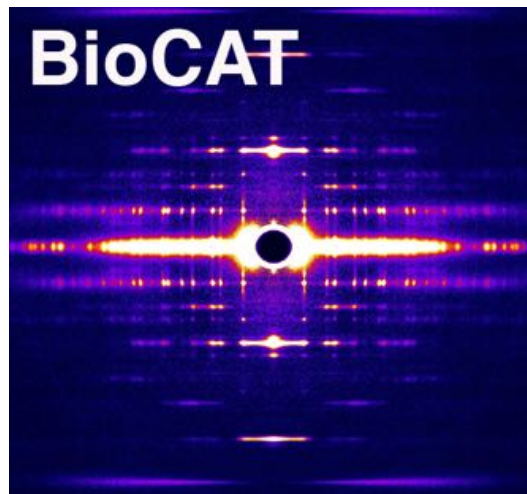


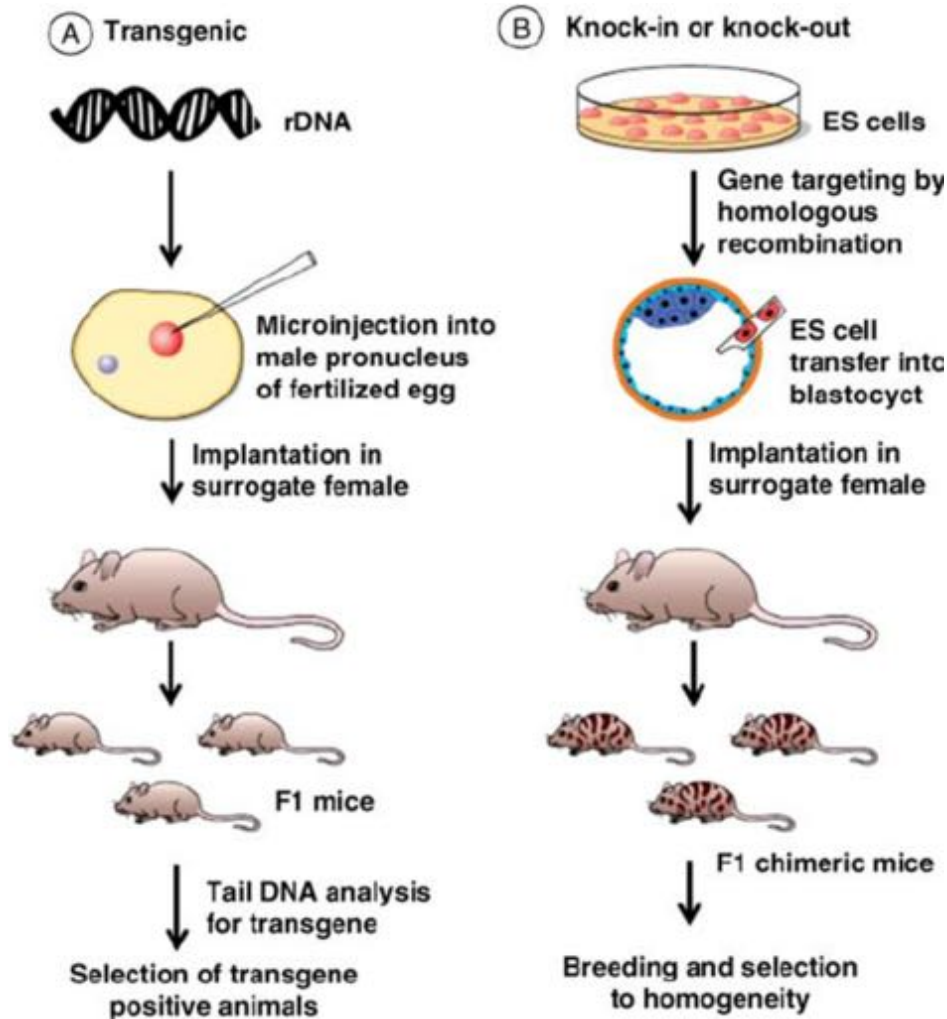
# Skeletal Muscle X-ray Diffraction at BioCAT



Weikang Ma

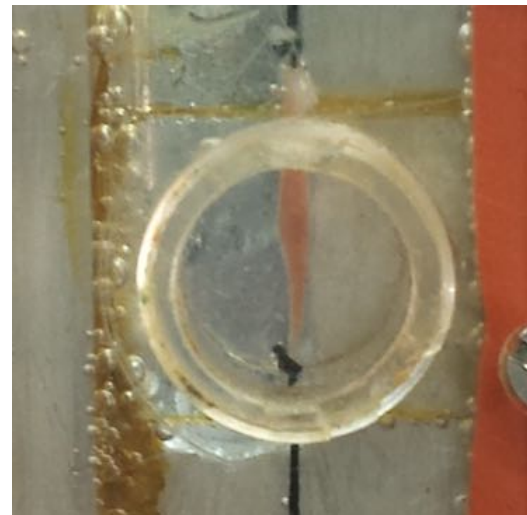
Illinois Institute of Technology, Chicago IL

# Mouse skeletal muscle XRD in BioCAT

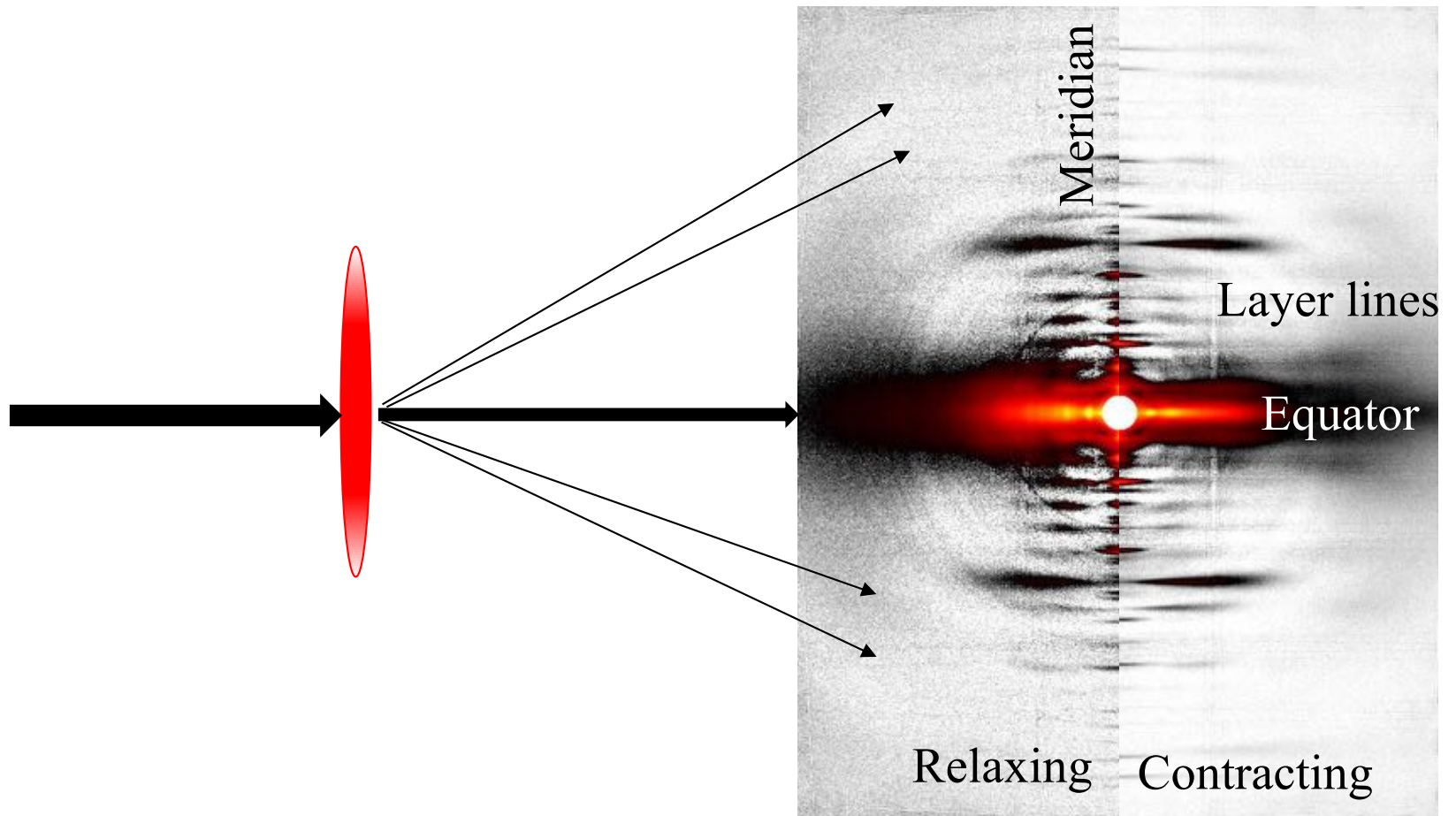


Soleus

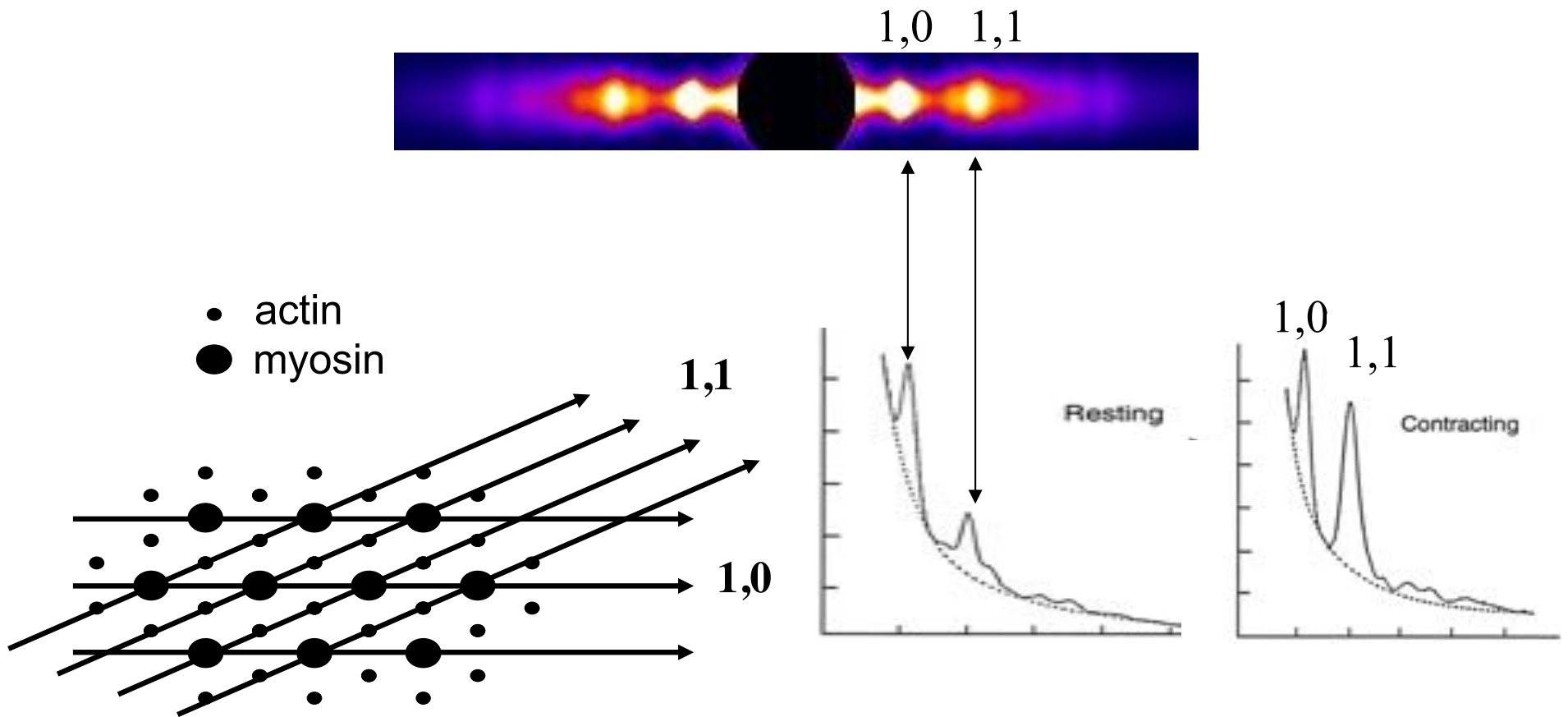
EDL



# Muscle X-ray pattern



# Equatorial reflections



The  $I_{1,1}/I_{1,0}$  intensity ratio will be correlated to the number of attached cross-bridges

# Concerns

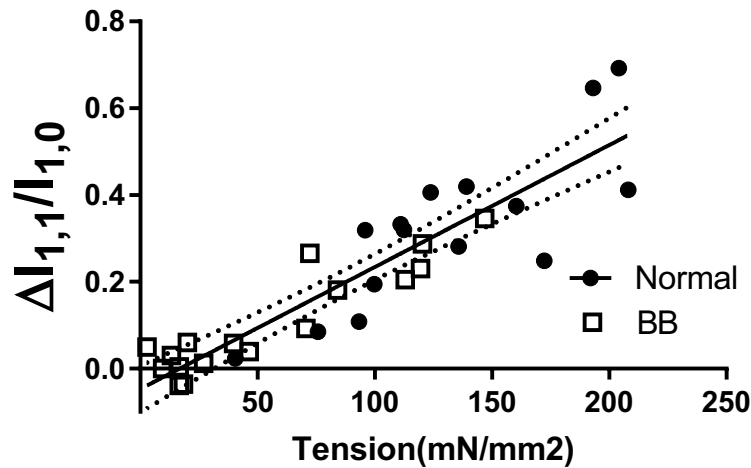
$I_{1,1}/I_{1,0}$  indicates how close of myosin heads to actin

But, close proximity doesn't mean bind to actin

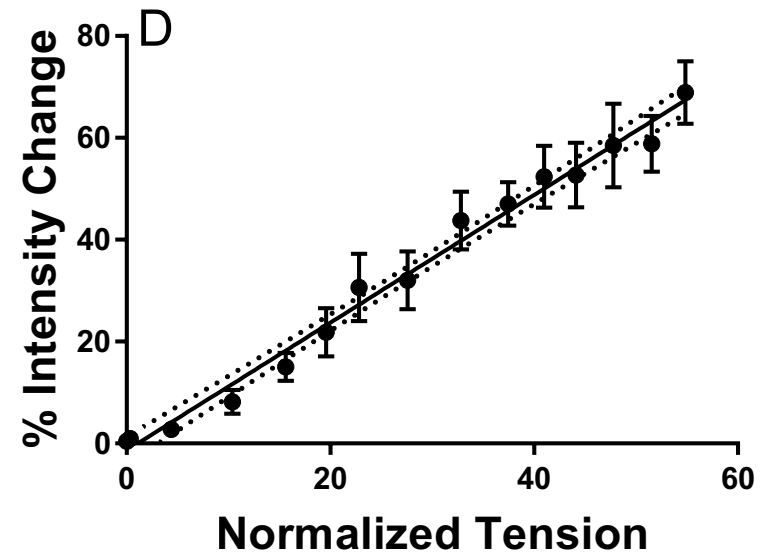
- In frog muscle,  $I_{1,1}/I_{1,0}$  indicates about 90% of myosin heads are in the vicinity of thin filament (Haselgrove, 1973; Huxley, 1985)
- But less than 30% of these myosin head binds to actin (Matsubara, 1975; Huxley, 1982; Huxley, 1985).

The discrepancy between  $I_{1,1}/I_{1,0}$  and the number of cross-bridges attached make the interpretation of problematic.

# $I_{1,1}/I_{1,0}$ in mouse skeletal muscle

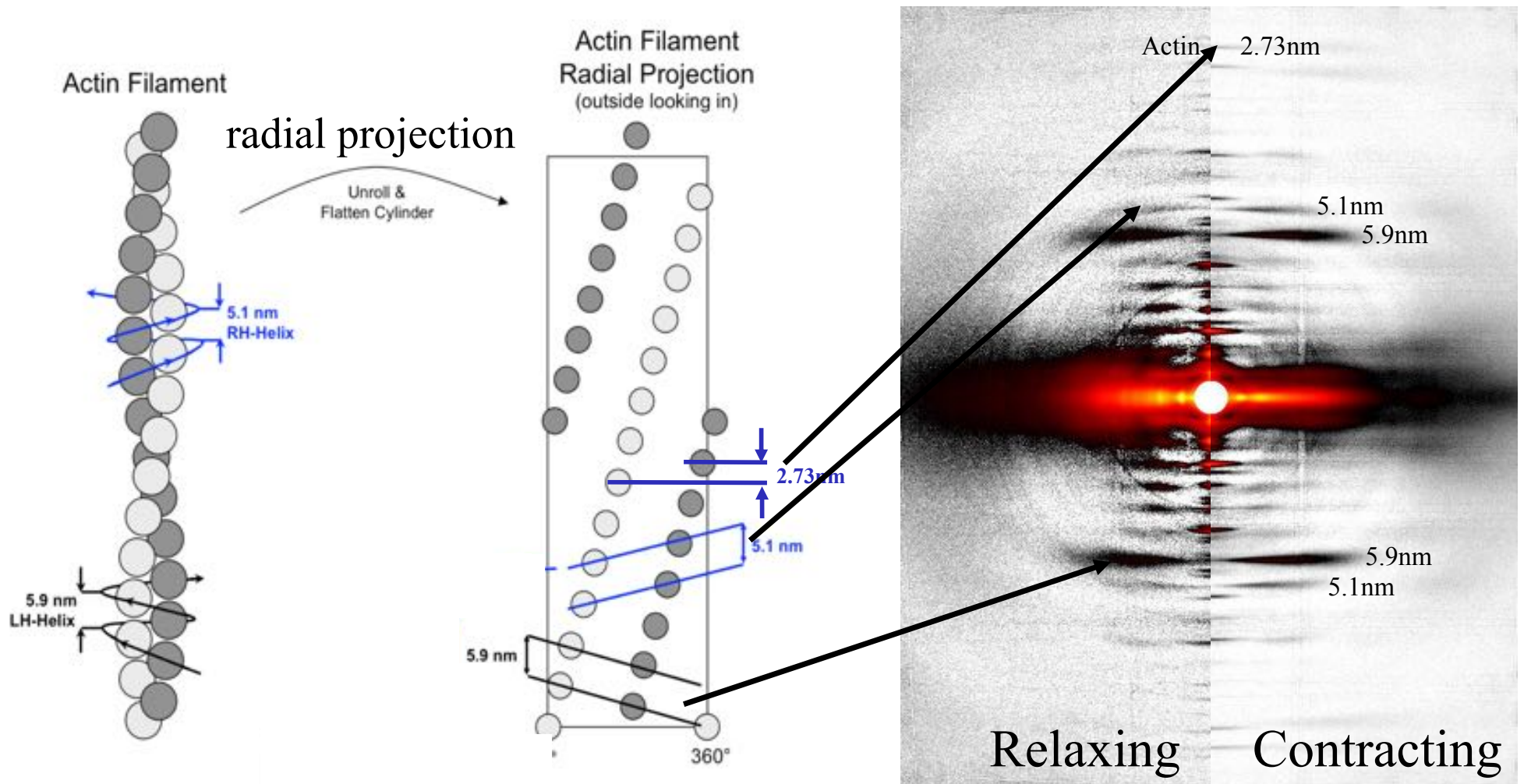


A linear relationship in equatorial intensity change to applied tension



Consistent with studies on cardiac muscle: intensity ratio correlates well with tension especially during the early phase of contraction

# Thin filament based reflections

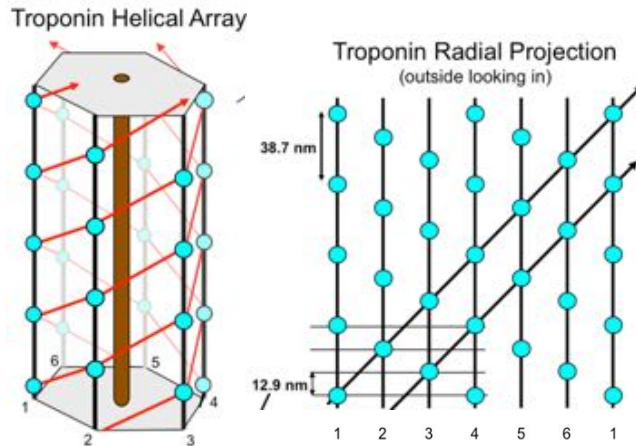


You could measure the thin filament extensibility from these reflections

# Tn/Tm reflections

When the muscle is activated

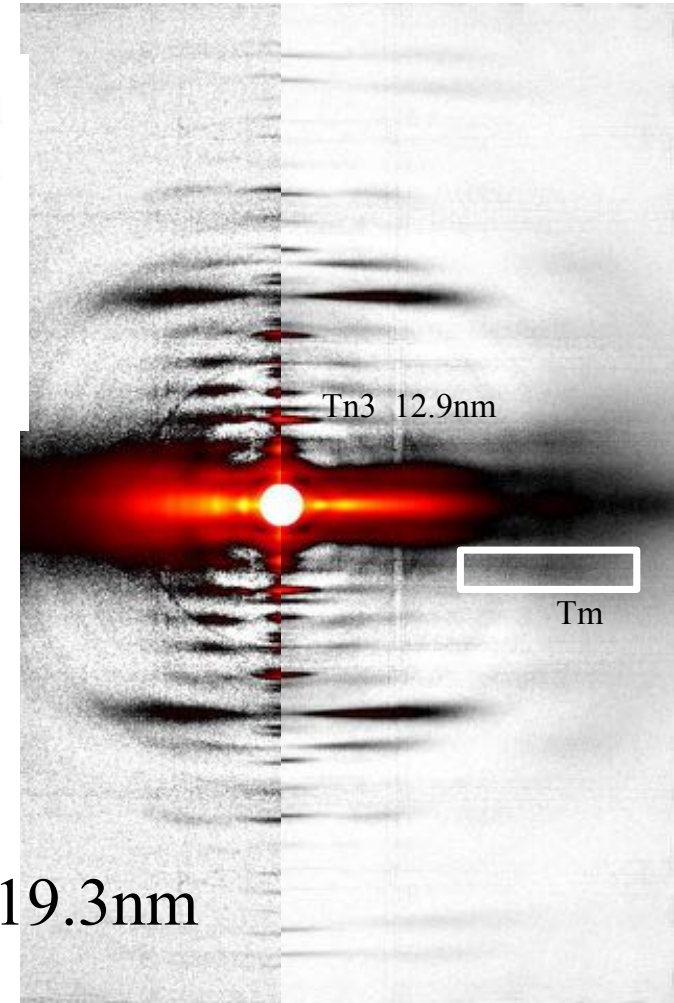
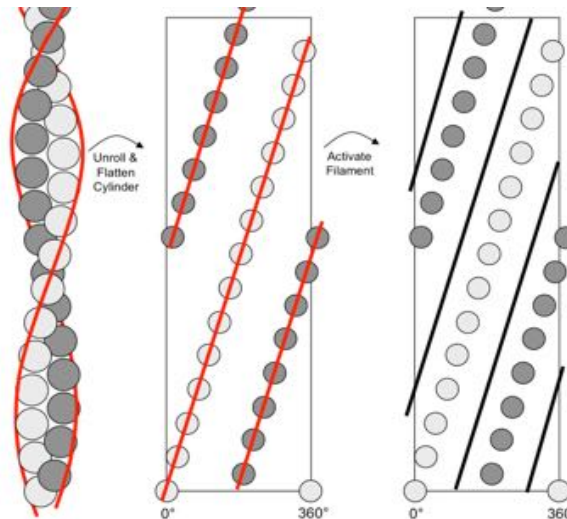
$$I_{Tn3} \sim 18\%$$



TM repeat 38.7 nm in a relaxed muscle

When the muscle is activated

19.3nm LL intensified

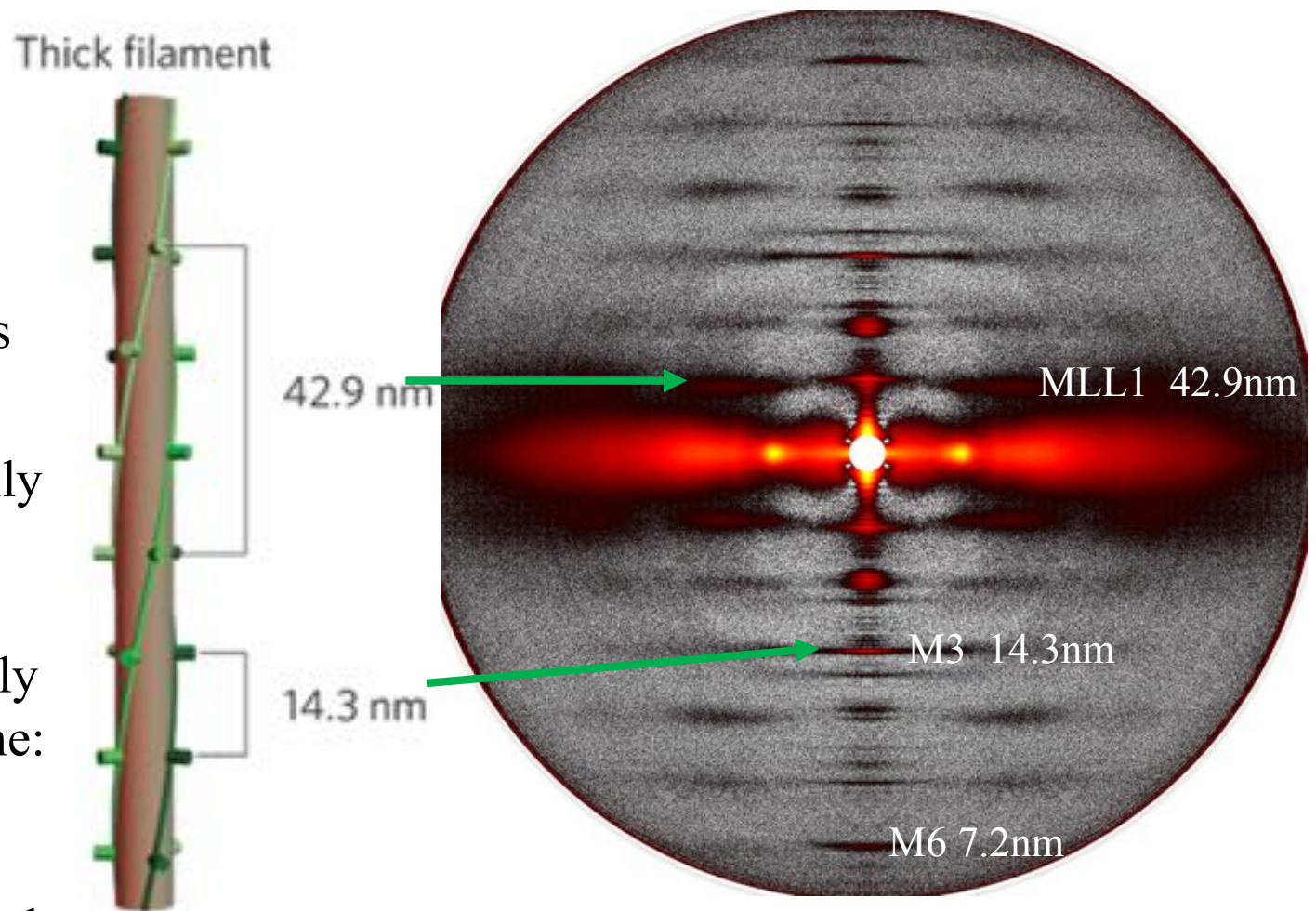


You could measure the intensity and the width of Tm reflection



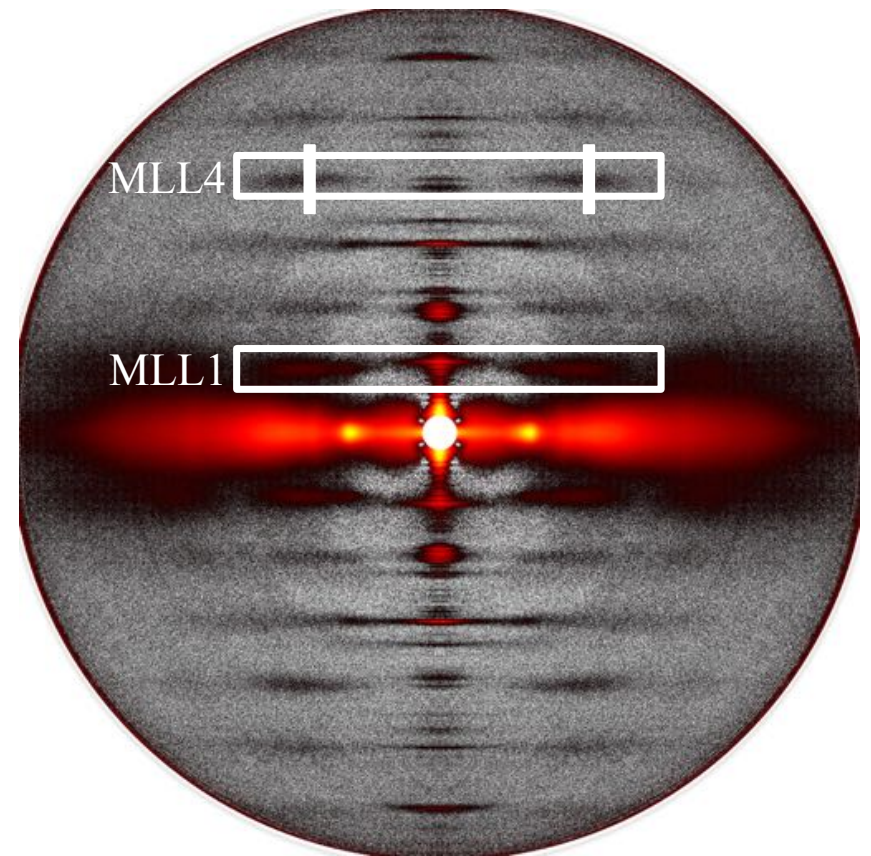
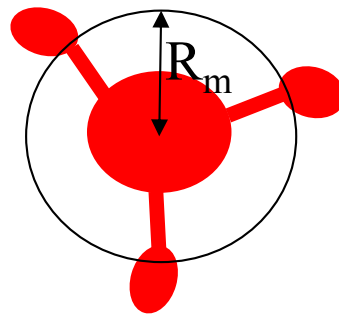
# Myosin filament based reflections

- MLL1: Helically ordered myosin heads
- M3: Intensity primarily from myosin heads
- M6: Intensity primarily from myosin backbone: The thick filament extensibility can be measured from M6 and higher meridional reflections



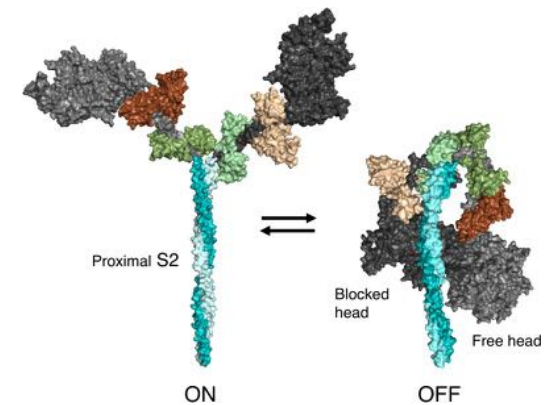
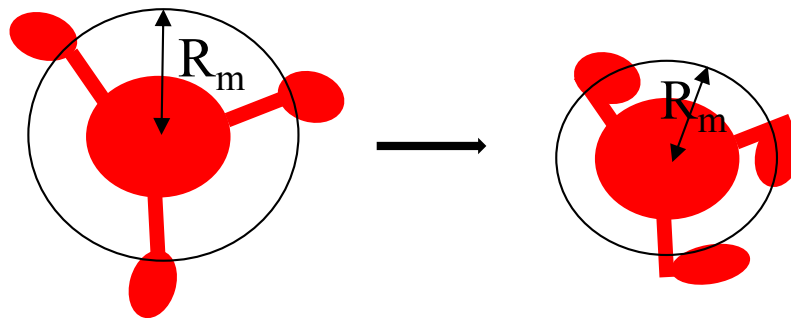
# Myosin filament based reflections

- The layer line intensity distribution is on the basis of Bessel function
- The distance between the first maximum layer is reversely related to the average myosin heads radius ( $R_m$ )



# SRX by Muscle X-ray Diffraction

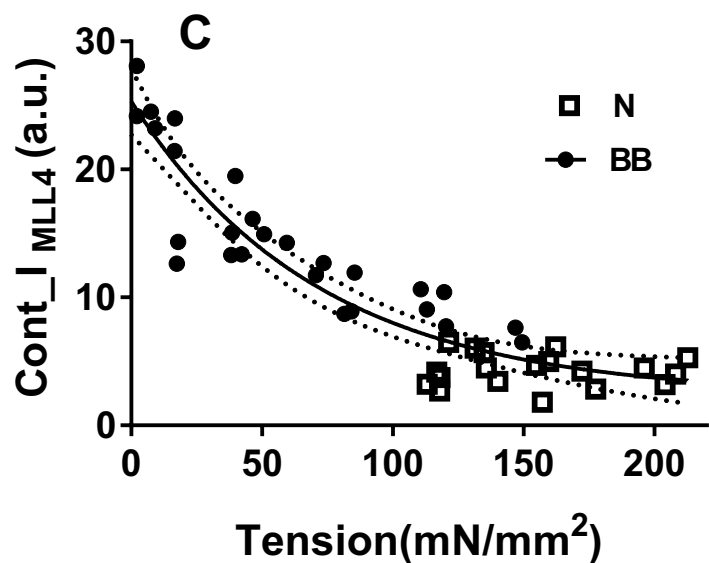
- MLL came from ordered myosin heads
- SRX was characterized structurally the heads are folded back on to its own coiled-coil S2 tail
- Smaller  $R_m$  when shifts more myosin heads to SRX



Trivedi, et al 2018 *Biophys Rev*

- ❖ Increased MLL intensity and/or smaller average myosin heads radius and changes in equatorial intensity ratio could be indicators of SRX in X-ray diffraction under stable temperature.

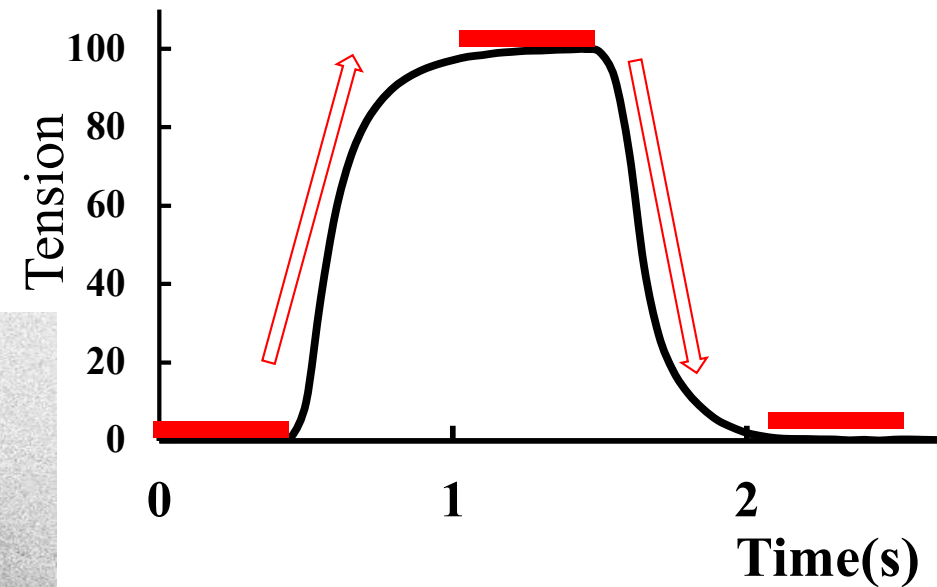
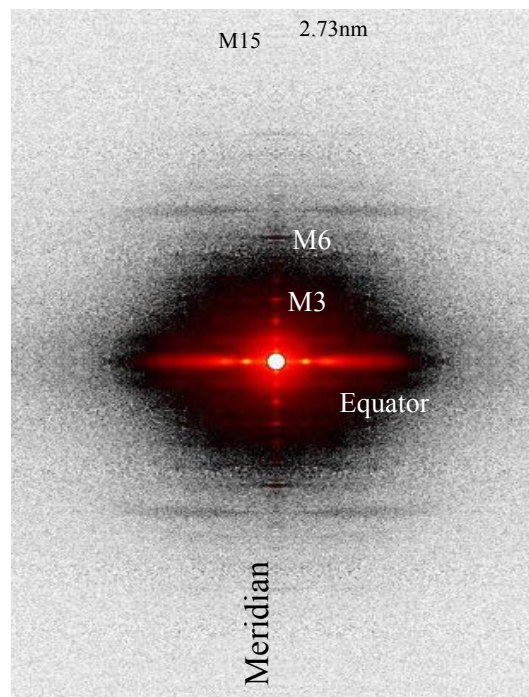
# MLL4 intensity Vs Tension



- MLL4 intensity residue is inversely related to applied tensions.
- MLL4 intensity residue can be used to estimate the number of myosin heads move when muscle was activated.

# Time-Resolved X-ray

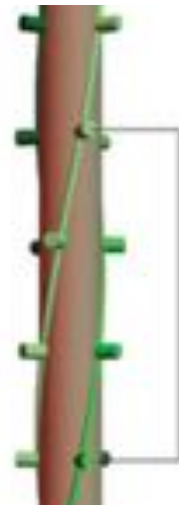
- Static shots at resting and plateau regions
- Take movie shots the whole time at up to 500Hz
- Activation
- Relaxation



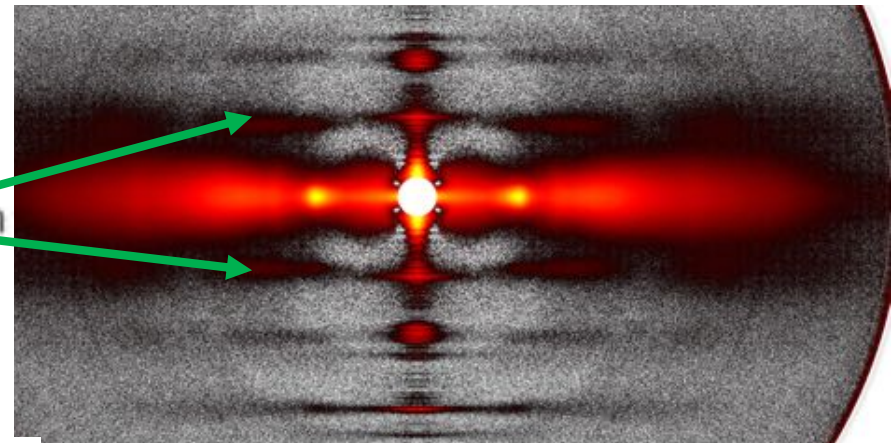
# Relaxation

- Activation/contraction had been heavily studied
- Relaxation studies were much less
  - Maeda 1983 *Nature*
  - Brunello et al 2009 *J Physiol*

- MLL1: Helically ordered myosin heads
- Indicator of myosin heads ON/OFF



42.9 nm



- Timing when MLL1 coming back after contraction can be used to study relaxation

# Conclusion

If you have mouse/rat models or reagents:

- Change thin/thick filament extensibility
- Change T<sub>n</sub>/T<sub>m</sub> movement
- Change activation/relaxation kinetics
- Study SRX state

Cool systems

Cool ideas



[Nature](#). 2005 Jan 20;433(7023):330-4.

**Molecular dynamics of cyclically contracting insect flight muscle in vivo.**

[Dickinson M](#)<sup>1</sup>, [Farman G](#), [Frye M](#), [Bekyarova T](#), [Gore D](#), [Maughan D](#), [Irving T](#).



[Proc Natl Acad Sci U S A](#). 2011 Jan 4;108(1):120-5. doi: 10.1073/pnas.1014599107. Epub 2010 Dec 9.

**X-ray diffraction evidence for myosin-troponin connections and tropomyosin movement during stretch activation of insect flight muscle.**

[Perz-Edwards RJ](#)<sup>1</sup>, [Irving TC](#), [Baumann BA](#), [Gore D](#), [Hutchinson DC](#), [Kržić U](#), [Porter RL](#), [Ward AB](#), [Reedy MK](#).

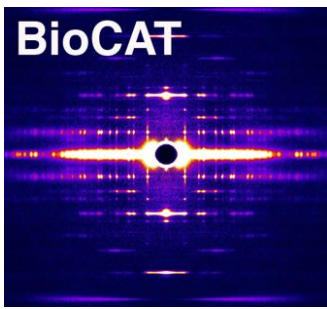


[Science](#). 2013 Jun 7;340(6137):1217-20. doi: 10.1126/science.1229573. Epub 2013 Apr 25.

**The cross-bridge spring: can cool muscles store elastic energy?**

[George NT](#)<sup>1</sup>, [Irving TC](#), [Williams CD](#), [Daniel TL](#).

For every hypothesis, there is a perfect critter out there to test it  
(With apologies to August Krogh)



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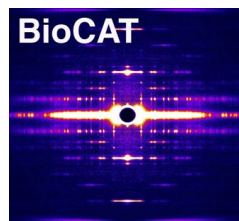
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