# IgDesign: Antibody inverse folding with *in vitro* validation

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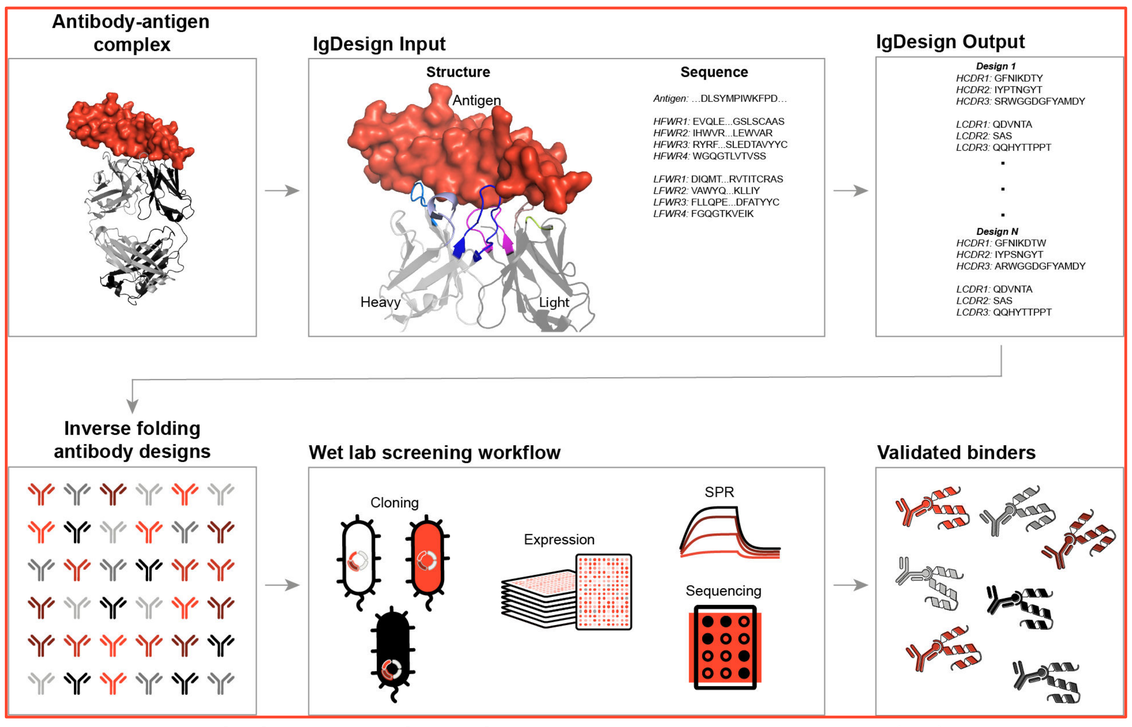
## Abstract

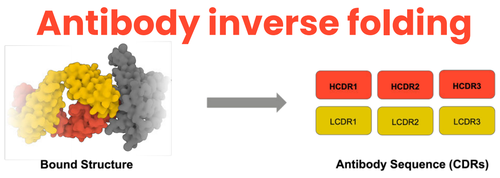
Deep learning approaches have demonstrated the ability to design protein sequences given backbone structures. While these approaches have been applied *in silico* to designing antibody complementarity-determining regions (CDRs), they have yet to be validated *in vitro* for designing antibody binders, which is the true measure of success for antibody design. Here we describe *IgDesign*™, a deep learning method for antibody CDR design, and demonstrate its robustness with successful binder design for 8 therapeutic antigens. The model is tasked with designing heavy chain CDR3 (HCDR3) or all three heavy chain CDRs (HCDR123) using native backbone structures of antibody-antigen complexes, along with the antigen and antibody framework (FWR) sequences as context. For each of the 8 antigens, we design 100 HCDR3s and 100 HCDR123s, scaffold them into the native antibody's variable region, and screen them for binding against the antigen using surface plasmon resonance (SPR). As a baseline, we screen 100 HCDR3s taken from the model's training set and paired with the native HCDR1 and HCDR2. We observe that both HCDR3 design and HCDR123 design outperform this HCDR3-only baseline. IgDesign is the first experimentally validated antibody inverse folding model. It can design antibody binders to multiple therapeutic antigens with high success rates and, in some cases, improved affinities over clinically validated reference antibodies. Antibody inverse folding has applications to both *de novo* antibody design and lead optimization, making IgDesign a valuable tool for drug development and enabling therapeutic design.




**Overview of *in silico* (top) and *in vitro* (bottom) workflow for antibody inverse folding.** (Top) Antibody-antigen complex structures are inputted to IgDesign which outputs CDR sequences. (Bottom) Libraries of inverse folding antibody designs are sent to the wet lab for screening. Designed binders are validated and their affinities are measured.

## Inverse folding


## Antibody inverse folding




## Existing models

- ProteinMPNN (Dauparas 2022) and its variants
- ESM-IF1 (Hsu 2022)
- PiFold (Gao 2022)
- LM-Design (Zheng 2023)
- AbMPNN (Dreyer 2023)

## IgMPNN

Based on ProteinMPNN (Dauparas 2022) architecture:

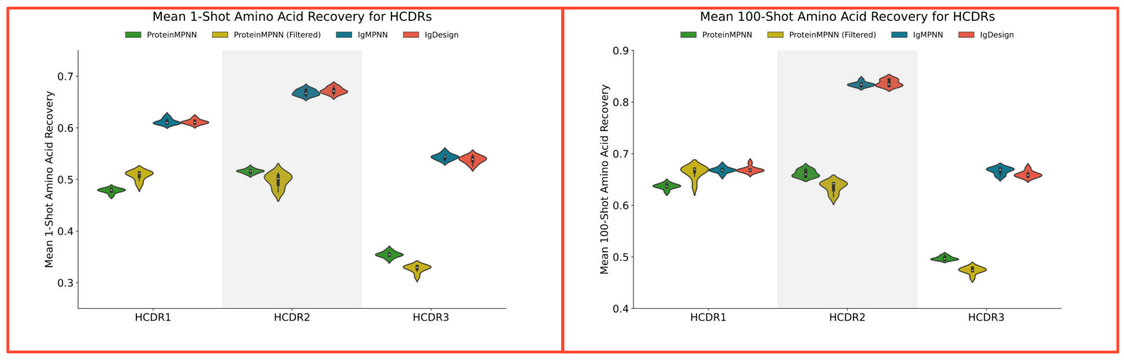
- Pretrained on general proteins, fine-tuned on antibody-antigen complexes (SAbDab)
- Antigen sequence and antibody framework sequences provided as context
- Antibody CDRs are predicted in order (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3)

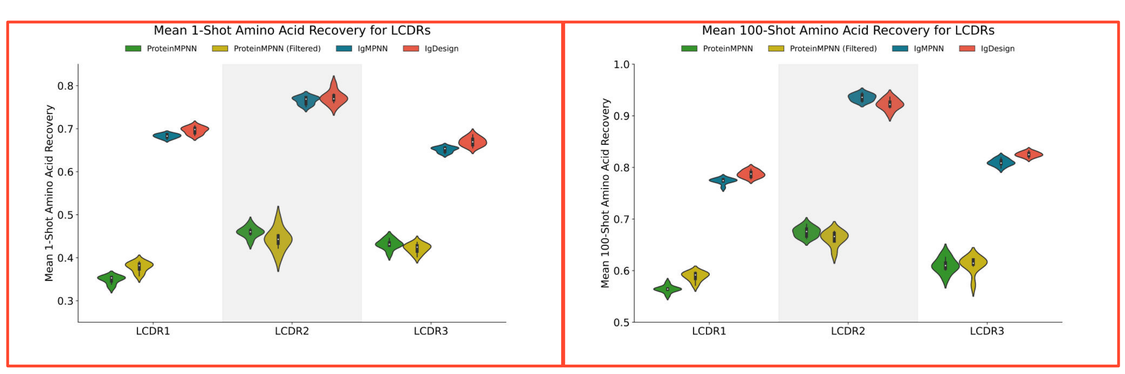
## IgDesign

Based on LM-Design (Zheng 2023) approach with IgMPNN as structural encoder and ESM2-3B as sequence decoder:

- Compute logits, final node embeddings, and MLE sequence from IgMPNN
- MLE sequence passed through ESM2-3B and embeddings extracted (before final projection head)
- Apply BottleNeck Adapter layer (Houlsby 2019) and compute cross-attention between IgMPNN embeddings (queries) and ESM2-3B embeddings (values)
- Pass above into ESM2-3B final projection head and sum logits with IgMPNN logits

## *In silico* results



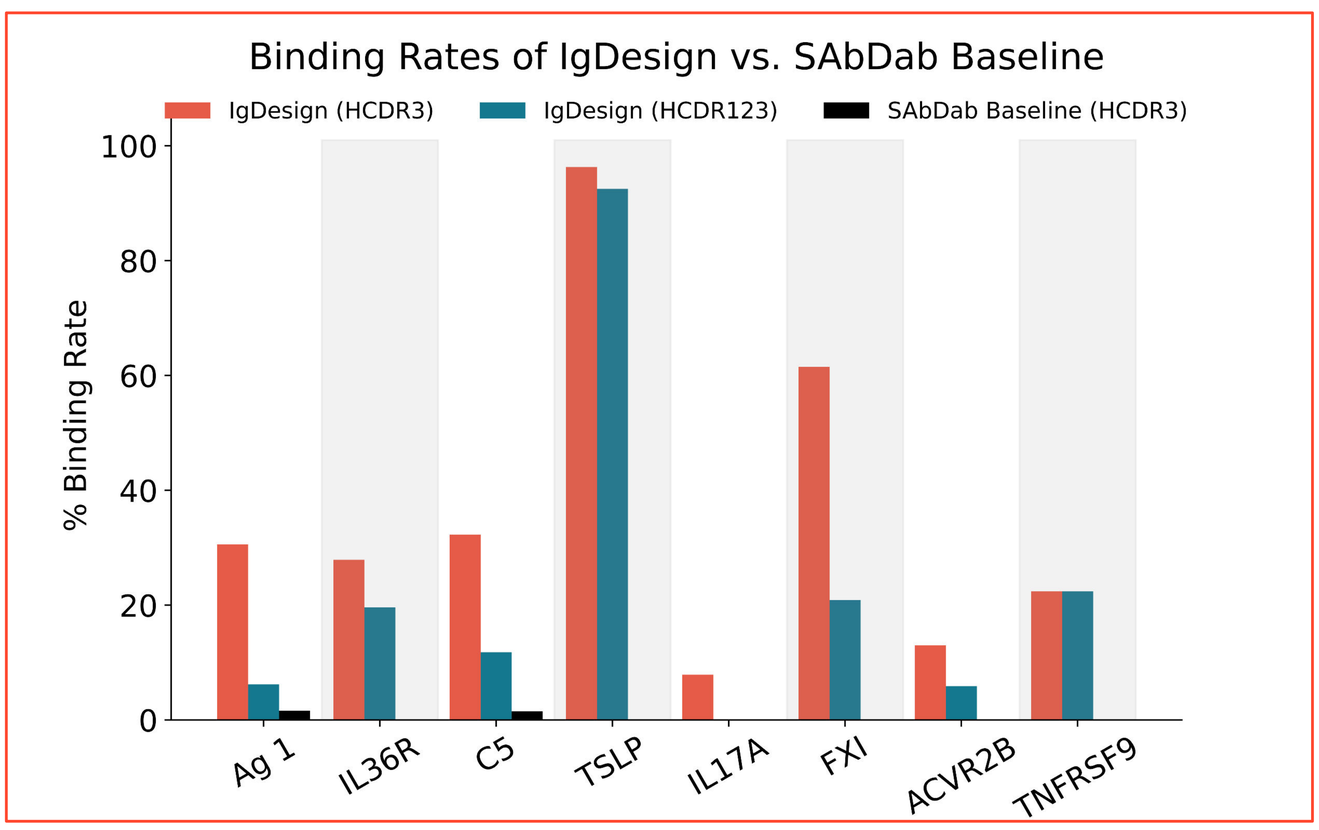

**Comparison between ProteinMPNN, IgMPNN, and IgDesign** on mean 1-shot (left) and 100-shot (right) amino acid recovery (AAR) for heavy chain CDRs [HCDRs] (top) and light chain CDRs [LCDRs] (bottom). Violin plots comparing distributions of mean AARs for ProteinMPNN (green), ProteinMPNN filtered to complexes not in its training set (yellow), IgMPNN (blue), and IgDesign (red) across 8 antigen test sets.

1-shot (100-shot) AAR is the maximum AAR over 1 (100) sample(s) from the model. Mean 1-shot (100-shot) AAR is the mean of the 1-shot (100-shot) AARs computed on each test set. The distribution captures the 95% interval, the white dot represents the median, and the box represents the interquartile range.

## Wet lab validation (*in vitro* results)

**Experimental design:**

- Used 8 different antibody-antigen complexes from SAbDab
- Models were trained (fine-tuned) on 40% antigen sequence identity holdout
- Each library contained:
  - 100 IgDesign HCDR3s
  - 100 IgDesign HCDR123s
  - 100 SAbDab HCDR3s (Baseline)
- IgDesign was sampled 1M times and filtered down to the top 100 sequences using perplexity
- Baseline HCDR3s were chosen from model training sets and with matching lengths to parental antibodies




**Binding rates across all antigens for IgDesign HCDR3 (red) and HCDR123 (blue) vs. SAbDab HCDR3 baseline (black).** Binding rate is defined as the percentage of sequences that bind to the target antigen as assessed by SPR. Baseline binding rate is 0% for all antigens except Ag 1 and C5. IgDesign significantly outperforms the SAbDab baseline at antibody binder design.

| Antigen | % Binding Rate (Binders / Observations) | | |
|---|---|---|---|
| | IgDesign (HCDR3) | IgDesign (HCDR123) | SAbDab (HCDR3) |
| Antigen 1 | 30.6% (22 / 72) | 6.3% (4 / 64) | 1.6% (1 / 64) |
| IL36R | 27.9% (17 / 61) | 19.6% (11 / 56) | 0.0% (0 / 59) |
| C5 | 32.3% (20 / 62) | 10.4% (7 / 67) | 1.5% (1 / 68) |
| TSLP | 96.3% (52 / 54) | 92.5% (62 / 67) | 0.0% (0 / 54) |
| IL17A | 7.9% (5 / 63) | 0.0% (0 / 65) | 0.0% (0 / 50) |
| FXI | 61.5% (24 / 39) | 20.9% (9 / 43) | 0.0% (0 / 33) |
| ACVR2B | 13.0% (10 / 77) | 5.9% (4 / 68) | 0.0% (0 / 66) |
| TNFRSF9 | 22.4% (15 / 67) | 24.4% (13 / 58) | 0.0% (0 / 59) |

**Binding rates across antigens for IgDesign on HCDR3 and HCDR123 as well as SAbDab HCDR3 baseline.**

| Antigen | IgDesign (HCDR3) | | IgDesign (HCDR123) | |
|---|---|---|---|---|
| | Ratio to Baseline | *p*-value | Ratio to Baseline | *p*-value |
| Antigen 1 | 19.6 | **2e-6** | 4.0 | 0.16 |
| IL36R | Inf | **3e-6** | Inf | **2.1e-4** |
| C5 | 21.9 | **8e-7** | 7.1 | 0.027 |
| TSLP | Inf | **1e-28** | Inf | **1e-28** |
| IL17A | Inf | 0.051 | N/A | 1.0 |
| FXI | Inf | **3.2e-9** | Inf | 4e-3 |
| ACVR2B | Inf | **1.6e-3** | Inf | 0.065 |
| TNFRSF9 | Inf | **3.4e-5** | Inf | **5.1e-5** |

**Fisher's exact tests across antigens for IgDesign on HCDR3 and HCDR123 vs. SAbDab HCDR3 baseline.** Significant *p-values* after Bonferroni correction are bolded. We note that IgDesign HCDR3 outperforms the baseline 8 out of 8 times and does so significantly 7 out of 8 times. IgDesign HCDR123 outperforms the baseline 7 out of 8 times and does so significantly 4 out of 8 times. We note that the baseline is only varying HCDR3 and keeping HCDR1 and HCDR2 fixed to the native sequence whereas IgDesign HCDR123 designs all three HCDRs.

## Conclusions

- IgDesign is able to design HCDR3 and HCDR123 loops using solved antibody-antigen structures
- Success rates are higher than a biological baseline (SAbDab HCDR3)
- Future Directions:
  - 6 CDR design (more challenging to screen *in vitro*)
  - Designing with noised or predicted antibody-antigen structures
  - Designing higher affinity binders

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