Supporting Material

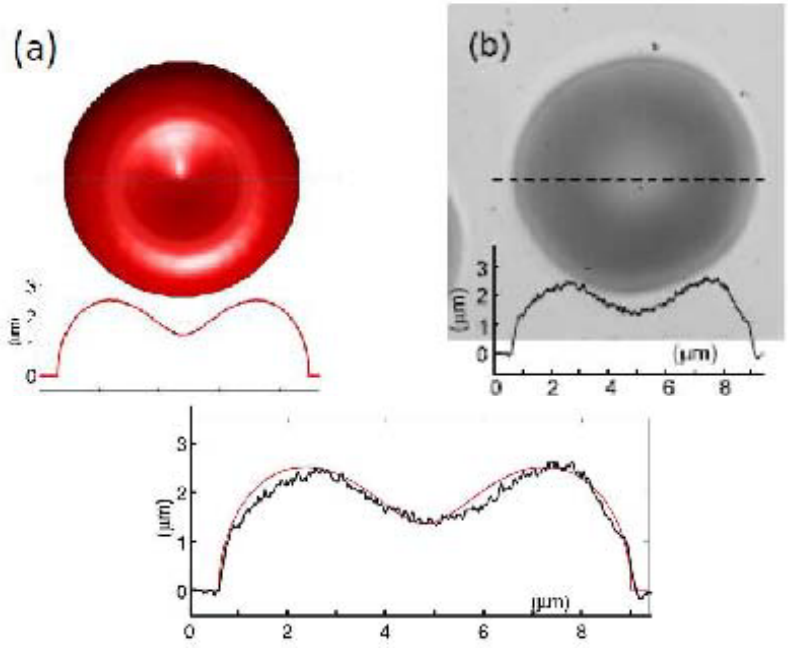

Figure S1: Discocyte shape generated by rotation of Cassini oval for (ε = 0.958), and profile (a); discocyte and experimentally determined profile (b, data from [1]); comparison of the experimentally determined is also shown, with the red line indicating the profile from the Cassini oval, and the experimentally determined contour shown in black. [1] A. Lewalle and K.H. Parker, *J. Biomech. Eng.*, 2011, 133, 011007 (with permission from ASME as original publisher).

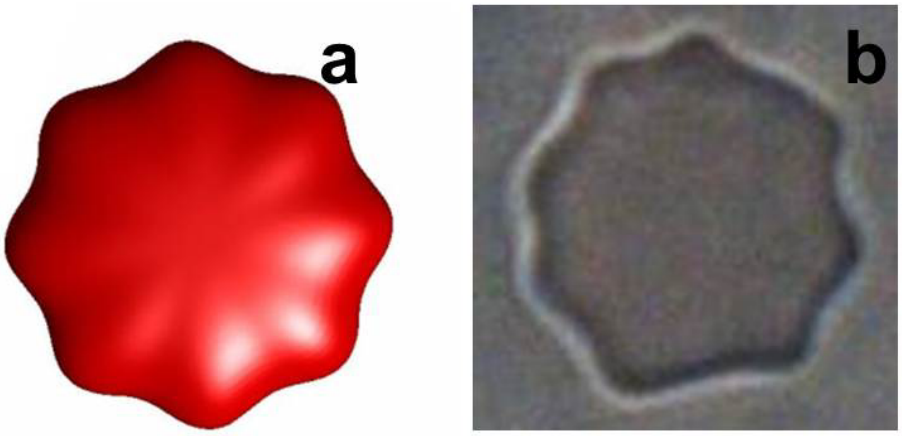

Figure S2: A comparison of a) the echinocyte model generated by rotation of the Cassini ovals with an added undulation (from Fig. 1c), and b) an observed echinocyte. The out-of-plane spicules that appear on the echinocytes were not considered here due to the complexity of the calculations involved, but will be considered in future work.

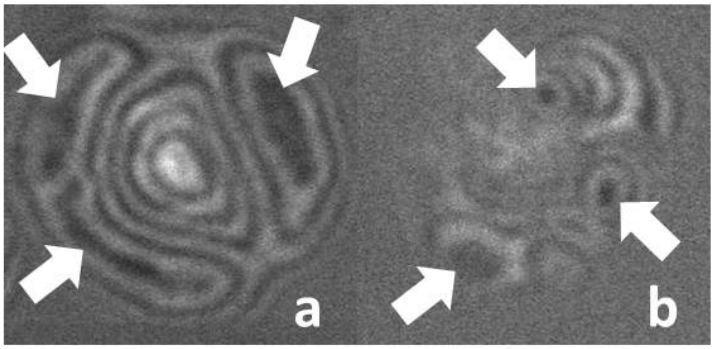

Figure S3: RICM images of a) a discocyte, and b) and echinocyte. The dark and light bands prepresent interference patterns between light reflecting off the surface of the PAH-coated slide and the bottom surface of the RBCs. The parts of the cells closest to the slide are identified by arrows. If the cells had flattened out to make a close contact with the substrate, the images would show a uniform dark patch rather than bands. These images show that discocytes retain their concave shape whenadhering to a substrate, and that the echinocytes retain their spicules.

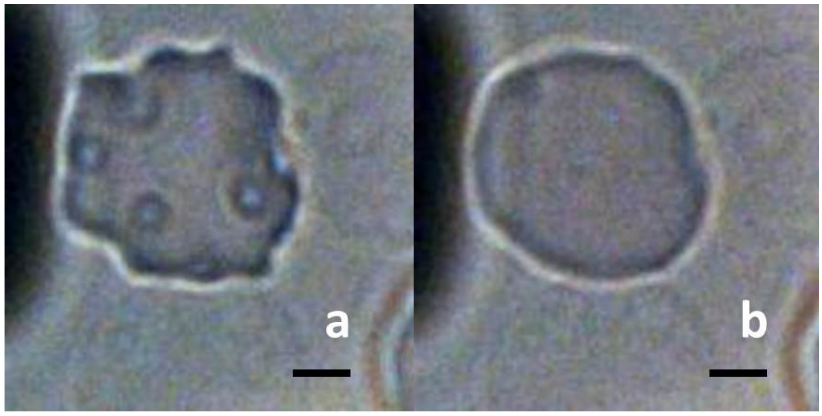

Figure S4: Images showing that sodium orthovanadate does not inhibit the ability of the cells to change shape, showing an RBC a)before and b)after AFM measurements in which 6 nN of pressure was applied to the cells for 10 s. Scale bars shown on the photographs represent 2.5 μm.

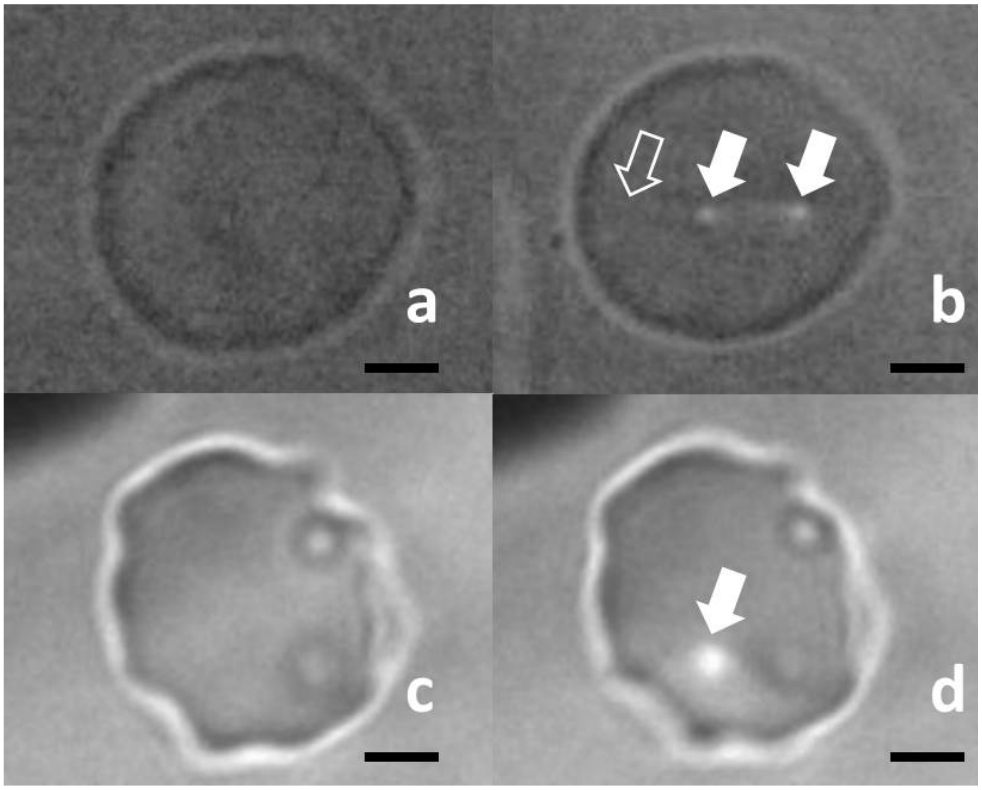

Figure S5: Images showing the marks that appeared sometimes after application of pressure with the AFM tip, showing a)a discocyte, before application of pressure; b)the same discocyte, after application of pressure at the three locations indicated by the arrows; the filled arrows indicate marks that were left at positions where the AFM tip was applied, while the open arrow indicates the location of a measurement with no resulting mark; c)an echinocyte is shown, before application of pressure, and d)the same echinocyte is shown, after the application of pressure. The resulting mark is again indicated by a filled arrow. Scale bars shown on the photographs represent 2.5 μm.

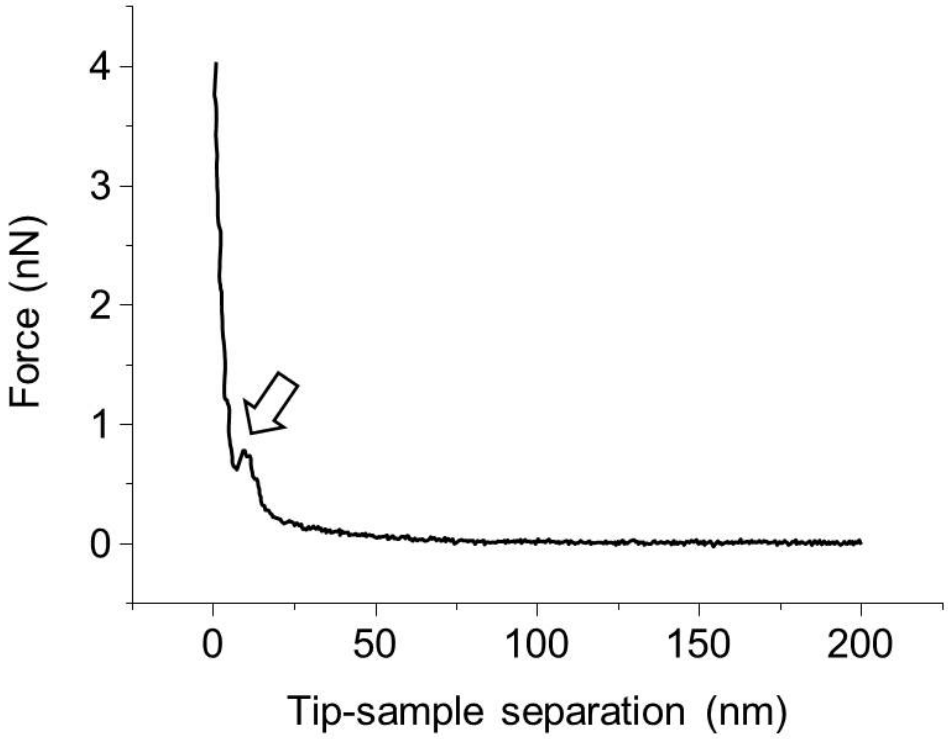

Figure S6: The force-distance curve acquired during extension of the AFM tip, in association with the images shown in Figure 6 indicates that the membrane can be punctured when there is no change in cell shape. The discontinuity shown here occurs over a length of 4.5 nm, which is characteristic of bilayer punctures .[2]

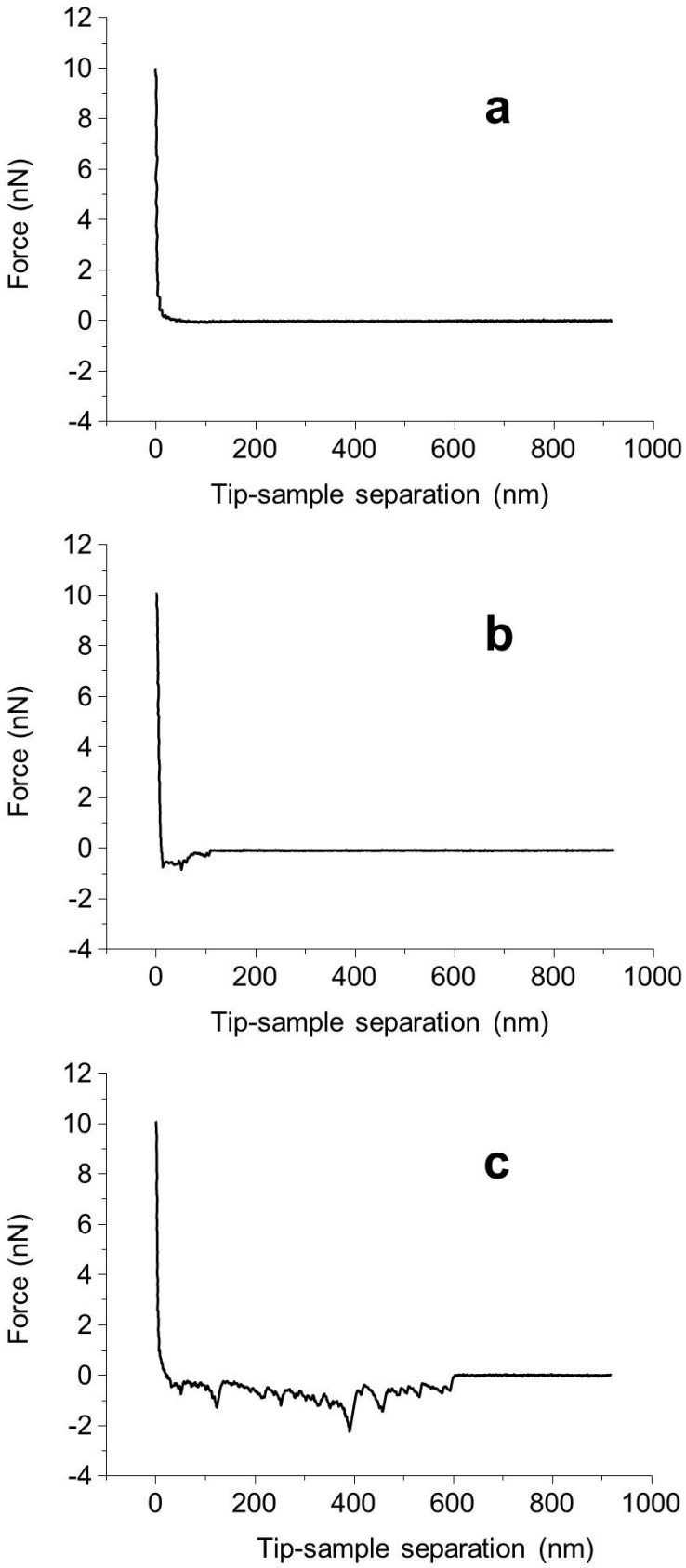

Figure S7: Force-distance curves acquired during retraction of the AFM tip are shown here to illustrate the three sorts of curves that were observed, showing a)a small adhesion event, b)no adhesion event, and c)features characteristic of protein unfolding events. Curves of the sort shown in S7c were seen in less than 1% of the measurements.

Summary of results shape changes and other events occurring at low rates.
A small proportion (1.8 %) of stage II echinocytes became ghosts within 2 minutes of the AFM measurement; an additional 0.7 % became spherical, and may have become ghosts later. Formation of ghosts was often associated with adhesion between the tip and the cell. Numbers for the anomalous results were calculated based on observations of AFM measurements on 1104 cells; for these calculations, all results over a one-month period were considered together, regardless of differences such as maximum load.

Of the cells that were investigated, 0.6 % showed a temporary positive change, and then reverted to the initial spiculated form; 1.4 % showed an additional spicule after the AFM measurement. Cells in both these categories were given a score of 0 when calculating the percentage of cells that changed shape. Control experiments in which AFM measurements were made on discocytes did not result in the formation of echinocytes- the cells retained their smooth discocyte shape.

Changing the AFM tip had a significant effect on the proportion of cells that changed shape; in one set of measurements, the proportion of cells changing shape at a given applied force was seen to double when the tip was changed (from 15 % at 10 nN to 32 %), while in a second set of measurements, no shape changes were observed at 3 or 4 nN with the first tip that was tried, although 20 and 55% shape change rates were observed with the second tip. In general, since the tip selected for the experiments affects the force required to cause the cells to change shape, the numerical values given here should be considered to be approximate. Within one set of measurements, however, values can be compared, in order to establish other factors that affect the results. Within one experiment, on one deposited batch of cells, using the same tip, values were seen to remain constant when three sets of measurements were made at 2 hr intervals. This held true if the tip was kept in buffer, or else rinsed immediately after removal from the sample medium; if the tip was allowed to dry without being rinsed, then the proportion of cells changing shape dropped, as is discussed in the text. The tip was therefore kept in buffer or else rinsed immediately after use.

References:

[1] A. Lewalle and K.H. Parker, *J. Biomech. Eng.*, 2011, 133, 011007.

[2] I. Pera, R. Stark, M. Kappl, H.-J. Butt and F. Benfati, *Biophys. J.*, 2004, 87:2446-2455.