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Volatile Diffusional Character of Cytoplasm

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The fluctuating extracellular environment of microbial organisms influences diffusional mobility of macromolecules in the cytoplasm. This effect may be measured experimentally on the one hand by directly tracking the trajectories of individual macromolecules. We discuss how it may also be indirectly estimated from fluctuations in the amount of cytoplasmic water and from the varying patterns of protein expression seen in 2d gel analysis.

The smooth running of systems−biological networks depends ultimately on the ability of enzymes and their substrates to find each other across the physical space of the in vivo environment, a phenomenon which can be aligned to some extent with the classical random-thermal Brownian motion of colloidal particles in a fluid solvent. Experiments monitoring the trajectories of individual labeled proteins, RNAs or inert tracer particles in the cytoplasm of microbial organisms seem to largely confirm this view,[1−5] although with the qualification that there is some deviation from the expected linear scaling with time of mean-squared distance traveled, the hallmark of a classical Brownian particle. These deviations have been attributed to factors such as active transport and the presence of effective traps, e.g., transient cages formed by percolation of the surrounding cytostructure.

A second complicating feature concerns the diffusion coefficient D, which is observed to fluctuate considerably in response to the extracellular environment; that is, in response to growth medium, to temperature, and so forth; an effect which presumably challenges overall robustness of the complex systems−biological network of interactions between cytoplasmic macromolecules, since D sets the time scale for diffusion-limited biochemical events. It is difficult to comprehensively address this "volatile" diffusional character of the cytoplasm at the fundamental level of molecular statistical mechanics. However, an intuitive and widely acknowledged starting point is that diffusion is sensitive to the degree of so-called "crowding" in the cytoplasm. We envisage a causal thread: (i) extracellular stimuli induce (ii) fluctuating crowding conditions induce (iii) fluctuating diffusional properties.

Crowding relates essentially to the amount of cytoplasmic water in the cell, call this V_w, and to the overall size distribution of macromolecules present in the cell. Large fluctuations in V_w have been reported in bacterial cells, associated with osmotic regulation.[6,7] The relation between extracellular environment and intracellular size distribution is rather more obscure; however, quantitative data concerning such a relation are amply available from proteomic investigations. To illustrate, Table 1 presents results extrapolated from a seminal early 2d gel analysis of Pedersen et al.,[8] for *E. coli* in five different growth media. We show the mean abundance N_i for each size class, averaged over the five growth conditions (slightly rounded), along with its noise, defined $\eta_i = N_i^{-1}\sigma_i$, where $\sigma_i^2 = \langle \delta N_i^2 \rangle$ is the variance over the five growth conditions. The term "noise" is appropriate here insofar as we can regard the data effectively as a simulation of those temporal fluctuations in the size distribution which would occur when the growth medium is the fluctuating natural environment.

TABLE 1: Fluctuating Macromolecule Size Distribution in a Bacterial Cytoplasm, Based on 2d Gel Data of Petersen et al. (ref 8)[a]

size class s_i	2d gel range	abundance N_i ($\times 10^4$)	noise η_i
200 aa	100−300 kDa	25	0.33
400	300−500	40	0.35
600	500−700	20	0.08
800	700−900	10	0.22
1000	>900	5	0.16

[a] Coarse-divided into size classes. The size unit is number of amino acid residues (aa). Note that the ribosome complement of the cytoplasm is not included in this dataset. There also exist some further ambiguities in the interpretation of the data which are glossed over here. For example, protein subunits which are oligomerized in the cell may break up in the gel, compromising reconstruction of the size distribution. In the development of the text we assume moreover that each size class fluctuates independently, whereas in reality they may be coupled, for example by a limited overall reservoir of amino acids available to the cell.

Clearly the size distribution undergoes quite large swings in response to growth medium, confirming and quantifying the connection (i)−(ii) above. To complete the connection (ii)−(iii), we propose the following framework. We first define diffusional volatility according to the noise paradigm, i.e., $\eta_D^2 = D^{-2}\langle \delta D^2 \rangle$, distinguishing the respective contributions of cytoplasmic water (w) vs macromolecule expression (e).

$$\eta_D^2 = \eta_{D,w}^2 + \eta_{D,e}^2 \tag{1}$$

Here, for the present purpose, it is convenient to neglect expression of macromolecules other than proteins, i.e., those

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TABLE 2: Quantitative Calculation of Diffusional Volatility[a]

		noise, η	diffusion coupling, Ω
cytoplasmic water, V_w	10^{-12} mL	0.16	1.29 ± 0.05
expression			
total no. of proteins, M_0	10^6	0.17	1.4 ± 0.2
total weight, M_1	460×10^6 aa	0.14	-5.6 ± 1.5
polydispersity, M_2	2.6×10^{11} aa^2	0.11	3.8 ± 1.6
diffusional volatility, $\eta_D = [\sum (\Omega\eta)^2]^{1/2}$			**0.94 ± 0.19**

[a] The value $\eta_w = 0.16$ in the first row is estimated from the variance in cytoplasmic water per cell measured by Cayley et al. for *E. coli* cultures over six different growth osmolalities in the range 0.03−1.0 Osm (Table 1, ref. 7). The $\eta_{(k)}$ are estimated by mapping from the Petersen et al. data of Table 1 (9); coupling coefficients are calculated by molecular dynamics simulation (Supporting Information).

not included in the Petersen et al. dataset, perhaps most importantly ribosomes, although it is understood that these contribute in principle.

Next, it is expedient to transform to a moment representation of the expression profile, $M_k = \sum_i s_i^k N_i$, as shown in the first column of Table 2. The zeroth and first moments (k=0,1) are respectively the total number and total weight of proteins expressed in the cytoplasm, while the second moment (k=2) is related to the width ("polydispersity") of the size distribution. To the kth moment we can attribute corresponding noise $\eta_{(k)}$, by mapping straightforwardly from the η_i of Table 1.[9] The respective contributions to diffusional volatility are then defined

$$\eta_{D,e}^2 = \sum_k (\Omega_k \eta_{(k)})^2; \quad \eta_{D,w}^2 = (\Omega_w \eta_w)^2 \tag{2}$$

where $\eta_w^2 = V_w^{-2}\langle \delta V_w^2 \rangle$, and where we have introduced coupling coefficients

$$\Omega_k = \frac{M_k}{D}\frac{\partial D}{\partial M_k}; \quad \Omega_w = \frac{V_w}{D}\frac{\partial D}{\partial V_w} \tag{3}$$

These latter parameters may be calculated via statistical mechanical methods, to varying degrees of sophistication. We choose here to implement a molecular dynamics simulation treating the proteins as compact colloidal-like hard spheres, following in the spirit of Weiss et al.[3] Details are given in the Supporting Information provided.

By combining the simulation results with the proteomic data of Table 1, and with cytoplasmic water data from Cayley et al.,[7] we arrive in Table 2 at an estimate $\eta_D \approx 1$ for the overall diffusional volatility of bacterial cytoplasm. It is noteworthy, as shown by the breakdown of the table, that the effect is not dominated by any single moment or by the volume term. Indeed, by neglecting higher moments, we have only estimated a lower bound on magnitude of the effect. The present estimate compares favorably, nonetheless, with the recent experimental investigation of Golding and Cox monitoring RNA mobility in *E. coli*,[5] where the diffusion coefficient is seen to effectively double when growth conditions are switched from rich to minimal. Interestingly, the variance in diffusivity seen between different cells of the same culture is markedly lower, as measured for example in the protein-tracking experiments of Elowitz et al.[1] A relevant distinction exists between "intrinsic" vs "extrinsic" contributions to noise in systems biological circuits.[10,11] The noise of our Table 1 is nominally of the "extrinsic" type. However, this data arguably also hides an "intrinsic" component occurring independently of the growth medium, and it is this which is responsible for between-cell variance.

In conclusion, we have outlined a crowding-based interpretation of the globally volatile diffusional character of microbial cytoplasm. We have left open some further lines of enquiry. For example, it will be interesting to determine whether diffusion-coupling to other environmental stimuli beyond the growth medium may be similarly rationalized, temperature in particular. Another avenue concerns the ongoing debate over whether and to what extent cytoplasmic biochemistry generally favors 1D diffusional processes over 3D processes.[12] Our initial impression here is that quite apart from its relative slowness, 3D diffusion may also be further handicapped by a stronger sensitivity to environment.

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Supporting Information Available: Details pertaining to how we have used molecular dynamics simulations and experimental data to estimate the diffusional noise of cytoplasm. This material is available free of charge via the Internet at http://pubs.acs.org.

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