Harnessing Yeast-Derived VLPs for Enzyme Encapsulation and Biocatalysis

Felix Fronek*[,1], Adrian Köber[1], Matthias Schittmayer-Schantl[2], Ruth Birner-Grünberger[2], Matthias G. Steiger[1]

1) Institute of Chemical, Environmental and Bioscience Engineering, Research Group for Biochemistry, TU Wien, Gumpendorfer Straße 1a, 1060, Vienna, Austria,
2) Institute of Chemical Technologies and Analytics, Research Group Bioanalytics, TU Wien, Getreidemarkt 9, 1060, Vienna, Austria
*presenting author, E-mail: felix.fronek@tuwien.ac.at

BACKGROUND

Subcellular compartmentalization enables eukaryotic cells to carry out complex, multistep, or energetically unfavorable reactions by preventing side reactions and creating chemical gradients. Enveloped virus-like particles (VLPs) contain a lipid bilayer in addition to their protein shell, resulting in a separate internal reaction volume. This feature can be utilized to mimic subcellular compartmentalization of eukaryotic cells.

By further functionalizing these particles with specific transporters, as well as targeted loading with proteins of interest by post-translational fusion with an improved SpyTag/SpyCatcher system[1], we aim to generate **artificial liposomal particles** (**ALPs, Figure 1**). Here we show that we can produce VLPs in *Saccharomyces cerevisiae* by overexpressing the HIV-1 Gag protein[2], load them with target proteins such as lactate dehydrogenase (LDH) and sGFP, and finally purify and concentrate these VLPs by density gradient ultracentrifugation.

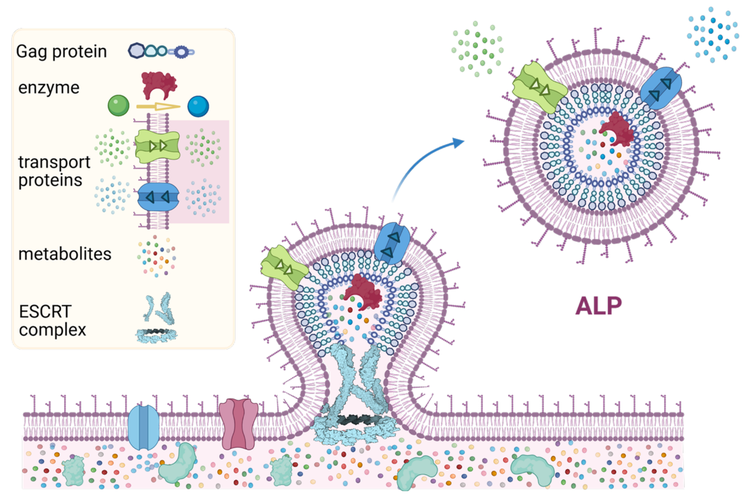

Figure 1: Schematic view of the budding process of an ALP from the *Saccharomyces cerevisiae* plasma membrane.

METHODS & STRAIN GENERATION

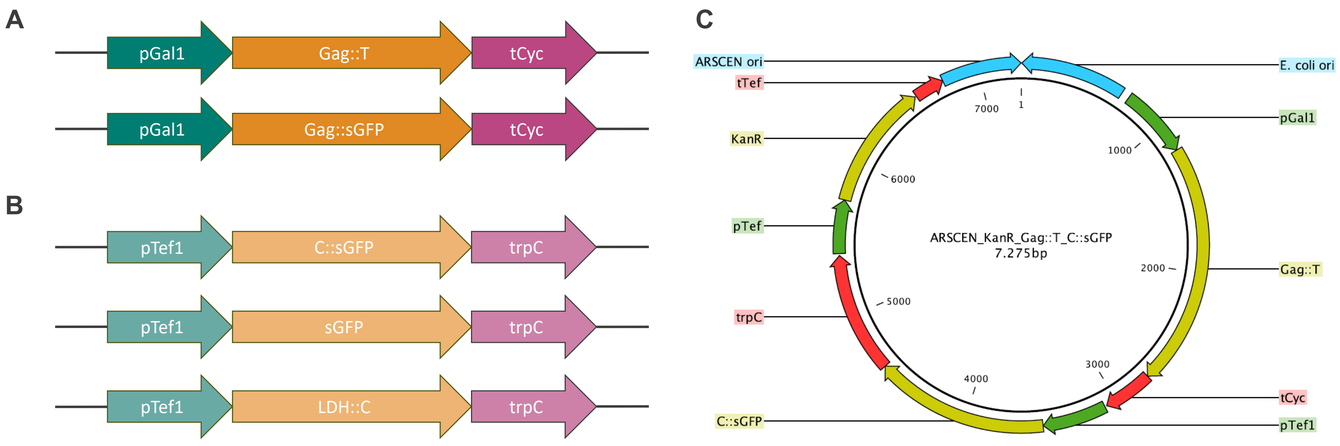

Figure 2: Expression cassettes and plasmids used for *S. cerevisiae* strain generation. **(A)** Expression cassettes for Gag, fused to SpyTag (T) or sGFP, were used with the inducible *gal1* promoter. **(B)** LDH and sGFP with or without SpyoIPD (C) were expressed under the constitutive *tef1* promoter. **(C)** The final plasmids used for ALP production contained the ARS/CEN origin of replication and a kanamycine resistance selection marker.

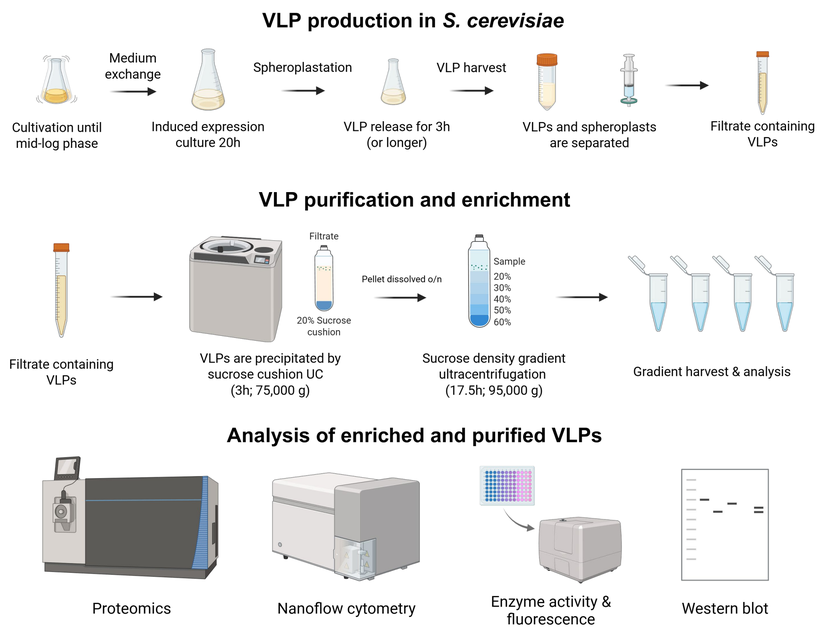

Figure 3: Production and purification process of Gag HIV-1 VLPs in *Saccharomyces cerevisiae,* as well as the performed analyses.

RESULTS

WESTERN BLOT & LDH ACTIVITY

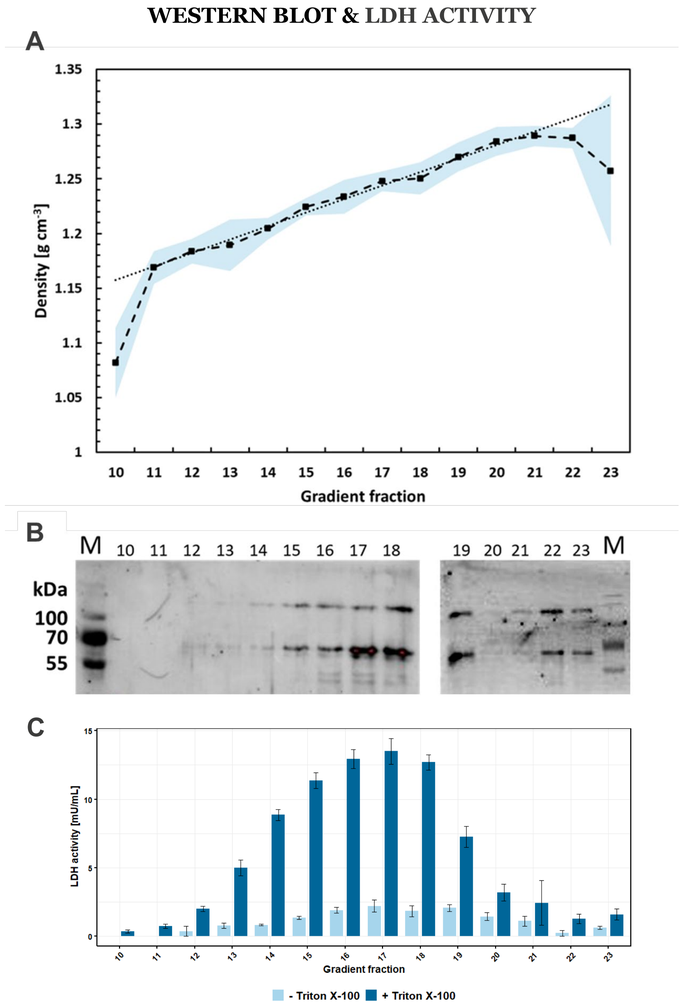

Figure 4: **(A)** Mean density of each fraction from the sucrose density gradient ultracentrifugation (n = 7). **(B)** Western blot results using Gag HIV-1 specific antibodies. The numbers shown represent the density gradient fractions. The strain shown here expresses Gag::sGFP. **(C)** Result of the LDH activity assay with and without the addition of Triton X-100. The numbers represent the density gradient fractions, and the strain shown expresses Gag::T and LDH::C.

FLOW CYTOMETRY

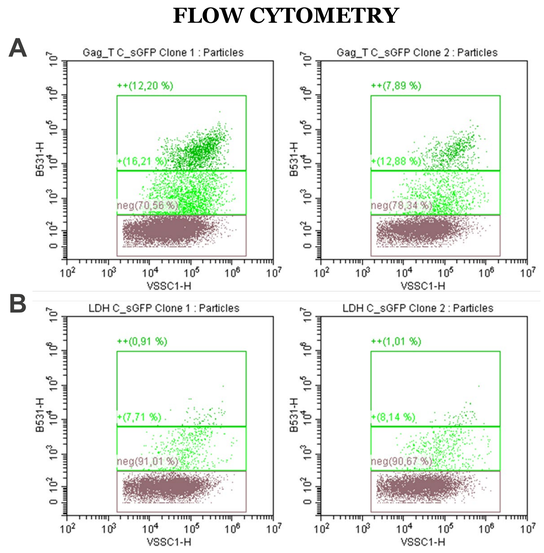

Figure 5: Flow cytometer data of the sGFP fluorescence (B531-H) over the side scatter (VSSC1-H) confirms targeted loading of the ALPs. **(A)** Purified and enriched particle fractions produced by the Gag::T C::sGFP strain, biological duplicates. **(B)** Purified and enriched particle fractions produced by the control strain, expressing no Gag, biological duplicates.

PROTEOMICS

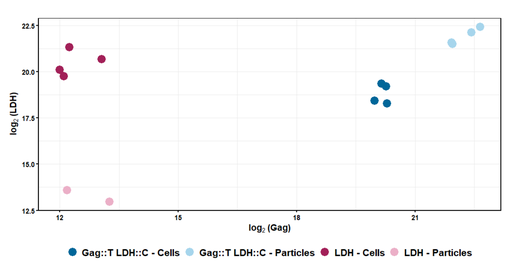

Figure 6: Protein intensities of Gag and LDH from the proteomics data set revealed that Gag::T and LDH::C were enriched in VLP-containing fractions relative to the cells, while the control strain lacking Gag showed minimal LDH in these enriched fractions.

CONCLUSION

We demonstrated that we can generate functionalized VLPs, here named **ALPs,** loaded with target proteins, including the enzyme LDH, in a functional state.

Purification and enrichment of these ALPs by density gradient ultracentrifugation were confirmed by Western blot and LDH activity measurements (**Figure 4**).

In addition, flow cytometry (**Figure 5**) and protein intensity of the proteomics data set (**Figure 6**) confirmed the loading of the ALPs with target proteins. Furthermore, the proteomics data set revealed a general enrichment of membrane proteins in the VLP-containing fractions compared to the cells.

ACKNOWLEDGEMENTS

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REFERENCES

[1] Hinrichsen et al. A new method for post-translationally labeling proteins in live cells for fluorescence imaging and tracking. Protein Eng Des Sel. 2017 Dec 1;30(12):771-780.

[2] Sakuragi et al. HIV type 1 Gag virus-like particle budding from spheroplasts of Saccharomyces cerevisiae. Proc Natl Acad Sci U S A. 2002 Jun 11;99(12):7956-61