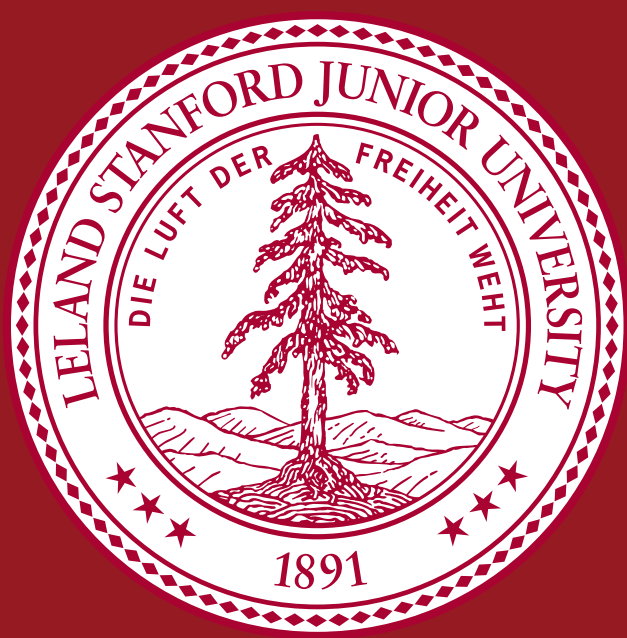




A complete protein suite to program compact secretion

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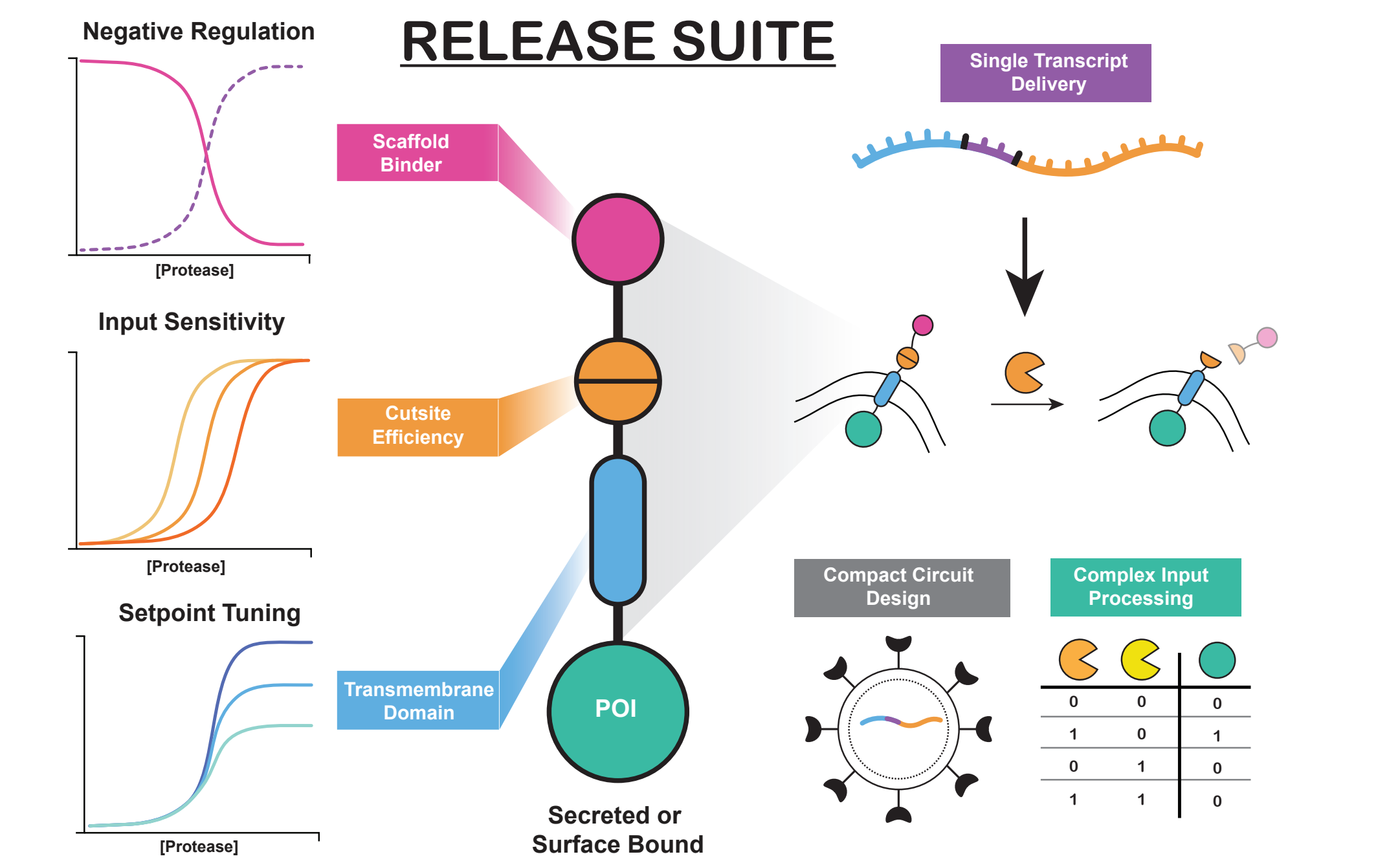
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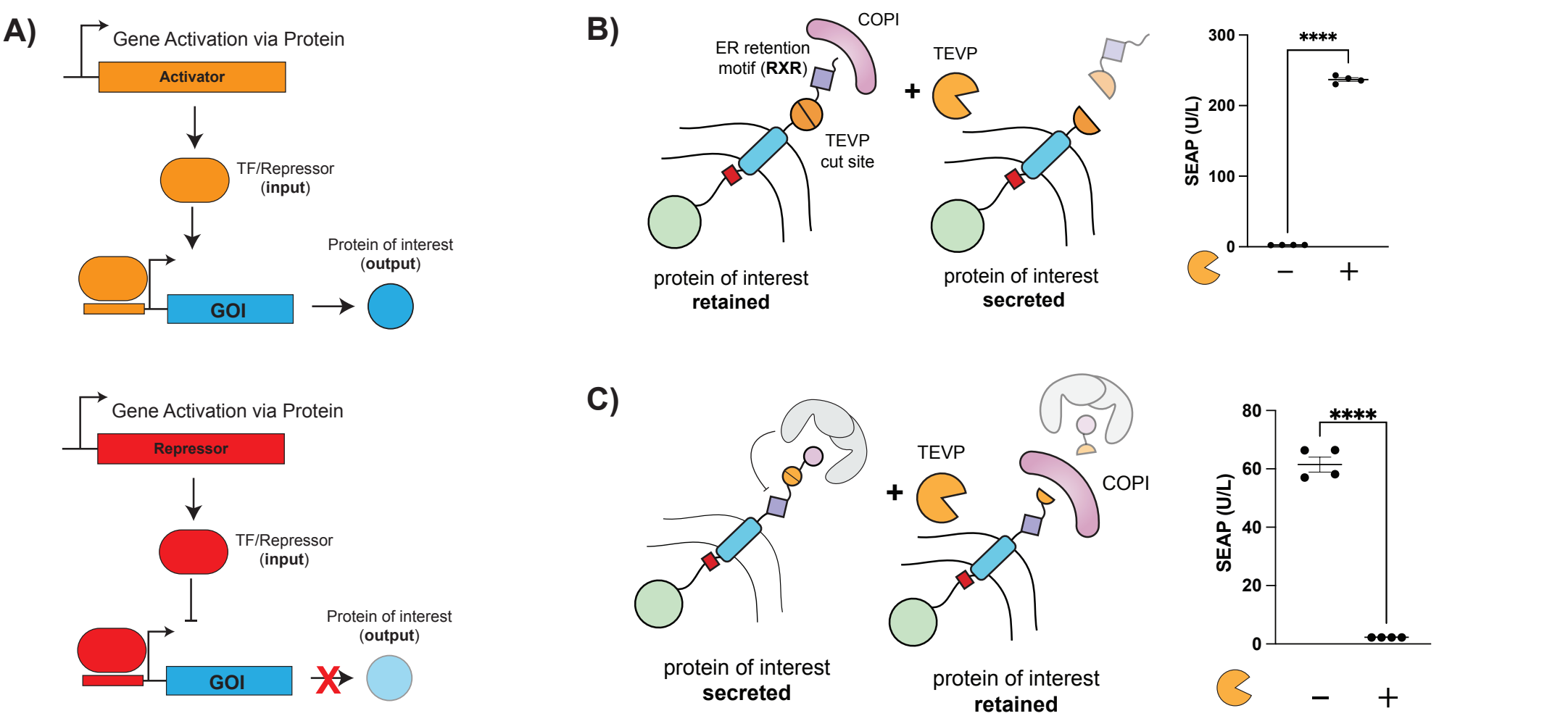
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Background

Proteins have been a basis of synthetic biology, allowing for precise modulation of gene expression. In recent years, protein circuits have leveraged these protein interactions to engineer cellular behavior purely at the protein-level.¹ Further improvements have expanded these tools to include intercellular communication by regulating protein secretion post-translationally using tools such as RELEASE² and LumER³. However, many of the capabilities of traditional gene circuits have been out of reach for secretion protein circuits, including negative repression and expression tuning. Here, we introduce a complete suite of protein engineering approaches to enable this level of programmability completely at the protein level, opening up multiple levels of the central dogma to program cell function and exemplify use in translational delivery cases such as AAV vectors for use in neurons and mRNA for use in transient therapeutics.

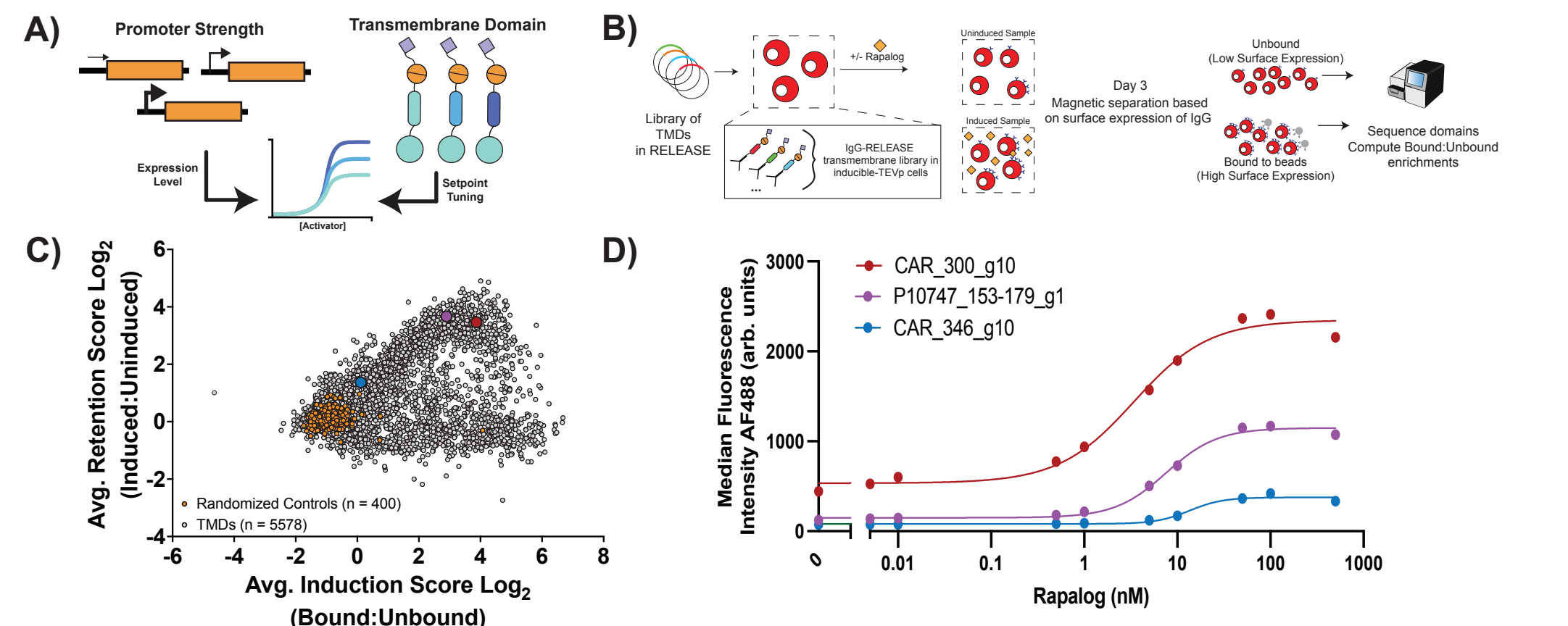


ER-retention domain modification enable negative regulation via 14-3-3 scaffold



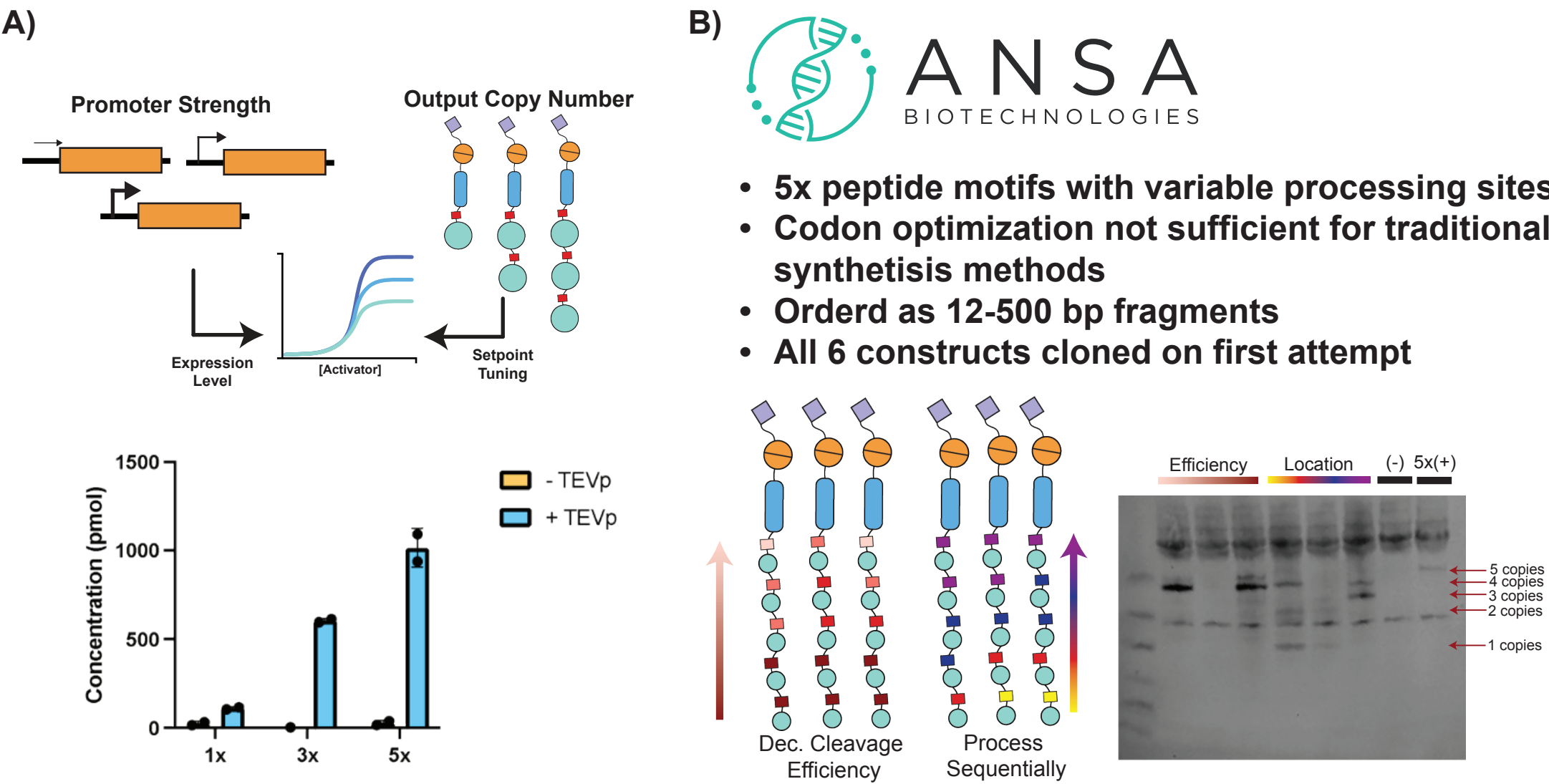
Retained Endoplasmic Cleavable Secretion (RELEASE) leverages a cytoplasmic C-terminal tail that can be modified for expanded programming. **A)** Genetic circuits allow for straightforward positive and negative regulation via enhancing or repressing protein domains. **B)** RELEASE ER-retention via COPII mediation is achieved by a diarginine motif (RXR) which can tolerate additional C-terminal residues while maintaining retention function. Cleavage of RXR domain leads to protein secretion. **C)** Addition of an R18 peptide to recruit native 14-3-3 scaffold proteins⁴ can sterically inhibit an RXR motif, allowing for protein trafficking through the secretion pathway. By cleaving the R18 domain, retention is restored, creating a functional NOT gate.

Transmembrane domain tunes setpoint secretion levels



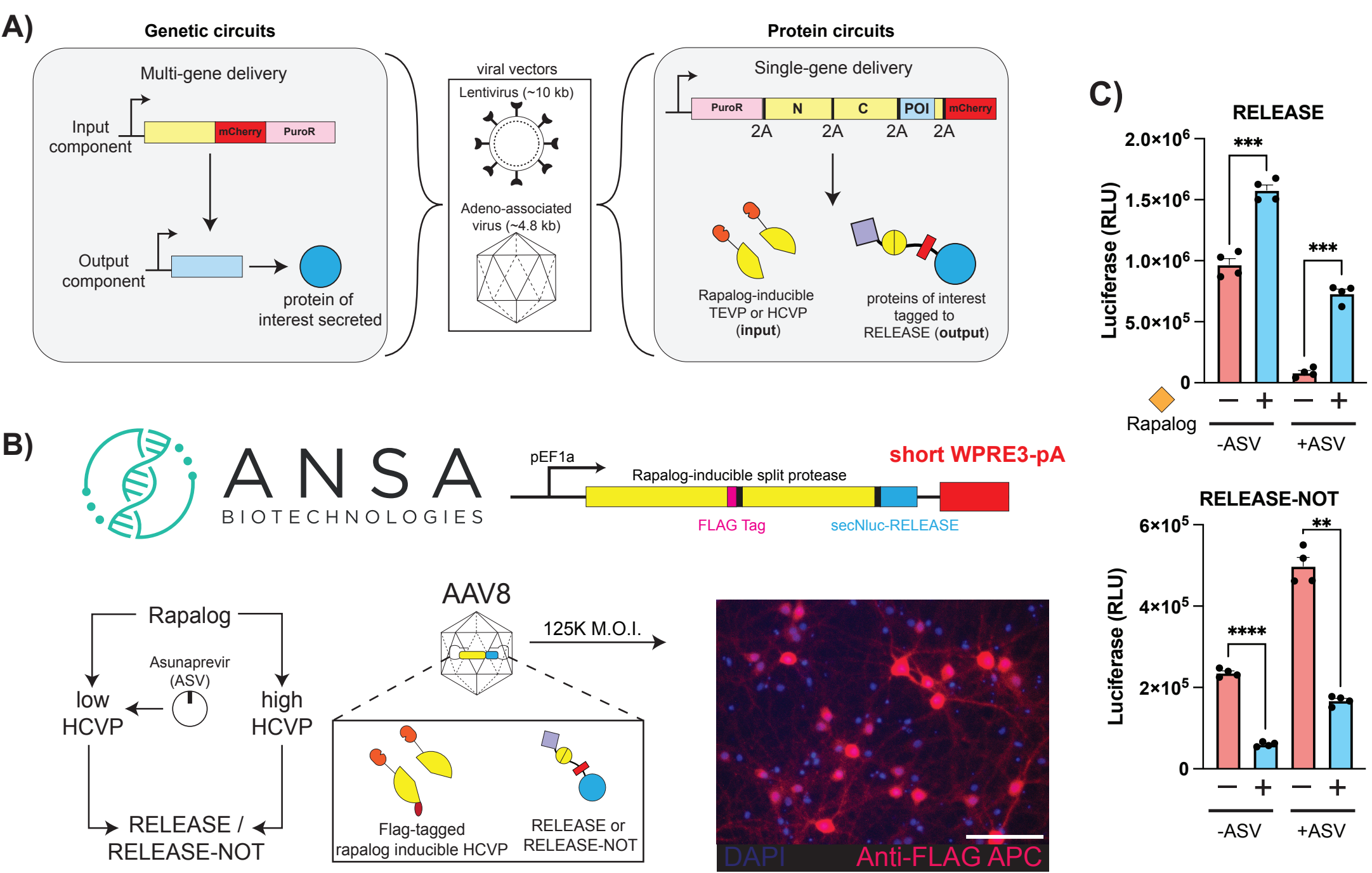
Transmembrane domains (TMDs) tune RELEASE setpoint expression levels. **A)** Genetic circuits can modify overall expression profiles by using promoters of various strengths, while protein secretion circuits can be tuned similarly by swapping TMDs. **B)** Schematic of a high-throughput screen of human TMDs from proteins with signal peptides using surface- IgG-RELEASE⁵. **C)** Compiled data plotted as retention score vs. induced surface display measured via enrichment in protein G binding assay. **D)** Example setpoint tuning demonstrated by selecting 3 TMDs of various expression.

Stoichiometric control of outputs achieved by protein repeats



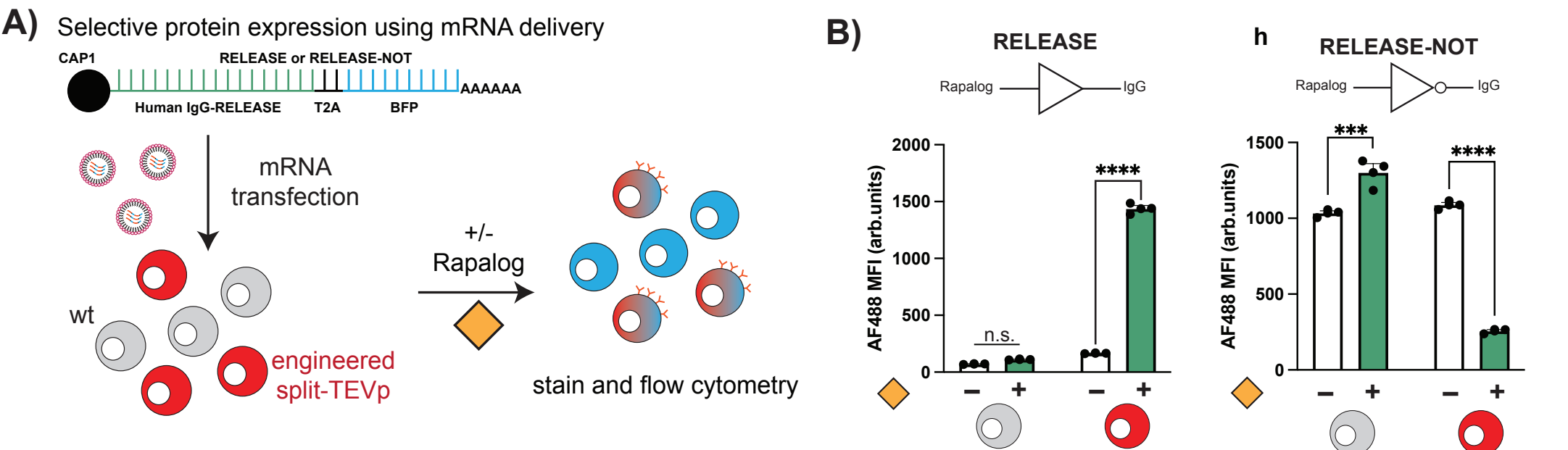
Stoichiometric control is an alternative methods to tune output signal strengths **A)** Control utilizing cleavable copies of the output protein can tune signal in proportion to copy number of protein. **B)** ANSA DNA synthesis was key in producing cleavage optimizing constructs (lower left) with variable processing domains to ensure proper processing. Constructs tested in HEK293 cell culture, and resulting cleavage products evaluated using low MW western blot to identify properly fragmented products.

Polycistronic transcript design for viral vector delivery



Protein circuits allow for the ability to create single-gene circuits, making them ideal candidates for viral vector delivery. **A)** Schematic demonstrating multi-gene designs of traditional gene circuits vs polycistronic, single-gene design of a RELEASE-based circuit. **B)** Design of AAV vector (transcription regulatory elements optimal for AAV packaging size limitations synthesized by ANSA) encoding rapalog-induced split HCV protease and secNLuc-RELEASE in single transcript and delivery to primary rat cortical neurons. Microscopy of neurons demonstrates strong transcript expression (anti-FLAG). **C)** Nanoluciferase signal from RELEASE (top) and RELEASE-NOT circuits (bottom) tuned using protease inhibitor (ASV) to modulate circuit strength.

Protein circuits provide selective secretion via mRNA delivery



Protein circuits leverage post-translational modifications to transmit information, making them compatible with mRNA based applications. **A)** Schematic of mRNA delivery to engineered cell models to demonstrate cell selectivity. **B)** Activation of RELEASE and RELEASE-NOT delivered via N1mΨ modified mRNA only in engineered cells. Cellular outputs modulated by tuning using variable transmembrane domains.

References and Funding

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