Supplementary text

Filament lattice spacing

The hexagonal lattice of thick and thin filaments is compressed as sarcomere length is increased. In resting fibre bundles the spacing between the (1,0) planes of the lattice, $d_{1,0}$, decreased linearly from 35.11 ± 0.12 nm (mean ± SE, n=25 X-ray measurements from 12 bundles) at sarcomere length 2.1 μm to 28.64 ± 0.11 nm (n=7 measurements from 2 bundles) at sarcomere length 3.5 μm (Fig. S2*A*). The volume of the filament lattice per thick filament is $2/\sqrt{3} \times (d_{1,0})^2 \times$ sarcomere length (Fig. S2*B*) and this parameter increased slightly with increasing sarcomere length, from 3.05. 10^6 nm^3 at sarcomere length 2.1 μm to 3.36. 10^6 nm^3 at 3.5 μm.

Thus filament lattice volume determined from $d_{1,0}$ is not independent of sarcomere length in the conditions of these experiments, in contrast with the conclusions of early experiments using laboratory X-ray sources (Elliott *et al*., 1963; Millman, 1998). The volume change found here, an increase of about 10% over the sarcomere length range 2.1 to 3.5 μm, is relatively small compared with the resolution of the older studies. However regression of the lattice volume data in Fig. 6 of Millman (1998) against sarcomere length in the range 2.1 to 3.5 μm gives a slope of 476 ± 134 nm^2, and the corresponding value on inclusion of data from sarcomere lengths up to 4.3 μm is 197 ± 60 nm^2. Thus the present results are not inconsistent with the older literature, and suggest that cytoplasmic fluid enters the filament lattice at longer sarcomere lengths. This in turn implies that the transverse compressive forces on the filament lattice in highly stretched muscle fibres are not quite sufficient to reduce the inter-filament spacing to the value corresponding to constant lattice volume.

$d_{1,0}$ during isometric contraction at sarcomere length 2.1 μm (mean ± SE, n=25 measurements from 12 bundles) was 34.68 ± 0.03 nm, slightly smaller than the value

measured at rest. $d_{1,0}$ during isometric contraction decreased with increasing sarcomere length (Fig. S3*A*), to 28.62 ± 0.16 nm at sarcomere length 3.5 µm (n=7 measurements from two fibre bundles). The volume of the filament lattice (Fig. S3*B*) increased apparently linearly with increasing sarcomere length, from 2.88 x 10^6 nm^3 at sarcomere length 2.1 µm to 3.28 x 10^6 nm^3 at 3.5 µm. $d_{1,0}$ and lattice volume were slightly smaller during isometric contraction than at rest at all sarcomere lengths, but the difference was smaller at longer sarcomere length and was almost abolished at sarcomere length 3.5 µm, consistent with the presence of radial compressive forces due to actin-attached myosin heads in the region of overlap between thick and thin filaments during contraction.

References

Elliott GF, Lowy J & Worthington CR (1963). An X-ray and light diffraction study of the filament lattice of striated muscle in the living state and in rigor. *J Mol Biol* **6**, 295-305.

Millman B (1998). The filament lattice of striated muscle *Physiol Rev* **78**, 359-391.

Supplementary Figure captions

Figure S1. The cross-meridional width of the M3 reflection (detector pixel units) at rest (*A*) and during isometric contraction (*B*) plotted against sarcomere length; mean ± SE from a total of 10-14 bundles.

Figure S2. Equatorial lattice spacing ($d_{1,0}$; *A*) and lattice volume ($2/\sqrt{3} \times (d_{1,0})^2 \times$ sarcomere length; *B*) in resting fibres plotted against sarcomere length. Mean ± SE from a total of 10 bundles (2.2 m camera; triangles) and 4 bundles (6 m camera; circles), n=1-4 bundles for each point. Regression lines in *A* and *B* have slopes of -4.75 (± 0.19) x 10^{-3} and 212 ± 40 nm^2 respectively.

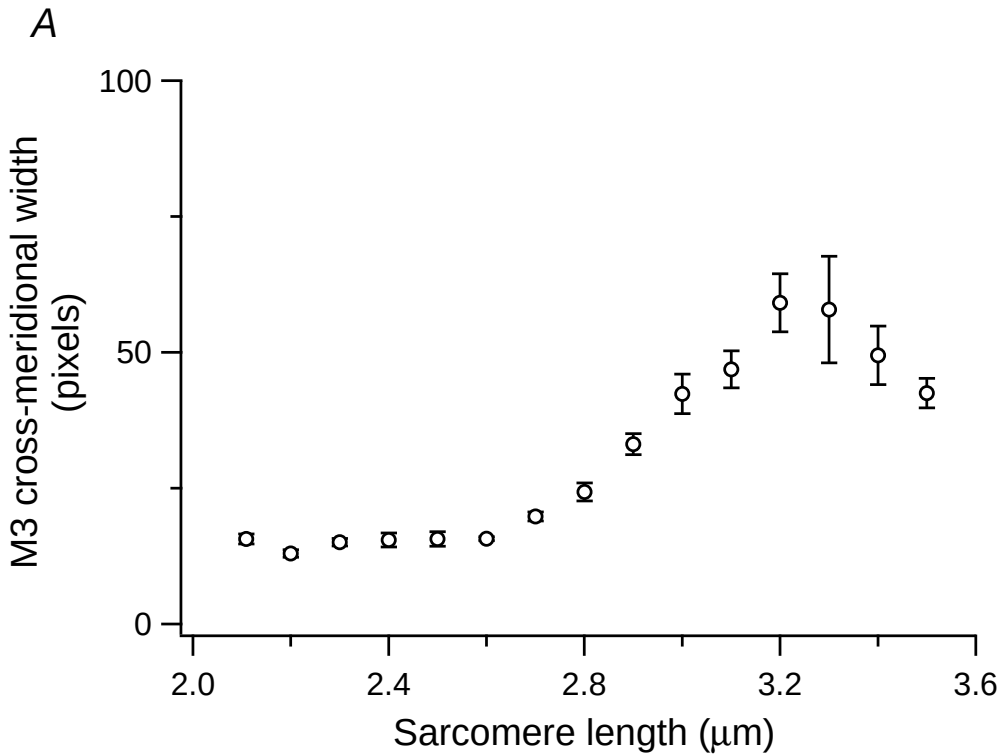
Figure S3. Equatorial lattice spacing ($d_{1,0}$; *A*) and lattice volume ($2/\sqrt{3} \times (d_{1,0})^2 \times$ sarcomere length; *B*) during active isometric contraction plotted against sarcomere length. Mean ± SE from a total of 10 bundles (2.2 m camera; triangles) and 4 bundles (6 m camera; circles); n=1-4 bundles for each point. The continuous regression lines fitted to the data in *A* and *B* have slopes of -4.28 (±0.15) x 10^{-3} and 289 ± 25 nm^2 respectively; the dashed lines show the results of the corresponding fits to data from the same set of fibre bundles at rest.

Figure S4. Sarcomere reflections in resting fibres corresponding to periodicities in the spacing range 160 nm to 80 nm. (*A*) Example profiles at sarcomere lengths 2.1, 2.65 and 3.3 µm. (*B*) Periodicity of the sarcomere reflections plotted against sarcomere length. Mean ± SE from a total of 5 bundles. Linear regression (continuous line) of black data points gave a slope of 0.48 ± 0.02 and ordinate intercept 20 ± 57 nm, consistent with the expected values 0.5 and 0 nm respectively corresponding to the dashed line. (C) Dependence of resting tension on sarcomere length; triangles: *Rana temporaria* (from Linari *et al*. 2000); circles: *Rana esculenta*.

Figure S5. Periodicity of the very low-angle reflections associated with the sarcomere repeat during active isometric contraction, plotted against resting sarcomere length. Mean ± SE from a total of 5 bundles. Linear regression (continuous line) of the black circles gave a slope of 0.509 ± 0.030 and ordinate intercept -42 ± 79 nm, consistent with the expected values 0.5, and 0 nm respectively corresponding to the dashed line.

Figure S6. Fractional intensities of the low-angle (LA, open circles), mid-angle (MA, gray), and high-angle (HA, black) components of the M3 meridional reflection in resting muscle fibres in the sarcomere length range 2.1 – 2.9 µm.

Figure S1


A
100
50
0
M3 cross-meridional width (pixels)
2.0
2.4
2.8
3.2
3.6
Sarcomere length (μm)

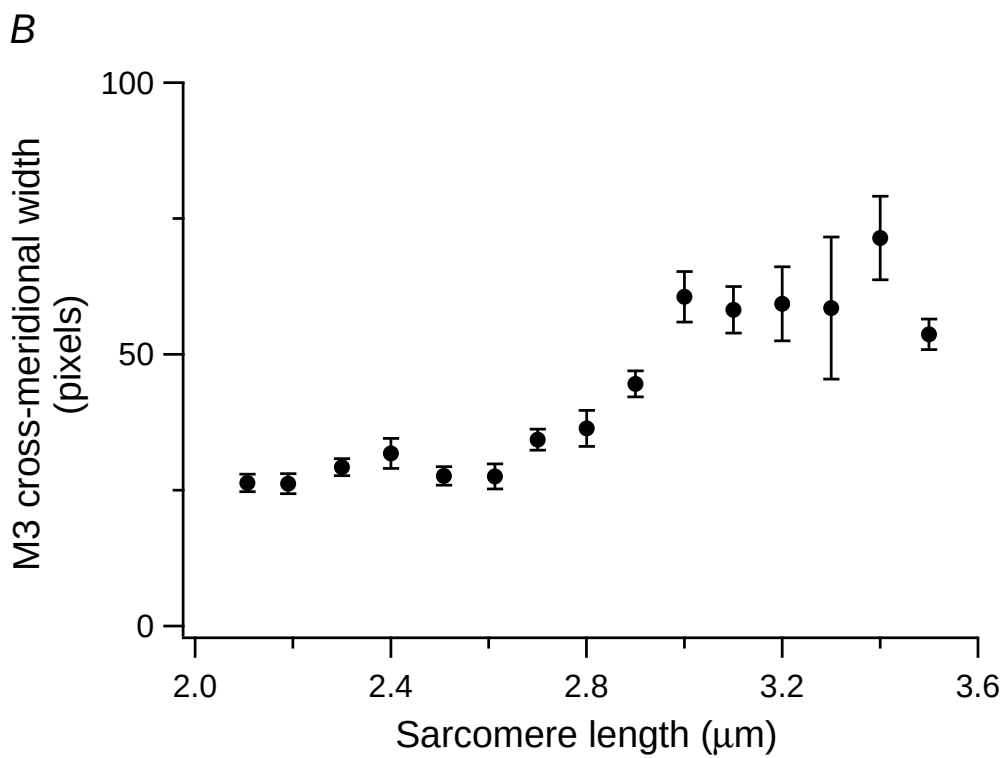
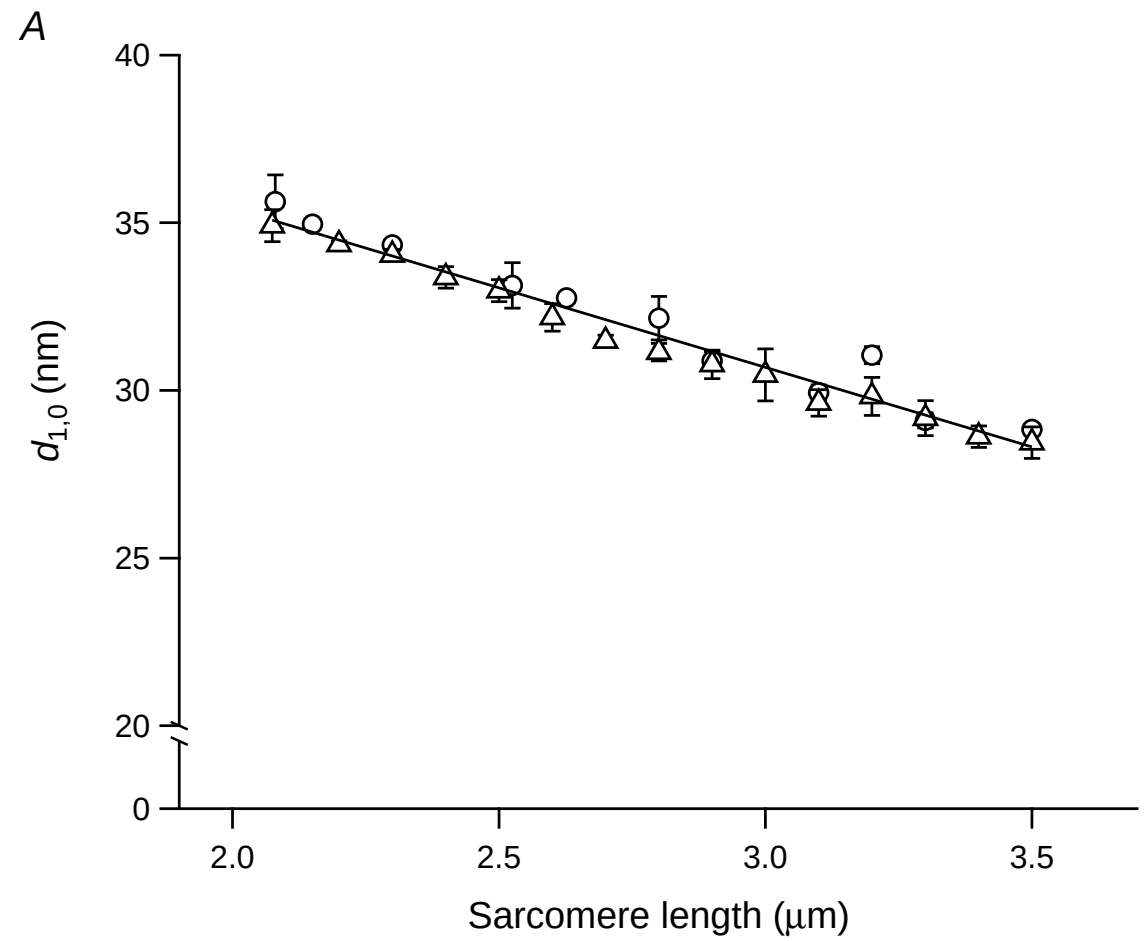

B
100
50
0
M3 cross-meridional width (pixels)
2.0
2.4
2.8
3.2
3.6
Sarcomere length (μm)

Figure S2


A
40
35
30
25
20
0
$d_{1,0}$ (nm)
2.0
2.5
3.0
3.5
Sarcomere length (μm)

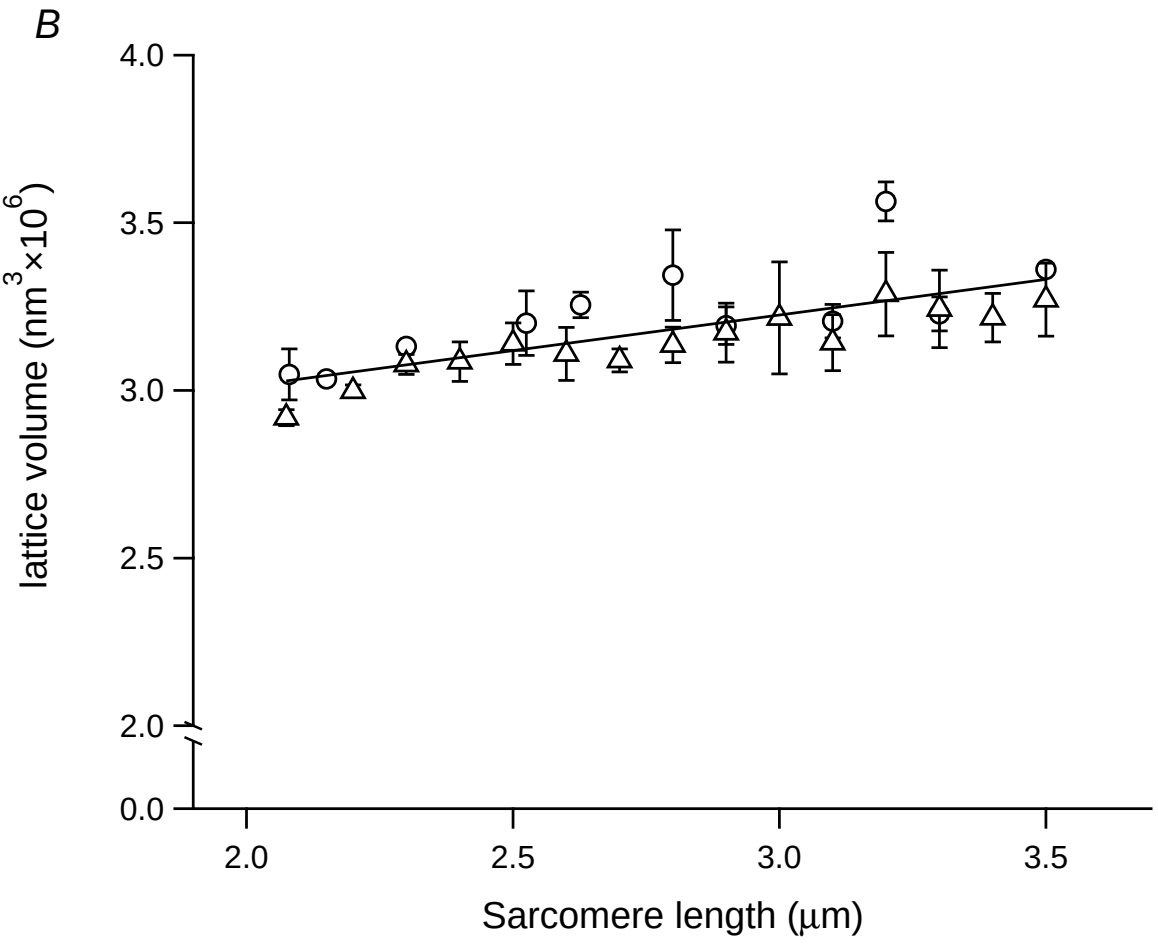
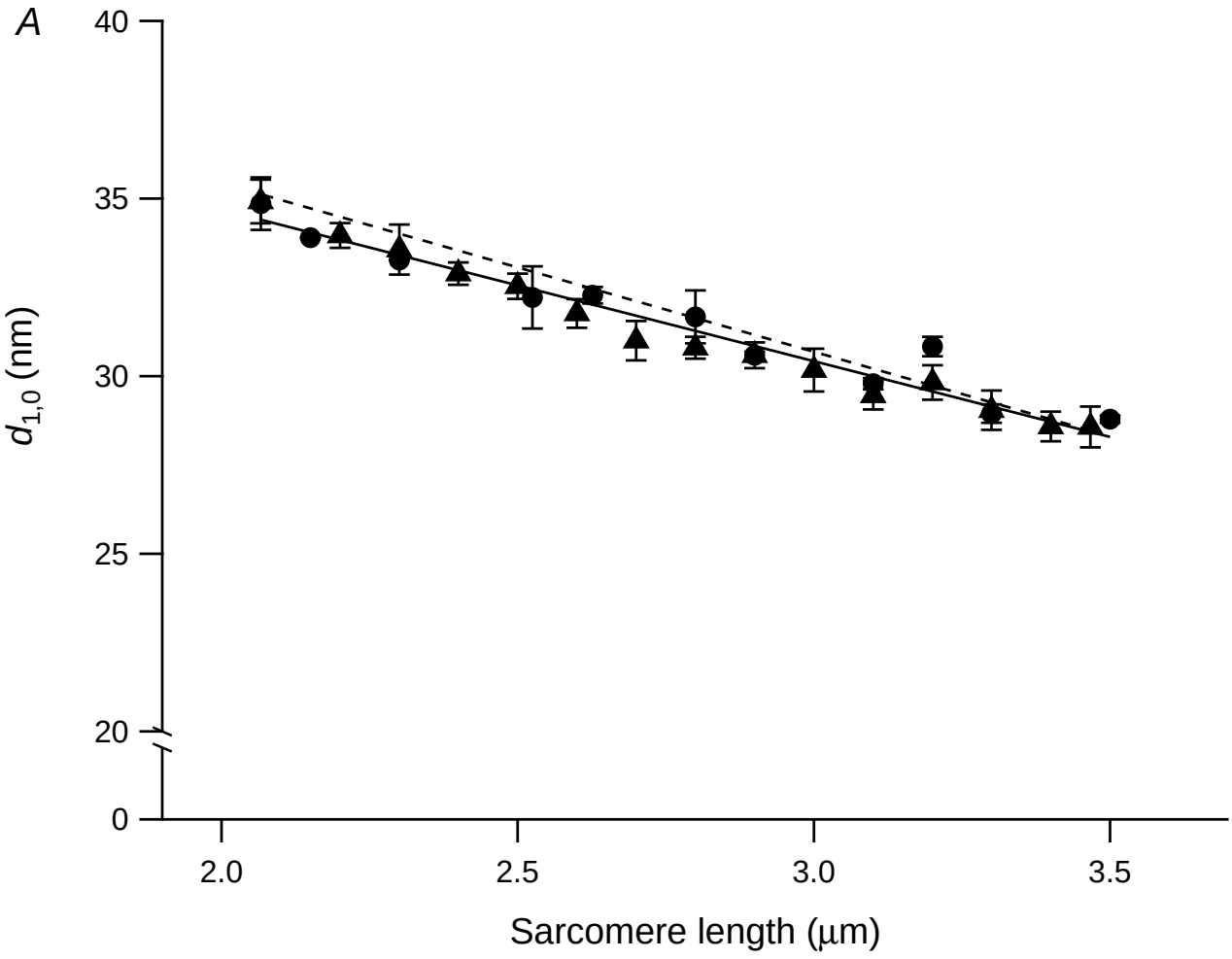

B
4.0
3.5
3.0
2.5
2.0
0.0
lattice volume ($nm^3 \times 10^6$)
2.0
2.5
3.0
3.5
Sarcomere length (μm)

Figure S3


A
40
35
30
25
20
0
$d_{1,0}$ (nm)
2.0
2.5
3.0
3.5
Sarcomere length (μm)

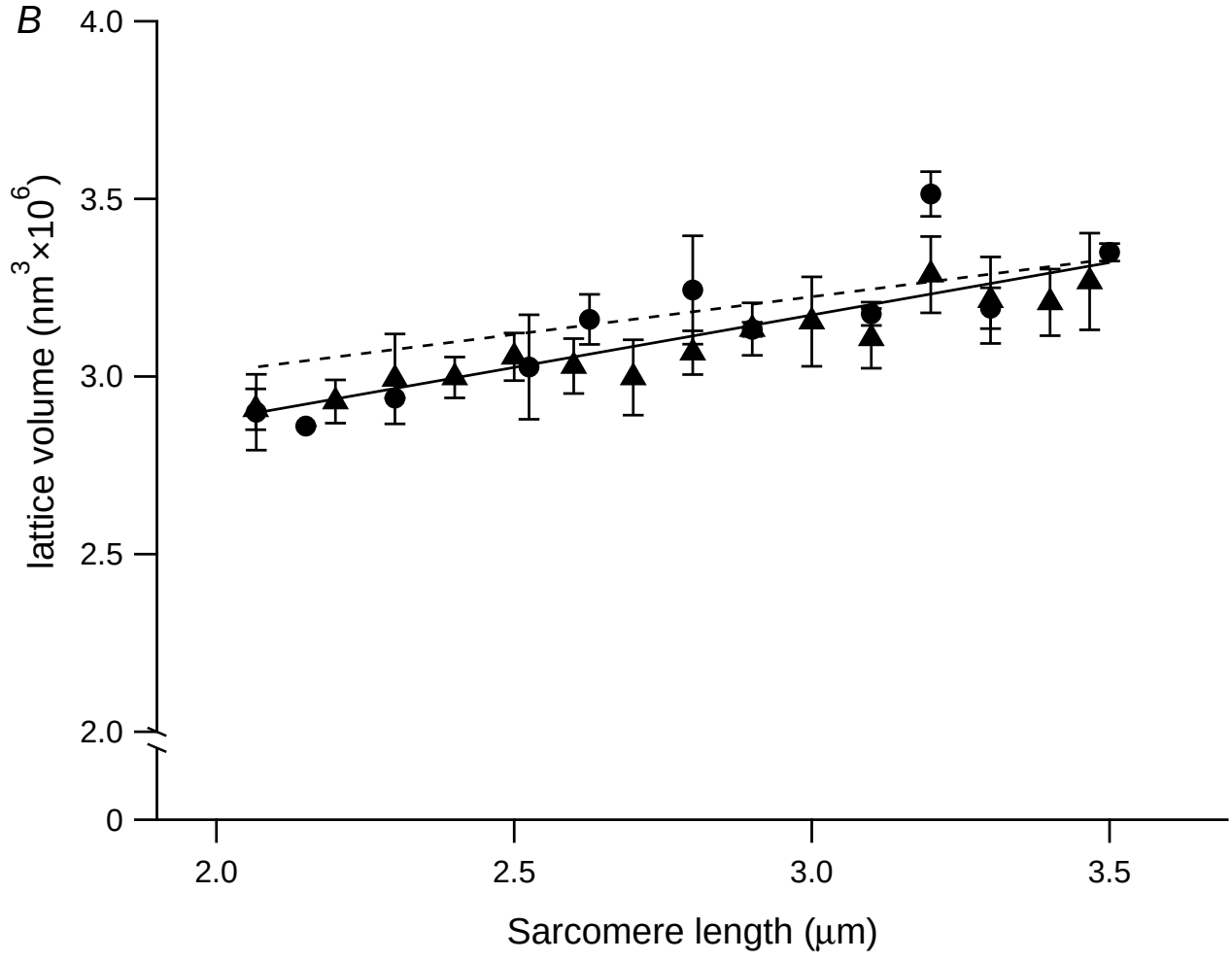

B
4.0
3.5
3.0
2.5
2.0
0
lattice volume ($nm^3 \times 10^6$)
2.0
2.5
3.0
3.5
Sarcomere length (μm)

Figure S4

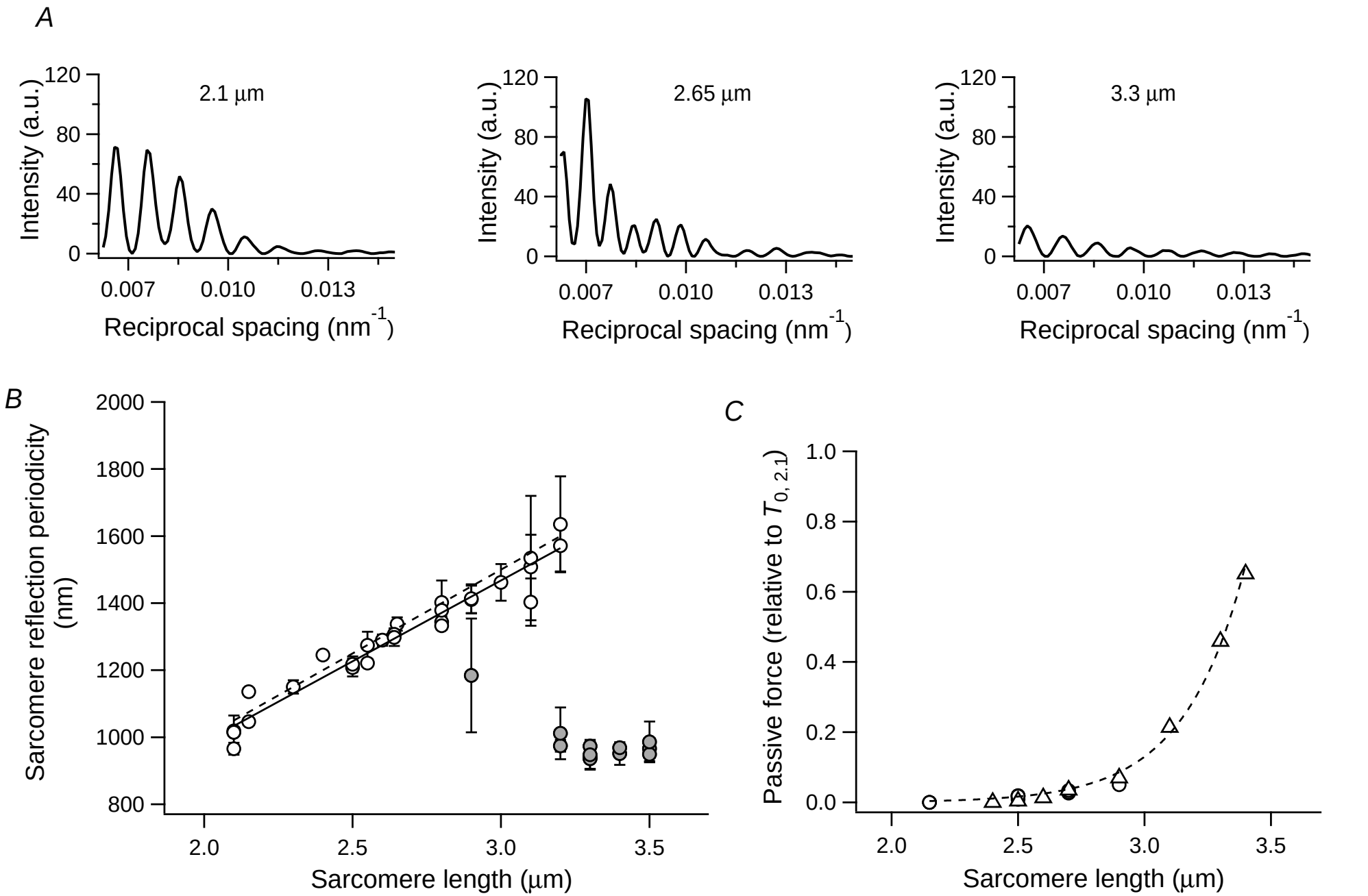

A
2.1 µm
2.65 µm
3.3 µm
Intensity (a.u.)
120
80
40
0
0.007
0.010
0.013
Reciprocal spacing (nm-1)
B
Sarcomere reflection periodicity (nm)
2000
1800
1600
1400
1200
1000
800
2.0
2.5
3.0
3.5
Sarcomere length (µm)
C
Passive force (relative to T0, 2.1)
1.0
0.8
0.6
0.4
0.2
0.0
Sarcomere length (µm)

Figure S5

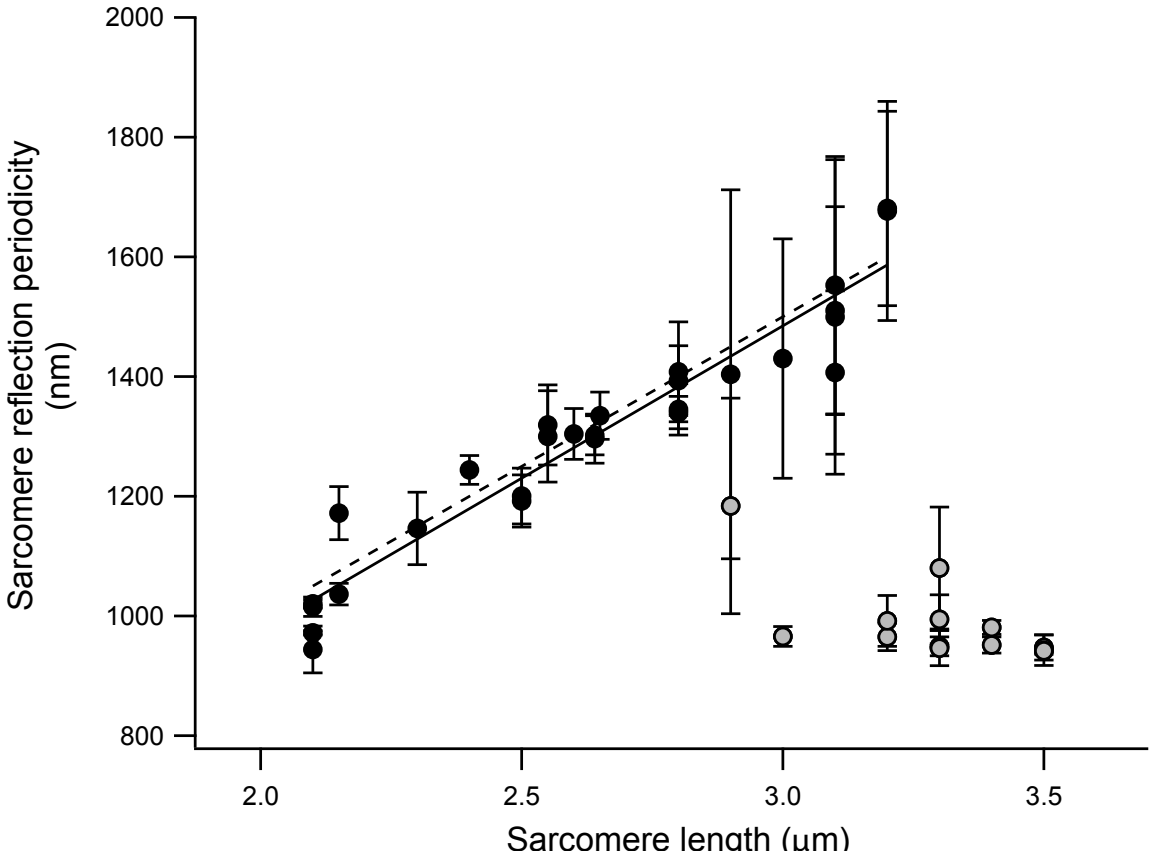
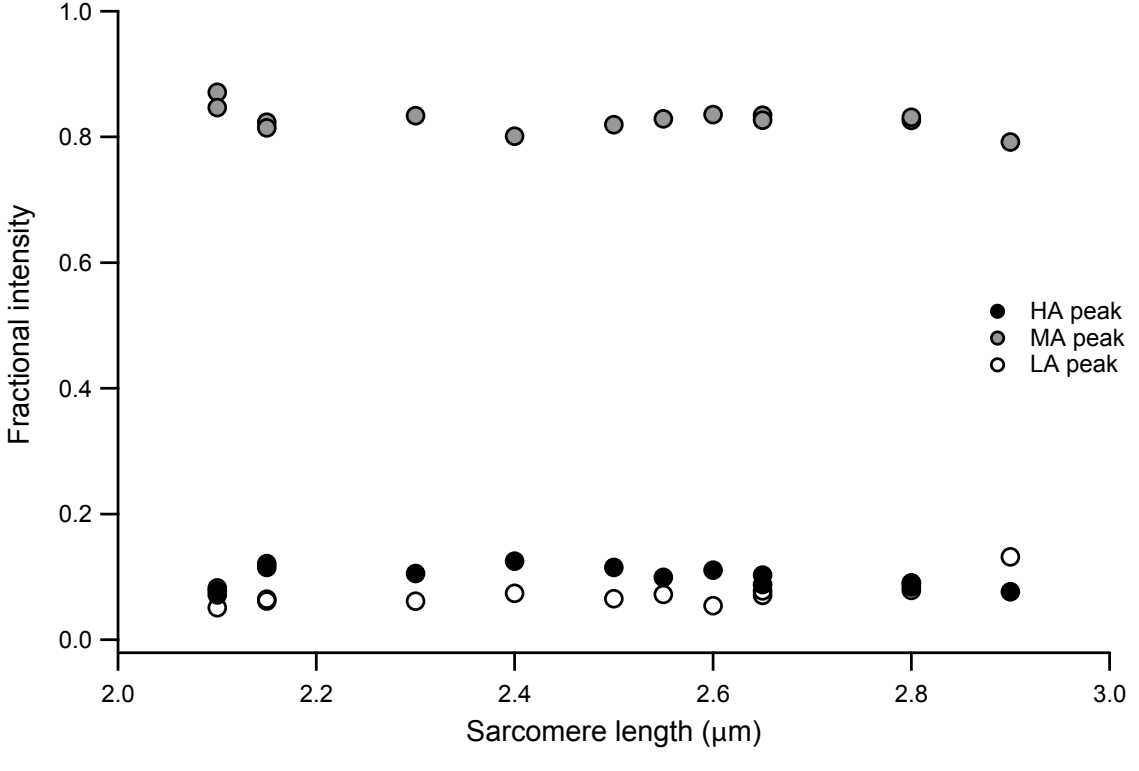

2000
1800
1600
1400
1200
1000
800
Sarcomere reflection periodicity (nm)
2.0
2.5
3.0
3.5
Sarcomere length (μm)

Figure S6


1.0
0.8
0.6
0.4
0.2
0.0
Fractional intensity
HA peak
MA peak
LA peak
2.0
2.2
2.4
2.6
2.8
3.0
Sarcomere length (μm)