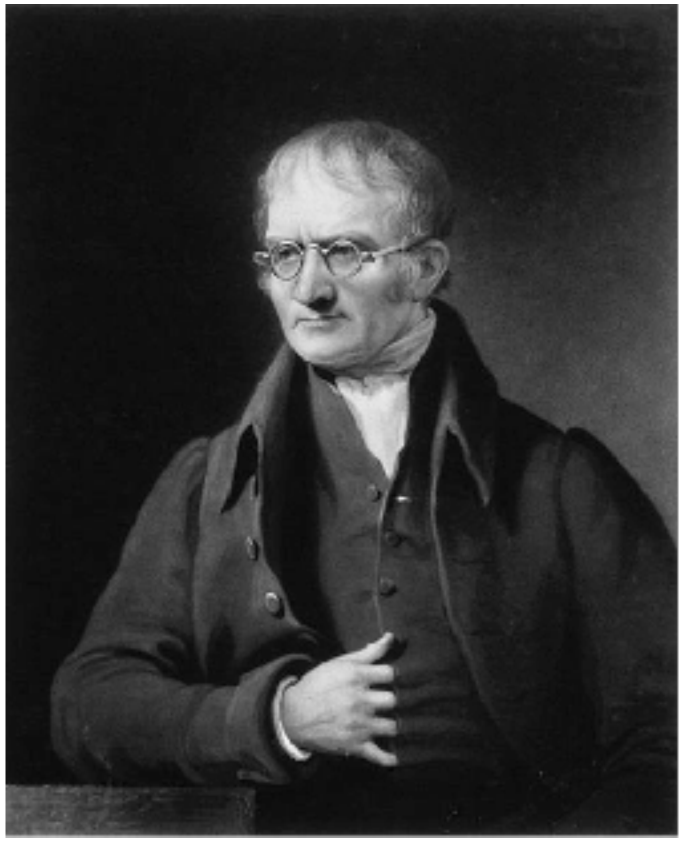
HOGYAN FOGJUNK MEG EGYETLEN MOLEKULÁT?

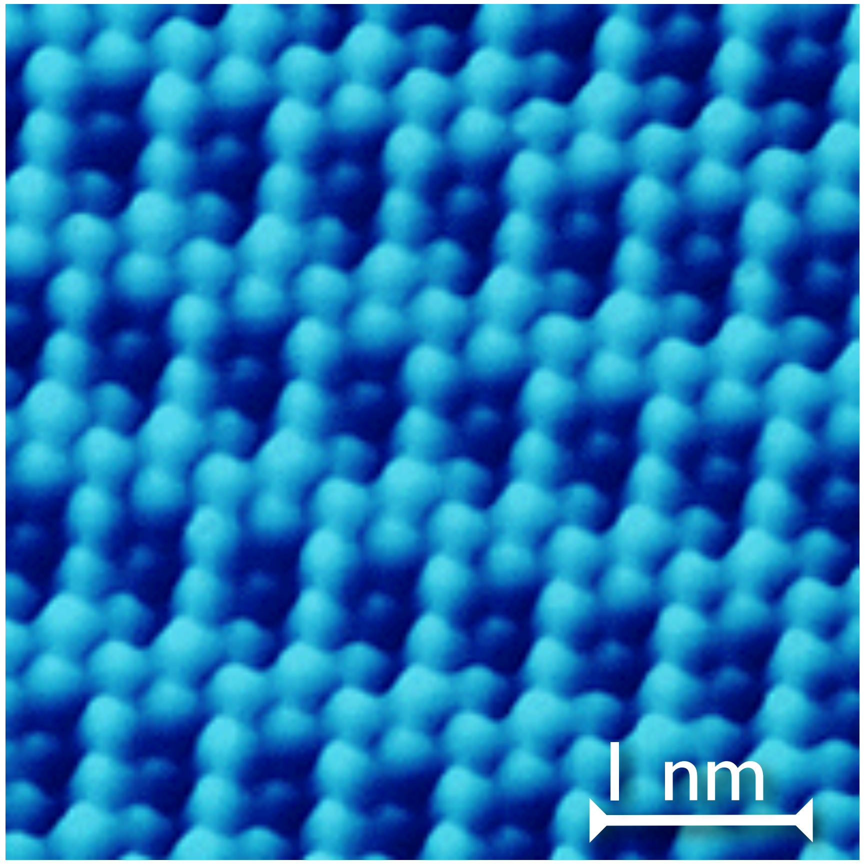
Kellermayer Miklós

Oktatás, kutatás, gyógyítás: 250 éve az egészség szolgálatában

Semmelweis Szenior Akadémia

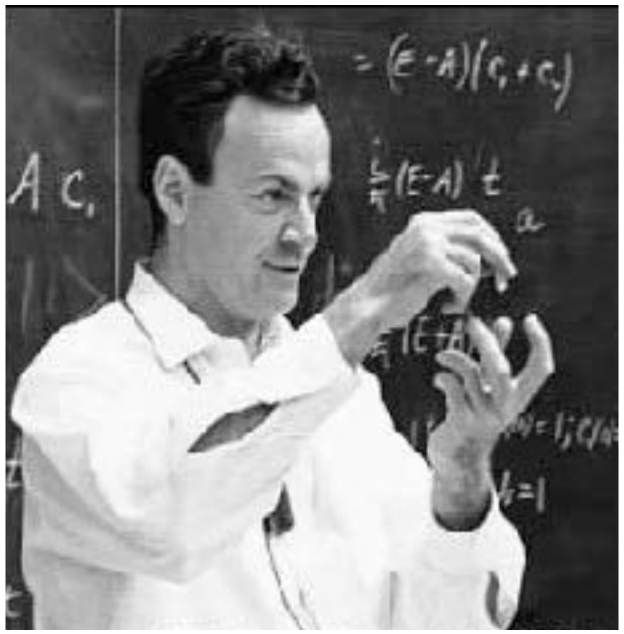
2020. március 2.


John Dalton (1766-1844)



Oxigén atomok rhodium egykristály felületén

A nanovilág

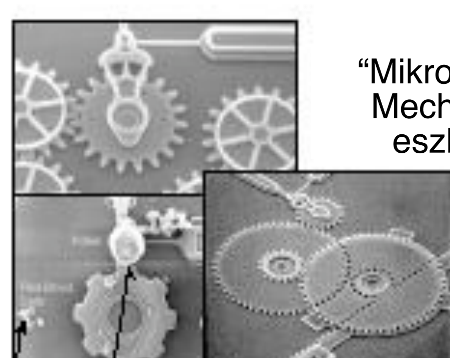


1959: Richard P. Feynman "There is plenty of room at the bottom”

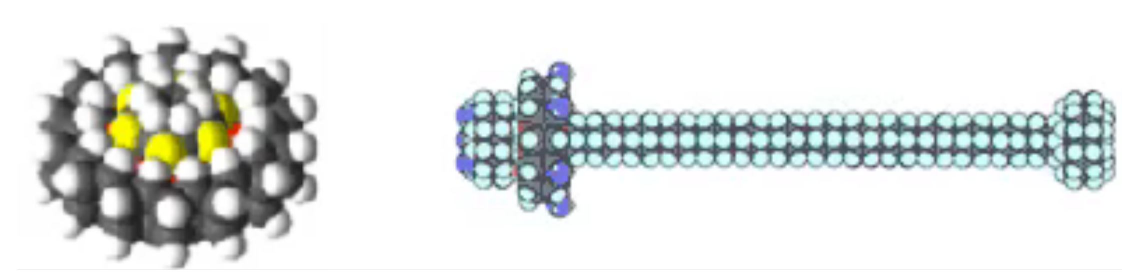
Nanotechnológia: az anyag megértése és manipulálása az 1-100 nanométeres méretskálán, ahol új és egyedi jelenségek lépnek fel és új alkalmazások kínálkoznak.

Nanotechnológiai elvek

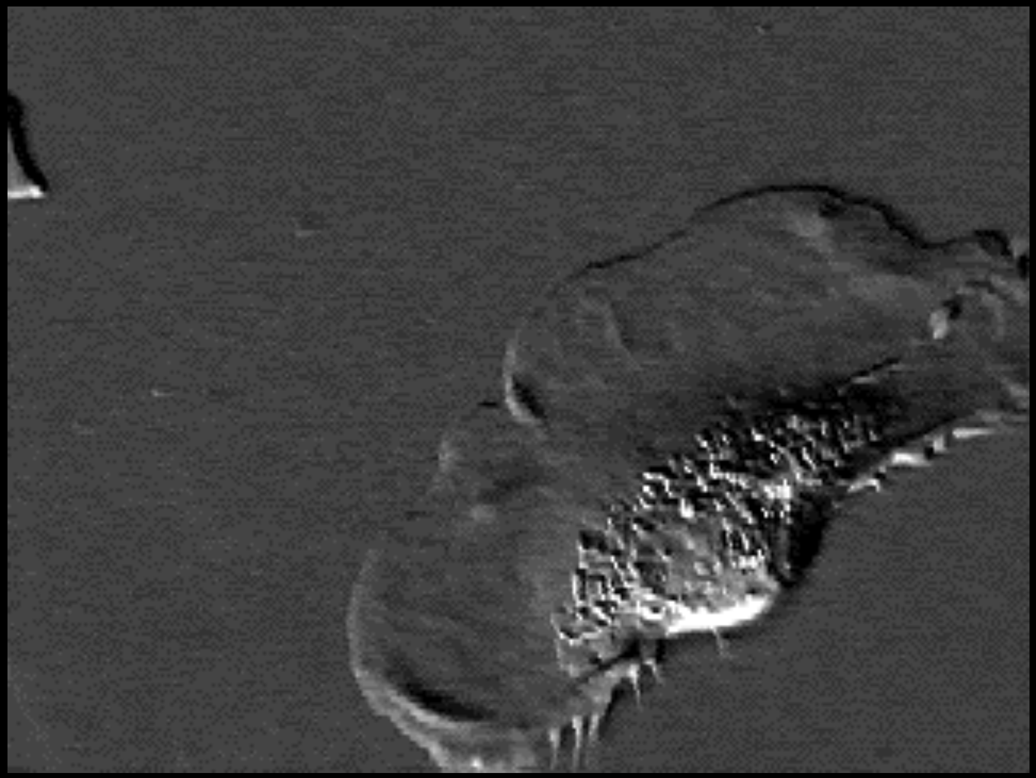
“Top-to-bottom”: miniatürizálás



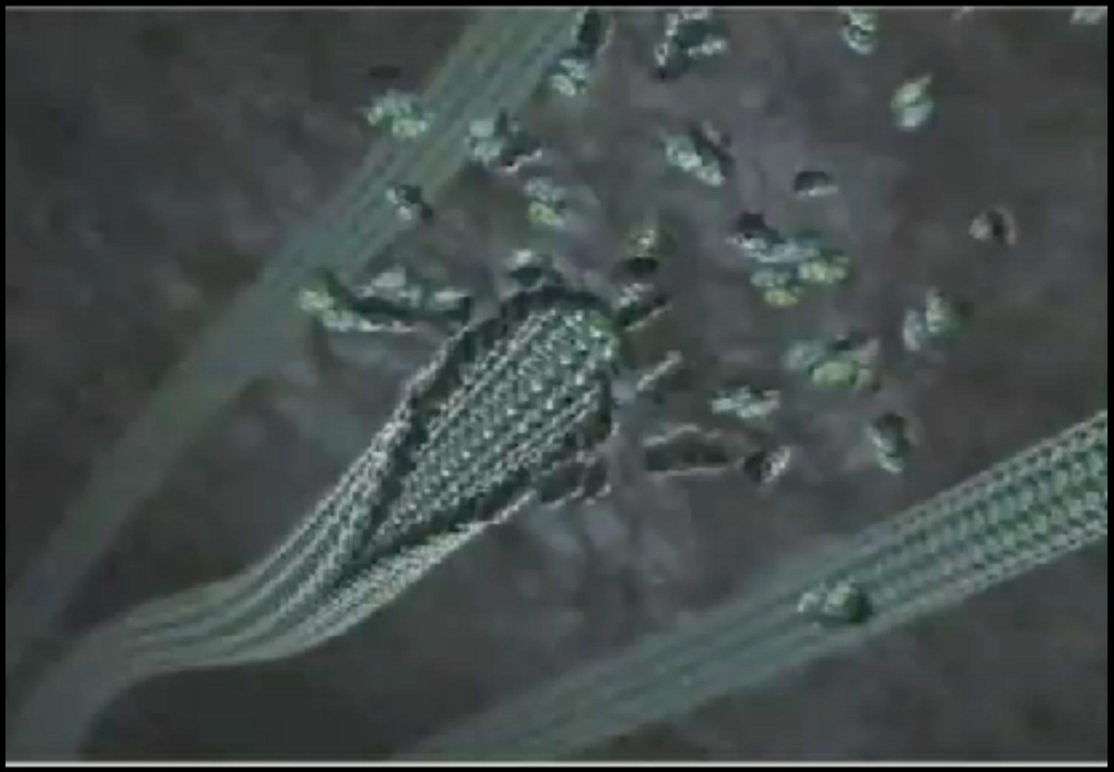
“Bottom-up”: gépezetek építése atomonként (Eric Drexler 1986)



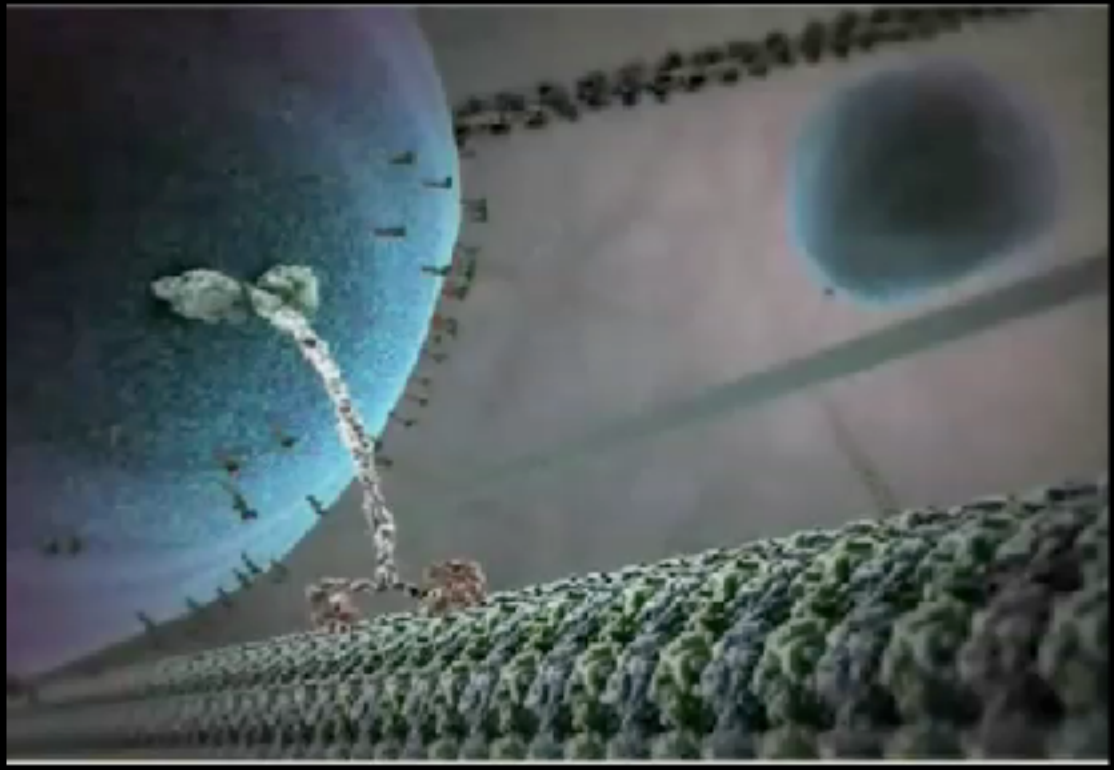
Élő sejtben: nanoméretű gépezetek sokasága



Tovakúszó keratinocita



Mikrotubulus dinamikus instabilitás



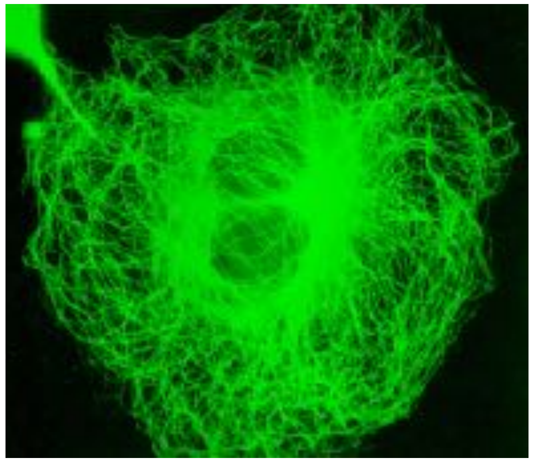
Vezikulum transzport kInezinnel

Fehérjeszintézis riboszómán

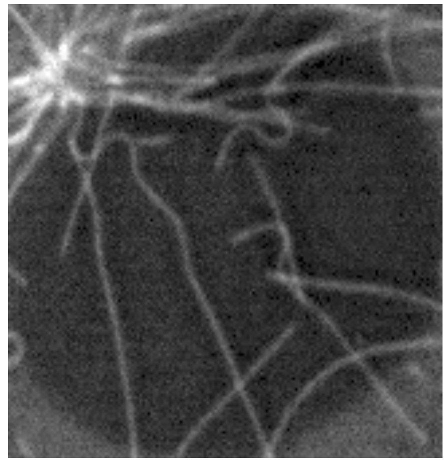
http://multimedia.mcb.harvard.edu

Molekulák: miért egyenként?

1. Egyéneket azonosíthatunk sokaságban

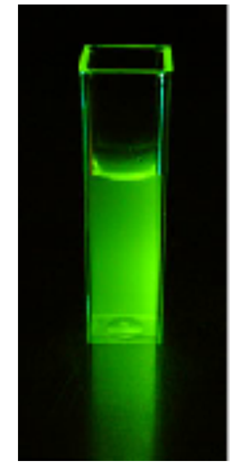


Sokaság - mikrotubuláris rendszer

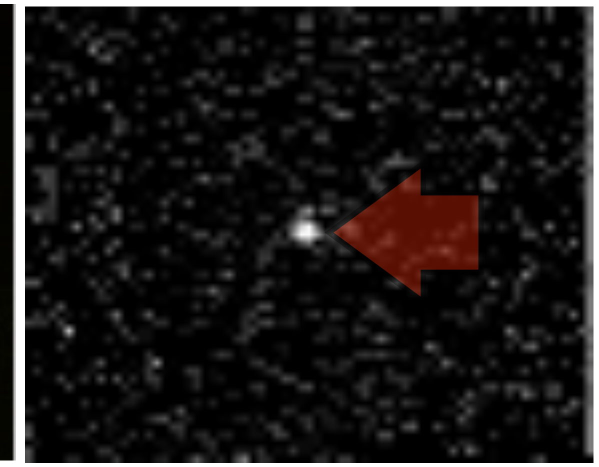


Egyedi mikrotubulusok - treadmilling

2. Véletlenszerű folyamatokat ismerhetünk meg

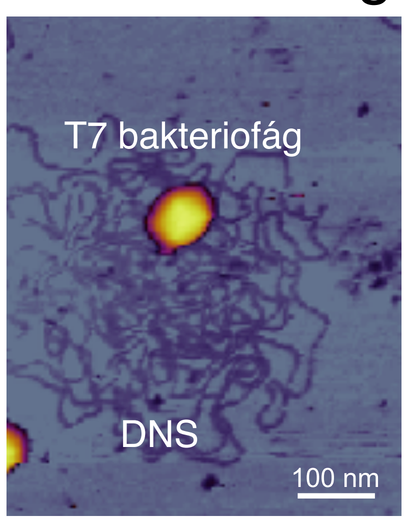


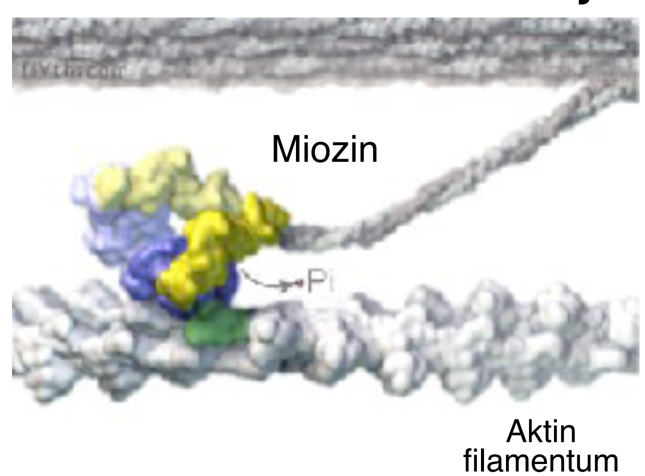
Sokaság - intenzitás

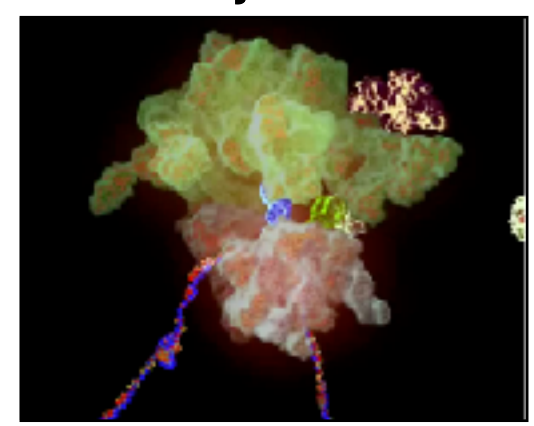


Egyedi kvantumpont - pislogás ("blinking")

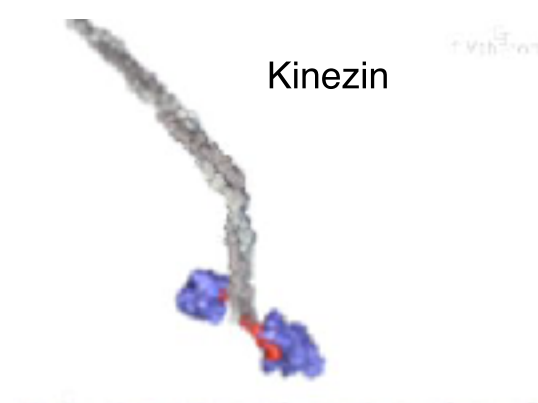
3. Biológiai molekulák mechanikáját jellemezhetjük

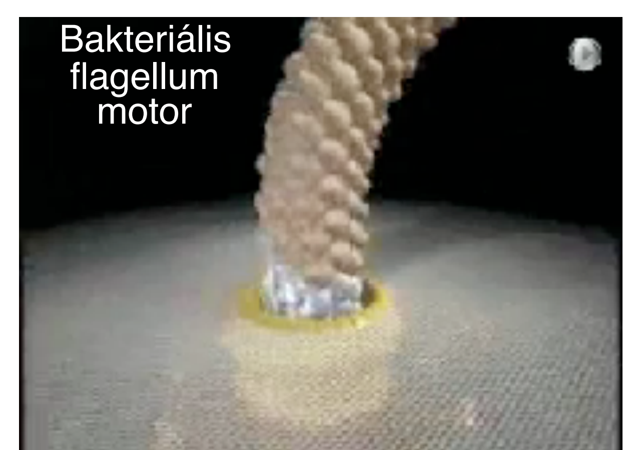






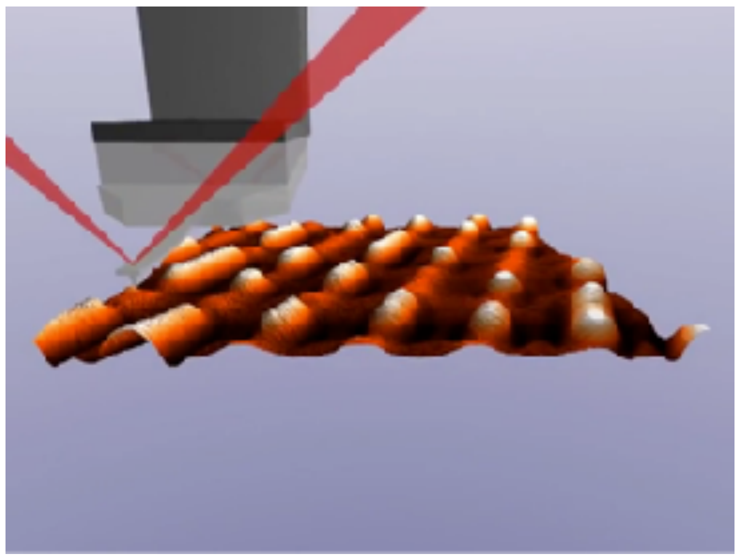
Fehérjegombolyodás riboszómán

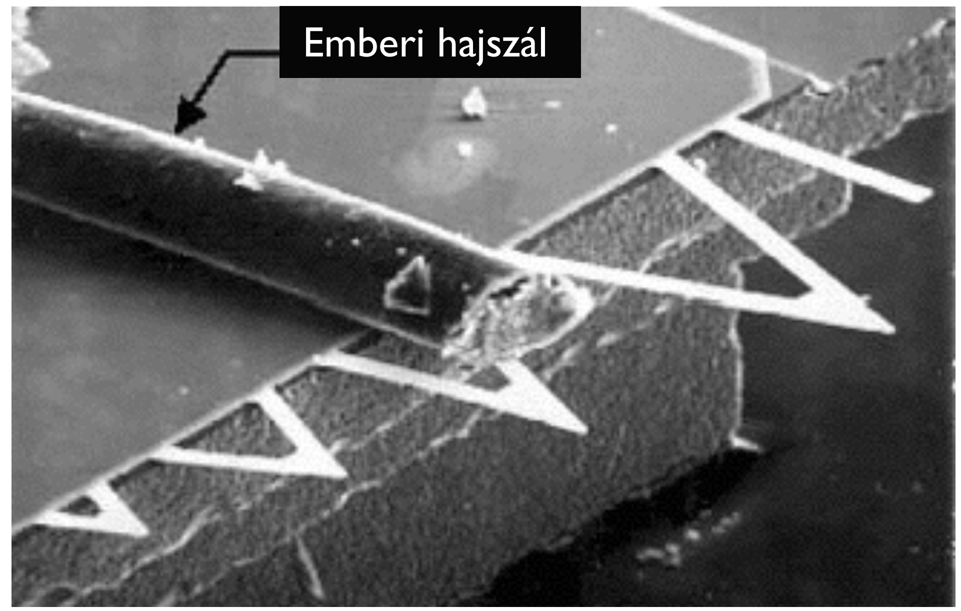


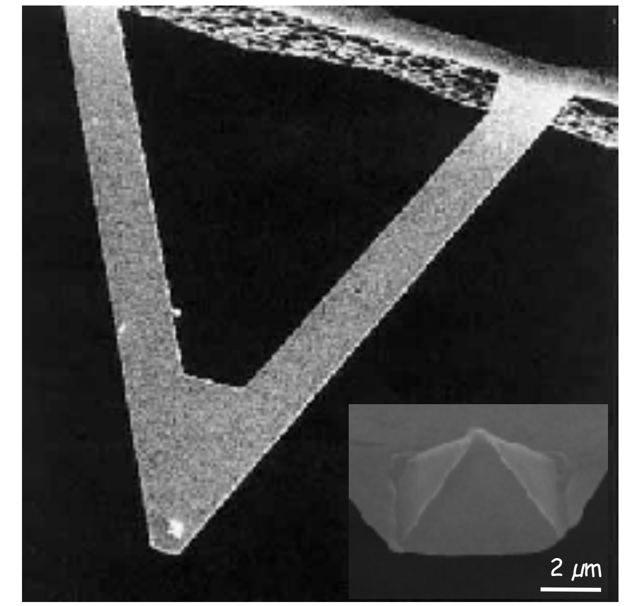


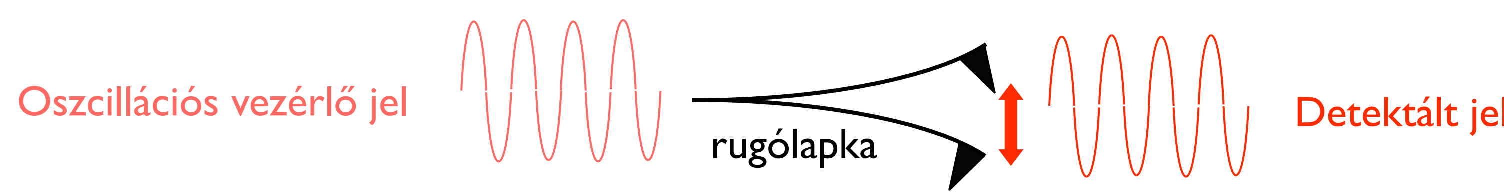
A nanovilág mikroszkópja: atomi erőmikroszkóp (AFM)

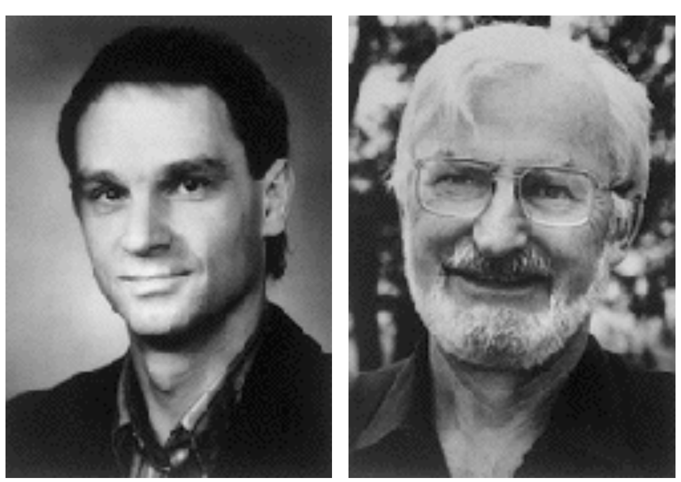
AFM működése









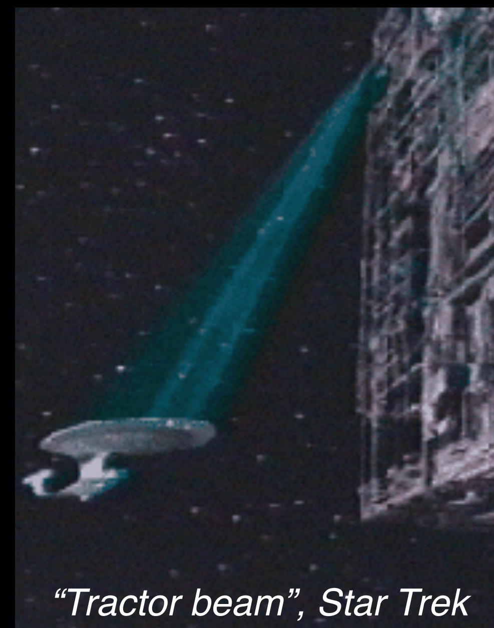


Gerd Binnig
Heinrich Rohrer
Nobel-díj 1986

Magasság kontraszt
Amplitudó kontraszt
Fázis kontraszt

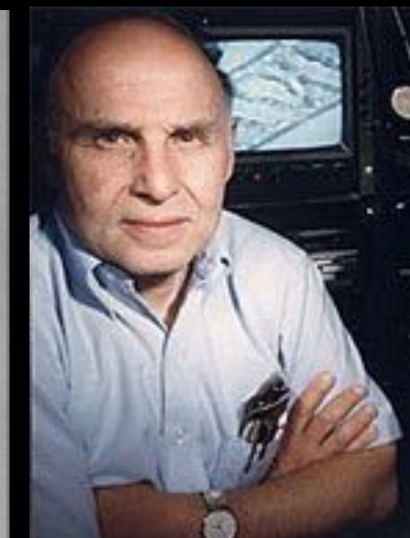
Nanomanipulálás fénnyel

“Tractor beam”, *Star Trek*



“Tractor beam”, *Star Trek*

Lézercsipesz



Arthur Ashkin
(Nobel-díj 2018)

E. coli baktériumsejt

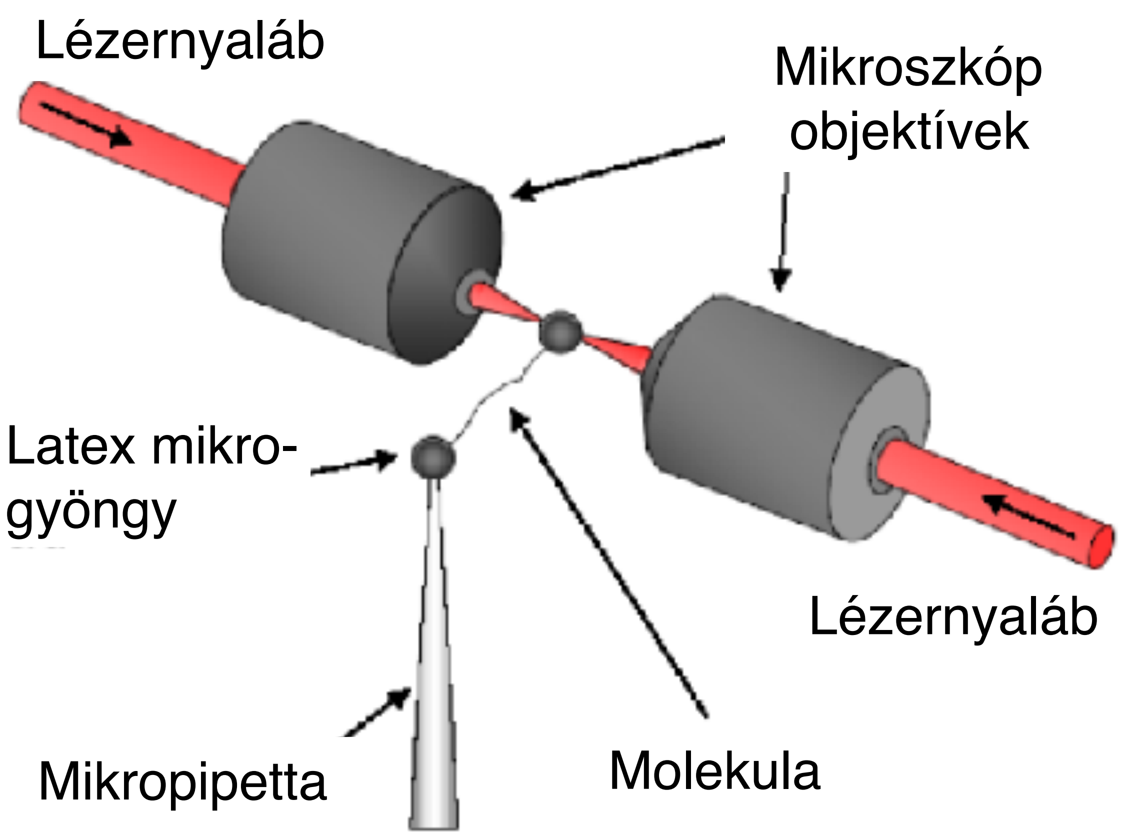
Aktin filamentum

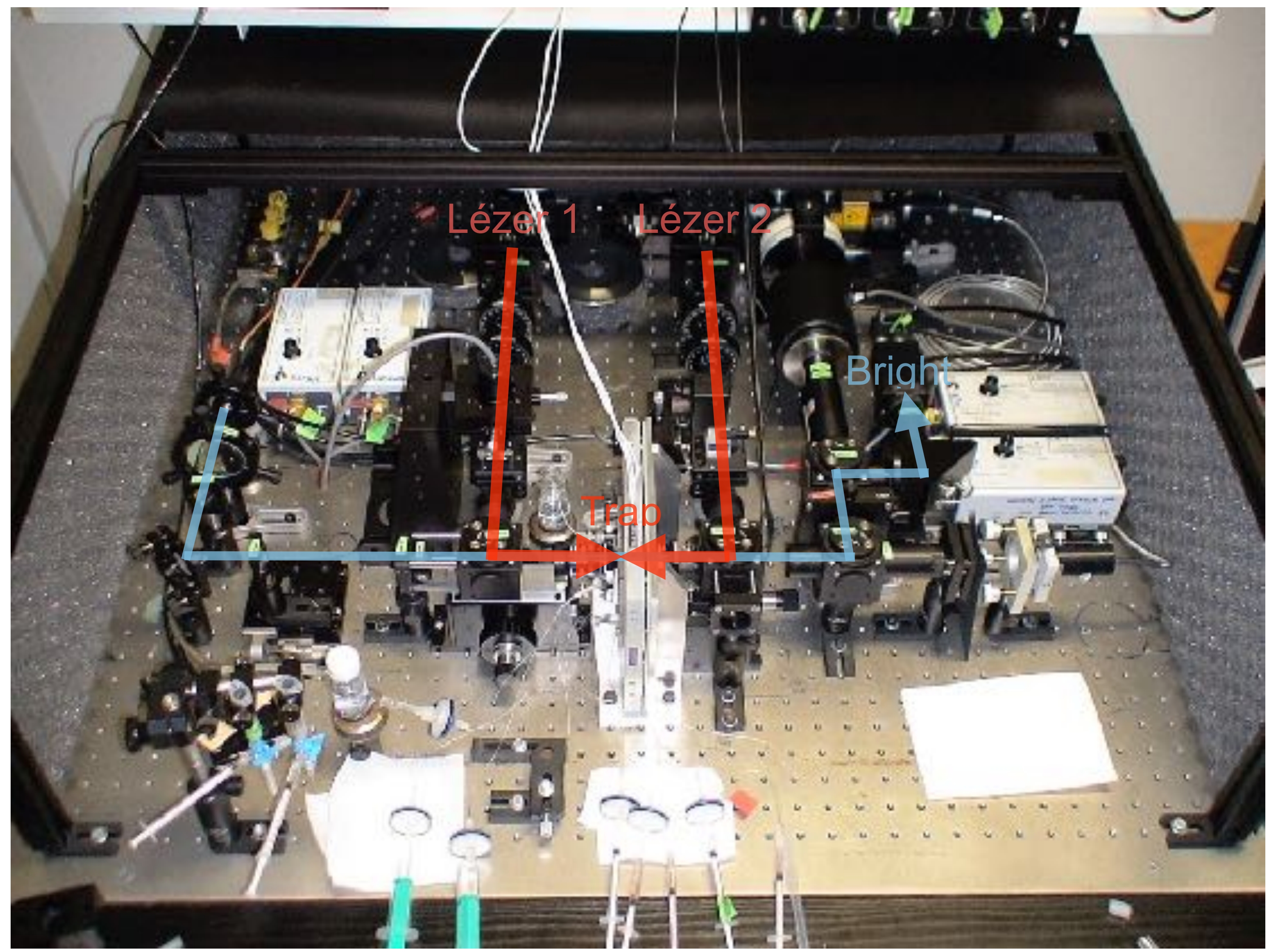
DNS

Fáziskontraszt kép

Fluoreszcencia kép

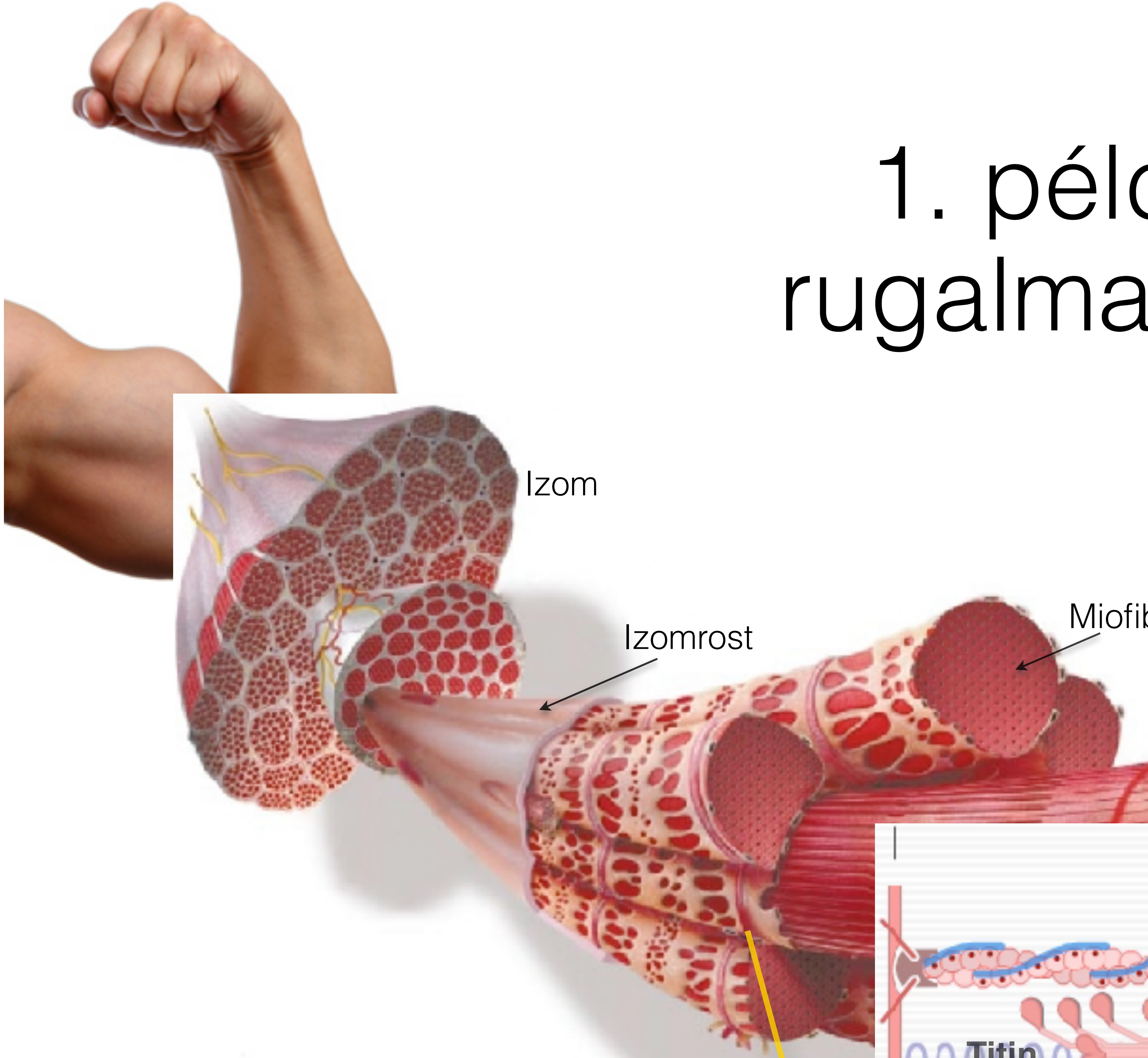
A lézercsipesszel erőt is lehet mérni!

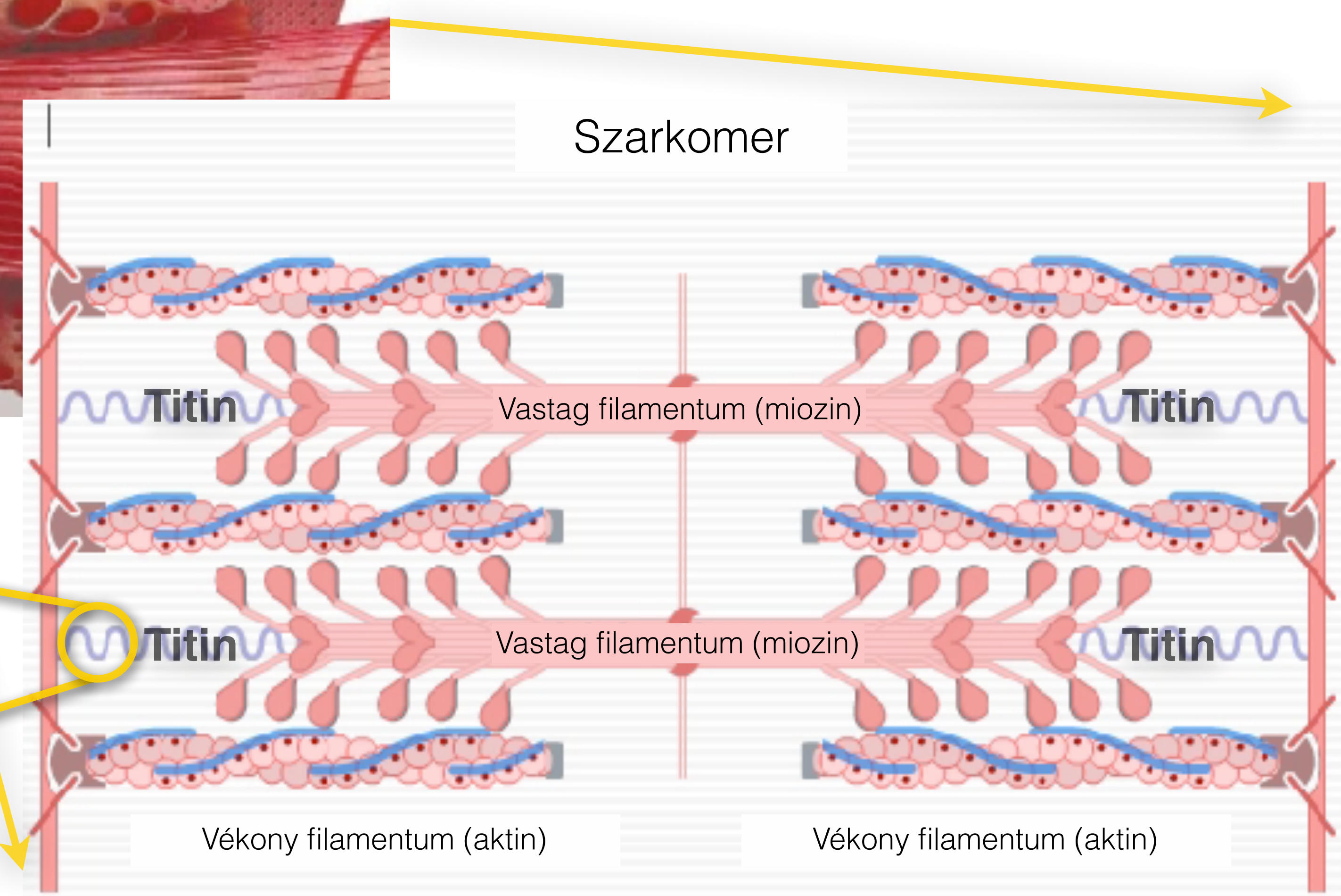


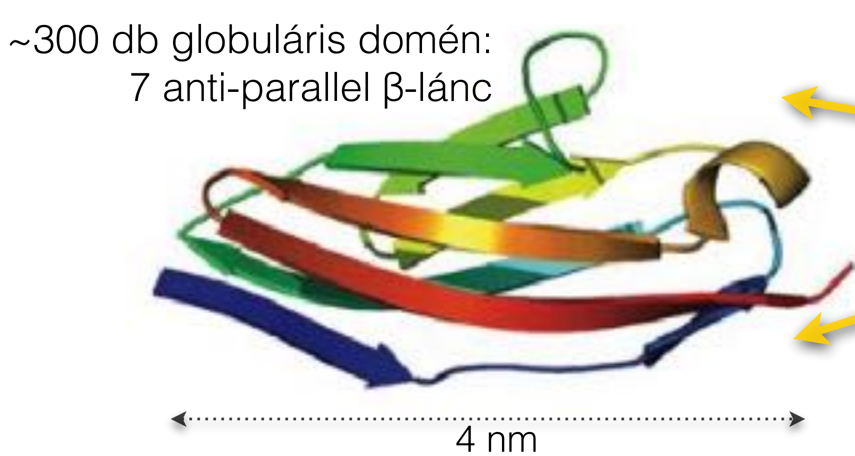


Két lézersugaras optikai csipesz berendezés

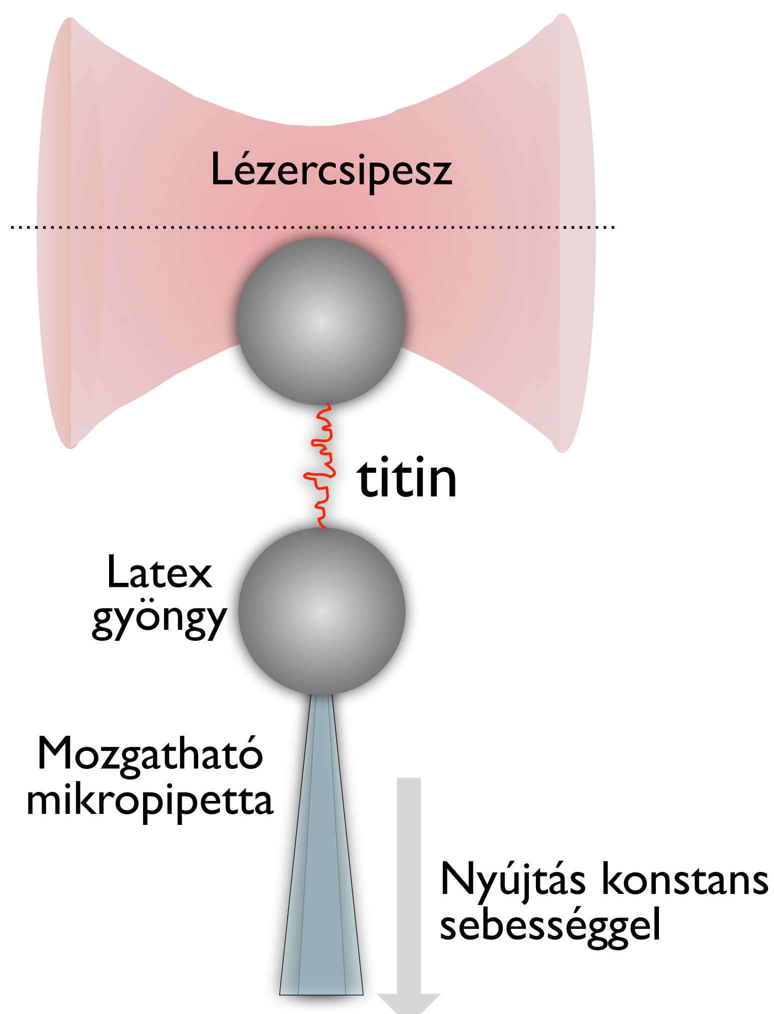
1. példa: az izom rugalmas fehérjéje, a **titin**

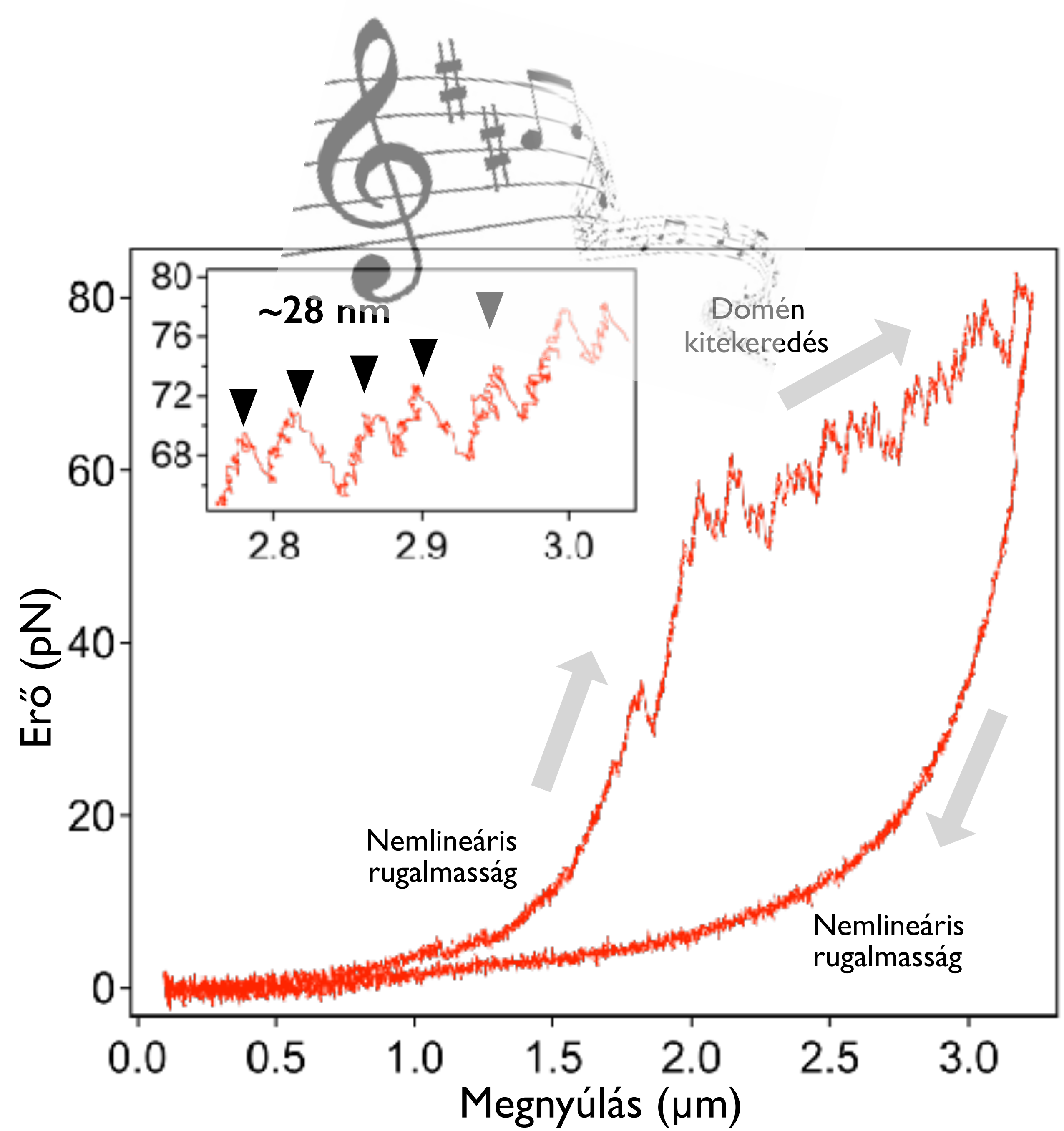




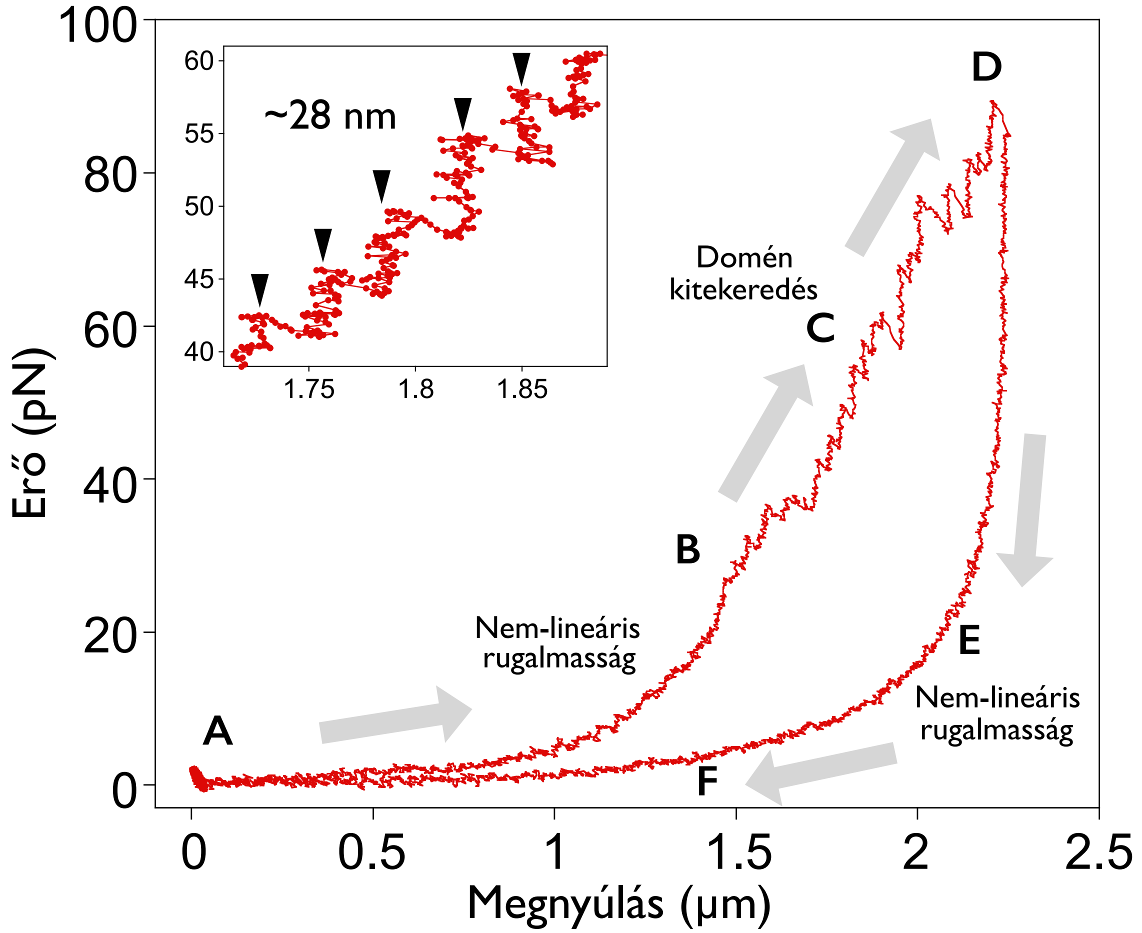


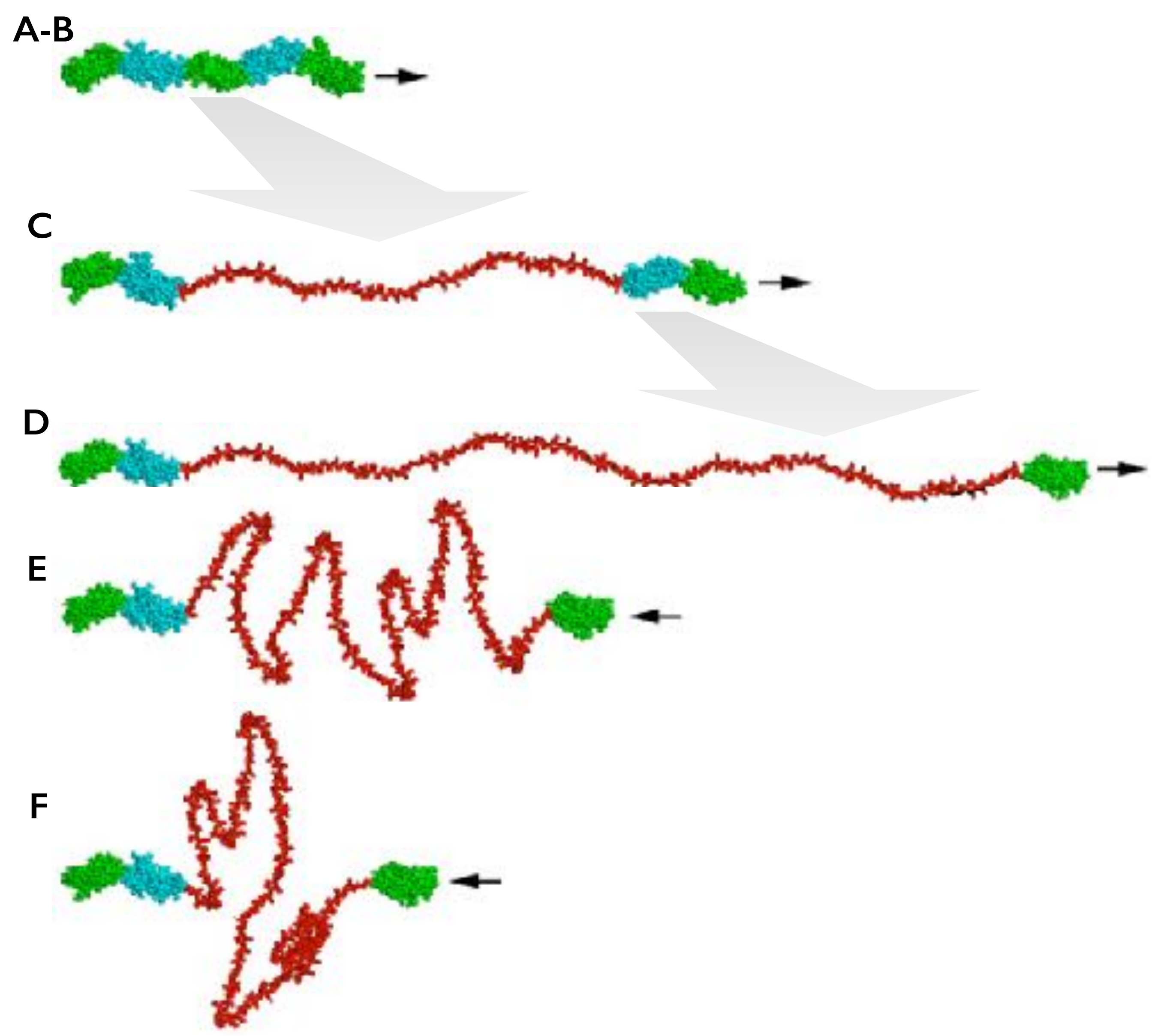
A titin nanomuzsikája



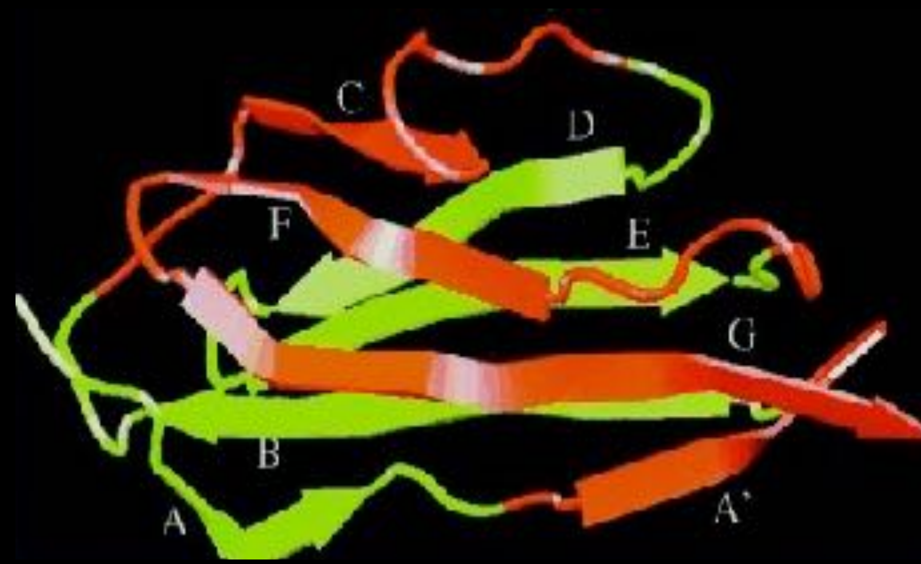


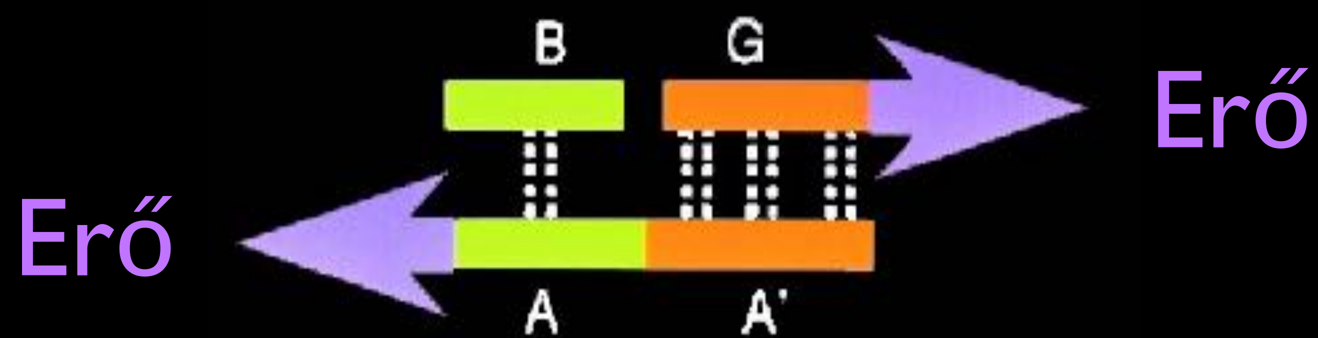
Erőhatásra a titin kitekeredik

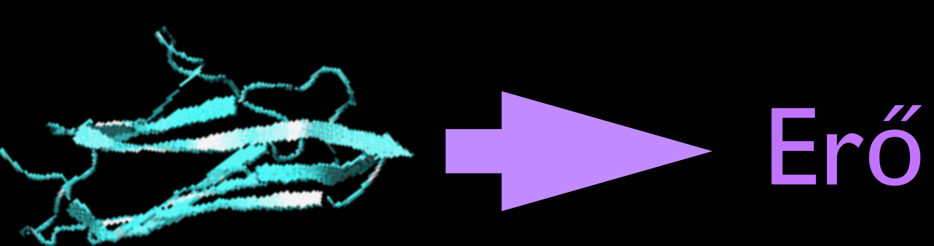




Vajon miért olyan stabilak titin doménjei?



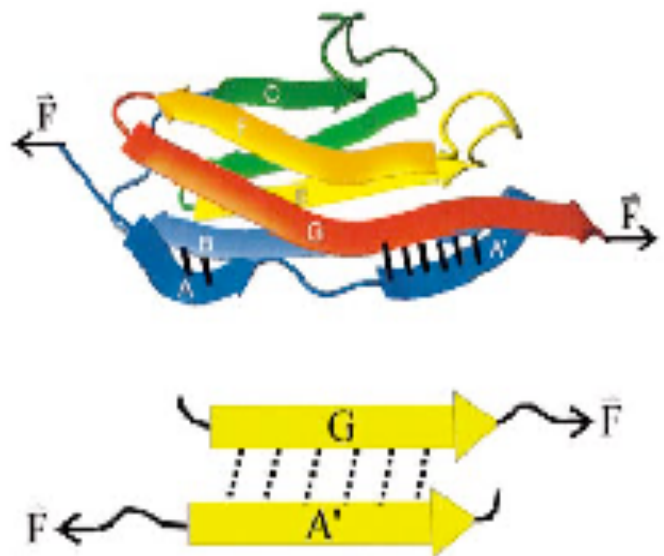


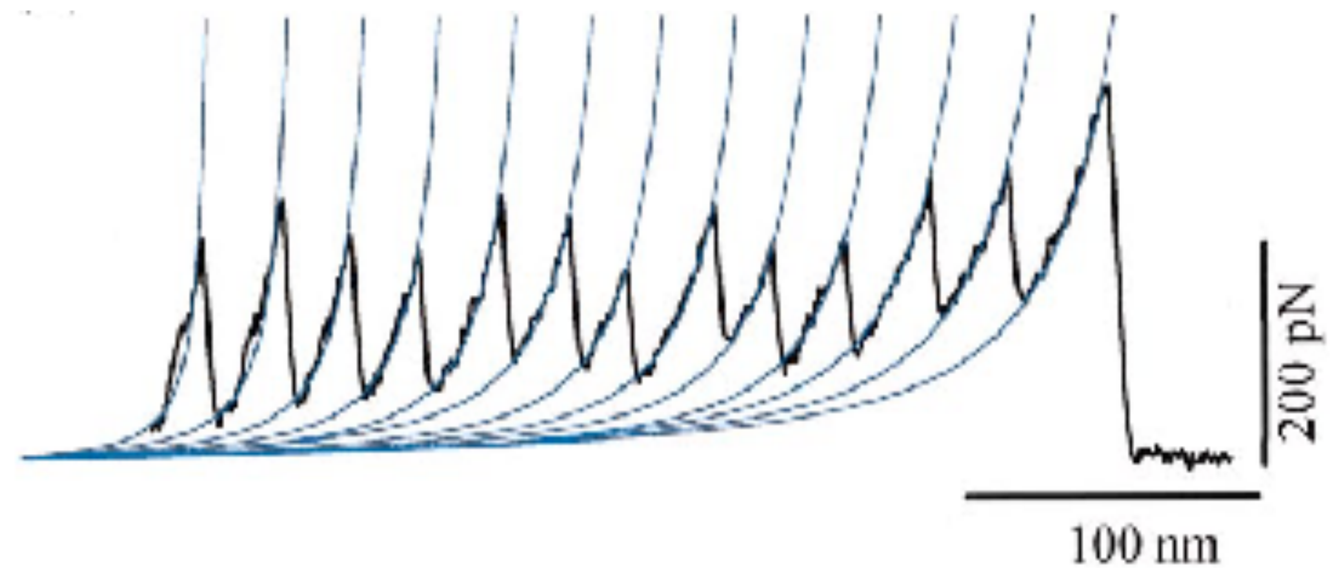


A mechanikai stabilitás biológiai logikája

Szerkezetet összetartó H-hidak párhuzamos csatolása

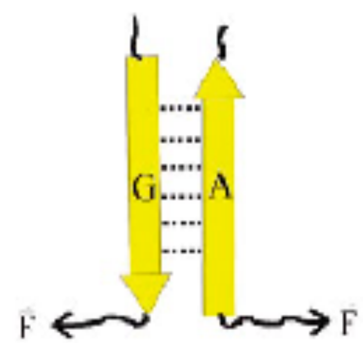
Nagy kitereredési erő

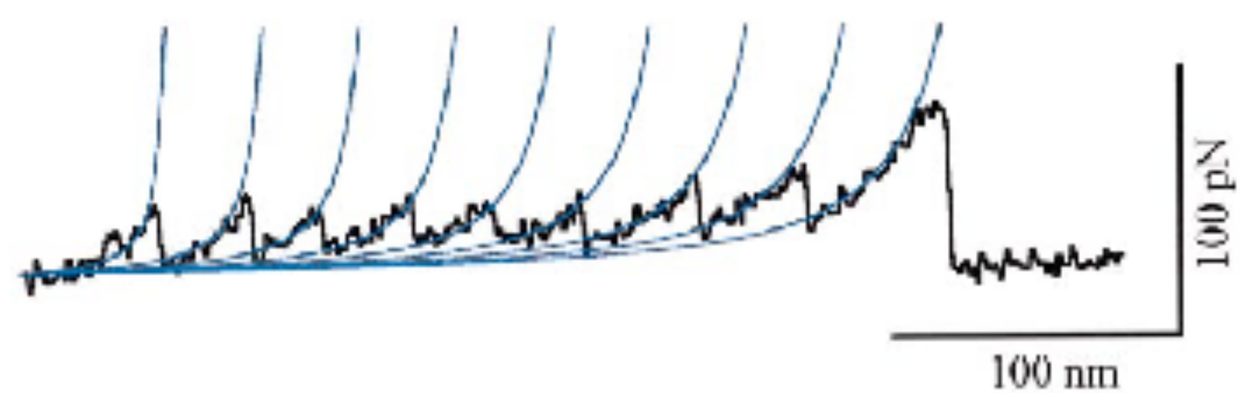




Szerkezetet összetartó H-hidak soros csatolása

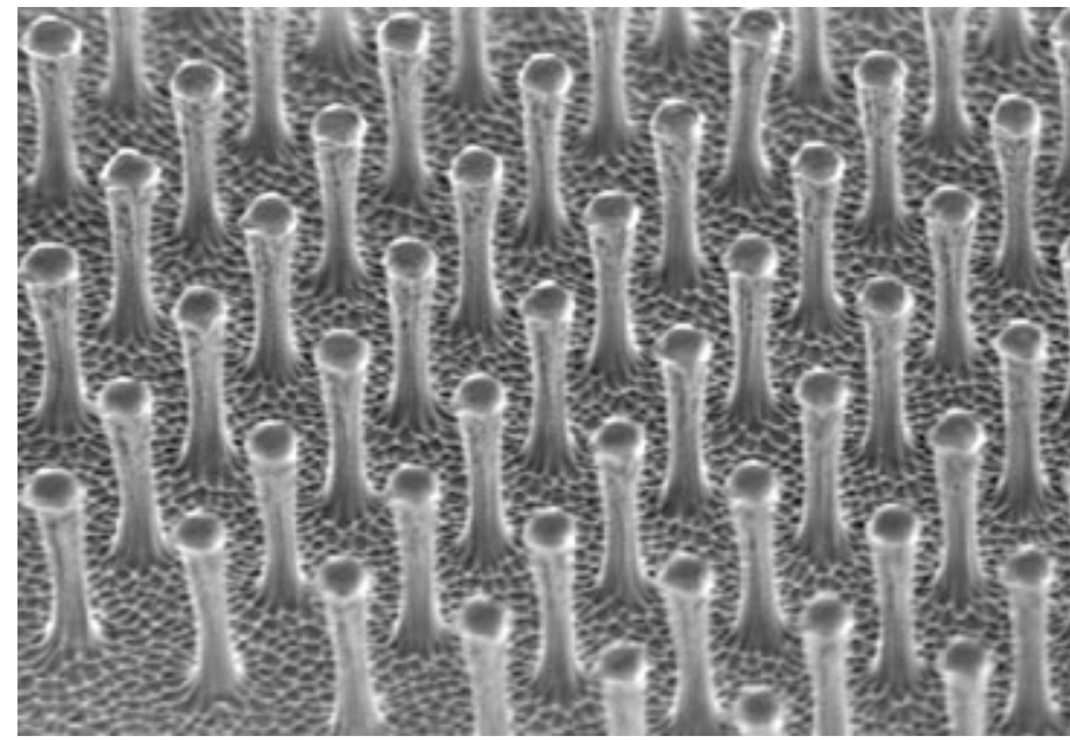
Alacsony kitereredési erő





Makroszkópikus mechanikai stabilitás

Effektív ragasztóanyag a párhuzamos csatolás elvén

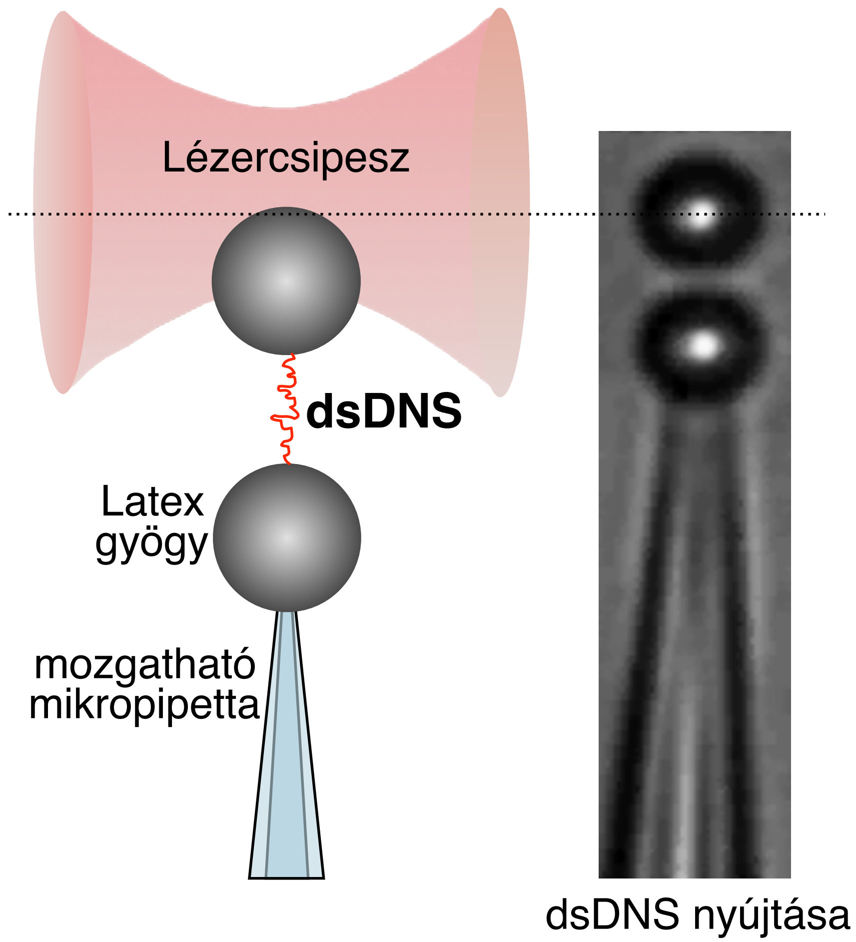


Mesterséges gecko talp
Nanotechnológiával készítve

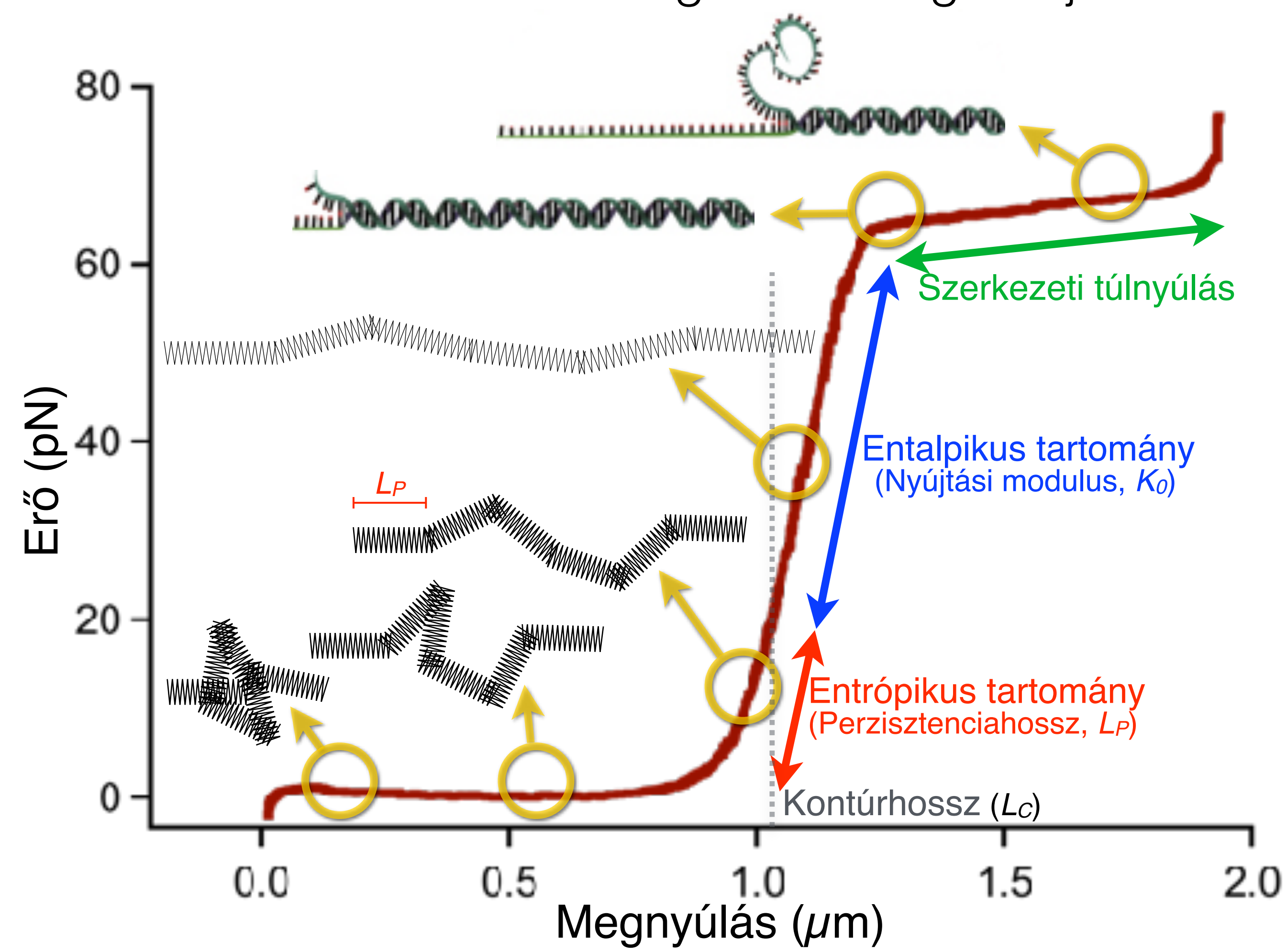
Gecko talp felületi tapadása:
Párhuzamosan csatolt Van der Waals kötések a serték és a felület között

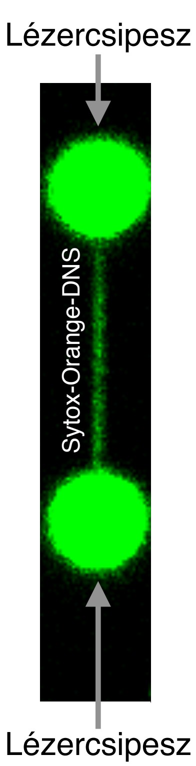
2. példa: a dsDNS molekula mechanikája

Rugalmasságmérés: lézercsipesszel

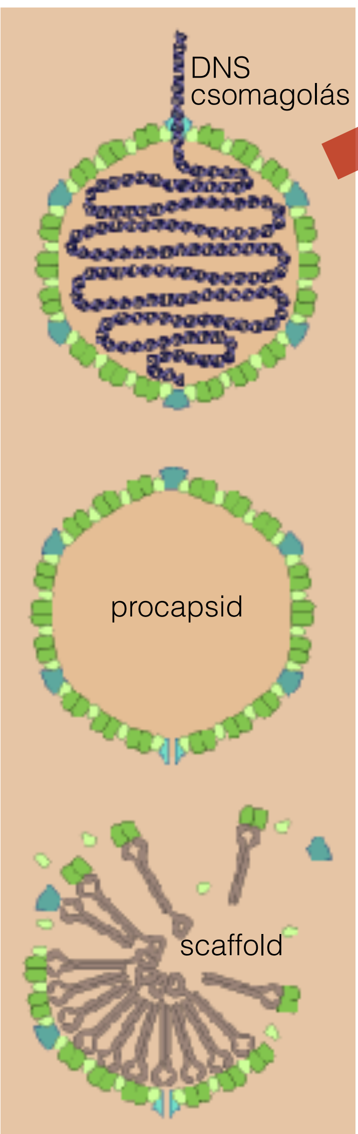


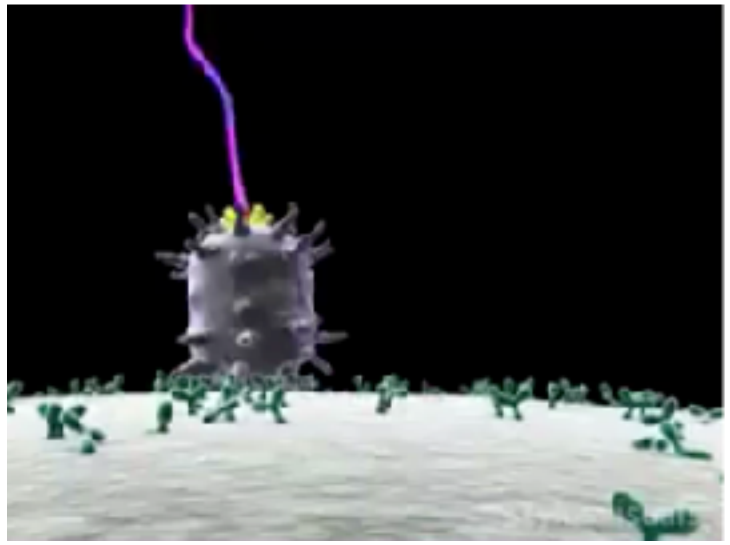
A dsDNS rugalmas erőgörbéje



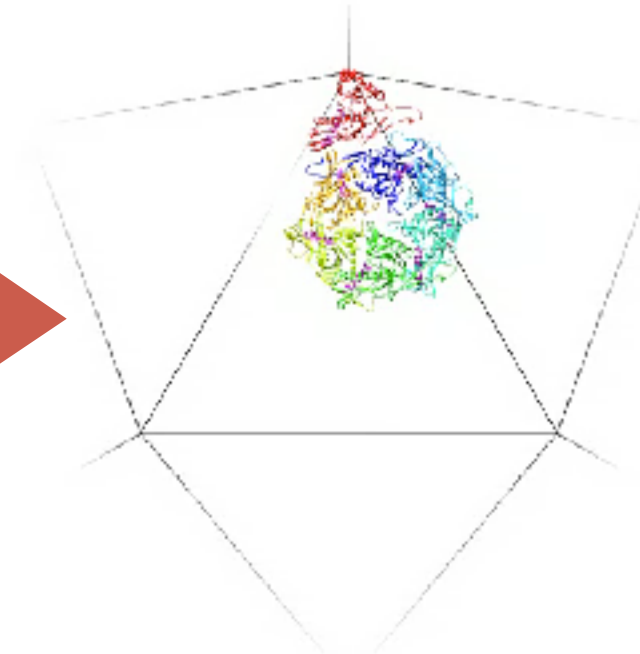


3. példa: egy DNS vírus működése



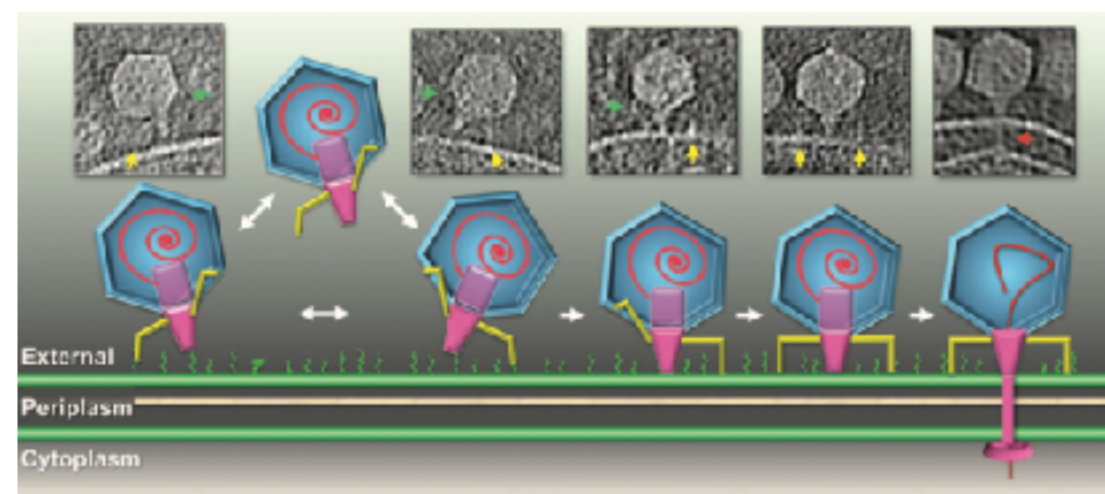


- **DNS becsomagolása** (ATP-függő, motor-vezérelt folyamat, kialakuló DNS nyomás ~60 atm!)



Szerkezeti érés:

- Kapszid expanszió, fal vékonyodás
- *gp10* N-terminális hélix kitekeredik, átlendül a kapszidfalon, majd felgombolyodik.
- Nem-kovalens, szerkezet-stabilizáló kötések alakulnak ki.



Fertőzés:

- receptor (LPS) felismerés
- trigger
- injektor komplex kialakulása
- DNS ejekció

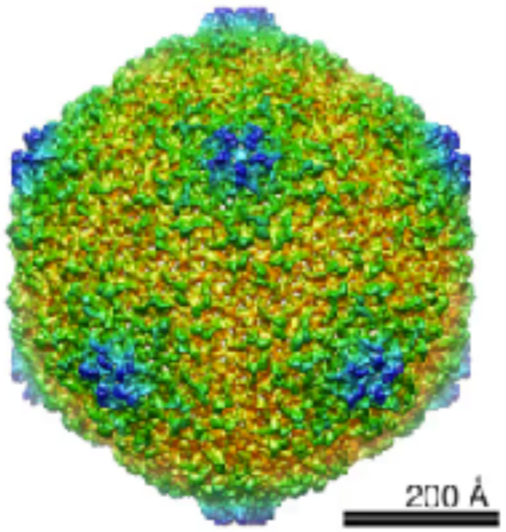
LamB (maltoporin) által kiváltott λ-fág DNS ejekció; Gyors DNS jelölés SYBR Golddal

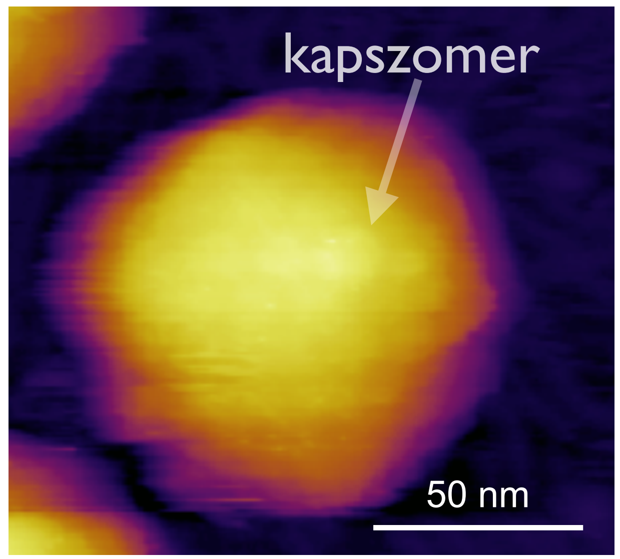
- **Vírus fehérjék szintézise** a gazdasejtben

- **DNS-mentes prokapszid kialakulása** (*"scaffold" fehérje-*függő)

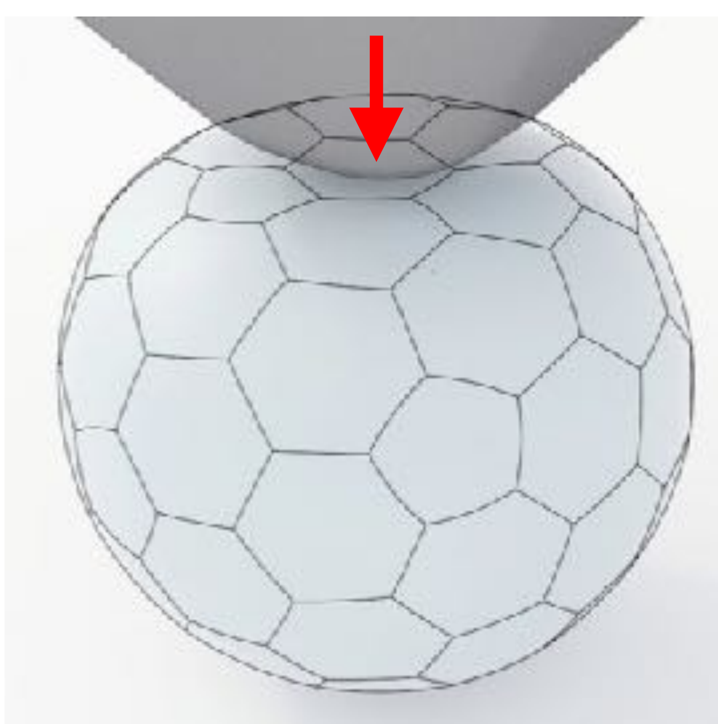
Vírus vizsgálat AFM-mel

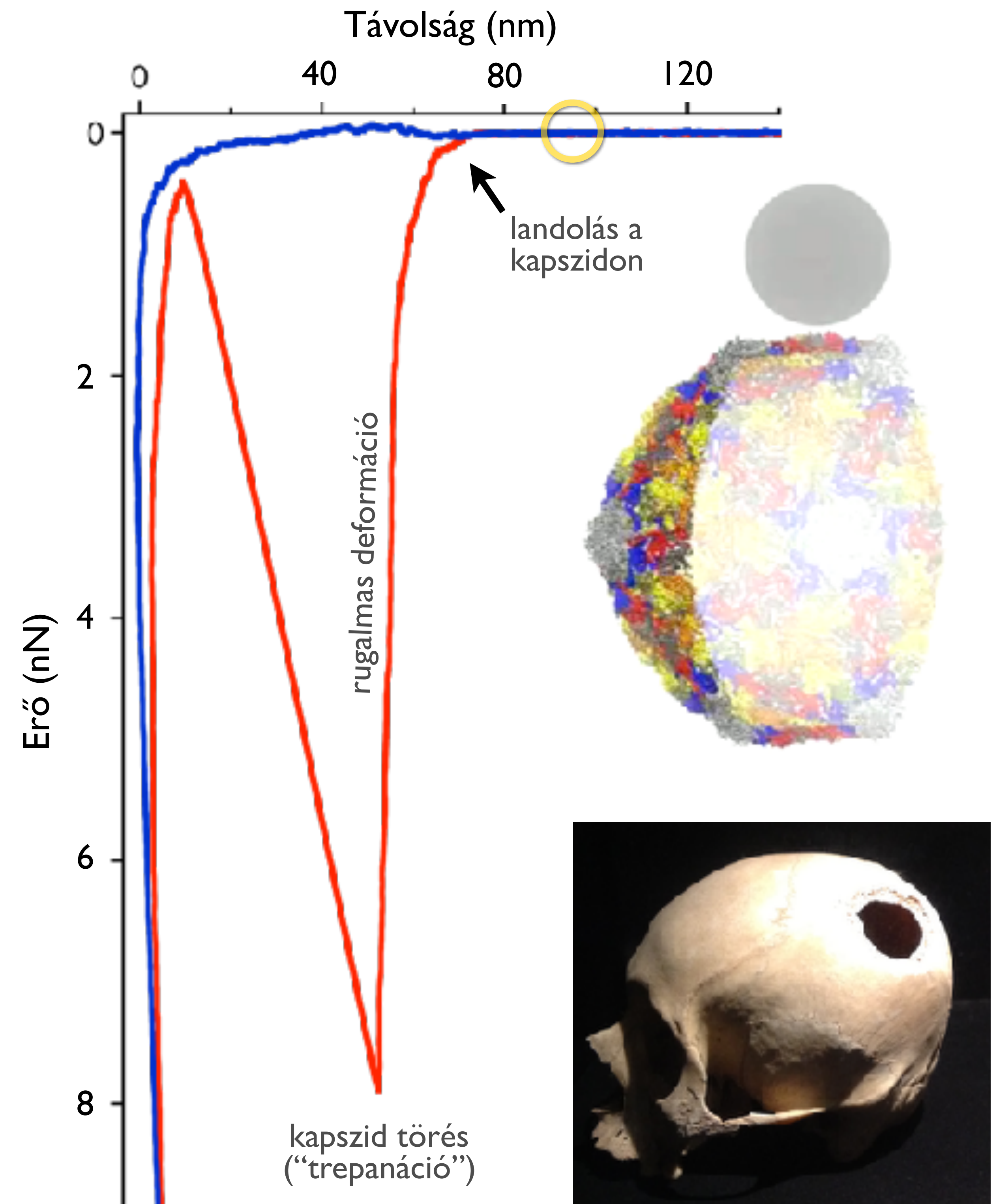
T7 bateriofág





Csillám felületen glutáraldehiddel immobilizált T7 fág partikulumok

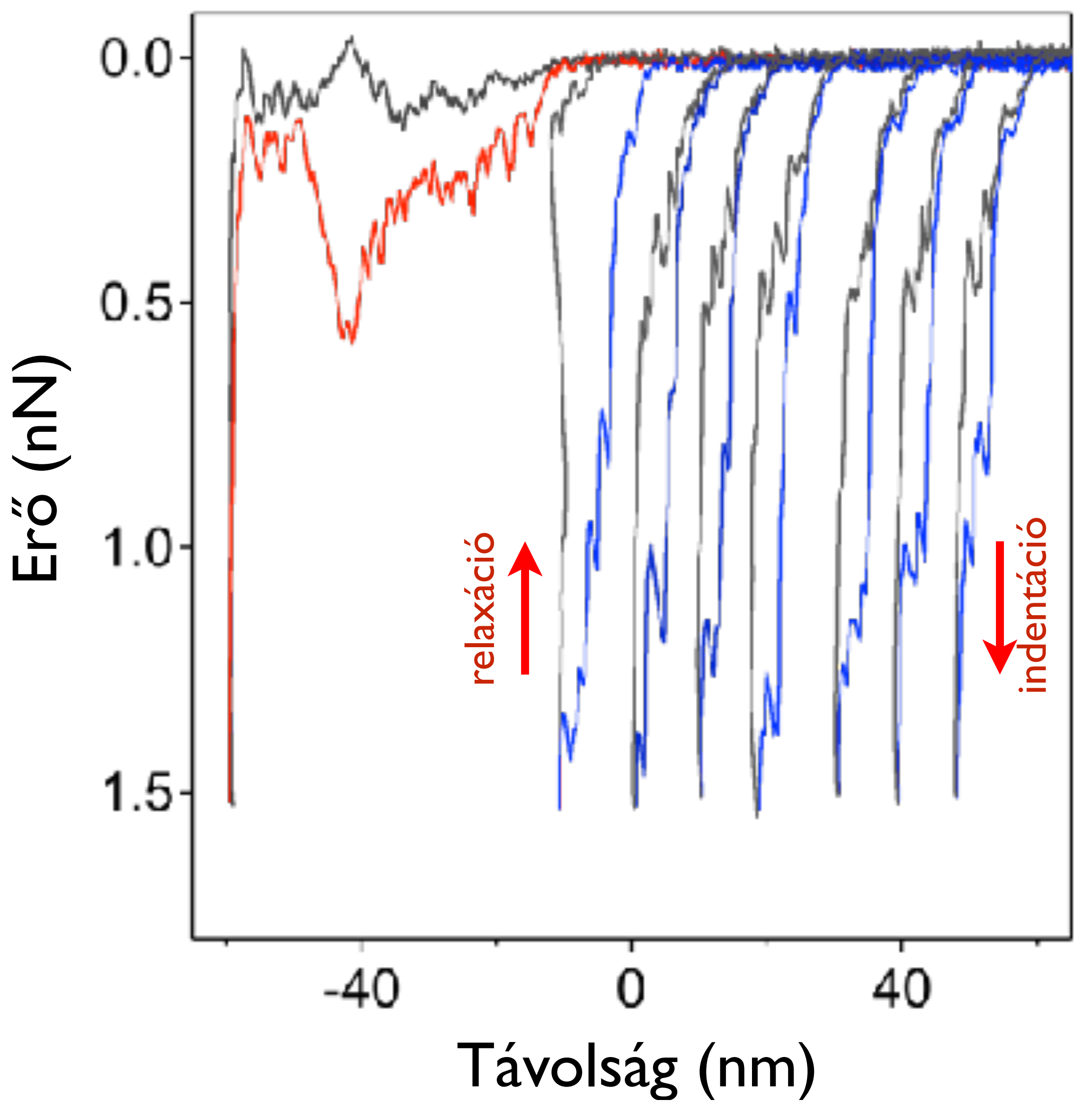


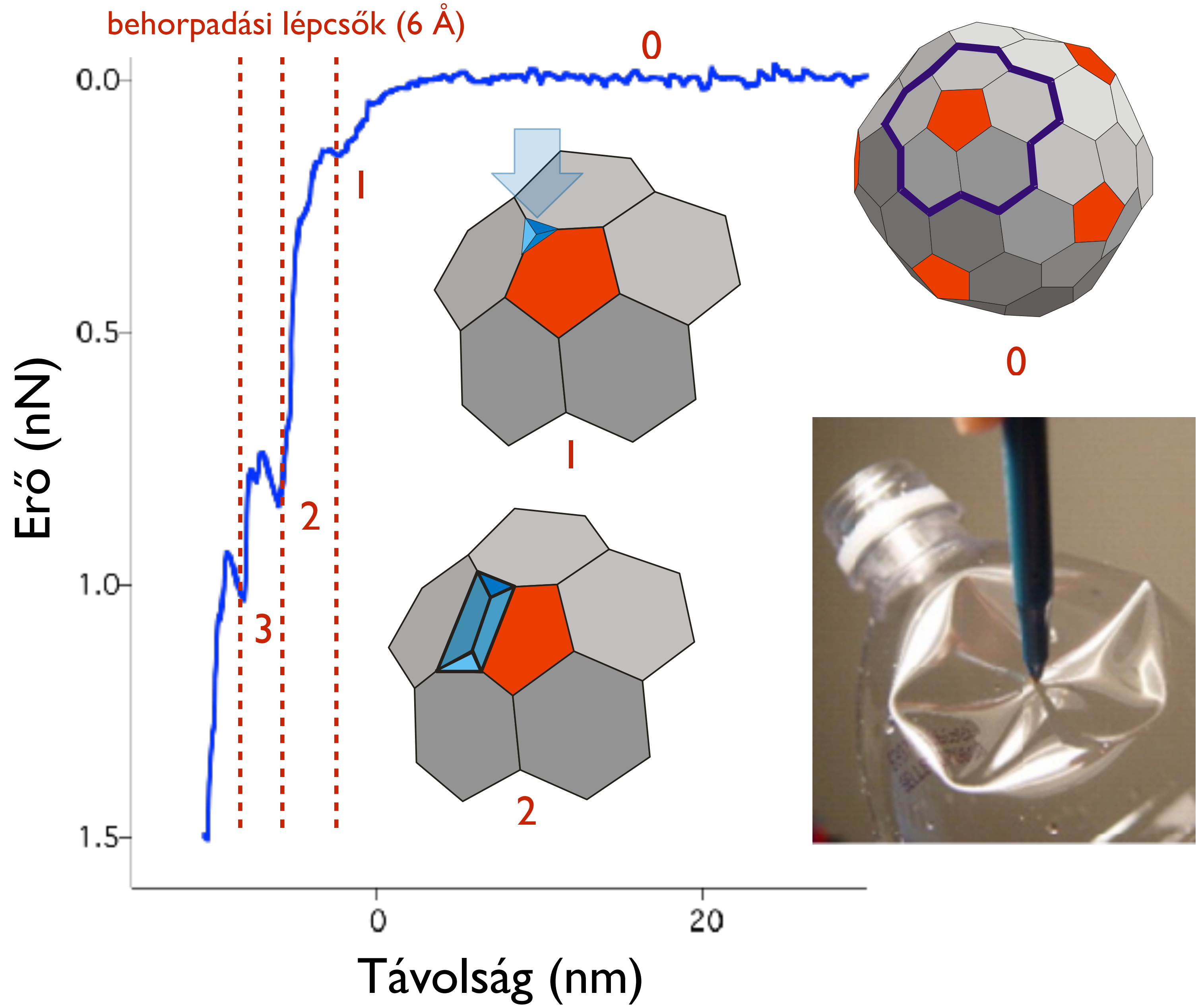


Trepanált inka koponya, Museo Rafael Larco Herrera, Lima

Erőhatásra a T7 fág lépcsőzetesen behorpad, de gyorsan regenerálódik

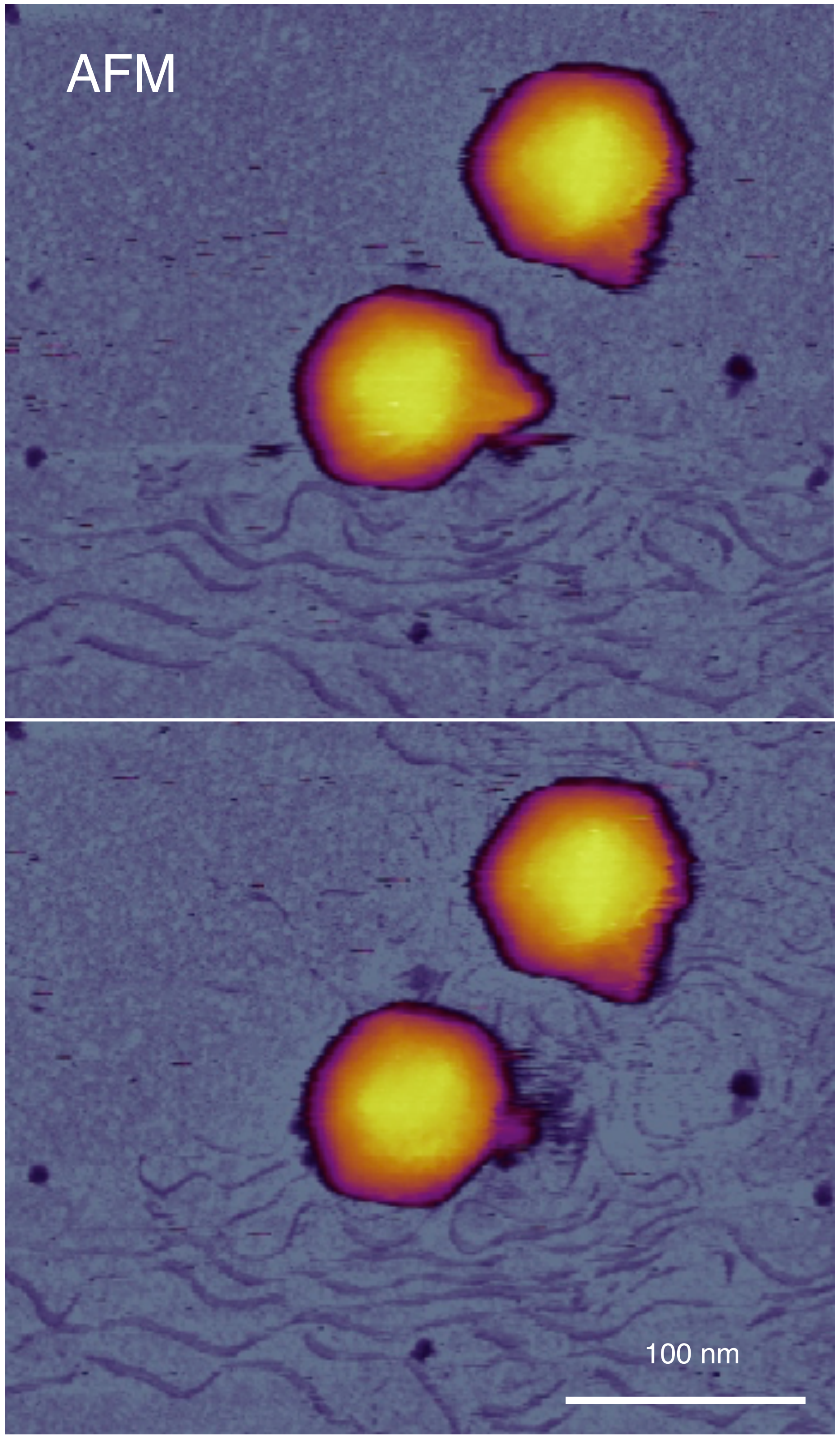
Ismétlődő mechanikai ciklusok

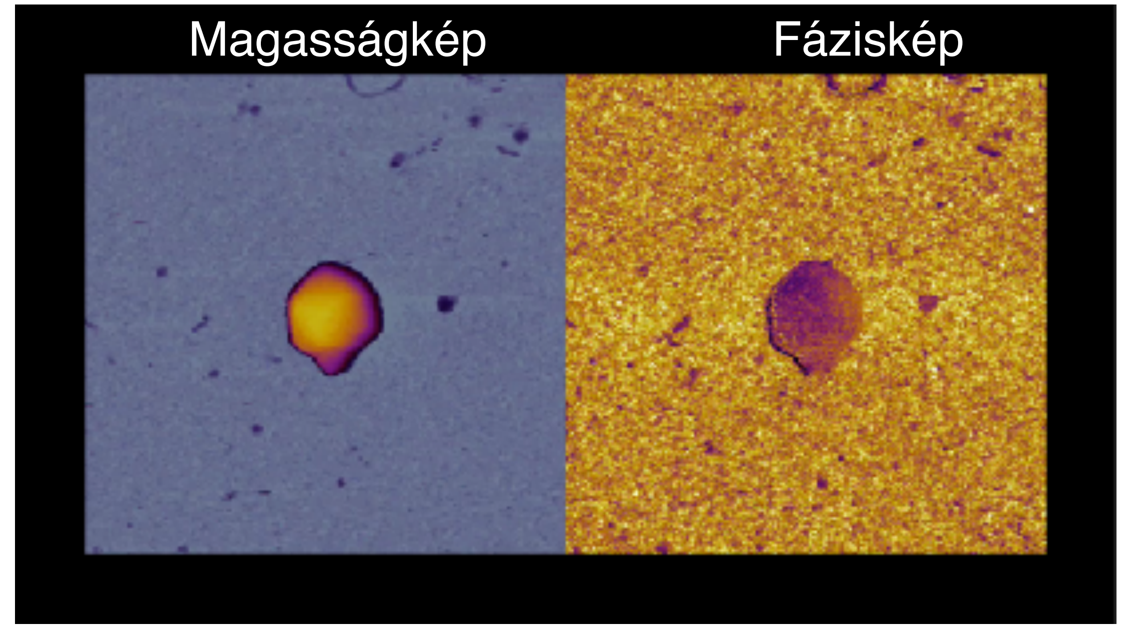


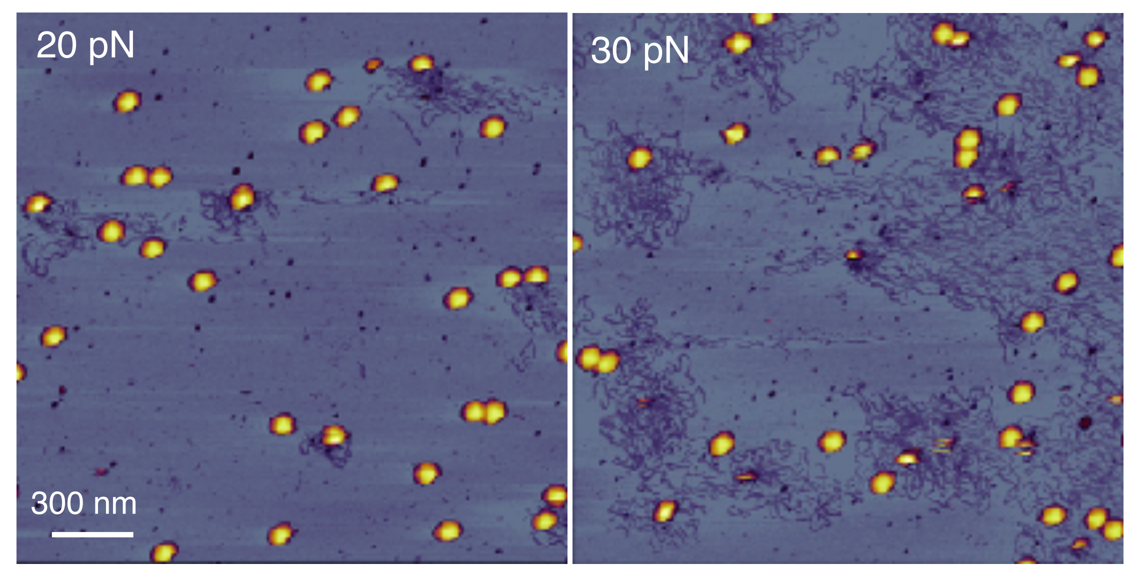


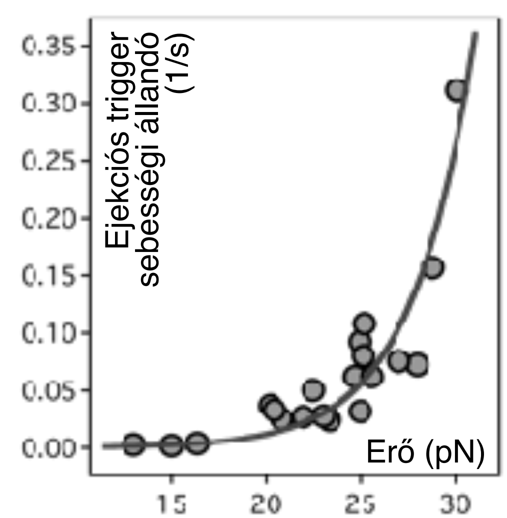
Vörös et al. Nanoscale **9**, *1136, 2017.*

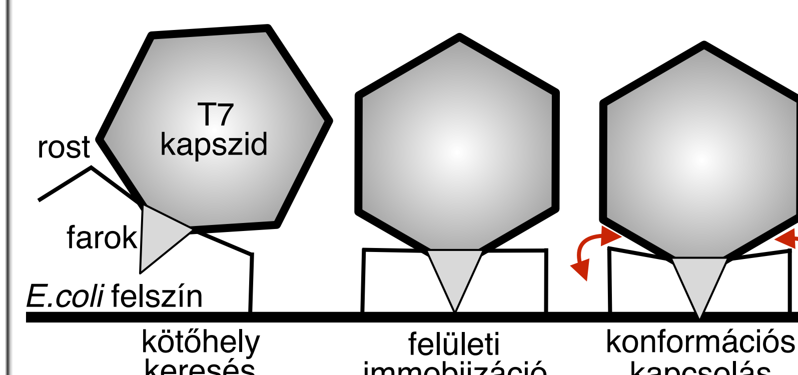
Vírus DNS kilökődés mechanikai erővel kiváltható!



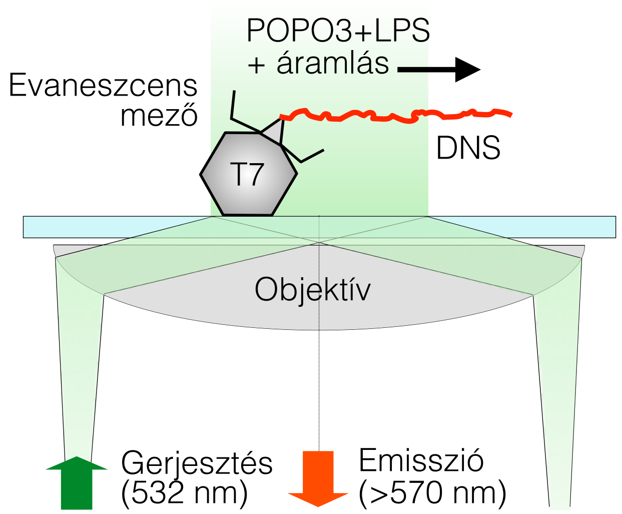


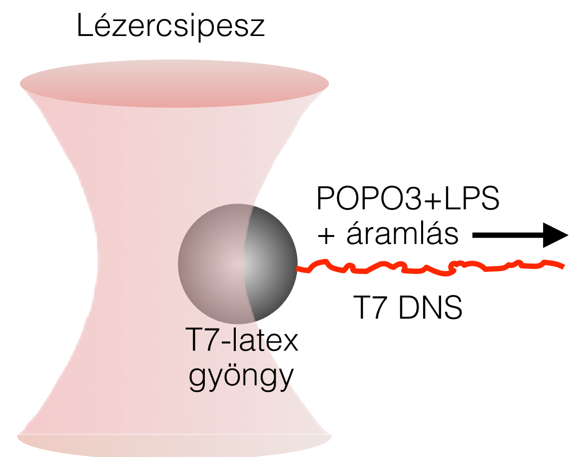


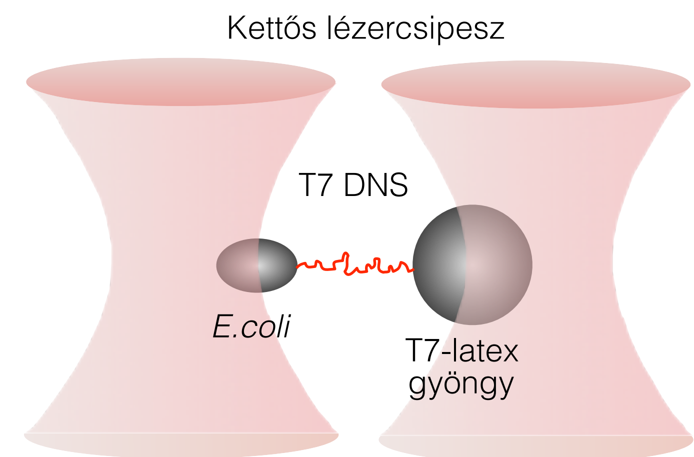


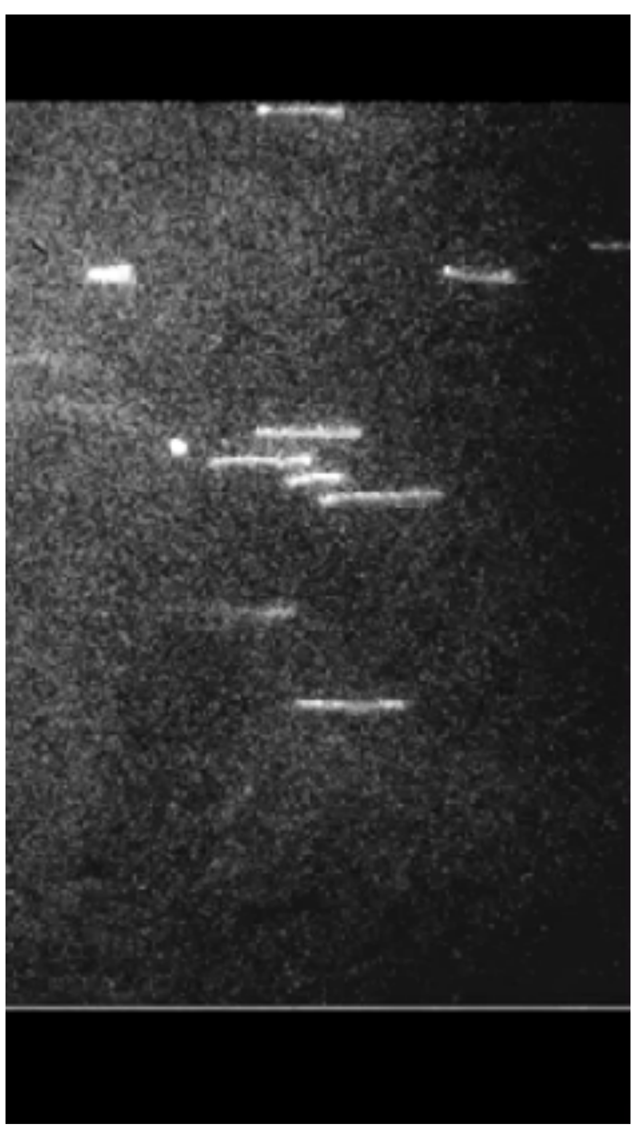


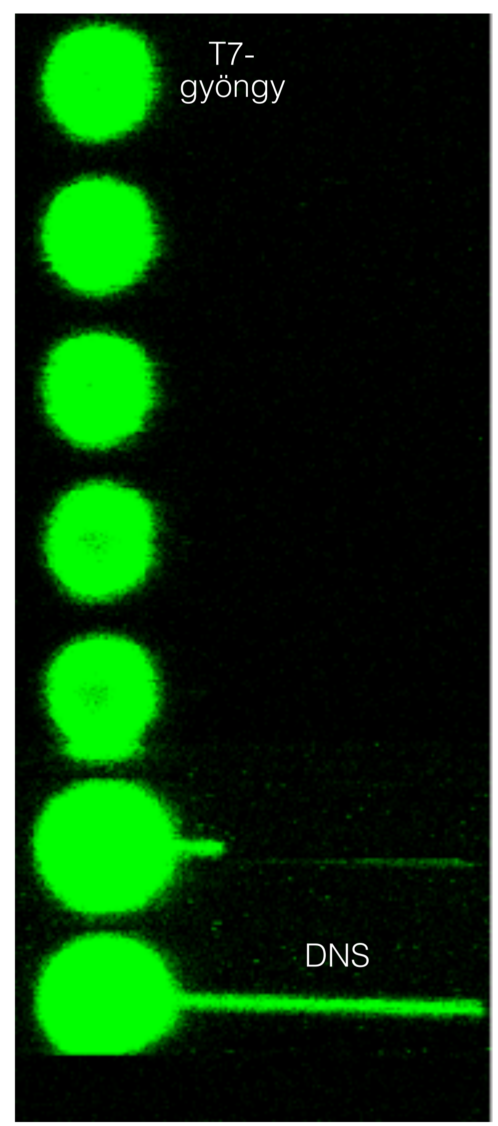
T7 DNS kilökődés és bakteriális transzfer nanomechanikája

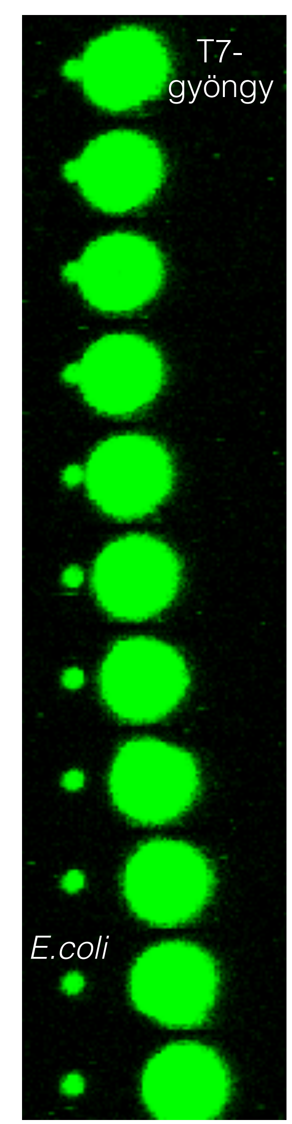


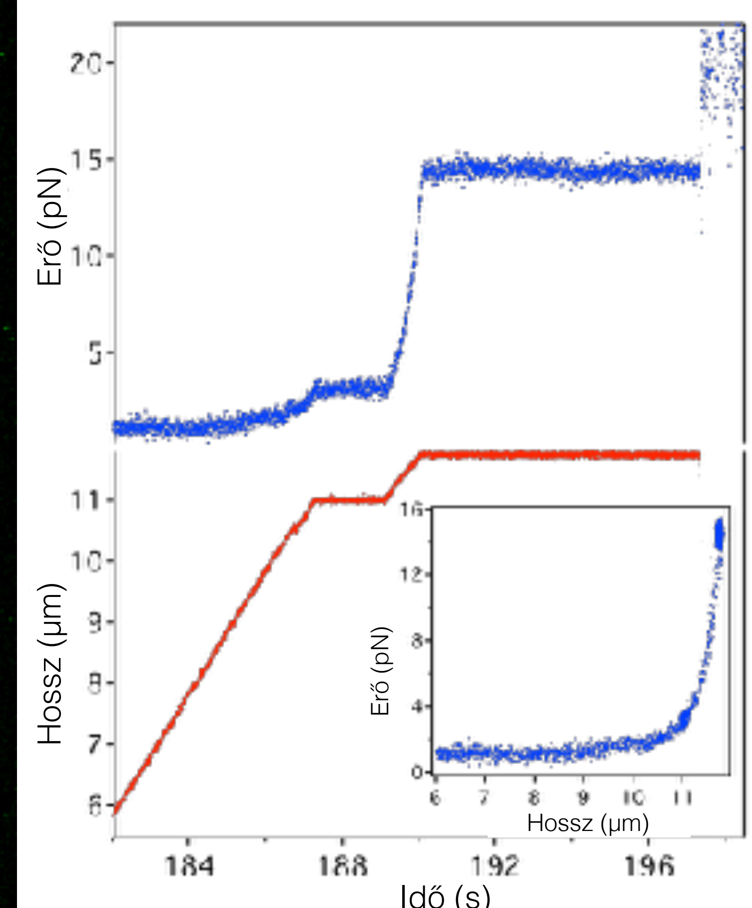




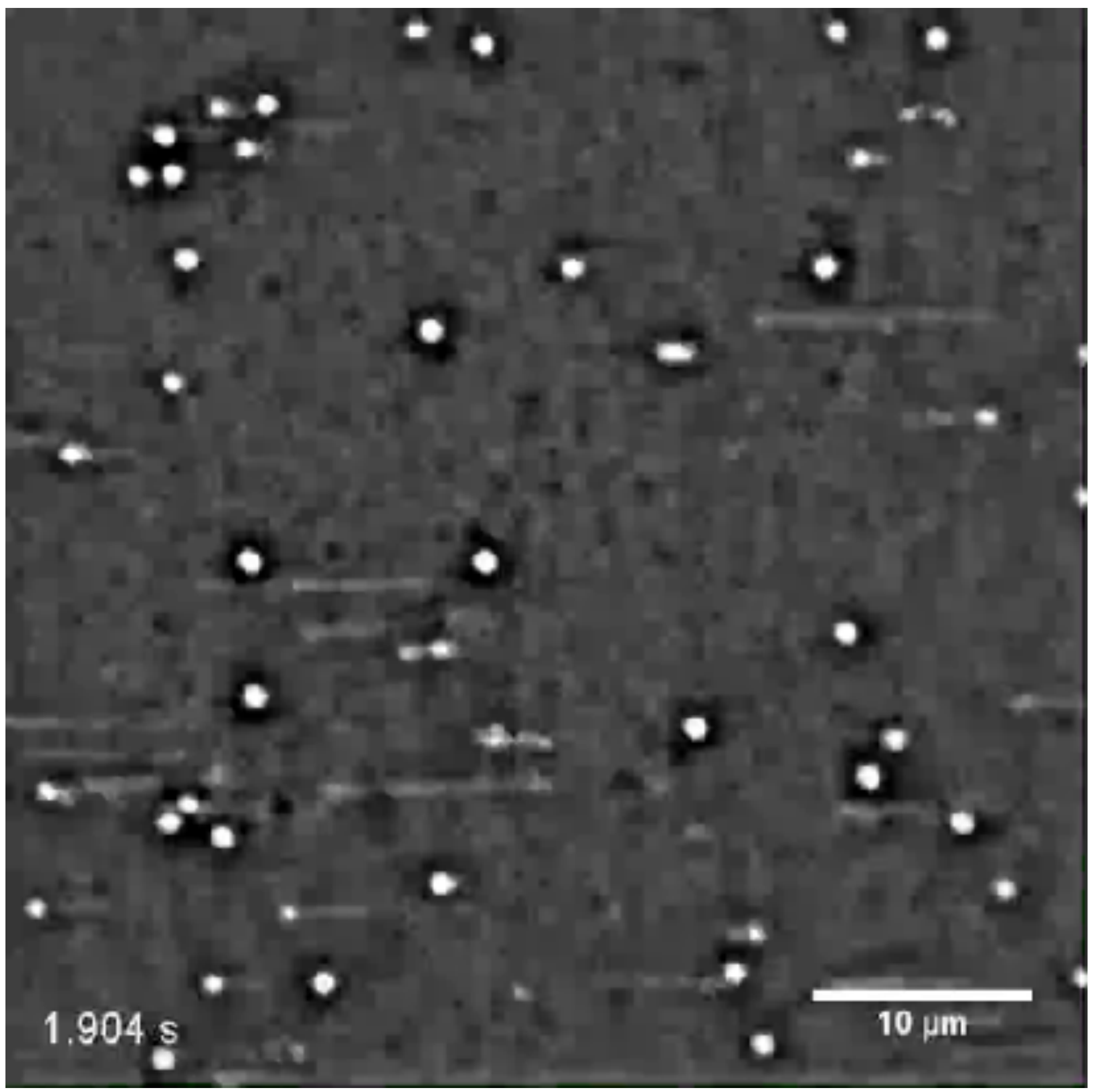


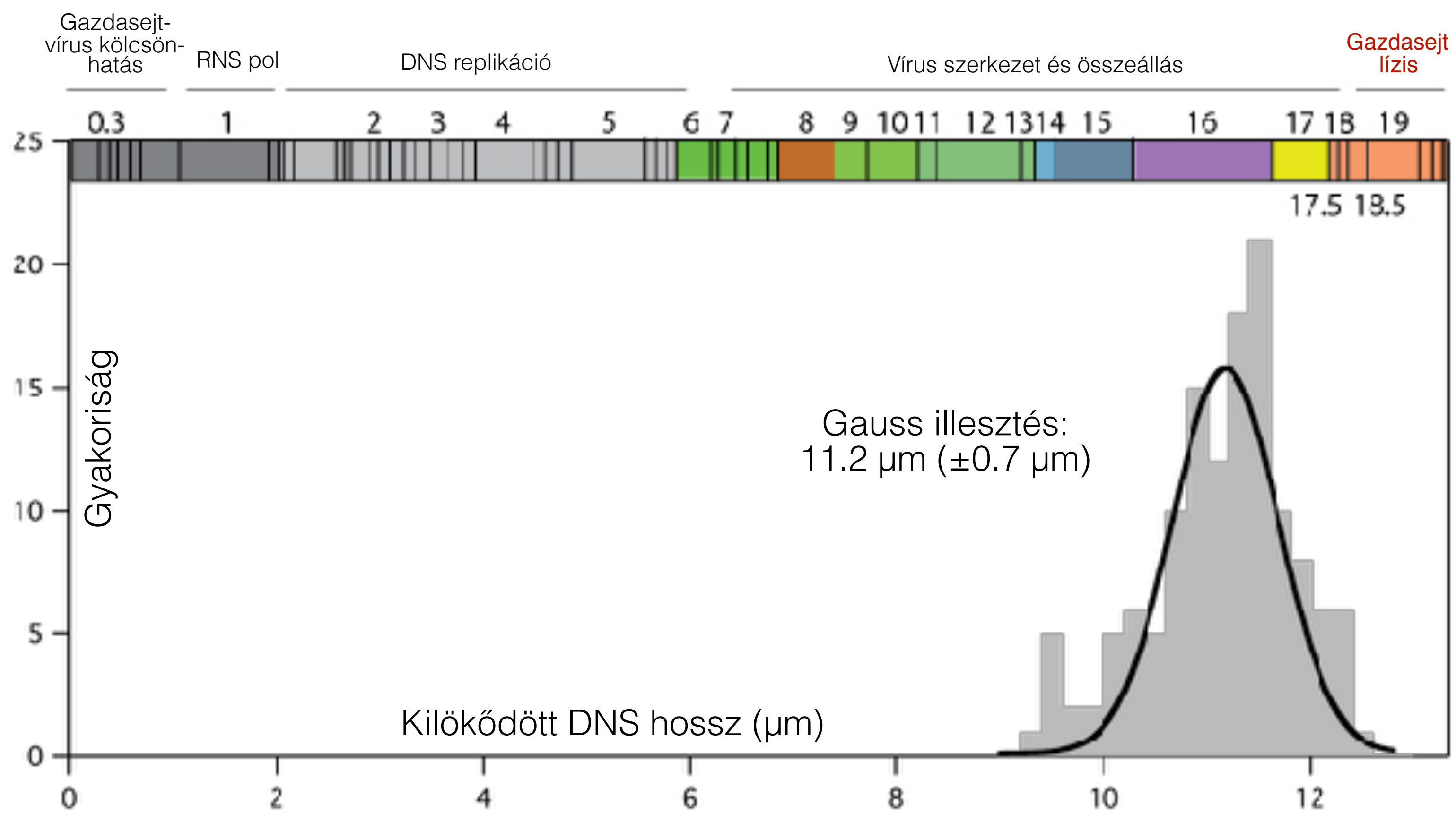


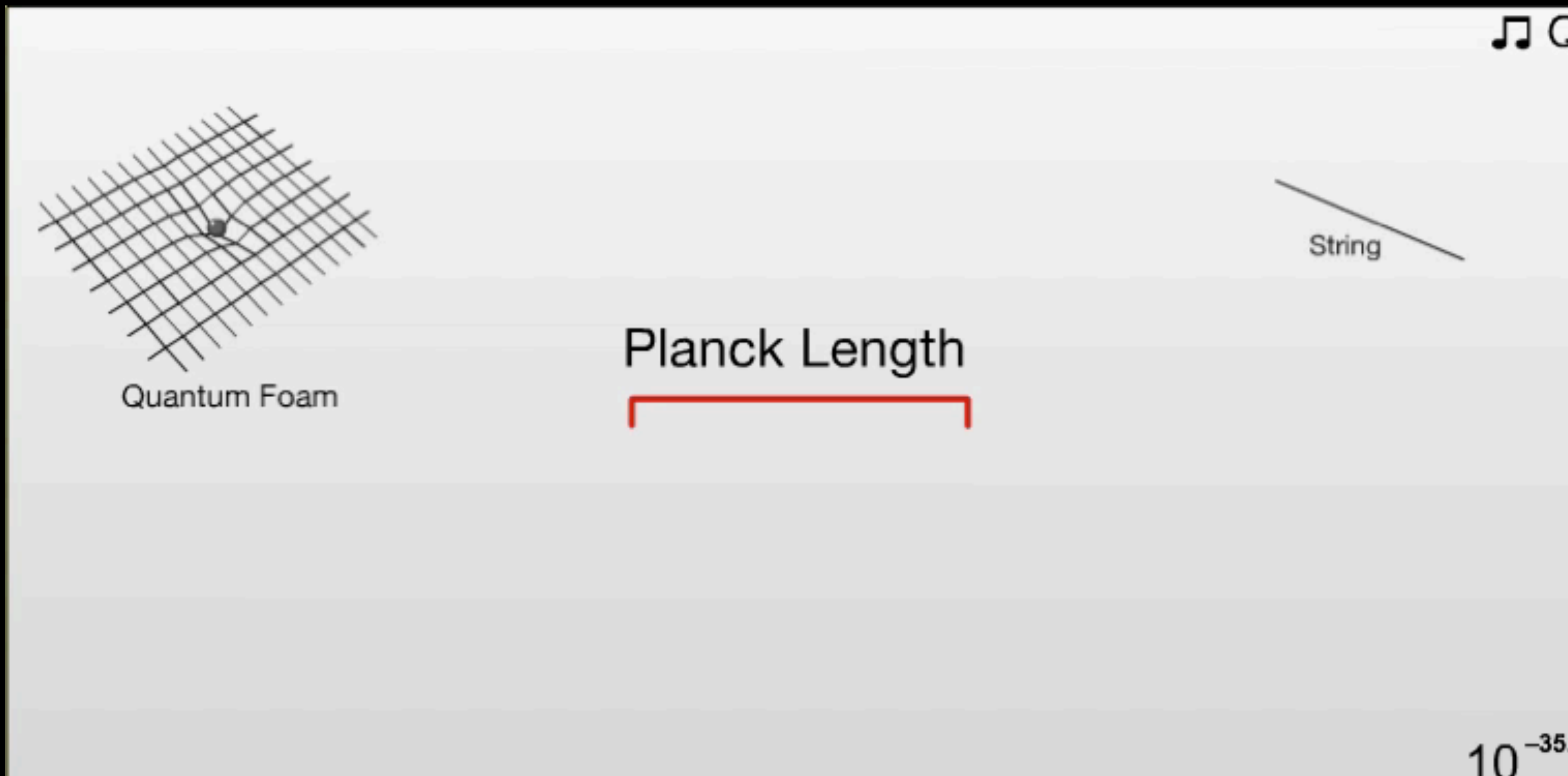




A T7 DNS kilökődés mechanikai szabályozás alatt áll







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