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Comment

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Comment on "Mechanical Properties of Giant Liposomes Compressed between Two Parallel Plates: Impact of Artificial Actin Shells"

Schäfer and coauthors[1] reported an extensive study on the mechanical characterization of giant vesicles, using a scanning force microscope and an ingenious experimental strategy. By means of an appropriate mechanical model based on Yoneda's work,[2] the authors were able to obtain mechanical parameters of the vesicle membrane, namely, the in-plane tension, σ_0, and the area compressibility modulus, K_A, from experimental force-compression curves. Although detailed in the description of the model, the article shows little if nothing about the method of data analysis. The latter poses a nontrivial mathematical issue, which is worth not only mentioning but also optimizing to some extent. In this Comment, we thus aim to complement the work of the authors by introducing a mathematical procedure to calculate membrane parameters from experimental force-compression data that involves the use of reduced variables and one single curve fitting.

To obtain σ_0 and K_A, Schäfer and coauthors fit the following equation to the experimental data:

$$F = \frac{2\pi R_0 R_i^2}{R_0^2 - R_i^2}\left(\sigma_0 + \frac{A_{cl} - A_0}{A_0}K_A\right), \tag{1}$$

where F is the force, R_0, R_i, and R_2 are the radii that characterize the shape of the compressed vesicle, and A_{cl}, A_0 are the surface areas of the compressed and uncompressed vesicle, respectively.[1] To do that, however, it is necessary to find expressions that relate R_0, R_i, R_2, as well as A_{cl}, to the extent of compression. The authors show in detail that this is possible by assuming that both the vesicle volume and the membrane curvature remain constant during compression. As a result, they obtain the following set of equations:

$$\begin{aligned}
&2\frac{R_0}{R_0^2 - R_i^2} = \frac{1}{R_0} + \frac{1}{R_2}\\
&\frac{4}{3}\pi R_v^3 = 2\pi(R_0 - R_2)\left\{\frac{z}{2}\sqrt{R_2^2 - \frac{z^2}{4}} + R_2^2 \arcsin\frac{z}{2R_2}\right\}\\
&\qquad + \frac{\pi z(12(R_0 - R_2)^2 + 12R_2^2 - z^2)}{12}\\
&R_i = R_0 - R_2 + \sqrt{R_2^2 - \frac{z^2}{4}}\\
&A_{cl} = 2\pi R_i^2 + 2\pi\left(2(R_0 - R_2)R_2 \arcsin\frac{z}{2R_2} + R_2 z\right),
\end{aligned} \tag{2}$$

where z is the height of the compressed vesicle such that compression = $2R_v - z$, with R_v being the radius of the vesicle. At this point, the analysis turns to be nontrivial insofar as it is necessary to solve the complicated system of highly-coupled, nonlinear algebraic eqs 2 for each z and for a given vesicle of radius R_v. Moreover, the system (eq 2) contains algebraic expressions that may introduce singularities in the equations and in their derivatives, which makes its resolution especially troublesome. Nonetheless, the system can be formulated in such a way that most of the expressions leading to singularities can be removed. If expressed in terms of the dimensionless variables $\bar{R}_0 = R_0/R_v$, $\bar{R}_i = R_i/R_v$, $\bar{R}_2 = R_2/R_v$, $\bar{z} = z/R_v$, and $\bar{A}_{cl} = A_{cl}/R_v^2$, the system (eq 2) takes the form

$$\begin{aligned}
&\bar{R}_0^2\bar{R}_2 + \bar{R}_i^2(\bar{R}_2 + \bar{R}_0) - \bar{R}_0^3 = 0\\
&\frac{4}{3} - 2(\bar{R}_0 - \bar{R}_2)\left(\frac{\bar{z}}{2}\sqrt{\bar{R}_2^2 - \bar{z}^2/4} + \bar{R}_2^2 \arcsin\left(\frac{\bar{z}}{2\bar{R}_2}\right)\right)\\
&\quad - \frac{\bar{z}}{12}(12(\bar{R}_0 - \bar{R}_2)^2 + 12\bar{R}_2^2 - \bar{z}^2) = 0\\
&\bar{R}_i^2 - 2\bar{R}_i(\bar{R}_0 - \bar{R}_2) + \bar{R}_0(\bar{R}_0 - 2\bar{R}_2) + \bar{z}^2/4 = 0\\
&\bar{A}_{cl} = 2\pi\bar{R}_i^2 + 2\pi\left(2(\bar{R}_0 - \bar{R}_2)\bar{R}_2 \arcsin\frac{\bar{z}}{2\bar{R}_2} + \bar{R}_2\bar{z}\right)
\end{aligned} \tag{3}$$

and can then be easily solved with standard iterative methods, such as the Levenberg-Mardquart[3,4] or the Trust-Region[5] algorithms. It is noteworthy that the dimensionless radii (Figure 1, left) and the area ratio A_{cl}/A_0 (Figure 1, right) can then be calculated for every realistic value of the compression once and for all; that is, relative to R_v, the latter can take any value from 0 (uncompressed vesicle, $\bar{z} = 2$) to 1 (vesicle compressed to half its diameter, $\bar{z} = 1$).

The membrane parameters σ_0 and K_A can thus be obtained from any set of F − compression or $F - z$ data and the solutions of Figure 1, by fitting the following expression to the reduced magnitudes $\bar{F} = F/R_v$ and $\bar{z} = z/R_v$,

$$\bar{F} = \frac{2\pi\bar{R}_0\bar{R}_i^2}{\bar{R}_0^2 - \bar{R}_i^2}\left(\sigma_0 + \frac{\bar{A}_{cl} - \bar{A}_0}{\bar{A}_0}K_A\right) \tag{4}$$

The model predicts that σ_0 and K_A do not depend on R_v, and therefore all vesicles with the same membrane should display identical $\bar{F} - \bar{z}$ behavior. This enormously simplifies the analysis, especially in the case of a large number of data sets. The experimental data can then be reduced to a single $\bar{F} - \bar{z}$ trend, and so can the number of curve fittings. The upper row of Figure 2 shows an example of how σ_0 and K_A are calculated out of a single curve fitting with no loss of statistical significance. To fit the data from four vesicles of different radii but the same membrane parameters, one can either employ eq 1 four times or eq 4 once. In both cases, we obtain exactly the same values for σ_0 and K_A (i.e., σ_0 = 0.11 mN/m and

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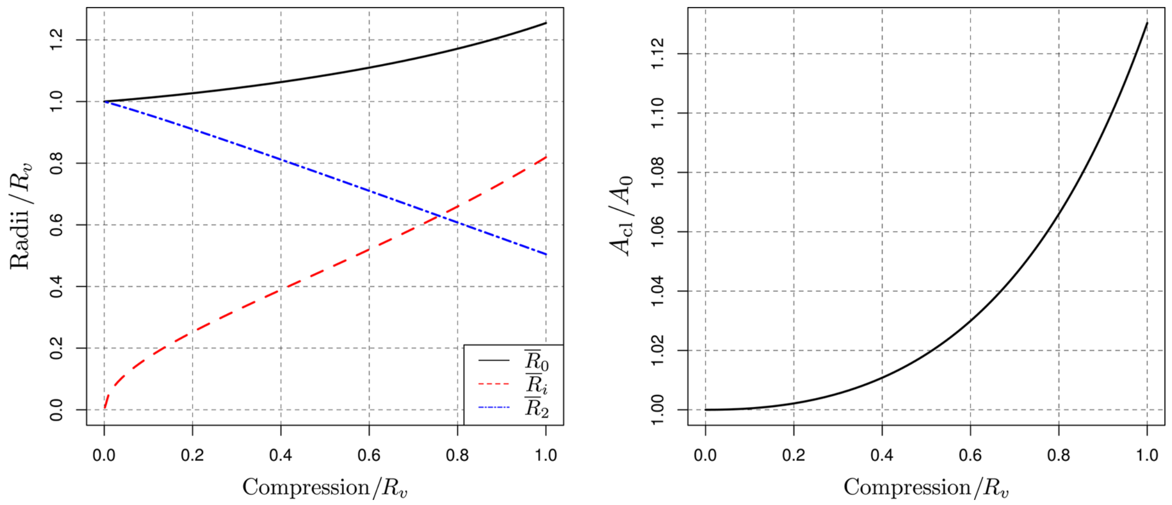

Figure 1. Dimensionless radii, $\overline{R}_0$, $\overline{R}_i$, and $\overline{R}_2$, of the vesicle (left), and area of the compressed vesicle relative to that of the uncompressed vesicle, A_{cl}/A_0, ($A_0 = 4\pi R_v^2$, right) as functions of the dimensionless compression, compression/R_v.

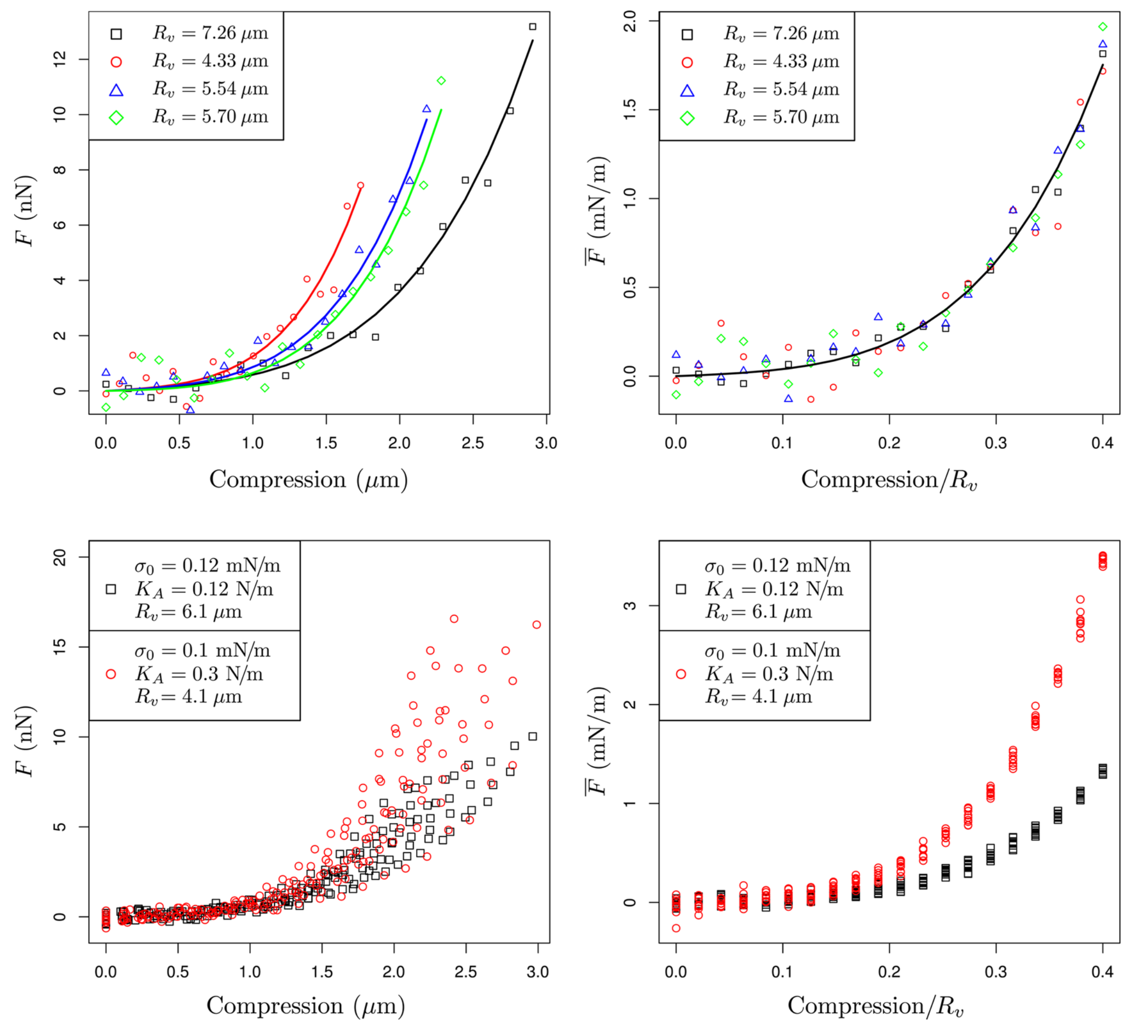

Figure 2. Example of reduced data analysis. Upper row: Data of vesicles with different R_v but same σ_0 and K_A. On the left, the force-compression graph shows four curve fits. On the right, the reduced force-compression plot shows one single curve fit. Lower row: Data of vesicles with two different σ_0's and K_A's. On the left, the force-compression graph displays highly dispersive mechanical behavior to compression. On the right, the graph of reduced magnitudes shows two distinct, low-dispersed mechanical trends. (Monte Carlo simulated data from the experimental curve and parameters of Figure 10b in ref 1. White noise $N(0,\mathrm{sd})$ with a standard deviation of 0.1 nN was added to the force values to simulate random data dispersion. Likewise, white noise with a standard deviation of 1 μm was used to generate different R_v's within the range 4.33−7.3 μm).

K_A = 0.12 N/m) within a statistical uncertainty that differs by less than 0.1% when both methods are compared. The graphical representation of reduced magnitudes is also advantageous in the sense that it provides a means to rapidly identify vesicles with different membrane properties out of a large number of data sets. The lower row of Figure 2 shows two groups of 10 simulated force-compression data sets from vesicles of varying sizes whose membranes are characterized by either one of two possible values for σ_0 and K_A. Unlike the force-compression representation (Figure 2, left), the representation of reduced magnitudes (Figure 2, right) arranges the otherwise highly dispersive data into two clearly distinguishable graphical patterns that display different mechanical behavior toward compression.

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Notes

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