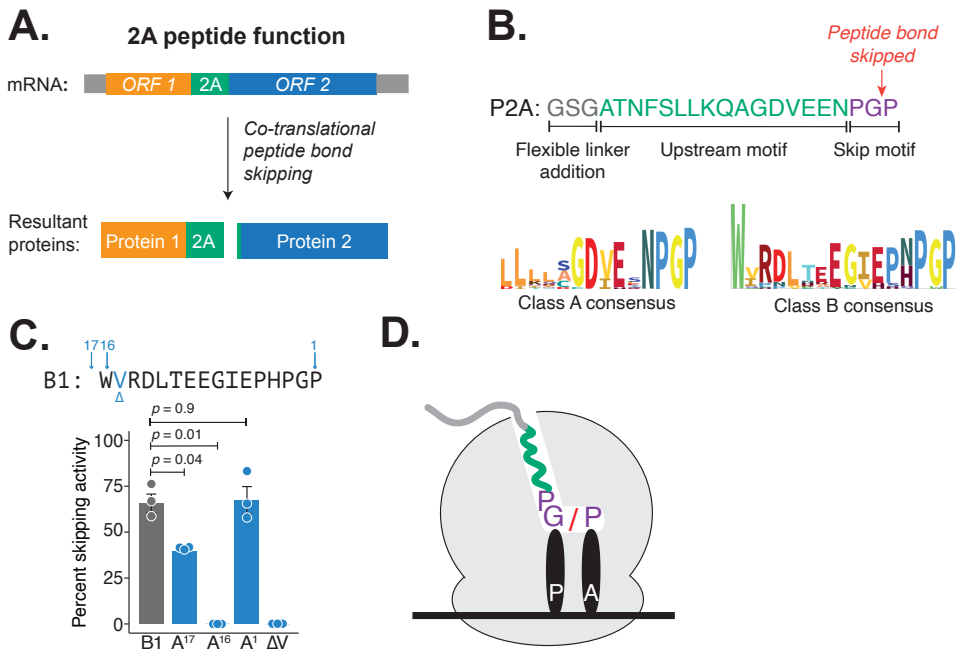

Figure 1. Background. A, Simple schematic of 2A peptide function. **B,** Top: sequence of a Class A (P2A) sequence. When a peptide bond is skipped, the Upstream motif must be in the ribosome exit tunnel, the Skip motif in the ribosome's catalytic center. Bottom: Consensus motifs of the two classes of 2A peptides, the Class B were discovered by the Rissland Lab. **C,** Mutation of conserved residues in the 2A sequence results in a loss of activity, demonstrating the exquisite specificity of the mechanism, which remains a mystery. **D,** Simple cartoon of the tRNA/peptide/ribosome configuration during bond skipping. The red slash represents the location of the skipped bond.

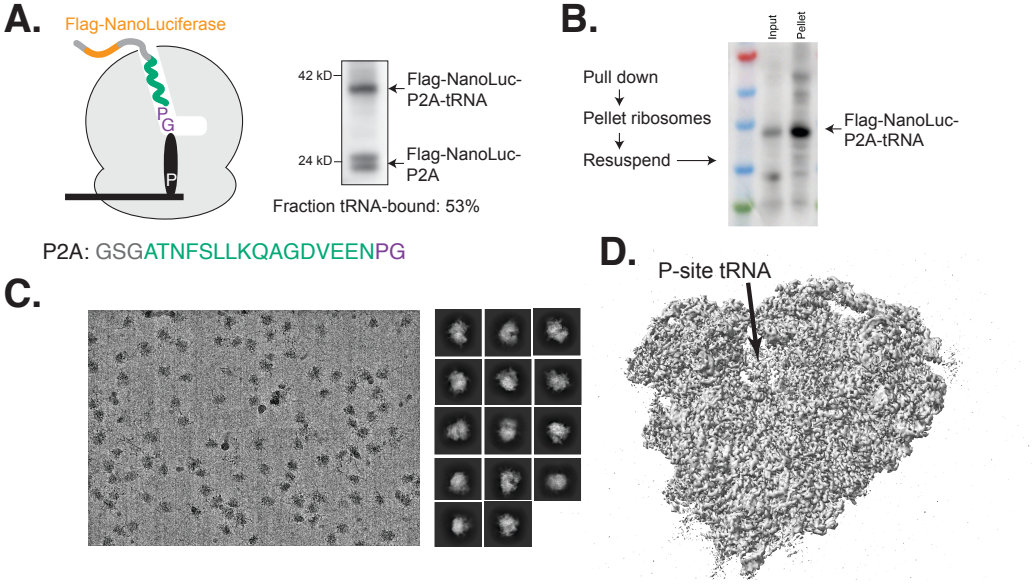

Figure 2. Sample preparation and preliminary cryoEM analysis. A, Left: Schematic of the first structural target, which uses a truncated mRNA; the primary goal of this structure is to observe interactions between the P2A sequence (below) and the ribosome exit tunnel. A FLAG tag uncoded upstream of the P2A sequence is used to pull down the complex, the NanoLuciferase is used to assess if translation is occurring. Right: Western blot of the pulled down sample (anti-FLAG) shows free nascent peptide and peptide that remains covalently linked to tRNA). **B.** Pulled down ribosome-containing complexes were pelleted by ultracentrifugation and resuspended. Western analysis with anti-FLAG shows a strong enrichment for tRNA-linked peptide within the ribosomes. **C.** Complexes from panel B were used to prepare grids at NYSBC by Dr. Wimberly using graphene oxide coated gold grids. Example micrograph from a Krios collection is shown with resultant 2-D class averages. **D.** Preliminary cryoEM map from these micrographs. Currently resolution is better than 3 Angstroms and P site tRNA is visible, as is peptide in the exit tunnel.