

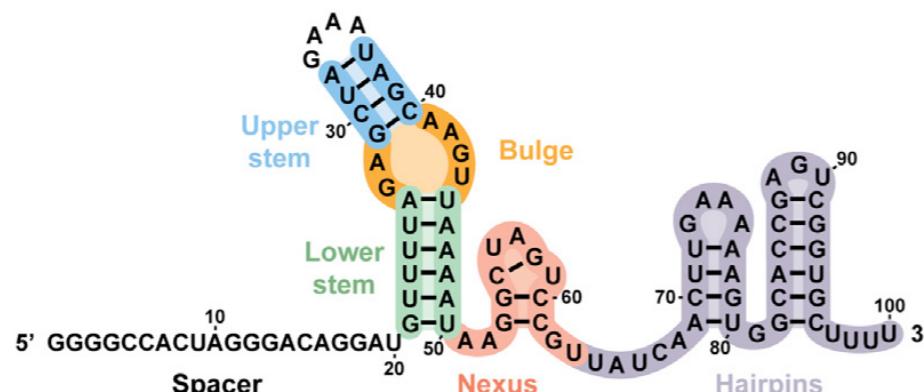
gRNA/Cas9

Molecular Dynamics

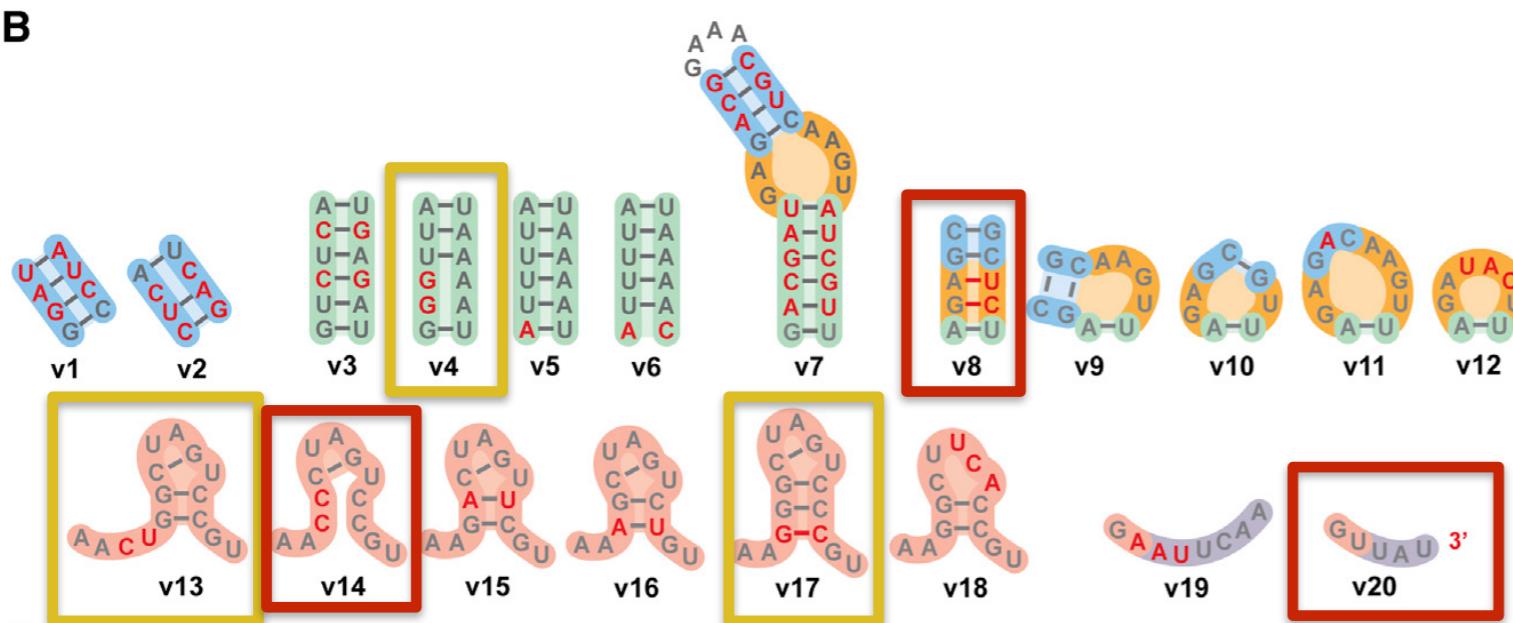
Zhewei Chen
Ch121 Final Project
04/06/2018

gRNAs have structure and function

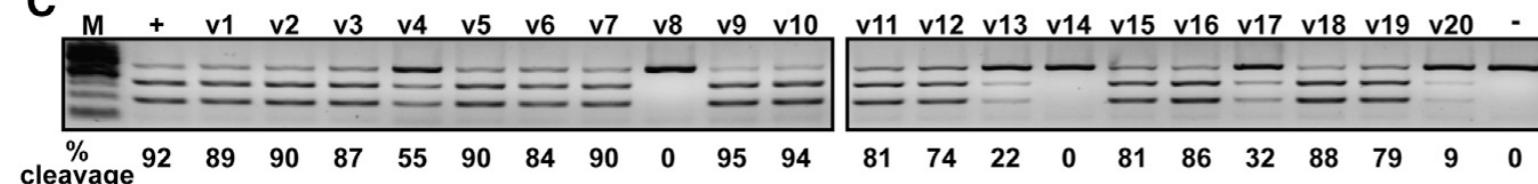
A



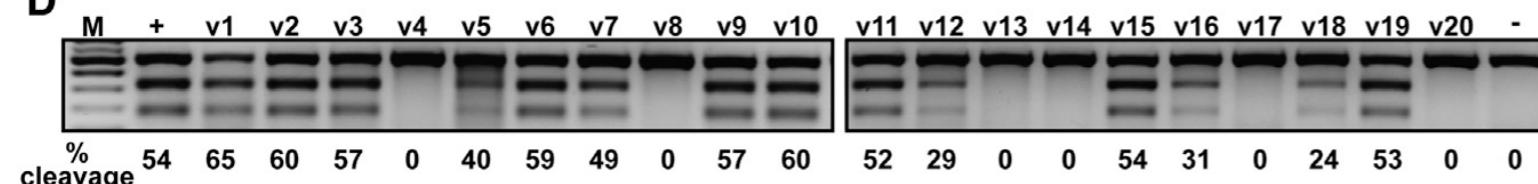
B



C



D



Mutations boxed in red cause complete loss of gRNA activity

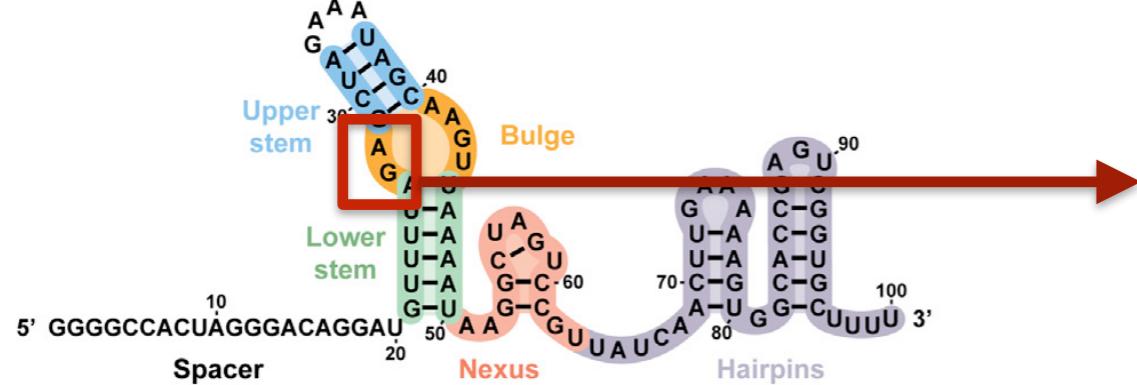
Mutations boxed in yellow significantly decreases gRNA activity

Nexus region and bulge loops are most important gRNA binding motifs

Cas9 handle stem needed for dCas9 binding

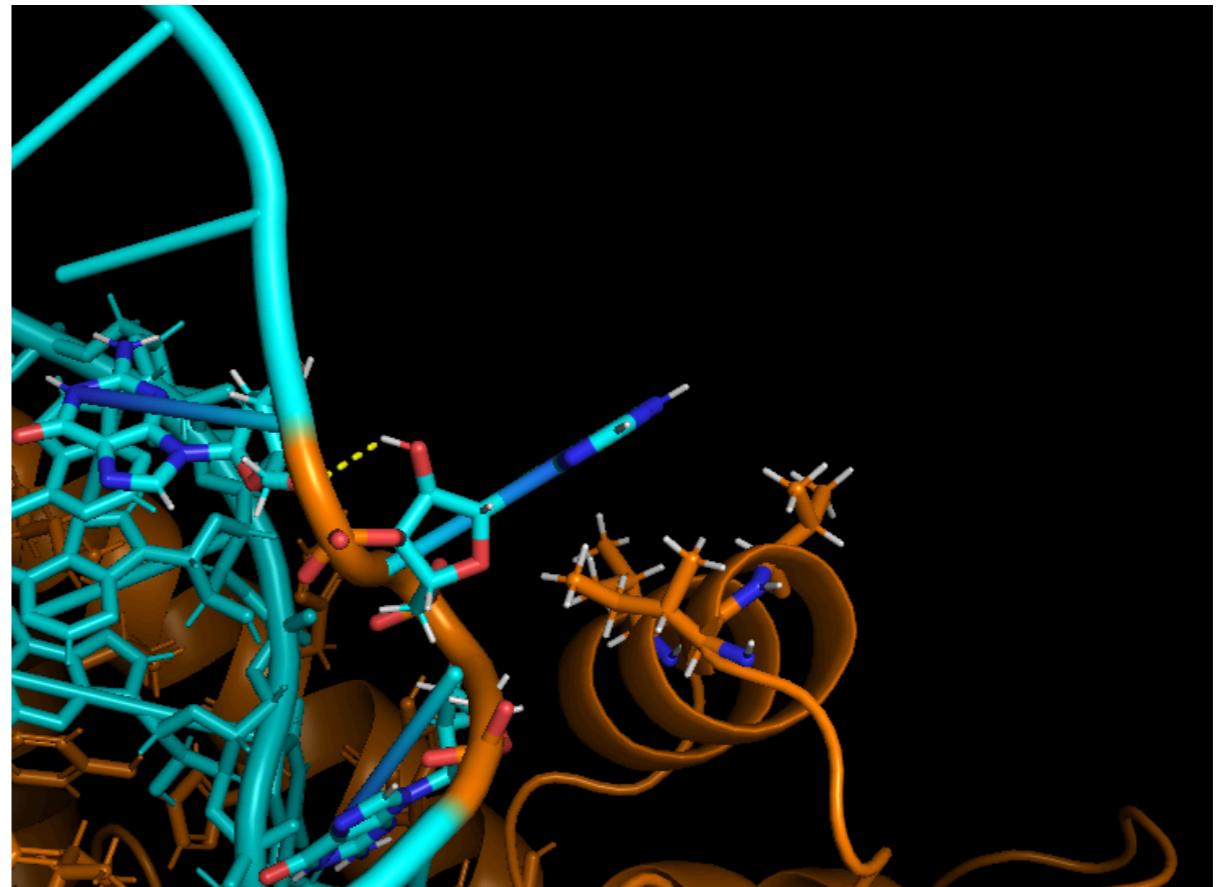
Terminator hairpins needed for termination and maybe function?

But are there salt bridges? In dCas9 handle?



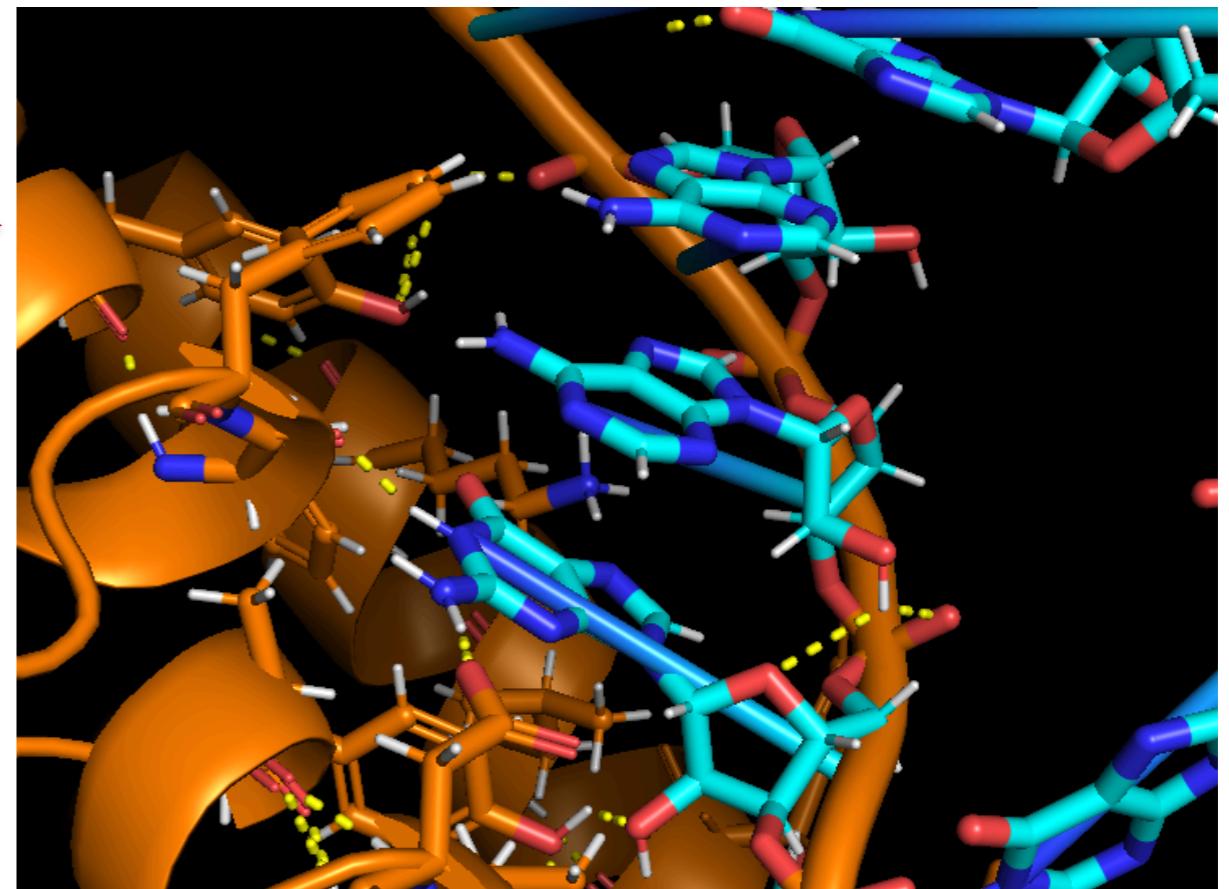
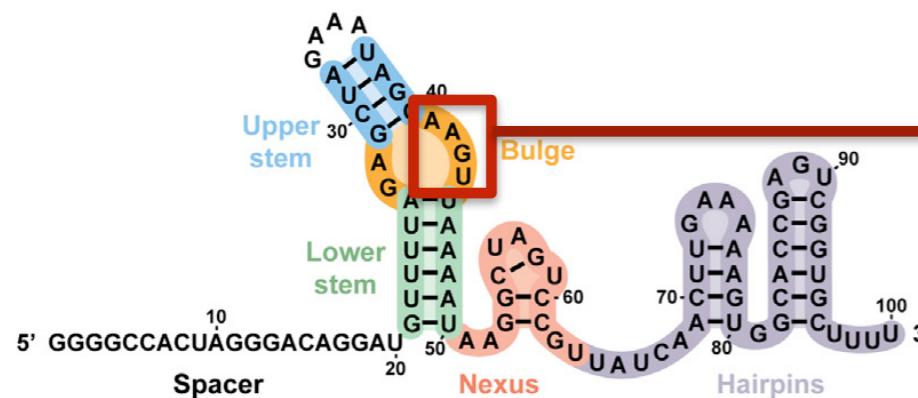
Left bulge is important for function,
but does not have any apparent salt
bridges with dCas9.

No interactions with the protein at
all. The nearby side chains are all
non-polar.



Yellow dashed lines =
all polar contacts between residues
4A away from the left bulge of gRNA

But are there salt bridges? In dCas9 handle?

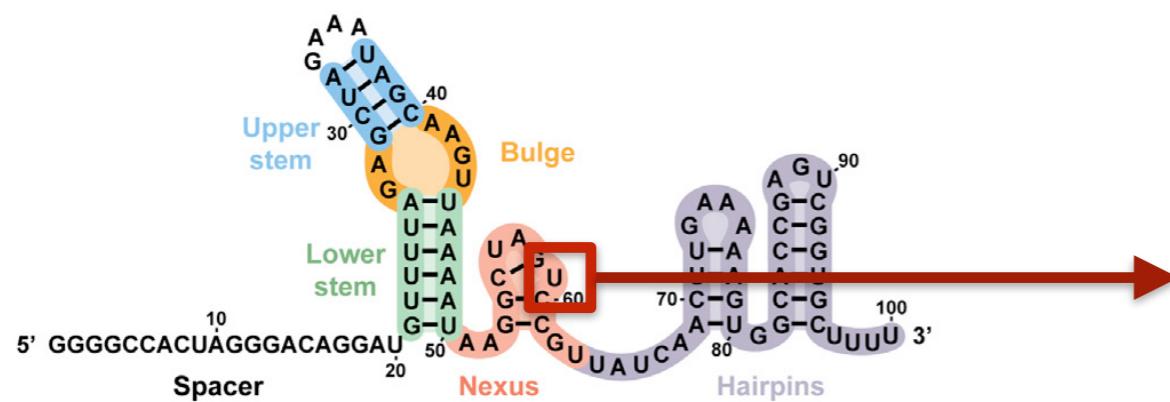


Right side bulge is important for function, but does not have any apparent salt bridges with dCas9.

Most salt bridges involve contact with the phosphate backbone of the gRNA

Yellow dashed lines =
all polar contacts between residues
4A away from the left bulge of gRNA

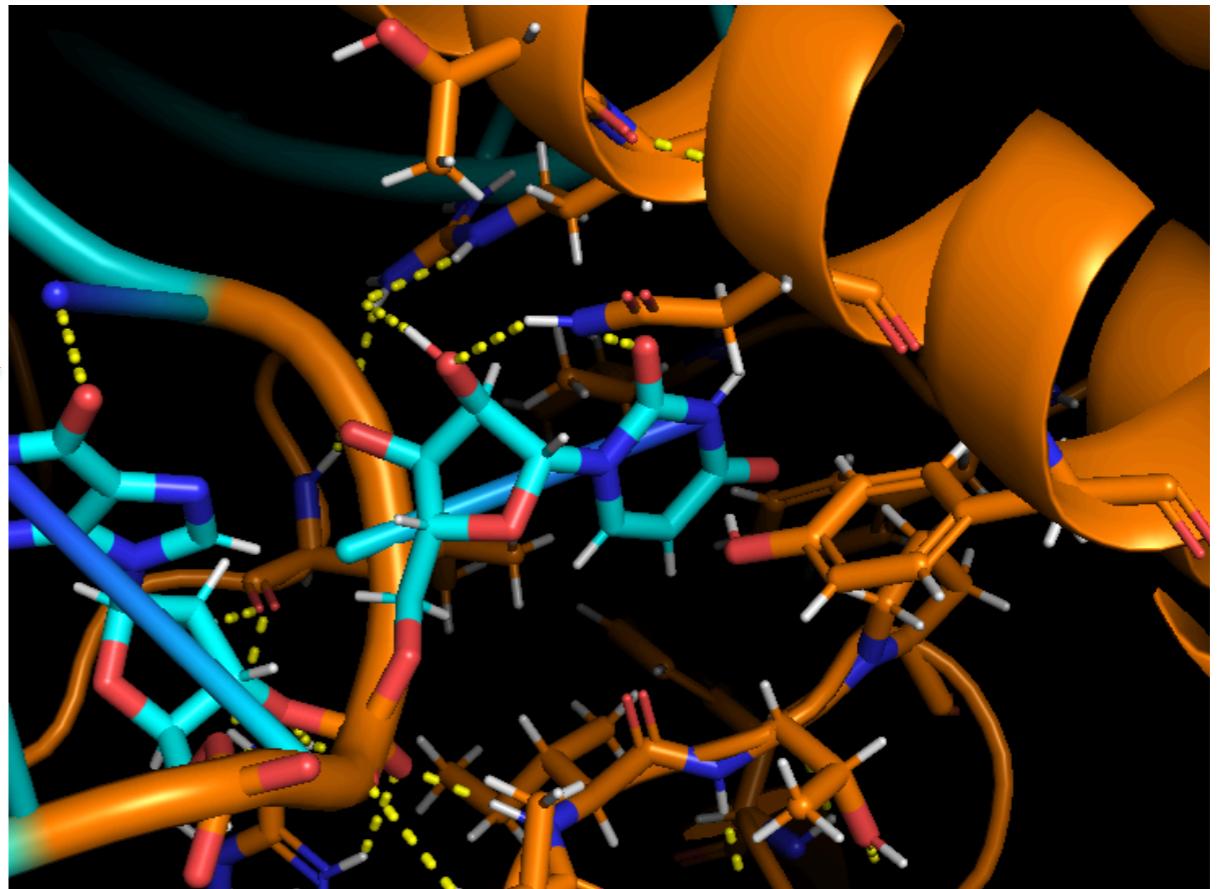
But are there salt bridges? In Nexus domain?



Nexus loop is a very conserved domain.
If there are salt bridges, it should be
here.

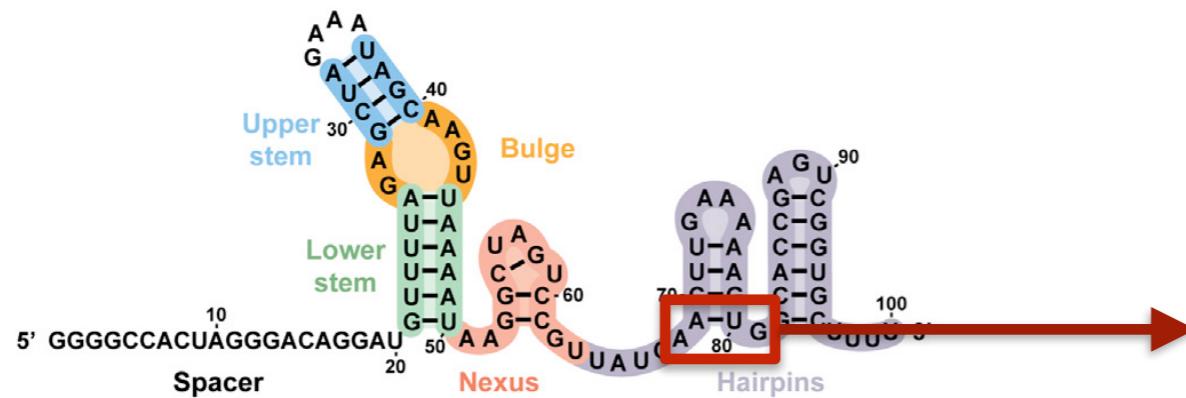
Here a single nucleotide fits inside some
kind of binding pocket. However, this
pocket is mostly non-polar.

Again, most salt bridges involve contact
with the phosphate backbone of the
gRNA.



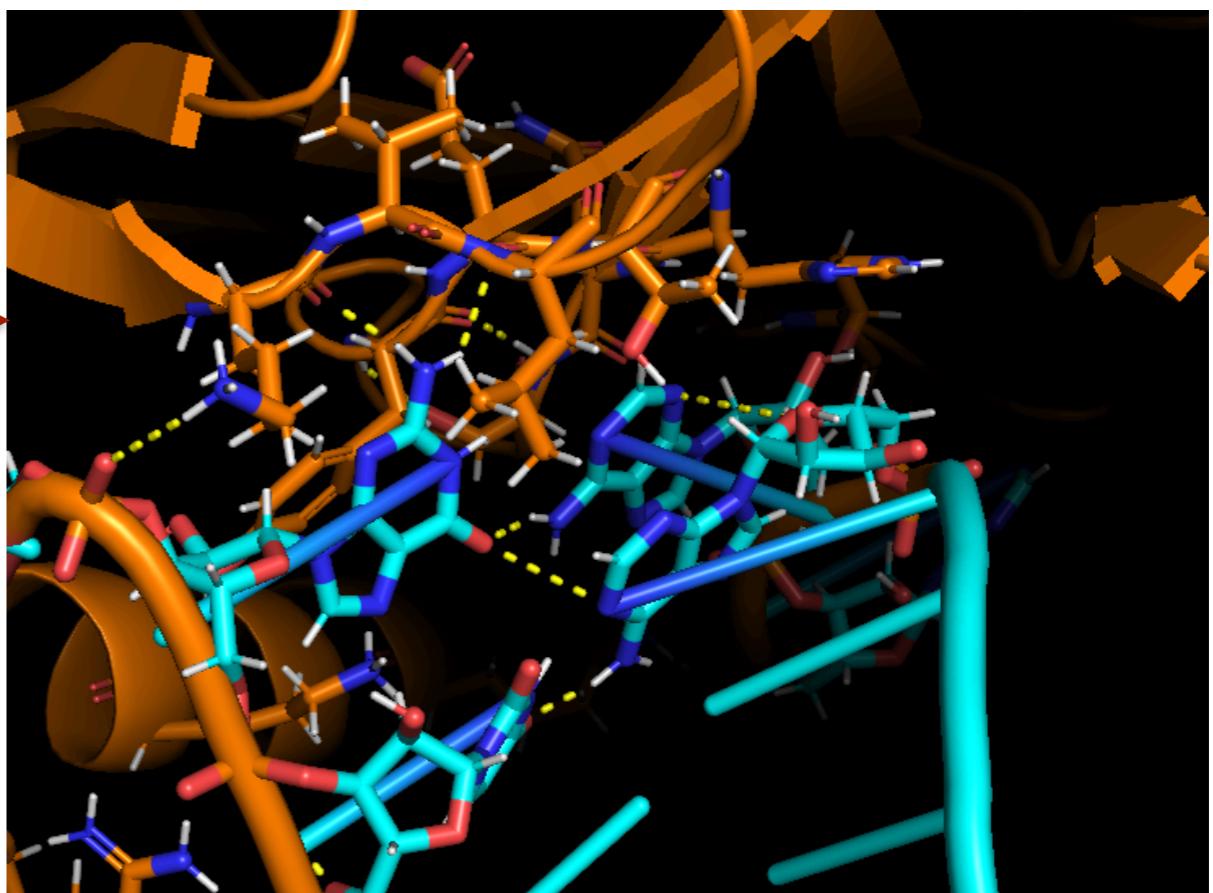
Yellow dashed lines =
all polar contacts between residues
4A away from the left bulge of gRNA

But are there salt bridges? Near the Terminator stem?



The terminator loop is important for function. Breaking this loop enables the cgRNA activity switch.

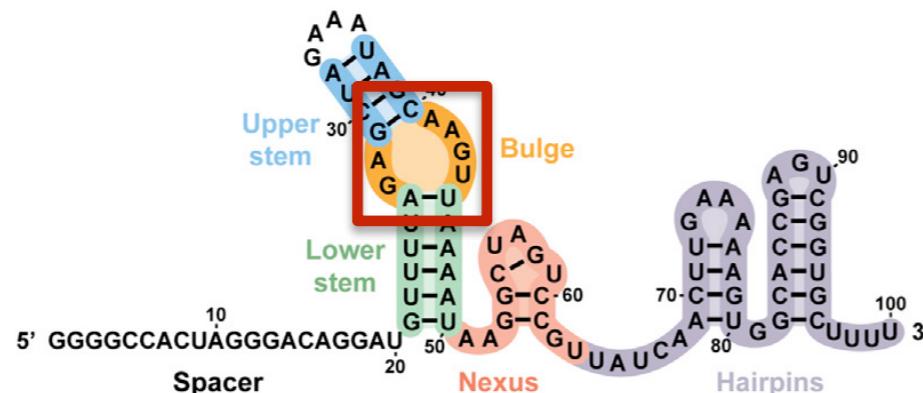
Again, no significant hydrogen bonding with the protein. :??



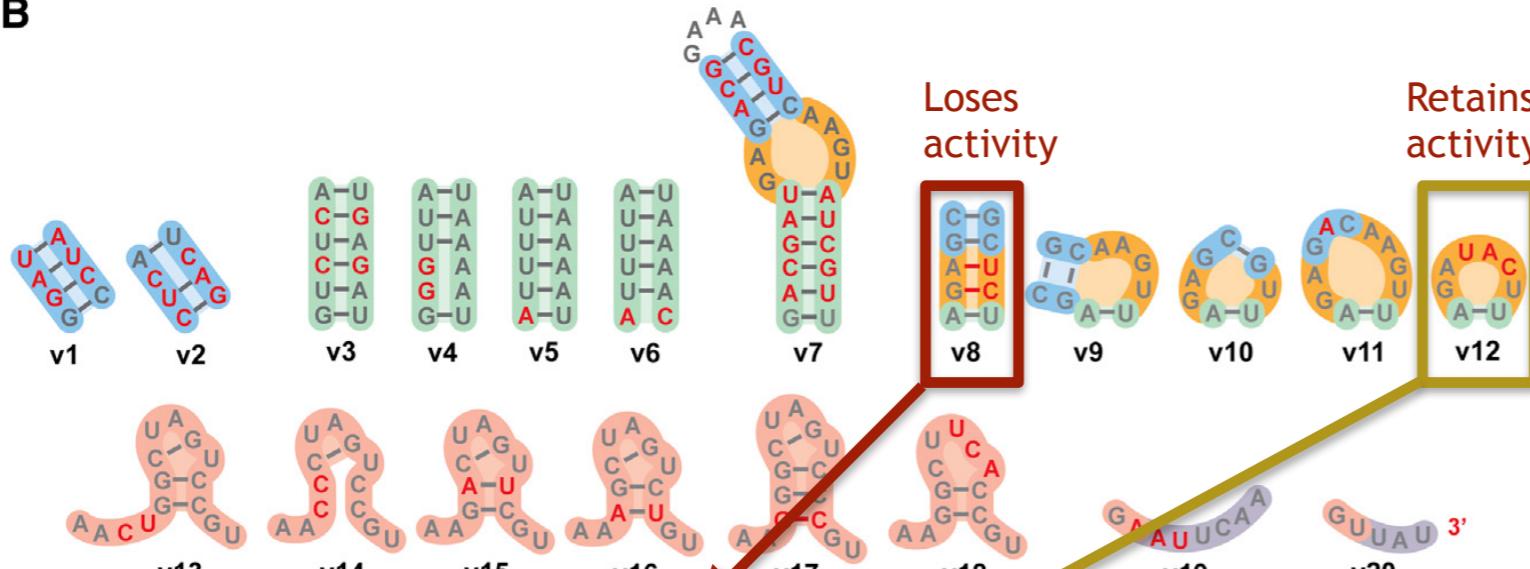
Yellow dashed lines =
all polar contacts between residues
4A away from the left bulge of gRNA

Build some homology models

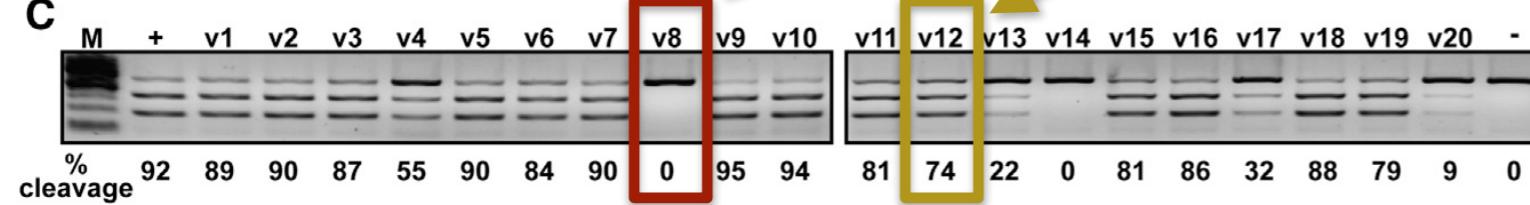
A



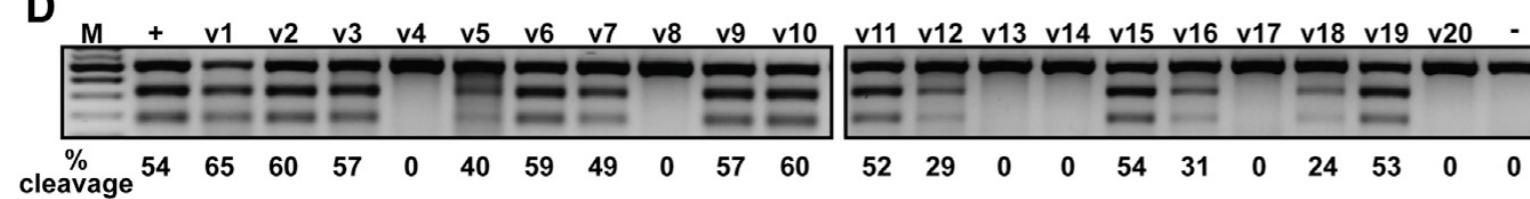
B



C



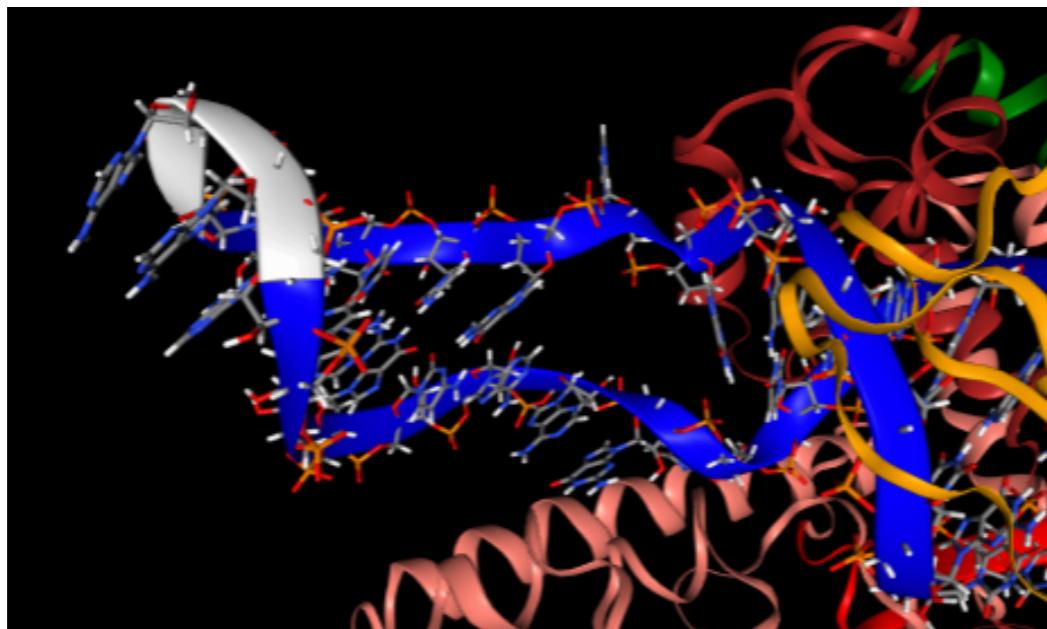
D



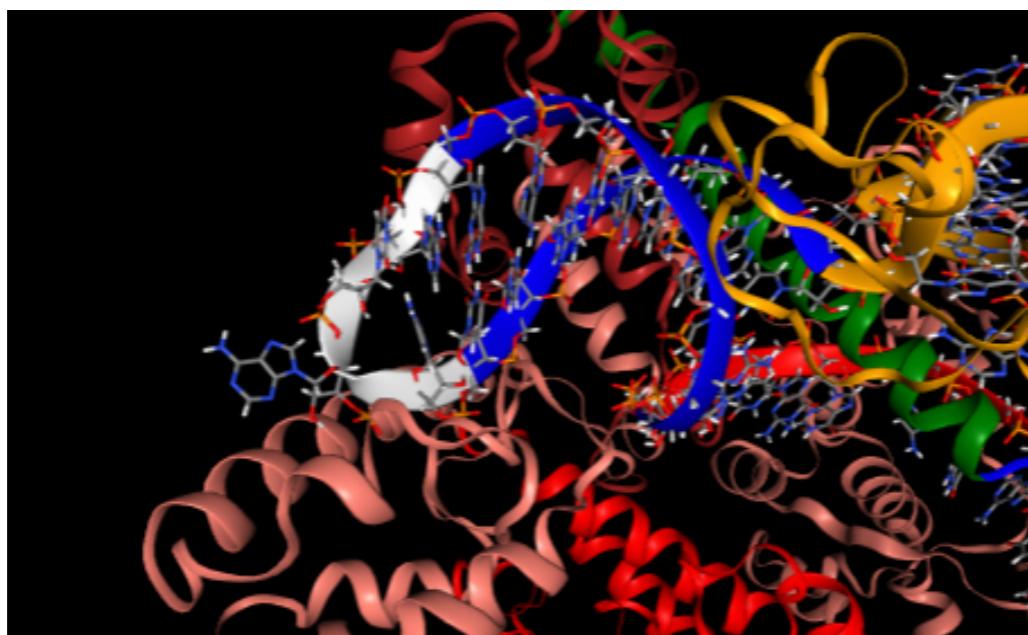
Approach

- 1) Introduce mutations to gRNA via RNA homology modeling
- 2) See how residues and domains move before and after mutations
- 3) If important interactions exist, we expect to see some diffusional shift during molecular dynamics simulation

Build some homology models



Unmodified gRNA

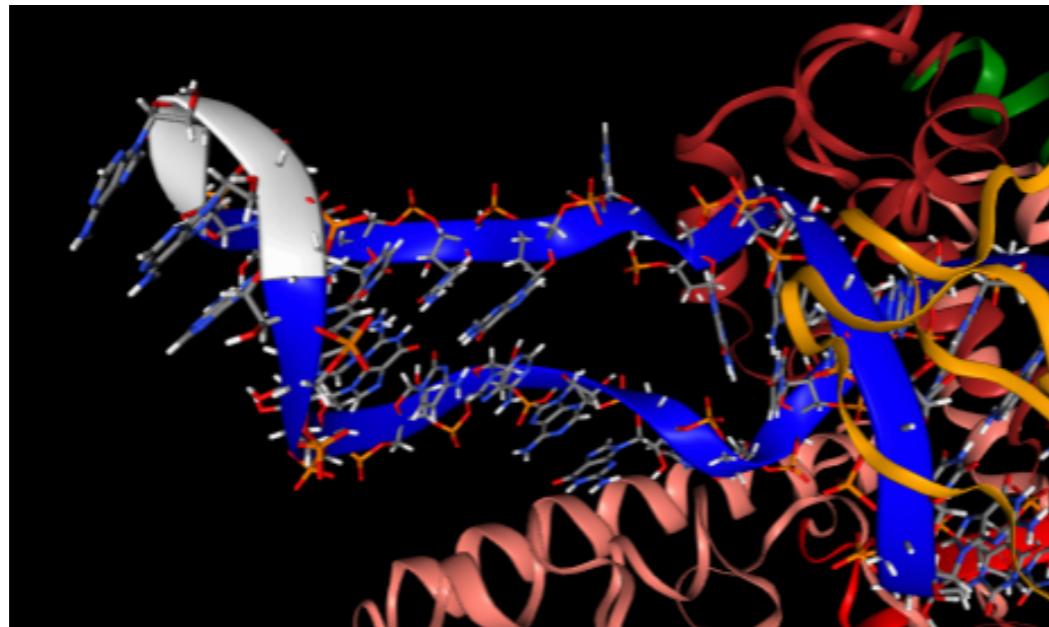


gRNA with deleted upper dCas9 handle

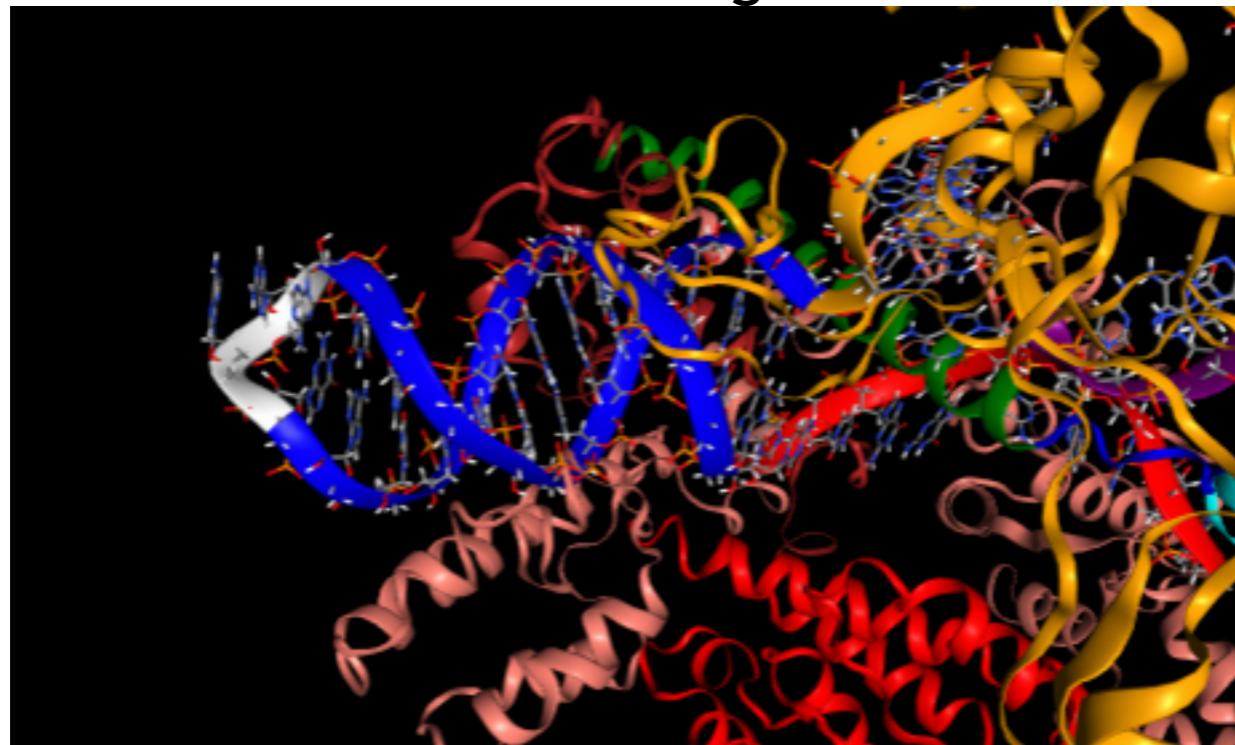
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Unmodified gRNA

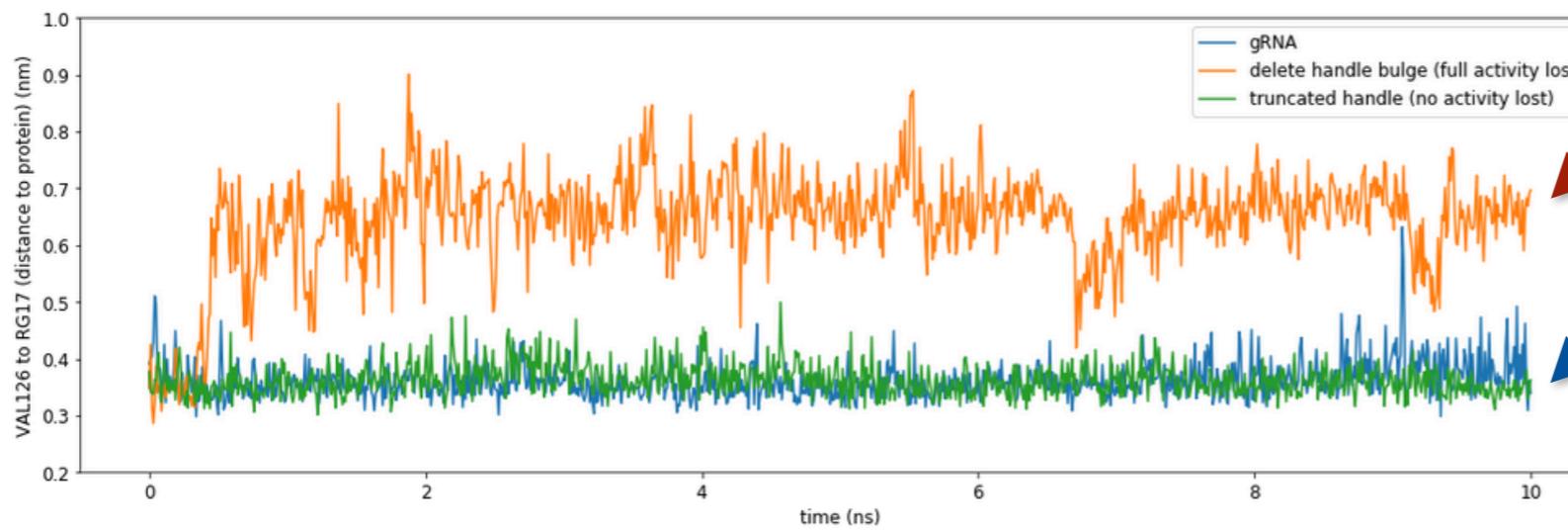
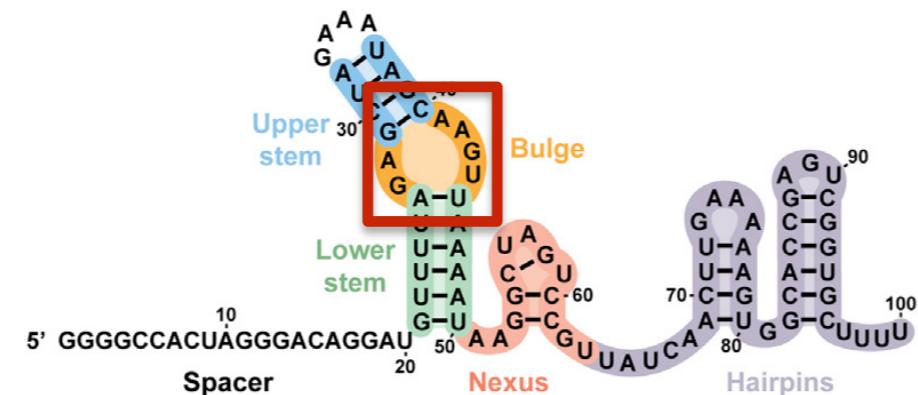
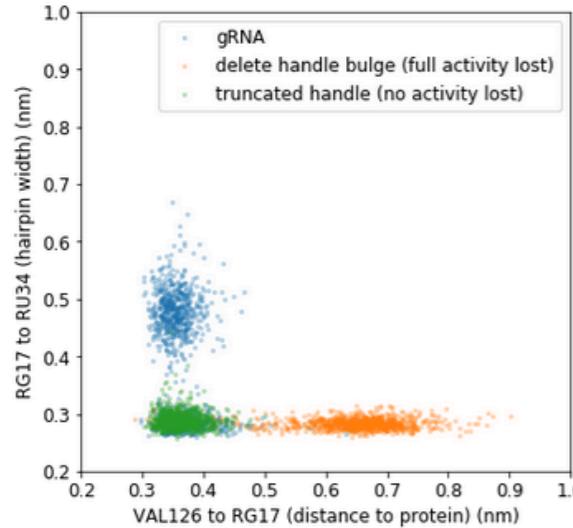


gRNA with deleted bulges

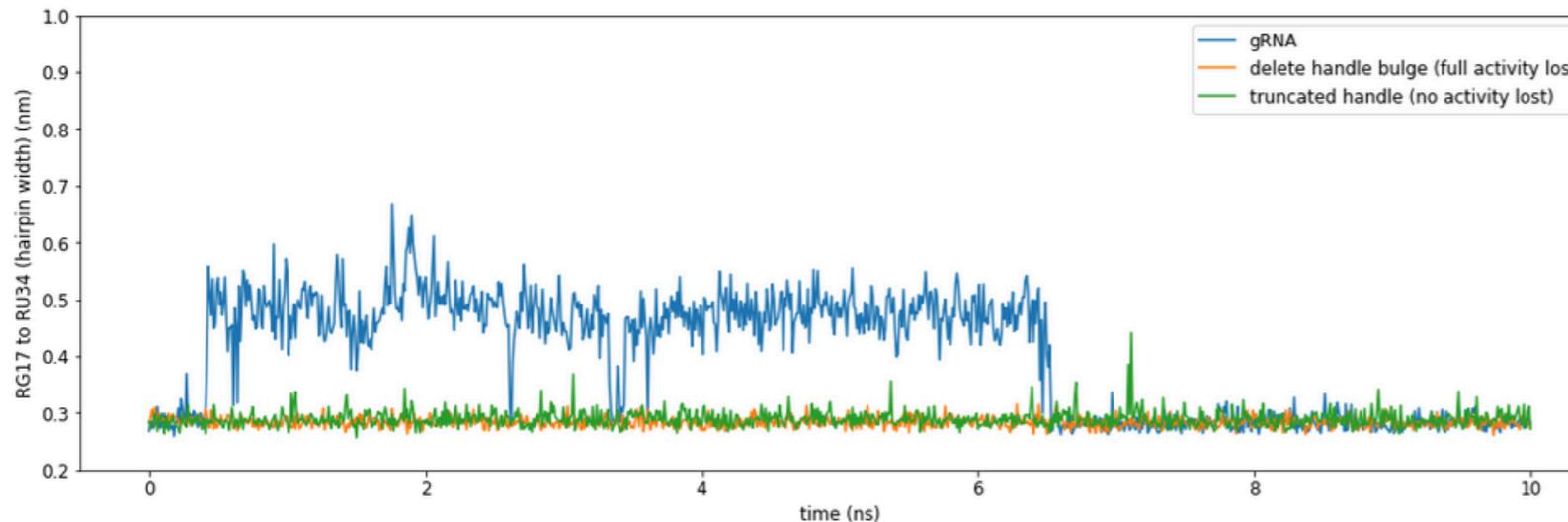
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If we mutate, does it wiggle?



Deleting the bulge causes gRNA to wiggle more?

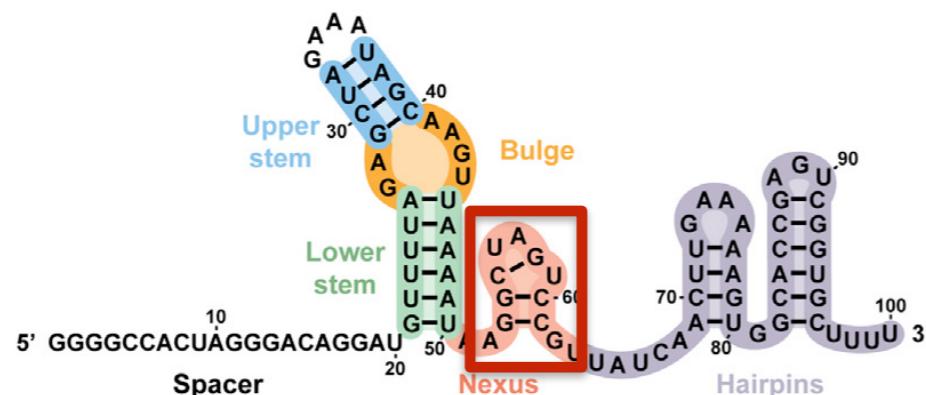


Truncated handle and regular gRNA do no wiggle much

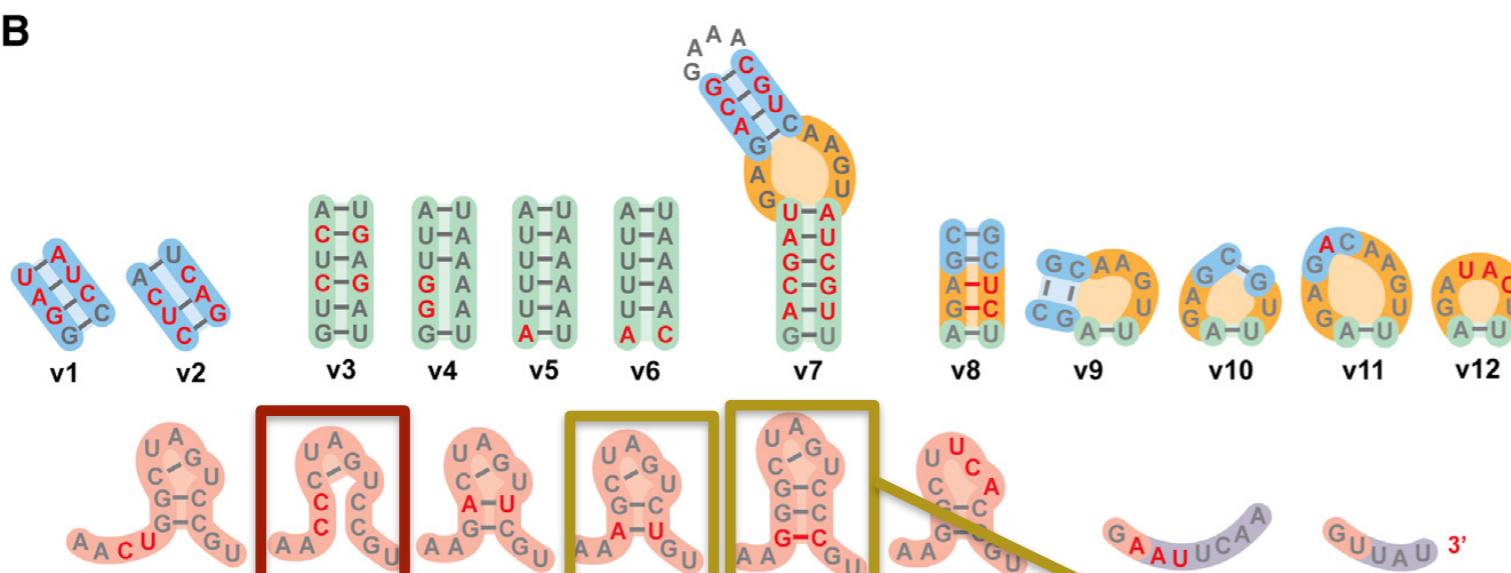
Unclear if this would cause gRNA to fall out binding pocket in a longer trajectory simulation

Build some homology models

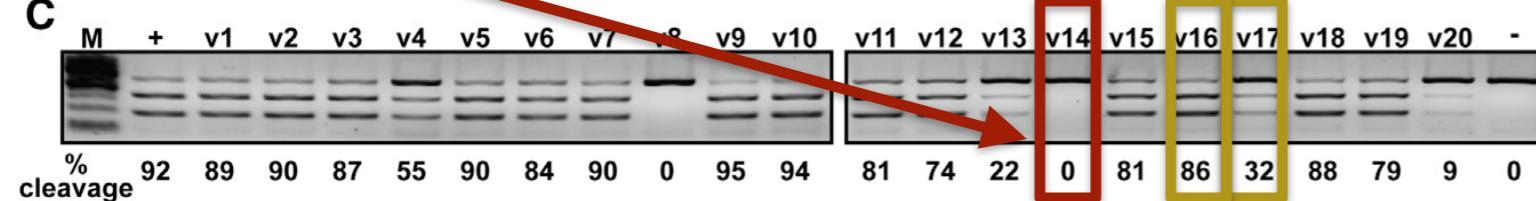
A



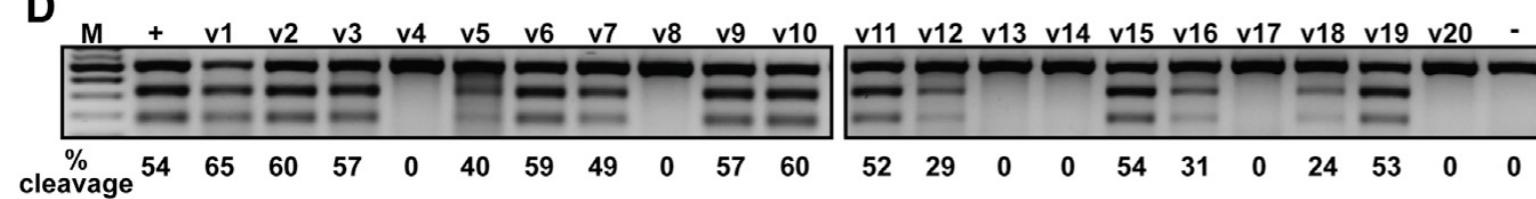
B



C



D

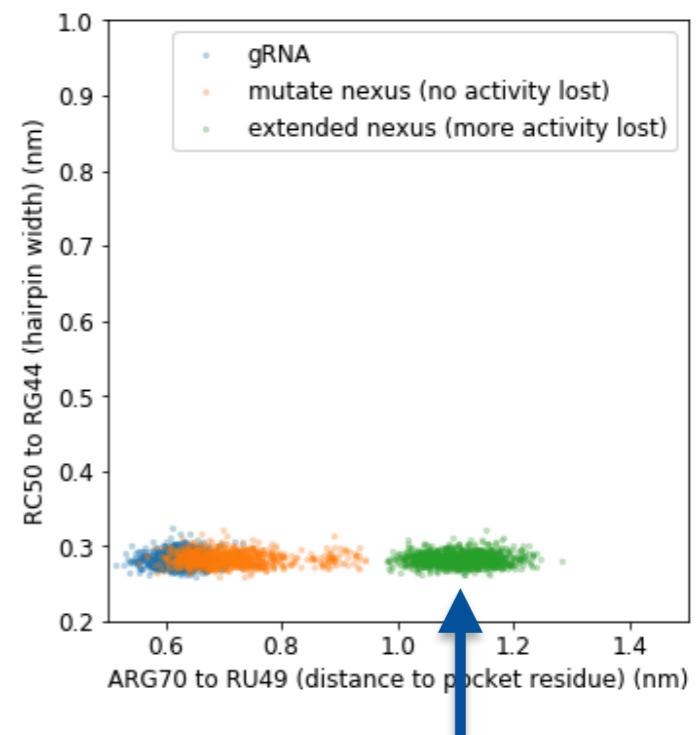
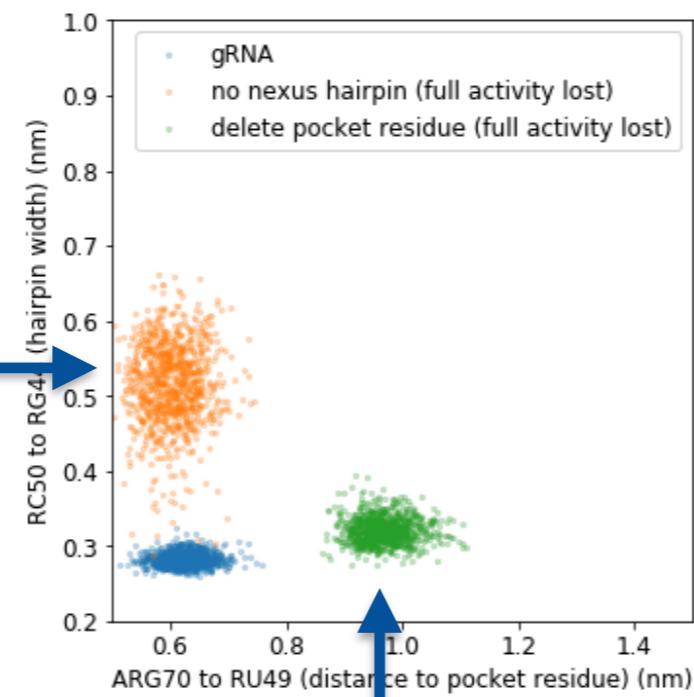


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If we mutate, does it wiggle?

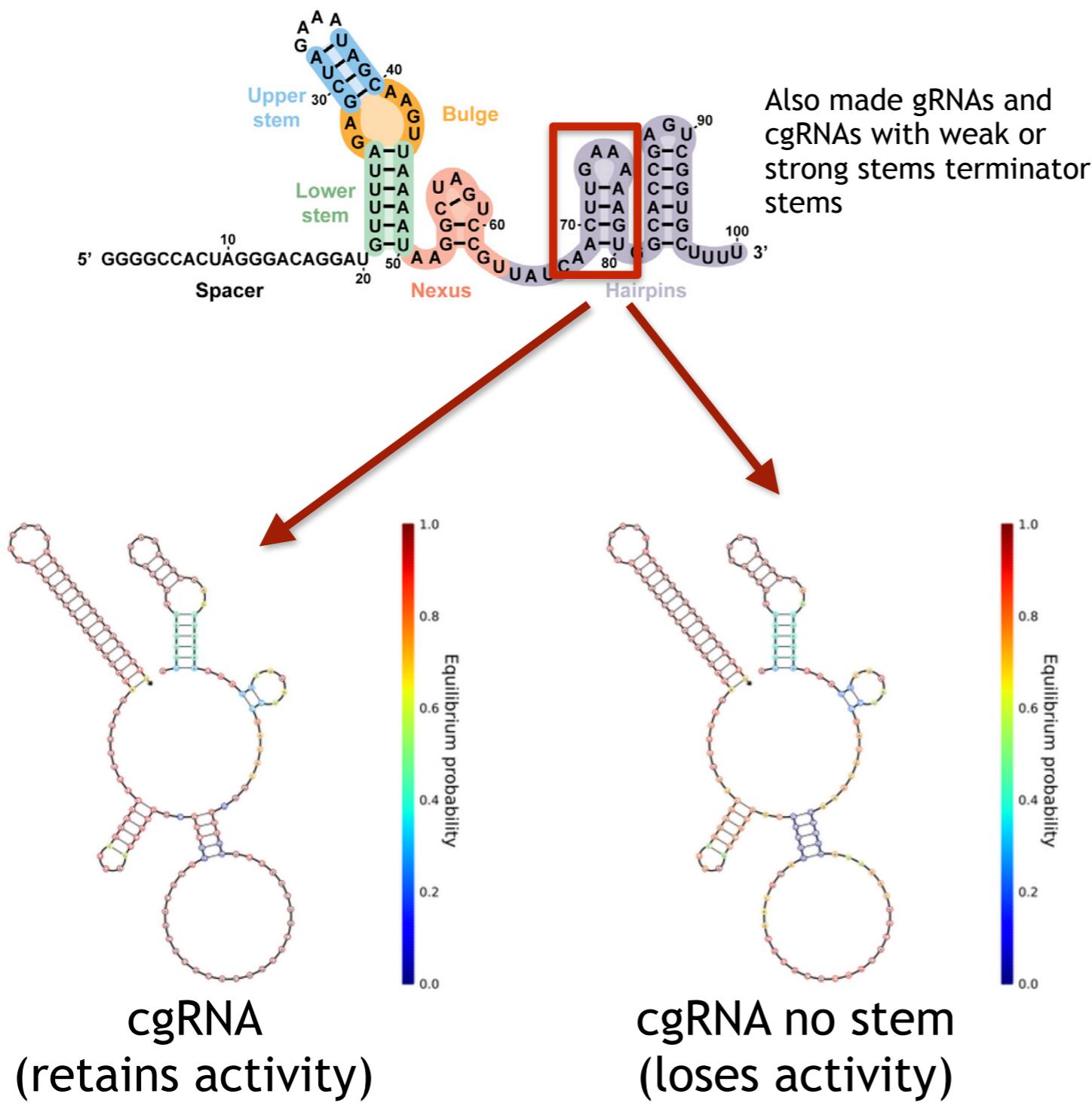
Hairpin starts to unfold, as expected



Still unclear how this affects protein activity?

Distance to pocket residue starts to increase as with mutations that disrupt function

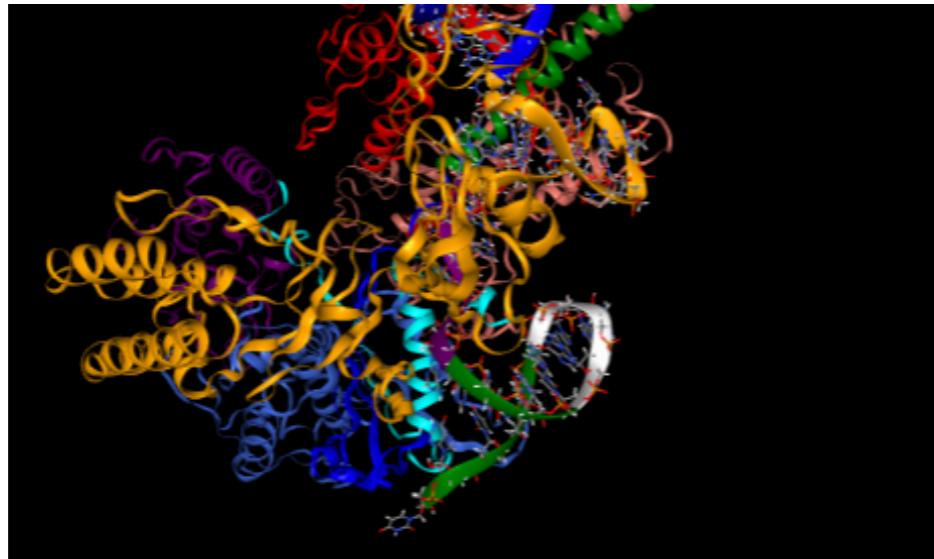
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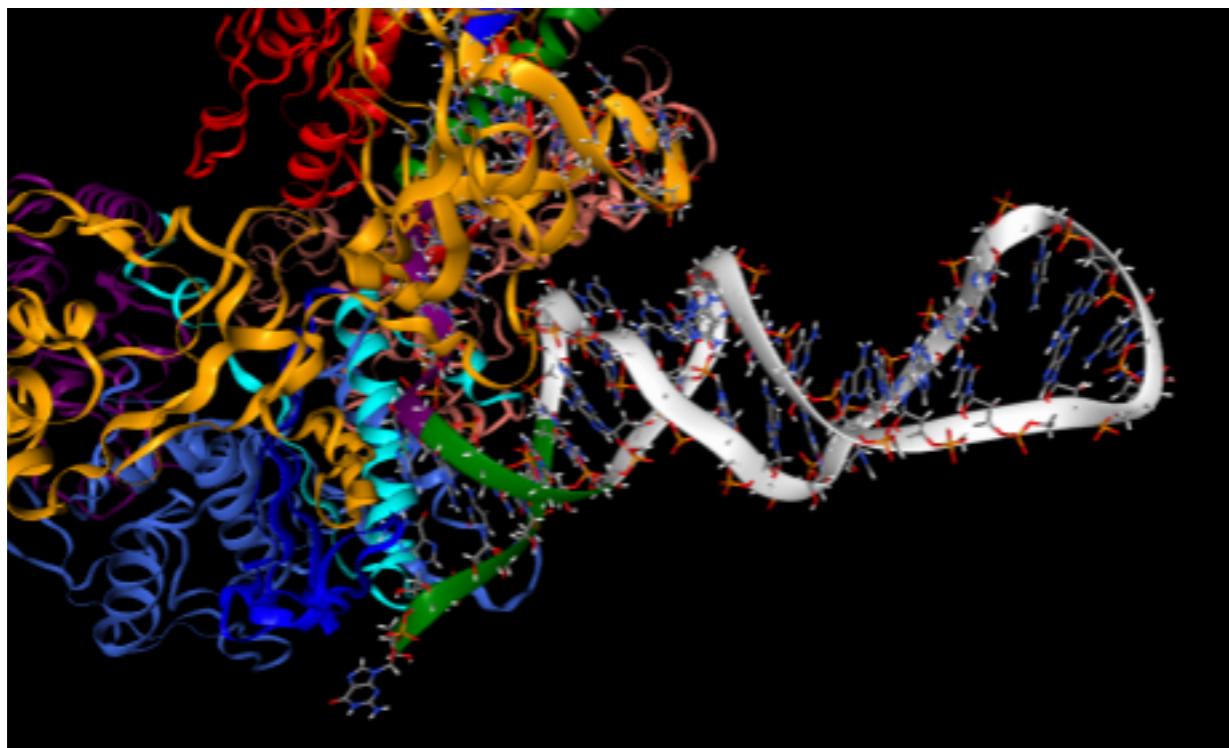
Can also make cgRNAs via Rosetta



Unmodified gRNA

Approach

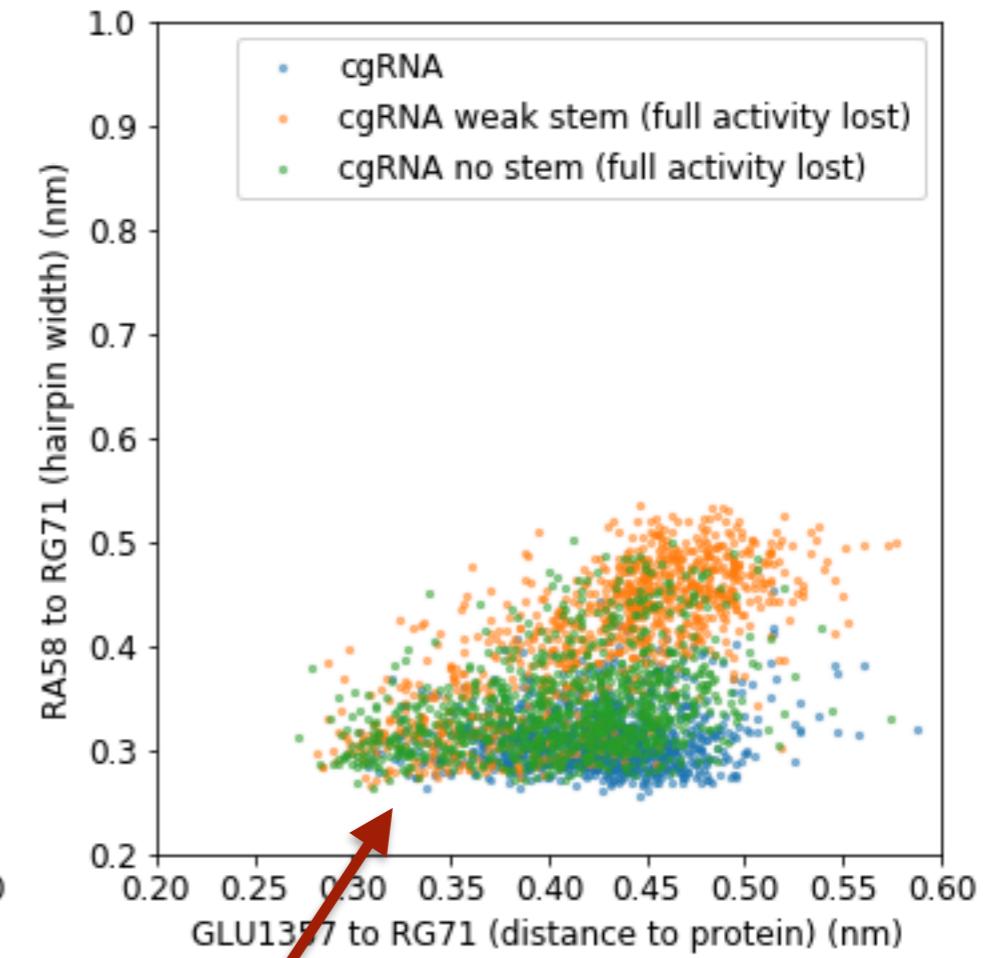
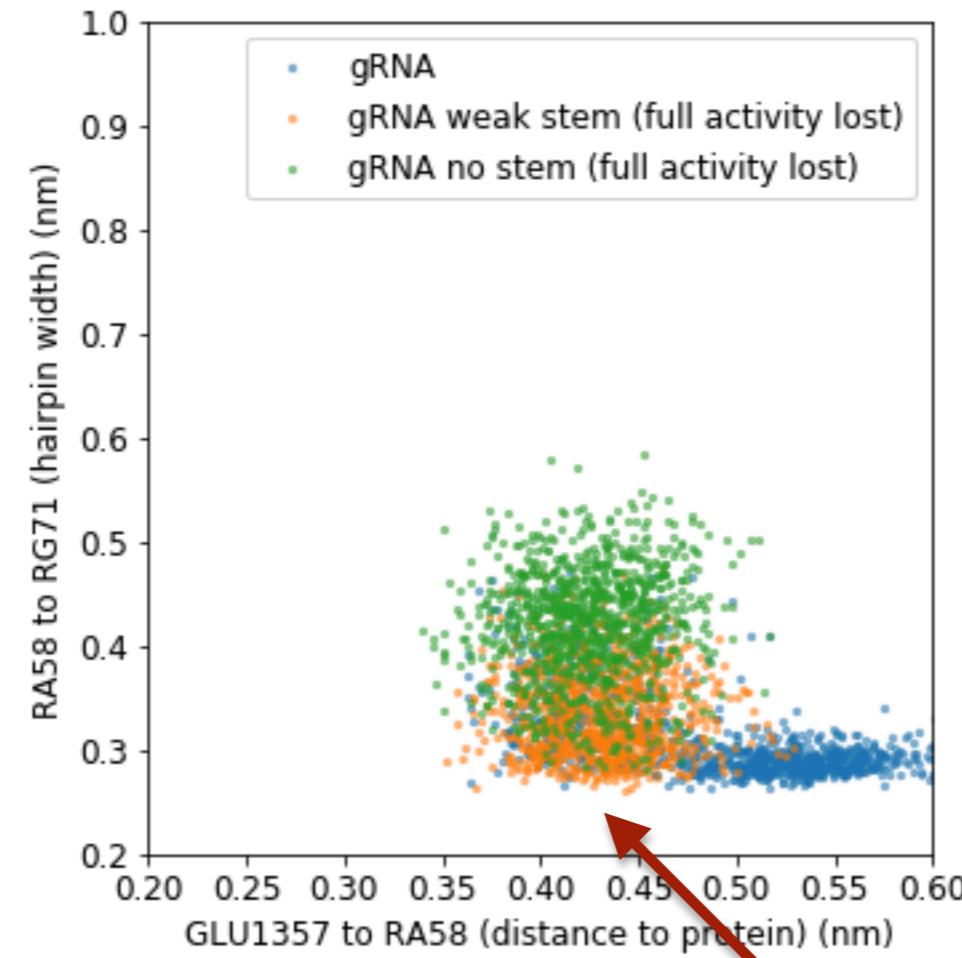
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cgRNA

If we mutate, does it wiggle?

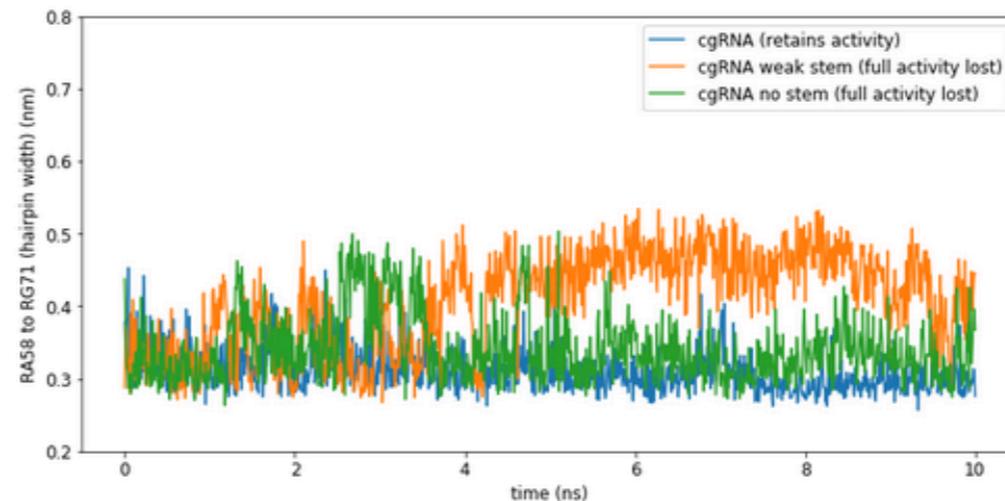
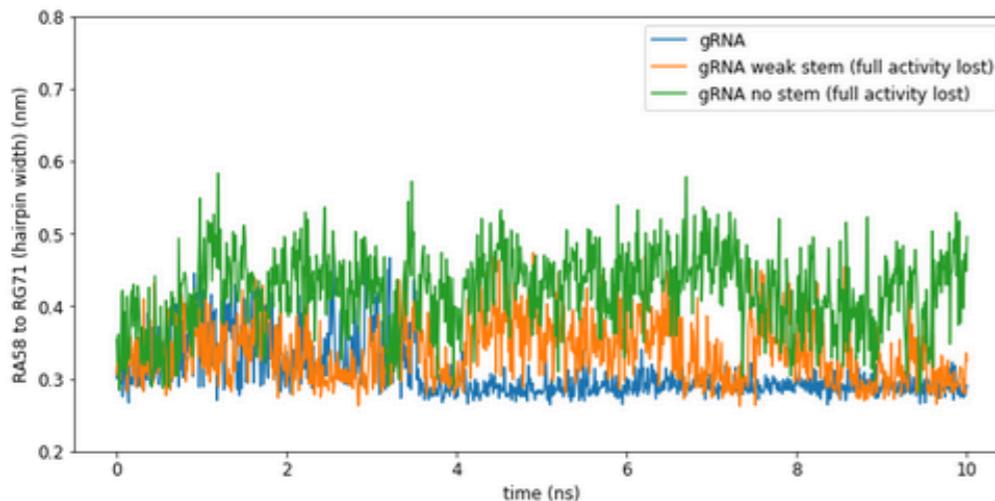
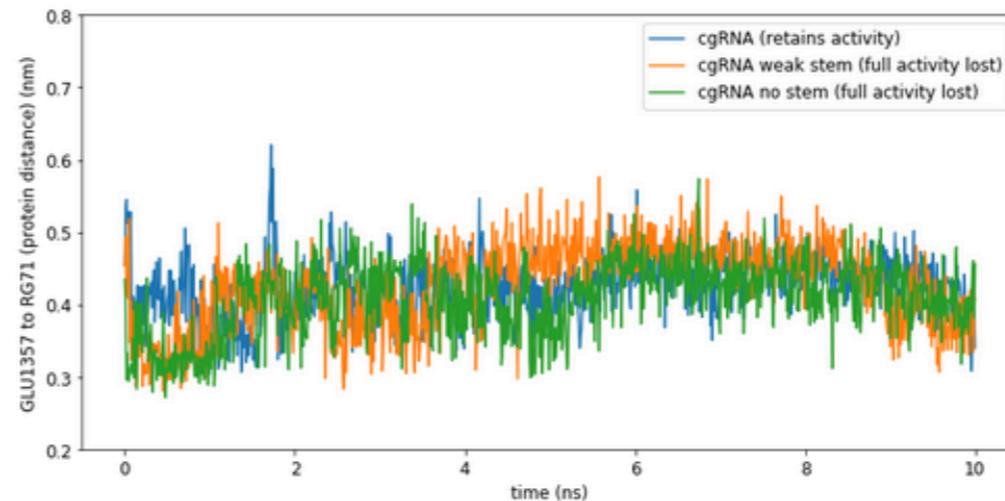
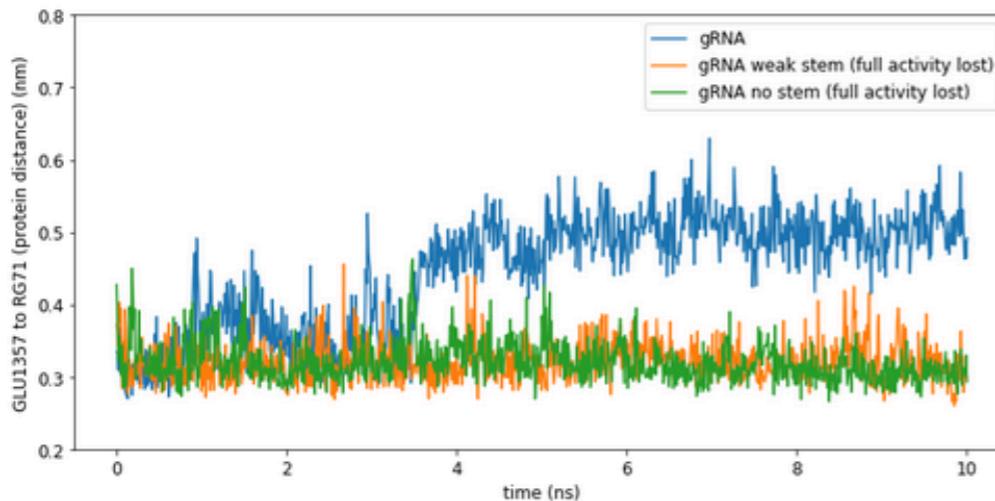
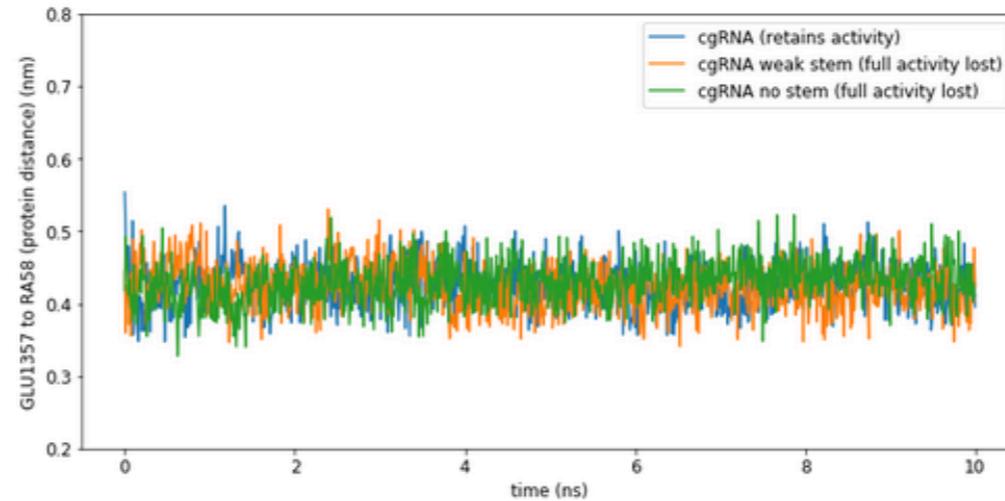
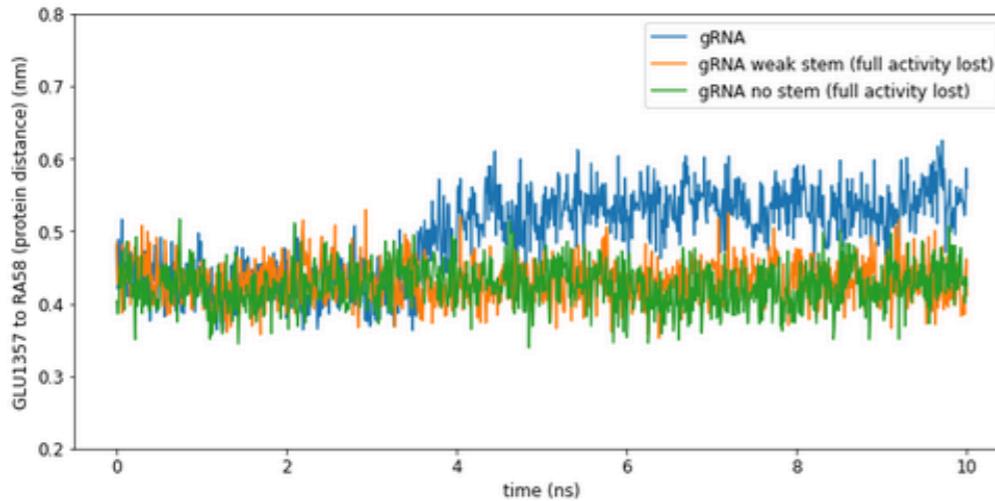
Hairpin width increases with hairpin breaking mutations (expected)



Distance to protein decreases for loss of function mutations :?

Molecular dynamics is not really informative on why cgRNA work

If we mutate, does it wiggle?



No interesting correlation with binding to protein surface



Hairpin width increases with hairpin breaking mutations (expected)

Challenges

- Hard to simulate trajectories long enough to see something interesting
- Most simulation effort wasted on water vibrations
- Initial states are usually stuck in local energy minima

Possible next steps

Coarse graining with cgmartini and elastic network

Current system:

Total # of atoms = ~220k

Run time = 1 ns/hr

Coarse grained:

Total # of atoms = ~220k/50 = 4400 atoms?

Run time = 1ns/hr * 50 = 40ns/hr = ~1us per day

Adaptive weighted histogram sampling

Try to bias trajectories to sample under represented ensemble conformations

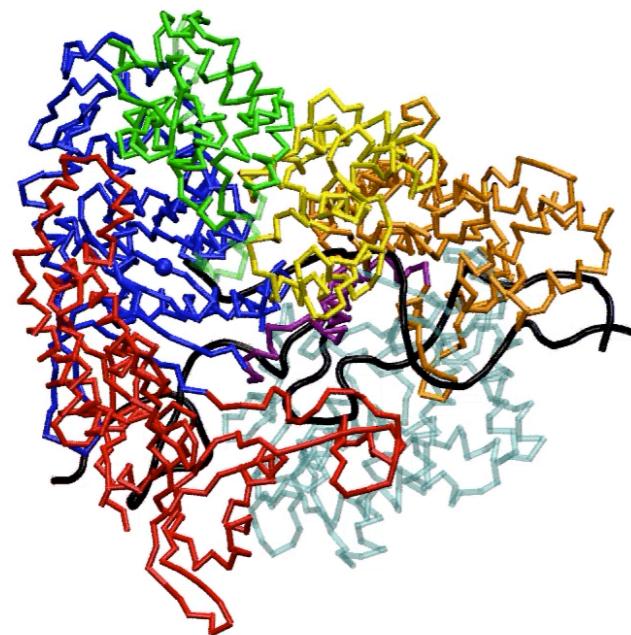
Can provide more efficient sample of reaction coordinate

Gaussian Accelerated molecular dynamics

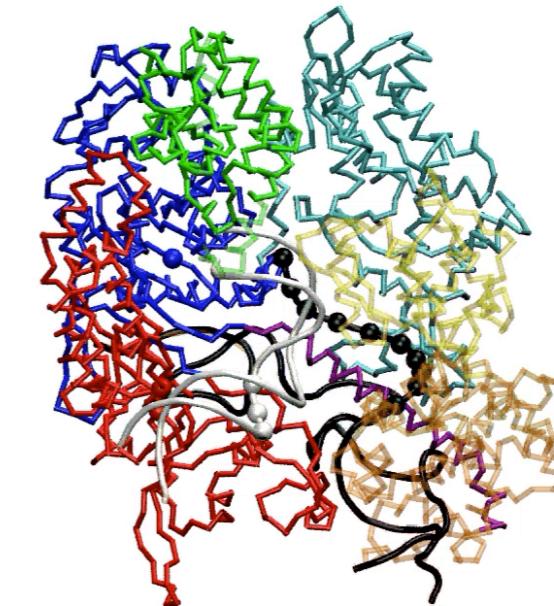
Similar to AWH but only implement in Amber MD

Tries to use boost potentials to escape energies wells and do better sampling

Coarse grain gRNA + Cas9 dynamics



Cas9 → Cas9:gRNA

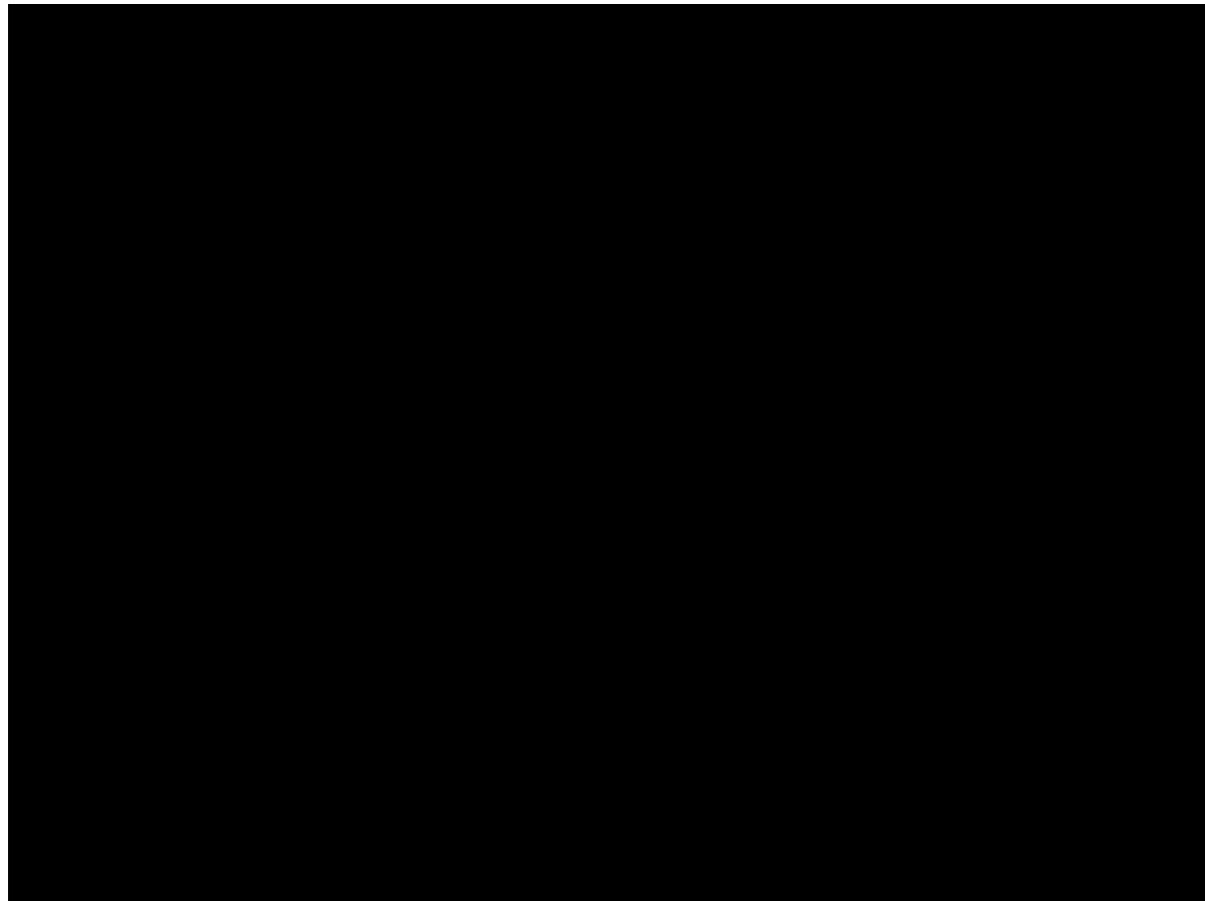


Cas9:gRNA → Cas9:gRNA:DNA

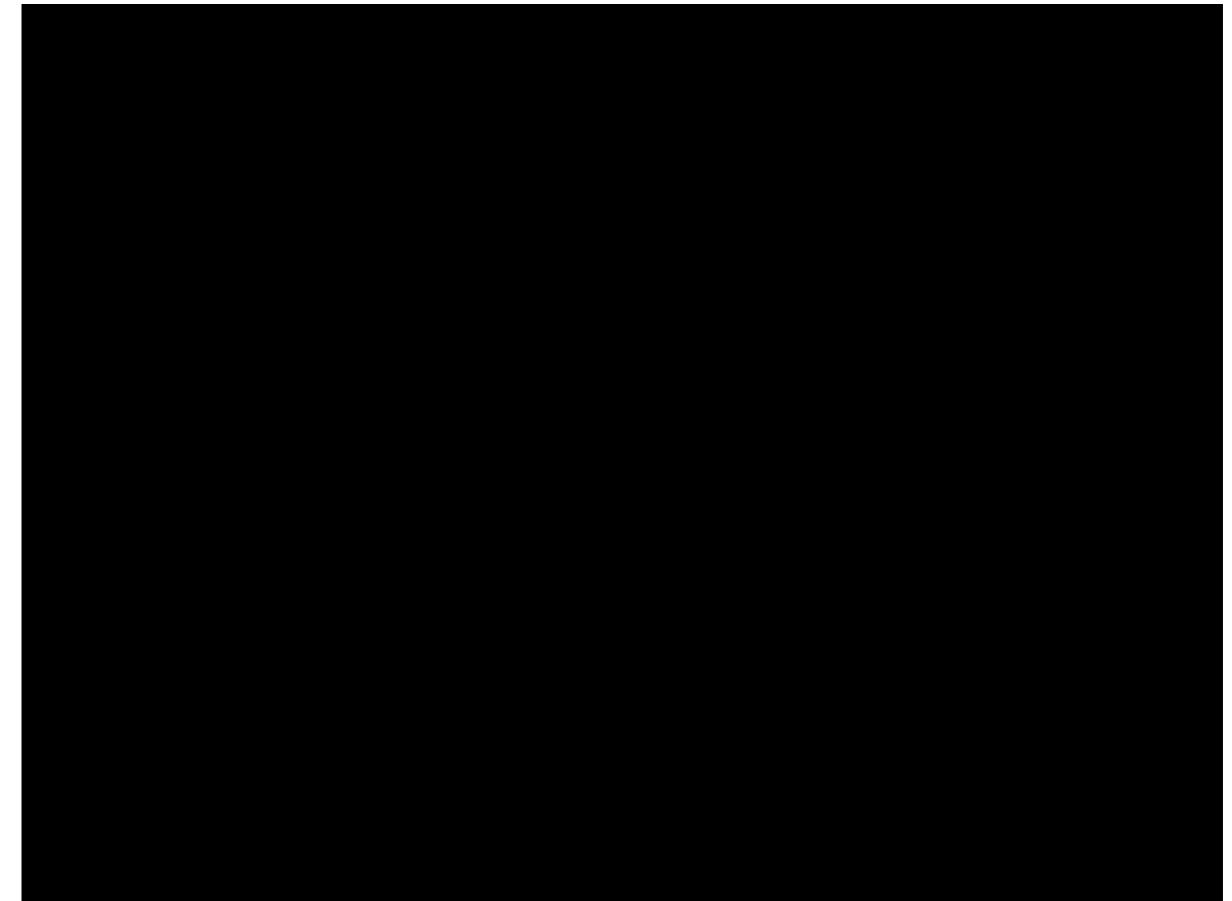
Can be done with limited compute resources! :)
But results may not always be accurate

Results from <https://doi.org/10.1002/prot.25229>

Or accelerated full atom MD?



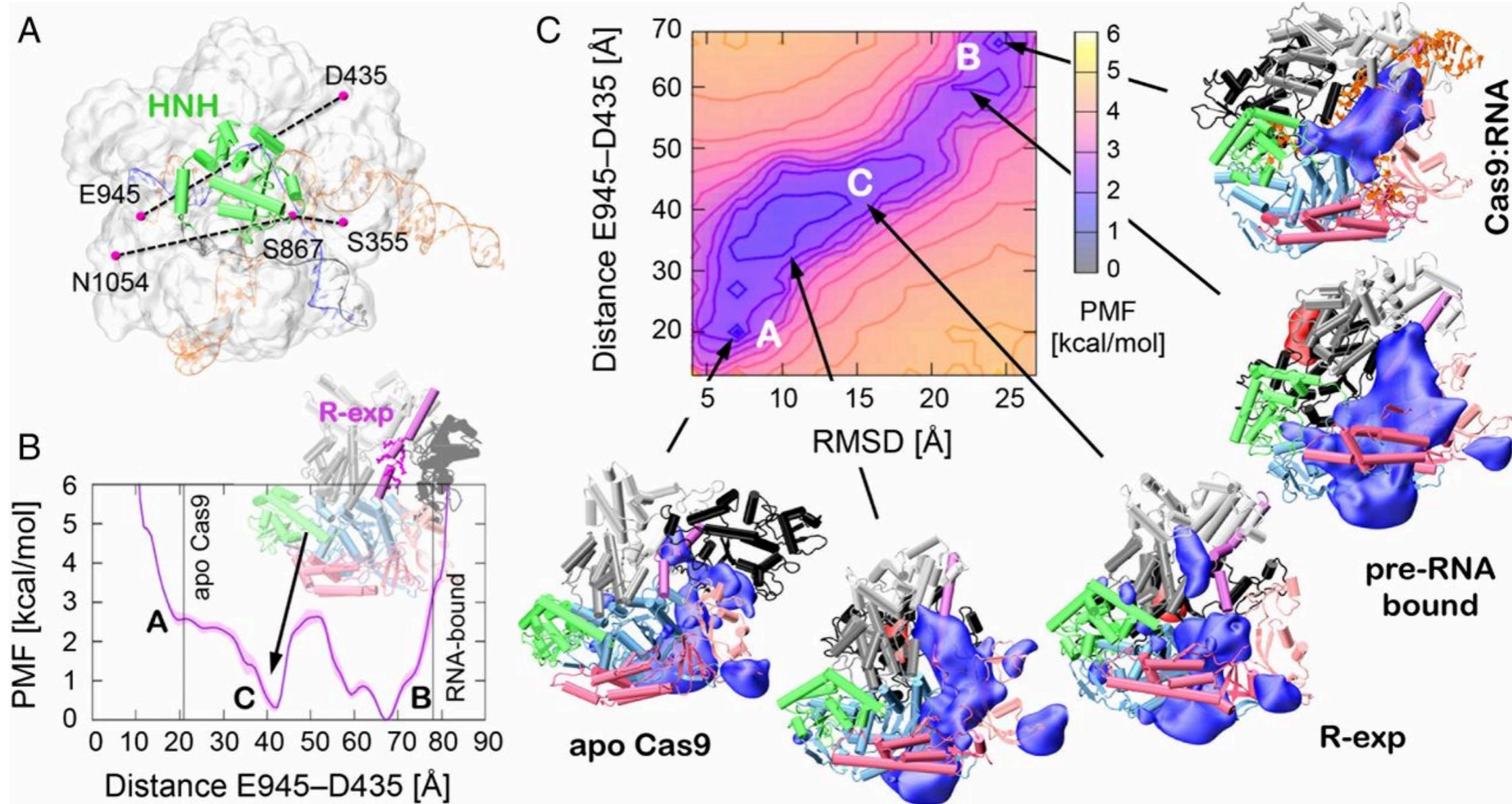
Cas9 \leftarrow Cas9:gRNA



Cas9:gRNA:DNA \rightarrow Cas9 + DNA cleaved

Gaussian accelerated full atom trajectories run for 15us \rightarrow too long to be practical

Mapping out the RNA docking energy landscape



If we could do gaMD, we can sample out the energy landscape for gRNA/Cas9 docking
—> use information to design better cgRNAs