"Why use SAS in my project?"

EMBO SAXS/SANS course

Grenoble 2022

EMBO

excellence in life sciences

María J. Marcaida López
Laboratory for Biomolecular Modeling
Institute of Bioengineering
School of Life Sciences

LBM

EPFL

Education

Imperial College of London - Physics (1997-2001)
University of Cambridge - PhD in Biochemistry (2001-2006)

Imperial College London

UNIVERSITY OF CAMBRIDGE

Experimental structural biology

Prof. Silvia Onesti

Prof. Ben Luisi

Professional experience

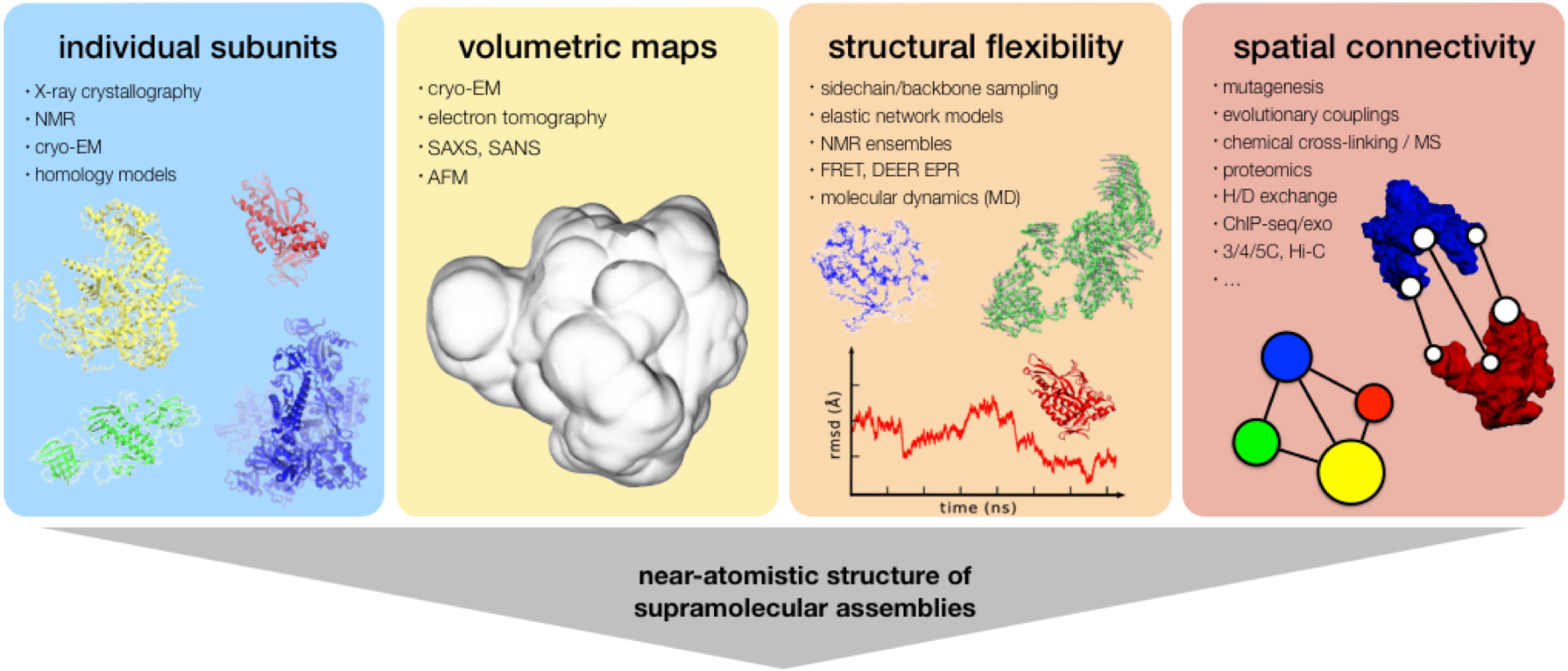
Postdoc at the Spanish National Cancer Research Center (2006-2010)
Postdoc at the Dept. Chemistry and Biochemistry Bern (2011-2014)

cnio CENTRO NACIONAL DE INVESTIGACIONES ONCOLÓGICAS

UNIVERSITÄT BERN

EPFL

Integrative modeling at LBM

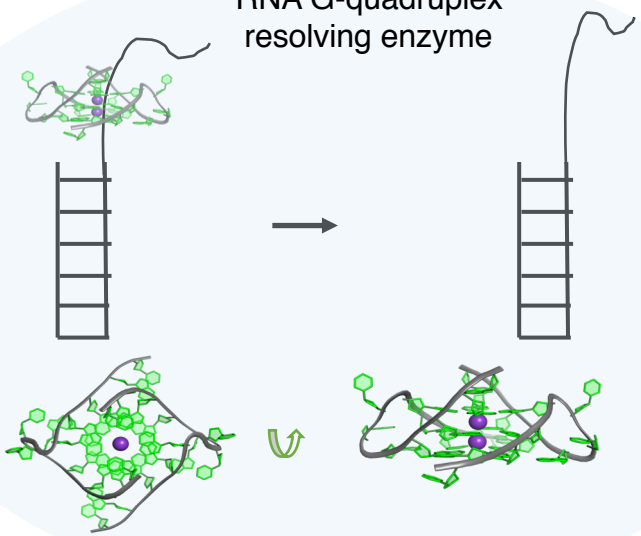


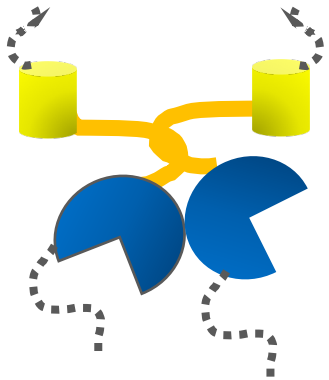
Projects where SAXS is/was used

- Structural biology projects dealing with flexible proteins
  - The human transcription regulator KAP1 (Fonti et al., Life Sci Alliance (2019))
  - **The human RNA helicase DDX21** (Marcaida et al., iScience (2020))

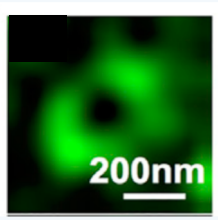
- CryoEM and SAXS
  - The human Huntingtin protein (Jung et al., Structure **28,** 1035-1050 (2020))
  - **The mosquito Toll5a and its ligand Spätzle** (M. Gangloff, Nat. Com. under review)

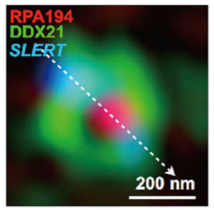
The human RNA helicase DDX21



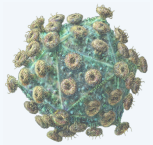


RNA Pol I & II
regulation

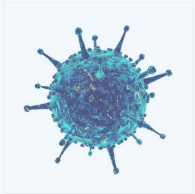




Ribosomal RNA
biogenesis

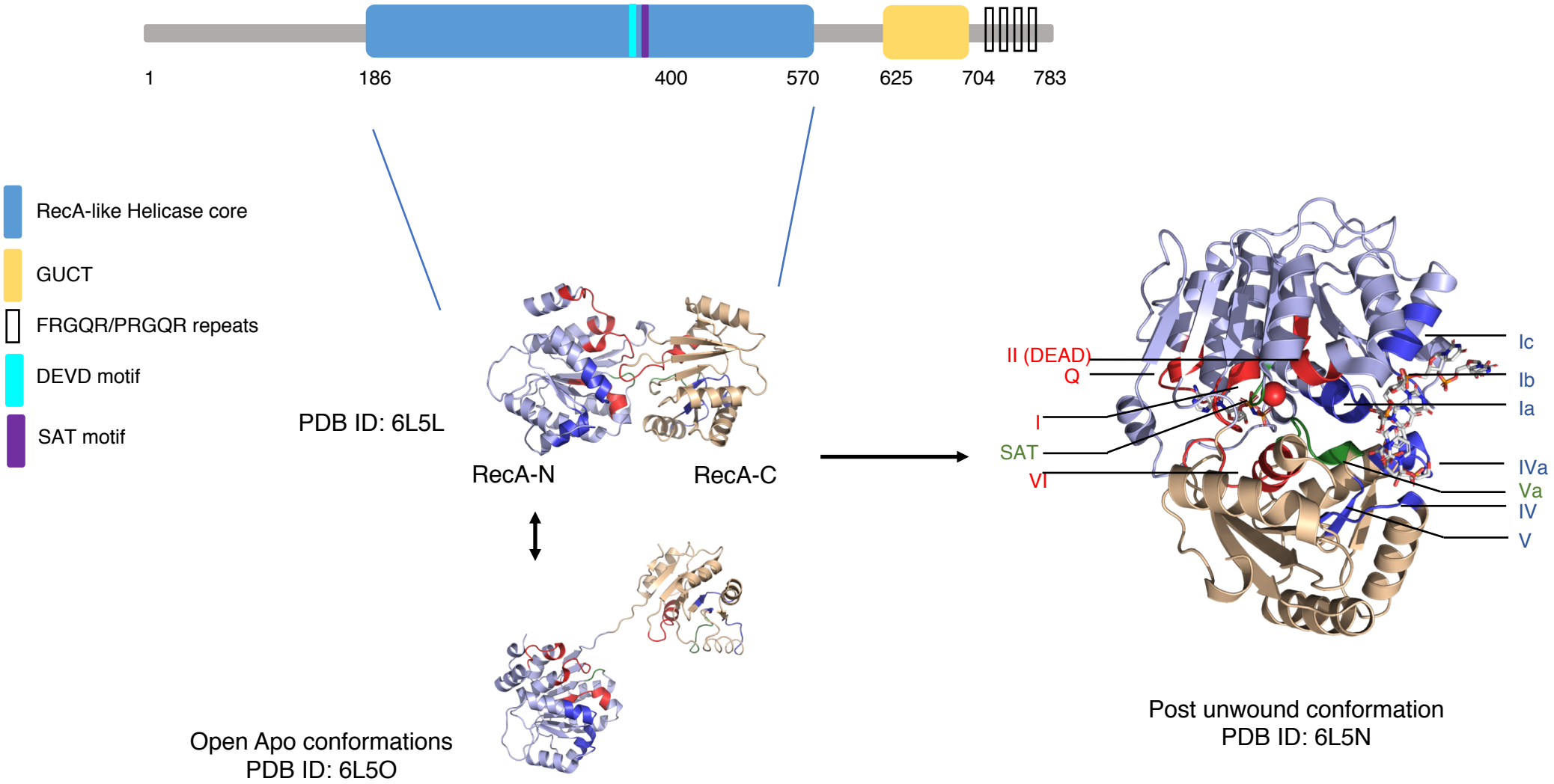


HIV, influenza and dengue virus
pathogenesis

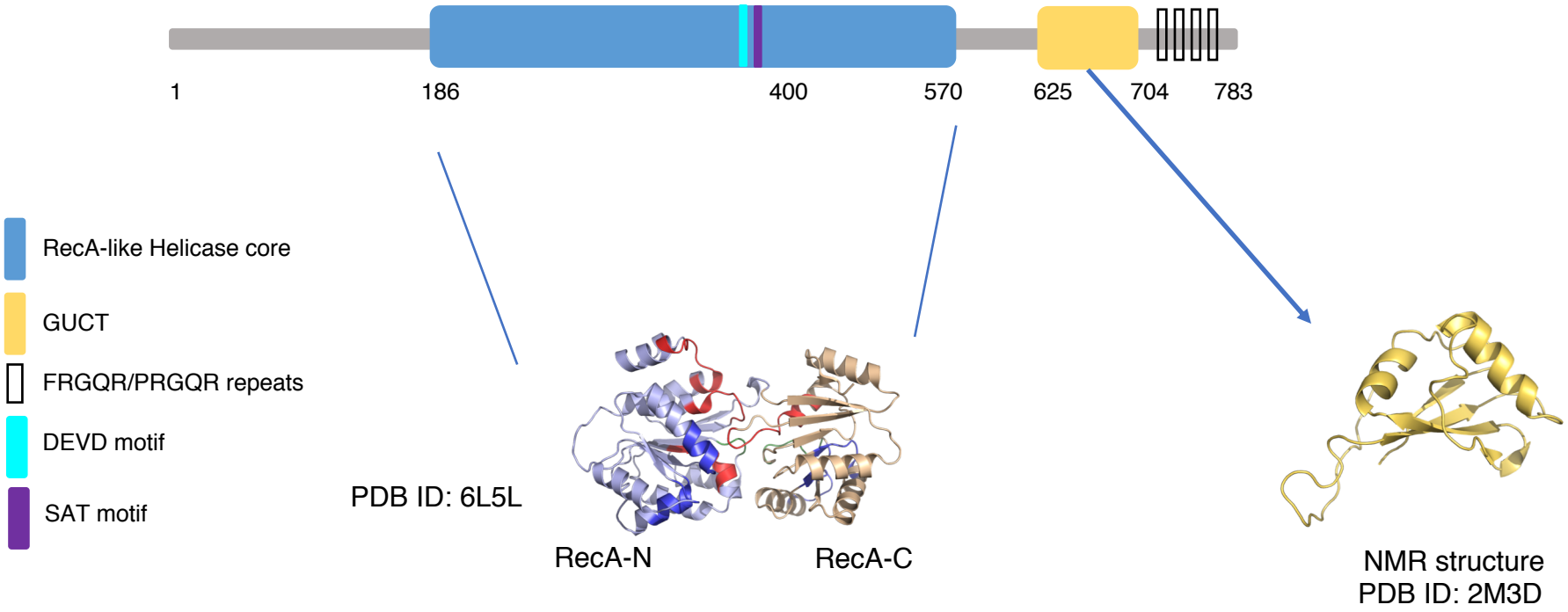


Sloan et al 2015, Xing et al 2017, Hammond et al 2018

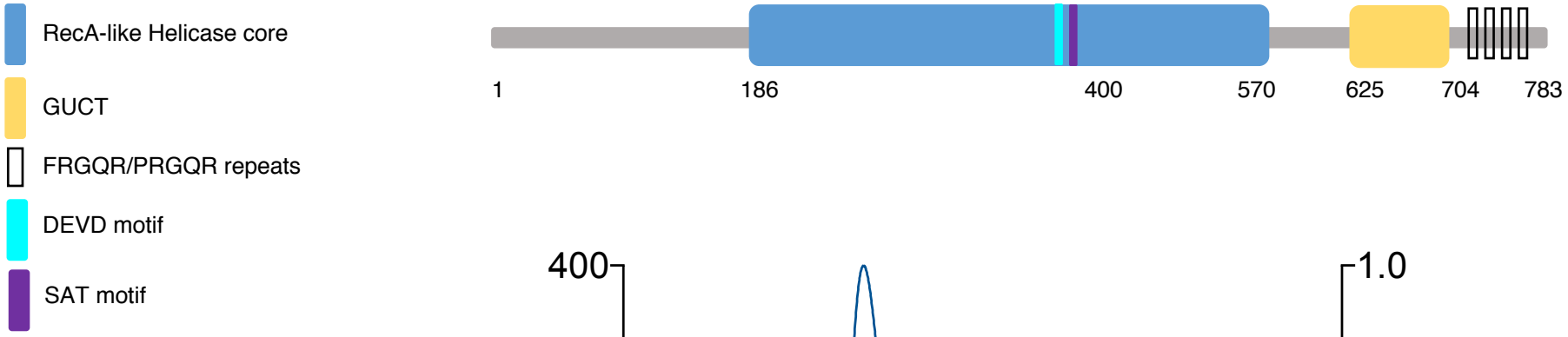
DDX21 is a DEAD box RNA helicase



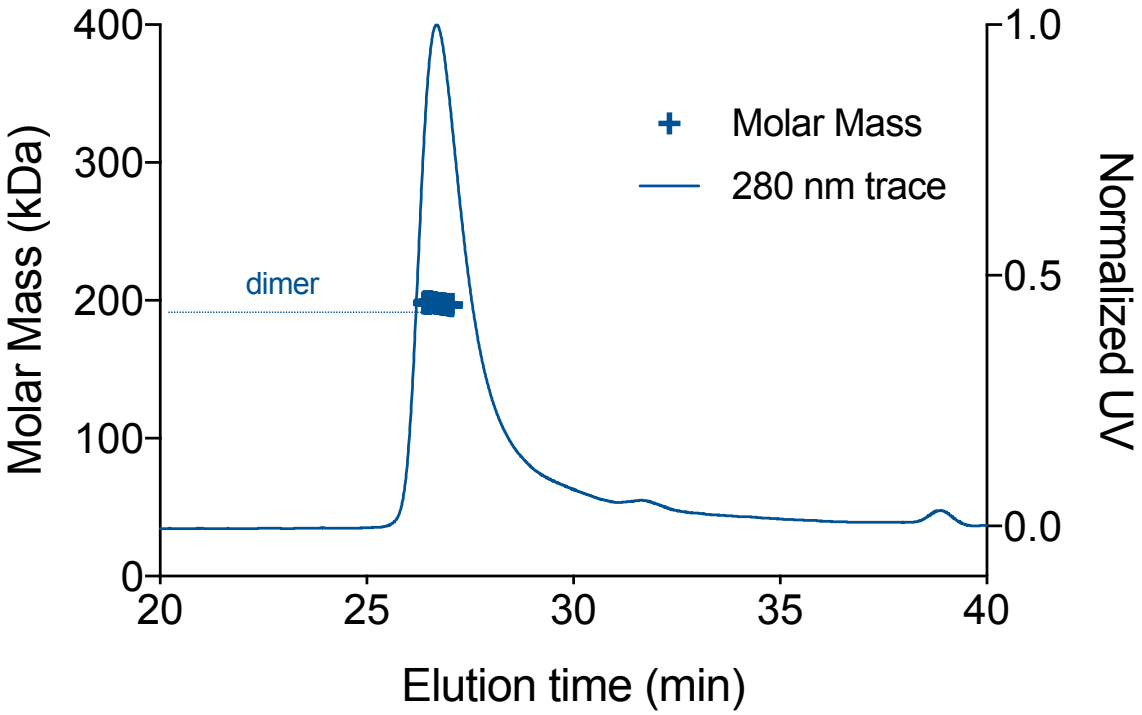
DDX21 has a C-terminal GUCT domain



DDX21 is a ‘well behaved’ dimer in solution



Theoretical MW = 100 kDa



MALS (Multi Angle Light Scattering)

Determines:

- -MM (shape independent)
- -Oligomerization state
- -Stoichiometry (complexes)

Also used **AUC** (analytical ultra centrifugation) and **crosslinking**

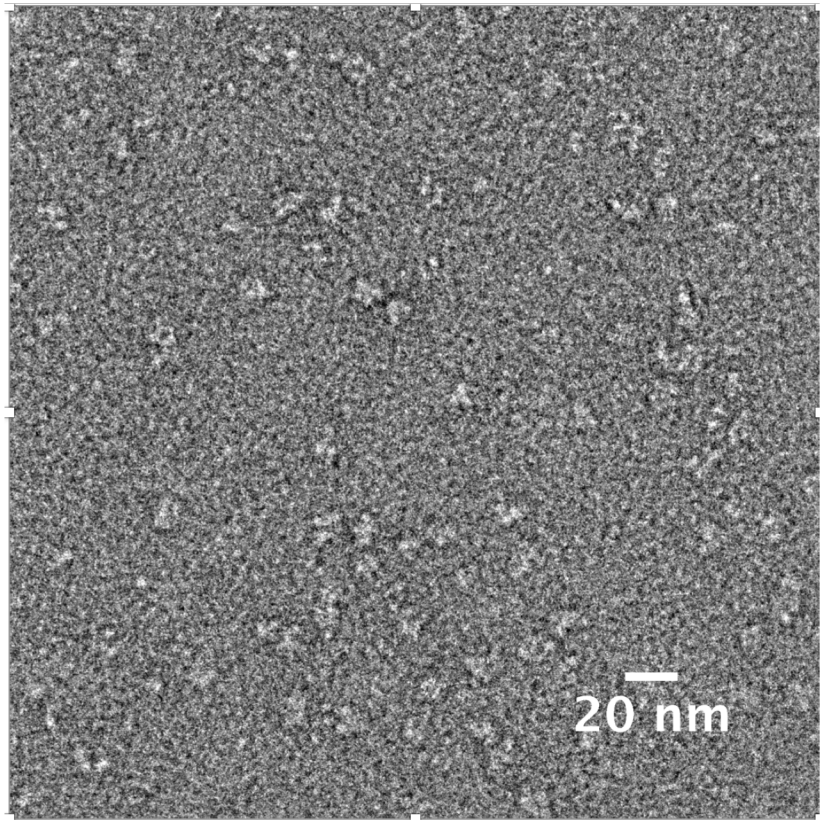
DDX21 is a ‘well behaved’ dimer in solution

BUT…

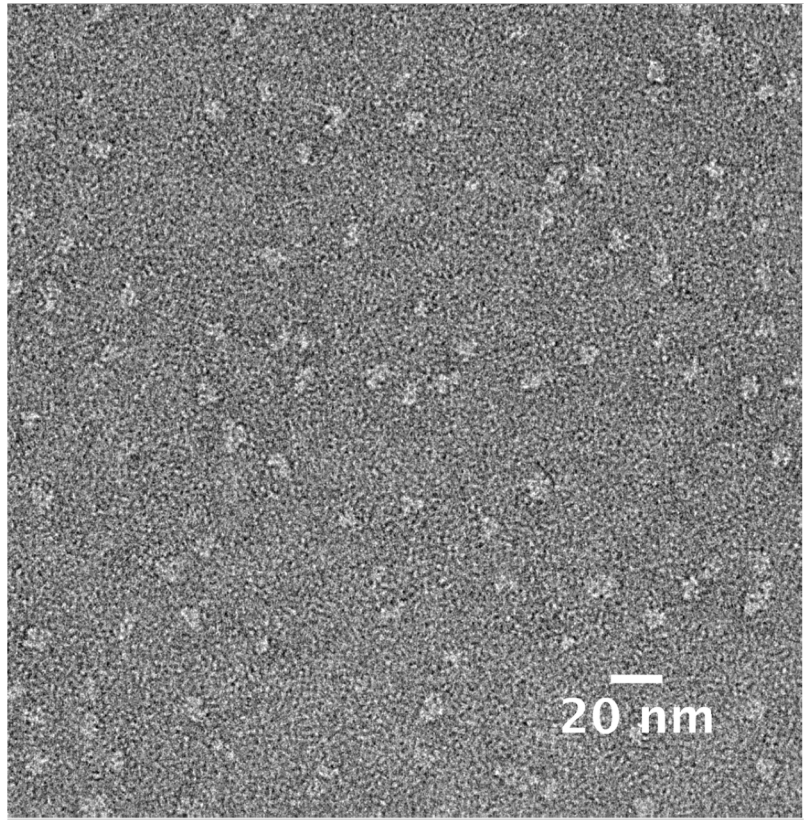
It does not crystallize

It has conformational heterogeneity by cryoEM

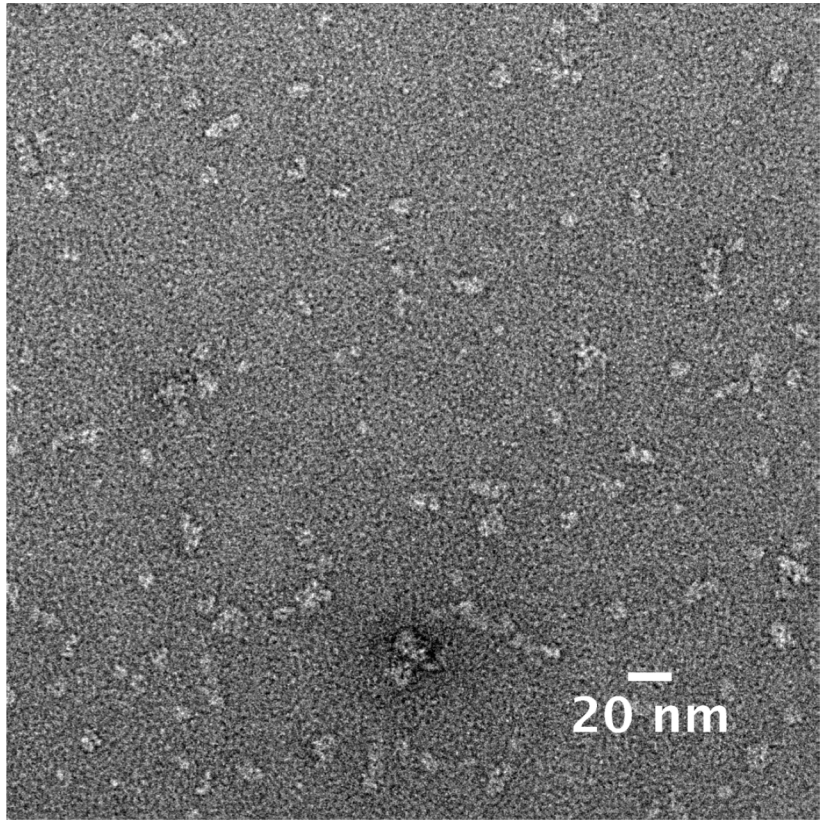
Negative stain EM on DDX21 variants



FL 0.1% GA



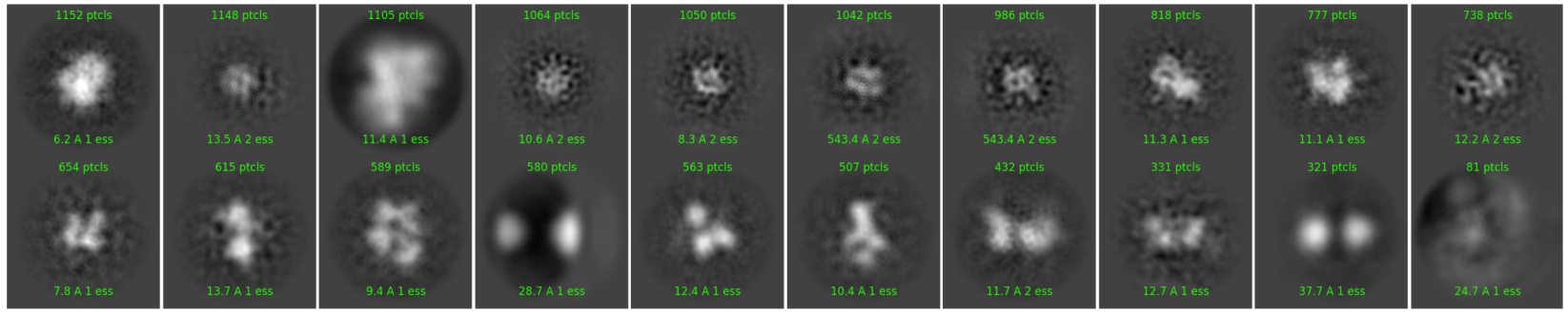
DeltaN 0.1% GA

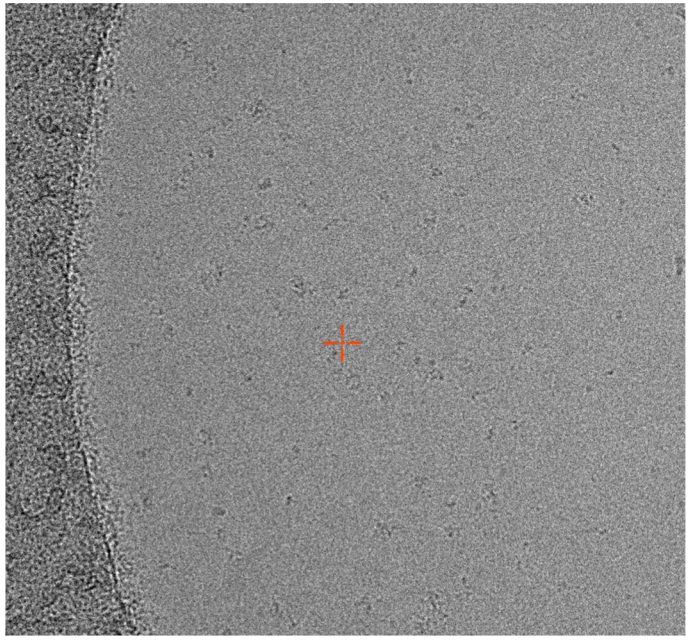


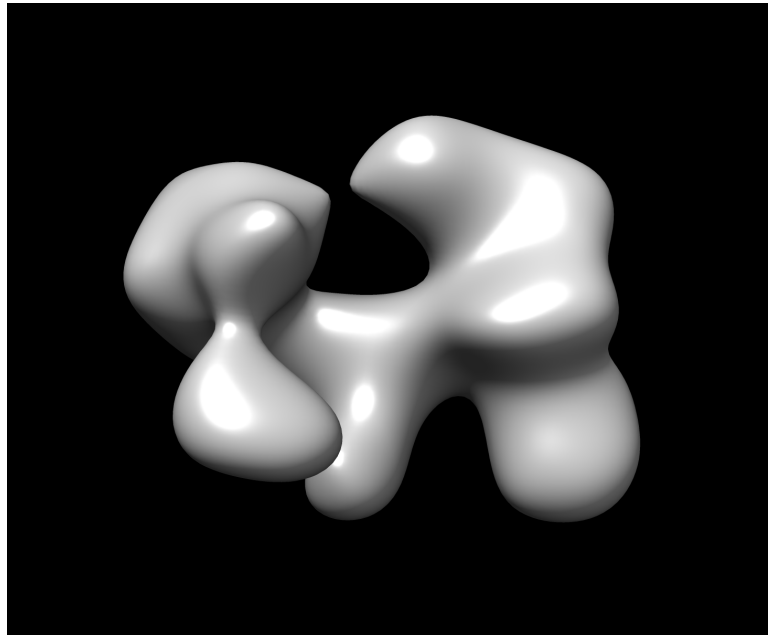
Core-DD 0.1% GA

0.05mg/ml in 20mM Hepes, 300 mM NaCl, 2mM TCEP using NanoW (organotungsten compound pH 6.8) and uranyl acetate

DDX21 has conformational heterogeneity by cryoEM



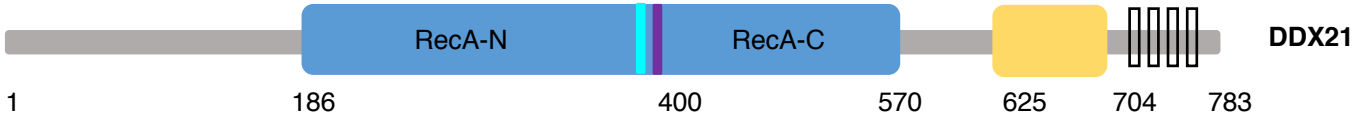




Flexibility – conformational heterogeneity – does not allow to reach atomic resolution

With DCI Lausanne

Identification of the dimerization domain



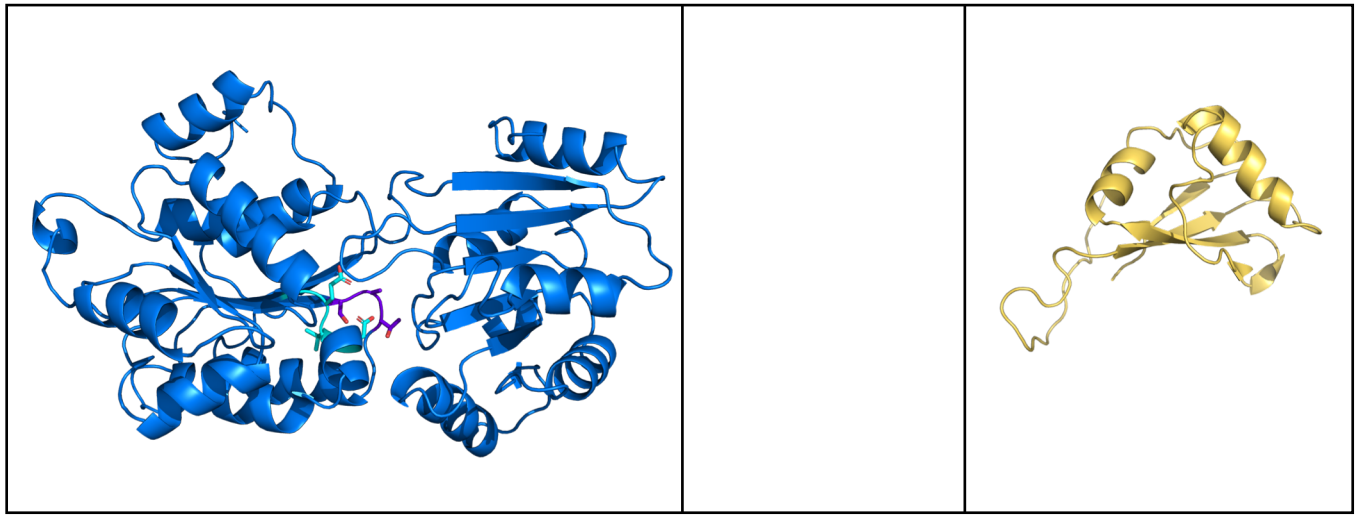
RecA-like Helicase core

GUCT

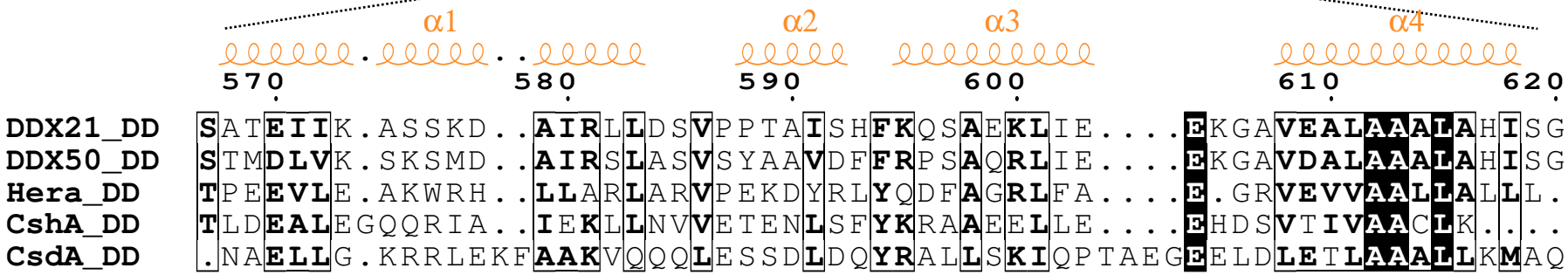
FRGQR/PRGQR repeats

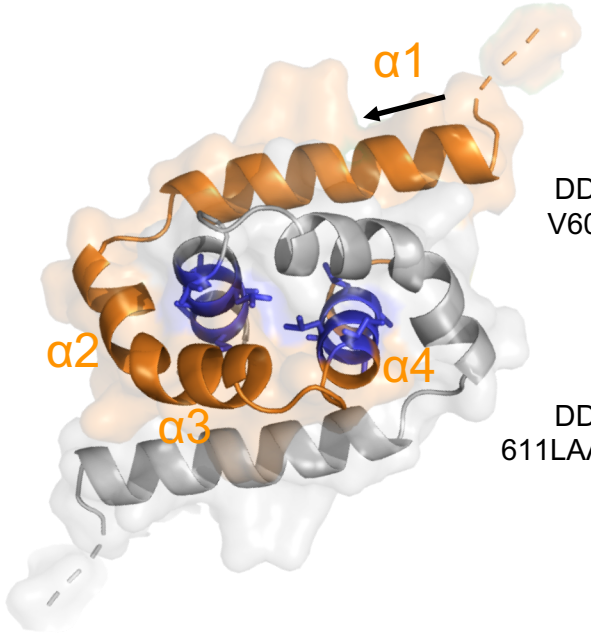
DEVD motif

SAT motif



Identification of the dimerization domain

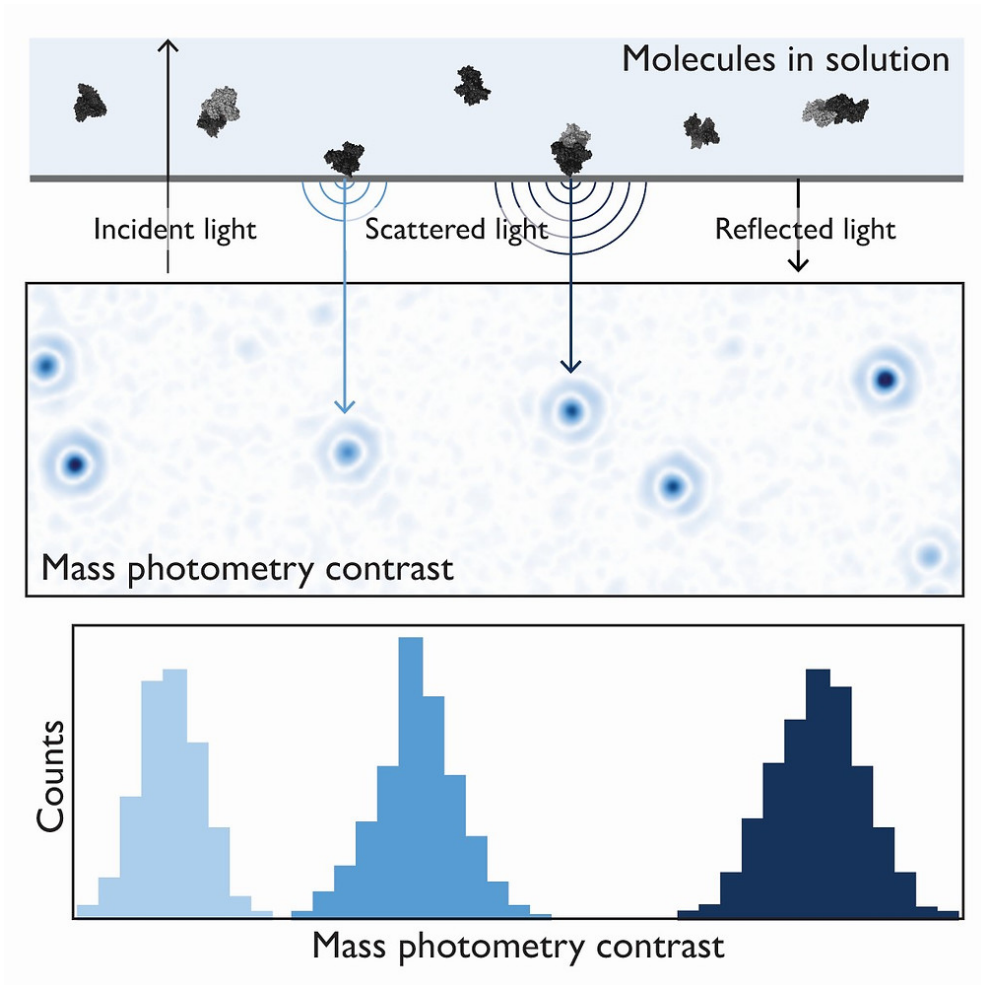


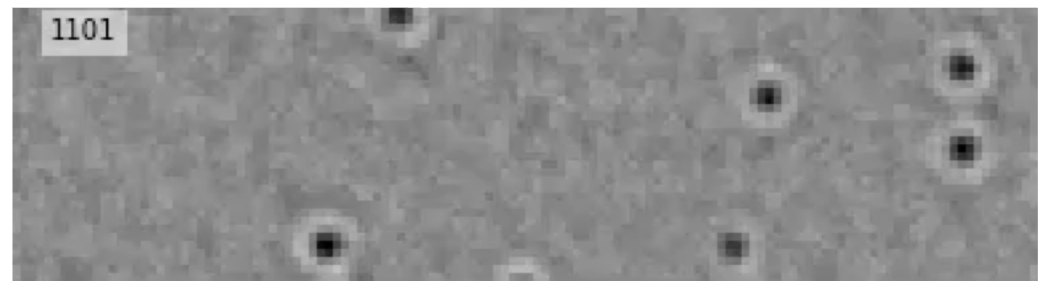


DDX21 ΔN mutant 1
V608Q/L611E/L615Q

DDX21 ΔN mutant 2
611LAAALA616 > RRRRRR

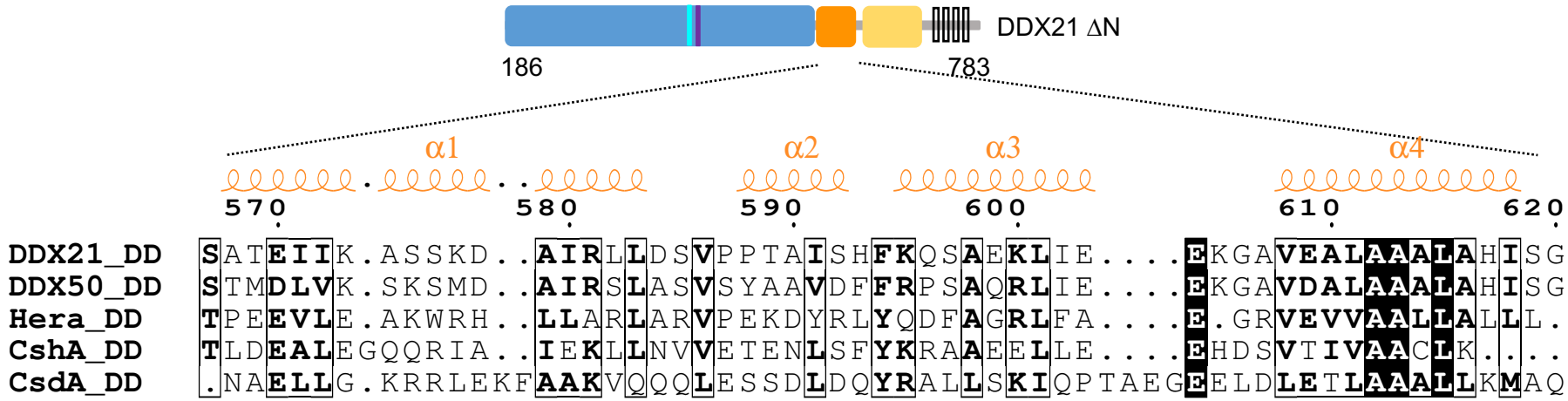
Mass photometry

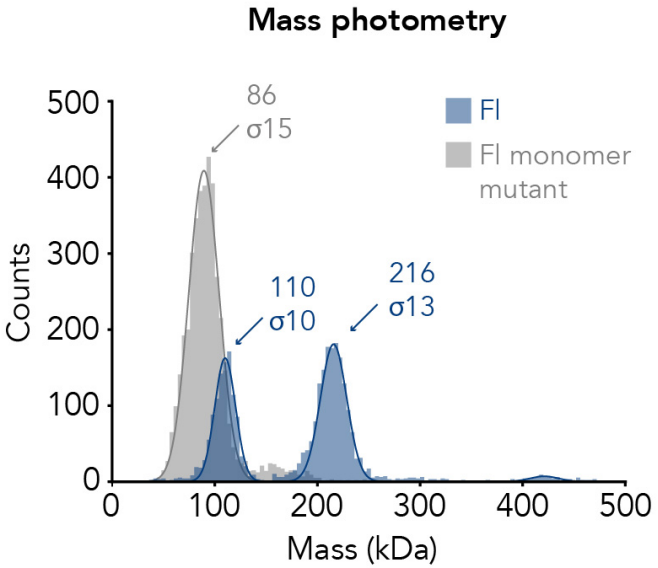




Refeyn
Jenny Sülzle (Manely Lab, EPFL)

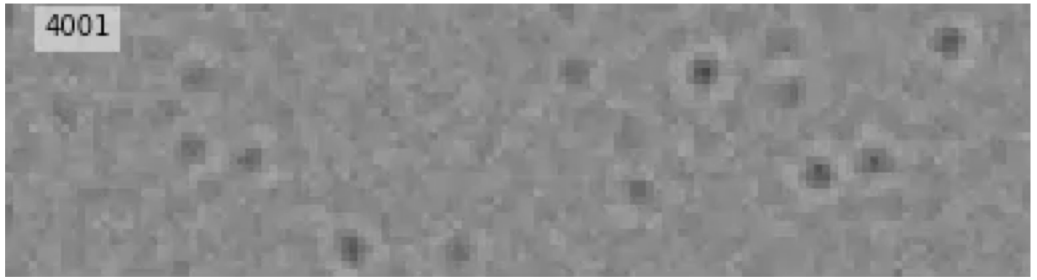
Identification of the dimerization domain





DDX21 30 nM

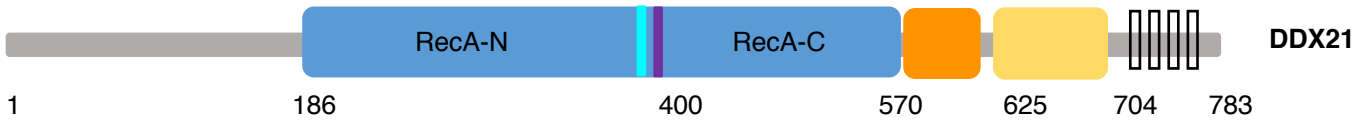
DDX21 monomer mutant 30nM



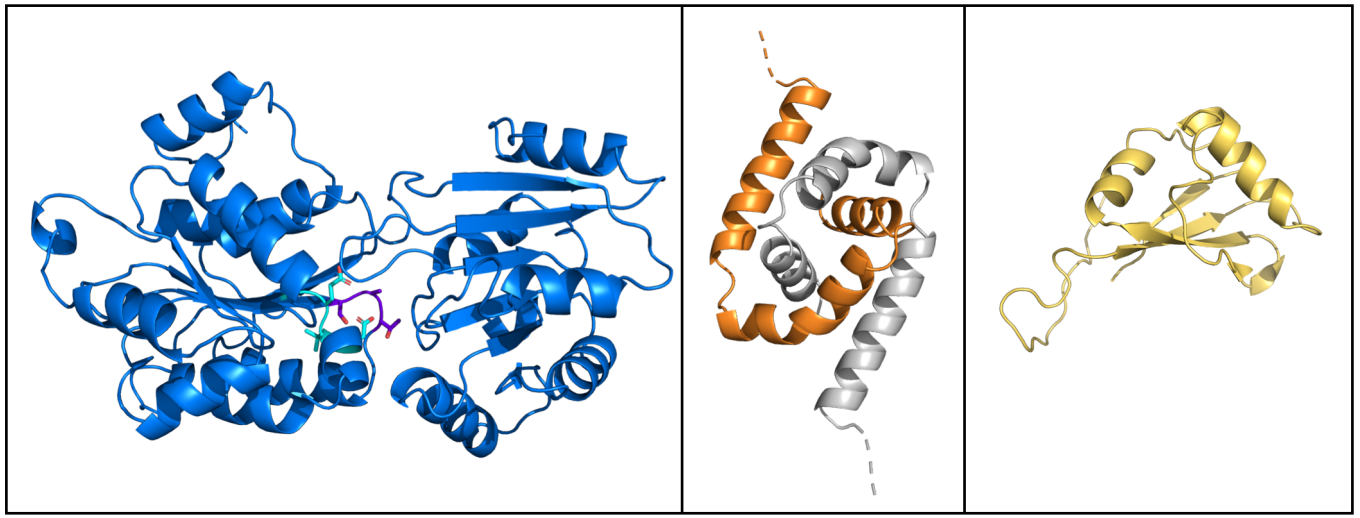
Conclusions from biochemistry

- DDX21 is dimeric and its oligomerization state influences its helicase activity.
- The Arg/Gly rich C-termini, rather than dimerization, are key to maintaining high affinity for RNA substrates
- In vitro helicase assays indicate that an intact dimer is essential for both DDX21 ATP-dependent double-stranded RNA unwinding.

Homology modeling of DDX21



What is the domain organization in the dimer?



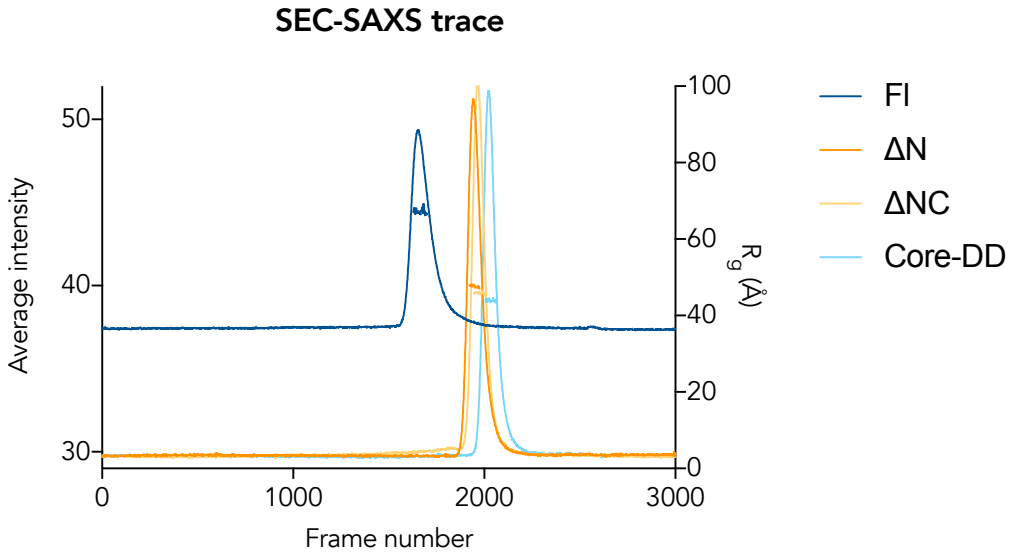
RecA-like Helicase core

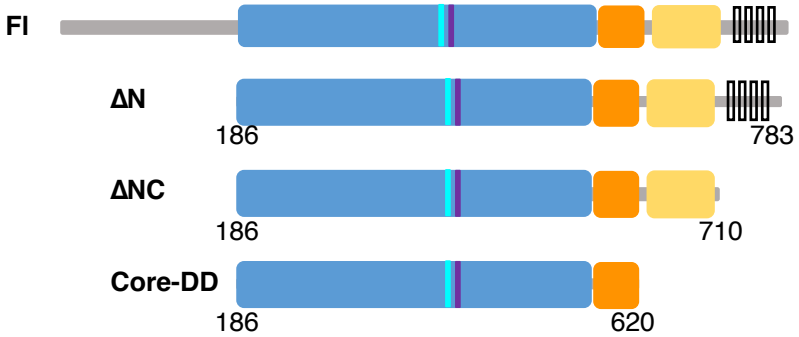
Dimerization domain

GUCT

FRGQR/PRGQR repeats

SAXS analysis of the DDX21 variants



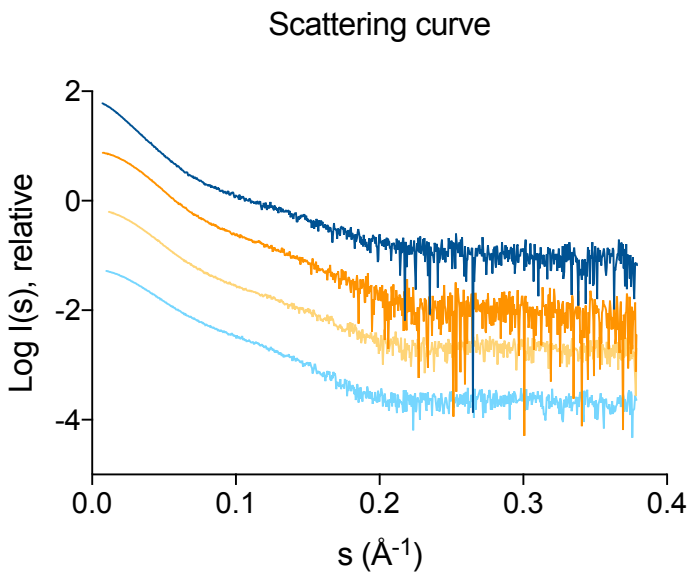


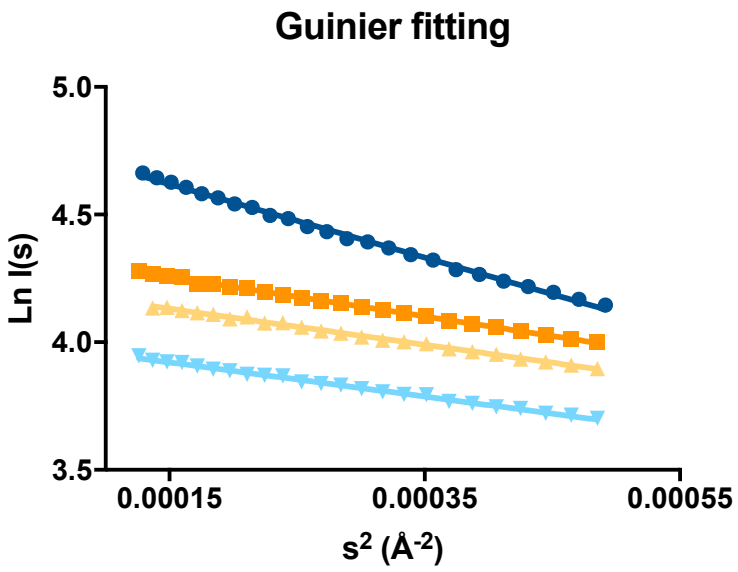
SEC-SAXS @ ESRF BM29

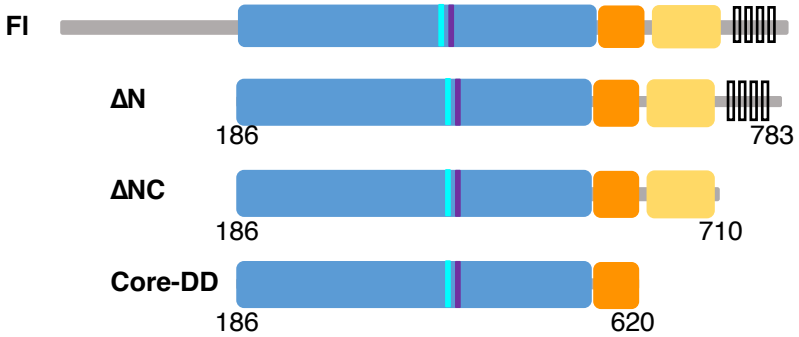
100ul of sample @ 10mg/ml

Superose 6 equilibrated in 20 mM HEPES, pH 7.5, 500 mM NaCl, 10% (v/v) glycerol, 2 mM TCEP

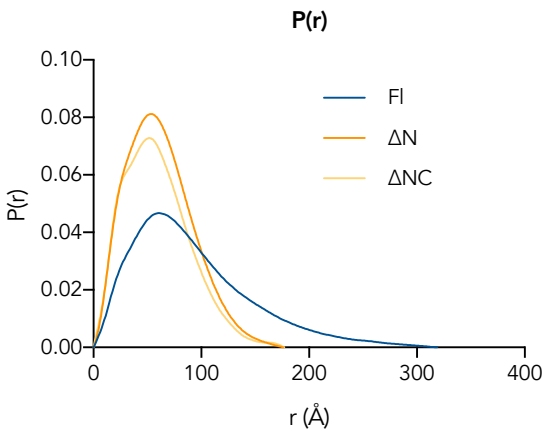
SAXS analysis of the DDX21 variants

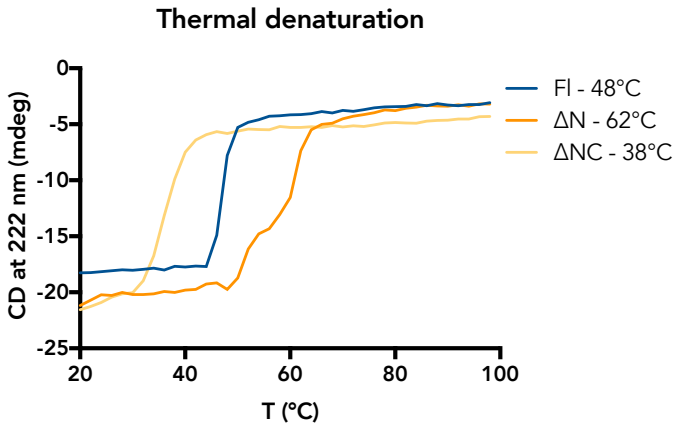


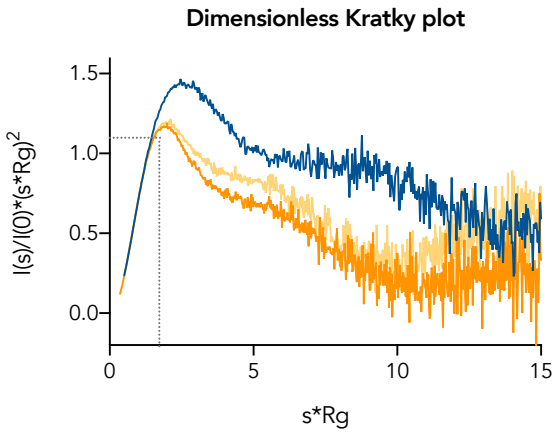


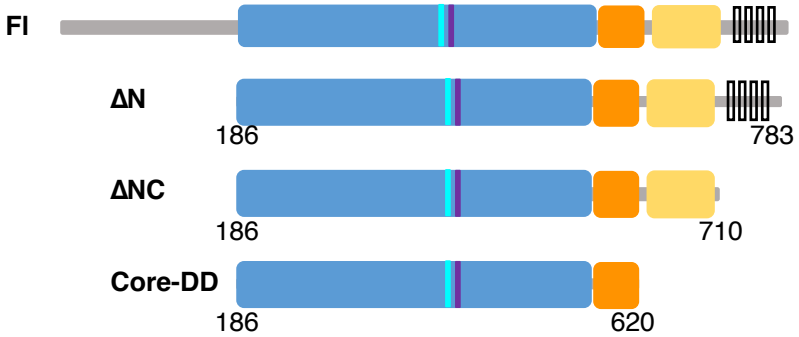


C-terminal 90 aa stabilize the GUCT domain



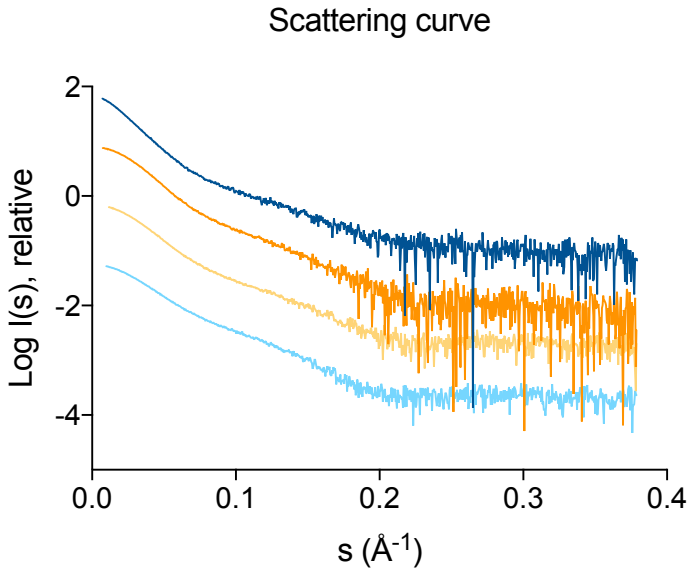


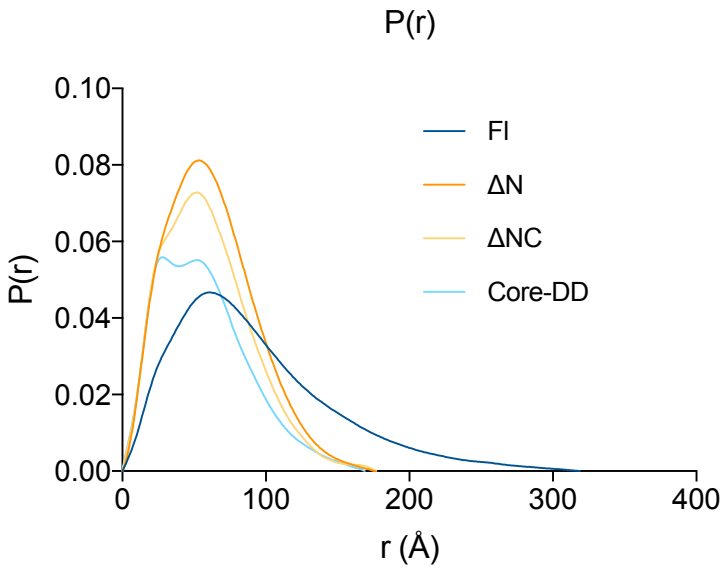


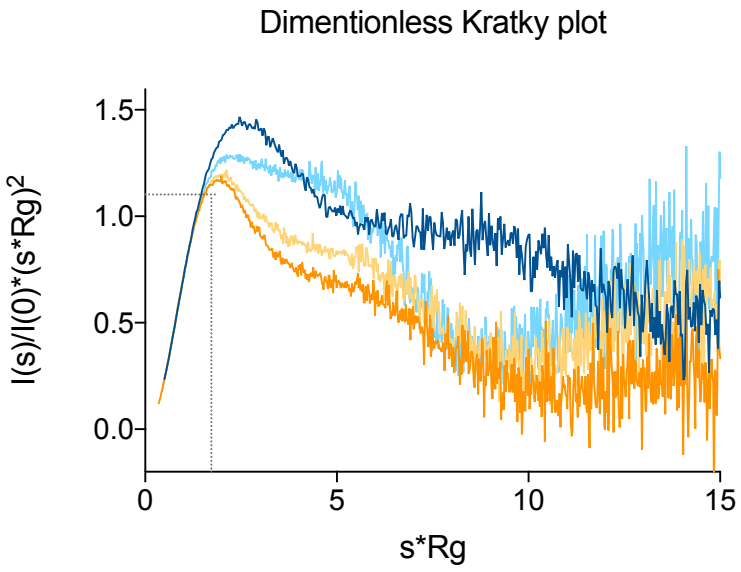


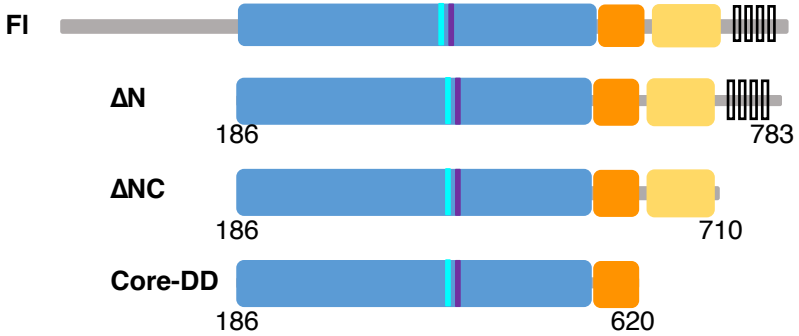
	FL	ΔN	ΔNC	
Rg (Å)	70	48	46	
Dmax (Å)	319	176	177	
Porod Volume (Å^3)	681000	297000	238000	
Mw (kDa)	227	160	120	

The C-terminal GUCT/tail domain stabilizes the dimer





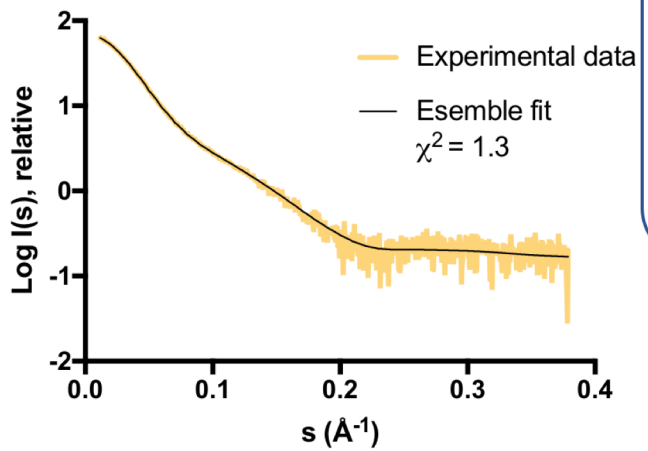




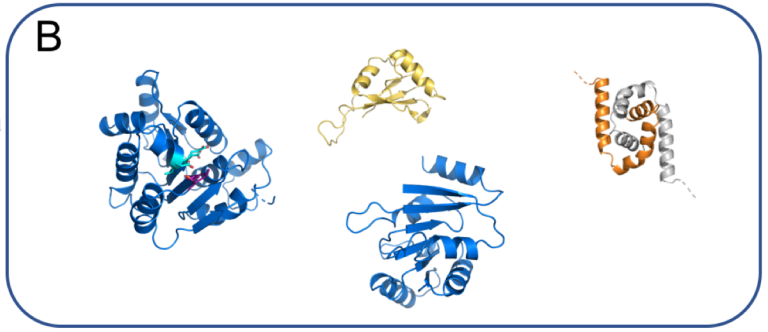
	FL	ΔN	ΔNC	Core-DD
Rg (Å)	70	48	46	44
Dmax (Å)	319	176	177	169
Porod Volume (Å^3)	681000	297000	238000	148000
Mw (kDa)	227	160	120	90

EOM analysis

A

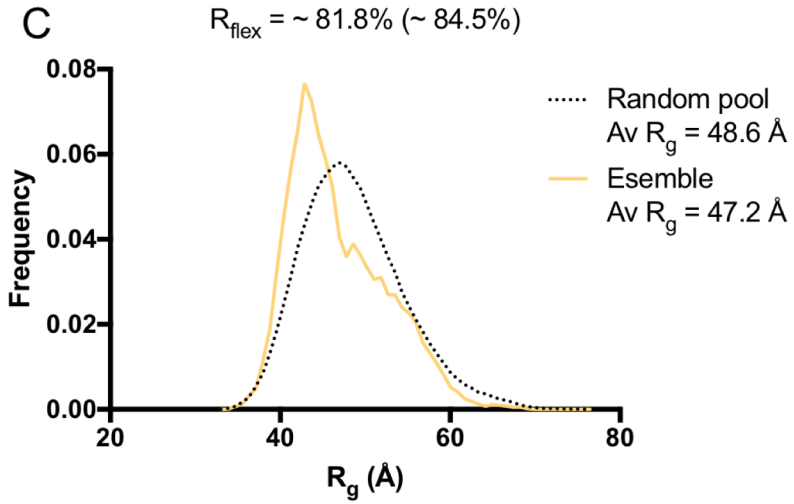


B

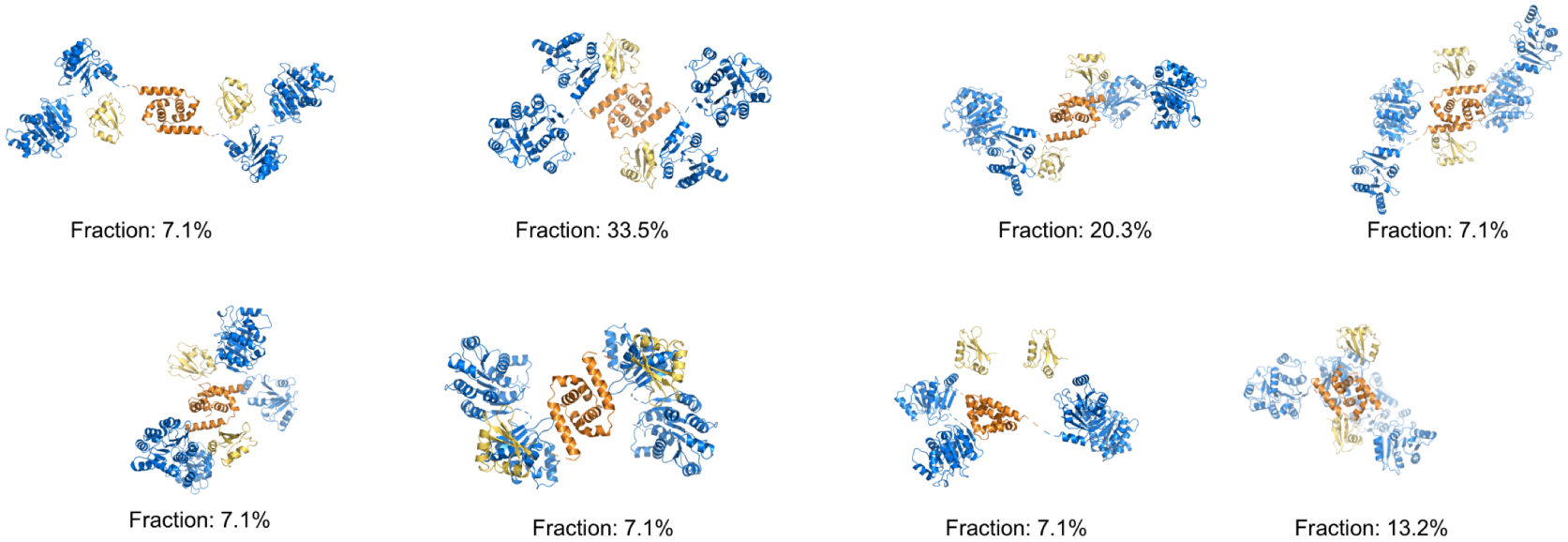


Ensemble optimization method

C

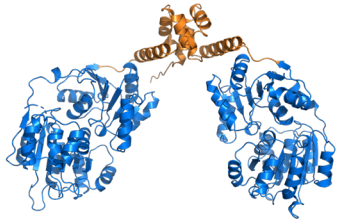


D

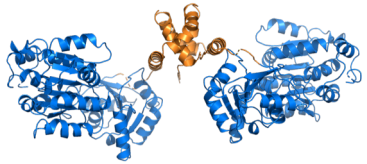


Model validation/elimination

Inverted V-shaped

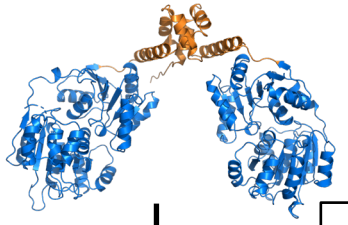


Flat



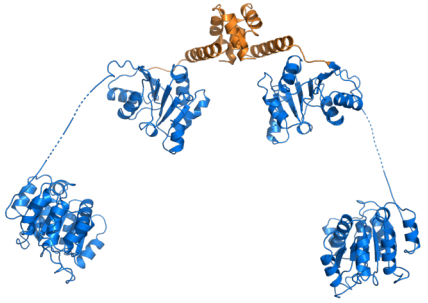
Model validation/elimination

Inverted V-shaped



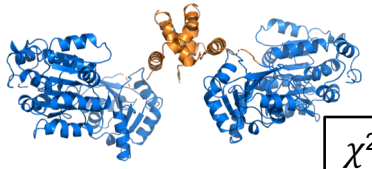
Open

χ^2=15



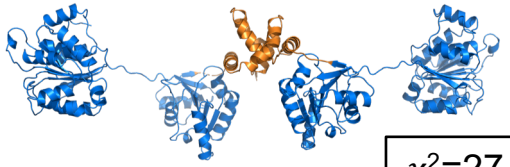
χ^2=107

Flat

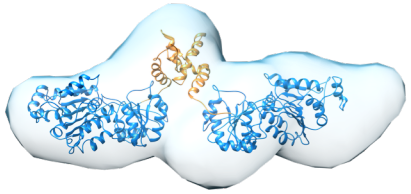


χ^2=13

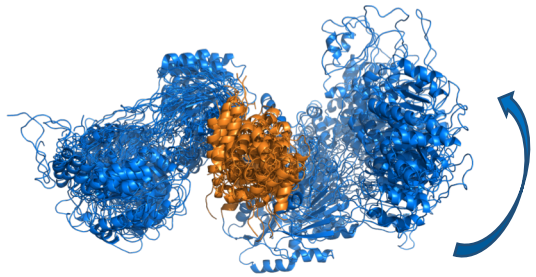
Open



χ^2=27.6



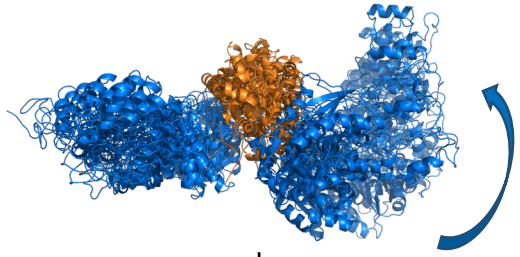
Ab initio DAMMIF envelope



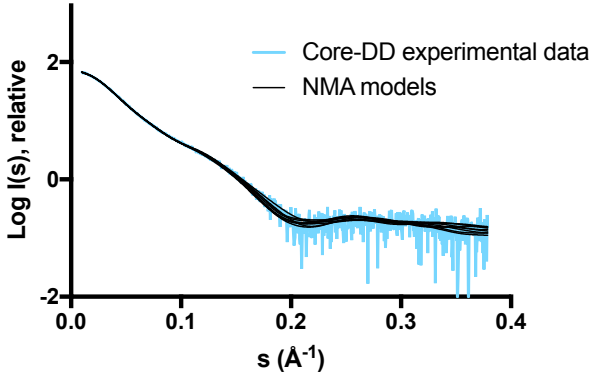
90°

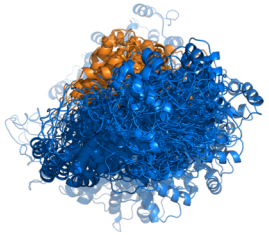
Flexible fitting to SAXS data

χ^2=1.4

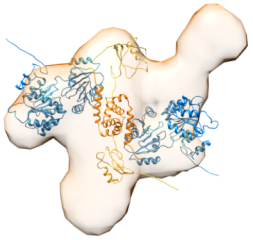


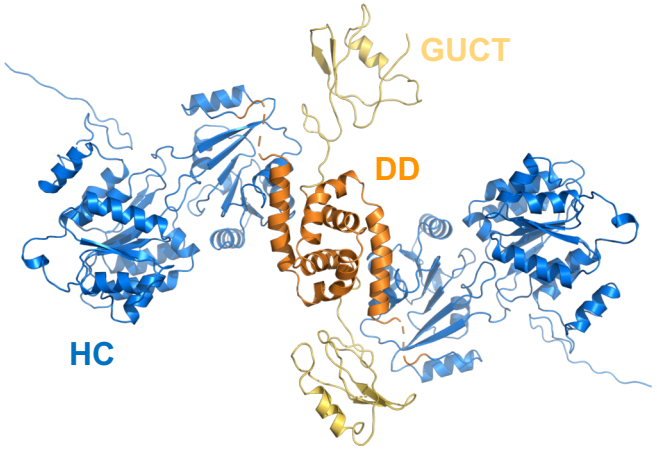
90°





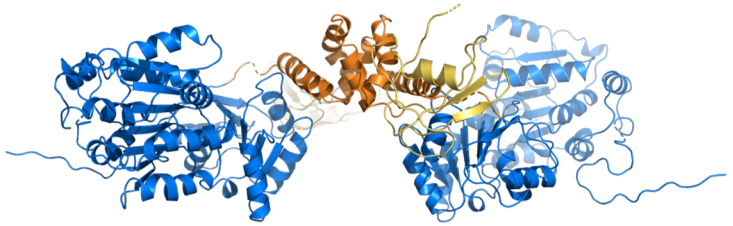
Model validation





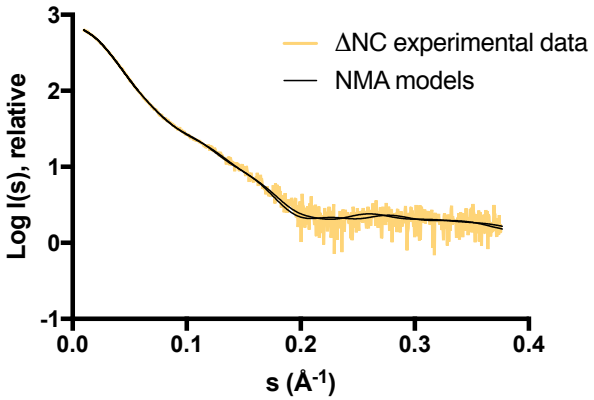
90°

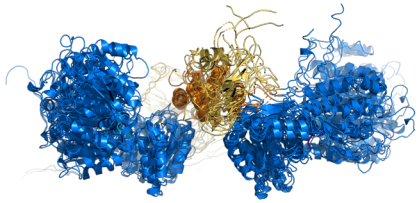
90°



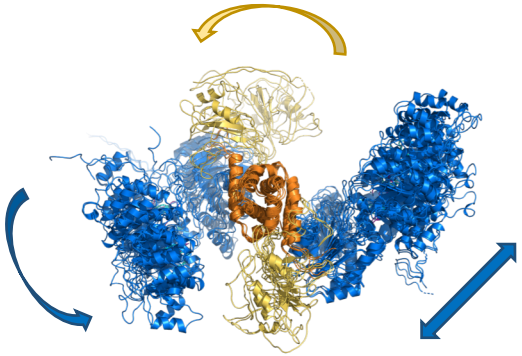
Flexible fitting to SAXS data

χ^2=1.3

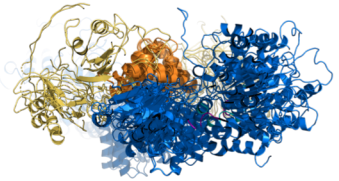




90°

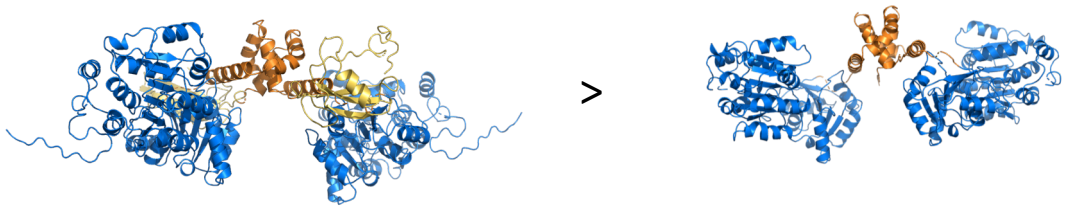


90°



Conclusions from SAXS experiments

- SAXS analysis uncovers a flexible multi-domain protein with a central dimerization domain.
- The GUCT domain stabilizes the core-DD dimer



- The C-terminal tail maybe compact around the GUCT domain
- The protein mostly adopts a flat extended conformation in solution (as opposed to the inverted V-shape conformation)

Mosquito Toll5A and its ligand Spz1C

Motivation

- Insect Toll receptors perform critical functions in both embryogenesis and innate immunity.

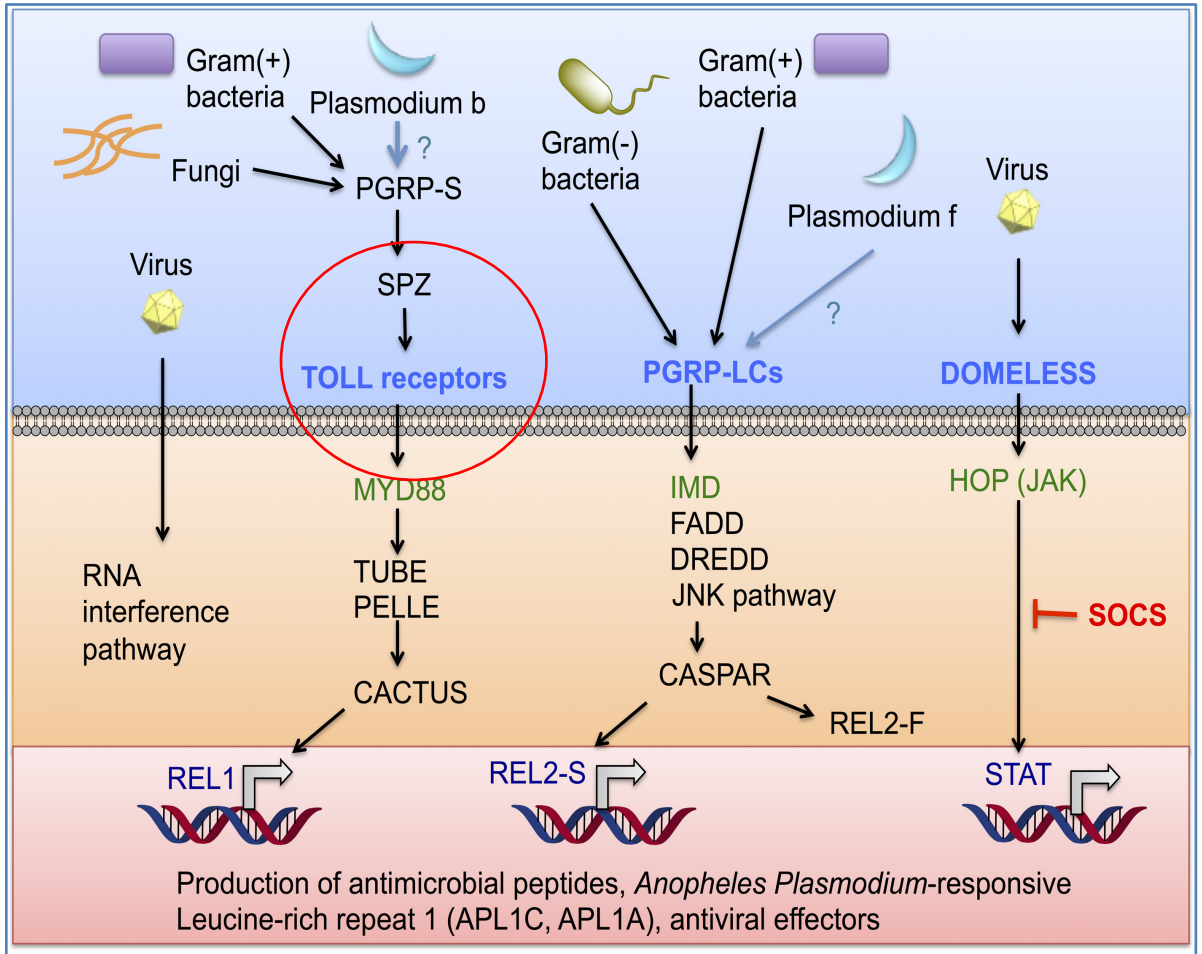


Figure 1: Overview of the four major signalling pathways involved in mosquito innate immunity.

M. Gangloff, personal communication

Motivation

- Mosquitos are (vectors) able to transmit diseases while being resistant to them.
- The mosquito innate immunity may be the key to counteract the spread of vector-borne diseases.

Project in collaboration with Monique Gangloff

Using a **multidisplinary approach** studies the **structure** and **function** of mosquito immune receptors that

- are implicated in **resistance** to pathogens
- have undergone **gene duplication** in disease-carrying mosquitoes.

UNIVERSITY OF CAMBRIDGE

Motivation

- Mosquitos are (vectors) able to transmit diseases while being resistant to them.
- The mosquito innate immunity may be the key to counteract the spread of vector-borne diseases.

Structural and functional characterisation of duplicated **Toll5A** and **Spz1C** in the viral mosquito vector *Aedes aegypti*

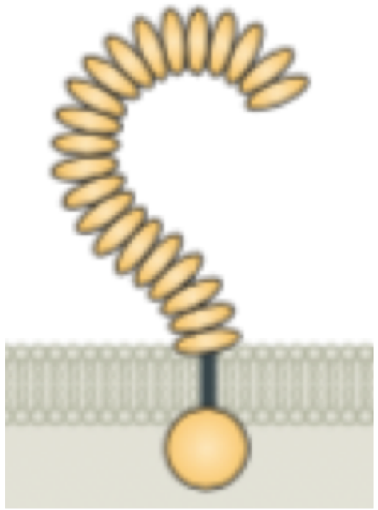
Project in collaboration with Monique Gangloff

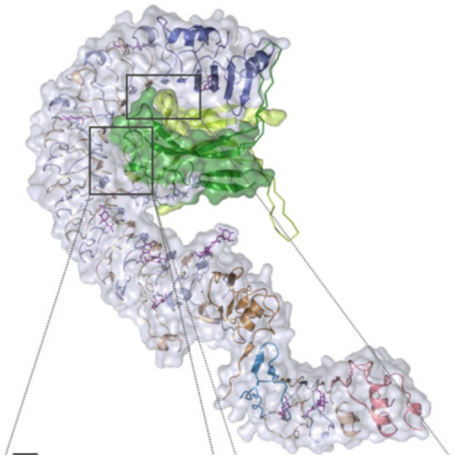
Using a **multidisplinary approach** studies the **structure** and **function** of mosquito immune receptors that

- are implicated in resistance to pathogens
- have undergone gene duplication in disease-carrying mosquitoes.

Toll receptors

Spätzle





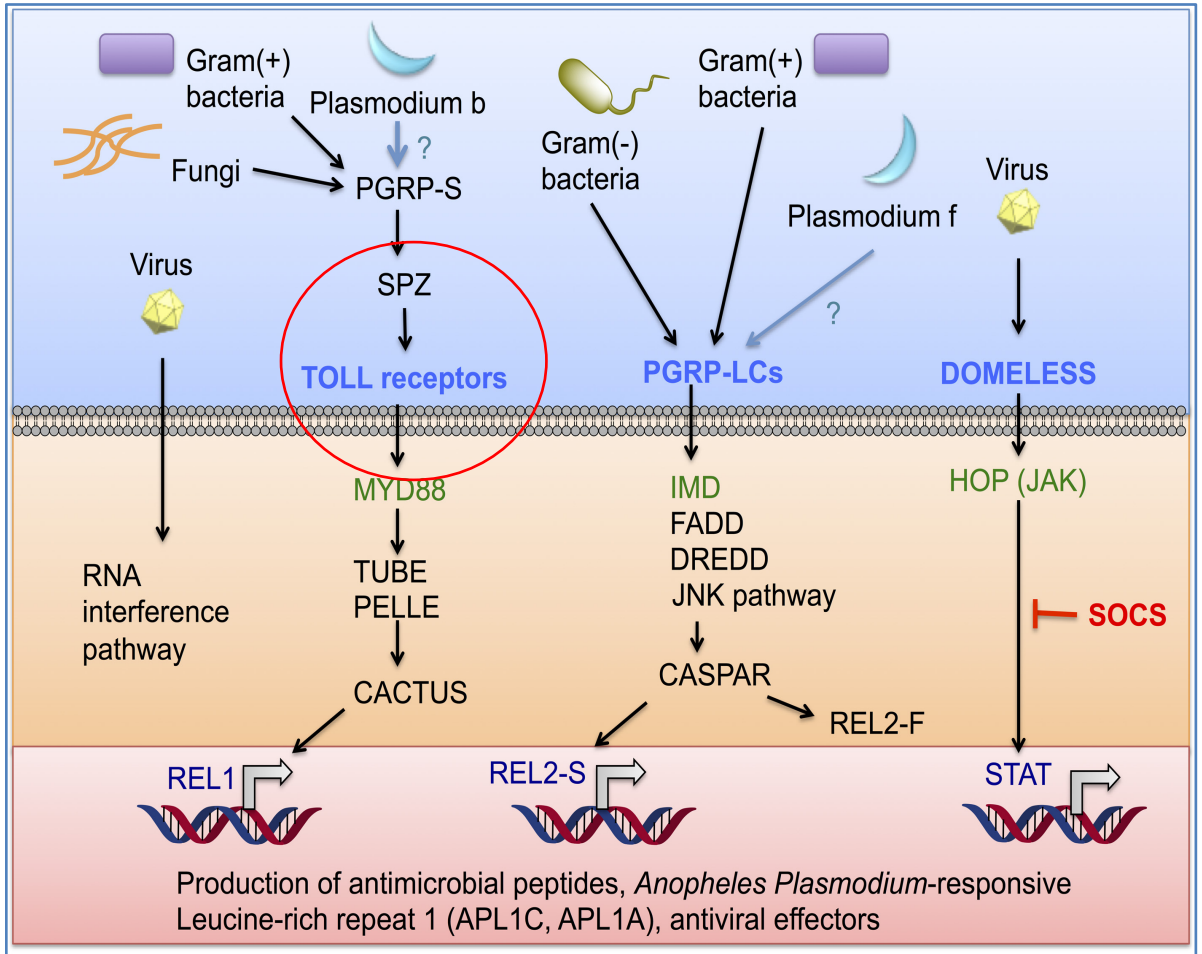
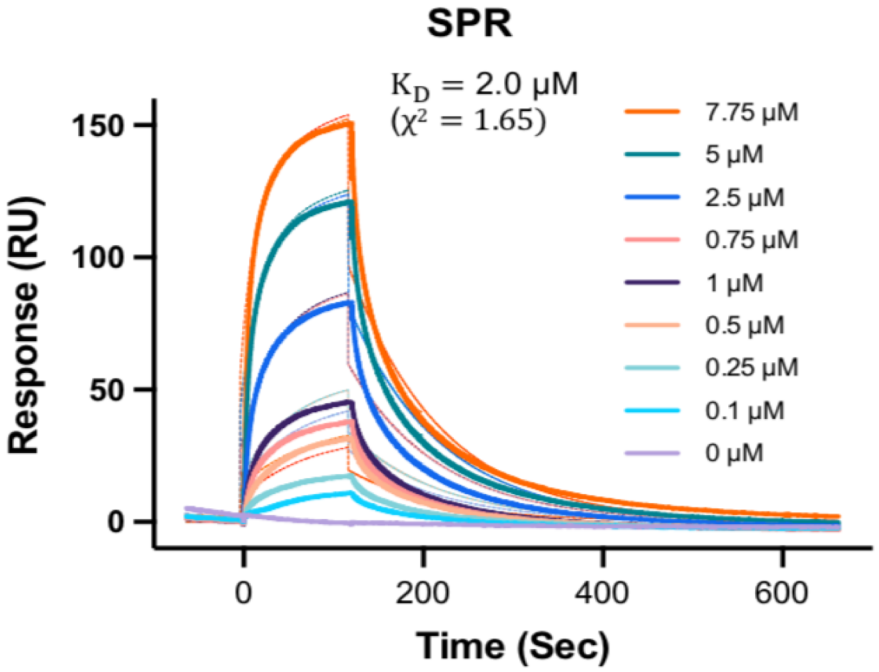


Figure 1: Overview of the four major signalling pathways involved in mosquito innate immunity.

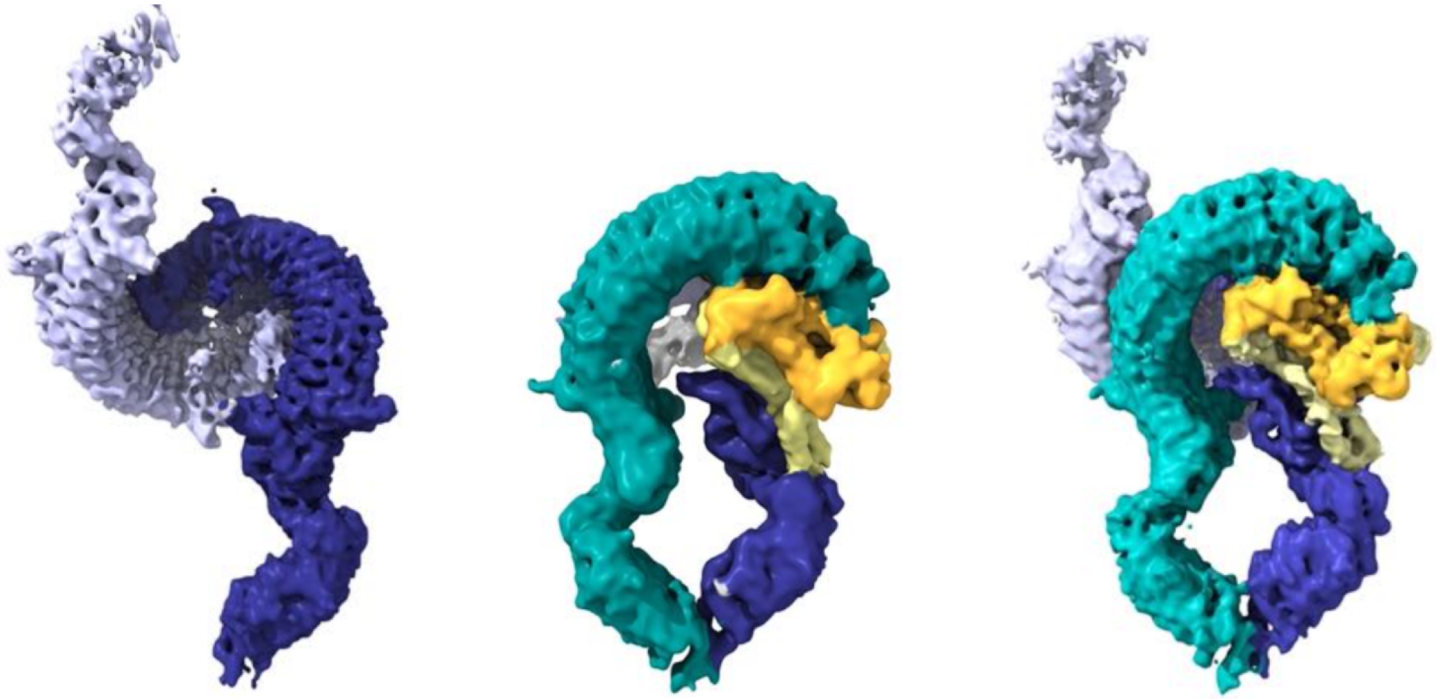
Gay *et al* 2014 Nature Immunol Rev

Receptor specificity is achieved with low ligand binding affinity.



Saucereau et al, under review

Cryo-EM capturing Toll5A-Spz1C binding intermediates.



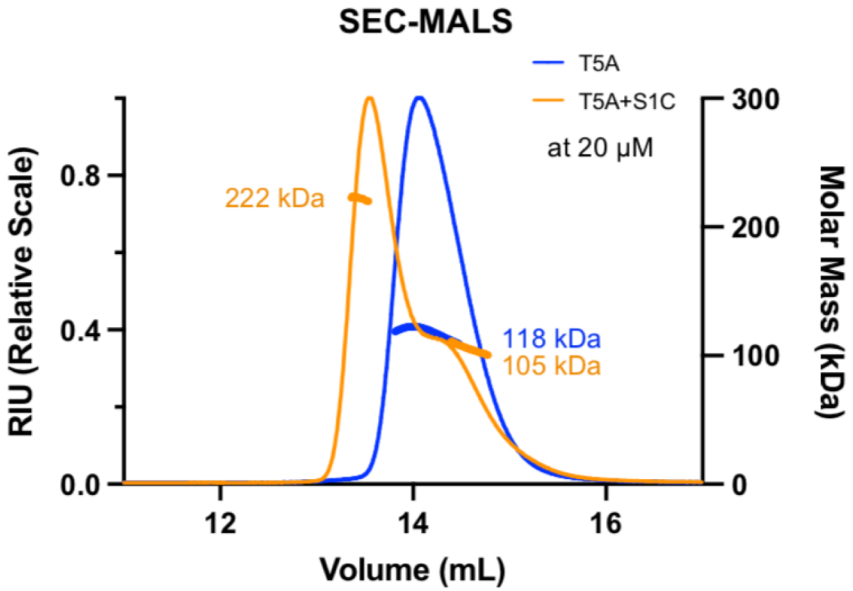
Homo-dimer
~3.4 Å
85,810
particles

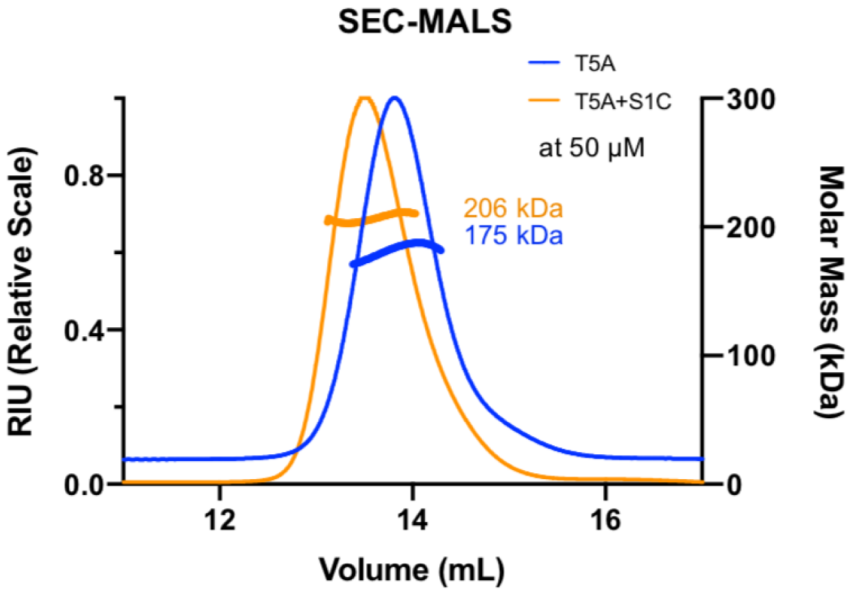
Hetero-dimer
bound to Spz1C
~4.2 Å
40,153
particles

Hetero-trimer
~3.7 Å
42,866
particles

Saucereau et al, under review

Ligand binding triggers a conformational change within Toll5A dimers in solution.





Saucereau et al, under review

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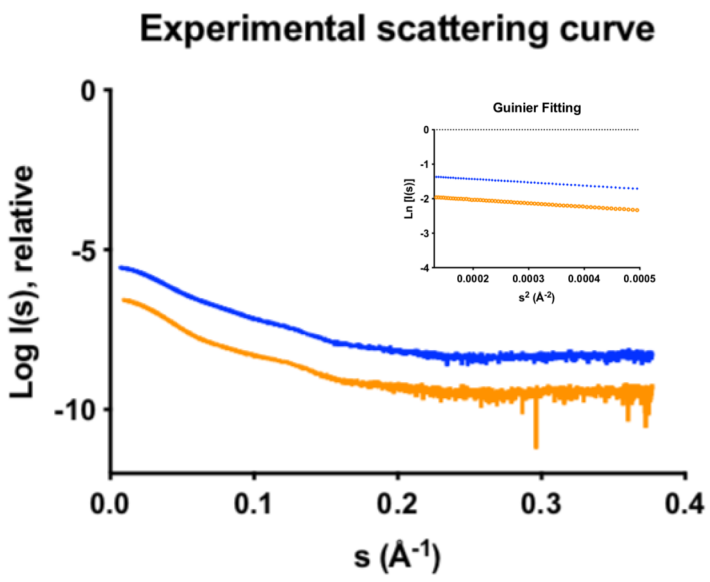
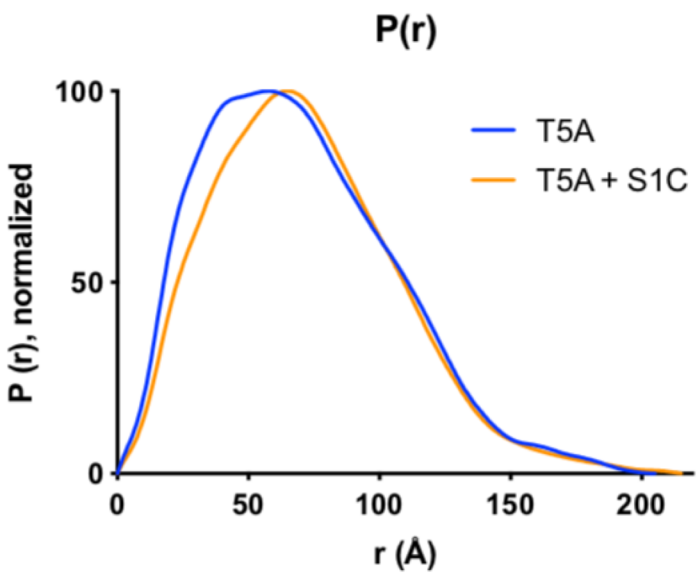
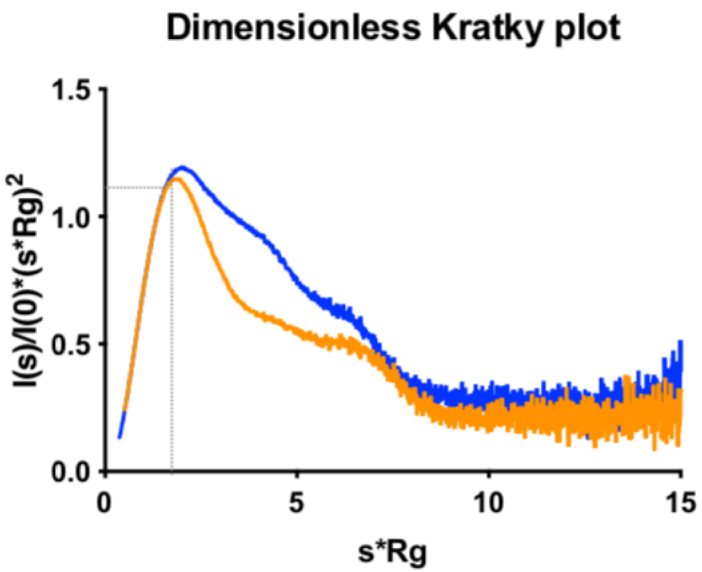






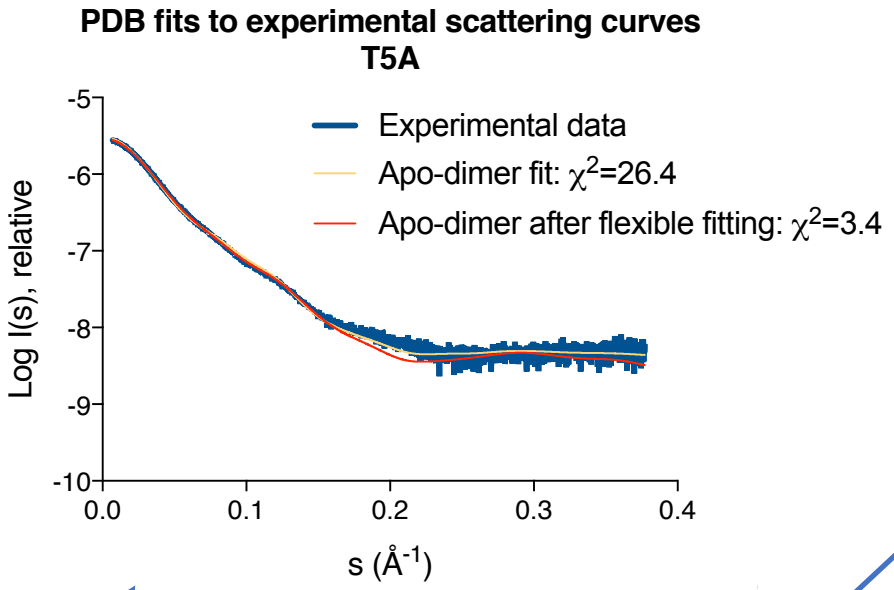
SEC-SAXS @ BM21 Diamond Light Source

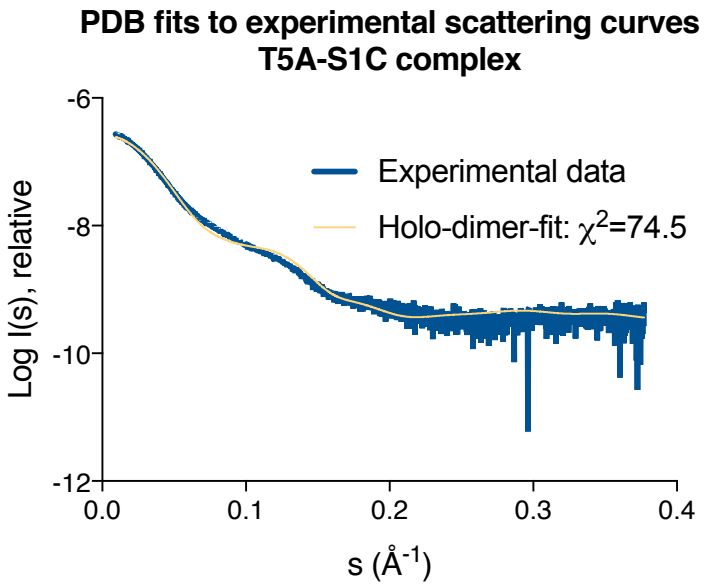
55ul of sample @ 5mg/ml

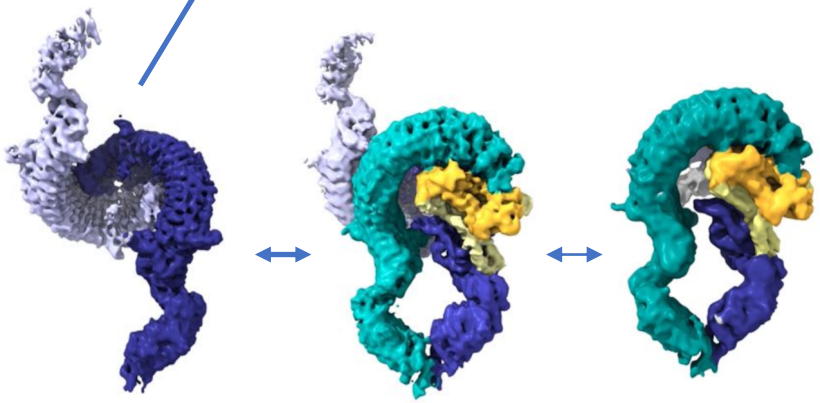
Superose 6 Increase 3.2/300 equilibrated in 50 mM NaCl, 50 mM Tris-HCl, pH 7.5

	T5A	T5A + S1C
Rg (Å)	53.5	55.3
Dmax (Å)	205	215
Porod Volume (Å^3)	370000	567000
Mw (kDa)	200	267

SAXS vs cryoEM models







PDB	.dat file (R_g(Å))	R_g (Å)	χ^2
Apo-monomer	T5A (53.5)	48.54	1149.9
Apo-dimer	T5A	56.65	26.35
Holo-dimer	T5A	49.37	257.47
Apo-monomer	Complex (55.3)	48.54	2107.23
Apo-dimer	complex	56.81	217.68
Holo-dimer	complex	50.43	74.51

Conclusions

- Low affinity and high specificity of Toll5A to Spz1C.
- Reversible binding.
- Cryo-EM single particle analysis capturing Toll5A-Spz1C binding intermediates.
- SAXS analysis suggests that the 2:1 complex is less flexible than the homodimer, despite its slightly larger dimensions.

Saucereau et al, under review

Thanks to

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Alice Duperrex
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BioEM Facility
DCI

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Cy Jeffries

@University of Cambridge, U.K.
Monique Gangloff
Martin Moncrieffe
Ben Luisi
EM facility

@BM29 ESRF Grenoble
Mark Tully

@B21 Diamond Light Source

@University Grenoble Alpes
Sergei Grudinin

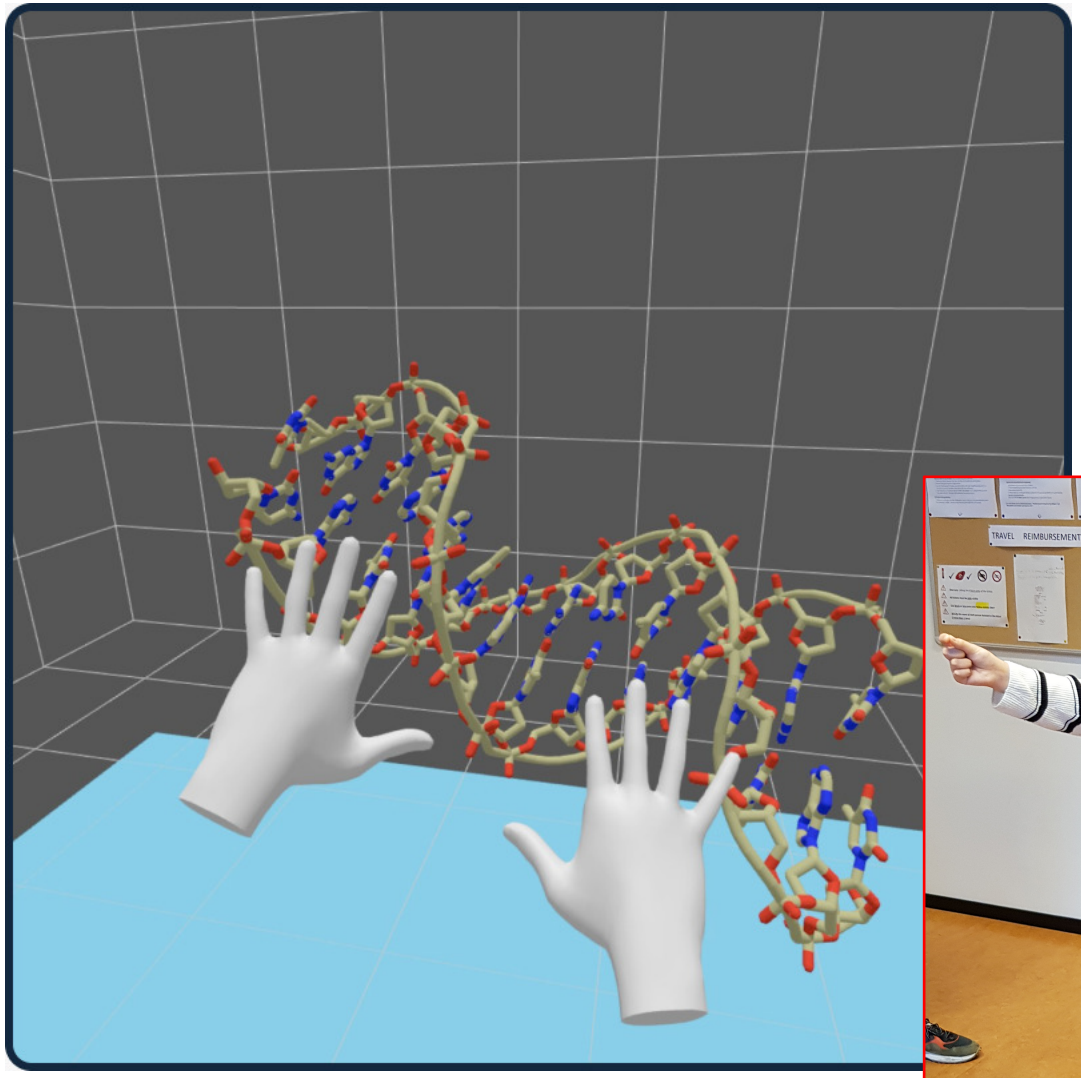
Luciano Abriata

Fabio Cortés

MoleculARweb

A website for chemistry and biology education through augmented reality, available out of the box in commodity devices

Luciano Abriata



Fabio Cortés

Demonstration
Tomorrow at the poster session

Thank you!

Table S3. SAXS parameters (Related to Figure 5).

	FI	ΔN	ΔNC	Core-DD	ΔN-monomer
SASPDB accession code	SASDGU9	SASDGV9	SASDGW9	SASDGX9	SASDGY9
Data collection					
Instrument			ESRF BM29		
Beam size at sample (µm)			700x700		
Wavelength (Å)			0.992		
s range ($Å^{-1}$)			0.0025-0.500		
Detector			Pilatus 1M		
Detector distance (m)			2.867		
Exposure (s per image)			1		
Column			Superose 6 Increase 10/300		
Flow rate (ml/min)			0.5		
Sample volume (µl)			100		
Sample concentration (mg/ml)			9-12		
Temperature (K)			293		
Structural parameters					
R_g (Å) Guinier	70	48	46	44	41
R_g (Å) P(r)	76	49	47	46	44
D_{max} (Å)	319	176	177	169	177
Porod volume ($Å^3$)	681000	297000	238000	148000	111000
Molecular mass determination					
Theoretical MW (kDa)	90	68.7	60.6	49	69
MALLS MW (kDa)	197.7	143.2	120.5	91.4	68
DATPOROD MW (kDa)	400	174	140	87	65
DATVC MW (kDa)	227	156	122	88	68
DATMOW MW (kDa)	232	165	120	91	74
Data analysis software					
Data reduction			PRIMUS & ScÅtter		
Ab initio modelling			DAMMIF		
Homology modelling			Swiss Model		
Theoretical scattering curve calculation from models			Pepsi-SAXS		
3D graphics representation			Pymol/Chimera		