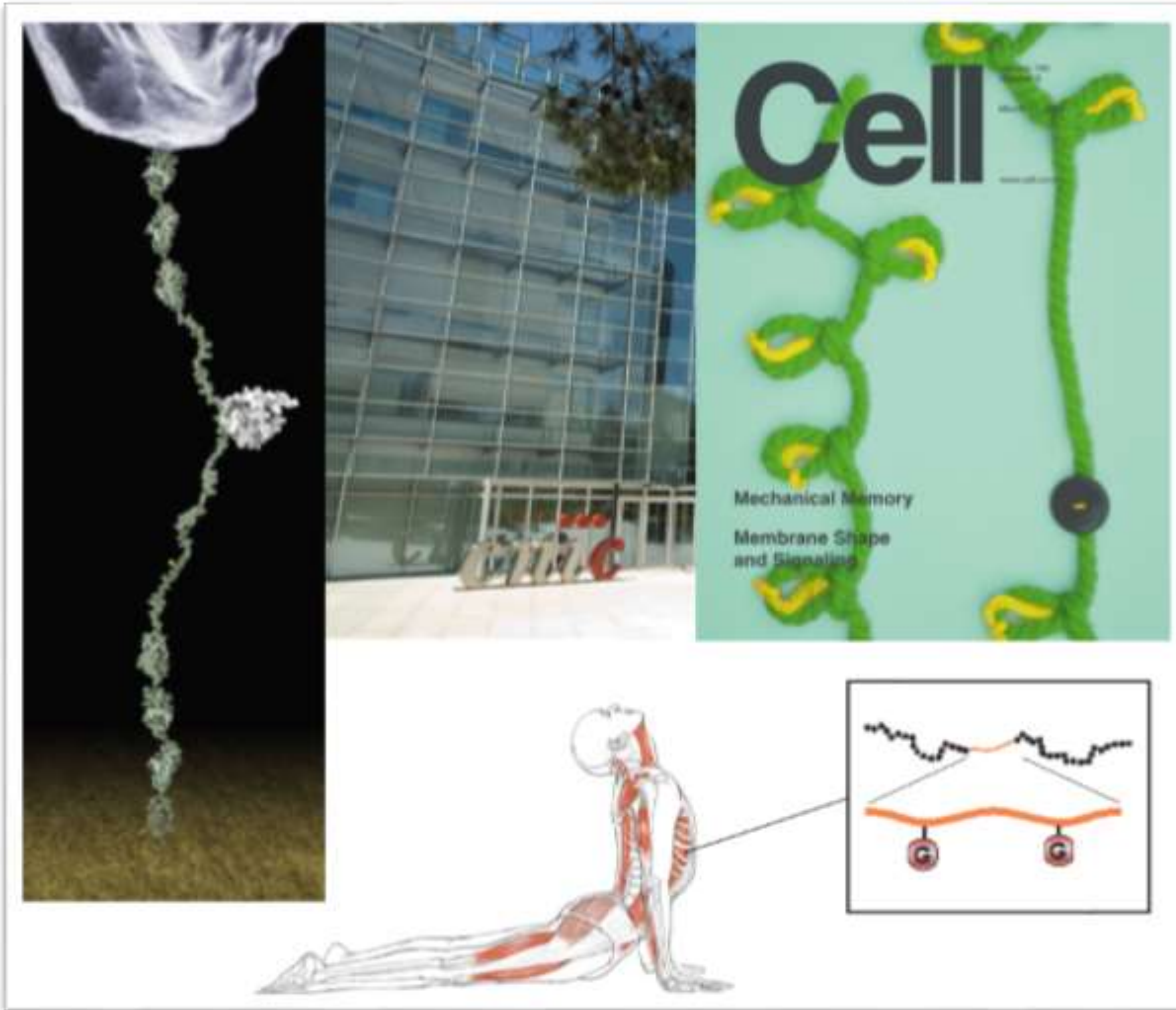
Mechanobiochemistry

Jorge Alegre-Cebollada

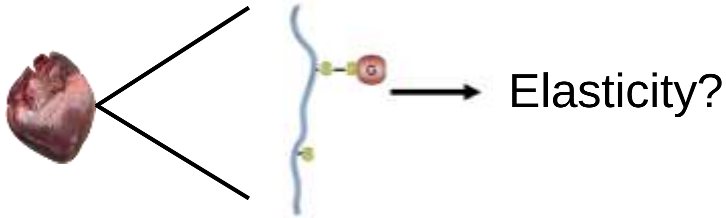


EXCELENCIA
SEVERO
OCHOA

cnic
Fundación
Centro Nacional de
Investigaciones
Cardiovasculares
Carlos III

Single-molecule Mechanobiochemistry at CNIC
Bridging Molecular Mechanobiology and Cardiac Disease

i. How do posttranslational modifications (PTMs) regulate elasticity *in vivo*?



Elías Herrero

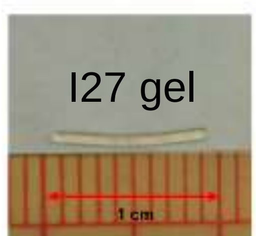
Cristina Sánchez

ii. Measure the mechanical properties of mutant proteins causing cardiomyopathy

Mechanical phenotype?

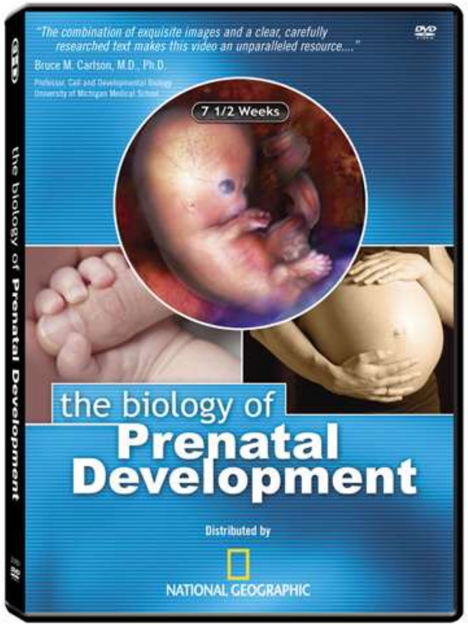
Carmen Suay

iii. Production of biomaterials whose mechanical properties are regulated by physiological cues

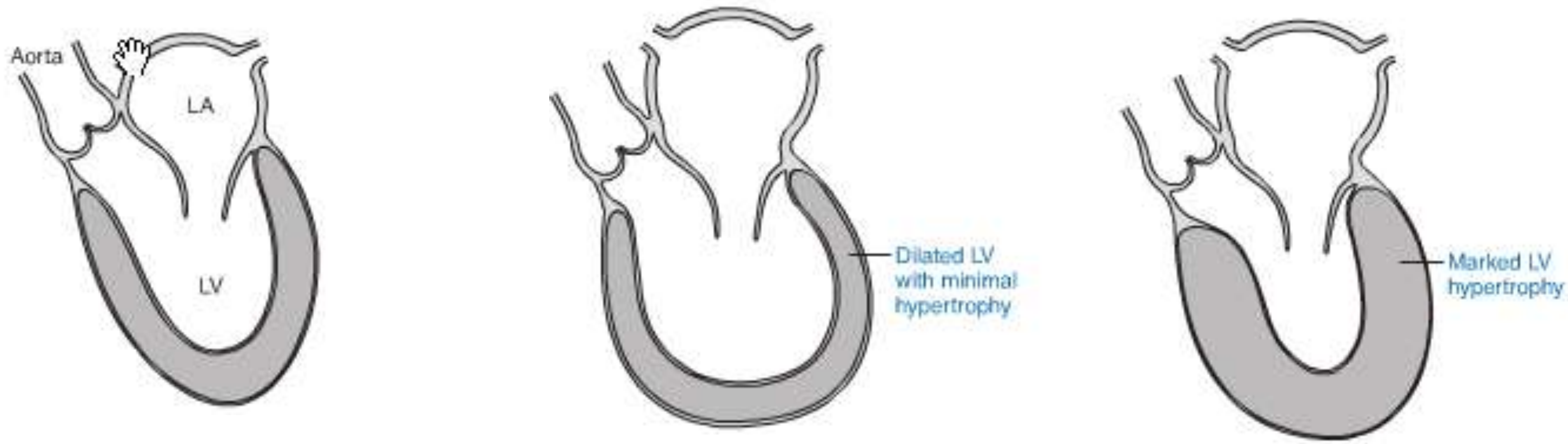


Carla Huerta

The elasticity of the myocardium is key to an efficient heart



The mechanics of the myocardium is defective in cardiomyopathies



Dilated cardiomyopathy (DCM)

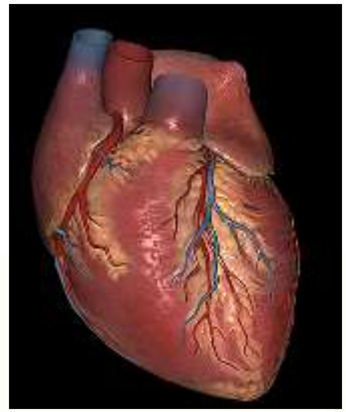
Defective contraction:
impaired systole

Hypertrophic cardiomyopathy (HCM)

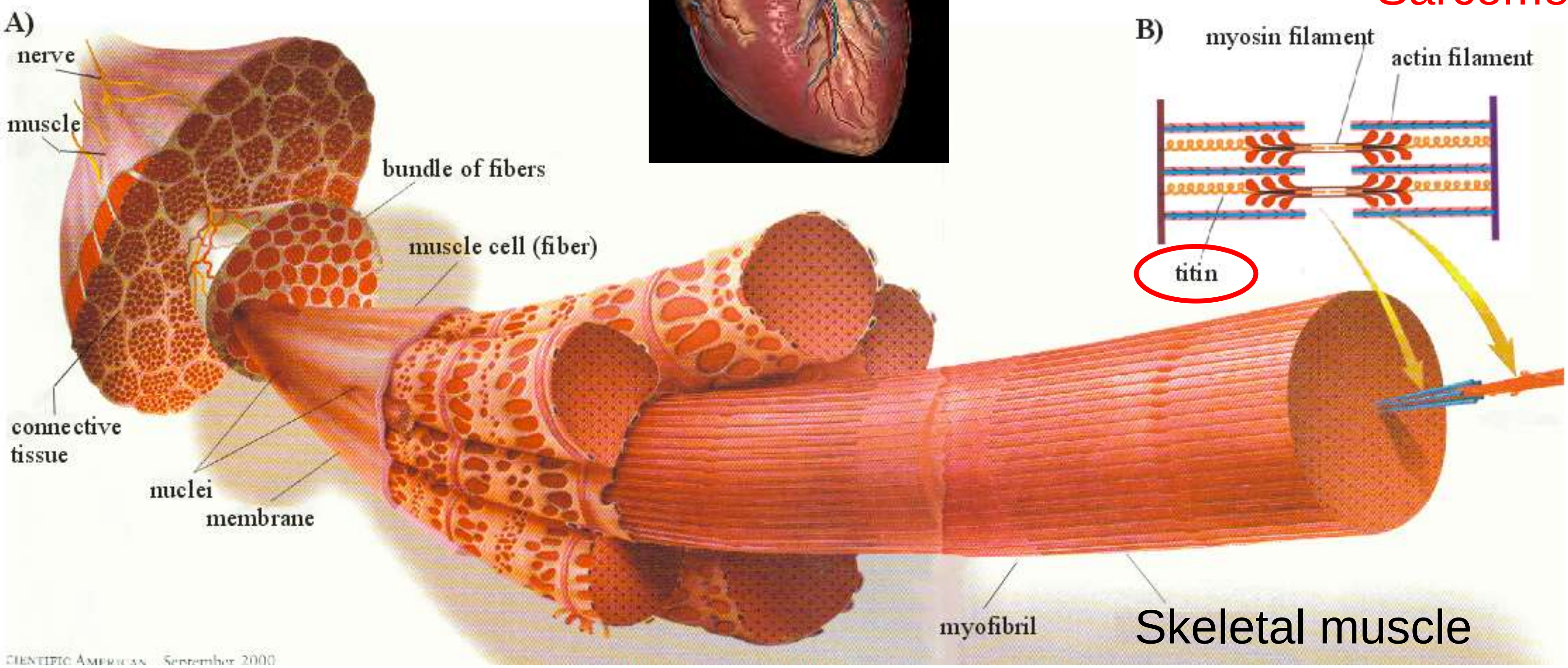
Defective relaxation:
impaired diastole

From "Pathophysiology of Heart Disease", 5th Edition, Ed. Leonard S. Lilly

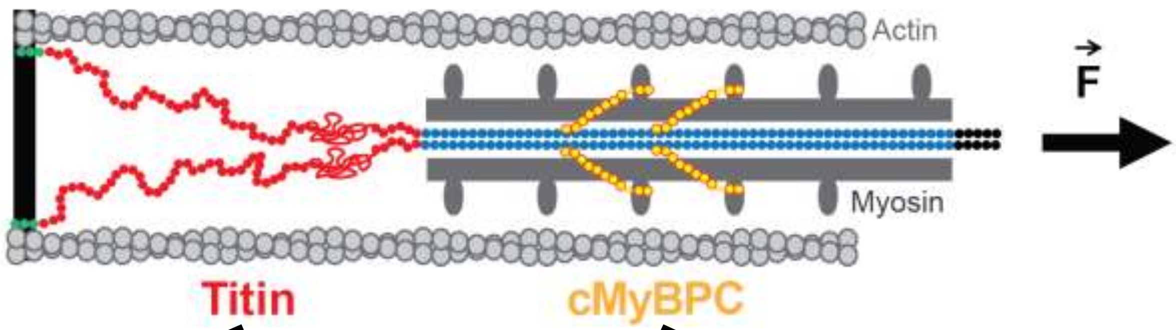
The sarcomere is the functional unit of striated muscle



Cardiac muscle

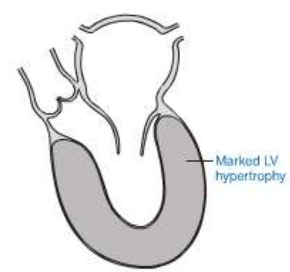


Mutations in structural proteins lead to familial cardiomyopathy



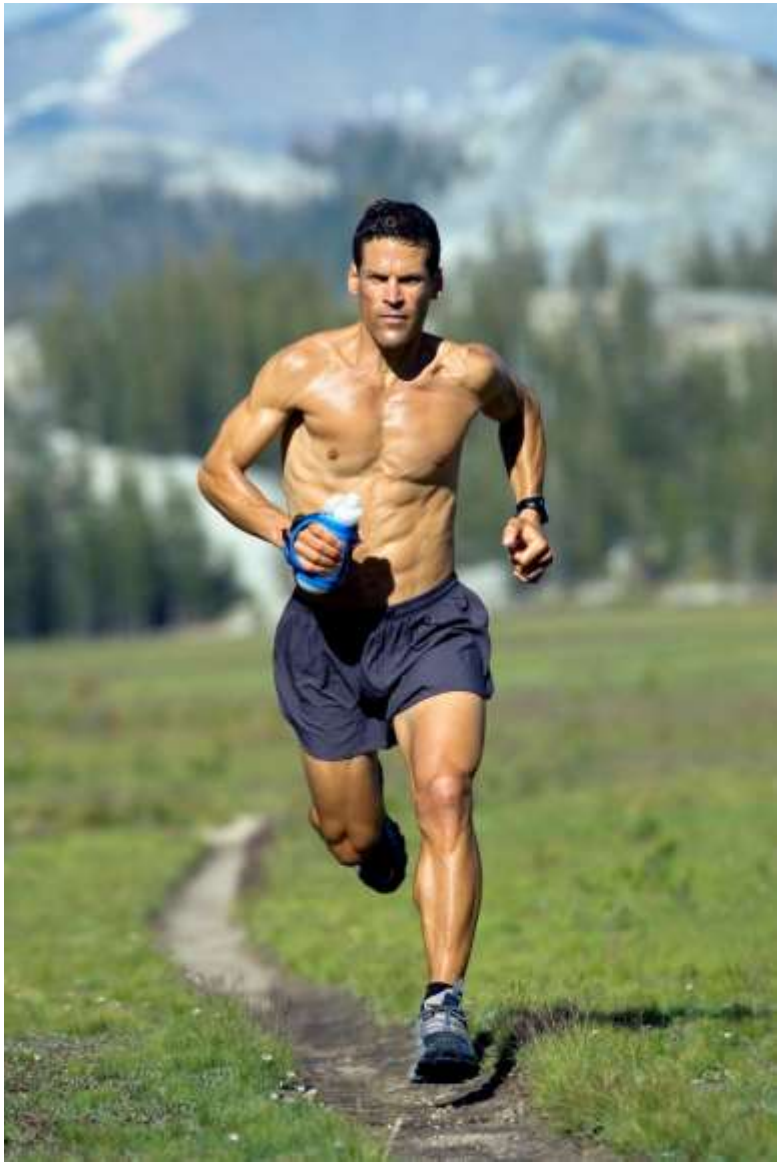
Dilated cardiomyopathy
(~ 25% of cases)

Hypertrophic cardiomyopathy
(~ 35% of cases)

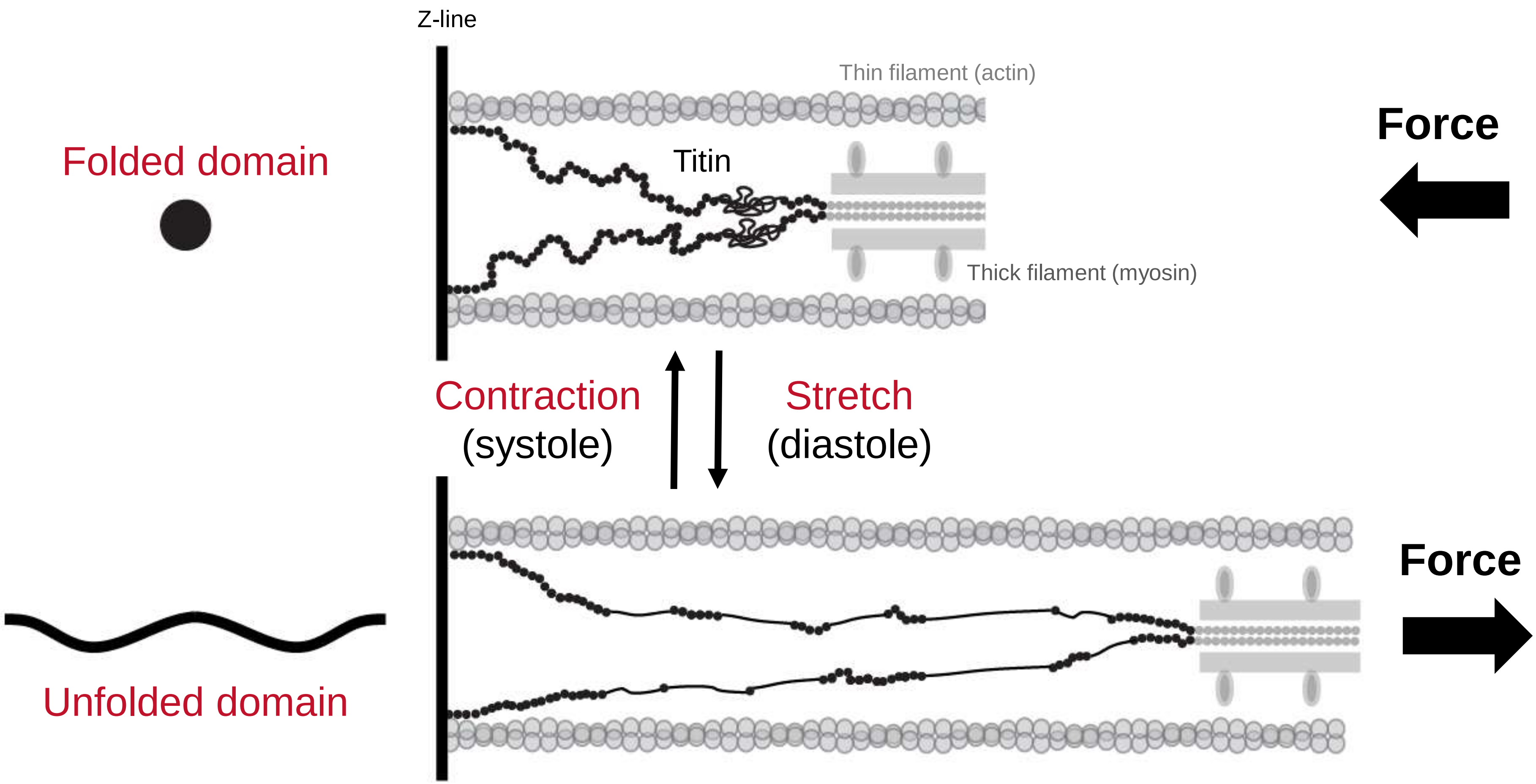


Genotype to phenotye?

The elasticity of striated muscle can be modulated



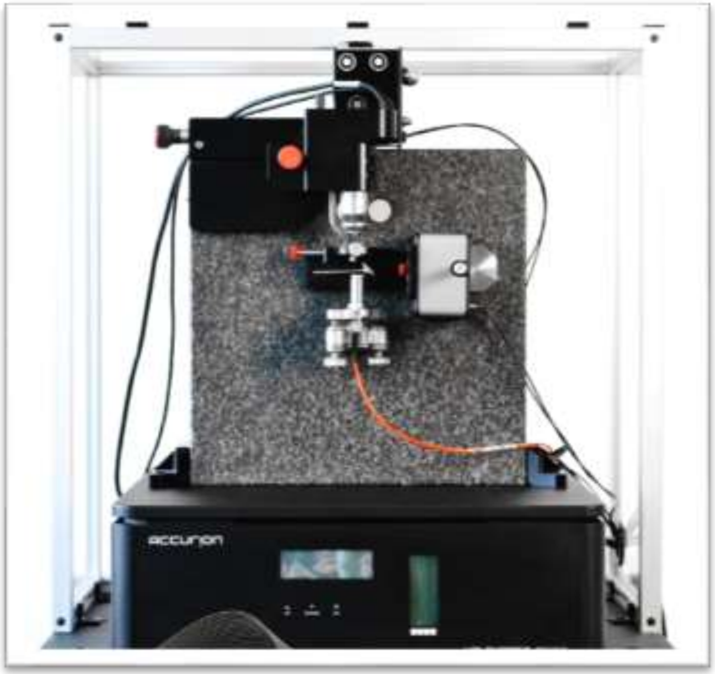
Protein elasticity is determined by protein unfolding/refolding

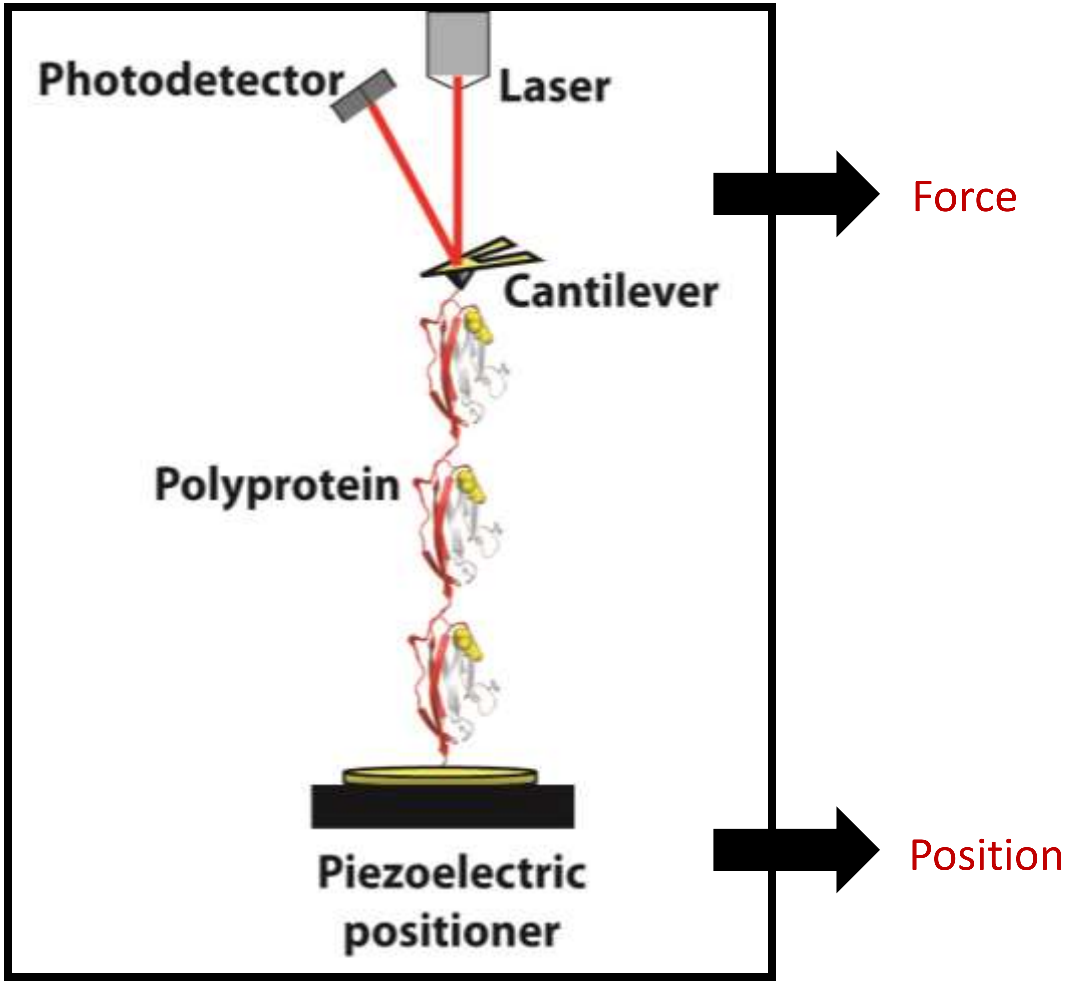


Novel technology

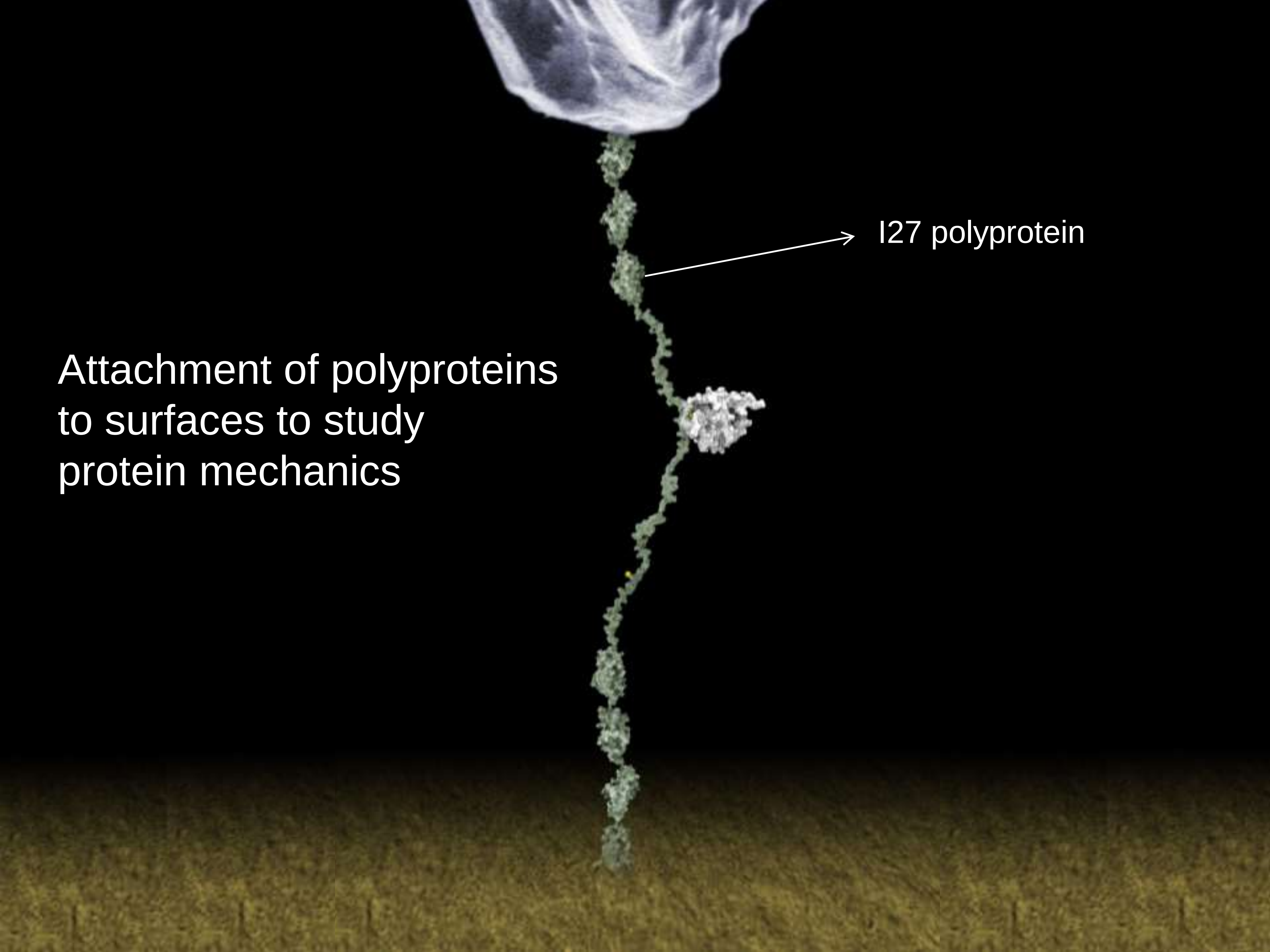
Single-molecule Atomic Force Microscopy (AFM)

Atomic Force Microscope

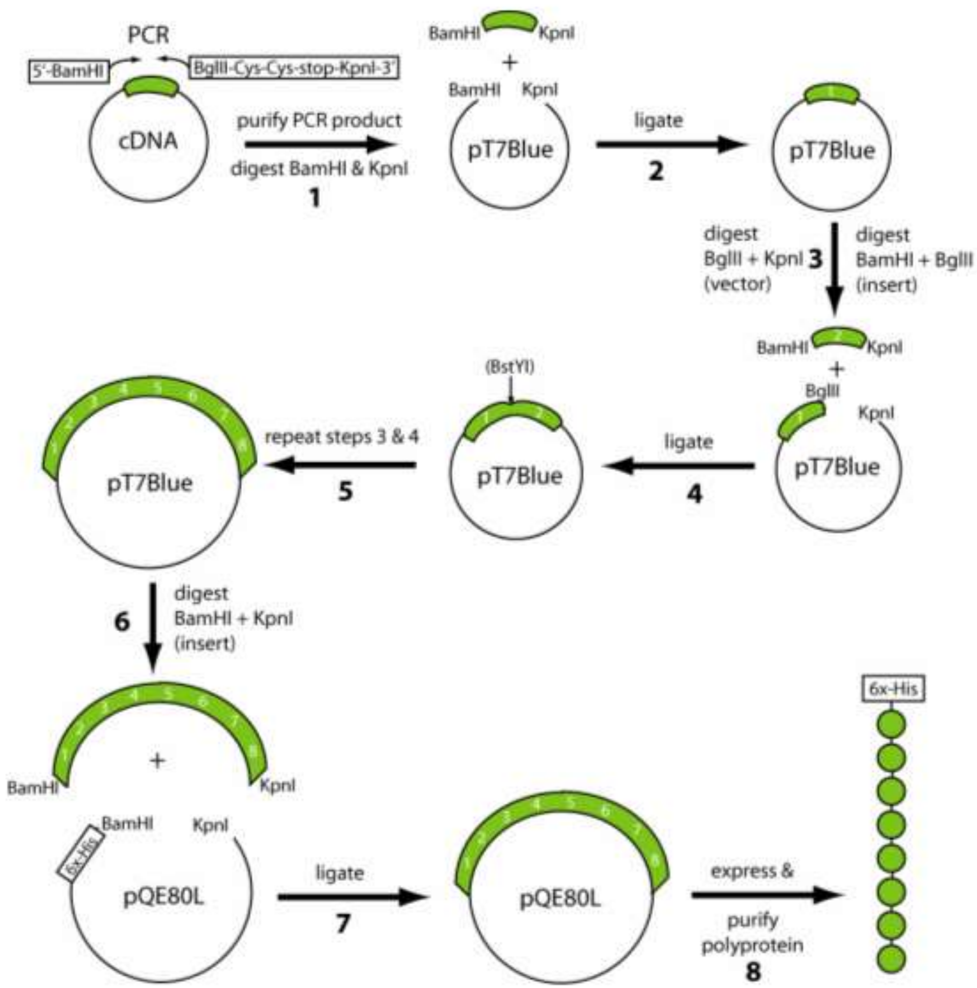


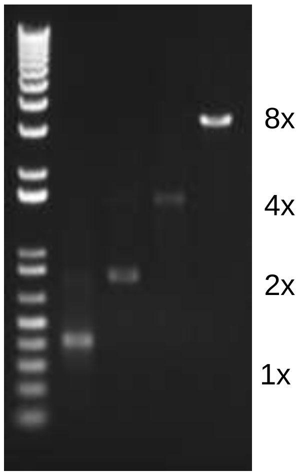


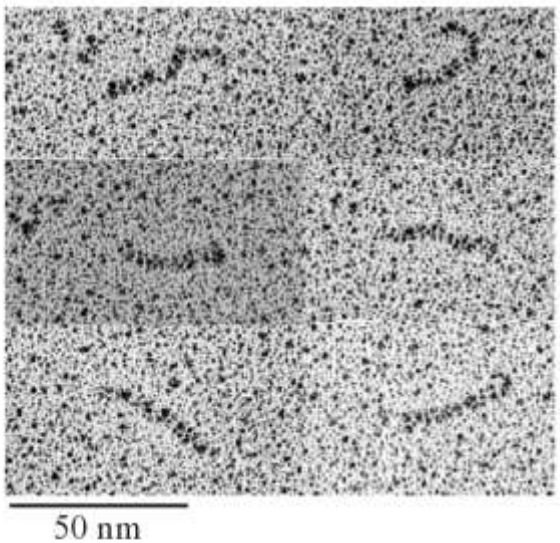
Popa*, Kosuri*, Alegre-Cebollada, *et al.* **Nat Prot**, 8, 1261 (2013)


Attachment of polyproteins to surfaces to study protein mechanics
I27 polyprotein

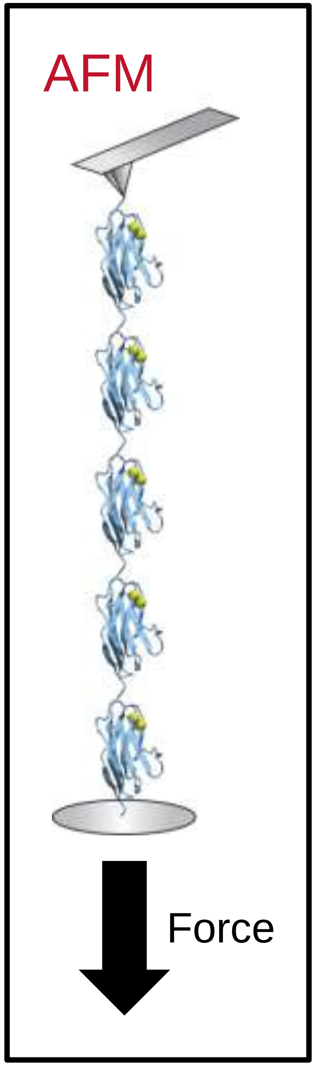
Polyprotein engineering for force spectroscopy

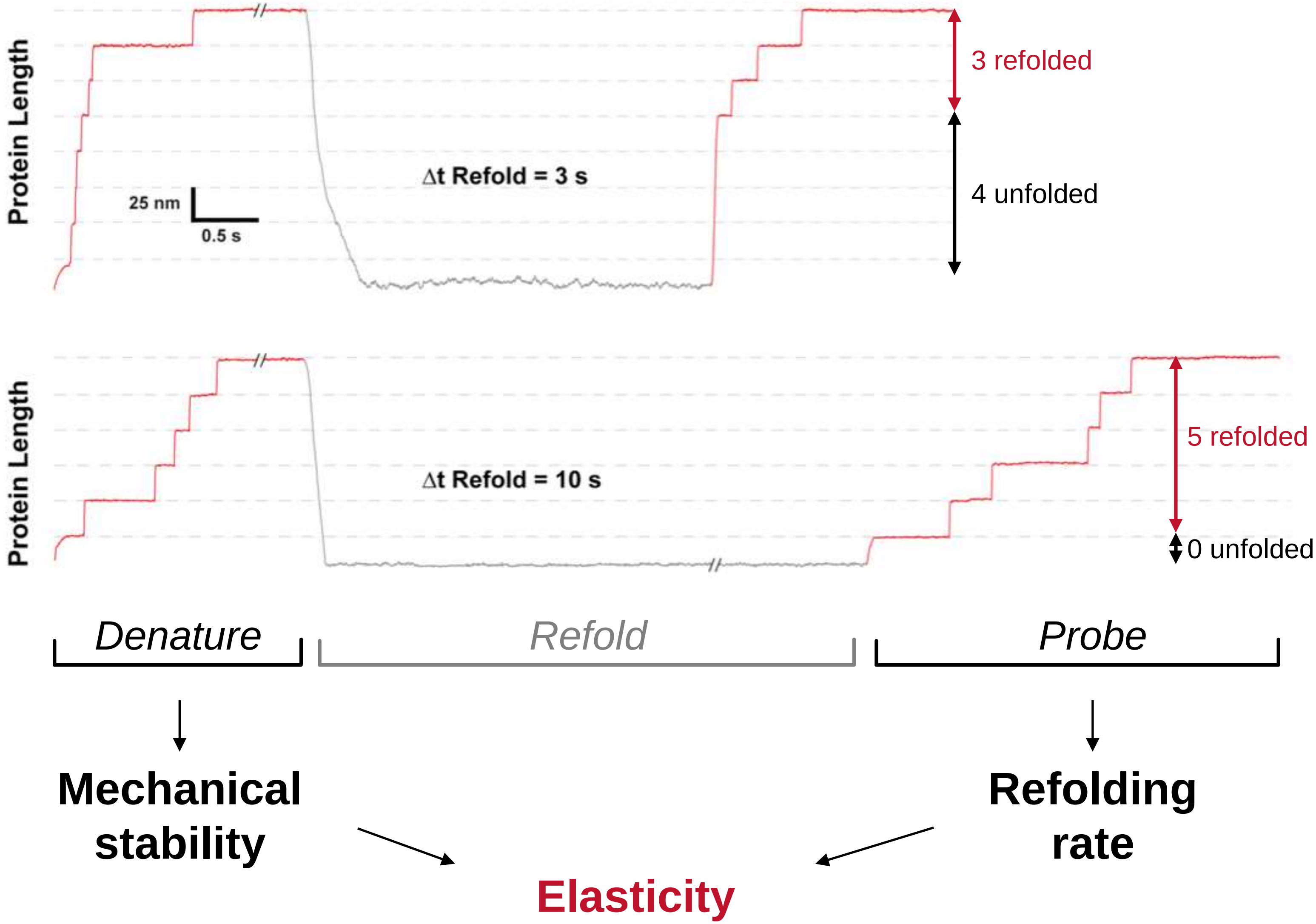




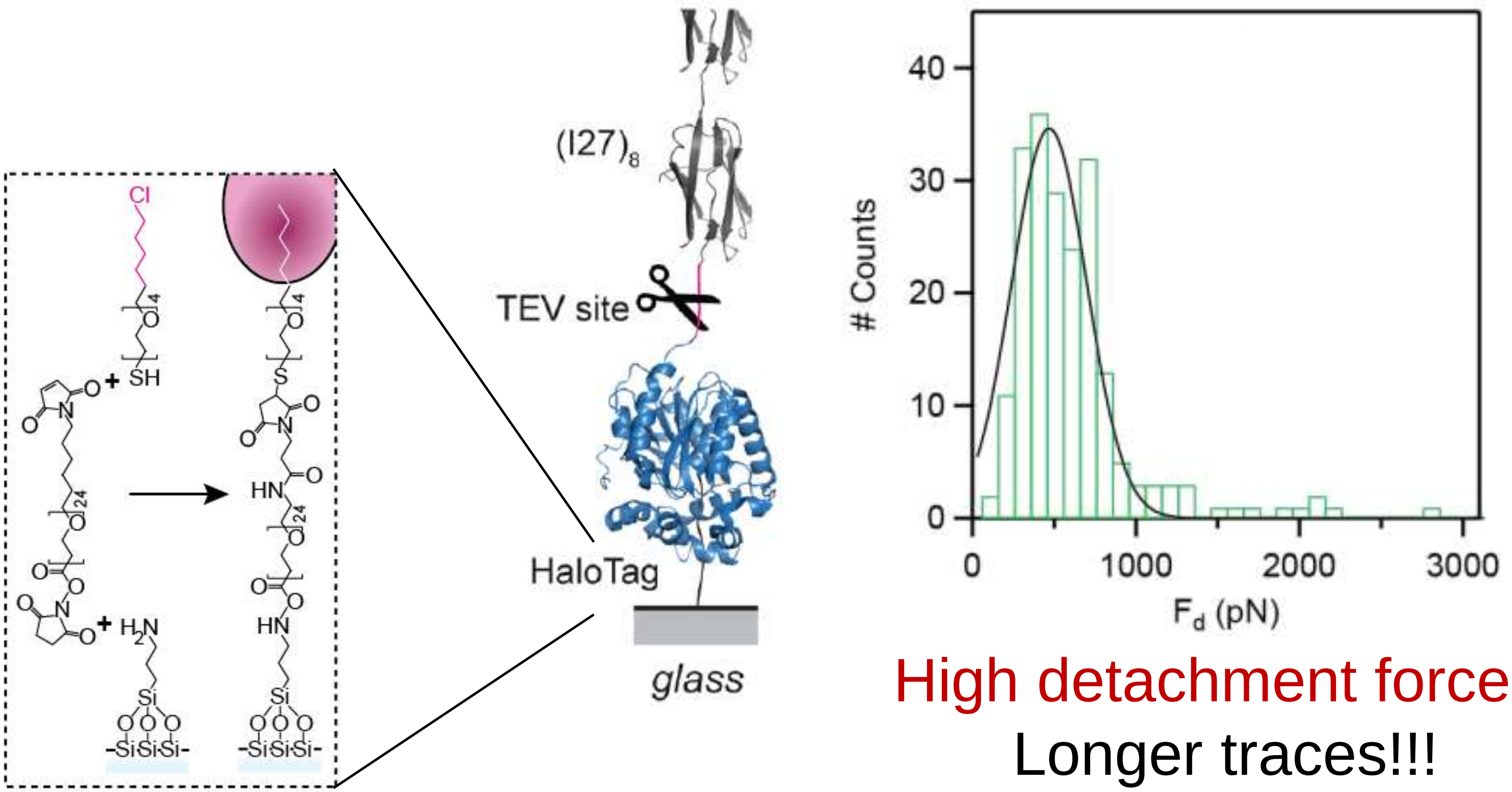


Single-molecule AFM
Mechanical unfolding and refolding





Covalent anchoring of polyproteins using HaloTag chemistry



High detachment forces:
Longer traces!!!

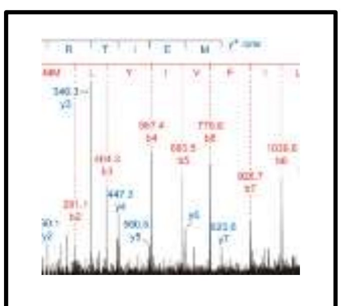
Popa, Berkovich, Alegre-Cebollada et *al.* **JACS** 135, 12762 (2013)

Experimental objectives (i)

How do redox posttranslational modifications regulate elasticity?

Native tissue

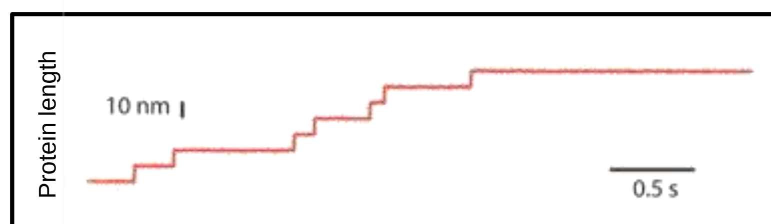
LC-MS/MS



Posttranslational modifications

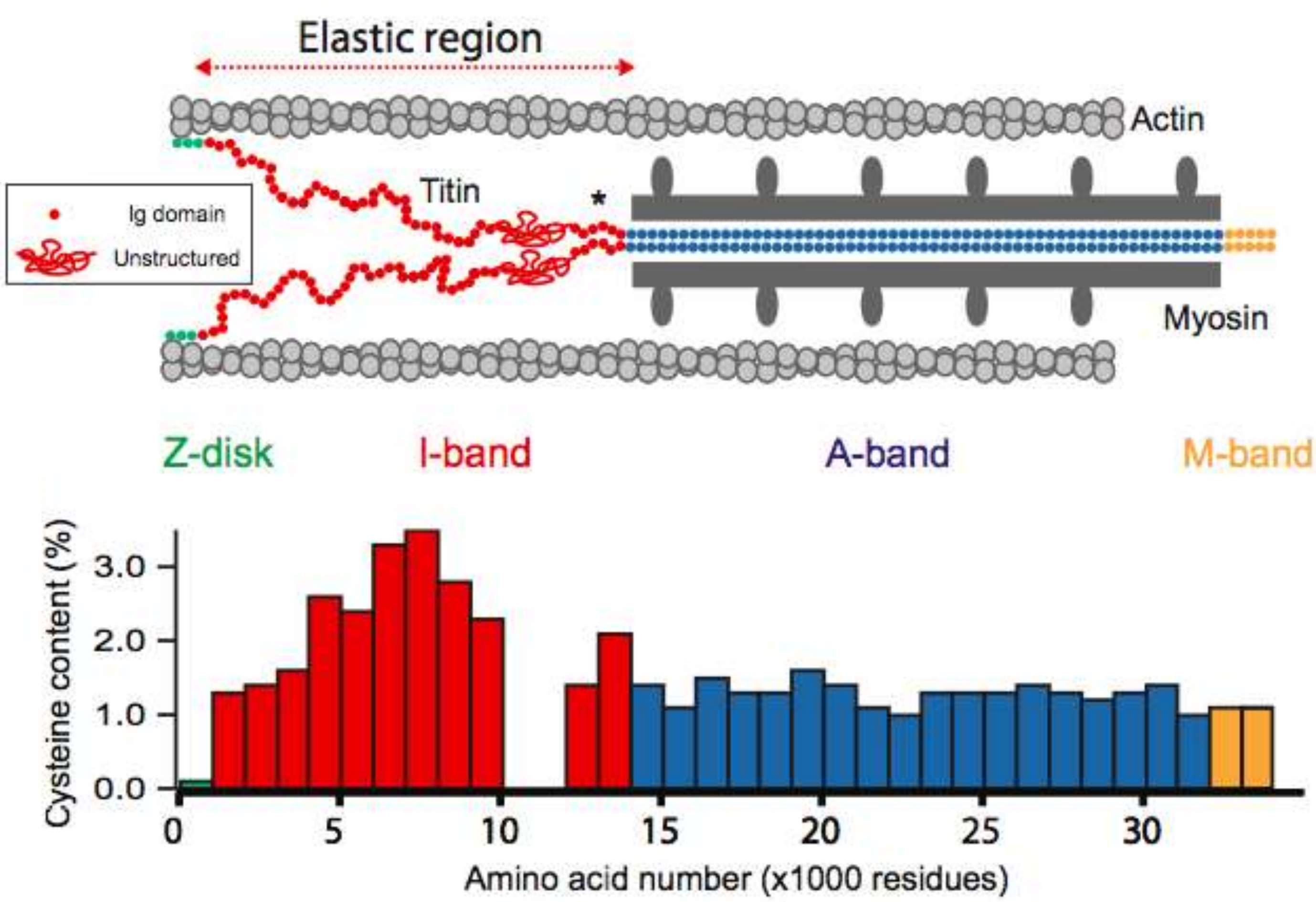
Recombinant polyprotein

AFM



Mechanical properties

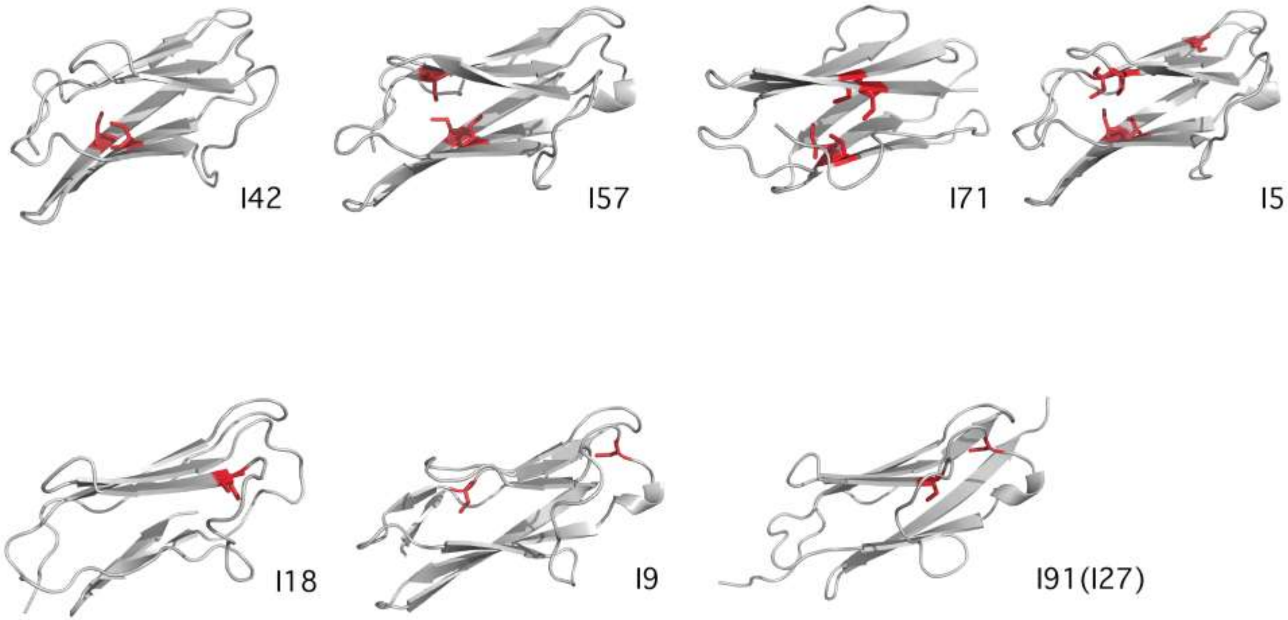
Thiol chemistry controlling titin elasticity



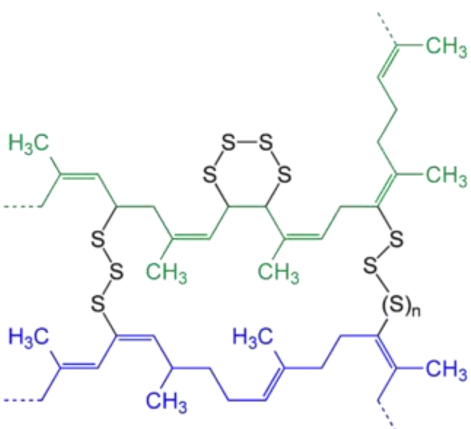
Titin's buried (cryptic) cysteines

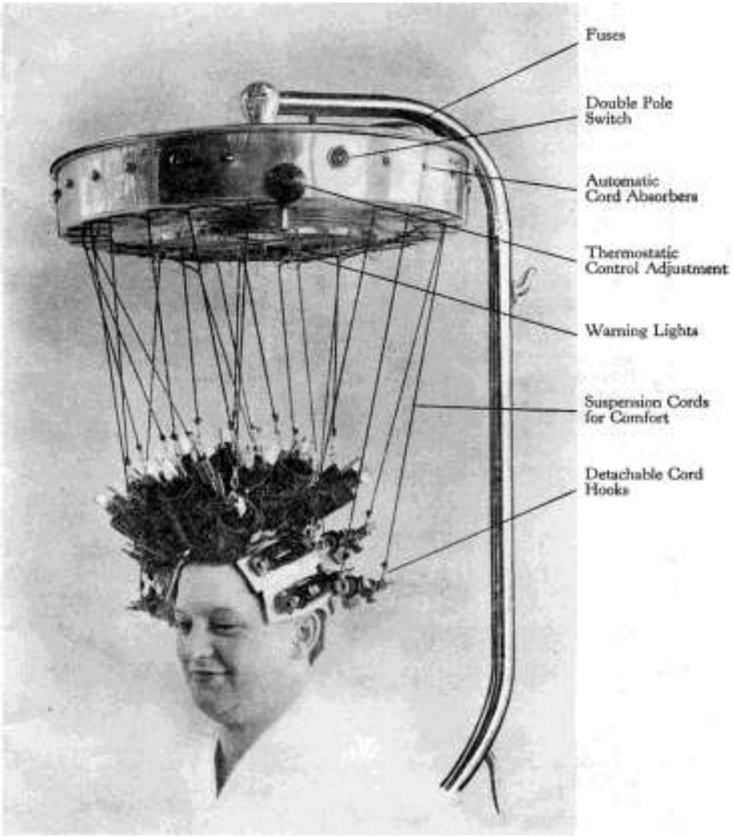
Paired

Unpaired



Disulfide bonds as regulators of polymer elasticity

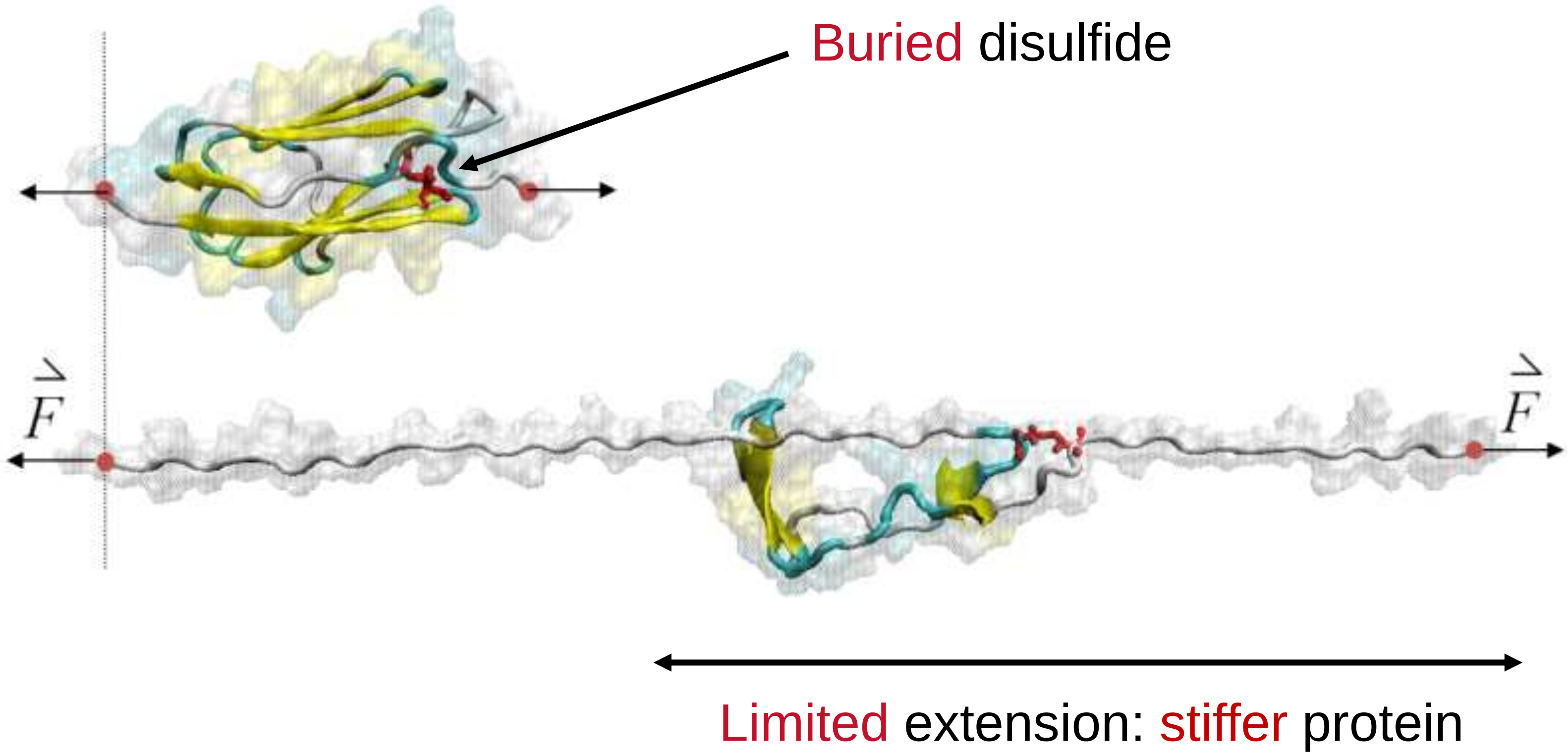




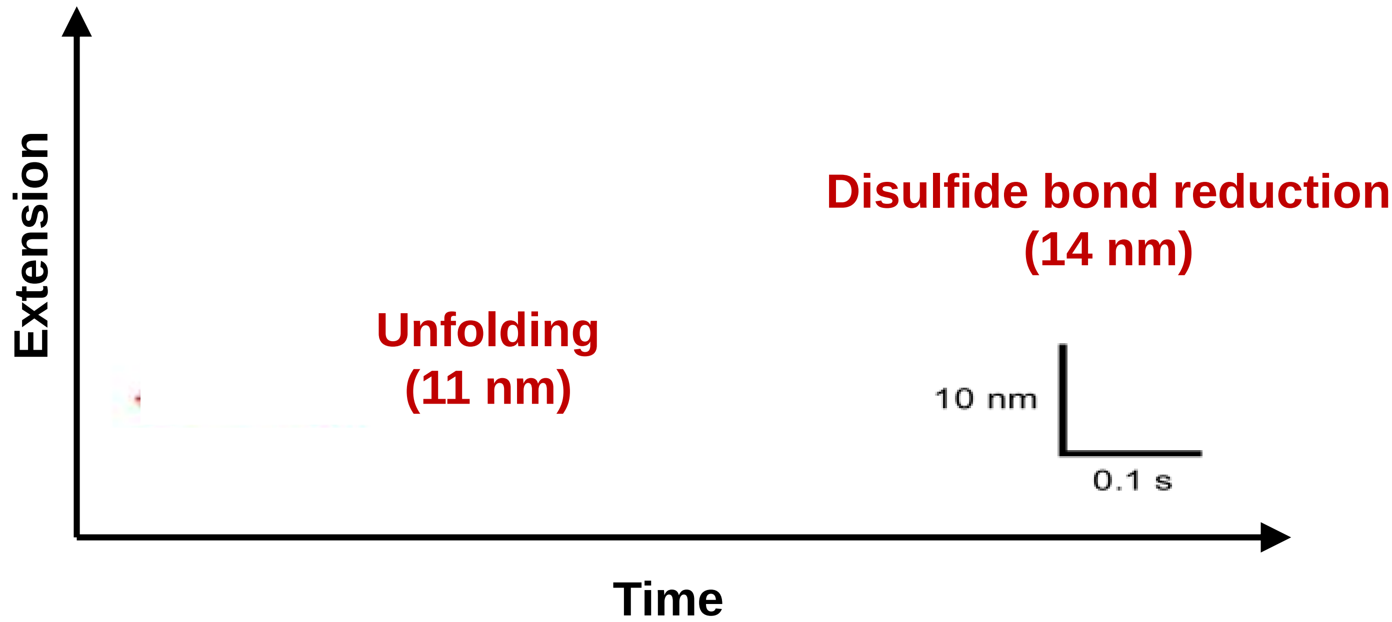
Vulcanization

Perm (1930’s)

Disulfide bonds as regulators of protein elasticity



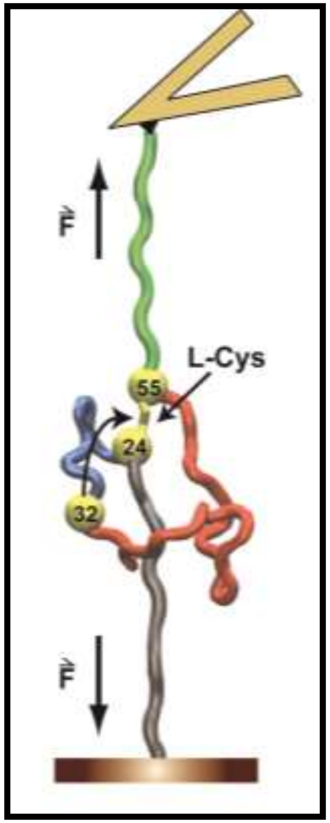
Detection of cryptic disulfides in titin Ig domains

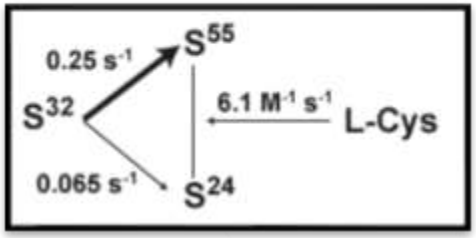


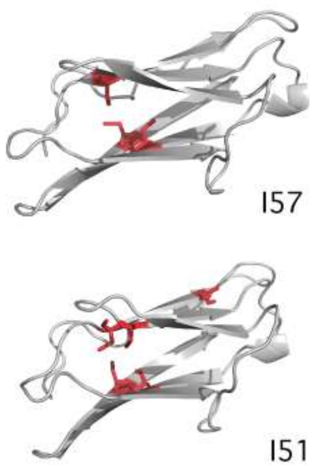
Wiita *et al*. **PNAS** 103, 7222 (2006)
Kosuri, Alegre-Cebollada *et al*. **Cell**, 151, 794 (2012)

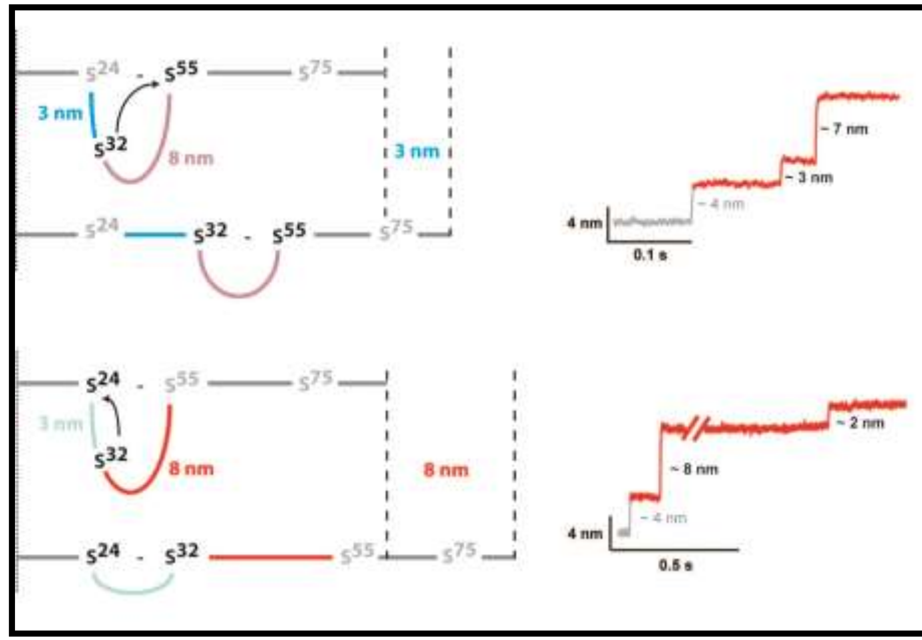
We pull from an Ig domain of cardiac titin.

Direct observation of disulfide isomerization









Alegre-Cebollada *et al*. **Nature Chemistry** 3, 882 (2011)
Solsona *et al*. **J Biol Chem**, 289, 26722 (2014)

What about unpaired cysteines?
S-glutathionylation of titin

Xanthine oxidase
NAD(P)H oxidase

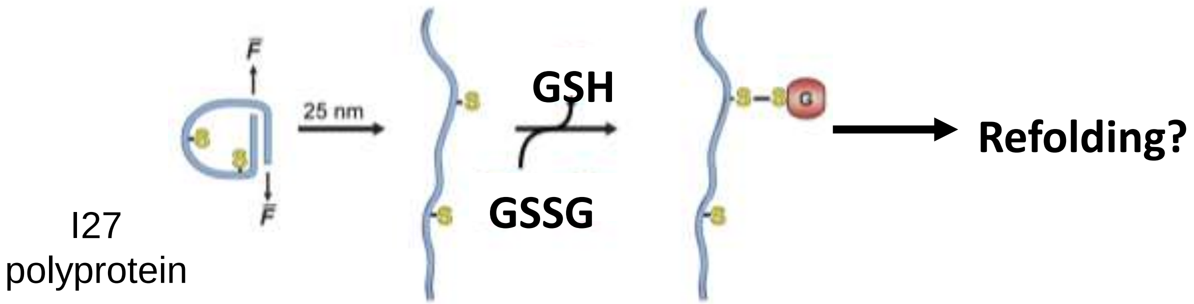
ROS (superoxide, H_2O_2)

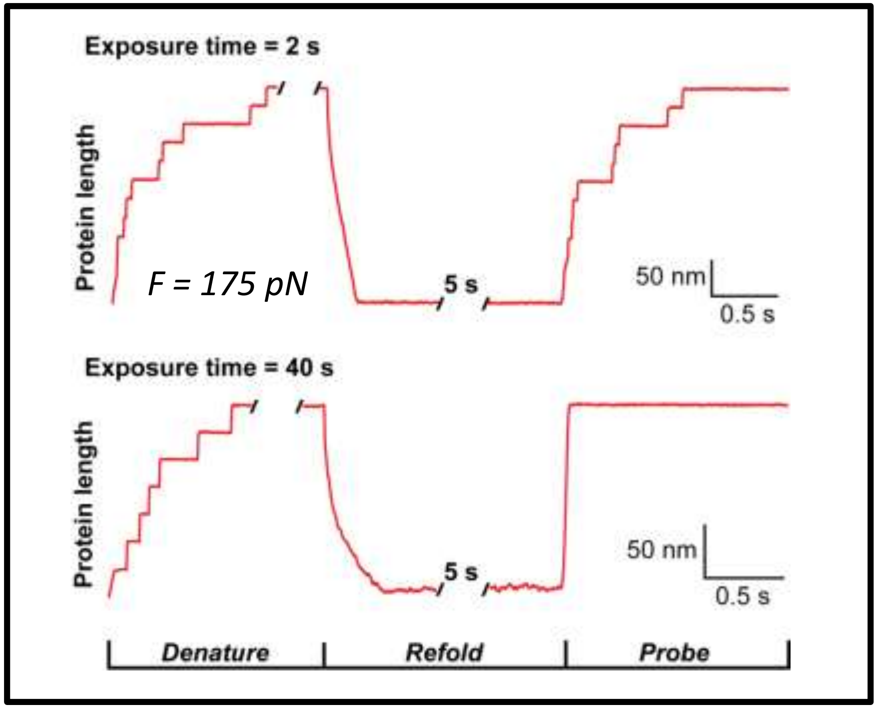
Oxidative modification of cysteines

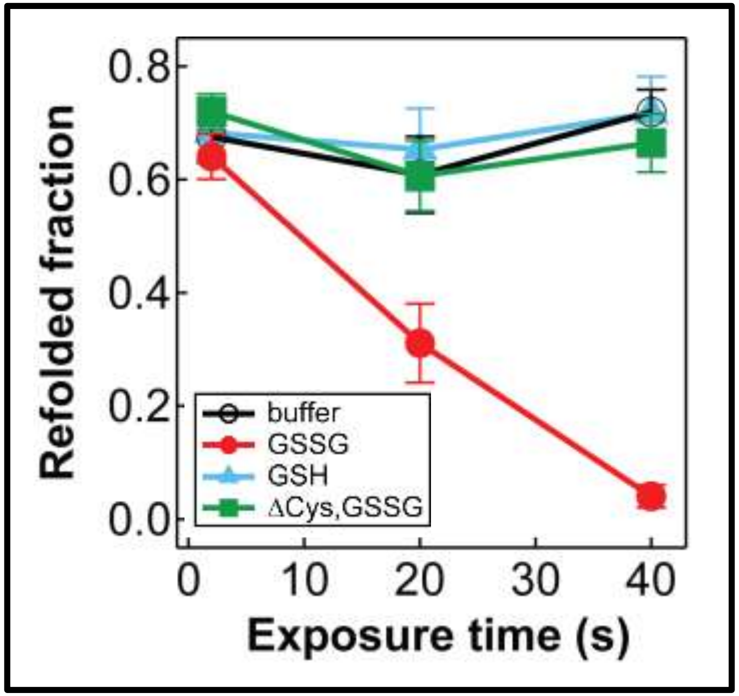
NO

Nitric oxide synthase

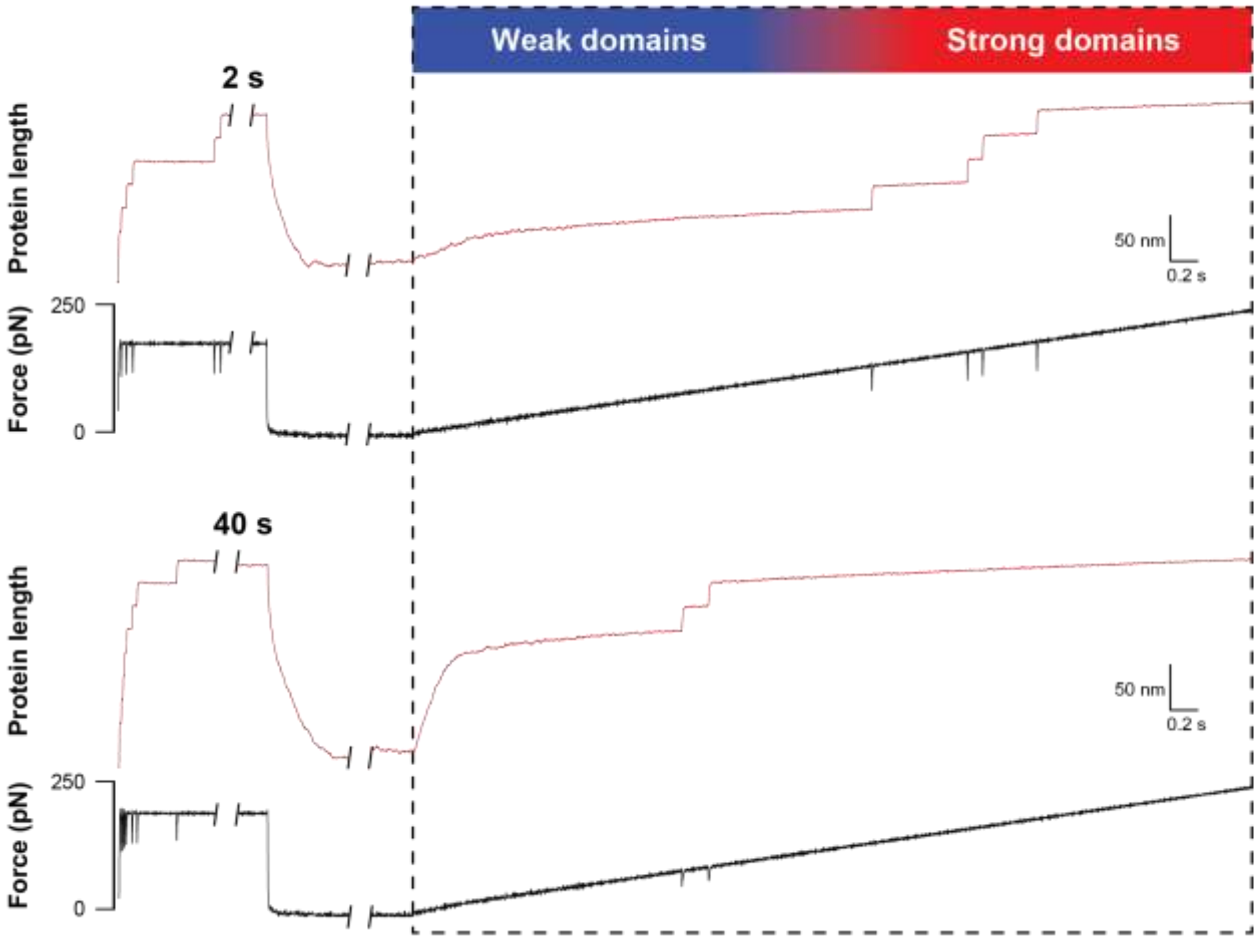
S-glutathionylation inhibits protein folding





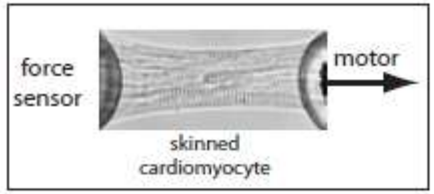


S-Glutathionylation decreases mechanical stability

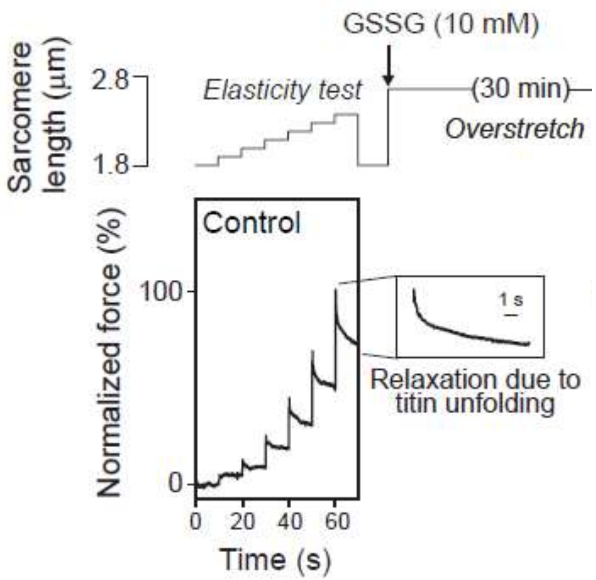


Inhibition of folding + weakening
↓
Increased elasticity

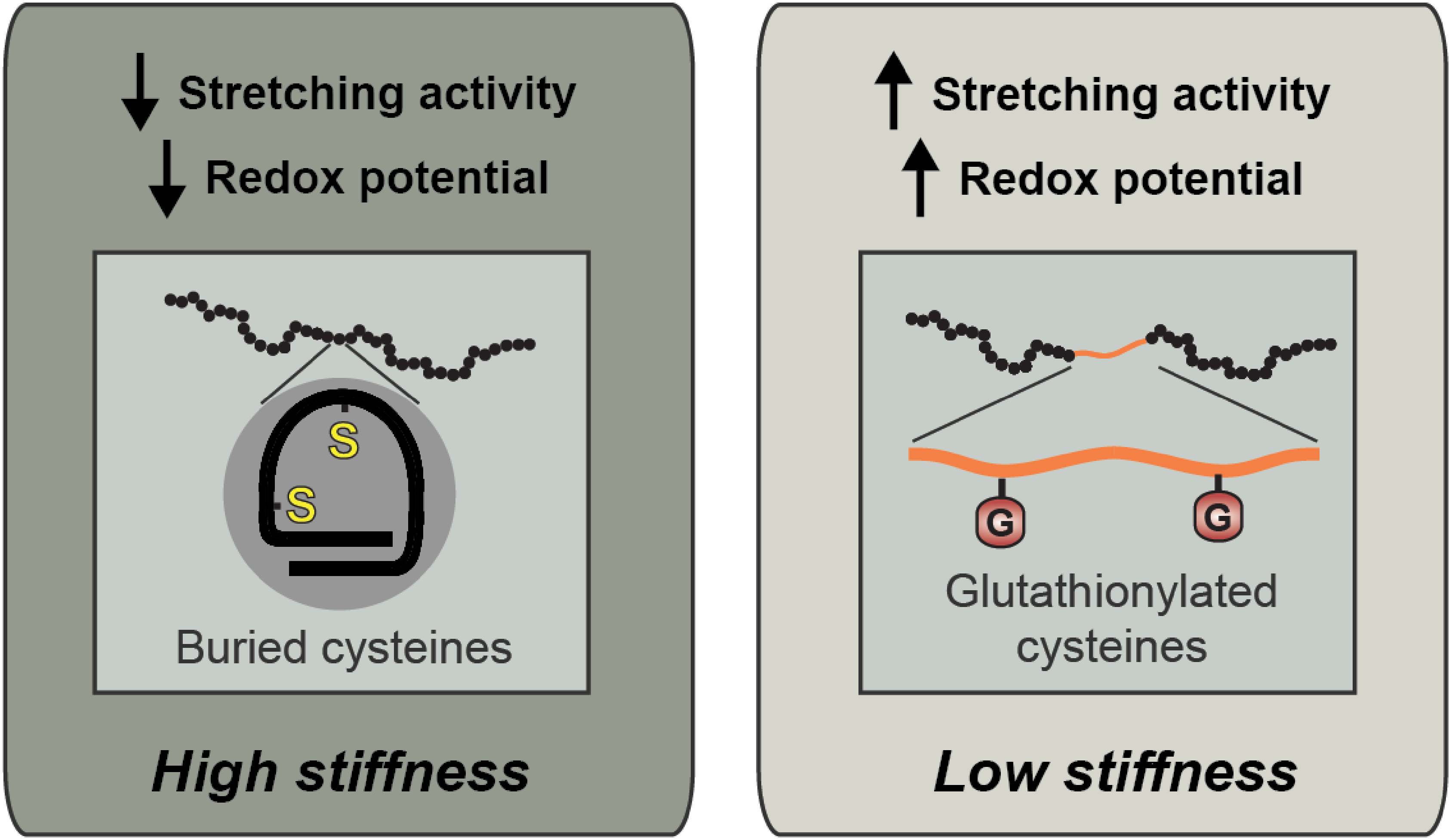
The elasticity of cardiomyocytes is modulated by S-glutathionylation of titin's cryptic cysteines



In collaboration with Nazha Hamdani and Wolfgang Linke (Bochum University, Germany)



Molecular yoga: a novel role for buried residues in proteins



Alegre-Cebollada*, Kosuri* *et al.* **Cell**,156, 1235 (2014)

Mass spectrometry to determine posttranslational modifications

Native tissue homogenization in **denaturing** conditions

Reduced: -SH
Oxidized: -SR

Protein extraction in N-ethylmaleimide (NEM)

Red: -SNEM
Ox: -SR

Band slicing

DTT + Iodoacetamide (IAM)

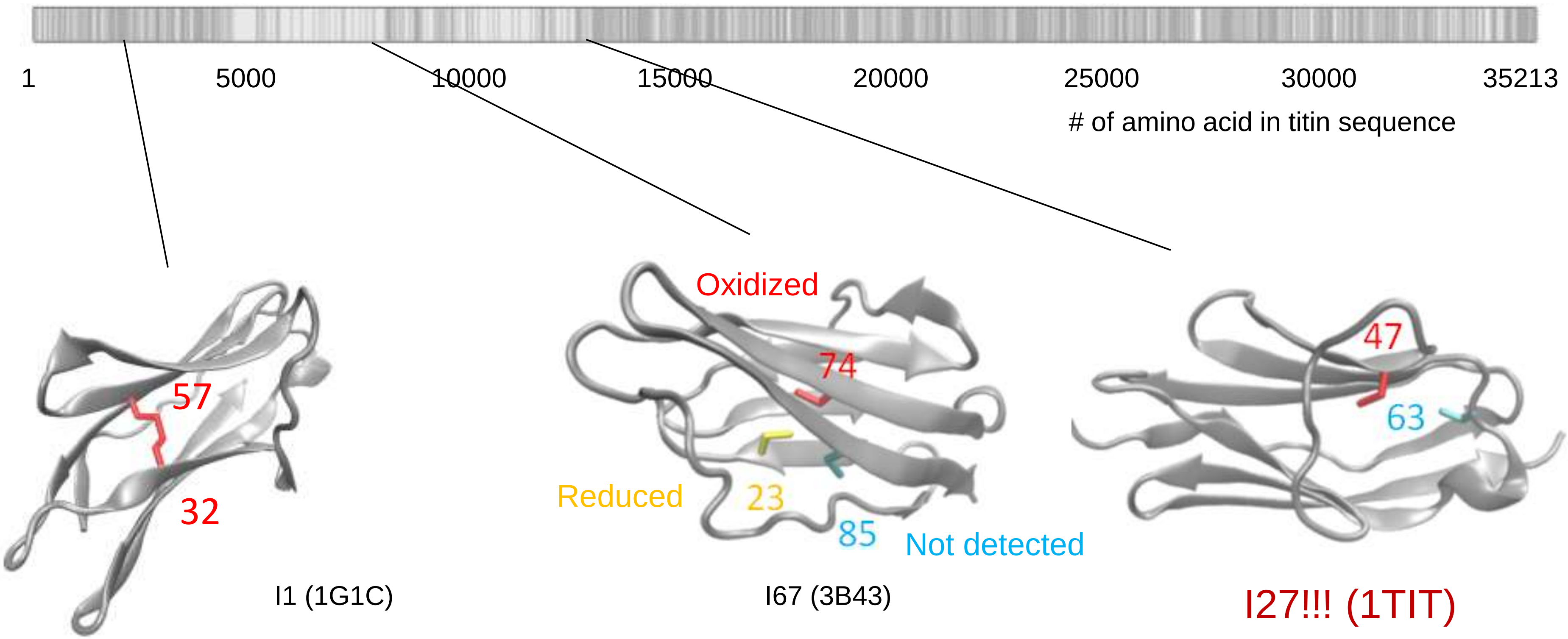
Automatic peptide identification

Red: -SNEM
Ox: -SIAM

In collaboration with Jesús Vázquez (Cardiovascular Proteomics group, CNIC)

Preliminary results
Posttranslational modifications of buried cysteines in titin

Over 1500 identified peptides (70% coverage)

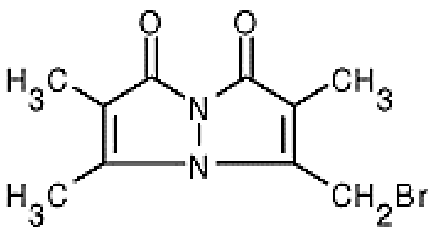


Disulfide bonds

Other redox modifications

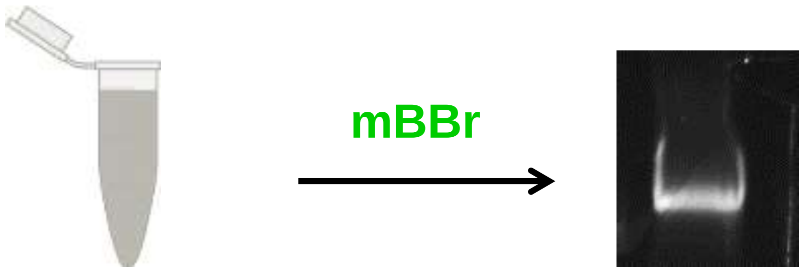
- S-glutathionylation
- S-nitrosylation
- Sulfenylation

In-gel detection of reduced thiols



Monobromobimane
(**mBBr**)

R-SH + mBBr →
Fluorescent derivative



Reduced: -SH
Oxidized: -SR

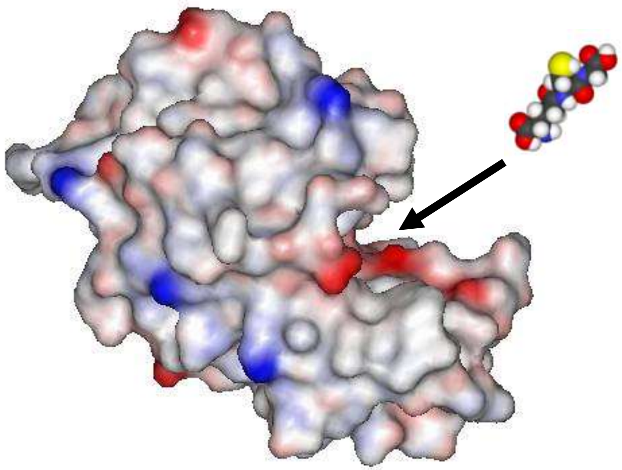
Red: -SmBBr
Oxidized: -SR

Experimental objectives (ii)
Mechanical properties of mutant proteins causing cardiomyopathy

Diagnostic reasons

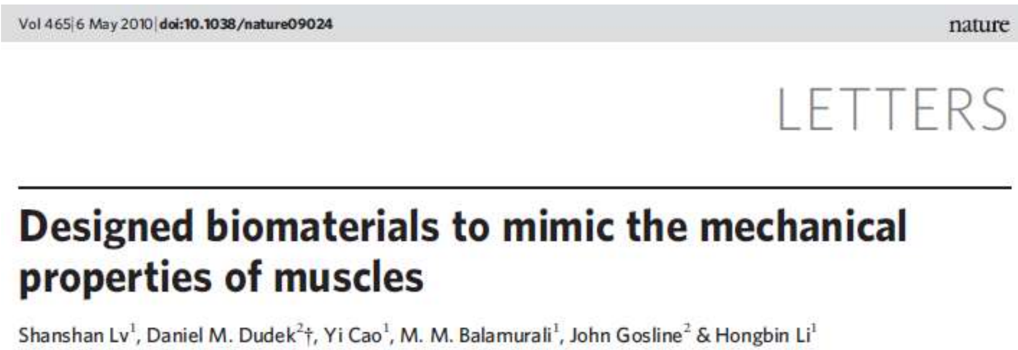
SNP or pathogenic mutation?

Therapeutic reasons



Drugs that restore healthy phenotype

Experimental objectives (iii)
Biomaterials that mimic the elasticity of muscle

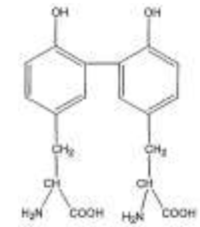

Vol 465 | 6 May 2010 | doi:10.1038/nature09024 nature

LETTERS

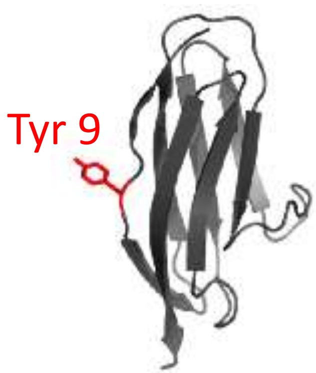
Designed biomaterials to mimic the mechanical properties of muscles

Shanshan Lv[1], Daniel M. Dudek[2]†, Yi Cao[1], M. M. Balamurali[1], John Gosline[2] & Hongbin Li[1]

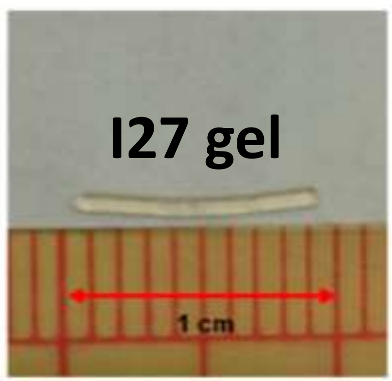
Young's modulus ~100 KPa

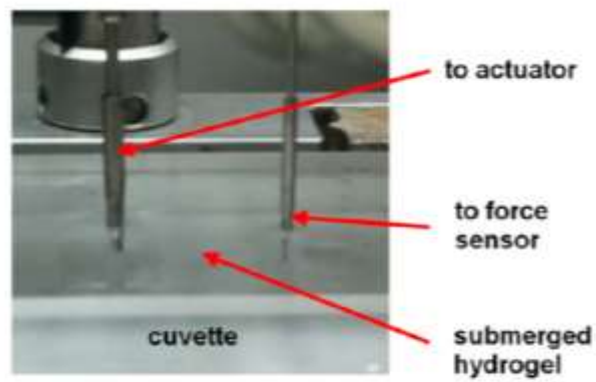


Dityrosine crosslinks



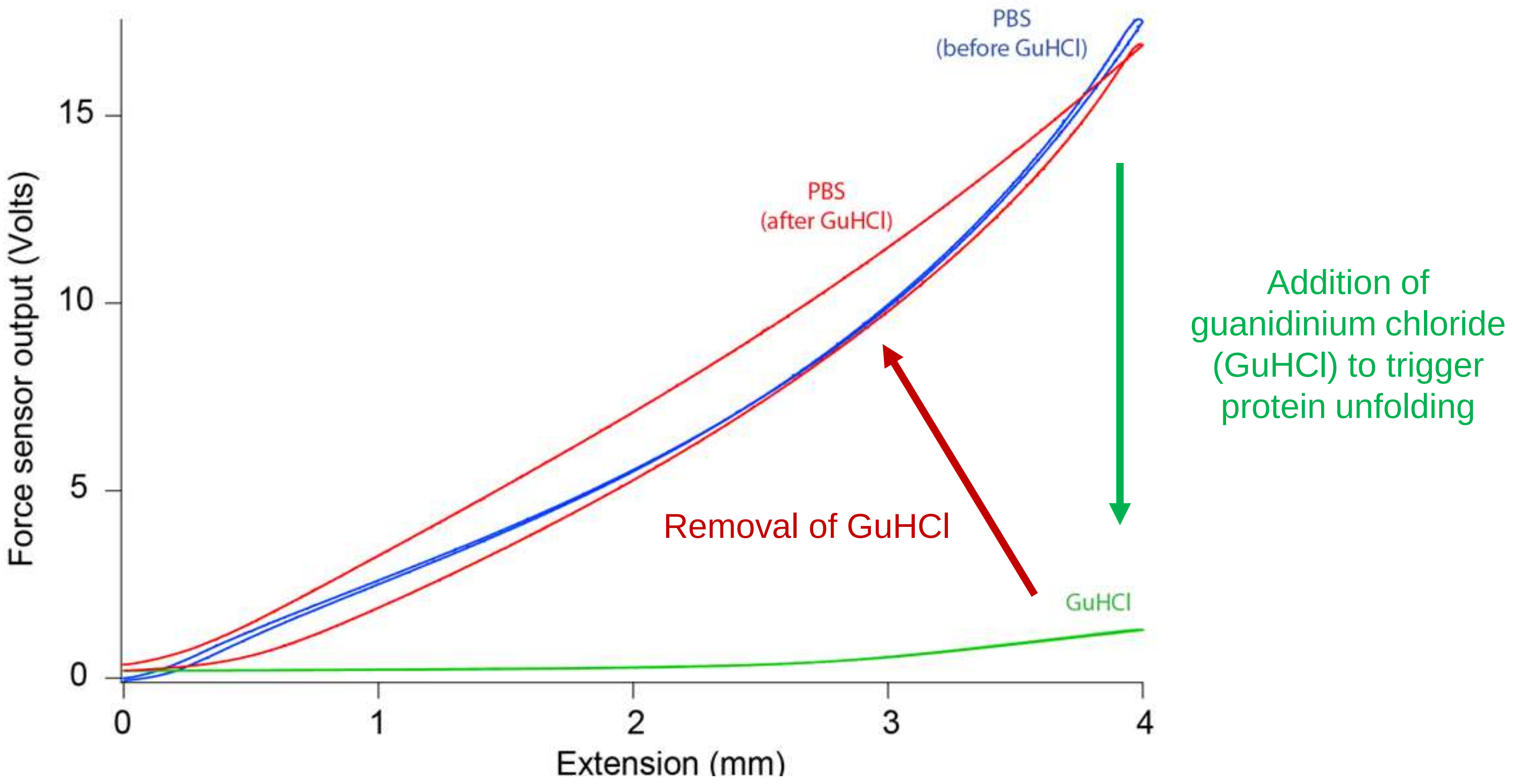
Titin's I27 domain





Saqlain *et al*. **Macromol Mater Eng 300**, 369 (2015)

Stiffness depends on reversible protein unfolding/refolding

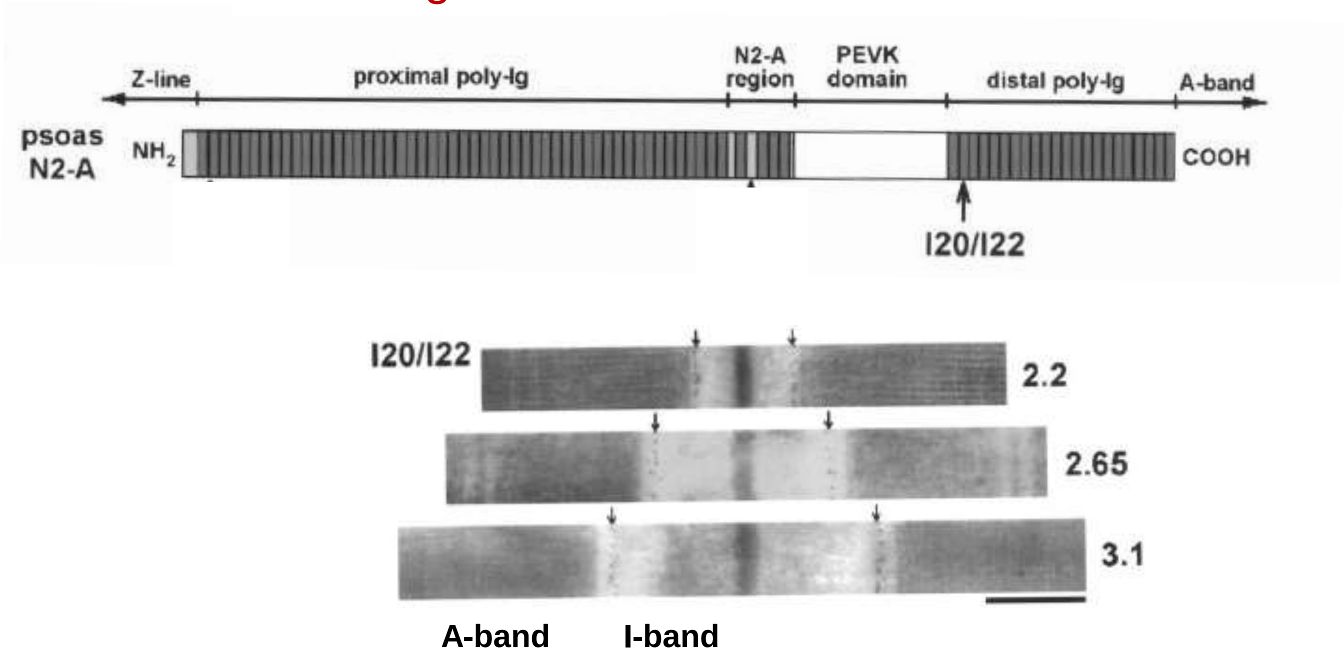


Saqlain *et al*. **Macromol Mater Eng 300**, 369 (2015)

Mechanobiology Seminar Series at CNIC. If interested, send an e-mail to jalegre@cnic.es

The length of titin changes during contraction/extension cycles

Folded Ig domains Random coil



Linke et *al.* J Cell Sci 111, 1567 (1998)