Epitope-scaffold immunogen design Torben Schiffner



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Diversity of HIV Env dwarfs that of SARS-CoV-2 spike

HIV Env Worldwide 2013

SARS-CoV-2 spike Worldwide Oct 29, 2022



Trees show amino acid substitutions. 5000 sequences each from LANL and GISAID.

Prototype HIV broadly neutralizing antibodies (bnAbs)



- bnAbs neutralize diverse isolates, some up to 99% of all isolates
- bnAbs can provide sterilizing immunity in NHP models
- bnAbs can protect in humans
- If vaccine can elicit bnAbs, could prevent HIV infection
- Vaccine responses must be broad, potent, and durable

MPER bnAb 10E8

- 10E8 is very broad (>98%) with good potency (geomean IC₅₀: 0.22 µg/mL)
- Not or weakly polyreactive
- MPER not part of most soluble nativelike trimers
- Epitope sterically occluded in Env trimer context
- 10E8 engages the epitope via a long (22aa) HCDR3 with a YxFW binding motif



De novo epitope-scaffold design



RFDiffusion

Article

Denovo design of protein structure and function with RFdiffusion

https://doi.org/10.1038/s41586-023-06415-8	Joseph L. Watson ^{12,15} , David Juergens ^{12,35} , Nathaniel R. Bennett ^{12,36} , Brian L. Trippe ^{2,45,15} , Jason Yim ^{28,5} , Helen E. Eisenach ^{12,16} , Woody Ahern ^{12,35} , Andrew J. Borst ¹² , Robert J. Ragotte ¹² , Lukas F. Milles ¹² , Basile I. M. Wicky ¹² , Nikita Hanikel ¹² , Samuel J. Pellock ¹² , Alexis Courbet ^{12,8} , William Sheffler ² , Jue Wang ¹² , Preetham Venkates ^{12,9} , Isaac Sappington ^{12,9} , Susana Vázquez Torres ^{12,8} , Anna Lauko ^{12,8} , Valentin De Bortoli ⁸ , Emile Mathieu ¹⁰ , Sergey Ovchinnikov ¹¹² , Regina Barzilay ⁶ , Tommi S. Jaakkola ⁶ , Frank DiMaio ¹³ , Minkyung Baek ¹⁵ & David Baker ¹²⁴⁴
Received: 14 December 2022	
Accepted: 7 July 2023	
Published online: 11 July 2023	
Open access	

Check for updates





57% of motif grafting designs bound to target sequence!!!

https://www.bakerlab.org/wpcontent/uploads/2023/03/RFDiffusion_animati on_by_lan_C_Haydon.gif

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Sequence design

- Designs goals:
 - High expression level
 - Stable protein
- Interface is kept intact
- Buried graft residues to be designed
- Suitable algorithms:
 - Rosetta Design
 - ProteinMPNN
 - ESM
 - Others
 - Combinations of the above
 - Multi-state design



Structure prediction

Requirements:

- Multimer protein structure prediction
- Acurate
- Fast!

OPEN ColabFold: making protein folding accessible to all

Check for update

Milot Mirdita^{[],10}, Konstantin Schütze^[], Yoshitaka Moriwaki^[], Lim Heo^[], Sergey Ovchinnikov^[], and Martin Steinegger^[],^{8,9,10}

- ColabFold is a variant of AlphaFold 2 that uses much faster homology search and redundancyreduced search database
- Colabfold achieves comparable accuracy to AlphaFold 2
- Colabfold can be used locally or on Google Colab



The antibody problem

• "As a limitation, we observe anecdotally that AlphaFold-Multimer is generally not able to predict binding of antibodies and this remains an area for future work."

Evans et. al; bioRxiv "Protein complex prediction with AlphaFold-Multimer"

• Possible solution: Alphafold with "initial guess":

Article https://doi.org/10.1038/s41467-023-38328-5 Improving de novo protein binder design with deep learning

Received: 29 July 2022	Nathaniel R. Bennett © ^{1,2,3,8} , Brian Coventry ^{1,2,4,8} , Inna Goreshnik ^{1,2} , Buwei Huang ^{1,2,5} , Aza Allen © ^{1,2} , Dionne Vafeados © ^{1,2} , Ying Po Peng ^{1,2} , Justas Dauparas © ^{1,2} , Minkyung Baek © ^{1,2} , Lance Stewart © ^{1,2} , Frank DiMaio ^{1,2} , Steven De Munck © ^{6,7} , Savvas N. Savvides © ^{6,7} & David Baker © ^{1,2,4}
Accepted: 24 April 2023	
Published online: 06 May 2023	

Filtering

- Graft accuracy:
 - Predict structure
 - Align on the antibody
 - Measure RMSD of the graft
 - Cut-off: <3Å
- Prediction accuracy: pLDDT>90
- Interface accuracty: pTM>0.85



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- To find designs for which structure and sequence predictions converge, we iteratively design the sequence and predict the structure
- Sequence recovery increases substantially after the first iteration, but converges after 2-3 iterations
- Target: 95%

Rational design of epitope-scaffold immunogens can specifically activate 10E8-class responses in vivo

- Non-human primates immunized with an epitopescaffold nanoparticle
- BCRs from epitope-specific B cells were sequenced
- BCRs with 10E8-like HDRH3s were detected in all animals

nature immunology

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https://doi.org/10.1038/s41590-024-01833-w

Vaccination induces broadly neutralizing antibody precursors to HIV gp41

Received: 24 February 2024

A list of authors and their affiliations appears at the end of the paper

Accepted: 4 April 2024

Article

Schiffner et al. Nature immunology 2014



Ivy Phung Shane Crotty



Today's tutorial



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