Pacman percolation: a model for enzyme gel degradation

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We study a model for the gel degradation by an enzyme, where the gel is schematized as a cubic lattice, and the enzyme as a random walker, that cuts the bonds over which it passes. The model undergoes a (reverse) percolation transition, which for low density of enzymes falls in a universality class different from random percolation. In particular we have measured a gel fraction critical exponent $\beta = 1.0 \pm 0.1$, in excellent agreement with experiments made on the real system.

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The extracellular matrix (ECM) is a gel composed by various proteins, including collagen, elastin, fibronectin and laminin, connected to form an elastic network that extends macroscopically. This gel is normally impermeable to cell passage, and ensures organ integrity by isolating organs and preventing cell dissemination. Moreover it is the support of cell adhesion and regulates cell proliferation, differentiation and locomotion. During specific processes, the ECM can be degraded by a variety of proteolytic enzymes, especially metalloproteinases, that catalyze the hydrolysis of the peptide bonds, increasing the permeability of the ECM to the passage of cells.

This degradation process can at some point solubilize the gel, realizing a reverse “gel-sol” transition, and bringing the ECM to a liquid state, in which cells are no longer confined and can freely diffuse. This solubilizing transition is especially connected with tumor invasiveness, in which some cells access the lymphatic and blood circulation, and disseminate to distant organs (metastasis).

In this view, beyond the biochemical processes involved at molecular level, the understanding of the physical mechanisms of the ECM degradation is of great importance.

The passage from a liquid to a gel state, is a critical phenomenon\textsuperscript{1, 2}, in which soluble monomers bind to form larger and larger clusters. At some point, when the bond density $p$ becomes greater than some critical threshold $p_c$, an “infinite” cluster extending macroscopically is formed, and the network becomes a solid gel with an elastic response. The reverse transition, in which bonds are removed, and the system goes from a gel to a liquid state, can be clearly described in the same framework. The theory of critical phenomena predicts that, near the transition, the macroscopic quantities describing the system are related to the distance from the transition $(p-p_c)$ by power laws. The average cluster diameter diverges as $|p-p_c|^{-\nu}$, while the weight average mass as $|p-p_c|^{-\gamma}$. The viscosity diverges as $|p-p_c|^{-\kappa}$ below the transition ($p < p_c$), and stays infinite above it, while the gel fraction (the density of the infinite cluster) and the elastic modulus, that are zero below the transition, grow above it respectively as $|p-p_c|^\beta$ and $|p-p_c|^t$. It is important to point out that exponents $\nu$, $\beta$, $\gamma$, etc. . . are universal, that is they do not depend on the microscopic details of the system, but only on characteristics like the dimensionality of the system, or whether or not there is a long range correlation in the distribution of the bonds.

Recently, a series of interesting experiments have been realized to study the in vitro degradation of protein gels by exogenous proteinases, under cell-free conditions\textsuperscript{3}. In particular, in\textsuperscript{3} it was shown that a gel-sol transition adequately describes the degradation of the gel. Two kinds of gel, fibronectin and ECM gel, and three kinds of enzyme, thermolysin, trypsin and proteinase K, were used in various combinations. An enzyme solution was added to a certain quantity of gel, and the solubilized fraction $X_{sol}(t)$ of peptides was measured as a function of time. The balance between enzyme diffusion inside the matrix, and proteolysis reaction, was such that the enzyme reached an homogeneous distribution inside the gel before the reaction had proceeded to a notable extent, so that the experiments studied the volume, and not surface, degradation of the gel. The gel-sol transition is reached at some time $t_c$, when $X_{sol}(t)$ becomes equal to one, or when the gel fraction $X_{gel}(t) = 1 - X_{sol}(t)$ becomes zero. With various gel-enzyme combinations, and different enzyme concentrations, it was found that $X_{gel} \propto |t-t_c|^\beta$, with $\beta \approx 1$, for $t < t_c$. For ECM gel and trypsin for example $\beta = 1.01 \pm 0.03$\textsuperscript{3}. It may be assumed that the density of hydrolyzed bonds is a linear function of time, $(p-p_c) \propto (t-t_c)$, at least near the transition threshold, so that $X_{gel} \propto |p-p_c|^\beta$, with $\beta \approx 1$, for $p > p_c$.

This result is quite unexpected, because sol-gel transition is usually well described by random percolation, which is obtained when each bond between two monomers is present with probability $p$, and there is no correlation between different bonds. Random percolation in three dimensions gives a critical exponent $\beta = 0.41$, very different from the one measured in the gel degradation experiments. In Ref.\textsuperscript{3} different possible explanations of this discrepancy are proposed, including the fact that the proteins of the gel may be considered as interpenetrated polymer coils, that not all the solubilized fraction may be measured in the experiments, or that bonds of the gel phase may be catalyzed more efficiently than those of the liquid phase.

Another possibility, in order to explain the change in the universality class with respect to random percolation,
is the presence of a long range correlation in the distribution of non-hydrolyzed bonds. The correlation function is defined as $G(r) = \langle \rho(r') \rho(r+r') \rangle - \langle \rho(r') \rangle^2$, where $\rho(r)$ is the density of bonds, and the average is done over the reference position $r'$. It was shown by Weinrib and Halperin that if the correlation obeys a power law $G(r) \propto r^{-\alpha}$ at long distances with $a < d$ (where $d$ is the dimensionality of the system), then the percolation transition falls in a universality class different from the random percolation, in particular with a correlation length exponent $\nu = 2/a$. In the case of enzyme catalyzed gel proteolysis, the density of non-hydrolyzed bonds is certainly correlated, and if the enzyme concentration is small it may be correlated also at large distances.

In this paper, we study a very simple model, which we call "pacman percolation model", in which the protein gel is schematized as a cubic lattice of $N = L^3$ sites, where each site represent an (exavalent) monomer. At time $t = 0$, all the bonds between nearest neighbor monomers are present. One enzyme is then introduced in the system, that at each step goes from one site to a nearest neighbor site, chosen randomly between the six possible neighbors, and hydrolyzes (deletes) the corresponding bond if not yet hydrolyzed. Periodic boundary conditions are chosen. In Fig. 1i, it is shown the two-dimensional version of the model, after the enzyme has walked around for some time (roughly at the percolation threshold). Note how the remaining non-hydrolyzed bonds are spatially correlated, with respect to a random percolation model (Fig. 1b). At each time step, there will be a distribution of clusters, where two sites belong to the same cluster if there is a path of non-hydrolyzed bond between them. We measure then, as a function of the density $p$ of bonds: a boolean variable equal to one if there is a percolating cluster, to zero otherwise; the size of the percolating cluster, if any; the mean cluster size, that is $\sum n_s s^2$, where $n_s$ is the number of clusters of size $s$, and the percolating cluster is excluded from the sum. We perform the experiment many times with different random numbers, and average the above mentioned quantities, obtaining respectively the percolating probability $\Pi(p, L)$, the percolating cluster density $\rho(p, L)$, the mean cluster size $\chi(p, L)$, where we have exploited the dependence on the size $L$ of the lattice.

From these quantities, it is possible to evaluate the percolation density $p_c$ and the critical exponents $\nu$, $\beta$ and $\gamma$. Plotting the percolating probability $\Pi(p, L)$ as a function of $p$ for different lattice sizes $L$, it is possible to measure the percolation threshold density $p_c$, as the point in which the different curves intersect, for $L \rightarrow \infty$. In Fig. 2 it is shown the measured $\Pi(p, L)$ for cubic lattices of size $L = 30, 40, 50,$ and $60$. The intersection point (upper inset) is $p_c = 0.139 \pm 0.001$. Plotting then $\Pi(p, L)$ as a function of $(p-p_c)L^{1/\nu}$, one can measure the correlation length exponent $\nu$ as the value that gives the best collapse of the curves. In the lower inset of Fig. 2 it is shown the obtained data collapse, that gives $\nu = 1.8 \pm 0.1$. The confidence interval is defined by looking when the curves, in the interval shown, do not collapse anymore within the error bars. In Fig. 3 it is shown the percolating cluster density for the same system sizes. Plotting $\rho(p, L)L^{\beta/\nu}$ as a function of $(p-p_c)L^{1/\nu}$, one can measure the exponent $\beta$ (see inset). We find $\beta = 1.0 \pm 0.1$, in excellent agreement with the experimental result of Ref. 3. Finally, in Fig. 4 we measure the mean cluster size exponent $\gamma$, finding $\gamma = 3.5 \pm 0.2$. Note that $\nu$, $\beta$ and $\gamma$ satisfy well the hyperscaling relation $2\beta + \gamma = \nu d$, expected on general grounds. In Tab. 1 the critical exponents found are

| $p_c$ | experiment | random percolation |
|------|------------|--------------------|
| 0.139 ± 0.001 | 0.2488 |
| $\nu$ | 1.8 ± 0.1 | 0.88 |
| $\beta$ | 1.0 ± 0.1 | 1.0 ± 0.1 |
| $\gamma$ | 3.4 ± 0.2 | 1.80 |
| $\mu$ | 3.5 ± 0.1 | 2.0 |
| $s$ | 1.1 ± 0.1 | 0.73 |

TABLE I: Percolation density and critical indices in the pacman percolation model, and in random percolation, in three dimensions.
FIG. 2: Percolation probability $\Pi(p, L)$ as a function of the bond density $p$, for cubic lattices of size $L = 30, 40, 50, 60$. Upper inset: the point of intersection of the curves. Lower inset: data collapse obtained plotting $\Pi(p, L)$ versus $(p - p_c)L^{1/\nu}$, with $\nu = 1.8$.

FIG. 3: Density $\rho(p, L)$ of the percolating cluster as a function of the bond density $p$, for the same lattice sizes of Fig. 2. Inset: data collapse obtained plotting $\rho(p, L)L^{\beta/\nu}$ versus $(p - p_c)L^{1/\nu}$, with $\nu = 1.8$ and $\beta = 1.0$.

compared with those of random percolation. These results show that the model of “pacman percolation” falls into another universality class with respect to random percolation.

We have also tried to verify the relation predicted by Weinrib and Halperin between the exponent $\nu$ and the power law governing the decay of correlations. In Fig. 4 the correlation $G(|i - j|) = \langle n_i n_j \rangle - \langle n_i \rangle \langle n_j \rangle$ in the occupation of the bonds $i$ and $j$, where $|i - j|$ is the distance between the centers of the bonds, is shown for a system of size $100^3$ at the percolation threshold $p = 0.139$. The correlation obeys a power law $G(r) \sim r^{-a}$ with $a = 1.15\pm0.05$, with an exponential cut-off, presumably due to finite size effects, at distances larger than $r \sim 30$. The relation $\nu = 2/a$ predicted by Weinrib and Halperin, is quite well verified within the errors. It has been recently argued that for such a model the correlation between bonds should decay as $1/r$, implying $a = 1$ and $\nu = 2$. The prediction, however, is valid only if some conditions are verified, such as long times and large distances. The discrepancy between this prediction and our results may be due to the fact that these asymptotic regimes are not reached in our simulations.

To complete our study, we have analyzed the critical indices of the conductivity in the random resistor and conductor-superconductor networks. In the first case each present bond of the model is substituted with a resistor of unitary conductance, while absent bonds have zero conductance. The total conductivity $\Sigma$ of the model is then measured as a function of bond density, and it is zero for $p < p_c$, while it grows as $|p - p_c|^\mu$ for $p > p_c$. Using finite size scaling as usual (see Fig. 5) we find...
FIG. 6: Conductivity of the random-resistor network.

$$\mu = 3.5 \pm 0.1$$. In the second case each present bond of the model is substituted with a superconductor of infinite conductance, while absent bonds are substituted with resistors of unitary conductance. In this case the total conductivity $\Sigma$ diverges as $|p - p_c|^s$ for $p < p_c$, and stays infinite for $p > p_c$. In this case we find $s = 1.1 \pm 0.1$ (see Fig. 7).

It was proposed some time ago \[8, 9\], that the exponents $\mu$ of the resistor network, and $s$ of the conductor-superconductor network, could be in correspondence respectively with the exponents $t$ of the elastic modulus, and $k$ of the viscosity. The first correspondence is based on the analogy between the equilibrium condition of sites under elastic tension ($\sum_j k_{ij}(r_i - r_j) = 0$) and the Kirchhoff equations for the current conservation ($\sum_j \sigma_{ij}(V_i - V_j) = 0$). However, the electrical problem is a pure scalar problem, while the elastic one has a vectorial nature, so the macroscopic elastic response is in principle determined by more than one elastic modulus.

It is worth to notice that, if the density of enzyme increases, the process of degradation of the gel becomes closer to a random degradation. Indeed, in the limit in which each bond is hydrolyzed by a different enzyme, there will be no correlation between them. This is confirmed by our simulations. We have analyzed the behavior of the model with a finite density $\rho_E$ of enzymes (defined as the ratio between the number of enzymes present and the number of sites of the lattice), and we have found that for densities greater than $\rho_E \simeq 0.8$ the system falls in the universality class of random percolation. For $\rho_E = 0.8$ we find $\nu = 0.88 \pm 0.02$ and $\beta = 0.41 \pm 0.01$, in excellent agreement with random percolation \[1\].

In conclusion, we have used a percolation model to study the degradation process of ECM due to the action of enzymes. Our results shows that, for low density of enzymes, our model belongs to a different universality class from random percolation. The change in the critical indices may be due to long range correlation. If the density of enzymes is sufficiently high, the correlation between bonds disappears and there is a crossover to random percolation.

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