Exploring and expanding the CRISPR-Cas enzyme toolbox for genome editing

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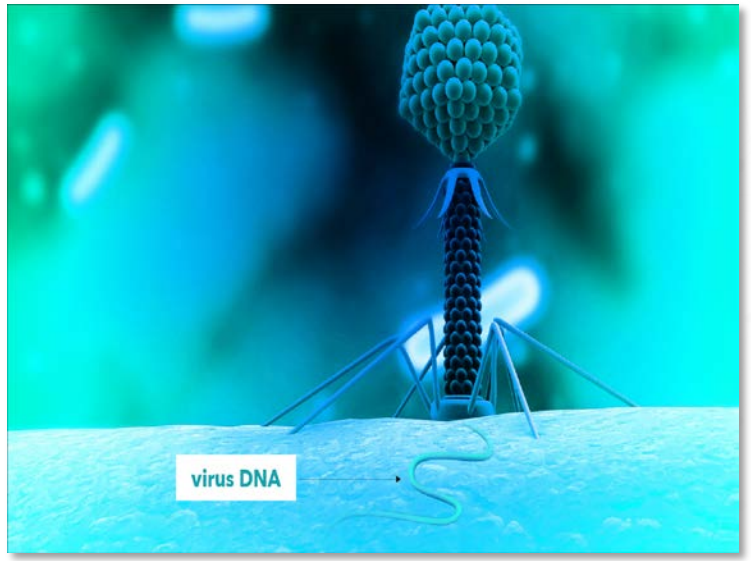
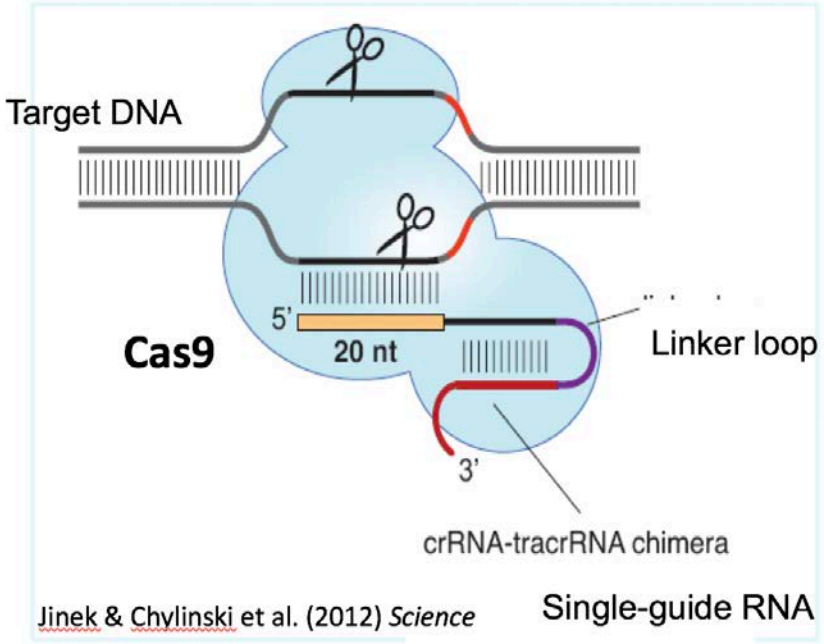
Innovative Genomics Institute

Company connections

Co-founder:	Caribou Biosciences
	Editas Medicine
	Intellia Therapeutics
	Mammoth Biosciences
SAB member:	Caribou Biosciences
	Intellia Therapeutics
	eFFECTOR Therapeutics
	Mammoth Biosciences
	Synthego
	Inari
Director:	Johnson & Johnson

Biology → biochemistry → applications





- Research
- Healthcare
- Agriculture
- Diagnostics
- Synthetic biology
- Therapeutics

Cas9 as an *in vivo* therapeutic: considerations

- Multiple clinical trials for somatic therapies will begin in 2019-20
- The most widely used enzymes for genome editing come from pathogenic bacteria
- Cas9 and Cas12a/Cpf1 are large proteins, complicating delivery
- Avoiding genome damage due to sustained activity is desirable

↓

MINING NATURAL DIVERSITY OF CRISPR SYSTEMS TO ENHANCE IN VIVO *APPLICATIONS*

New small CRISPR-Cas enzymes from non-pathogenic bacteria

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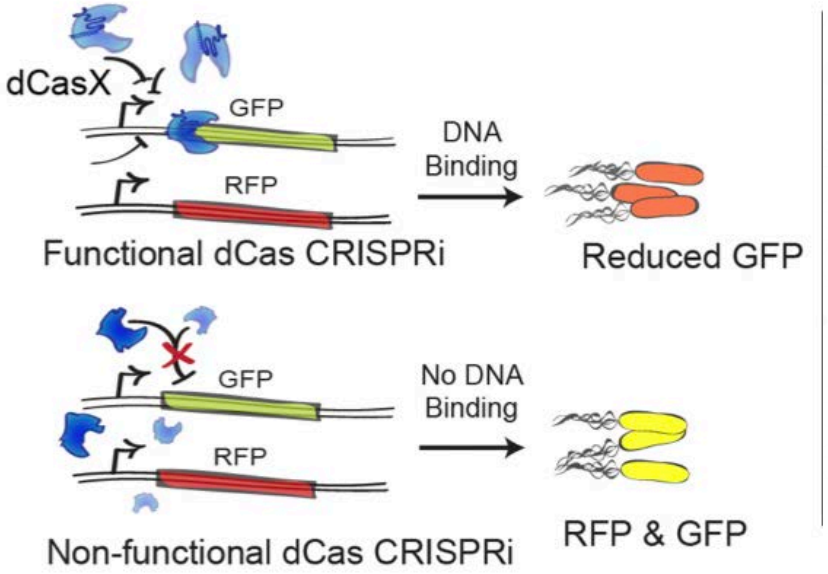
CasX is part of a new kind of CRISPR locus

?

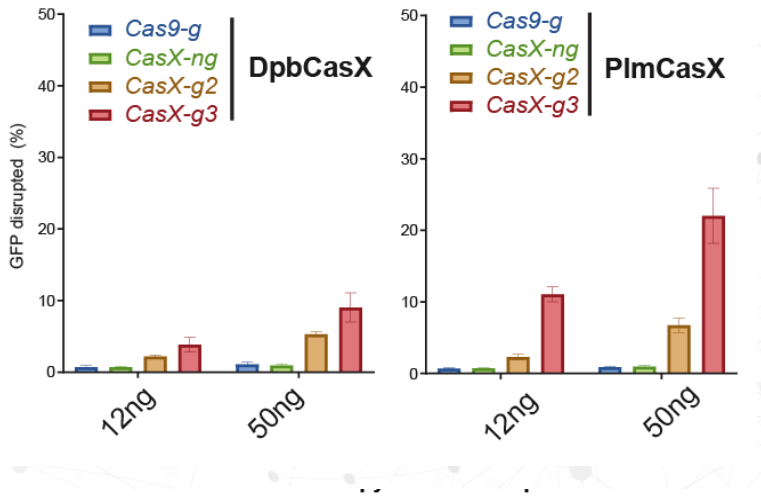
Adaptation/DNA integration

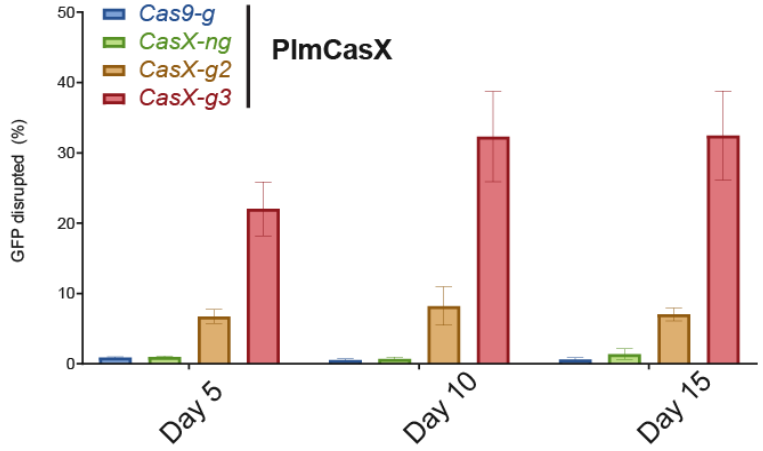
Burstein, Harrington, Strutt et al. (2017) *Nature*

CasX-mediated transcriptional control in bacteria



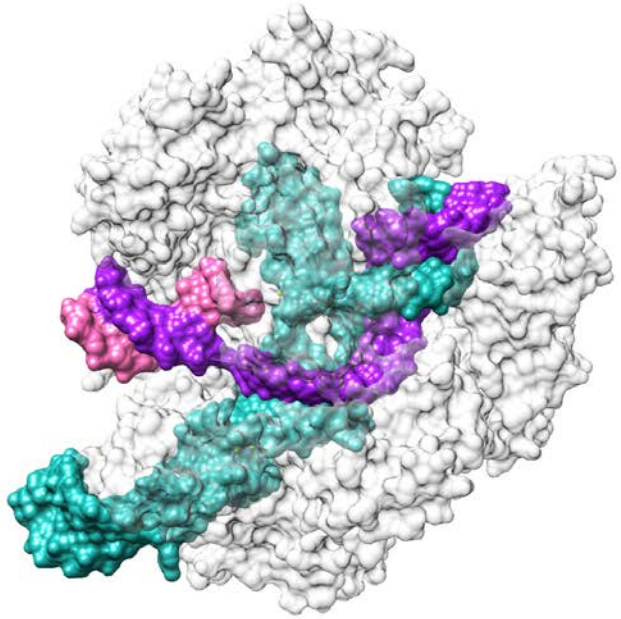
CasX-mediated gene knockout in human cells

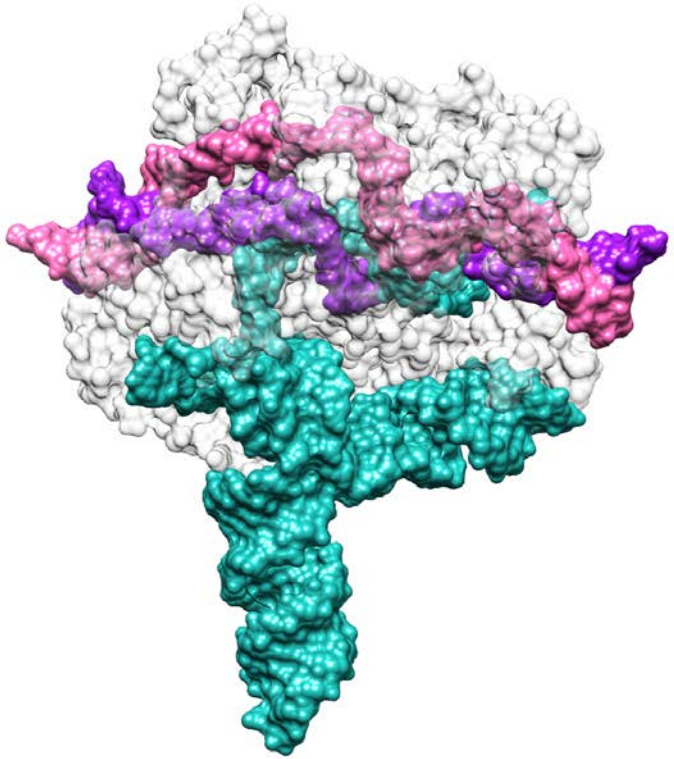




CasX makes 10-bp staggered dsDNA cut

CasX: smaller protein, larger sgRNA





1368aa 83nt

SpyCas9

986aa 122nt

CasX

Systematic discovery and validation of CRISPR-Cas inhibitors

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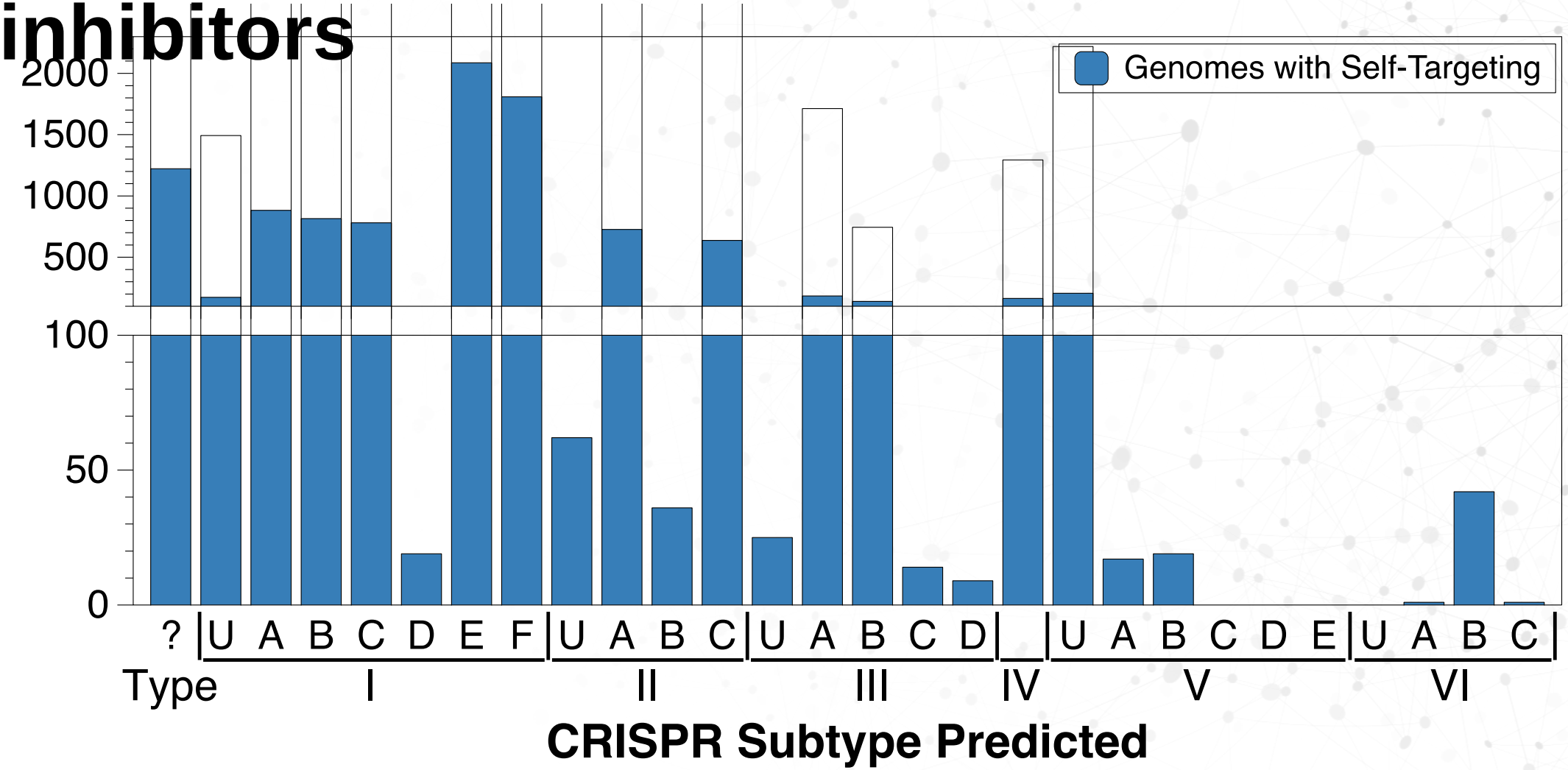
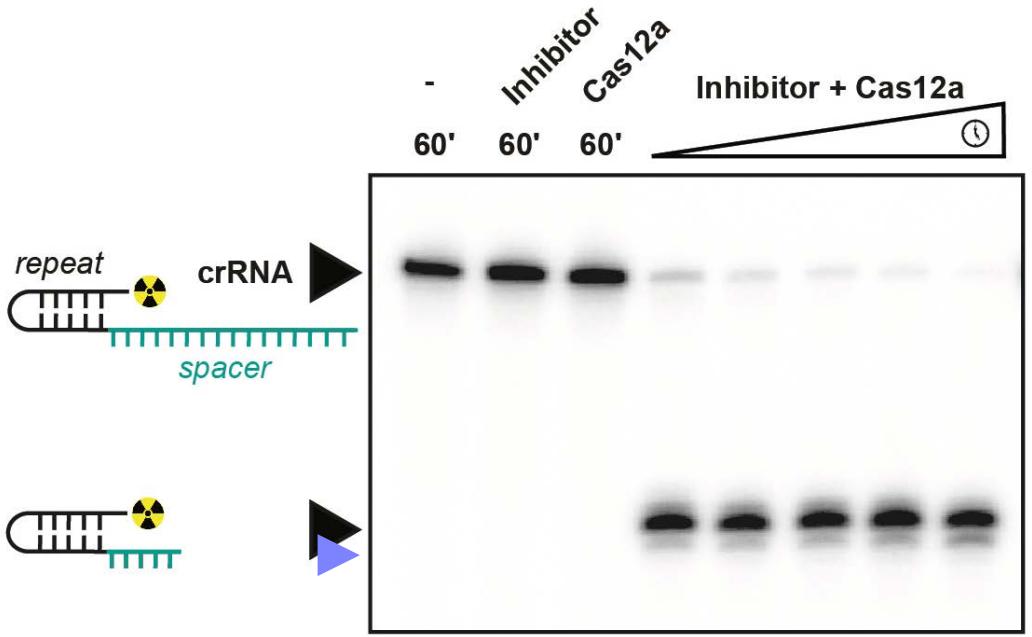
Most CRISPR-Cas systems have natural inhibitors

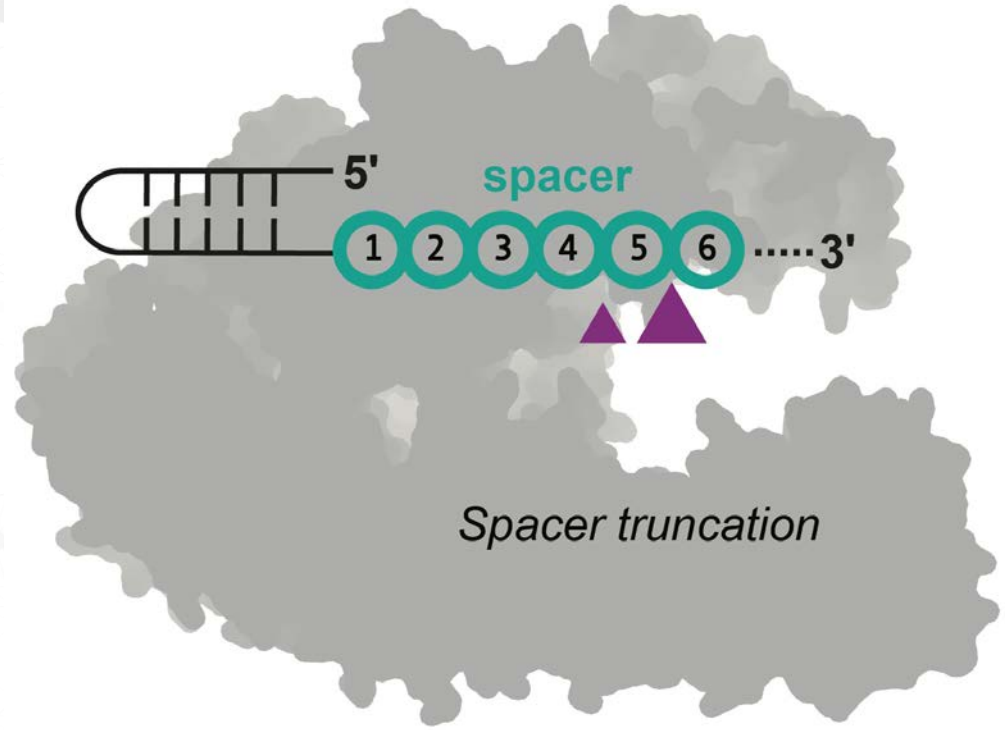


Watters et al. (2017) *Science*

Watters et al. (2018) Science

A multiple-turnover inhibitor of Cas12a





Gavin Knott, Brittney Thornton, Kyle Watters
Watters et al. (2017) *Science*

Conclusions

- New CRISPR-Cas enzymes: alternative tools for *in vivo* genome editing and control

- Multiple-turnover CRISPR inhibitor could prevent genome damage

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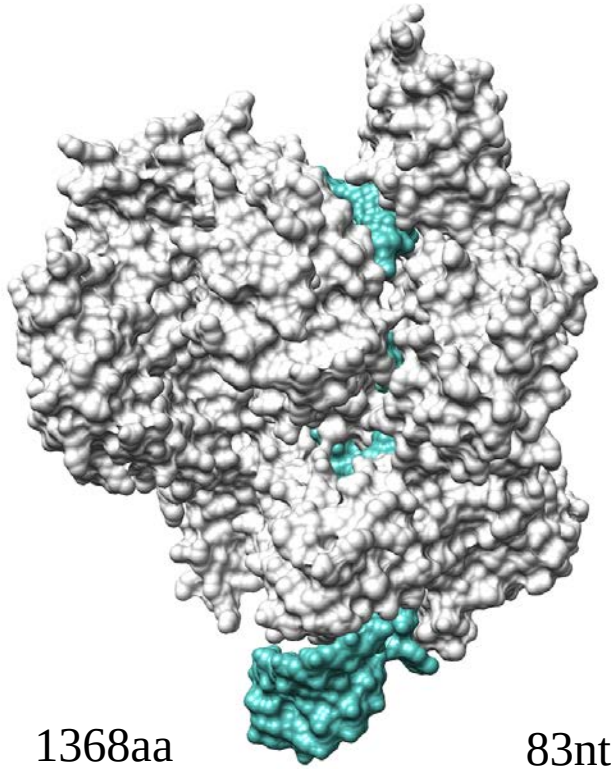
Brittney Thornton

CasX: smaller protein, larger sgRNA



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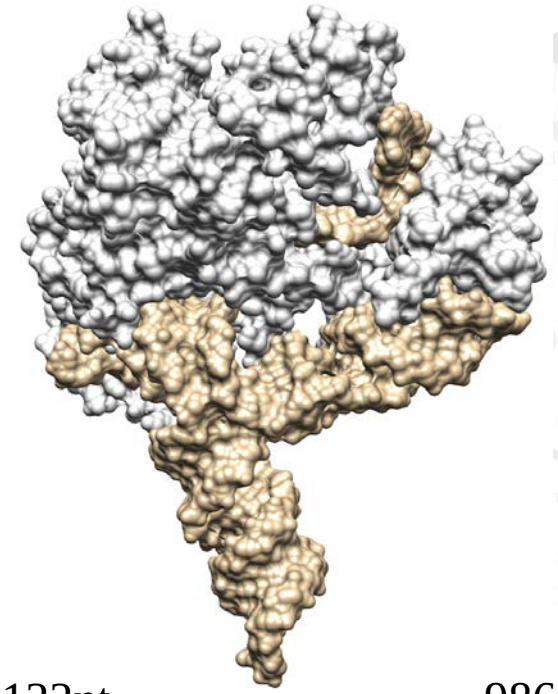
SpyCas9



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CasX