Single-molecule detection or “There’s more room at the bottom”

Image by Vladislav Kunetki

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The middle way

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Contributed by David Pines, October 29, 1999

Mesoscopic organization in soft, hard, and biological matter is examined in the context of our present understanding of the principles responsible for emergent organized behavior (crystallinity, ferromagnetism, superconductivity, etc.) at long wavelengths in very large aggregations of particles. Particular attention is paid to the possibility that as-yet-undiscovered organizing principles might be at work at the mesoscopic scale, intermediate between atomic and macroscopic dimensions, and the implications of their discovery for biology and the physical sciences. The search for the existence and universality of such rules, the proof or disproof of organizing principles appropriate to the mesoscopic domain, is called the middle way.

Limits of Understanding

Seeing is the beginning of understanding. This may seem an obvious truism, yet it conflicts with a dogma central to much of science, that knowledge of the underlying physical laws alone is sufficient for us to understand all things, even ones that cannot be seen. But the conflict is only apparent, for the dogma is false. Although behavior of atoms and small molecules can be predicted with reasonable accuracy starting from the underlying laws of quantum mechanics, the behavior of large ones cannot, for the errors always eventually run out of control as the number of atoms increases because of exponentially increasing computer requirements. At the same time, however, very large aggregations of particles have some astonishing properties, such as the ability to levitate magnets when they are cooled to cryogenic temperatures, that are commonly acknowledged to be "understood." How can this be? The answer is that these properties are actually caused by collective organizing principles that formally grow out of the microscopic rules but are in a real sense independent of them.

We say that superfluidity, ferromagnetism, metallic conduction, hydrodynamics, and so forth are "protected" properties of matter—generic behavior that is reliably the same one system to the next, regardless of details (1). There are more sophisticated ways of articulating this idea, such as stable fixed point of the renormalization group, but these all boil down to descriptions of

the very large and the very small. But, as we all know, there is life in the desert.

The miracles of nature revealed by modern molecular biology are no less astonishing than those found by physicists in macroscopic matter. Their existence leads one to question whether as-yet-undiscovered organizing principles might be at work at the mesoscopic scale, at least in living things. This is by any measure a central philosophical controversy of modern science, for a commonly held view is that there are no principles in biology except for Darwinian evolution. But what if this view is just a consequence of our inability to see? Indeed the rules of self-organization at macroscopic length scales were not self-evident at the time of their discovery and were accepted as true only after repeated confrontations with experiment left no alternative. The existence of similar rules at the mesoscopic scale would have profound implications for all of science, not just biology, for noncrystalline matter often has curious and poorly understood behavior suggestive of mesoscopic organization. It is thus a question worth asking. We call the search for the existence of mesoscopic protectorates—the proof or disproof of organizing principles appropriate to the mesoscopic domain—the middle way.

Life in the Desert

Twentieth-century science has uncovered the fact that there are numerous large molecules that carry out the processes of life. Although the functions carried out by these molecules are still very incompletely understood, they are amazing to an extent rarely appreciated by physical scientists and engineers. Proteins can catalyze a vast number of unrelated chemical reactions. They can pick out one substrate from thousands of chemically similar ones. They can act like computers executing a sequence of instructions. They can alter their activity through the presence of specific affector molecules in their environments. They can function as signals or receptors for these signals. They can be poisons. They can assemble together spontaneously to form mechanical structures like the cytoskeleton or viruses. The precedent of life allows no other conclusion than that mesoscopic objects organize themselves and function in ways unlike

PNAS **97** : 32—37 (2000)

Les questions qui nous animent

Comment fonctionnent les machineries complexes du vivant?

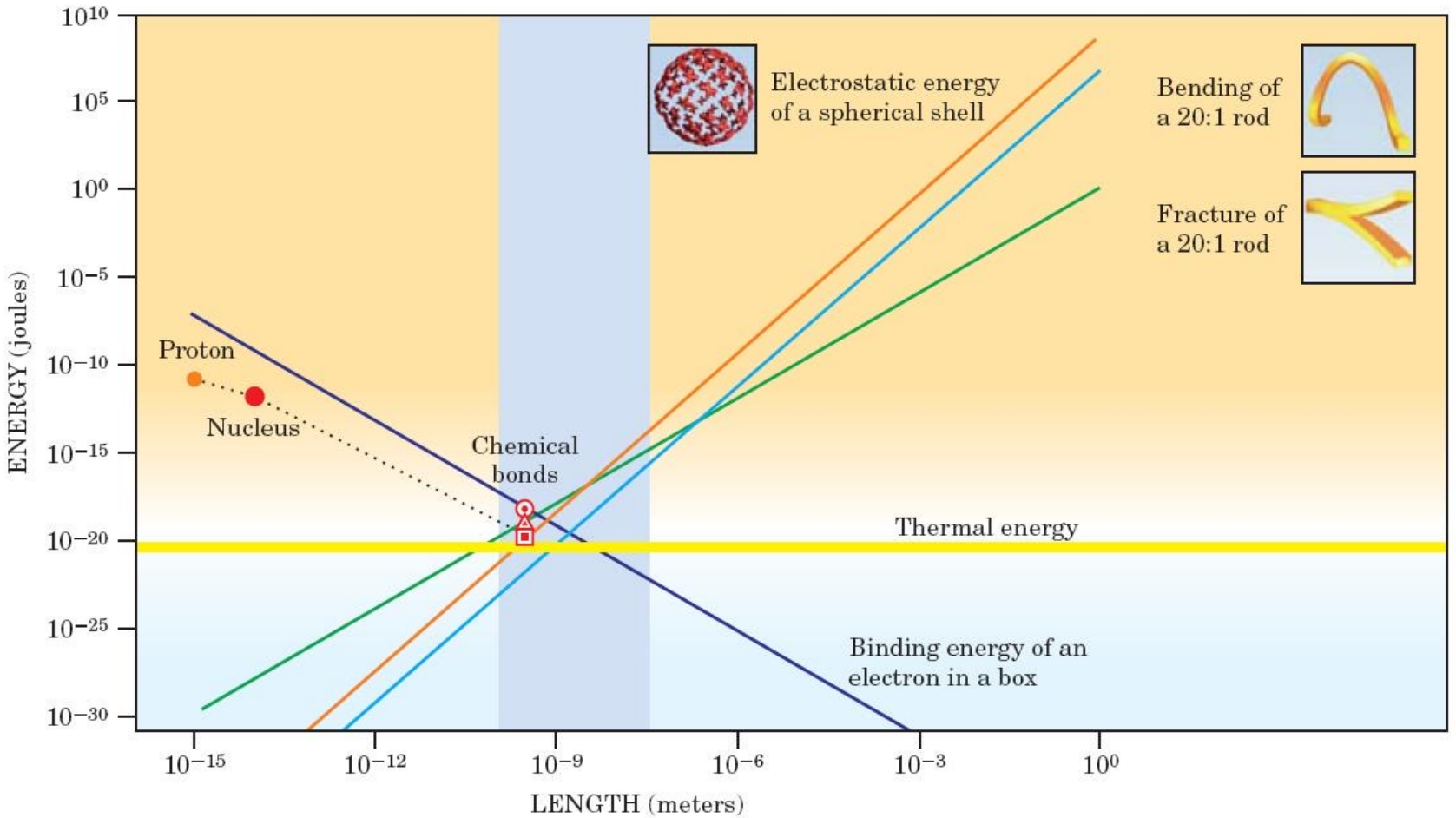
En quoi leurs propriétés mécaniques sont-elles mobilisées?

Comment s'assemblent et se désassemblent-elles en fonctionnant?

Tolèrent-elles des variations en leur nombre de sous-unités?

En quoi est-ce que tout ceci peut illustrer les propriétés uniques de la matière organique moléculaire?

Pourquoi la mécano-chimie?



Philips & Quake, Physics Today Oct. 2005

Echelles de Forces Moléculaires

ENERGIE = FORCE x DISTANCE et FORCE = ENERGIE/DISTANCE

- Energie thermique ~ $k_BT = 4 \times 10^{-21}$ J
- Distance ~ nm = 10^{-9} m
- Force caractéristique ~ k_BT /nm = 4 x 10^{-12} N, ou 4 picoNewtons (pN)
- Le pN est l'unité relevante de force à l'échelle moléculaire
- k_BT = 4 pN·nm

L’expérimentation molécule-unique

- Accès temporel au monde moléculaire par

Observation

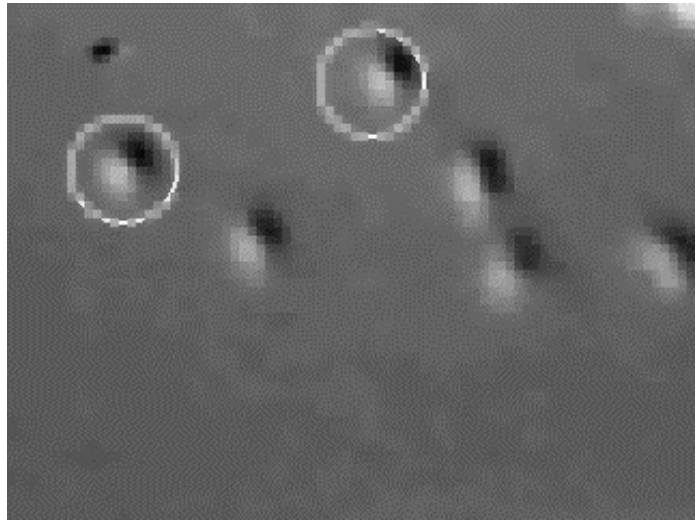
Suivi de mouvement Brownien, colocalization par fluorescence *in vitro* et *in vivo*, FRET, super-résolution, cryoEM et crystallographie…

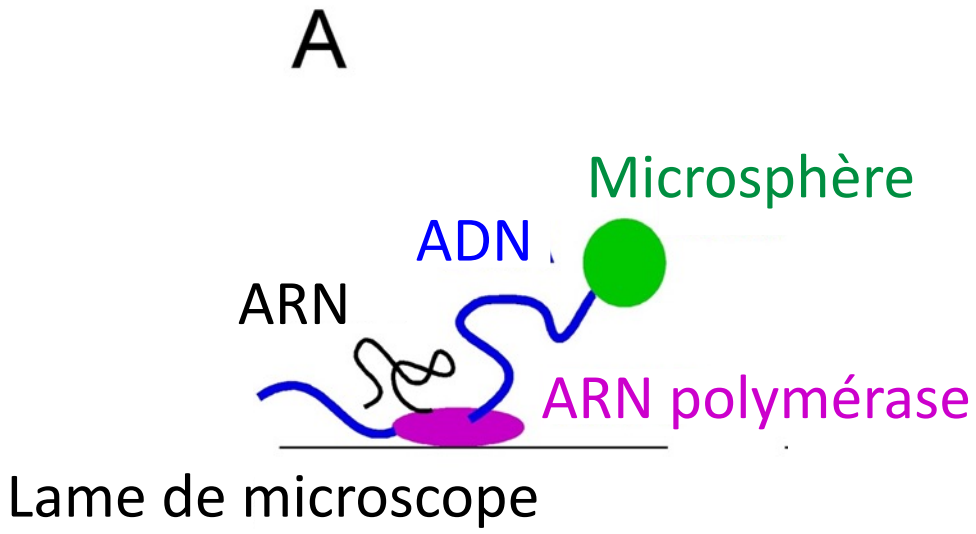
Manipulation

Piège optique (OT), piège magnétique (MT), microscope à force atomique (AFM), origamis ADN

Observation de la synthèse d'ARN (transcription)

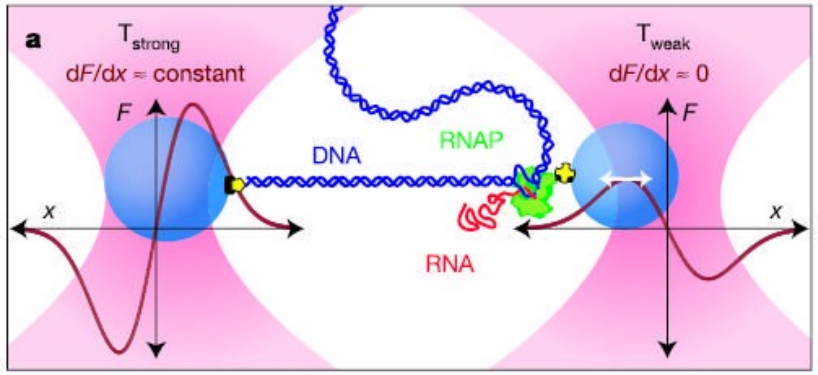
ADN→ARN→Protéine



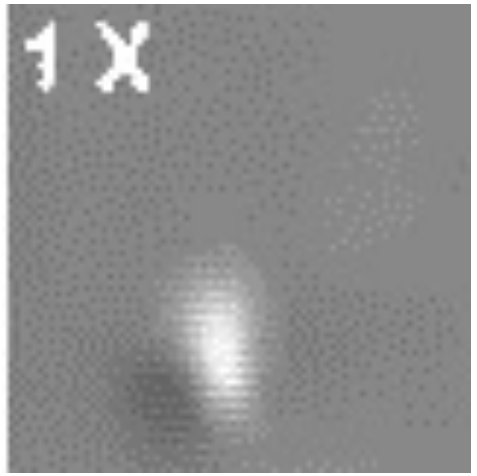


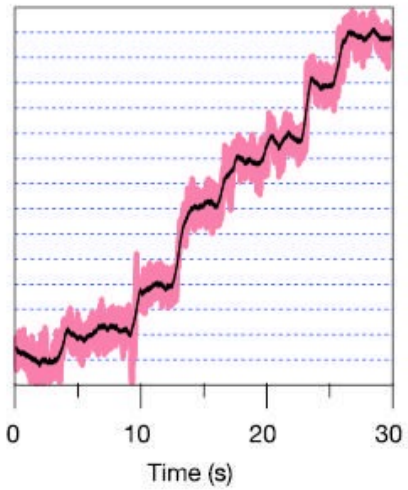
Schafer, Gelles, Sheetz & Landick, « Transcription by single molecules of RNA polymerase observed by light microscopy »
Nature **352** 444—448, 1991

Piègeage Optique d'ARN Polymérase

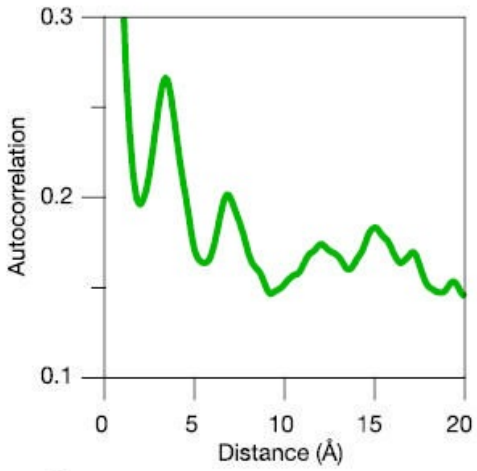


Pas Individuels

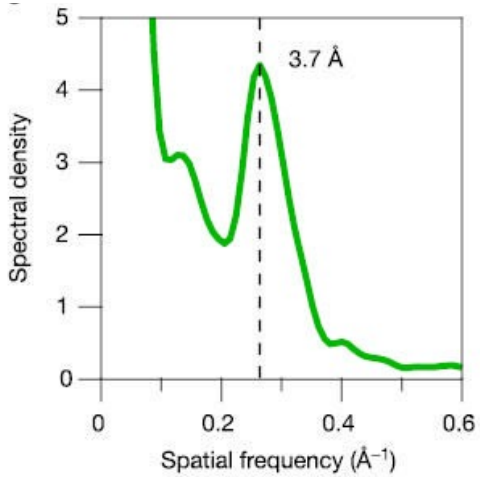




Espace Direct



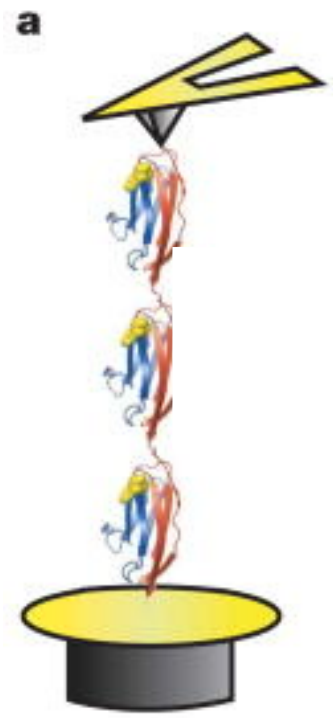
Distribution
« Pairwise »

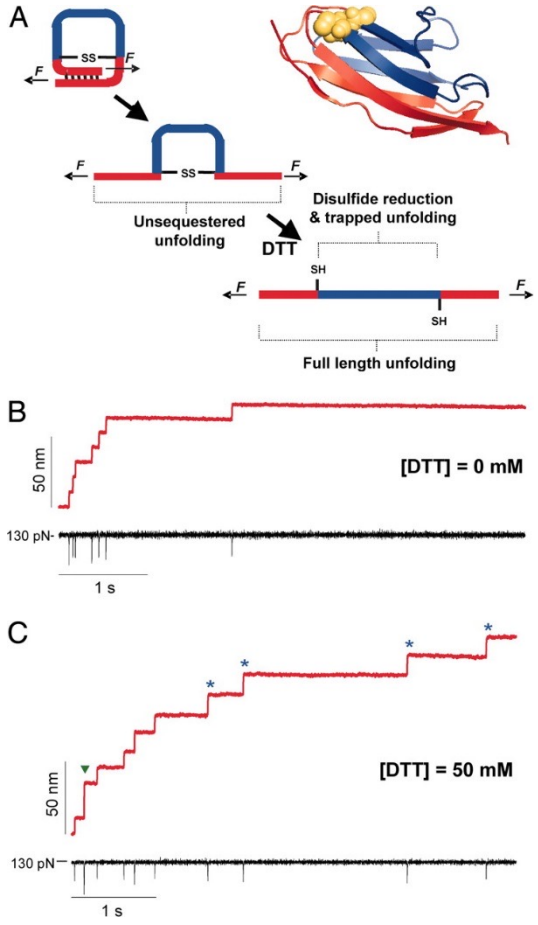


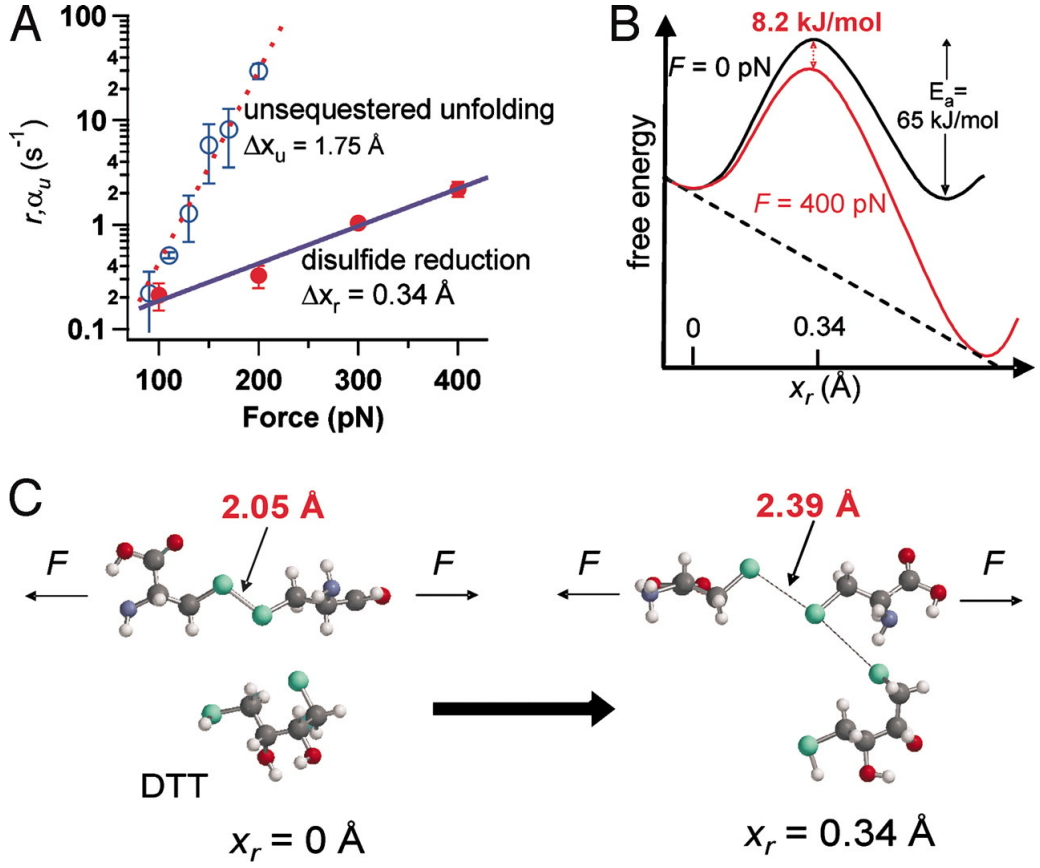
Espace Fourier

Abbondanzieri, *et al*. "Direct observation of base-pair stepping by RNA polymerase" *Nature* **438**: 460-465, 2005

Réduction de liaison di-sulfure et cadre théorique







Wiita, Ainavarapu, Huang & Fernandez « Force-dependent chemical kinetics of disulfide bond reduction observed with

Advantages et Désavantages de la Manipulation

<u>Pour</u>

Observation directe des interactions d'association/dissociation, catalyse, etc. (1-10 ms).

Analyse cinétique détaillée, observation d'évènements rares et transitoires, états de transition, etc.

Très faible consummation de réactifs (qq femtomoles/mesure)

Analyse « modèle-independente » analysis

<u>Contre</u>

Attaches moléculaires à optimizer, interactions non-spécifiques…

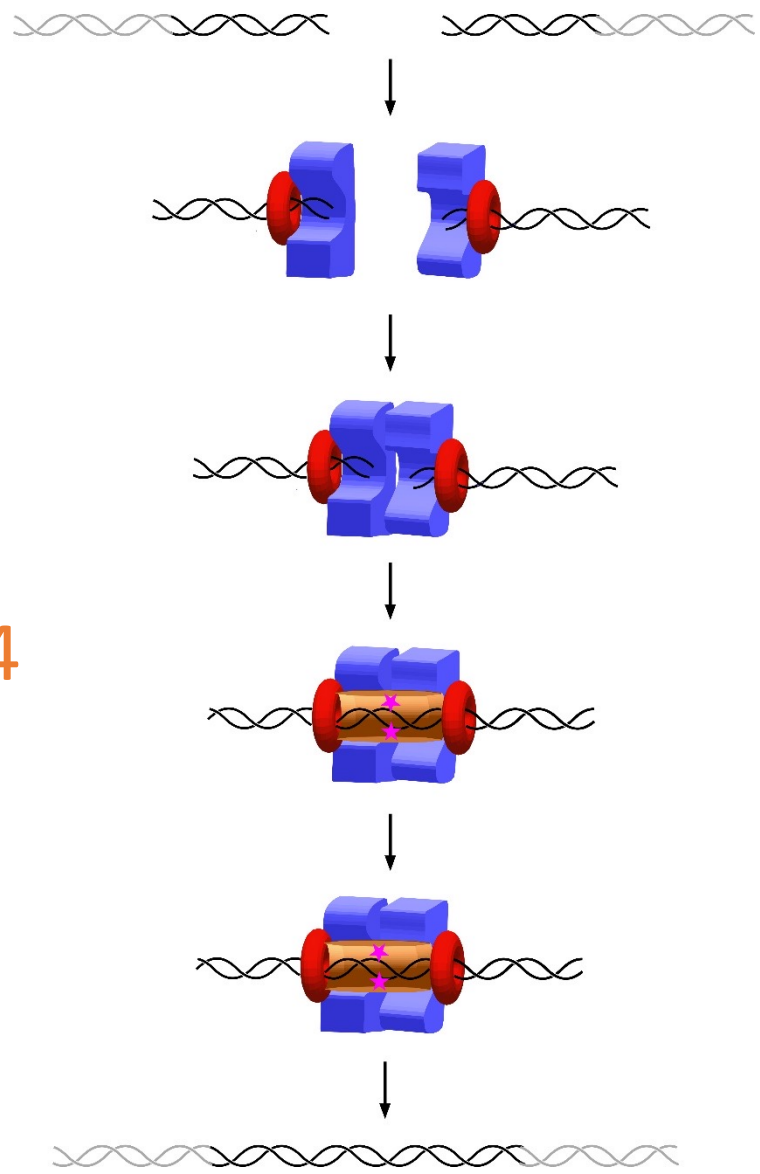
Collecte de données répétitive

Réparation des Cassures d'ADN

CDB

+Ku

+DNA-PKcs



+PAXX ??

Ochi et al. Science 2015

+XLF-XRCC4

+LigIV

ADN intact

Des Questions qui Perdurent

Cassure Double Brin

+Ku

+DNA-PKcs

+PAXX

+XLF-XRCC4

+LigIV

Pourquoi tant de composantes?

Lesquelles sont redondantes?

Lesquelles sont essentielles?

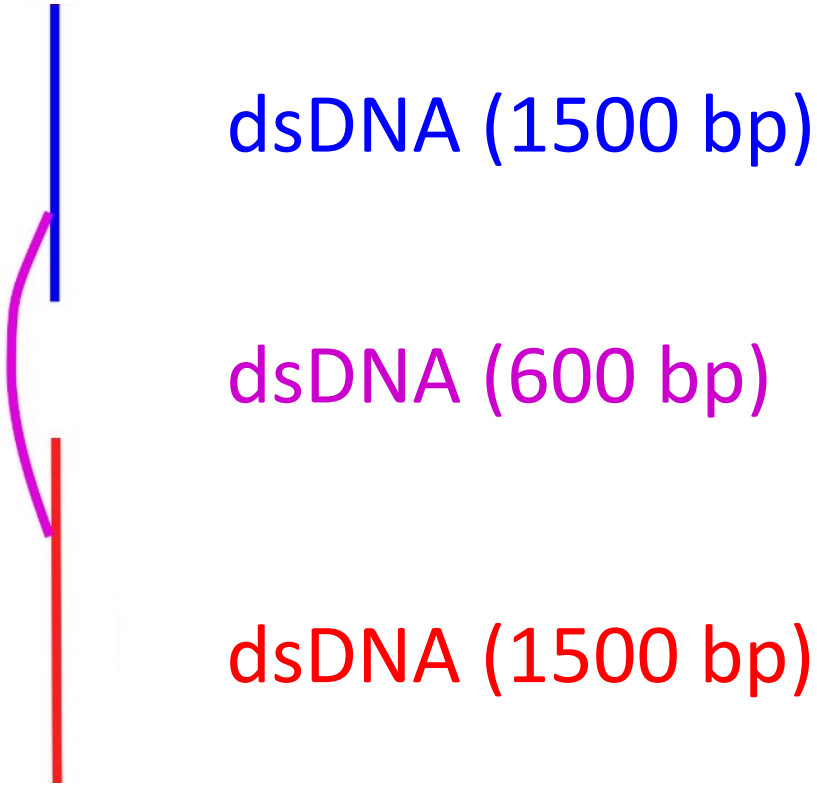
Quelle est leur stoichiométrie?

Les complexes tolèrent-ils les fluctuations de composition?

Les chemins réactifs sont-ils linéaires ou en embranchement?

Quelles sont les échelles temporelles relevantes?

Un ADN synthétique mimant une cassure

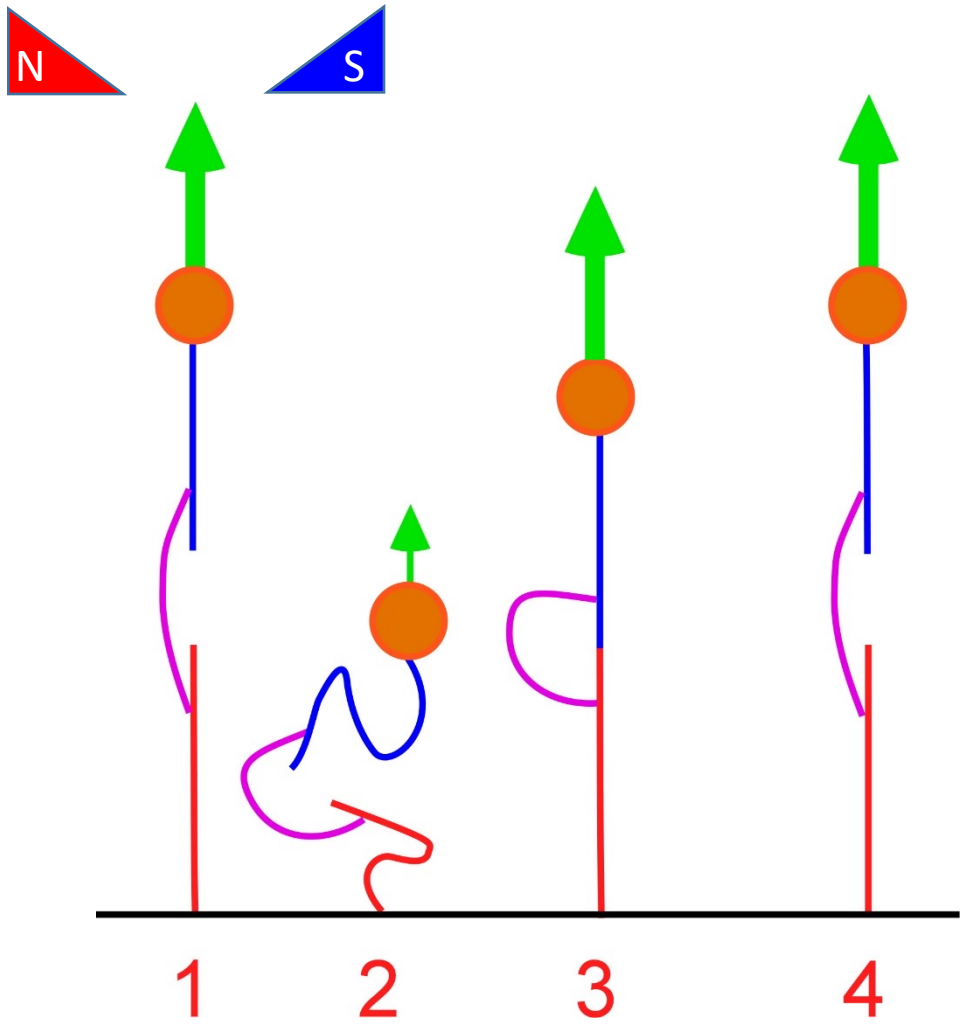


Emploi d'un forceps moléculaire

dsDNA (1500 bp)

dsDNA (600 bp)

dsDNA (1500 bp)



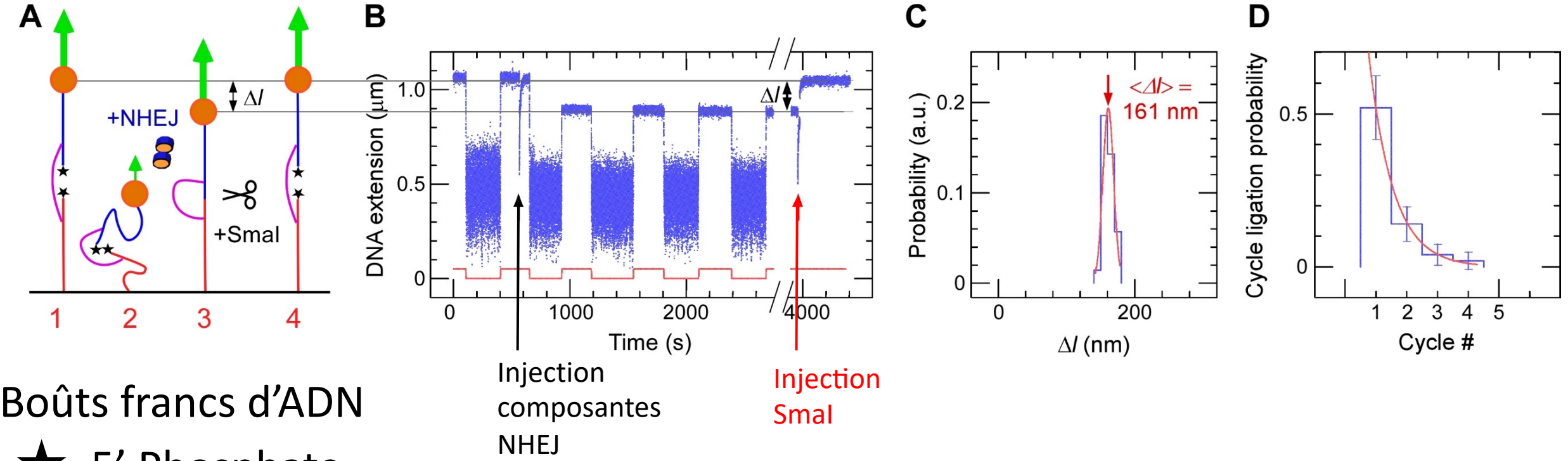
Force

Bille Magnétique

Step 1 : Calibrer
Step 2: Permettre aux extrémités de se rencontrer
Step 3: Sonder
Step 4: Durée de vie obtenue au retour à la position initiale.

Ku
DNA-PKcs
PAXX
XLF
XRCC4
LigIV

Réparation non-homologue des extrémités cassées



Boûts francs d'ADN

★ 5' Phosphate

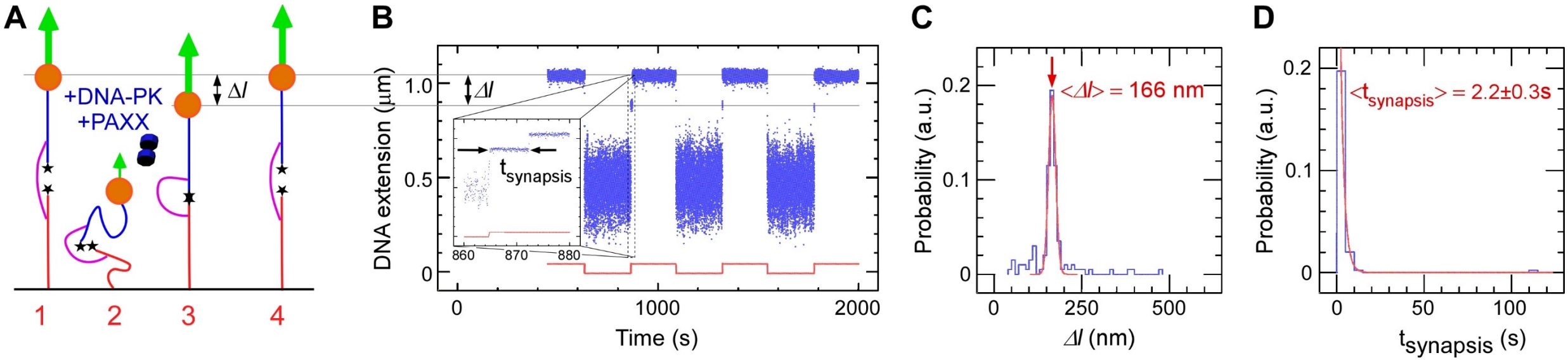
Injection composantes NHEJ

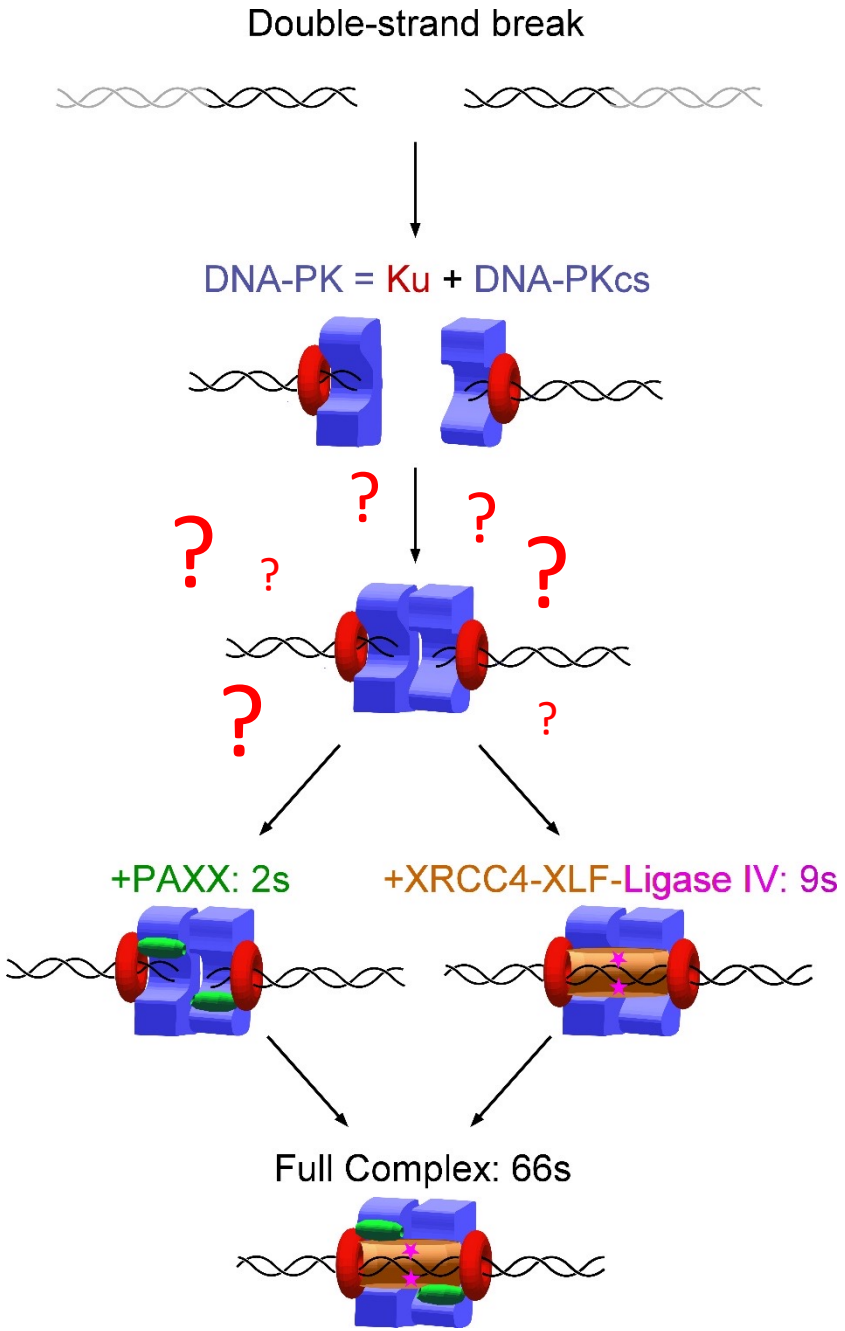
Injection SmaI

Definir 3 groupes de composantes

- Ku + DNA-PKcs
- PAXX
- XLF/XRCC4/LigaseIV

DNA-PK + PAXX médie une interaction synaptique de ~2 secondes



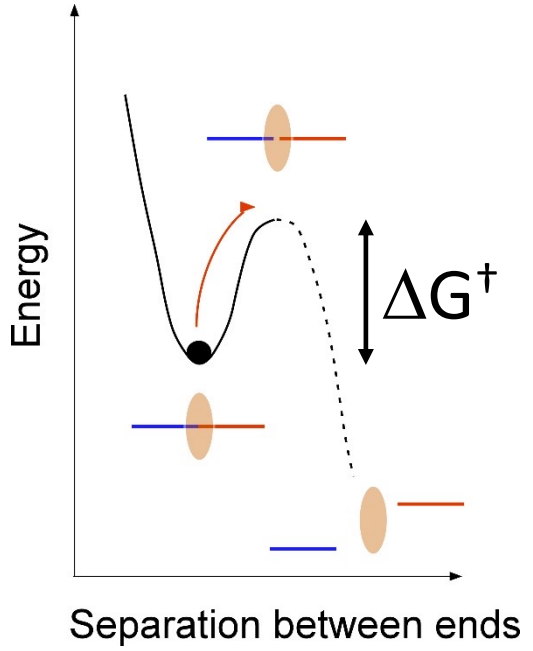

Double-strand break
DNA-PK = Ku + DNA-PKcs
?
?
?
?
?
?
+PAXX: 2s
+XRCC4-XLF-Ligase IV: 9s
Full Complex: 66s

La Loi de Boltzmann relie le temps et l'énergie

Probabilité d'être à un niveau d'énergie ($\Delta G^{\dagger}$):

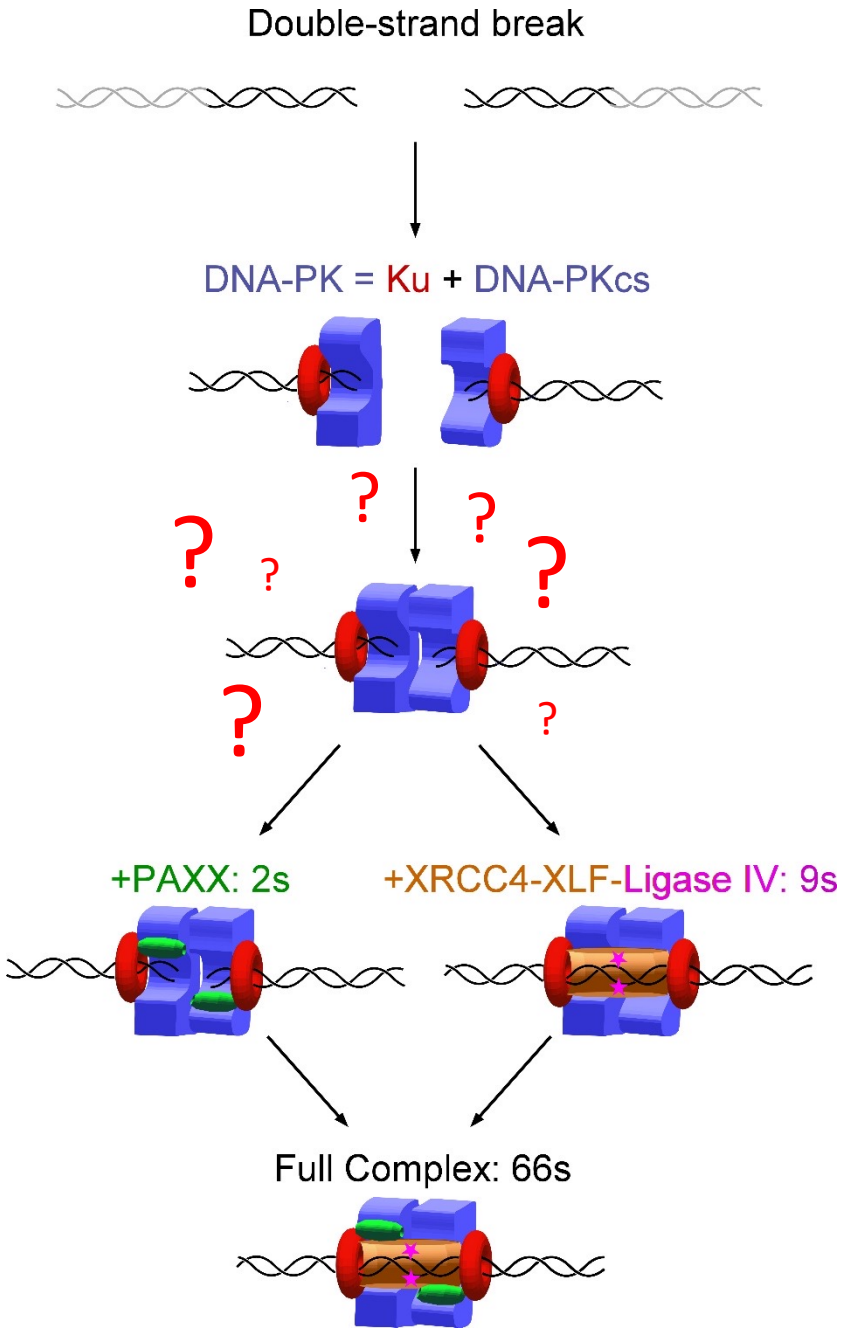
$$P(\Delta G^{\dagger}) = \exp(-\Delta G^{\dagger}/k_B T)$$

→ Durée de l'état lié $t \sim 1/P(\Delta G^{\dagger})$

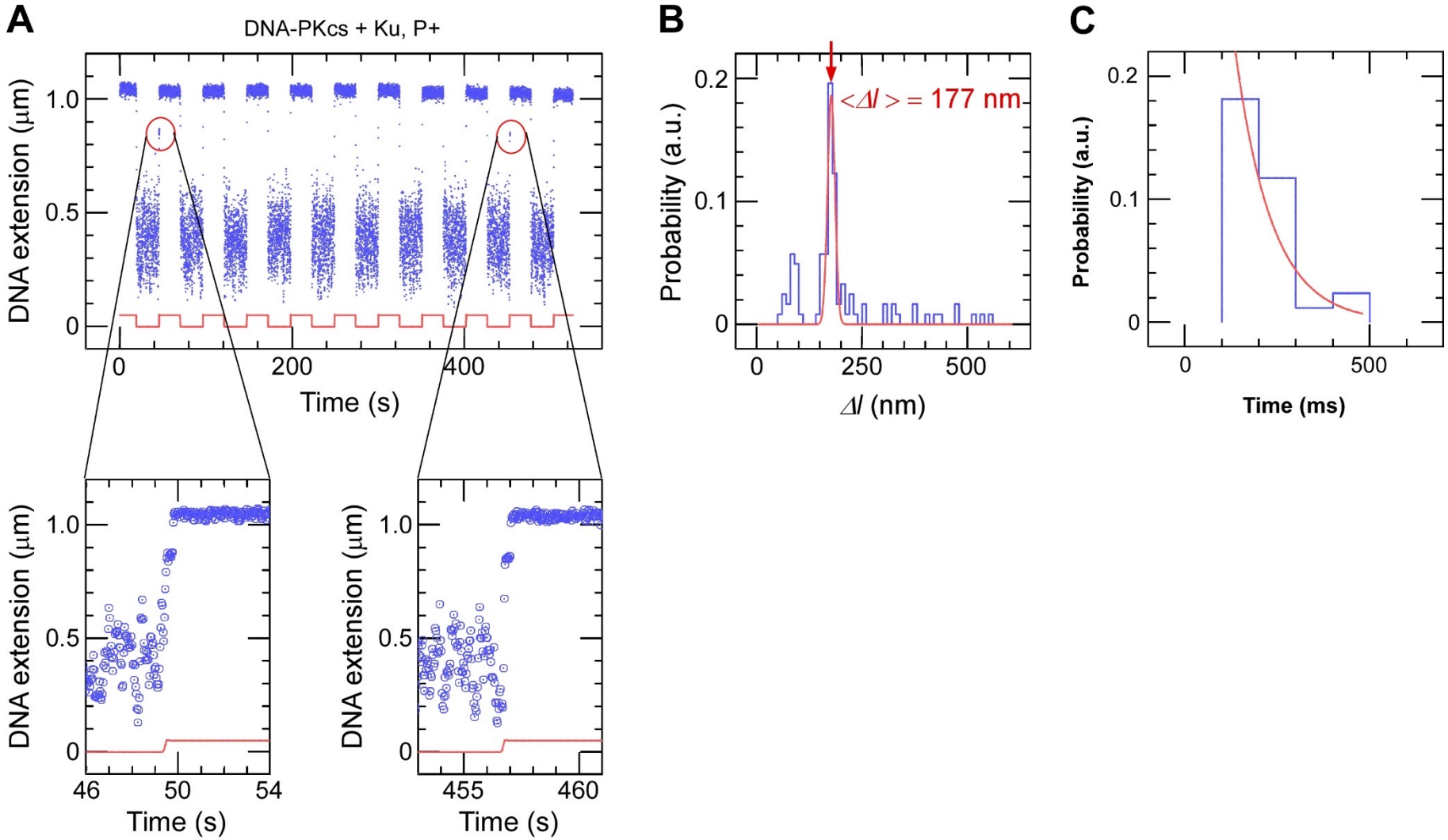


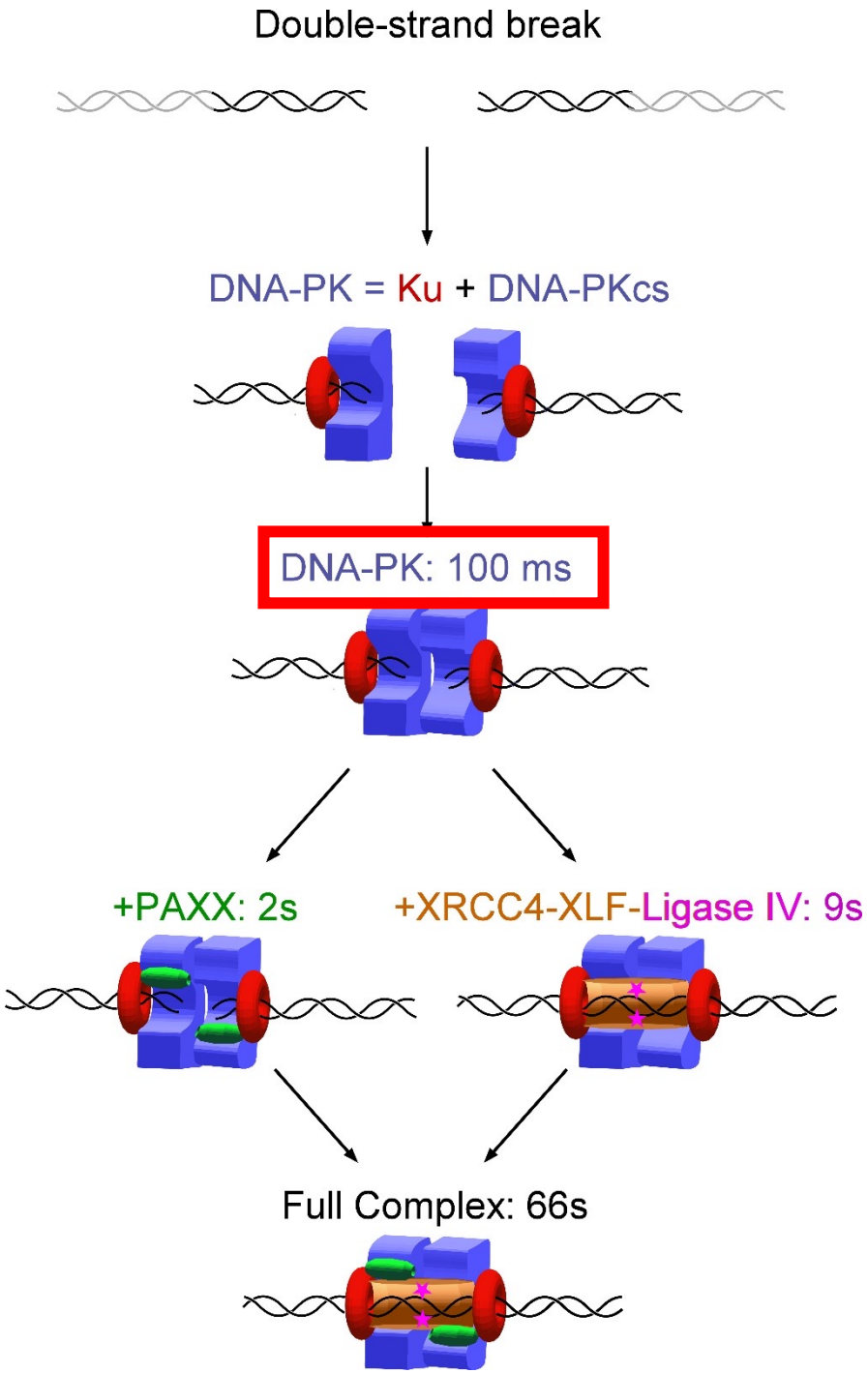
$$\Delta G^{\dagger}_{\text{complèxe1}} - \Delta G^{\dagger}_{\text{complèxe2}} = k_B T \ln (t_1/t_2)$$

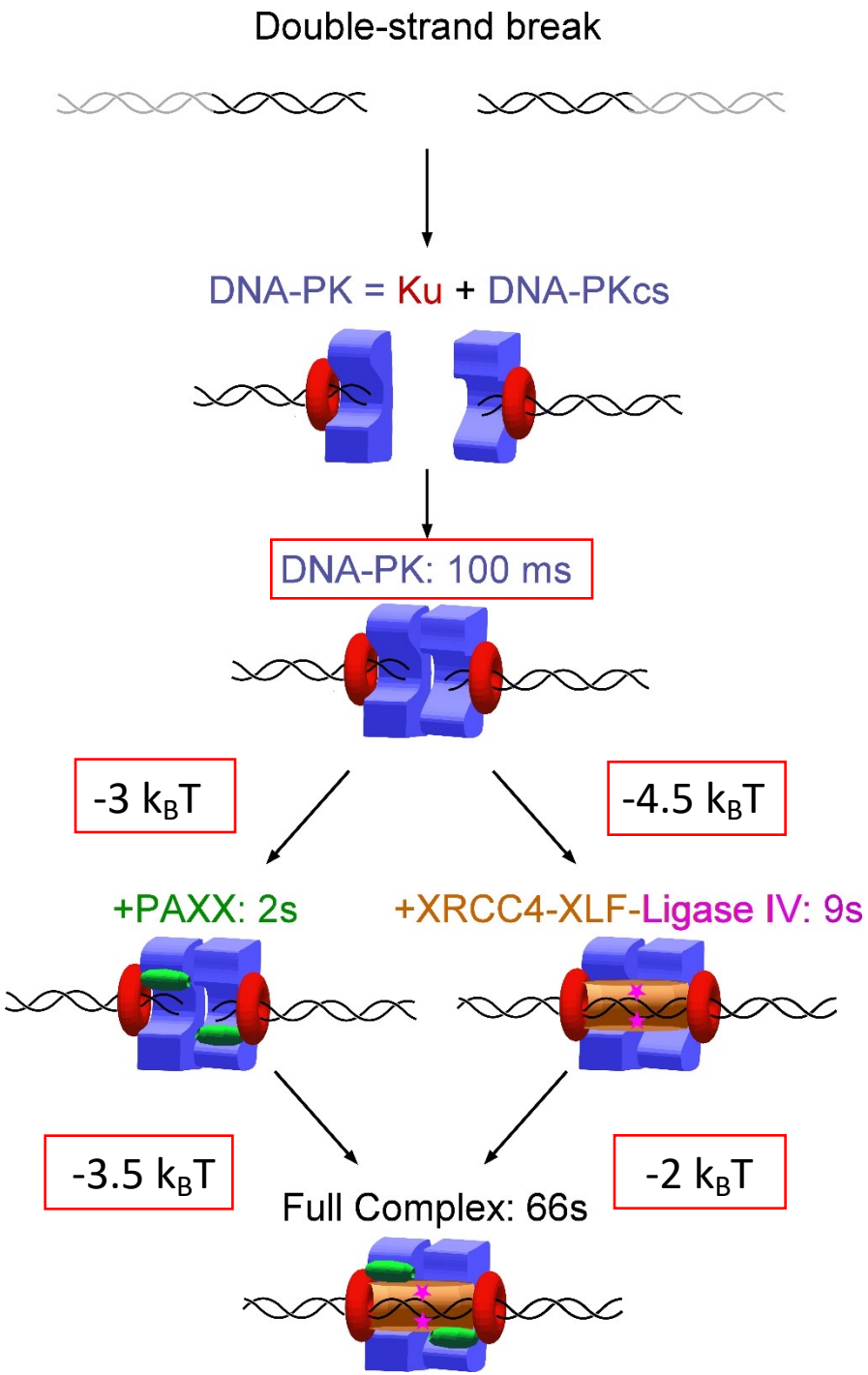
Différence d'énergie d'activation des déux états liés → Log du rapport des durées Des déux états liés


Double-strand break
DNA-PK = Ku + DNA-PKcs
?
?
?
?
?
?
+PAXX: 2s
+XRCC4-XLF-Ligase IV: 9s
Full Complex: 66s

DNA-PK médie une synapse de 100 milisecondes




Double-strand break
DNA-PK = Ku + DNA-PKcs
DNA-PK: 100 ms
+PAXX: 2s
+XRCC4-XLF-Ligase IV: 9s
Full Complex: 66s



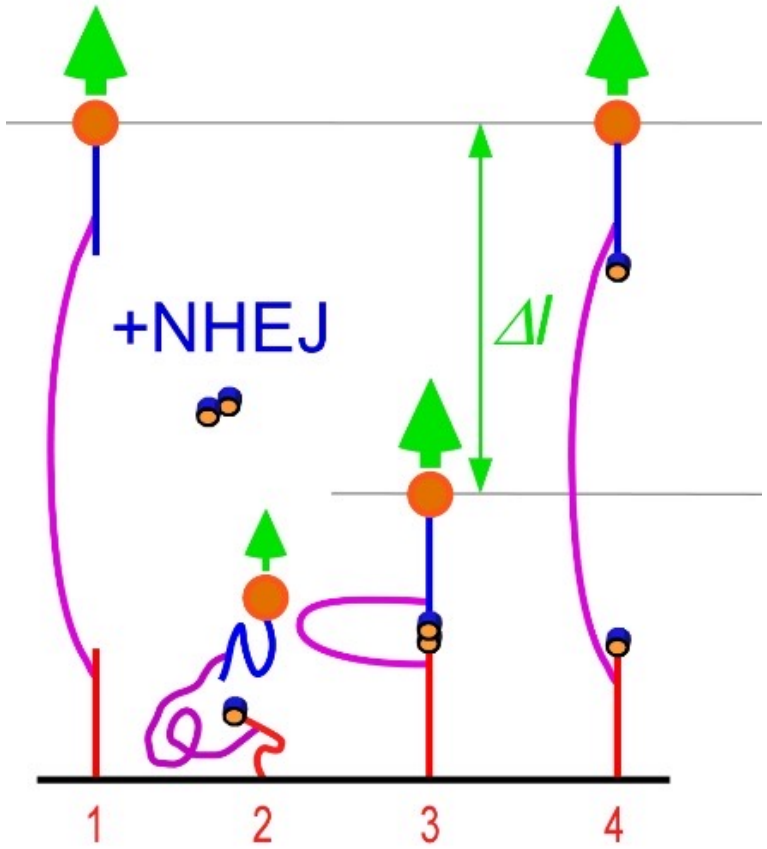
$k_BT = 4x10^{-21}$ Joules
= 2.4 kJ/mol
= 0.6 kCal/mol

Conclusions

- Reconstruction fonctionnelle de la reparation des cassures en temps reel et à échelle de la molecule individuelle
- Description cinétique de la synapse primaire formée par Ku et DNA-PKcs
- Characterization de quatre complexes distincts avec des durées de vie couvrant presque trois orders de grandeurs (de 0.1 secondes à 60 secondes)
- La stabilité émerge de la somme de nombreuses interactions faibles
- Role unique de PAXX comme interacteur précoce

→PAXX est une mimique fonctionnelle du complexe XLF/XRCC4/LigIV

L’ARN long non-codant LINP1 ponte des extrémités distantes d’ADN



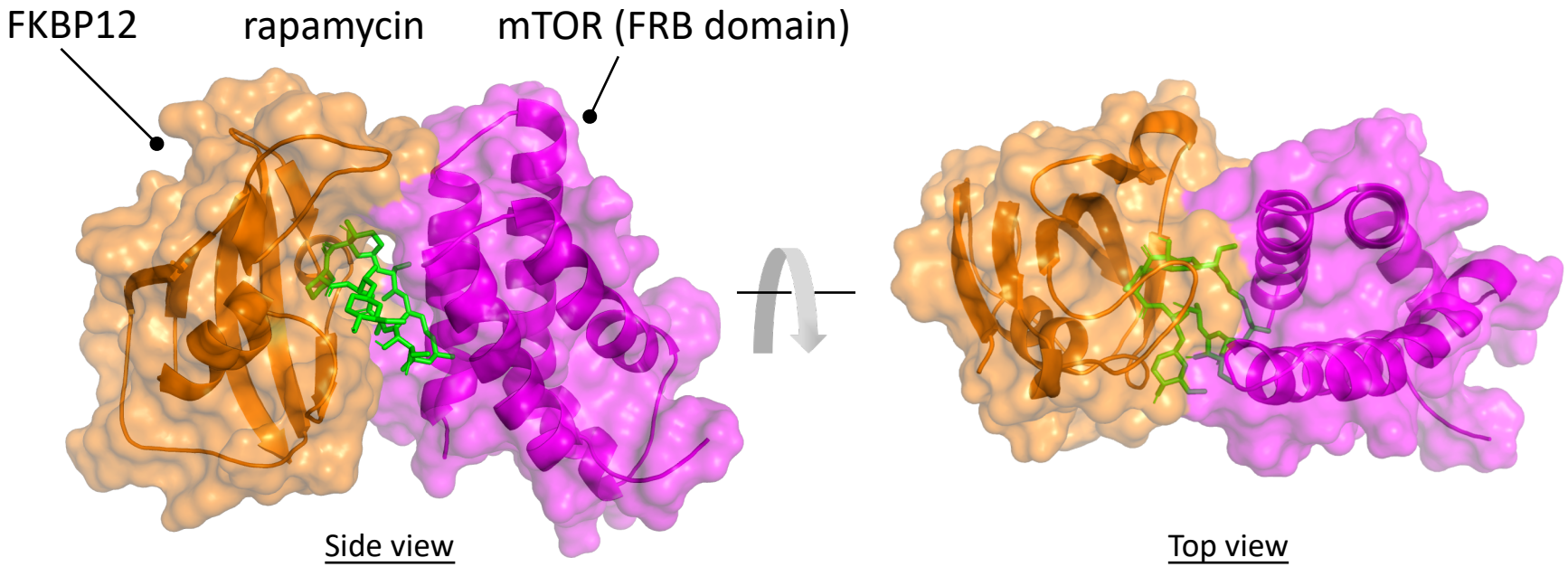
LINP1 est associé à de mauvais prognostiques dans les cancers du sein

-surexprimé dans les forms triples-négatives (ER-,HER-, BRCA-)

-associé à la résistance des tumeurs aux traitements hormonaux (Tamoxifène)

Thapar, Wang…Strick, Tainer et al.
Nucleic Acids Res. 2020

Inhibition par la rampamycine de la signalisation mTOR



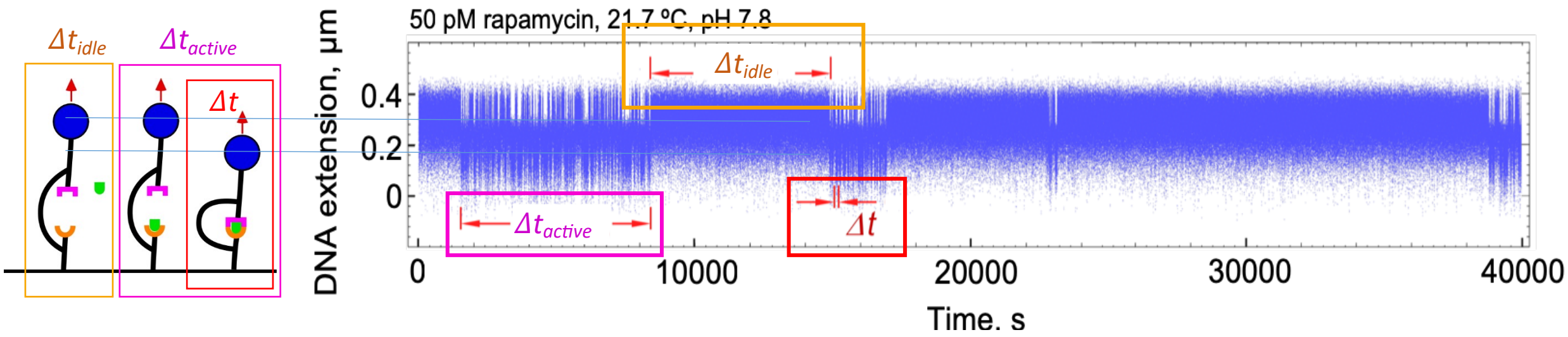
[FKBP12·rapamycin] · FRB

Dr. Dorota Kostrz (Gosse + Strick lab)

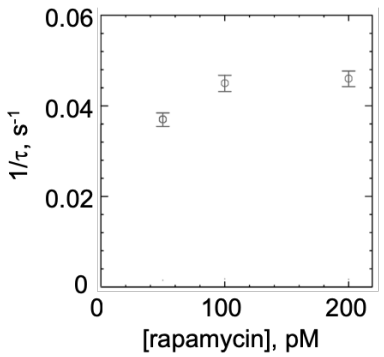
J. Am. Chem. Soc., 2005, 127, 4715-4721

pdb file: 1FAP, Science, 1996, 273, 239-242

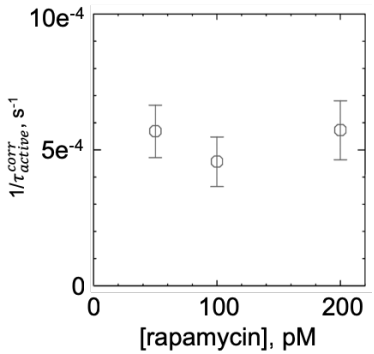
Analyse de l'interaction FKBP12-rapamycine-FRB



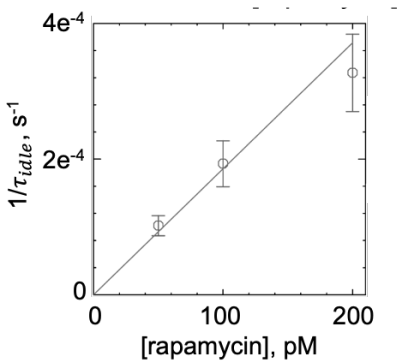
1/<Δt>
independent of
[rapamycin]



1/<Δt_{active_corr}>
independent of
[rapamycin]



1/<Δt_{idle}>
proportional to 1/[rapamycin]



$k_A = (1.9 \pm 0.18) \times 10^6\ M^{-1}s^{-1}$

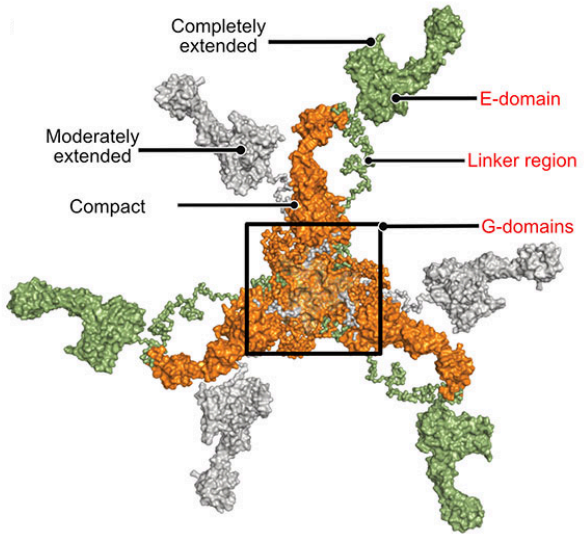
$k_D = (1/\tau^{corr}_{active}) = (5.3 \pm 0.57) \times 10^{-4}\ s^{-1}$

$K_D = 0.28 \pm 0.04$ nM

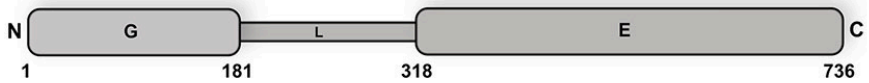
D. Kostrz *et al.*, **Nature Nanotechnology** 2019

Extension à la neuropharmacologie: Interactions entre le récepteur inhibiteur de la glycine et la gephyrine

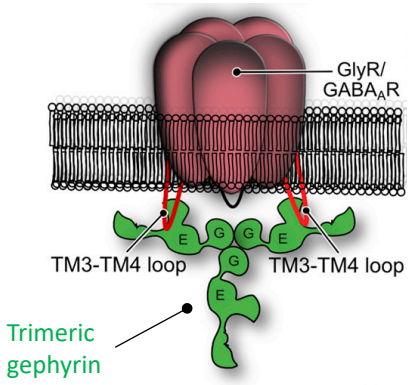
Dans la densité post-synaptique la boucle cytosolique des récepteurs inhibiteurs GlyR et GABAR interagit avec la protéine d'échafaudage gephyrin, qui sert à recruiter les récepteurs et réguler leur temps de residence à la synapse



Gephyrine trimérique



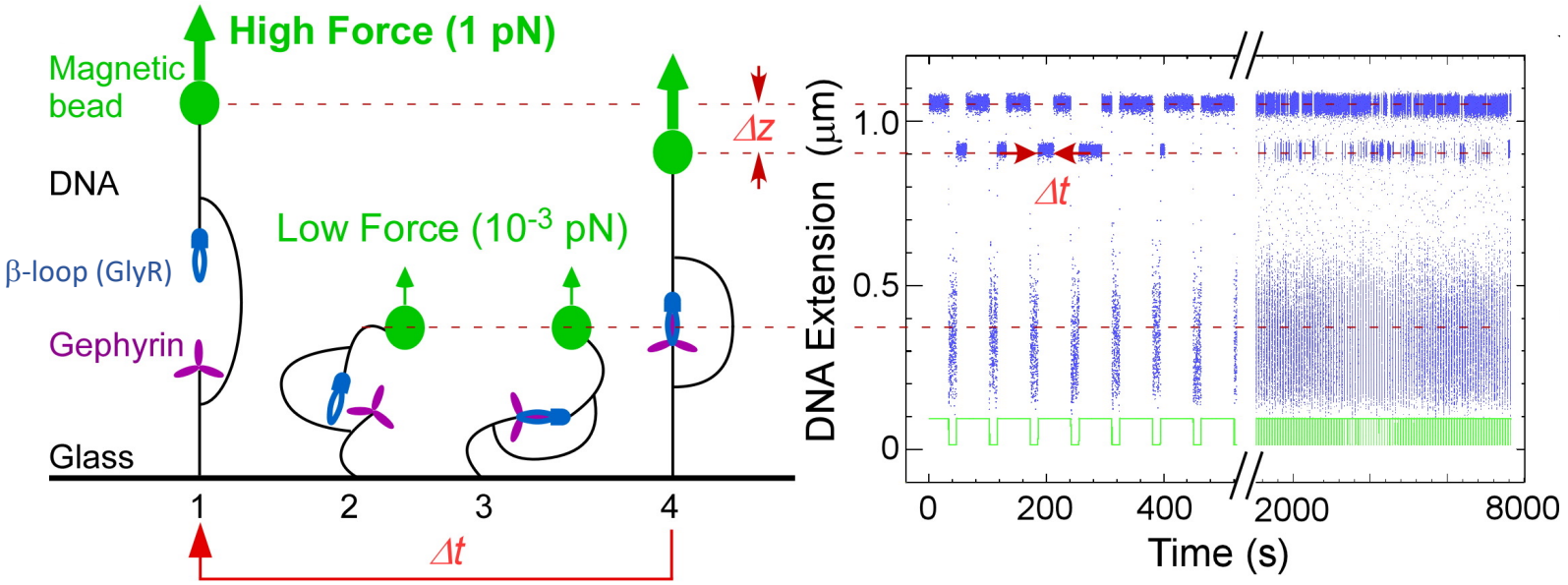
La β-loop (GlyR) interagit avec la **gephyrine**



Collaboration avec:

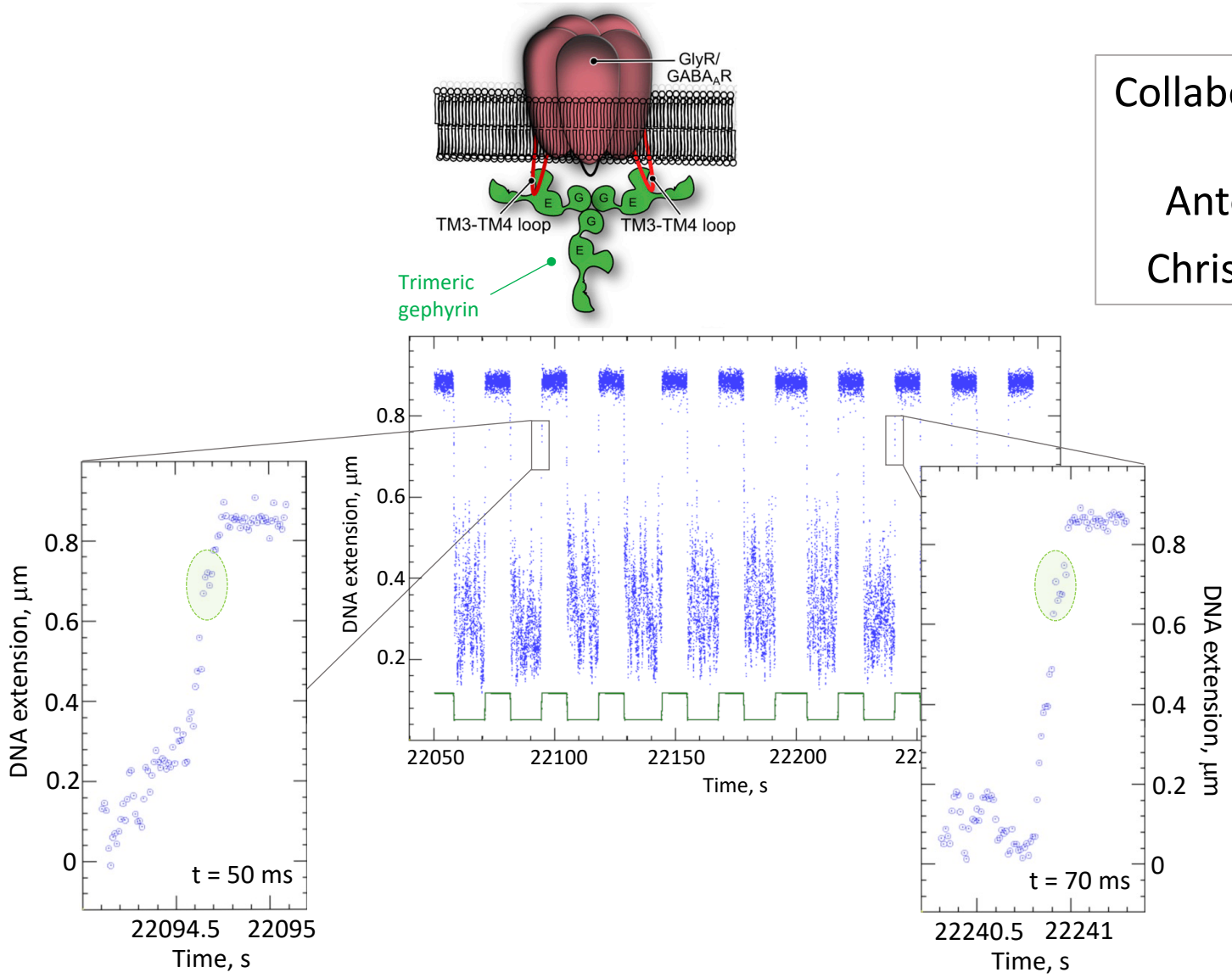
Antoine Triller

Christian Specht



[1] Kasaragod *et al.*, *Neuron*, **101**, 673-689 (2019); [2] Tretter *et al.*, *Front Cell Neurosci.*, **6**, 23 (2012)

La boucle3α **($GABA_aR$)** interagit également avec la **gephyrine**



Collaboration avec :

Antoine Triller

Christian Specht

[1] Kasaragod *et al.*, *Neuron*, **101**, 673-689 (2019); [2] Tretter *et al.*, *Front Cell Neurosci.*, **6**, 23 (2012)

Un portfolio d'intearctions moléculaires

- Neurobiologie: recrutement des récepteurs inhibiteurs GlyR and GABAR à la gephyrine
  (with A. Triller and C. Specht, iBENS, Paris, FR)

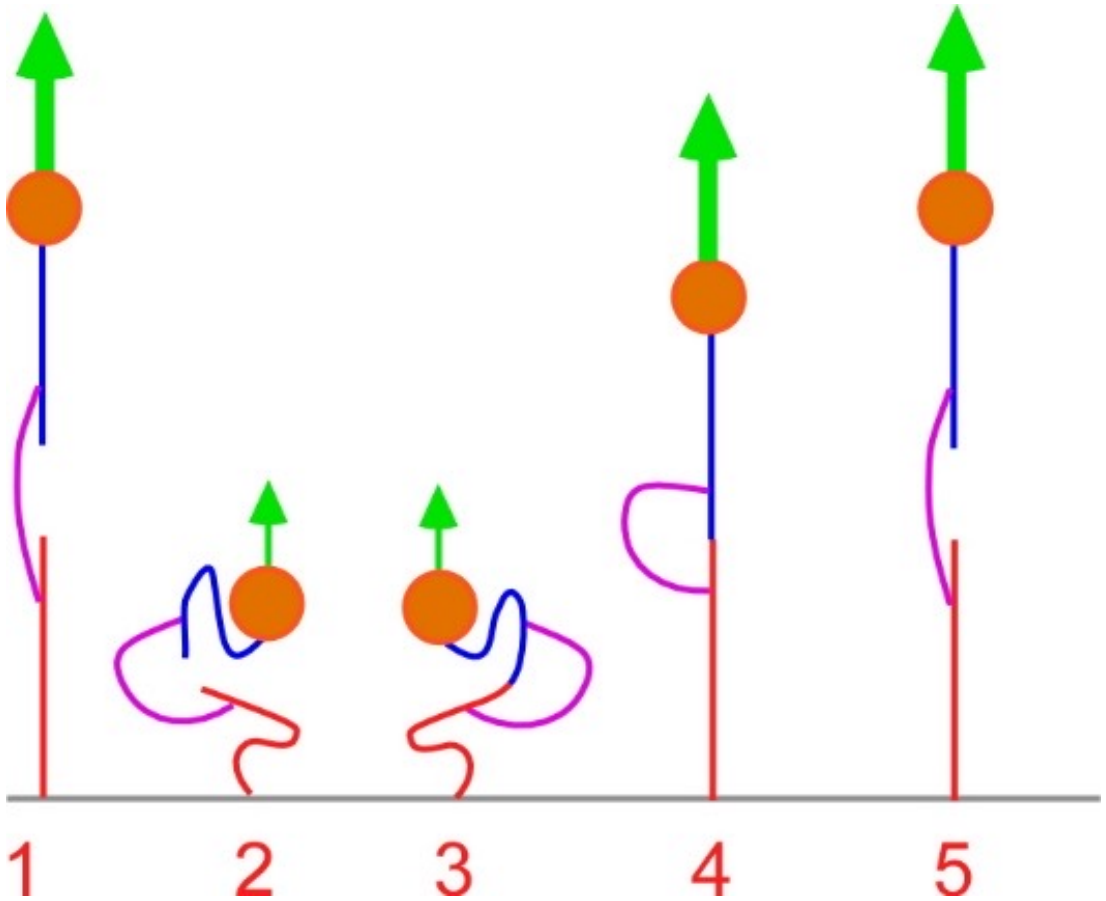
- GPCRs: couplage d'un récepteur à la grehlin solubilisé dans un nanodisque
  (with L. Catoire, IBPC, Paris, FR and J.-L. Banères, IBMM, Montpellier)

- Interactions nanobody-antigen pour immunothérapies CAR-T (PDL-1/anti-PDL-1)
  (with L. Limouzin, LAI, Marseille, FR and P. Chames, CRCM, Marseille)

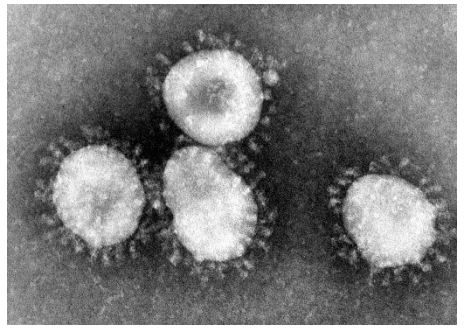
- Intearctions entre Spike du SARS-nCOV2 et ses récepteurs
  (with F. Rey, Pasteur Institute, Paris, FR, and F. Rico, LAI, Marseille)

- Characterisation de librairie de drogues encodées par l'ADN
  (with F. Hausch, TU Dresde, Germany and DyNAbind GMBH)

Le forceps moléculaire en tant que détecteur universel?



Convertit toute interaction en une mesure physique en temps reel?



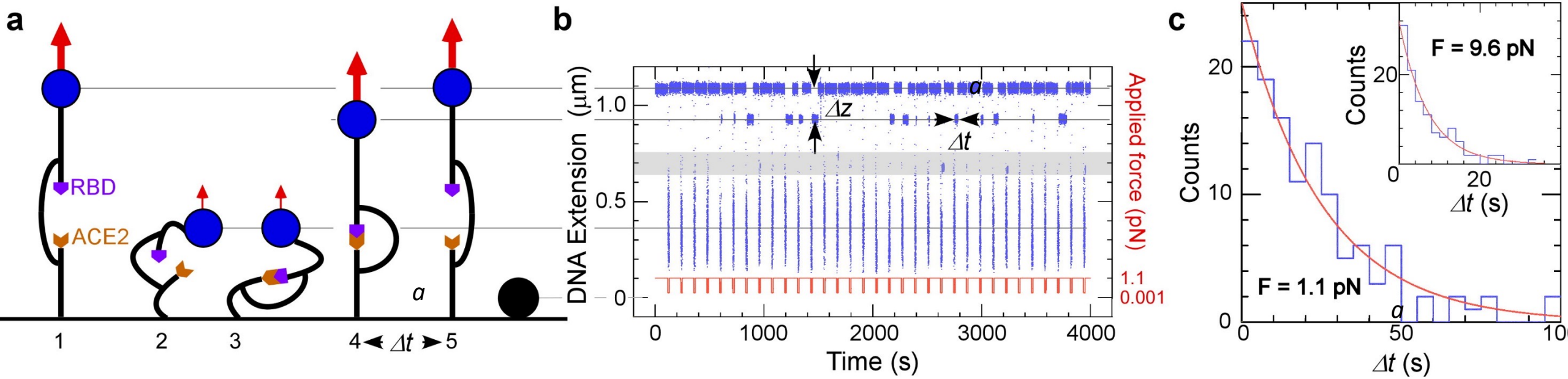
Patent Pending (PSL-Valo), Pre-Maturation/Maturation Phases (Qlife)

CDC/Dr. Fred Murphy

Interactions entre SARS-CoV-2 Spike RBD et ACE2

Spike à trois sites de liaison à l"ACE2; le site de liaison est le RBD

T = 30 ℃

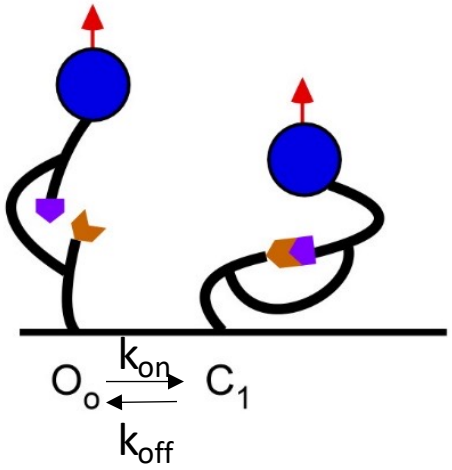


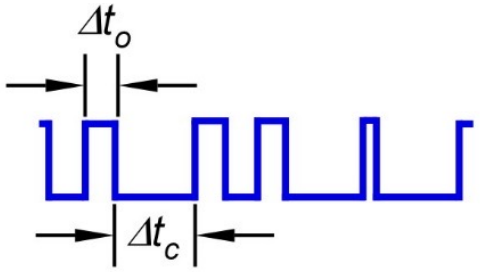
F = 1.1 pN: τ_A = 25 ± 3 s

F = 9.6 pN: τ_A = 7 ± 0.9 s

Dorota Kostrz, PhD (Strick Team)

Titration *en trans* de hACE2

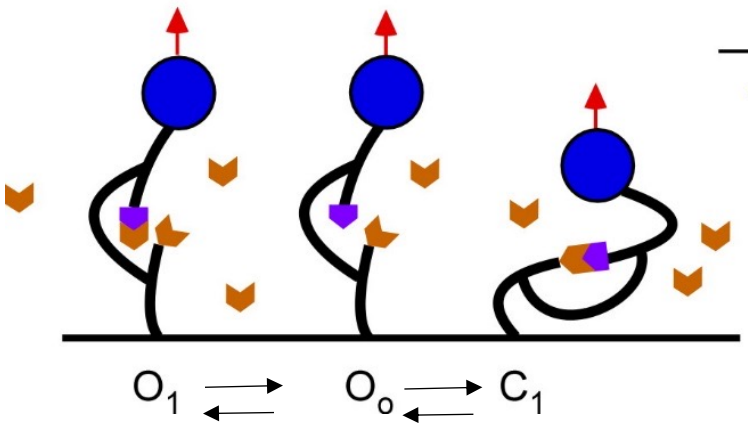


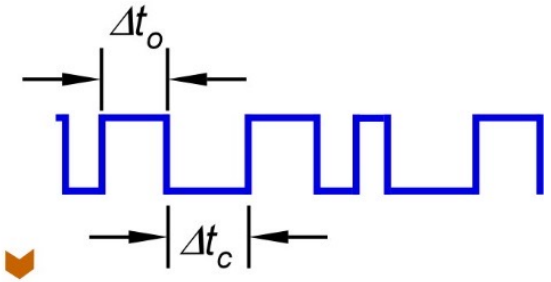


$1/<\Delta t_o> = k_{on}$

$$\frac{<\Delta t_O>}{<\Delta t_c>} = \tilde{K}_c$$

In trans concentration of hACE2



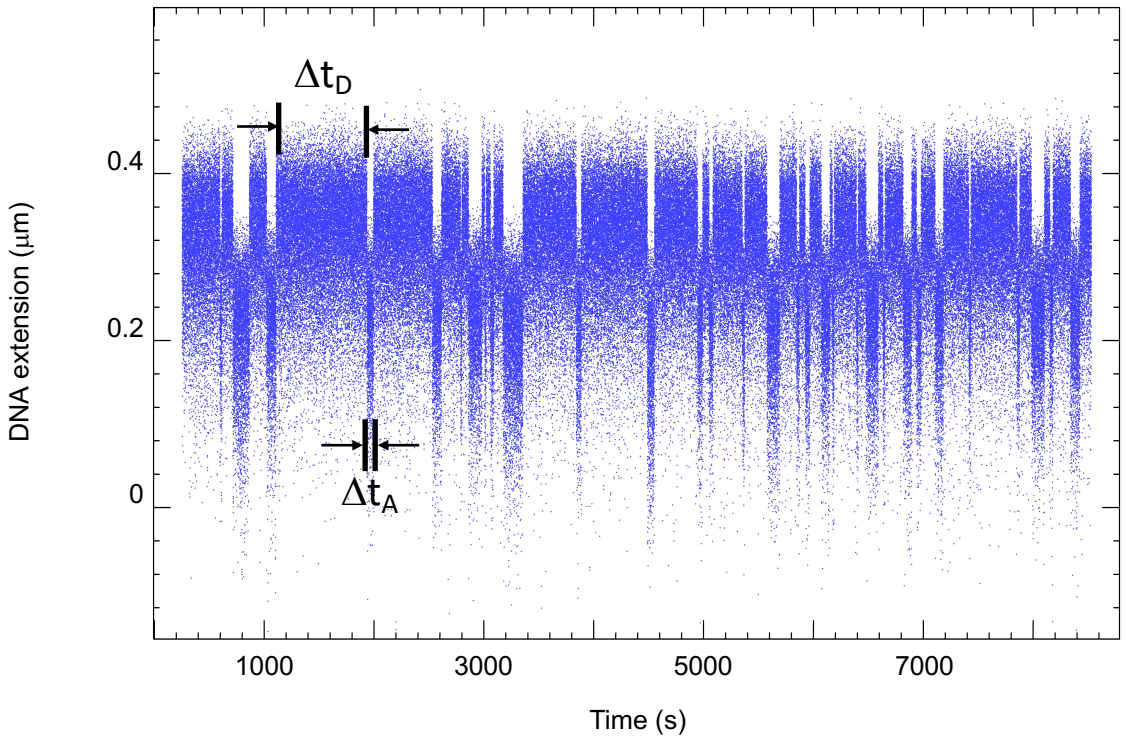


$$\frac{<\Delta t_O>}{<\Delta t_c>} = \tilde{K}_c\left(1 + \frac{[hACE2_t]}{K}\right)$$

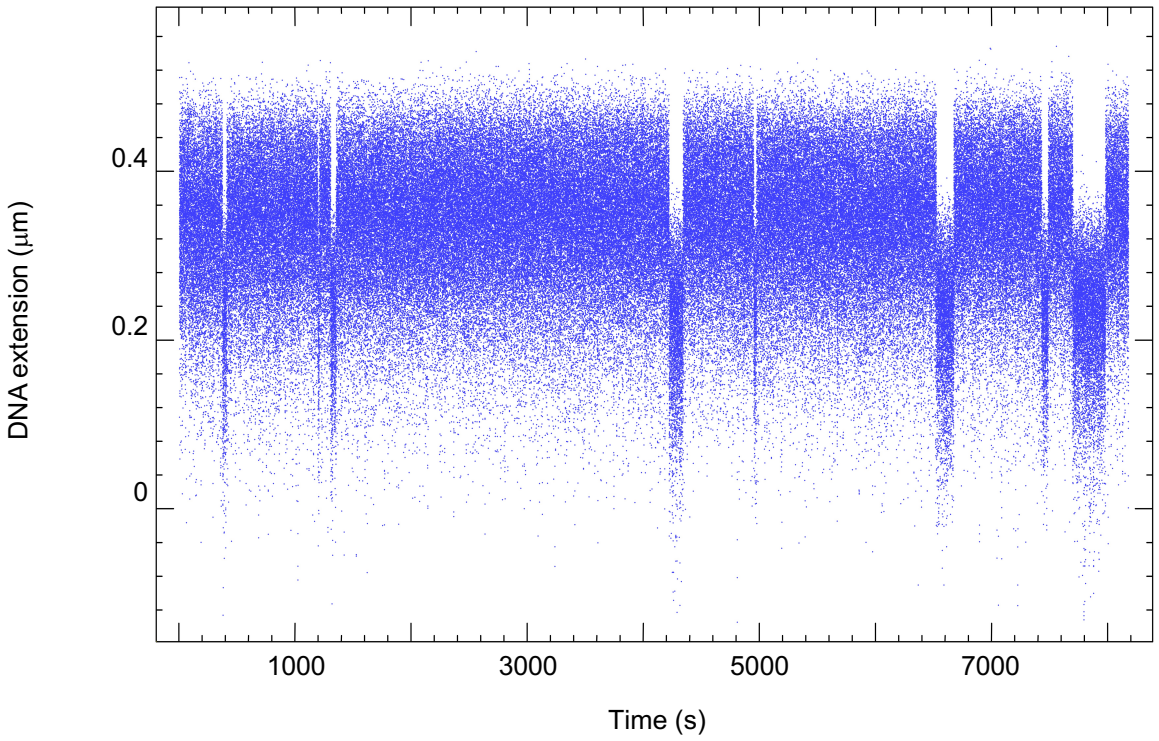
$K = k_{on}/k_{off}$

In trans titration of hACE2 against RBD

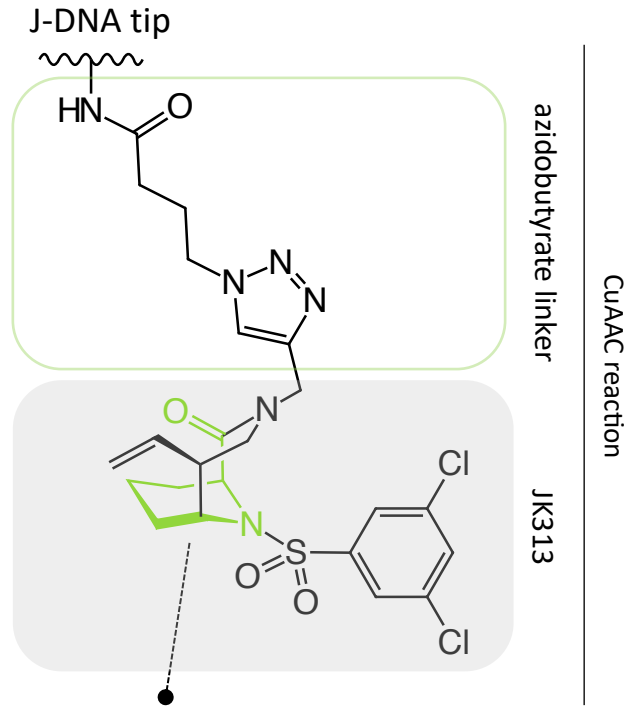
0 nM hACE2



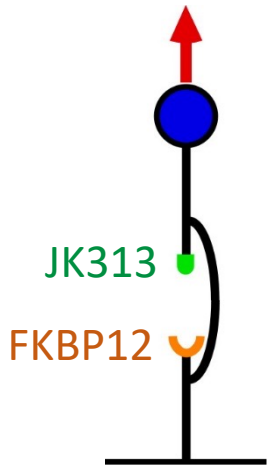
60 nM hACE2

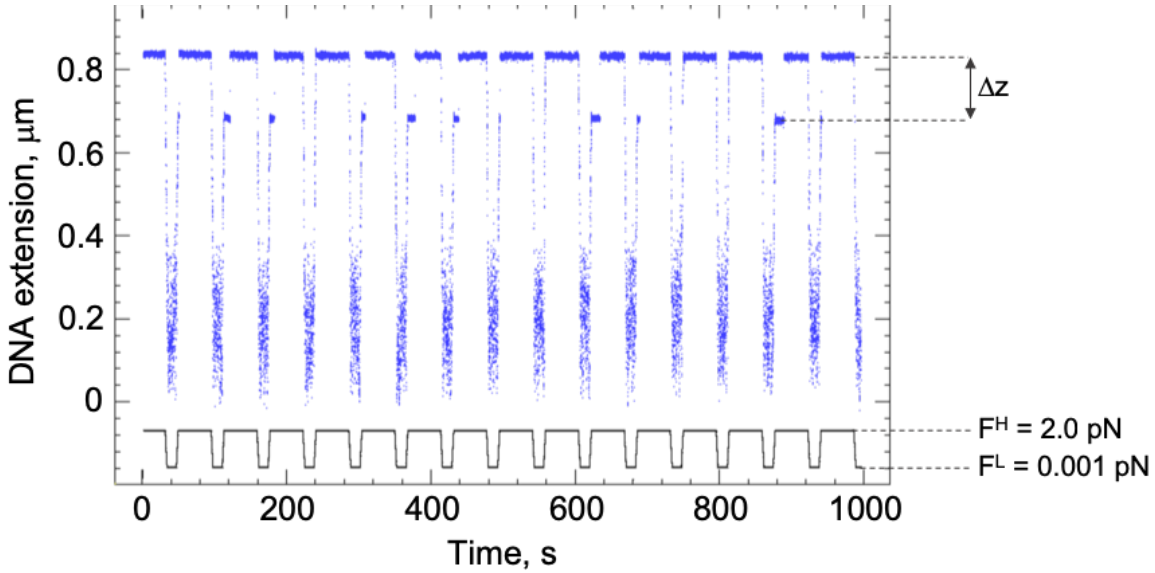


Interaction entre une molécule médicamenteuse et sa sible



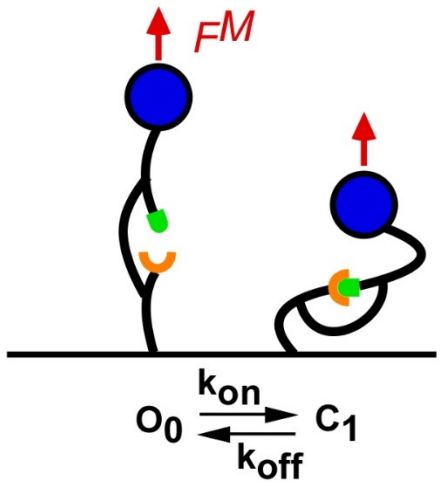
Pipecolate core:
shared structure with rapamycin

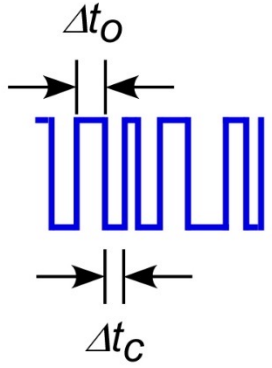




Pomplun *et al.*, *J. Med. Chem.*, **61**, 3660-3673 (2018)

Titration *en trans* de JK313

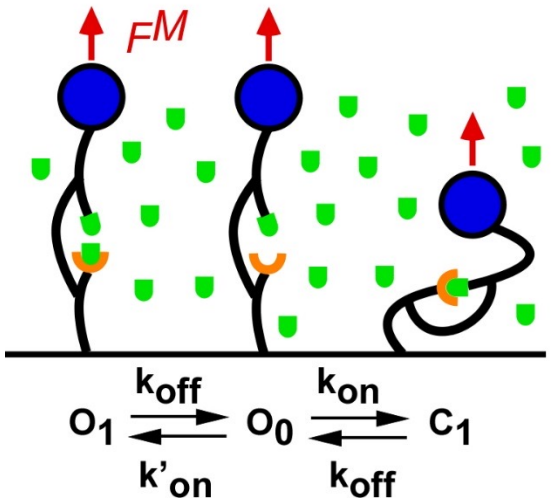


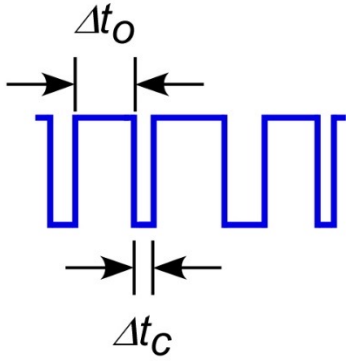


$1/<\Delta t_o> = k_{on}$

$$\frac{<\Delta t_O>}{<\Delta t_c>} = \tilde{K}_c$$

In trans concentration of hACE2





$$\frac{<\Delta t_O>}{<\Delta t_c>} = \tilde{K}_c\left(1 + \frac{[hACE2_t]}{K}\right)$$

$K = k'_{on}/k_{off}$

L'équipe

Marc Nadal, Professor Paris 7
Charlie Gosse, CNRS CR1
Helene Debat, Asst. Prof. UVSQY
Florence Garnier, Asst. Prof. UVSQY

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- Long Yun, PhD student
- Fianzo Smith-Clarke, PhD student
- Julien Le Gall, PhD student
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- S. Weiss (UCLA)
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- A. Weixlbaumer (IGBMC)
- A. Poterszman (IGBMC)
- F. Hausch (Dresden)
- F. Rey (I. Pasteur)
- F. Rico (Aix-Marseille Uni.)

- Horizons 2020 ITN Consortium
  - T. Sixma, NKI
  - J. Lebbink, Erasmus MC
  - P. Friedhoff, Geissen
  - N. Savery, Bristol
  - M. Lamers, Leiden
  - Elvesys (FR)
  - Future Synthesis (PL)

The Team

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- Xi Yang, PhD student
- James Portman, PhD student
- Katryna Nitsenko, PhD student

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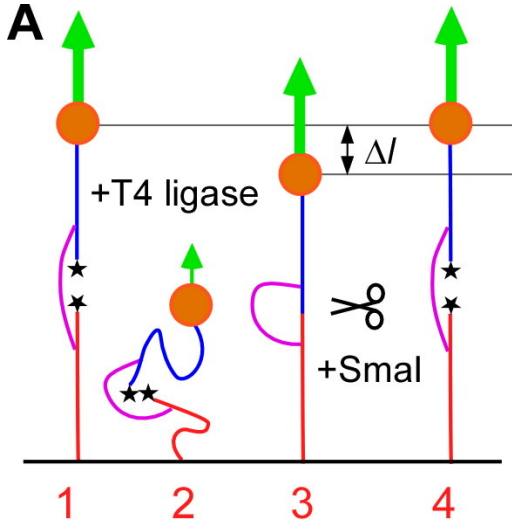
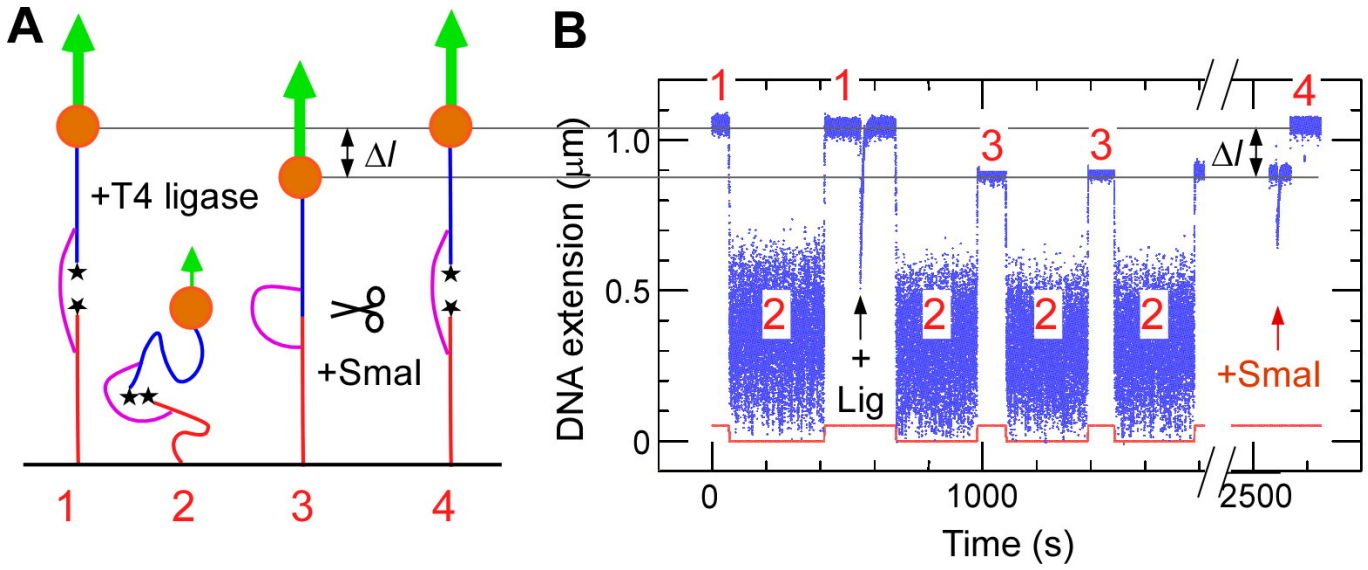
Testing the Molecular Forceps with T4 DNA Ligase



★ 5’ Phosphate

Testing the Molecular Forceps with T4 DNA Ligase



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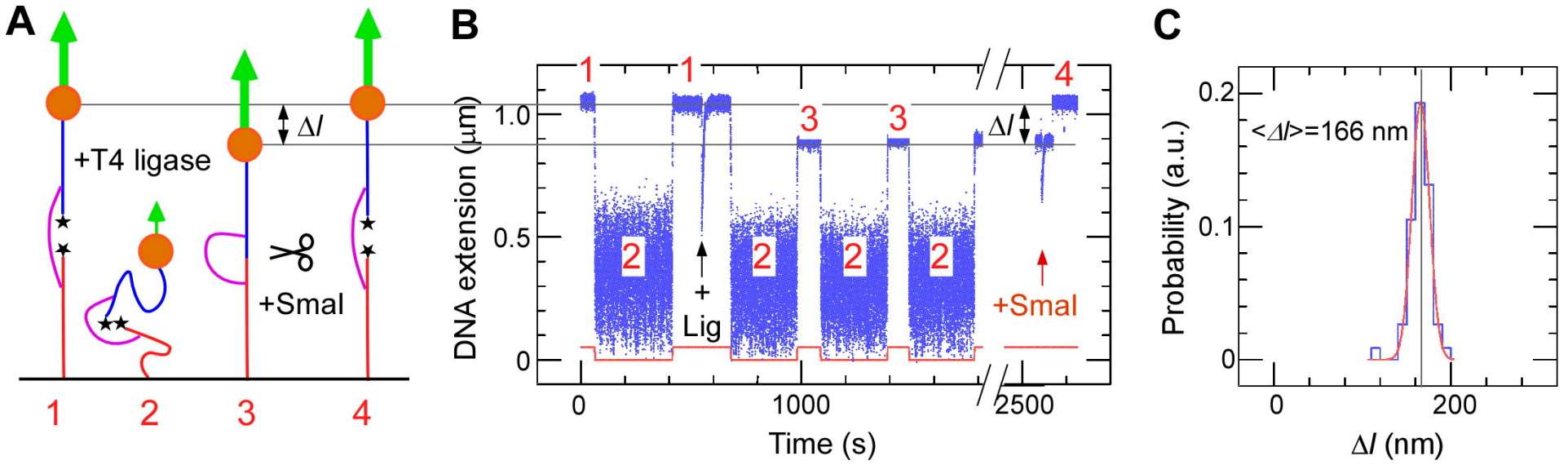
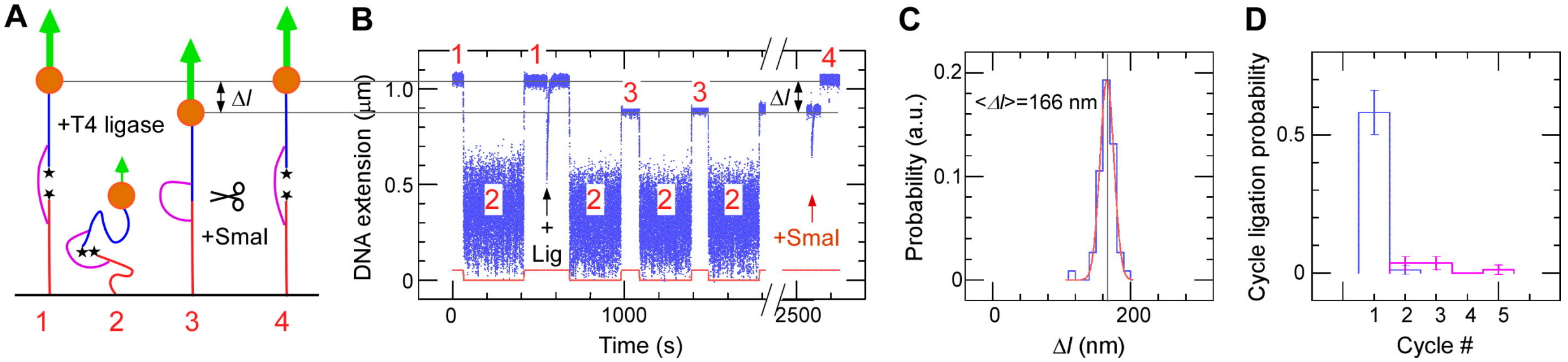
Testing the Molecular Forceps with T4 DNA Ligase



★ 5’ Phosphate

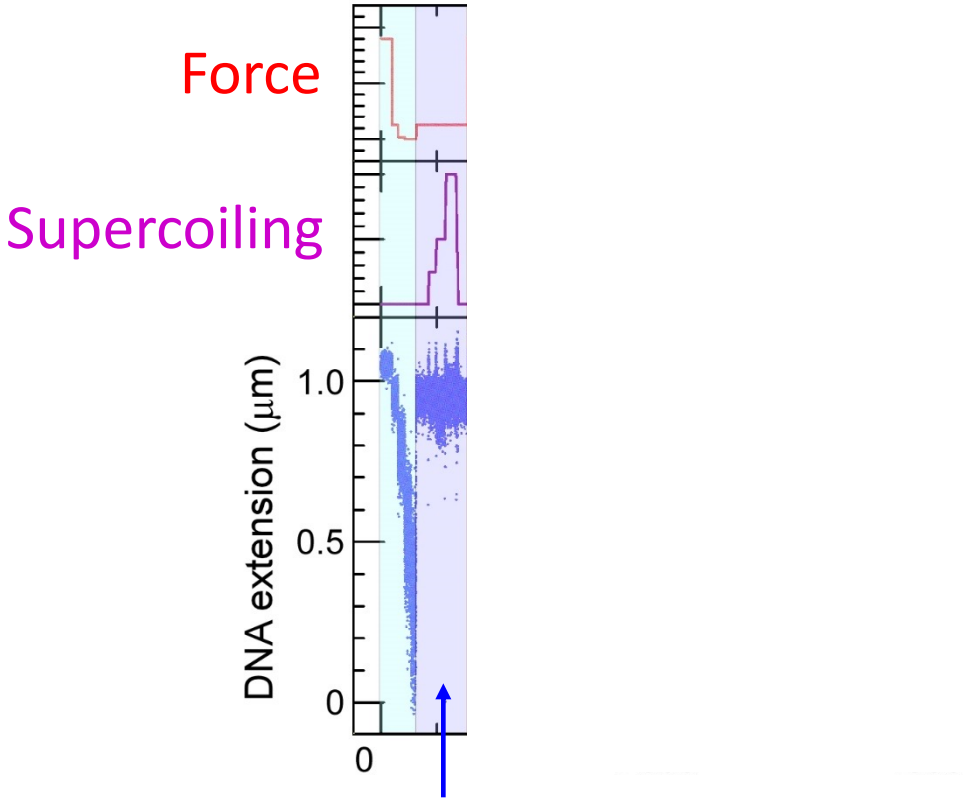
Testing the Molecular Forceps with T4 DNA Ligase

Restriction digest of DNA "ends" : overhang (XmaI)
blunt (SmaI)



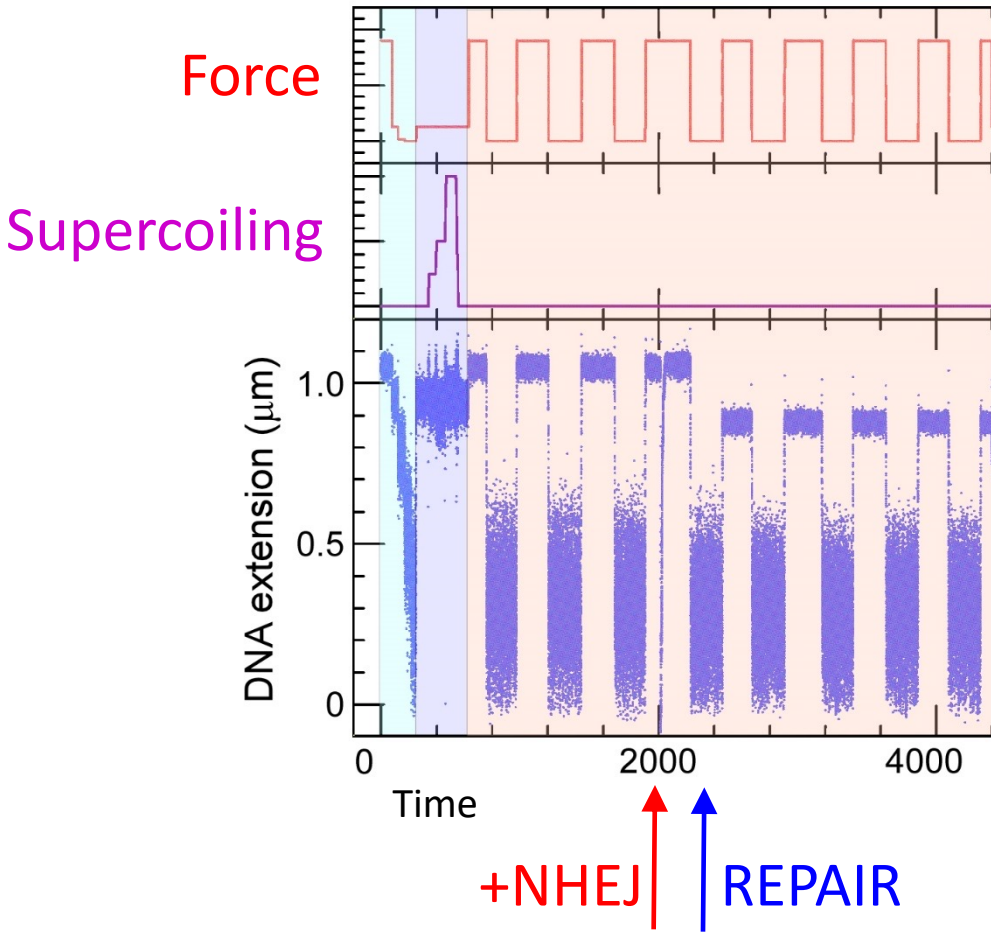
★ 5' Phosphate

Repaired DNA can be supercoiled: repair occurs on both strands



DNA cannot supercoil

Repaired DNA can be supercoiled: repair occurs on both strands



Repaired DNA can be supercoiled: repair occurs on both strands