Using AI and Twist's Multiplexed Gene Fragments in the Hunt for the “Holy Grail” in De Novo Antibody Design

INTRODUCTION

Monoclonal antibodies (mAbs) have become a prominent therapeutic modality, with more than 160 therapeutic mAbs currently licensed worldwide. Their target binding specificity and ability to engage a diverse range of therapeutic targets make them a uniquely valuable component of modern medicine. However, isolating a mAb for a target epitope of interest remains a laborious process requiring extensive experimentation and screening.

Because antibody functionality is dictated by both structural and amino acid-level interactions, researchers must navigate a vast protein sequence space to identify optimal designs. Traditional antibody discovery methods often rely on animal immunization or the screening of vast libraries of randomly generated variants. Even among promising candidates, further optimization of the mAb sequence is often required to improve epitope specificity, affinity and developability. These approaches can be both costly and inefficient, requiring the iterative testing of tens to hundreds of thousands of variants to identify promising candidates.

To address these limitations, a research team at the University of Washington, led by Nobel Laureate David Baker, leveraged artificial intelligence, multiplex gene synthesis, and de novo protein design software to rationally design and validate single-domain antibodies known as variable heavy-chain domains (VHHs). Not only does their approach represent a breakthrough in antibody development, it highlights a way for researchers to overcome a fundamental bottleneck in AI-driven protein engineering—translating in silico designs into experimentally testable sequences at scale. In this case, a solution was found in Twist Bioscience’s Multiplexed Gene Fragments (MGFs).

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THE POTENTIAL OF DE NOVO ANTIBODY DESIGN

There are many reasons to be excited about de novo antibody design. Traditional antibody development has involved extensive protein engineering, wherein naturally existing antibodies are methodically altered to produce a molecule with desired properties (high affinity, easy to manufacture). While effective, the process is severely limited by only sampling sequences closely related to the input sequence of the natural antibody. To introduce new functionality into these proteins without affecting stability, binding, or other existing properties requires a deep understanding of how the entire protein sequence interacts. Such an understanding is beyond reach in most cases, leaving researchers to take the arduous and often fruitless approach of empirically testing specific variants.

De novo protein design takes a different approach, one where proteins are purpose-built. This is enabled by the application of diffusion models, similar to those used in image generation like DALL-E, for the generation of new proteins. These models can design protein backbones, or in this case antibody loops, whose structure and composition is likely to produce the desired functions. Tools such as RFdifusion have been developed for this purpose and have successfully produced de novo protein binders. If applied to antibody design, researchers may be able to forgo experimental screening approaches, and instead build a library with rationally designed antibody variants.The result could be a significant improvement in efficiency and productivity.

In a recent pre-print study, the Baker lab developed a novel, fine-tuned RFdiffusion model that is specifically trained to design de novo VHHs, an antibody-like molecule consisting of only one antibody chain instead of two. This method enabled the creation of completely novel VHHs with structurally accurate binding interfaces.

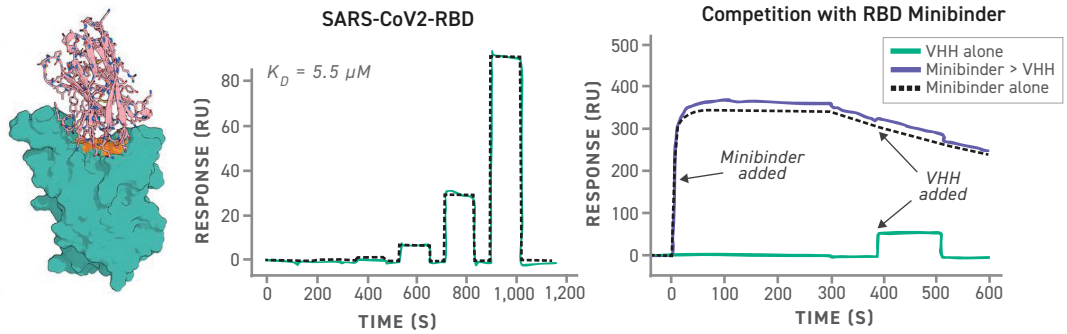

Figure 1. 9000 VHH designs were tested against SARS-CoV-2 receptor binding domain (RBD), and after soluble expression, SPR confirmed an affinity of 5.5μM to the target. Importantly, binding was to the expected epitope, confirmed by competition with a structurally confirmed de novo binder (AHB2, PDB: 7UHB).

Figure adapted from Baker et. al. (2025)

THE CHALLENGE OF BRIDGING AI-DRIVEN DESIGN AND EXPERIMENTAL VALIDATION

A key limitation of AI-designed VHHs is that they remain purely theoretical unless researchers can synthesize and experimentally validate them. For AI-designed antibodies, this requires producing large numbers of precise gene sequences that encode the computationally derived proteins. In a recent study (now published as a pre-print), the team needed to test roughly 36,000 VHH designs (9,000 for each target), a scale that would be unfeasible using traditional gene synthesis methods.

Until recently, DNA synthesis technology has struggled to accurately produce DNA fragments longer than 250bp. As VHH proteins tend to be 120 to 130 amino acids long, their underlying DNA had to be assembled from multiple DNA fragments, increasing the risk of errors and sequence dropouts. Additionally, assembling thousands of unique sequences individually is both costly and time-consuming, further limiting the scale of screening efforts. Lastly, researchers often have to skip DNA sequences containing repetitive elements or high GC content due to the difficulties of assembling these components.

Without a means to efficiently produce these sequences, AI-driven antibody design may remain an academic exercise rather than a practical tool for drug development.

PAIRING AI WITH TWIST'S MULTIPLEXED GENE FRAGMENTS

To overcome these challenges, the Baker lab utilized Twist's MGFs, a powerful DNA synthesis technology that enables the cost-effective and precise fabrication of thousands of gene fragments in parallel. Unlike traditional synthesis methods, which struggle with long and complex sequences, MGFs allow for the direct synthesis of up to 500 base pair sequences with high fidelity. This made it possible to encode thousands of entire VHH domains—including their crucial complementarity-determining regions (CDRs)—without compromising sequence integrity.

The ability to rapidly synthesize and test AI-designed sequences provided a crucial advantage. Rather than relying on a random selection process, the team could computationally design an entire screening library and directly synthesize every candidate for experimental validation. This eliminated the need for speculative screening and allowed for a more targeted and efficient approach to antibody discovery.

A NEW ERA IN VHH DESIGN

Using this AI and synthetic biology-driven workflow, the team successfully identified multiple VHHs that bound to four disease-relevant epitopes with high specificity. One of the most compelling validations came from cryo-electron microscopy (cryo-EM), which confirmed that a designed VHH bound to influenza hemagglutinin in a configuration nearly identical to the AI-predicted structure. This atomic-level accuracy underscores the transformative potential of AI-driven antibody design.

Additionally, cross-reactivity studies confirmed that the AI-designed VHHs were highly specific, binding only to their intended targets without off-target interactions. This level of precision is critical for therapeutic applications, where unintended interactions could lead to safety concerns.

Twist Multiplexed Gene Fragments made it possible to encode thousands of VHH domains without compromising sequence integrity.

THE PROMISE OF MGFS IN ANTIBODY ENGINEERING

The success of this study highlights the valuable role of Twist Bioscience's Multiplexed Gene Fragments in accelerating the transition from computational design to experimental validation. By providing precise, long synthetic DNA sequences in a multiplexed format, MGFs empower researchers to:

- Rapidly synthesize and screen thousands of AI-designed, de novo antibody variants, regardless of sequence complexity
- Build better ground-truth datasets for AI training, improving predictive accuracy
- Identify promising antibody candidates with greater efficiency

As AI-driven protein engineering continues to evolve, the need for scalable and accurate gene synthesis technologies will only grow. Twist's MGFs provide a critical link between computational models and real-world application, enabling the next generation of biologics to be developed with unprecedented speed and precision. For researchers working at the cutting edge of antibody discovery, MGFs offer an invaluable tool for translating innovative designs from concept to reality.

REFERENCE
Bennet, R. N. et al (2025) Atomically accurate de novo design of antibodies with RFdiffusion. BioRxiv. doi: https://doi.org/10.1101/2024.03.14.585103