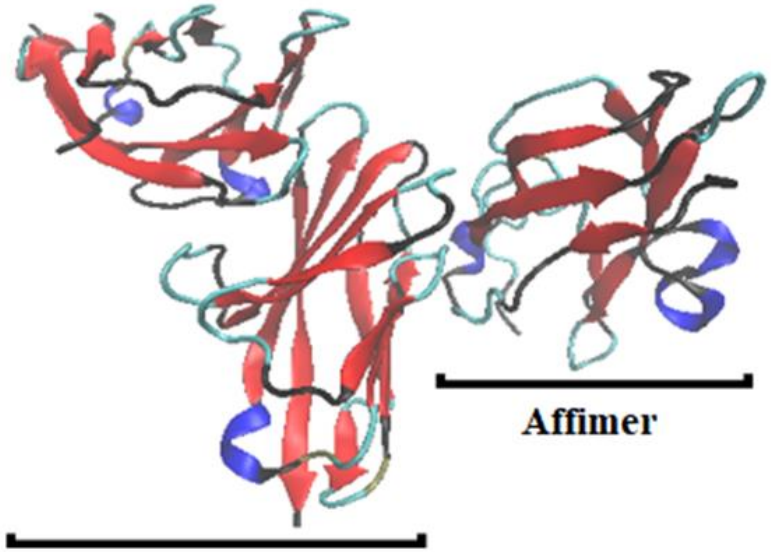
Hydrogen bonds



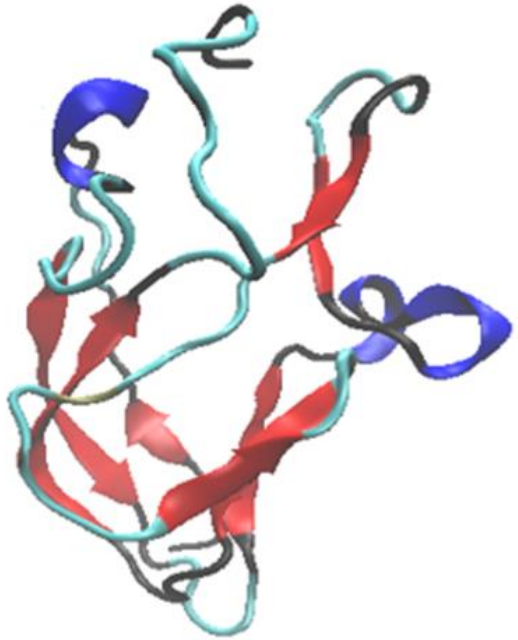
Hydrophobic interactions

Results

- The most stable truncated protein containing this sequence had 110 amino acids length.
- This sequence contains residues 17 to 126 (Glu17... Gly126) of Dr adhesin.
- The root mean square deviation (RMSD) of this interaction was 3.95.



The interaction of affimer with CEA N-domain



3D structure of affimer

Discussion

- De Melo et al. and Lee et al. introduced two DNA aptamers and one RNA aptamer for CEA, respectively.

- Wang and colleagues built an anti- CEA nanobody.

- Shamsuddin et al. Introduced three affimers for CEA by phage display method.

Discussion

- In this study, a pseudo affimer consisting of 110 amino acids that have a high binding power to CEA was designed by in-silico method.

- In-silico selection helps to solve problems related to insolubility and low affinity **before** experimental laboratory work.

- Increased production of soluble proteins is associated with decreased disulfide bonds as well as α helix secondary structures.

Conclusion

- The main purpose of this study:

- designing a pseudo affimer with
- high binding capacity to the target molecule
- and high production efficiency (increasing protein solubility).

References

- **Groff, K. (2015).** Modern affinity reagents: Recombinant antibodies and aptamers. Biotechnology advances.
- **Hosseini-Zijoud. (2013).** Aptamers and their biological-therapeutical applications: A review article. Pajouhan Scientific Journal.
- **Sellmann. (2020).** A one-step process for the construction of phage display scFv and VHH libraries. Molecular biotechnology.
- **de Melo (2020).** DNA aptamers selection for carcinoembryonic antigen (CEA). Bioorganic & Medicinal Chemistry Letters, 30(15), 127278.
- **Lee, Y. J. (2012).** An RNA aptamer that binds carcinoembryonic antigen inhibits hepatic metastasis of colon cancer cells in mice. Gastroenterology, 143(1), 155-165. e158.
- **Wang, H. (2017).** A nanobody targeting carcinoembryonic antigen as a promising molecular probe for non-small cell lung cancer. Molecular medicine reports, 16(1), 625-630.
- **Shamsuddin, S. H. (2021).** Selection and characterization of Affimers specific for CEA recognition . Scientific reports, 11(1), 1-10.
- **Arsphreet Bhatwa. (2021).** Challenges Associated with the Formation of Recombinant Protein Inclusion Bodies in *Escherichia coli* and Strategies to Address Them for Industrial Applications. Frontiers in Bioengineering and Biotechnology.

CERTIFICATE
OF POSTER PRESENTATION

ISC : 00210 - 89644 SN : 22-8-21/7616123 ISSUE DATE : August 22 , 2021

This is to certify that

Mohammad Babashamsi

presented a **poster** titled:

In silico Design of an Affimer Specific for Carcinoembryonic Antigen

Authors:

Mohammad Babashamsi, Mohammad Mahdi Babashamsi*

at the 12 th National & 4 th International Biotechnology Congress of the Islamic Republic of Iran

(Biotechnology for Food Safety and Health)

Organized by the Iranian Biotechnology Society

August 22nd-24th, 2021(online)

Tehran, Iran

Prof. Sirous Zeinali

Congress Head and Head of the Society

You can check the accuracy of this certificate at www.BiotechSociety.ir

Affimer by Phage display

- Affimers are engineered proteins that act like antibodies.
- Affimers are produced by **phage display** method.