Measuring the sequence-affinity landscape of antibodies

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To ensure a fast and reliable immune response upon infection, B-cells rapidly mutate the sequence of their antibodies to improve their binding affinity to invading pathogens. However, little is known quantitatively about the precise relationship between the antibody sequence and its binding properties. To study this question, we developed a high-throughput method combining experiments with advanced statistical analysis to measure antibody affinity with high precision. We used this method to measure the effects of a wide variety of mutations in complementary determining regions (CDR) 1 and 3 of the antibody heavy chain (H). We find that the CDR1H domain tends to have fewer severe mutations than CDR3H, but has a higher tendency to affect antibody expression for non-severe mutations.

Keywords **— yeast display, protein affinity landscape, immunology, antibody, statistical analysis.**

I. Introduction

A successful immune response is based on the recognition of antigens by binding them to antibodies.

Antibody binding affinity is largely determined by 6 domains called complementarity determining regions (CDR). Mutating these CDRs affect antibody stability and affinity. Since measuring antibody stability and affinity is a labor intensive and expensive process it is not know how they quantitatively depend on the CDR region sequences. To overcome these limitations, we developed a method to quantitatively measure the landscape of functional antibody properties directly from their sequences.

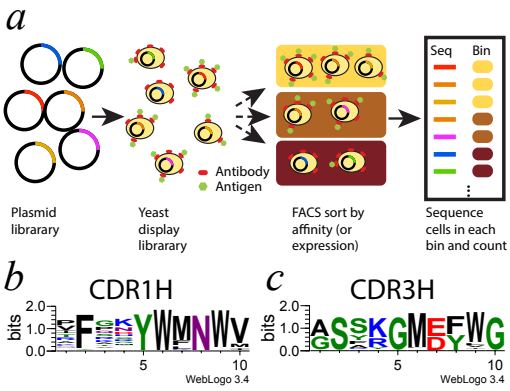
Our method combines a novel high throughput sequencing approach with advanced statistical analysis to quantitatively map out the binding landscape. It combines a sequencing based method for simultaneously measuring functional protein-protein interactions in a large sample, called Sort-seq [1], with yeast display [2]. Sort-seq uses FACS armed with a statistical analysis to en masse sort cells based on their binding affinity and expression. Yeast display has the advantage that it allows for disentangling protein expression and affinity. Yeast display expression levels also correlate highly with protein thermostability.

II. Methods and Results

We designed yeast plasmid libraries of CDR1H and CDR3H mutants for the fluorescein binding 4-4-20 scFv antibody using microarray oligonucleotides. Each CDR library consisted of 1950 codon mutations including synonymous mutation controls. We FACS separated the mutants and counted the frequencies of mutants in each bin. Using statistical techniques and experimental validation, we mapped mutant frequencies to affinity and expression measurements.

Because affinity measurement is tightly related to both antigen and antibody expression, we show that explicitly deconvolving expression from affinity is essential for accurately measuring affinity. This deconvolution, along with the set of synonymous mutations in our library, allow us to precisely measure antibody properties with an estimated (50:1) signal to noise ratio.

Our initial results clearly show different effects of mutations in CDR1H and CDR3H. CDR3H mutants are more likely to destroy antibody affinity. CDR1H mutants affect expression independently of affinity, while CDR3H mutations tend to affect expression only if affinity was abolished.



a) Schematic for sort-seq yeast display experiment. Weblogos are generated from the effects of single amino acid mutations of the 4-4-20 antibody on b) CDR1H and c) CDR3H affinity to fluorescein.

III. Conclusion

By designing libraries and measuring both affinity and expression, we can precisely and accurately simultaneously measure the biophysical properties of thousands of antibodies. This work gives us a basis for studying the effects of mutations on immunological recognition.

References

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