

Customized Biothreat Detection Reagents: Computer Design of Proteins Recognizing Smallpox Variant *Vaccinia*

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Abstract

Background and potential impact to mission:

Mitigation strategies against bioterrorism critically depend on the accuracy, sensitivity, and robustness of the systems used to identify biothreats. Antibody-based approaches, one of the most prevalent classes of biothreat sensing devices used today, depend on time-consuming and expensive animal protocols for production. Once developed, deployment of these devices is hampered by the limitations of tissue culture scale-up and the "cold-chain" demands of distribution. Challenges such as antigen contamination, toxicity and immunodominance, along with labor-intensive screening requirements continue to compound the significant complications associated with antibody production.

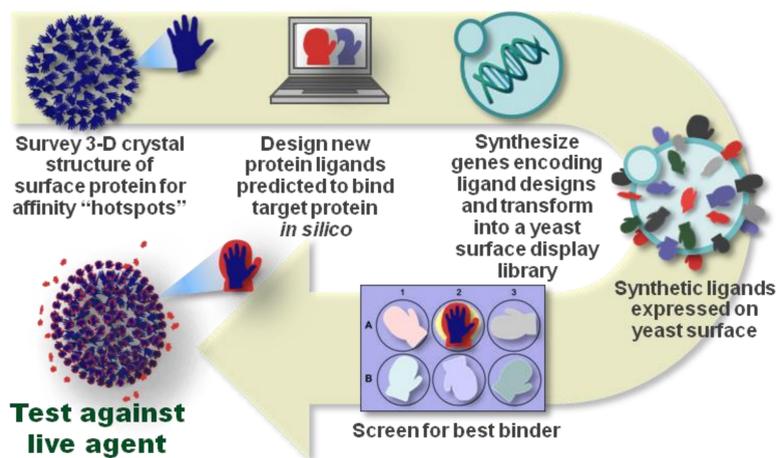
Our project transitions the DARPA-funded *in silico* protein folding algorithm, Rosetta, to design recognition reagents that can complement or replace antibody-derived reagents, yet are readily generated using industry standard, significantly cheaper and more rapid recombinant production methods. This technology platform provides a flexible means to design and manufacture multifunctional materials, *potentially providing detection reagents not otherwise available*. The synergy of computer-aided *de novo* design and recombinant protein production offers a highly flexible, easily deployed approach to create robust detection reagents at a fraction of the cost and time required for antibody development. Our multi-agency effort will offer robust alternative bioagent detection reagents that are compatible with existent antibody-based biosensors, next-generation point-of-care devices, and diagnostic platforms for use in austere environments.

Methods:

This proof-of-principle study targets the development of detection reagents against *Vaccinia* by recognizing the L1 protein, a component of the mature virion membrane that is conserved in all sequenced poxviruses. Protein sequences predicted to bind the L1R crystal structure were generated by Rosetta, then synthesized and expressed in a yeast display library to screen for binders using purified recombinant L1 protein as "bait." Candidates were sorted by flow cytometry based on expression and affinity for down-selection. Affinity maturation offers a path forward to optimize binding characteristics.

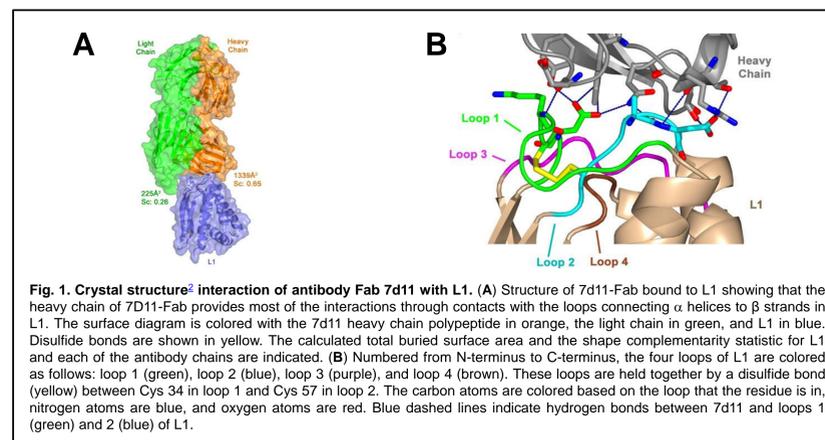
Preliminary results/conclusions:

Recombinant L1 protein has been successfully cloned, expressed and validated. All methods have been established and implemented. Screening of a new structure library, which was built leveraging results from preliminary binding studies, was screened against the recombinant L1R target. From these studies, a number of promising binding candidates were identified for further development such as affinity maturation.

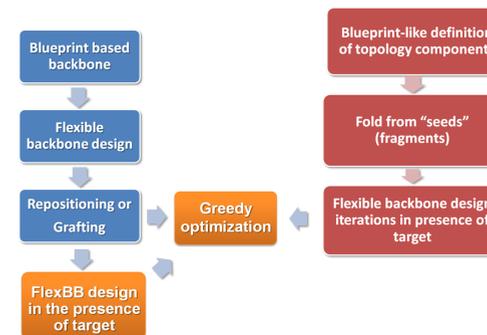


Background

L1 is a myristoylated, a highly conserved transmembrane protein found on the surface of the mature virion form of poxviruses, is essential for rendering the virus particle infectious¹. Several neutralizing antibodies against L1 have been identified that prevent cell entry. L1 contains 3 intramolecular disulfide bonds that are essential for infectivity, and is only recognized by these antibodies in its oxidized state. L1 has been co-crystallized with one of these neutralizing antibodies (7d11, PDB ID: 2I9L)², pointing out a crucial conformational epitope that is a target of high interest for novel recognition reagents. Interestingly, this structure demonstrates that the neutralizing antibody 7d11 uses predominantly heavy chain residues to bind its epitope, similar to other neutralizing antibodies that have been recently isolated to bind to envelope surface proteins such as hemagglutinin of Influenza and gp120 of HIV³.



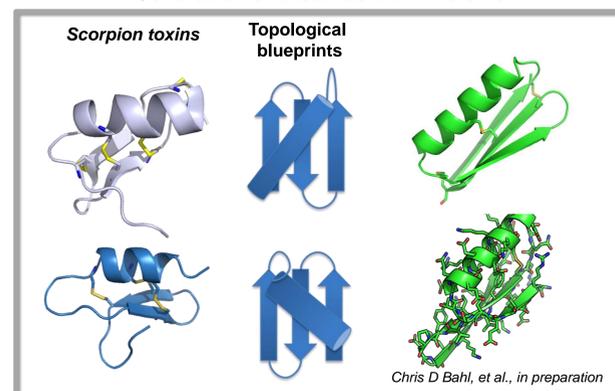
Re-Design with Flexible Backbone in the Presence of Target



7d11 uses residues of its complementarity determining region (CDR) to form a β -hairpin-like structure that wraps around Asp35 in the center of the L1 epitope (Fig. 1). It is extremely unfavorable to bury highly charged residues within a protein-protein interface unless each polar group is coordinated through a hydrogen bond or salt bridge. Therefore, various parts of the CDR loop of 7d11 were utilized to identify and design new recognition reagents, thus maintaining energetically valuable contacts to Asp35.

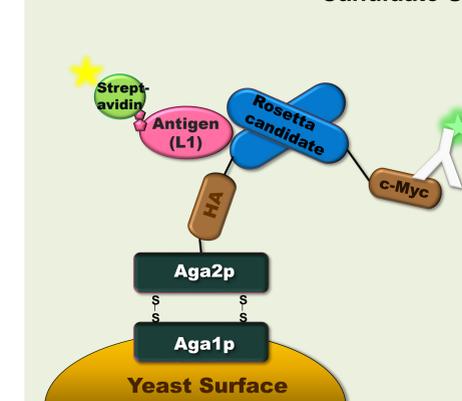
The original library of decoys developed as part of the first generation of L1 binders was used to source 18,000 backbones containing disulfide bonds with different topologies but still had the three sheets and one helix motif. This library was used to design an interface with L1, along with the redesign of the core of the starting decoys (blue path on the workflow above). Additionally, a second computational approach (the red path in the workflow) is concurrently being pursued which builds a mini-protein around the binding epitope taken from the neutralizing antibody 7d11.

Computer Modeling Generation of Disulfide Mini-Proteins



Similar to the Koga Baker *de novo* design approach⁴, permutations of mini-proteins were designed with a simple topology of three β -sheets and one α -helix, originally inspired by structures of scorpion toxins. These small proteins (less than 42 amino acids) are disulfide cross-linked and extremely stable. The Baker laboratory (Chris D. Bahl) pioneered this approach and has since structurally verified some of the computational designs made using Rosetta.

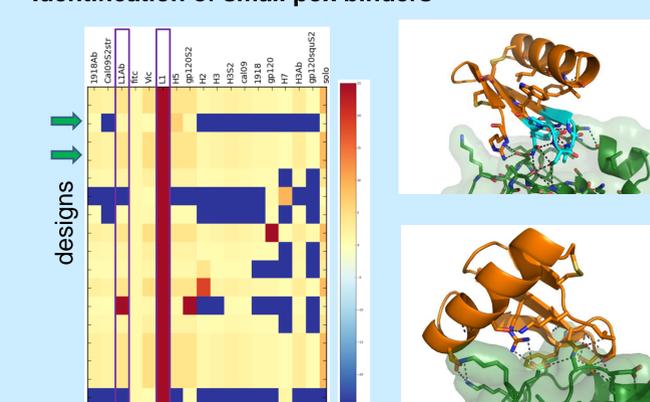
Candidate Screening



Since the disulfide linked mini-proteins are small, their genes can be synthesized as single strand by chip oligonucleotide synthesis. The mini-genes were amplified and linker regions were added that allow recombination into a yeast-surface display expression vector. Genes encoding the Rosetta designs were transformed as a pool into yeast cells and evaluated for binding as a pool. Cells were induced for expression, mixed with 1 μ M biotinylated L1 protein that was pre-incubated with the chromophore streptavidin-phycoerythrin (SAPE), washed and probed with fluorescein-labeled (FITC) anti-Myc antibody. These chromophores were used to interrogate surface-displayed Rosetta designs by flow cytometry, sorting cells based on their capability to retain either fluorophore conjugate. FITC-fluorescence reports expression levels via anti-Myc antibody binding to the c-Myc sequence tagging each Rosetta design, whereas SAPE-based fluorescence monitors binding through the L1-biotin-streptavidin interaction.

Cells were sorted once to collect the subset which expressed designs that displayed on the yeast surface, then sorted again for L1 binders based on increased SAPE signal. After selecting for binding, DNA from each pool was isolated and PCR-amplified to attach adapter sequences that would allow next generation sequencing of the barcodes. The frequency of a given gene found within a sample pool was tracked by the number of occurrences of its barcode. The propensity of a design to bind L1 is evaluated by its "enrichment value," defined as the ratio of a construct's surface display frequency versus the subpopulation that both displays and binds to SAPE. Several specific binders to L1 were identified. Two of these hits were chosen for optimization by mutagenesis and selections (green arrows at left, below).

Identification of small pox binders



The heat map above indicates the enrichment values for candidates selected during screening, using the proteins listed across the top as "bait." Blue indicates that the candidate is not represented in a given pool, yellow indicates neutral or depleted levels, and red indicates enrichment. Any score above 25 is shown as 25. All constructs shown bind L1 (purple box). "L1Ab" reflects enrichment with L1 which has been pre-incubated with antibody 7d11. Binding of a candidate to L1 alone, but not to L1Ab, is suggestive that the candidate binds on the same face as the 7d11 antibody. All other recombinant bait molecules were used as negative controls. Green arrows indicate two candidates moved forward for affinity maturation. Rosetta-calculated structures (above right) show predicted binding modes of each of these two designs (orange) to L1 (green), with the 7d11 binding interface shown in cyan (top).

References

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