**Biophysical Journal, Volume 114** 

### **Supplemental Information**

### Active Prestress Leads to an Apparent Stiffening of Cells through Geo-

#### metrical Effects

Elisabeth Fischer-Friedrich

## I. GEOMETRICAL COUPLING WITH PHASE-SHIFTED, FREQUENCY-DEPENDENT ACTIVE FORCE CONTRIBUTIONS

In the first and second example in the main text, geometrical coupling of active prestress has contributed only a frequency-independent addition to the measured storage modulus of the system. In the following, we will present an example where geometrical coupling gives rise to a complex-valued, frequency-dependent addition to the effective elastic modulus of the system for the case of a deflected prestressed fibre with a viscoelastic connector between the bead and the fiber (see Fig. 1B, main text). The bead is deflected in a vertical manner. The combined system has a (complex) spring constant

$$k_{comb} = 1/(1/k_{conn} + 1/k_{fibre})$$

where  $k_{conn}$  and  $k_{fibre}$  are the effective (complex) spring constants of the connector and the fibre with respect to vertical deflection. One finds

$$k_{fibre}(\sigma_{\rm act},\omega) = 16h_0^4/(4h_0^2 + L^2)^{3/2}G_{\rm meas}^*(\sigma_{\rm act},\omega),$$

where  $G_{\text{meas}}^*$  equals the r.h.s of formula (6), main text. Thus, in the stiffness of the combined system  $k_{comb}$ , the active term in  $G_{\text{meas}}^*$  contributes in general also to the imaginary part of the system and is frequency-dependent.

# II. DETERMINING THE GEOMETRICAL FACTOR FOR THE CASE OF A DEFLECTED PRESTRESSED CYTOSKELETAL FIBRE

In the main text, we where discussing the example of geometrical coupling of active stress in a prestressed cytoskeletal fibre tethered at its end points at a distance L (see Fig. 1A, main text). Height oscillations are imposed on the fibre center with  $h(t) = h_0 + \tilde{h} \exp(i\omega t)$ . Here, we derive the factor of geometrical coupling  $1/g_0 \cdot \tilde{g}/\tilde{\epsilon}$  for this specific example.

We make a perturbation calculation determining the fibre oscillation dynamics up to first order in height amplitude h. We make the following expansions of the dynamic fibre length l(t) and the dynamic angle  $\alpha(t)$  between the substrate and the fibre

$$l(t) = l_0 + \tilde{l} \exp(i\omega t) + \mathcal{O}(\tilde{h}^2),$$
  

$$\alpha(t) = \alpha_0 + \tilde{\alpha} \exp(i\omega t) + \mathcal{O}(\tilde{h}^2).$$
  
Using the geometrical relations  $\sin(\alpha(t)) = h(t)/(l(t)/2)$  and  $h(t)/(L/2) = \tan(\alpha(t))$ , we obtain  

$$l_0 = \sqrt{4h^2 + L^2}$$

$$\begin{aligned} u_0 &= \sqrt{4h_0 + L^2}, \\ \alpha_0 &= \arctan(2h_0/L), \\ \tilde{\alpha} &= (2\tilde{h}L)/(4h_0^2 + L^2), \\ \tilde{l} &= (4h_0\tilde{h})/\sqrt{4h_0^2 + L^2}. \end{aligned}$$
(1)

We can thus calculate the time variation of the geometrical factor  $g(t) = 2\sin(\alpha(t))$  to first order

$$g(t) \equiv g_0 + \tilde{g} \exp(i\omega t) + \mathcal{O}(h^2)$$
  
=  $2\sin(\alpha_0) + 2\cos(\alpha_0)\tilde{\alpha}\exp(i\omega t) + \mathcal{O}(\tilde{h}^2)$   
=  $\frac{2\tan(\alpha_0)}{\sqrt{1+\tan(\alpha_0^2)}} + \frac{2\tilde{\alpha}}{\sqrt{1+\tan(\alpha_0^2)}}\exp(i\omega t) + \mathcal{O}(\tilde{h}^2)$   
=  $\frac{4h_0}{\sqrt{4h_0^2 + L^2}} + \frac{4\tilde{h}L^2}{(4h_0^2 + L^2)^{3/2}}\exp(i\omega t) + \mathcal{O}(\tilde{h}^2),$ 

where we have used Eqn. (1) in the last transformation step. We conclude that  $g_0 = \frac{4h_0}{\sqrt{4h_0^2 + L^2}}$  and  $\tilde{g} = \frac{4\tilde{h}L^2}{(4h_0^2 + L^2)^{3/2}}$ . The strain amplitude of the fibre is  $\tilde{\epsilon} = \tilde{l}/l_0$ . We thus obtain the coupling geometrical factor as

$$\frac{1}{g_0} \frac{\tilde{g}}{\tilde{\epsilon}} = \frac{1}{\frac{4h_0}{\sqrt{4h_0^2 + L^2}}} \frac{\frac{4h_L^2}{(4h_0^2 + L^2)^{3/2}}}{\frac{\tilde{l}}{l_0}}$$
$$= \frac{L^2}{4h_0^2}.$$