Development and characterization of vault-based nanocarriers in *Pichia pastoris*

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Abstract:

Vault is the largest known ribonucleoprotein particle, naturally occurring in higher eukaryotic cells[1,2]. It is involved in several cellular functions. Many of its features, such as its non-immunogenic structure and its huge internal cavity, make it a suitable nanovector for drug delivery[3]. Recombinant vaults are produced by expression of the major vault protein, occurring in 78 copies that assemble into a barrel-like "nanocapsule".

Baculovirus-insect expression is the most used system for recombinant vault synthesis. However, it suffers from low scalability and slow production rates. Thus, the yeast *Pichia pastoris* (reported to enable vault expression at lower costs and in higher yields[4]) has been chosen to constitutively express human recombinant vaults. Recombinant vaults from *Pichia,* purified by size exclusion chromatography, display the same morphology and size of authentic vault as shown by transmission electron microscopy, dynamic light scattering and nanoparticle tracking analysis.

Vault engineered variants containing a Cys-rich stabilizing domain or carrying a membrane-lytic peptide enhancing endosomal escape have been constructed. Their characterization and the construction of a variant allowing antibody-direct conjugation is in progress. Future steps, involving vault-mediated targeting of active molecules to specific cancer cell lines, in particular melanoma cell lines, will be conducted at the *Université de Paris* in the framework of a *cotutelle* agreement.

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