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# The bio-physics of condensation of divalent cations into the bacterial wall has implications for growth of Gram-positive bacteria

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**Abstract**

**Background:** The anionic-polyelectrolyte nature of the wall of Gram-positive bacteria has long been suspected to be involved in homeostasis of essential cations and bacterial growth. A better understanding of the coupling between the biophysics and the biology of the wall is essential to understand some key features at play in ion-homeostasis in this living system.

**Methods:** We consider the wall as a polyelectrolyte gel and balance the long-range electrostatic repulsion within this structure against the penalty entropy required to condense cations around wall polyelectrolytes. The resulting equations define how cations interact physically with the wall and the characteristic time required for a cation to leave the wall and enter into the bacterium to enable its usage for bacterial metabolism and growth.

**Results:** The model was challenged against experimental data regarding growth of Gram-positive bacteria in the presence of varying concentration of divalent ions. The model explains qualitatively and quantitatively how divalent cations interact with the wall as well as how the biophysical properties of the wall impact on bacterial growth (in particular the initiation of bacterial growth).

**Conclusion:** The interplay between polymer biophysics and the biology of Gram positive bacteria is defined for the first time as a new set of variables that contribute to the kinetics of bacterial growth.

**General significance:** Providing an understanding of how bacteria capture essential metal cations in way that does not follow usual binding laws has implications when considering the control of such organisms and their ability to survive and grow in extreme environments.

**Keywords:** Gram-positive bacteria; teichoic acid; cell wall; metal cations; polyelectrolytes; Manning's Theory.

## Introduction

The bacterial cell wall is formed by a rigid network of carbohydrates (peptidoglycan) and amino acids that are responsible for many cellular functions and protect bacteria against external physical stresses [1]. In Gram positive bacteria, in addition to peptidoglycan, the cell wall contains the highly charged anionic polyelectrolytes, teichoic acid (TA), which may constitute up to 60% of the wall's mass [2]. At physiological pH, the chemical groups (phosphoryl, hydroxyl and amino) composing the wall are deprotonated [3] and the bacterial wall can be considered as a negatively charged polyelectrolyte gel.

Two types of TAs have been described depending on their attachment to the bilayer membrane or the cell wall [2]: The lipo-TAs (LTAs), anchored to the cytoplasmic membrane, extend into the peptidoglycan layer whereas the wall TAs (WTAs) are attached directly to peptidoglycan and extend through the cell wall. Given that TAs are anionic polyelectrolytes embedded in the peptidoglycan, the repulsion between TAs can only be balanced by binding cationic groups. The electrostatic interactions involved in the wall play a fundamental role as they define the wall volume and rigidity [4-7]. Beside their involvement in the physical electro-mechanics of the wall, LTAs/WTAs are thought to be important for cation homeostasis, which is essential to the physiology of Gram positive bacteria [8]. The morphology of strains lacking LTAs, is altered, and results in swelling and aggregation of bacterial cells [9] with strains lacking both LTAs and WTAs not viable [10].

The importance of cation homeostasis is well documented. For example, calcium ($Ca^{2+}$) participates in synergistic interactions with enzymes to facilitate the anchoring of surface proteins involved in bacterial adhesion [11, 12], whereas magnesium ($Mg^{2+}$) plays a fundamental role in peptidoglycan biosynthesis, wall strength, prevention of cell lysis and growth [13-15]. The impact of the deletion of WTAs on growth can only be partially rescued by increasing the magnesium in the growth medium [9], demonstrating how central wall

polyelectrolytes are for growth. The importance of the wall composition is also highlighted when phosphate availability is limited or when external $Mg^{2+}$ is reduced as in this case, the phosphate content of WTA is exchanged with uronic acids and the WTA is transformed to teichuronic acid, thus maintaining the overall anionic properties of the wall [16, 17]. In this situation, bacteria synthesize more WTAs and in doing so increase the probability of attracting divalent cations, such as $Mg^{2+}$ [18]. While the retention of divalent cations by the cell wall is essential, specific transporter proteins embedded in the underlying cytoplasmic membrane are required for their uptake by the bacterial cell [19, 20]. This indicates that there must be transport of divalent cations across the bacterial wall; from the outside world to the membrane bound receptor. The flux of cationic substances through the wall most likely accounts for the ability of cationic antimicrobial peptides (CAMPs) to access the cell membrane; intrinsic sensitivity to CAMPs is dependent on the amount of negatively charged groups in the cell wall [21, 22].

A biochemical model concerning divalent cations transport has recently been suggested [23] . In this model, when metals cations are sparse chelation appears but weakens when their concentrations increase. It is proposed that this permits the ability of divalent cations to be released and slide along the molecules of the wall before finally being absorbed by the bacterium [24]. This model suggests/requires the presence of an undefined cooperativity to explain the switch between these two behaviours (cation attraction vs. cation release). What is probably more intriguing in this study is that at low concentration of divalent cations, chelation is total [23, 24]; which seems to contradict usual laws of thermodynamics and statistical physics upon which cooperativity phenomena are usually based. In classical thermodynamics, regarding binding sites and involving bulk concentrations of cations, the entropy should dominate at low concentration of divalent cations always leaving free cations in solution that should be detected experimentally meaning that total chelation should not be an option [23,

24]. It does seem, therefore, that another explanation of the mechanism needs to be invoked to explain the binding behaviour the cell wall towards divalent cations.

In another field seemingly distant from bacteriology, soft matter physics (also known as, condensed matter physics), neutral polymers and charged polymers (polyelectrolytes) have been studied along with their interactions with counterions. From the point of view of physics, the presence of both short range entropic interaction and long range electrostatic interaction (coulomb force) define the physical mesoscopic properties of polyelectrolytes [25]. Those interactions have an impact on polyelectrolyte structures. In addition, unique physical properties emerge due to the quasi-linear structure of polyelectrolytes within ionic solutions that are not apparent when only binding affinities, and related cooperativity, are considered. The physical behaviour of solutions containing a mixture of gel polyelectrolytes immersed in electrolyte solutions was first highlighted using physics by Gerald S. Manning in 1969 [26-29]. It is the aim of this manuscript to underline how the understanding of the physical biology or biophysics of a system composed of polyelectrolyte gels and electrolytes mixed together can provide insight into the attraction and movement of across the bacterial wall.

In order to introduce how we envisage the mechanisms underlying this process, the paper is divided in several parts. In the first part, we underline the main/critical physical parameters of the Gram positive bacterial cell wall. The second part, provides a synopsis of Manning's theory with particular reference to the notion of the condensation of ions on polyelectrolytes. In the third part, we suggest a condensation theory for the bacterial wall that will, in turn, be directly compared to: (i) recent data produced by Thomas III and Rice [23] regarding calcium binding to bacterial wall material (part four) and (ii), to data produced by Webb [30] regarding the role of magnesium in bacterial growth (part five).

**Part 1 – Sketch and notation of the bacterial cell wall**

The wall of Gram positive bacteria is composed of a gel of polyelectrolytes (figure 1). The typical mesh size of length $L$ defines the spatial location of a single polyelectrolyte that can be treated independently of its junction with other polyelectrolytes. We shall assume that the length $L$ is constant across the wall. As the single polyelectrolyte is composed of $N$ monovalent charges, $q$, the line density of charge of a single polyelectrolyte is simply $Nq/L$. The monovalent charges are surrounded by cations that are restricted within a volume $V_{poly}$ around the polyelectrolyte. This restriction is the result of charge condensation derived from Manning's theory (see below). Considering single divalent cations the concentration shall be noted $C_0$.

**Part 2 - Manning theory in the case of polyelectrolytes**

The essential ingredients regarding Manning theory [26-29] with particular reference to the notion of the condensation of ions on polyelectrolytes are given below.

Let us assume that a single polyelectrolyte can be treated as a rod carrying $N$ negative charges each noted, $q$, over an average length, $L$; and that no extra salt is added to the solution or equivalently that, Debye length is larger than Manning length [31]. These assumptions provide the charge line density, $Nq/L$ (figure 1A). Neglecting the extremities of a single polyelectrolyte for simplicity (or equivalently concentrating on the determination of electrical properties within the bacterial wall) and considering Gauss theorem, the radial electric field, $E$, can be deduced as: $E = Nq/2\pi\varepsilon_r\varepsilon_0 Lr$. Where $\varepsilon_r$ and $\varepsilon_0$ are the relative permittivity and the vacuum permittivity, respectively. Still using the radial symmetry, as the electric potential $V$ is linked to the electric field under the form, $E = -\vec{\nabla}V$, one finds: $V = A - Nq\ln(r)/2\pi\varepsilon_0 L$ where, $A$, is an integration constant. Next to the polyelectrolyte, the potential energy, $E_p$, of

a counterion of valence $Z$ and hence total charge, $Zq$ is: $E_p = ZqV$. Using Boltzmann theory, the probability to find a counterion at a distance $r$ from the polyelectrolyte is thus: $\sim \exp\left(-E_p / k_B T\right)$; where $k_B T$ is the thermal energy. As a result, using the electric potential one finds: $\exp\left(-E_p / k_B T\right) \sim 1/r^{2\xi}$, where $\xi = ZNl_B / L$ and $l_B = q^2 / 4\pi\varepsilon\varepsilon_0 k_B T$ is the Bjerrum length, i.e. the distance at which the electrostatic energy is comparable to the thermal energy. If one determines the amount of counterions located within a distance $r_0$ from the polyelectrolyte, result given by the integral $\int_0^{r_0} \exp\left(-E_p / k_B T\right) 2\pi r dr \sim \left[r^{2(1-\xi)}\right]_{r=0}^{r=r_0}$, one sees that the integral diverges at $r = 0$ if $\xi = ZNl_B / L > 1$. This is unrealistic physically and therefore Manning suggested that a certain amount of counterions would necessarily condense onto the polyelectrolyte to drop the value of $N$, and therefore brings $\xi$ toward unity. As a conclusion, within a solution containing polyelectrolytes there are always two populations of ions, namely free and condensed counterions. Note that the condensed ions are not fixed onto the polyelectrolyte but can move between charges.

**Part 3 - Fraction of charges condensed onto bacterial cell wall polyelectrolytes**

To determine the fraction of charges on the polyelectrolytes being compensated by condensed counterions, one needs to determine the energy required to partially discharge the polyelectrolyte and compare this energy to the entropy penalty linked to concentrating counterions within a volume similar to the polyelectrolyte volume, $V_{poly}$ (figure 1B). To do so, we use a mean field theory. Let us assume that each monovalent charge on the polyelectrolyte is partially compensated by an average factor $\theta Z$ ($Z$ being the valence of the condensed counterion and $\theta$ the probability that a counterion is present) leading to a new charge value: $q' = q(1 - \theta Z)$. Let us assume also that those charges aligned onto the polyelectrolyte are

indexed by the letter "$i$", where $i$ varies between 0 and $N$, and interact together via a Debye-Huckel potential, $V_{DH}(r_i)$. So the electric potential felt by a given charge on the polyelectrolyte is a function of all the other charges on the same polyelectrolyte. In this case, the energy $du_i$ required to change the charge indexed by the letter "$i$" by a value $dq_i$ is: $du_i = \sum_{\substack{j=1 \\ j\neq i}}^{N} V_{DH}(r_i - r_j) dq_i$; where $\sum_{\substack{j=1 \\ j\neq i}}^{N} V_{DH}(r_i - r_j) = \sum_{\substack{j=1 \\ j\neq i}}^{N} \frac{q_j}{4\pi\varepsilon\varepsilon_0} \frac{e^{-|r_i - r_j|/l_D}}{|r_i - r_j|}$ is the electric potential felt by the $i^{th}$-charge at the position "$r_i$" to which contribute all the other charges located at "$r_j$". The total electric energy required to partially discharge the polyelectrolyte is therefore: $\Delta U_{elec} = \frac{1}{2}\sum_{i=1}^{N}\int_{q}^{q'} du_i$. Note that the pre-factor is included to avoid counting twice pairwise interactions. As we assume that all charges on the polyelectrolytes are identical, one can remove the subscript on the charge, i.e. $q_i$ becomes $q$. Consider that the charges on the polyelectrolyte are periodically separated by a distance $L/N$, namely $r_i = i \times L/N$, using the Debye-Huckel approach the potential felt by the $i^{th}$-charge at the position "$r_i$" is therefore: $V_{DH}(r_i) = \frac{q}{4\pi\varepsilon\varepsilon_0}\frac{N}{L}\left[\sum_{k=1}^{i-1}\frac{e^{-\alpha k}}{k} + \sum_{k=1}^{N-i}\frac{e^{-\alpha k}}{k}\right]$, where $\alpha = L/l_D N$. Finally, the electric energy linked to the condensation can be determined as: $\Delta U_{elec} = -\theta Z(2-\theta Z)\frac{q^2}{4\pi\varepsilon\varepsilon_0}\frac{N}{L}\sum_{i=1}^{N}\left[\sum_{k=1}^{i-1}\frac{e^{-\alpha k}}{k} + \sum_{k=1}^{N-i}\frac{e^{-\alpha k}}{k}\right]$. The double summation can be simplified and for long enough polyelectrolytes, i.e. $N >> 1$, one finds (see appendix):

$$\Delta U_{elec} \cong \theta Z(2-\theta Z) k_B T \frac{N l_B}{L} N \ln\left(1 - e^{-\alpha}\right) \quad (1)$$

Note that as we have focused on quasilinear portions of crosslinked anionic polyelectrolytes corresponding to the mesh size, Eq.1 is also valid for treating linear LTAs. As the electric

energy has been determined, one can also assume, as a first approximation, that the entropic penalty, $\Delta S_{cond}$, linked to charge condensation is only related to the change in entropy associated with the concentrations of charges from being free in the bulk solution at a concentration $C_0$, to within a volume $V_{poly}$ near the polyelectrolyte. Assuming the ideal gas assumption valid, the entropic penalty is therefore:

$$T\Delta S_{cond} = -k_B TN\theta \ln\left(\frac{N\theta}{V_{poly}C_0}\right) \quad (2)$$

It is now possible to determine the probability that a particular site on the polyelectrolyte is occupied by a condensed ion with a probability $\theta^*$ as a function of the external concentration of ions by considering that the drop in electric energy (Eq.1) has to match the increase in entropy linked to condensation (Eq.2), i.e. $\Delta U_{elec} \sim T\Delta S_{cond}$. It is worth noting here that $l_D$ is a function of the electrolyte concentration in solution. In particular, if one assumes a single ionic species at concentration $C_0$ in solution: $l_D \propto 1/\sqrt{C_0}$. The later scenario is typical of experiments that have been carried out with wall material from Gram positive bacteria to measure the ionic absorption of divalent cation onto the bacterial wall (see thereafter). Finally, we shall use the following notations: $C_0^* = C_0 V_{poly}/N$, $l_D^* \propto \sqrt{V_{poly}/N}$ and $\alpha^* = L/Nl_D^*$. In those conditions one finds:

$$C_0^* \sim \theta^*\left(1 - e^{-\alpha^*\sqrt{C_0^*}}\right)^{Z\left(2-\theta^* Z\right)\frac{Nl_B}{L}} \quad (3)$$

Eq.3 links the amount of condensed charges onto the polyelectrolyte, $\theta^*$, to the amount of charge in solution, $C_0^*$. It is now essential to compare Eq.3 against experimental data.

**Part 4 - Apparent "cooperativity" is linked to condensation of charge at the cell wall**

Consider a divalent cation, i.e. $Z = 2$, Eq.4 is represented in Figure 2A. The figure demonstrates the presence of two phases; initially, condensation in the cell wall occurs at low concentrations of cations, and subsequently a near-linear acquisition of cations is seen. During this second phase one sees that as $\sqrt{C_0^*} >> 1/\alpha^*$, a linear dependency exists under the form: $C_0^* \sim \theta^*$; i.e. with a slope related to the charge density of the polyelectrolyte: $N/V_{poly}$. These two phases are also very clearly visible in the data from the experimental study by Thomas III and Rice [23], who studied the association of magnesium and calcium divalent cations with the bacterial wall using experimental conditions similar to those considered in our model (Figure 2B) showing the interaction of calcium with the cell wall.

In their study, the authors discuss metal binding in line with the notion of negative cooperativity to explain the two phases seen in their experimental data, without explaining the nature of such cooperativity. We suggest that the negative cooperativity is not required as the condensation of charges in line with Manning's theory pertaining to polyelectrolytes can explain why those two regions exist. A nonlinear fit of Eq.4 against Thomas III and Rice data [23], using the Matlab fitting toolbox (version R2015a) and Trust-Region algorithm, provided an adjusted $R^2$ value of $R_{adj}^2$=0.998 (Table 1). While we agree that our model remains minimalistic and as a result is a simplified version of reality (as we have considered only one type of rod-like polyelectrolyte with fixed physical parameters including its length, total charge and volume), the similarity between the curve regions is clearly visible.

**Part 5- Magnesium condensation tunes bacterial growth**

Seminal studies on magnesium acquisition have shown that while this divalent cation is essential to Gram positive bacteria to grow [14, 30, 32], there exists a lag-time between the incubation of magnesium and growth that is not observed in Gram-negative bacteria that are similarly dependent on magnesium for growth [32]. The interesting observation is that the lag-

time is only measurable at low concentration of magnesium (Figure 3A). We know, from the condensation theory developed earlier, that the bacterial wall will retain counterions. This suggests that at low cation concentration the bacterial wall could be considered as a trap for divalent cations, but that this behaviour disappears at higher concentrations. In this scenario, the initiation of magnesium dependent bacterial growth is likely associated with the escape rate of magnesium from the wall into the bacteria.

To model this effect let us consider a steady state condition in which the flux of magnesium coming from the wall to the space between the wall and the cytoplasmic membrane is identical to the flux of magnesium entering the cell (i.e. magnesium ions released at the inner surface of the wall are instantaneously assimilated by the bacterium). In this case the flux of magnesium, $J$, from the wall into the bacteria is: $JS_{bact} \sim \rho V_{wall} N\theta^* / \tau$; where, $S_{bact}$, is the membrane surface area available for exchanges between the wall and the bacterium's cytoplasm; $\tau$, the characteristic time of magnesium to detach from the wall; $\rho$, the density of polyelectrolytes of length $N$ and; $V_{wall}$, the volume of the wall. The amount of magnesium $\Delta Mg^{2+}$ entering the cell over a time $\Delta t$ is thus: $\Delta Mg^{2+} \sim \rho V_{wall} N\theta^* \Delta t / \tau \; S_{bact}$. It is very likely that not all the magnesium will be used directly for growth and that a certain amount will be "mopped up" in other non-productive or maintenance processes within the bacterium. Let us note $\left(\Delta Mg^{2+}\right)_0$ the amount of magnesium not involved in growth, it follows that the amount of magnesium specifically involved in growth can be written as: $\left(\Delta Mg^{2+}\right)_{growth} \sim \rho V_{wall} N\theta^* \Delta t / \tau - \left(\Delta Mg^{2+}\right)_0$.

If one introduces the following notation: $\Delta t_c \sim \left(\Delta Mg^{2+}\right)_0 \tau / \rho V_{wall} N\theta^*$, it follows:

$$\left(\Delta Mg^{2+}\right)_{growth} \sim \frac{\rho V_{wall} N\theta^*}{\tau}\left(\Delta t - \Delta t_c\right) \qquad (4)$$

To determine the bacterial growth rate, one notes $B(t)$ the amount of bacteria measured at time "$t$". The amount of bacteria at time $t+\Delta t$, i.e. $B(t+\Delta t)$, is therefore:

$B(t+\Delta t) = B(t) + \Phi\left[\left(\Delta Mg^{2+}\right)_{growth}\right]B(t)\Delta t$; where $\Phi\left[\left(\Delta Mg^{2+}\right)_{growth}\right]$ is a function that defines the growth kinetic as a function of the amount of magnesium inside the bacteria. Note that in our model, the function $\Phi\left[\left(\Delta Mg^{2+}\right)_{growth}\right]$ that is intimately related to Eq.4 encompasses both the initiation of bacterial growth and the growth rate. This new function is dependent on how the bacterium manages its growth internally, namely involving processes largely independent of polyelectrolyte physics. As there is no bacterial growth in the absence of magnesium [30], i.e. $\Phi[0] = 0$, we make the simplest assumption possible (we have no reason to think otherwise) that $\Phi\left[\left(\Delta Mg^{2+}\right)_{growth}\right]$ is a linear function of magnesium concentration, namely: $\Phi\left[\left(\Delta Mg^{2+}\right)_{growth}\right] = \gamma\left(\Delta Mg^{2+}\right)_{growth}$; where $\gamma$ is a kinetic constant involving the subcellular growth processes. Noting: $\Delta B(t) = B(t+\Delta t) - B(t)$. It follows therefore that:

$$\frac{\Delta B(t)}{\Delta t} \sim B(t)\gamma \frac{\rho V_{wall} N\theta^*}{\tau}\left(\Delta t - \Delta t_c\right) \qquad (5)$$

Eq.5 imposes that bacterial growth is only possible if $\Delta t \geq \Delta t_c$ and that there exists a lag-phase $\Delta t_c \sim \tau$ for bacteria to grow that is proportional to the required time for any counter ion to leave the bacterial wall. The essential physical parameter for this system is therefore: $\tau$. Using Kramer's theory regarding individual escape rates, it is possible to link this parameter to the energy required for one magnesium ion to leave the wall under the form: $\frac{1}{\tau} \sim \frac{1}{\tau_0}\exp\left(-\frac{\mu}{k_B T}\right)$ where $\frac{1}{\tau_0}$ is typical of a diffusion kinetic, i.e. without energy barrier. The energy, $\mu$, that a condensed charge needs to acquire to leave the polyelectrolyte is identical to the energy required for the polyelectrolyte wall to lose one charge and as such increase its self-repulsion. Eq.1 provides the electrolyte energy and the energy that will be required by magnesium to

"jump" over this attractive energy barrier is $\mu \sim -\Delta U_{elec} / N\theta^*$. Finally, Using Eq.1 together with Eq.3 and Eq.5 leads to Eq.6:

$$\frac{\Delta B(t)}{\Delta t} \sim B(t)\gamma \frac{\rho V_{wall} N}{\tau_0} C_0{}^* (\Delta t - \Delta t_c) \quad (6)$$

Eq.6 is only valid once the concentration of magnesium in solution is high enough, i.e. beyond the condensation regime when the wall can release magnesium and suggests that the wall is involved in bacterial growth and, following our linear assumption, that the growth kinetic should be a linear function of the magnesium concentration in solution. In addition, Eq.6 suggests that the relative bacterial growth is not a simple exponential-like function of time but a quadratic function of time. To demonstrate the coherence of our model, we have demonstrated in Figure 3B the quadratic dependence of the bacterial growth, namely $\Delta B / \Delta t(\Delta t - \Delta t_c)$, against the original data by Webb [30] to confirm with a good agreement ($R^2$=0.952) the linear dependence of the bacterial growth with regard to the magnesium concentration in solution.

## Discussion

One needs to start by underlining the minimalistic aspect of our model describing the bacterial wall compared to the real bacterial wall. Again, our model encompasses a number of constant parameters which we have imposed including the polyelectrolyte length (i.e. the mesh size), its linearity, charge line density and volume. While our model is minimalist, it captures the simplest form of the expected physical interactions between divalent cations, which are essential for bacterial physiology, and the integrity and function of the bacterial wall. Despite these caveats a good agreement was found between published data and our theory suggesting, in turn, that fundamental physical principles from soft matter physics linked to the notion of condensation are at play in the physiology of assimilation of divalent metal ions by Gram positive bacteria.

'Naturally, the applicability of our model relies on the presence of negative charges in the wall polyelectrolytes and it is important to highlight the limitation of our model. This point is essential as bacteria respond to their environment and that, as a result, the wall composition may change due to the depletion of essential chemicals. For example, when phosphorus, which is electronegative and is a major component of the wall teichoic acids is depleted, teichoic acid is transformed to teichuronic acid that does not contain phosphorus [33]. As a result, it has been demonstrated that the divalent cation magnesium has a lower 'affinity' for the wall [34], suggesting that the wall charge density and the distribution of negative charges on wall polyelectrolytes is fundamental. Likewise, the condensation of ions is a phenomenon that relies fundamentally on the distribution of polyelectrolyte charges, as it is the magnitude of self-repulsion between native charges composing the polyelectrolyte that results in the condensation of ions (the stronger the self-repulsion the stronger the condensation). The condensation of ions and related apparent 'affinity' measured is therefore a function of the distance between charges on the polyelectrolyte. As a result, the condensation of ions is unlikely once the polyelectrolyte

charges are separated by a critical distance above which they do not repulse each other efficiently. This critical distance between polyelectrolyte charges can be estimated using the Debye-Huckel potential when the repulsion energy between two consecutive charges on the polyelectrolyte is similar to the thermal energy, $k_B T$. Consider that the charges on the polyelectrolyte are periodically separated by this critical distance, $b_c$, using the Debye-Huckel potential one finds: $b_c \sim l_B \exp(-b_c / l_D)$. Note that in the latter relation Debye's length, $l_D$, is a function of the external concentration of free ions (or ionic strength) meaning that the critical distance, $b_c$, is impacted by the external environment. Let us assume an external solution containing only monovalent electrolytes in water, for ionic strengths $\sim 10^{-1} - 10^{-4} M$, one finds $b_c \sim 0.5 - 1nm$.'

While previous models have been published to explain and assess the electrical properties of the cell wall, see for example [35-38], none of the studies have focused specifically on polymer physics. Furthermore, and as pointed out by Thomas III and Rice [23], most studies use too large concentration of divalent cations or complex medium to study bacterial growth, and as a result the observation of the condensation phenomenon is hindered.

One essential result from our study is the simple connection that could exist between a lag-phase involved in bacterial growth and the wall electrostatic properties. However, it is essential to remember here that the lag-phase that is defined in our work is directly related to the low concentration of magnesium used. As a result the lag-phase mediated by low concentration of magnesium may not be similar to the usual lag-phase discovered in late 19$^{th}$ century that was suggested, by J. Monod, to result of a process of equilibration controlled by an unknown regulatory mechanism [39], whereby the bacteria would start exploiting its environment to grow [40]. However, it is interesting to note that a recent study has demonstrated that the lag-phase involves transient metal accumulation [40] that is in line and supported by our theory.

While more work is required to clarify a possible link, the lack of full biochemical criteria defining the usual lag-phase could result from the interplay between polymer physics, the cell wall and the bacterium's physiology and genetics.

Biomathematical continuous models have been suggested to explain the growth of bacteria including those from Verhulst [41], Gompertz [42], Baryani and Robert [43, 44] and Horowitz [45]. These models can either be empirical, based on differential equations or stochastic. In those models the lag-phase is an adjustable variable that is introduced without clear physical justification. Our model can be considered as continuation of the previous modelling works performed in which our unique point lies in the physical explanation of the lag-phase and the exponential-quadratic nature of the initial growth as a function of time. The lag-phase that was previously an adjustable variable in mathematical models of bacterial growth can now be explained and rooted in bacteriology using polymer physics.

## Conclusion

We present a physics-based model to understand the interaction between divalent cations and the cell wall and suggest that the physical characteristics of the cell wall are very likely to be central to understand the concepts and dynamics of lag-phase.

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## Appendix

The first step is to prove that: $\sum_{i=1}^{N}\left[\sum_{k=1}^{i-1}\frac{e^{-\alpha k}}{k}+\sum_{k=1}^{N-i}\frac{e^{-\alpha k}}{k}\right]=(N+1)\sum_{k=1}^{N-1}\frac{e^{-\alpha k}}{k}$.

To do so, the left-hand term is split under the form of two sums: $A=\sum_{i=1}^{N}\sum_{k=1}^{i-1}\frac{e^{-\alpha k}}{k}$ and $B=\sum_{i=1}^{N}\sum_{k=1}^{N-i}\frac{e^{-\alpha k}}{k}$. Let us introduce the Kronecker symbol namely: $\delta(x)=\begin{cases}1\,if\ \ x\geq 0\\ 0\,if\ \ x<0\end{cases}$. It then follows that both sums can be rewritten as $A=\sum_{i=1}^{N}\sum_{k=1}^{N-1}\frac{e^{-\alpha k}}{k}\delta((i-1)-k)$ and $B=\sum_{i=1}^{N}\sum_{k=1}^{N-1}\frac{e^{-\alpha k}}{k}\delta((N-i)-k)$. Inverting the summation for both sum it follows: $A=\sum_{k=1}^{N-1}\sum_{i=1}^{N}\frac{e^{-\alpha k}}{k}\delta(i-(k+1))$ and $B=\sum_{k=1}^{N-1}\sum_{i=1}^{N}\frac{e^{-\alpha k}}{k}\delta((N-k)-i)$. The summation can now performed transforming $A$ into $A=\sum_{k=1}^{N-1}\sum_{i=k+1}^{N}\frac{e^{-\alpha k}}{k}=\sum_{k=1}^{N-1}\frac{N-k}{k}e^{-\alpha k}$; and $B$ into $B=\sum_{k=1}^{N-1}\sum_{i=N-k}^{N}\frac{e^{-\alpha k}}{k}=\sum_{k=1}^{N-1}\frac{k+1}{k}e^{-\alpha k}$. Finally on finds:

$$A+B=\sum_{k=1}^{N-1}\left(\frac{N-k}{k}+\frac{k+1}{k}\right)e^{-\alpha k}=(N+1)\sum_{k=1}^{N-1}\frac{e^{-\alpha k}}{k} \qquad \text{(a1)}$$

For the second step, recalling that $\ln(1-x)=-\sum_{i=0}^{+\infty}\frac{x^i}{i}$ if $x<1$; as $e^{-\alpha k}<1$ and for long polyelectrolyte, i.e. $N>>1$, one finds:

$$A+B=-(N+1)\ln(1-e^{-\alpha}) \qquad \text{(a2)}$$

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