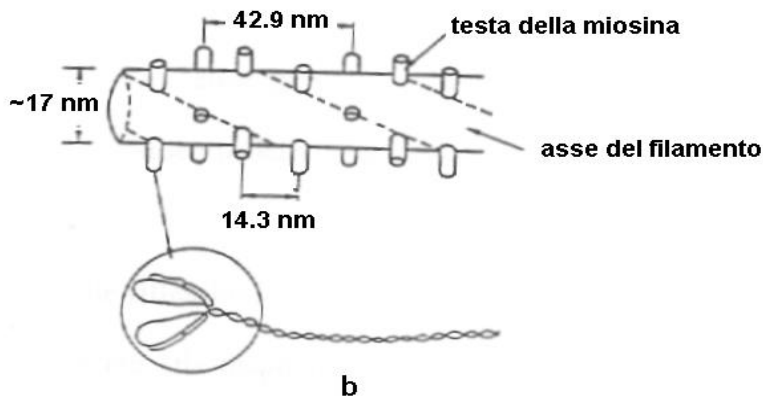
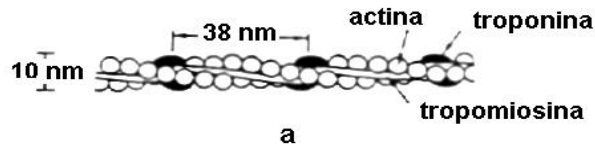
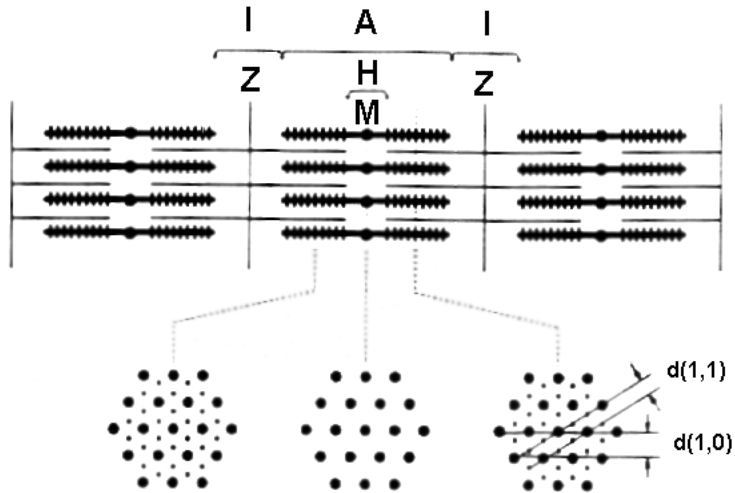
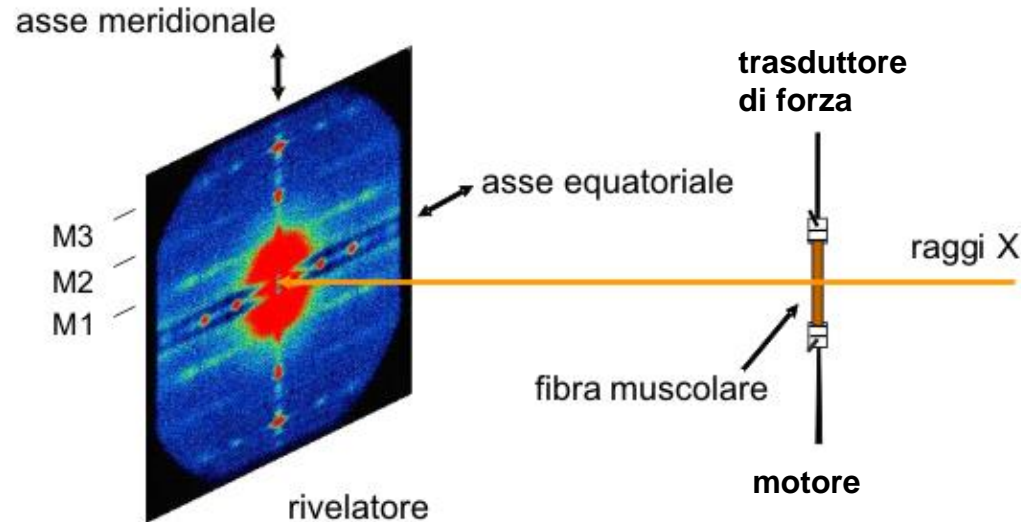


Metodi strutturali per la misura del working stroke *in situ*

La diffrazione a raggi X a piccolo angolo

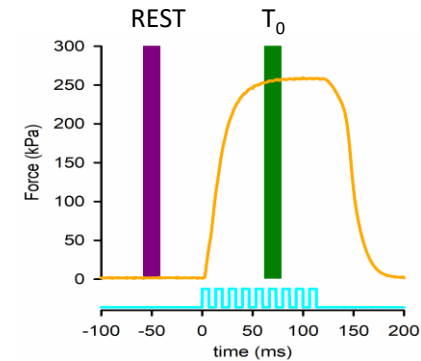
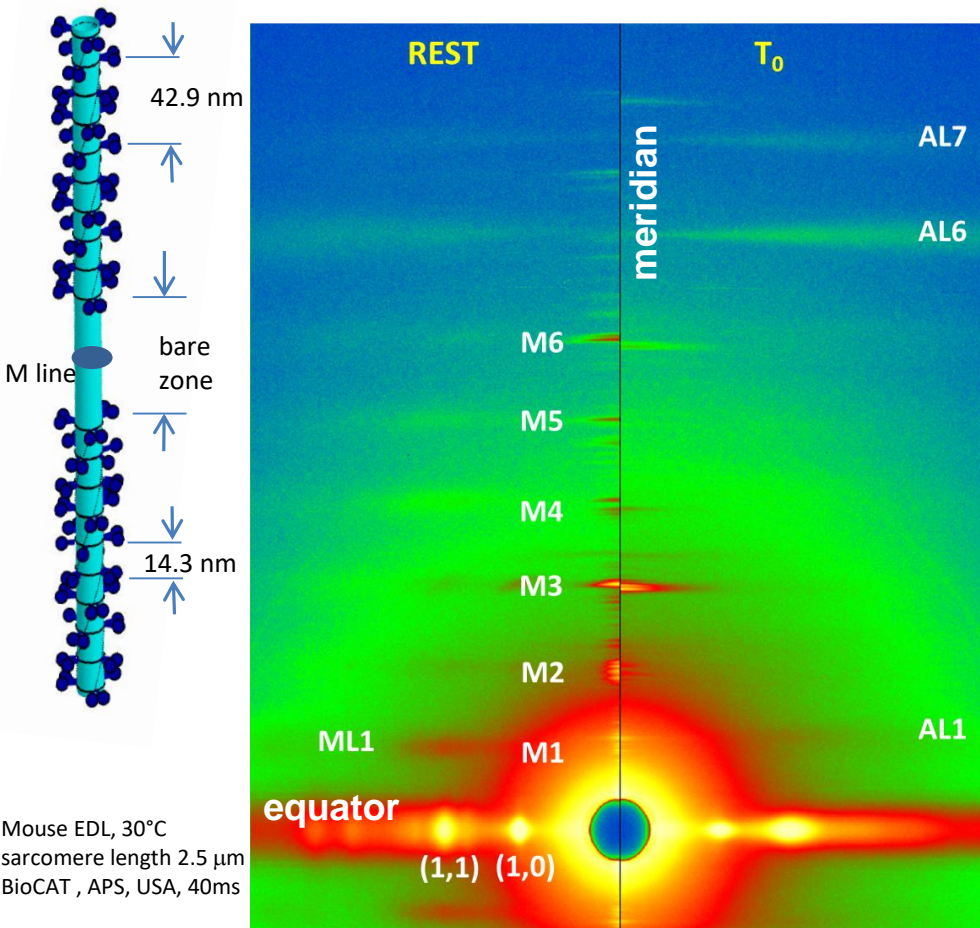


Quando un fascio di fotoni con lunghezza d'onda di circa 1 Å colpisce una fibra muscolare, la regolare disposizione delle proteine nei filamenti e dei filamenti nel reticolo tridimensionale producono uno scattering della luce che su un rivelatore messo a distanza opportuna (L) forma un diagramma di diffrazione a piccolo angolo. L'angolo di diffrazione (θ) dipende dal periodo della sorgente di diffrazione (d) secondo la legge: $\sin \theta = n\lambda/d$.



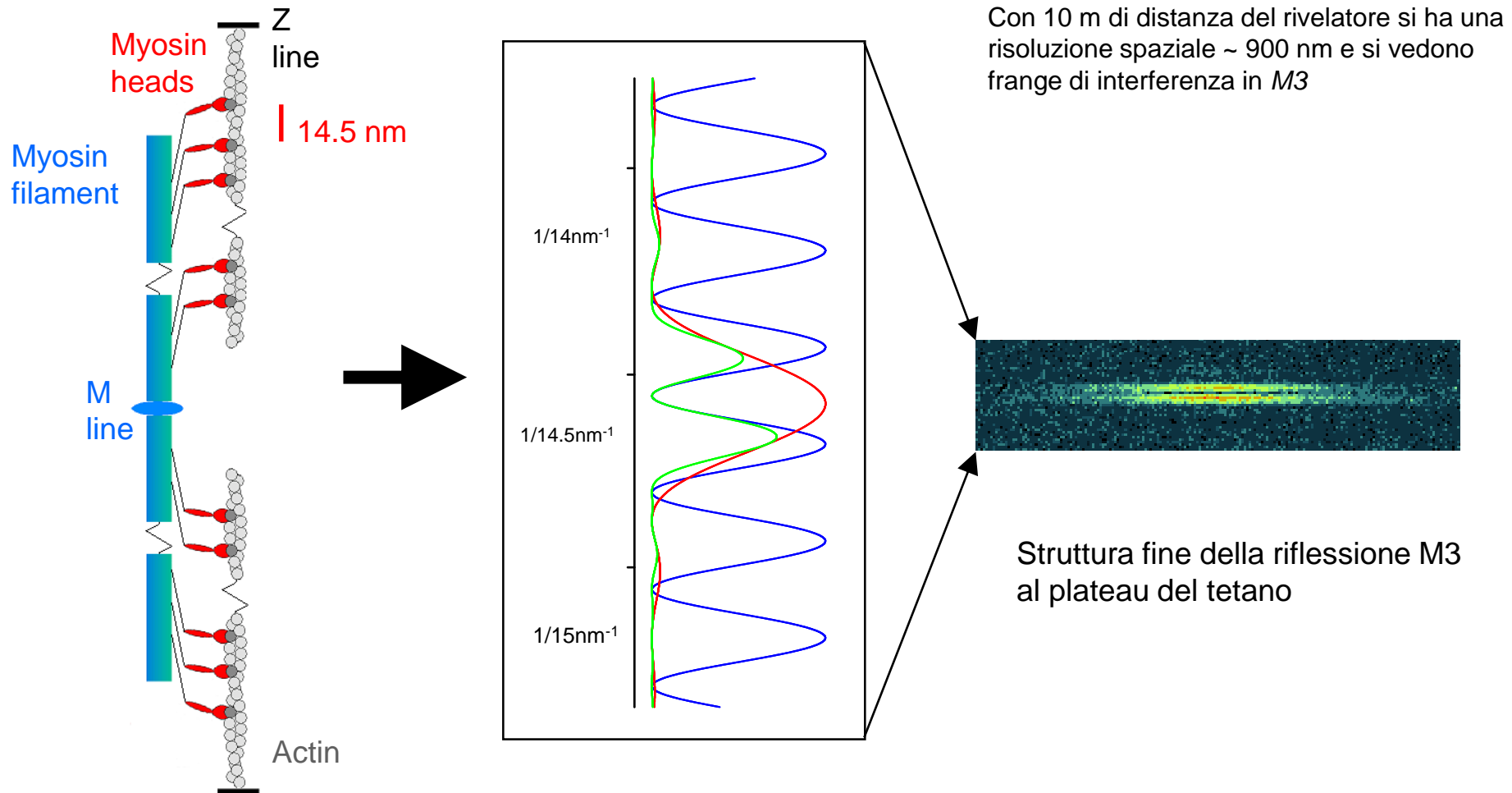
La riflessione $M3$, che rimane molto intensa durante la contrazione, è originata dalla periodicità di 14.3 nm con cui si ripetono le teste della miosina lungo l'asse del filamento e la sua intensità è sensibile alle variazioni di forma della testa.

X-ray patterns from the EDL muscle of mouse at 30°C at rest and at the plateau of the isometric tetanus (T_0)



- At rest the intense layer line reflection at 43 nm (ML1) is due to the myosin motors forming a three stranded helix with 43 nm periodicity on the surface of the thick filament.
- At T_0 IML1 is zero because of the loss of helical order as the heads move away from their tracks on the thick filament and the intensity of the actin layer line at 38 nm (AL1) is increased as the attached motors take the helical periodicity of the double stranded actin filament
- The intensity of the M3 reflection remains high at T_0 but its spacing is increased and its fine structure is changed from one to two peaks as a result of the change in conformation of attached heads that are the main contributor

Interferenza a raggi X tra le due schiere di motori in ciascun filamento spesso

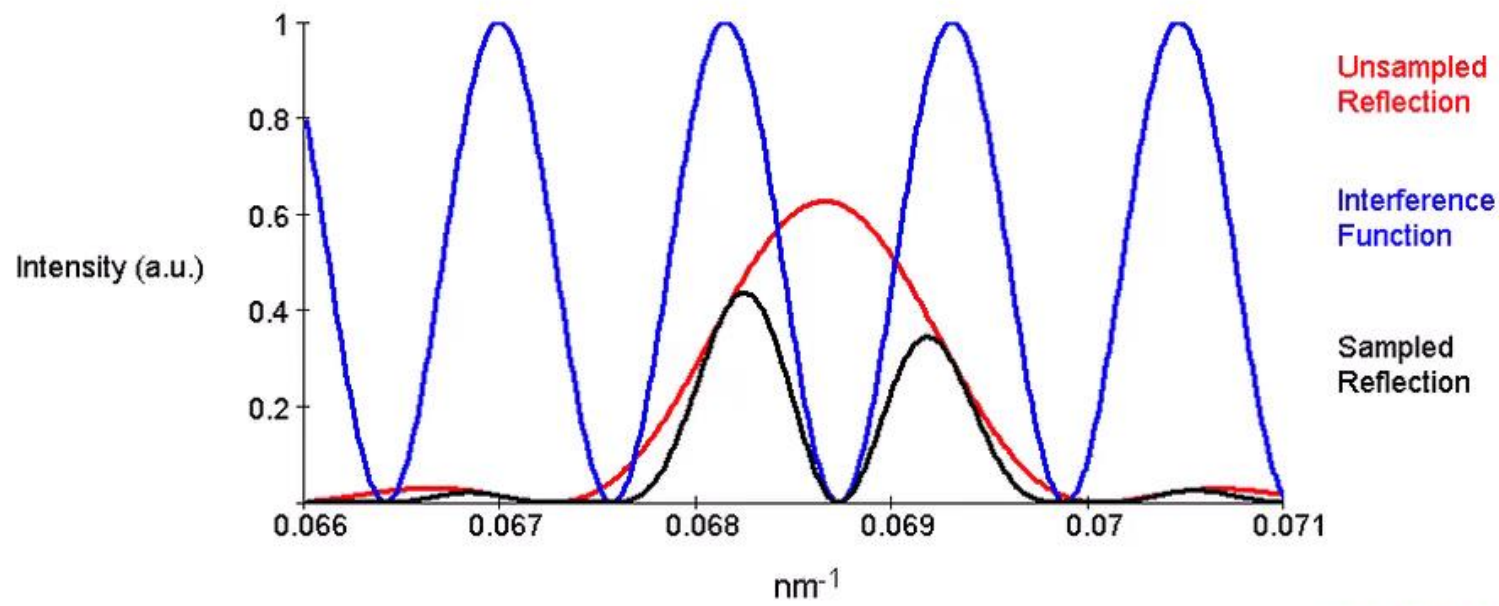


Due schiere di motori in ciascun filamento spesso con distanza centro-centro ~900 nm

Il pattern osservato (verde) è il prodotto di quello generato da una singola schiera di motori (rosso) con la frangia di interferenza generata dalle due schiere (blu)

X-ray interference between the two arrays of myosin heads allows direct Å scale measurements of axial motion of the motors in situ

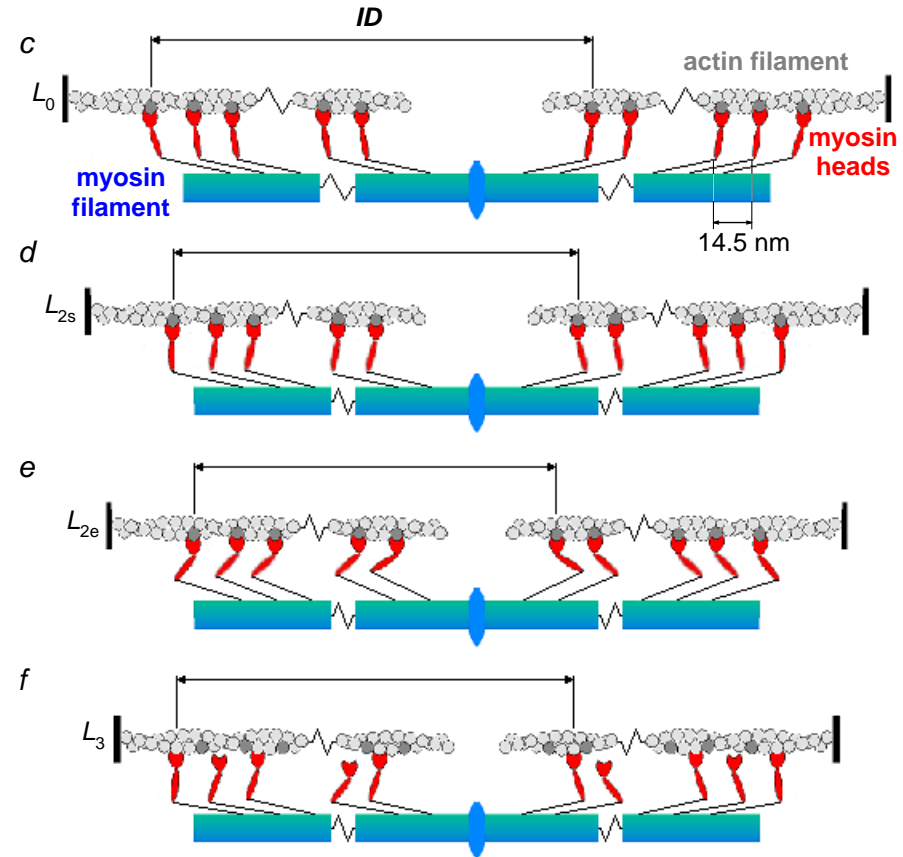
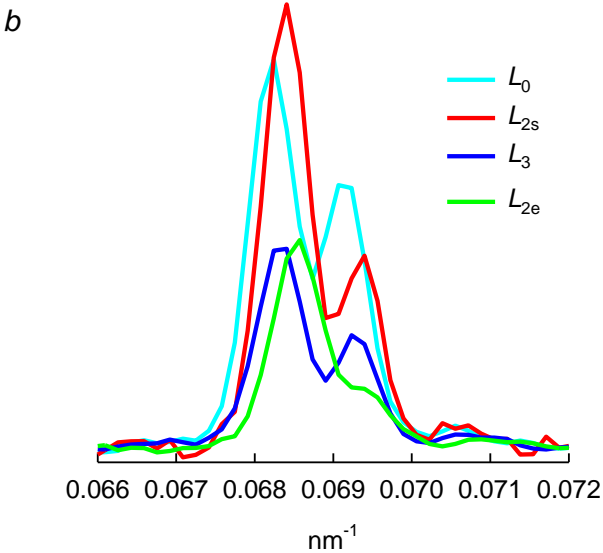
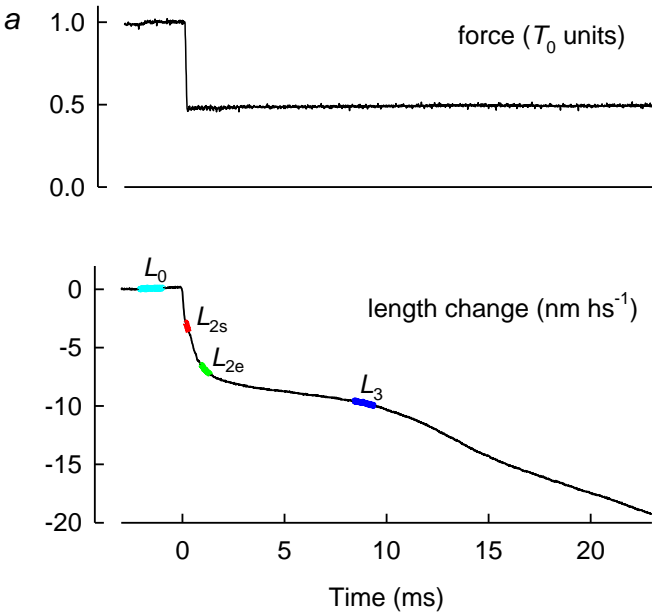
angle = 60 deg



by MassimoReconditi

Change in the interference splitting from T_0 to 'rigor' conformation ($\Delta z = 6$ nm)

La variazione dell'interferenza a raggi X durante il transiente isotonicico di velocità misura il movimento del motore durante il working stroke

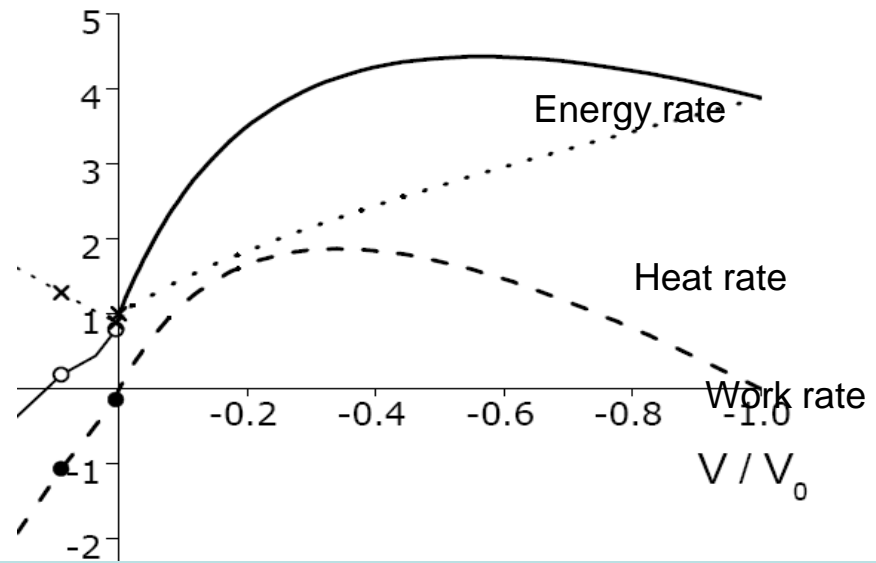


Le teste di miosina si muovono verso il centro del sarcomero in fase 2 e si staccano dall'actina in fase 3

Integrating mechanical and structural parameters reveals the molecular basis of muscle energetics

Muscle energetics

Frog, 0 °C	T_0	$0.5 T_0$
Heat+work rate	12 mW/g	40 mW/g
Work rate		18 mW/g
efficiency		0.45



Efficiency

0.45 @ $0.5 T_0$

$$\Delta G_{ATP} = \Delta G_{0,ATP} + k_b T_K \times \ln \left\{ \frac{[P_i][ADP]}{[ATP]} \right\} \sim 85 \text{ zJ } (21 k_b T_K)$$

Assume tight coupling between chemical and mechanical cycle:

Molecular level

Expected work/cycle: $85 \times 0.45 = 38 \text{ zJ}$

Work/cycle from half-sarcomere mechanics/X-ray in intact fibres:

$6 \text{ pN} \times 6 \text{ nm} = 36 \text{ zJ}$