

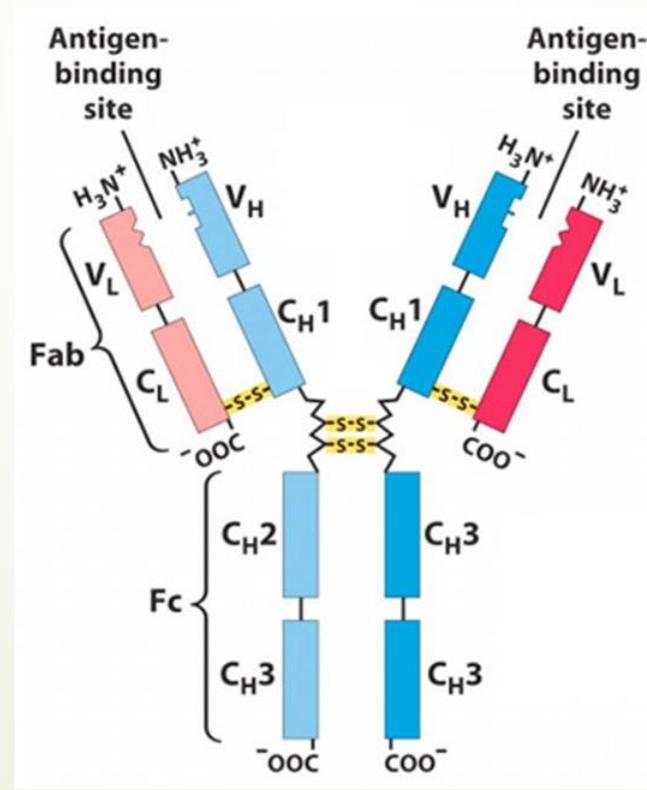




In-silico pseudo affimer selection

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

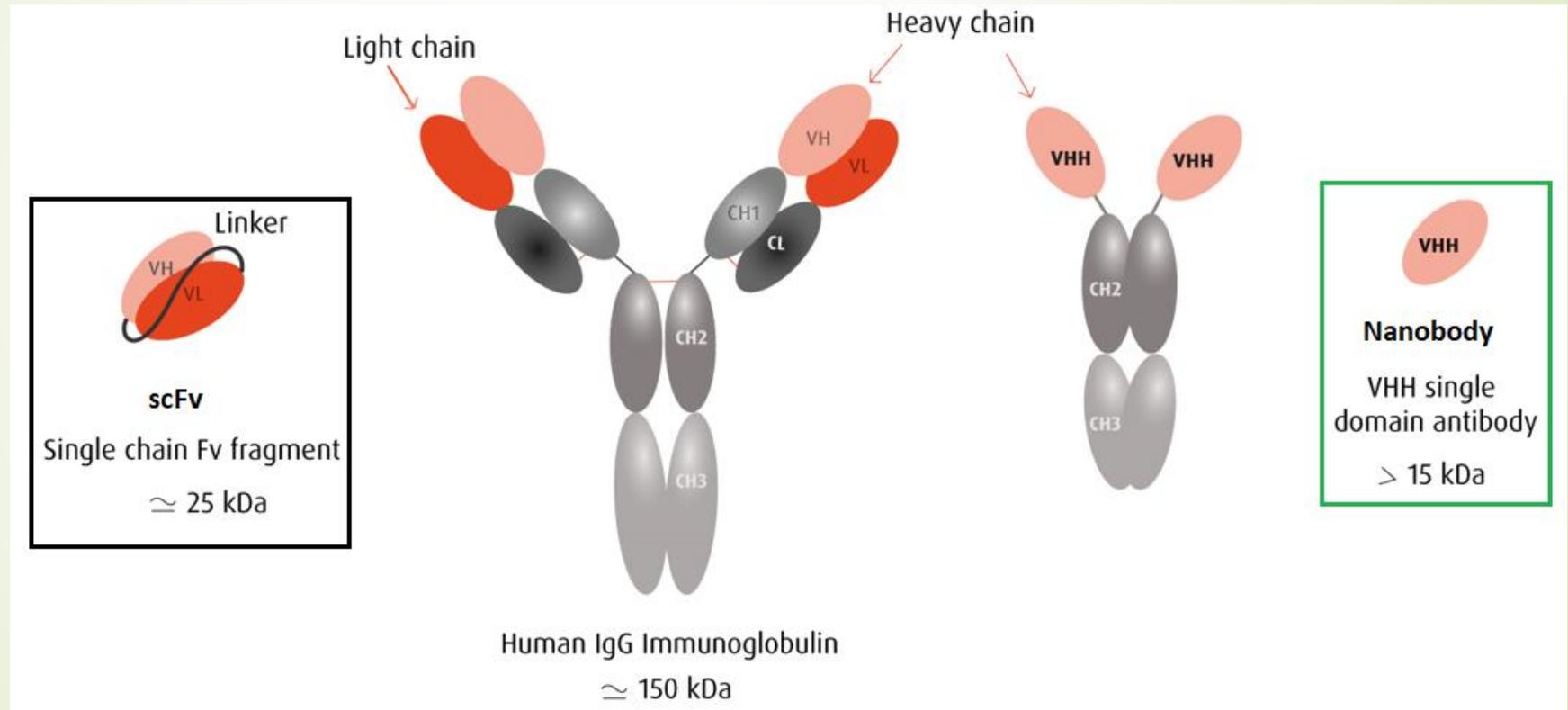
- Establish an **immune response** in human.
 - **Costly** and **time** consuming (Specially the **humanized** form).
 - Cell **penetration** limitation.
 - **Ethical** problems (Killing animals).
- 



mAb alternatives

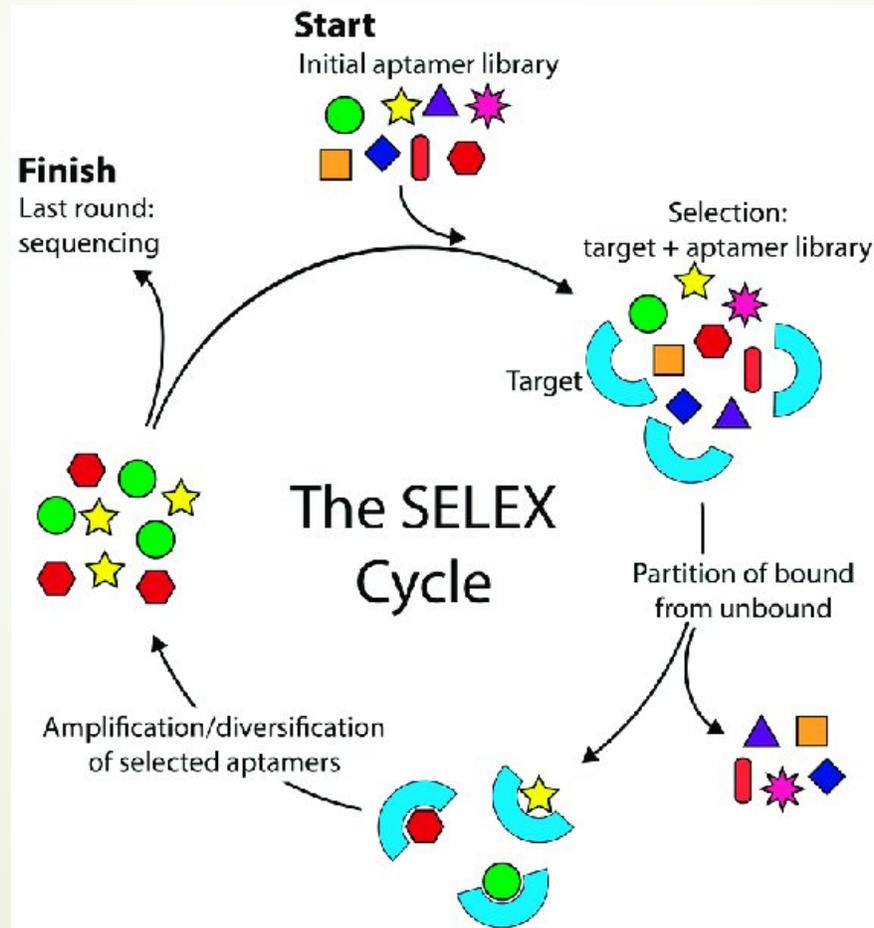
- Single-chain fragment variable (scFv)
 - Nanobody
 - Aptamer
 - Affimer
- 

scFv and Nanobody

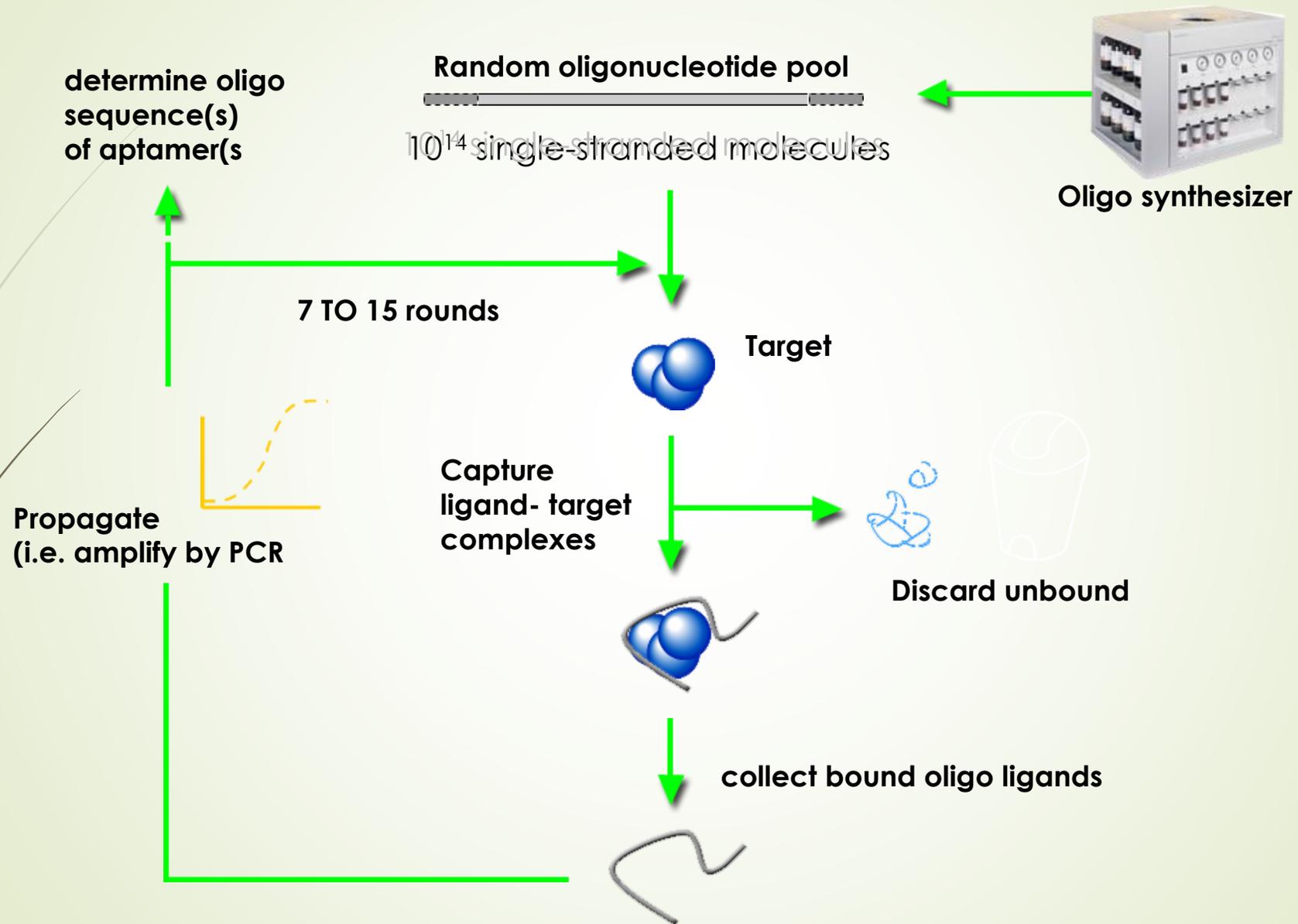


Aptamer

- ▶ 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.

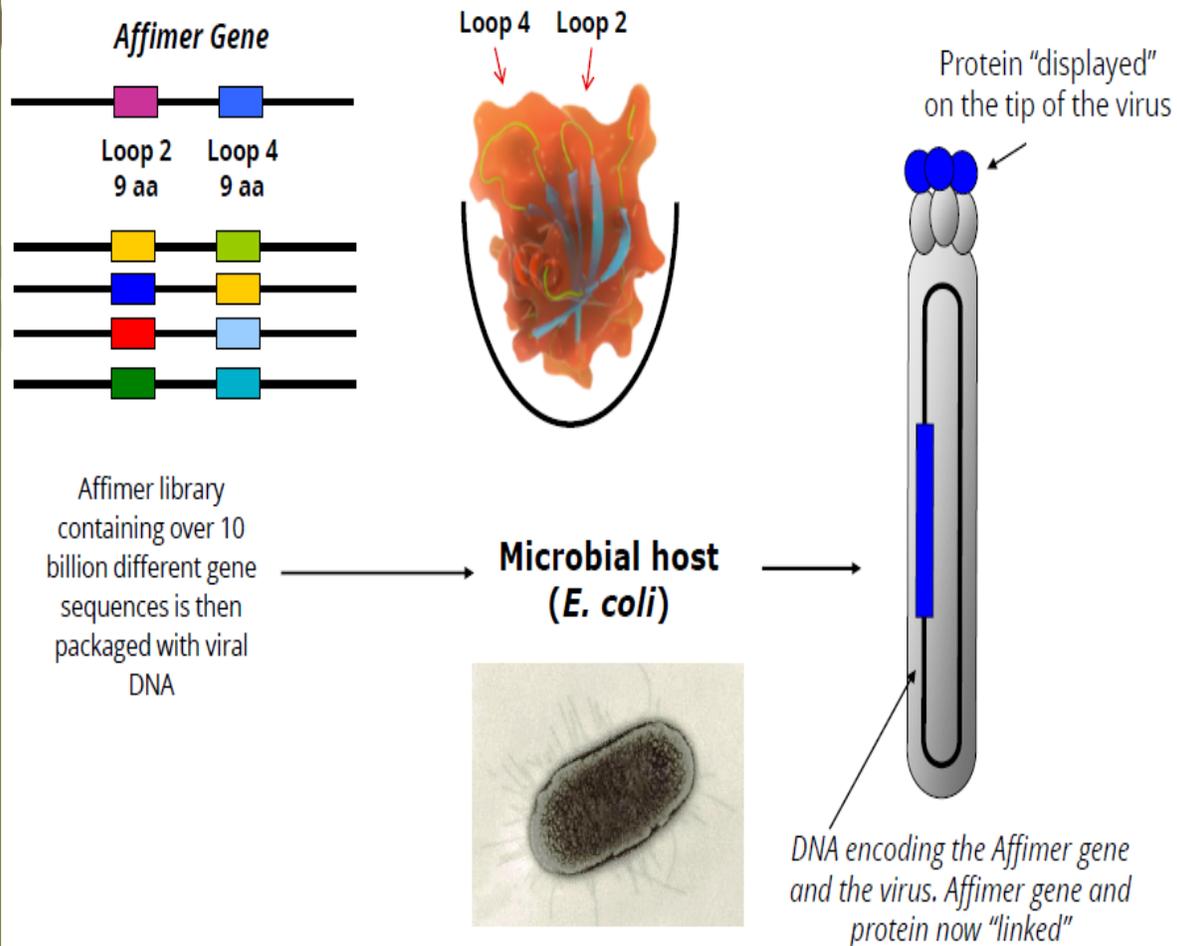


General Aptamer Selection Scheme

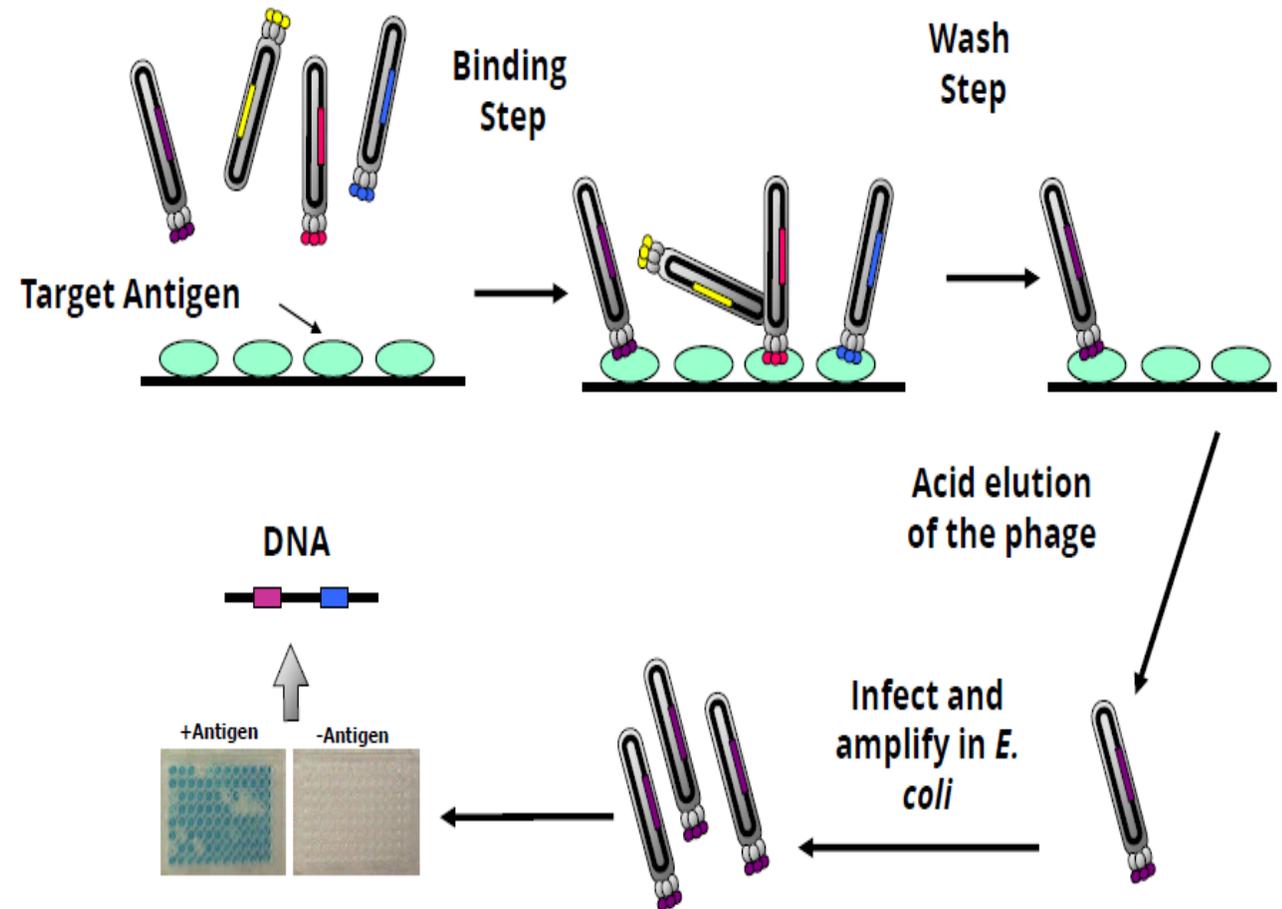


Affimer: Phage display and selection

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

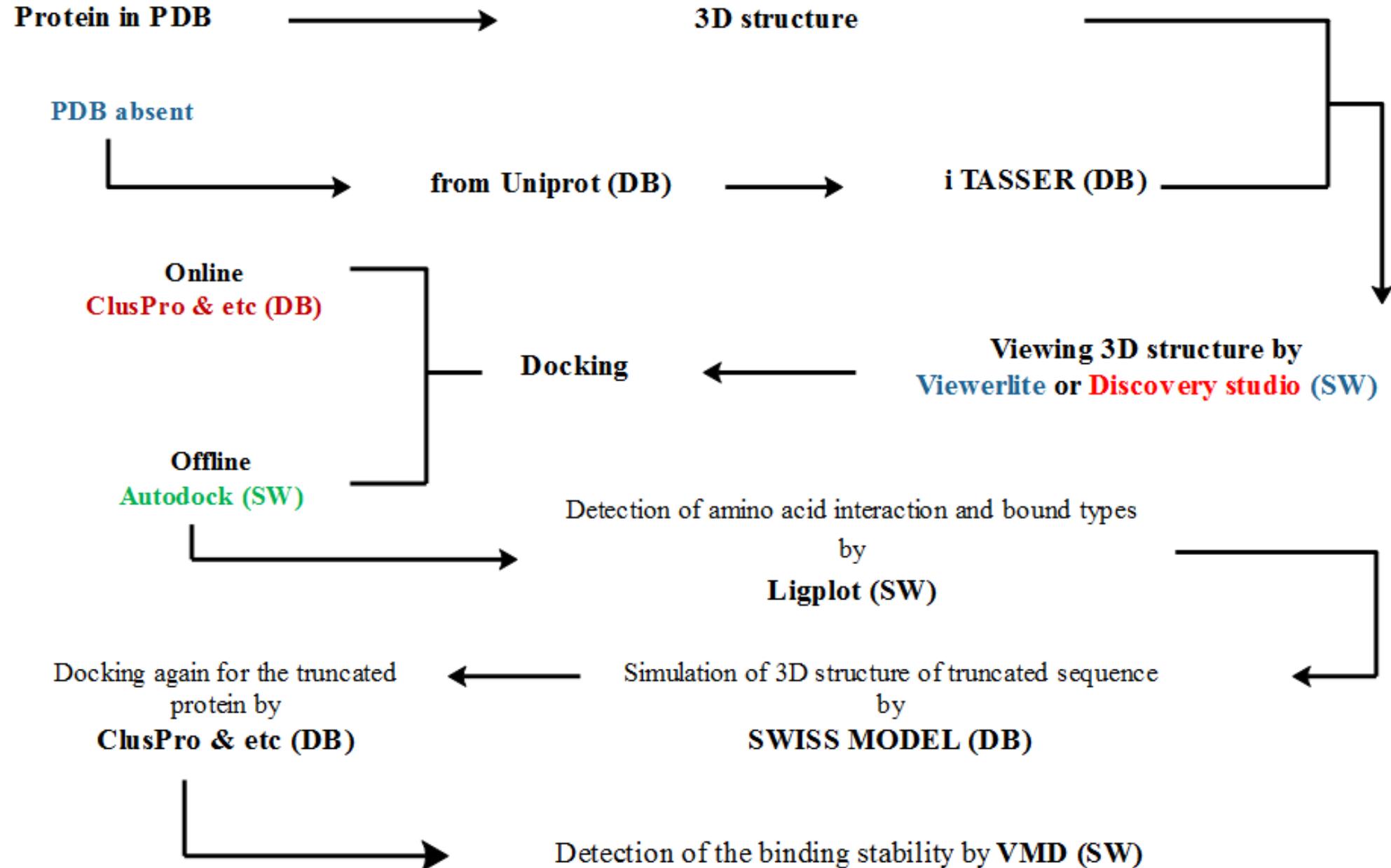
Softwares (SW)

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

Databases (DB)

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

Methods



Selection of 3D structure of proteins

RCSB PDB Deposit Search Visualize Analyze Download Learn More Documentation Careers MyPDB

RCSB PDB PROTEIN DATA BANK 184202 Biological Macromolecular Structures Enabling Breakthroughs in Research and Education

Enter search terms or PDB ID(s)

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Worldwide Protein Data Bank Foundation

EMDataResource Unified Data Resource for 3DEM

Worldwide Protein Data Bank Foundation

101 PDB-101

Worldwide Protein Data Bank

EMDataResource Unified Data Resource for 3DEM

NUCLEIC ACID DATABASE

Worldwide Protein Data Bank Foundation

Celebrating 50 YEARS OF Protein Data Bank

Facebook Twitter YouTube

Absent of protein structure in PDB



UniProt UniProtKB

BLAST Align Retrieve/ID mapping Peptide search SPARQL

sp | P06731 | 35-685

```
KLTIESTPFENVAEGKEVLLLVHNLPOHLFGYSWYKGERVDGNROIIGYVIGTQOATPGPA
YSGRELIYPNASILLIQNIIONDTGFYTLHVIKSDLVNEEATGQFRVYPELPKPSISSNNS
KPVEDKDAVAFTCEPETODATYLVWVNNOSLPVSPRLOLSNGNRTLTLEFNVRNDTASYK
CETONPVSARRSDSVILNVLYGPDAPTISPLNTSYRSGENLNLSCHAASNPPAQYSWFVN
GTFOOSTOELFIPNITVMNNSGSYTCQAHNSDTGLNRRTVTTITVYAEPPKPFITSNNSNP
VEDEDAVALTCEPEIQNTTYLWVWVNNOSLPVSPRLOLSNDNRITLTLVTRNDVGPYECG
IQNKLSVDHSDPVIILNVLYGPDPTISPSYTYRPGVNLNLSCHAASNPPAQYSWLDIGN
IQOHTOELFISNITEKNSGLYTCQANNSASGHSRTTVKTI TVSAELPKPSISSNNSKPVE
```

FASTA format

I-TASSER On-line Server (View an example of I-TASSER output):

Copy and paste your sequence within [10, 1500] residues in FASTA format. [Click here for a sample input:](#)

Or upload the sequence from your local computer:

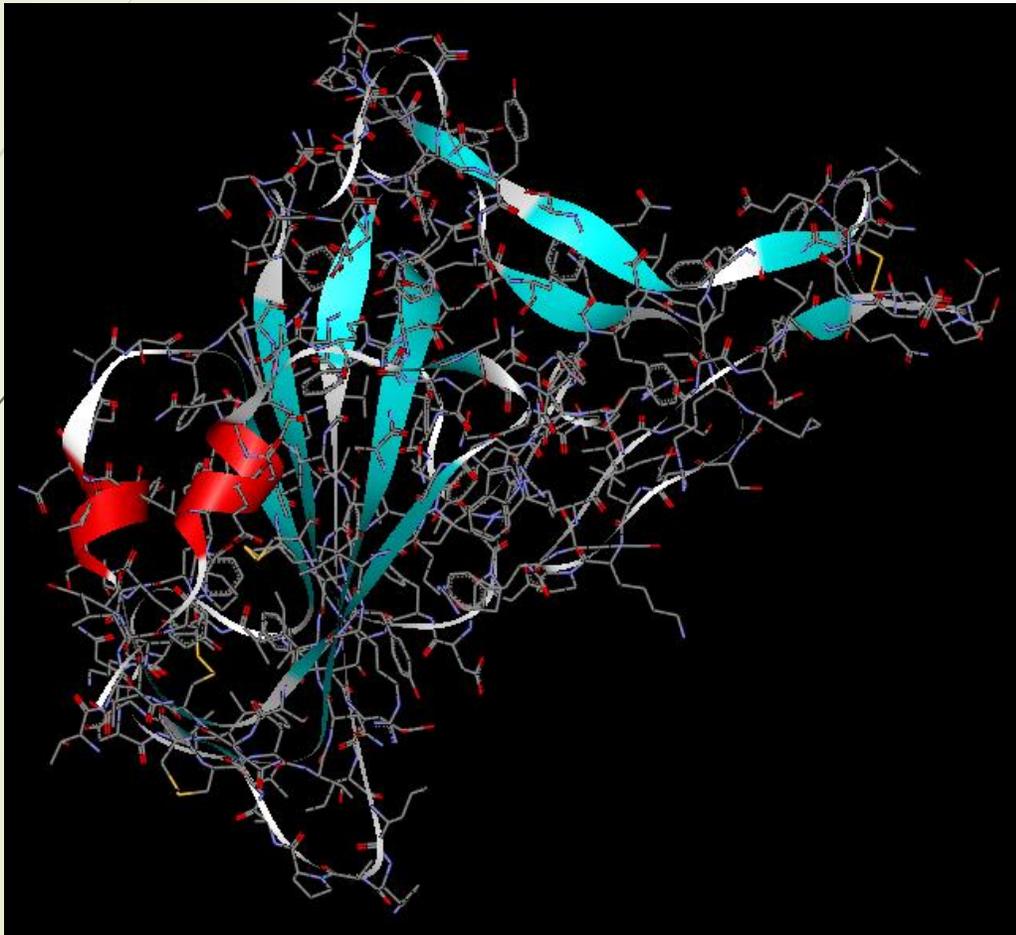
Browse... No file selected.

Run I-TASSER Clear form

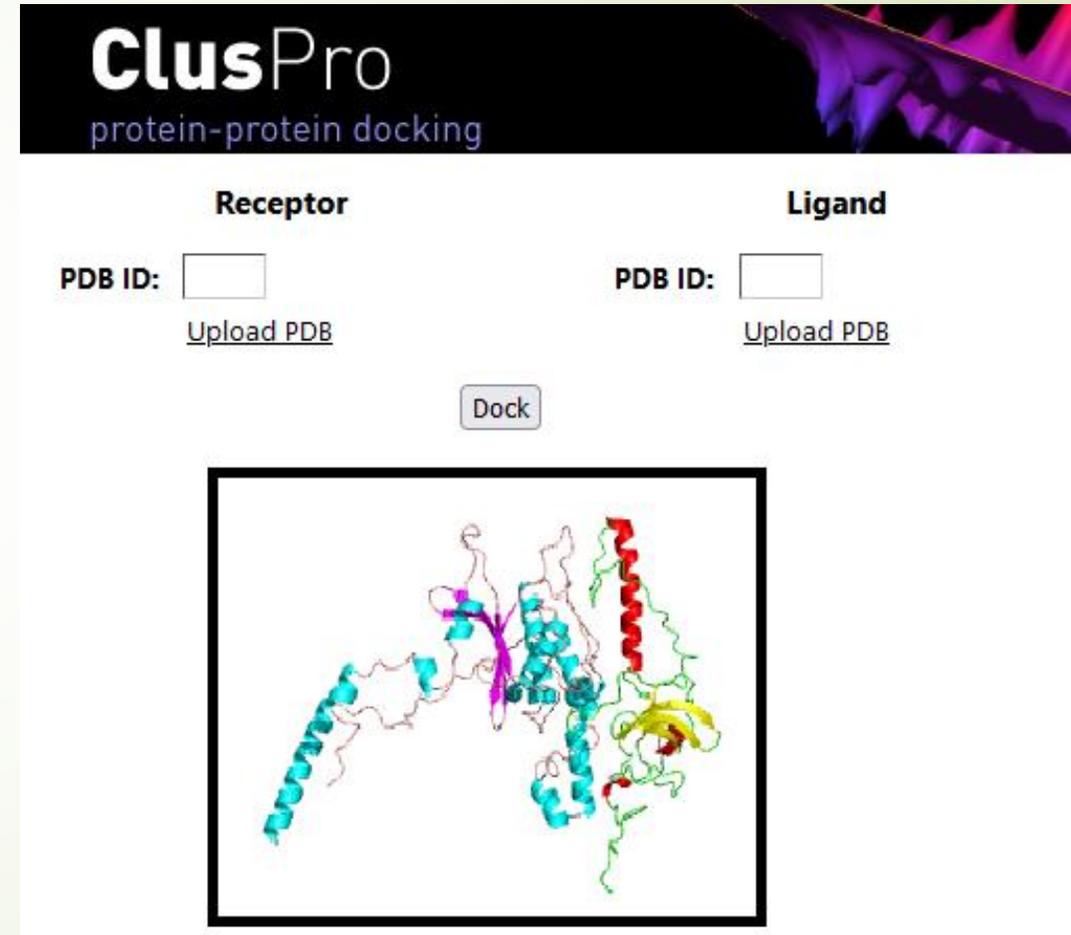


Viewing and Docking

View 3D structures

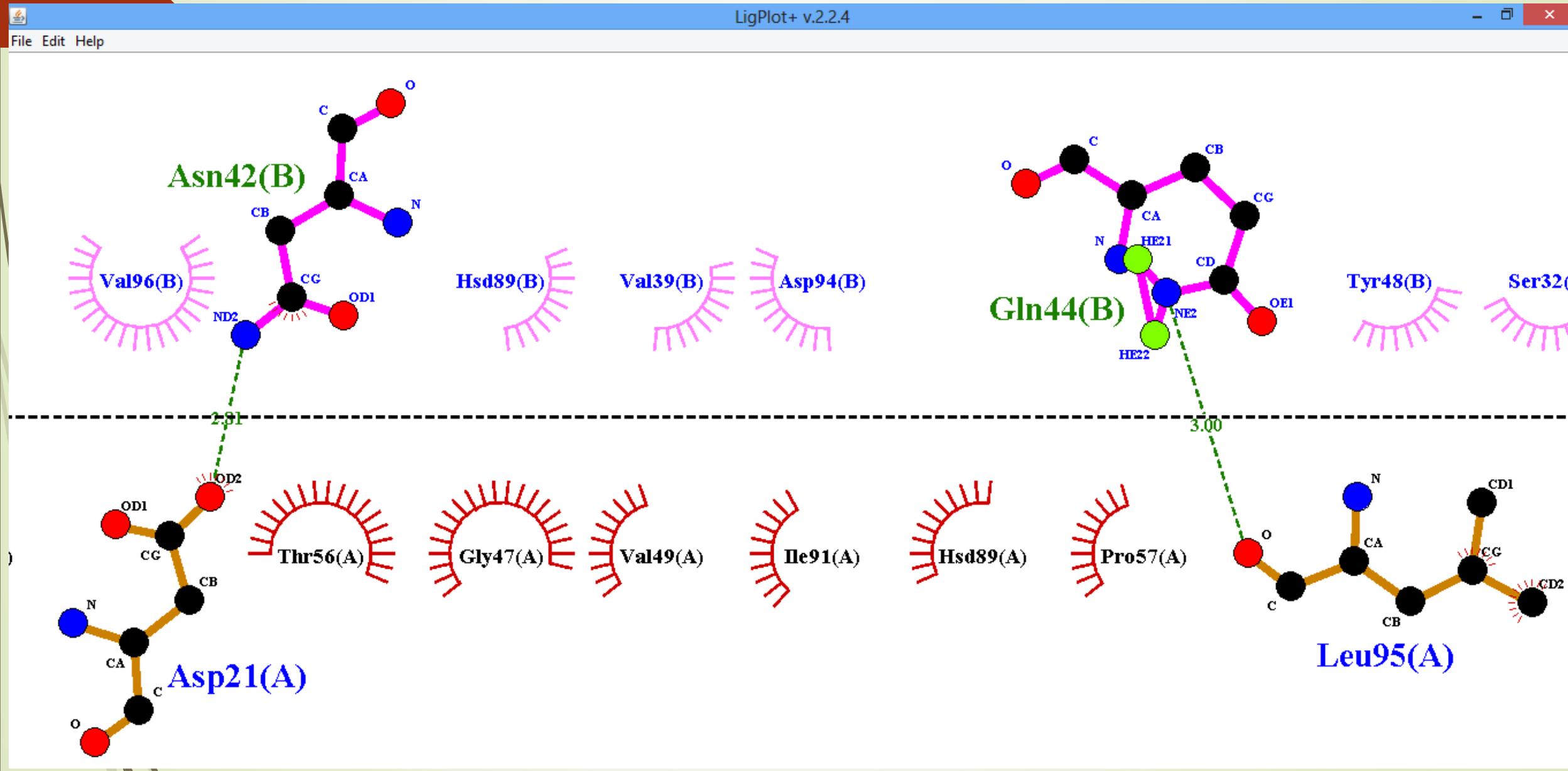


Docking



The ClusPro protein-protein docking interface. The header features the ClusPro logo and the text "protein-protein docking". Below the header, there are two input fields for "Receptor" and "Ligand", each with a "PDB ID:" label and an empty text box. Underneath each input field is a link labeled "Upload PDB". A "Dock" button is positioned below the input fields. At the bottom of the interface, there is a preview window showing a 3D molecular structure of a protein complex, rendered in various colors (cyan, purple, red, yellow, green) to represent different parts of the protein.

Determination of amino acids interaction with Ligplot



Simulation of 3D structure of truncated sequence

Start a New Modelling Project

Target Sequence(s):

*(Format must be FASTA,
Clustal,
plain string, or a valid
UniProtKB AC)*



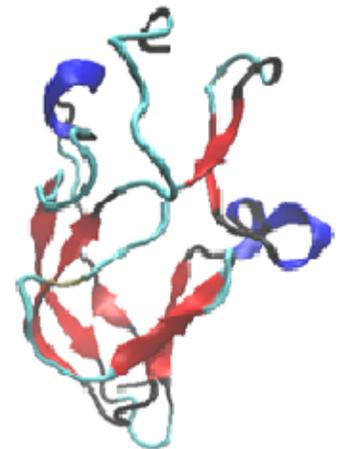
Paste your target sequence(s) or UniProtKB AC here

 Upload Target Sequence File...

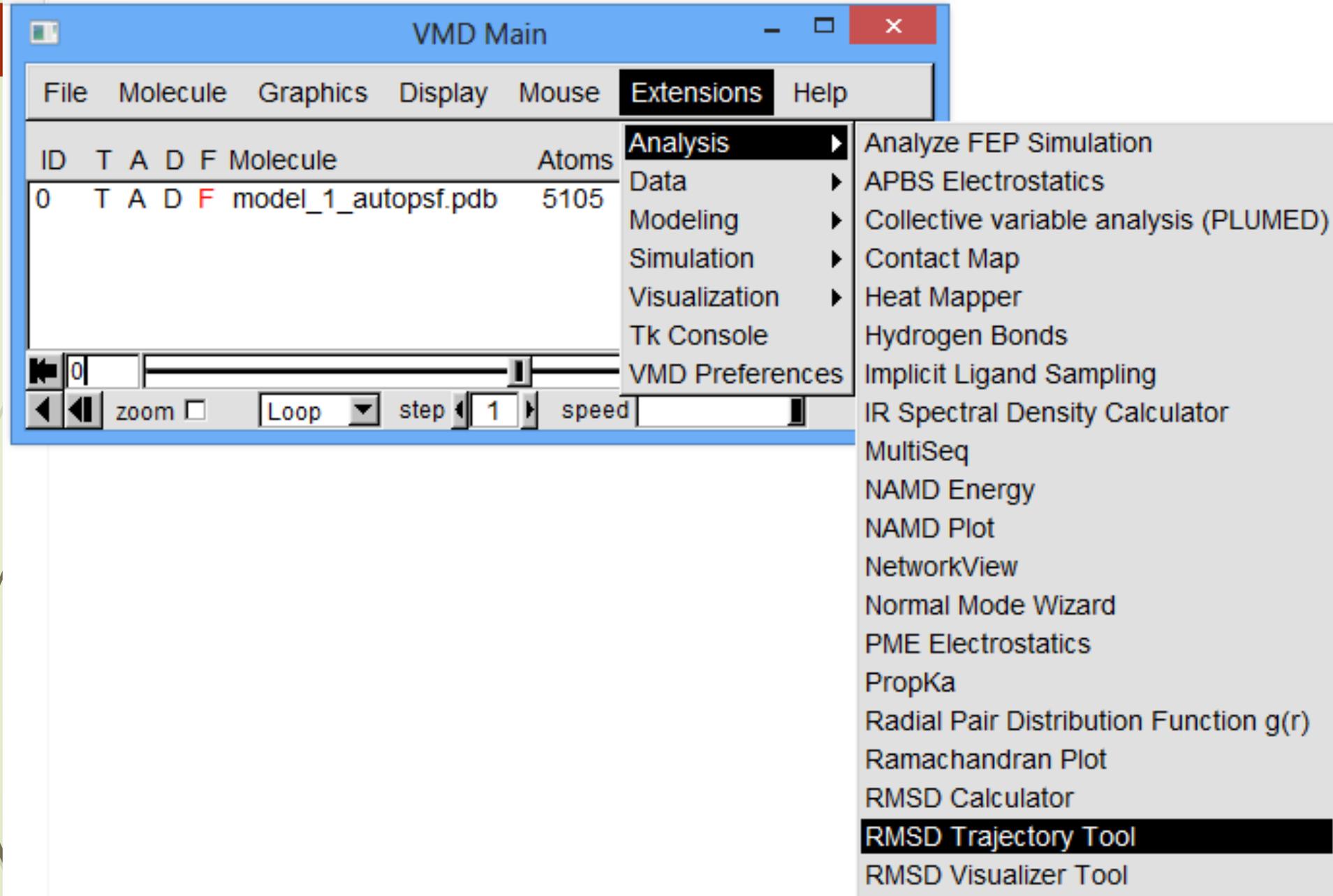
 Validate

Search For Templates

Build Model



Detection of binding stability



The screenshot shows the VMD Main window with the Extensions menu open. The menu is organized into sub-sections: Analysis, Data, Modeling, Simulation, Visualization, Tk Console, and VMD Preferences. The 'RMSD Trajectory Tool' option is highlighted in the Analysis sub-section.

ID	T	A	D	F	Molecule	Atoms
0	T	A	D	F	model_1_autopsf.pdb	5105

0

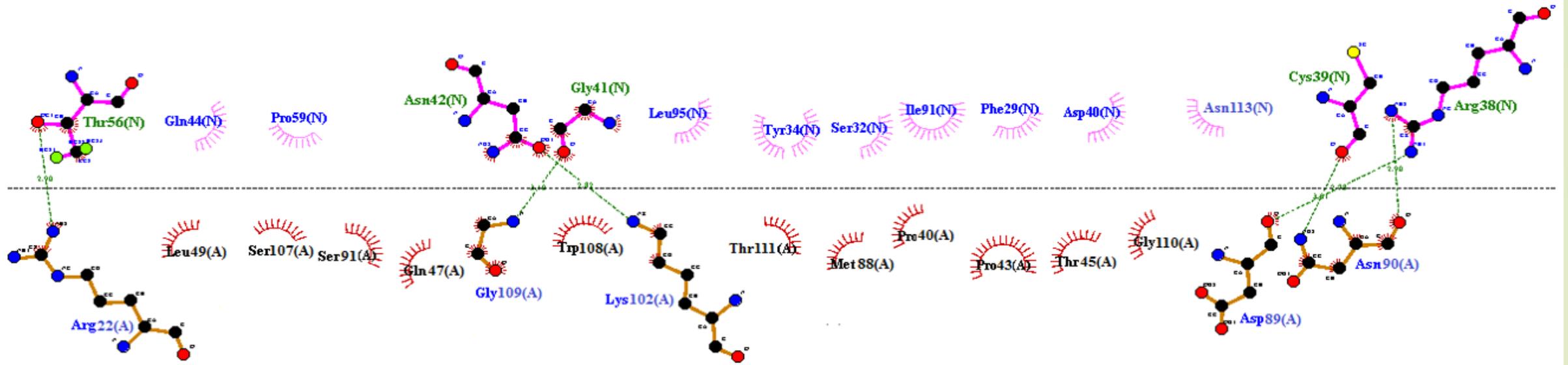
zoom Loop step 1 speed

- Analysis
 - Analyze FEP Simulation
 - APBS Electrostatics
 - Collective variable analysis (PLUMED)
 - Contact Map
 - Heat Mapper
 - Hydrogen Bonds
 - Implicit Ligand Sampling
 - IR Spectral Density Calculator
 - MultiSeq
 - NAMD Energy
 - NAMD Plot
 - NetworkView
 - Normal Mode Wizard
 - PME Electrostatics
 - PropKa
 - Radial Pair Distribution Function $g(r)$
 - Ramachandran Plot
 - RMSD Calculator
 - RMSD Trajectory Tool**
 - RMSD Visualizer Tool
- Data
- Modeling
- Simulation
- Visualization
- Tk Console
- VMD Preferences

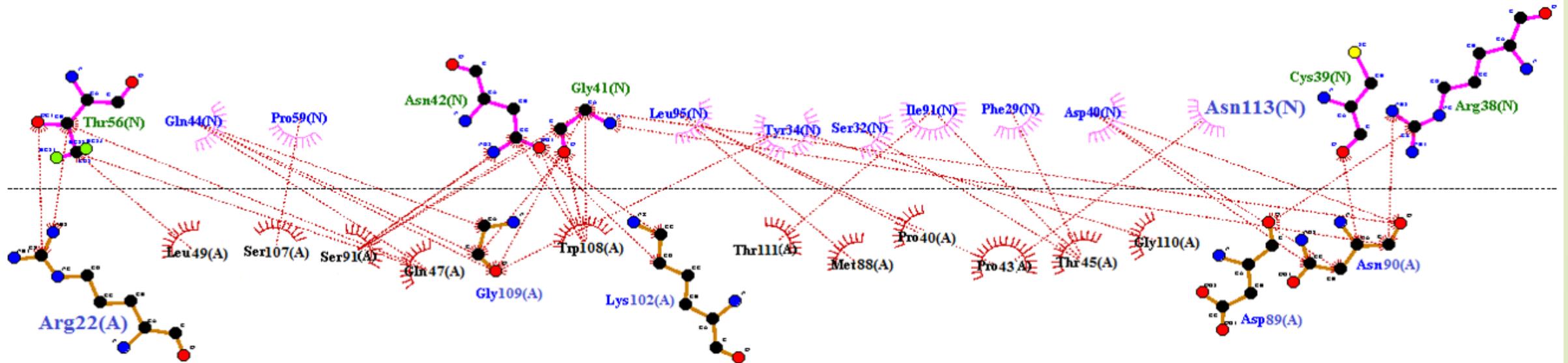
Results

- ▶ 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



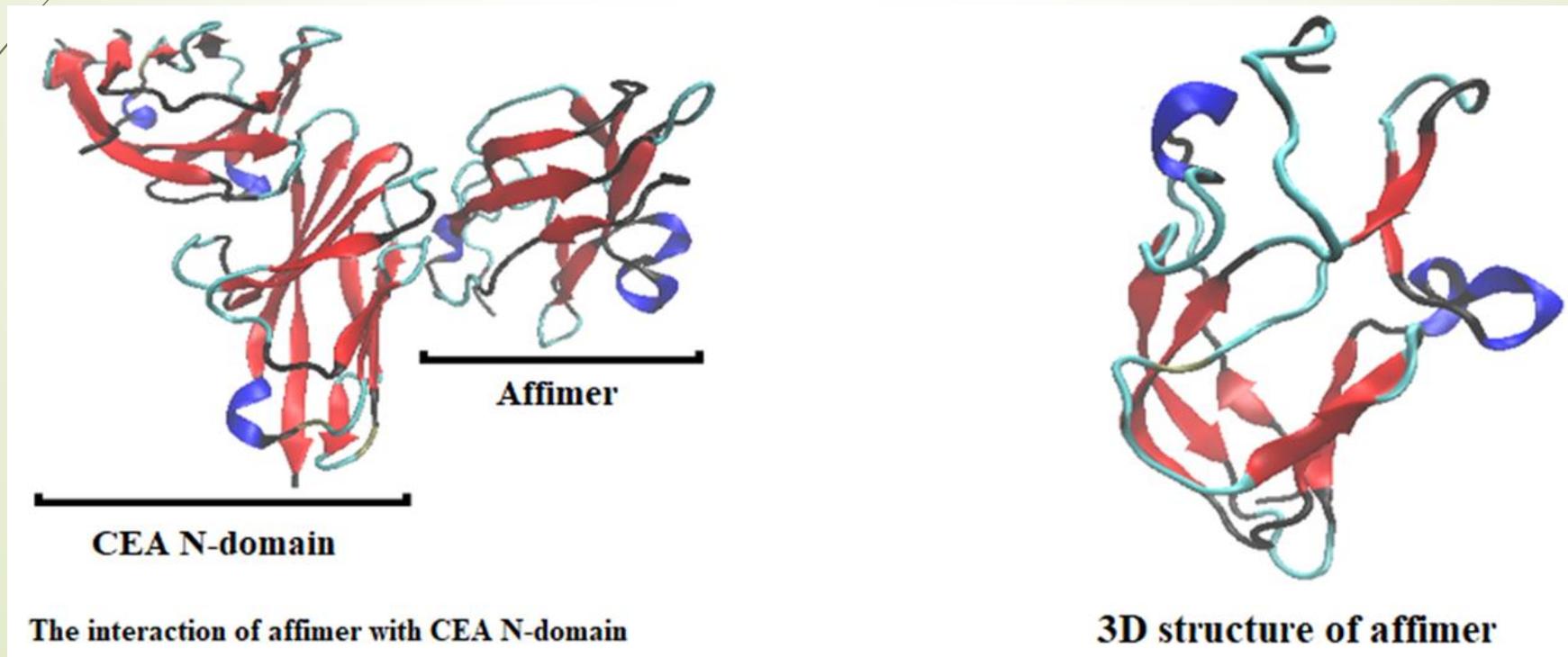
Hydrogen bonds



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.



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.
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- **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

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Authors:

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Organized by the Iranian Biotechnology Society

August 22nd-24th, 2021(online)

Tehran, Iran



Prof. Sirous Zeinali
Congress Head and Head of the Society

Affimer by Phage display

Affimers are engineered proteins that act like antibodies.

- Affimers are produced by **phage display** method.

