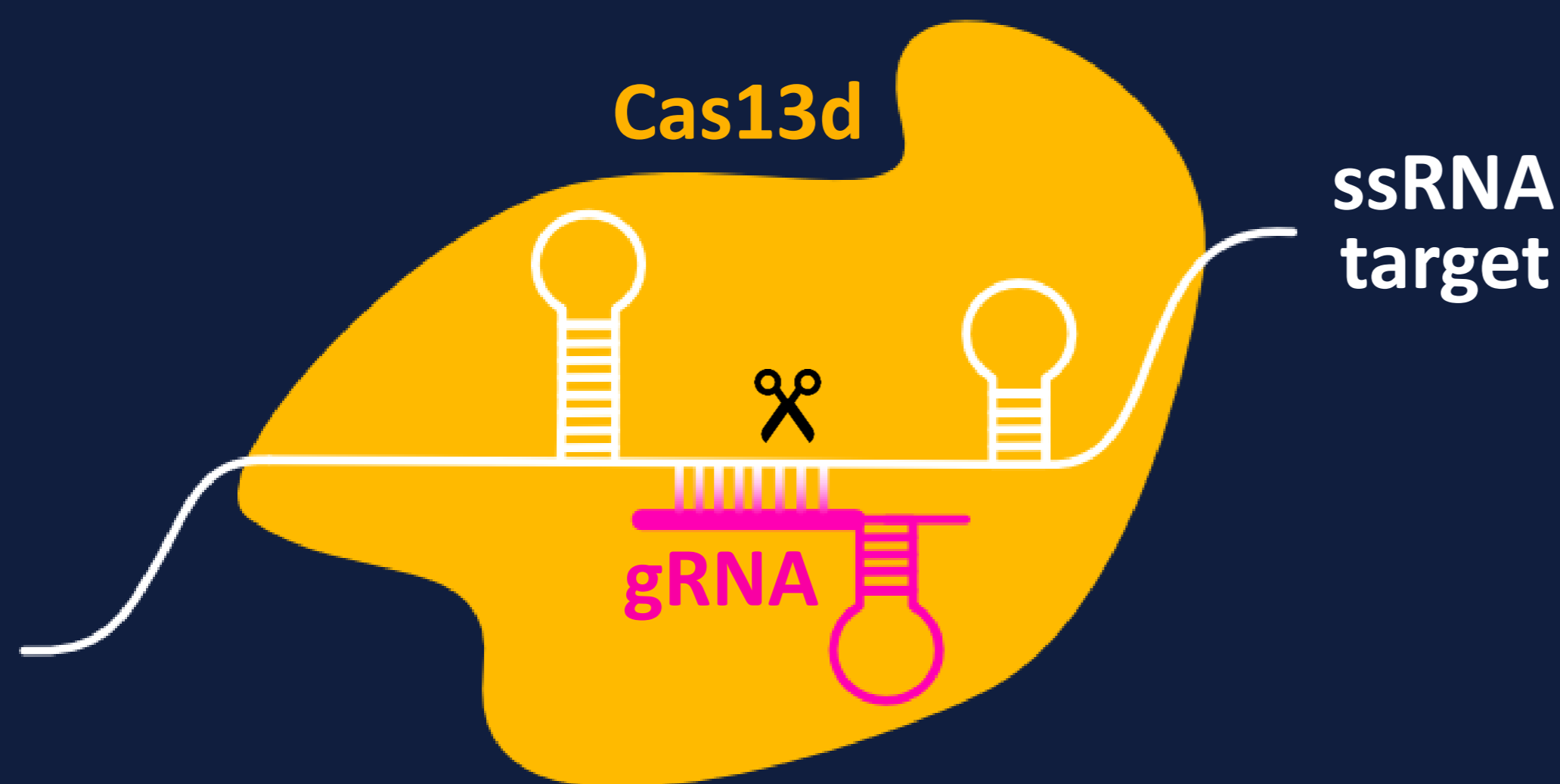


# Cas13d

## Tiny but mighty!

Cas13 is a family of enzymes that can target and cut RNA. There are 4 known members: Cas13a, Cas13b, Cas13c, and Cas13d. All Cas13 enzymes have 2 HEPN domains, which acts to cleave RNA. Cas13 enzymes can be programmed to cleave any RNA of choice by designing a guide RNA that is complementary to the target RNA sequence. At 2.8kb and ~930 amino acid long, Cas13d is a whopping 33% smaller than Cas9!

### RNA knockdown with Cas13d



Cas13d binds to a guide RNA that is complementary to the single-stranded RNA target.

Cas13d cuts the target RNA, which is then degraded. If the target is an mRNA transcript, this results in decreased protein production.

### Our Project: RNA editing with dCas13d-ADAR

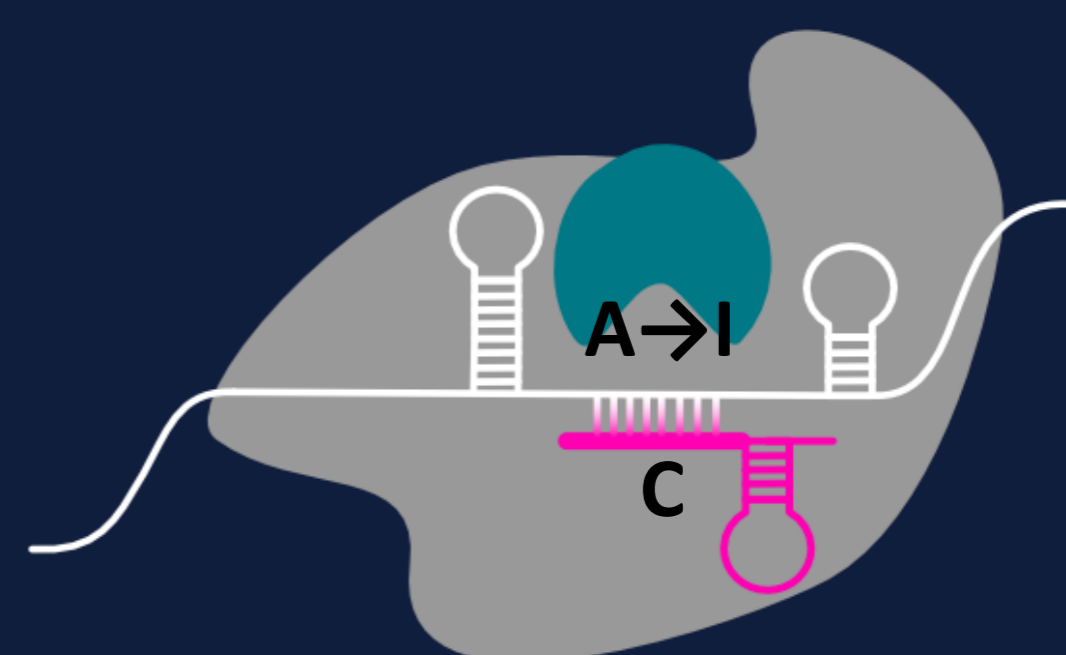
RNA editing occurs naturally in cells to regulate gene expression after transcription. An example is adenosine deamination (adenosine → inosine) which is catalyzed by ADAR1 and ADAR2 in humans. This results in changes to the mRNA transcript, which changes the resulting protein sequence.



Catalytically inactive/dead Cas13d (dCas13d) is first fused to ADAR to form a fusion protein.

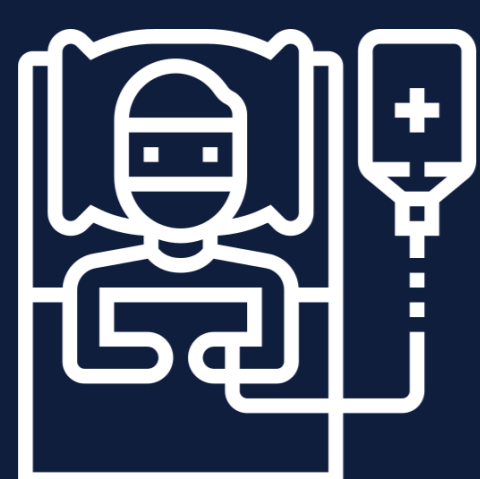


A guide RNA complexes with the fusion protein and directs it to the RNA target.



The ADAR on dCas13d then converts adenosine → inosine, which acts like a guanosine. These changes are **non-permanent**, making RNA editing safer than DNA editing!

### Other applications of dCas13d



Cure genetic diseases



Genetic screens



RNA imaging



RNA regulation

#### References:

- [1] Granadas-Riveron, et al. (2018). *Cancer Res.* 78(15): 4107-4113.
- [2] Noack, et al. (2018). *Front Neurosci.* 12: 85.