12-9-2021

Determining the Nucleic Acid Binding Affinities of CRISPR-Associated DinG (CasDinG)

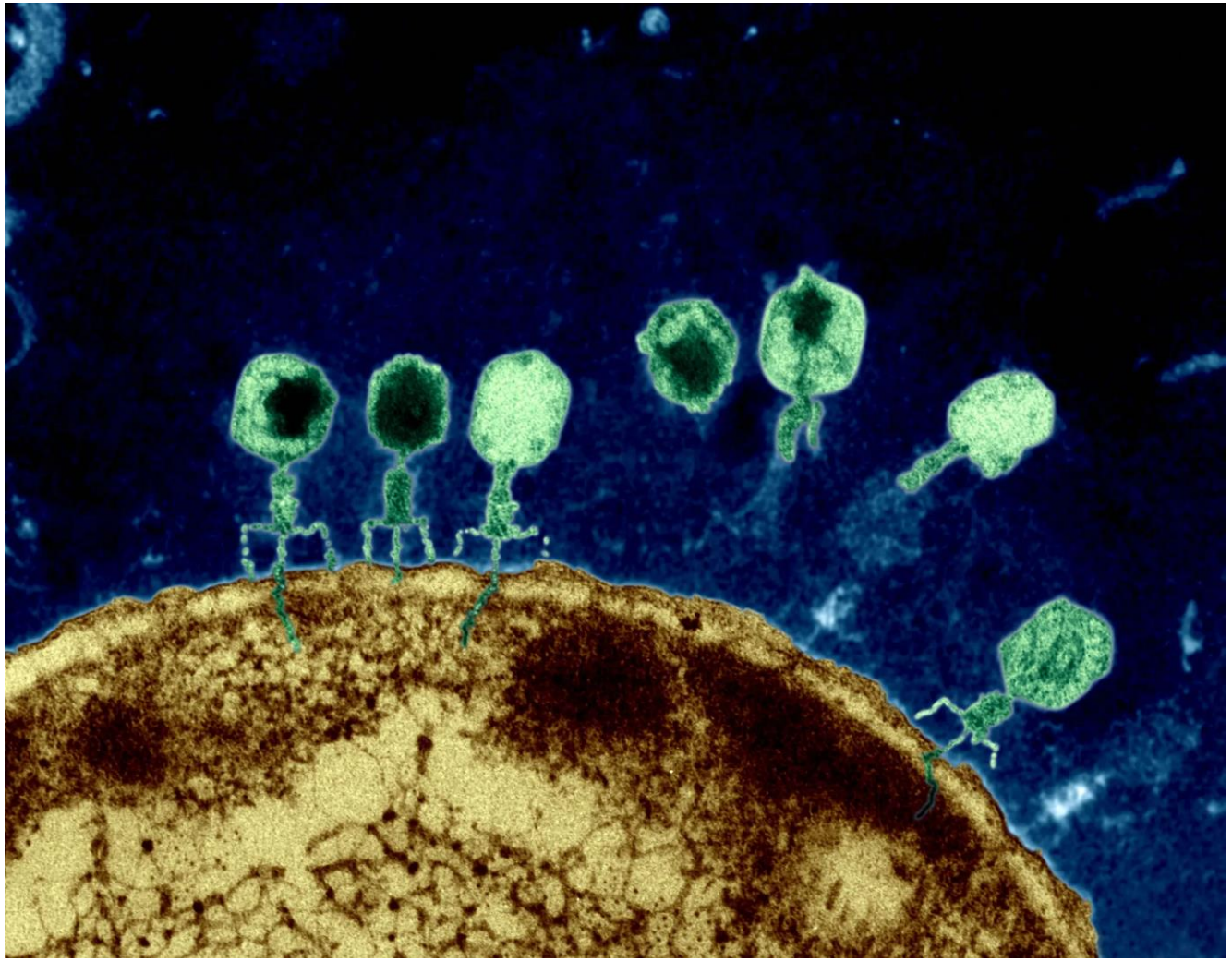
Matt Armbrust
Utah State University, matt.armbrust5@gmail.com

Determining the nucleic acid binding affinities of CRISPR-associated DinG (CasDinG)

Matt Armbrust

Dr. Ryan Jackson Research

Phage infections drive bacterial evolution



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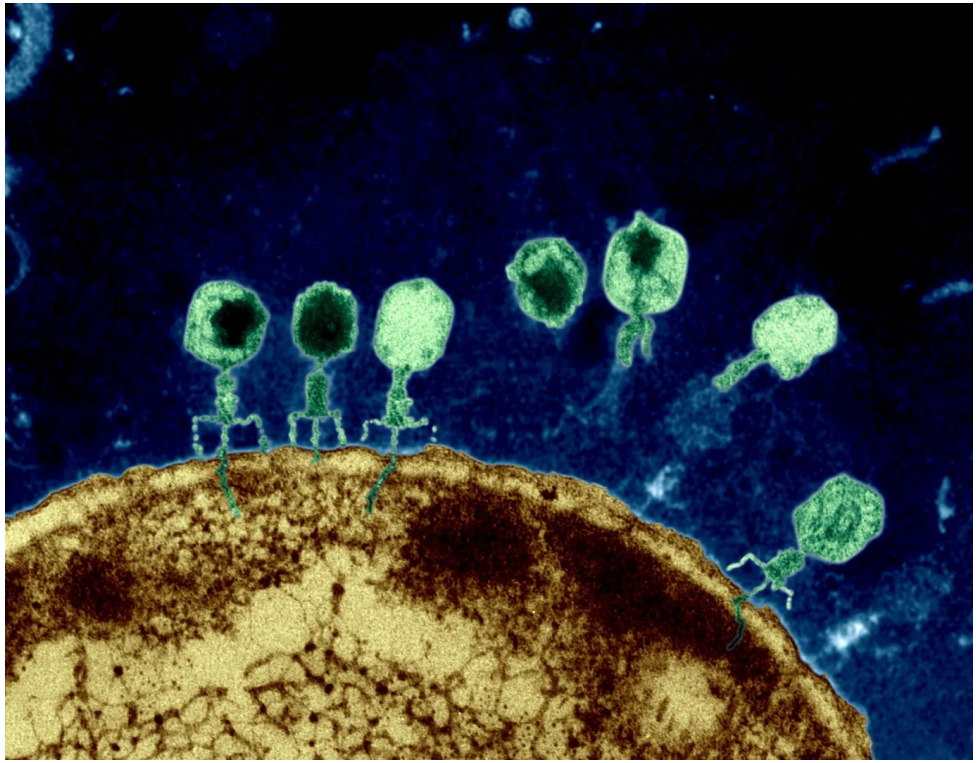
Phage infections drive bacterial evolution



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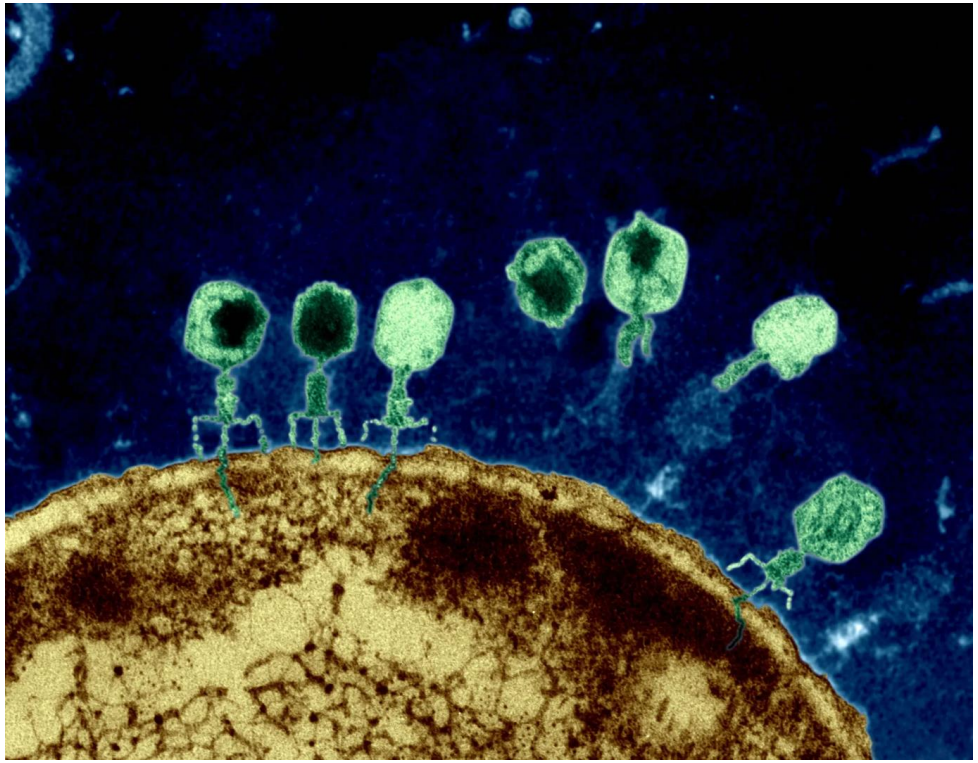

- Phage outnumber bacteria 10:1

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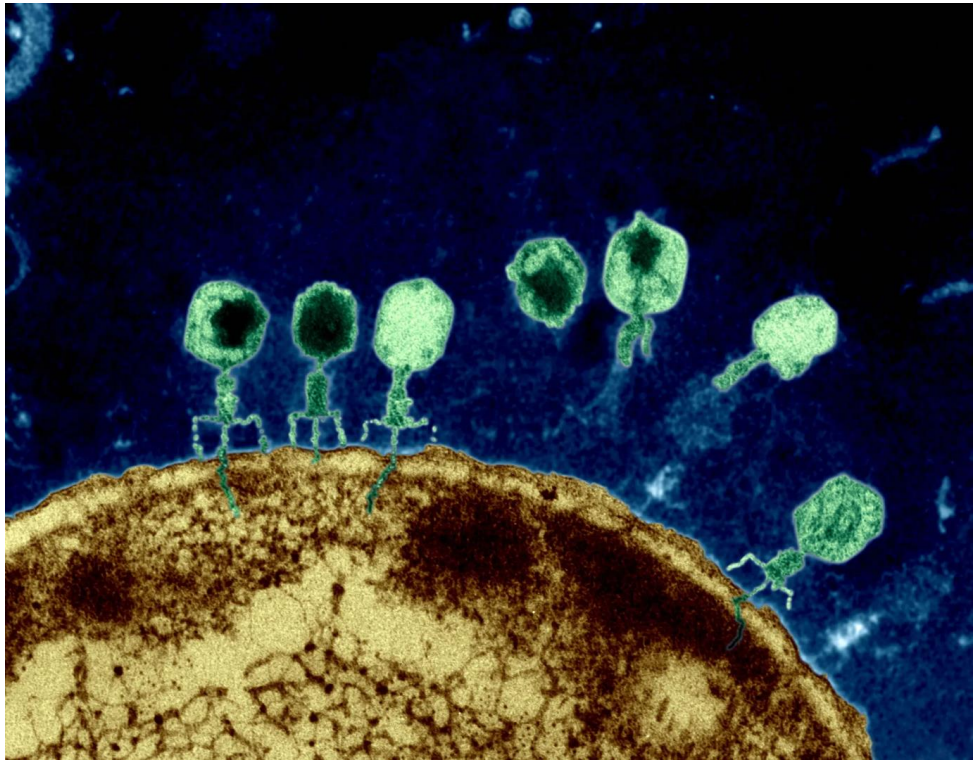

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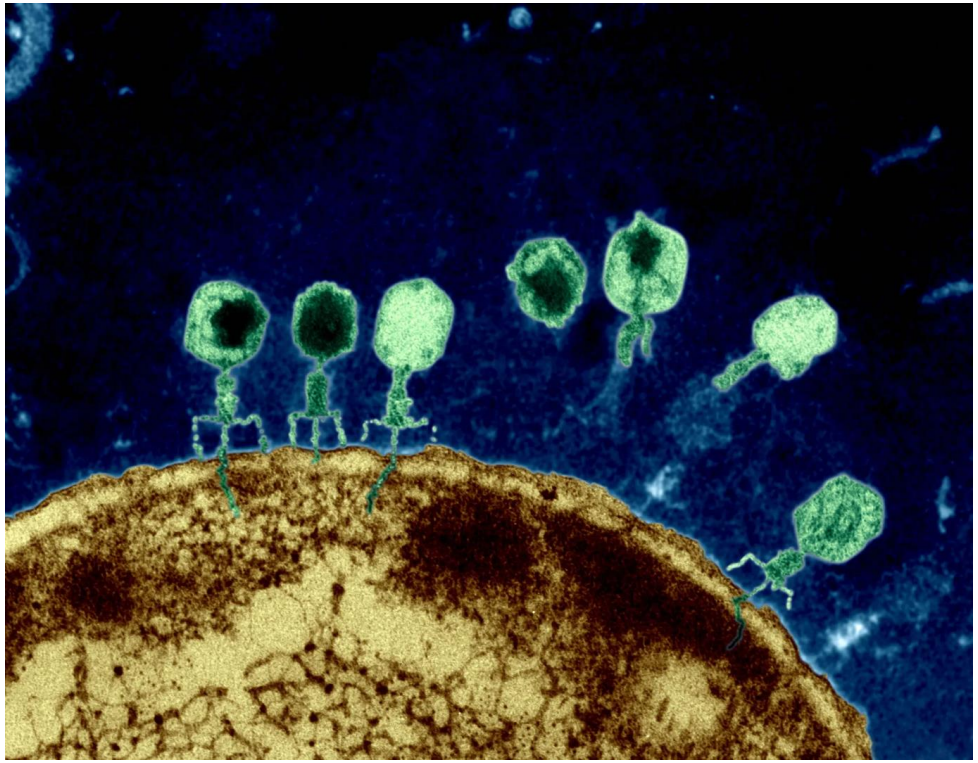

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Major question: how do bacteria protect themselves?

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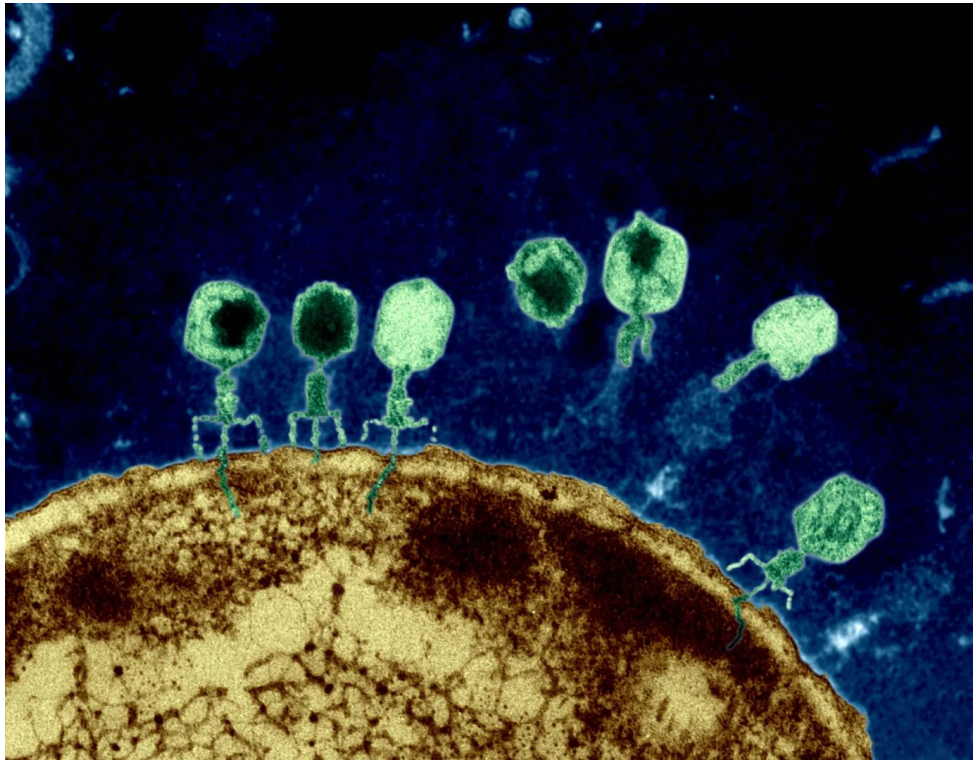

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CRISPR-Cas

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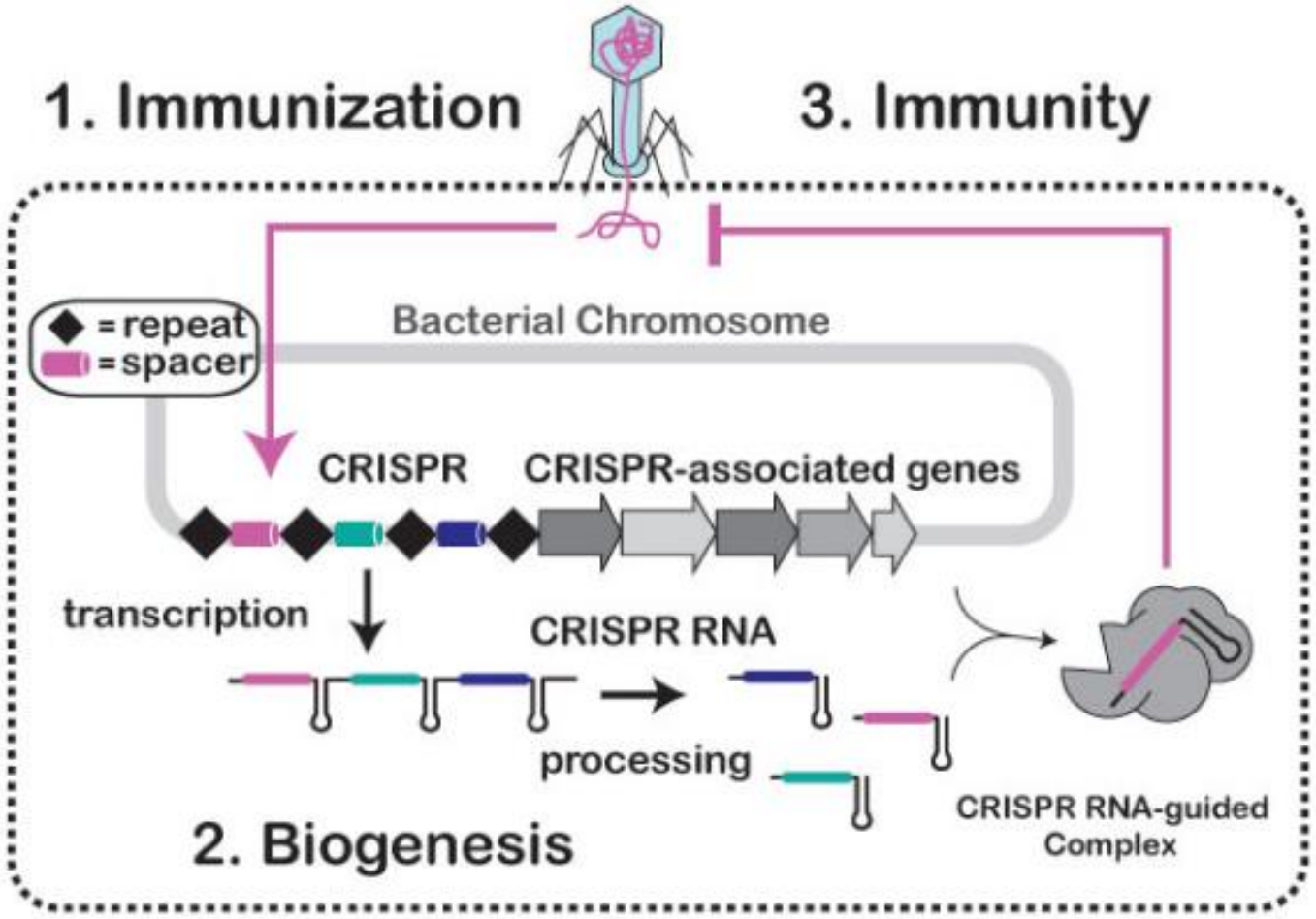
CRISPR-Cas systems provide adaptive immunity



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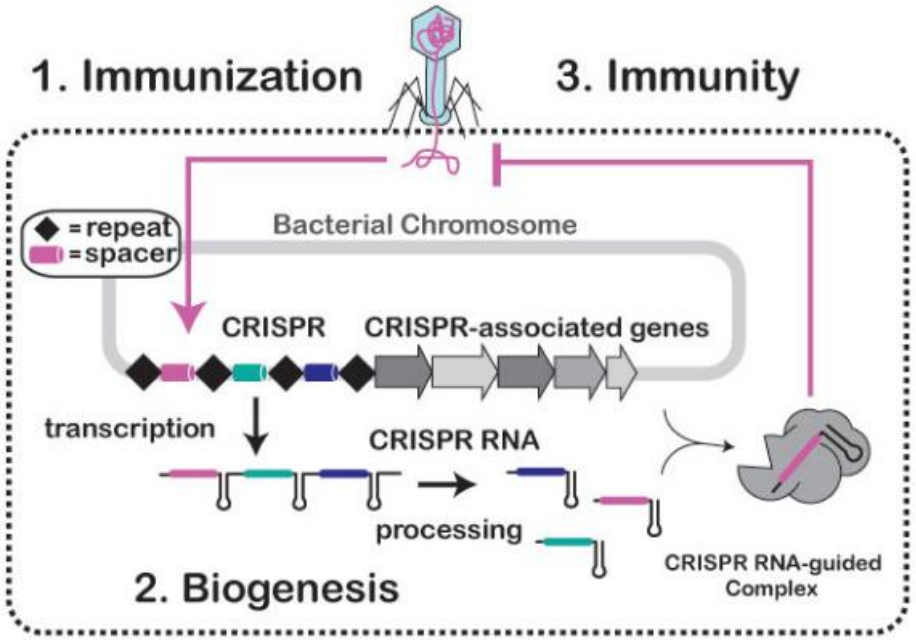

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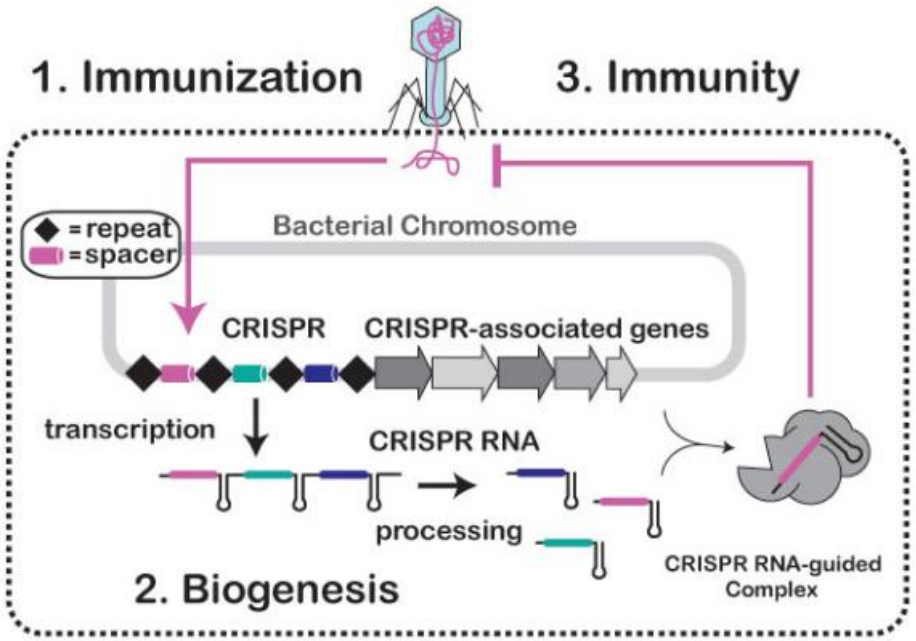
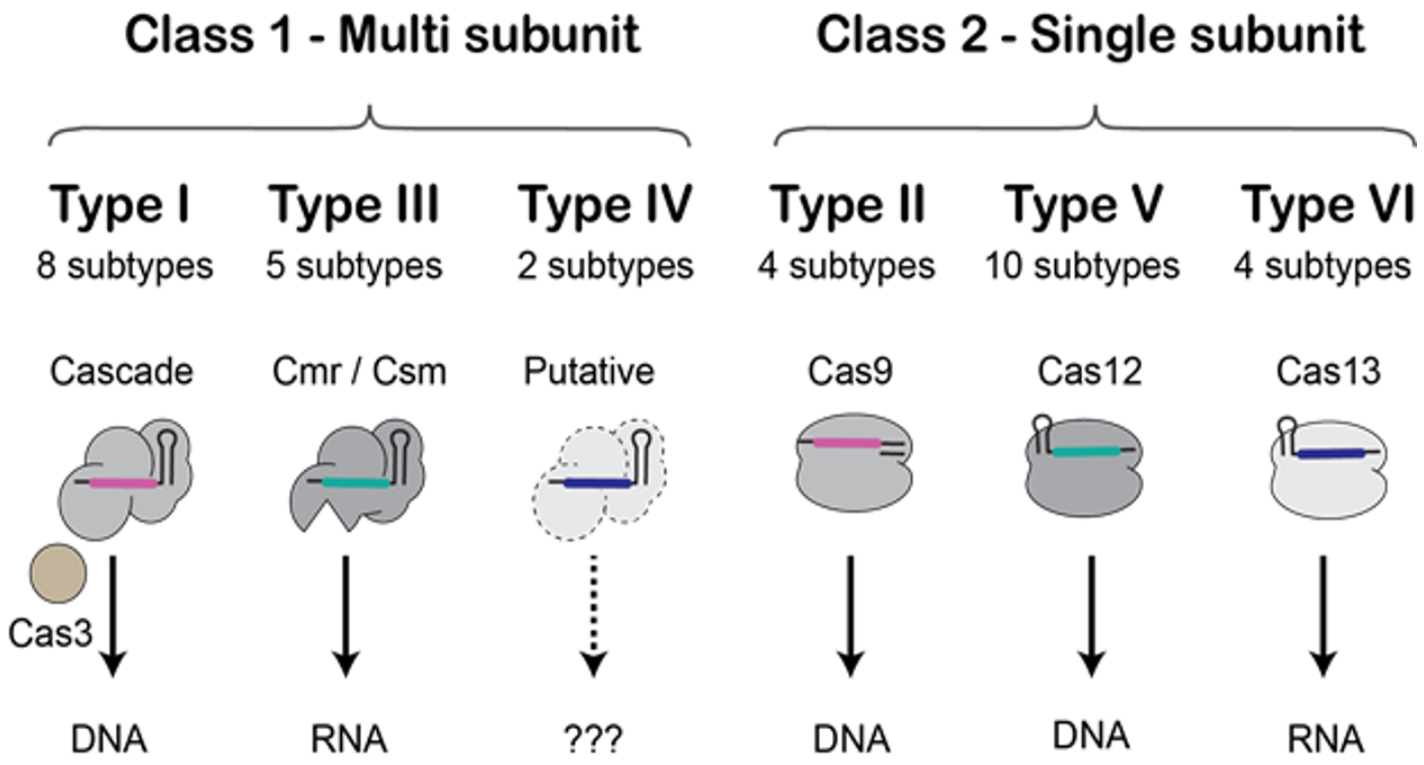


- Biochemical characterization of Cas genes is necessary for understanding mechanisms
  - How are spacers integrated?
  - How are crRNA libraries processed?
  - **What is the molecular mechanism of immunization?**

CRISPR-Cas systems are diverse



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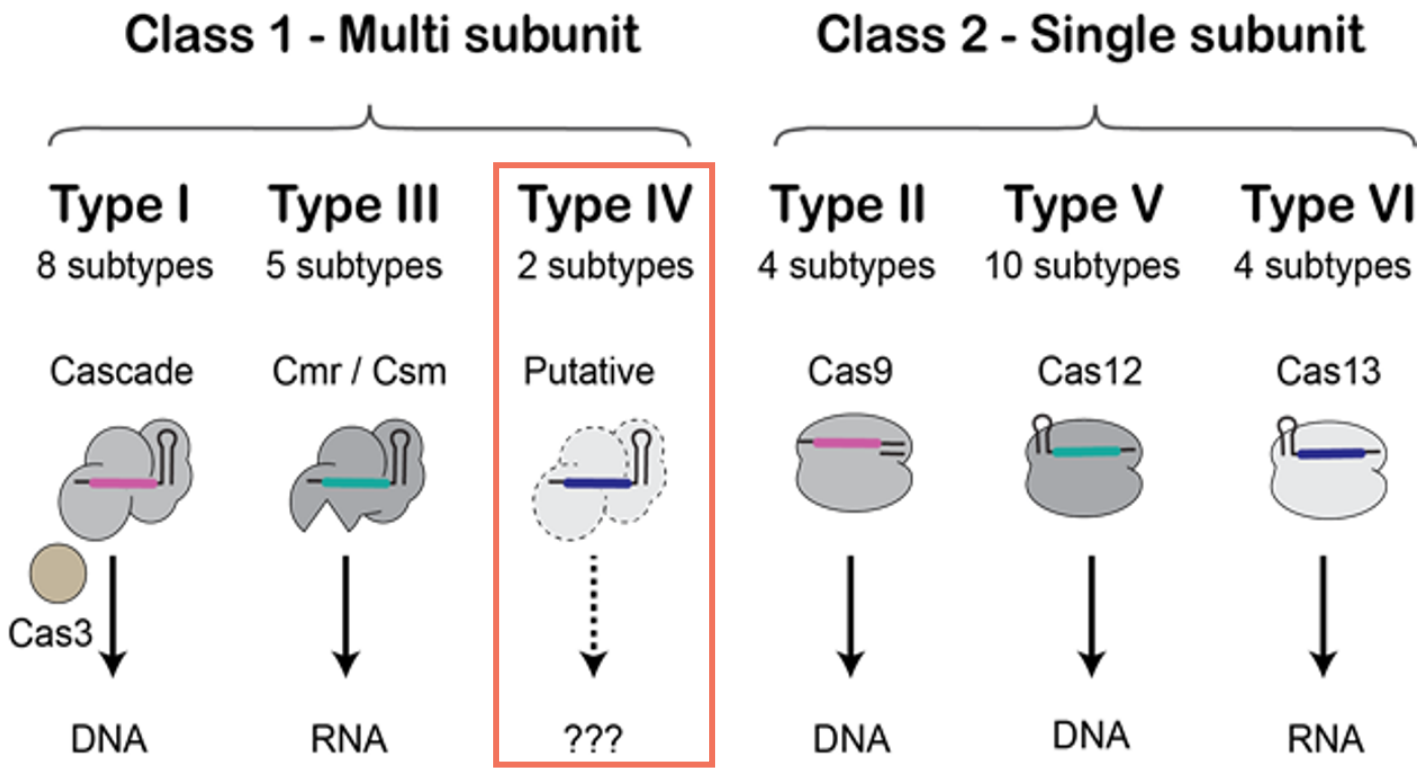
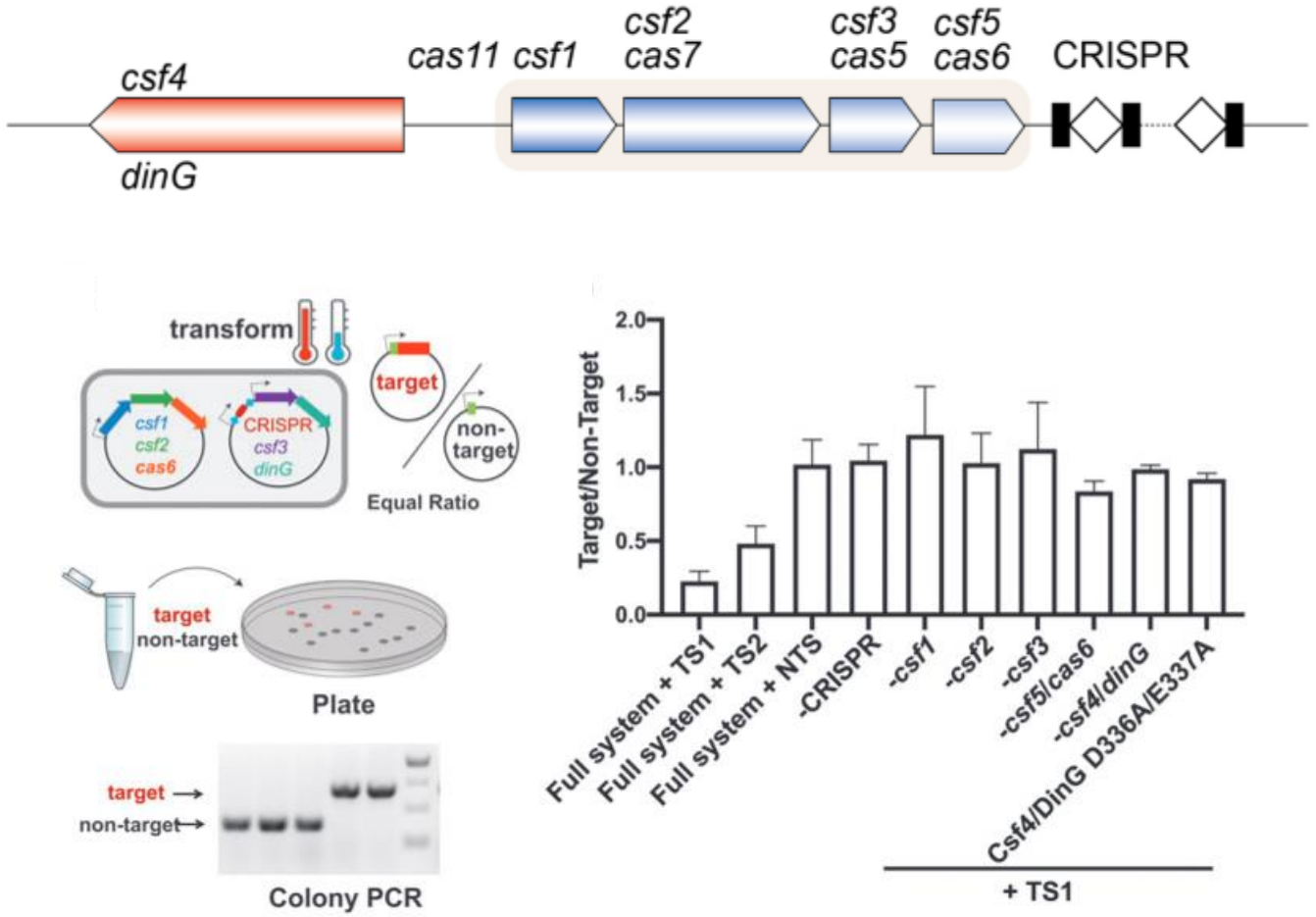


Type IV-A CRISPR-Cas systems are novel, functional immune systems *in vivo*



Crowley et. al. 2019

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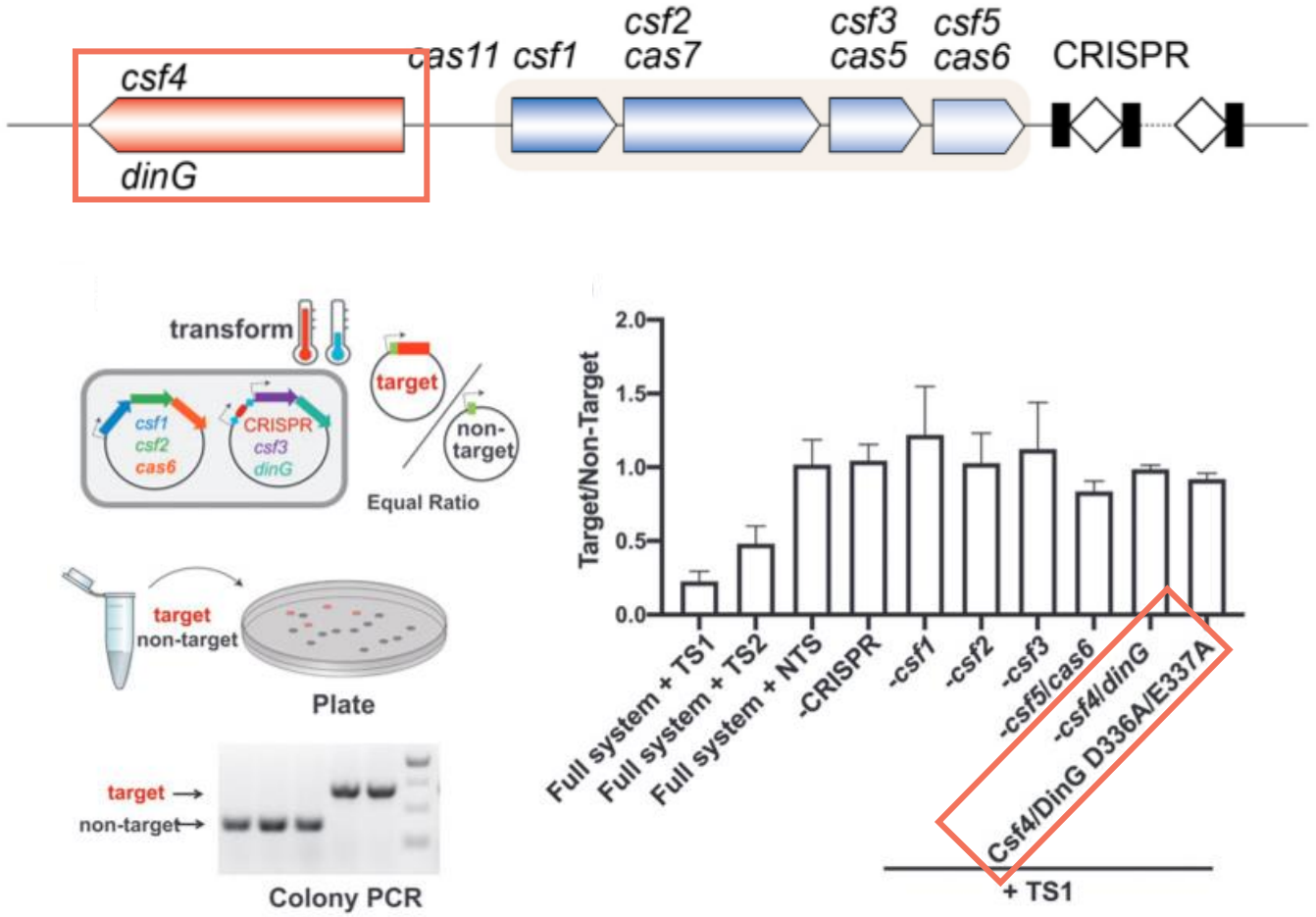

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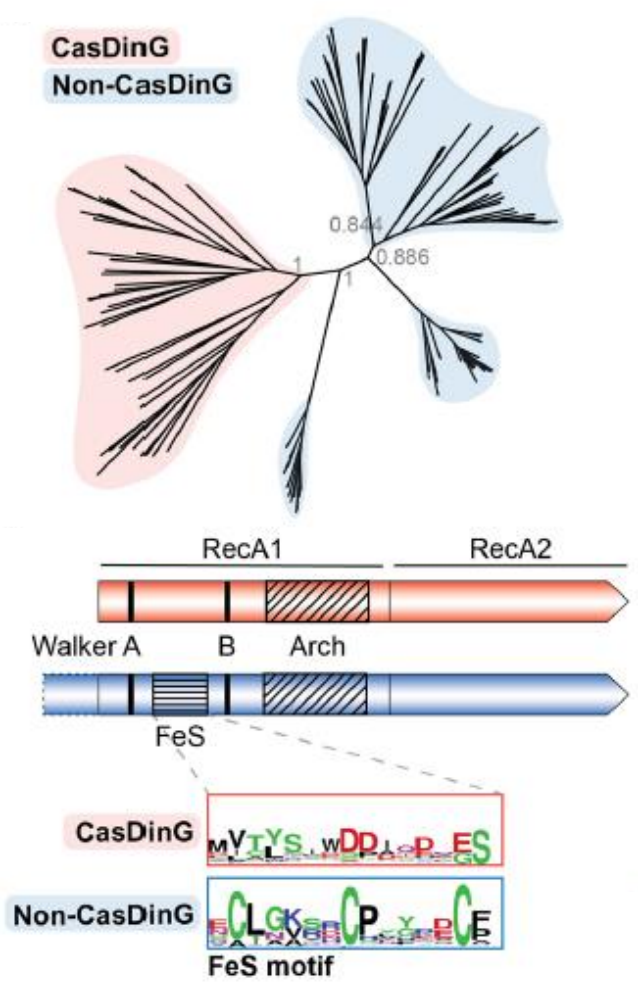
Cas *dinG* genes are phylogenetically unique from non-Cas *dinG* analogues



Taylor et. al. 2021

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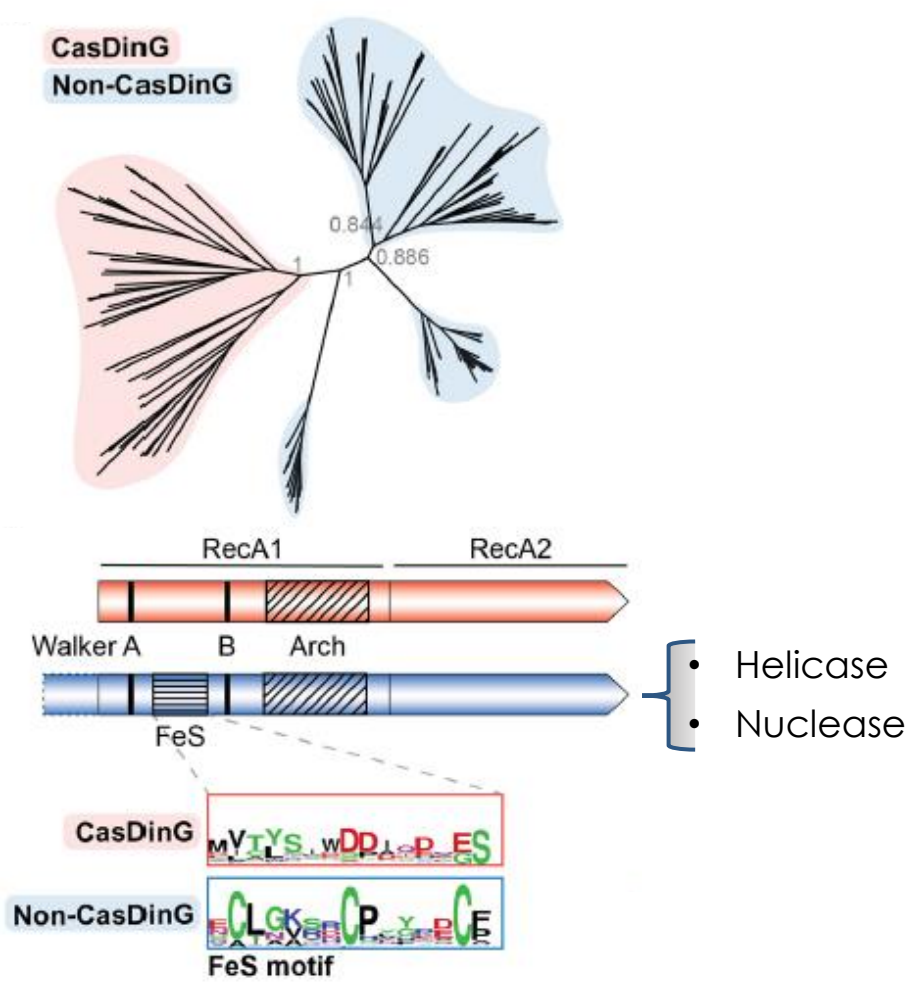

- Helicase
- Nuclease

Taylor et. al. 2021

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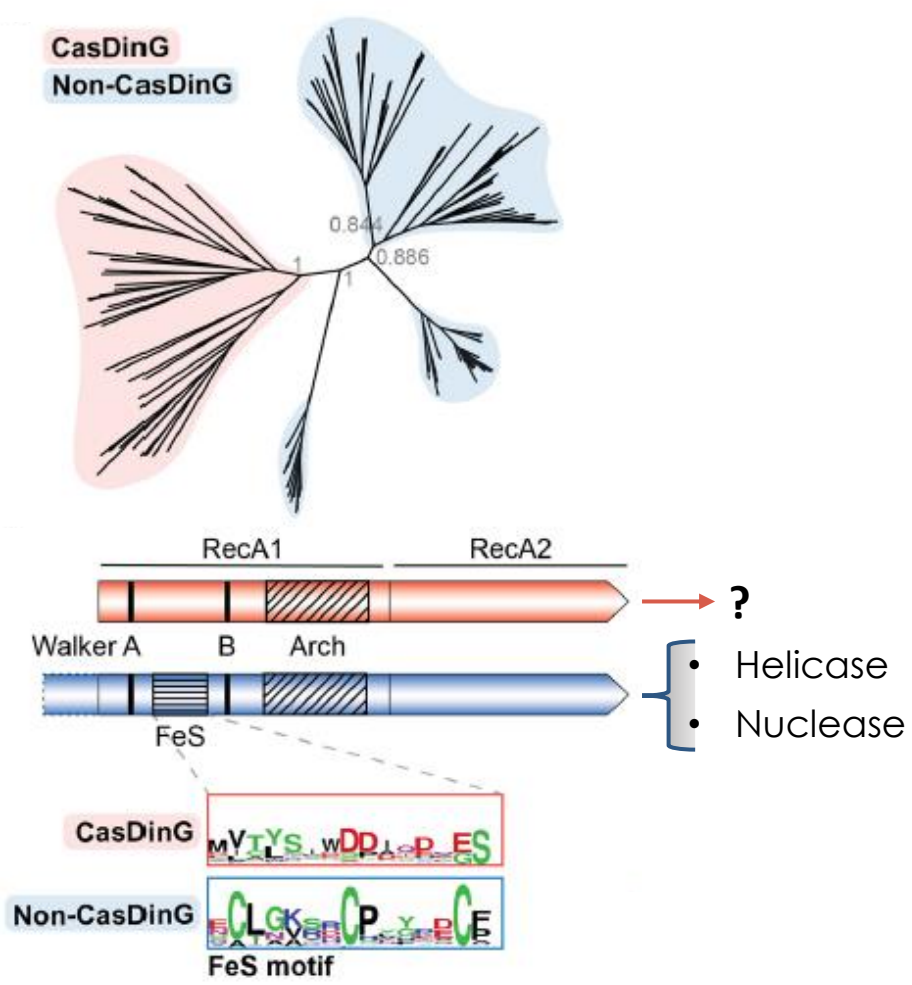

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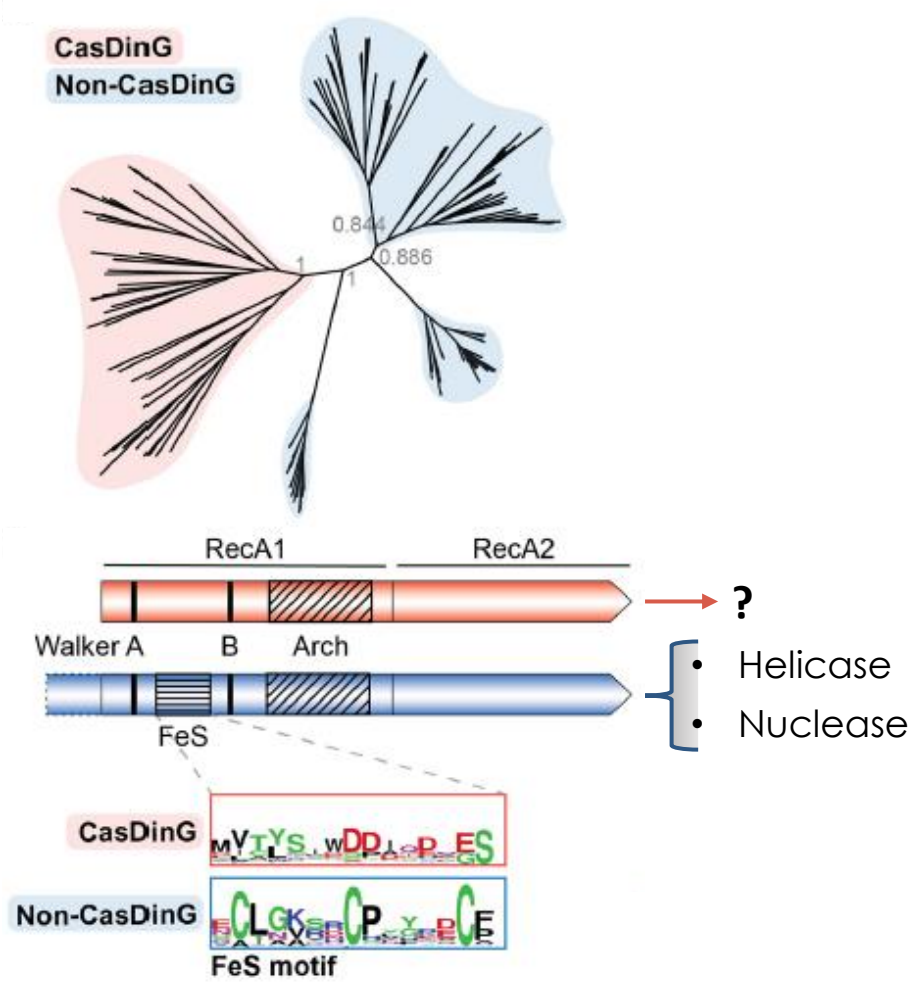

Primary Structure Alignment to *P. aeruginosa* CasDinG:

	***E. coli* DinG**	***S. aureus* DinG**
Amino Acid Identity	20.0 %	13.3 %
Amino Acid Similarity	32.0%	24.5 %

Questions to answer:

Taylor et. al. 2021

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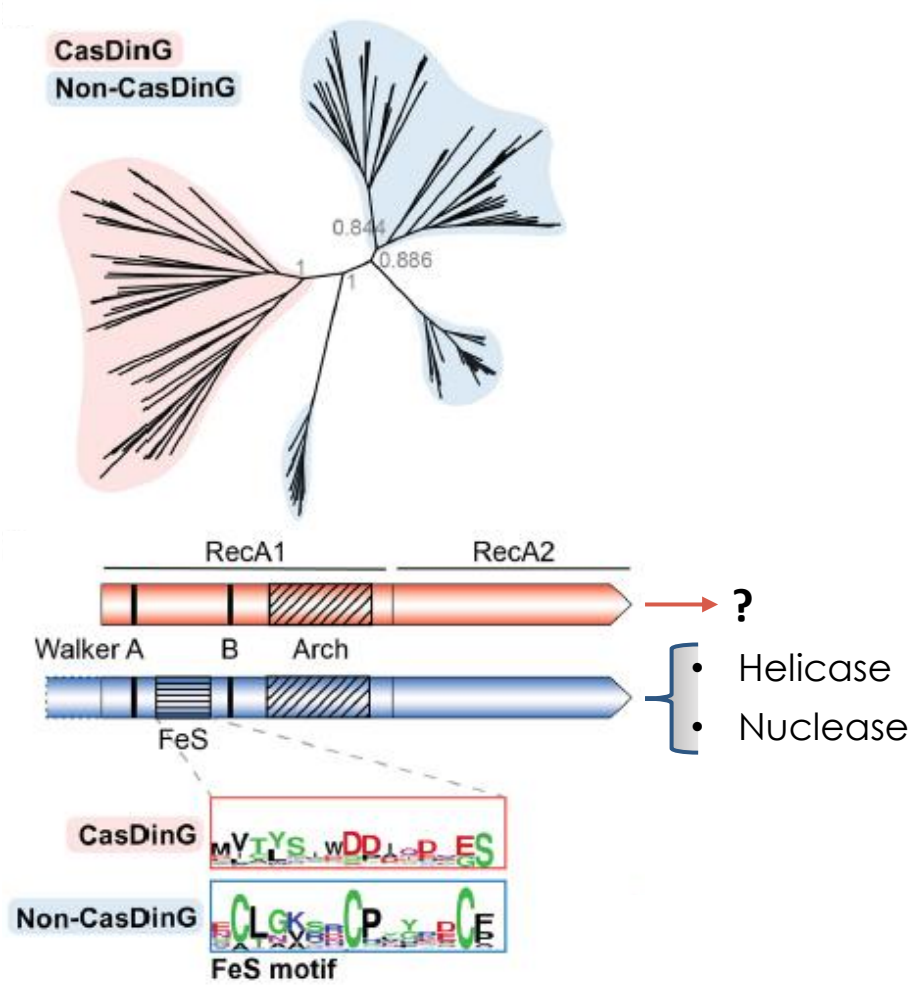

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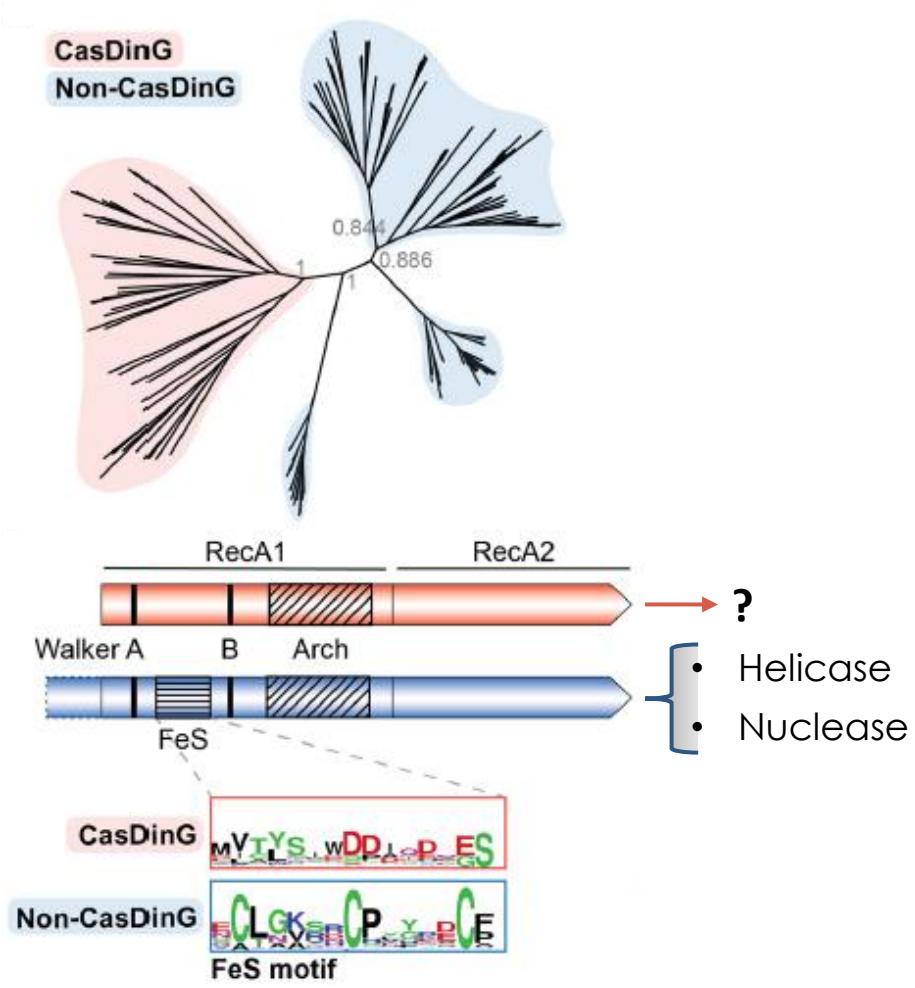

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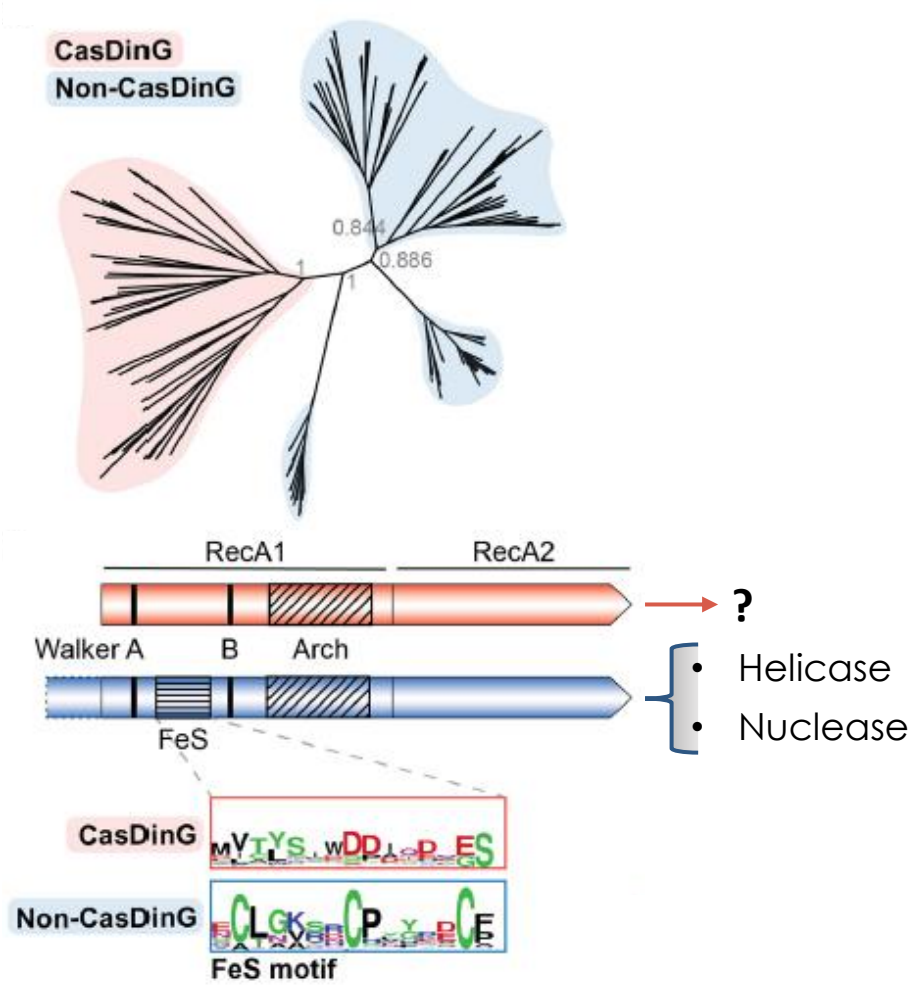

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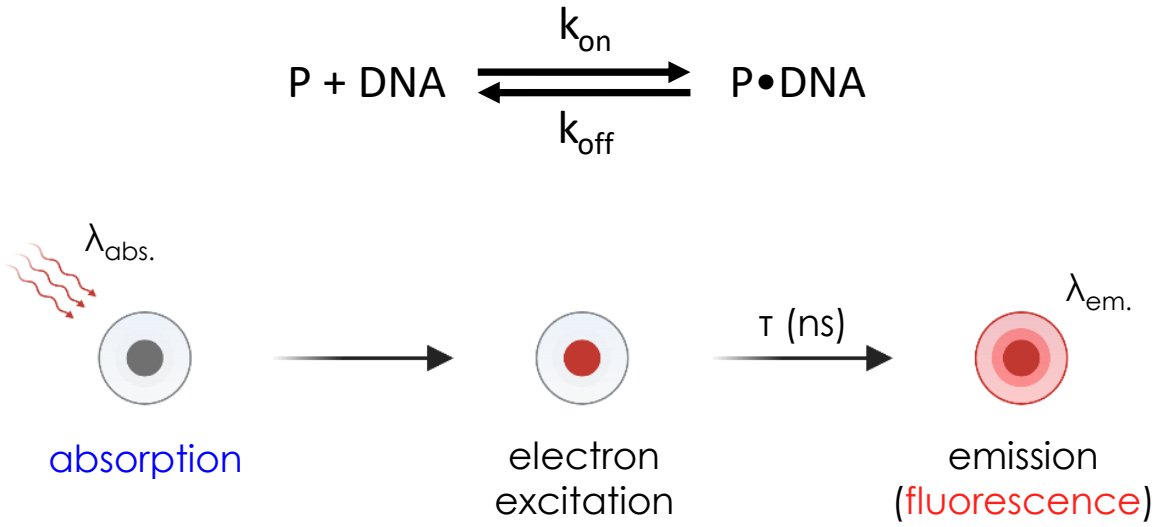
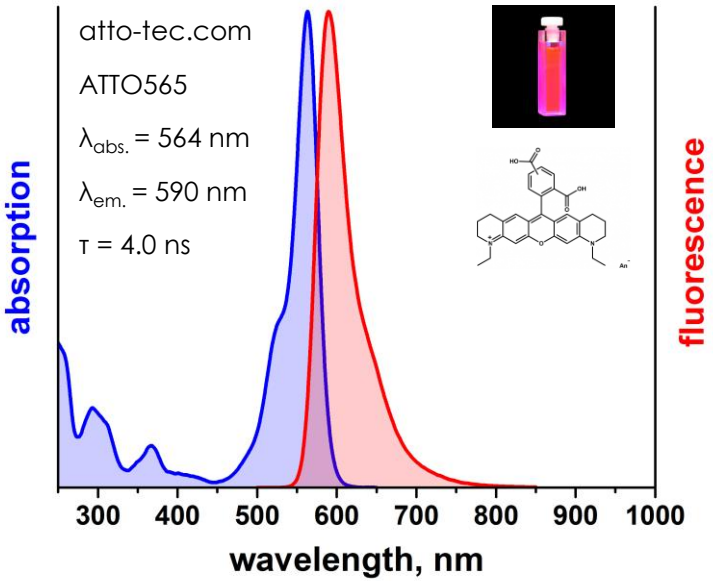
Taylor et. al. 2021

Quantifying CasDinG binding to nucleic acid with fluorescence anisotropy





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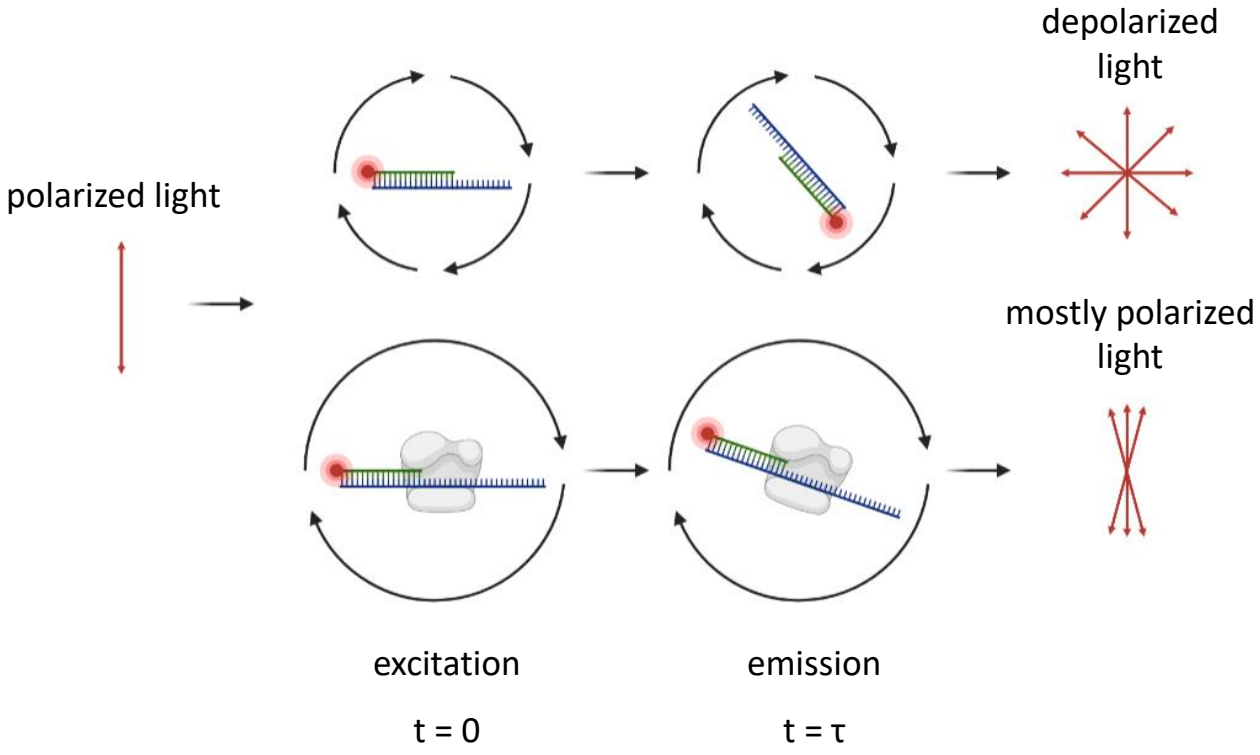
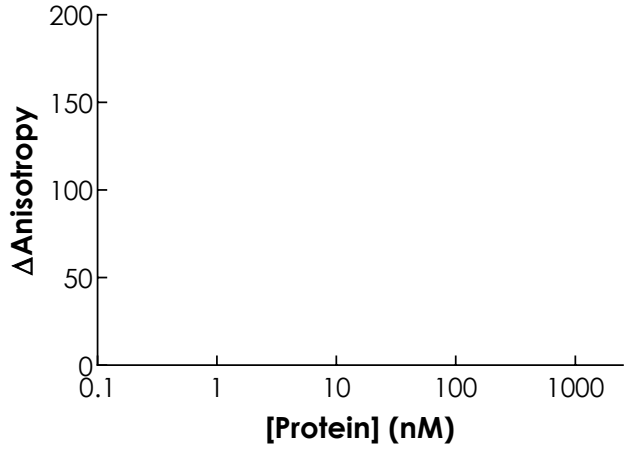


Titrate protein into ligand, calculate r:

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}}$$

Plot [Protein] vs. Δr:



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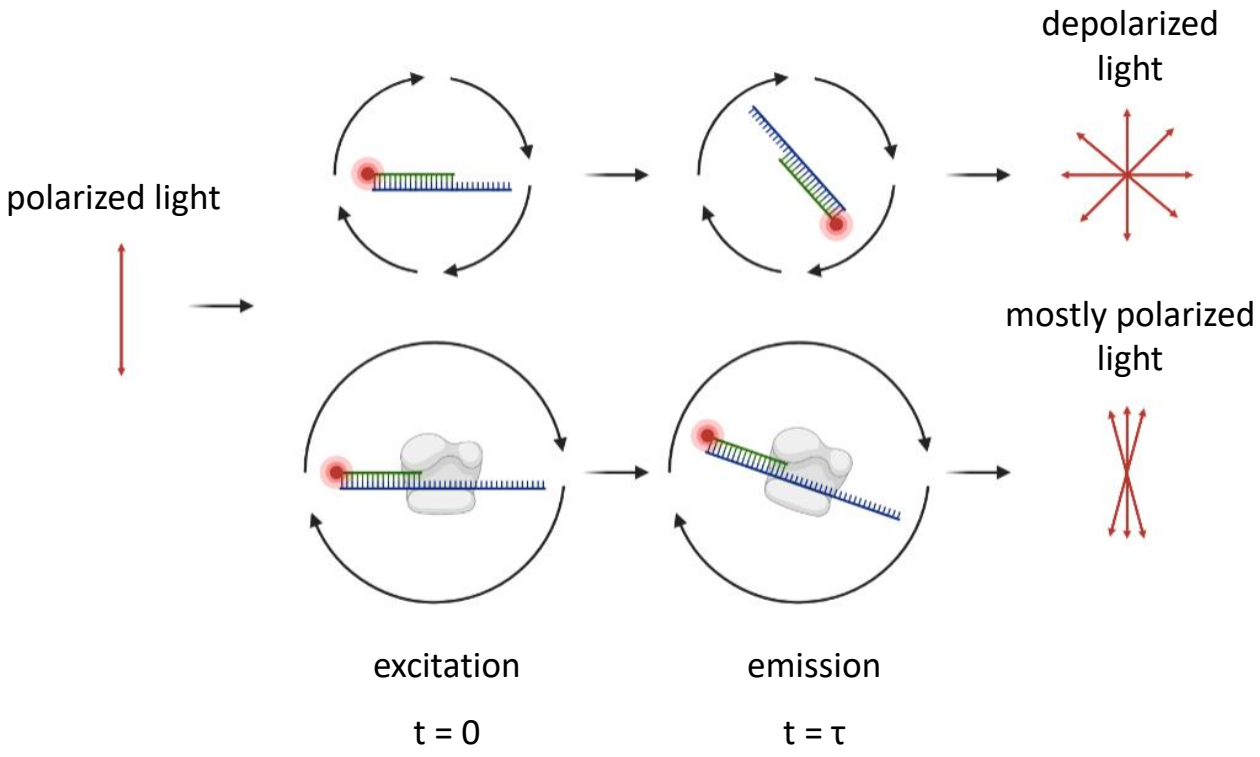

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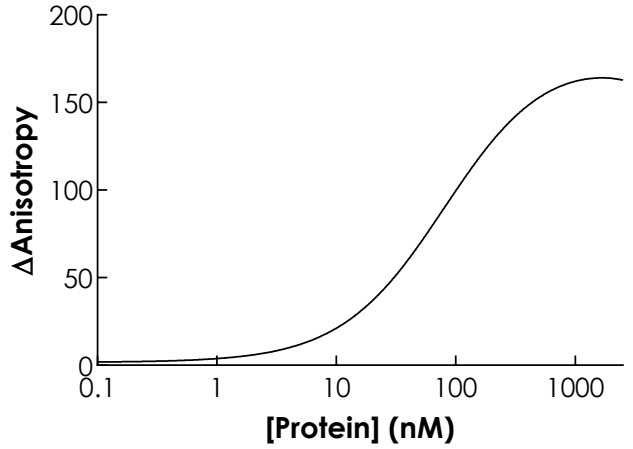
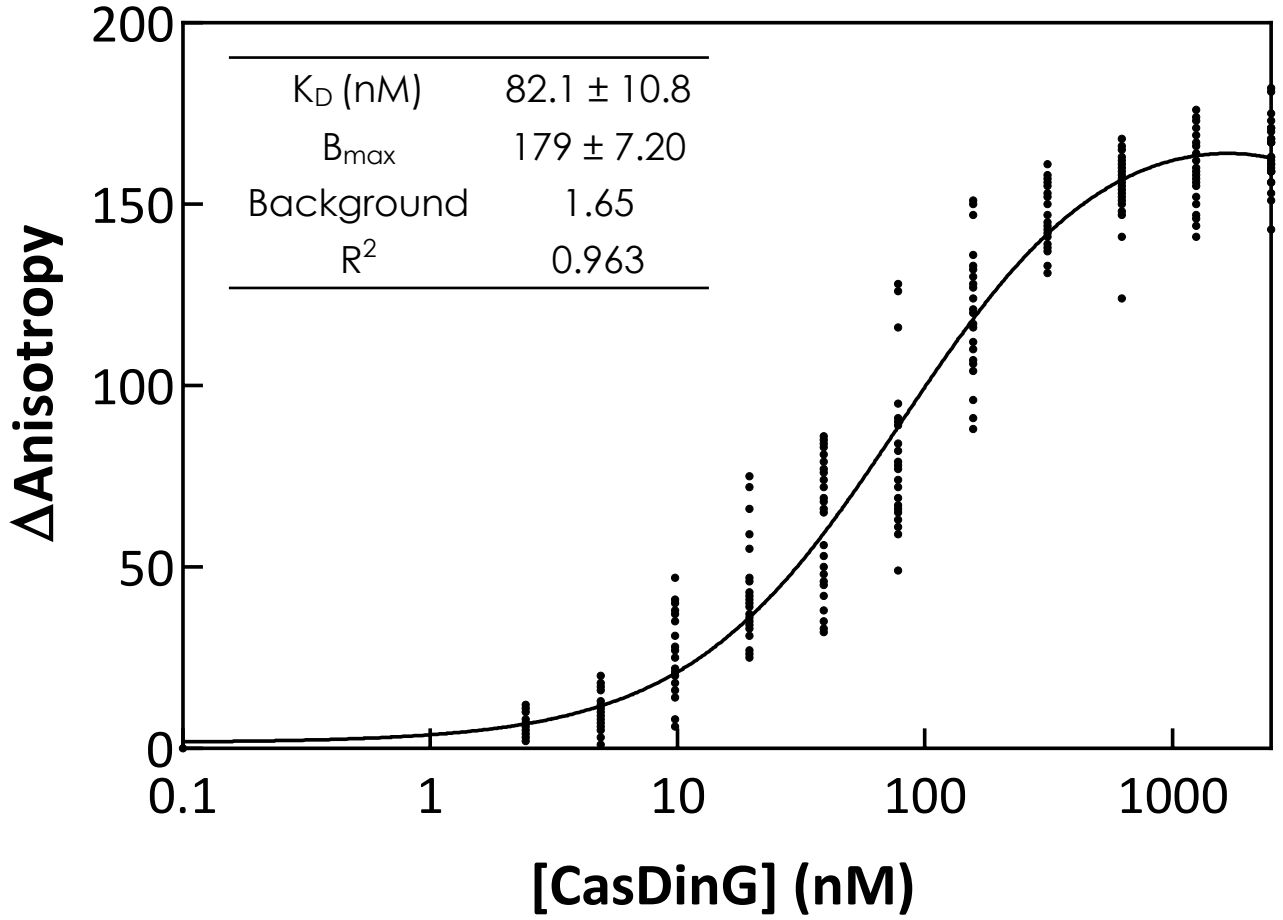


CasDinG binds to ssDNA with high affinity



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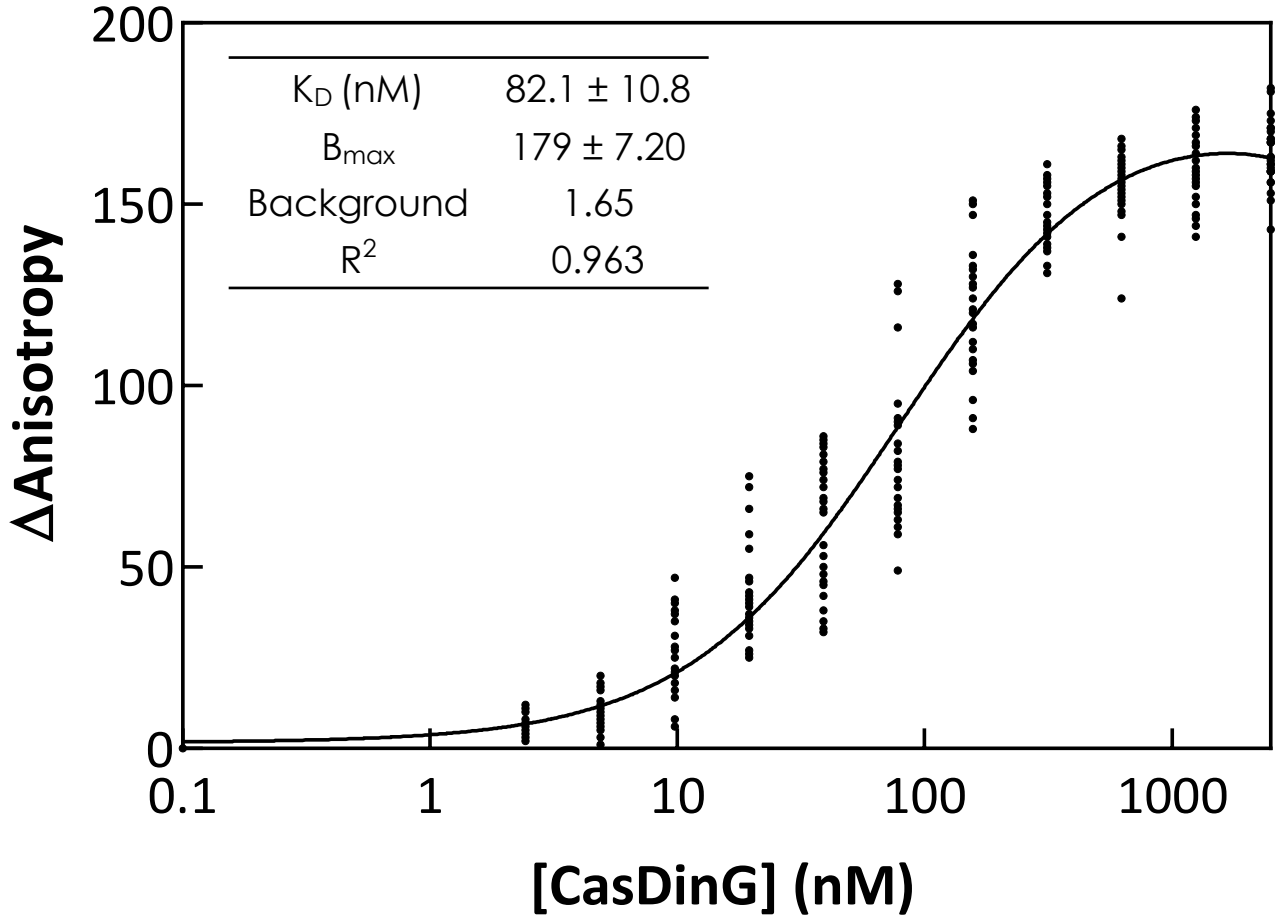
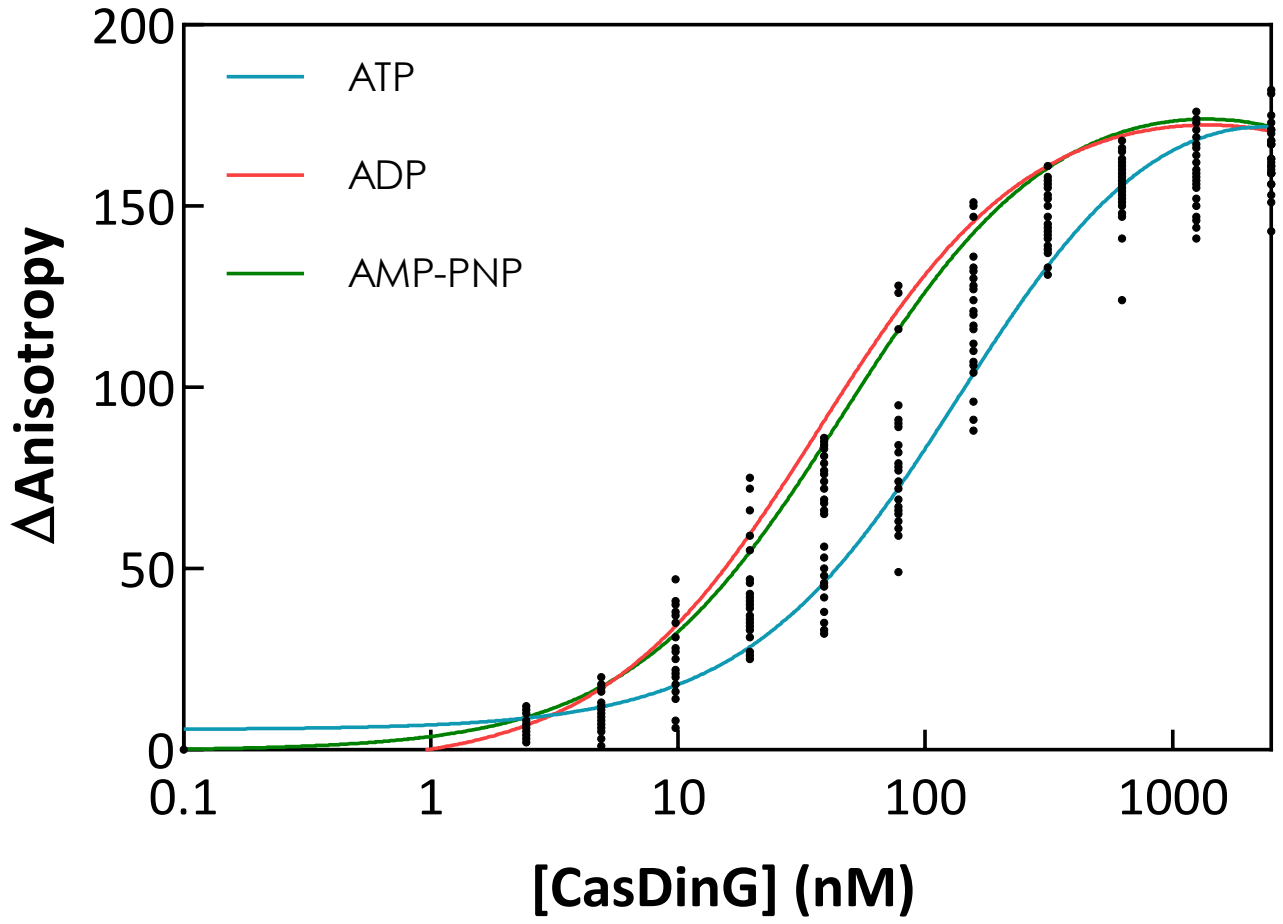


What about ribonucleotides?

Presence of an ATP analogue does not alter CasDinG binding affinity for ssDNA



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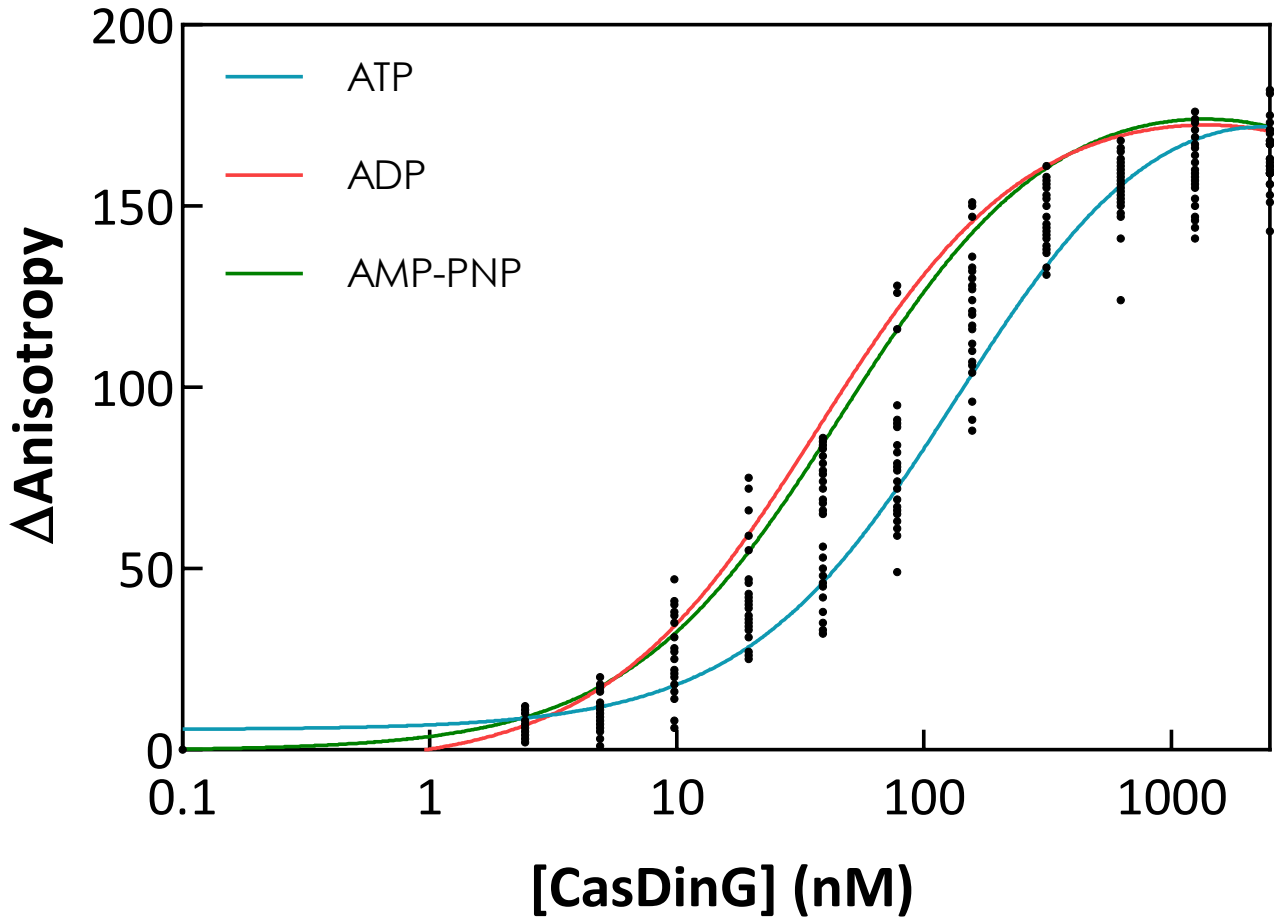
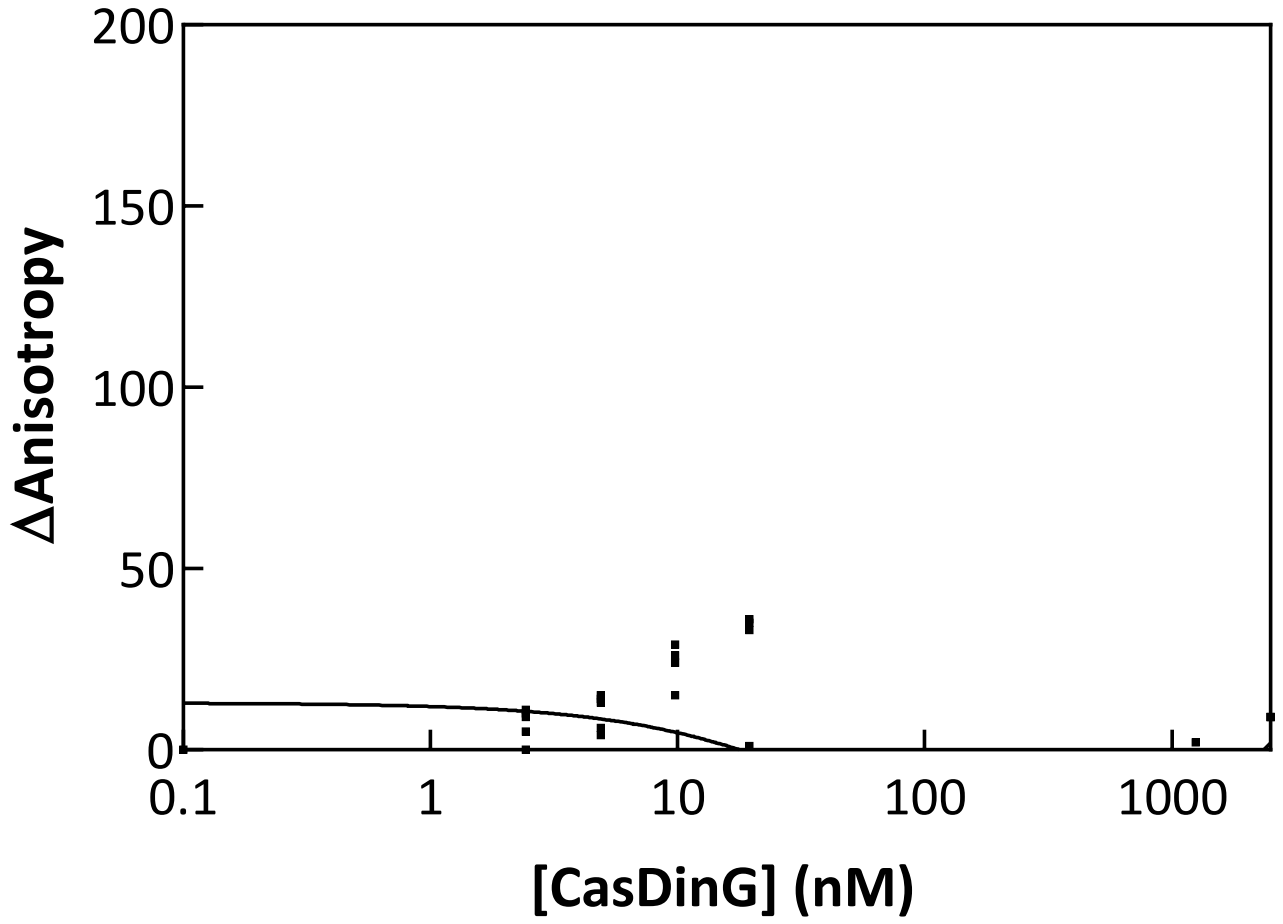


Can CasDinG bind to ssRNA substrates?

CasDinG does not appear to bind ssRNA



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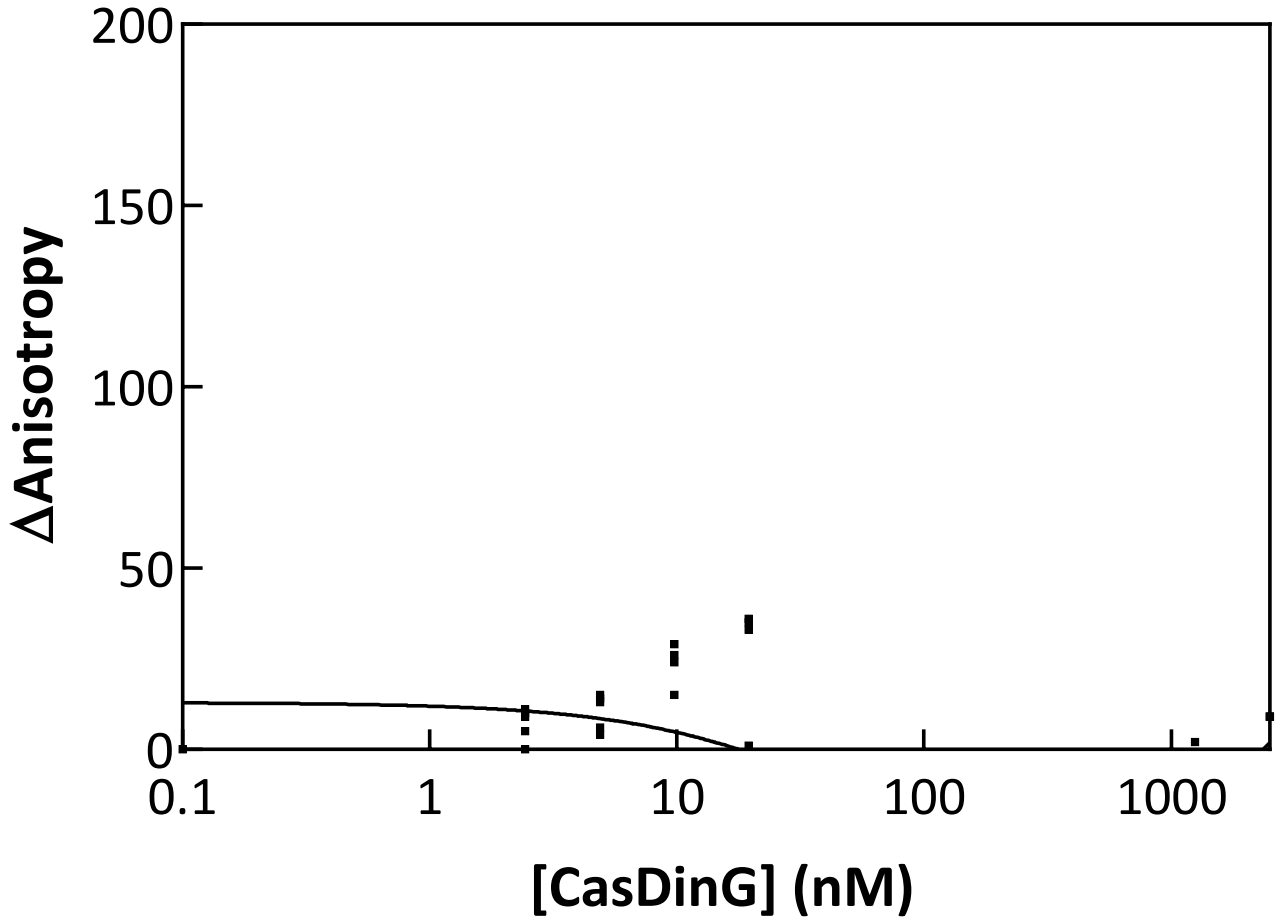
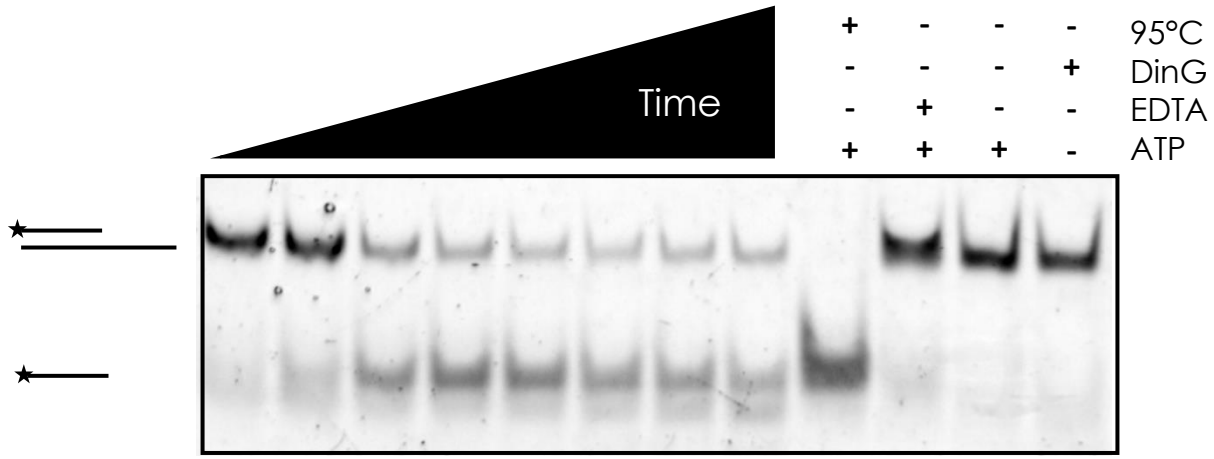


Can CasDinG unwind double-stranded substrates?

CasDinG unwinds DNA-RNA hybrids and may possess RNase activity



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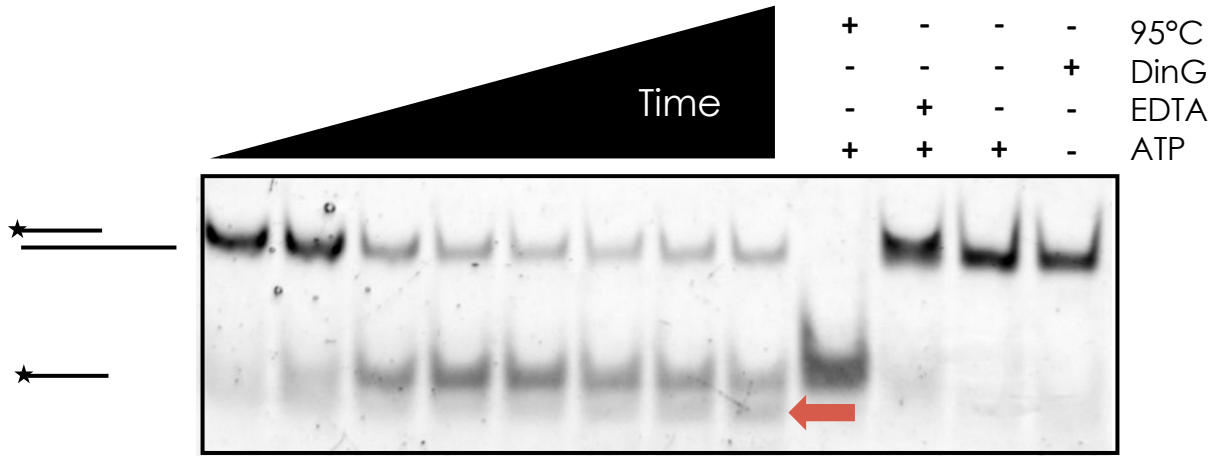

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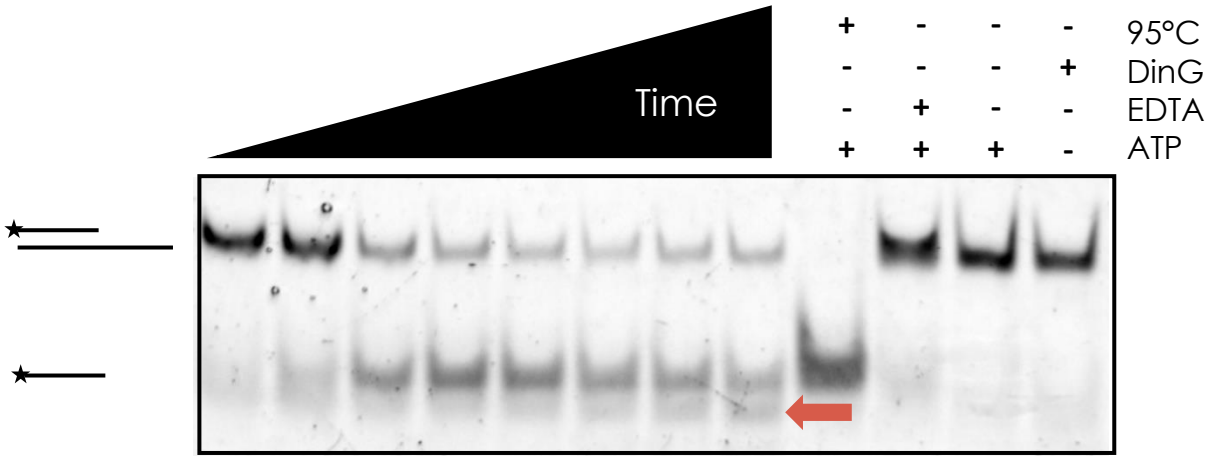
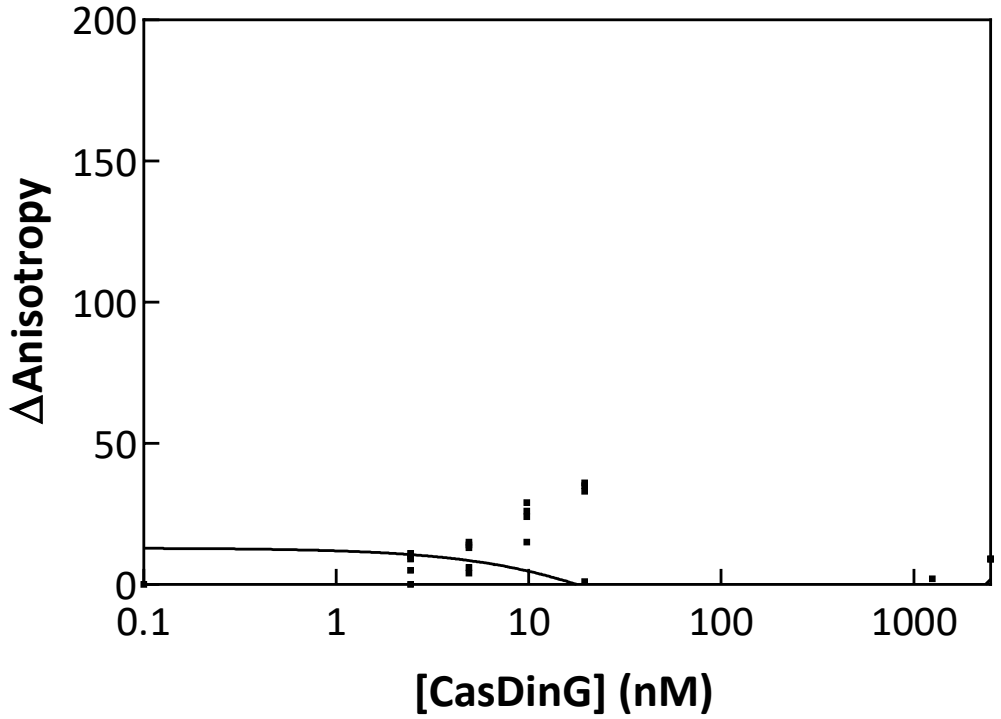
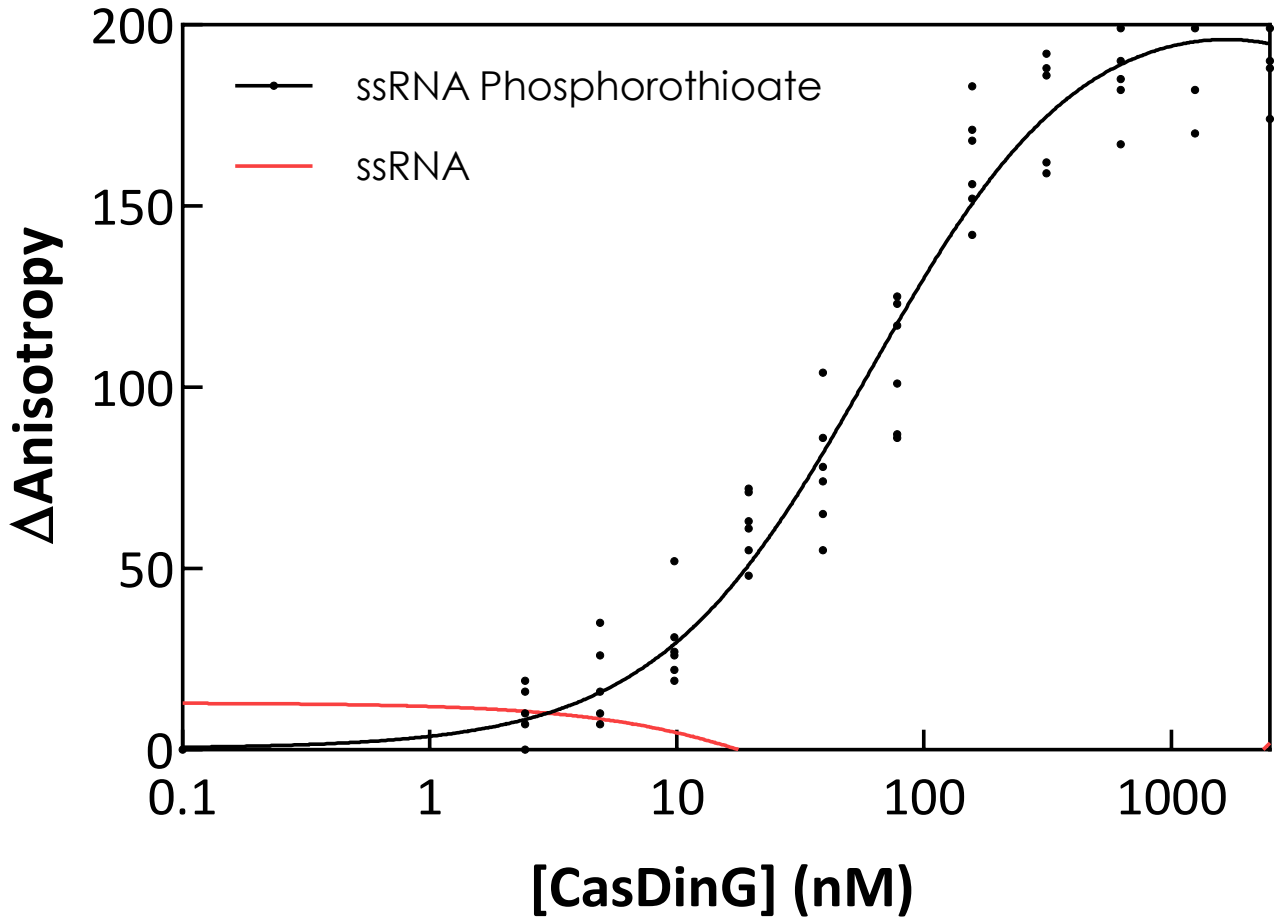
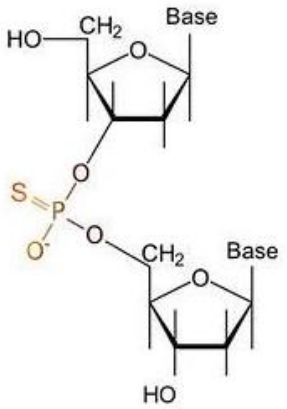




CasDinG binds to non-hydrolyzable ssRNA





Elucidating the role of CasDinG in CRISPR-mediated immunity

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Does the presence of ATP alter binding affinities?

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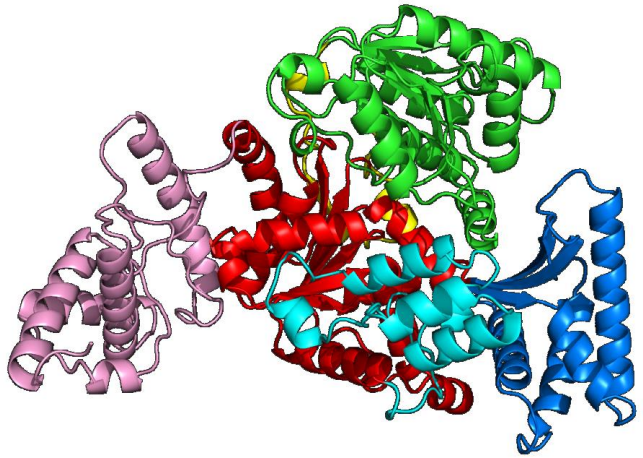
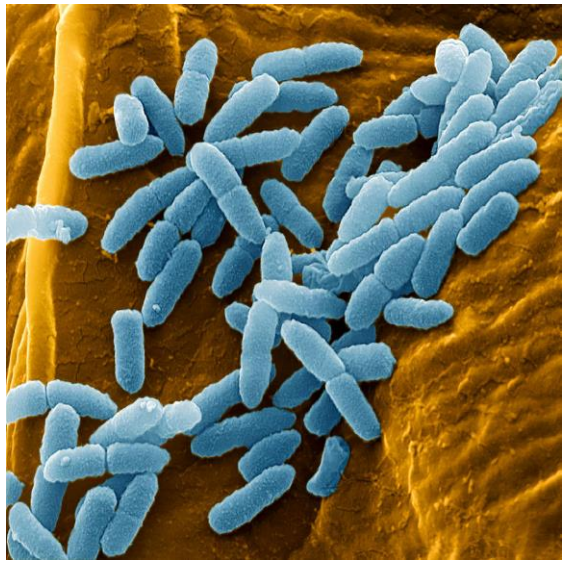


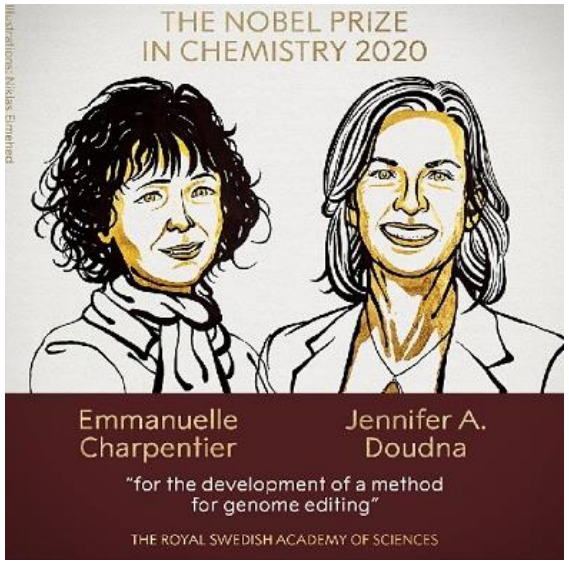
More work to be done…

Does CasDinG interact with the other Type IV-A gene products?

What is the atomic structure of CasDinG?

Insights from this research provide crucial groundwork for human therapeutics





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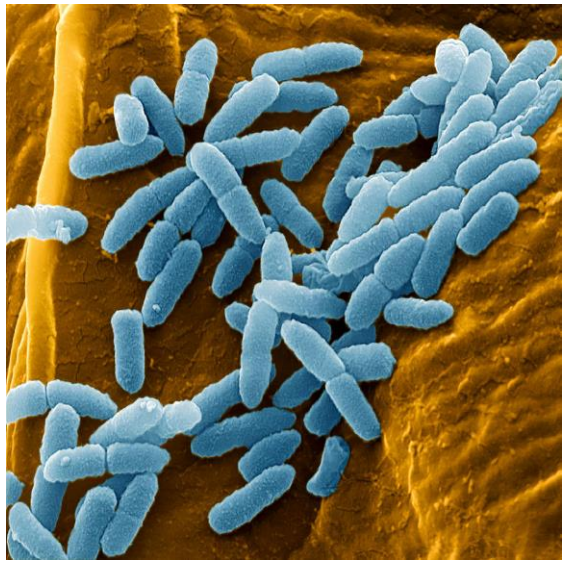
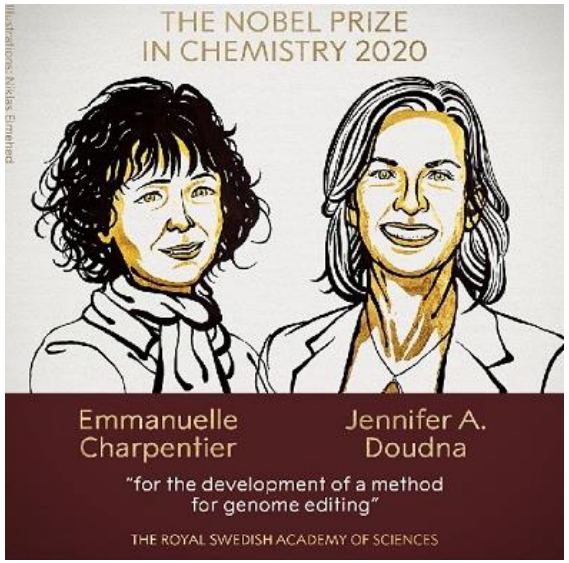
Insights from this research provide crucial groundwork for human therapeutics

- Understanding of bacterial immune systems and pathogenesis





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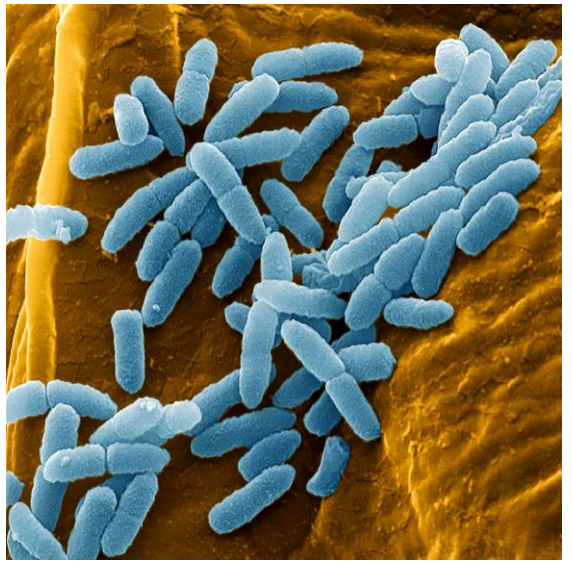

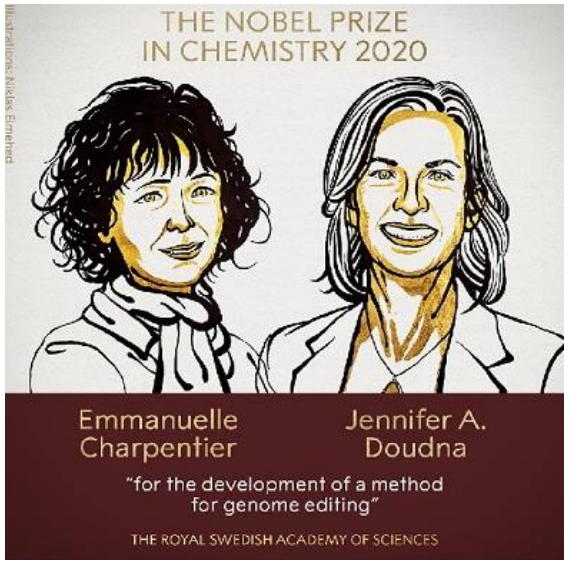

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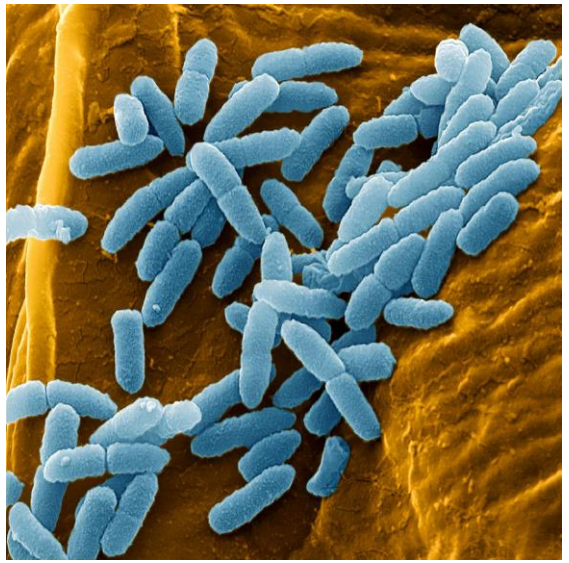
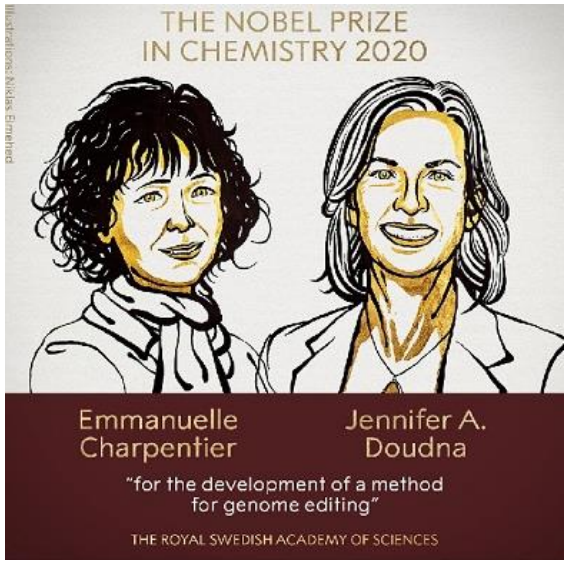
- Cas proteins have been used to detect COVID-19





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Thank you!
Any questions?

Poster Session: 1 PM