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1 **Titin-induced force enhancement and force depression:**

2 **A ‘sticky-spring’ mechanism in muscle contractions?**

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8
9 **The sliding filament and crossbridge theories do not suffice to explain a**
10 **number of muscle experiments. For example, from the entire muscle to myofibrils,**
11 **predictions of these theories were shown to underestimate the force output during**
12 **and after active tissue stretch. The converse applies to active tissue shortening.**

13 **In addition to the crossbridge cycle, we propose that another molecular**
14 **mechanism is effective in sarcomere force generation. We suggest that, when due**
15 **to activation, myosin binding sites are available on actin, the giant protein titin’s**
16 **PEVK region attaches itself to the actin filament at those sites. As a result, the**
17 **molecular spring length is dramatically reduced. This leads to increased passive**
18 **force when the sarcomere is stretched and to decreased or even negative passive**
19 **force when the sarcomere shortens. Moreover, during shortening, the proposed**
20 **mechanism interferes with active-force production by inhibiting crossbridges.**

21 **Incorporation of a simple ‘sticky-spring’ mechanism model into a Hill-type**
22 **model of sarcomere dynamics offers explanations for several force-enhancement**
23 **and force-depression effects. For example, the increase of the sarcomere force**
24 **compared to the force predicted solely by the sliding filament and crossbridge**
25 **theories depends on the stretch amplitude and on the working range. The same**

26 **applies to the decrease of sarcomere force during and after shortening. Using only**
27 **literature data for its parameterization, the model predicts forces similar to**
28 **experimental results.**

29

30 **Key words:** molecular mechanism, model, stretch, shortening, sarcomere, myofilament
31 interaction

32

33 **1. Introduction**

34 From whole muscle to myofibrils, the steady-state force after an active stretch can
35 exceed the force measured in an isometric contraction at the same final length (Abbott
36 and Aubert, 1952; Edman et al., 1982) and can even exceed the maximum isometric
37 force (Herzog et al., 2008; Lee and Herzog, 2008). While the first result might be
38 explained with non-uniform sarcomere lengthening (Morgan, 1990), the latter cannot be
39 explained solely within the framework of the sliding filament (Huxley and Niedergerke,
40 1954; Huxley and Hanson, 1954) and the crossbridge theories (Huxley, 1957). The
41 physiological mechanism(s) underlying enhanced forces have not been understood to
42 date (see Herzog et al., 2008 for a review). However, it has been suggested that residual
43 force enhancement (the force difference between the two measured steady-state forces)
44 can be separated into active and passive parts (Herzog et al., 2006) related to the
45 crossbridge mechanism and to titin, respectively (Herzog et al., 2008). In contrast,
46 Pinniger et al. (2006) provided evidence suggesting that force enhancement during and
47 after stretch results from a mechanism independent of the crossbridges and may be
48 related to titin.

49 Stretching active fibers from the same initial length (throughout the manuscript
50 initial refers to the length at which activation occurs) with different constant velocities,
51 Edman et al. (1982) found constant stiffness greater than zero after a short transient

52 phase, suggesting a spring-like characteristic (elongation-dependent) of force
53 enhancement. Moreover, muscles stretched on the descending limb of the active force-
54 length relationship exceeded the isometric force at the stretched length by a larger
55 amount than muscles stretched the same distance on the ascending limb (Morgan et al.,
56 2000; Peterson et al., 2004). These effects are hard to explain with enhanced crossbridge
57 forces. Not only would the crossbridge have to exert force rising with stretch amplitude,
58 but it would also have to ‘know’ about the initial filament overlap. This substantiates
59 the presence of a force-producing mechanism independent of the crossbridge force-
60 generation producing increasing forces with increasing initial sarcomere length.

61 Another reported phenomenon of muscular contraction is the decreased steady-state
62 force after active muscle (e.g. Abbott and Aubert, 1952) or fiber (e.g. Edman, 1975,
63 1996; Edman et al., 1993; Sugi and Tsuchiya, 1988) shortening compared to the force
64 measured in an isometric contraction at the same final length. Residual force depression
65 (force difference between these two measured steady state forces) was found to be
66 proportional to the shortening distance (Marechal and Plaghki, 1979; Edman, 1975;
67 Herzog and Leonard, 1997). Mechanisms proposed in this context include stress-
68 induced inhibition of crossbridges (Marechal and Plaghki, 1979).

69 Physiological explanations, as well as most phenomenological descriptions, deal
70 with either force enhancement (the increase in experimental force compared to the force
71 expected from valid sliding filament and crossbridge theories; e.g. Morgan et al., 1982)
72 or with force depression (the converse of the above; e.g. Marechal and Plaghki, 1979;
73 Meijer et al., 1998). Forcinito et al. (1998) showed that a physiologically non-motivated
74 recruitable elastic rack conceptually explains force enhancement and force depression
75 effects. However, in contradiction to experimental results (Herzog and Leonard, 2000),
76 such modeling predicts commutativity of shortening-stretch vs. stretch-shortening
77 experiments with respect to the subsequent steady-state muscle force.

78 In this study, we propose and model a physiological mechanism related to titin
79 (connectin) to describe both force-enhancement and force-depression effects. The giant
80 protein titin is a structural part of myosin with a free part acting as a molecular spring
81 connecting myosin to the Z-disc near the actin filament (Linke et al., 1998; Prado et al.
82 2005). This molecular spring consists of three morphologically distinct regions: 1) the
83 proximal Immunoglobulin (Ig) region connecting to the Z-disc, 2) the adjacent PEVK
84 region (rich in proline (P), glutamic acid (E), valine (V) and lysine (K)), and 3) the
85 distal Ig region attached to myosin. Interaction with actin seems possible when calcium
86 is present (Kellermayer and Granzier, 1996) and the PEVK region can attach to the
87 actin filament (Bianco et al., 2007). Such attachments may occur at myosin binding
88 sites on actin (Niederlander et al., 2004). Here, we assume that the PEVK region
89 attaches itself to the myosin binding sites on actin during activation (Fig. 1, top),
90 thereby altering titin's behavior during subsequent length changes compared to passive
91 length changes. This 'sticky-spring' mechanism might induce considerable forces
92 during and after sarcomere stretch, as well as during and after sarcomere shortening.

93 By dramatically reducing titin's free spring length in the active state (i.e. when
94 calcium concentration is high), the 'sticky-spring' mechanism may lead to 1) increased
95 passive half-sarcomere force during and after active stretch and 2) decreased or even
96 negative passive half-sarcomere force during and after active shortening. Moreover,
97 during shortening, the proposed mechanism may interfere with active-force production
98 by inhibiting crossbridges. Resultant, the mechanism might offer an explanation for
99 several force-enhancement and force-depression effects including the non-
100 commutativity (Herzog and Leonard, 2000) of those effects.

101

102 **2. Methods**

103 2.1 Model

104 We restricted our modeling to the half-sarcomere as the force-producing unit of the
 105 muscle (Fig. 1) and neglected elasticity in series to the crossbridges such as elasticity of
 106 the actin and myosin filaments. In addition, we normalized forces to the maximum
 107 isometric active force $F_{[im]}$ (throughout the manuscript, values subscripted with brackets
 108 were constant for at least one experiment) produced at optimal thin (actin) and thick
 109 (myosin) filament overlap and assumed that all forces would act along the myofilament
 110 axis.

111 The normalized half-sarcomere force f_{hs} equaled the sum of an active (crossbridges)
 112 component f_{act} and a passive (titin) component f_{pass} :

113

$$114 \quad f_{hs} = F_{hs} / F_{[im]} = f_{act} + f_{pass} . \quad (1)$$

115

116 A common Hill-type separation approach matching the sliding filament and crossbridge
 117 theories defined the active force:

118

$$119 \quad f_{act} = F_{act} / F_{[im]} = f_v(v_{hs}) \cdot f_l(l_{hs}, l_{hs[0]}) . \quad (2)$$

120

121 The f_v is a factor due to the force-velocity (Hill, 1938; Abbot and Aubert, 1952) relation
 122 (shortening: $f_v < 1$; stretch: $f_v > 1$) and depends on the half-sarcomere velocity v_{hs} . The f_l
 123 is a factor due to the force-length (Gordon et al., 1966) relation ($f_l \leq 1$). According to the
 124 sliding filament theory, f_l depends on the number of effective crossbridges. Moreover,
 125 on the steep part of the ascending limb of the active force-length relationship, it also
 126 assumedly depends on counteracting compressive forces. Following the proposed
 127 mechanism, however, f_l can be decreased due to inhibited crossbridges; it not only

128 depends on the half-sarcomere length l_{hs} , but also on the initial half-sarcomere length
129 $l_{hs[0]}$ (please see appendix A).

130 We modeled titin's molecular spring region as an elastic spring. In the passive state
131 (calcium concentration is low, i.e. the sarcomere is deactivated), f_{pass} is a function of l_{hs} .
132 In the active state (calcium concentration is high, i.e. the sarcomere is activated), the
133 PEVK region attaches itself to the actin filament. Hence, titin's distal Ig region can be
134 pulled not only in the same direction (Fig. 1, middle) as the crossbridges ($f_{pass} > 0$), but
135 also in the opposite (Fig. 1, bottom) direction ($f_{pass} < 0$). Resultant, f_{pass} is a function of
136 l_{hs} and $l_{hs[0]}$ as described in the next section.

137 We assumed that skinned myofibril passive tension is induced by titin (Linke et al.,
138 1998). With a typical value of maximum active muscle tension of 20 N/cm^2 , we
139 translated measured myofibril tension (Linke et al., 1998) into normalized tension
140 corresponding to normalized titin force. Further length data (Linke et al., 1998) yielded
141 force-elongation data for the entire molecular spring and for the distal Ig and PEVK
142 regions (Fig. 2). We used lookup tables and linearly interpolated desired values from
143 these essentially nonlinear data which were extrapolated linearly when necessary.

144

145 2.2 The titin-induced 'sticky-spring' mechanism

146 Consider a rectangular adhesive tape stuck on a plain surface. It can bear high forces
147 if pulled along its length axis away from its adherend (stretch), but is easily peeled off
148 in the opposite direction (shortening). We suggest that titin's PEVK region may
149 function similarly during muscular contraction.

150 Along its length, the PEVK region seems to be able to attach to the actin filament, to
151 quickly reattach itself when the bond is broken, and moreover, the attachment may bear
152 an average maximum force $F_{[am]}$ of 8 pN (Bianco et al., 2007). In the active state, we
153 assume that PEVK-actin attachment occurs spontaneously when the PEVK region

154 approaches a myosin binding site on actin, and that the attachment can bear $F_{[am]}$ in any
 155 direction (i.e. in our model in any of the two longitudinal half-sarcomere directions).

156 To normalize the force induced by the ‘sticky-spring’ mechanism, we related $F_{[am]}$
 157 to $F_{[im]}$. With assumed 20 N/cm^2 maximum isometric active muscle tension and a 42 nm
 158 distance between myosin filaments in the crystalline structure of skeletal muscle, the
 159 maximum active force related to one myosin is 300 pN. Six titin molecules connect
 160 each myosin filament to the Z-disc at the corresponding actin filaments. Hence the
 161 maximum isometric force related to one titin molecule is 50 pN, and $F_{[am]} = 8 \text{ pN}$
 162 corresponds to $f_{[am]} = 0.16$ normalized maximum attachment force.

163

164 *2.2.1 Single active stretch*

165 First, assume a high calcium concentration and that the PEVK region is attached to
 166 actin thereby dramatically reducing the free molecular spring length to the distal Ig
 167 region length (Fig. 1, top). At the initial half-sarcomere length $l_{hs[0]}$, the initial passive
 168 half-sarcomere force $f_{pass[0]}$ acts in the PEVK region as well as in the proximal and
 169 distal Ig regions. However, no force pulls on the PEVK-actin attachments. When the
 170 active sarcomere is stretched, the force in the distal Ig region increases due to a pull on
 171 the most distal (nearest to myosin) PEVK-actin attachment. As soon as the increase in
 172 force exceeds the normalized attachment force $f_{[am]}$, the attachment may break and
 173 PEVK may reattach at the same binding site, thus reducing the PEVK chain length
 174 between the two most distal PEVK-actin attachments and leading to an additional force
 175 bearing of the second (from distal) attachment site. If the stretch continues, more and
 176 more of the initial PEVK-actin attachments will bear force, and the elongating PEVK
 177 region will approach additional binding sites (Fig. 1, middle). Hence, during the stretch,
 178 the PEVK region may be seen as a ‘sticky-spring’, and its distal-end force f_{sticky} is the

179 sum of the constant $f_{pass[0]}$ (compare appendix B) and the cumulated force pulling on the
 180 PEVK-actin attachments f_{att_cum} (Fig. 1, middle). Hence

181

$$182 \quad f_{pass} = f_{sticky} = f_{pass[0]} + f_{att_cum} . \quad (3)$$

183

184 Note that the sum of these forces does not indicate simultaneous parallel action, but a
 185 sequential increase of passive force.

186 We derived a constant stiffness $k_{[sticky]}$ dependent on $l_{hs[0]}$ to characterize the increase
 187 in force due to ‘sticky-spring’ elongation (see appendix B). The half-sarcomere length
 188 change equals the sum of the PEVK region’s elongation Δl_{PEVK} equaling Δl_{sticky} and the
 189 distal Ig region’s elongation Δl_{dist_Ig} (Fig. 1, middle):

190

$$191 \quad l_{hs} - l_{hs[0]} = \Delta l_{sticky} + \Delta l_{dist_Ig} = f_{att_cum} / k_{[sticky]}(l_{hs[0]}) + l_{dist_Ig} - l_{dist_Ig[0]} , \quad (4)$$

192

193 where l_{dist_Ig} is the length of the distal Ig region and $l_{dist_Ig[0]}$ is its length attained under
 194 $f_{pass[0]}$.

195

196 2.2.2 Single active shortening

197 Here, we only consider the case where the initial passive force is lower than $f_{[am]}$
 198 which, according to the used literature data, corresponds to half-sarcomere lengths
 199 below $1.66 \mu\text{m}$ ($f_l = .31$, descending limb of active force-length relation). Such lengths
 200 are exceeded only by a few muscles within their operating ranges (Burkholder and
 201 Lieber, 2001). Again, assume that the PEVK region is attached to actin (Fig. 1, top) and
 202 further, that the most distal PEVK-actin attachment coincides with the distal end of the
 203 PEVK region. The following shortening can be separated into three distinct length

204 ranges with ‘sticky-spring’ effects on active and passive half-sarcomere forces. In the
 205 first phase (Fig. 3, top), shortening leads to a stronger decrease in passive force
 206 compared to passive shortening since the molecular spring length is reduced to the
 207 length of the distal Ig region. Hence

$$209 \quad l_{dist_Ig} = l_{dist_Ig[0]} - (l_{hs[0]} - l_{hs}). \quad (5)$$

210
 211 The ‘sticky-spring’ force equaling the passive force is then given by the force-length
 212 data of the distal Ig region. Note that the PEVK region is pre-stretched by $f_{pass[0]}$ before
 213 attaching itself to actin. Therefore, as f_{sticky} decreases to zero during shortening to the
 214 first shortening phase’s final length $l_{hs[1]}$, the resulting force pulling on the most distal
 215 PEVK-actin attachment towards the Z-disc increases from zero to $f_{pass[0]}$.

216 During the second shortening phase (Fig. 3, middle), a passive tensile force
 217 counteracting the active force develops in the distal Ig region. Notably, it also pulls
 218 solely on the most distal PEVK-actin attachment towards the Z-disc. Consequently, the
 219 PEVK-actin attachments break consecutively whenever this force exceeds $f_{[break]} = f_{[am]}$
 220 $- f_{pass[0]}$. Since the mechanical energy expended to peel off the PEVK region during
 221 half-sarcomere shortening increased approximately with the square of the displacement
 222 (Fig. C.1B), we assumed the ‘sticky-spring’ peel-off force to increase linearly with half-
 223 sarcomere shortening $l_{hs[1]} - l_{hs}$ with a slope of $k_{[short_2]}$ depending on $l_{hs[0]}$ (see appendix
 224 C):

$$226 \quad f_{pass} = -f_{sticky} = -k_{[short_2]}(l_{hs[0]}) \cdot (l_{hs[1]} - l_{hs}). \quad (6)$$

227

228 The negative sign indicates that the ‘sticky-spring’ force opposes the active force f_{act} .

229 Myosin entering the range of the attached PEVK region at the length $l_{hs[1]}$ reduces
 230 the number of effective crossbridges, thereby decreasing active forces compared to the
 231 crossbridge and sliding filament theories. The f_i value decrease is defined by the region
 232 l_{block} (compare appendix A) where potential crossbridges are inhibited by the attached
 233 PEVK region (Fig. 3, middle):

$$235 \quad l_{block} = \begin{cases} l_{hs[1]} - l_{hs} - \Delta x_{PEVK_dist}, & x_{PEVK_prox[0]} < l_{hs} - l_{[myosin]} \\ l_{hs[1]} - l_{hs} - \Delta x_{PEVK_dist} - (x_{PEVK_prox[0]} - (l_{hs} - l_{[myosin]})), & x_{PEVK_prox[0]} \geq l_{hs} - l_{[myosin]} \end{cases} \quad (7)$$

236
 237 The proximal displacement of the attached PEVK region's distal end Δx_{PEVK_dist} (Fig. 3,
 238 middle) is given by

$$239 \quad \Delta x_{PEVK_dist} = \begin{cases} 0, & l_{hs[1]} - l_{hs} < l_{[dist_Ig_break]} \\ s(l_{hs[0]}) \cdot (l_{hs[1]} - l_{hs} - l_{[dist_Ig_break]}), & l_{hs[1]} - l_{hs} \geq l_{[dist_Ig_break]} \end{cases} \quad (8)$$

240
 241 where $s(l_{hs[0]})$ (see appendix C) is a gearing < 1 relating the length change of the half-
 242 sarcomere $l_{hs[1]} - l_{hs}$ offset by $l_{[dist_Ig_break]}$ (the length of the distal Ig region strained by
 243 $f_{[break]}$) to Δx_{PEVK_dist} . The constant length $x_{PEVK_prox[0]}$ corresponds to the distance
 244 between the Z-disc and the proximal end of the PEVK region, and $l_{[myosin]}$ (0.8 μm) is
 245 the length of half a myosin molecule.
 246

247 The third shortening phase (Fig. 3, bottom) begins when the distal Ig region's distal
 248 end passes the PEVK region's proximal end at the corresponding length $l_{hs[2]}$. In this
 249 situation the distal Ig region is pre-stretched to $l_{dist_Ig[2]}$ with the force $f_{sticky[2]} (< f_{[break]})$.
 250 We assume that during half-sarcomere shortening $l_{hs[2]} - l_{hs}$ the PEVK region attaches
 251 as described in section 2.2.1 and that similar equations apply, however, resulting here in
 252 passive force counteracting the active force. Remaining initial PEVK-actin attachments

253 continue to break consecutively during shortening, thus, contrary to stretch, no initial
 254 PEVK-actin attachments can be recruited permanently for force bearing of the ‘sticky-
 255 spring’. Thus, ‘sticky-spring’ force only increases due to approaching new binding sites
 256 on actin at intervals $c_{[bind]}$ (0.038 μm), and $k_{[sticky]}$ equals $f_{[am]}/c_{[bind]}$ (compare Fig.
 257 B.1A). Neglecting the decrease in force distal to the first newly approached binding site
 258 due to its minor role in passive force generation, the passive force equals (compare Eq.
 259 (3))

260

$$261 \quad f_{pass} = -f_{sticky} = -(f_{sticky[2]} + f_{att_cum}). \quad (9)$$

262

263 The proximal displacement of the PEVK region’s proximal end Δx_{PEVK_prox} (Fig. 3,
 264 bottom) is defined by $k_{[sticky]}$. Hence (compare Eq. (4)),

265

$$266 \quad l_{hs[2]} - l_{hs} = \Delta x_{PEVK_prox} + \Delta l_{dist_Ig} = f_{att_cum} / k_{[sticky]} + l_{dist_Ig} - l_{dist_Ig[2]}. \quad (10)$$

267

268 For simplicity, we assumed that the attached PEVK-region’s distal end moved
 269 towards the Z-disc by half Δx_{PEVK_prox} until it reached the third-phase initial locus of the
 270 proximal PEVK end. Hence

271

$$272 \quad l_{block} = \begin{cases} l_{block[2]} + .5 \cdot \Delta x_{PEVK_prox} & \Delta x_{PEVK_prox} < 2 \cdot l_{block[2]} \\ \Delta x_{PEVK_prox} & \Delta x_{PEVK_prox} \geq 2 \cdot l_{block[2]} \end{cases}, \quad (11)$$

273

274 where $l_{block[2]}$ corresponds to the length l_{block} at the end of the second shortening phase.

275

276 **Results**

277 The ‘sticky-spring’ mechanism as modeled is independent of stretch or shortening
278 velocity. Consequently, we show forces during active stretch (Fig. 4) and during active
279 shortening (Fig. 5) from different initial lengths in force vs. half-sarcomere length plots.
280 The f_v value resulting from the force-velocity relation modulating the active half-
281 sarcomere force component is, however, set to 1 (isometric condition). This approach
282 enables the reading of force enhancement during stretch and force depression during
283 shortening, as well as the reading of residual force enhancement and residual force
284 depression at any half-sarcomere length.

285 ‘Sticky-spring’ stiffness in stretch experiments increases with increasing initial half-
286 sarcomere length (Fig. B.1A).

287

288 **Discussion**

289 In addition to the crossbridge cycle, we proposed a titin-induced molecular ‘sticky-
290 spring’ mechanism to contribute to sarcomere force generation. When compared to
291 passive half-sarcomere length changes, it increases passive force during and after active
292 stretch (Fig. 4, top), and it produces decreased or negative passive force during and after
293 active shortening (Fig. 5, top). Moreover, during shortening, the ‘sticky-spring’
294 mechanism interferes with active force production by inhibiting crossbridges (Fig. 5,
295 middle). The mechanism accounts for many experimental observations concerning
296 force-enhancement and force-depression effects leaving the sliding filament and the
297 crossbridge theories intact (i.e. without increasing or decreasing the rate functions of
298 attachment or detachment of myosin heads to actin). Notably, we used only
299 experimental data from the literature to derive ‘sticky-spring’ forces.

300 Residual force enhancement and residual force depression depend on the muscle’s
301 working range. More precisely, no residual force enhancement (Edman et al., 1982;
302 Morgan et al., 2000; Peterson et al., 2004) and no residual force depression (Gordon et

303 al., 1966; Morgan et al., 2000) were found in the steep range of the ascending limb of
304 the active force-length relationship. Furthermore, residual force enhancement increased
305 from the upper part of the ascending limb to longer lengths (e.g. Herzog et al., 2008;
306 Lee and Herzog, 2008). Predictions of the ‘sticky-spring’ mechanism reflect these
307 findings. There is no titin length change in the range of the steep part of the ascending
308 limb of the active force-length relationship (myosin hits Z-disc), and thus neither
309 residual force enhancement nor residual force depression are predicted for contractions
310 within this length range. Predicted residual force enhancement increases from the upper
311 part of the ascending limb to longer lengths (Fig. 4) due to increasing ‘sticky-spring’
312 stiffness (Fig. B.1A, inset).

313 Measured residual force depression D (Edman, 1975; 20 % for $.15\mu\text{m}$ half-
314 sarcomere shortening to plateau) is lower by an order of magnitude than residual force
315 enhancement (Herzog et al., 2008; 170 % for $.35\mu\text{m}$ half-sarcomere stretch from
316 plateau). The ‘sticky-spring’ mechanism predictions are similar to these experimental
317 results (Figs 4, 5).

318 Sarcomere lengths, though inhomogeneous before stretch, were shown to
319 homogenize during and after stretch on the descending limb of the active force-length
320 relationship (Herzog et al., 2008). Stunningly, this is contrary to what would be
321 expected according to the sliding-filament and the crossbridge theories. The longer the
322 sarcomere, the weaker it should be and should elongate more rapidly until it is ‘caught’
323 by passive forces at long lengths which is not seen in the cited experiments. In general,
324 the ‘sticky-spring’ mechanism leads to higher passive forces during and after active
325 stretch compared to during and after passive stretch. The higher passive stiffness in
326 initially longer sarcomeres, and accordingly the higher passive force during and after
327 stretch in those sarcomeres could result in a compensation of lower active forces

328 compared to initially shorter sarcomeres and lead to homogenization of sarcomere
329 lengths as observed.

330 For large magnitude stretches, residual force enhancement can reach a plateau
331 (Bullimore et al., 2007) whose development is within the framework of the proposed
332 mechanism. During a stretch, the force gradient in the PEVK region might reach the
333 proximal (to Z-disc) PEVK-region end. Further stretch would then lead to movement of
334 the entire PEVK region along actin at a rather constant force (neglecting the increase in
335 passive force in the proximal Ig region). The lower the bearable PEVK-actin attachment
336 force and the shorter and the stiffer the PEVK region, the lower the stretch magnitude at
337 which the plateau occurs and the lower the plateau force.

338 Moreover, residual force enhancement (Edman et al., 1982) as well as residual force
339 depression (Herzog and Leonard, 1997) do not disappear if force temporarily decreases
340 to zero during stretch or shortening, but vanish if activation temporarily decreases to
341 zero after the final stretch or shortening length is reached. With regards to the ‘sticky-
342 spring’ mechanism, this also makes perfect sense because it is independent of a decrease
343 in force to zero induced by a quick shortening step. On the other hand, when the
344 sarcomere deactivates, the PEVK-actin attachments must break according to our theory,
345 otherwise the proposed mechanism would be a one-way street. We expect tropomyosin
346 to break the PEVK-actin attachments while covering the myosin binding sites on actin.

347

348 *Model limitations*

349 Though many experimental results might be explained qualitatively with the mechanism
350 proposed in its current form, a well-documented feature of force depression is not yet
351 included. Similar to experiments by Marechal and Plaghki (1979), the ‘sticky-spring’
352 mechanism leads to residual force depression which linearly depends on the shortening
353 distance in a number of ranges (Fig. 6). However, residual force depression not only

354 depends on shortening distance, but also on shortening velocity (Marechal and Plaghki,
355 1979).

356 Considering the narrow interfilamentary space, it may be possible that instead of
357 sliding past them, the myosin filaments knock off a fraction of PEVK-actin attachments
358 during shortening. The impulse exerted on the PEVK-actin attachment increases with
359 increasing contraction velocity. Hence, the probability of knock-off might increase with
360 velocity resulting in decreased force depression. To incorporate the speculated knock-
361 off into the model in a simple manner, we could assume that if the most distal PEVK-
362 actin attachment is knocked off at the beginning of the second shortening phase (Fig. 3,
363 middle), all following PEVK-actin attachments of this particular titin molecule will also
364 be knocked-off. Then, a factor decreasing from one towards zero with increasing
365 velocity can simply be multiplied with the passive force in the second and the third
366 shortening phases as well as with the length l_{block} of PEVK-inhibited binding sites to
367 account for velocity-dependent force depression. Interestingly, the fraction of knocked-
368 off PEVK-actin attachments might increase with a decreasing $f_{[break]}$. Thus, the current
369 model yields the maximal force depression to be expected (Fig. 5), which may decrease
370 with increasing velocities as stated in the literature, and moreover with decreasing $f_{[break]}$
371 (i.e. with increasing initial length or after an active pre-stretch).

372 During active isovelocity stretches, muscle stiffness was constant and greater than
373 zero, independent of stretch velocity (Edman et al., 1982) and also independent of initial
374 length (Till et al., 2008). The widely accepted product approach for the active force (Eq.
375 (2)) leads to different active force slopes for different constant velocities in ranges of
376 non-zero force-length relationship slopes, whereas the ‘sticky-spring’ mechanism in its
377 current form yields passive forces which are independent of stretch velocity. Half-
378 sarcomere force is the sum of active force and ‘sticky-spring’ force. Thus the
379 experiments in the range of the plateau conducted by Edman et al. (1982) might be

380 explained, but those by Till et al. (2008) cannot be explained without accounting for a
381 stretch-velocity dependence in the ‘sticky-spring’ mechanism model.

382 The increase in passive force attributed to increased titin stiffness in the presence of
383 calcium was shown to be very small (e.g. Labeit et al., 2003; Joumaa et al., 2008) and
384 would not be able to predict residual force enhancement values observed in
385 experiments. However, Labeit et al. (2003) removed actin, and Joumaa et al. (2008)
386 removed the tropomyosin motor troponin C. As a result, no myosin binding-sites on
387 actin were available for the suggested PEVK-actin attachments which, in turn, are
388 crucial for the ‘sticky-spring’ mechanism. However, the ‘sticky-spring’ forces depend
389 on the force-elongation relations of distinct titin regions. Stiffness adaptations of these
390 regions due to calcium increase may be worth considering, but were not accounted for
391 in the model due to lack of data.

392

393 *Functional implications*

394 The proposed mechanism strongly links force enhancement with force depression
395 effects, thereby implying functional consequences.

396 Passive, elastic force enhancement might improve the muscle’s efficiency in cyclic
397 stretch-shortening situations by temporarily storing and subsequently releasing elastic
398 strain energy (Lindstedt et al., 2002; Pinniger et al., 2006). In a study with rats
399 conducted by Lindstedt et al. (2002), eccentric muscle training increased muscle
400 stiffness, but did not increase the rats’ maximum isometric force, thus the ability of
401 muscles to produce force enhancement (perhaps accompanied by force depression)
402 seemed to be a plastic feature of muscles depending on their use. In this context, force
403 depression could be seen as an unwanted by-product of force enhancement, and might
404 be circumvented by timely deactivation in the shortening phase.

405 If the muscle's primary function is to produce positive work (i.e. active fiber
406 shortening is the predominant action), the 'sticky-spring' mechanism is contra-indicated
407 since by decreasing the muscle's force it leads to a reduction of the positive work.
408 Muscle fibers and entire muscles showing no force enhancement exist (Pinniger et al.,
409 2006; Stienen et al., 1992) and, following the proposed mechanism, they might lack
410 force depression and might be adapted for production of positive work.

411 An intriguing aspect pointing to a possible source of the 'sticky-spring'
412 mechanism's speculated adaptiveness might be the structural difference of heart titin
413 when compared to skeletal muscle titin. The heart is one of the above mentioned
414 muscles whose primary function is to produce positive work. The PEVK region in heart
415 titin compared to that in selected skeletal muscles is very short (Linke et al., 1996),
416 which is appropriate since it reduces the number of possible PEVK-actin connections
417 and, therefore, reduces force depression.

418

419 *Active shortening-stretch and stretch-shortening experiments*

420 Non-commutativity of active shortening-stretch vs. stretch-shortening experiments
421 with respect to the final steady-state force was found in experiments conducted by
422 Herzog and Leonard (2000). Regarding the 'sticky-spring' mechanism, in such
423 experiments the initial conditions for the second part of the experiment differ from
424 single stretch or single shortening.

425 In an equidistant shortening-stretch experiment, shortening to an extent where the
426 distal end of the attached PEVK region remains in place leads to equal configuration of
427 the 'sticky-spring' at the final length compared to a purely isometric contraction at the
428 same final length. Therefore, the steady-state force for both experiments may remain
429 unchanged as reported (Herzog and Leonard, 2000). Greater shortening-stretch

430 amplitude can lead to the development of a force gradient in the PEVK region during
431 stretch and, therefore, to force enhancement.

432 However, the situation for an equidistant stretch-shortening experiment of
433 comparable amplitude would be different from the one already discussed. Due to
434 stretch, the bound PEVK region elongates distally. During the first shortening phase,
435 while the passive force decreases to zero, the force gradient in the PEVK region
436 reverses with only marginal proximal displacement of the PEVK-region's distal end.
437 Thus, the distal PEVK-region end is more distal when the final length is reached than it
438 is in a purely isometric contraction at the same final length. As a result, the steady-state
439 force at the final length is lower than the force resulting from the purely isometric
440 contraction due to decreased passive force or even negative passive force and inhibited
441 crossbridges (i.e. the 'sticky spring' introduces hysteresis).

442

443 *The force decay after active stretch seen in experimental data*

444 The force increase during active myofibril or muscle stretch can be separated into
445 two main parts with different slopes. Pinniger et al. (2006) related the first part to
446 increasing force-velocity values, and mainly associated the second part with increased
447 titin stiffness. The force decay in a subsequent active isometric phase exceeds the force
448 increase in the first part of the active stretch (e.g. Pinniger et al., 2006). This suggests
449 that the force output of the 'sticky-spring' mechanism should decay in isometric
450 conditions. It should be noted that this decay is incompatible with the 'sticky-spring'
451 behavior as modeled.

452 Interestingly, it has been reported that, depending on force, the Ig regions unfold and
453 refold in a statistical process (e.g. Linke and Grutzner, 2008). Assuming that the 'sticky-
454 spring' mechanism is valid, force levels in the distal Ig region can vastly exceed those
455 introduced by passive extension (Fig. 4) during and after active stretch. Therefore, the

456 unfolding of the distal Ig region might play a crucial role in the force decay after active
457 stretch. Accounting for such a process, the forces produced by the ‘sticky-spring’
458 mechanism would inherit a velocity-dependence and force would decay in the isometric
459 phase after stretch.

460 Finally, in our model we did not account for increased passive titin stiffness due to
461 high calcium concentrations (Labeit et al., 2003) since to date it is not clear to what
462 extent which titin region stiffens. However, stiffening of mainly the distal Ig or the
463 PEVK regions, for example, might have a profound effect on ‘sticky-spring’ force
464 output during active stretch which, in turn, might lead to changed folding and unfolding
465 ratios and thereby influence overall time constant(s) of force decay.

466

467 **Acknowledgement**

468 We thank the German Science Foundation (DFG) for support of work (SI841/2-2).

469

470 APPENDIX A Calculation of f_l

471 We used the rat’s actin length (1.04 μm ; Walker and Schrodt, 1974), the typical
472 myosin length (1.6 μm), the measured plateau width (0.25 μm), and the measured
473 length of zero active force (1.27 μm) of the active sarcomere force-length relationship
474 (Gordon et al., 1966) to calculate the potential range of crossbridges l_{bind_pot} for a given
475 half-sarcomere length. The effective myosin bare-region length was assumed to
476 correspond to half the plateau width, thus the maximal range of operative crossbridges
477 $l_{[bind_max]}$ per half-sarcomere is $1.6/2 - .25/4 = .7375$ [μm]. In the range of the upper part
478 of the ascending limb, actin filaments entering the range of myosin heads of the
479 adjacent half-sarcomere are supposed to reduce the number of potential crossbridges by
480 half, thus the fraction of l_{bind_pot} in this range is halved. Subtraction of the length l_{block} of

481 PEVK-inhibited binding sites (compare Fig. 3) from l_{bind_pot} , and division by $l_{[bind_max]}$
 482 yielded the corresponding f_l value.

483

484 APPENDIX B Derivation of ‘sticky-spring’ stiffness during stretch

485 Consider a long active stretch of the activated half-sarcomere. The attached PEVK
 486 region elongates along actin and the force pulling on the distal PEVK region end
 487 increases due to 1) consecutive force bearing from distal to proximal PEVK segments in
 488 between initial PEVK-actin attachments and 2) superimposed force steps induced by
 489 binding sites approached during elongation.

490 Using the given passive force at any initial half-sarcomere length, we determined the
 491 initial PEVK-region length, $l_{PEVK[0]}$. Dividing $l_{PEVK[0]}$ by $c_{[bind]}$, the distance between
 492 two binding sites on actin (0.038 μm), and rounding up to the next integer yielded the
 493 number of initial PEVK-actin attachments. For simplicity, we assumed that the
 494 proximal end of the PEVK region would coincide with a PEVK-actin attachment. This
 495 led to full loading of all initial PEVK-actin attachments from the distal to the proximal
 496 end only when ‘sticky-spring’ force exceeded $2.5 F_{[im]}$. Hence, the proximal Ig region
 497 did not elongate during stretch.

498 To calculate the ‘sticky-spring’ force-elongation relation (Fig. B.1A), we elongated
 499 the distal PEVK end by defined steps Δx_i and iterated the corresponding increased distal
 500 force f_i (Fig. B.1B). The length change Δl_k of the k -th recruited PEVK segment (i.e.
 501 PEVK segment loaded during active stretch) due to the segment-force increase from the
 502 force prior to the step $f_{i-1} - k \cdot f_{[am]}$ to the force after the step $f_i - k \cdot f_{[am]}$ is

503

$$504 \quad \Delta l_k = c_{[bind]} \cdot \left(\frac{l_{PEVK}(f_i - k \cdot f_{[am]})}{l_{PEVK}(f_{i-1} - k \cdot f_{[am]})} - 1 \right). \quad (B.1)$$

505

506 The expression in large parentheses yields the strain of the respective segment,
 507 which was calculated using the passive PEVK force-length relation (Fig. 2). Throughout
 508 appendices B and C, lengths depend on the forces written in parentheses. Each Δl_k
 509 migrates to the distal end and is finally strained by the force f_i . It is multiplied with a
 510 factor calculated again using the PEVK force-length relation to yield its final length. Δx_i
 511 is then expressed by

$$513 \quad \Delta x_i = l_{end}(f_{i-1}) \cdot \left(\frac{l_{PEVK}(f_i)}{l_{PEVK}(f_{i-1})} - 1 \right) + \sum_{k=1}^{recr_segs} \left(\Delta l_k \cdot \frac{l_{PEVK}(f_i)}{l_{PEVK}(f_i - k \cdot f_{[am]})} \right), \quad (B.2)$$

514
 515 where the first term describes the elongation of the PEVK segment between the
 516 most distal PEVK-actin attachment and the PEVK-region's distal end with length $l_{end} <$
 517 $c_{[bind]}$. The *recr_segs* is the number of segments recruited for force-bearing by the
 518 'sticky-spring' mechanism (Fig. B.1B).

519 Considering the thousands of titin molecules arranged in parallel in a sarcomere and
 520 the probability distribution of PEVK-actin attachment force, we approximated the
 521 'sticky-spring' force increase to depend linearly on elongation (Fig. B.1A), resulting in
 522 a constant stiffness $k_{[sticky]}$ for each initial half-sarcomere length (Fig. B.1A, inset).

523

524 APPENDIX C The second active shortening phase

525 We calculated discontinuous force-shortening relations during the second shortening
 526 phase resulting from 'sticky-spring' peel-off for different initial sarcomere lengths (Fig.
 527 C.1A). For this purpose, we used the passive force-length relations of titin, the distal Ig
 528 region, and the PEVK region (Fig. 2). The free spring consists of the distal Ig region
 529 and a fraction of the PEVK region in series. Its length, l_{spring} , is expressed by

530

$$l_{spring} = l_{dist_lg}(f_{sticky}) + n \cdot c_{[bind]} \cdot \frac{l_{PEVK}(f_{sticky})}{l_{PEVK}(f_{pass[0]})} \quad (C.1)$$

532

533 where n is the number of PEVK-actin attachments already broken. Force peaks
 534 occur due to the breaking of PEVK-actin attachments at $f_{[break]}$. The breaking of one
 535 distal PEVK-actin attachment induces a stepwise proximal displacement of the PEVK-
 536 region's distal end Δx_{PEVK_dist} (Fig. 3, middle) by $c_{[bind]}$. The half-sarcomere shortening
 537 distance from peak to peak $\Delta l_{[peak]}$ is expressed by

538

$$\Delta l_{[peak]} = c_{[bind]} \cdot \left(\frac{l_{PEVK}(f_{[break]})}{l_{PEVK}(f_{pass[0]})} + 1 \right) \quad (C.2)$$

540

541 Expected homogenization effects induced by the parallel arrangement of thousands of
 542 titin filaments with, for example, distributed PEVK-actin attachment strengths (Bianco
 543 et al, 2007), led us to translate the half-sarcomere length change to Δx_{PEVK_dist} via the
 544 gearing ratio $s(l_{hs[0]})$:

545

$$s(l_{hs[0]}) = \frac{c_{[bind]}}{\Delta l_{[peak]}} = \frac{l_{PEVK}(f_{pass[0]})}{l_{PEVK}(f_{pass[0]}) + l_{PEVK}(f_{[break]})} \quad (C.3)$$

547

548 Integration of the discontinuous force-shortening relation (Fig. C.1A) yielded an
 549 approximately quadratic function (Fig. C.1B). To derive $l_{hs[0]}$ -dependent $k_{[short_2]}$, the
 550 constant stiffness related to second-phase half-sarcomere shortening $l_{hs[1]} - l_{hs}$ (Fig.
 551 C.1A, inset), we fitted $k_{[short_2]} \cdot (l_{hs[1]} - l_{hs})^2 / 2$ to this function.

552

553

553 **List of symbols and abbreviations**

554	$c_{[bind]}$	distance of binding sites on actin (=0.038 μm)
555	f_{act}	normalized active half-sarcomere force component
556	$F_{[am]}$	maximum bearable PEVK-actin attachment force (=8 pN)
557	$f_{[am]}$	normalized $F_{[am]}$ (= .16)
558	$f_{[break]}$	force in distal Ig region at which PEVK-actin attachment breaks (= $f_{[am]}$ –
559	$f_{pass[0]}$)
560	F_{hs}	half-sarcomere force
561	f_{hs}	normalized half-sarcomere force
562	$F_{[im]}$	maximum isometric force
563	f_l	factor due to active force-length relationship
564	f_{pass}	passive half-sarcomere force
565	$f_{pass[0]}$	initial passive half-sarcomere force
566	f_{sticky}	‘sticky-spring’ force (sum of forces pulling on the PEVK region)
567	$f_{sticky[2]}$	‘sticky-spring’ force at the end of the second shortening phase
568	f_v	factor due to active force-velocity relationship
569	$k_{[short_2]}$	linear stiffness during second shortening phase
570	$k_{[sticky]}$	‘sticky-spring’ stiffness during stretch and during third phase shortening
571	l_{block}	length range with PEVK inhibited potential crossbridges
572	$l_{block[2]}$	length range with PEVK inhibited potential crossbridges at the end of the
573		second shortening phase
574	l_{bind_pot}	potential range of operative crossbridges
575	$l_{[bind_max]}$	maximal range of operative crossbridges
576	l_{dist_Ig}	length of titin’s distal Ig region
577	$l_{dist_Ig[0]}$	length of titin’s distal Ig region attained under $f_{pass[0]}$
578	$l_{dist_Ig[2]}$	length of titin’s distal Ig region attained under $f_{sticky[2]}$

579	$l_{[dist_Ig_break]}$	length of titin's distal Ig region attained under $f_{[break]}$
580	l_{end}	length of PEVK region end in between most distal PEVK-actin
581		attachment and distal PEVK region end
582	l_{hs}	half-sarcomere length
583	$l_{hs[0]}$	half-sarcomere length at begin of first shortening phase
584	$l_{hs[1]}$	half-sarcomere length at begin of second shortening phase
585	$l_{hs[2]}$	half-sarcomere length at begin of third shortening phase
586	$l_{[myosin]}$	half myosin length (0.8 μm)
587	l_{PEVK}	length of titin's PEVK region
588	$l_{PEVK[0]}$	length of titin's PEVK region attained under $f_{pass[0]}$
589	n	number of initially bound PEVK segments
590	$recr_segs$	number of PEVK segments recruited for 'sticky-spring' force bearing
591	s	slope of proximal PEVK end length change in second shortening phase
592	v_{hs}	half-sarcomere velocity
593	$x_{PEVK_prox[0]}$	distance between Z-disc and proximal PEVK end at $l_{hs[0]}$
594	Δl_{dist_Ig}	length change of the distal Ig region
595	Δl_k	k -th recruited (loaded during active stretch) PEVK segment in between
596		PEVK-actin attachments (Fig. B.1B, bottom)
597	Δl_{sticky}	length change of the 'sticky spring'
598	Δx_i	defined elongation steps for determination of 'sticky spring' force-
599		elongation relation
600	Δx_{PEVK_dist}	length change of the distal PEVK end during shortening
601	Δx_{PEVK_prox}	length change of the proximal PEVK end during shortening
602		

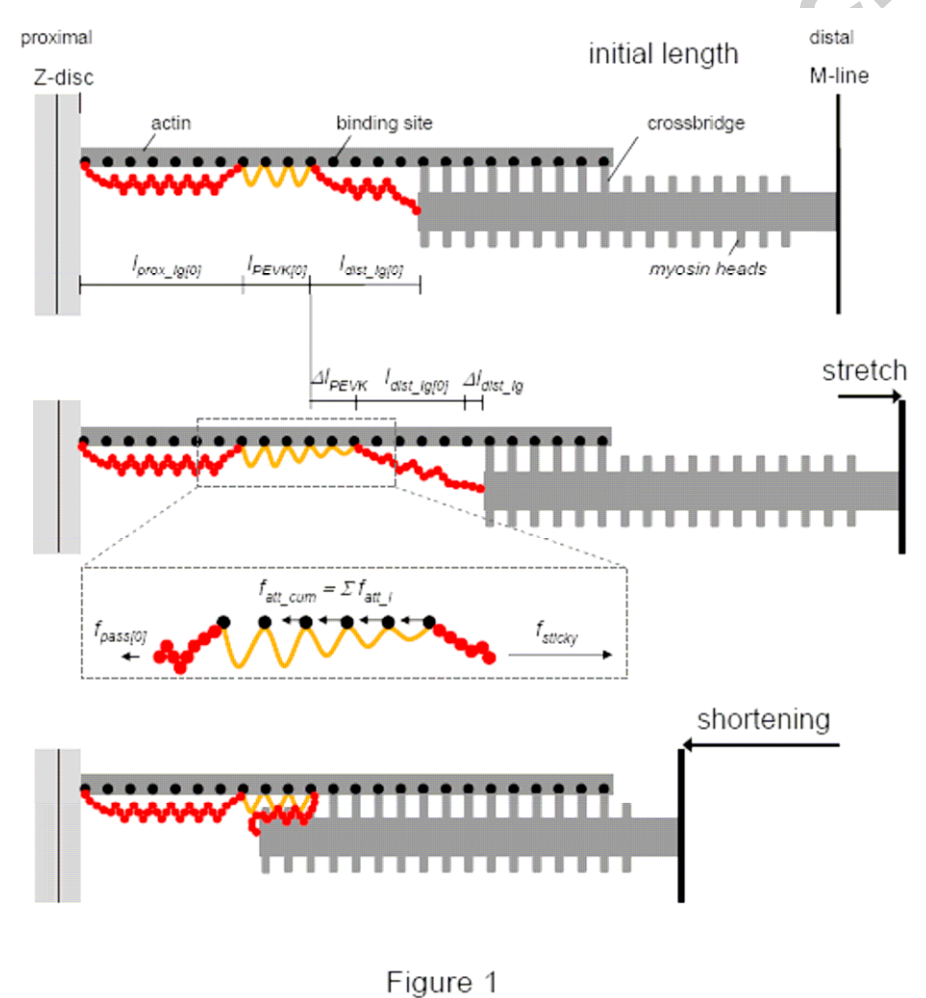
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724 FIGURE LEGENDS

725 **Fig. 1.** Schematic of the active (high calcium concentration) half-sarcomere: (top) at an initial length $l_{hs[0]}$
 726 on the descending limb of the active force-length relationship; (middle) after stretch from $l_{hs[0]}$; (bottom)
 727 after shortening from $l_{hs[0]}$. All actin, myosin and titin (its molecular spring part is colored) filaments are
 728 represented with one specimen each. The long axis length ratios, as well as the number of myosin heads
 729 correspond to physiological values. (top) The PEVK region (yellow) is attached to actin at myosin
 730 binding sites (black dots). The length of titin's proximal Ig region, PEVK region, and distal Ig region
 731 attained under the initial passive force $f_{pass[0]}$ is depicted. (middle) The sum of the length changes Δl_{PEVK}
 732 and Δl_{dist_Ig} equals the stretch distance. (middle, enlarged view) The 'sticky-spring' force f_{sticky} equals the
 733 sum of attachment forces f_{att_i} pulling on the PEVK-actin attachments and $f_{pass[0]}$. (bottom) Inhibition of
 734 crossbridges by PEVK-actin attachments is illustrated.

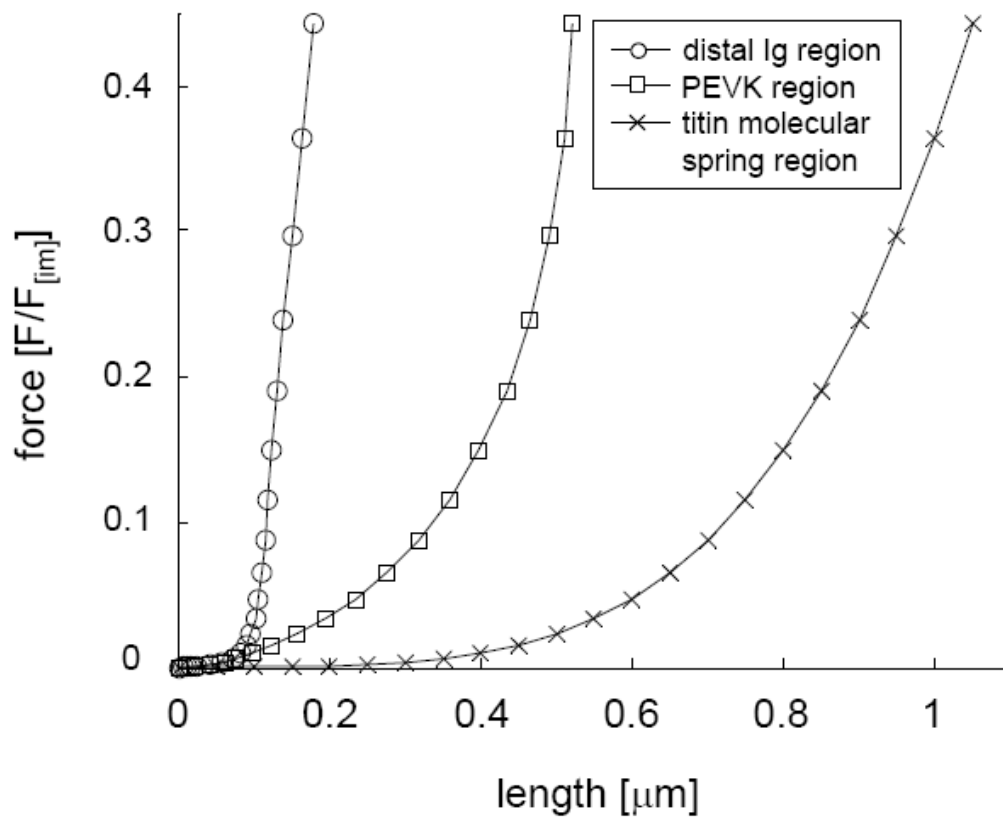


735

Figure 1

736

736 **Fig. 2.** Force-length relations (data adapted from Linke et al., 1998) of titin's entire molecular spring
737 region (x-mark), its PEVK region (square), and its distal Ig region (circle).



738

739

Figure 2

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739 **Fig. 3.** Schematic of three subsequent phases (from top to bottom) of active half-sarcomere shortening.
740 Only the vicinity of the myosin end and a part of the actin filament is depicted. The dark grey actin
741 (dotted with binding sites) and myosin segments depict initial loci of the respective phase, and the
742 transition between titin's PEVK region (yellow) and the distal Ig region (red) is marked with a circle
743 containing a dot. The myosin segments depicted in light grey correspond to their position in a later stage,
744 while the PEVK region and distal Ig region are now shown in grey. The arrows indicate length changes.
745 (top) Shortening from the initial length results in quick unloading of the distal Ig region and proximal
746 loading of the most distal PEVK actin attachment. (middle) The myosin end passes the distal end of the
747 attached PEVK region and the attached PEVK region begins to peel off. Furthermore, dependent on the
748 attached PEVK-region length and on the overlap with the myosin end, crossbridges are inhibited in the
749 range l_{block} . (bottom) The sufficiently lengthened, peeled PEVK segment is allowed to reattach behind the
750 attachment of its former proximal end. For further explanations see text.

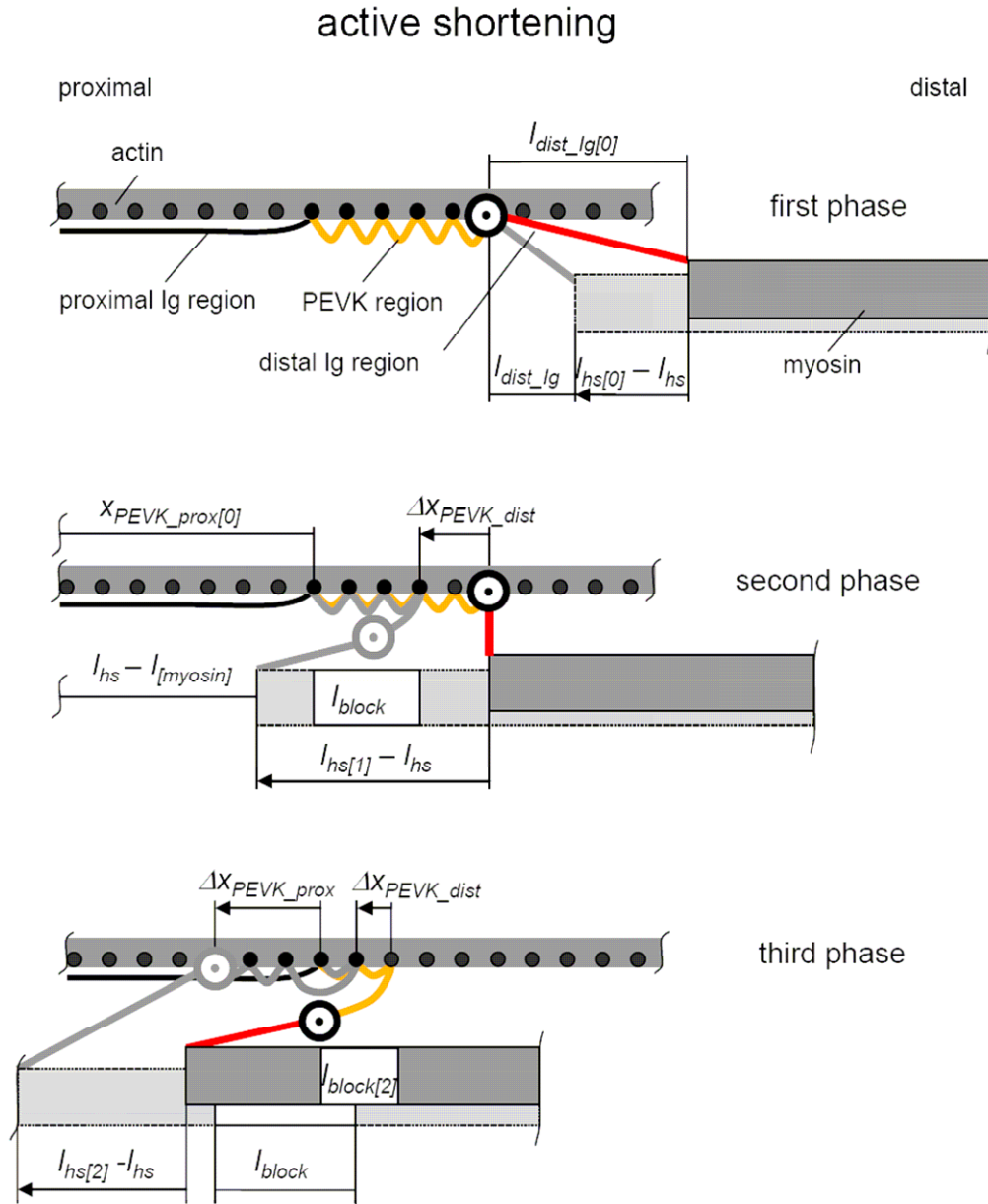


Figure 3

751

752

752 **Fig. 4.** Forces during quasi-isometric active stretch (thick lines). From top to bottom, the passive force,
753 the active force, and the sum of the two, the half-sarcomere force, is shown. For orientation, noted with
754 thin black lines, the passive (low calcium concentration) half-sarcomere force (top), the isometric active
755 half-sarcomere force (middle) and both as well as the sum of the two (bottom) are given.

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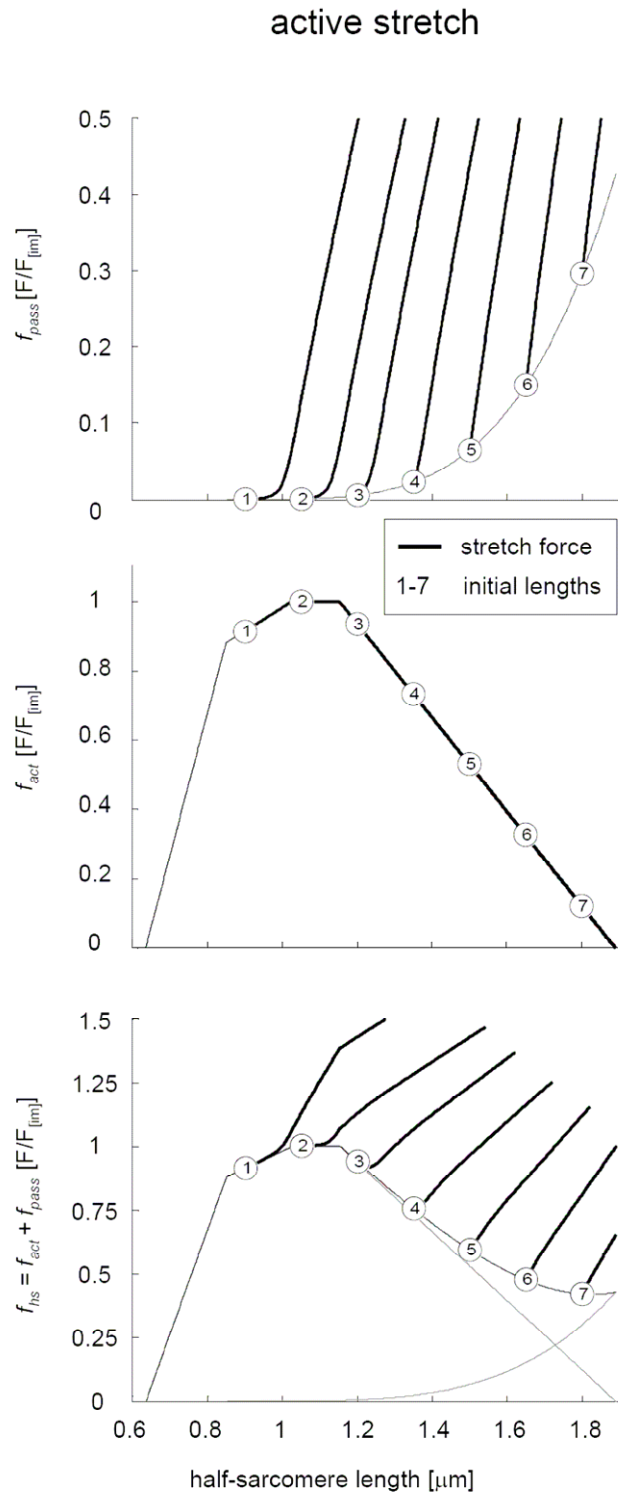


Figure 4

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757

757 **Fig. 5.** Forces during quasi-isometric active shortening (thick lines). From top to bottom, the passive
758 force, the active force and the sum of the two, the half-sarcomere force, is shown. The broken black line,
759 the thick black line, and the thick grey line indicate the first, the second, and the third shortening phases,
760 respectively. For orientation, noted with thin black lines, the passive (low calcium concentration) half-
761 sarcomere force (top), the isometric active half-sarcomere force (middle), and both, as well as the sum of
762 the two (bottom) are given. At the length $.85\mu\text{m}$, indicated by the vertical line, myosin hits the Z-disc.

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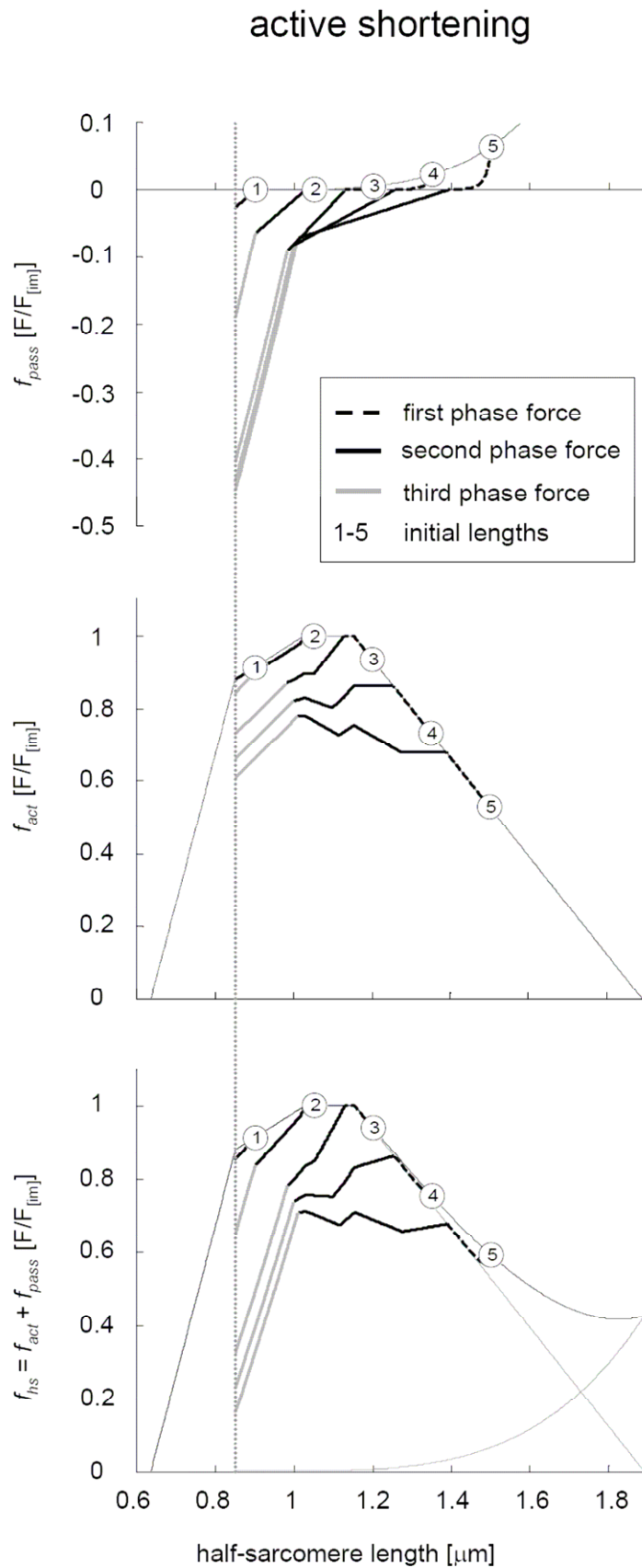


Figure 5

764 **Fig. 6.** Residual force depression (RFD). Thick lines show half-sarcomere forces due to quasi-isometric
 765 shortening from different (1-9) initial half-sarcomere lengths $l_{hs[0]}$. The vertical, broken line indicates a
 766 chosen, final half-sarcomere length. RFD (inset) depends almost linearly on the shortening distance
 767 (initial length – final length).

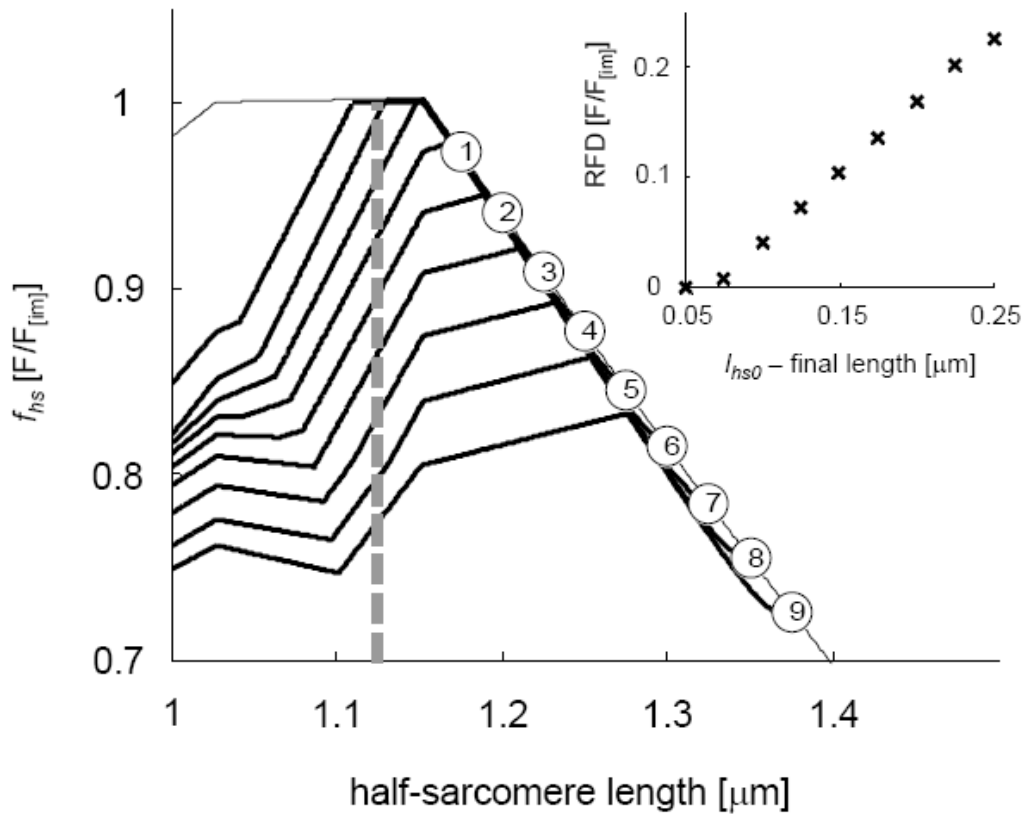


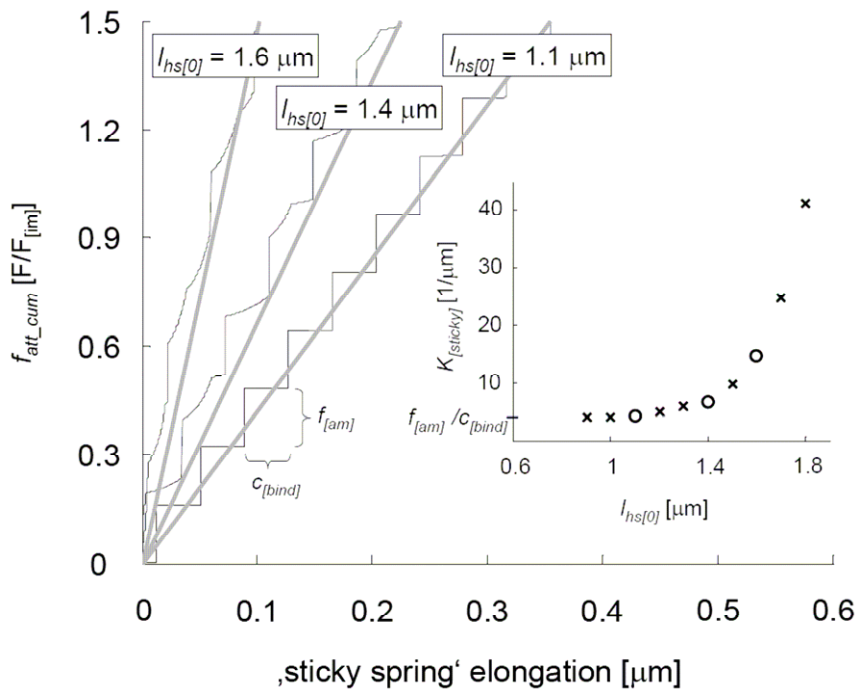
Figure 6

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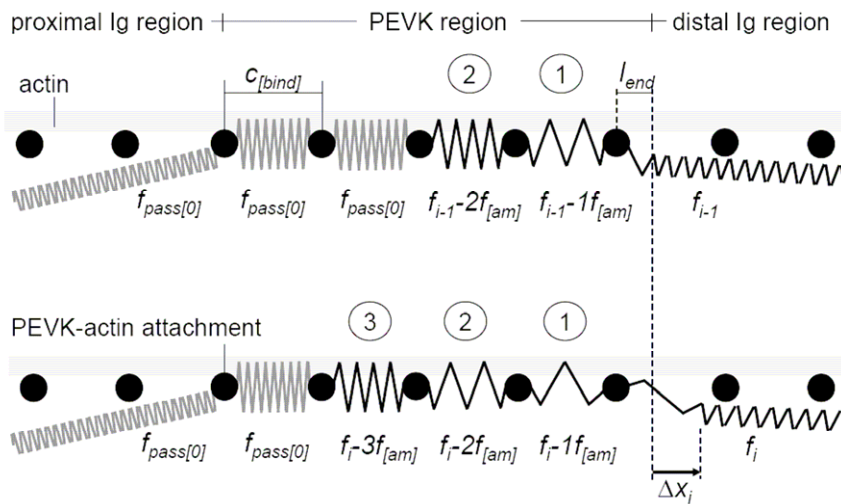
769

769 **Fig. B.1.** Linear approximation of ‘sticky-spring’ stiffness during stretch from different initial half-
 770 sarcomere lengths $l_{hs[0]}$. (A) Discontinuous force-elongation relations of the ‘sticky-spring’ resulting from
 771 stretch (black lines) are approximated linearly (grey lines). (A, inset) The linear ‘sticky-spring’ stiffness
 772 $k_{[sticky]}$ increases with increasing $l_{hs[0]}$. Circles correspond to the grey lines in A. (B) Schematic of ‘sticky-
 773 spring’ elongation by Δx_i . (B, top): The PEVK region is attached to actin. The distal Ig region and the
 774 PEVK segments 1 and 2 (encircled numbers) bear forces decreasing successively by the PEVK-actin
 775 attachment force $f_{[am]}$. The grey springs only bear the initial passive force $f_{pass[0]}$. (B, bottom) Stretch by
 776 Δx_i results in the same force increase in the distal PEVK-region end l_{end} and in segments 1 and 2. In this
 777 example, during stretch, the force difference between segments 2 and 3 exceeded $f_{[am]}$, and segment 3
 778 now bears force exceeding $f_{pass[0]}$.

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A



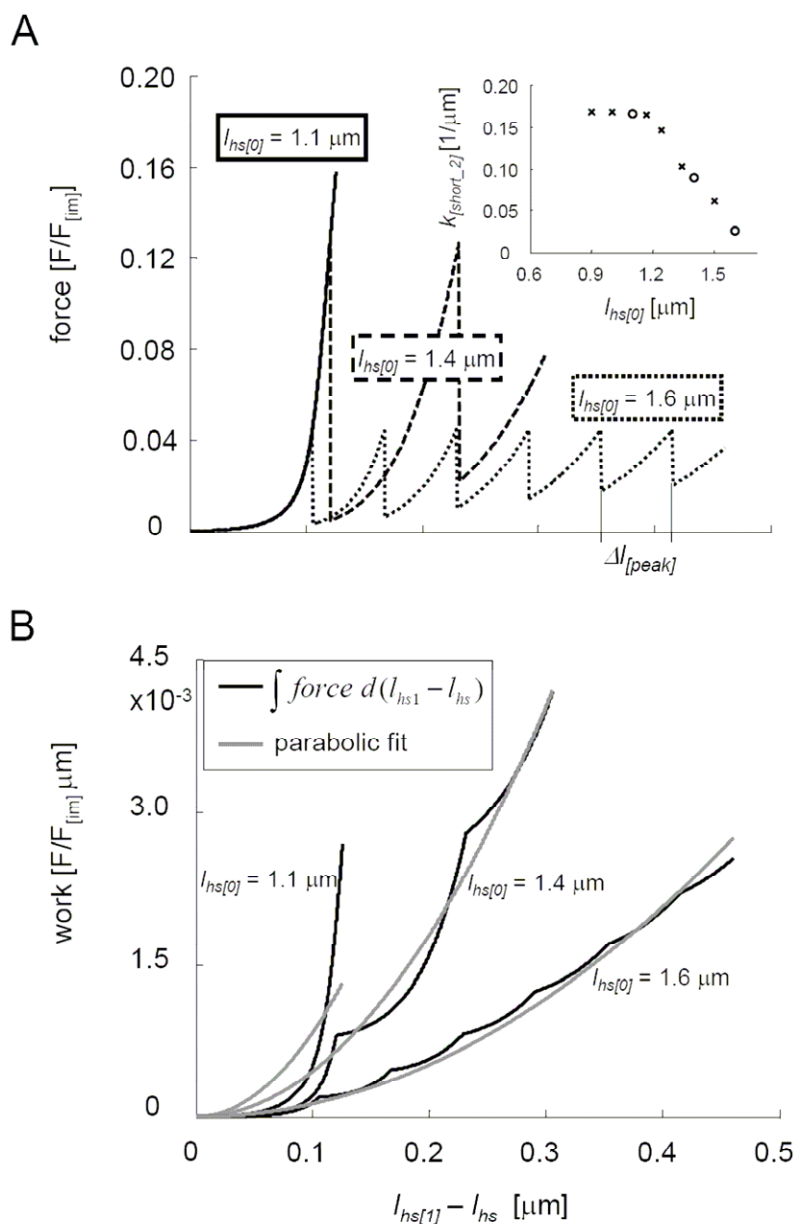
B

Figure B.1

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780

780 **Fig. C.1.** Linear approximation of ‘sticky-spring’ peel-off force during the second shortening phase. (A)
 781 Discontinuous peel-off force dependent on selected initial half-sarcomere lengths $l_{hs[0]}$ plotted vs. second-
 782 phase half-sarcomere shortening $l_{hs1} - l_{hs}$. The corresponding approximate linear stiffness $k_{[short_2]}$
 783 depending on $l_{hs[0]}$ is shown in the inset (circles). The constant distance from peak to peak $\Delta l_{[peak]}$ is
 784 indicated for shortening from $l_{hs0} = 1.6 \mu\text{m}$. (B) According to the model, the mechanical energy (black
 785 lines) expended to peel off the PEVK region changes approximately with the square of the displacement
 786 similar to a linear spring. Parabolic approximation (grey lines) yields $k_{[short_2]}$.



787

Figure C.1