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Hypothesis

How crowded is the prokaryotic cytoplasm?

Jan Spitzer [a,*], Bert Poolman [b,*]

[a] Mallard Creek Polymers, Inc., 14700 Mallard Creek Road, Charlotte, NC 28262, USA
[b] Department of Biochemistry, Groningen Biomolecular Sciences and Biotechnology, Institute & Zernike Institute for Advanced Materials, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

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ABSTRACT

We consider biomacromolecular crowding within the cytoplasm of prokaryotic cells as a two-phase system of 'supercrowded' cytogel and 'dilute' cytosol; we simplify and quantify this model for a coccoid cell over a wide range of biomacromolecular crowding. The key result shows that the supercrowded cytogel extends the vectorial character of the plasma membrane deeper into the cytoplasm by about 20–70 nm. We discuss useful physiological insights that this model gives into the functioning of a prokaryotic cell on the micrometer scale.

1. Introduction

Given the astonishing robustness and repeatability of the bacterial cell cycle, it has long been suspected [1] that bacterial cells in vivo have subcellular 'architectures' [2] that predictably reproduce with each cell cycle; they have been described as 'metabolons' [3], 'modules' [4] or 'hyperstructures' [5]. The discovery of cytoskeleton proteins has further strengthened the case for the transient subcellular architecture of bacterial cells [6–9], and the advent of system and network biology with many 'omes' (proteome, signalsome, etc.) also suggests functional localizations of a large number of biomacromolecules. Such localized biomacromolecular clusters have now been visualized by super-resolution optical microscopy (15–35 nm) using photoswitchable proteins or dyes to obtain high resolution images of a cell [10–12].

The clustering of biomacromolecules also becomes apparent by considering repulsive (stabilizing) and attractive (agglomerating) non-covalent forces between biomacromolecules under their high crowding in vivo [13–19]. On microsecond and longer timescales, biomacromolecular surfaces are 'smooth and patchy', with distributions of hydrophilic, hydrophobic, and negatively and positively charged surface areas giving rise to both stabilizing forces and agglomerating forces. The attractive forces bring about transient occurrences of protein clusters [19,20], within which biomacromolecules become crowded even higher than their average level of crowding. Consequently, 'uncrowded' reservoirs of dilute solutions of freely diffusing proteins, nucleic acids and metabolites appear elsewhere in the cytoplasm. This non-random cytoplasmic crowding is supported by spectroscopic data of slower protein diffusion in crowded systems [21–24]. Such data suggest 'diffusion-to-capture' mechanisms [25], when biomacromolecular clusters intermittently 'capture' freely diffusing proteins at their periphery by presenting suitable interaction surfaces for attractive forces to become operative. Protein diffusion is slowed down also by biomacromolecular clusters simply becoming 'obstacles to avoid', making the diffusion path longer than necessary, and by increased viscosity of the aqueous medium. Taken together, these considerations suggest that bacterial cytoplasm in vivo consists of reservoirs of dilute solutions of metabolites, nucleic acids and proteins, and of their 'supercrowded' subcellular clusters of transient sizes and longevity. How can we describe such unequal cytoplasmic crowding?

* Corresponding authors.
E-mail addresses: jspitz@mcpolymers.com, jan.spitzer@gmail.com (J. Spitzer), b.poolman@rug.nl (B. Poolman).

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2. A simple coccoid model

A generic model of a prokaryotic cell with subcellular architecture is shown in Fig. 1. In the middle, there is a replicating nucleoid (RNA, DNA and proteins) with excrescences [26] that reach all the way to the plasma membrane (red interrupted line). The ribosomes (blue circles) are transiently situated along the nucleoid periphery including its excrescences: this arrangement brings replication, transcription, ribosome biogenesis and translation into close molecular proximity, and enables localization and translocation of membrane and peripheral proteins [27–29]. The cell envelope is extended deeper into the cytoplasm with submembrane supercrowded protein clusters (different shapes and colors) of variable composition, crowdedness, and longevity, which extend the vectorial nature of membrane biochemical reactions deeper into the cytoplasm; not shown, and likely to exists on a smaller (ribosomal) scale, are biomacromolecular clusters along the nucleoid periphery. The remaining space (the lightest color) is occupied by reservoirs of dilute solutions of ions, metabolites, proteins and nucleic acids, where they diffuse as fast as they do in assays of classical 'dilute' biochemistry in vitro, and where other clusters may also assemble, including plasmids and bacteriophages.

The nucleoid and the cell envelope, being connected via DNA excrescences, define a prokaryotic 'cellular scaffold' that grows and morphs into two cells during the cell cycle. Such a model of a bacterial cell offers a great variety of 'designs', dependent on the size of nucleoids and the spatiotemporal expression of proteomes they encode. For a given genome (genotype), the transient structuring of the bacterial cytoplasm and the cell envelope (phenotype) depends on extracellular conditions: nutrients and their concentrations, the growth state of the cell (exponential, stationary, dormant or 'dead'), and on (fluctuating) physicochemical variables of the environment, such as temperature, water activity, pH or electromagnetic irradiation [30].

This generic model is simplified for coccoid geometry in Fig. 2, not showing the ribosomes and the extra-cytoplasmic layers of the cell envelope. In this example, we show submembrane supercrowdedness of 52% volume fraction that corresponds to a simple cubic packing of spheres, which though somewhat arbitrary, represents a suitable reference point (*cf.* Supplementary information). In this range of biomacromolecular supercrowding we can reasonably assume that the submembrane layer has a character of a (soft) viscoelastic gel. For clarity then, we use the following cellular terminology: the region between the nucleoid and the plasma membrane is the *cytoplasm* (P), which consists of the *cytogel* (G), and the *cytosol* (S). The cytogel represents the submembrane supercrowded regions of peripheral proteins, structural proteins (filaments) and nucleic acids. The cytosol represents liquid reservoirs of metabolites, proteins and nucleic acids; its low content of biomacromolecules at ~5% volume fraction reflects typical 'dilute' conditions of classical biochemical assays.

For a given size of the cell and of the nucleoid, we can calculate how the extent of the cytogel depends on the level of its supercrowding. We assume a uniform degree of supercrowding (no angular dependence, *cf.* Fig. 1) within the cytogel and neglect the volume of the DNA excrescences, and constrain the calculations by the typical average value of volume fraction of biomacromolecules within the cytoplasm as $f_P = 0.25$. Independent of geometry, the mass balance equation for the distribution of dry protein/nucleic acids within the cytoplasm is:

$$f_P V_P = f_G V_G + f_S V_S \tag{1}$$

where V_P is the volume of the cytoplasm between the cell membrane and the nucleoid, f_G is the dry fraction of protein/nucleic acids

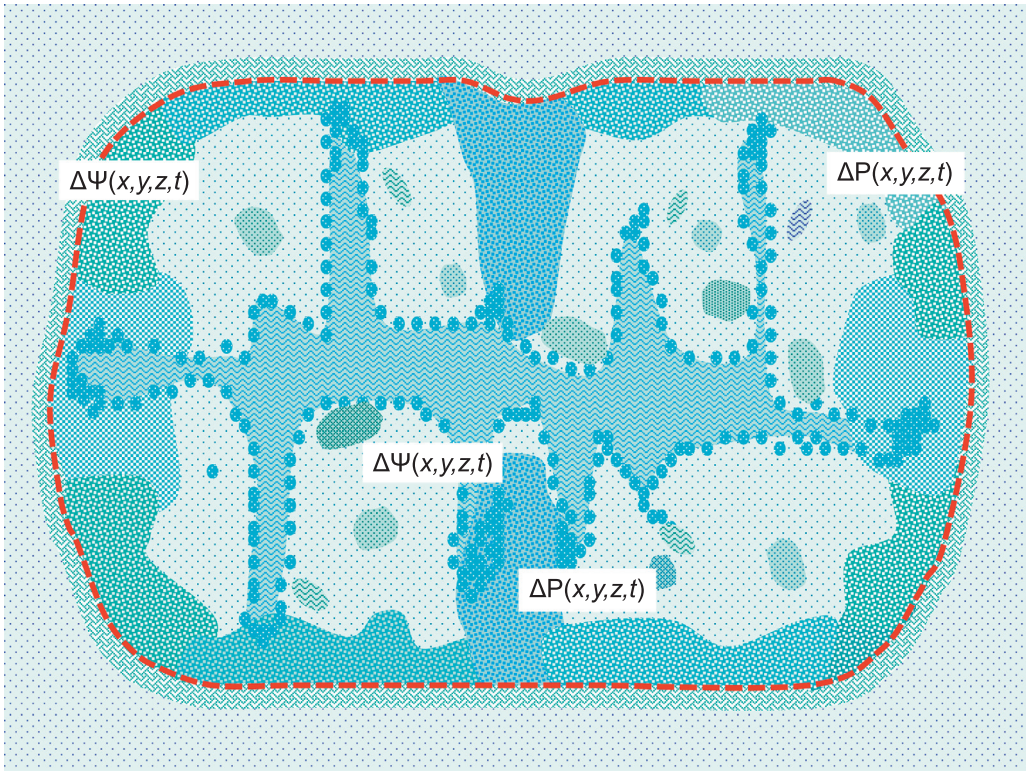

Fig. 1. The spatiotemporally structured bacterial cell is a sol/gel system of non-random protein crowding. The interrupted red line is the lipid–protein bilayer (plasma membrane), with extra-cellular biopolymers facing the nutrient solution (cross-hatched); gelled regions of variable shapes and compositions with estimated 40–90% volume fraction of biomacromolecules transiently 'grow and disappear' along the cytoplasmic side of the membrane; the replicating nucleoid, its excrescences and ribosomes (blue circles) are situated in the middle. Localized biochemical reactions control the dynamics of the gelled superclusters and of the resulting electrostatic and pressure fields, which act as 'cues' for the localization of biomacromolecules.