MOL231: **Identification of the stable Mlp1 interaction site at aged nuclear pore complexes of budding yeast**

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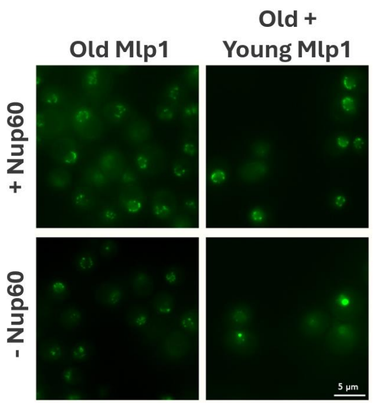
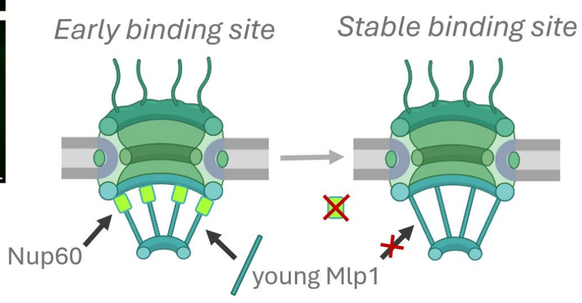
Background

The **nuclear pore complexes** (**NPCs**) conduct cellular communication between the nucleus and cytoplasm. Being one of the largest protein complexes the NPCs consist of multiple copies of ~30 different proteins, **nucleoporins** (**Nups**), that assemble into a pore-structure made of eight "spokes". In budding yeast (*S. cerevisiae*) the NPC can exist with or without a **nuclear basket**, whose main structural components are **Mlp1 and Mlp2.** Interestingly Mlp1 assembles into the NPC extremely late [3] which depends on another nucleoporin Nup60 akin to an assembly factor. **Mlp1 binds to an unknown stable binding site at the old NPC** which is no longer dependent on the presence of Nup60 [1]. This project aimed at identifying stable Mlp1 binding site at the NPC.



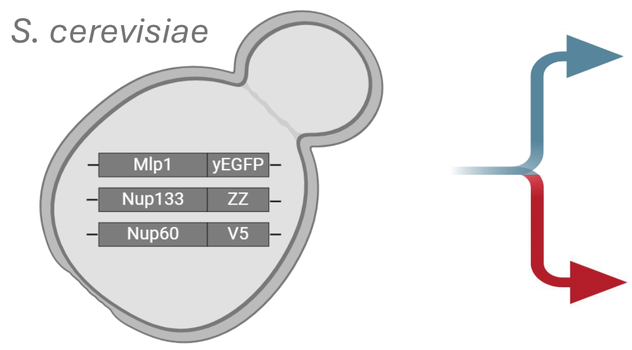
Onishchenko E. and Hamilton A. (personal communication)

Aim: to identify stable binding site of Mlp1 at the nuclear pore complex.

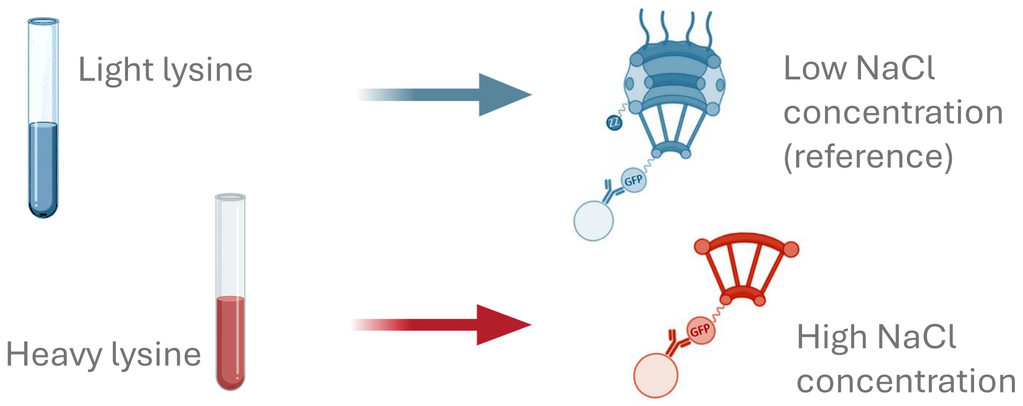


Experimental approach

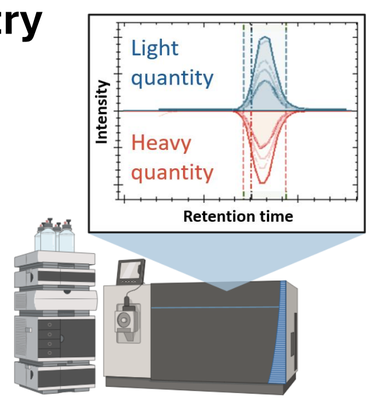
1 Epitope tagging of Mlp1 and reference nucleoporins



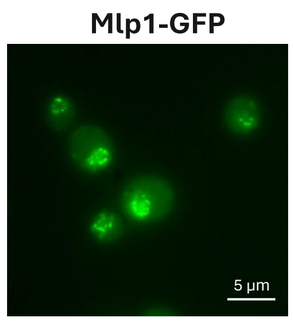
2 Affinity enrichment for stably Mlp1 associated nucleoporins

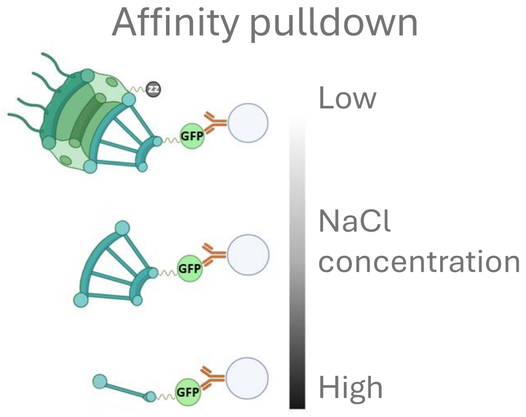


3 Analysis of relative nucleoporin enrichment by quantitative mass spectrometry

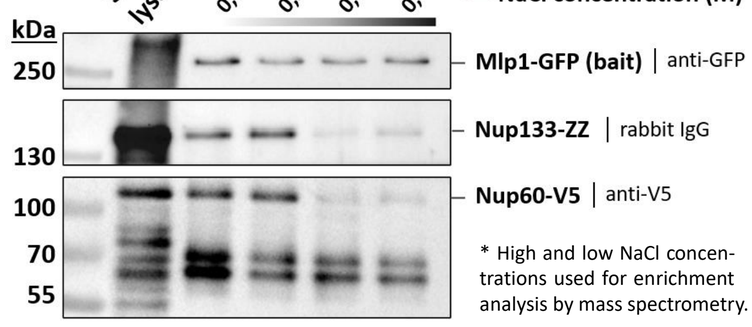


Results





Reference NUPs (Nup133 and Nup60) were used to determine stringency of affinity pull-down conditions before mass spectrometry analysis. The different stringencies were achieved via increasing concentration of NaCl in lysis and wash buffers in the affinity pulldowns with GFP-tagged Mlp1 as affinity bait.



* High and low NaCl concentrations used for enrichment analysis by mass spectrometry.

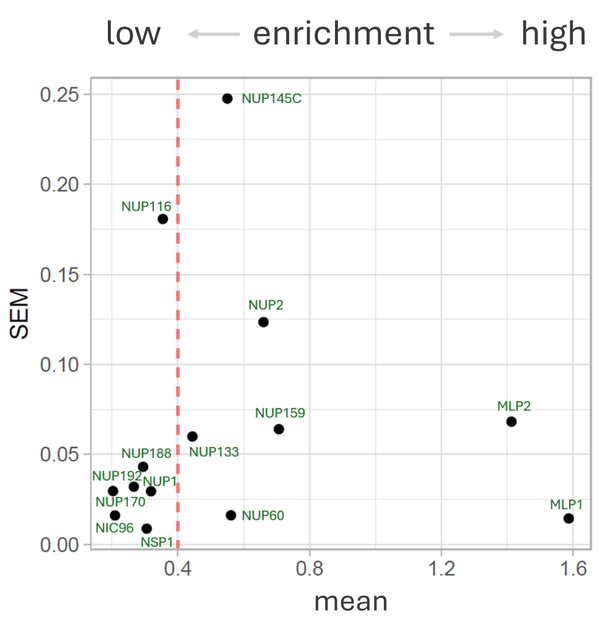


Figure 1: *NUP enrichments were quantified based on heavy and light lysine-labelled NUPs intensity ratios and plotted against the standard error of the mean (SEM) after quantification of respective signal intensities by mass spectrometry.*

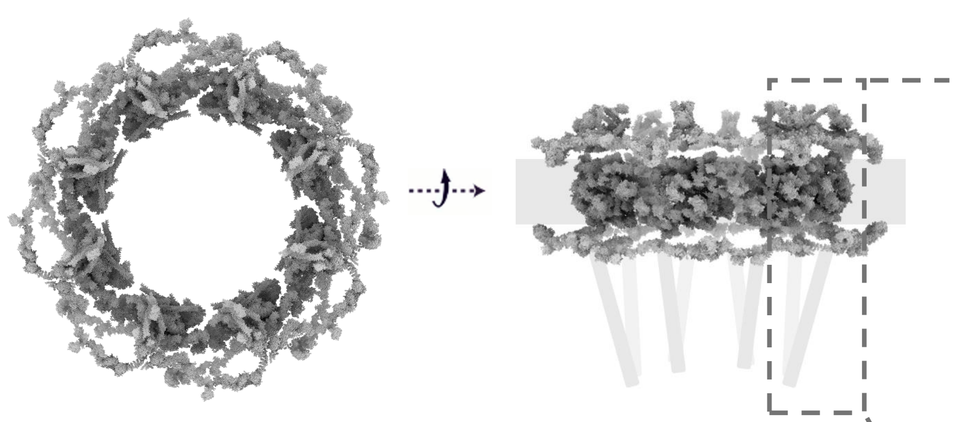


Figure 2: *3D architecture of the yeast nuclear pore complex (PDB: 7TBI) [2].*

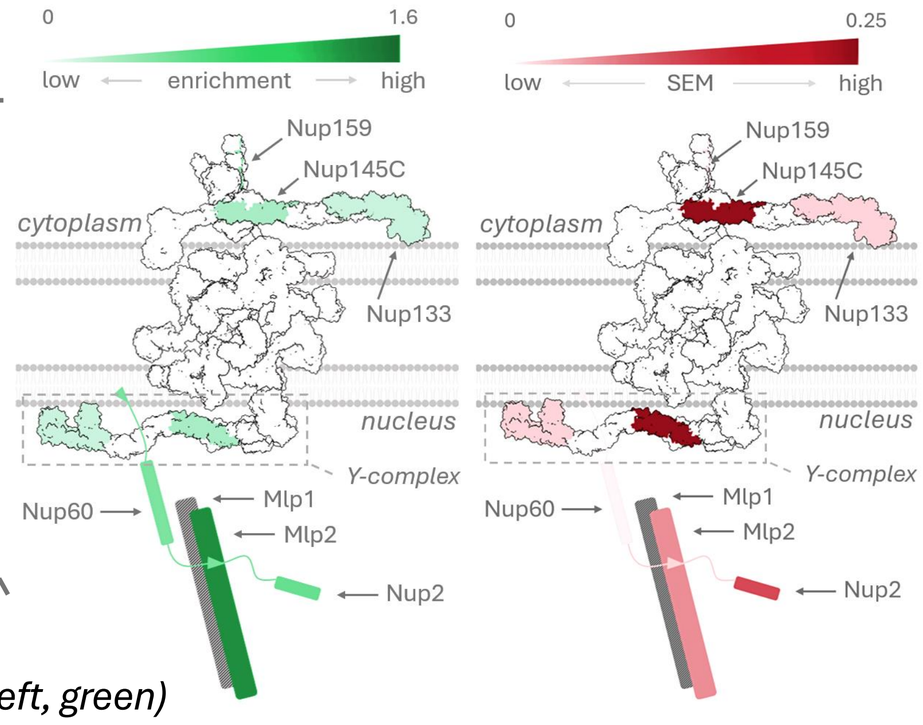


Figure 3: *Enrichment (left, green) and standard error of the mean (SEM) (right, red) for NUPs with enrichment level ≥ 0.4 projected as heat-map on the structure of single NPC spoke.*

Conclusions:

- Nuclear basket components Nup2, Nup60 and homo/hetero-oligodimerization between Mlp1 and Mlp2 are primary candidates for stable Mlp1 binding platform at the NPC.
- Nucleoporins of the Y-complex might be involved in the stable binding of Mlp1.

Perspectives:

- A genetic approach can be used to test the impact of identified Mlp1 interactions for its stable NPC association.
- A more precise enrichment-map is needed for better identification of the interactors at the stable binding site of Mlp1.

References:

1. Zsok, J., Simon, F., Bayrak, G., Isaki, L., Kerff, N., Wolstenholme, A., Weiss, L. E., & Dultz, E. (2023). *The nuclear basket regulates distribution and mobility of nuclear pore complexes in budding yeast*. Cold Spring Harbor Laboratory. https://dx.doi.org/10.1101/2023.09.28.558499
2. Petrovic, S., Samanta, D., Perriches, T., Bley, C. J., Thierbach, K., Brown, B., Nie, S., Mobbs, G. W., Stevens, T. A., Liu, X., Tomaleri, G. P., Schaus, L., & Hoelz, A. (2022). Architecture of the linker-scaffold in the nuclear pore. *Science*, *376*(6598). https://doi.org/10.1126/science.abm9798
3. Onischenko, E., Noor, E., Fischer, J. S., Gillet, L., Wojtynek, M., Vallotton, P., & Weis, K. (2020). Maturation Kinetics of a Multiprotein Complex Revealed by Metabolic Labeling. *Cell*, *183*(7), 1785-1800.e1726. https://doi.org/10.1016/j.cell.2020.11.001

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