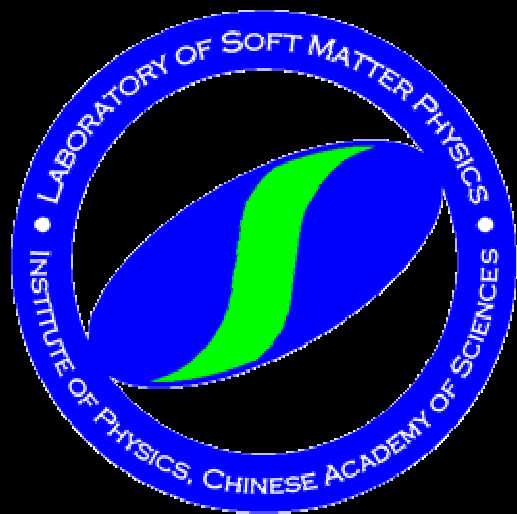


利用单分子和荧光方法研究DNA和蛋白质



王鹏业

中科院物理所，软物质物理实验室

网址：<http://sm.iphy.ac.cn>

物理学与分子生物学之总论

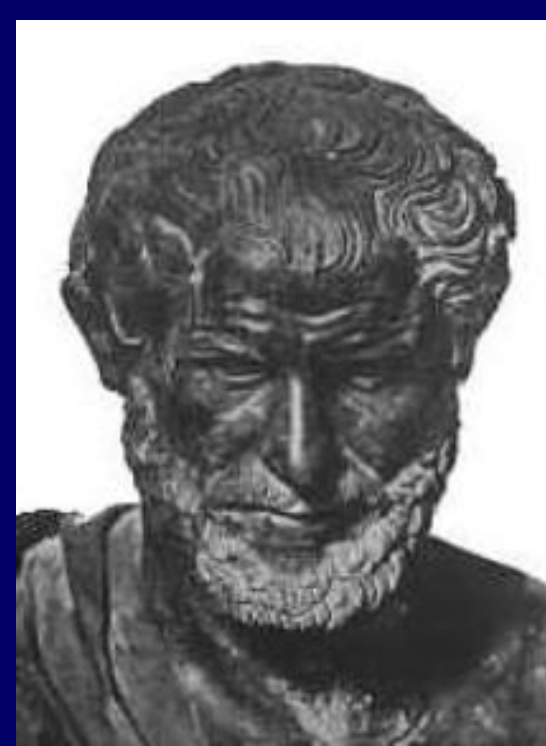
科学研究的还原论 (reductionism)方法:

还原论方法主张把研究对象分解成部分，把每个部分都研究清楚了，整体也就清楚了。这一思想从古希腊时期就存在了。是西方科学发展的主线。

举例来说，按照还原论方法，当今物理学对物质结构的研究已到了夸克层次。

天文学中也是如此向下分解：宇宙
→星系→天体→物质→能量。

无疑还原论方法在科学研究中取得了巨大的成功。



亚里士多德
(公元前384年—前322年)

开创了:

逻辑学

伦理学

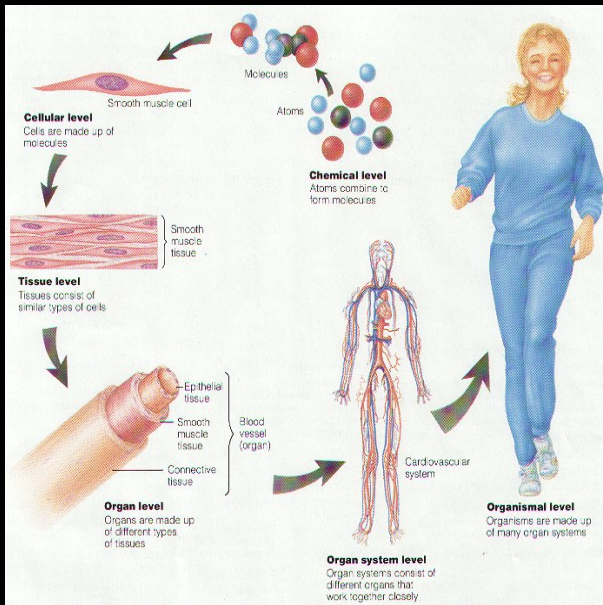
政治学

生物学

等学科的独立研究

还原论方法在生物学研究中同样取得了巨大的成功:

系统 (神经、吸吸、循环、消化、生殖.....)



组织 (神经、骨骼、结缔、肌肉.....)

器官 (脑、心、肺、肝、胃、肠.....)

细胞 (线粒体、核、质、壁、膜.....)

生物大分子 (DNA、RNA、蛋白质、肽.....)

小分子 (水、离子、氧、二氧化碳.....)



从物理学的视点上看生物大分子也已有较长的历史，薛定谔所著的 **《生命是什么？》** (**What Is Life?**) 的出版 (1944年) 是一个代表，这本书深深地影响了一批物理学家和生物学家。

对生命体引进了非周期性晶体、负熵、遗传密码、量子跃迁式的突变等概念。

薛定谔 (量子力学的创始人之一，1933年获得诺贝尔物理奖)

他的观点引导人们用物理学、化学方法去研究生命的本性，也使他成了今天蓬勃发展的分子生物学的先驱

伽莫夫（物理学家）

不但在宇宙学上最先提出了“大爆炸”宇宙模型，在生物学上他深刻分析了“遗传密码”（1954年），成为遗传密码研究的奠基人之一。

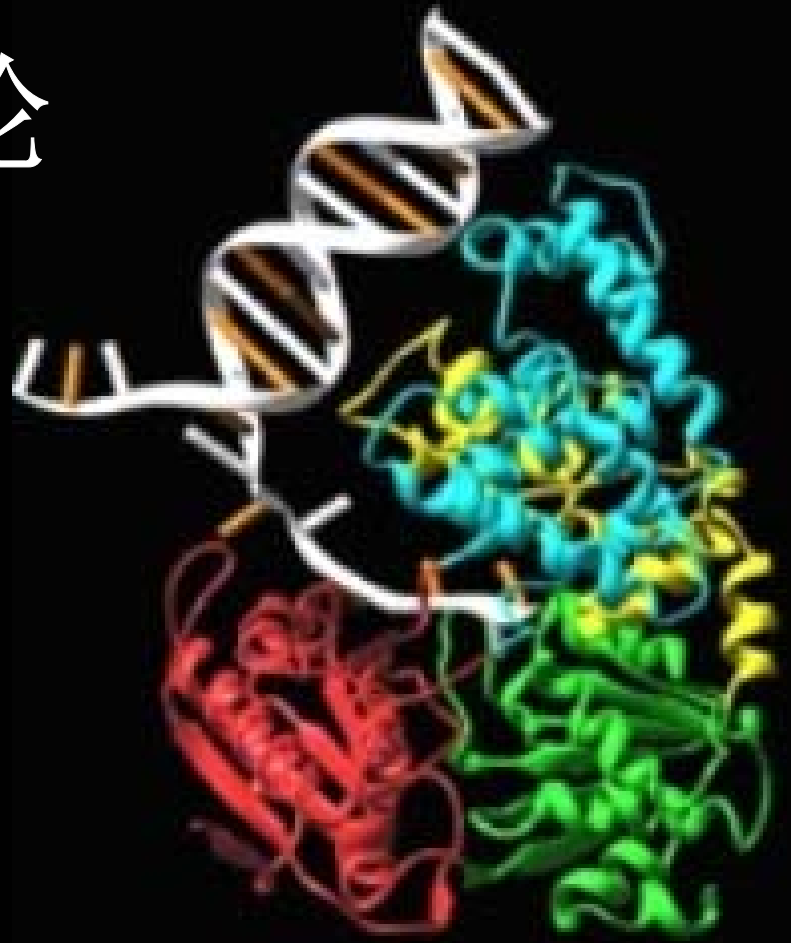
有关的趣事，建议大家有时间读读沃森写的
《基因·女郎·伽莫夫》
(Genes, Girls and Gamow) (3G)。



尽管生命科学的研究近来突飞猛进，其中仍存在许许多多未解之谜。因此，从物理学的视点上看生物大分子，运用物理学的概念和方法（例如，通过先进的技术手段获得更精确的实验数据和应用更准确定量模型来描述生物规律）研究生物物质及生命规律，将大有作为。

DNA 与蛋白质概论

知道，
去大麻花！



DNA : 遗传信息的携带者，基因的载体

蛋白质 : 生命体中的结构和功能材料

我们研究工作的空间尺度

Relative Sizes and Detection Devices

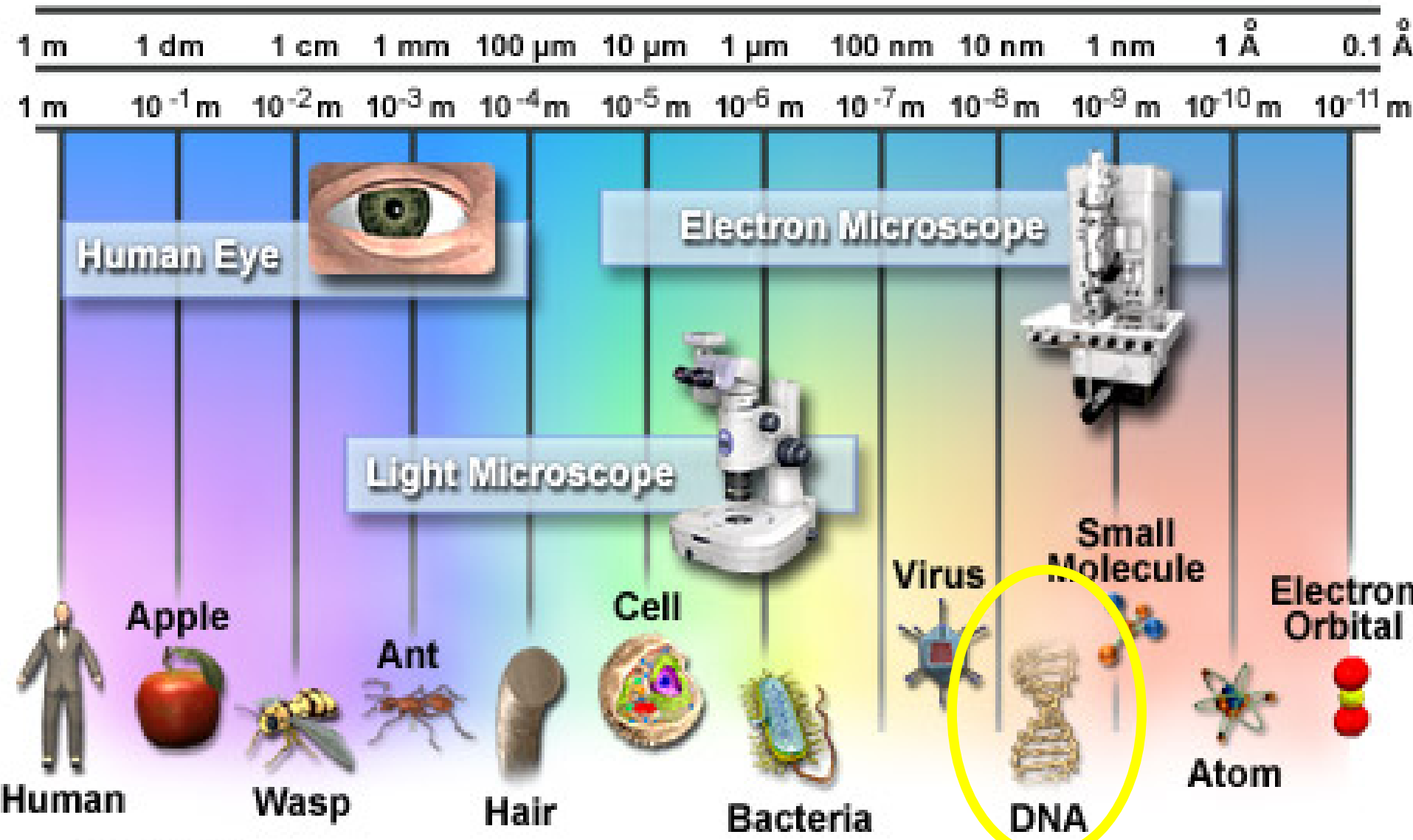
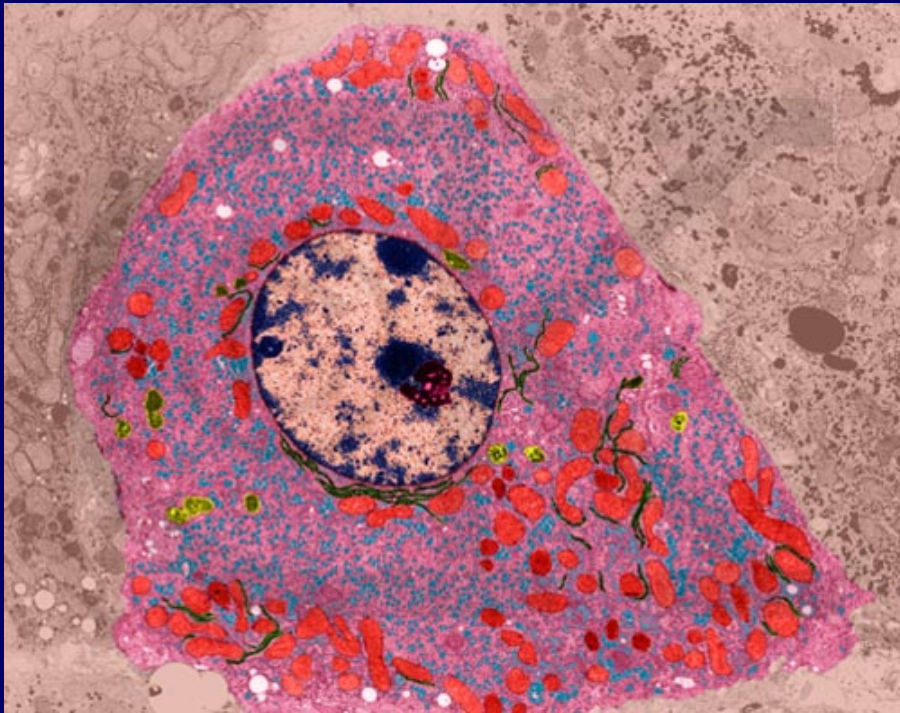
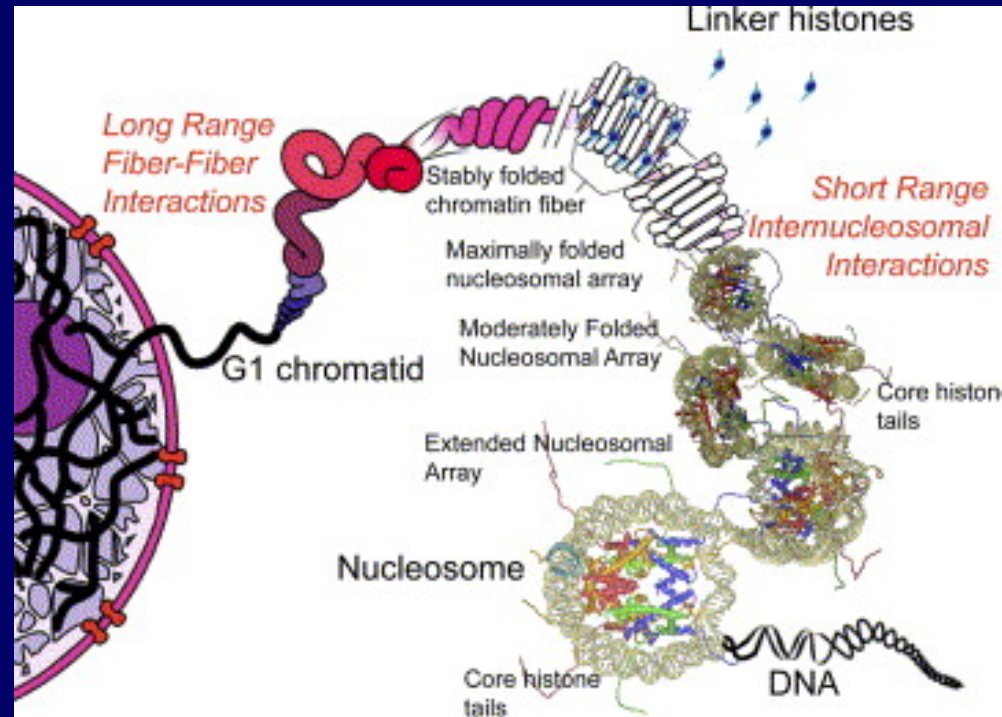


Figure 1

细胞结构

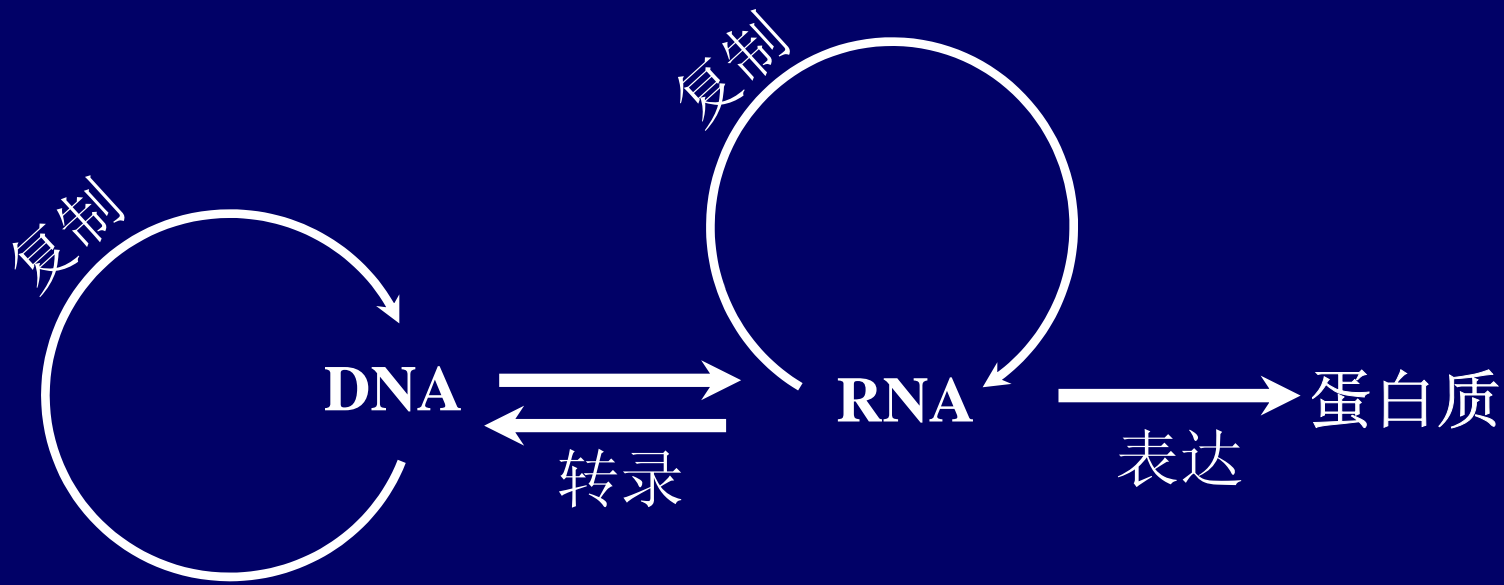


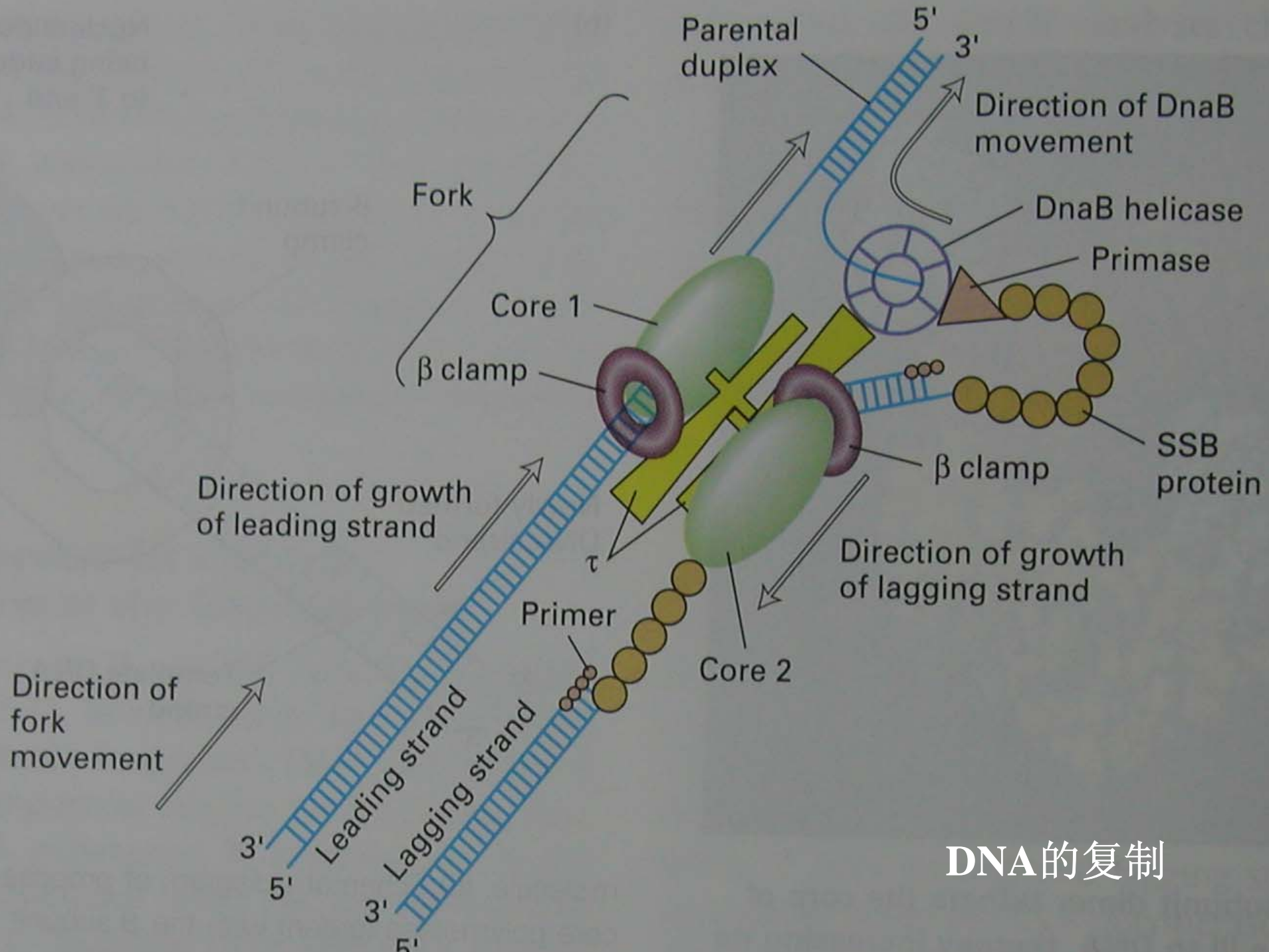
Liver cell 电镜照片 (放大约1万倍)



从细胞核到 DNA (未按比例)

从DNA到蛋白质 – “中心法则”（由 Crick 提出）： （基因的表达）





DNA的复制

DNA单分子荧光、组蛋白、分子马达

荧光法观察DNA分子

DNA特异染料:

从左到右:

POPO-1

BOBO-1

→ **YOYO-1**

TOTO-1

JOJO-1

POPO-3

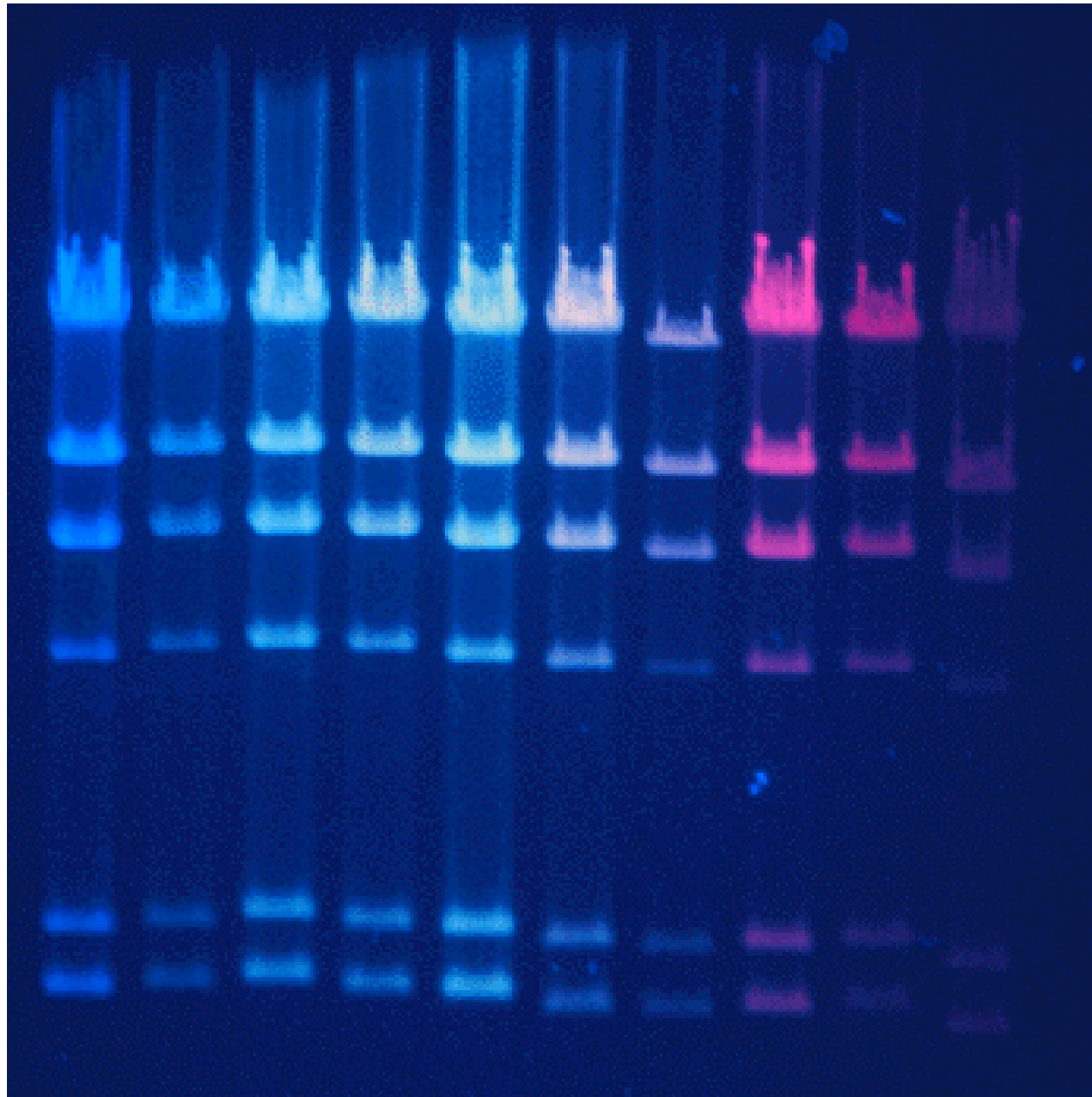
LOLO-1

BOBO-3

YOYO-3

TOTO-3

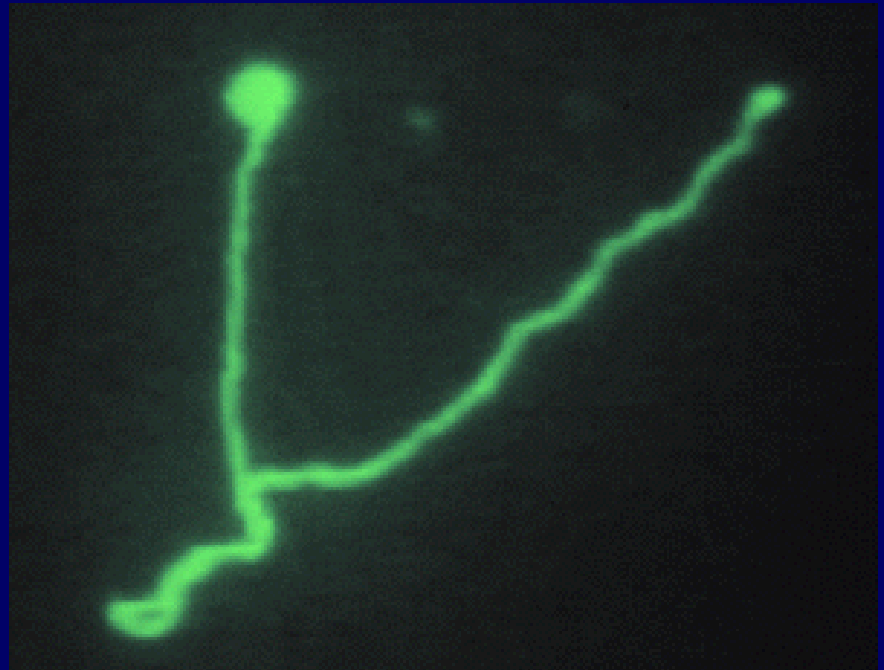
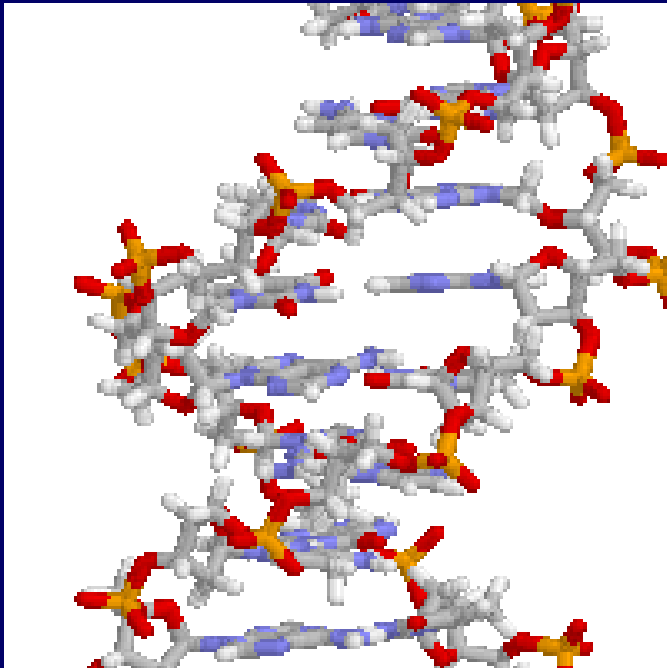
由紫外灯激发

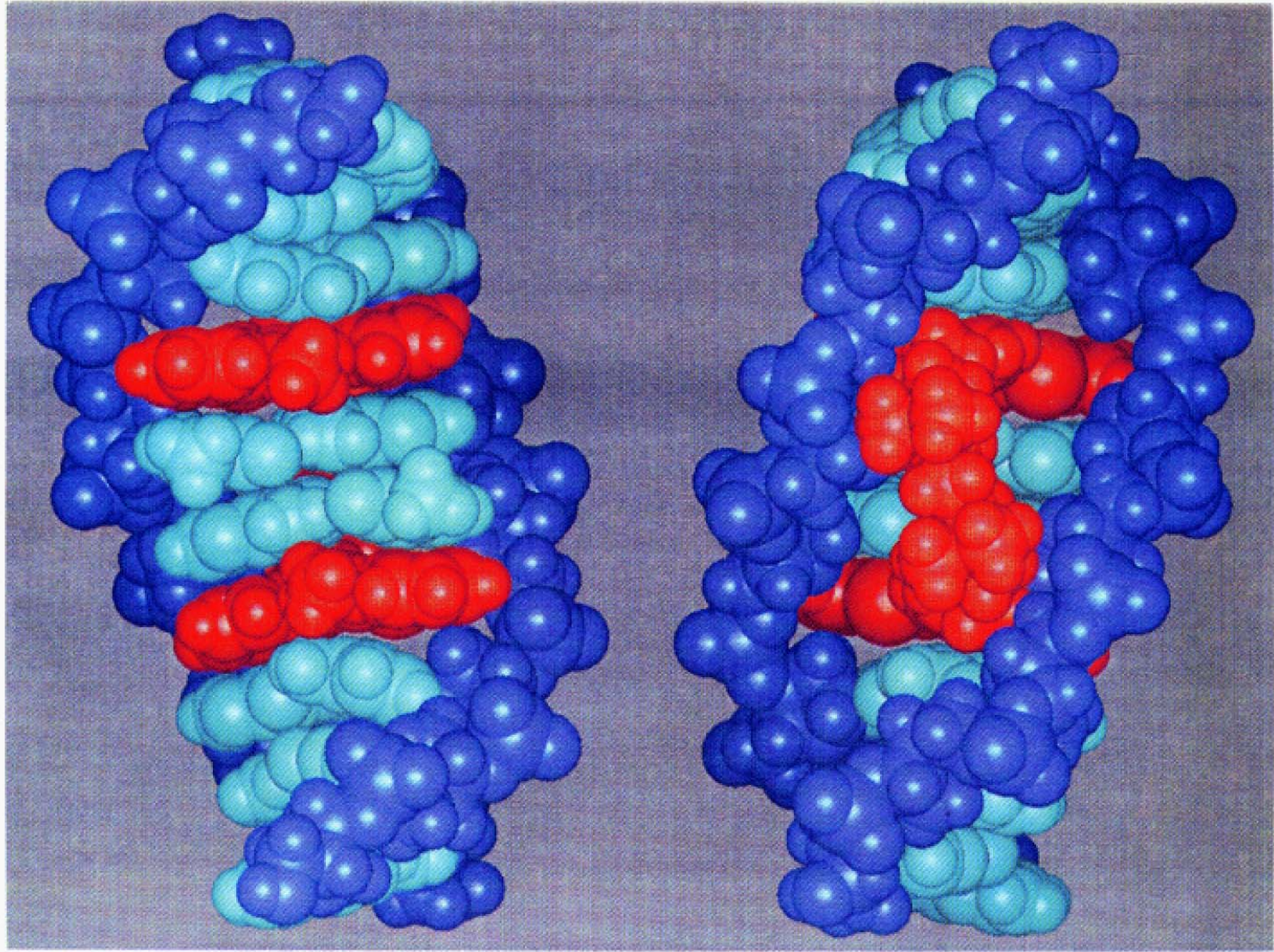


单个 DNA 分子的荧光观察

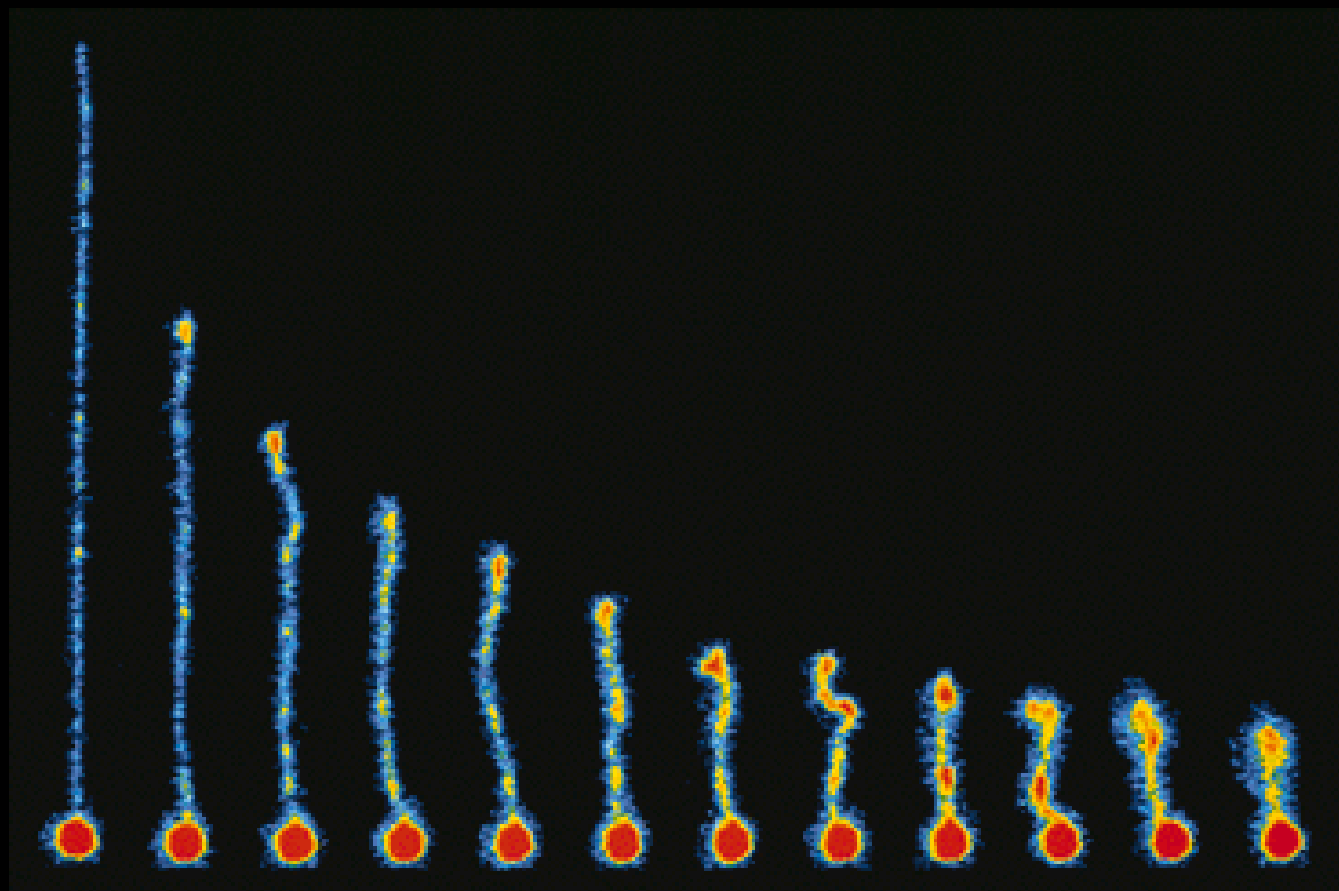
DNA 的特异染料 + 高灵敏 CCD

[用单分子荧光显微方法观测, (e.g. λ -phage DNA, $L=16.4 \mu\text{m}$)]





荧光法观察单个DNA分子

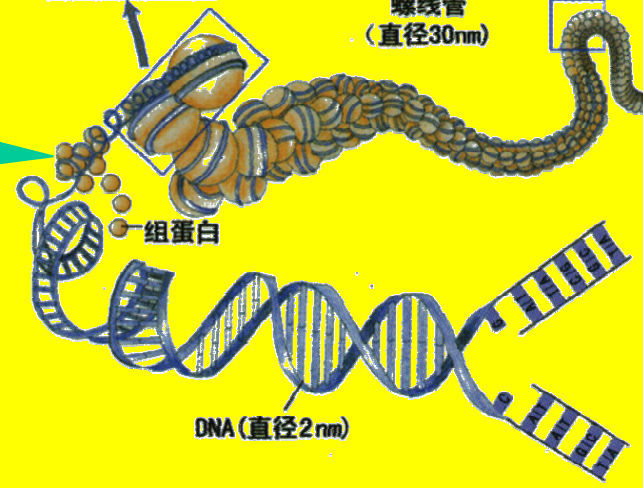
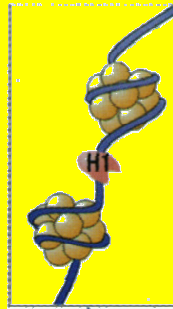
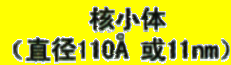
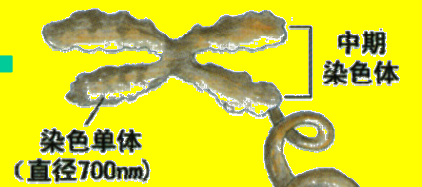
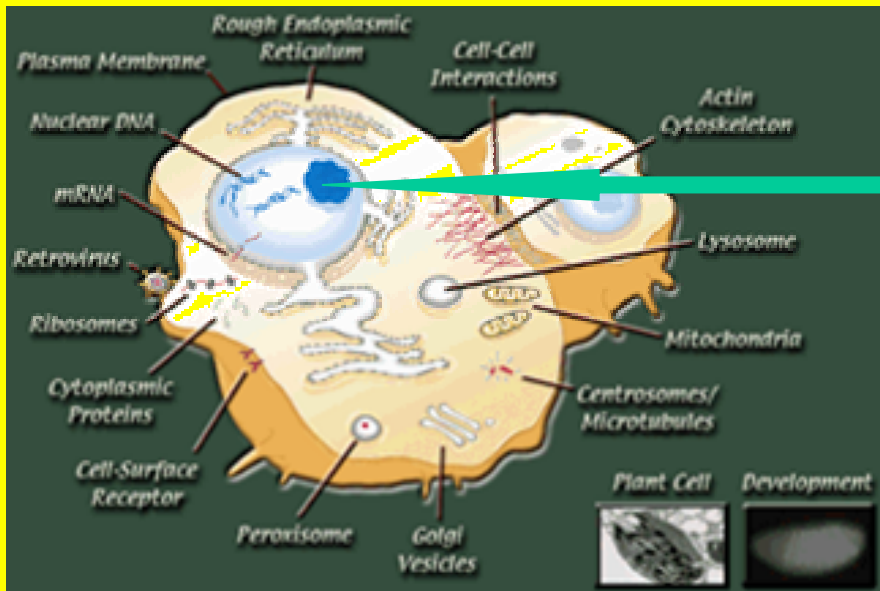


液体流动方向

Steven Chu (朱棣文, 1997年诺贝尔物理奖)

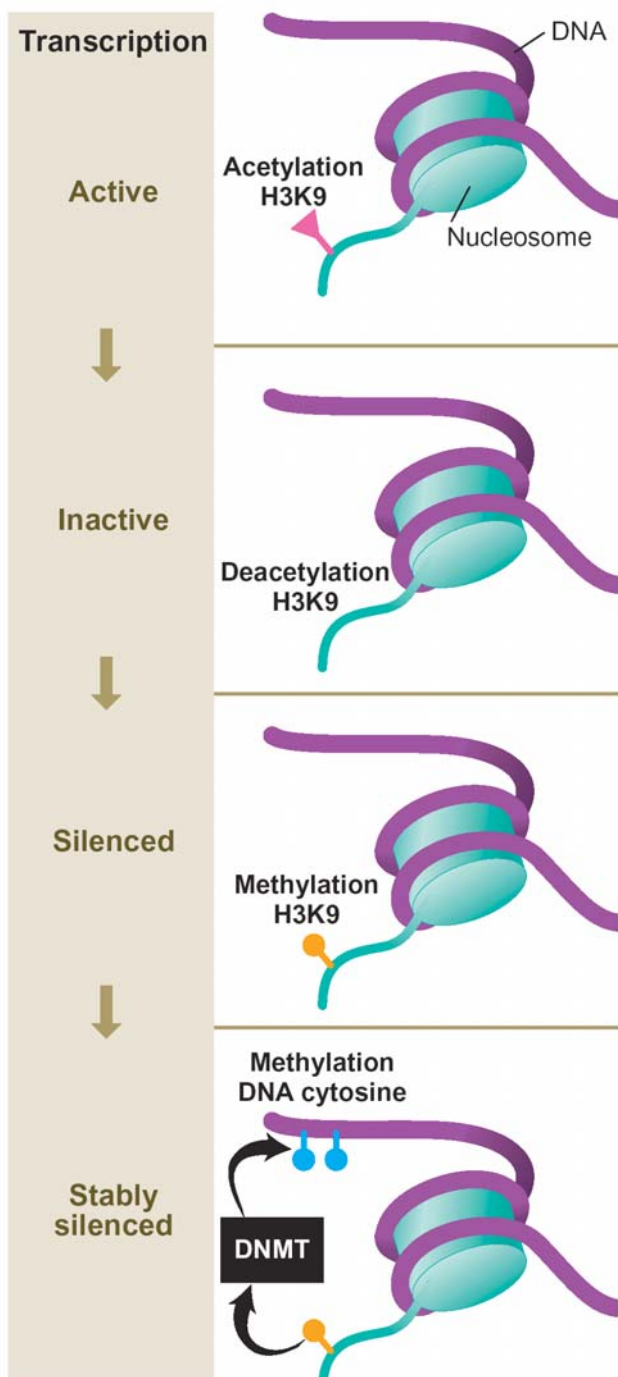
研究组的工作, 1994

流动池中, 时间间隔: 4.5s, DNA长度=39微米



组蛋白:

用于对 DNA 的压缩
(真核生物, 例如人)



组蛋白可以被修饰
用来控制基因表达

生物分子马达

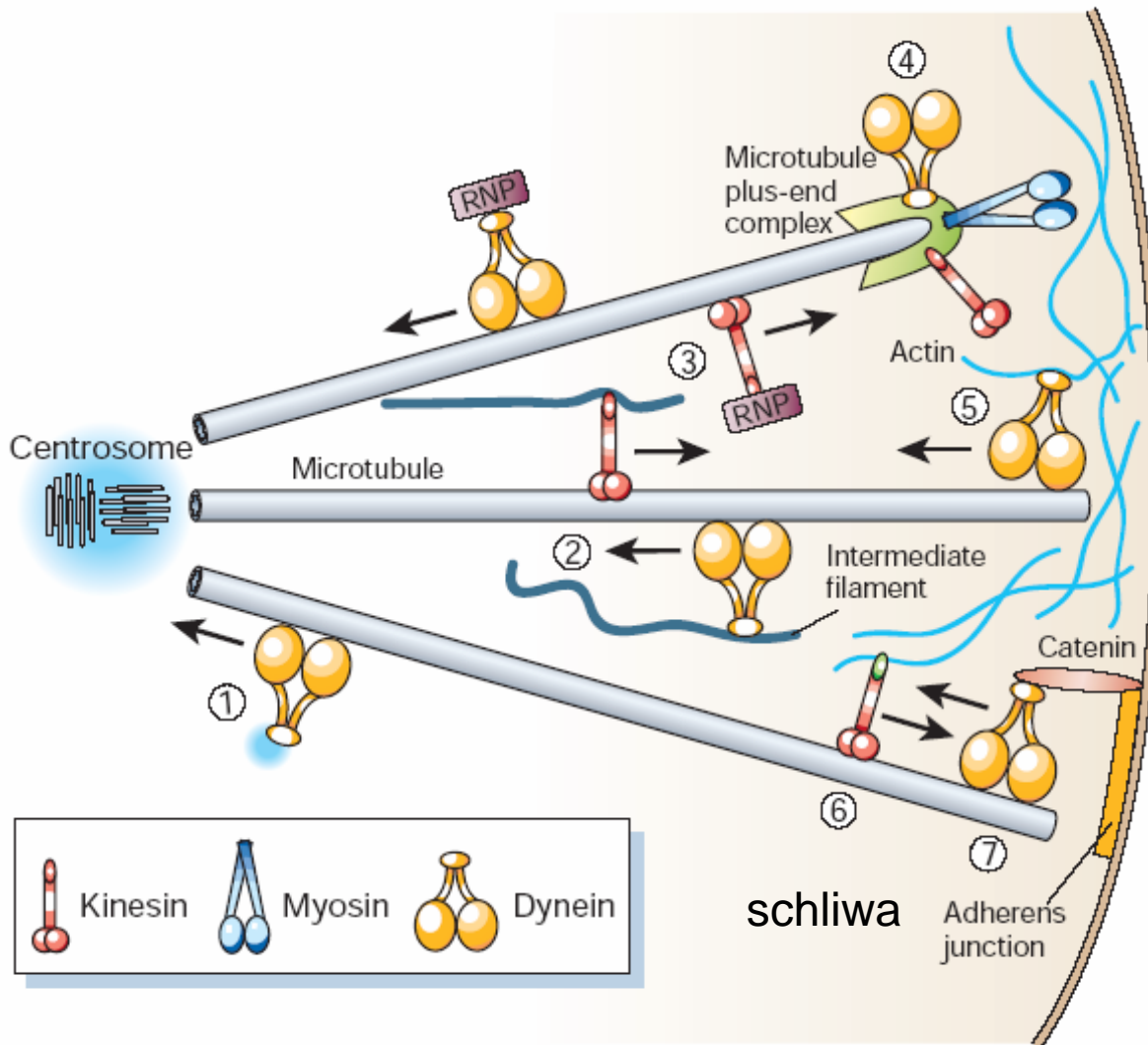
能够将化学能转变为机械力和机械运动的大分子

化学能来自于水解**ATP** (三磷酸腺苷)

ATP是通用的细胞燃料、

ATP → **ADP** + **Pi** + 能量

Transportation in cells



Motor Type

Track

Cytoskeletal motors (细胞骨架马达)

Myosin (肌球蛋白)

Kinesin (驱动蛋白)

Dynein (动力蛋白)

Actin (肌动蛋白丝)

Microtubule (微管蛋白)

Microtubule (微管蛋白)

Rotary motors (旋转马达)

F1 ATPase (F1三磷酸腺苷酶)

Bacterial flagellar (细菌鞭毛)

Nucleic acid motors (核酸马达)

Polymerase (聚合酶)

Helicase (解旋酶)

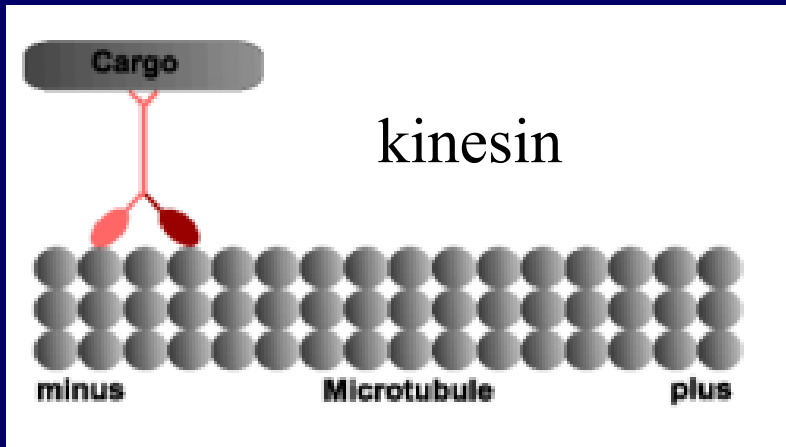
Terminase (末端酶)

DNA/RNA

DNA/RNA

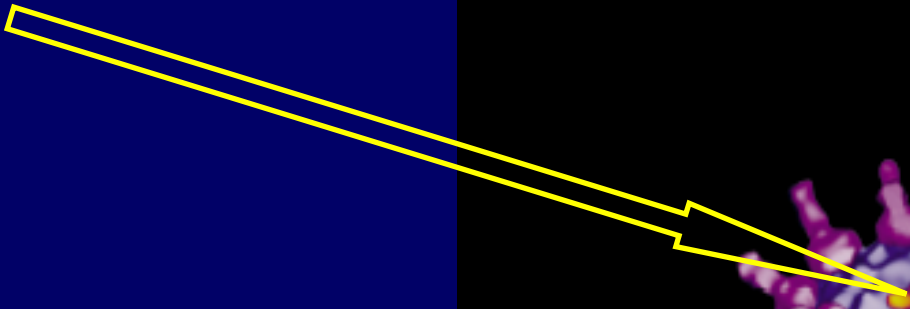
DNA

分子马达的一种运动形式：
交臂(hand-over-hand)模式



Φ29噬菌体:

病毒用来包装DNA
的马达



18 October 2001

International weekly journal of science

nature

£10.00

www.nature.com

Viral DNA packing unravelled

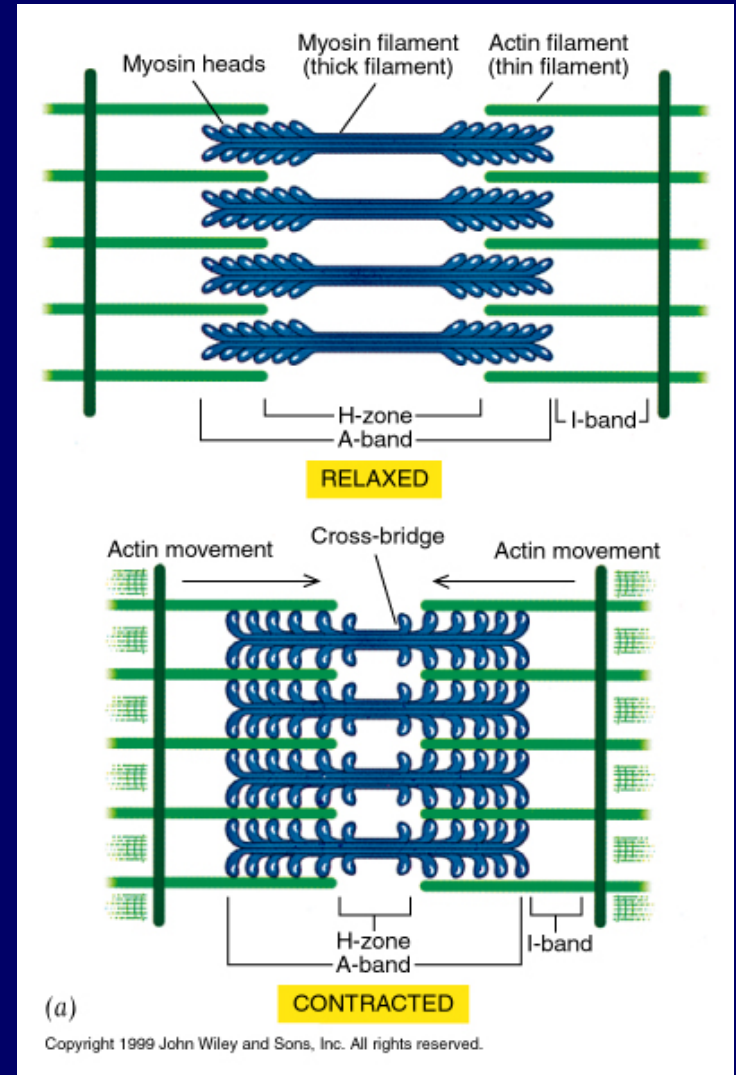
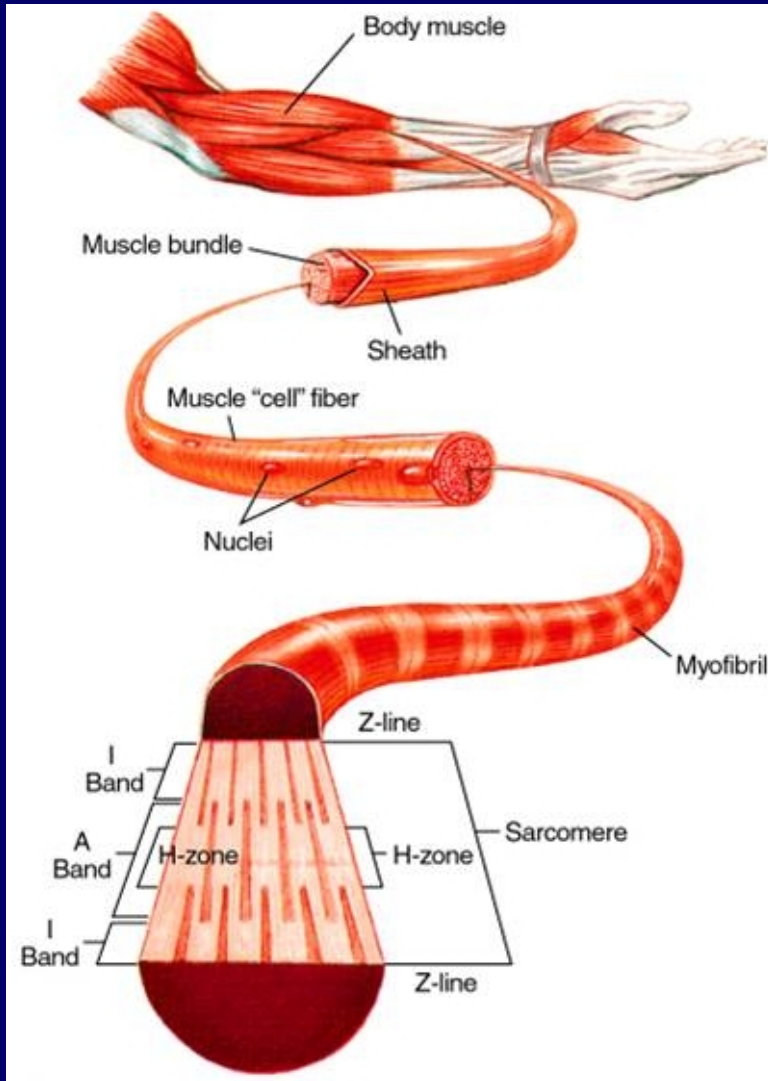
Polymerized C₆₀ A room-temperature ferromagnet

RNA splicing Catalysis by spliceosomal snRNAs

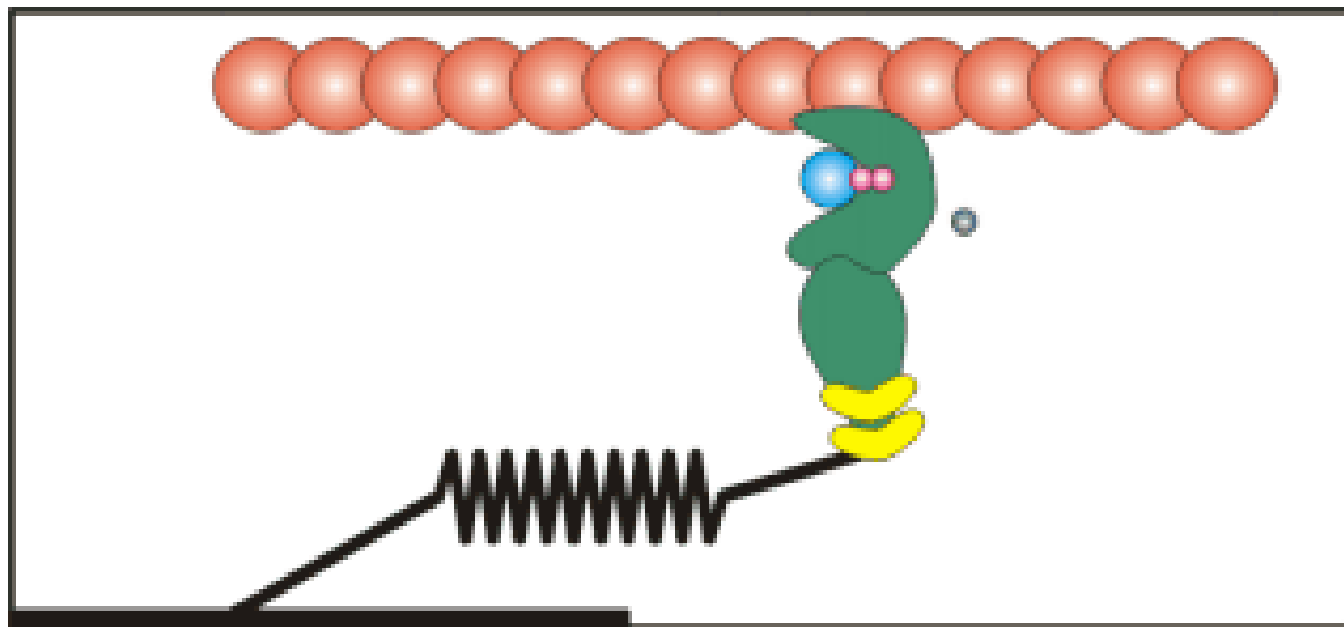
Herbig-Haro objects The X-ray perspective

new journals

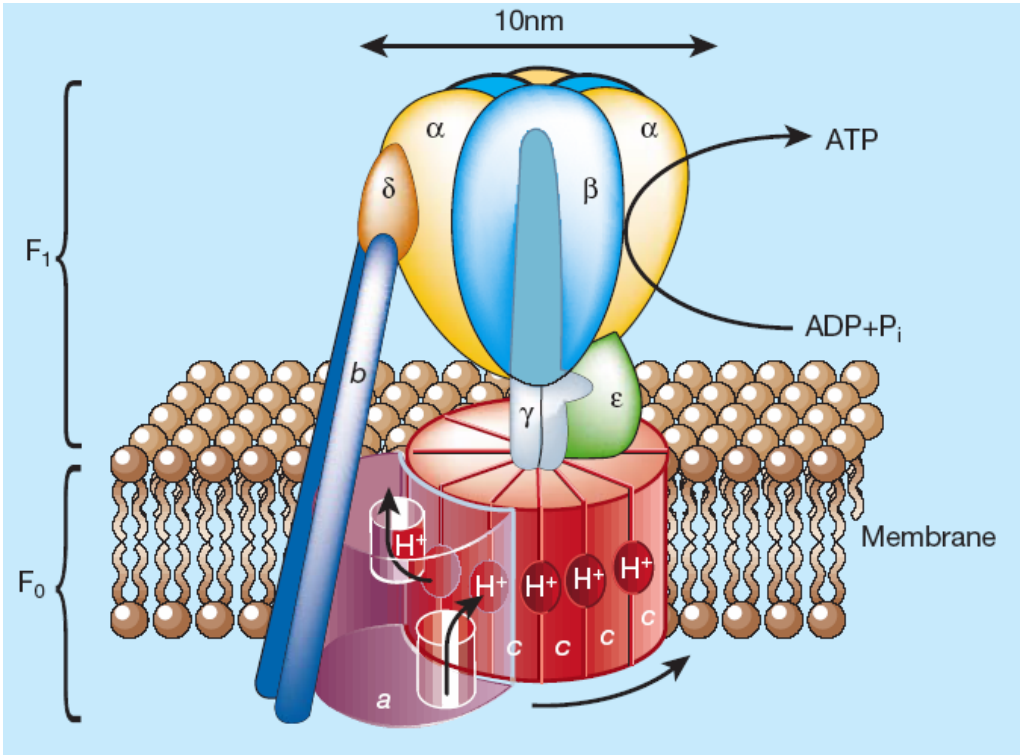
肌肉伸缩用的马达蛋白 myosin (肌球蛋白)



单个肌球蛋白的运动模型



F0-F1 ATPase (三磷酸腺苷酶)

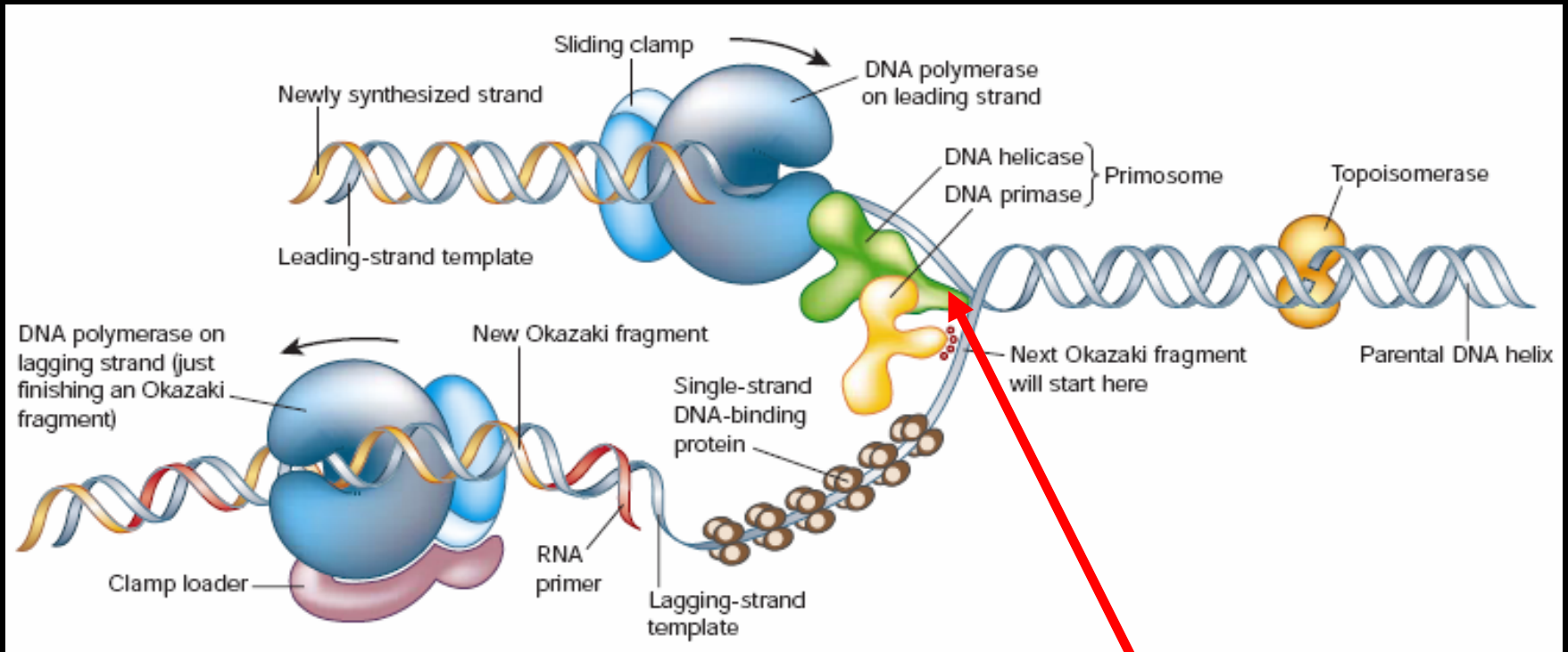


是一个能量转换器

利用离子梯度制造ATP (燃料)

或者反过来通过水解ATP泵离子

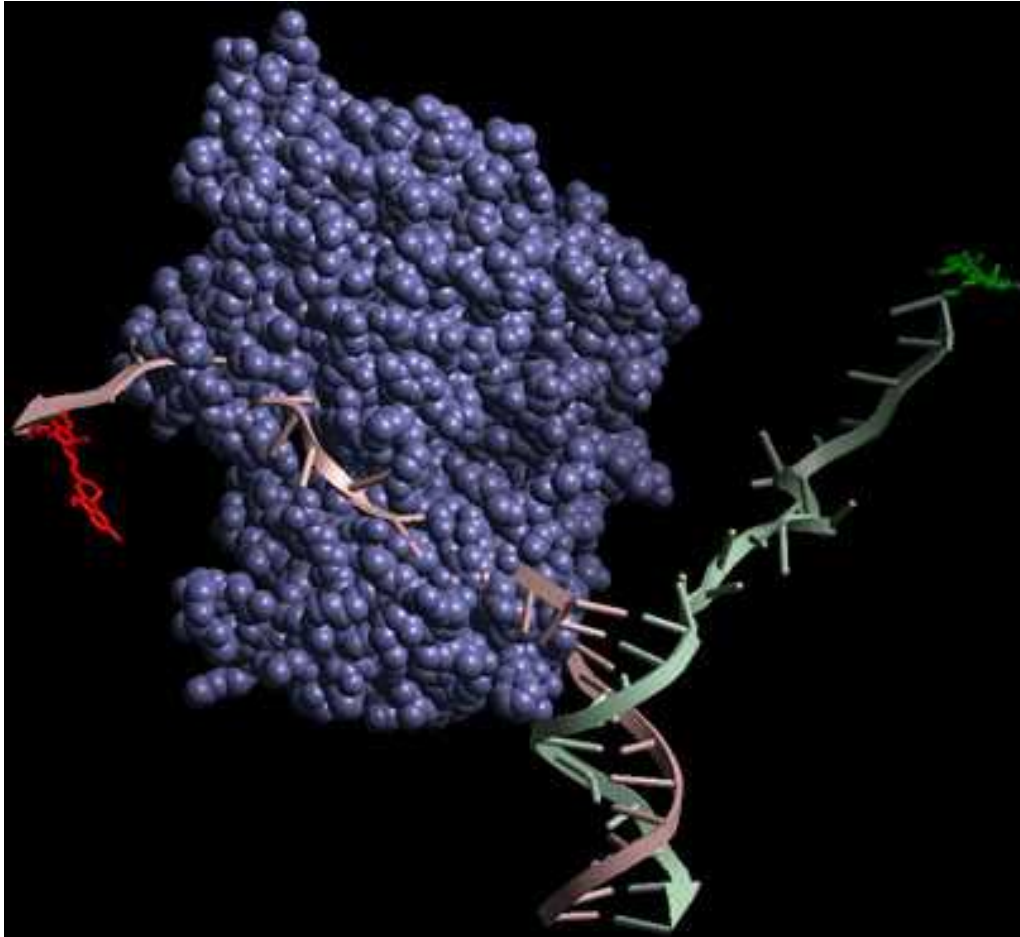
将双链DNA分子打开的马达



解旋酶在复制叉处使双螺旋DNA解旋，打开双链DNA

Helicase (解旋酶)

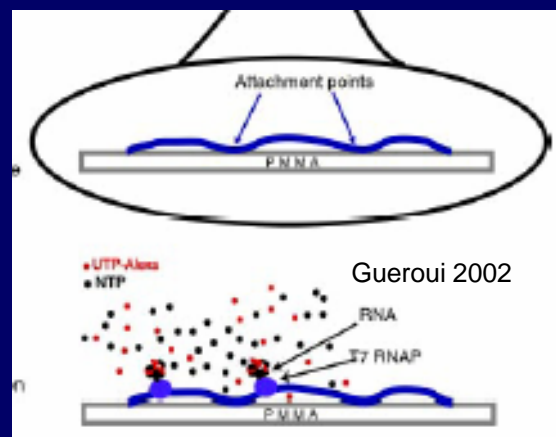
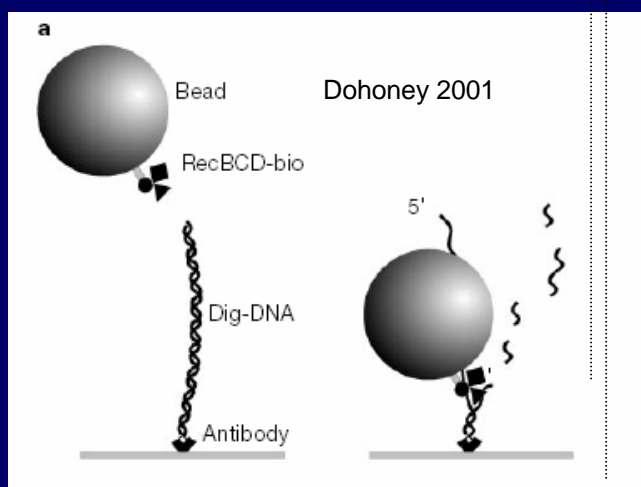
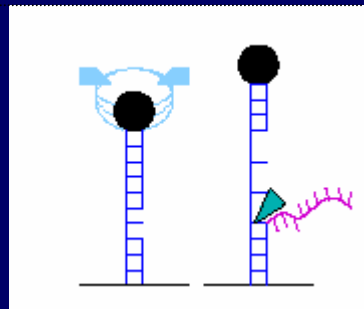
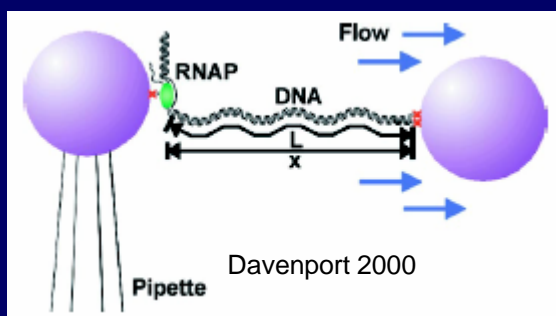
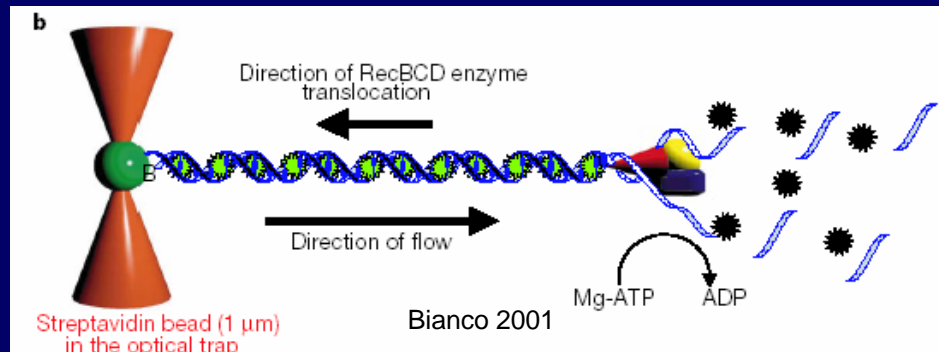
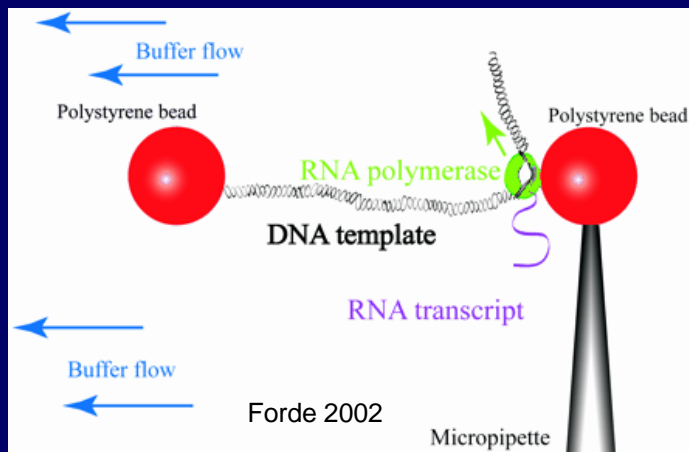
Unwinds double-stranded DNA to single-stranded DNA



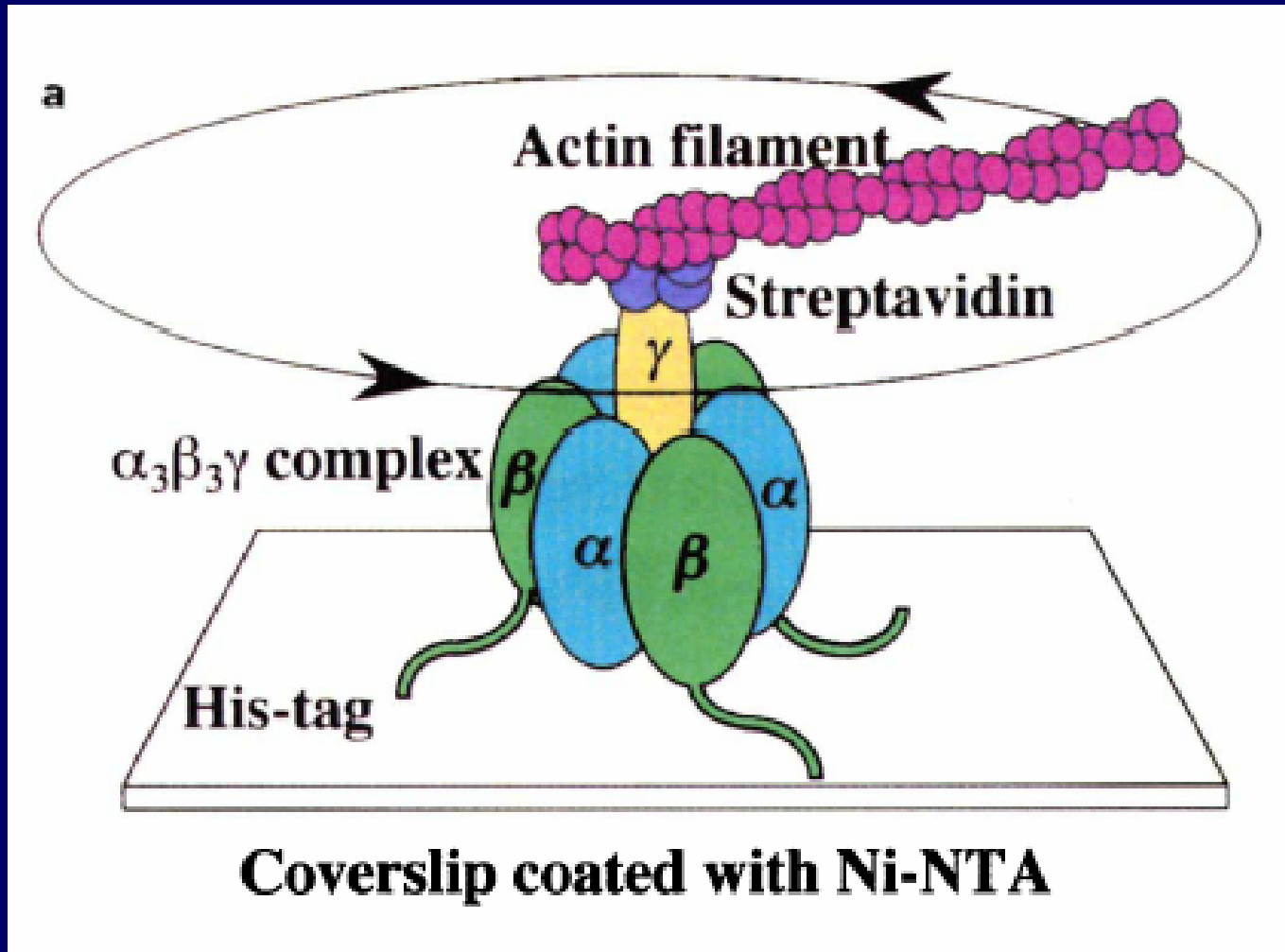
DNA replication
DNA repair
DNA recombination
RNA transcription
(转录)

.....

与DNA作用的一些马达蛋白单分子实验:

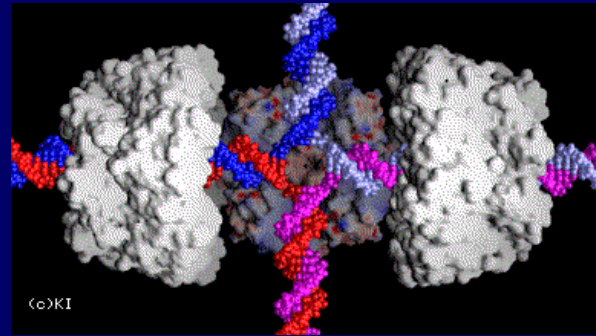
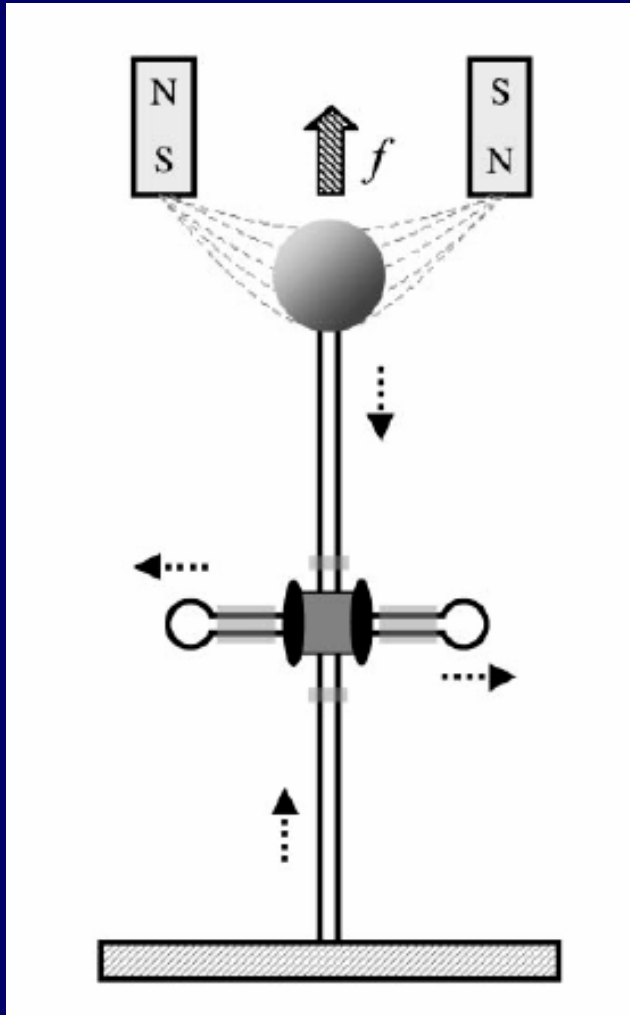


ATPase 的单分子实验

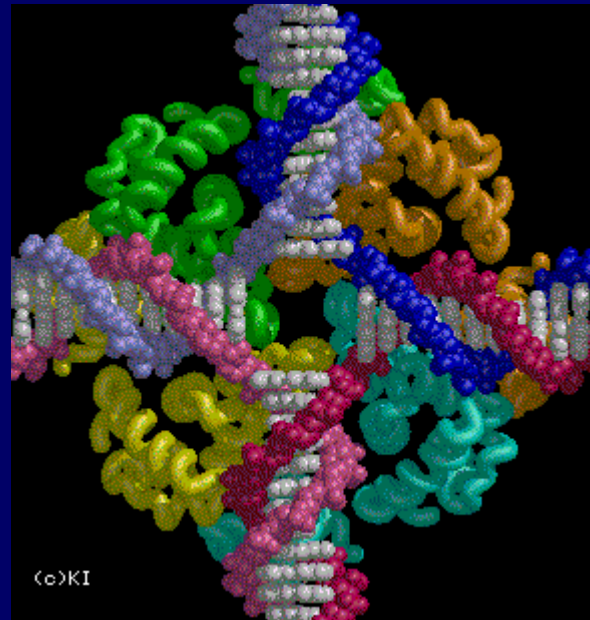


单分子方法可直接观察到转动

RuvAB / Holliday junction



RuvAB



RuvA

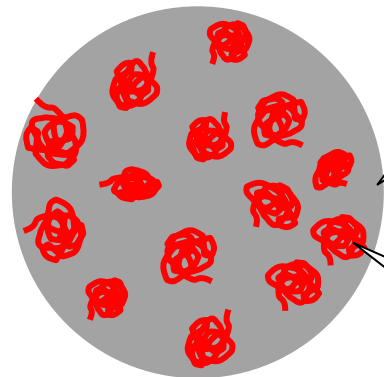
我们研究组的部分研究工作

分子梳技术拉伸DNA分子：

通过该方法使DNA分子的末端与固体表面结合，然后通过退却的液面对DNA进行拉伸，使DNA平行地排列在固体表面。

分子梳方法拉伸DNA原理（从顶面看）

玻片

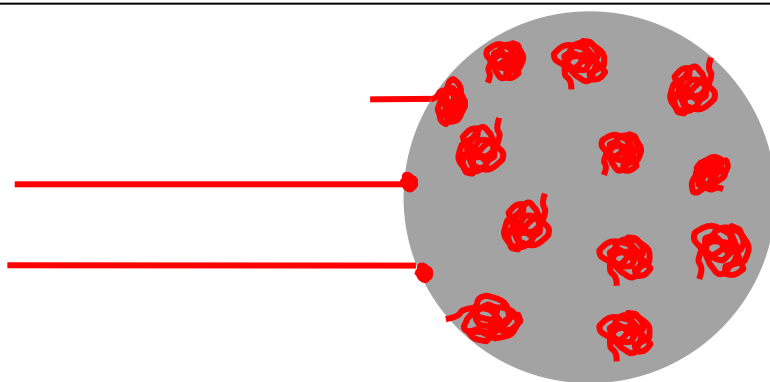


溶液

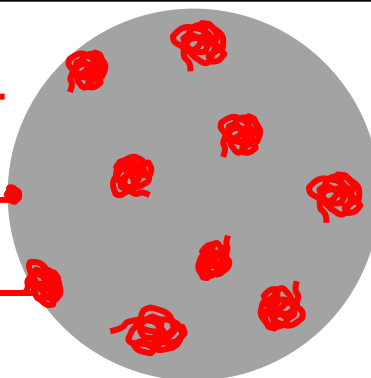
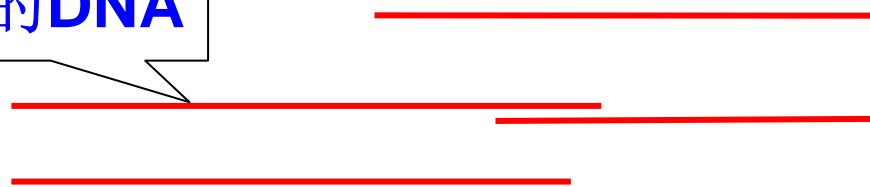
DNA

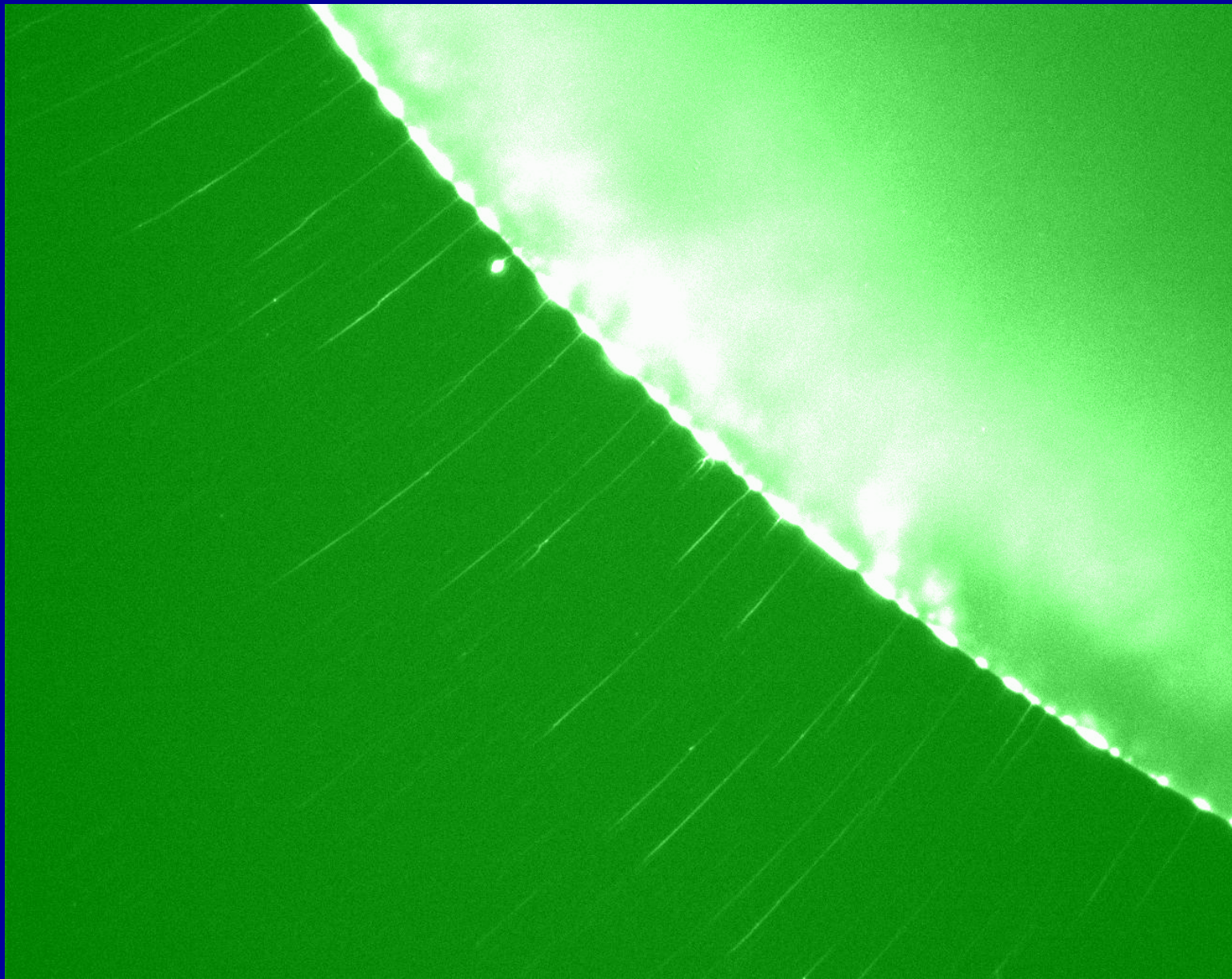


液滴移动方向

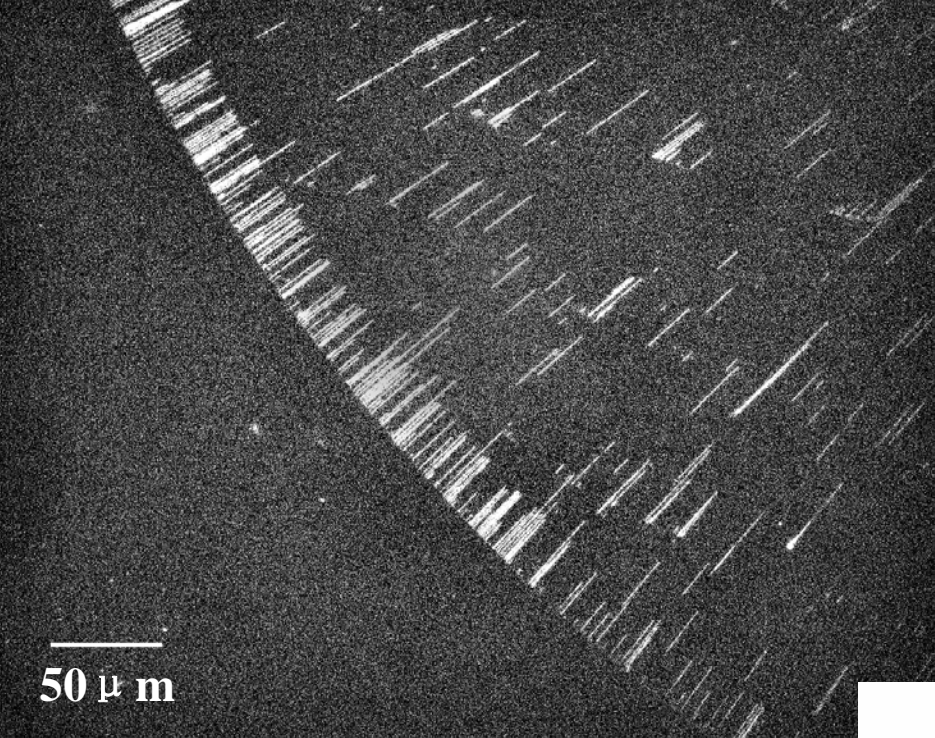


拉直的DNA





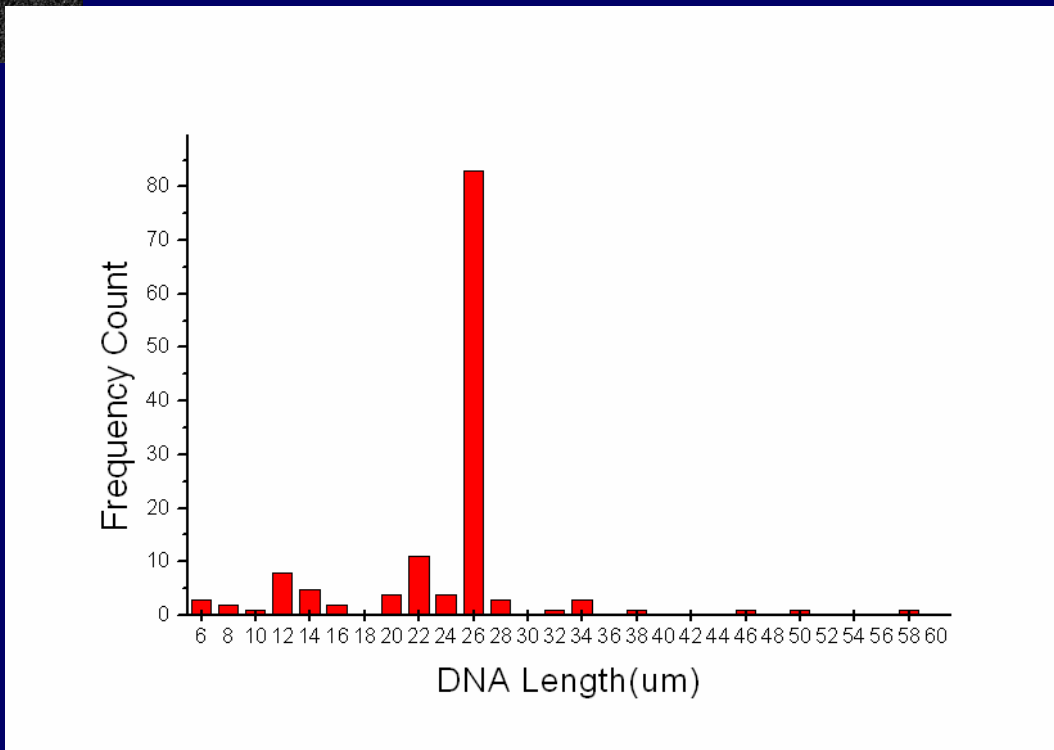
100µm



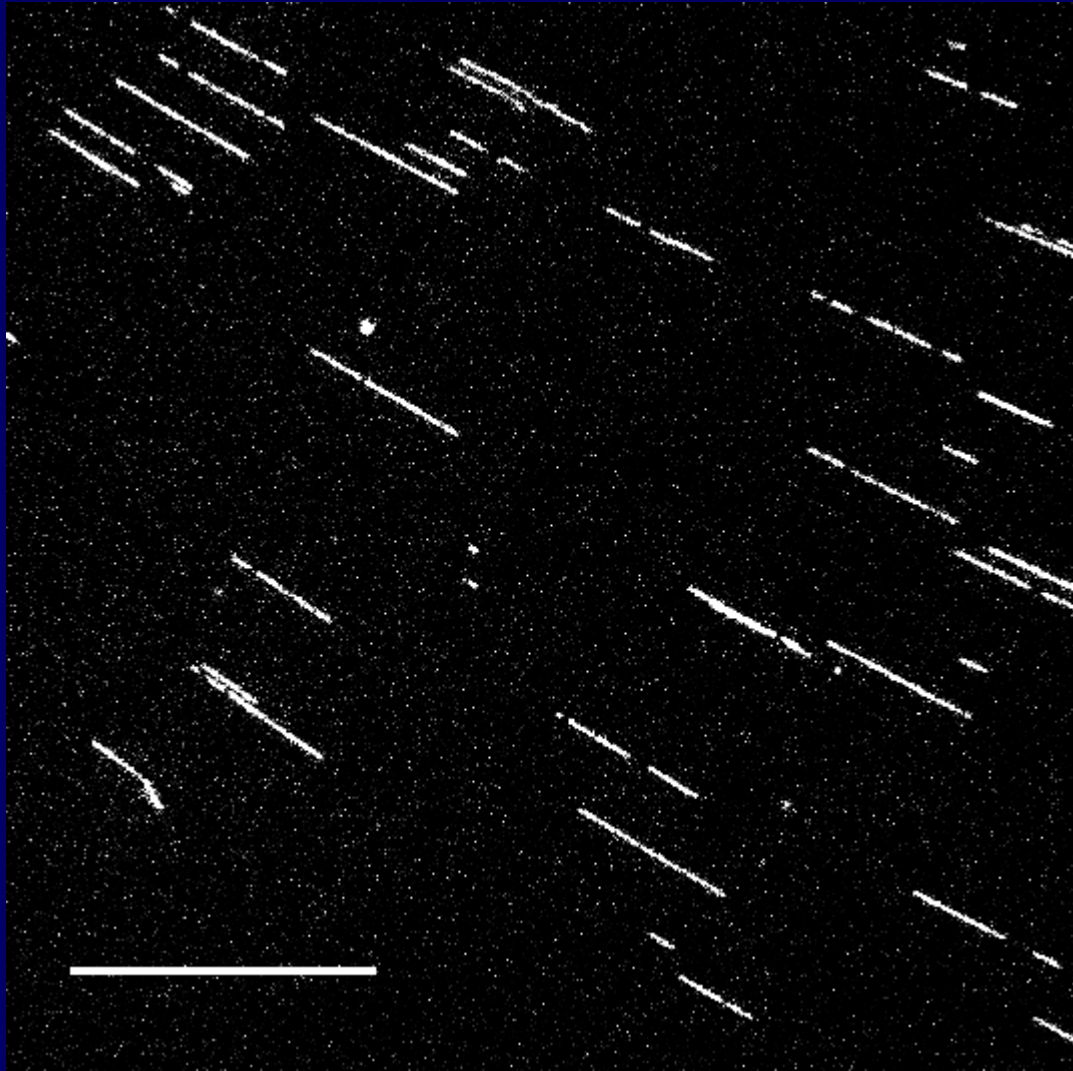
λ -DNA

不受力时伸直长度为 $16\mu\text{m}$

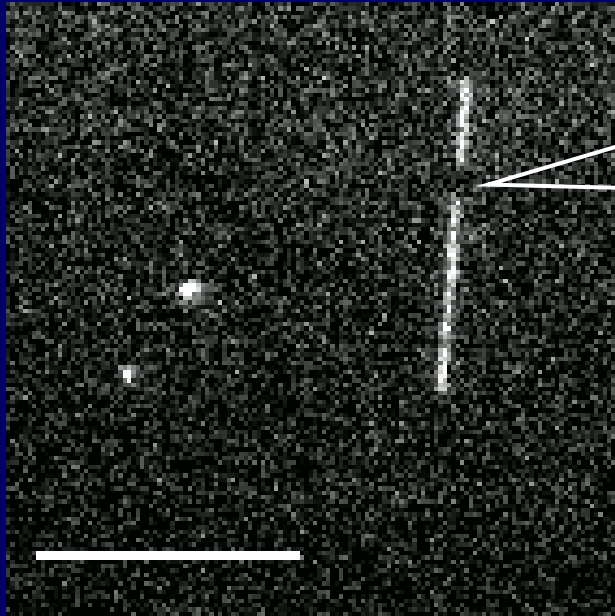
这里的伸直长度已达 $26\mu\text{m}$
证明已经受到较大拉伸力
(大约 60pN)



观察到的一个重要新现象：单分子 DNA 融化



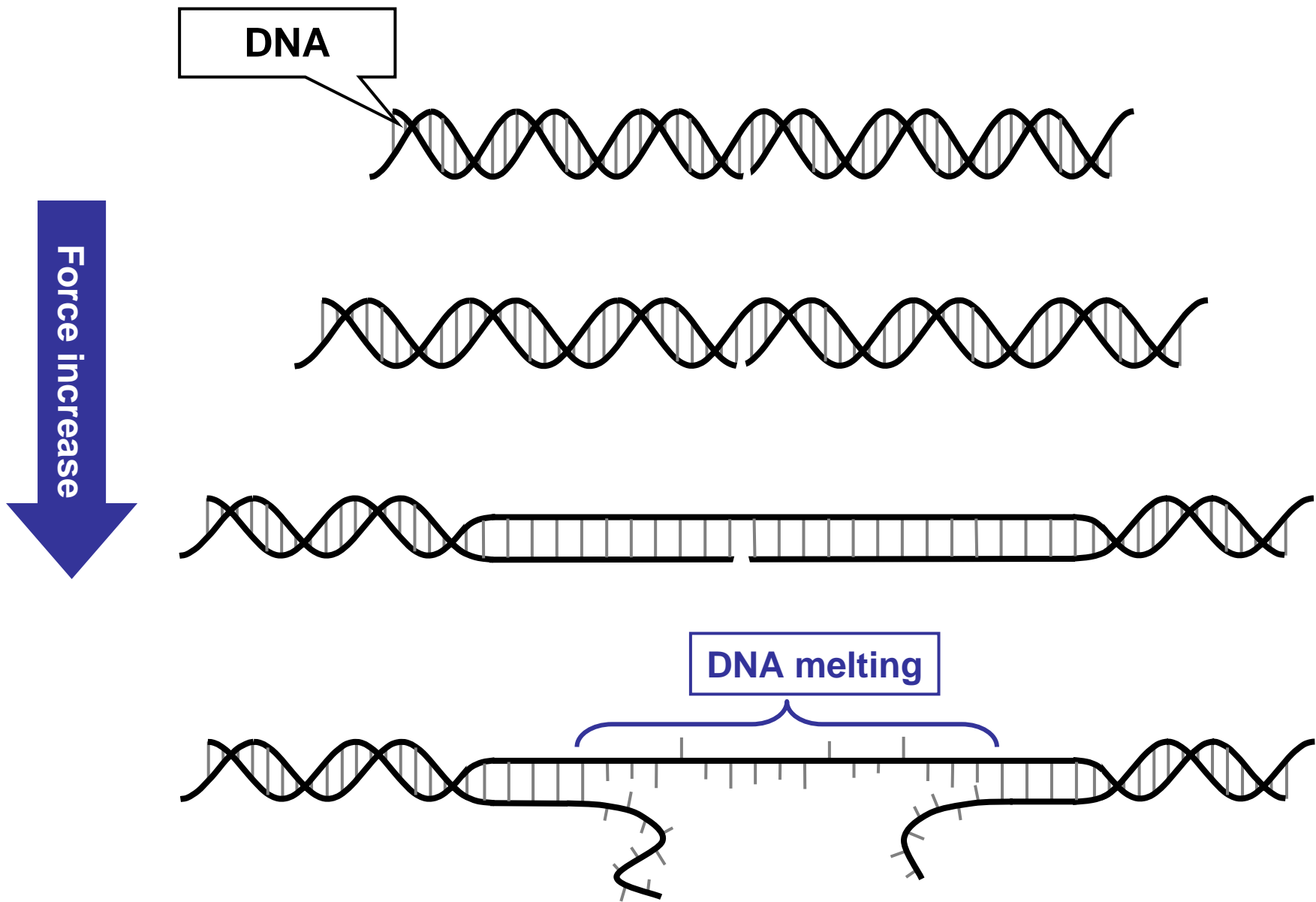
Bar = 50 μ m



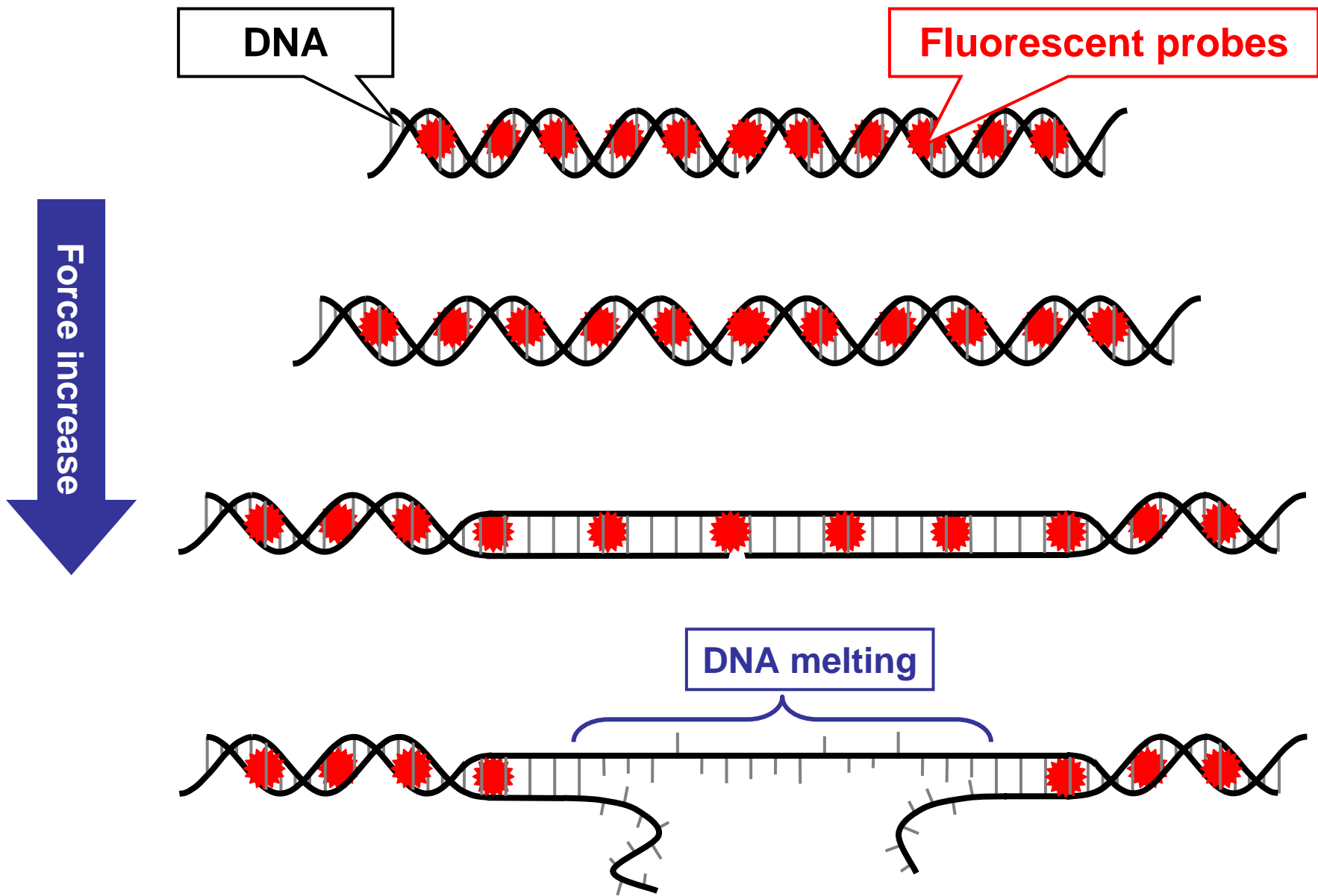
Force-induced melting
region of a single DNA
molecule

(Bar = $20 \mu\text{m}$)

我们直接观察到的**DNA**单分子融化

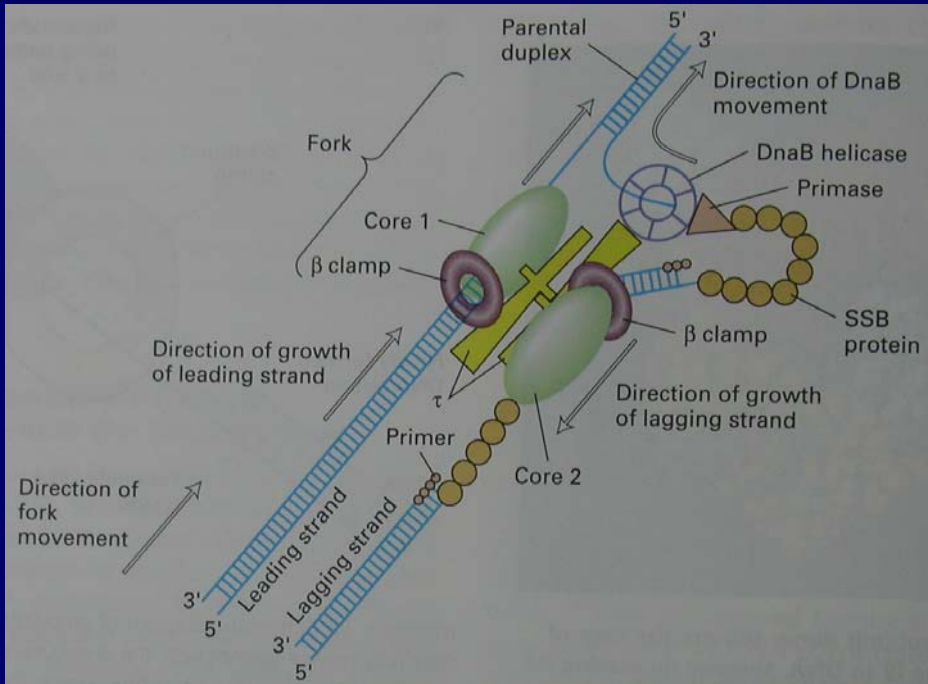


拉伸力引起的单分子**DNA**熔化模型

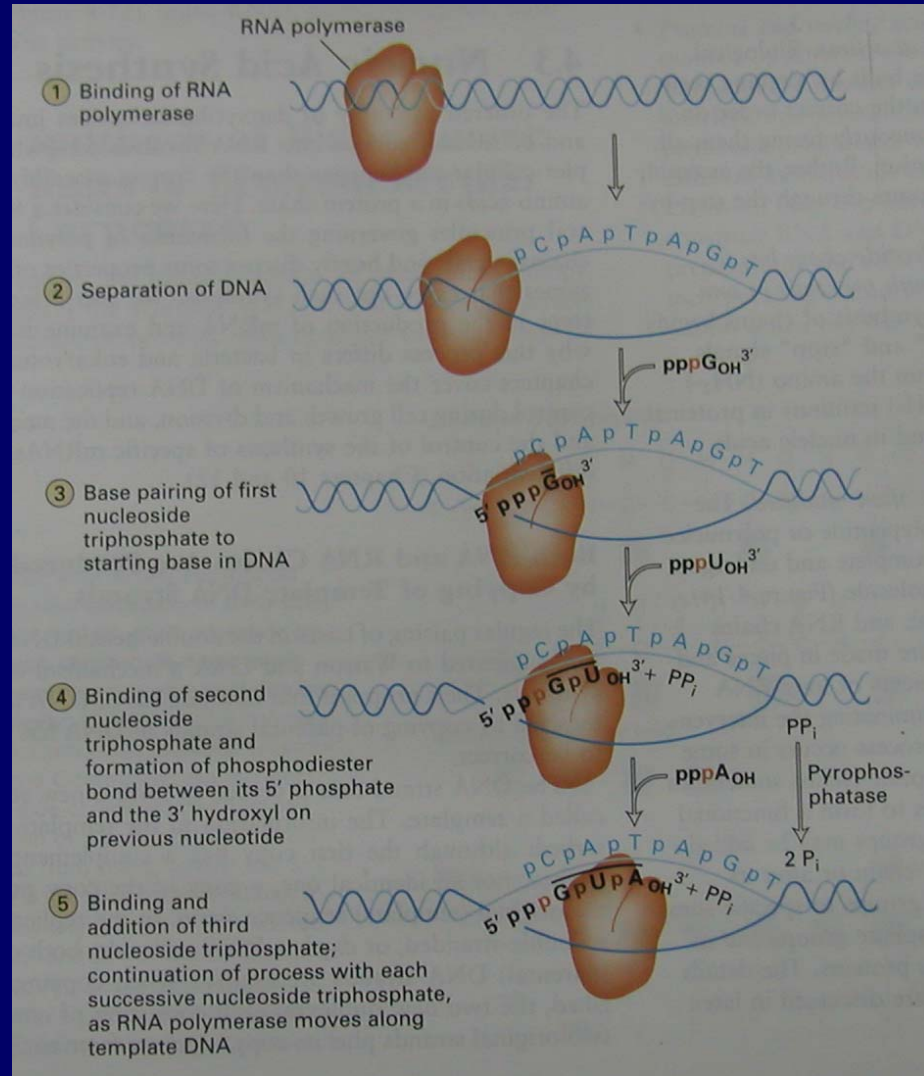


拉伸力引起的单分子DNA熔化模型

在生物体内(in vivo)将碱基对的氢键打开

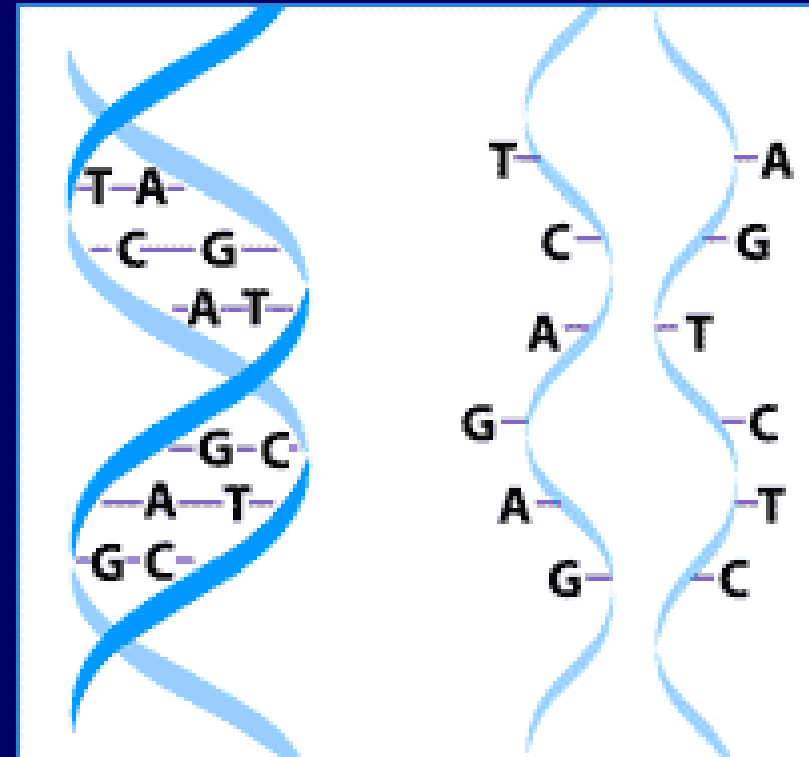
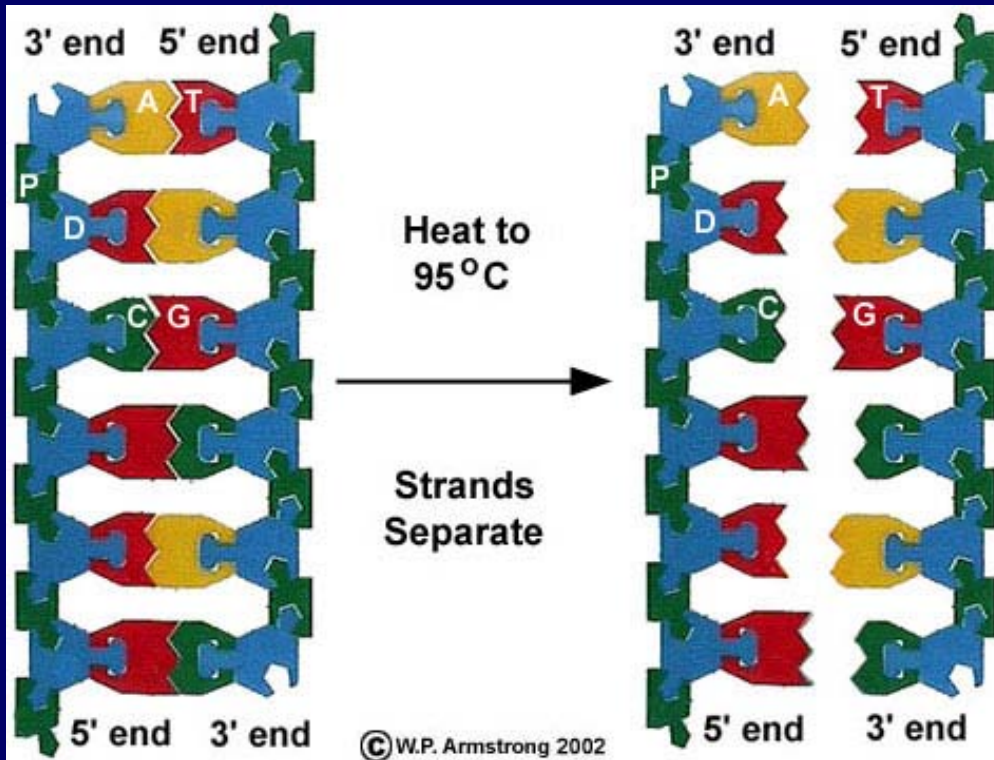


复制



转录

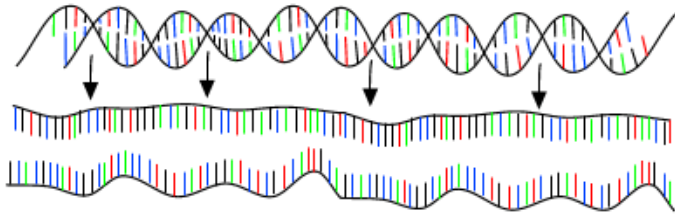
在体外(in vitro)将碱基对的氢键打开



加热到 90 度左右

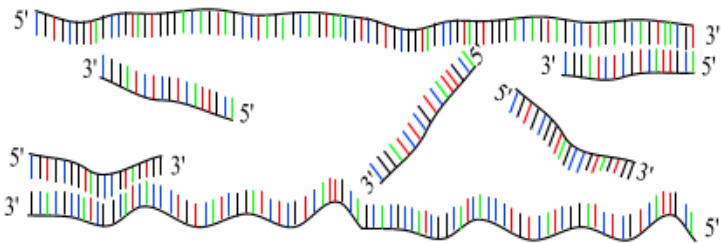
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

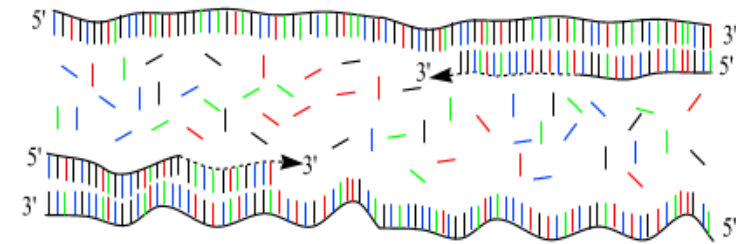
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!



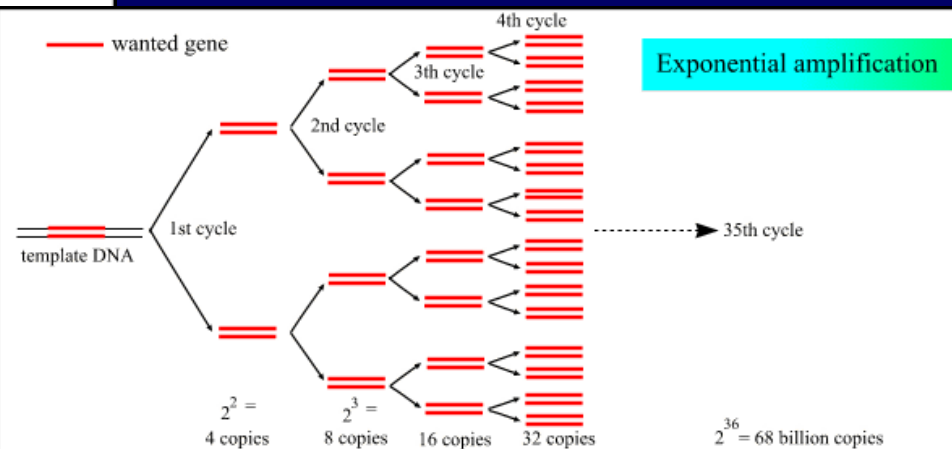
Step 3 : extension

2 minutes 72 °C
only dNTP

(Andy)

对DNA模板进行
融化（变性）- 退火（复性）
循环处理
DNA分子数目以指数速度增长
大量复制
例如36个循环后变为

$2^{36} \approx 68$ billion copies



DNA 与组蛋白的相互作用动力学研究

科学问题 (以人类本身为例)

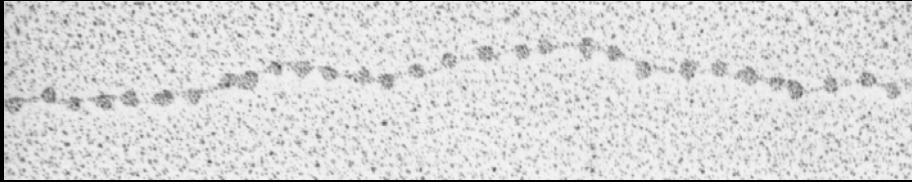
人的**DNA**分子长度近 2 米

人的细胞核直径只有 **10** 微米量级

从而提出问题：

如何折叠进去的？

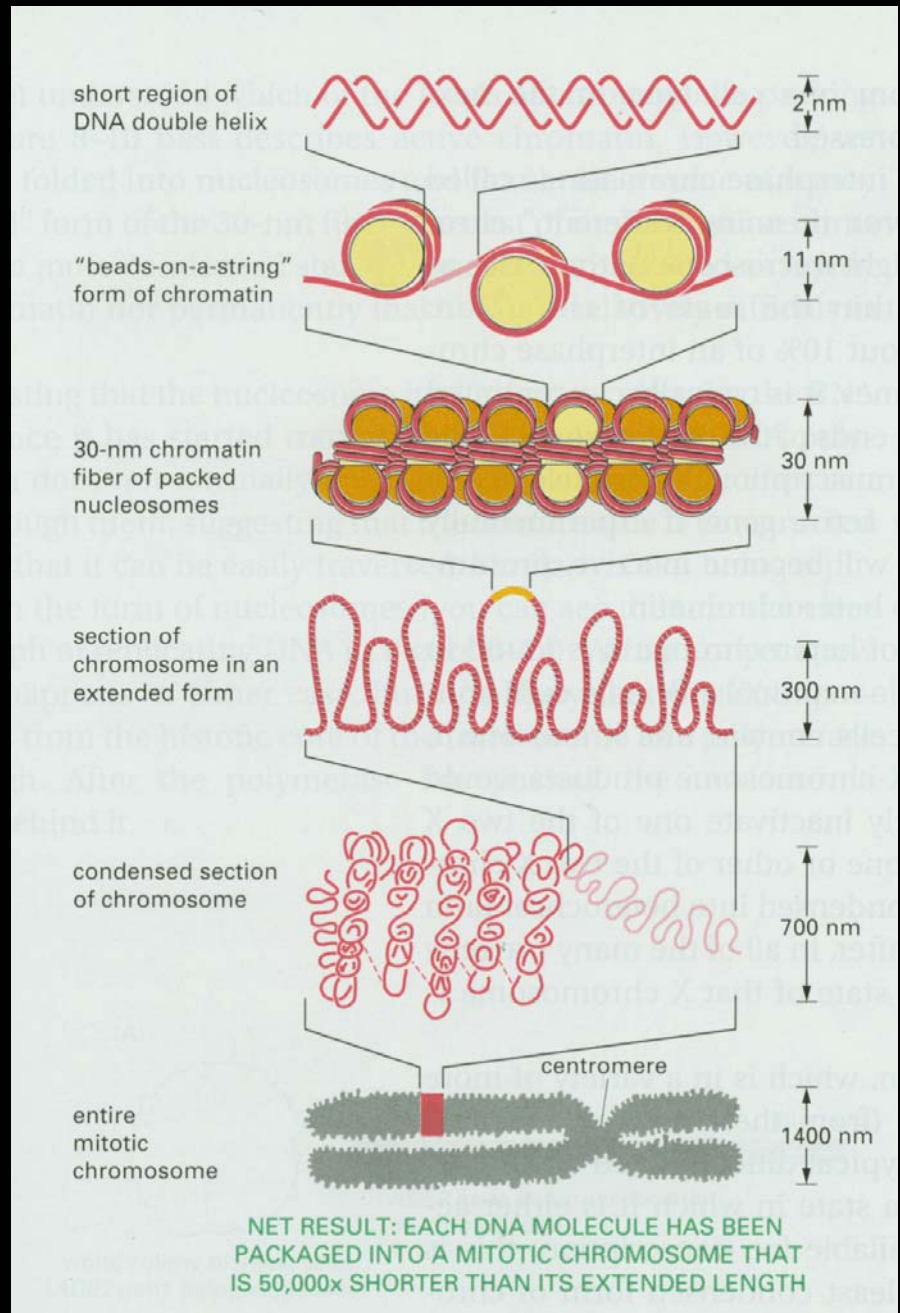
染色体的结构



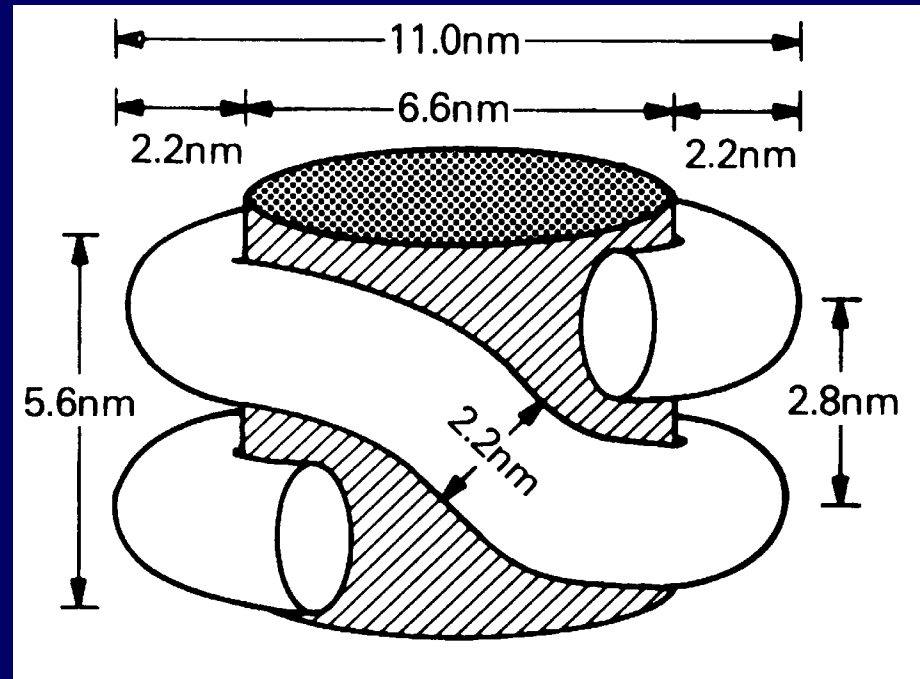
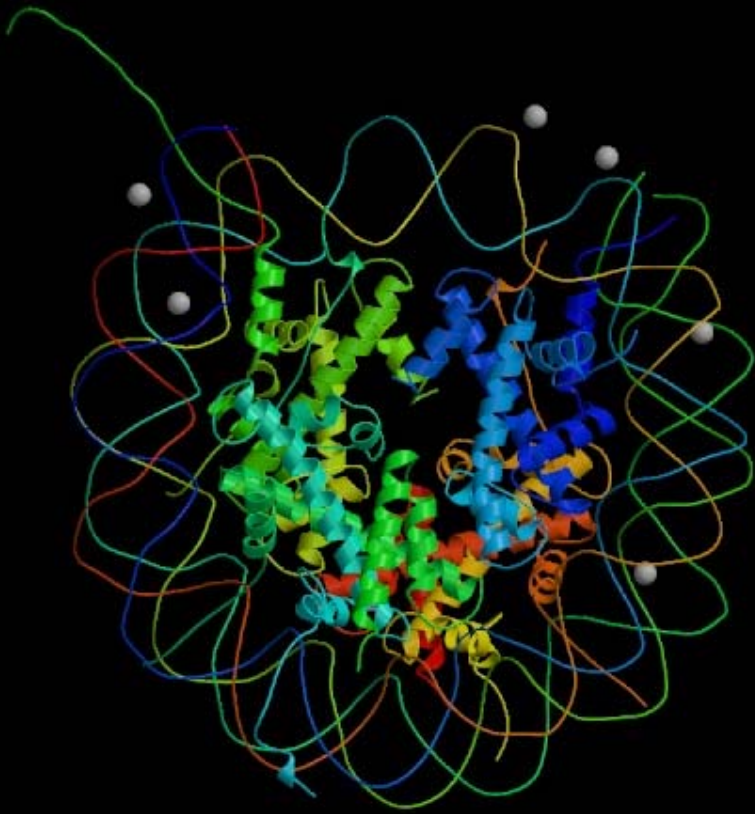
从DNA到染色体:

2nm DNA双螺旋⇒
由核小体组成的10nm纤维⇒
30nm粗纤维⇒
超粗纤维⇒ 染色体

通常分为 4 级结构



点击左图观看立体结构



核小体结构

核小体(DNA-histone)
的尺寸示意图

问题：核小体结构形成的动力学过程是怎样的？

我们利用布朗动力学进行分析

简化的模型：

DNA – 柔性串珠

histone – 球（非手征性和手征性）

势能函数的选择

i、DNA链的自我排斥能:

$$U_{m,rep} = \varepsilon_m k_B T \sum \exp \{-\alpha_m (r_{i,j} - \sigma_m)\}$$

ii、DNA小球间的键能:

$$U_{bond} = \frac{k k_B T}{2 \sigma_m^2} \sum (|\vec{r}_i - \vec{r}_{i+1}| - \sigma_m)^2$$

iii、DNA链的弯曲能:

$$U_{bend} = \kappa k_B T \sum \left(1 - \frac{(\vec{r}_{i-1} - \vec{r}_i) \cdot (\vec{r}_i - \vec{r}_{i+1})}{\sigma_m^2}\right)$$

iv、组蛋白与组蛋白之间的排斥能:

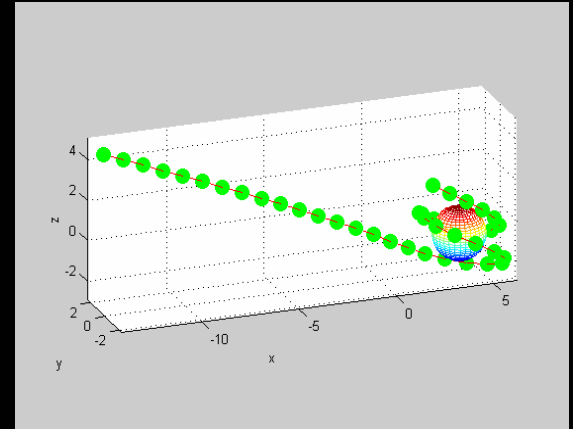
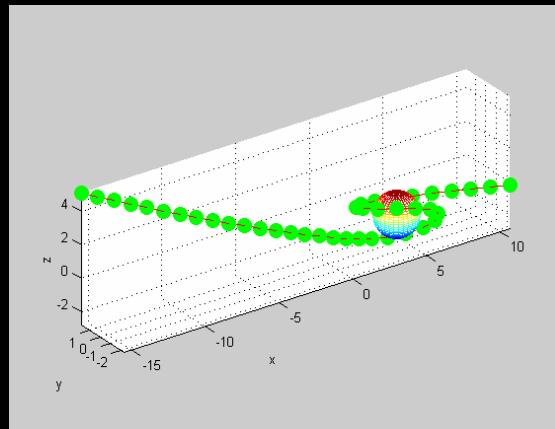
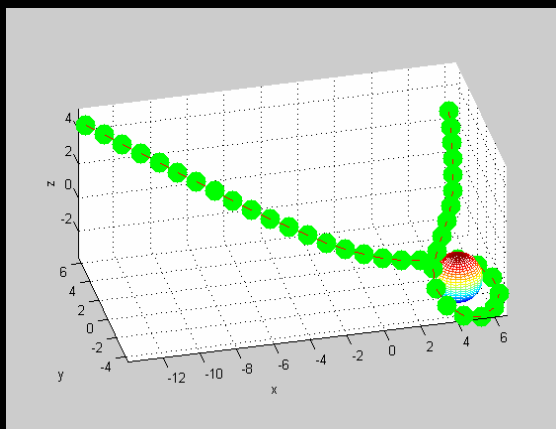
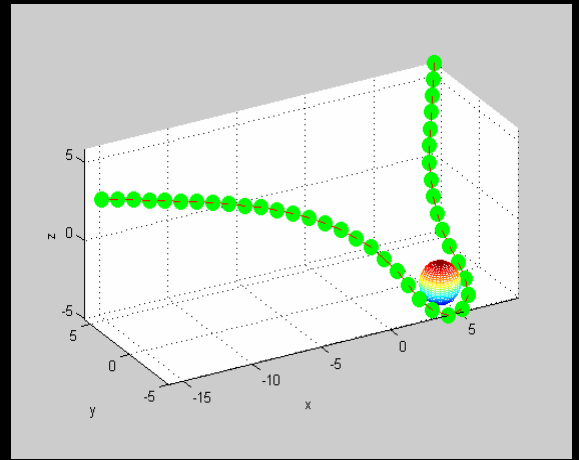
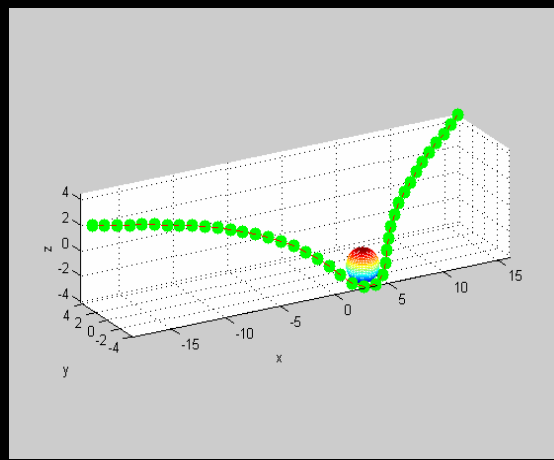
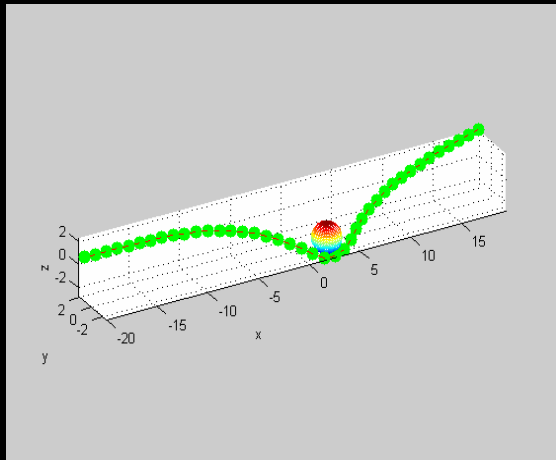
$$U_{M,rep} = \varepsilon_M k_B T \sum \exp \{-\alpha_M (R_{i,j} - \sigma_M)\}$$

v、DNA与组蛋白的相互作用势:

$$U_M = \varepsilon k_B T \sum [\exp \{-2\alpha (r_i - \sigma)\} - 2 \exp \{-\alpha (r_i - \sigma)\}]$$

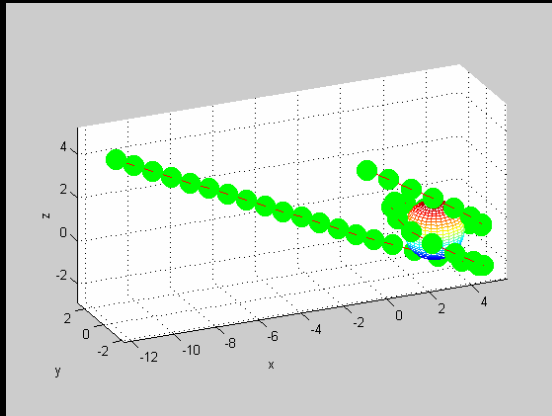
整个系统的运动由过阻尼朗之万方程描述

模拟结果 Snapshots

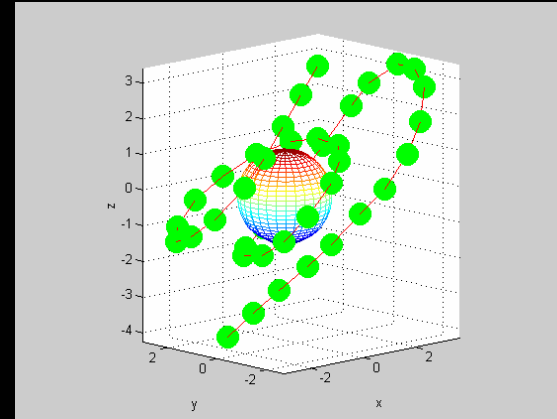


Temperature dependence

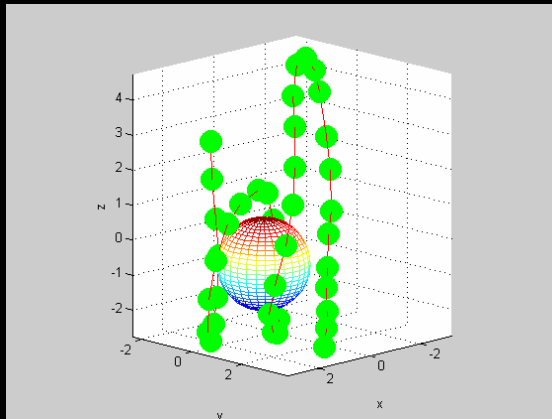
(A)



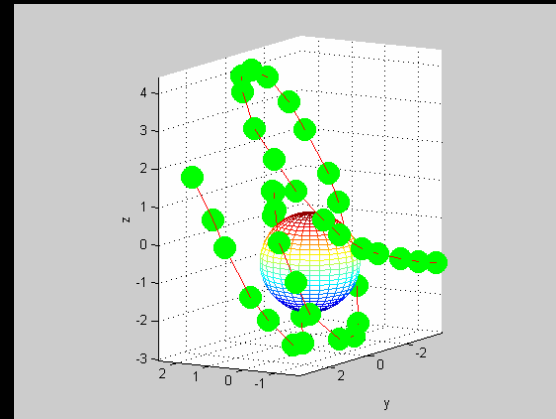
(B)



(C)



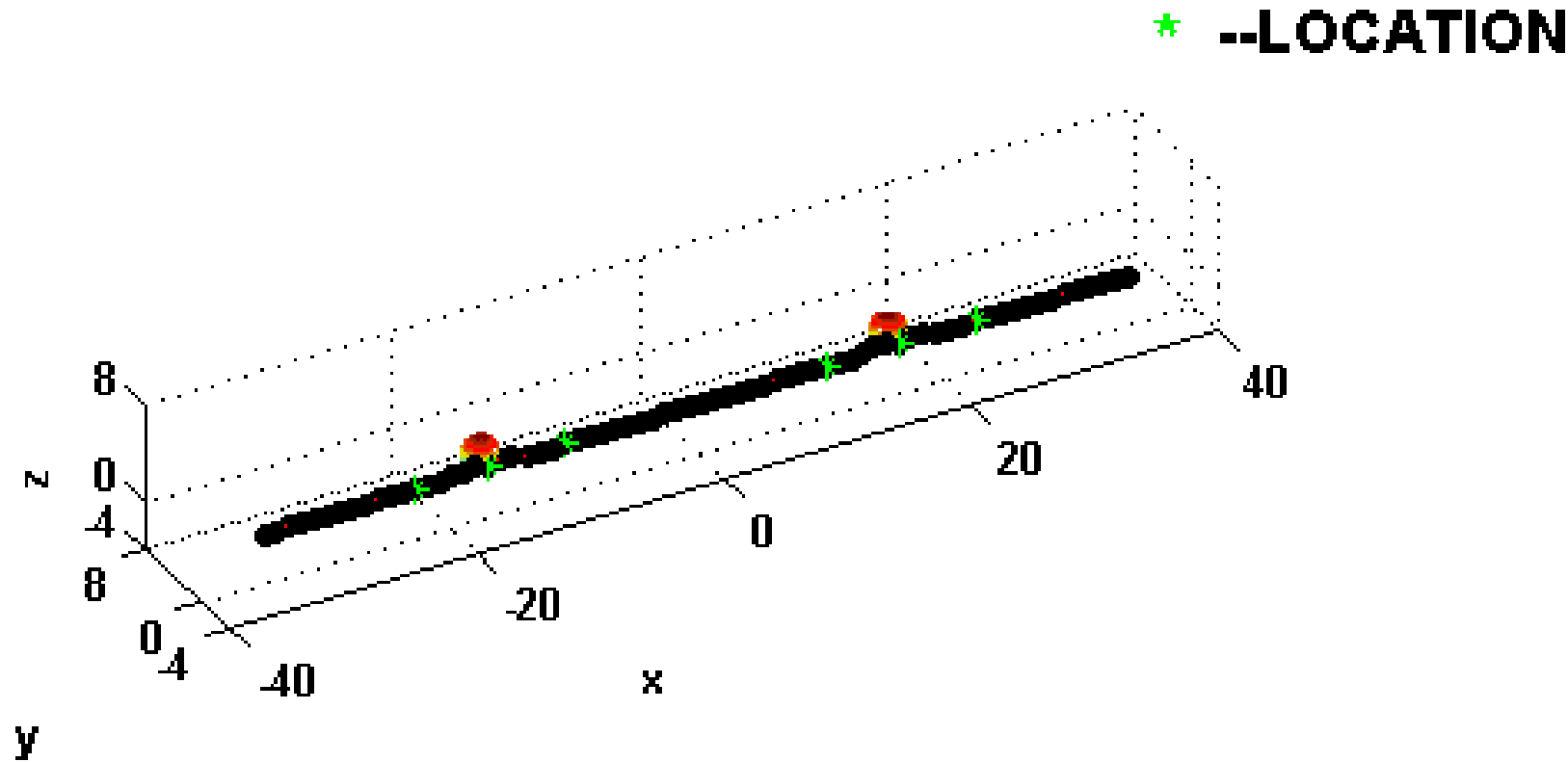
(D)



(A) $T=298\text{K}$; (B) $T=348\text{K}$; (C) $T=398\text{K}$; (D) $T=448\text{K}$

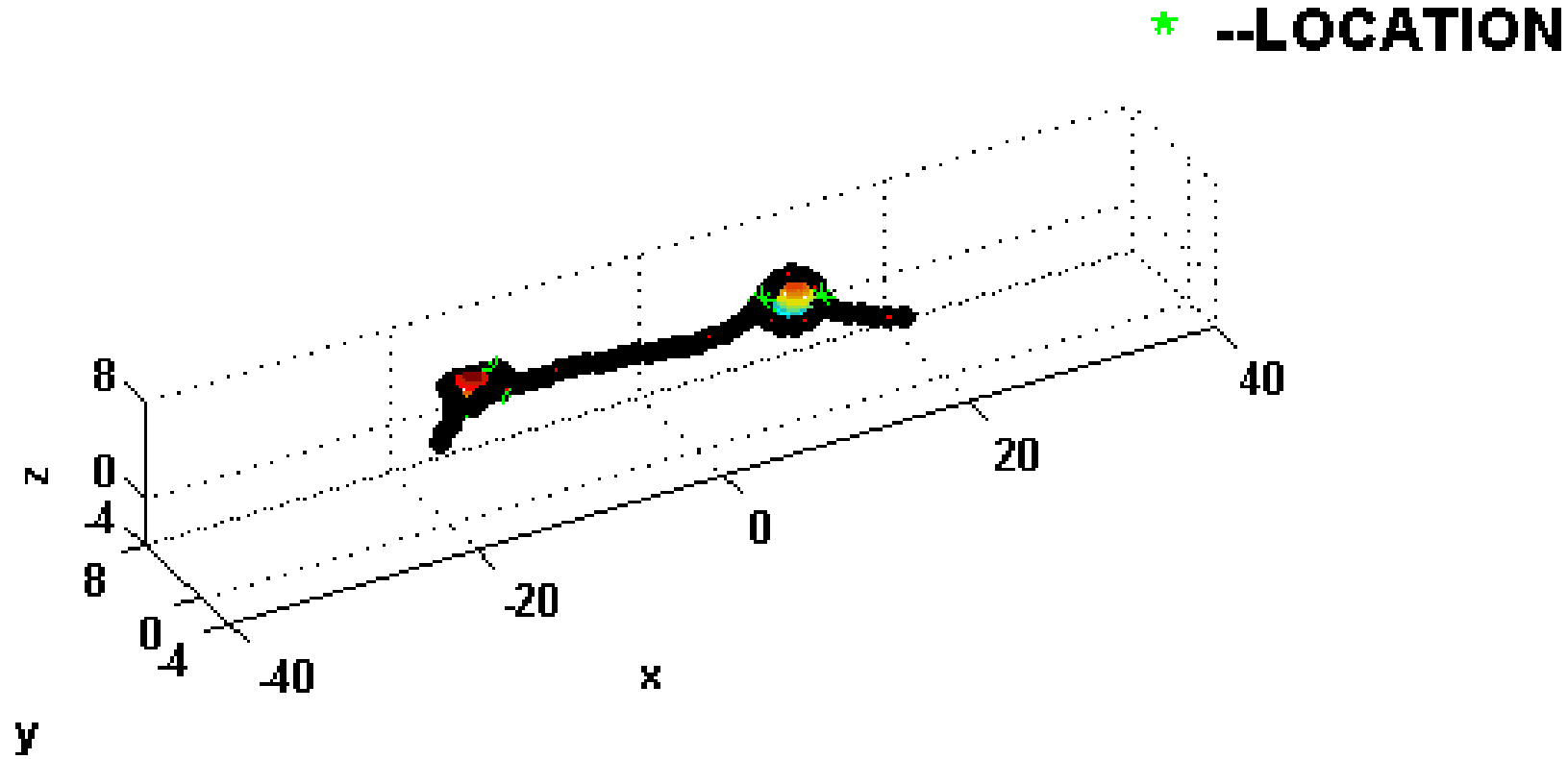
DNA与两个组蛋白结合的过程

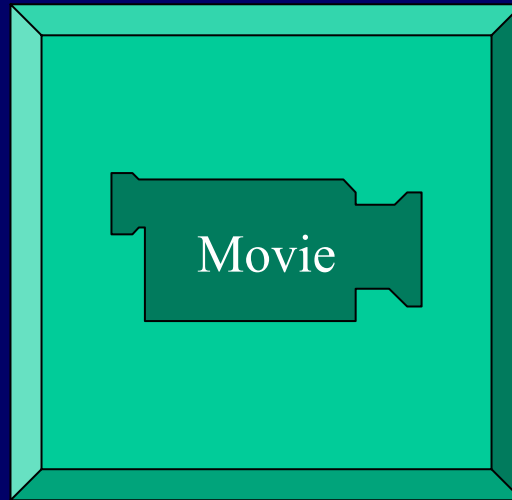
INTERACTION BETWEEN DNA AND HISTONES



在拉伸力作用下，DNA与两个组蛋白的解离过程

INTERACTION BETWEEN DNA AND HISTONES





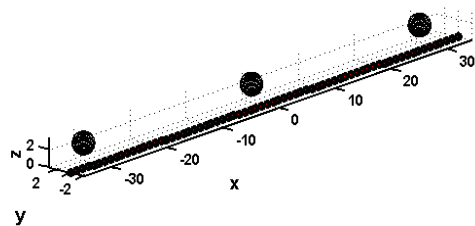
来自教科书的猜想动画

Lodish, Berk, Zipursky, Matsudaira, Baltimore, Darnell

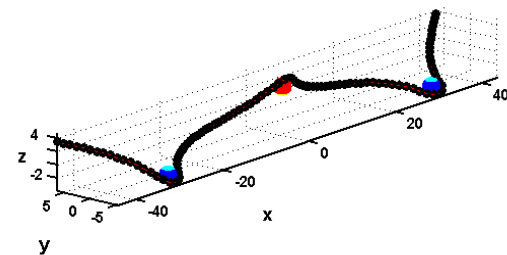
Molecular Cell Biology (4th Ed.) (Freeman, New York, 2001)

我们的结果:

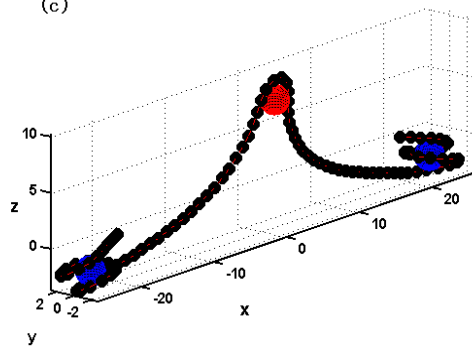
(a)



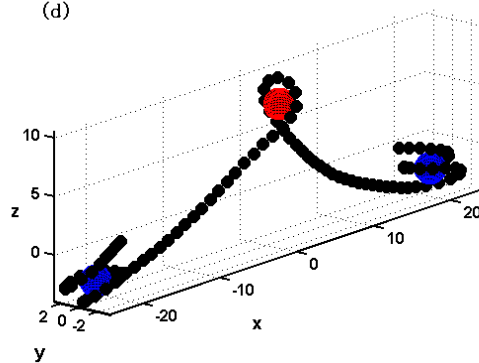
(b)



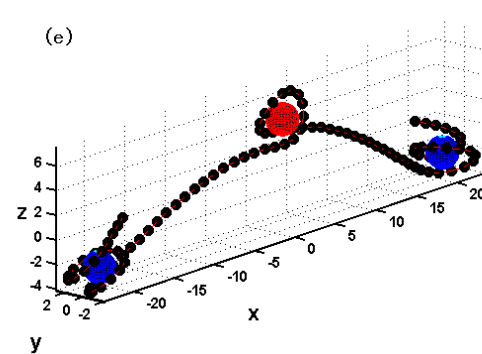
(c)



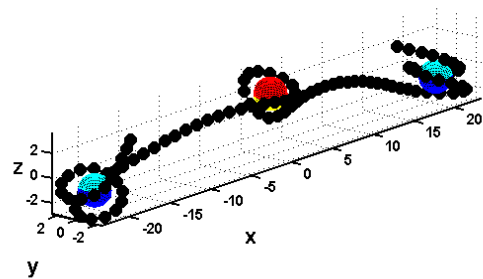
(d)



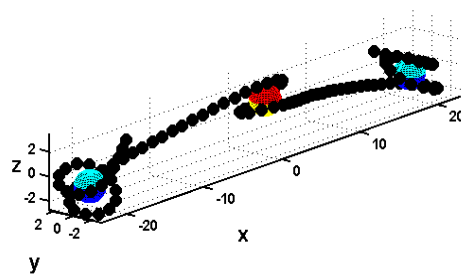
(e)



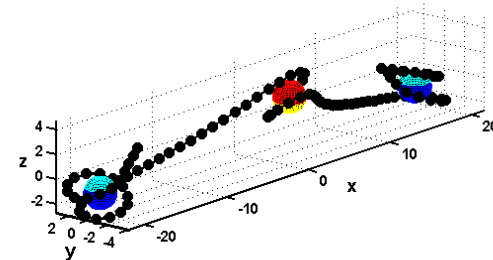
(f)

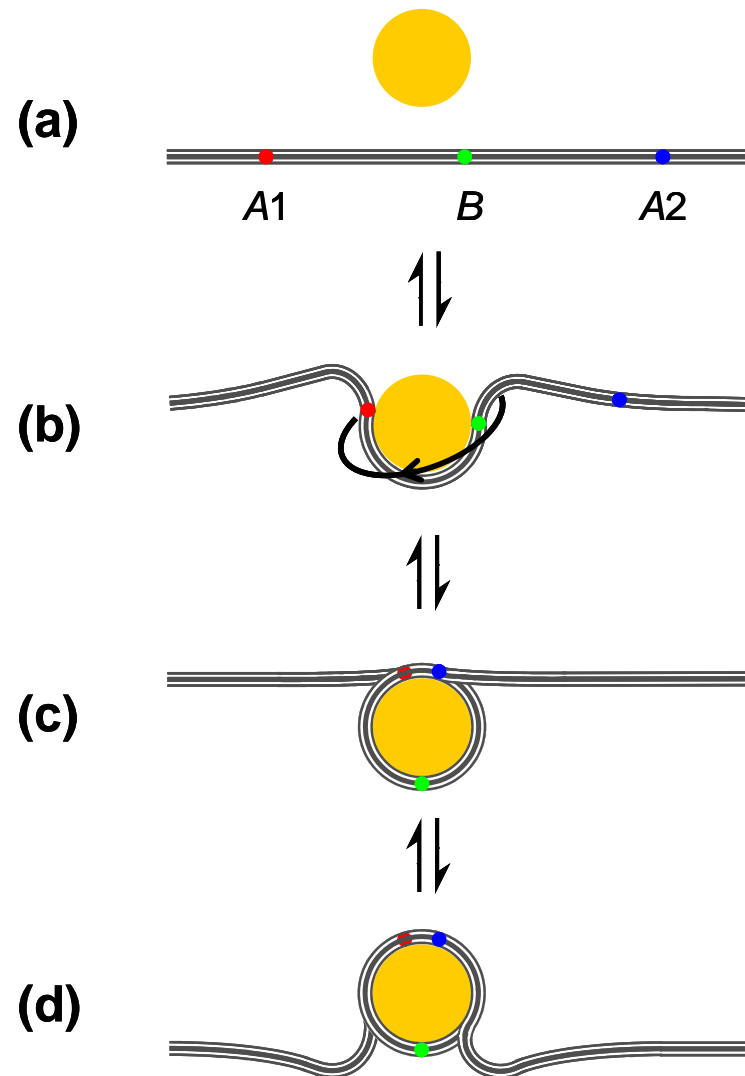


(g)



(h)

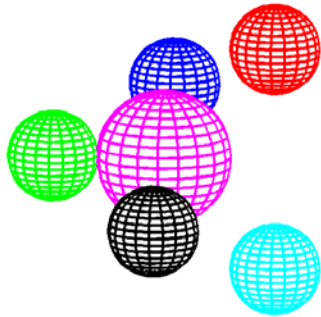




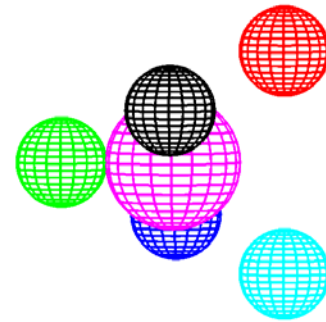
我们提出的 **DNA** 与组蛋白结合模式

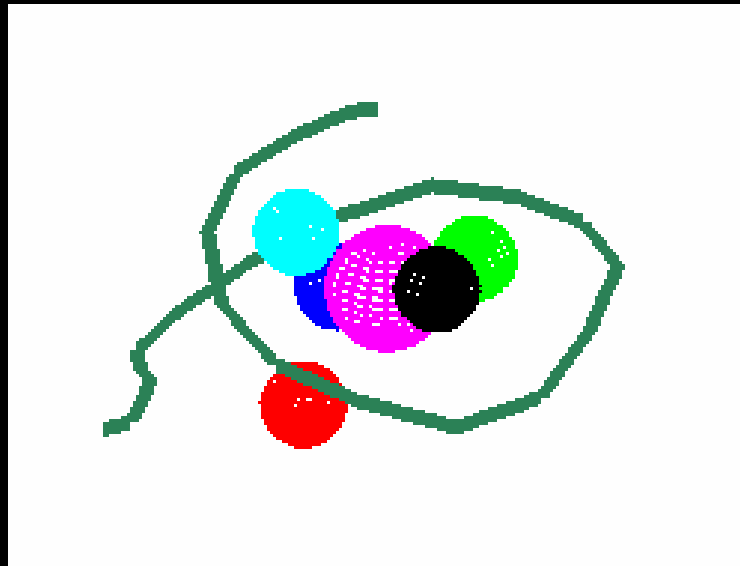
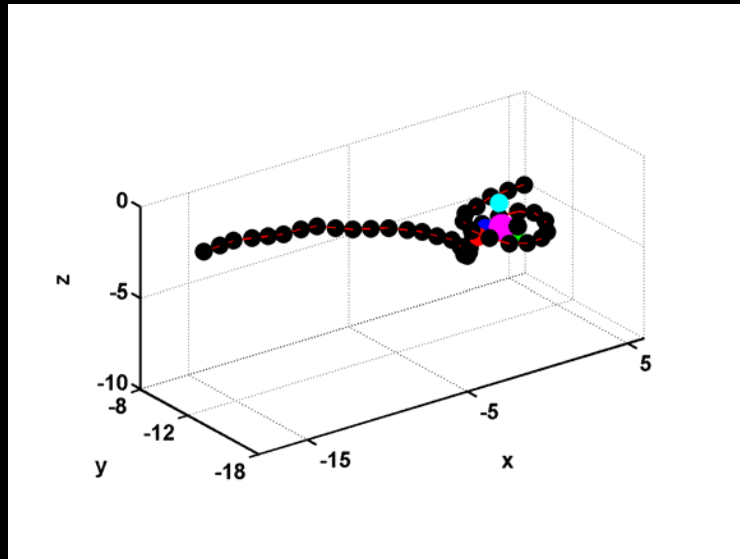
Models for a histone octamer with chirality 具有手征性的组蛋白八聚体模型

left-handed



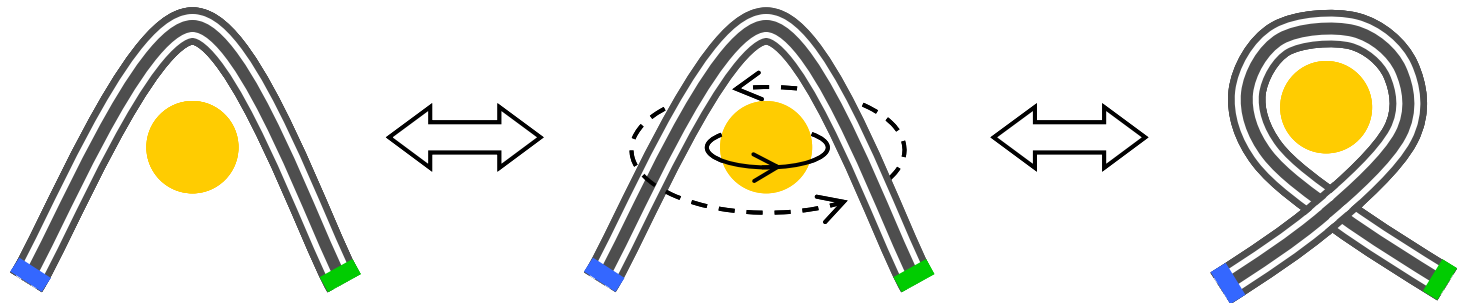
right-handed

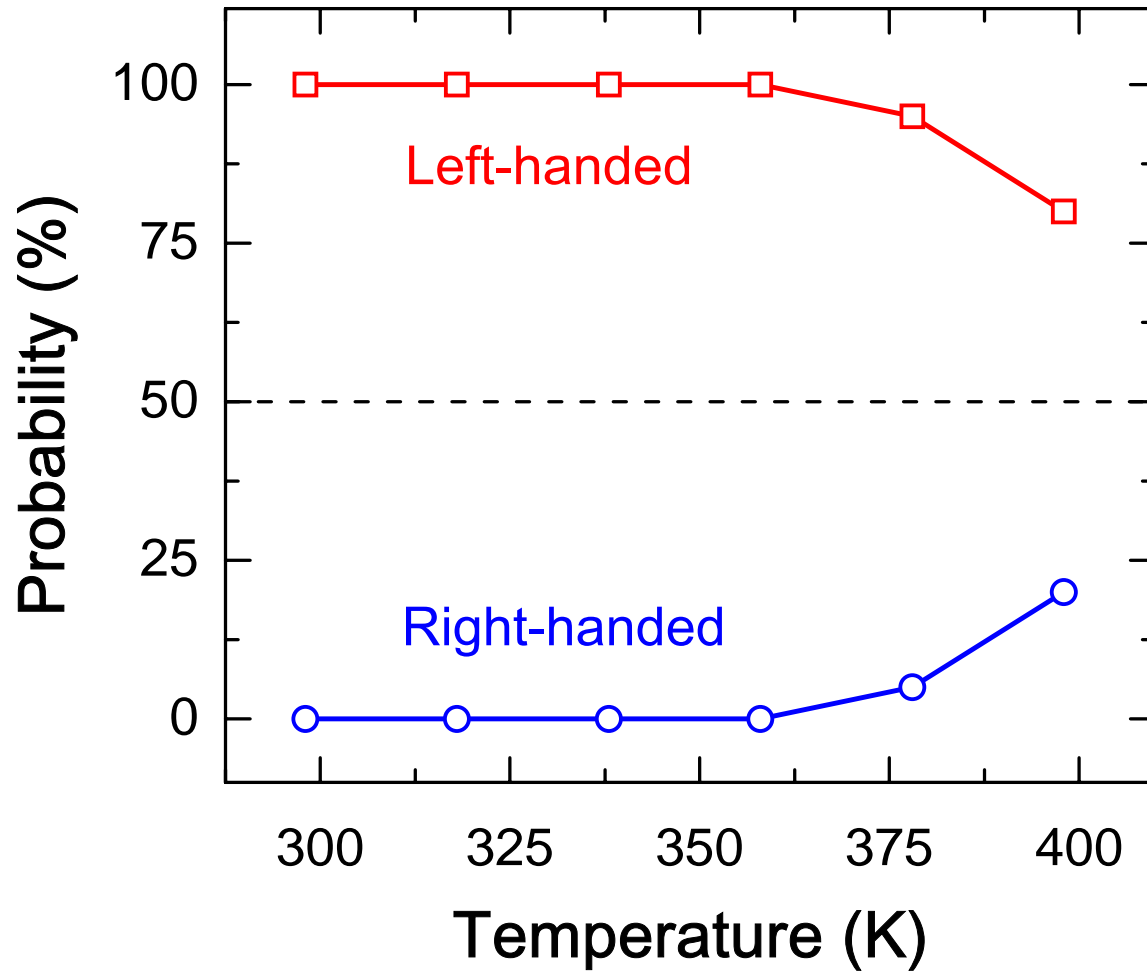




我们计算得出的 **DNA** 与组蛋白结合的手征性

Model for the formation of a natural nucleosome with a left-handed histone octamer

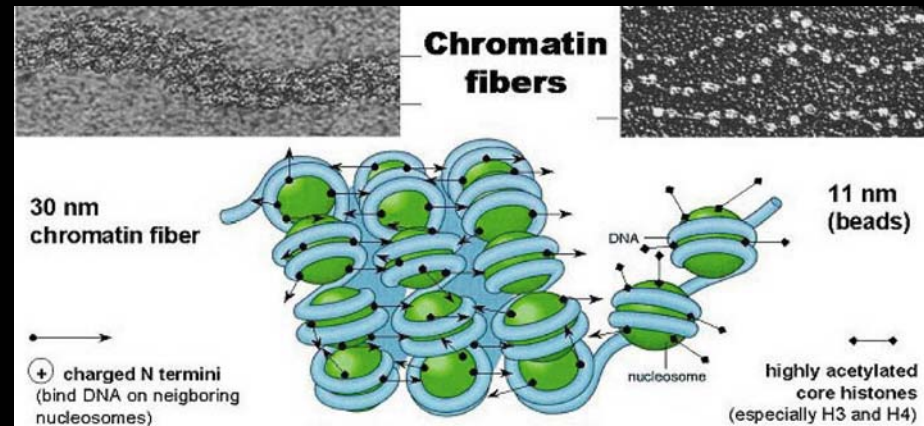




Probabilities of occurrence of left-handed and right-handed nucleosomes versus temperature

DNA 弯曲对组蛋白定向滑移的影响

最近，越来越多的证据表明，组蛋白在基因调控中起着至关重要的作用



染色体的结构

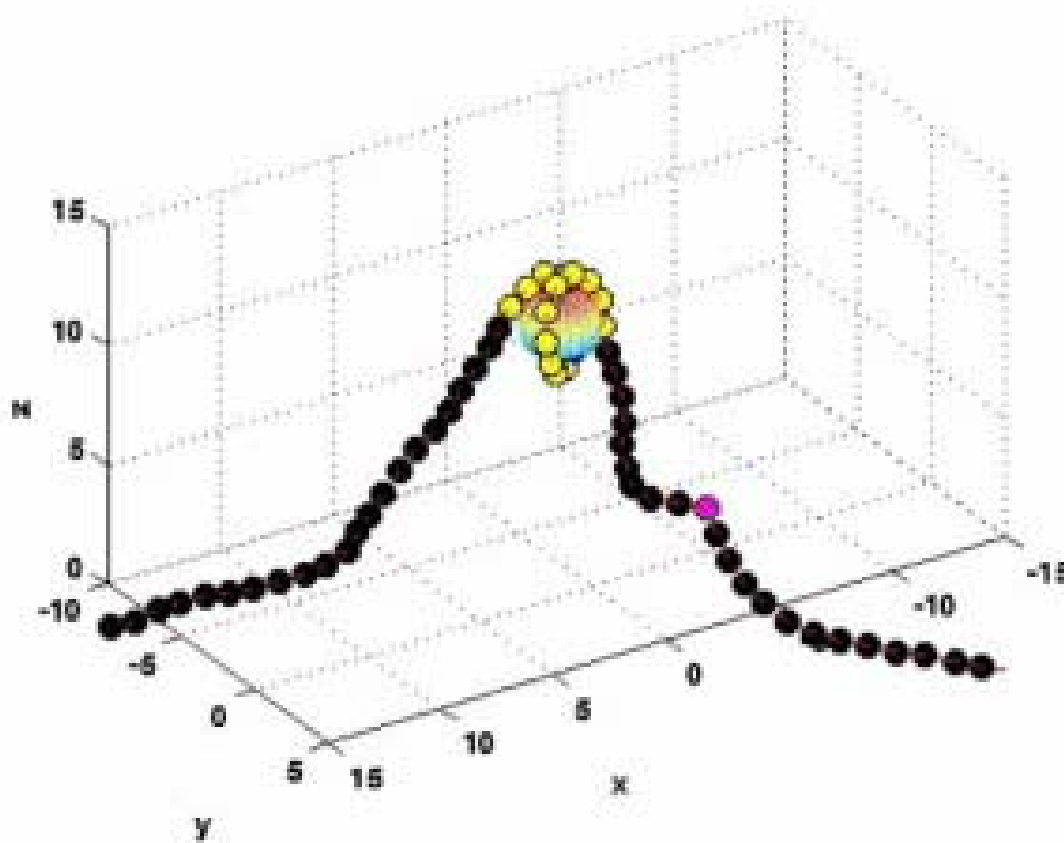
例如：母猫经常具有花的毛色，原因是它的一条**X染色体被静默**（基因不表达）。最近，有人发现X染色体静默与其中富集的**H2A**组蛋白有关。

基因静默有望成治疗一些严重疾病（艾滋病、肝炎、流感和恶性肿瘤等）的有力手段。

人们已经发现有一类蛋白质复合体 (e.g.: **SWI/SNF and RSC**) 通过水解**ATP**能够改变染色体的结构。包括**2006**年获得诺贝尔奖的 **Kornberg** 的研究组的研究工作发现，这些染色质重塑复合体(**chromatin-remodeling complex**) 能够导致组蛋白在 **DNA** 上滑移。

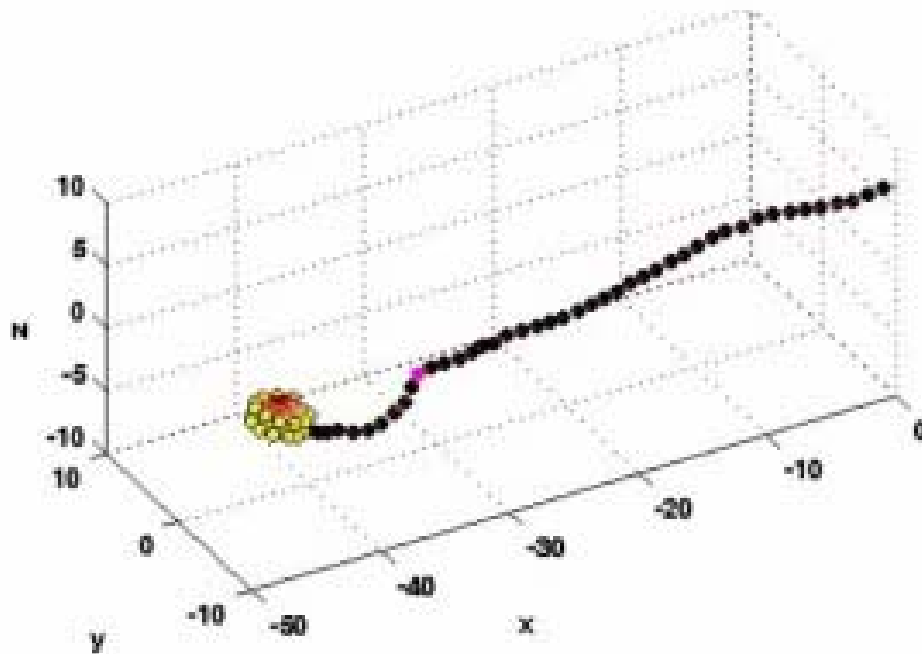
但这些染色质重塑复合体到底是如何工作的并不明了。有人认为是拆开-滑移-再绕上，有人认为是通过 **DNA** 扭曲。最近，**Kornberg** 研究组的在 **DNA** 上引入单链缺口，发现并不产生影响。因此，认为是通过**DNA** 弯曲，而不是 **DNA** 扭曲。至今，其动力学过程仍不清楚。

Histone octamer slides along DNA driven by a DNA bending



Movie

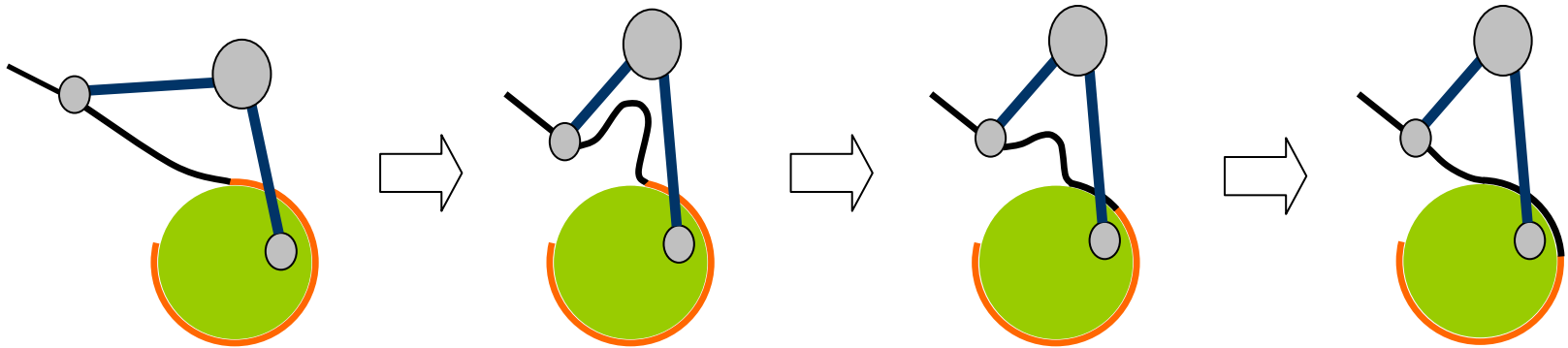
If more than one bending are generated one by one the histone octamer will slide for a longer distance



Movie

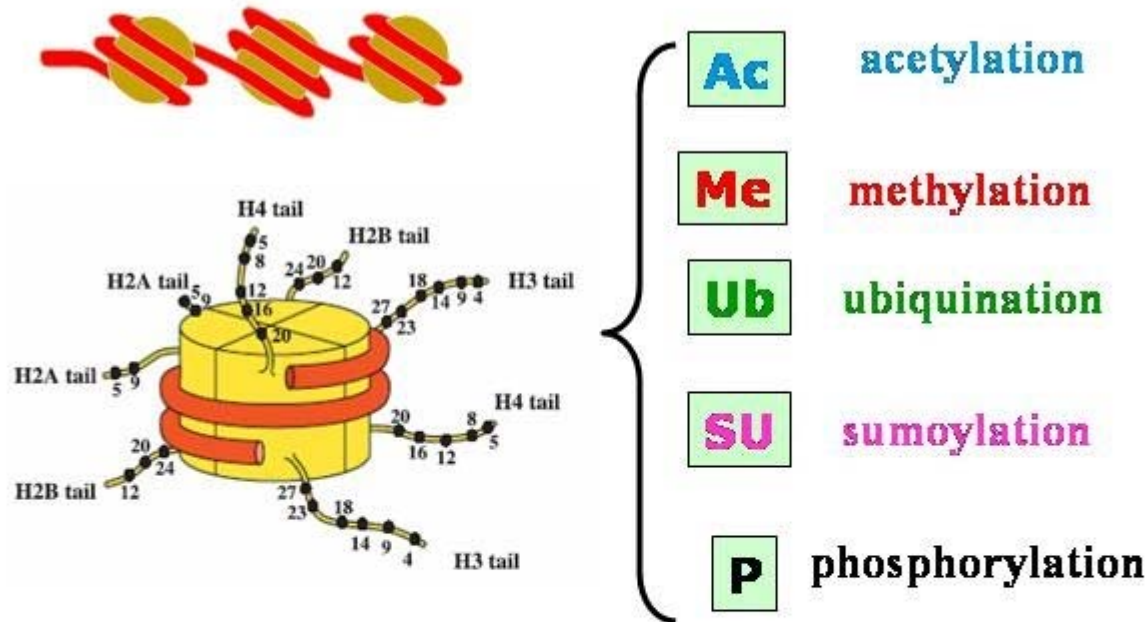
We proposed a new model:

(for the interaction between a nucleosome and a chromatin-remodeling complex)



In the model, the histone octamer (the large green sphere) slides after a DNA bending is produced by the chromatin-remodeling complex (gray balls connected with blue bars) at one side of the nucleosome. In the process of the histone octamer's sliding, the DNA loop is shrinking. At last, the DNA loop disappears and the histone octamer stops sliding.

组蛋白修饰对核小体结构的影响



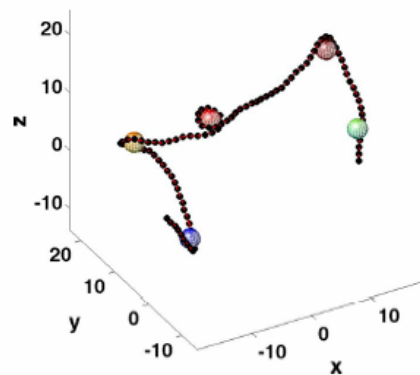
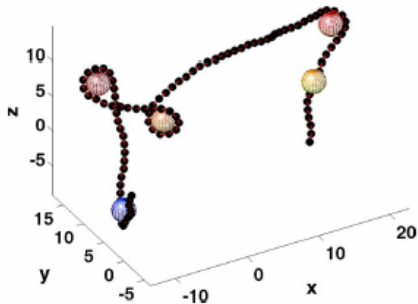
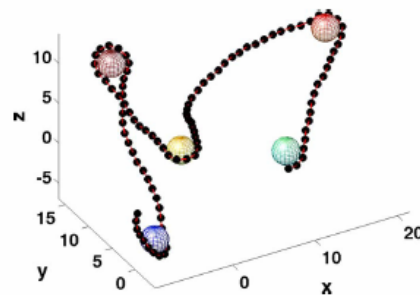
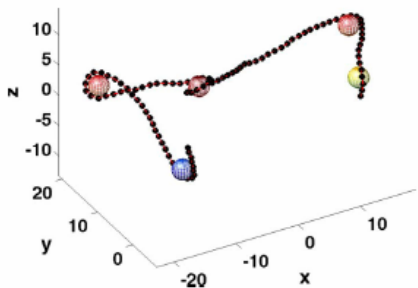
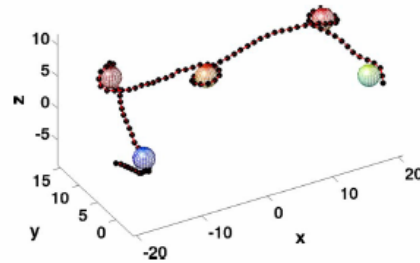
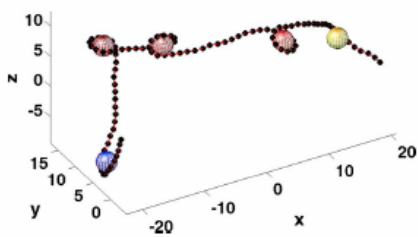
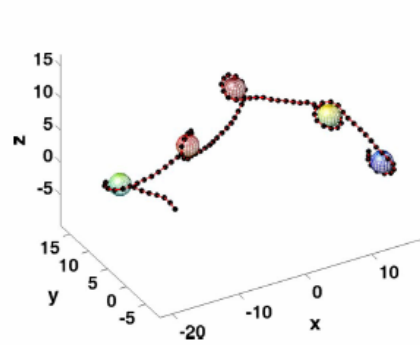
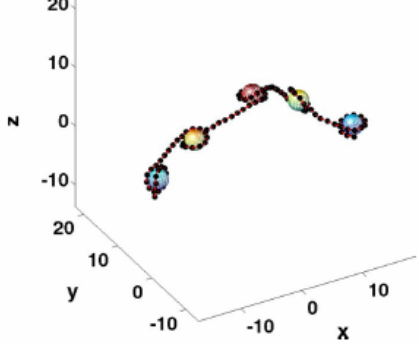
The figure illustrates nucleosome models and major posttranslational modifications which play essential roles in gene expression regulation and disease processes

我们通过改变DNA与组蛋白八聚体相互作用力的大小，描述组蛋白修饰的影响。

根据：组蛋白的磷酸化和乙酰化均改变组蛋白上带的电荷，使其正电荷减少（或增加负电荷），从而减弱了与DNA的结合力。

Modified histones

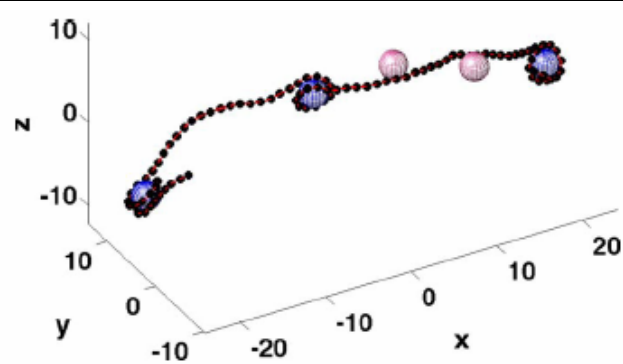
ε 值从 4.0 变为 3.0



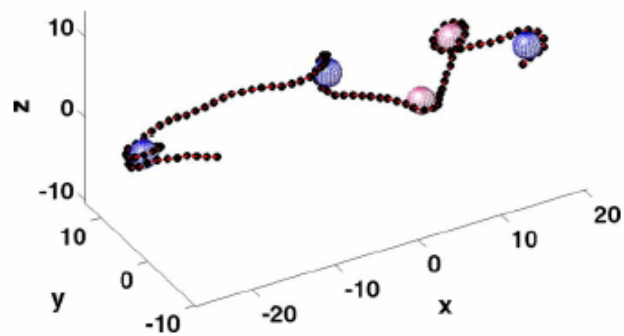
时刻:

(a) 0s; (b) 1.29s; (c) 3.86s; (d) 5.45s;
(e) 6.52s; (f) 9.26s; (g) 11.88s; (h) 12.87s

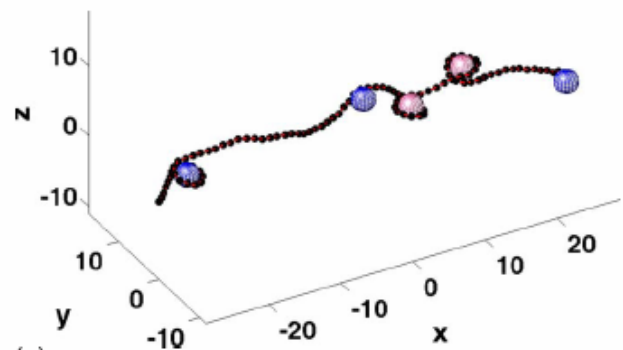
两种组蛋白共存情况



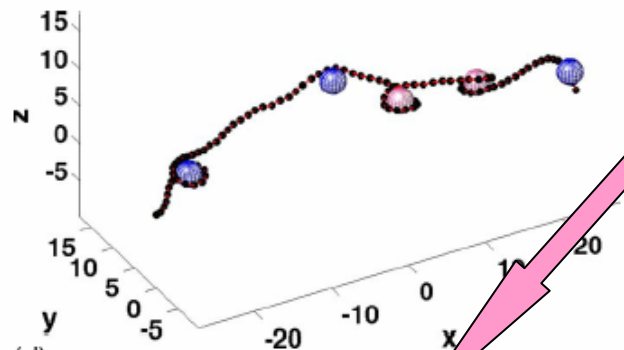
(a)



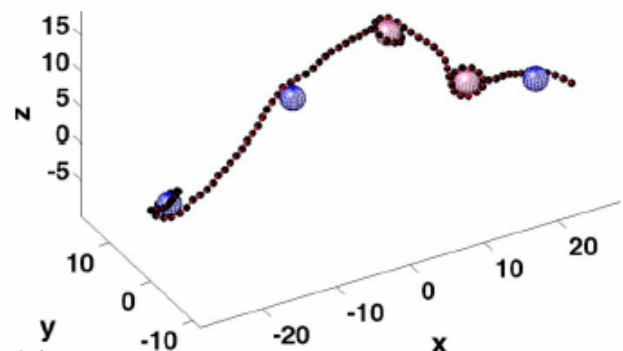
(b)



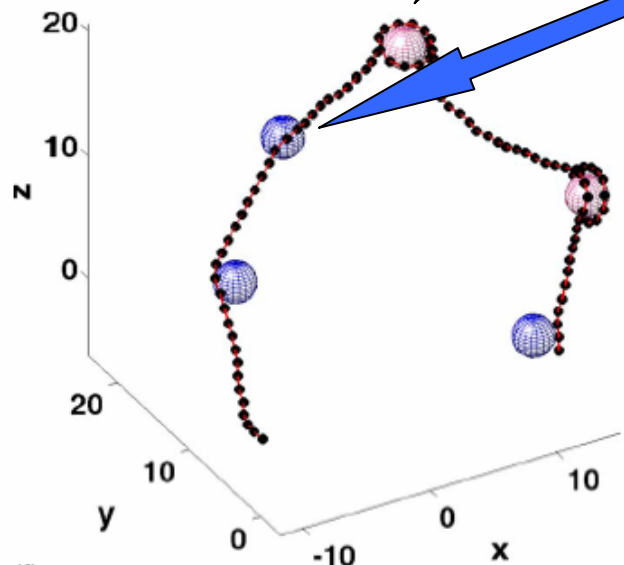
(c)



(d)



(e)



(f)

未修饰
 $\epsilon=4.0$

修饰
 $\epsilon=3.0$

时刻:

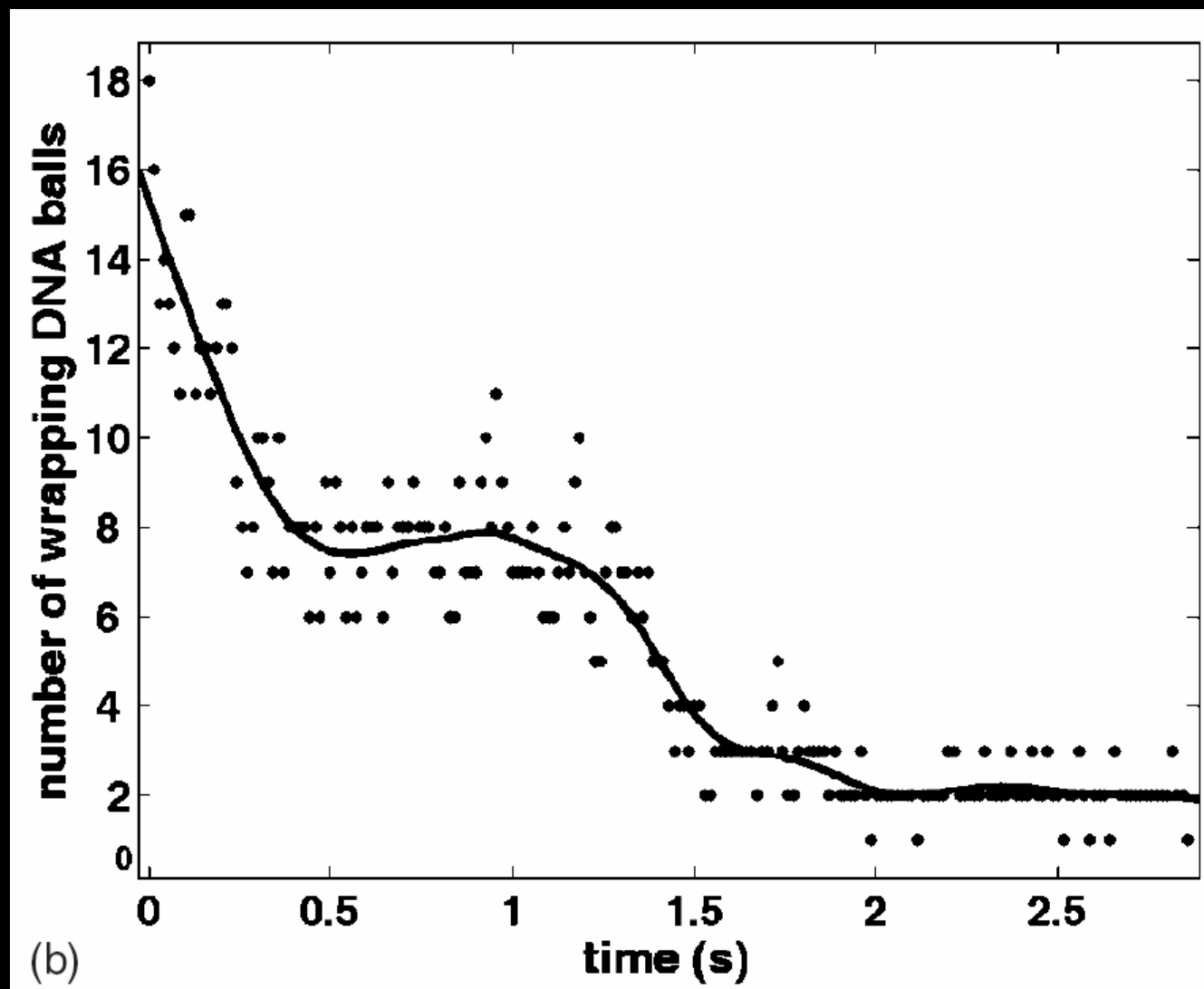
(a) 0s; (b) 1.22s;

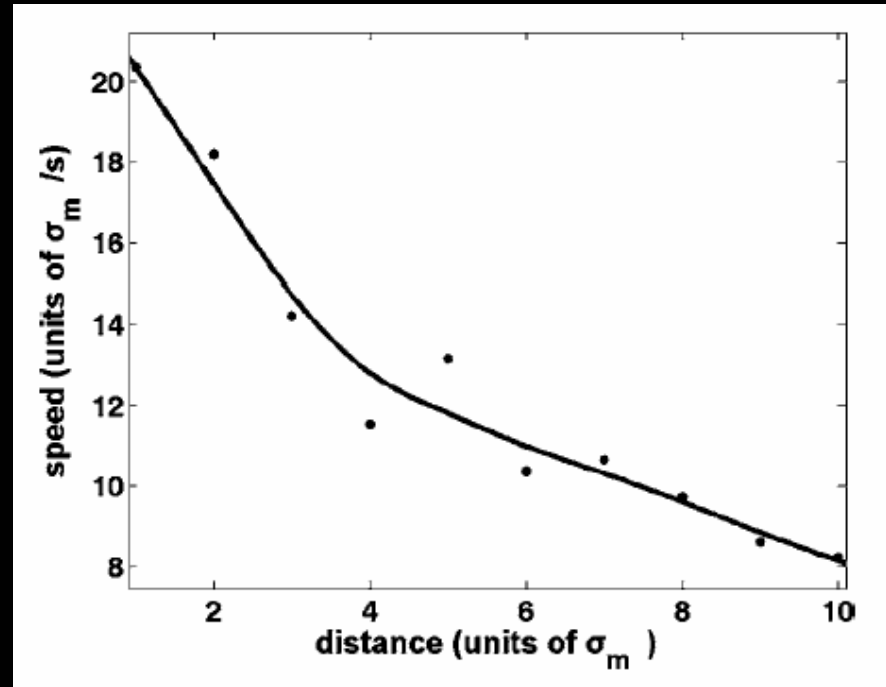
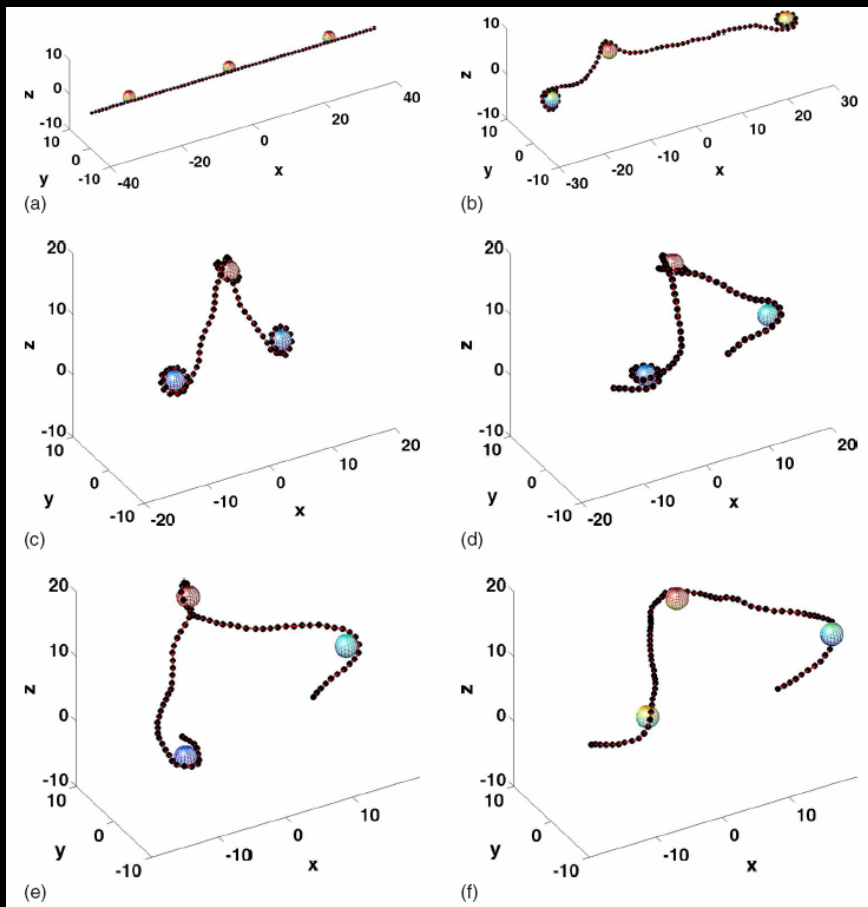
(c) 2.12s; (d) 3.22s;

(e) 5.08s; (f) 17.11s

DNA从修饰后的组蛋白上脱离过程有台阶效用

下图纵轴表示仍绕在组蛋白上的DNA长度，横轴是修饰后的时间



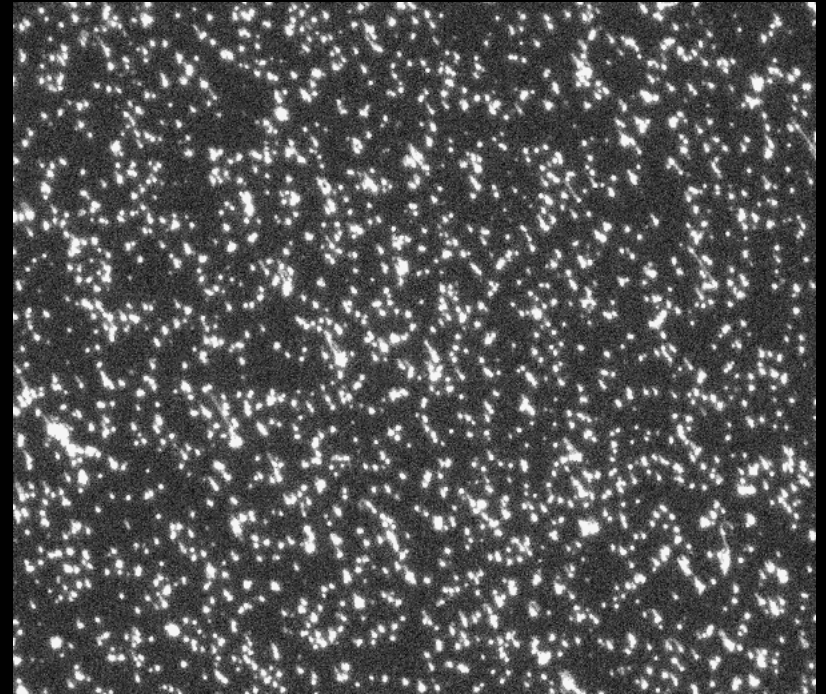
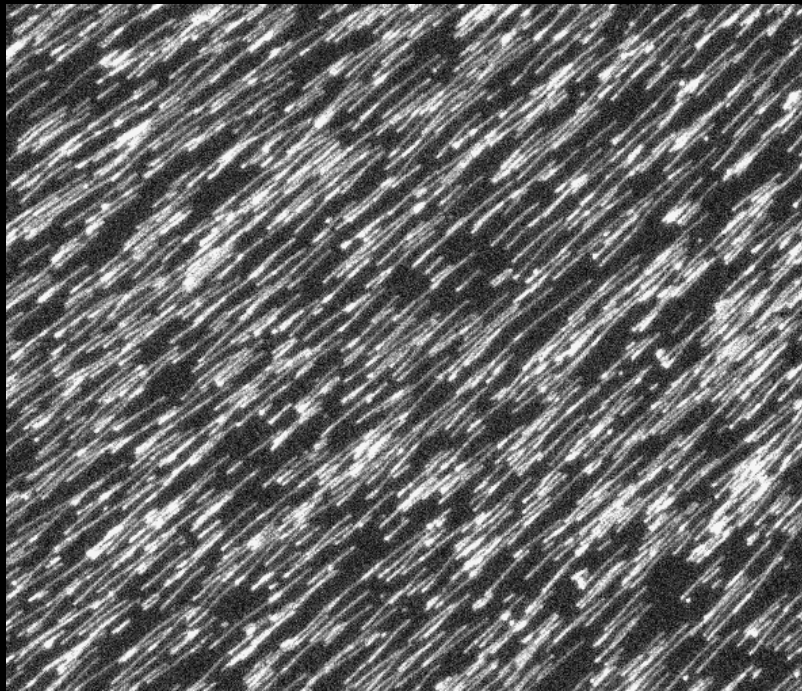


端点效用：接近DNA断裂点的组蛋白更快地结合和解离

生物学意义：DNA损伤(断裂)的快速修复

分子梳实验结果：

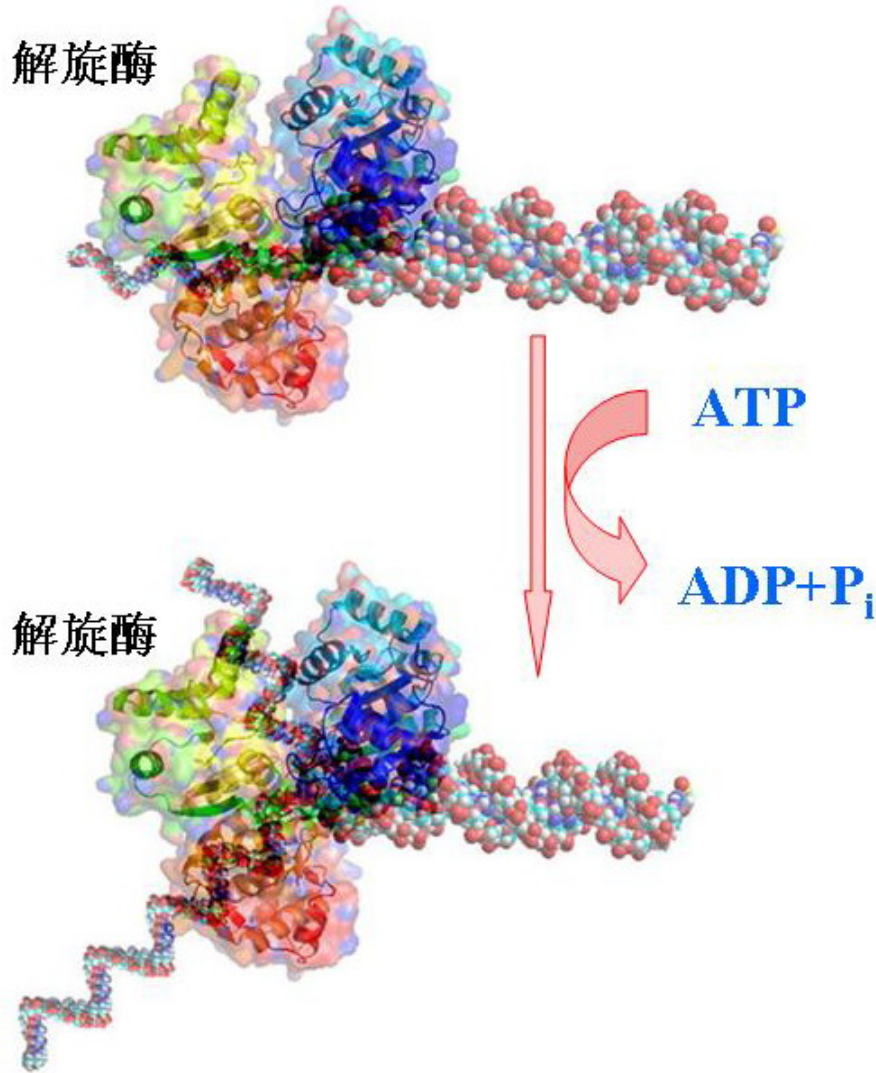
DNA与组蛋白结合后，利用分子梳方法展开



增加组蛋白的浓度

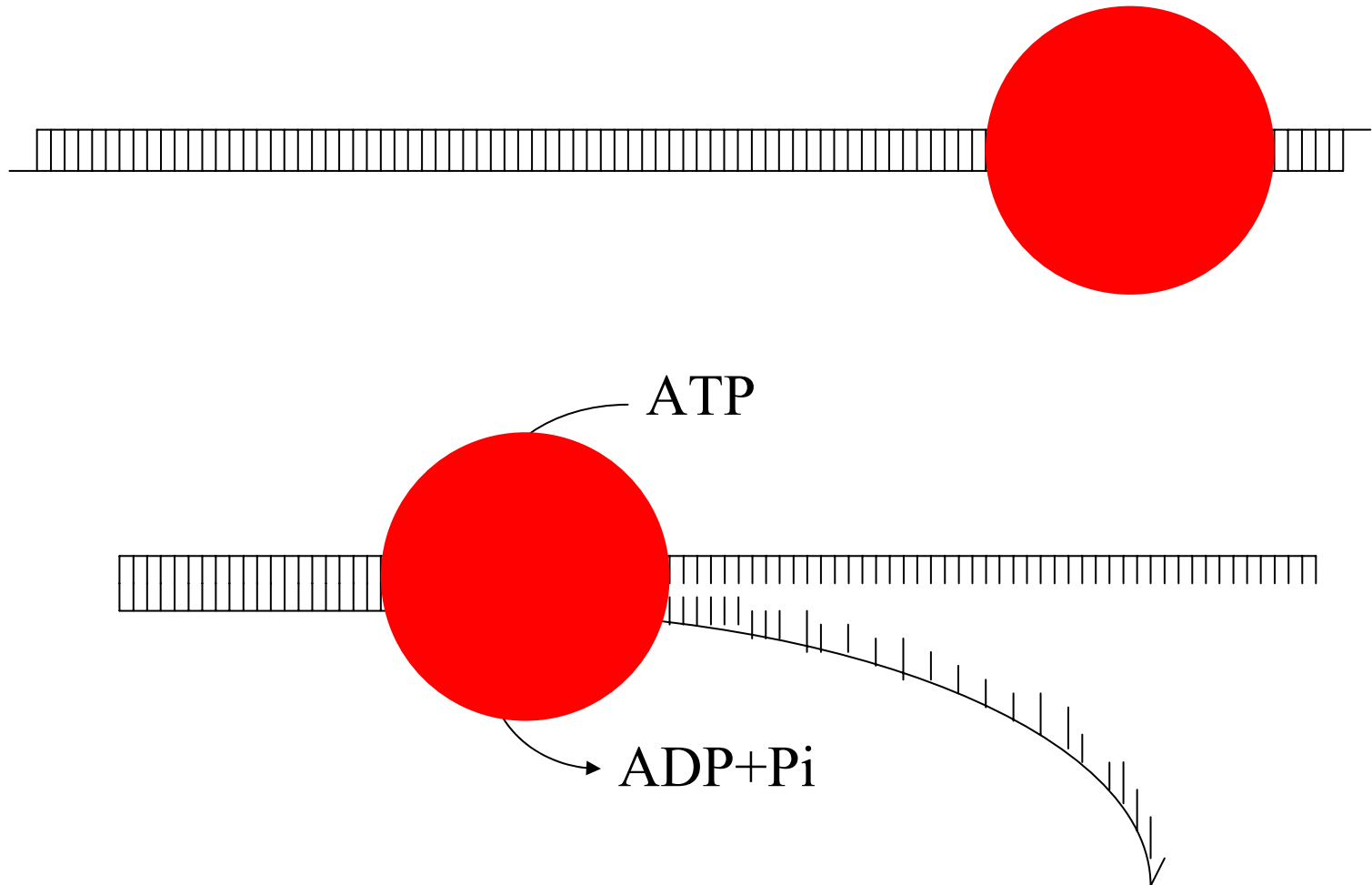
解旋酶的动力学研究

解旋酶是一种常见的马达蛋白



- 解旋酶在DNA的复制，修复，重组以及转录等代谢过程中也起着非常重要的作用
- 解旋酶广泛存在于原核、真核生物和病毒体内

解旋酶以ATP为燃料，通过水解ATP产生能量，打开DNA碱基对之间的氢键，并向前运动



解旋酶具有重要的生理学意义:

它在细胞的新陈代谢中起重要作用,
一旦出现问题, 将导致癌症和早老

RecQ 1 **unknow**

RecQ 2 **Bloom's syndrome** **Cancer**

RecQ 3 **Werner syndrome** **Cancer and aging**

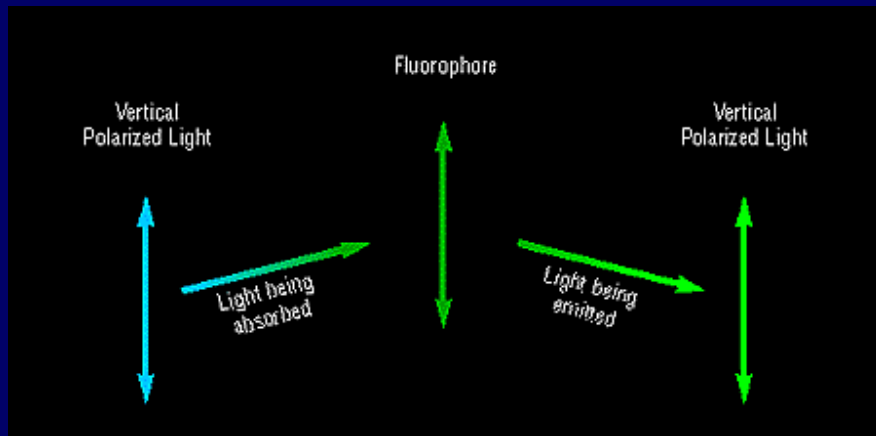
RecQ 4 **Rothmund-Thompson** **Cancer**



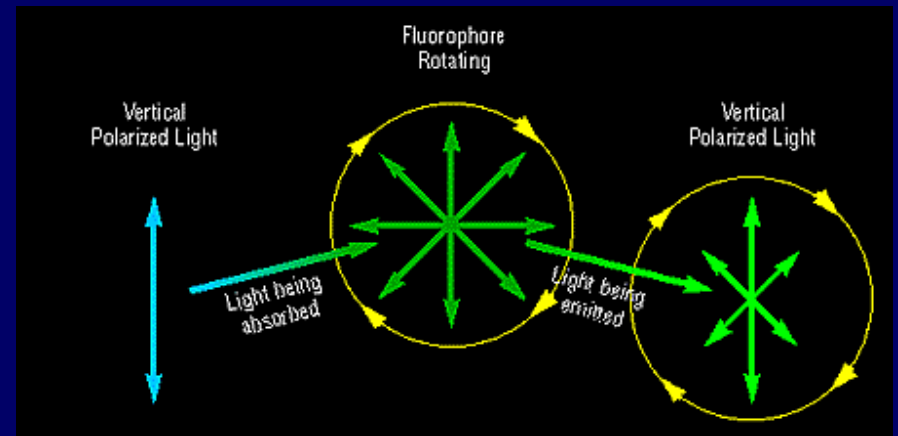
Werner's syndrome:
human premature aging



偏振荧光方法的原理

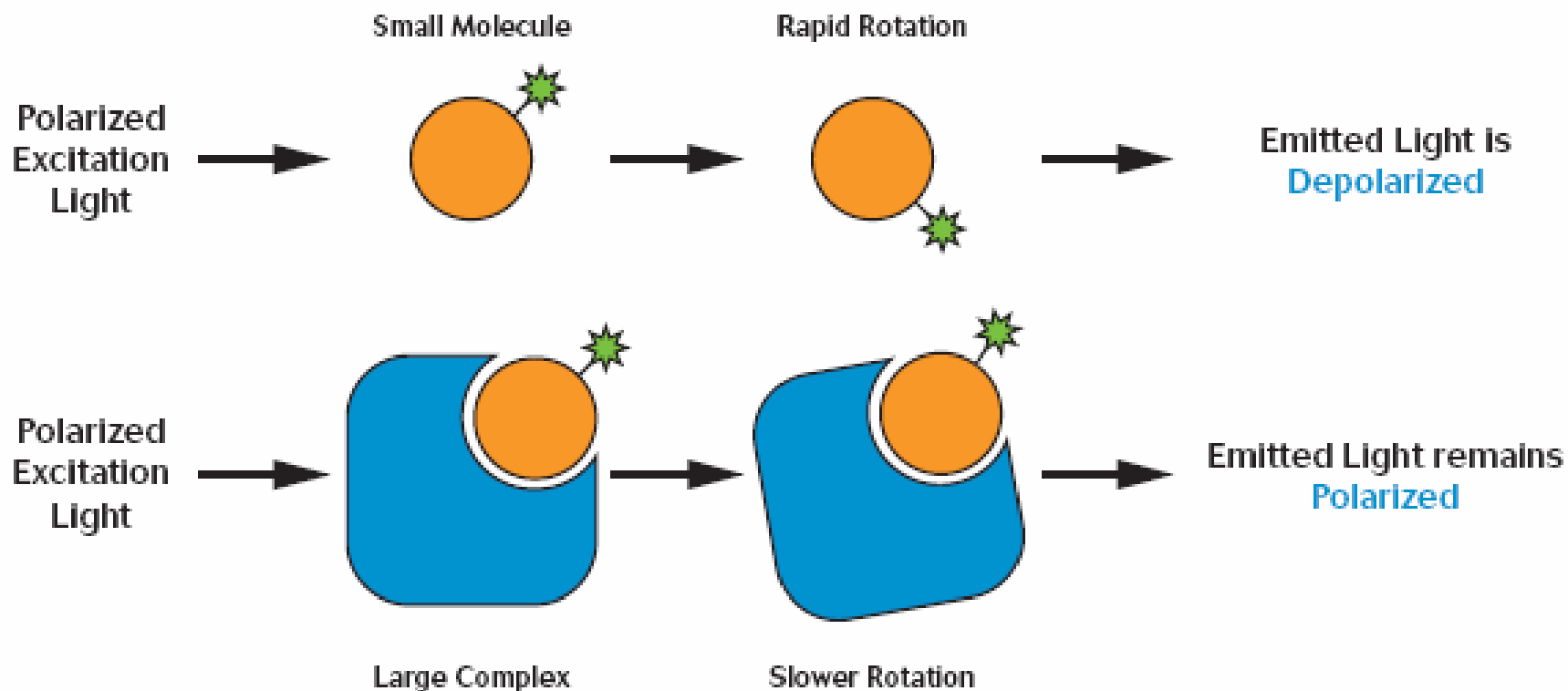


固定的分子

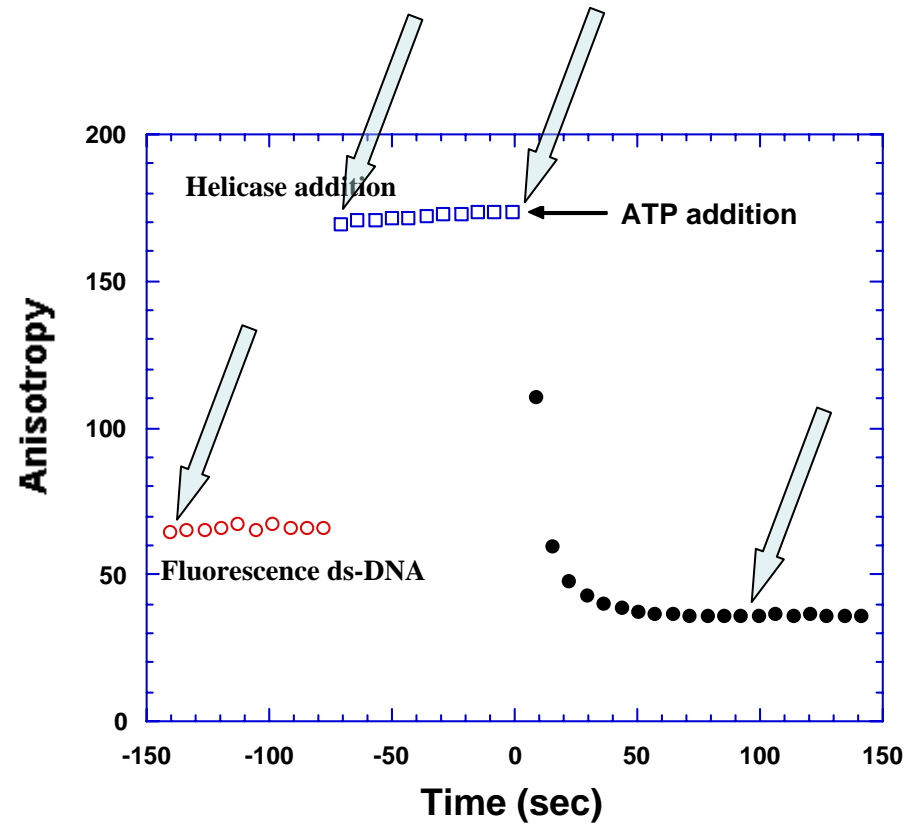
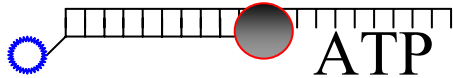


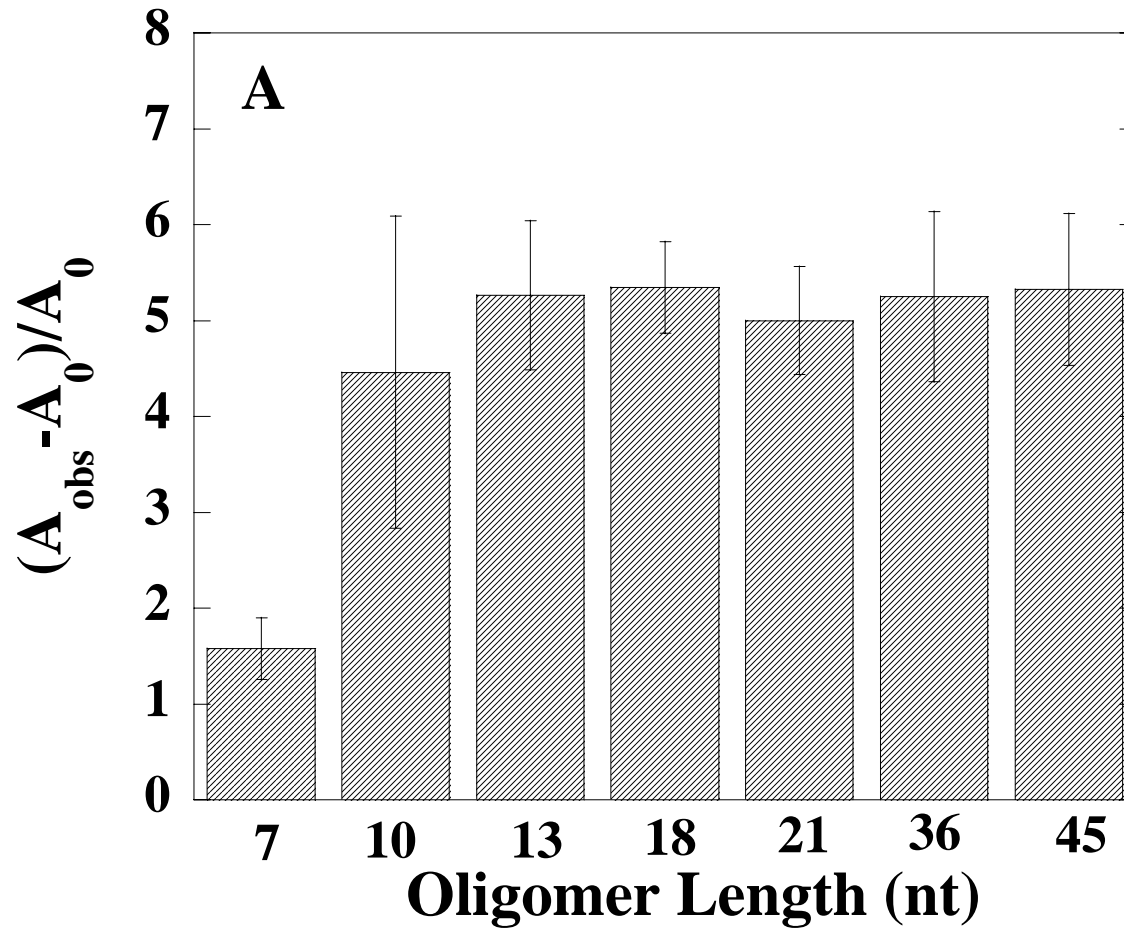
转动的分子

偏振荧光可给出大分子间的相互作用信息

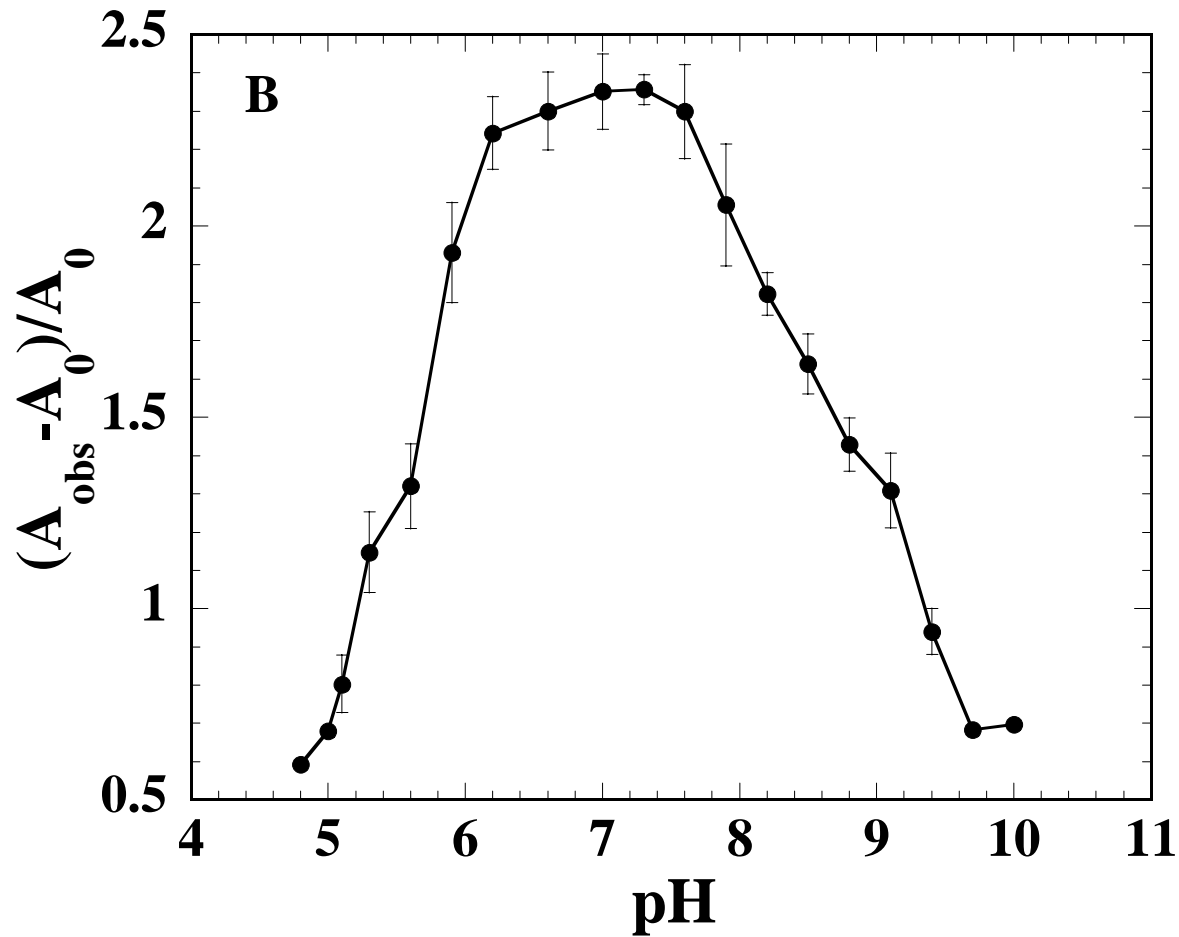


Studying DNA binding properties of RecQ with fluorescence anisotropy



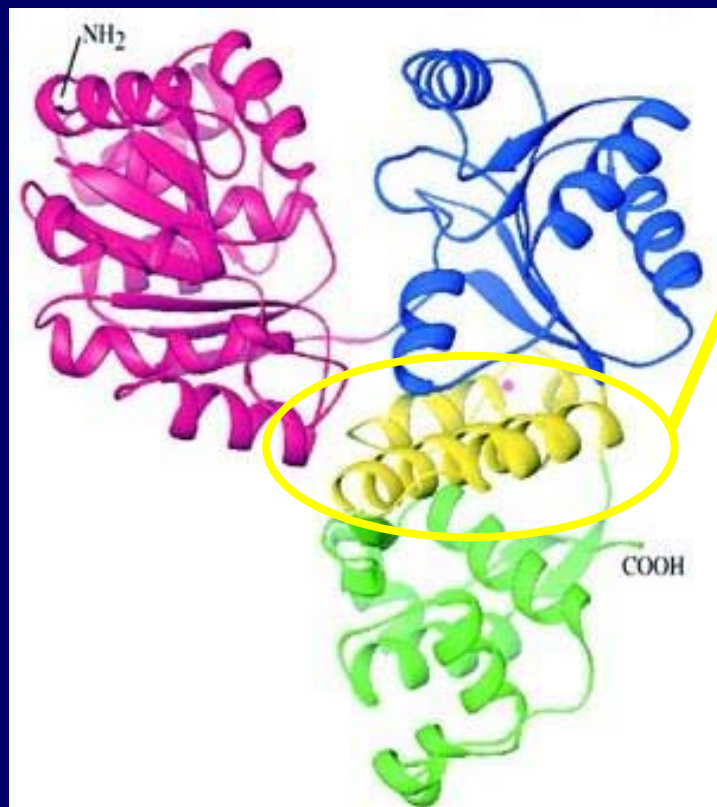
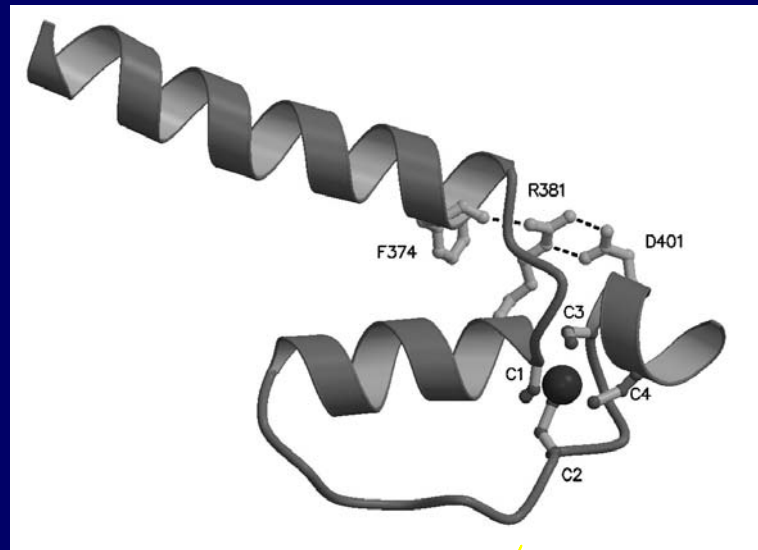
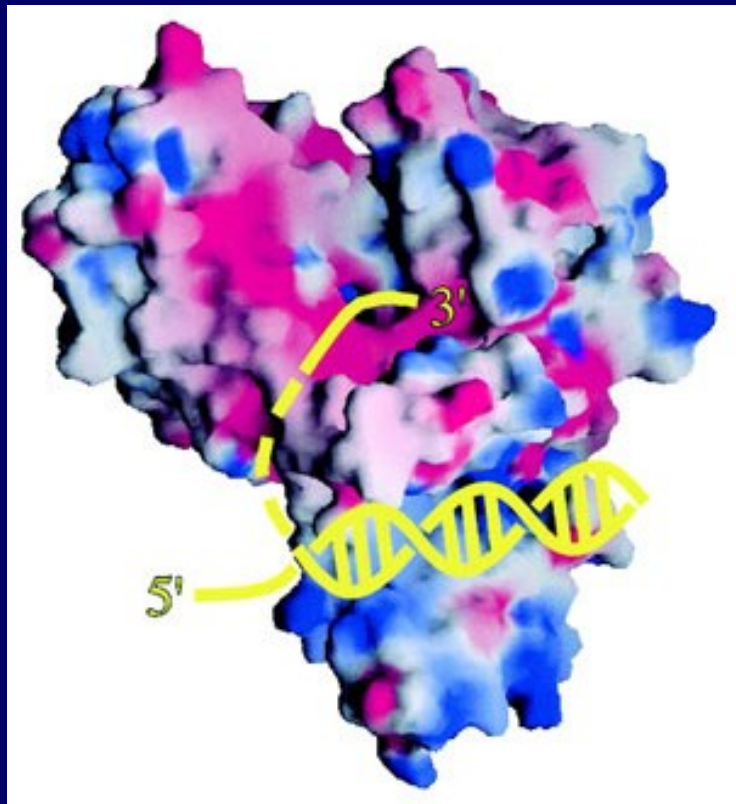


Binding of oligonucleotides (different lengths of ssDNA) by RecQ helicase.
 A_{obs} is the observed anisotropy, A_0 is the anisotropy of the oligonucleotide alone.



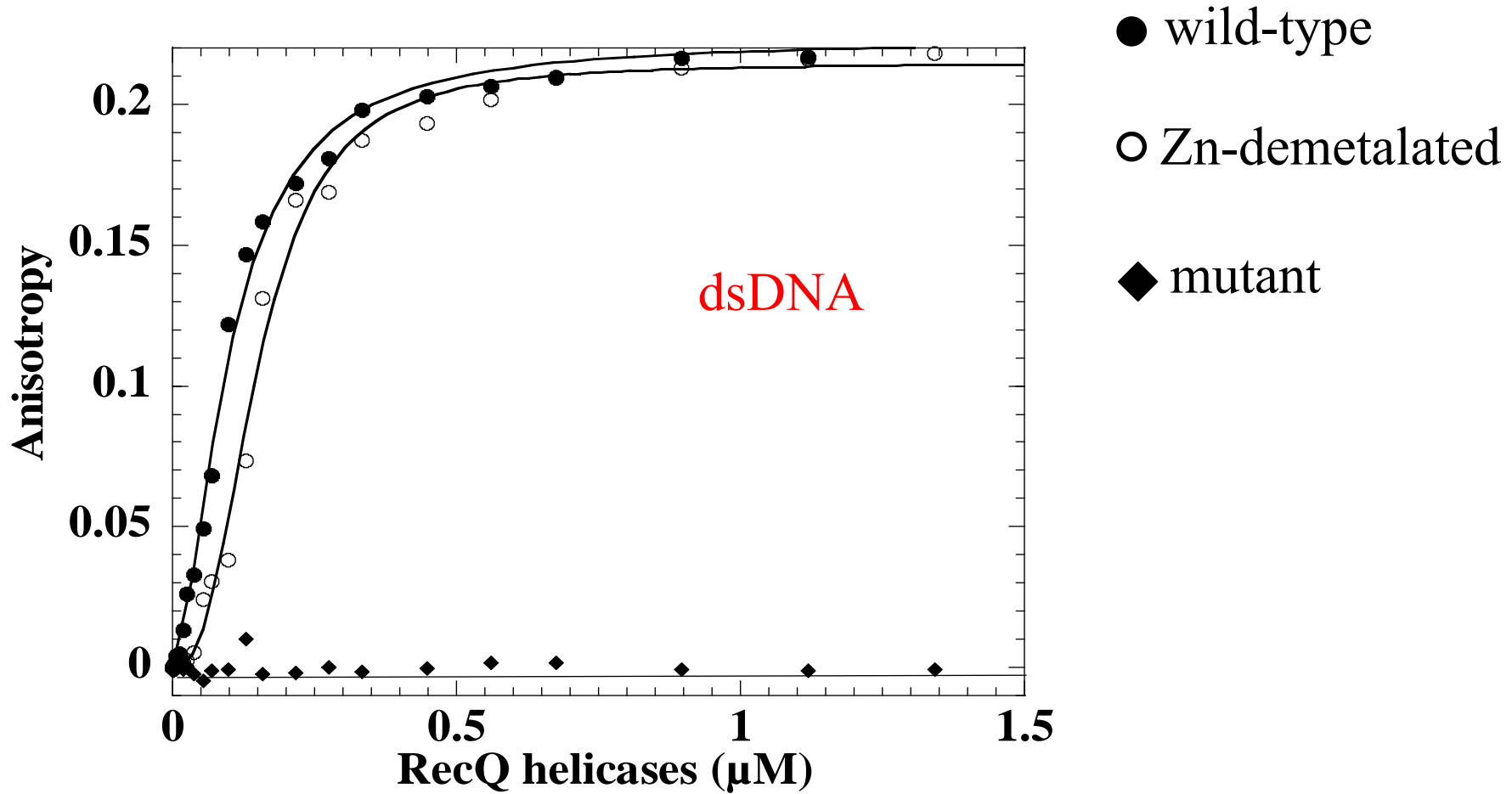
Effect of pH on the interaction of RecQ helicase with ssDNA.
5 nM 36-mer ssDNA and 200 nM RecQ helicase were used for each pH effect study.

RecQ的锌指结构域的影响

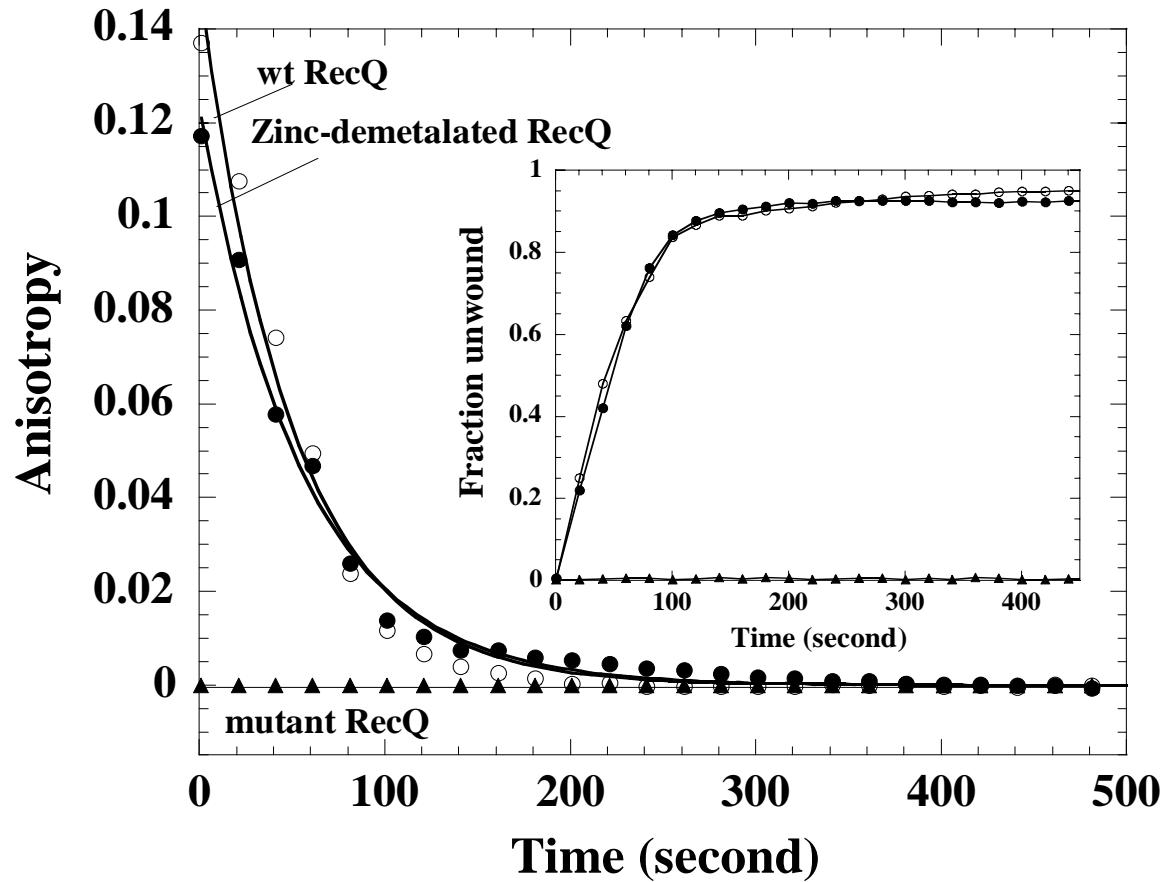


观察锌原子以及其邻近
氨基酸C2和C3的影响

◆ 对两个半胱氨酸残基Cys³⁹⁷ (C2) 和 Cys⁴⁰⁰ (C3) 残基进行了双突变，用天冬酰胺替代

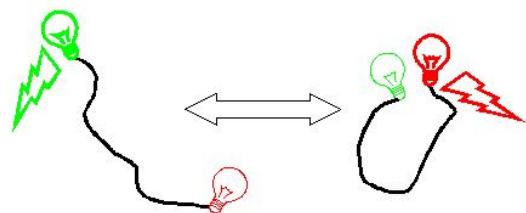


Binding equilibrium of different RecQ helicases to DNA



Kinetics of RecQ helicase-mediated DNA unwinding

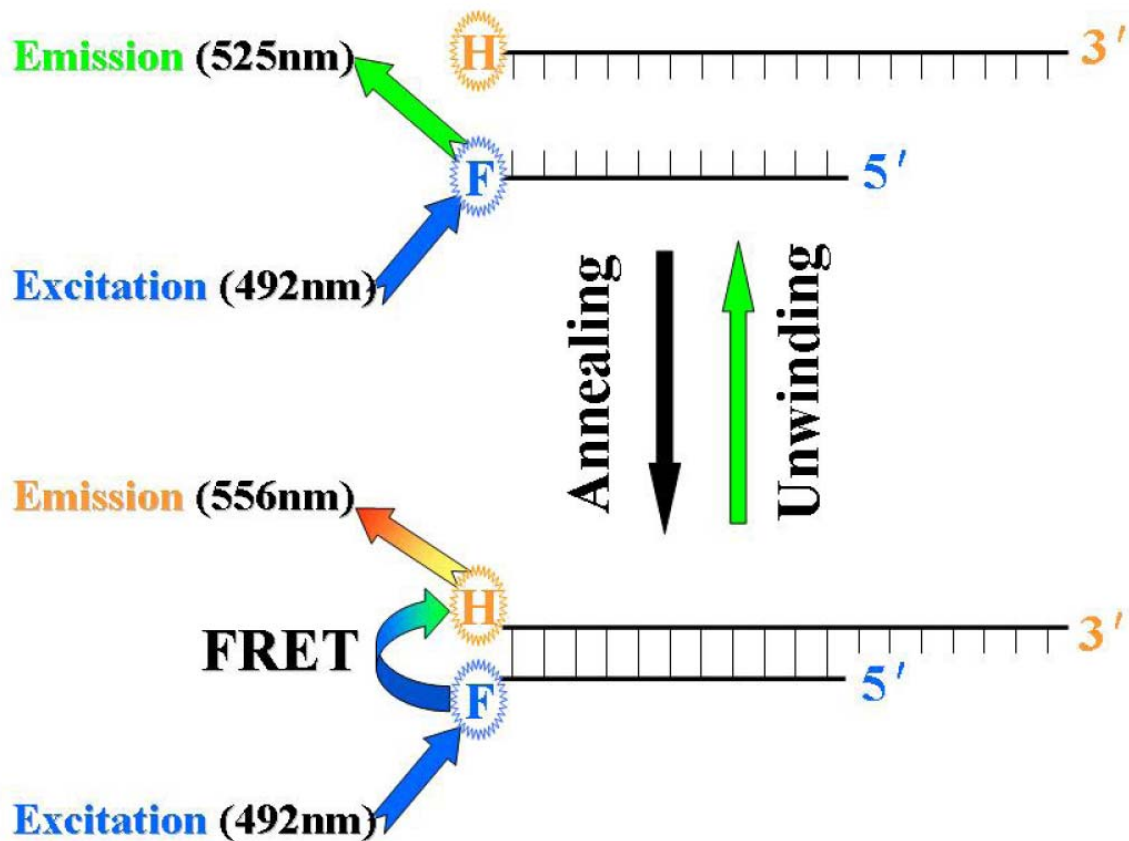
荧光共振能量转移(FRET)



Nanometer-scale Motion

Spectroscopic Change

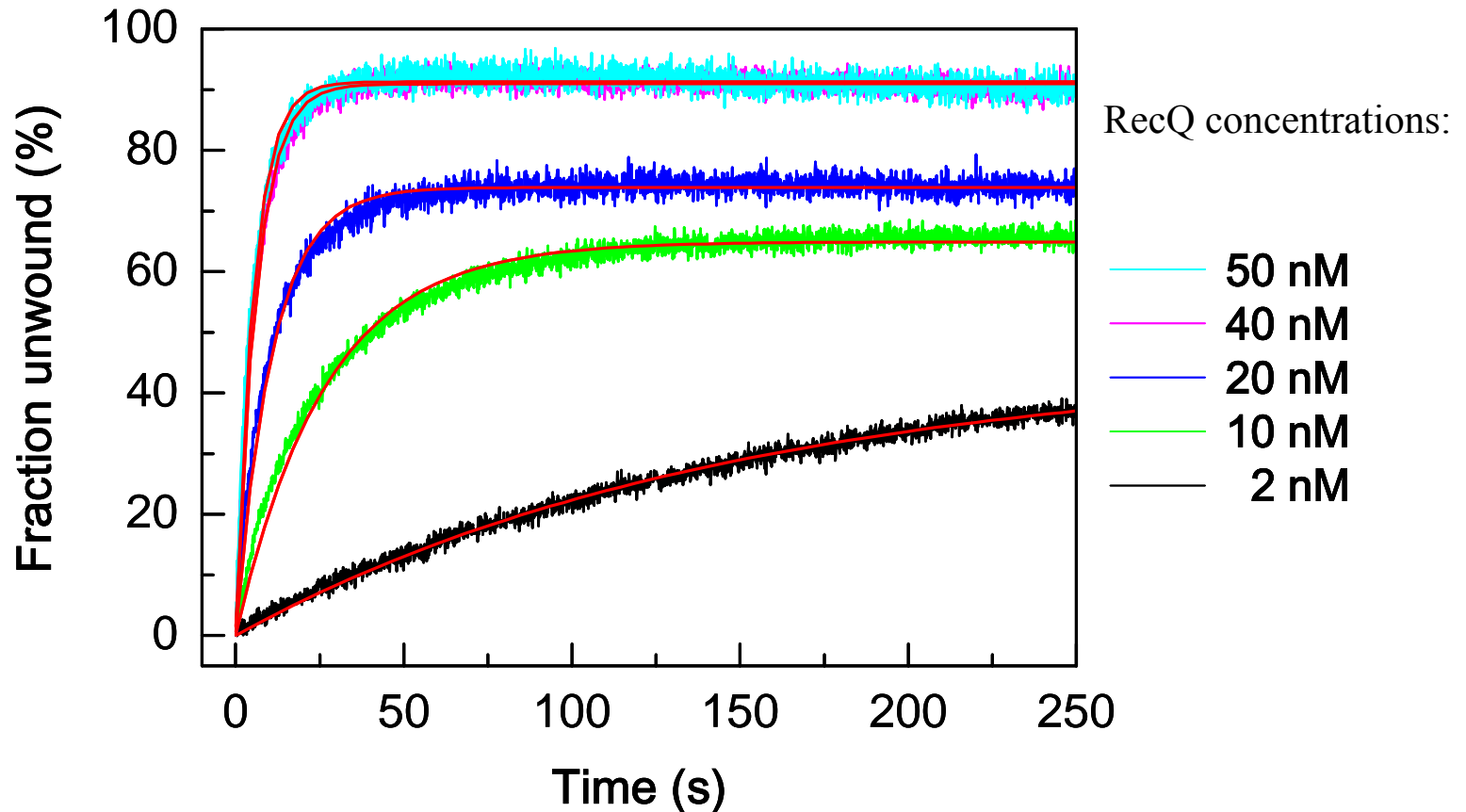
原理



我们的方法

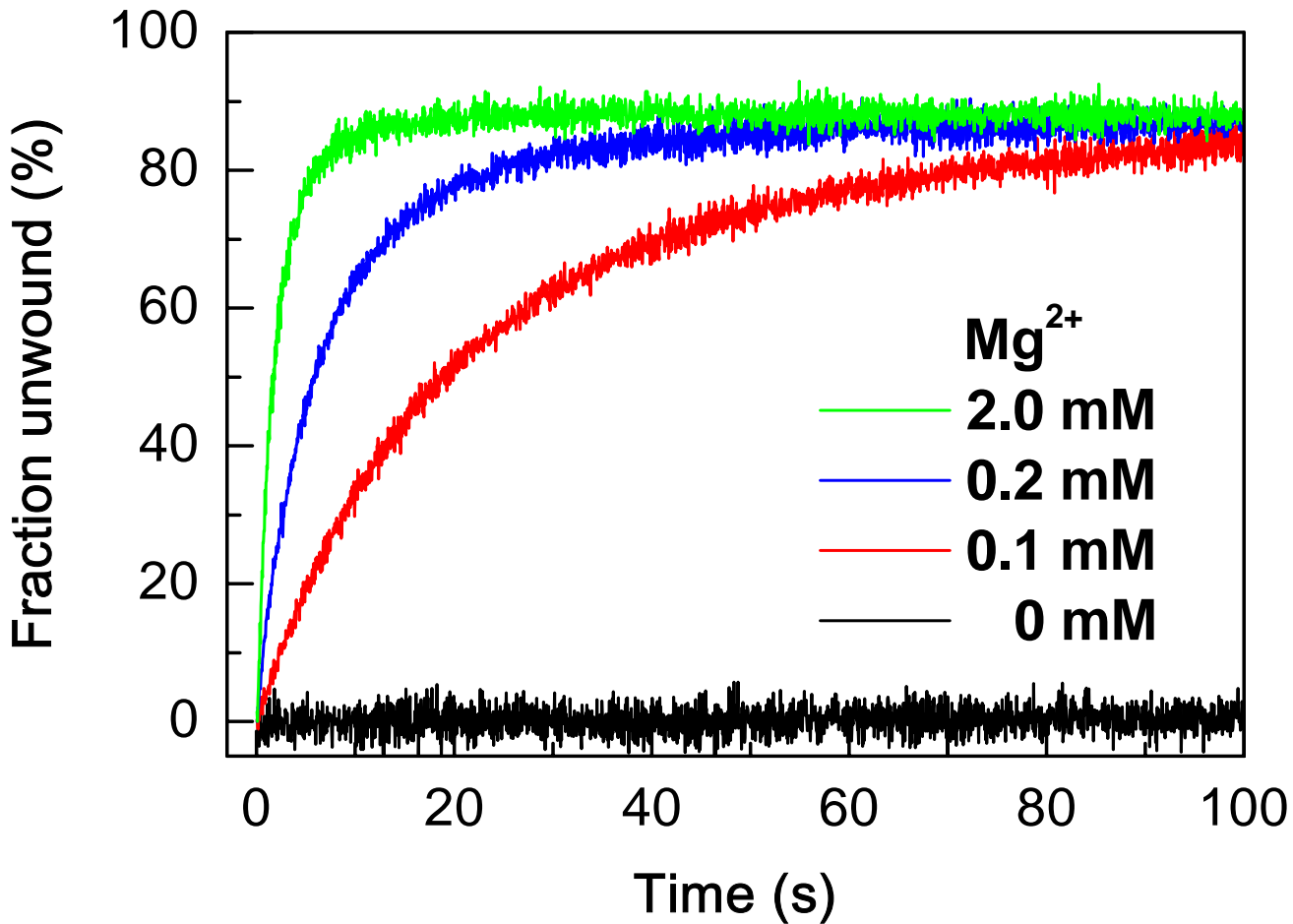
F: 荧光素 (辐射中心波长 525 nm)

H: 六氯荧光素 (辐射中心波长 556 nm)



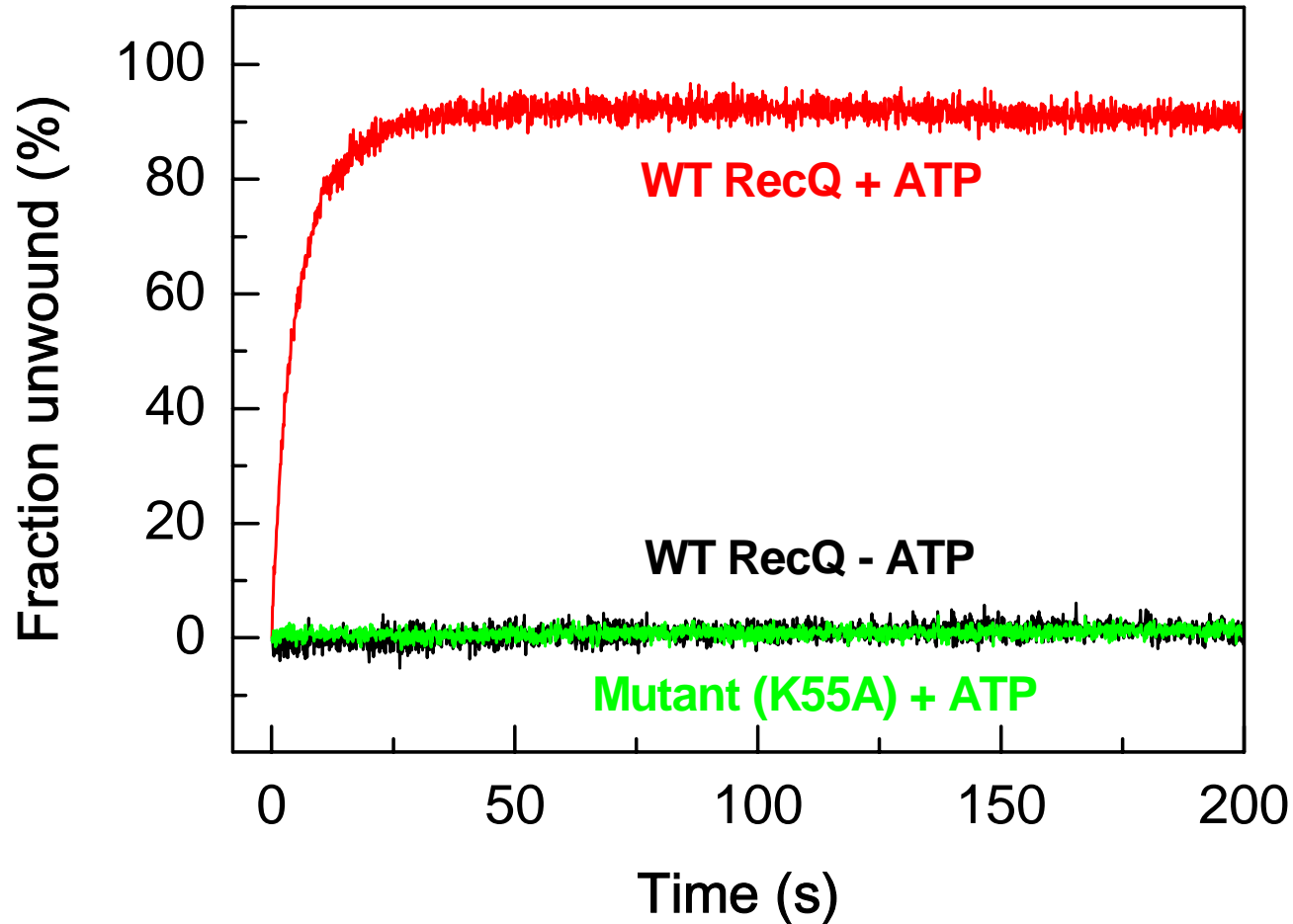
Time courses of DNA unwinding by RecQ helicase

DNA: 5'-AATCCGTCGAGCAGAGttagggtagggtagggtagctctagcagt (t_{18})
 3'-TTAGGCAGCTCGTCTC
 DNA concentration is 2 nM



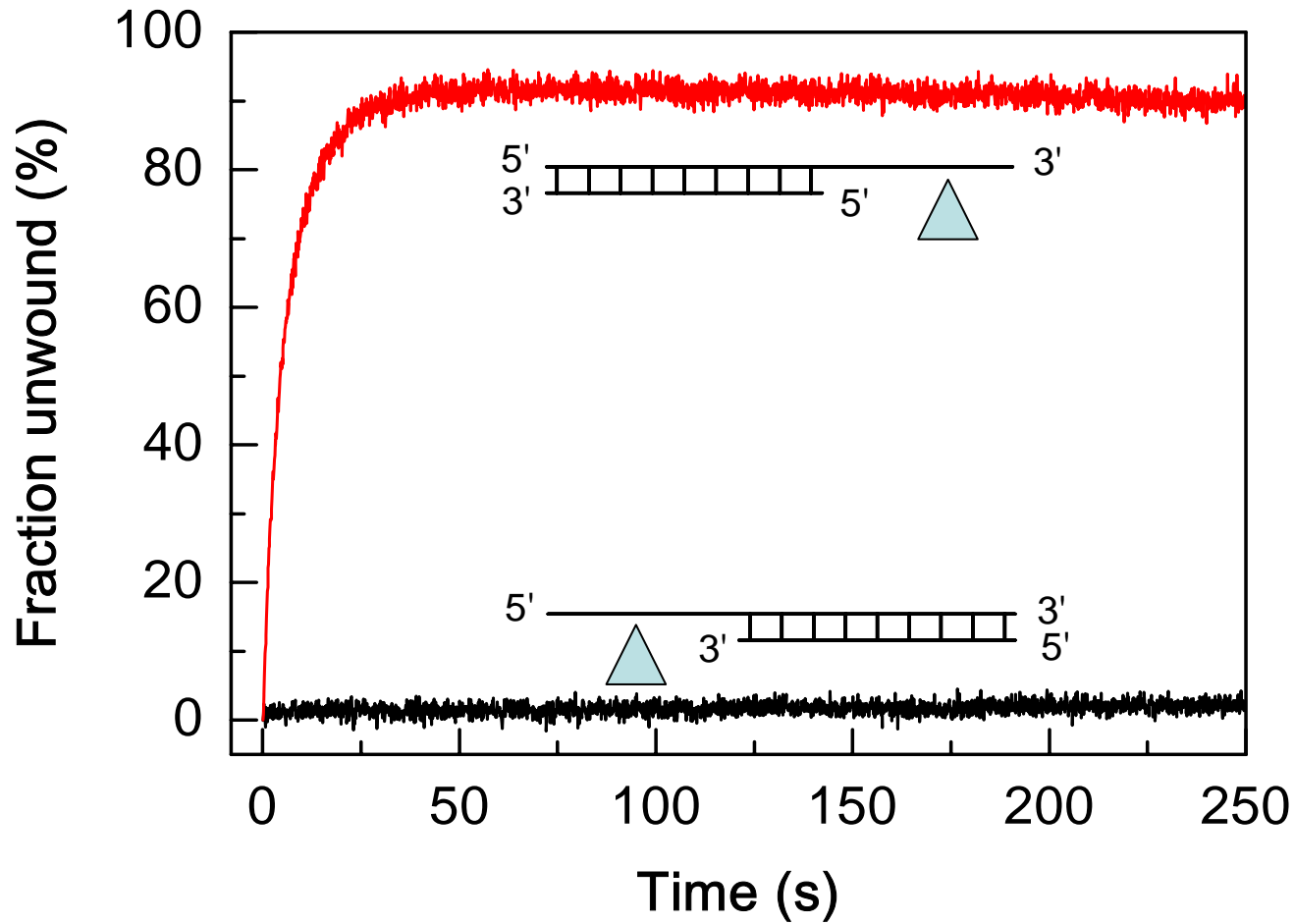
镁离子的影响巨大

**DNA unwinding by RecQ helicase
is dependent on Mg²⁺**



点突变的影响，ATP依赖性

Unwinding of the 3' → 5' substrate by RecQ helicase is absolutely dependent on ATP



马达运动的方向性

RecQ helicase displays 3' → 5' polarity in DNA unwinding

RecQ 解旋 DNA 的动力学参数测定

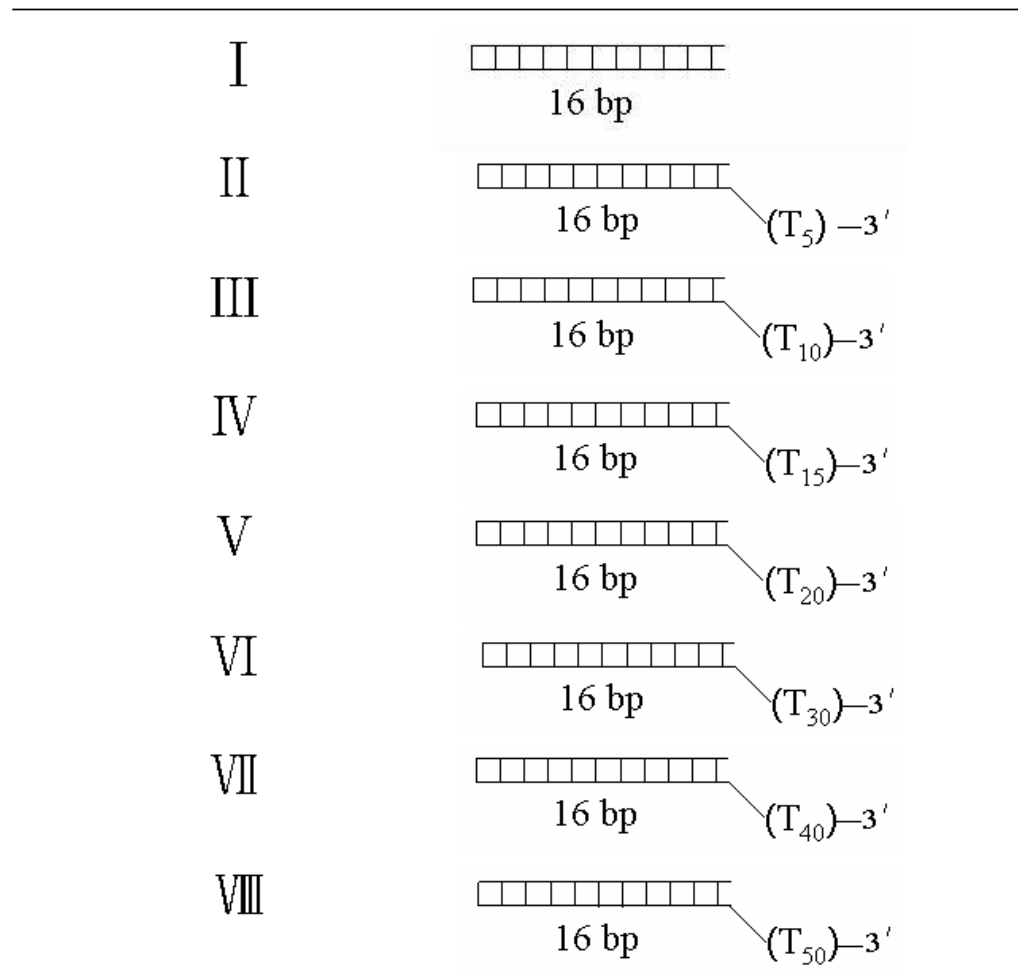
我们设计的DNA序列

Substrates used in DNA binding and unwinding assays

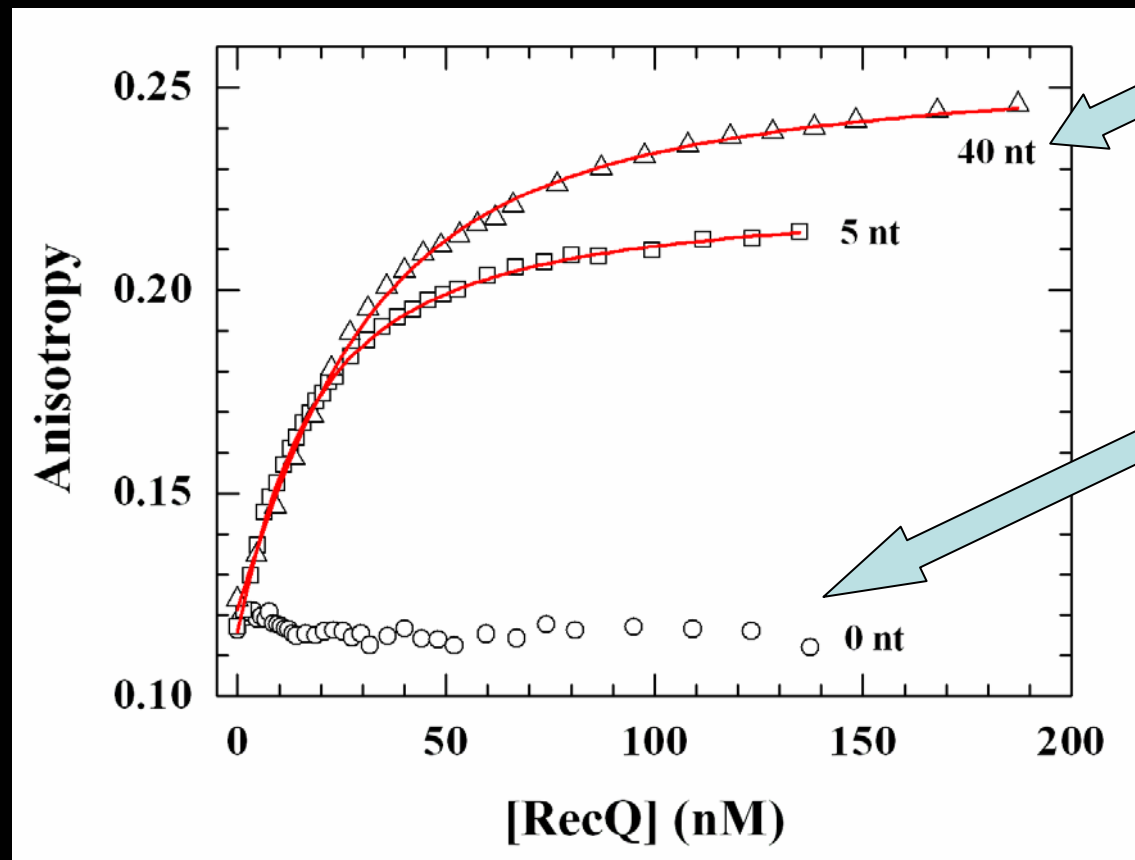
Substrate	Structure and sequence
16-bp duplex with 3'-ssDNA tail	3'-F-TTAGGCAGCTCGTCTC-5' 5'-H-AATCCGTCGAGCAGAG(dT _N)-3'
16-bp duplex with 5'-ssDNA tail	5'-F-AATCCGTCGAGCAGAG-3' 3'-TTAGGCAGCTCGTCTC(dT _N)-5'
12-bp duplex with 10-nt 3'-ssDNA tail	3'-F-GCAGCTCGTCTC-5' 5'-H-CGTCGAGCAGAG(dT ₁₀)-3'
20-bp duplex with 10-nt 3'-ssDNA tail	3'-F-CGGAGCGACGGCAGCGGTTT-5' 5'-H-GCCTCGCTGCCGTCGCCAAA(dT ₁₀)-3'
25-bp duplex with 10-nt 3'-ssDNA tail	3'-F-CGGAGCGACGGCAGCGGTTTGCTTC-5' 5'-H-GCCTCGCTGCCGTCGCCAAACGAAG(dT ₁₀)-3'
30-bp duplex with 10-nt 3'-ssDNA tail	3'-F-CGGAGCGACGGCAGCGGTTTGCTTCCAATG-5' 5'-H-GCCTCGCTGCCGTCGCCAAACGAAGGTTAC(dT ₁₀)-3'
35-bp duplex with 10-nt 3'-ssDNA tail	3'-F-CGGAGCGACGGCAGCGGTTTGCTTCCAATGTAAGG-5' 5'-H-GCCTCGCTGCCGTCGCCAAACGAAGGTTACATTCC(dT ₁₀)-3'
40-bp duplex with 10-nt 3'-ssDNA tail	3'-F-CGGAGCGACGGCAGCGGTTTGCTTCCAATGTAAGGGCGAC-5' 5'-H-GCCTCGCTGCCGTCGCCAAACGAAGGTTACATCCCGCTG(dT ₁₀)-3'

我们设计的DNA底物 3'单链尾部结构

16-bp duplex with 3' -ssDNA substrates



利用荧光偏振方法 测定RecQ对ss/dsDNA底物的结合

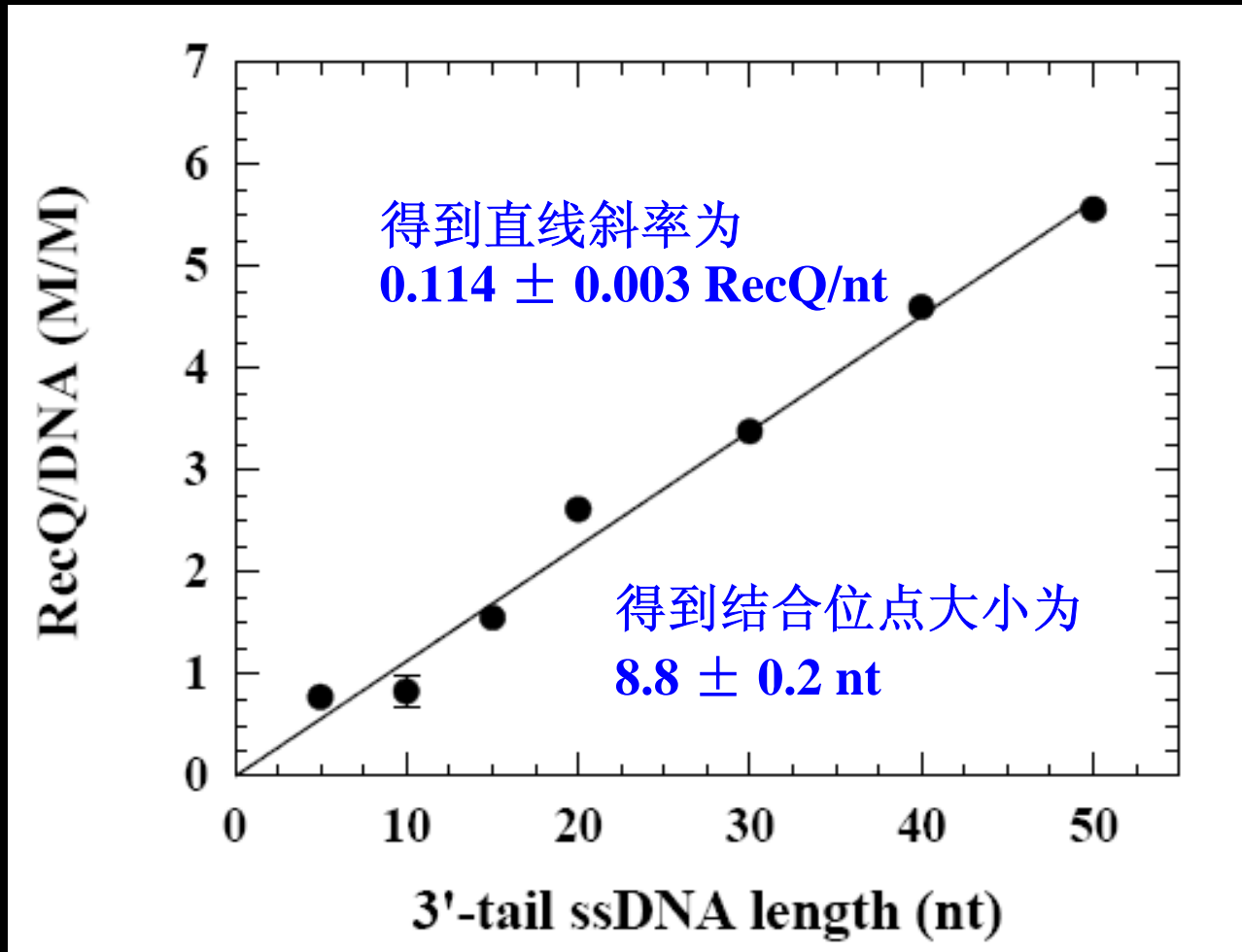


3'尾部ssDNA长度

说明没有单链DNA不行

16-bp DNA

RecQ结合ss/dsDNA的计量数 随单链尾部的长度呈线性递增关系



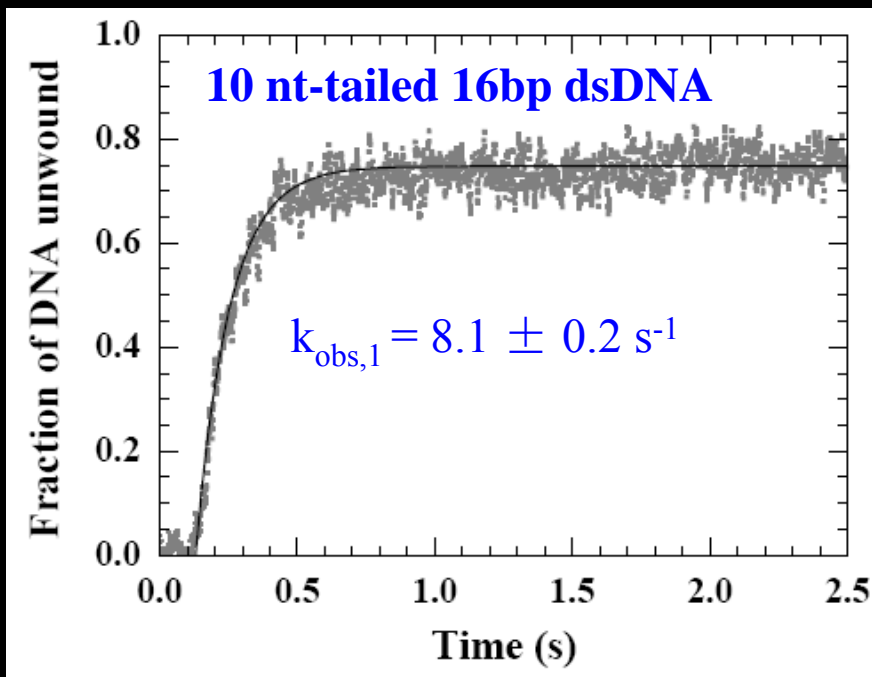
16-bp DNA

RecQ的结合位点大小为 **8.8nt**，但与3'末端单链尾部长度的 **5nt** 的DNA底物的结合仍然很强，**揭示一个事实：ss/dsDNA交叉结构对RecQ有更强的结合。**

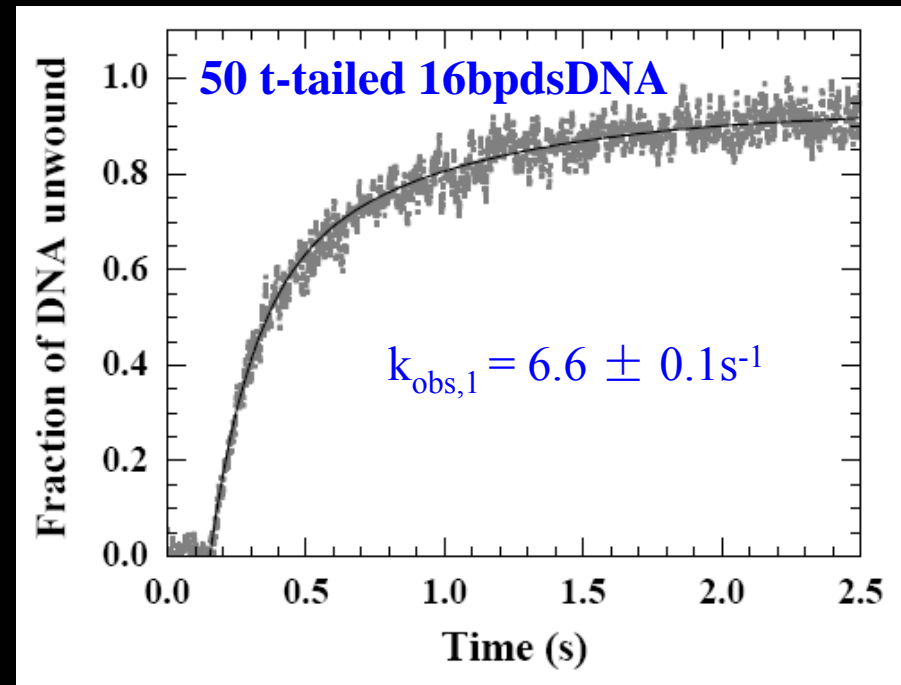
单转换动力学实验（加入过量的蛋白诱捕剂）表明
解旋效率不依赖于单链尾部长度的

证实RecQ以单体形式工作

◆ 短尾部的 DNA 底物



◆ 长尾部的 DNA 底物



对反应快相的解旋速率影响很小，只对慢相有影响

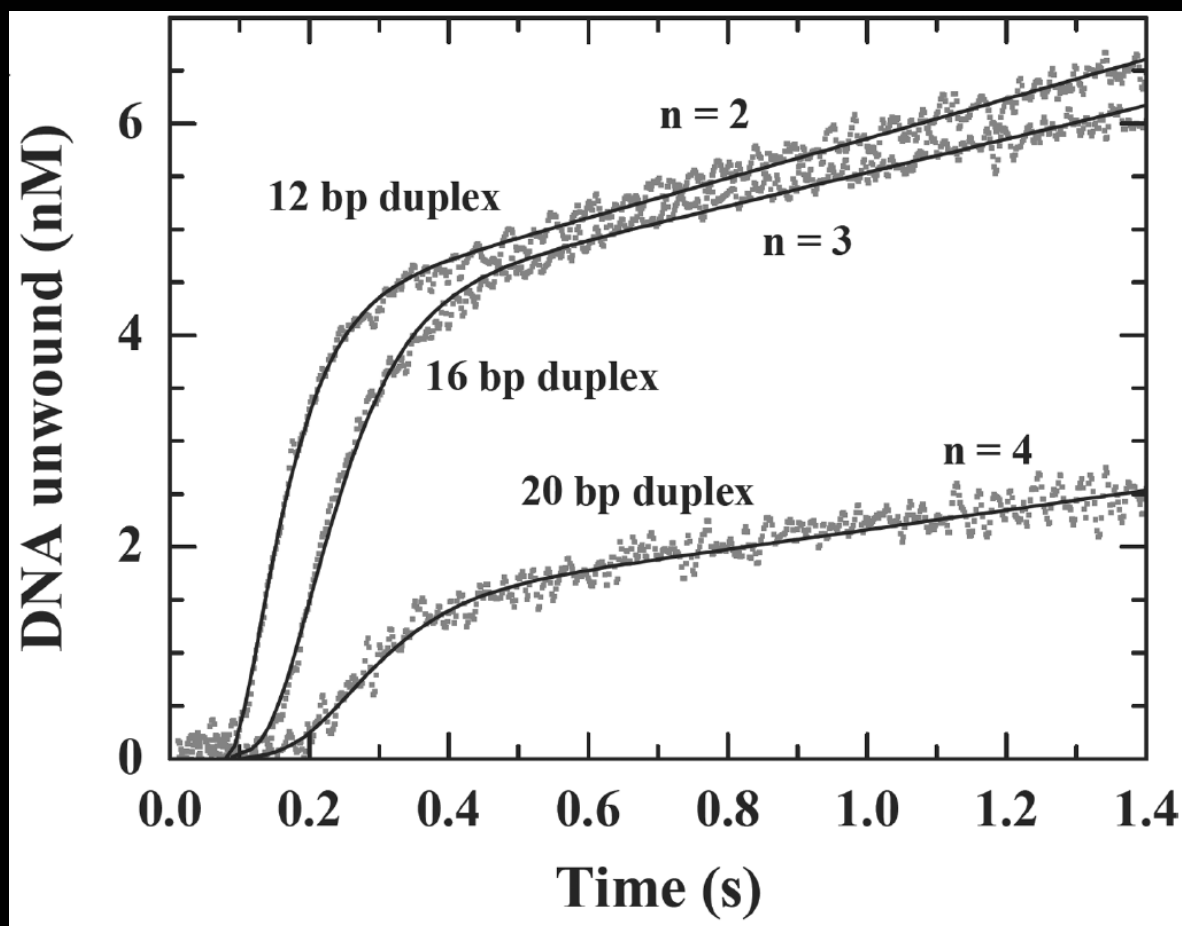
利用**FRET**进行预稳态动力学实验 (**Pre-steady-state kinetics**)

在极短的时间内，检测稳态过程之前第一步酶促反应的中间产物。可检测到 **RecQ** 对 **DNA** 的瞬时解旋过程。

要求的一个重要条件是 **DNA** 底物浓度必须大于或远大于酶的浓度，这样可以确保在反应开始时，几乎所用的活性 **RecQ** 分子都结合到 **DNA** 底物上。

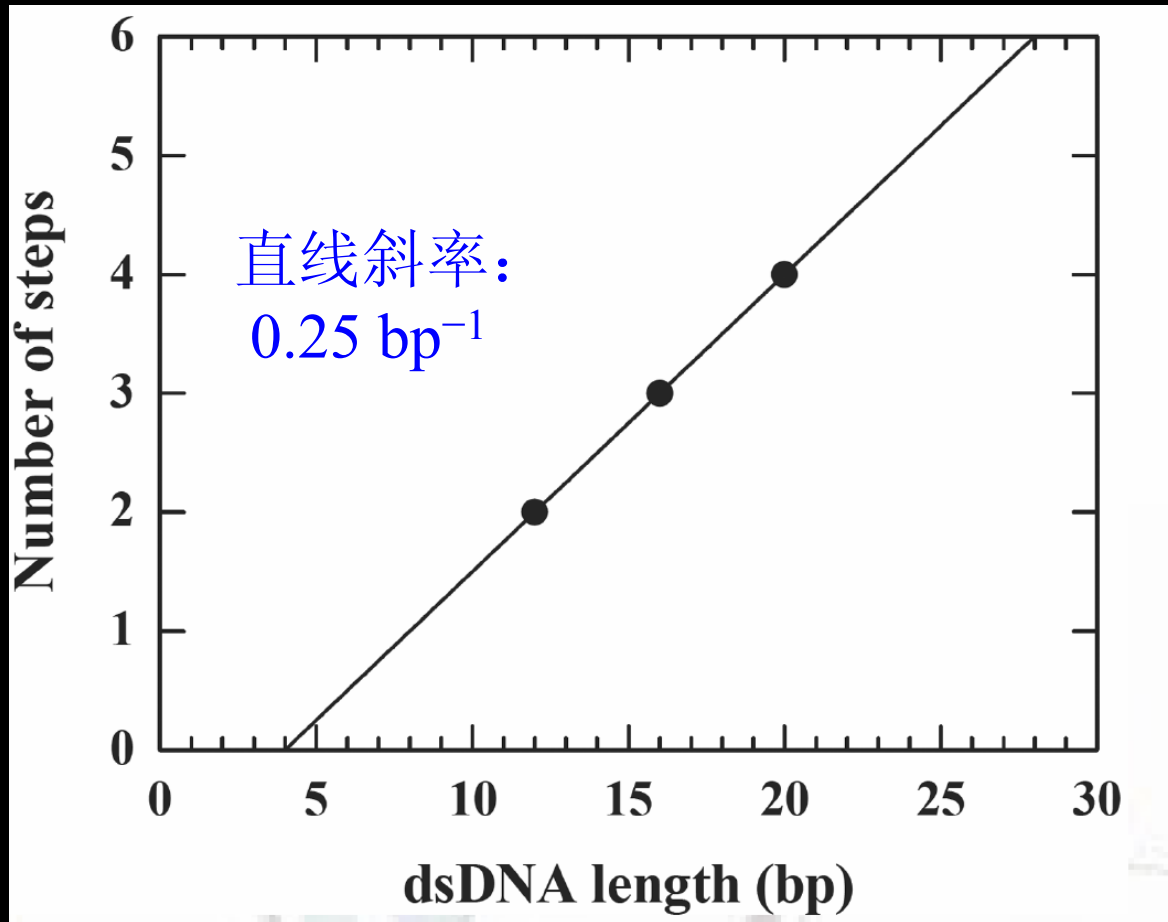
然后快速加入 **1mM ATP** 触发反应，就可以实时检测由 **RecQ** 解旋 **DNA** 导致的荧光素 (**F**) 荧光强度的增强。

利用不同 dsDNA 长度的预稳态动力学实验



得到RecQ的解旋速率为 84bp/s

利用步数与dsDNA长度的关系



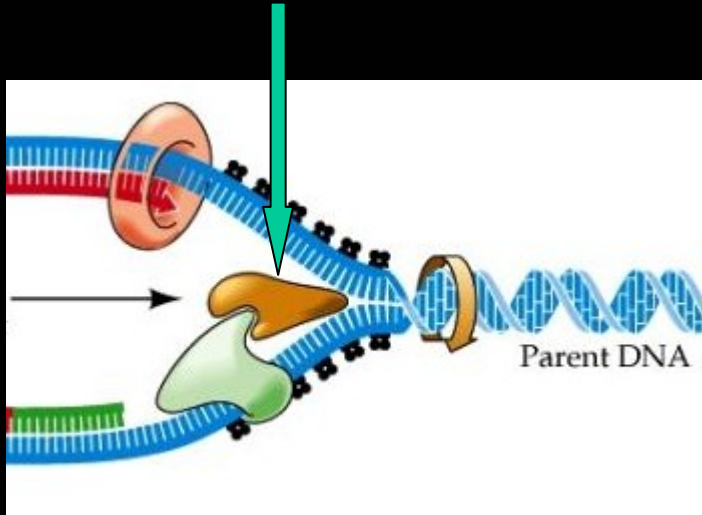
得到RecQ的解旋步幅大小为4 bp

Bloom综合征蛋白与 DNA 的作用动力学

正常的 **BLM** 蛋白是一种人类的解旋酶。

如果 *BLM* 基因产生变异，会导致一种疾病：**Bloom综合征**

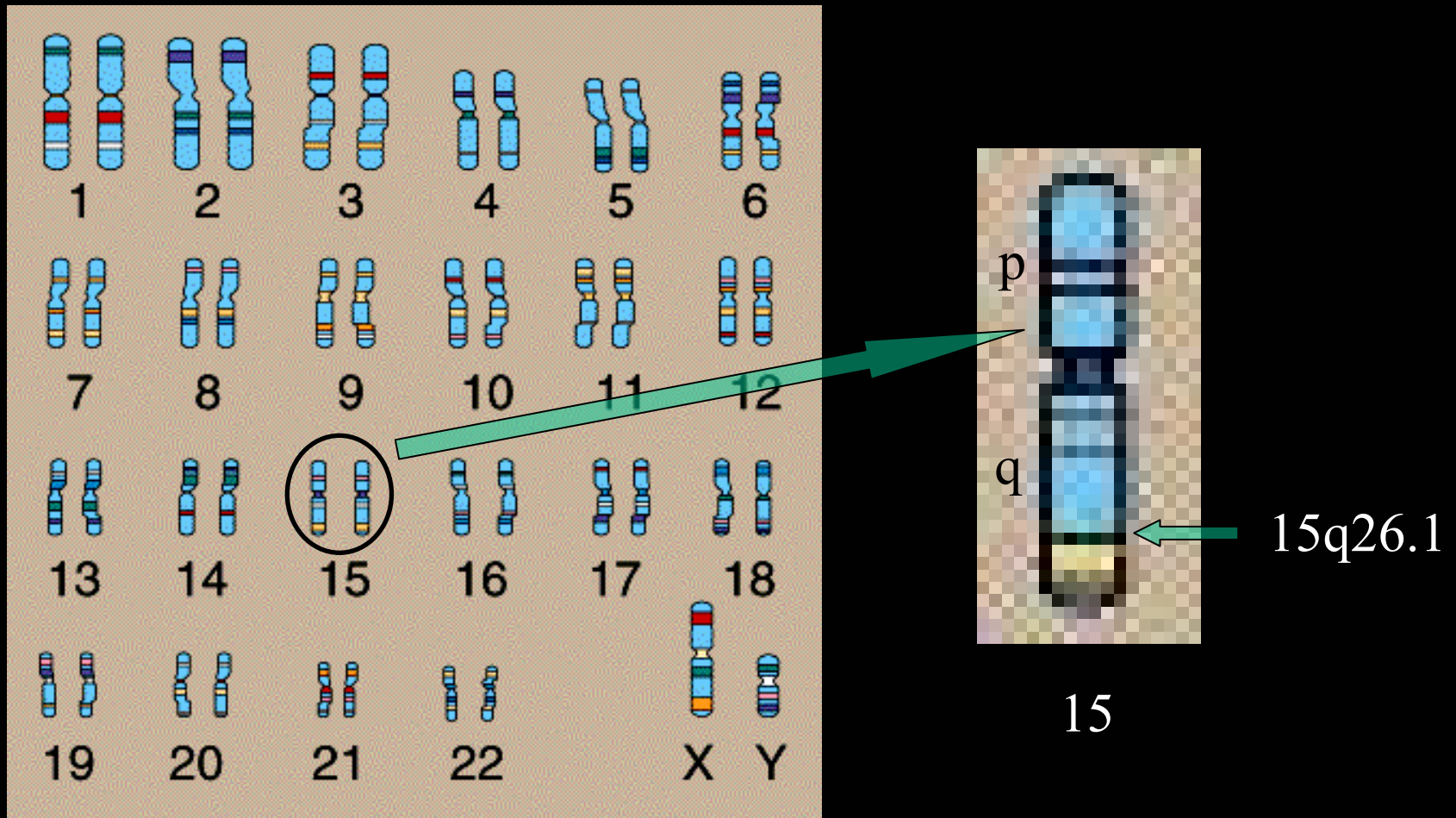
解旋酶



Bloom综合征：

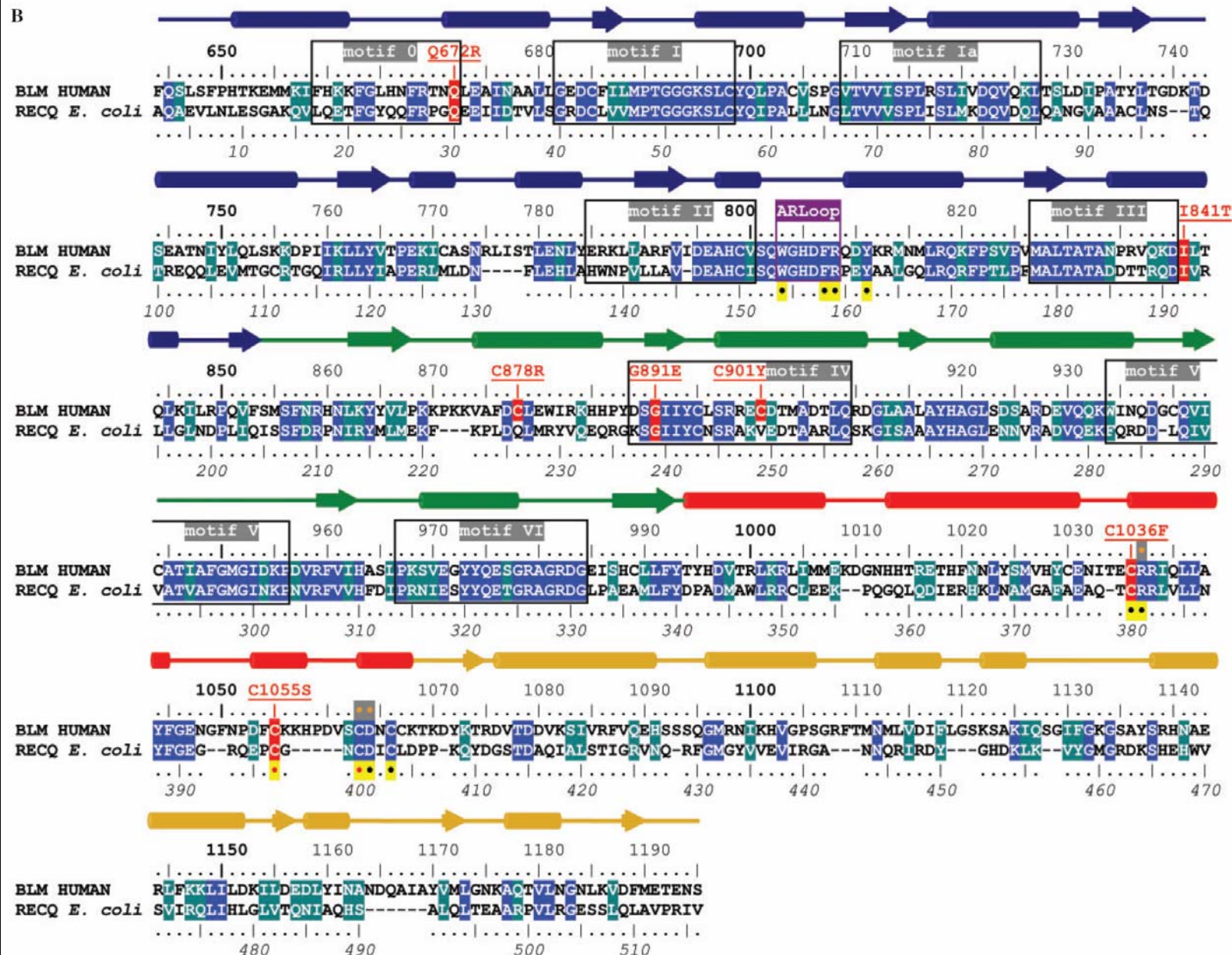
矮小，阳光诱导红斑
II型糖尿病，感染频繁
恶性肿瘤易感等

BLM 在人类基因组中的位置



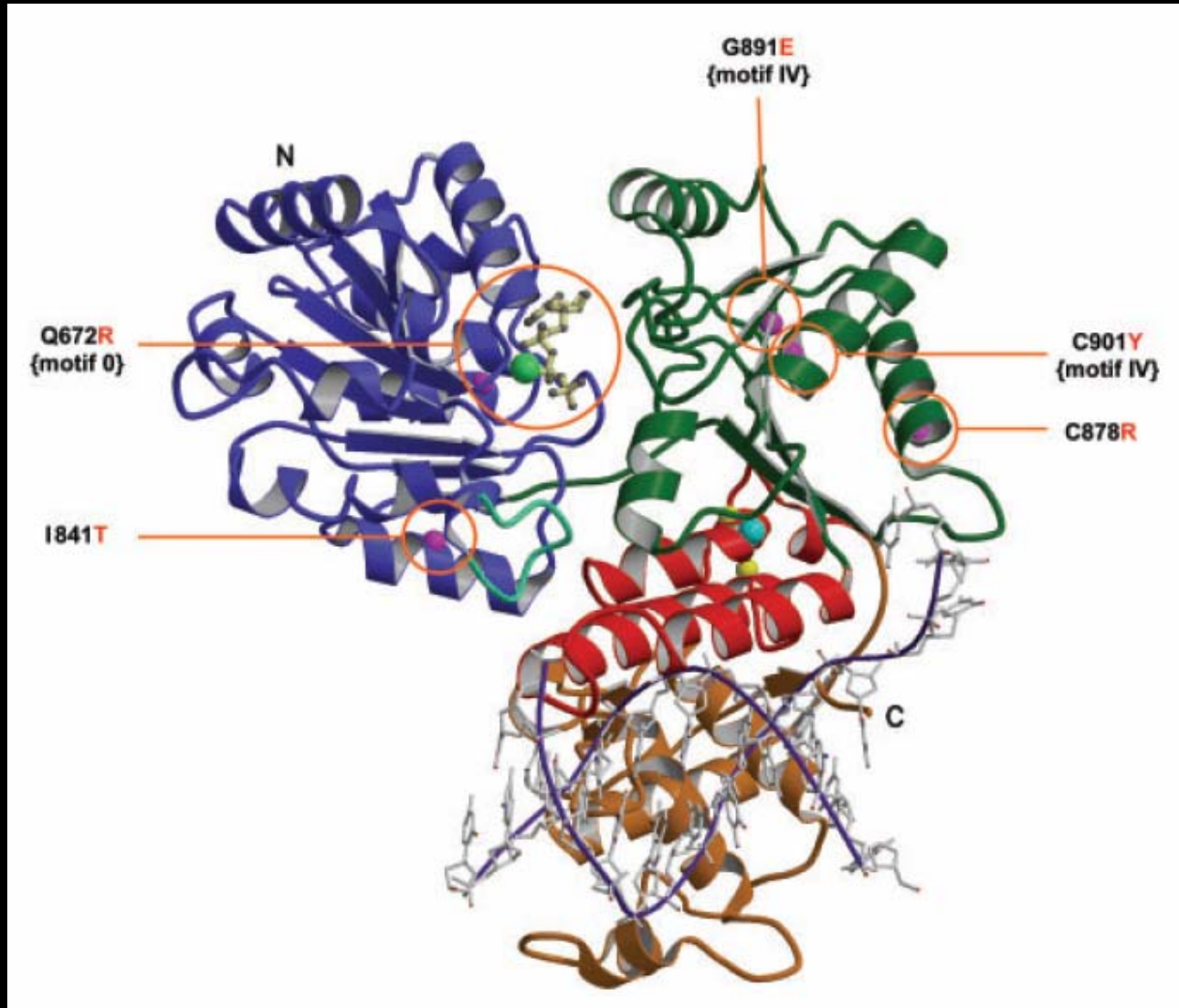
DNA长度为 4437 bp

BLM蛋白的核心氨基酸序列（全长1417个氨基酸, 159kD）



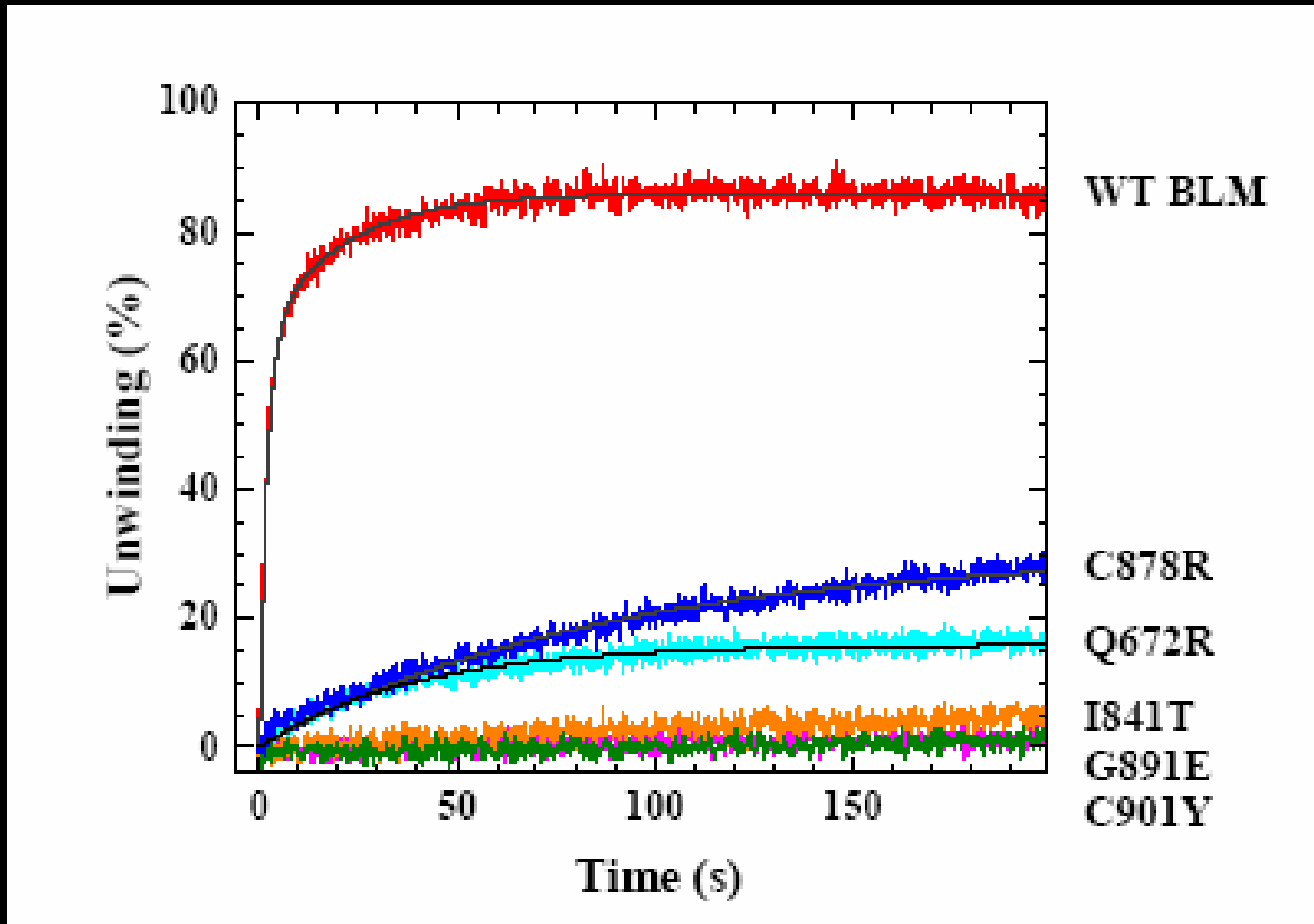
导致疾病的5个错义突变点

Five disease-causing missense mutations that are localized in the BLM helicase core region: **Q672R**, **I841T**, **C878R**, **G891E** and **C901Y**



Disease-causing mutations in Bloom syndrome helicase

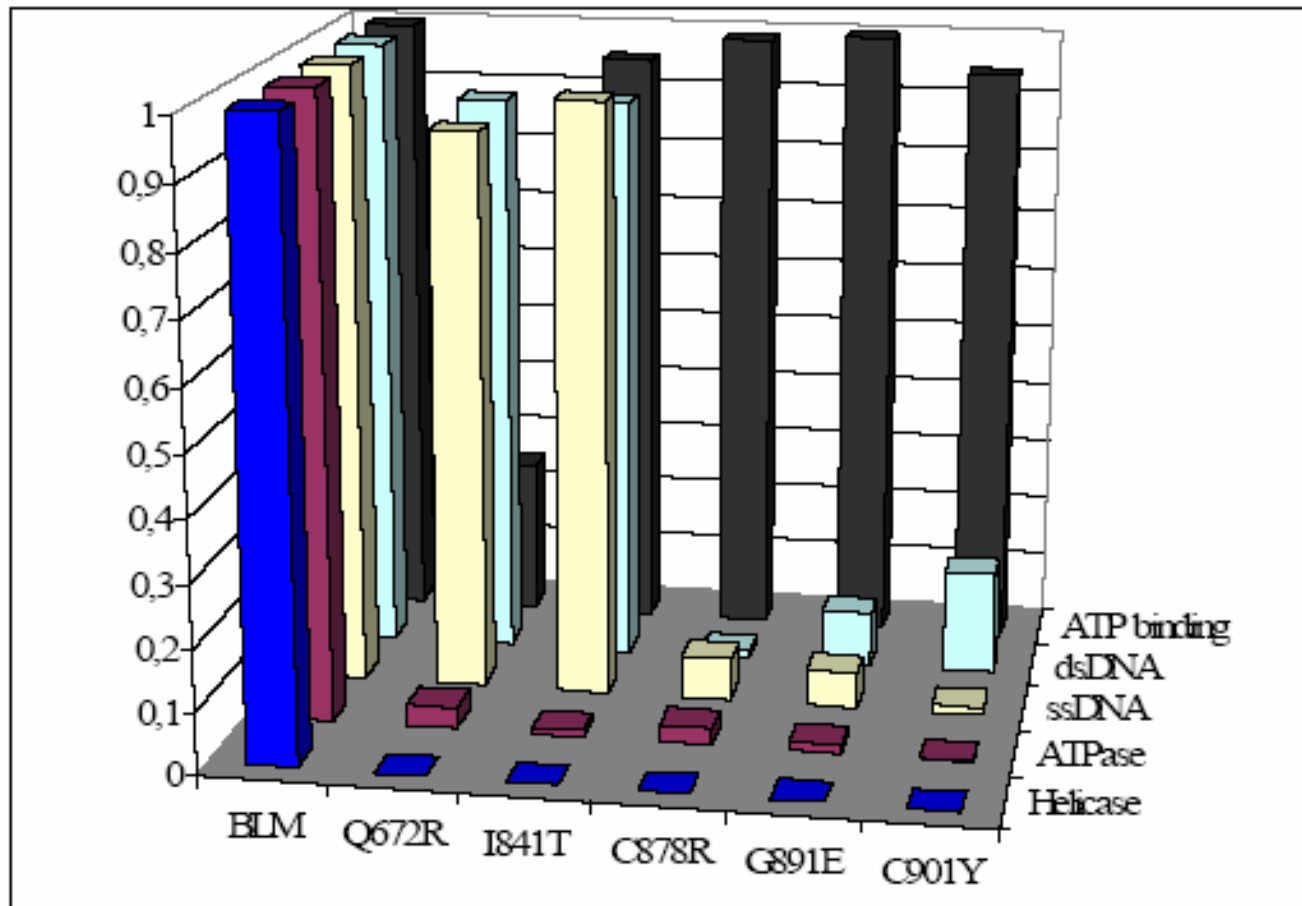
Unwinding ability 变异对解旋能力的影响



Disease-causing mutations in Bloom syndrome helicase

Enzymatic activity 各个致病突变对酶活性的影响

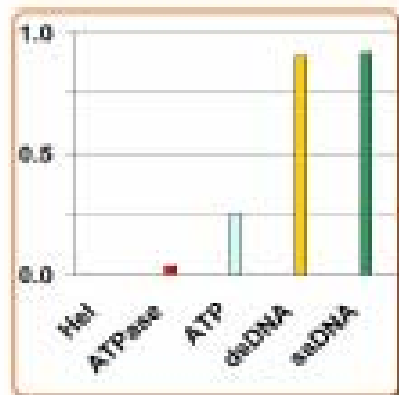
活性



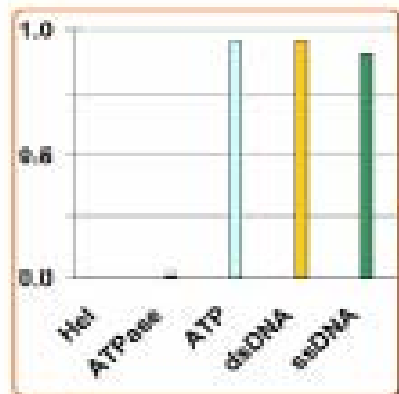
类型

突变

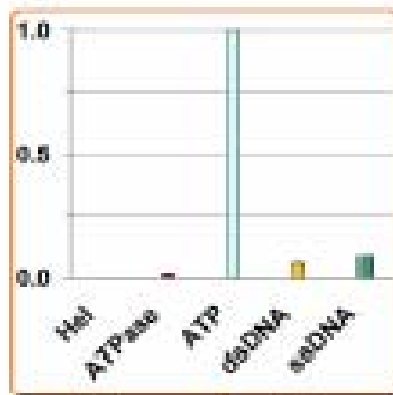
各个位点的影响



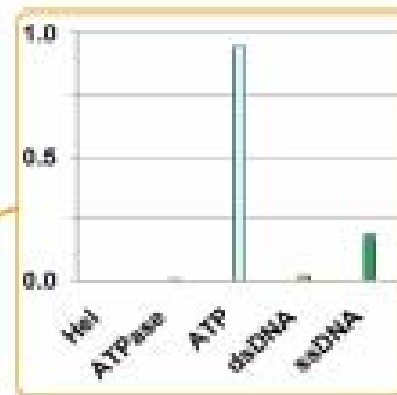
Q672R (motif 0)



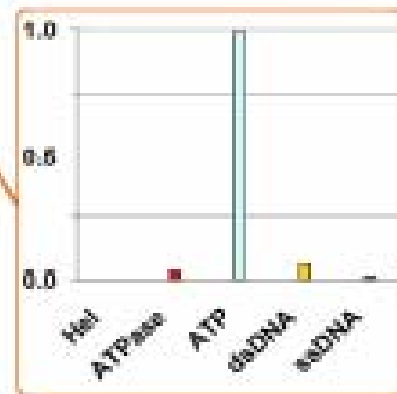
I841T



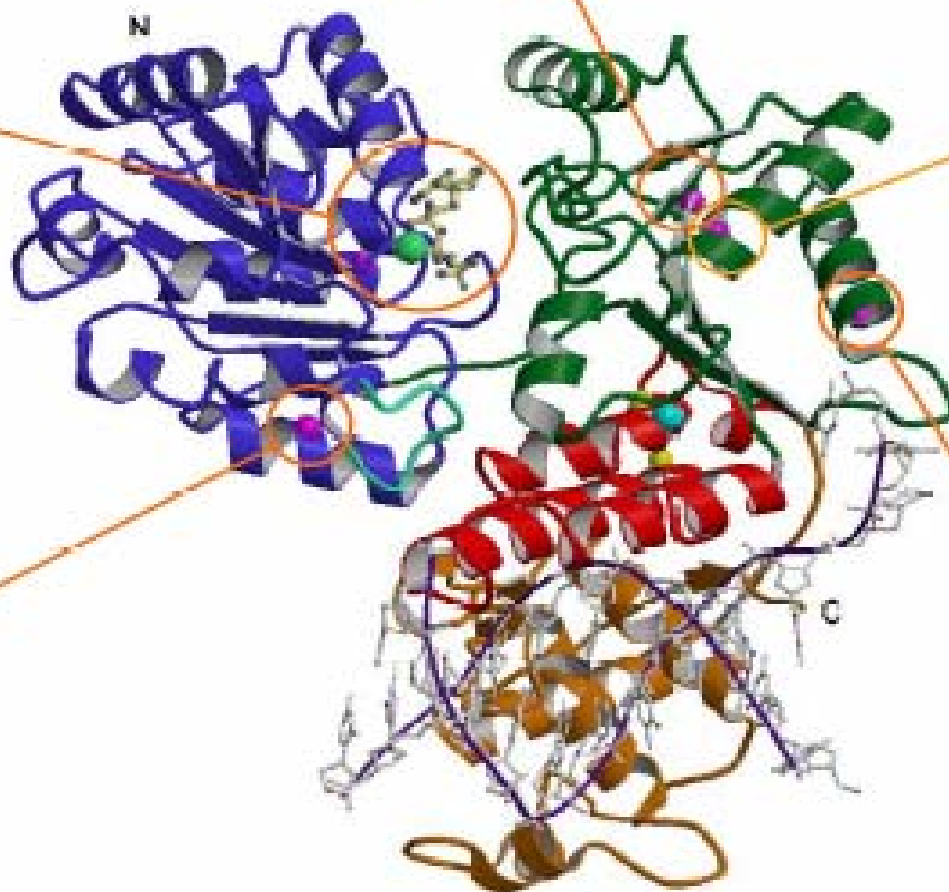
G891E (motif IV)



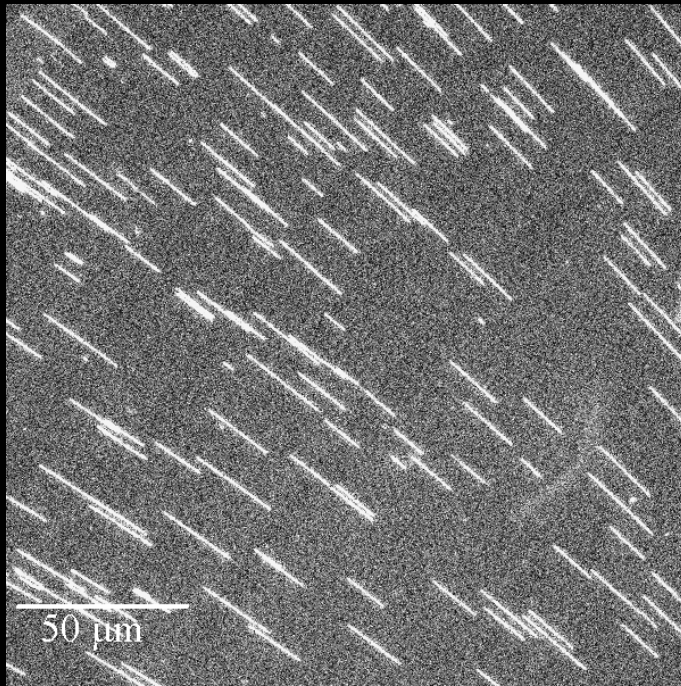
C901Y (motif IV)



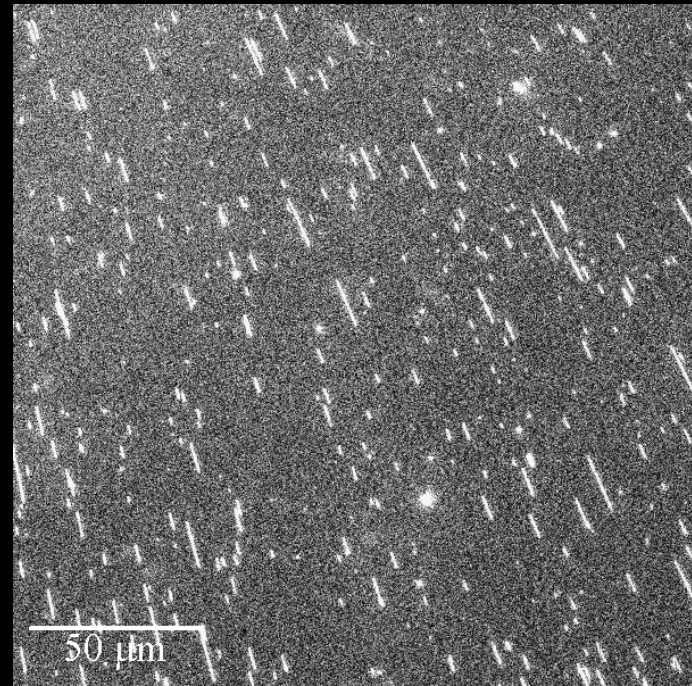
C878R



分子梳方法直接观察 DNA-RecQ complexes



(a)



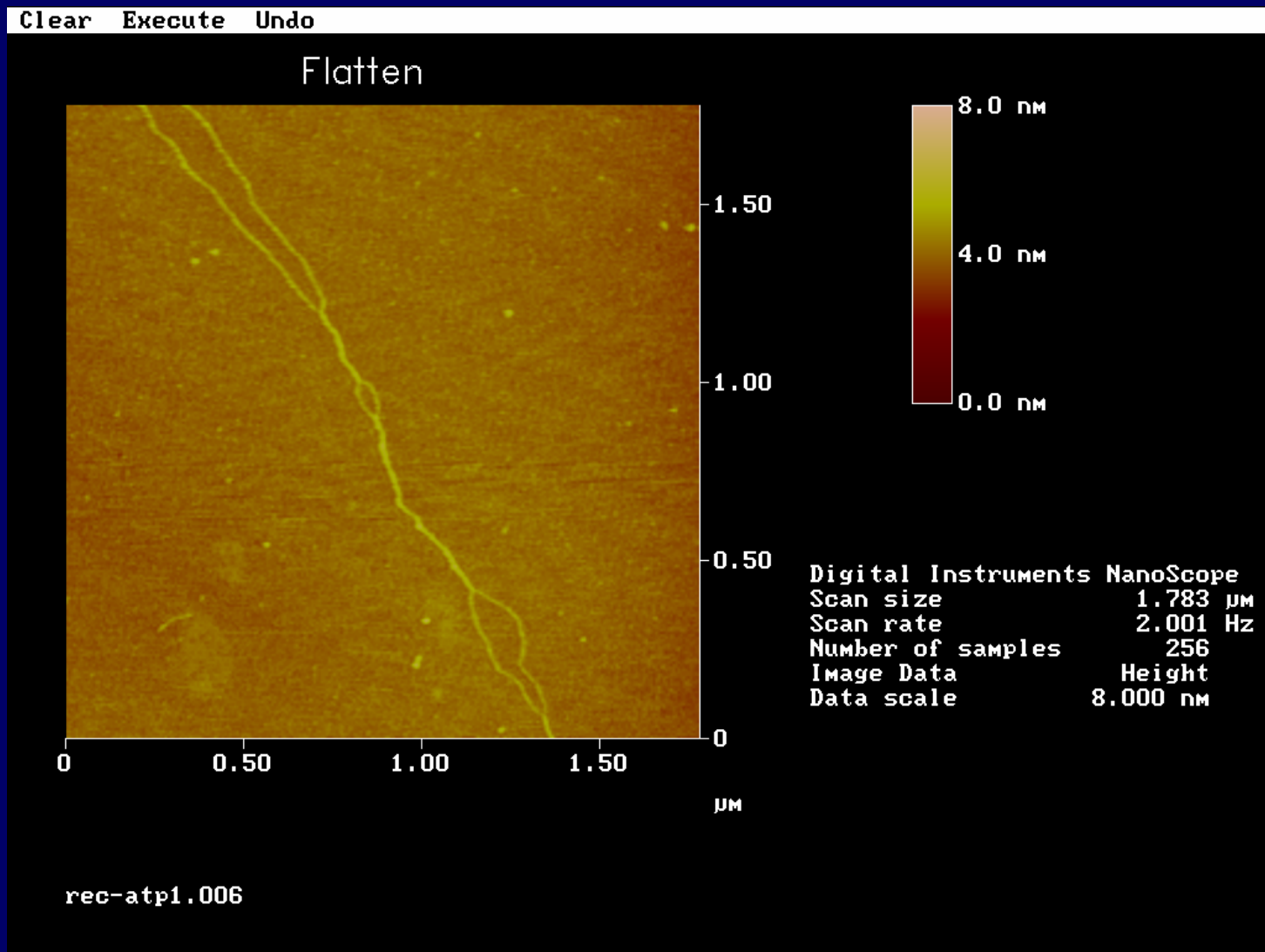
(b)

(a) $[\text{RecQ}] = 975 \text{ pM}$, $[\text{ATP}] = 0 \text{ mM}$

(b) $[\text{RecQ}] = 975 \text{ pM}$, $[\text{ATP}] = 1 \text{ mM}$

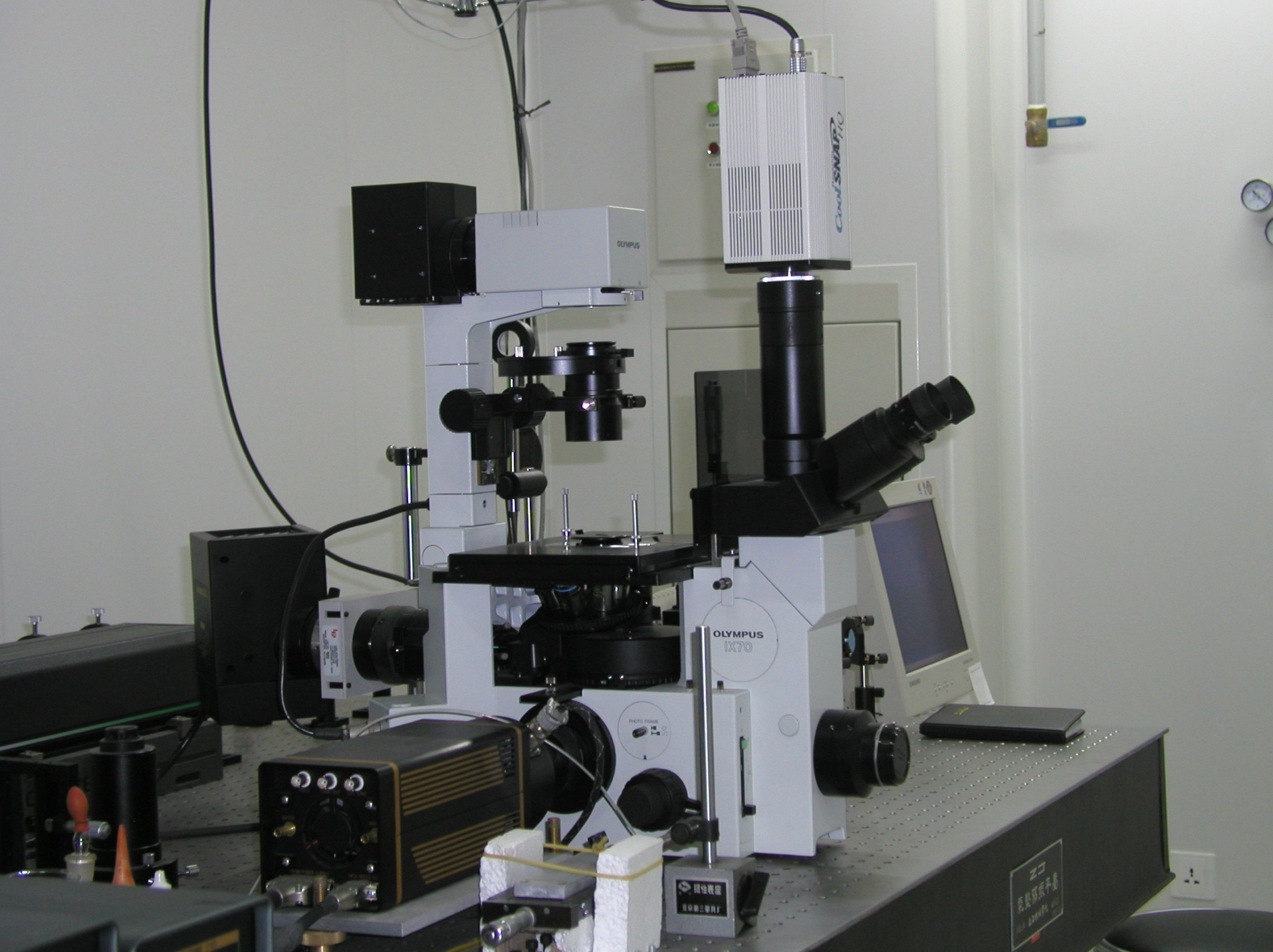
$[\text{DNA}] = 6.5 \text{ pM}$

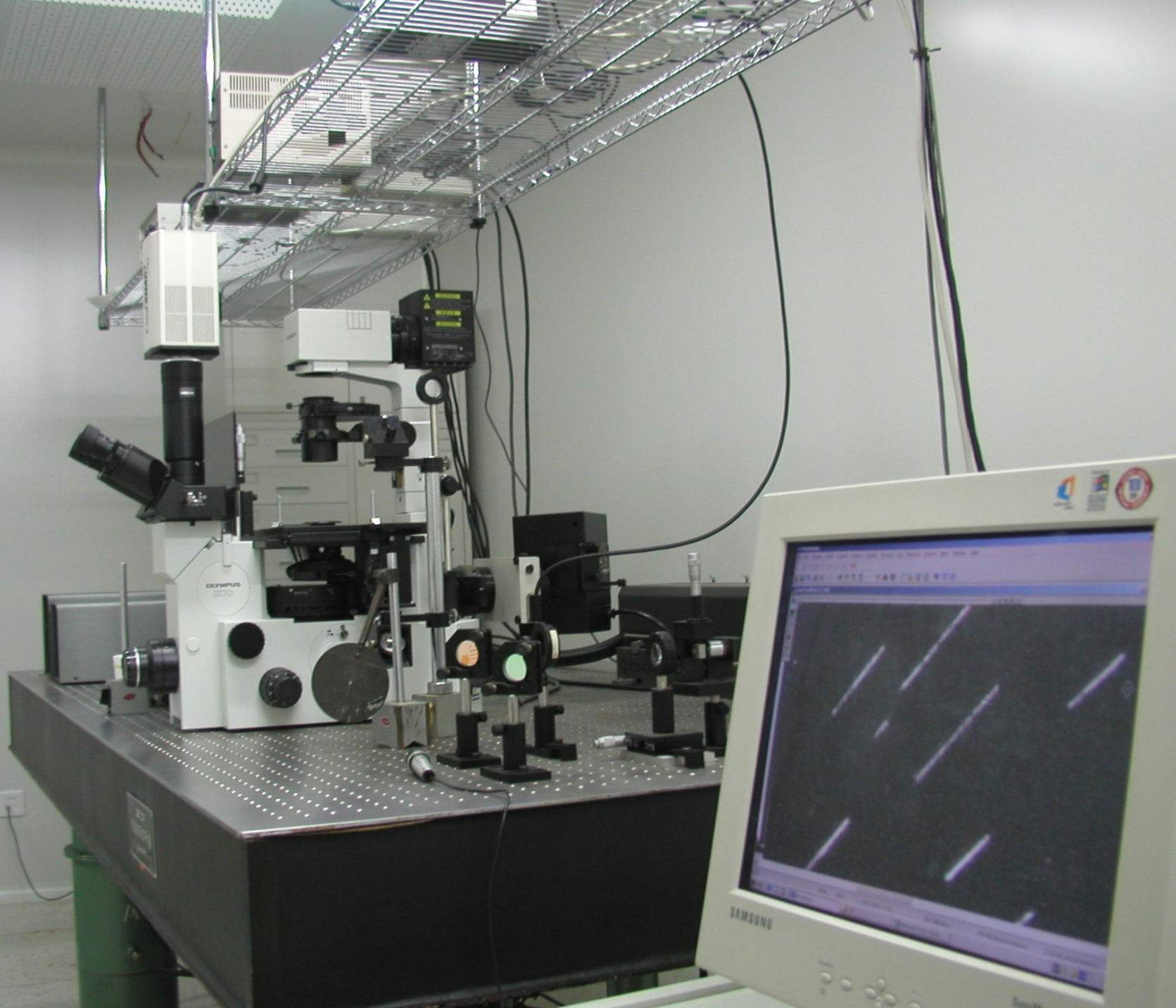
用原子力显微镜直接观察 DNA-RecQ complexes

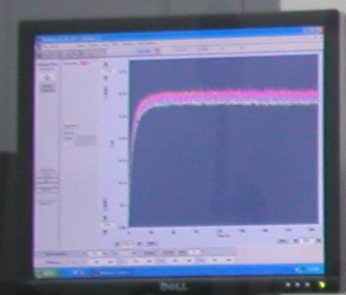


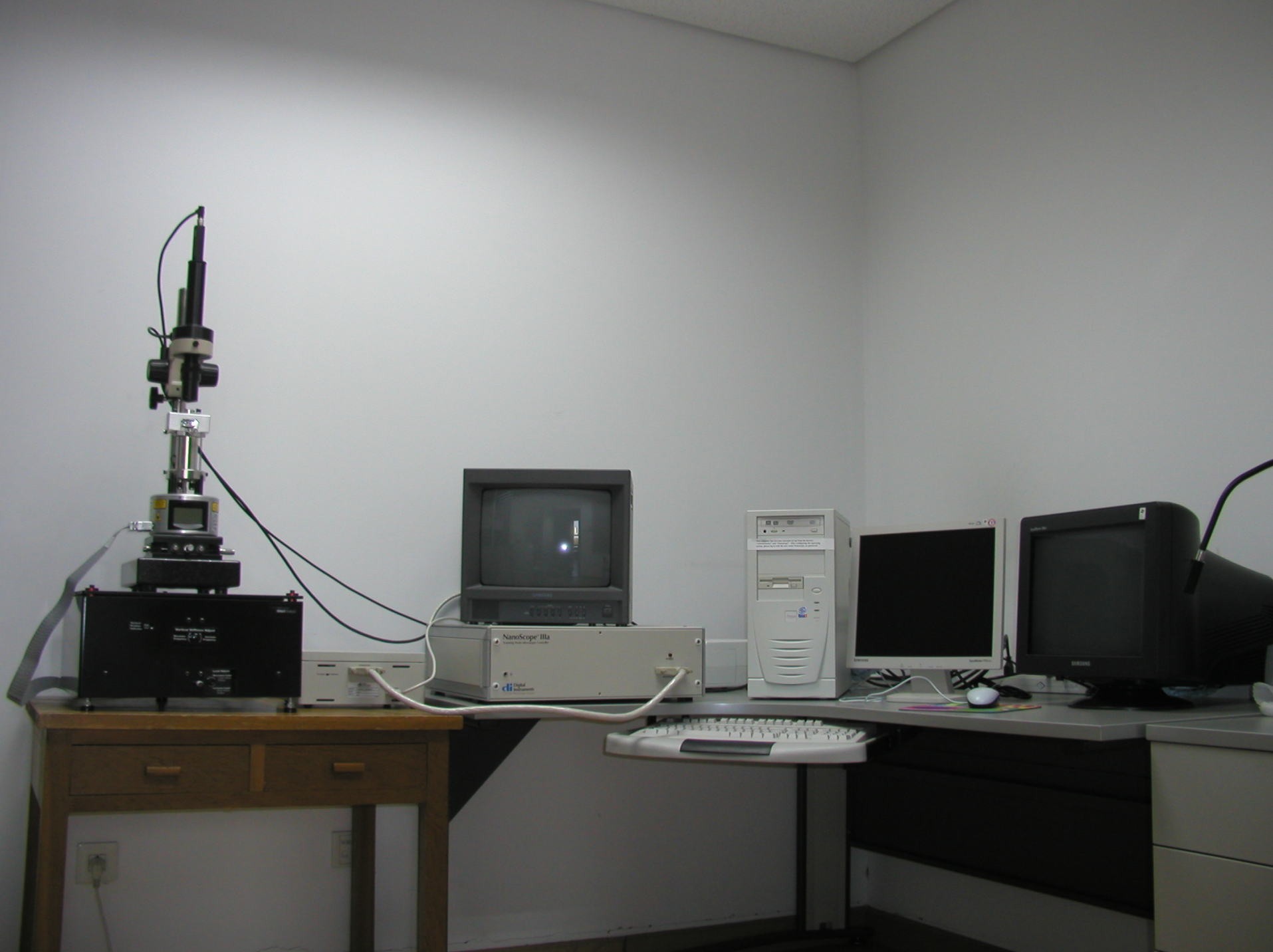
被 RecQ 解旋后的 DNA



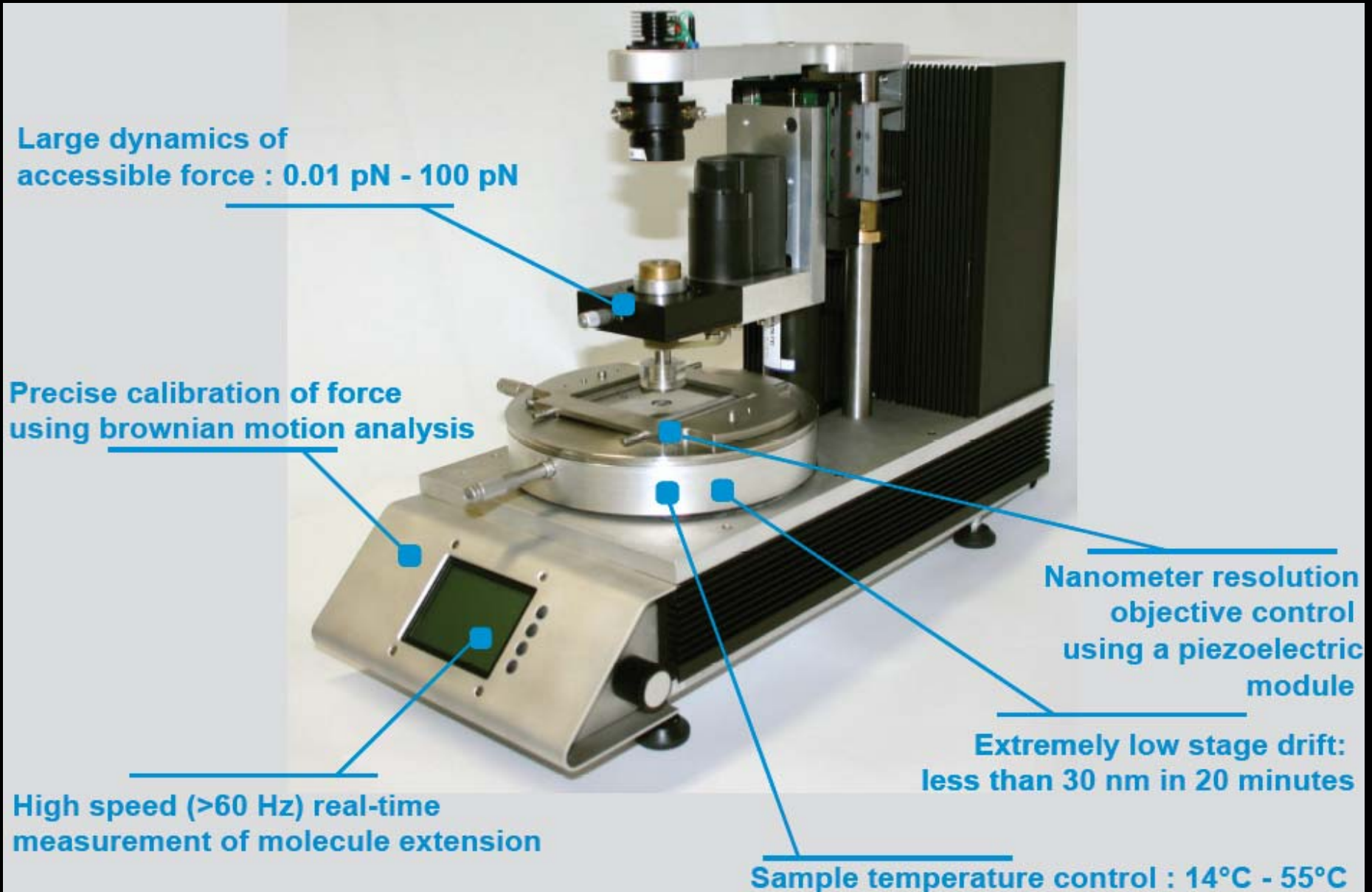








将于今年6月份到的磁阱，可用于DNA的单分子操纵(拉伸及扭转)





谢谢!

Thank you!

Group members, PhD students, postdocs, and collaborators:

Shuo-Xing Dou, Ping Xie, Wei-Chi Wang, Ling-Yun Zhang, Wei Li, Yu-Ying Liu, Xing-Dong Zhang, Jun Qian, Hong Wang, Zhi-Qiang Sun, Xi-Miao Hou, Bing-Yi Pan, Ye Yang, Chao Ji, Zhi-Qiang Zhang, Zhao-Wen Duan, Hui Li, Kai-Ge Wang, Ya-Xin Jiang, Yong Zhang, Xiao-Hong Fang, Xu Guang Xi

Supported by NSFC and CAS