A repurposed AMP binding domain reveals protein AMPylation as a regulator of cellular metabolism

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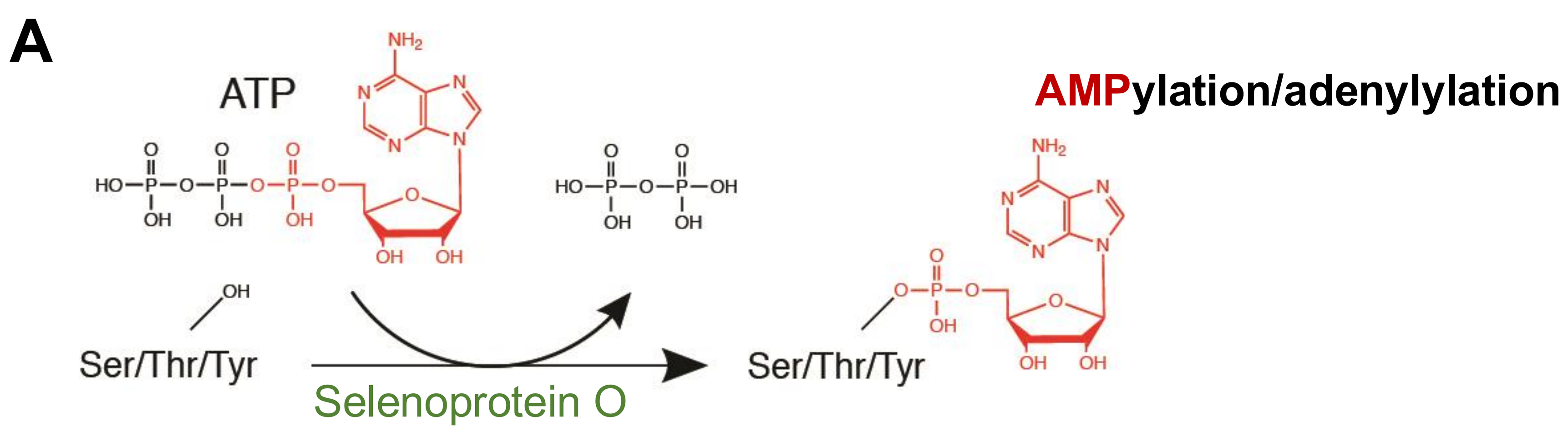
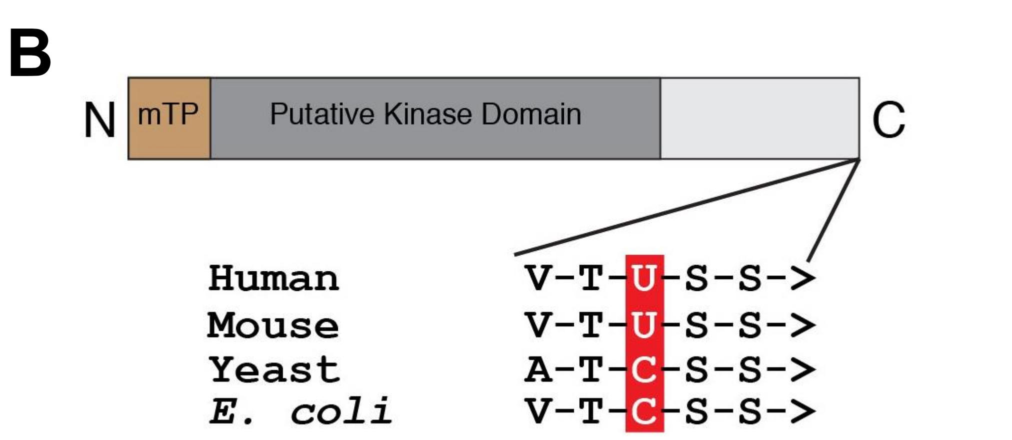
Abstract

Protein AMPylation, the covalent addition of adenosine monophosphate (AMP) to protein substrates, has been known as a post translational modification for over 50 years. Research in this field is largely underdeveloped due to the lack of tools that enable the systematic identification of AMPylated substrates. To address this gap, we developed an enrichment technique to isolate and study AMPylated proteins using a nucleotide-binding protein, hinT. Using structure guided mutagenesis, we optimized enrichment to identify novel substrates of the evolutionarily conserved AMPylase, Selenoprotein O. We show that mammalian Selenoprotein O regulates metabolic flux through AMPylation of key mitochondrial proteins including glutamate dehydrogenase and pyruvate dehydrogenase.

Our findings highlight the broader significance of AMPylation, an emerging post translational modification with critical roles in signal transduction and disease pathology. Furthermore, we established a powerful enrichment platform for the discovery of novel AMPylated proteins to study the mechanisms and significance of protein AMPylation in cellular function.

Selenoprotein O catalyzes protein AMPylation





What are the substrates?

What is the functional importance ?

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Methodology:

Enrichment of AMPylated proteins using engineered AMP binding protein, hinT

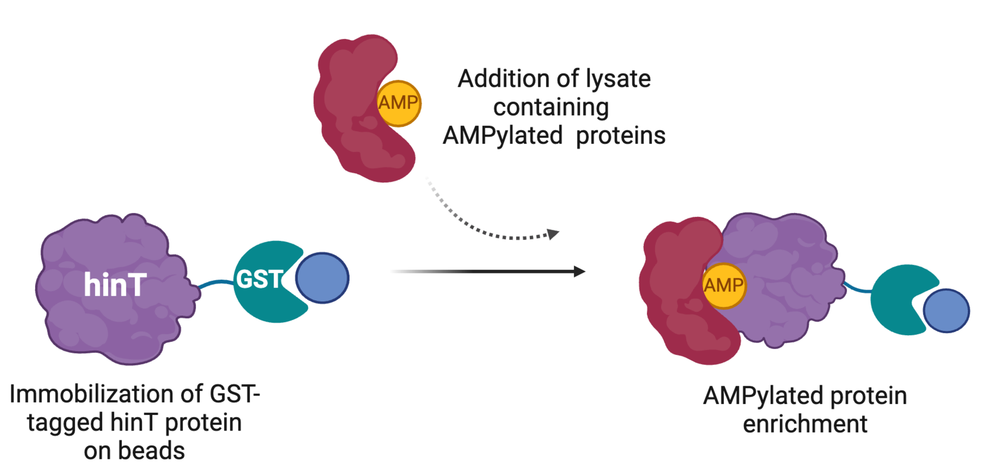

Figure 1. (A) Selenoprotein O (SelO) catalyzes the transfer of AMP from ATP to the hydroxyl side chain of protein substrates. **(B)** Schematic representation of the evolutionarily conserved SelO depicting the mitochondrial targeting peptide (mTP) and pseudokinase domain. The amino acid sequences at the C-terminus of the human, mouse, yeast and *E. coli* proteins are shown, highlighting the selenocysteine (U) and cysteine (C) residues. **(C)** Schematic representation of AMPylated protein enrichment strategy using GST-hinT immobilized on glutathione agarose as bait to identify AMPylated proteins in lysates.

Structural basis for AMP selectivity

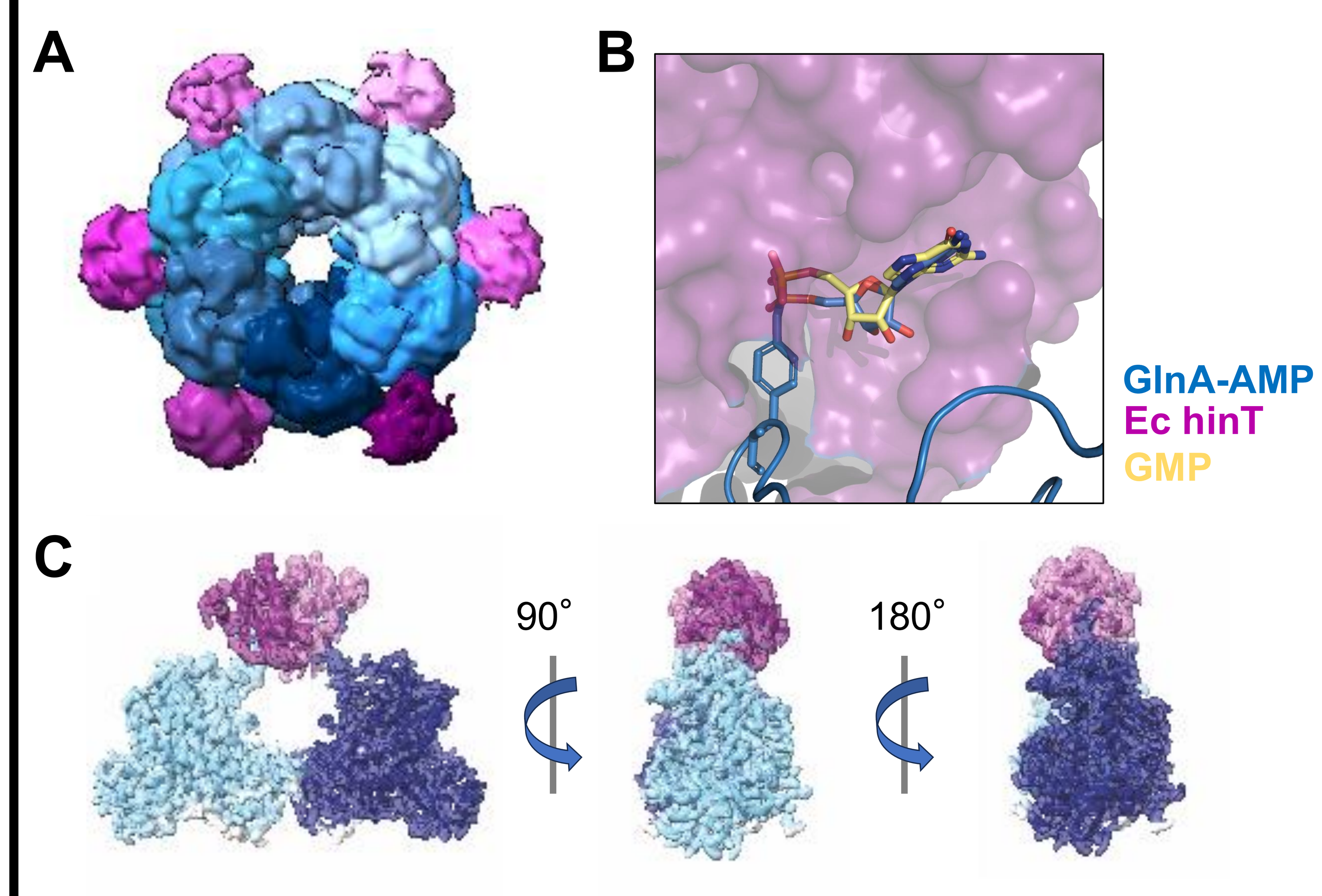

Figure 2. (A) Cryo-EM density map representation of the GlnA-AMP and hinT complex. The dodecamer GlnA is in blue, and the dimeric hinT is in pink. Map resolution 2.7 Å **(B)** Stick representation of superimposed nucleotide GMP with GlnA Y398-AMP in the binding pocket of hinT. Surface representation of hinT shown in pink, GMP shown in yellow, GlnA shown in blue **(C)** Enlarged image of cryo-EM density map representation of GlnA-hinT binding interface. AMPylated Tyr-398 present in each of the two GlnA molecules extends into active site of the dimeric hinT H101N. GlnA subunits colored in dark and light blue. hinT dimer is in magenta and light pink.

Identification of novel AMPylated proteins

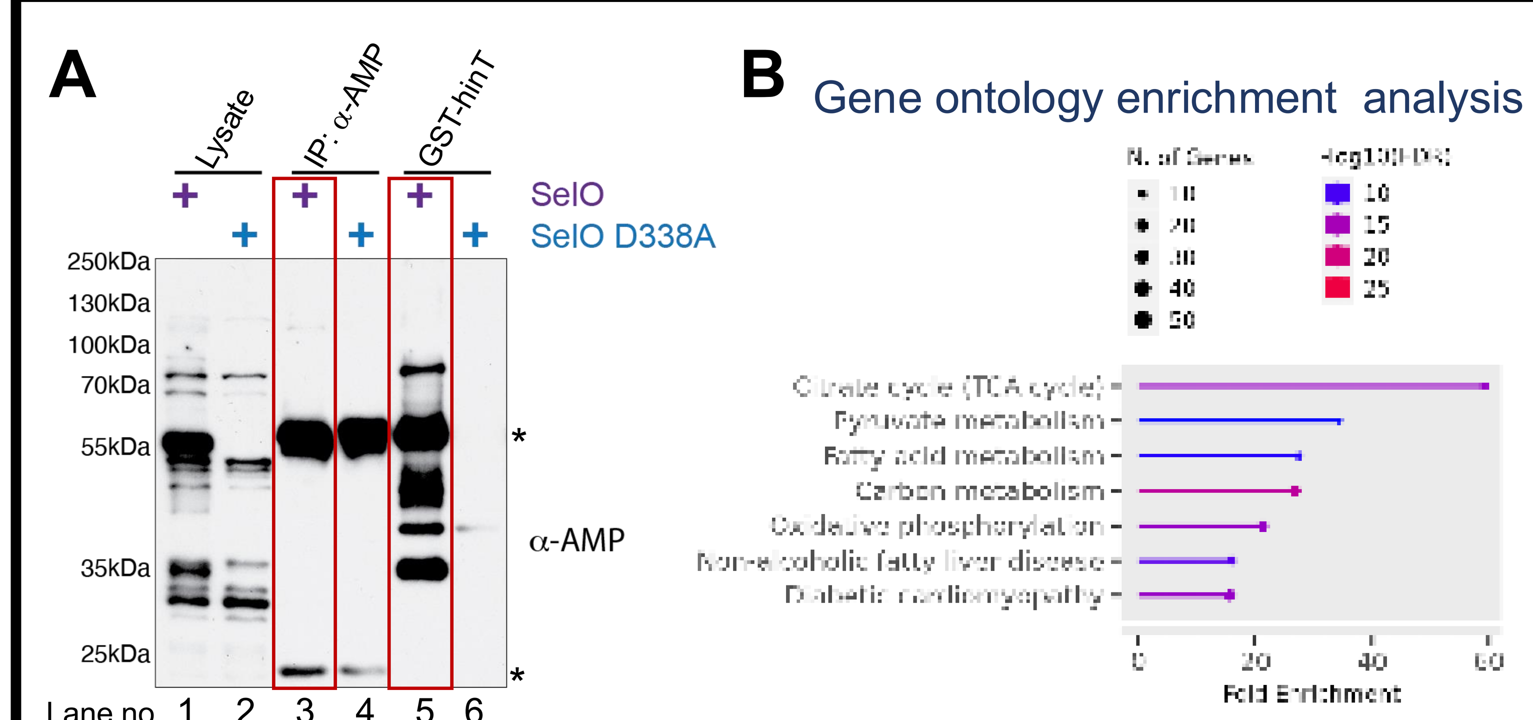

Figure 3. (A) α-AMP immunoblotting of cell lysates (lanes 1-2), anti-AMP immunoprecipitates (lanes 3-4), and GST-hinT enriched proteins (lanes 5-6) from mouse melanoma cell lines expressing SelO or inactive SelO, D338A. * denotes the positions of heavy and light chains of AMP antibodies used for immunoprecipitation. In contrast to immunoprecipitation (lane 3), enrichment using hinT revealed several AMPylated proteins (lane 5) **(B)** Enriched GO biological process terms for mitochondrial proteins enriched from melanoma cells expressing SelO but not inactive SelO DA.

AMPylation inhibits glutamate dehydrogenase

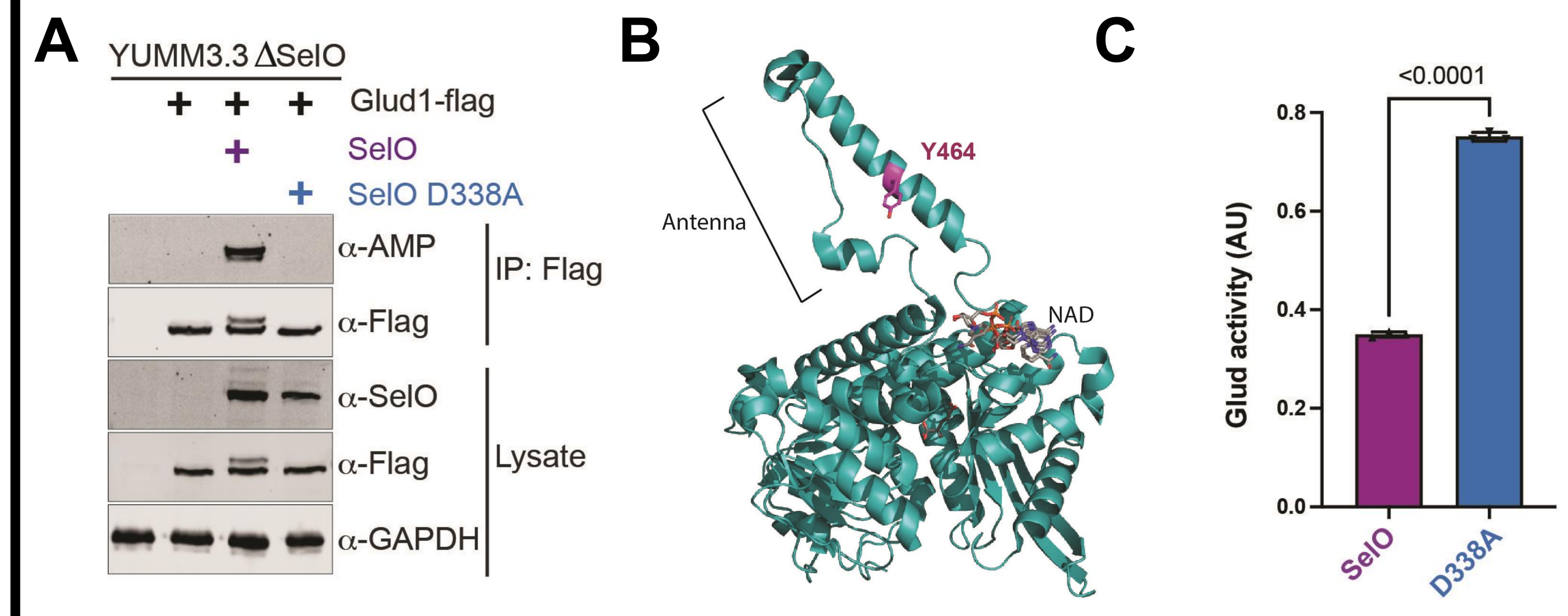

Figure 4. Among our top enriched proteins from Figure 3 is glutamate dehydrogenase (Glud1). **(A)** Protein immunoblotting of flag immunoprecipitates or cell lysates from mouse melanoma cells, YUMM3.3ΔSelO, expressing Glud1-flag or Glud1-flag and SelO or $SelO^{D338A}$. **(B)** Ribbon representation of bovine Glud1 (PDB: 1HWY). Putative site of AMPylation, Y464, as revealed by mass spectrometry analysis shown in magenta. NAD is shown in stick representation. **(C)** The activity of Glud1 in cells expressing SelO or $SelO^{D338A}$.

Conclusions and future directions

Utility of hinT as a new tool to study protein AMPylation

AMPylation is emerging as a prominent post translational modification in mammalian cells, and our knowledge is in its infancy. Currently, there are only a handful of enzymes known to catalyze AMPylation. There may be many more AMPylases and AMPylated substrates that have evaded detection due to the low abundance and stoichiometry of AMPylation. Akin to SH2 binding domains, which have been valuable tools for the analysis of protein tyrosine phosphorylation, our strategy to isolate endogenous AMPylated proteins will enhance our understanding of the fundamental mechanisms and the functional importance of AMPylation.

Acknowledgements and References

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