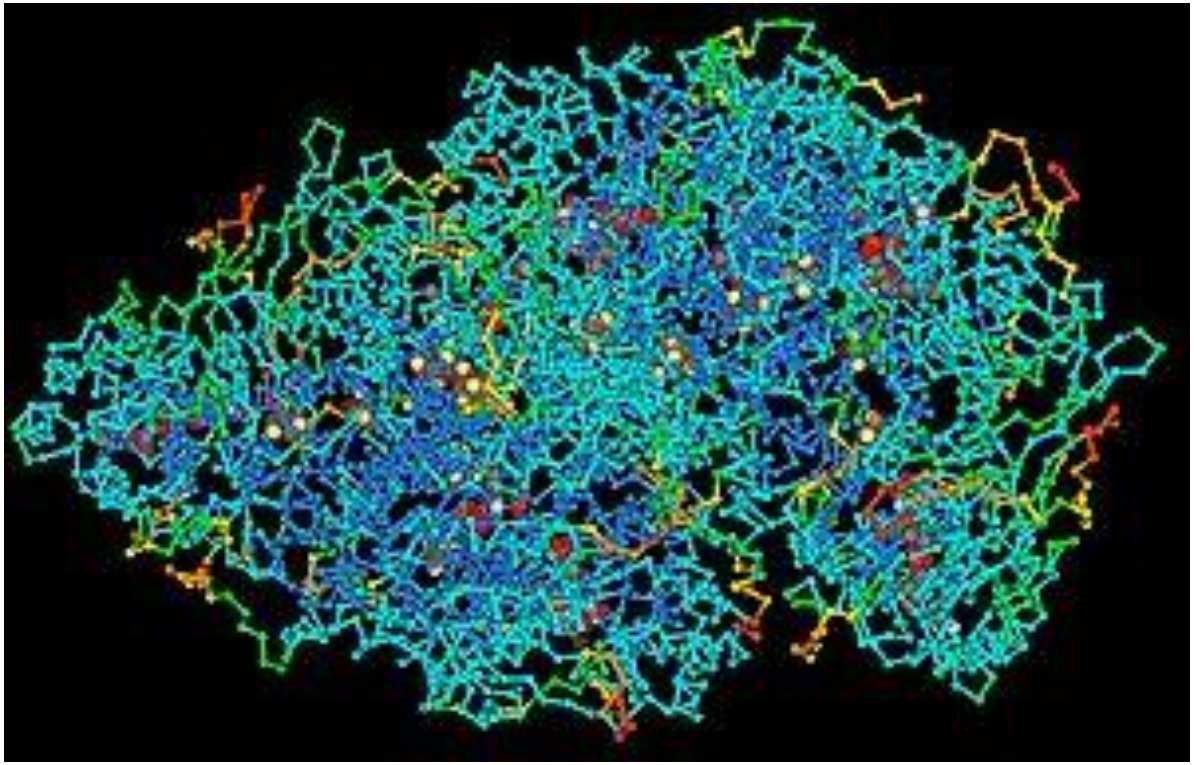
Introducción a la física de las macromoléculas

Curso general de Biofísica 2023

Andrés Pomi

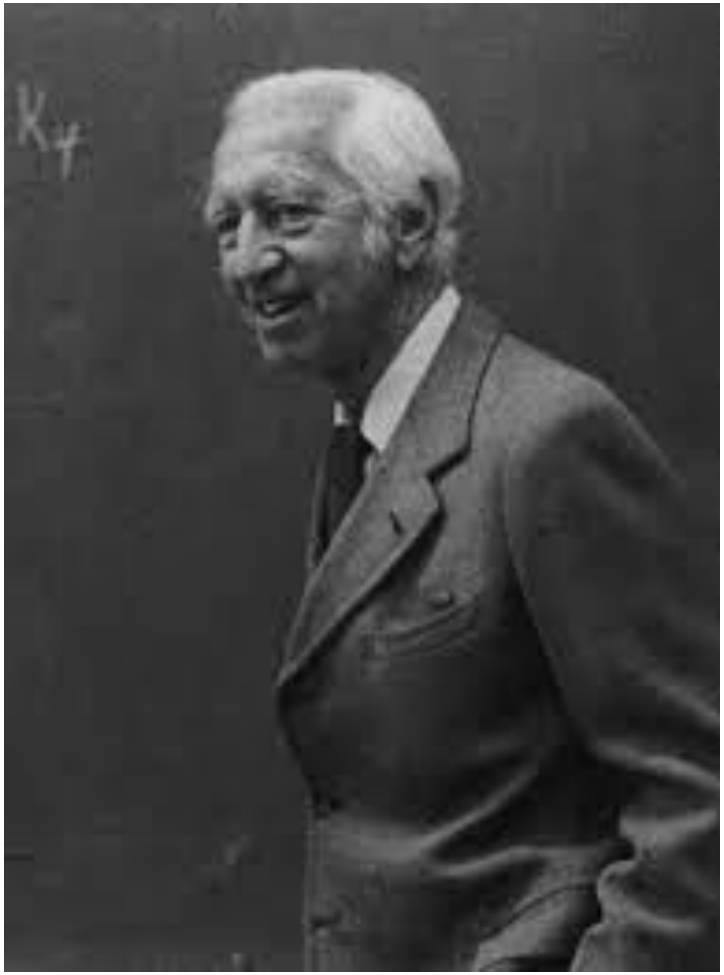
1. La macromolécula como sistema complejo



La visión de Jeffries Wyman

- "*Cuando consideramos las propiedades polifuncionales de las macromoléculas biológicas, nos sorprende la complejidad omnipresente que a menudo se asocia con estos sistemas deterministas relativamente simples*".

- Transducción ("linkage", enlace) de diferentes eventos, químicos o físicos, en el tiempo y el espacio. Asume diferentes características dependiendo si el sistema está en equilibrio, en estado estacionario o lejos del equilibrio"



- Existe una “cibernética” de las macromoléculas biológicas, desarrollada junto con Wyman por Jacques Monod.

- Las macromoléculas como “demonios de Maxwell”.

- Capacidad de armado, ensamblado, plegado.

2. Polímeros en solución

- Las macromoléculas biológicas son en general polímeros: cadenas de aminoácidos que constituyen la base de las proteínas, los ácidos nucleicos…

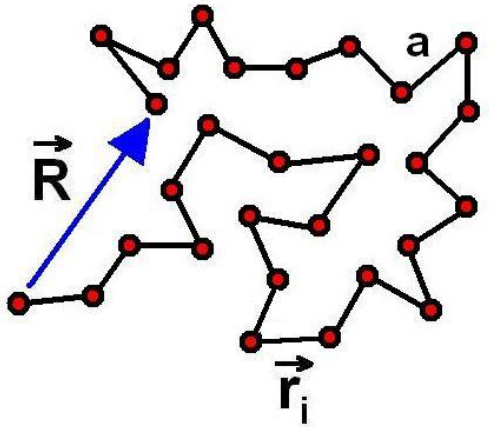
- Un área básica importante es el estudio de las propiedades de los (bio)polímeros en solución. Éstos, al igual que las partículas coloidales, están sujetas a la agitación térmica por los choques de las moléculas de solvente contra los eslabones de la cadena.

- Existen diversos tratamientos teóricos de esta situación. Uno de ellos es ver la dinámica de la cadena como un movimiento browniano.

a) Representación como marcha aleatoria

La noción de Cadena Ideal:
desplazamientos independientes en direcciones arbitrarias y con el mismo módulo

$$\vec{R} = \sum_{i=1}^{N} \vec{r}_i$$



L = N a

< R > = 0 media cero
Varianza = < $\mathbf{R}^2$> - <$\mathbf{R}$>^{2}
< R^2 > = N a^2 es decir que **R ~ $N^{1/2}$**

En cadenas suficientemente largas, con R<<L,
R se distribuye de acuerdo a función Gaussiana:

$P(R) \approx N^{-3/2} \cdot \exp\{ - (3 R^2) / (2Na^2)\}$

“cadenas Gaussianas”

b) Modelos más realistas

- Si se tiene en consideración el volumen de exclusión (excluded volume)

$$R \sim N^{3/5}$$

que se acerca bien a los datos experimentales

c) Consideraciones termodinámicas

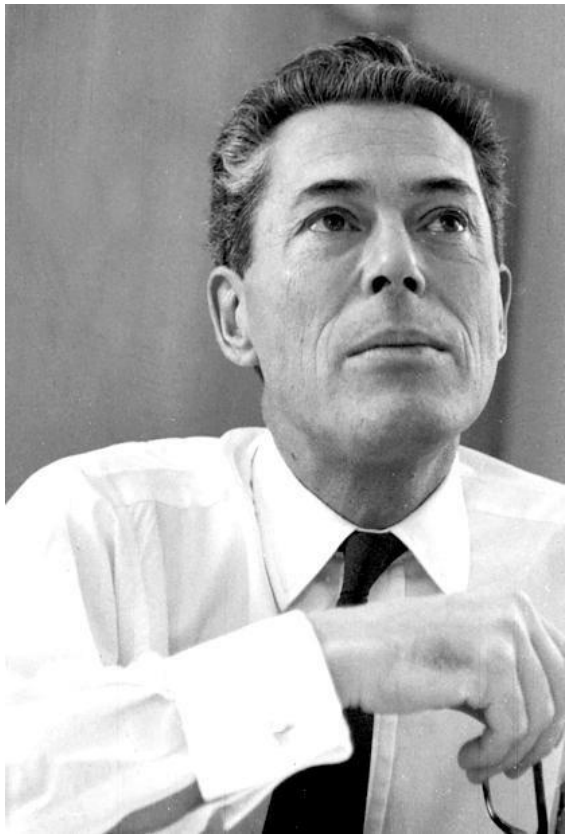
- A partir de otra visión simplificada de los polímeros en solución, que considera a la macromolécula como un hilo flexible elástico con coeficiente de elasticidad α, se obtiene:

$$< R^2 > = \frac{2\,L\,\alpha}{T}$$

No importa la composición química, una macromolécula larga se enrolla en un ovillo fluctuante al aumentar la temperatura, como consecuencia de la agitación térmica.

Corolario 1 - Una pregunta de Jacques Monod a Pierre Giles de Gennes

Monod



de Gennes

¿Cuántos aminoácidos debe tener una cadena polipeptídica para que aparezca un sitio activo?

Es decir: para que sea altamente probable que el extremo final se acerque al origen (y así se genere una conformación espacial necesaria para la existencia de un sitio activo).

Nótese la importancia evolutiva de esta pregunta, ya que conlleva la posibilidad de aparición de actividad catalítica en biomoléculas primordiales.

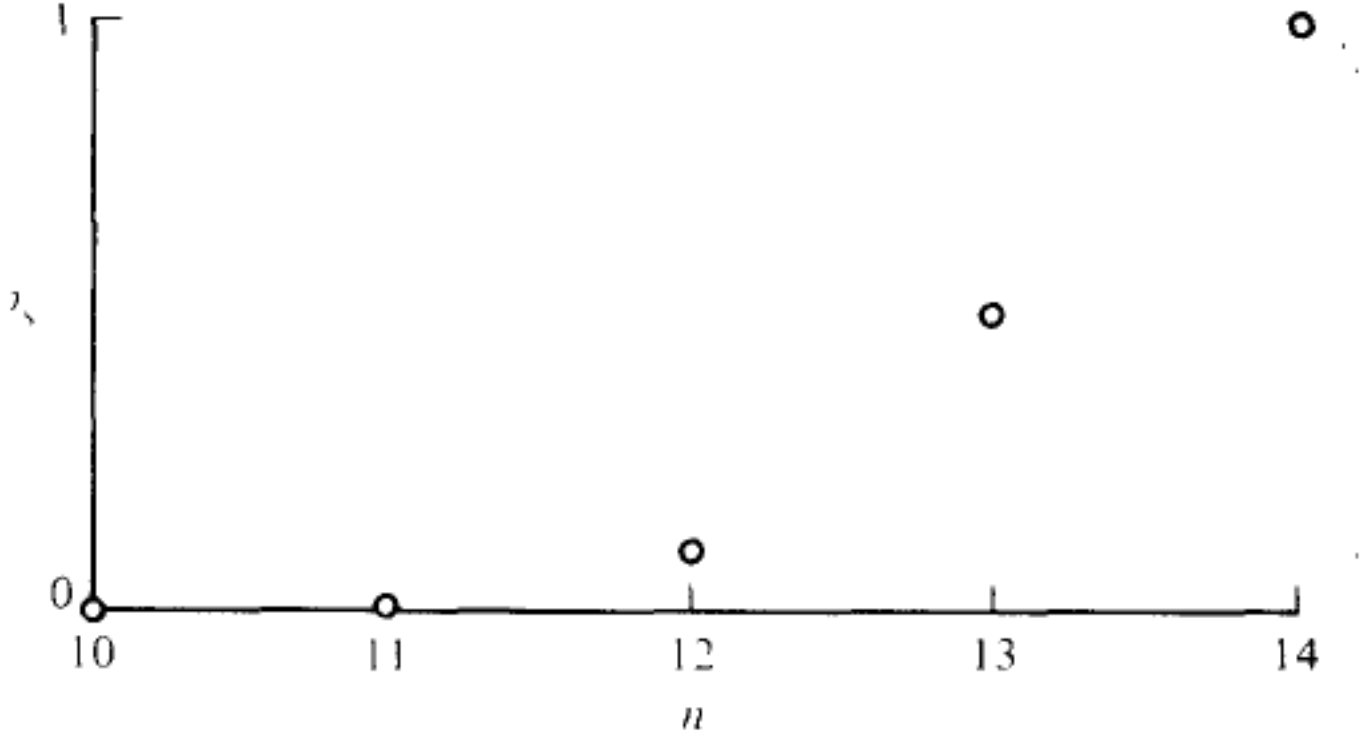
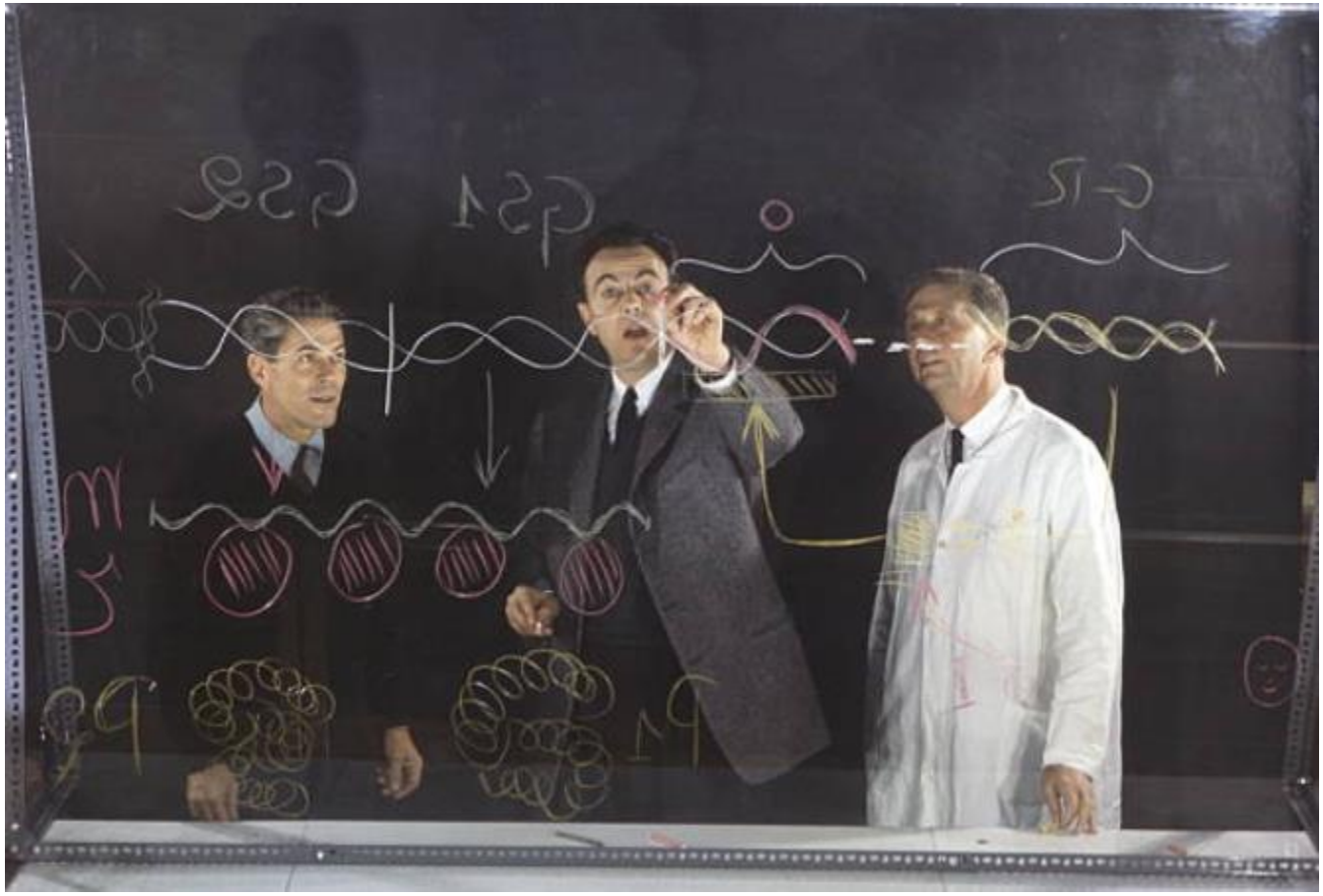


Fig. 2.3. Probability of success versus loop length. Note the abrupt variation near $n = 13$.

Premio Nobel de Fisiología 1965

- Jacques Monod, François Jacob, André Lwoff,

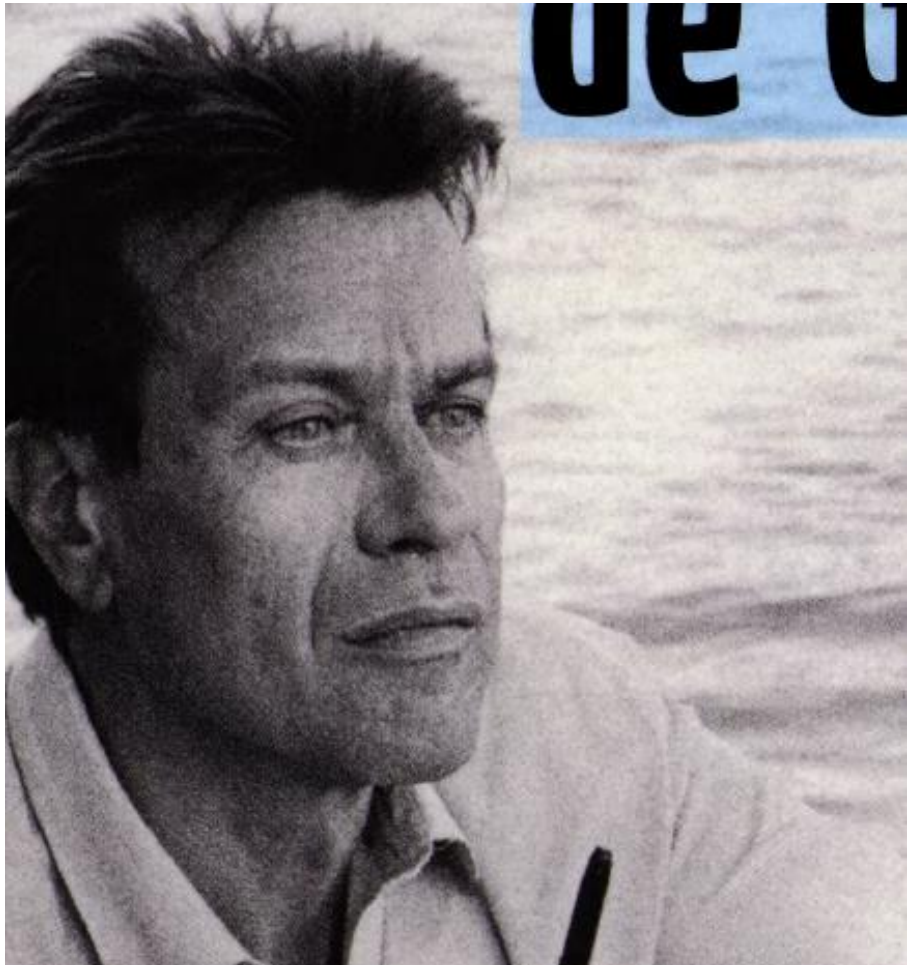
  por regulación génica (operón lac)



Premio Nobel de Física 1991

- Pierre Giles de Gennes

por sus trabajos sobre polímeros y cristales líquidos



Es una mirada '*a la Ramsey*'

- **La pregunta de Monod pertenece a un tipo de problemas muy interesantes del punto de vista epistemológico**, que generaron la rama de las Matemáticas conocida como *Teoría de Ramsey*.

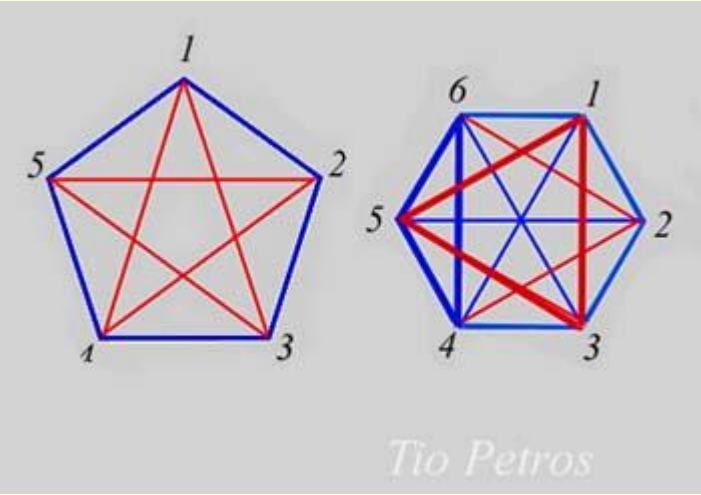
- Se trata de algo no intuitivo, pero muy importante para la biología: el hecho de que, al aumentar la complejidad de un sistema, hay estructuras que pasan "*necesariamente*" a existir.

Franck P. Ramsey

Estructuras que forzosamente deben existir al aumentar la complejidad

- Ej. 1 - ¿Cuántas cartas debo robar de un mazo para tener forzosamente dos del mismo palo? [*'pigeonhole principle'*]

- Ej. 2 - ¿Cuántos invitados debo tomar de los participantes de una fiesta para que necesariamente haya o tres mutuamente conocidos, o tres mutuamente desconocidos? (Teorema de Ramsey)



Si en un grafo completo K_6 coloreamos sus aristas de dos colores, siempre encontraremos un subgrafo K_3 monocolor.

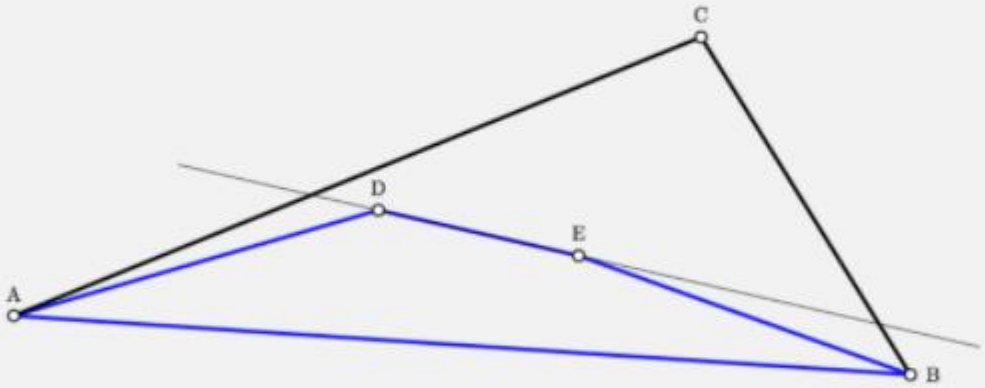
“Happy-end problem”

- Ej. 3 - ¿Cuántos puntos no-colineales debo tener en un plano para que forzosamente n de ellos formen los vértices de un polígono convexo de n-lados?

La observación de Eszter Klein.

Observación. *Dados 5 puntos del plano en posición general, siempre hay 4 de ellos que forman un cuadrilátero convexo.*

Si la envolvente convexa de los 5 puntos es un pentágono, cualesquiera cuatro puntos forman un cuadrilátero convexo. Si la envolvente convexa es un cuadrilátero, sus cuatro vértices forman el cuadrilátero convexo buscado.



Queda solo el caso de que la envolvente convexa sea un triángulo de vértices ABC, los otros dos puntos DE estarán en el interior del triángulo. Como no hay tres puntos en línea dos vértices del triángulo están al mismo lado de la recta DE. Esos dos vértices junto con DE forman un cuadrilátero convexo.

Eszter Klein y Gyorgy Szekeres

Corolario 2 – Una conjetura de Max Delbrück

Al aumentar la longitud de la cadena de un polímero en solución , la probabilidad de que esté anudada tiende a 1

Nudos primos

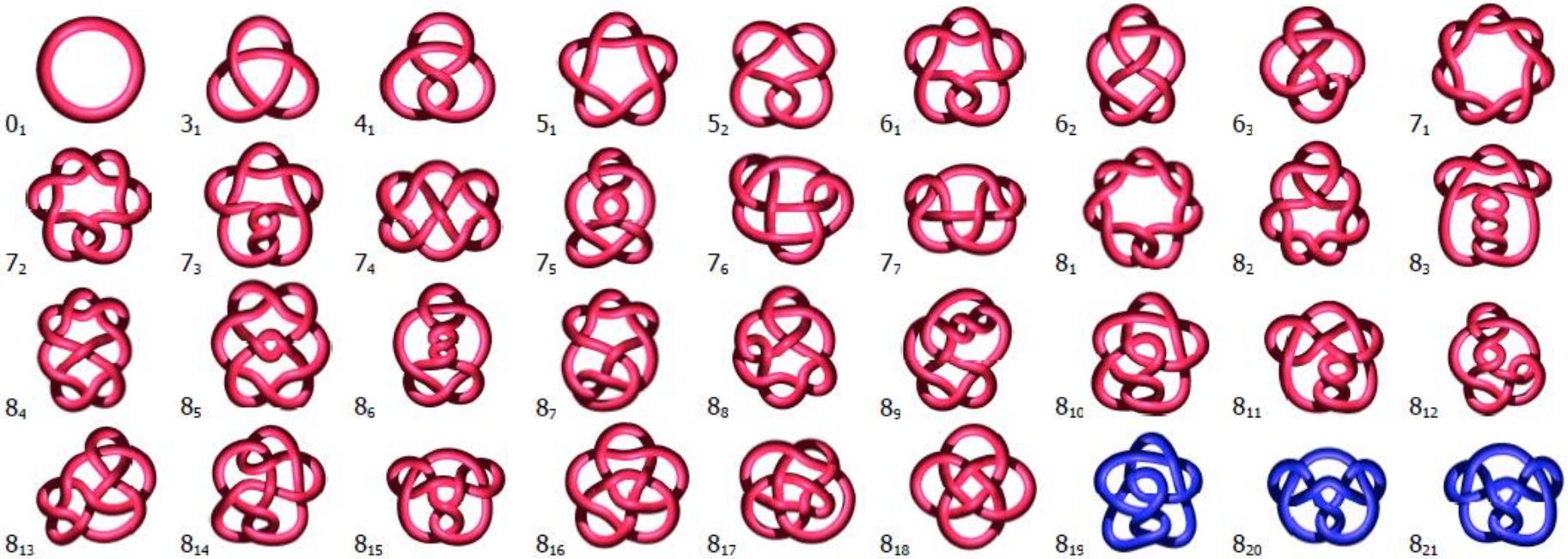


Figure 2. Knot table depicting all prime knots with up to eight crossings. Several prime knots also have common names; e.g., unknot (0_1), trefoil (3_1), figure eight (4_1), pentafoil (5_1), three-twist (5_2), and endless (7_4). Red knots are alternating and blue knots are nonalternating.

La probabilidad de **no** estar anudada, decrece exponencialmente con la longitud de la cadena

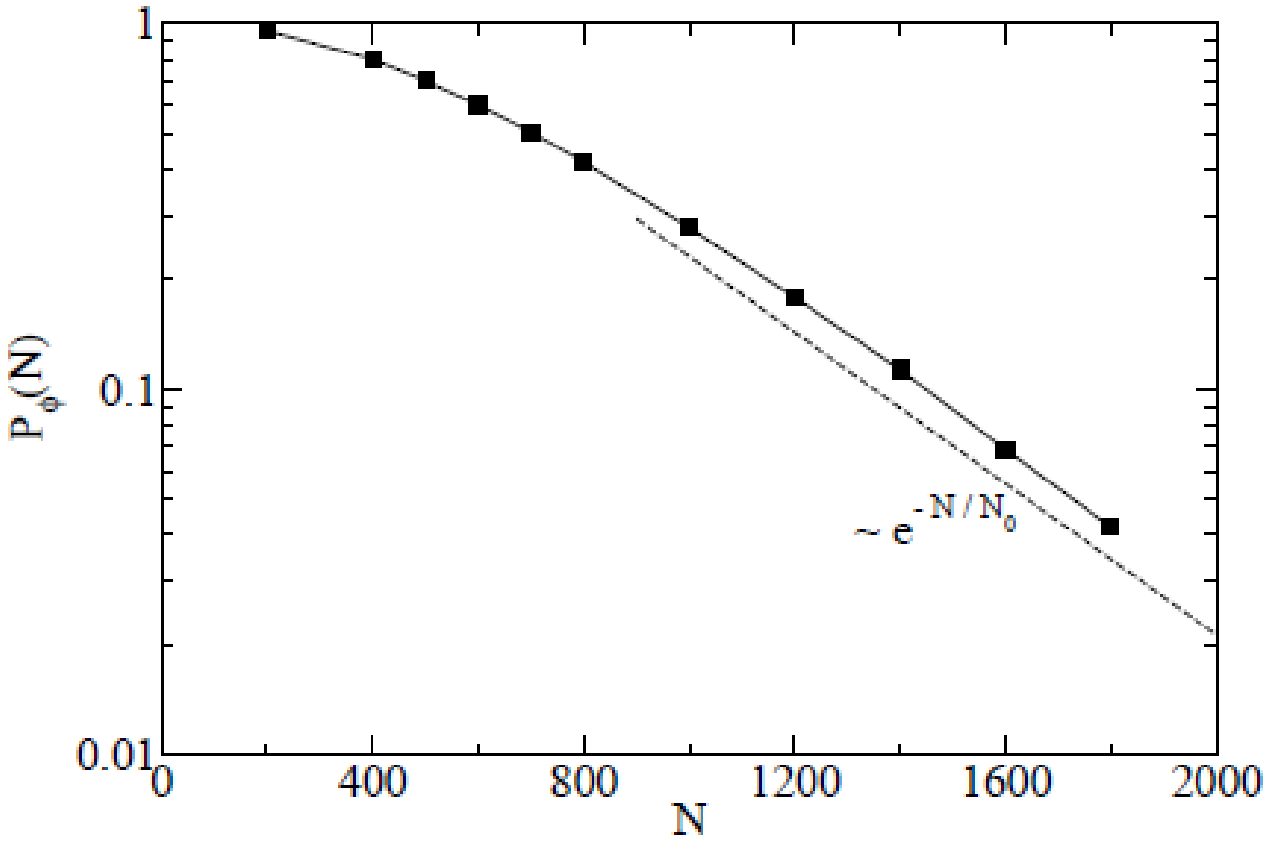


FIG. 1: Probability of finding an unknot. The straight dasl

Complejidad creciente de los nudos que aparecen al aumentar la longitud de la cadena

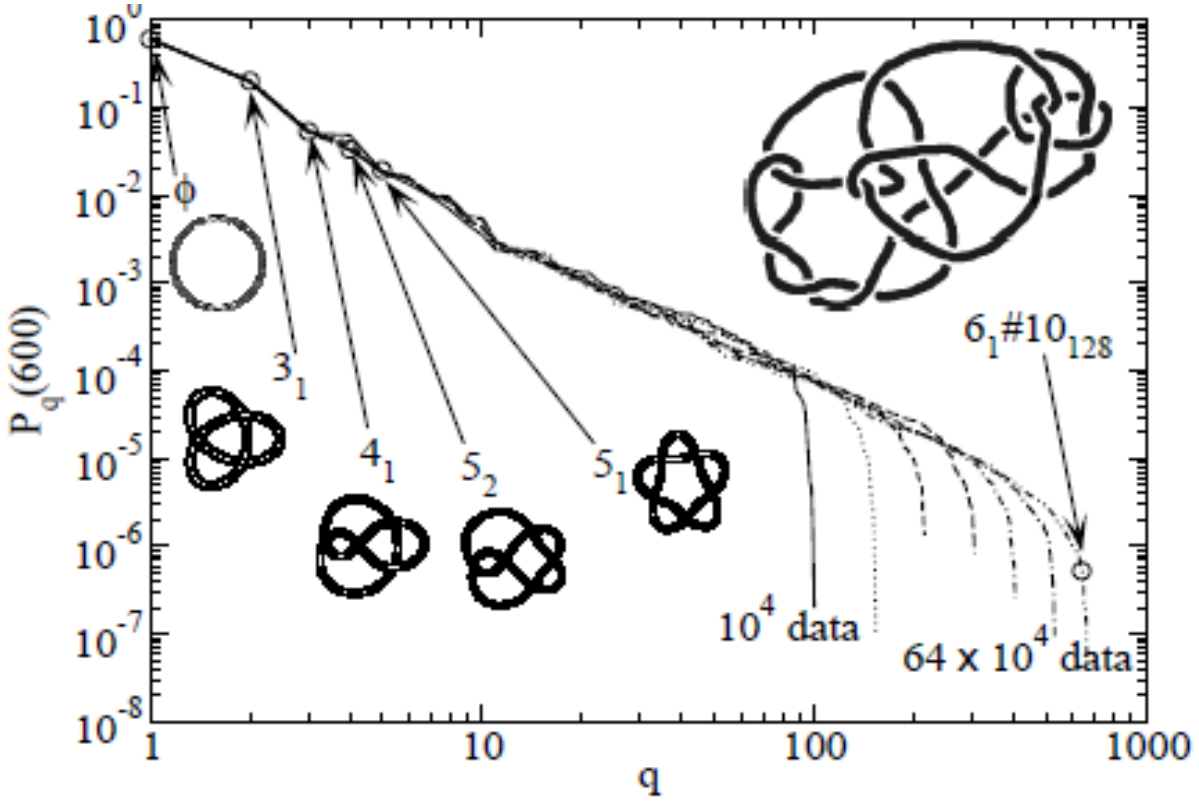
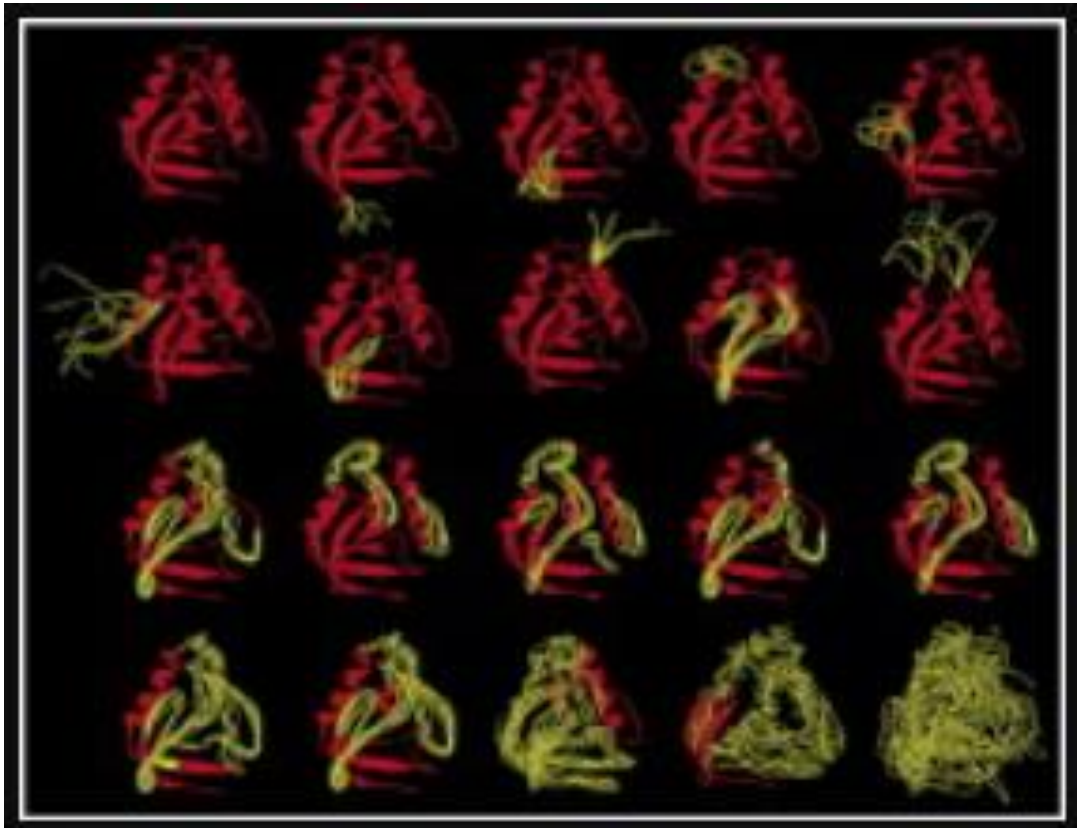
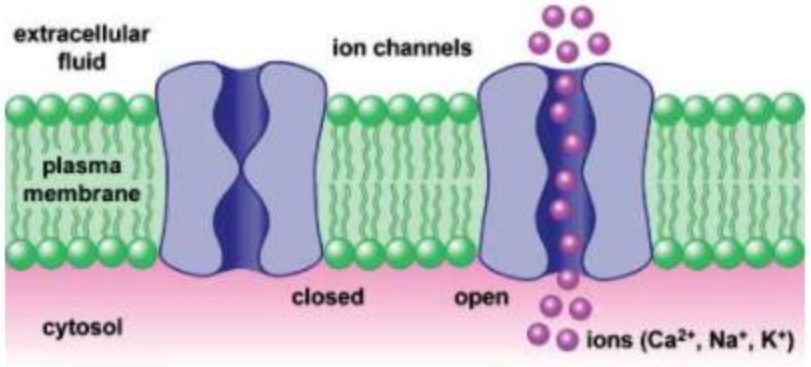


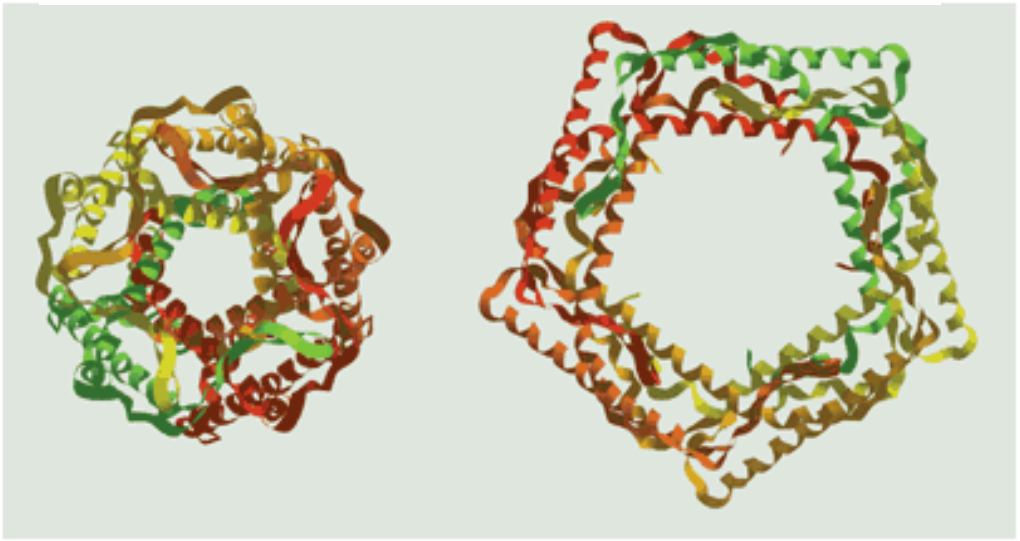
FIG. 2: Probability of rank-ordered knots for N=600. Cur

3. Fluctuaciones en proteinas



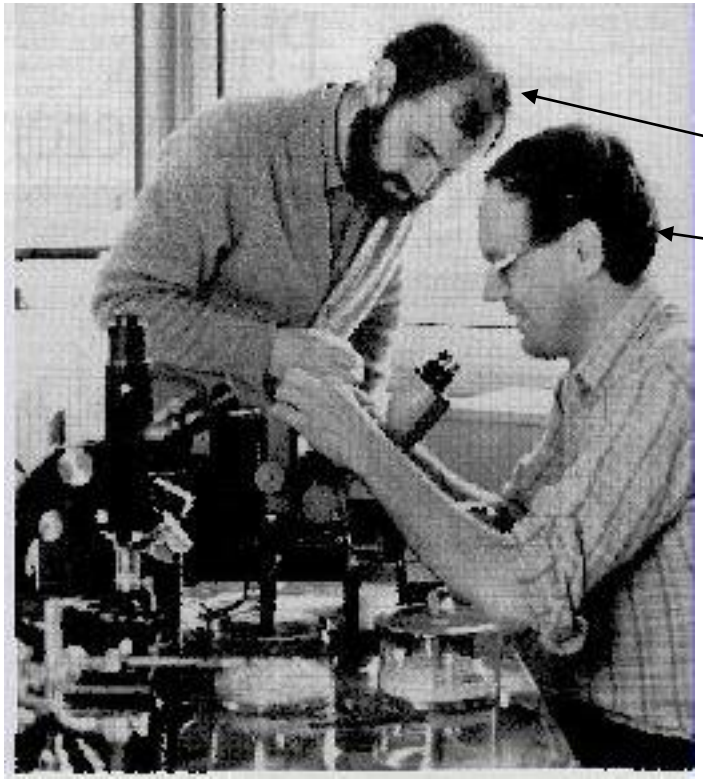
a) Apertura y cierre de canales iónicos





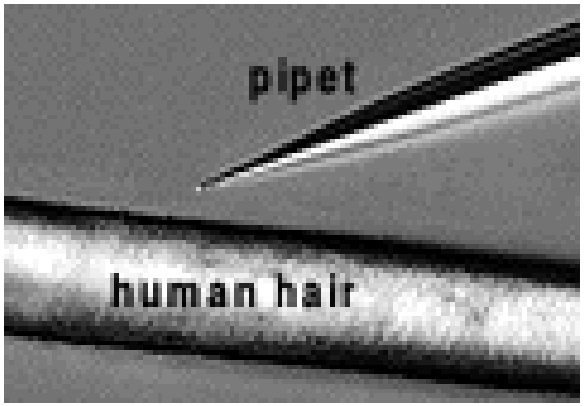
OPEN AND SHUT UV light prompts a modified MscL channel protein to switch from its closed form (left) to its open form (right). Visible light closes the channel back up. The channel is a pentamer made up of five identical helices (each shown in a different color).

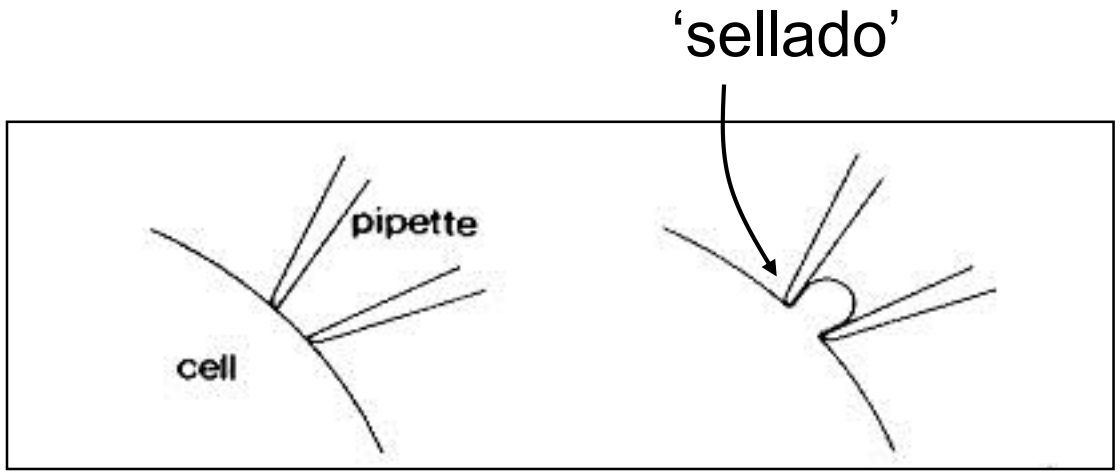
Técnica de “patch-clamp”



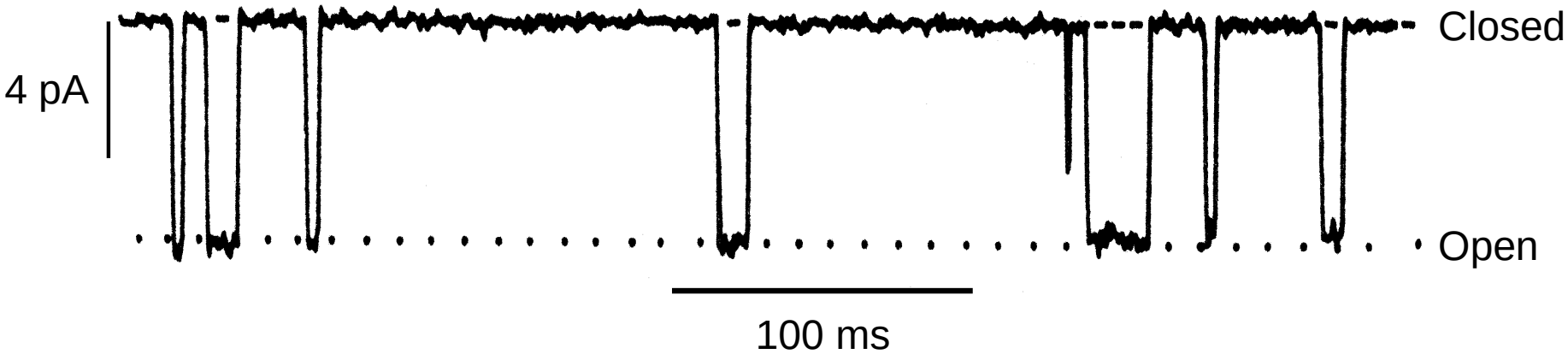
Erwin Neher
Bert Sakmann

(P. Nobel 1991)





Registro de canal único



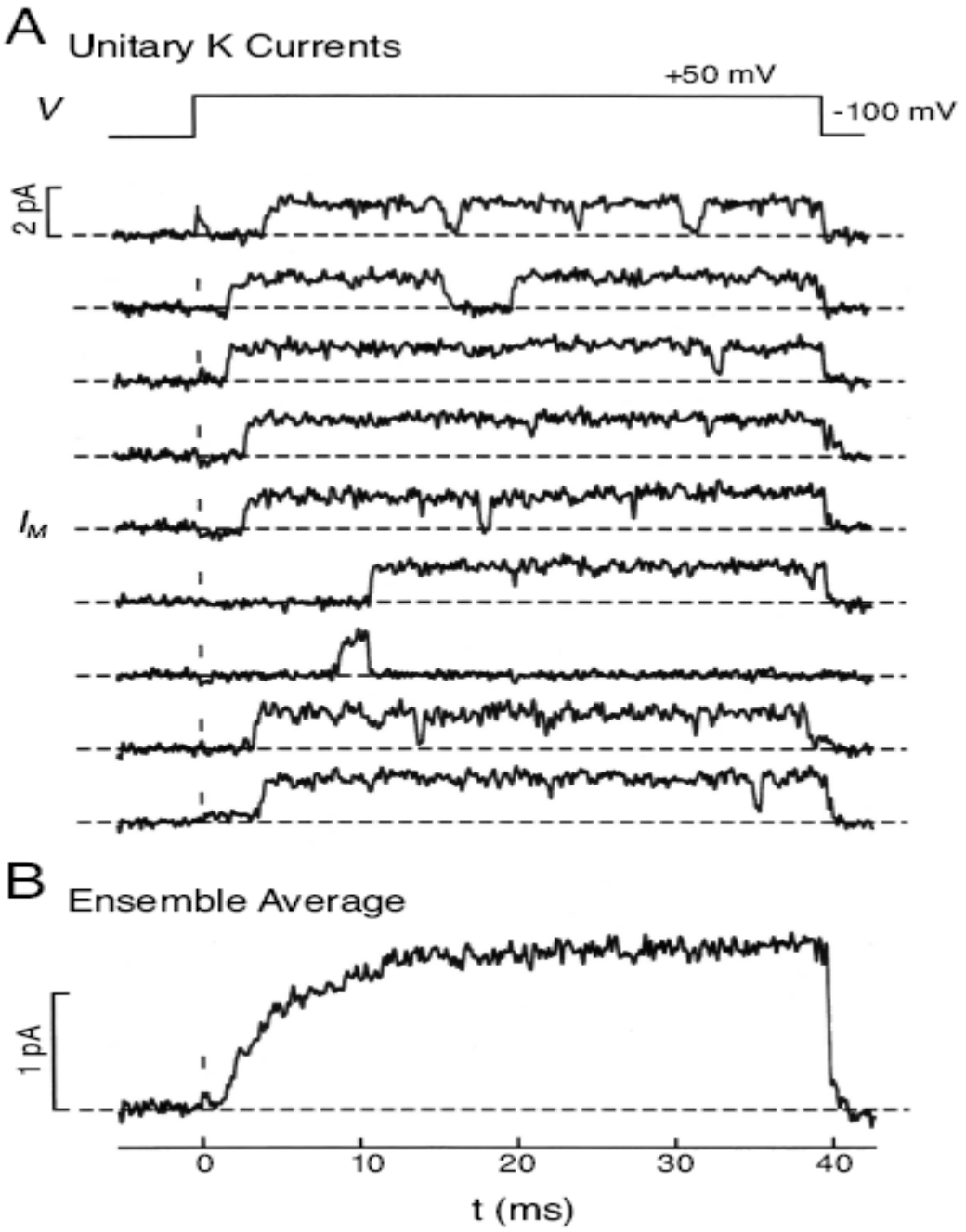
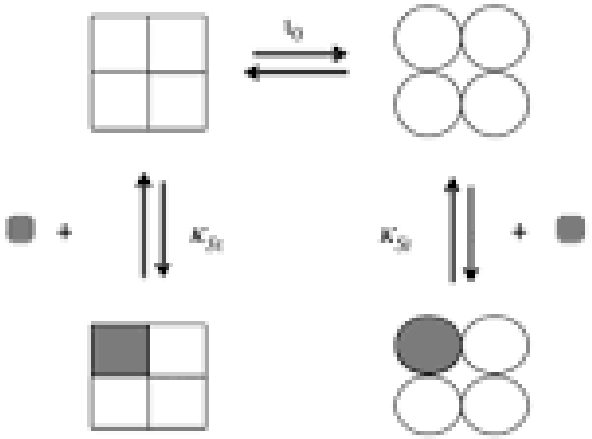


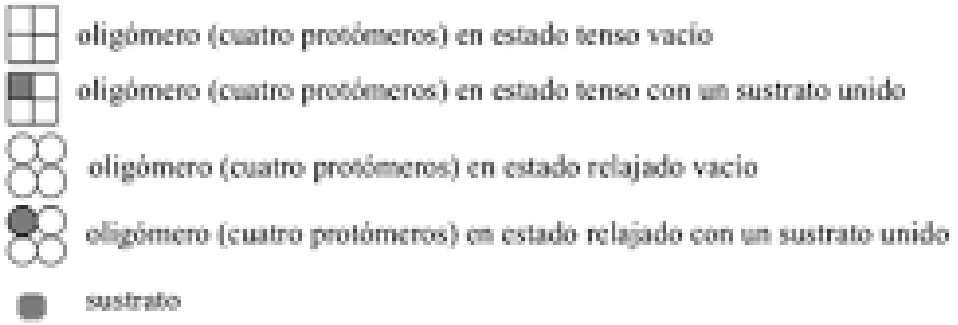
Figure 4.12. (A) Repeated voltage-clamp trials in single-channel recording mode for I_{K}. (B) Ensemble average of above recordings. Figure from F. Bezanilla.

b) Cambios conformacionales y transiciones entre estados

[Modelos alostéricos para explicar la cooperatividad]

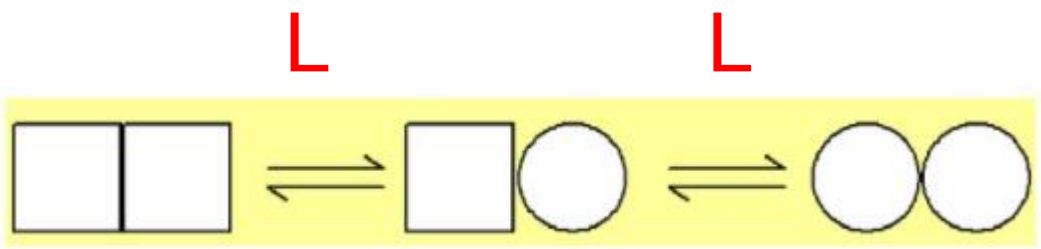
1. Modelo de Monod-Wyman-Changeux





El ligando fija una conformación: la “selecciona”

2. Modelo de Koshland-Némethy-Filmer



El ligando induce un cambio conformacional: lo “instruye”

Dos visiones: seleccionismo vs. instruccionismo

El debate se mapea también en otros campos de la biología:

- Evolución
- Teorías neurocognitivas

c) Motores moleculares

- Marcha aleatoria 1D sobre polímeros

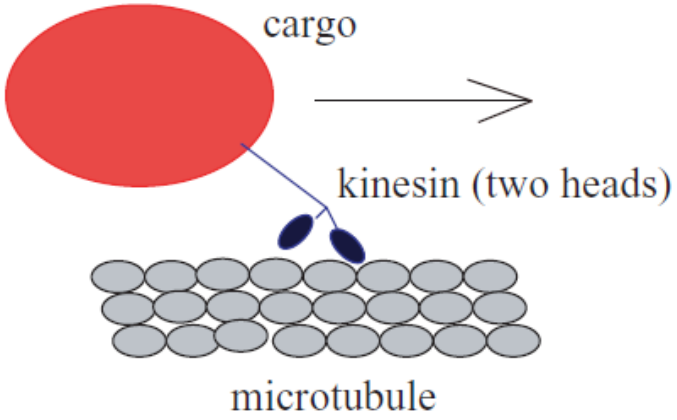
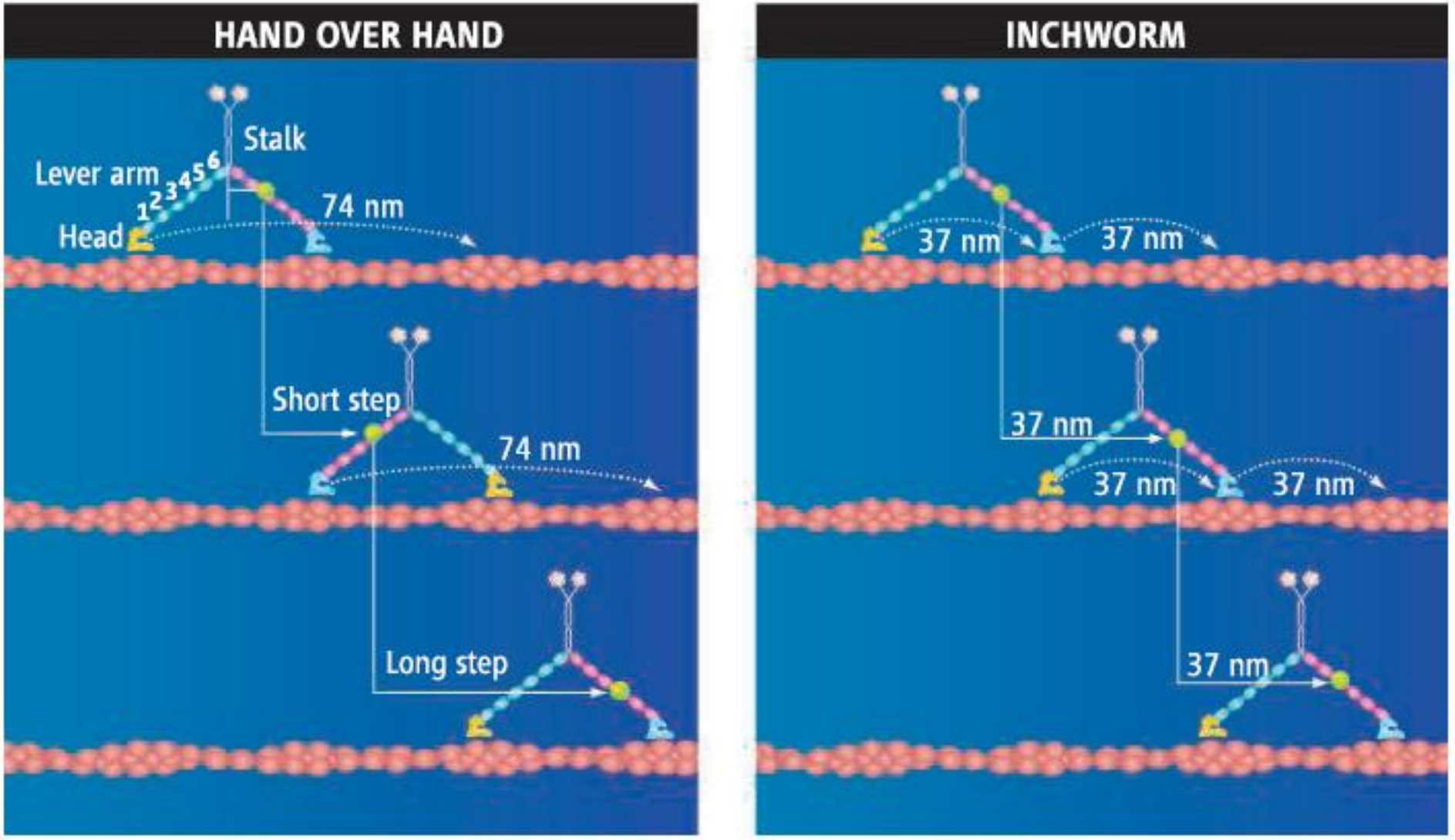


Figure 6.1. Kinesin walking on a microtubule. Kinesin forms dimers, consisting of two globular heads, a stretched stalk about 80 nm long, and a tail. The head and the tail domains contain the microtubule- and the cargo-binding sites, respectively. Microtubules are 25 nm thick and about 5–20 μm long hollow cylindrical fibers that are formed by tubulin dimers. Microtubules are polar; there are kinesins moving from + to −, and there are kinesins designed to move the opposite way. Each tubulin dimer is 8 nm long, and the kinesin moves in steps of 8 nm along the surface of microtubules.



Myosin V: Walking or inchworming? Predicted movement for the heads and a dye molecule label (green dot) on the lever arm in the hand-over-hand model (**left**) and the inchworm model (**right**). The FIONA assay has revealed that myosin V, along with kinesin and myosin VI, walks hand-over-hand.

Mecanismo: *Brownian ratchets*

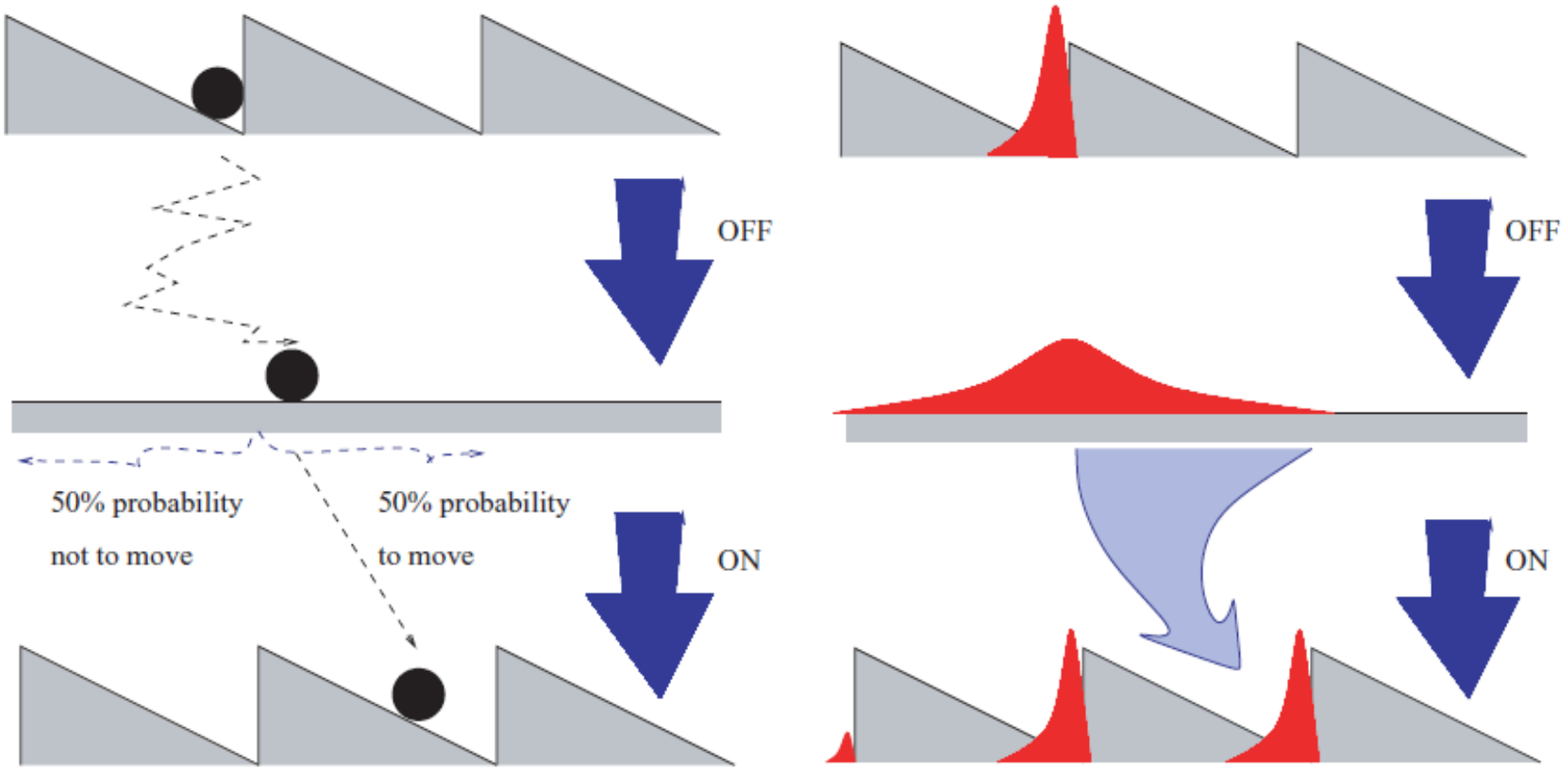


Figure 6.4. Particle in an idealized ratchet: a potential with large left–right asymmetry. When potential is on, the particle moves in a directed way until it is trapped. If potential is off, the particle diffuses freely in both directions. Consider the parti-

Miscelánea: *Paradoja de Parrondo*

Game theory

Losing strategies can win by Parrondo's paradox

In a game of chess, pieces can sometimes be sacrificed in order to win the overall game. Similarly, engineers know that two unstable systems, if combined in the right way, can paradoxically become stable. But can two losing gambling games be set up such that, when they are played one after the other, they becoming winning? The answer is yes. This is a striking new result in game theory called Parrondo's paradox, after its discoverer, Juan Parrondo[1,2]. Here we model this behaviour as a flashing ratchet[3], in which winning results if play alternates randomly between two games.

There are actually many ways to construct such gambling scenarios, the simplest of which uses three biased coins (Fig. 1a). Game A consists of tossing a biased coin (coin 1) that has a probability (p_1) of winning of less than half, so it is a losing game. Let $p_1 = 1/2 - \epsilon$, where ϵ, the bias, can be any small number, say 0.005.

Game B (Fig. 1a) consists of playing with two biased coins. The rule is that we play coin 2 if our capital is a multiple of an integer M and play coin 3 if it is not. The value of M is not important, but for simplicity let us say that $M = 3$. This means that, on average, coin 3 would be played a

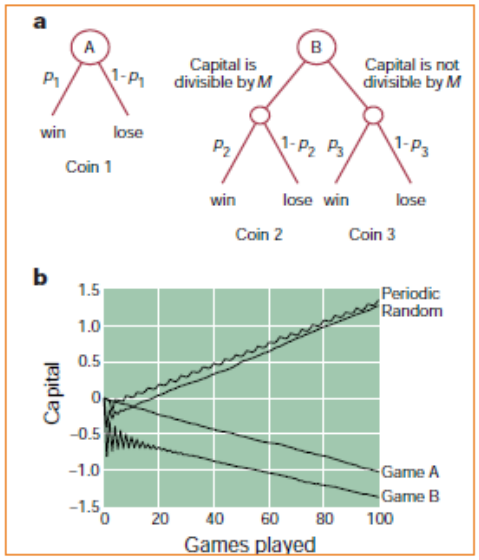


Figure 1 Game rules and simulation. **a,** An example of two games, consisting of only three biased coins, which demonstrate Parrondo's paradox, where p_1, p_2 and p_3 are the probabilities of winning for the individual coins. For game A, if $\epsilon = 0.005$ and $p_1 = 1/2 - \epsilon$, then it is a losing game. For game B, if $p_2 = 1/10 - \epsilon$, $p_3 = 3/4 - \epsilon$ and $M = 3$ then we end up with coin 3 more often than coin 2. But coin 3 has a poor probability of winning, so B is a losing game. The paradox is that playing games A and B in any sequence leads to a win. **b,** The progress of playing games A and B individually and when switching between them. The simulation was performed by playing game A twice and game B twice, and so on, until 100 games were played; this is indicated by the line labelled 'Periodic'. Randomly switched games result in the line labelled 'Random'. The results were averaged from 50,000 trials with $\epsilon = 0.005$.

asymmetrical in a way that favours particles spilling over a higher tooth.

The flat slope is like game A, where the bias ϵ is like the steepness of the slope. Game B is like the sawtooth slope, where the difference between coin 2 and coin 3 is like the asymmetry in the tooth shape. In the brownian ratchet case, there are two types of slope, with falling particles, but when they are switched the particles go uphill. Similarly, two of Parrondo's games have declining capital that increases if the games are switched or alternated. The games can be thought of as being a discrete ratchet and are known collectively as a parrondian ratchet.

Game theory is linked to various disciplines such as economics and social dynamics, so the development of parrondian-like strategies may be useful, for example for modelling cases in which declining birth and death processes combine in a beneficial way.

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2. McClintock, P. V. E. *Nature* **401**, 23–25 (1999).
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Teoría de Juegos en Biología

La Evolución como un juego

Maynard Smith
(1920-2004)

The Logic of Animal Conflict

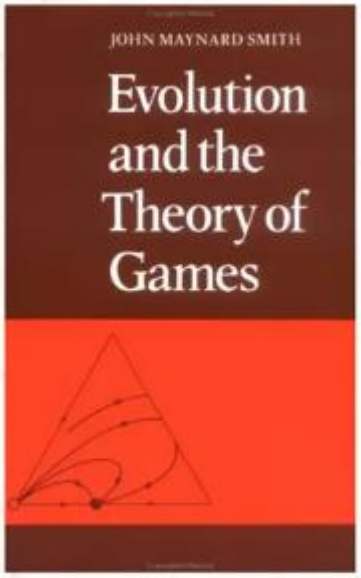
J. MAYNARD SMITH

G. R. PRICE

Conflicts between animals of the same species usually are of "limited war" type, not causing serious injury. This is often explained as due to group or species selection for behaviour beneficial to the species rather than individuals. Game theory and computer simulation analyses show, however, that a "limited war" strategy benefits individual animals as well as the species.

A Computer Model

Nature, 1973



	TJ clásica	TJ evolutiva
jugadores	racionales	irracionales
estrategias	elegibles	heredadas (fenotipos)
interacción	todos a la vez	muestreo aleatorio de una población
resultados	utilidad	capacidad reproductiva *(fitness)*

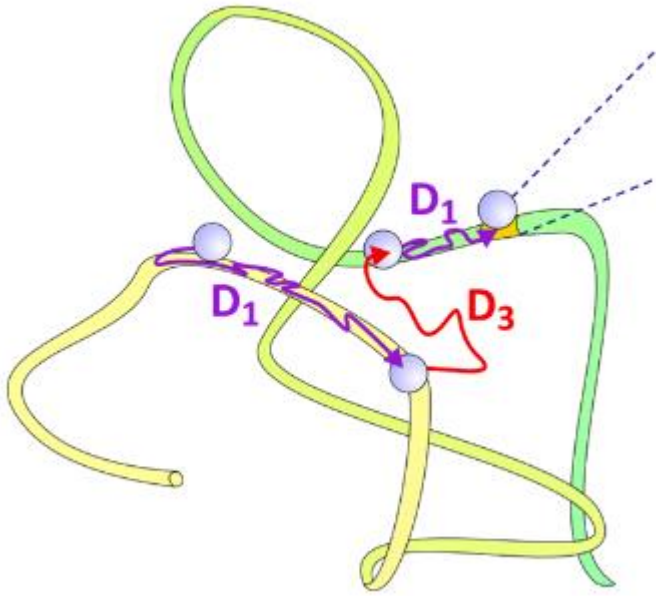
d) Búsquedas aleatorias

- Unión de proteínas reguladoras al DNA

Riggs et al. (1970) mostraron que el represor Lac de *E. coli* encuentra su sitio de unión aproximadamente cien veces más rápido de lo esperado por la difusión 3D.

Modelo con alternancia de
difusión 3D – 1D
(Berg y von Hippel, 1981),

+ dos conformaciones de
la proteína.



4. Nudos del DNA

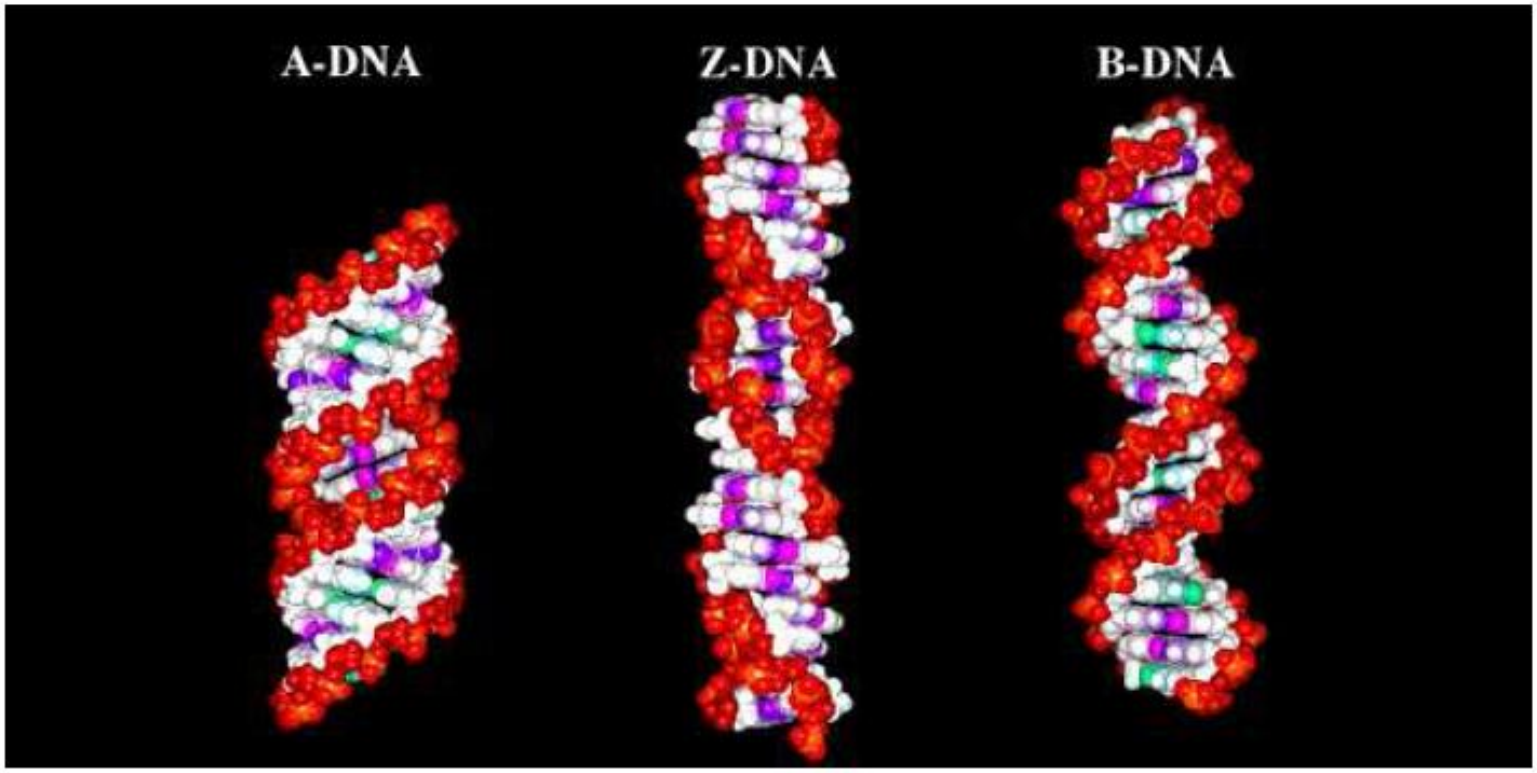
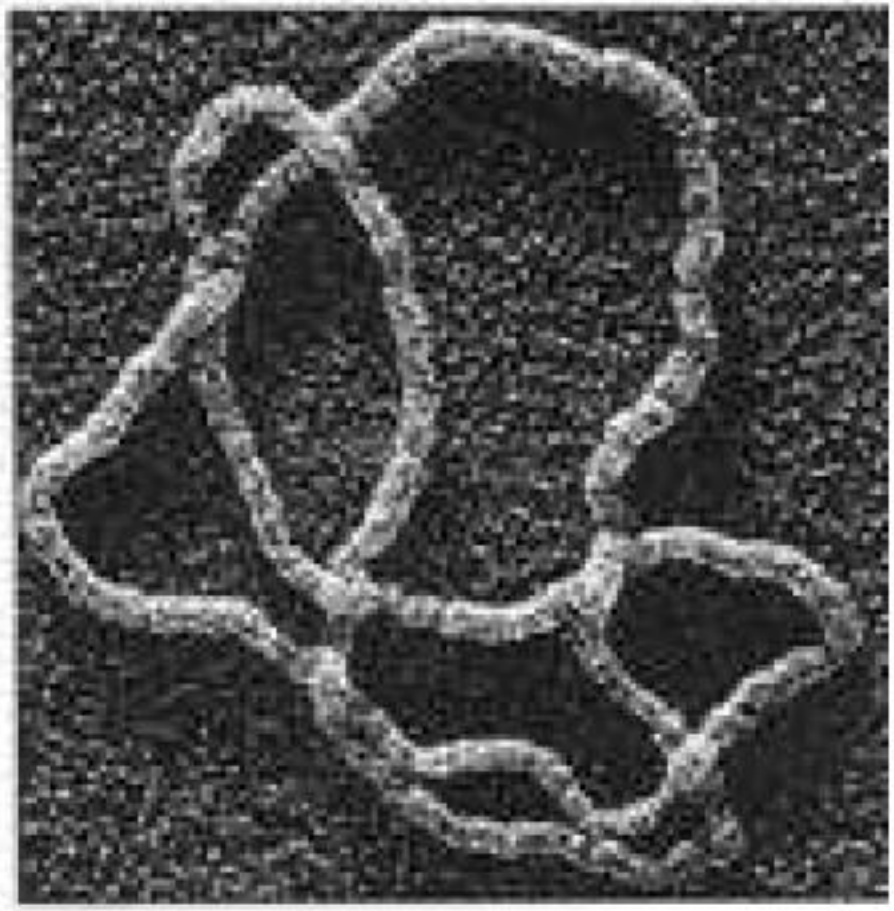
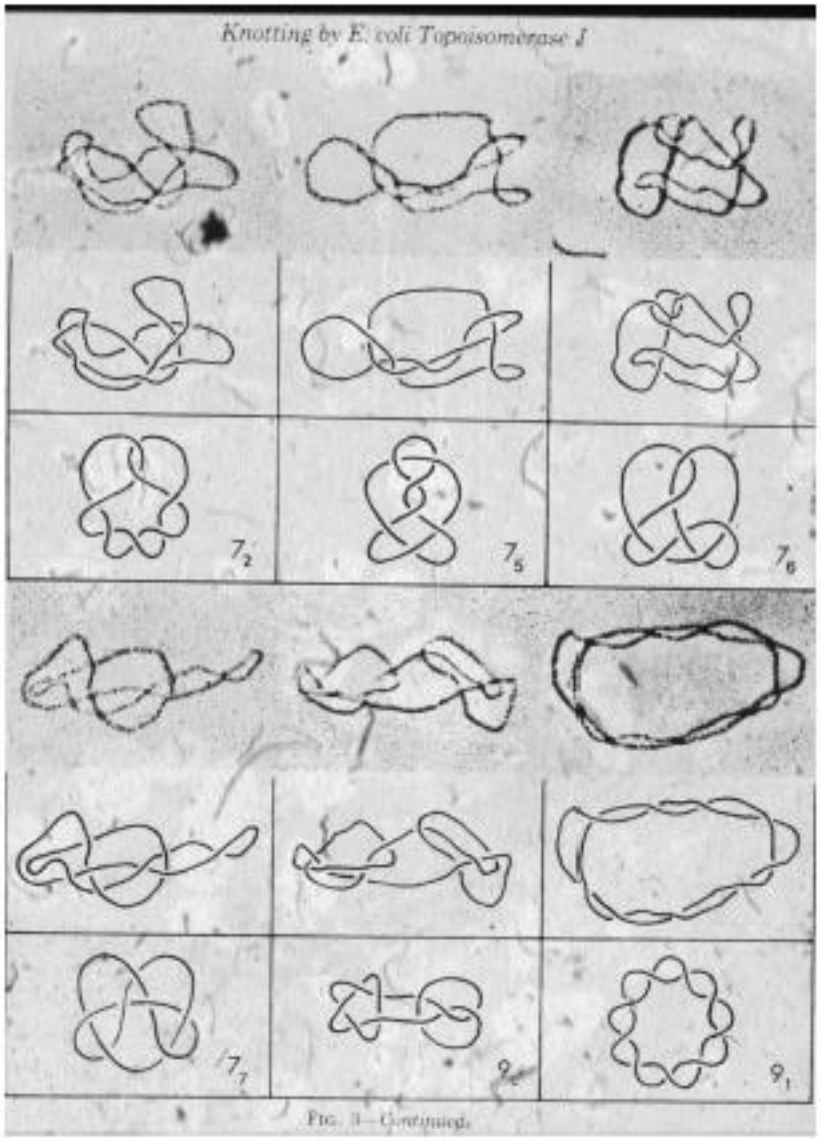


Figure 6 The three DNA configurations labelled A, B and Z. A-DNA occurs as a dehydrated form of DNA, the A-form is also the preferred conformation of RNA. Z-DNA can occur at stretches of alternating G and C bases and may be important for controlling replication. By far the most important physiological conformation is B-DNA (1; 6–8; 58).



re 1 Left: DNA knot. Electron microscope image



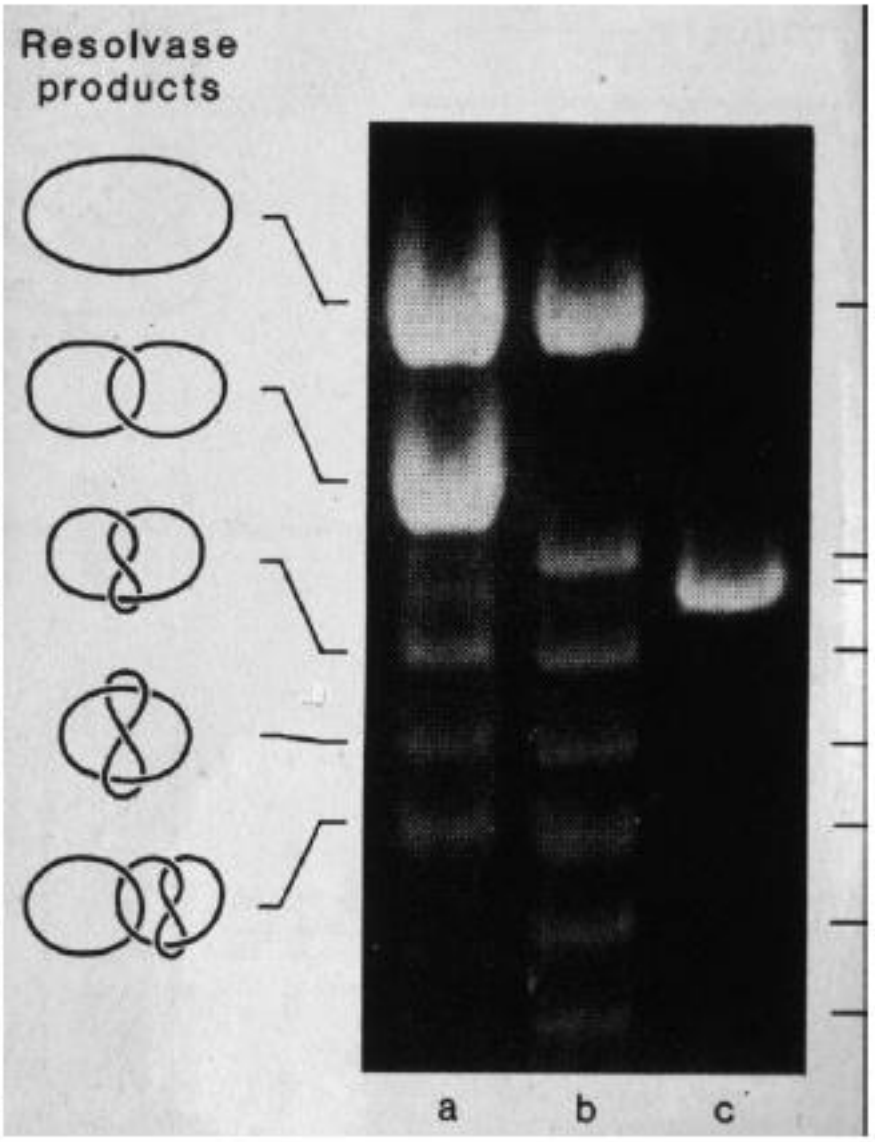


Figure 18 Various DNA knots created by topoisomerase I from a nicked ring DNA of unknot topology (11). Right: Different mobility of various DNA knots in gel electrophoresis. The more complex topologies are faster (12).

Topoisómeros

The gel demonstrates that it is possible to separate genetically identical DNA molecules that differ in their topologies.

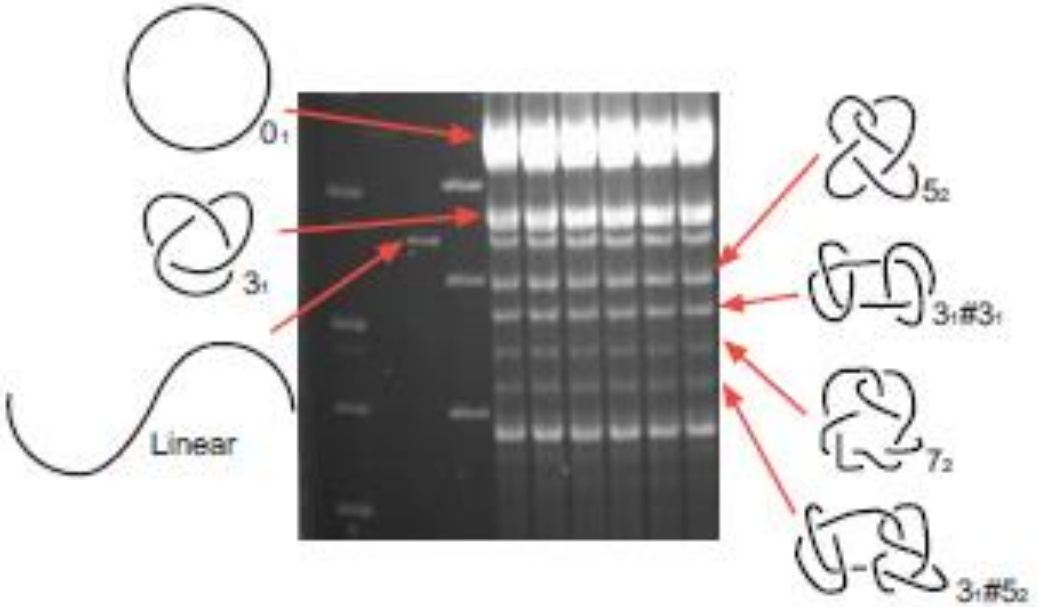


Figure 1.13. Gel Electrophoretic Separation of DNA Knots. High resolution agarose gel displaying topoisomers of a 5.4 kb plasmid. (Nicked monomer is topologically equivalent to the unknot 0_1.)

Teoría de nudos

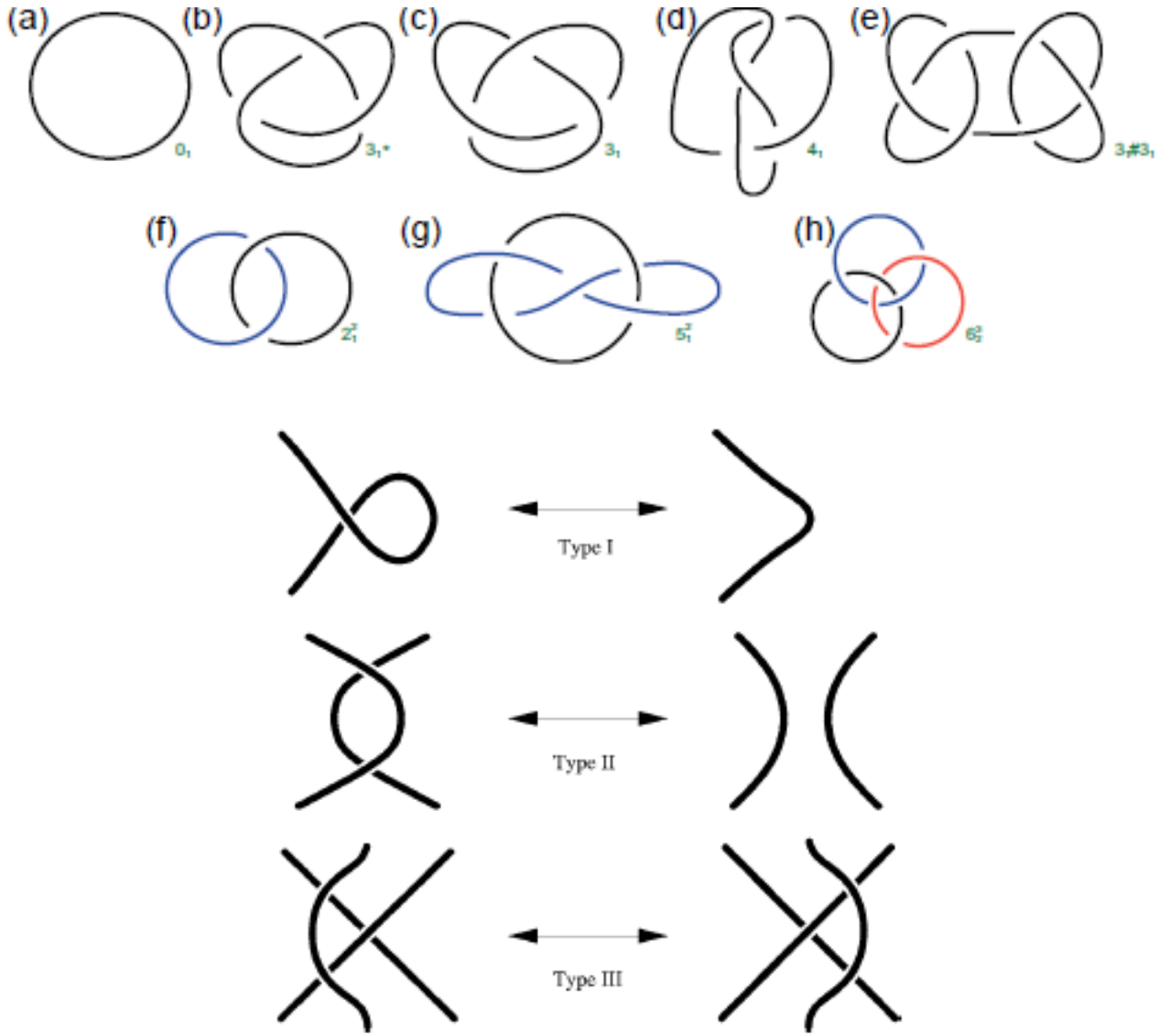


FIG. 2. The three Reidemeister moves.

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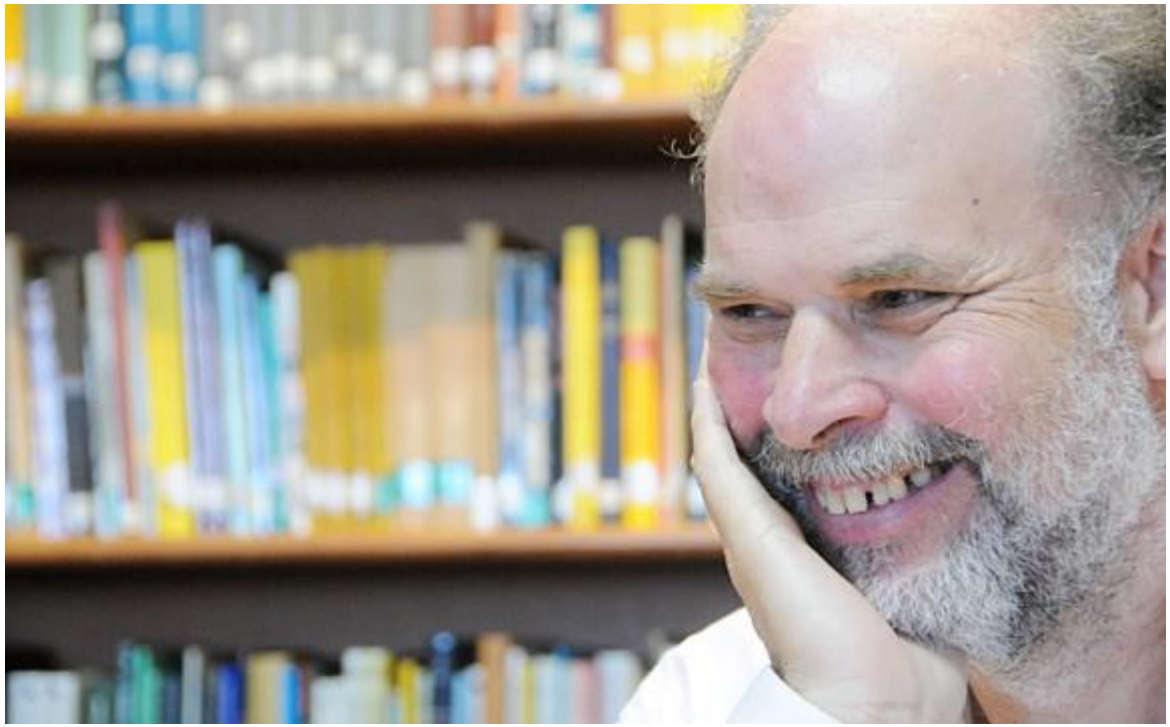

Búsqueda de invariantes de nudos. Polinomios

Más máquinas moleculares: las Topoisomerasas

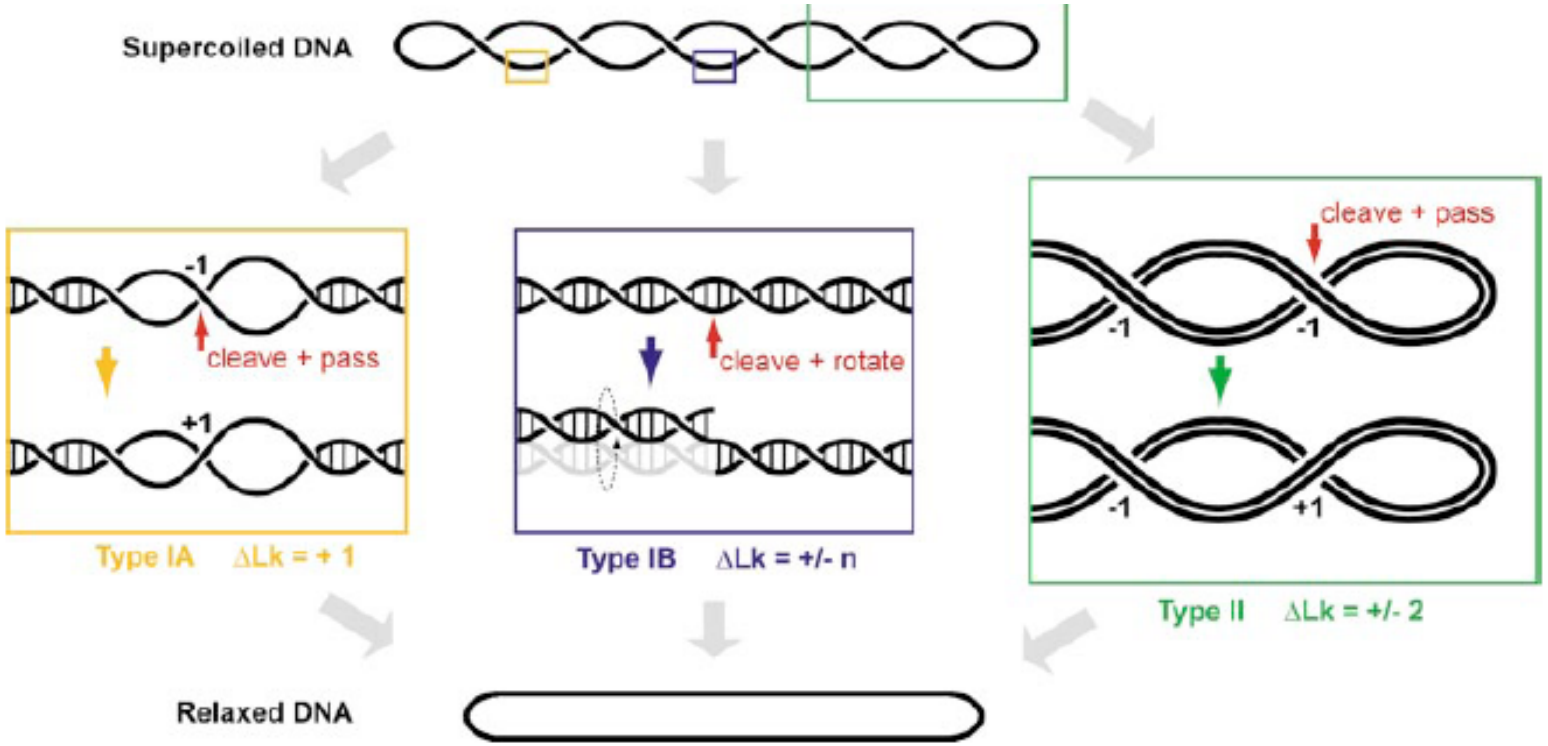

Figure 1 Activities of topoisomerases. The superhelicity of DNA can be altered by topoisomerases through three different mechanisms. Type IA topos (*yellow box*, *left*) pass one strand of a melted duplex DNA through the second strand, increasing DNA's linking number (Lk) in steps of +1. Type IB topos (*blue box*, *center*) cleave one strand of a duplex and allow rotation of the duplex around the remaining strand, changing the linking number by either a positive or negative integral number of turns. Type II topos (*green box*, *right*) pass one duplex DNA through another duplex, changing the linking number in steps of +/–2.

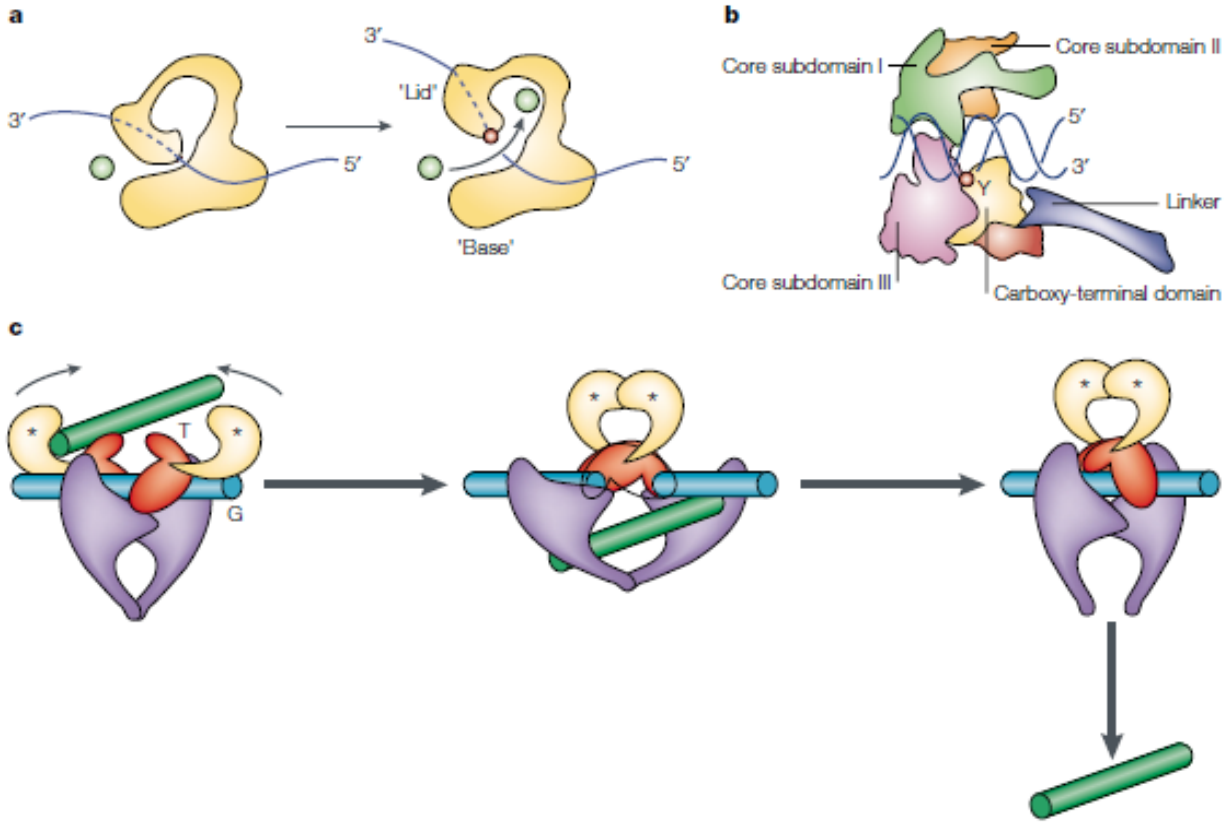

Figure 2 | **Molecular models for the passage of one DNA strand or double helix through another by different subfamilies of DNA topoisomerases. a** | Type IA topoisomerases. On transient breakage of a DNA strand (blue line), the 5′ end of the broken DNA strand is covalently attached to the active-site tyrosyl group (red circle) in the 'lid' of the enzyme, and the 3′ end is noncovalently bound to the 'base' of the enzyme. Lifting the lid away from the base opens a gate in the DNA for the passage of another strand (green circle). The location of the second strand, either before or after its passage through the DNA gate, is largely unknown. Once the second strand has entered the central cavity of the enzyme, it must exit the cavity, after the rejoining of the broken strand, without passing through the rejoined DNA strand[7,113]. **b** | Type IB enzymes. The covalent intermediate between a 22-base-pair DNA fragment and a type IB DNA topoisomerase is shown. The 3′ end of the broken DNA scissile strand is covalently linked to the active-site tyrosyl group (Y) of the enzyme (red circle). For clarity, a portion of the enzyme is sectioned off to reveal the entire DNA fragment. The enzyme-generated nick divides the DNA fragment into two segments: the DNA segment to the left of the nick is tightly held by the enzyme, but interaction between the enzyme and the DNA segment to the right of the nick is mostly ionic, so it permits rotation of the DNA segment to the right of the nick relative to the protein. The illustration is based on the crystal structures of several complexes that are formed between DNA and human DNA topoisomerase I (REF. 3). This DNA-rotation mechanism allows multiple strand-passage events for each strand breakage–rejoining cycle[114]. **c** | Type IIA enzymes. The protein structure shown is based on structures of the ATPase domain of *E. coli* GyrB protein[115] and a fragment of yeast DNA topoisomerase II containing the domains that are required for DNA breakage and rejoining[12]. The G-segment — the double-stranded DNA segment that contains the enzyme-mediated DNA gate — is depicted as a blue rod. The DNA T-segment being passed through the G-segment is depicted as a green rod. The asterisks represent the ATP-binding sites. Reproduced with permission from *Nature*[12] © (1996) Macmillan Magazines Ltd. See REFS 9 and 12 for further details.

- Sigue siendo una interrogante el rol de los nudos en el ADN, en la dinámica de la macromolécula, y su importancia en la lectura de la información genética.

Fin