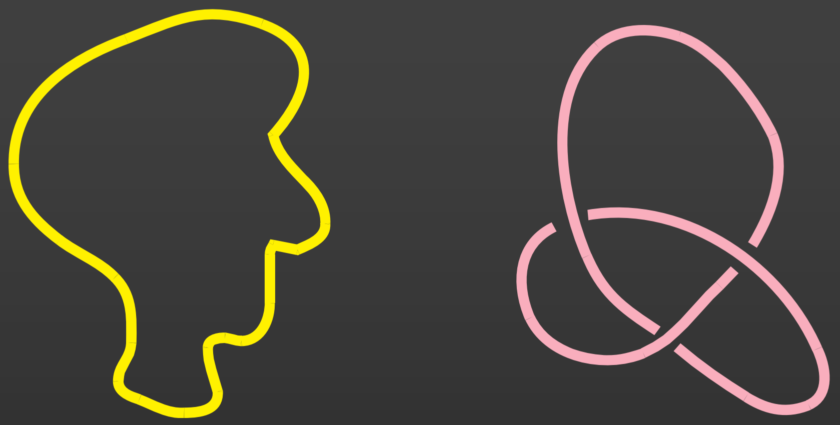

KNOT SHOP
KNOT SHOP
Collar Full

How Protein-induced Local DNA Bends Can Regulate Global DNA Topology

Alexander Vologodskii

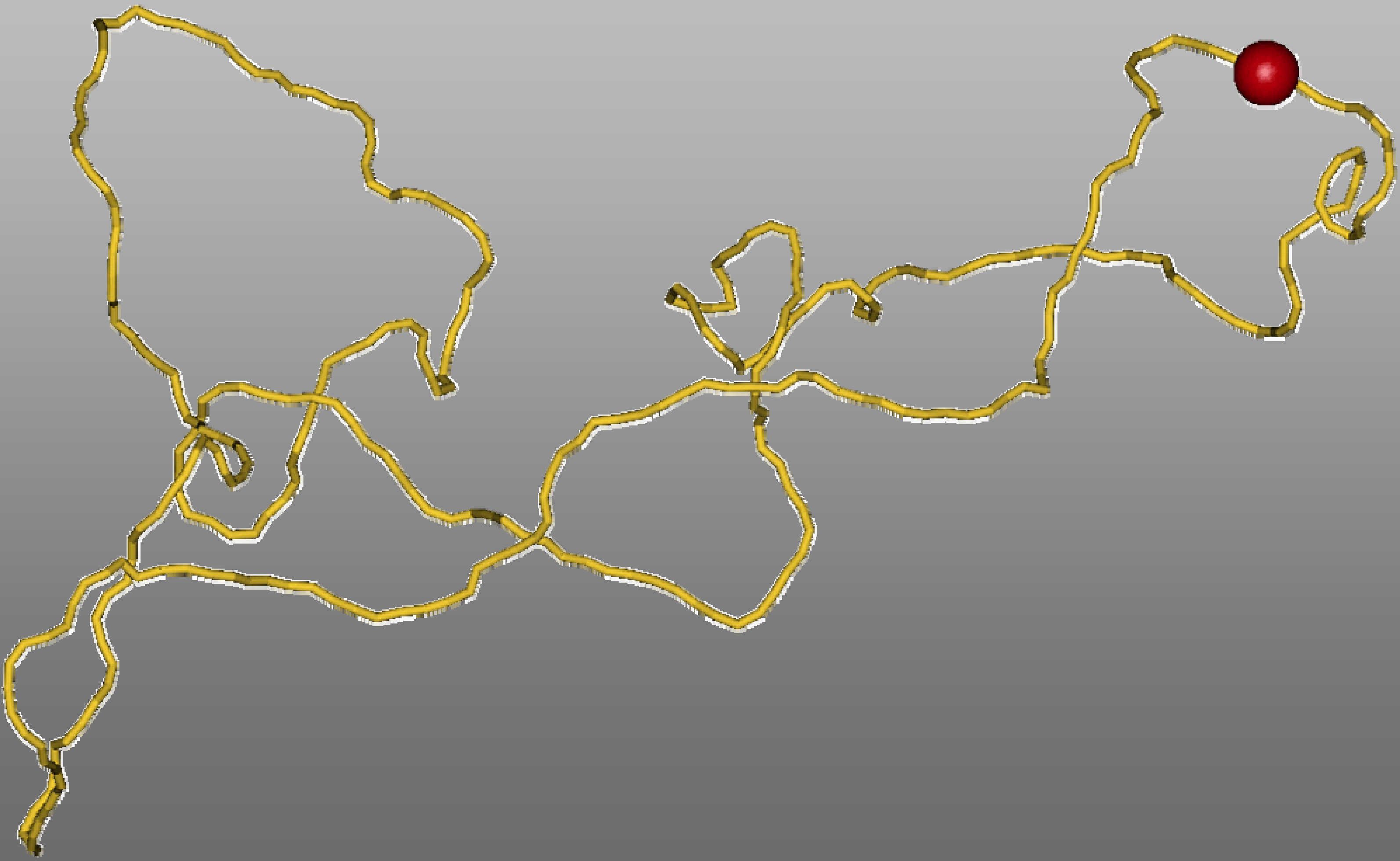
New York University

The axis of the double helix can be unknotted or form a knot



Two double-stranded DNA can be unlinked or linked

Enzymes can be very large proteins but they are very small in comparison with DNA coils

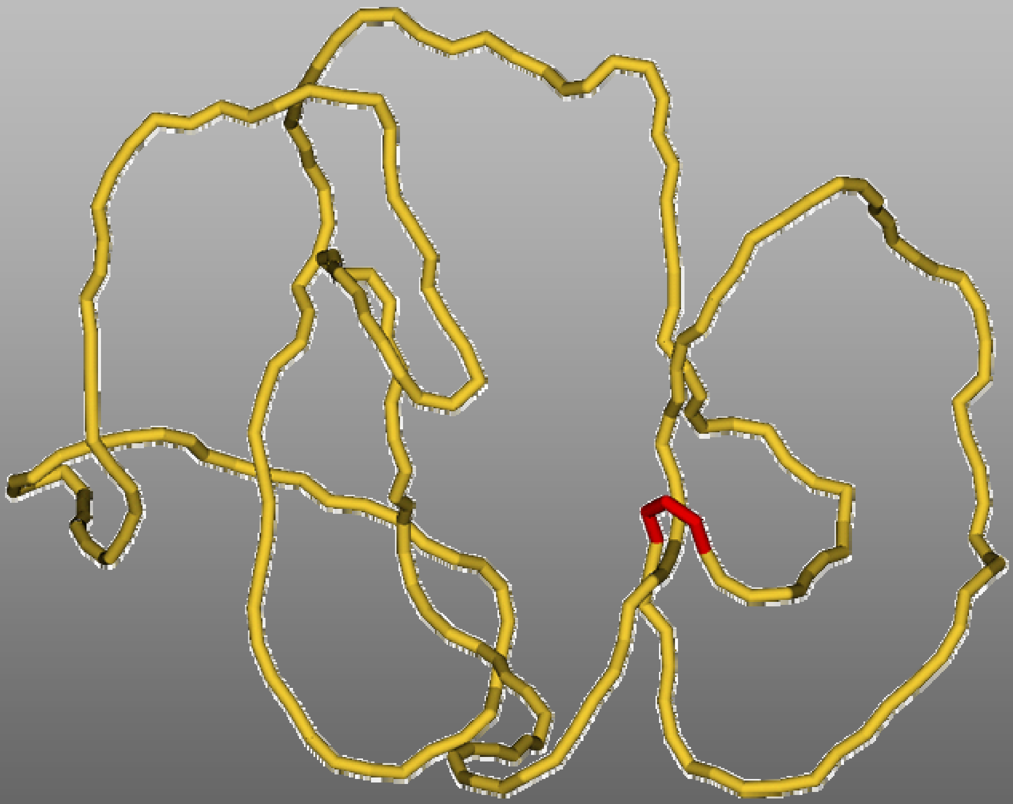


1. Unknotting and unlinking by DNA topoisomerases by local DNA bends

2. Regulating topology of recombination products by local DNA bends

3. Measuring protein-induced bends

DNA conformation with a small rigid hairpin



The probability to have a segment inside the hairpin depends on the DNA topology

Localized and distributed conformations of trefoil

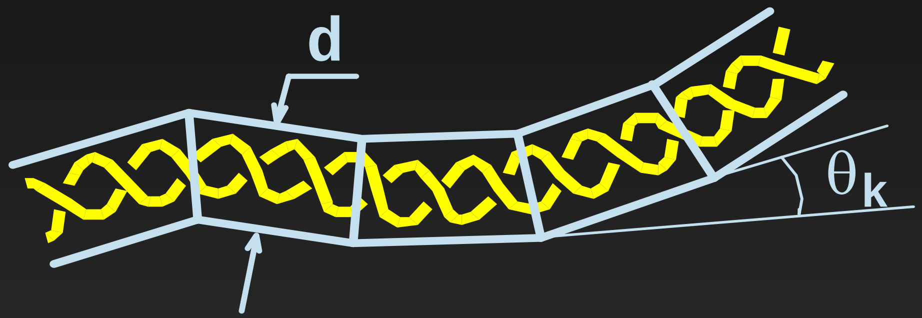
We assume that the rate of synapsis is not limited by the rate of diffusion of one site to another

Thus, the probability of synapse (formed reaction complex) with a particular DNA conformation is proportional to the equilibrium probability of the corresponding conformation with properly juxtaposed reaction sites

Each conformations of the molecule appears with probability specified by the Boltzmann distribution

$$P_i = \frac{\exp(-E_i / kT)}{\sum_k \exp(-E_k / kT)}$$

Discrete wormlike chain model of DNA



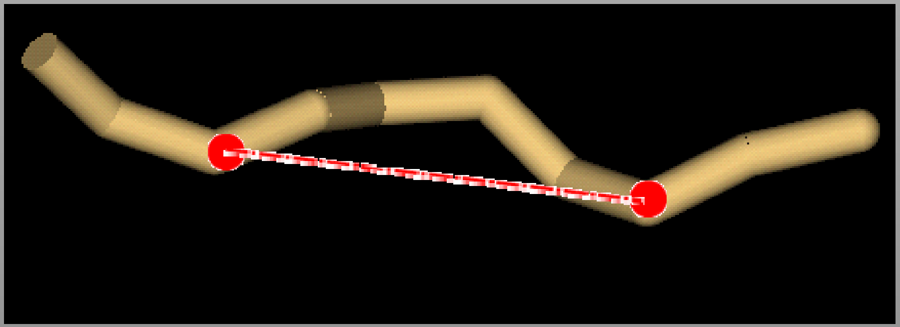
$$E_b = \sum_k g\theta_k^2 ,$$

where **g** is the bending rigidity constant.

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

Metropolis Procedure

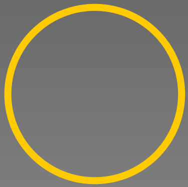
An initial conformation is deformed step by step



Trial conformation is accepted with probability

$$\begin{array}{lll} 1 & \text{if} & E_T < E_C \\ \exp\left[\left(-(E_T - E_C\right)/kT\right] & \text{if} & E_T > E_C \end{array}$$

Simplest knots and their Alexander polynomials



Unknot

1

3_1 (Trefoil)

$t^2 - t + 1$

4_1

$t^2 - 3t + 1$

5_1

$t^4 - t^3 + t^2 - t + 1$

5_2

$2t^2 - 3t + 2$

6_1

$2t^2 - 5t + 2$

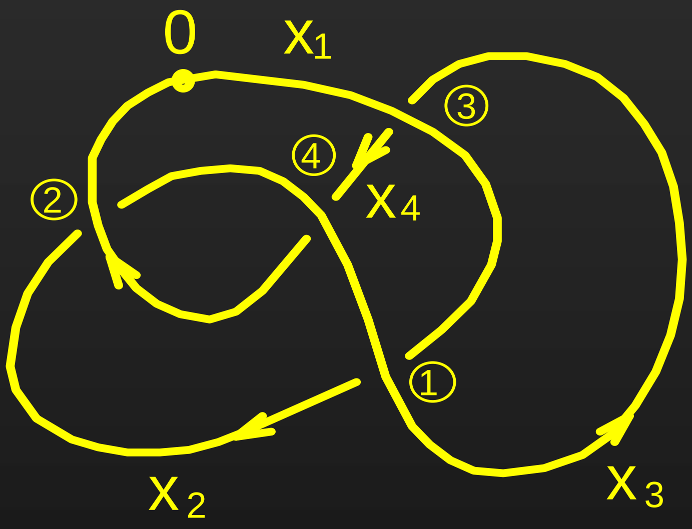
6_2

$t^4 - 3t^3 + 3t^2 - 3t + 1$

6_3

$t^4 - 3t^3 + 5t^2 - 3t + 1$

Calculation of the Alexander polynomial

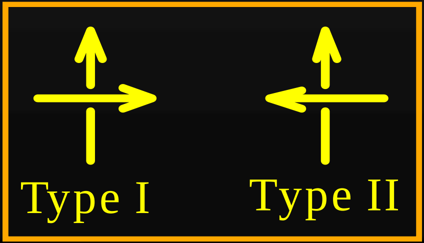


→

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

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

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

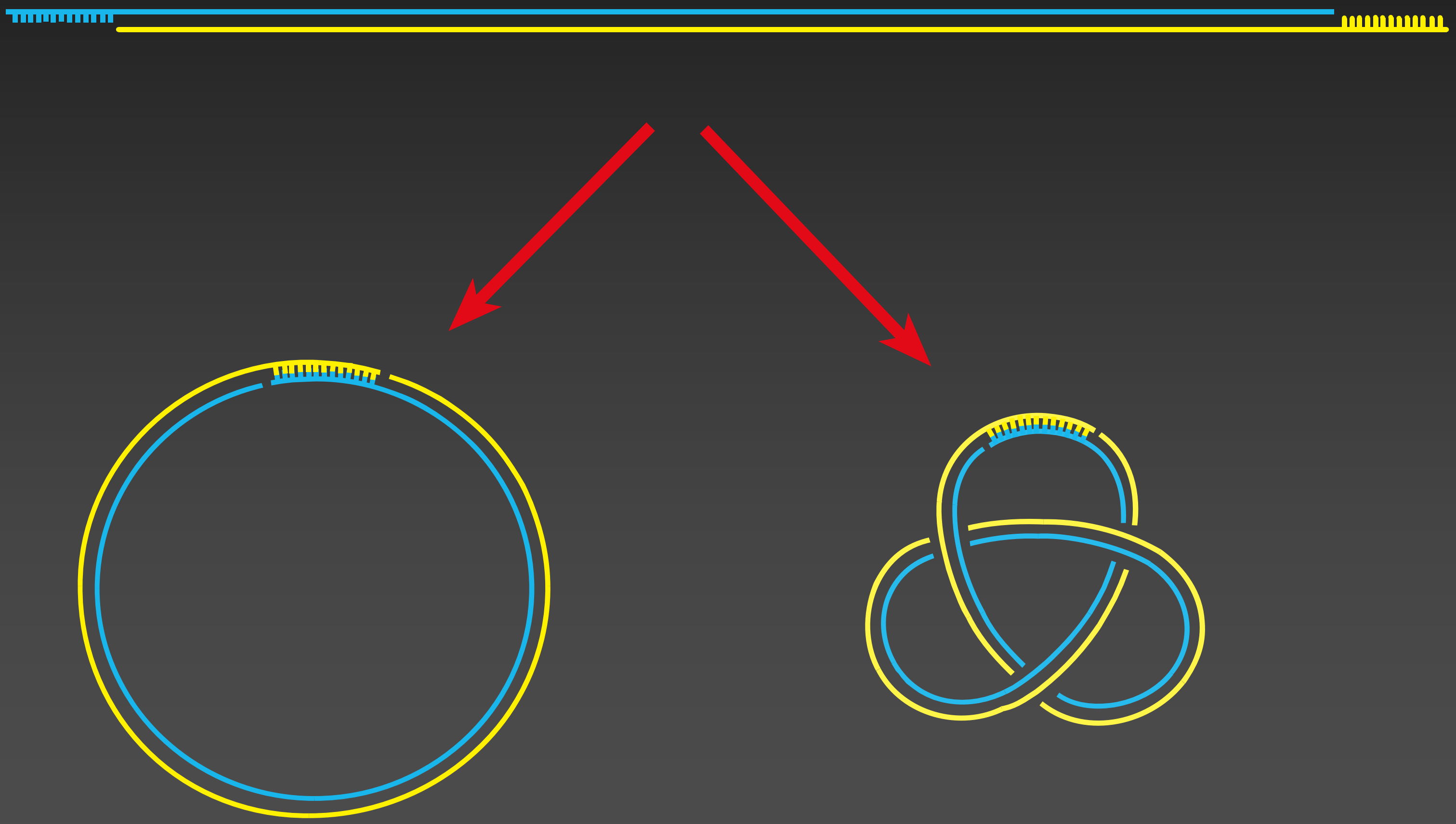


Equilibrium distribution of topological states

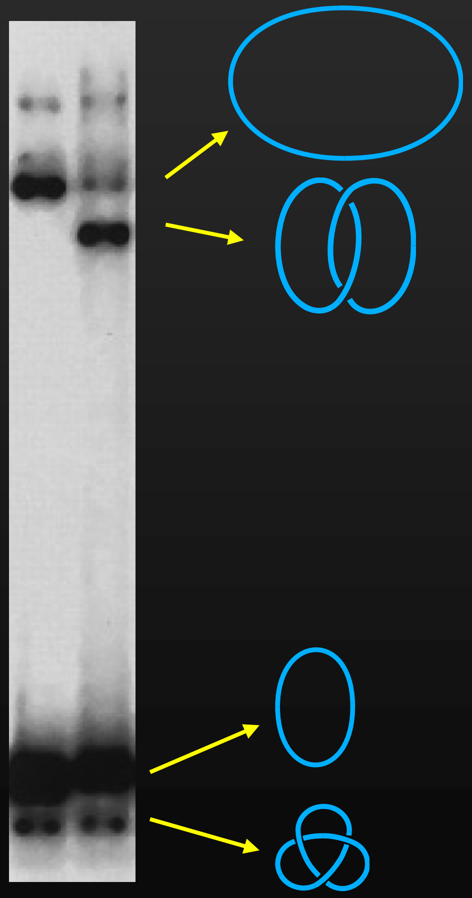
$$P_i = \frac{\sum\limits_{\text{Topology } i} \exp(-E_k / kT)}{\sum\limits_{\text{All conformations}} \exp(-E_k / kT)},$$

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



Separations of knots and links by gel electrophoresis



Circular dimmers ⇒

Catenanes ⇒

Unknotted circular DNA ⇒

Trefoils ⇒

Computed and measured equilibrium probabilities of knots are in good agreement

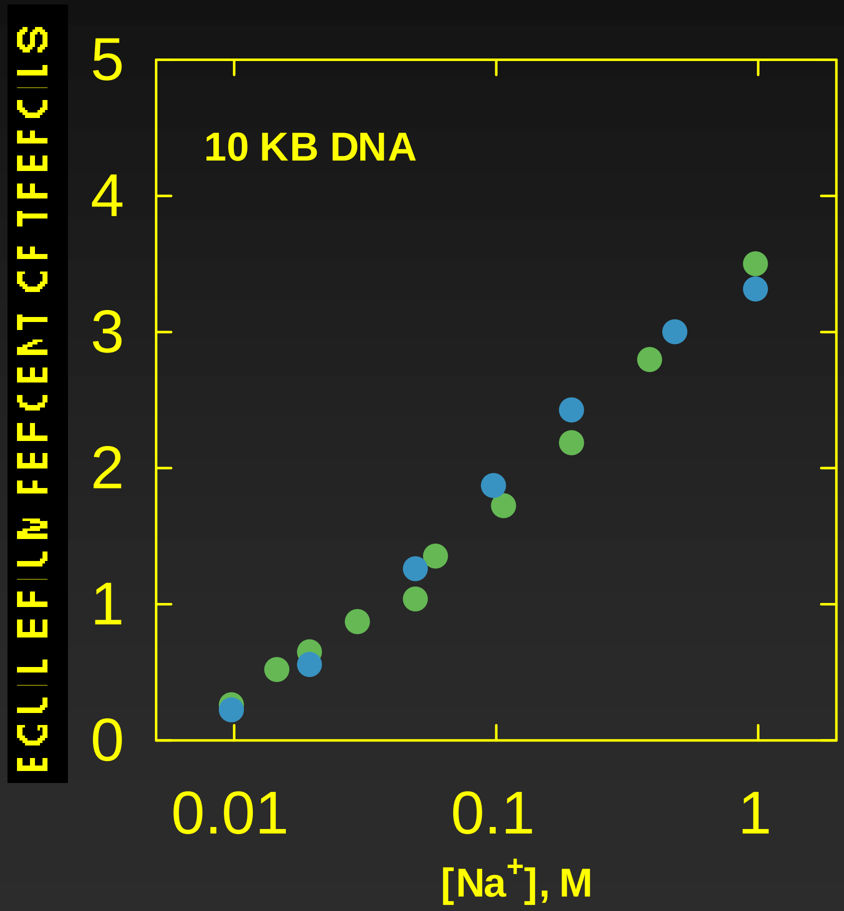
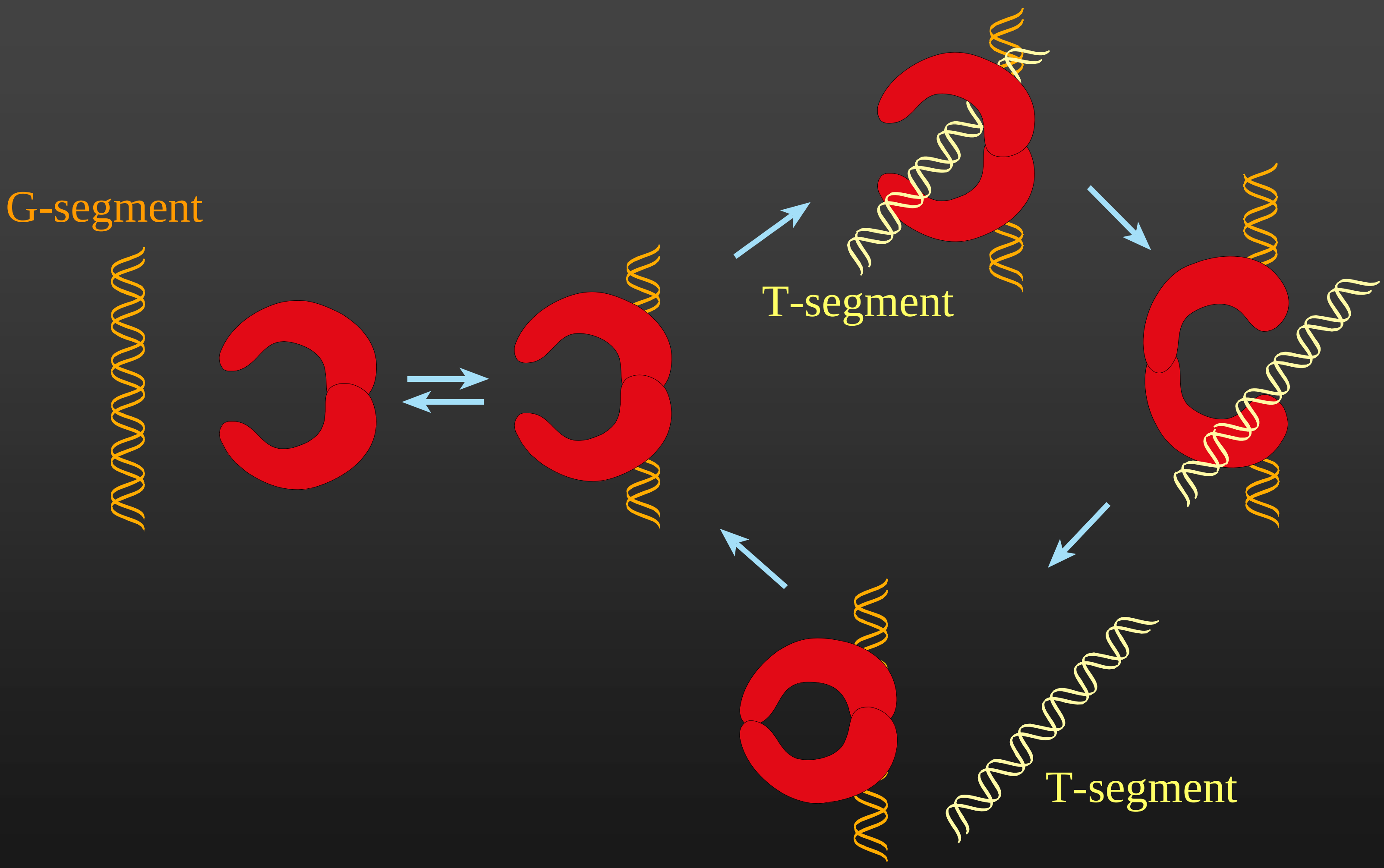
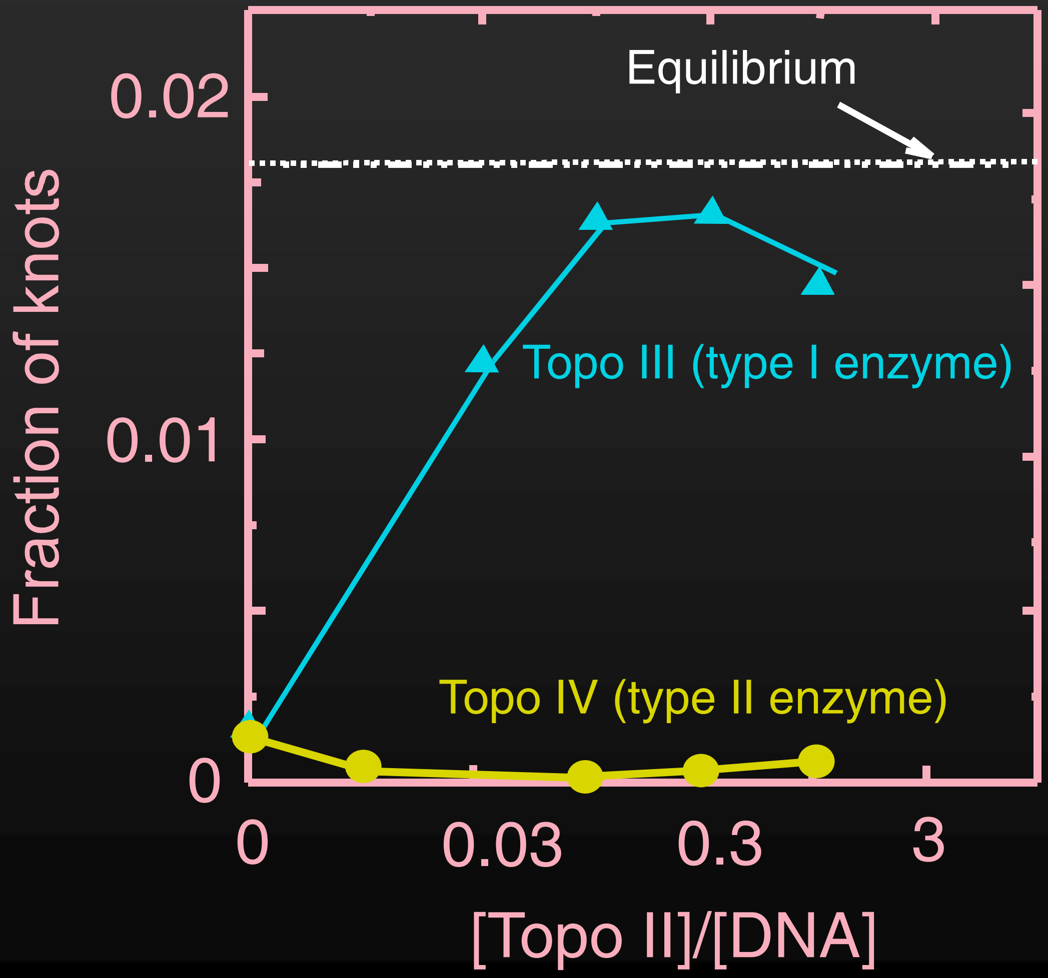


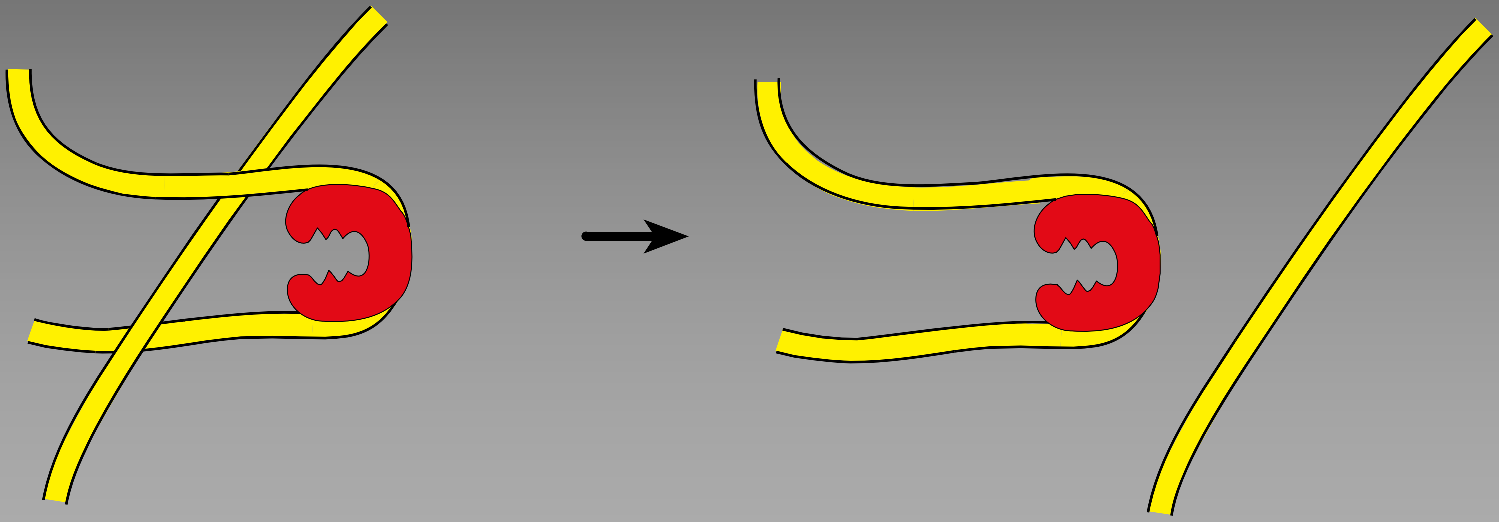
Diagram of type II topoisomerase action



Unknotting circular DNA by type II DNA topoisomerases



The model of topo II action



Calculation of the steady-state fraction of knots

$$0 \Leftrightarrow K$$

The steady state concentrations of knots and unknotted chains, C_k and C_o, are connected by equation

$$k_{ok} C_o - k_{ko} C_k = 0$$

where

k_{ok} is the rate constant of the conversion of unknotted chains into knotted ones,

k_{ko} is the reverse rate constant

Calculation of the steady-state fraction of knots

$$k_{ok} = A r_{ok} P^{o}_{jux}; \qquad k_{ko} = A r_{ko} P^{k}_{jux}$$

where

r_{ok}, r_{ko} are the coefficients which specify the efficiency of topology changes resulting from the strand passing

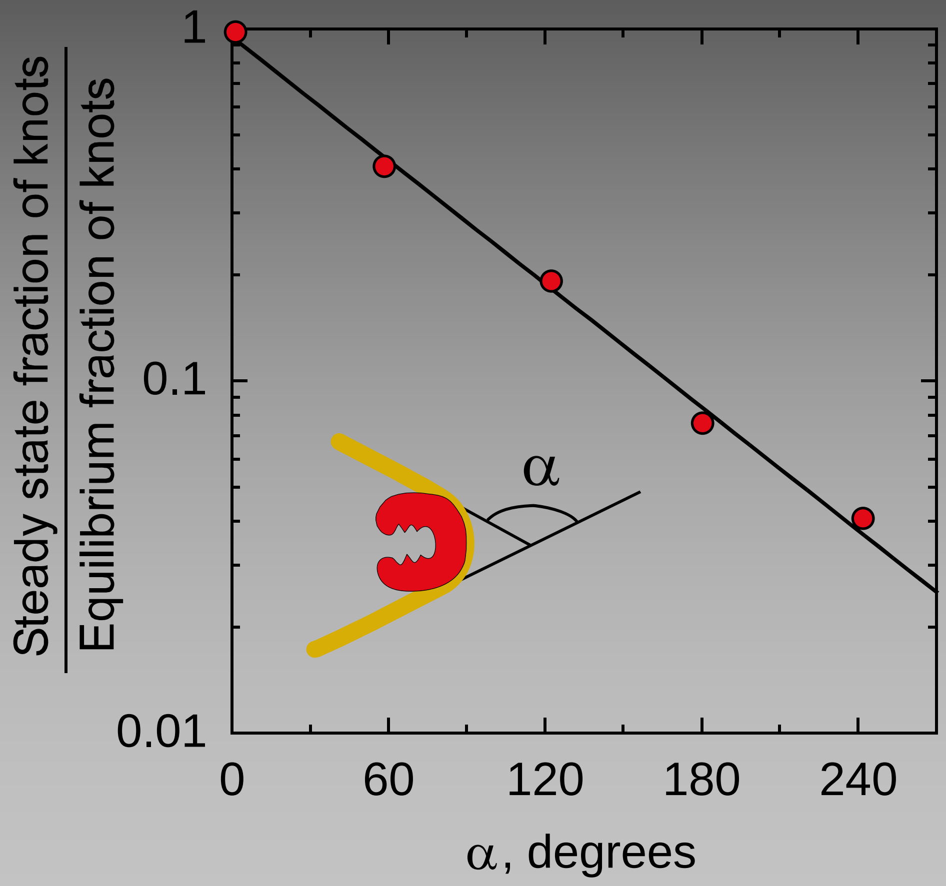
P^{o}_{jux}, P^{k}_{jux} are the probabilities of the juxtaposition for unknotted and knotted conformations;

A is a coefficient.

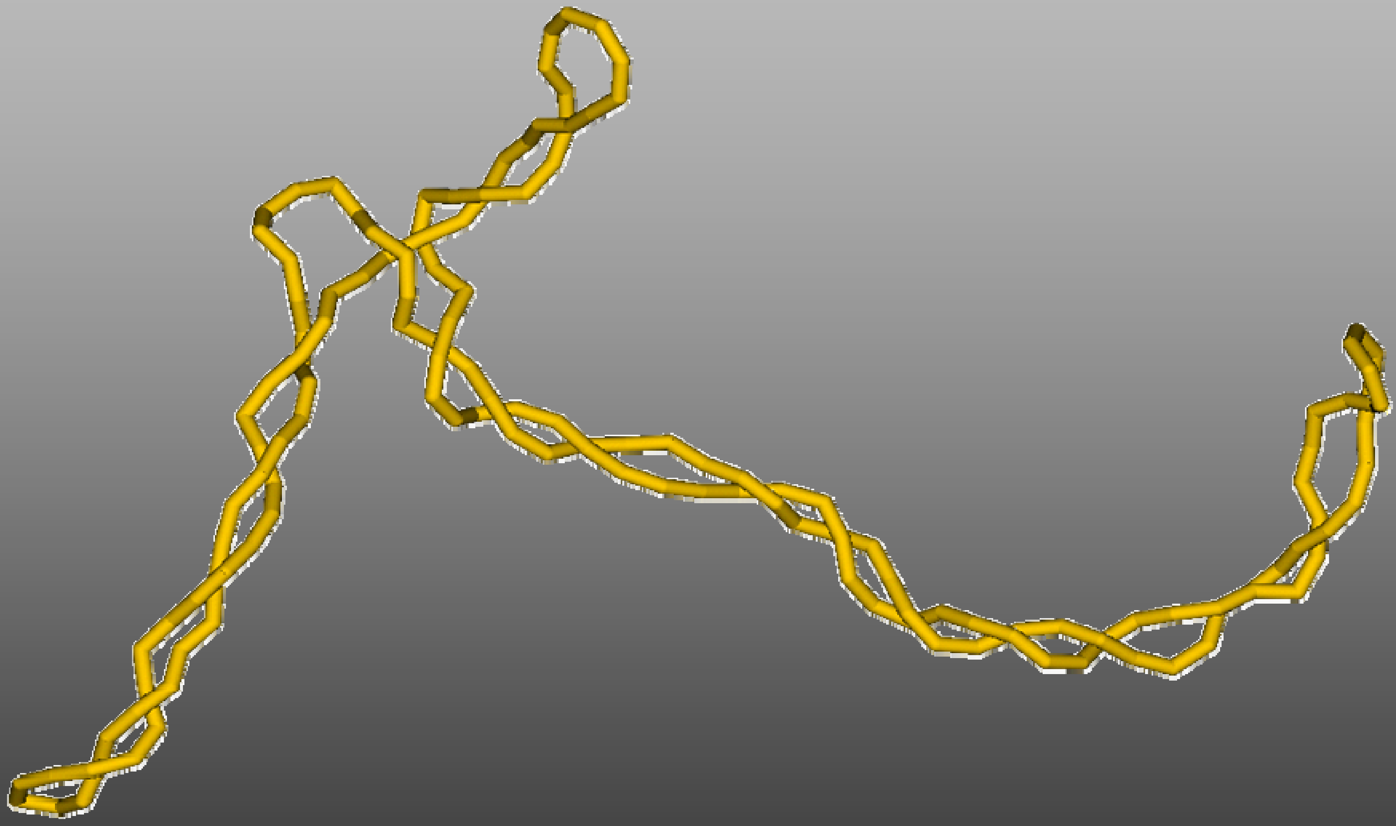
Thus

$$\frac{C_k}{C_o} = \frac{r_{ok} P^{o}_{jux}}{r_{ko} P^{k}_{jux}}$$

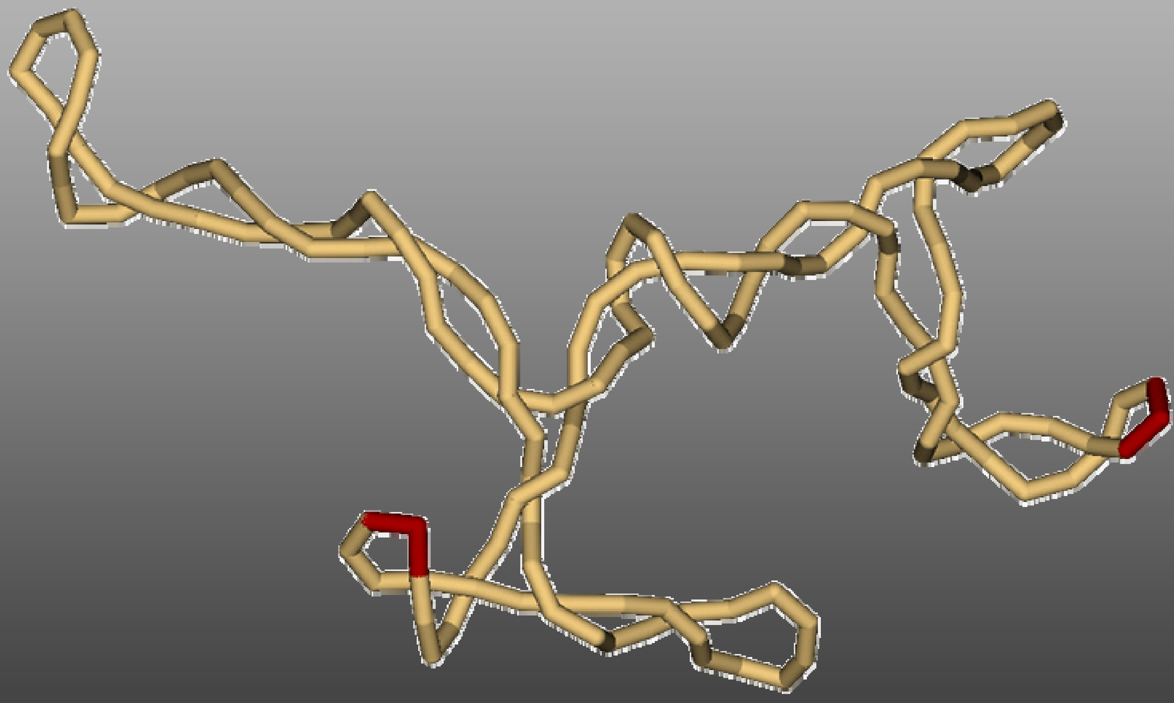
Calculated reduction of the steady state fraction of knots for DNA 7,000 base pairs in length



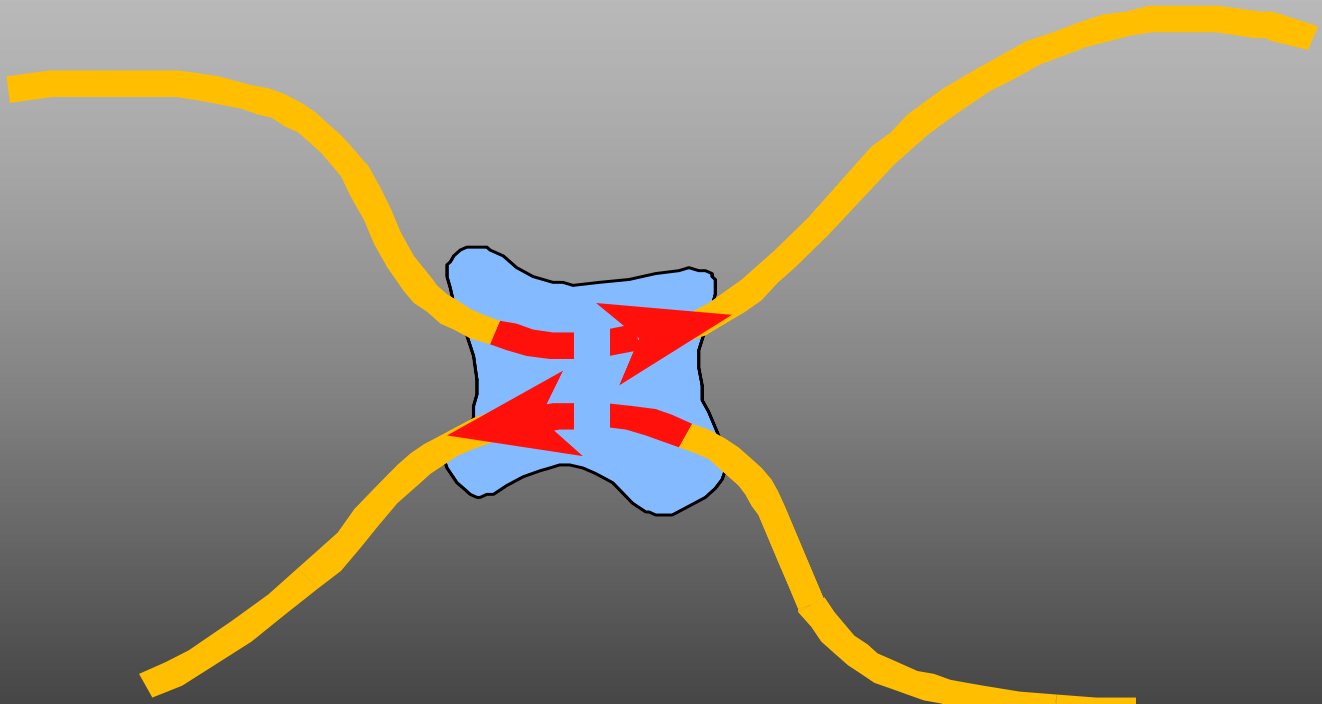
Typical conformation of supercoiled DNA



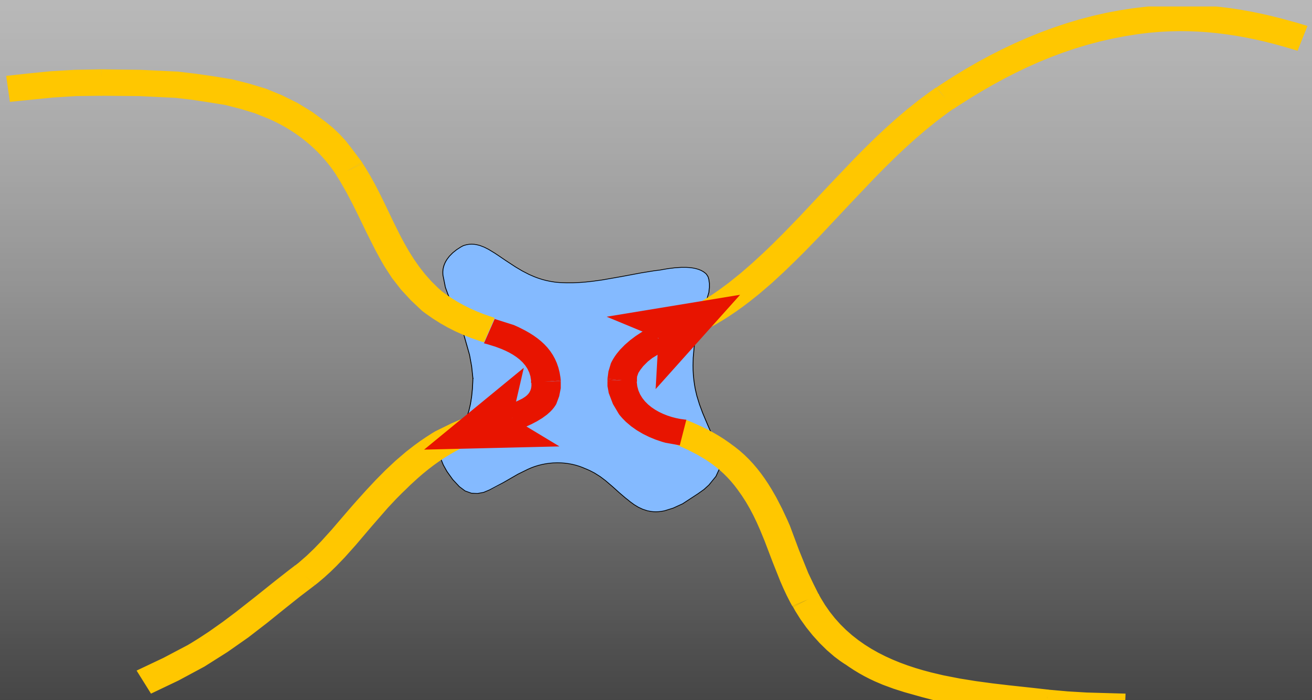
Bent sites are preferentially located in superhelix apices



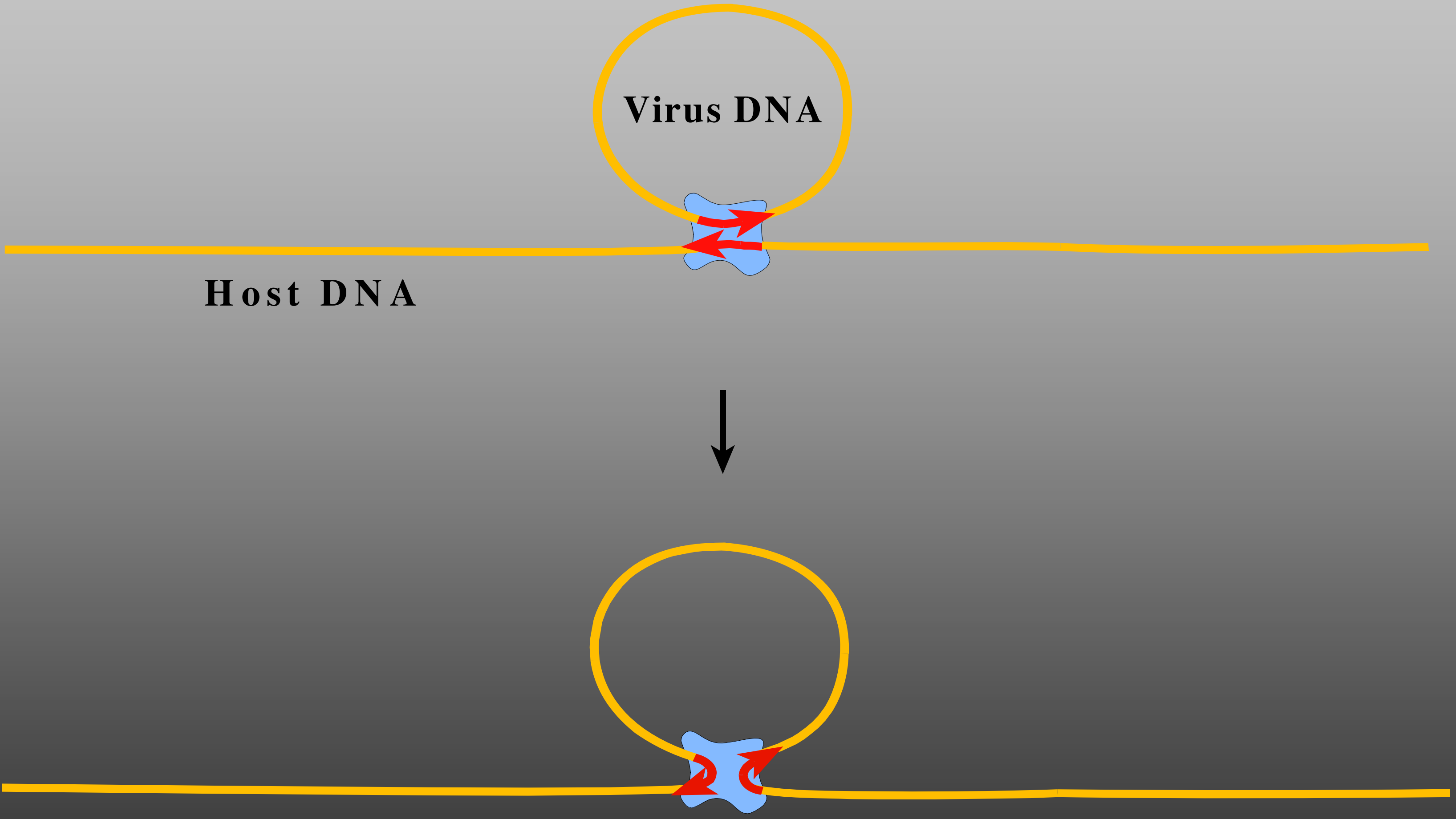
Site-specific recombination



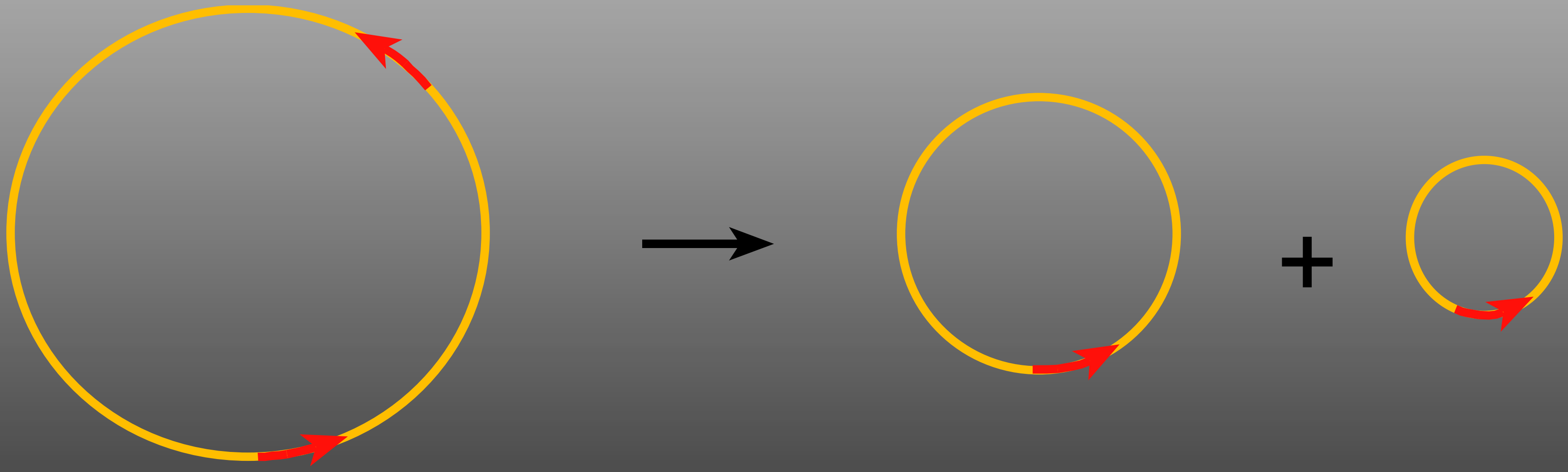
Site-specific recombination



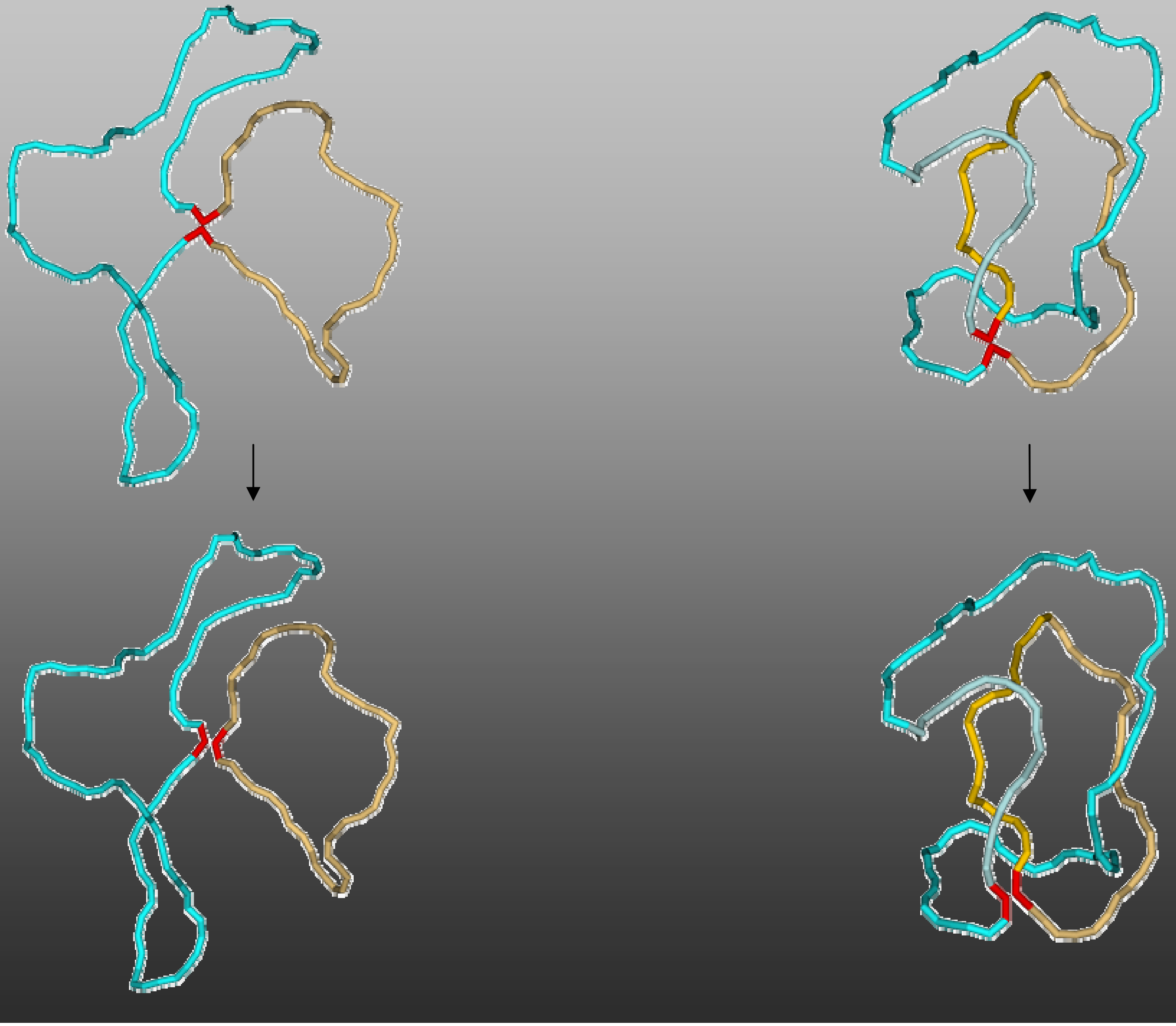
Integration of virus DNA into DNA of its host



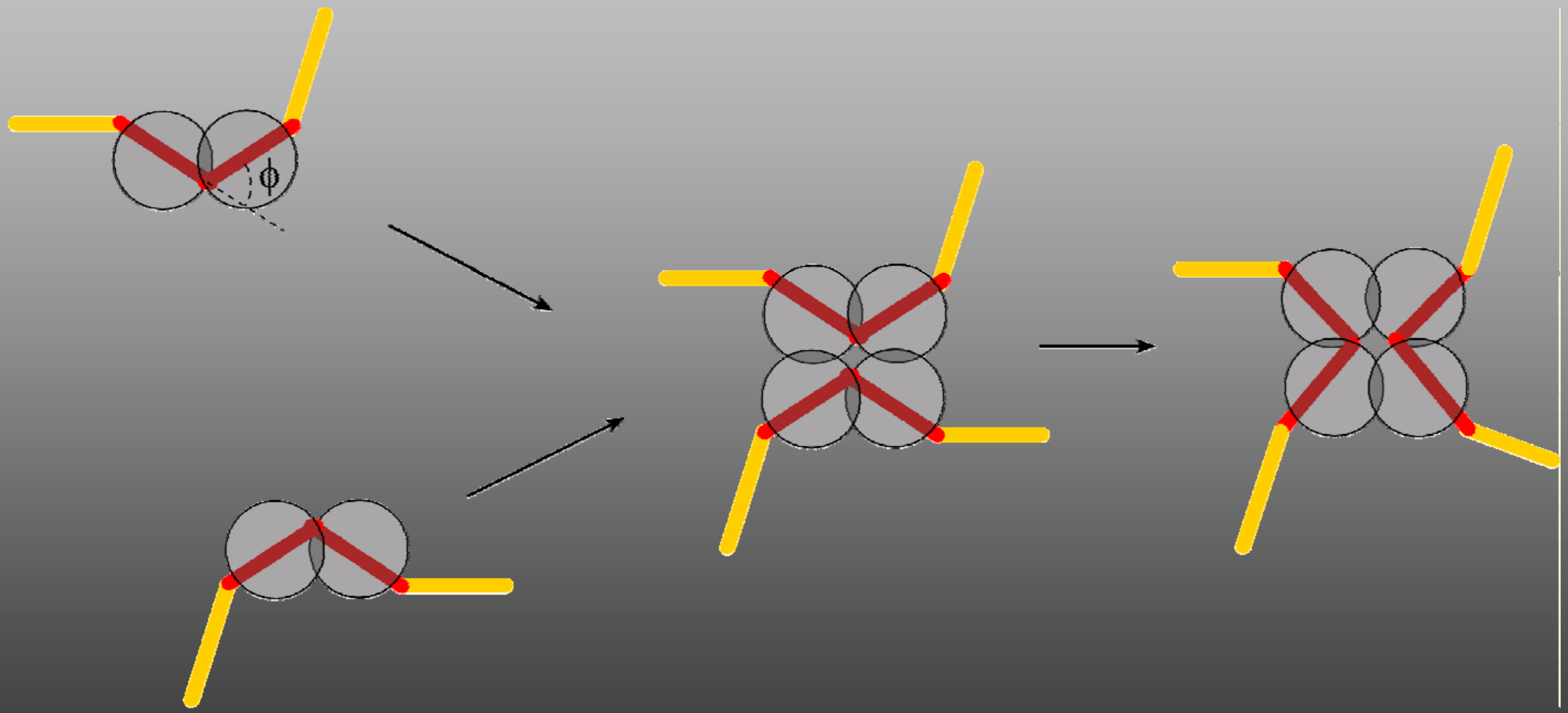
Circular DNA molecules a few thousands base pairs in length, with two specific sites, are usually used to study site-specific recombination



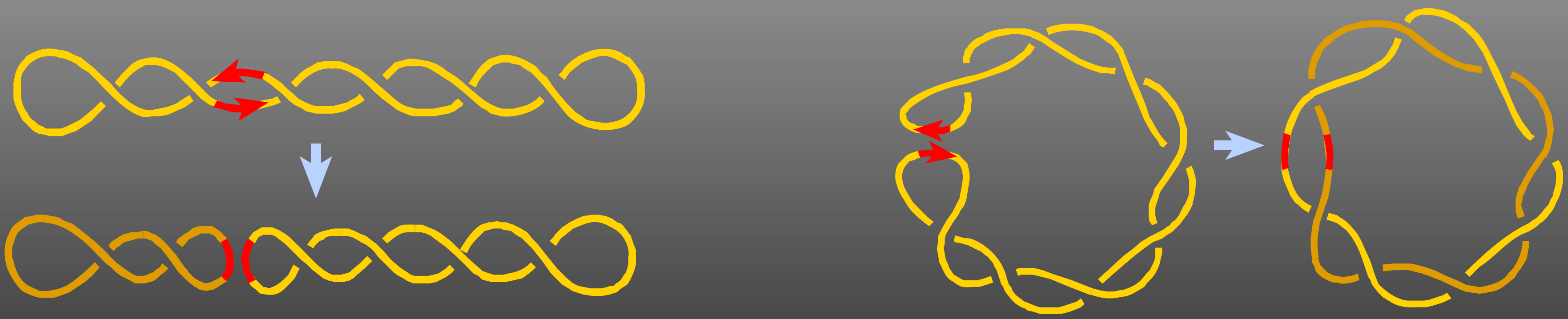
The topology of recombination products is determined when the synaptic complex is assembled



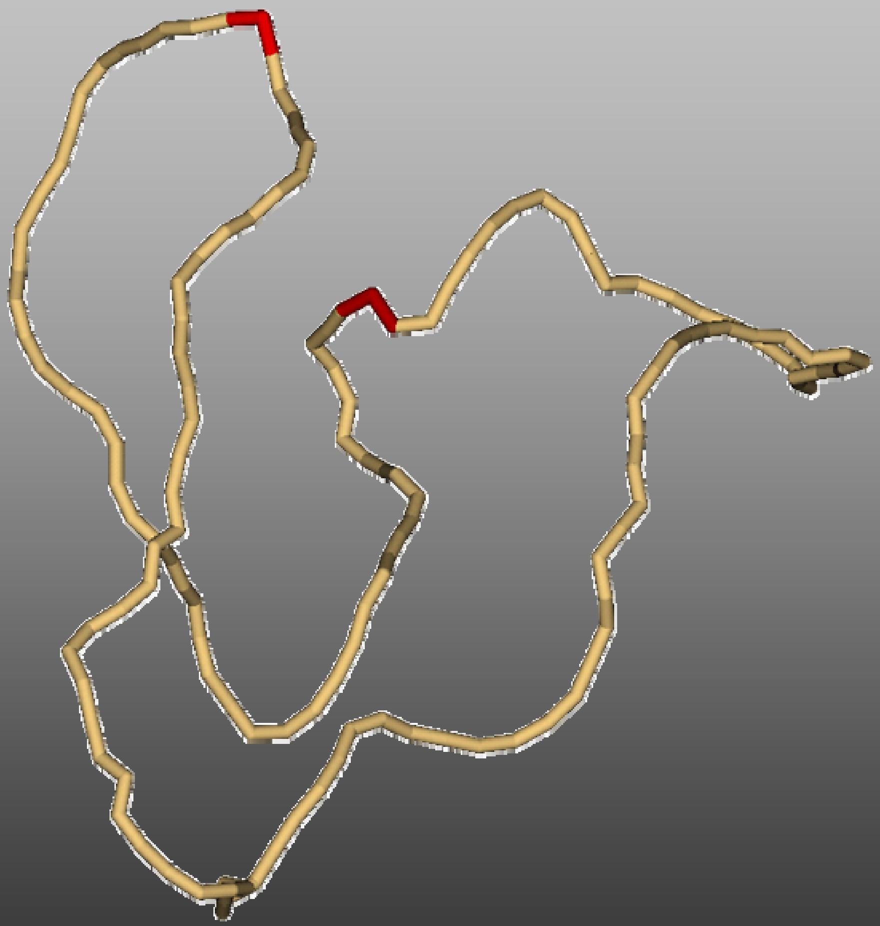
The model of the complex assembling in site specific recombination (integrase family)



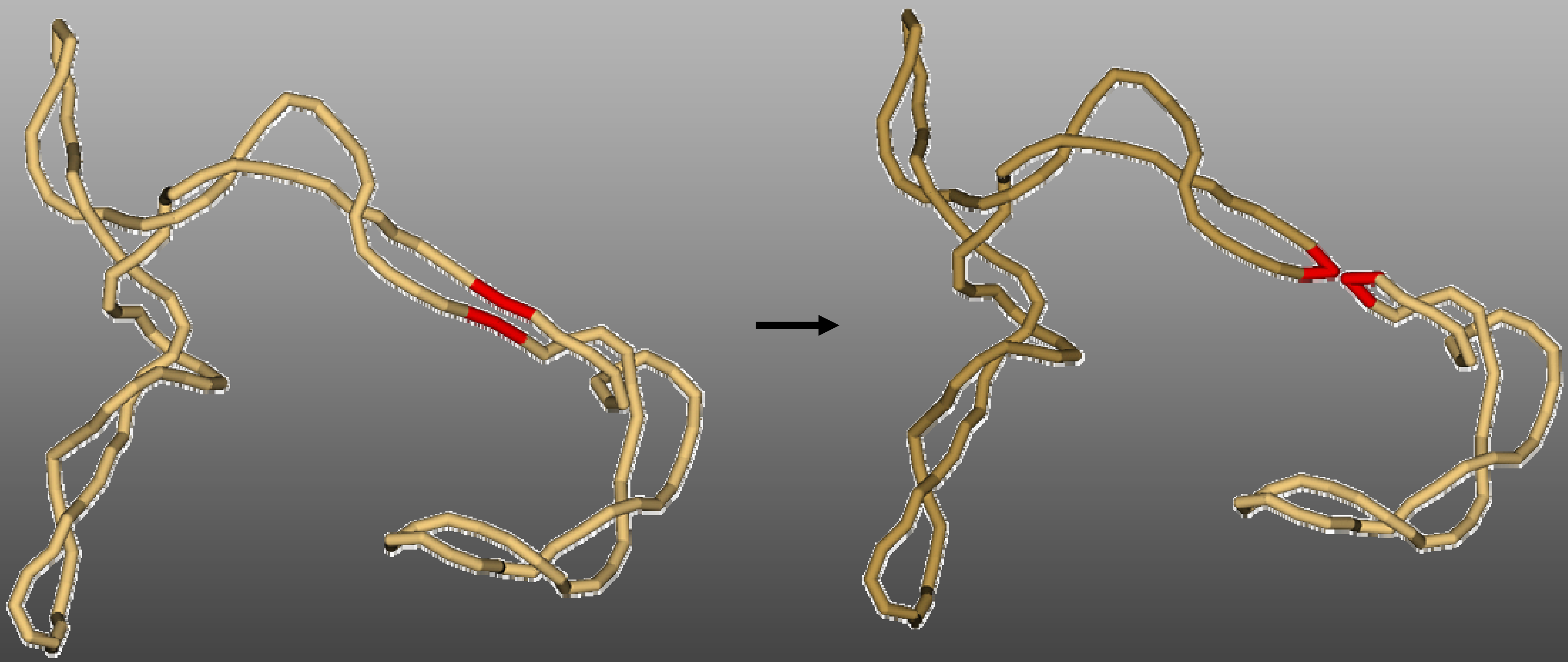
Two types of juxtaposition of the recombination sites and resulting recombination products



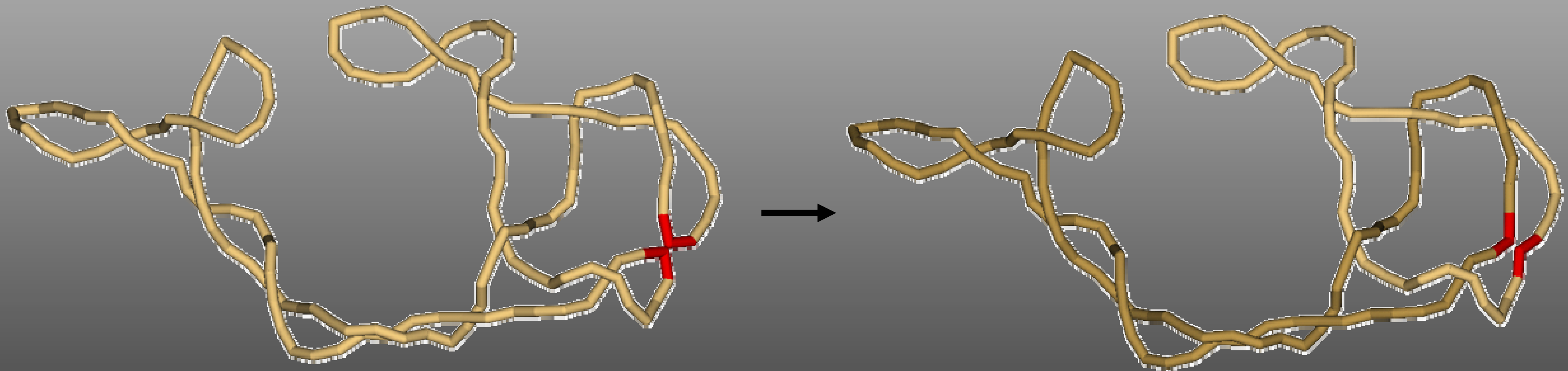
The conformation of the specific sites is held when equilibrium ensemble of chain conformations is simulated



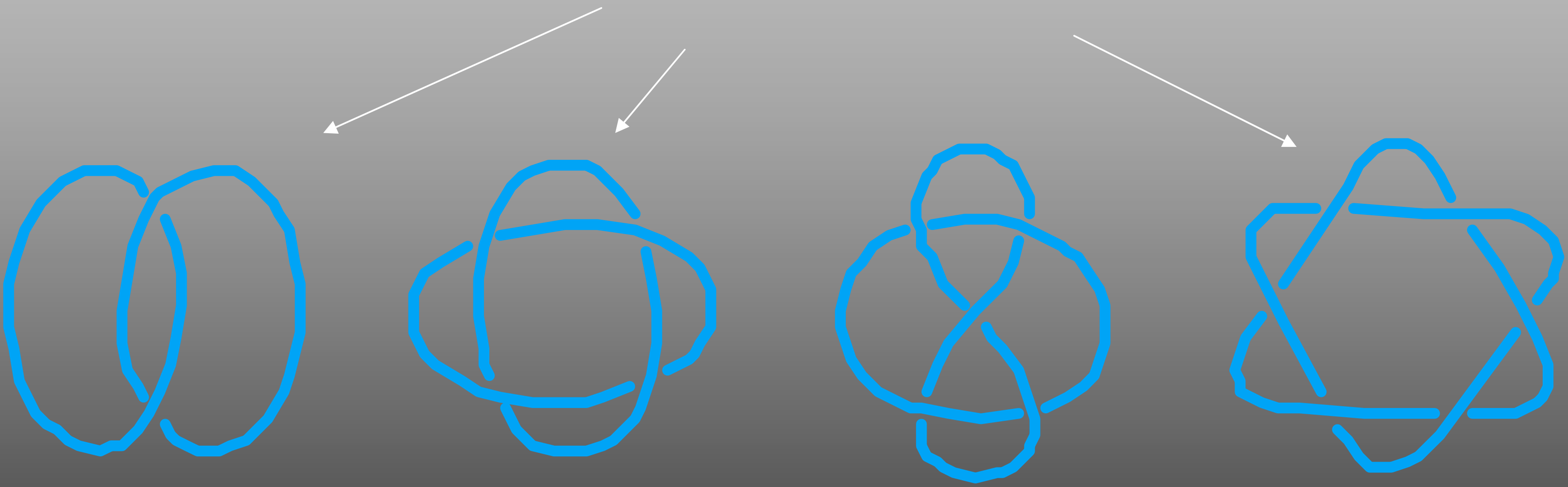
Juxtaposition of the specific sites inside a superhelix branch results in unlinked recombination products



Juxtaposition of the specific sites by collision of superhelix branches results in highly linked recombination products



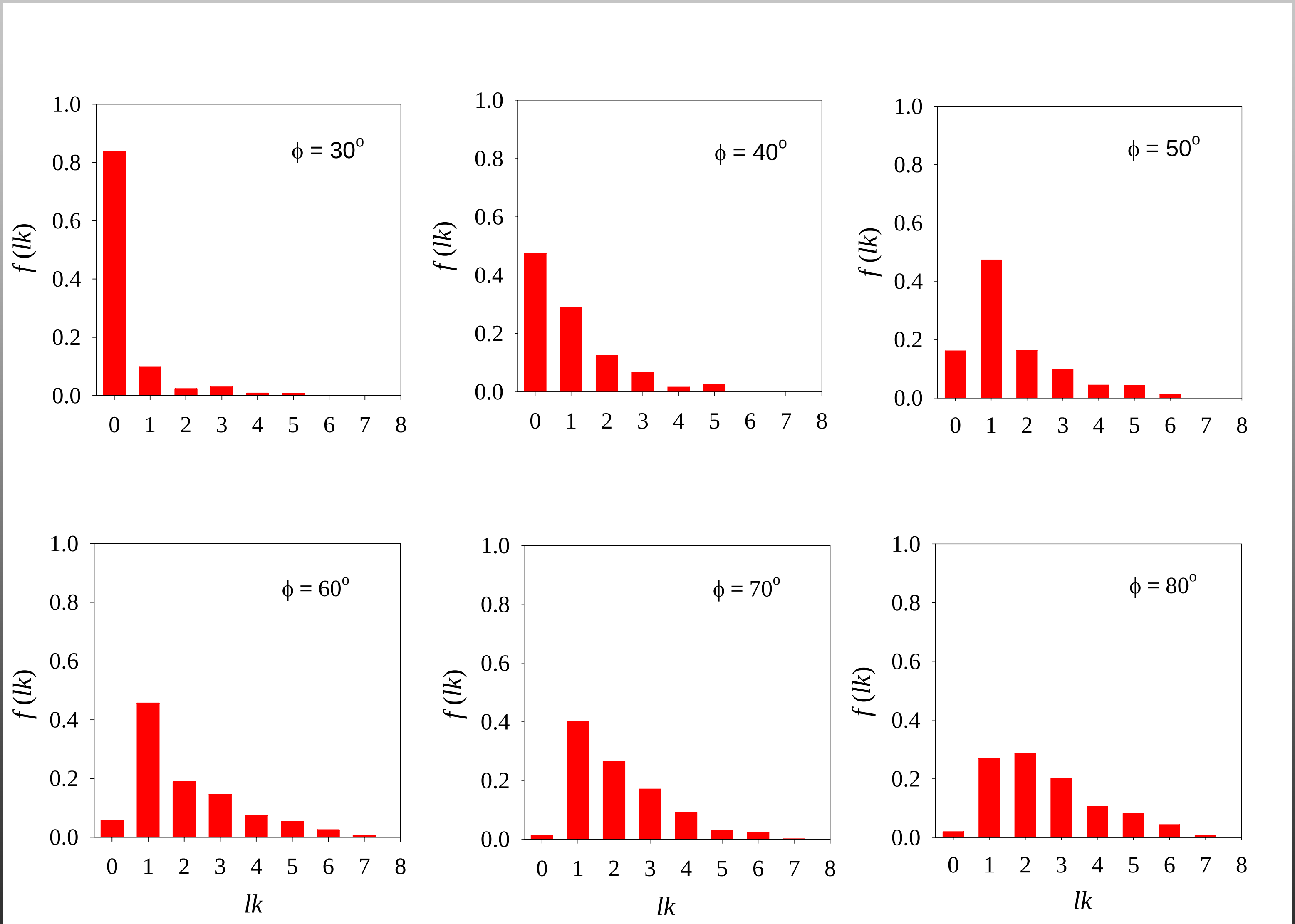
The simplest links



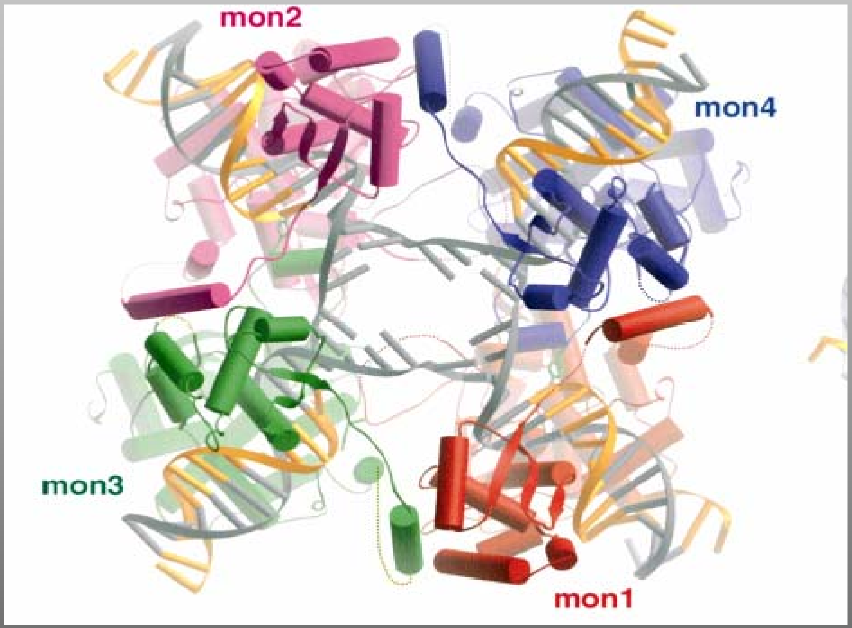
The torus links

$lk = 1$ $lk = 2$ $lk = 0$ $lk = 3$

Computed distribution of the recombination product for different values of the bend angle

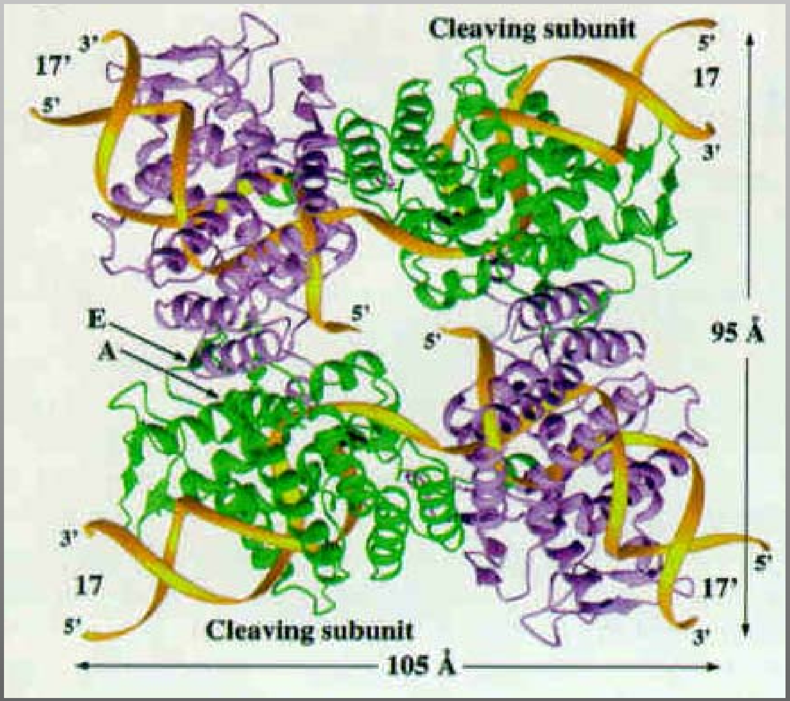


Structure of a Flp recombinase-Holiday junction complex



Y. Chen, U. Nerendra, E. L. Iype, M. M. Cox, A. P. Rice. *Mol. Cell* **6**, 885-897 (2000)

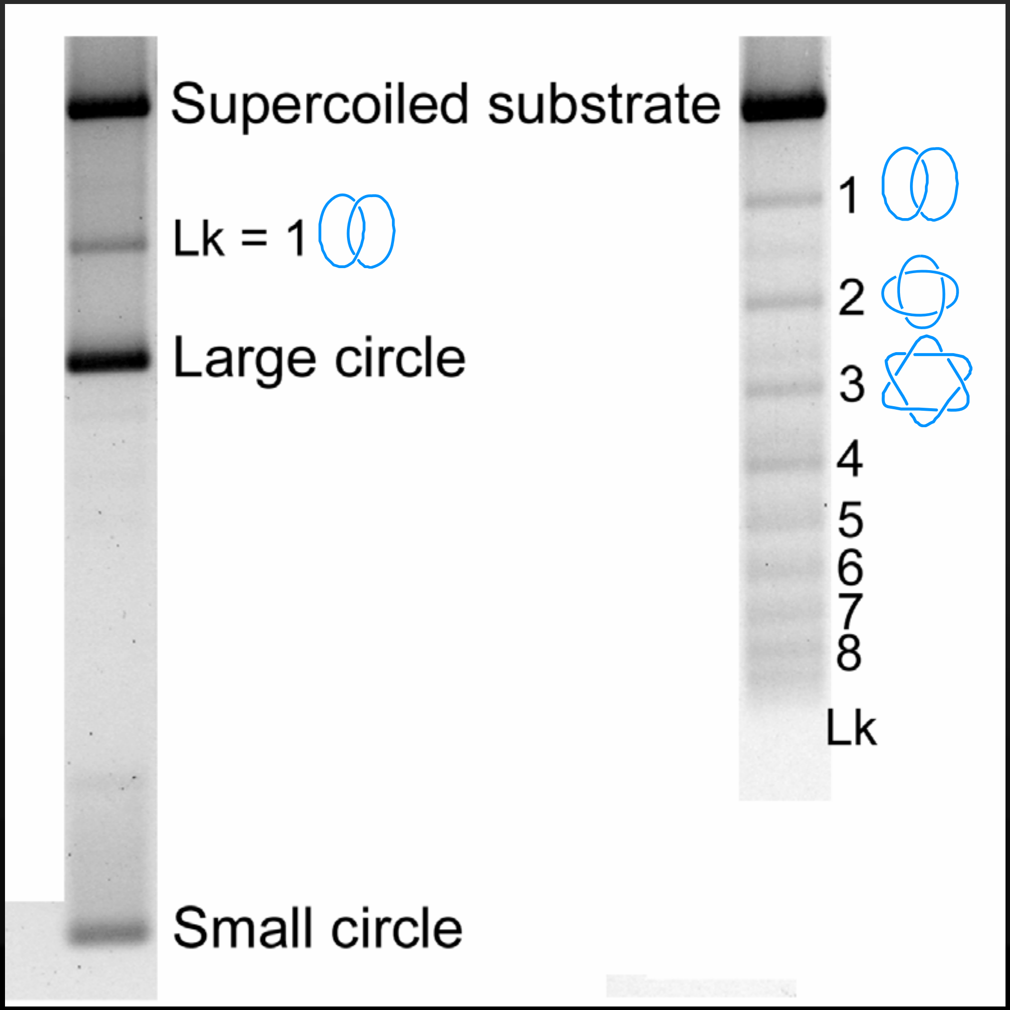
Structure of a Cre recombinase-Holiday junction complex



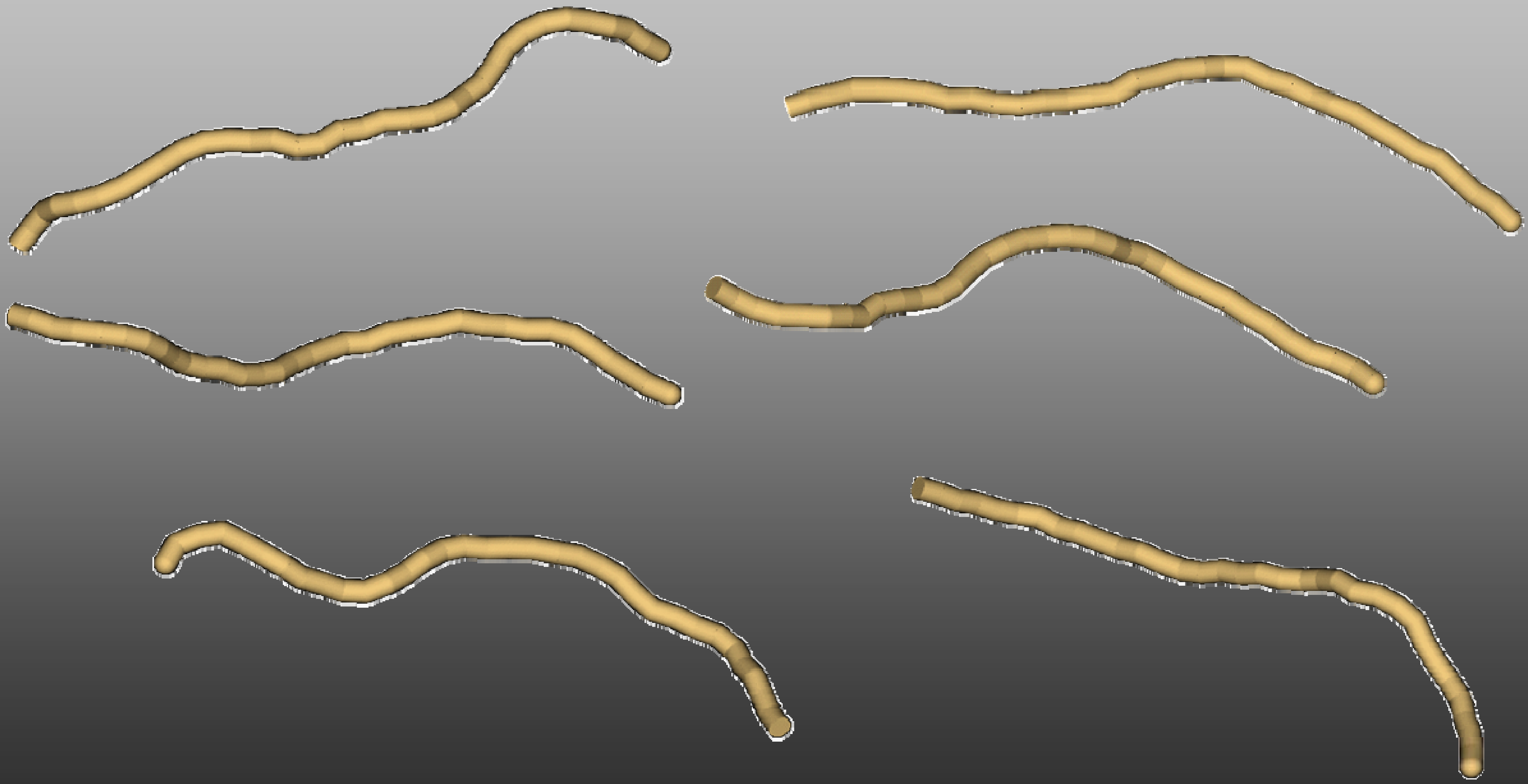
F. Guo, D. N. Gopaul, G. D. Van Duyne. *Nature, 389, 40-46 (1997)*

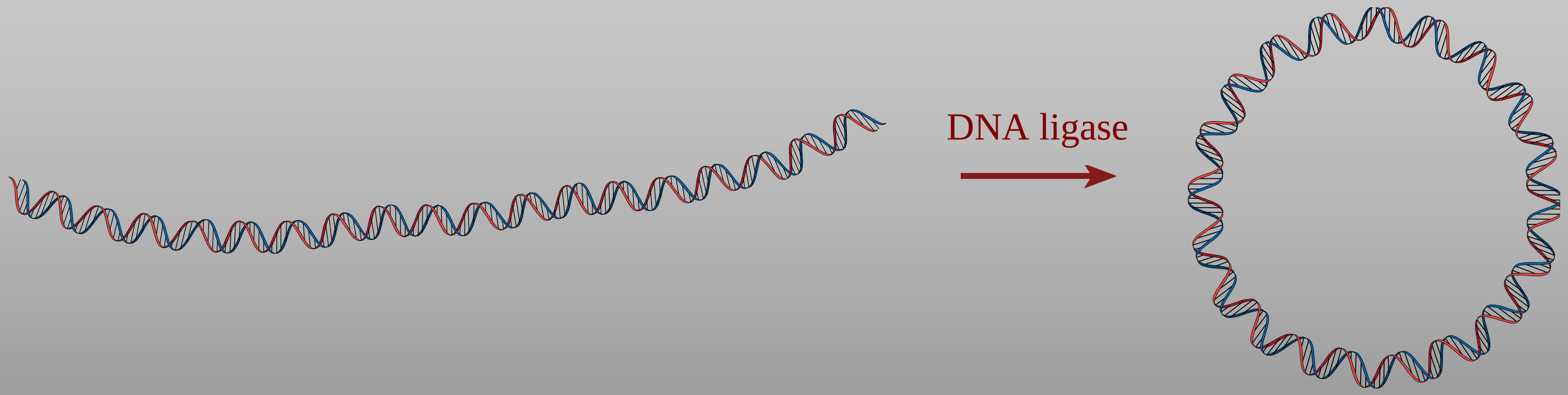
j-Factor measurements for Cre- and Flp-bound fragments

Cre/*lox*P **Flp/*FRT***

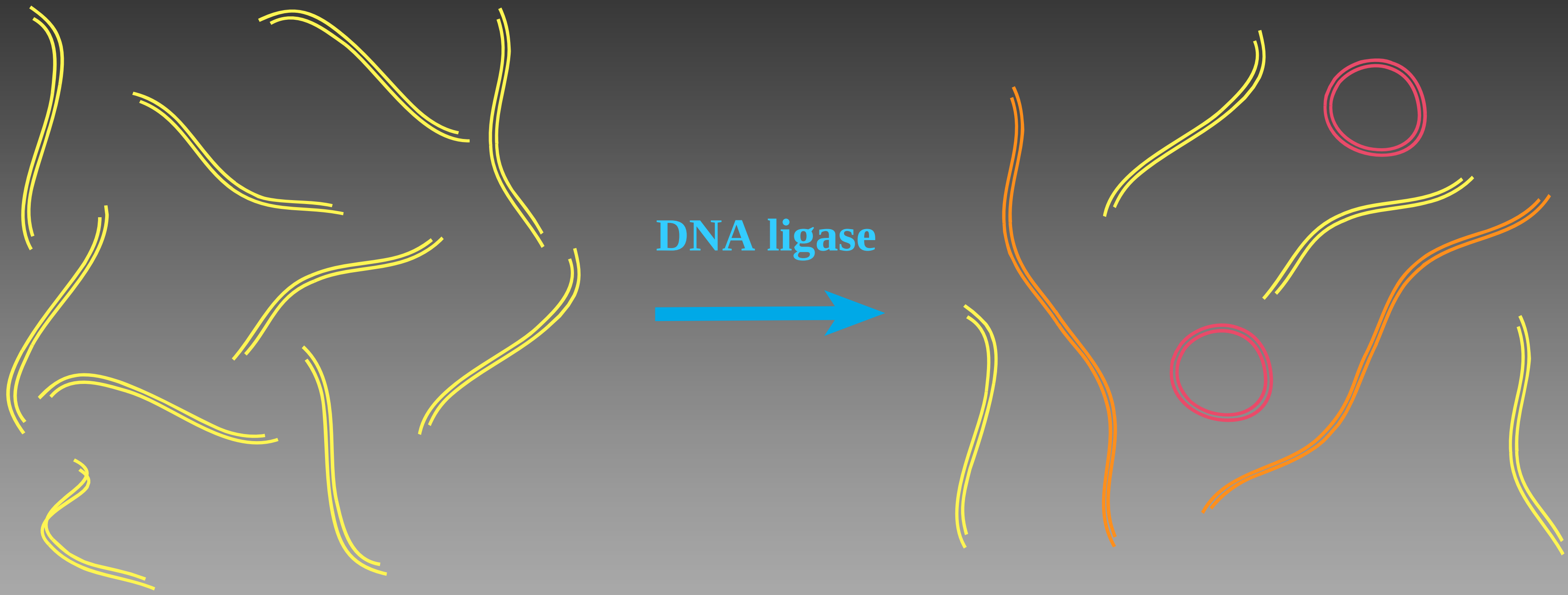


Typical conformations of DNA fragments 200 base pairs in length are more or less straight



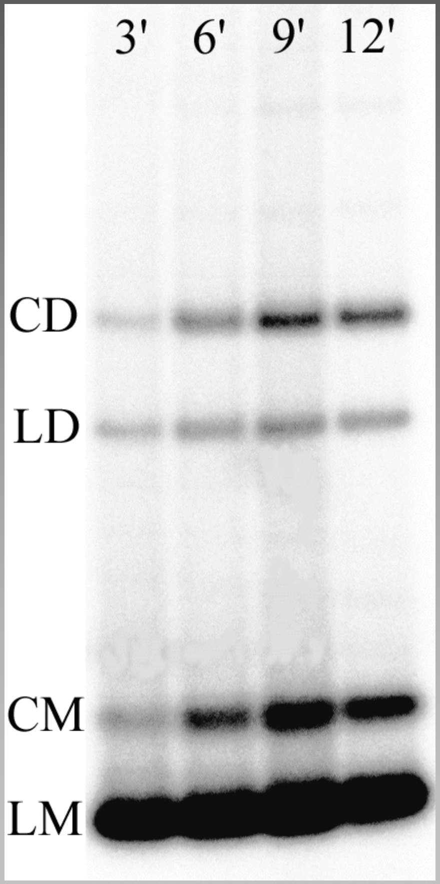


Kinetics of cyclization of short DNA fragments provides an opportunity to measure DNA properties with remarkable accuracy



The rate of cyclization is proportional to the local concentration of one end of the chain in the vicinity of the other end, *j*-factor.

Different reaction products can be separated by gel electrophoresis

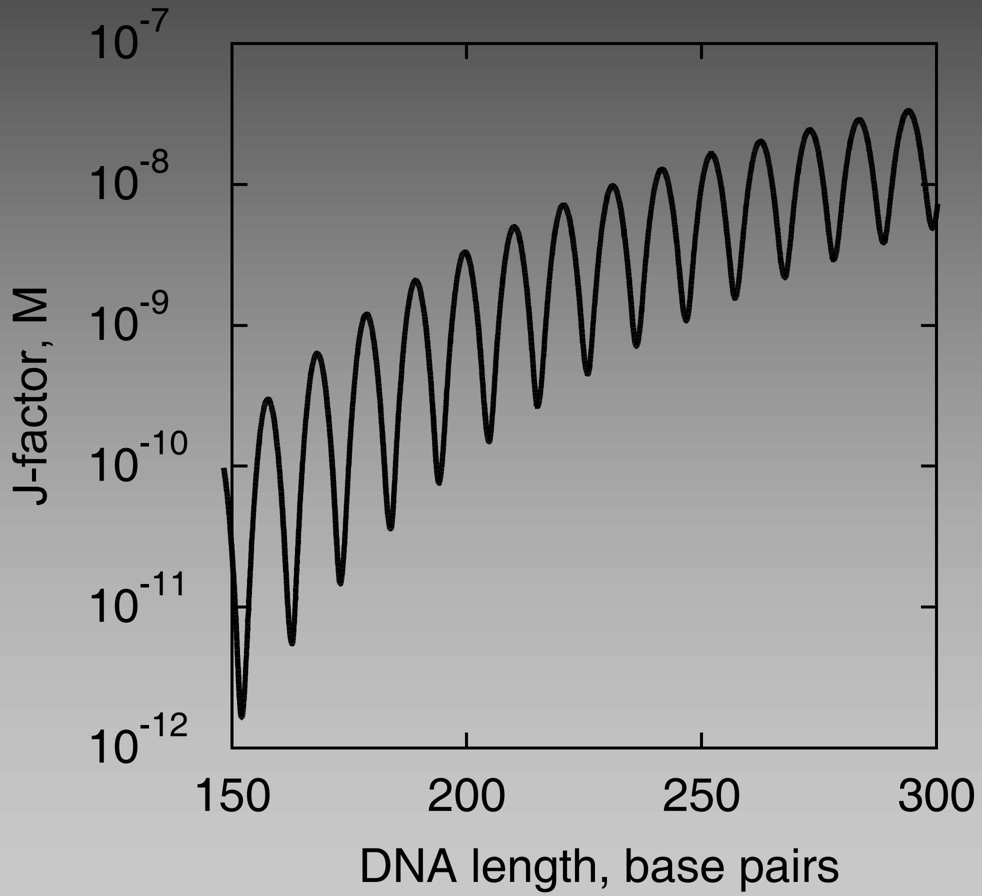


If *C(t)* and *D(t)* are the concentrations of circles and linear dimers formed in the ligation reaction, than

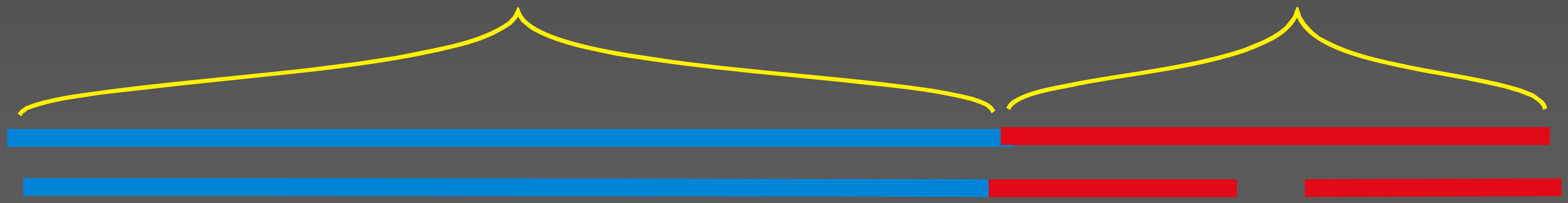
$$j = 2M_0 \lim_{t \to 0} \left[C(t) / D(t) \right]$$

where M_0 is the initial concentration of the fragments.

There is one complication: j-factors for short DNA fragments also depend on their equilibrium twist



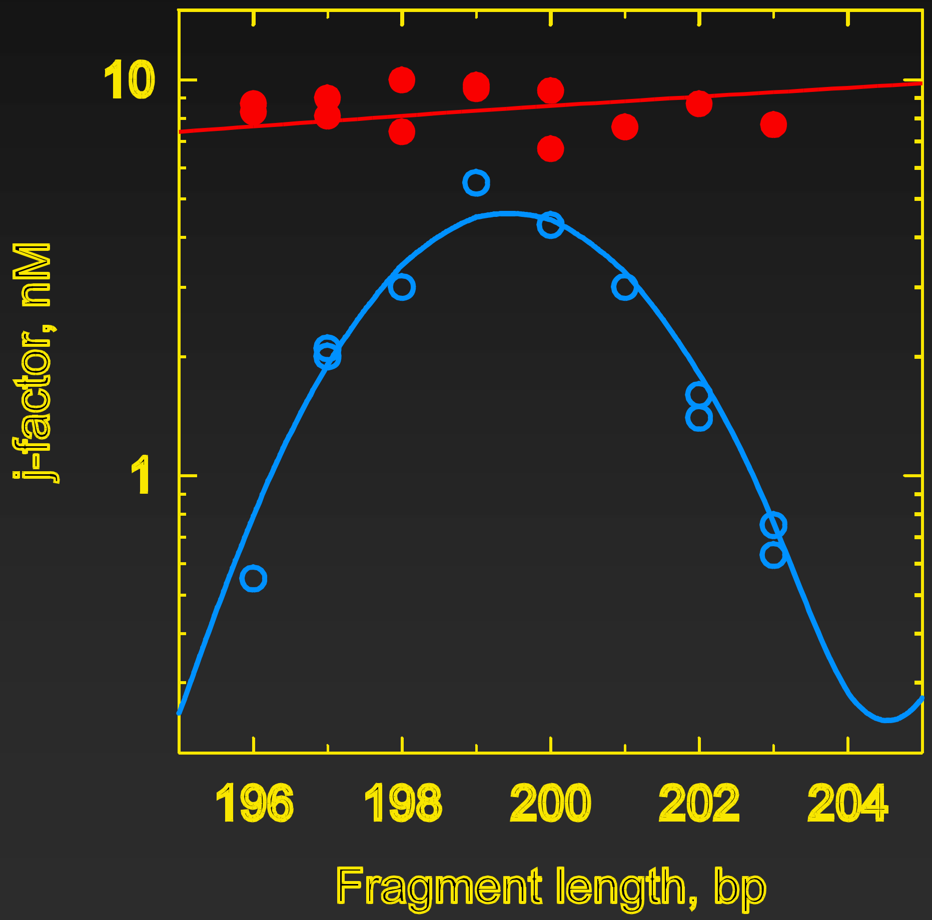
DNA fragments with a gap can be assembled from universal linker carrying the gap and DNA fragment with a specific sequence cloned into a plasmid



170 bp fragment with a desired sequence which has a specific binding site in the middle

30 bp DNA duplex assembled from synthetic oligonucleotides

DNA fragments with a gap of four nucleotides in length do not have preferable torsional orientation

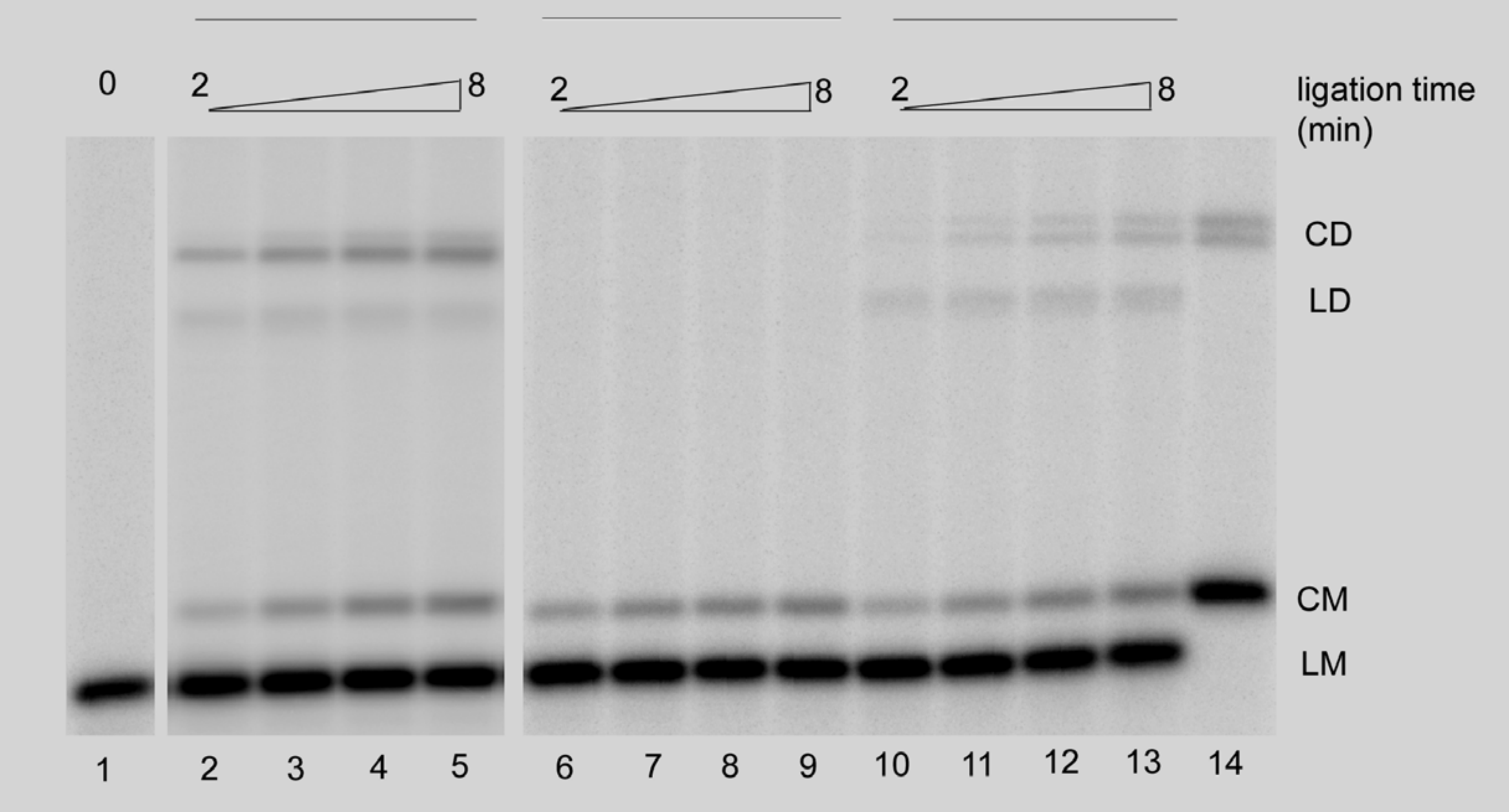


Fragments with gap

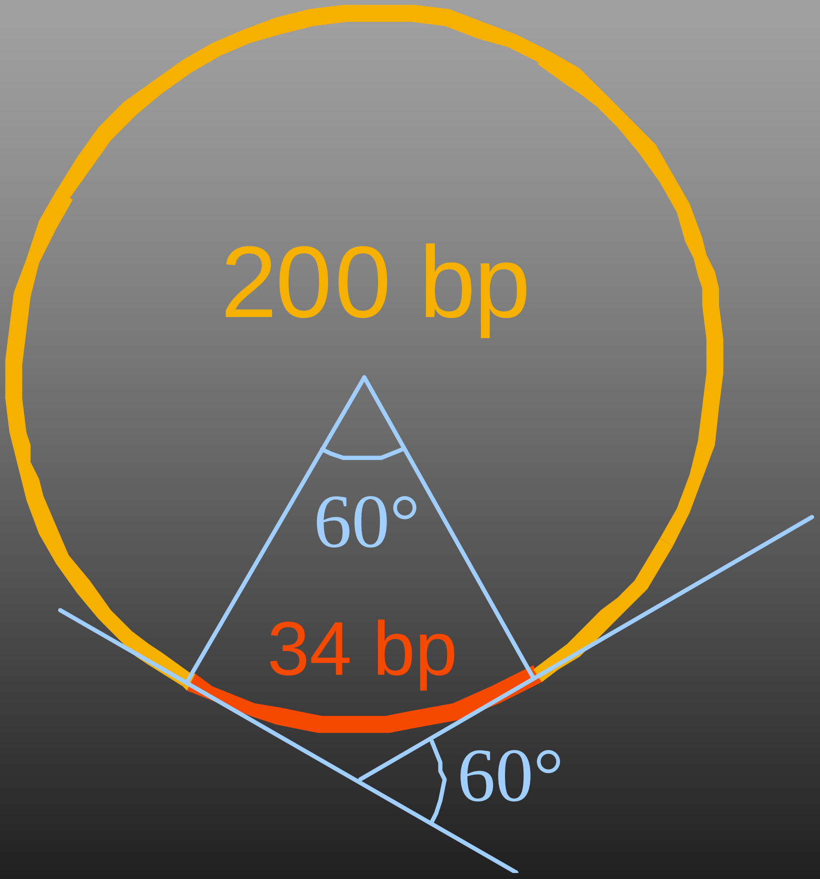
Fragments without gap

j-Factor measurements for Cre- and Flp-bound fragments

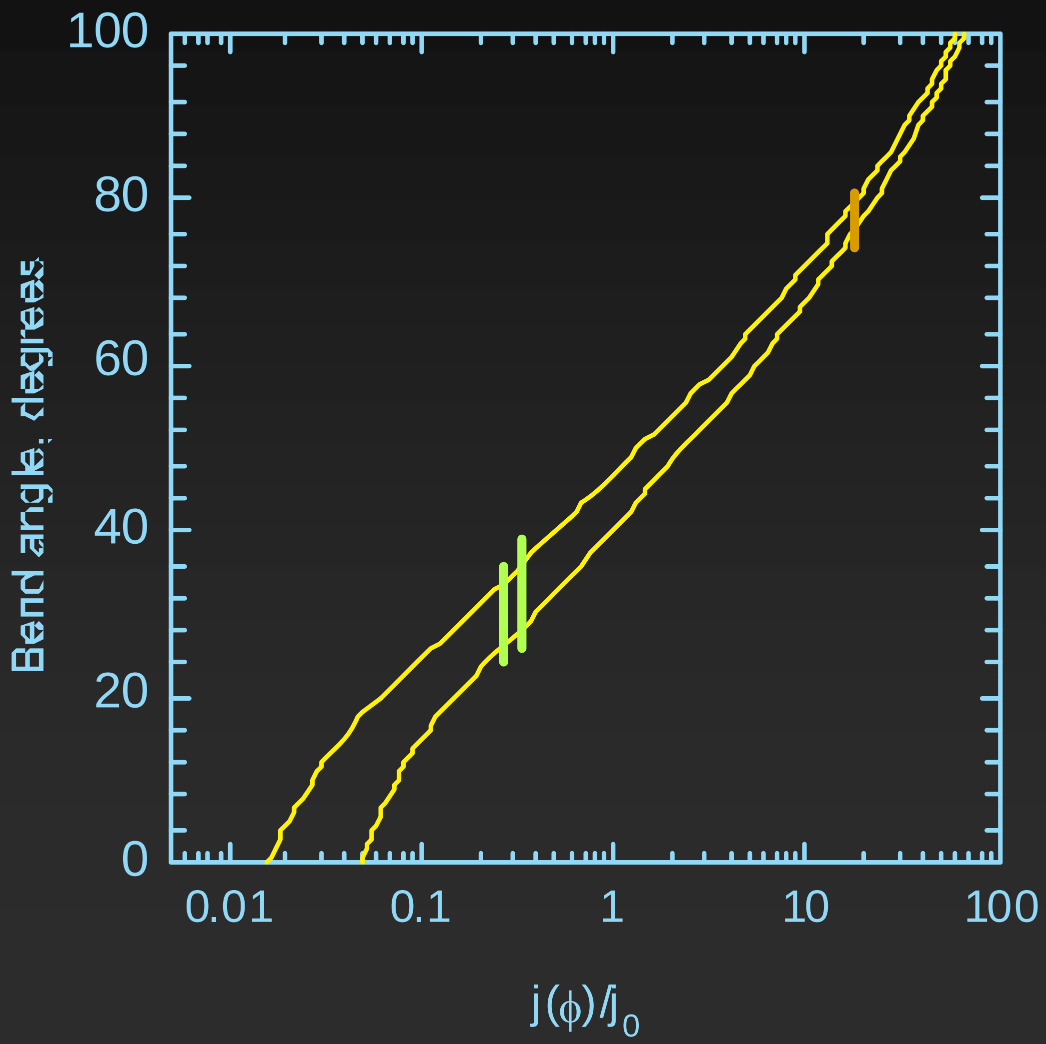
Fragment+Cre **Fragment+Flp** **Fragment only**



Naked *lox*P site is bent by 60° in the 200 bp DNA circle

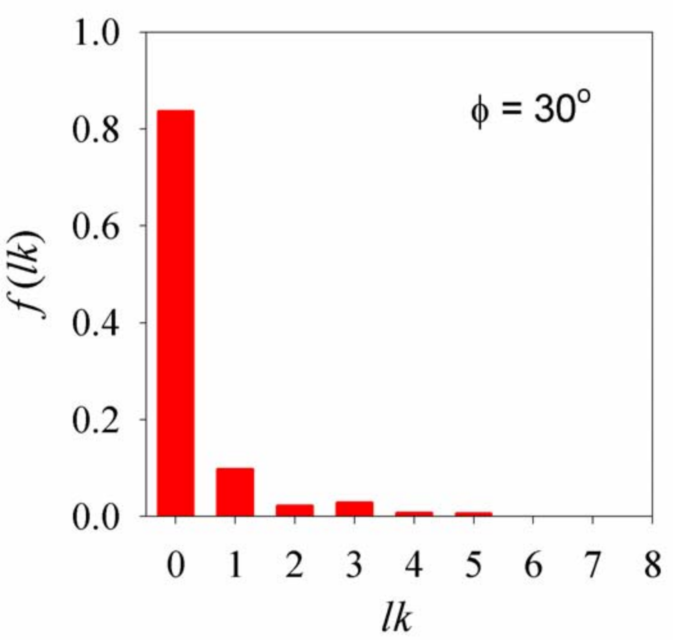


The bend angles induced by Cre and Plp binding with their specific sites

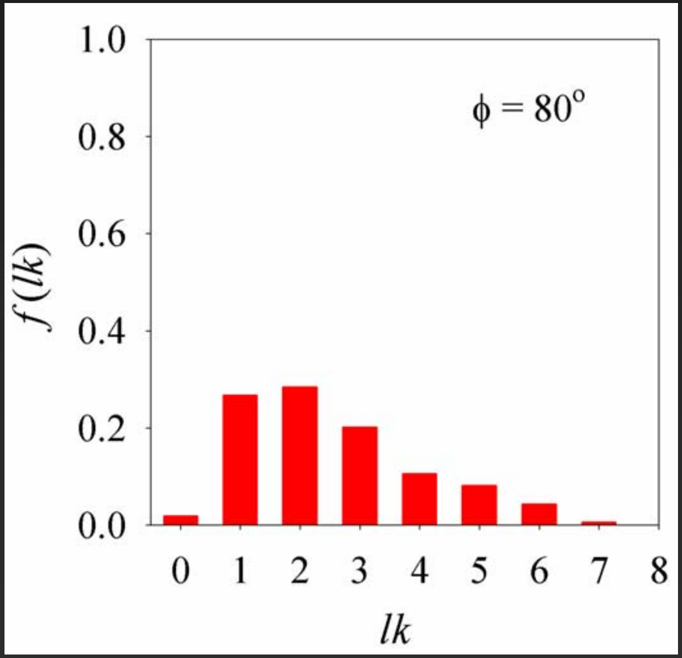


The computed product distributions for Cre and Flp recombinases

***Cre/lox*P: $\phi = 30^\circ \pm 4^\circ$**



Flp/FRT: $\phi = 78^\circ \pm 6^\circ$



Quan Du & Alexei Podtelezhnikov
New York University

Valentin Rybenkov & Nicholas Cozzarelli
University of California, Berkeley

Makkuni Jayaram
University of Texas at Austin

The work is supported by NIH