Mapping the Minimal MEA1 Binding Region for AP1 and AP2

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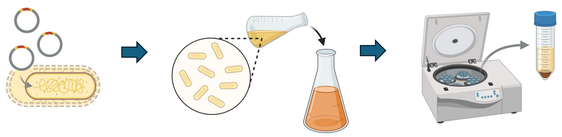
Introduction

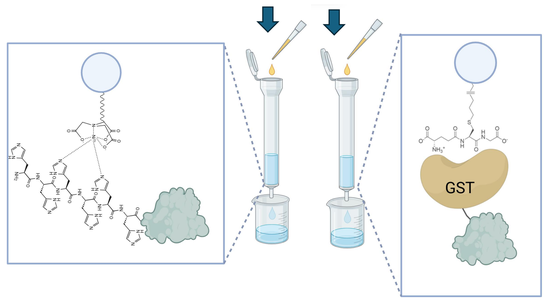
- Adaptor protein complexes regulate vesicular trafficking, but the mechanisms governing their assembly remain incompletely understood.
- AP1 and AP2 are heterotetrameric adaptor complexes composed of β, μ, σ, and large α/γ subunits.
- The chaperone Male Enhanced Antigen 1 (MEA1) facilitates assembly of the β and μ subunits into mature adaptor complexes.
- The C-terminal domain (CTD) of MEA1 interacts with the β subunit of AP1 and AP2.
- Objective: define the minimal MEA1 region required for adaptor binding using GST pull-down assays with MEA1 truncations.

Methods

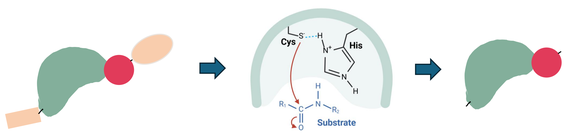
Gene of interest cloned into E. coli



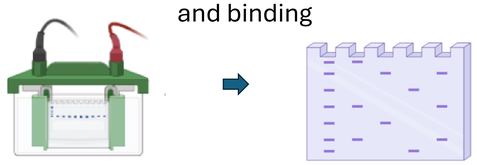
Supernatant split between Ni-NTA and GSH affinity columns



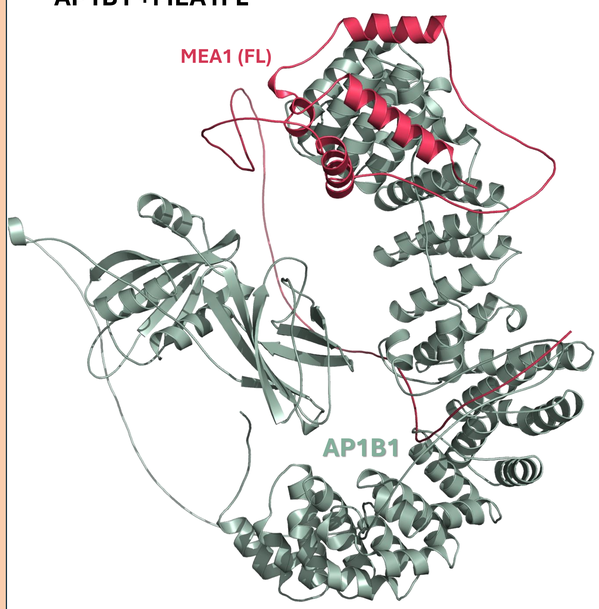
Purified protein treated with ULP1 and 3C protease to cleave affinity tags



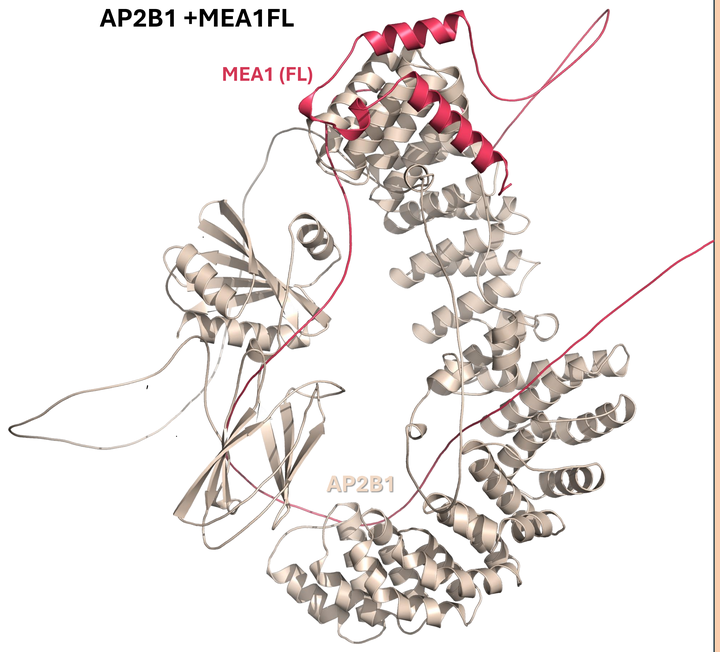
SDS-page run to confirm purification and binding



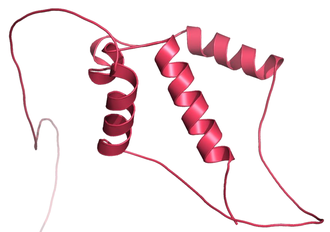
AP1B1 +MEA1FL



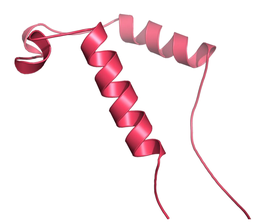
AP2B1 +MEA1FL



MEA1 aa 1-185



MEA1 aa 122-185



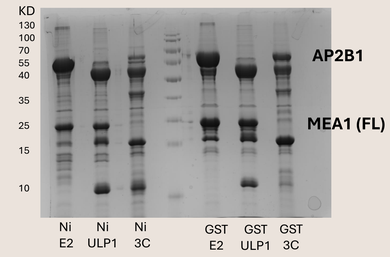
MEA1 aa 154-185

His-SUMO-AP1B1+GST-MEA1 (FL)

His-SUMO-AP1B1+GST-MEA1 (122E)

His-SUMO-AP1B1+GST-MEA1 (154E)

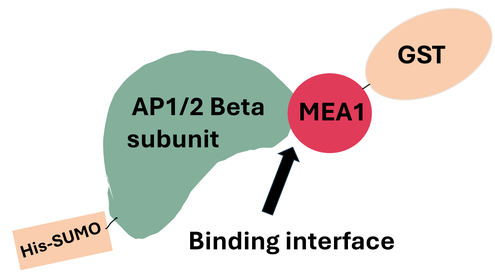
His-SUMO-AP2B1+GST-MEA1 (FL)



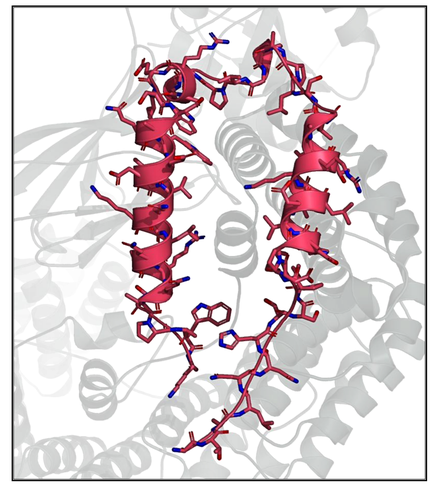
His-SUMO-AP2B1+GST-MEA1(122E)

His-SUMO-AP2B1+GST-MEA1 (154E)

Truncation Constructs



Results



MEA1 (122-185) + AP1B1

- MEA1 residues 122-185 pulled down AP1β1 and AP2β1 subunits
- MEA1 truncations 154-185 and 164-185 did not pull down AP1β1 or AP2β1
- These results suggest the critical adaptor-binding region lies between residues 122-154 of MEA1

References