Supplemental Materials

Molecular Biology of the Cell

Guillou et al.

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY FIGURE S1: Equations for computing the area and volume of a cell aspirated into a micropipette.

We assume that micropipette aspiration of a cell constitutes an axisymmetric system around the axis of the micropipette.

The initial cell volume before aspiration is:

$$V0 = \frac{4}{3}\pi(\frac{D0}{2})^3$$

During aspiration, the height and volume of the small dome that needs to be subtracted are:

$$hdome = \frac{Df}{2} - ((\frac{Df}{2})^2 - (\frac{Dp}{2})^2)^{1/2}$$

$$Vdome = \frac{hdome}{6}\pi(3(\frac{Dp}{2})^2 + hdome^2)$$

The final volume during aspiration is:

$$Vf = \frac{4}{3}\pi(\frac{Df}{2})^3 - Vdome + \pi(\frac{Dp}{2})^2 L + \frac{2}{3}\pi(\frac{Dp}{2})^3$$

The initial cell area before aspiration is:

$$A0 = \pi(D0)^2$$

During aspiration, the area of the small dome that needs to be subtracted is:

$$Adome = 2\pi(\frac{Df}{2})hdome$$

The final cell area during aspiration is:

$$Af = \pi(Df)^2 - Adome + \pi(Dp)L + 2\pi(\frac{Dp}{2})^2$$

SUPPLEMENTARY FIGURE S2: Fluorescent staining of T lymphocytes used to estimate cell volume. F-actin is in red and nucleus is in blue. Scale bar is 5 µm. We find V0 = 274 ± 75 µm^3 (n = 298), 473 ± 136 µm^3 (n = 143) and 797 ± 250 µm^3 (n = 68) (mean ± SD) for resting T lymphocytes, lymphoblasts and Jurkat cells respectively. This is slightly higher than the apparent volume we estimated using optical microscopy of cells in suspension, but still in good agreement.

SUPPLEMENTARY FIGURE S3: Compressive force F exerted on a cell during microindentation, as a function of the indentation δ. The raw data, in grey, are acquired at a frequency of ~350 Hz. In blue we plot a moving average of the raw data over 50 points. In red, we overlay the best fit found using the Hertz model.

SUPPLEMENTARY FIGURE S4: The relationship between the apparent stiffness and the apparent membrane surface area is reversible. (A) Plot of the apparent stiffness E as a function of the normalized apparent membrane surface area A/A0, where A0 is the initial membrane surface area and A the membrane surface area at the time where the apparent stiffness E is measured. We have N = 5 cells (resting T lymphocytes) and n = 201 microindentations. Black dots represent measurements for which ΔP = - 15 Pa, green dots those for which ΔP = - 50 Pa and red dots those for which ΔP = - 100 Pa. The dotted line represents the best fit using the phenomenological relation $E = E0$ for $A/A0<(1+\varepsilon)$ and $E = E0 + k(A/A0-(1+\varepsilon))$ for $A/A0>(1+\varepsilon)$.

SUPPLEMENTARY FIGURE S5: Myosin-II activity softens resting T lymphocytes. (A) Normalized apparent membrane surface area A/A0, where A0 is the initial membrane surface area and A the membrane surface area, as a function of time T. An aspiration pressure of ΔP = -300 Pa is applied for T between 220 and 440 seconds. Before and after that, we have ΔP = -30 Pa. Bars represent the standard deviation. In black, control cells (N = 13 cells, n = 429 microindentations) and in blue, blebbistatin treated cells (N = 8 cells, n = 212 microindentations). Membrane surface area was measured at every indentation

cycle. (B) Apparent stiffness E, as measured by microindentation, as a function of time T. These data show the apparent stiffness of the cells in panel A. Therefore, the same profile of aspiration pressure over time applies here. Also, the same color code and conventions apply as in panel A.

SUPPLEMENTARY FIGURE S6: Effect of aspiration pressure on Jurkat cell membrane rupture. (A) Plot of the membrane expansion at rupture A*/A0, as a function of the absolute value of the aspiration pressure ΔP for Jurkat cells (n = 22 ruptured cells). (B) Plot of the duration T of micropipette aspiration before rupture, as a function of the absolute value of the aspiration pressure ΔP for Jurkat cells (n = 22 ruptured cells). The two aspiration pressures with the most ruptured cells (ΔP = -300 and -1000 Pa), which account for over 80% of the total number of Jurkat cell ruptures, were selected for this plot.

SUPPLEMENTARY TABLES

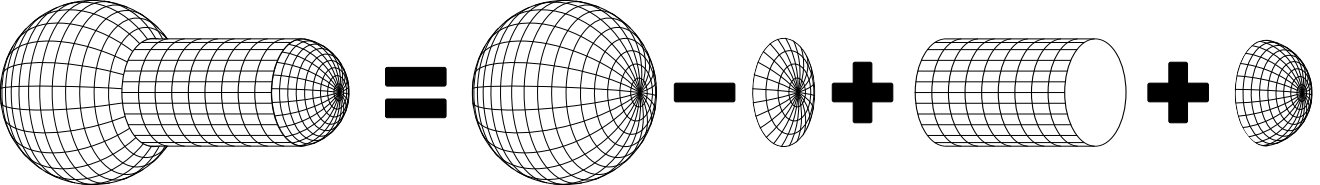
	Membrane slack ε	Expansion modulus k	Method of measurement for k
Resting T lymphocytes	12 ± 2%	660 ± 80 Pa	Profile microindentation
Neutrophils	6%	0.16 mN/m for A/A0 < 1.25 2.14 mN/m for A/A0 > 1.30	Micropipette aspiration at equilibrium

SUPPLEMENTARY TABLE T1: Fitting parameters in the following phenomenological law linking the effective stiffness E and apparent membrane surface increase A/A0:

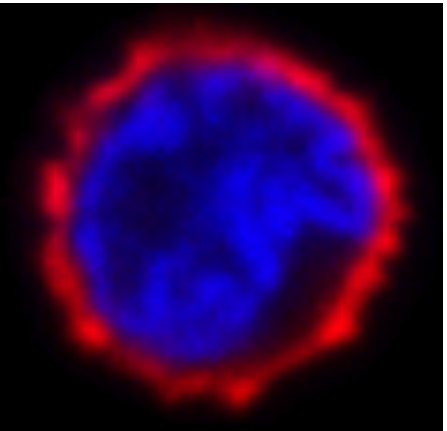
$$\begin{cases} E = E0 \text{ for } A/A0 < (1+\varepsilon) \\ E = E0 + k\,(A/A0 - (1+\varepsilon)) \text{ for } A/A0 > (1+\varepsilon) \end{cases}$$

The values for resting T lymphocytes come from our own measurements and are given as mean ± standard error of the mean. The comparative values come from

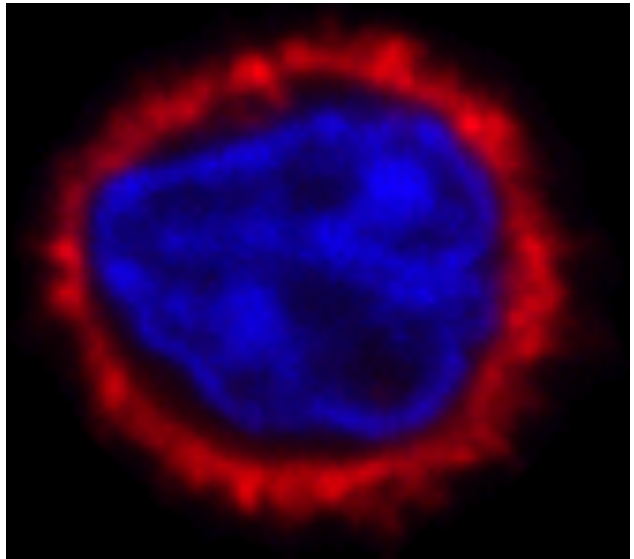
a previous study by Herant *et al.* which looked at the cell tension and the membrane expansion of neutrophils at equilibrium (Herant *et al.*, 2005).



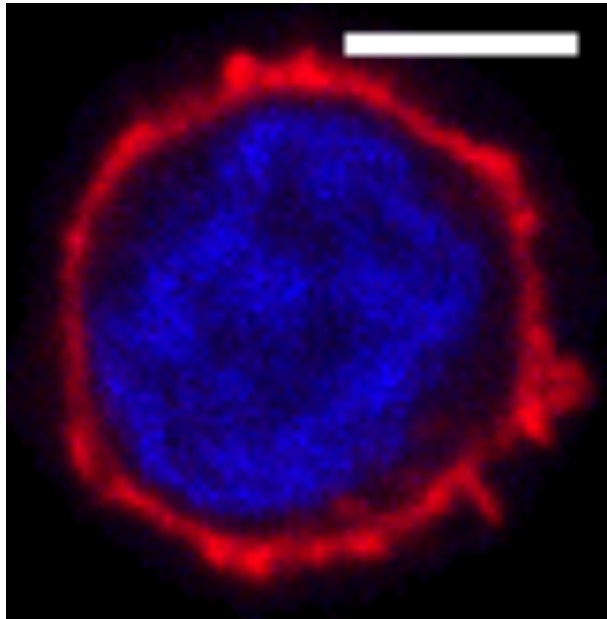
Resting T lymphocyte

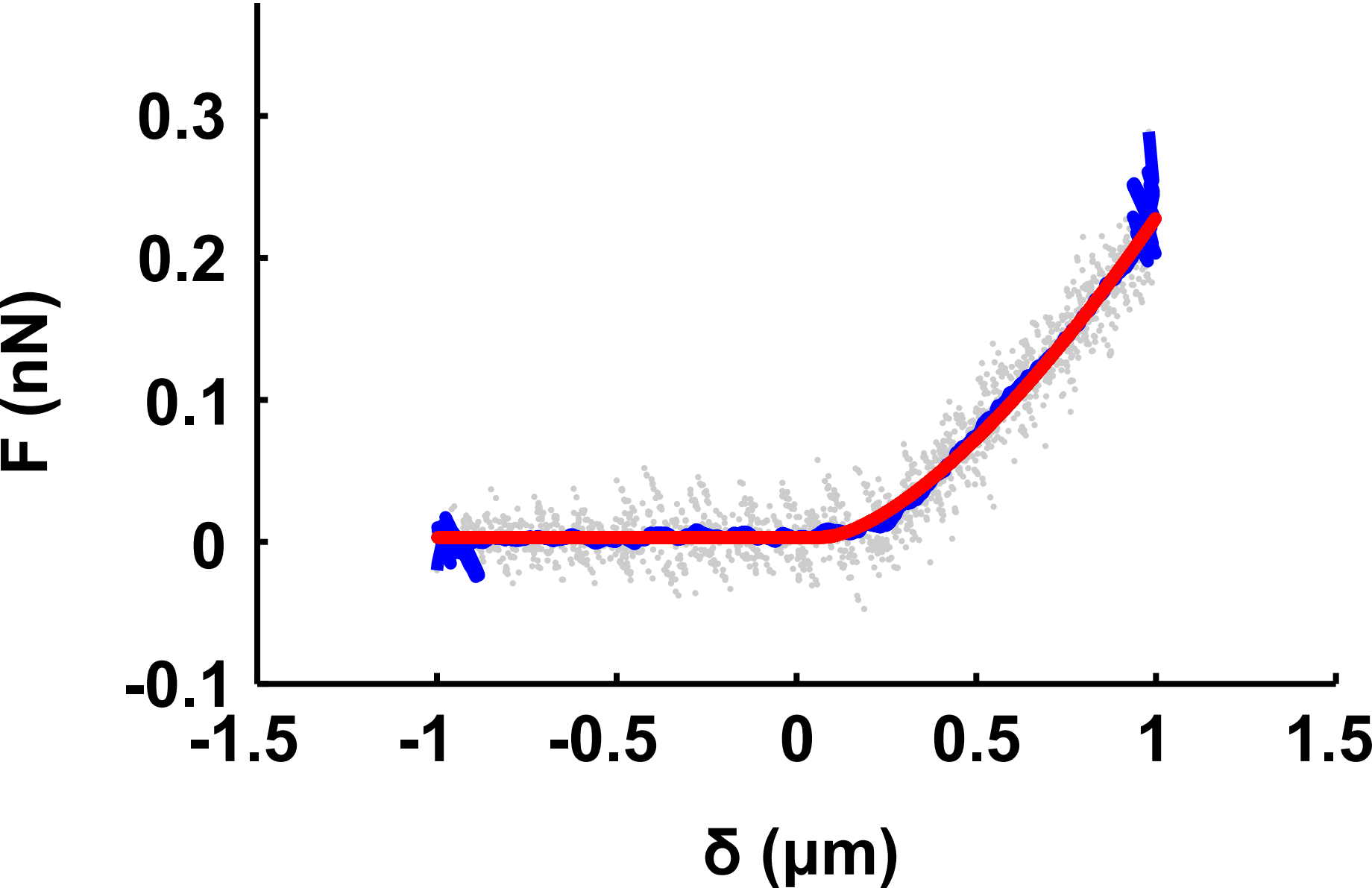


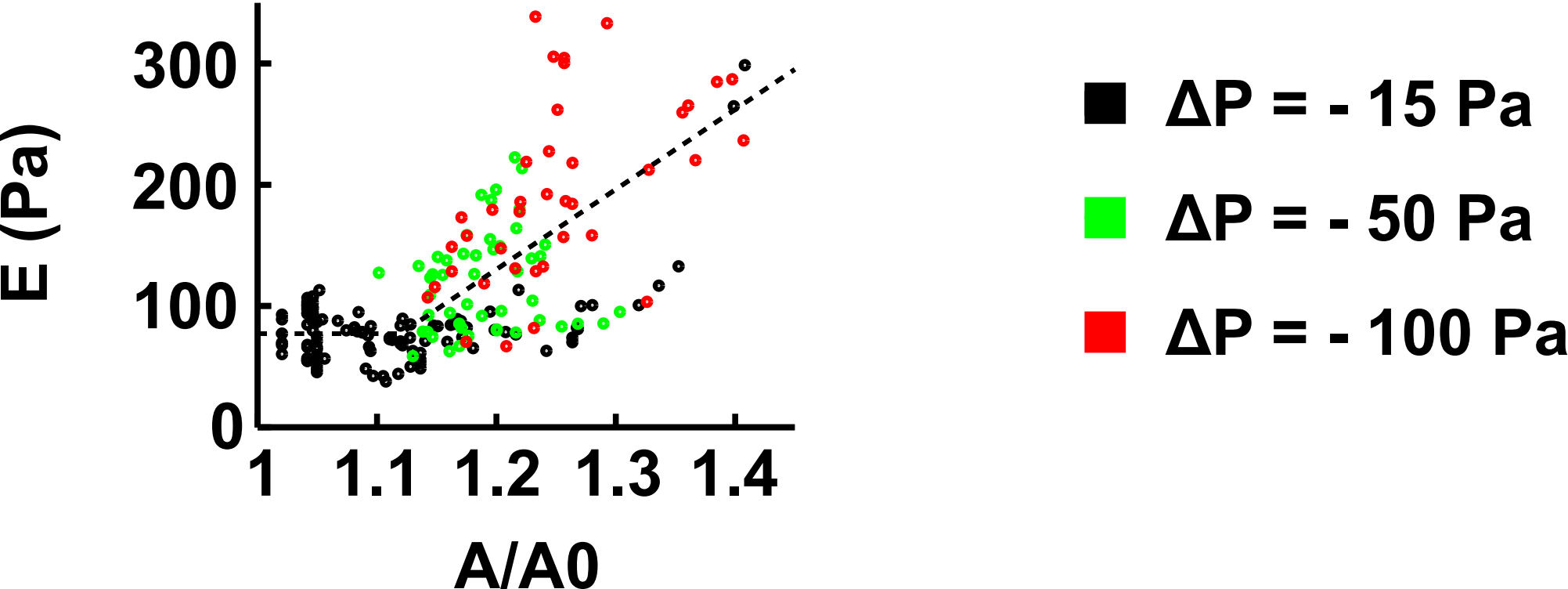
Lymphoblast

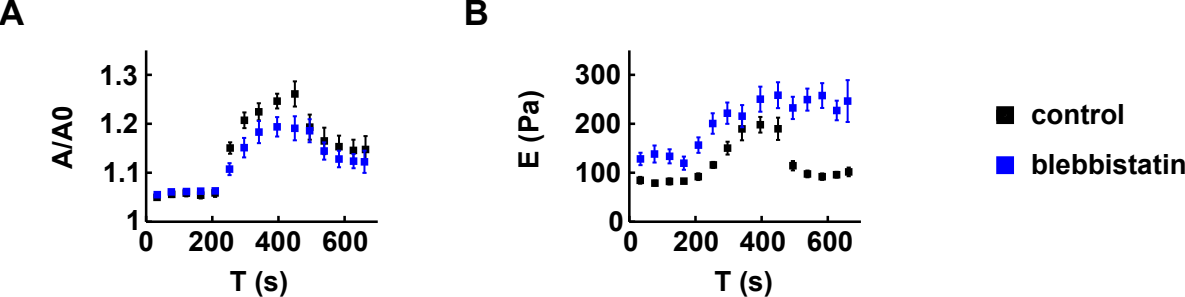


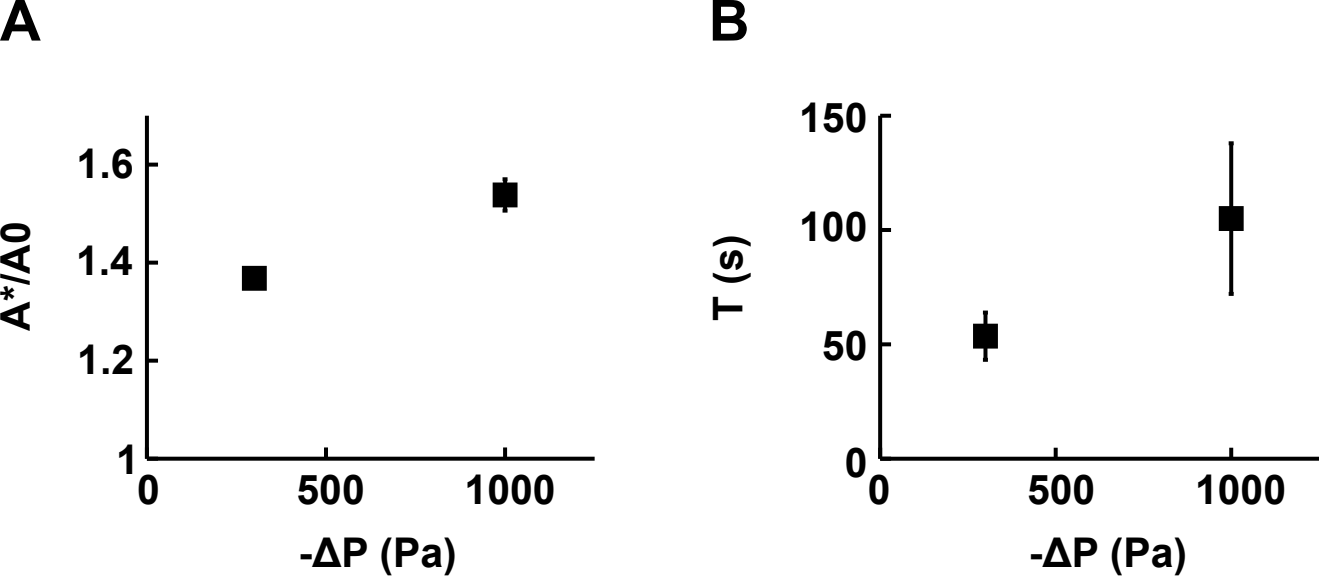
Jurkat cell




0.3
0.2
0.1
0
-0.1
F (nN)
-1.5
-1
-0.5
0
0.5
1
1.5
δ (μm)


E (Pa)
300
200
100
0
1
1.1
1.2
1.3
1.4
A/A0
ΔP = - 15 Pa
ΔP = - 50 Pa
ΔP = - 100 Pa


A
A/A0
1.3
1.2
1.1
1
0
200
400
600
T (s)
B
E (Pa)
300
200
100
0
0
200
400
600
T (s)
control
blebbistatin


A
A*/A0
1.6
1.4
1.2
1
0
500
1000
-ΔP (Pa)
B
T (s)
150
100
50
0
0
500
1000
-ΔP (Pa)