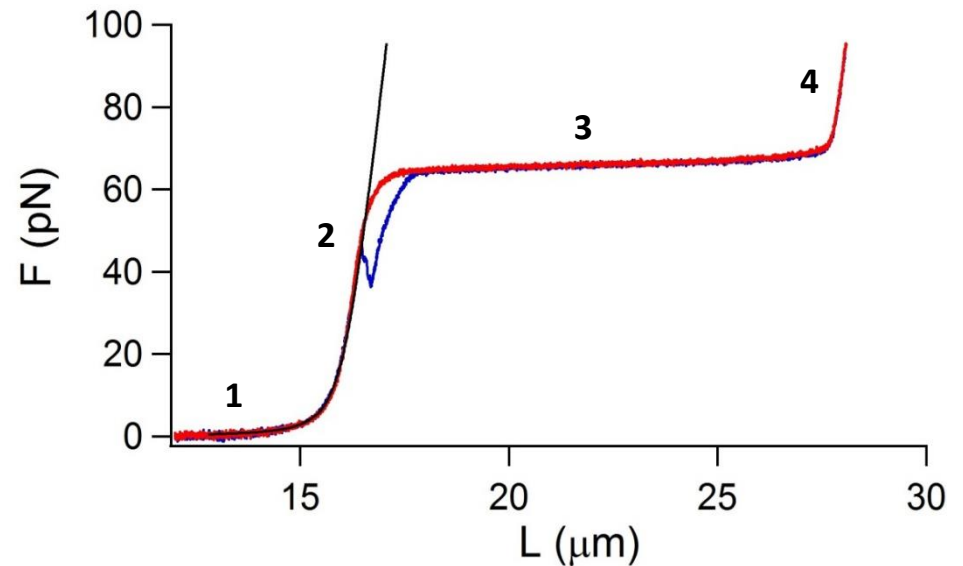
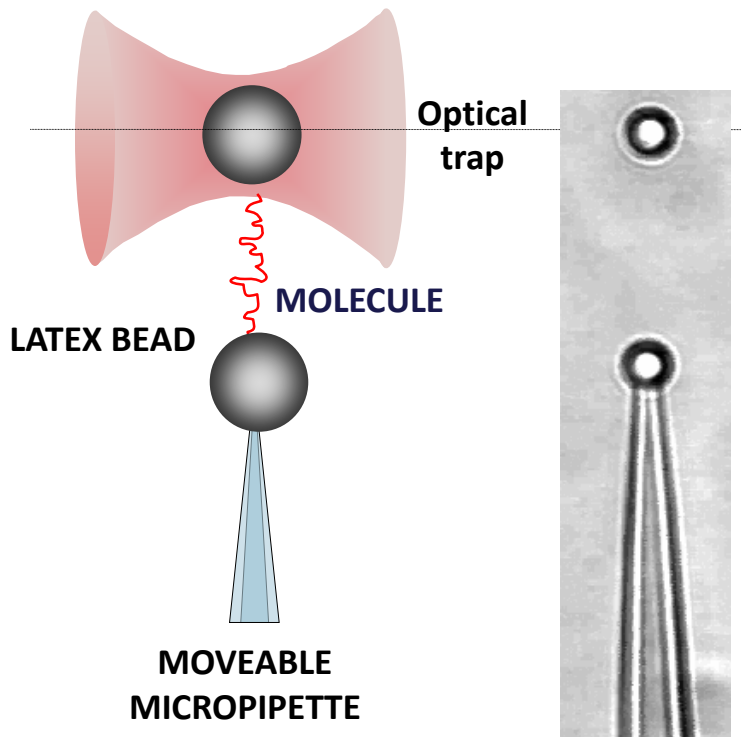
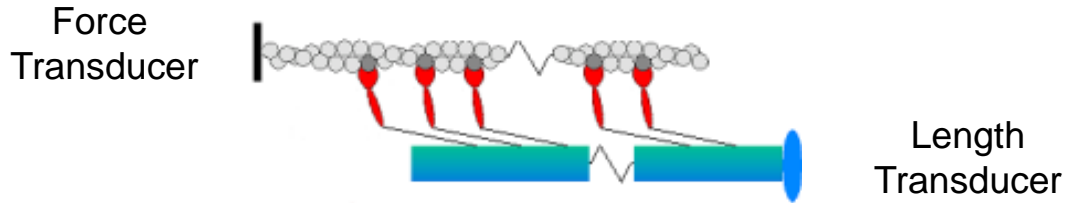


Misure di meccanica con la trappola ottica a doppio laser

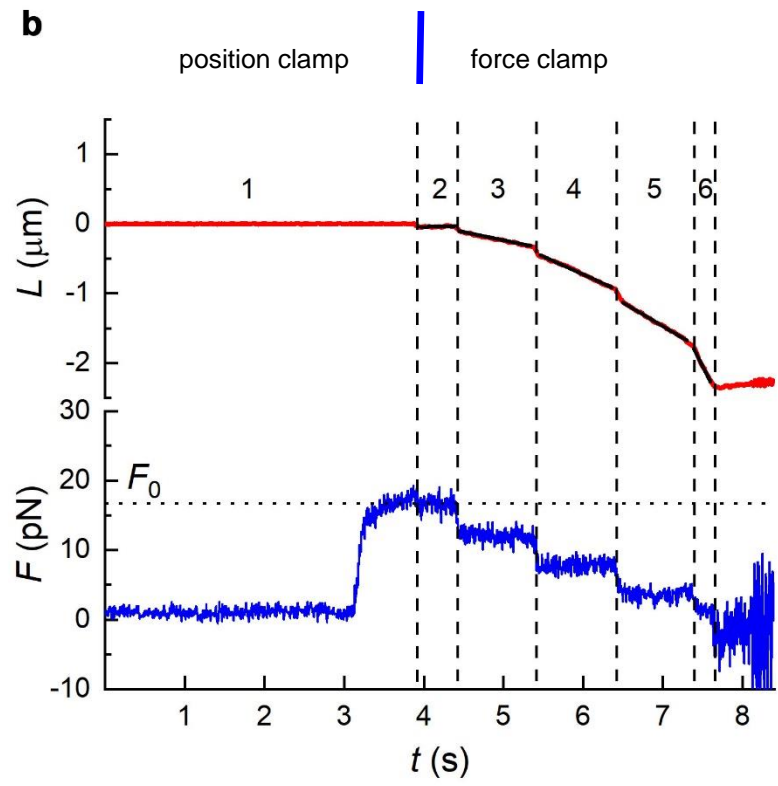
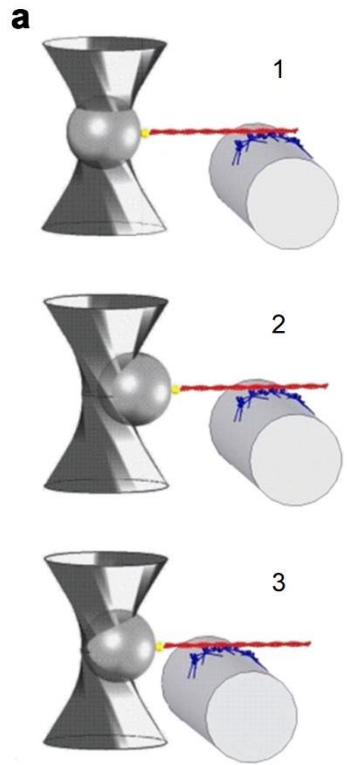
La trappola ottica a doppio laser espande l'ambito dinamico della forza di dieci volte (0.5-200 pN) e permette di registrare la dinamica strutturale della transizione di superallungamento del DNA (che avviene a circa 70 pN) o la forza stabile generata da una schiera di motori che agiscono in parallelo su un filamento di actina come nell'emisarcomero.



Mechanics of a synthetic myosin-based machine able to recover the half-sarcomere processivity



Half-sarcomere mechanics in a single muscle cell



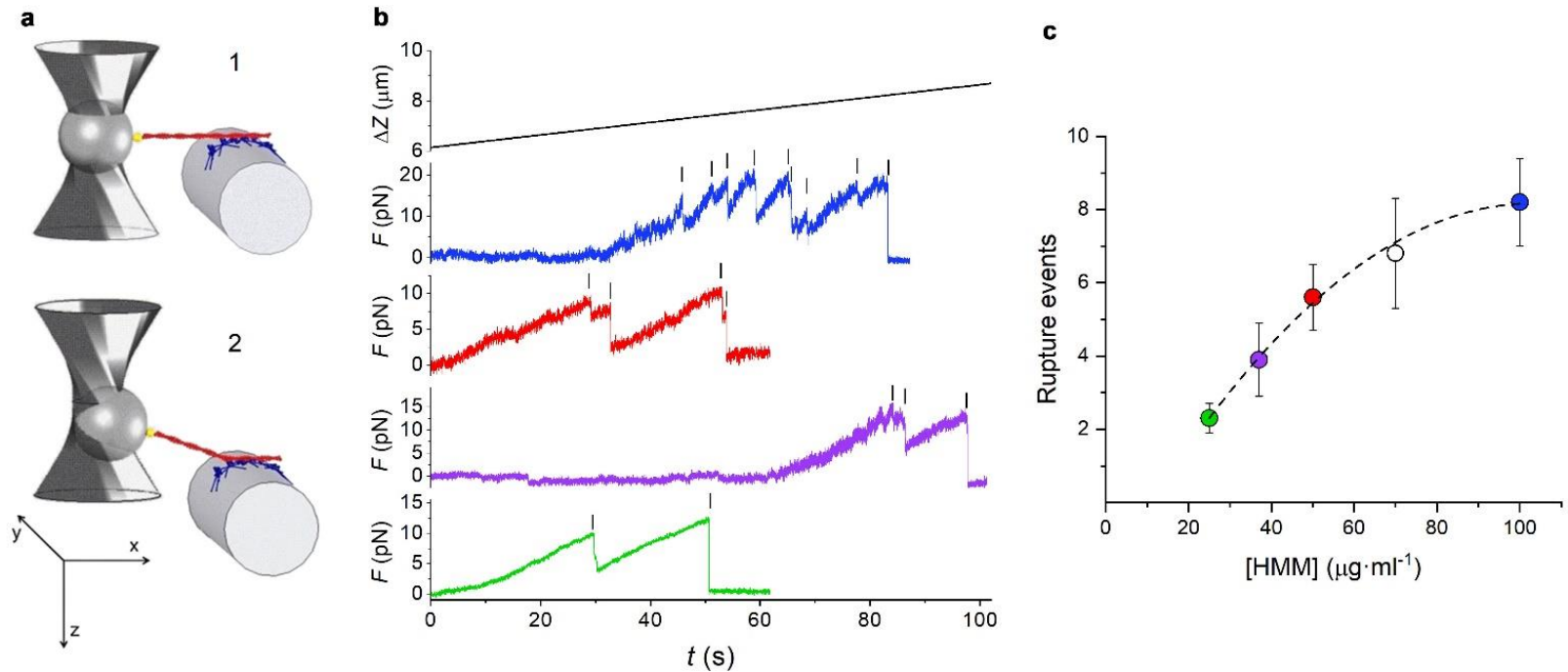
Synthetic one-dimensional machine: an array of myosin motors deposited on a support carried by a three-ways nanopositioner is brought to interact with the actin filament attached with the correct polarity to the trapped bead via gelsolin.

Perfusion solution with with 2 mM ATP:

In **position clamp**, force develops up to a steady maximum value (F_0), as in an **isometric contraction**.

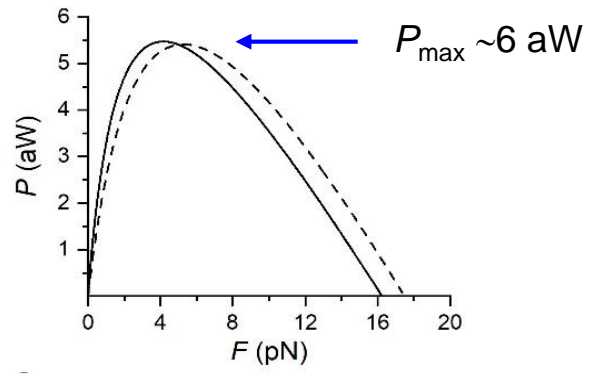
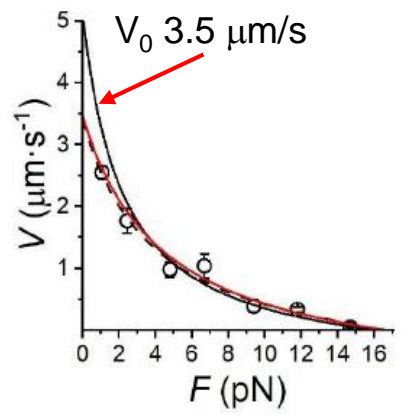
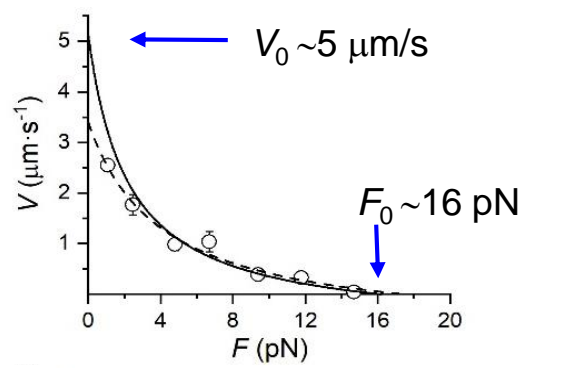
In **force clamp**: steady shortening at velocity dependent on the load (**isotonic contraction**)

Number of available motors estimated from rupture events in rigor

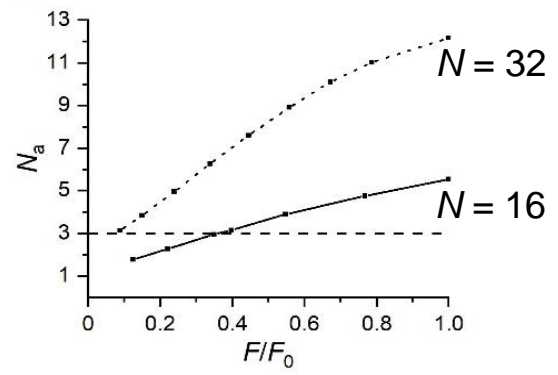
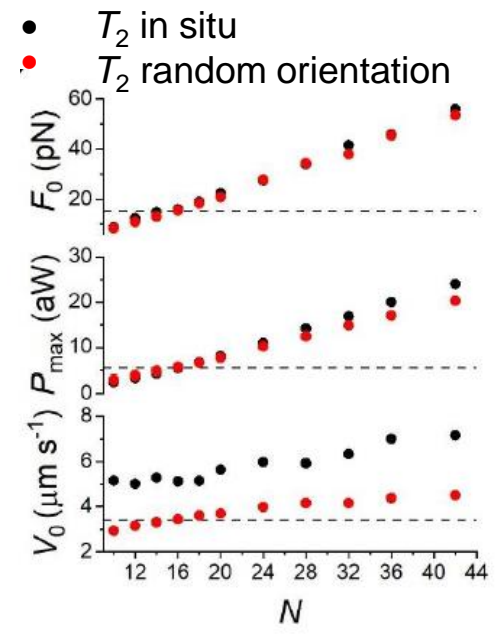
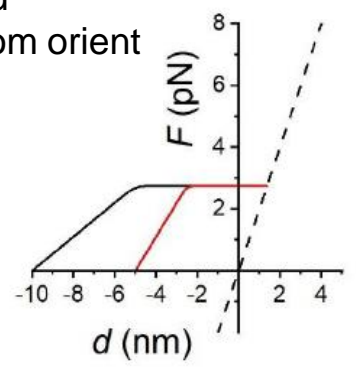


After the actin-myosin bond formation in ATP-free solution, motor support moved away in direction orthogonal to the actin myosin interface to avoid reattachment. Rupture of a bond (=detachment of a HMM dimer) indicated by the abrupt force drop. Number of ruptures increases with [HMM] to a maximum of 8. Thus, assuming that in physiological conditions each motor of a dimer acts independently, the support has 16 motors available for actin interaction.

The experimental F - V relation is fitted by using the kinetic scheme of the intact fibre mechanics when the number of motor interacting with actin (N) is dropped from that in the half-sarcomere (294) to 16



- T_2 in situ
- T_2 random orient



The random orientation of motors reduces to $\frac{1}{2}$ the step size ($= T_2$ curve intercept) and this emerges in the collective motor as a corresponding reduction in V_0 : compare the simulated F - V curves, black continuous line with T_2 as in situ, red with T_2 adapted for the random orientation step size.

With $N = 16$, due to the reduction of duty ratio with V , the number of attached motors (N_a) drops below 3 during shortening against a load $< 0.4 F_0$

Motori processivi e non processivi

Meccanica ed energetica per la massima velocità (bassi carichi)

La **distanza di scorrimento** del filamento durante un ciclo completo di idrolisi, è :

$$\Delta = V \cdot \tau_0 = V / k_{\text{cat}} .$$

La **distanza dovuta al *working stroke*** è:

$$\delta = V \cdot \tau_{\text{on}} .$$

dato che $\tau_0 = \tau_{\text{on}} / r$ ne deriva: $\Delta = (V \cdot \tau_{\text{on}} / r) = \delta / r$

→ Δ aumenta riducendo r

Miosina muscolare: $\delta = 10 \text{ nm}$ e $r = 0.05 \rightarrow \Delta = 200 \text{ nm}$.

Chinesina: $\delta = 8 \text{ nm}$ e $r = 0.5 \rightarrow \Delta = 16 \text{ nm}$

$V = \Delta \cdot k_{\text{cat}}$ → aumenta con l'aumento di Δ (riduzione di r) e/o con l'aumento di k_{cat}

Comparative kinetics of molecular motors

Motor	Function	V_F (nm/s)	ATPase <i>in vitro</i> (s ⁻¹)	Δ (V/kcat) (nm)	τ_{on} (ms)	r ($\tau_{on}k_{cat}$)	δ (= $\Delta \cdot r$) (nm)	δ_{oss} (nm)
Miosin								
Miosin I	Ameboid motility, hair cell adaptation	200	6	33				
Miosin II	Fast skeletal muscle	6000	25	240	1	0.025	6	5-10
Miosin II	Smooth muscle	250	1.2	210	25	0.03		
Miosin V	Vescicle transport	350	5	70	100	0.5	35	35
Dynein								
Axonemal	Movement of cilia and sperm	-4500	10	450		0.02	8	
Cytoplasmic	Retrograde axonal transport	-1250	2	625				
Kinesin								
Conventional	Anterograde axonal transport	840	44	19	10	0.44	8.3	8
Nkin	Secretory vescicle transport	1800	78	23				
BimC	Mitotic spindle	60	2	30				

Nei **motori non processivi**, come la miosina muscolare, velocità di scorrimento elevate possono essere raggiunte con bassi valori della velocità di idrolisi dell'ATP grazie al basso valore del *duty ratio*.

Nei **motori processivi** costituiti da dimeri, il *duty ratio* è 0.5 e una velocità di trasporto alta è ottenuta aumentando la velocità di idrolisi dell'ATP (chinesina) o l'ampiezza del passo (miosina V)

Testi consigliati:

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Edi-ermes Milano (2006).

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