

# Type II topoisomerase challenges

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Institut Pasteur

Réunion MASIM 2017





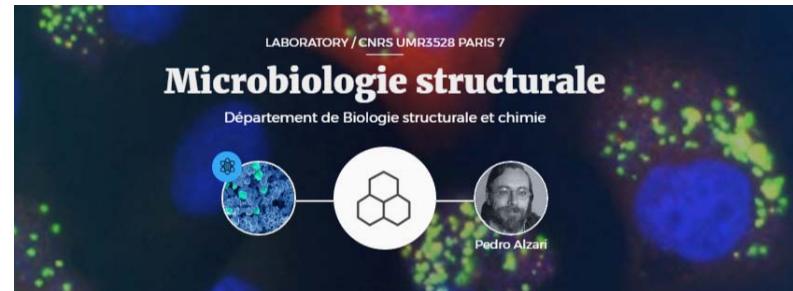
# Presentation of the team

DEPARTMENT



Institut Pasteur, CNRS UMR 3528, University Paris Diderot

LABORATORY / UNIT



PARIS  
DIDEROT  
université  
PARIS 7

**X-ray crystallography**  
**Molecular biophysics and biochemistry**  
**Bacterial genetics**  
**Structural and mechanistic enzymology**  
**Mycobacterial proteins**

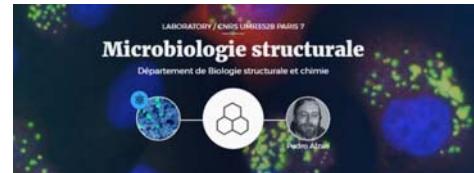


# Presentation of the team

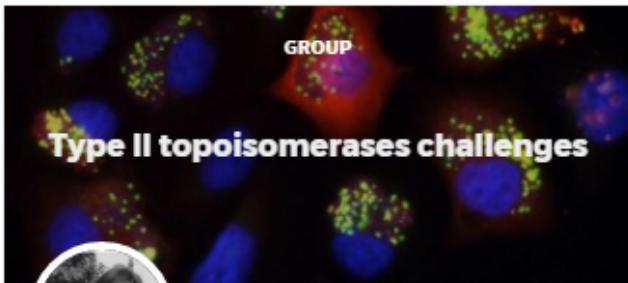
université  
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DIDEROT  
PARIS 7



Institut Pasteur

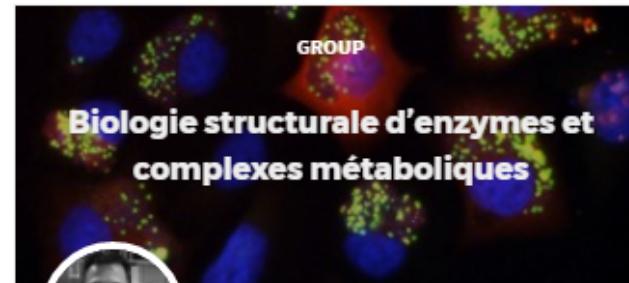


**Head:** Pedro M. Alzari (PR IP)



Type II topoisomerases challenges

Claudine Mayer



Biologie structurale d'enzymes et  
complexes métaboliques

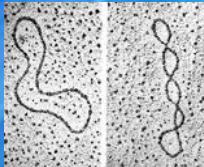
Marco Bellinzoni



Biologie de Cibles du Paludisme et  
Antipaludiques

Jean-Christophe Barale

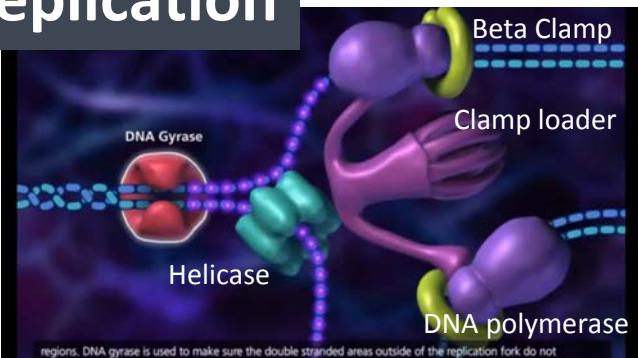
**Structural studies of type II topoisomerases  
Crystallography, biophysics, cryoEM,  
modeling**



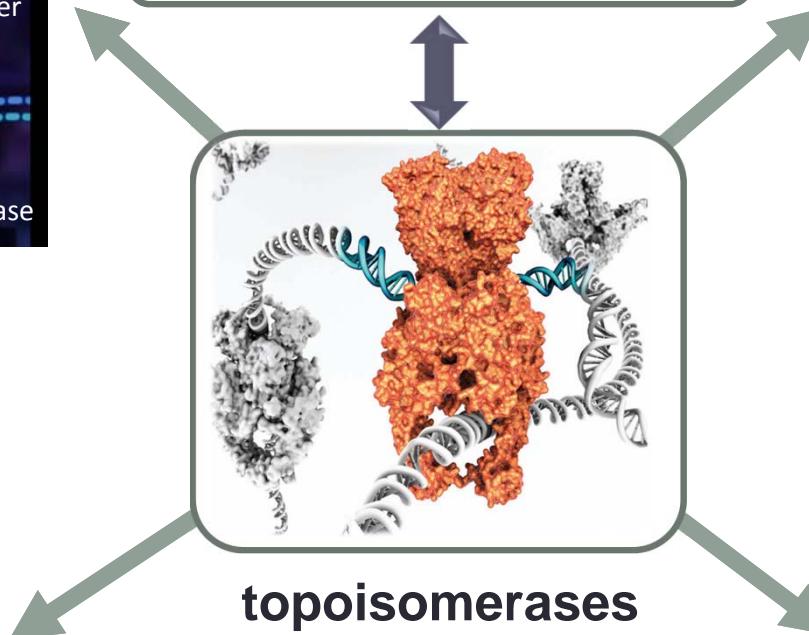
# DNA topoisomerases

In the cell, DNA is subjected to bending, folding, overwinding and underwinding

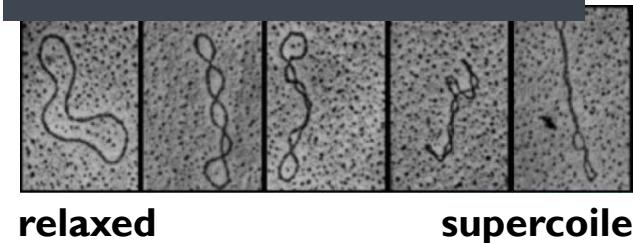
# Replication



# Manipulation and control of DNA topology



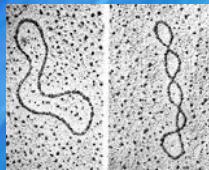
# DNA condensation



# Transcription

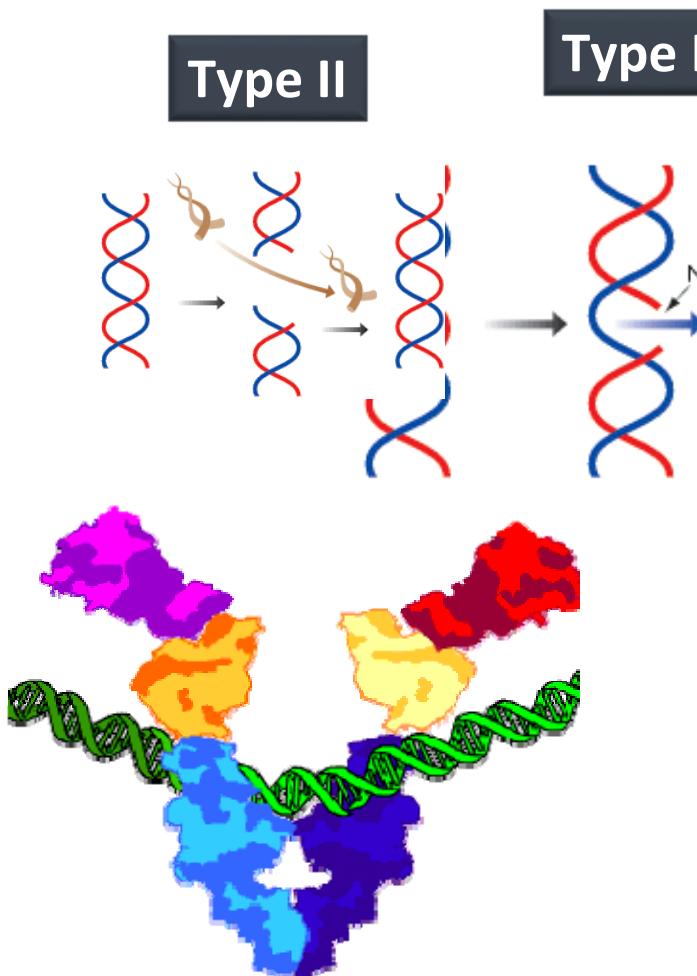
# Cell division

- Unwinding to gain access
  - Removing supercoils
  - Separation of daughter chromosomes



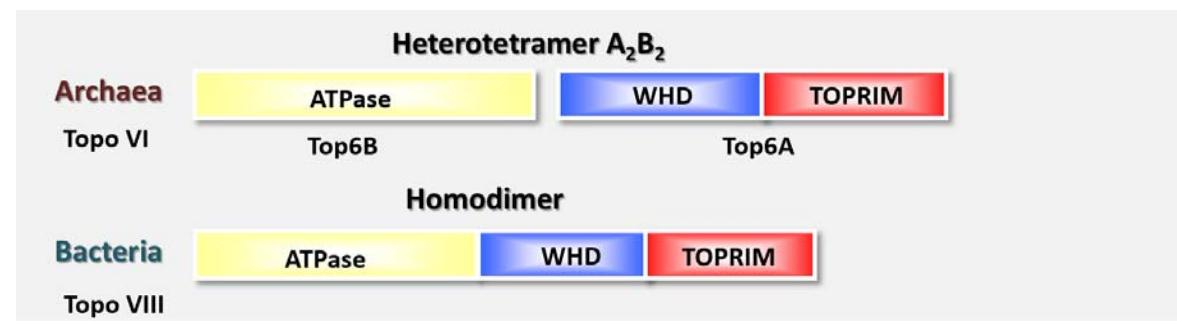
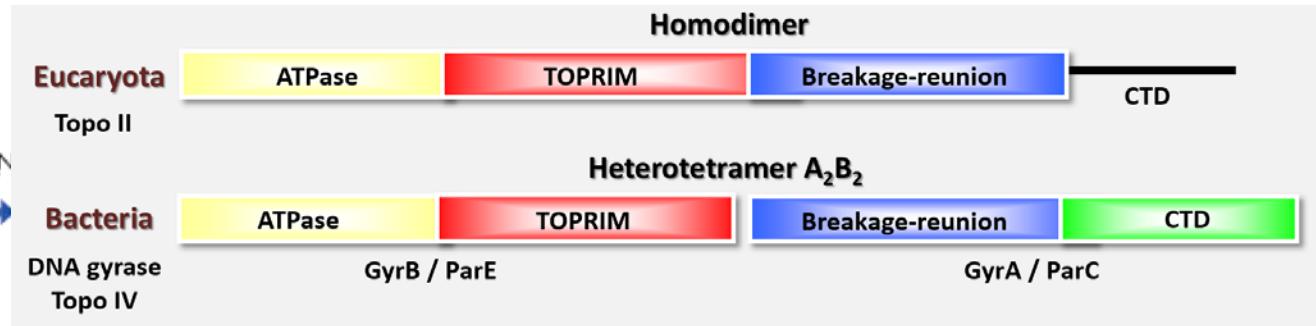
# DNA topoisomerases

There are two types of topoisomerases



## Modular dynamic nanomachines

### Type II





# *M. tuberculosis* DNA gyrase

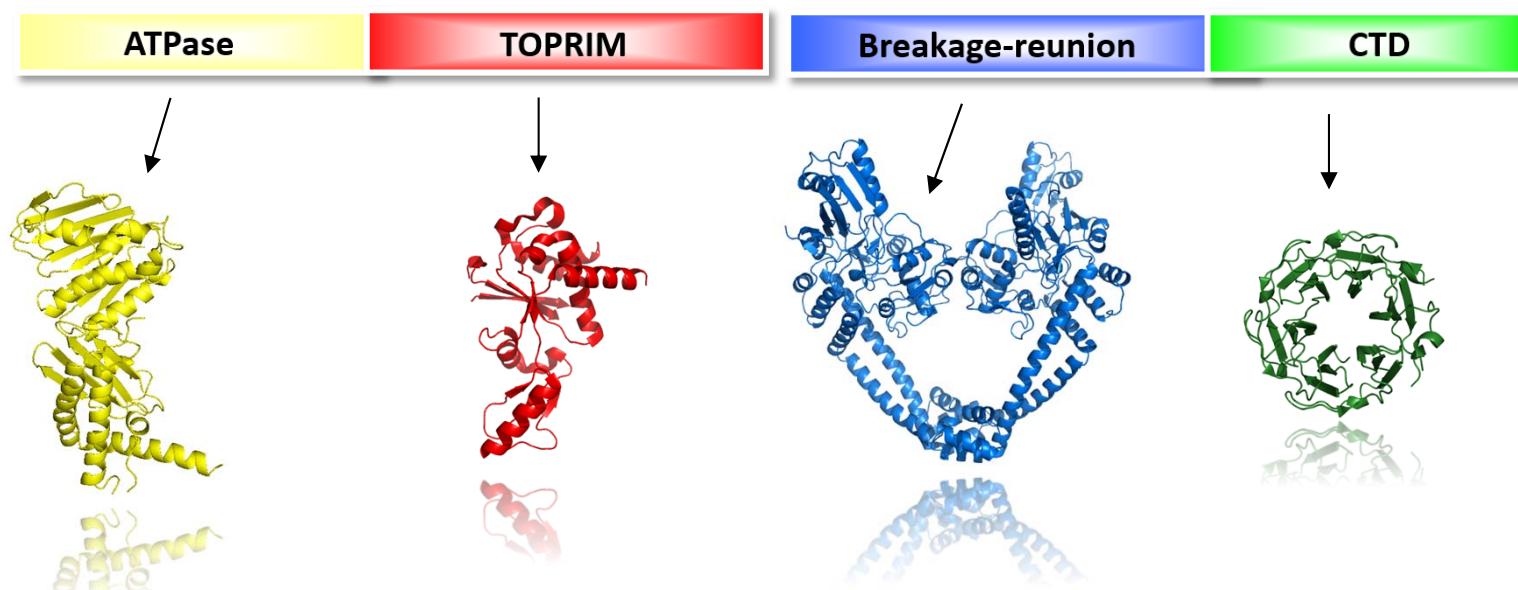
The unique type II topoisomerase in *Mycobacterium tuberculosis*

Resistance to fluoroquinolones

collaboration A. Aubry, UPMC

10 years

Structure-function relationships studies of the 4 isolated domains



Roué et al, Acta cryst F 2013  
Agrawal et al, Biochem J 2013

3ZM7

Piton et al, PlosOne 2010

3IG0

Piton et al, Acta cryst F 2009  
Piton et al, PlosOne 2010

3M4I

Darmon et al, Acta cryst F 2012  
Bouige et al, Biochem J 2013

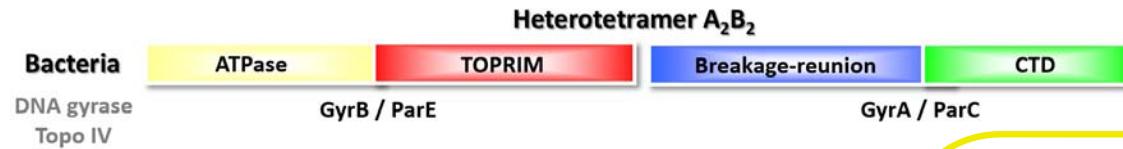
3IFZ

4G3N



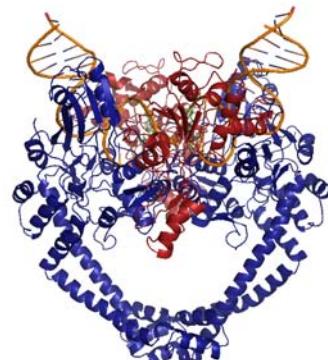
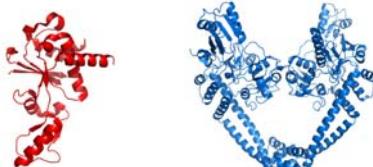
# *M. tuberculosis* DNA gyrase

Structure-function relationships of the 3 functional domains



## Catalytic core

Jérémie PITON  
Thèse, 2010



Structural insights into quinolone resistance mechanism

## ATPase domain

Mélanie ROUE  
ATER, 2011



Structural insights into ATP hydrolysis and its inhibition

## CTD

Amélie DARMON  
Thèse, 2013



Structural insights into DNA wrapping mechanism



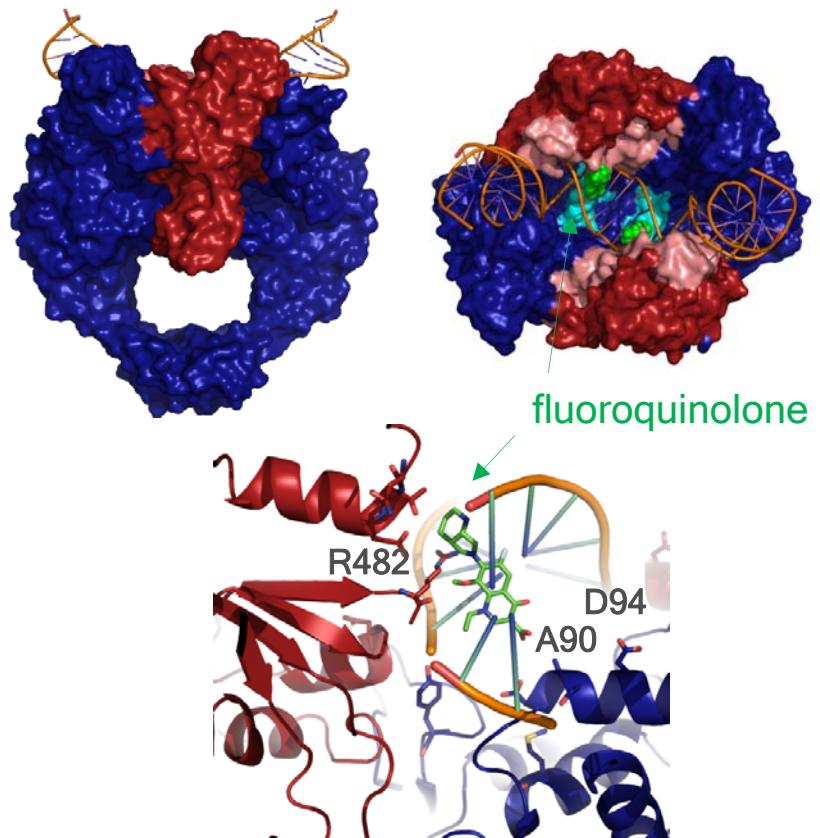
# *M. tuberculosis* DNA gyrase

The unique type II topoisomerase in *Mycobacterium tuberculosis*

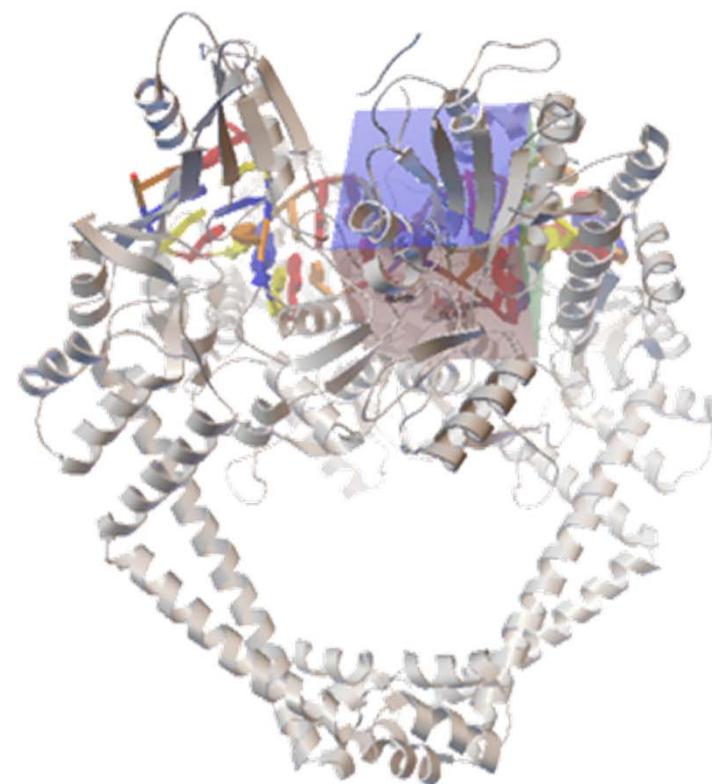
*In silico* studies of FQR mechanisms

collaboration G. André-Leroux, INRA

Binding energies as a tool to predict resistance phenotype



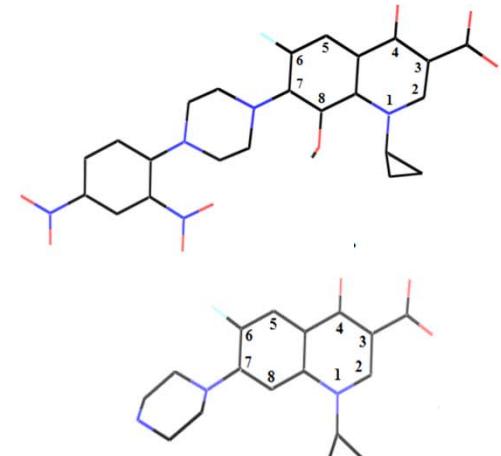
Atypical binding mode



Wild type or mutants



AutoDock 4

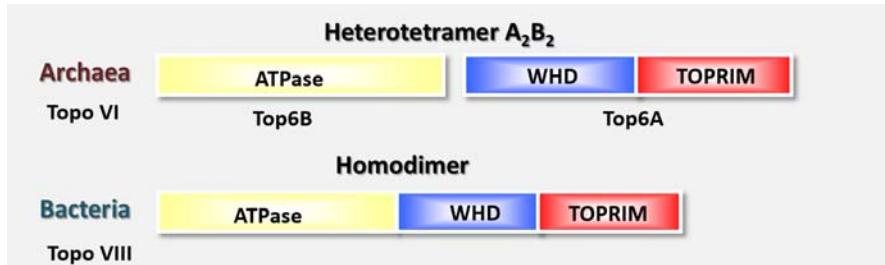




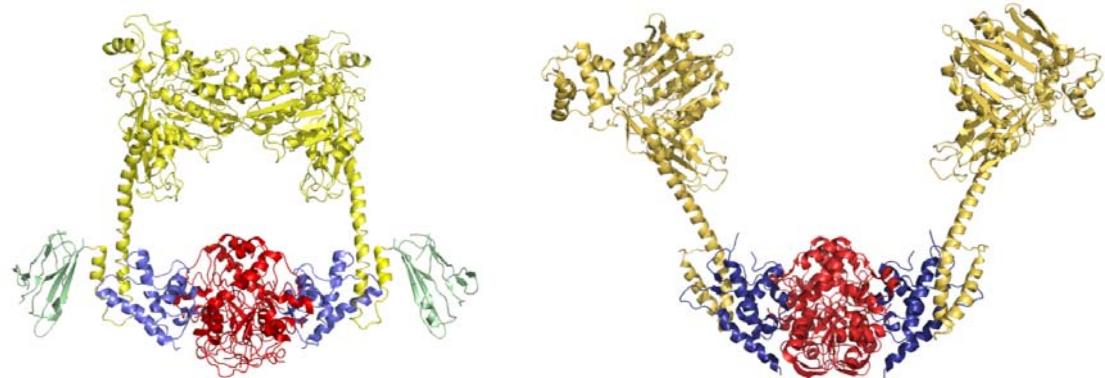
# Type IIB topoisomerases

Topoisomerase VIII, a new member of the type IIB family

Homology modeling

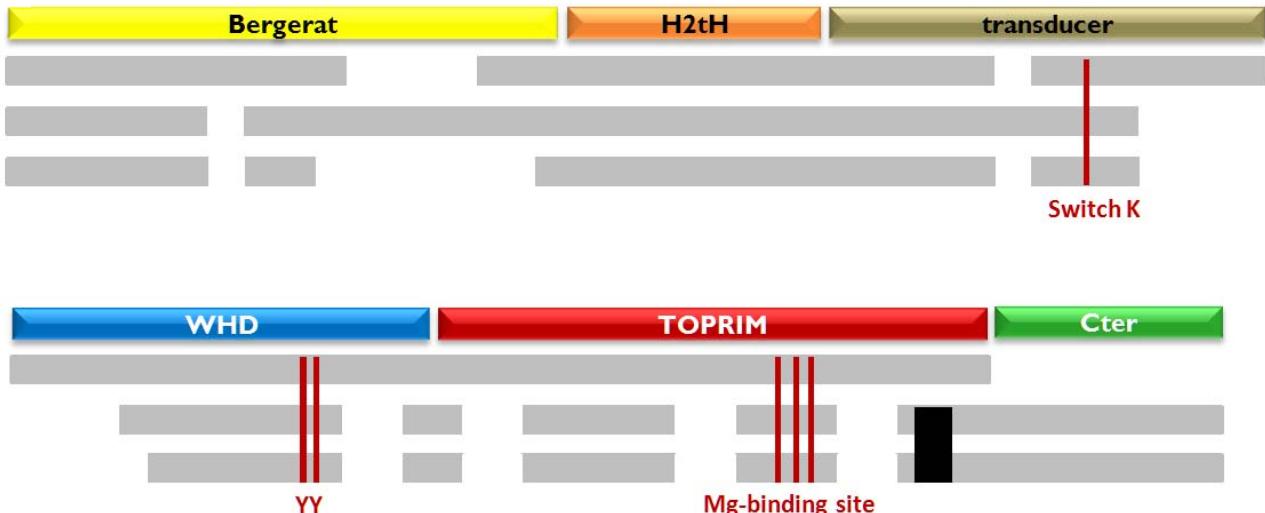


collaboration P. Forterre, UPSud & IP



Plasmid maintenance and transfer

	bergerat	H2TH	transducer	WHD	TOPRIM	Cter
Top6SS	229	78	222	157	232	-
Adeg	243	78	161	80	200	111
Mmar	187	74	144	82	207	89
pPpol	179	75	141	80	185	90

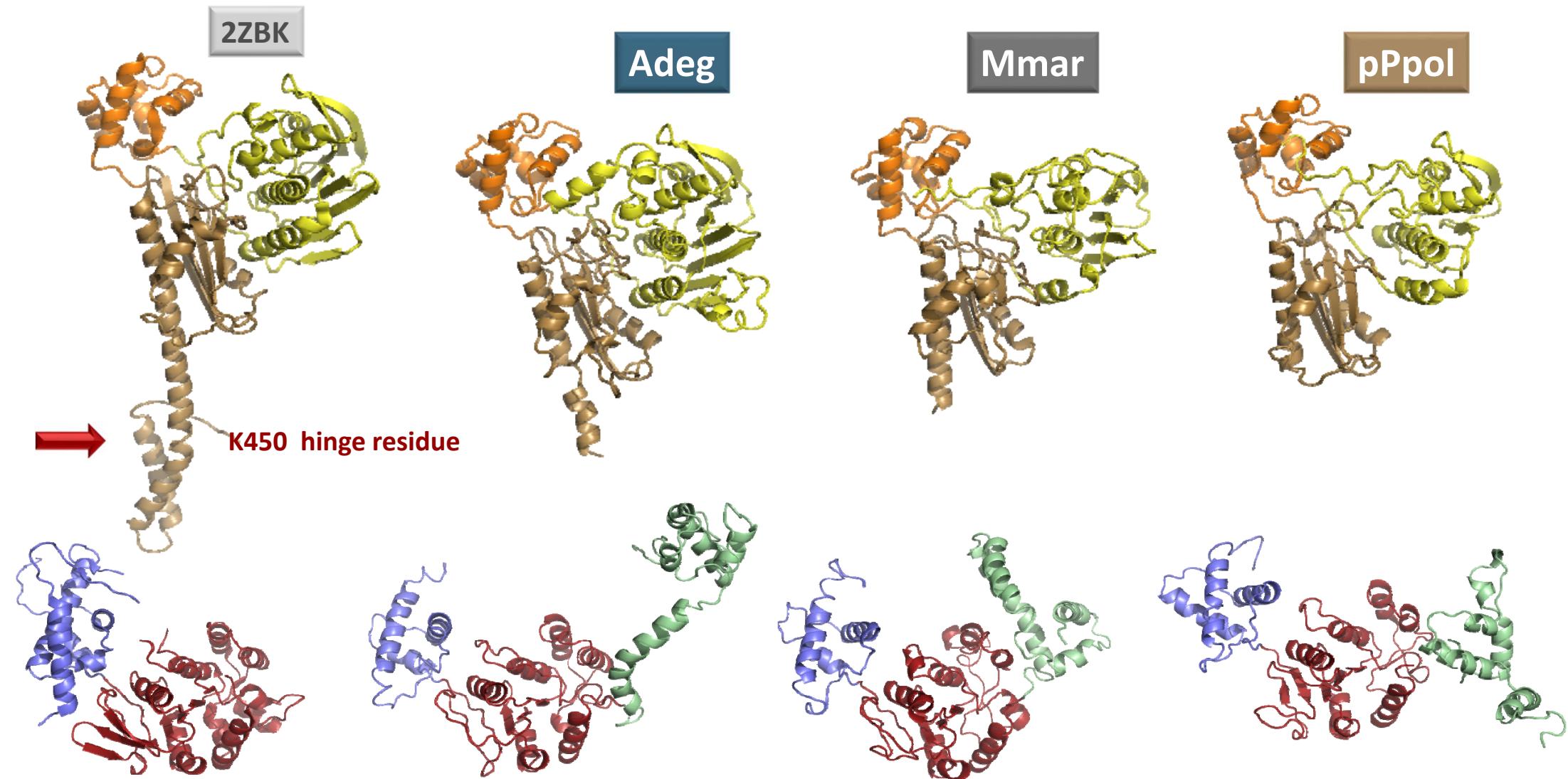


Schematic multiple alignment



# Type IIB topoisomerases

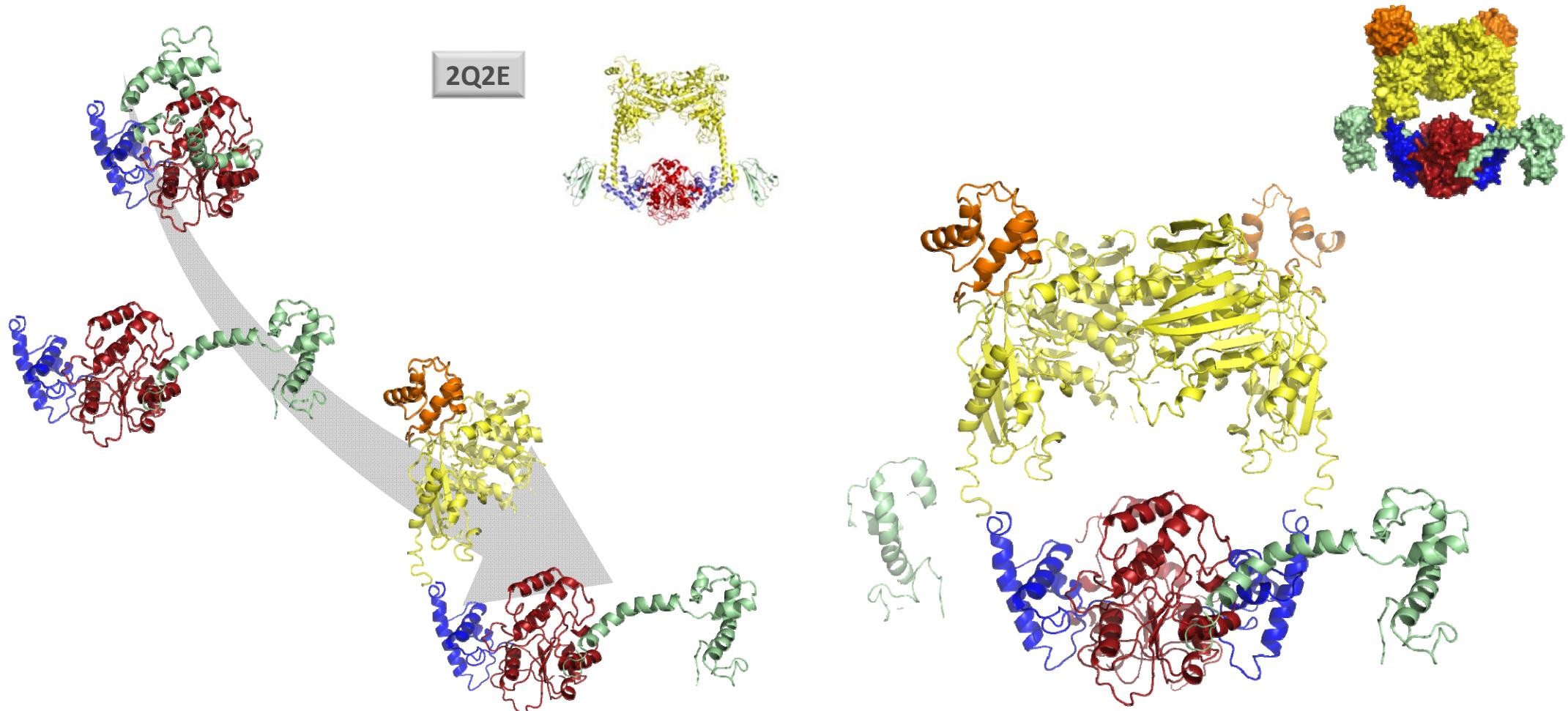
3D-models built using homology modeling





# Type IIB topoisomerases

Modeling of the heterotetramer



**Many uncertainties concerning domain-domain and subunit-subunit interfaces!**



# Type IIB topoisomerases

The meiotic topoisomerase-like complex

Remote homology modeling

collaboration M. Grelon, INRA



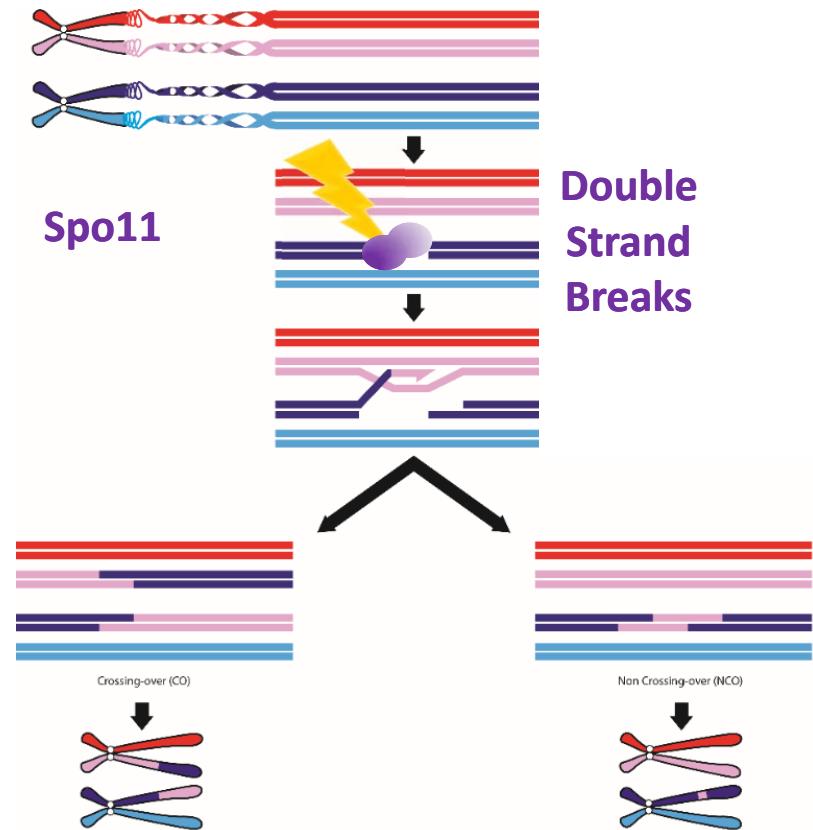
letters to nature  
**An atypical topoisomerase II from archaea with implications for meiotic recombination**

Agnès Bergerat\*, Bernard de Massy†, Danielle Gadelle\*,  
Paul-Christophe Varoutas†, Alain Nicolas†  
& Patrick Forterre\*

\* Institut de Génétique et Microbiologie, Université Paris Sud, CNRS URA 1354,  
GDR 1006, 91405 Orsay Cedex, France

† Institut Curie, Section de Recherche, CNRS UMR144, 26 rue d'Ulm,  
75248 Paris Cedex 05, France

The DSB are catalyzed by Spo11

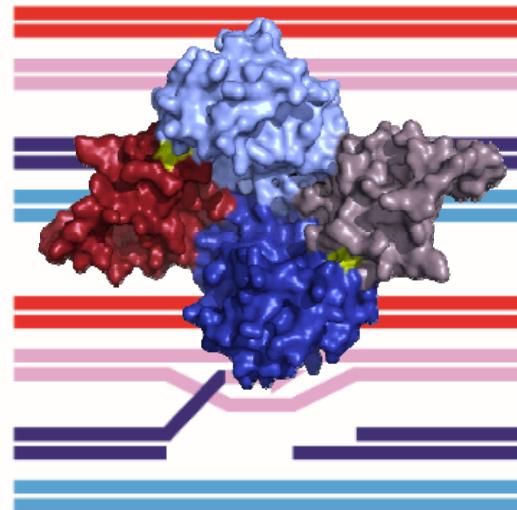




# Type IIB topoisomerases

## The meiotic topoisomerase-like complex

In *A. thaliana*, 2 Spo11 proteins form a heterodimer that catalyse meiotic DSB

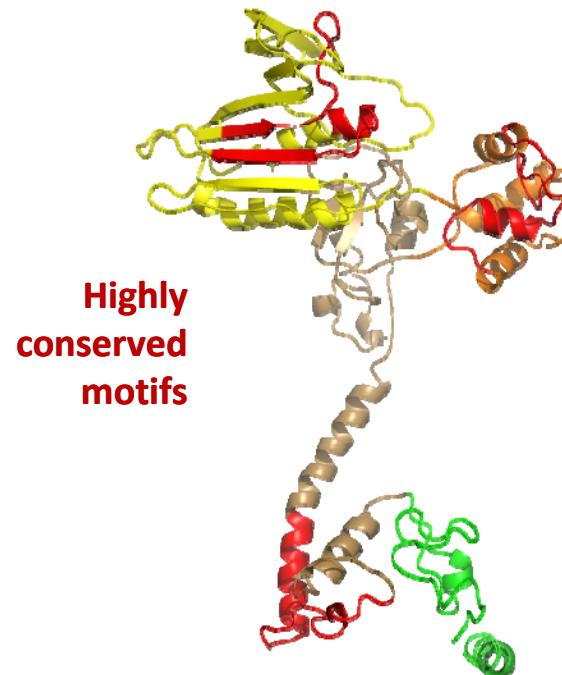


Does the Spo11  
heterodimer need a  
partner?

## Genetic screen

A 493 aa protein highly conserved in  
flowering plant (*Magnoliophyta*)

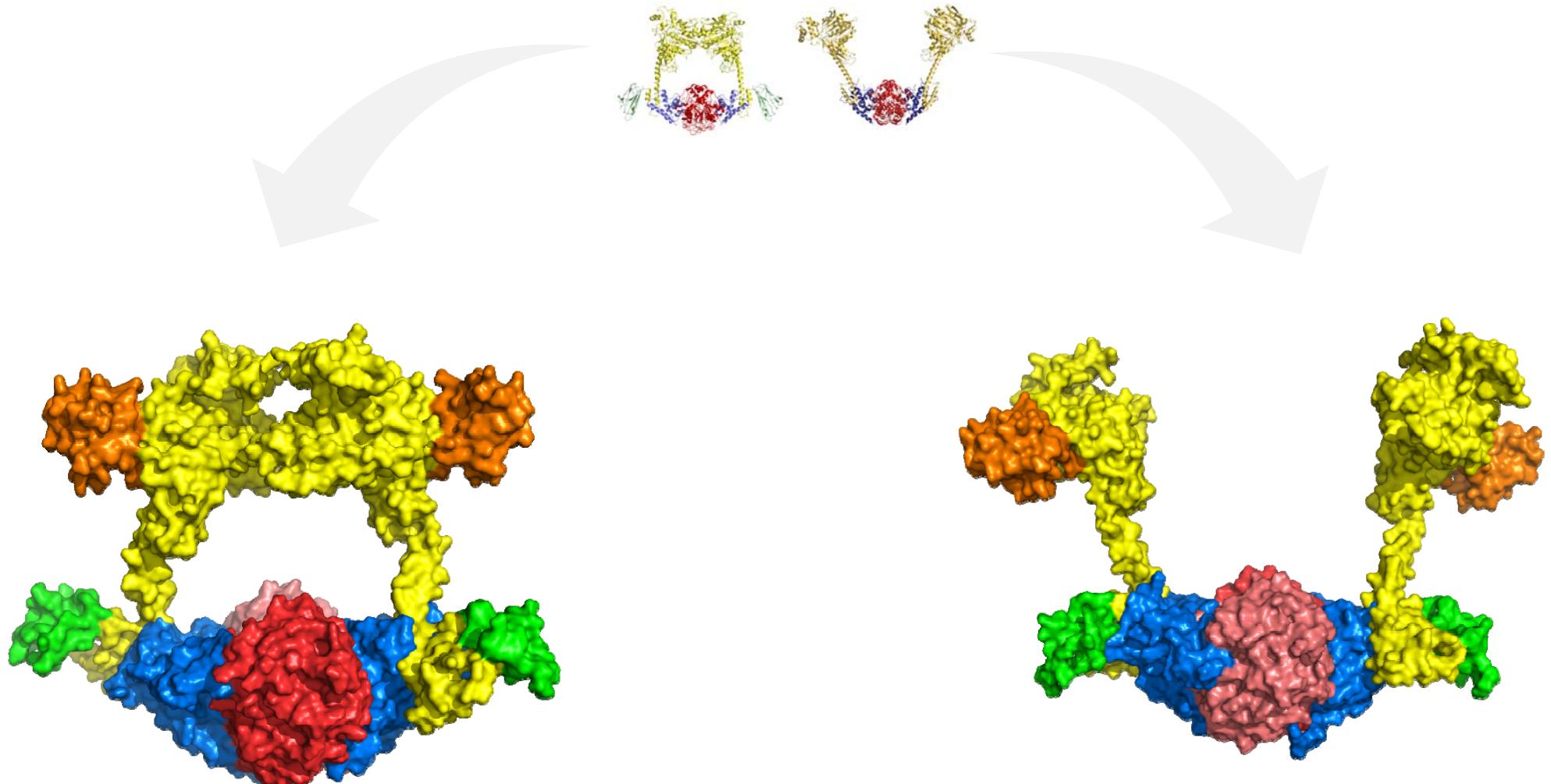
HHPred searches detected structural homology Top6B





# Type IIB topoisomerases

The meiotic topoisomerase-like complex





# Conclusion and perspectives

Experimental and *in silico* structural studies are highly complementary to gain insight into the structure of complex macromolecular assemblies

- **Sequence-structure-solubility relationships**

- Spo11 vs archaeal Top6A

- **Sequence-structure-interaction relationships**

- Spo11 homodimerisation vs heterodimerisation
  - Subunit A - subunit B interactions

- **Sequence-structure-pocket geometry relationships**

- ATP-binding pocket and inhibition mechanisms

- **Sequence-structure-resistance phenotype relationships**

- Resistance phenotype = f(aa substitution, fluoroquinolone)

# Thank you

Jérémie Piton  
Mélanie Roué  
Amélie Darmon  
Marcela França Penna  
Alexandre Guignard  
Thomas Zapf  
Cédric Pissis  
Geneviève Janvier  
Elodie Leroy  
Elisa Quiot  
Stéphanie Petrella

UMS  
Pedro Alzari

Laboratoire de  
Bactériologie  
UPMC  
Alexandra Aubry  
and coworkers



Mti, Université Paris Diderot  
Leslie Regad

MaIAGE, INRA  
Gwenaëlle André-Leroux

BMGE, IP-Université Paris Saclay  
Patrick Forterre and coworkers

Institut Jean-Pierre Bourgin, INRA  
Mathilde Grelon and coworkers

ANR

Institut Pasteur

PTR n°367

Île de France

DIM Malinf

PFBMI

Bertrand Raynal, Bruno Baron, Patrick England

PF6

Patrick Weber, Rafael Navaza, Ahmed Haouz



# Main publications

- A. Bouige, A. Darmon, J. Piton, M. Roué, S. Petrella, E. Capton, P. Forterre, A. Aubry, and C. Mayer. (2013). *Mycobacterium tuberculosis* DNA gyrase possesses two functional GyrA-boxes. *Biochem J.*, 455 (3), 285-294.
- A. Agrawal, M. Roué, C. Spitzfaden, S. Petrella, A. Aubry, MM. Hann, B. Bax, and C. Mayer. (2013). *Mycobacterium tuberculosis* DNA gyrase ATPase domain structures suggest a dissociative mechanism that explains how ATP hydrolysis is coupled to domain motion. *Biochem J.*, 456 (2), 263-273.
- C. Mayer and Y.L. Janin. (2014). Non-quinolone inhibitors of bacterial type IIA topoisomerases: a feat of bioisosterism. *Chem Rev.*, 114 (4), 2313-2342.
- D. Gadelle, M. Krupovic, K. Raymann, C. Mayer, and P. Forterre. (2014). DNA topoisomerase VIII: a novel subfamily of type IIB topoisomerases encoded by free or integrated plasmids in Archaea and Bacteria. *Nucleic Acids Res.*, 42 (13), 8578-8591.
- N. Vrielynck, A. Chambon, D. Vezon, L. Pereira, L. Chelysheva, A. De Muyt, C. Mézard, C. Mayer, and M. Grelon. (2016). A DNA topoisomerase VI-like complex initiates meiotic recombination. *Science*, 351 (6276), 939-943.