

Finding an Antibody Mimetic Peptide to HCV's Nucleocapsid Protein

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Abstract

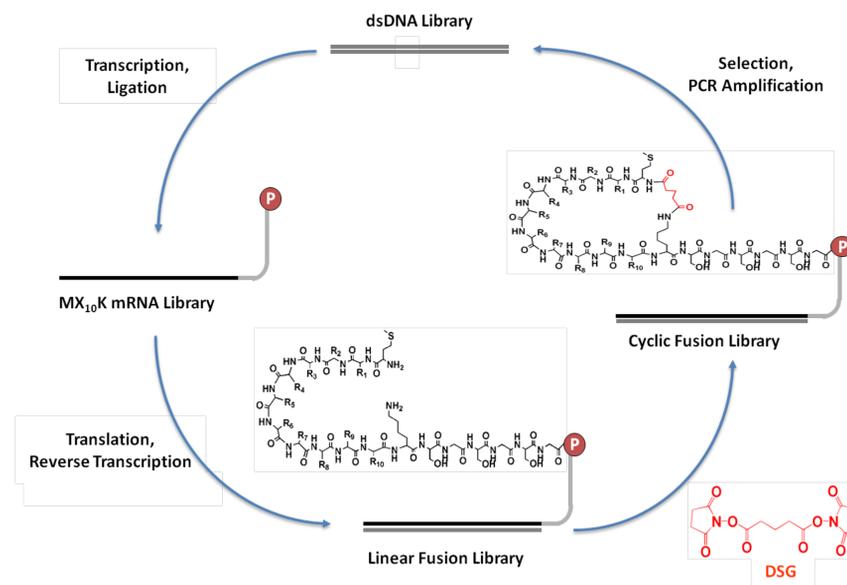
The Hepatitis C Virus (HCV) is one of the most common blood-borne infectious agents in the United States and there are currently no effective treatments available. Current research has used mRNA display to generate an enriched pool of Peptides which specifically bind the peptide target with low affinity. From this pool, a top peptide binder was selected and the amino acid sequence modified with the goal of enhancing its binding ability. The effects of various modifications on the peptide's binding were measured and the best sequence was sent away to a collaborator for in vivo testing.

Introduction

The project's goal is the discovery and improvement of peptides that bind the nucleocapsid protein of the virus, which may be useful for treatment or diagnosis of HCV. The nucleocapsid protein was chosen as a target for mRNA display due to the high amino acid conservation between the different genotypes of the disease (greater than 90%). Since the expression of the nucleocapsid protein itself is very difficult, amino acids 16-40 of the protein were chosen as the target for selection. The peptide was made synthetically and was immobilized on either agarose or acrylamide beads using hydrazide chemistry.

The pool of sequences was characterized and the top binder chosen. Based on the sequences obtained, there appeared to be some evidence of helical structural motifs in the enriched pool. Modifications were designed to test this hypothesis and potentially enhance binding affinity.

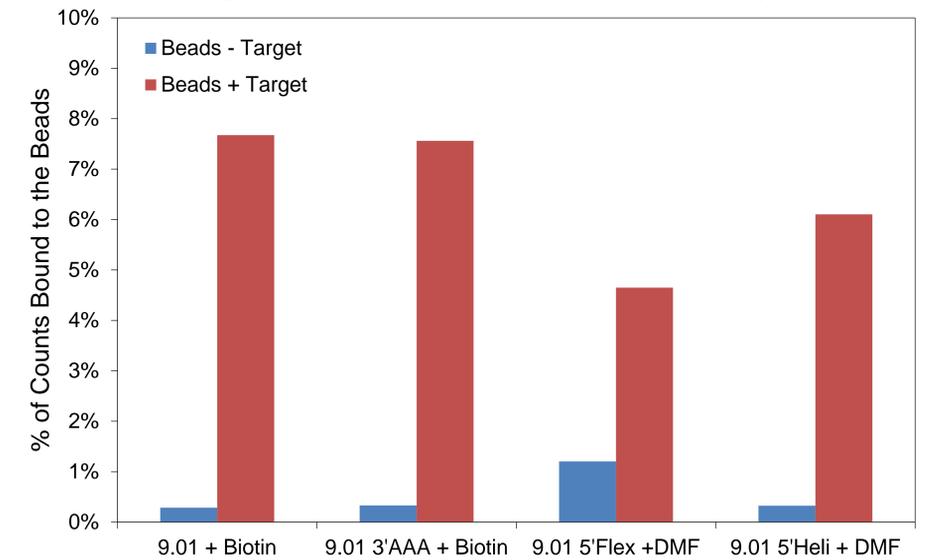
In Vitro Selection using mRNA display



In vitro selection cycle with mRNA display. A double stranded DNA library (top) is generated from synthetic oligonucleotides. Transcription and ligation of a puromycin linker (P) results in the mRNA library (left). Translation and reverse transcription leads to the formation of a cDNA/mRNA linear fusion library (bottom). Addition of DSG will result in a cyclic fusion library (right). This product is then enriched for functional proteins using affinity chromatography.

Binding Data

Binding of 3' and 5' Modified versions of 9.01 Sequence



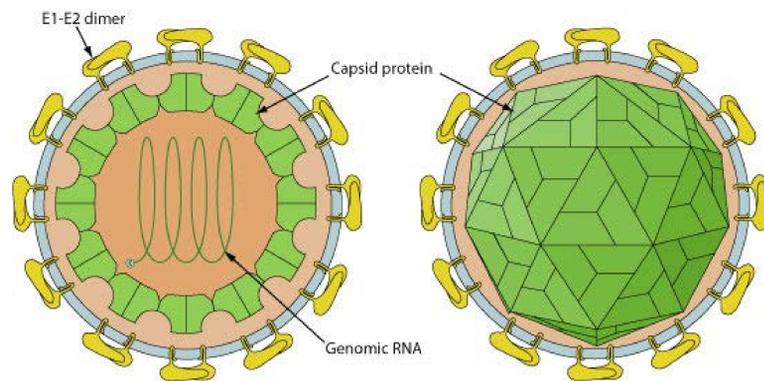
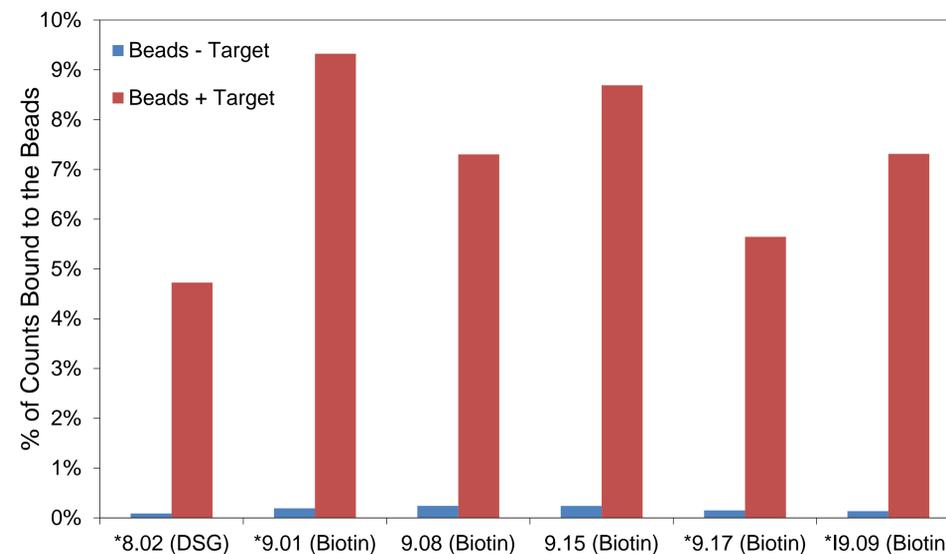
Results and Conclusions

Clone 9.01 was chosen for modification as it showed the overall highest binding to the target. Modifications were made at both the 3' (C terminal) and 5' (N terminal) end and compared to a biotinylated version of the original sequence, which had previously demonstrated the maximum binding. All of the C terminal modifications made expression of the peptide (which is necessary to measure binding) difficult, so only one modification, AAA, was successfully measured. The AAA-modified peptide showed binding comparable to biotinylated 9.01 but the minor change in binding ability was not useful given the increased difficulty of expression.

A helical and flexible linker were added at the N terminal end. The flexible linker consistently decreased binding compared to the original sequence. The helical linker had slightly lower binding ability than the biotinylated 9.01, but was higher than the non-biotinylated form.

While none of the modifications significantly improved the peptide's binding ability, this data provided other useful information about the project. It confirmed previous inferences about the helical nature of the original peptide—the flexible linker destabilized the binding while the helical linker stabilized it. The peptide has now been sent away for in vivo testing including the helical linker. This is useful because the cells in which the peptide will be expressed cannot perform post-translational modifications like biotinylation. Therefore, the binding of the helical-modified peptide will be comparable to the biotinylated form but does not require biotinylation.

Binding of Clones to Hydrazide HCV Core Peptide Beads



Schematic of Hepatitis C Virus