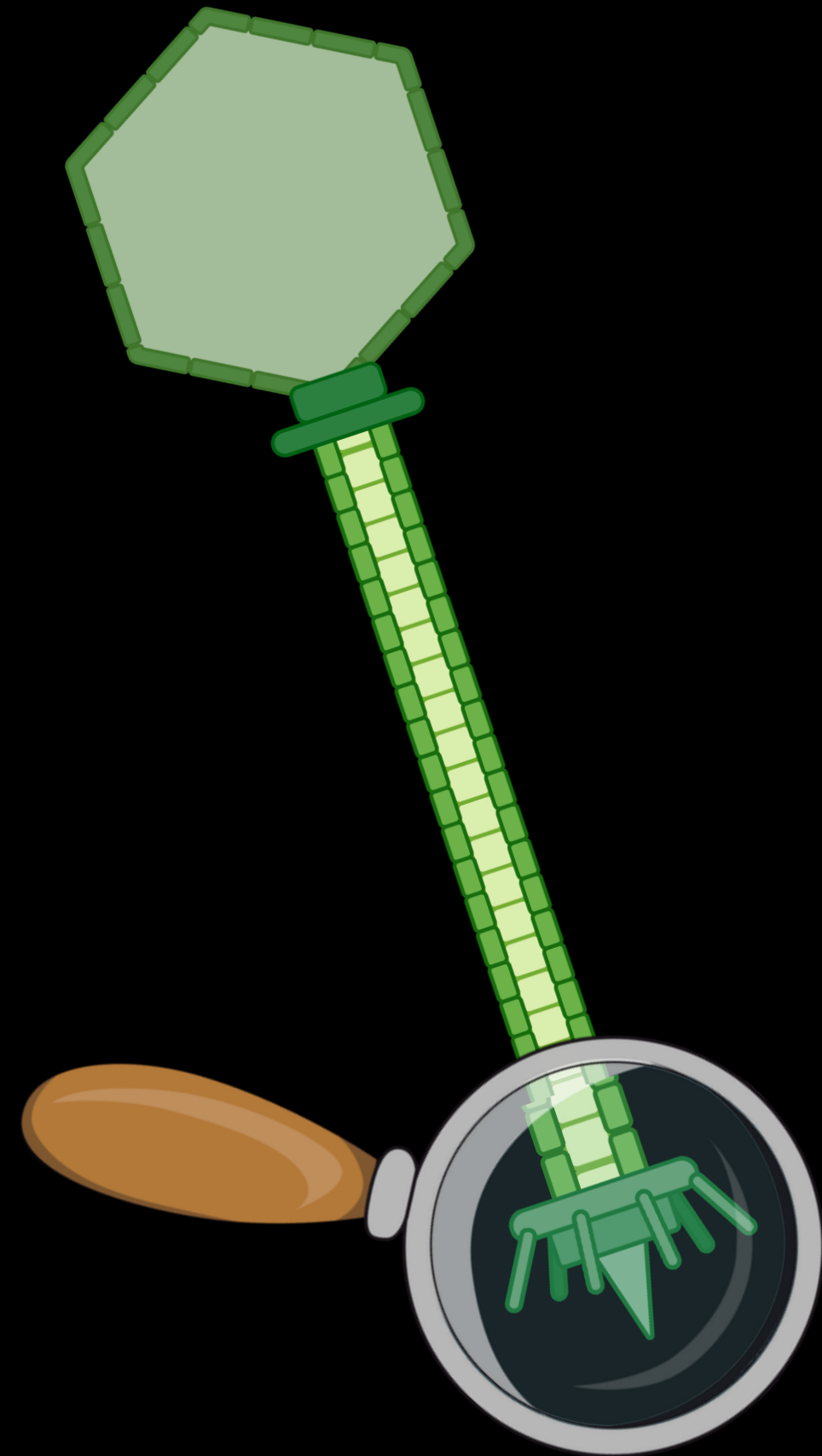


Dissecting siphophage *modus operandi* : focus on host binding



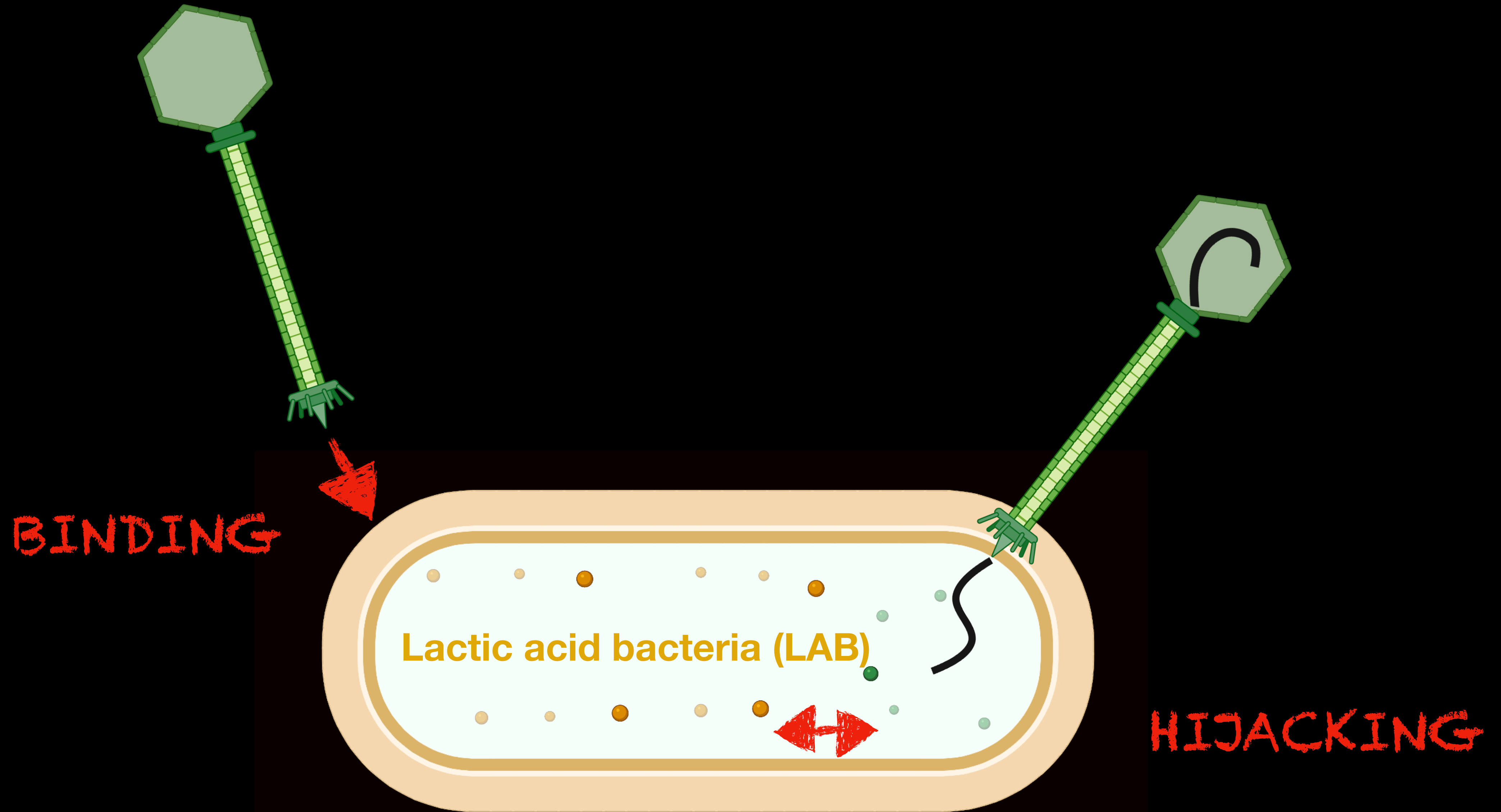
PHAGES in Roscoff, 11-12 October 2021

Adeline Goulet



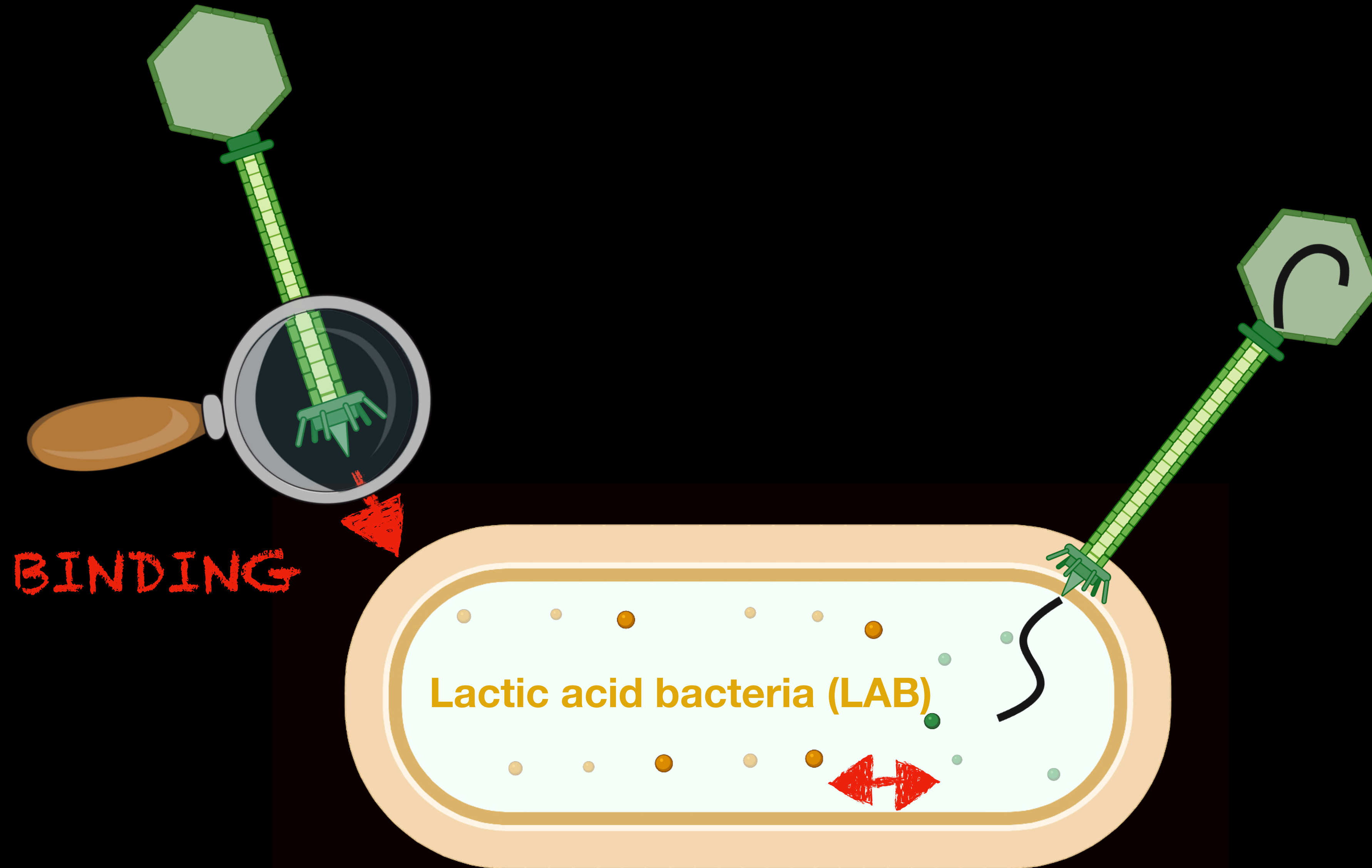


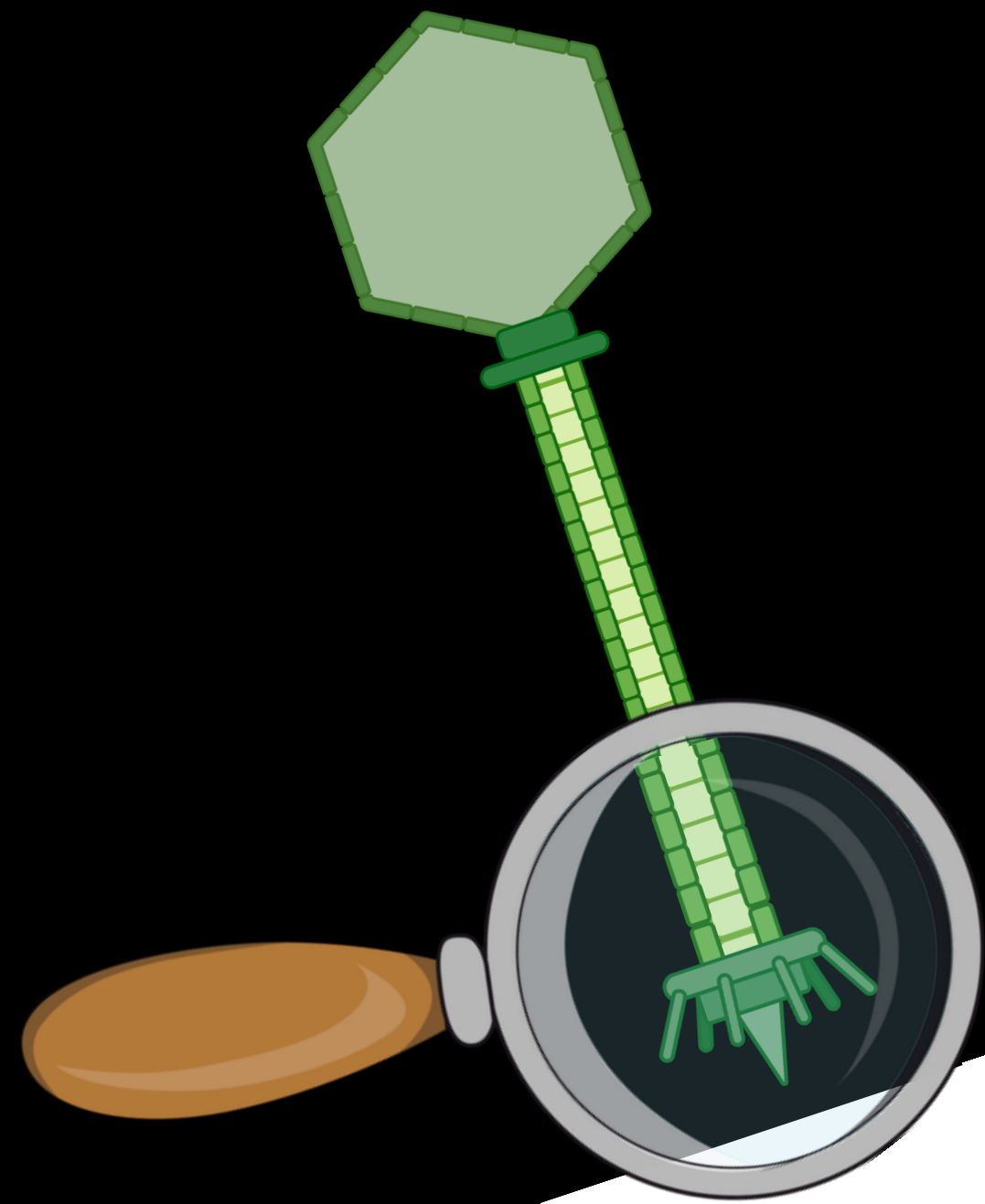
Unraveling molecular mechanisms of LAB siphophage infection





Unraveling molecular mechanisms of LAB siphophage infection





under review



microorganisms



Article

Structure and topology prediction of phage adhesion devices using AlphaFold2: the case of two *Oenococcus oeni* phages

Adeline Goulet^{1,2*} and Christian Cambillau^{1,2}



Host-binding machineries are structurally and functionally diverse

Lactococcus lactis
phages

Streptococcus thermophilus
phage

Lactobacillus casei
phage

Capsid

p2

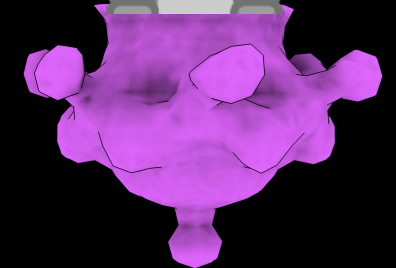
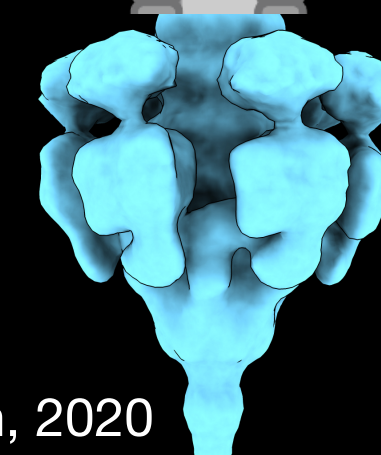
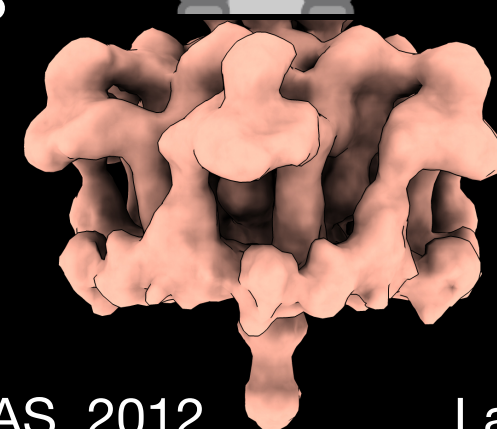
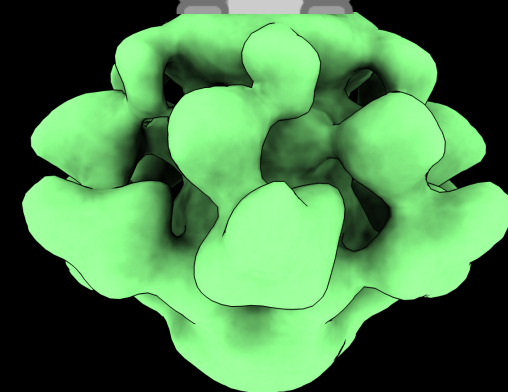
TP901-1

STP1

J-1

Tail

Host-binding
machinery



Sciara et al. PNAS, 2010

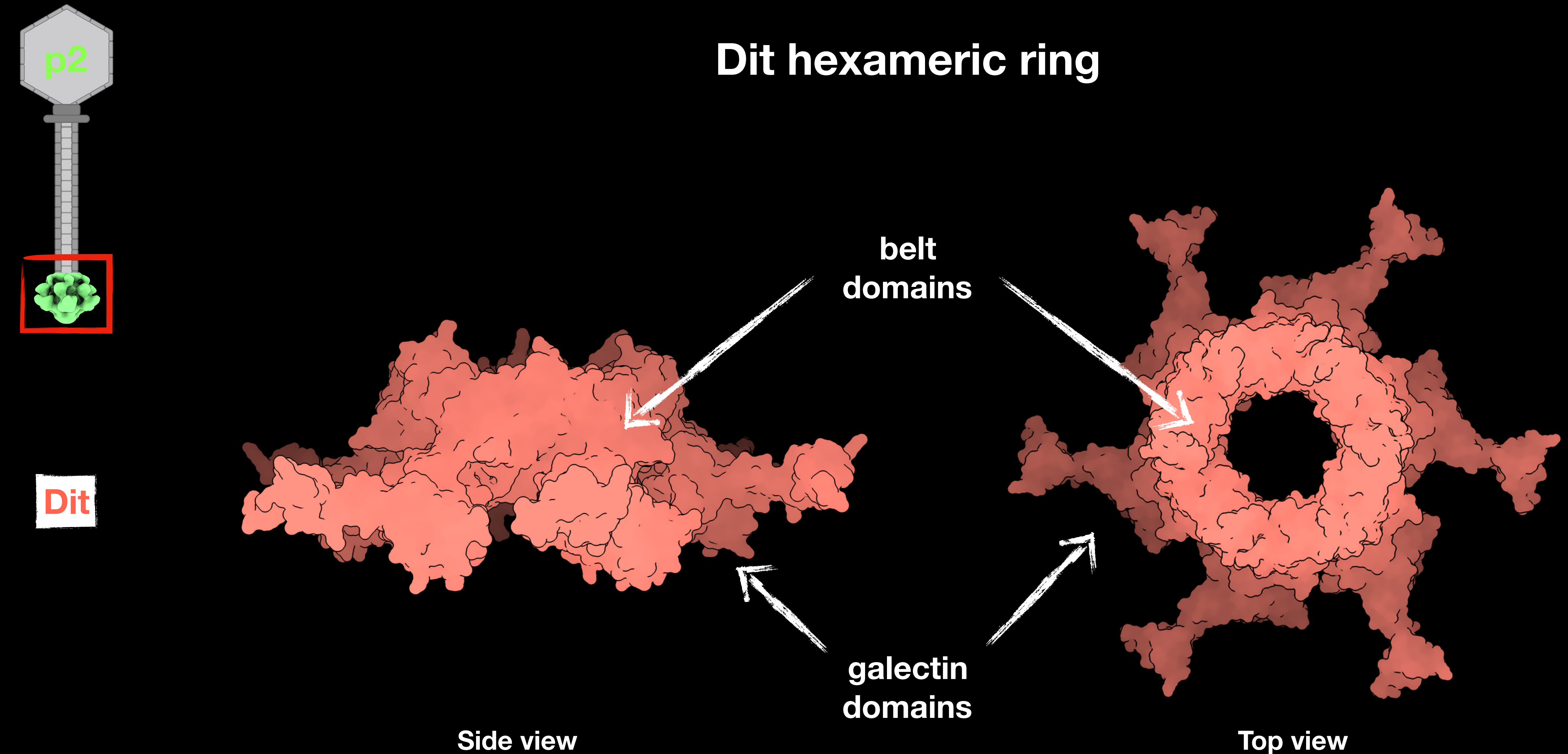
Veesler et al. PNAS, 2012

Lavelle et al. Microbiol Biotech, 2020

Dieterle, Mol. Microbiol. 2017



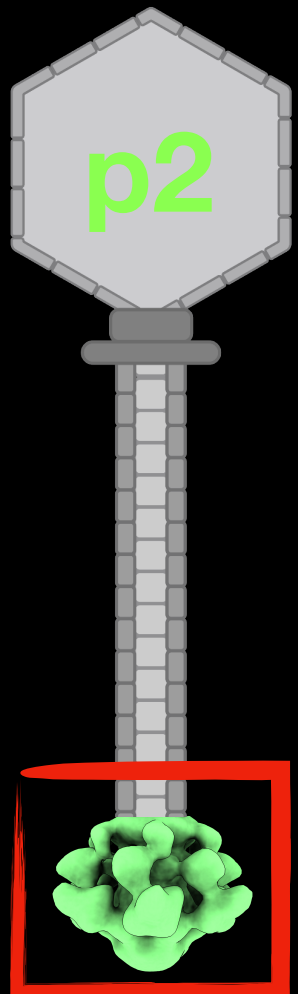
Host-binding machineries are structurally and functionally diverse



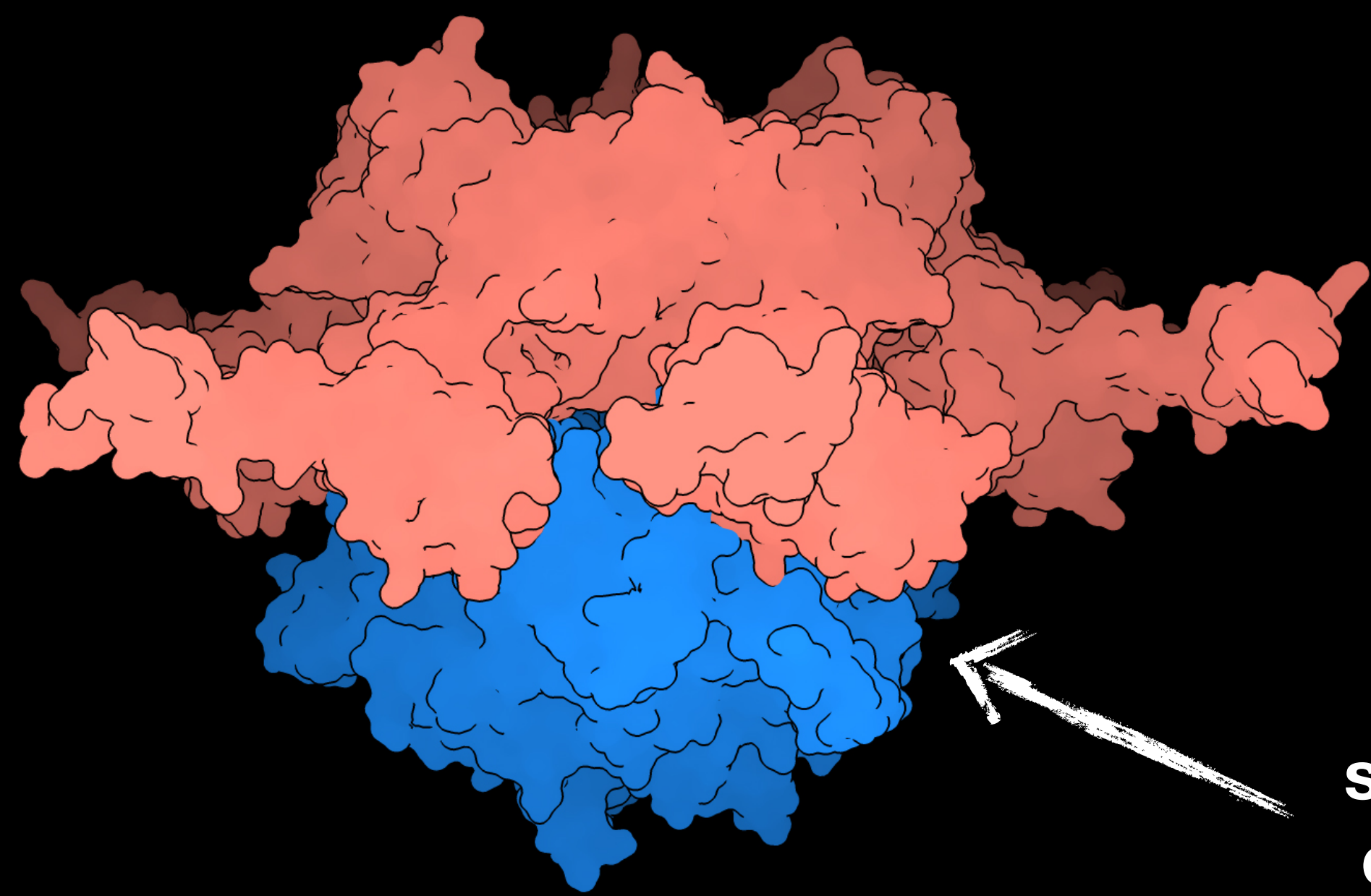
Dit: Distal tail protein



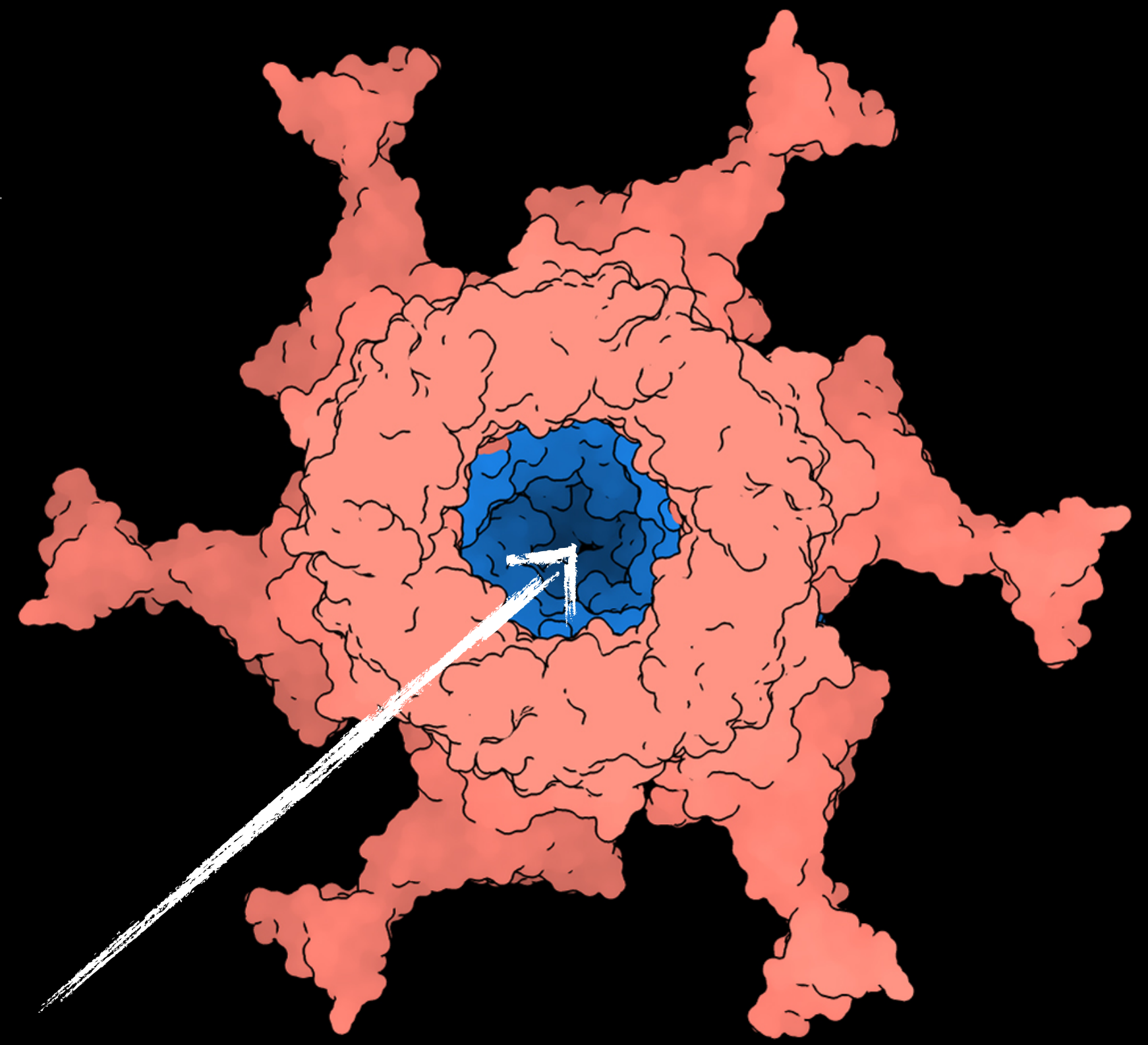
Host-binding machineries are structurally and functionally diverse



Tal trimeric plug



Side view



Top view

Dit

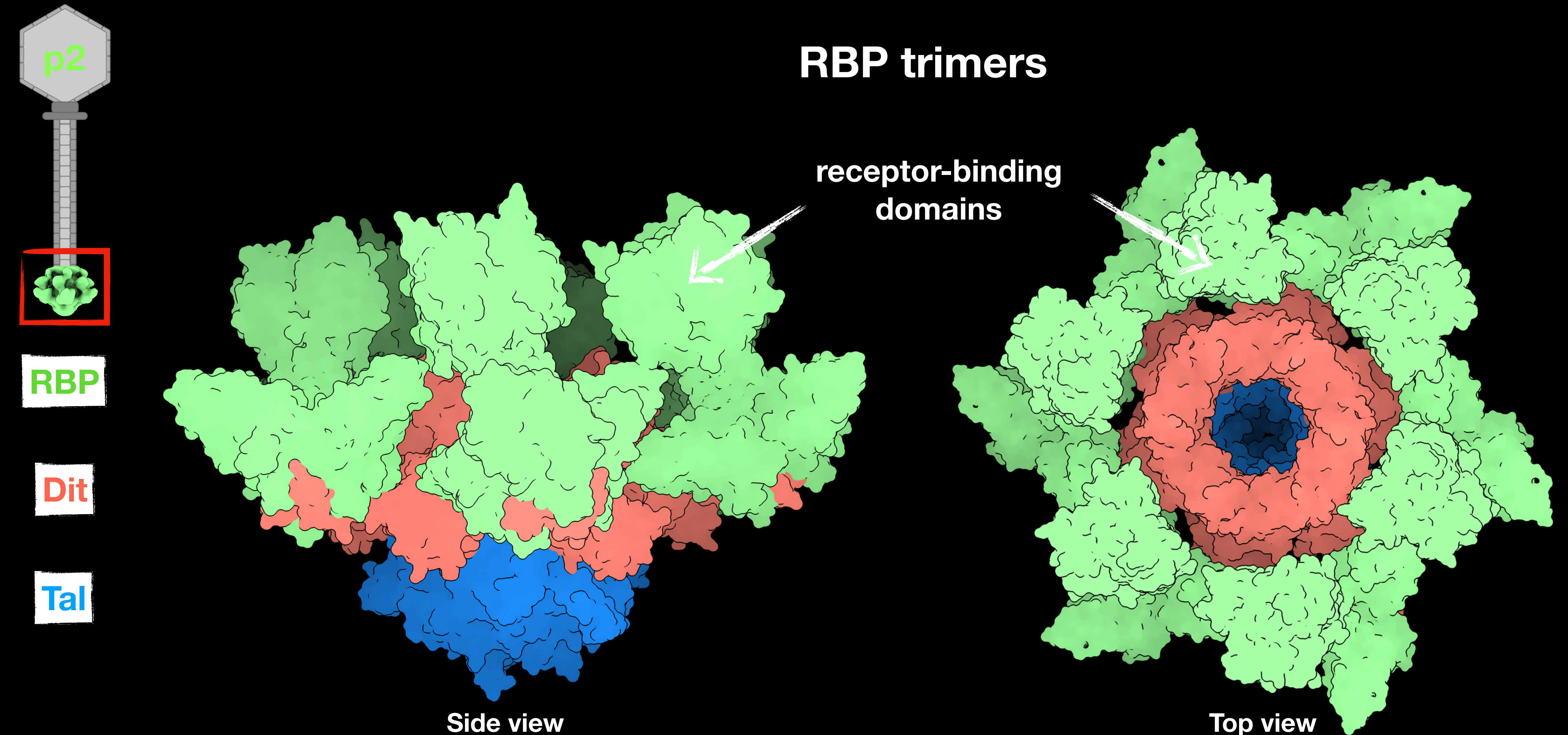
Tal

structural domains

Tal: Tail-associated lysin



Host-binding machineries are structurally and functionally diverse

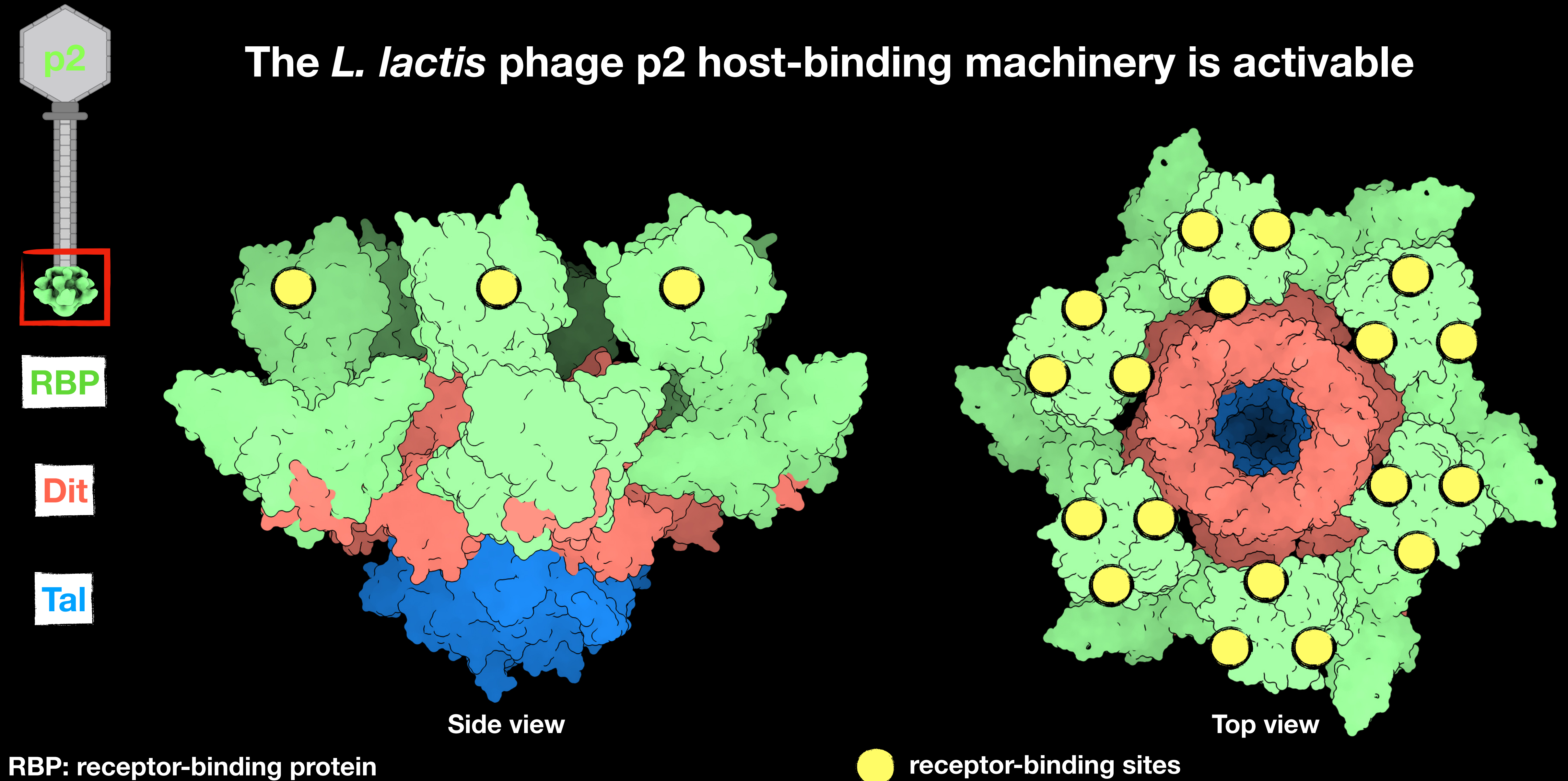


RBP: receptor-binding protein



Host-binding machineries are structurally and functionally diverse

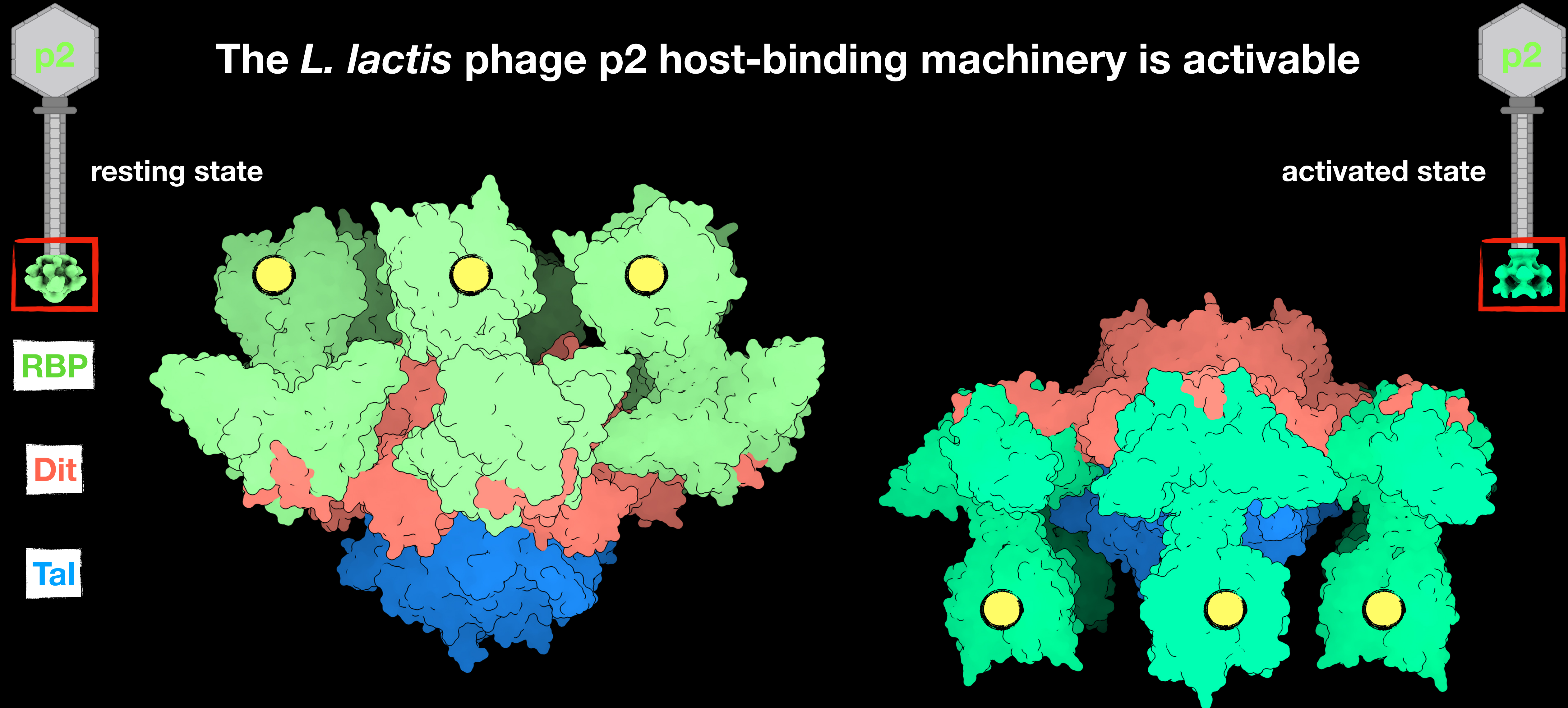
The *L. lactis* phage p2 host-binding machinery is activable





Host-binding machineries are structurally and functionally diverse

The *L. lactis* phage p2 host-binding machinery is activable



RBP

Dit

Tal

RBP: receptor-binding protein

Sciara et al. PNAS, 2010

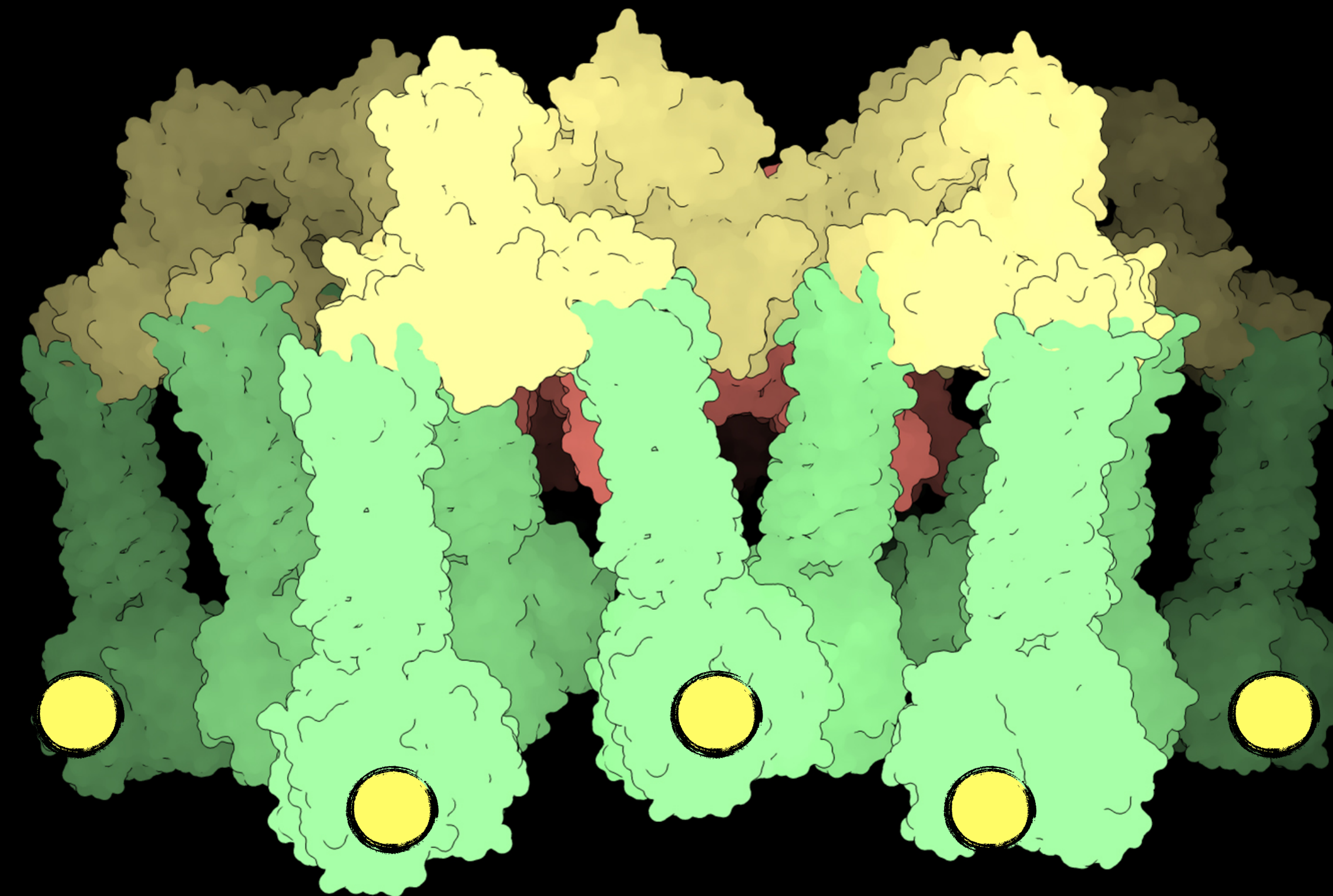
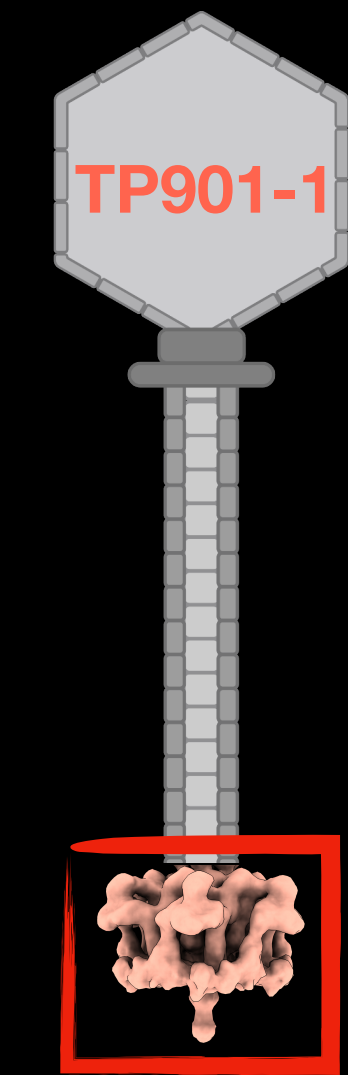
● receptor-binding sites



Host-binding machineries are structurally and functionally diverse

The *L. lactis* phage TP901-1

host-binding machinery is constitutively active



BppU

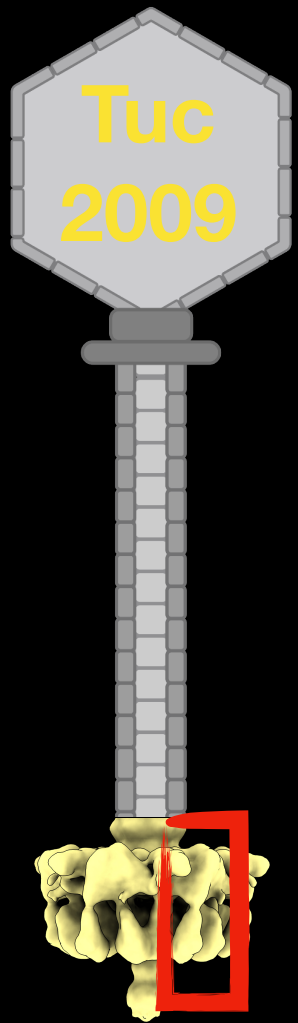
Dit

RBP

 receptor-binding sites

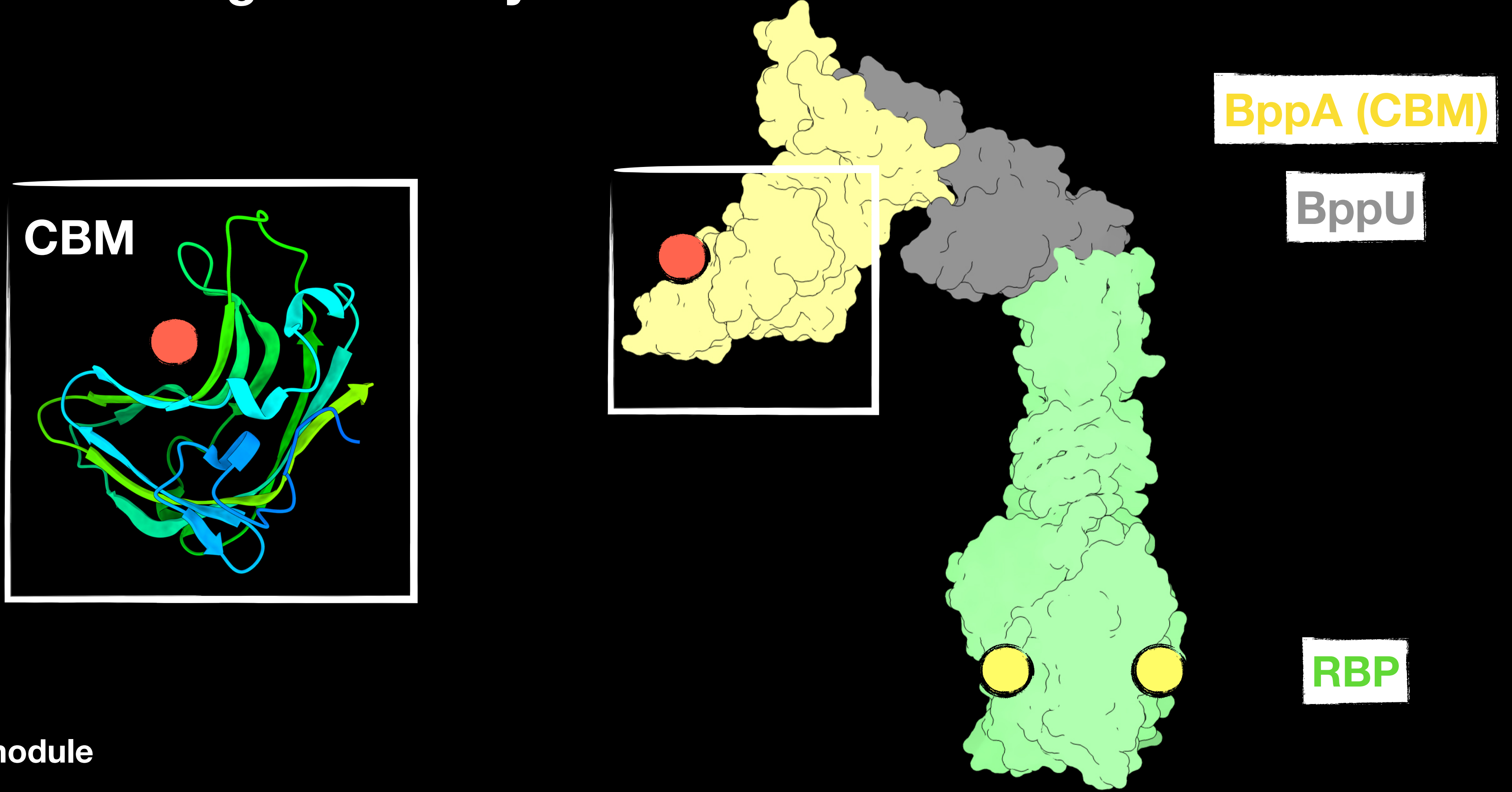


Host-binding machineries are structurally and functionally diverse



The *L. lactis* phage Tuc2009

host-binding machinery contains RBPs and CBMs



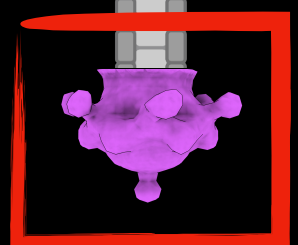
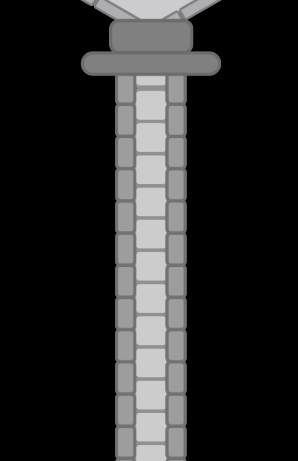
CBM: carbohydrate-binding module

● carbohydrate-binding site

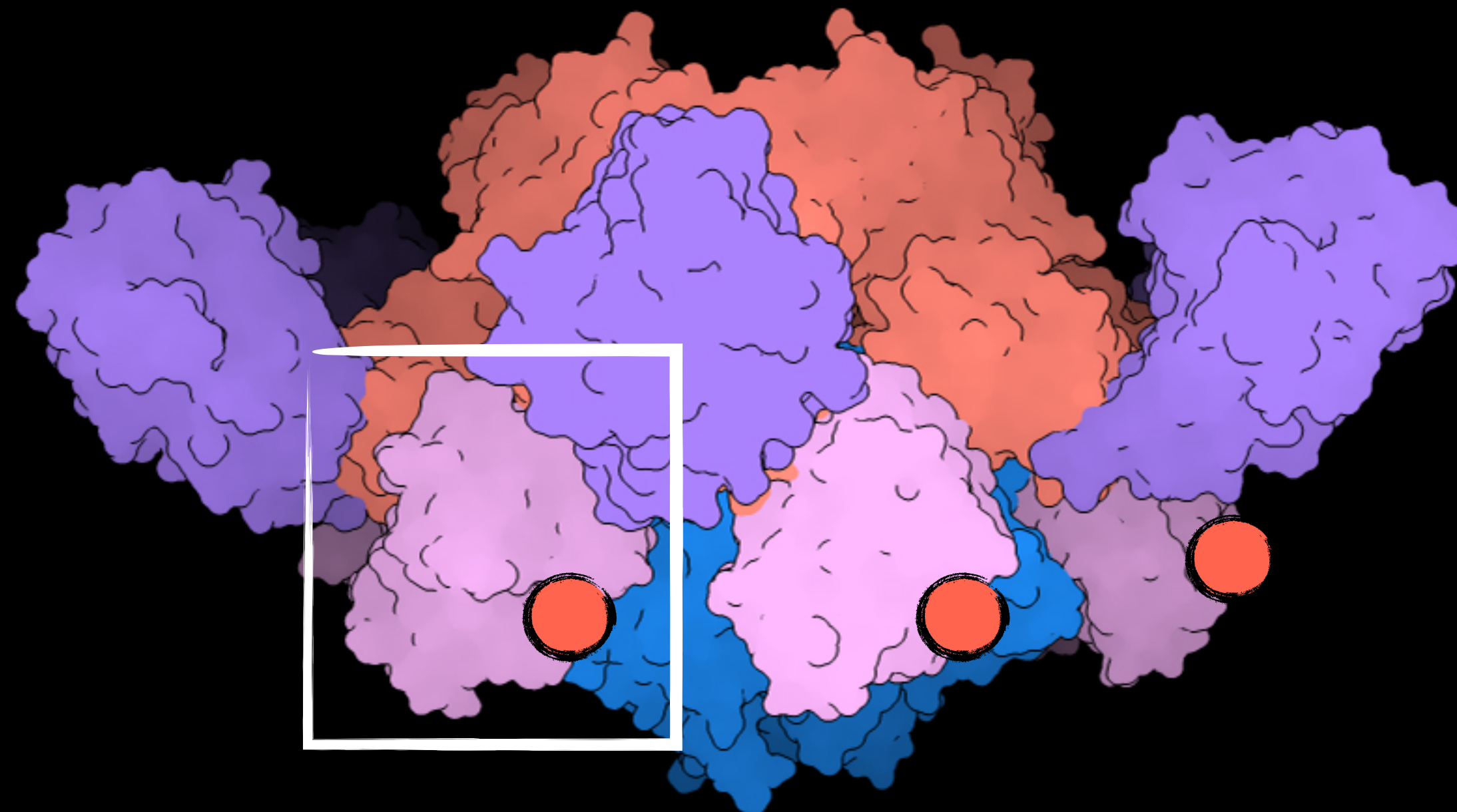
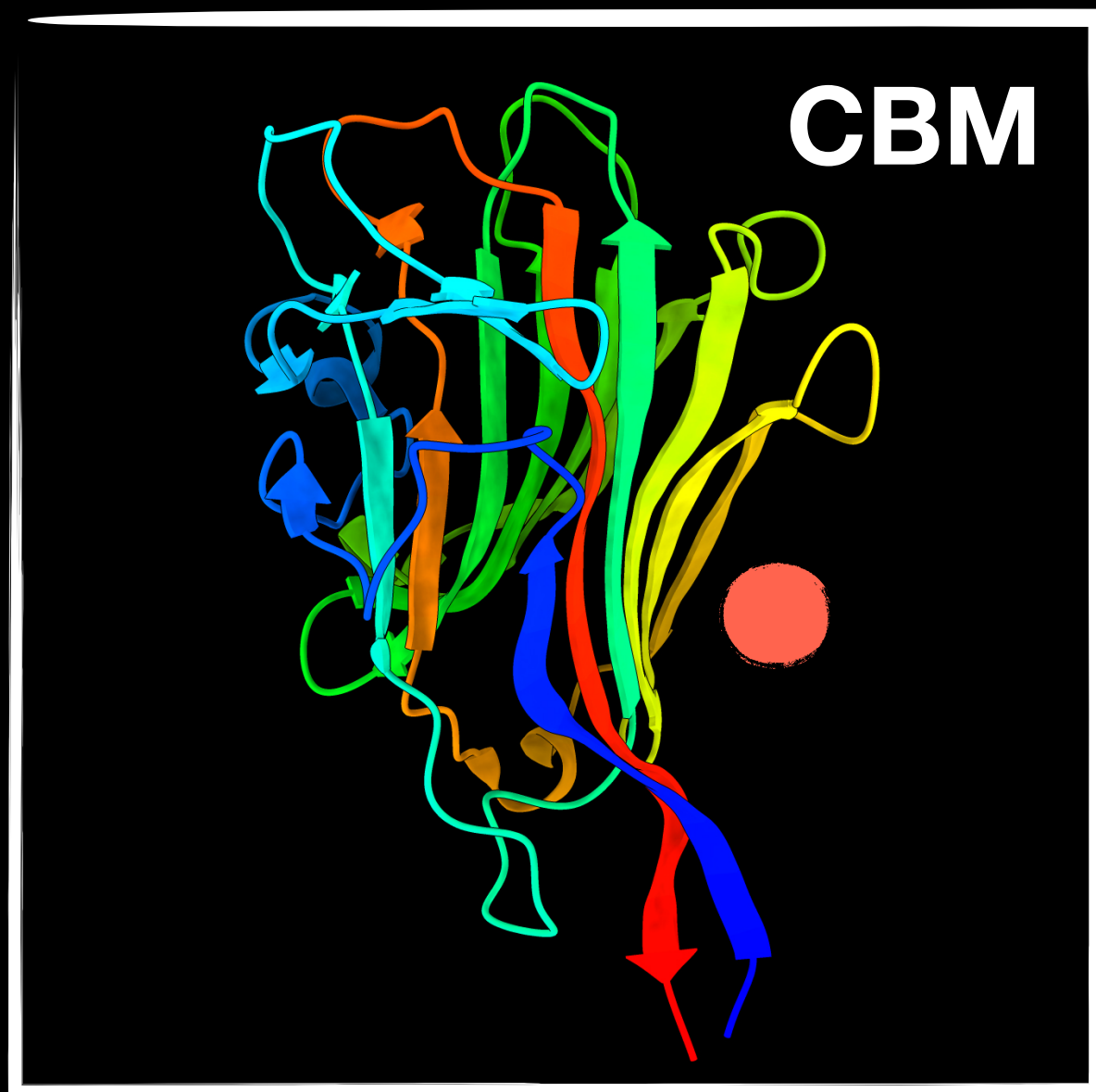


Host-binding machineries are structurally and functionally diverse

J-1



The *L. casei* phage J-1
host-binding machinery contains only CBMs



- CBM
- Dit
- CBM
- Tal

CBM: carbohydrate-binding module

● carbohydrate-binding site



CBMs are widespread in Gram+ infecting phages

Molecular Microbiology (2018) 110(5), 777–795

doi:10.1111/mmi.14124
First published online 30 October 2018

Functional carbohydrate binding modules identified in evolved dits from siphophages infecting various Gram-positive bacteria

Classical Dit

Belt domain

Galectin domain

Evolved Dit

Belt domain

CBM(s)

Galectin domain



CBMs are widespread in Gram+ infecting phages

microbial biotechnology

Open Access

Revisiting the host adhesion determinants of *Streptococcus thermophilus* siphophages

Katherine Lavelle,¹ Adeline Goulet,^{2,3} Brian McDonnell,¹ Silvia Spinelli,^{2,3} Douwe van Sinderen,^{1,4} Jennifer Mahony^{1,4*} and Christian Cambillau^{1,2,3**}

¹School of Microbiology, University College Cork, Cork, Ireland.

²Architecture et Fonction des Macromolécules Biologiques, Aix-Marseille Université, Campus de Luminy, Marseille, France.

³Architecture et Fonction des Macromolécules Biologiques, Centre National de la Recherche Scientifique (CNRS), Campus de Luminy, Marseille, France.

⁴APC Microbiome Ireland, University College Cork, Cork, Ireland.

RBP CBMs and observed by fluorescence microscopy that both modules bind to their corresponding St host, the putative RBP CBM with higher affinity than the Tal-associated one. The large number of CBM functional domains in St phages suggests that they play a contributory role in the infection process, a feature that we previously described in lactococcal phages and beyond, possibly representing a universal feature of the siphophage host-recognition apparatus.

Introduction

The dairy fermentation industry depends on robust starter cultures of lactic acid bacteria (LAB). Strains of *Lac-*

Short Tal

Structural domain

Evolved Tal

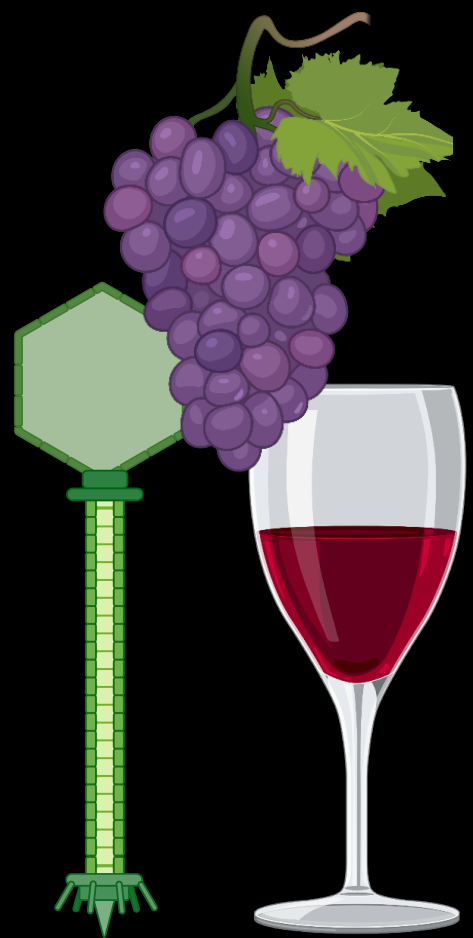
Structural domain

CBM(s)

**Are CBM insertions a universal feature
of siphophages infecting LAB?**

**Are CBM insertions a universal feature
of siphophages infecting LAB?**

**What is the extent of the structural and functional
variety of LAB phages' host-binding machineries?**



Unraveling molecular interactions between wine LAB, their viral predators, and polyphenols



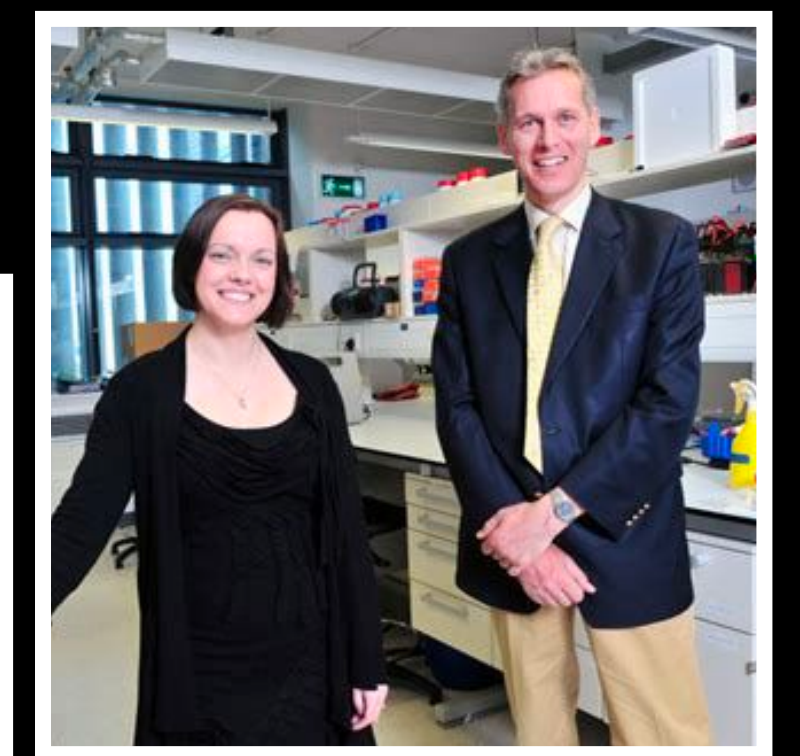
WINE project

2022-2024

Marie-Pierre Chapot-Chartier
INRAE, Micalis Institute



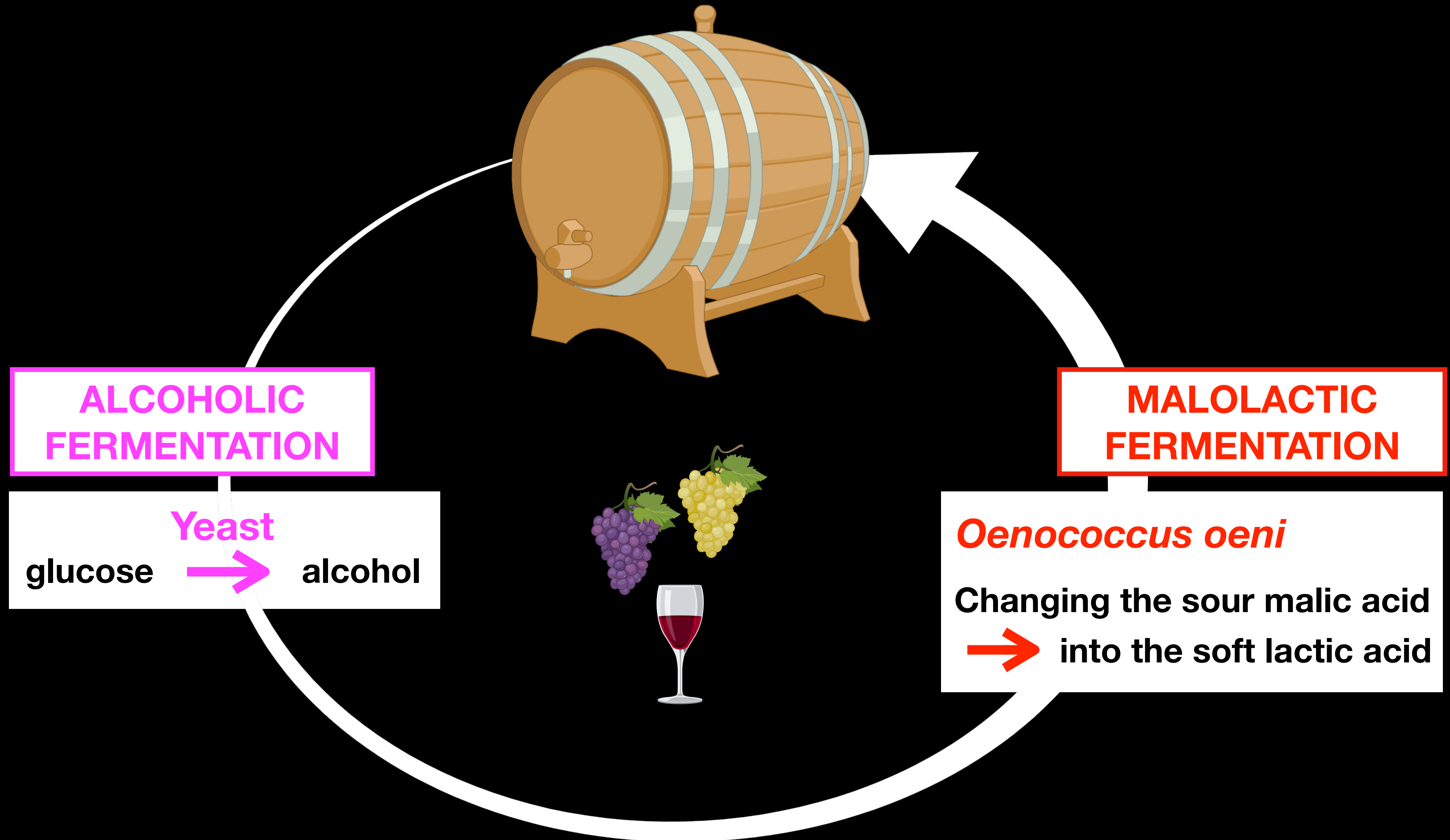
Claire Le Hénaff
Université de Bordeaux



Jennifer Mahony
Douwe van Sinderen
University College Cork

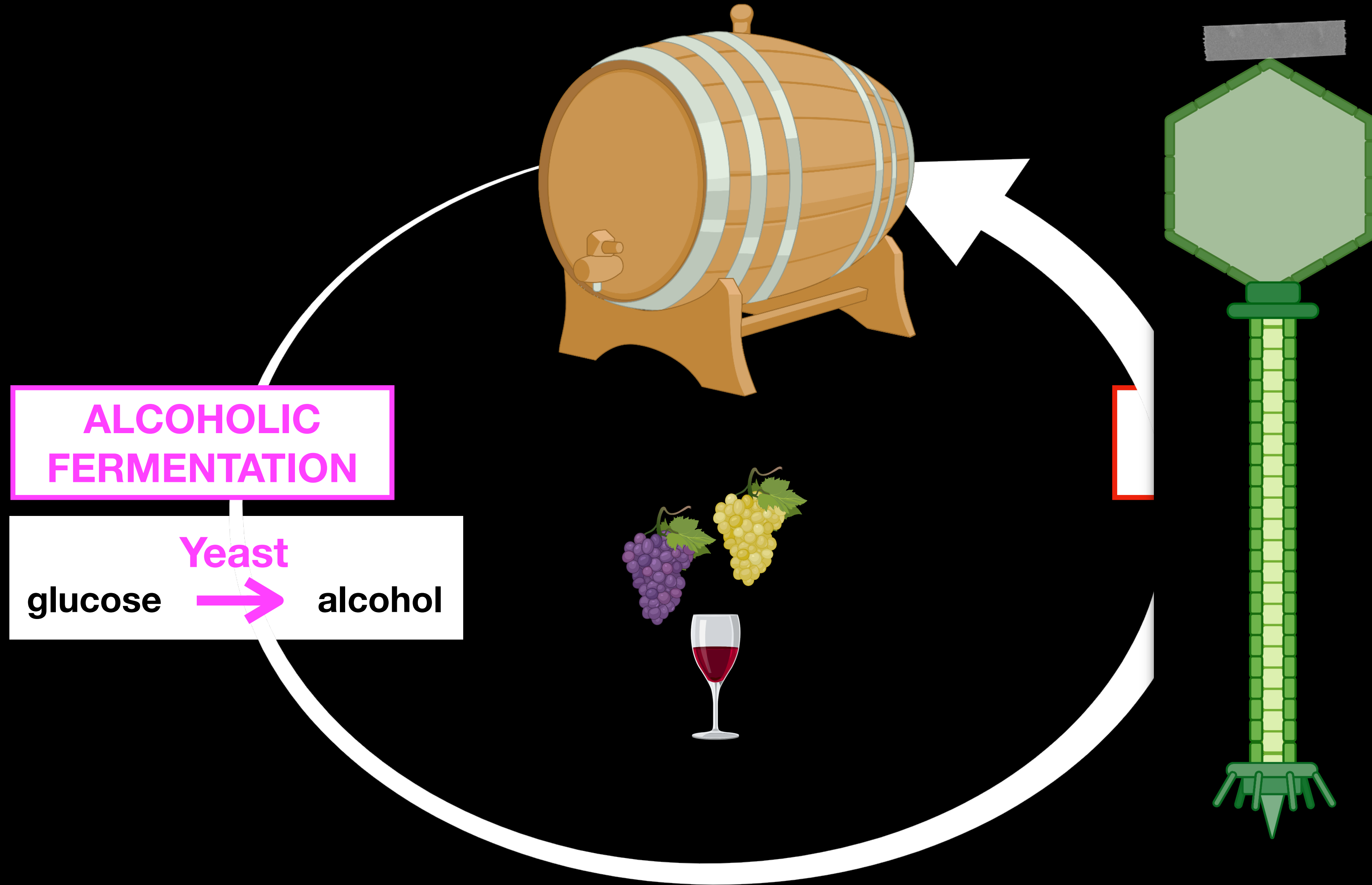


O. oeni is the main LAB involved in the wine malolactic fermentation



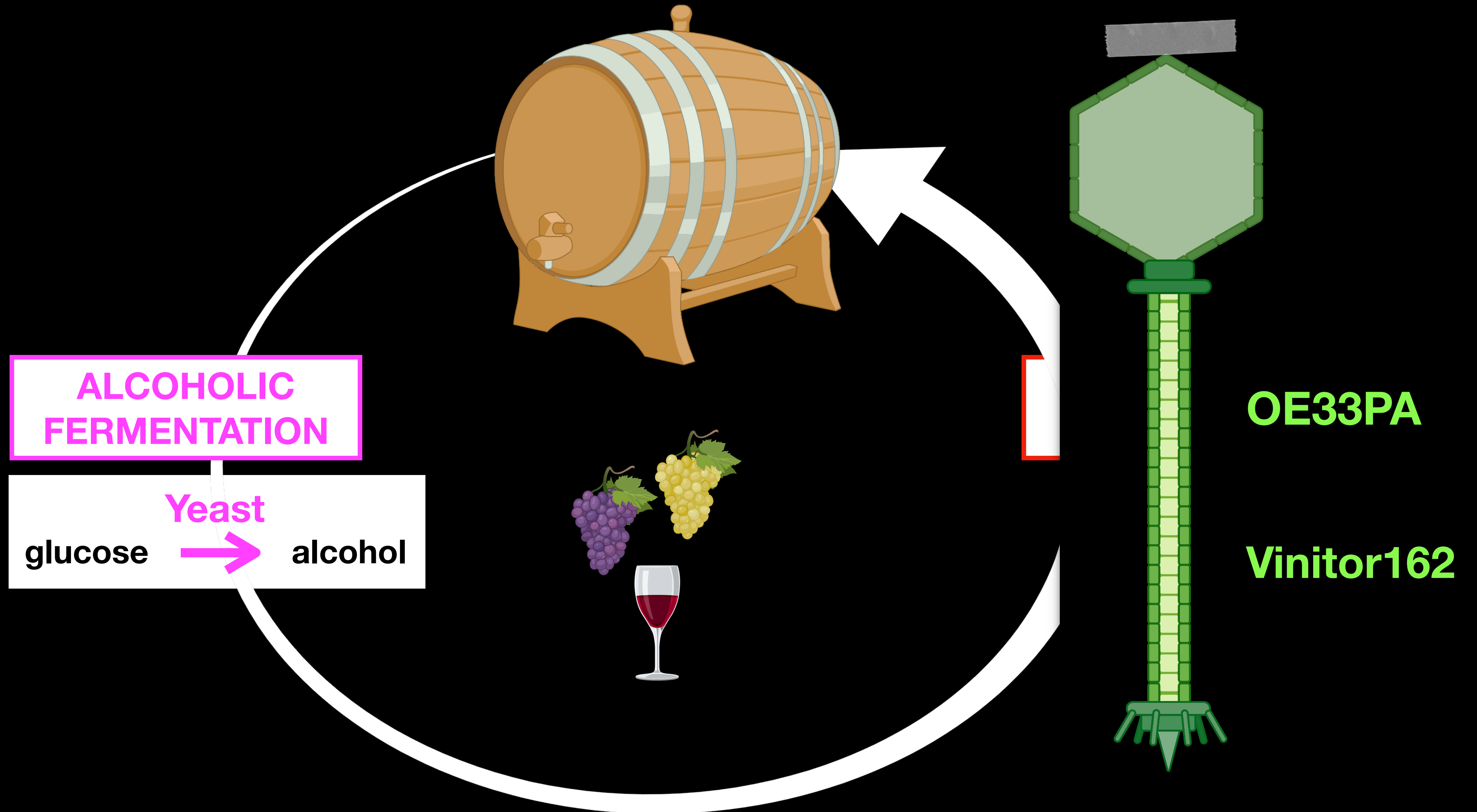


OE33PA and Vinitor162 are representative oenophages



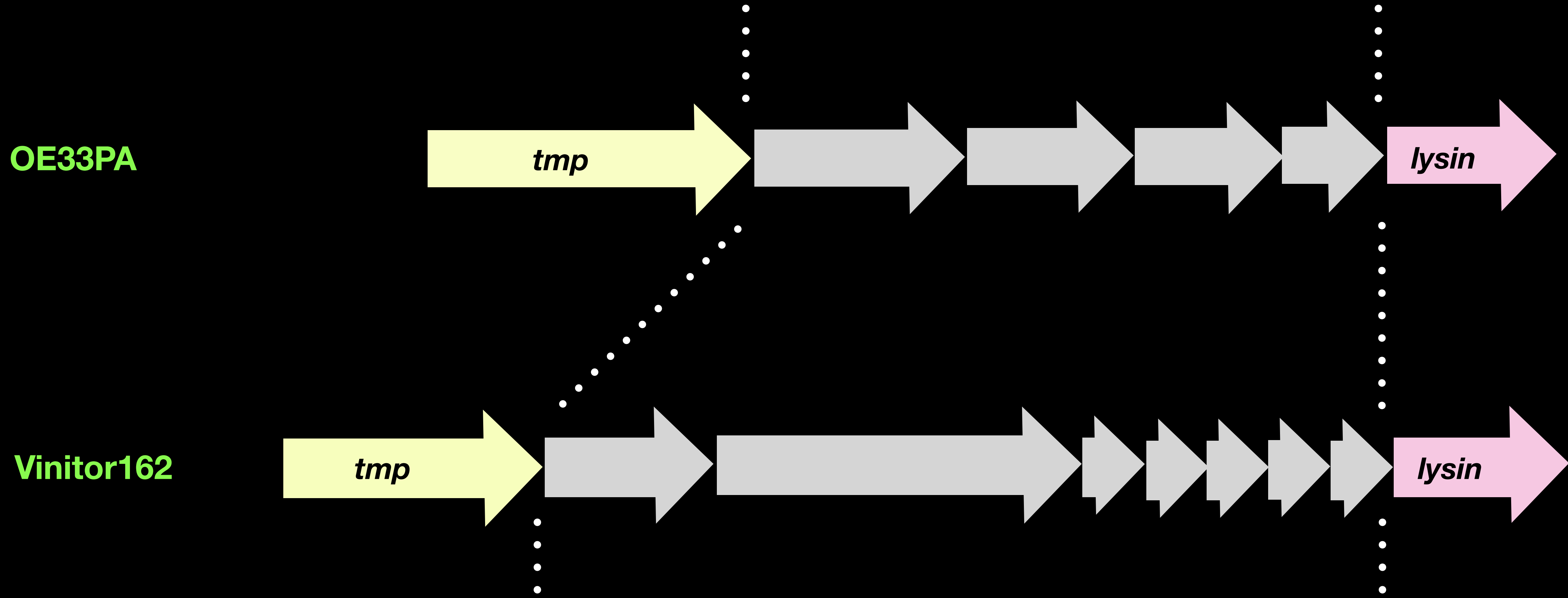


OE33PA and Vinitor162 are representative oenophages





OE33PA and Vinitor162 possess distinct host-binding machineries



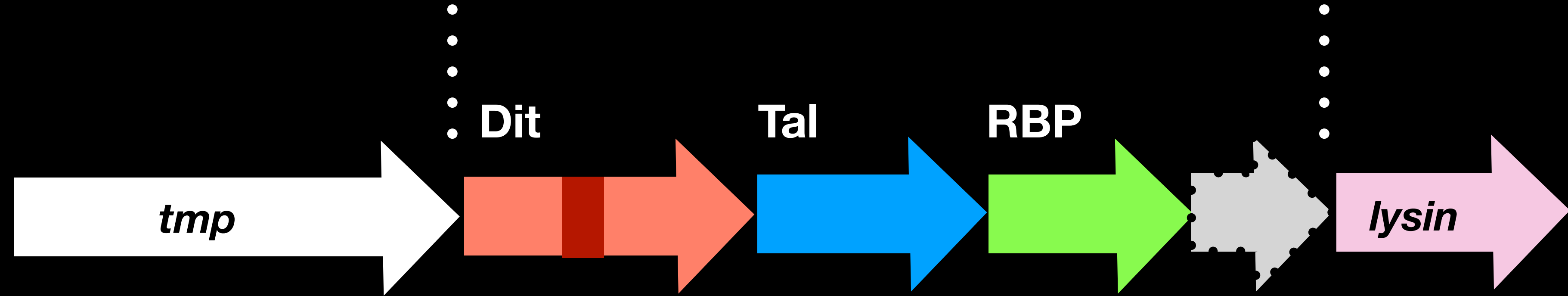
HHPred analyses of genomic regions coding for host-binding machinery components



OE33PA and Vinitor162 possess distinct host-binding machineries

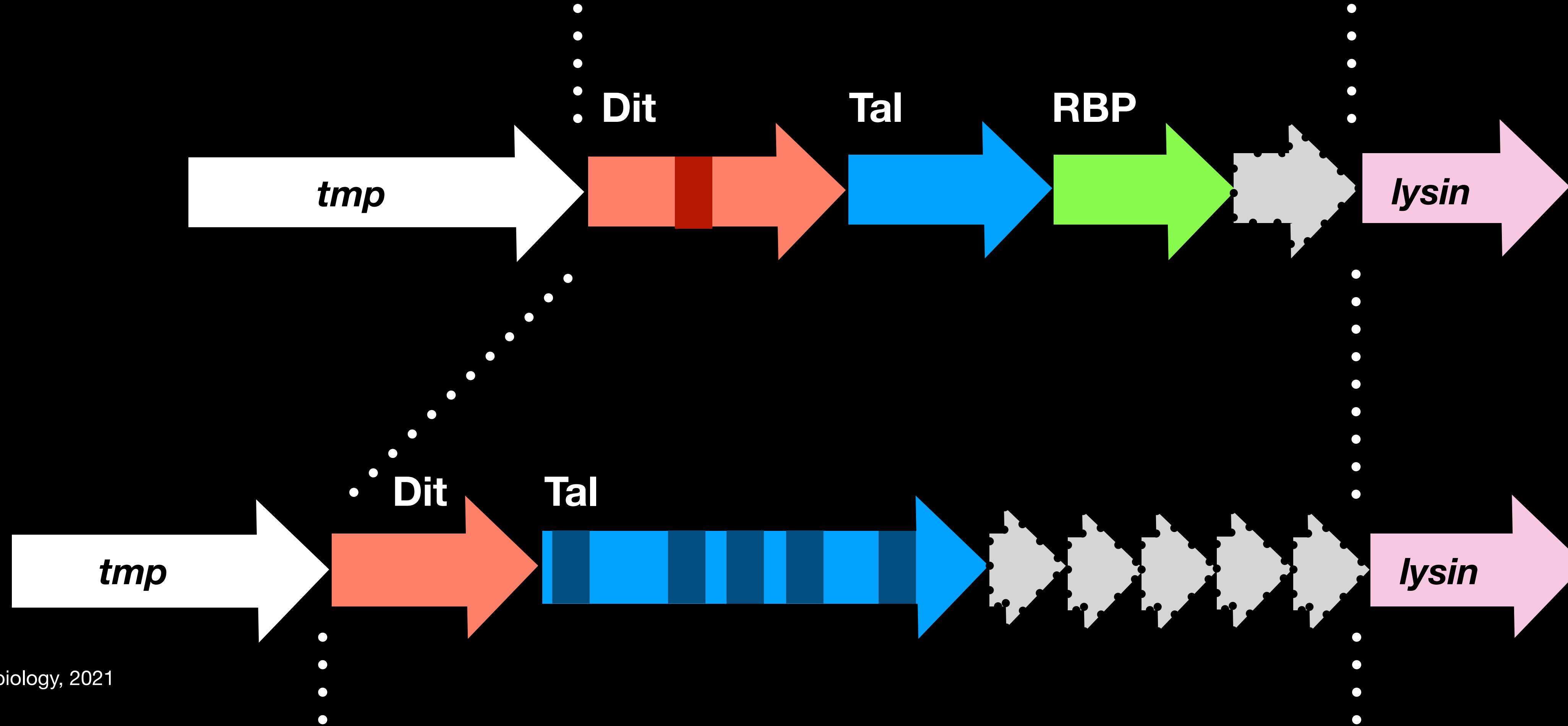
OE33PA

Philippe C et al., Viruses, 2020



Vinitor162

Philippe C et al., Frontiers in Microbiology, 2021



 predicted CBMs



PCs prevent OE33PA, but not Vinitor162, from host binding



Article

Wine Phenolic Compounds Differently Affect the Host-Killing Activity of Two Lytic Bacteriophages Infecting the Lactic Acid Bacterium *Oenococcus oeni*





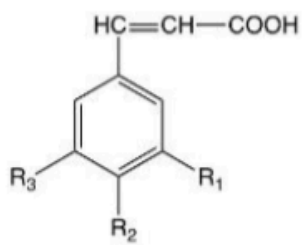
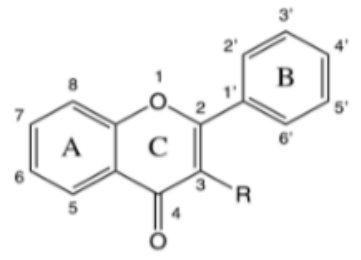
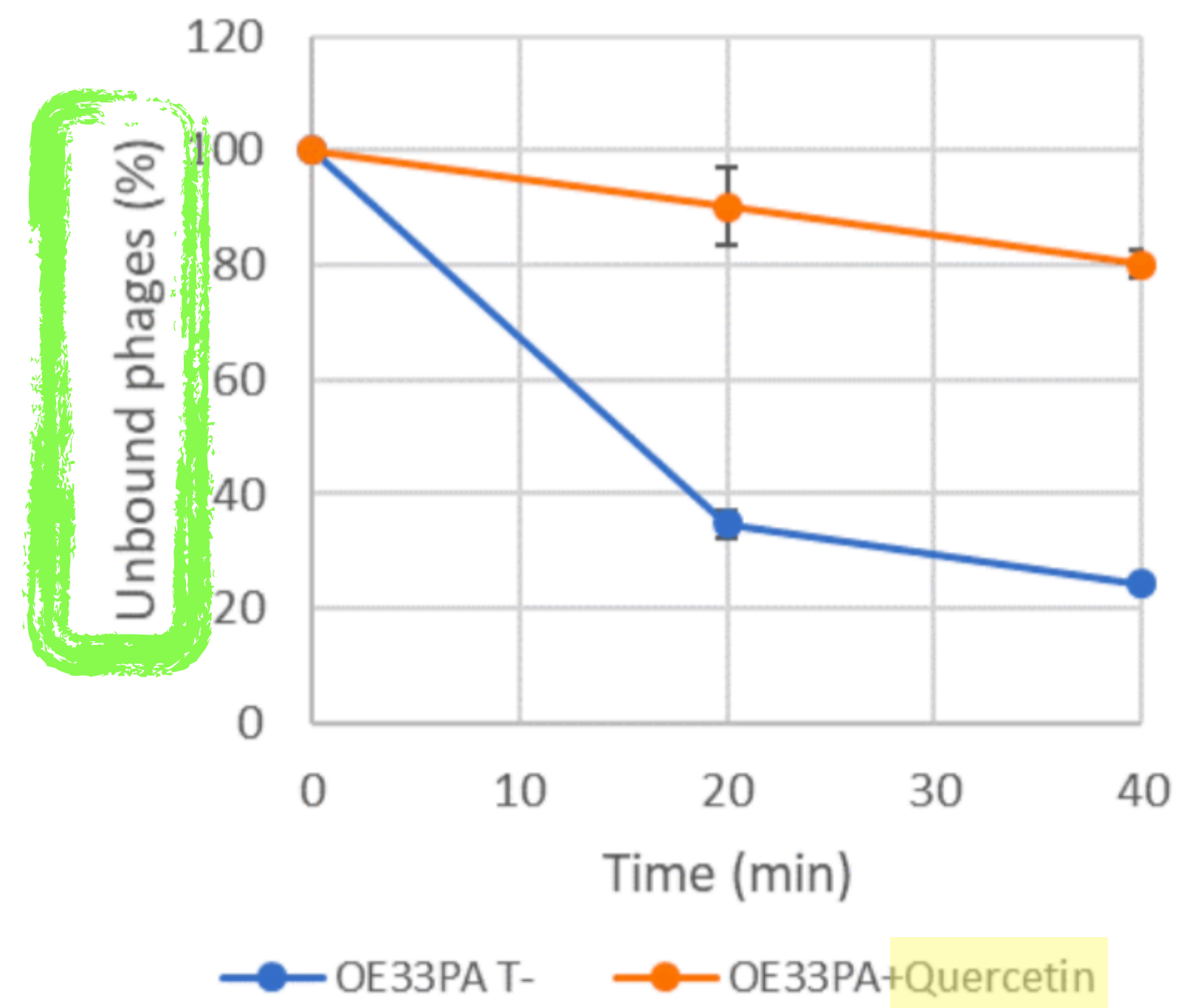
Cécile Philippe ¹, Amel Chaïb ¹, Fety Jaomanjaka ¹ , Stéphanie Cluzet ¹, Aurélie Lagarde ¹, Patricia Ballestra ¹, Alain Decendit ¹, Mélina Petrel ², Olivier Claisse ^{1,3} , Adeline Goulet ^{4,5}, Christian Cambillau ^{4,5}  and Claire Le Marrec ^{1,6,*} 

Table 1. Name and chemical structures of the hydroxycinnamic acids and flavonoids tested in killing assays with the lytic oenophages OE33PA and Vinitor.

Chemical Structure	PC Family and Functional Groups							Inhibition of Phage-Killing Activity	
	Hydroxycinnamic acids	R1	R2	R3				OE33PA	Vinitor 162
	Coumaric acid	H	OH	H				+	-
	Caffeic acid	H	OH	OH				+	-
	Cinnamic acid	H	H	H				+	-
	Flavonols	2'	3'	4'	5'	R	7		
	Quercetin	H	OH	OH	H	OH	OH	+	-
	Quercetin 3-O-glucuronide	H	OH	OH	H	Gluc. acid	OH	-	-
	Myricetin	H	OH	OH	OH	OH	OH	+	-
	Kaempferol	H	H	OH	H	OH	OH	-	-
	Morin	OH	H	OH	H	OH	OH	+	-
	Retusin	H	OCH3	OCH3	H	OCH3	OCH3	+	-

The intensity level reductions of phage killing activity are indicated (++, +). The absence of impact is shown (-).





AlphaFold2 predicts protein structure with unprecedented accuracy

The AlphaFold2 craze

nature
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nature > news & views > article

NEWS AND VIEWS | 23 August 2021

Protein-structure prediction revolutionized

The full might of a world-leading artificial-intelligence laboratory has been brought to bear on protein-structure prediction. The resulting method, AlphaFold2, promises to transform our understanding of proteins.

nature
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NEWS | 15 July 2021

DeepMind's AI for protein structure is coming to the masses

Machine-learning systems from the company and from a rival academic group are now open source and freely accessible.

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'It will change everything': DeepMind's AI makes gigantic leap in solving protein structures

Google's deep-learning program for determining the 3D shapes of proteins stands to transform biology, say scientists.

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Highly accurate protein structure prediction for the human proteome

Kathryn Tunyasuvunakool ✉, Jonas Adler, [...] Demis Hassabis ✉

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Highly accurate protein structure prediction with AlphaFold

John Jumper ✉, Richard Evans, [...] Demis Hassabis ✉

Nature 596, 583–589 (2021) | Cite this article

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1. AlphaFold2 structure prediction

3D models (monomers & oligomers)



2. Structure comparisons (PDB) using the **Dali** server

Structural homologs

3. Calculation of oligomeric assemblies using **SymmDock**

oligomers with cyclic symmetries

4. Topology prediction based on prior knowledge

Topology of host-binding machineries



The evolved Dit of OE33PA

1

659

Full-length Dit

Domain 2

Domain 1

Domain 3

Domain 4

Domain 5



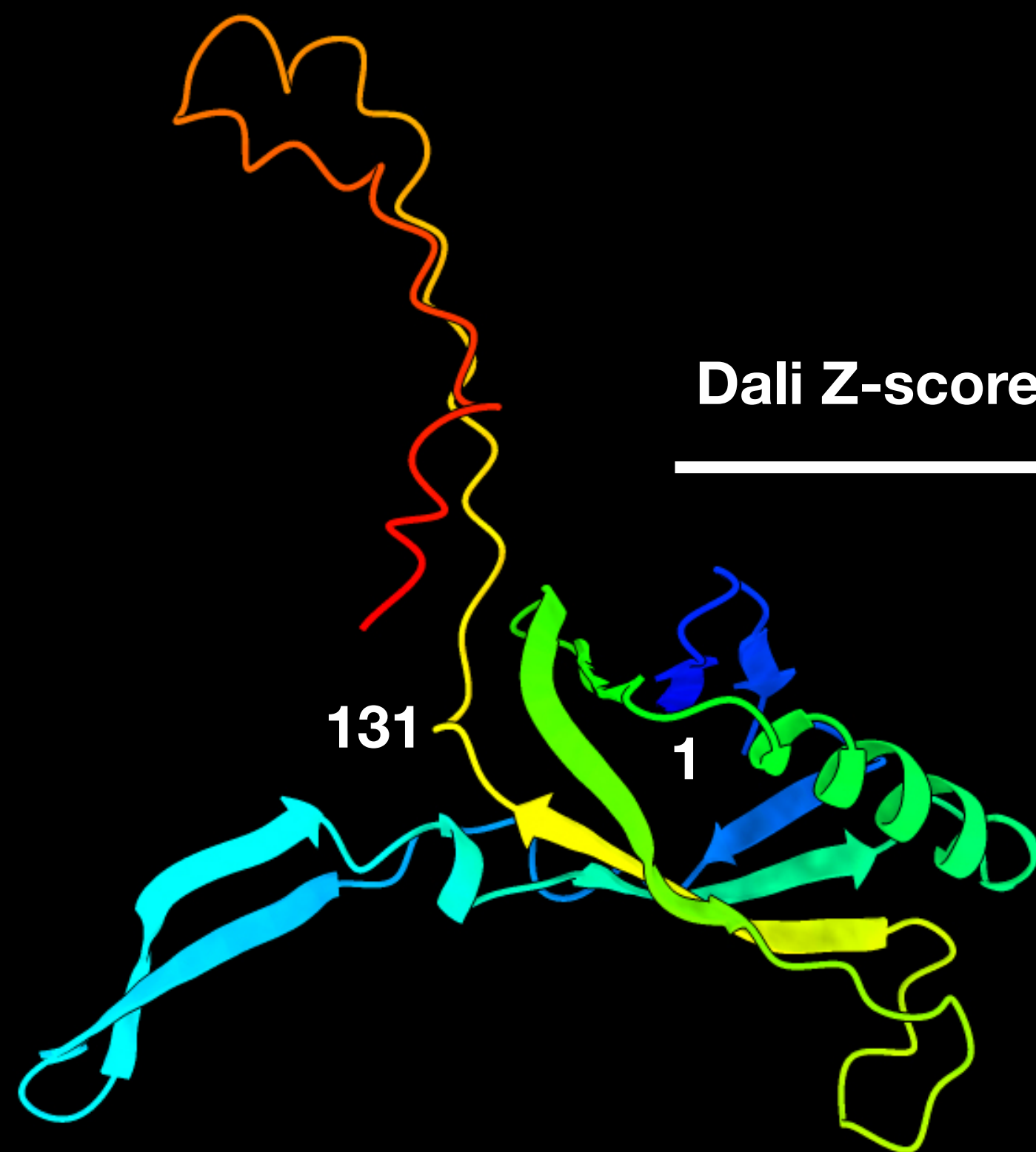


The evolved Dit of OE33PA

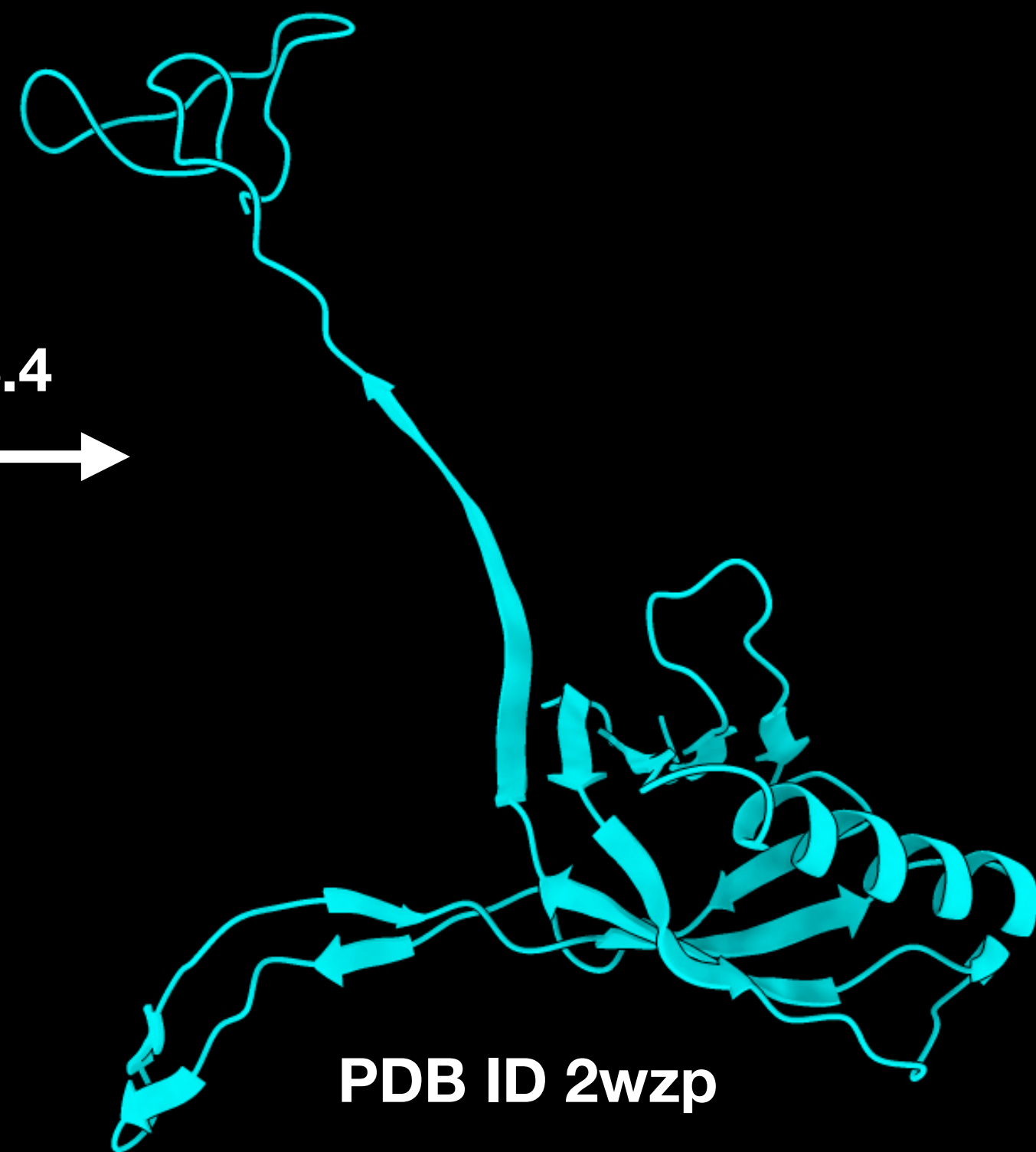
1

131

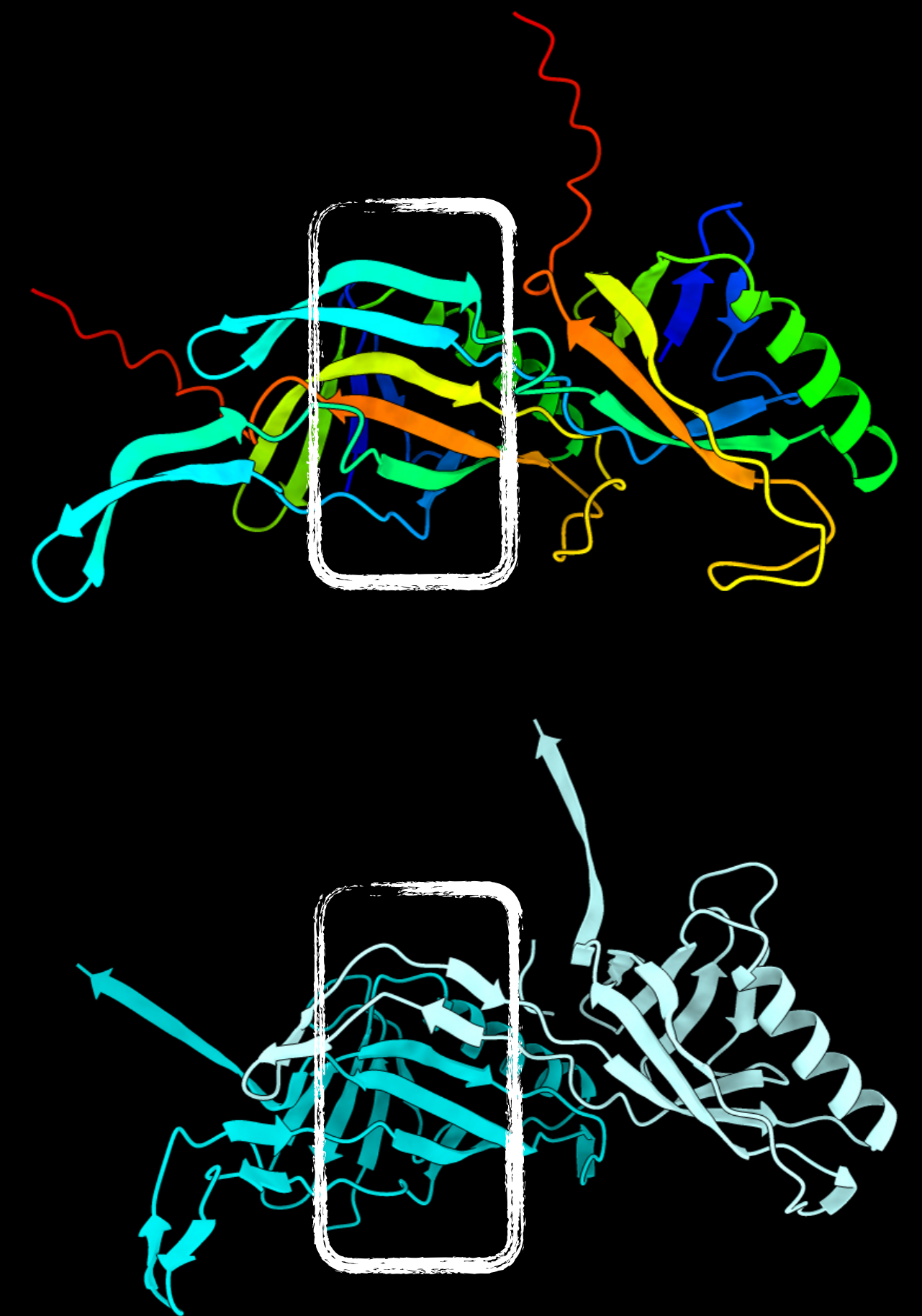
belt domain



Dali Z-score=13.4



PDB ID 2wzp
(phage p2)





The evolved Dit of OE33PA

1

131

520

659

belt domain

galectin domain



PDB ID 2x8k

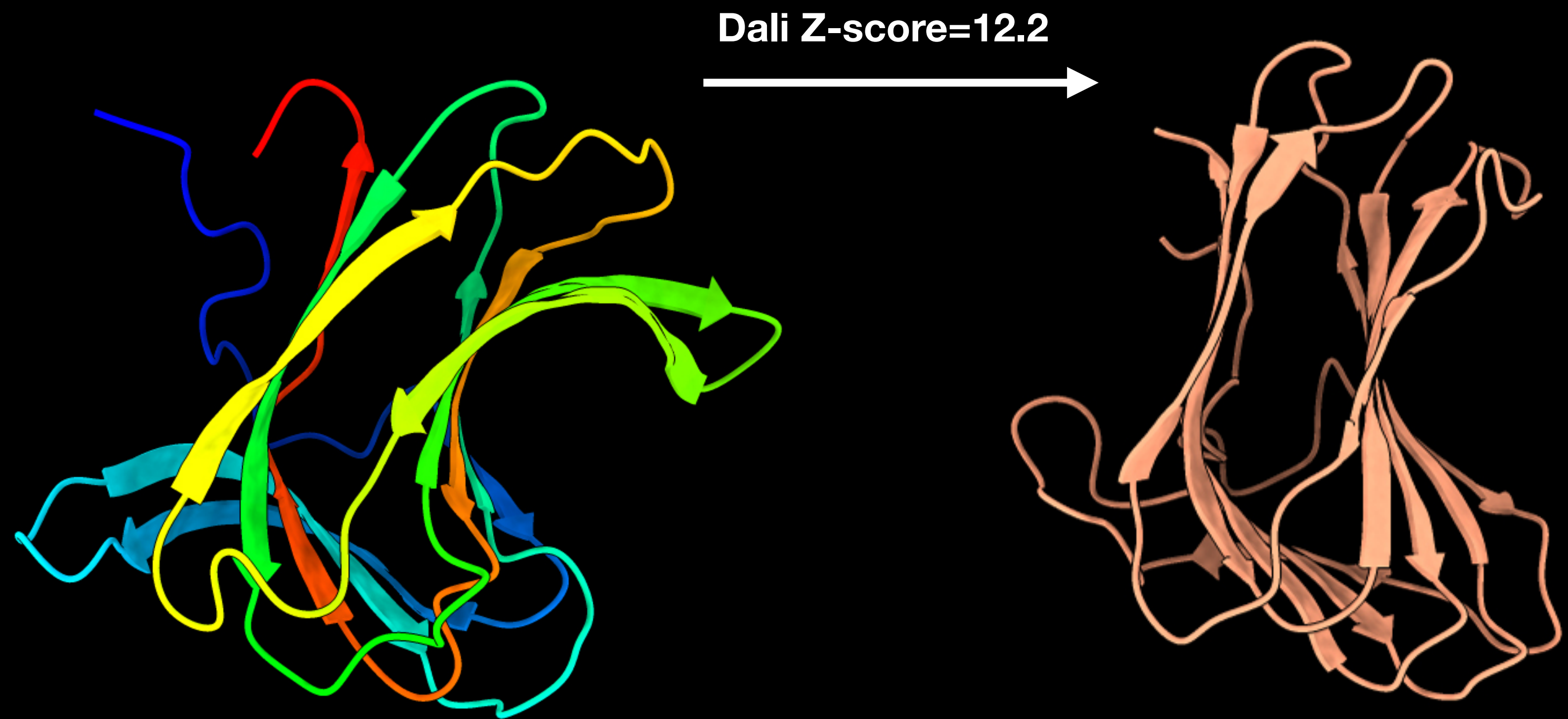
(*Bacillus subtilis* phage SPP1)

Dali Z-score=12.3





The evolved Dit of OE33PA

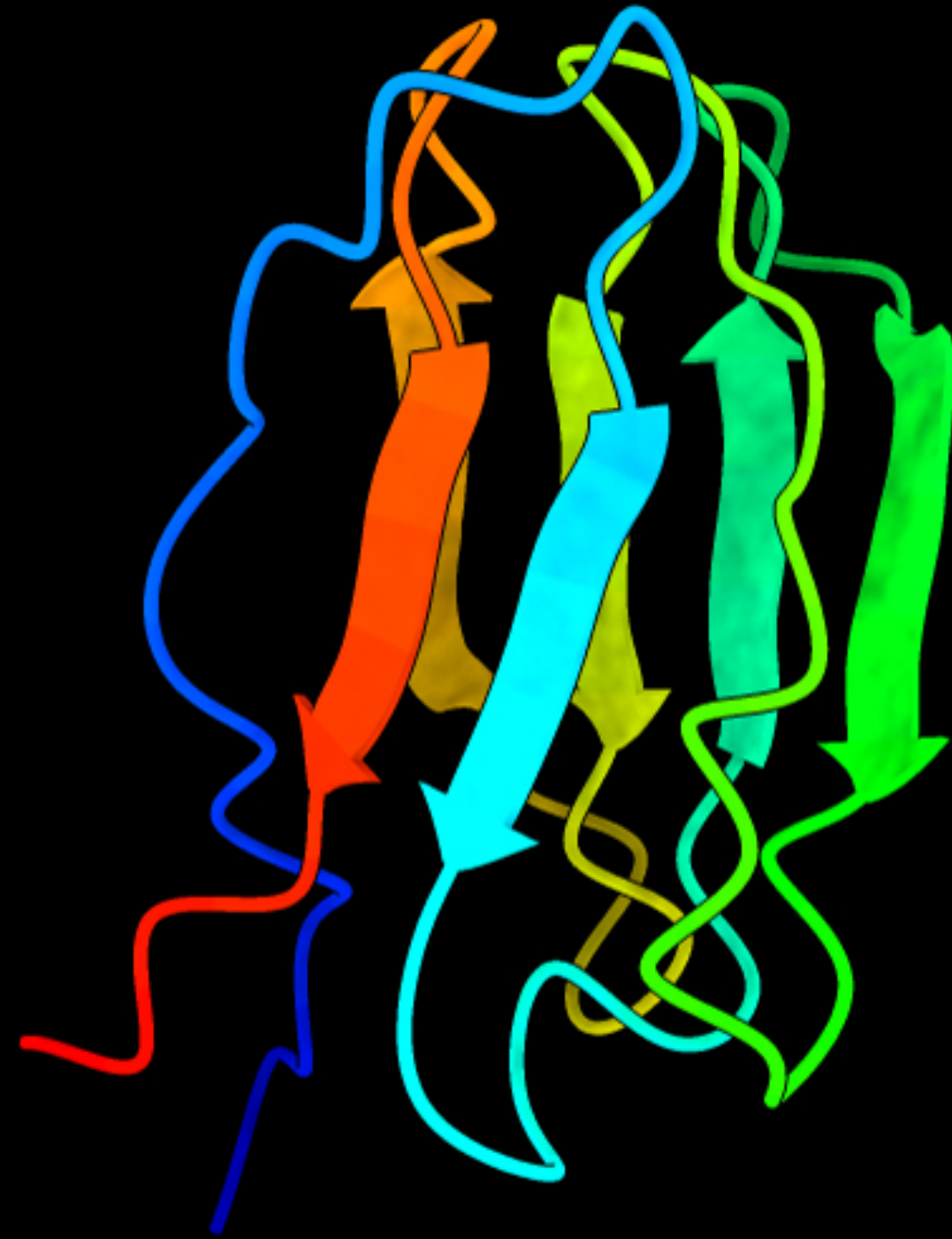


PDB ID 5w6h

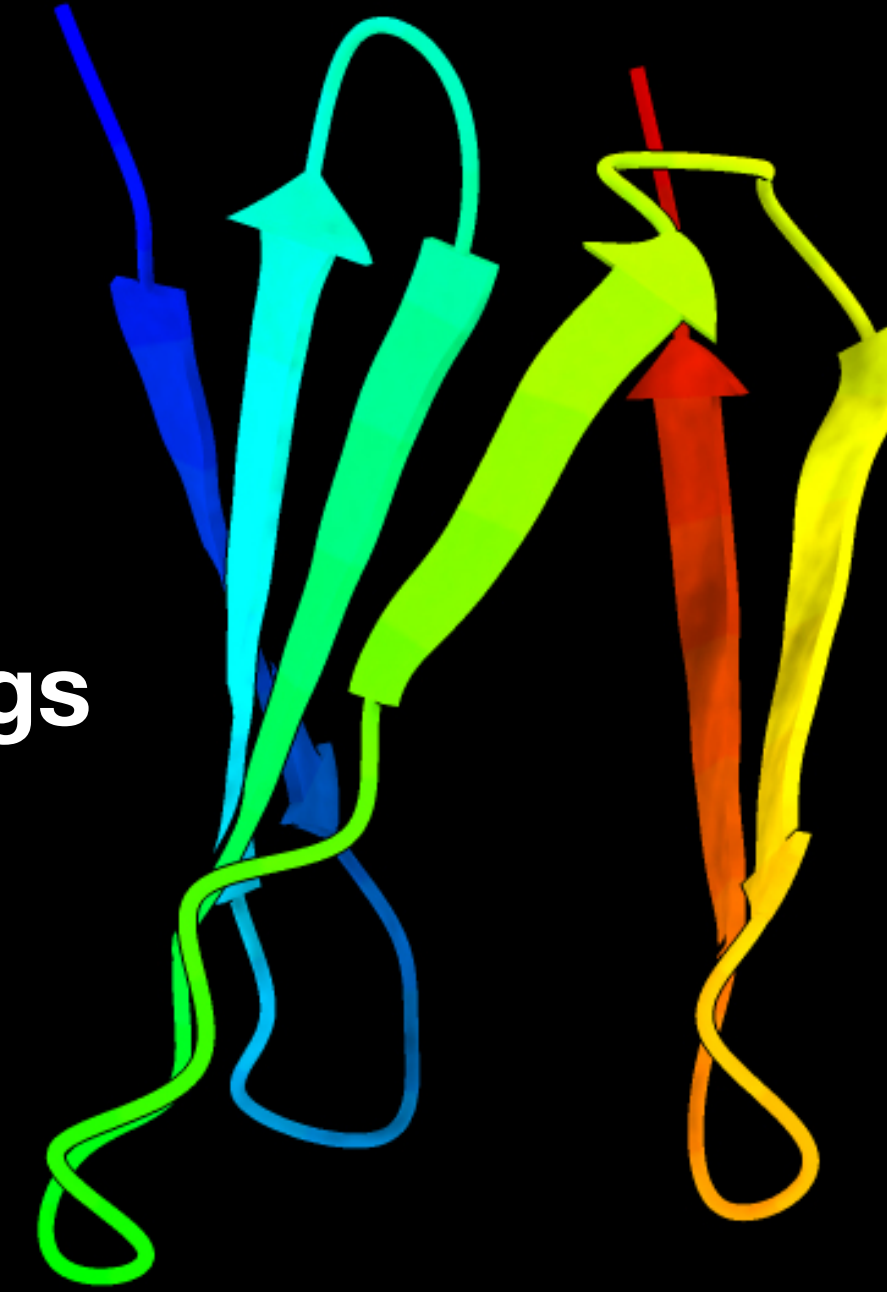
(phage tail spike, CBM4 @ CAZyDB)



The evolved Dit of OE33PA



No significant structural homologs



Potential structural domains contributing to the Dit overall fold



The short Tal of OE33PA

1

362

structural domain (phage T4 gp27-like)



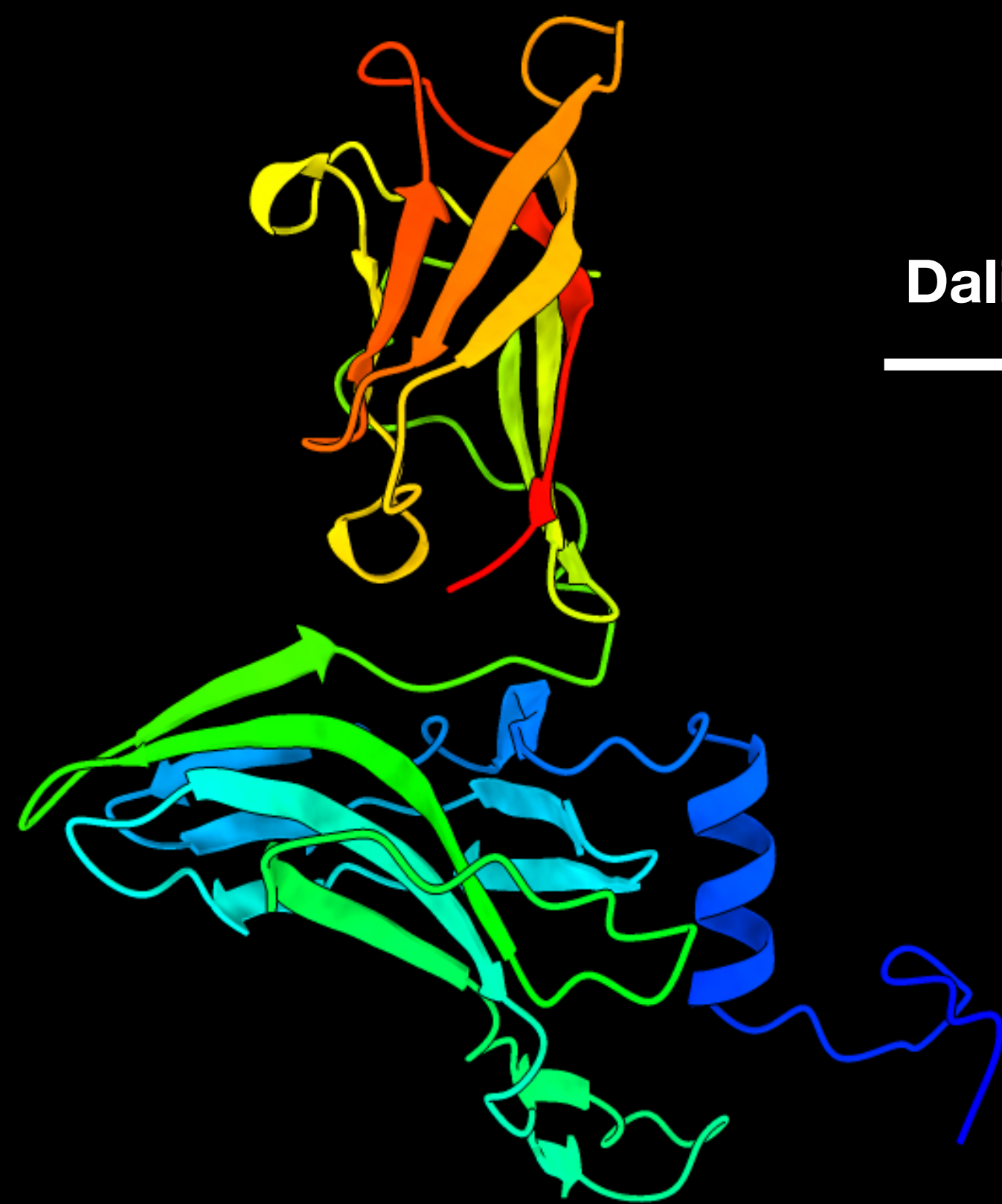
Dali Z-score=19.7



PDB ID 2wzp
(phage p2)



The chimeric RBP of OE33PA



Dali Z-score=11.9



PDB ID 2fsd
(phage Bil170)

Dali Z-score=14.9

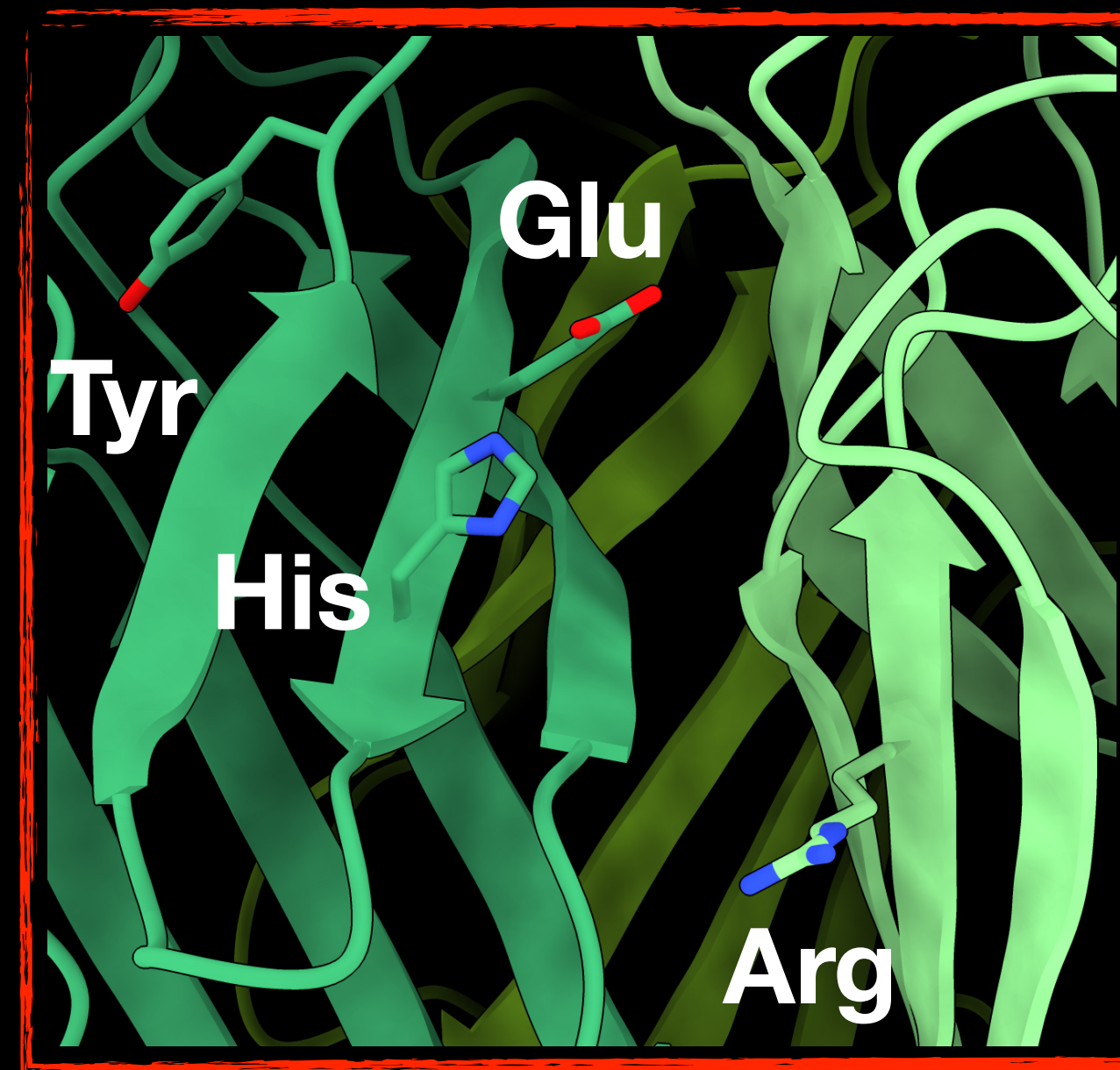
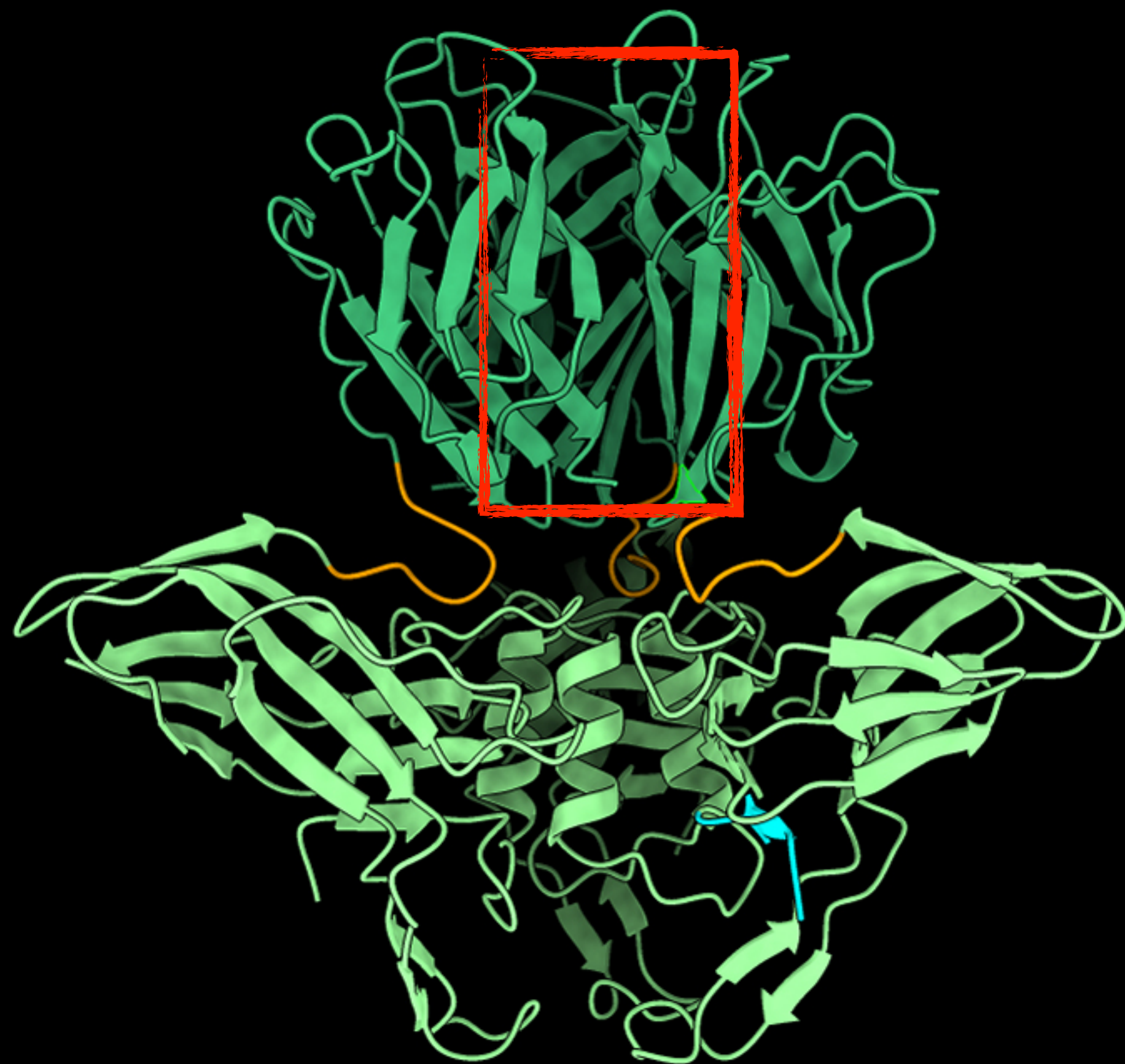


PDB ID 4l92
(phage 1358)

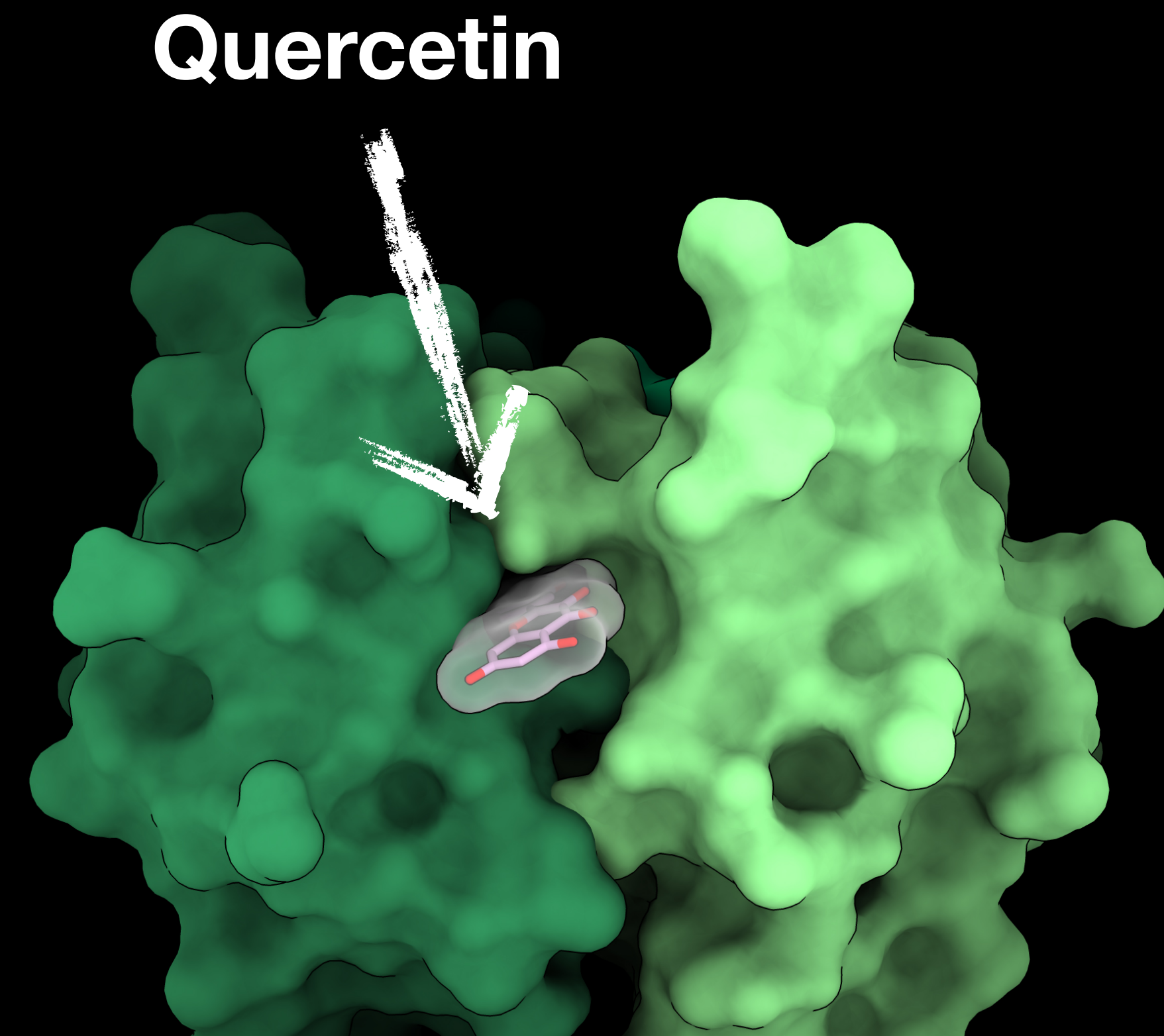


The chimeric RBP of OE33PA

PCs may occupy receptor-binding sites thereby preventing host binding



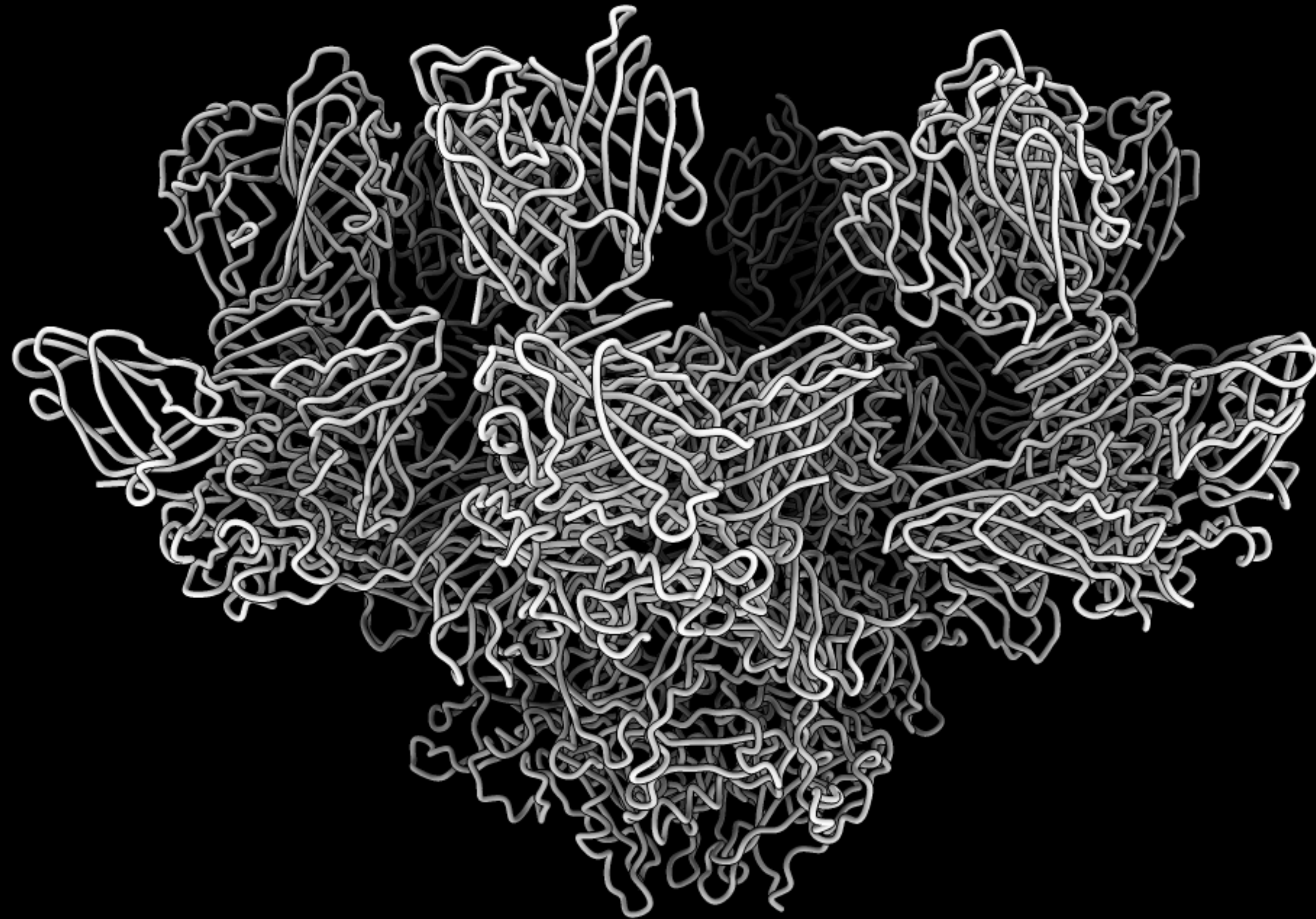
Potential sugar-binding residues



Potential PC binding site



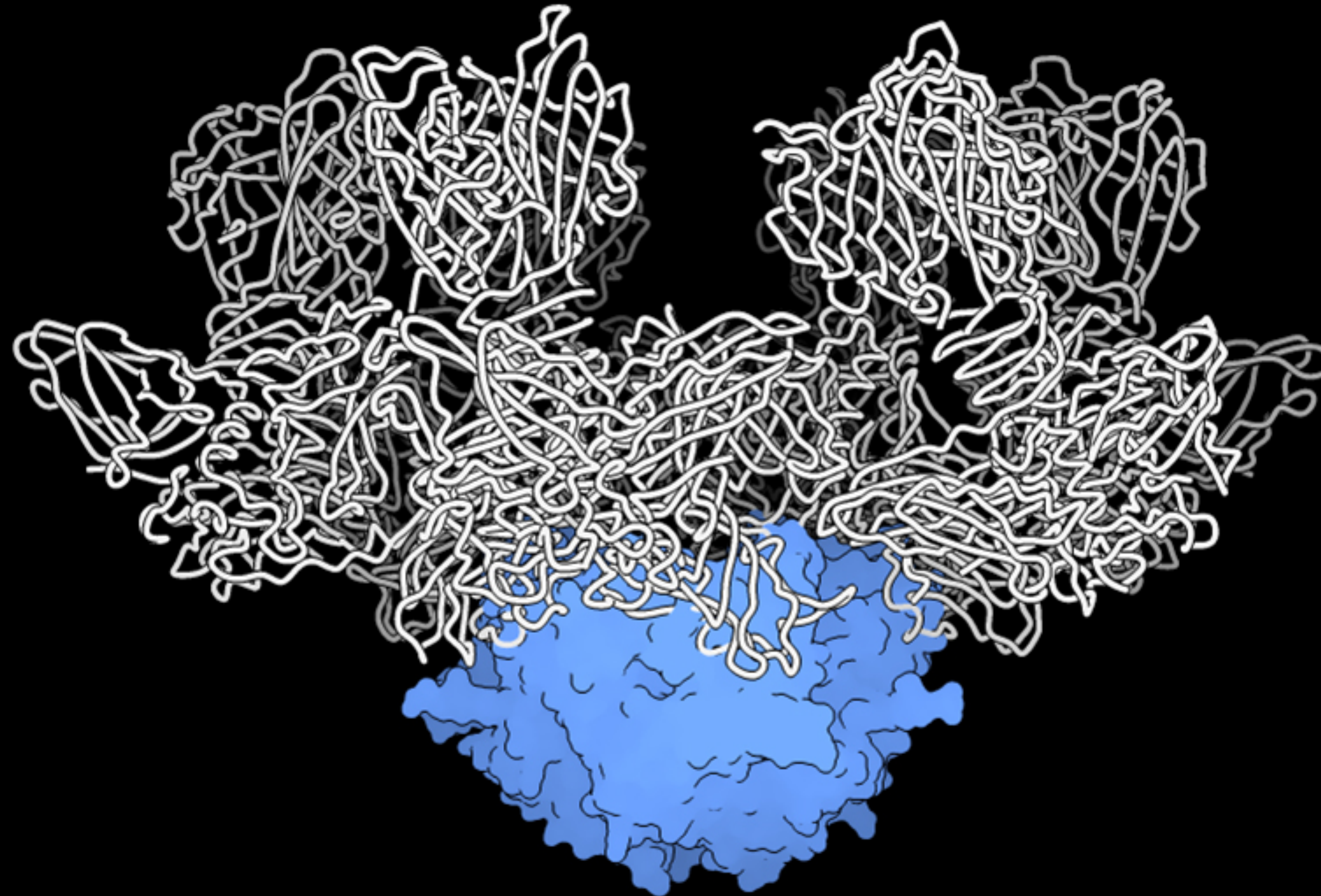
A bulky, star-like host-binding machinery



L. lactis phage p2 (resting state)



A bulky, star-like host-binding machinery

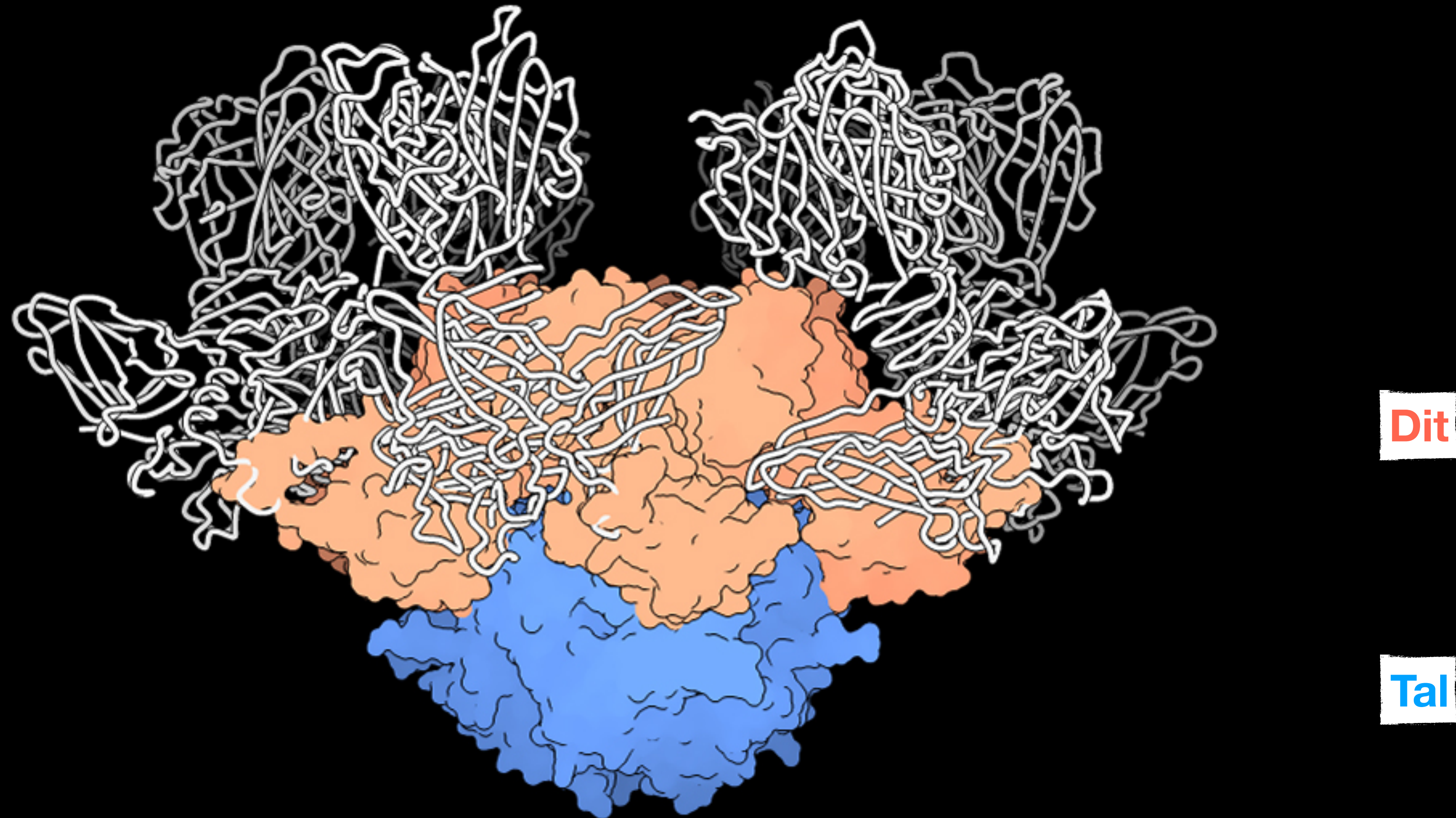


Tal

L. lactis phage p2 (resting state)



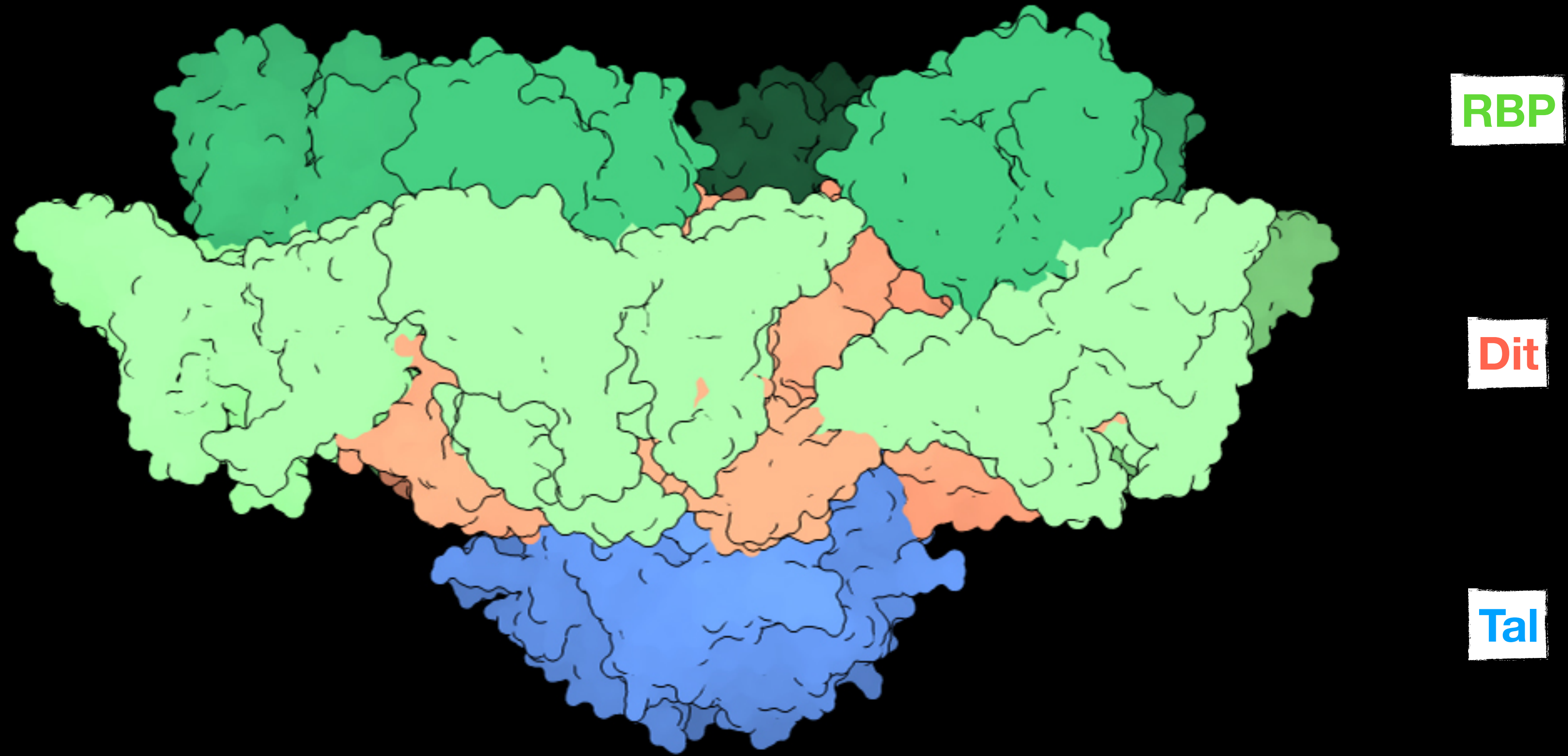
A bulky, star-like host-binding machinery



L. lactis phage p2 (resting state)



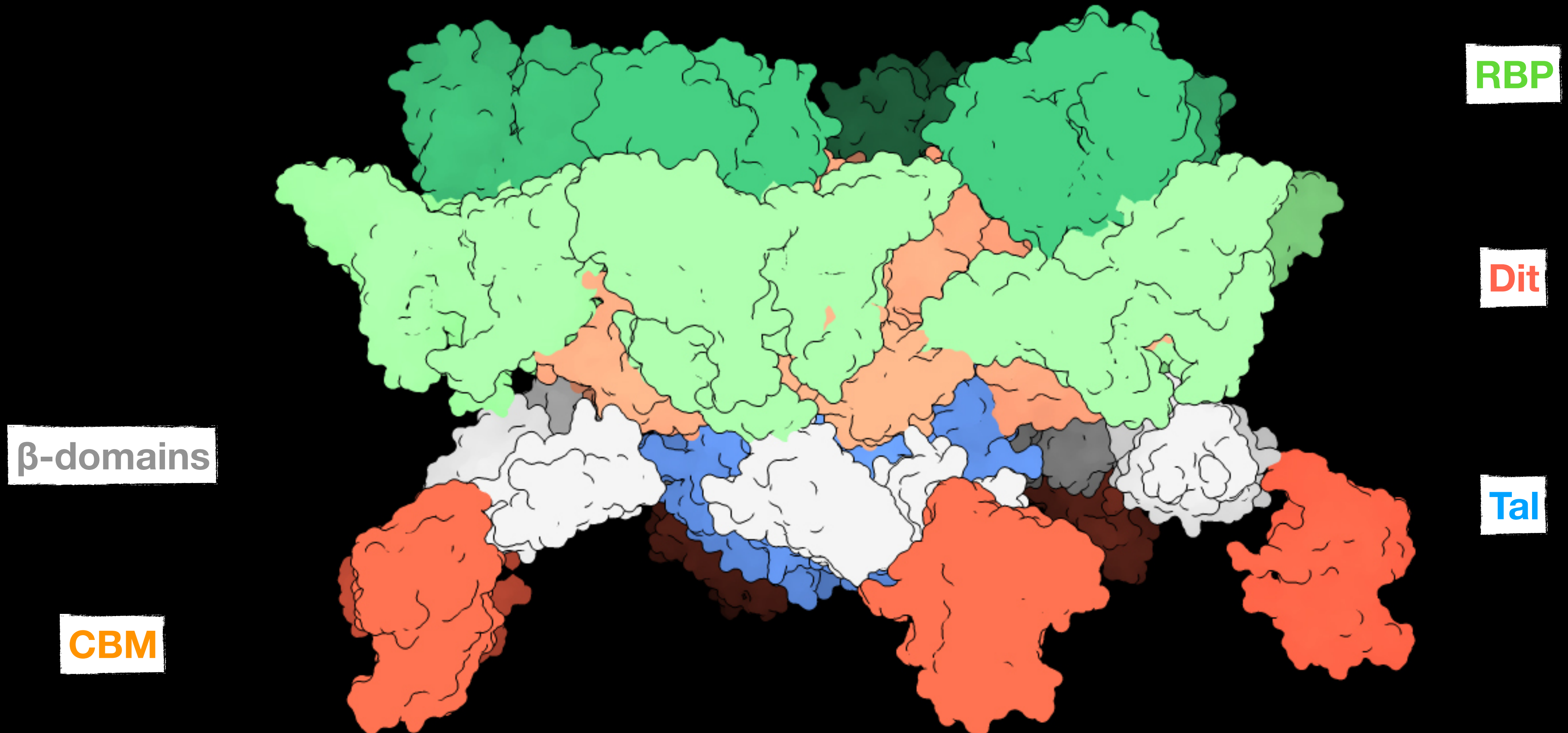
A bulky, star-like host-binding machinery



L. lactis phage p2 (resting state)



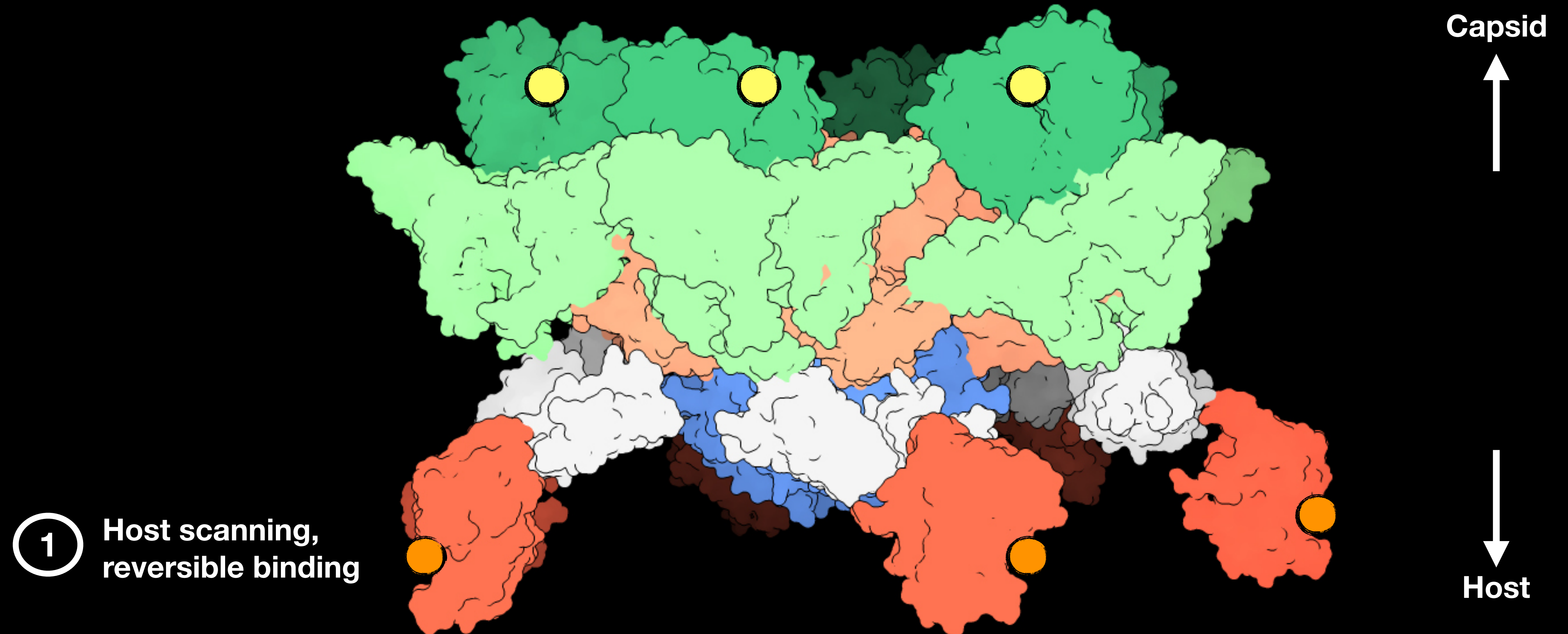
A bulky, star-like host-binding machinery





A bulky, star-like host-binding machinery

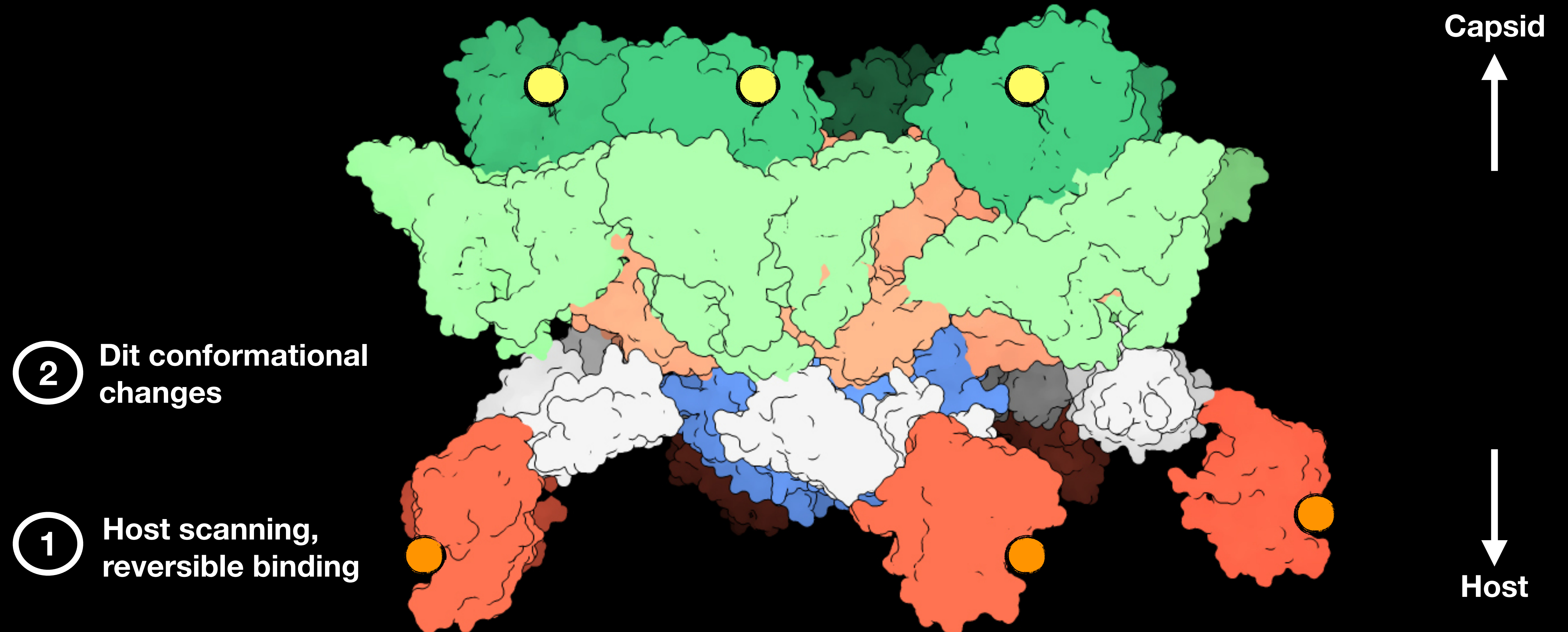
CBMs may induce RBP conformational changes upon preliminary, reversible binding to host polysaccharides





A bulky, star-like host-binding machinery

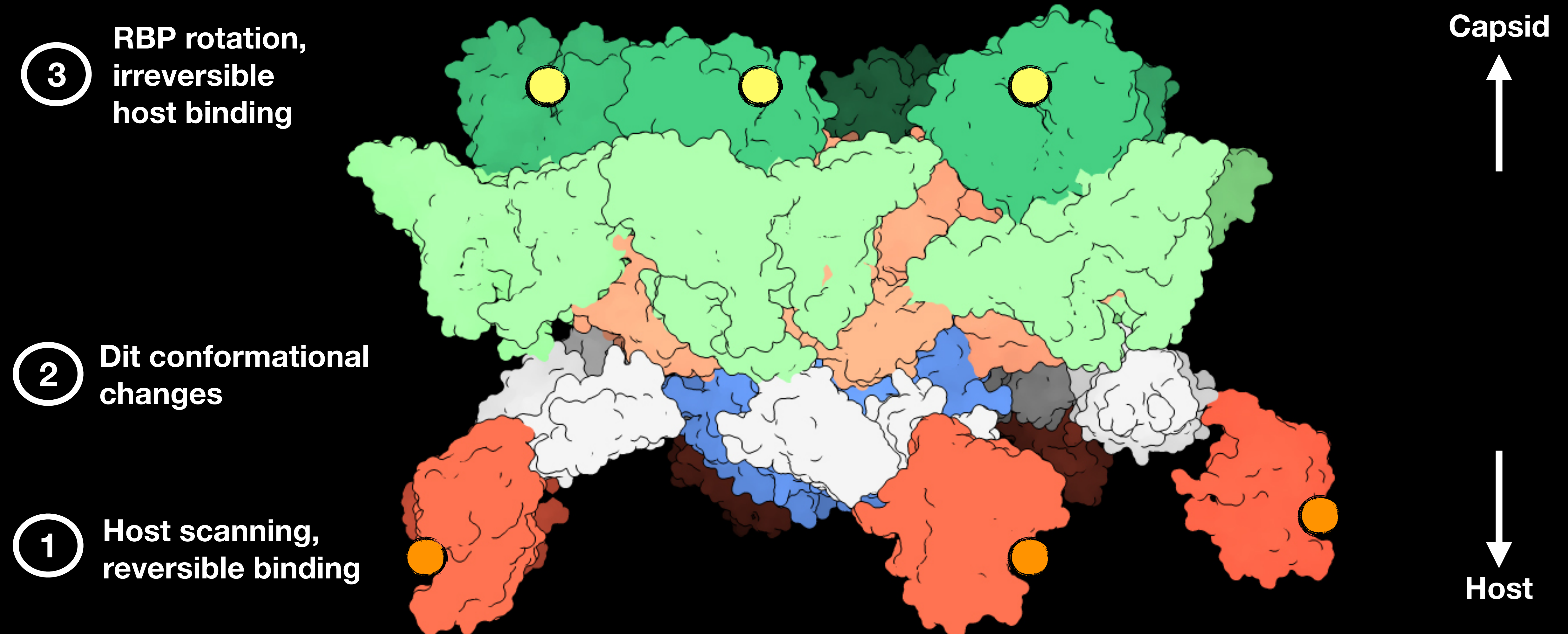
CBMs may induce RBP conformational changes upon preliminary, reversible binding to host polysaccharides





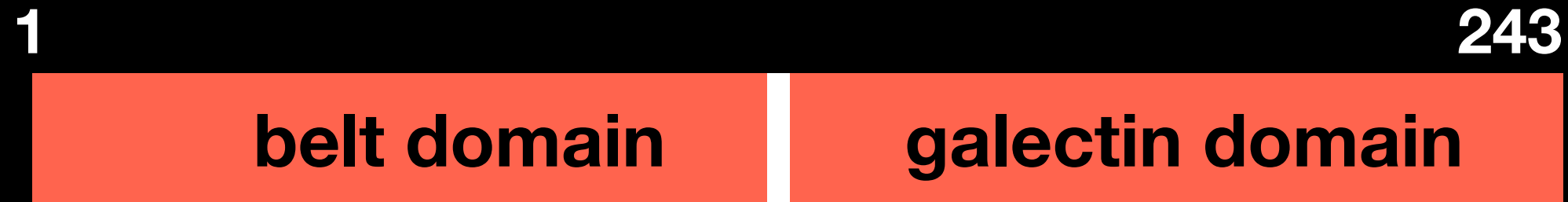
A bulky, star-like host-binding machinery

CBMs may induce RBP conformational changes upon preliminary, reversible binding to host polysaccharides

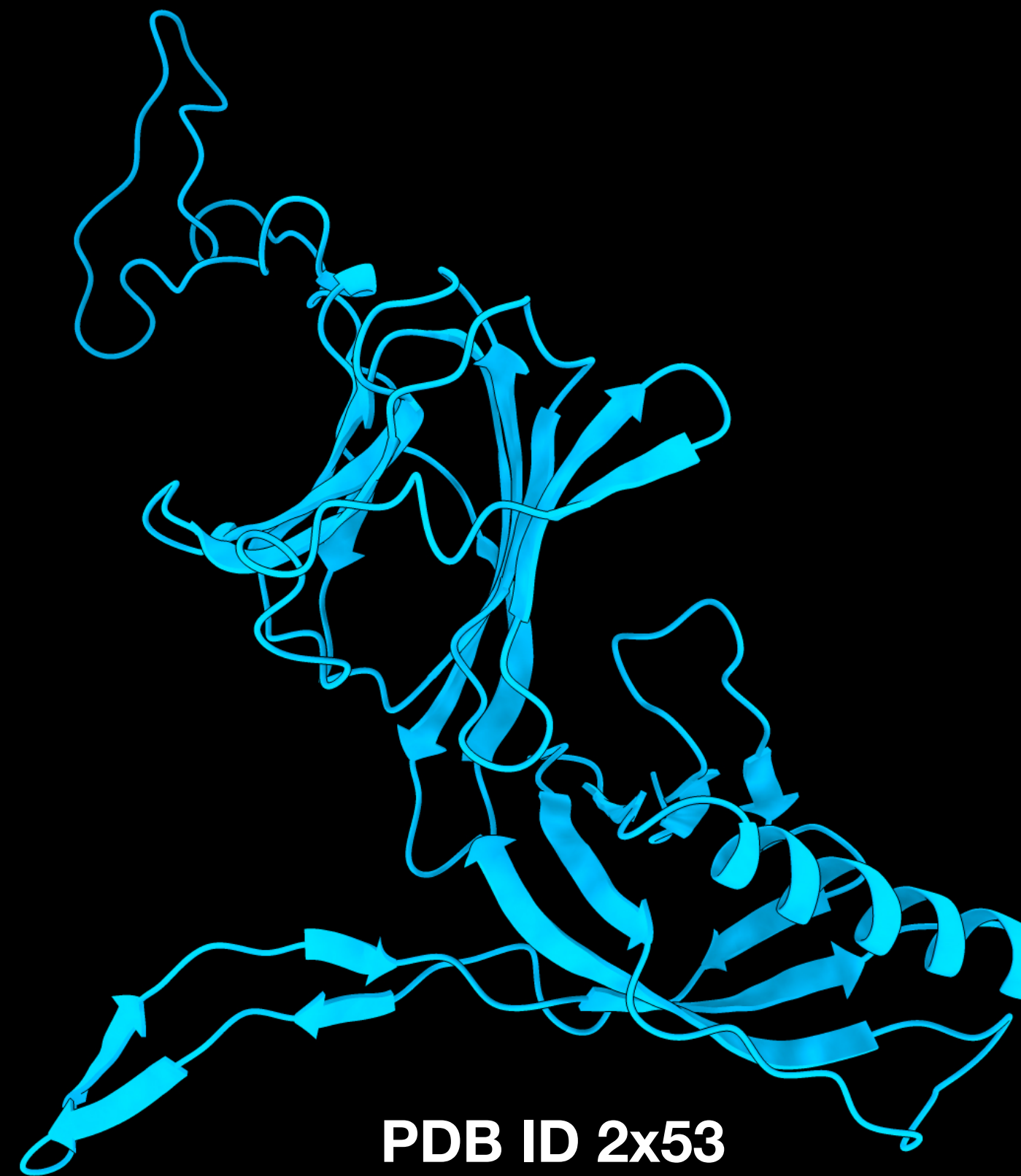




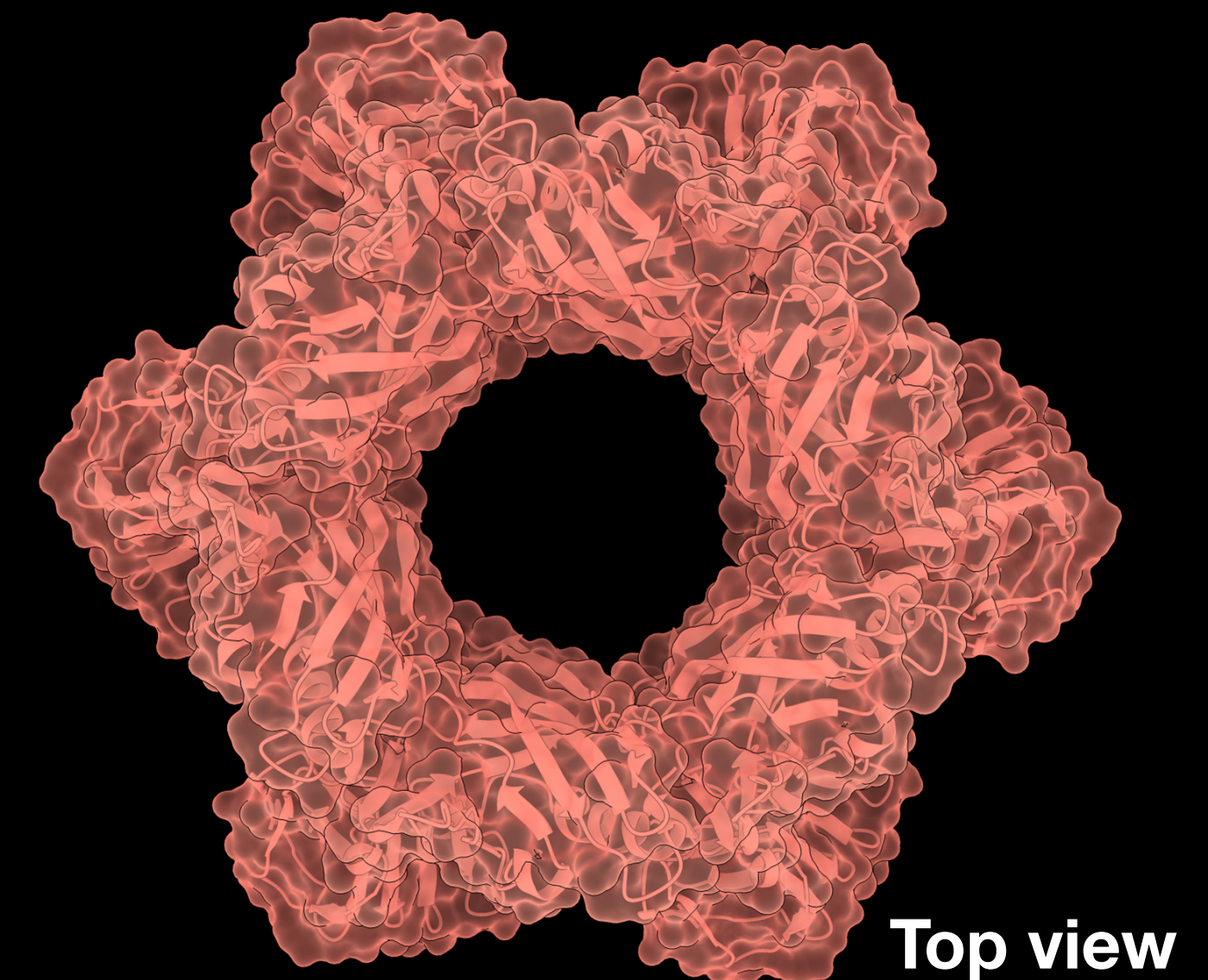
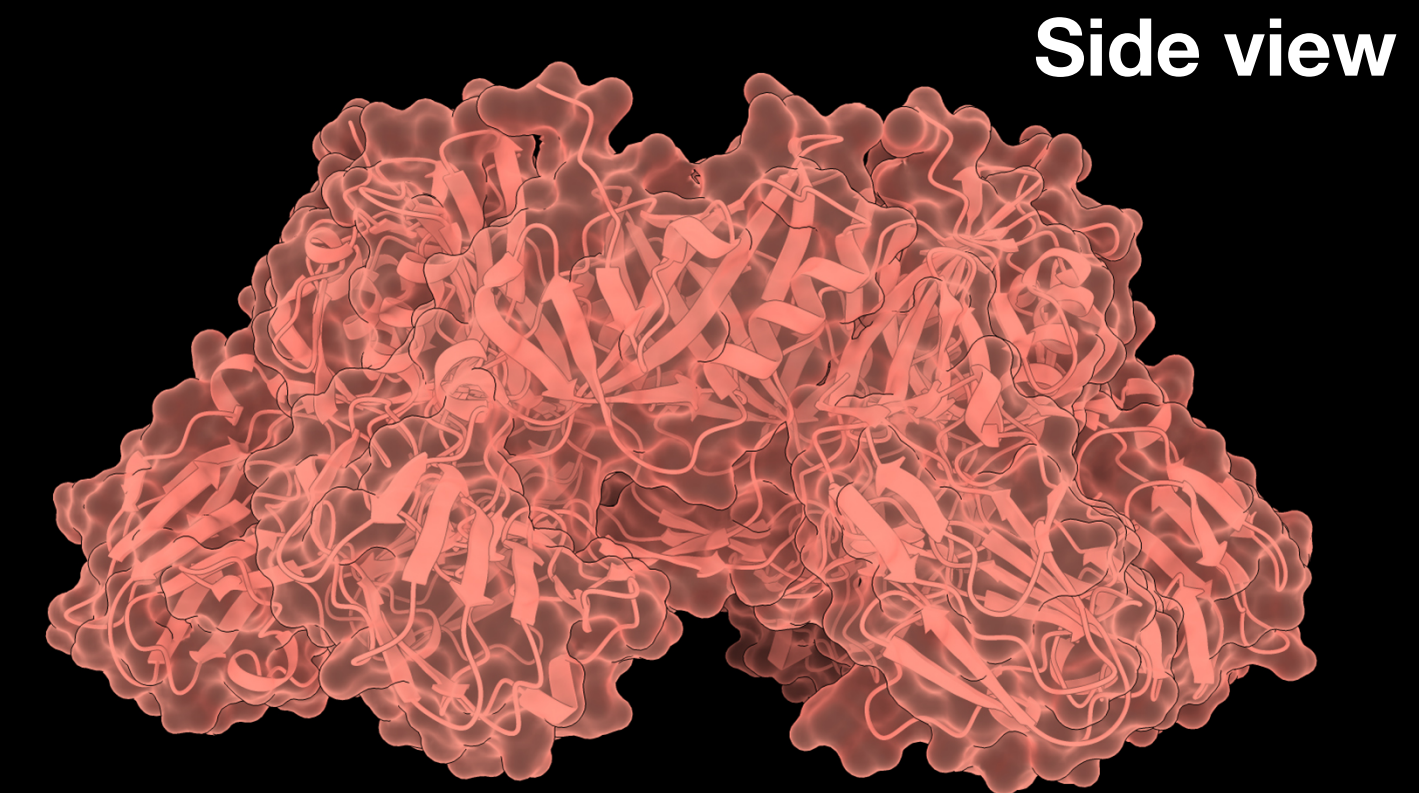
The classical Dit of Vinitor162



Dali Z-score=12.9



PDB ID 2x53
(phage p2)





The evolved Tal of Vinitor162

1

489

CBM_1

structural domain (phage T4 gp27-like)

...





The evolved Tal of Vinitor162

1

489

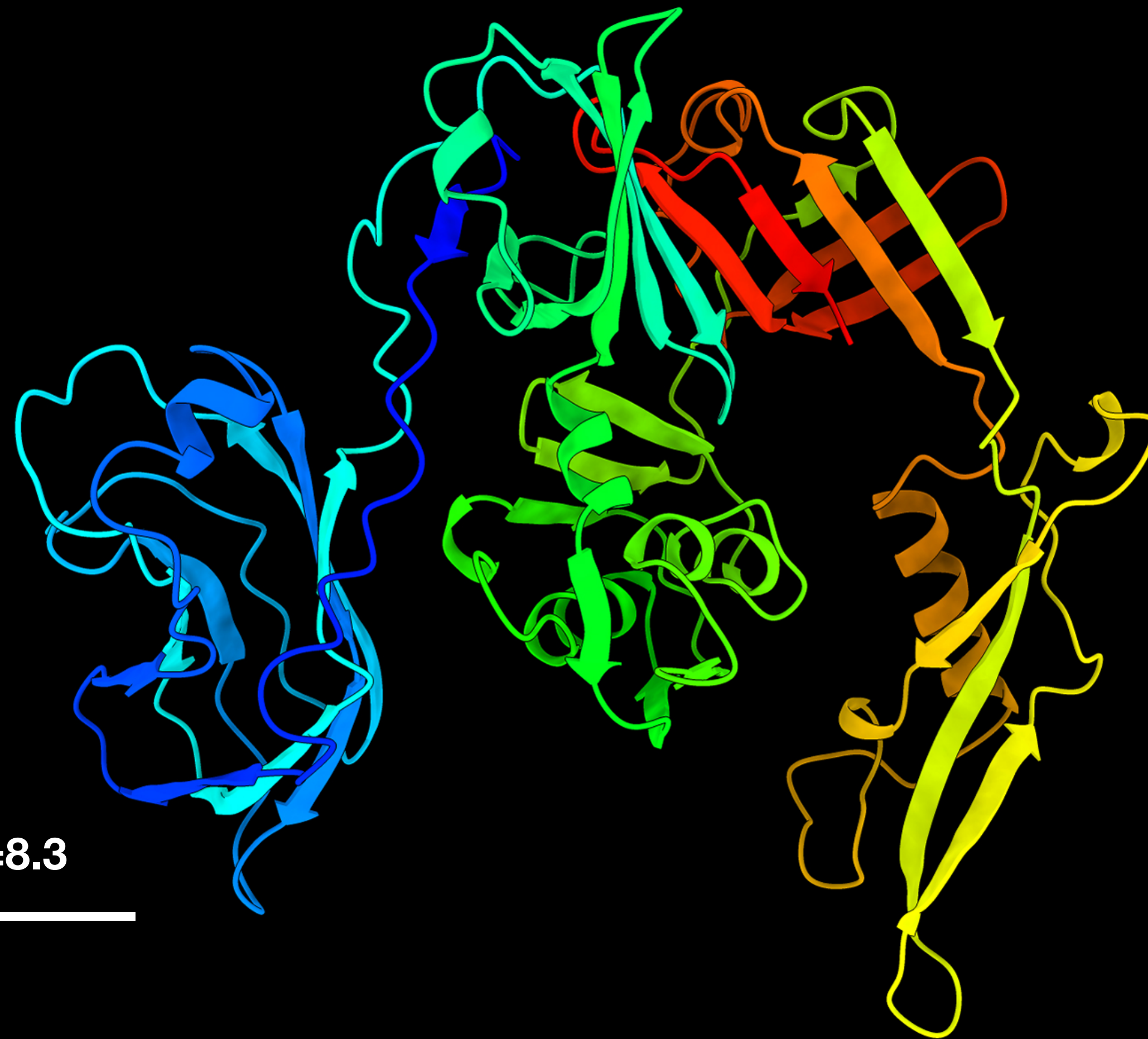
CBM_1

structural domain (phage T4 gp27-like)

...



Dali Z-score=8.3



PDB ID 1us2

(CBM15 @ CAZyDB)



The evolved Tal of Vinitor162

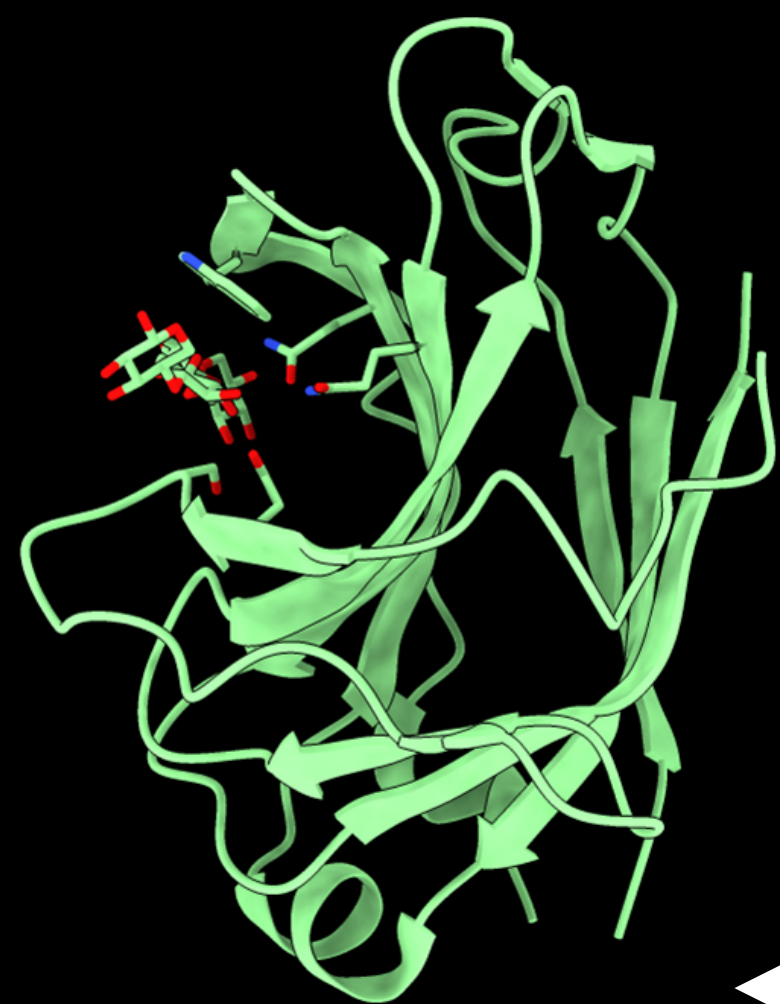
1

489

CBM_1

structural domain (phage T4 gp27-like)

...

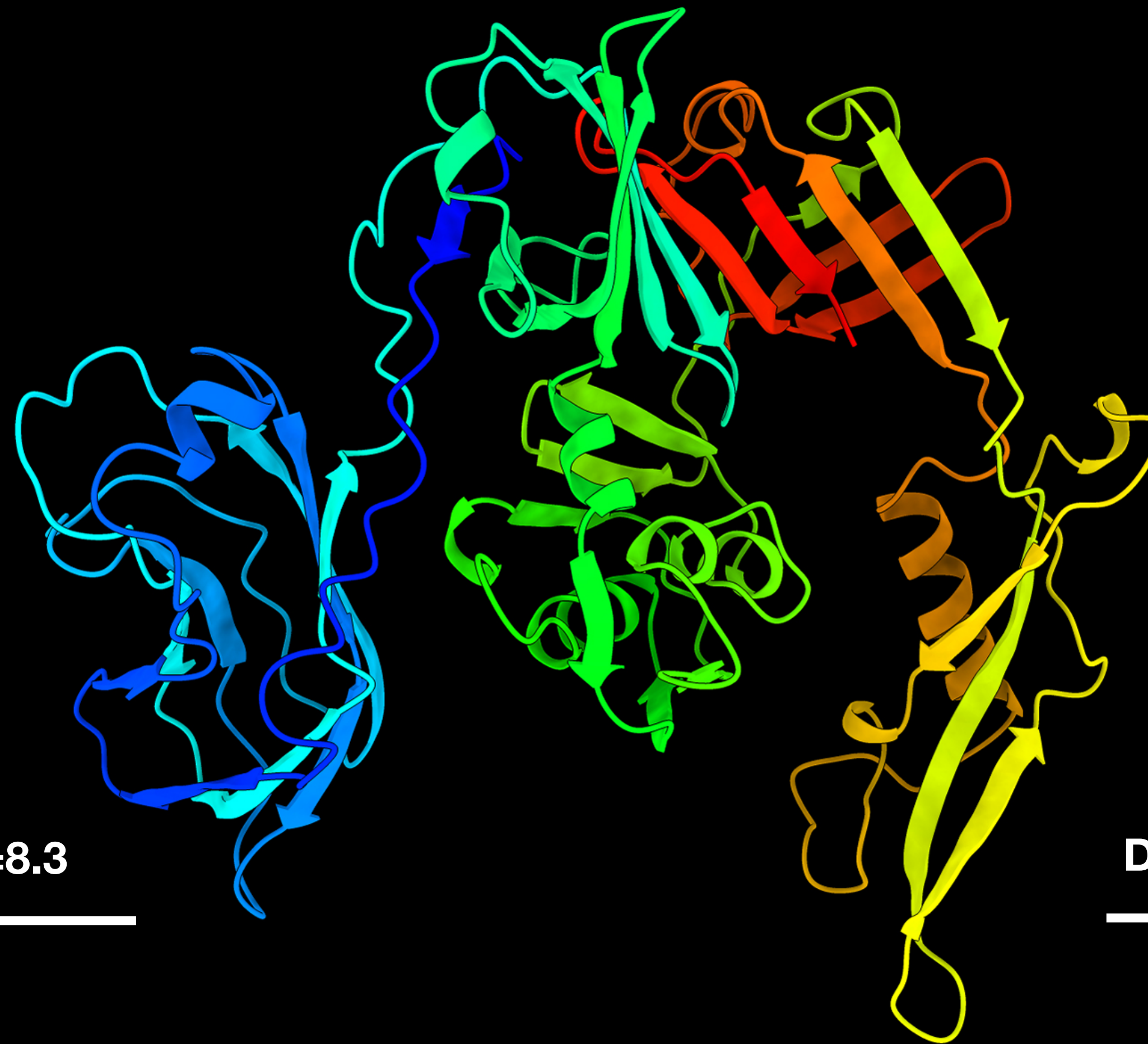


Dali Z-score=8.3



PDB ID 1us2

(CBM15 @ CAZyDB)

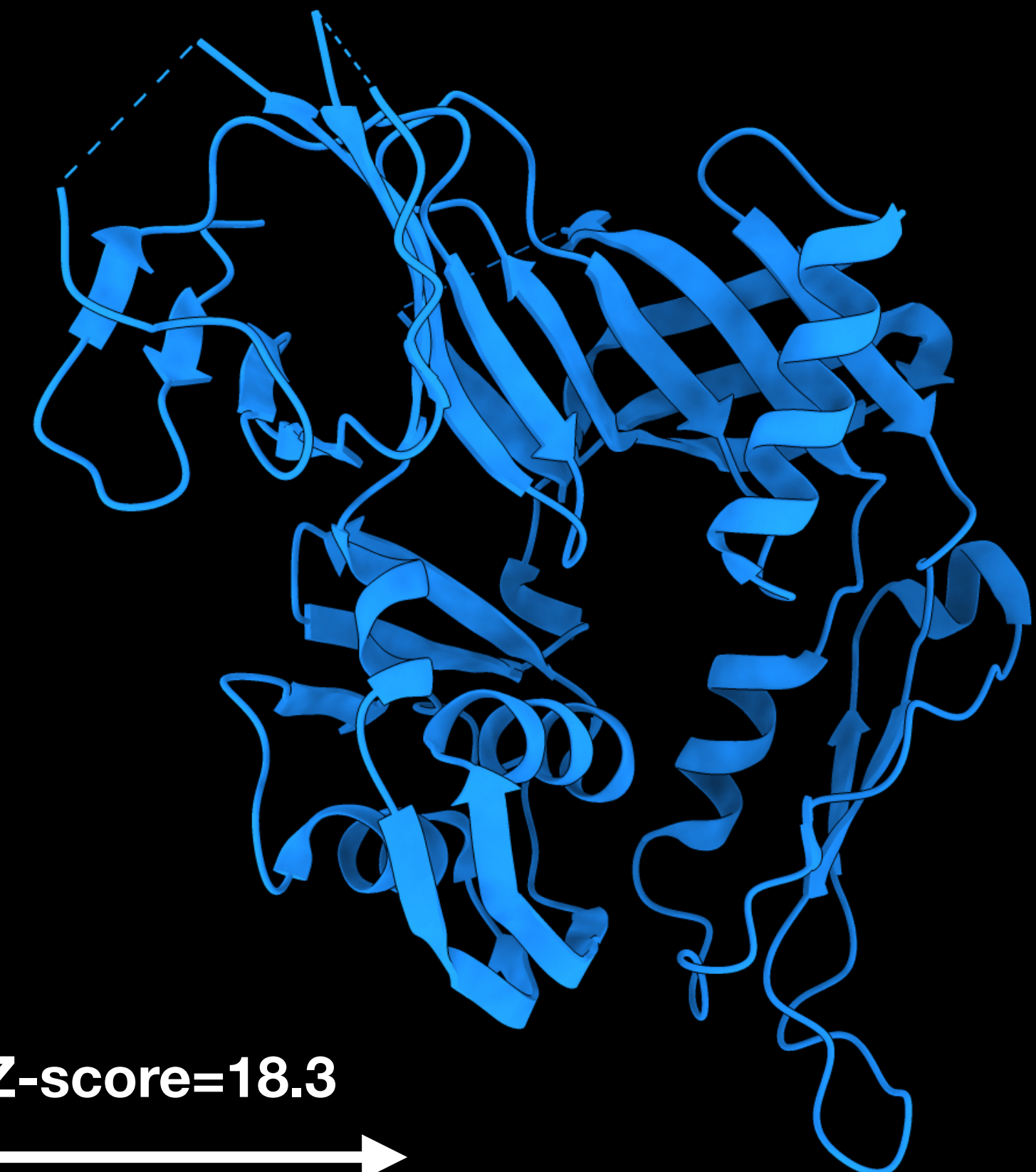


Dali Z-score=18.3



PDB ID 3gs9

(Listerial phage)





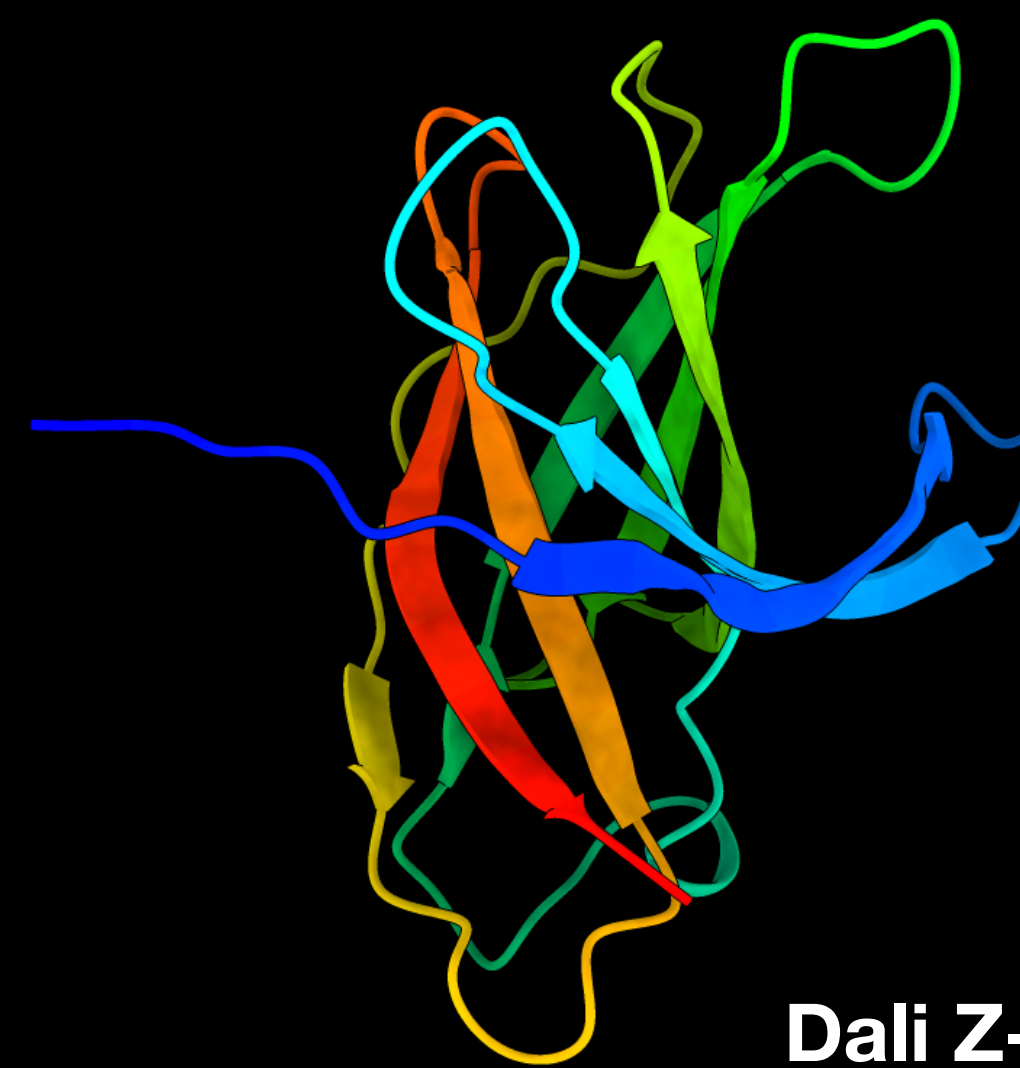
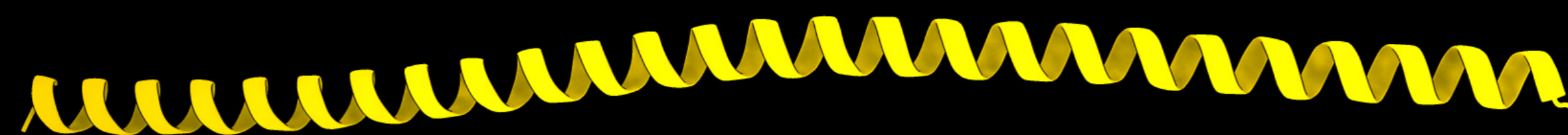
The evolved Tal of Vinitor162

500

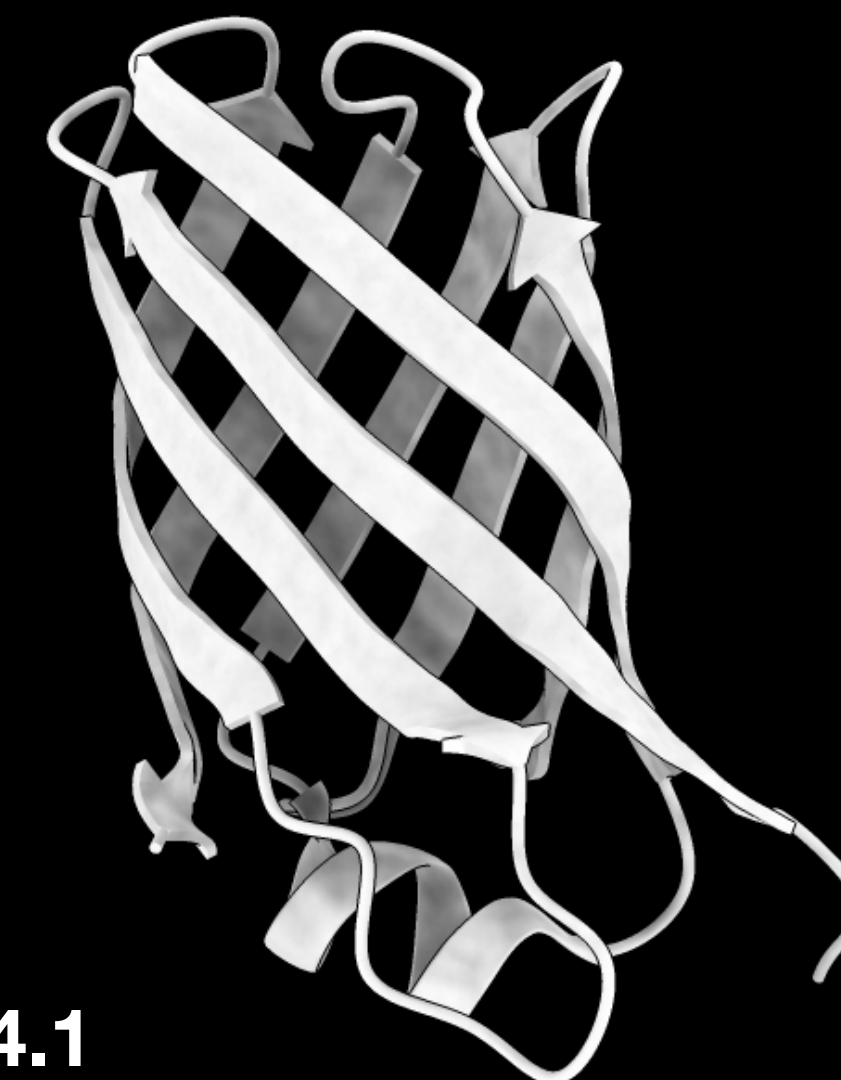
946

α -helix

CBM_2?



Dali Z-score=4.1



PDB ID 4csb
(bacterial β -barrel)



The evolved Tal of Vinitor162

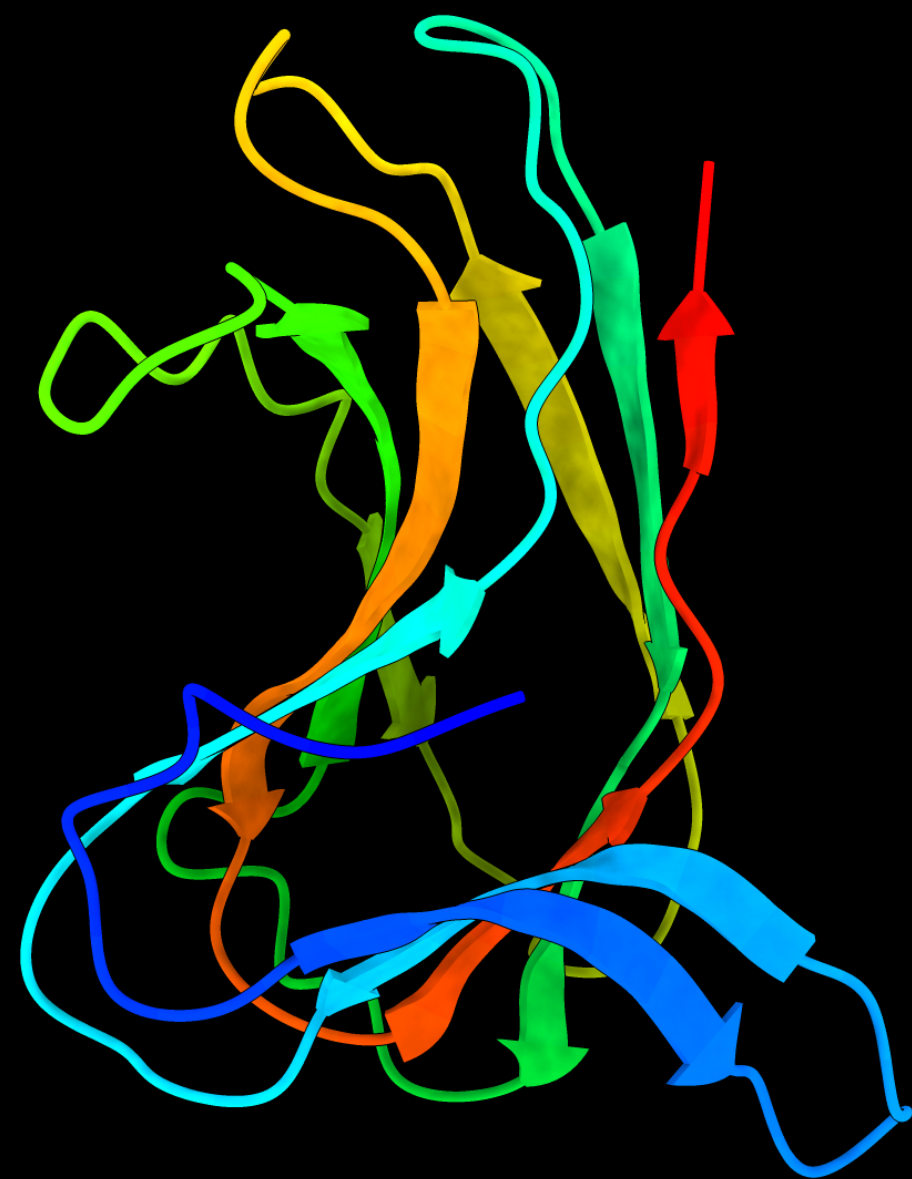
1194

1524

CBM_3

CBM_4

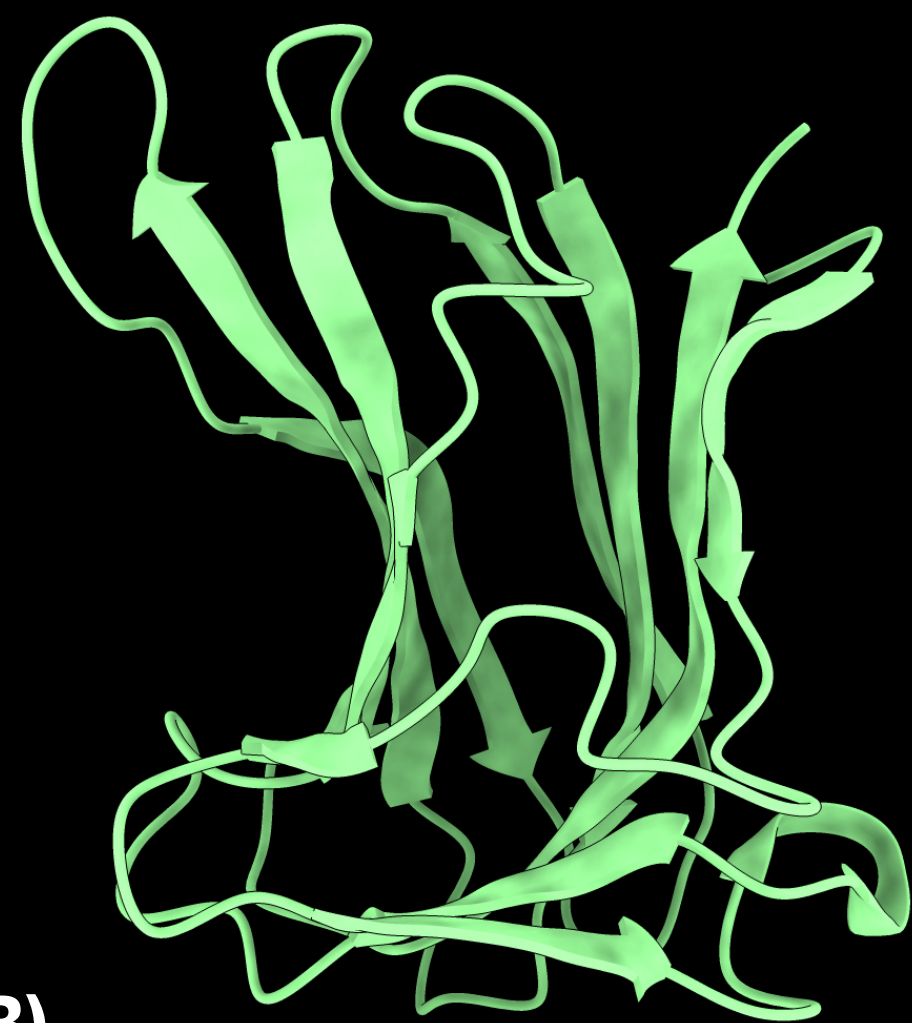
...



Dali Z-score=14.3



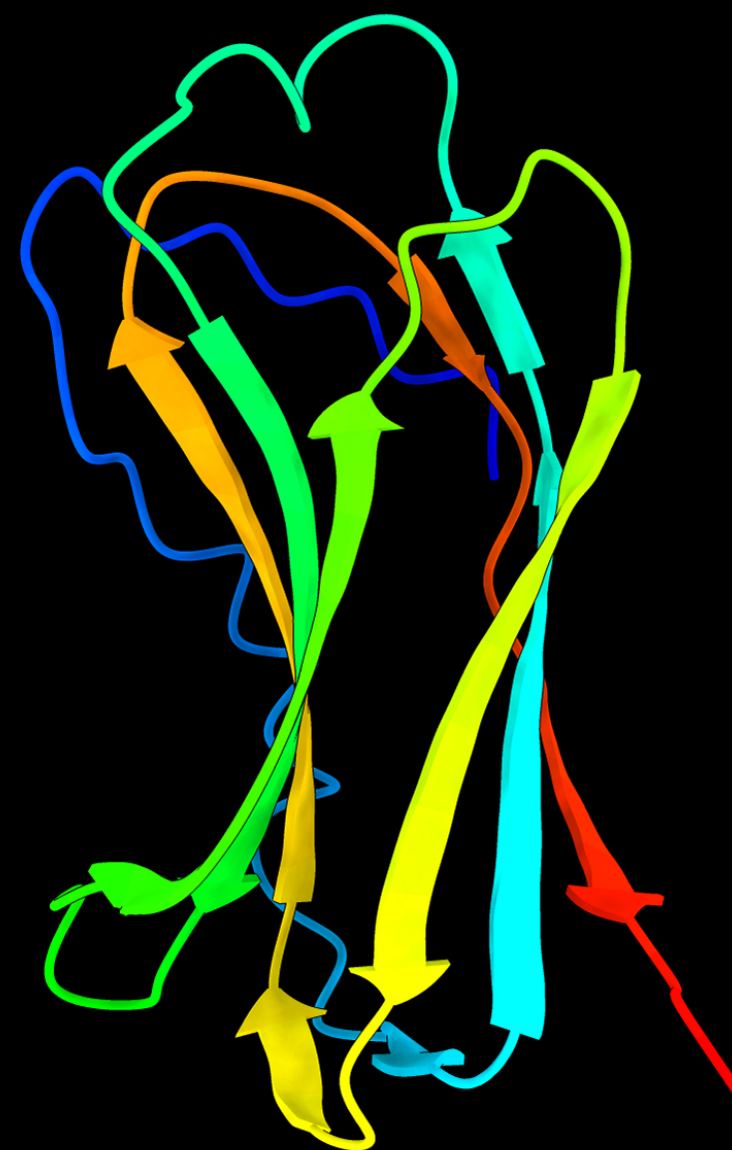
PDB ID 2wze
(CBM22 @ CAZyDB)



Dali Z-score=8.8



PDB ID 4bj0
(CBM4 @ CAZyDB)

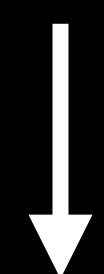
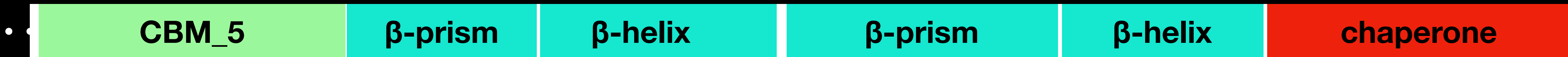




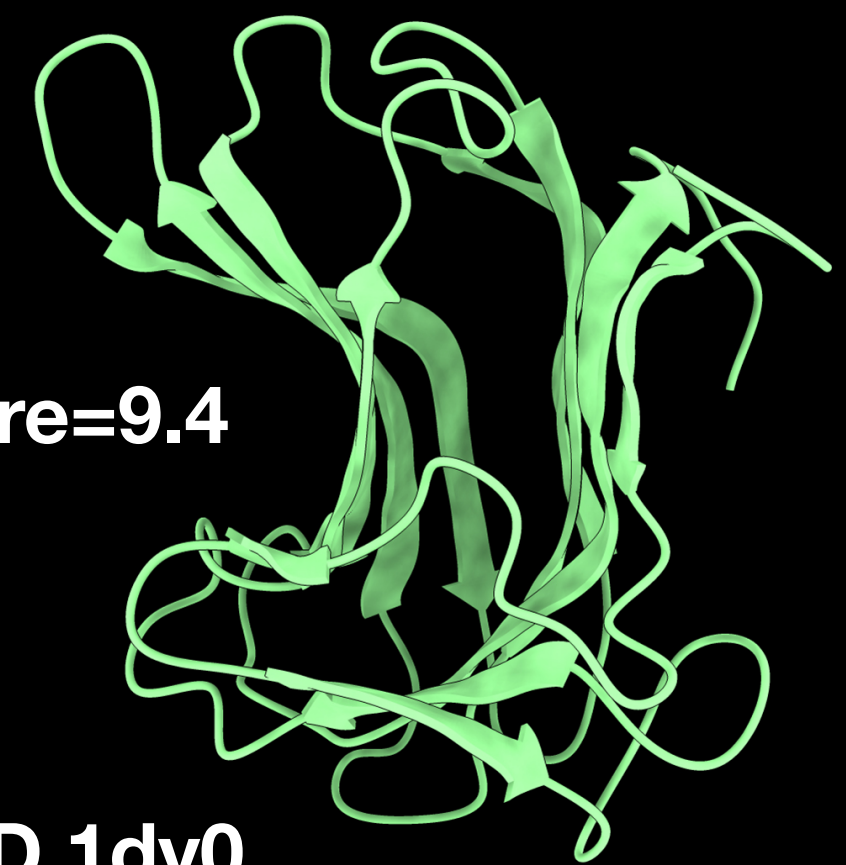
The evolved Tal of Vinitor162

1637

2170



Dali Z-score=9.4



PDB ID 1dy0

(CBM22 @ CAZyDB)



The evolved Tal of Vinitor162

1637

2170

CBM_5

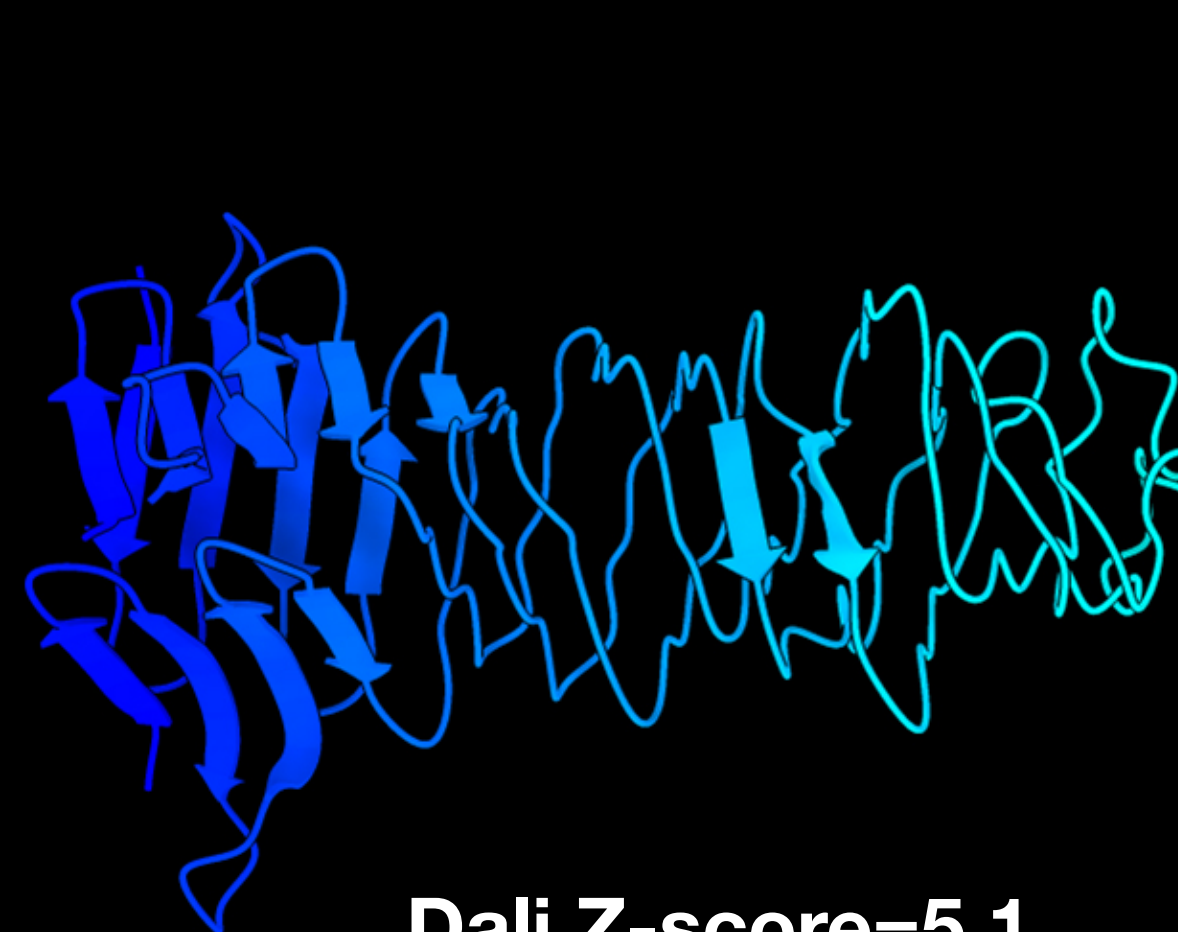
β -prism

β -helix

β -prism

β -helix

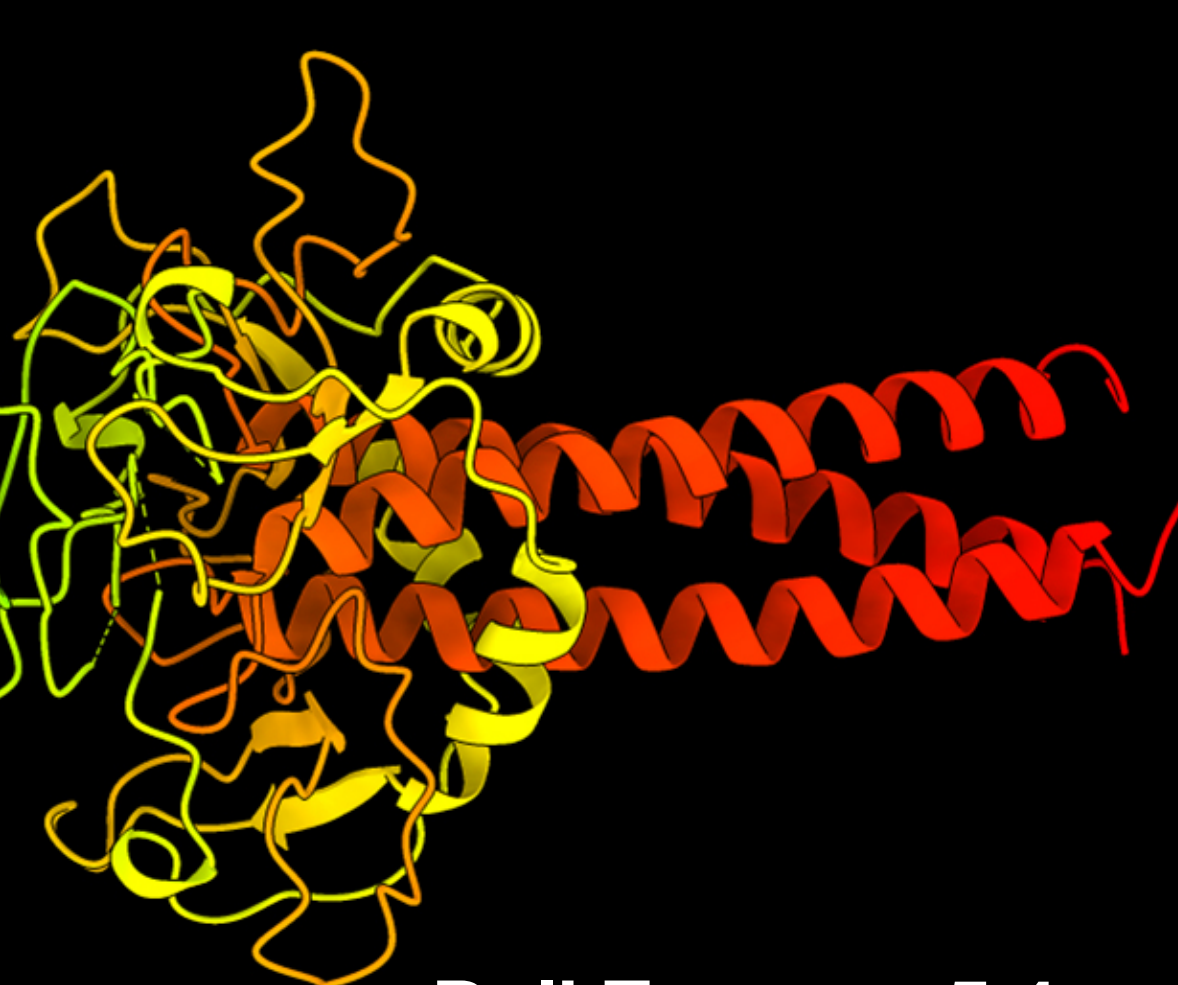
chaperone



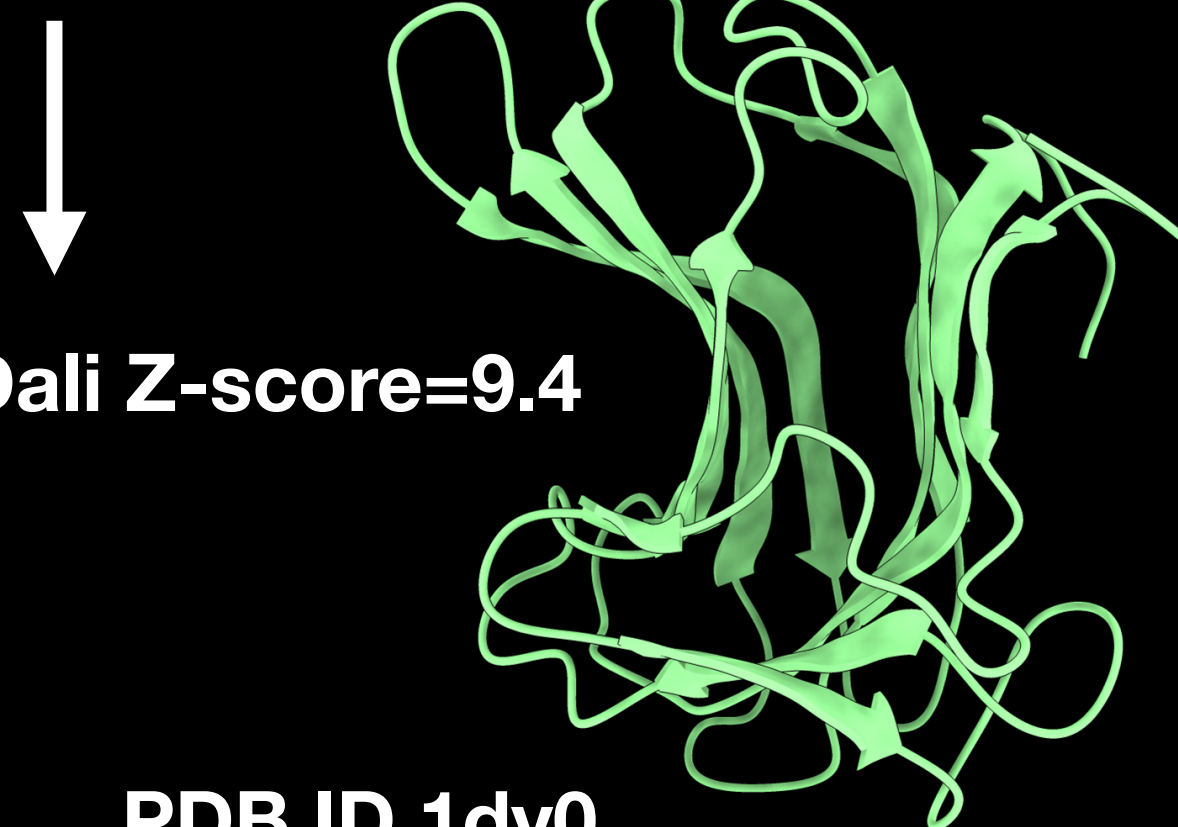
Dali Z-score=5.1



Dali Z-score=5.7



Dali Z-score=5.4

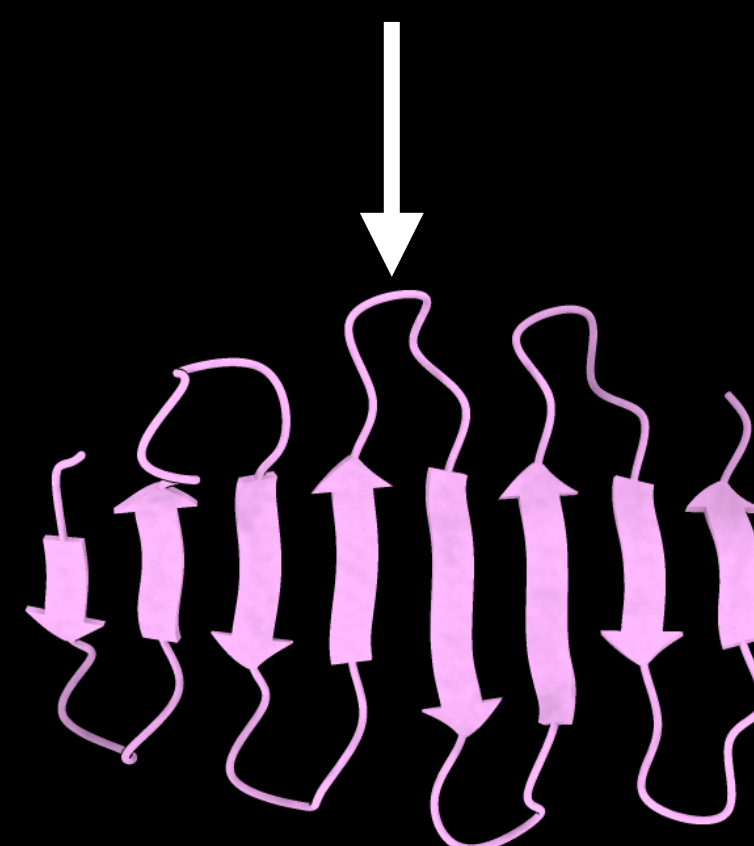


Dali Z-score=9.4

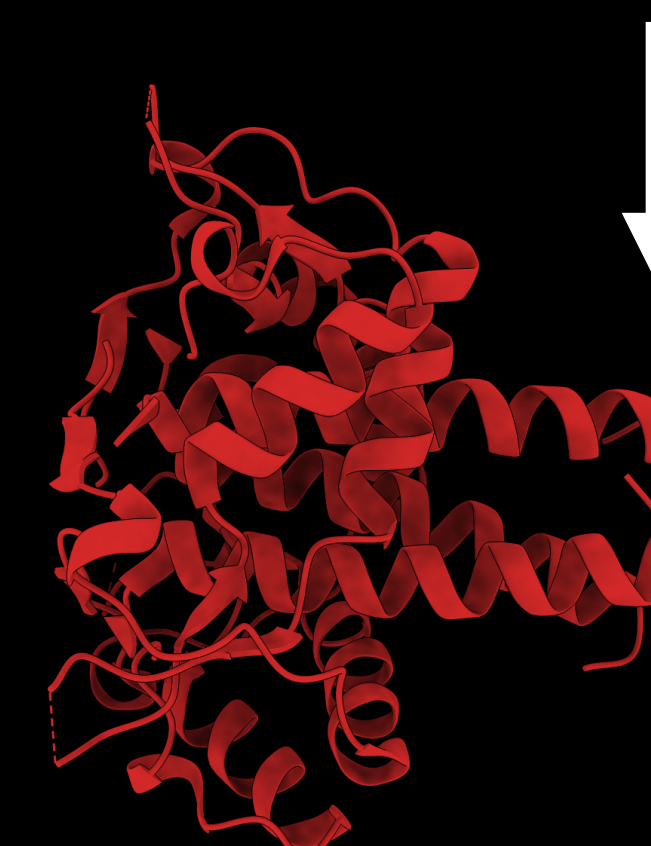
PDB ID 1dy0



PDB ID 4s37



PDB ID 4bxq



PDB ID 4uw8

(CBM22 @ CAZyDB)

(R2 pyocin spike)

(T5 tail fiber chaperone)



An elongated, CBM-rich host-binding machinery

Capsid

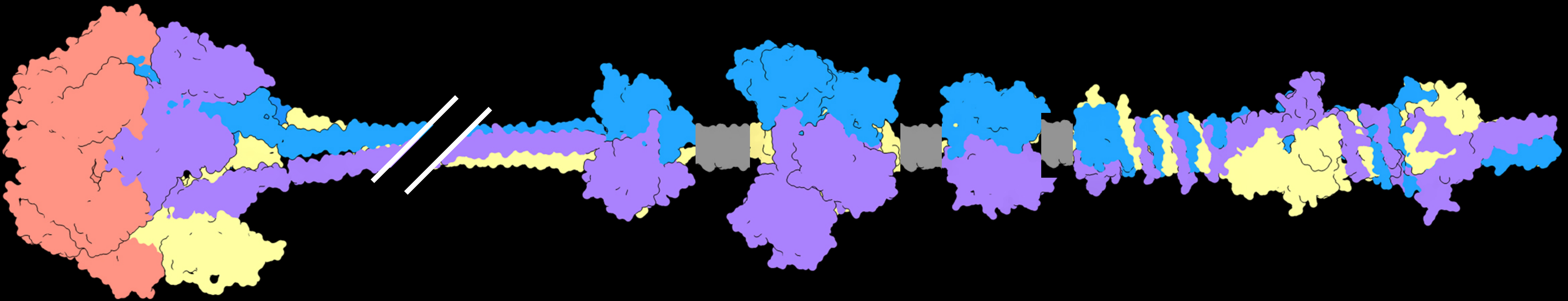


Host



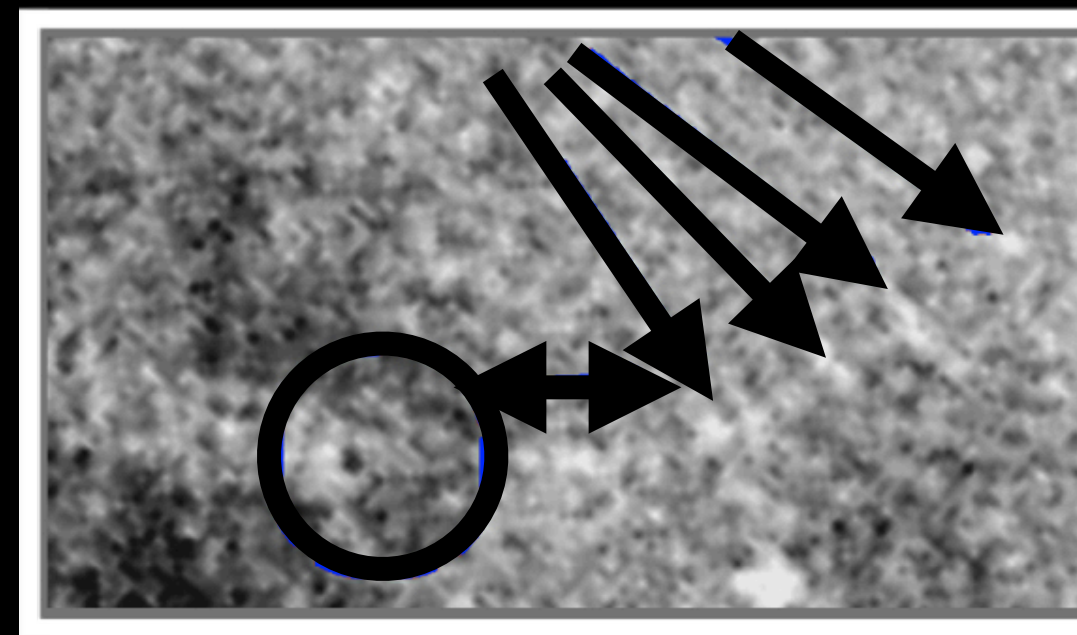
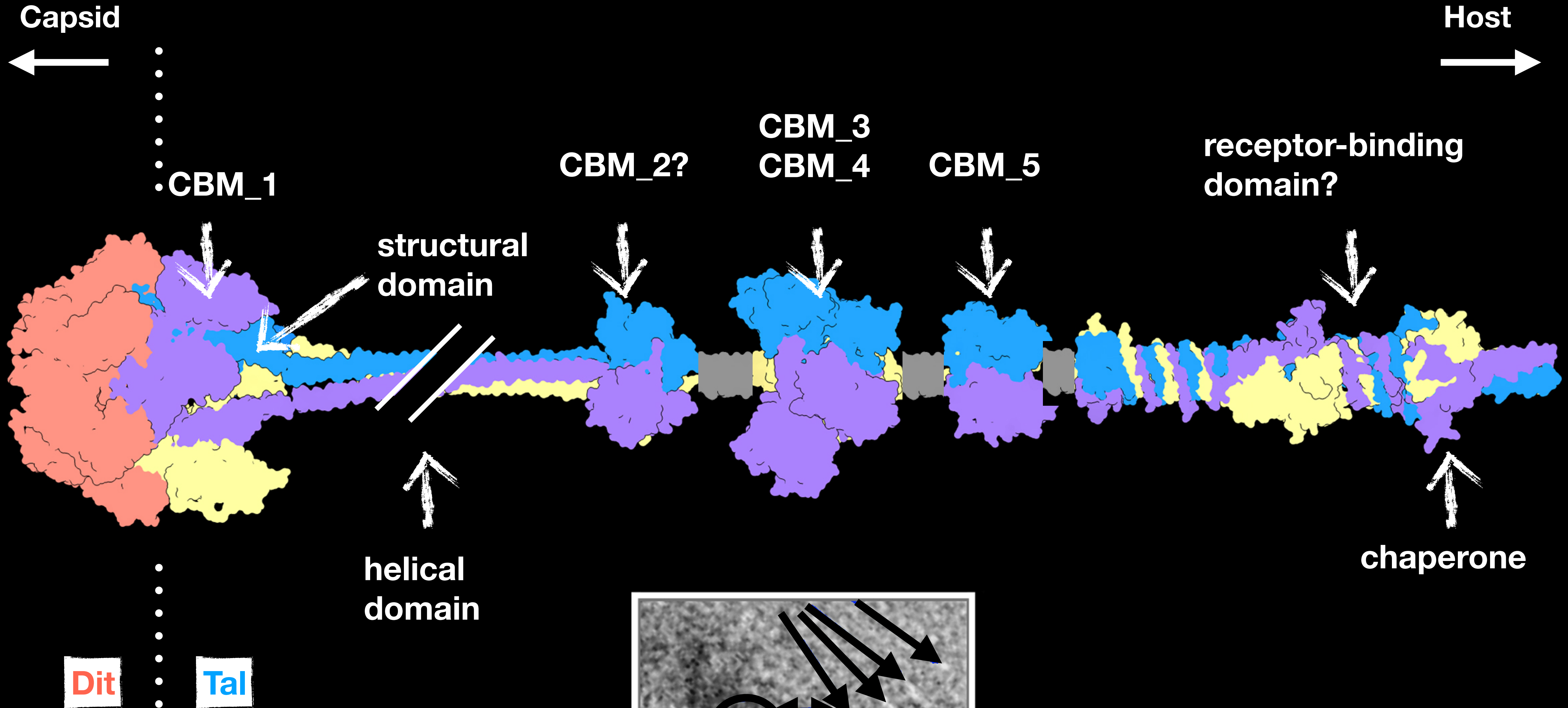
Dit

Tal





An elongated, CBM-rich host-binding machinery



Philippe et al. Frontiers in Microbiology, 2021



An elongated, CBM-rich host-binding machinery

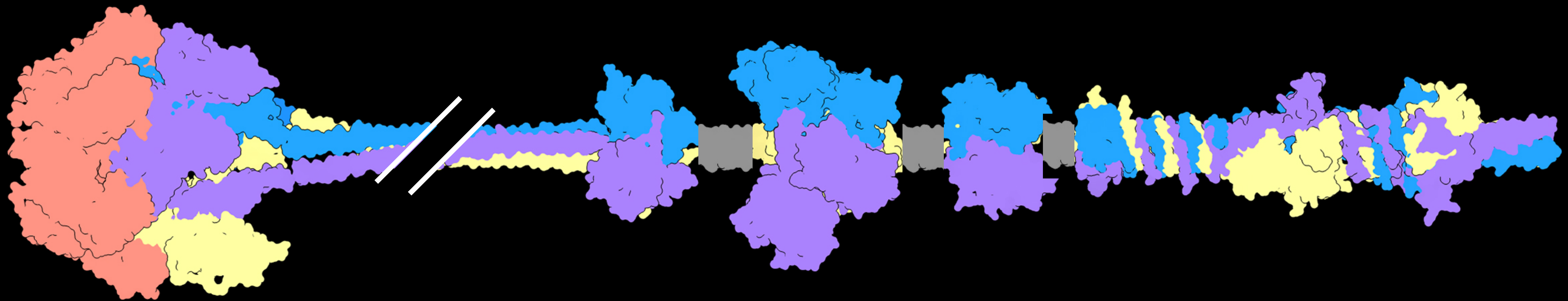
Capsid



Host



CBMs may induce a multi-step host-binding process



CBM_1



Host scanning,
reversible binding



An elongated, CBM-rich host-binding machinery

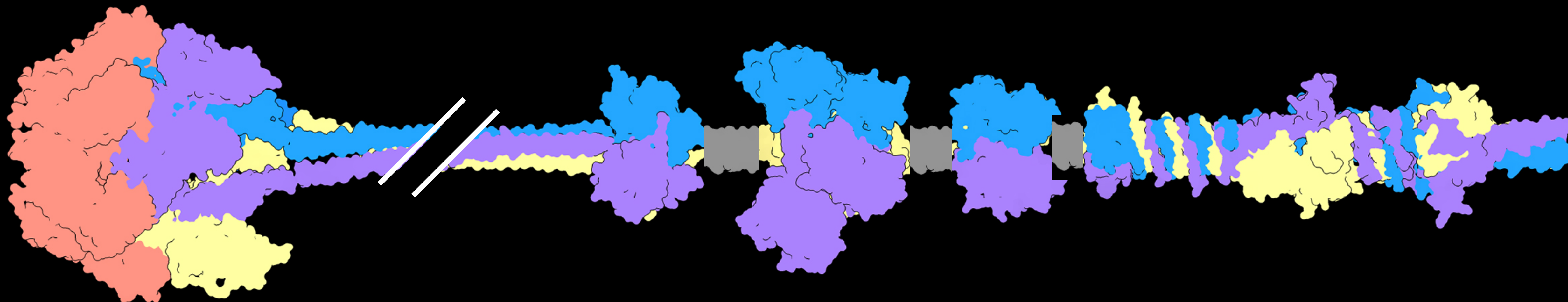
Capsid



Host



CBMs may induce a multi-step host-binding process



CBM_1

CBM_2?

CBM_3/4

CBM_5

1

Host scanning,
reversible binding

2

Specific & stronger host
binding of a CBM cluster



An elongated, CBM-rich host-binding machinery

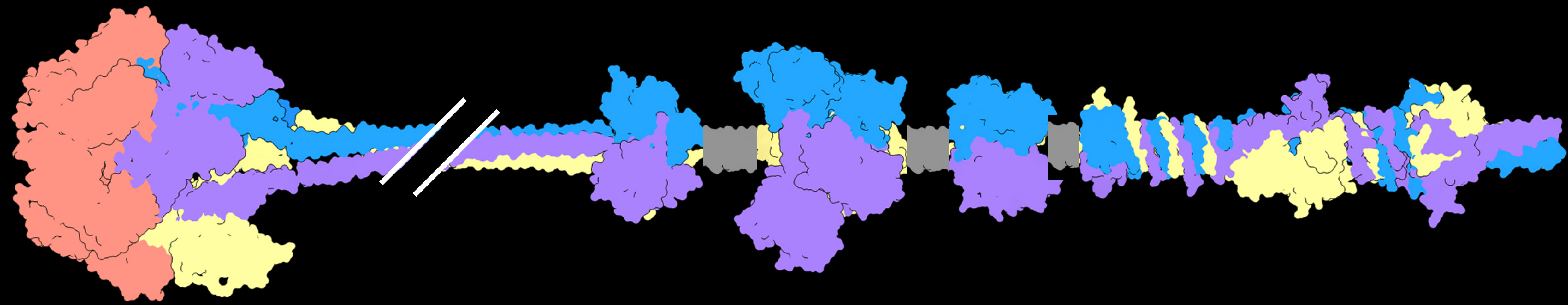
Capsid



Host



CBMs may induce a multi-step host-binding process



CBM_1

CBM_2?

CBM_3/4

CBM_5

receptor-binding domain?

1

Host scanning, reversible binding

2

Specific & stronger host binding of a CBM cluster

3

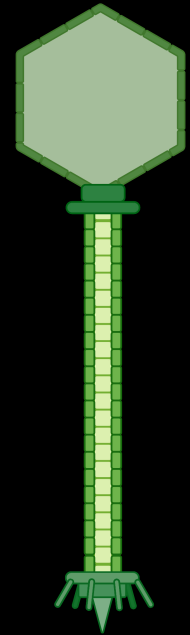
Host binding deeper in the host cell wall



- **The structural repertoire of LAB phages' host-binding machineries keeps expanding**
- **AlphaFold2 is powerful to predict accurate 3D structures. Try it for you projects!**

Acknowledgments

Phage team



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Researcher



**Olivier
Fuchsbauer**
Engineer



**Laura
Schmitt**
Engineer



HPI head: Alain Roussel

