Exploiting the Vault Nanoparticle as a Tool for siRNA Delivery

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Abstract:

Thanks to their molecular mass of 13 MDa and size of 72.5 × 41 × 41 nm, vaults are the largest cytosolic eukaryotic ribonucleoprotein particles ever described. The vault nanoparticle consists of 78 copies of the 99-kDa major vault protein, which assemble to generate two cup-shaped symmetrical halves.

This peculiar structure makes vault suitable for the delivery of different types of molecules, i.e. drugs, proteins and nucleic acids. This is also supported by its ability to encapsulate cargo molecules by fusing them to the INT peptide (a vault-targeting domain from the vPARP protein, which tightly binds to the inner surface of the nanoparticle). Here, we have explored the encapsulation of the cargo model protein GFP-INT into the inner cavity of the vault particle.

Firstly, we expressed the His_6-tagged GFP-INT in M15 *E. coli* strain and purified through IMAC. We next performed the conjugation of the cargo protein with a crosslinker and determined the binding stoichiometry and stability of the final complex with vault.

We are now exploring the possibility of using vault as a tool for siRNA delivery. The strategy consists in GFP-INT crosslinking via its amino groups with the sulfo-LC-SPDP crosslinker. Next, a modified siRNA carrying a sulfhydryl group will be directly reacted with the 2-pyridyldithiol group of the sulfo-LC-SPDP crosslinked with GFP-INT.

Our attempts are aimed at loading vault with different siRNAs to evaluate their silencing activity against different pathological traits of tumor cell lines.