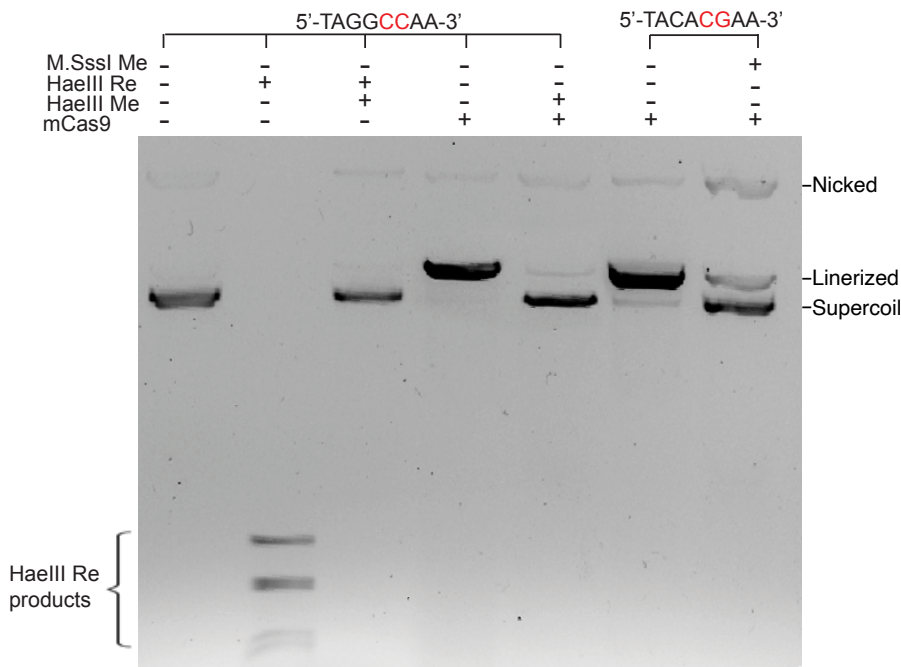
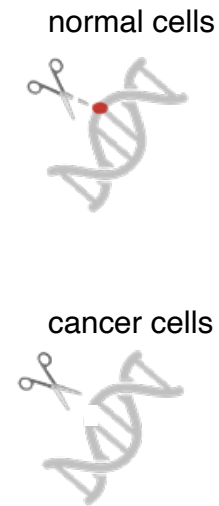


a. mCas9 is inhibited by a single methylation



b. mCas9 applications



c. mCas9 structures when bound with unmethylated DNA

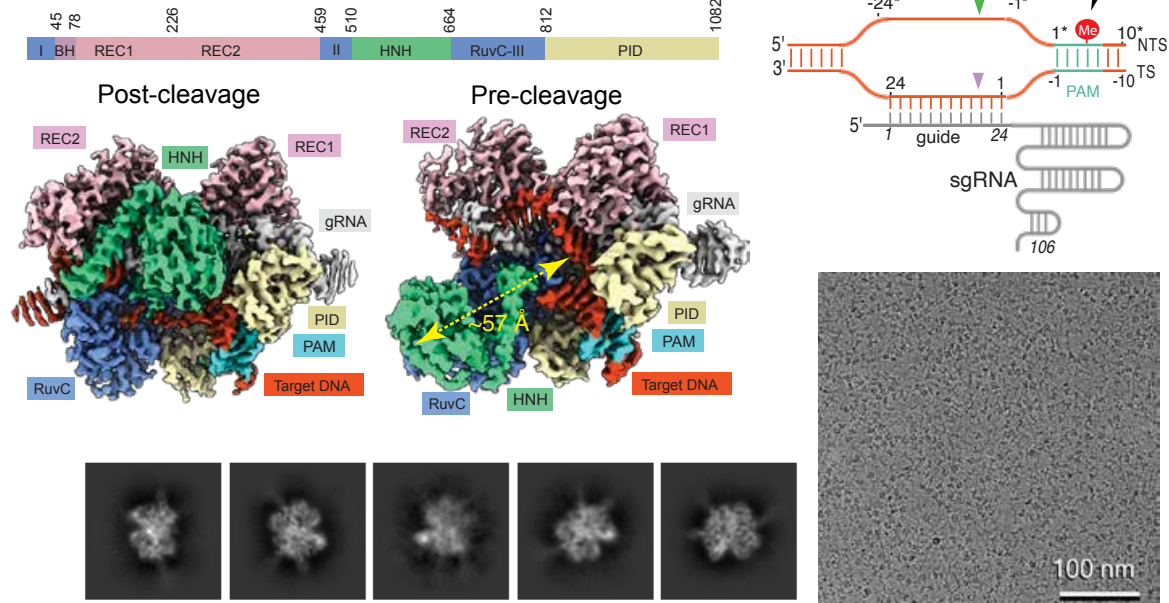
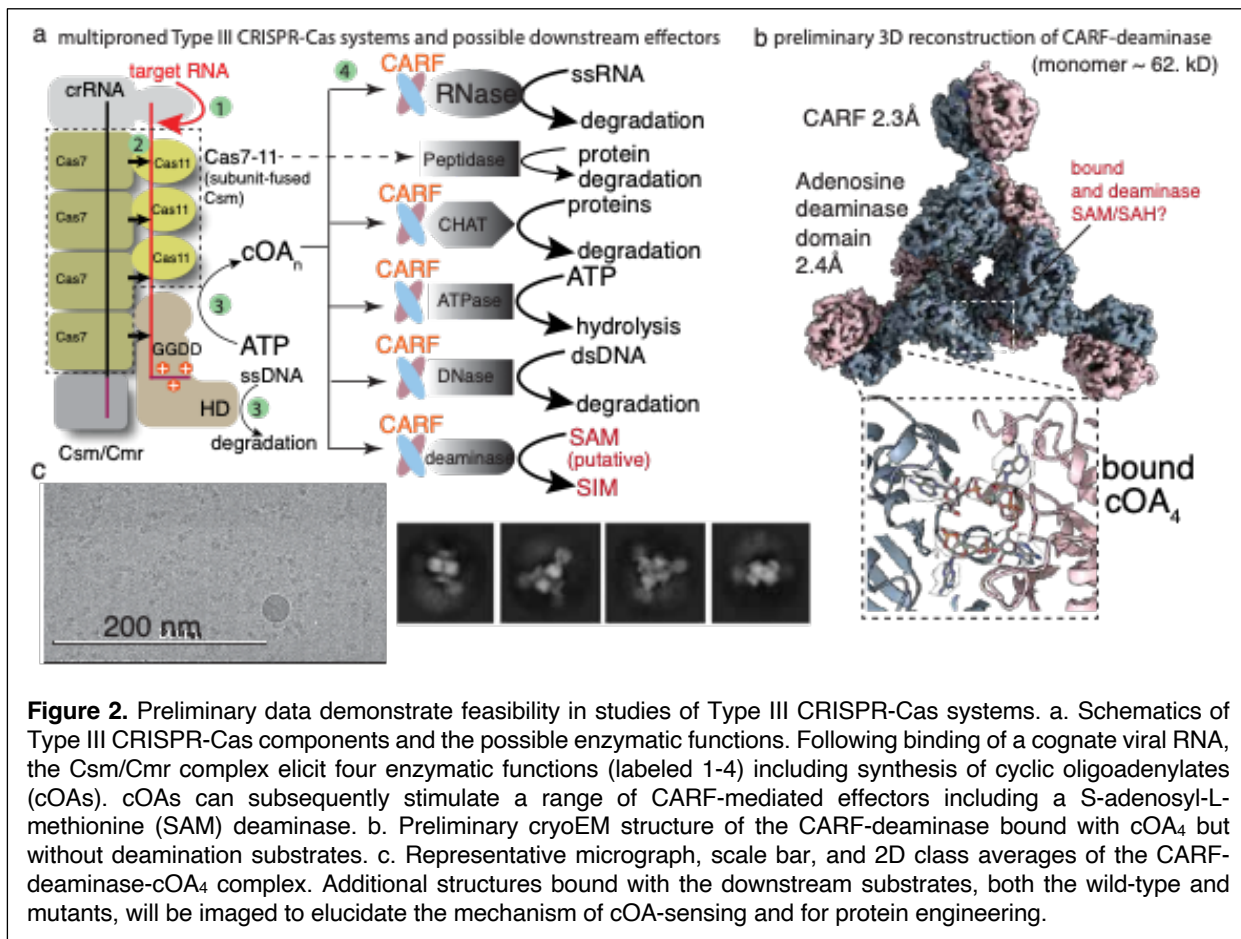


Figure 1. Preliminary data demonstrate feasibility in studies of methylation-sensitive Cas9 (mCas9). a. mCas9 activity is sensitive to DNA methylation. “HaeIII Me”= HaeIII methyltransferase that methylates GGCC sequence. “HaeIII Re”=HaeIII restriction enzyme that cleaves non-methylated GGCC sequence. “M.SssI”= M.SssI methyltransferase that methylates CpG sequence. Cleavage results of non-methylated and methylated DNA by mCas9 are analyzed on agarose gel. The DNA containing 5'-NNGGCCA-3' PAM is methylated by HaeIII methyltransferase. The HaeIII-methylated DNA is examined by HaeIII restriction endonuclease. The DNA containing 5'-NNGGCCGA-3' PAM is methylated by Msssl methyltransferase. b. possible application of mCas9 in detecting and eradicating cancer cells. c. Preliminary cryoEM structures of mCas9 bound with non-methylated DNA in post- and pre-cleavage states. d. Representative micrograph with scale bar and 2D class averages. When mixing mCas9 with methylated DNA, we anticipate similar quality of grids and images.

Project 2: Structural Mechanisms of Type III CRISPR-Cas Systems



Project 3: Structural Mechanism of Ribosome Modifications

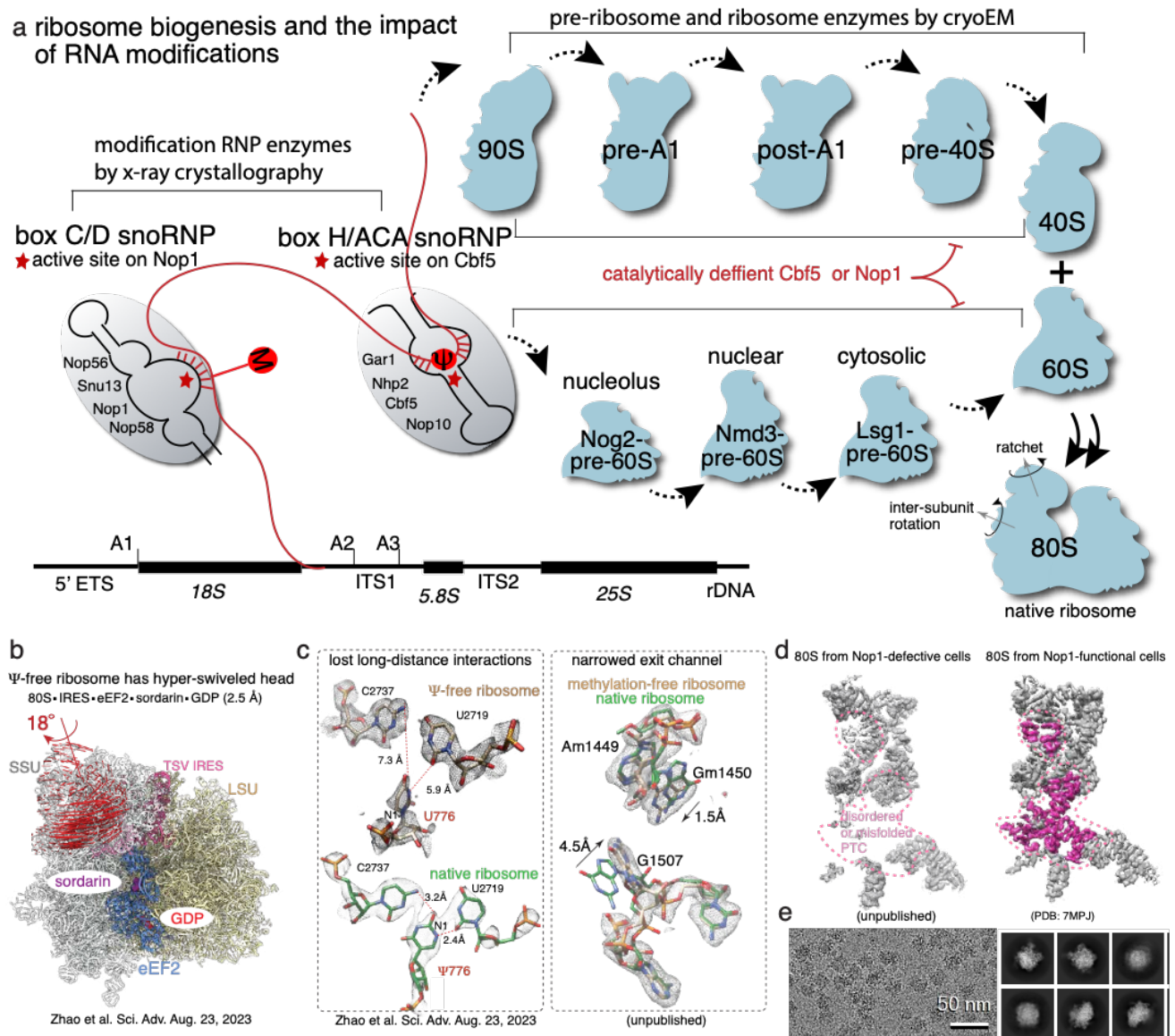


Figure 3. Preliminary data demonstrate feasibility in studies of ribosome modification studies. **a.** Simplified schematic processes of ribosome biogenesis with emphasis on ribosomal RNA (rRNA) modifications. The components of the rRNA modification enzymes are depicted including the catalytic subunits. We have created catalytically deficient yeast cells and will elucidate pre-ribosome and ribosome from these cells for structural studies. Typical structural methods used to analyze the depicted components are indicated. Note that cryoEM is the only method for analyzing the pre-ribosome complexes. **b.** A representative result of our recent study of the hypopseudouridylated ribosome that indicates defect in ribosome dynamics. **c.** A representative result of our recent study of the hypopseudouridylated (left) and hypomethylated (right) ribosome that indicate defect in ribosome local structures. **d.** A representative result of our recent study of the hypomethylated ribosome indicate misassembly of the peptidyl transferase center (PTC). **e.** Representative micrograph and 2D class averages of the hypomethylated pre-60S complex purified using Nmd3 from yeast. Current reconstruction at 2.7 Å has revealed structural changes in RNA due to the pathological loss of methylation.