RéNaFoBiS Réseau National de Formation en Biologie Structurale Intégrative

Spécificité et Complémentarité des méthodes de la biologie structurale
Illustration par un exemple

Type II topoisomerases

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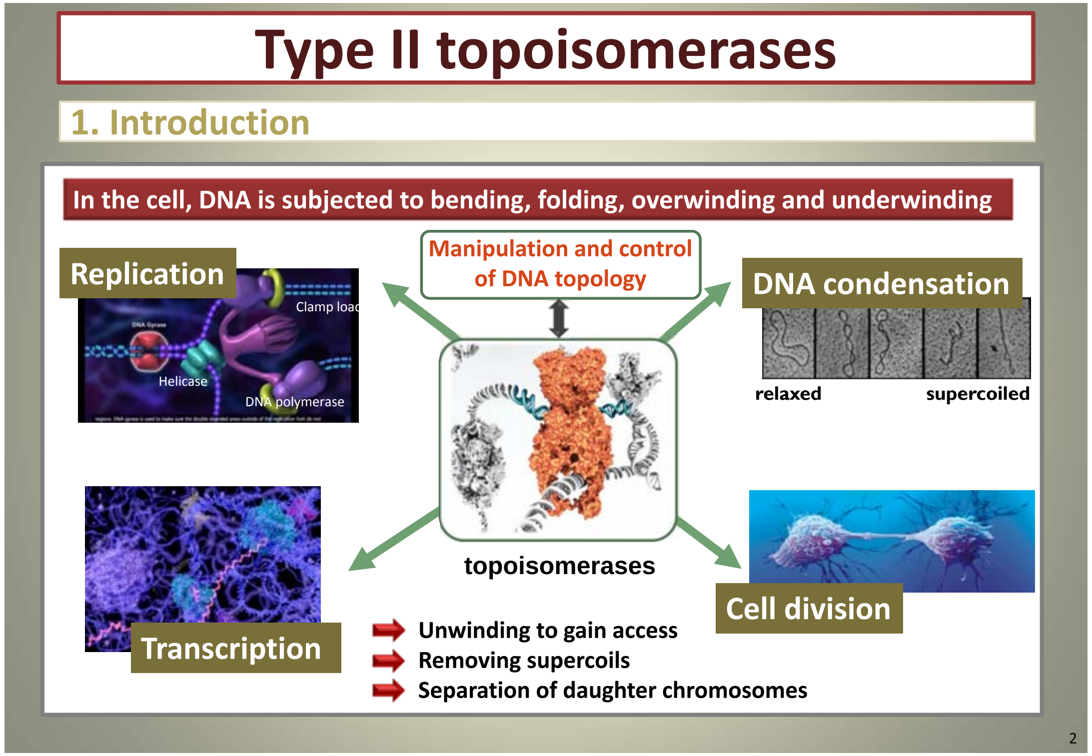
20-27 mai 2016



Type II topoisomerases

1. Introduction

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

Manipulation and control of DNA topology

Replication

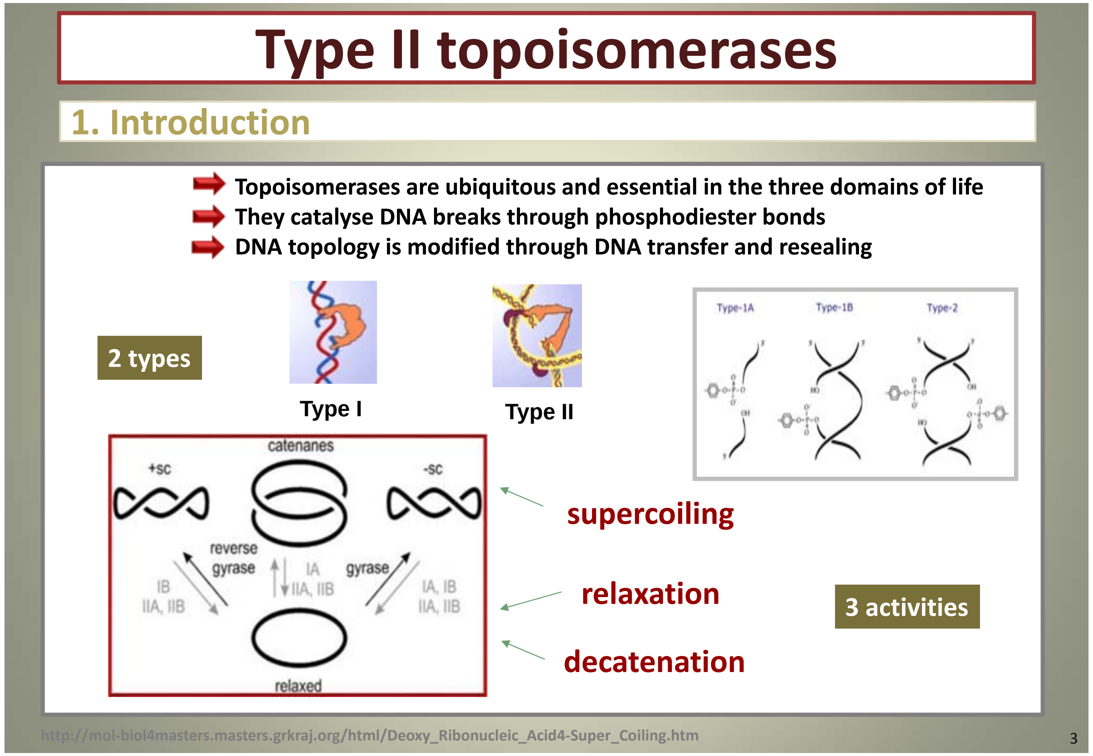
DNA condensation

topoisomerases

Transcription

Cell division

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



Type II topoisomerases

1. Introduction

- Topoisomerases are ubiquitous and essential in the three domains of life
- They catalyse DNA breaks through phosphodiester bonds
- DNA topology is modified through DNA transfer and resealing

2 types

Type I

Type II

supercoiling

relaxation

decatenation

3 activities

http://mol-biol4masters.masters.grkraj.org/html/Deoxy_Ribonucleic_Acid4-Super_Coiling.htm

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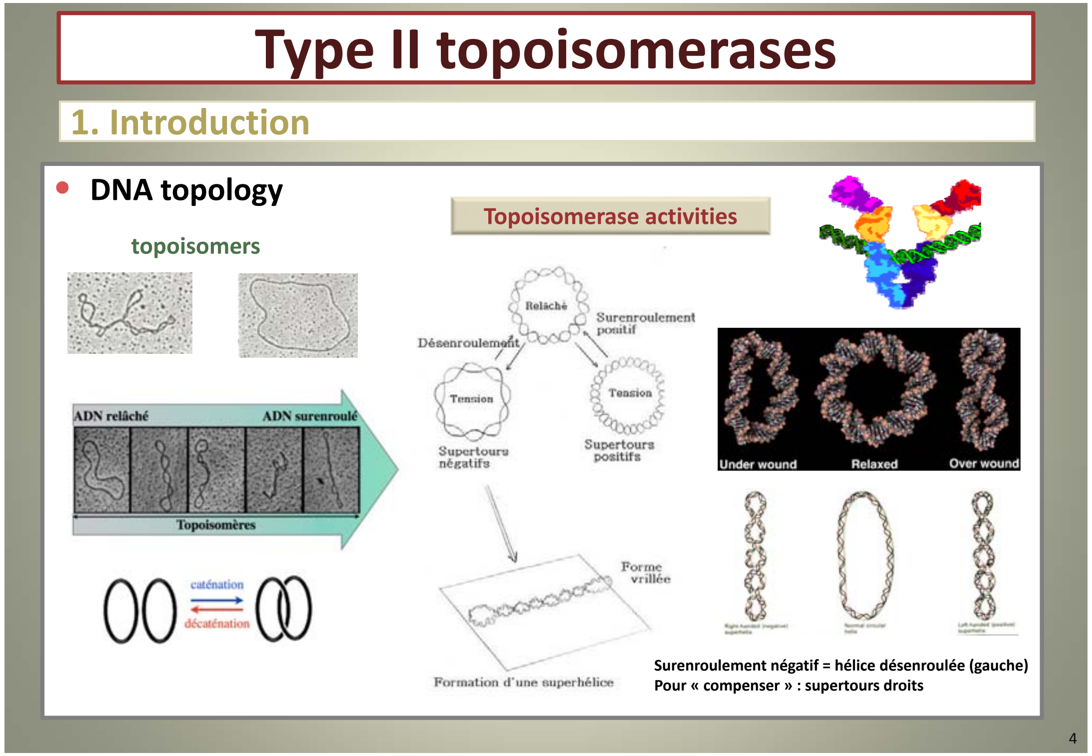


Type II topoisomerases

1. Introduction

- DNA topology

topoisomers

Topoisomerase activities

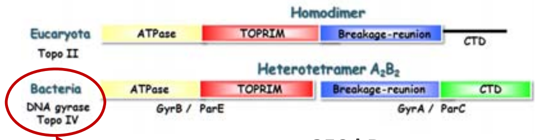
Surenroulement négatif = hélice désenroulée (gauche)
Pour « compenser » : supertours droits

Type II topoisomerases

1. Introduction

- **Phylogenetic distribution**

Type IIA



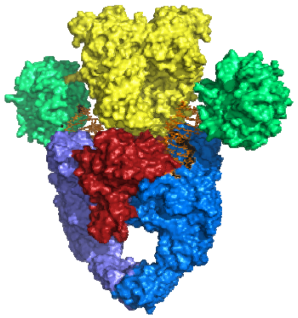
350 kDa

2 type II topoisomerases

DNA gyrase
negative supercoiling

Topo IV
decatenation

Topo II
relaxation & decatenation



Manual modeling of the global architecture

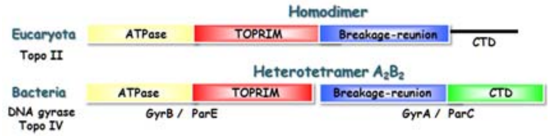
DNA gyrase
also found in plants, some
protists and archaea

Type II topoisomerases

1. Introduction

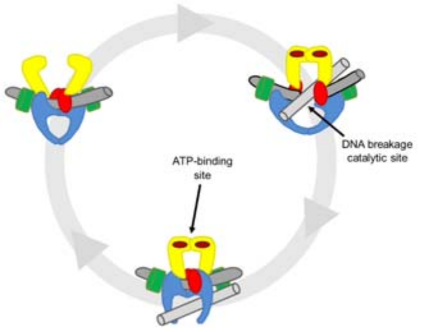
- **Architecture and catalytic cycle**

Type IIA

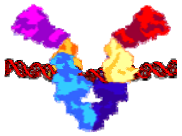


350 kDa

DNA gyrase	negative supercoiling
Topo IV	decatenation
Topo II	relaxation & decatenation



intramoléculaire



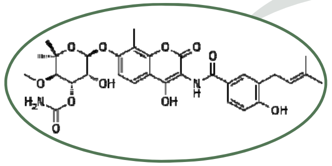
intermoléculaire

Type II topoisomerases

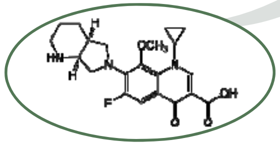
1. Introduction

Type II topoisomerases are drug targets

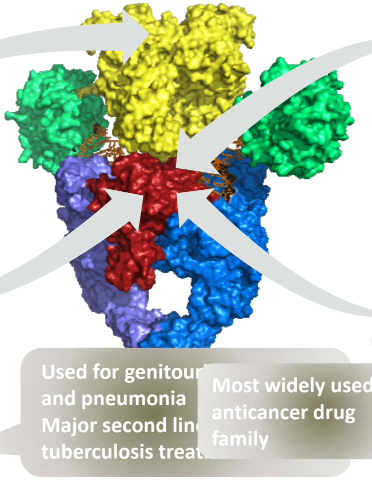
Antibiotics



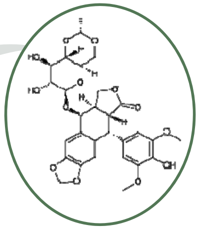
coumarines



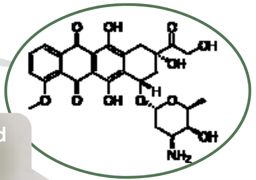
quinolones



Anticancer drugs



podophyllotoxines



anthracyclines

Used for genitouri
and pneumonia
Major second lin
tuberculosis treat

Most widely used
anticancer drug
family

Type II topoisomerases

1. Introduction

- **Problematics**

Multi-domain enzyme

Large size protein

Highly flexible et dynamic

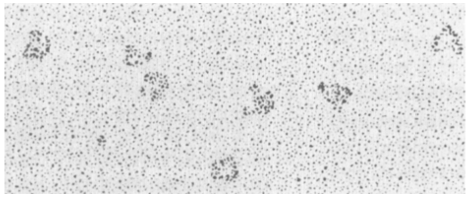
Obtain a maximum of structural data combining several methods from different sources (time, groups, …) in order to understand and to explain in 3D how this enzyme family is working

Type II topoisomerases

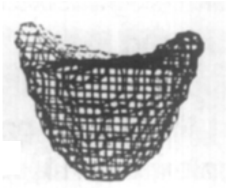
2. Premières informations structurales

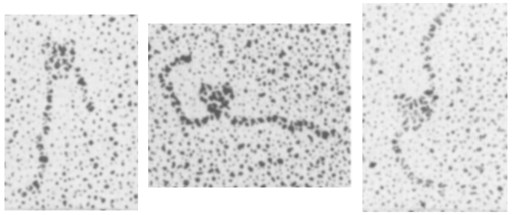
- **Electron Microscopy**

Kirchhausen et al, Cell 1985

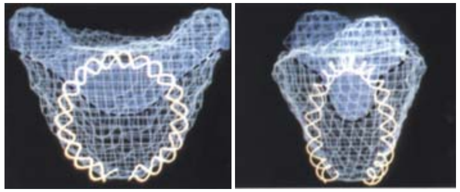


Heterotetramer of *M. luteus* DNA gyrase





DNA gyrase in complex with DNA (541 pb)



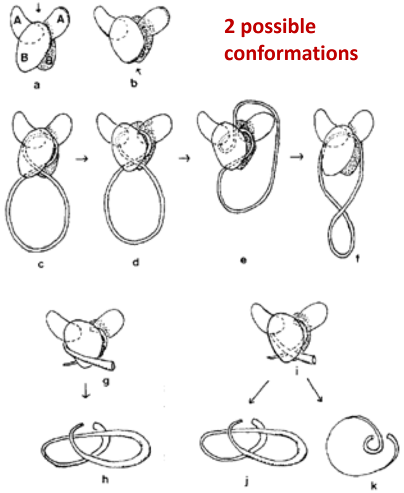
First envelop proposing a 3D model for the entire DNA gyrase

Type II topoisomerases

2. Premières informations structurales

- **Electron Microscopy**

Kirchhausen et al, Cell 1985



2 possible conformations

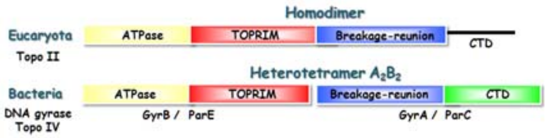
Possible Conformational States of DNA Gyrase and of the Gyrase-DNA Complex

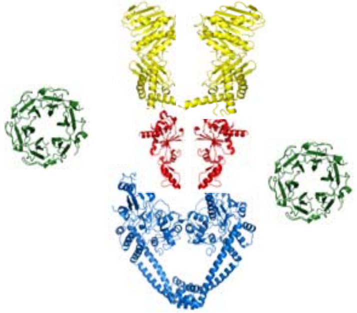
Schematic representation of arrangement of A and B subunits in DNA gyrase
(a) (c) closed A-A contact and a open B-B contact (c) + DNA
(b) (d) open A-A contact and a closed B-B contact (c) + DNA
Note that opening the A-A contact in (d) involves double-strand scission

The loop of DNA is shown contained between lobes of the two B subunits. Alternatively, in (g), the arms of the DNA loop pass across the outer surface of the B subunits. (g-h) show one way in which strand passage can occur. A segment of the closed circular DNA passes through the open A-A contact and through the double-strand break. The gyrase molecule is omitted in (h) for clarity. This picture is consistent with either mode of B subunit/DNA interaction, as illustrated in (i) and (j). Completion of a reaction following this course would require closing the A-A contact and opening the B-B contact to allow exit of the inserted segment.
(d-f) show an alternative picture, consistent only with the S subunit/DNA binding mode shown in (d) and (i). One of the two arms of the DNA loop, constrained between B subunits and trapped by closure of the B-B contact, diffuses through the double-strand break (e). Completion of the reaction cycle requires rearrangement of the DNA after strand passages, closure of the A-A contact to open the B-B contact (f), and reformation of a bound DNA loop as in (d).

Type II topoisomerases

First step : crystal structure of isolated domains





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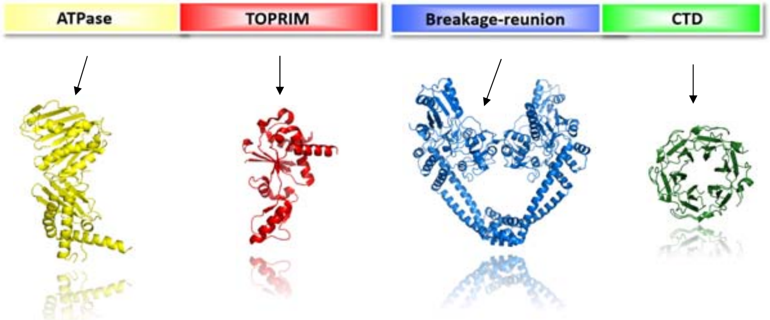
Type II topoisomerases

Structure-function relationships of the *M. tuberculosis* DNA gyrase isolated domains



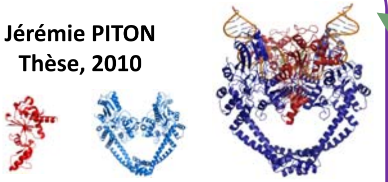
Roué et al. 2013 Agrawal, Roué et al. 2013	Piton et al. 2010	Piton et al. 2009 Piton et al. 2010	Darmon et al. 2012 Bouige, Darmon et al. 2013
3ZM7	**3IG0** **3M4I**	**3IFZ**	**4G3N**

Type II topoisomerases

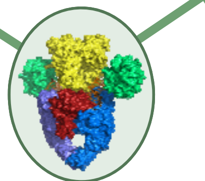
Structure-function relationships of the *M. tuberculosis* DNA gyrase isolated domains

Catalytic core

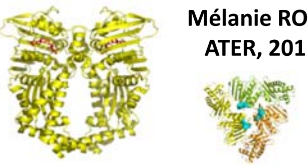
Jérémie PITON
Thèse, 2010



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

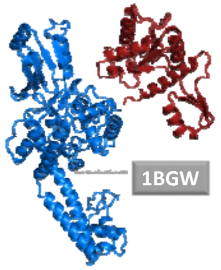
Type II topoisomerases

3. Crystallography : structures of the isolated domains

- **Breakage-reunion and TOPRIM domains**

1st structures

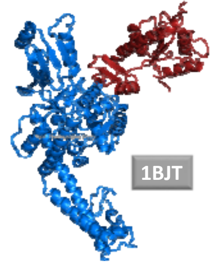
1996 : BRD + TOPRIM of *S. cerevisiae* topo II 1BGW
1997 : BRD *E. coli* de DNA gyrase 1AB4
1999 : BRD + TOPRIM of *S. cerevisiae* topo II 1BJT



Berger et al, Nature 1996

Cabral et al, Nature 1997

Fass et al, NSB 1999

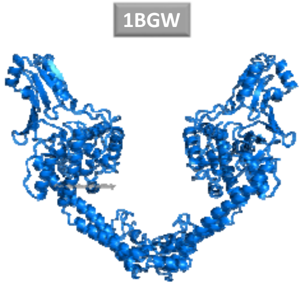


Conserved structure of the BRD organised in 4 regions

Type II topoisomerases

3. Crystallography : structures of the isolated domains

- **Breakage-reunion and TOPRIM domains**



Structural dynamics of the gates

The BRD is a heart-shaped dimer with 2 gates and a cavity for DNA passage

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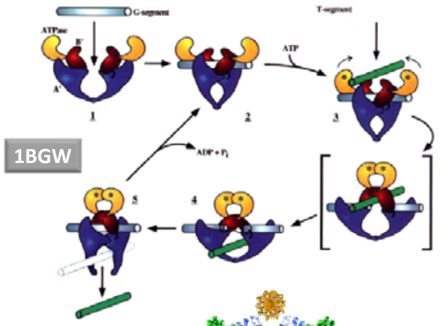
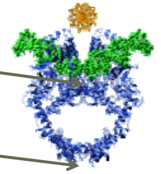
Type II topoisomerases

3. Crystallography : structures of the isolated domains

- **Breakage-reunion and TOPRIM domains**



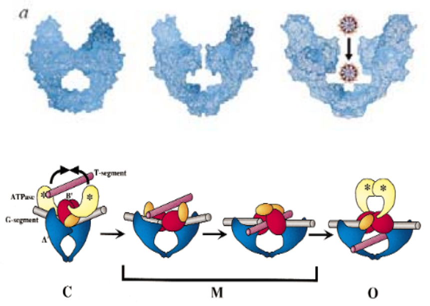


Porte ADN

T-DNA segment transfert

Porte C

Evacuation of T-DNA segment



Closing/opening mecanism of the DNA gate and DNA passage

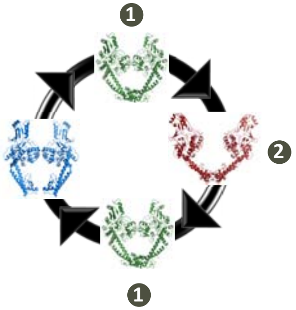
Type II topoisomerases

3. Crystallography : structures of the isolated domains

- **Breakage-reunion and TOPRIM domains**

Dynamique structurale

Drug design

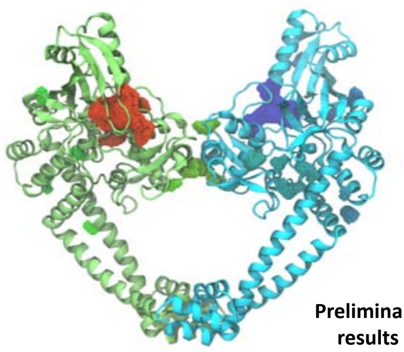


Modeling of the 3 conformations for *Mtb* gyrase

Calculations of transition path using the Path Optimization and Exploration approach

Calculations of the cavities during the structural transitions

Structural insights into DNA- and C-gate opening/closing



Preliminary results

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Thesis Nathan DESDOUITS

Type II topoisomerases

3. Crystallography : structures of the isolated domains

- **The ATPase domain**

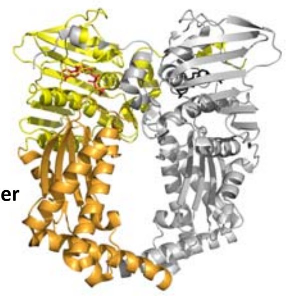
2000 : ATPase of *E. coli* DNA gyrase 1EI1

2002 : ATPase of *T. thermophilus* DNA gyrase 1KIJ

1EI1

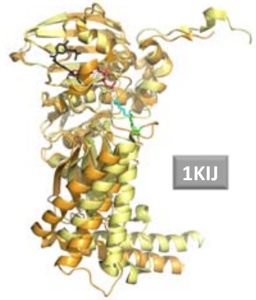
GHKL

transducer



Brino et al, JBC 2000

ANP



1KIJ

Lamour et al, JBC 2002

novobiocin

- Today, > 80 structures of ATPase domains or GHKL
- ***Structure-based drug design*** : new inhibitors targeting ATP hydrolysis

Structural dynamique of GHKL/transducer

The ATPase dimer is a dimer and includes the N-gate

Type II topoisomerases

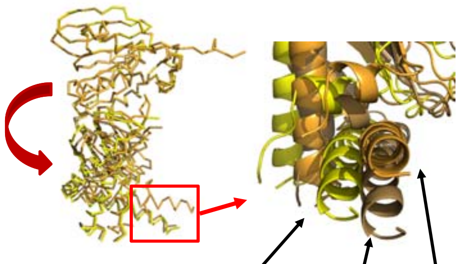
3. Crystallography : structures of the isolated domains

- **The ATPase domain**

2013 : ATPase of *M. tuberculosis* DNA gyrase 3ZM7

3ZM7

The ATP-relaxed conformational state



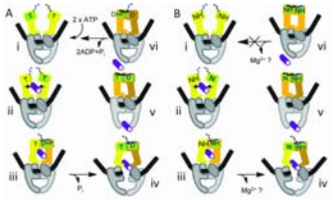
New conformational state ATP-relaxed (RelaxT) ?

Agrawal et al, Biochem J 2013

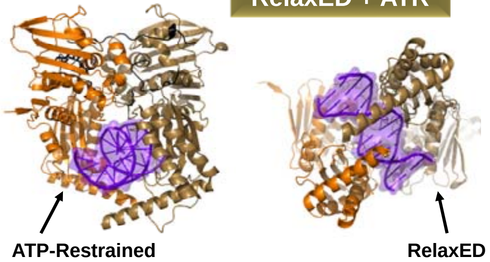
Type II topoisomerases

3. Crystallography : structures of the isolated domains

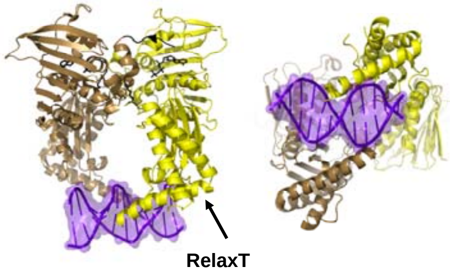
- **The ATPase domain**



RelaxED + ATR



RelaxT + RelaxED



The RelaxT conformation allows T-segment passage

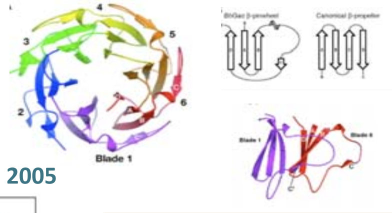
Type II topoisomerases

3. Crystallography : structures of the isolated domains

- **CTD**

1SUU

2004 : CTD of *B. burgdorferi* DNA gyrase 1SUU
2005 : CTD of *E. coli* topo IV 1ZVT



DNA gyrase : 6 blades
Topo IV : 4 to 8 blades

Corbett et al, PNAS 2004

Corbett et al, JMB 2005

ADN gyrase Topo IV

1ZVT

Responsible for the DNA gyrase / topo IV function specificity

The CTD has a β-propeller fold

Type II topoisomerases

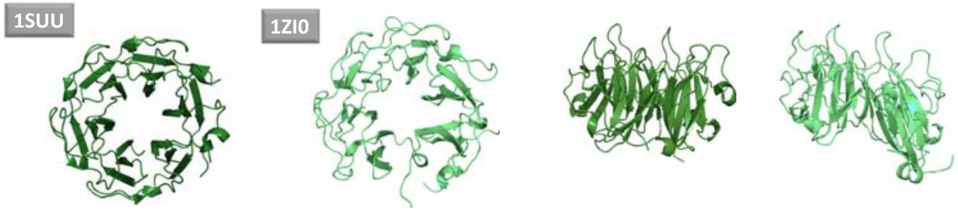
3. Crystallography : structures of the isolated domains

- **CTD**

2005 : CTD of *E. coli* DNA gyrase 1ZI0

1SUU

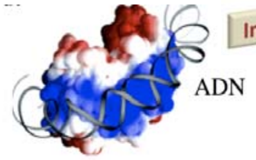
1ZI0

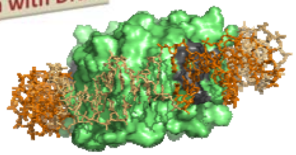


Ruthenburg et al, JBC 2005

2 conformations or 2 structures ?

Interaction with DNA

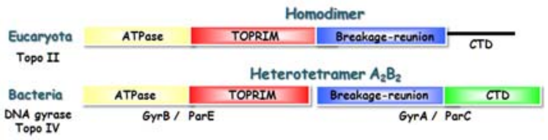




The CTD exists under 2 forms (flat and et spiral)

Type II topoisomerases

Second step : crystal structure of associated domains



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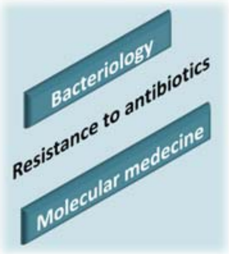
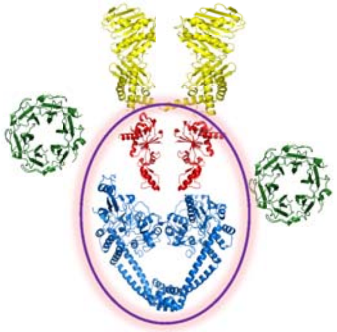




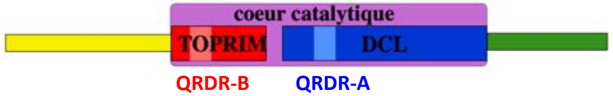
The catalytic core

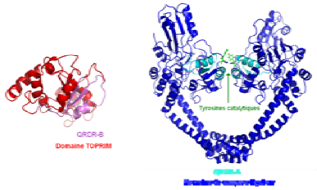
The catalytic core

Type II topoisomerases

4. Crystallography : structures of « associated » domains

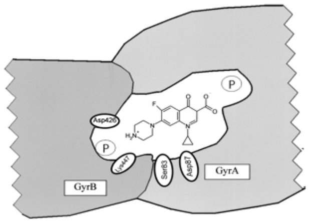
- **The DNA and quinolone binding pocket**





Yoshida *et al.*, 1990, 1991

Sequencing of DNA gyrase from clinicla strains resistant to quinolones : mutations are localised in 2 specific regions named Quinolone Resistant Determining Regions



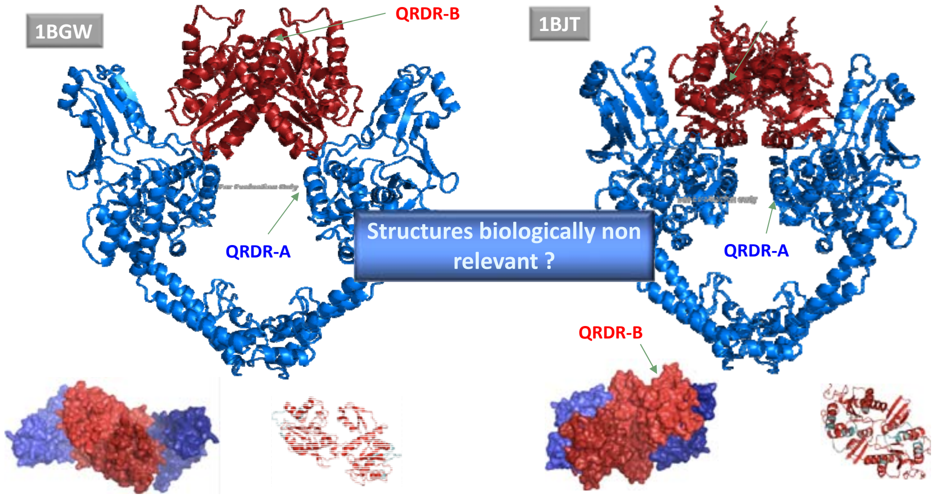
There should be a unique quinolone binding pocket localised at the 2 subunit interface

Heddle & Maxwell, 2002

Type II topoisomerases

4. Crystallography : structures of « associated » domains

- **The DNA and quinolone binding pocket**



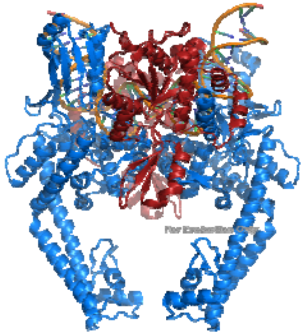
Structures biologically non relevant ?

Type II topoisomerases

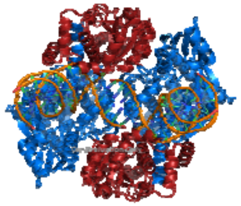
4. Crystallography : structures of « associated » domains

- **The catalytic core**

The 2 structures BRD+TOPRIM did not bring any information with respect to QBP
2007 : 1st structure of BRD+TOPRIM in complex with DNA



Topo II of *S. cerevisiae*



DNA is highly curved

2RGR

The 2 TOPRIM are on each side of the BRD dimer
the QRDR are spatially close

Dong & Berger, Nature 2007

Type II topoisomerases

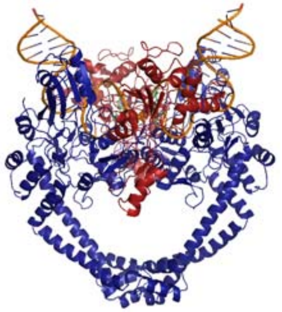
4. Crystallography : structures of « associated » domains

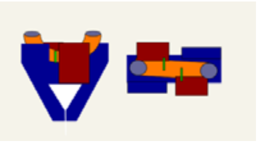
- **The catalytic core**

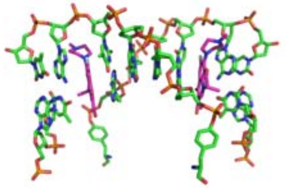
2009 : 1st structures of BRD+TOPRIM with DNA and fluoroquinolones

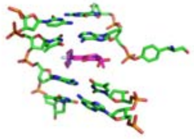
Topo IV of *S. pneumoniae*

3FOF









Quinolones inhibit DNA religation

Same organisation in presence of quinolone
Mode of action of quinolones

Laponogov et al, NSMB 2009

Type II topoisomerases

4. Crystallography : structures of « associated » domains

- **Le domaine TOPRIM**

2007 : 1st structure of DCL+TOPRIM with DNA
2009 : structure of TOPRIM alone : non biological dimer !!

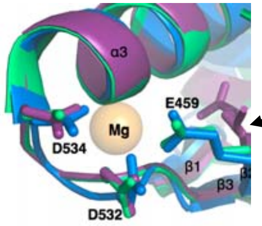
Fu et al, NAR 2009

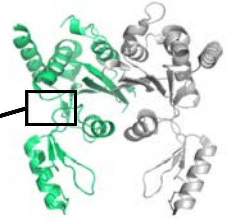
2ZJT

Toprim fold

Specific to topoisomerase

Mg binding site





Yeast Toprim TopBK / Ca TopBK / Mg

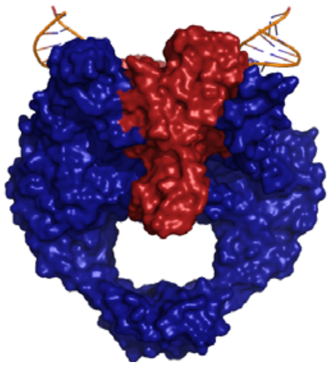
M. tuberculosis DNA gyrase

Magnesium binding site and mechanism

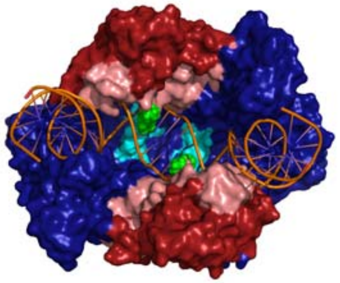
Type II topoisomerases

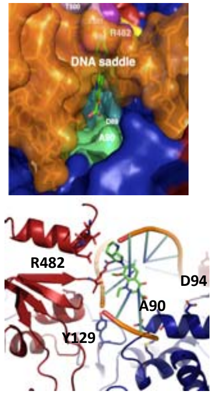
4. Crystallography : structures of « associated » domains

- **The catalytic core**



Clinical applications





QBP = QRDR-A + QRDR-B + ADN

Piton et al, Plos One 2010

Better understanding of quinolone resistance mechanism

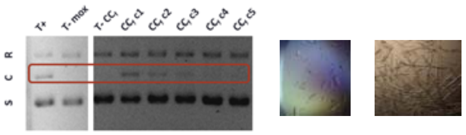
Type II topoisomerases

4. Crystallography : structures of « associated » domains

- **The catalytic core**

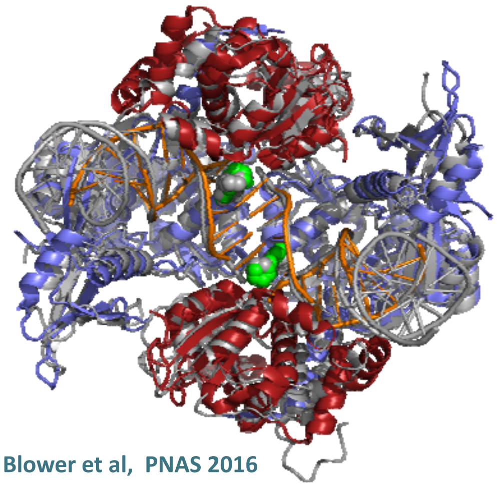
Stéphanie PETRELLA
Marcela FRANÇA

GyrB TopRIM (448-675) + GyrA BRD (1-502)
i.e. **730 aa for the fusion construct**



The CC_f possesses cleavage activity

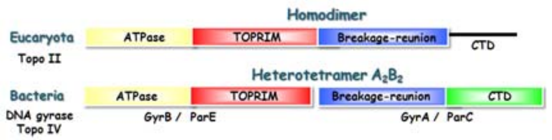
- ≈ 700 crystallization conditions tested
- 8 Å resolution max
- Instability of the ternary complex



Blower et al, PNAS 2016

Type II topoisomerases

Third step : SAXS enveloppe of entire subunits



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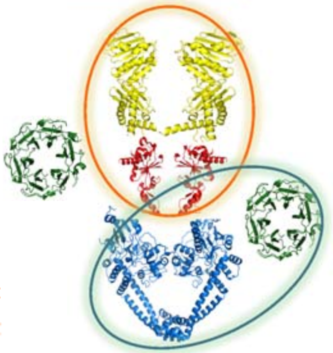


B subunit

A subunit

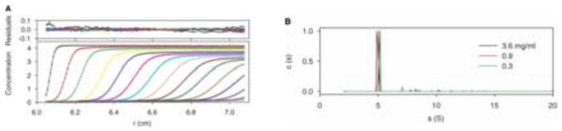
Type II topoisomerases

5. SAXS data on individual subunits

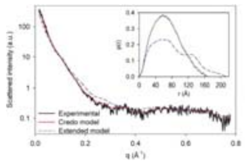
- **The A subunit**

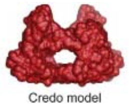
Costenaro et al, Structure 2005

2005 : 1st SAXS envelop of an entire subunit



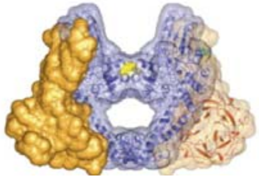
La sous-unité A est dimérique

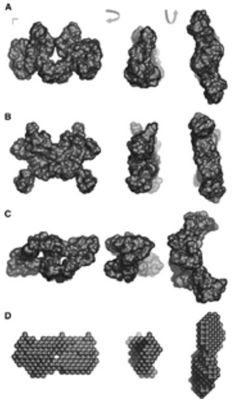




Credo model

Extended model





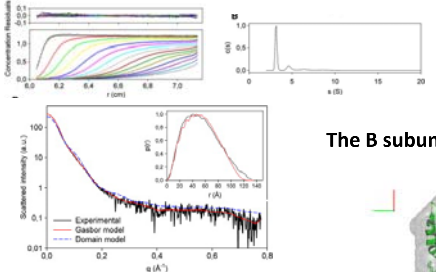
CTD/BRD orientation ??

Type II topoisomerases

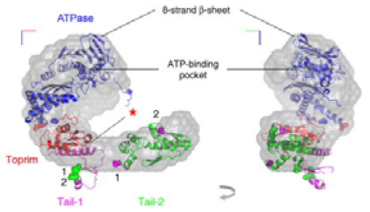
5. SAXS data on individual subunits

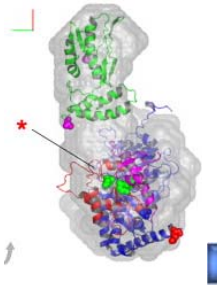
- **The B subunit**

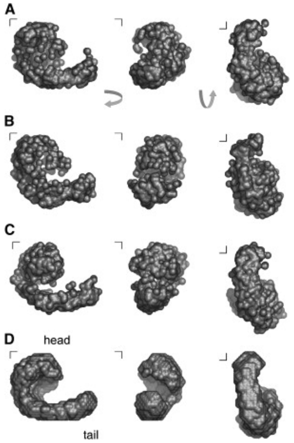
Costenaro et al, Structure 2007



The B subunit is a monomer



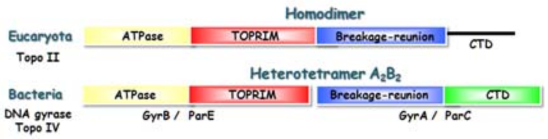


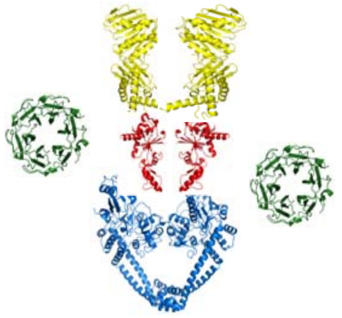


TOPRIM/ ATPase orientation ??

Type II topoisomerases

Fourth step : toward the structure of an entire enzyme ?





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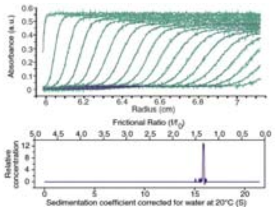
Type II topoisomerases

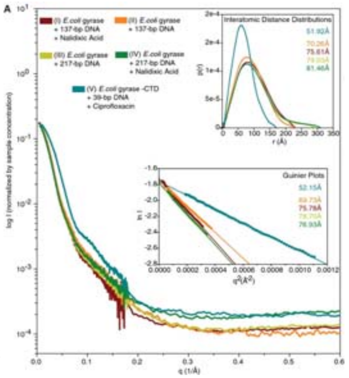
6. SAXS data of the entire enzyme



ADN 137 pb

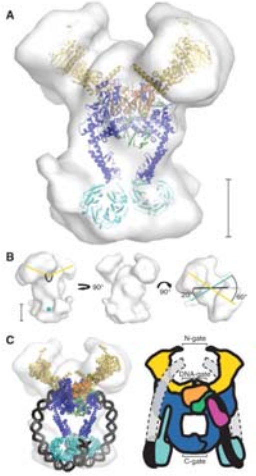
The full-length enzyme is dimeric

Baker et al, NAR 2010



R_H = 70-80 Å

CTD localised at the C-gate : wrong position !!

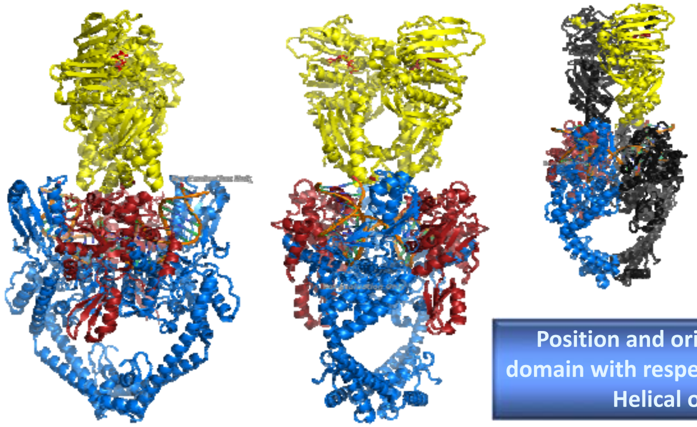


Type II topoisomerases

7. Towards a crystal structure of the entire enzyme ?

- **The 3 domains : ATPase + TOPRIM + BRD**

Schmidt et al, NSMB 2012



Low resolution

4.4 Å

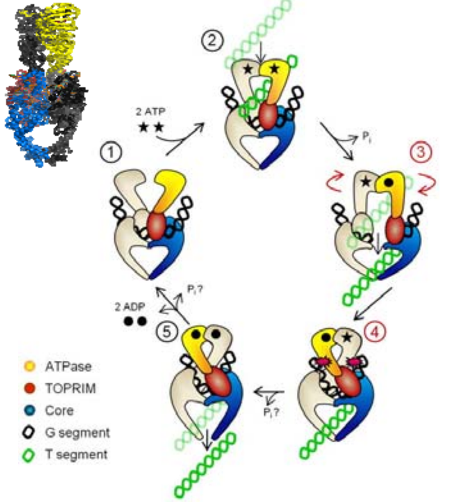
24.1 % / 27.5 %

Position and orientation of ATPase domain with respect to catalytic domain
Helical organisation

Type II topoisomerases

7. Towards a crystal structure of the entire enzyme ?

- **The 3 domains : ATPase + TOPRIM + BRD**



Unexpected complexities in the type IIA topoisomerase catalytic cycle

(1) Homodimer at the beginning of cycle
The enzyme binds and bends a G-segment (black)
(2) The binding of two ATP (stars) promotes T-segment capture (green).
(3) Hydrolysis of one ATP to ADP (circle) leads to G-segment cleavage and T-segment transport through the break
Following strand transport the ATPase domains swivel about each other to impede backward translocation of the T-segment
(4) G-segment ends are brought back together for relegation
K-loop contacts are proposed to trigger hydrolysis of the second ATP
(5) T-segment escape, followed by ADP release, resets the enzyme

Steps (3) and (4) are highlighted in red to denote the new findings from this work

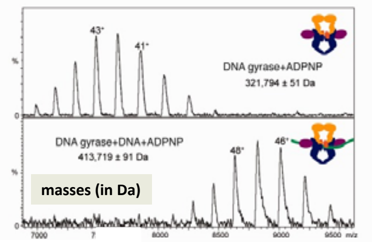
- **as many prokaryotic type IIA topoisomerases appear to lack a K-loop, interactions between this region and G-segment DNA (step 4) may be limited to eukaryotic topo II homologs**
- **The precise point of P_i release following the second hydrolysis step is not known (question marks).**

Type II topoisomerases

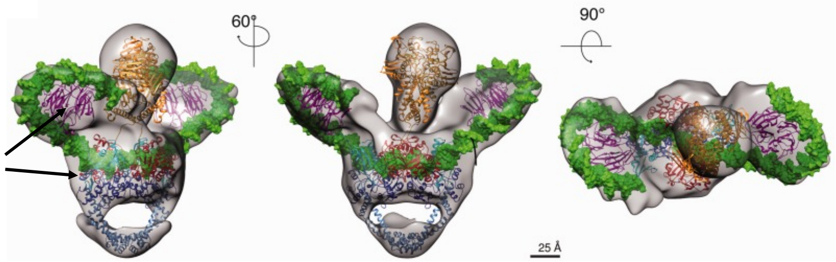
8. Electron microscopy électronique : structure entière

- **Electron microscopy, 30 years later …**

Papillon et al, NAR 2013



Native MS on the holoenzyme and DNA-bound complex showing the presence of the dimeric A_2B_2 form of the fusion DNA gyrase in presence of ADPNP and formation of the complex with DNA (green) as shown by the total shift of the mass spectra.



Structure of isolated domains

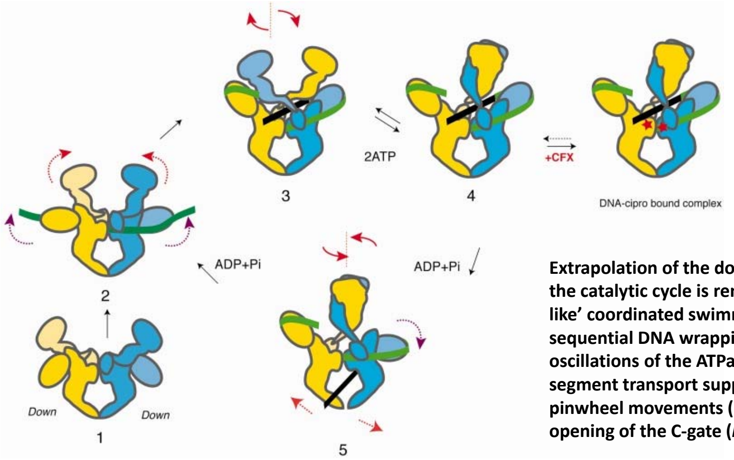
Type II topoisomerases

8. Microscopie électronique : structure entière

- **Electron microscopy, 30 years later …**

Papillon et al, NAR 2013

Catalytic cycle during the supercoiling activity



Extrapolation of the domains positions during the catalytic cycle is reminiscent of a ‘crawl-like’ coordinated swimming movement with sequential DNA wrapping guided by oscillations of the ATPase domain (*head*), T-segment transport supported by the β-pinwheel movements (*arms*) and orthogonal opening of the C-gate (*legs*).

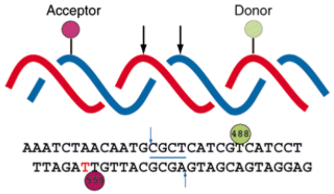
Type II topoisomerases

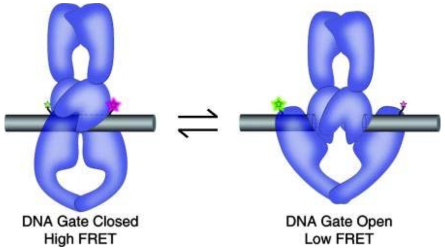
9. Autres techniques biophysiques

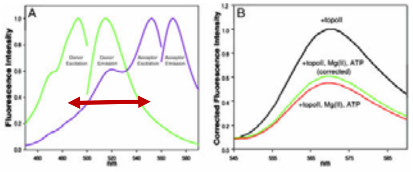
- **Single-molecule FRET**

Smiley et al, PNAS 2007

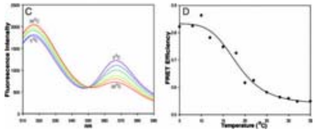
2007 : 1st smFRET data on drosophile topoisomerase II







Spectrum overlap



FRET signal in fonction of temperature

Following of the closing/opening of the DNA-gate by fluorescence

Type II topoisomerases

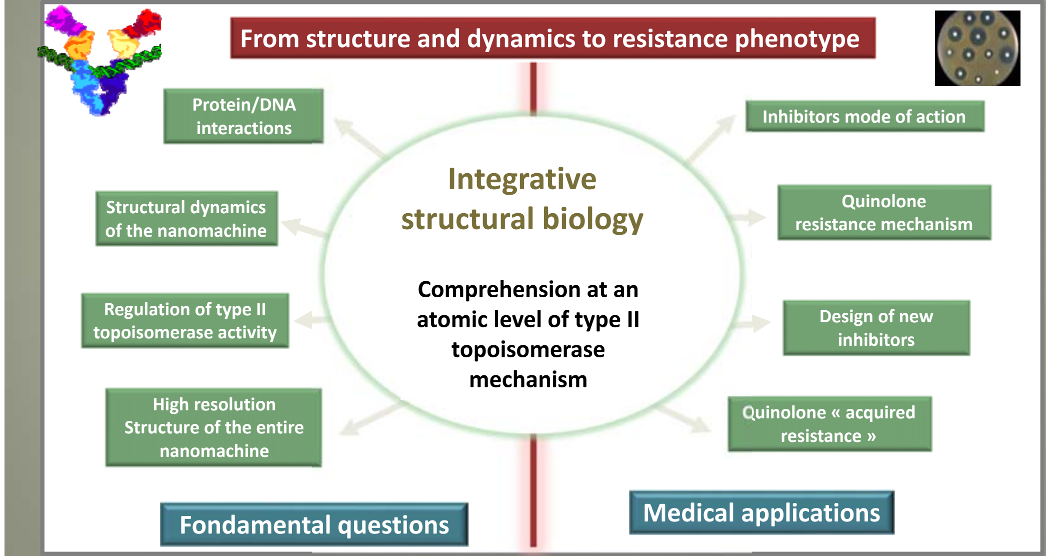
9. Autres techniques biophysiques

- **Single-molecule FRET**

***B. subtilis* DNA gyrase**	**Nucleotide cycle**	**Göttler et al, JMB 2007**
***B. subtilis* DNA gyrase**	**DNA-gate predominantly in the closed conformation**	**Gubaev et al, PNAS 2009**
***B. subtilis* DNA gyrase**	**Role of CTD in supercoiling cycle**	**Lanz et al, NAR 2011**
***B. subtilis* DNA gyrase**	**Role of N-gate in T-segment capture and strand passage**	**Gubaev et al, PNAS 2011**
***B. subtilis* DNA gyrase**	**Role of K^+ in nucleotide induced closure of N-gate**	**Gubaev et al, JBC 2012**
***B. subtilis* DNA gyrase**	**Role of GyrA-box in DNA binding**	**Lanz et al, NAR 2012**
***E. coli* topo IV**	**Role of CTD in substrate discrimination**	**Vos et al, JMB 2013**
***B. subtilis* DNA gyrase**	**Spectrum of conformational states of DNA- and C-gates**	**Rudolph et al, JMB 2013**
***B. subtilis* DNA gyrase**	**Mechanism of DNA supercoiling**	**Gubaev et al, DNAR 2014**
***B. subtilis* DNA gyrase**	**C-terminal tail moderates DNA supercoiling activity**	**Lanz et al, JBC 2014**

Type II topoisomerases

8. Conclusion - perspectives



From structure and dynamics to resistance phenotype

Integrative structural biology

Comprehension at an atomic level of type II topoisomerase mechanism

- Protein/DNA interactions
- Structural dynamics of the nanomachine
- Regulation of type II topoisomerase activity
- High resolution Structure of the entire nanomachine
- Inhibitors mode of action
- Quinolone resistance mechanism
- Design of new inhibitors
- Quinolone « acquired resistance »

Fondamental questions

Medical applications

Type II topoisomerases

8. Conclusion - perspectives

A 3D description at atomic scale of each step of the catalytic cycle