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# Validation of the 3-under-2 principle of cell wall growth in Gram-positive bacteria by simulation of a simple coarse-grained model

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## Abstract

The aim of this work is to propose a first coarse-grained model of *Bacillus subtilis* cell wall, handling explicitly the existence of multiple layers of peptidoglycans. In this first work, we aim at the validation of the recently proposed “3-under-2” principle.

## 1 Introduction

The determination of cell shape is one of the fundamental question in bacterial cell biology. Bacterial cells are surrounded by boundary layers that may confer shape and rigidity and that allows protection and communication with the environment. However, little is known about the mechanisms determining cellular shape. Furthermore the larger questions concerning morphogenesis are the same in prokaryotic and eukaryotic systems: how is cell shape regulated temporally and spatially?

Bacterial cell wall (CW) synthesis and structure have been studied for decades and it is admitted for a long time that the CW is a highly dynamic structure, constantly synthesized and remodeled as the cell cycle progresses and as the bacterium adapts to its environment, see e.g. [20, 21, 4, 8, 24, 14, 22]. However, until recently, there are major gaps in our knowledge of the CW ultra-structure and in the molecular mechanisms that control

its morphogenesis. These gaps explain why the CW synthesis remains a challenging problem in bacterial cell biology.

Recent advances have opened new perspectives in this field. The first one concerns the architecture of the CW ultra-structure, see e.g. [24] for a review, and in particular for the Gram-negative model bacterium *Escherichia coli*. The second one concerns a fine characterization of the peptidoglycan (PG) features, which is a key element of the CW, see e.g. [16, 10, 13, 2, 12]. The last one is related to *in vivo* observations of the molecular actors involved in the CW synthesis, see e.g. [7, 11, 5, 25], thanks to progresses in single cell imaging.

These recent advances have allowed the community to take a decisive step towards the development of coarse-grained models of the CW morphogenesis<sup>1</sup>. Such a coarse-grained model makes it possible to couple global aspects of the morphogenesis with more local molecular mechanisms related to the wall synthesis. The first models were centred around *E. coli* for which it is reasonable to assume that the CW is composed of only one layer of PG, and thus for which the CW can be considered as a 2D elastic network composed of polymers of PG, with long strands of glycan, cross-linked by stretchable peptides, see e.g. [15, 9, 19]. In contrast, to our knowledge, such a coarse-grained model does not exist for Gram positive bacteria, in particular for its model *Bacillus subtilis*. The lack of such a model is clearly linked to the more complex nature of *B. subtilis*' CW since it is composed of multiple layers of PG. This specific feature has numerous consequences both from biological and experimental points of view.

A first consequence, known for a long time, concerns the general principles allowing the CW elongation during the cell cycle progression. In *E. coli*, the elongation is obtained through the insertion of new PG materials in the existing layer (see e.g. [4]); in *B. subtilis*, it is obtained through the application of an inside-to-outside principle: new layers of PG are added to the innermost face of the CW, pushing outwards the previous layers which will be eventually degraded, accounting for the observed PG turnover (see [20, 21, 6, 18, 8]). A second important consequence concerns the application of high-resolution atomic force microscopy to the study of the CW structure. Indeed, thanks to this approach, it is possible to obtain a direct evidence on the molecular structure of *E. coli*'s CW. In contrast, this approach leads to unclear conclusions on the wall's molecular structure for *B. subtilis*.

This lack of direct knowledge at the molecular level explains to some ex-

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<sup>1</sup>There also exist models where the CW is described as a global (and continuous) elastic structure comparable to some extent to an elastic balloon with a nonuniform thickness inflated by the osmotic pressure, see e.g. [23, 17].

tent why it remains difficult to build a coarse-grained model for *B. subtilis*. To bypass this limitation, it is necessary to combine several recent advances to propose a realistic hypothesis. Obviously, this hypothesis has to be compatible with the set of all indirect experimental evidences about the CW, as for instance the circumferential movement of the synthesis machines [7, 11] or the existence of very long strands of glycan [15] to cite a few.

Following this hypothesis, the aim of our work is to propose a first coarse-grained model of *B. subtilis*' cell wall, handling explicitly the multiple layers of PG. Our main goal for this first step is to validate the synthesis principle, the so called "3-under-2" principle, recently proposed in [3]. Here, we restrict ourselves to a short bi-layers CW as it is both enough for the purposes of this paper and allows for a simpler presentation while saving computation time for the simulation.

## 2 Consequence of the morphogenesis on the ultra-structure

For the sake of clarity, we first assume that a PG has 4 potential bonds as represented in Figure 1. The CW ultra-structure is obtained by the super-

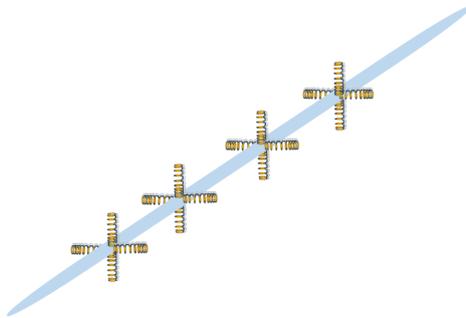


Figure 1: Assumption on a PG strand structure used for explanation

position of several layers, with the same structure as the one used in [15, 19]. Consequently, it is a planar model with a 90 degree angle between two successive peptidic cross-links. For sake of simplicity, we assume that the CW ultra-structure is regular.

With the above assumptions on the PG strand, it is possible to obtain different configurations for a bi-layers CW as illustrated in Figure 2. In order

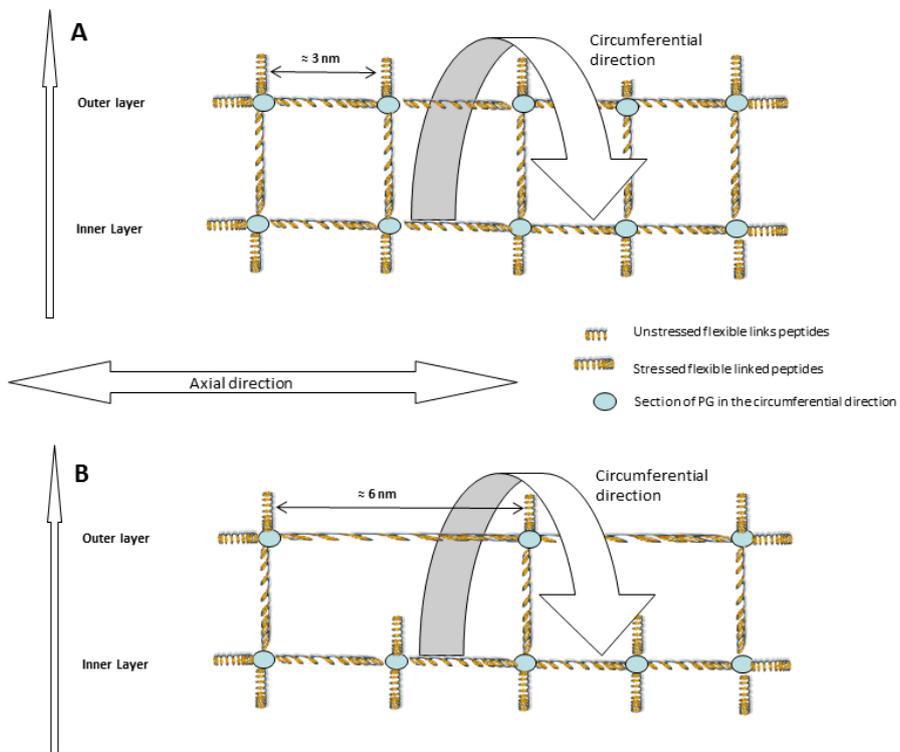


Figure 2: (A) A potential bi-layers ultra-structure. (B) A bi-layers CW ultra-structure with the “3-under-2” morphogenesis principle.

to ensure that a PG layer insertion leads to the same ultra-structure, we have introduced the “3-under-2” principle leading to the addition of a new layer as described in Figure 3. The application of this principle leads to the structure

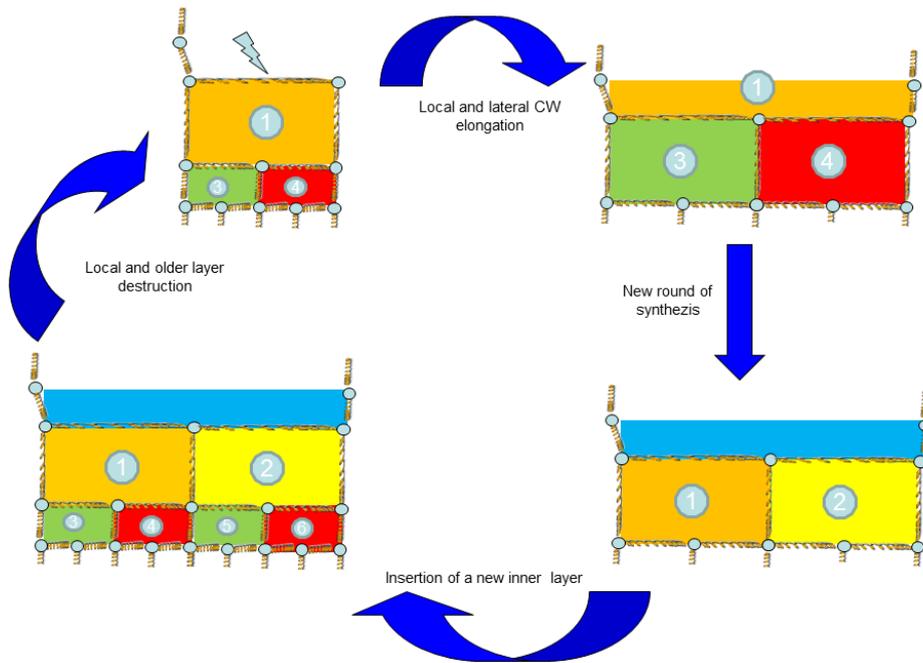


Figure 3: Insertion cycle of PG strands with the “3-under-2” morphogenesis principle.

of configuration **B** in Figure 2, the configuration **A** on the same figure is not reachable by the chosen principle.

The previous derivations can be made with the usual model of the PG, depicted in Figure 4. Under this more realistic description, we obtain the ultra-structure depicted in Figure 5 and we will show by simulation that the “3-under-2” principle guarantees the regularity of the CW structure.

### 3 Simulation

For a mono-layer CW, all the pressure is transferred, through the membrane, to the only one existing layer which dissipates all the pressure energy. For a multi-layers CW, the situation is more complex as each layer dissipates only a fraction of the pressure energy. The problem is well summarized in the

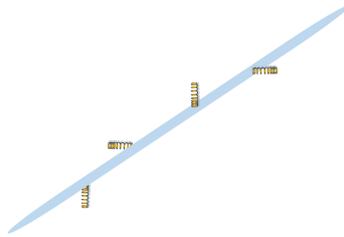


Figure 4: A realistic model of a PG strand

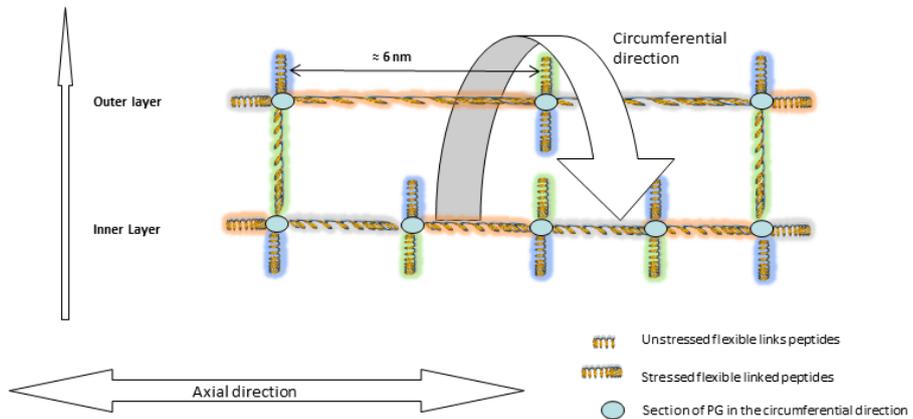


Figure 5: Hypothesized bi-layers CW: a colored aura represents a depth.

case for a bi-layers CW. The pressure is transferred to the innermost layer which bears a fraction of it via its internal structure; since both layers do not penetrate each other, the remaining part of the pressure is transferred to the outermost layer via an interaction of repulsive nature, this outermost layer bearing this remaining part. To simulate such features, there is a need for a dedicated and complex algorithm such as the ones used in clothes simulation where the clothes should not penetrate the person's body wearing them [1].

Nonetheless, our objective here is less demanding, we would like to validate the "3-under-2" principle which has been proposed only recently and not yet validated by simulation. Indeed, if for a complete layer the proposed model is coherent, the dynamic part of the CW elongation calls for a validation. In Figure 6, it is shown that inserting a patch in a regular structure results in some PG strands to be mono-layered and that this strands are the only ones with all peptidic bonds actually connected to another PG. This is a dynamical feature which disappears when the new layer has been completed as the regular nature of the CW structure will result in a two times longer structure with the same motif. Moreover the number of layer to layer peptidic bridges was shown above to be two times less than expected. This could lead to a potential fragility of the structure. Therefore, these layer to layer peptidic bridges may play an important role in the dynamical process of elongation.

### 3.1 Simulation characteristics

We propose a simple simulation on a regular structure in order to keep the simulation as simple as possible.

Since the circumference of the outermost strands is necessarily greater than the one of the innermost strands: they are more stressed in the radial direction and bear a greater fraction of the radial pressure. Due to their regularity, it is likely that each layer bears a constant fraction of this radial pressure. Moreover, the bacteria is assumed to regulate its osmotic pressure so that its value is constant in our simulations. The pressure has thus been split and applied on both layers separately saving the computation time of the interactions between the membrane and the innermost layer and between the layers themselves. From a computational point of view, the mono-layered strands belong to both layers so that they effectively bear all the pressure.

A PG is modelled as a mass that has potentially three bonds to other PG: 2 glycosidic bonds and 1 peptidic bridge (see Figure 7). The chain of glycosidic bonds forms a PG strand.

Moreover, as the insertion is not performed on the both poles, they are not simulated. We assume that the first and last strands of CW lie in a plane

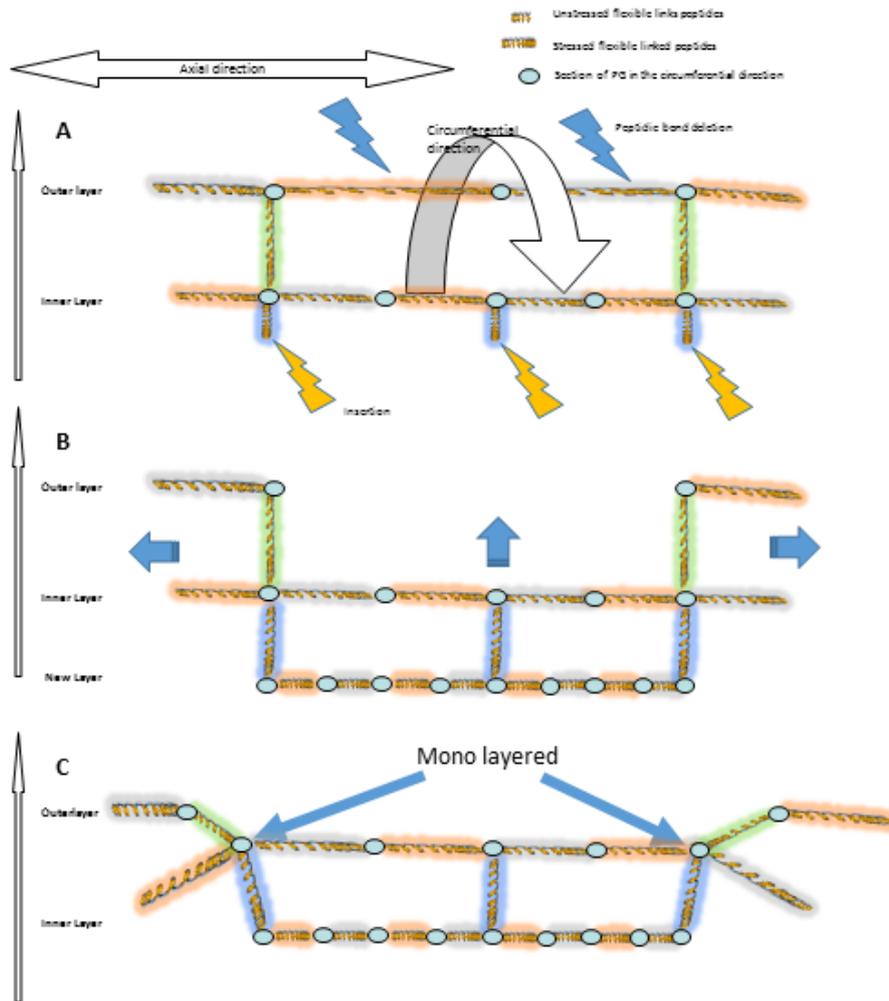


Figure 6: An elongation cycle showing two mono-layered strands (all the unnecessary potential peptidic bonds were deleted for clarity). (A) The original structure with the insertions and deletions. (B) The resulting CW ultra-structure: the newly PG strands and the ones above them are pushed up by the osmotic pressure while the CW elongates. (C) The extreme strands becomes mono-layered during the process even if a completed new layer would result in a bi-layers CW everywhere.

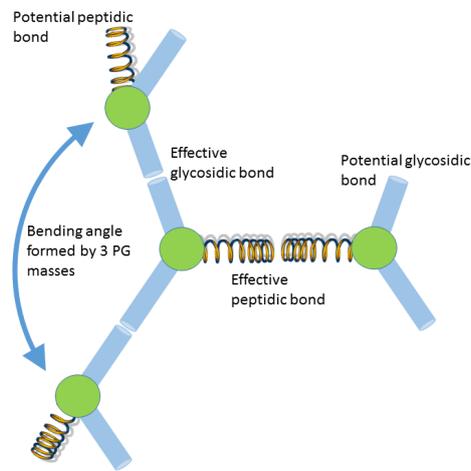


Figure 7: A PG is modelled as a mass (green) with two potential glycosidic bonds (blue) and one potential peptidic bond (spring). Whenever two potential glycosidic bonds match, they form an effective glycosidic one, likewise when two potential peptidic bonds match, they form an effective peptidic one. Each effective bond (either glycosidic or peptidic) stores an energy that creates a force applied on the two PG masses forming the bond. Whenever a PG has its two effective glycosidic bonds, a bending angle is created which stores an energy and creates a force applied on the three PG masses forming the angle.

described by a mechanical frame which can translate and rotate with respect to a reference (inertial) frame. Axial pressure acts on the surface delimited by the PGs belonging to a pole.

The simulation is further detailed in Appendix A.

### 3.2 Swelling up of initial structure

We describe here the results of an initial swelling up (see Figure 8 and Figure 9 for a zoom) with two sets of parameters values with the initial CW shown in panel **A**. The first set of values (see panel **B**) is the ones of [15] and [19]. The peptidic force was fitted with a polynomial of degree 9. We also used a pressure of 10 bars. In the second set of values (see panel **C**), we used a nonlinear force for the glycosidic bond equal to 5 times the one of the peptidic bond and increased the bending force by a factor 100. As can be

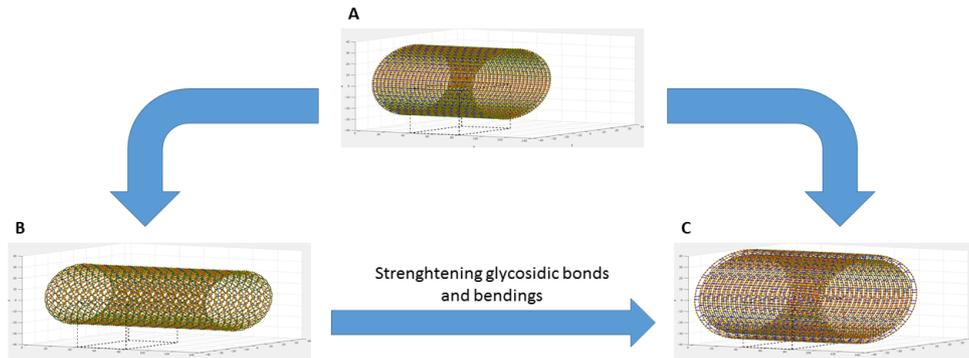


Figure 8: **(A)** Initial CW (PG mass in green, peptidic bonds in blue, glycosidic bonds in brown/red). **(B)** CW swelled up with the first parameter set. **(C)** CW swelled up with the second parameter set.

seen, for both sets, the CW ultra-structure is regular. With the second set, the bacteria is shorter and the hexagons are less pronounced due to a higher bending force, the overall structure being still regular. Note also that due to this higher bending force, the bacteria is larger since the circumference of a straighter strand is higher.

### 3.3 Insertion of PG strands

From the results of Figure 8, panel **(C)**, Figure 10 depicts the resulting CW structure after strands insertion. In both panels **B2** and **C2**, the bacteria should be longer than in panel **A**. The length ratio can be approximated

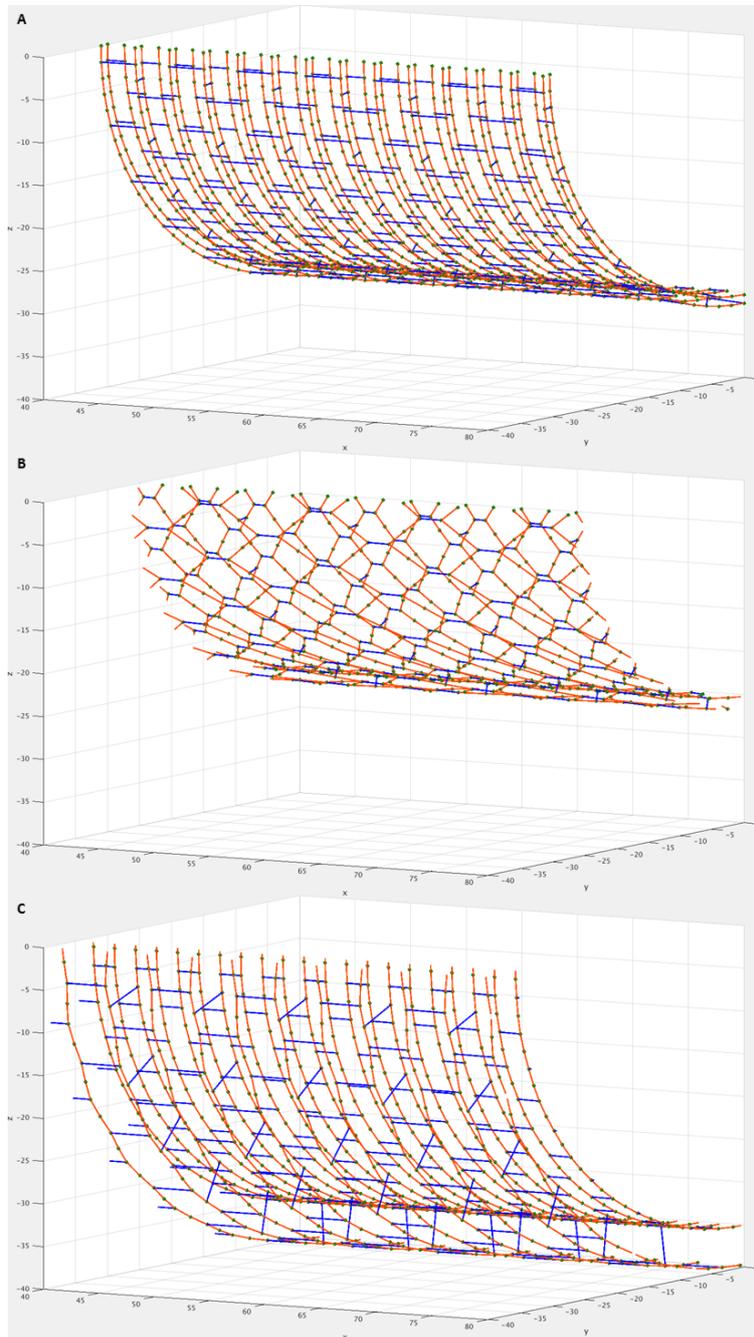


Figure 9: Zoom on the part of the space delimited by the dark dotted lines in Figure 8. **(A)** Initial CW (PG mass in green, peptidic bonds in blue, glycosidic bonds in brown/red). **(B)** CW swelled up with the first parameter set. **(C)** CW swelled up with the second parameter set.

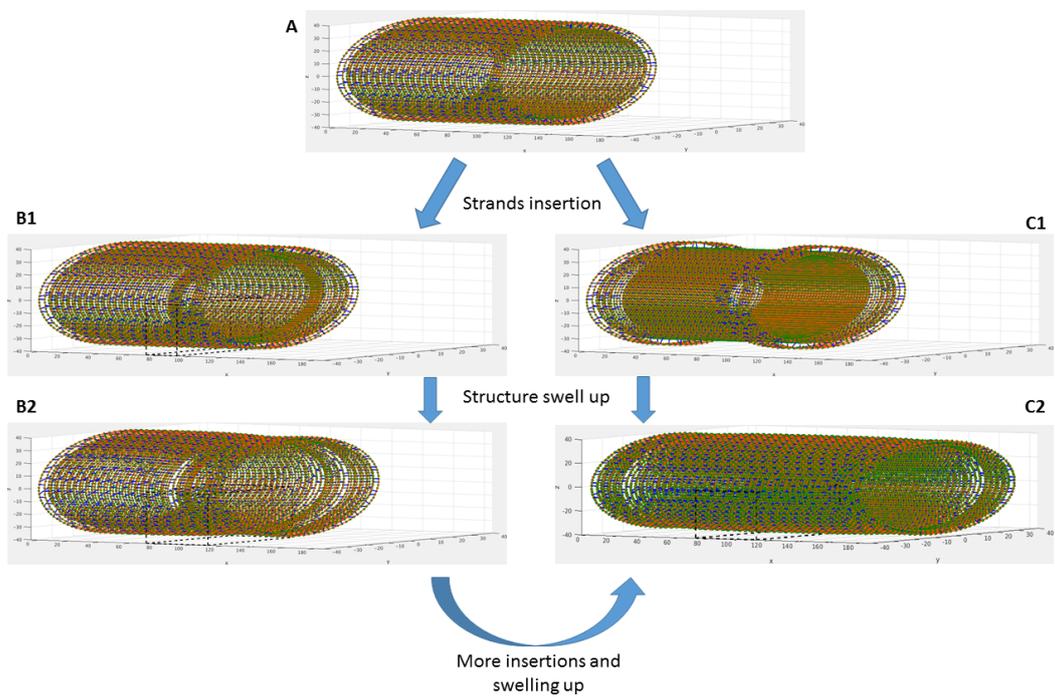


Figure 10: CW after strands insertion. (A) CW before insertion, from Figure 8, panel (C), rescaled for the need of this illustration. (B1) CW after a “short” insertion of 9 strands. (B2) CW after swell up of B1. (C1) CW after a “long” insertion of 81 strands. (C2) CW after swell up of C1.

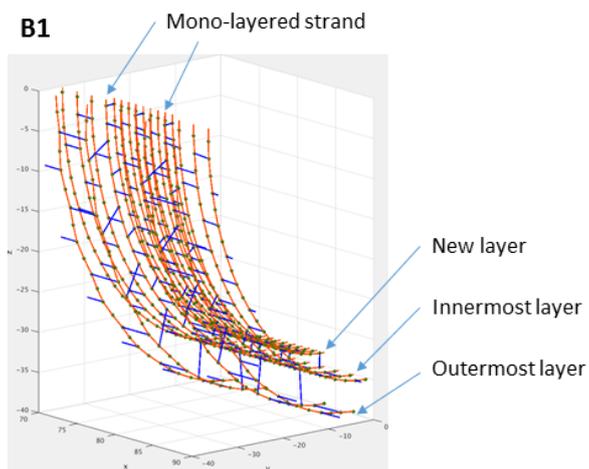
by the ratio of strands. The outermost layer of panel **A** is composed of 27 strands while it is composed of respectively 31 and 47 for respectively panel **B2** and **C2**, which gives a length ratio of around 1.1538 and 1.7692. With the length of the bacteria computed as the  $x$  coordinate of the origin of the second pole, the obtained ratio are 1.1518 and 1.7427 which are consistent values.

Figure 11 showing the insertion zone in the “short” insertion case confirms the displacement of the new layer as depicted in Figure 6 and the role of the layer to layer peptidic bridge; for clarity, Figure 12 displays the same figure but only for the innermost layer (likewise Figure 13 displays the innermost layer for both insertions showing at the same time the elongated CW). The radius at the mono-layered strands are higher since they bear all the radial pressure alone. It can also be seen (especially in Figure 12, panel **B2**) that these strands are less straight than the others since they also bear the axial pressure. Finally, two successive strands are closer together in the newly inserted part than in the old one. Indeed, when both layers are complete there are twice the number of peptidic bridges bearing the axial pressure in the innermost layer than in the outermost one. For the “short” insertion, inside the newly inserted part (see Figure 6, panel **C**), there are 10 peptidic bridges bearing the axial pressure in the innermost layer for only 4 bridges in the outermost layer: the innermost bears, comparatively to complete layers, less of the axial pressure. The effect is less pronounced in the “long” insertion case as they are 82 peptidic bridges bearing the axial pressure in the innermost layer for 40 bridges in the outermost layer.

Finally, we illustrate that the “3-under-2” principle leads to the regularity of the structure. Even if this can be figured out in Figure 11, it is clearer when more insertions are performed as depicted in Figure 14.

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The zoom is two times larger in panel B than in panel A.

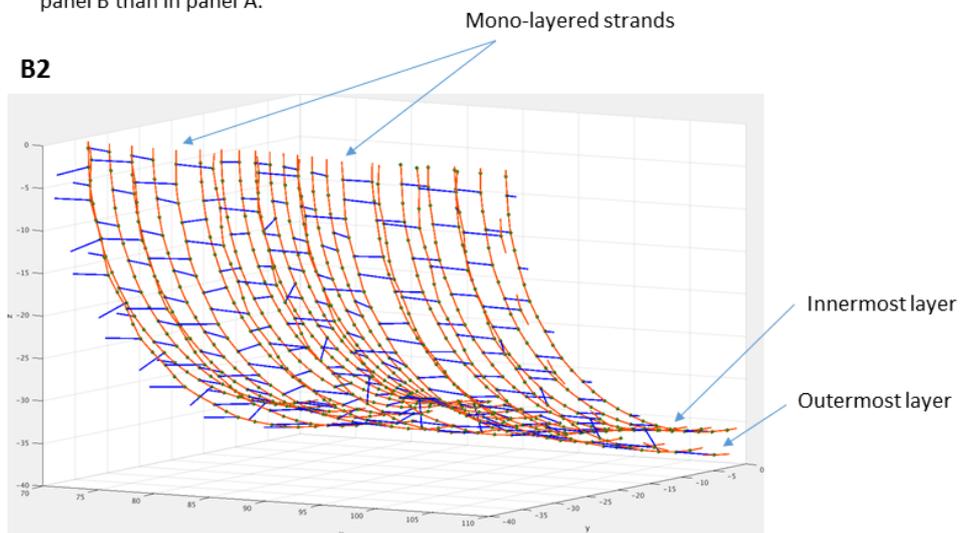


Figure 11: Zoom on the part of the space delimited by the dark dotted lines in Figure 10. **(B1)** CW after insertion of 9 strands. **(B2)** CW after swell up of **B1**.

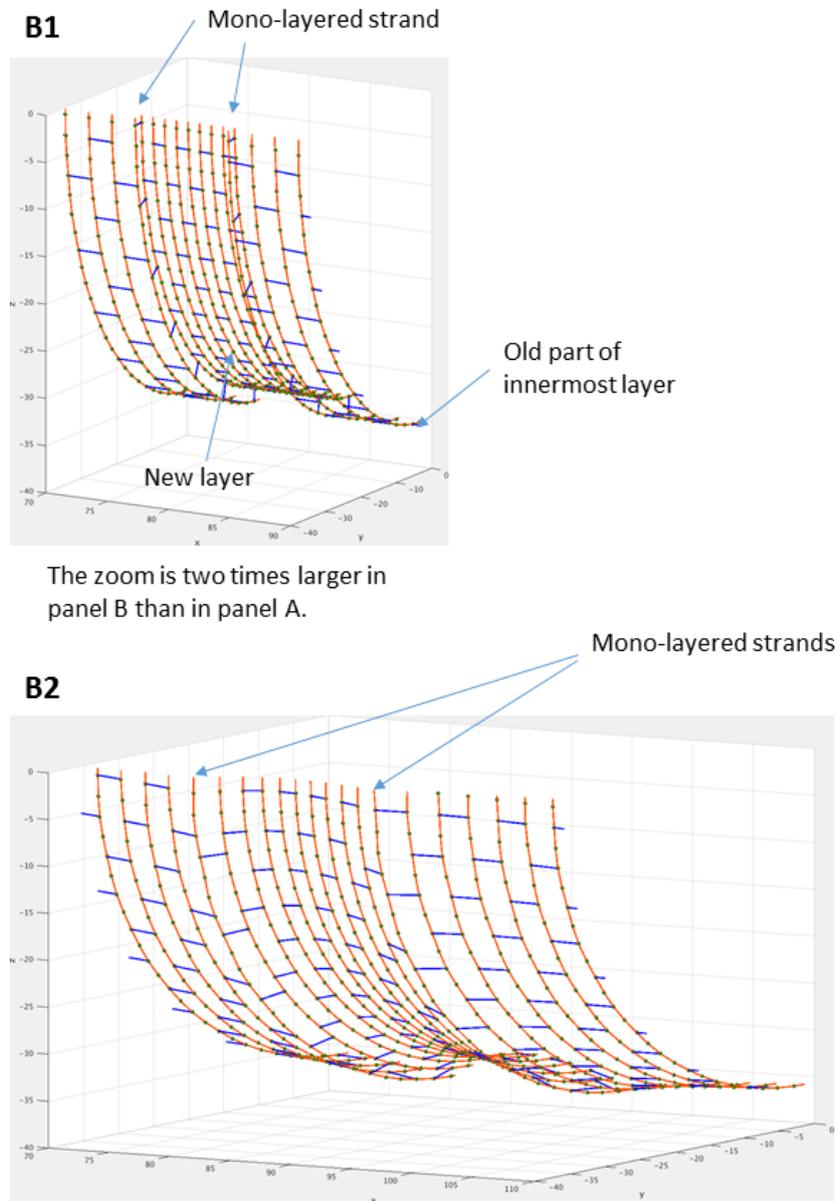


Figure 12: Zoom on the part of the space delimited by the dark dotted lines in Figure 10 for the innermost layer. **(B1)** CW after an insertion of 9 strands. **(B2)** CW after swell up of **B1**.

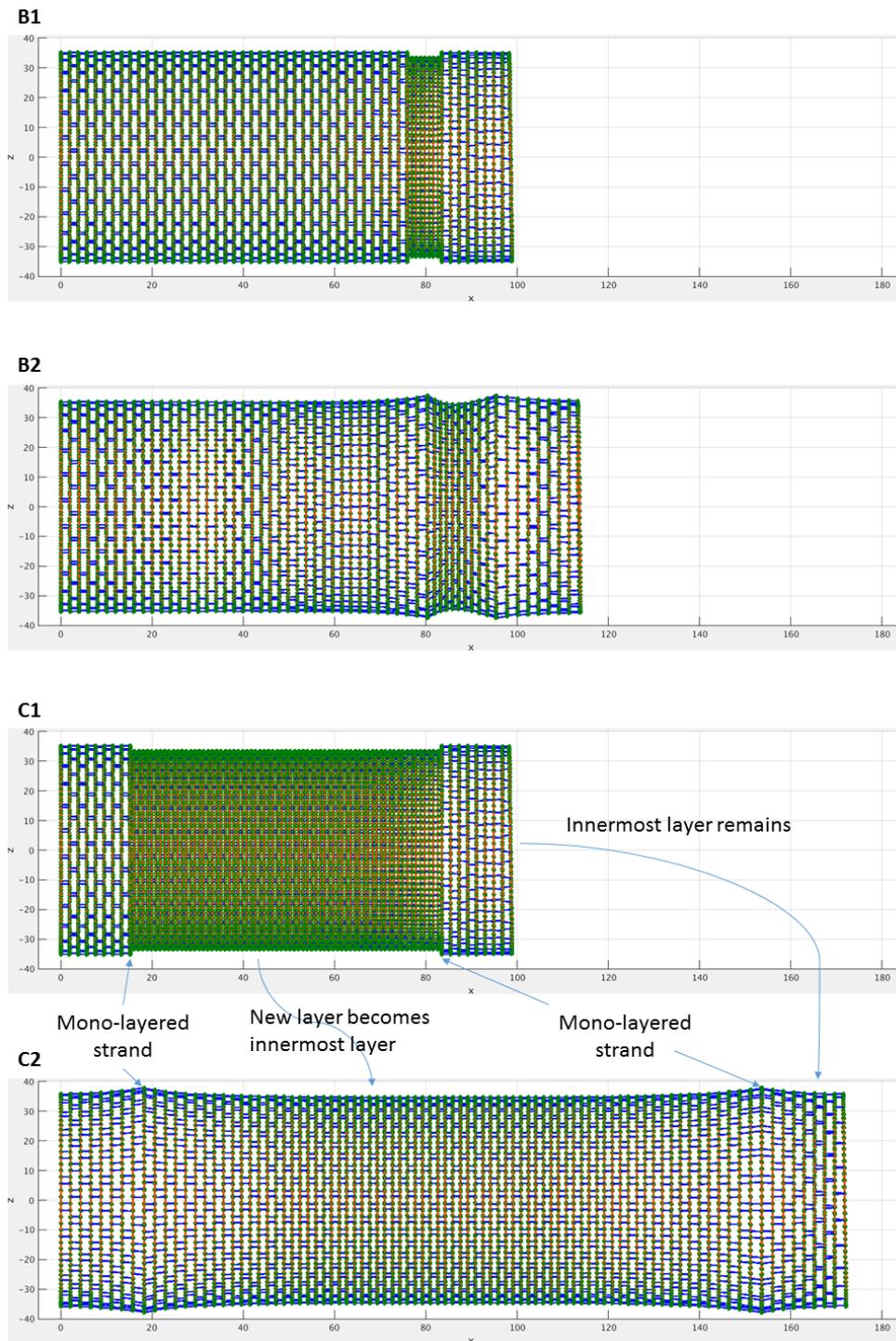


Figure 13: Innermost layer after insertion of 9 strands (panel **B1**) and swelling up (panel **B2**). Innermost layer after insertion of 81 strands (panel **C1**) and swelling up (panel **C2**).

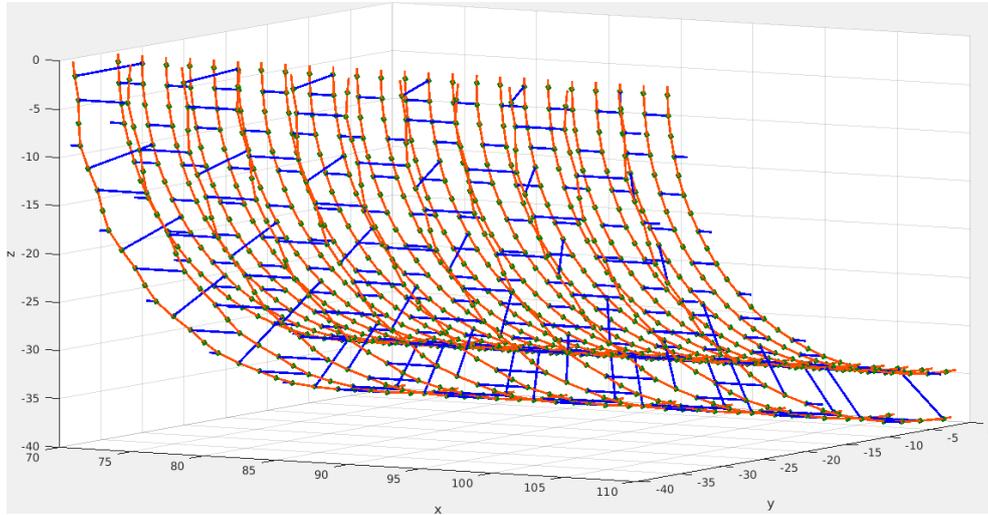


Figure 14: Zoom on the part of the space delimited by the dark dotted lines in Figure 10, panel **C2**. After insertion cycles, the CW structure is the same as in the original one as shown in Figure 9, panel **C**.

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## A Simulation Description

### A.1 Frames description

We consider an absolute (inertial) frame  $\mathcal{R}_0$  and two frames  $\mathcal{R}_1$  and  $\mathcal{R}_2$  linked to the poles. Each frame is described by an origin  $o_r$  and three axes  $\vec{i}_r$ ,  $\vec{j}_r$  and  $\vec{k}_r$ ,  $r = 0, 1, 2$ . The frames are orthonormal and direct. In the simulation, the frame  $\mathcal{R}_1$  linked to the first pole is set and does not change. For convenience, this frame is equal to  $\mathcal{R}_0$  and is thus also inertial. The second frame  $\mathcal{R}_2$  can move and rotate with respect to  $\mathcal{R}_0$ . Let  $p$  be a mass and its coordinates in  $\mathcal{R}_0$  denoted by

$$p|_{\mathcal{R}_0} = \begin{bmatrix} x_{p|\mathcal{R}_0} \\ y_{p|\mathcal{R}_0} \\ z_{p|\mathcal{R}_0} \end{bmatrix}.$$

Likewise, we denote

$$p|_{\mathcal{R}_1} = \begin{bmatrix} x_{p|\mathcal{R}_1} \\ y_{p|\mathcal{R}_1} \\ z_{p|\mathcal{R}_1} \end{bmatrix} \text{ and } p|_{\mathcal{R}_2} = \begin{bmatrix} x_{p|\mathcal{R}_2} \\ y_{p|\mathcal{R}_2} \\ z_{p|\mathcal{R}_2} \end{bmatrix}$$

the coordinates of  $p$  in  $\mathcal{R}_1$  and  $\mathcal{R}_2$ . We have

$$p|_{\mathcal{R}_0} = p|_{\mathcal{R}_1} \text{ and } p|_{\mathcal{R}_0} = o_{2|\mathcal{R}_0} + x_{p|\mathcal{R}_2} \vec{i}_{2|\mathcal{R}_0} + y_{p|\mathcal{R}_2} \vec{j}_{2|\mathcal{R}_0} + z_{p|\mathcal{R}_2} \vec{k}_{2|\mathcal{R}_0}$$

where  $o_{2|\mathcal{R}_0}$  is the coordinate of the origin of  $\mathcal{R}_2$  in  $\mathcal{R}_0$  and where  $\vec{i}_{2|\mathcal{R}_0}$ ,  $\vec{j}_{2|\mathcal{R}_0}$  and  $\vec{k}_{2|\mathcal{R}_0}$  are the coordinates of the axes of  $\mathcal{R}_2$  in the frame  $\mathcal{R}_0$ . Considering a cell axis along  $\vec{i}_0$ , we have  $x_{p|\mathcal{R}_1} = 0$  for a mass in the first pole; likewise we have  $x_{p|\mathcal{R}_2} = 0$  for a mass in the second pole.

The movement of the frame  $\mathcal{R}_2$  with respect to the frame  $\mathcal{R}_0$  is governed by the equations of kinematics of classical mechanics and can be decomposed into a translation (of the origin) and a rotation (of the frame around its origin).

## A.2 Overview of the algorithm

At the beginning of the simulation, we assume that the origin of the second frame is the center of mass of the peptidoglycans in the second pole and that the first pole is in the plane formed by  $(o_0, \vec{j}_0, \vec{k}_0)$ . The algorithm is: at iteration  $t$ ,

1. compute forces (see below for the forces computation) in the frame  $\mathcal{R}_0$  denoted  $\vec{F}_{p|\mathcal{R}_0}(t)$ :
  - (a) compute all forces – except the axial pressure force on the second pole: glycosidic and peptidic bonds, glycosidic bendings and radial pressure;
  - (b) add the above forces to obtain the resultant on all PGs;
  - (c) set the  $x$  component of force on all masses in the first pole to 0 (the first pole does not move);
  - (d) compute axial pressure force on the second pole (no computation on the first pole as it does not move);
  - (e) add this longitudinal pressure force (divided by the number of PG in the second pole);
2. update coordinates in the frame  $\mathcal{R}_0$ :

- (a) for all PGs *not* in the second pole, update the coordinates:  $p|_{\mathcal{R}_0}(t+1) = p|_{\mathcal{R}_0}(t) + \alpha \vec{F}_{p|\mathcal{R}_0}(t)$  where  $\alpha$  is the algorithm step length;
- (b) update the frame  $\mathcal{R}_2$  and the coordinates of PGs in the second pole:
- project all the forces into the frame  $\mathcal{R}_2(t)$ , that is compute  $F_{px2}(t) = \vec{F}_{p|\mathcal{R}_0}(t) \cdot \vec{i}_2|_{\mathcal{R}_0}(t)$ ,  $F_{py2}(t) = \vec{F}_{p|\mathcal{R}_0}(t) \cdot \vec{j}_2|_{\mathcal{R}_0}(t)$  and  $F_{pz2}(t) = \vec{F}_{p|\mathcal{R}_0}(t) \cdot \vec{k}_2|_{\mathcal{R}_0}(t)$ ;
  - update the frame  $\mathcal{R}_2$ :
    - i. update the origin:  $o_{2|\mathcal{R}_0}(t+1/2) = o_{2|\mathcal{R}_0}(t) + \alpha \sum F_{pz2}(t) \vec{k}_2|_{\mathcal{R}_0}(t)$ ;
    - ii. update the axes:
      - compute the moment vector:  $\vec{\omega}|_{\mathcal{R}_0}(t) = \sum \left( F_{pz2}(t) \vec{k}_2|_{\mathcal{R}_0}(t) \right) \wedge \left( F_{px2}(t) \vec{i}_2|_{\mathcal{R}_0}(t) + F_{py2}(t) \vec{j}_2|_{\mathcal{R}_0}(t) \right)$ ;
      - update the axes: compute  $\left( \vec{i}_2|_{\mathcal{R}_0}, \vec{j}_2|_{\mathcal{R}_0}, \vec{k}_2|_{\mathcal{R}_0} \right) (t+1)$  from a rotation of  $\left( \vec{i}_2|_{\mathcal{R}_0}, \vec{j}_2|_{\mathcal{R}_0}, \vec{k}_2|_{\mathcal{R}_0} \right) (t)$  around  $\alpha \vec{\omega}|_{\mathcal{R}_0}(t)$ , that is a rotation around  $\frac{\vec{\omega}|_{\mathcal{R}_0}(t)}{\|\vec{\omega}|_{\mathcal{R}_0}(t)\|}$  of angle  $\alpha \|\vec{\omega}|_{\mathcal{R}_0}(t)\|$ ;
  - update the coordinates of all PGs in the second pole in  $\mathcal{R}_2$ :

$$\begin{aligned}
 x_{p|\mathcal{R}_2}(t+1/2) &= 0 \\
 y_{p|\mathcal{R}_2}(t+1/2) &= y_{p|\mathcal{R}_2}(t) + \alpha F_{py2}(t) \\
 z_{p|\mathcal{R}_2}(t+1/2) &= z_{p|\mathcal{R}_2}(t) + \alpha F_{pz2}(t)
 \end{aligned}$$

- update the coordinates of all PGs in the second pole in  $\mathcal{R}_0$ :  $p|_{\mathcal{R}_0}(t+1) = o_{2|\mathcal{R}_0}(t+1/2) + x_{p|\mathcal{R}_2}(t+1/2) \vec{i}_2|_{\mathcal{R}_0}(t+1) + y_{p|\mathcal{R}_2}(t+1/2) \vec{j}_2|_{\mathcal{R}_0}(t+1) + z_{p|\mathcal{R}_2}(t+1/2) \vec{k}_2|_{\mathcal{R}_0}(t+1)$ ;
- finish update: compute  $o_{2|\mathcal{R}_0}(t+1)$  as the center of mass of  $p|_{\mathcal{R}_0}(t+1)$  of all PGs in the second pole and update their coordinates in  $\mathcal{R}_2(t+1)$  to obtain  $y_{p|\mathcal{R}_2}(t+1)$  and  $z_{p|\mathcal{R}_2}(t+1)$  and set  $x_{p|\mathcal{R}_2}(t+1) = 0$ , that is compute  $y_{p|\mathcal{R}_2}(t+1)$  and  $z_{p|\mathcal{R}_2}(t+1)$  such that  $(y_{p|\mathcal{R}_2}(t+1) - y_{p|\mathcal{R}_2}(t+1/2)) \vec{j}_2|_{\mathcal{R}_0}(t+1) + (z_{p|\mathcal{R}_2}(t+1) - z_{p|\mathcal{R}_2}(t+1/2)) \vec{k}_2|_{\mathcal{R}_0}(t+1) = o_{2|\mathcal{R}_0}(t+1) - o_{2|\mathcal{R}_0}(t+1/2)$ .

### A.3 Forces computation

The following derivations of forces are very similar to the ones found in [15, 19].

### A.3.1 Useful formulae

Let A and B be two masses, then

$$\frac{\partial \|\vec{AB}\|}{\partial A} \Big|_{\mathcal{R}_0} \stackrel{\triangle}{=} \begin{bmatrix} \frac{\partial \|\vec{AB}\|}{\partial x_A |_{\mathcal{R}_0}} \\ \frac{\partial \|\vec{AB}\|}{\partial y_A |_{\mathcal{R}_0}} \\ \frac{\partial \|\vec{AB}\|}{\partial z_A |_{\mathcal{R}_0}} \end{bmatrix} = - \frac{\vec{AB} |_{\mathcal{R}_0}}{\|\vec{AB}\|}$$

with  $\stackrel{\triangle}{=}$  meaning equal by definition and

$$\frac{\partial \|\vec{AB}\|}{\partial B} \Big|_{\mathcal{R}_0} = \frac{\vec{AB} |_{\mathcal{R}_0}}{\|\vec{AB}\|} = - \frac{\partial \|\vec{AB}\|}{\partial A} \Big|_{\mathcal{R}_0}.$$

## A.4 Bond

We consider two masses (A and B) linked by a spring, that is A and B are linked by either a glycosidic or a peptidic bond) whose energy is given by

$$E = \frac{1}{2} \sum_{i=2}^n k_i \left( \|\vec{AB}\| - l_0 \right)^i$$

where  $\|\vec{AB}\|$  is the euclidean distance between A and B (independent of the chosen frame) and where  $l_0$  is the spring length at rest.

Then the force applied by the spring stiffness on A is given by

$$\vec{F}_{A|\mathcal{R}_0} = - \frac{\partial E}{\partial A |_{\mathcal{R}_0}} = - \frac{1}{2} \sum_{i=2}^n i k_i \left( \|\vec{AB}\| - l_0 \right)^{i-1} \frac{\partial \|\vec{AB}\|}{\partial A} \Big|_{\mathcal{R}_0}$$

leading to

$$\boxed{\vec{F}_{A|\mathcal{R}_0} = \left( \sum_{i=2}^n \frac{i k_i}{2} \left( \|\vec{AB}\| - l_0 \right)^{i-1} \right) \frac{\vec{AB} |_{\mathcal{R}_0}}{\|\vec{AB}\|}.}$$

Likewise, the force applied by the spring stiffness on B is given by

$$\boxed{\vec{F}_{B|\mathcal{R}_0} = - \left( \sum_{i=2}^n \frac{i k_i}{2} \left( \|\vec{AB}\| - l_0 \right)^{i-1} \right) \frac{\vec{AB} |_{\mathcal{R}_0}}{\|\vec{AB}\|} = - \vec{F}_{A|\mathcal{R}_0}.$$

#### A.4.1 Bending

We consider three masses (A, B and C, with C in between of A and B) with C and A having a glycosidic bond as well as C and B. The bending energy is given by

$$E = \frac{1}{2} \sum_{i=2}^n k_i (\theta - \theta_0)^i$$

where

$$\theta = \widehat{\vec{CA}, \vec{CB}} = \arccos \left( \frac{\vec{CA} \cdot \vec{CB}}{\|\vec{CA}\| \|\vec{CB}\|} \right)$$

is the angle formed by the three masses (independent of the chosen frame) and where  $\theta_0$  is the angle at rest.

Then the force applied by the bending on A is given by

$$\vec{F}_{A|\mathcal{R}_0} = -\frac{\partial E}{\partial A|\mathcal{R}_0} = -\frac{1}{2} \sum_{i=2}^n i k_i (\theta - \theta_0)^{i-1} \frac{\partial \theta}{\partial A|\mathcal{R}_0}$$

Since

$$\begin{aligned} \frac{\partial \cos(\theta)}{\partial A|\mathcal{R}_0} &= -\sin(\theta) \frac{\partial \theta}{\partial A|\mathcal{R}_0}, \\ \vec{F}_{A|\mathcal{R}_0} &= \left( \sum_{i=2}^n \frac{i k_i}{2 \sin(\theta)} (\theta - \theta_0)^{i-1} \right) \frac{\partial \cos(\theta)}{\partial A|\mathcal{R}_0} \end{aligned}$$

And since

$$\frac{\partial \cos(\theta)}{\partial A|\mathcal{R}_0} = \frac{\vec{CB}_{|\mathcal{R}_0}}{\|\vec{CA}\| \|\vec{CB}\|} + \frac{\vec{CA} \cdot \vec{CB}}{\|\vec{CB}\|} \left( -\|\vec{CA}\|^{-2} \frac{\vec{CA}_{|\mathcal{R}_0}}{\|\vec{CA}\|} \right)$$

leading to

$$\boxed{\vec{F}_{A|\mathcal{R}_0} = \left( \sum_{i=2}^n \frac{i k_i}{2 \sin(\theta)} (\theta - \theta_0)^{i-1} \right) \frac{1}{\|\vec{CA}\|} \left( \frac{\vec{CB}_{|\mathcal{R}_0}}{\|\vec{CB}\|} - \cos(\theta) \frac{\vec{CA}_{|\mathcal{R}_0}}{\|\vec{CA}\|} \right).}$$

Likewise, the force applied by the bending on B is given by

$$\boxed{\vec{F}_{B|\mathcal{R}_0} = \left( \sum_{i=2}^n \frac{i k_i}{2 \sin(\theta)} (\theta - \theta_0)^{i-1} \right) \frac{1}{\|\vec{CB}\|} \left( \frac{\vec{CA}_{|\mathcal{R}_0}}{\|\vec{CA}\|} - \cos(\theta) \frac{\vec{CB}_{|\mathcal{R}_0}}{\|\vec{CB}\|} \right).}$$

To obtain the force applied by the bending on C, we need

$$\frac{\partial \cos(\theta)}{\partial C|\mathcal{R}_0} = \frac{-\vec{CB} - \vec{CA}}{\|\vec{CA}\| \|\vec{CB}\|} - \frac{\vec{CA} \cdot \vec{CB}}{(\|\vec{CA}\| \|\vec{CB}\|)^2} \left( -\|\vec{CB}\| \frac{\vec{CA}}{\|\vec{CA}\|} - \|\vec{CA}\| \frac{\vec{CB}}{\|\vec{CB}\|} \right)$$

so that

$$\vec{F}_{C|\mathcal{R}_0} = \left( \sum_{i=2}^n \frac{ik_i}{2\sin(\theta)} (\theta - l_0)^{i-1} \right) \times \dots \frac{1}{\|\vec{C}\vec{A}\| \|\vec{C}\vec{B}\|} \left( \cos(\theta) \left( \|\vec{C}\vec{B}\| \frac{\vec{C}\vec{A}|_{\mathcal{R}_0}}{\|\vec{C}\vec{A}\|} + \|\vec{C}\vec{A}\| \frac{\vec{C}\vec{B}|_{\mathcal{R}_0}}{\|\vec{C}\vec{B}\|} \right) - \vec{C}\vec{A} - \vec{C}\vec{B} \right).$$

#### A.4.2 Pressure

Let  $P$  denote the (osmotic) pressure in the bacteria, more precisely the difference of pressure between the inside of the bacteria and its outside. Let  $p_1$ ,  $p_2$  and  $p_3$  denote three points forming a surface of pressure. Then the pressure force is computed as

$$\vec{F}|_{\mathcal{R}_0} = \frac{P}{2} \overrightarrow{p_1 p_2} \wedge \overrightarrow{p_1 p_3}.$$

The actual force applied on each PG depends on the actual number of physical masses forming the surface. The triangles chosen for the radial and axial pressure are plotted in Figure 15 so that the actual number of PGs is 3 for the radial pressure. For the axial pressure, see the algorithm overview in Appendix A.2.

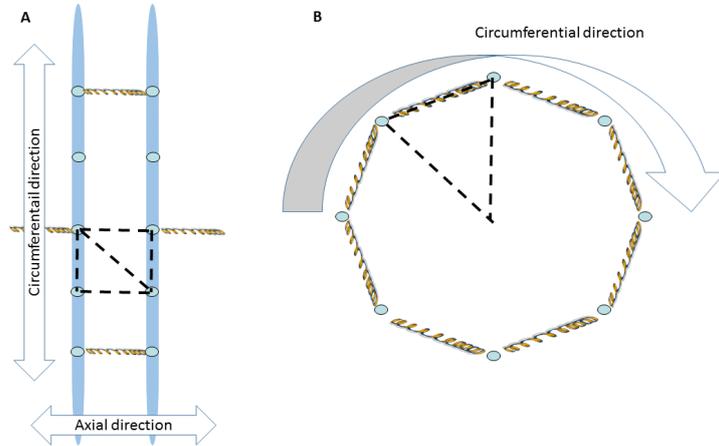


Figure 15: Surface for pressure computation. (A) Radial pressure. (B) Axial pressure.