
KNOT SHOP
Collar Full

Unlinking of Newly Replicated DNA Molecules

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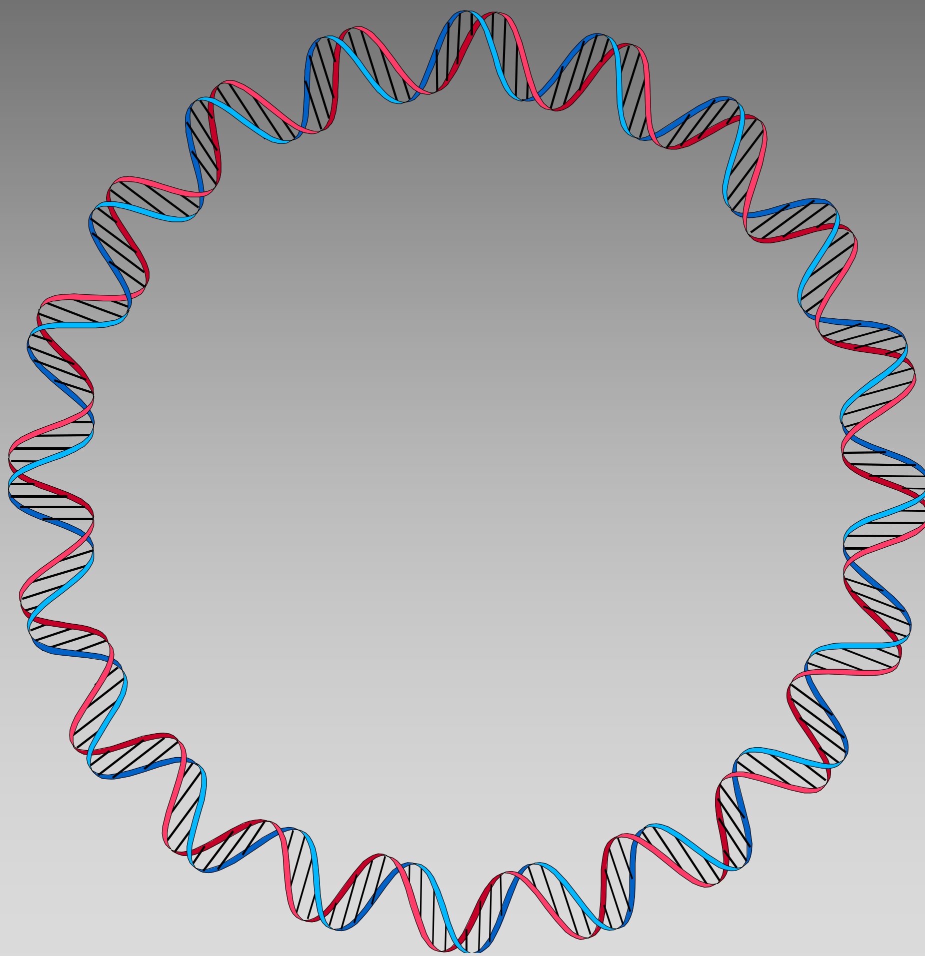
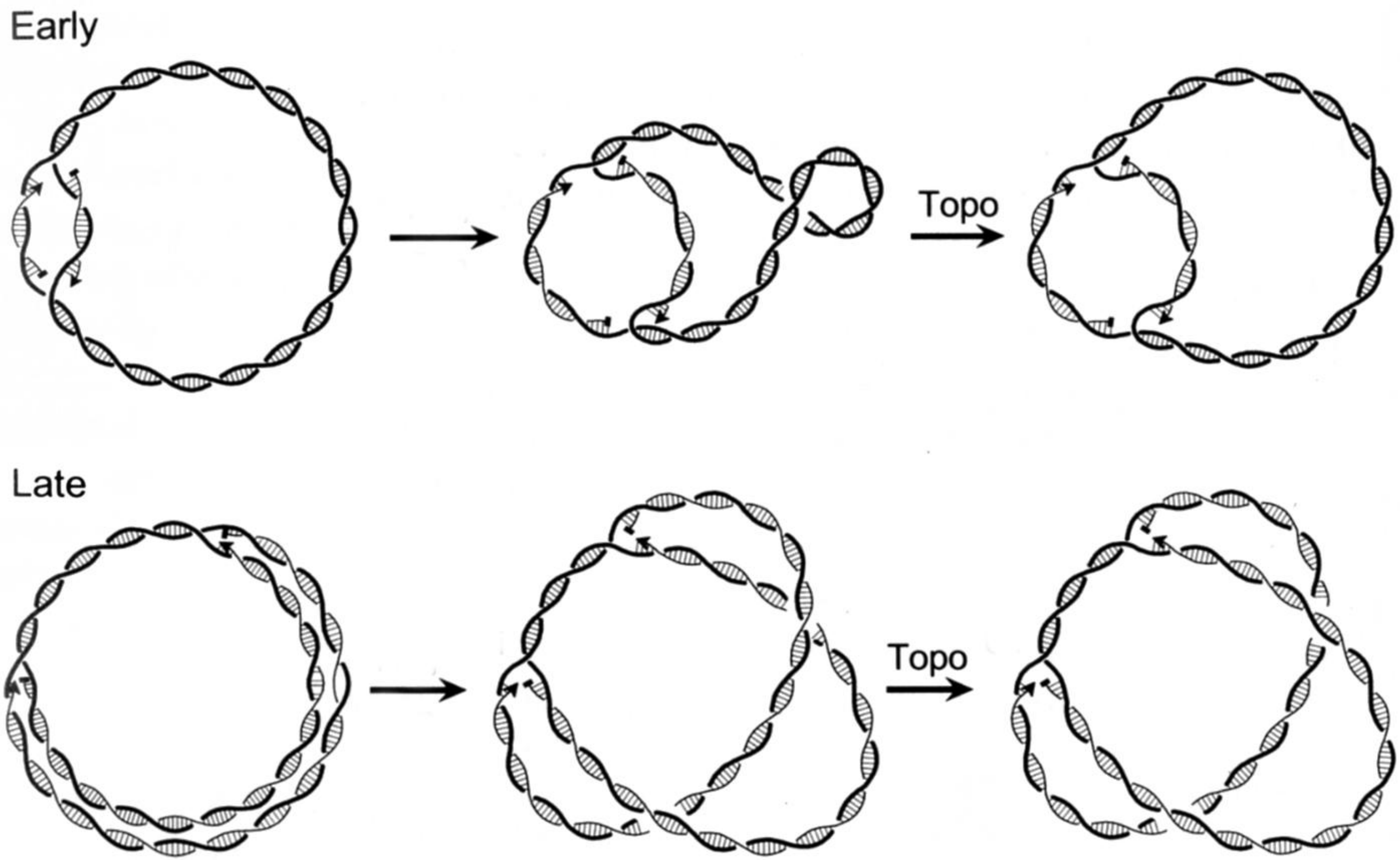
New York University

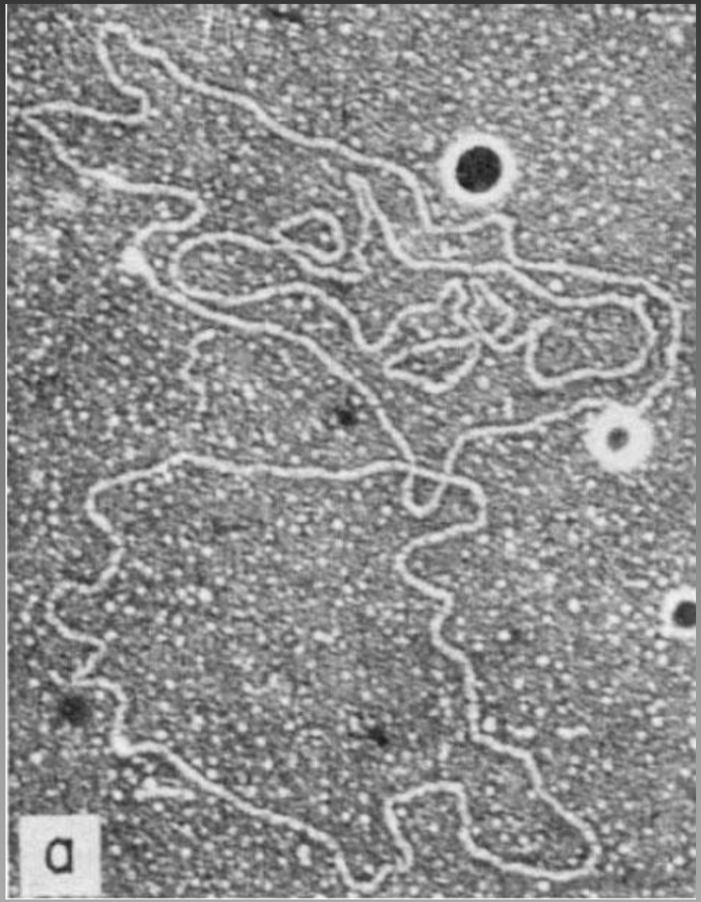
In circular DNA complementary strands are linked



DNA replication stalls without topoisomerases

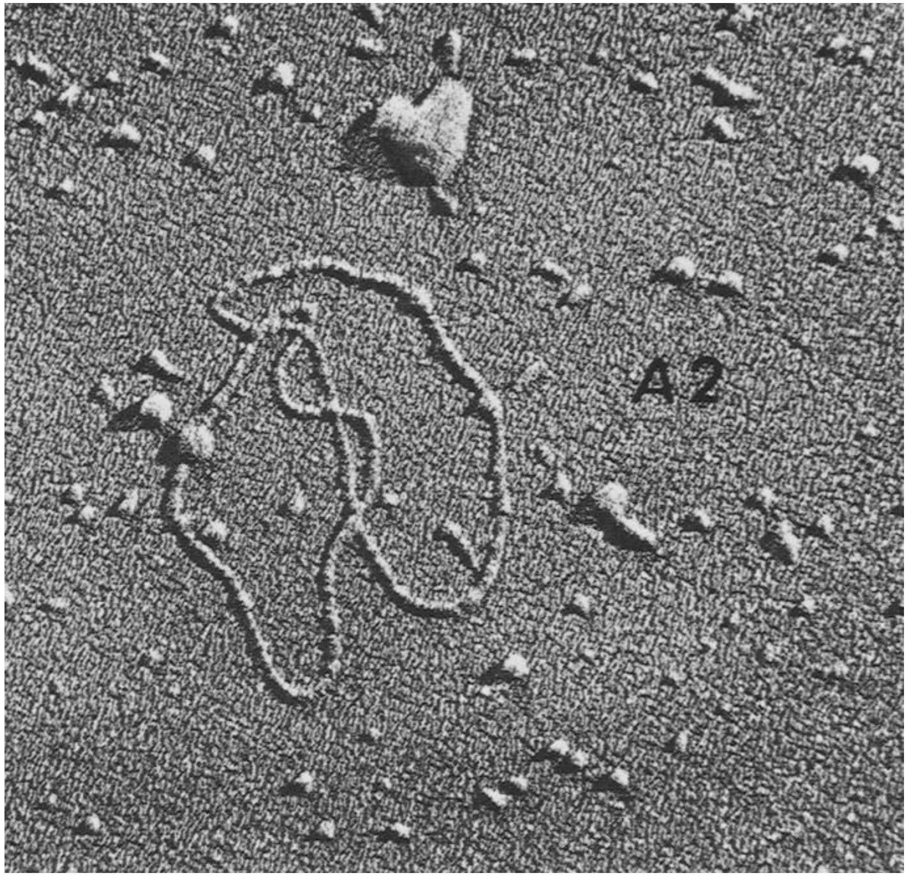


DNA catenanes



B. Hudson & J. Vinograd. Catenated Circular DNA Molecules in HeLa Cell Mitochondria. *Nature*, **216**, 647-652 (1967).

Replication Catenanes

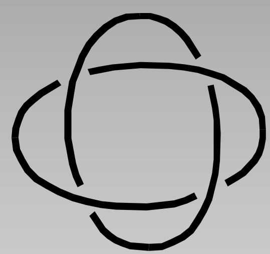


Sundin, O. & Varshavsky, A. (1980). Terminal stages of SV40 DNA replication proceed via multiply intertwined catenated dimers. *Cell* **21**, 103-114.

Simplest links and their Alexander polynomials

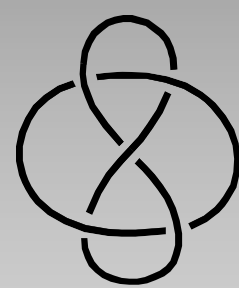
2_1

1



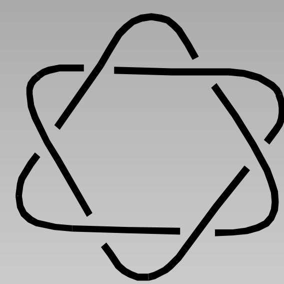
4_1

$s+t$



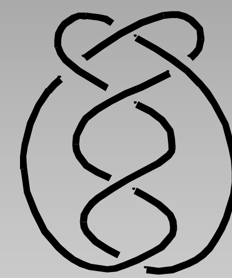
5_1

$(s-1)(t-1)$



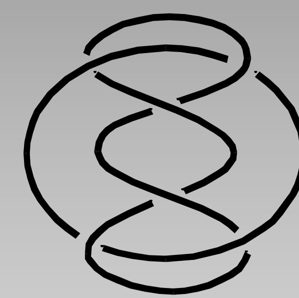
6_1

s^2+st+t^2



6_2

$st(t+s)-st+s+t$



6_3

$2st-t-s+2$

A few mechanisms facilitating unlinking were developed by nature

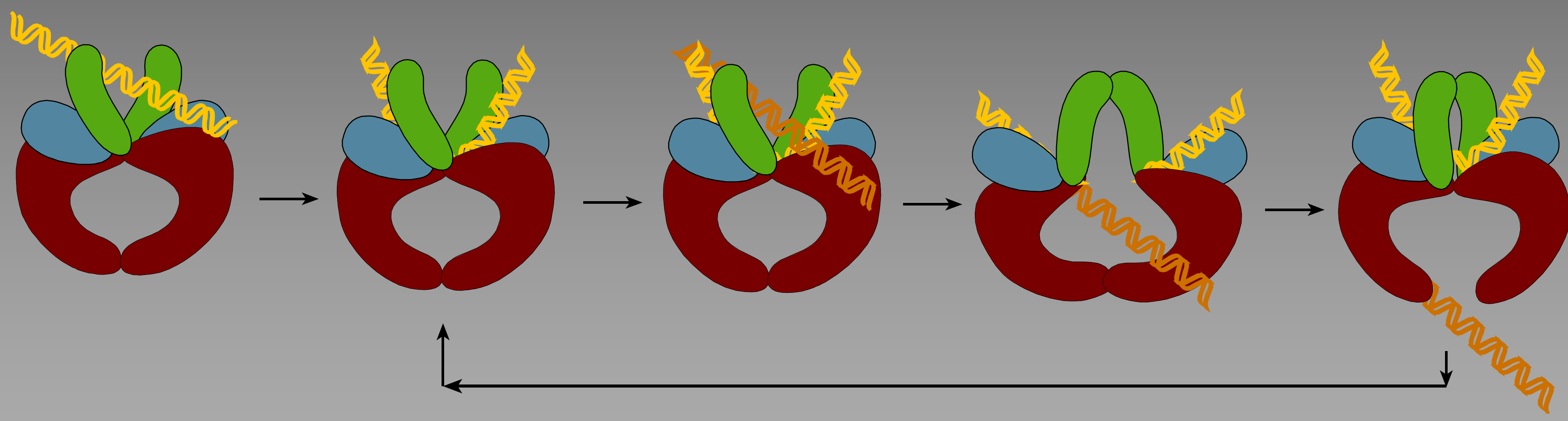
1. Chromosomes compactization.

2. Mechanical forces which stretch linked daughter chromosomes during mitosis.

3. DNA supercoiling (prokaryotes).

4. Special mechanism of topology simplification encoded in type IIA DNA topoisomerases.

Diagram of type IIA topoisomerase action

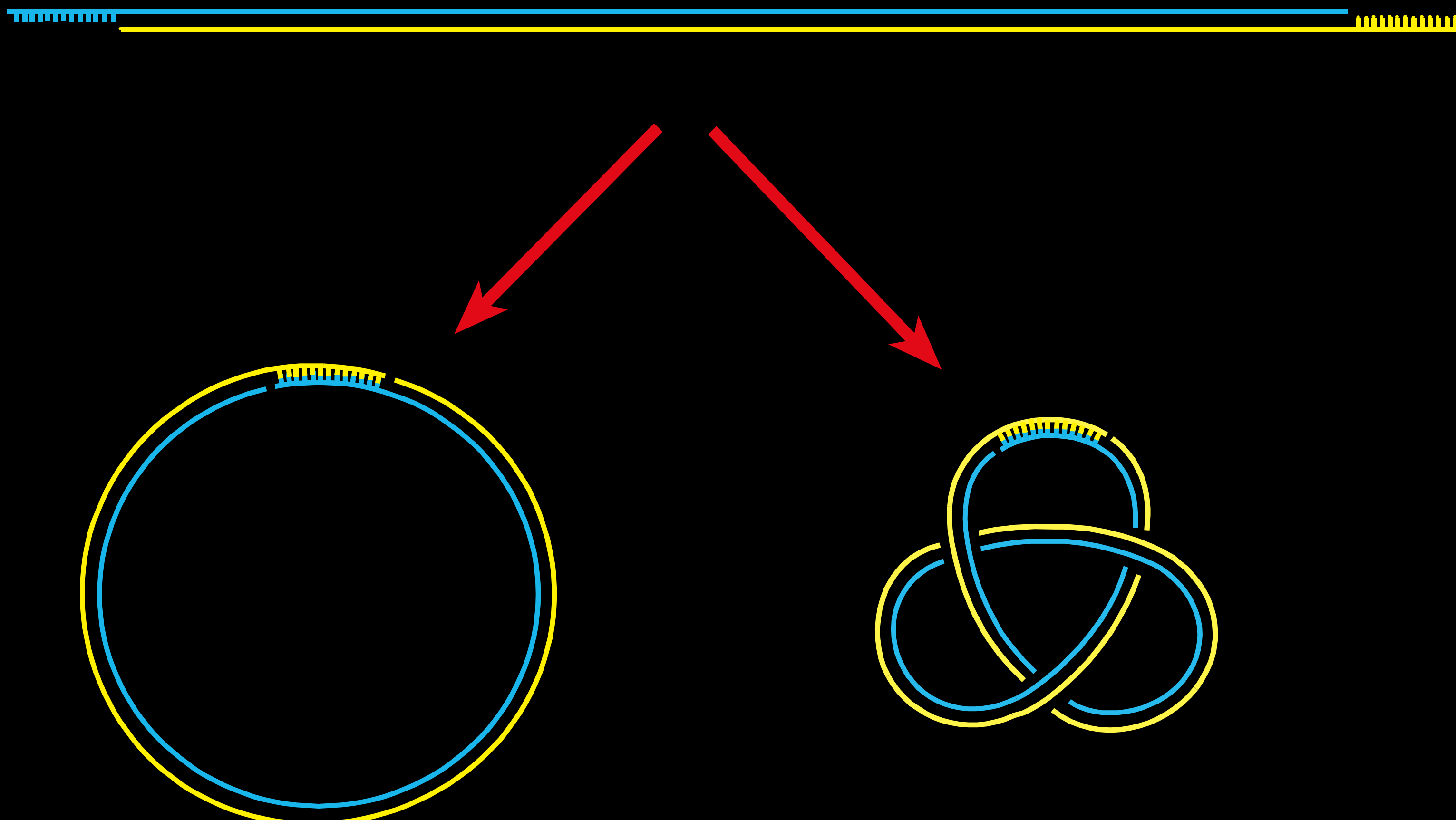


Equilibrium distribution of topological states

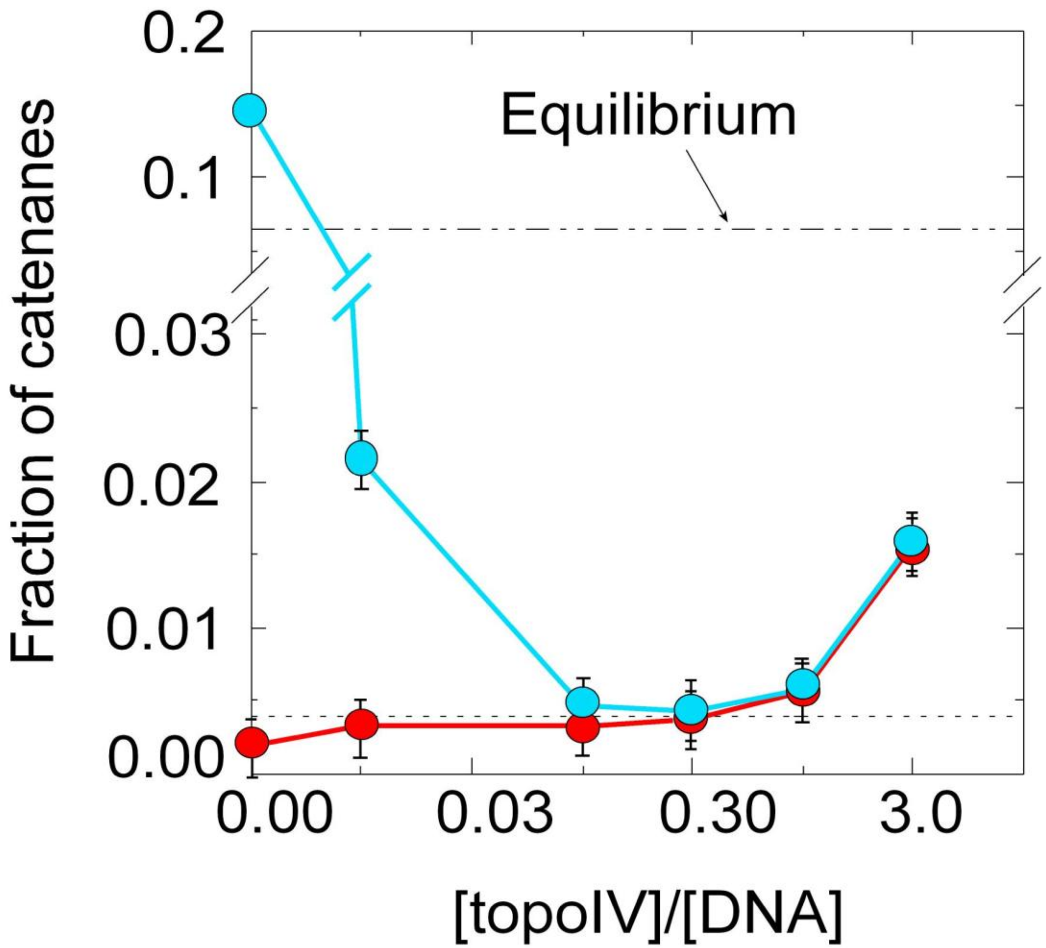
$$P_i = \frac{\sum\limits_{\text{Topology } i} \exp(-E_k / kT)}{\sum\limits_{\text{All conformations}} \exp(-E_k / kT)},$$

where P_i is the equilibrium probability of topological state i, E_k is the energy of the conformation k

Slow cyclization of DNA molecules in solution results in the equilibrium distribution of topological states

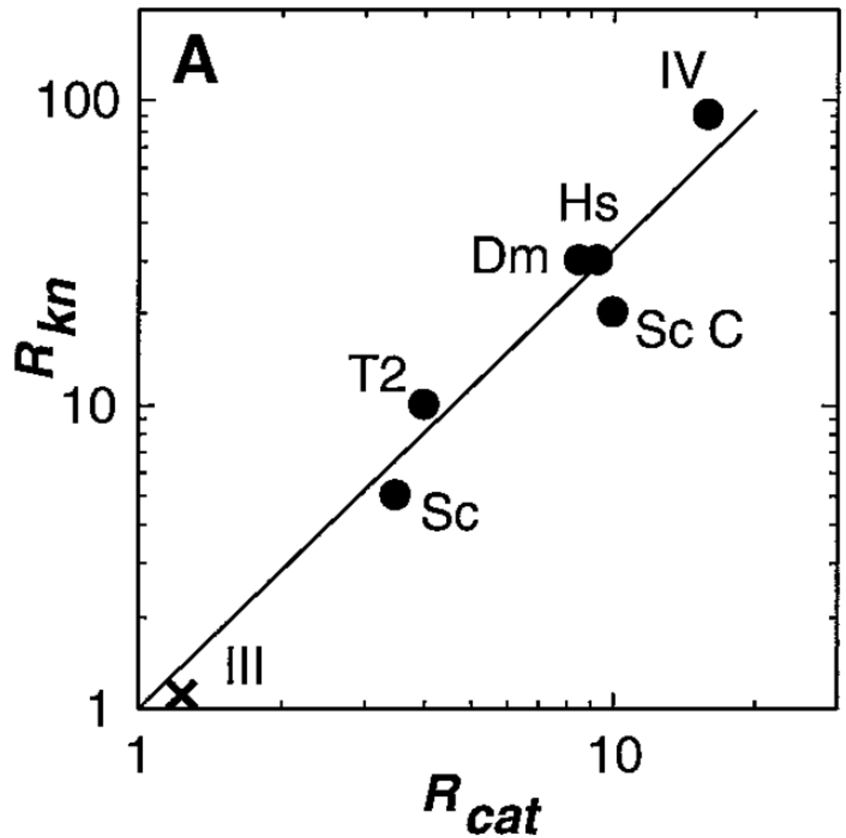


Unlinking of DNA catenanes by type II DNA topoisomerases



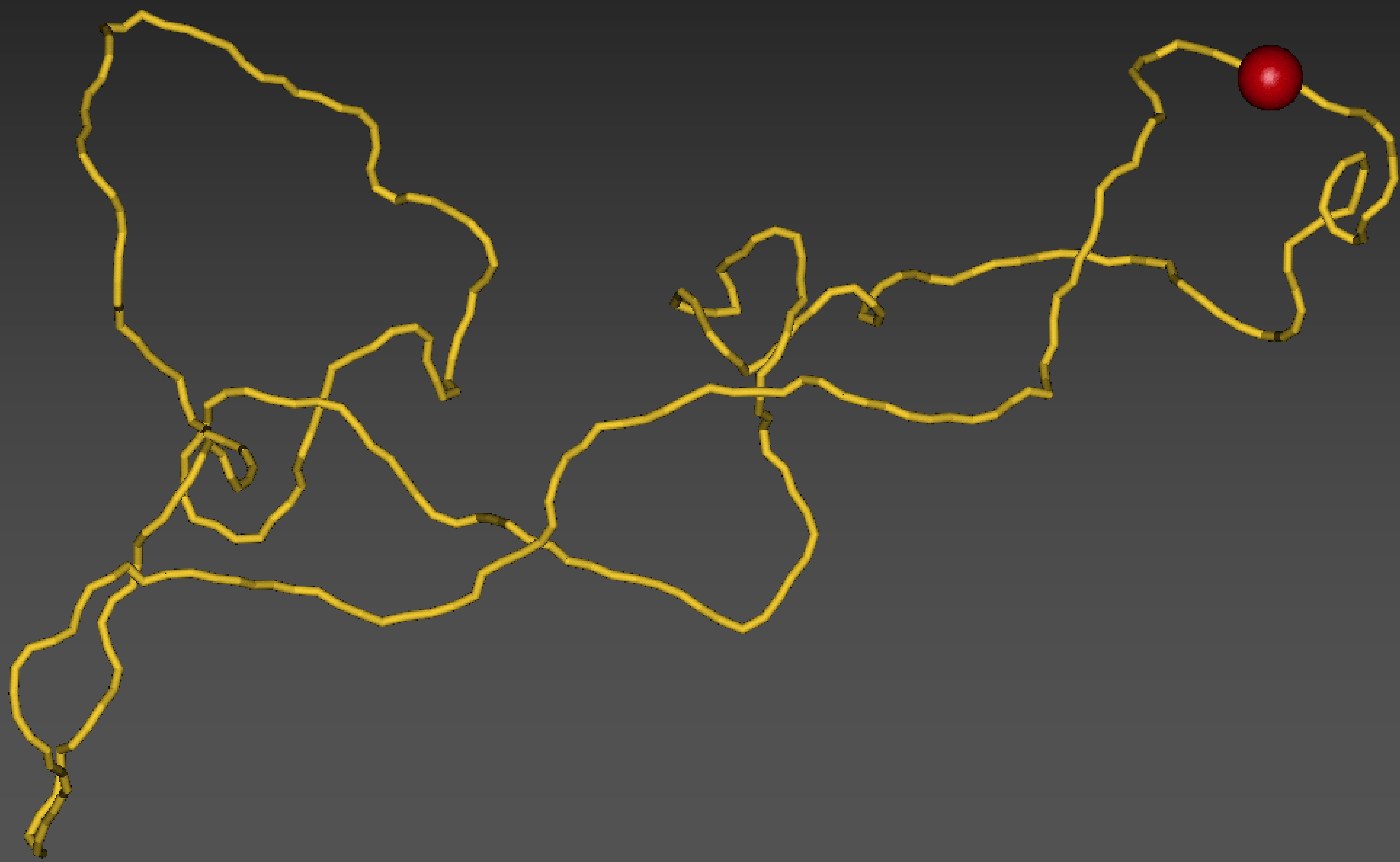
V. V. Rybenkov, C. Ullsperger, A. V. Vologodskii, N. R. Cozzarelli, *Science* **277**, 690-693, (1997)

Topo IIA can reduce the catenane concentration up to 20 times relative to the equilibrium level

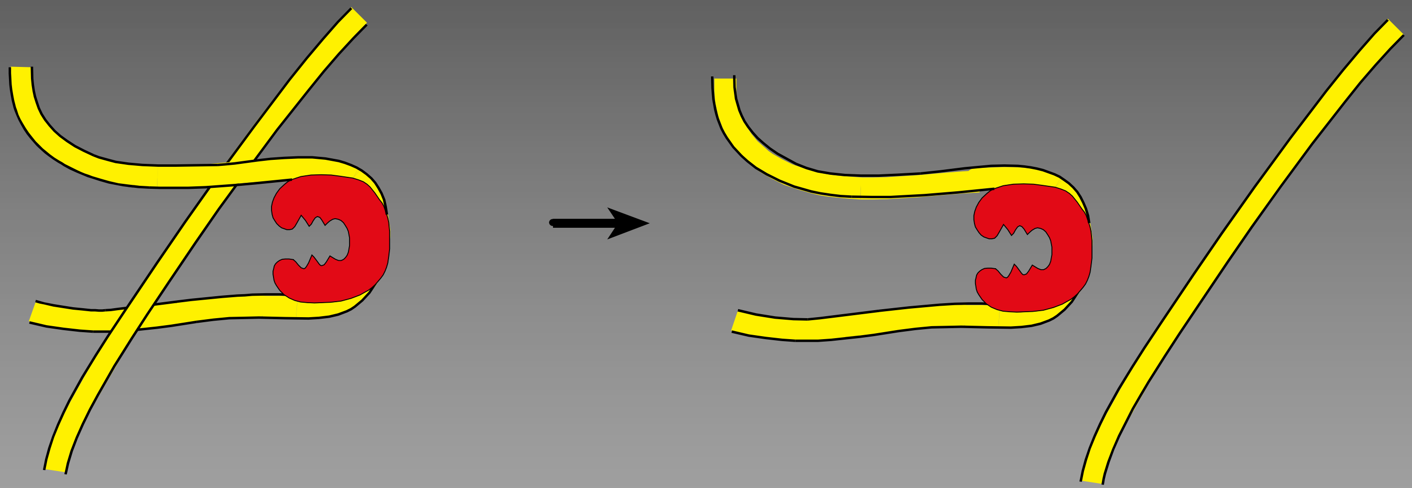


V. V. Rybenkov, C. Ullsperger, A. V. Vologodskii, N. R. Cozzarelli, *Science* **277**, 690-693, (1997)

Type II topoisomerases are very large proteins but they are very small in comparison with DNA coils

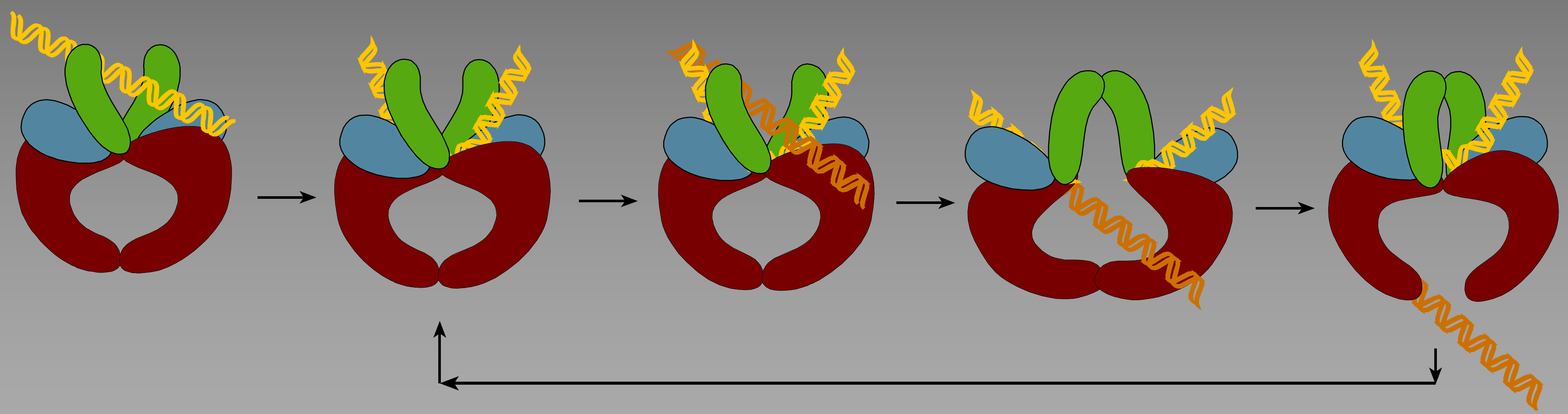


The model of topo II action

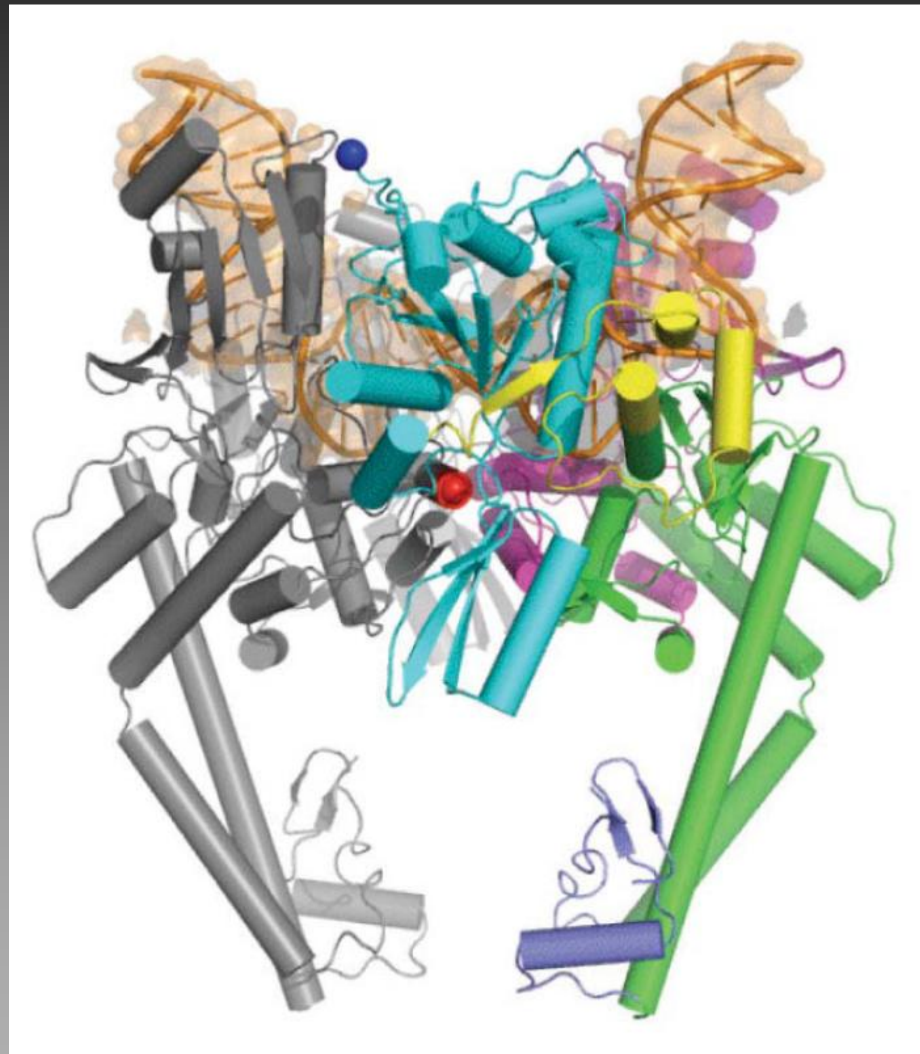


A. V. Vologodskii, W. Zhang, V. V. Rybenkov, A. A. Podtelezhnikov, D. Subramanian,
B. J. D. Griffith & N. R. Cozzarelli, *PNAS* **98**, 3045-3049 (2001)

Diagram of type IIA topoisomerase action



Structure of yeast topo II enzyme bound with G segment



K. C. Dong & J. M. Berger, *Nature* **450**, 1201-1205 (2007)

The steady-state fraction of links can be calculated as

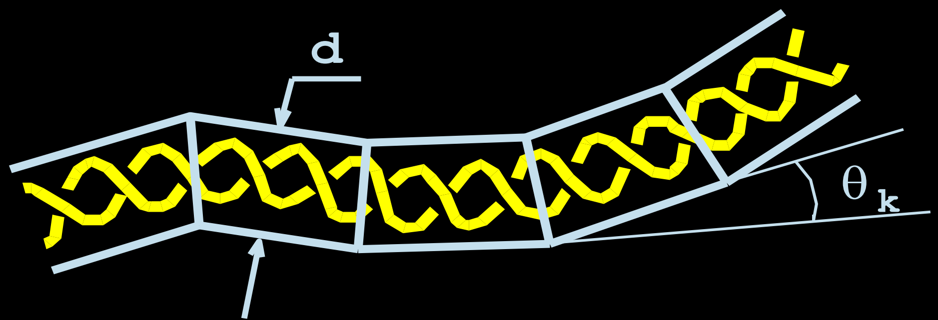
$$\frac{c_c}{c_u} = \frac{P(u|j)}{P(c|j)} B_2 c,$$

where c_c and c_u are the steady state concentrations of catenanes and unlinked chains,

$P(u/j)$ and $P(c/j)$ are the probability that a second chain is unlinked/linked with the first chain under condition that its segment is properly juxtaposed with the G segment,

B_2c is the equilibrium fraction of catenanes at DNA concentration c.

Discrete wormlike chain model of DNA



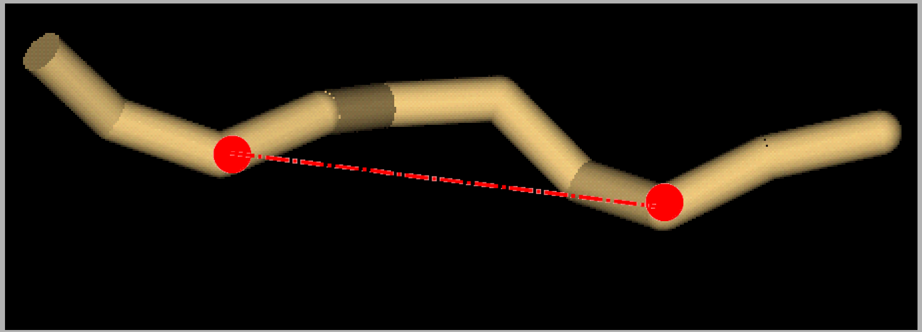
$$E_b = \sum_k g\,\theta_k^2 ,$$

where g is the bending rigidity constant.

DNA effective diameter, d, is a strong function of ionic conditions.

Metropolis Procedure

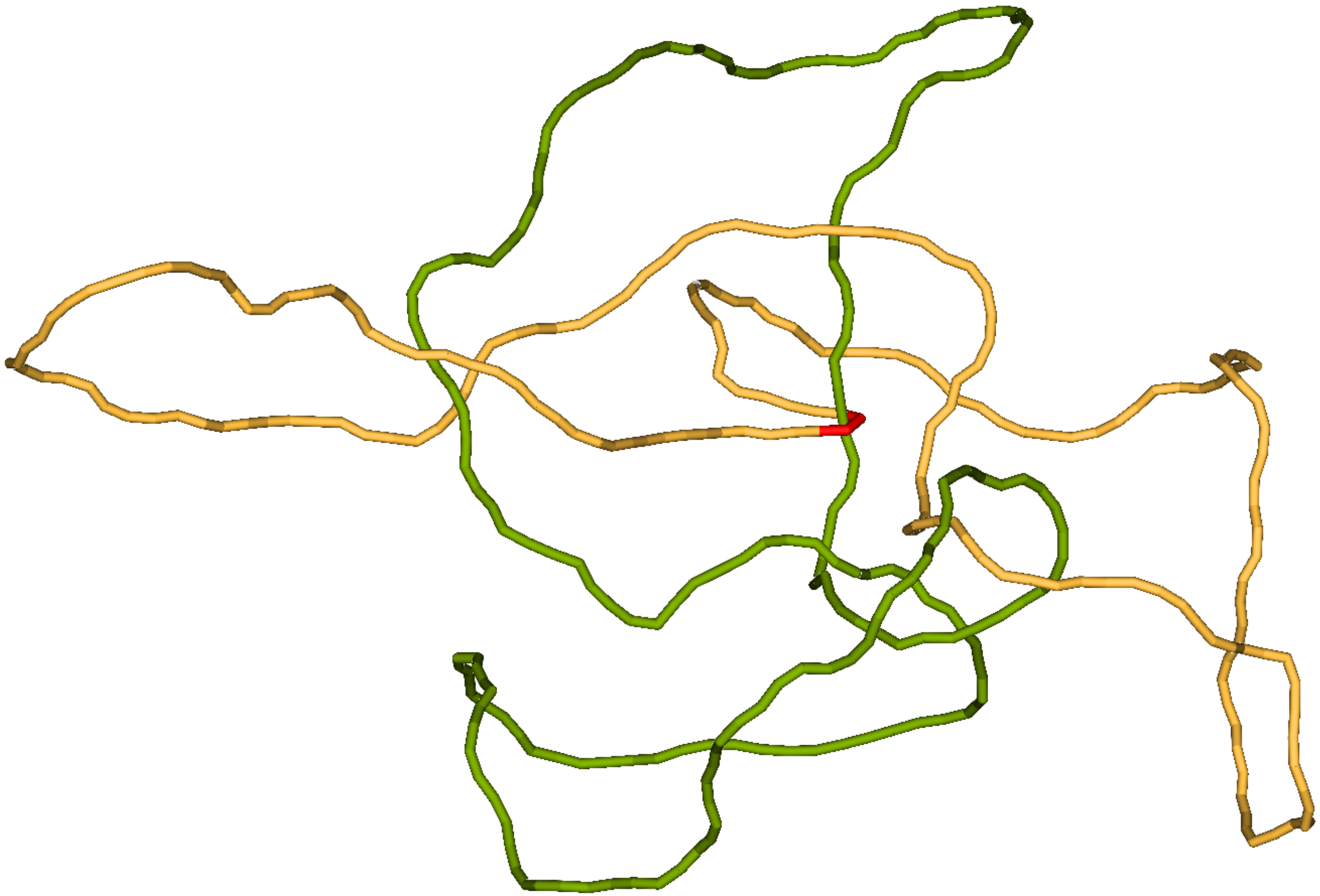
An initial conformation is deformed step by step



Trial conformation is accepted with probability

1	if	$E_T < E_C$
$\exp\left[\left(-\left(E_T - E_C\right)/kT\right]\right.$	if	$E_T > E_C$

The ratio of the conditional probabilities is easy to calculate



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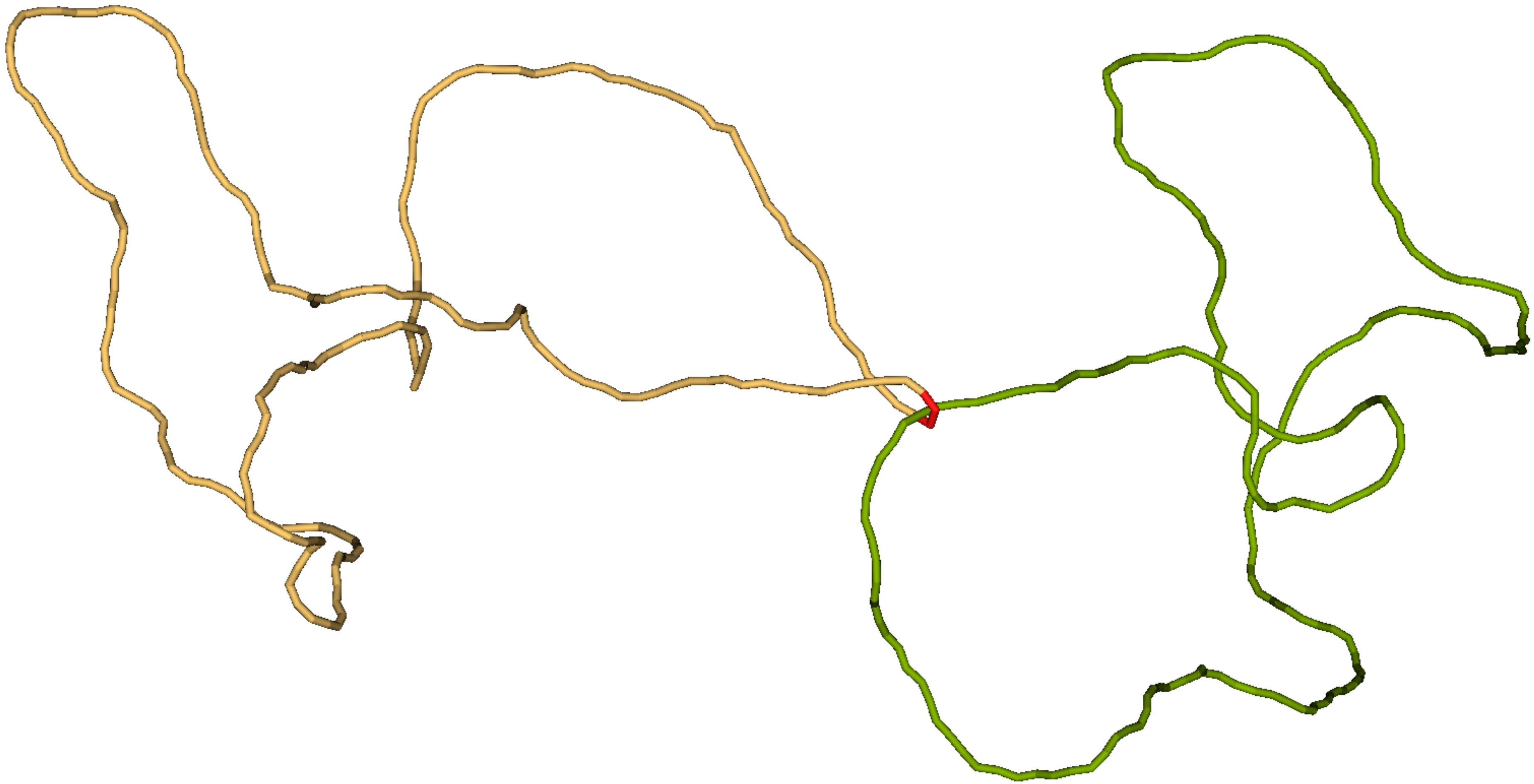
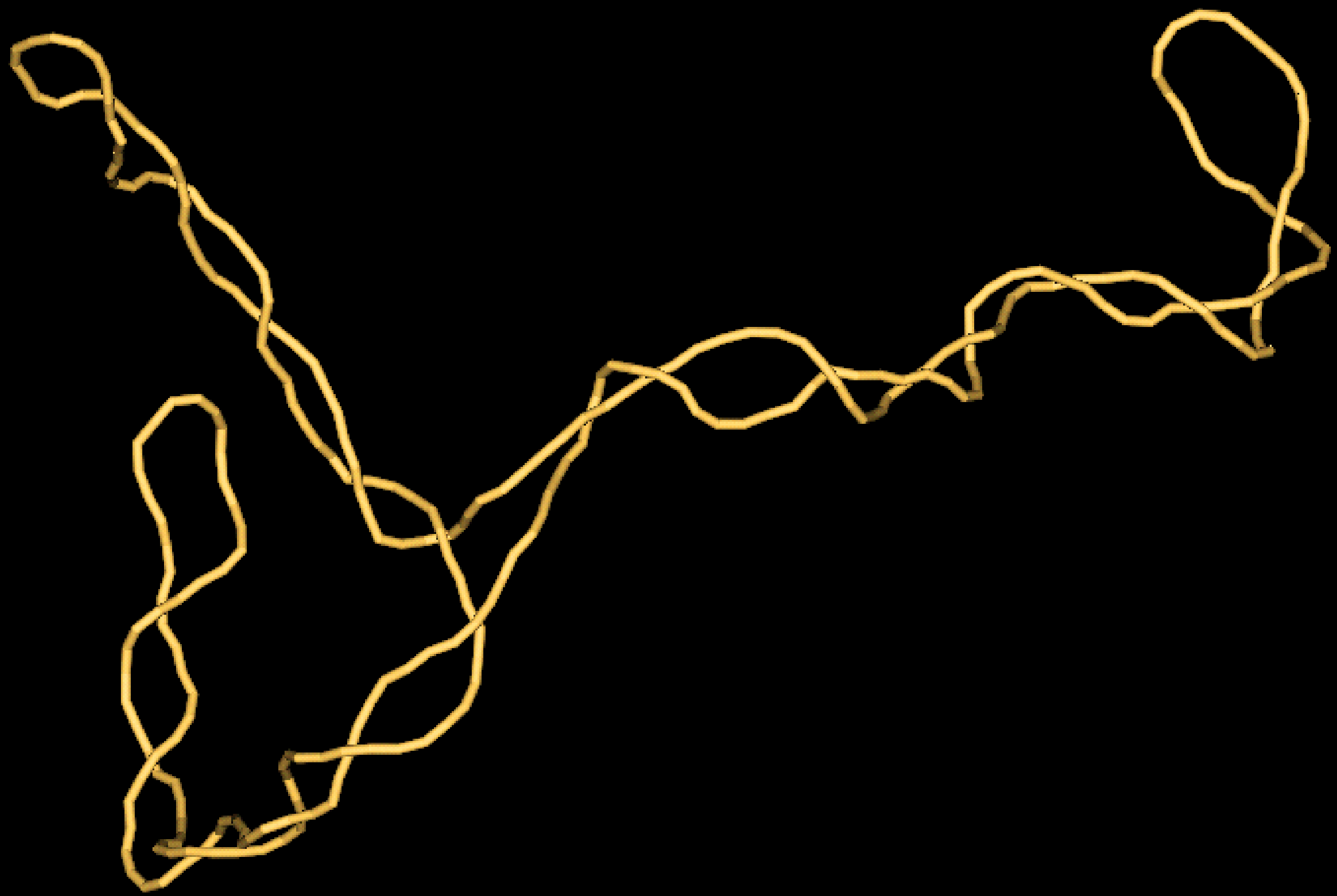
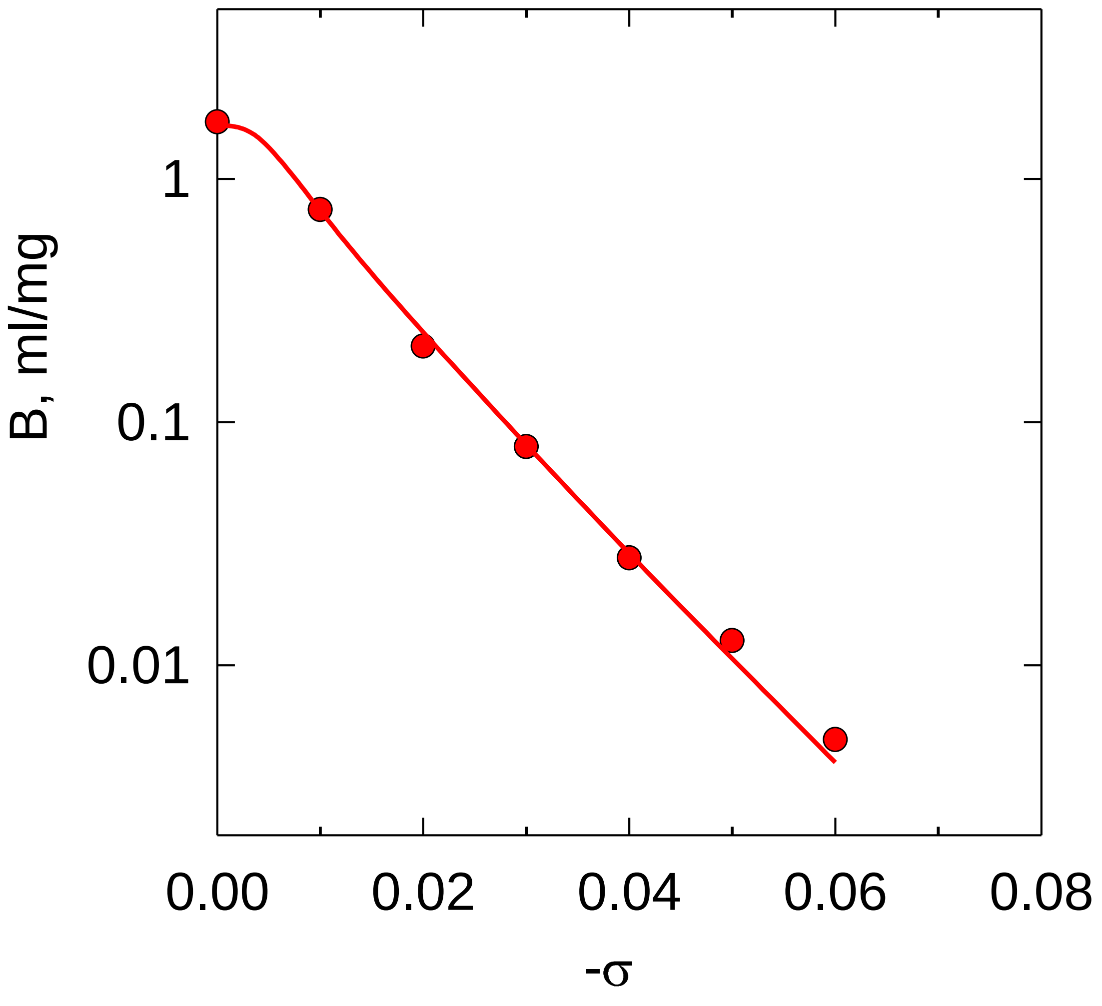


Supercoiled DNA

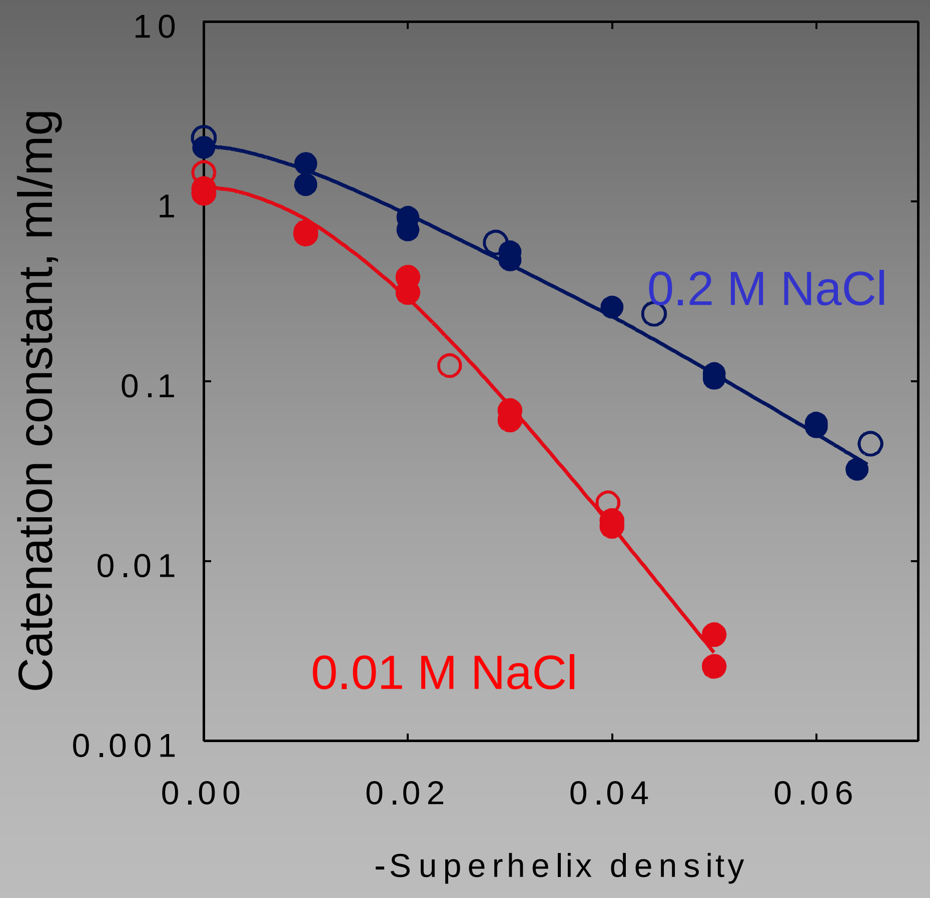


Supercoiling greatly decreases the probability of linking



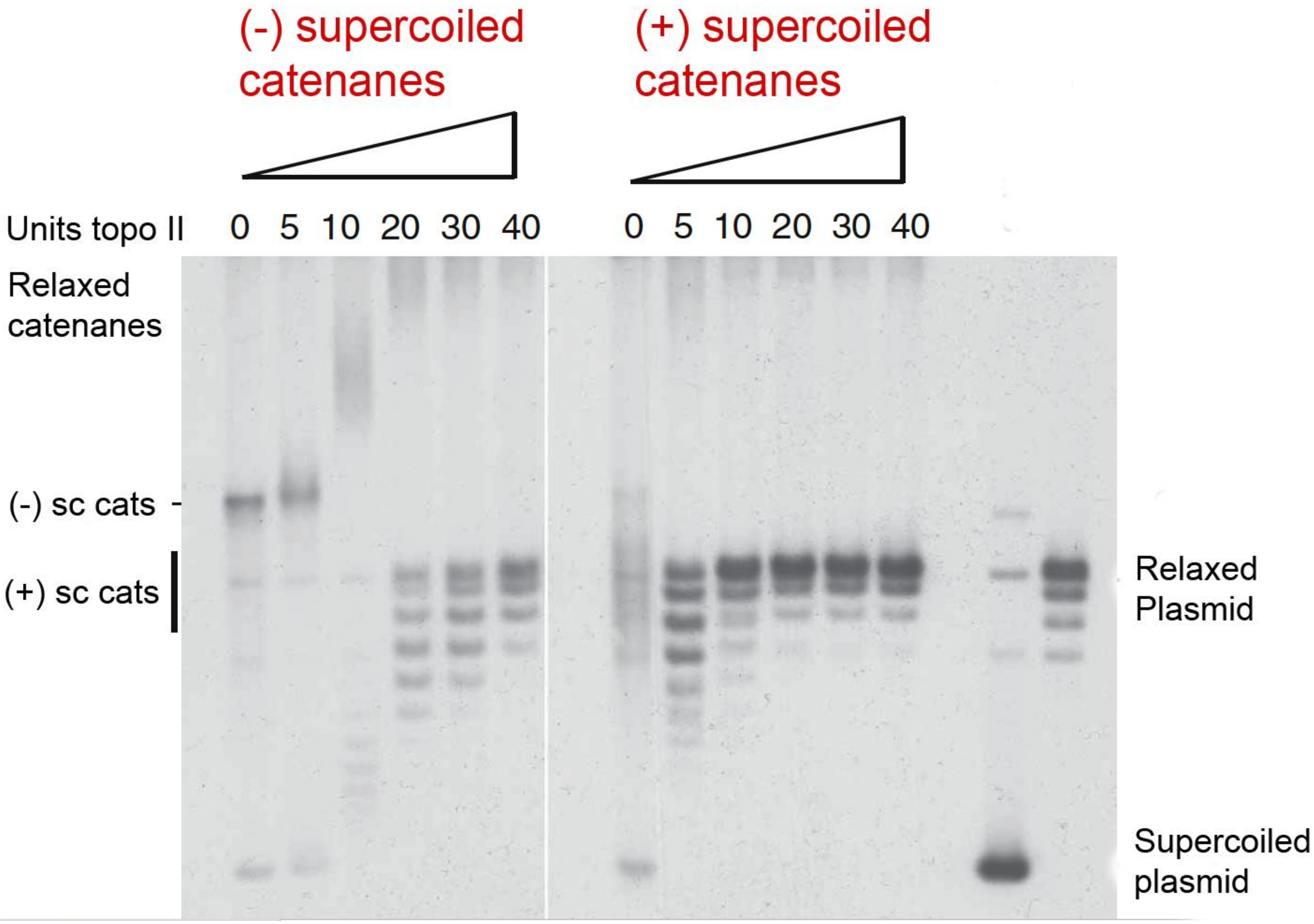
Rybenkov, V. V., Vologodskii, A. V. & Cozzarelli, N. R. (1997). *J. Mol. Biol.* **267**, 312-323

Catenanes formed by supercoiled and relaxed DNA molecules



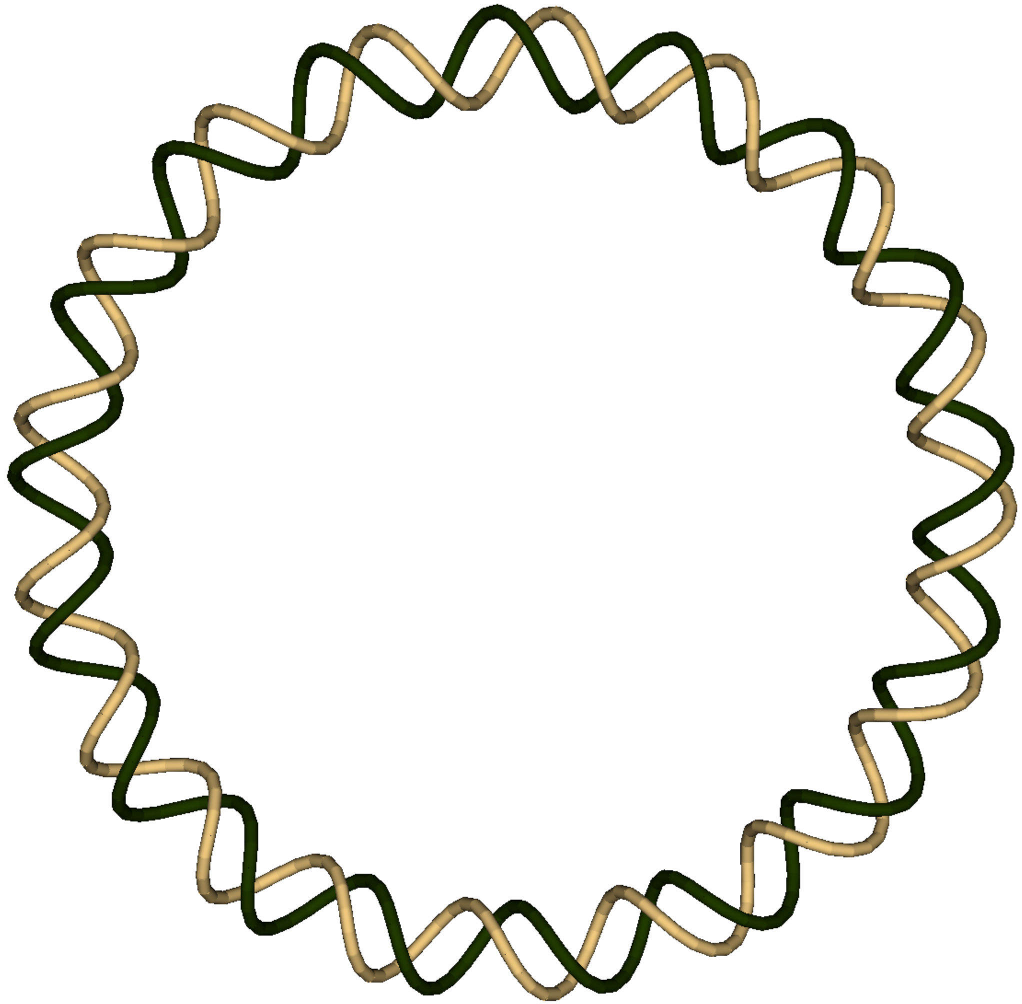
Rybenkov, V. V., Vologodskii, A. V. & Cozzarelli, N. R. (1997). *J. Mol. Biol.* **267**, 312-323

It was found that unlinking of (+) supercoiled catenanes proceeds much faster than unlinking of (-) supercoiled catenanes



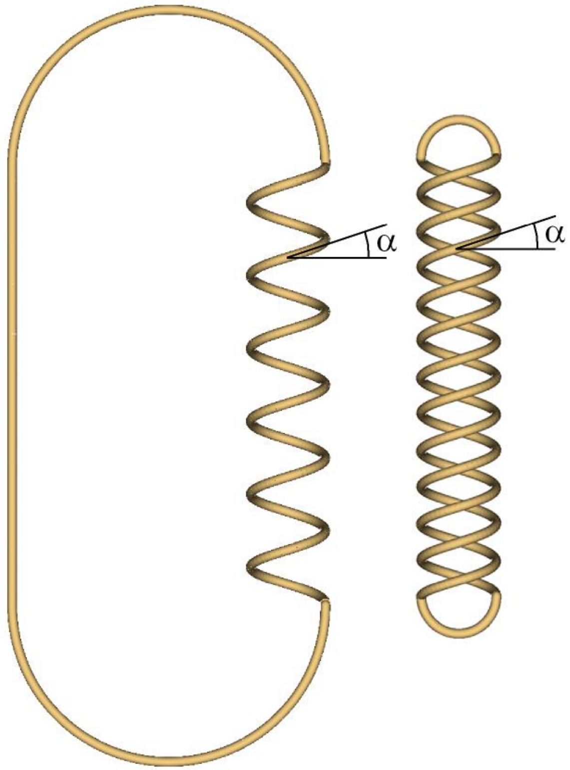
Baxter, J., Sen, N., Martinez, V. L., De Carandini, M. E., Schvartzman, J. B., Diffley, J. F. & Aragon, L. (2011) *Science* **331**, 1328-1332

Replication catenanes are (+) torus links



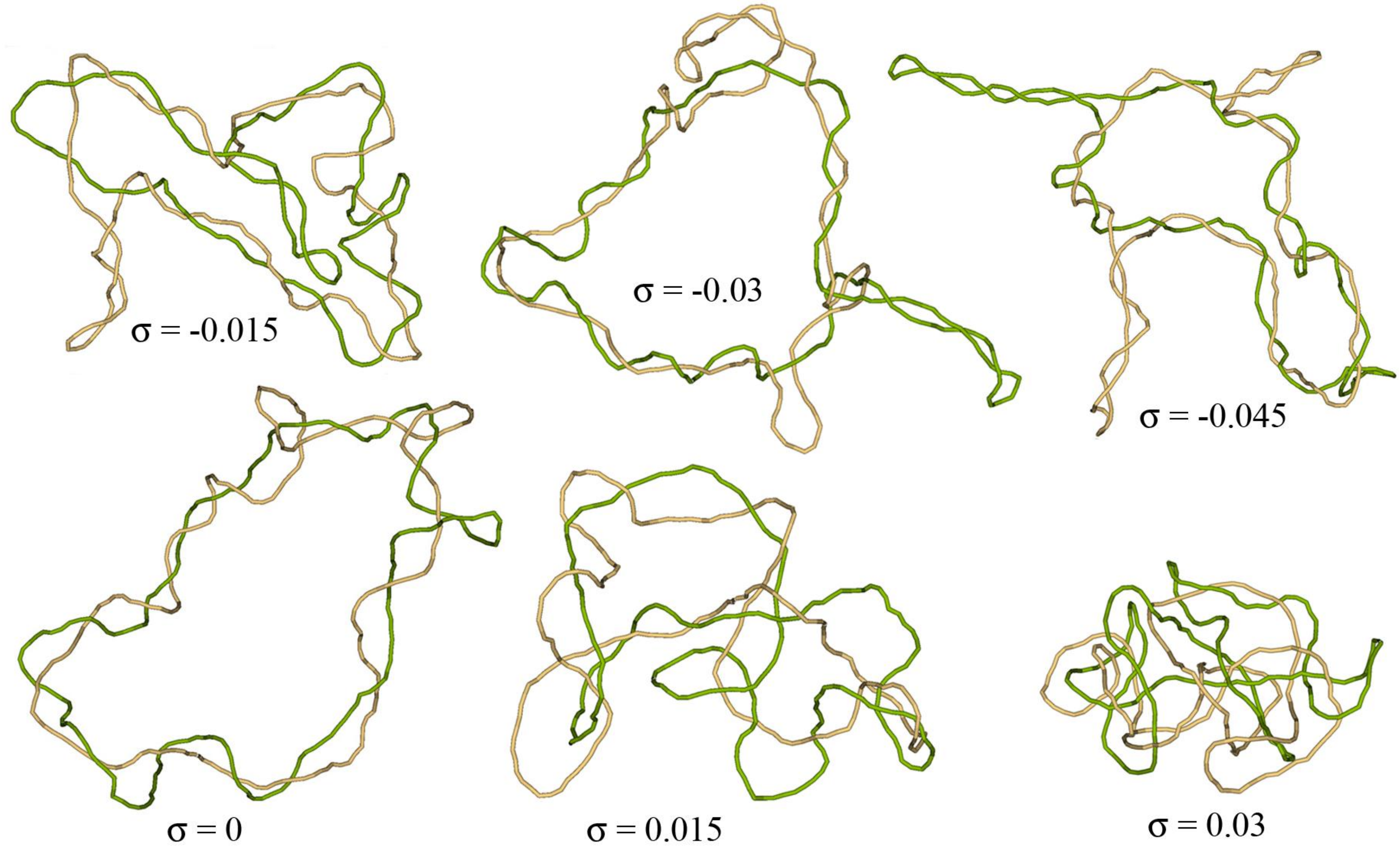
In their regular conformations each contour has a positive writhe

Simple right-handed helix has positive writhe

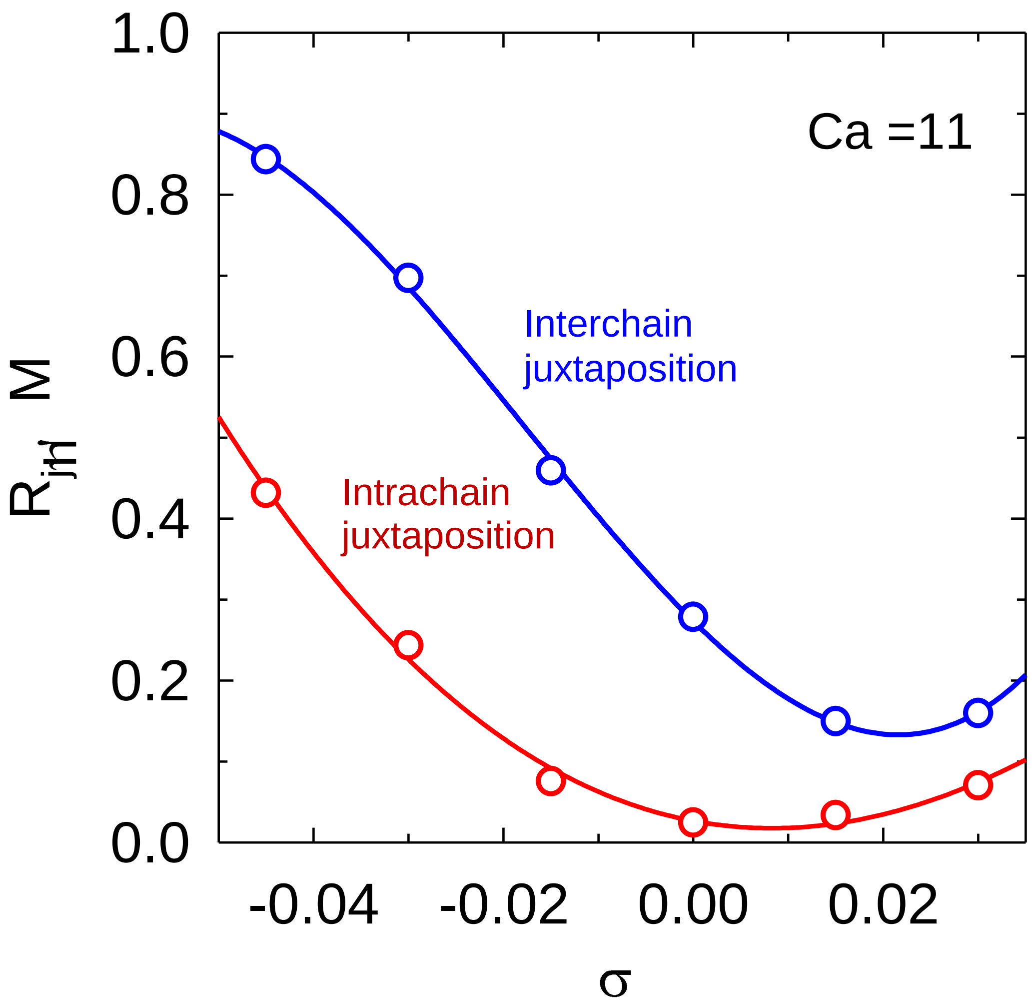


Interwound right-handed helix has negative writhe

Typical simulated conformations of the replication catenanes



Segment juxtaposition in the replication catenanes



The distributions of angles between the juxtaposed segments

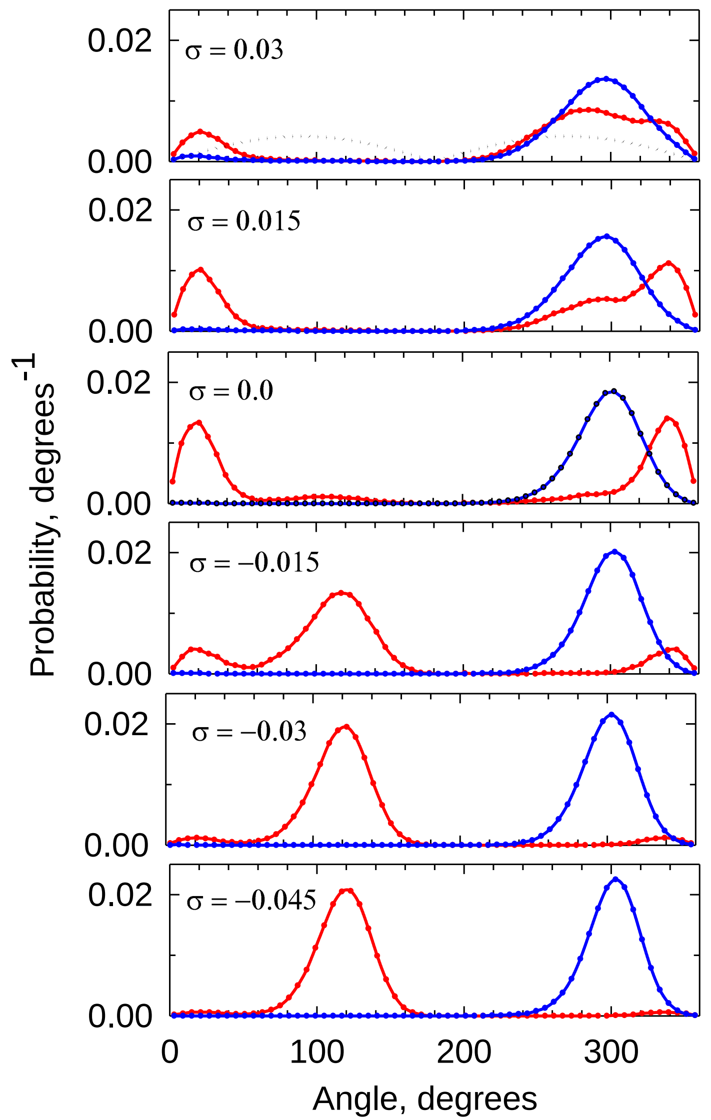
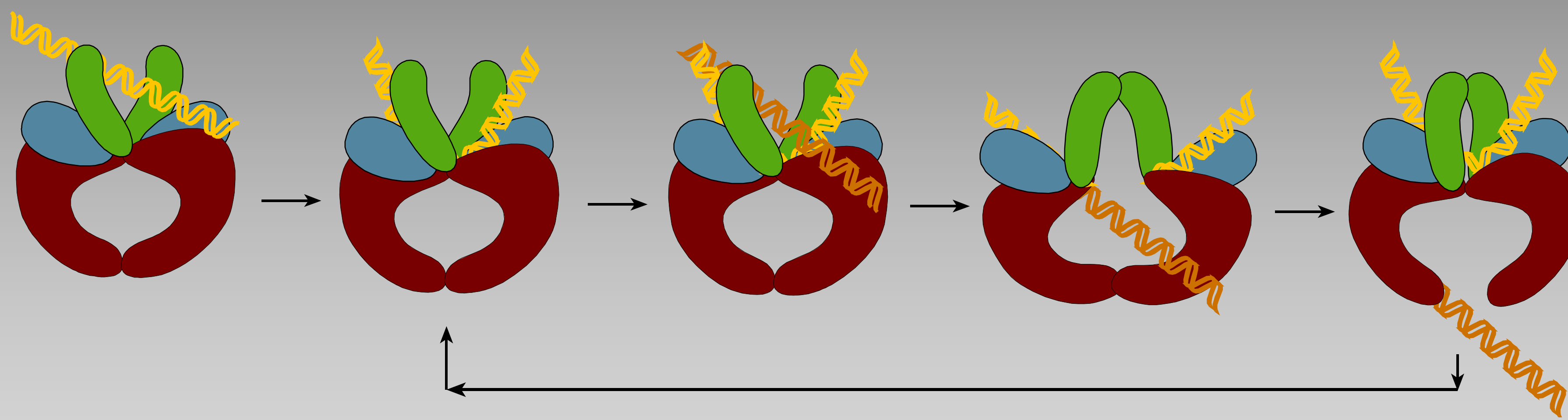
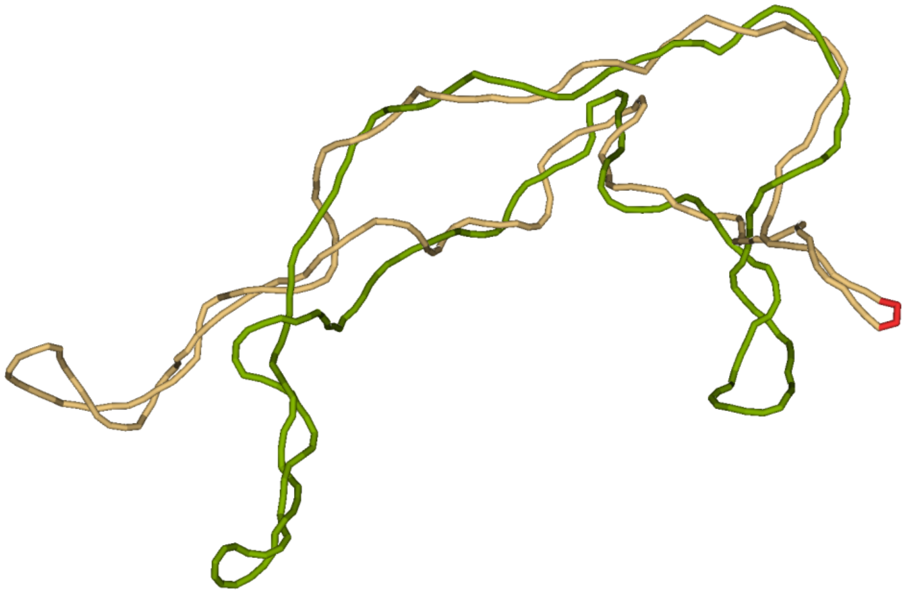
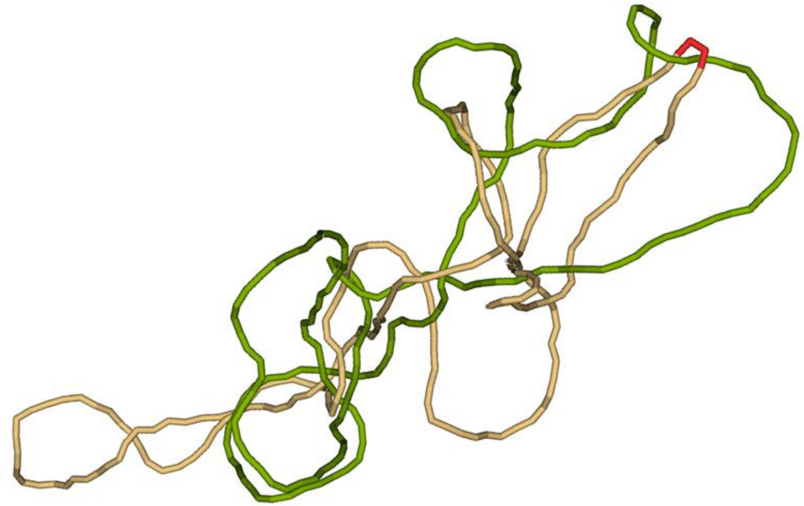


Diagram of type IIA topoisomerase action







In (-) supercoiled catenanes G segment is strongly bent and therefore is preferentially located at the superhelix apices; it is hardly accessible for other segments there.

$P_{\text{jux}} < 0.002$

In (+) supercoiled catenanes G segment is easily accessible for other segments of the chains.

$P_{\text{jux}} = 0.05 \pm 0.01$

Bending of the G segment by the enzyme strongly affects kinetics of DNA topological transformations

Collaborators

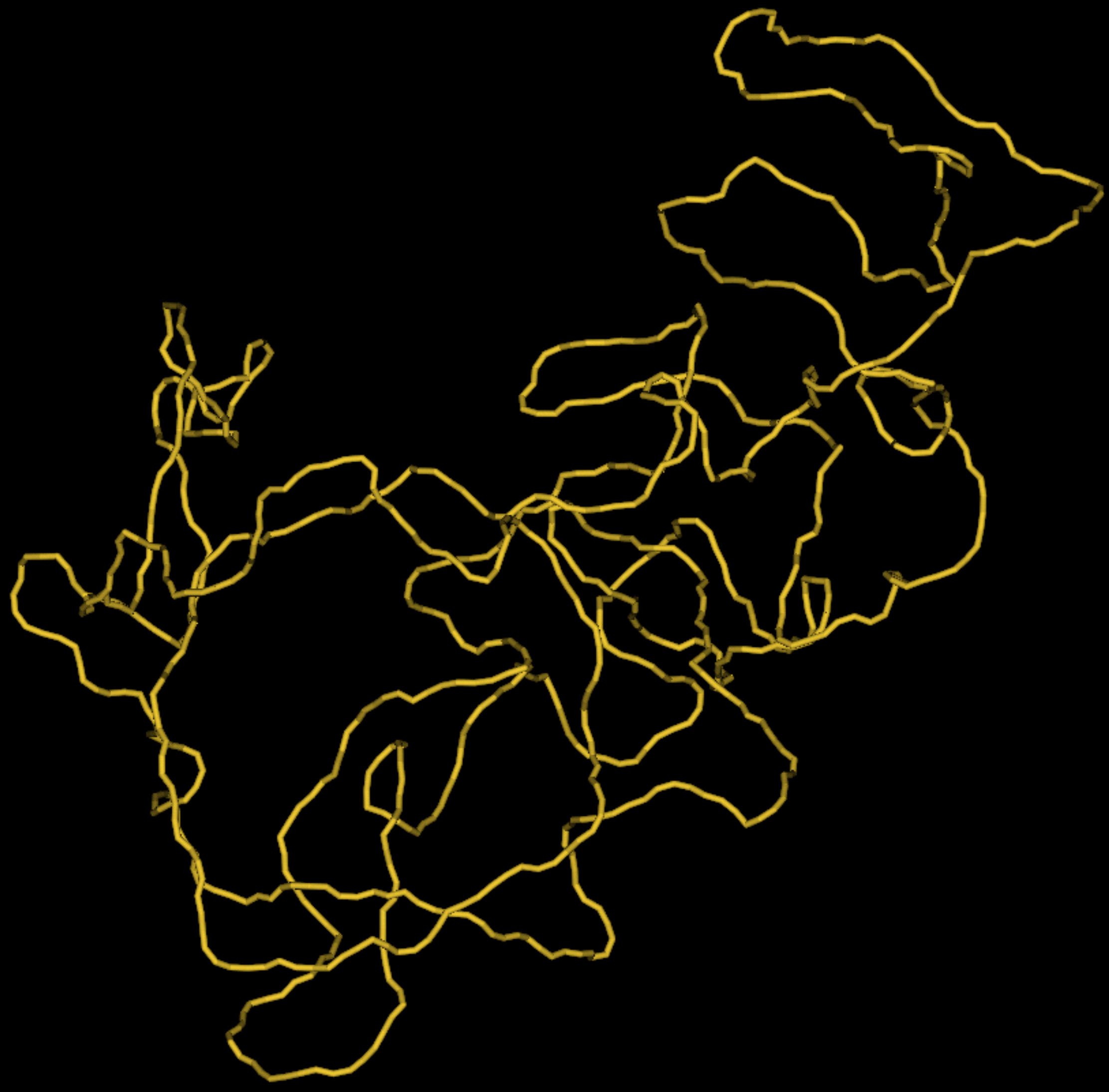
UC, Berkeley

Nicholas Cozzarelli

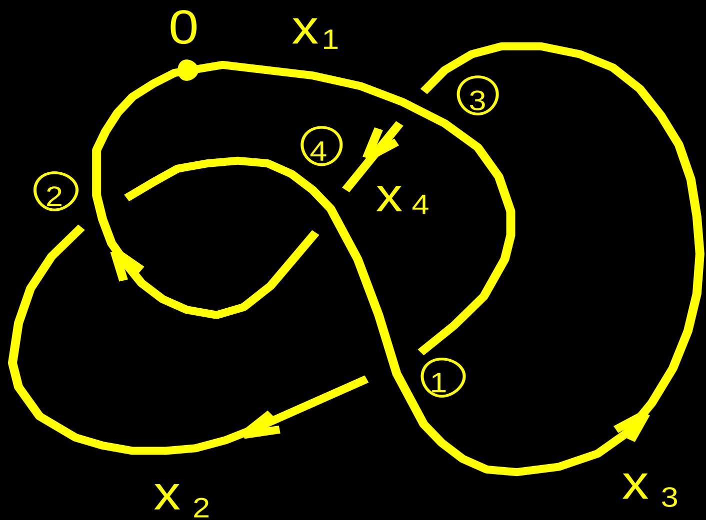
Valentine Rybenkov

The work is supported by NIH

Typical conformation of 30 kb DNA



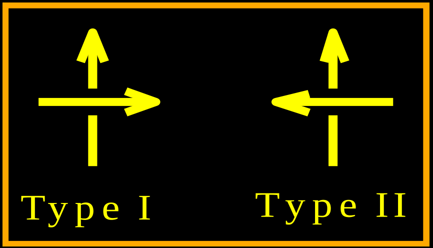
Calculation of the Alexander polynomial



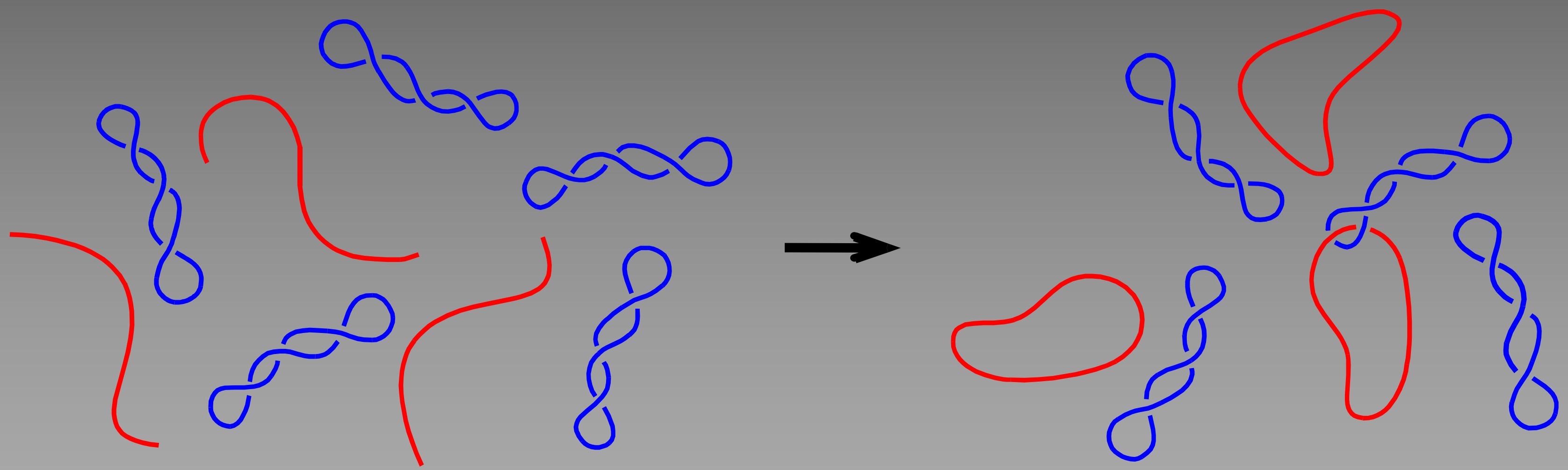
i	1	2	3	4
k	3	1	1	3
Intersection type	II	II	II	II

$$\begin{Vmatrix} 1 & -t & t-1 \\ t-1 & 1 & -t \\ t-1 & 0 & 1 \end{Vmatrix}$$

$$\Delta(t) = t^2 - t + 1$$

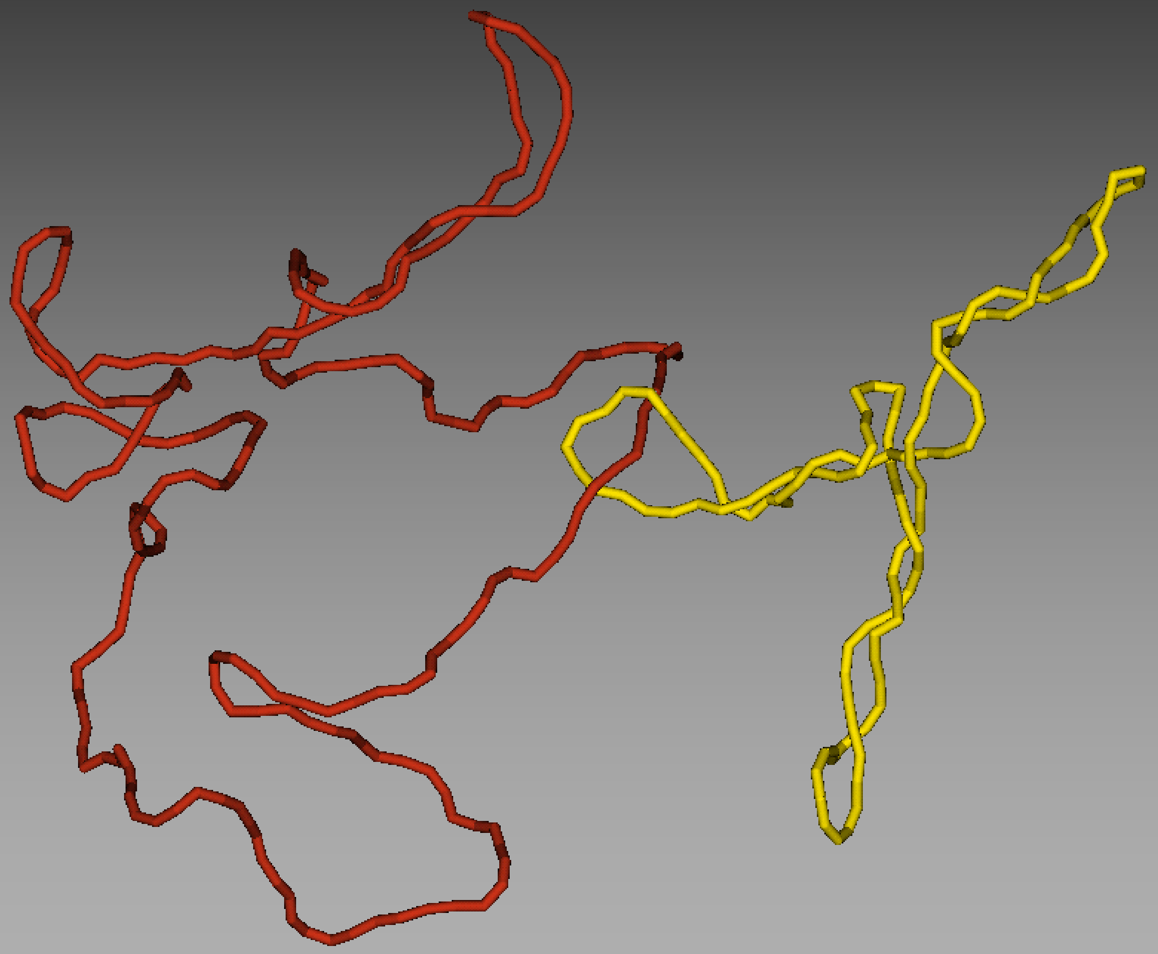


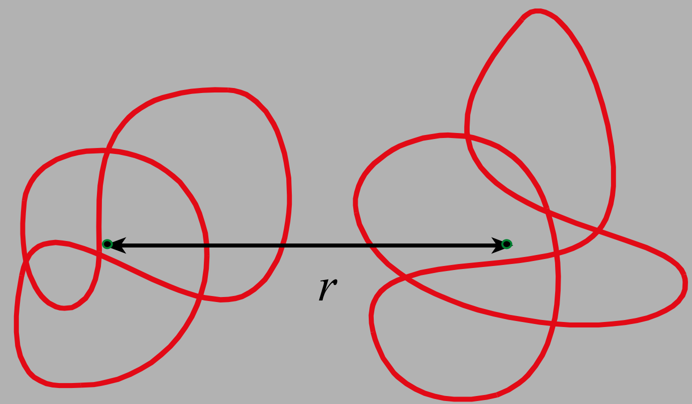
Probing conformations of supercoiled DNA by forming catenanes with relaxed molecules



A link between two relaxed DNA molecules

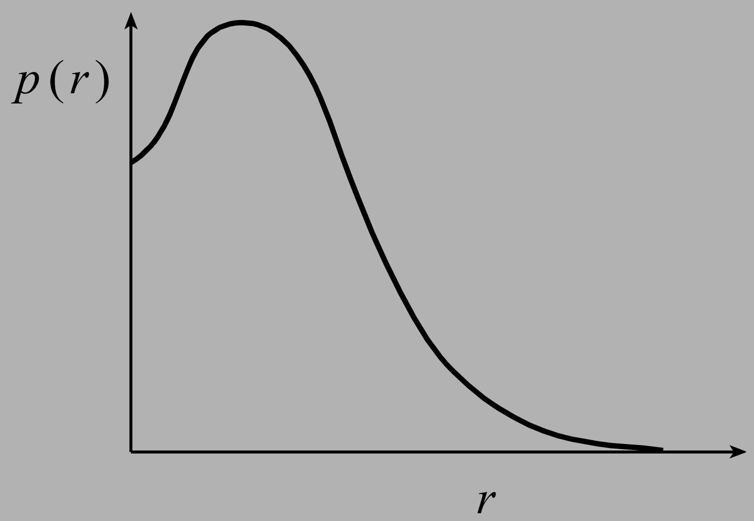
A link between supercoiled and relaxed DNA molecules





The probability P_{cat} that a molecule is linked with another one is equal to

$$P_{cat} = c \int_0^{\infty} p(r) 4 \pi r^2 dr \ ,$$



We can rewrite this as

$$P_{cat} = Bc \ ,$$

where

$$B = \int_0^{\infty} p(r) 4 \pi r^2 dr$$