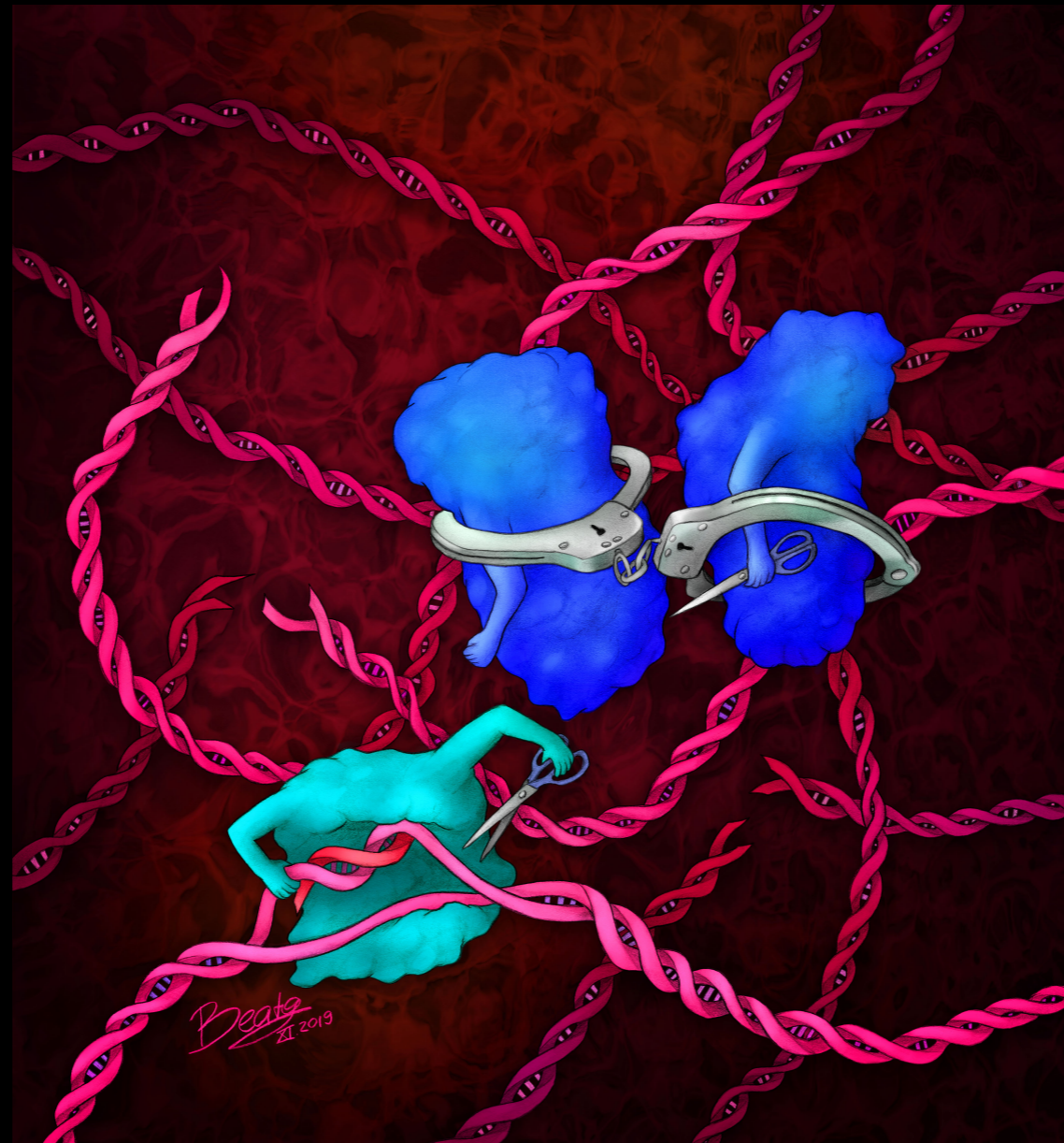


CRISPR-Cas9 allosteric inhibition by the viral anti-CRISPR protein AcrIIA6



Adeline Goulet
ESRF User Meeting - 10/02/2021

The CRISPR-Cas and anti-CRISPR craze

LETTER

doi:10.1038/nature11723

Bacteriophage genes that inactivate the CRISPR/Cas bacterial immune system

Joe Bondy-Denomy¹, April Pawluk², Karen L. Maxwell³ & Alan R. Davidson^{1,2}

Molecular Cell

Short Article

Inhibition Mechanism of an Anti-CRISPR Suppressor AcrIIA4 Targeting SpyCas9

Graphical Abstract

Authors

Hui Yang, Dinshaw...

Article

Molecular Cell

Diverse Mechanisms of CRISPR-Cas9 Inhibition by Type IIC Anti-CRISPR Proteins

Abstract

Authors

Yalan Zhu, Ang Gao, Qi Zhan, ..., Guangxia Gao, Alexander Serganov, Pu Gao

nature **methods**

Anti-CRISPR protein applications: natural brakes for CRISPR-Cas technologies

Nicole D. Marino¹, Rafael Pinilla-Redondo^{1,2,3,7}, Bálint Csörgő^{1,4,7} and Joseph Bondy-Denomy^{1,5,6}

REVIEW ARTICLE

<https://doi.org/10.1038/s41592-020-0771-6>

Check for updates

RESEARCH ARTICLE

A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity

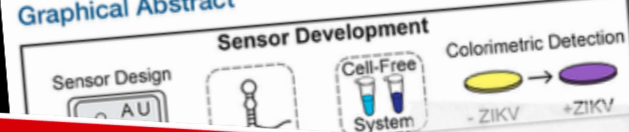
Martin Jinek^{1,2*}, Krzysztof Chylinski^{3,4*}, Ines Fonfara⁴, Michael H... Jennifer A. Doudna^{1,2,5,6,†}, Emmanuelle Charpentier^{4,‡}

Resource

Cell

Rapid, Low-Cost Detection of Zika Virus Using Programmable Biomolecular Components

Graphical Abstract



Authors

Keith Pardee, Alexander A. Green, Melissa K. Takahashi, ..., David H. O'Connor, Lee Gehrke, James J. Collins

LETTERS

<https://doi.org/10.1038/s41587-020-0513-4>

nature **biotechnology**

Check for updates

CRISPR-Cas12-based detection of SARS-CoV-2

James P. Broughton^{1,7}, Xianding Deng^{2,3,7}, Guixia Yu^{2,3}, Clare L. Fasching¹, Venice Servellita^{2,3}, Jasmeet Singh¹, Xin Miao¹, Jessica A. Streithorst², Andrea Granados^{2,3}, Alicia Sotomayor-Gonzalez^{2,3}, Kelsey Zorn⁴, Allan Gopez², Elaine Hsu², Wei Gu², Steve Miller², Chao-Yang Pan⁵, Hugo Guevara⁵, Debra A. Wadford⁵, Janice S. Chen^{1,8} and Charles Y. Chiu^{2,3,6}

The CRISPR-Cas and anti-CRISPR craze

Nobel Prize in Chemistry 2020 :

« for the development of a method for genome editing »



Emmanuelle
Charpentier

Jennyfer
Doudna



The CRISPR-Cas9 genetic scissors

Phages versus bacteria : the battle for survival

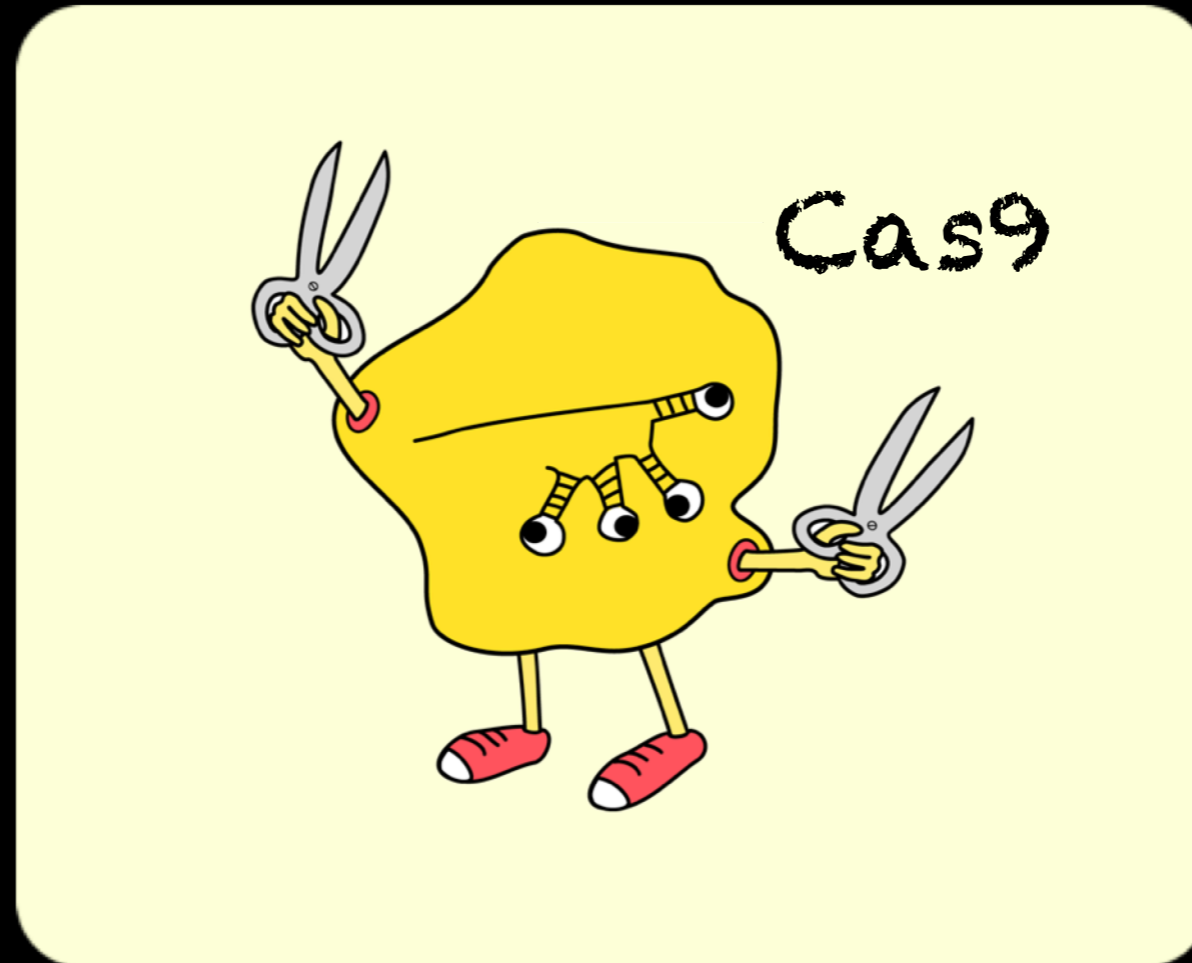


Image courtesy of Graham Beards

co-adaptation of defense and attack mechanisms

Bacteria : CRISPR-Cas9 immunity

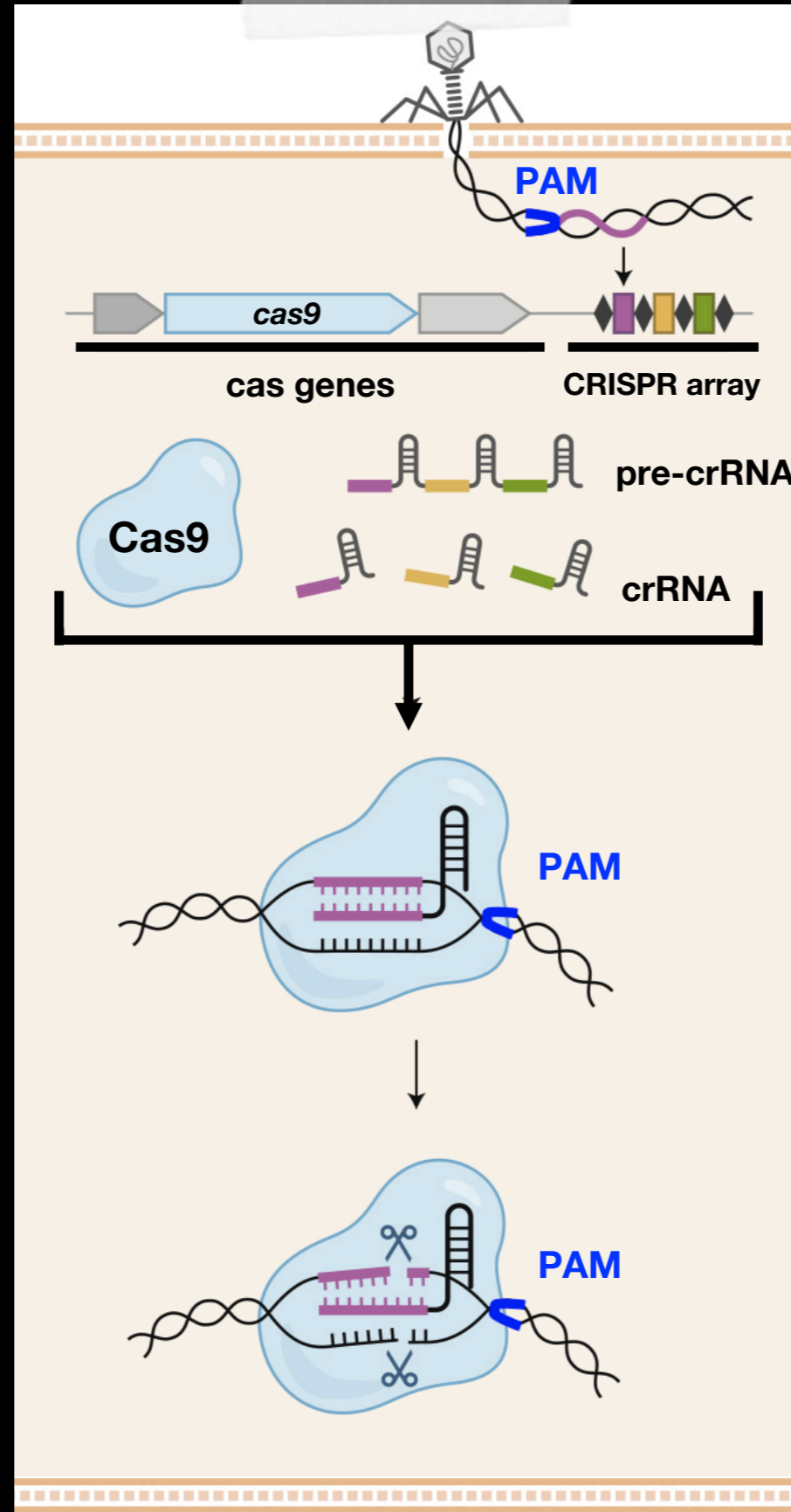
Adaptive CRISPR-Cas9 immune system



Sequence specific cleavage of foreign nucleic acids

Bacteria : CRISPR-Cas9 immunity

1. Acquisition

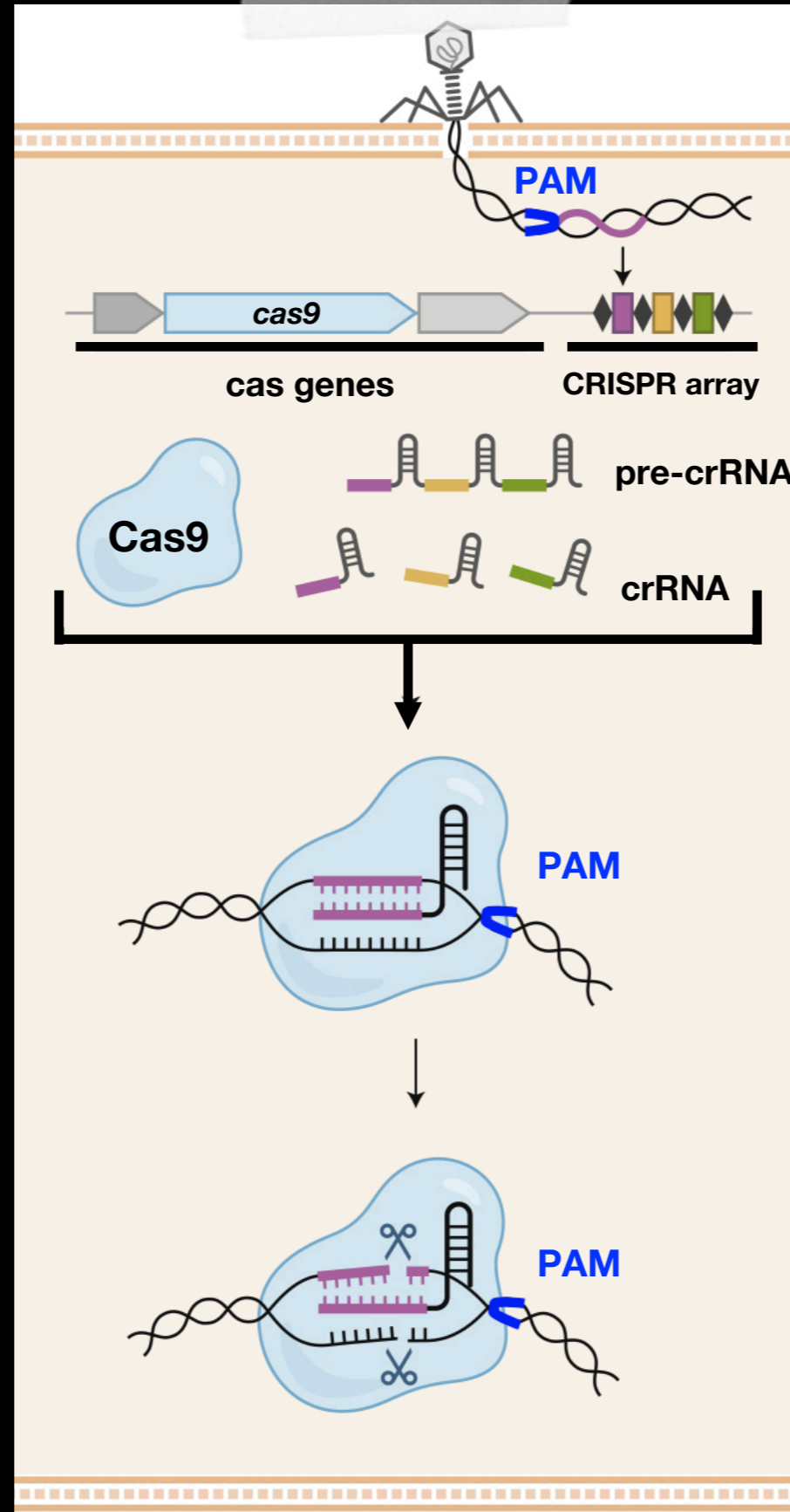


viral infection memory

Bacteria : CRISPR-Cas9 immunity

1. Acquisition

2. Biogenesis



viral infection memory

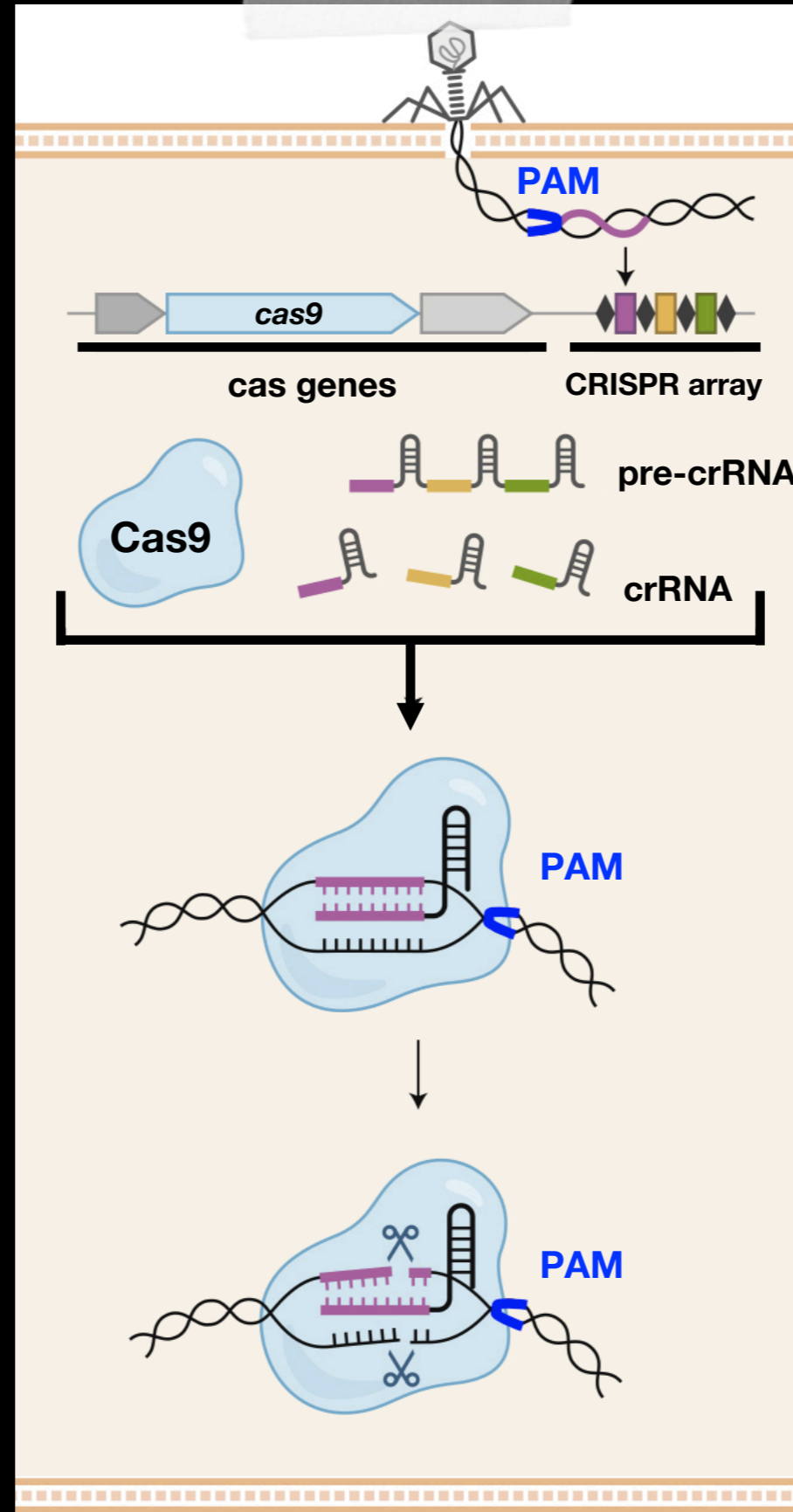
surveillance complex
(Cas9-RNA)

Bacteria : CRISPR-Cas9 immunity

1. Acquisition

2. Biogenesis

3. Interference



viral infection memory

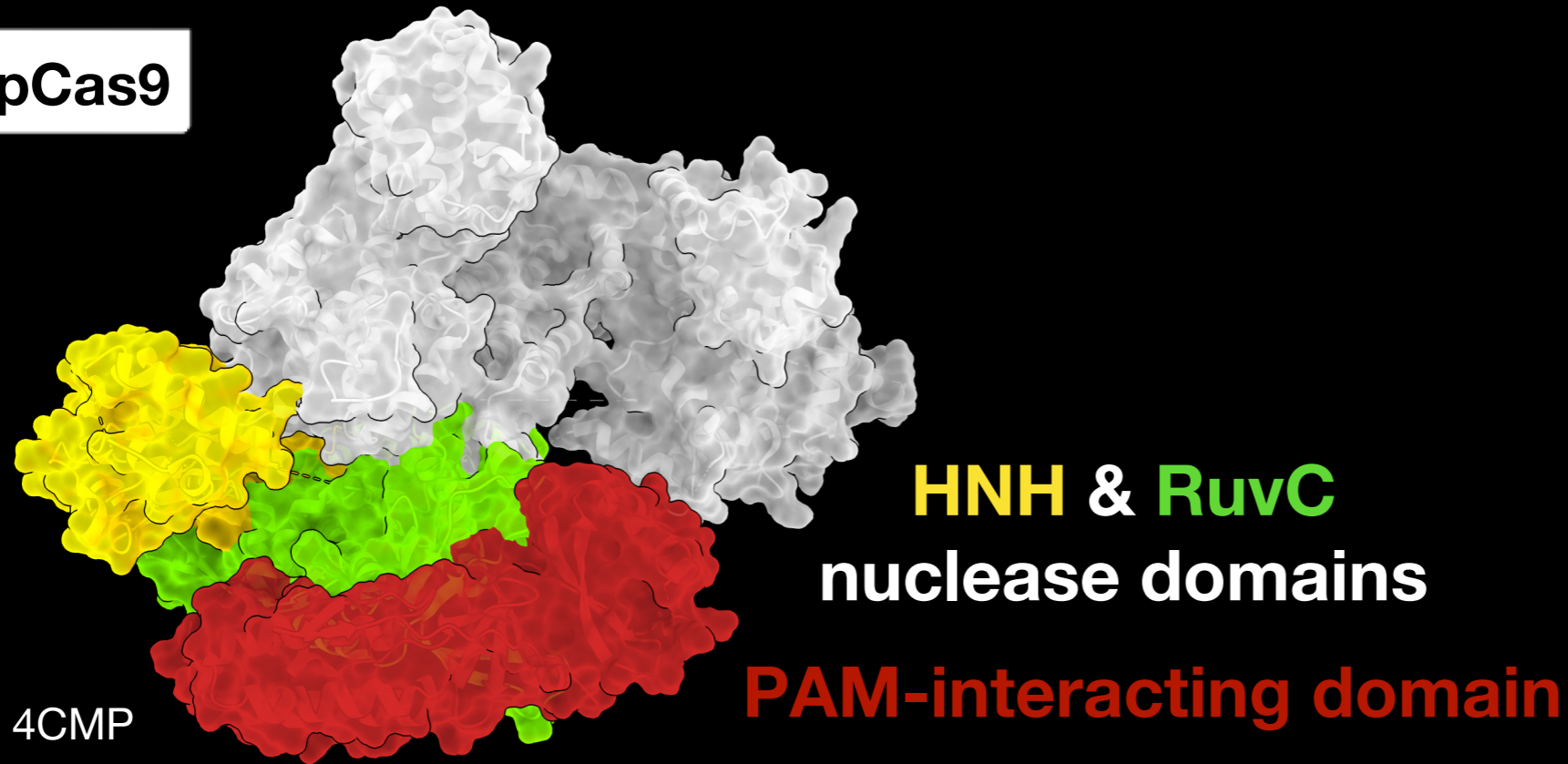
surveillance complex
(Cas9-RNA)

target DNA binding

target DNA cleavage

Bacteria : CRISPR-Cas9 immunity

apo SpCas9



PDB ID 4CMP

HNH & RuvC
nuclease domains

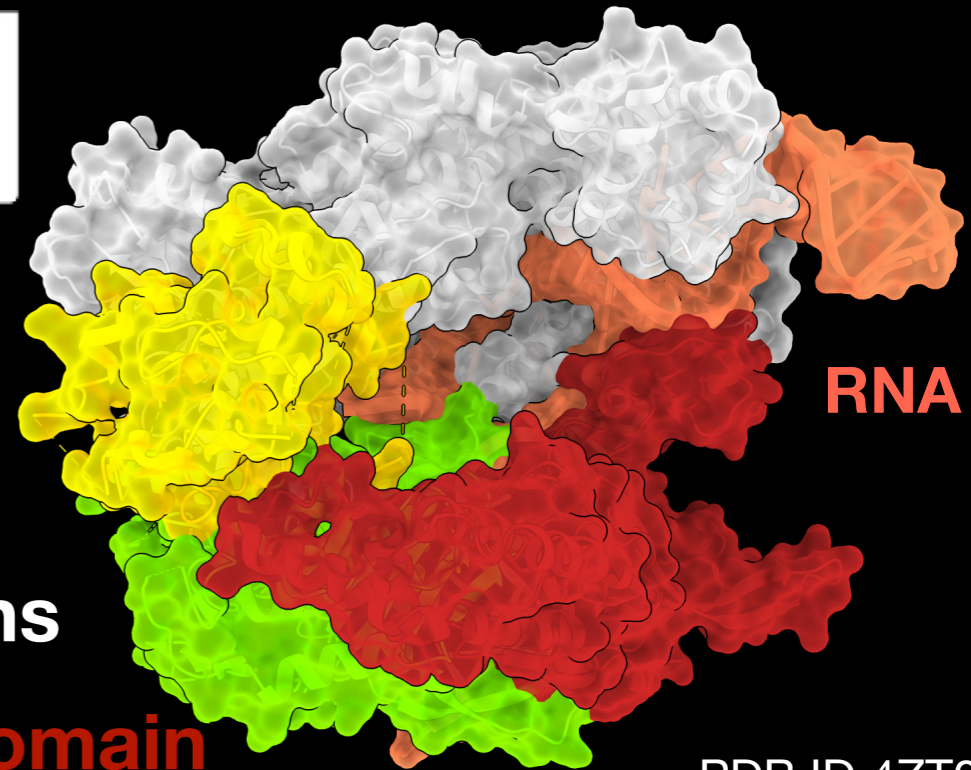
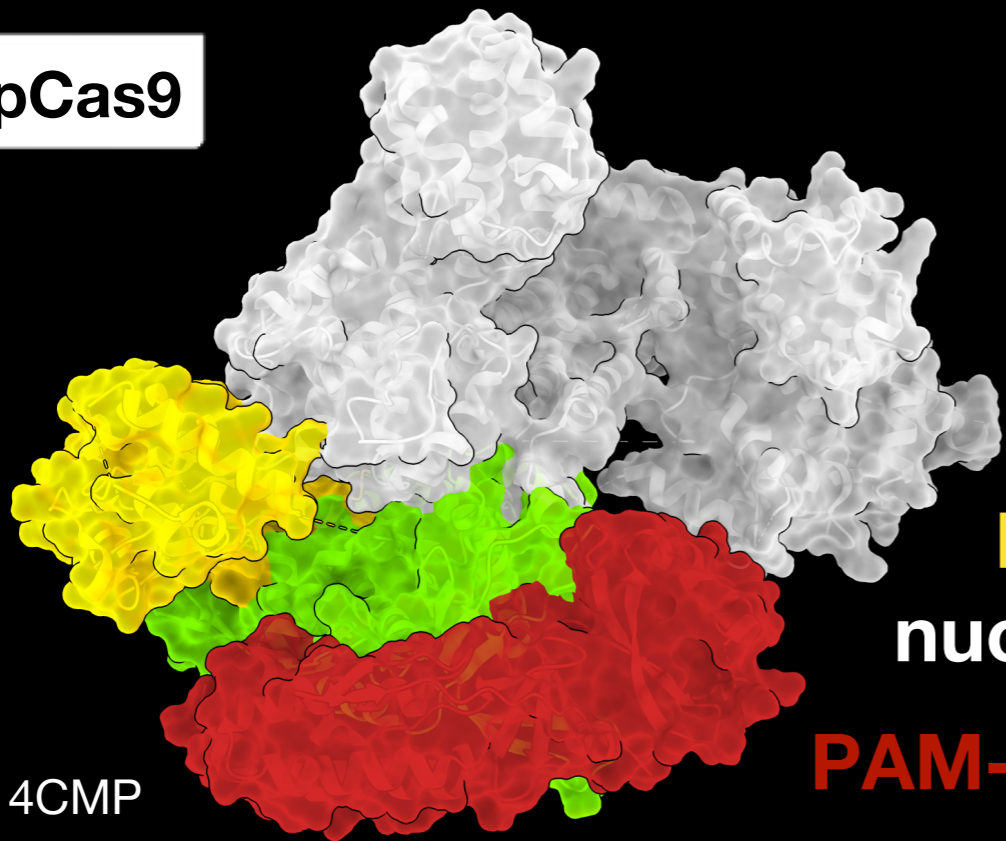
PAM-interacting domain

Streptococcus pyogenes Cas9 (SpCas9)

Bacteria : CRISPR-Cas9 immunity

apo SpCas9

surveillance complex



PDB ID 4CMP

PDB ID 4ZT0

HNH & RuvC
nuclease domains

PAM-interacting domain

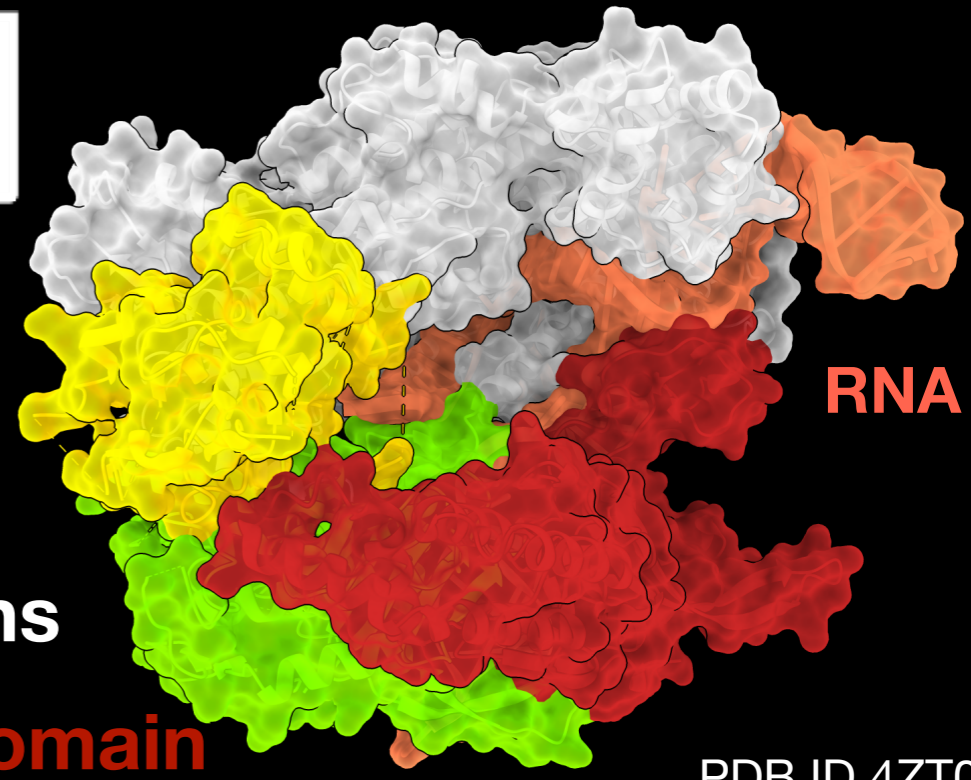
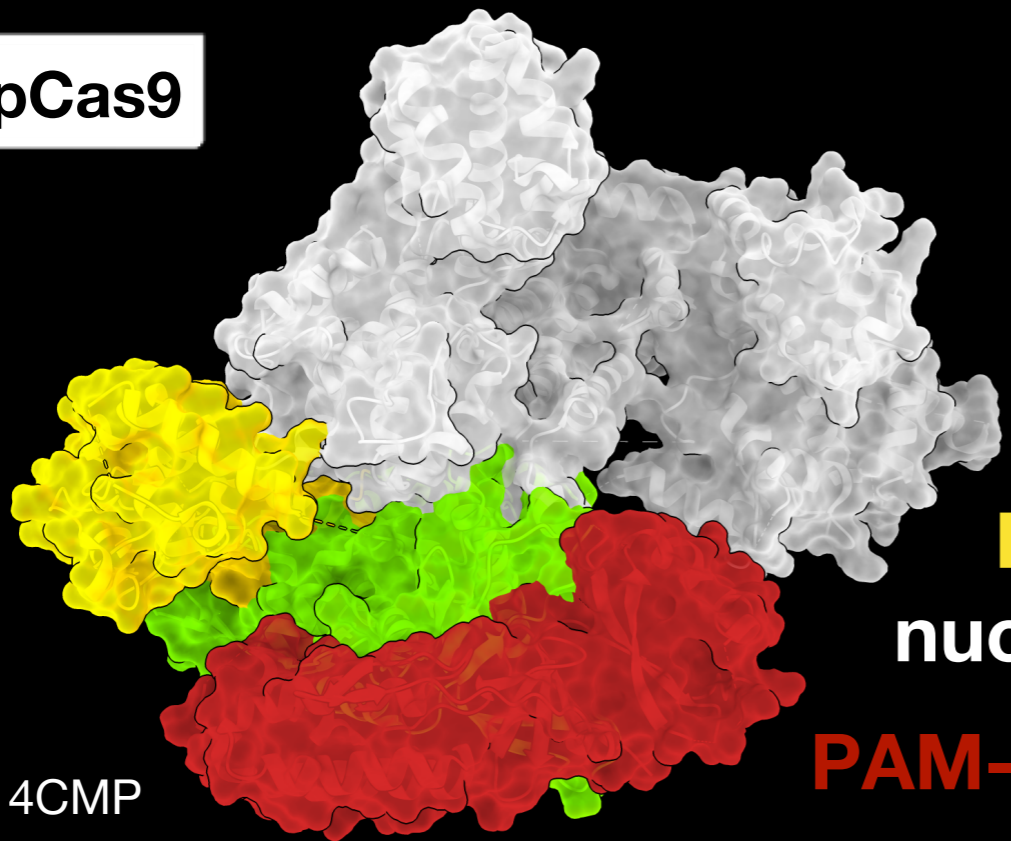
RNA

Streptococcus pyogenes Cas9 (SpCas9)

Bacteria : CRISPR-Cas9 immunity

apo SpCas9

surveillance complex



HNH & RuvC
nuclease domains

PAM-interacting domain

RNA

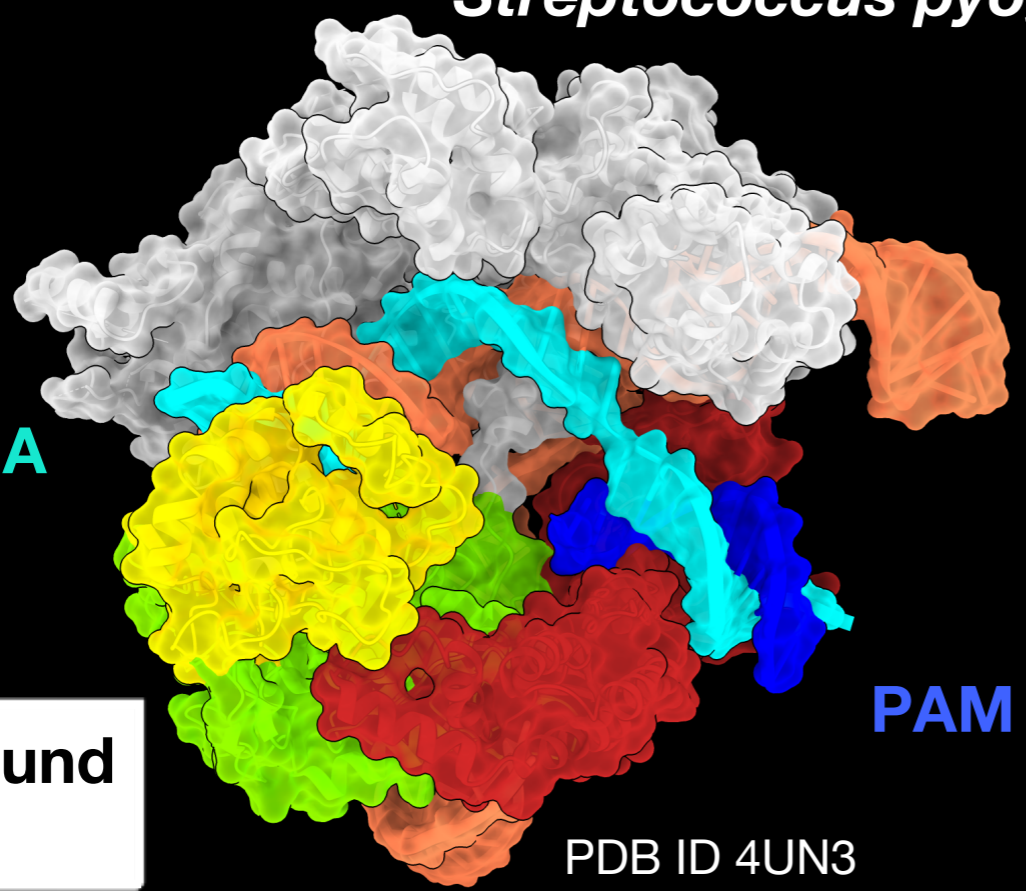
PDB ID 4CMP

PDB ID 4ZT0

Streptococcus pyogenes Cas9 (SpCas9)

target DNA

target-bound state



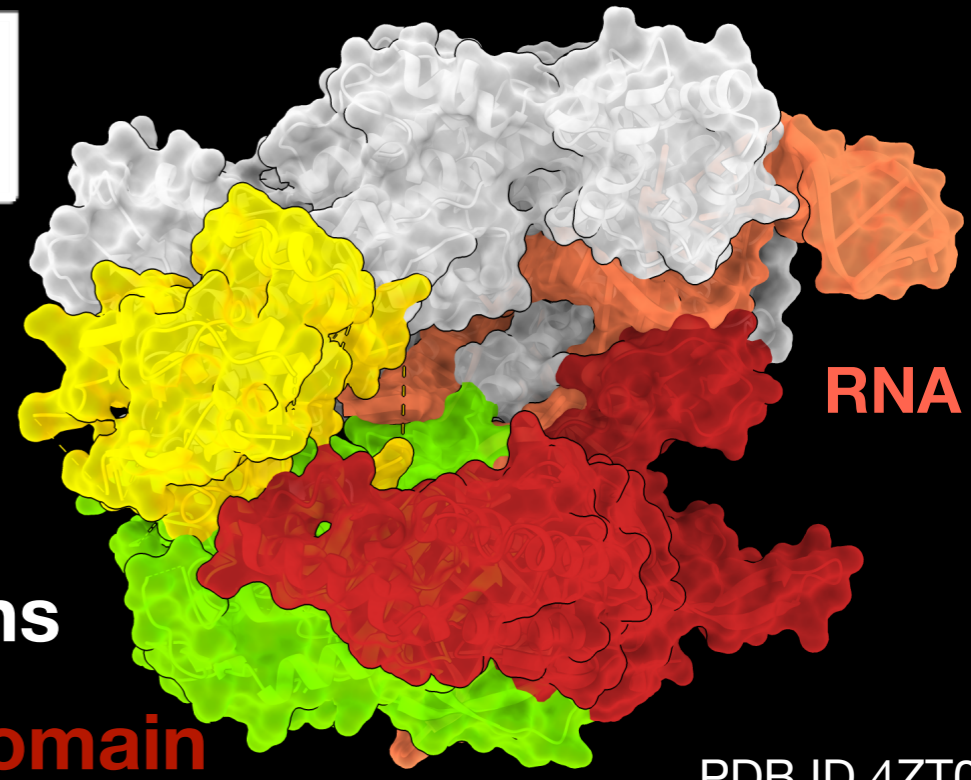
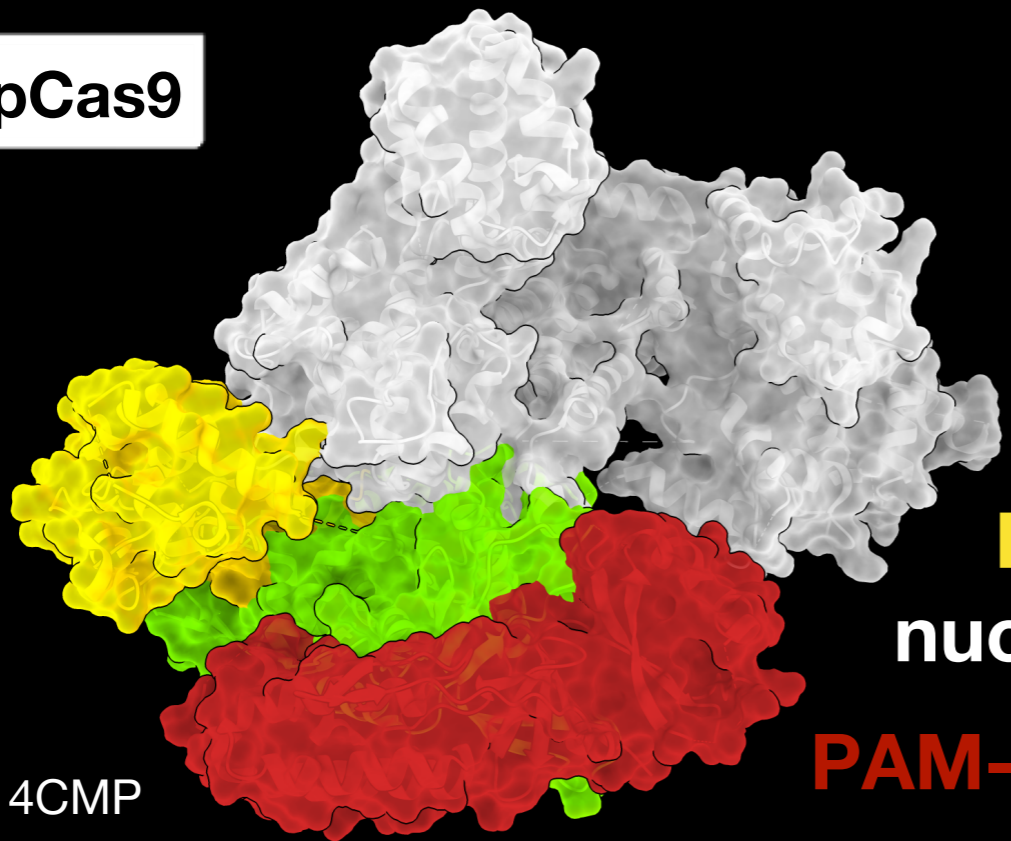
PAM

PDB ID 4UN3

Bacteria : CRISPR-Cas9 immunity

apo SpCas9

surveillance complex



PDB ID 4CMP

PDB ID 4ZT0

HNH & RuvC
nuclease domains

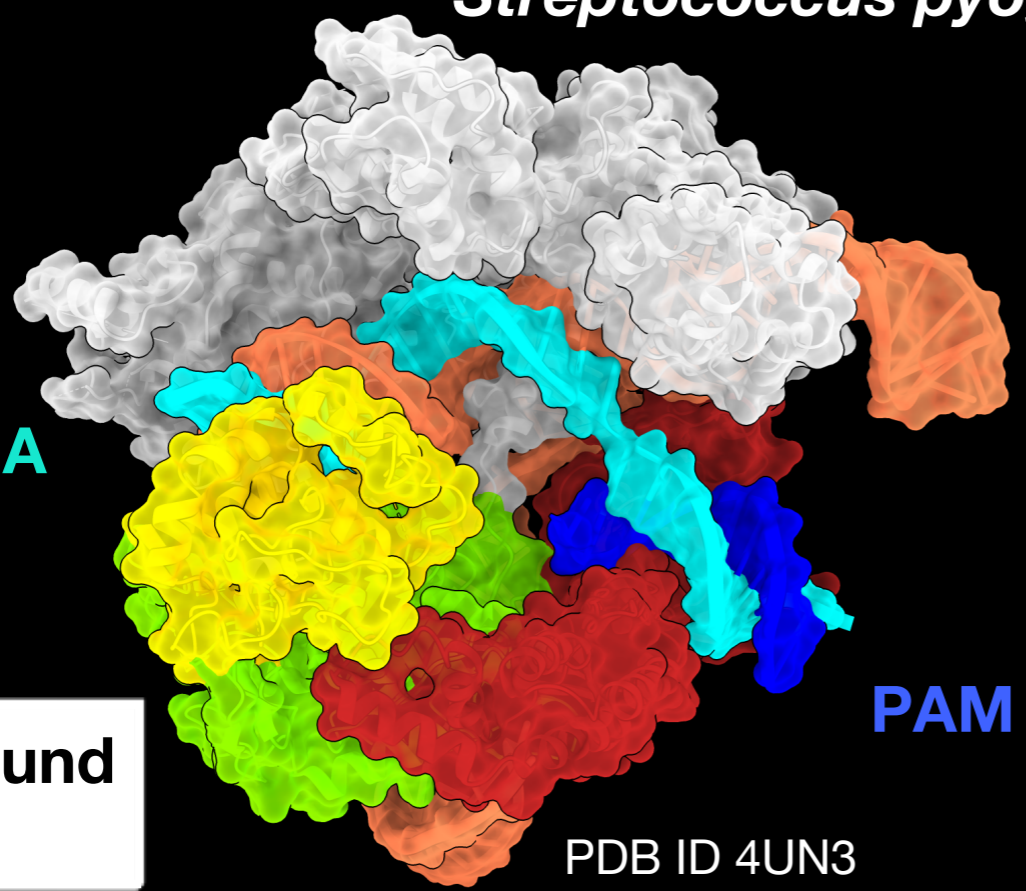
PAM-interacting domain

RNA

Streptococcus pyogenes Cas9 (SpCas9)

target DNA

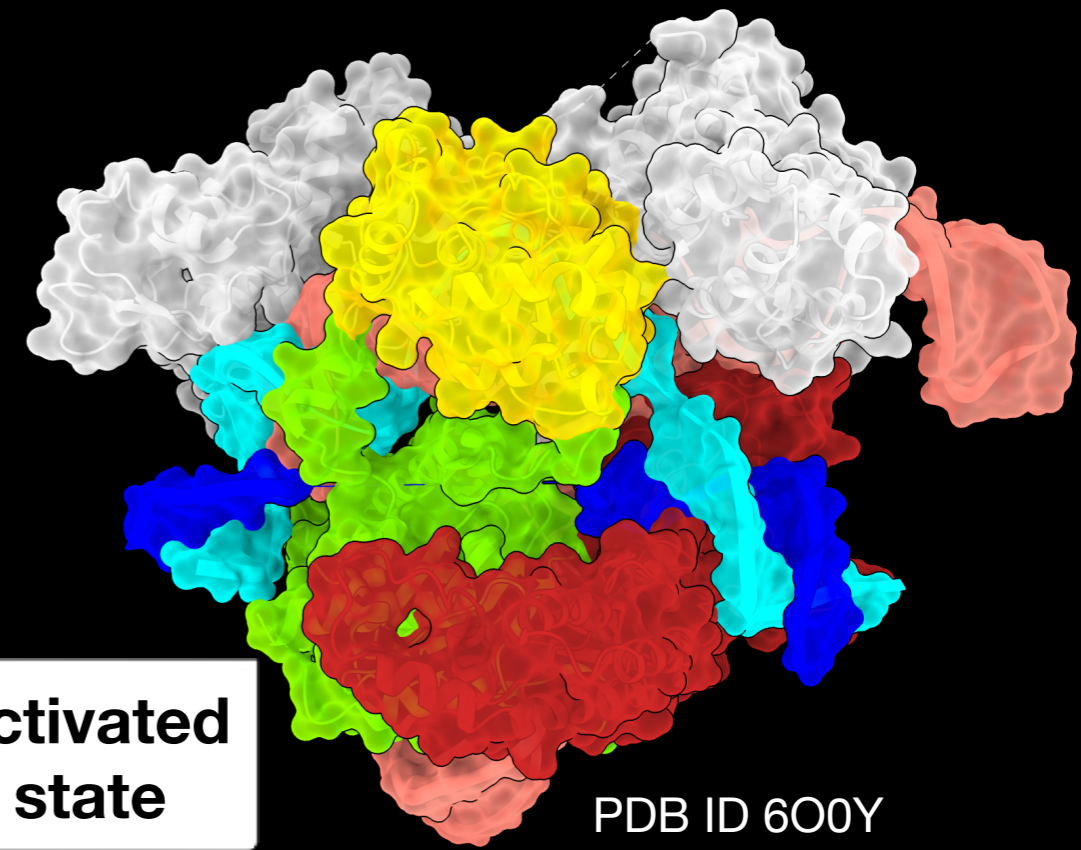
target-bound state



PDB ID 4UN3

PAM

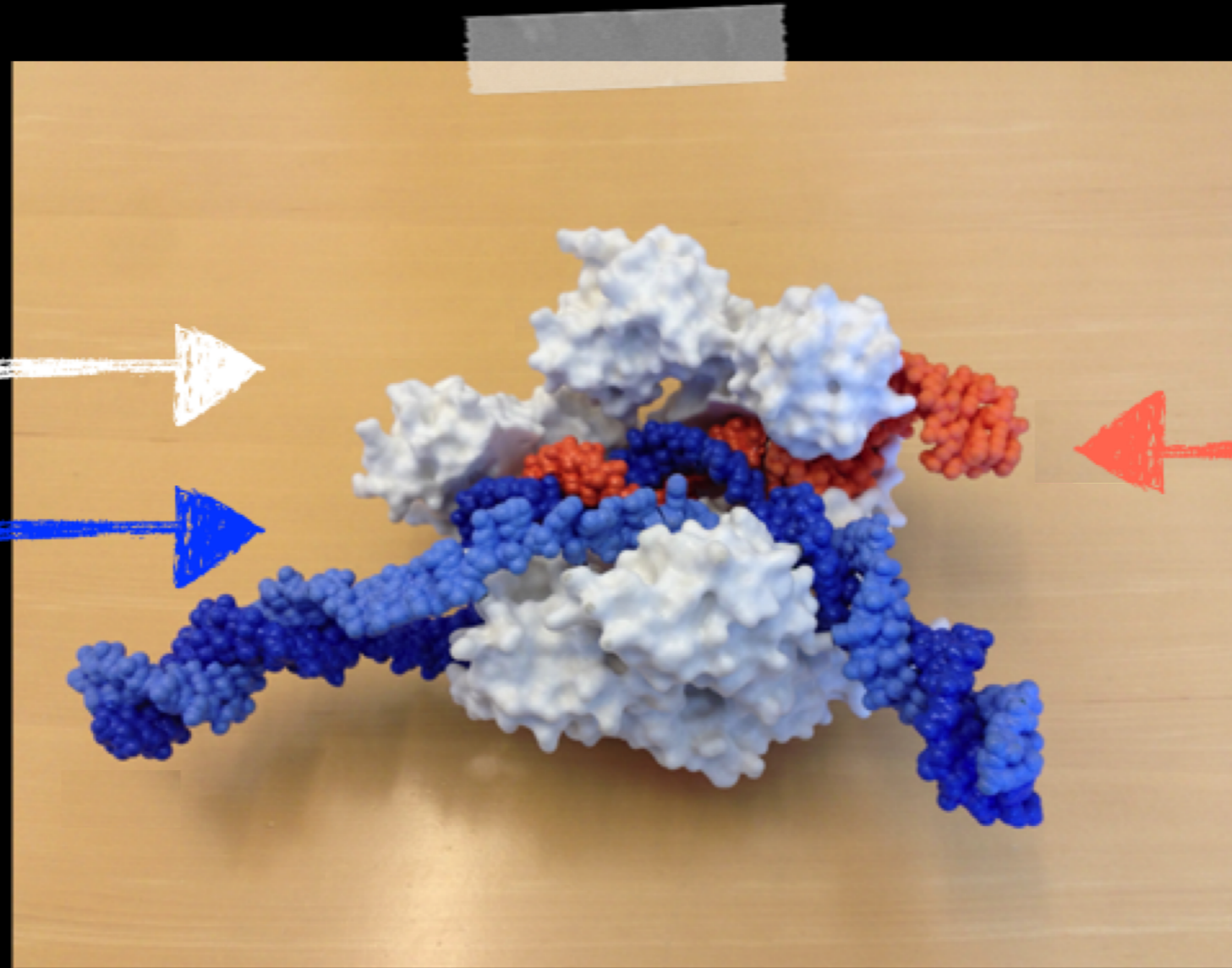
activated state



PDB ID 6O0Y

Bacteria : CRISPR-Cas9 immunity

Cas9
Target
DNA

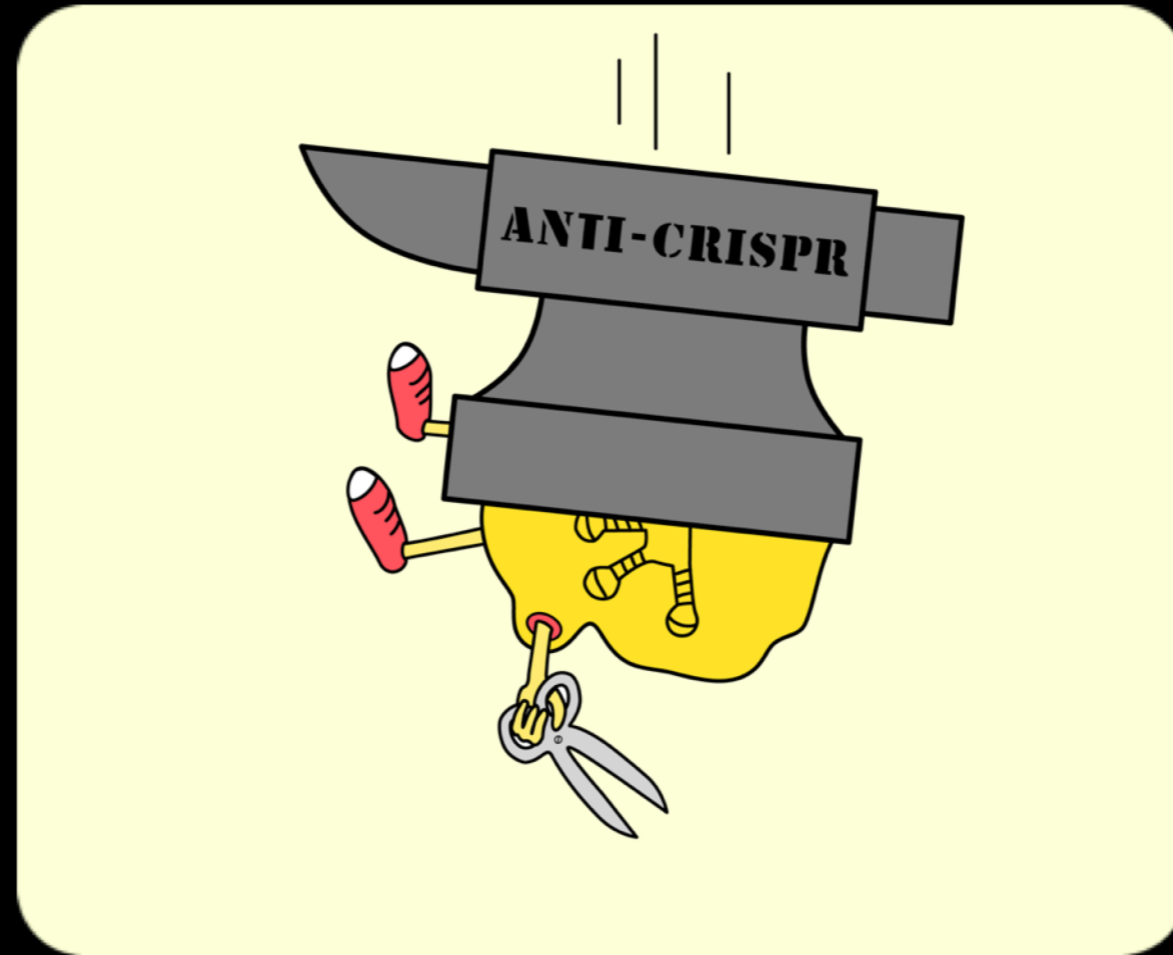


RNA

Streptococcus pyogenes Cas9 (SpCas9)

Phages : anti-CRISPR-Cas9 proteins

Phage anti-CRISPR proteins



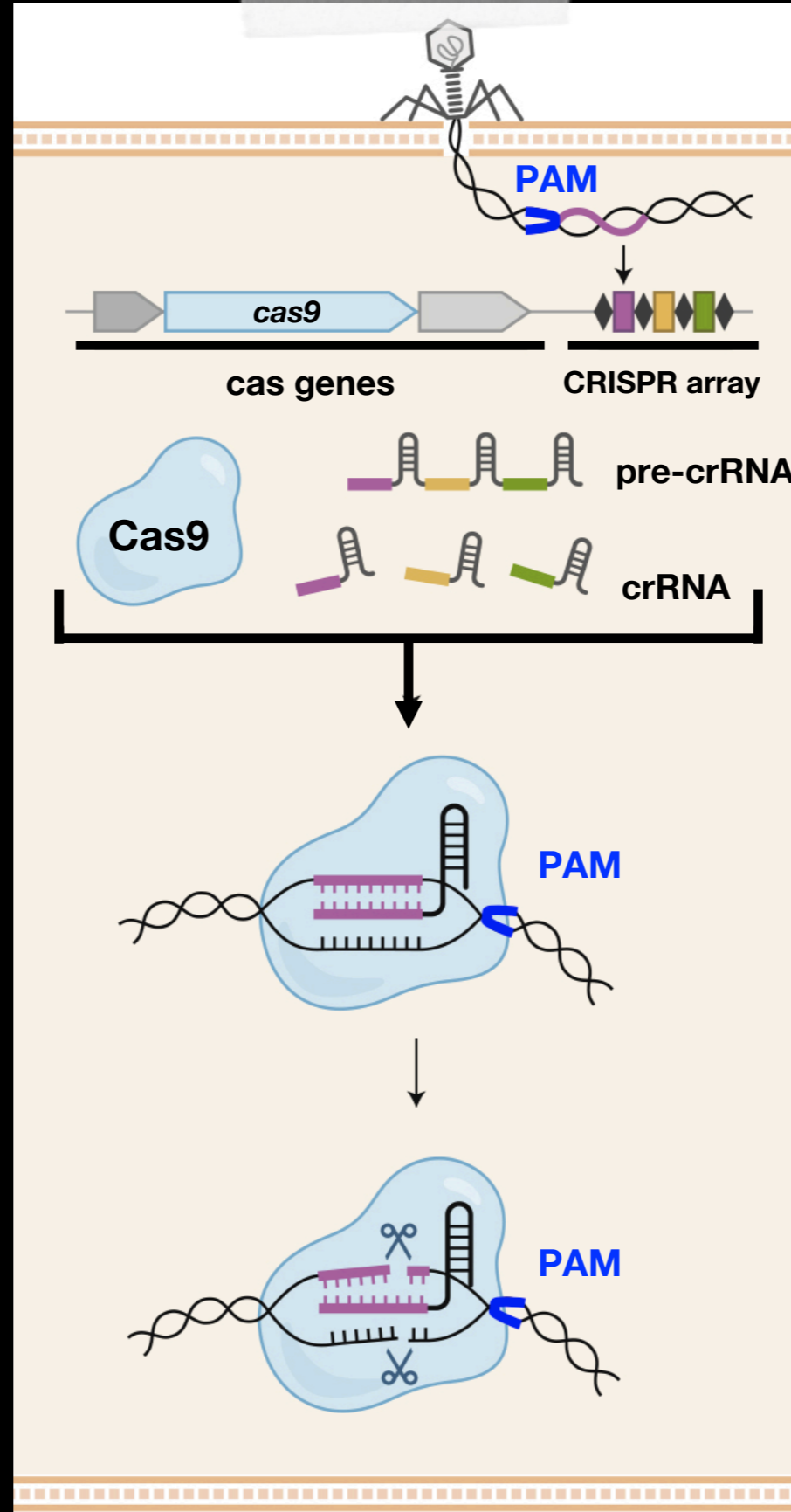
Inactivation of CRISPR-Cas9 immunity

Phages : anti-CRISPR-Cas9 proteins

1. Acquisition 

2. Assembling 

3. Interference 



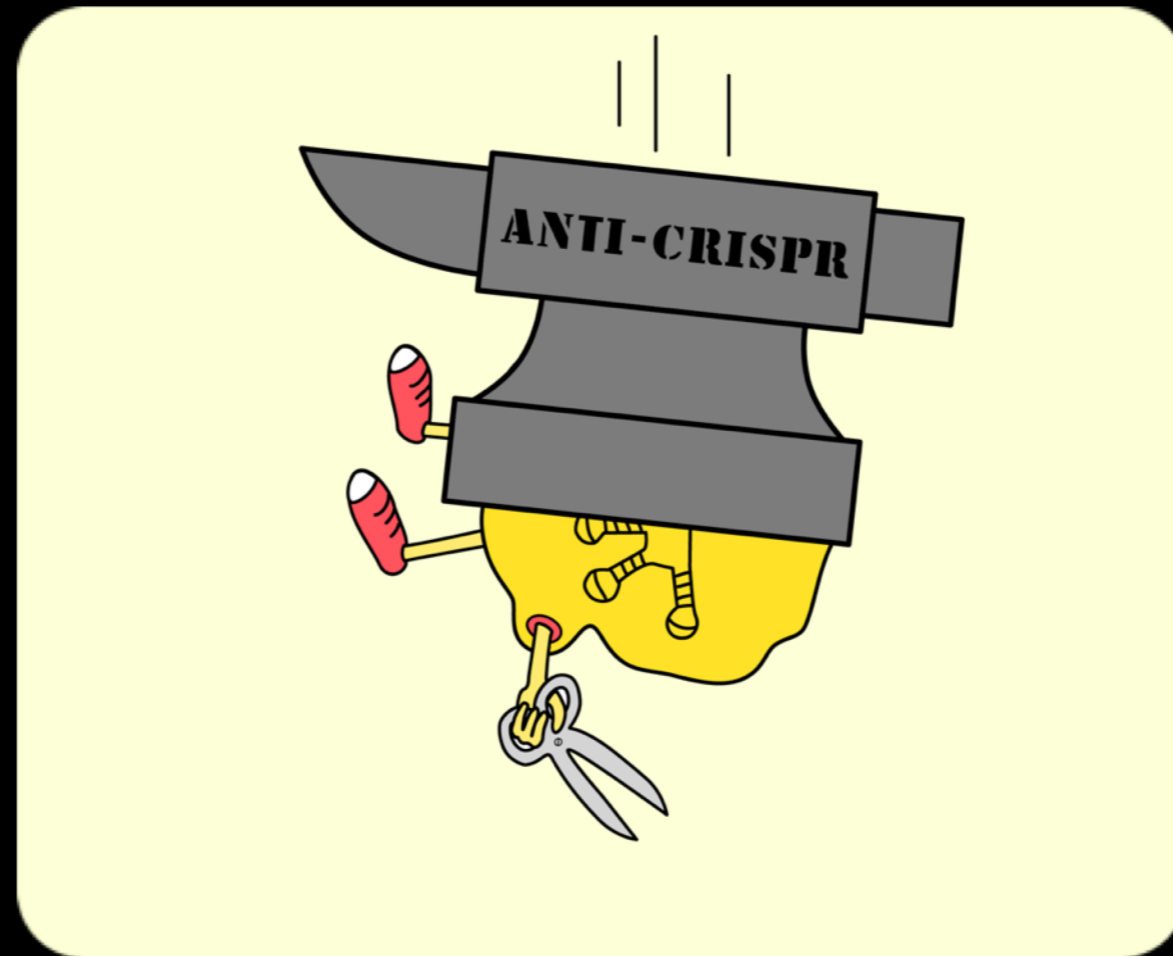
viral infection  memory

surveillance  complex (Cas9-RNAs)

target DNA  binding

target DNA  cleavage

Phages : anti-CRISPR-Cas9 proteins



- Widespread in prophages, virulent phages, MGEs
- 90 anti-CRISPR families
- No resemblance with proteins of known function

What are their modes of action?

What are their modes of action?

What are their molecular mechanisms ?

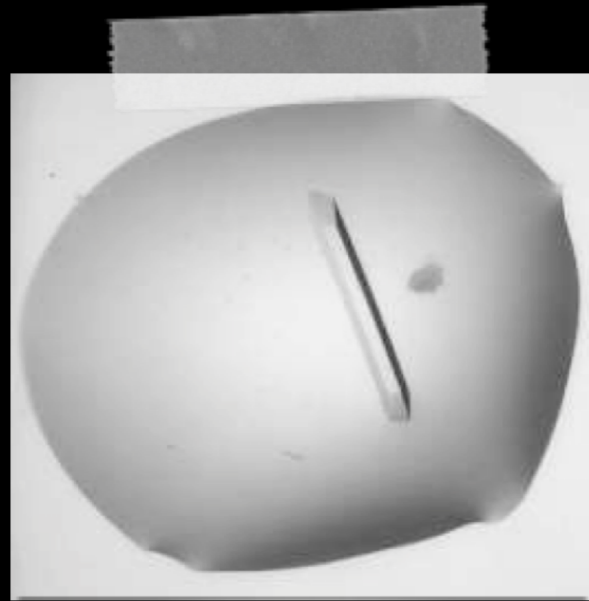
Allosteric inhibition of *Streptococcus thermophilus* Cas9 (St1 Cas9) by the anti-CRISPR protein AcrIIA6



Sylvain Moineau

Geneviève Rousseau

AcrIIA6 shows a novel α/β fold



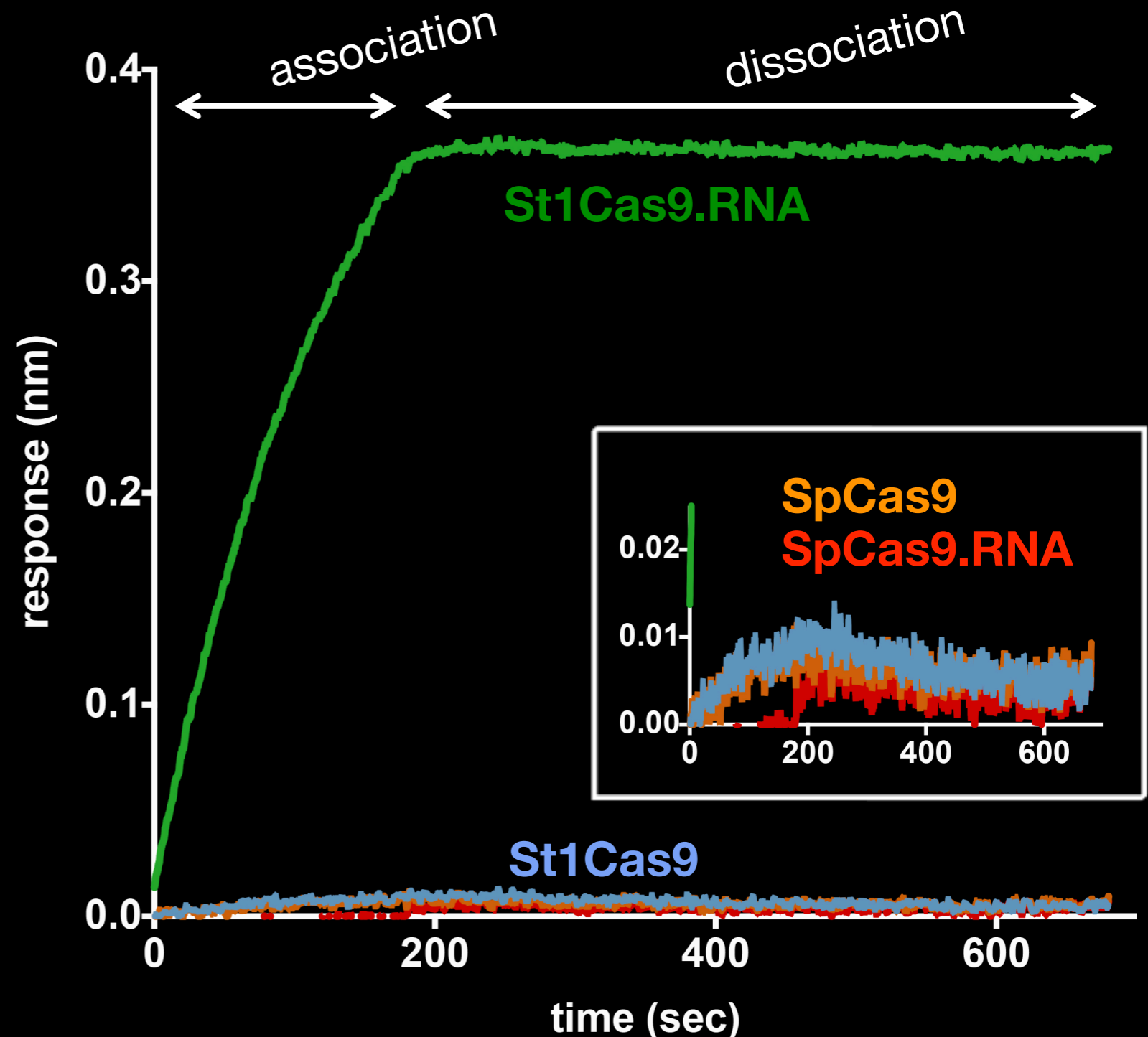
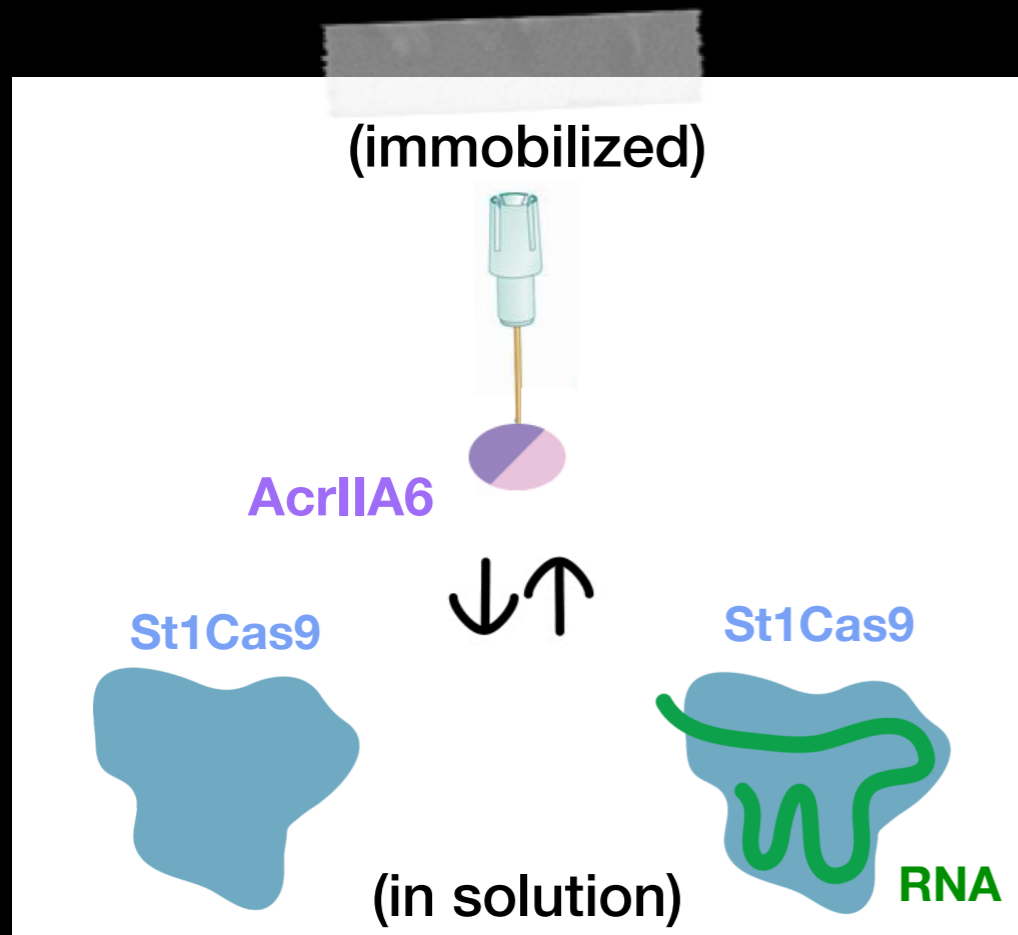
SAD phasing
Proxima-1 @ SOLEIL (Fr)

1.96 Å resolution



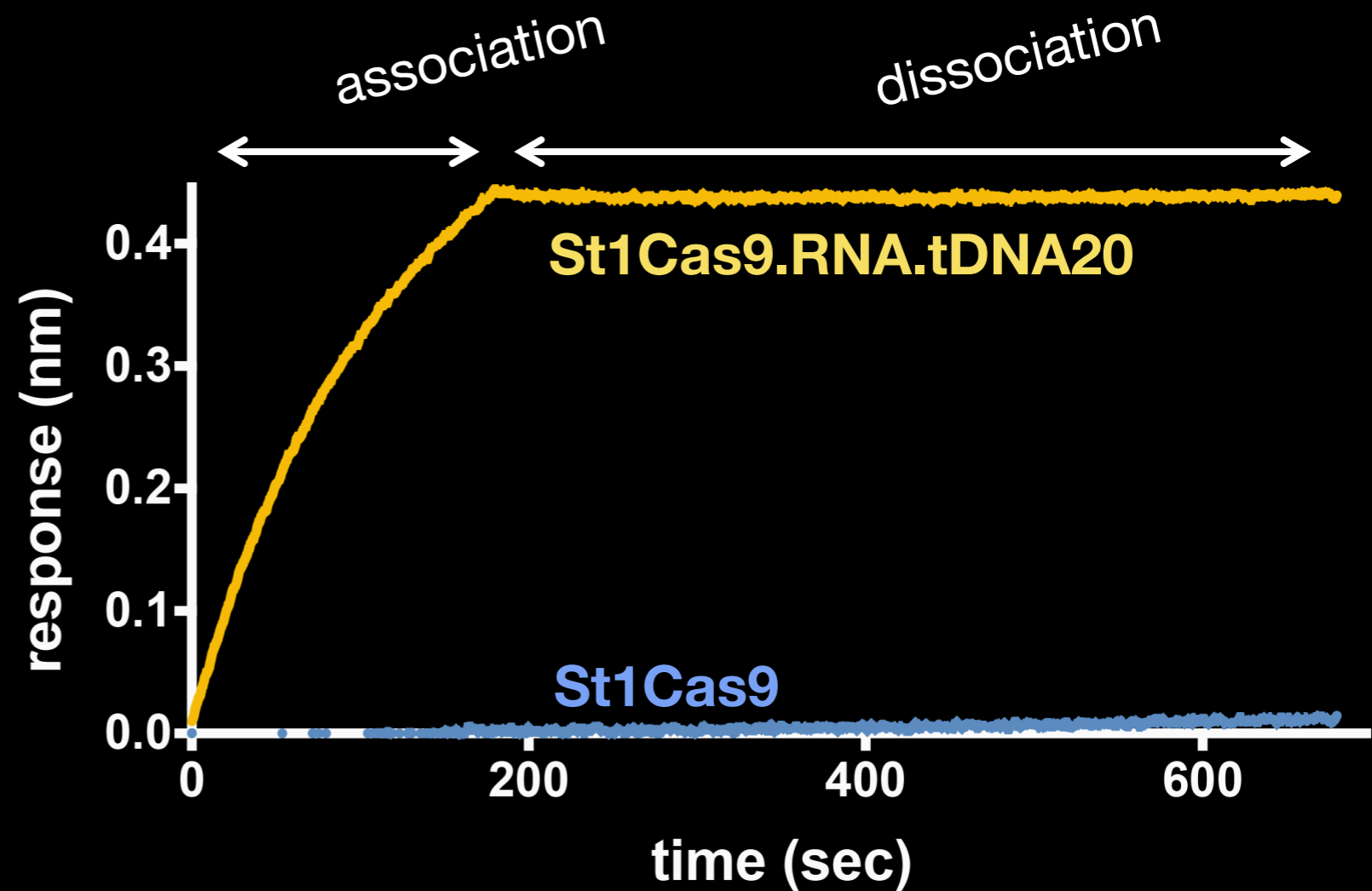
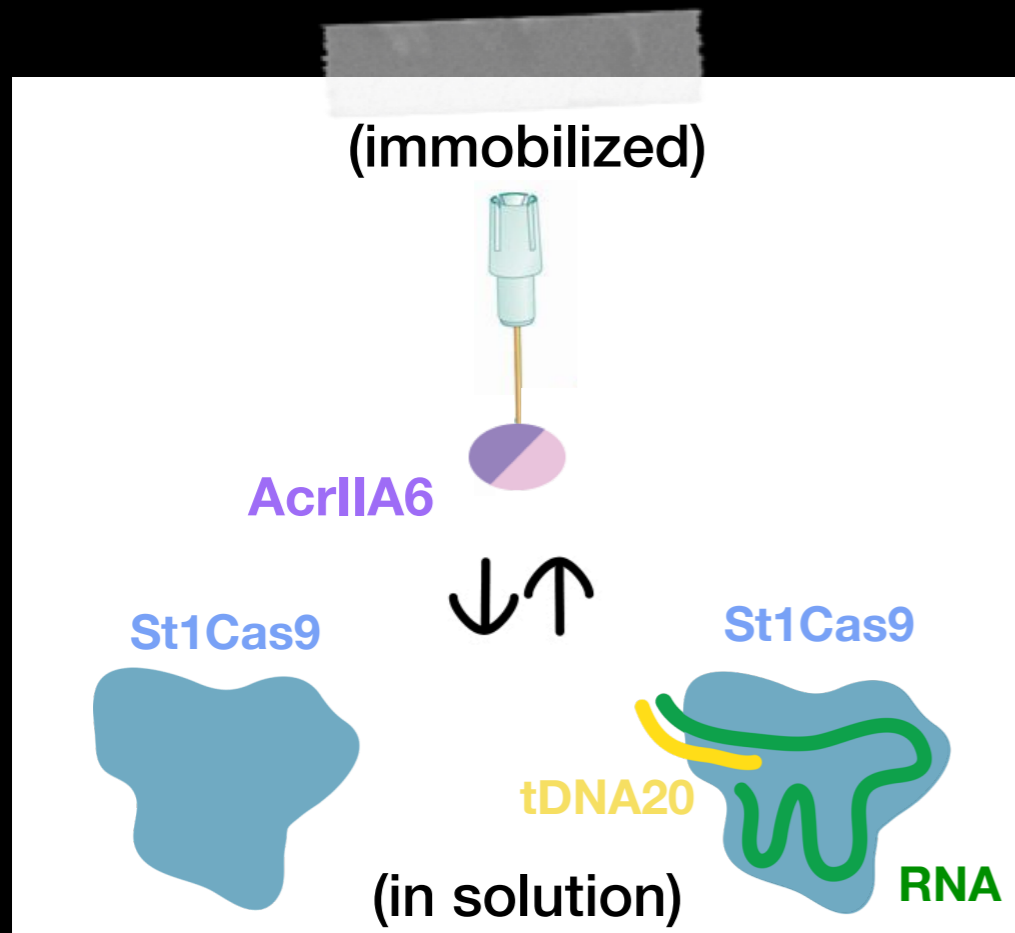
AcrIIA6 stably binds to St1Cas9 surveillance complex

BioLayer Interferometry (BLI)

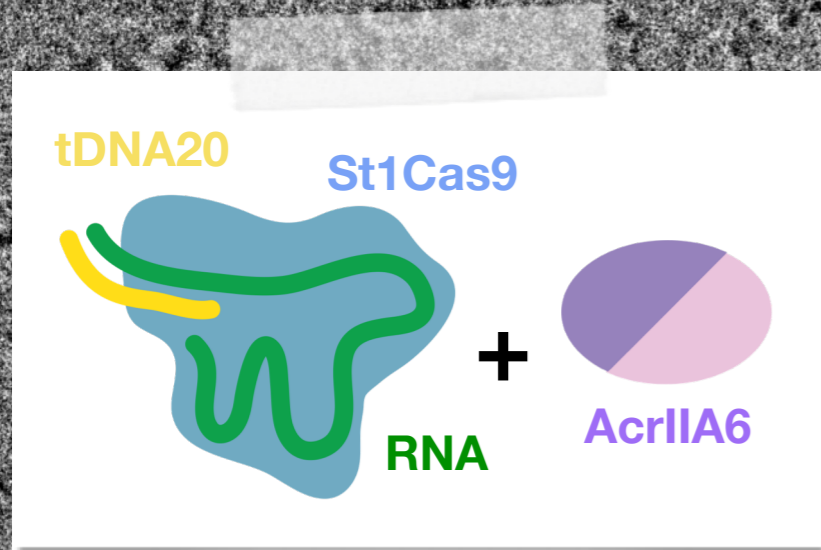


AcrIIA6 stably binds to St1Cas9 surveillance complex

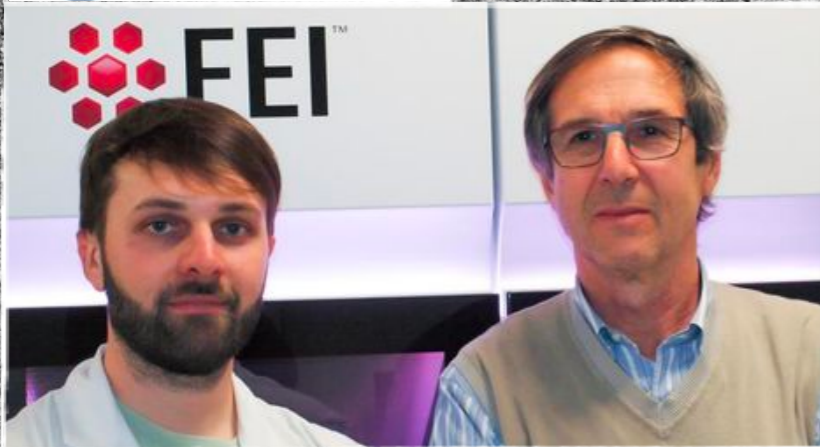
BioLayer Interferometry (BLI)



The AcrIIA6 binding mode revealed by cryoEM



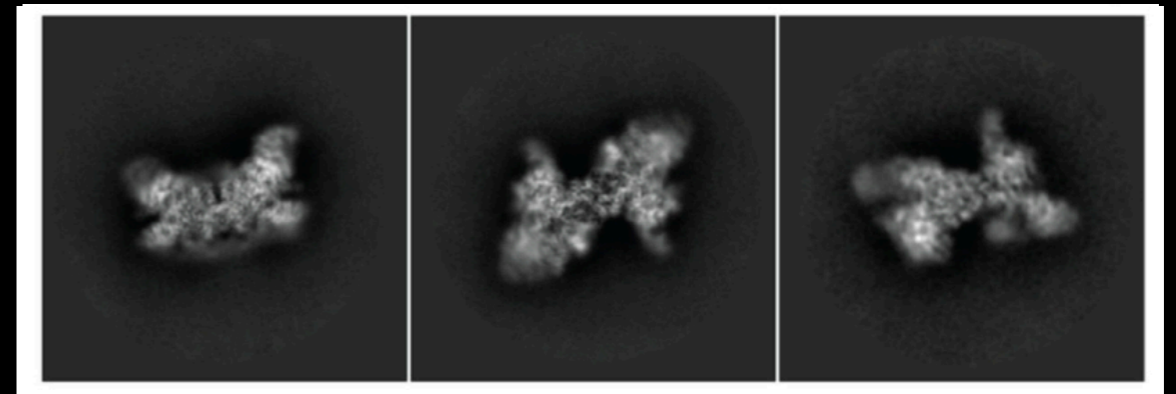
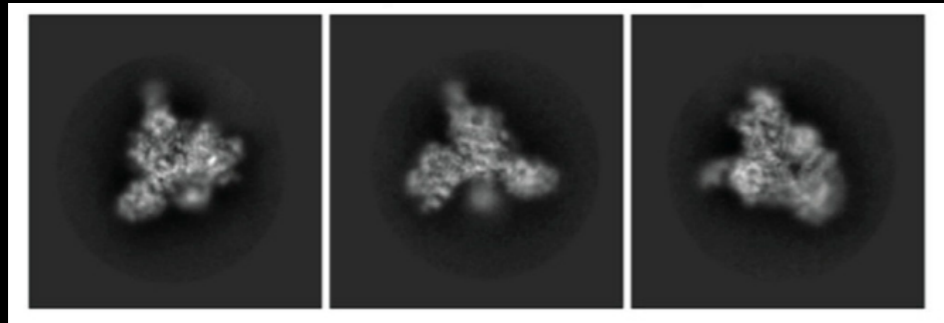
UNIVERSITÀ
DEGLI STUDI
DI MILANO



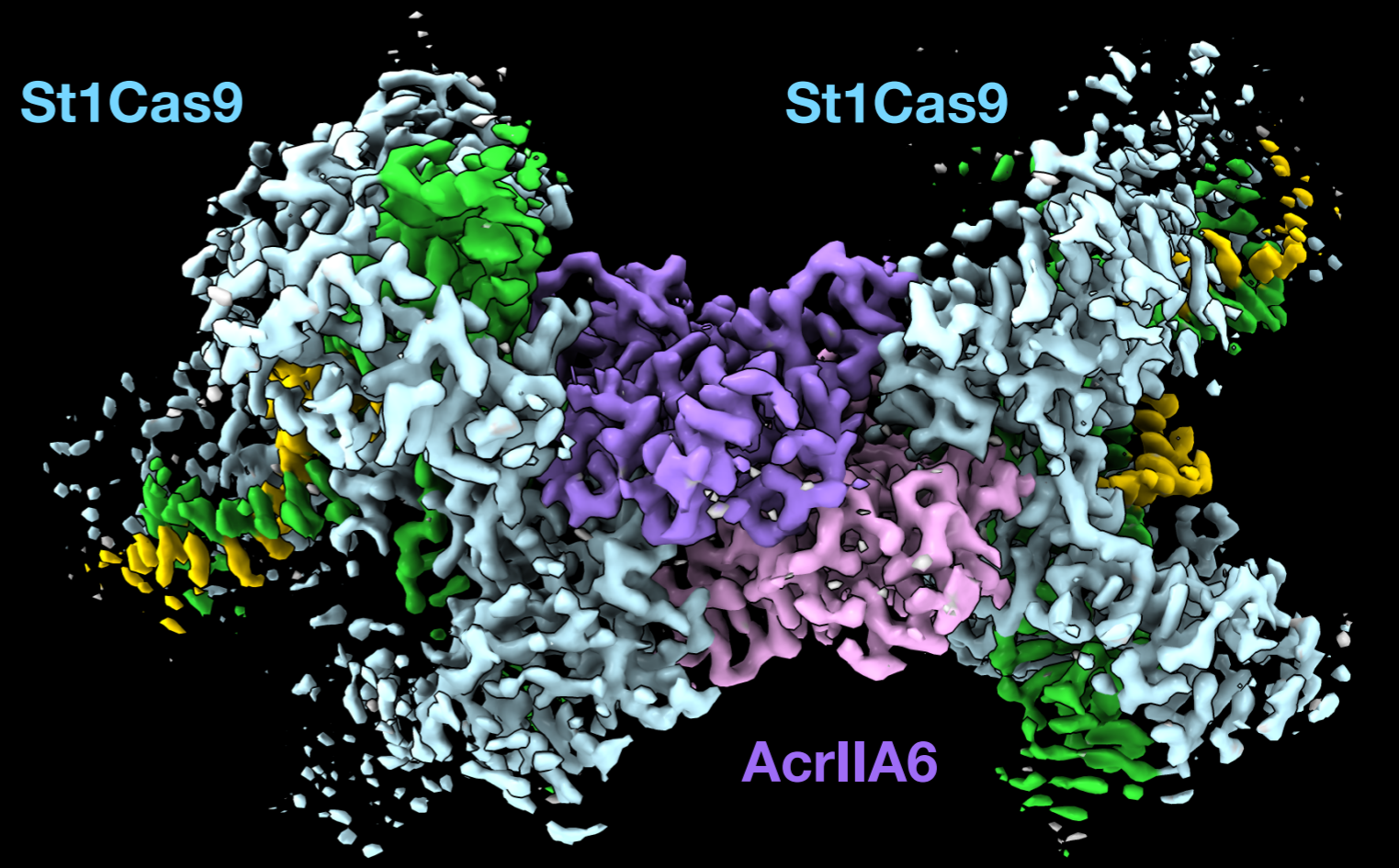
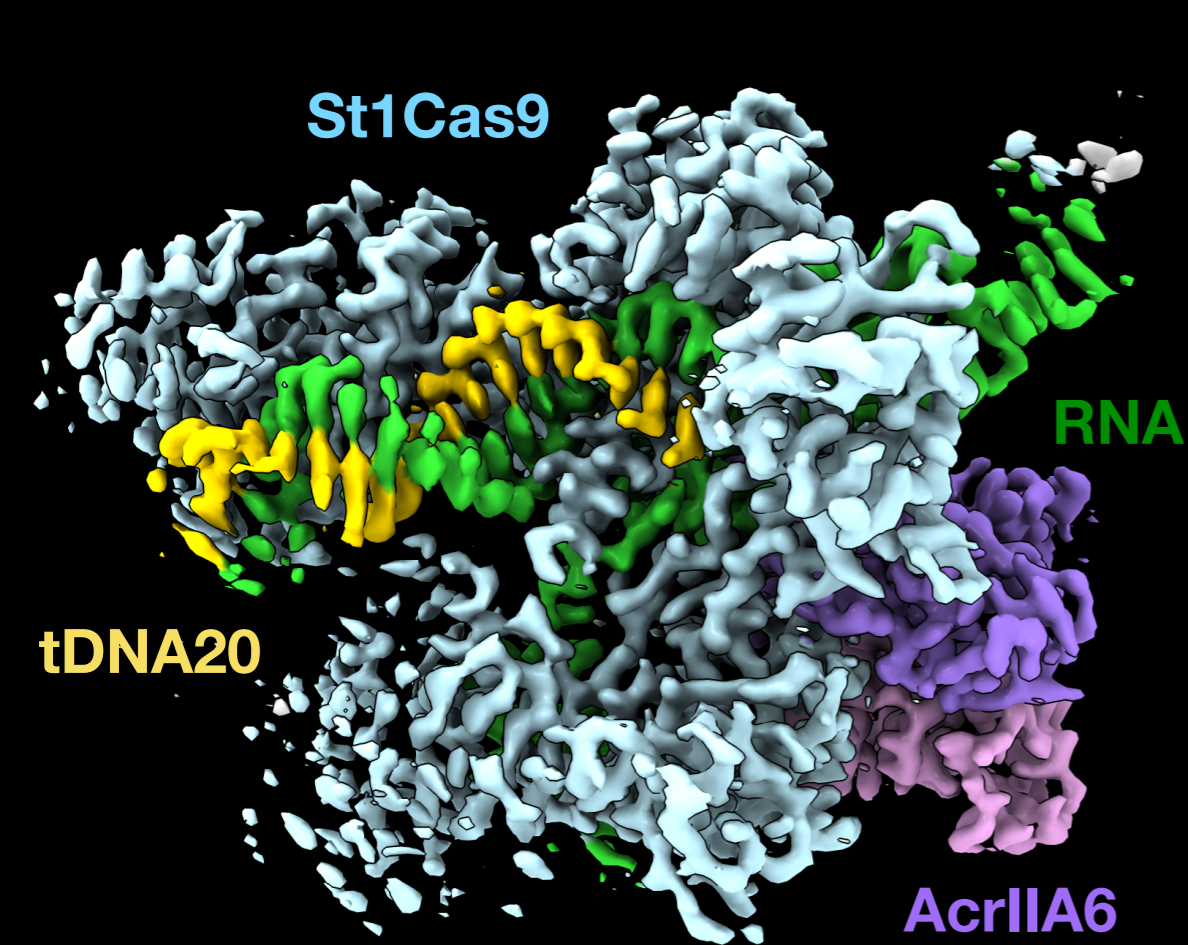
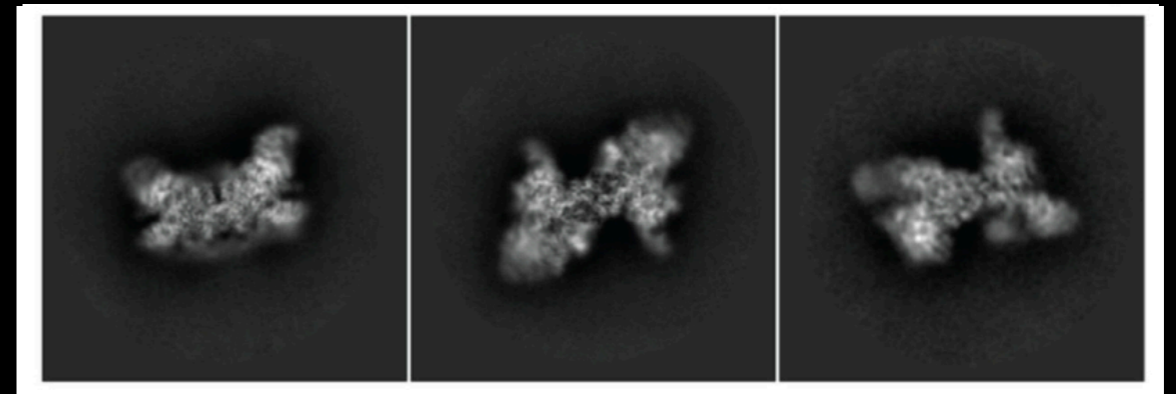
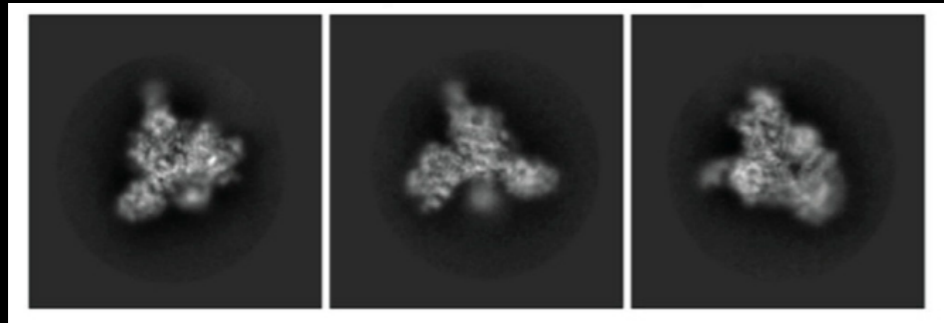
Talos Arctica

Martino Bolognesi
Paolo Swuec

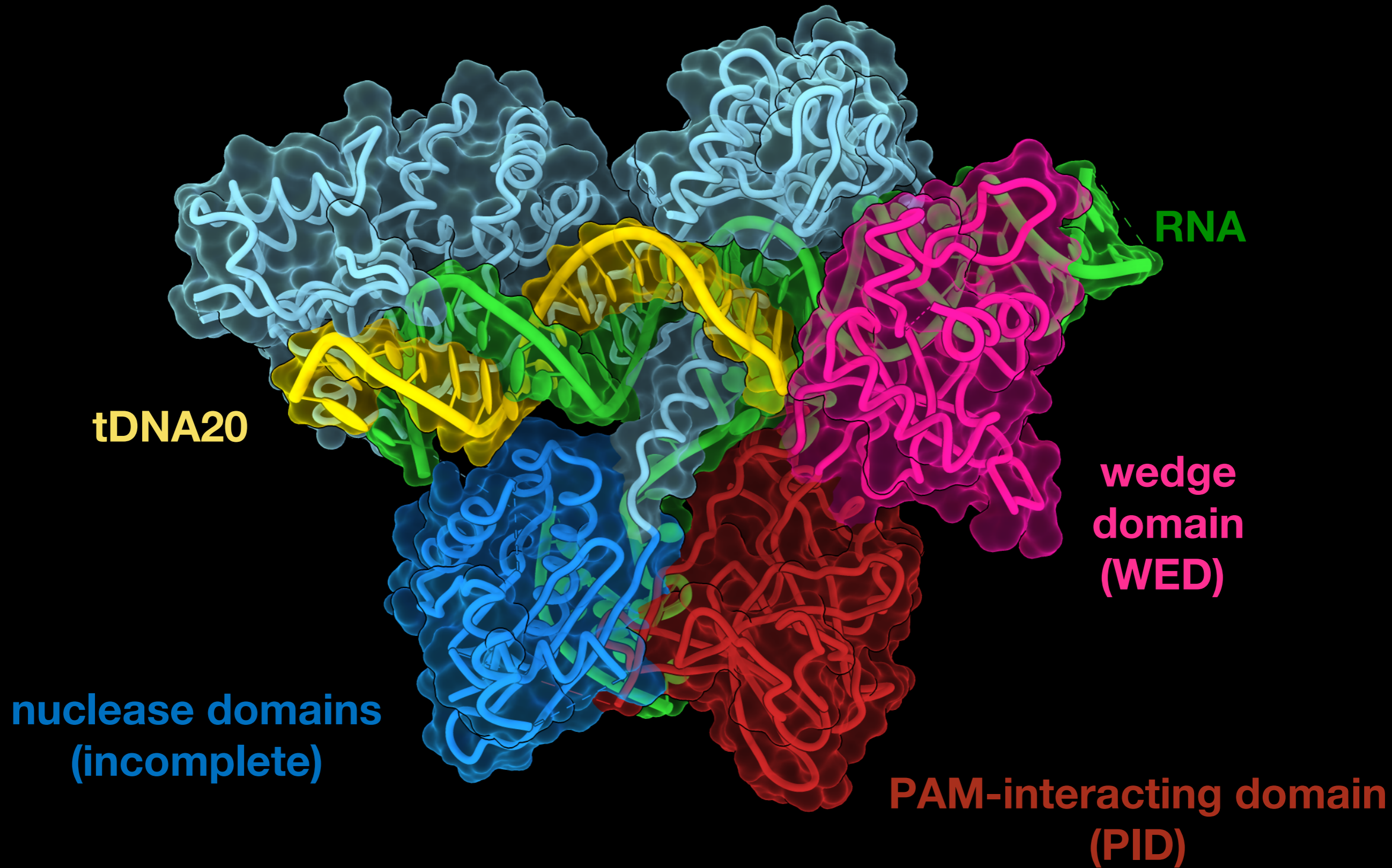
The AcrIIA6 binding mode revealed by cryoEM



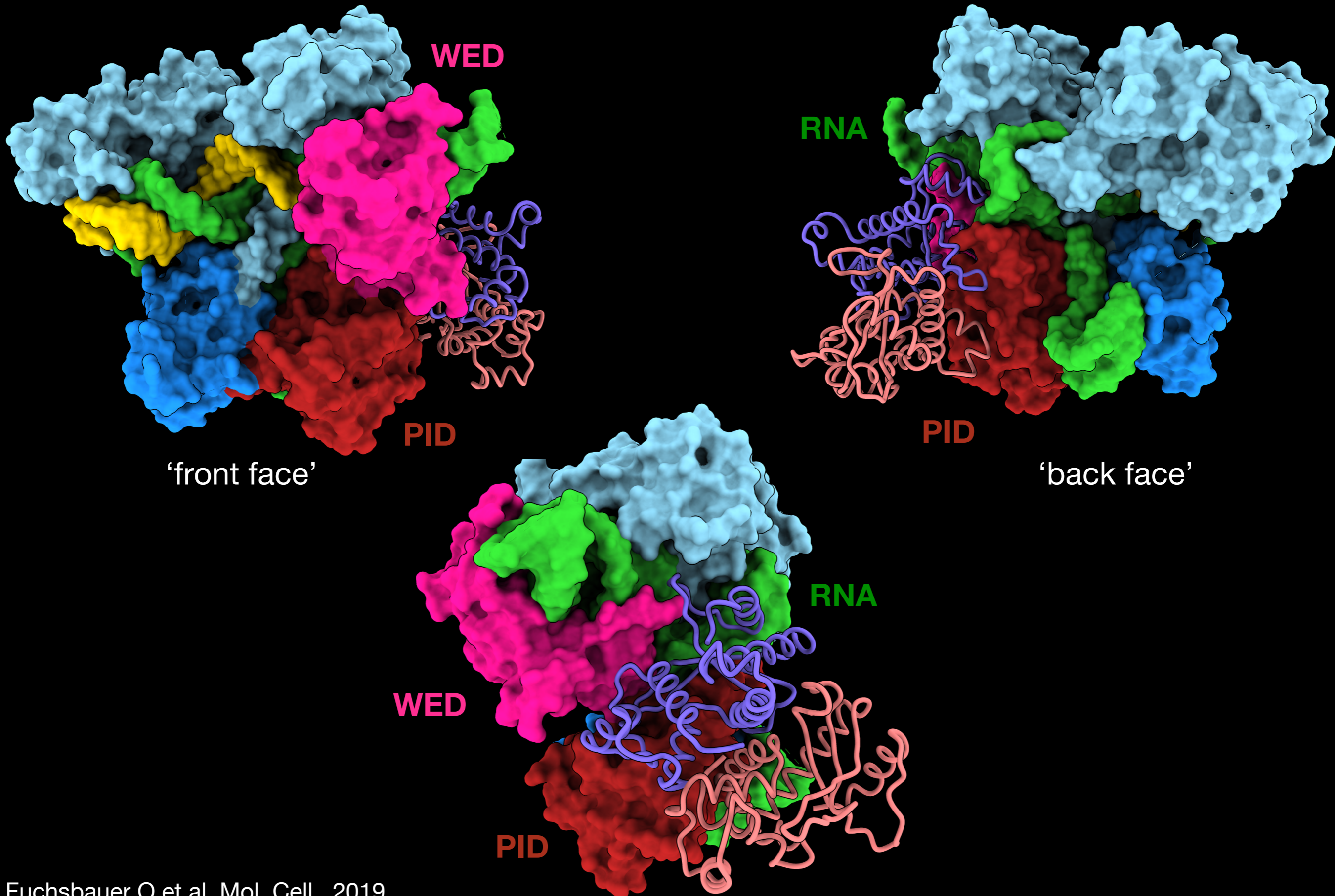
The AcrIIA6 binding mode revealed by cryoEM



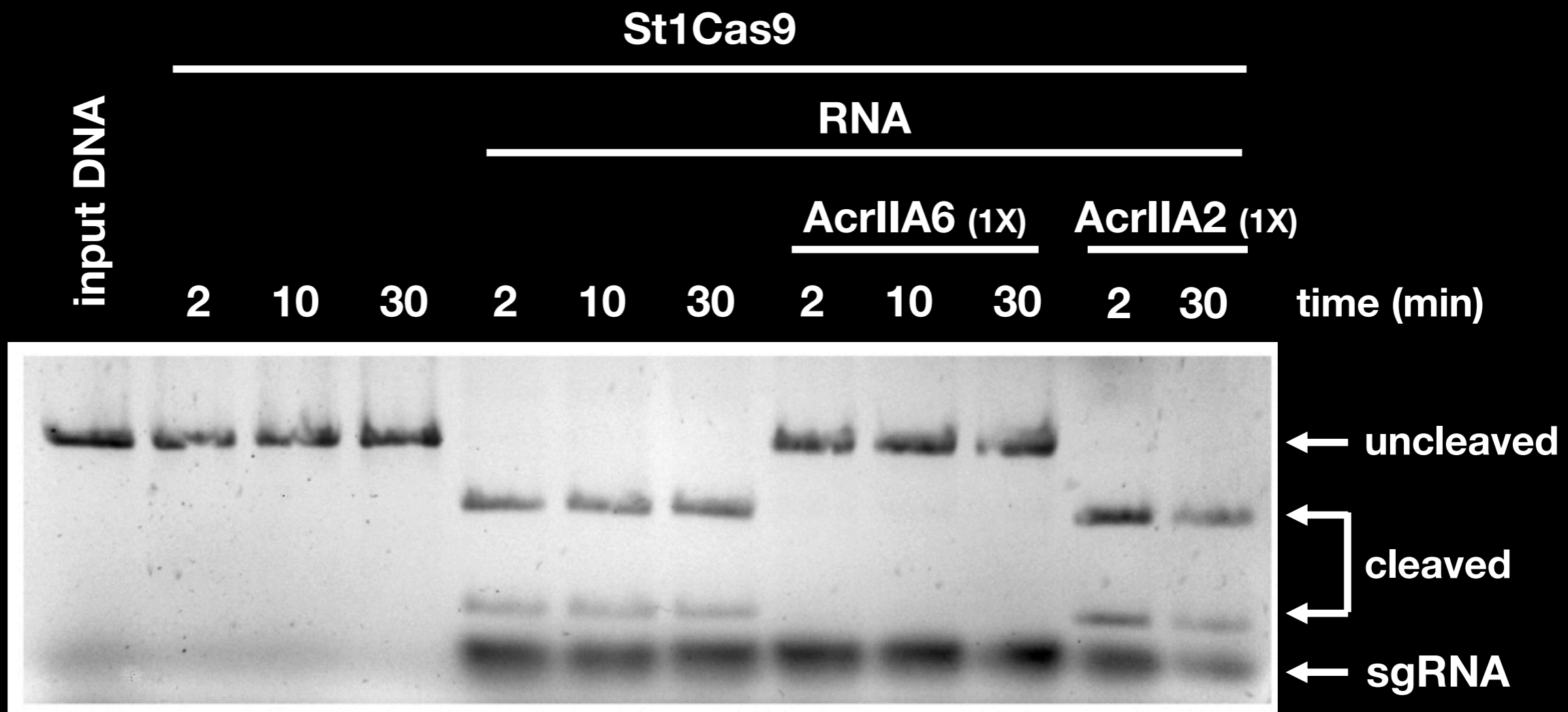
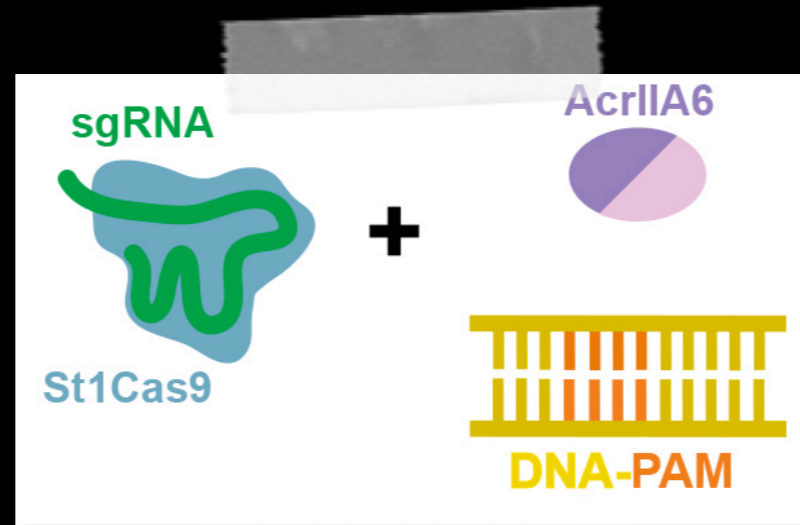
AcrIIA6 binds to an allosteric site on St1Cas9



AcrIIA6 binds to an allosteric site on St1Cas9

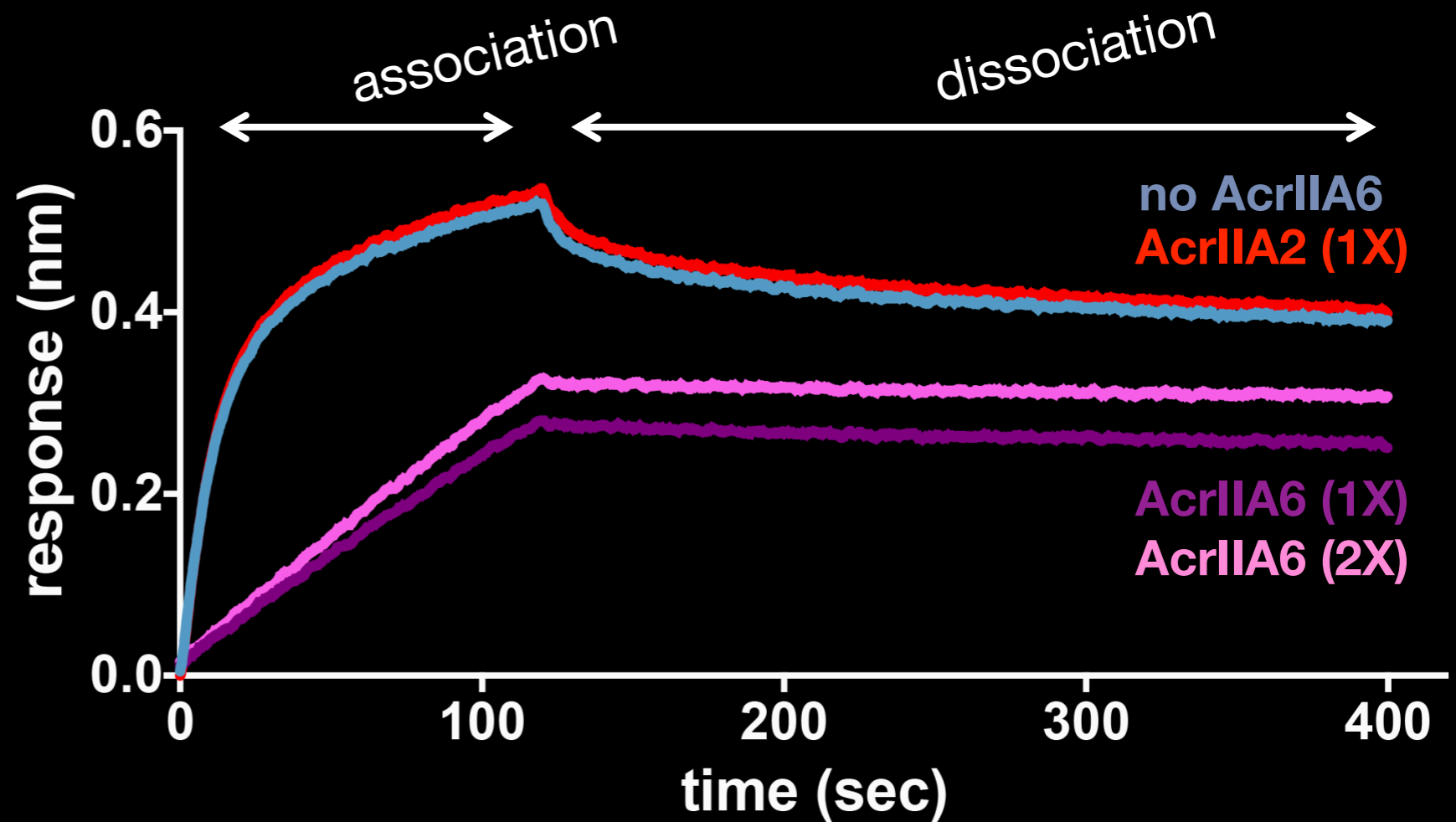
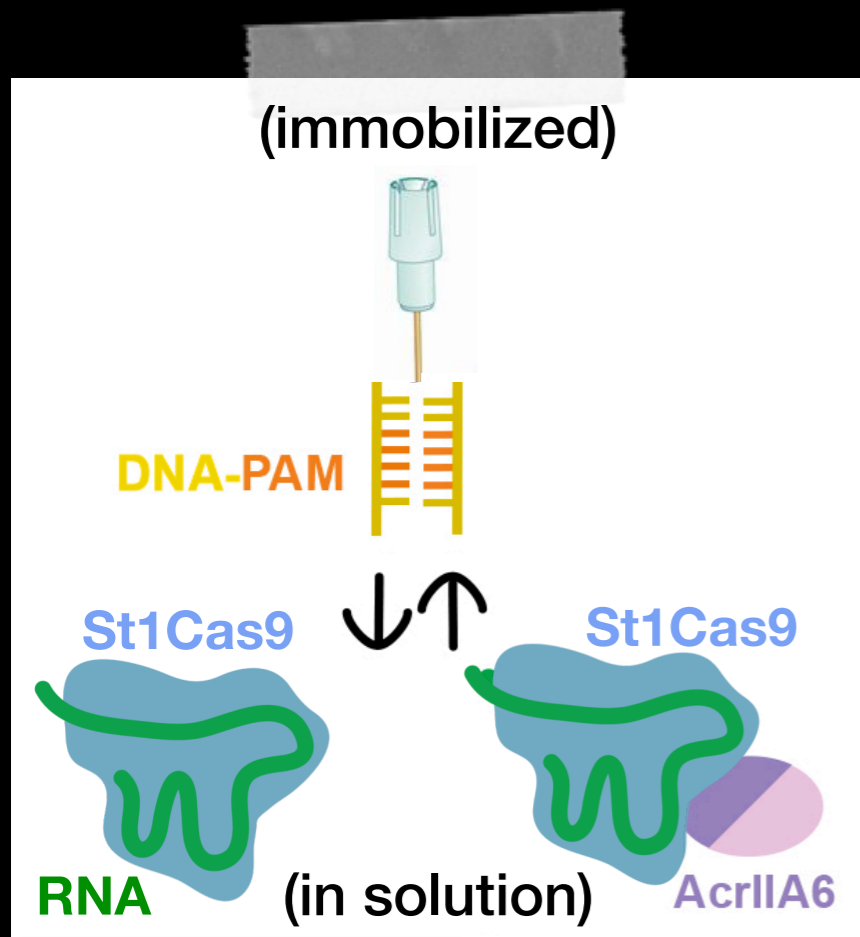


AcrIIA6 blocks St1Cas9 activity



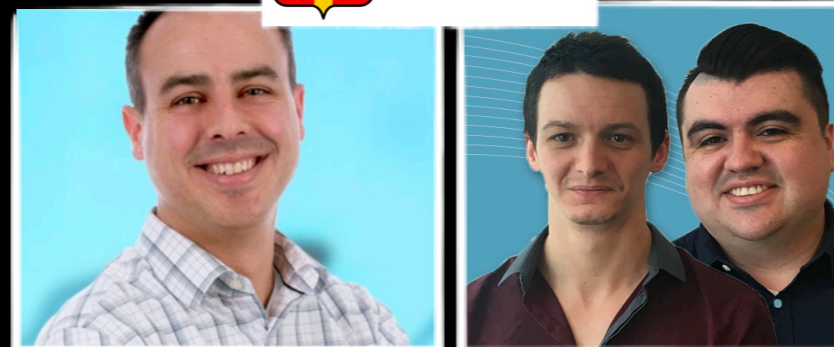
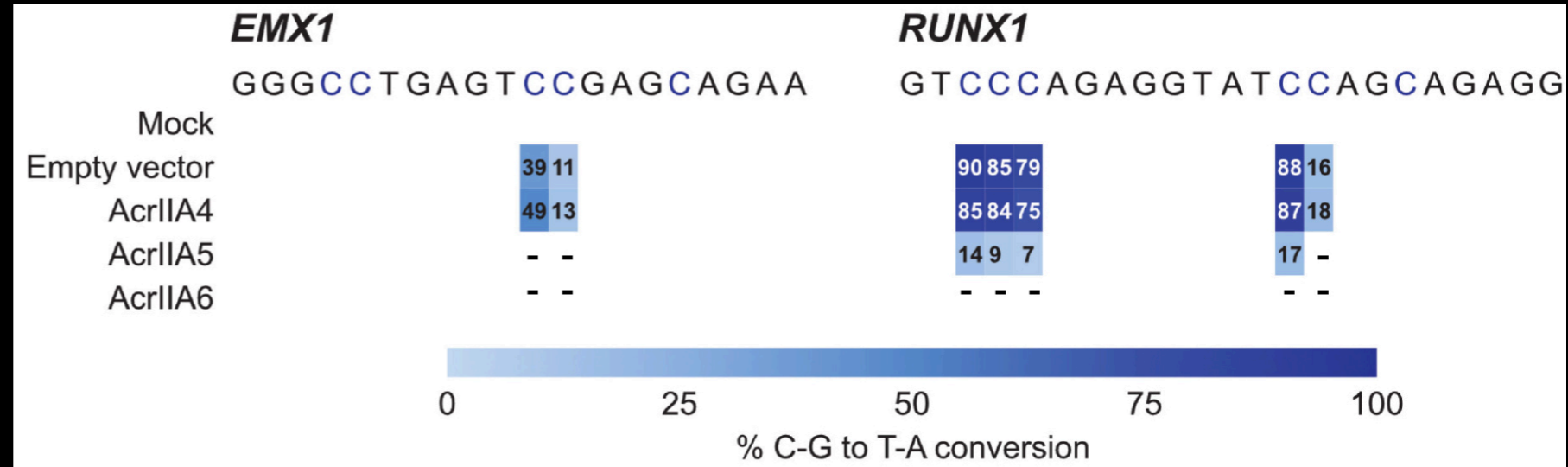
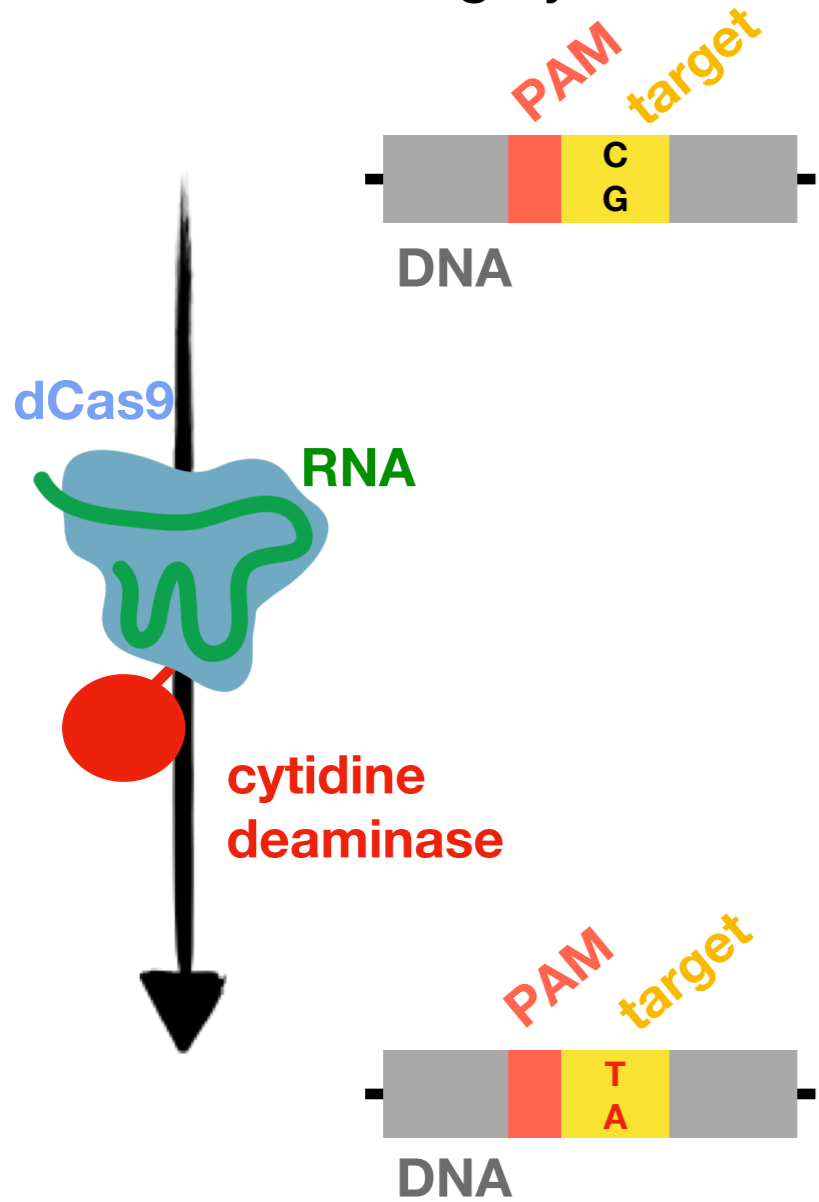
AcrIIA6 reduces target binding affinity

BioLayer Interferometry (BLI)



AcrIIA6 prevent target binding within cells

Base editing system

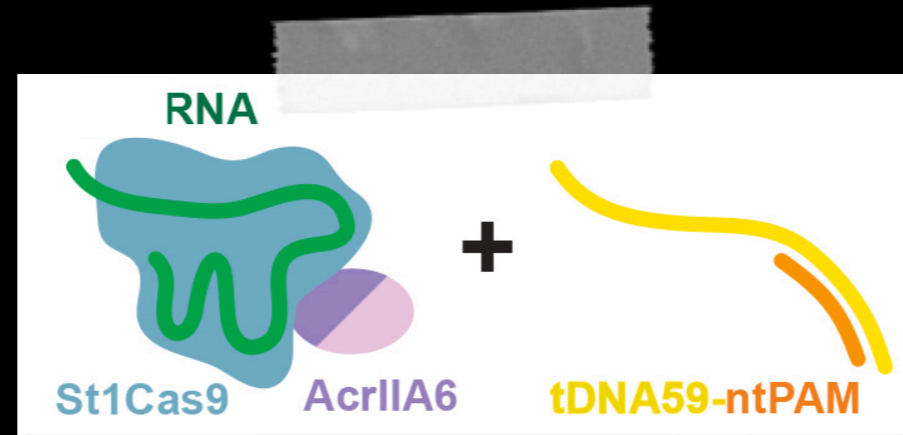


Alexis Duringer
Daniel Agudelo

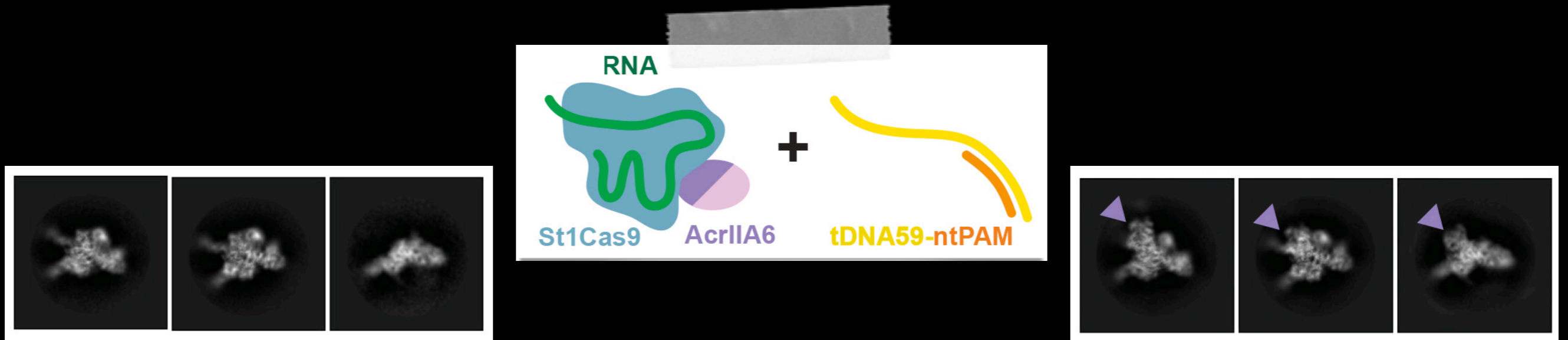
Sébastien Levesque
Minja Velimirovic

Yannick Doyon

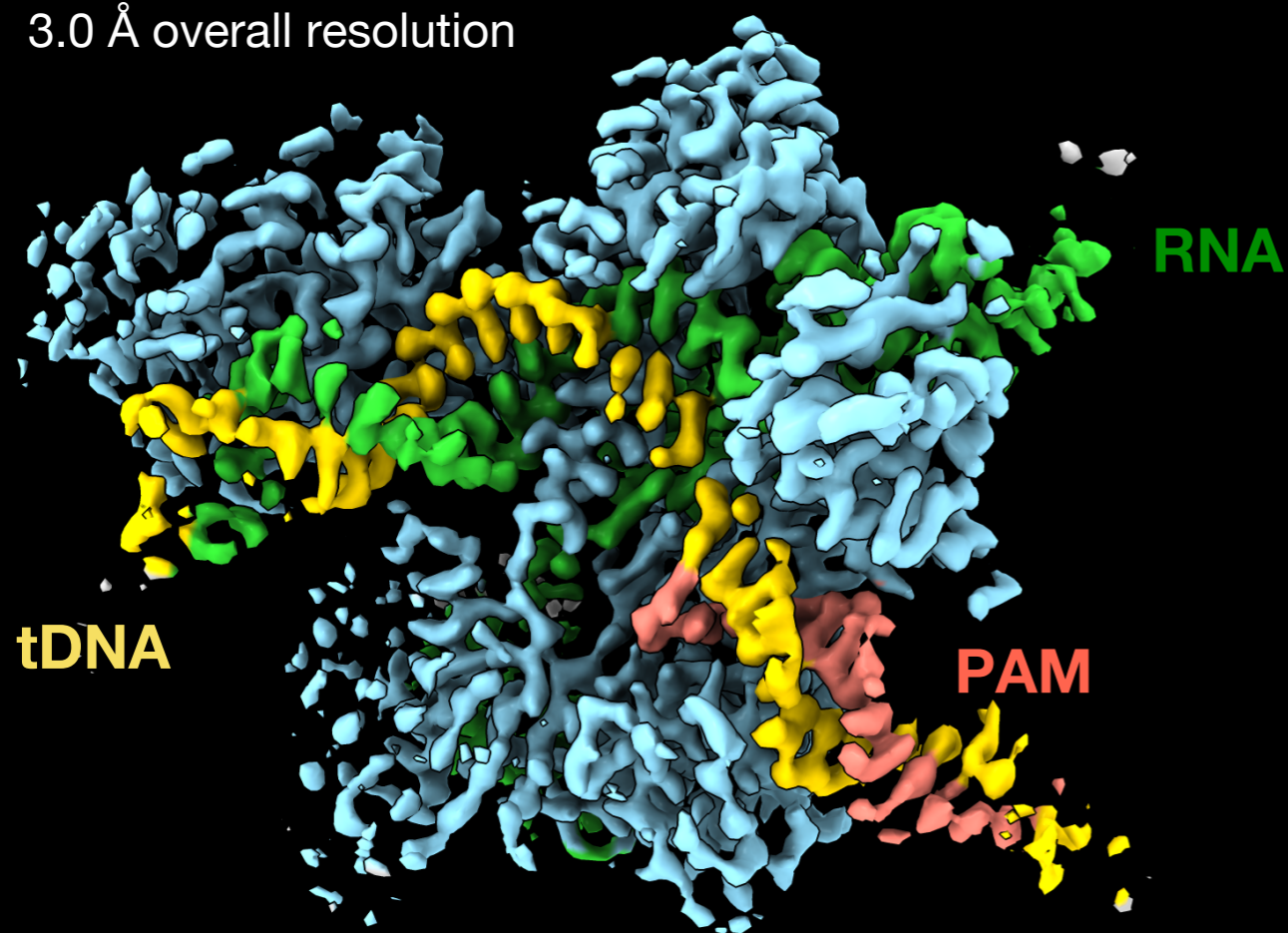
AcrIIA6 alters St1Cas9 conformational dynamics associated with PAM binding



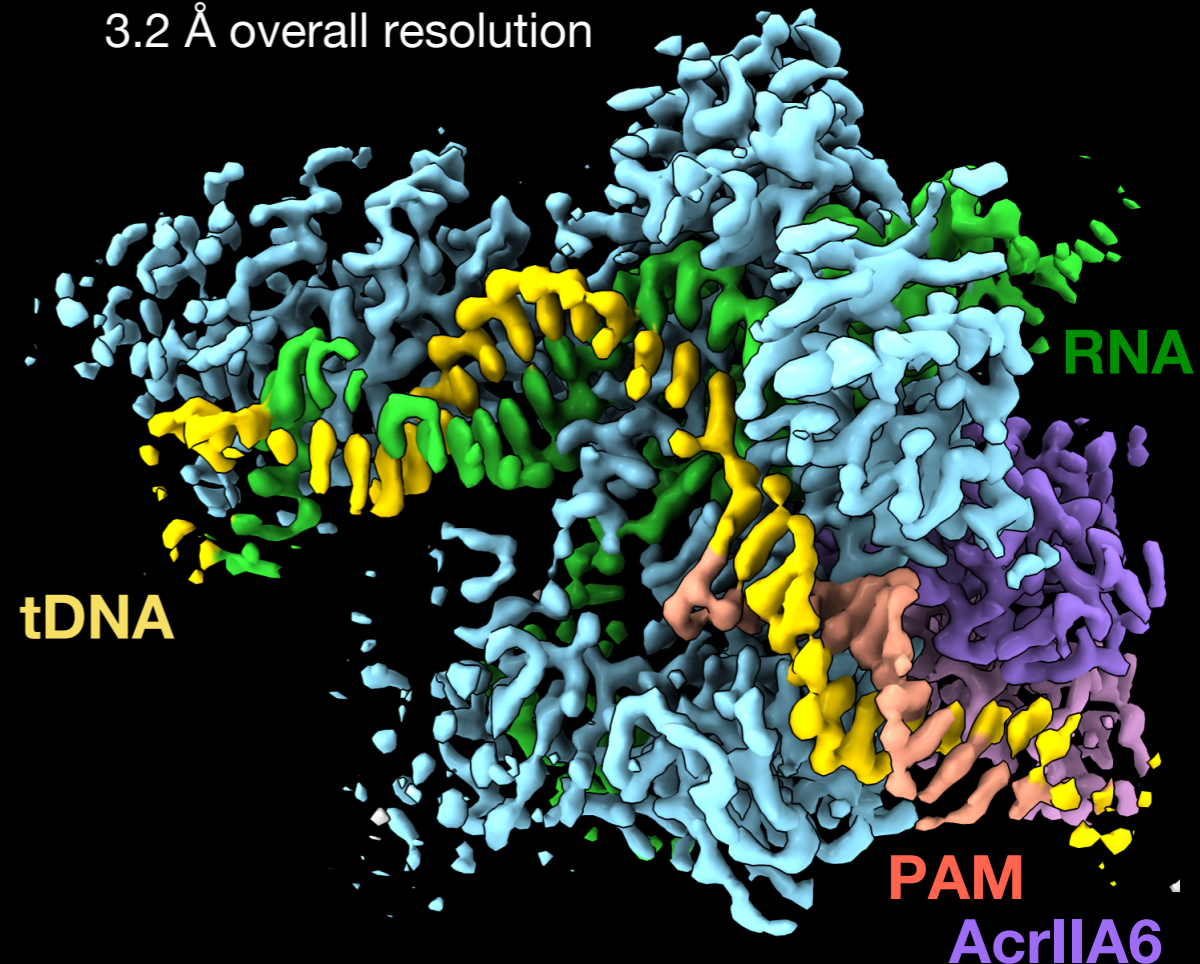
AcrIIA6 alters St1Cas9 conformational dynamics associated with PAM binding



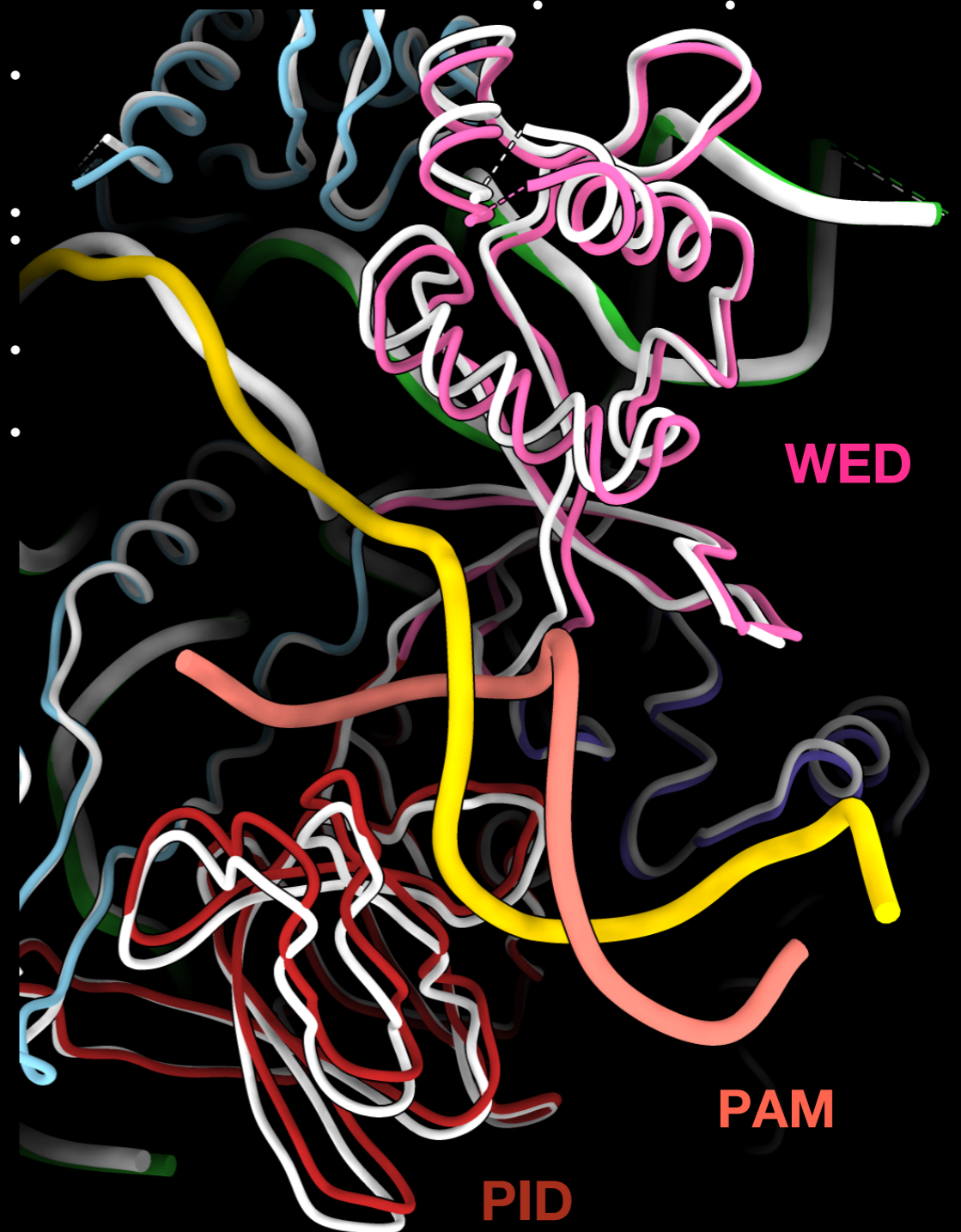
3.0 Å overall resolution



3.2 Å overall resolution



AcrIIA6 alters St1Cas9 conformational dynamics associated with PAM binding

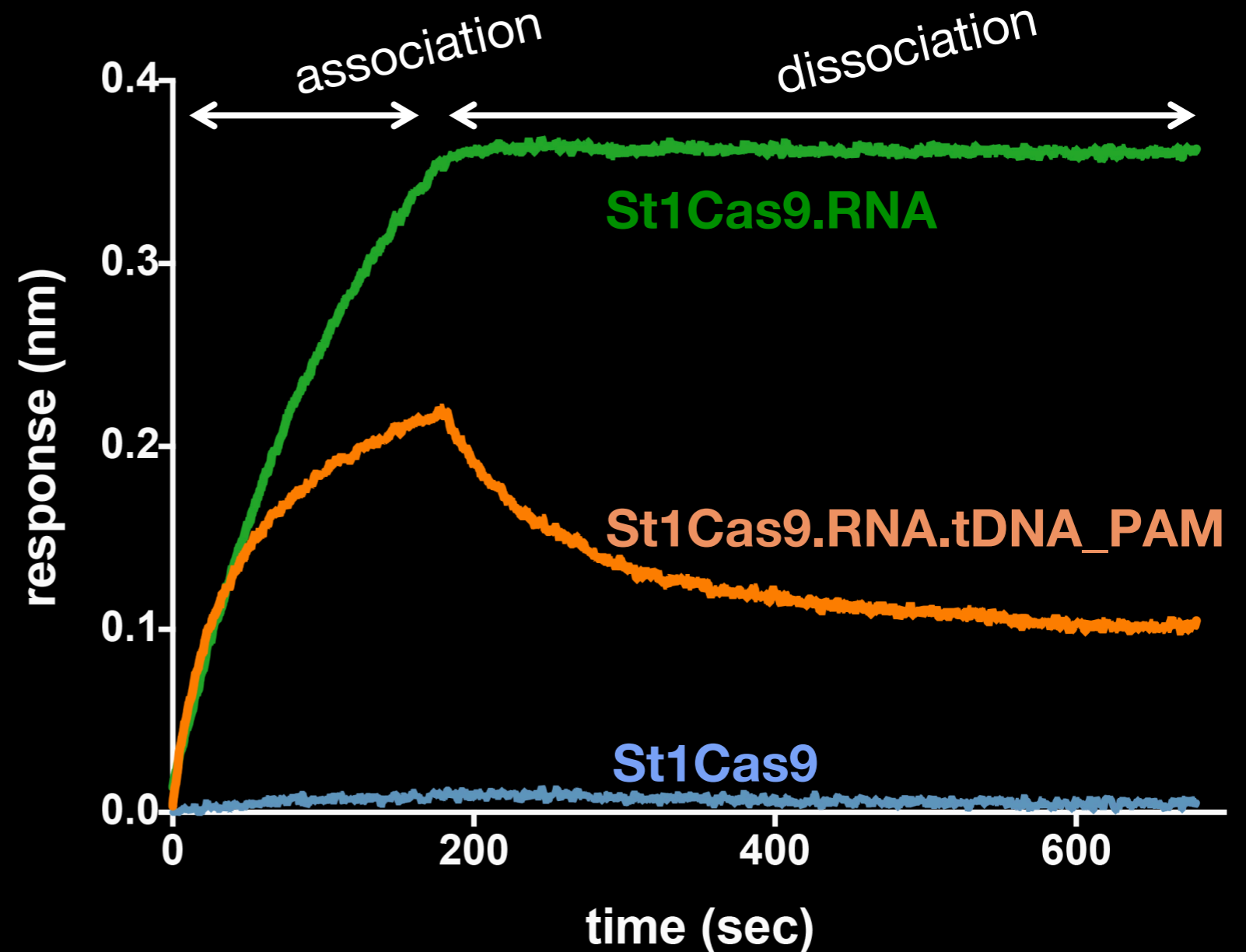
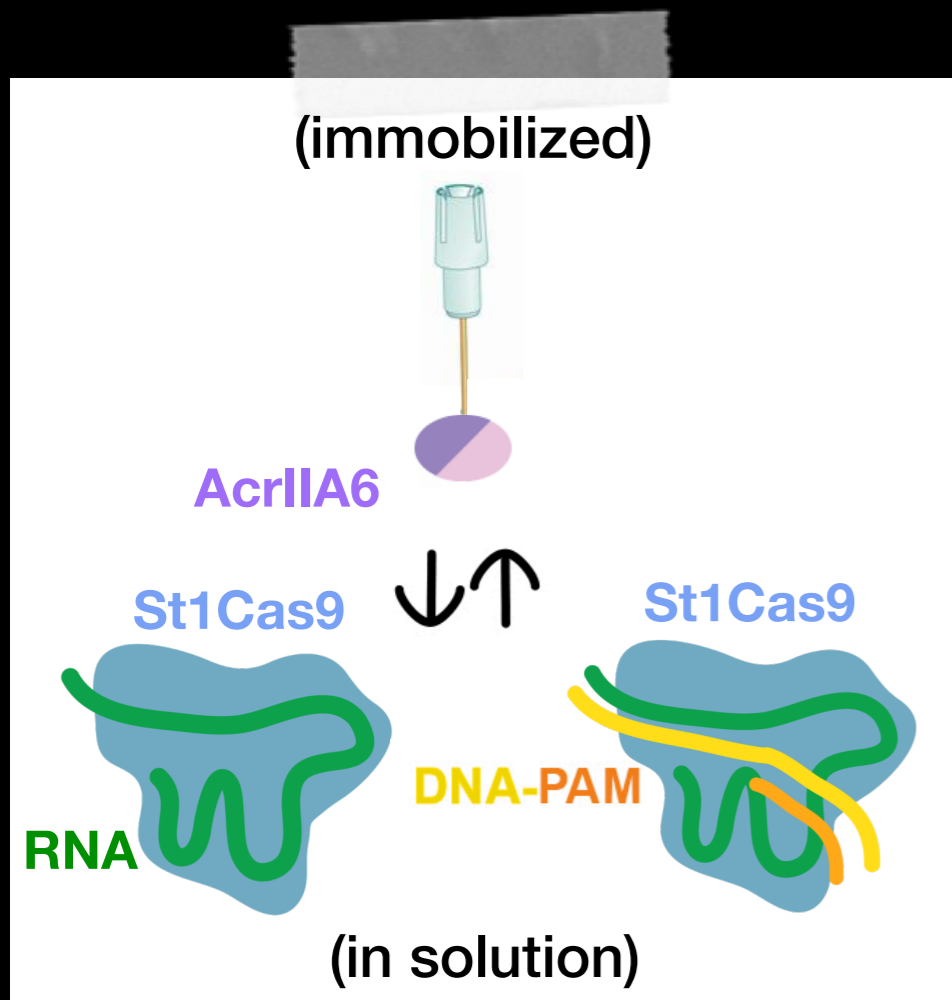


AcrIIA6 alters St1Cas9 conformational dynamics associated with PAM binding



AcrIIA6 alters St1Cas9 conformational dynamics associated with PAM binding

BioLayer Interferometry (BLI)



AcrIIA6 alters St1Cas9 conformational dynamics associated with PAM binding



Take home messages

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- AcrIIA6 uses a unique allosteric inhibition mode to prevent St1Cas9 target DNA binding

Take home messages

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- AcrIIA6 inactivates two St1Cas9 molecules at a time, which makes it a potent inhibitor

Take home messages

- AcrIIA6 uses a unique allosteric inhibition mode to prevent St1Cas9 target DNA binding
- AcrIIA6 inactivates two St1Cas9 molecules at a time, which makes it a potent inhibitor
- The anti-CRISPR-Cas9 modes of action are highly diverse

Acknowledgments



ANR 'PHARE'
JCJC 2019-2021

Alain Roussel
Christian Cambillau

Olivier Fuchsbauer
Claire Zimberger
Pierre Hardouin
Béatrice Amigues
Silvia Spinelli



Sylvain Moineau



Yannick Doyon