# Burkholderia projection stresses

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## 1 Fluorescent microscopy data

Nora previously did live-cell fluorescent imaging of *Burkholderia thailandensis* (green) infecting A549, human lung alveolar epithelial cells. The A549 cells carry a fluorescent membrane marker (red). Figure 1 shows a still from one of the movies.

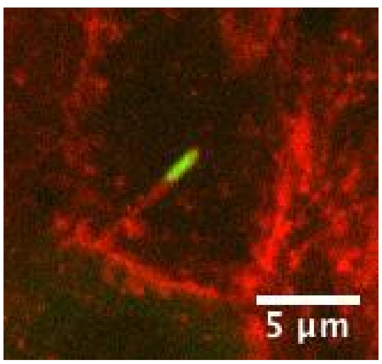



Figure 1: Burkholderia thailandensis in a double-membrane protrusion just prior to induction of cell-cell fusion and escape of the bacterium into the neighboring cell

I estimated generic protrusion dimensions via ImageJ measurements of protrusions just before the fusion event in a few of the movies. **Protrusion length 5 $\mu m$, and protrusion diameter 0.5 $\mu m$.** Protrusions often curved and were therefore measured in 3-4 segments. Note that these 2D measurements don't capture any bending that might have occurred above or below the focal plane.

Temporal resolution was limited to 1 frame every 15 seconds or so, but *Burkholderia* typically took 5 min to form then escape from a protrusion into the neighboring cell's cytosol. Just from looking at the video set, my impression is that protrusions grow at constant speed until just before bacterial escape and cell-cell fusion. Bacteria sometimes pause for a few frames at the protrusion apex before actual escape, but this doesn't seem to be particularly consistent. However, I've not spent any great deal of time analyzing the videos for these specific parameters, so these are just my general impressions.

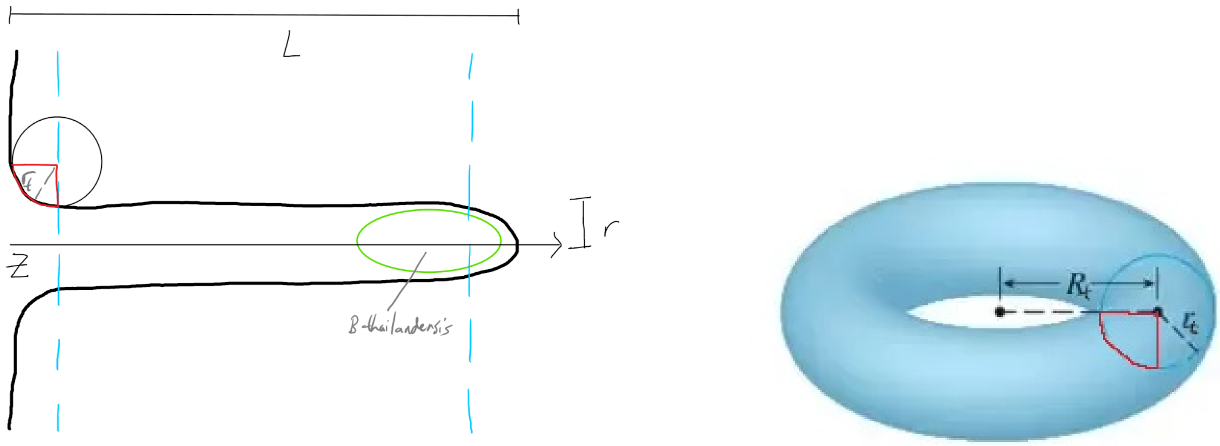
The image in figure 1 clearly doesn't have very high spatial resolution, so I'm currently working on imaging the protrusions via transmission electron microscopy. That could take a while though, so this is the best we have on *Burkholderia* protrusions for the time being.

## 2 Geometric model of the protrusion

To make it easier to examine the membrane bending stress over the protrusion's profile, I assumed the protrusion was rotationally symmetric and broke it into three sections as shown in figure 2. Each section is modeled as a simple geometric shape. The protrusion tip (the "cap") is modeled as a hemisphere; the main body of the protrusion is made an open-ended cylinder; and the base is taken as a quarter circle revolved around the protrusion's main axis to product a torus segment. Each segment's surface is continuous with neighboring ones so that a single protrusion lumen is produced.

Note that the radius of the torus tube, $r_t$, is a free parameter.

While the protrusion is a double-membrane structure, I treated it as a single-membrane structure for simplicity since our current goal is to figure out where the highest mechanical stresses are, not a precise calculation of bending stresses.




(a) Segmentation of protrusion into *base* (left), *body* (center), and *cap* (right). L = 5 $\mu m$, r = 0.5 $\mu m$.

(b) Torus with labeled dimensions

Figure 2: Model of the protrusion symmetric about the indicated z axis. The *base* has been modeled as if the quarter circle outlined in red in (b) has been rotated about the protrusion's longitudinal axis as shown by the matching red quarter circle in (a). The base is thus the bottom inner portion of a torus centered where the protrusion's longitudinal axis intersects the blue dashed line on the left of (a). Meanwhile, the body is taken to be a cylinder, and the cap is taken to be a hemisphere

# 3 Calculation of bending energy and stress

Membrane bending energy was calculated in cylindrical coordinates using the Helfrich-Canham-Evans free energy as presented in *Physical Biology the Cell* (2e), equation 11.7:

$$G_{bend}[h(z,\theta)] = \frac{K_b}{2}\int da[\kappa_1(z,\theta) + \kappa_2(z,\theta)]^2 \tag{1}$$

where h is the distance between the membrane and the z axis while $\kappa_1$ and $\kappa_2$ are the membrane's principal curvatures at that point. $K_b$ was set as 15 kT.

The $G_{bend}$ per unit area is thus

$$G_{bend}[h(z,\theta)] = \frac{K_b}{2}[\kappa_1(z,\theta) + \kappa_2(z,\theta)]^2 \tag{2}$$

We can obtain the bending stress, $\sigma_{bend}$, by dividing equation 2 by the local membrane thickness. I assumed membrane thickness was uniform at 5 nm over the whole protrusion.

Since the principal curvatures are uniform over the entire surface of a sphere and of the sides of a cylinder, the bending stresses will be the same everywhere within the protrusion cap and within the protrusion body. We can intuitively predict that the cap will have a higher bending stress than the body since it is more curved than the body. More quantitatively, their stresses are given by

$$\sigma_{bend}^{cap} = G_{bend}^{cap}\frac{1}{5nm} = \frac{K_b}{2}[\frac{2}{r}]^2\frac{1}{5nm} \tag{3}$$

$$\sigma_{bend}^{body} = G_{bend}^{body}\frac{1}{5nm} = \frac{K_b}{2}[\frac{1}{r}]^2\frac{1}{5nm} \tag{4}$$

On the other hand, the toroidal base has constant curvature parallel to the protrusion's longitudinal axis, but the base's curvature going along the protrusion's circumference is a function of z, the distance along the protrusion. For the base, $\kappa_1$ is constant at $\frac{1}{r_t}$. However, $\kappa_2 = \frac{1}{h(z,\theta)}$ within the base and is therefore a function of z. I calculated $h(z,\theta)$ as

$$h(z,\theta)_{base} = r + r_t - r_t cos(arcsin(\frac{r_t - z}{r_t})) \tag{5}$$

Using equation 5, $\sigma_{bend}$ for the base was calculated over z via

$$\sigma_{bend}^{base} = G_{bend}^{base} \frac{1}{5nm} = \frac{K_b}{2} [\frac{1}{r_t} + \frac{1}{h(z,\theta)}]^2 \frac{1}{5nm} \tag{6}$$

Since $r_t$ is a free parameter, the $\sigma_{bend}$ profile over z was calculated for various values of $r_t$. Using ImageJ and trying to make a circle following the base's membrane, I estimated $r_t$ to be 200 nm and therefore constructed the range of $r_t$ values around 200 nm.

# 4 Results

The graph of $\sigma_{bend}$ vs z for various $r_t$ choices is shown below in figure 3.

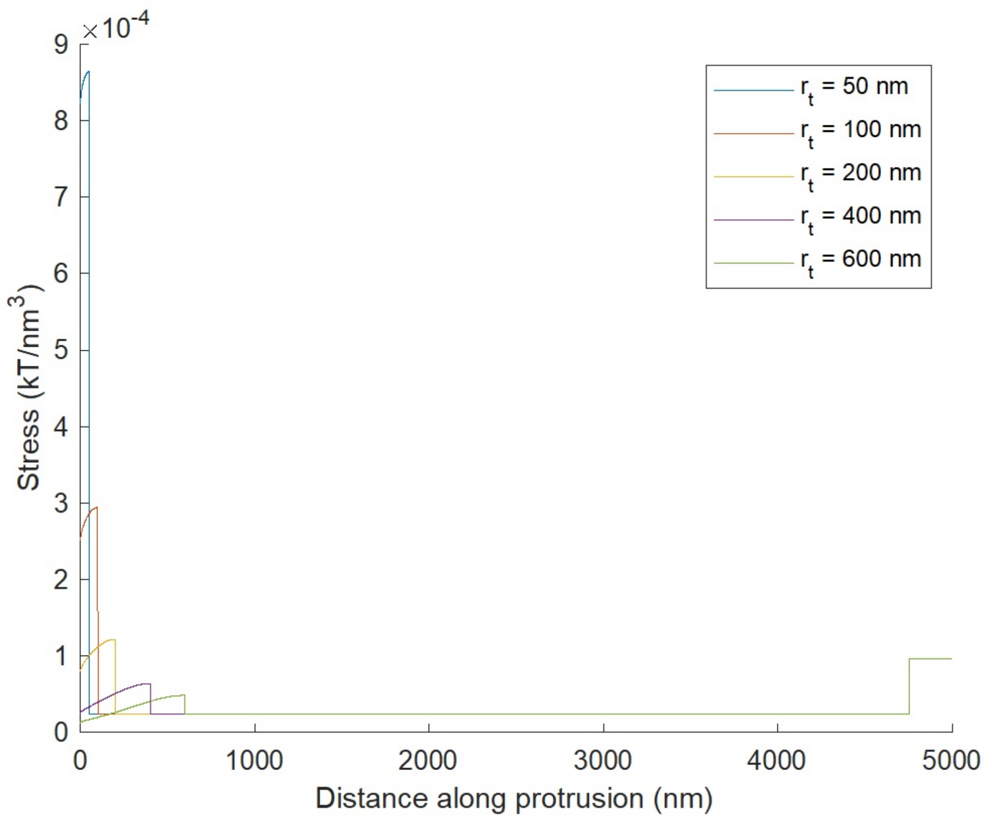



Figure 3: Plot of membrane bending stress vs distance vs z, the distance along the protrusion.