AI-Powered De Novo Design of GPCR Protein Antigen

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Booth #519

Illuminating Discovery

Preparation of full-length GPCR protein antigen

Opportunities

Preparing pure GPCR antigens with native conformation is crucial for the isolation of therapeutic antibody molecules.

Because of the large hydrophobic transmembrane domains and limited exposure epitopes, various antigen formats are developed for GPCR antibody discovery.

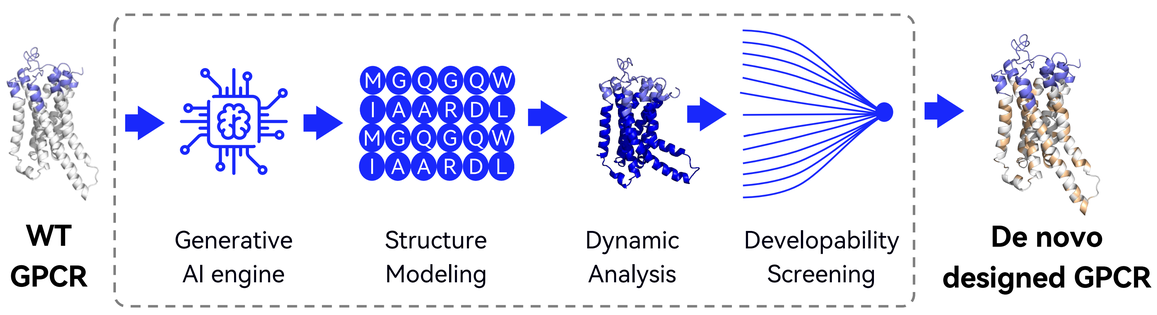
Purified protein antigens, as opposed to DNA and overexpressed cells, usually induce superior immunization effects and allow for exclusion of impurities, which will facilitate the isolation of target-specific antibodies.

Challenges

Full-length protein antigens are rarely used in antibody discovery[1] due to
(1) low expression level on cell membranes,
(2) low solubility and stability of expressed proteins, and
(3) complex and time-consuming purification procedures.

Solution: AI-powered de novo GPCR antigen design

Figure 1. Schematic diagram of AI-powered de novo GPCR antigen design workflow.



- ✓ **Shorter time** for R&D
- ✓ **High** yield rate
- ✓ **Comparable** binding affinity
- ✓ **High** thermostability

Step 1
A generative AI model were trained using all available data from solved GPCR and non-GPCR structures in the Protein Data Bank (PDB).

Step 2
The wild-type GPCR structure was constructed, and potential epitopes were analyzed.

Step 3
The generative AI model were employed to generate new sequences with large-scale mutations in the non-epitope transmembrane domains.

Step 4
The structures of the new GPCRs were modeled and their structural stability were analyzed using molecular dynamics (MD) simulations.

Step 5
The de novo designed GPCR sequences were obtained for wet-lab verification after conducting developability screening.

Result: In silico screening & generation of GPCR sequences

Over 160,000 unique sequences that preserved the pointed epitope of GPCR were generated. Following sequence rationality screening, approximately 10,000 sequences were subjected to structural modeling, with 100 of them undergoing dynamic analysis based on several criteria. Ultimately, 15 sequences were selected for expression and functional verification. The entire in silico process was completed within **5 weeks**.

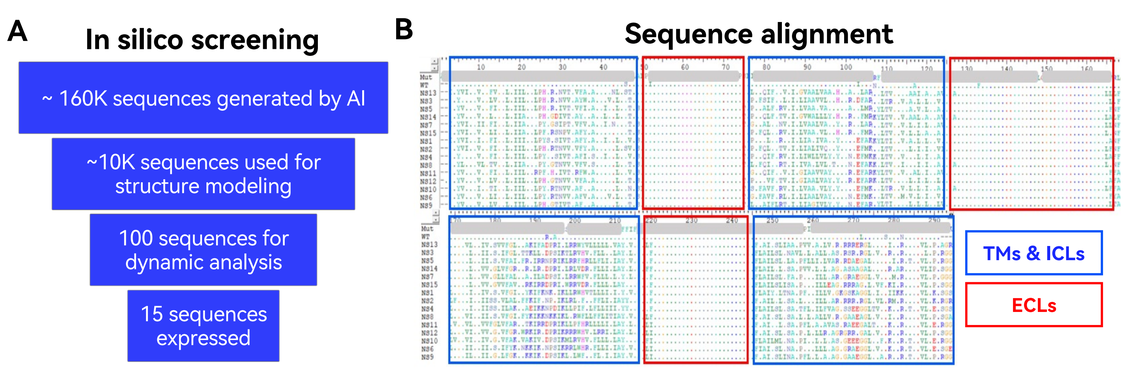


Figure 2. (**A**) In silico screening generated 15 new ready-to-express GPCR sequences. (**B**) Sequence comparison with wild-type and mutant GPCRs. About **53.2%-69.7%** of residues in transmembrane domains (TMs) and intracellular loops (ICLs) were replaced. Extracellular loops (ECLs) were spared from mutagenesis.

Result: Wet-lab evaluation on AI generated GPCR antigen

All 15 sequences, along with the positive control mutant from a previous study, were expressed in an insect system. Out of the 15 sequences generated, 13 (**86.7%**) exhibited expression on the cell membrane, and 11 (**73.3%**) demonstrated binding affinity to the known GPCR antibody.

- ✓ **Successful expression of antigen with high yield & biological activity comparable to the control GPCR mutant**

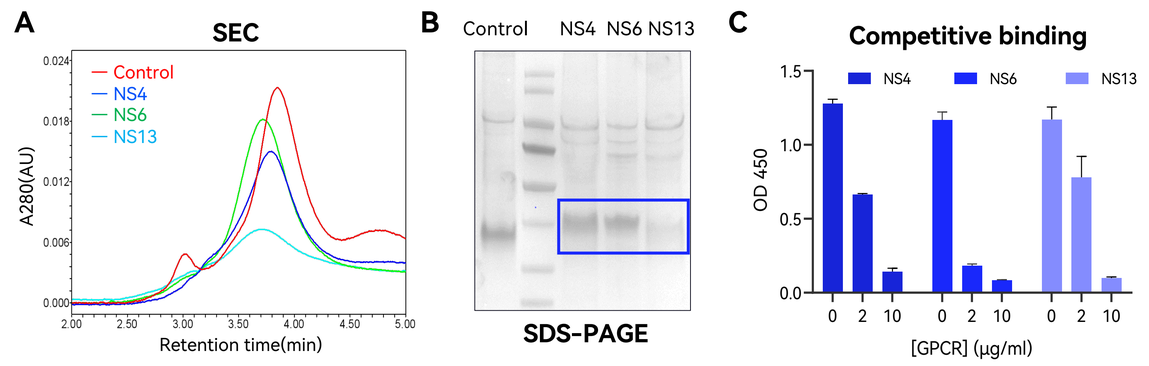


Figure 3. (**A**) Size-exclusion chromatography. (**B**) SDS-PAGE results of three new sequences (NS4, NS6, NS13) and control protein purified by DDM. (**C**) ELISA results showed that binding between control protein and a benchmark antibody was competed by GPCR mutants at different concentrations.

- ✓ **Enhanced thermostability of de novo designed GPCRs compared to the control**

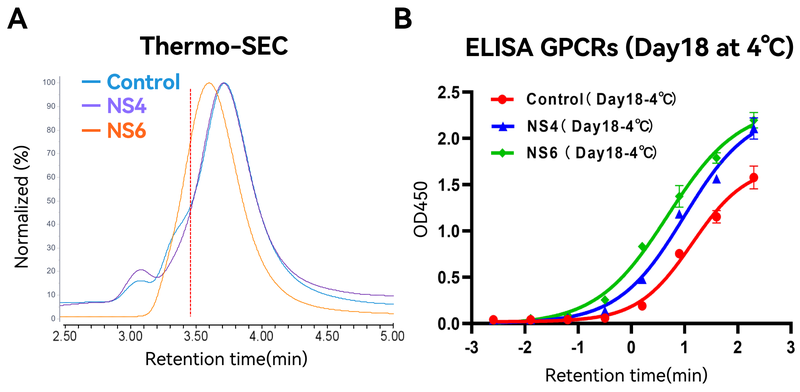


Figure 4.
(**A**) Normalized thermo-SEC results for NS4, NS6, and control after heating at 40 ℃ for 10 min.

(**B**) ELISA binding affinity results for GPCR proteins stored at 4℃ for 18 days.

- ✓ **After immunization and a small-scale hit generation …**

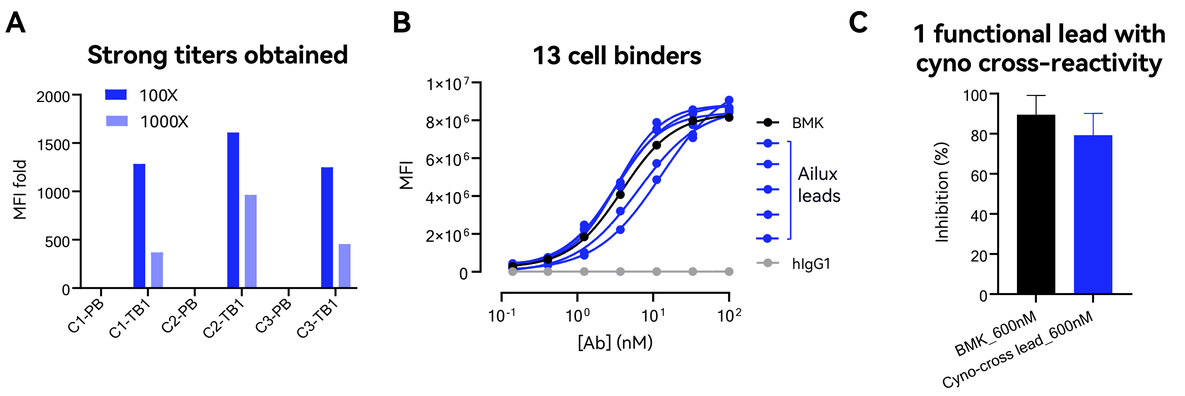


Figure 5. (**A**) Serum titers of SJL mice immunized with NS6 nanodisc protein, as measured by FACS binding to CHOK1 GPCR+ cell. PB: primary blood. TB: test blood. (**B**) Antibody binding to CHOK1 GPCR+ cells. (**C**) Inhibitory activity of benchmark antibody and cyno cross-reactive lead at 600 nM.

AI-generated GPCRs demonstrated excellent properties

Conclusion

We developed an AI-driven, computationally assisted solution that rapidly generates high-quality GPCR protein antigens, with demonstrated excellent homogeneity, high yield rates, comparable binding affinity, and immune response.

Our innovative dry-lab screening and generation of GPCR sequences was completed within a span of 5 weeks, which significantly reduced the time required for GPCR protein optimization.

This approach offers a novel perspective on de novo protein antigen design for antibody discovery, with the potential to revolutionize the field of GPCR-targeted therapeutics.

References

1. Ju MS, Jung ST. Antigen design for successful isolation of highly challenging therapeutic anti-GPCR antibodies. *Int J Mol Sci.* 2020 Nov 3;21(21):8240.

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