CEEPUS, Budapest, 24 May, 2024

From single molecules to complex systems

Passion for discovery and value-driven leadership

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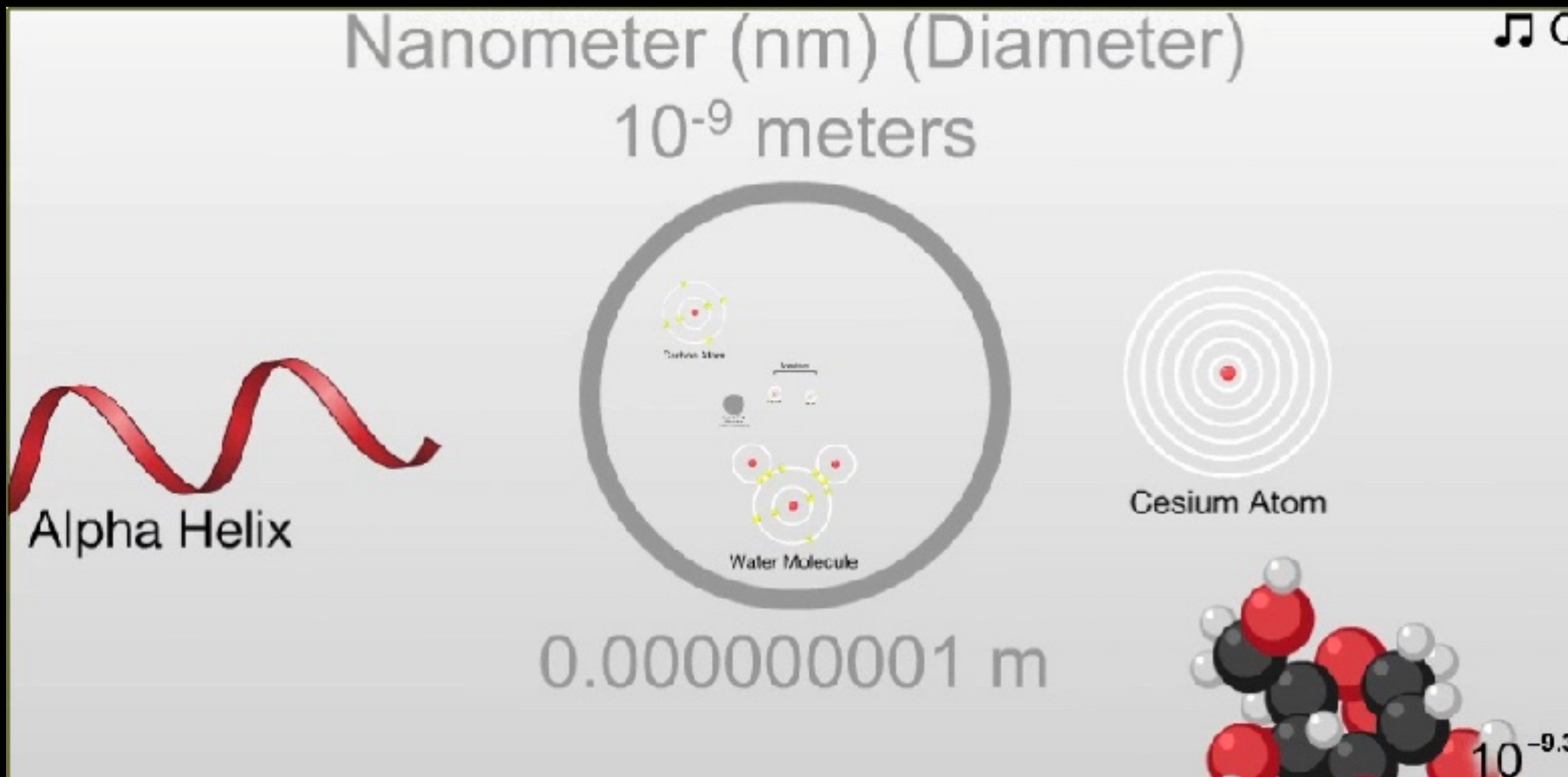
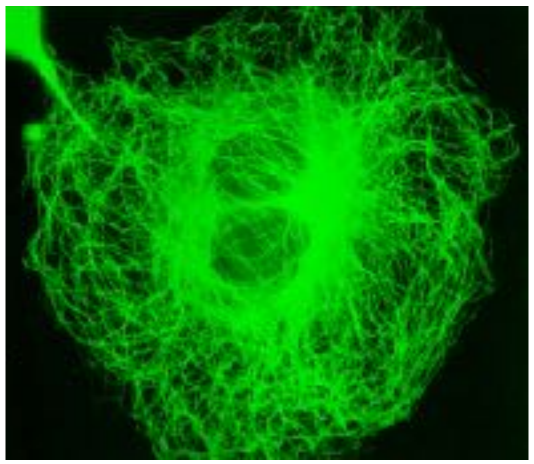


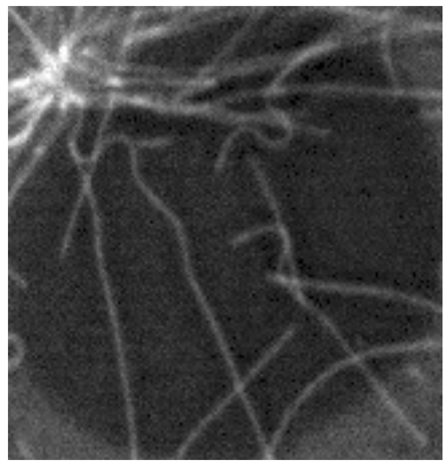
Cary and Michael Huang (http://htwins.net)

Why single molecules?

1. Individuals (spatial and temporal trajectories) may be identified in a crowd



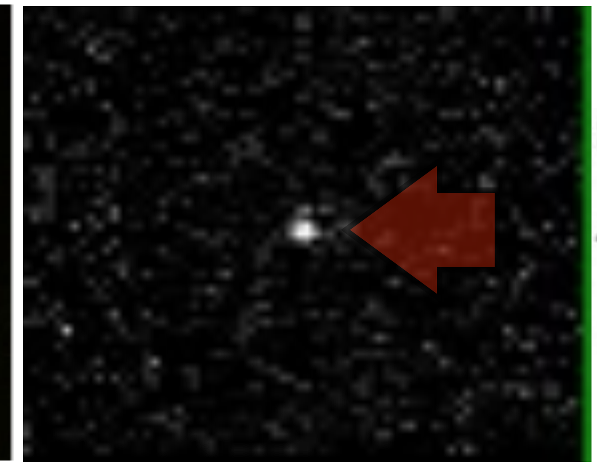
Ensemble - microtubular system

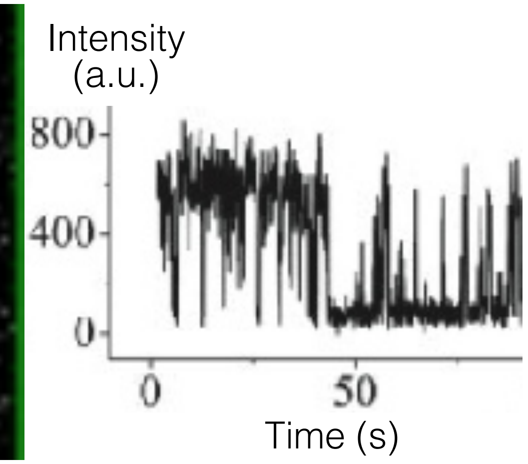


Single microtubues - treadmilling

2. Stochastic processes may be uncovered

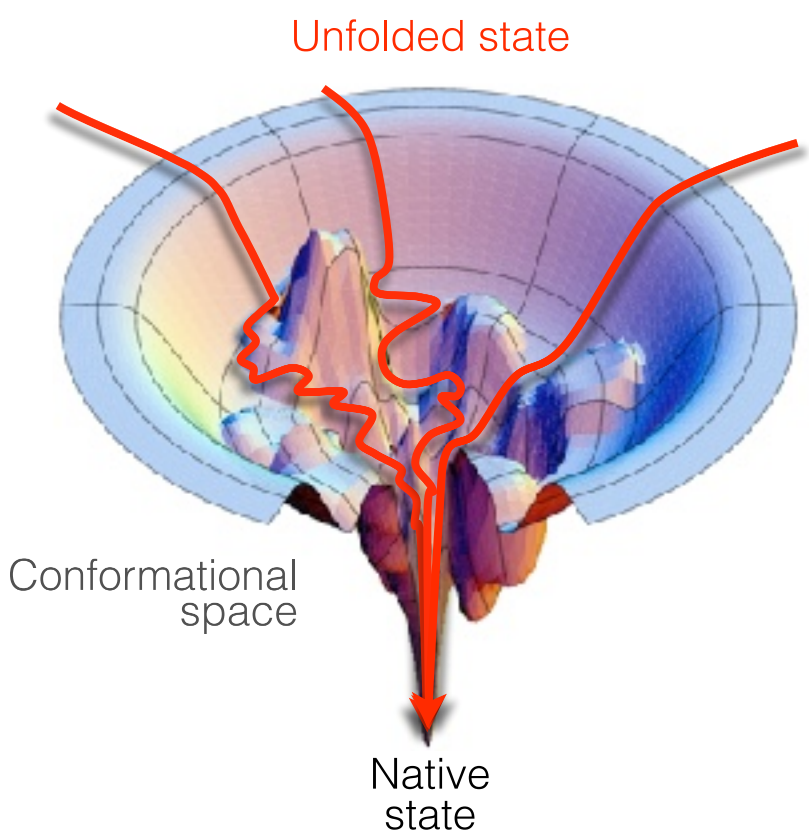
Ensemble - intensity



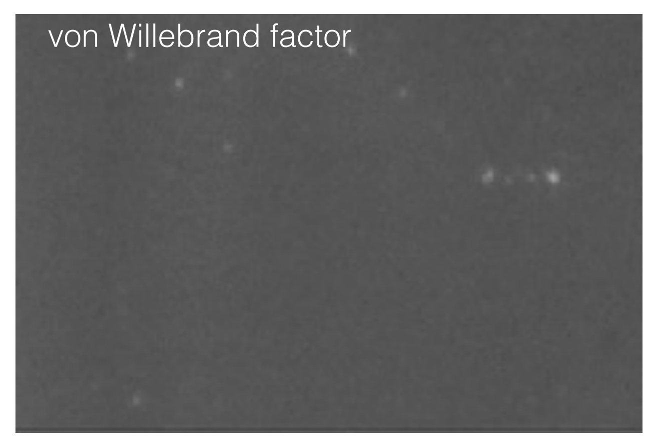


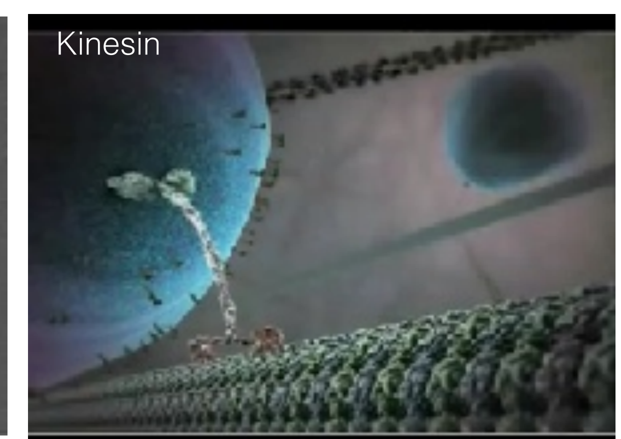
Single quantum dot - blinking

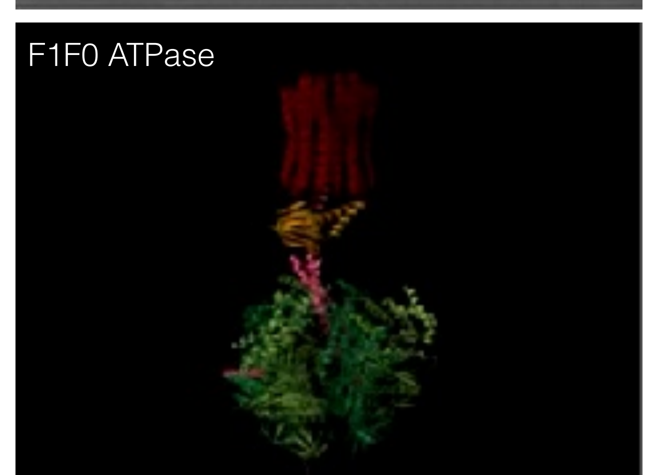
3. Parallel-pathway events may be identified



4. Mechanics of biomolecules may be characterized

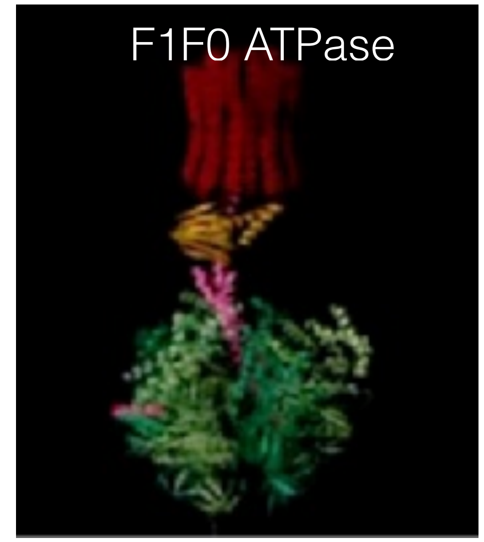


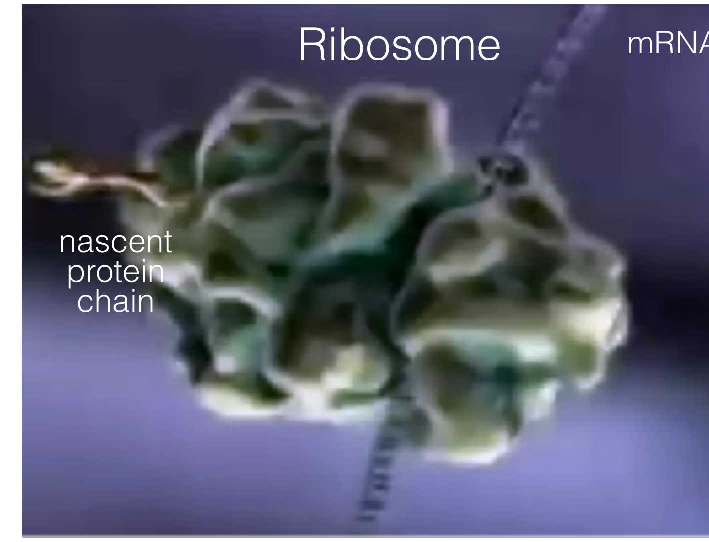




Role and use of mechanical force

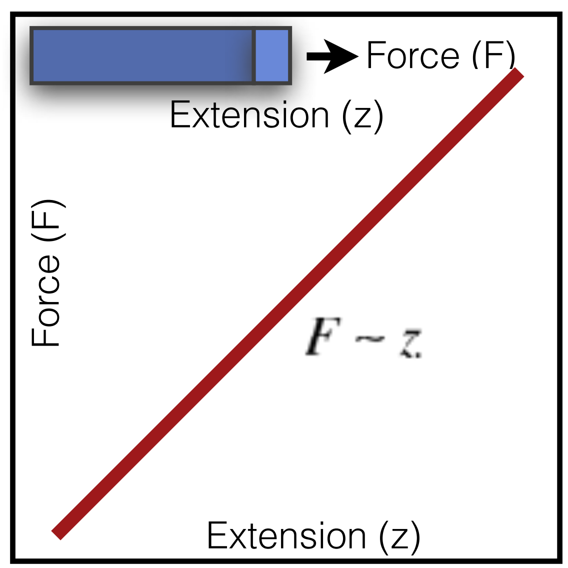
1. Force: *develops*



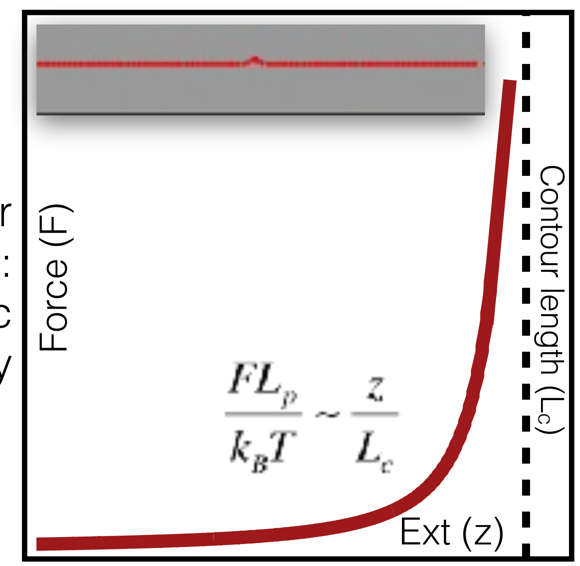


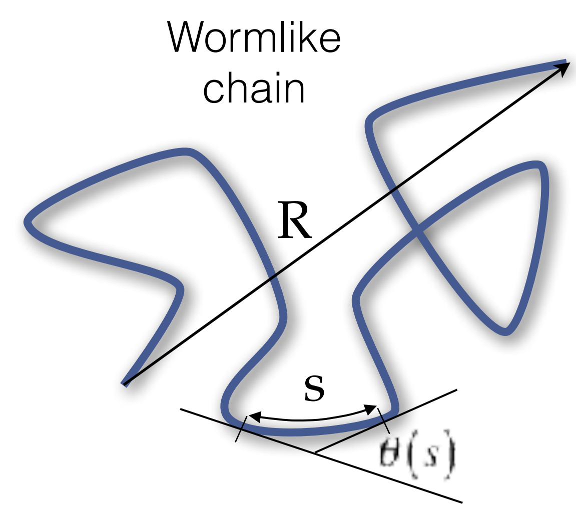
2. Force: *deforms* shape

Rigid body: Hooke's law

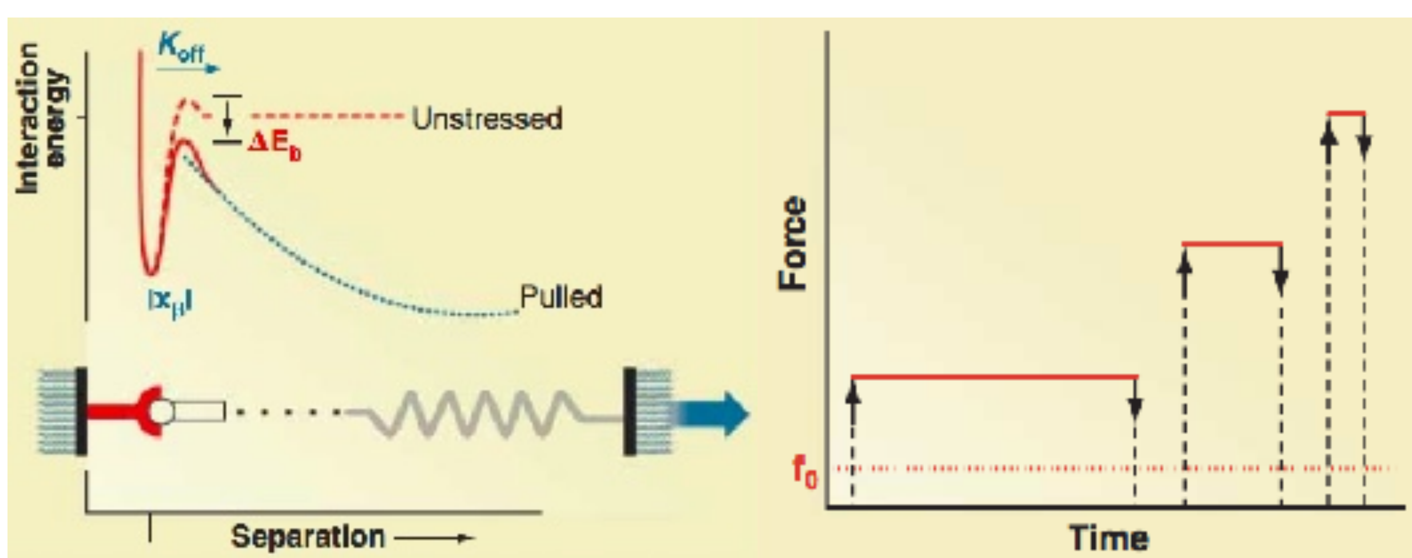


Polymer chain: entropic elasticity





3. Force: reduces bond *lifetime*



$$\tau(F) = \omega e^{\frac{E_a - F\Delta x}{k_BT}} = \tau(0) e^{-\frac{F\Delta x}{k_BT}}$$

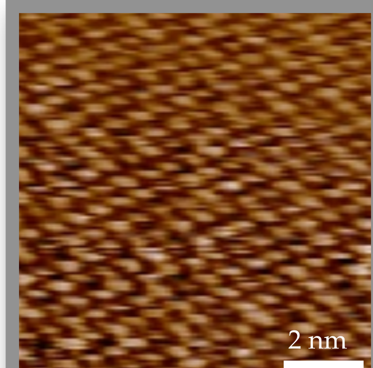
ω = characteristic time
E_a = activation energy
Δx = distance between bound and transition states

“3M” ... ...”molecule - mouse - man”

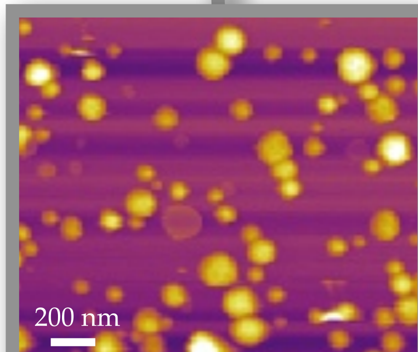
Nanobiotechnológiai és In Vivo Képalkotó Központ
Semmelweis
NIVIC
Nanobiotechnology and In Vivo Imaging Center

AFM
(AR MFP1D,
MFP3D,
Cypher)

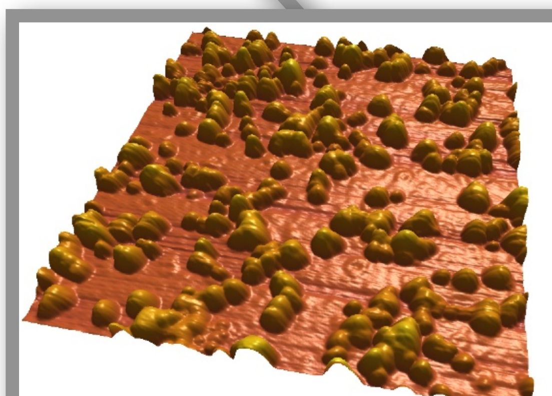
optical
tweezers



atoms on mica

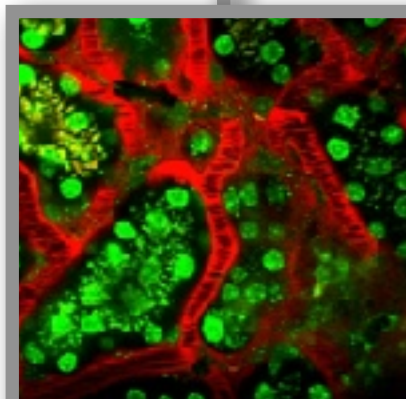


liposomes



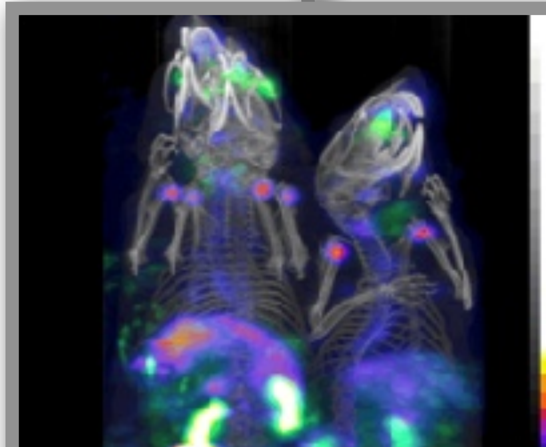
Prussian-blue multi-modal contrast nanoparticles

multi-
photon
microscopy

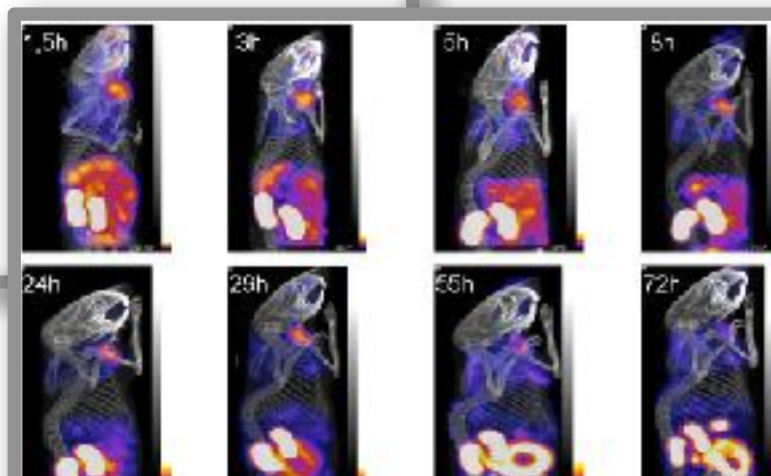


kidney cortex

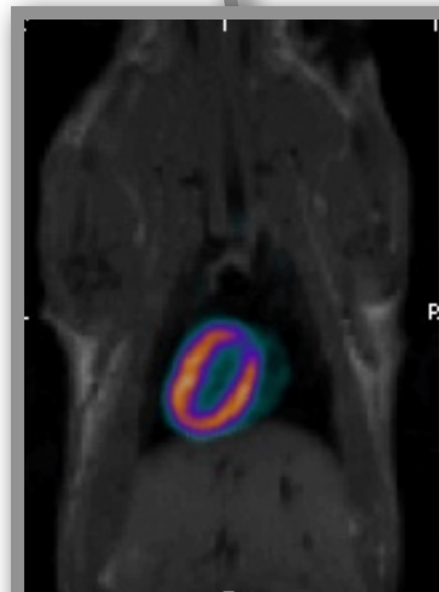
nanoSPECT/CT



^{99m}Tc-DTPA: BBB - blue/red)
^{201}Tl-DDC: perfusion - green



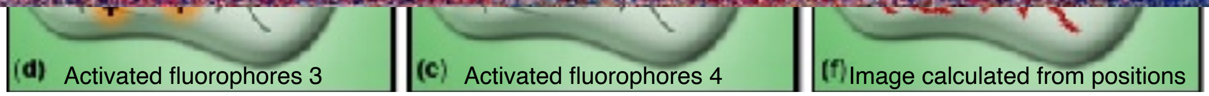
nanoPET/MRI

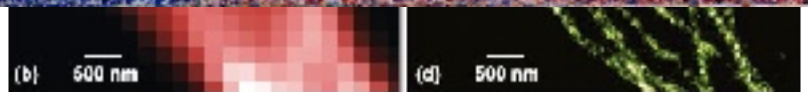


EKG-gated ^{18}FDG PET/MRI

Imaging nanoscale structures:

Data collection process

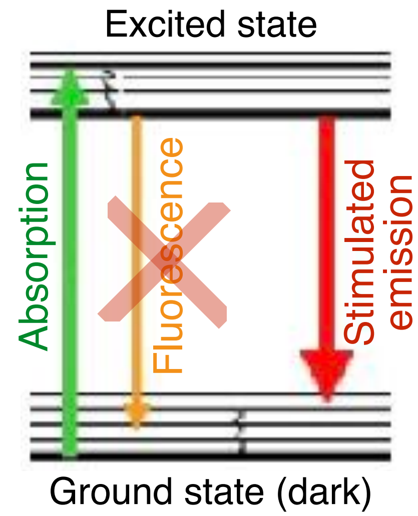


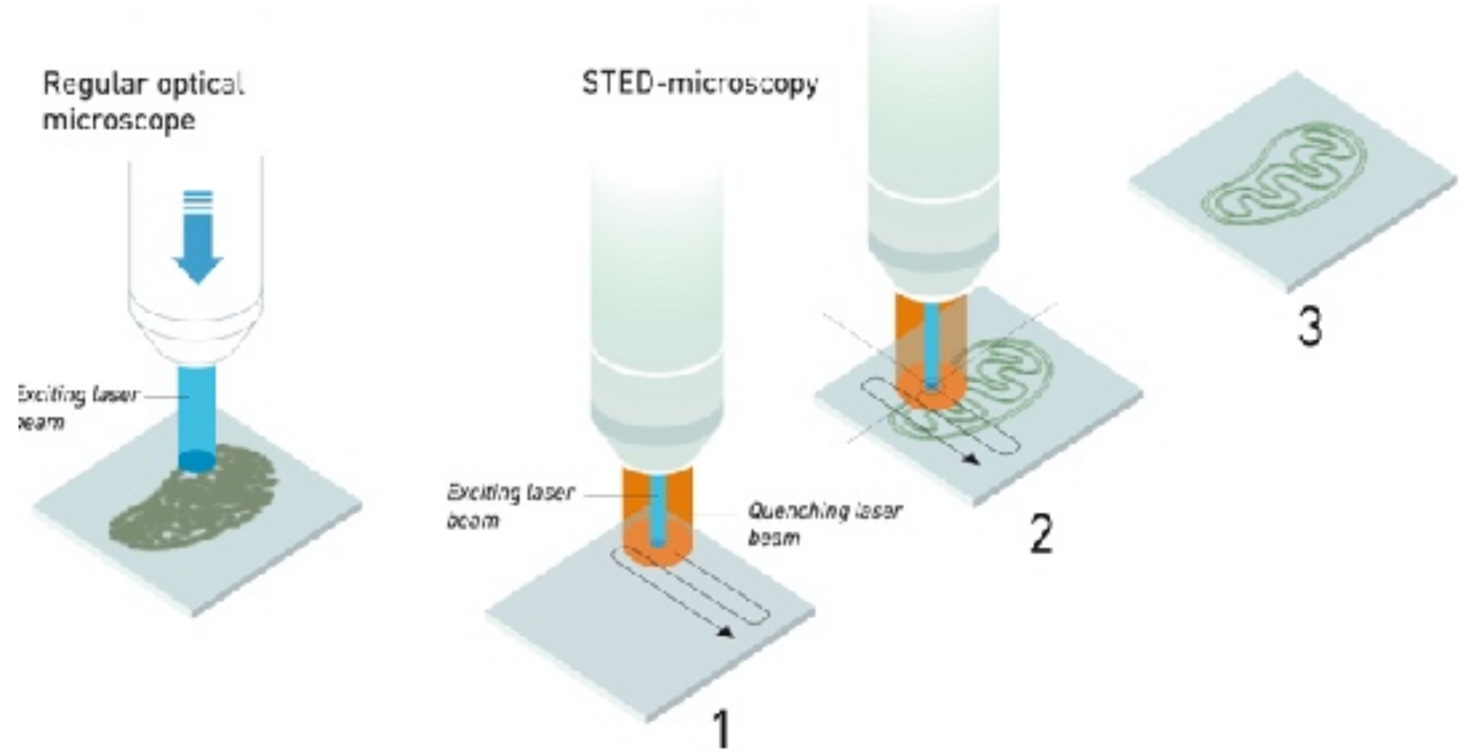


Microtubular system

STED microscopy
(STimulated Emission Depletion)

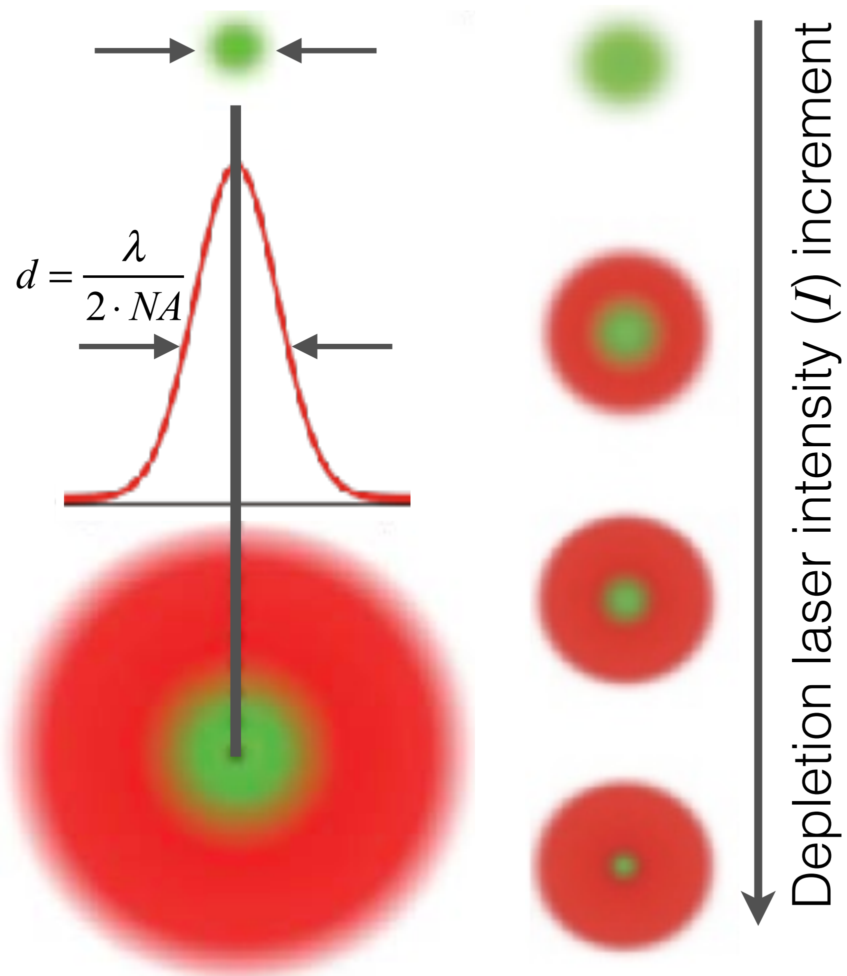
Stefan Hell (Nobel-prize 2014)

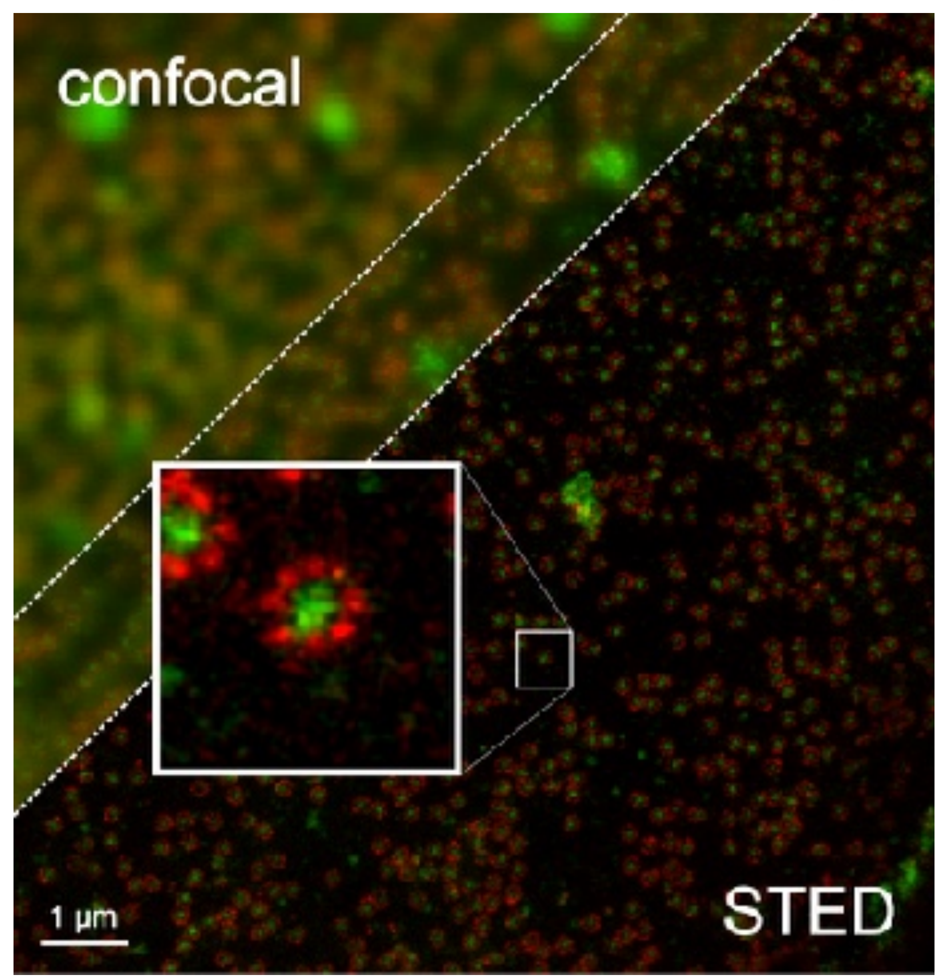


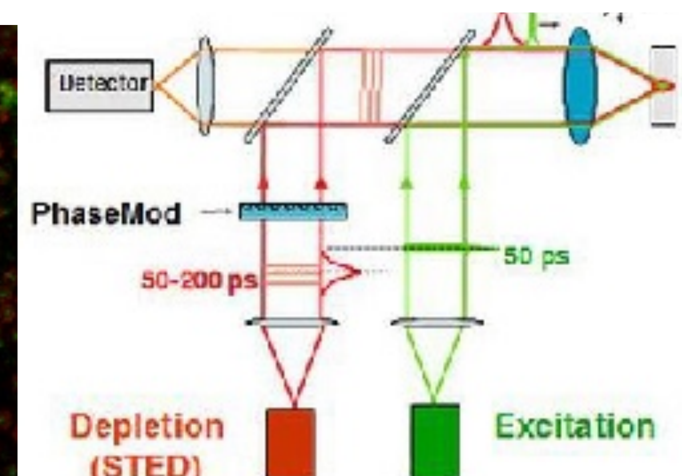


Hell: $d = \dfrac{\lambda}{2 \cdot NA\sqrt{1+I/I_S}}$

Abbé: $d = \dfrac{\lambda}{2 \cdot NA}$

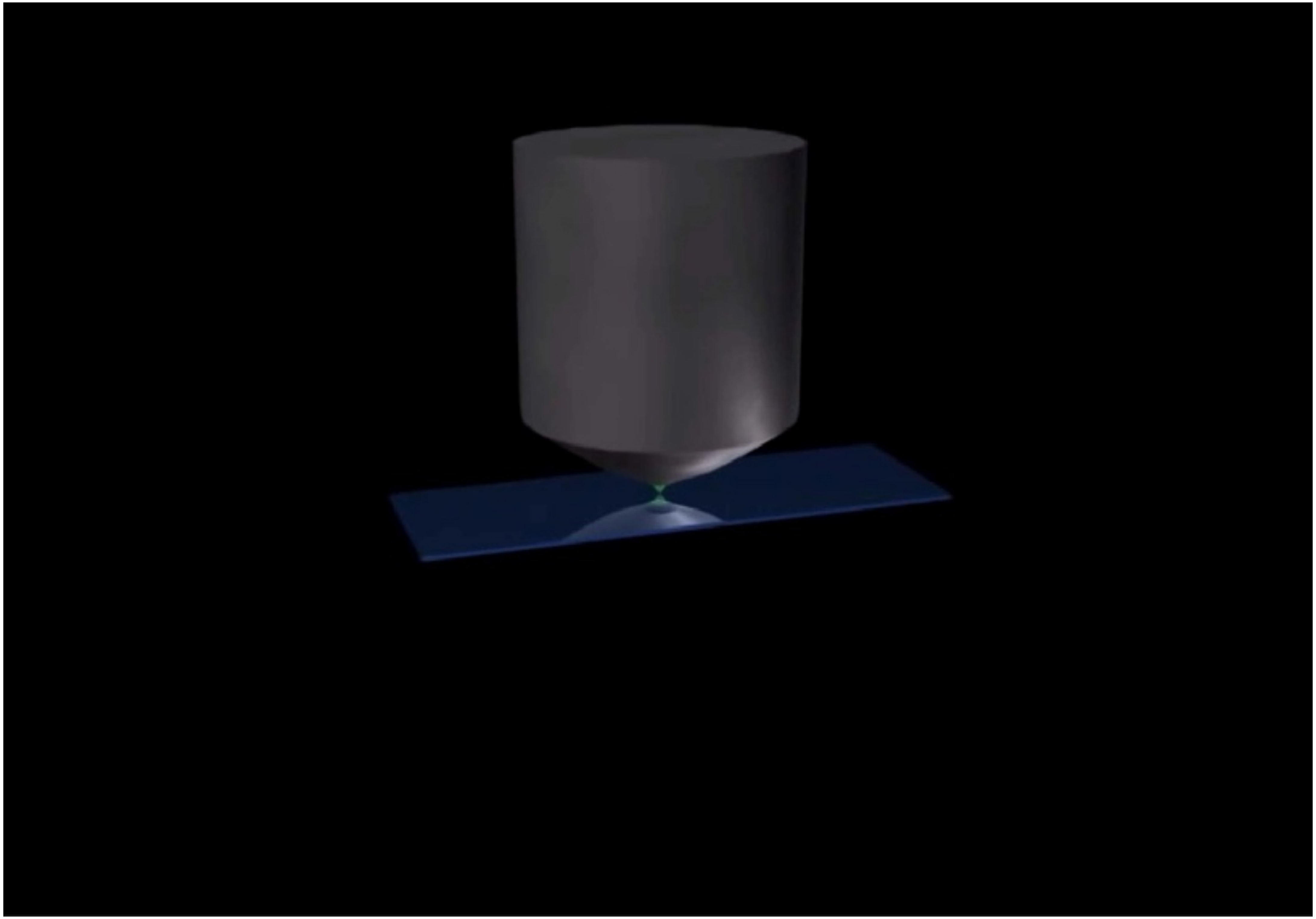






Nuclear pore complexes with STED microscopy

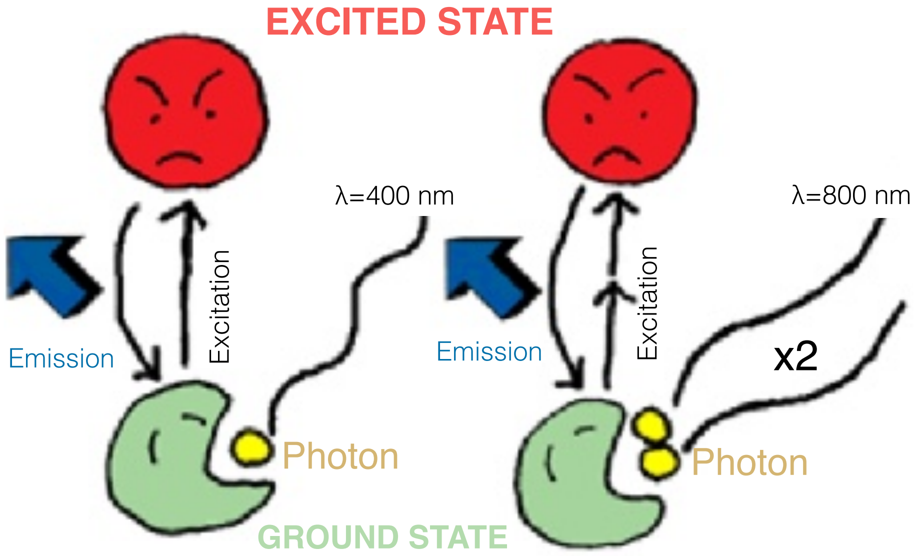
STED microscopy

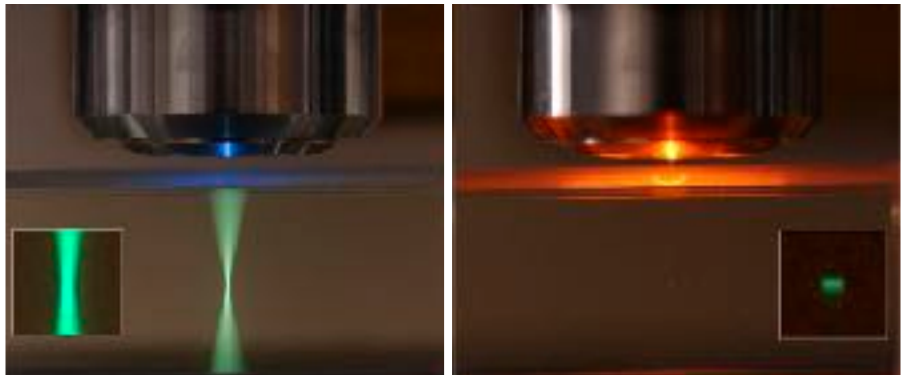


Jakub Chojnacki

Imaging and manipulating femtoliter volumes: Multiphoton microscopy

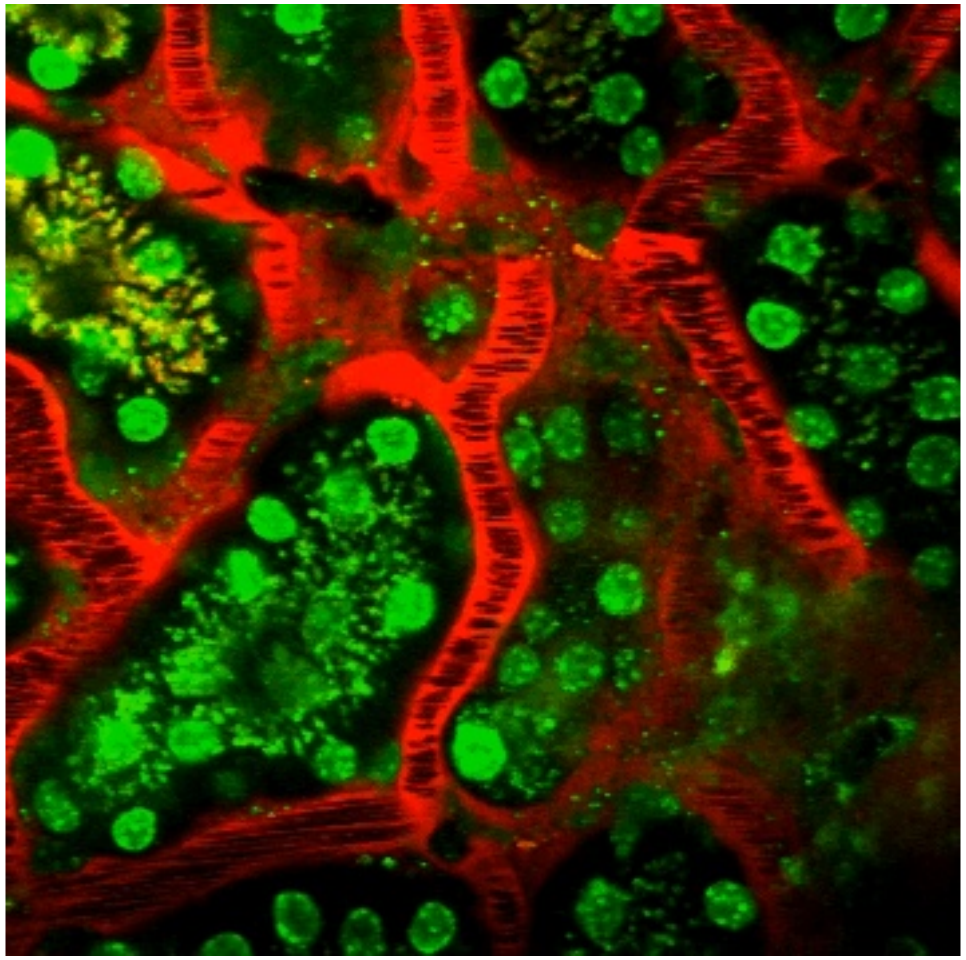
- Energy of two (or more) photons are added during excitation
- Excitation (hence emission) only in focal point (limited photodamage)
- Excitation with long wavelength (near-IR), short (fs) light pulses
- Large (up to 2 mm) penetration due to long wavelength
- Possibility of launching light-sensitive reactions



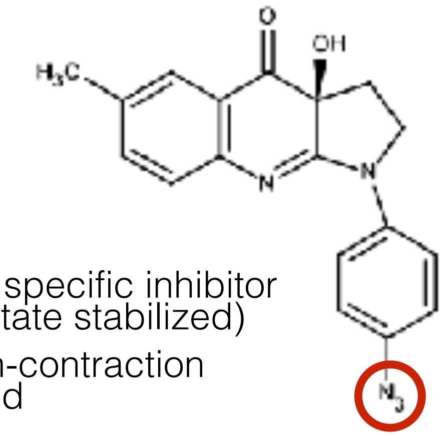


Single-photon fluorescence

Two-photon fluorescence

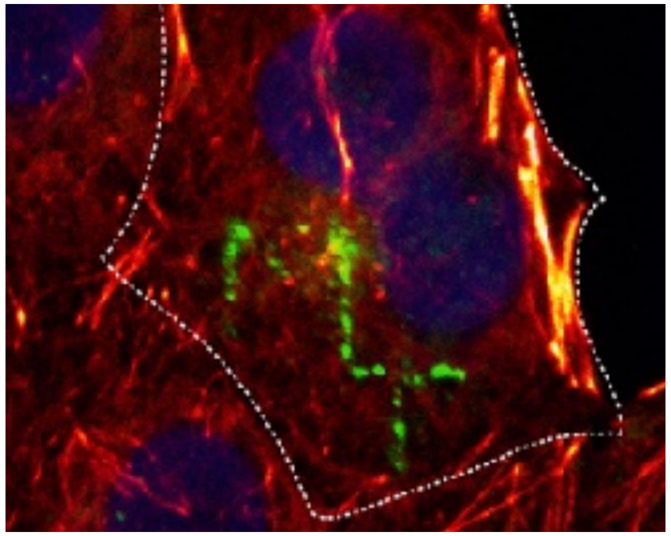


Green: proximal kidney tubules; Red: albumin (plasma)



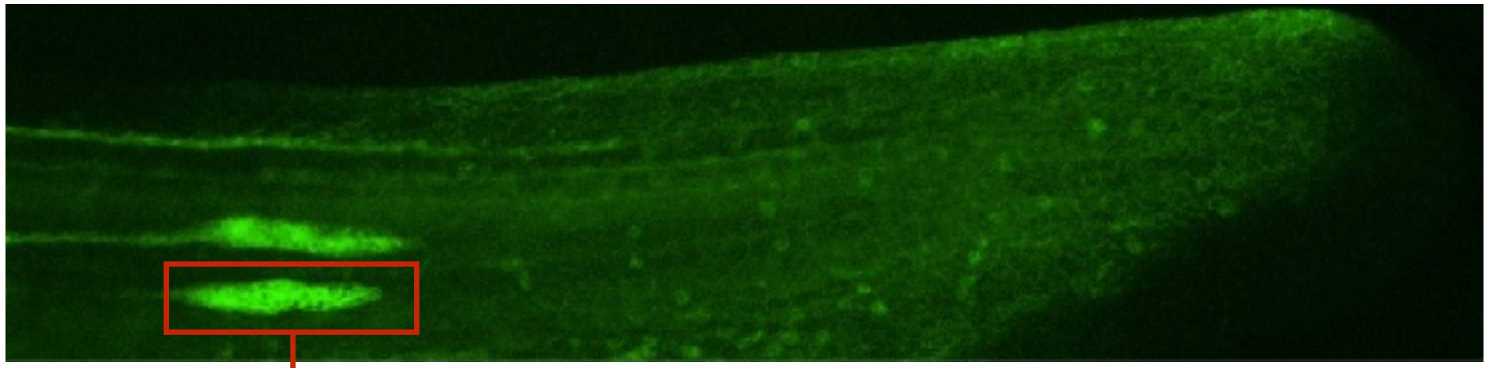
Blebbistatin:

- Myosin II specific inhibitor (ADP-P_i state stabilized)
- Excitation-contraction decoupled



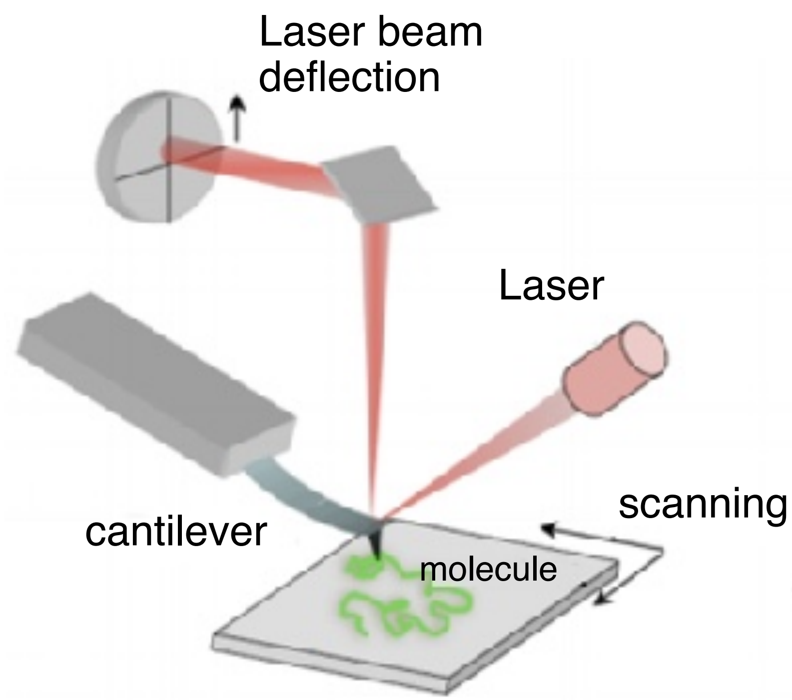
Molecular tattoo: azido-blebbistatin photoactivated with spatial localization (HeLa)

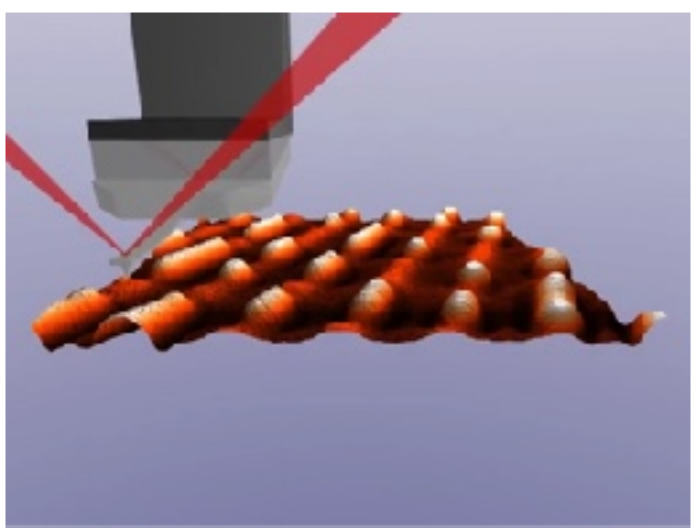
Zebrafish lateral line organ development stops upon 2P exposure



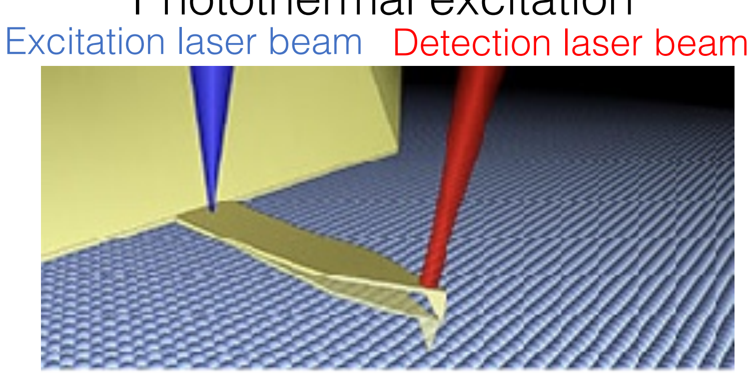
Képiró et al. Chem. Biol. 22, 548, 2015

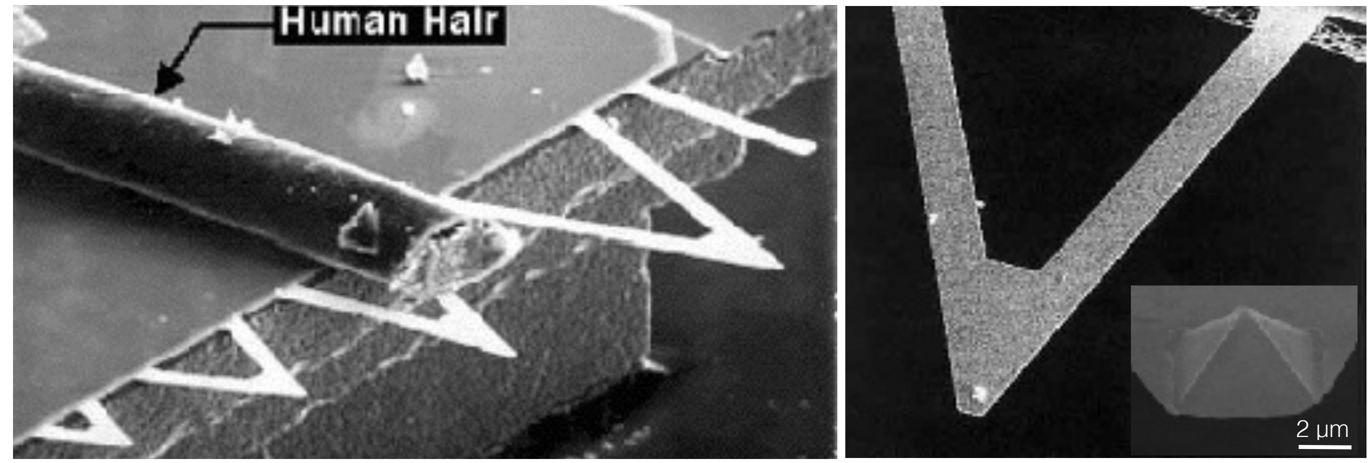
Atomic Force Microscopy (AFM)

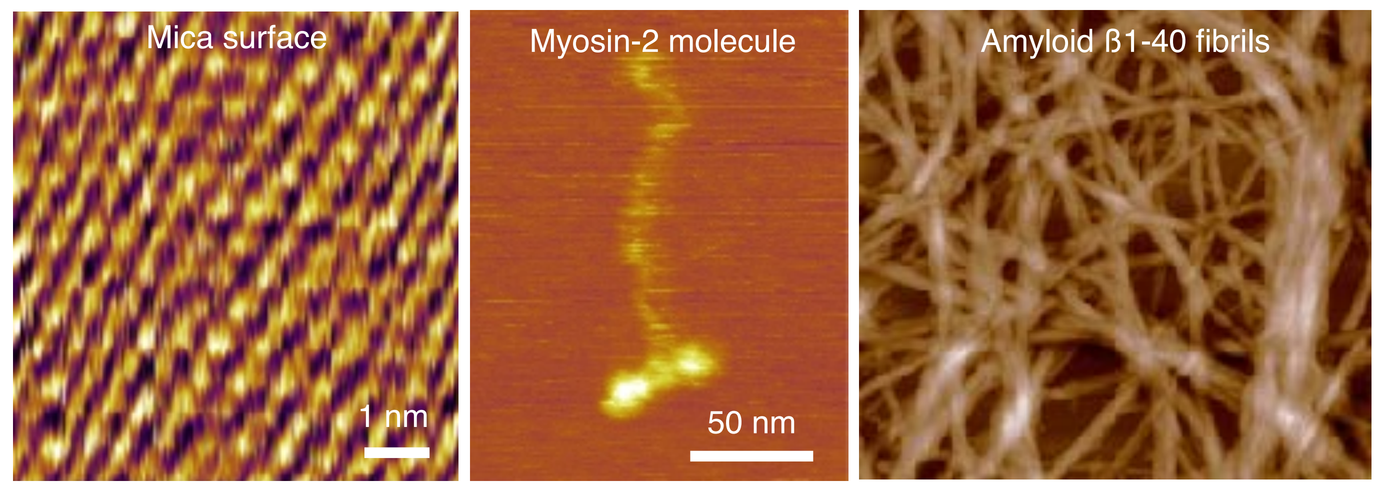


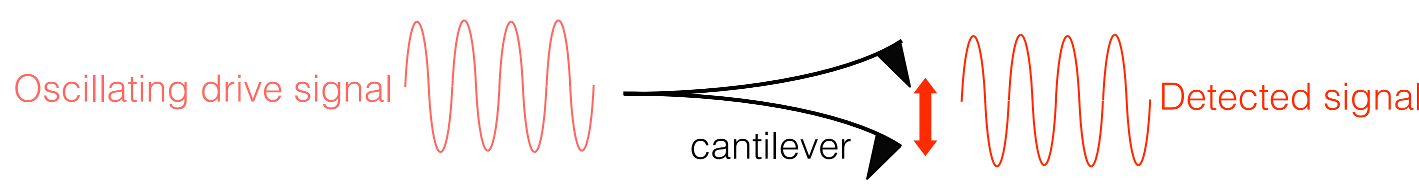


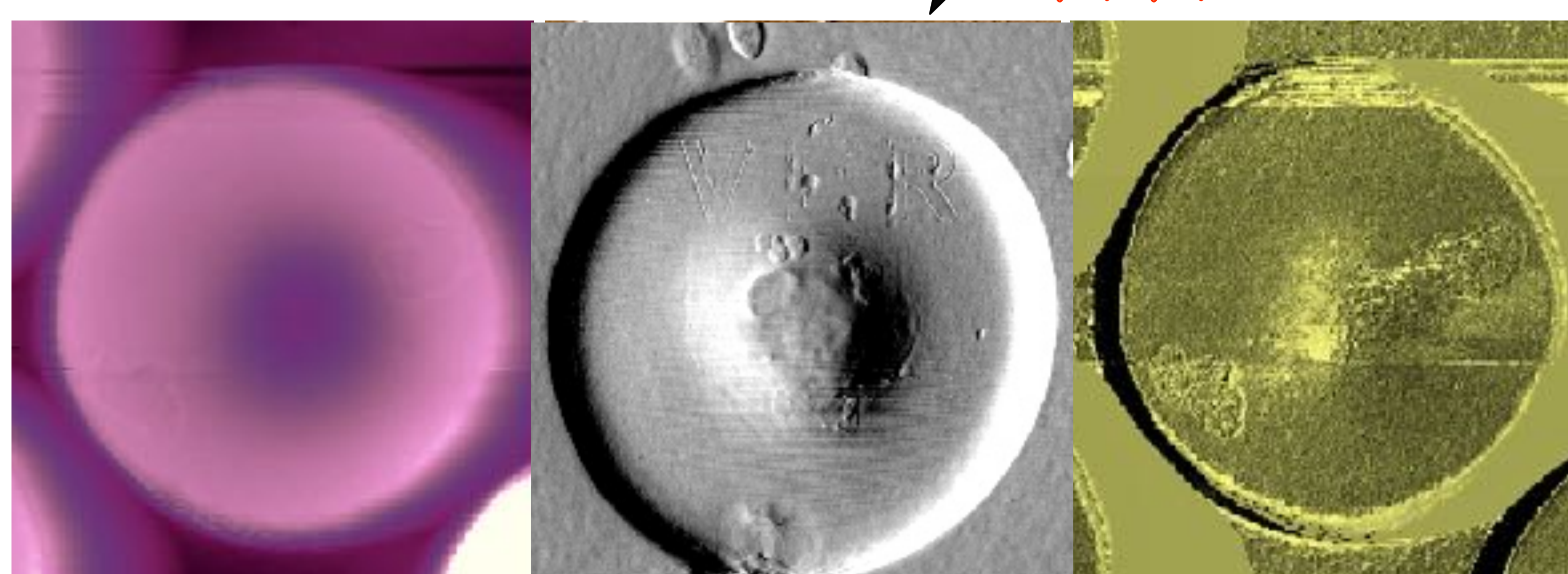
Photothermal excitation







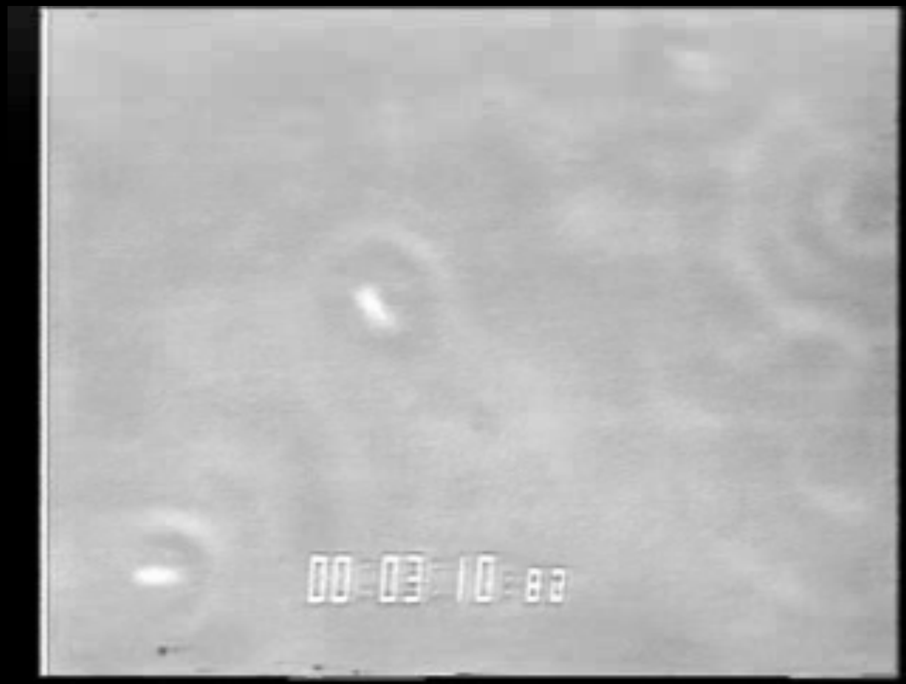




Height contrast Amplitude contrast Phase contrast

Nanoscale manipulation with light

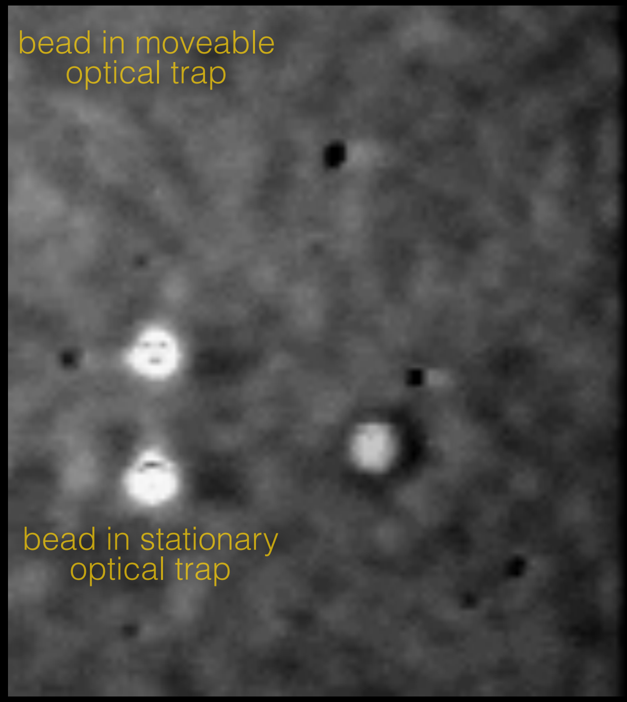
E. coli bacterium



Actin filament

DNA

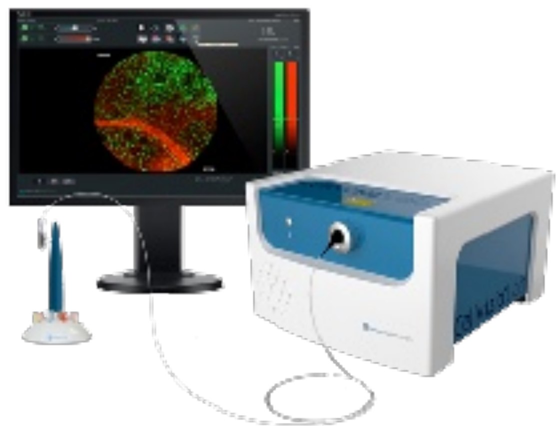
Phase contrast image

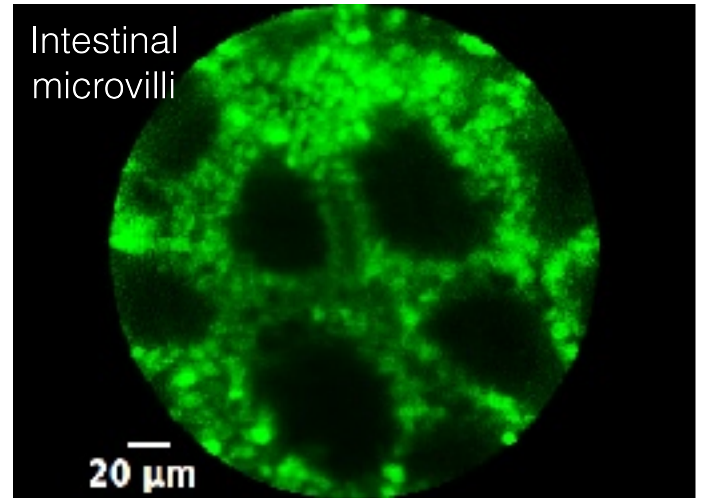


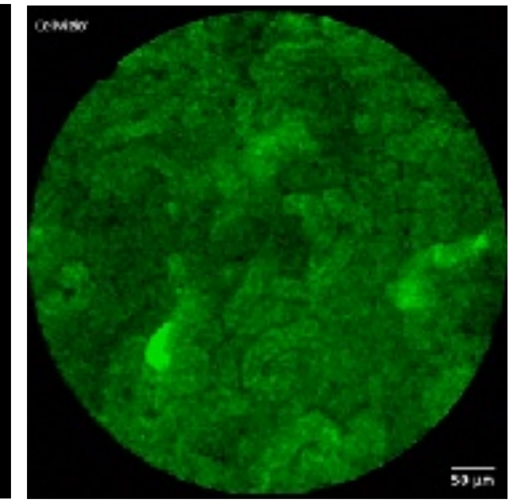
Fluorescence image

In vivo small-animal imaging

Fluorescence endoscopy

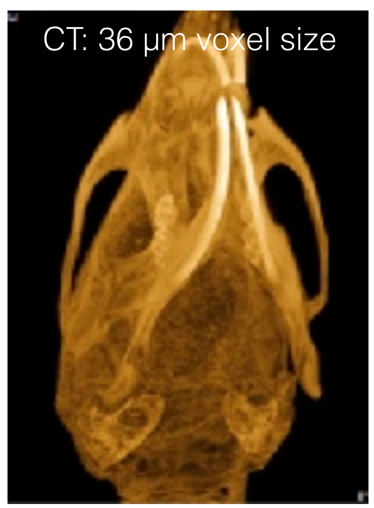


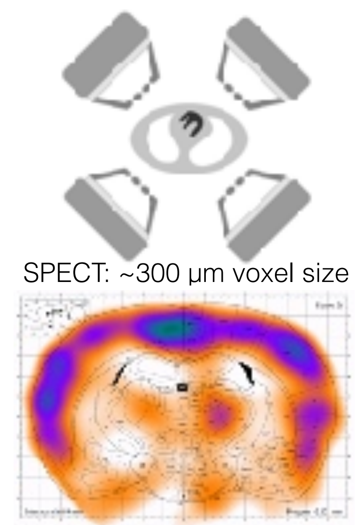


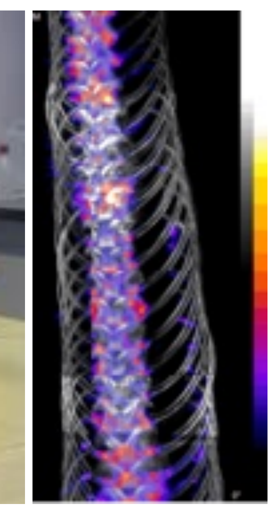


Kidney cortex transgenic mouse expressing calcium-binding fluorescent protein

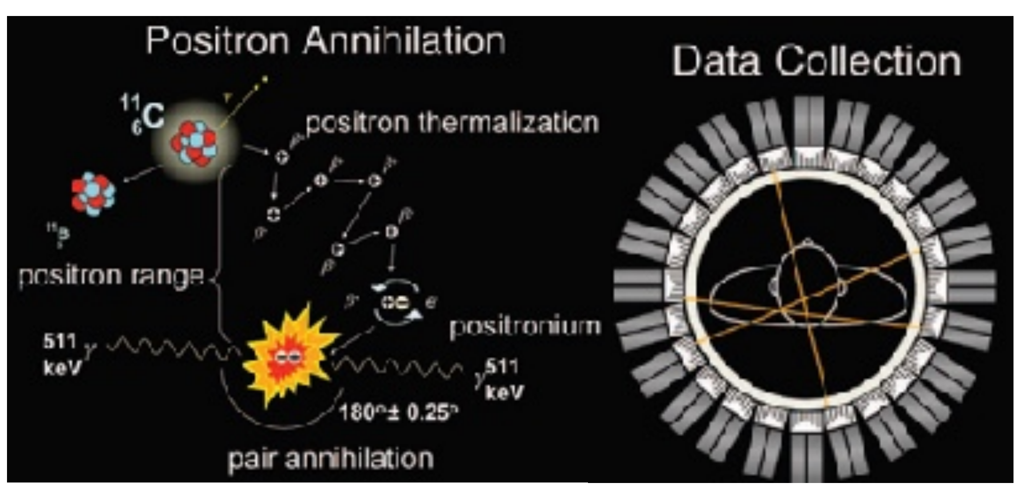
Small animal CT, SPECT, SPECT/CT

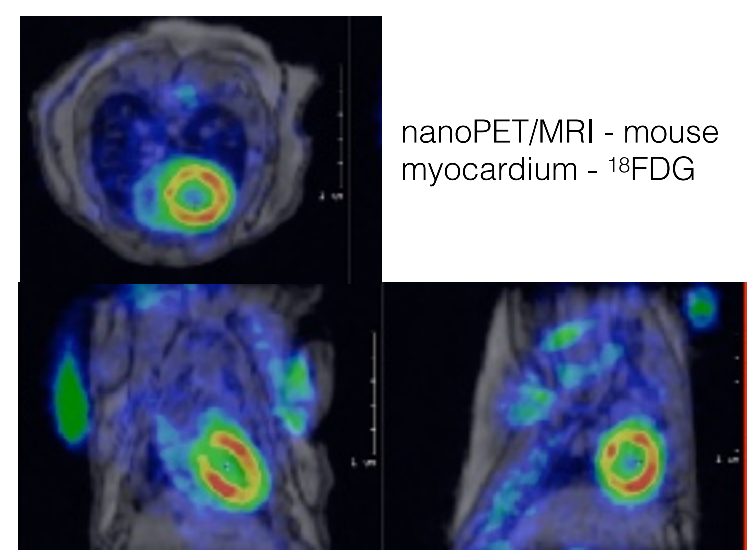




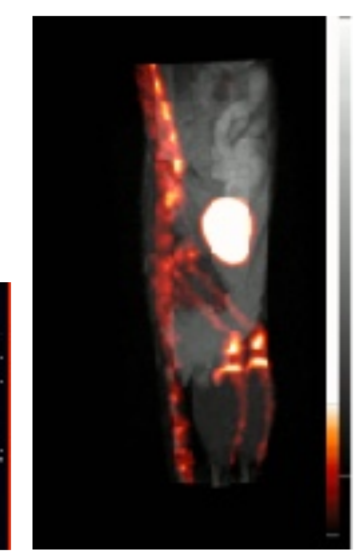


Small animal PET/MRI



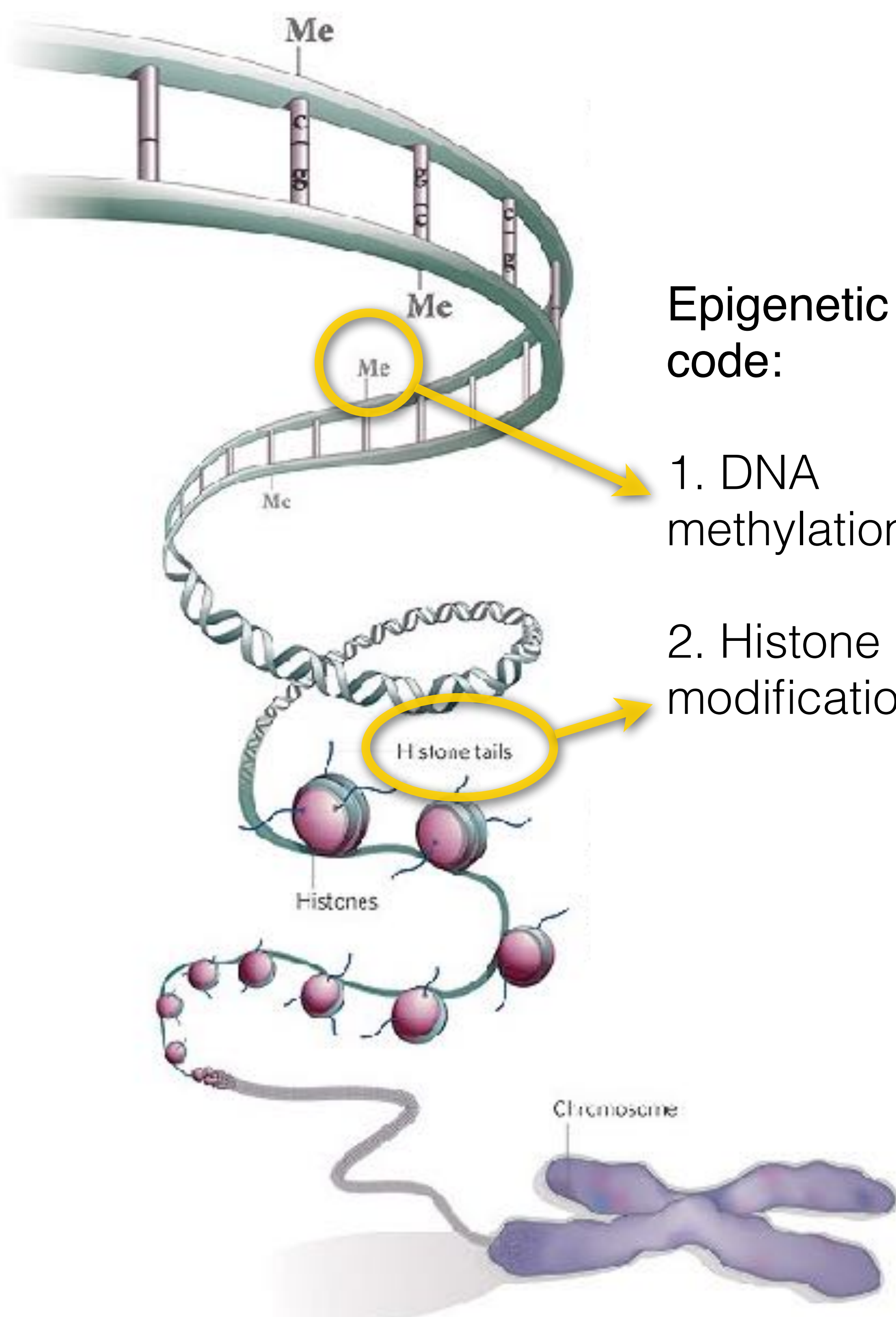


nanoPET/MRI - mouse myocardium - ^{18}FDG



5.8MBq Na^{18}F, mouse, 90 min, bone incorporation

1. From single DNA to epigenetics



Epigenetic code:

1. DNA methylation

2. Histone modification

Functions of DNA methylation:

1. Transcriptional gene silencing

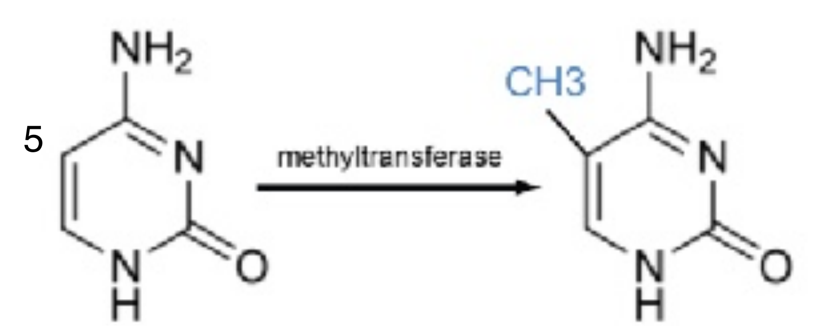
Prader-Willi syndrome

Juan Carreño de Miranda: The Nude Monster (1680)

Angelman syndrome

Giovanni Francesco Caroto: Boy with a Puppet (1555)

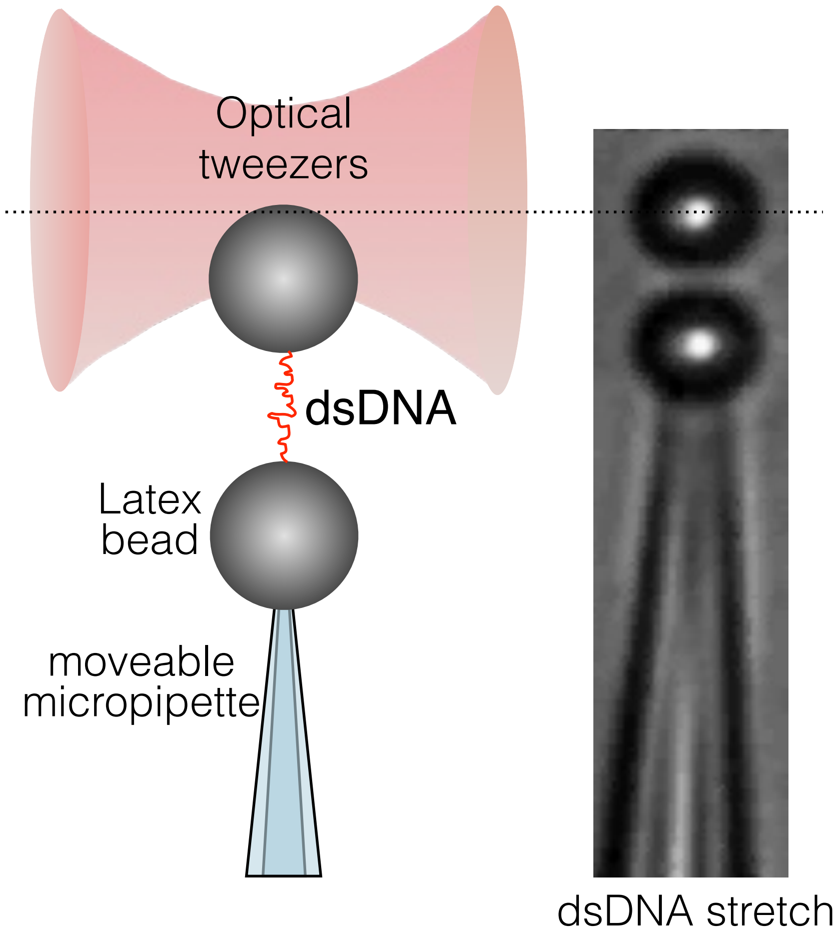
2. Genomic stability and protection
3. Chromatin compaction
4. Suppression of homologous recombination
5. X-chromosome inactivation (in women)

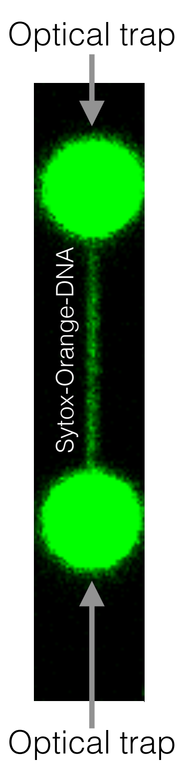


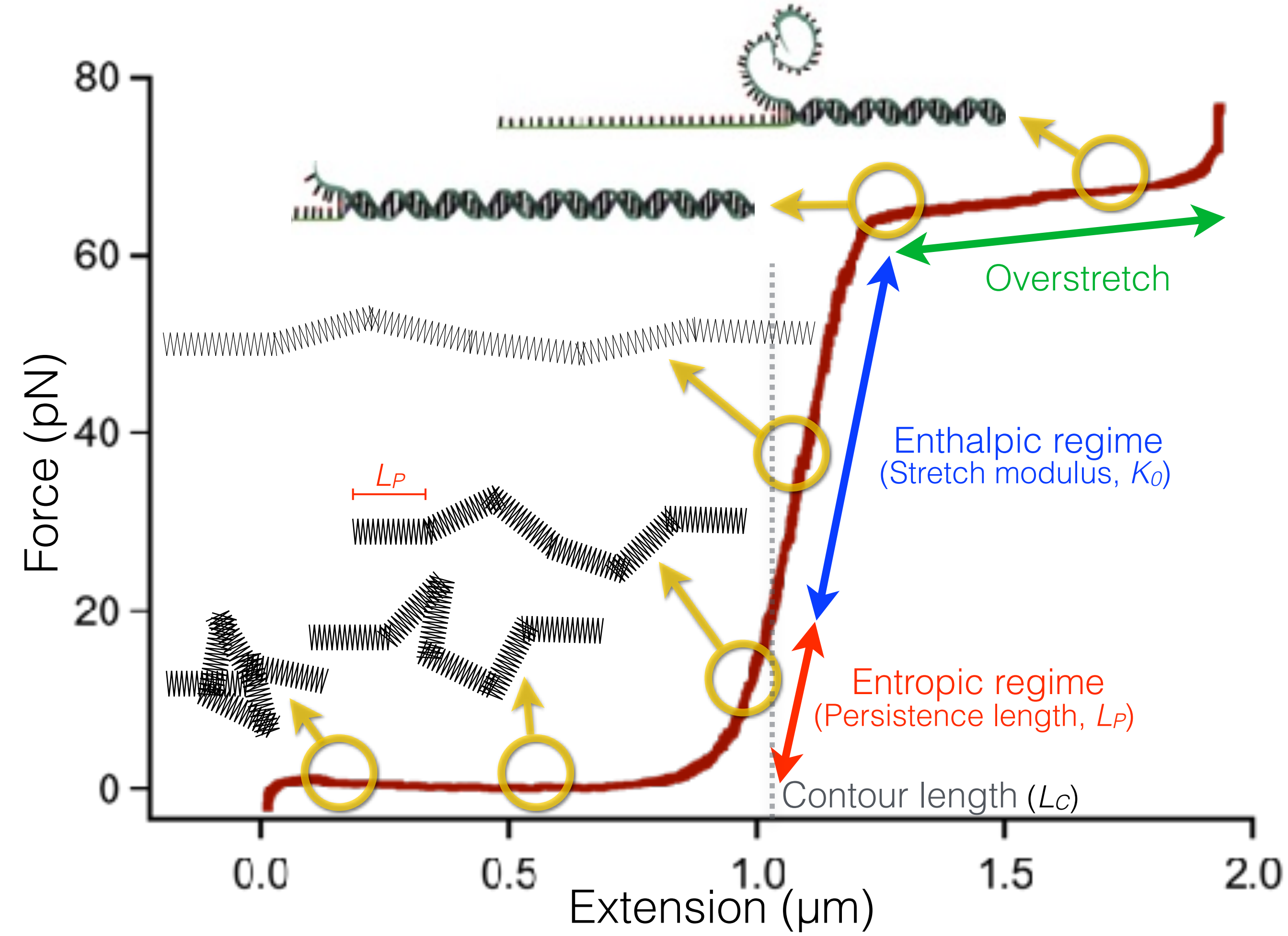
Cytosine in CpG islands

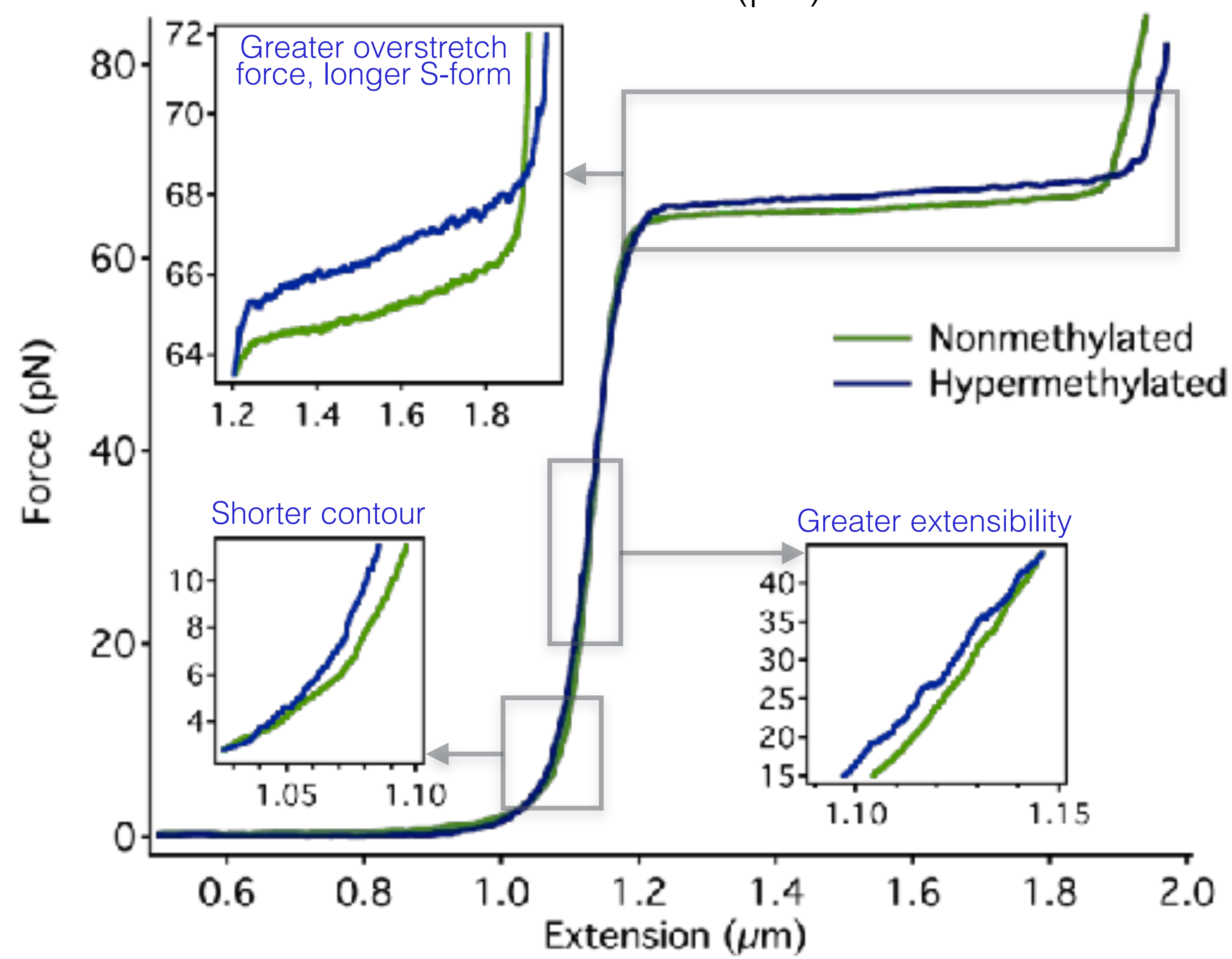
Methyl groups in the large groove of DNA

Nanomechanics of methylated DNA



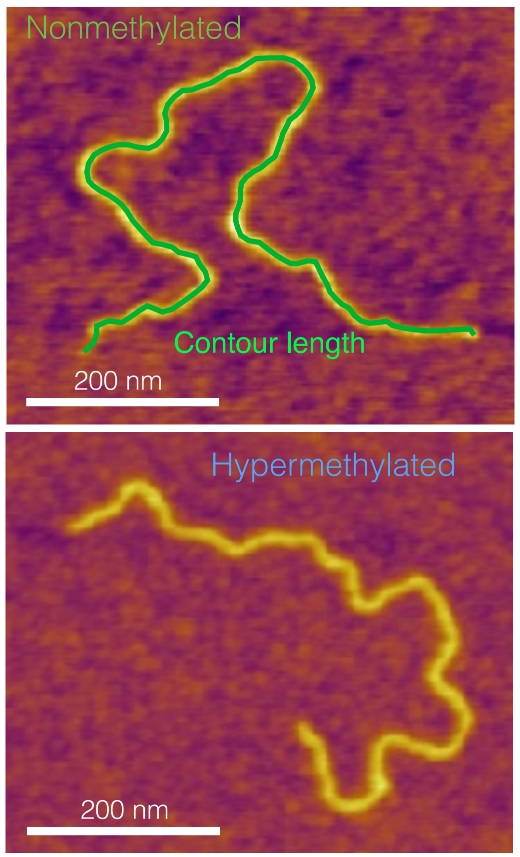


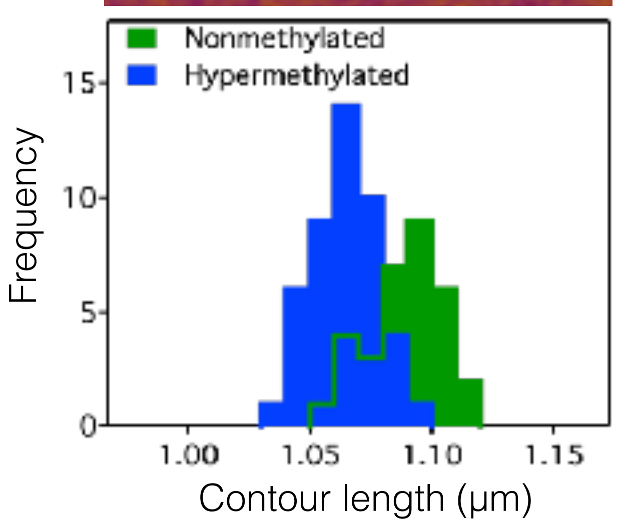




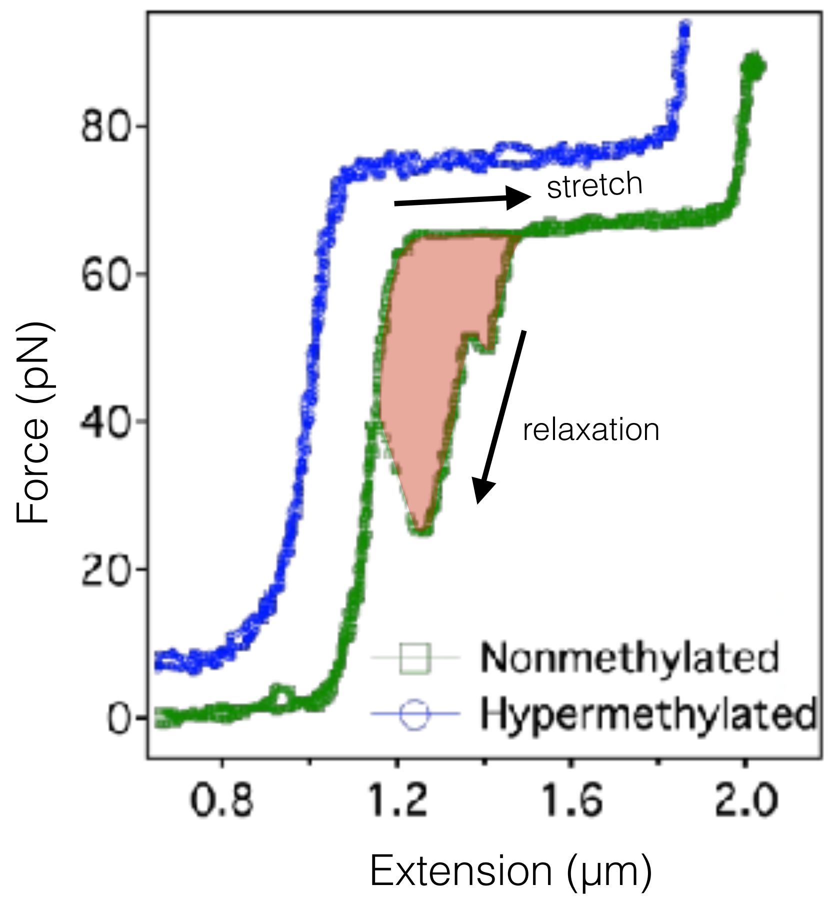
Hypermethylated dsDNA is more compact, and structurally and mechanically more stable

Structural contour length of hypermethylated dsDNA is shorter



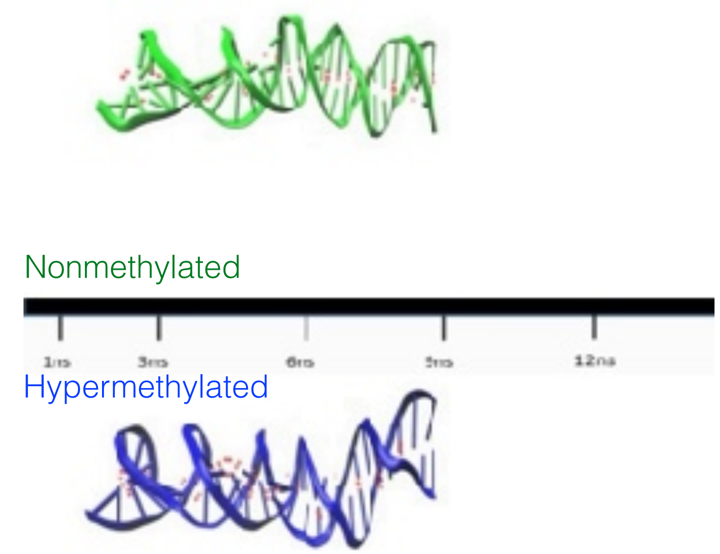


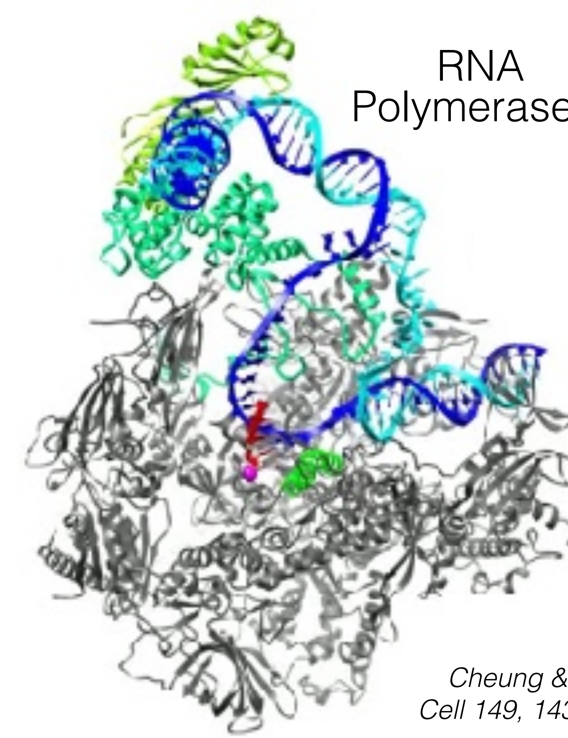
Hypermethylated dsDNS recovers faster from overstretch



Force hysteresis, present in nonmethylated dsDNA, disappears in the hypermethylated form

Helicity is retained in hypermethylated dsDNA



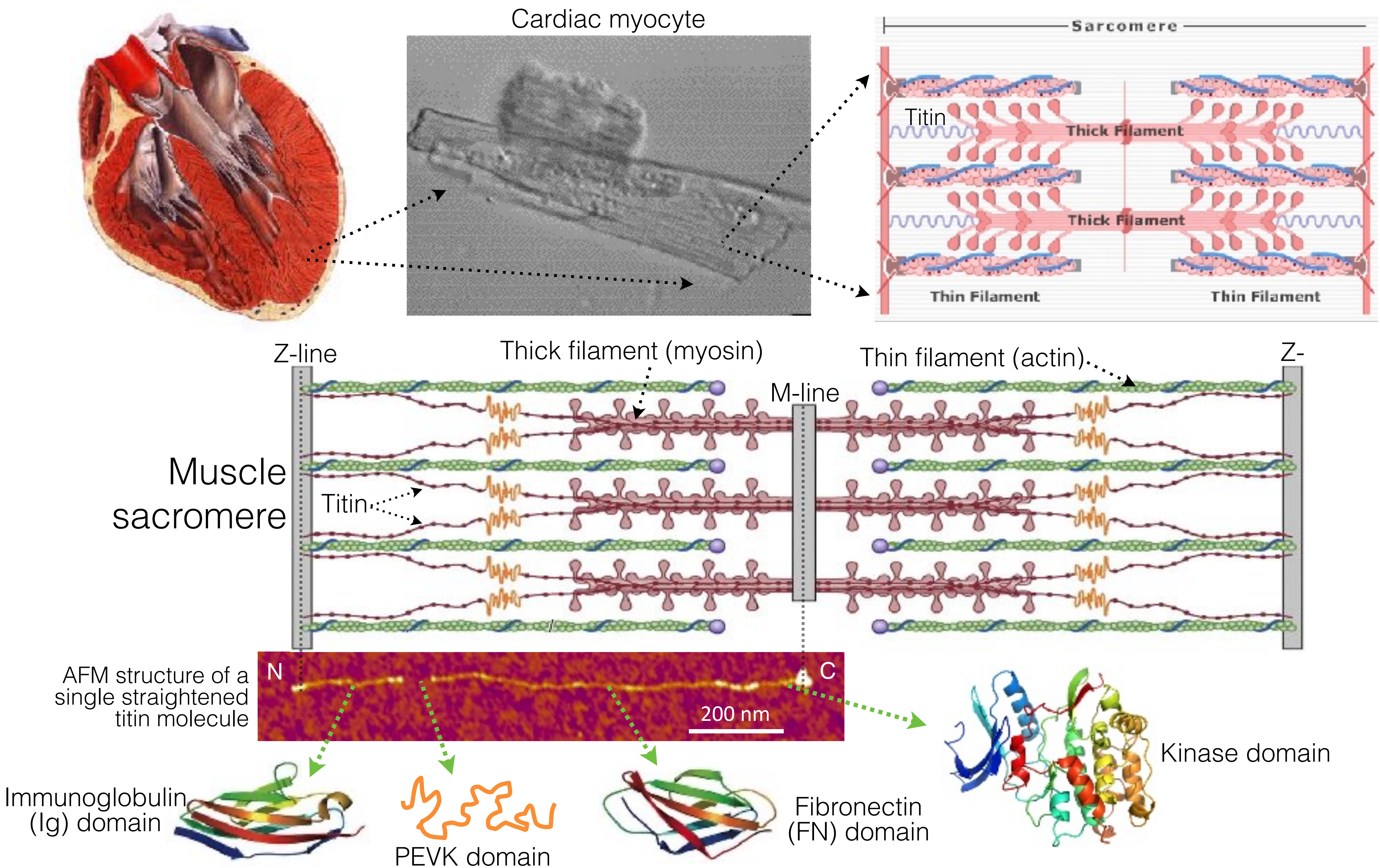


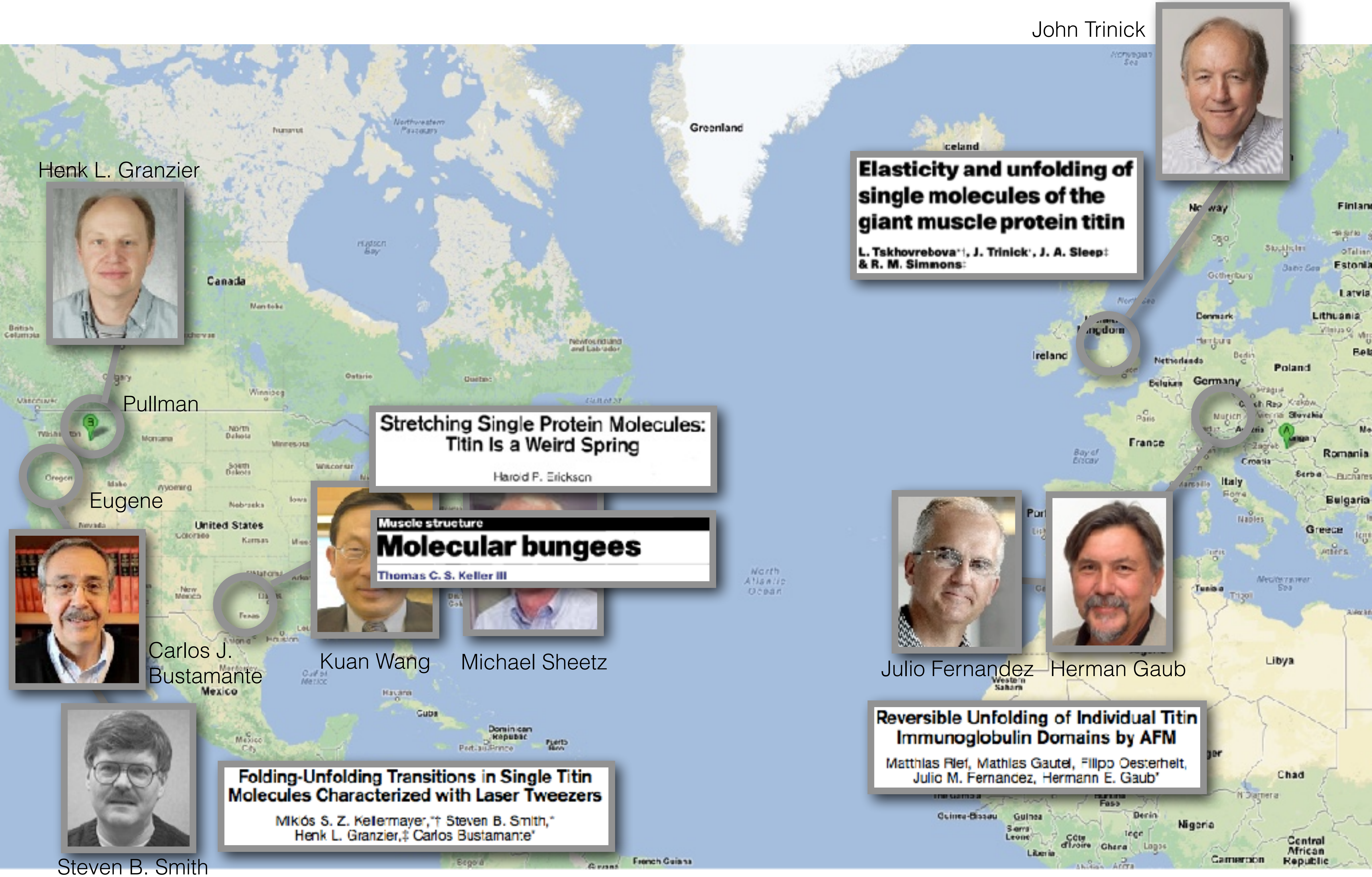
RNA Polymerase II

Cheung & Cramer Cell 149, 1431, 2012

Pongor et al. Biophys.J. 112, 512, 2017.

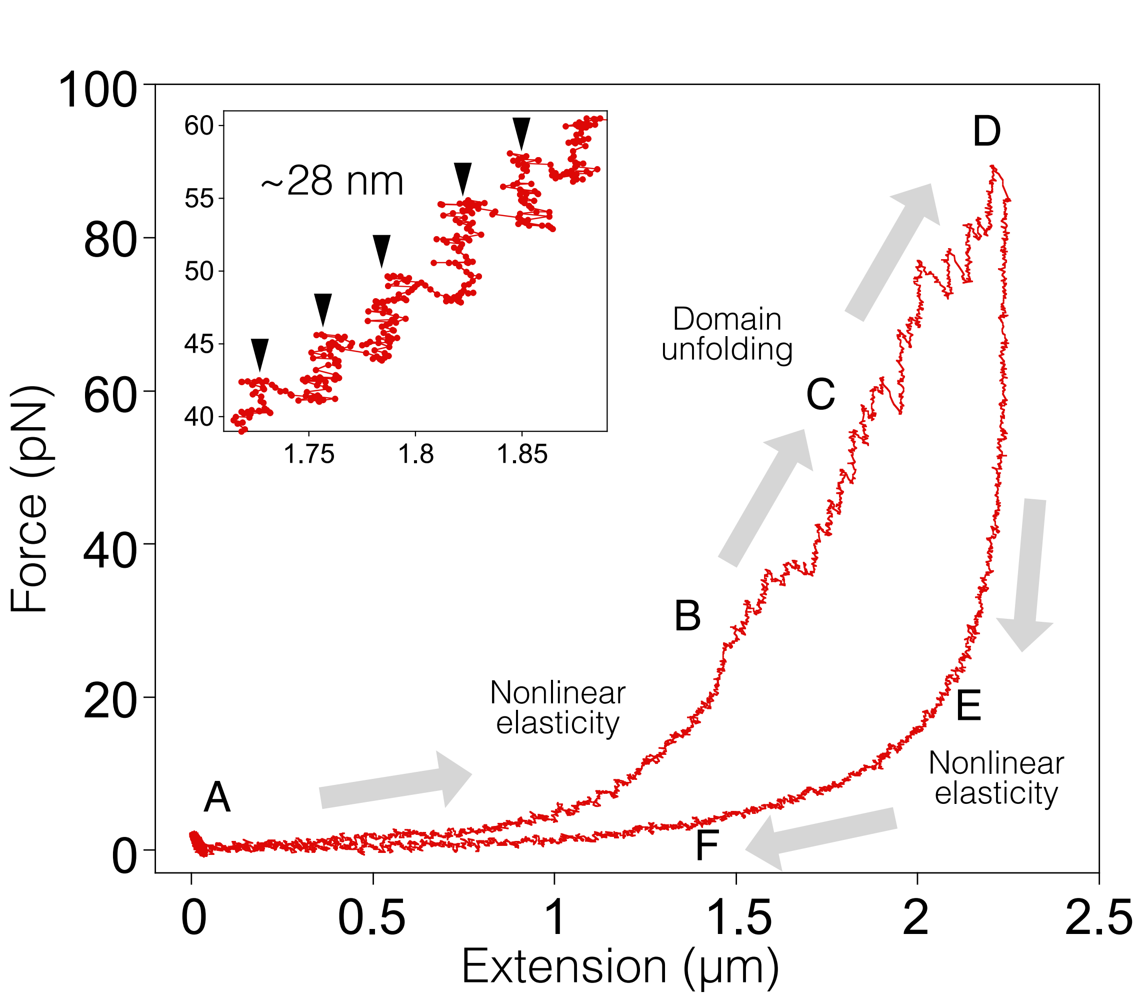
2. From single titin to DCM
Giant modular protein traversing the sarcomere

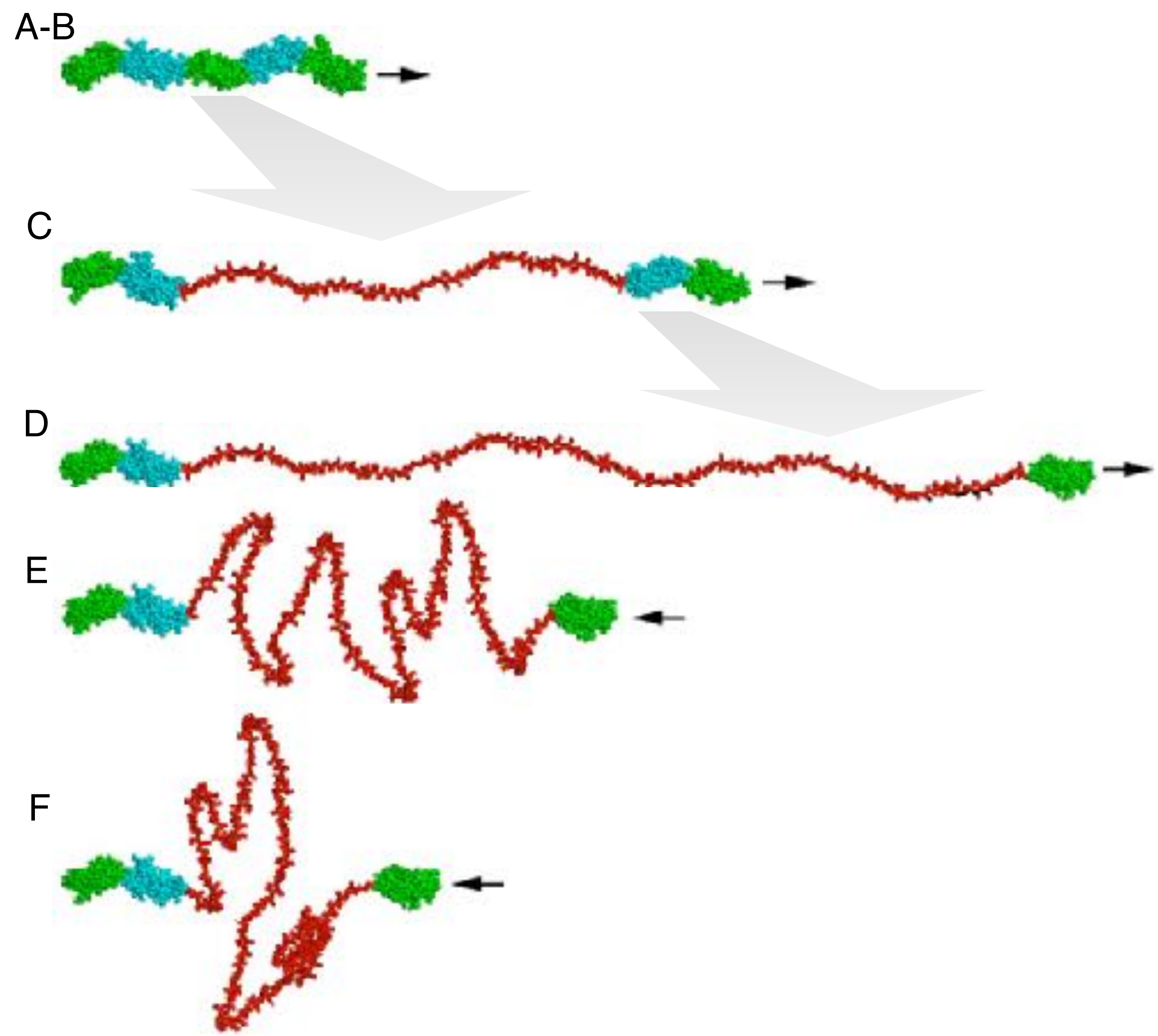


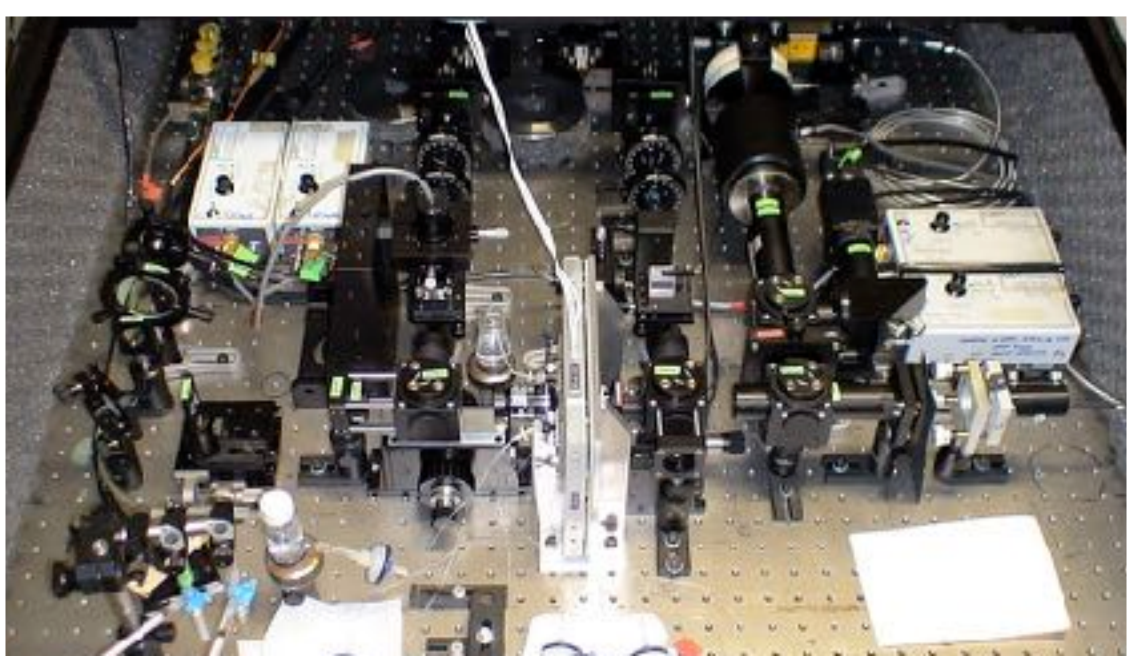


It is good to be first, but it is better to be right!

Stretching a single titin molecule





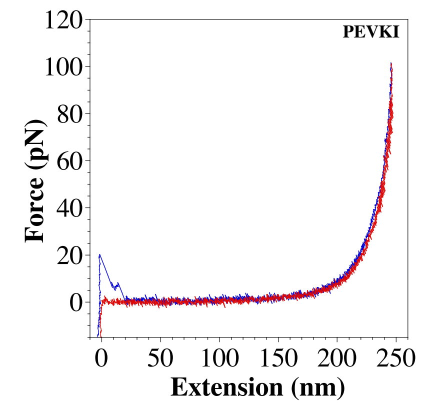


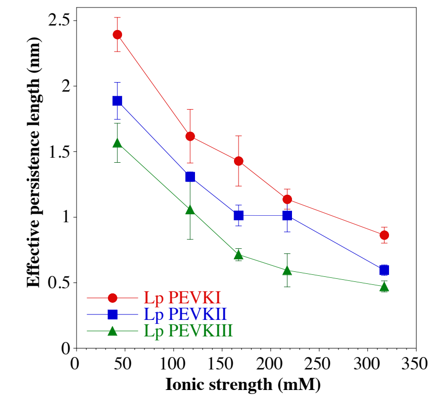
Sherwin S. Lehrer
Boston Biomedical Research Institute

"…aren't you Hungarian…?

Titin is a multi-functional molecular hub

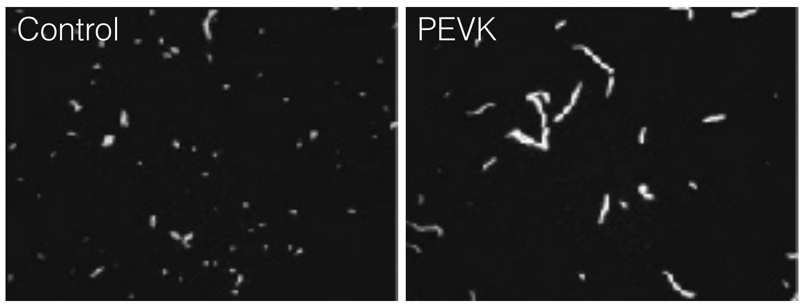
- Provides tunable non-linear elasticity





Nagy et al. Biophys. J., 2005.

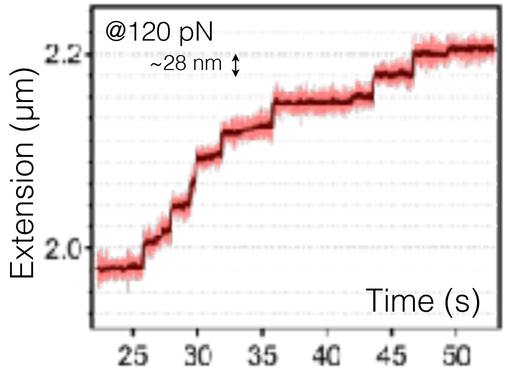
- Interacts with actin to serve as a viscoelastic shock-absorber



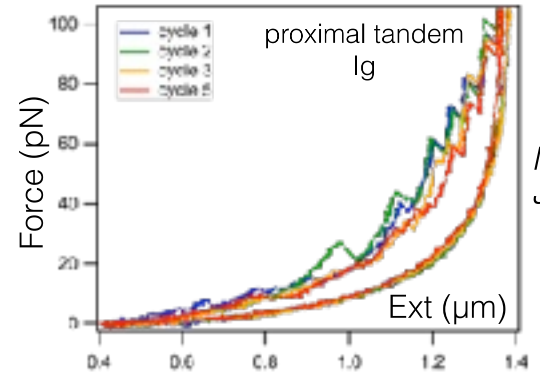
Nagy et al. J. Cell. Sci., 2004

Bianco et al. Biophys. J., 2007.

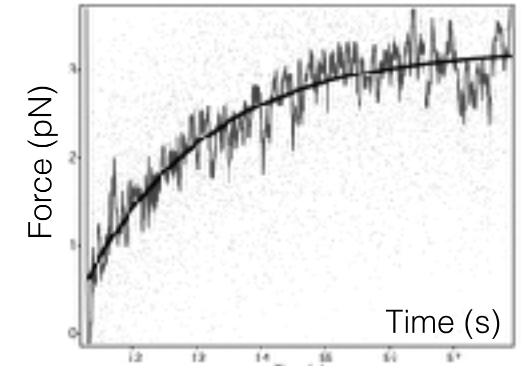
- Lends extensibility via domain unfolding



Bianco et al. Biophys. J., 2015



Mártonfalvi et al. J. Cell Sci., 2014



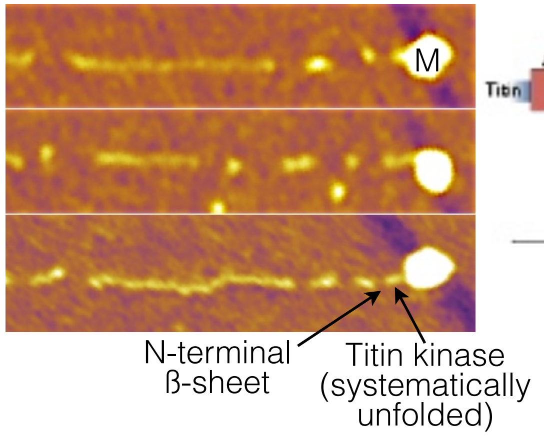
Mártonfalvi et al. Prot. Sci., 2017

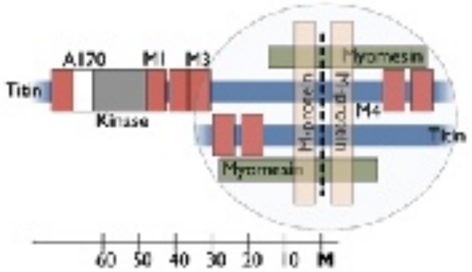
1000 nm

Mártonfalvi and Kellermayer PLoS ONE, 2014

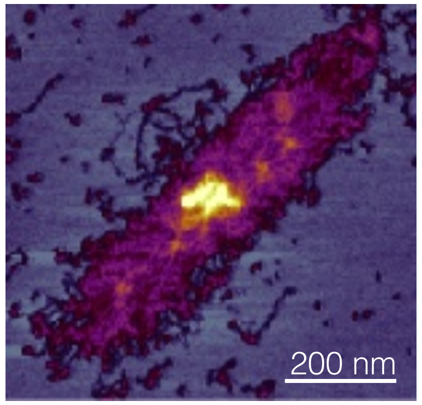
- Mechanosensor *via* the kinase domain

- Binds along the thick filament (regulatory blueprint rather than stiff template)





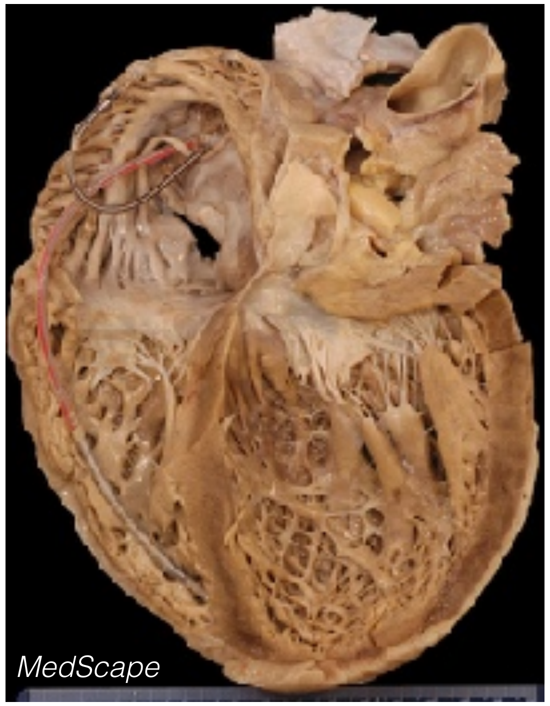
Mártonfalvi and Kellermayer PLoS ONE, 2014



Kellermayer et al. J. Struct. Biol., 2018

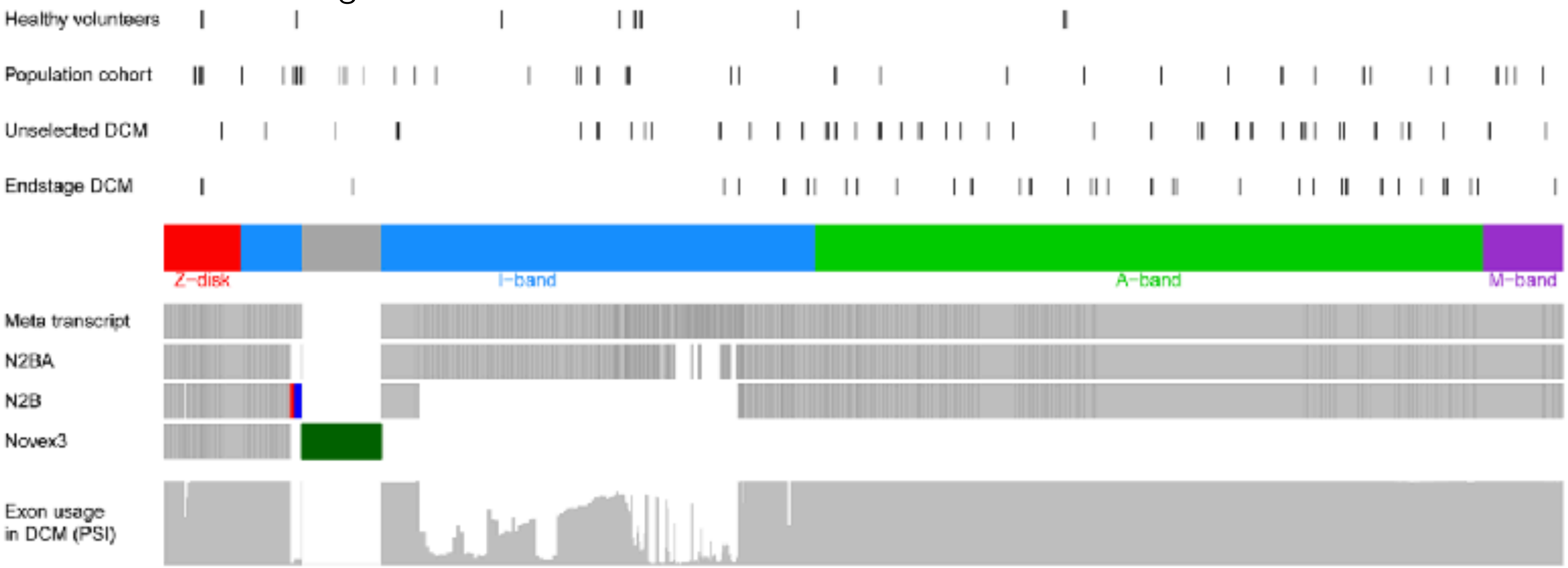
Titin is a major disease protein: **neuromuscular** and **cardiac** pathologies

Dilated cardiomyopathy (DCM)



- Left ventricular dilatation and wall thinning
- Systolic dysfunction
- ~20% of DCM caused by mutations in *TTN*
- Splicing, copy number, nonsense and frameshift mutations cause transcript **truncation** (TTNtv+)

- Herman *et al. NEJM*, 2012: TTNtv is common cause of DCM. Pathogenic TTNtv's overrepresented in A-band.
- Roberts *et al. Science Trans Med*, 2015: possible dominant negative mechanism.



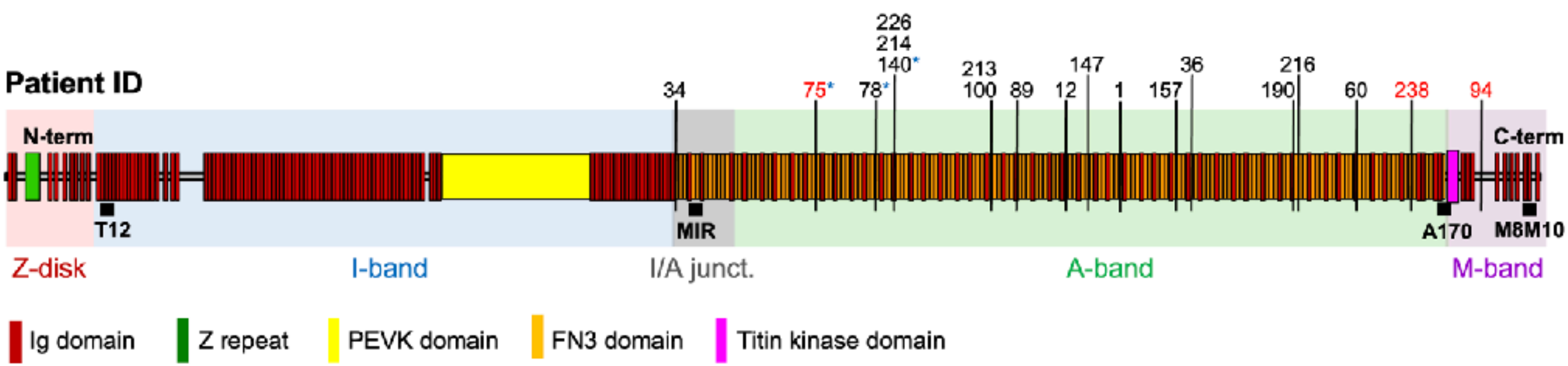
Roberts et al, 2015

- Fomin *et al. Science Trans Med*, 2021: Haploinsufficiency, truncated proteinin aggregates, deregulated protein quality control.
- McAfee *et al. Science Trans Med*, 2021: truncated protein present in the heart, combined dominant-negative/haploinsufficiency.

Whether/how truncated titin is structurally incorporated into the sarcomere is unknown.

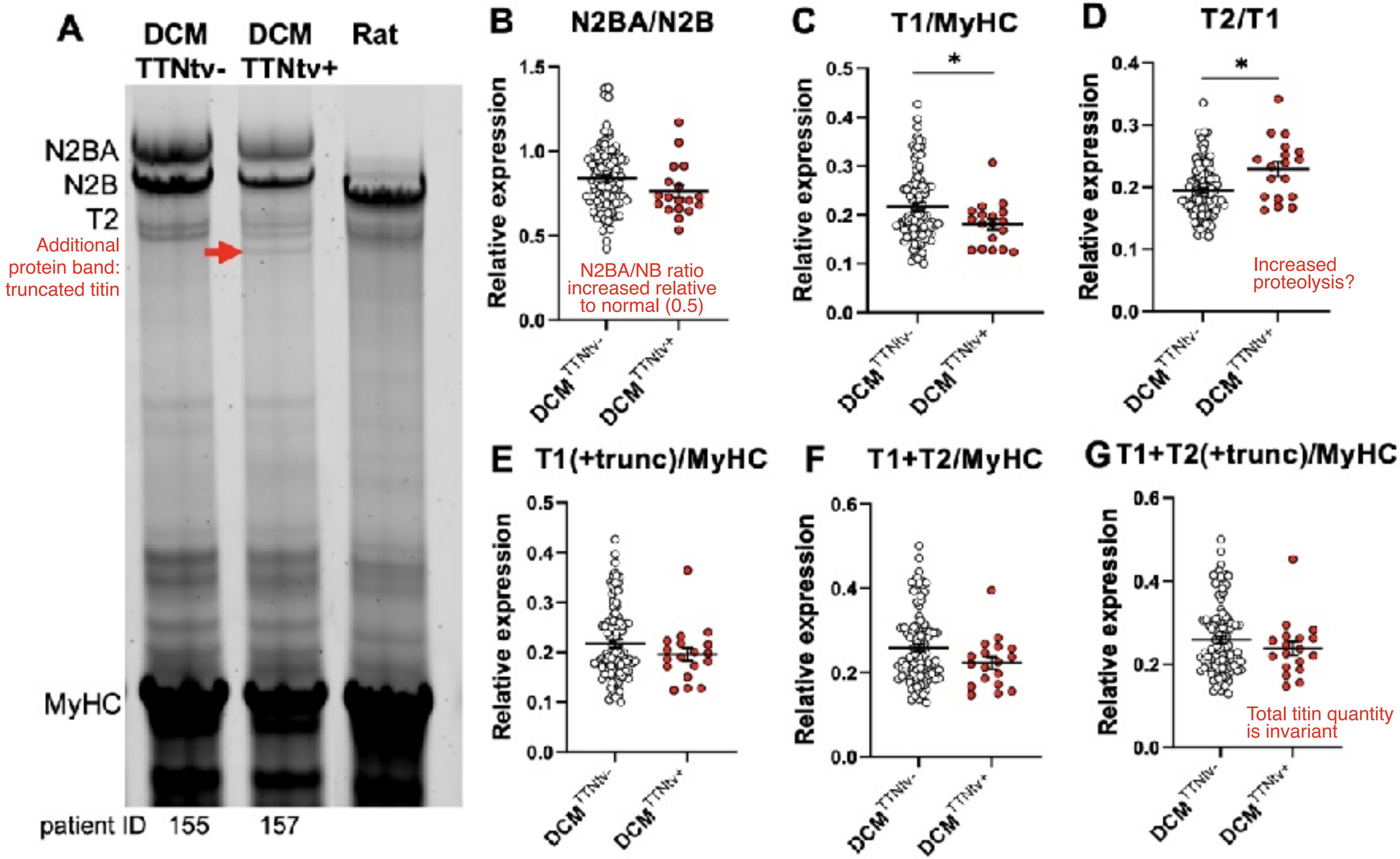
The patient cohort

- 127 clinically identified end-stage DCM patients.
- Orthotopic heart transplantation.
- 35 samples (27.5%): mutations in DCM genes.
- 19 TTN truncation variants (15%) identified with NGS (8 frameshift, 11 nonsense mutations); 4 LMNA, 4 DSP, 2 BAG3, 1 FKTN, LAMA2, MYBPC3, MYH6, MYH7, PLN, RBM20 and TNNI3.
- ~95% of TTNtv+ were male.
- No differences in functional parameters between TTNtv+ *versus* TTNtv-.
- Truncations in I/A junction, A-band, M-band.



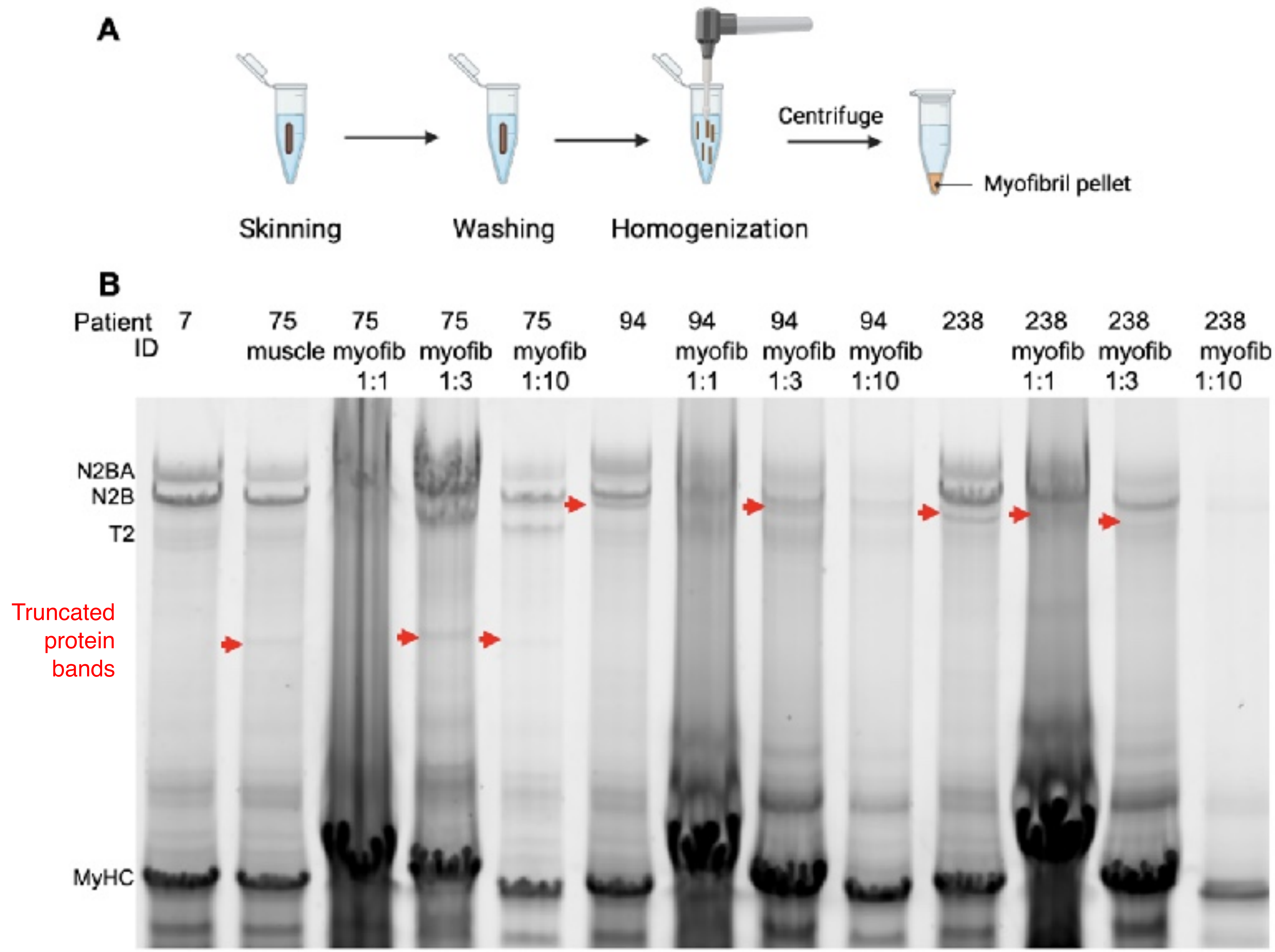
Kellermayer D et al. J. Clin. Invest., 2024

Truncated proteins are detectable: total titin similar in TTNtv+/-



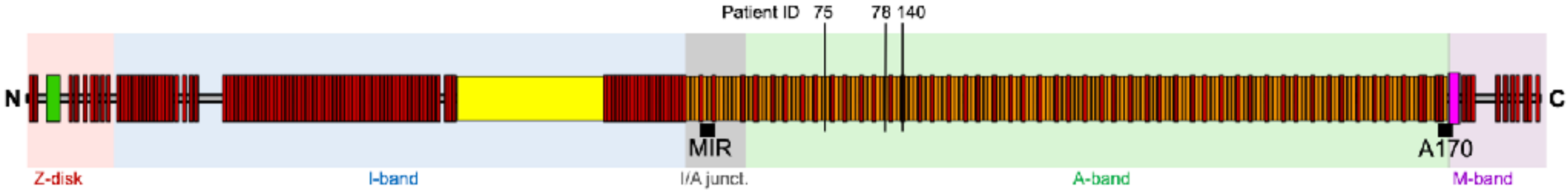
Kellermayer D et al. J. Clin. Invest., 2024

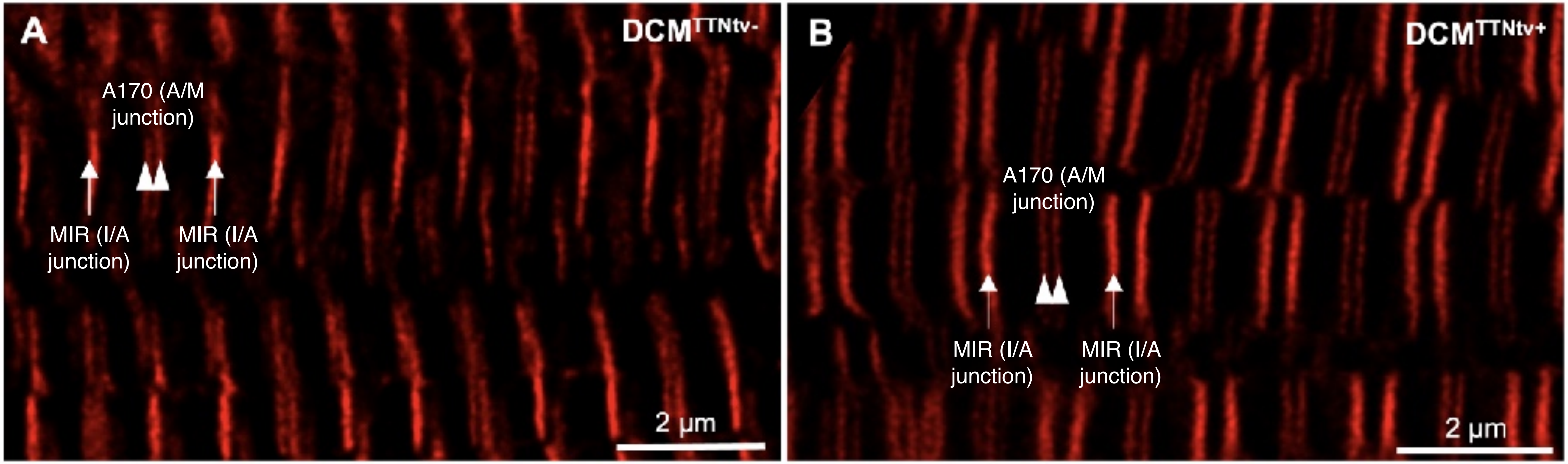
Truncated titin is present in the myofibril fraction…



Kellermayer D et al. J. Clin. Invest., 2024

TTNtv+ sarcomeres retain structural integrity

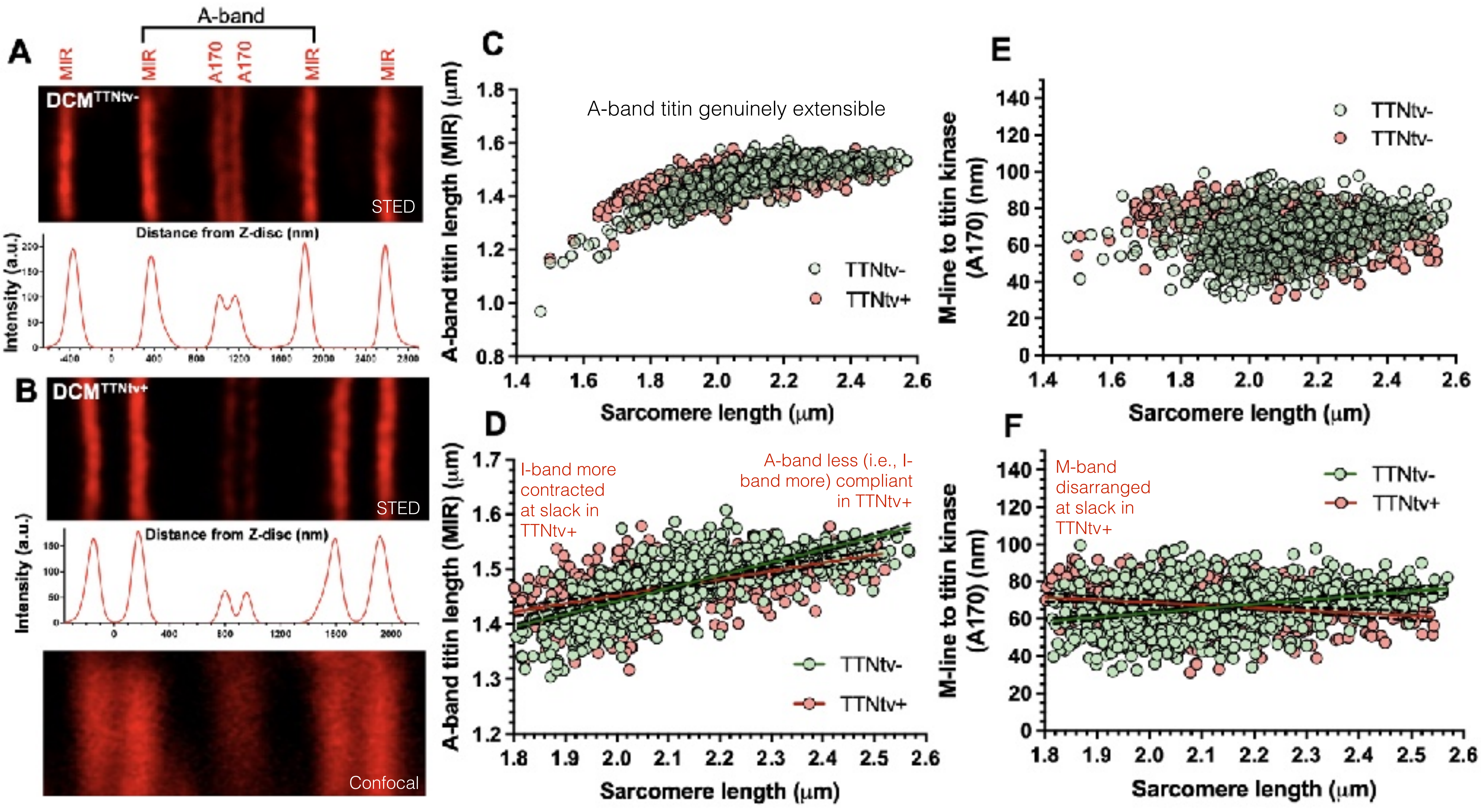




- A170 epitope intensity reduced
- No doubling of the MIR epitope

Kellermayer D et al. J. Clin. Invest., 2024

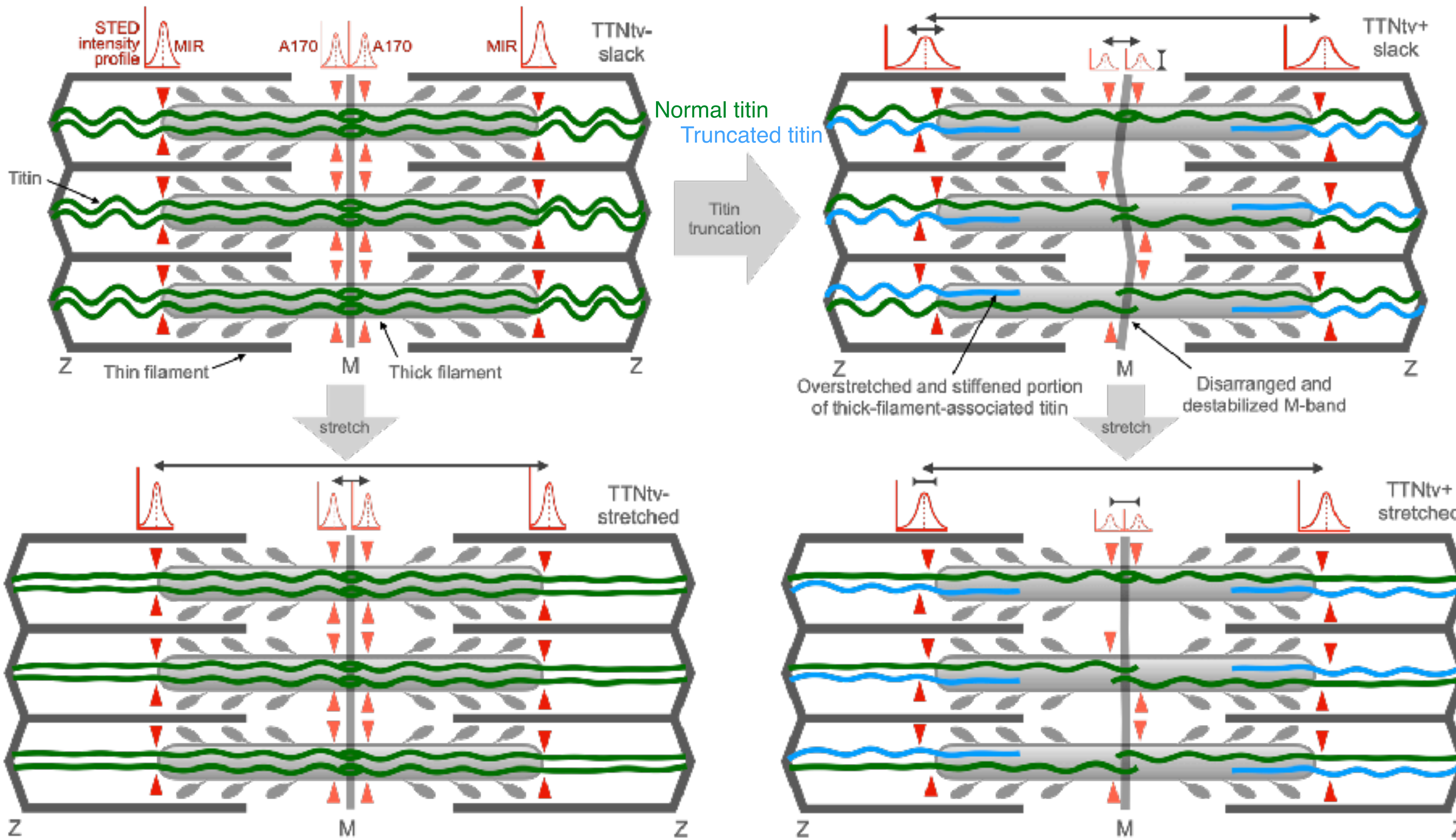
Epitopes respond differently to stretch in TTNtv+/- sarcomeres



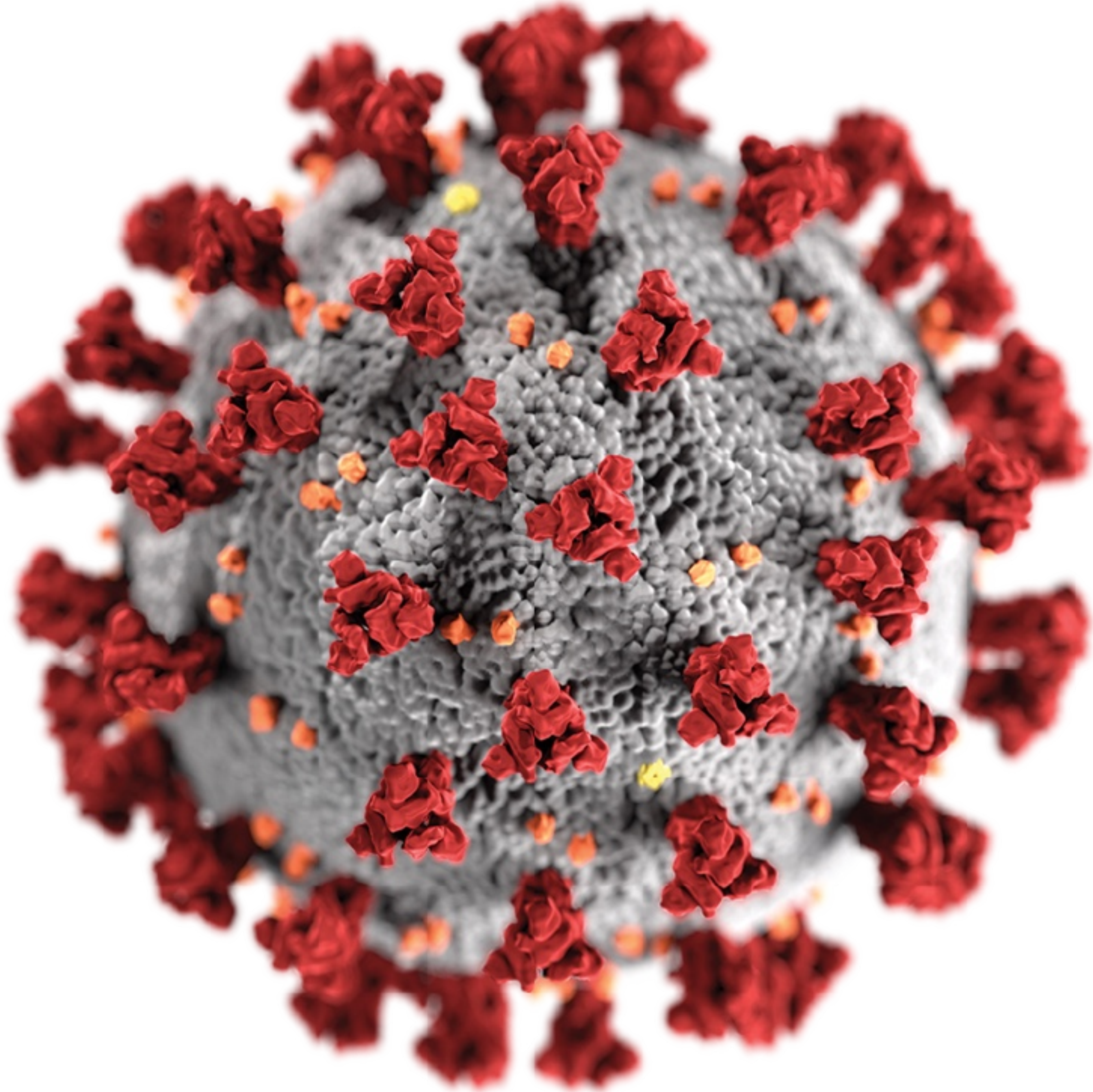
Kellermayer D et al. J. Clin. Invest., 2024

Model

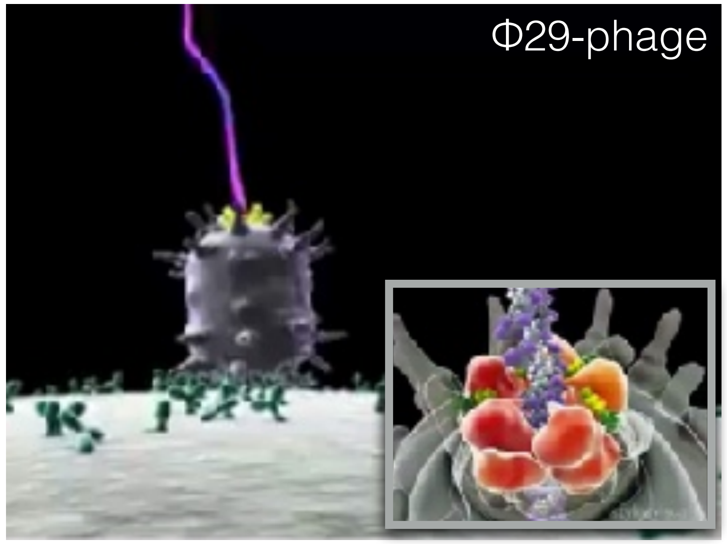
Truncated titin causes structural and mechanical perturbation in the sarcomere



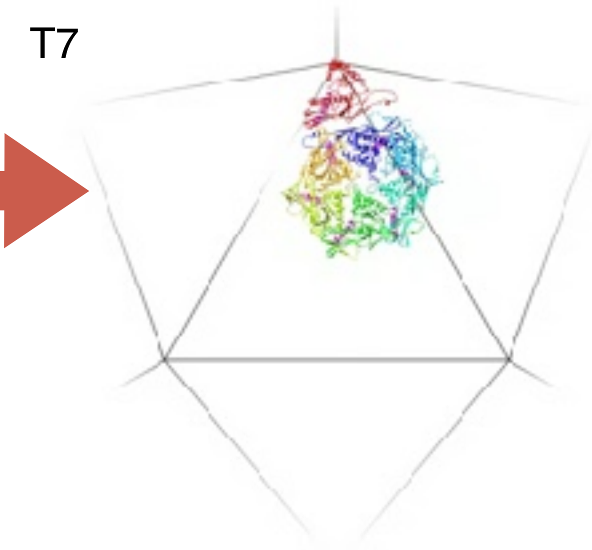
Pathomechanistic factors leading to DCM:
1) reduced M-band mechanosensor function; 2) internal mechanical load generated by truncated titin.



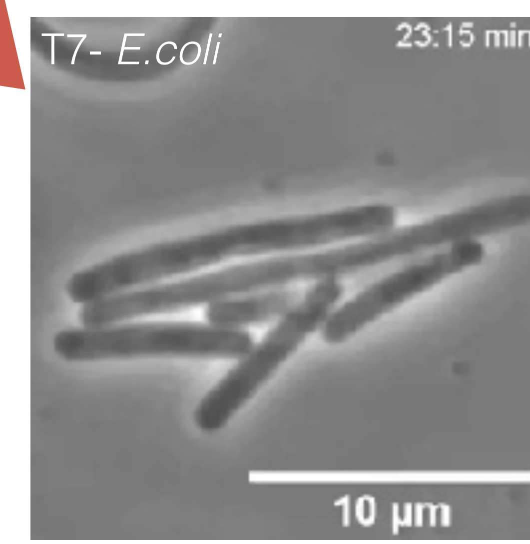
dsDNA virus (bacteriophage) life cycle



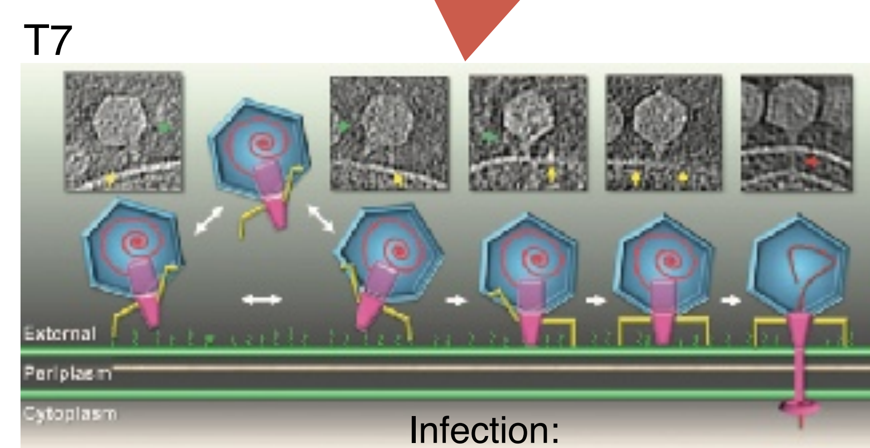
• **DNS packaging** (ATP-dependent, motor-driven process, DNA pressure generated ~60 atm!)
Animation by Eric Keller based on work by the Bustamante Group



Maturation:
• Capsid expansion
• Wall thinning
• *gp10* N-terminal helix unfolds, swings through shell, refolds.
• Stabilizing non-covalent interactions are formed.
Guo et al. PNAS 111, E4606, 2014.



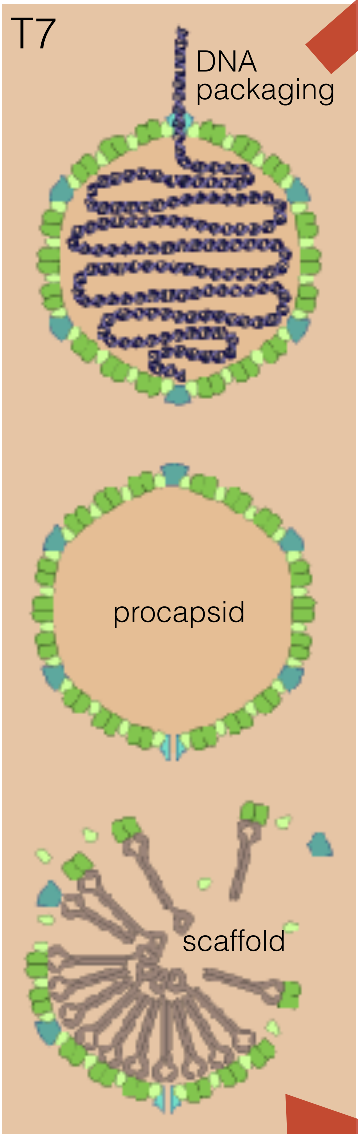
Host cell lysis:
• Phase contrast



Infection:
• receptor (LPS) recognition
• trigger
• injector complex formation
• DNA ejection
Hu et al. Science 339, 576, 2013.

• LamB (maltoporin)-induced **DNA ejection** *in vitro.* Rapid DNA labeling by SYBR Gold.
Grayson et al. PNAS 104, 14652, 2007.

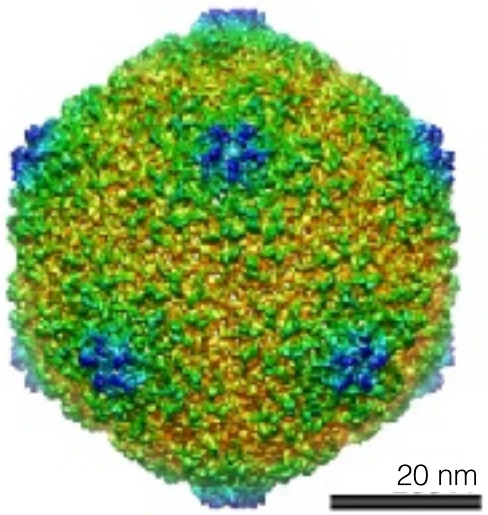
• **Viral protein synthesis** in host cell



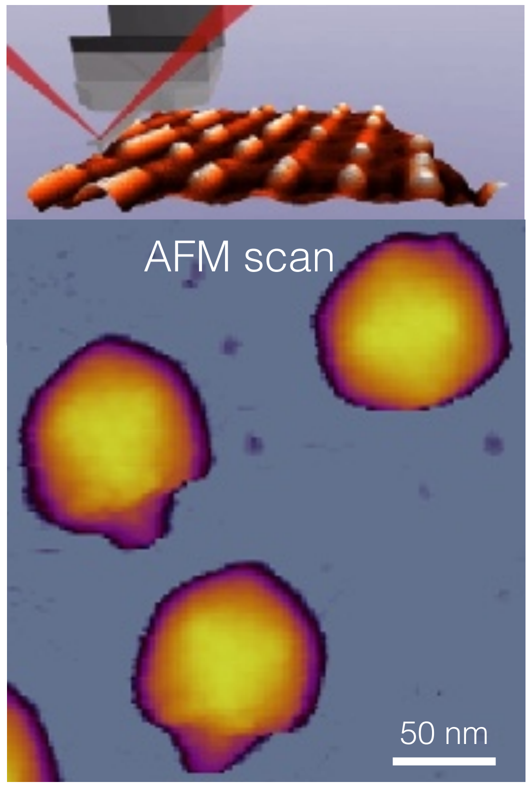
• **Assembly of DNA-free procapsid** (*scaffold protein*-dependent)

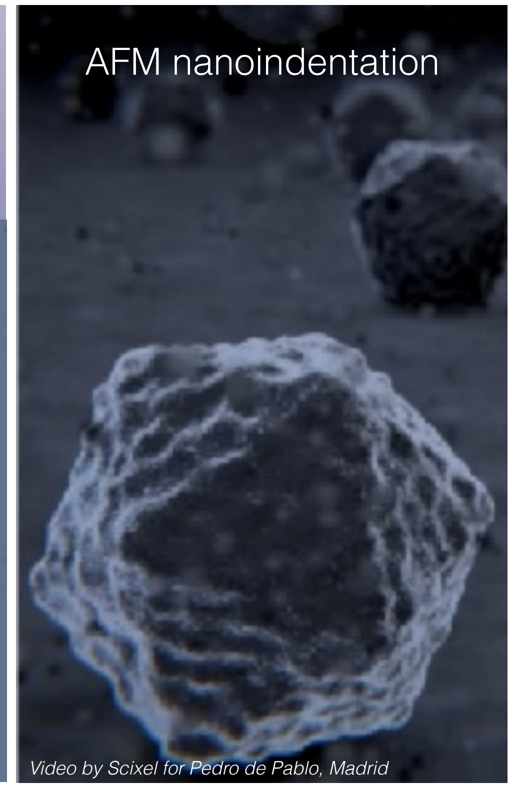
T7 capsid buckles and breaks under force

- Short-tailed DNA virus (~40 kbp)
- *Podoviridae* family
- Infects *E.coli*
- Icosahedral capsid

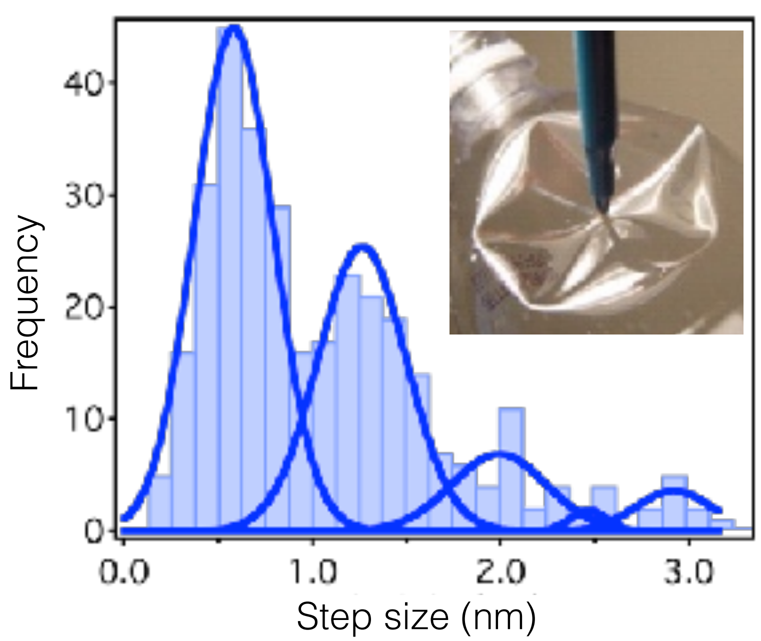


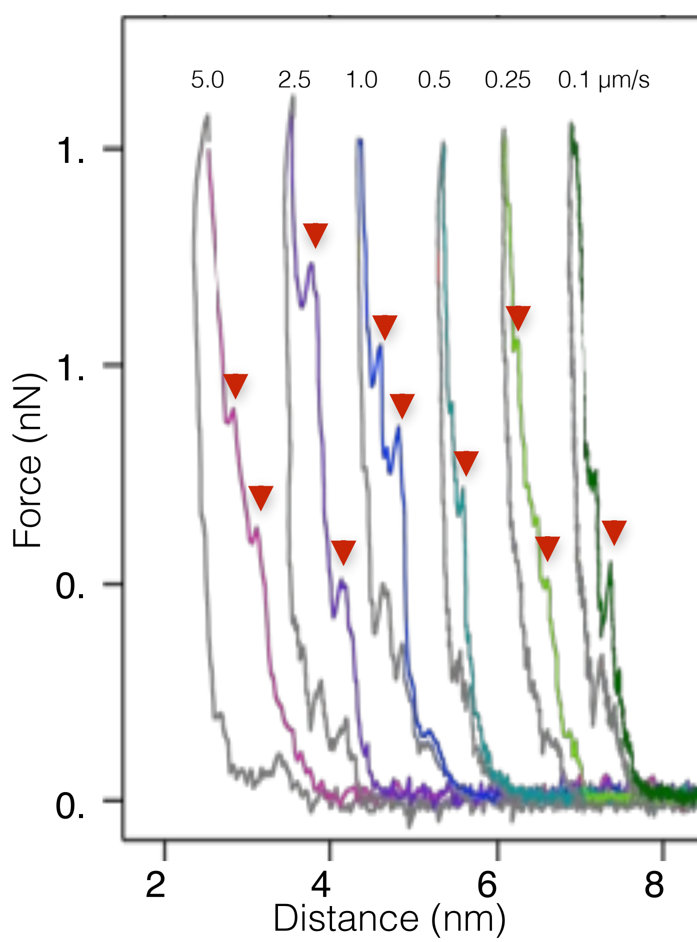
Ionel et al. J.Biol.Chem. 286, 234, 2010.

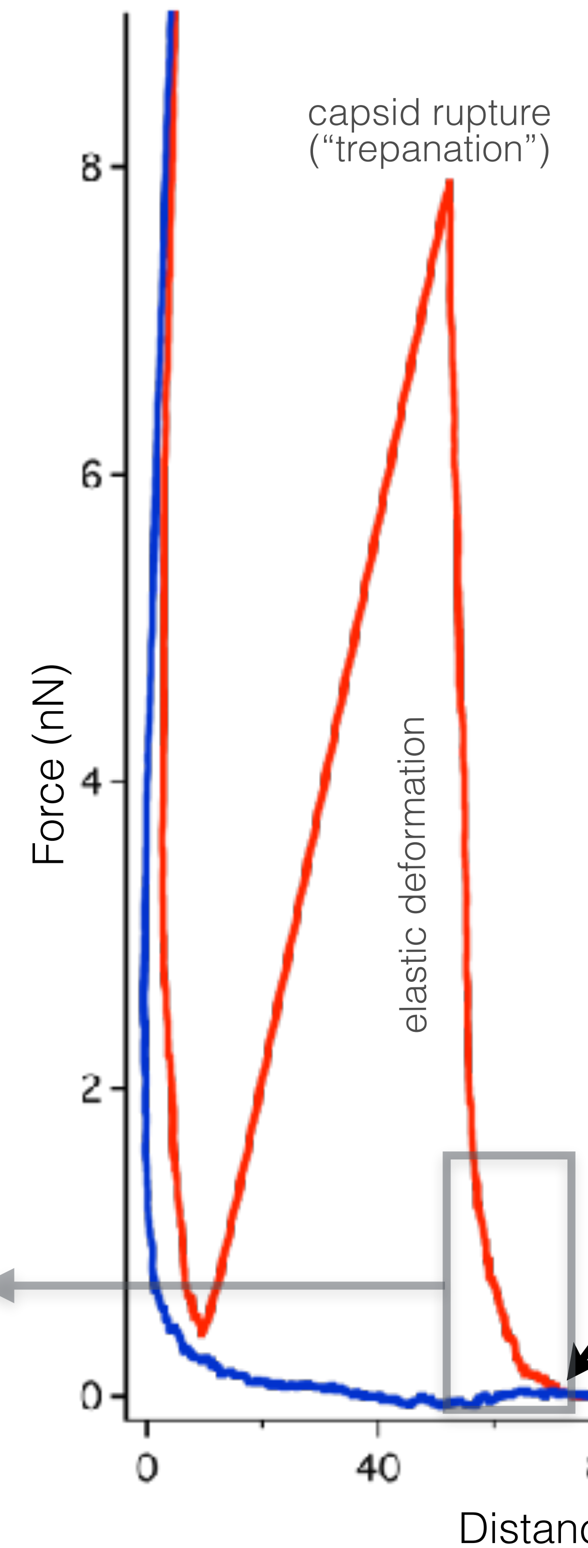




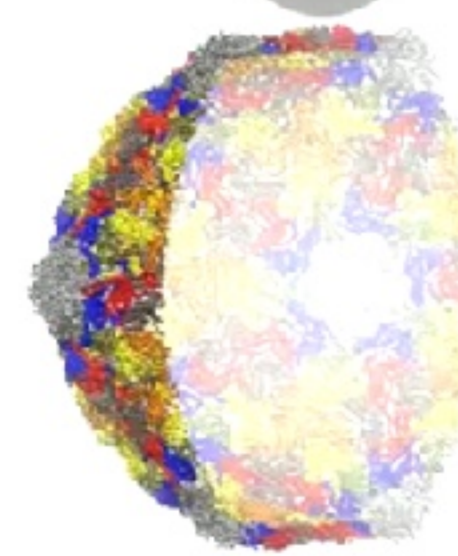
Reversible indentation
("buckling") steps (~6 Å)







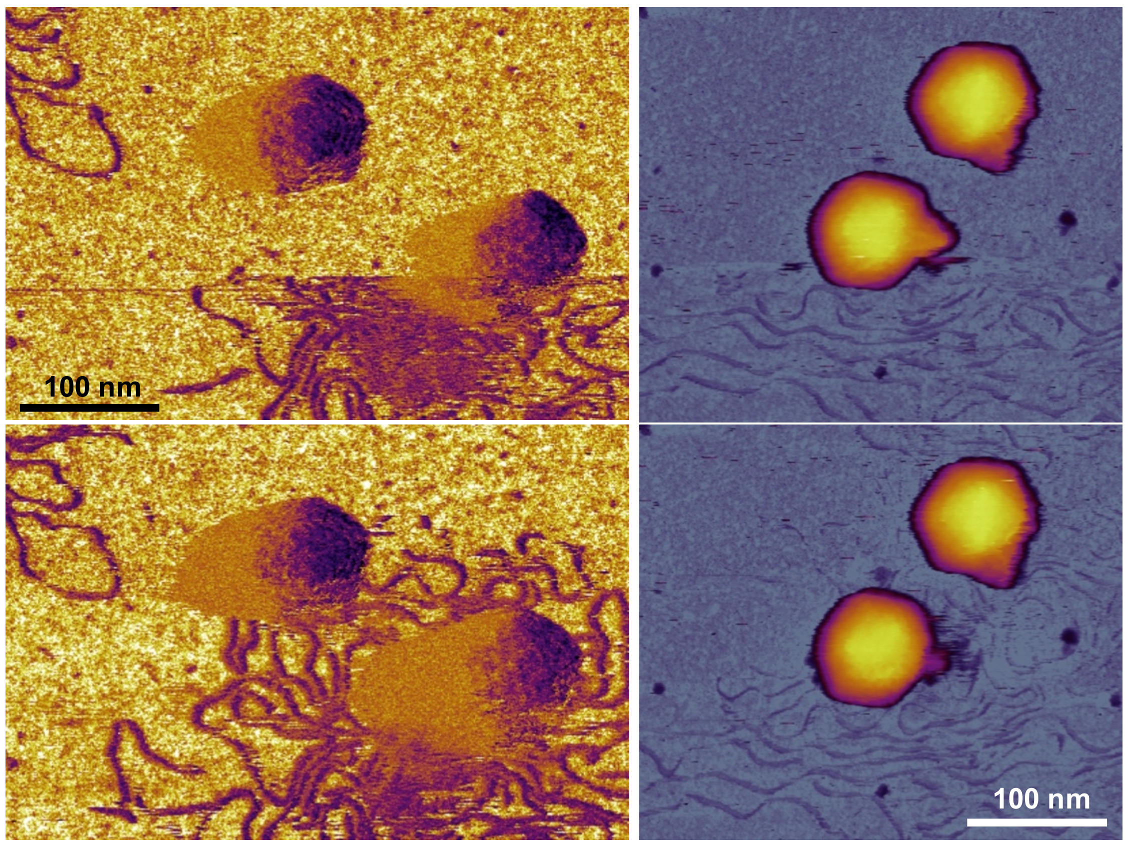
Trepanated inca skull,
Museo Rafael Larco Herrera, Lima

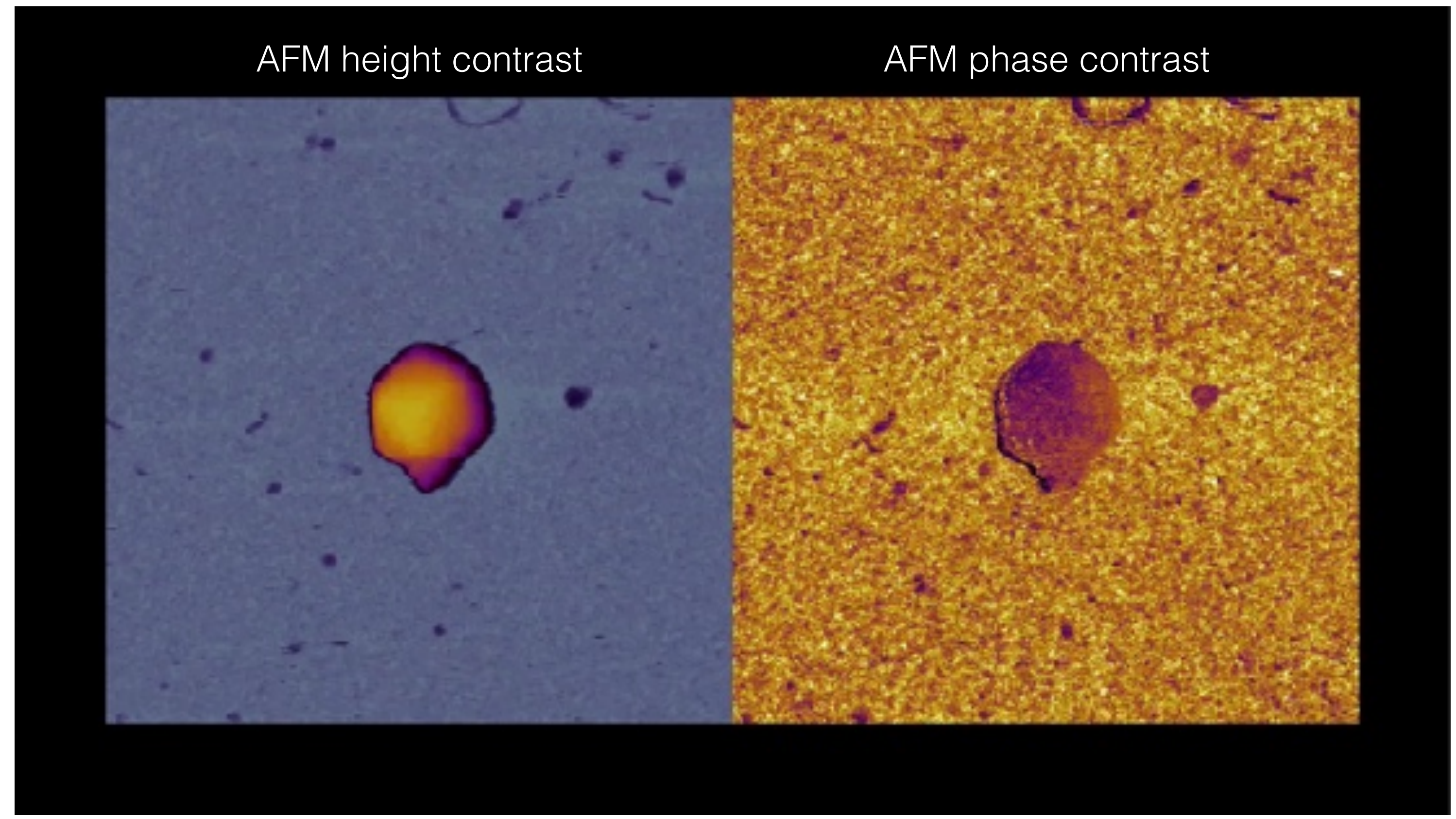


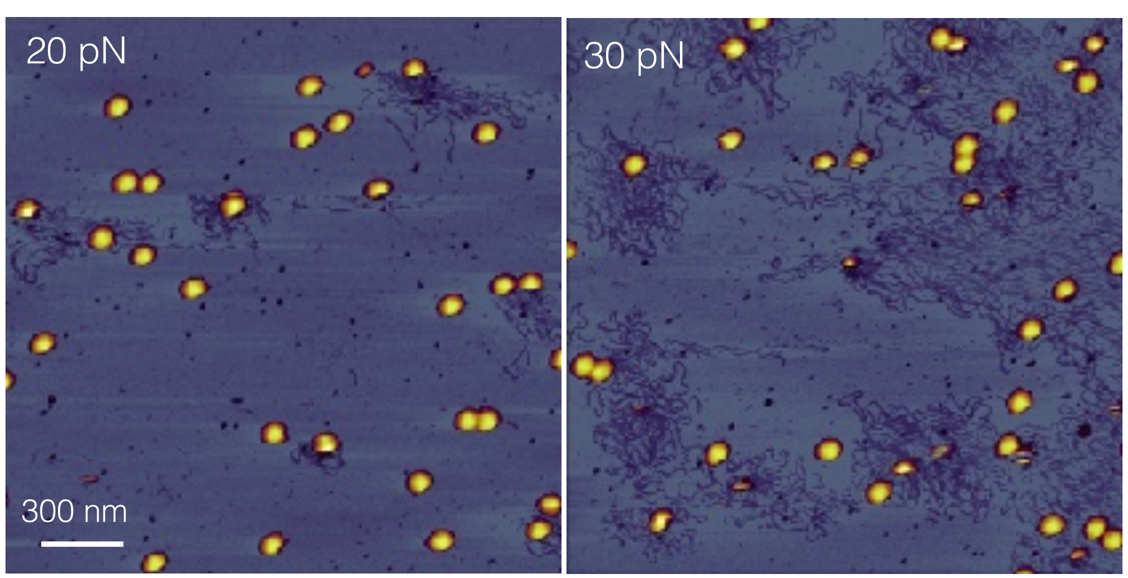
Zhmurov et al. J.Phys.Chem.B 115, 5278, 2011.

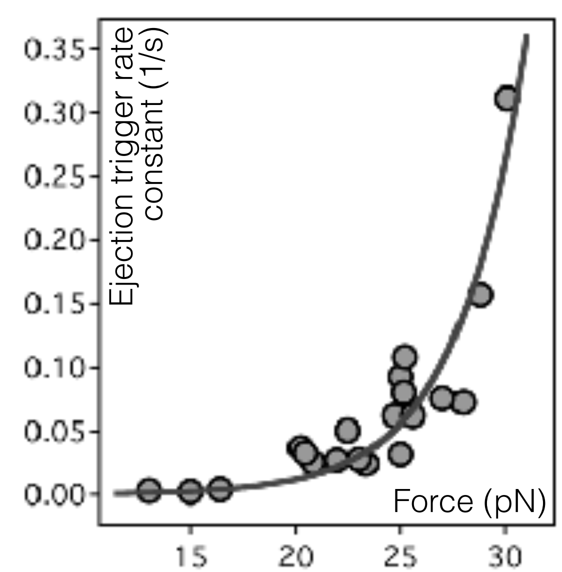
Vörös et al. Nanoscale 2017

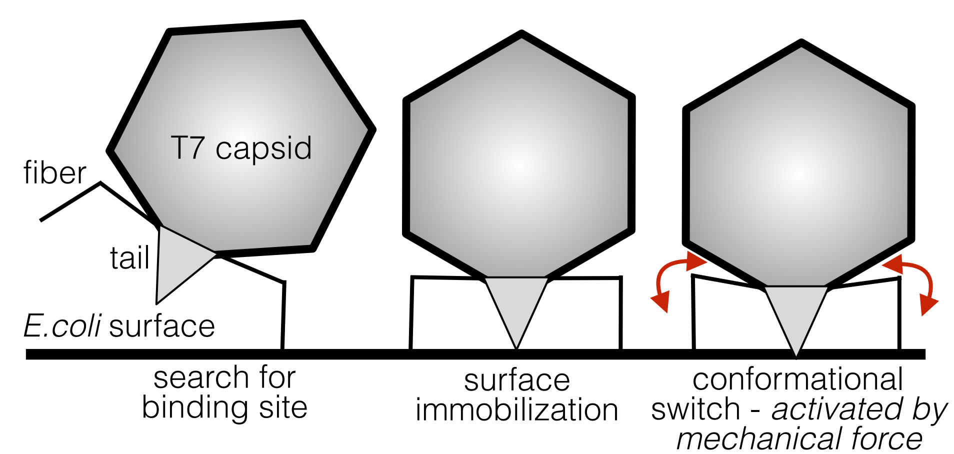
DNA ejection from T7 phage can be triggered by mechanical force





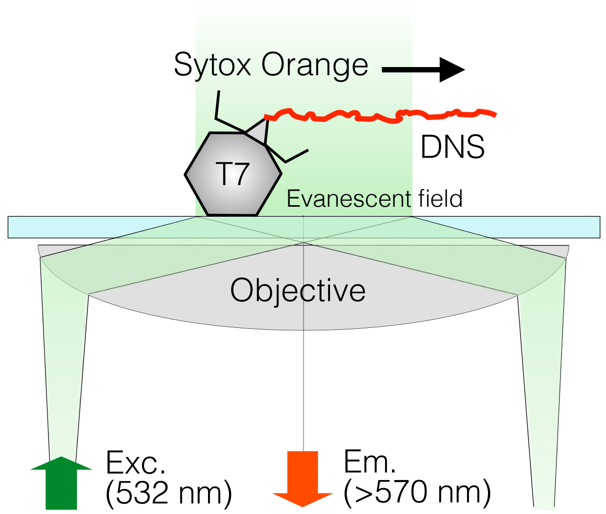


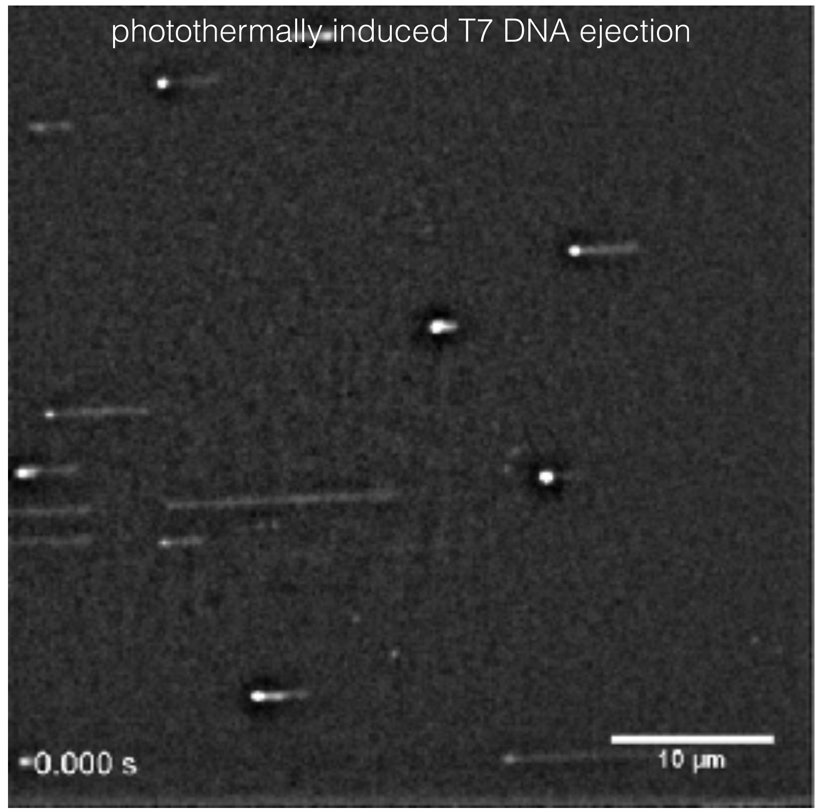


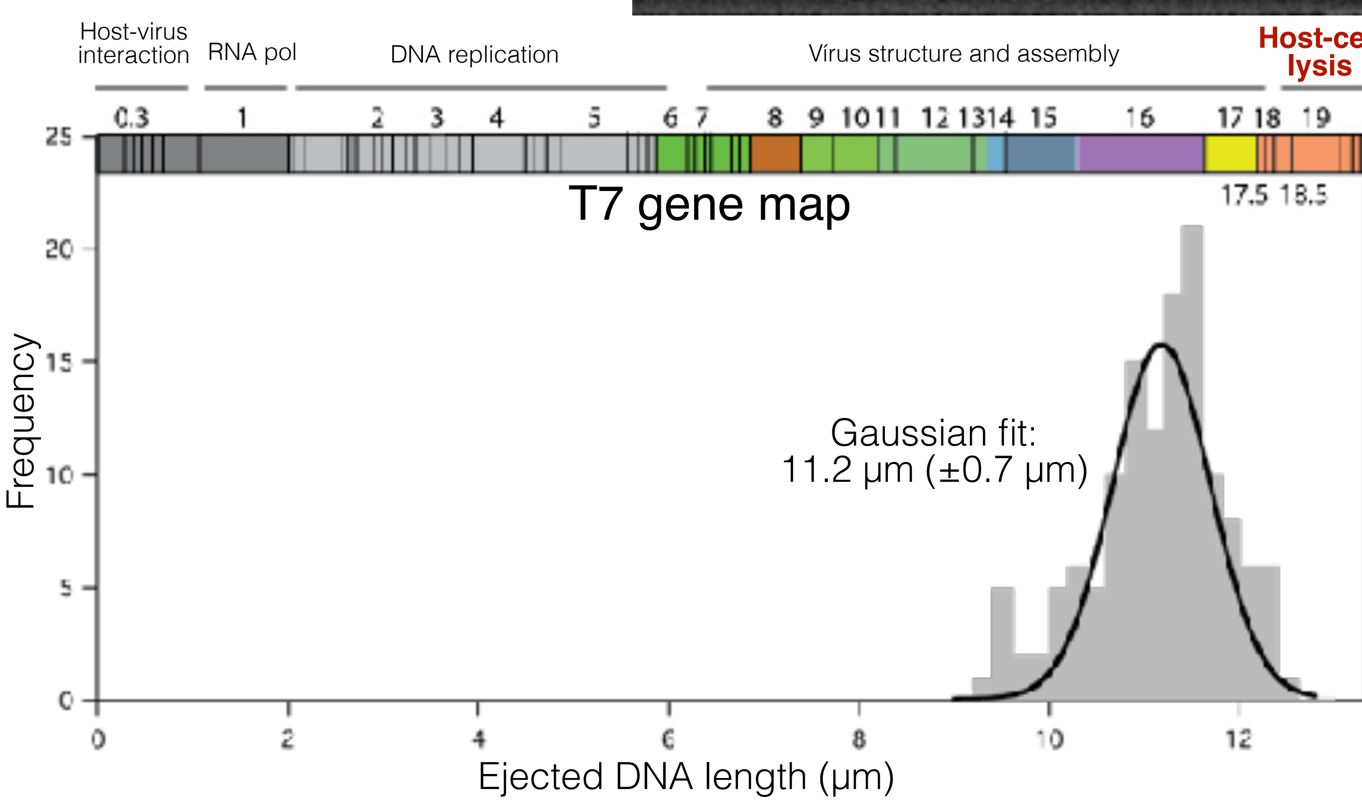


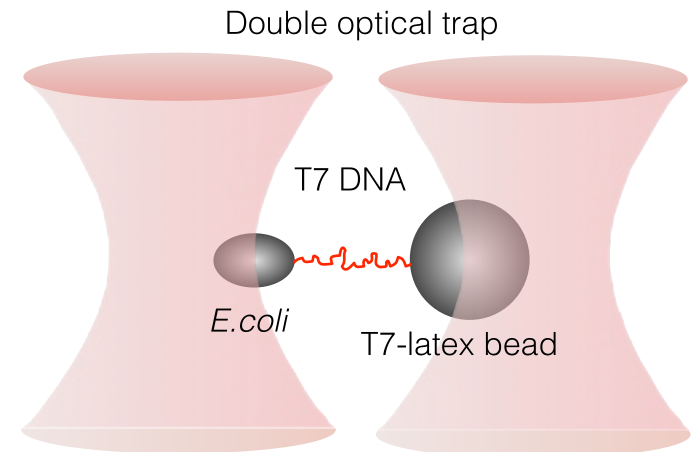
Kellermayer et al. Nanoscale 2018

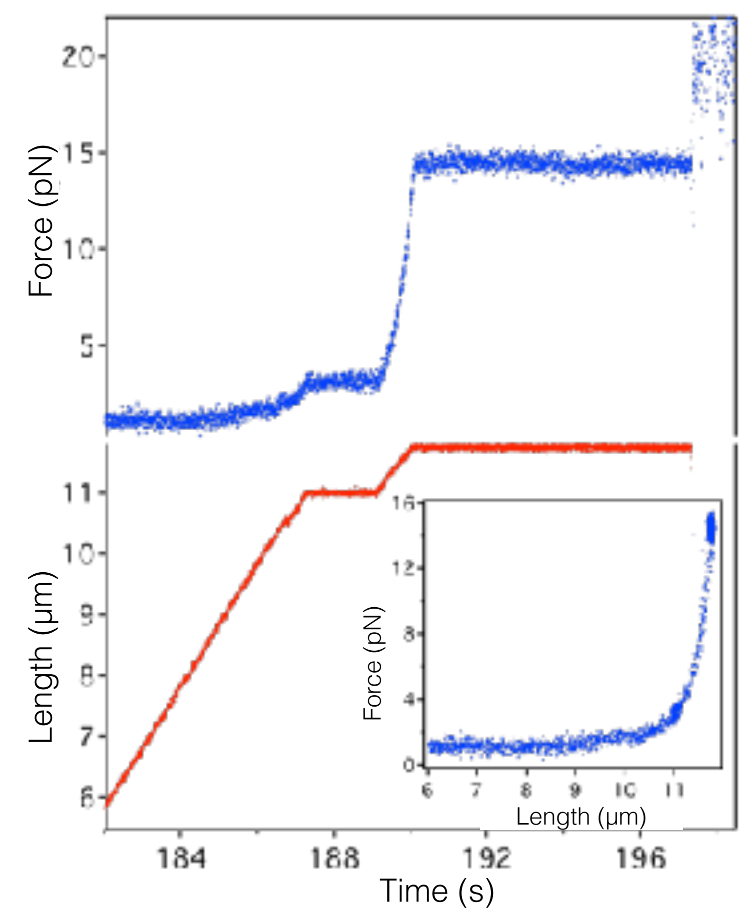
T7 DNA release may be under mechanical control

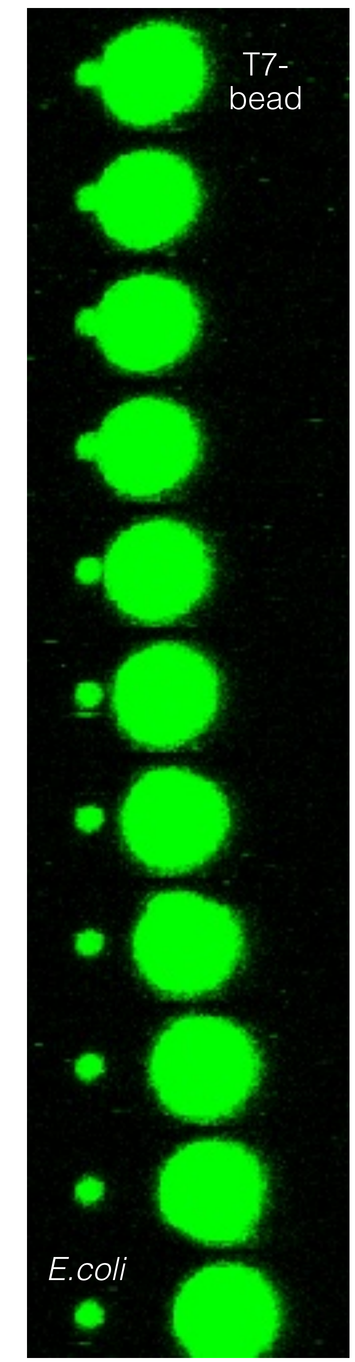






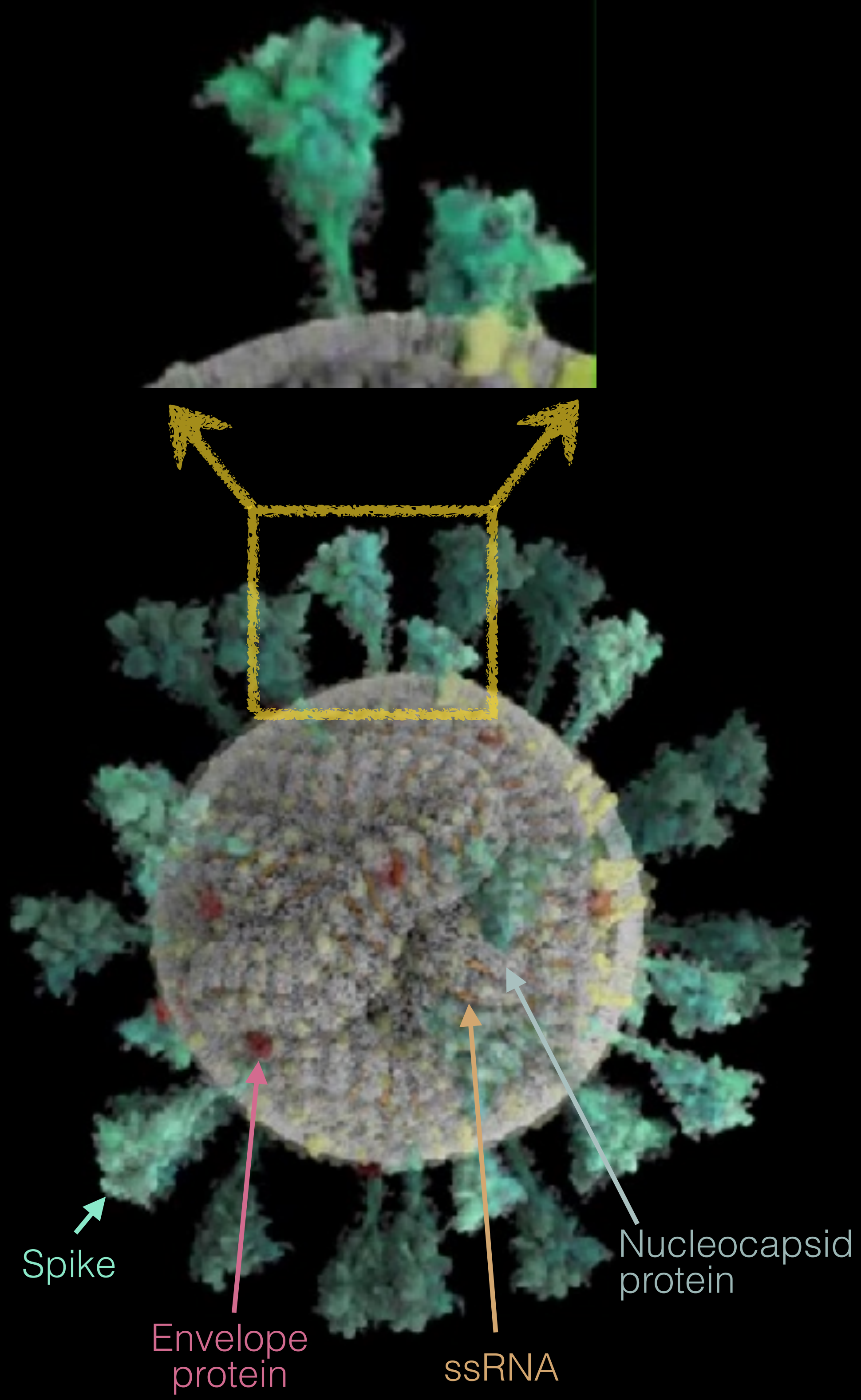


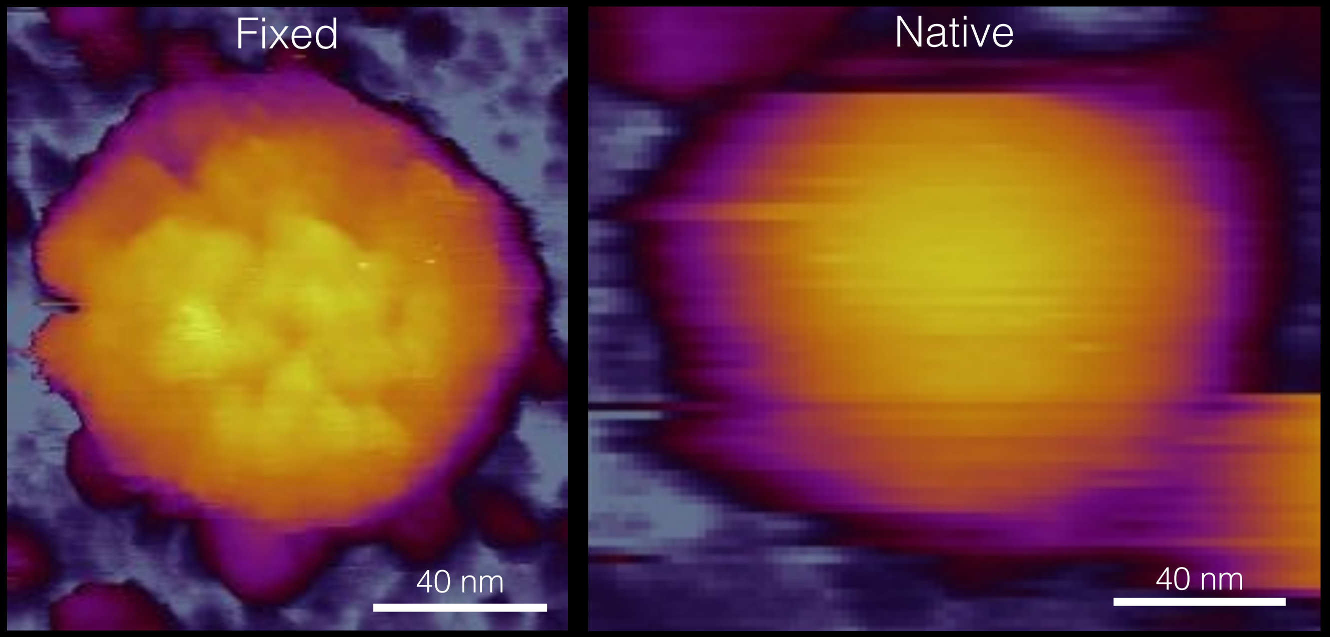


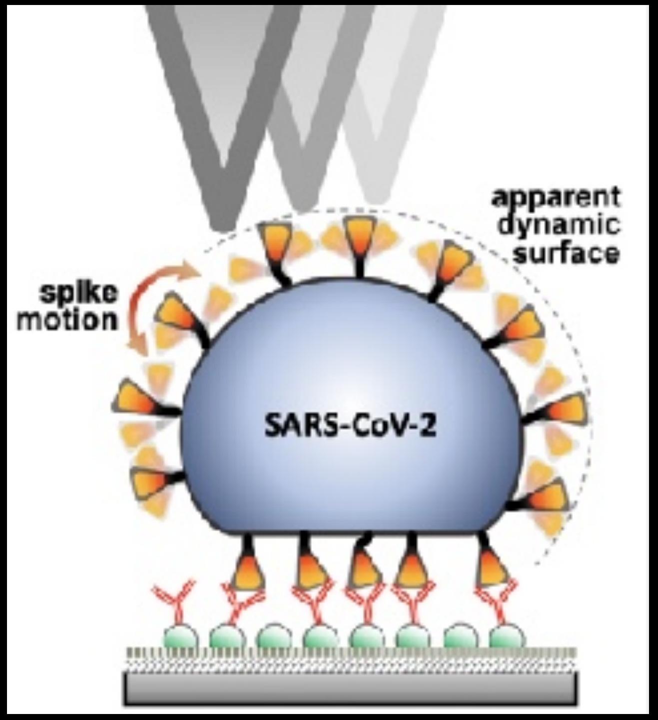


Kiss et al. IJMS 2022

Spike dynamics of SARS-CoV-2

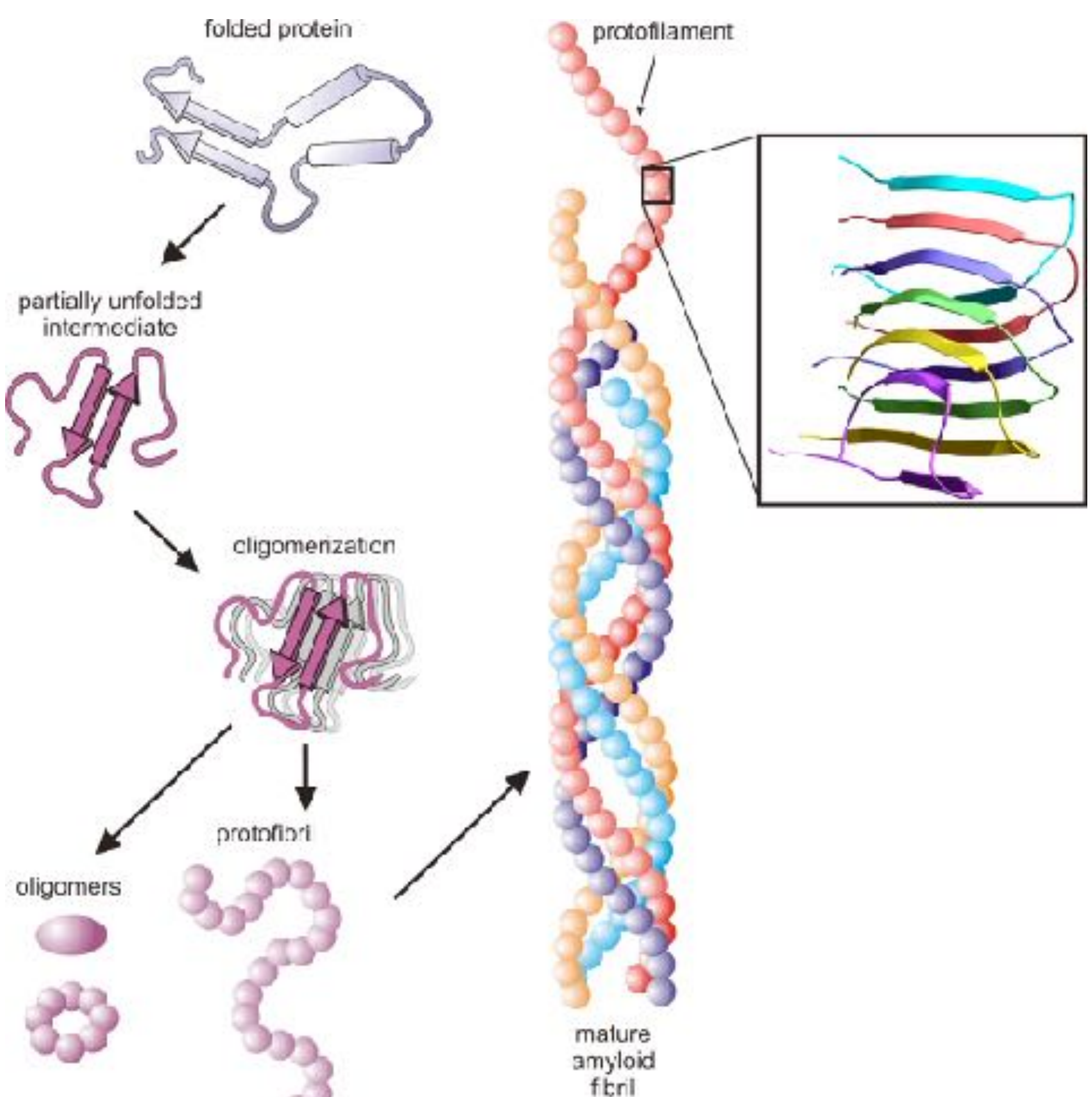




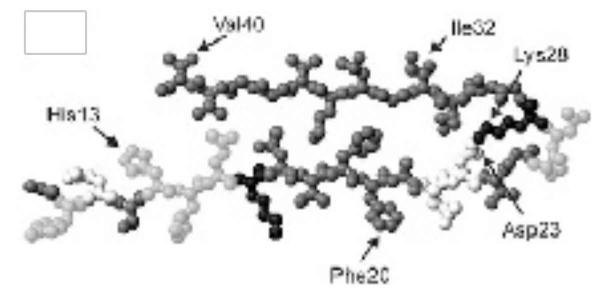


4. From amyloid to nanotechnology

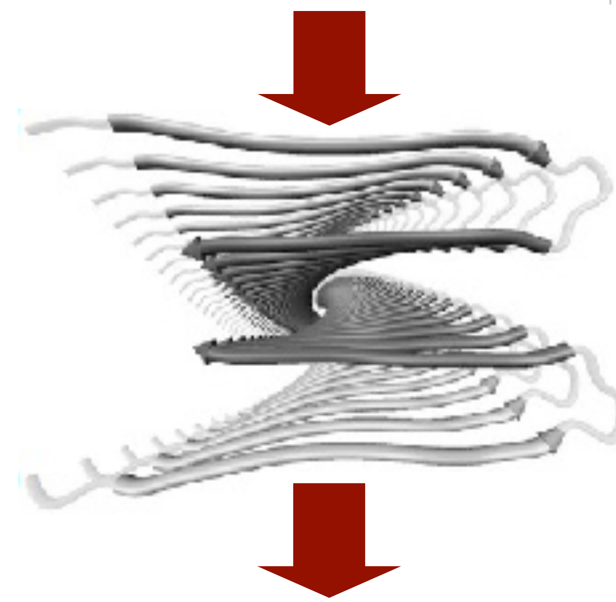
General fibrillogenesis scheme



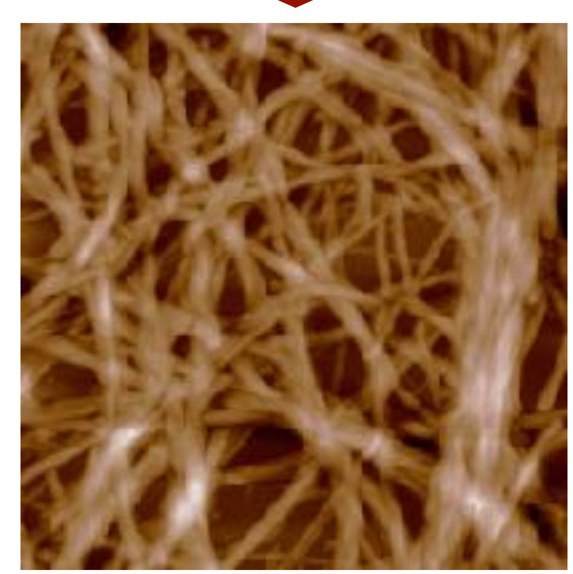
Alzheimer ß fibril formation



Amyloid ß1-40 peptide

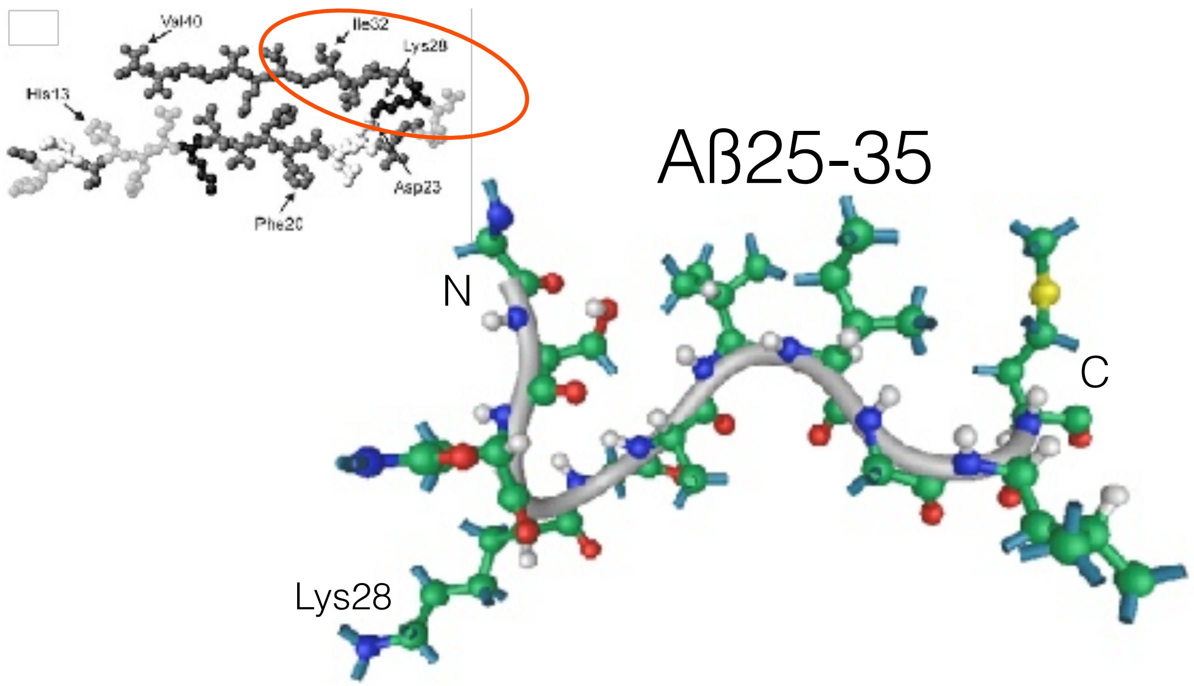


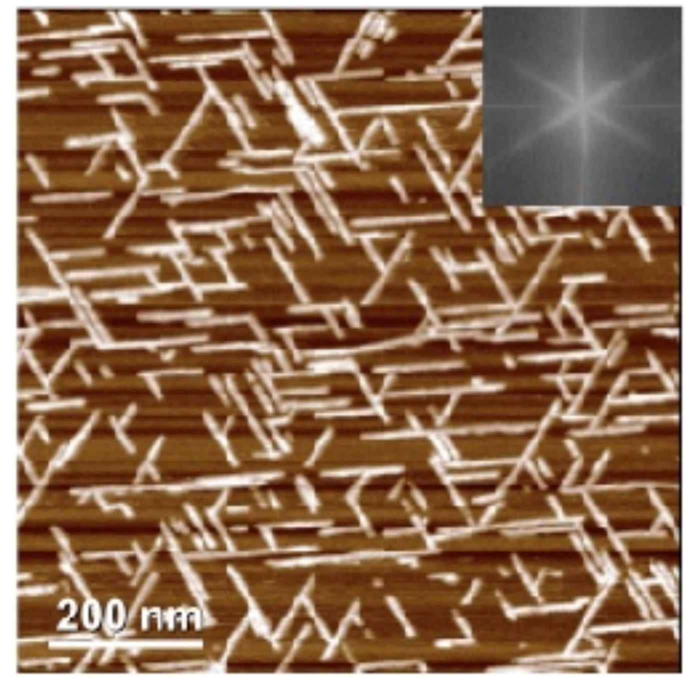
Structural model of the Aß1-40 protofilament
Tycko, R. Progr. NMR Spectr. 42, 53, 2003.

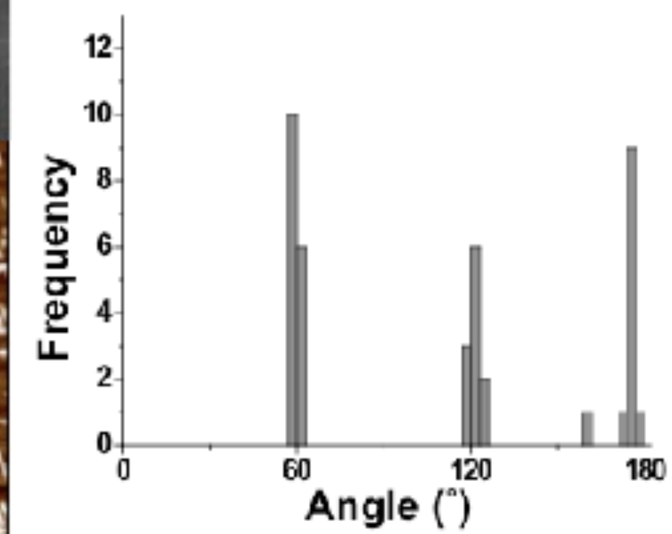


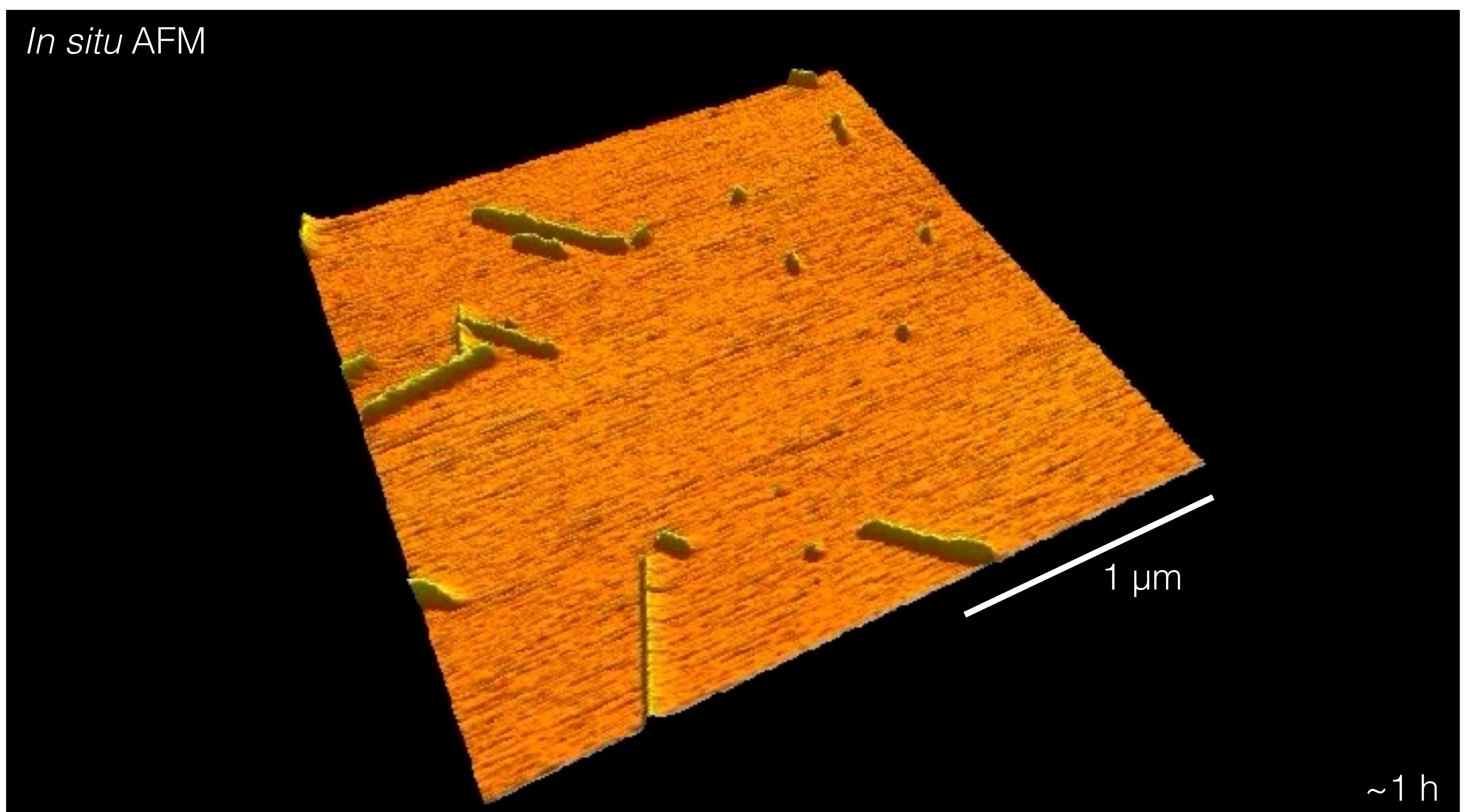
Amyloid ß1-40 fibril meshwork (AFM)

Oriented epitaxial assembly of amyloid fibrils

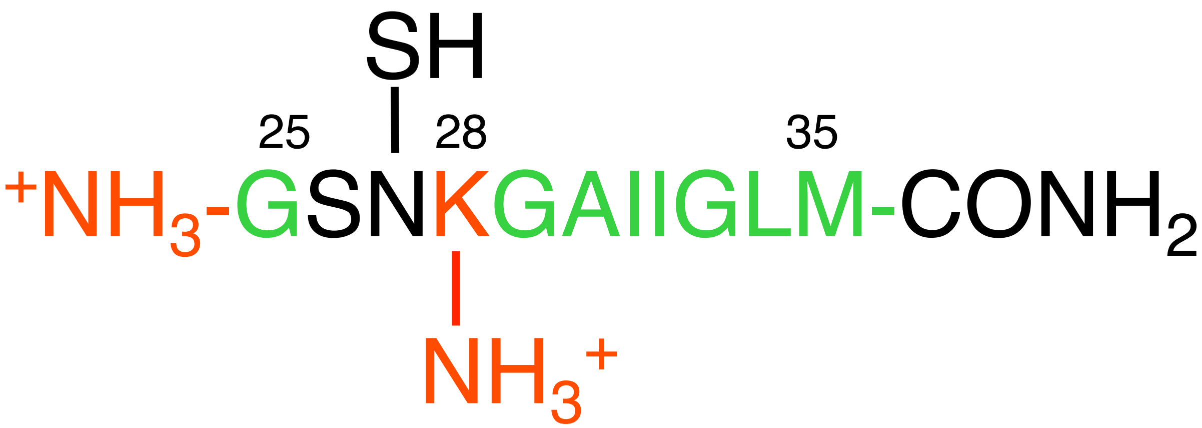




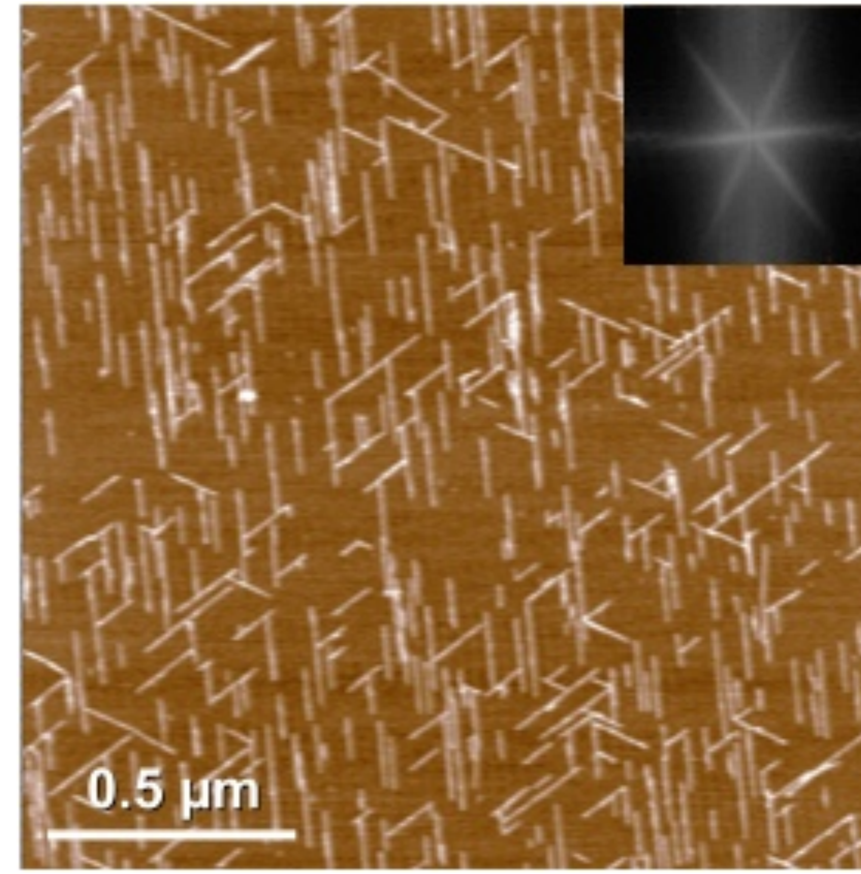


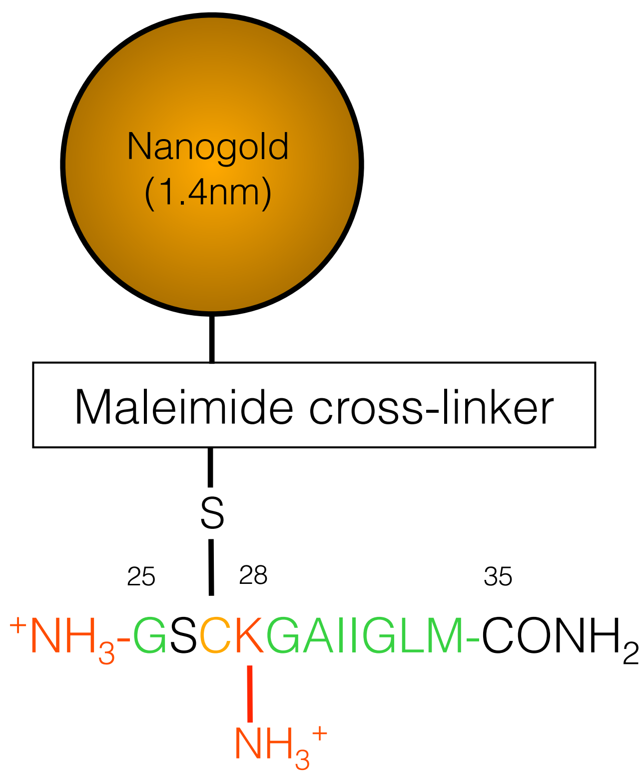


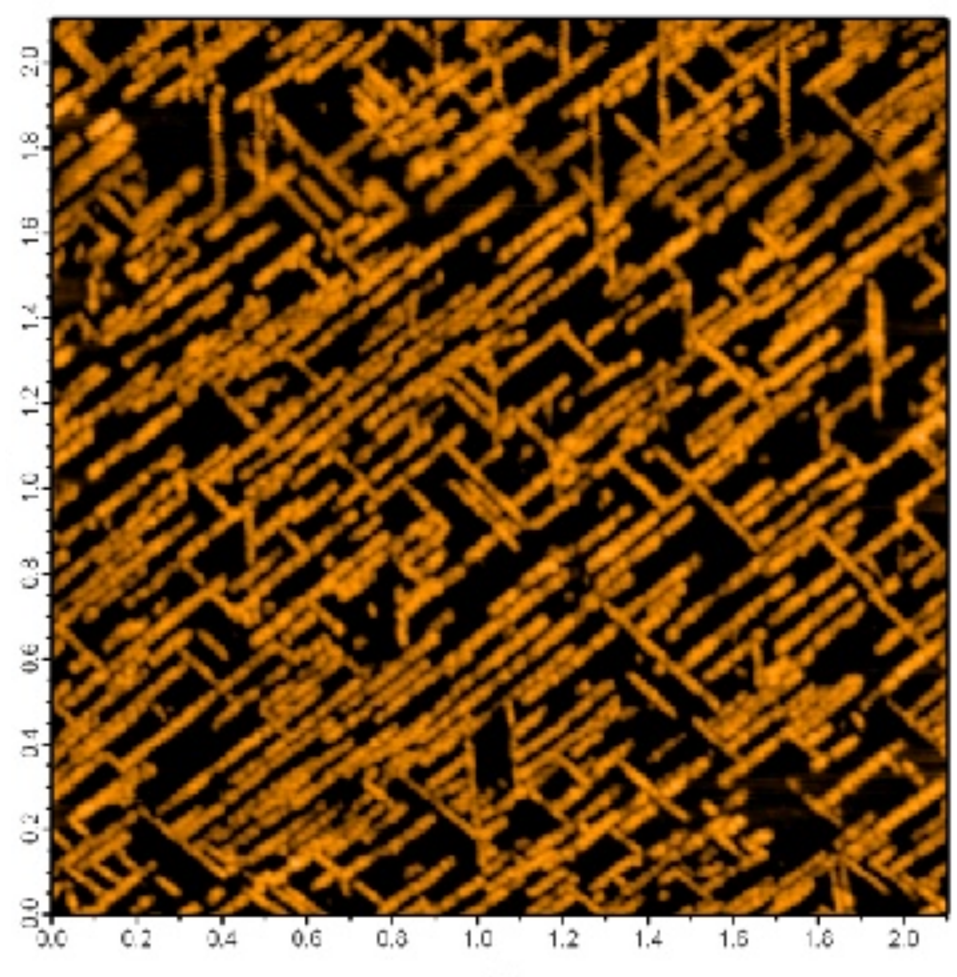
Functionalized oriented nano-network built from a mutant Aß25-35 peptide



Orientation symmetry of Aß25-35_N27C is trigonal



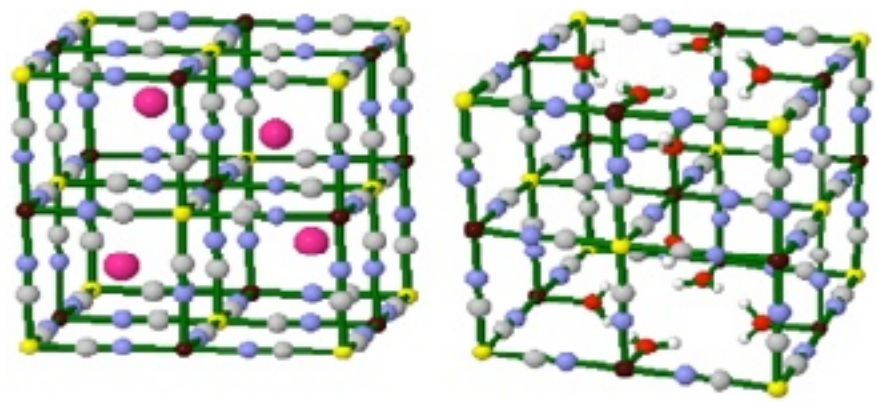




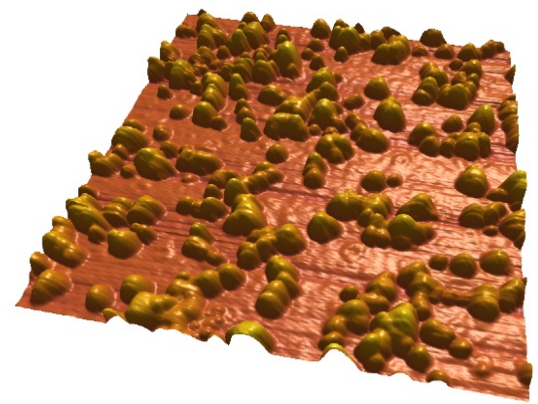
Nanogold particles line up along the oriented Aß25-35_N27C fibrils

5. From nanotechnology to *in vivo* imaging

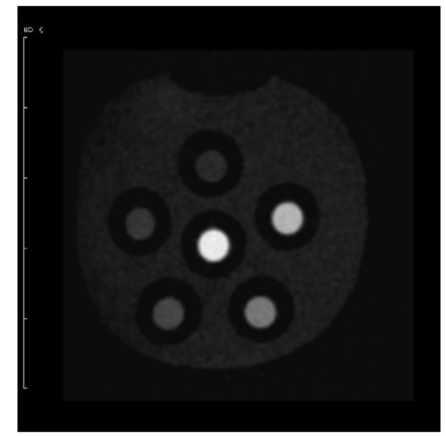
Nanoparticle-based multimodal contrast agent (CT, MR, SPECT)



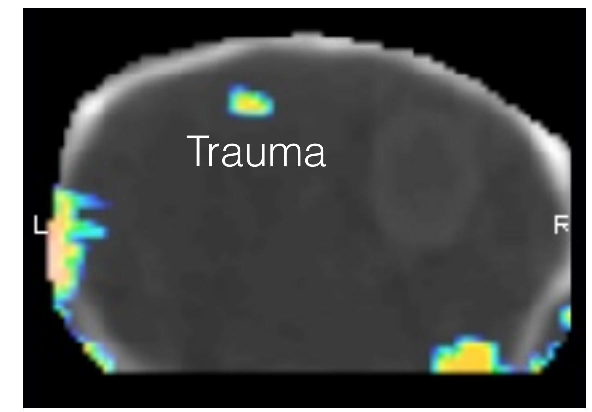
Prussian blue ion chelator crystal structure



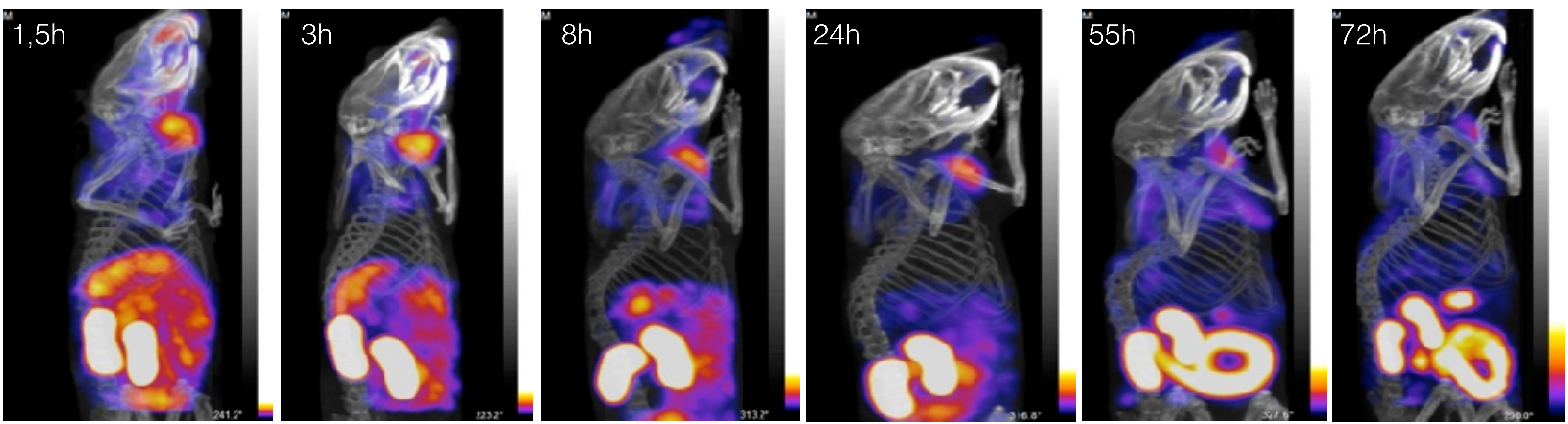
AFM: ~60 nm nanoparticles



MRI, T1



BBB-damage (^{201}Tl)



Biodistribution and excretion of Prussian blue nanoparticles

6. Towards more complex systems

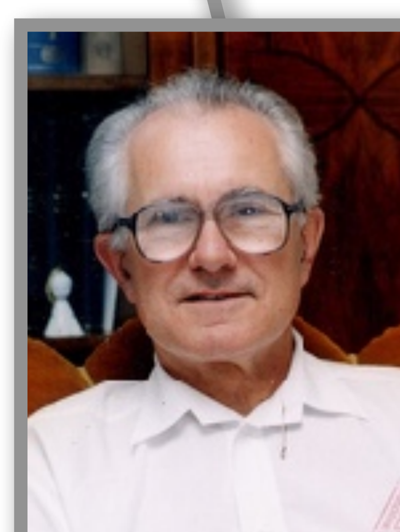
Seek role models


György Romhányi
Pathology


Károly Méhes
Pediatrics


Béla Flerkó
Anatomy


Endre Grastyán
Physiology


József Czopf
Neurology

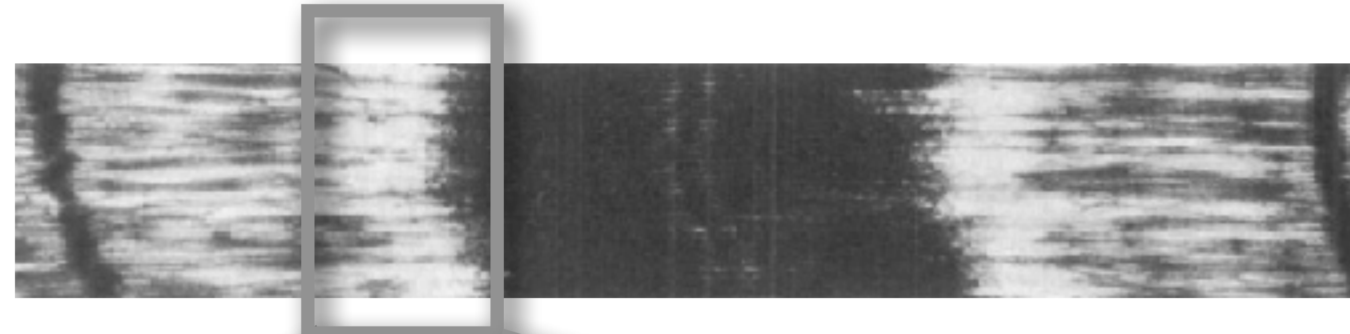

Ibolya Nagy
Internal medicine

Pursue interesting and valuable goals

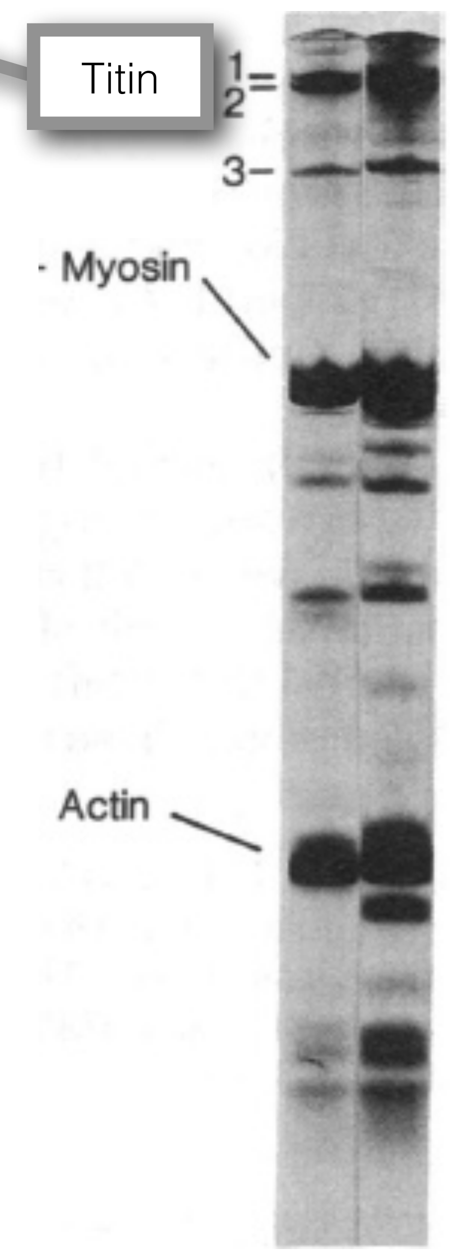
Research trainee (1983-1988)

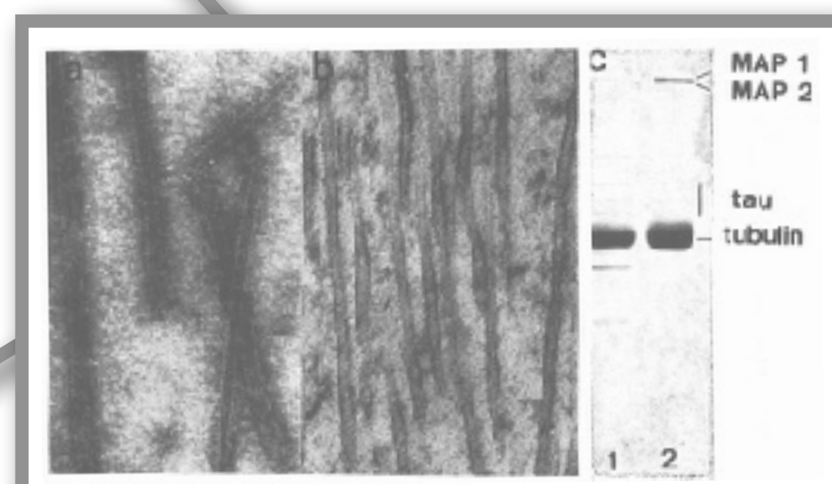
József Belágyi

Károly Trombitás

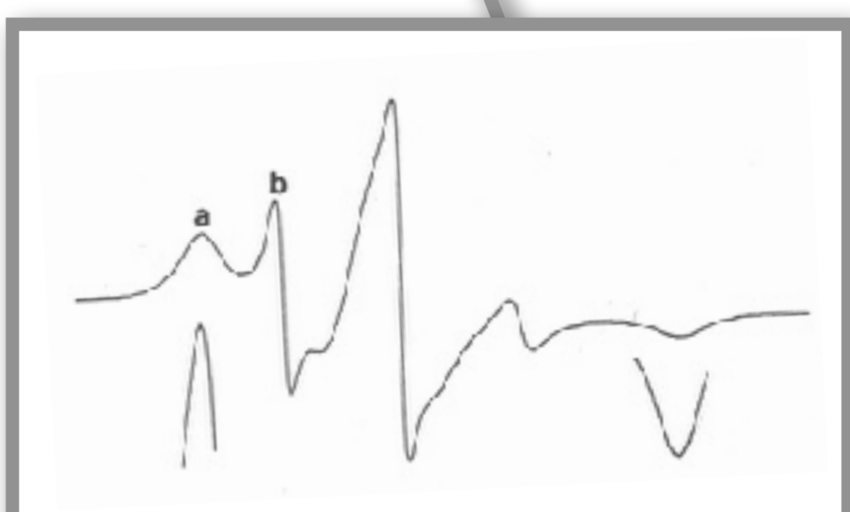


connecting
filaments





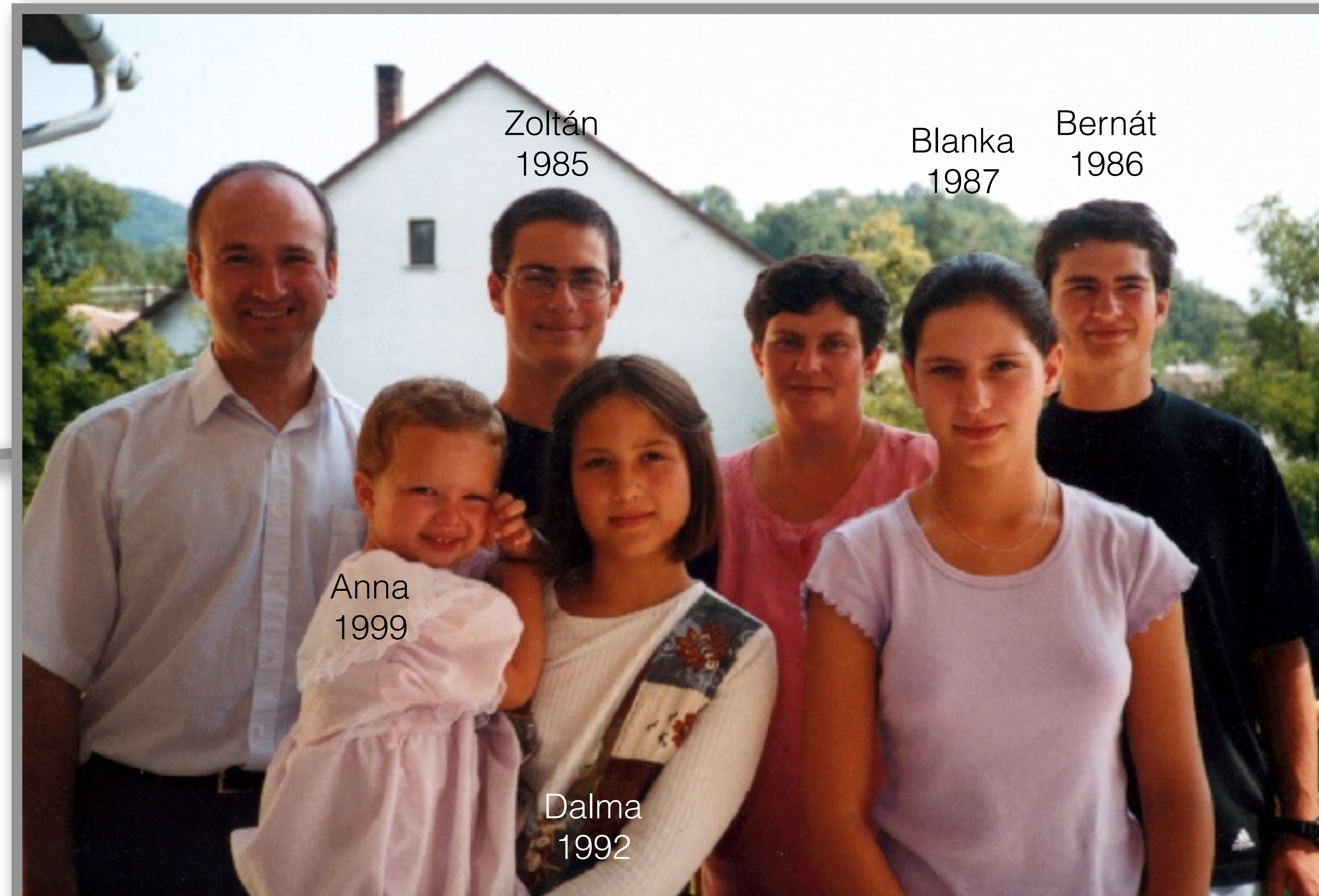
negatively stained
microtubules



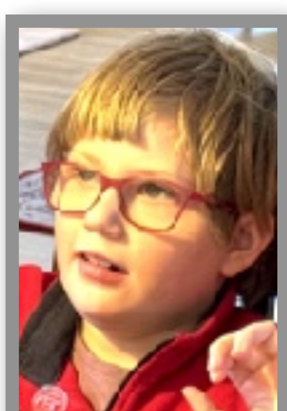
EPR spectrum of spin-labeled
microtubular protein

Build strong and passionate relationships

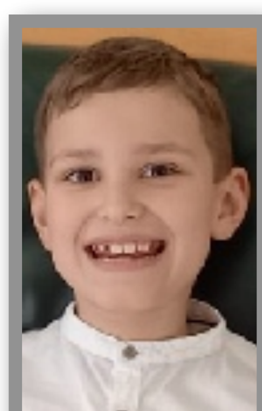
Gyöngyi Szántó
1985



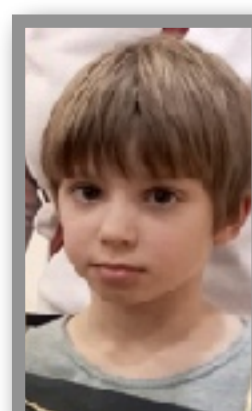
Írisz 2012



Sári 2016



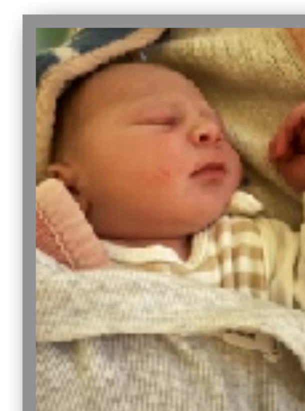
Ákos 2016



Ábel 2016

Ervin 2020

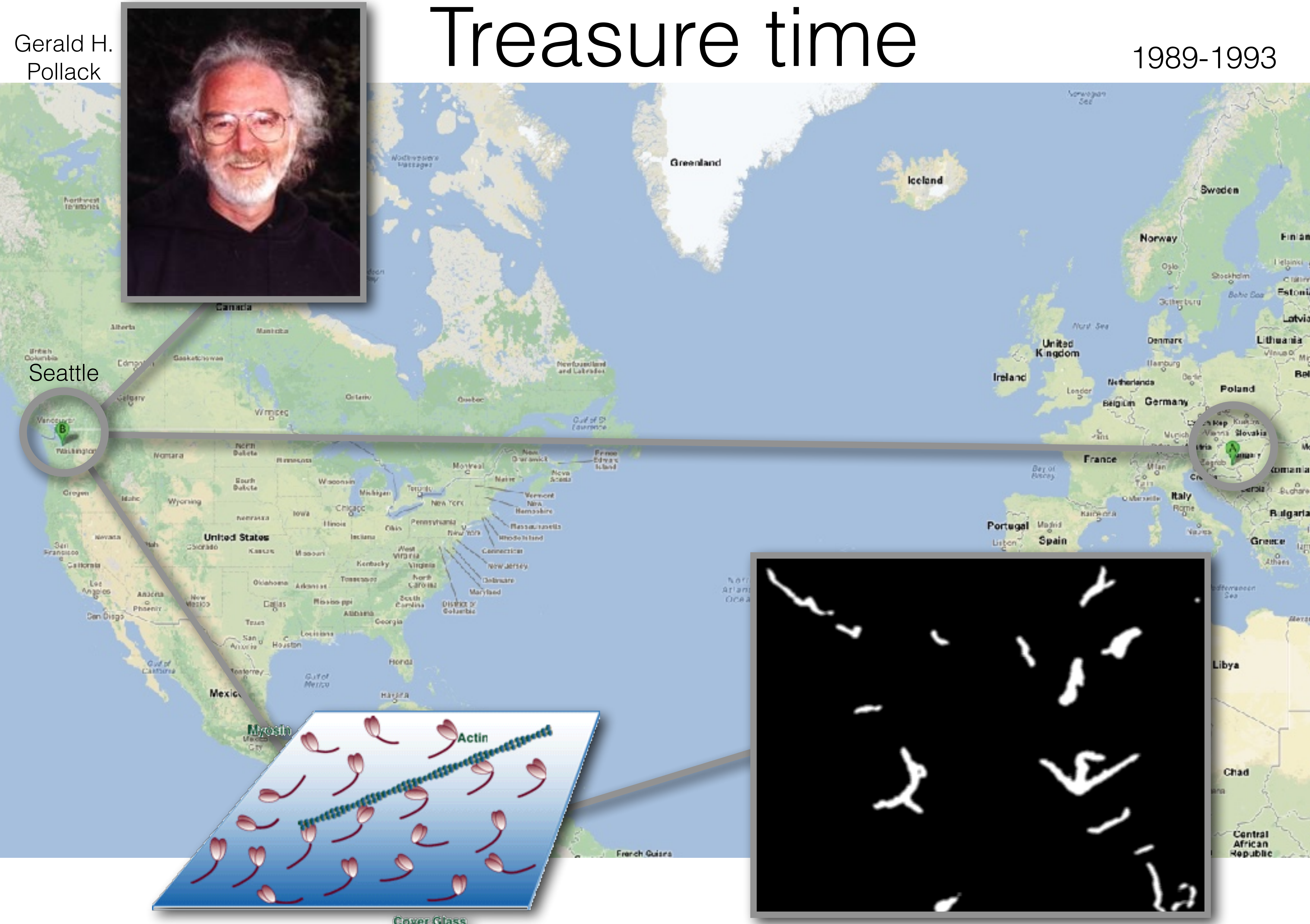
Bercel 2020



Palkó 2024

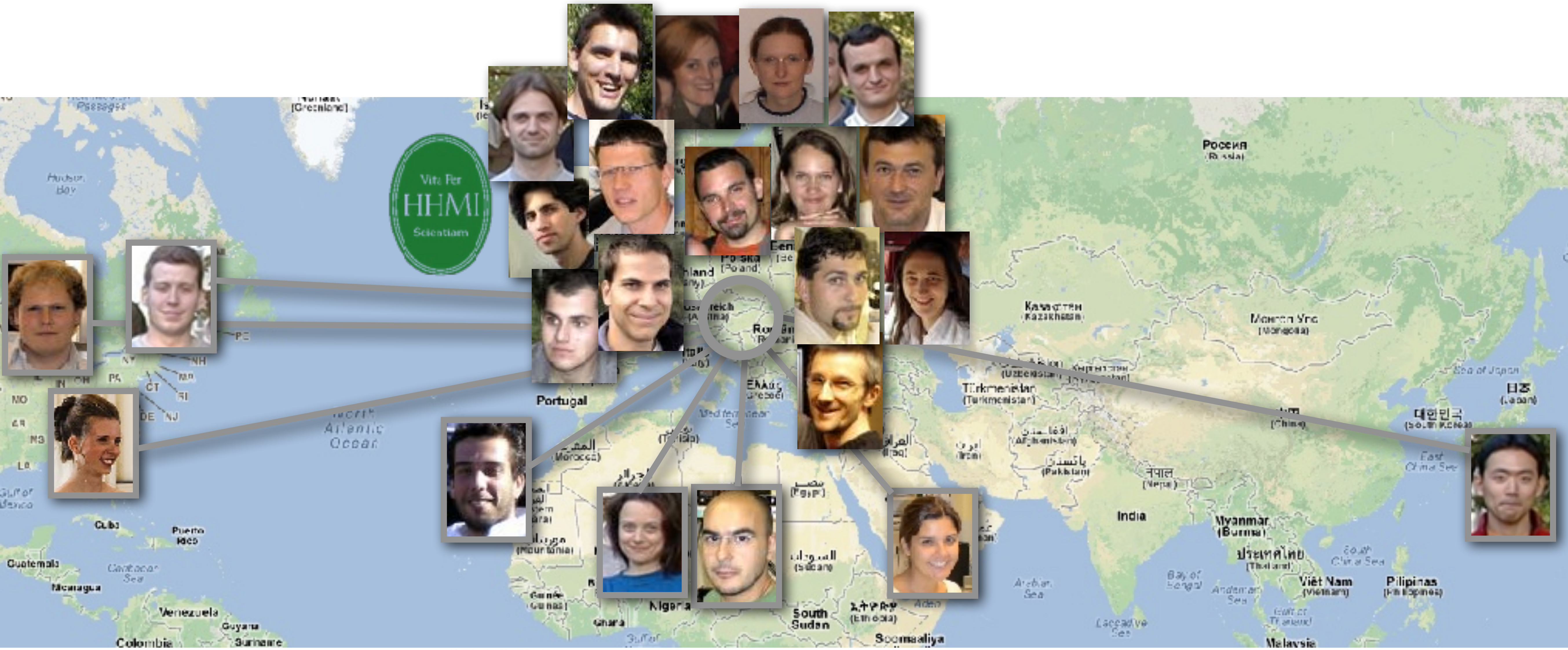
Treasure time

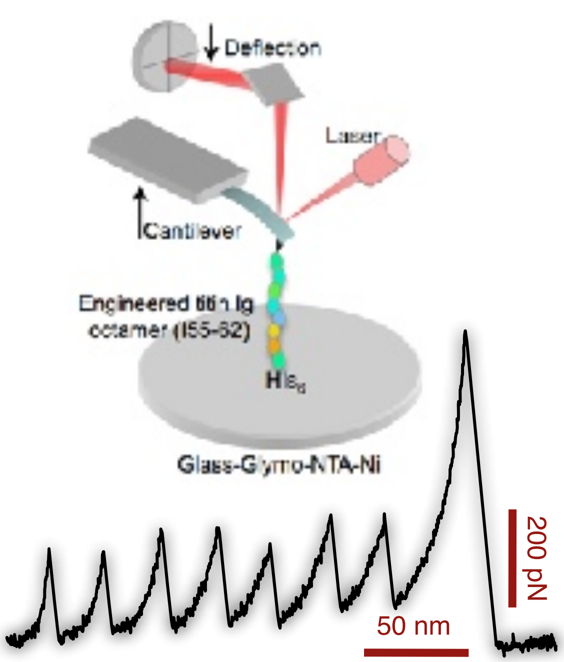
1989-1993

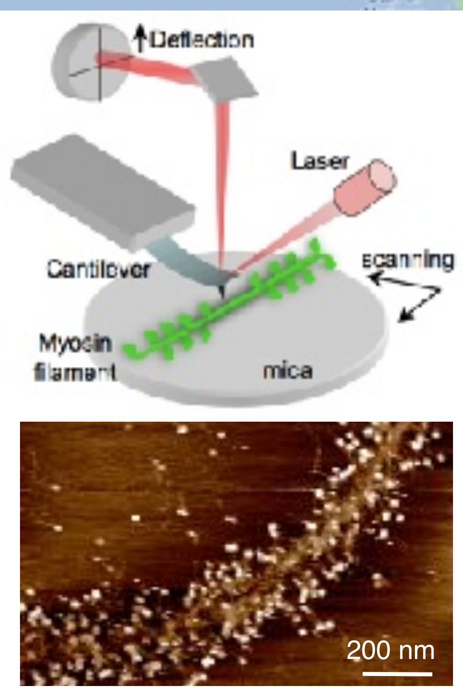


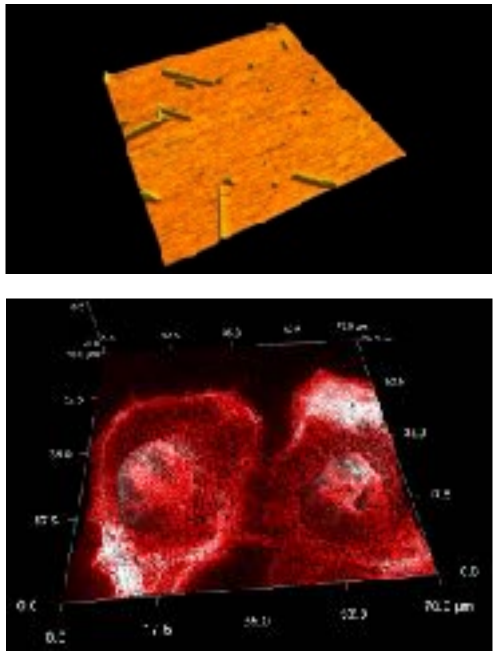
Catalyze your community

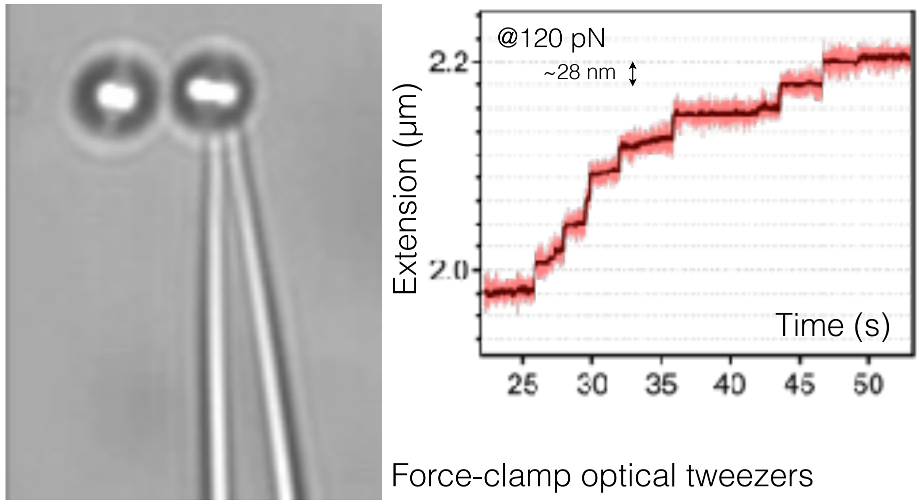
Nanobiotechnology and single-molecule biophysics











Force-clamp optical tweezers

Dare to lead

Semmelweis University, Department of Biophysics and Radiation Biology, Faculty of Medicine

Gyula Koczkás
(1948–1950)

Imre Tarján
(1950–1982)

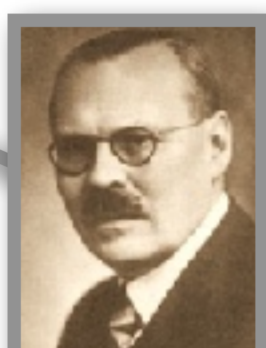
Györgyi Rontó
(1982-1999)

Judit Fidy
(1999-2008)

2010

2018

Albert Szent-Györgyi



István Rybár

Károly Tangl

Loránd Eötvös

Be motivated, and motivate

In pursuit of the scientific truth

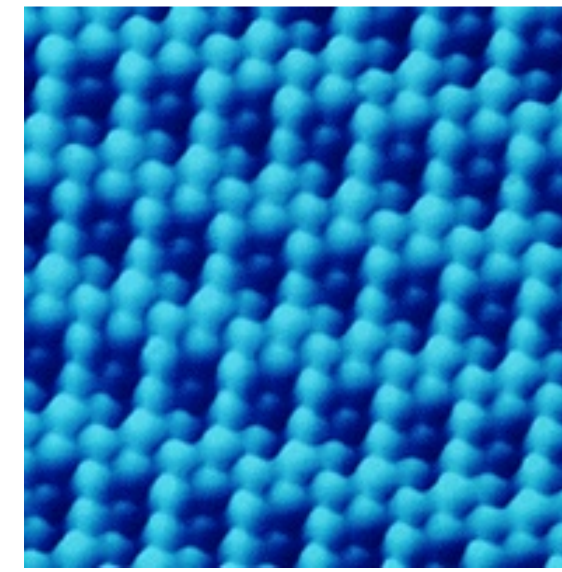
- **Truth**
  Ontologic truth (well tested statement, fact; BUT must not forget about logical and ethical truth!)

- **Is scientific truth constant?**
  May hypotheses become facts?

- **Knowledge and belief?**
  Knowledge rests on belief.

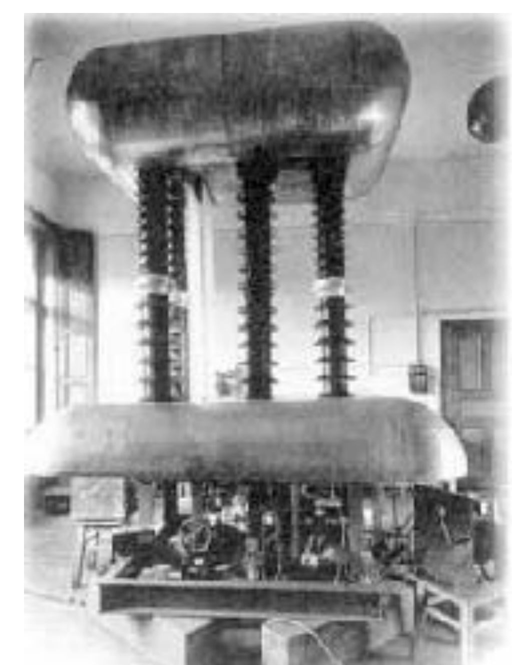
  Do you believe in our knowledge so much that you bet your life on it?

John Dalton (1766-1844)

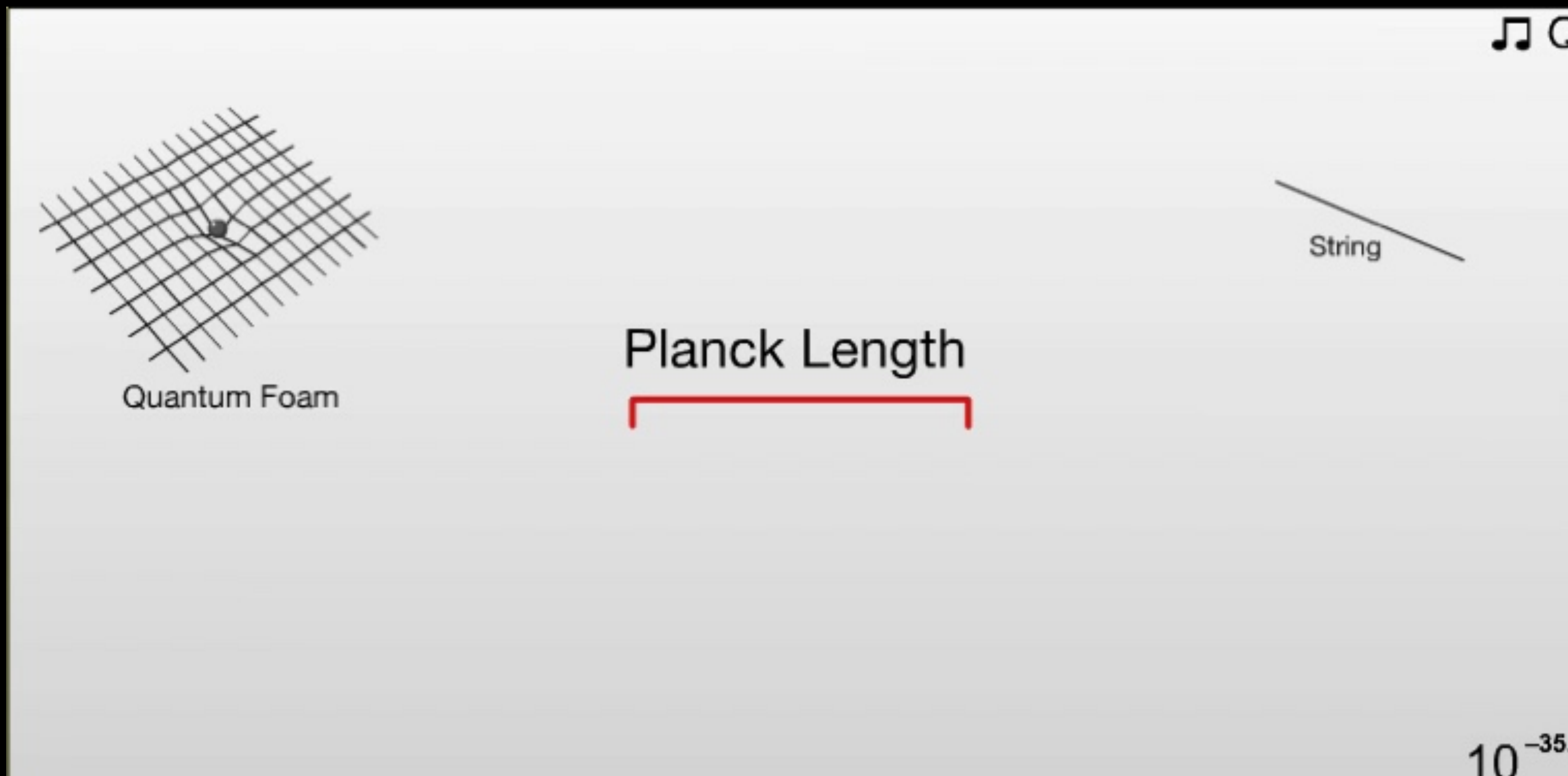


Oxygen atoms on rhodium single crystal

Károly Simonyi (1916-2001)



Van de Graaff particle accelerator (Sopron, 1951)



Cary and Michael Huang (http://htwins.net)