13th PhD meeting 2021
September 21-23, 2021
University of Milan Bicocca

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

Giulia Tomaino*[1], Marco Vanoni[1,2], Paolo Tortora[1], Gianni Frascotti[1]

*g.tomaino3@campus.unimib.it
https://campus-unimib.webex.com/meet/g.tomaino3

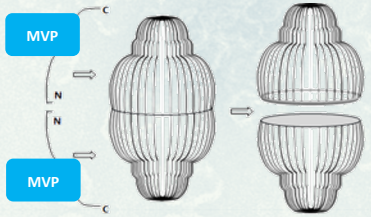
1 Department of Biotechnology and Biosciences, University of Milano-Bicocca, Italy
2 SYSBIO – Centre of Systems Biology, Italy

Supported by CHRONOS project

Introduction - VAULT NANOPARTICLES

Vault is the largest **RIBONUCLEOPROTEIN PARTICLE** known,
- Found in nearly all **higher eukaryotic** cells (10^4 to 10^5 particles per cell in human cell)[1]
- Involved in **a broad range of cellular functions**: nuclear-cytoplasmic transport, innate immunity, MDR[2]

MW: **13 MDa**
Dimension: **72.5 × 41 × 41 nm**



Mrazek, J., et al., 2014

78 copies of **Major Vault Protein** (**MVP**) 96 kDa generate an **ovoidal, hollow, barrel-like "nanocapsule"** formed by two symmetrical halves

Other components (< 25% of the total protein mass):
- ~ 8 copies of **vPARP** (vault poly-ADP ribose polymerase; 193 kDa)
- ~ 2 copies of **TEP1** (telomerase associated protein; 290 kDa)
- 1-6 untranslated **RNAs** (88-141 bases)

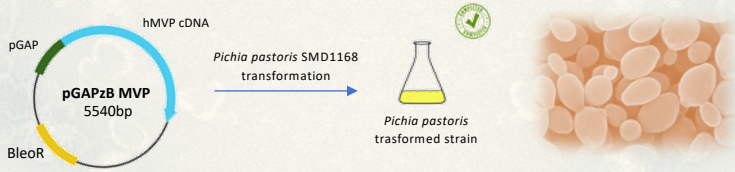
Main features
- **Dynamic structure**
- Large internal cavity (5 × 10^4 nm³)
- **Non-immunogenic**, **non-toxic**
- It can be **chemically and genetically modified**

→ Recombinant vault as an ideal **tool** for the targeted delivery of therapeutic molecules

VAULT PRODUCTION

Previously Expression of MVP sequence in **insect cells (**baculovirus-insect expression system)
→ Low scalability and slow production rates

In this project Human recombinant vaults has been constitutively expressed in the methylotrophic yeast ***Pichia pastoris***, previously reported to enable vault expression at lower cost and in higher yields[3]



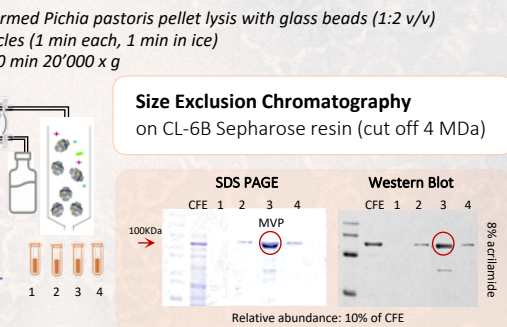
VAULT PURIFICATION & CHARACTERIZATION

- *MVP-transformed Pichia pastoris pellet lysis with glass beads (1:2 v/v)*
- *Vortex 10 cycles (1 min each, 1 min in ice)*
- *Centrifuge 20 min 20'000 x g*

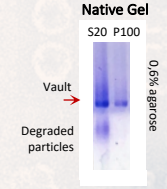
Cell free extract (CFE)

Inlet pump, linear flow rate 30 cm/h

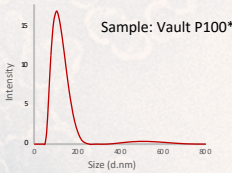
Size Exclusion Chromatography
on CL-6B Sepharose resin (cut off 4 MDa)



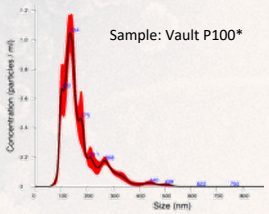
- *Centrifuge 20 min 20'000 x g*
→ discard pelleted aggregates (P20), keep the supernatant (S20)
- *Ultracentrifugation 2 h 100'000 x g*
→ pellet folded vault (P100), discard S100



Dynamic Light Scattering

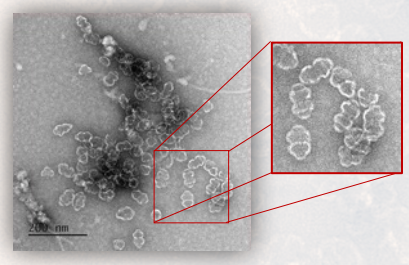


Nanoparticle Tracking Analysis



* mean of three technical replicates

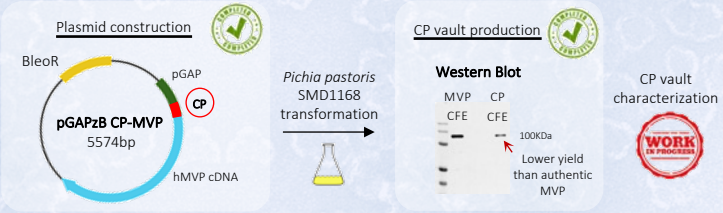
Transmission Electron Microscopy (TEM)



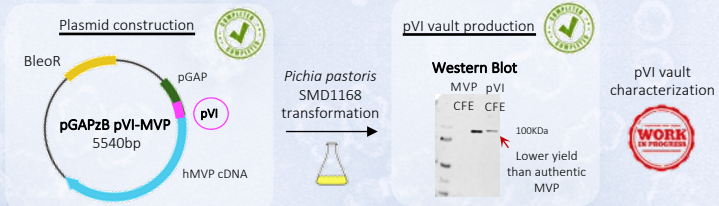
Recombinant vaults from *Pichia pastoris* show the **same morphology and size of authentic vault**

VAULT ENGINEERED VARIANTS

12 aa Cys-rich stabilizing domain (CP) fused at MVP N-terminus

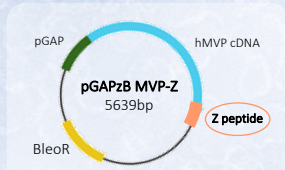


pVI-derived membrane-lytic peptide promoting endosomal escape fused at MVP N-terminus



Vault engineered variant construction in progress:

Vault variant carrying **Protein A derived Z33 peptide** allowing vault-antibody direct conjugation fused at MVP C-terminus



Work in progress & Future perspectives
- Vault variants characterization
- Vault-mediated targeting of active molecules to specific cancer cell lines e. g. **nucleic acid**
Cotutelle with *Université de Paris*
- Vault receptor identification

References: [1] Kedersha, N. L., & Rome, L. H. 1986, *The Journal of cell biology*, *103*(3), 699–709 . [2]Frascotti, G. et al.; 2021. *Cancers*, *13*(4), 707. [3]Wang, M., et al., 2018, *Biotechnology and bioengineering*, *115*(12), 2941–2950.