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antibodies MDPI

A Computational Workflow for The Prediction of Epitope/Paratope Regions and Antibody–Antigen Binding Poses

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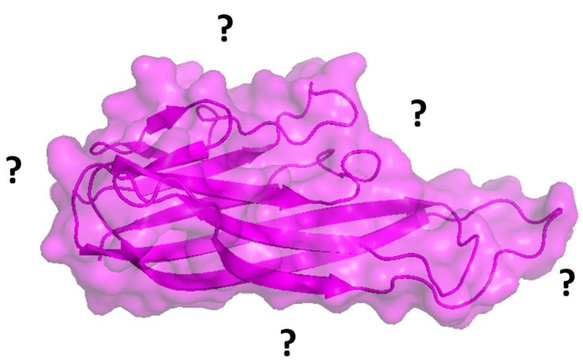
INTRODUCTION & AIM

Predicting the accurate binding pose of an **antibody-antigen** complex is essential for rational drug design.

Antigens often have multiple epitopes; standard docking struggles to pinpoint the correct site.

Standard docking (Blind Docking) searches the entire protein surface, leading to:

1. Excessive computational expense.
2. Low accuracy when multiple epitopes exist.
3. Antibody often binds to the incorrect epitope.



- **Time-Consuming**
- **Inaccurate**
- **High False Positives**

Figure 1. TNF-α protein surface (PDB ID: 4g3y)

MATERIALS & METHODS

Structure Selection

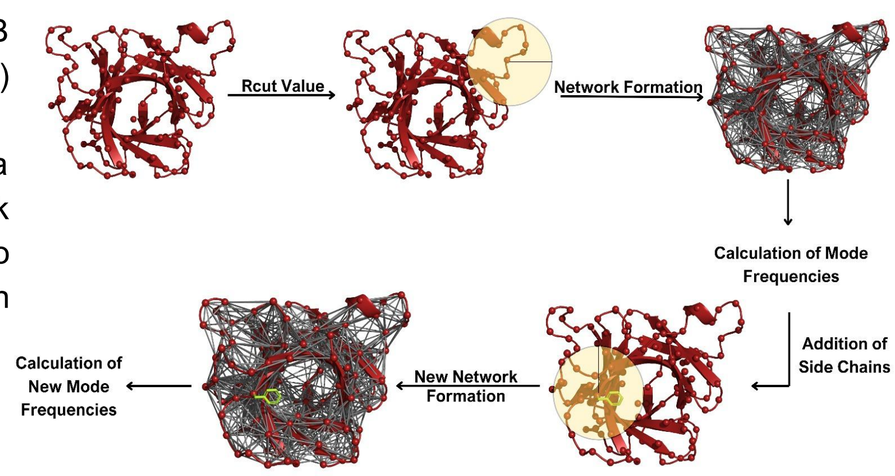
Our dataset includes TNFα, IL1β, and PD-L1 antibody:antigen crystal structures. For **TNFα** (PDB ID: 4G3Y[1]), for **IL-1β** (PDB ID: 4G6M[2]), and for **PD-L1** (PDB ID: 7C88[3]) were selected and analyzed.

Essential Site Scanning Analysis (ESSA)[4], a fast and effective elastic network model-based method, was used to determine essential residues in antibody-antigen structures.

LightDock

Essential Site Scanning Analysis (ESSA)

ESSA
Essential Site Scanning Analysis



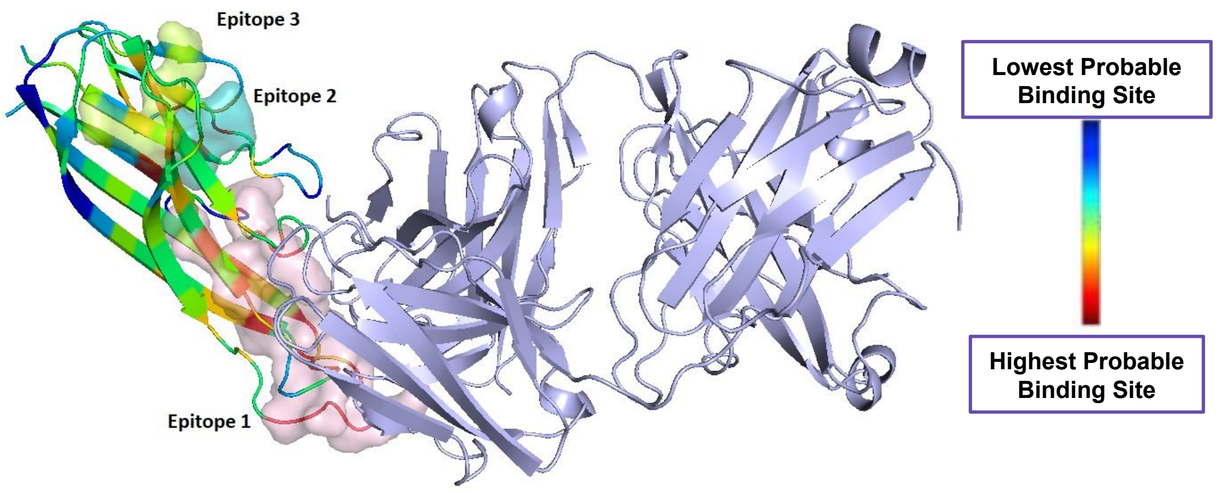
ESSA-Guided LightDock-ing

1. **Antigen Preparation:** ESSA-detected residues were **clustered to define specific Epitopes**.
2. **Docking Tool: LightDock**[5] (flexible protein-protein docking framework) was used for all calculations.
3. **Constraint:** ESSA-defined Epitope/Paratope residues were used as **constraints** to focus LightDock's sampling space, eliminating irrelevant poses for speed and accuracy.
4. **Analysis:** Docking was run **with/without the Antibody mode**. The **top three poses** (highest score) from each case were analyzed.

RESULTS & DISCUSSION

ESSA-guided docking significantly outperforms blind docking. The correct binding poses were consistently found in the top-ranked models when using our workflow.

TNFα: INFLIXIMAB (4G3Y) Epitope Mapping Result



Clustering Output: Three potential epitopes
Epitope 1 -> 18 Residues
Epitope 2 -> 5 Residues
Epitope 3 -> 2 Residues

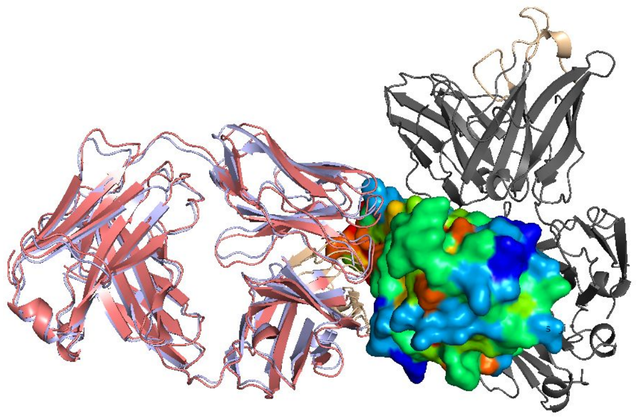
ESSA Guided Restraint Mode
Ag+Ab
Ag (Antibody Mode On)
Blind
Semi – Blind (Antibody Mode On)

Type of Protein	PDB ID	Identified Epitope #
TNF-α	4g3y-ag	3
PD-L1	7c88-ag	2
IL1B	4g6m-ag	3

•For each **ESSA-detected epitope** in each antigen (TNF-α, IL-1β and PD-L1) we performed docking calculations in **four different restraint modes**. In total we obtained 22 different docking results. ***Ag:** Antigen, ****Ab**: Antibody

Highest-Performing Pose for 4G3Y - Epitope 1

Original Conformation



Blind

Epitope 1-Guided Best Docking Result

TNFα

CONCLUSION

- **ESSA successfully detects multiple epitope regions** in complex antigens.
- **ESSA-guided docking significantly improves prediction accuracy** over blind docking.
- This workflow reliably **pinpoints the correct epitope** and refines the antibody-antigen pose.
- **Impact:** A possible new tool for **rational antibody design**.

FUTURE WORK / REFERENCES

Broad Validation: Applying the workflow to a **larger, diverse dataset** to validate its broad applicability.

Workflow Automation: Developing a **single, automated tool** to streamline ESSA extraction and LightDock setup for easier research access.

[1]Liang et al. (2013). J. Biol. Chem. PDB ID: 4G3Y.
[2]Blech et al. (2012). J. Mol. Biol. PDB ID: 4G6M.
[3]Liu et al. (2020). Signal Transduct. Target. Ther. PDB ID: 7C88.
[4]Kaynak et al. (2020). Comp. Struct. Biotechnol. J. DOI: 10.1016/j.csbj.2020.06.020
[5]Jiménez-García et al. (2017). Bioinformatics. DOI: 10.1093/bioinformatics/btx555

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