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

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

![Diagram showing a heart and a question mark labeled Elasticity?]

ii. **Measure the mechanical properties** of mutant proteins causing cardiomyopathy

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

![Image of I27 gel]

Carmen Suay, Carla Huerta, Elías Herrero, Cristina Sánchez
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.
Mutations in structural proteins lead to familial cardiomyopathy

- Dilated cardiomyopathy (~25% of cases)
- Hypertrophic cardiomyopathy (~35% of cases)

Genotype to phenotype?
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

Attachment of polyproteins to surfaces to study protein mechanics
Polyprotein engineering for force spectroscopy
Single-molecule AFM
Mechanical unfolding and refolding

AFM

Protein Length

Denature
Refold
Probe

Force

Mechanical stability

Refolding rate

Elasticity

25 nm
0.5 s

$\Delta t$ Refold = 3 s

$\Delta t$ Refold = 10 s

3 refolded
4 unfolded
5 refolded
0 unfolded
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?
Thiol chemistry controlling titin elasticity
Titin’s buried (cryptic) cysteines

Paired

Unpaired

I27 + 5 mM GSSG

<table>
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<th>+DTT</th>
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α-GSH

Ponceau S
Disulfide bonds as regulators of polymer elasticity

Vulcanization

Perm (1930’s)
Disulfide bonds as regulators of protein elasticity

Buried disulfide

Limited extension: stiffer protein
Detection of cryptic disulfides in titin Ig domains

We pull from an Ig domain of cardiac titin.

Wiita et al. PNAS 103, 7222 (2006)
Direct observation of disulfide isomerization

What about unpaired cysteines?

S-glutathionylation of titin

ROS (superoxide, H$_2$O$_2$)

Xanthine oxidase
NAD(P)H oxidase

Oxidative modification of cysteines

NO

Nitric oxide synthase
S-glutathionylation **inhibits** protein folding

I27 polyprotein

GSSG

GSH

Refolding?

\[ F = 175 \text{ pN} \]
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

Mass spectrometry to determine posttranslational modifications

Native tissue homogenization in denaturing conditions

Protein extraction in N-ethylmaleimide (NEM)

Band slicing

DTT + Iodoacetamide (IAM)

Automatic peptide identification

Reduced: -SH
Oxidized: -SR

Red: -SNEM
Ox: -SR

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
  - I1 (1G1C) 57 32
  - I67 (3B43) 74 23 85
  - I27!!! (1TIT) 47 63

- 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

Your kids are going to be OK!

Therapeutic reasons

SNP or *pathogenic* mutation?

Drugs that *restore* healthy phenotype
Experimental objectives (iii)

**Biomaterials that mimic the elasticity of muscle**

**Experimental objectives (iii)**

Biomaterials that mimic the elasticity of muscle

Young’s modulus ~100 KPa

Dityrosine crosslinks

Stiffness depends on reversible protein unfolding/refolding.

Addition of guanidinium chloride (GuHCl) to trigger protein unfolding.

Removal of GuHCl.

Mechanobiology Seminar Series at CNIC. If interested, send an e-mail to jalegre@cnic.es
The length of titin changes during contraction/extension cycles.