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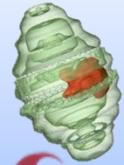
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Modify the "Vault" for a Better Drug Delivery Capsule

The "Vault" Can Be a Good Drug Delivery Capsule

The vault is a protein naturally occurring in most eukaryotic-cell organisms, except for fruit fly, a type of worm and yeast. It is most frequently suggested with some transportation function within the cell. The key components in the vault we are manipulating are the major vault proteins (MVPs) (green) and the minimal interacting domain (mINT) (red). Naturally the vault can disassemble under a pH lower than 4.

The vault can be a good drug delivery capsule, due to its large capacity and bio-compatibility. Therapeutic molecules can be fused to mINT and easily enclosed into the vault shell.



Modification Is Needed for Proper Drug Release

The mINT-fused drug molecule needs to be released from the vault shell (96 copies of MVPs) to carry out its mission on the targeted site within a cell. During its entrance into the cell (endocytosis), a vesicle (the lysosome) can provide a low-pH environment (pH ≈ 5). In addition, acidic environment is characteristic of cancer tissue. Thus we want to weaken the interaction between the MVP and mINT by introducing clusters of histidine residues at critical sites so that they dissociate at the lysosomal pH of 5. Histidine is positively charged at pH < 6 while remains uncharged at pH 7. This characteristic is hypothesized to result in repulsion between the MVP and mINT at pH 5.

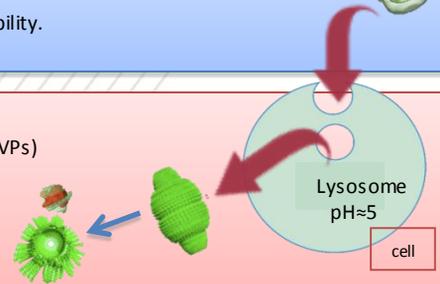


Figure 1: Schematic drawing of the vault as a drug delivery capsule showing the vault entering the cell through endocytosis and disassembled in lysosome, releasing the drug molecule

Modification of the Protein Was Done by Site-directed Mutagenesis The Mutated Protein Was Produced by Bacteria

The major vault protein (MVP) was modified through mutating its encoding DNA sequence.

The simplified *site-directed mutagenesis* procedure is shown in Figure 2 on the left.

Based on the wild-type DNA sequence, we designed a primer with the desired mutation;

Then the mutated primer was extended into a full sequence with Polymerase Chain Reaction (PCR). The sequencing results below showed the correct mutations we have done for MVP (Figure 3).

E. coli DH5α was transformed with plasmids containing the mutated genes for storage..

Having got the desired mutation, *E. coli* (BL21) was used to produce the protein by expressing these mutated genes.

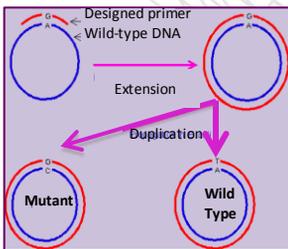
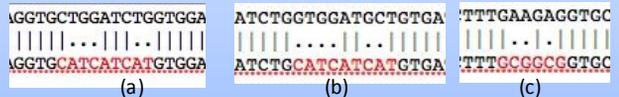


Figure 2 (above): Site-directed Mutagenesis

Figure 3 (right): Sequence alignment showing the mutated fragments
Top row: wild-type AA sequence; bottom row: mutated sequence
(a) LDL → HHH (b) VDA → HHH (c) EE → AA
Histidine (H) is (+)charged at pH < 6; Alanine (A) is small non-polar AA



Result and Future Work

The protein production was done at different conditions (SDS-PAGE gel in Fig. 4) and was optimized at 20° C with 0.1 mM IPTG induction at OD600 0.6-0.8.

After production, the protein was purified by HisTrap FPLC, an affinity liquid chromatography that traps the His-tagged protein and eludes it lastly in the Presence of 500mM imidazole.

The protein produced in the first round degraded after an overnight storage, due to improper dialysis in a salt solution.

The study of the affinity between two types of protein require intact structures. Hence more rounds of protein production need to be done.

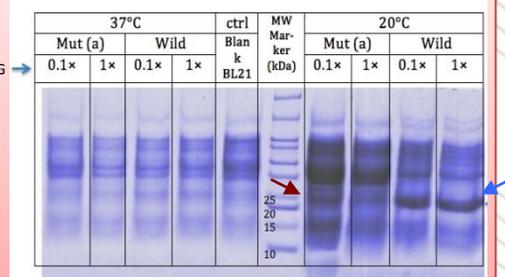


Figure 4 (Right): SDS-PAGE gel of the protein produced at different conditions, as indicated on top of the gel picture. Bacteria cells with no target genes were cultured as a negative control, to rule out the undesired proteins. Our target protein, MVP domains 345, has a molecular weight of around 21 kDa. The wild-type is pointed out by the small blue arrow and the mutant by the red arrow. The production of the mutated protein was not as effective as the wild-type.