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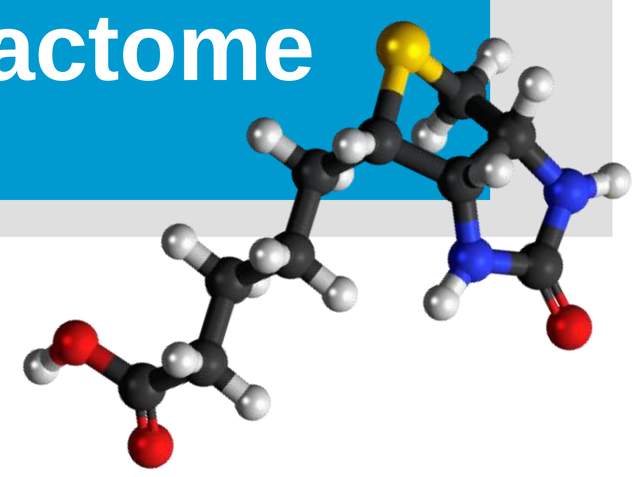
A Promiscuous Snatch Towards the B56ε Interactome



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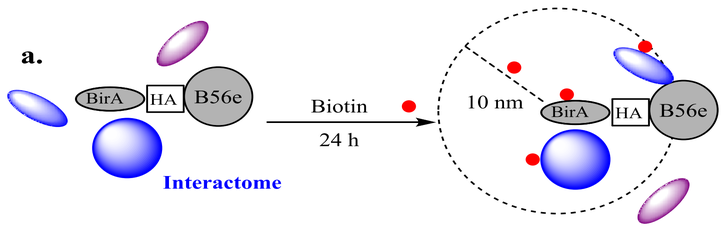
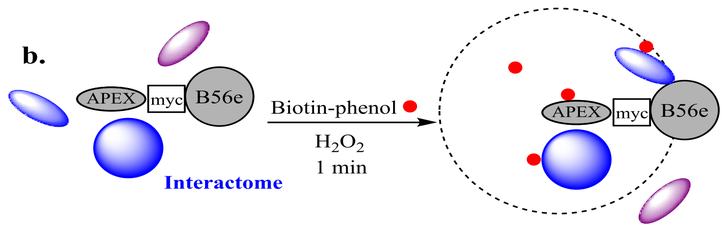
Introduction

Protein phosphatase 2A (PP2A) is an important enzyme which dephosphorylates myriads of proteins, being involved in cytoskeleton organization, protein synthesis, gene regulation and cell division. Its heterotrimeric structure comprises the B-type subunit which is imperative for substrate selectivity. In order to study the protein-protein interactions of the B-type subunit B56ε, proximity-based labeling of proteins in living cells was used. Thereby the bait protein B56ε was fused to enzymes that generate reactive biotin molecules, enabling the promiscuous labeling of the proteins' interactome. In this study, engineered ascorbate peroxidase (APEX) and proximity-dependent biotin identification (BioID) using BirA as fusion proteins were harnessed to tag the B56ε interactome.

Proteomic Mapping

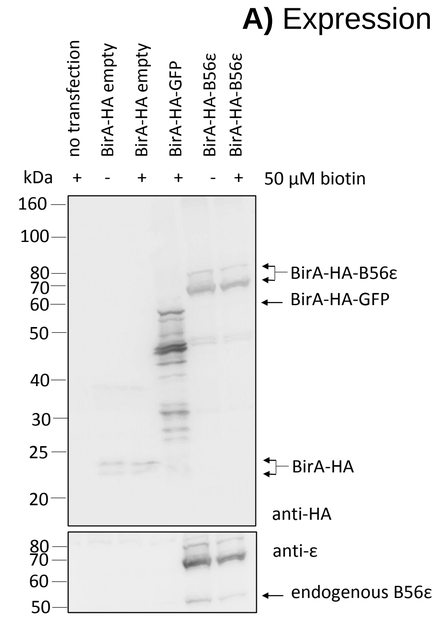


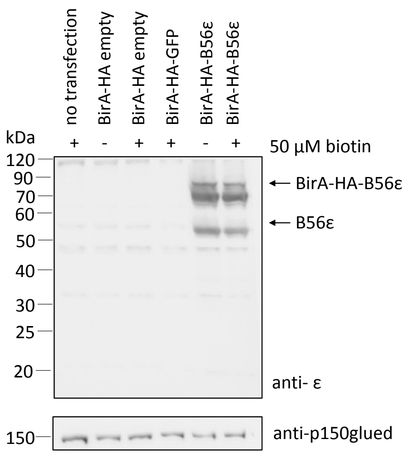
- Fusion of BirA-HA and APEX-2myc to B56ε
- Transfection of fusion constructs in HEK293 cells
- Supplementation with biotin (**a.** BirA protocol) or biotin-phenol and H_2O_2 (**b.** APEX protocol)
- Covalent labeling of lysine residues (BirA) or electron-rich aa (APEX)



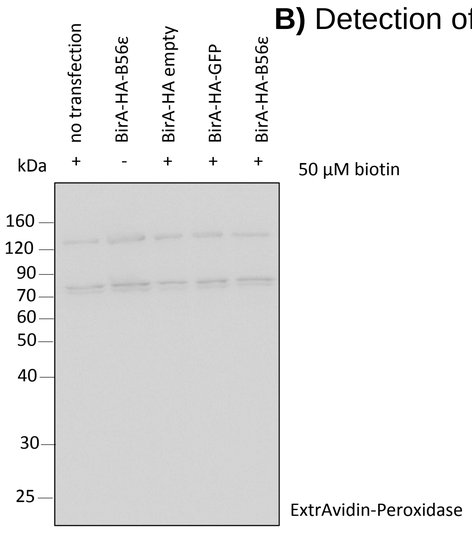
Results

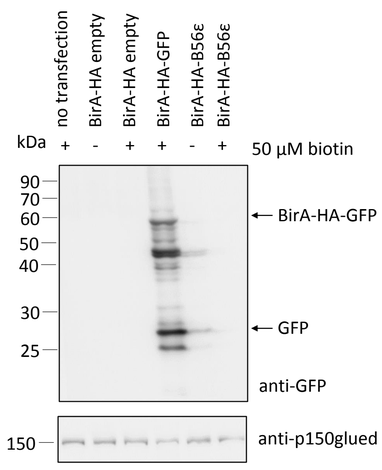
A) Expression of BirA fusion constructs



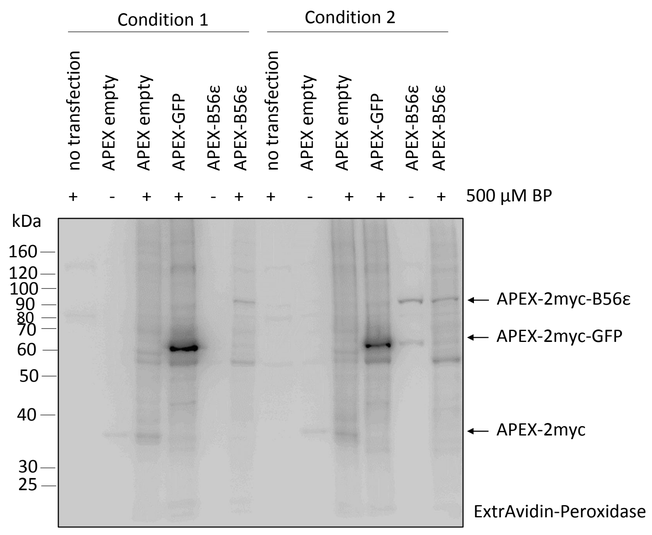


B) Detection of biotinylation

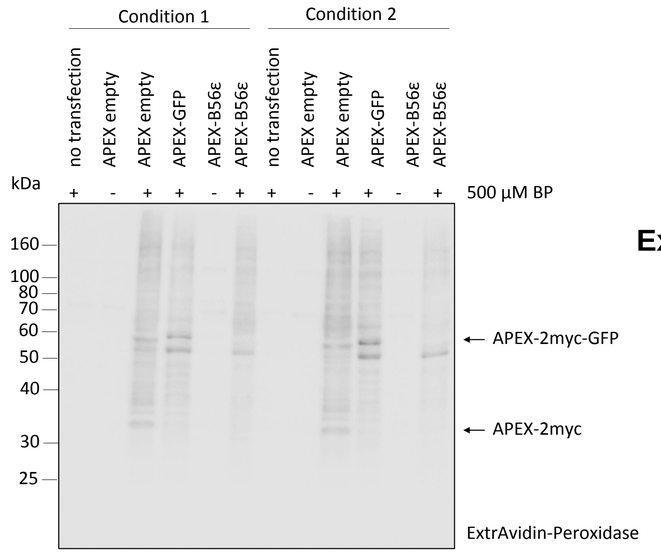




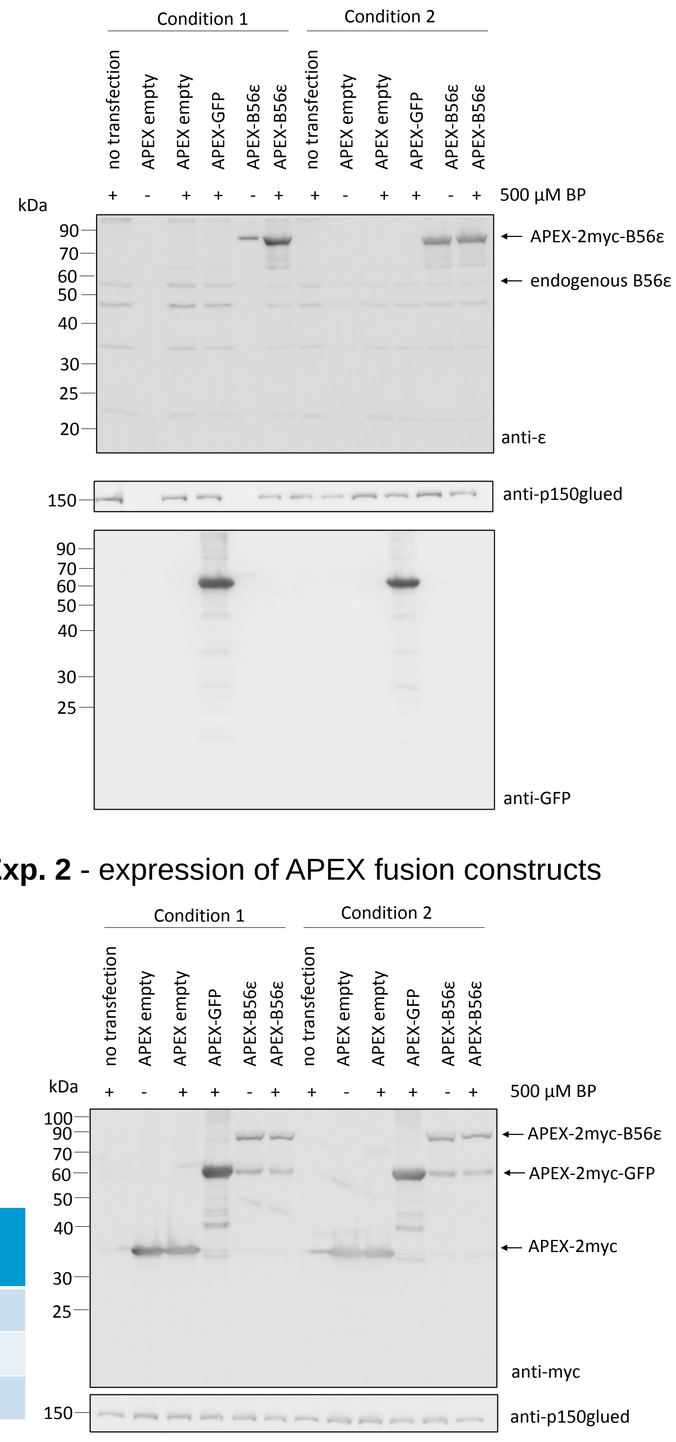
Exp. 1 - detection of biotinylation



Exp. 2 - detection of biotinylation



Exp. 1 - expression of APEX fusion constructs



Exp. 2 - expression of APEX fusion constructs

- Upon transfection and supplementation with biotin or BP, the cells were harvested
- Harvested cells were lysed in Lämmli buffer, the proteins were separated *via* SDS-PAGE and transferred to a membrane
- Ctrl: GFP constructs and empty vector were chosen for unspecific biotinylation
- Reactions in absence of supplemented biotin or BP show background biotinylation
- Loading control: p150glued and tubulin

	Condition 1 [µg DNA]	Condition 2 [µg DNA]
APEX empty	5	3
APEX-GFP	5	3
APEX-B56ε	7.5	5

Acknowledgement

I would like to express my sincere gratitude to Beata Rymarczyk for her continuous support and Prof. Dr. Thomas U. Mayer for the interesting project and the provision of all necessary facilities for the reasearch.

Outlook

- Test if BirA and APEX-B56ε fusion constructs interact with PP2A A and C subunits to give the heterotrimeric holoenzyme
- Pull-down assay with BirA and APEX-B56ε to verify known B56ε interaction partners and identify new ones

References: Janssens, Veerle, Sari Longin, and Jozef Goris. "PP2A holoenzyme assembly: in cauda venenum (the sting is in the tail)." *Trends in biochemical sciences*33.3 (2008): 113-121. Kim, Dae In, and Kyle J. Roux. "Filling the Void: Proximity-Based Labeling of Proteins in Living Cells." *Trends in Cell Biology* (2016) https://upload.wikimedia.org/wikipedia/commons/thumb/3/32/Biotin-3D-balls.png/1280px-Biotin-3D-balls.png