Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary information:
Membrane rolling induced by bacterial toxins

Martin Berg Klenow[1,*], Jonas Camillus Jeppesen[1], and Adam Cohen Simonsen[1]

[*]Corresponding author: klenow@sdu.dk

[1]Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark.

1 Theoretical model for membrane rolling dynamics

1.1 Energy change during membrane rolling

The theoretical model presented here builds upon previous work showing that rolling of a membrane patch is energetically favored[1]. We consider a membrane patch on top of a supporting membrane. The initial state is a flat membrane patch. Upon binding of a protein (which induces negative curvature) a spontaneous curvature c_0 of the membrane will be induced. If the spontaneous curvature is sufficiently large the membrane patch will roll up, this rolled configuration is the final state. We consider a roll with a width w which have rolled a length x. We would like to find the energy difference between the initial state and the final state. The energies considered are the adhesion energy, E_{ad} and the curvature elastic energy, E_{c}. The adhesion energy can be described as

$$E_{\mathrm{ad}} = A \cdot w_{\mathrm{ad}} \tag{1}$$

where A is the area and w_{ad} is the adhesion energy per area. The curvature elastic energy originates from the Helfrich Hamiltonian[2] and is given by the sum of a mean curvature term E_{mc} and a gaussian curvature term E_{gc}:

$$E_{\mathrm{c}} = E_{\mathrm{mc}} + E_{\mathrm{gc}} = \int_A \left[\frac{1}{2}k_c(\overline{c} - c_0)^2 + k_G\overline{c_G}\right] dA \tag{2}$$

where k_c is the mean curvature elastic modulus, $\overline{c}$ is the mean curvature, k_G is the gaussian curvature elastic modulus and $\overline{c_G}$ is the gaussian curvature. The mean curvature is given by $\overline{c} = \frac{1}{R_1} + \frac{1}{R_2}$ where R_1 and R_2 are the radii of curvature. The gaussian curvature is given by $\overline{c_G} = \frac{1}{R_1} \cdot \frac{1}{R_2}$. In the case of cylinder corresponding to a linear roll, $R_2 = \infty$ and $R_1 = R(s)$ where $R(s)$ is the local radius of curvature in the roll at arc length s. That is the mean curvature reduces to $\overline{c} = \frac{1}{R(s)}$ and the gaussian curvature term drops out. The energy of the initial flat state is given by:

$$E_0 = E_{\mathrm{ad}} + E_{0\mathrm{c}} = A\left(\frac{k_c}{2}c_0^2 - w_{\mathrm{ad}}\right) \tag{3}$$

While the energy in the final rolled state is:

$$E_1 = \int_A \left[\frac{k_c}{2}(\overline{c} - c_0)^2\right] dA \tag{4}$$

The energy change for a roll of width w from the flat to the rolled state is:

$$\Delta E = E_1 - E_0 = w\int_0^{s_{\max}} \left[\frac{k_c}{2}\left(\frac{1}{R(s)} - c_0\right)^2 - \frac{k_c}{2}c_0^2 + w_{\mathrm{ad}}\right] ds \tag{5}$$

$$\Delta E = w\int_0^{s_{\max}} \left[\frac{k_c}{2}\left(\frac{1}{R(s)^2} - \frac{2c_0}{R(s)}\right) + w_{\mathrm{ad}}\right] ds \tag{6}$$

This is the general energy expression for the formation of a roll, rolled to the distance $s_{\max}$. Rolling is energetically favored when $\Delta E < 0$ or correspondingly the condition for the initiation of rolling is given by[1]:

$$\frac{k_c}{2}c_0^2 > w_{\mathrm{ad}} \tag{7}$$

Let us assume that the shape of the roll is an Archimedean spiral. Then the radius of the spiral as a function of the rolling angle θ will be given by $r(\theta) = a + b\theta$, where a is the radius of the inner roll and $2\pi b$ is the distance between the layers in the roll. For $r >> b$ the radius of curvature can be approximated by the radius of the spiral: $R \approx r$, which have been validated to be a very reasonable estimation[1]. From the same approximation we have $ds \approx rd\theta = \frac{r}{b}dr$. Applying this approximation we get:

$$\Delta E = \frac{wk_c}{b}\int_a^{a+b\theta} \left(\frac{1}{2r} - c_0 + \frac{w_{\mathrm{ad}}}{k_c}r\right) dr \tag{8}$$

Let us define dimensionless variables by measuring lengths in terms of b. For the radius of the spiral we get:

$$\tilde{r} = \frac{r}{b} = \frac{a}{b} + \theta \qquad dr = bd\tilde{r} \tag{9}$$

Similarly we can define:

$$\tilde{c_0} = c_0 b \qquad \tilde{w} = \frac{w_{\mathrm{ad}}}{k_c} b^2 \tag{10}$$

By writing the integrand in terms of these dimensionless variables we get:

$$\frac{1}{2r} - c_0 + \frac{w_{\mathrm{ad}}}{k_c} r = \frac{1}{b}\left[\frac{1}{2\tilde{r}} - \tilde{c_0} + \tilde{w}\tilde{r}\right] \tag{11}$$

Inserting the integrand yields:

$$\Delta E = \frac{wk_c}{b} \int_{\frac{a}{b}}^{\frac{a}{b}+\theta} \left[\frac{1}{2\tilde{r}} - \tilde{c_0} + \tilde{w}\tilde{r}\right] d\tilde{r} \tag{12}$$

Applying $dr = bd\theta = bd\tilde{r}$ we can change the integration variable:

$$\Delta E(\theta) = \frac{wk_c}{b} \int_0^{\theta_m} \left[\frac{1}{2\tilde{r}} - \tilde{c_0} + \tilde{w}\tilde{r}\right] d\theta \tag{13}$$

where θ_m is the maximum roll angle.

1.2 Equation of motion for membrane rolling

In order to find the equation of motion for membrane rolling we will need the force on a roll from the curvature energy. We can find the rolling force by treating the curvature energy as a potential energy:

$$F_x = -\frac{\partial \Delta E}{\partial x} = -\frac{\partial \Delta E}{\partial \theta}\frac{\partial \theta}{\partial x} \tag{14}$$

$$-\frac{\partial \Delta E}{\partial \theta} = -\frac{wk_c}{b}\left[\frac{1}{2\tilde{r}} - \tilde{c_0} + \tilde{w}\tilde{r}\right] \tag{15}$$

Then we need to find $\frac{\partial \theta}{\partial x}$. The rolled distance is given by $x = \int ds = \int r d\theta$. That is $\frac{\partial x}{\partial \theta} = r$. By inverting the expression we get:

$$\frac{\partial \theta}{\partial x} = \frac{1}{r} = \frac{1}{b\tilde{r}} \tag{16}$$

Inserting the inverted expression we get

$$F_x = -\frac{wk_c}{b^2}\left[\frac{1}{2\tilde{r}^2} - \frac{\tilde{c_0}}{\tilde{r}} + \tilde{w}\right] = -\frac{wk_c}{b^2} f_x(\tilde{r}) \tag{17}$$

This is the force on a roll in the x-direction as a function of the roll radius. We would like to convert the $\tilde{r}$-dependence to a $\tilde{x}$-dependence. In order to do so we start by rewriting the rolled length:

$$x = \int ds = \int r d\theta' = \int_0^{\theta} (a + b\theta') d\theta' = a\theta + \frac{1}{2} b\theta^2 \tag{18}$$

The dimensionless rolled length is then:

$$\tilde{x} = \frac{x}{b} = \frac{a\theta}{b} + \frac{1}{2}\theta^2 \tag{19}$$

Solving for the rolling angle yields:

$$\theta = -\frac{a}{b} + \sqrt{\left(\frac{a}{b}\right)^2 + 2\tilde{x}} \tag{20}$$

Now we can rewrite the dimensionless roll radius:

$$\tilde{r} = \frac{a}{b} + \theta = \sqrt{\left(\frac{a}{b}\right)^2 + 2\tilde{x}} \tag{21}$$

Applying the conversion we can rewrite the rolling force as

$$F_x = -\frac{wk_c}{b^2}\left\{\frac{1}{2\left[\left(\frac{a}{b}\right)^2 + 2\tilde{x}\right]} - \frac{\tilde{c_0}}{\sqrt{\left(\frac{a}{b}\right)^2 + 2\tilde{x}}} + \tilde{w}\right\} = -\frac{wk_c}{b^2} f_x(\tilde{x}) \tag{22}$$

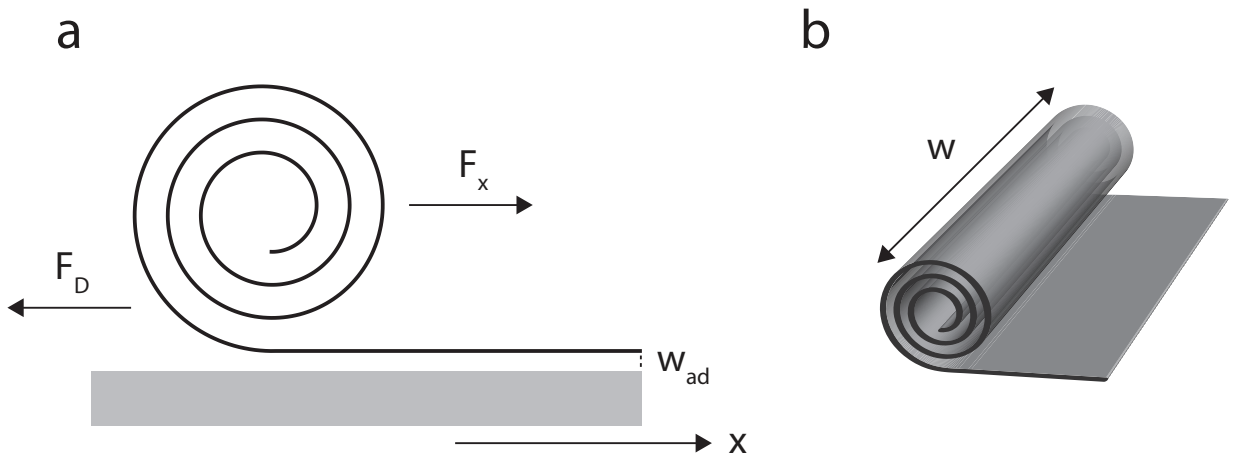

Figure S1: Illustration of the forces on a membrane roll (**a**). F_x denotes the rolling force in the x-direction, F_D is the drag force and the adhesion energy per area is given by w_{ad}. 3-dimensional illustration of the membrane roll (**b**). The width of the roll w is indicated.

Furthermore there is a drag force on the roll, F_D, which can be approximated by the drag force on a cylinder. Similar to the more common geometry of a sphere, we assume a laminar flow around the cylinder, i.e. at low Reynolds number the friction will be proportional to the velocity $v = \dot{x}$:

$$F_D = -\gamma w \dot{x} = -\gamma w \dot{\tilde{x}} b \tag{23}$$

where γ is the friction coefficient on a cylinder. The total force on the roll is the sum of the rolling force, F_x and the drag force, F_D. All forces on the roll are illustrated in Figure S1. The adhesion energy per area w_{ad} is also shown, this term is included in the rolling force F_x. Now we would like to find the equation of motion for membrane rolling. From Newton's second law we have:

$$F_{\mathrm{tot},x} = F_x + F_D = \frac{d}{dt}(m\dot{x}) \tag{24}$$

We consider now small objects experiencing a laminar flow, that is at low Reynolds numbers. In the laminar flow regime the rolling force will be in equilibrium with the drag force. The drag force will continuously slow down the rolling motion of the membrane. That is we can neglect the inertial term such that we have

$$F_x = -F_D \tag{25}$$

$$-\frac{wk_c}{b^2} f_x(\tilde{x}) = \gamma w \dot{\tilde{x}} b \tag{26}$$

Solving for the dimensionless velocity:

$$\dot{\tilde{x}} = -\frac{k}{\gamma b^3} f_x(\tilde{x}) = -\frac{1}{\tau} f_x(\tilde{x}) \tag{27}$$

Where the time constant τ is defined as:

$$\tau = \frac{\gamma b^3}{k} \tag{28}$$

We can insert $f_x(\tilde{x})$:

$$\dot{\tilde{x}} = -\frac{1}{\tau}\left[\frac{1}{2\left(\frac{a^2}{b^2} + 2\tilde{x}\right)} - \frac{\tilde{c_0}}{\sqrt{\left(\frac{a}{b}\right)^2 + 2\tilde{x}}} + \tilde{w}\right] \tag{29}$$

By scaling all times in terms of τ we get:

$$\dot{\tilde{x}}\tau = -\left[\frac{1}{2\left(\frac{a^2}{b^2} + 2\tilde{x}\right)} - \frac{\tilde{c_0}}{\sqrt{\frac{a^2}{b^2} + 2\tilde{x}}} + \tilde{w}\right] \tag{30}$$

This is the equation of motion for rolling of a membrane patch.

1.3 Results

In order to obtain theoretical values for the rolling velocity and the rolled length as a function of time we have to use specific values for the parameters: b, k_c, c_0, w_{ad} and γ. Below we state the parameter values we have used for our calculations for the theoretical model together with estimates from the literature based on similar lipid systems:

- $b = 2$ nm. The value is based on AFM measurements of roll diameters for annexin A4[3].
- $c_0 = 0.04\ \text{nm}^{-1}$. Computer simulations of Shiga toxin by Pezeshkian et. al. yield a spontaneous curvature of $c_0 = 0.033\ \text{nm}^{-1}$[4]. Similarly for cholera toxin the spontaneous curvature have been estimated by computer simulations to be $c_0 = 0.028\ \text{nm}^{-1}$[5]. As mentioned we find experimental estimates to be in the same range for both toxins.
- $k_c = 2.0 \cdot 10^{-20}$ J. Reported values for POPC are from $k_c = 2.5\text{-}8.5 \cdot 10^{-20}$ J, e.g. Marsh[6], table II.10.4.1, page 474 reports $k_c = 4.0 \cdot 10^{-20}$ J.
- $w_{\text{ad}} = 3.3 \cdot 10^{-6} \frac{\text{J}}{\text{m}^2}$. Literature values for neutral SOPC bilayers in 0.1 M PBS report a value of $w_{\text{ad}} \simeq 1.0 \cdot 10^{-5} \frac{\text{J}}{\text{m}^2}$[7].
- $\gamma = 0.32 \frac{\text{N·s}}{\text{m}^2}$. γ can be estimated for a cylinder[8]. Inserting realistic values for our system yields $\gamma = 0.8 \cdot 10^{-3} \frac{\text{N·s}}{\text{m}^2}$, which is far below our value, however the drag for a membrane roll must be considered to be much larger since the liquid can not flow underneath as would be the case for a cylinder.

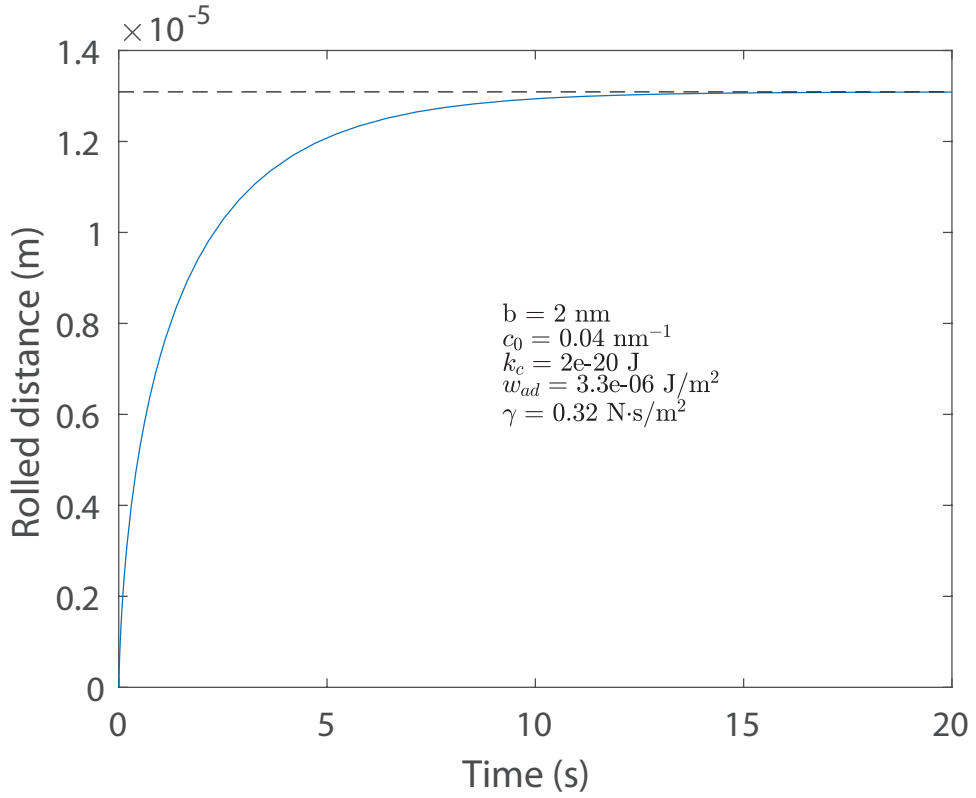

Figure S2: The cumulative rolled distance as a function of time for the theoretical model. The dashed line marks the maximum rolled distance.

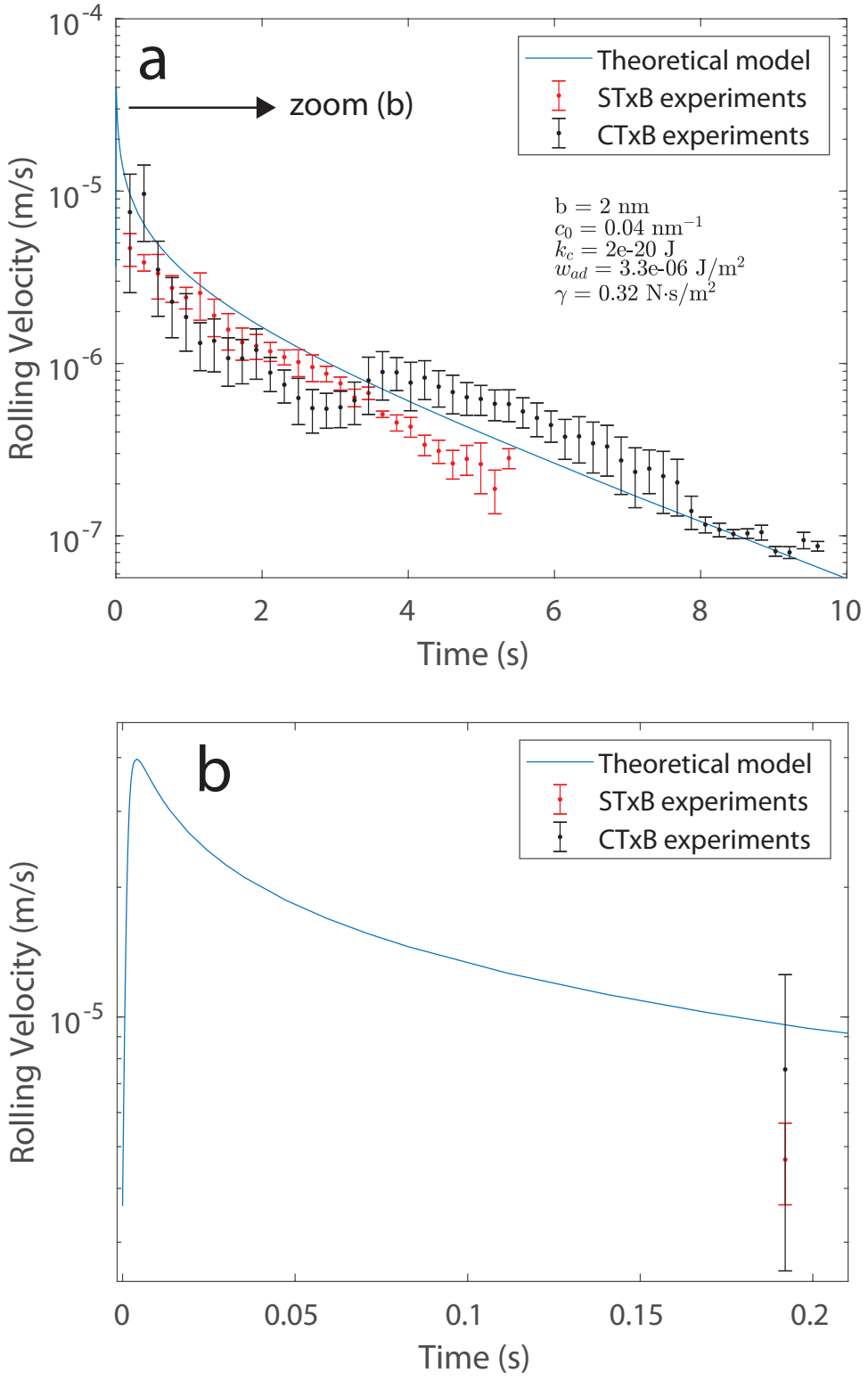

Figure S3: The rolling velocity as a function of time. The blue line shows the theoretical model, while the experimental data for STxB and CTxB are shown in red and black respectively (**a**). By performing a zoom the initial theoretical peak of the rolling velocity can be viewed (**b**).

2 Controls for toxin experiments

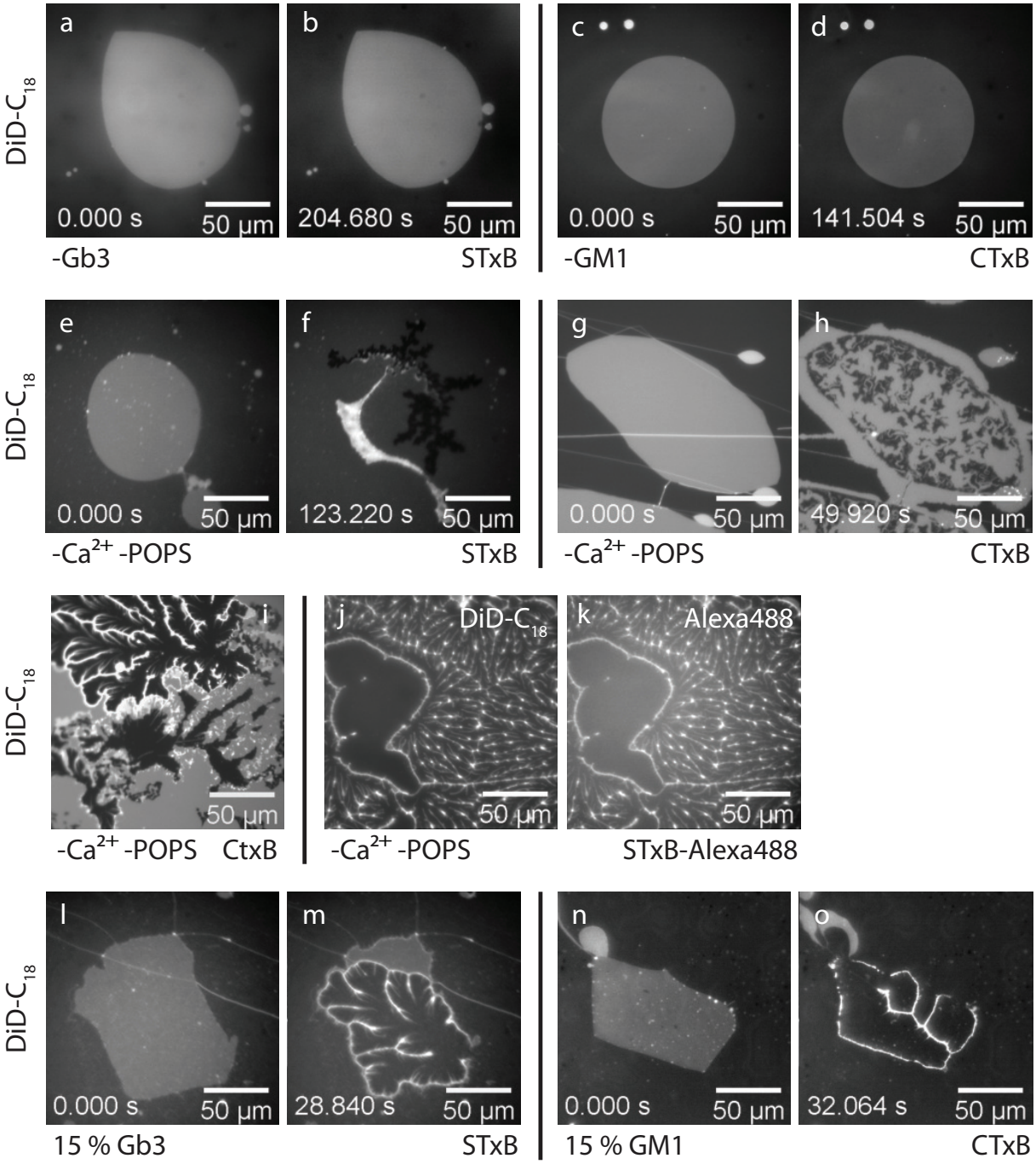

Figure S4: Control experiments for STxB and CTxB. No response of the membrane patch is observed when the glycosphingolipids are removed for both STxB (**a, b**) and CTxB (**c, d**). In the absense of Ca^{2+} and POPS rolling is only occuring in some cases whereas in most cases the membrane patch is dissolved and goes into solution over time as observed for both STxB and CTxB (**e-k**). When the content of glycosphingolipids in the supported membranes is increased to 15 % roll-up of the membrane patch is still observed for both STxB (**l, m**) and CTxB (**n, o**). Cell concentrations of proteins for all experiments: STxB: 40 nM, STxB-Alexa488: 40 nM, and CTxB: 40 nM. The experiments were performed with at least n = 5 repetitions.

References

1 T. L. Boye, J. C. Jeppesen, K. Maeda, W. Pezeshkian, V. Solovyeva, J. Nylandsted and A. C. Simonsen, *Scientific reports*, 2018, **8**, 1–14.

2 W. Helfrich, *Z. naturforsch*, 1973, **28**, 693–703.

3 T. L. Boye, K. Maeda, W. Pezeshkian, S. L. Sønder, S. C. Haeger, V. Gerke, A. C. Simonsen and J. Nylandsted, *Nature communications*, 2017, **8**, 1–11.

4 W. Pezeshkian, A. G. Hansen, L. Johannes, H. Khandelia, J. C. Shillcock, P. S. Kumar and J. H. Ipsen, *Soft matter*, 2016, **12**, 5164–5171.

5 W. Pezeshkian, L. J. Nåbo and J. H. Ipsen, *FEBS open bio*, 2017, **7**, 1638–1645.

6 D. Marsh, *Handbook of Lipid Bilayers*, CRC Press, Boca Raton, 2nd edn, 2013.

7 E. Evans and D. Needham, *Macromolecules*, 1988, **21**, 1822–1831.

8 B. Huner, *Cylinder Drag at Low Reynolds Number*, PhD thesis, Louisiana State University and Agricultural & Mechanical College, 1977.