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High throughput ligand binding mapping of HCV protease mutations using phage display system

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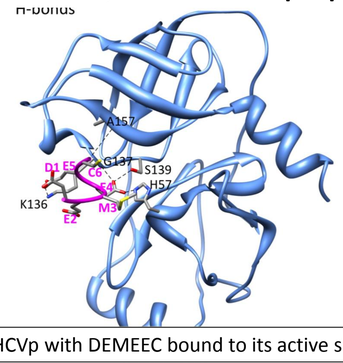
INTRODUCTION

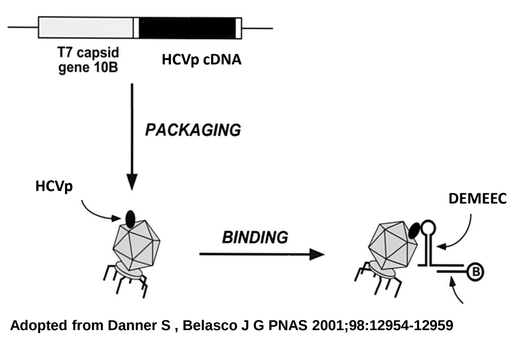
To study molecular recognition between protein and ligand, high throughput mutants to ligand binding mapping system was developed by utilizing T7 phage display system. In this study, mutations in active site of Hepatitis C virus protease (HCVp) was mapped against one of known HCVp inhibitors, DEMEEC peptide.

HCV protease mediates the cleavage of the HCV polyprotein to release the functional proteins that are essential for viral propagation. The inhibition of this protease activity is expected to block HCV replication in infected host cells.



HCVp with DEMEEC bound to its active site

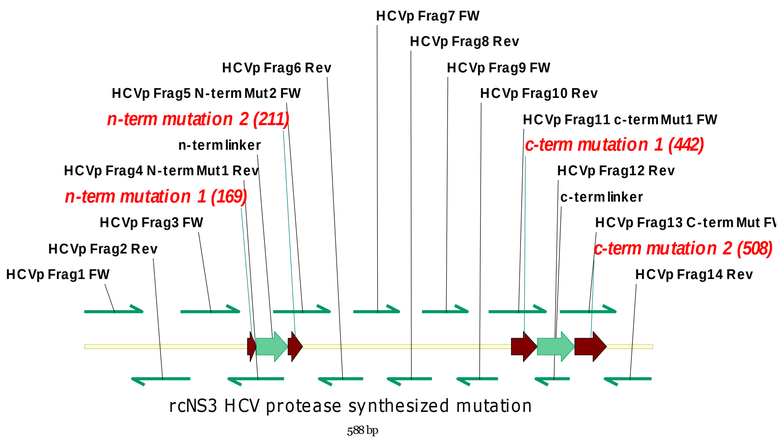
T7 Phage display system is a method for the study of protein–protein, protein–peptide, and protein–DNA interactions that uses bateriophage, T7 to connect proteins with the genetic information that incodes the proteins. In this study, Wild type or mutated HCVp cDNA is inserted into a T7 cloning vector and packaged in a phage capsid to generate a recombinant phage in which the HCVp is displayed on the surface as a carboxyl-terminal fusion to the T7 capsid protein. The resulting phage is allowed to bind to its ligand such as DEMEEC peptide.

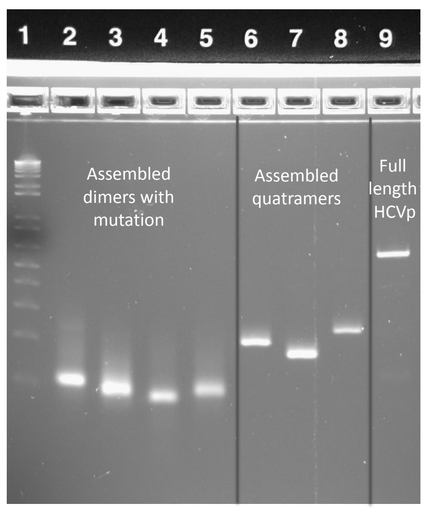


Adopted from Danner S , Belasco J G PNAS 2001;98:12954-12959

APPROACHES AND RESULTS

Mutation generation in HCVp gene by assembling HCVp DNA fragments with mutated DNA oligos

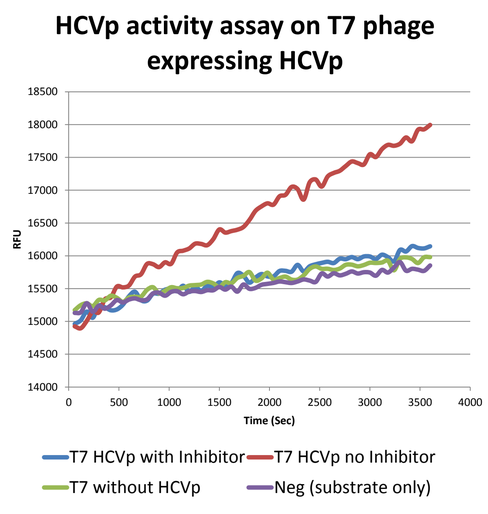




DNA fragments containing targeted 2 random mutations in HCVp active site were synthesized and incorporated by stepwise gene assembly (dimer → quatramer → full length).

APPROACHES AND RESULTS

Mutant library generation using T7 phage and activity assay of HCVp displayed on T7 phage



A library of phages displaying mutated HCVp (<= 1 protein copy per phage) was created by cloning the assembled HCVp genes into T7 phage vector followed by packaging and amplification of the phage particles. HCVp activity assay using the phage library showed that the protease displaying on the phages has activity.

Attachment of DEMEEC to Fractogel to generate affinity column for HCVp

DEMEEC peptide was attached to Epoxy activated Fractogel via amine coupling and the coupling efficiency was determined by BCA assay. The DEMEEC conjugated Fractogel was then packed into FPLC column for affinity chromatography.

Work needs to be done: The mutant library will be sorted out based on affinities to immobilized DEMEEC, selecting weak, medium and strong binders by step gradient affinity FPLC. Next generation sequencing of each fraction will be performed to determine which mutation effects binding affinity to DEMEEC. These sequence analysis of mutants will allow to identify key residues in interaction with DEMEEC.

CONCLUSION

This system allows to simultaneously identify large number of mutants with their ligand binding selectivity, leading to predict new ligands and mutant protein structures with binding selectivity.

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